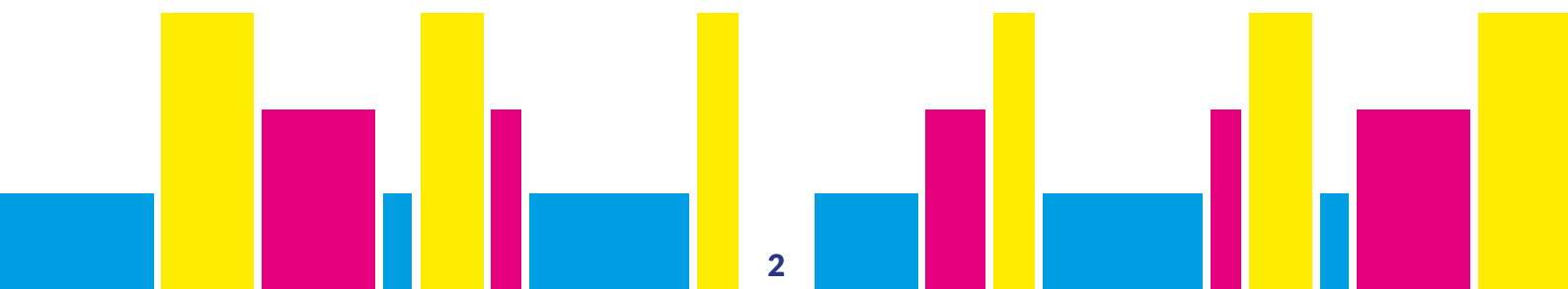# ICAVS12

12th International Conference on Advanced Vibrational Spectroscopy

27.08-01.09.2023, Krakow, Poland

ABSTRACT BOOK

Perspective lectures

Perspective 1

Title: *Progress in infrared spectroscopy*

Author: Kathleen Gough[1]

[1]University of Manitoba

Abstract:
In her scientific biography of her husband, Pierre Curie, Marie Curie described some of her early efforts to become scientifically educated as a young woman in Warsaw, writing "I was taught that the way of progress is neither swift nor easy".[1] Fortunately for those of us using vibrational spectroscopy as our method of choice, infrared spectroscopic tools are constantly being enhanced with myriad innovative ways of getting our data and of correlating it with other techniques, to create deeply informative pictures of our targets. In this talk, I will focus on a few of these targets and approaches: (Figure 1, Panel A) near field infrared (nano-FTIR) via sSNOM methods applied to collagen fibrils;[2] (Figure 1, Panel B) far field Optical PhotoThermal IR (O-PTIR) at ~400 nm resolution imaging of cells, correlated with super resolution fluorescence (SRF) images, layers deconvolved and overlaid, resolution X, Y: 102 nm, Z-stack: 200 nm;[3] and (Figure 1, Panel C) the more conventional wavelength-dependent far field FTIR imaging with a Focal Plane Array and 5x magnification optics, illustrating the spectroscopic analysis of Arctic sea ice diatoms from the Northwest Passage, Canada.[4] Both near field and O-PTIR methods inherently involve polarized light, while IR polarizers can be used with FTIR instruments. Burgeoning developments in the field are constantly being announced. The need for these advances is immediately evident in the applications that they enable, from disease diagnostics to novel materials to climate change.

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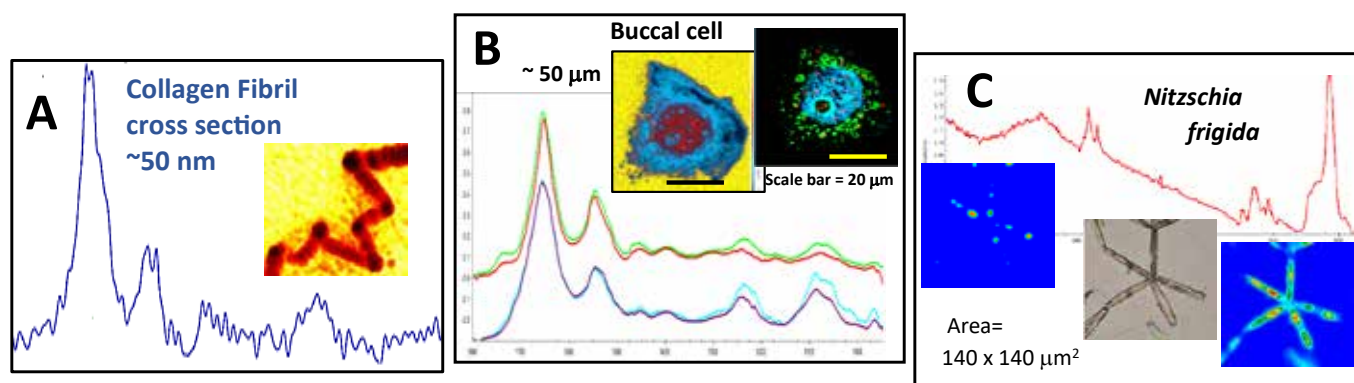
Figure captions:
Figure 1. A: nano-FTIR and heat map B: O-PTIR, ratio images show nucleus (red) and cytoplasm (blue) matching correlated SRF image C: FPA FTIR. Left: lipid, Right: protein; Centre: diatom colony.

Keywords: nano-FTIR, O-PTIR, SRF, FTIR FPA

Perspective 2

Title: *Frontiers of Advanced Vibrational Spectroscopy: The Molecular Chirality Perspective*

Author: Laurence Nafie[1]

[1]Syracuse University

Abstract:
The intersection of vibrational spectroscopy and molecular chirality is embodied principally by two phenomena, one infrared and one Raman, collectively called vibrational optical activity (VOA).[1] As is now well known, the infrared case is vibrational circular dichroism (VCD) the difference in the absorbance of left minus right circularly polarized infrared radiation that results in a vibrational change of state. The Raman case is simply Raman optical activity (ROA), the difference in Raman scattered intensity for right minus left incident circularly polarized radiation (ICP), scattered CP radiation (SCP) or dually CP, either in-phase (DCP$_I$) or out-of-phase (DCP$_{II}$) that likewise results in a vibrational transition. Both VCD and ROA were discovered experimentally in the early to mid 1970s, and as such preceded what is now a frontier of vibrational spectroscopy. In this perspective talk, the current frontiers of VOA will be described with an eye to where future advances are likely to occur.[2] These frontier VOA topics include, absolute configuration determination, modeling effects of solvent and anharmonicity, visualization of vibrational electron transition current density (TCD), resonance ROA and surface enhanced ROA, nuclear velocity perturbation theory of VCD, comparisons of VCD and ROA for the same systems, and supramolecular chirality in amyloid fibrils and related biological molecules.

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1. Vibrational Optical Activity: Principles and Applications by Laurence A. Nafie, John Wiley & Sons, Ltd., Chichester, (2011).
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Keywords: vibrational, optical, activity, chirality, Raman

Perspective 3

Title: *Strategies and perspectives to investigate the heme-enzymatic mechanism by resonance Raman spectroscopy*

Author: Giulietta Smulevich[1]

[1]Dipartimento di Chimica "Ugo Schiff" (DICUS), Università di Firenze

Abstract:

Fifty years ago, Thomas Spiro applied for the first time resonance Raman spectroscopy (RR) to study oxyhemoglobin [1]. The selective enhancement of the Raman scattering from specific modes under resonance conditions of chromophores embedded in a complex structure, enabled him to conclude that RR could be used to monitor the structural consequences of ligation or electron transfer in heme proteins.

Heme proteins perform a wide variety of essential biological functions, including oxygen storage and transport, oxygen reduction, electron transfer, catalysis, gas sensing, etc. In other words, biological systems rely on heme-proteins to carry out basic functions essential for their survival.

I don't know whether it would have been possible to predict the amazing development in the use of resonance Raman spectroscopy as a tool in biochemistry and biology to understand heme function at the molecular level could have been predicted. Certainly, the use of micro-RR as well as the time-resolved approach, together with the constant development of new technologies and biochemical techniques, such as site directed mutagenesis, ensured that RR would become a very important tool to dig out the structure-function relationship in heme proteins [2-4].

In this presentation, I will attempt to delineate different applications of RR in this field and demonstrate how subtle structural features allowed us to disclose enzymatic mechanisms.

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1. T.G. Spiro, T.C. Strekas, Resonance Raman Spectra of Hemoglobin and Cytochrome c: Inverse Polarization and Vibronic Scattering, Proc. Nat. Acad. Sci. USA 69 (1972) 2622-2626.
2. G. Smulevich, A.Feis, B.D. Howes, Fifteen years of Raman spectroscopy of engineered heme containing peroxidases: what have we learned? Acc. Chem. Res. 38 (2005) 433-440.
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Keywords: RR-Microscopy, Structure-Function Relationship.

Plenary lectures

Plenary 1

Title: *Molecular Optomechanics Approach to Surface-Enhanced Raman Scattering*

Author: Javier Aizpurua[1]

[1]Center for Materials Physics (CSIC-UPV/EHU)

Abstract:
The possibility to exploit the interaction between molecular vibrations and localized plasmons in nanoscale cavities has set a new regime of optomechanics, where near-strong optomechanical coupling has been identified in surface-enhanced Raman spectroscopy (SERS) of organic molecules. The use of light localized onto atomic protrusions in metallic cavities produces extremely small effective mode volumes, down to the atomic scale, in the so-called picocavities, which enhance the interaction between cavity photons and molecular vibrations. To capture the complexity of modes of typical plasmonic nanocavities used in SERS configurations, a complete continuum-field model based on the description of the nanocavity Green's function is needed, beyond the single optical mode approximation. Within this molecular optomechanics context, one can identify and theoretically describe strong nonlinearities of the Stokes and anti-Stokes photons emitted from nano- and pico-cavities as a function of incident laser intensity. Moreover, a shift of the frequencies of the molecular vibrational fingerprints, analog to the optical spring effect in standard optomechanics can also be identified, and experimentally validated by annalysing the Raman signal from molecules in such nanocavities. Finally, collective effects, where the vibrations of different molecules coherently couple to each other building up a bright collective vibrational supermode, are also explored to quantitatively explain the Stokes signal from molecular self-assemblies. Molecular optomechanics enables a new regime of interactions between nanocavity photons and mechanical vibrations at room temperature, which could be used to control molecular reactivity on demand, and exploit vibrational states of matter for quantum technology applications.

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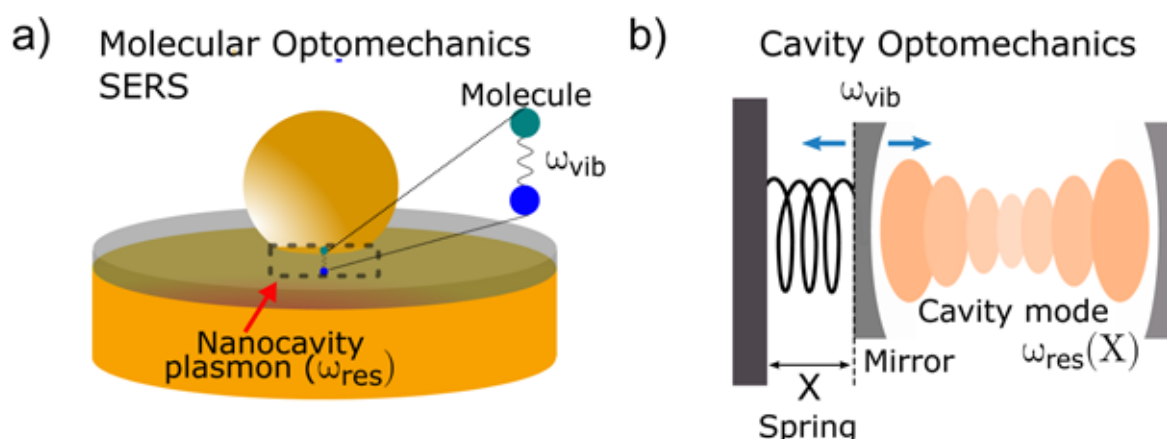
Figure captions:
Analogy between a typical SERS configuration (a) and a macroscopic optomechanical system (b). Both systems show a resonance cavity frequency (res) and a vibrational frequency (vib).

Keywords: SERS, Molecular optomechanics, picocavity, nonlinearities

Plenary 2

Title: *Increasing the utility of infrared spectroscopic imaging by high performance instrumentation and AI*

Author: Rohit Bhargava[1]

[1]Departments of Bioengineering, Electrical & Computer Engineering, Mechanical Science & Engineering, Chemical and Biomolecular Engineering, and Chemistry, Beckman Institute for Advanced Science and Technology, Cancer Center at Illinois, University of Illinois at Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801 USA

Abstract:
Infrared spectroscopic imaging combines the ability to record molecular content with the ability to visualize its spatial diversity. Arising from fundamental vibrational modes of molecules, IR absorption is the strongest optical signal indicative of composition and provides extensive, rich data that has the potential to be rapidly recorded. In an imaging format, it represents a unique opportunity to understand the chemical composition of tissue and used artificial intelligence (AI) workflows to study disease progression.[1] However, given the need to record a significantly larger quantity of data than a typical microscopy image (MB vs. GB) and the extensive bandwidth of the spectra (~10 um), trade-offs often have to be made between the closely related considerations of signal to noise ratio, spatial-spectral coverage, resolution and optical arrangements. Here, we present a path from rigorous theory to modeling with design that builds in the ability to integrate AI. This approach offers to realize new advantages in fundamentally changing traditional trade-offs and provide new capability. We first describe a new microscope design for increased speed and rapid coverage that is useful for biomedical and clinical tissue imaging. Next, we describe a configuration to measure chirality in samples that promises higher spectral information that present methods.[2] We show a progression of capabilities that allow for increasingly precise, localized and high quality mapping. Finally, we present a new approach to nanoscale IR imaging that provides greater fidelity and speed at unprecedented levels of signal to noise ratio, allowing cellular imaging in detail.[3] For each instrumentation advance, examples of use cases will be presented and emerging directions discussed.

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3. S. Kenkel, M. Gryka, L. Chen, M.P. Confer, A. Rao, S. Robinson, K.V. Prasanth, R. Bhargava, Chemical imaging of cellular ultrastructure with null-deflection infrared spectroscopy, Proc. Natl. Acad. Sci. 119 (2022) e2210516119.

Keywords: Infrared spectroscopy, imaging, instrumentation, artificial intelligence, pathology

Plenary 3

Title: *Raman Imaging of Plant Cells: probing distribution and orientation of molecules*

Author: Notburga Gierlinger[1]

[1]University of Natural Resources and Life Sciences Vienna (BOKU)
Austrian Science Fund (FWF): START Y-728-B16
European Research Council: ERC consolidator grant 681885

Abstract:
Plants are the most important natural resource to make the transition to a sustainable environment and society. We aim to deepen our understanding of intrinsic properties of the plant cell walls and surfaces as well as cell morphogenesis by Confocal Raman microscopy. Our plant samples include strong lignified materials like wood and nutshells, plant model systems like Arabidopsis and algae and important economic fruits like tomato.
The outer plant surface, the cuticle, fulfils many physiological and protective functions. Raman imaging revealed distinct patterns of lipids, carbohydrates and phenolic components of the cuticle and transition to the epidermal layer. Based on Raman spectra we distinguished phenolic acids and flavonoids and their different distribution suggested different roles in mechanical support and UV-protection [1-2].
Cell morphogenesis was studied in the star-shaped algae Micrasterias and puzzle cells of walnut shells by Raman imaging. Spectral differences between the indent and tip region of the primary cell walls of the Micrasterias lobes were scarce, but a spectral unmixing approach pointed to more cellulose fibrils deposited in the indent region. Therefore, we suggested that cell wall thickening together with a denser network of cellulose microfibrils stiffens the cell wall at the indent and induces different cell wall extensibility to shape the lobes [3]. A similar lobe formation mechanism was revealed in the 3D-puzzle cells of walnut shell [4]. Raman imaging opens the chemical view on plant cell walls on the micro- and nano-level in the native plant cell walls during growth. Based on these in-depth insights we can retrieve structure-function relationships and come up with a better understanding of properties and development of plant cell walls – one of our most important sustainable resources for food, materials and energy.

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Figure captions:
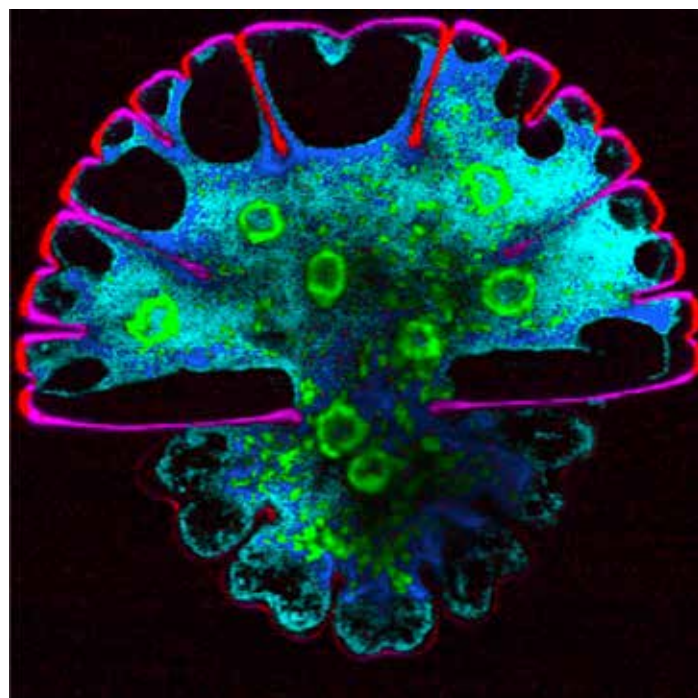Fig. 1: Raman image of Micrasterias: cellulose orientation (red, pink), starch (green), proteins and lipids (blue, cyan) [3]

Keywords: cell wall, cellulose, multivariate, unmixing,

Plenary 4

Title: *What we learn with new time-resolved Raman spectrometers*

Author: Koichi Iwata[1]

[1]Gakushuin University

Abstract:

Picosecond time-resolved Raman spectroscopy is an effective and attractive method when studying the structure and rise/decay time courses of short-lived chemical species. We have developed new time-resolved Raman spectrometers for spontaneous and non-linear Raman measurements.

A technical difficulty for picosecond time-resolved Raman spectroscopy is to secure a stable light souse that produces near Fourier-transform limited picosecond pulses. We convert the output from a femtosecond laser system, which tends to be better in its output stability than a picosecond one, to picosecond pulses by using a volume grating notch filter [1]. With this spectrometer, the power stability of the probe radiation is 0.8 %RMS for a time period of 100 minutes. The widths in wavenumber and time are 2.0 ps and 8.6 cm^{-1} in FWHM for 532 nm, and 3.2 ps and 6.0 cm^{-1} for 633 nm. Thermal diffusivity of lipid bilayer membranes dispersed in water has been estimated with picosecond time-resolved Raman spectroscopy.

Spontaneous resonance Raman spectroscopy in the near-infrared region beyond the detection limit of silicon-based detectors including CCDs is still a challenging task because of large noises produced by near-infrared detectors. We have developed a time-resolved multiplex stimulated Raman spectrometer with a picosecond Raman pump pulse of 1190 nm and femtosecond broadband probe covering the near-infrared spectral range of 900 to 1500 nm [2]. The intensity change of the probe radiation induced by the Raman pump is monitored by an InGaAs array detector. In the stimulated Raman detection scheme, shot noises from the probe radiation is dominant over noises from the near-infrared detectors. We have applied the time-resolved multiplex stimulated Raman spectrometer for various short-lived species including electronically excited states of C=C conjugated systems, carotenoids and organic semiconductors in particular.

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2. T. Takaya, K. Iwata, Development of a femtosecond time-resolved near-IR multiplex stimulated Raman spectrometer in resonance with transitions in the 900–1550 nm region, Analyst 141 (2016), 4283-4292.

Keywords: time-resolved spectroscopy, spontaneous Raman, stimulated

Plenary 5

Title: *Advances and applications in FTIR spectroscopic imaging for studies of dynamic systems*

Author: Sergei Kazarian[1]

[1]Imperial College London

Abstract:

FTIR spectroscopy combined with the imaging capability of infrared array detectors provides a powerful tool to obtain both chemical and spatial information about the sample. The use of micro ATR approach to acquire FTIR spectra offers an increased numerical aperture due to the high refractive index of the ATR crystal. This leads to significantly improved spatial resolution being achieved with ATR-FTIR imaging compared to imaging in transmission or reflection modes. The improved spatial resolution allows one to obtain chemical images of many samples without recourse to a synchrotron source of infrared radiation.[1] This makes enhanced chemical imaging previously hampered by the inadequate spatial resolution, possible for many samples.[2] We have demonstrated applications of micro ATR-FTIR imaging to polymer blends, biomaterials, including biopsies, pharmaceuticals, hair and artwork.[3]

This talk will demonstrate new opportunities and applications of ATR-FTIR imaging with macro ATR accessories. For example, we developed ATR-FTIR imaging into a powerful analytical tool for analysis of materials and biomedical samples, and for studies of dynamic systems, such as tablet dissolution and drug release. Other new opportunities using this approach include imaging of medical and forensic samples, biomaterials, ionic liquids, high-throughput analysis, stone conservation.[3] It is particularly suited to studying microfluidics due to its high chemical specificity and ability to study dynamic systems. Microfluidics facilitate a high degree of control of chemical reactions with only small quantities of reagents. ATR-FTIR spectroscopic imaging was used to investigate secondary structure of model proteins under freeze thaw cycling stress conditions, which can lead to a substantial loss of product, and contributes to the high cost of antibody production. This research also aims to demonstrate the suitability of FTIR spectroscopic imaging for biopharmaceutical process monitoring.

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3. GL Liu and S G Kazarian, Recent advances in studies of cultural heritage using ATR-FTIR spectroscopy and ATR-FTIR spectroscopic imaging, Analyst, 147 (2022) 1777.
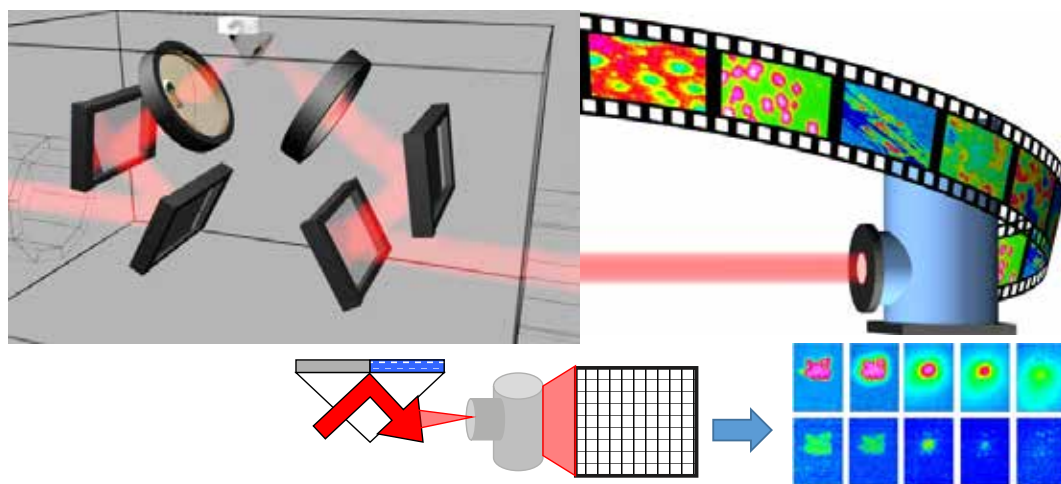
Figure captions:

ATR-FTIR spectroscopic imaging enables a variety of dynamic phenomena to be simultaneously observed due to chemical specificity and spatial resolution (e.g. tablet dissolution and drug release).

Keywords: ATR, FTIR, imaging, microfluidics, monoclonal-antibody

Plenary 6

Title: *Ultrafast Structural Dynamics in Various π-Conjugated Molecular Systems Probed by Time-resolved Electronic and Vibrational Spectroscopy*

Author: Dongho Kim[1]

[1]Department of Chemistry, Yonsei University, Seoul 03722, Korea
E-mail:dongho@yonsei.ac.kr

Abstract:
Aromaticity, the special energetic stability derived from cyclic [4n+2]π-conjugation, has been the topic of intense interest in chemistry. Recently, the pioneering work by Colin Baird on aromaticity reversal, postulating that aromatic (antiaromatic) character in the ground state reverses to antiaromatic (aromatic) character in the lowest excited triplet state, has attracted much attention. The completely reversed aromaticity in the excited states provides direct insight into understanding the properties of photoactive materials. However, most studies on excited state aromaticity have been based on the theoretical point of view. Time-resolved optical spectroscopies can provide a new and alternative avenue to experimentally evaluate excited state aromaticity. With monitoring ultrafast changes in the excited states, they can provide valuable information for excited state aromaticity. In this regard, recent breakthroughs in experimentally assessing aromaticity reversal in the excited states with time-resolved optical spectroscopic measurements are introduced. To scrutinize this intriguing and challenging scientific issue, expanded porphyrins have been utilized as the ideal testing platform because they exhibit perfect aromatic and antiaromatic congener pairs, having the same molecular framework but with different numbers of π-electrons, which facilitates the study of the pure effect of aromaticity. Time-resolved electronic and vibrational absorption spectroscopies capture the change of electronic structure and molecular conformations driven by the change of aromaticity and provide clear evidence for aromaticity reversal in the excited states. These approaches will pave a way for the development of new experimental indices for the evaluation of excited state aromaticity and its applications.
Here, we will also discuss the ultrafast coherent exciton dynamics in a series of cofacially stacked perylene bisimides (PBIs). First, we present coherent exciton transport and excimer formation dynamics from the Frenkel state of PBI dimeric and oligomeric H-aggregates. From the vibronic peak ratio analysis in the early-time transient fluorescence spectra obtained by femtosecond broadband fluorescence upconversion spectroscopy, the initial spatial coherence and its evolution are directly unraveled. Second, we introduce symmetry breaking charge separation dynamics via excimer intermediate state in a cyclophane bridged PBI dimer. Based on our observation that the rise time of PBI anion and cation bands in the transient absorption spectra is equivalent to the decay time of the excimer fluorescence, we suggest that the excimer state can effectuate the charge transfer dynamics in the cofacially stacked PBI dimer. Our findings on ultrafast coherent exciton dynamics in various PBI aggregate systems will provide valuable insights into future applications in the field of molecular optoelectronic materials to achieve long-range coherent energy transfer and superb charge transfer efficiency.

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[6] Y. Hong et al. J. Am. Chem. Soc. 142, 7845–7857 (2020)
[7] Y. Hong et al., Nat. Commun. 14, 4488 (2022).

Plenary 7

Title: *Can attenuated total reflectance infra red spectroscopy (ATR-IR) be used with polarised light?*

Author: Alison Rodger[1], Paul Wormell[2], Jun Koshubu[3], Junya Kitamura[3], Akihiro Sato[3]

[1]Macquarie University
[2]Western Sydney University
[3]Jasco International

Abstract:
CAN ATTENTUATED TOTAL REFLECTANCE INFRARED SPECTROSCOPY (ATR-IR) BE USED WITH POLARISED LIGHT?
Attenuated total reflectance (ATR), where the light beam is totally internally relected by a crystal and the sample interacts with the electric fields of the evanescent light above the surface, has transformed the way routine infrared (IR) spectroscopy is performed in most laboratories, whether the application is for research, quality control or training. The simplicity of the ATR sample presentation, where a solid or a liquid is placed in contact with the surface of the ATR crystal, and the well-defined nature of the effective pathlength means that reproducible spectra can be measured quickly. We have found ATR sample presentation and reproducibility of the spectra is particularly useful when we are trying to collect aqueous protein spectra since the overlay of a large water absorbance signal on key protein bands means that baseline subtraction needs to be carried out very carefully. However, ATR has the disadvantage of having the effective path length (the penetration depth of the evanescent light) being dependent on the refractive index of the sample which in turn depends on the absorbance of the sample. For isotropic protein samples we have developed a theoretical approach to transform ATR spectra into the equivalent transmission spectrum [1,2]. In this talk we will share how far we have proceeded on the journey to extend ATR-IR spectroscopy into vibrational circular dichroism and vibrational linear dichroism, where the challenge is that not only does the sample determine the penetration depth of the light but it does so differently for the x-, y-, and z- components of the light.

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Keywords: ATR, VCD, VLD

Plenary 8

Title: *Theory is dead, long live theory: Hypothesis-centric machine learning in vibrational spectroscopy*

Author: Axel Mosig[1]

[1]Ruhr University Bochum, Center for Protein Diagnostics

Abstract:

The rapid advancement of deep learning in biomedical applications has been a key driver of breakthrough developments in computational pathology and other image based approaches that support medical diagnosis and treatment. This development has also reached vibrational spectroscopy, in particular in the field of infrared microscopy, where quantum cascade lasers facilitate rapid measurement of large number of tissue samples recruited in clinical studies. In this context, our work adresses a key problem: Training deep neural networks to localize patterns of disease in imaging data conventionally requires pixel precise annotations, which are notoriously difficult to obtain for vibrational microspectroscopic images. To overcome this problem, we introduce an approach based on the so-called comparative segmentation network (CompSegNet) that requires weak, coarse grained labels only, while pixel-precise localization patterns are being inferred during the process of training the neural network. We demonstrate the validity of our approach in several applications, where the CompSegNet successfully localizes disease patterns in infrared microscopic images. The CompSegNet can be understood as an explainable neural network approach: While deep neural networks are inherent black boxes that lack transparency, the localization map inferred by the CompSegNet makes its output interpretable and explainable. Driven by the question of what constitutes a scientifically valid explanation, we introduce a hypothesis-based framework for falsifiable explanations of machine learning models. In this framework, a falsifiable explanation is a hypothesis that connects the interpretable output inferred by a neural network with the sample from which the data originate. As we demonstrate, this framework provides answers to some fundamental questions in machine learning, and identifies explainable machine learning as the missing link between machine learning and the scientific method.

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Keywords: Infrared Microscopy, Deep Learning, Pathology

Plenary 9

Title: *In-Operando Magneto-Raman Study of Graphene in the Quantum Hall Regime*

Author: Angela Hight Walker[1]

[1]National Institute of Standards and Technology (NIST)

Abstract:
Raman spectroscopy, imaging, and mapping are powerful non-contact, non-destructive optical probes of fundamental physics in graphene and other related two-dimensional (2D) materials, including layered, quantum materials. An amazing amount of information can be quantified from the Raman spectra, including layer thickness, disorder, edge and grain boundaries, doping, strain, thermal conductivity, magnetic ordering, and unique excitations such as magnons and charge density waves. Most interestingly for quantum materials is that Raman efficiently probes the evolution of the electronic structure and the electron-phonon, spin-phonon, and magnon-phonon interactions as a function of laser energy and polarization, temperature, and applied magnetic field. Our unique magneto-Raman spectroscopic capabilities will be detailed, enabling spatially resolved optical measurements while simultaneously measuring electrical transport in a back-gated graphene Hall bar device. Raman and electrical data from an hBN-graphene-hBN device operating in the quantum Hall regime will demonstrate our novel capabilities. In addition, unconventional quantization plateaus from a PNP junction created via spatial photodoping by the Raman laser will be presented.
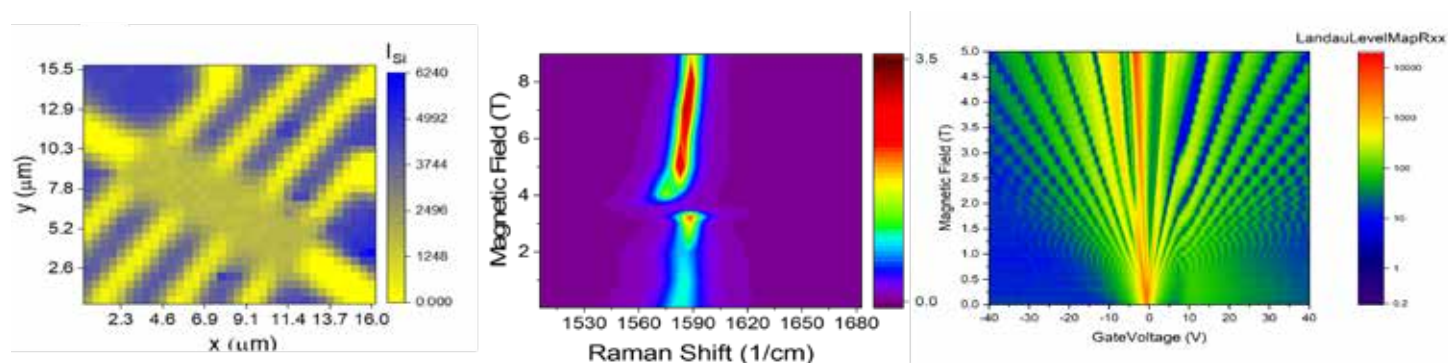
Figure captions:
Raman map of silicon peak highlighting device geometry. Raman graphene G peak as a function of the applied magnetic field. Magneto-transport; Graphene Fan Diagram

Keywords: Raman, magnetic, graphene, electrical, magneto-optical

Plenary 10

Title: *IR-control of ultrafast excited state dynamics in transition metal complexes*

Author: Julia Weinstein[1], Iona Ivalo[1], Rory Cowin[1], Martin Appleby[1], Catherine Royle[1], Igor Sazanovich[2], Dimitri Chekulaev[3], Anthony Meijer[1], Alexander Auty[1], Guaznhi Wu[1], Tao Cheng[1], James Shipp[1]

[1]University of Sheffield
[2]Central Laser Facility, Rutherford Appleton Laboratory
[3]Lord Porter Laser Laboratory, University of Sheffield

Abstract:
One of the fascinating challenges in the field of photoinduced charge separation is how to control reaction pathways, and direct reactivity "at will". Nuclear-electronic (vibronic) coupling is of particular interest in this regard since the Born-Oppenheimer approximation is not valid on the ultrafast timescales intrinsic to photo-processes. Perturbing vibronic coupling may thus offer a way to affect photochemical reactions.[1,2] Such perturbation can be achieved by introducing a narrow-band IR pulse after initial population of an excited state to selectively affect vibration(s) that are coupled to electron transfer processes; the overall sequence of ultrafast pulses used is {UVpump-"narrow"IRpump-IRprobe}, thereafter "IR-control".
Our work on IR-perturbation of electron transfer explores Pt(II) complexes as chromophores and/or bridges in the Donor-Bridge-Acceptor (DBA) systems. Selective excitation of bridge vibrations can change the yield of the product states, up to 100%.[3,4] To further develop potential for IR-control, several new series of DBA-systems have been synthesized. Their excited state dynamics was characterised by ultrafast transient absorption, time-resolved IR, 2DIR, and fluorescence upconversion[6] spectroscopies; the latter also allowing us to investigate the role of intersystem crossing in the branching process. In cases when a branching excited state was detected, IR-control studies were undertaken, to discover the role of several factors in the IR-control effect:
(i) Strong- vs. weak coupling regimes between Donor/Bridge and Bridge/Acceptor.
(ii) Investigation of the mechanism of the effect using isotopic substitution[5] of the bridge.
(iii) Tuning the energetics of the different steps in charge separation/recombination process.
The suggested mechanisms of the interplay between the acceleration and deceleration of individual processes and the yields of resulting states, and potential applications of the fundamental effects observed will be discussed.

References:
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[2] Yue, Y. et al, Dalton Trans., 2015, 8609.
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[4] Delor, M. et al, Nature Chem., 2015, 7, 689.
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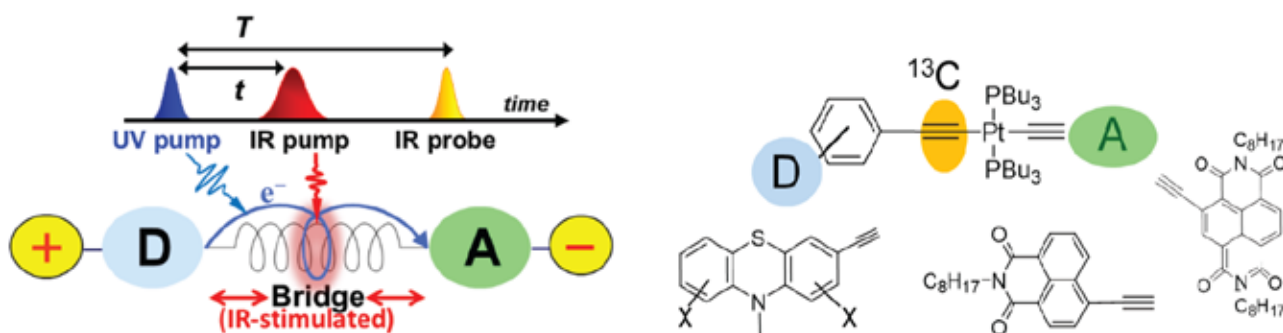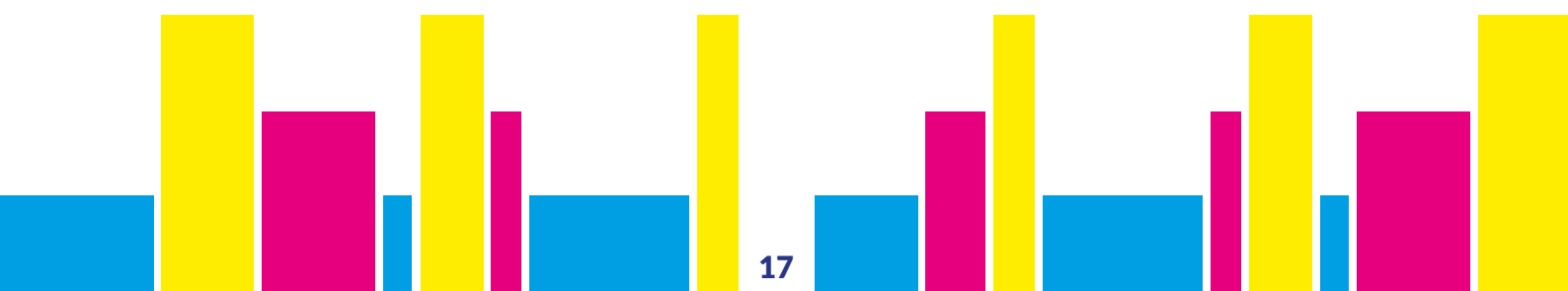[7] Yuly, J.L. et al. Biochim. Biophys. Acta, 2022, 148737.

Figure captions:
Schematic of the experiments performed and of the D-B-A molecules

Keywords: ultrafast, electron transfer, vibronic, 2DIR

Abstracts invited & oral

A-I.1

Title: *Inside block copolymer micelles – An AFM-TERS study on the interfacial influences on the core crosslinking efficiency*

Author: Christiane Höppener[1], Xinyue Wang[2], Johanna Elter[3], Felix Schacher[3], Volker Deckert[1]

[1]Leibniz Institute of Photonic Technologies (IPHT)
[2]Institute of Physical Chemistry, Friedrich Schiller University
[3]Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University

Abstract:
Amphiphilic block copolymers unify different chemical and mechanical properties in a single macromolecular structure, and can assemble in selective media into core-corona or core-shell-corona micelles. These nanostructures have received high attention for the development of new functional nanoscale biomedical materials,[1] and particularly, are of interest for the design of drug delivery systems. Therefore, understanding the property-structure relation of such systems is crucial. Tip-enhanced Raman scattering (TERS) paired with advanced AFM spectroscopy modes, which access nanomechanical properties of matter, can provide this information [2]. Here we investigate micelles formed from the amphiphilic diblock terpolymer poly(ethylene oxide)-*block*-polyfurfuryl glycidyl ether-*co-tert*-butylglycidyl ether) (PEO-*b*-P(FGE-co-*t*BGE). The FGE enables core crosslinking by initiating a furan-maleimide Diels-Alder reaction in the core region. TERS investigations of these micelles can clearly discriminate the different monomers. Furthermore, TERS enables distinguishing unreacted and crosslinked core regions by the detection of the Diels-Alder product. While the central core region shows a high degree of crosslinking, the crosslinking efficiency breaks down at the PEO-P(FGE-*co-t*BGE) interface. The sharpness of this transition zone is determined to ~5nm, which is also confirmed by supplementary AFM investigations probing the nanomechanical properties. Utilizing the insights provided by both techniques, it is coroborated that the breakdown of the crosslinking process at the interfacial region is caused by an exclusion of the hydrophobic crosslinker from the interfacial region due to the formation of a mixed PEO-FGE transition zone. Furthermore, the nanomechanical properties of this transition zone seem to impart the obtainable core-crosslinking efficiency in the central core region.[3]

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1. F. H. Schacher, P. A. Rupar, I. Manners, Functional block copolymers: nanostructured materials with emerging applications, Angew. Chem., Int. Ed. 2012, 51, 7898
2. C. Höppener, F.H. Schacher, V. Deckert, Multimodal Characterization of Resin Embedded and Sliced Polymer Nanoparticles by Means of Tip-Enhanced Raman Spectroscopy and Force–Distance Curve Based Atomic Force Microscopy, Small 2020, 16, 1907418
3. Höppener, J.K. Elter, F.H. Schacher, V. Deckert, nside Block Copolymer Micelles-Tracing Interfacial Influences on Crosslinking Efficiency in Nanoscale Confined Spaces, Small 2023, 2206451

Figure captions:
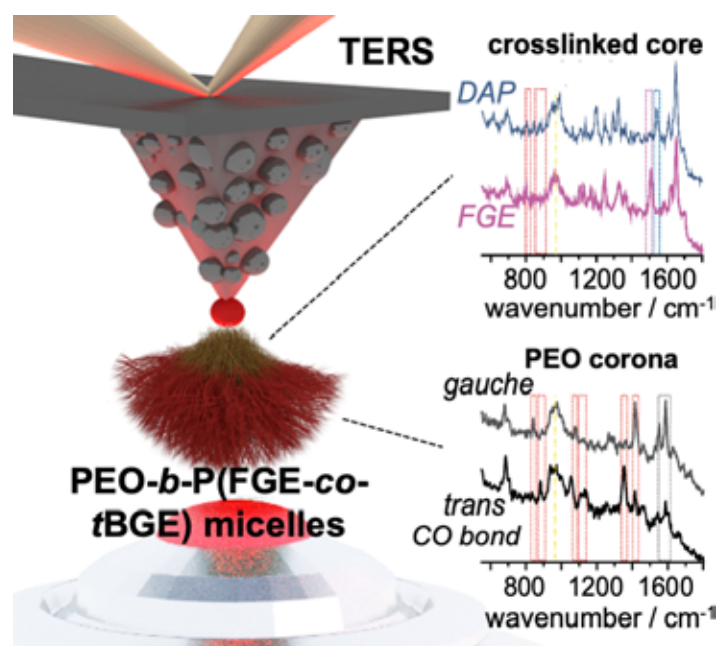Outline of the TERS study on fractured block copolymer micelles. TERS spectra represent typical peak pattern found in the crosslinked core and the PEO corona region.

Keywords:
TERS, polymer micelles, nanomechanics, crosslinking

A-I.2

Title: *Towards the compactness and permeability of the polymer brushes studied by surface-enhanced Raman spectroscopy*

Author: Marek Procházka[1], Monika Spasovová[2], Markéta Vrabcová[2], Josef Štěpánek[1], Ondřej Kylián[3], Hana Vaisocherová-Lísalová[4]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University
[2]Department of Optical and Biophysical Systems, Institute of Physics of the Czech Academy of Sciences; Institute of Physics, Faculty of Mathematics and Physics, Charles University
[3]Department of Macromolecular Physics, Faculty of Mathematics and Physics, Charles University
[4]Department of Optical and Biophysical Systems, Institute of Physics of the Czech Academy of Sciences

Abstract:
Zwitterionic carboxybetaine (CB)-based polymer brushes (PBs) are bio-compatible, functionalizable, and furthermore, represent the state-of-the-art in suppressing nonspecific interactions with biomolecules (fouling). We prepared hybrid copolymers combining poly(carboxybetaine methacrylamide – CBMAA/ hydroxypropyl methacrylamide – HPMAA) brushes in different concentrations [1]. The samples covered a wide scale of CBMAA/HPMAA ratios, which significantly affect the brush layer properties, namely its hydrophobicity and antifouling capability [2].
We used the original method enabling us to investigate the permeability of PBs with respect to small molecules. It is based on rafting the polymer brush to a custom-made surface-enhanced Raman scattering (SERS) substrate prepared by magnetron sputtering of Ag nanoislands on the Au surface. After deposition of about 1 mL droplet of a 10^{-5} M concentrated solution of reporter molecule (methylene blue-MB), a spectroscopic scan via a confocal Raman microscope reveals clearly distinct points exhibiting SERS signal of MB penetrated to the vicinity of the SERS-active layer. MB reporter molecule provides an intensive signal, exhibits high stability, and allows distinguishing whether the MB molecule is in direct contact with the SERS-active substrate or only situated in its proximity. The obtained SERS maps were (after prior simple background correction) subjected to factor analysis. The results allow us to compare the permeability of different polymer brushes. An automatic procedure was then employed to identify outlying points in the SERS maps and to analyze them via the correlation of their spectral shapes with a reference spectral set. Statistics of the detected "deep holes" (MB molecule in direct contact with the SERS-active surface) and "shall holes" (MB molecule in the proximity but not in direct contact) were found to depend on the CBMAA/HPMAA ratio in the way correlating with the known properties of these brushes.

References:
1. I. Víšová et al., Modulation of Living Cell Behavior with Ultra-Low Fouling Polymer Brush Interfaces, Macromol. Biosci. 20 (2020), e1900351.
2. H. Vaisocherová-Lísalová et al., Copolymer Brush-Based Ultralow-Fouling Biorecognition Surface Platform for Food Safety, Anal. Chem. 88 (2016) 10533-10539.

Keywords: zwitterionic, polymer brushes, permeability, SERS

A-I.3

Title: *Operando IR spectroscopic investigations of (hybrid) porous materials*

Author: Marco Daturi[1]

[1]Laboratory of Catalysis and Spectrochemistry, ENSICAEN, UNICAEN, CNRS

Abstract:

Vibrational spectroscopies are particularly adapted for the investigation of functional material properties, notably in conditions close to those of their use.

In situ and operando Raman and infrared allow at understanding the working mode of adsorbers, catalysts, membranes, … and can help into design new generations of technical materials.

In the present talk, a few examples will be provided on the development and use of advanced infrared and Raman tools, in specifically made reactor-cells, to study zeolites and Metal-Organic Frameworks in action, in environments relevant for fundamental or industrial investigations. In particular, the harvesting of quantitative data will be highlighted, as a fundamental approach for this kind of analyses.

Keywords: Infrared, insitu, operando, zeolites, MOFs

A-I.4

Title: *In situ FTIR, RS and coupled RS/AFM methods for surface understanding of metal oxide materials applied as catalysts for methane abatement*

Author: Joanna Profic-Paczkowska[1]

[1]Faculty of Chemistry Jagiellonian University

Abstract:

The article is an attempt to summarise the application of the vibrational spectroscopic methods for surface analysis of catalytic materials. A question that arises considering it is that both FTIR and Raman are not yet the surface methods whatsoever, thus how they can be applied to overcome this incompatibility.

In this study in situ and operando FTIR and Raman spectroscopic techniques have been utilised to look into the metal oxides surface functioning as catalyst for methane combustion. To be able to limit the analyses to the very surface of the materials different probe molecules were chosen in terms of their affinity to the surface and to be able to understand reaction mechanisms by assuming possible reaction intermediates.

The results provided the foundation for understanding fundamental correlations amongst preparation conditions of the materials, their structure and activity. They also allowed for deepening the discussion over chemical nature of active centres and the mechanism of the reactions under scrutiny. [1]

Apart from in situ and operando techniques a new approach to study the materials surface have been developed based on the combination of two techniques at a time: atomic force and Raman microscopy. A marriage of this kind gives an outstanding opportunity to simultaneously ascertain the texture, morphology and structure of materials surface. However, to fully synchronise the measurements the method of calibration of the coupled instrument needed to be developed and used to study the surface of the complex metal oxide materials for methane catalytic combustion. Also the effort was made to increase special resolution of the mapping especially elaborating a new method for AFM tip preparation. The results were used to evaluate the catalyst preparation procedures leading to the bimetallic Pd-Co materials as well as to understand the phenomenon of the enhanced activity of the catalyst in comparison with its pure noble metal counterpart (Fig. 1). [2]

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[2] J. Łojewska, A. Kołodziej, A. Knapik, P. Jodłowski, T. Łojewski, Topography and morphology of multicomponent catalytic materials based on Co, Ce and Pd oxides deposited on metallic structured carriers studied by AFM/Raman interlaced microscopes, Catal. Today 216 (2013) 11-17
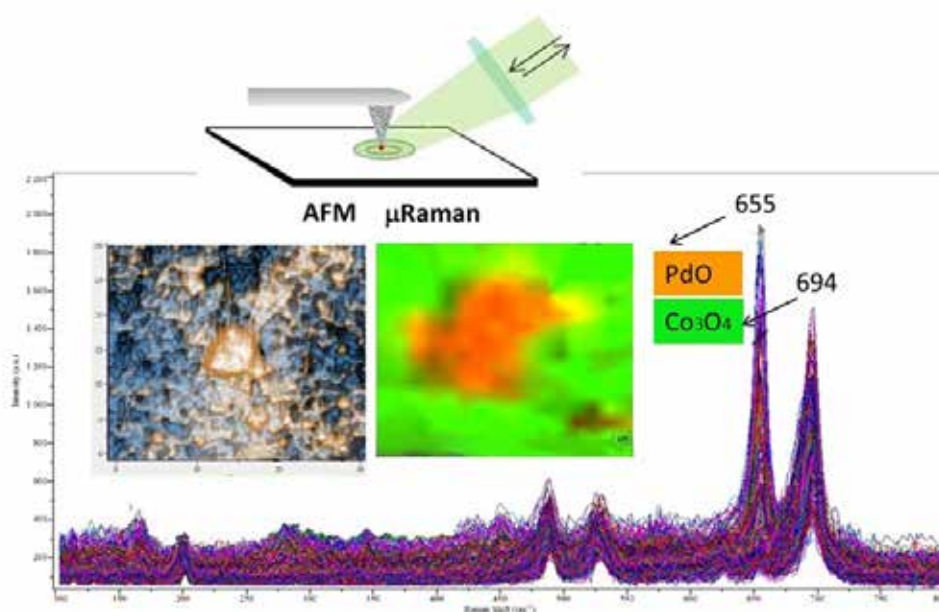
Figure captions:
Fig. 1 Example of AFM/uRaman coupled maps of PdO/Co3O4/Al2O3 catalyst

Keywords: in situ, operando, Raman/AFM, FTIR,mapping

A-O.1

Title: *Characterisation and evaluation of molecularly imprinted polymers using surface enhanced infrared absorption (SEIRA) spectroscopy.*

Author: Armel F. T. Waffo[1], Sagie Katz[1], Giorgio Caserta[1], Aysu Yarman[2], Bettina Neumann[3], Ulla Wollenberger[3], Frieder W. Scheller[3]

[1]Technische Universität Berlin
[2]Turkish-German University
[3]University of Potsdam

Abstract:
Molecularly imprinted polymers (MIP's) are synthetic receptors with high affinity and selectivity towards a specific analyte. For their formation functional monomers are polymerised in the presence a template. The latter is subsequently removed to produce a polymer matrix containing cavities, which are complementary to the target in regard to their shape and electrostatic properties. By means of this technique, artificial receptors for chemical or biological molecules can be synthesized. Such devices have been widely used as sensors or as platforms for separation approaches, purification or catalysis[1]. Scropoletin is a water soluble coumarin derivative, which can easily be polymerised on top of conductive surfaces using electrochemical procedures to create a thin isolating film. Here, we present a full characterisation of the synthesis of a polyscropoletin MIP. Using surface enhanced infrared absorption (SEIRA) spectroscopy, the entire workflow was evaluated allowing optimisation of parameters such as template loading/removal as well as providing insights into the polymerisation reaction mechanism. Different synthesis strategies were examined and their efficacy was assessed[2]. Additionally, affinity tags were used as template to create a universal platform for recombinant proteins. Thereby, overcoming an inherit difficulty related to the preparation of such MIP's, namely the time demanding screening for suitable epitopes. The spectroscopic data is complemented with results obtained from atomic force microscopy as well as electrochemical and computational methods[3]. Such MIP's can be used in future for the coupling of redox enzymes or for producing regenerative bioanodes. The herein conducted experiments demonstrate the applicability of SEIRA spectroscopy for studying surface chemistry and to serve as a tool to analyse thin films for (bio)chemical applications.

References:
[1] G. Vasapollo, R.D. Sole, L. Mergola, MR. Lazzoi, A. Scardino, S. Scorrano, G. Mele, Molecularly Imprinted Polymers: Present and Future Prospective. Int. J. Mol. Sci. 12(9) (2011) 5908-5945
[2] G. Caserta, X. Zhang, A. Yarman, E. Supala, U. Wollenberger, R.E. Gyurcsányi, I. Zebger, F.W. Scheller, Insights in electrosynthesis, target binding, and stability of peptide-imprinted polymer nanofilms. Electrochim. Acta. 381 (2021) 138236
[3] A. Yarman, A.F.T. Waffo, S. Katz, S. Frielingsdorf, E. Supala, J. Dragelj, C. Bernitzky, S. Kurbanoglu, B. Neumann, M.A. Mroginski, R.E. Gyurcsányi, U. Wollenberger, F.W. Scheller, G. Caserta, I. Zebger, A Strep-tag based Imprinted Polymer Platform for Heterogenous Bio(electro)catalysis. To be submitted

Keywords: SEIRA, MIP's

A-O.2

Title: *Enhancement of the E12g and A1g Raman modes and Layer Identification of 2H-WS2 Nanosheets With Metal Coatings*

Author: Bharathi Rajeswaran[1], Rajashree Konar[1], Gilbert Daniel Nessim[2], Yaakov Raphael Tischler[1]

[1]Bar-Ilan University, Israel
[2]Bar-Ilan University, Ramat Gan, Israel

Abstract:

Raman spectroscopy in Transition Metal Dichalcogenides (TMDCs) helps determine their structural information and layer dependency. Because it is non-destructive and fast, it is an archetypal technique to investigate the structure and defects in TMDCs. In our earlier study, we used a metal-dielectric coating to enhance the Raman signal of WS_2 the Raman Spectra measured from WS_2 coated on the standard Si/SiO_2 was significantly lower. Therefore, we used a quarter-wave thick dielectric coating on a metal substrate utilizing interference-enhanced Raman scattering to enhance the Raman signal. This metal-dielectric coating allowed access to the otherwise unavailable E^1_{2g} and A_{1g} modes of WS_2. In the current study, we observe a factor of enhancement of 11 times for the quarter wave thick metal-dielectric coating(80nm) compared to 30nm thick dielectric, for a laser power of ~2mW. We then compare the Raman Spectra of WS_2 on the quarter metal-dielectric stack of Al/Al_2O_3 to the Raman spectra of WS_2 on metal layers on either Au(10nm) or Al(200nm). A significant enhancement in the Raman signal of WS_2 is observed for both the metal coating. When a metal surface like Au (10nm) is being used, we report a higher enhancement compared to Al (200 nm) in the Raman scattering of a few layered WS_2. This enhancement not only allows us to quantify the number of layers but also investigate the presence of defects.

References:

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2. McCreary, A. et al. Distinct photoluminescence and Raman spectroscopy signatures for identifying highly crystalline WS2 monolayers produced by different growth methods. J. Mater. Res. 31, 931–944 (2016).
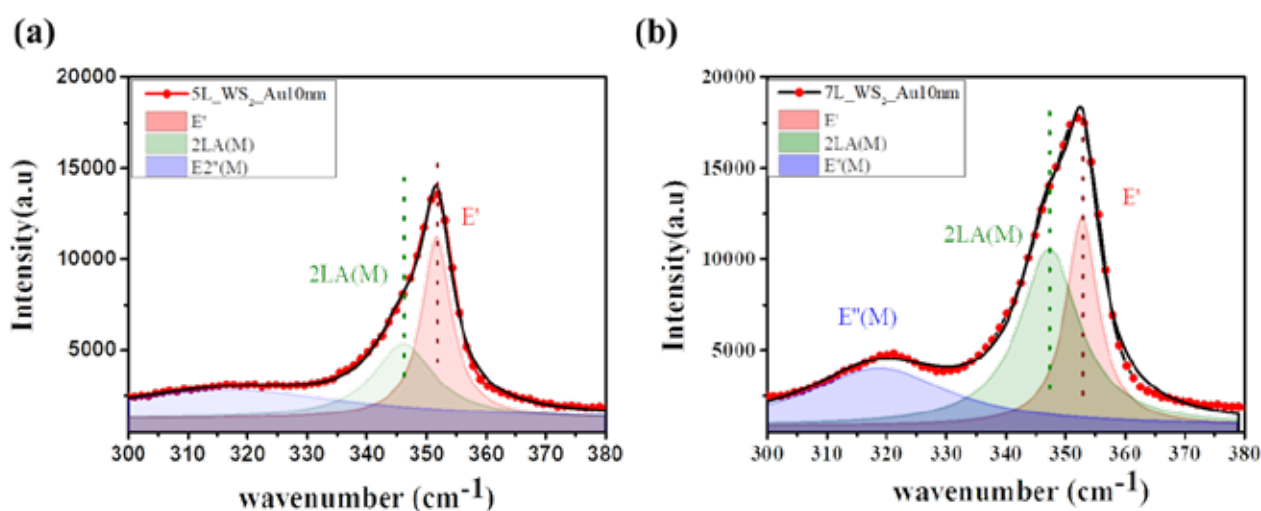
Figure captions:
Deconvoluted Raman spectra of 5L and 7L WS2 on Au (10nm) to study the presence of defects.

Keywords: Raman Enhancement, WS2, Defects, Layeridentification

A-O.3

Title: *Ibuprofen/chitosan matrices as a promising base for intestinal soft capsules*

Author: <u>Barbara Gieroba</u>[1], Maryna Khalavka[2], Olena Mozgova[3], Paulina Kazimierczak[4], Grzegorz Kalisz[1], Izabela S. Pięta[5], Liudmyla Nosach[6], Vladyslav Vivcharenko[4], Agata Przekora[4], Anna Sroka-Bartnicka[1]

[1]Independent Unit of Spectroscopy and Chemical Imaging, Faculty of Biomedical Sciences, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland

[2]Independent Unit of Spectroscopy and Chemical Imaging, Faculty of Biomedical Sciences, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland; Department of Industrial Technology of Drugs, National University of Pharmacy, Push-kins'ka 63 St., 6100

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Abstract:
Soft capsules should disintegrate quickly in the stomach and upper part of the intestine, providing the body with a precisely defined amount of the active substance [1]. Ibuprofen is a substance from the group of non-steroidal anti-inflammatory drugs (NSAIDs), which poses analgesic, antipyretic and anti-inflammatory effects. Ibuprofen refers to compounds practically insoluble in water, which causes some problems in ensuring bioavailability, in particular in the speed of reaching therapeutic concentrations in the blood [2, 3].
Immobilization of ibuprofen in chitosan matrices made of low- or high-molecular weight (MW) chitosan and their physico-chemical analysis and evaluation of the release profile in PBS will allow to select a matrix with the best potential for pharmacological applications. The maximum durability of the drug substance, relatively cheap and effecient production and ease of taking the drug can be provided by using chitosan matrices for the production of soft capsules [4].
The purpose was to investigate chitosan matrices with ibuprofen (concentration 400 µg/mL) as a potential base for soft capsules for oral use. The particular aim of this study was to evaluate the physico-chemical features and molecular arrangements of chitosan films consisting of molecules with various molecular weight (low MW- 2wt% and 4wt% in 1% acetic acid, and high MW- 2wt% in 1% acetic acid) with and without ibuprofen (also after drug relase).
These properties were determined by combining a few analytical techniques – Fourier transform infrared spectroscopy in attenuated total reflection mode (ATR FT-IR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, and atomic force microscopy (AFM), In specific, the assessment of these properties will be helpful to comprehend the possible applications of the chitosan gels in the biomedical field, especially as a drug delivery systems.

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2. Motov, S.; Masoudi, A.; Drapkin, J.; Sotomayor, C.; Kim, S.; Butt, M.; Likourezos, A.; Fassassi, C.; Hossain, R.; Brady, J.; Rothberger, N.; Flom, P.; Marshall, J., Comparison of Oral Ibuprofen at Three Single-Dose Regimens for Treating Acute Pain in the Emergency Department: A Randomized Controlled Trial. Ann Emerg Med 2019, 74, (4), 530-537
3. Bushra, R.; Aslam, N., An overview of clinical pharmacology of Ibuprofen.Oman Med. J. 2010, 25, (3), 155-1661
4. Muxika, A.; Etxabide, A.; Uranga, J.; Guerrero, P.; de la Caba, K., Chitosan as a bioactive polymer: Processing, properties and applications. Int. J. Biol. Macromol. 2017, 105, 1358-1368

Keywords: FT-IR spectroscopy, Raman spectroscopy, Chitosan, ibuprofen

A-O.4

Title: *Low frequency Raman spectroscopy for characterization of amorphous and crystalline variably substituted hydroxyapatites*

Author: Joshua Kirkham[1], Tim Kortner[2], Kārlis Bērziņš[1], Cushla McGoverin[3], Keith Gordon[1], Sara Miller[1]

[1]Te Whai Ao – The Dodd-Walls Centre for Photonic and Quantum Technologies and Department of Chemistry, University of Otago
[2]Department of Chemistry, Syracuse University, Center for Science and Technology
[3]Te Whai Ao – The Dodd-Walls Centre for Photonic and Quantum Technologies, and Department of Physics, University of Auckland

Abstract:

Hydroxyapatite is a hydroxyl calcium phosphate apatite ($Ca_5(PO_4)_3OH$)[1] which can undergo substitution in the Ca^{2+}, OH^- and PO_4^{3-} sites by other species. Carbonate ions are substituted in the hydroxyapatite present in bone and teeth.[1] Substitutions alter the physical properties and order/disorder of the mineral is associated with different pathologies in teeth and bone. Raman spectroscopy has shown promise for diagnostic applications based on bioapatite spectral signatures, including spatially offset and transmission Raman methods for non-invasive detection of breast tissue microcalcifications[2] and assessment of bone health.

Low frequency Raman spectroscopy (LFR) is the study of low wavenumber vibrations (10 to 300 cm^{-1}) arising from low energy phonon modes associated with long range order within a sample.[3] LFR is particularly sensitive to the solid state form of materials and its use is growing, particularly in the field of pharmaceutics.[3]

In this work we characterize a range of variably substituted apatite's (CO_3^{2-}, F^- and Sr^{2+} substitutions) in the amorphous and crystalline form with LFR and solid state density functional theory calculations. We also propose a crystallinity index using the low wavenumber region. This forms a base understanding for future work exploring the incorporation of LFR for sub-surface detection of bioapatites where order/disorder in a structure is related to certain pathologies.

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2. P. Matousek, N. Stone, Prospects for the diagnosis of breast cancer by noninvasive probing of calcifications using transmission Raman spectroscopy. J. Biomed. Optics. 12 (2007).
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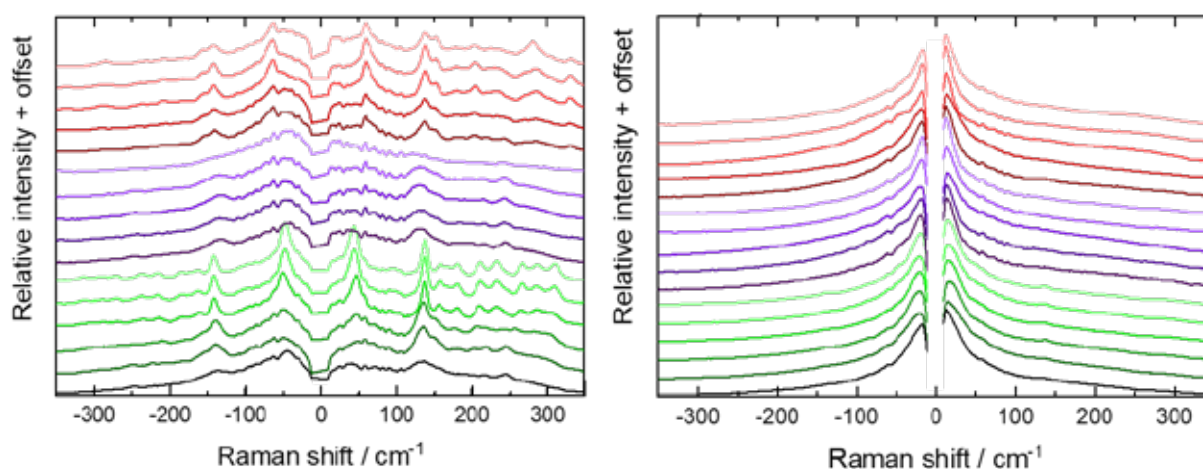
Figure captions:
Low frequency Raman spectra of crystalline (left) and amorphous (right) variably substituted hydroxyapatites. Red = CO32- substituted, purple = Sr2+ substituted and Red = F- substituted.

Keywords: Low-frequency Raman, apatite, crystallinity, PBC-DFT

A-O.5

Title: *Exploring the glycosaminoglycan structure: does it fold and how?*

Author: <u>Gergo Peter Szekeres</u>[1], Jan Horlebein[2], Jerome Riedel[1], Gert Von Helden[2], Mark Mero[3], Kevin Pagel[1], Zsuzsanna Heiner[4]

[1]Freie Universität Berlin, Fritz-Haber-Institut der Max-Planck-Gesellschaft
[2]Fritz-Haber-Institut der Max-Planck-Gesellschaft
[3]Max Born Institute for Nonlinear Optics and Short Pulse Spectroscopy
[4]School of Analytical Sciences Adlershof, Humboldt-Universität zu Berlin

Abstract:
Glycosaminoglycans (GAGs) are essential for a multitude of physiological processes ranging from regulation to host-pathogen interactions and infection prevention. Irregularities in the GAG chains have been linked to disease development and -progression, such as cancer. Therefore, the in-depth characterization of their sequence and structure is crucial for the understanding of GAG function and their application as pharmaceuticals.[1]
While there are several approaches targeting GAG sequencing, the attention paid to the three-dimensional structural description of these molecules is insufficient. In this work, we approach the structural aspects of GAGs with both broadband vibrational sum-frequency generation (VSFG) spectroscopy [2,3] and cryogenic gas-phase messenger-tagging infrared (cryo-IR) spectroscopy. Due to its unique selection rules, VSFG spectroscopy allows for the understanding of molecular order and interactions at interfaces in-situ and real-time, thus allowing for studying GAG-model membrane interactions. The results of VSFG experiments prove that GAGs can adopt chiral secondary structural motifs under physiologically relevant conditions. [4] GAGs are highly flexible molecules; therefore, to retain such a well-defined folding, intramolecular interactions must play a role. To expand the understanding of these interactions, short GAG structures were studied in the gas phase by cryo-IR. The results conclusively show strong interactions within GAG homodimers and exemplify the importance of intramolecular hydrogen bonding in the retention of folded GAG structures.

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[3] Z. Heiner et al., Broadband vibrational sum-frequency generation spectrometer at 100 kHz in the 950-1750 cm−1 spectral range utilizing a LiGaS2 optical parametric amplifier, Opt. Express 27 (2019) 15289-15297.
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Keywords: Glycosaminoglycans, VSFG spectroscopy, cryo-IR spectroscopy

A-O.6

Title: *Phosphine Halogen-Bonded Complexes: Investigated Using Matrix Isolation IR Spectroscopy*

Author: Elliot Tay[1], Corentin Grassin[1], Clemens Müller[1], Christian Merten[1]

[1]Organische Chemie II, Fakultät für Chemie und Biochemie

Abstract:
Halogen bonding is an exciting non-covalent intermolecular interaction due to its high directionality and degree of variability in halogen bond strength.[1] With interest in its varied uses, including catalysis, drug design,[2] and biological application, understanding how strong various halogen bonds are is critical. While halogen bond strength has been well characterized for iodine-nitrogen complexes,[4,5] analogous phosphine-derived complexes remained unexplored, with few examples elucidated in the literature and only by crystallographic[6] or computational methods.[7] We report complexes of iodotrifluoroethylene (ITFE) with three phosphine acceptors explored using matrix-IR: triphenylphosphine (TPP), tris(2,4,6-trimethoxyphenyl)phosphine (TTMPP), and DIPAMP. We demonstrate that there are multiple binding motifs for ITFE and each phosphine acceptor, and the importance of sterics for forming halogen-bonded complexes. We also discuss work towards investigating these complexes in cryosolution media.

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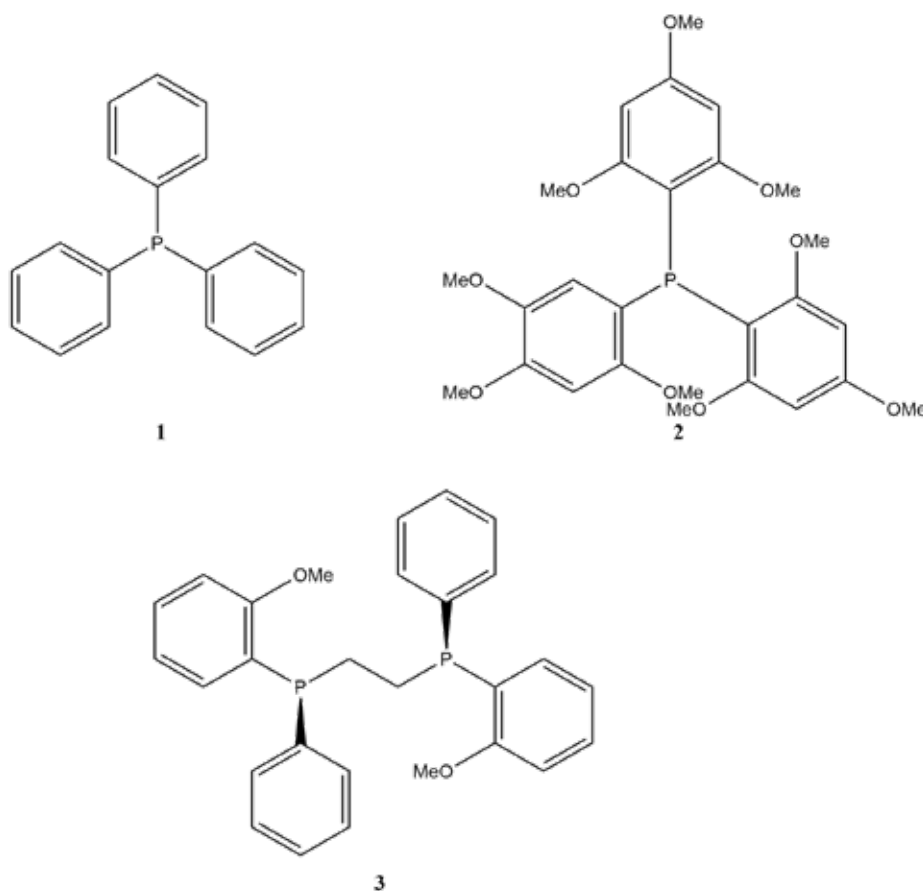
Figure captions:
Structure of the acceptors investigated: triphenylphosphine (1), tris(2,4,6-trimethoxylphenyl)phosphine (2), and S,S-DIPAMP (3).

Keywords: Halogen Bonding, Cryosolution, Matrix Isolation

A-O.7

Title: *Raman spectroscopy for investigation of interaction within polymer based magnetic multicomponent scaffolds*

Author: Anna Kołodziej[1], Małgorzata Świętek[2], Anna Hlukhaniuk[2], Daniel Horák[2], Aleksandra Wesełucha-Birczyńska[1]

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Abstract:
Polymer-based scaffolds have been extensively studied for the bone tissue regeneration applications. In the presented study, the polymer based magnetic multicomponent scaffolds were subjected for spectroscopic study. Poly(ε-caprolacton) (PCL) was chosen as an matrix, because of its relatively long biodegradation time corresponding to the average time of bone tissue regeneration.[1] Modifications took place in several stages. They concerned iron oxide nanoparticles (MNPs) that are characterised by peculiar physical, especially magnetic, properties which make them possible to use in various biomedical applications (magnetic resonance imaging, drug delivery, hyperthermia treatment).[2] The MNPs were also proven to enhance and accelerate the proliferation of osteoblast cells and new extracellular matrix (ECM) secretion.[3] Phenolic compounds (tannic acid – TA, gallic acid – GA) that are antioxidants of natural origin, exhibits antimicrobial and anti-inflammatory properties as well as up-regulates bone formation markers.[4]
The set of studied materials includes PCL, PCL+MNP@SiO$_2$, PCL+TA/GA, PCL+MNP@SiO2+TA/GA and were manufactured by combined solvent casting and particulate leaching techniques. The scaffolds prepared in this way were subjected for Raman spectroscopy measurements. A laser line of 785 nm and a microscope objective magnification of x50 were selected. For the PCL+MNP@SiO2+TA sample, Raman bands originating from the components of the PCL matrix, and TA and MNP nanoadditives were identified (Figure 1). Spectroscopic investigation have revealed that the highest critical concentration of TA in composite is 20% above which the material became nonhomogeneous.

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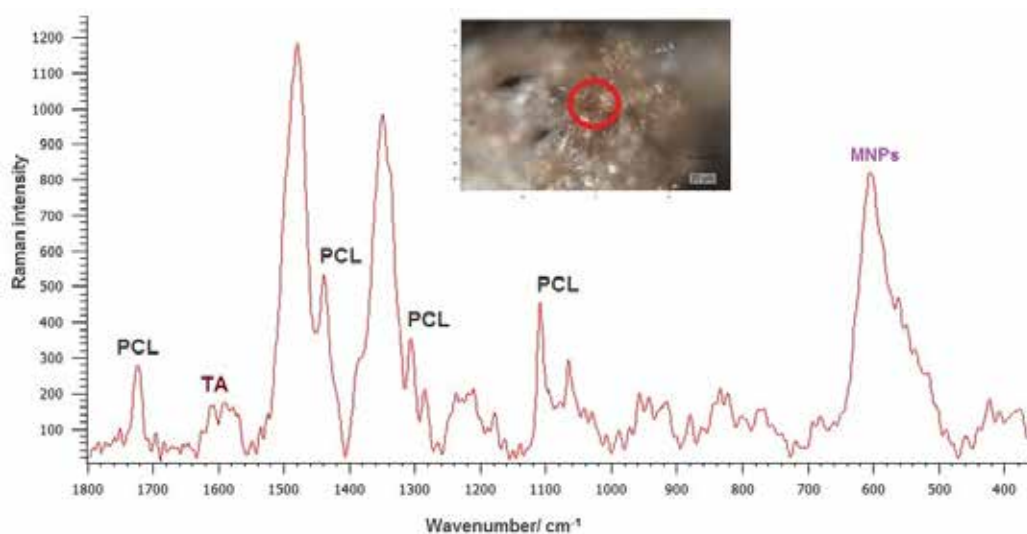
Figure captions:
Figure 1. Raman spectra of PCL+MNP@SiO2+TA scaffold.

Keywords: Raman spectroscopy, poly(ε-caprolacton), iron oxide

A-O.8

Title: *Which method will distinguish nanofibrous carbon materials?*

Author: Aleksandra Wesełucha-Birczyńska[1], Maria Pajda[2], Elżbieta Długoń[3], Krzysztof Morajka[1], Marek Michalec[1], Marta Błażewicz[4]

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[3]AGH – University of Science and Technology, Faculty of Materials Science and Ceramics,
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Abstract:
Carbon nanofibres are of great interest for biomedical and industrial applications [1,2]. Their properties and functionality depend on additional modification of their structure. Although research on these materials is already being carried out, there are still many unresolved issues. X-ray diffraction, Raman microspectroscopy and 2D correlation analysis, and also nanomechanical studies were used to unravel some structural details at the molecular level.

Materials in the form of thin flakes were made of carbon nanofibers obtained by electrospinning (ESCNF) from a polyacrylonitrile (PAN) precursor. The reference sample was obtained by carbonization, which consisted in heating the nanofibers to a temperature of 1000°C. The reference material was functionalized by oxidation of CNFs in a hot 65% nitric acid solution and then carbonized again by heating to 1000°C. CNFs were also graphitized by heating to 2800°C.

The diffractograms obtained indicate the influence of chemical and thermal treatment on the tested materials. Oxidation leads to a partial amorphization of the material, while graphitization induces the formation of a new, different crystalline phase.

The Raman spectra are distinctive with prominent Raman G and D bands, appearing near 1580 cm^{-1} and 1350 cm^{-1} respectively. The degree of crystallinity of the analyzed materials was estimated as the ratio of the intensity of the respective D and G Raman bands. Graphitization leads to significant ordering of the molecular structure [3].

The nanoindentation method clearly differentiates the tested samples, e.g. the instrumented hardness is the highest for chemically modified flakes, and the lowest for the initial, reference material.

2D-COS correlation of the Raman spectra leads to the unraveling of the hidden phasesand the determination of the mechanical properties characterizing the tested materials [4,5].

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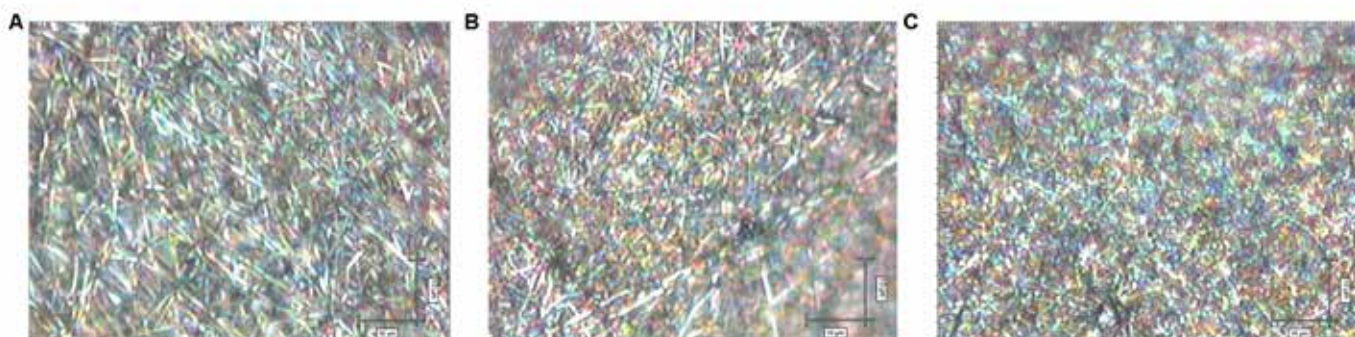
Figure captions:
Microphotographs of nanofibrous carbon materials: (A) reference, (B) oxidized, and (C) graphitized (objective magnification 50x).

Keywords: CNFs, Raman spectroscopy, PXRD, 2D-COS,

A-O.9

Title: *Raman Confocal Imaging for materials at high temperatures*

Author: Maciej Bik[1], Piotr Jeleń[1], Maciej Sitarz[1]

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Abstract:
High-temperature oxidation is a very detrimental phenomenon that limits a further development of many metallic materials including alloys and steels. Apart from new solutions in the form of protective coatings or modified chemical compositions, it is also very important to efficiently determine the phase composition of the surface and near-surface layer independently on area and oxidation time [1]. The method that provides such unique possibility is Raman spectroscopy. Moreover, in last years a significant boost in designing powerful spectrometers took place, which resulted in spectral and lateral resolution at levels as low as 1 cm-1 and hundreds of nanometers, respectively [2].

The main idea of this work was to present versatile capabilities of Raman Confocal imaging technique for post-mortem analysis of numerous metallic materials (ferritic steel, TiAl alloy, High-Entropy Alloy (HEA), pure Cr) oxidized at high temperatures with or without protective coatings (Polymer Derived Ceramics, Mn-Co and Mn-Cu spinels). Results of SEM+EPMA/EDS, as well as XRD and TEM studies will be showed to supplement the results, as well as to highlight the advantages of Raman spectroscopy over these methods, currently most often used in case of high-temperature oxidation topic.

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Keywords: Raman imaging, oxidation, phase composition

A-O.10

Title: *Automated Quantitative Analysis of (Microplastic) Particles and Fibers down to 1 μm by Raman Microspectroscopy*

Author: Oliver Jacob[1], Alejandro Ramírez-Piñero[1], Natalia Ivleva[1]

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich

Abstract:
Microplastics (MPs, synthetic polymer fragments in size range of 1μm–1mm) are found in the environment all around the globe as well as in drinking water and food. Since more hazardous effects are expected from smaller MPs, reliable quantitative analysis is required. Here, Raman microspectroscopy (RM) is suitable for the chemical identification and quantification of MPs down to 1μm (Ivleva 2021). Our open-source program *TUM-ParticleTyper* (von der Esch 2020 et al.) enables the automated detection, quantification, and morphological characterization of (plastic) fragments in dark-field images of optical microscopy, followed by the automated RM-based identification of MPs and non-plastic fragments of up to 7000 particles/fibers down to 10μm, randomly selected on the entire filter. Since the number of particles increases with decreasing the particle size, it become nearly impossible to detect all particles down to 1μm on the entire filter. Therefore, we proposed alternative strategy – *random window subsampling*, where the automated acquisition of optical image and localization of (MP) fragments are followed by RM measurements from window to window. We also introduced a bootstrap method, to provide an error quantification with confidence intervals from the available window data. Ultimately, we developed and implemented in *TUM-ParticleTyper 2* new RM measurement algorithm that computes confidence intervals *on-the-fly* during the analysis using bootstrap and by checking whether given precision requirements are already met, automatically stops if an appropriate number of fragments is identified, thus improving time efficiency (Schwaferts et al. 2021). Furthermore, we implemented advanced image processing for better recognition and morphological characterization of MPs.
In this presentation we will discuss our new software program *TUM-ParticleTyper 2*, which enables automated analysis of (MP) particles and fibers down to 1μm, with the focus on the analysis of environmental samples.

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N. P. Ivleva, Chemical Analysis of Microplastics and Nanoplastics: Challenges, Advanced Methods and Perspectives, Chemical Reviews 2021 121, 11886–11936
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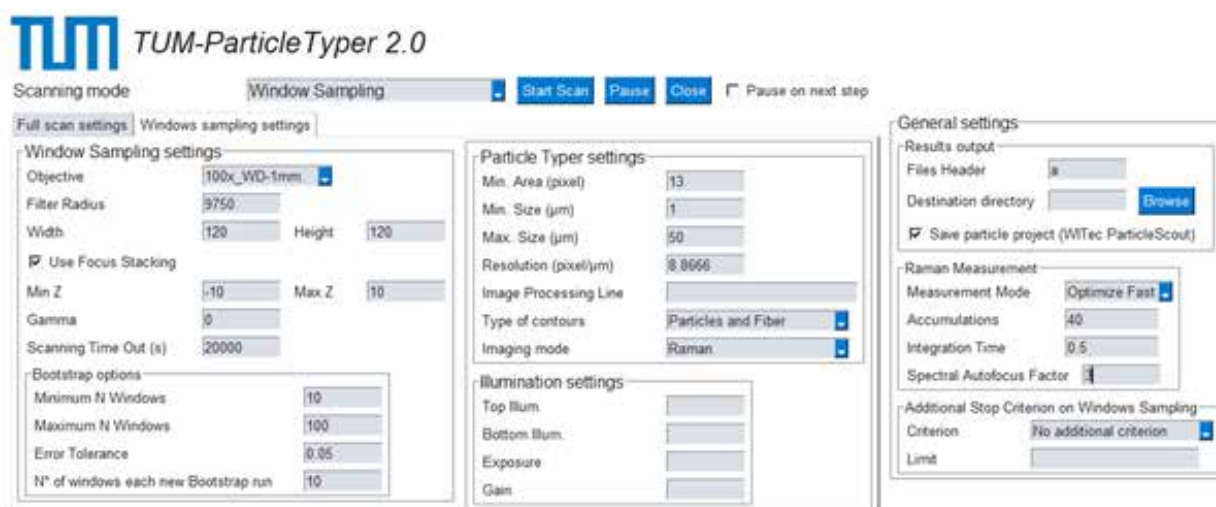
Figure captions:
Screenshot of the graphical user interface of the software TUM-ParticleTyper 2

Keywords: Automated Raman Analysis, Microplastics, Identification

A-O.11

Title: *Investigating Degradation of Poly(vinyl chloride) by Spectroscopic Methods*

Author: Marwa Saad[1], Krzysztof Kruczała[1], Marek Bucki[1], Karol Górecki[1], Sonia Bujok[2], Łukasz Bratasz[2]

[1]Jagiellonian University, Faculty of Chemistry,
[2]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences

Abstract:
The durability of art objects made of plasticized poly(vinyl chloride) (PVC) is one of the current interests of conservators due to the rapid deterioration of PVC, leading to dramatic alterations that were unanticipated by artists and collectors. According to current knowledge, around 15–30% of plastic objects in memory institutions across Europe are in poor or unacceptable condition for display [1]. The role of preventive conservation is to propose strategies for such objects that can be planned based on the study of their degradation [2]. The deterioration of plasticized PVC objects proceeds through polymer degradation and/or plasticizer loss. PVC matrix primarily degrades through the dehydrochlorination reaction, in which conjugated polyene structures appear, and HCl is released. The formed hydrochloric acid catalyzes a further degradation process while the conjugated polyene structure absorbs longer wavelengths of light, causing a yellowing of the object over time. The migration of plasticizers from PVC objects results in the stiffening of the object and plasticizer accumulation on the surface, which becomes sticky [3]. In this work, we focused on the evaluation of the PVC deterioration process by Raman spectroscopy, ATR-FTIR, UV-Vis, and Color quest spectrophotometry. The Raman spectrum of PVC aged at 60°C showed a small increase of resonance of $\nu(C-C)$ vibration at 1120 cm^{-1} and $\nu(C=C)$ vibration around 1500 cm^{-1} corresponding to the polyene structure formation. However, the experiment performed at 80°C resulted in the fast formation of the polyene structures. In addition, the presence of a new band at 2600 cm^{-1} was observed and assigned to the cyclic products of the PVC chain degradation. Obtained results indicated that the rate of PVC degradation depends on the experimental conditions and the analyzed material (e.g. amount of thermal stabilizer). This investigation will allow proposing a preventive conservation strategy reflecting the conditions in museums.

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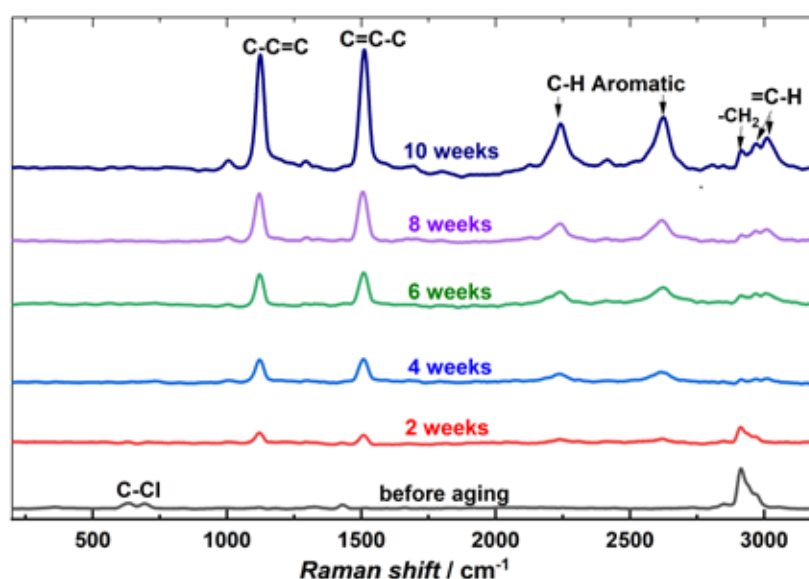
Figure captions:
Raman Spectra of stabilized PVC sheet aged at 80°

Keywords: Raman, spectroscopy, PVC, plasticizers, degradation

A-O.12

Title: *Visualization of Intermolecular Hydrogen Bonding of Poly(ε-caprolactone) during Marine Degradation using Low-frequency Raman Spectroscopy*

Author: Harumi Sato[1], Tomoaki Segawa[1], Kohei Ito[1], Yota Maruyama[1], Masahiro Hatayama[1], Gao Jiacheng[1]

[1]Kobe University

Abstract:

The Raman spectra are in the low-frequency region includes modes of intermolecular vibrational modes derived from the crystal structure of polymers and vibration caused by intermolecular hydrogen bonding. Therefore, we have tried to directly observe intermolecular hydrogen bonding and crystallinity of polymer films non-destructively and non-contact by Raman mapping measurement in the low to high frequency region. The assignment of Raman spectra in the low-frequency region was performed using quantum chemical calculations (QCC) based on the Cartesian Coordinate Tensor Transfer (CCT) method. Raman mapping measurements were performed at a total of 121 locations over an area of 100 μm x 100 μm in 10 μm steps, before, during, and after marine degradation. For 3D mapping, measurements were taken from the surface down to 10 μm in 2 μm steps in the depth direction.

The low–frequency Raman spectra of PCL film showed that PCL has peaks at 58, 32, and 21 cm^{-1} in the low-frequency region. The peak at 58 cm^{-1} reflects intermolecular hydrogen bonding of PCL, and the wavenumber position of the peak indicates the strength of the intermolecular hydrogen bonding, while the intensity ratio of amorphous (1730 cm^{-1})/crystalline (1720 cm^{-1}) in the high wavenumber region (C=O stretching region) provides information on the degree of crystallinity. Figure 1 shows the 2D Raman mapping of the PCL film before and after marine degradation, based on the wavenumber position of the Raman band at around 60 cm^{-1} in the low-frequency region. As marine degradation progresses, the wavenumber position of the peak at 60 cm^{-1} shifts toward the low frequency region, indicating that the intermolecular hydrogen bonds on the surface of PCL film weaken as marine degradation progresses. Depth profiling analysis also confirmed that degradation was more enhanced at the film surface.
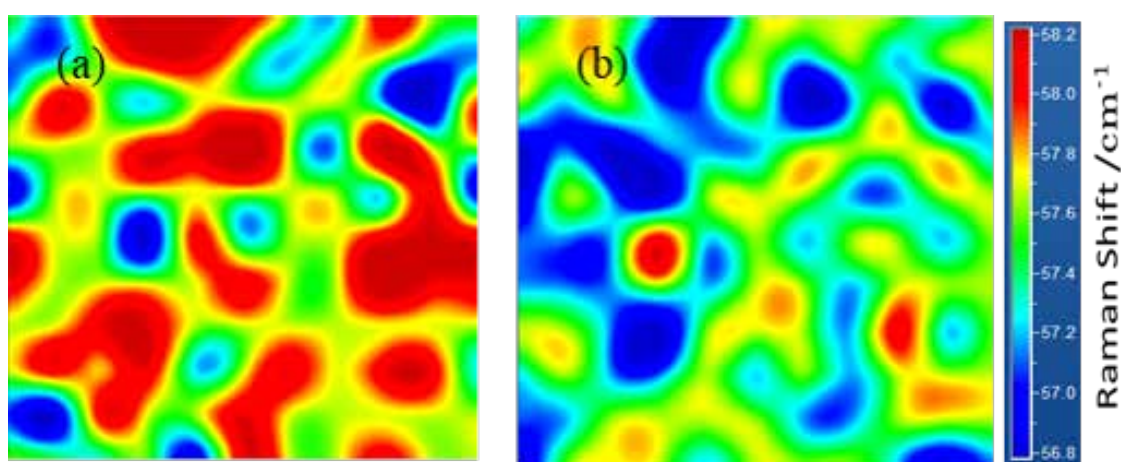
Figure captions:
Fig. 1 The 2D Raman mapping of the PCL film (a) before and (b) after marine degradation, based on the wavenumber position of the peak at 60 cm-1 in the low-frequency region.

Keywords: Hydrogen Bonding, Marine Degradation, Low-frequency

A-O.13

Title: *Imaging of Three-dimensional Molecular Orientation Using FT-IR, Raman, and O-PTIR Microspectroscopies of various samples*

Author: Tomasz Wrobel[1]

[1]Jagiellonian University

Abstract:
Fourier transform infrared microspectroscopy (FT-IR) is a nondestructive, information-rich, and label-free technique successfully applied for years in material science. The introduction of linear polarization enriches the technique with the possibility of studying the orientation of macromolecules. Until now, experiments focused on using the absorptance of a single band to retrieve the in-plane orientation and the degree of order. The extended four-polarization (4P) method, which enables the visualization of the macromolecule orientation regardless of the choice of the direction of polarization, was proposed by Hikima et al. for polymers [1]. The application of IR imaging with 4P on heterogeneous structure, human tissue microarrays, was presented for the first time by our team in 2020 [2], [3].
A deeper characterization of the sample structure is the next step. Simultaneous analysis of two bands of roughly perpendicular transition moment orientations was proposed by Lee in 2018 as a method of determining the orientation of the molecule in three-dimensional space, which was recently approached by Lee [4]. The first application of "concurrent analysis" (4P-3D) to infrared spectromicroscopic data and obtaining orientation angles of a model polycaprolactone spherulite sample was presented by our team in 2021 [5]. The applicability of this method ranges from high-resolution, diffraction-limited FT-IR and Raman imaging to super-resolution O-PTIR imaging. The results obtained in these studies were groundbreaking, we proved that this method can be easily applied not only to FT-IR but also to O-PTIR and Raman imaging.
Spatial, non-destructive orientation studies are expected to have a profound impact on materials and life sciences as a method of extracting previously unattainable information from complex systems.

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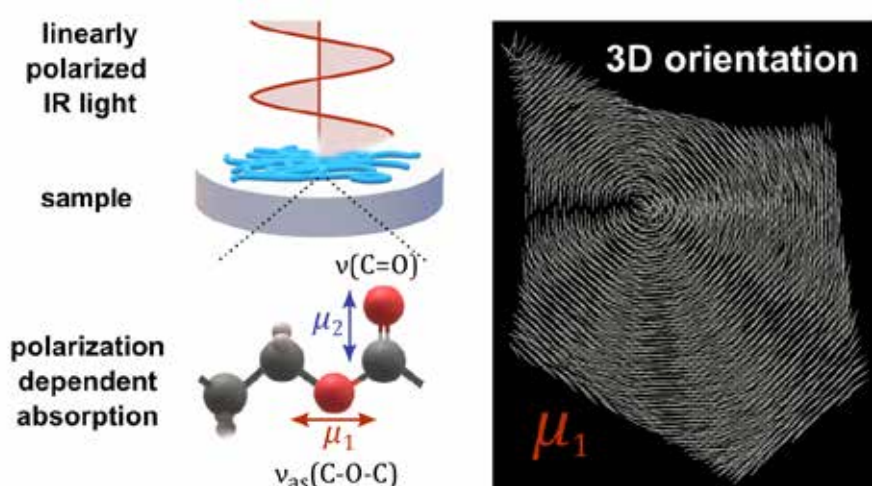
Figure captions:
A schematic of the 4P-3D approach

Keywords: infared, raman, photothermal, macromolecule, polarization

A-O.14

Title: *Structural characterization of amorphous silica coatings combining specular reflectance (SR) and attenuated total reflectance (ATR) infrared spectroscopic techniques*

Author: Brenda Bracco[1], Helios Vocca[2], Silvia Corezzi[2], Alessandro Di Michele[2], Laura Silenzi[3], Angela Trapananti[3], Flavio Travasso[3], Stefano Colace[4], Michele Magnozzi[5], Paola Sassi[1]

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[2]Department of Physics and Geology, University of Perugia and Istituto Nazionale di Fisica Nucleare, Sezione di Perugia
[3]School of Science and Technology – Physics Division, University of Camerino and Istituto Nazionale di Fisica Nucleare, Sezione di Perugia
[4]Department of Physics, Università di Genova
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Abstract:
Amorphous silica coatings are important mirror components of gravitational wave detectors currently used in the Virgo experiment hosted by the European Observatory of Cascina (PI-Italy). Exceptionally high sensitivity is required for the detection of gravitational waves and thermal noise from mirror coatings is one of the main limiting factors in the spectral region where the detector is most sensitive1. The main challenge for many research teams around the world is to identify new materials and define the deposition and post-deposition procedures that ensure thermal noise reduction. Possible tools to obtain structural information and detect the presence of impurities on thin films are specular reflection (SR) and attenuated total reflection (ATR) IR spectroscopic techniques2. In our work, these two approaches are employed to analyze amorphous silica coatings produced by ion-beam spattering on a silicon and SiO2 substrates, and treated after deposition at different annealing temperatures, from 500°C to 1000°C. We highlight how such techniques can be applied to the structural analysis of thin films of nanometer thickness. In particular, we demonstrate how the spectroscopic techniques are powerful tools to detect impurities and inhomogeneities. In addition, they can provide estimates of optical properties, such as the refractive index, and of the effective thickness which are sensitive to annealing. To this end, the results of IR measurements are discussed and compared to those obtained by Brillouin scattering, Scanning Electron Microscopy and Spectroscopic Ellipsometry.

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Keywords: Amorphous silica coatings, IR spectroscopy

A-O.15

Title: *Can elevated temperatures in HTGR nuclear reactors reverse irradiation damage in graphite? – high-temperature in-situ Raman spectroscopy study*

Author: Magdalena Gawęda[1], Piotr Jeleń[2], Małgorzata Frelek-Kozak[1], Łukasz Kurpaska[1], Jacek Jagielski[3]

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Abstract:
Generation IV high-temperature gas-cooled reactors (HTGR) are of great interest due to their high level of safety, reliability, and efficiency, all of which are crucial factors in addressing the current energy crisis. This is guaranteed by using helium as a coolant and graphite as a moderator, both chemically stable at high temperatures of up to 900°C [1]. In the reactor, graphite also serves as the construction material for hexagonal prisms with vertical tunnels provided for control rods and helium gas coolant flow [2]. As materials used in the reactor are exposed to the radiation for extended periods, detailed knowledge is required of processes concerning the structural deterioration of graphite, which may lead to element failure.
Previous studies have shown that structural deterioration is not a continuous process but characterized by a threshold value of fluence when rapid deterioration occurs [3, 4]. However, those studies only considered one of the hostile environmental factors of the reactor. The core of this study was to provide detailed information on the structural behaviour of ion-irradiated damaged nuclear graphite under high temperatures. Commercial IG-110 and NBG-17 graphites, along with in-home laboratory material, were irradiated with He$^+$ and Ar$^+$ ions at 400°C with 150 keV and 2E17 ion/cm^2 fluence. In-situ HT-Raman spectroscopy at temperatures up to 900°C showed a similar level of structural deterioration and types of defects in room temperature measurements for all samples. However, their high-temperature behaviour differed significantly, especially for reversing damage caused by Ar$^+$ ion irradiation. In all cases, the level of irradiation-induced defects decreased with increasing temperature, most distinctly at the upper limit. Nevertheless, the effect was significantly stronger for NBG-17 and in-home specimens. The IG-110 graphite remained more damaged after the high-temperature procedure, and structural ordering was slower and less pronounced.

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Keywords: HT-Raman, HTGR, graphite, ion irradiation

B-I.1

Title: *Probing the active site structural changes in P450/P420 forms of CYP121*

Author: Piotr Mak[1]

[1]Saint Louis University

Abstract:
Cytochrome P450 121 (CYP121) is essential for Mycobacterium tuberculosis (Mtb) vitality, as shown in knock out experiments, and is therefore considered a prime anti-tubercular drug target.[1] CYP121 catalyzes a carbon-carbon coupling reaction between two Tyr aromatic rings of its dipeptide cyclo-*L*-Tyr-*L*-Tyr (cYY) substrate to form a mycocyclosin product. Several crystal structures of CYP121 were solved, indicating a relatively large and rigid active site filled with water molecules that form an extensive H-bonding network with the S237, Q385, and R386 residues.[2] Interestingly, crystallographic comparison of the cYY-bound with its substrate-free structure shows that no major conformational changes occur upon cYY binding.[2] Another interesting aspect of the CYP121 function is that it can undergo a reversible and pH-dependent conversion to its inactive P420 form.[3] While such conversion is typically irreversible, the CYP121 is an ideal model for investigating the structural changes accompanying P420 formation. In this work, we employ resonance Raman (rR) and UV-Vis spectroscopies, mutagenesis, and enzymatic activity assays to investigate the active site structural response to substrate binding, pH changes, and mutation of the catalytically critical R386 residue in the ferric resting state and ferrous-CO ligated forms. Additionally, we characterize the effects of these perturbations on the enzymatically inactive P420 form.

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Keywords: P450, P420, CYP121, resonance Raman

B-I.2

Title: *Insights into molecules structure and dynamics by multi-wavelengths UV Resonance Raman spectroscopy*

Author: Barbara Rossi[1]

[1]Elettra Sincrotrone Trieste

Abstract:
Raman spectroscopy is a well-assed tool from several years for the investigation of the vibrational dynamics of several kind of systems, including condensed matter, liquids, gels, aqueous solutions, polymers and bio-macromolecules. Thanks to the resonance effect, UV Resonance Raman (UVRR) spectroscopy enables to overcome some of the limitations of conventional visible Raman technique, offering a significant increment of the detection limit and the selectivity needed to incisively monitor specific chromophoric segments within the sample. However, the full exploitation of UVRR has so far been limited by the lack of extended and tuneable excitation sources in the UV range that allow to finely approach the resonance conditions of specific targeted molecular groups. In this contribution, we would like to give an overview on the opportunities offered by the unique in the world UVRR setup working with the synchrotron radiation (SR) source available at the BL10.2-IUVS beamline (Elettra synchrotron facility, Trieste, Italy). The SR-based UVRR set-up at Elettra enables to perform multi-wavelengths UVRR experiments with a fine tuneable source in the deep UV range of excitation wavelengths (200-270 nm), resulting in an innovative spectroscopy facility for approaching open issues in physics, chemistry, materials and life sciences. Some examples of applications of UVRR spectroscopy to provide insights in the structural dynamics of simple and more complex molecular systems will be presented and discussed, in order to highlight the opportunities offered by this technique.

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2. B. Rossi, M. Tortora, S. Catalini, A. Gessini, C. Masciovecchio, Synchrotron based UV Resonance Raman Spectroscopy for Polymer Characterization, book chapter (eds Y. Ozaki, H. Sato, Wiley (2021): Spectroscopic Techniques for Polymer Characterization. Methods, Instrumentation, Applications, pages 183-225

Keywords: Raman spectroscopy, UV resonance, synchrotron

B-I.3

Title: *Electrostatic and electrodynamic fields in lipid bilayer membranes*

Author: Lauren Webb[1]

[1]The University of Texas at Austin

Abstract:
Lipid bilayer membranes are complex, dynamic, and functional structures composed of a wide diversity of lipids, proteins, small molecules, and water organized in heterogeneous domains through noncovalent interactions. The structure and motion of these molecules generate large electric fields within the interior of the membrane that are critical to membrane structure and function. Here, we describe how vibrational spectroscopy of unnatural nitrile chromophores places throughout the membrane structure is used to measure electrostatic fields in peptides intercalated in free-standing lipid bilayer membranes of increasing chemical complexity. In combination with electrodynamics simulations, these experiments highlight how common small molecules such as cholesterol dramatically affect membrane structure and dynamics through large changes to membrane electric fields.

Keywords: Membrane biophysics, membrane electrostatics

B-I.4

Title: *Probing protein structure on nanoparticle surfaces using theoretical and experimental sum frequency scattering spectroscopy*

Author: Tobias Weidner[1]

[1]Department of Chemistry, Aarhus University, Denmark, email: weidner@chem.au.dk

Abstract:
When nanoparticles enter a biological host, the surface of the particle will be immediately covered by a dense layer of proteins. The structure and orientation of these proteins define the chemical identity of the nanoparticle and, thereby, dictate any biological response. Information about the structure and orientation of proteins at nanoparticle surfaces is of tremendous importance for our understanding of nanoparticle toxicity and foreign body reactions, but also for new strategies in nanomedicine. For example, designing effective medical drug particles for controlled release must take into consideration the binding of proteins to the drug nanocontainers. Another important matter are nanoplastics, which are present in our everyday lives – e.g. in personal care products and the degradation of larger plastics. Here, the toxicity or biological activity of a nanoplastic particle is strongly affected by the type, structure, and orientation of proteins bound to its surface. While significant progress has been made over the past years to identify binding proteins, determining their orientation and folding at particle surfaces is still challenging. We have recently developed sum frequency scattering (SFS) spectroscopy into an effective tool to provide this information. Using well-defined model leucinelysine (LK) peptides we have observed that binding to nanophase particles leads to peptide structure and orientation, which are markedly different from those observed on flat surfaces.
This was surprising since it had been assumed that in view of the small size of the peptide in comparison with the particle curvature, flat surfaces would be reasonable model interfaces for studying protein orientation. We found that Coulombic charge interactions across the droplet volume play a dominant role in how proteins bind, fold and orient. Consequently, as we begin to study human proteins known to bind to nanoplastic materials, we find markedly different binding poses compared with flat material interfaces. Using SFS spectra calculations and molecular dynamics simulations, we find aggregated protein folds at several nanoparticle interfaces, which could explain the tight binding of these human proteins to nanoplastics in vivo.

B-I.5

Title: *Domain movements and conformational changes in large membrane proteins identified by combined SEIRAS and IR labelling approach.*

Author: Petra Hellwig[1], Tatjana Gerasimova[2], Ana Filipa Seica Santos[3], Thorsten Friedrich[4]

[1]University of Strasbourg CNRS, UMR 7140
[2]University of Strasbourg and University of Freiburg
[3]University of Strasbourg, UMR 7140
[4]University of Freiburg, Institute for Biochemistry

Abstract:
Surface-enhanced infrared absorption spectroscopy (SEIRAS) is a powerful tool that allows studying the reactivity of protein monolayers at a very low concentration and independent from the proteins size. The reorganization on the surface takes place and the changes of the nanostructures (NP) after adding the protein have been identified. It was found that the interaction between proteins and nanoparticles is determined by the surface heterogeneity of the NPs, but also depends on the protein heterogeneity as well as its size. [1]
Once optimized we used SEIRAS to enhance the signal of single IR probes introduced into large membrane proteins. IR probes such as nitrile- and thiocyanate-derivatized amino acids have been first described for peptides and soluble proteins. [2] They have been found to give specific information on the local environment of the probe, because their IR absorption frequencies strongly depend on the hydrogen bonding with the surrounding protic solvent molecules, backbone, or amino acids. The protein studied is the respiratory complex I, the entry point for electrons into most respiratory chains that generates the proton motive force required for cellular energy consuming processes. The three largest subunits in the membrane arm show primary sequence similarity to one particular class of antiporters and are thus predicted to play a role in the proton translocation machinery. The substrate binding site is more than 200 A away from the proton pathways and the coupling of these sites is highly discussed. IR probes were introduced to individual cysteine mutants. [3,4] The spectral signature in the presence of different substrates was then monitored. Information on the reorganization of the introduced IR label upon the induced reaction was obtained. An opening of the structure in the membrane arm upon addition of NADH was demonstrated providing evidence for the long range conformational arrangements taking place in the enzyme during catalysis.

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Keywords: SEIRAS, Nitrile label, Complex I,Conformation

B-I.6

Title: *Local Structural Dynamics of Membrane Protein Bacteriorhodopsin Revealed by 2D Vibrational Spectroscopy*

Author: Jianping Wang[1]

[1]Institute of Chemistry

Abstract:
Bacteriorhodopsin (bR) is a light-driven proton pump found in the purple membrane of Halobacterium salinarum. It transfers protons against the pH gradient across the cell membrane from the cytoplasmic (CP) side to the extracellular (EC) side through a photocycle to synthesize ATP from ADP. In this work, by utilizing a ^{13}C, ^{15}N labeled lysine (K) as an isotope probe, we created a network of site-specific amide-I vibrational signatures (backbone carbonyl stretch) to identify the frequency contribution of the labeled residues to the amide-I excitonic band structure. The red-shifted amide-I frequency in the ^{13}C, ^{15}N lysine labeled bR (uK-bR) to the unlabeled bR (WT-bR) could be differentiated and examined by ultrafast two-dimensional vibrational echo infrared (2D IR) spectroscopy. Our results showed that the backbone carbonyl of K159 is located at the high frequency side and possess a hydrogen-bonded gamma-turn structure with E161, one of the carboxylate residues on the cytoplasmic surface of bR. The result is supported by 2D NMR study and is in agreement with early electron crystallographic structure of bR. The local structure and dynamics of K159, and its role in proton recruiting in the E-F loop region, are discussed.

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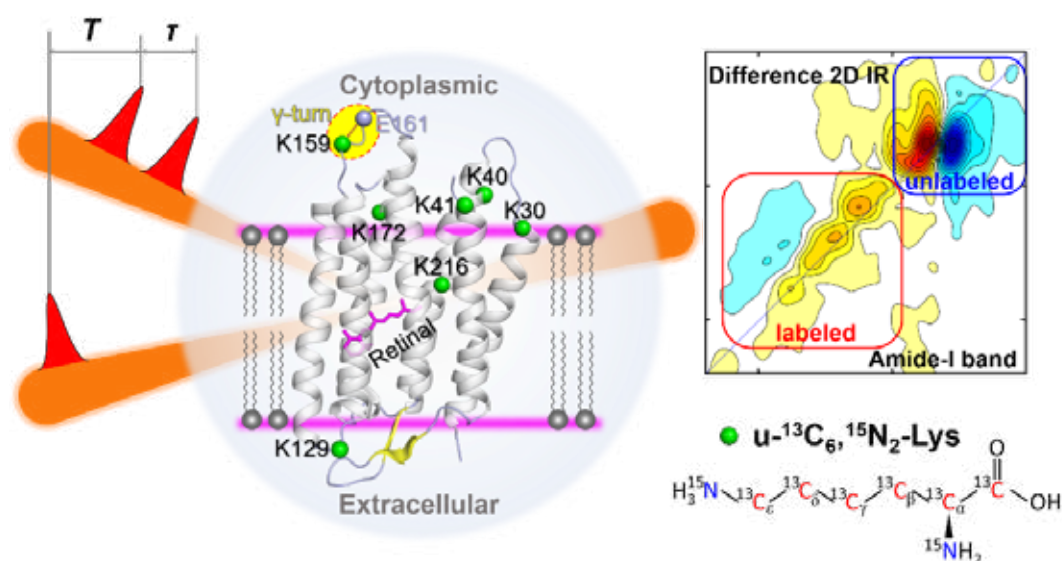
Figure captions:
Figure 1. IR excitation pulses and 2D IR spectra in the amide-I region of bR with 13C, 15N-labeled lysine residues.

Keywords: 2D IR, bacteriorhodopsin, local structure

B-O.1

Title: *Detection, characterization, and differentiation of SHb and HbFeIII–SH adducts inside functional erythrocytes*

Author: Jakub Dybaś[1], Tetiana Stepanenko[2], Grzegorz Zajac[1], Katarzyna M. Marzec[3]

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[3]AGH University of Science and Technology

Abstract:
An overdose of sulfur-containing drugs can lead to sulfhemoglobinemia, which is characterized by the presence of sulfhemoglobin in the bloodstream.[1,2] The diagnosis and treatment of this medical condition can be challenging due to the ambiguities concerning the structure of sulfhemoglobin. Usually, it's defined as a hemoglobin (Hb) adduct with a sulfur atom connected to the porphyrin ring (SHb).[3] However, sulfur-containing drugs possess oxidizing properties, which may lead to the accumulation of ferric Hb and the formation of ferric-SH ($HbFe^{III}–SH$) adduct in consequence. Herein, we employed a set of spectroscopic techniques to characterize the sulfhemoglobin structure formed inside functional erythrocytes.[1]
Experiments were conducted on the specially designed model of sulfhemoglobinemia developed on erythrocytes isolated from human whole blood. The formation of adducts was monitored after the administration of sodium sulfide. Raman measurements were conducted on the confocal CRM alpha 300 Raman microscope (WITec GmbH, Ulm, Germany) with 488 nm excitation, UV–Vis spectra recorded on the Lambda 950 spectrophotometer (PerkinElmer, Massachusetts, USA), and ECD spectra on J-1500 spectrometer (Jasco, Tokyo, Japan).
In the study, we confirmed the formation of two different Hb adducts in the sulfhemoglobinemia human blood model, SHb and $HbFe^{III}–SH$, when erythrocytes were exposed to sodium sulfide, and their ratio was dependent on the presence of oxidative conditions. Raman spectroscopy was the sole technique that allowed us to distinguish between both adducts. Importantly, ECD spectra delivered evidence that Hb sulfuration evoked changes in the protein packing or led to distinct Hb aggregation inside erythrocytes. Differentiation between SHb and $HbFe^{III}–SH$ is of great importance in sulfhemoglobinemia treatment, as SHb is an irreversible adduct incurable without blood transfusion, while $HbFe^{III}–SH$ can be removed with methylene blue.

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Keywords: sulfhemoglobin, erythrocytes, Raman spectroscopy

B-O.2

Title: *Revealing the problem of the effective charge of iron ion in oxy-haemoglobin molecule*

Author: Katarzyna Dziedzic-Kocurek[1], Jakub Dybaś[2], Jan Stanek[1]

[1]Faculty of Physics, Astronomy and Applied Computer Science, M. Smoluchowski Institute of Physics, Jagiellonian University
[2]JCET, Jagiellonian University

Abstract:
The search for a convincing description of the oxygen transport mechanism by heamoglobin has been the subject of multiple studies performed for a century [1]. The crucial., still not solved problem is the iron charge and spin state in oxyhemoglobin. There are basic discrepancies between the interpretation of results in such assignments that have been obtained using different spectroscopic methods (i. e. vibrational spectroscopy, X-ray absorption spectroscopy, and Mossbauer spectroscopy [2-4]). Each method is constrained to its time observation scale, energy sensitivity, and spatial resolutions, promoting different perspectives of the iron oxidation state description. There have been also many theoretical models used to calculate the effective charge of Fe ion [1, 5-6]. The result of these cross-studies is the widely accepted opinion, that it is hard to determine a value if any, electron transfer among Fe-O2 bonds.
One of the spectroscopic method which enables truly microscopic insight into the local electronic states of iron ions is Mössbauer spectroscopy. In the 60-ties of the previous century, the Mössbauer spectrum recorded at low temperatures and in an external magnetic field of 3T confirmed the low spin state of Fe and pointed out its diamagnetic state, i.e. its +2 charge state; the last conclusion is still debated since decades [5-6].
Here we present the results of the first Mössbauer measurements performed on native oxyhaemoglobin in red blood cells, in an external magnetic field of 8Tat 4K. That experiment finally enabled the determination of a slightly paramagnetic characteristic of the iron ion electronic state and the estimation of iron ion oxidation and spin state.

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Keywords: oxyhaemoglobin, Mössbauer spectroscopy, effective charge,magnetic-moment

B-O.3

Title: *Understanding Hydrogenases by 2D-IR Spectroscopy and Vibrational Perturbation Theory*

Author: Marius Horch[1], Yvonne Rippers[1], Cornelius Bernitzky[1], Solomon Wrathall[2], Barbara Procacci[2], Janna Schoknecht[3], Claudia Schulz[3], Christian Lorent[3], Catharina Kulka-Peschke[3], James Birrell[4], Ingo Zebger[3], Gregory Greetham[5], Oliver Lenz[3], Neil Hunt[2]

[1]Freie Universitaet Berlin
[2]University of York
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[4]University of Essex
[5]Rutherford Appleton Laboratory

Abstract:
[NiFe] hydrogenases catalyze the conversion of dihydrogen – an ideally clean fuel – by utilizing protein-embedded metal centers that carry biologically uncommon CO and CN⁻ ligands. The function of these diatomics is not well understood, but they can serve as structurally sensitive vibrational probes. Thus, IR absorption spectroscopy and harmonic vibrational analyses have long been used for studying hydrogenases. Recently, we have expanded this strategy by introducing 2D-IR spectroscopy and complementary anharmonic analyses:[1-4] (1) Probing sequences of vibrational transitions, solution-phase insights into bond lengths, strengths, and energies were gained. Besides their relevance for understanding hydrogenases, these data revealed new prospects for the vibrational spectroscopic analysis of chemical bonding. (2) Detailed information on structural dynamics, intramolecular energy transfer, and protein-cofactor interactions was obtained by analyzing vibrational energy transfer and the time evolution of coherent states. Results differ markedly from those obtained for synthetic metal carbonyls, highlighting the importance of the protein matrix for tuning the properties of biological metal sites. (3) Utilizing quantum beats and 2D-IR cross peaks, strategies for the analysis of spatial proximity and complex catalytic mixtures were established. (4) Based on vibrational perturbation theory, structural information encoded in 2D-IR spectra of [NiFe] hydrogenases and experimentally inaccessible insights into (resonant) vibrational interactions were obtained. All these strategies were also applied to [FeFe] hydrogenases, whose extended set of CO and CN− ligands leads to a more complex vibrational manifold. Insights into these enzymes indicate that the bonding situation and vibrational structure of the [FeFe] active site is dictated by multiple molecular factors. In total, these findings and strategies provide new perspectives for understanding complex (bio)organometallic targets.

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Keywords: 2D-IR, vibrational-perturbation-theory, hydrogenases, metalloenzymes, metal-carbonyls

B-O.4

Title: *FTIR studies of mutual interaction in PLL-doped DPPC/DPPG membranes: a powerful insight by chemometric analysis*

Author: Paulina Trombik[1], Mirosław Czarnecki[1], Katarzyna Cieślik-Boczula[1]

[1]Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw

Abstract:
It is commonly known that mutual interaction in protein/peptide – lipid membrane systems plays a crucial role in many biological processes [1]. The most important outstanding problem is that these systems are not one- but multicomponent systems, in which protein folding can involve transformation from one structure to another one in a non-one-step manner. Additionally, membrane structure may be heterogeneous and the phase separation can take place.
FTIR spectroscopy is known as one of the most powerful methods to study the secondary structure of proteins and peptides as well as many different structural properties of lipid membranes [2,3]. It will be shown how we can increase quality of abstained results and rise structural information about peptide – lipid membrane system under study by using chemometric methods such as PCA, MCR-ALS, 2D COS and mowing window 2D COS.
Phase separation will be detected and characterised in details in both pure and poli-L-lysine (PLL) – doped DPPC/DPPG membranes. Additionally, the effect of two different domains in DPPC/DPPG membranes on the secondary structure of PLL will be shown. A type of secondary structure of PLL studied in the presence of negatively charged DPPC/DPPG membranes and how they can transform in a function of temperature will be discussed too.

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Keywords: Lipid membranes, poli-L-lysine, FTIR, chemometry

B-O.5

Title: *Crystalline purines in microalgae: Surprising robustness of the biosynthesis of crystalline guanine in dinoflagellates*

Author: Peter Mojzeš[1], Maxim Bokov[1], Radek Bura[1], Jana Pilátová[2]

[1]Charles University, Faculty of Mathematics and Physics, Institute of Physics
[2]Charles University, Faculty of Science, Department of Experimental Plant Biology

Abstract:
Recently, it has been shown that the biosynthesis and accumulation of microcrystalline purines is a highly widespread property shared by a great part of protists from all major eukaryotic supergroups including photosynthetic microalgae [1]. Considering that evolutionarily very distant eukaryotic microorganisms are able to accumulate, store and reuse in the absence of other nitrogen sources [2] large amounts of purines in crystalline form, this property was probably already present in their last common ancestor. It was shown that eukaryotic microorganisms are able to store purines in various derivatives, most often in two forms of crystalline guanine, but also as crystalline uric acid or xanthine [1]. Using Raman microscopy and *Amphidinium carterae* as a model organism, in this work we have studied the de novo biosynthesis of crystalline guanine from different nitrogen sources under various cultivation conditions. We have found that the biosynthesis of crystalline guanine takes place even under conditions of extreme stress, for example in media with a high content of heavy water, when the number of other biochemical processes is reduced or completely stopped [3, 4]. Using Raman microscopy, we have revealed that even under these extremely unnatural conditions, A. *carterae* is able to biosynthesize crystalline guanine from a wide range of nitrogen sources and create large reserves of crystalline guanine with a total degree of deuteration exactly corresponding to the fraction of heavy water in the medium.

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Keywords: Raman, microscopy, microalgae, purines, inclusions

B-O.6

Title: *In-cell IR Difference Spectroscopy as a Time-resolved Method to Study Proteins in Living Cells*

Author: <u>Lukas Goett-Zink</u>[1], Anna Toschke[1], Eileen Baum[1], Tilman Kottke[1]

[1]Bielefeld University / Biophysical Chemistry and Diagnostics

Abstract:
Structural changes of proteins can be elucidated by FT-IR difference spectroscopy. The proteins are studied in vitro under defined buffer condition optimized for protein stability. However, in vitro conditions differ to the environment inside cells. Crowding and small molecules in a cell can influence the mechanism of proteins. Recently, we developed an approach of in-cell infrared difference spectroscopy (ICIRD) to study the response of soluble photoreceptors with concentration of ~200 µM in living bacterial cells [1]. ICIRD was established in transmission mode and in the attenuated total reflection configuration. Studying light, oxygen, voltage (LOV) receptors with ICIRD revealed an altered activation mechanism between *in vitro* and in cells that appeared in specific suppression of structural elements. These deviations were rationalized by emulating the cellular environment. In addition, ICIRD is applicable to proteins that cannot be isolated. The plant cryptochrome pCRY is a photoreceptor of which isolation fails due to degradation. Here, we used ICIRD to investigate the full-length pCRY directly in living cells. Comparison of the sensor domain and the full-length of pCRY in cells revealed a shift of a beta-sheet signal resolving the domain arrangement [2]. Next, we extended ICIRD to a time-resolved method with a resolution of 7.6 ms after laser excitation by applying the rapid-scan technique, a home-build sample changer and synchronization of the laser with the interferometer (Fig. 1). Time-resolved ICIRD was applied to aureochrome1a, a light regulated transcription factor. The signal progression from the sensory domain to the effector of aureochrome1a was time-resolved in cells, providing structural insights into the activation mechanism [3]. Static and time-resolved ICIRD expands the range of in-cell methods and will contribute to a better understanding of the structural response of proteins in a cellular environment.

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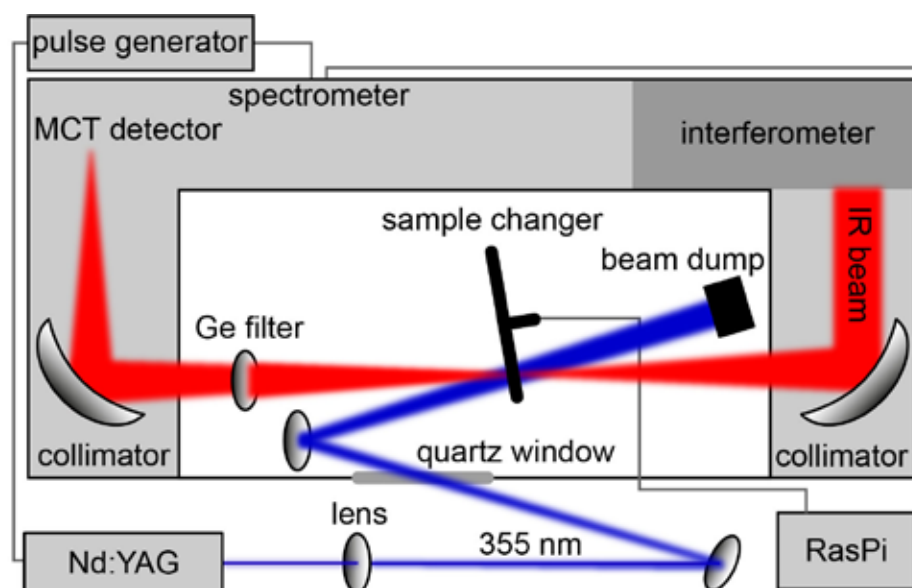
Figure captions:
Figure 1: Scheme of the setup for time resolved ICIRD spectroscopy. A pulsed laser was used for excitation. Synchronization of the interferometer and the laser yielded a time resolution of 7.6 ms.

Keywords: time-resolved, in-cell, spectroscopy, ICIRD, FT-IR

B-O.7

Title: *Nanosecond time-resolved IR spectroscopy on proteins using quantum cascade laser setups*

Author: Jessica Klocke[1], Tilman Kottke[1]

[1]Biophysical Chemistry and Diagnostics, Bielefeld University

Abstract:
The application of quantum cascade lasers (QCL) as mid-infrared probe lights significantly advances the investigation of mechanisms of proteins in H_2O. In particular, irreversible reactions in proteins have been a real challenge for time-resolved spectroscopy because of the high sample consumption. One approach to address this challenge is to employ single shots of acquisition that provide both temporal and spectral resolution at the same time. We demonstrated using QCL frequency combs that protein reactions can be resolved with sub-microsecond resolution requiring only few excitations on bacteriorhodopsin, a light-driven proton pump [1]. Characteristic kinetic traces at different wavenumbers were even obtained after a single shot.
Alternatively, sample consumption can be minimized by focusing the QCL to the diffraction limit. We developed a setup using a tunable QCL to record time-resolved difference spectra from 20 nanoseconds to 1 second of irreversible photoreactions of flavin in H2O. The combination of the focused QCL with a pressure-tolerant flow cell and a micrometer stage orthogonal to the flow allowed us to drastically reduce the sample consumption to a few microliters for a complete dataset [2]. A continuous dataset in the spectral dimension was generated and treated with global kinetic analysis. Moreover, the comparison of kinetics acquired with the setup on bacteriorhodopsin with those from FTIR step-scan spectroscopy highlights the advantages of QCL-based approaches. These studies have been extended by us to irreversible reactions in soluble photoreceptors providing new insight into the mechanisms.

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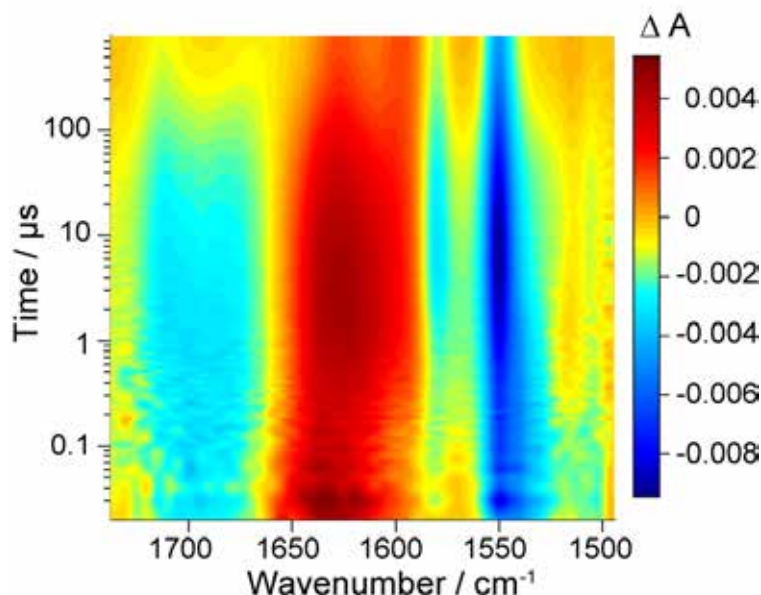
Figure captions:
Contour plot of light-induced difference spectra generated from raw data after 100 averages on the irreversible reaction of flavin in H2O.

Keywords: QCL, frequency-comb, biomolecules, irreversible, kinetics

B-O.8

Title: *Rapidly determining the 3D structure of proteins by Surface-enhanced Raman spectroscopy*

Author: Hao Ma[1], Bin Ren[1]

[1]Xiamen University

Abstract:
Despite great advances in protein structure analysis, label-free and ultrasensitive methods to obtain the natural and dynamic 3D structures are still urgently needed. Surface-enhanced Raman spectroscopy (SERS) can be a good candidate,[1] whereas the lack of deciphering strategies makes it extremely challenging to determine the protein structure. Herein, we developed a deciphering strategy based on well-defined SERS detecting configuration and a novel algorithm for rapid and accurate determination of protein structure from the experimentally obtained SERS spectra, as shown in Figure 1. On one hand, we constructed a gap-mode configuration with well-defined local gradient field based on iodide-modified nanoparticles over single crystal surface. The strongly adsorbed monolayer iodides not only prevent strong chemical interactions between the protein and metal surfaces, but also remove the impurities on the surface, enabling highly reproducible and reliable SERS spectra to be obtained.[2] On the other hand, we innovatively introduced a coarse-grained model to isolate and obtain the polarizability derivatives (PDs) of each amino acid in protein structures.[3] The complex interactions between protein and the electric field for calculating SERS spectra can be simplified into simple matrix computation of PDs, rotation matrix, and gradient local electric field.[4] As a result, the 3D protein structure can be reconstructed by comparing the experimental spectra obtained in a well-defined gap-mode SERS configuration with the simulated spectra. Armed with the proposed strategy, SERS will no longer be limited to the interfacial systems of small molecules but becomes competent for exploring dynamics of interfacial protein. Moreover, the possible adsorption configuration of surface proteins can be predicted with its SERS spectra, which provides a new approach for structural biology.

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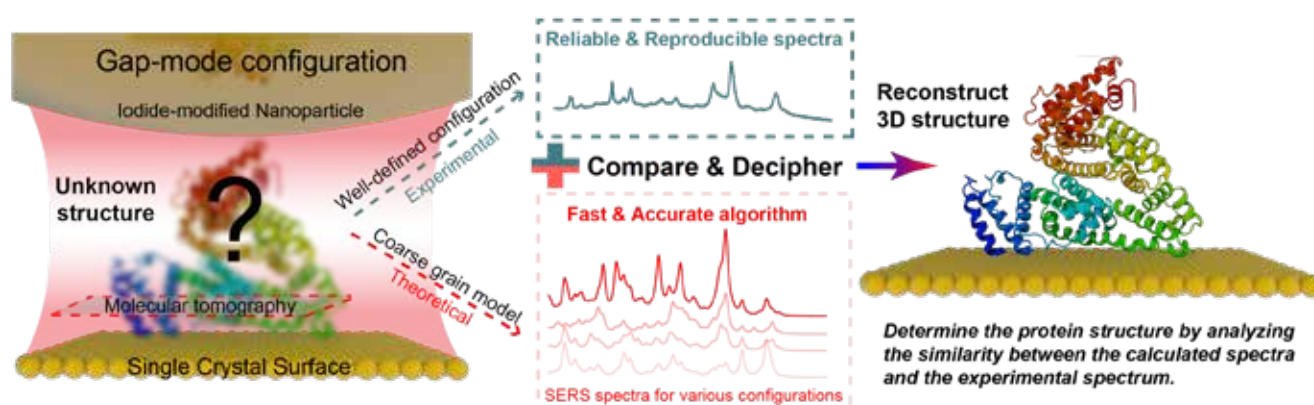
Figure captions:
The deciphering strategy to extract 3D protein structure from the experimental SERS spectra in seconds with a high accuracy.

Keywords: Protein, Structural biology, SERS, Polarizability

B-O.9

Title: *Decoding early signs of erythrocyte pathology through analysis of protein secondary structure alterations*

Author: Tetiana Stepanenko[1], Katarzyna Bułat[2], Natalia Wilkosz[2], Fatih C. Alcicek[3], Jakub Dybas[4], Katarzyna M. Marzec[5]

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Abstract:
Early detection of erythrocyte, red blood cells (RBCs) pathology is critical for timely intervention and effective treatment. The analysis of protein secondary structures in both cell cytosol and cell membranes can serve as a potential diagnostic tool in this regard. Fourier transform infrared (FTIR) spectroscopy is a non-destructive technique widely used to investigate protein structural changes [1]. This study aims to explore alterations in protein secondary structures in erythrocytes as an initial marker of erythrocyte pathology using FTIR.

Our findings suggest that modifications in the way proteins are structured within intact RBCs could serve as biomarkers for certain diseases or aging. The storage of human RBCs for an extended period leads to a shift from α-helix structures to β-sheets related to the aggregation process stabilized by strong intermolecular hydrogen bonding, indicating aging and was followed by irreversible changes in the quaternary structure of Hb [2]. In mice, the changes in protein secondary structure within the intact RBCs of D-gal-treated mice were similar to those observed during natural aging of C57BL/6J mice, and a reduced ratio of turns to α-helices indicated protein aggregation [3]. Similarly, an increase in unordered conformations relative to α-helical structures is observed in RBCs as atherosclerosis progresses. Our results showed more pronounced secondary structure alterations within the RBCs of 8-week-old ApoE/LDLR-/- mice than in the age-matched control, and sex-related differences were observed solely in 24-week-old male ApoE/LDLR-/- mice [4]. Moreover, our study proved a higher resistance of female RBCs comparing to male RBCs, to secondary structure changes with progression of atherosclerosis [4].

Our multimodal studies demonstrate the usefulness of FTIR for the analysis of protein secondary structure alterations and provide a better understanding of the underlying molecular mechanisms of erythrocyte pathology.

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Keywords: erythrocyte pathology, proteins alterations, FTIR

B-O.10

Title: *Hydration Structure of Biomaterials Studied by Infrared Spectroscopy and Chemometrics*

Author: Shigeaki Morita[1]

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This work was supported by JSPS KAKENHI Grant Number JP22H04563.

Abstract:
Water structure at the biointerface between artificial materials and living body plays an important role for their biocompatibility. We have investigated hydration structure of biomaterials which shows excellent blood compatibility such as poly(2-methoxyethyl acrylate) (PMEA).[1, 2] In the present study, hydration structure of polyvinylpyrrolidone (PVP) compared to that of PMEA is reported based on infrared spectroscopy combined with multivariate curve resolution (MCR) [3].

A polymer film was prepared on a calcium fluoride substrate. The polymer sample on the substrate was mounted in a flow cell designed for transmission spectroscopy. The polymer sample was sufficiently dried by nitrogen gas flow before the spectroscopic measurement. Time-dependent infrared spectra during a sorption process of water vapor were obtained using an FT-IR. Figure 1 shows the time-dependent infrared spectra during a sorption process of water vapor into a PVP film collected every 0.16 s. A gradual increase of the O-H stretching band around 3600-3000 cm^{-1} having a spectral waveform variation was observed, representing change of water structure in the PVP matrix. It was revealed from the MCR in the O-H stretching region that there are at least three different types of water structure in the PVP matrix. In contrast, our previous study reported only one type of water of non-freezing water is hydrated to PMEA by the water vapor feeding.

A positive and negative intensity splitting in the C=O stretching region around 1800-1600 cm^{-1} representing C=O ••• HOH type of hydrogen bonding. The positive increase represents the structure of the hydrogen bonded C=O and the negative decrease that of the free C=O not involved in the hydrogen bonding. Similar signal intensity splitting was observed in the C-N stretching region. Detailed hydration structure of PVP compared to that of PMEA will be discussed.

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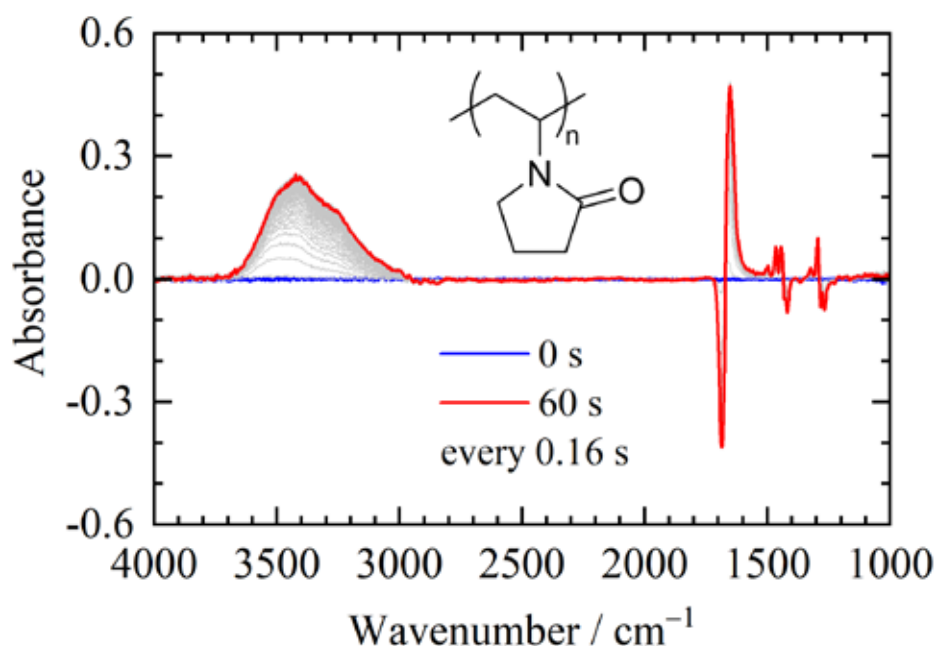
Figure captions:
Time-dependent IR spectra of PVP during a sorption process of water vapor.

Keywords: Hydration structure, Biomaterials, IR, MCR

B-O.11

Title: *Plasmonic infrared study of SARS COV-2 mPro dimerization and its inibition*

Author: <u>Federica Piccirilli</u>[1], Giovanni Birarda[1], Lisa Vaccari[1], Hendrik Vondracek[1], Lucia Silvestini[2], Francesco Spinozzi[3], Paolo Mariani[3], Antonio Palumbo Piccionello[4], Vincenzo Aglieri[5], Andrea Toma[5], Maria Grazia Ortore[3]

[1]Elettra Sincrotrone Trieste
[2]Università politecnica delle Marche
[3]Università Politecnica delle Marche
[4]Università degli studi di Palermo
[5]Istituto Italiano di tecnologia

Abstract:
We present the experiments performed at SISSI-Bio beamline of Elettra Sincrotrone Trieste[1] addressing the structural study of SARS CoV-2 main protease (mPro) through PIR-SEIRA microspectroscopy. The mPro is an essential enzyme for the replication of the virus since it processes most of the viral polyproteins. It is, thus, a key target for drugs development. The most functional form for Mpro enzymatic activity is associated to mPro homodimer, thus compounds inhibiting dimerization should also inhibit mPro catalytic activity. However, shifting the dimer-monomer equilibrium towards monomers does not directly lead to a loss in the enzymatic activity. Based on this evidence and given the importance of efficently block SARS-CoV-2 replication, it is of outmost importance to highlight the structural differences alternatively produced on Mpro upon dimerization or upon binding to inhibitors.
Recently PIR-SEIRA spectroscopy has proven to be the perfect technique to study proteins subtle structural variations associated to inhibitors binding2. Nanoantennas arrays can selectively enhance IR signals and, in addition, confine the fields to the surface to a monolayer protein thickness. In PIR-SEIRA, reflection measurements conducted under back illumination of nanoantennas allows thus to probe anchored proteins monolayers, with minimum contribution of environmental buffer molecules2. In the presented experiments, PIR-SEIRA spectroscopy on Mpro was carried out thanks to ad hoc designed devices, resonating in the spectral region of Amide I and Amide II bands. We evaluated here the structure of anchored mPro monomers and dimers in buffered environment. Our results show that dimerization is not associated to relevant backbone rearrangements of the protein at secondary structure level. Moreover, we obtained a direct evidence of mPro monomers binding to a selected inhibitor and observed that even if the compound inhibits the dimerization it is not effective on breaking preformed dimers.

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Keywords: sars-Cov-2, PIR-SEIRA, dimerization, inhibition

B-O.12

Title: *The chemical structure and conformation of tau protein aggregates at the growth phase*

Author: Kamila Sofińska[1], Sara Seweryn[1], Katarzyna Skirlińska-Nosek[1], Piotr Batys[2], Jakub Barbasz[2], Ewelina Lipiec[1]

[1]Jagiellonian University, Faculty of Physics, Astronomy, and Applied Computer Science, M. Smoluchowski Institute of Physics
[2]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences

Abstract:
Neurodegenerative disorders with accompanying abnormal aggregation of proteins are one of the most serious health and social problems worldwide today.[1,2] The histopathological hallmark of Alzheimer's disease (AD) is the abundance of intraneuronal tau neurofibrillary tangles (NFTs) and extra-neuronal amyloid-β (Aβ) plaques in the neocortex.[3,4] The presence of tau lesions clearly correlates with cognitive impairments. Thus, tau protein became the target of potential therapeutic regimes against AD.5 In AD, several forms of tau protein aggregates can be recognized long before the onset of dementia symptoms, i.e. phosphorylated pre-tangles and neuropil threads. Tau neurofibrillary tangles (NFTs) appear in neurons at the late stage of the disease.[5] It suggests that inhibiting tau aggregation at the early stages of the tau aggregation process could prevent neurodegenerative symptoms characteristic for the late stages of AD. However, while the structure of tau monomers (Figure 1) or fibrils is thoroughly investigated, still little is known about the structure of tau aggregates occurring in the curse of aggregation.
In the presentation, the research at the nanoscale into the chemical structure and conformation of tau protein aggregates at the growth phases of the aggregation process will be discussed. We incorporated tip-enhanced Raman spectroscopy (TERS) to capture spectra of protofibrils and young fibrils at the early aggregation stage to reveal their structure and to gain insights into TER signature related to structural transitions resulting from the ongoing aggregation. We applied multivariate data analysis to treat the data acquired from protofibrils and young fibrils to reveal discrepancies in the structure of tau aggregates in time. Moreover, the results of molecular dynamics (MD) simulations revealing the structure of tau aggregates as well as the molecular interactions governing the aggregation at the initial aggregation stage will be presented.

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Figure captions:
TER and Raman spectra of individual tau protein monomers. The locations of TER spectra acquisition are marked on the AFM topography image.

Keywords: tau, protein, TERS, AFM, MD
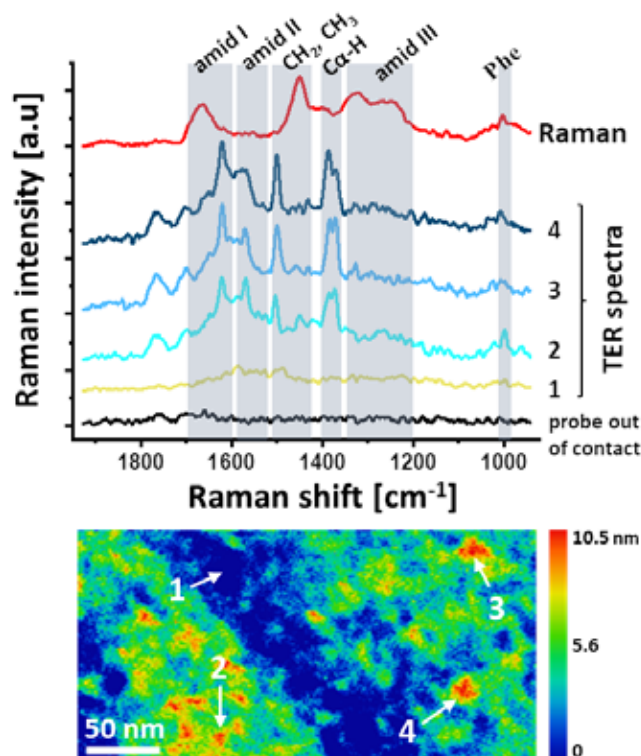
B-O.13

Title: *Raman Spectroscopic Investigations of the Mechanisms of Inhibition of Protein Fibrils by Novel Spirooxindole Compounds*

Author: Anthony Dahdah[1], Subashani Maniam[1], Nilamuni De Silva[1], Helmut Huegel[1], Ewan Blanch[1]

[1]RMIT University

Abstract:
The formation and accumulation of β-sheet rich amyloid fibrils is a complex multi-step process which results in the formation of toxic plaques that are implicated in the cause of various neurodegenerative diseases, such as Alzheimer's disease [1]. The fibrilogenesis mechanism and cellular toxicity of these aggregates is still relatively unclear, however, complicating the search for effective therapies for these diseases. Xian et.al [2] explored the protective effect of the oxindole alkaloid Isorhycnchophylline, which displayed a neuroprotective effect against the neurotoxicity of Aβ25-35 in PC12 cells by inhibiting oxidative stress and suppressing the mitochondrial pathway of cellular apoptosis. This motivated the current work which involves introducing various synthesised spirooxindole compounds to study their inhibitory effect against the formation of amyloid fibrils. Conventional Raman spectroscopy is a widely applicable and non-destructive method that can provide important information on changes in the secondary structure of proteins during complex processes such as fibrilogenesis. Significant changes in the Amide I and III band profiles are widely used to characterize the transition from α-helix to β-sheet. In this study, we have used Raman specectroscopy in combination with Fluorescence spectroscopy, Circular Dichroism and TEM to investigate the mode of interaction of a range of novel spirooxindole compounds as potential inhibitors of the formation of protein fibrils. The combination of spectroscopic techniques has revealed that the inhibitor compounds which were introduced to hen egg white lysozyme solutions produced off-pathway oligomers which are far more disordered than the toxic stacked β-sheet strands that are found in most fibrils. In addition several of these spirooxindole derivatives were able to dissociate the highly ordered structure of preformed β-sheet fibrils.

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Keywords: Raman spectroscopy, fibrils, inhibitors, spirooxindoles

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B-O.14

Title: *State of water in various environments: aliphatic ketones. MIR/NIR spectroscopic, dielectric and theoretical studies.*

Author: Mirosław Czarnecki[1], Krzysztof Beć[2], Justyna Grabska[2], Christian Huck[2], Sylwester Mazurek[1], Kazimierz Orzechowski[1]

[1]University of Wrocław
[2]University of Innsbruck

Abstract:
This work provides new insight into the state and interaction of water in a series of aliphatic ketones. [1] For studies we selected 2-heptanone, 4-heptanone, cyclopentanone, cyclohexanone, cycloheptanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and 2,6-dimethylcyclohexanone. Selection of these ketones permitted us to examine the effect of various structural motives on behavior of water in the mixtures. Our results evidence that the solubility of water in ketones depends mainly on accessibility of the carbonyl group, while the other factors are less important. Conformational flexibility of aliphatic chains allows for effective shielding of the carbonyl group in linear ketones, and is the main reason of poor solubility of water in these ketones. Due to low water content, in the linear ketones molecules of water are involved mostly in ketone-water interactions, while the water-water interactions are insignificant. In contrast, better solubility of water in the cyclic ketones enables creation of bulky clusters of water, where most of molecules are in water-like environment. In water-saturated cyclic ketones, the temperature rise increases population of ketone-water interactions at the expense of the water-water ones. On the other hand, in saturated solutions of linear ketones and 2,6-dimethylcyclohexanone at higher temperatures increases the population of singly bonded water at the expense of the doubly bonded ones (Figure 1). Comparison of the experimental and theoretical results reveals that the substitution of the cyclic ketones by a single methyl group does not affect the strength of the ketone-water interactions, while it has a significant impact on the solubility of water in ketone. This is a strong evidence that the steric effect is the most important factor determining the intermolecular interactions and the solubility of water in aliphatic ketones.

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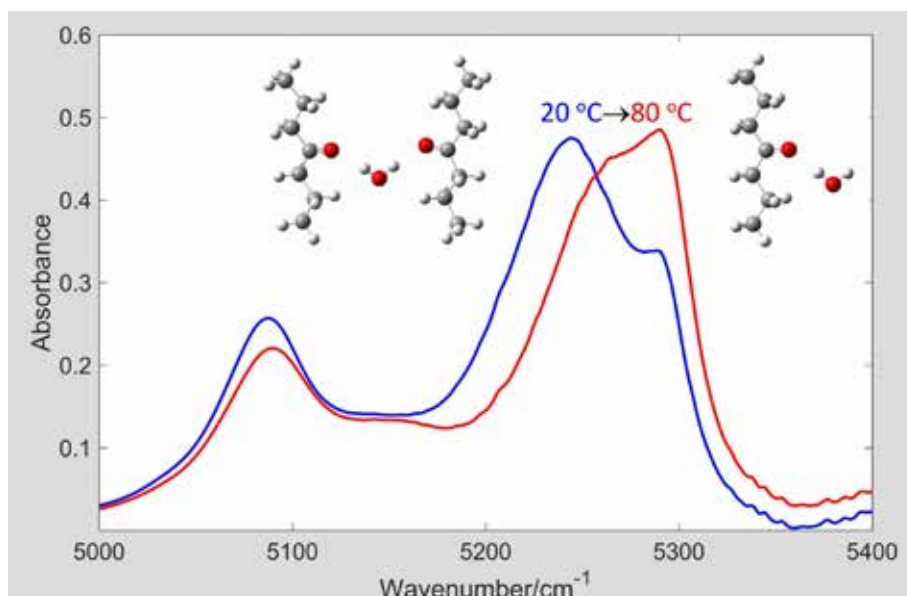
Figure captions:
Figure 1. Effect of temperature on structure of 4-heptanone/water complex.

Keywords: ketone/water mixtures, solubility, hydrogen bonding,

B-O.15

Title: *Near-Infrared and visible excited Raman optical activity in the study of B12 derivatives: far-from-resonance vs strong resonance approach*

Author: Ewa Machalska[1], Grzegorz Zając[1], Monika Halat[2], Takumi Tani[3], Tomotsumi Fujisawa[3], Masashi Unno[3], Malgorzata Baranska[1]

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[2]Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture
[3]Department of Chemistry and Applied Chemistry, Faculty of Science and Engineering, Saga University

Abstract:
Raman optical activity (ROA) is based on measuring small Raman scattering intensity difference between right- and left-circularly polarized light (CPL) from chiral samples. Recently, resonance Raman optical activity (RROA) together with electronic circular dichroism (ECD) have proved to be effective spectroscopic tools in examination of the vitamin B12 molecular structure and its derivatives. As a structure-sensitive spectroscopic technique RROA enables to explore subtle alteration in a structure of truncated vitamin B12 analogs [1], and derivatives with different upper axial substituents or ring modifications [2] by providing bisignate and enhanced spectra due to resonance via more than one electronic state. Also, for different vitamin B12 variations, that exhibit relatively strong ECD signal close to the ROA excitation wavelength (532 nm), a valid phenomenon masking natural RROA spectra has been discovered. Briefly, so-called ECD-Raman effect results from a combination of electronic circular dichroism and circularly polarized Raman [3]. In this study, we present the RROA study of native vitamin B12 and its four chemical modifications that differ in structure around the chromophoric corrin ring and central cobalt atom (Figure 1), being fully aware appearance of ECD-Raman contribution on RROA spectra and knowing strategies of its elimination. Without a doubt, the different substitutions lead to large variations in the intensity and shape of UV-Vis and ECD absorption bands, and they also determine the spectral profiles in RR/RROA experiments. To more in-depth study of vibrational properties and corrin ring conformations of cobalamin analogs, among the visible excited ROA (λex=532 nm) we included also near infrared (λex=785 nm) ROA along with the DFT calculations.

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Keywords: corrinoids, ROA, ECD-Raman effect, simulation

B-O.16

Title: *Evaluating the acidity levels in super-acidic ionic liquids by Raman Spectroscopy*

Author: Cedric Malherbe[1]

[1]University of Liege

Abstract:
Understanding fundamental levels of acidity accessible in non-aqueous solvents, often expressed as the proton transfer activity coefficient, which is related to the free energy of transfer when the proton is transferred from one solvent to another, experiencing difference in solvation[1], is critical for developing new chemical syntheses and applications in non-aqueous media[2]. For the proton, values for the transfer activity coefficient can only be experimentally estimated from extra-thermodynamic models applied to electrode potentials, solubility or spectroscopic measurements[3,4]. Experimental values for the transfer activity coefficient of proton are essential to confront the computed values, as determining free energies of proton solvation, either experimentally or theoretically, in non-aqueous solvents is one of the most discussed questions in physical chemistry[5]. Proton solvation are particularly interesting to study in stable room temperature ionic liquids (RTILs), resulting from the combination of organic cations (often derivatives of N,N′-substituted imidazolium, N-substituted pyridinium, quaternary ammonium or phosphonium) and inorganic or organic anions (e.g. carboxylates, sulphates, halogens), which emerged as an interesting alternative to volatile organic solvents for inorganic and organic syntheses, catalyses, electrolytes and microextraction, especially for their capacity to dissolve sugar-based biopolymers such as lignin[6–10]. The presence of small fractions of molecular impurities in RTILs has major impacts on their physico-chemical properties, especially the acidity levels accessible in these solvents, which need to be addressed to develop more robust applications. Here we report on the determination of the free energy of solvation for proton in stable N,N′-substituted imidazolium ionic liquids using far-field classical Raman spectroscopy and the acidity function proposed by Hammett[11].

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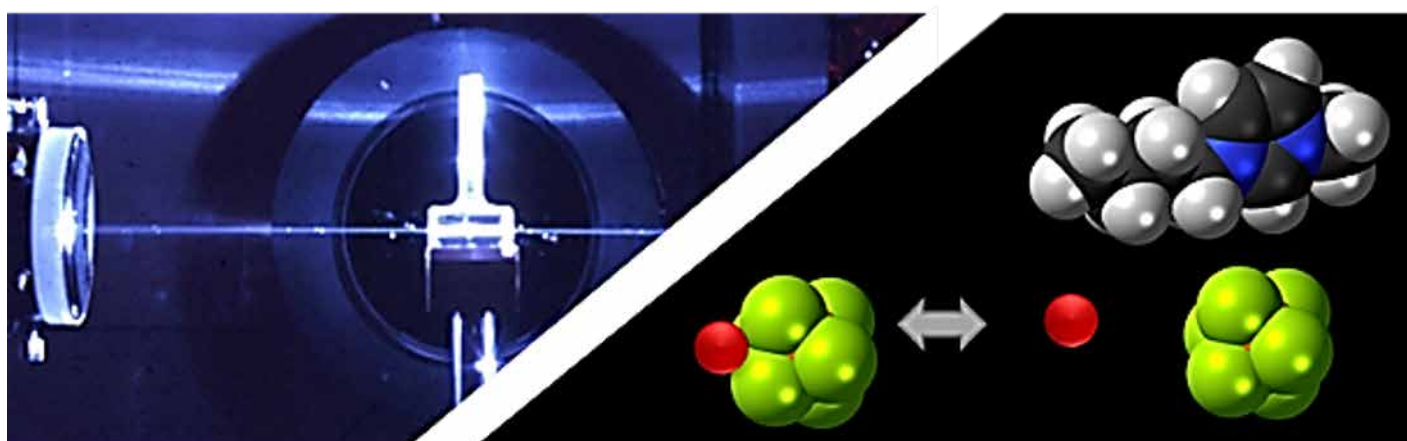
Figure captions:
Fundamental acidity level in ionic liquids by Raman spectroscopy

Keywords: acidity levels, ionic liquids, Hammett

B-O.17

Title: *Unraveling the Structural Polymorphism of Mononucleotide G-Quadruplexes via Raman Optical Activity*

Author: Štěpán Jílek[1], Josef Kapitán[2], Mohammed Siddique Para Kkadan[1], Ivan Barvík[1], Václav Profant[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University
[2]Department of Optics, Faculty of Science, Palacký University Olomouc

Abstract:
In the last 20 years, G-quadruplexes (G4), which are self-associating nanostructures of guanine nucleotides, started to attract considerable research interest due to their newly discovered significance in genomics and potential applications in bionano-technology.[1] Despite significant advances in the field, many fundamental aspects of the structural, functional, and dynamical properties of G4 remain inadequately understood. This knowledge gap has crucial implications for understanding the G4 formation and stability. Notably, the relation between the G4 topology and the size of the stabilizing cations is not well explained, and the hydrogen bonding structural patterns in the outer regions of agglomerates, as well as the stacking patterns and helicity of G-quartets in different mG4s are still being debated.[2,3]

In this study, we demonstrate the use of Raman optical activity (ROA) to monitor the association and dynamics of mono-nucleotide G4s (mG4s). We obtained well-resolved Raman and ROA spectra and found that ROA is more sensitive to mG4 formation than Raman scattering. Agglomeration was reflected by sharpening and double intensity increase of vibrational features in the fingerprint region and striking magnitude signal increase in the terahertz region, reflecting the higher-order arrangement of systems in the presence of different stabilizing cations (Fig. 1, left). Furthermore, we observed a significant loss in G4 stability (melting temperature dropped by ~30°C) when ribose was changed for 2´-deoxyribose, highlighting the important role of H-bond networking in the outer part of agglomerates. In the case of Li-stabilized agglomerates, we observed a reversible transition between two types of mG4 arrangement, which has not been previously reported (Fig. 1, right). Our experimental observations were accompanied by molecular dynamics simulations and advanced quantum mechanics simulations of spectral profiles, providing a more detailed analysis of the observed phenomena.

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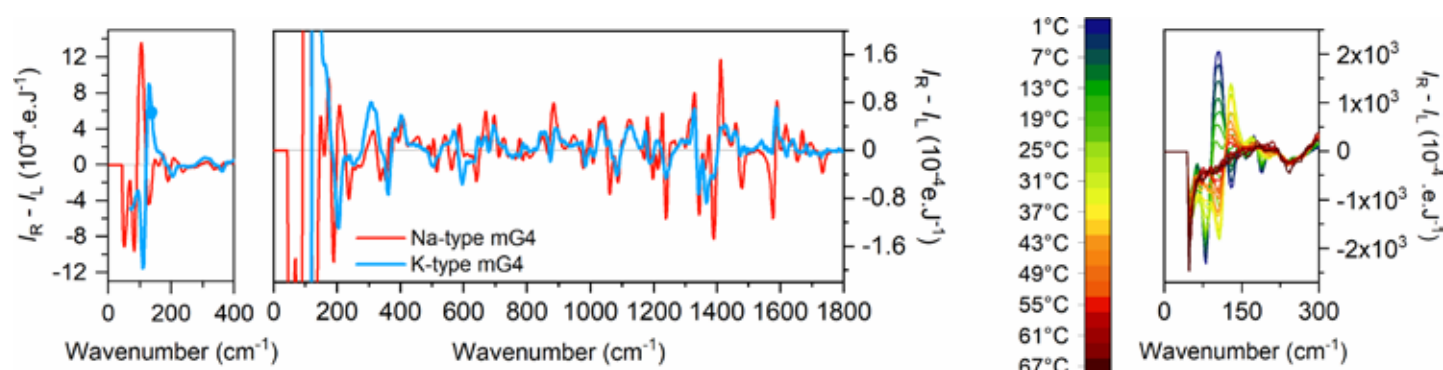
Figure captions:
ROA spectra of self-associated mG4s stabilized by Na+ and K+ ions (left). The temperature evolution of ROA of mG4 with Li+ ions in the THz region evidencing the structural transition (right).

Keywords: G-quadruplex, self-associtaion, GMP, Raman, ROA

C-I.1

Title: *Probing protein conformations at the nanoscale by means of IR nanospectroscopy*

Author: Antonia Intze[1], Maria Eleonora Temperini[1], Raffaella Polito[2], Michele Ortolani[2], <u>Valeria Giliberti</u>[3]

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[2]Department of Physics, Sapienza University of Rome
[3]Istituto Italiano di Tecnologia, Center for Life Nano- and Neuro-Science

Abstract:
Assessing the protein conformation, and possibly the slight conformational changes that accompany the protein function, is key to understanding their role in a variety of physiological and pathological processes. Despite this, the study of transitions of protein conformation is still demanding [1,2]. Experimental tools capable to monitor protein conformation, and possibly its modifications, at the nanoscale and ideally at the single molecule level, are therefore of paramount relevance to address this issue. Here we present two applications of atomic force microscopy-assisted (AFM-assisted) infrared (IR) nanospectroscopy [3] (Fig. 1a) where the unprecedented capability to monitor the protein conformation at the individual nanometer-sized biological specimen represents a powerful tool enabling to tackle still unresolved issues. The first case study is protein aggregation, which takes place upon modification of the protein native tridimensional conformation, and it is attracting an ever-growing interest due to the connection that has been identified with many neurodegenerative diseases [4]. In our recent experiments, we focused on the alphasynuclein protein (alphaS), considered the main pathological protein associated with Parkinson's disease, and more specifically on the interaction with RNA. The possibility to combine morphological and structural information at the nanoscale (Fig. 1b-d) has proven to be crucial to gain insights into the effect of RNA on the formation of protein amyloid fibrils. The second application deals with the study of membrane proteins. We have pioneered the first application of IR difference nanospectroscopy to monitor the light-induced functional conformational changes of proteins embedded in individual patches of cell membranes [5,6]. These results open the way towards possible combination of the electrical and spectroscopic capabilities of AFM so as to gain insights into the voltage-dependent membrane protein dynamical behavior.

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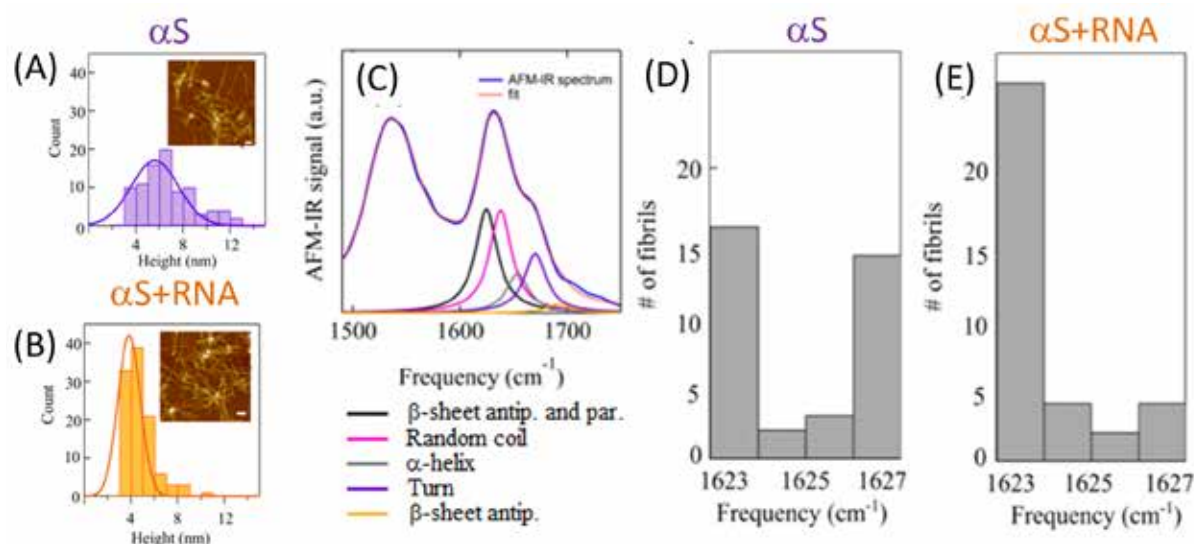
Figure captions:
A Sketch of the technique. B Example of deconvolution of the amide-I band sensitive to protein conformation. C-D Histogram of the central frequency of the component considered marker of aggregation.

Keywords: IR nanospectroscopy, protein conformation, AFM

Title: *Nanophotonic platforms for enhanced chirally sensitive vibrational spectroscopy*

Author: Malcolm Kadodwala[1]

[1]University of Glasgow

Abstract:
Chirally sensitive, optically active, vibrational techniques based on the absorption and scattering of circularly polarised light (vibrational circular dicroism (VD) and Raman Optical Activity (ROA)) rely on measuring very small differential signals (< 0.01%) and are thus extremely insensitive (~mM range). I will present work[1,2] which demonstrates the potential to massively increase the sensitivity of chirally dependent vibrational measurments using nanophotonic platforms based on lithographically created plasmonic chiral structures. Specifically this strategy allows chirally sensitive vibrational measurments to be made from femtomole quantities of material.

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Keywords: chiral nanophotonics metamaterials

C-I.3

Title: *Surface-Enhanced Anti-Stokes Intensity Fluctuations at High Speed*

Author: Alexandre Brolo[1], Nathan Lindquist[2]

[1]University of Victoria
[2]Bethel University

Abstract:
Strong surface-enhanced Raman scattering (SERS) intensity fluctuations (SIFs) are typically observed from diluted solutions, and they have traditionally been assigned to evidence of single-molecule detection by SERS [1]. Recently, we have developed new experimental methods capable of monitoring those fluctuations at high speed (sub-ms time scale) [2]. Surprisingly, we observed those fluctuations even from fully coated nanostructures (i.e., not in "single molecule regime"). It is then clear that, at those high-speed time scale, the SERS fluctuations seems to be a general phenomenon. In fact, we observed fast SIFs from different molecular probes adsorbed on single nanoparticles of different shapes, aggregated structures and deposited metal films [3-5]. The challenging in our previous experiments was to obtain a fully spectral signature of the SIFs at those high acquisition speeds. This challenge has been addressed by the development of an acquisition procedure that captures the full SERS spectrum, including both the Stokes and the anti-Stokes region, with microseconds time resolution, as shown in Figure 1 [6]. We found these high-speed SIF events occur over a broad spectral range, covering both the anti-Stokes and the Stokes sides of the spectrum. This means that some of the events produces anomalously large anti-Stokes peaks. These results seems to support the idea that transient hotspots, with narrow resonances either centered in the Stokes or the anti-Stokes side, drive the SIFs at extremely high speeds.

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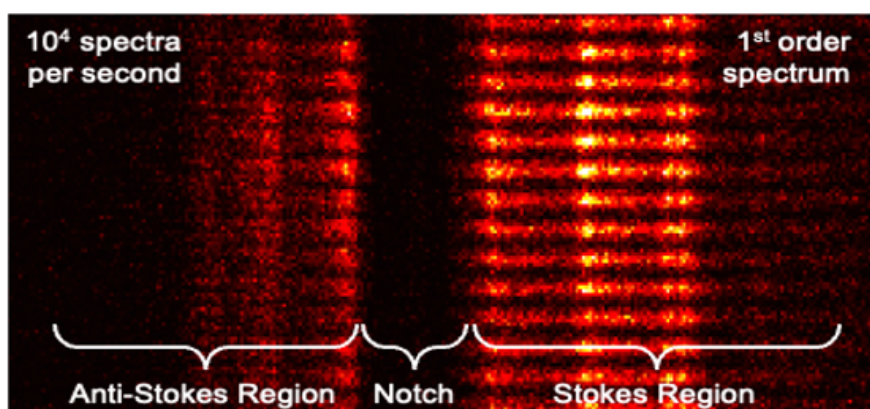
Figure captions:
Figure 1: Raw data from the high-speed acquisition system, showing the spectral acquisition and intensity fluctuations in both the Stokes and anti-Stokes regions.

Keywords: SERS, single-molecule, plasmonics, hotspots, nanophotonics

C-I.4

Title: *Spectrally Resolved Super-Resolution Surface Enhanced Raman Scattering Imaging*

Author: Zachary Schultz[1]

[1]The Ohio State University

Abstract:
The enhanced Raman signal arising from the interaction between plasmonic nanostructures and molecules, Surface enhanced Raman scattering (SERS), has demonstrated the ability to detect Raman signals from low concentrations and even single molecules. The ability to detect the Raman signal from molecules on individual nanostructures has enabled high resolution imaging experiments. It has been shown that localization algorithms, common for super-resolution fluorescence imaging, can be applied to SERS signals to enable super-resolution SERS imaging.[1,2] These initial super-resolution SERS imaging experiments we not able to record the spectral response with the same spatial resolution, either requiring a follow-up measurement or recording the spectrum from the diffraction limited area. The ability to correlate the Raman spectrum with same spatial resolution as the image opens new possibilities to understand chemical properties on the nanometer length scale in a variety of applications. We have developed technology to enable super-resolution spectral SERS imaging of plasmonic nanoparticles, where the fluctuations in the surface enhanced Raman scattering (SERS) signal can be analyzed with localization microscopy techniques to provide nanometer spatial resolution of the emitting molecules location and the spectrum associated with each signal can be recorded simultaneously (Figure).[3,4] This spectrally resolved SERS imaging provides two spatial dimensions, a frequency dimension, and a time dimension, providing increased information characterization of molecules interacting with plasmonic nanoparticles. In this presentation we will discuss the instrumentation, nanoparticles, and data illustrating the imaging of the Raman signal from nanoparticle probes to understand activity in biological and other systems.

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3. de Albuquerque, C.D.L.; Zoltowski, C.M.; Scarpitti, B.T.; Shoup, D.N.; Schultz, Z.D. Spectrally Resolved Surface-Enhanced Raman Scattering Imaging Reveals Plasmon-Mediated Chemical Transformations. ACS Nanosci Au 1 (2021) 38-46.
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Figure captions:
Illuminating an area and transmitting the image through a diffraction grating records the image and spectrum simultaneously and localization algorithms to produce super-resolved results.

Keywords: Raman, SERS, microscopy, super-resolution, plasmonics

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2. de Albuquerque, C.D.L.; Schultz, Z.D. Super-Resolution Surface-Enhanced Raman Scattering Imaging of Single Particles in Cells. *Anal. Chem. 92* (2020) 9389-9398.

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4. Shoup, D.N.; Scarpitti, B.T.; Schultz, Z.D. A Wide-Field Imaging Approach for Simultaneous Super-Resolution Surface-Enhanced Raman Scattering Bioimaging and Spectroscopy. *ACS Measurement Science Au 2* (2022) 332-341.

Title: *In-situ study of nanocatalytic reactions using surface-enhanced Raman spectroscopy*

Author: Hua Zhang[1]
[1]College of Materials Xiamen University

Abstract:
Developing in-situ characterization methods with high-sensitivity and temporal-spatial resolution resolution is of great significance for the deep understanding of catalytic reaction mechanisms and the rational design of efficient catalysts. Raman spectroscopy can provide rich structural information, especially for species located in the low-frequency region of the spectrum, thus is one of the most commonly used techniques in heterogeneous catalysis. However, its sensitivity is too low to achieve in-situ monitoring of the catalytic reaction process and trace surface intermediate species. To address this bottleneck, we have developed a highly sensitive SHINERS-satellite strategy by assembling nanocatalysts on the surface of nanoparticles with Au cores and oxide shells. In the SHINERS-satellite strategy, the Raman signals of surface species of the nanocatalyst (or even single-atom catalysts) can be greatly amplified, thus realizing in-situ Raman studies of the catalytic process and reaction intermediates. Using this strategy, we successfully achieved in-situ Raman characterization of species such as oxygen species in the catalytic oxidation process and revealed the influence of the catalyst's interfacial structure on oxygen activation. In addition, to address the low spatial resolution of conventional Raman spectroscopy, we developed a nanogap-enhanced Raman strategy, which enabled the in-situ characterization of hydrogen spillover at the nanometer scale. The results show that the spillover distance of active hydrogen species on the TiO_2 surface is about 50 nm, and hydrogen spillover is carried out by the formation and breaking of surface O-H bonds. Similar strategies have also been further extended to in-situ studies of photocatalysis and electrocatalysis.

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Keywords: Surface-enhanced Raman spectroscopy, In-situ characterization, Nanocatalysis, Intermediates

C-I.6

Title: *Precision reuseable flow SERS for Healthcare BioSensors 2.0*

Author: Jeremy Baumberg[1]

[1]University of Cambridge

We acknowledge a decade of work from researchers in the NanoPhotonics Centre, University of Cambridge, and collaborators.

Abstract:
Precision healthcare is an essential societal priority, in order to improve early-detection of disease across populations and drive down healthcare costs, but it rests on a new generation of Biosensors 2.0 that are sensitive, informative, quantitative and low-cost. Utilising surface-enhanced Raman scattering (SERS) for such practical bioanalyte detection in continual monitoring has been hampered by the inability for reuse of the metal nanostructures involved, and the challenge of quantitative measurement in complex biofluids.
Here we show how nanoscale plasmonic gaps that can detect small-molecule bioanalytes such as hormones and neurotransmitters using SERS, can be repeatedly cleaned and the 0.5-2nm gaps rescaffolded in-situ. This is a surprising result since previous methods have always destroyed nanostructures. We show two methods for completely cleaning the metal surfaces of all molecules, and that a variety of alternative molecules can then be re-inserted into the nanogaps, for subsequent sensing. This introduces wide opportunities to create non-specific binding of different classes of bioanalytes, which previously have been difficult to concentrate into optical hotspots. We show the ability for multiplexed detection of neurotransmitters and hormones at clinical concentrations in urine, opening the path to 'intelligent toilet' concepts. We also show volatile organic vapour sensing, thus underpinning 'electronic nose' capabilities.

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Plasmonic Sensing Assay for ... Neurotransmitters in Urine, DOI: 10.1021/acsnanoscienceau.2c00048
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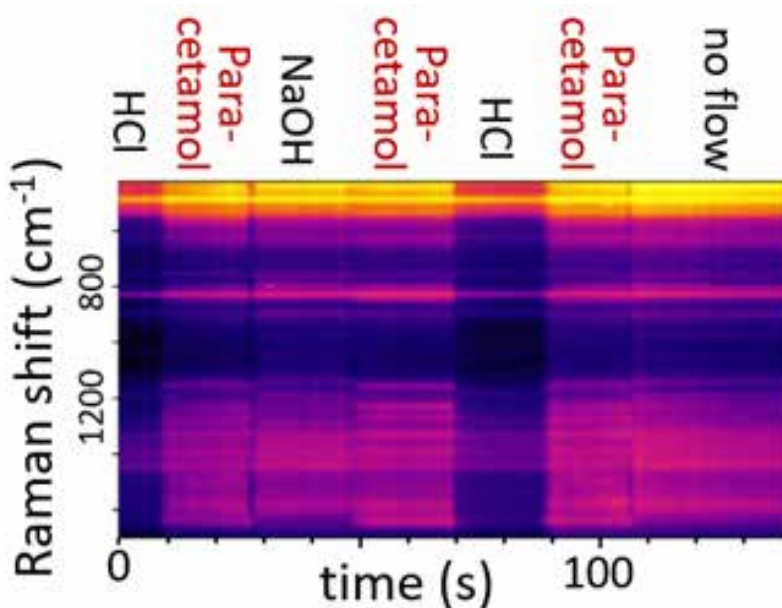
Figure captions:
Flow SERS sensing of paracetamol, comparing cleaning strategies.

Keywords: plasmonics, nanogap, SERS, toilet, nose

C-I.7

Title: *Studying Metal-Molecule Interactions to Improve SERS Sensor Performance*

Author: <u>Laura Fabris</u>[1], Chiara Deriu[1], Kaleigh Scher[2], Shaila Thakur[1]

[1]Politecnico di Torino
[2]Rutgers University

Abstract:
The integration of biosensors into various industries can transform the ability to monitor personal and public health, food safety, and the environment. Nanostructured sensors, in particular, have pushed detection limits down to femtomolar and even attomolar concentrations by utilizing diverse sensing modalities. In particular, high sensitivity and specificity have been realized using surface enhanced Raman spectroscopy (SERS), which has been proven very useful in biomarker analysis. SERS sensors can be implemented both in direct and indirect modality; in the first approach the SERS signal collected is that of the analyte under investigation, while in the second the SERS signal of a Raman reporter is leveraged as a proxy to the analyte recognition event. Regardless of the chosen approach, metal-molecule interactions are key to achieving an optimal sensor performance. Yet, these interactions are very complex to monitor, especially when the SERS substrate used is in form of a colloidal plasmonic nanoparticle. In my talk, I will report on our work aiming at understanding, through SERS and other analytical techniques, the interplay between the molecular structure of the analyte or the Raman reporter molecule and the properties of the nanoparticles, including the surface chemistry, the crystal structure, and the shape. These results can inform us on the parameters we need to take into consideration when implementing a SERS sensor, in particular if it is aimed at answering medical or biological questions.

Keywords: SERS, nanoparticles, surface interactions

Studying Metal-Molecule Interactions to Improve SERS Sensor Performance

C-I.8

Title: *Comparative study of p-Aminothiophenol adsorption by Surface-Enhanced Raman Spectroscopy*

Author: María Rosa López-Ramírez[1], María De la Cabeza Fernández[2], Alexis Alvear-Fernández[2], Rafael Contreras-Cáceres[3]

[1]Department of Physical Chemistry, Faculty of Science, University of Málaga
[2]Department of Chemistry in Pharmaceutical Sciences, Faculty of Pharmacy, Universidad Complutense de Madrid
[3]Department of Chemistry and Physics, University of Almería

Abstract:
The organic compound p-aminothiophenol (PATP, HS-Ph-NH$_2$) has become very popular for checking the enhancement capability of novel substrates due to its very intense SERS spectra. SERS of PATP on metal nanoparticles is significantly different from its ordinary Raman spectra and it is very dependent on the particular experimental conditions. It has been demonstrated that PATP molecule can chemically transform to 4,4′-dimercaptoazobenzene (DMAB) upon adsorption, being this new compound the responsible of giving rise to new SERS bands [1]. In this work, we have studied the adsorption behavior of PATP on different metal substrates: silver electrode [2], silver colloids and bimetallic nanoparticles made of gold and silver. Additionally, theoretical DFT calculations have been performed for supporting the experimental data.
The analysis of the SERS results of the PAPT adsorbed on this type of nanoparticles leads us to deduce a very different catalytic capability in the dimerization of this adsorbate that depends directly on the morphology of the nanoparticle. These preliminary but fascinating results on these bimetallic systems are going to be the focus of further experiment in order to quantify the catalytic capabilities of these interesting nanoparticles.

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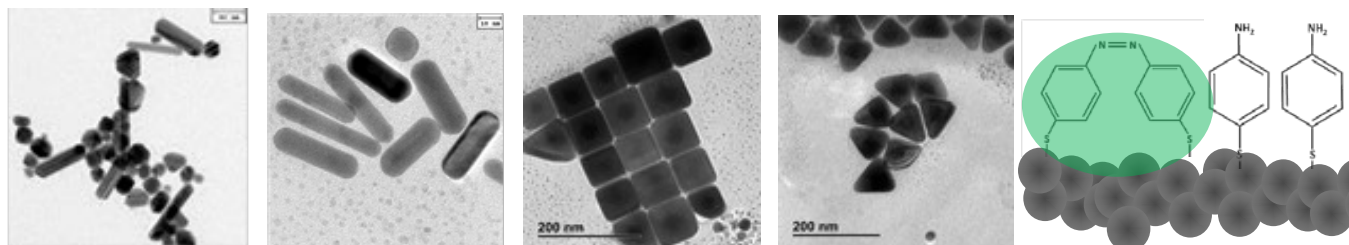
Figure 1. Different types of metallic and bimetallic nanoparticles and molecular adsorption configurations of PATP on the surface.

Figure captions:
Figure 1. Different types of metallic and bimetallic nanoparticles and molecular adsorption configurations of PATP on the surface.

Keywords: metal electrodes, metal nanoparticles, bimetallic

C-O.1

Title: *Viewing interfacial chemistry through a graphene window with broadband infrared nanospectroscopy*

Author: Hans Bechtel[1], Jonathan Larson[2], Xiao Zhao[3], Xin He[2], Dong Li[4], Behzad Rad[4], Chunsheng Yan[4], Paul Ashby[4], Stephanie Gilbert Corder[1], Robert Kostecki[2], Miquel Salmeron[4]

[1]Advanced Light Source, Lawrence Berkeley National Laboratory
[2]Energy Storage & Distributed Resources Division, Lawrence Berkeley National Laboratory
[3]Materials Sciences Division, Lawrence Berkeley National Laboratory
[4]Molecular Foundry, Lawrence Berkeley National Laboratory

Abstract:
The chemical interactions that occur at an interface of two or more materials often determine the how natural and engineered systems function. Characterizing these interfaces in their native environment, however, can be extremely challenging because the interfacial region is typically buried by the bulk. Here, we exploit the nanoscale spatial resolution, chemical selectivity, and surface sensitivity of near-field infrared nanospectroscopy (nano-FTIR) at the Advanced Light Source (ALS) to characterize chemical processes at interfaces [1]. Here, we use graphene as an ultra-thin IR transparent "coverslip" that serves as an impermeable barrier to protect the interfacial region from contamination or oxidation and allow ambient conditions on the probe side while enabling direct access to the interfacial region between liquid or solid-state solutions [2]. *In-vitro* measurements using the liquid cell show the formation of S-layer protein lattices by monitoring the response of Amide-I and Amide-II absorption bands to environment variables, including ionic strength and solvent [3]. The non-linear growth of these bands mirrors the increase in the percentage of the ordered protein domains obtained from AFM images but with inhomogeneous spatial distributions not previously seen. In-situ measurements of the electrochemical cell reveal that intrinsic molecular, structural, and chemical heterogeneities in the solid polymer electrolyte lead to nonuniform Li plating and formation of a mosaic-like solid electrolyte interphase of similar length scales [4]. These studies provide a unique insight into the mechanisms of interfacial chemistry and an experimental diagnostic means to aid in the development of methods to control local nanoscale variations in biological and electrochemical systems.

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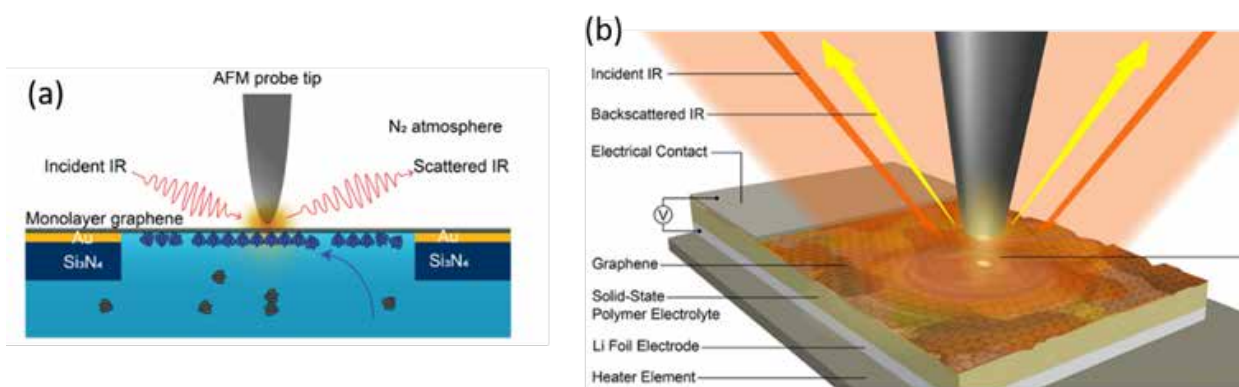
Figure captions:
Schematics of (a) the aqueous cell for in vitro protein self-assembly measurements and (b) the solid-state battery cell for in situ electrochemical measurements of the graphene/ electrolyte interface

Keywords: Nano-FTIR, synchrotron, near-field, infrared, interface

C-O.2

Title: *Comparison of resonant and non-resonant reporter for the selection of brightest gold nanoparticles for Surface-enhanced Raman spectroscopy.*

Author: Megha Mehta[1], William Skinner[1], Sara Mosca[2], Benjamin Gardner[1], Francesca Palombo[1], Pavel Matousek[2], Nicholas Stone[1]
[1]University of Exeter
[2]STFC Rutherford Appleton Laboratory

Abstract:
The choice of Raman reporter is a significant aspect for improving the imaging sensitivity and multiplexing capabilities of SERS nanoparticles, particularly when attempting to read out Raman signals from NPs deeply buried in tissues[1-2]. In this study, we have investigated the combination of three AuNPs with a range of different Raman reporter molecules. Three resonant reporters, IR-125, IR-820, IR-797 and three non-resonant reporters (2-bi-(4-pyridyl) ethylene (BPE), biphenyl-4-thiol (BPT) and 4-mercaptobenzoic acid (MBA) bound to gold nanoparticles of different morphologies – nanospheres and nano-raspberries. We used commercially available AuNPs and in-house synthesised gold nano-raspberries (AuNRBs) using the green chemistry method[3] of reduction of gold ion by 2-[4-(2-hydroxyethyl)-1-piperazyl] ethane sulfonic acid (HEPES). The method carried out limits the need for extensive post-synthesis routines of biofunctionalization to improve sensitivity. The appropriate reporter concentration, and volume ratio of reporter to nanoparticle concentration parameters were analysed to provide a valuable assessment of the reporter molecule that gives maximum SERS enhancement for these AuNPs. We have used 785 nm laser excitation to find the brightest 'Raman reporter – gold nanoparticle' combination for further use in deep Raman multiplexed imaging. We have demonstrated that AuNRBs provide significant SERS enhancement with better sensitivity for Raman resonant reporters due to strong label binding affinity of dye to gold surface as compared to non-resonant dyes. It also explains inherently stronger signals generated by surface-enhanced resonance Raman scattering (SERRS), as opposed to surface-enhanced Raman scattering (SERS). These simple, scalable and tunable size AuNRBs are excellent candidates for predicting which Raman reporters could improve sensitivity and be used for deep Raman multiplexed imaging.

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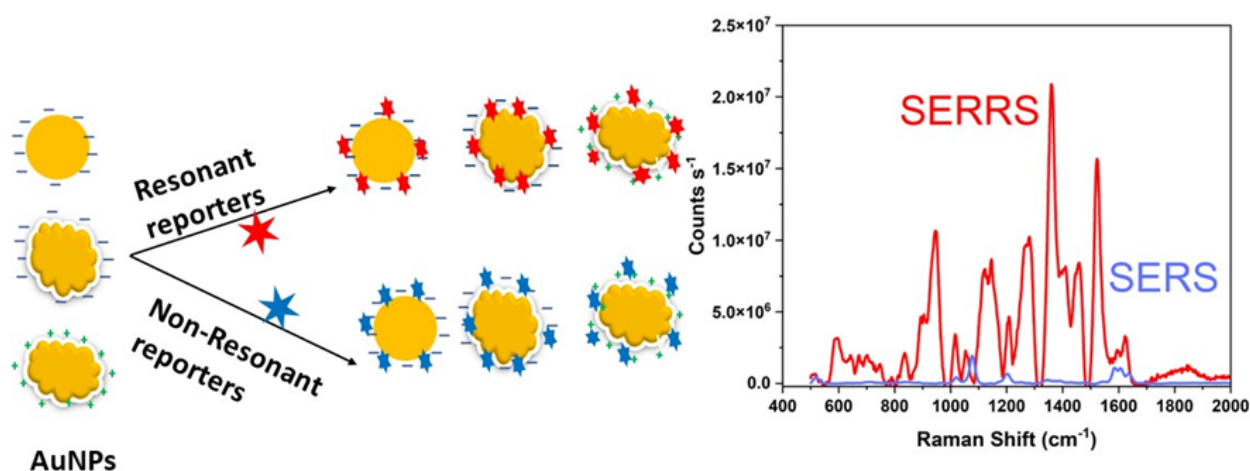
Figure captions:
Schematic representation of different morphology gold nanoparticles after tagging with resonant and non-resonant reporter showing stronger SERRS enhancement than SERS

Keywords: surface-enhanced Raman spectroscopy, surface-enhanced resonance

C-O.3

Title: *Beyond the metal core: leveraging stabilizer-metal interactions for direct SERS detection*

Author: Chiara Deriu[1], Laura Fabris[1]

[1]Politecnico di Torino

Abstract:

In a SERS measurement, nanostructured substrates amplify the inherently weak Raman scattering of an adsorbed analyte, enabling its trace detection. Because the observed signal enhancement is the result of a plasmonic, near-field effect, conditions for signal observation, and consequent low limits of detection, are achieved when analyte adsorption on the substrate is thermodynamically favored.

When implementing SERS analytical protocols that are based on the use of colloidal nanoparticles as the enhancing substrate, one must reflect on the fact that these are never "chemically clean"; rather, they always bare a population of adsorbed species on their surface. As a result, the association constants that must be considered when performing SERS measurements in colloidal sols are not limited to those between the analyte and the plasmonic surface but should also include those between the surface and pre-adsorbed species, and those between the analyte and pre-adsorbed species. The latter can be synthesis by-products, such as oxidized or unreacted reducing agent molecules, or intentionally added colloidal formulation improvers, such as stabilizers. Because stabilizers typically represent most of the surface adsorbed species in a colloidally stable sol, failure to consider their energetics when implementing direct SERS protocols might result in failure to observe the desired SERS signal.

Despite its importance, the study of the interaction between colloidal sol stabilizers and plasmonic surfaces is an under-explored area of SERS research. In this presentation, different examples of such studies will be discussed, with a focus on how they can be leveraged for *ad hoc* SERS substrate and analytical protocol design. These include, but are not limited to, stabilization of AuAg nanostars for tailored, class-specific analyte detection, determination of experimental and theoretical thermodynamic parameters of stabilizer-metal systems, and their computational modeling.

Keywords: SERS, colloidal nanoparticles, stabilizers

C-O.4

Title: *Exploring and Optimizing Factors Influencing Surface-Enhanced Raman Scattering (SERS) Performance*

Author: Sylwester Gawinkowski[1]

[1]Institute of Physical Chemistry, Polish Academy of Sciences

Abstract:
Surface-enhanced Raman scattering (SERS) spectroscopy has risen to prominence as a powerful analytical instrument with extensive applications in a variety of scientific and industrial domains, such as chemistry, biology, medicine, and materials engineering. Due to its remarkable sensitivity, SERS allows for the detection and analysis of individual molecules [1,2], making it essential for scientific investigations and practical applications alike. Nonetheless, despite the vast potential of SERS, a comprehensive understanding of the fundamental mechanisms is still lacking, and this knowledge gap could potentially impact the effectiveness and precision of results derived from SERS measurements [2,3].
Our research delves into the factors impacting signal strength and stability in single-molecule SERS spectra measurements, emphasizing the significance of precisely calibrating the laser wavelength to the electronic resonance of the analyte to enhance SERS signal strength (Fig. 1). Through the investigation of porphyrin derivatives, we uncover a robust correlation between SERS excitation spectra and absorption spectra in solution [4]. We demonstrate that even minor deviations in laser excitation from the electronic transition energy can substantially reduce SERS signal intensity, thereby affecting the detection of single molecules. Furthermore, we assess the influence of factors such as sample temperature, excitation power, and molecular substituents on the temporal dynamics of single-molecule SERS spectra.

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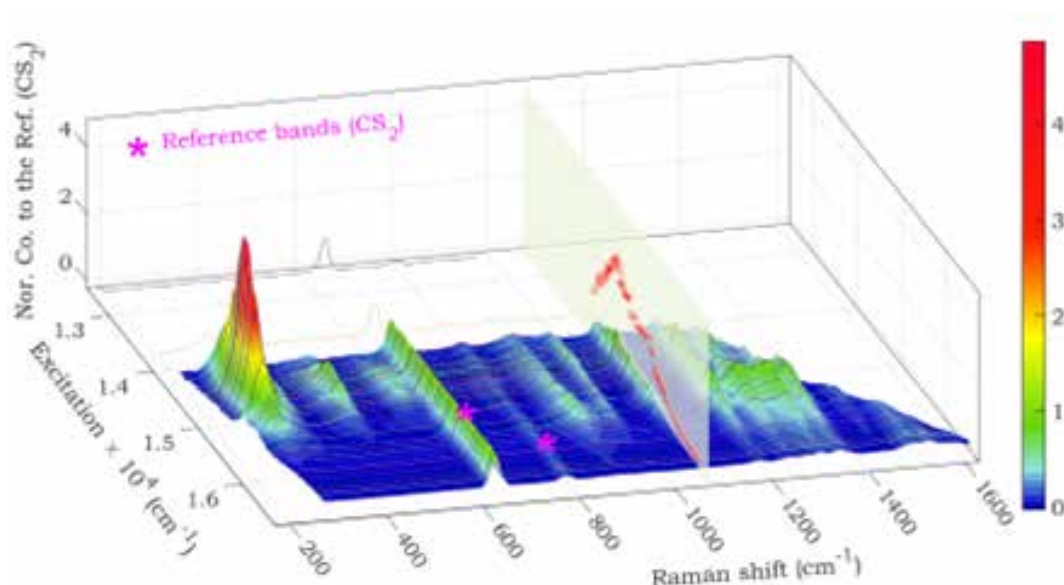
Figure captions:
The 3D SERS excitation map of porphycene derivative. The asterisk shows the location of reference band (CS2). The light green plane shows a profile of the band 1194 cm-1.

Keywords: SERS, SM-SERS, single-molecule, porphyrin

Title: *In vivo Real-time Multiplex Detection of Plant Signalling Molecules Using Surface-Enhanced Raman Scattering Nanosensor*

Author: Won Ki Son[1]

[1]Seoul National University

Abstract:
Environment pollution and climate changes makes plant management one of the most immediate task in global world. And these current trends consequently demand new innovate monitoring technology and many reserachers have studied nanobiosensors such as carbon nanotubes and metal organic framework. However, they have a crucial drawback in that they usually rely on phenotypic information or indirect information of in vivo dynamics. 'Signalling molecules' released against adverse stimuli on plants are emerging as key biomarkers to understand plant's biological status. As one of the analytical approaches to detect the signalling molecules, surface-enhanced Raman scattering (SERS)-based optical nanosensor has shown strong potential for its non-invasiveness, water-transparency and capability of real-time detection of chemical dynamics from fingerprint spectra.
In our research, PDDA-capped Ag nanoshell (AgNS@PDDA) was fabricated and had high enhancement factor of ca.10^7, infiltrated into plants through stoma and localized in intercelluar space. Thanks to the plasmonic properties of AgNS@PDDA in NIR window, it was possible to evade chlorophyll's autofluorescence and obtain strong SERS signals. PDDA polymer attracted signalling molecules most of which are negative conjugate base form by coulombic interaction. Moreover, since the interaction between the polymer chain and analytes was reversible, AgNS@PDDA was found to be effective to monitor the change of the concentration of signalling molecules in real time. Then, we studied the plant's reaction against biotic and abiotic stress with SERS signal monitoring. Nasturlexin B, glutathione, salicylic acid and eATP were detected and the change of chemcal dynamics were explored during under stress situations such as wounding, cold damage and fungal disease. Based on our research, we expect that the SERS nanosensors provide novel approach for plant stress diagnosis and early diagnosis of diseases.

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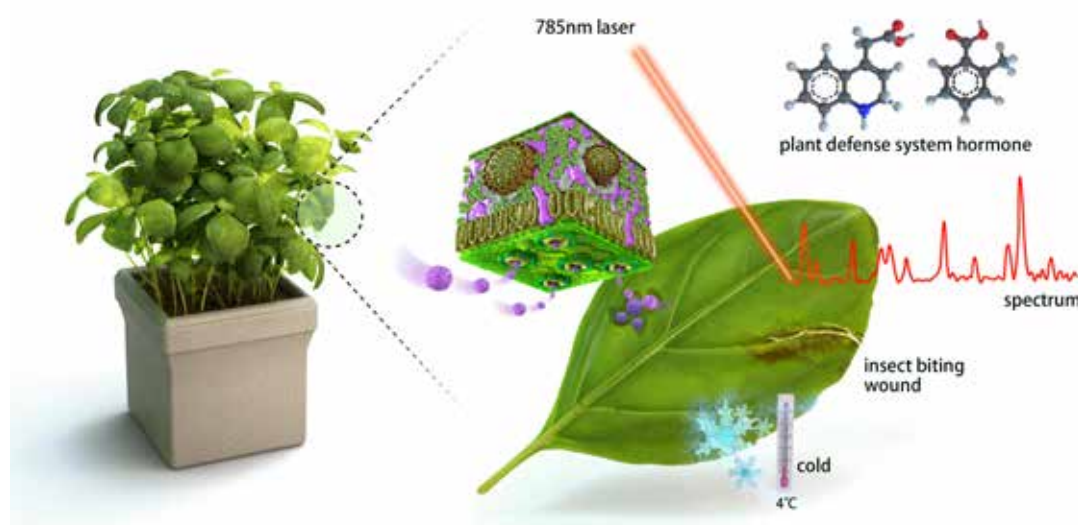
Figure captions:
Illustration describing SERS detection from living plants under adverse stresses.

Keywords: SERS, Plant, in vivo monitoring

Title: *Exciton-Phonon Coupling in MoSe2/WSe2 Heterobilayers Probed Using Resonant Raman Spectroscopy*

Author: Oisín Garrity[1], Thomas Brumme[2], Annika Bergmann[3], Tobias Korn[3], Patryk Kusch[1], Stephanie Reich[1]

[1]Freie Universität Berlin
[2]Technische Universität Dresden
[3]Universität Rostock

Abstract:
Transition metal dichalcogenides (TMDCs) are a class of materials that host an array of frontier physical phenomena such as room temperature excitonic states with binding energies of 40 meV to 100 meV [1], valley pseudospin effects, and moiré physics to name a few. An elegant way to characterise these materials and explore their unique physical properties is through spectroscopic and microscopic methods. They reveal their optoelectronic and vibrational properties while also revealing information on the TMDC crystal quality. In previous work we showed how the dual scattering type near field optical microscope (dual s-SNOM) can be used to study dielectric disorder in TMDCs and that the obtained near-field images are correlated with tip enhanced photoluminescence (TEPL) peak position [2][3][4].
In this work we present a resonant Raman study of exciton-phonon coupling in $MoSe_2$/WSe_2 heterobilayers. We show that the A_{1g} mode of both layers couple to their own intralayer excitons as well as the intralayer excitons in the adjacent layer, effectively exhibiting an interlayer phonon-exciton interaction. This can be seen in an additional $MoSe_2$ A1g resonance appearing at the A excitonic energy of WSe_2 with a resonance profile two times the size of the resonance with the A exciton of $MoSe_2$. In contrast the E_{2g} mode in both layers don't show any effective exciton-phonon coupling. This can be understood by considering that the A_{1g} mode generates a deformation potential out-of-plane which can then couple to the intralayer excitonic wavefunction extending out from the other layer. With the E_{2g} mode being an in-plane motion of atoms prevents coupling to adjacent layers This coupling effect is present in both the hybridised and non-hybridised samples making it clear that for these materials to be used in novel optoelectronic applications, exciton-phonon interactions need to considered not only in their respective layers, but also with respect to the layers surrounding them.

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Figure captions:
Inelastic light scattering on TMDCs: (a) Photoluminescence intensity map (b) electronic band alignment showing staggered band gap configuration (c) Exemplary Raman spectra of MoSe2/WSe2 heterobilayer

Keywords: Resonant Raman scattering, exciton-phonon coupling

C-O.7
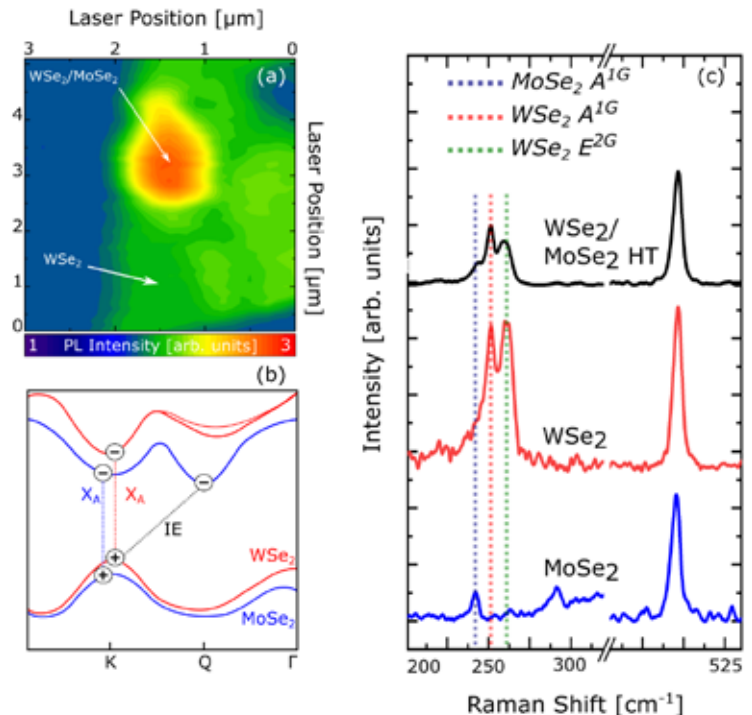
Title: *In-Situ Cost-effective Methods for Fabricating SERS Substrates using Polydopamine*

Author: <u>Ahmed Mahmoud</u>[1], Alexandra Teixeira[1], Maria Sousa-Silva[1], Sara Abalde-Cela[1], Lorena Diéguez[1]
[1]The International Iberian Nanotechnology Laboratory (INL)

Abstract:
Surface-enhanced Raman scattering (SERS) technique has emerged as a powerful chemical and biological analytical tool in many fields, such as environmental monitoring, disease diagnosis, and food safety.[1,2] A key component in any SERS platform is the SERS substrate, where the intrinsic Raman scattering signals get amplified by various orders of magnitude mainly due to electromagnetic and chemical enhancement mechanisms. Most of SERS substrates are fabricated by micro/nanofabrication techniques, which can provide SERS substrates with high enhancement and reproducible nanostructures.[2] However, these techniques require well-equipped laboratories, highly trained technicians, and hence a high cost to fabricate and use, which can be a burden in resource-limited areas.[2] Therefore, there is a crucial need to develop facile and cost-effective alternative methods to fabricate SERS substrates. Here, we present in-situ methods for fabricating SERS substrates on different materials based on utilizing the adhesive and reducing powers of polydopamine. Polydopamine can offer a sustainable and cost-effective approach to synthesizing plasmonic nanostructures, as it is biodegradable and biocompatible.[3] We systematically investigated how we can optimize and tune the SERS performance of these substrates by varying experimental parameters and, consequently, their plasmonic behavior. We employed this method to fabricate different SERS substrates on porous membranes, glass vials, microfluidic devices, etc. We will show and discuss some environmental and biomedical applications of these substrates. Developing inexpensive, sustainable, sensitive, and user-friendly SERS substrates can open new avenues for SERS applications at points of need, using portable and handheld Raman devices.

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3. H. Lee, S. M. Dellatore, W. M. Miller, P.B. Messersmith, Mussel-Inspired Surface Chemistry for Multifunctional Coatings, Science. 318 (2007) 426-430.

Keywords: SERS, Plasmonics, Polydopamine, Microfluidics, Biomedical

C-O.8

Title: *Vanadium oxide nanoparticles as non-plasmonic platforms for surface-enhanced Raman spectroscopy*

Author: Eva Kočišová[1], Anna Kuzminova[2], Marek Procházka[1], Ondřej Kylián[2]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University
[2]Department of Macromolecular Physics, Faculty of Mathematics and Physics, Charles University
Support by grant 22-16667S from the Czech Science Foundation.

Abstract:
The metal-oxides (MeO) are promising non-plasmonic platforms for surface-enhanced Raman spectroscopy (SERS). In contrast to conventionally used plasmon metallic SERS-active platforms, MeO were reported to offer good signal uniformity, spectral reproducibility, and reduced local heating upon laser irradiation [1]. However, the main limiting factor of MeO-based SERS is its lower signal enhancement as compared to plasmonic-based metallic SERS. Because of this, new concepts are still to be developed to improve the performance of MeO-based SERS-active materials, such as vanadium oxide nanoparticles (NPs) [2,3].
We developed a new approach for the synthesis of vanadium oxide (V2O5) NPs that employs magnetron-based gas aggregation sources of vanadium NPs (Fig. 1a) and thermally induced transformation of deposited vanadium nanoparticle films into vanadium pentoxide ones (Fig.1b) [4]. The solution of the analyte is then dropped to the surface (Fig. 1c) and dried in the air. Finally, SERS spectra are measured using a confocal Raman system and 623.8 nm excitation (Fig. 1d).
We studied the impact of process parameters on the properties of produced nanomaterials and their applicability to SERS spectroscopy. SERS spectra of various model molecules have been obtained. Spectral detection limits were determined as 5×10^{-8} M, 1×10^{-6} M, and 4×10^{-6} M for methylene blue, crystal violet, and triphenylphosphine oxide, respectively. Spectral mapping over the surface proved excellent spectral reproducibility (RSD less than 10%). The enhancement factor was determined as 10^{5}-10^{6} primarily due to the charge transfer mechanism.

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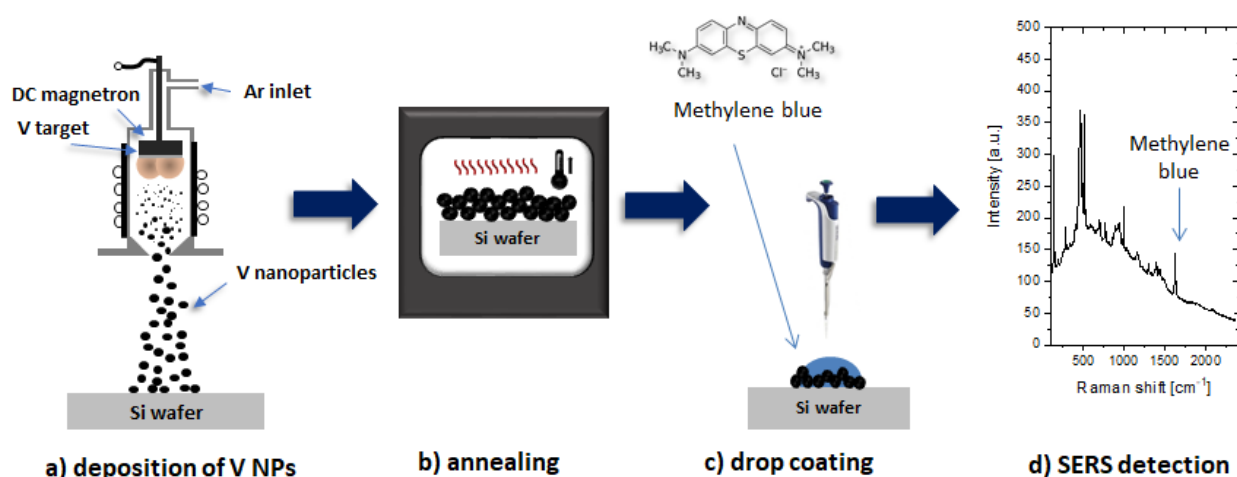
Figure captions:
Figure 1. Investigated strategy: a) Deposition of vanadium NPs films, b) heat-induced formation of vanadium oxide NPs, c) drop coating of the analyte, and d) SERS measurements.

Keywords: vanadium oxide, nanoparticles, SERS

C-O.9

Title: *Searching for one-armed thiol bandit – SERS and DFT studies on adsorption modes of cyclo(L-Cys-D-Cys) on silver*

Author: Agata Królikowska[1], Marcin Witkowski[1], Lasse Jensen[2], Wojciech Dzwolak[1]

[1]Faculty of Chemistry, University of Warsaw, Pasteura 1
[2]Department of Chemistry, Penn State University, 101 Chemistry Building, University Park, 16802, PA

Abstract:
Peptides are promising biorecognition elements selectively interacting with variety of chemical targets. This makes them excellent candidates for the development of novel stimuli-responsive materials and design of new highly accurate and reliable biosensors [1]. Coupling peptide receptor to plasmonic nanostructures paves the way for the use of surface-enhanced Raman scattering (SERS) spectroscopy, a technique well-known for its exceptional sensitivity and flexibility of design [2], as the means to transduce the target-peptide interactions into a measurable signal.
Cyclic dipeptides containing a 2,5-diketopiperazine ring are appealing in this field, due to their structural rigidity, outstanding hydrogen bonding affinity and often improved stability compared to their linear counterparts, resulting in superior self-assembly properties and ability to bind a variety of targets [3].
In this work, we performed SERS analysis on cyclo(L-Cys-D-Cys); (diCys), with the thiol functional groups located at opposite sides of the diketopiperazine ring. Cysteine was chosen to provide a thiolate unit, chemically anchoring the bioreceptor to Ag nanoparticles (AgNPs). In contrast to other dithiol peptides, diCys cannot form a disulfide bridge between the two thiol groups within one molecule. Thus, when adsorbed on metal surface, one of the -SH groups is expected to remain solvent-accessible and therefore may be utilized for ion coordination or chemical reactions. Our SERS results suggest that we can guide the availability of the free thiol groups by controlling the relative amount of diCys and AgNPs. Density functional theory(DFT) calculations were performed to get deeper insight into the adsorption modes of diCys on Ag. Our findings show the potential of tailoring diCys adsorption properties with an experimental protocol.

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Keywords: SERS, biosensors, dipeptides, adsorption, DFT

C-O.10

Title: *A newly recognized chemically stable surface bound thiolate intermediate in plasmon-induced catalysis*

Author: Xiaobin Yao[1], Sadaf Ehtesabi[2], Christiane Höppener[1], Tanja Deckert-Gaudig[1], Henrik Schneidewind[3], Stephan Kupfer[2], Stefanie Gräfe[2], Volker Deckert[1]

[1]1. Friedrich Schiller University Jena, Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, Jena 07743, Germany; 2. Leibniz Institute of Photonic Technology, Albert-Einstein-Str.9, Jena 07745, Germany
[2]1. Friedrich Schiller University Jena, Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, Jena 07743, Germany
[3]2. Leibniz Institute of Photonic Technology, Albert-Einstein-Str.9, Jena 07745, Germany

Abstract:
Specific redox reactions involved in the conversion of p-aminothiophenol (ATP) and p-nitrothiophenol (NTP) resulting from plasmon-induced catalysis have been intensively investigated in multiple studies leading to the assignment of the dimer product to dimercaptoazobenzene (DMAB) (dimer pathway)[1] and also to the assignment of the reduction of NTP to ATP in low pH HCl solutions (monomer pathway)[2]. However, the mechanism of the monomer pathway and the connection between the two pathways are still unclear. In our recent joint spectroscopic-theoretical study of NTP and ATP in surface enhanced Raman spectroscopy (SERS), a new chemically stable intermediate p-nitrosothiophenol (TP*) has been found experimentally in the monomer pathway (Scheme 1) and the connection between the two pathways was explored.[3]
Motivated by the influence of water, HCl vapor instead of aqueous HCl solution was used, and a new band at ~1355 cm^{-1} clearly distinct from the nitro group in NTP (~1330 cm^{-1}) was found. We assume this signal to a N=O vibration of a so-far undetected intermediate TP*. Quantum chemical simulations to evaluate the reaction mechanism and thermodynamics support the existence of TP*. Time-dependent experiments further clarified the connection between TP*, ATP, NTP and DMAB, and allowed to propose different reaction routes (Scheme 1), in which TP* plays an important role as a stable intermediate in the overall reaction scheme.

References:
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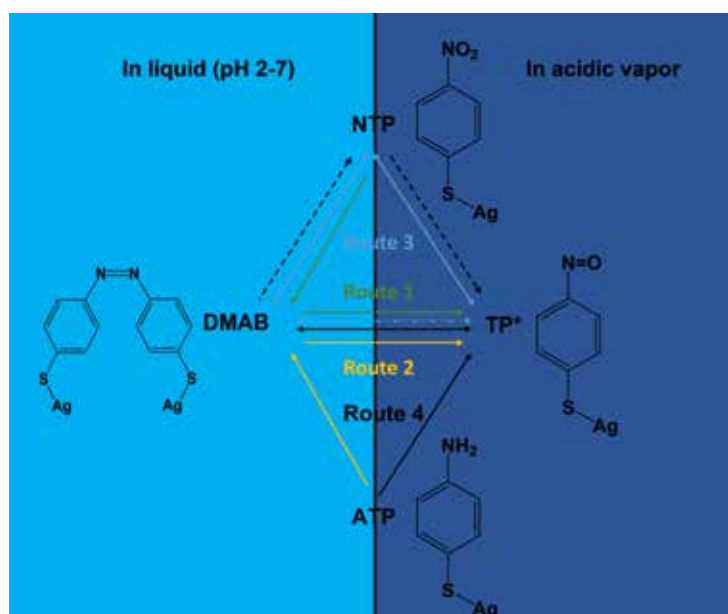
Figure captions:
Scheme 1. Sketch of the plasmon-induced reactions of ATP and NTP via DMAB and TP* on Ag island SERS substrates depending on the experimental conditions. Routes are labelled by corresponding colors.

Keywords: p-aminothiophenol, p-nitrothiophenol, p-nitrosothiophenol, density-functional theory

C-O.11

Title: *Pushing the limits of Raman Spectroscopy: Photo-induced enhanced Raman Spectroscopy on Ag-TiO2 hybrid nanoplatforms.*

Author: <u>Łukasz Pięta</u>[1], Aneta Kisielewska[2], Ireneusz Piwoński[2], Kamilla Małek[1]

[1]Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
[2]Department of Materials Technology and Chemistry, Faculty of Chemistry, University of Lodz, Pomorska 163, 90-236 Lodz, Poland

Abstract:
Photo-Induced Enhanced Raman Spectroscopy (PIERS) arrived as a successor of surface-enhanced Raman spectroscopy (SERS), pushing further the threshold of the detection limit compared to SERS. PIERS enhancement in noble metal-semiconductor hybrids arises from electromagnetic (EM) and chemical enhancement (CE), caused by localized surface plasmon resonance (LSPR) of metal nanoparticles (NPs) and charge transfer (CT) between the analyte molecules and the substrate after its photoactivation with UV light, respectively. The magnitude of PIERS enhancements for analyte molecules under non-resonant conditions typically ranges between 2 to 10 times greater than SERS. However, for certain analyte molecules such as organic dyes, the PIERS phenomenon can lead to significant signal amplification, up to 50 times. Despite numerous scientific reports, much remains to be explained and discovered about the PIERS effect, as its origin is still not well understood.
In our experiments, we utilized sol-gel and photoreduction methods to create stable and reproducible Ag-TiO^2 nanoplatforms for PIERS measurements. Various Ag-TiO^2 nanoplatforms were synthesized under controlled conditions with localized surface plasmon resonance in the UV (325 nm, 370 nm) and Vis (415 nm) regions and different surface coverage. Two UV lamps (254 nm and 365 nm) were used to investigate whether photon energy played a role in the enhancement mechanism. Our results showed that the PIERS enhancement depends strongly on the structure and plasmonic features of the Ag – TiO^2 interface and photo-irradiation conditions. All prepared nanoplatforms are characterized by a high PIERS gain factor, up to 20 times higher than SERS. Additionally, in each case, the PIERS enhancement was maintained for at least 4 hours after the photoactivation process, which is one of the highest results obtained so far in the literature.

References:
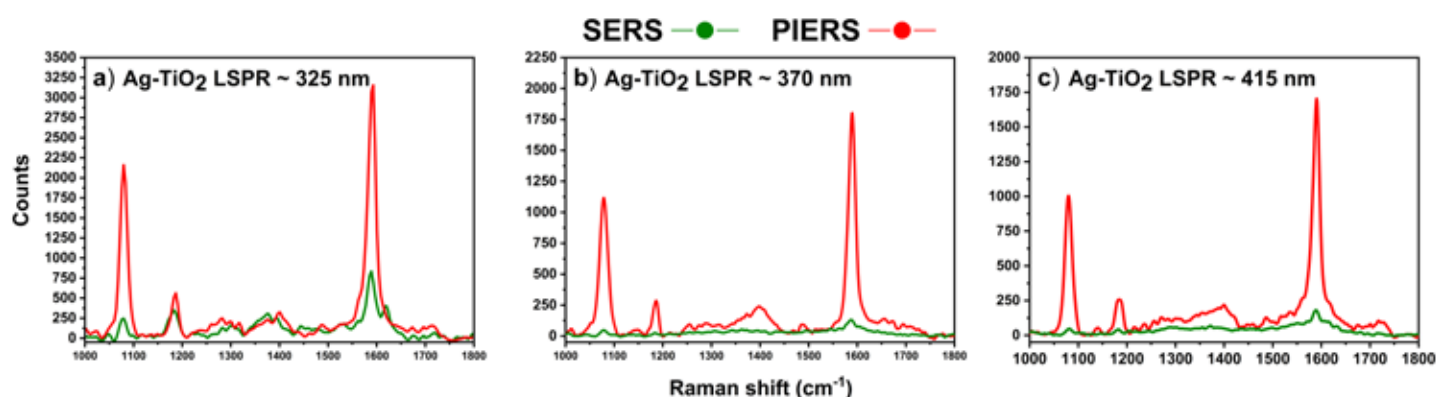
Figure captions:
Averaged PIERS and SERS spectra of 1.0 M 4-MBA for Ag-TiO2 nanoplatforms with LSPR maximum at a) 325 nm, b) 370 nm, and c) 415 nm.

Keywords: SERS, PIERS, TiO2, AgNPs, Plasmonics

D-I.1

Title: *Surface-enhanced resonance Raman spectro-electrochemistry as a tool to study redox-related structural changes in (bio)chemistry in-situ*

Author: Michelle Mahler[1], Patrycja Kielb[1]

[1]University of Bonn

Abstract:
Protein engineering and the development of artificial metalloproteins that could catalyze new types of transformations emerge as promising fields in biocatalysis for technological or medical applications. To accomplish their timely progress, it is indispensable to use experimental tools that can correlate the structural or molecular information of the system of interest with its function or performance. Combination of electrochemistry with surface-enhanced resonance Raman spectroscopy stands out as a method detecting chromophore-selective vibrational spectrum under the control of applied potential. Particularly, resonance Raman provides an elegant way of decongesting Raman spectra through selective and amplified detection of the vibrational fingerprint of the chromophore. Nanostructured metal surface serves as surface-enhanced Raman active support on which the biomolecule of interest is interfaced, and electrochemistry provides means to inject or remove an electron from a molecule to drive a redox reaction. In this talk, the application of SERR spectro-electrochemistry and its benefits are presented on the example of a rationally-engineered, redox-active multimeric heme-containing protein (HTHP). HTHP variants have been modified to harbor a variety of coordination patterns of hemes which influence its oxidation, spin state, and redox properties laying the ground for supporting new catalytic reactions.
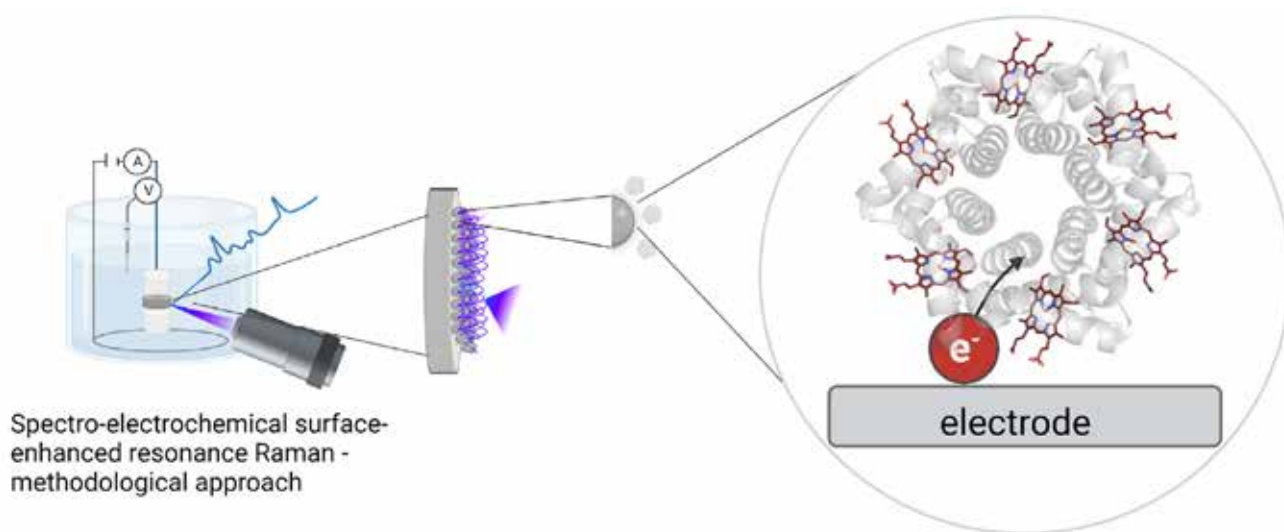
Figure captions:
Surface-enhanced resonance Raman spectro-electrochemical approach to study interfaced proteins on nanostructured electrodes.

Keywords: Spectro-electrochemistry, surface-enhanced resonance Raman, heme-protein

D-I.2

Title: *Tip-enhanced Raman spectroscopy for nanoscale studying of catalytic. systems*

Author: <u>Bin Ren</u>[1], Xiang Wang[1], Tengxiang Huang[1], Huishu Feng[1]

[1]Xiamen University

Abstract:
Tip-enhanced Raman spectroscopy (TERS), which combines scanning probe microscopy and plasmon-enhanced Raman spectroscopy, is capable of simultaneously obtaining the topographical and Raman fingerprint information at nanometer spatial resolution. Such a spatial resolution allows the identification of the local physicochemical properties of different sites on the nanocatalysts without bothering the averaged effect in ensemble measurements. This advantage makes it possible to disentangle the electronic effect and geometric effect with the change of the size. We visualized the size-specific electronic, geometric and catalytic properties of the different sites located on the individual Pd nanocatalyst by real-space TERS imaging with 3 nm spatial resolution. We further developed electrochemical tip-enhanced Raman spectroscopy (EC-TERS) for in situ monitoring the geometric and electronic evolution of individual active sites of MoS_2 during hydrogen evolution reaction (HER) and revealed the progressive generation of active sites during the electrochemical activation and reaction processes. These discoveries offer new insights into our understanding of the active site and its dynamics during electrocatalytic processes.
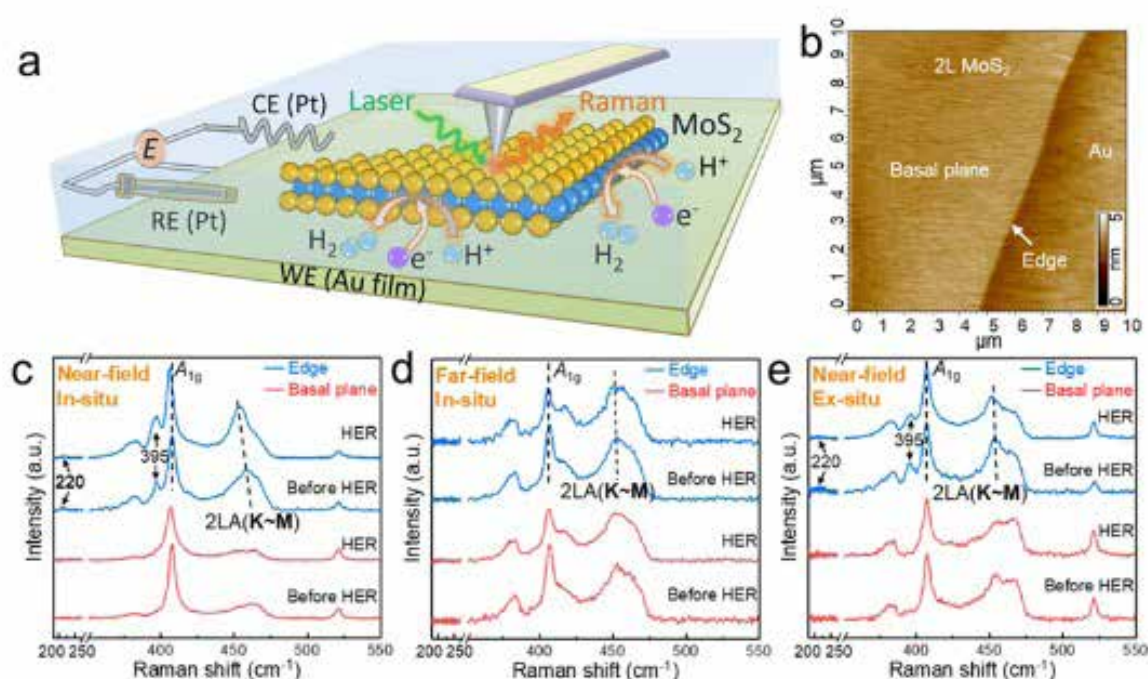
Figure captions:
Electrochemical TERS for in situ studying of MoS2 during hydrogen evolution and a comparison of in situ TERS, ex situ TERS and in situ confocal Raman.

Keywords: Tip-enhanced Raman, nanoscale, catalyst

D-I.3

Title: *Quantifying Large-Scale Structural Changes During pH-Induced Channel Opening of Influenza A M2 using Surface-enhanced Infrared Absorption Spectroscopy*

Author: Ronja Paschke[1], Swantje Mohr[2], Sascha Lange[2], Adam Lange[2], Jacek Kozuch[1]

[1]Freie Universität Berlin
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie Berlin

Abstract:
Viroporins are small ion/proton channel-forming proteins in membranes of enveloped viruses that play important roles at multiple stages of the viral life cycle. In particular, the M2 proton channel from Influenza A virus (IAV) has garnered much interest for this family of proteins, when it was shown that its pH-activated proton conductance can be inhibited, thereby, stopping infection with the flu. With the scarcity of potent treatments against viral infections, this demonstrated that viroporins present a promising target for antiviral therapy. As such, a detailed understanding of their mechanisms of action and inhibition is required and, consequently, methods that enable their investigation under in-situ conditions. Towards this goal, we utilize surface-enhanced infrared absorption (SEIRA) spectroscopy, which enables us to monitor the function of the membrane proteins and membrane-active peptides within a single, planar solid-supported bilayer lipid membrane (ssBLM) via its specific vibrational fingerprint.[1,2] After reconstituting the IAV M2 proton channel into the ssBLM, we monitored the structural changes upon pH-activation and noticed signals characteristic of a reorientation of the transmembrane α-helices associated with channel opening, which vanished after exposure to the inhibitor rimantadine. To test this interpretation, we calculated computational IR spectra based on a structural model from nuclear magnetic resonance and obtained an excellent match with the experimental picture. Based on the combination of experimental and computational spectra, we quantifed the mechanical opening of the transmembrane helices to refine the picture of how IAV M2 transports protons into the virus during infection. In the future, we aim to utilize this approach to enable a combined structural and functional analysis of viroporins of current relevance and contribute to the discovery of new antiviral drugging strategies.

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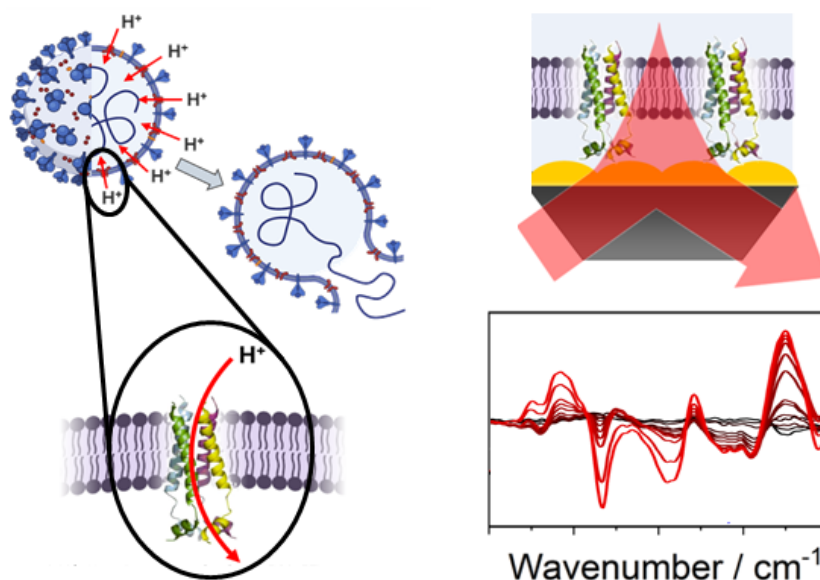
Figure captions:
Viroporins, like the Influenza A M2 proton channel, are targets for antiviral drugs. We monitor function and inhibition of M2 in model membranes using surface-enhance infrared absorption spectroscopy.

Keywords: SEIRA, membranes, viroporins, DFT, M2

D-I.4

Topic: *Mechanistic insights into the electrosynthesis of chemical feedstocks by in situ Raman and ATR-FTIR spectro-electrochemistry*

Author: Dr. Khoa H. Ly[1]

[1]Fakultät für Chemie und Lebensmittelchemie, Technische Universität Dresden, Andreas-Schubert-Bau, Zellescher Weg 19, 01069 Dresden, Germany

Abstract:

Renewable electricity-driven electrochemical synthesis of feedstocks from inexpensive and abundant small molecule precursors is widely considered an emerging sustainable alternative to current chemical processes. Recent studies have demonstrated, that organo-nitrogen compounds of high relevance for the chemical industry, such as urea and acetamide, as well as organo-sulphur compounds, such as sulfoacetate, hydroxymethane sulfonate, and methane sulfonate, have been demonstrated to be produced by co-electrolysis of N/S-substrates and CO_2, CH_3OH, H_3CCOH etc. as carbon source.[1,2] Electrosynthesis of basic chemicals is still a largely unexplored field. Rational development of the method requires now detailed understanding of the reaction steps taking place on the electrocatalyst enabling identification structure-activity relationships, efficient operation parameters on the one hand and to open avenues to novel coupling reactions on the other.

This presentation highlights the application of resonance Raman, SERS, and ATR-FTIR spectroscopy coupled with electrochemistry for the mechanistic investigation of electrosynthesis reactions by heterogeneous and molecular electrocatalysts. The talk provides a brief overview of experimental approaches and discusses the application of the methods specifically for understanding N-C and S-C coupling reactions on Cu oxide and phthalocyanine electrocatalysts.

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D-I.5

Title: *Surface-enhanced Raman Scattering in scaffolds for 3D cell cultures*

Author: Judith Langer[1], Javier Plou[2], Clara Clara García-Astrain[1], Beatriz Molina-Martínez[3], Luis M. Liz-Marzán[4]

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Abstract:
The Raman scattering from molecules close or bound to plasmonic nanoparticles (NP) can be significantly enhanced due to the electromagnetic field enhancement at the NP surface. This effect known as surface-enhanced Raman scattering (SERS) can be particularily enhanced by the excitation of localized surface plasmon resonances (LSPRs) in anisotropic AuNPs such as nanostars or nanorods, whose LSPRs can be tuned over the entire visible up to the IR range leading large electromagnetic fields localized at the tips [1] by modifying the aspect ratio. Especially for biological applications, certain prerequisites must be met; (1) excitation wavelength and LSPR should match the biologically transparent window, (2) adsorption of molecules on the NP surface must be effective to overcome the detection limit, (3) NP and bound molecule must be stable in present conditions.
In this presentation, we show that Au nanorods and stars with LSPRs tailored for the 785 nm excitation wavelength cannot only act as "sensors" for monitoring dynamic processes in 2D cell cultures, e.g., the intracellular pH [3] or the NP fate after multiple cell division cycles [4] but also be implemented in complex, up to 1 mm thick matrices such as 3D-printed hydrogel-based scaffolds as support for controlled cell growth and finally used for detection of diffusing drug molecules [5]. Alternatively, these embedded nanoparticles can be also decorated previously with an efficient Raman scatterer as messenger and then applied as SERS marker for 3D bioimaging applications. The big challenge of these applications is to match all necessary parameters and requirements to the system be able to detect the SERS signal in such highly complex constructions. This ranges from the synthesis of nanoparticles, the development of suitable, printable nanoparticle-incorporated biopolymer inks to the choice of cells and growth matrix and ends with ensuring the stability of the system over the necessary time period.

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Keywords: SERS, printed 3D scaffolds

D-O.1

Title: *Mechanistic insights of conjugated acetylenic polymers for the photoelectrochemical nitrogen reduction reaction to ammonia*

Author: Mino Borrelli[1], Agnieszka Kuc[2], Xinliang Feng[1], Inez Weidinger[1]

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Abstract:
Sustainable alternatives to the energy-consuming Haber-Bosch process for ammonia production are highly appealing. (Photo) electrocatalysis for the nitrogen reduction reaction (NRR) to ammonia is a promising approach as it directly enables ammonia production from the air with a zero-carbon footprint.[1-2] For this purpose, we developed thiophene-based conjugated acetylenic polymers (CAPs), able to produce ammonia under electrochemical bias and light irradiation.[3] Their catalytic properties and mechanism(s) are extensively investigated by vibrational spectroelectrochemistry. Electrochemical-operando resonance Raman (RR) and attenuated total reflectance IR (ATR-IR) spectroscopy, in combination with density functional theory (DFT), are employed to identify active sites and photoinduced conformational changes, assess the catalytic role of the CAPs flexibility and reveal rate-limiting steps.[4]

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2. MacFarlane, D. R. et al. A Roadmap to the Ammonia Economy. Joule 4, 6 (2020) 1186–1205.
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Keywords: resonance Raman, ATR-IR, nitrogen reduction

D-O.2

Title: *Revealing the Size Effect of Pd/Au Bimetallic Catalysts at Nanoscale with Tip-enhanced Raman Spectroscopy*

Author: Xiang Wang[1], Hui-shu Feng[1], Hai-sheng Su[1], Ya-qiong Su[2], Bin Ren[1]

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Abstract:
The size of nanocatalysts is a key factor that significantly determines the catalytic performance.[1,2] However, conventional ensemble measurements can hardly study the size- specific electronic properties and activities for individual nanocatalysts. Tip-enhanced Raman spectroscopy (TERS), which combines scanning probe microscopy and plasmon-enhanced Raman spectroscopy, can simultaneously obtain the topographical and chemical fingerprint information with a nanometer spatial resolution. [3]Here, we combined TERS with density function theory (DFT) calculations to investigate the size effect of bimetallic catalysts consisting of monolayer Pd nanoislands on Au(111), which shows a superior performance in various catalytic reactions. 4-chlorophenyl isocyanide (CPI) with a N≡C group that is sensitive to metal surface[4] was utilized to probe the electronic properties of Pd nanoislands with different sizes in real space. The high spatial resolution of TERS (ca. 3 nm) allows us to extract electronic properties for different sites with varied sizes. The catalytic efficiency for hydrogenation reaction of p-nitrothiophenol (PNTP) was further imaged with TERS. It is interesting to find that both electronic property and catalytic activity show a dramatic change near 10 nm for Pd nanoisland/Au(111) bimetallic catalysts. In particular, the nanoscale resolved surface structure, electronic properties and catalytic behavior enables a clear understanding for the role of geometric and electronic effect at different sites for varied sizes during catalytic processes, which can be a guide for rational design of catalysts.

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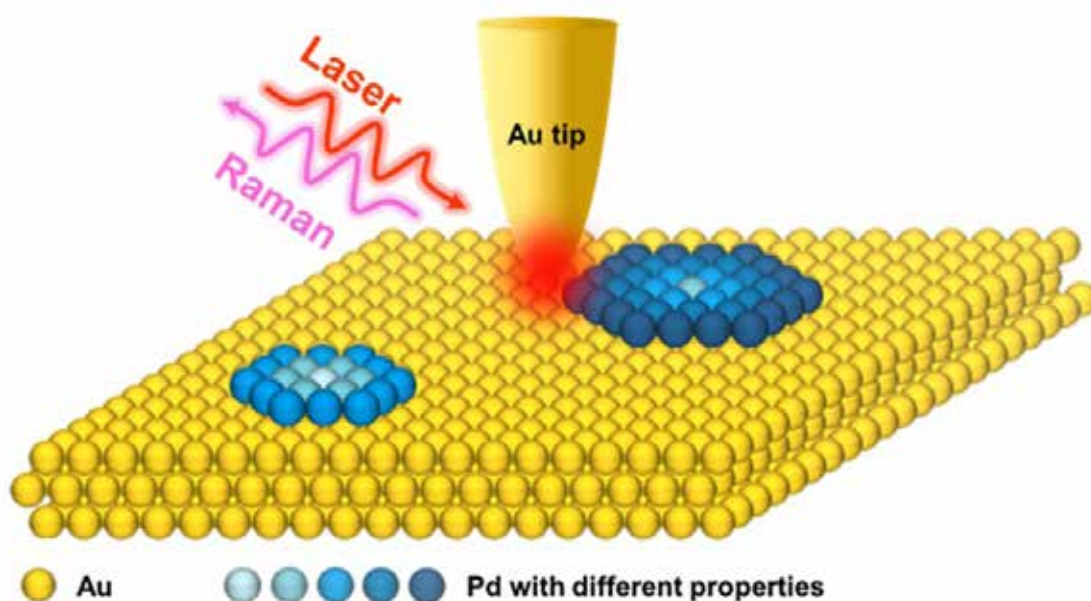
Figure captions:
Schematic illustration of TERS using Au tip and Pd/Au(111) substrate for studying size effect of nanocatalysts

Keywords: Size effect, Tip-enhanced Raman spectroscopy

D-O.3

Title: *The study of correlated Stokes-and-anti-Stokes in normal Raman and in Surface-Enhanced Raman Scattering (SERS)*

Author: Filomeno Aguiar Junior[1], Sahar Milani[1], Sanker Timsina[2], Stanislav Konorov[1], Michele L. de Souza[1], Rogério De Sousa[3], Alexandre Brolo[1]

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[3]Department of Physics, University of Victoria – BC

Abstract:
The inelastic scattering is known to exhibit two components, the Stokes (S) and the anti-Stokes (aS). Those components are extensively explored in spectroscopy as vibrational fingerprint of materials. In the Stokes scattering a photon from the laser loses energy creating a vibrational excitation, while in the anti-Stokes scattering, the vibrational energy from the molecule is consumed scattering a high-energy photon. As proposed by Klyshko in 1977 [1], these two processes can also occur simultaneously, within the vibrational lifetime to produce a correlated Raman scattering (SaS effect). In this case a correlated S-aS photon pair is created by an exchange of energy between the S and aS scattering, that can be mediated by a real excitation, or by a virtual excitation (Fig. 1) in an analogy to the Cooper pair in superconductivity [2]. The "real" SaS process, and the "virtual" SaS related to a counterpart of photonic Cooper pair, has been observed in many transparent materials, including solids, like diamond [3], and liquid samples such as water [4] and decane [2]. From a spectroscopic point of view, an efficient SaS processes can lead to the observation of a nonlinear dependence of anti-Stokes signal with the laser power. However, the normal Raman signal intensity of the SaS effect is very weak. The Surface-enhanced Raman scattering (SERS) technique shows great potential for generating correlated SaS photon pairs by enhancing SaS scattering. However, the use of the metallic surface to intensify the SaS process brings some challenges in the direct measurement of the phenomena due to the strong interaction of light with the metal, originating a wide range of optical phenomena. In this presentation, we are going to show some recent results from the Study of the SaS process in transparent samples, the challenges, and the preliminary results for the study of quantum correlations from stokes and anti-Stokes surface enhancement Raman scattering.

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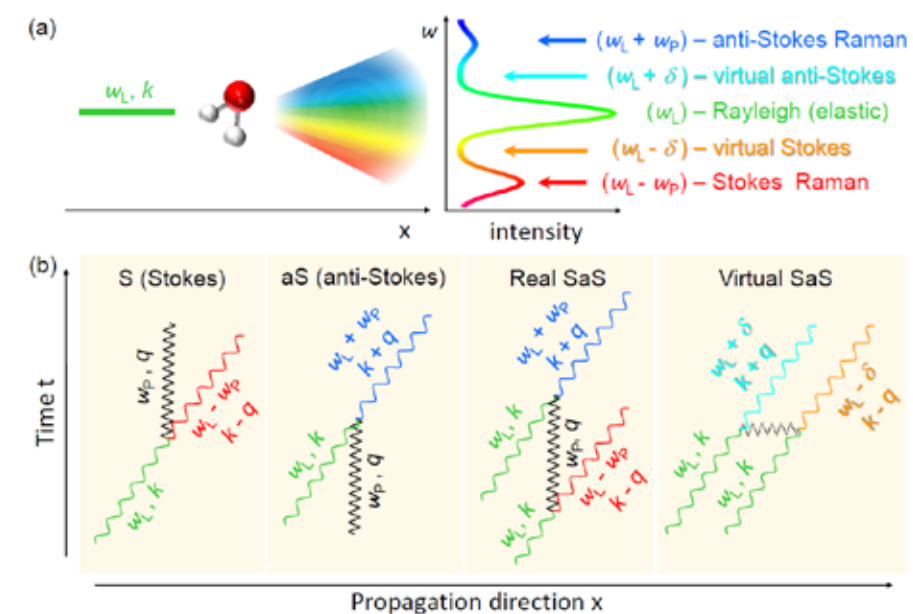
Figure captions:
(a) Spectral representation of the real and virtual process. (b) Schematically represented Raman processes interactions of: Stokes (S) anti-Stokes (AS), and a correlated real SAS or virtual SAS. [2]

Keywords: Stokes, anti-Stokes, quantum correlation, SERS

D-O.5

Title: *Nanoscale hyperspectral imaging of biologically relevant molecules*

Author: Ewelina Lipiec[1], Michał Czaja[2], Anna Chachaj-Brekiesz[3], Adrian Cernescu[4], Dhiman Ghosh[3], Dawid Lupa[1], Roland Riek[3], Sara Seweryn[2], Katarzyna Skirlińska-Nosek[2], Kamila Sofińska[1], Anita Wnętrzak[3], Marek Szymoński[1]

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[2]1) Jagiellonian University, Faculty of Physics, Astronomy, and Applied Computer Science, M. Smoluchowski Institute of Physics, Cracow, Poland, 2) Jagiellonian University, Doctoral School of Exact and Natural Sciences, Cracow, Poland
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Abstract:
One image is worth a thousand words. Nanoscale hyperspectral imaging is of particular importance for biospectroscopy, since the local molecular structure determines the chemical activity of biologically-relevant molecules. Nanoscale structural alternations allow several processes essential to life, including DNA conformational change related to DNA damage and its repair [1], formation of domains in lipid membranes [2], and secondary structure transformations in aggregating proteins [3]. Despite the best scientific efforts, efficient chemical imaging of biomoleculecules at the nanoscale is still challenging.Tip-enhanced Raman spectroscopy (TERS) and Fourier transform infrared nano-spectroscopy (nano-FTIR) are appropriate tools for achieving this goal because such methodology combines nanometric spatial resolution of scanning probe microscopy and chemical selectivity of Raman or infrared spectroscopies respectively, providing information on the chemical structure of nano-volumes of samples. Here several applications of molecular nanospectroscopic imaging of DNA, proteins, and lipids that reveal unique properties of the investigated systems will be presented:
i) a comparison of AFM-TERS and STM-TERS hyperspectral mapping obtained of DPPC/DPPE/cardiolipin monolayers for comprehensive characterization of artificial membranes, revealing local molecular distribution, orientation, phase separation, and formation of domains (Fig. 1A),
ii) application of AFM-TERS and STM-TERS in imaging of the β-sheet secondary structures distribution in individual aggregates of neurodegenerative peptides to follow aggregation pathways,
iii) nano-FTIR in following the effect of anti-aggregation agent called bexarotene on the local distribution of secondary structures in individual fibrils and oligomers of β-amyloid (Fig. 1B),
iv) pioneering imaging of individual DNA strands by means of nanoFTIR as a first step for the characterization of nucleic acid local conformational transition (Fig 1C).

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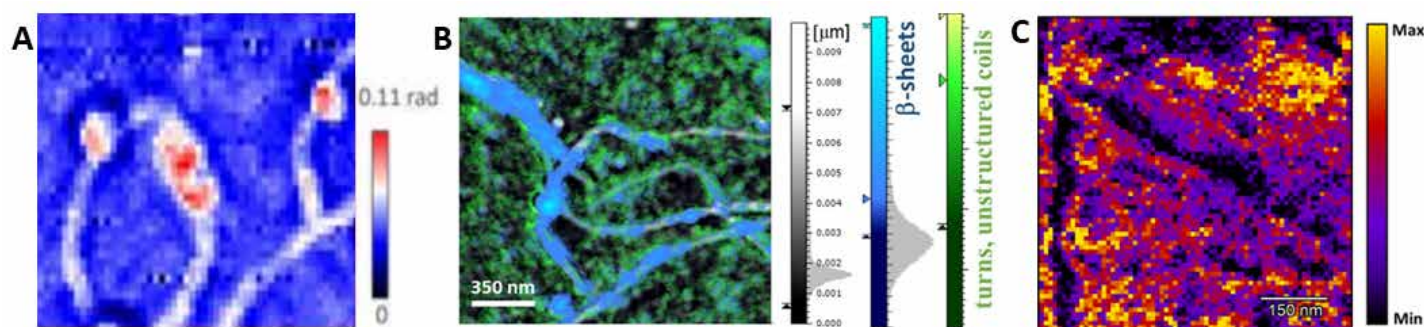
Figure captions:
Nanospectroscopic imaging of selected biologically relevant samples: DNA (A), β-amyloid treated with Bexarotene (B), DPPC monolayer (C)

Keywords: TERS, infrared nanospectroscopy, DNA, amyloids

D-O.4

Title: *Nanospectroscopy imaging of the molecule/metal interaction*

Author: Natalia Piergies[1], Dominika Święch[2], Magdalena Oćwieja[3], Czesława Paluszkiewicz[1], Wojciech M. Kwiatek[1]

[1]Institute of Nuclear Physics Polish Academy of Sciences
[2]AGH University of Science and Technology, Faculty of Foundry Engineering
[3]Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences

Abstract:
Metal nanoparticles due to their unique properties have become very interesting materials that found application in many fields, such as material science, bioengineering, medicine, industry, and so on.[1] Quite uncomplicated synthesis, functionalization, and also optical behavior are only a few that determine the importance of metal nanoparticles.[2] Special attention deserves the surface plasmon resonance (SPR) phenomenon that occurs on metal nanoparticle surfaces and which laid the foundations for the surface–enhanced vibrational spectroscopies such as surface–enhanced Raman spectroscopy (SERS) and surface–enhanced infrared absorption spectroscopy (SEIRA). By these techniques together with the surface selection rules the adsorption structure of biologically active molecules on metal surfaces can be clearly defined.

In recent years, our studies have been extended by the application of atomic force microscopy in combination with infrared spectroscopy (AFM–IR) for characterization of the molecule/metal nanoparticle systems with ultra–high spatial resolution. The performed investigations allowed us to prove the presence of the surface enhancement effect in the photothermal AFM–IR technique (AFM–SEIRA).[3,4]

In these studies, we discuss the utility of the AFM–SEIRA for characterization of the interactions between different molecules (drugs and amino acids) and metal nanoparticles (silver, gold, platinum, and copper). Figure 1 shows the experimental scheme, AFM–SEIRA spectrum, topography, and the spectral signal distribution of the erlotinib drug/silver nanoparticle (AgNP) system. The obtained results indicate that erlotinib binds to AgNPs through the phenylacetylene, ethoxy, and methoxy moieties. Moreover, the data favor the postulate that this type of infrared nanospectroscopy technique is a powerful tool providing full nanoscale insight not only into the molecule/metal interaction but also into the surface enhancement phenomenon visible in the intensity maps.

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Figure captions:
AFM–SEIRA experimental scheme (A). The AFM–SEIRA spectrum (B) and the AFM topography of the erlotinib/AgNP system (C). The AFM–SEIRA intensity map of the 1573 cm-1 band [ν(CC)Phe] of erlotinib (D).

Keywords: Infrared nanospectroscopy, drugs' adsorption
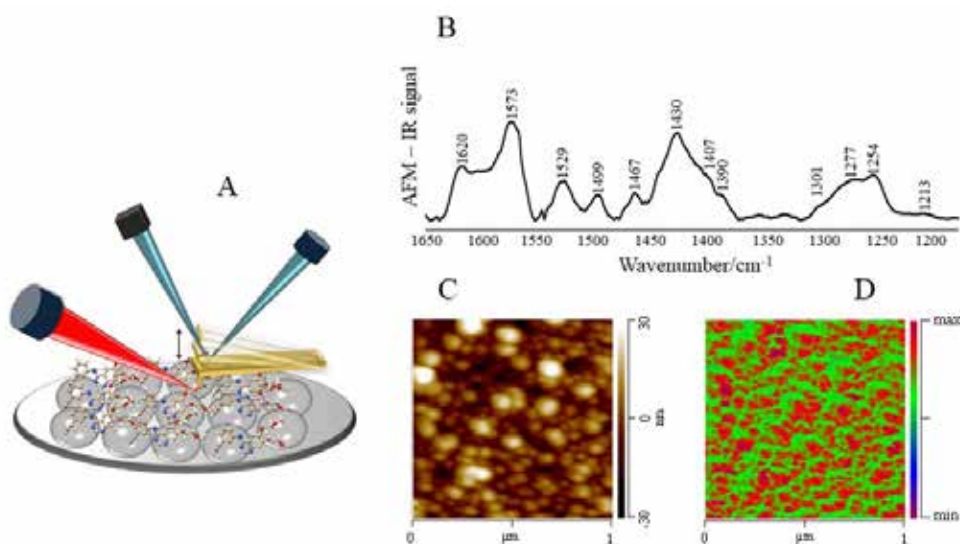
D-O.6

Title: *Spectroscopic study of extracellular vesicles using plasmonic nanoobjects*

Author: Tímea Bebesi[1], Marcell Pálmai[1], Anikó Gaál[1], Imola Csilla Szigyarto[1], Orsolya Bálint-Hakkel[2], Zoltán Varga[1], Judith Mihály[1]

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Abstract:
Extracellular vesicles (EVs), spontaneously released by cells, play an important role in intercellular communication. Due to their special size and composition (lipid bilayer-bounded nanosystems, usually smaller than 200 nm, containing both proteins and RNA), they can be used even in diagnostics as "new generation" biomarkers of various diseases.
IR spectroscopy, especially attenuated total reflection (ATR), is rapidly emerging as a label-free promising tool for molecular profiling of EVs [1–2]. However, the relative low number of extracellular vesicles (~10^{10} particle/mL) and possible impurities (protein aggregates, lipoproteins, buffer molecules, etc.) present in EV samples might result in poor signal-to-noise (S/N) ratio. The plasmonic properties of gold nanoparticles (AuNPs) are used in many characterization techniques, inclusive characterization and testing of EVs. Surface-enhanced infrared spectroscopy (SEIRA – Surface-enhanced IR absorption) using plasmonic nanoparticle, however, is still an unexploited method.
He we report SEIRA of model-EVs (EV-like liposomes) and red blood cell derived EVs using gold nanoparticles and tailored nanostructures with confined electromagnetic near-fields. Using the most common citrate-stabilized gold nanoparticles (c-AuNPs), a concentration dependent interaction was established between c-AuNPs and the lipid bilayers, affecting strongly both the plasmonic behaviour of AuNPs and the bilayers lipid organization. At appropriate extracellular vesicle:gold nanoparticle ratio a 6-fold maximum enhancement was obtained.To improve reproducibility and applicability, different structured nanoobjects and different surface modifications were also tested. Due to the structural and compositional complexity of EVs, the interaction between vesicle and the plasmonic nanoobject is the key factor obtaining SEIRA effect.

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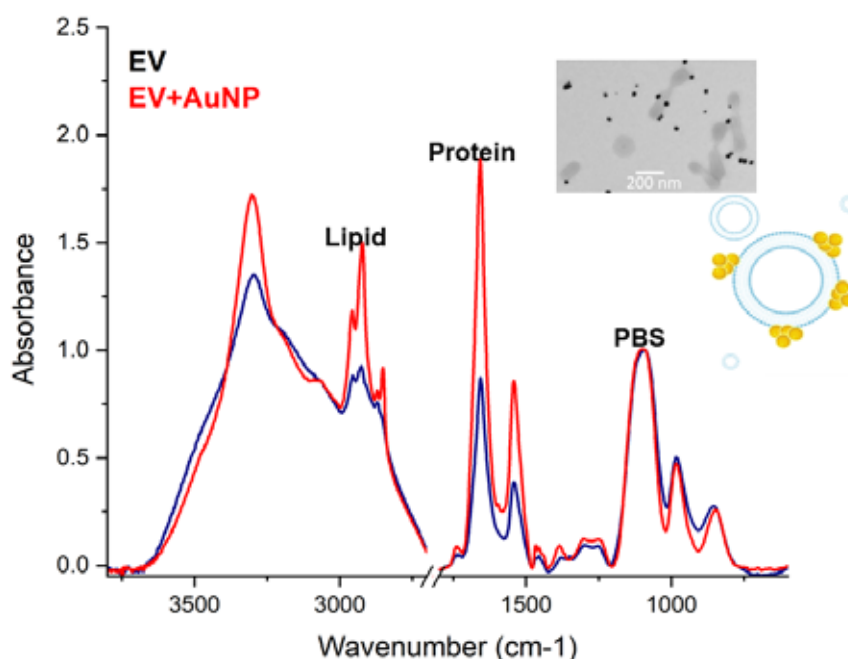
Figure captions:
SEIRA spectrum of extracellular vesicles

Keywords: extracellular vesicles, SEIRA, gold nanoobjects

D-O.7

Title: *Giant plasma membrane vesicles as the model systems to resolve nanoscale heterogeneity of native lipid membranes*

Author: Katarzyna Pogoda[1], Klemencja Berghauzen-Maciejewska[2], Natalia Piergies[2], Karolina Chrabąszcz[2], Czesława Paluszkiewicz[2], Wojciech Kwiatek[2]

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Abstract:
The study of the structure and function of cellular membranes has experienced rapid progress over the last years. There are many powerful bioanalytical tools used in lipid membrane research, but they lack the information about structural organization and molecules orientation within the cell membrane at the nanoscale. The combination of atomic force microscopy with infrared spectroscopy (AFM-IR) can provide simultaneous information about the nanoscale physical properties of the membranes – its topography and roughness, as well as biochemical composition through collection of local absorption spectra (1). Isolation of giant plasma membrane vesicles (GPMVs) by chemical vesiculation from a variety of cell types enables elucidation of native membrane structure and behavior and observation of lateral heterogeneity and segregation in the plane of the plasma membrane (2). In our study, we have used GPMVs isolated from normal microglia cells and cancerous glioblastoma cells. As presented on the figure A, the production of the GPMVs can be controlled through optical microscopy in the real time. Collected vesicles can be subsequently deposited on gold sputter-coated mica sheets and imaged using atomic force microscopy (figure B and C). Combination of AFM with infrared spectroscopy allows the collection of the local absorption spectra (figure D) that resolve their nanoscale heterogeneity, and these measurements are beyond conventional IR spectroscopy due to sample nanoscale thickness and spatial resolution that is diffraction limited.
Extensive studies on nanoscale organization of biomolecules that built native lipid membranes in normal and cancerous cells can explain the differences in drug-native lipid membrane interactions as well as to follow molecular changes of the membrane structure during multiple drug treatment and drug-resistance development.

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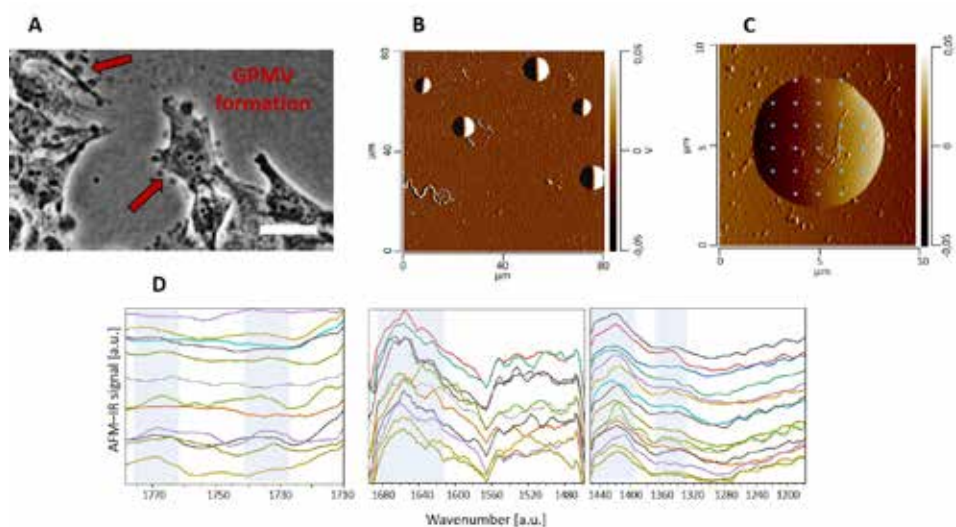
Figure captions:
(A) GPMVs formation in live microglia cells (bar=30μm). (B) 80μmx80μm and (C) 10μmx10μm AFM topographies of GPMVs. (D) Local AFM–IR spectra collected on single vesicle from points marked in C.

Keywords: nanospectroscopy, FT-IR, AFM, lipid membranes

D-O.8

Title: *SERS based detection of cytosine methylation in genomic DNA*

Author: <u>Stefania D. Iancu</u>[1], Vlad Moisoiu[1], Adrian B. Tigu[2], Andrei Stefancu[1], Zoltán Bálint[1], Ciprian Tomuleasa[2], Nicolae Leopold[1]

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[2]Medfuture Research Center for Advanced Medicine, Iuliu Hatieganu University of Medicine and Pharmacy

Abstract:
The most common epigenetic changes in cancer are global DNA hypomethylation and clustered hypermethylation in CpG islands[1]. Optical biosensors, based on the interaction between DNA and metallic nanoparticles, have been developed to detect DNA methylation and to distinguish between cancerous and non-cancerous DNA[2,3].
In this study, we used surface-enhanced Raman scattering (SERS) to quantify the levels of methylated cytosine (5mC) in genomic DNA (gDNA), and thus to detect the early onset of cancer. gDNA was extracted from five cell lines with different methylation levels in the range of 0.16-0.91%. We found a strong positive correlation ($R_{Pearson}$=0.94, p=0.005) between the intensity of the SERS band at 790 cm^{-1} and the level of 5mC in the cell lines (Figure A), highlighting the potential of SERS for quantifying the level of methylated gDNA.
To obtain the SERS spectrum of gDNA, we used Ca^{2+} to favor the binding between DNA and silver nanoparticles (AgNPs). Additionally, we evaluated the effects of different bivalent cations with the same charge and found that Ca^{2+}, Mg^{2+}, Be^{2+}, and Zn^{2+} ions promote the switch-on of the SERS spectrum of DNA, while this effect was absent by supplementing the solution with Cu^{2+} or Fe^{2+}.
Fluorescence spectroscopy was used to complement the SERS spectra and monitor the adsorption of gDNA on the silver surface. We found that, with increasing 5mC levels, the gDNA adsorbs weaker to the AgNPs surface (Figure B).
We noticed that non-cancerous gDNA has a greater tendency to bind to AgNPs than malignant gDNA. The findings suggest that the affinity of gDNA adsorption to nanoparticles is dependent on global 5mC levels, which could lead to the development of nanoparticle-based biosensors for cancer detection.

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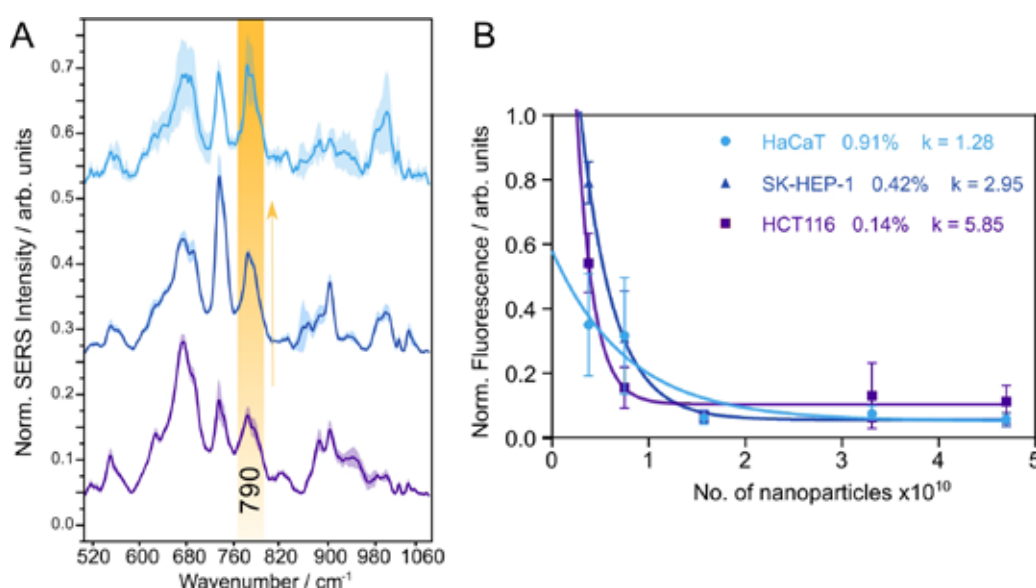
Figure captions:
(A) Mean SERS spectra and standard deviation of gDNA samples extracted from cultured cell lines with different levels of 5 methylcytosine. (B) The adsorption rate of gDNA onto AgNPs

Keywords: SERS, DNA methylation, fluorescence, cytosine

E-I.1

Title: *Proteins at charged biointerfaces as revealed by nonlinear vibrational spectroscopy*

Author: Zsuzsanna Heiner[1]

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Abstract:
Characterization of the adsorption and self-assembly of proteins and peptides at macromolecular interfaces is a prerequisite for understanding fundamental physiological processes and is also crucial when designing biomaterials for implants, medical devices, and bioelectronics, or photosensitive coatings for conversion of light to electrical energy [1-3]. To this end, in situ determination of the orientation and secondary structure of the adsorbed biomolecules is required with combined chemical and surface specificity [4]. Vibrational sum-frequency generation (VSFG) spectroscopy is a powerful label-free technique especially suited for such investigations, as it is sensitive only to anisotropic molecular structures characteristic to the immediate vicinity of macromolecular interfaces, and, at the same time, it retains the chemical sensitivity of infrared spectroscopy. Here we report on model studies carried out by using high-resolution VSFG spectroscopy on the structure of photoactive yellow protein (PYP) at nanometer-thick, positively and negatively charged self-assembled poly-electrolyte surfaces typically used for anchoring bioactive molecules in biomaterials. The interpretation of chiral and achiral VSFG spectra acquired in the 1400-1700 and 2800-3800 cm-1 spectral regions were aided by molecular dynamics (MD) simulations yielding protein conformation and orientation distributions, which in turn were used to calculate VSFG spectra. We show that chiral VSFG spectra reveal a wealth of information on secondary and tertiary protein structure and provide deeper insight into protein interaction processes at biological interfaces.

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Keywords: VSFG, photoactive yellow protein, polyelectrolyte

E-I.2

Title: *Time-domain Raman spectroscopy for large-scale cell screening*

Author: Kotaro Hiramatsu[1]

[1]The University of Tokyo

Abstract:
High-speed Raman spectroscopy has revolutionized high-dimensional cellular phenotyping. Fourier-transform coherent anti-Stokes Raman scattering (FT-CARS) stands out due to its rapid spectral acquisition, extensive spectral sensitivity in the fingerprint region, and elimination of nonresonant background. In this talk, we showcase recent advancements in FT-CARS spectroscopy:

(1) Color-scalable Flow Cytometry[1]
Flow cytometry is vital for counting and analyzing heterogeneous cell populations in biology and medicine. However, it faces a significant hurdle: the color barrier. We introduce advanced multiplex flow cytometry, utilizing FT-CARS flow cytometry and Raman tags to overcome this limitation. We achieved multiplex flow cytometry on MCF-7 breast cancer cells stained with 12 distinct Raman tags, achieving a 98% classification accuracy.
(2) Broadband Raman-Activated Cell Sorting[2]
Raman-activated cell sorting (RACS) has gained attention for its capacity to differentiate cells based on their intracellular chemical content without labeling. Despite this, RACS's broader application is restricted by a compromise between throughput and measurement bandwidth. We address this issue by demonstrating high-throughput, broadband RACS in the fingerprint region, processing approximately 50 cells per second.
(3) Dual-Band FT-CARS Spectroscopy[3]
Ultrafast coherent Raman spectroscopy techniques typically only obtain Raman spectra in either the fingerprint or high-frequency region. We present a groundbreaking dual-band (200 – 1600 and 2800 – 3200 cm-1) FT-CARS spectroscopy method that is ultrafast (24,000 spectra/s) and highly sensitive without the need for ultrashort pulses. This innovation is made possible by using rapid-scan FT-CARS based on passively synchronized Ti:Sapphire and Yb-doped fiber lasers.

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Keywords: Flow cytometry, Nonlinear Raman spectroscopy,

Title: *Specific Ion Effects in the Electrical Double Layer Structure at the Silica/Aqueous Interface*

Author: Julianne Gibbs[1], Nathaniel Tetteh[1], Shyam Parshotam[1]

[1]University of Alberta

Abstract:
Understanding the structure and properties of charged interfaces is important to a variety of fields, from catalysis to environmental transport modeling. Yet for many natural solid materials such as oxides, their charging behavior is difficult to monitor over a wide range of relevant pH conditions owing to the instability of oxide colloid dispersions and the challenges in using electrochemistry on oxide insulators. Recently, we have shown that the water structure of the electrical double layer (EDL) at the silica/water interface can be determined by a combination of vibrational sum frequency generation (SFG) and streaming potential measurements oft aided by complementary second harmonic generation measurements [1,2]. These results have revealed that the Stern layer of the EDL exhibits significant changes when the pH or salt concentration are varied suggesting that static ideas about the EDL structure for a given oxide are incorrect. Here I will discuss our exploration of how cation identity affects the EDL structure using vibrational SFG and streaming potential measurements. Consistent with our earlier work[3], we find that the specific ion (also referred to as Hofmeister) trends exhibit pH dependence at the silica/water interface. However, now this work allows us to distinguish the indirect effects of the cations on the EDL structure that stem from the zeta potential (the potential at the edge of the Stern layer) and the direct effects based on the influence of the different ions on the water structure in the Stern layer. Ultimately, with this molecular information we can improve electrical double layer models, thereby improving the accuracy of the many models that depend on them.

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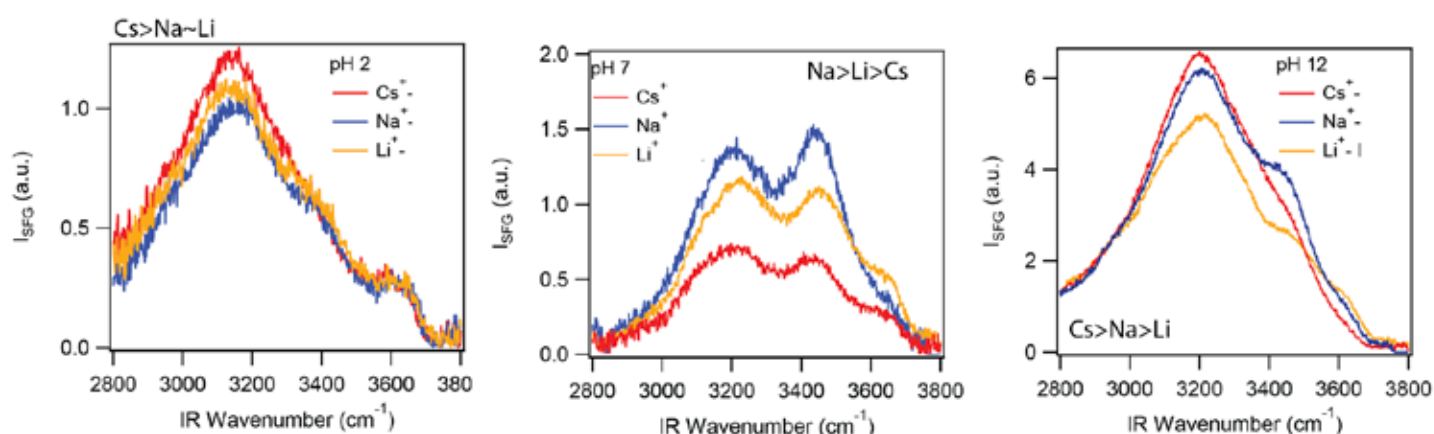
Figure captions:
Vibrational sum frequency intensity at different pH which reveals the amount of net ordered water at the silica/aqueous interface in the presence of 50 mM sodium, lithium, or cesium chloride.

Keywords: Environmental interfaces, electrical double layer,

E-I.4

Title: *Nonlinear Vibrational Spectroscopy as an Orientation-Independent Probe of Molecular Environments at Interfaces*

Author: Dennis Hore[1], Aruna Kumarasiri[1], Peter Yang[1]

[1]University of Victoria

Abstract:
Measurement techniques that probe the second-order susceptibility, such as electronic second-harmonic generation and vibrational sum-frequency generation are recognized for their ability to study environments with broken centrosymmetry.[1,2] As a result, they serve as an excellent reporter of molecules at surfaces since the second-order susceptibility is often zero in the adjacent bulk media, but non-zero for those same molecules that are ordered at the interface between the two bulk phases. Although the signals measured in such experiments carry unique information on the interfacial environment, the challenge is to disentangle properties related to the electronic structure as they are wrapped up in the orientation distribution. Over the past 30 years, this challenge has been turned into an opportunity, as many studies seek to learn about the orientation distribution of molecules at surfaces.[3,4] Here we demonstrate that the flipped case is possible, where fundamental clues about the interfacial environment can be extracted in a manner that is completely independent, and therefore oblivious to, the orientation distribution. Using para-cyanophenol adsorbed at the air–water interface as an example, we illustrate that the cyano group polarizability varies less along the direction of the C–N bond when at the surface, than when the same molecules are in the bulk aqueous phase.

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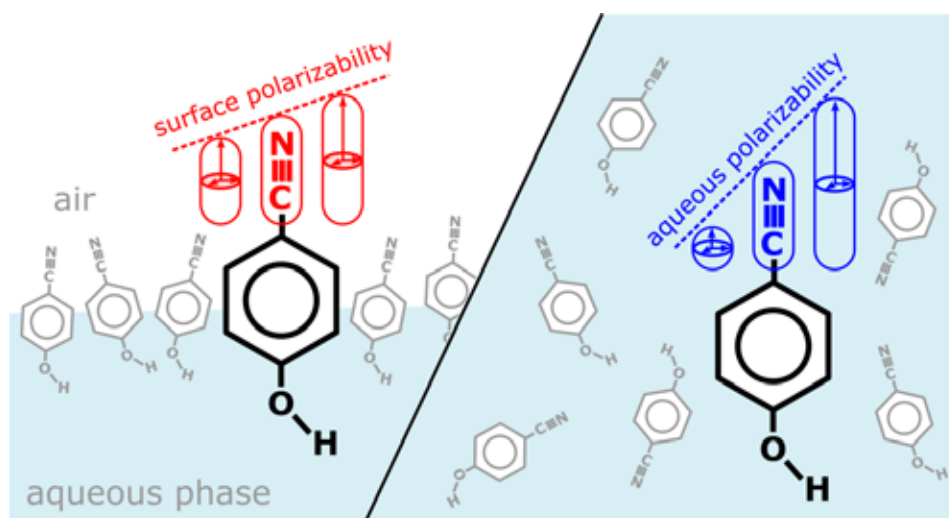
Figure captions:
The change in polarizability along the C–N bond is less when p-cyanophenol is adsorbed at the air-water interface, in comparison to the bulk aqueous environment.

Keywords: SFG, Raman, depolarization, hyperpolarizability

E-I.5

Title: *Molecular-Level Elucidation of Buried Solid/Liquid Interfaces by the Use of Heterodyne-detected Vibrational Sum Frequency Generation*

Author: Satoshi Nihonyanagi[1]

[1]Molecular Spectroscopy Lab., RIKEN

Abstract:

Molecular-level elucidation of the structures of solid/liquid interfaces is one of the central issues in the research fields related to interface science, battery, wet control, and so on. However, it is still challenging because of the technical difficulties in observing the solid/liquid interface in situ. Vibrational sum frequency generation (VSFG) is a powerful technique for studying the structure and orientation of molecules at interfaces, owing to its intrinsic interface selectivity based on second-order nonlinear optics.[1] Furthermore, the phase-resolved version of VSFG technique, Heterodyne-detected VSFG (HD-VSFG) spectroscopy, enables us to obtain real (Re) and imaginary (Im) parts of a second-order nonlinear susceptibility ($X^{(2)}$) spectrum separately. From the sign of $ImX^{(2)}$ spectrum, one can determine the up/down orientation of interfacial molecules.[2] Although the application of HD-VSFG had been limited to simple exposed interfaces, we have recently succeeded in applying this advanced technique to buried solid/liquid interfaces, which include both transparent[3] and non-transparent solids.[4,5].

In the present talk, I will first explain the methodology of HD-VSFG spectroscopy for buried solid/liquid interfaces and talk about in-situ HD-VSFG study of a model battery interface: the interface of a platinum electrode and acetonitrile solutions containing either dilute or super-concentrated Lithium bis(trifluoro methanesulfonyl)imide (LiTFSI). Super-concentrated electrolyte solutions have attracted attention in developing tolerant electrolyte solutions for high-voltage rechargeable batteries because of their ability to stabilize solvent molecules. The in-situ HD-VSFG measurements can clearly distinguish the structures of two electrode interfaces, demonstrating the powerfulness of this technique for elucidating the structure at an electrolyte /electrode interface.[6]

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Keywords: non-linear vibrational spectroscopy, Sum Frequency

Title: *Mechanistic Approach to Investigate the Water Evaporation Process at Air/Water Interface using Hofmeister Ions*

Author: Bhawna Rana[1], David J. Fairhurst[2], Kailash C. Jena[1]

[1]Indian Institute of Technology Ropar
[2]Nottingham Trent University

Abstract:
Ions at the air/aqueous interface determine various interfacial reactions and phenomena which are imperative in atmospheric and environmental chemistry. The thermodynamics and kinetics of such interfacial processes are controlled by the conformation and hydrogen bonding environment of the surface water molecules. Therefore, we have probed the hydrogen bonding environment of water molecules at the air/aqueous interface in the presence of Hofmeister salts. In order to probe the interface, we have used sum frequency generation (SFG) vibrational spectroscopy [1-3]. SFG is a surface-specific spectroscopic tool based on second-order nonlinear optical process. Here, two pump beams are used one is vis (532 nm) and another is of tunable IR wavelength to produce the SFG signal after satisfying the spatial and temporal overlap at the interface. The SFG signal carries the vibrational signature of the molecules residing at the interface. It is observed that the presence of chaotropes brings more weakly bonded water molecules at the interface whereas the presence of kosmotropes creates more enhanced strongly hydrogen-bonded water molecules. It is also noticed that the orientation of the free-OH gets affected by the presence of ions at the interface [4]. In my talk, I will provide a detailed qualitative and quantitative analysis of the hydrogen bonding network of water molecules which gets perturbed in the presence of kosmotropic and chaotropic ions at the air/aqueous interface and their influence on the evaporation process of water molecules.

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Keywords: Nonlinear Vibrational spectroscopy, Evaporation Processes,

E-O.1

Title: *GOOD VIBRATIONS: SMALL MOLECULE RAMAN OPTICAL PROBES TO IMAGE METABOLISM IN TISSUE MICROENVIRONMENTS*

Author: Ailsa Geddis[1], Fabio De Moliner[1], Colin Campbell[1], Marc Vendrell[1]

[1]University of Edinburgh

Abstract:
Imaging of small molecules in biological systems is complex. Fluorescence imaging of biomacromolecules is common; however the size of fluorescent tags and other available imaging tools is often too large to retain the biophysical properties of the native substrates. Small vibrational tags have been developed to image small molecules, such as metabolites, significantly improving the biorthogonality of the probes. These tags can consist of a non-common bond or an isotope that resonates within the biologically silent window of the Raman spectrum (1800-2800 cm^{-1}). Previous research within our group focussed on the design of optical Raman analogues of sucrose, the most abundant nutrient in plants, and we demonstrated its application for imaging of metabolism in live plant cells using Stimulated Raman Scattering (SRS) spectroscopy.[1] SRS irradiates a specific wavelength improving spatiotemporal resolution and Raman intensities.[2]
This approach has vast potential for biorthogonal real-time in vivo imaging and can be, in principle, extended to many other metabolites and small molecules. Current research is focused on the development and synthesis of a vibrationally active citric acid Raman probe for Raman imaging. These probes are highly versatile and have applications in many areas of research – in metabolomics, for acting as biomarkers in oncology and for determination of citrate transport mechanisms in osteology; this will be demonstrated in a zebrafish embryo model.
Further research is focusing on synthesis of a Raman active Estrogen probe to determine abnormalities in Estrogen receptors for development of an endometriosis sensor with other potential applications in breast cancer research. Preliminary studies show strong Raman activity of estrone backbone with various vibrational tags attached, with current research into attaching tags to other more biomimetically appropriate regions of the structure.

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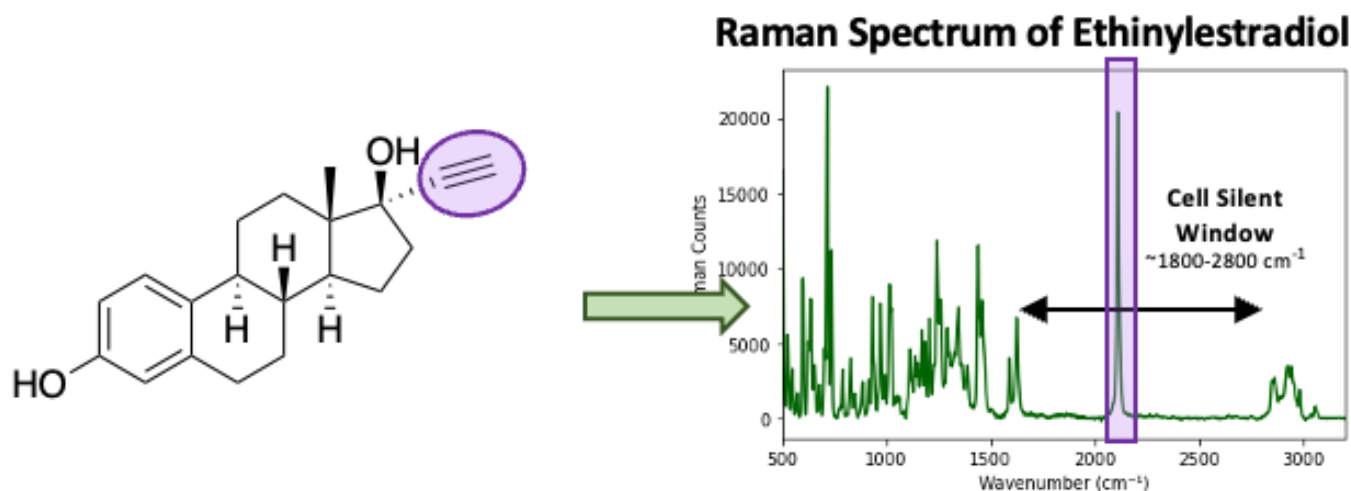
Figure captions:
Figure 1: Estrogen analogue, Ethinylestradiol, has a strong alkyne signal in the "cell-silent" window of a Raman spectrum

Keywords: Vibrational, Small Molecule, Biomimetic, Probe

E-O.2

Title: *Probing amide I-water vibrational coupling in α-helical and β-strand protein structures with two-color two-dimensional infrared spectroscopy*

Author: Fani Madzharova[1], Adam Chatterley[1], Steven Roeters[1], Tobias Weidner[1]

[1]Aarhus University

Abstract:
Protein hydration plays an important role in biology. Protein structure and dynamics are stabilized and driven by hydrophobic interactions and hydrogen bond networks. [1] Characterizing biomolecule-water interactions will be fundamental for providing mechanisms of biocatalytic processes. Here, we use two-dimensional infrared spectroscopy (2D IR) to probe protein – water coupling. We have constructed a two-color experiment, in which the pump and probe pulses are generated by two synchronized laser systems. [2] We use a pump frequency centered at 1650 cm−1 to excite amide modes and a probe frequency near 2400 cm−1 to observe cross peaks with water (D2O) stretching modes. The cross peaks in this experiment should originate directly from hydration water molecules. This allows for direct probing of protein-water interactions in contrast to prior 2D IR work that has focused on indirectly assessing hydration shells using spectral features of probes.
We chose a set of peptides that were designed to fold into well-defined secondary structures, depending on the periodicity of hydrophobic and hydrophilic side chains. We used the α-helix sequence Ac-LKKLLKLLKKLLKL (LKa14) and the β-strand sequence LKLKLKLKLKLKLKL (LKb15). We also studied analog sequences in which leucine was replaced either by tyrosine or phenylalanine to vary the side chain chemistry. To avoid the strong bulk water absorption, we prepared the samples as solid-state thin films. First, we record 2D IR spectra of the peptides in the amide region to confirm their secondary structure. Then, we show amide I – OD stretch coupling in the corresponding two-color 2D IR spectra and discuss its relation to secondary structure. Finally, we present two-color 2D IR data from bovine serum albumin and concanavalin A, which provides an insight into the powerful capabilities of 2D IR to probe hydration water within separate protein domains.

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Keywords: 2D-IR, vibrational coupling, water, protein

E-O.3

Title: *Molecular structure, surface charge and dissolution of the MgO-water interface influenced by liquid flow*

Author: Moritz Zelenka[1], Ellen H. G. Backus[1]

[1]University of Vienna

Abstract:
Understanding the dissolution and surface adsorption processes of magnesium oxide (MgO) in aqueous solutions has motivated various studies in the past [1]. However, knowledge of the molecular processes and interfacial water structure at the interface is still limited. Furthermore, an important aspect neglected so far is the distinctive difference of flowing versus static liquid at the interface.

To investigate these issues, we apply sum frequency generation spectroscopy at the MgO-water interface. Due to its selection rule, no signal is generated in centrosymmetric media. Therefore, this technique is capable of retrieving vibrational information of selectively interfacial molecules even if bulk amounts of liquid are present at the interface. Using this method, the interface of single crystalline MgO and aqueous solutions is probed in the OH-stretching region. The interfacial structure is assessed in the range between pH 3 and pH 11, both under flowing and static liquid condition.

In the case of flowing liquid, large sum frequency signals of the MgO-water interface were recorded when using aqueous solutions below pH 5. This signal originates from the breaking of the centrosymmetry of the interfacial water molecules, which stems from a charged MgO surface due to the presence of protons at or near the surface. Surprisingly, the sum frequency signal intensity is vanishing when turning off the liquid flow. The decrease of the intensity is linked to a decreasing surface charge. This behaviour is explained by the acid mediated MgO dissolution process. Additionally, investigating the dynamics of the sum frequency signal after switching off the flow allows for new insights into the dissolution process and kinetics based directly on the response of interfacial molecules.

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Keywords: Nonlinear spectroscopy, MgO-water interface

E-O.4

Title: *Ultrafast decay of coupled molecule-plasmon nanogap structure*

Author: <u>Fiona Bell</u>[1], Lukas Jakob[1], Ishaan Lohia[1], Rakesh Arul[1], Jeremy Baumberg[1]

[1]University of Cambridge

Experimental apparatus designed and built with Lukas Jakob, Ishaan Lohia developed gold disk fabrication techniques. All authors contributed to project discussions with special thanks to Jeremy Baumberg.

Abstract:
Understanding the ultrafast vibrational dynamics of molecule-plasmon coupled systems is crucial within the growing field of plasmonic-driven chemistry. This aims to elucidate the reaction mechanics and energy transfer of molecules experiencing extreme electromagnetic fields with high spatial confinement. Here, we study the vibrational decay of coupled molecules within a single plasmonic nanogap using ultrafast mid-IR (MIR)-pumped surface-enhanced Raman scattering (SERS). Simultaneous excitation with MIR ps-pulses modifies the measured SERS spectrum, upconverting vibrational information to the visible (Fig.1). Utilising this technique – termed MIR molecular upconversion – we present the first reported measurements of molecular vibrational decay within a plasmonic nanogap under MIR excitation.

MIR upconversion has been extensively used to measure the vibrational dynamics of molecules coupled to metallic surfaces, which averages over many conformations [1]. There have been no previous reports observing dynamics within a single plasmonic nanogap as the simultaneous confinement of MIR and visible wavelengths proves challenging. The nanoparticle-on-resonator (NPoR) geometry (Fig.1a) supports simultaneous MIR and visible plasmonic resonances, confined within a 1nm plasmonic gap. Thus upconversion within a single NPoR structure is possible even under continuous wave excitation [2]. Using the NPoR structure, vibrational MIR upconversion is demonstrated from a coupled ensemble of <100 4-nitrothiophenol (4NTP) molecules in a self-assembled monolayer within a single nanogap. Confocal pump-probe microscopy experiments track the decay of the upconverted signal, extracting the coherent dephasing time (T2) (Fig.1c) and providing additional insights into energetic transfer dynamics within the molecular-plasmon coupled system [3]. This cascade of vibrational energy on ps-scales couples to chemical reactivity, and is crucial to resolve across a wide range of molecule-metal landscapes.

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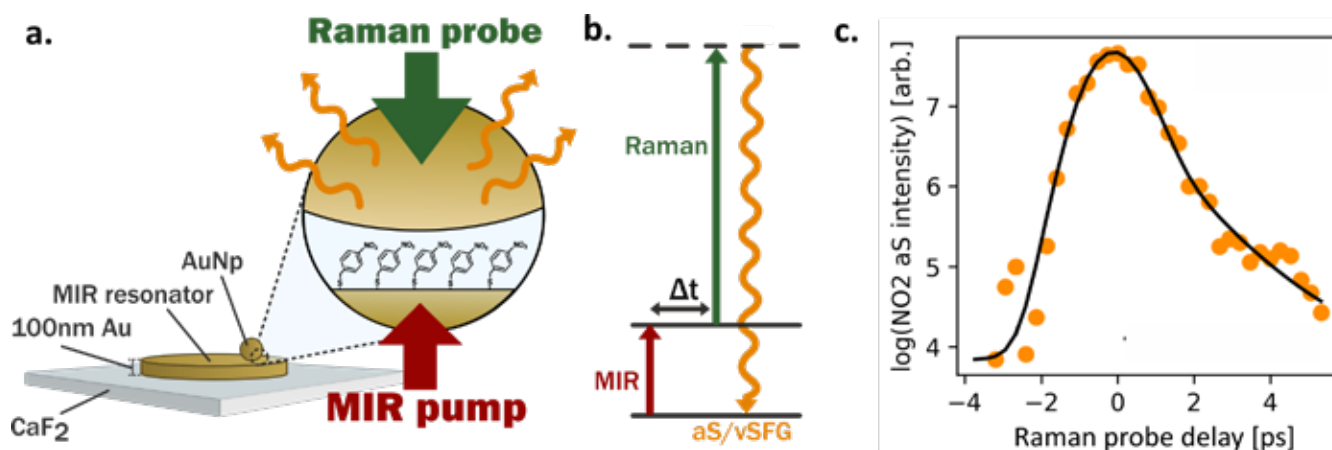
Figure captions:
(a) NPoR formed of gold disk, molecular monolayer and gold nanoparticle (AuNP). (b) Upconversion scheme yielding enhanced antiStokes (aS) signal. (c) Pump-probe measurements of 4NTP NO2 stretch decay.

Keywords: Plasmonic nanogaps, upconversion, vibrational dynamics

E-O.5

Title: *How and when does the collapse of a macromolecule in water start? From time-resolved Raman to elastic light scattering viewpoint.*

Author: Marcin Pastorczak[1], Michał Nejbauer[1], Naoki Shinyashiki[2], Masanobu Takatsuka[2], Gonzalo Angulo[1], Yuriy Stepanenko[1], Czesław Radzewicz[3]

[1]Institute of Physical Chemistry Polish Academy of Sciences
[2]Department of Physics, School of Science, Tokai University
[3]Institute of Experimental Physics, Faculty of Physics, University of Warsaw

Abstract:
The temperature-induced coil-to-globule (c-t-g) transition of a macromolecule in water is responsible for the collapse of proteins, peptides, or thermosensitive polymer gels. Moreover, this kind of transition in polymer solutions has usually been regarded as the simplest model of protein folding [1] and henceforth attracted plenty of researchers' attention. However, the observation of the initial stages of such transition was hampered not only by the limited time resolution of applied methods but also by the too-slow heating of a macromolecule/water mixture. [2] Hence, up to recently, the nature of the primary stage of the transition was speculative. In this study, we applied a new experimental approach– heating the sample with a femtosecond near-infrared pulse to trigger the c-t-g transition with subsequently following the evolution of a polymer/water system with optical methods – femtosecond stimulated Raman (FSRS, to monitor intermolecular interactions) and time-resolved elastic light scattering to observe nanoscopic phase-separations in a system. [3] As a model system, we used poly(vinyl methyl ether), PVME, a simple thermo-responsive homopolymer with a lower critical solution temperature (associated with c-t-g transition) located at 35-37°C. We observed signal changes 300–400 ps after the temperature jump that triggered the c-t-g transition with both experimental methods. That time coincides with the time of segmental relaxation of PVME, determined by broadband dielectric spectroscopy in the temperature range of the c-t-g of the PVME/water mixture. This coincidence strongly suggests that the observed stage of the c-t-g transition is the rapid formation of local nuclei on the polymer chain. The nucleation and growth of the defects also go along with the autocatalytic kinetic model we used to fits the time-resolved light scattering data. Our findings could be crucial for designing fast, thermoresponsive materials and understanding protein collapse process.

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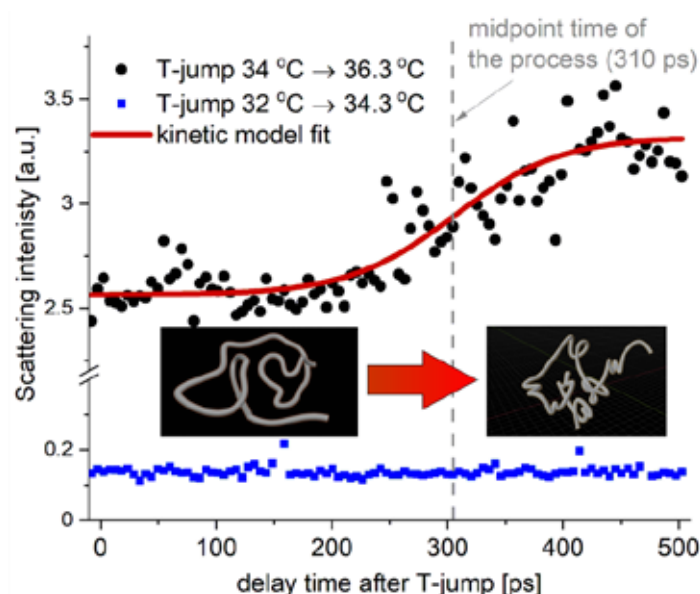
Figure captions:
Time-traces of Rayleigh spectrum of 8 wt. % PVME in water for T-jump: 32°C → 34.3°C and 34°C → 36.3°C with the kinetic model fit in red. Inset: scheme of the earliest chain rearrangement during c-t-g.

Keywords: coil-to-globule transition, FSRS, BDS, light-scattering

E-O.6

Title: Taking Advantage of High Sensitivity Enabled by Stimulated Raman Scattering: Multi-Parameter Analysis of Nanoplastics in Flow

Topic: (E) nonlinear vibrational spectroscopies, (E) nonlinear vibrational spectroscopies

Author: Maximilian Huber[1], Liron Zada[2], Freek Ariese[2], Natalia P. Ivleva[1]

[1]Technical University of Munich, Institute of Water Chemistry, Chair of Analytical Chemistry and Water Chemistry, School of Natural Sciences (Dep. Chemistry)
[2]Vrije Universiteit Amsterdam, LaserLaB Amsterdam, Department of Physics and Astronomy

Abstract:
The characterization of nanoplastics is still quite challenging since most analytical techniques can deliver only limited information on these complex analytes. Many different properties, like size, concentration, and chemical composition, must be considered for a proper characterization. [1] A technique that can deliver multiple parameters – mainly size and chemical information - from one measurement is online-coupled field flow fractionation (FFF) – Raman microspectroscopy. [2] However, this hyphenated technique still has some limitations, e.g., low sensitivity and dependency on optical trapping, and cannot deliver particle concentrations. Therefore, a coherent Raman technique, called stimulated Raman scattering (SRS), was tested for its potential hyphenation with FFF. This technique employs two different laser wavelengths. Their difference in frequency must match a vibrational transition of the target compound to result in an enhanced signal. [3] Compared to spontaneous Raman, measurement times can be significantly reduced from 10 s to 60.5 µs. Nanoplastics (PS, PE, PMMA) in a size range from 100 nm to 5 µm could be detected in flow with this setup using a flow cell with either a reflective or a transparent base. Due to the increased time resolution, individual signals per particle could be observed with SRS rather than an average sum signal for spontaneous Raman. (Figure 1A, B) Therefore, this method can be used to count particles and quantify nanoplastics while also giving chemical information on the material. (Figure 1C) The peak shape of the individual signals reveals that not all detected particles were trapped in the focus of the laser. In case of untrapped particles the mean peak intensity and width can be used for size estimation. Overall, with this method a broad characterization of nanoplastics is possible since information on size, concentration and chemical composition can be obtained within one measurement.

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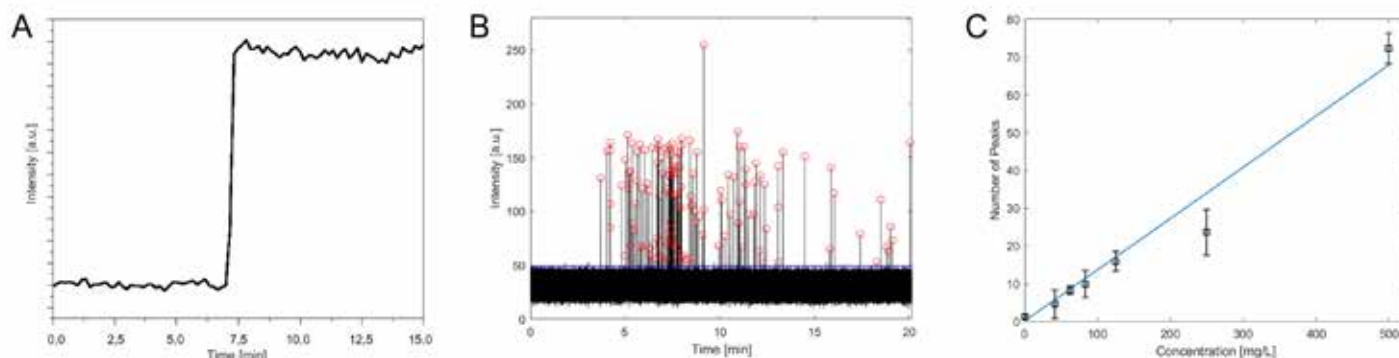
Figure captions:
Comparison of spontaneous Raman (A) and SRS (B) for polydisperse PS particles with an average diameter of 250 nm. C: Calibration curve for 600 nm PS particles using SRS data.

E-O.7

Title: *Raman and Stimulated Raman Scattering characterization of carotenoids and amyloid beta deposits in Alzheimer's Disease brain samples*

Author: Freek Ariese[1], Benjamin Lochocki[2], Liron Zada[1], Loes Ettema[1], Can Keskin[1], Jinke Van der Sluis[1], Robert W. Schmidt[1]

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[2]ARCNL

Abstract:
Alzheimer's disease (AD) is considered the main cause of dementia. It is characterized by extracellular deposits (plaques) of aggregated amyloid peptides. We developed a workflow of label-free and non-invasive techniques to study post mortem AD human brain tissue, as much as possible in its native form [1]. Working with snap-frozen, unstained tissue slices, amyloid plaques are first localized by means of their green autofluorescence. The same area is then Raman mapped at 532 nm excitation. The spectra are preprocessed and clustered using Vertex Component Analysis (VCA) [2]. In all (cored) plaques we found strong Raman signatures around 1150 and 1514 cm^{-1}, indicating the presence of carotenoids (see Figure). With 785 nm excitation (i.e., in absence of pre-resonance enhancement) these compounds were not detected. Reference spectra of six carotenoid compounds were recorded adsorbed on aggregated Abeta42 peptides [3]. Lastly, Stimulated Raman Scattering (SRS) microscopy was performed using two synchronized picosecond NIR laser beams. By repeating the SRS images over a range of wavenumber differences we observed a blueshift of the Amide I peak, typical for a beta-sheet conformation. However, with our NIR-SRS system the relatively low levels of carotenoids could not be detected. Several studies have shown correlations between dietary or blood levels of carotenoids and the development of AD. Carotenoids are powerful antioxidants that can protect against inflammation, but their role in the brain deserves further study.
Most recently, in order to enhance the SRS sensitivity and selectivity for carotenoids, a frequency doubling unit was added to the OPO signal and idler beams. This resulted in a tunable blue output for SRS under resonance conditions, or in a tunable yellow output for pre-resonance SRS (both in combination with the 532-nm beam). Preliminary findings with these visible-SRS approaches in comparison with NIR-SRS will also be discussed.

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Figure captions:
Raman spectra of plaque from AD brain sample (orange) and from surrounding tissue areas (grey). Note the presence of carotenoids (1514 cm-1) and the blueshift of the amide I peak; exc. 532 nm. [3]

Keywords: Pre-resonance Raman, SRS, Alzheimer's

E-O.8
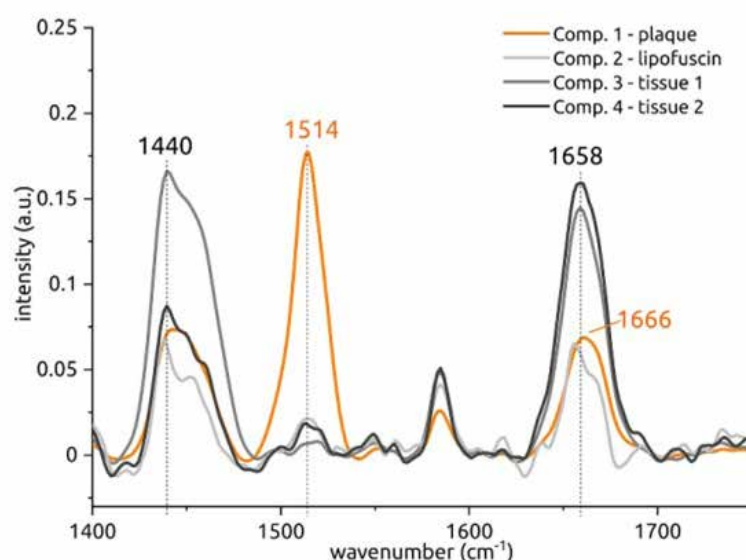
Title: *Glucose and lipid metabolism in endothelium inflammation studied by Raman microscopy*

Author: Aleksandra Borek-Dorosz[1], Anna Pieczara[2], Jagoda Orleańska[3], Krzysztof Brzozowski[1], William Tipping[4], Duncan Graham[4], Malgorzata Baranska[5], Katarzyna Majzner[1]

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Abstract:
Endothelial cell (EC) metabolism depends on the availability of energy substrates. Since the EC is the first line of defence against inflammation in the cardiovascular system and its dysfunction can lead to the development of cardiovascular diseases, it is important to understand how endothelial dysfunction impacts glucose metabolism[1]. Particularly important in these are the correlations between lipid and glucose during inflammation and metabolic reprogramming. Endothelial metabolism depends on the availability of energy substrates and involves four major metabolic pathways: oxidative phosphorylation, glutamine metabolism, fatty acid oxidation, and glycolysis[2]. Glycolysis is the primary energy-generating process in ECs, producing more than 85% of ATP molecules under normal conditions[3,4].
Raman microscopy techniques enter the study of cellular metabolism due to their uniqueness, which lies in the fact that the analysis can be performed non-destructively from single living cells. In this study, glucose and lipid metabolism was studied in human microvascular endothelial cells (HMEC-1) in high glucose (HG) conditions, and additionally in the inflammatory state of the cell. The HG state was induced by the incubation of ECs with a d7-glucose, while the inflammation of ECs was caused by TNF-α pre-treatment. Spontaneous and stimulated Raman scattering microscopies enable one to provide comprehensive information on biochemical changes, which included the alteration in the lipid content and composition. The results show that ECs, due to limited access to glucose, can change their metabolism to fatty acids. Inflammatory state enhances the uptake of glucose. Metabolic changes were also correlated with a strong increase in the ratio of the intensity of lipid/protein bands and an increase in the level of lipid unsaturation. Furthermore, an increase of the cytochrome signal from the mitochondrial area indicates accelerated mitochondrial activity.

References:
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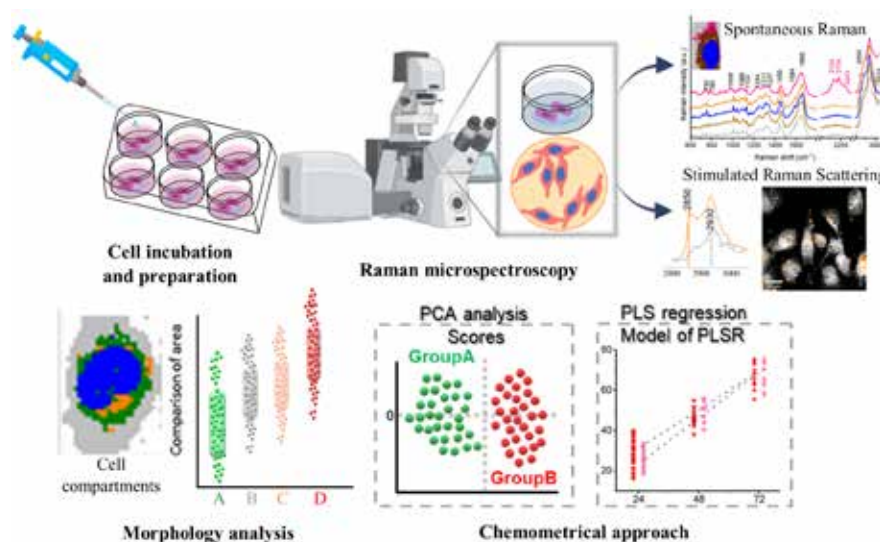
Figure captions:
Schematic representation of the experiment.

Keywords:
high glucose, cellular metabolism, inflammation,

E-O.9

Title: *Stimulated Raman scattering imaging – 3D spatial generation*

Author: Ronja Eriksson[1], Per Gren[1], Mikael Sjödahl[1], Kerstin Ramser[1]

[1]Department of Engineering Sciences and Mathematics, Luleå University of Technology

Abstract:

Stimulated Raman scattering1 (SRS) is an effective imaging method in areas such as biomedicine2. SRS allows for the targeting of a molecular vibration ω_m by the selection of the frequencies of the two illumination beams, a Stokes beam, and a pump beam with the frequencies ω_S and ω_P respectively, so that $\omega_m = \omega_P - \omega_S$. To be able to image samples in 3D without point scanning, knowledge about spatial generation is necessary.

The investigation of spatial generation was based on a comparison between simulations and experiments. The simulations were based on diffraction theory3 and the induced Kerr effect4. The principle of the sample setup is shown in Figure 1 a) where culminated Stokes light passes through the sample and the pump beam is focused into the sample.

In Figure 1 b) the result of the simulated spatial generation of SRS along the propagation axis for five different pump energies can be seen. The vertical black line marks the position of the pump beam focus. The results show a rapid increase in intensity as the light approaches the position of the pump beam focus. The maximum in Stokes intensity is reached after passing the pump beam position. In the experiments, the beam profile of the Stokes light for different interaction lengths, LInt, between the laser beams and sample where investigated. In Figure 1 c) the experimental (Exp) and simulated (Sim) profiles for two interaction lengths can be seen. For LInt = 58.4 mm SRS is generated in small amounts in a larger area, see the light gray and white areas. For LInt = 152.4 mm a bright SRS spot at the center can be seen. The experiments show areas around the center containing an SRS signal.

The results show that most of the SRS signal is generated close to the pump beam focus, with the peak in Stokes intensity reached after the pump beam.

Future work revolves around integrating a speckle imaging system together with the SRS setup to generate 3D species-specific images of samples.

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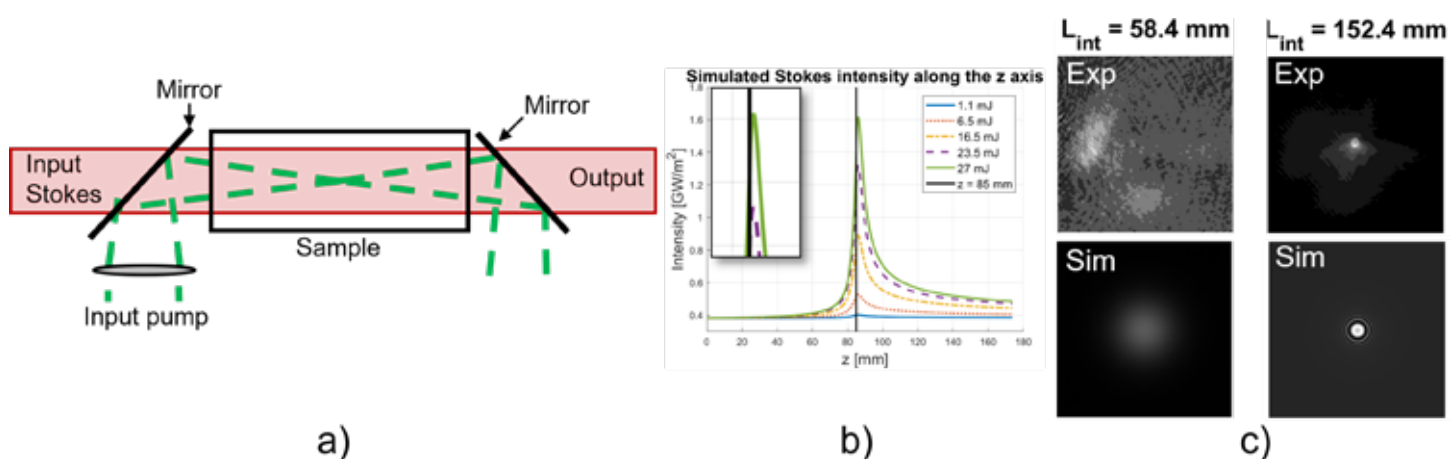
Figure captions:
a) The principle of the SRS imaging, b) Stokes intensity along the propagation direction, c) the Stokes light profile at different interaction lengths.

Keywords: Stimulated Raman scattering, imaging, spatial

E-O.10

Title: *Modified glucose as a probe to track the metabolism in single endothelial cells – observation of the 1602 cm-1 band called "Raman spectroscopic signature of life"*

Author: Anna Pieczara[1], Aleksandra Borek-Dorosz[1], Szymon Buda[1], William Tipping[2], Duncan Graham[2], Robert Pawlowski[3], Jacek Mlynarski[3], Malgorzata Baranska[1]

[1]Jagiellonian University
[2]University of Strathclyde
[3]Polish Academy of Sciences

Abstract:
A relatively new approach to subcellular research is Raman microscopy combined with Raman probes. [1]. ECs play a significant role in the healthy and dysfunctional state, the latter being correlated with a number of lifestyle diseases, particularly cardiovascular disorders. Glucose metabolism and uptake may reflect the physiopathological conditions and cellular activity correlated with energy utilization. [2,3]. To study metabolic changes at the subcellular level, the sensitive and specific Raman probe of glucose analogue, 3-OPG was used, which displays a characteristic and intense Raman band at 2124 cm^{-1}. [4] 3-OPG was applied as a sensor to track both glucose accumulation and metabolism, in live and fixed ECs normal and inflamed. Two spectroscopic techniques were used in the research, i.e. spontaneous and stimulated Raman scattering microscopy. The results indicate that 3-OPG is a sensitive sensor to follow glucose metabolism, which is manifested by the Raman band of 1602 cm^{-1}. The 1602 cm^{-1} band has been called the *"Raman spectroscopic signature of life"* in the cell literature [5], and here we demonstrate that it is attributed to glucose metabolites. Additionally, we have shown that glucose metabolism and its uptake are slowed down in inflammation. We showed that Raman spectroscopy can be classified as a metabolomic method, and its uniqueness lies in the fact that it allows the analysis of the processes occurring in a single living cell. Gaining further knowledge on metabolic changes in the endothelium, especially in pathological conditions, may help in identifying markers of cell dysfunction, and more broadly, in cell phenotyping, a better understanding of the mechanism of disease development and searching for new treatments.

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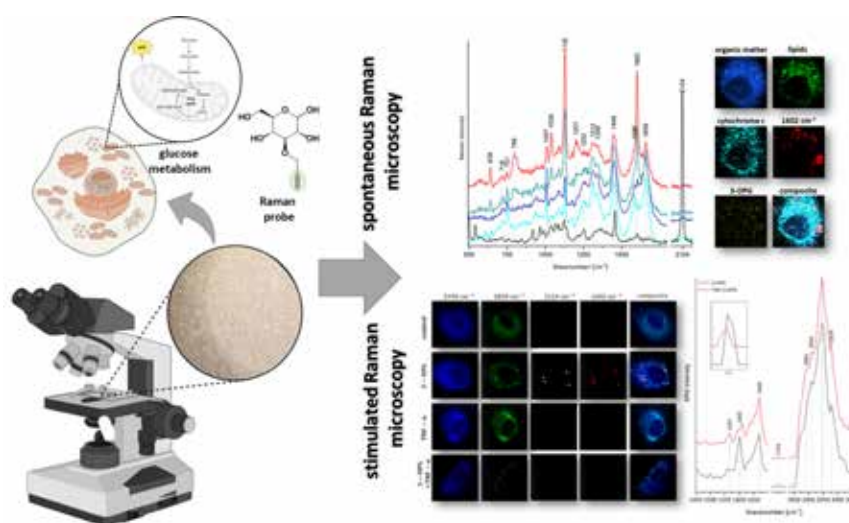
Figure captions:
Schematic representation of the experimental with the microscopic techniques used in the study.

Keywords: 3-OPG glucose, endothelium, spontaneous Raman

E-O.11

Title: *Stimulated Raman scattering (SRS) microscopy to investigate pharmaceutical co-crystal formation*

Author: Oona Auvinen[1], Alba Arbiol[1], Tom Konings[1], Teemu Tomberg[1], Leena Peltonen[1], Clare Strachan[1], Jukka Saarinen[1]

[1]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki

Abstract:
One method to improve the solubility of poorly water-soluble drugs is to use co-crystals [1]. In this study, stimulated Raman scattering (SRS) microscopy, supported with complementary analytical techniques, was used to investigate and image the formation of co-crystals of indomethacin (IND) and nicotinamide (NIC).

Co-crystalline systems were prepared by liquid-assisted milling. Samples at different time points of milling were analyzed with X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy, as well as SRS. SRS analyses were performed with an in-house built SRS microscope with fast spectral focusing.

Co-crystals were formed as proven by XRPD and DSC after 16 min. Co-crystals showed changes in the FTIR spectra when compared to physical mixtures of the two components, including a shift of C=O stretching to lower wavenumbers and C=C stretching to higher wavenumbers, indicating intermolecular interactions between the two components.

SRS microscopy was used to obtain spatially-resolved spectroscopic insights into the co-crystallization process. It was seen that early in the milling process (time point 4 min) the particle morphology changed from prismatic to needle-like particles (Figure 1 A). The SRS spectra also showed heterogeneity between different spatial regions (Figure 1 B). After 28 min of milling, the particles were again more prismatic and the SRS spectra between different regions were more similar to each other. SRS microscopy demonstrated its potential as a solid-state specific imaging tool to study co-crystallization. In this case, the co-crystallization process consisted of different stages, including morphological changes from prismatic crystals to needle-like crystals and back. In future, SRS microscopy will be used to study the co-crystal formation in the earlier stages of milling and potentially to image co-crystals during dissolution as a part of drug release studies.

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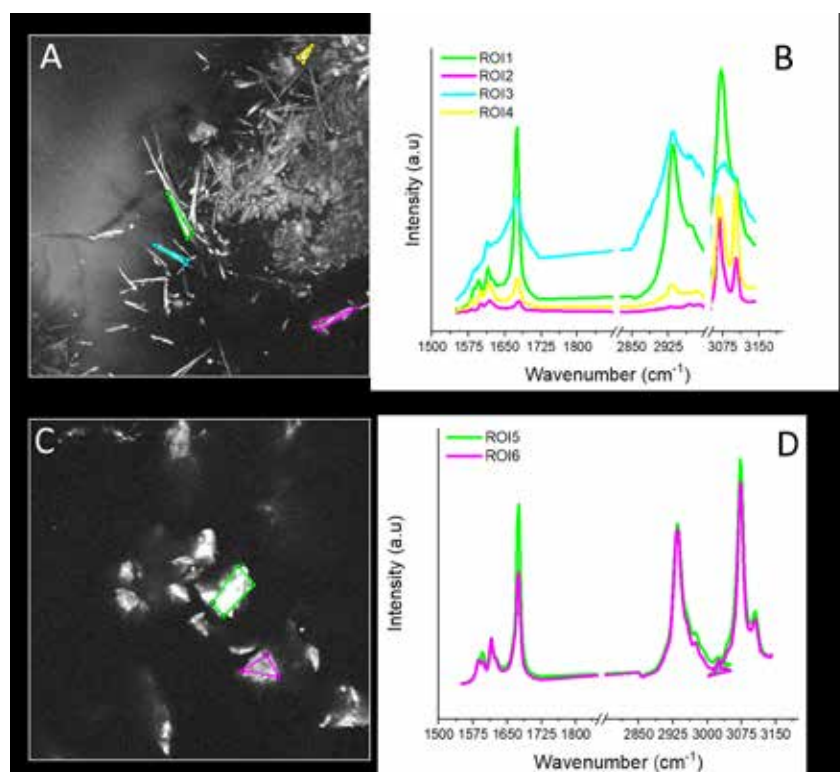
Figure captions:
Figure 1. SRS images at 3066 cm-1 (A) and at 3069 cm-1 (C) and spectra from different regions indicated with polygons (B and D) at time point of 4 min (A and B) and 28 min (C and D) of co-crystals.

Keywords: Stimulated Raman scattering, SRS, co-crystals

E-O.12

Title: *Investigating Viscoelastic Induced Nature at Air-Aqueous Interface by Nonlinear Laser Vibrational Spectroscopy*

Author: Sarabjeet Kaur[1], Kailash Chandra Jena[1]

[1]Indian Institute of Technology Ropar

Abstract:

Polymer surface viscoelasticity on multivalent-ions incorporation have drawn a lot of attention along with Weissenberg's effect appearance/disappearance in fluid dynamic processes, which directly influence rheology control field domain.[1, 2] In order to determine the existence and correlation between macroscopic and microscopic viscoelastic properties by gaining molecular insight at interface, we combined sum frequency generation (SFG) vibrational spectroscopy with viscosity and surface tension measurement.

In our present study, we majorly have employed sum frequency generation vibrational spectroscopy (SFG) to elucidate the Weissenberg effect at the air-aqueous interface. [3, 4] SFG is a potent second-order nonlinear optical technique that due to its extreme surface sensitivity detects interfacial active chemical processes. It has the potential to examine the molecular structure and composition of molecules present at light-accessible surfaces. We investigated the OH-stretch region from 3000 to 3800 cm^{-1} in order to monitor the interfacial water structure. The structural conformation of the alkyl chains of the polymer illustrates the CH-stretch region from 2800-3000 cm^{-1}. According to our findings, polymer-metal ion interaction influences molecular interfacial configuration. At the conference, we will give a detailed analysis of our studies on investigating the interfacial structure that could explain the appearance or disappearance of Weissenberg phenomenon.

References:

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4. Kaur H, Verma M, Kaur S, Rana B, Singh N, and Jena K C, Langmuir 38 (2022), 13456-13468.

Keywords: Nonlinear Laser Vibrational Spectroscopy, Viscoelasticity

Title: *Unraveling the sign of excited-state molecular displacements via broadband impulsive Raman spectroscopy*

Author: Giovanni Batignani[1], Emanuele Mai[1], Giuseppe Fumero[2], Shaul Mukamel[3], Tullio Scopigno[4]

[1]Physics Department, Sapienza University of Rome, Rome, Italy; Italian Institute of Technology, Center for Life Nano Science @Sapienza, Rome, Italy
[2]Physics Department, Sapienza University of Rome, Rome, Italy
[3]Department of Chemistry, University of California, Irvine, CA, USA
[4]Physics Department, Sapienza University of Rome, Rome, Italy; Italian Institute of Technology, Center for Life Nano Science @Sapienza, Rome, Italy; Italian Institute of Technology, Graphene Labs, Genoa, Italy

Abstract:
Ultrafast photoreactions are governed by the multidimensional excited state potential energy surface (PES), which describes how the molecular potential varies with the nuclear coordinates. Nature has tailored electronically excited PESs, in which the molecular geometry is specifically modified from the ground state (GS) equilibrium configuration to efficiently convert the absorbed light energy into nuclear rearrangements, driving the system photochemistry and determining the biological functions by bond length modifications, torsional re-orientations, formation or rupture of chemical bonds. This can be rationalized by the displacements between PESs, i.e. the positional shift between the excited-state (ES) and the GS minima along specific GS eigenvectors. Critically, their sign determines if these changes move closer or away two functional groups, ruling ES properties and dynamics. Their knowledge is thus of utmost importance.

Such displacements are encoded in the Franck-Condon overlap integrals, which in turn determine absorption spectra and complex-valued Raman excitation profiles (REP). Conventional spectroscopic approaches probe transition amplitudes [1-2], only accessing the REP's absolute value, and hence are not effective for determining the sign of ES displacements. Herein we introduce an experimental technique [3], based on the detection of the broadband impulsive Raman response at selected temporal delays and probe chirps, to directly measure complex REPs along desired normal modes. The key to achieve this task is in the linear dependence of the signal on the Frank-Condon overlaps, brought about by resonant probe and off-resonant pump pulses, which critically enables time domain sensitivity to the phase of the stimulated vibrational coherences. Our results, demonstrated on Rhodamine B [3], provide the tool to unambiguously determine the sign of ES molecular displacements, ultimately revealing the first steps of photoreaction processes [3-4].

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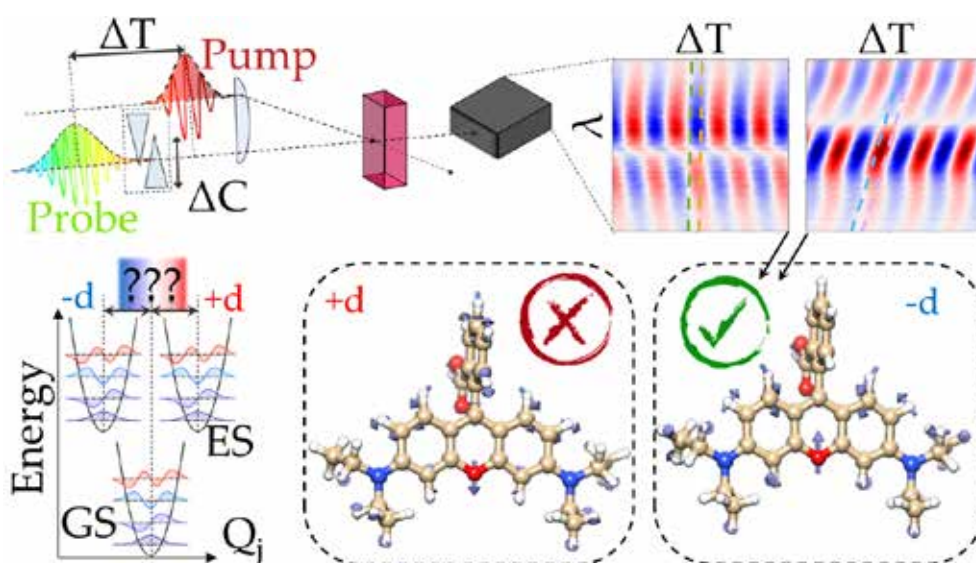
Figure captions:
Sketch of the non-degenerate pump-probe experimental scheme which, by measuring impulsive Raman excitations, accesses the sign of Rhodamine B excited-state displacements along its normal modes.

Keywords: Impulsive Vibrational Spectroscopy, molecular displacement, Raman

E-O.14

Title: *Charge Transfer Across Hydrophobic Interfaces*

Author: Saranya Pullanchery[1], Sergey Kulik[1], Benjamin Rehl[1], Ali Hassanali[2], Sylvie Roke[1]

[1]Laboratory for Fundamental BioPhotonics, Institute of Bioengineering (IBI), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL)
[2]The Abdus Salam International Centre for Theoretical Physics

Abstract:
Hydrophobic surfaces acquire a negative charge when in contact with water. The origin of this negative charge has been debated for over a century. Using vibrational sum frequency scattering spectroscopy (SFS), an inherently interface-specific technique, we investigated the interfacial molecular structure of a hydrophobic/water interface. We used 200-nanometer hexadecane oil droplets in water as a model system and measured the vibrational spectra of water and oil molecules at the interface using polarimetric SFS. The SFS spectrum of interfacial water revealed that water molecules form an overall stronger hydrogen bonding network at the oil droplet surface compared to a planar air/water interface. The role of intramolecular and intermolecular vibrational coupling on the interfacial water spectra was determined using isotopic dilution. Interestingly, an unusually broad and red-shifted (compared to the air/water interface) spectral distribution of interfacial water molecules that were not hydrogen bonded to other water molecules was observed at the oil droplet surface. The vibrational frequency of interfacial oil molecules that interact with water exhibited a blue shift compared to the oil molecules in a completely hydrophobic medium. Oil and water frequency shifts revealed the occurrence of a charge transfer from interfacial water to oil. Molecular dynamics simulations demonstrated that these unexpected strong charge-transfer interactions involved the formation of interfacial C–H···O hydrogen bonds.

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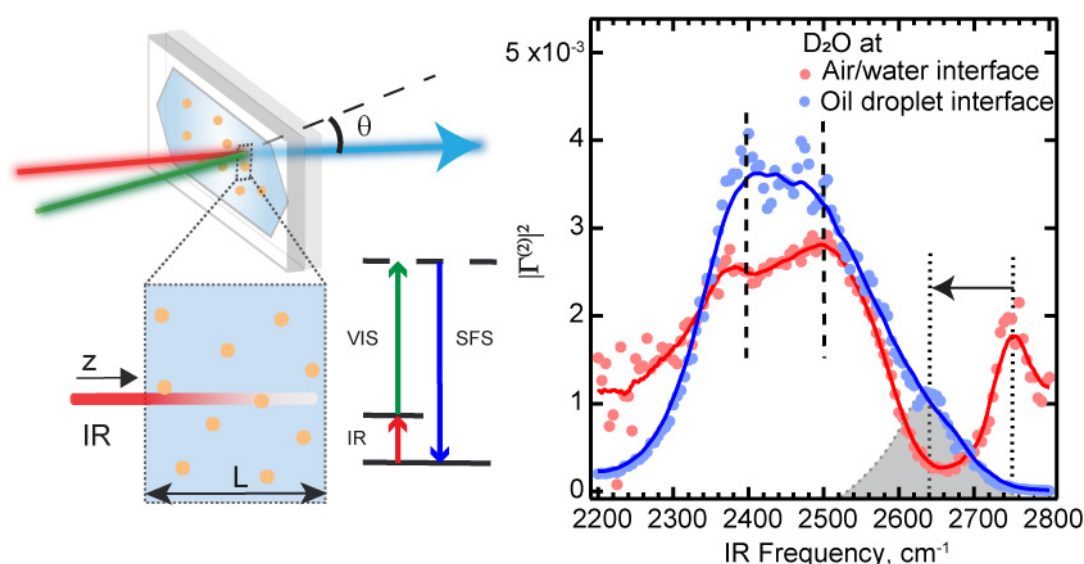
Figure captions:
Vibrational sum frequency scattering spectroscopy of hydrophobic droplets in water

Keywords: Hydrophobicity, SFS spectroscopy, interface

F-I.1

Title: *Comparison of ATR-FTIR and O-PTIR techniques at ISMI beamline for the characterisation of biological and cultural heritage samples*

Author: Krzysztof Banas[1], Agnieszka Banas[1], Mark Breese[1]

[1]Singapore Synchrotron Light Source

Abstract:

ISMI (Infrared Spectro Microscopy) beamline is one of the end-stations available at the Singapore Synchrotron Light Source (SSLS). The use of synchrotron radiation as a source of infrared radiation brings additional tangible benefits. However diffraction-limited spatial resolution cannot be overcome with any traditional approach used in (micro)-spectroscopy. The Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) technique is a widely used method for the characterization of various materials, particularly in chemistry, biology, and materials science. Recently, a novel method known as Optical Photothermal Infrared (O-PTIR) spectroscopy has been introduced, which offers several advantages over the classic ATR-FTIR. In this contribution, we will compare these two methods for characterising biological and cultural heritage samples.

One of the main advantages of the O-PTIR method is wavenumber-independent spatial resolution (around 500 nm). Another benefit of the O-PTIR microscopy lies in its ability to measure the samples in a contactless mode, without any sample preparation or modification. This is particularly advantageous for materials that are soft or brittle and may be damaged by direct contact with ATR crystal. However, there are also some disadvantages associated with the O-PTIR method. It requires pre-experiment system optimisation and finding the correct settings for the power of IR and green lasers. Another disadvantage may be the restricted spectral range available for the experiments that is limited by the tunability of IR pulsed laser. The most often used is the fingerprint region (1900-800 cm^{-1}) laser source.

In conclusion, while both the ATR-FTIR and O-PTIR methods are useful for the characterization of biological and cultural heritage materials, the O-PTIR method offers several advantages over the classic ATR-FTIR method, particularly in terms of non-destructiveness and is substantially better than traditional ATR-FTIR spatial resolution.

Keywords: O-PTIR, ATR-FTIR, cultural heritage

F-I.2

Title: *Emerging Trend in AFM-IR: Surface-sensitive mode to probe sample's very surface*

Author: Ariane Deniset-Besseau[1], Jérémie Mathurin[2], Alexandre Dazzi[1]

[1]Institut de Chimie-Physique, Université Paris-Saclay
[2]Institut de Chimie-Physique, CNRS

Abstract:
In the last decade, the AFM-IR technique has become step by step a reference technique for infrared analysis at the nanoscale [1]. This technique combines the high spatial resolution of an AFM (Atomic Force Microscope) with the vibrational analysis capabilities of infrared spectroscopy. The field of application is extremely vast and covers fields as diverse as molecular biology, polymer science, microbiology, medicine, geology, ancient materials and astrochemistry [2,3]. Currently, the AFM-IR measurements are implemented with 3 different AFM modes (contact, peak force, tapping [4,5])) and allow the analysis of many types of samples in terms of hardness and geometry. Recently, our team have developed and patented a new acquisition mode called 'sensitive surface mode' [5]. It uses the AFM in its classical contact mode to probe the first tens of nanometers of a sample surface instead of the usual few micrometres. We highly hope it will help the AFMIR analysis of samples with nanometric active coating as well as surfaces with wanted or unwanted deposits (contaminant, protective layer, active layers or oxidative products). During the oral presentation, the general theoretical background, as well as some experimental constraints, will be discussed and results obtained on two different samples will be presented and commented on.

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Keywords: IR nanospectroscopy, surface analysis

Title: *Through the looking glass: Raman imaging through the bottle*

Author: Kishan Dholakia[1]

[1]University of Adelaide

Abstract:

To determine the composition of a sealed container, without opening the contents, is of great interest from forensics to product quality control especially the food and drinks industry. The approach of inverse spatially offset Raman spectroscopy (ISORS) illuminates the smapel under evaluation with an annular beam of light and collects Raman scattering from the center of the ring, thereby retrieving the chemical signature of the contents while suppressing signal from the container. Here we explore in detail the relative benefits of a newly developed version of ISORS, which we term focus-matched ISORS [1,2].

In our geometry we too illuminate the sample with an annular beam, and collects scattered light through the dark center of this beam, to selectively suppress signal from the glass. However, our route focusses the annular beam to take advantage of the Fourier relationship between the annular beam formed by an axicon and a Bessel beam, creating an intense focus inside the sample of interest. Similarly to conventional Raman spectroscopy, the Raman scattered light is collected from this excitation focal point to maximize the total Raman signal collected.

The advantages of the approach and the potential for using machine learning are described. In particular we explored machine learning methods without manual preprocessing for application to the brand identification of a variety of whisky. Methanol and ethanol concentrations, and identification performance was evaluated.

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Keywords: Raman, Bessel, machine learning

F-I.4

Title: *New Approaches for Raman Spectroscopic Imaging and High-Throughput Monitoring in Biomedical Applications*

Author: Torsten Frosch[1]

[1]Technical University Darmstadt

Abstract:
Raman spectroscopy provides excellent molecular selectivity for biomedical applications. The sensitivity can be tremendously enhanced with help of elaborate hollow fibers, which guide the light with minimal losses [1]. Besides the chemical information, the spatial distribution of analytes is often of major interest. Conventional Raman imaging relies on mapping (slow) or tunable filters (pure spectral resolution). We developed approaches for wide-field Raman spectroscopic imaging [2-5] which facilitate a good spectral and spatial resolution and allow simultaneous acquisition of all spectra of an area. The new device for hyperspectral Raman imaging was used for analyzing the spatial heterogeneity of biomedical and pharmaceutical samples, e.g., hemozoin in malaria infected red blood cells [2], gas analytes [3], and the distribution of active ingredients in pharmaceutical formulations [4].
Recently, we designed an approach for highly parallelized Raman difference spectroscopy, to investigate subtle changes in the Raman spectra due to biochemically important molecular interactions, which opens new avenues to perform drug binding assays and to monitor highly parallelized biochemical reactions [5, 6].

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[6] Sebastian Wolf, Robert Domes, Andreas Merian, Christian Domes, and Torsten Frosch; Analytical Chemistry (2022); 94, 10346–10354

Keywords: Raman hyperspectral imaging, high throughput

F-I.5

Title: *New Perspectives for Mid-IR Spectroscopy of Liquids as Enabled by Quantum Cascade Lasers*

Author: Bernhard Lendl[1], Alicja Dabrowska[1], Daniel-ralph Hermann[1], Giovanna Ricchiuti[1], Gustavo Lukasievicz[1], Georg Ramer[1]

[1]TU Wien

Abstract:
Mid-IR Quantum Cascade Laser (QCL) provide coherent, polarized radiation with spectral power densities in the tens to hundred mW range. These semiconductor based sources are small in size and can be operated in pulsed as well as CW mode at room temperature with ample possibilities for realizing frequency and intensity modulation schemes. These properties are very different from those of thermal sources, thus enabling the development of new sensing schemes that go well beyond classical absorption spectroscopy based on Beer´s law.

This short presentation will provide an overview on the new perspectives for mid-IR spectroscopy enabled by mid-IR QCLs. In particular mid-IR dispersion spectroscopy employing a Mach Zehnder Interferometer (MZI) will be shown and it´s advantageous features as opposed to absorption spectroscopy discussed. Furthermore, advances in photothermal spectroscopy for the analysis of solutes in liquids will be shown. Here the focus will be given on novel concepts such as photothermal lens and photothermal mirror spectroscopy. Finally recent advances in QCL based Vibrational Circular Dichroism (VCD) spectroscopy will be reported.

Keywords: QCL, Dispersion Spectrscopy, Photothermal Spectroscopy

F-I.6

Title: *Stimulated Raman scattering and resonance Raman spectroscopy combined with holography, interferometry and video imaging*

Author: Kerstin Ramser[1]

[1]Department of Engineering Sciences and Mathematics/Luleå University of Technology

Abstract:
In our lab, we combine stimulated Raman scattering (SRS) and resonance Raman spectroscopy with holography, interferometry, or image sequences to achieve direct imaging. We aim to achieve fast, species-specific Raman imaging without the need to scan the sample. In this talk, I will first present experiments where SRS was combined with holography. The beam of an Nd:YAG laser was split into three parts, an one was frequency tripled and guided to an OPO to be tuned to the desired Stokes wavelength, the second and third beam were frequency doubled and either sent to a Michelson interferometer to create a spatial striped pattern, or to be used as a reference beam, respectively. The reference beam was steered directly onto a cooled PCO camera, whilst the other beams were guided through the sample. Sequential imaging, fast Fourier transform, and calculations of the phase shift enabled the creation of holographic images of the object and the phase shifts. These were used to determine gas mixtures in a vessel [1] and to calculate the number of methane molecules in a burning flame [2]. In the second part of my talk, the investigation of vasoconstriction of pulmonary arterial smooth muscle cells (PASMCs) will be presented. The mitochondrial action of PASMCs was surveilled by online resonance Raman spectroscopy, while the vasoconstriction was captured by a video film [3]. The measurements showed that the oxygenation state of cytochrome c could be followed reversibly when going between normoxic to hypoxic oxygen content of a flowing buffer solution, while the vasoconstriction continued throughout the process. Finally, our future plans and development of an imaging technique termed InFeRa will be presented. Here we intend to combine interferometry with SRS using speckled laser beams to get two-, and three-dimensional imaging of semitransparent objects.

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Keywords: SRS, holography, interferometry, video

F-O.1

Title: *SR-FTIR Imaging of Live Cells Using a Novel Demountable Flow System to Study Phospholipidosis*

Author: <u>Ohood Alshareef</u>[1], K.L Andrew Chan[1], Ben Forbes[1], Mohamed Alhnan[1], Gianfelice Cinque[2]

[1]Institute of Pharmaceutical Sciences, King's College London
[2]Diamond Light Source, Harwell Science and Innovation Campus

Abstract:
FTIR spectroscopic imaging is a well-established technique for studying biochemical changes of proteins, lipids, and DNA in tissues and cells without requiring labelling for molecular tracking. These research were initially dominated by fixed samples studying macroscale but with the integration of advanced instrumental development and increasing complexity in cell biology research, there has been an interest to shift toward live samples at the micro- or even nano-scale. Measuring live cells has many benefits including the ability to study real-time responses to a stimulus that cannot be captured in-vitro.
In this work, we demonstrate a custom-built flow system that can obtain high-quality FTIR images with an increased spatial resolution of 2.25-fold coupled with the IR Synchrotron radiation. The demountable system was made from two ZnS hemispheres sandwiching the sample providing a path length of 6-10 µm to obtain a subcellular level with 1 µm pixel resolution. Live cells were incubated on the ZnS hemisphere and then assembled into the flow system just before the experiment.
The combined system allows in situ live cell imaging for over a duration of 24 h with high signal-to-noise ratio to resolve subcellular features. We have demonstrated the system by the study of phospholipidosis induce drug, amiodarone, on single living cells, which has shown spectral changes in lipid bands, consistent with our previous work [1] that shows the capability of inducing cell response and measuring biochemical changes simultaneously.
The current set-up is a significant improvement from our static approach [2], which can widen the range of possible experiments in the study of drug–cell interactions

References:
[1] K.L.A. Chan, I. Lekkas, M.D. Frogley, G. Cinque, A. Altharawi, G. Bello, L.A. Dailey, Synchrotron Photothermal Infrared Nanospectroscopy of Drug-Induced Phospholipidosis in Macrophages, (2020).
[2] K.L.A. Chan, P.L.V. Fale, A. Atharawi, K. Wehbe, G. Cinque, Subcellular mapping of living cells via synchrotron microFTIR and ZnS hemispheres, Anal. Bioanal. Chem. 410 (2018) 6477–6487.
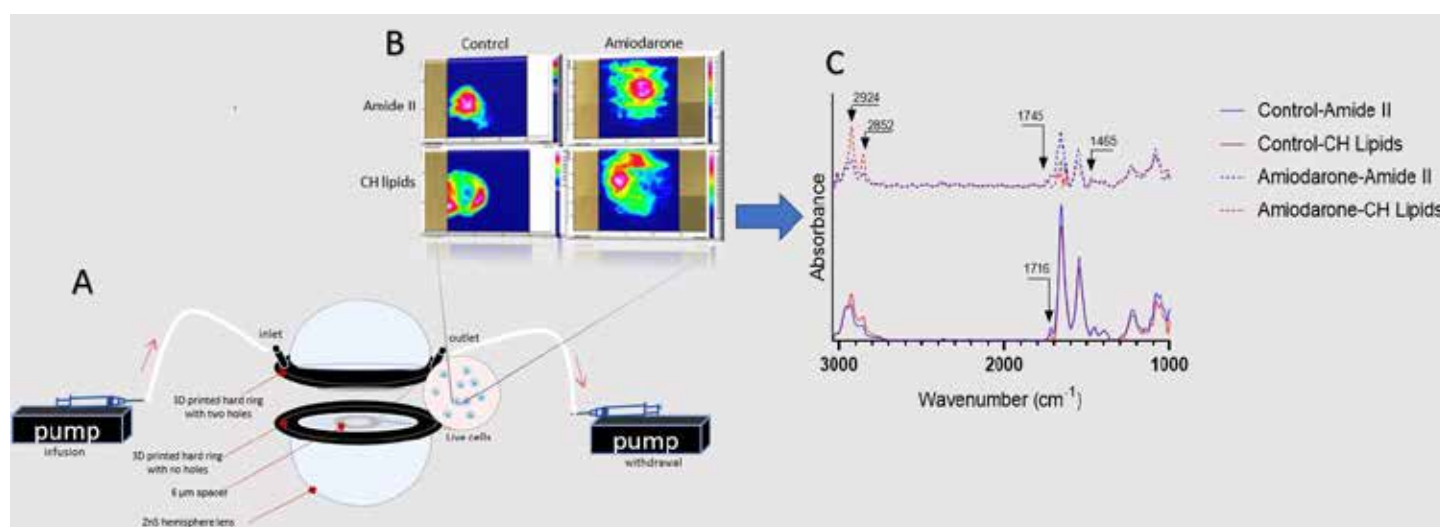
Figure captions:
A- Flow system diagram, B- Chemical image of amide II & CH integration maps of control & amiodarone treated cells, C- Averaged absorbance spectra of amide II & CH region for control & amiodarone

Keywords: Flow system, SR-FTIR, ZnS hemispheres

F-O.2

Title: *Infrared spectroscopy at the user facility ELI Beamlines*

Author: <u>Nils Lenngren</u>[1], Mateusz Rebarz[1], Jakob Andreasson[1], Miroslav Kloz[1]

[1]The Extreme Light Infrastructure ERIC

Abstract:

The infrared (IR) spectroscopy setup at ELI Beamlines is getting ready to receive its first users. ELI Beamlines is a user facility based on high-energy lasers in Dolní Břežany outside Prague. As a user facility, we support visiting researchers with their experiments and data analysis, at levels adapted to user requests and needs.

The core of our infrared spectroscopy facility is the time-resolved setup, based on [1]. IR pump–IR probe, visible pump–IR probe and 2DIR operation modes will be available. Mid-IR pump and probe light is provided by a Topas Twins optical parametric amplifier (OPA)/difference frequency generator system (Light Conversion), with two outputs independently tuneable from 2 to 12 μm (5000–833 cm^{-1}). Ultraviolet, visible and near-IR pump light comes from a Topas Prime OPA system (Light Conversion), tuneable from 250 to 2500 nm. Pulse pairs for 2DIR experiments will be created by a pulse shaper (PhaseTech). Two delay lines create pump–probe time intervals up to a few nanoseconds, with visible pump delays up to microseconds planned. For fragile and otherwise demanding samples, we have or develop a large variety of sample delivery systems, such as flow cells, regular cuvettes, closed capillaries and a jet with sub-millimetre path length [2], and sample actuators moving the samples during measurements. We can also measure solid-state samples such as films. Sample temperatures from 5.5 to 350 K will be possible with a closed-cycle helium cryostat. To minimize absorption of the selected wavelengths, we can purge the setup with various gasses.

The setup is supported by steady-state IR, UV–visible and fluorescence spectrometers and complemented by other time-resolved spectroscopy methods such as UV–visible transient absorption and ellipsometry, femtosecond stimulated Raman spectroscopy, as well as state-of-the-art laser-driven femtosecond vacuum ultraviolet and X-ray sources for diffraction, imaging and spectroscopy measurements.

References:

[1] S.-H. Shim, M. T. Zanni, How to turn your pump–probe instrument into a multidimensional spectrometer: 2D IR and Vis spectroscopies via pulse shaping, Phys. Chem. Chem. Phys. 11 (2009) 748–761.

[2] A. Picchiotti, V. I. Prokhorenko, R. J. D. Miller, A closed-loop pump-driven wire-guided flow jet for ultrafast spectroscopy of liquid samples, Rev. Sci. Instrum. 86 (2015) 093105.

Keywords: time-resolved infrared spectroscopy, user facility

F-O.3

Title: *Current status of Chemical Infrared Imaging (CIRI / SOLAIR) beamline in Solaris*

Author: Maciej Roman[1], Danuta Liberda[1], Paulina Koziol[1], Karolina Kosowska[1], Tomasz P. Wrobel[1]

[1]SOLARIS National Synchrotron Radiation Centre, Jagiellonian University

Abstract:
The Chemical Infrared Imaging (CIRI) /Solaris Advanced InfraRed beamline (SOLAIR) is currently under construction. The large radiation extraction from a bending magnet will allow the collection of a very wide wavelength range (0.4 – 500 µm), covering the near (NIR), mid (MIR), and far (FIR) infrared spectral range. The extraction of the infrared range of synchrotron radiation will be achieved using a flat and slotted mirror (M1), which will be located inside the dipole chamber located at the bending magnet in the storage ring. Then the M2-M6 mirror system, which is designed in-house, will direct the beam out of the ring wall.

The presentation will showcase the current status of the project along with the expected IR beam parameters. It will also highlight microscopic techniques (FT-IR, s-SNOM, and O-PTIR) planned to be used at the beamline with potential applications (with special emphasis on biomedical samples).

Keywords: IR imaging, synchrotron, biomedical samples

F-O.4

Title: *Dxcover® Platform: The next generation of ATR-FTIR spectroscopy*

Author: Holly Butler[1], Loren Christie[1], Matthew J. Baker[2]

[1]Dxcover Ltd
[2]School of Medicine, University of Central Lancashire

Abstract:
ATR-FTIR spectroscopy is a powerful technique without the need for extensive sample preparation or use of reagents. However, the ATR method is reliant upon an internal reflection element (IRE), which is often a single fixed point of analysis that requires mandatory cleaning steps to avoid cross contamination. We present an alternative ATR-FTIR platform that allows improved sample throughput, and batch processing without affecting spectral quality[1].

The Dxcover® Sample Slides are made from microfabricated silicon wafers and replace the single IRE with four sampling areas for one background measurement and three sample measurements. The format means that multiple Sample Slides can be prepared in batches prior to sample analysis which can improve sample throughput. Slides can subsequently be stored after analysis for applications where archiving may be beneficial. The Dxcover Autosampler can automate Sample Slide analysis, indexing the slide across the infrared beam without user interaction.

High quality spectra can be obtained from a range of biofluids, such as blood products, without loss of information in the fingerprint region. It has been shown that analysis of patient blood serum can be reduced from ˜1 hour to less than ˜15 minutes, based on triplicate measurements of 9 spectra per patient [2,3]. This has enabled translation of the technology to the clinic as part of large scale prospective clinical studies [4]. As well as applications in biodiagnostics, the system can be applied to process analytics where it has been shown to be able to monitor culture and product for quality and infection risk. This high-throughput ATR-FTIR platform allows faster sample analysis that is more efficient for researchers and is better suited to industrial or clinical applications.

References:
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[2] J. Hands, et al. Brain tumour differentiation: rapid stratified serum diagnostics via attenuated total reflection Fourier-transform infrared spectroscopy, Neurooncol., 127: 463-472 (2016)
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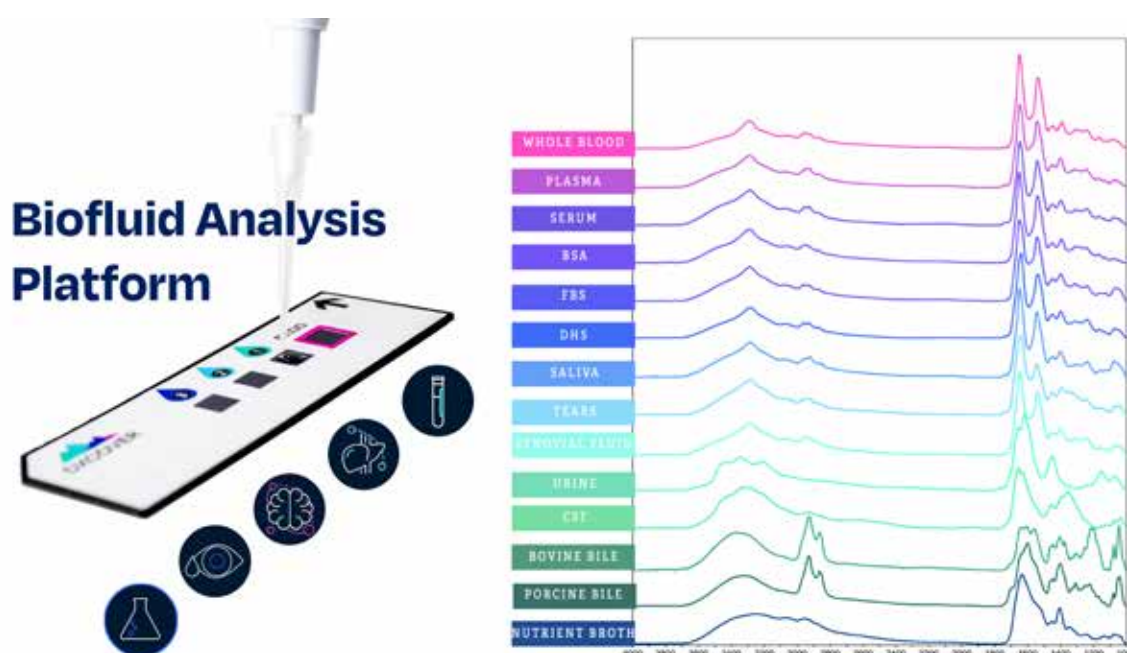
Figure captions:
Characterisation of various biological fluids using the Dxcover Infrared Platform.

Keywords: ATR-FTIR, Diagnostics, Biofluids, IRE

F-O.5

Title: *Infrared nanoimaging and nanospectroscopy – emerging tools for physical and (bio)chemical nanoanalytics*

Author: Adrian Cernescu[1]

[1]attocube systems AG

Abstract:
Nano-FTIR has become a key technology to study the chemical composition of organic and inorganic materials at the nanoscale. This AFM-based technology exploits the strong confinement of light at the end of a sharp, metallic AFM tip to generate a nanoscale optical hotspot at the sample surface. The use of broadband or tunable IR sources combined with a Michelson interferometer enables optical spectroscopy with <10 nanometer precision, as well as nanoscale mapping of the sample chemical composition.
With tremendous sensitivity compared to classical vibrational spectroscopy techniques, nano-FTIR allows chemical identification of any nanomaterial based on their spectroscopic fingerprint. It further reveals encoded information such as secondary structure in proteins or local chain orientation within highly oriented polymer samples. Various applications of nanoFTIR for nanomaterials characterization will be presented.

Keywords: nanoFTIR, nanomaterials, bio-chemical, nanoimaging, nanospectroscopy

F-O.6

Title: *Most recent advances of QCL-IR microspectroscopy*

Author: Matthias Godejohann[1]

[1]MG Optical Solutions

Abstract:

Mid-infrared microscopes using quantum cascade lasers (QCL-IR) as a light source were developed simultaneously in several labs [1, 2, 3] and are commercially available since 2014. The spectral and spatial power density of the laser illumination accelerates data acquisition by orders of magnitude compared to Raman- or even FTIR-systems. Improvement factors up to 160x shorter acquisition times were reported.[4] This is expected to lead soon to solutions for clinical or environmental routines, which could not be addressed in the past, because the data acquisition times exceeded routinely available time slots.

The spectral results are very similar to commonly used techniques and allow the use of former data sets to make classifiers as well appropriate for QCL-IR-micro spectroscopy results. Under certain circumstances the detection of particles, which are slightly smaller than the diffraction limit at the wavelength in use, e.g., a PMMA particle in fibrocytes.[5] might be detected. Especially life science and environmental applications profit from the feature being able to measure even liquids in transmission with longer absorption lengths up to a 25 μm. The restrictions in image and spectrum quality of being forced to use attenuated total reflection cells (ATR-cells) can be avoided.

References:

4 Claus Kuepper, Angela Kallenbach-Tieltges, Hendrik Juette, Andrea Tannapfel, Frederik Grosserueschkamp, Klaus Gerwert, Fast Quantum Cascade Laser-Based Infrared Microscopy for label-Free and Automated Cancer Classification in Tissue Sections, Scientific Reports, 8:7717, (2018)

5 Alvaro Barroso, Steffi Ketelhut, Matthias Godejohann, Björn Kemper, Jürgen Schnekenburger, Optical imaging methods for label free detection of microplastics in cells, tissues and environmental organisms, SPIE Photonics West BIOS 2020 Conference BO500 Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVIII
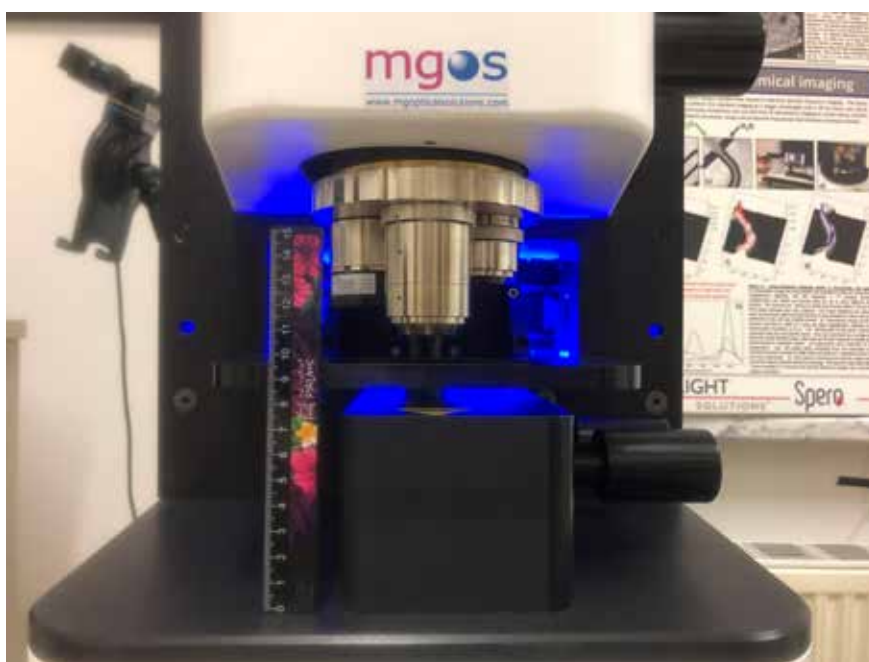
Figure captions:
Figure 1: Probe Chamber of SPERO QT 340-microscope from DRS Daylight Solutions

Keywords: microscopy, MIDIR, QCL-IR, microspectroscopy, Chemical

F-O.7

Title: *Widefield Super-Resolution IR Imaging with Fluorescence Enhanced Photothermal Infrared*

Author: Miriam Unger[1], Mustafa Kansiz[1]

[1]Photothermal Spectroscopy Corp.

Abstract:

Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman and fluorescence imaging, in non-contact mode with high sensitivity without any dispersive scattering artefacts. O-PTIR has generated significant research interest and publications, however there still exists a demand for rapid, high sensitivity and high resolution widefield IR imaging. To this end, we have developed a novel widefield super-resolution IR imaging approach that utilizes the fluorescent signal directly for IR signal extraction. As the fluorescent signal is captured with a 2D fluorescence camera, this generates, simultaneously, widefield IR imaging as well as widefield fluorescence images. We have termed this - Fluorescence-Enhanced Photothermal Infrared (FE-PTIR) spectroscopy.

The key enabling factor here, is that when the wavelength of the IR pulses is tuned to a molecular vibration of fluorescently labeled molecules, the absorbed heat causes a modulation in the amount of fluorescent light emitted from the fluorophores and it's surrounds. Coupled with the parallel data acquisition via the 2D (megapixel) visible fluorescence camera, using a standard glass objective of 50x, 0.8NA, single field of view for IR of 70x70um with 200nm pixels are possible. Compatibility with other standard visible glass objectives such like those with higher NA, or even immersion objectives opens up further possibilities for widefield super-resolution IR imaging.

FE-PTIR thus allows the IR spectroscopic analysis of specifically labeled regions of biological cells and tissue, for example to study conformational stages of a specifically labeled class of target proteins. FE-PTIR can enable the study protein misfolding associated with neurodegenerative diseases. Various examples from these applications will be provided.

Keywords: IR imaging, Fluorescence imaging, super-resolution

F-O.8

Title: *Mode Optimized Tip-Enhanced Raman Scattering*

Author: Tao Chen[1], Wei Wang[1], <u>Volker Deckert</u>[1]

[1]Friedrich-Schiller University

Abstract:
Tip-enhanced Raman scattering (TERS) utilizes the strong and extremely confined plasmonic enhancement at the apex of a metallized tip. The optimum shape of the tip is a matter of discussion since the advent of TERS and recently several papers indicate that a grainy structure is advantageous [1,2] and that also the atomic structure of the apex has an influence [3-5]. Less well investigated is the effect of the focus itself on the enhancement. However, if minor nanoscale changes can dramatically affect the TERS signal, those should in turn also have a strong effect on the optimal coupling of the electric field and a particular tip. Efficient coupling is realized mostly by orienting the electric field parallel to the tip axis. For an epi illumination, as used in our setup, this either result in optimized illumination if the tip is slightly laterally offset with respect to the focus center for a linearly polarized beam, or for a radially polarized beam if the tip is directly in the center. Obviously, this does not account for specific coupling efficiencies. To investigate this in more detail we initially spatially modulate the phase of the incoming beam to generate an optimized focus. The field contribution orthogonal to the sample surface was tested via scattering scanning near-field microscopy or photo-induced force microscopy, both providing comparable results and indicating a radially polarization. This optimization already yielded an enhancement of the TERS signal by up to a factor of 10.
Interestingly, a further optimization could be achieved when a Raman signal of the sample was used as feedback. This indicates that a simple optimization with respect to the tips silicon band or the excitation will not necessarily lead to an optimal TERS illumination.
In summary we will provide a method to optimize TERS via mode optimization of the illumination laser and discuss the consequences of the results with respect of new potential applications of TERS.

References:
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2. S. Trautmann et al., Plasmon response evaluation based on image-derived arbitrary nano structures, Nanoscale 2018, 10, 9830–9839.
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5. K. Fiederling et al., The chemical effect goes resonant – a full quantum mechanical approach on TERS, Nanoscale 2020, 12, 6346–6359.

Keywords: Tip-enhanced Raman scattering, TERS, Plasmonics

F-O.9

Title: *Electric-field-dependent infrared nanospectroscopy of PVDF with an atomic force microscope*

Author: Maria Eleonora Temperini[1], Valeria Giliberti[2], Tommaso Venanzi[2], Raffaella Polito[1], Antonia Intze[1], Michele Ortolani[1]

[1]Sapienza University of Rome
[2]Istituto Italiano di Tecnologia

Abstract:
We have customized an atomic force microscope (AFM), equipped with conductive probes and a widely tinable mid-infrared quantum cascade laser beam focused on the probe tip, to simultaneously apply a DC voltage to nanometric samples and perform tip-enhanced infrared nanospectroscopy.

The AFM works in contact mode and records IR absorption spectra based on the photothermal expansion effect (Anasys NanoIR2).

Here we first demonstrate the operation of our novel nanospectroscopy setup by studying the photo-thermoelectric effect as a function of wavenumber (910-1900 cm-1) in graphene stripes connected to a lithographc gold electrode on one side and to the AFM probe on the other side. We then move to spectroscopy as a function of applied electric field of thin films of the piezoelectric polymer PVDF deposited on metallic substrates, where we observe signatures of both the vibrational Stark effect and the electric-field-induced orientational deformations of the polymer chains.

The electric-field dependent nanospectroscopy data are compatioble with those obtained by FTIR on extended samples [1], but in this case we could highlight the effect of inhomogeneities in the PVDF films (e.g. multiple crystal phases). Also, with our voltage-dependent nanospectrosocpy technique one could study much thinner films and/or nanopatterned films.

In the Figure, we show the nano spectra in the 920-1350 cm-1 range of a spin-cast 100-nm thick film of PVDF. Here we selected a quasi-monofasic domain (alpha phase of PVDF) and we performed voltage-dependent spectroscopy. At least four voltage-dependent absorption features are observed, in agreement with FTIR measurements of homogenous PVDF films. Some of these features can be attributed to the vibrational Stark effect (C-F2 stretching at 1180 cm-1) while other features are related to variations of the polymer chain arrangement unders the static electric field that approaches 1 MV/cm.

References:

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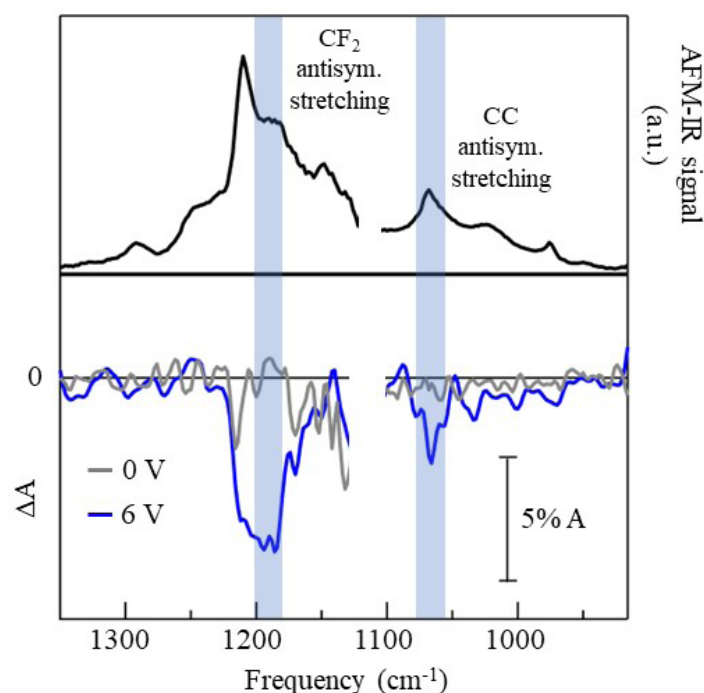
Figure captions:
Top: nano-infrared absorption spectrum of a quasi-monofasic nanoscale region of a PVDF film. Bottom: voltage-induced absorption difference under 6 V bias and after reset to 0 V (baseline).

Keywords: nanospectroscopy, quantum-cascade-laser, Stark-effect, atomic-force-microscopy

F-O.10

Title: *Detection of microplastics using optical manipulation techniques and Raman spectroscopy*

Author: Silvie Bernatová[1], Martin Kizovský[1], Antonino Foti[2], Maria Donato[2], Pavel Zemánek[1], Ota Samek[1], Onofrio Maragò[2], Jan Ježek[1], Pietro Gucciardi[2]

[1]Institute of Scientific Instruments of the Czech Academy of Sciences
[2]Istituto per Processi Chimico-Fisici – Consiglio Nazionale delle Ricerche

Abstract:
Degradation of plastic waste items results in the generation of various types of microplastic and nanoplastic particles that represent high-concern environmental pollutants. Detection of these particles suspended in marine and fresh water still faces major challenges due to the limitations of the current detection methods, especially for the elusive sub-1 µm nanoplastic fraction. Our detection approach is based on optical manipulation and Raman spectroscopy, allowing to overcome the technological gap in the detection of plastic debris with size ranging from tens of nanometers up to 20 microns, see Fig. 1. We summarize recent advances obtained in our lab regarding the optical trapping of microplastics and the optical manipulation of plasmonic nanoparticles, enhancing the detection of plastic objects at the nanometer scale[1,2].

References:
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2. R. Gillibert et al., "Raman tweezers for tire and road wear micro- and nanoparticles analysis, " Environ. Sci.: Nano 9 (2022) 145-161.

Acknowledgments:
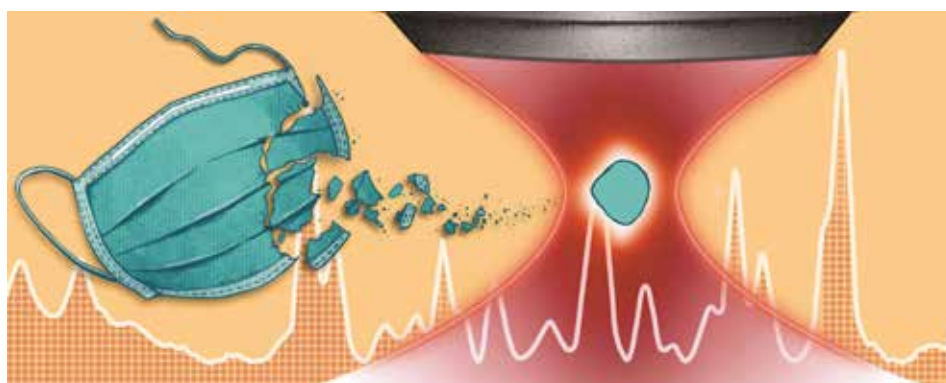
Figure captions:
Fig. 1: The degradation of plastic item leads to fragmentation into small micrometric particles. The fragments can be identified due to Raman spectroscopy and optical manipulation techniques.

Keywords: Microplastics, Raman spectroscopy, Optical manipulation

F-O.11

Title: *Simultaneous SERS & SEIRA with Single Molecule Detection – The Application and Characterization of Plasmonically Resonant Structures with Sub-Micron Optical Photothermal Infrared and Simultaneous Raman spectroscopy*

Author: Mustafa Kansiz[1], Miriam Unger[2], Deepthy Kavungal[3], Felix Richter[4], Hatice Altug[3], Mark Anderson[5]

[1]Photothermal Spectroscopy Corp
[2]Photothermal Spectroscopy Corp GmbH
[3]Bionanophotonic Systems (BIOS) Laboratory & Lashuel Lab, EFPL
[4]Bionanophotonic Systems (BIOS) Laboratory & Lashuel Lab, EFPL,
[5]Caltech, Jet Propulsion Labs, NASA

Abstract:
Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman.

In a first, the combined and correlative IR+Raman microspectroscopic approach was used to explore Simultaneous Surface Enhanced Infrared Absorption (SEIRA) and Surface Enhanced Raman Spectroscopy (SERS). Both SERS and SEIRA were simultaneously measured from the same location, at the same time at the same resolution on plasmonically active substrates. The sensitivity of this approach enabled very small quantities of molecules, even to the single molecule level to be interrogated while providing complementary information from both infrared and Raman spectroscopy. This arrangement provides additional improvement of the SEIRA sensitivity through the enhancement of both the optical photothermal detector signal and the infrared absorption. The plasmonic substrates tested were silver nanospheres and a gold coated atomic force microscope tip. The concurrent acquisition of SEIRA and SERS is further demonstrated by nano-sampling material onto an atomic force microscope tip. The analytes, Buckminsterfullerene and 1,2-bis(4-pyridyl) ethylene, were analyzed individually and as mixtures. The concurrent acquisition of SERIA and SERS is a unique approach. It has general applications in trace surface analysis and for the analysis of returned planetary samples.

In a further application of the ultra-high far-field IR spatial resolution properties (<500nm), the newly engineered, counter-propagating mode was utilized to characterize, both spatially via single frequency IR imaging and spectrally, plasmonic structures consisting of patterned gold on 500micron thick CaF2 windows. Exceptional IR images utilizing a 100x, 0.9NA glass objective were collected showing unprecedented spatial detail of structures <500nm at different IR wavelengths.
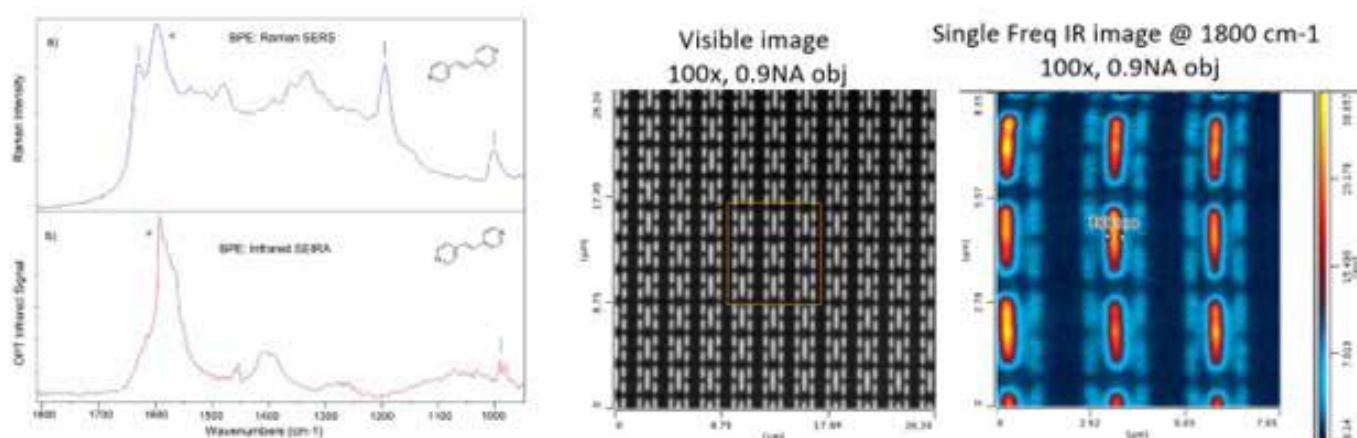
Figure captions:
(a) Raman (SERS) and (b) infrared (SEIRA) spectra from a single molecule of BPE. Centre & Right : Visible image (centre) and IR image at 1800cm-1 (right) of patterned gold on CaF2 structures

Keywords: SERS, SEIRA, O-PTIR, Plasmonics

F-O.12

Title: *Raman optical activity as a sensitive tool in analytical chemistry*

Author: <u>Josef Kapitán</u>[1], Pavel Michal[1], Jana Hudecová[1], Petr Bouř[2]

[1]Palacký University Olomouc, Department of Optics
[2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences

Abstract:
In this review lecture, we will outline areas of analytical chemistry where Raman optical activity can be a useful tool in providing information not readily available by other methods.
The optical purity of a chiral sample is of particular importance to the analytical chemistry and pharmaceutical industries. In recent years, the Raman optical activity (ROA) has become established as a sensitive and nondestructive technique for the analysis of chiral molecules in solution. A methodology for the determination of enantiomeric excess using ROA achieved an accuracy better than 0.1 % for neat liquid and 0.2 % for aqueous solution.[1]
ROA is also increasingly used to describe the 3D structures of many molecules of biological significance, from illicit substances[2] to metal complexes.[3] ROA in combination with ab initio and molecular dynamics simulations with a realistic description of solvent, provides not only population weights for individual conformer groups but also detailed insight into the structure of the molecules and their interaction with the solvent.
To fully exploit the potential of this technique, it is necessary to suppress the presence of artefacts in the spectra and control and compensate for other external influences that may affect the accuracy of the measurements. For Raman optical activity, it is also possible to use the large spectral range of 50-4500 cm^{-1}, which makes it possible to observe anharmonic effects and distinct features of intermolecular interactions.[4]
These findings show the great potential of ROA spectroscopy for the quantitative analysis of chiral molecular systems.

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4. P. Michal, J. Kapitán, J. Kessler, P. Bouř, Low-frequency Raman optical activity provides insight into the structure of chiral liquids, Phys. Chem. Chem. Phys. 24 (2022) 19722–19733

Keywords: Raman optical activity, enantiomeric excess

F-O.13

Title: *A novel wide-field Raman imaging setup*

Author: B. J. A Mooij[1], R. W. Schmidt[1], W. A. J. Vijvers[2], F. Ariese[1]

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[2]Chromodynamics B.V.

Abstract:
Raman spectroscopy is a versatile technique for molecular identification with a wide variety of possible applications, including mineralogy, art analysis, biomolecular imaging and pharmacology. Raman spectroscopy has many advantages over other techniques such as high chemical specificity and minimal sample preparation requirements, but there are limitations. One of the main limitations is mapping speed. To scan a 1.0 x 1.0 mm surface with traditional point scanning Raman spectroscopy would take several hours, or even days, depending on the sample and step size. Measurement of a similar area for this abstract only took 10 seconds, with as little as 1 second showing promising results. To overcome the speed limitation, we have developed a wide-field Raman spectroscopy setup that simultaneously images the sample with 4 cameras, each with a dedicated band-pass filter.

The setup (Figure 1) uses a power-adjustable laser source (max. 20 W, 532 nm, 80 MHz) to illuminate a roughly 1 mm^2 surface containing aspirin, caffeine, and paracetamol. The goal was to determine the spatial distribution of the different compounds in a measurement lasting no more than 10 seconds. First, calibration samples were measured for each pure compound. Based on these calibration measurements, we were able to successfully determine the paracetamol, aspirin and caffeine distributions in a mixed sample. The data analysis followed a partial least squares method based on Zada et al. 2018.

Figure 2 shows the image with the identified compounds: paracetamol (blue), aspirin (red) and caffein (green). We can successfully distinguish these compounds using our novel setup and method. This supports applications for instance in pharmaceutical quality control, where the potential studied compounds are typically known. The system can then distinguish between desired and undesired compounds in a mixture within a practical timeframe.

References:
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Acknowledgments:

Figure captions:
A) schematic of the setup. DFL:lens; DM:dichroic; OL:objective; S:sample; EF:edge filter; BS: beam splitter F:bandpass; C:camera. B) processed data: caffeine(green); paracetamol(blue); aspirin(red)

Keywords: Raman spectroscopy, wide-field, global imaging

F-O.14
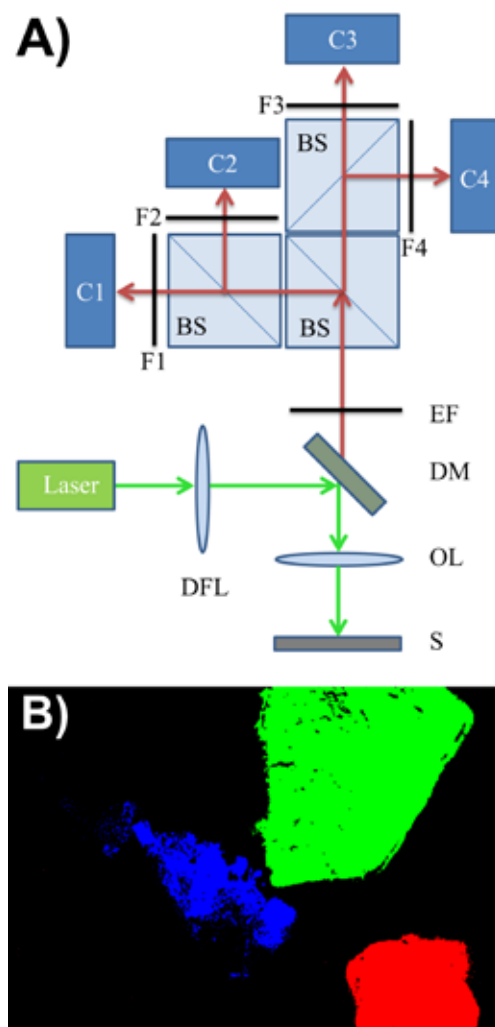
Title: *Simultaneous co-located Raman and SEM imaging for correlated SEM microscopy*

Author: <u>Jorge Diniz</u>[1], Agnieszka Sozanska[2], Tim Batten[3]

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[2]Renishaw Spzoo
[3]Renishaw PLC

Abstract:
Scanning electron microscopy (SEM) is a powerful technique for structural and elemental analysis of a wide range of materials. In contrast, Raman spectroscopy is a complimentary optical technique that provides information on the chemical bonding and structure. When used together, SEM analysis can be used to locate structures or defects at high spatial resolution, while Raman spectroscopy can be used to determine chemical information with high specificity. Correlating the information from these techniques leads to a greater understanding of the specimen. It is important however, that the data and images from the different modalities can be directly and accurately aligned without positional errors that could be caused when moving samples to different analysis positions or equipment. In this work, we demonstrate the inLux SEM Raman interface which, when attached to a SEM chamber is capable of simultaneous and co-incident SEM and Raman imaging from the same sample position thus avoiding correlation errors.

 Figure 1 shows SEM and Raman images acquired from single and multi-layer graphene. Over 11,000 spectra were collected by moving the inLux probe, while the sample remains stationary inside the SEM. In contrast to the SEM image, the information rich Raman images of the carbon G band and 2D bands visualise layer thicknesses and strain within material.

The application potential of co-incident SEM-Raman imaging will be demonstrated as well on the example of the mineral section where clear and accurate overlay between SEM and Raman features highlights presence of additional components (biotite) in a sample which were not detected by the SEM image.

We demonstrate with these examples how Raman and SEM can increase understanding of materials, the complementary nature of the techniques, and their power when combined.
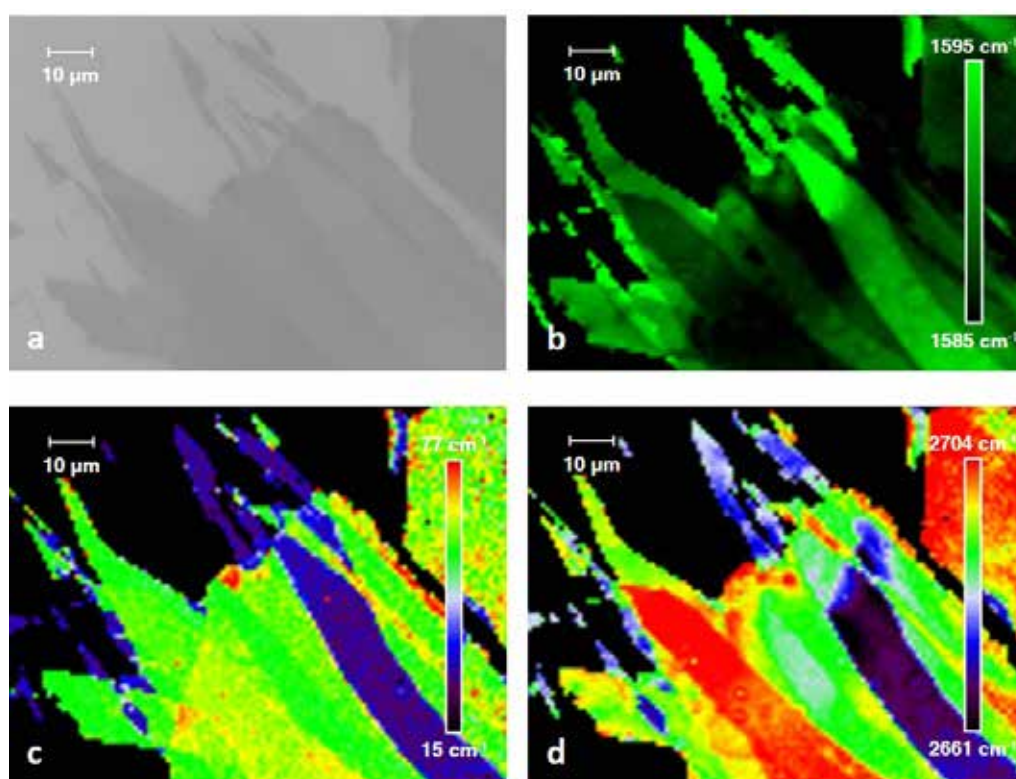
Figure captions:
SEM (a) and Raman (b) image of graphene G band position. (c) Raman graphene 2D band width image. Single layer (blue), few layer (green), multi layer (yellow/red). (d) Raman image of 2D band position

Keywords: co-incident Raman-SEM imaging, graphene, geology

F-O.15

Title: *Reducing frequency fluctuations induced by back-reflected light into a non-stabilized low cost laser diode.*

Author: <u>Konstantinos Stergiou</u>[1], Oleksii Ilchenko[2], Yurii Pilhun[1], Andrii Kutsyk[2]

[1]Lightnovo ApS
[2]Technical University of Denmark

Abstract:
Raman spectroscopy is one of the most well established non-invasive techniques of studying the vibrational properties of molecules and crystals. Despite their necessity in a broad range of applications related with the identification of the chemical composition of materials, realization of modern Raman spectroscopy devices still requires bulky and costly Raman instrumentation. One of the main sources of cost is the frequency and power stabilized lasers. In order to provide low cost but high quality Raman spectrometers Lightnovo ApS developed miniRaman, a compact spectrometer which operates with non-stabilized AlGaAs laser diode in a 5.6 mm TO package with Fabry–Perot resonator. The drifting of frequency and the occurring mode hops are treated with real-time post processing analysis. The beam of the laser is divided into a main and a reference channel. The reference channel constantly provides Raman spectrum of polystyrene which is used for the real-time correction of spectrum collected from the main channel [1]. In this work we present a method of reducing the frequency fluctuations caused by the back-reflected light from the sample into a low cost laser diode at a central wavelength of 785nm and maximum power of 200mW used in the miniRaman spectrometer. For that purpose, we developed an affordable solution for optical isolation. Such a solution can be a polarization beam splitter (PBS) combined with a quarter wave plate (QWP). Figure 1 presents time maps of the Raman spectrum of polystyrene ("reference channel") acquired with miniRaman with and without optical isolation. The red line indicates the time when a sample of paracetamol (Figure 1a,c) or Si (Fig.1b,d) are inserted in front of the surface probe of the device. When the sample is inserted, at absence of optical isolation, frequency fluctuations occur due to the back-reflected light (Figure 1a,b). The fluctuations are significantly reduced when PBS and QWP are inserted in the optical path (Figure 1a,b).

References:
[1] Ilchenko, O. et al. An apparatus for carrying out Raman spectroscopy. WO2019145005 (2019).
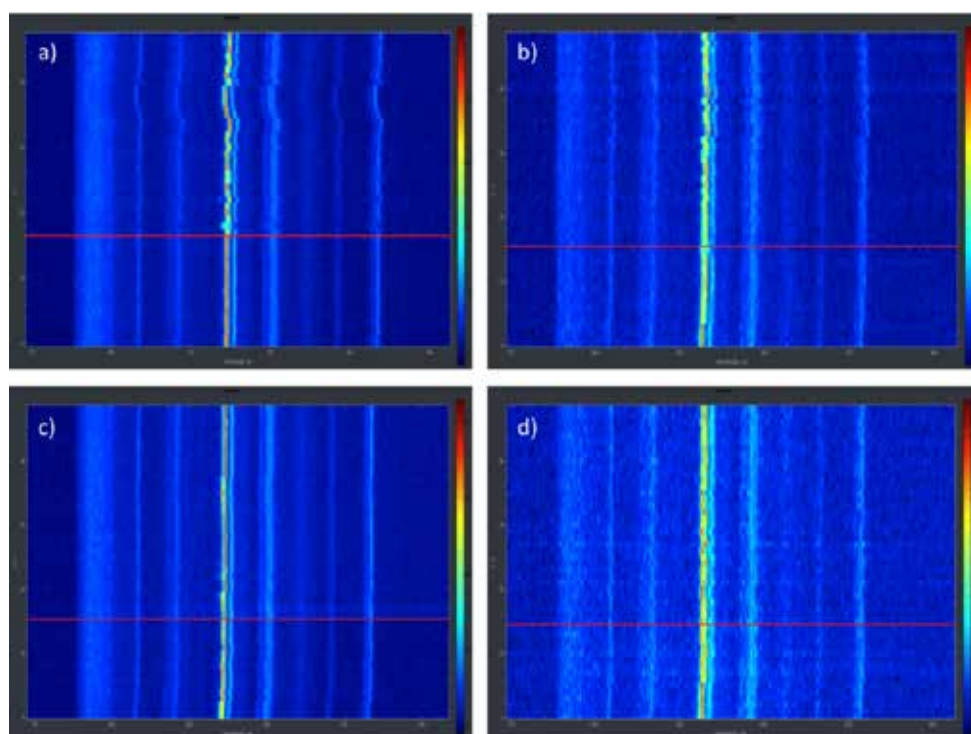
Figure captions:
Figure 1: Time mapping of Raman spectrum of reference channel. a) Paracetamol, no optical isolation. b)Si, no optical isolation. c) Paracetamol, with optical isolation. d) Si, with optical isolation.

Keywords: Raman spectrometer, miniaturization, optical isolation

F-O.16

Title: *Maximizing Positive Microplastic Particle Identification and Provenance Through Optimized Optical and Raman Microscopy – Particle-Correlated Raman Spectroscopy (PCRS)*

Author: Andrew Whitley[1], Eunah Lee[1], Massimiliano Rocchia[1], Sebastien Laden[1]

[1]HORIBA

Abstract:
In recent years Raman microscopes have been developed that provide rapid, fully automated and accurate particle characterization and identification of large numbers, 1000's to 10,000's, of particles, over extreme size ranges down to 0.5 microns or less. This methodology is often referred to as Particle-Correlated Raman Spectroscopy (PCRS). The hardware and software of these systems take advantage of good spatial and spectral resolution Raman data and combine this with optical characteristics of each particle e.g. morphology and color, to accurately identify the chemical type and aid provenance for all particles in a sample. Combining the software and hardware to fully automate this process, including automation of the focusing steps, is by no means trivial, especially for certain particle types where contrast and size ranges are more challenging. We will describe the important steps in both the software and method development, using microplastics as an example of one of the most challenging but now successful applications. Much of the work on microplastics was driven by legislation in both the US and EU. In the US this was driven by Senate Bill 1422: California Safe Drinking Water Act – Microplastics; and Senate Bill 1263: Ocean Protection Council – Statewide Microplastics Strategy; these bills mandated that methodologies and strategies be developed for monitoring and tracking the concentration of microplastics. We will explain how this work has developed and driven improvements in hardware, software and methods for automated particle analysis. We will also discuss future developments and opportunities.

Keywords: Microplastics, Raman, Particle Correlation, Microscopy

F-O.17

Title: *Developing Sensitive Stimulated Raman Scattering (SRS) Microscopy*

Author: <u>Krzysztof Brzozowski</u>[1], Anna Pieczara[2], Malgorzata Baranska[3]

[1]Jagiellonian University
[2]Jagiellonian Centre for Experimental Therapeutics
[3]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics

Abstract:
We present the first in Poland, a newly developed by our group, stimulated Raman scattering (SRS) microscope for sensitive investigations of biological samples. SRS microscopy is a technique that enables ultra-fast chemical imaging and was first performed in 2008 [1]. Since then, SRS microscopy has become one of the most promising imaging techniques in biomedicine. Breakthroughs in microscopy development have revolutionized our ability to study biological systems. Still, what is really crucial in living systems is happening below the micron scale, at the level of organelles, unfortunately, the available research methods have their limitations, i.e. they are not sufficiently sensitive, are sensitive but destructive, or are non-specific.
The main goal of our project was to overcome these technical limitations in detecting small and specific organelles in cells by applying sophisticated measurement methodologies in SRS microscopy. Our SRS system is characterized by high sensitivity while maintaining the low power of laser beams, so the system is not destructive for biological materials. A prototype laser with a low repetition rate (20 MHz) of picosecond pulses (2 ps) is used allowing for high pulse energy maintaining low average laser power [2]. The built system using simple homodyne detection allows for measurements in the range of 1000-3600 cm-1 being very sensitive not only in the C-H stretching range (typical SRS characteristics for biological materials) but also in the "silent" range. We also use Raman tags in our research, breaking the stereotype that Raman microscopy must be label-free, which allows for better specificity by measuring well-resolved signals in a „silent" region.
Here we present data on endothelial cells stimulated *in vitro* with deuterium-labeled saturated FA. Cells were measured both by RS and SRS imaging to extract detailed information about uptaken FA, whereas CARS and fluorescence imaging showed the global content of FA in cells.

References:
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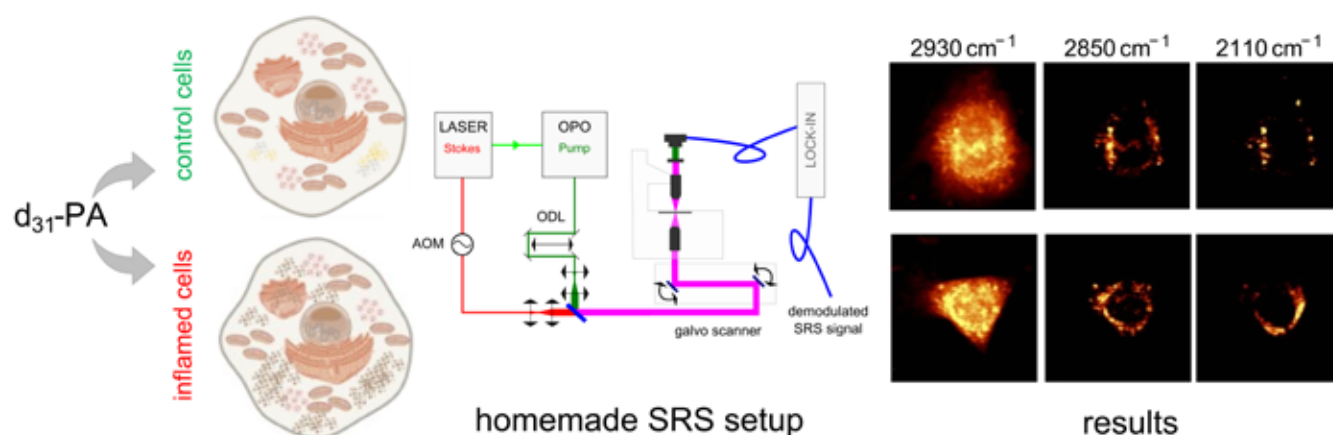
Figure captions:
Schematic representation of the homemade SRS setup.

Keywords: Stimulated Raman, Enhanced Raman, Raman

F-O.18

Title: *Rapid field-resolved infrared fingerprinting and discrimination of particles in flow*

Author: Marinus Huber[1], Daniel Gerz[1], Holger Mirkes[2], Florian Lindinger[2], Yannick Münzenmaier[2], Alexander Weigel[3], Mark Kielpinski[1], Thomas Henkel[1], Mihaela Zigman[3], Ferenc Krausz[3], Jürgen Popp[1], Ioachim Pupeza[1]

[1]Leibniz Institute of Photonic Technology
[2]Ludwig Maximilians University
[3]Max Planck Institute of Quantum Optics

Abstract:
In infrared (IR) spectrometry, the achievable spectral acquisition rate at a given signal-to-noise ratio is fundamentally linked to detection sensitivity. For example, a 10-fold increase in sensitivity affords a reduction of the required measurement time by a factor of 100. This makes sensitive spectrometers essential for high-throughput measurements or for investigating the dynamics of irreversible events. In recent years, field-resolved spectroscopy (FRS) [1] employing bright, femtosecond-laser-based IR sources, along with field-sensitive detection approaching ultimate sensitivity [2] has shown the capacity to surpass the sensitivity of conventional infrared spectroscopies.
We leverage this advantage by combining FRS with rapid spectral acquisition at 38 kHz [3] to investigate individual particles in flow. Broadband, waveform-stable IR pulses with 70 mW of average power are focused to a 40-μm spot ($1/e^2$-intensity diameter) onto a microfluidic chip. A stream of beads suspended in buffer (7-μm-diameter PMMA or PS beads in water) is hydrodynamically focused, continuously transporting particles through the IR focus (see Fig. 1a). Within a total averaging time of less than 1 ms, we record the spectra of each particle (see Fig. 1b). Principal component analysis confirms that spectra belonging to PS and PMMA beads are well separated (see Fig. 1c)
Our findings showcase the potential of FRS in realizing IR-based, label-free flow cytometry of human cells for the first time. This technique could significantly expand the scope of vibrational fingerprinting of biological systems and enable high-throughput screening for low-abundance circulating tumor cells [4]. Furthermore, this technology opens up new possibilities for applications demanding short acquisition times such as micro-spectroscopy of organic samples in aqueous environments at video rate or the monitoring of fast chemical processes with an excellent signal-to-noise ratio.

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2. C. Hofer et al., Quantum-Efficiency and Bandwidth Optimized Electro-Optic Sampling, EPJ Web of Conf. 243, 16001 (2020).
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4. I. W. Schie et al., High-Throughput Screening Raman Spectroscopy Platform for Label-Free Cellomics, Analytical Chemistry, 90, 3 (2018).
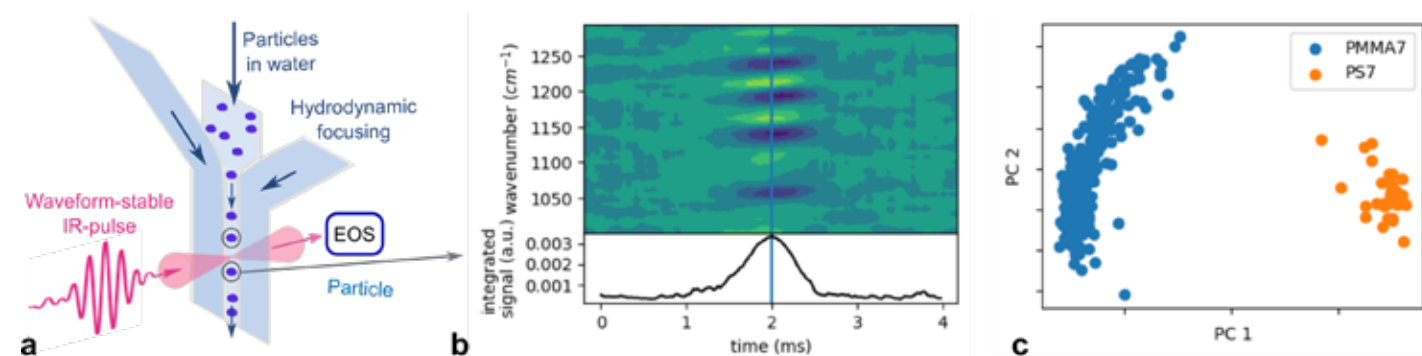
Figure captions:
a Schematic of the channel geometry transporting particles through the IR focus. b Spectral change over time for a typical event. c Principal component analysis of the recorded particle spectra.

Keywords: Infrared spectroscopy, field-resolved spectroscopy, microfluidics

F-O.19

Title: *Current state of spectrometer miniaturization: synergy with analytical potential of NIR spectroscopy*

Author: Christian W. Huck[1], Justyna Grabska[1], Krzysztof B. Bec[1]

[1]University of Innsbruck

Abstract:
Vibrational spectroscopy (mid-infrared, MIR; near-infrared, NIR; and Raman) has become increasingly important tool of analytical chemistry. Past decade was a particularly vivid period of miniaturization of spectrometers with aim reaching the concept of a 'lab on a chip'. In this context, particular attention should be directed at near-infrared (NIR) spectroscopy. It offers especially potent suite of qualities forming rapid, non-destructive, and cost-effective analytical tool. It has widespread over wide field of applications, with the most prominent ones including agriculture, food analysis, forensics, security, and industry, where it often serves as the primary quality control tool [1].

However, the portability and miniaturization of the spectrometers forms particularly strong bond with the conventional advantages of NIR spectroscopy that have opened a new era in its analytical applications [2]. It enabled bringing all the practical qualities of NIR analysis directly to the measurement site making it possible to perform analysis in the field and in real-time. Overall, this new technology has expanded the potential applications of NIR spectroscopy analysis far beyond traditional laboratory-based systems [3].

The dynamic development of the synergy between miniaturized, on-site capable NIR spectrometers and new tools for spectral analysis has increased the potential and reliability of NIR spectroscopy in various applications. Current trends favor development of devices, which can be used by non-experts for rapid and routine analyses. This has expanded the use of NIR spectroscopy beyond research laboratories and into industries where quick and accurate analysis is critical, such as food and agriculture. This progress revolutionizes NIR spectroscopy, making it a more versatile and accessible analytical technique with a wide range of potential applications including everyday life use.

References:
[1] Beć, K.B.; Grabska, J.; Huck, C.W. Miniaturized near-infrared spectroscopy in current analytical chemistry: from natural products to forensics. In: Molecular and laser spectroscopy - Advances and
applications. Vol. 3, Gupta, V.P. Ed.; Elsevier, 2022, pp. 141-188. DOI: 10.1016/B978-0-323-91249-5.00009-0
[2] Beć, K.B.; Grabska, J.; Huck, C.W.; Principles and applications of miniaturized near-infrared (NIR) spectrometers, Chem. Eur. J. 2021, 27, 1514-1532. DOI: 10.1002/chem.202002838
[3] Beć, K.B.; Grabska, J.; Siesler, H.W.; Huck, C.W. Handheld near-infrared spectrometers: Where are we heading? NIR News 2020, 31(3–4), 28–35. DOI: 10.1177/0960336020916815

Keywords: portable/miniaturized instruments, NIR spectroscopy, analytical

F-O.20

Title: *Mid-IR Dispersion Spectroscopy – A Powerful Tool for Liquid-Phase Chemical Analysis*

Author: Alicja Dabrowska[1], Bernhard Lendl[1]

[1]Technische Universität Wien

Abstract:

Mid-infrared (mid-IR) dispersion spectroscopy is an attractive, novel approach to liquid phase analysis that overcomes the limitations of conventional mid-IR absorption spectroscopy. This technique detects inherent refractive index fluctuations (phase shifts) caused by IR absorption, rather than measuring changes in intensity. It delivers quantitative and qualitative information about the sample equivalent to absorption spectroscopy with the advantages of immunity to source intensity fluctuations, constant sensitivity, baseline-free detection, and high dynamic range beyond the capabilities of the Beer-Lambert's law. [1]

In this work, we discuss the theoretical principles of the technique and experimentally demonstrate the advantages of dispersion spectroscopy over conventional absorption spectroscopy. Moreover, we present the latest configuration of the developed spectroscopic instrument for dispersion sensing in liquids. In brief, it consists of a Mach-Zehnder interferometer illuminated by a tunable quantum cascade laser. The sample is introduced to an instrument via a custom-made dual-channel transmission flow cell, placed between the interferometric arms, which is filled with a reference solution (solvent) and a sample solution (solvent + analyte) prior analysis. IR absorption in the sample solution introduces phase shifts between the interferometric arms proportional to the sample's refractive index allowing the dispersion spectrum to be recorded and analyzed. Our example applications demonstrate the power of our technique and the developed setup for analysis of various analytes (i.e., proteins, carbohydrates), complex mixtures, and chemical reaction monitoring. [2-3]

In summary, the presented work illuminates the potential of dispersion spectroscopy as an upcoming robust and sensitive way of recording IR spectra of liquid samples, which can be of interest for many applications across various industrial areas.

References:

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Mid-IR Laser-Based Dispersion Spectroscopy of Liquids

Experimentally demonstrated advantages (*vs.* absorption spectroscopy):
- ✓ Extended dynamic range
- ✓ Background-free
- ✓ Constant sensitivity
- ✓ Immunity to intensity fluctuations
- ✓ Extended analytical path-lengths for strong absorbers
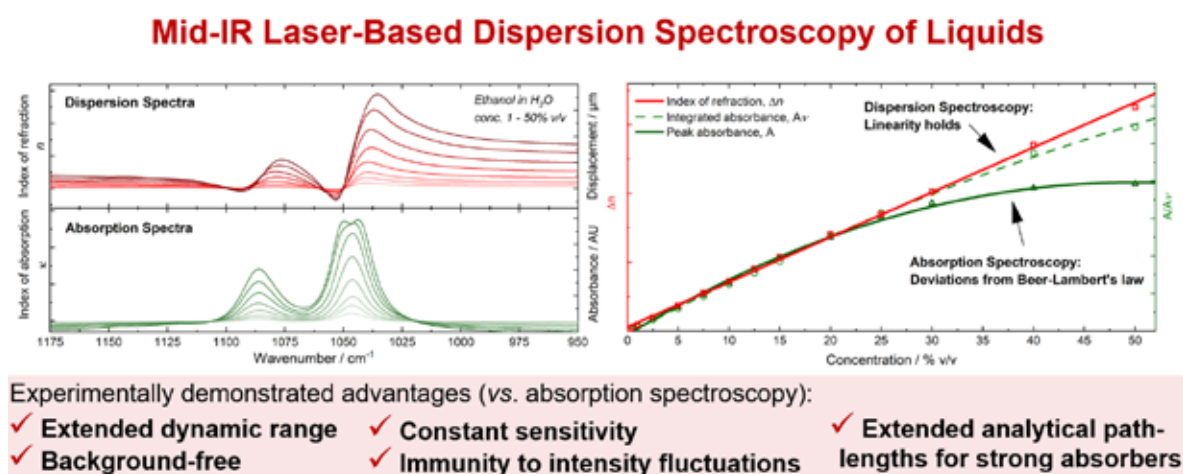
Figure captions:
Demonstrated advantages of mid-IR dispersion spectroscopy of liquids over conventional absorption spectroscopy.

Keywords: Mid-InfraredDispersionSpectroscopy, Liquid-PhaseAnalysis, QuantumCascadeLaser, RefractiveIndexSensing, Mach-ZehnderInterferometer

F-O.21

Title: *Raman spectrometer with vertical flow method for organic solvents*

Author: Ting-hao Chen[1], Hirotsugu Hiramatsu[1]

[1]Department of Applied Chemistry and Institute of Molecular Science, National Yang Ming Chiao Tung University

Abstract:

Raman spectroscopy provides information about molecular vibrations and structure. Improving the signal-to-noise (S/N) ratio of the spectroscopic data increases the precision and reliability of the discussion. To this end, we developed a vertical flow (VF) method to enhance the signal intensity. The VF method enhances the non-resonance Raman signals of any molecules. This paper reports the combination of the VF method with a Raman spectrometer that consists of a common monochromator and detector. We examine the performance of the constructed system.

In the VF method, a sample solution is spout from a pinhole, forming a laminar flow liquid column. The Raman excitation beam is introduced from the pinhole. The excitation beam and a part of the generated Raman signals are confined to the column due to the total reflection and returned to the pinhole. The Raman signal that escapes from the pinhole is gathered and delivered to the Raman spectrometer. Thus, the VF method improves the Raman signal generation and collection efficiency. The VF method was combined with the Raman spectrometer equipped with a 660 nm cw laser using a round-to-linear optical fiber. The Raman signal gathered at the pinhole, having a circular shape, is reformed into a linear image and introduced to the rectangular entrance slit. By doing so, the signal was efficiently delivered to the detector. We also examined the pinhole size dependence of the signal enhancement factor. The highest signal enhancement, up to 168 times, was achieved with a pinhole size of 60 μm. The signal enhancement resulted in a limitation of the choice of the solvent, i.e., the Raman signals of organic solvents easily saturated. We propose an improved design of the VF sampling unit and a method to reduce the Raman signal intensity of the solvent, which overcomes this problem.

References:

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Keywords: non-resonance Raman, signal enhancement

F-O.22

Title: *High-performance miniaturized Raman systems for challenging Raman spectroscopy and microscopy applications*

Author: Oleksii Ilchenko[1], Yurii Pilhun[2], Andrii Kutsyk[1], Yaman Goksel[1], Elodie Dumont[1], Thomas Andersen[3], Mikael Lassen[4], Hemanshu Mundhada[5], Christian Jendresen[5], Anja Boisen[1]

[1]Technical University of Denmark
[2]Lightnovo ApS
[3]Odense University Hospital
[4]Danish National Metrology Institute
[5]Cysbio ApS

Abstract:
We present a novel miniaturization strategy that allows us to create versatile compact Raman spectrometers and microscopes based on cheap non-stabilized laser diodes, densely-packed optics, and non-cooled small pixel size sensors. We demonstrate that the achieved performance is comparable with expensive and bulky research-grade Raman systems. Our miniaturization concept is based on real-time calibration of Raman shift and Raman intensity using a built-in reference channel that is independent of the main optical path1. We have demonstrated the miniaturization of the whole device dimensions down to several centimeters and achieved excellent sensitivity, low power consumption, perfect wavenumber and intensity calibration combined with high spectral resolution of around 7 cm^{-1} within the spectral range of 400-4000 cm^{-1}. We demonstrate possible solutions to the most critical Raman miniaturization challenges: need for laser temperature and power stabilization, reduction of sensor dark noise, compensation on pixel-to-pixel quantum efficiency variation, laser optical isolation and achieving high spectral resolution. Moreover, the proposed miniaturization strategy provides shifted-excitation Raman difference spectroscopy and spatially offset Raman spectroscopy functions as a derivative of the working principle.
The high performance and vast versatility offered by our strategy facilitate simple integration into various applications. As examples, we show the quantification of methanol in alcoholic beverages through a glass bottle, in-vivo Raman measurements of human skin, quantification of p-coumaric acid and serine during fermentation by E. coli bacteria, high resolution Raman mapping, quantitative SERS mapping of the anti-cancer drug methotrexate and in-vitro bacteria identification by Raman mapping. We foresee that the proposed miniaturization strategy will allow realization of super-compact Raman spectrometers for integration in e.g. smartphones and medical devices.

References:
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3. Mosca S, Conti C, Stone N, Matousek P. Spatially offset Raman spectroscopy. Nat Rev Methods Prim (2021) 1: 21.

Figure captions:
Figure 1. (a) Raman system, (b) sensor image that demonstrates acquisition of main and reference signals, Raman spectra variation in (c) reference and (d) main channel, (e) spectra after calibration.

Keywords: Raman spectrometer, miniaturization, real-time calibration

F-O.23
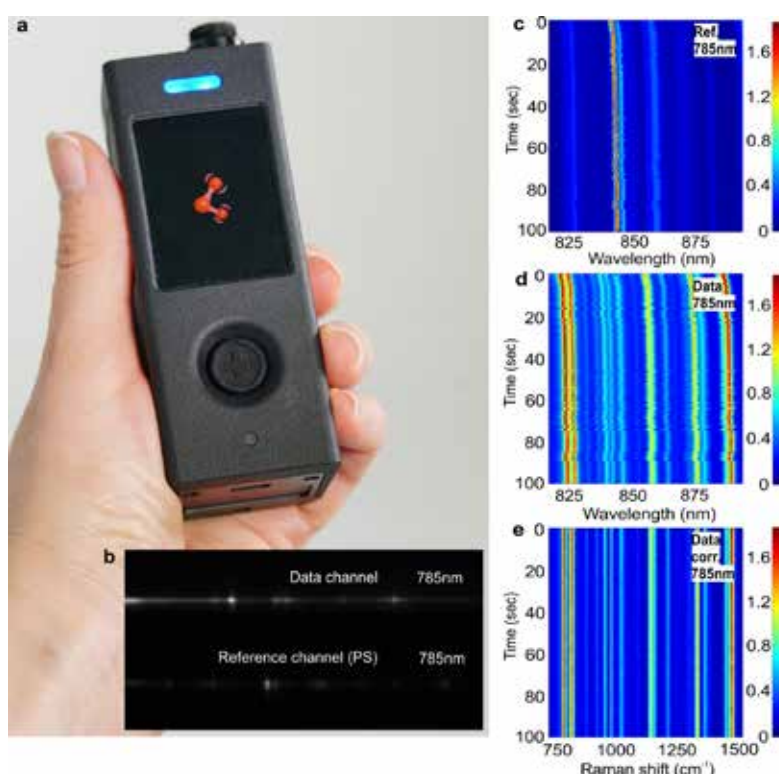
Title: *A correlated OF2i®-Raman method for micro- and nanoparticle detection and chemical analysis in liquids*

Author: Christian Neuper[1], Marko Šimić[2], Christian Hill[3], Werner Grogger[4], Harald Fitzek[5]

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[2]Brave Analytics GmbH, Austria / Gottfried Schatz Research Center, Division of Biophysics, Medical University of Graz, Neue Stiftingtalstraße 2, Graz 8010, Austria / Insitute of Physics, University of Graz, Universitätsplatz 5, Graz 8010, Austria
[3]Brave Analytics GmbH, Austria / Gottfried Schatz Research Center, Division of Biophysics, Medical University of Graz, Neue Stiftingtalstraße 2, Graz 8010, Austria
[4]Graz Centre of Electron Microscopy, Steyrergasse 17, Austria / Institute of Electron Microscopy and Nanoanalysis, NAWI Graz, Graz University of Technology, Steyrergasse 17, Austria
[5]Graz Centre of Electron Microscopy, Steyrergasse 17, Austria

Abstract:

Micro- and nanoparticles have increasingly received public attention, posing a potential threat to ecosystems and human health due to their widespread presence in the environment [1]. As part of the recently launched Nano-VISION project, a novel technique is being developed to detect and identify nano- and microplastics in liquids, thereby enabling the quantification of the amount and types of plastics present in a fluidic environment. The technique is based on a correlation between an optofluidic force induction method (OF2i®) and Raman spectroscopy.

OF2i® is a novel counting technique that combines optical and fluidic forces to characterize nanoparticles with single-particle sensitivity and high throughput [2]. The particles are transported through a microfluidic flow channel alongside a weakly focused vortex beam. Based on the principle of optical tweezers and microfluidics, the single particles become optically trapped, and experience size-dependent velocity changes through photon momentum transfer between light and matter. An ultramicroscope setup captures scattered light and the position of individual particles at a 90° angle, from which number-based particle size, size distribution, and concentration can be determined [3].

In this work, we demonstrate the extension of the OF2i® system by chemical analysis through Raman spectroscopy with single particle sensitivity. We explore the measurement capabilities of the OF2i®-Raman technique to analyze single particles. One of the most promising advantages of this novel technique is the intensive excitation of particles by a 2 W laser in a constant water flow that helps mitigate beam damage. Combining the OF2i® technology with Raman spectroscopy could enable the chemical analysis of nanoparticles with unmatched speed and versatility.

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Figure captions:
Schematics of OF2i®-Raman based on [4]. (Top) Representation of the microfluidic channel and the weakly focused laser beam. (Bottom) Illustration of the OF2i® and Raman signals of the particles
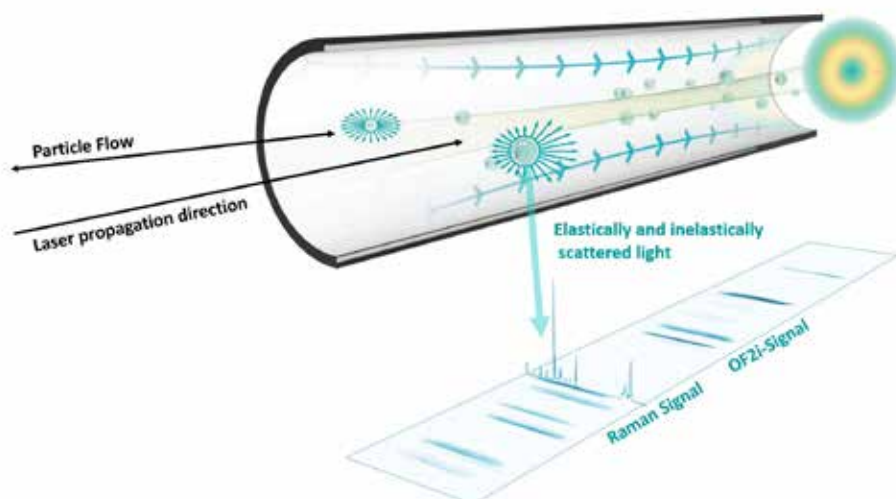
Keywords: Instrument development, Nanoparticles, Microplastic, OF2i

F-O.24

Title: *Dielectrophoresis for Raman analysis in liquid: towards a rapid and label-free platform for virus identification*

Author: Alessio Sacco[1], Giulia Barzan[1], Slavica Matić[2], Chiara D'Errico[2], Marta Vallino[2], Marina Ciuffo[2], Emanuela Noris[2], Andrea Mario Giovannozzi[1], Chiara Portesi[1], Andrea Mario Rossi[1]

[1]National Metrology Research Institute (INRiM)
[2]Institute for Sustainable Plant Protection, National Research Council of Italy (CNR)

Abstract:
Raman microspectroscopy offers several potential advantages over conventional methods for the characterization of biological and environmental specimens. Thanks to its speed and non-destructivity, and its compatibility with water matrices, it is suitable for real-time measurements, also on living samples directly in suspension, without any labelling or time-consuming sample preparation process. Dielectrophoresis (DEP) — the electric field-induced motion of non-conductive particles by non-uniform electric fields [1] — was already employed in combination with Raman spectroscopy to manipulate biological samples dispersed in their liquid environment, such as bacteria, to locally concentrate them and maximizing the Raman signals to obtain their specific chemical fingerprint [2].
In this work, the Raman-DEP technique was applied, using a specifically designed device optimized by measuring polymer beads, to characterize five different purified plant viruses' suspensions, creating a Raman spectral library. Raman-DEP revealed similar Raman features of the viruses within the same genus, but also identified differences at a molecular level. Furthermore, different Raman spectral profiles were obtained for the viruses that were indiscernible by transmission electron microscopy. Additionally, this method was successfully applied to follow the capsid proteins denaturation of viruses subjected to thermic stress: it was demonstrated that Raman-DEP is sensitive enough to detect the critical temperature of proteins unfolding.
This demonstrates the viability of this new method for rapid characterization of viruses, the identification of different viral pathogens in agricultural and human medical fields and in the biotechnological exploitation of viruses. Furthermore, it can be applied for the study of efficacy and mechanism of action of new virucidal agents that target the viruses' capsid proteins, paving the way to the use of Raman to new frontiers.

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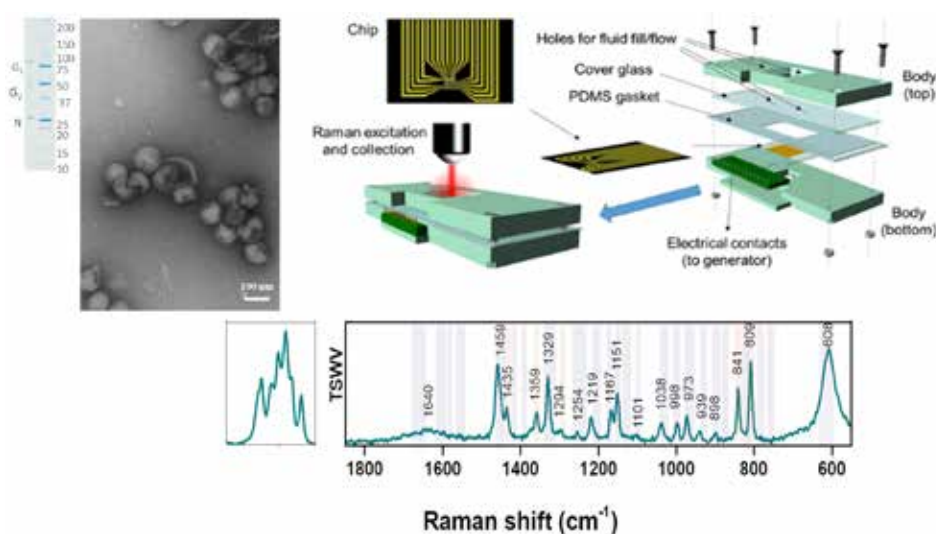
Figure captions:
Raman-DEP device schematic representation for the determination of TSWV Raman fingerprint.

Keywords: Raman spectroscopy, virus, dielectrophoresis, TMV

F-O.25

Title: *A Tailored Setup for Multiphase In situ Spectroscopy on Gas-processing Metalloenzymes*

Author: Christian Lorent[1], Sagie Katz[1], Vladimir Pelmenschikov[1], Giorgio Caserta[1], Stefan Frielingsdorf[1], Maria Alessandra Martini[2], Konstantin Bikbaev[3], Ingrid Span[3], James A.F. Birrell[4], Oliver Lenz[1], Marius Horch[5], Ingo Zebger[1]

[1]Technische Universität Berlin, Insititut für Chemie
[2]Max-Planck-Institut für Chemische Energiekonversion
[3]Friedrich-Alexander-Universität Erlangen-Nürnberg
[4]University of Essex, School of Life Sciences
[5]Freie Universität Berlin, Institut für Physik, Biophysik

Abstract:
Gas-converting metalloenzymes like hydrogenases are potential blueprints for bioinspired chemistry, targeting sustainable alternatives for nobel metal-based catalysts. The development of efficient and long-term stable synthetic analogues requires the fundamental understanding of the structural and functional relationships in the biological models. In this context, we established a novel experimental platform for studying catalysis of gas-processing metalloenzymes under in situ conditions at various temperatures and gas atmospheres by infrared and other spectroscopies using two oxygen-tolerant [NiFe] hydrogenases as model systems.[1] Redox transitions in protein lyophilizate[1], single crystals[1-3] or solution phase[1] can be followed via the characteristic stretches of Fe-ligated CO and CN- by exchanging the atmosphere to substrate or inhibitors, e.g. H_2 or O_2, facilitating an enrichment of certain redox species. Further, reaction intermediates can be trapped under cryogenic conditions to explore e.g. their photochemistry. Especially in combination with protein crystallography this toolbox proved beneficial. Using resonance Raman (to monitor metal ligand modes) and IR spectroscopy the structural determinants of the hydrogen-binding and a carbon monoxide inhibited intermediate in a F420-reducing [NiFe] hydrogenase had been deciphered.[2] Furthermore, the structural key aspects of oxygen-resistant redox states, stabilized by a glutamate-coordinated high-valent nickel, in a soluble [NiFe] hydrogenase were elucidated. Studying two model [FeFe] hydrogenases details on the structure of oxygen-stable, sulfide- and cyanide-inhibited redox states were obtained.[3,4] Solution phase experiments shed light on recently discovered catalytic hydride intermediates.[4] These examples demonstrate how our advanced experimental approach for vibrational spectroscopy can provide detailed insights into structure-function relationships of gas-converting metalloenzymes.

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[4] C. Lorent et al. Shedding Light on Proton and Electron Dynamics in [FeFe] Hydrogenases, J. Am. Chem. Soc. 2020, 142, 5493-5497

Figure captions:
An advanced experimental approach for studying solvated, lyophilized and crystallized metalloenzymes under varying gas atmospheres as function of temperature and optional under light exposure

Keywords:
Gas-processing-enzymes, in-situ-IR-spectroscopy, Resonance-Raman-spectroscopy, single-crystal-spectroscopy

G-I.1
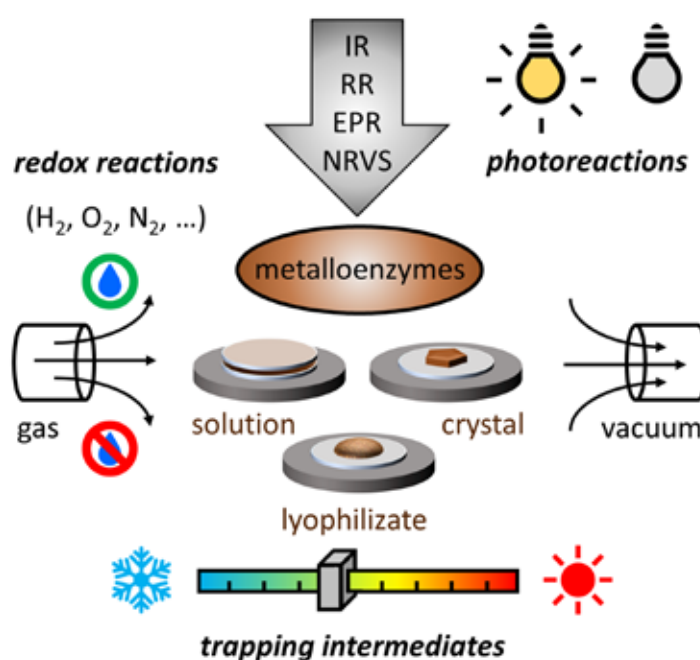
Title: *SERS combined with chemometric analysis for detection and identification of microorganisms: viruses and bacteria.*

Author: Agnieszka Kamińska[1], Krzysztof Niciński[1], Sylwia Berus[1], Dorota Korsak[2], Tomasz Szymborski[1], Beata Młynarczyk-Bonikowska[3], Monika Adamczyk-Popławska[2], Evelin Witkowska[1]

[1]Institute of Physical Chemistry, Polish Academy of Sciences
[2]University of Warsaw, Faculty of Biology, Institute of Microbiology
[3]Department of Dermatology and Venerology, Medical University of Warsaw,

Abstract:

Surface–enhanced Raman spectroscopy provides a unique vibrational signature of the scattered molecules. SERS as an ultrasensitive, label-free and non-destructive technique reveals a specific information down to the molecular level and thus will offer valuable information for biological systems analysis and monitoring. We present its application for detection and identification of pathogenic bacteria from clinical and environmental samples, viruses including SARS-CoV-2 [1-3]. The proposed SERS-based method for bacteria identification challenges the standard biochemical methods in terms of simplicity, specificity and rapidity (maximum 60 s for single SERS measurement). The direct SERS analysis of bacteria (even a single bacteria cell) is performed directly from SERS-active nanostructures incorporated into a microfluidic module. The recorded SERS data of bacteria are categorized (assigned to particular bacterial species) using data analysis software based on a SERS database created for bacteria. The long-time of incubation of bacteria was eliminated and the total analysis including numerical analysis of recorded SERS data not exceed 15 minutes. Coupling of plasmonic nanostructures with microfluidic systems ensures miniaturization of the developed methods for their further applications. Presented approach opens a new path in microbiological diagnostics for sensitive, simple, quick, and on-site detection of pathogenic microorganisms including environmental and clinical microbiology (hospitals, health centre), food industry and environmental protection.

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Acknowledgments:

Figure captions:

The averaged SERS spectra (A), 2D-PCA (B), 3D-PCA (C), and loading plot (D) calculated for 20 different samples of male urethra swabs (infected and uninfected samples).

Keywords: SERS, bacteria, chemonetric, identification, viruses
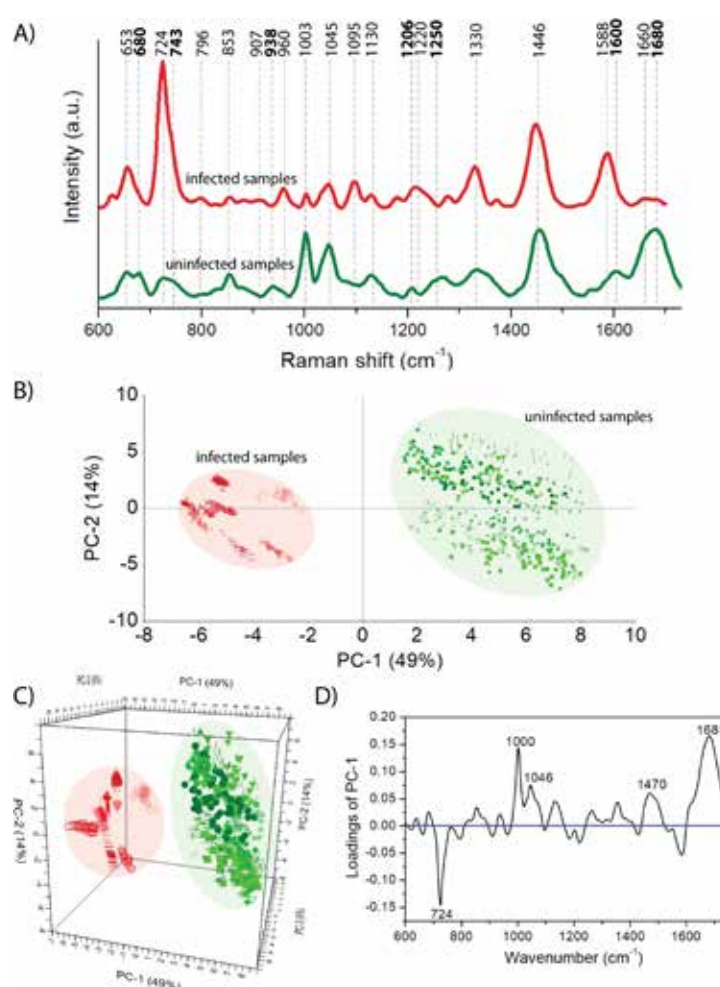
G-I.2

Title: *SISSI-Bio: the multipurpose infrared laboratory at Elettra synchrotron facility*

Author: <u>Lisa Vaccari</u>[1], Giovanni Birarda[1], Federica Piccirilli[1], Diana Eva Bedolla[2], Chiaramaria Stani[3]

[1]Elettra Sincrotrotrone Trieste
[2]Area Science Park
[3]CERIC-ERIC

Abstract:
SISSI-Bio is the Chemical and Life Sciences branch of the infrared beamline SISSI (Synchrotron Infrared Source for Spectroscopy and Imaging) at Elettra. The laboratory is up-to-date with the latest equipment for Fourier Transform InfraRed (FTIR) analysis and currently hosts endstations for spectroscopy, microscopy and nanospectroscopy, that can alternatively be operated with benchtop sources, increasing the usage time of the setups beyond the infrared synchrotron radiation (IRSR) availability. Additional new endstations for IR tomography and IR microscopy with nanometric and sub-micron spatial resolution will be installed in the next months. In this contribution, the laboratory will be presented highlighting its multipurpose capabilities and emphasizing the present opportunities for facility users through selected examples, focused on multi-scale and correlative analyses. The foreseen upgrades will be also discussed in an integrated manner with the upgrade plan of Elettra to the next-generation Diffraction Limited Storage Rings (DLSR) Elettra 2.0. The destiny of worldwide IR beamlines DLSRs is a challenge associated to the efficient extraction of bending magnet (BM) IRSR. Nevertheless, the conceived Elettra 2.0 design offers the possibility of a suitable extraction port for BM. In this contribution the results of the more recent simulations run in SRW on the expected performances of SISSI-Bio in Elettra 2.0. will be also presented, highlighting the aspects that will allow for both continuing the spectroscopy and microscopy program and promoting the IRSR nano-spectroscopy program at SISSI-Bio@Elettra2.0.

Keywords: Synchrotron Radiation, DLSR, IR nanoscopy

G-I.3

Title: *Probing chemical speciation with low-frequency Raman spectroscopy*

Author: Keith Gordon[1]

[1]University of Otago and Dodd Walls Centre – Te Whai Ao
Dodd-Walls Centre Te Whai Ao

Abstract:
Low frequency Raman spectroscopy is very sensitive to crystalline states because of the presence of intense phonon modes in the spectra.[1] This makes it possible to readily determine differing polymorphs and detect rapid solid state transformations such as dehydration.[2] We have recently coupled low frequency Raman with a spatially offset optical configuration.[3] This allows one to observe sub-surface species and to utilise the power and speed of low frequency Raman to measure solid state transformations below surface levels. This would permit one to characterise and determine the nature of degradation processes in a completely non-invasive fashion in tableted systems.[4]

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3. K. Berzins, S. J. Fraser-Miller, K. C. Gordon Anal. Chem. 2021, 93, 3698-3705
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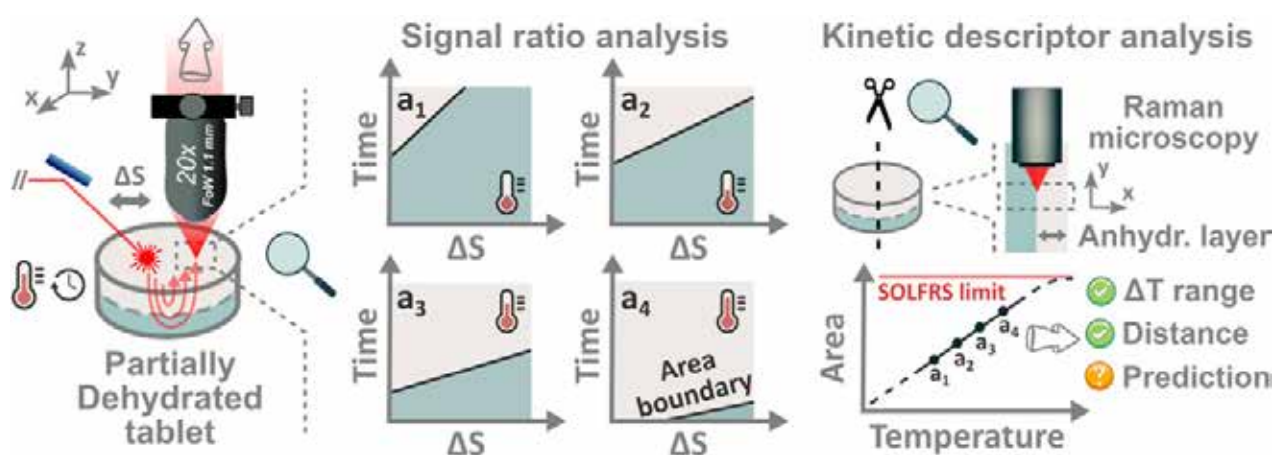
Figure captions:
Spatially offset low frequency Raman configuration for analysis of subsurface features

Keywords: Low frequency Raman, pharmaceuticals, dehydration,

G-I.4

Title: *Profiling of Human Bones by Vibrational Spectroscopy*

Author: <u>Maria Paula Marques</u>[1], David Gonçalves[2], Stewart F. Parker[3], Winfried Kockelmann[3], Giulia Festa[4], Luís Batista de Carvalho[1]

[1]Univ. Coimbra, Molecular Physical-Chemistry R&D Unit
[2]Archaeosciences Lab., Directorate General Cultural Heritage
[3]ISIS Facility, STFC Rutherford Appleton Laboratory
[4]CREF – Museo Storico della Fisica e Centro Studi e Ricerche Enrico Fermi

Abstract:
Burned human bones are often the only remains found in forensic scenarios (*e.g.* terrorist attacks, fires or criminal burning of victim´s corpses) and archaeological settings, from which the bioanthropologists aim to identify victims or obtain information on past populations. To achieve this goal, it is essential to understand the effect of the heating conditions on the skeletal remains, and to accurately characterise the heat-induced structural and chemical changes. Complementary optical and neutron-based vibrational spectroscopy techniques – infrared (FTIR-ATR), Raman and inelastic neutron scattering (INS) – were applied to the study of human bones burned in a wide range of temperatures (200 to 1000 °C), under aerobic or anaerobic conditions. Clear changes in bone´s chemical composition were unveiled upon heating: the lipids and protein constituents being found to disappear at *ca.* 550 °C/aerobic burning or *ca.* 800 °C/anaerobic burning (Fig. 1). Furthermore, alterations in the microcrystallinity of hydroxyapatite´s framework were detected upon a temperature increase. Archaeological skeletal remains were also analysed (from the Roman-period sites Guidonia-Montecelio and Grottaferrata, Italy), based on the data previously gathered for the modern bones. The results obtained allow a thorough understanding of the heat impact on bone´s constituents, thus contributing to an accurate characterisation of both forensic and archaeological human skeletal remains found in distinct scenarios.

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5. G. Festa et al. Anal. Chem. 95 (2023) 2469.
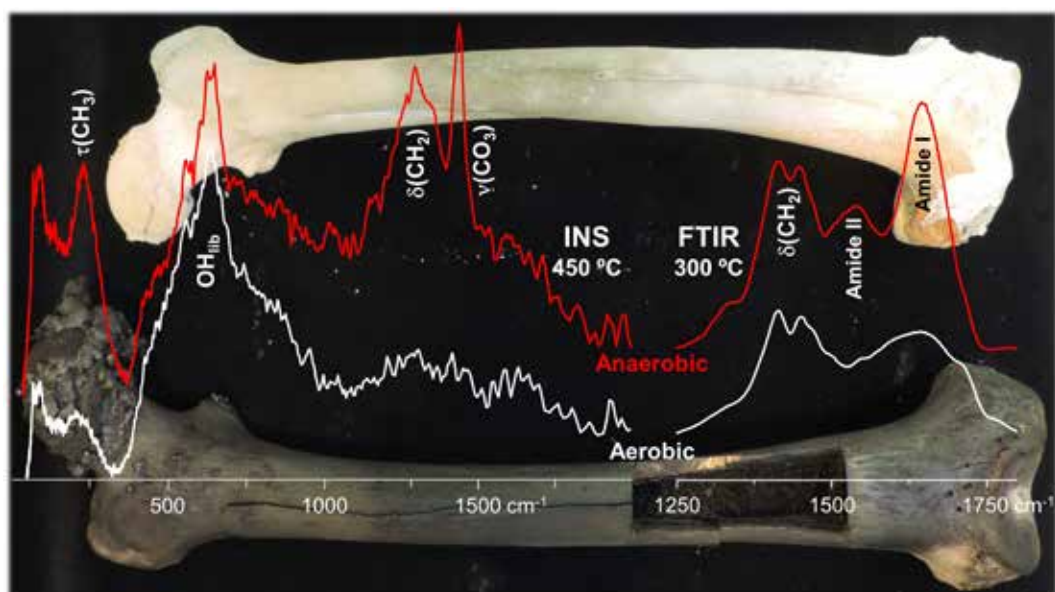
Figure captions:
FTIR spectra of burned human bones, under aerobic and anaerobic conditions.

Keywords: burned-human-bone, FTIR-ATR, Raman, INS, forensics-archaeology

G-I.5

Title: *Insights into forensic analysis of peripheral blood stains on natural and synthetic fabrics using ATR-FTIR spectroscopy*

Author: Entesar Al-Hetlani[1], Zainab Husain[1], Mohamed Amin[1]

[1]Kuwait University

Abstract:
Peripheral blood in forensic investigations is related to identifying the origin of the blood evidence particularly in missing/murdered women and trauma from sexual and violent assault cases.[1] Herein, we propose the use of non-destructive attenuated total reflectance Fourier infrared (ATR-FTIR) spectroscopy for the analysis of whole peripheral blood stains deposited on different fabric. Both natural and synthetic fabrics contaminated with blood were investigated by obtaining the blood from different female donors and investigate the effect of substrate (fabric) type on the detection of the blood stain. 20 μL blood samples obtained from four female donors analysed on cotton, wool, satin, linen, organza, polyester, modal and chiffon substrates. The analysis showed promising results in terms of detecting the two main bands related to blood on all substrates, namely, the presence of amide I and amide II positioned approximately at 1640 cm^{-1} and 1533 cm^{-1} in blood-stained fibres.[2] Interestingly, detection of blood traces on wool fabric was challenging which could be due to it highly absorbing nature. Thus, given the proven track record of ATR-FTIR in forensic analysis, it is a promising strategy to expand the application of ATR-FTIR for detection of peripheral whole blood on natural and synthetic fabric substrates.

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Keywords: ATR-FTIR; peripheral whole blood, fabric,

G-I.6

Title: *Revealing the Secrets of Graeco-Roman Egyptian Mummies Using Vibrational Spectroscopic Techniques*

Author: <u>Bayden Wood</u>[1], Callum Gassner[1], Magdalena Giergiel[1], Ankit Dodla[1], Janet Davey[2]

[1]Monash University
[2]Victorian Institute of Forensic Medicine

Abstract:

Vibrational spectroscopic techniques, including Fourier-transform infrared (FTIR) imaging and Raman spectroscopy, have emerged as powerful tools for the analysis of ancient materials. Here we apply FTIR and Raman spectroscopy to study the chemical composition of Graeco-Roman Egyptian mummies to gain insights into their preservation.

One of the key findings of the study was the discovery of a 150 nm layer of a highly pure crystallized calcium soap complex in a 4-micron thick dewaxed tissue section from the mummies' neck. This layer was detected using FTIR imaging and indicates the presence of highly pure adipocere, a substance that forms during a process called saponification, which occurs after death and causes chemical changes in the body, converting body fat into adipocere, also known as grave wax.

Infrared spectroscopy has previously been applied to identify calcium soaps in adipocere (1,2). The mean extracted spectrum of adipose tissue (Fig 1), shows evidence of highly crystalline calcium soap with a characteristic $v_{as}(COO^-)$ doublet at 1575 cm^{-1} and 1539 cm^{-1} along with the vs(COO-) doublet appearing at 1434 cm^{-1} and 1420 cm^{-1}. The band at 1575 cm^{-1} is associated with unidentate carboxylate coordination with the Ca^{2+} ion, while the band at 1539 cm^{-1} is characeristic of bidentate coordination. Both unidentate and bidentate coordination are expected for 3-dimensional calcium soap crystal structures.(3) Surprisingly the H&E section shows clearly discernible crystallised adipocytes that have not degraded over time. Besides the calcium soap bands observed in the calcified adipose tissue, bands from collagen can be clearly seen above and below the crystallised adipocytes, indicating a high degree of tissue preservation. The incredible preservation of the adipocyte membranes and collagen in Graeco-Roman mummies brings into question the dogma that Old Kingdom mummification was more advanced than mummification that occurred during the New Kingdom.

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Figure captions:
Figure 1. A. H&E stained section of mummified tissue. B. FTIR image processed using UHCA. C.Mean extracted spectra from the UHCA image. D. Labelled spectrum of the calcium soap complex.

Keywords: Egyptian mummification, adipocere, calcium soap,

Figure 1. A H&E stained section of mummified tissue form the neck of a Graeco-Roman mummy. **B** FTIR image processed using UHCA from the region highlighted by the square in the H&E section. **C** Mean extracted spectra for each of the clusters in the UHCA image. **D** Labelled spectrum of the calcium soap complex.

G-O.1

Title: *SERS-based detection schemes in complex biological matrices*

Author: Dana Cialla-May[1], Natalia E. Markina[2], Alexey V. Markin[2], Juergen Popp[1]
[1]Leibniz Institute of Photonic Technology
[2]Saratov State University

Abstract:
Raman spectroscopy is known as powerful analytical tool in biomedical application schemes. Its limitation due to the intrinsic weak Raman effect is overcome by applying powerful plasmonic nanostructures creating the surface enhanced Raman spectroscopy (SERS) technique. SERS became attractive to identify and estimate the trace concentration of biomolecules even in complex matrices. [1] To perform SERS investigations, we applied as sensing principle the label-free or direct approach, which allows all molecules to contribute to the overall SERS spectrum. The specificity is increased for those molecules with high affinity towards the metal surface. To illustrate the potential of SERS in bioanalysis, we will introduce within this contribution various application scenarios. The bacterium *S. multivorans* is known to form PCE reductive dehalogenase (PceA) within the membrane, which is the key enzyme in respiration of a major groundwater contaminant, perchloroethylene (PCE). PceA harbors B12 which was detected by means of SERS after coating the SERS-active surface with the bacterial membrane. [2] Further on, the antibiotic sulfamethoxazole was spiked in human urine samples. In order to allow for quantification, a liquid-liquid extraction protocol in combination with SERS was established. Thus, background contributions could be suppressed and a satisfactory limit of detection of 1.7 µg mL^{-1} was achieved. [3] Finally, silver nanoparticles were modified with cyclodextrins (CD) to improve the performance of the SERS-based detection in body fluids due to a reduced interaction of matrix molecules with the sensing surface. CD-coated SERS-active nanostructures were applied for the estimation of fluoroquinolones, i.e. ciprofloxacin, norfloxacin, pefloxacin, and levofloxacin, in urine and blood plasma samples. Limit of detection values were determined for the tested antibiotics with 2.9–5.8 and 0.05–0.34 µg mL^{-1} in spiked urine and blood plasma, respectively. [4]

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Keywords: SERS,B12, antibiotics, body fluids, bioanalysis

G-O.2

Title: *Quantitative Raman Analysis of Carotenoid Protein Complexes in Aqueous Solution*

Author: Joy Udensi[1], Ekaterina Loskutova[1], James Loughman[1], Hugh Byrne[1]

[1]Technological University Dublin

Abstract:
Carotenoids are naturally abundant, fat-soluble pigmented compounds with dietary, antioxidant and vision protection advantages [1-3]. The dietary carotenoids, Beta Carotene, Lutein, and Zeaxanthin, complexed with in bovine serum albumin (BSA) in aqueous solution, were explored using Raman spectroscopy to differentiate and quantify their spectral signatures. UV visible absorption spectroscopy was employed to confirm the linearity of responses over the concentration range employed (0.05–1 mg/mL) and, of the 4 Raman source wavelengths (785 nm, 660 nm, 532 nm, 473 nm), 532 nm was chosen to provide the optimal response. After preprocessing to remove water and BSA contributions, and correct for self-absorption, a partial least squares model with R^2 of 0.9995, resulted in an accuracy of the Root Mean Squared Error of Prediction for Beta Carotene of 0.0032 mg/mL and Limit of Detection 0.0106 mg/mL. Principal Components Analysis clearly differentiated solutions of the three carotenoids, based primarily on small shifts of the main peak at ~1520 cm^{-1}. Least squares fitting analysis of the spectra of admixtures of the carotenoid:protein complexes showed reasonable correlation between norminal% and fitted%, yielding 100% contribution when fitted with individual carotenoid complexes and variable contributions with multiple ratios of admixtures. The results indicate the technique can potentially be used to quantify the carotenoid content of human serum and to identify their differential contributions for application in clinical analysis.

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Keywords: DietaryCarotenoids, Bovine Serum Albumin, RamanSpectroscopy

G-O.3

Title: *Towards a SERS electronic nose: VOC and gas sensing*

Author: Elle Wyatt[1], Marika Niihori[1], Sarah Sibug-Torres[1], Rakesh Arul[1], David- Benjamin Grys[1], Bart De Nijs[1], Jeremy Baumberg[1]

[1]University of Cambridge

Abstract:
Canines can smell cancer or COVID-19, however electronic noses are not yet able to replicate this multiplexed continuous sensing of volatile organic compounds (VOCs). Current sensing technologies such as GCMS are highly sensitive, but have high complexity and cost. Here we show that surface-enhanced Raman spectroscopy (SERS) can deliver an in-situ inexpensive sensing methodology with high sensitivity and specificity for trace VOC concentrations, which is enabled by repeated removal of all organics from the plasmonic hotspots followed by precision re-scaffolding.[1]

We have developed a reproducible SERS substrate that exploits self-assembly of gold nanoparticles (AuNPs) into 2D close-packed aggregate arrays with precision (0.9nm) plasmonic nanogaps defined by cucurbit[n]uril molecular spacers.[2] These aggregates can be deposited onto a range of substrates, and allow for backside illumination and integration in liquid/gas flow systems for real time measurement.

Immobilization of the AuNP aggregates as a monolayer on the substrate provides direct access to the nanogaps for analytes (Fig.1a), but also access to the scaffolding molecules in the gaps for further treatment. These SERS substrates can be regenerated by stripping organic molecules from the nanogaps and oxidizing the surface of the nanoparticles, providing a clean and metastable substrate that can be re-scaffolded with a wide variety of alternative molecules. This process allows for tuning the hotspot chemistry through choice of each new scaffold and for reuse of substrates for many cycles while retaining high SERS signals (Fig.1b). We successfully demonstrate the static sensing of a range of VOCs and gases using such functionalized SERS substrates, and their integration into gas flow cells. We monitor the binding and unbinding of small gas molecules such as CO_2 and ammonia to demonstrate their capability for sensing gases and VOCs in real time.

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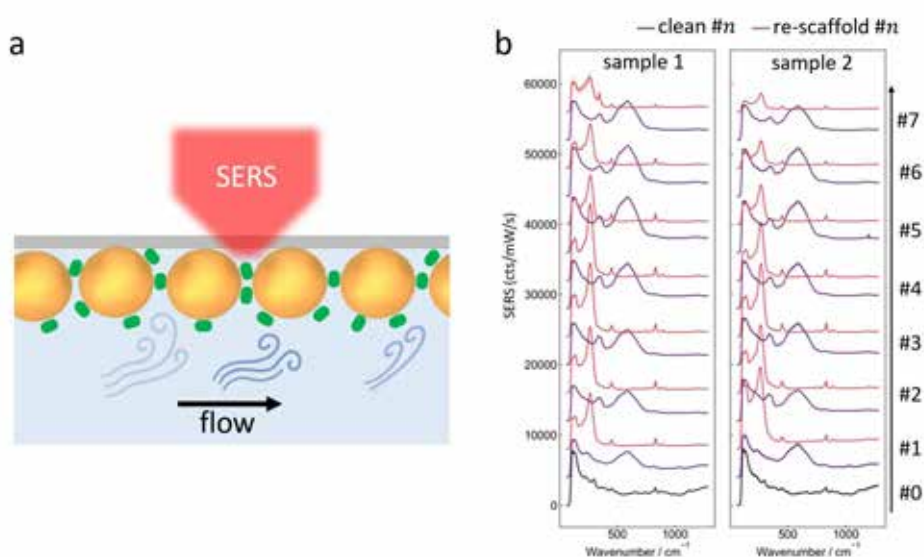
Figure captions:
Fig 1. a) Schematic of immobilised AuNP monolayer aggregate for in-flow gas sensing. b) Cleaning and re-scaffolding of the monolayer, demonstrating cucurbituril SERS signal recovery after each cycle.

Keywords: SERS, VOC, electronic-nose, sensing, plasmonics

G-O.4

Title: *Correlation analysis of spectroscopic and biological features to follow mesenchymal stem cell differentiation*

Author: Karolina Augustyniak[1], Hubert Latka[1], Monika Lesniak[2], Jacek Z. Kubiak[2], Robert Zdanowski[2], Kamilla Malek[1]

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Abstract:
Mesenchymal stem cells (MSCs) are the core of currently proposed cell-based therapies in regenerative medicine[1]. To recognize and characterize phases of their dynamic cellular transition, innovative and sensitive methods must be developed[2]. Among several transformation processes of MSCs, adipogenesis has been already introduced into clinics and regenerative medicine.
In this study, we employed High-Resolution Raman and FTIR Imaging, delivering complementary hyperspectral databases, to give a detailed insight into chemism of adipogenesis. We aimed to find spectral biomarkers of this cellular transformation and evaluate the potential of label-free imaging to distinguish the early and late phases.
For this purpose, primary MSCs isolated from adipose tissue of C57BL6 mice were cultured (in the Osteogenic/Adipogenic Base Media + Adipogenic Supplement) and the differentiation process towards adipocytes was observed from 6 h up to 14 days (four time points) by Raman (a Witec Alpha 300 microscope) and FT-IR imaging (an Agilent a 620-IR microscope with an FPA detector). Oil Red and fluorescence staining were used as the reference method. Statistical and chemometric methods were used for data analysis. Exemplary results from FTIR spectra are shown in Fig. 1. The major spectral features were observed in the lipid-specific region indicating alternations in their content, saturation, and acyl chain shortening and for bands of protein and nucleic acids. Importantly, the overall spectral region allowed discrimination of the early and late phases of MSC differentiation. We suggest the phase of clonal expansion, which leads to the formation of preadipocytes3, appears 48 h after induction of trans-formation. HCA and PCA results also indicated unique spectral characteristics of this stage. Finally, we employed the PLS-DA method to classify the degree of the formation of adipocytes with an accuracy higher than 90%.

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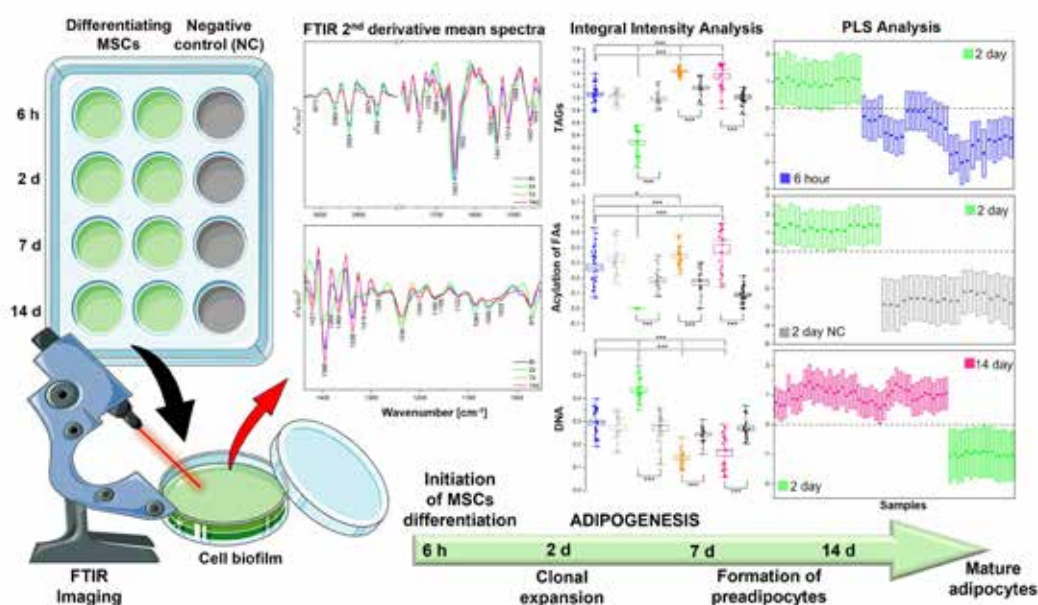
Figure captions:
Fig. 1 Scheme and exemplary results of spectroscopic analysis to follow mesenchymal stem cell differentiation

Keywords: Stem Cells, Adipogenesis, FTIR, Imaging

G-O.5

Title: *Thriving Advantages of Drug Combination in Osteosarcoma Treatment – A Vibrational Microspectroscopy Study*

Author: Raquel C. Laginha[1], Jéssica D. Silva[1], Maria Paula M. Marques[1], Gianfelice Cinque[2], Luís A. E. Batista de Carvalho[1], Ana L.M. Batista de Carvalho[1]

[1]Molecular Physical-Chemistry R&D Unit
[2]Diamond Light Source

Abstract:
Osteosarcoma (OS) is the most common primary malignant bone cancer with a poor prognosis for patients with metastatic or recurrent disease, with a greater incidence in children and young adults.[1] Hence, there is an urge to develop new and more effective anticancer agents while having minimal effects on healthy tissues. In the last 30 years, some progress has been achieved regarding OS therapy and survival rates have increased from less than 20% to 65-70% with the postoperative multidrug regimen designated as MAP (methotrexate (MTX), doxorubicin (DOX)) and cisplatin). However, since the severe toxicity associated with MAP is a limiting factor the currently ongoing European and American Osteosarcoma Study (EURAMOS-1) phase III clinical trial seeks to improve survival rate of OS patients through MAP concentration adjustments.[2] The advantage of a combined therapy is to be able to deliver the same or an enhanced cytotoxic effect relative to the one attained with each drug individually, with less deleterious side effects. Vibrational microspectroscopy – both FTIR with synchrotron radiation and Raman – were used to assess drug's bioavailability, biodistribution, metabolic impact and cellular response to treatment., Newly synthesized cisplatin-like compounds were tested (Pd_2Spm and Pd_3Spd_2), both alone and in combination using the MAP regimen, against both osteosarcoma (cancer cells) and osteoblasts (healthy cells) cell lines.[3,4]
The results thus gathered clearly evidenced a spectral differentiation between the control cells, the cells treated with the MAP combination according to the EURAMOS-1 protocol – incubation with Pt(II)/Pd(II) drug at 4.8 μM + DOX at 3 μM for 72 h after which a dose of MTX at 4.8 μM was added for an additional 24 h period – and cells treated with Pt(II)/Pd(II) drugs at their IC_{50} – cisplatin (12 μM), Pd_2Spm and Pd_3Spd_2 (14 μM). Interpretation of the data was carried out through unsupervised PCA analysis of the spectra.

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Figure captions:
Schematic representation of the experiment.

Keywords:
Osteosarcoma, microRaman, microFTIR

G-O.6
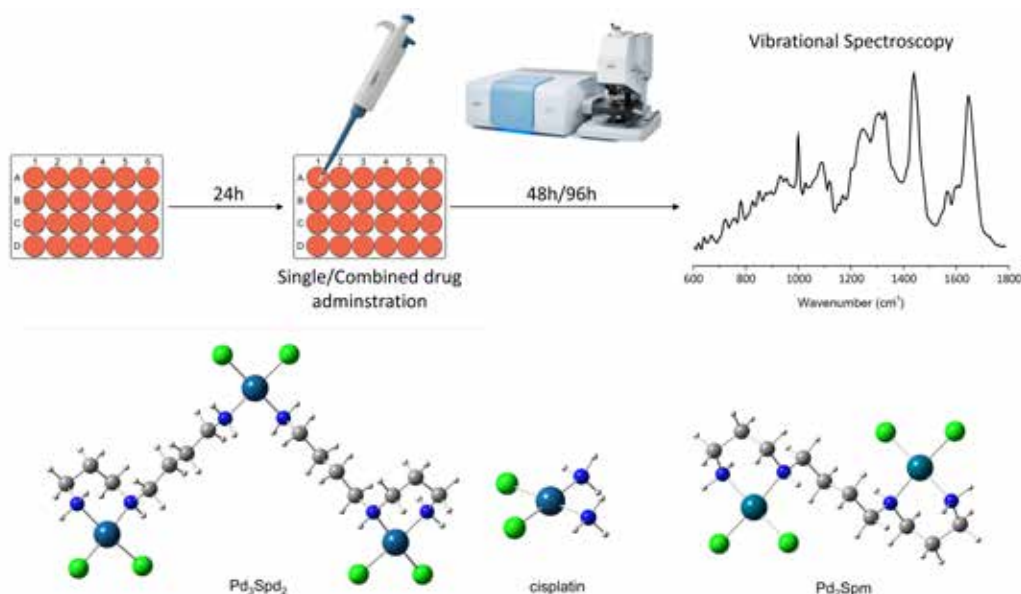
Title: *ATR-FTIR spectroscopic study of cells from the human monocytic cell line MONO-MAC-6 with stimulation by insulin*

Author: H. Michael Heise[1], Jacinta Tomas Borges[1], Yannik Merx[1], Saskia Simon[1], Sandra Stoppelkamp[1]
[1]SOUTH-WESTPHALIA UNIVERSITY OF APPLIED SCIENCES

Abstract:
FTIR-spectroscopy has been applied to various fields of biospectroscopy with the advantages of being non-destructive and label free [1]. Our interest was in monocytes as part of white blood cells, which play essential roles in inflammation and adaptive immunity. Glucose is a major energy source for activated monocytes while its uptake is facilitated by glucose transporters. We hypothesized that insulin dependent monocytes [2] could be used as tools to study insulin action at the cellular level and facilitate the investigation of insulin activity. Cells from the human monocytic cell line MONO-MAC-6 [3] were cultivated in a very-low endotoxin RPMI cell medium, enriched with amino acids and fetal bovine serum.
ATR-spectroscopy has been utilized to monitor transient states of the cells under treatment or activation. Spectra were recorded from samples at the interface as the evanescent wave exponentially decays within a few micrometers. Many of the reported IR- spectra have been measured from dried cells. The hydration level was monitored to support our spectral interpretations. Initially, influences of medium additives on the cells were analyzed and supplements optimized. For sample preparation, the isolated cells were re-suspended in physiological NaCl solution. Either cell pellets or cell suspensions were placed on the ATR-diamond element. Cells were dried with repeat recordings after reaching stability. The spectral interpretation in the fingerprint region is complicated, as bands of biological macromolecules have features heavily overlapped. Main interest was in the protein amide I and II bands. Cells were monitored over five days of cultivation with insulin addition at the first day. Further experiments were carried out for investigating the dynamic cellular response within 2 h after adding insulin. Fifteen minutes after stimulation, spectral features not apparent in resting MM6 cells became obvious. Further studies and spectral interpretation are ongoing.

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Keywords: ATR-spectroscopy, monocytes, insulin stimulation

G-O.7

Title: *Shedding new light on the action of cisplatin, 5-fluorouracil, and 5-azacytidine on primary Oral Squamous Carcinoma Cells by Raman Microspectroscopy coupled with multivariate statistical analyses*

Author: Valentina Notarstefano[1], Alessia Belloni[1], Paolo Mariani[1], Elisabetta Giorgini[1], Hugh J. Byrne[2]

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[2]Technological University Dublin

Abstract:
The effects of three nuclear targeting chemotherapeutic agents, cisplatin (cisPt)[1,2], 5-fluorouracil (5FU)[1], and 5-azacytidine (5aza)[3], on primary Oral Squamous Carcinoma Cells were *in vitro* monitored by exploiting Raman Microspectroscopy. The three drugs have distinct modes of action: cisPt forms inter- and intra-strand DNA cross-linkages; 5FU acts by misincorporation into RNA and DNA; 5aza is a demethylating agent. Hence, the aim of this work was to elucidate subtle and specific spectral changes related to peculiar aspects of their mechanisms of action.

Towards this aim, a variety of multivariate analysis techniques, notably Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) regression and Principal Components Analysis (PCA) was employed. MCR-ALS regression was used to unmix the cell population and follow its progression, from viable to apoptotic, as a consequence of the action of cisPt, 5FU or 5aza, by decomposing the Raman spectral profiles acquired from cells after 16-72 hours of exposure.

In cisPt exposed cells, MCR-ALS regression associated the early stage (16h) spectral profile with that of viable cells, while those of the intermediate (24h) and late (48h) stages with early and dead cells, respectively. As regards the cell populations exposed to 5FU and 5-aza, PCA elucidated spectral differences *indicative* of the initial mode of action of the drugs, and also evidenced late stage differences, particularly for the case of 5-azacytidine, possibly related to a differing cell death pathway. The study contributes to the body of evidence supporting the applications of Raman MicroSpectroscopy, in conjunction with multivariate analysis techniques, for label-free *in vitro* pre-clinical screening of drugs, given its ability not only to show the effects of a treatment, but also to shed new light on its mode of action.

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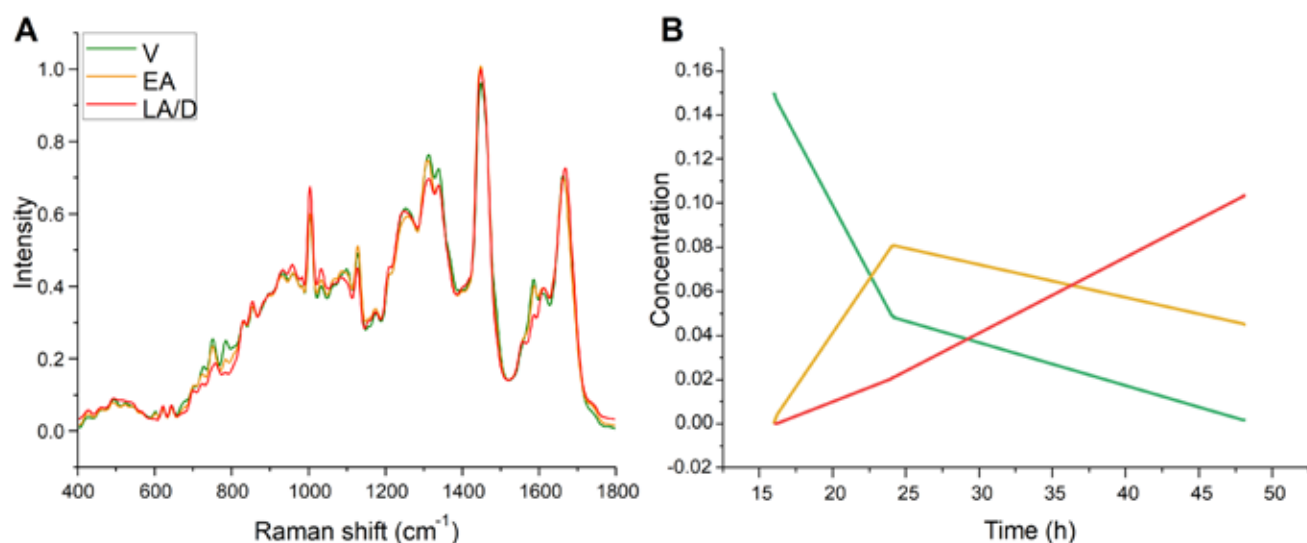
Figure captions:
(A) MCR-ALS Component spectra and (B) kinetic evolution for cisPt treated cell populations. V=viable cells; EA=early apoptotic cells; LA/D=late apoptotic/dead cells.

Keywords: Cisplatin, 5-fluorouracil, 5-azacytidine, Raman MicroSpectroscopy,

G-O.8

Title: *Multimodal Spectroscopic Imaging (MALDI MSI vs Raman imaging / FTIR) in the analysis of the secondary metabolites*

Author: Mikolaj Krysa[1], Katarzyna Suśniak[2], Monika Szymańska-Chargot[3], <u>Anna Sroka-Bartnicka</u>[1]

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[2]1Independent Unit of Spectroscopy and Chemical Imaging, Biomedical Faculty, Medical University of Lublin; 2 Department of Genetics and Microbiology, Institute of Biological Sciences, Maria Curie- Sklodowska University
[3]Institute of Agrophysics, Polish Academy of Sciences

Abstract:

Usually the investigation using spectroscopic imaging focuses on one particular technique (eg. **FTIR imaging Raman spectroscopic imaging or MALDI mass spectrometric imaging**), however simultaneous use of variety of spectroscopic techniques can provide a wider insight to the metabolic changes occurring in the tissue. The use of MALDI mass spectrometric imaging can provide an information about the distribution and concentration of small, easily ionisable molecules eg. flavonoids or plant hormones[1]. The use of Raman spectroscopic imaging on the other hand can provide an information on the concentration and distribution of substances that are not easily ionised eg. structural polymers such as cellulose, hemicellulose and lignin[2]. The use of infrared spectroscopy, however provides and insight on the concentration and distribution of proteins, lipids and carbohydrates with an emphasize on the dominant secondary protein structure[3]. **In the present study, three of the above-mentioned techniques were used to evaluate the metabolic changes** in the pea root nodule to assess the efficiency of atmospheric nitrogen fixation process.
Within this study, it was demonstrated that both purified and non-purified biofertilizers stimulate the growth and development of pea plants; however, the purified preparation is more effective.
The higher amount of α-helical proteins correlated with the lower amount of basic amino acids, possibly due to the higher efficiency of nitrogen fixation and higher efficiency of the nitrogen species transport (higher amount of basic amino acid transporters) in the Nod-factor-based biofertilizer treated plants.
We can state that this study demonstrated that different chemical imaging spectroscopic methods can be used as effective tools to study the molecular distribution of nitrogen fixation metabolites.

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Acknowledgments:

Keywords: MALDI MSI, FTIR, Raman, metabolites

G-O.9

Title: *Spectroscopic analysis of cancer-derived small extracellular vesicles for in vitro cancer diagnosis*

Author: Yuling Wang[1], Wei Zhang[1]

[1]Macquarie University

Abstract:
Cancer-derived small extracellular vesicles (sEVs) carry various messages and signal biomolecules to constitute the key features of cancer cells, which make them as highly competitive non-invasive biomarkers for cancer diagnosis/prognosis.[1] However, the sEVs are highly heterogeneous that the molecular signatures of sEVs may vary from different isolation approaches, original resources and concentrations. In this contribution, we have applied a variety of spectroscopic methods including Raman, surface-enhanced Raman scattering (SERS), AFM-IR to characterize the cancer-derived sEVs.[2] Figure 1 shows the schematic illustration of sEVs, and the typical SERS spectra of sEVs from different cell lines, indicating the different SERS spectral features of sEVs from different cell lines. To improve the specificity for the detection of cancer-derived sEVs, we have proposed a few specific approaches by taking the advantages of magnetic beads[3-4] and microfluidic devices[5] to specifically enrich the target sEVs on the surface, as well as the use of SERS nanotags for specific reading of the surface biomarkers on sEVs.

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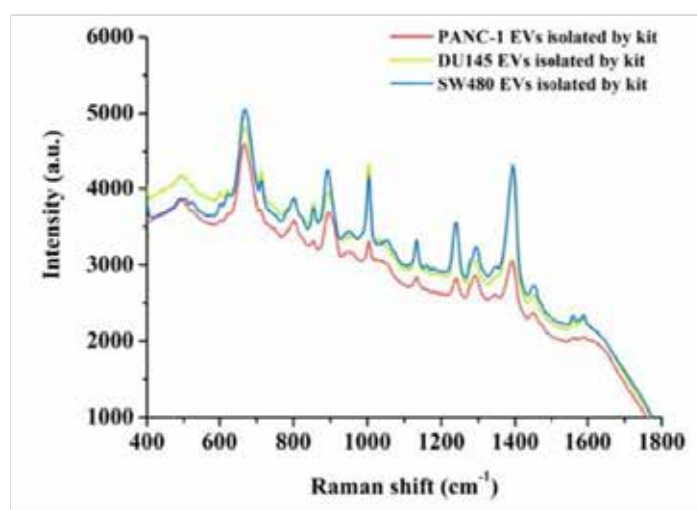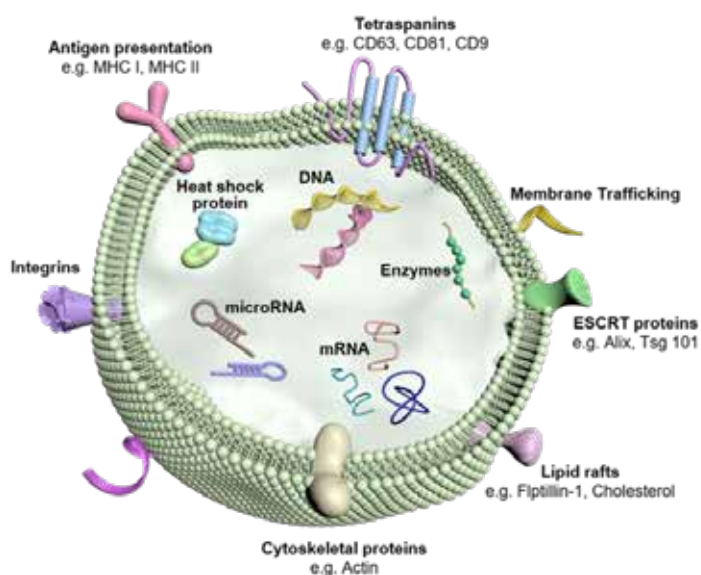
Figure captions:
Scheme (left) of sEVs composed of a lipid bilayer vesicle containing nucleic acids, proteins, lipids and other small molecules; and typical SERS spectra (right) of sEVs from different cell lines.

Keywords: Extracellular Vesicles, SERS, cancer diagnosis

G-O.10

Title: *Chemically-specific in situ coherent Raman imaging of liquid-liquid phase separation in the crystallization process of pharmaceutical solids*

Author: Alba Arbiol[1], Laurin Zöller[2], Teemu Tomberg[1], Jukka Saarinen[1], Tom Konings[1], Sara Carlert[3], Eva Karlsson[3], Anders Borde[2], Quentin Vicentini[2], Christoph Saal[3], Jennifer Dressman[2], Clare Strachan[1]

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[3]AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

Abstract:

The crystallization mechanisms of organic molecules in solution are still not fully understood. Non-classical crystallization (NCC) theory has emerged as an alternative possibility to the well-established classical nucleation theory (CNT). According to NCC, crystallization occurs in two steps involving the initial formation of phase separated prenucleation clusters, known as liquid-liquid phase separation (LLPS) (1-3). To date, characterization of LLPS and NCC has been limited to non-spatially or non-chemically-resolved analyses. The aim of the present study, was to investigate the potential of stimulated Raman spectroscopy (SRS) for chemically-specific real-time imaging of LLPS and NCC in a pharmaceutical context.

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that is often formulated as a salt. Several studies have suggested NCC of ibuprofen using indirect detection and non-chemically specific microscopy (4-6). In the present study, crystallization after initial dissolution of two racemic ibuprofen salts (lysine and sodium) was chemically-specifically imaged *in situ* in real time using SRS. SRS analyses were performed with an in-house built coherent Raman imaging microscope that allowed rapid recording of chemically-specific vibrational hyperspectral images.

The SRS images showed distinct processes over time upon dissolution of the ibuprofen salts (Figure 1). First, the formation of dense liquid intermediate droplets enriched with ibuprofen, representing LLPS were successfully imaged. Subsequently, solid needle-shaped crystals corresponding to the free acid form were identified.

The results are, to our knowledge, the first demonstration of chemically-specific and label-free real-time imaging of LLPS. The study demonstrates the suitability of SRS for label-free imaging and elucidation of complex crystallization processes in real-time, with the potential to provide deeper insights into LLPS and NCC in the pharmaceutical and other industrial sectors.

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Acknowledgments:

Figure captions:

Figure 1. (A) Schematic representation of the observations upon dissolution of ibuprofen salts. (B-G) SRS images of identified LLPS and free acid crystals. (H) SRS spectra of different species.

Keywords: Stimulated-Raman-scattering-microscopy, hyperspectral-imaging, ibuprofen, cystallization, liquid-liquid-phase-separation

G-O.11
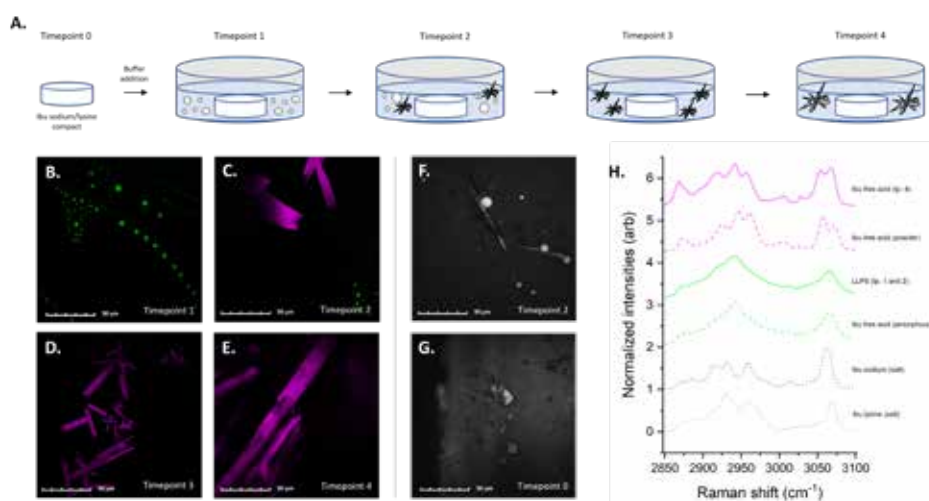
Title: *Spectral identification of therapeutic allergen products*

Author: Christian Ickes[1], Pirya Rani[2], Kristiyana Tsenova[3], Johanna Rost[1], Frank Führer[1], Detlef Bartel[1], Christel Kamp[1]

[1]Paul-Ehrlich-Institut
[2]Saarland University
[3]Goethe University

Abstract:
Raman spectroscopy is a widely used technique in the quality control of pharmaceutical products. Inelastic scattering of laser light generates unique fingerprints of chemical compounds which allows for identification of products and potentially quantification of active components.

The spectroscopic analysis of biomedicines like vaccines or therapeutic allergen products introduces new challenges as these products show inherent variability and contain excipients that strongly contribute to the spectral signal [1-3]. Therefore, standardization in experimental and analytical protocols is particularly relevant. Spectral pre-processing affects each analysis's outcome. We show that Raman spectroscopy can distinguish between near-related bee and wasp therapeutic allergen products from different manufacturers with varying sensitivity and specificity depending on the details of prior pre-processing. Using machine learning and statistical techniques based on different models, we found that completely processed Raman spectra from bee and wasp venoms can be differentiated with accuracies above 95%. While baseline correction had a major impact on the separability of spectra, unprocessed spectra showed a high variance obfuscating relevant spectral differences. Our results demonstrate that Raman spectroscopy can serve as a method to distinguish between therapeutic allergen products and offers a proof-of-concept for the applicability of Raman spectroscopy in the quality assurance of biomedicines. Further improvements and standardization in experimental protocols and spectral analysis are required to ensure robust and reliable predictions.

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Keywords: Raman spectroscopy, machine learning, biomedicines

G-O.12

Title: *Raman-based Detection of Antibiotics and Metabolites in Pharmaceutical Formulations and Clinical-relevant Matrices*

Author: Chen Liu[1], Jürgen Popp[1], Dana Cialla-May[2]

[1]Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP), Friedrich Schiller University Jena, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Helmholtzweg 4, 07743 Jena, Germany
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Abstract:
Raman spectroscopy is a powerful tool for bioanalytical detection methods due to its molecular-specific fingerprint information. The inherent weak Raman signal can be enhanced by several orders of magnitude by applying plasmonic-active nanostructured surfaces, creating surface-enhanced Raman spectroscopy (SERS). As a consequence, analyte molecules in the µM range or lower can be detected with high specificity. A Raman-based label-free analytical method was developed to detect the antibiotic ciprofloxacin (CIP) in various pharmaceutical formulations. [1] The Raman spectral analysis is performed for semi-quantification in the case of a low background Raman signal, i.e., the signal originating from the excipient and carrier substance of the formulation does not interfere with the fingerprint spectrum of CIP. In the case of a background spectrum rich in Raman modes originating from the excipient and carrier substance of the formulation, the pharmaceutical formulation is diluted 1:5000, and thus, the background signal is undetectable. Due to the high affinity of CIP towards metallic surfaces, SERS is applied to allow for the sensitive detection of this antibiotic even in complex pharmaceutical formulations with high dilution ratios. Moreover, pyrazinoic acid (POA) is detected in cultural supernatants by using specially designed gold nanoparticles coated with Prussian blue. POA is the metabolite of the drug pyrazinamide (PZA), applied in tuberculosis treatment. [2] POA can be detected by means of SERS applying Prussian blue coated SERS probes in a complex biological matrix. This illustrates the potential of the proposed detection scheme for an assessment of the resistance of M. tuberculosis in cultures as only sensitive strains allow the metabolism from PZA to POA. Finally, we developed a SERS-based detection scheme using silicon nanowires decorated with silver nanoparticles to detect the antibiotic ceftriaxone (CRO) in spiked blood plasma and microdialysis solutions.

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Keywords: Raman spectroscopy, SERS, ciprofloxacin, pyrazinoic acid, ceftriaxone

G-O.13

Title: *Insights into triglycerides removal: Study using FTIR and Raman imaging in flow and static conditions*

Author: Gunjan Tyagi[1], Zain Ahmed[1], Joao Cabral[1], Sergei Kazarian[1]

[1]Imperial College London

Abstract:
Understanding and controlling the adhesion and cohesion, delamination and dissolution, and eventual removal of triacylglycerols (TAG) films as a surrogate for organic soil from solid surfaces is important in both industrial and household environments[1] impacting food manufacturing, hygiene, environmental safety and health[2]. Vibrational spectroscopic imaging has been shown to be a powerful label-free and nondestructive chemical imaging method[3]. We have utilized the potential of FTIR and Raman imaging to understand the mechanism of removal of triacylglycerol films under flow and static soak conditions.
We employed a customized microfluidic flow device in the laminar regime and a model, binary, surfactant solution to monitor the removal of a model ternary TAG mixture composed of triolein, tripalmitin, and tristearin utilizing an ATR macro imaging system. Our preliminary results suggests that the TAG film spatially receded over time, with an exponential decrease in the absorbance of the carbonyl band , corresponding to the film's slow erosion or dissolution. Raman imaging in static soak conditions shows the heterogeneous distribution of triolein-specific bands (C=C at 1650 cm^{-1} and 3005 cm^{-1}) with their intensities increasing along the depth of TAG film. However, after 20 minutes of soaking triolein was found to have a higher concentration at the surface as compared to tripalmitin and tristearin indicating toward its preferential elimination over time.
Overall, our findings demonstrate the potential of FTIR and Raman imaging to provide the spatial and temporal resolution necessary to unravel the complicated chemistry of adhesion and cohesion implicated in TAG film removal. The findings contribute to the evaluation of organic soil properties, interactions, and underlying mechanisms, which is essential for the development of high-performance and environmentally friendly formulations that meet the specific cleaning and environmental criteria.

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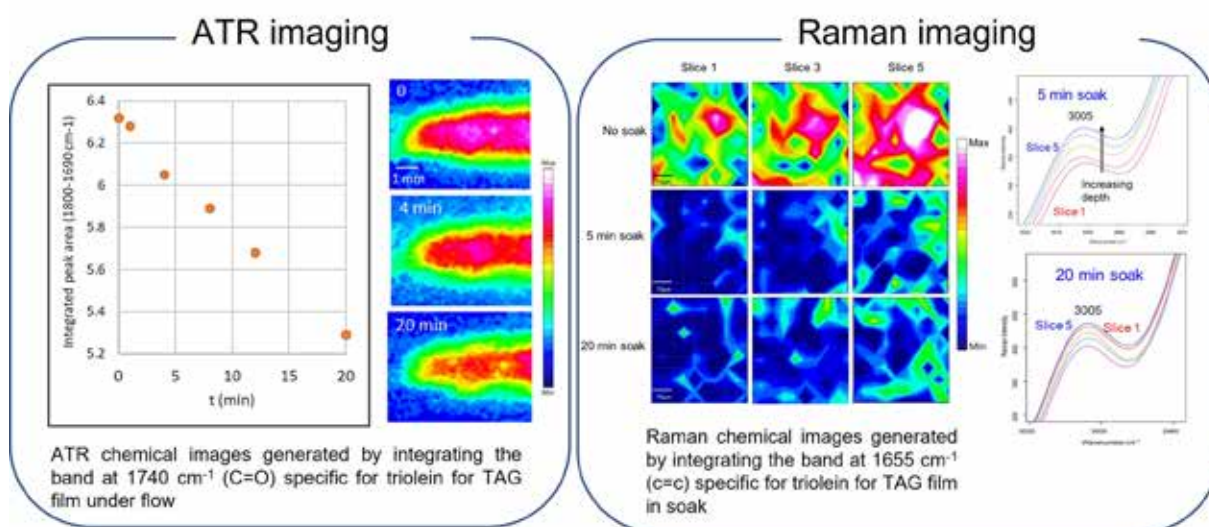
Figure captions:
Figure 1: Left; time series ATR images and C=O intensity change of TAG removal under flow, Right; Raman Images and spectra showing the variation of triolein bands with time in soak.

Keywords: Triglycerides, FTIR, Raman imaging, microfluidics

G-O.14

Title: *Rare earth-citrate complexes study using surface-enhanced Raman scattering spectra*

Author: Hao Jin[1], Tamitake Itoh[2], Yuko. S. Yamamoto[1]

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[2]Nano-Bioanalysis Research Group, Health Research Institute, National Institute of Advanced Industrial Science and Technology

Abstract:
Rare earth (RE) elements possess a unique 4f electron configuration, resulting in distinctive optical, magnetic, and spin physical properties that make RE-molecular complexes highly promising for functional materials research and applications [1]. Despite these benefits, the similar chemical properties of RE elements and difficulties in measuring RE^{3+} ions have posed challenges for RE analytical studies[2]. However, surface-enhanced Raman scattering (SERS) offers a viable solution for studying specific physical properties of low-concentration RE-molecular complexes in aqueous solutions, as it significantly enhances Raman signals, enabling detection of analytes at low concentrations ($<10^{-5}$M) [3-4]. Thus, establishing the correlation between physical properties of RE elements and SERS spectra is of significant importance for RE analytical chemistry.
In this study, we utilized citrate-capped silver nanoparticles to investigate the spin effect of two non-fluorescent RE^{3+} ions, lanthanum (La) and gadolinium (Gd), on the enhancement and characteristic differences of SERS spectra. We observed changes in the intensity of SERS characteristic peaks around 1070 cm^{-1} and 1315 cm^{-1} upon incorporation of RE^{3+} ions into citrate molecules. Density functional theory (DFT) calculations revealed that these characteristic peaks were related to the coordination of carboxylic and hydroxyl groups of the citrate and RE^{3+} ions, indicating the presence of spin-correlated bands of the RE^{3+} ions. Additionally, by analyzing the relative intensity ratios of the SERS peaks, we could qualitatively differentiate between La^{3+} and Gd^{3+}.

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Figure captions:
Figure 1. SERS spectra of (a) La-citrate and (b) Gd-citrate excited at 488 nm (red line) and 532 nm (black line) with the schematics of RE-citrate complexes adsorbed on the silver cluster's surface.

Keywords: Surface-enhanced Raman scattering, Rare earth

G-O.15
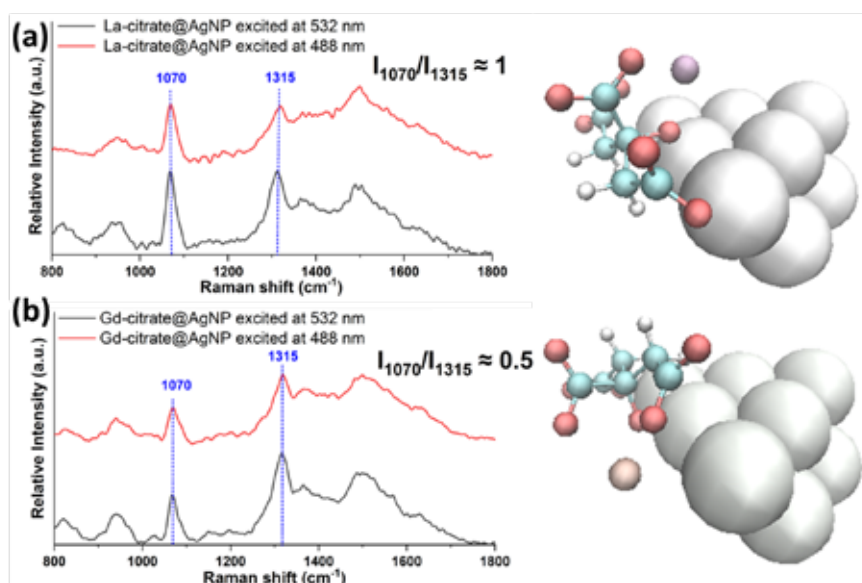
Title: *Silicon within fossil and cultivated coccoliths of Helicosphaera carteri: new insights from Infrared Spectromicroscopy and X-ray Fluorescence analyses*

Author: Giovanni Birarda[1], Manuela Bordiga[2], Diana Eva Bedolla[3], Alessandra Gianoncelli[1], Simone Pollastri[1], Valentina Bonanni[1], Gianluca Gariani[1], Lisa Vaccari[1], Federica Cerino[2], Marina Cabrini[2], Alfred Beran[2], Mario Zanoni[4], Maurizio Zuccotti[4], Giulia Fiorentino[4], Miriam Cobianchi[5], Andrea Di Giulio[5], Claudia Lupi[5]

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[2]National Institute of Oceanography and Applied Geophysics OGS
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Abstract:

Coccolithophores, one of the main marine calcifiers, significantly affect the global carbon cycle since geological time by capturing CO2 through photosynthesis and permanently fixing it in their shells composed of micrometrical plates, i.e. coccoliths.[1] However, the physiology and proliferation strategies of coccolithophores are still poorly known, especially at species-specific level.
Monospecific cultures of *Helicosphaera carteri* were grown under 290 ppm of CO_2 mimicking the conditions recorded during Marine Isotope Stage (MIS) 5. These samples were compared to species-specific fossil coccoliths isolated from two deep-sea sediment samples of the NW Pacific (Ocean Drilling Program Site 1209) and deposited during MIS 5, a good analogue of modern warming, and during the foregoing glacial phase MIS 6. The coccoliths were then analyzed at three beamlines of Elettra Sincrotrone Trieste: XRF, TwinMic, and SISSI-Bio.
The infrared beamline capabilities allowed at first acquiring infrared images with FPA detector, and then, by exploiting the brightness of IRSR, collecting vibrational spectra on single coccoliths with high S/N ratio. The strongest peaks recorded were the ones related to $CaCO_3$, but it was possible to observe also the presence of Si-X signal and some organic residues. Recent studies on DNA sequences proved that some living species need silica-transporters (SIT) or silica-like transporters (SILT) to build their mineralized shell [2,3]. After IR, the presence of silicon has been confirmed by the two X-ray beamlines. XRF beamline detected Si in samples at macroscale, whereas TwinMic returned Si distribution maps on single coccoliths at sub-micrometric resolution. Thanks to the high number of coccoliths measured at SISSI-Bio, it waspossible to add the key statistical value to the findings, that could be deemed pivotal in coccolithophore studies, since monospecific chemical analyses in the fossil record are extremely complex, if not almost impossible.

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[2] Durak G.M., Taylor A.R., Walker C.E., Probert I., de Vargas C., Audic S., Schroeder D., Brownlee C., Wheeler G.L., 2016. A role for diatom-like silicon transporters in calcifying coccolithophores. Nat. Commun. 7, 1, 10543, doi: 10.1038/ncomms10543.
[3] Langer G. Taylor A.R., Walker C.E. Meyer E.M., Ben Joseph O., Gal A., Harper G.M., Probert I., Brownlee C., Wheeler G. L., 2016. Role of silicon in the development of complex crystal shapes in coccolithophores. New Phytol. 231, 5, 1845-1857.

Acknowledgments:

Keywords: Coccolithophores, Carbon cycle, FTIR, Synchrotron

G-O.16

Title: *Methods of vibrational microspectroscopy for the assessment of the internalization, biodistribution, fate and toxicity of nano- and microparticles at in vitro and in vivo conditions*

Author: Joanna Chwiej[1], Natalia Janik-Olchawa[2], Agnieszka Dróżdż[3], Aleksandra Wajda[2], Maciej Sitarz[1], Daniel Horak[4], Michal Babic[4], Jolanta Gol[1], Zuzanna Setkowicz-Janeczko[2], Aleksandra Wilk[1], Marzena Rugieł[1], Katarzyna Matusiak[1], Christoph Sandt[5], Ferenc Borondics[5], Magdalena Wytrwał-Sarna[1]

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[4]Czech Academy of Sciences
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Abstract:

Nano- and microparticles can come from natural or anthropogenic sources, and those of anthropogenic origin can be a by-product of human activity or be designed and manufactured for specific applications [1]. Regardless of the source of origin, however, such small materials may have an adverse effect on human health and life [2]. The toxicity/biocompatibility studies of nano- and microparticles are carried out primarily in vitro on cell cultures, using both classical and modern methods of cell biology [3]. Although such investigations are the source of valuable mechanistic information on the harmfulness of various materials, do not allow for the assessment of their systemic impact on a living organism. For this purpose, in vivo research basing on laboratory animals are typically used [4].

As part of the presentation, examples of own research will be presented, in which the methods of vibrational microscopy (FTIR and Raman) were utilized to assess the toxicity of nano- and microparticles (respectively, superparamagnetic iron oxide nanoparticles and polystyrene microparticles) both at in vitro and in vivo conditions. Using the above mentioned methods of molecular spectroscopy, it was possible to confirm the internalization of the tested materials into cells, to assess their biodistribution and fate, as well as the pathological processes induced by them [5],[6].

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[6] Drozdz A, et al.: Spectrochim Acta A Mol Biomol Spectrosc 2020, 236, 118355.

Acknowledgments:

Keywords: FTIR, Raman, SPIONs, microplastics, toxicity

G-O.17

Title: *The increase of fibres and flavonoids concentration in the Zea mays stem treated with Nod-factor-based biofertilizer. A multimodal imaging study.*

Author: Mikolaj Krysa[1], Katarzyna Susniak[2], Cai Li Song[3], Monika Szymanska-Chargot[4], Artur Zdunek[4], Izabela S. Pieta[5], Janusz Podlesny[6], Anna Sroka-Barnicka[1], Sergei G. Kazarian[3]

[1]Medical University of Lublin, Independent Unit of Spectroscopy and Chemical Imaging,
[2]Maria Curie-Sklodowska University, Department of Genetics and Microbiology
[3]Imperial Collage London, Department of Chemical Engineering
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[5]Institute of Physical Chemistry, Polish Academy of Sciences
[6]Institute of Soil Science and Plant Cultivation, State Research Institute

Abstract:
Maize (*Zea mays*) is a plant that have the highest production weight from all other plants species all over the world , therefore the need for new fertilizers that do not pollute the environment is high. One of good candidates for such are Nod-factor-based biofertilizers. The aim of this study was evaluation of Nod-factor-based biofertilizer on the growth, yield and metabolic changes in the maize. The grains of maize were covered with biofertilizer and the field study was conducted. The grain yield and the whole plant was weighted. The stem was then used for the MALDI mass spectrometry imaging, Raman spectroscopic imaging and FTIR spectroscopic imaging, ATR-FTIR spectroscopy and DRIFT spectroscopy. The field study showed the statistically significant increase of the yield by 4.22% and the increase in the mass of the whole plant by 11.25%. MALDI mass spectrometry imaging showed increase in the flavonoid concentration (namely rutin, myasin and quercetin), while vibrational spectroscopy showed increase in the fibres concentration. These results indicate that the Nod-factor-based biofertilizer might be used for increasing the yield of maize, the mass of the whole plant, for increasing the concentration of flavonoids (that protect the plant from herbivores and UV radiation)[1,2] and to increase the amount of fibres, which are essential for the endurance of stem and therefore increase the lodging resistance, which decreases the amount of the yield up to 25% [3,4].

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Keywords: Maize, Nod factor, Spectroscopic imaging

Title: *Development of Raman Spectroscopic analysis techniques to assess quality biomarkers in fish*

Author: Jeremy Landry[1], Peter Torley[1], Ewan Blanch[1]

[1]RMIT University

Abstract:
Fish have been a major source of food for the world's population for thousands of years. As fish production trending is directed away from wild capture and more towards aquacultural practises, the analytical techniques used to measure and monitor fish quality will be required to be further developed, improved, and optimised. The development of rapid, non-destructive analysis techniques is highly sought after in food production, and which is well suited to the use of Raman Spectroscopy coupled with chemometrics.

We present here our results highlighting several ways this technology can used to measure quality parameters, several different Raman techniques have been used to develop to analytic methods targeting quantitation of biomarkers in fish. Spatially Offset Raman Spectroscopy was utilised to determine if biomarkers could be measured in the tissues of whole fish using sub-surface measurements; a method to quantitate carotenoid concentration through-skin in Atlantic Salmon with a portable Raman instrument using defocused measurements; and a SERS based method for detection of histamine, which is linked to immunogenic responses in seafood.

References:
Landry, JD, Torley, PJ & Blanch, EW 2020, 'Detection of Biomarkers Relating to Quality and Differentiation of Some Commercially Significant Whole Fish Using Spatially Off-Set Raman Spectroscopy', Molecules, vol. 25, no. 17 Landry, JD, Torley, PJ & Blanch, EW 2022, 'Quantitation of carotenoids and fatty acids from Atlantic salmon using a portable Raman device', Analyst, vol. 147, no. 19, pp. 4379-4388.
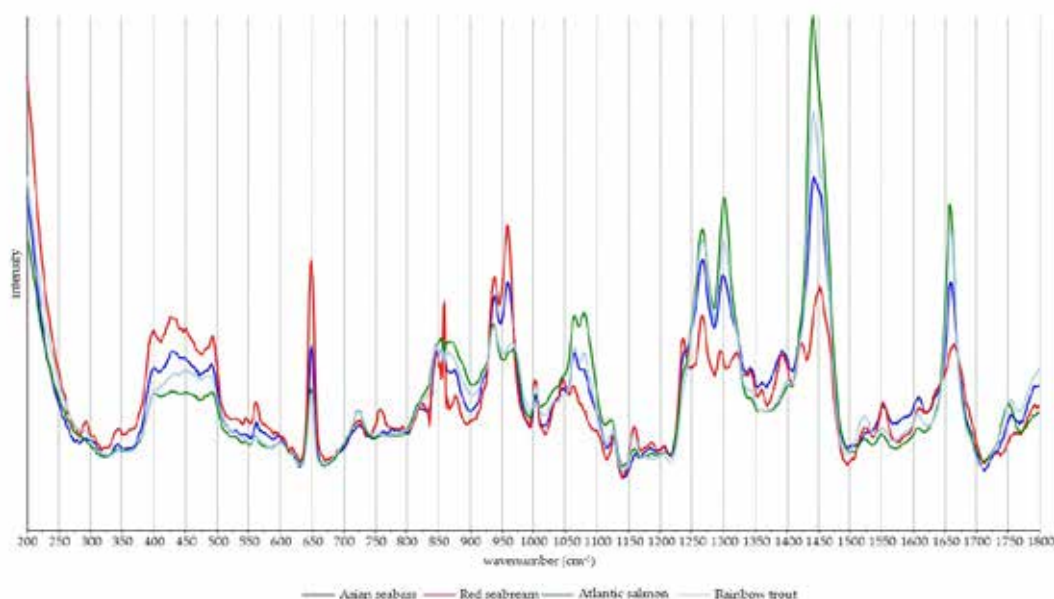
Figure captions:
Mean process SORS spectra of some commercial fish

Keywords: SORS, SERS, food analysis,

G-O.19

Title: *Visualization and identification of components in a gigantic spherical dolomite concretion by Raman imaging and MCR analysis*

Author: Ryosuke Kitanaka[1], Motohiro Tsuboi[2], Tomoko Numata[3], Yusuke Muramiya[4], Hidekazu Yoshida[5], Yukihiro Ozaki[2]

[1]Kewansei Gakuin University
[2]Kwansei Gakuin University
[3]HORIBA Techno Setvice Co. Ltd.
[4]Fukada Geological Institute
[5]Nagoya University

Abstract:

We have investigated visualization and identification of components in a gigantic spherical dolomite concretion by Raman imaging and MCR analysis. This study had dual purposes. One is to demonstrate the potential of the combination of Raman imaging system (LabRAM Soleil, HORIBA) and MCR analysis (MVA Plus in LabSpec6, HORIBA). Another purpose is to explore the distribution and identification of components in a concretion with particular interest in investigating if there are components with biological origin or not.

The sample was taken from the gigantic spherical dolomite concretion in the Miocene Morozaki group, Chita Peninsula, southwestern Japan. The diameter of the concretion is approximately 170 cm, and the analyzed sample is located 79 cm from the exposed sample surface. Raman imaging of 101-point square with 4 µm step was developed from the collected Raman spectra by use of the Raman imaging system. The excitation wavelength used was 785 nm. The obtained Raman spectra of the fossil suffered from severe fluorescence, and thus we treated the spectra with SVD first, and then the smoothing was applied. Finally, baseline correction was carried out.

Figure 1 (a) displays one of the Raman images obtained by 0326 CLS and (b) exhibits loading spectra calculated by MCR. It is noted that MCR have nicely provided the loading spectra which are assigned to each component in the fossil. The colors of the spectra in Figure 1(b) correspond to those in the Raman image. The results in Figure 1(b) demonstrates the potential of MCR analysis algorism which we have developed. Figure 1(b)(B) shows three bands at 1101, 300, and 178 cm^{-1}. These are characteristic of dolomite. Figure 1(b) (D) and (G) depict characteristic bands of anatase, and quartz, respectively. Of interest is that Figure 1(b) (C) gives broad bands at around 1205 and 1284 cm^{-1} assignable carbon materials. Thus, it is very likely that the concretion investigated the components with biological origin.

(a)

(b)
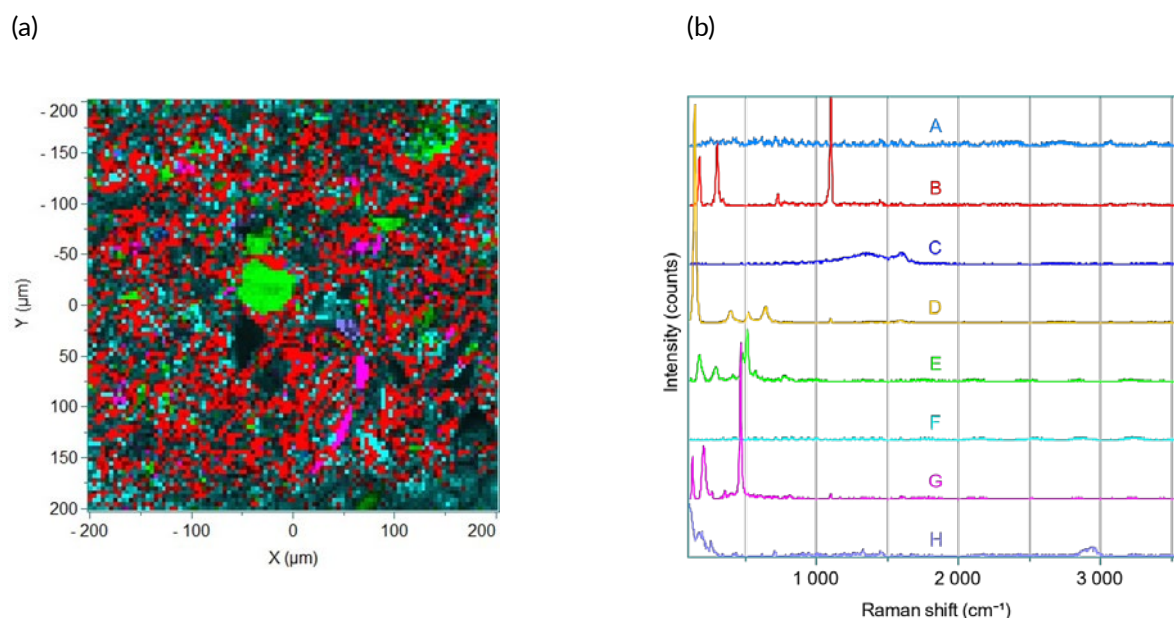
Figure captions:
Figure 1(a) One of the Raman images obtained by 0326 CLS.
(b) Loading spectra calculated by MCR.

Keywords: Raman imaging, MCR, mineralogy, visualization,

G-O.20

Title: *SIP vibrational microspectroscopy in micro-structured chips reveals single-cell metabolic dynamics of soil microbes*

Author: Milda Pucetaite[1], Edith C. Hammer[1], Louise C. Andresen[2], Sofía Gabriela Rodas Samayoa[2]

[1]Department of Biology, Lund University
[2]Department of Earth Science, University of Gothenburg

Abstract:
Large scale ecosystem processes, and especially many of those governing terrestrial carbon cycle, are driven by soil microbes. Because they differ in their function and activity levels, and individually respond to the conditions in their immediate microenvironment that trigger specific biogeochemical and metabolic reactions, these processes can be better understood if we also study them at microscale[1]. Emergent applications of microfluidic technology in soil microbial ecology have opened up possibilities for studying growth and behavior of living soil microbes[2], including studies of communities from real soil inocula[3]. Such microstructured *soil chips* serve as habitats for the microbes inoculated within and can be set up at a structural and chemical complexity level which allows mimicking real soil. Here, we further propose the use of vibrational microspectroscopy to be applied in soil chips to analyze the chemistry of the microbial processes in vitro. For instance, microbial metabolic activities can be studied via stable-isotope probing (SIP), and microbial identification and phenotypic characterization can be performed via signatures of their intrinsic chemistry[4]. In our pilot study we used confocal Raman and optical-photothermal infrared microspectroscopy to analyze uptake and transport rates of deuterium-labeled glucose and amino acid L-alanine both by the hyphae of laboratory grown soil fungus *P. subviscida* (Fig. 1) and by organisms from a natural microbial community from arctic biological soil crusts. The results demonstrate the potential of the approaches to monitor microbial responses in terms of metabolic activity and phenotypical (e.g. build-up of pigments) changes to various biotic (microbial interactions) and abiotic (temperature change, exposure to pollutants) triggers. Ultimately, this will be crucial for pin-pointing key factors affecting microbial processes under changing environmental conditions and their feedbacks on global nutrient cycles.

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4. M. Pucetaite et al., Shining new light into soil systems: Spectroscopy in microfluidic soil chips reveals microbial biogeochemistry, Soil Biol. Biochem. 153 (2021) 108078.
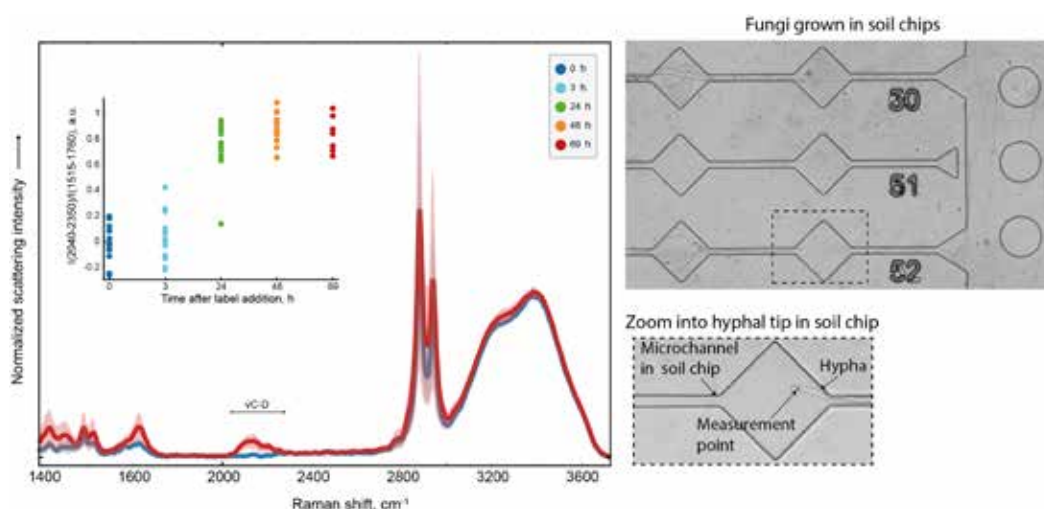
Figure captions:
Fig. 1. Raman spectra (left) of fungal hyphae collected at 0 h and 69 h after addition of D-labeled glucose into the soil chip (right). Inset shows intensity evolution of C-D related spectral bands.

Keywords: Raman microspectroscopy, micro-structured chips, soil

G-O.21

Title: *Fusion of IR and RS spectral data in 2D and 3D in vitro studies for the spheroid blood-brain barrier model.*

Author: Anna Antolak[1], Aleksandra Pragnąca[2], Zuzanna Krysiak[3], Monika Leśniak[3], Joanna Korszun[4], Robert Zdanowski[3], Kamilla Małek[1]

[1]Jagiellonian University
[2]Jagiellonian University, Doctoral School of Exact and Natural Sciences
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[4]Military Institute of Medicine National Research Institute; Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institute of the Polish Academy of Sciences

Abstract:
As the central nervous system drug screening is limited by lack of the appropriate system covering the complexity of drug transport to the brain, there is an ongoing need to develop a functional model of the human blood-brain barrier (BBB). The 3D spheroids, composed of brain endothelial cells, pericytes, and astrocytes, which mimic the spatial architecture and cell-cell interactions in BBB are concerned the most reliable model of the in vivo conditions[1]. However, these multicellular structures require rapid and non-destructive methods for complex analysis of the internal structure, biochemical characteristics, and biological effects within the sphere. The combination of Raman (RS) and Fourier Transformed Infrared (FTIR) spectroscopies provides a powerful tool for overall spheroids' characteristics, as the complementarity of both techniques enhances their detection capability. With the support of the multivariable analysis, those methods allow for the recognition of cell phenotype and discrimination between cell classes[2,3]. Applying a spectroscopic approach to probe the cell lines in the presented research, is a first step to extract substantial biological information from the multicellular BBB model and to standardize the methodology for further preclinical assays. The goal of the following research was to obtain spectroscopic characterization (fusion of IR and RS spectral datasets) of the 2D cell cultures in normal, toxic, and hypoxic states, which mimic perturbated conditions of the BBB functioning, to establish preliminary experimental conditions for the 3D spheroids. IR and RS imaging parameters were adjusted at different levels and spectral profiles of 2D and 3D cell cultures were evaluated to provide a distinction between cells in spherical models. Also, spheres were constructed from individual cell lines in order to optimize culturing conditions, cells' performance, and viability, as well as, spheres' fixation and sectioning for further analysis.

References:
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3. Szafraniec E , Wiercigroch E , Czamara K , Majzner K , Staniszewska-Slezak E , Marzec KM , Malek K , Kaczor A , Baranska M . Diversity among endothelial cell lines revealed by Raman and Fourier-transform infrared spectroscopic imaging. Analyst. 2018 Sep 10;143(18):4323-4334.

Figure captions:
Spectroscopic characterization of blood-brain barrier components in 2D cell cultures (modeling of brain injury and disease) and 3D spheroids.

Keywords:
Blood-brain barrier, spheroids, RS/FTIR fusion

G-O.22
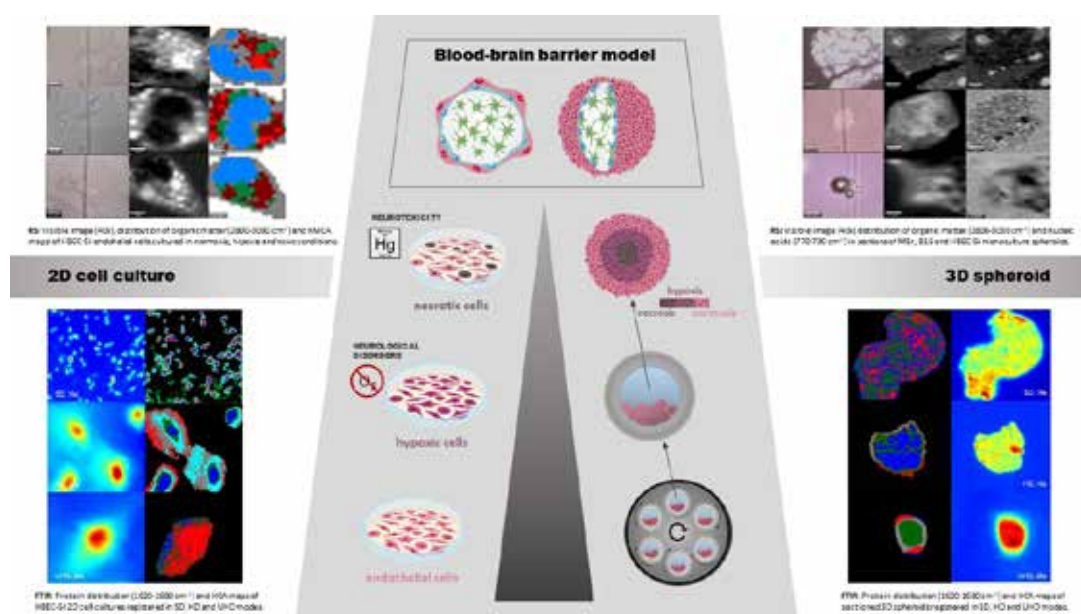
Title: *Aging in coronal dentine of the human tooth seen at the sub-micron resolution in non-contact IR spectroscopy*

Author: Agnieszka Banas[1], Krzysztof Banas[1], Chin-ying, Stephen Hsu[2], Guang Rong Tang[2], Mark B.H. Breese[1]

[1]Singapore Synchrotron Light Source NUS
[2]National University of Singapore, Dentistry Department

Abstract:
By 2050, a significant portion of the world's population will be over 65 years old, and as many people will likely retain their natural teeth, it is increasingly important to understand how ageing affects dental health. Ageing is a physiological process that has a profound impact on various biological systems, including human dentition. However, the underlying mechanisms of tooth ageing are not well understood.

The impact of ageing on dentin is that the ability of coronal dentin to absorb and dissipate energy during chewing or other mechanical stresses is reduced. This means that as people age, their teeth may become more susceptible to damage and cracking, which can lead to tooth decay or even tooth loss.

One reason for this reduced ability of ageing dentin to dissipate energy is thought to be a decrease in the mineral content of the dentin, which can affect the mechanical properties of the tissue. Additionally, changes in the organic matrix of dentin, such as alterations to collagen fibres or proteoglycans, may also contribute to decreased biomechanical performance.

To investigate this further, the chemical composition of young and old coronal dentin was analyzed using Optical Photothermal Infrared Spectroscopy. This analysis aimed to identify any links between mineral and matrix components and the biomechanical performance of ageing dentin.

Various structural and compositional parameters were evaluated in the mineralized tissues using O-PTIR spectra, including carbonate substitution indices (MQ-1,2), mineral-to-matrix indices (MMR-1,2), collagen post-translational modification (PTM-1,2), proteoglycan content, and glycosaminoglycan (GAG) sulfation pattern.

Understanding how ageing affects dentin is important for maintaining good dental health in the elderly population. It can help in the development of new preventative and therapeutic approaches to address age-related changes in dentin and improve overall dental function and quality of life.

Keywords: dentine, sub-micron resolution, O-PTIR

G-O.23

Title: *Micro and nano-spectroscopic studies of modified metallic surface for implantology application*

Author: Dominika Święch[1], Gaetano Palumbo[1], Natalia Piergies[2], Kamila Kollbek[3], Czesława Paluszkiewicz[2]

[1]AGH University of Science and Technology, Faculty of Foundry Engineering, av. Mickiewicza 30
[2]Institute of Nuclear Physics Polish Academy of Sciences
[3]AGH University of Science and Technology, Academic Centre for Materials and Nanotechnology, av. Mickiewicza 30

Abstract:
Metallic biomaterials (i.e. stainless steel, titanium) are widely used in many applications of implantology (for dental and orthopedic implants) due to their unique combination of physical properties, corrosion resistance, and biocompatibility [1]. However, contact with body fluids that contain inorganic and organic compounds can have many adverse effects on the human body, for example, toxicity, the corrosion process, and inflammation [1]. The surface modification of metallic biomaterials can be a good way to improve anticorrosion properties. In our studies, we deposited nanoparticles (copper and gold) on the metallic surface using magnetron sputtering combined with the Inert Gas Condensation technique, which allowed fine–tuning of NPs size and chemical composition [2]. For example, the deposition of a thin CuNPs layer on the titanium substrate improves the corrosion resistance of the substrate [2]. Furthermore, the analysis of the processes that take place on metallic surfaces in the presence of biological compounds is very important. For example, after implementation, the plasma protein adsorption occurs. This is a very complex process that depends on many parameters, and the amino acid sequence affects the protein-metal interaction. The adsorption behaviour of amino acids, which play many biological functions, such as tryptophan (Trp) and cysteine (Cys) onto metallic surfaces is a topic of interest [2-4]. The application of spectroscopic methods such as Raman (RS) and Fourier-transform infrared (FT-IR) spectroscopy and techniques based on surface-enhanced effects such as surface-enhanced Raman spectroscopy (SERS), surface-enhanced infrared absorption spectroscopy (SEIRA), and technique which combines atomic force microscopy (AFM) with infrared spectroscopy (nano-SEIRA) gives us an opportunity to track the conformational changes occurring during the adsorption process of amino acids onto the corroded metallic surface at micro and nanoscale (see Figure 1).

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4. D. Święch, et. al., In Situ and Ex Situ Raman Studies of Cysteine's Behavior on a Titanium Surface in Buffer Solution, Coatings, 13 (2023) 175.
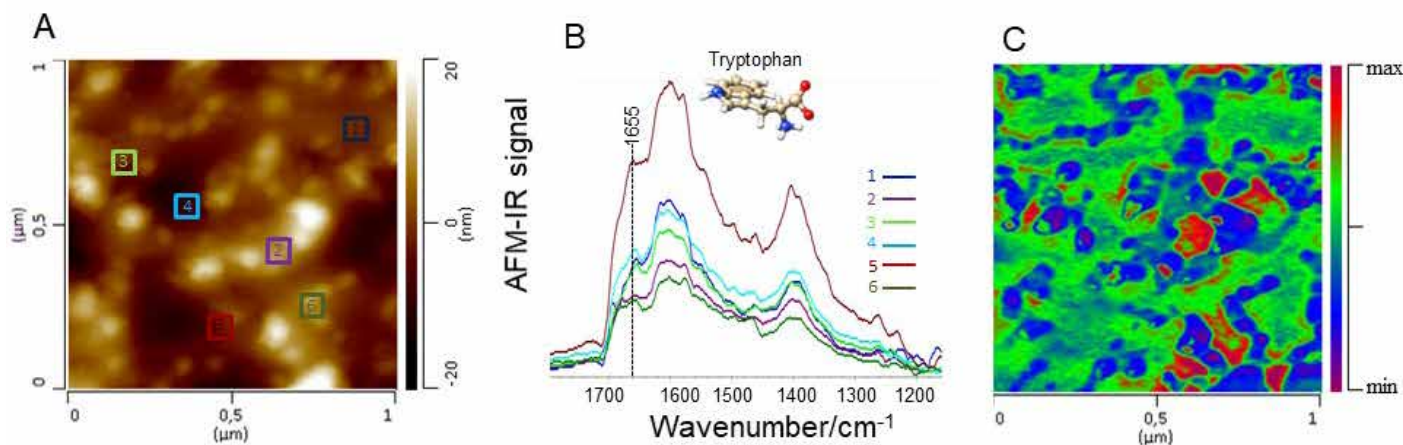
Figure captions:
The AFM image (A) and nano-SEIRA spectra of the corroded CuNPs-Ti surface with deposited Trp (B) collected from the spot marked in A (B). The nano-SEIRA intensity map of the bands at ~1655 cm−1(C)[2].

Keywords: metallic biomaterials, surface-enhanced vibrational spectroscopy

G-O.24

Title: *Novel Analytical Approach for Rapid Detection and Characterization of Microplastics in Environmental Samples: Utilizing MIR Spectroscopy's Silent Region for Enhanced Structural Information*

Author: Krzysztof B. Bec[1], Justyna Grabska[1], Jovan Badzoka[1], Christian W. Huck[1]

[1]University of Innsbruck

Abstract:
Micro- and nanoplastics are small plastic particles, with sizes ranging from less than 5 mm (large MPs) to below 100 nm, respectively. They are present in various environmental matrices and their analysis poses a challenge for analytical methods, leading EU and US authorities urging for developing feasible ways to address this issue. In recent years, vibrational spectroscopy techniques such as micro-FT-IR and Raman imaging have gained popularity in analyzing MNP for their high sensitivity, spatial resolution, and specificity. However, the analysis faces significant challenges that can reduce its throughput and practicality, such as low sampling throughput and the difficulty of performing spectral analysis of environmental samples directly on the filter.

In this study, a novel analytical approach is presented for the improved detection and characterization of microplastic particles in practical scenarios where high throughput and efficiency are crucial. A high-pressure, closed filtration and fractionation system is developed to rapidly filter and fractionate microplastics, reducing the risk of sample contamination. An innovative method for MIR imaging spectroscopy is also demonstrated, directly performed on the filter, which focuses on the "silent region" of the MIR spectrum, yielding rich and specific structural information on the polymer material. The mechanistic background of the identification process is explained through anharmonic quantum chemical calculations, highlighting the specificity of the processed spectral information. By combining the novel filtration system with MIR imaging spectroscopy, the approach achieves improved throughput, minimized sample preparation, and direct measurement on the filter, making it practical, cost-effective, and accessible for wider analysis of microplastics. The proposed method aligns with the current trend of moving towards more practical approaches to detecting and identifying microplastics in environmental samples.

Keywords: micro-/nanoplastics, MIR imaging, silent region

G-O.25

Title: *Quantification of microplastics in environmental samples through a combination of optical and FTIR- and Raman microspectroscopy enhanced by Machine Learning evaluation*

Author: Dieter Fischer[1], Kristina Enders[1], Robin Lenz[1], Franziska Fischer[2], Elisavet Kanaki[1], Julia Muche[1], Benedikt Hufnagl[3]

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[2]Advanced Mask Technology Center GmbH Dresden
[3]Purency GmbH Wien

Abstract:
The analysis of microplastic particles (MP) in different environment areas is a complex process that includes sampling, sample treatment, measurement and data analysis. We will focus here to measurement and data analysis of MP by FTIR- and Raman microspectroscopy. For the measurement of MP < 500 μm, we filter all particles on silicon filters. The measurement of the filter is done by a combination of optical particle detection and FTIR and Raman. The comparability of the results of different labs is an important issue than there can be very many particles, up to several thousands, on a filter. It is necessary to have an interlaboratory test that compares the results. We developed such a test by fixing defined particles in a defined area on a filter that can be sent from laboratory to laboratory. First results will be presented. Furthermore, we will show statistical subsampling strategies [1] and their error limits. For the data analysis, we use the open source program GEPARD [2]. For the assignment of the spectra, a common method is an automated library search that uses comparison algorithms to assign the spectrum with the best match. Hence, the results often requires a manual follow-up control, which is time-consuming and prone to introducing human bias. As an alternative, we developed a machine learning-based approach for the assignment of the spectra. We trained a model to identify several of the most common polymer types and compare its performance to the routinely used spectral database analysis. First results will be shown.
In the second part of the lecture, we show results on selected samples. These are the determination of MP along a river, in a sewage treatment plant (influent, sludge, effluent), in an agricultural soil (Fig. 1), in the atmosphere and along a production line for mineral water (influent, filling, product). All these examples show that the presented combination of methods is very well suited to determine MP in various environmental areas.

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2. J. Brandt, J. et al., High-throughput analyses of microplastic samples using fourier transform infrared and raman spectrometry, Appl. Spec., 74 (2020), 1185. doi.org/10.1177/0003702820932926

Figure captions:
Fig.1: Microplastic pollution in agriculture soil from contaminated sewage sludge

Keywords: FTIR, Raman, microplastics, environment, machine

Microplastic pollution in agriculture soil from contaminated sewage sludge

G-O.26

Title: *Comparison of raman- and fluorescence techniques for detection and identification of microplastics in environmental samples*

Author: Merel Konings[1], Liron Zada[1], Robert Schmidt[1], Freek Ariese[1]

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Abstract:
To assess the potential toxicological effects of microplastics on the environment, the type, size, surface, additives, and shape of the polymer must be characterized [1]. To successfully detect and identify microplastics, while gaining information about their shape and size, laser-based imaging techniques such as Raman, Stimulated Raman Scattering Microscopy (SRS) and fluorescence microscopy can be used after Nile Red staining [2]. The challenges, however, are specifically the detection of particles of <10 μm, finding an optimum in terms of measurement speed, chemical specificity, minimum particle size, quantification, and sample preparation. Comparisons between these different methods on the same samples have not yet been published. During this project, sample preparation, spontaneous Raman, SRS, Deep-UV Raman, fluorescence staining and (fluorescence) microscopy and combinations of these techniques were optimized and evaluated for the detection, identification and quantification of micro-plastics based on suspended matter samples from the Rhine, the Maas, and the port of Rotterdam. Based on the first results, all techniques can detect MP in the matrix, but sample preparation has been proven to be important for the success of quantification. The combination of spontaneous Raman spectroscopy and fluorescence microscopy with Nile Red staining is shown below. It enables fast detection of microplastics based on the fluorescent signal and eases selection of regions of interest for identification with spontaneous Raman spectroscopy. Deep-UV Raman microscopy can be applied to characterize strongly pigmented plastics. SRS microscopy was found to be 3-4 orders of magnitude faster than conventional Raman and can be used to map larger areas [3].The advantages and disadvantages of the different techniques will be discussed.

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Figure captions:
Fluorescence microscopic image (A) of detected particle after Nile Red staining (λexc 340-380 nm, λem. >425 nm) and Raman spectrum overlay (B) of the particle (PoI) with ref. polystyrene (λexc 785 nm)

Keywords: Microplastics, Spontaneous Raman, SRS, fluorescence

G-O.27

Title: *Applications of optical photothermal infrared spectroscopy (O-PTIR) in plastic pollution research: from detecting microplastics to monitoring the production of microbial bioplastic*

Author: Cassio Lima[1], Howbeer Muhamadali[1], Royston Goodacre[1]

[1]University of Liverpool

Abstract:
In recent decades, plastics have become indispensable products of daily life [1]. Currently, the majority of plastics are synthetic polymers derived from petroleum-based sources, which takes hundreds of years to decompose due to their physicochemical properties. The mismanagement of plastics has led to a significant environmental burden of growing concern as plastics have been spotted on different places around the globe from the poles to the tropics including seas, oceans, mountains, and urban environments [2]. Recent reports have shown the negative impacts due to the ingestion of nano/microplastics in a wide range of organisms and there is an increasing concern about their effects on human health as recent studies have reported the presence of plastics in daily life consumer products such as bottled water, teabags, baby bottles, food containers, among others [2]. Biopolymers such as poly-3-hydroxybutyrate (PHB) represent a good alternative to replace synthetic polymers as living microorganisms in the environment can easily degrade it into water, CO_2, and biomass [3]. Several analytical techniques have been proposed to study plastics; however, no single approach has been successful on fully describing their complexity. Here we discuss the applications and limitations of infrared and Raman spectroscopy acquired simultaneously via O-PTIR system to detect nano/microplastics commonly found in the environment as well as to monitor the production of PHB within microbial populations at single-cell level.

References:
[1] M.O. Rodrigues, N. Abrantes, F.J.M. Goncalves, H. Nogueira, J.C. Marques, A.M.M. Goncalves, Impacts of plastic products used in daily life on the environment and human health: What is known?, Environ Toxicol Pharmacol, 72 (2019) 103239.
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G-O.28

Title: *Nanoscale chemical characterization is crucial for polymer recycling*

Author: Georg Ramer[1], V. D. Dos Santos A. Catarina[1], Lena Neubauer[2], Bernhard Lendl[2]

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Abstract:
One of the challenges in moving to a carbon neutral economy lies in the recycling of polyolefins, i.e. the conversion of polymer waste into materials with similar properties as virgin polymers currently made from fossil resources. One of the limiting steps is the sorting of polyolefin waste into its individual polymer types - polyethylene (PE), polypropylene (PP) and other polymers and contaminants. Impurities in the sorted fractions lead to undesired mechanical properties of the blends, while higher purity through more accurate sorting means increased recycling costs and decreased recycling rates. Therefore, recycled blends that retain the desired mechanical properties, but avoid the separation step of the recycling process are cost-advantageous. To understand and improve the properties of recycled polymers a look at their nanoscale chemical composition is indispensable. Currently, the standard techniques used to analyze polymers at the nanoscale (TEM, SEM, and AFM), provide no direct chemical information, and are thus dependent on prior knowledge of the polymer´s composition which may not be available for polymers with unknown and hard to characterize contaminants and composition.
In this presentation we show how we use AFM-IR for nanoscale chemical characterization of polymer recyclates to generate information that helps designing the next generation of polymer materials. AFM-IR is a scanning probe technique that combines the resolution of AFM with the possibility to obtain IR spectra at the at a spatial resolution of 10 nm. Using AFM-IR, we are able to detect and identify different polyolefine phases as well as the presence of sub-micrometer impurities in a post-consumer recyclate. More importantly, the technique also allows to directly analyze the chemistry at the interphase between different materials – crucial information for optimizing polymer recycling procedures.

References:
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Keywords: AFM-IR, nanoscale spectroscopy, chemometrics

G-O.29

Title: *In-line near-infrared spectroscopic monitoring for injection molding of biodegradable polymer blends*

Author: Itsuki Yoshikawa[1], Yuta Hikima[1], Masahiro Ohshima[1]

[1]Kyoto University

Abstract:
Polymeric materials must possess more sophisticated properties and various functionalities as our society diversifies. Due to the abundance of plastic in the environment, biodegradable polymers are strongly desired to broaden their applications with the improvement of their properties. In addition to synthesizing new biodegradable polymers, it is also essential to investigate the polymer processing techniques for polymer composites or polymer blends, which are potential solutions to meet these desires. An injection molding (IM) process is the most popular processing technique for plastic parts production. However, there are some challenges in handling and determining the optimum processing condition of the biodegradable polymer due to their slow crystallization rate, the necessity of moisture control, and heat degradation. The lack of in-line chemical sensing in the polymer processing process has further complicated the situation. We developed an in-line near-infrared spectroscopic sensing system to measure chemical composition during polymer processing. We applied the system to an IM of biodegradable polymer blends, specifically the blends of poly(lactic acid) and poly(butylene succinate adipate). We fabricated a high-pressure and high-heat resistant probe in-house to withstand 100 MPa and 200°C and higher. The sensing system comprises a pair of probes, optical fibers, and a NIR spectrometer, as illustrated in Figure 1. The probes, with the same geometrical shape as a typical polymer pressure transducer, were placed in the nozzle zone, where the molten polymer is stored just before injection into the mold. NIR spectra were collected during the IM to measure the blend ratio and moisture content. A calibration curve was constructed to relate the blend ratio and the spectra. The variation in the blend ratio was successfully measured in real-time. The developed sensing system can monitor composition and reduce polymer waste during the grade changeover operation.
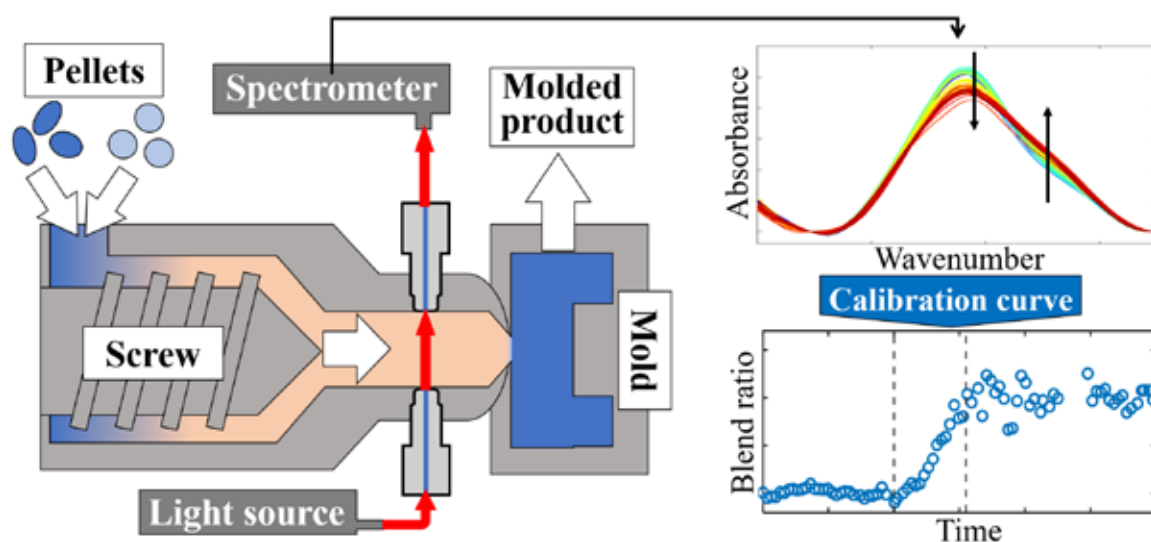
Figure captions:
Figure 1 Near-infrared spectroscopic monitoring system for an injection molding process using transmission and diffuse reflection methods with high resistance fiber optic probes

Keywords: NIR, In-line, Injection molding, Biodegradable

G-O.30

Title: *Fingermark analysis utilizing ATR-FTIR spectroscopy for forensic discrimination of smoker and nonsmoker*

Author: Mohamed O. Amin[1], Entesar Al-Hetlani[1], Igor K. Lednev Lednev[2]

[1]Kuwait University
[2]University at Albany

Abstract:
Chemical analysis of latent fingermark (LFM), with particular reference to "touch chemistry," offers additional intelligence for forensic examination. Continuous improvements in the versatility and sensitivity of detection of the molecular makeup of fingermark (FM) is of considerable importance [1]. Particularly, extracting phenotypical information such as age and sex can be significantly beneficial in criminal investigation. However, chemical profiling of FMs using vibrational spectroscopy has been limited to age and sex determination, while other significant traits can also aid in narrowing down the suspect pool [2-3]. In the current proof-of-concept study, ATR-FTIR spectroscopy combined with partial least squares discriminant analysis (PLSDA) have been employed for determining smoker from non-smoker donors from their FM residues. Genetic algorithm (GA) was applied to improve the discrimination rate of the developed model by selecting regions that are significant for the distinguish between the two classes. A subject wise leave-one-out cross-validation (LOOCV) was initially used to evaluate the performance of the binary PLSDA classifier for each donor. The binary model showed 84% correct classification at spectral level and 92% correct classification at donor level in subject wise LOOCV. In addition, receiver operating characteristic (ROC) curve analysis was constructed to establish a threshold for differentiating between the smoker and non-smoker FMs, this resulted in 100% accuracy at a donor level for external validation test. This preliminary study shows a great promise for identifying smoker donors from non-smoker individuals based on chemical analysis of FM residues. After fully developed, we believe the method will offer significant potential for real crime scene investigation due to its simplicity, non-destructive nature and its prospective for in-field analysis

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Figure captions:
Analysis of smoker and non-smoker fingermarks using ATR-FTIR spectroscopy and chemometrics

Keywords: Vibrational spectroscopy, Fingermark, Forensics

Schematic illustration of external validation of the PLS-DA model. The percentage of samples classified as a nonsmoker fingermark is plotted as the bar height for each sample.

G-O.31

Title: *Deep UV Raman spectroscopy for post-mortem interval determination*

Author: Anna Wójtowicz[1], Luis Perez Almodovar[2], Igor K. Lednev[2], Renata Wietecha-Posłuszny[1]

[1]Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University
[2]Department of Chemistry, University at Albany, SUNY,

Abstract:
Determining the time that elapsed since death (PMI – post-mortem interval) is one of the most important and at the same time the most difficult tasks of forensic analysis. Many internal and external factors that affect post-mortem processes, such as circumstances of death, body position, ambient temperature, and humidity, require the conduct of research under strictly controlled conditions to fully understand the mechanism of the processes that take place in body tissues and their correlation with PMI [1]. Such controlled conditions can be met when an animal experiment is performed [2].
For the detection of post-mortem changes in tissues, it is advantageous to use Raman spectroscopy, which is fast, does not require complicated sample preparation, and allows the measurement of samples in aqueous solutions. In addition, the use of lasers in the UV range allows the avoidance of fluorescence and, as a result of the resonance effect, the selective enhancement of the intensity of the bands assigned to the main biomolecules [3].
The aim of this study was to detect post-mortem changes in rabbit liver samples collected 0, 12, and 24 hours after animal sacrifice using Raman spectroscopy with a laser wavelength in the UV range. Lyophilized samples after dispersion in distilled water were analyzed with the use of a deep UV Raman spectrometer constructed by the group of Dr. Igor K. Lednev at the University at Albany, SUNY [4]. Two lasers were applied: 199 and 239 nm.
The laser wavelengths used allowed the registration of resonance spectra with the enhancement of, respectively, protein bands for 199 nm and aromatic amino acid bands for 239 nm. The differences between individual PMIs for spectral data obtained with both lasers were evaluated and compared. Three class PLS-DA classification models were built and proposed to distinguish between samples taken immediately, 12, and 24 hours after death.

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Keywords: Deep-ultraviolet resonance Raman spectroscopy, PMI

H-I.1

Title: *High-resolution Raman imaging of >300 cells from human patients affected by nine different leukemia subtypes: a global clustering approach*

Author: Renzo Vanna[1], Andrea Masella[2], Manuela Bazzarelli[2], Paola Ronchi[3], Aufried Lenferink[4], Cristina Tresoldi[3], Carlo Morasso[5], Marzia Bedoni[6], Dario Polli[7], Fabio Ciceri[3], Giulia De Poli[2], Matteo Bregonzio[2], Cees Otto[4]

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Abstract:
Background: Leukemia defines a large group of tumors characterized by the proliferation of immature cells ("blasts") in the bone marrow. According to specific morphological and genetic features, more than forty leukemia subtypes should be recognized to define diagnosis and treatment. The visual assessment of bone marrow (BM) smears is still a fundamental step of diagnosis and classification, and it is based on morphology. This process is subjective, time-consuming, may require specific immunostaining and suffers from intra- and inter-observer variability. Here we report the characterization of 9 leukemia subtypes using a global clustering approach.
Methods: BM samples from 19 patients affected by 9 different leukemias, including 6 acute myeloid leukemia (AML) subtypes (0, 1, 2, 3, 5a and 6) and 3 acute lymphoid leukemia (ALL) subtypes (BPh+, BPh-, T) were selected. A total of 319 cells were studied by high-resolution Raman imaging using a home-built confocal Raman microscope (647 nm laser, 63xW). Each cell was scanned by 64x64 Raman spectra with 180 nm step size and 100 ms/pixel. After applying a 9-step automatic pre-processing pipeline, the entire dataset (>667k spectra) was retrieved and clustered (n. clusters = 17) using a global clustering approach, and then processed to obtain pseudo-stained images.
Results: The global clustering approach applied to the 319 Raman maps allowed to automatically identify five components (i.e., nucleus, cytoplasm, myeloperoxidase, carotenoids, hemoglobin) over the 9 leukemia subtypes. The resulting information was used 1) to produce pseudo-stained images, resembling those used in clinics, by an unsupervised method and 2) to use per-cell cluster distribution to characterize leukemia subtypes and to automatically classify them.
Conclusions: This study demonstrates the potential of Raman imaging for the study of leukemia and reports the advantages and challenges associated with global clustering approaches.

Figure captions:
This figure shows representative Raman images of 9 leukemia subtypes and associated clusters, after global cluster analysis of the entire (>300 cells) dataset.

Keywords: Raman, imaging, leukemia, myeloperoxidase, clustering
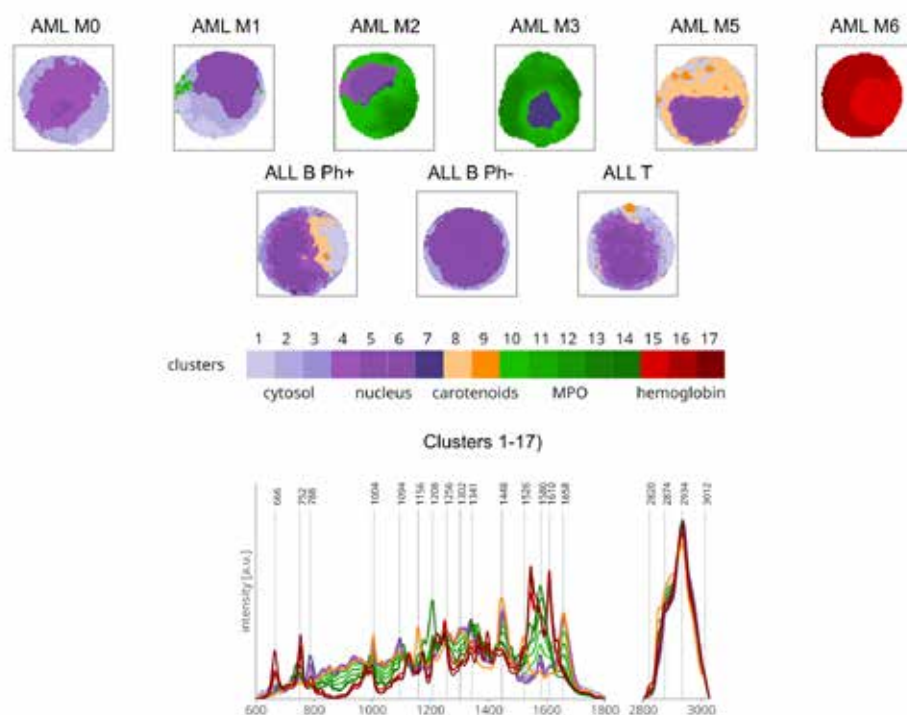
Title: *Surface Enhanced Spatially Offset Raman Spectroscopy: A Promising Optical Imaging Modality in Preclinical Cancer Imaging*

Author: Fay Nicolson[1], Eunah Lee[2], Andrew Whitely[2], Bohdan Andreiuk[3], Scott Rudder[4], Samuel Mabbott[5], Kevin Haigis[1]

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Abstract:
Here, we combine the use of "spatially offset Raman spectroscopy" (SORS) with that of Surface Enhanced Raman Scattering (SERS) nanoparticles in a technique known as "surface enhanced spatially offset Raman spectroscopy" (SESORS) to image deep-seated tumors. We will discuss the optimization of SORS instrumentation and imaging approaches, and subsequent application of SESORS to pre-clinical cancer imaging and delineation of tumor margins in $Apc^{fl/+}$, $Apc^{fl/+};Kras^{G12D/+}$, and GL261 mouse models of colorectal cancer and glioblastoma respectively. We demonstrate that our approach enables improvements in the non-invasive detection of these cancers due to improvements in SNR, spectral resolution, and depth acquisition, and can complement clinically approved radiographic techniques.

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Figure captions:
Conceptual figure outlining the detection of glioblastoma multiforme (GBM) through the use of cRGDyK-conjugated SERRS NPs. Following injection of NPs, in vivo SESORRS imaging of GBM was achieved.

Keywords: SERS, SORS, SESORS, Cancer, Imaging

Conceptual figure outlining integrin-based detection of glioblastoma multiforme (GBM) through the use of cRGDyK-conjugated SERRS nanostars. Following injection of integrin-targeting nanoparticles, in vivo SESORRS imaging of GBM was performed using a custom-built SORS system

H-I.3

Title: *Portable Raman spectroscopy for in-clinic skin and prostate cancer diagnosis*

Author: Suse J. Van Breugel[1], Hannah Matthews[1], Kamran Zargar-Shoshtari[2], Paul Jarret[3], Michelle Locke[4], Cather Simpson[1], Michel Nieuwoudt[1], Claude Aguergaray[1]

[1]The University of Auckland
[2]Counties Manukau District Health Board
[3]Department of Dermatology, Middlemore Hospital
[4]Department of Plastic Surgery, Middlemore Hospital

Abstract:
In a research environment, Raman spectroscopy has been extensively used to study biological systems as it is non-destructive and provides highly specific molecular information in vivo and in situ. The main challenges to its application in clinical settings for identifying and measuring diseases like cancer are the weak Raman scattering cross section for biological tissue and sensitivity to environmental light. Here, we applied a portable Raman system in-clinic to diagnose two highly prevalent cancers; skin cancers (in vivo) and prostate cancer (ex vivo)[1, 2]. We show that portable Raman systems with fiber optic probes can provide high-quality Raman spectra even when high levels of background noise are present. Using partial least squares-discriminant analysis, we classified malignant skin lesions from benign lesions with positive likelihood ratio (+LR) of 4.8 and prostate cancer tissue from benign prostate tissue with +LR of 6.8[1, 2]. We also show ability to grade prostate cancers. The ROC and bee swarm plots for the classification of malignant and benign prostate tissue are shown in Fig1. The diagnostic accuracy of the classification model was evaluated in terms of the AUC of the ROC. The optimized trade-off between sensitivity and specificity is denoted by the red mark and resulted in 82% sensitivity and 88% specificity. When sensitivity is set to 90%, a specificity of 80% is achieved. With 95% sensitivity, specificity was 64%[2].
As will be discussed, it is important to achieve high sensitivity to ensure no cancers are missed. However, a high specificity is still desirable to ensure clinicians do not excise unnecessary healthy prostate tissue and induce trauma.
The high classification accuracy achieved and streamlined integration into clinical workflow will allow easy uptake of this rapid technique in clinical settings.

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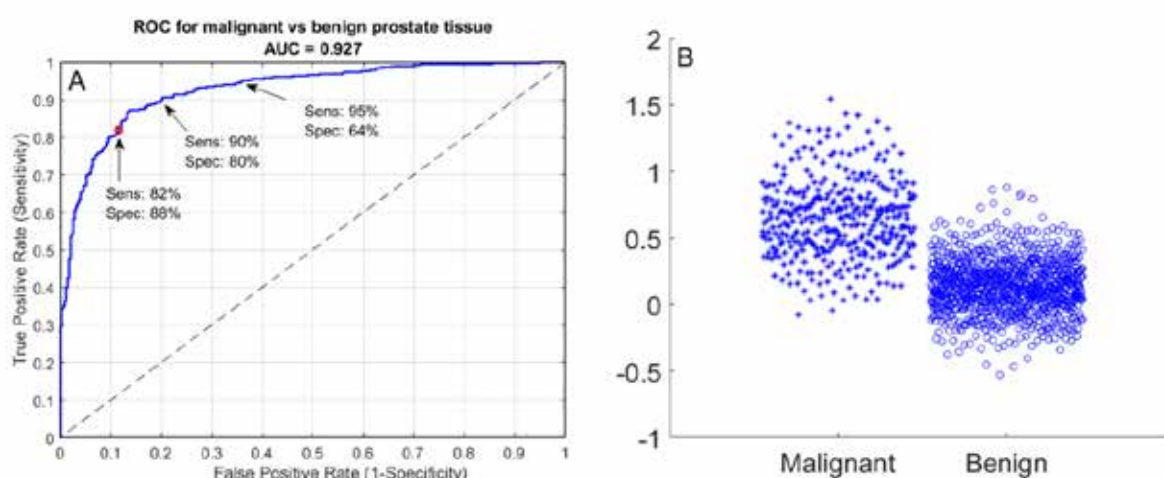
Figure captions:
Fig. 1: ROC for classification of malignant and benign prostate tissue at different thresholds (A), and associated Bee swarm plots for malignant and benign prostate tissue (B).

Keywords: Raman spectroscopy, chemometrics, biomarkers, skin

Title: *Self-assembled nanogap arrays of gold nanoparticles in dimple nanopores induced by DNA hybridization*

Author: Hajun Dang[1], Jaebum Choo[1]

[1]Chung-Ang University

Abstract:
The fabrication of reproducible plasmonic subatrates with a high density of hot spots in a large area has been a technically challenging issue for the commercialization and clinical application of surface-enhanced Raman scattering (SERS) biosensors. In this work, we present a new SERS substrate with a high density of hot spots that has been fabricated through the uniform assembly of gold nanoparticles inside nanopores of nanodimple-shaped substrates using DNA hybridization [1]. We utilized a SERS-based imaging method through a fast mapping technique to identify uniform nanogap formations and electromagnetic enhancement effects when gold nanoparticles are uniformly anchored in nanopores. Finite-difference time-domain (FDTD) simulations between gold nanoparticles and nanopores have been performed to investigate a gap distance-dependent collective plasmonic resonance. This nanodimple plasmonic substrate, including a high density of hot spots induced by the uniform self-assembly of gold nanoparticles, provides new insights for its application as a plasmon-enhanced biosensor [2].

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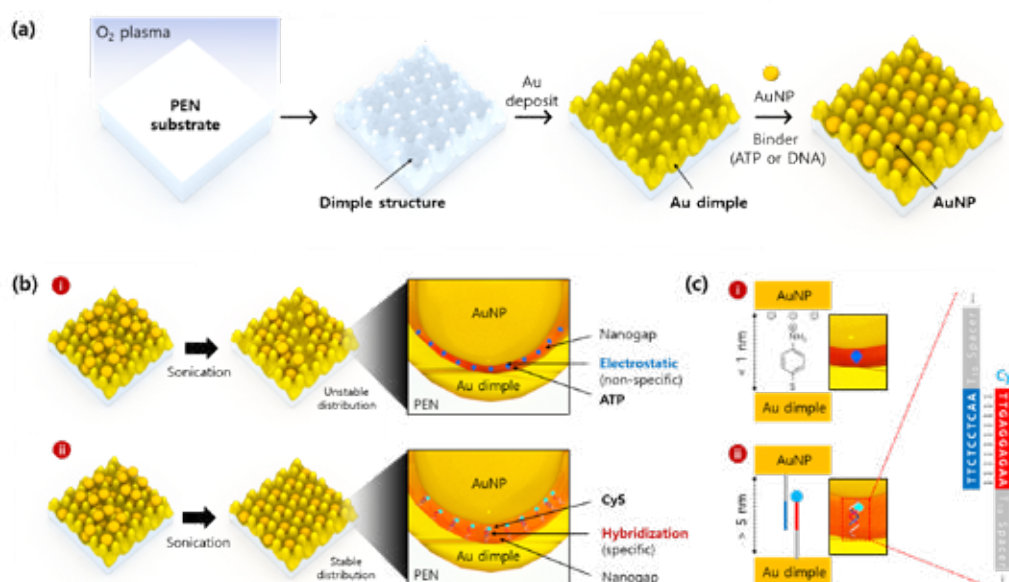
Figure captions:
Schematic of (a) the fabrication of a nanodimpled Au substrate and the internalization of AuNPs, (b) the internalization of AuNPs in the cavities, and (c) their binding interactions.

Keywords: Au nanodimple, Nanogap, SERS-PCR, SARS-CoV-2

H-I.5

Title: *Raman imaging and AFM studies of human colon tissues and cells – cholesterol impact on CRC development*

Author: Beata Brozek-Pluska[1], Karolina Beton-Mysur[1]

[1]Lodz University of Technology, Faculty of Chemistry, Laboratory of Laser Molecular Spectroscopy

Abstract:
Colorectal cancer (CRC) is the third most common cancer worldwide. Obesity, alcohol consumption, smoking, high consumption of red or processed meat and a diet with low fibre, fruit, and vegetables intake increase CRC risk. The surgery is the first-line treatment to restore to health. Despite advances in surgical techniques, chemotherapy, and radiotherapy CRC remains the second leading cause of cancer-related deaths in the world. The social importance of this problem is therefore a stimulus for undertaking research aimed at developing new tools for rapid CRC diagnosis and analysis of CRC risk factors.
We used Raman spectroscopy and imaging to analyse the cholesterol content in human colon tissues and cells to prove the increased cholesterol level in human colon cancer samples. Moreover, we used Raman techniques to study the impact of mevastatin to cholesterol biosynthesis. We have shown that: Raman spectroscopy and imaging allow to study cholesterol content in human colon tissues and human colon single cells of both types: normal and cancer and allow to prove the effectiveness of mevastatin in the mevalonate pathway modulation and disruption of the cholesterol level. All observations have been confirmed by chemometric analysis including PCA and PLSDA. The positive impact of statin on cholesterol content was observed not only analysing vibrational features but also by using AFM technique. The significant increasing of Young modulus as mechanomarker for CaCo-2 human cancer colon cells upon mevastatin supplementation compared to CCD18-Co human normal colon cells was confirmed.

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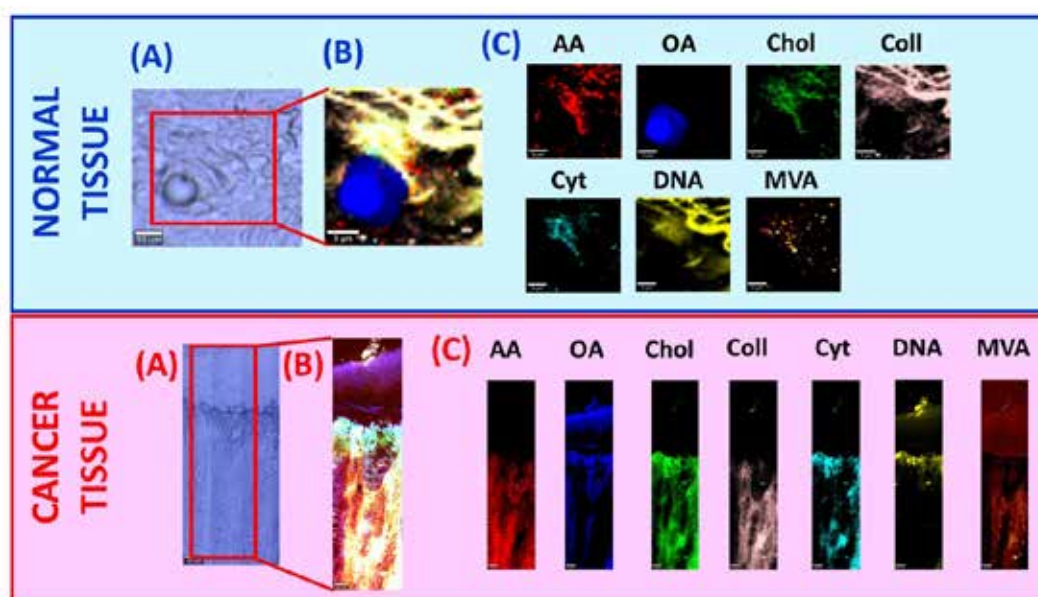
Figure captions:
Fig. 1: Microscopy image (A), Raman image (B), distribution of: arachidonic acid, oleic acid, cholesterol, collagen, cytidine, DNA, mevalonic acid for normal and cancer human colon tissues (C)

Keywords: Raman imaging, Raman spectroscopy, CRC, FM

H-I.6

Title: Raman Spectroscopy for Pre-Disease Analysis

Author: Pradjna Paramitha[1], Keita Iwasaki[1], Kosuke Hashimoto[1], Bibin Andriana[1], Hidetoshi Sato[1]

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Abstract:
A person does not get a disease without any cause. The signs of a disease may be found in the body or in the environment surrounding a person. If it was possible to detect the cause of diseases that does not have any subjective symptoms in advance, it would reduce the personal risk as well as the social cost for medicine. Hence, we have 2 directions in studies to develop pre-disease detection analyses. One is the study on noninvasive body fatty chain analysis.1-4 Multivariate analytical techniques and Raman probe were developed for semi-quantitative analysis of fatty chains in subcutaneous adipose tissues. The result suggested that the adipose tissue preferred to accumulate linoleic acid chain. In contrast, liver model cells had different reactions to various fatty acids Although the cells preferred to uptake linoleic acid, they induced apoptosis. The other direction is the study on human infectious virus detections in environment.5,6 As the first discovered virus was tobacco mosaic virus which infected a plant, numerous viruses exist in the nature and their hosts are also various. Consequently, it would be quite difficult to identify human infectious viruses, even if there was a way to visualize viruses in our surrounding environment. A method to use a human cell was developed to detect only human infectious viruses. The result suggested that a small modification took place in molecular composition in the virus infected cells within only 2-3 hours after the virus invasion. The molecular changes took place much earlier than that had estimated from the known mechanisms of the virus invasion. Although these studies stay at preliminary stages of developments, their results have already demonstrated technically high viability of Raman spectroscopy in pre-disease analyses.

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Keywords: Raman, Noninvasive, Chemometrics, Fat, Virus

H-I.7

Title: *Finding a Needle in a Haystack: Transmission Raman Spectroscopy (TRS) for Detecting Micro Calcifications in Breast Tissue*

Author: Benjamin Gardner[1], Jennifer Haskell[1], Adrian Ghita[2], Charlotte Ives[3], Douglas Ferguson[3], Pavel Matousek[4], Nick Stone[1]

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[4]STFC

Abstract:
Breast micro calcifications are routinely observed in mammography, yet are currently underutilized in clinical decisions, due to incomplete scientific understanding (an active area of research[1-3]). Mammographic screening routinely offered has limitations, including the level of false positive screens (9-13%), therefore would benefit from adjunct techniques to support clinical decision making. Raman spectroscopy has been shown able to distinguish between types of breast micro calcifications, and correlate this information with overall breast pathology[3]. This offers the potential of new diagnostic opportunities using Raman spectroscopy, especially when coupled with deep Raman approaches. The aim of this work is to demonstrate the capability of transmission Raman (TRS) to detect calcifications in bulk excised breast tissue, as a prelude to non-invasive *in vivo* whole breast scanning.
Participants identified with invasive or in-situ breast disease were targeted for this study, who were undergoing surgical intervention. Once diseased tissue was removed, it followed a protocol of interoperative X-ray, imaging, TRS scanning, then returned to the clinical pathway for pathology assessment i.e. H&E.
The work to date shows that high quality spectra can be acquired rapidly (seconds) from breast tissue ˜1 – 5 cm thickness, and importantly that the non-ideal sample quality is not prohibitive, i.e. specimens contains surgical dyes, tissue burning (diathermy). Through robust development of analysis protocols, better understanding the origins of signals and the causes of spectral distortions; while, simultaneously developing analytical methods, it is possible to demonstrate calcified signals within the bulk breast tissue with transmission Raman.
This ongoing work shows the great potential of Raman spectroscopy for future *in vivo* rapid non-invasive measurements of bulk breast tissue, with the first in vivo whole breast scanning planned in the near future.

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3)Cancer research, 80 (8), 1762-1772

Keywords: Clinical, Micro-Calcification, Raman Spectroscopy

H-O.1

Title: *Surface-enhanced Raman Spectroscopy in tumor detection*

Author: Aneta Kowalska[1], Marta Czaplicka[1], Ariadna Nowicka[2], Tomasz Szymborski[3], Izabela Chmielewska[4], Wojciech Kukwa[5], Agnieszka Kamińska[3]

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Abstract:
One of the biggest challenges for modern medicine is distinguishing between healthy and cancerous tissues. Therefore, a major effort by researchers is currently devoted to finding a new way to diagnose as quickly as possible with the greatest possible accuracy in distinguishing between healthy and cancerous tissues. These issues are probably most relevant to human health, where the timing of diagnosis plays a huge role in patient treatment.
Surface-enhanced Raman spectroscopy (SERS) is presented as new method for tumor discrimination and differentiation, used in a direct way, of several different types of cancer e.g. to study salivary glands [1], brain [2], lungs [3], and also as indirect method, using L-selectin levels for cancer detection. It should be highlighted, that the quality of SERS spectra of cancerous tissues is much worse than that of healthy tissues. Therefore, in order to overcome this problem and establish SERS as a new method of diagnosis, additional approaches must be introduced, e.g., in the case of salivary tissues, homogenization of samples before measurement makes it possible to obtain a good quality SERS signal, comparable to that from healthy samples. Moreover, due to complexity of SERS data a multivariate dimensionality-reduction tool e.g. Partial Least-Squares Discriminant Analysis (PLS-DA) should be applied. The diagram below (Figure 1) shows one of the ideas used when studying salivary tissues, which ultimately allow significant discrimination and differentiation results between healthy and diseased samples, compared to the standard and the most common histology technique, also in terms of time.
The data presented demonstrate the significant potential of SERS combined with multivariate analysis for distinguishing cancer or tumor from normal tissues as a rapid, label-free cancer detection tool for oncology diagnosis, which in the future may add value to the development of personalized medicine.

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Figure captions:
Figure 1. The schematic idea of applied method that allows to register the spectra of normal and neoplastic tissues of the salivary glands samples in comparable spectral quality.

Keywords: SERS, tumor, multivariate analysis

H-O.2
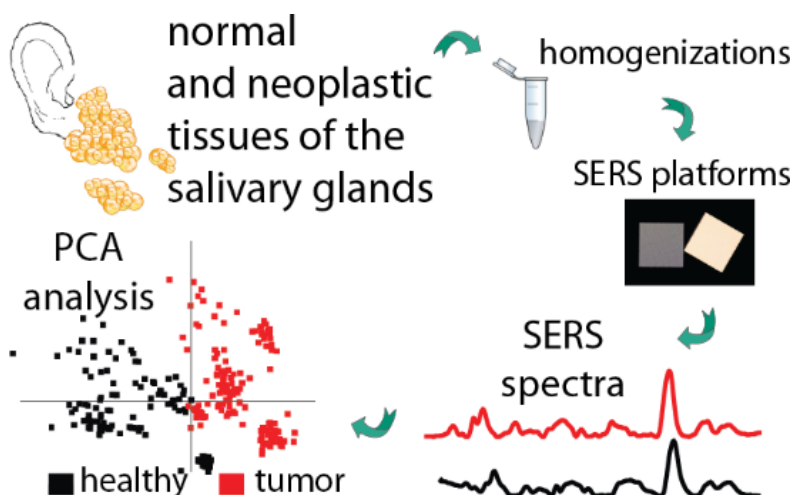
Title: *FTIR Spectroscopy for Bladder Carcinoma Detection and Prediction of Grade, Invasion, and Lymph Nodes Metastases*

Author: Monika Kujdowicz[1], David Perez-Guaita[2], Piotr Chlosta[3], Krzysztof Okon[4], Kamilla Malek[5]

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Abstract:
Bladder carcinoma (BC) due to the high incidence and recurrence rate, and the complicated diagnostics, is the most cost-effective malignant neoplasm worldwide. The prognosis and treatment depend on the depth of invasion into the bladder wall, the morphology (grade) of the cells, and the presence of metastases (most common to lymph nodes). Early diagnosis can save the lives of patients and reduce unnecessary suffering. The BC clinical diagnosis includes urine cytology and bladder biopsies. Early management involves transurethral resection and BCG therapy, while advanced BC stage needs cystectomy and severe chemotherapy in case of metastasis. We have recently reported several prediction models for the detection of BS staging and grading from TTIR spectra acquired in imaging and ATR models of urine sediment, urine cytology and bladder biopsies [1-3]. The accuracy determined from PLSDA modeling was from 80 to 95% depending on the type of the experimental group and the spectrum. To achieve high-quality parameters of classification, we proposed solutions in data reduction before approaching the prediction models, e.g., IR imaging of epithelial and subepithelial layers of the bladder or segregation of cells from urine based on glycogen level [1-3]. Finally, we investigated FTIR spectra of bladder tumors to predict their metastatic potential to lymph nodes [4]. Even though multiple changes in biochemical composition were observed among the samples, BC is accompanied by a decreased level in glycogen and collagens and an elevated content of proteins, nucleic acids, and lipids. Our results showed the high efficiency of label-free and rapid RTIR spectroscopy to be a supportive tool for clinical diagnosis (Fig.1)

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Figure captions:
Proposed workflow of the FTIR-based diagnosis of bladder carcinoma.

Keywords: Infrared Spectroscopy, Raman Spectroscopy, Bladder
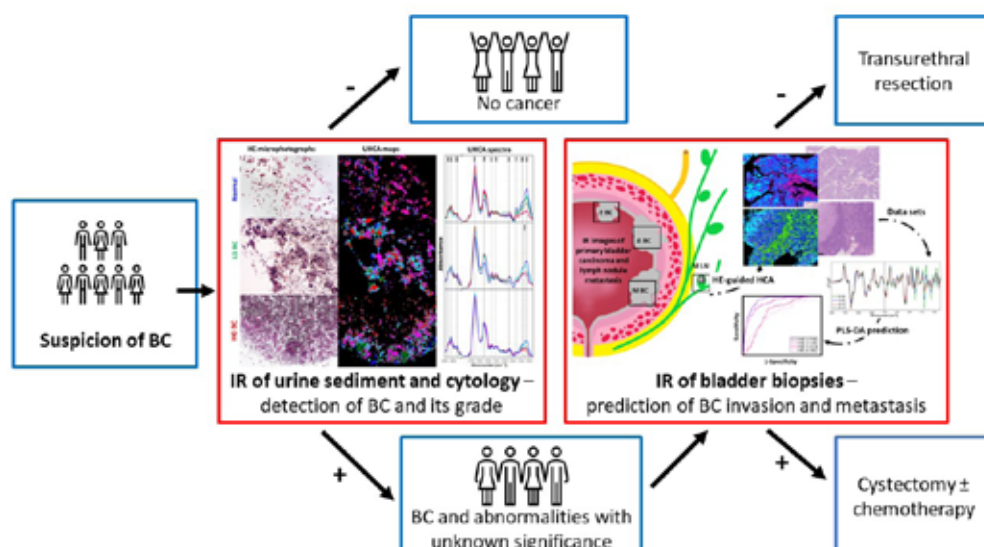
H-O.3

Title: *Raman Spectroscopic application in cervical cancer screening*

Author: <u>Rubina Shaikh</u>[1], Aoife Mc Guinness[2], Alison Malkin[3], John O'Leary[4], Cara Martin[4], Fiona Lyng[2]

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Abstract:
The talk will focus on the future perspectives of using Raman spectroscopy for cervical cancer screening. Cervical cancer is the fourth most common women's cancer globally, and unfortunately mainly affects younger women. Cervical cancer is treatable if detected early. High-quality cervical screening programmes and the introduction of the human papillomavirus (HPV) vaccine are reducing the incidence of cervical cancer in many countries. However, screening is still crucial for all women. Current gold standard methods include HPV testing and cytology for screening, followed by colposcopy and histopathology for diagnosis. However, these methods are limited in sensitivity/specificity, cost, and time. New methods are required to aid clinicians in the early detection of cervical precancer. Over the past 20 years, the potential of Raman spectroscopy, together with multivariate statistical analysis, has been shown to detect cervical cancer. The cervical cancer screening landscape is evolving, and so are the research questions. This talk will discuss issues such as cervical cytology, HPV-based cervical cancer screening and self-sampling, its future role in cervical cancer screening programs and Raman spectroscopy.

Keywords: Cervical cancer, Cytology, Raman Spectroscopy

H-O.4

Title: *An injectable biosensor for continuous remote monitoring of patients with prostate cancer*

Author: <u>Marta Aranda Palomer</u>[1], Maria S. Relvas[2], Sergio Quintero[1], Jason B. King[3], Mengkun Chen[3], James W. Tunnell[3], Ana Oliveira[4], Pedro Costa[5], Rui Sousa[5], Adriana Mendes[6], Olga Martinho[6,] Fatima Baltazar[6], Lorena Dieguez[1], Sara Abalde-Cela[1]

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[3]University of Texas at Austin (UTA)
[4]Stemmaters Biotecnologia e Medicina Regenerativa SA
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Abstract:
Remote cancer progression monitoring has the potential to increase current predictive rates of recurrence, while contributing for a more accessible diagnosis and treatment. Patients at high risk of relapse would benefit from improved cancer monitoring tools based on nanobiomedical devices providing higher sensitivity. These novel tools should have the ability to remotely monitor patients, collecting data that can be used to detect disease progression earlier. Herein, we present an implantable biosensor, developed by combining biocompatible hydrogels with plasmonic nanoparticles, to be applied in the context of prostate cancer monitoring, through surface-enhanced Raman scattering (SERS) spectroscopy. SERS is an ultrasensitive technique with many applications in the biomedical field, offering high selectivity and sensitivity, multiplexing capabilities and label-free detection (1). A handheld Raman probe is being applied to acquire the signal from the implanted biosensor which, in combination with a machine learning algorithm, has the capacity to classify patients in a label-free manner according to the recorded SERS patterns. A highly controlled data analysis workflow is being developed in parallel to normalise signals acquired through different skin types and from complex biological matrices (2). Initially, the biosensor was tested in artificial interstitial fluid to build a library of the expected Raman signals (Figure 1). Later, the system was able to discriminate cellular supernatants from cancerous and healthy prostate cell lines, upon incubation with the nanobiosensor. Next steps will consist of testing the biosensor in clinical samples and performing *in-vivo* tests after implantation of the biosensor in mice, in order to detect the metabolite signals under the dermis.

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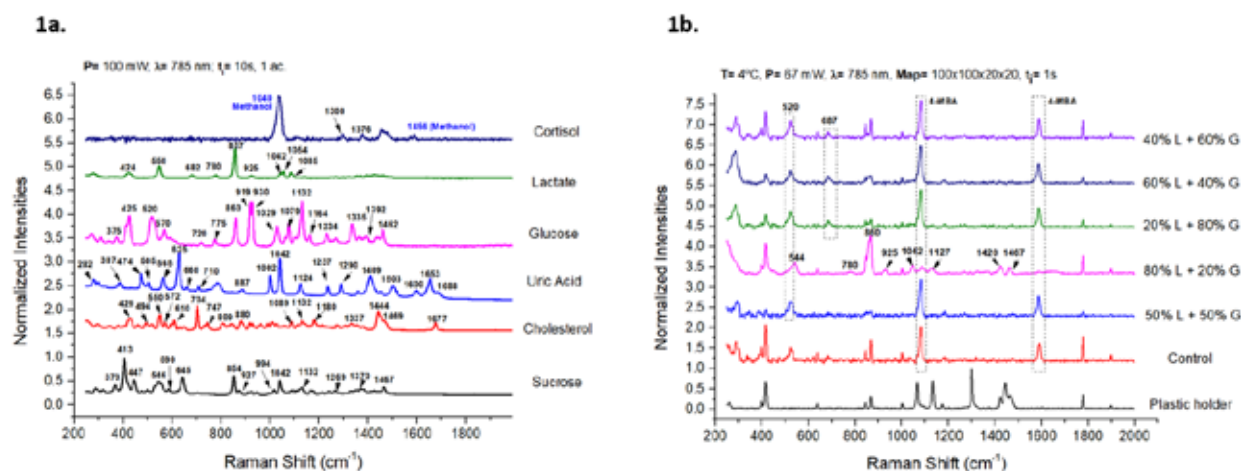
Figure captions:
Figure 1a: Raman analysis in artificial interstitial fluid compounds. 1b: SERS analysis in biosensor sample to detect two interstitial fluid combined compounds: glucose (G) + lactate (L).

Keywords: SERS, biosensor, nanoparticles, machine learning

H-O.5

Title: *Dual nano-heater and SERS temperature sensor for cancer photothermal therapy*

Author: William H. Skinner[1], Renata L. Sala[2], Kamil Sokolowski[2], Jeremy J. Baumberg[2], Oren A. Scherman[2], Benjamin Gardner[1], Pavel Matousek[3], Nicholas Stone[1]

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[2]University of Cambridge
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Abstract:
Photothermal therapy induces tumour cell death by elevating local tissue temperature via the conversion of near-infrared (NIR) radiation into heat. A key parameter during photothermal therapy is the temperature increase inside the tumour: too low and cell death does not occur; too high and necrotic cell death occurs.[1] However, non-invasive techniques to monitor tissue temperature during photothermal therapy are currently lacking. In this talk, I will discuss the application of surface-enhanced Raman spectroscopy (SERS) to optically measure temperature increase during photothermal therapy. Raman spectra encode information about thermally excited states in the ratio of the Stokes and anti-Stokes vibrational modes. However, the intensity of anti-Stokes peaks in spontaneous Raman spectra are prohibitively low for in vivo sensing applications. Gold nanoparticle (AuNPs) clusters with a surface plasmon resonance (SPR) in the NIR region can boost the spectral intensity of surface-bound molecules via SERS and have also been explored as potential photothermal therapy agents (PTAs).[2] We leveraged the optical properties of AuNPs to create a dual nano-heater and SERS temperature sensor to enable local temperature measurements during photothermal heating. Our PTA agent is fabricated by clustering AuNPs to shift their SPR towards the NIR region and optimise both heating and SERS response. In this way, we show AuNPs clusters can be simultaneously heated and report on local temperature via the ratio of the Stokes and anti-Stokes vibrational models of surface-bound biphenyl-4-thiol. This work demonstrates the use of a single PTA agent to heat and report on local temperature and in future will be combined with Surface-Enhanced Spatially Offset Raman Spectroscopy (SESORS) to simultaneously heat and measure temperature at depth in tissues.[3]

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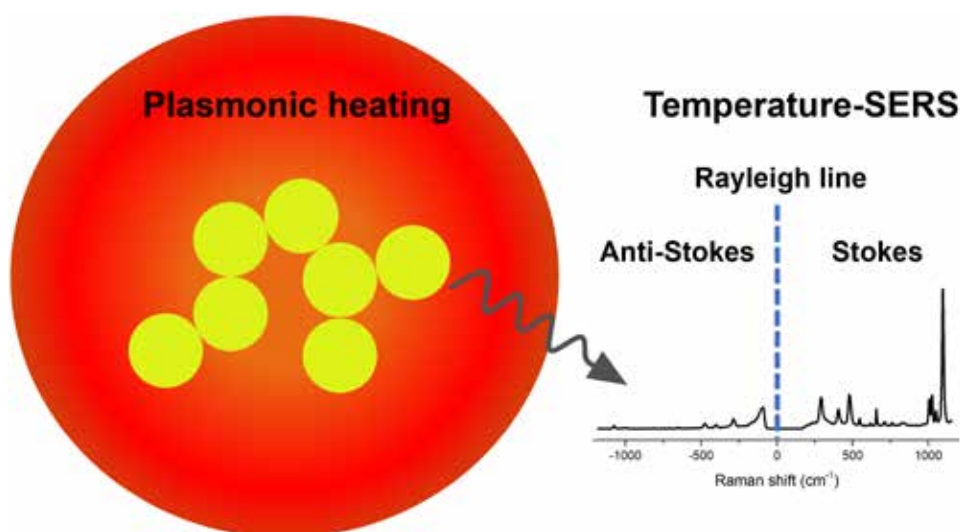
Figure captions:
AuNPs clusters convert NIR radiation into heat and the resulting temperature increase is measured via the ratio of anti-Stokes and Stokes vibrational modes in the SERS spectrum of biphenyl-4-thiol.

Keywords: SERS, plasmonic heating, nanoparticles, cancer

H-O.6

Title: *Blood pulse dynamics investigation with non-invasive Raman spectroscopy*

Author: Maciej Wróbel[1]

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Abstract:

Monitoring physical and chemical parameters of blood is crucial in medicine, both during surgery and general hospital treatment, and for individuals who monitor themselves at home, such as diabetics measuring their glucose levels. Accurate and minimally invasive methods for these measurements are highly desirable. Light-based techniques, particularly spectroscopy, show great promise due to their accuracy, specificity, and speed. Among these, Raman spectroscopy is especially promising as it enables both qualitative and quantitative measurements.

This paper presents the application of Raman spectroscopy for non-invasive monitoring of blood parameters by utilizing the intrinsic dynamics of an individual's blood pulse as a reference signal. The developed signal processing scheme separates the signal from bulk tissues and the signal from blood, eliminating the individual's background signal characteristics in the Raman spectrum, thus enabling correction and clearing of the blood's spectrum. These spectra can then be used for machine learning-based classification algorithms to establish relationships between the spectra and the actual physical and chemical parameters of blood.

The measurement scheme is based on a phase-sensitive or lock-in amplifier that uses the individual's own blood pulse signal as a reference while the time-resolved Raman spectra are measured. Here, we present time-resolved Raman spectra measured non-invasively from a fingertip during natural blood pulsation, with recording speeds from 10 Hz up to 80 Hz. We evaluate the metrological characteristics of the developed algorithm for blood signal retrieval versus measurement speed and noise influence.

Overall, this work demonstrates the potential of Raman spectroscopy as a non-invasive and accurate method for monitoring blood parameters, with implications for both medical and personal use.

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Keywords: non-invasive Raman spectroscopy, blood pulse,

H-O.7

Title: *Rapid identification of bacteria isolated directly from patient urine and diagnosis of their antibiotic susceptibility using infrared spectroscopy-based machine learning*

Author: George Abu-Aqil[1], Manal Suleiman[1], Uraib Sharaha[1], Lior Nesher[2], Itshak Lapidot[3], Ahmad Salman[4], Mahmoud Huleihel[1]

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[3]Afeka Tel-Aviv Academic College of Engineering
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Abstract:
Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide, affecting people of all ages, genders, and races. UTIs are mainly caused by *E. coli*. Antibiotics are thought to be the most effective treatment for bacterial infections. However, the vast majority of bacteria are now resistant to most of the widely used antibiotics. Therefore, it is crucial to identify the infecting bacteria and to determine its susceptibility to antibiotics for prescribing effective treatment. Conventional methods require at least 48 hours to determine the susceptibility of the infecting bacterium. Thus, it is crucial to develop a rapid technique that may drastically shorten the time needed to identify the infecting bacterial species and its antibiotic susceptibility. Fourier-Transform Infrared (FTIR) spectroscopy is a sensitive and rapid method that can detect minor bacterial molecular changes associated with the development of resistivity to antibiotics. Examining the potential of FTIR spectroscopy in combination with machine learning techniques is the primary objective of this study to identify the infected bacterium, isolated directly from patients' urine, as E. coli and determine its susceptibility to different antibiotics in a few minutes.
Analyzing the spectra by RF achieved a 96% success rate in the identification of *E. coli* and an 85% accuracy in susceptibility determination.

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Keywords: UTI, E.coli, infrared-spectroscopy, machine-learning

H-O.8

Title: *Supplementation of vitamin C and E – an effect on human gastrointestinal tract tissues and cells: Raman spectroscopy and imaging*

Author: Karolina Beton-Mysur[1], Beata Brożek-Płuska[1]

[1]Lodz University of Technology, Faculty of Chemistry, Institute of Applied Radiation Chemistry, Laboratory of Laser Molecular Spectroscopy

Abstract:
Cancer of gastrointestinal tract, such as colorectal cancer (CRC) and gastric cancer (GC), are common types of cancer globally and their origin can be linked to oxidative stress conditions. Commonly available antioxidants, such as vitamins C and E, are widely considered as potential anti-cancer agents. Raman spectra have great potential in the biochemical characterization of matter based on the fact that each molecule has its own unique vibrational properties. Raman spectroscopy allows to precisely characterized cell substructures and components.

This study shows the differences between healthy and cancerous tissues from the human digestive tract and human normal and cancer colon and gastric cell lines. The research includes the spectroscopic characterization of normal colon cells - CCD-18 Co in physiological and oxidative conditions and effect of oxidative injury of normal colon cells upon supplementation with vitamin C at various concentrations based on Raman spectra. The obtained results were related to the Raman spectra recorded for human colon cancer cells - Caco-2. In addition, the effect of the antioxidant in the form of vitamin E on gastric cancer cells - HTB-135 is presented and compared with normal gastric cells - CRL-7869.

Results, as well as the statistical analysis, made us concluded that Raman spectroscopy enables the detection and tracking of cancerous changes in the human colon tissues and cells based on the identification of characteristic, unequivocal vibrational bands of nucleic acids, proteins and lipids, including unsaturated fatty acids. Obtained results may be a prelude to the preparation of anti-cancer dietary recommendations for patients.

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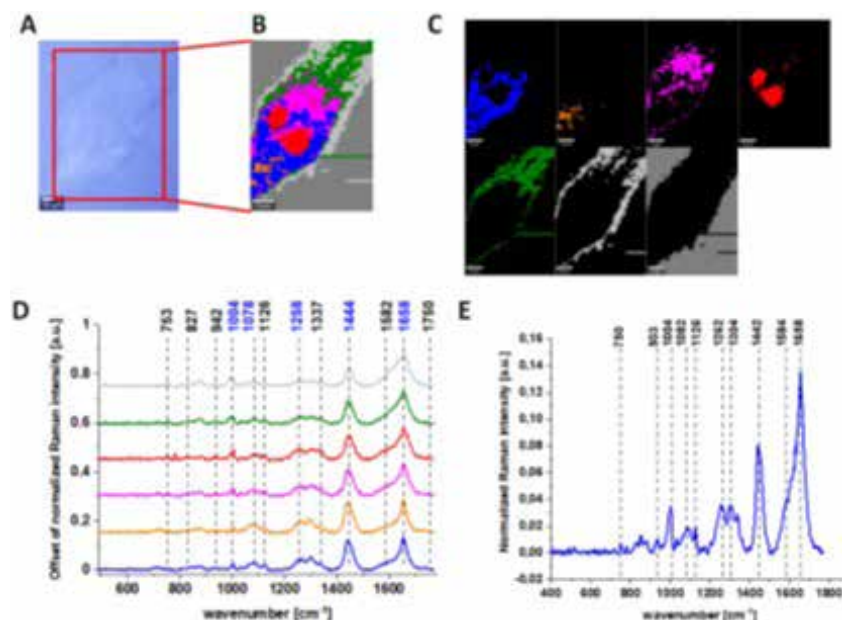
Figure captions:
FIG 1. Microscopy image of human normal gastric CRL-7869 cell and Raman images of cell substructures obtained using Raman spectroscopy and Cluster Analysis method (CA).

Keywords: cancer, antioxidants, Raman spectroscopy, supplementation

H-O.9

Title: *Molecular Characterisation of T-cell acute lymphoblastic leukemia using Raman spectroscopy*

Author: Patrycja Dawiec[1], Patrycja Leszczenko[1], Anna Nowakowska[2], Karolina Czuja[2], Justyna Jakubowska[3], Marta Zabczyńska[3], Agata Pastorczak[3], Kinga Ostrowska[3], Wojciech Mlynarski[3], Malgorzata Baranska[4], Katarzyna Majzner[2]

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Abstract:
The hallmark of acute lymphoblastic leukemia (ALL) are chromosomal abnormalities and genetic alterations developed in lymphocyte precursor cells that alter the normal development, function, and phenotype of cells. Depending on the type of cell lineage, two main types of this blood malignancy can be distinguished: B-cell ALL (B-ALL) and T-cell ALL (T-ALL). The diagnosis of ALL subtypes is time-consuming and relies on cytomorphology, cytochemistry, immunophenotyping, cytogenetics, and molecular genetics. Therefore, it is important to look for new, rapid, and molecular-orientated approaches to encourage the development of new diagnostic methods. Here, we present an attempt to identify and characterise T-ALL cells in paediatric patients using Raman imaging.
The study was carried out using clinical samples collected from children suffering from T-ALL and B-ALL. Normal T cells were isolated from peripheral blood of healthy donors. Cell Raman imaging was performed using a WITec Alpha 300 confocal Raman system using two excitation lasers (633 and a 532 nm) and water dipping objective (63x, NA=1).
To identify the spectroscopic profile of T-ALL leukemic cells (blasts), a chemometric approach was applied. Conducted analysis revealed spectroscopic Raman features allowing for efficient discrimination between normal T cells and their leukemic counterparts. Consistent with the literature1, characteristic marker bands attributed to carotenoids were observed in the T cell spectra. In contrast, clinical T-ALL samples from patients showed a higher contribution of bands assigned to proteins and lipids. A developed model based on a supervised principal least squares discrimination (PLS-DA) method enabled the prediction and appropriate classification of Raman spectra of normal T cells and T-ALL cells.
In conclusion, Raman imaging combined with multivariate chemometric analysis is a powerful tool with potential application in the clinical diagnosis of T cell-derived leukemia.

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Keywords: Raman spectroscopy, leukemia, T-ALL, chemometrics

H-O.10

Title: *Raman-based assessment of the endothelial response to antiretroviral drugs: in vitro studies on NN-RTI-treated human endothelial cells*

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Abstract:
The vascular burden of drugs is an important challenge in the area of drug development and the clinical practice. Highly active antiretroviral drugs used in HIV therapy have a long plasma half-life and can be a source of drug-induced endothelial dysfunction [1]. The endothelium plays a key role in homeostasis maintenance in the cardiovascular system, and its dysfunction can lead to the onset and / or progression of various diseases. Direct protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitor (NRTIs) may induce endothelial dysfunction [2]. Mechanisms involved in chronic antiretroviral-mediated endothelial dysfunction include mitochondrial impairment and increased oxidative stress [3].
Efavirenz (EFV) and etravirine (ETV) belong to non-nucleoside reverse transcriptase inhibitors (NNRTIs) with a steady-state high plasma concentration [2]. They may also have an impact on endothelial cell homeostasis and condition, which form the innermost part of blood vessels. EFV was found to generate oxidative stress in endothelial cells (ECs) [4] and has been associated with endothelial and cardiovascular dysfunction [4].
In this study, we use Raman imaging to track the biochemical alterations in single ECs caused by selected NNRTIs. Human aorta endothelial cells (HAECs) were treated for 24 hours with different concentrations of EFV and ETV (1, 10 and 50 µM). Raman-based biochemical analysis was carried out on spectra from several subcellular compartments: nucleus, perinuclear area and cytoplasm. Applied chemometrics of the spectral data revealed progressive and concentration-dependent changes in the drug-treated HAECs. Changes in lipid composition and cytochrome signals were further confirmed by fluorescence staining. Furthermore, for a 10 µM ETV concentration, the uptake and accumulation by living cells was observed within the perinuclear area. Obtained results may be associated with the generation of early stage of the ECs dysfunction, caused by NNRTIs.

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Keywords: Raman imaging, endothelium, antiretroviral drugs

H-O.11

Title: *Bladder Cancer detection by Fourier Transform Infrared Spectroscopy (FTIR) using urine samples.*

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Abstract:
Aim: Diagnosis and monitoring of Bladder Cancer (BCa) are mainly based on cystoscopy, which is invasive, high-cost and patient discomfort[1]. We attempt to detect BCa non-invasively by a combination of Fourier Transform Infrared Spectroscopy (FTIR) technique with machine learning algorithms using urine as proximal biofluid of tumor site.
Methods: Random total urine samples of 50 BCa patients and 53 healthy volunteers were collected from Urology department (Reims University Hospital) and directly analyzed by high-throughput FTIR. In total 295 spectra were included in the study. After specific chemometric processing, Support Vector Machines (SVM) and Random Forest (RF) methods were used to construct models in order to develop a binary classification to distinguish between BCa and normal control groups. Clinico-biological characteristics of the two groups were also collected.
Results: Preliminary result obtained by using the two developed models: SVM and RF gave the same specificity (64%), whereas the sensitivity of SVM (75.5%) was better than the RF (66%). These results, reflect moderate sensitivity and specificity values of the current approach, which can be explained by the existence of high intra and inter variability in urine samples.
Conclusion: For now, the obtained results are insufficient to include our method in BCa diagnosis strategy, but they show that infrared spectroscopy may provide some discriminative information. To improve method's specificity other machine learning models will be tested, and the use of more targeted urinary components such as extracellular vesicles will be also investigated.

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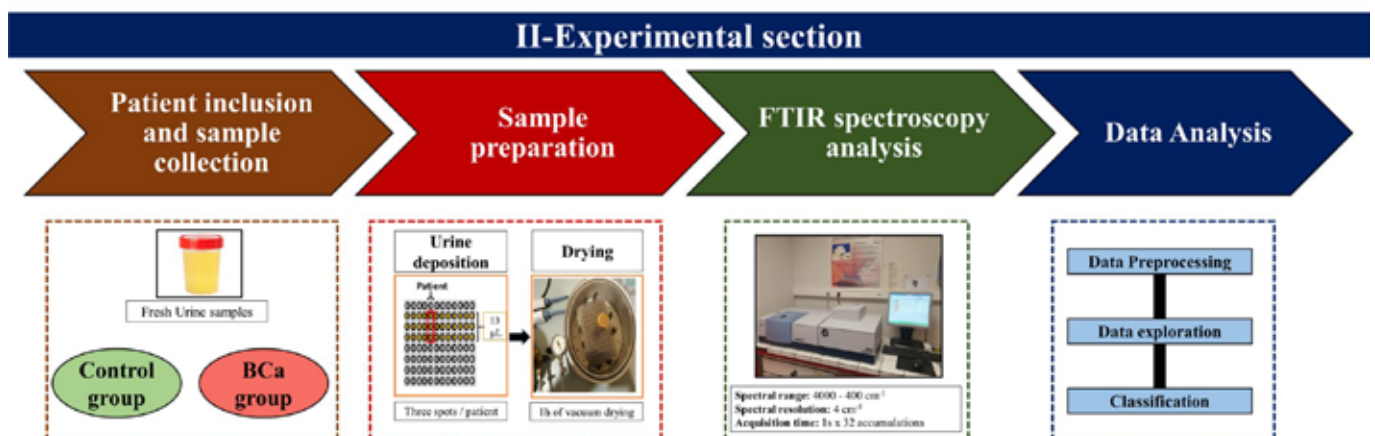
Figure captions:
Fig. Experimental steps of bladder cancer diagnosis: FTIR spectroscopy-based method using total urine samples.

Keywords: FTIR spectroscopy, Urine, Bladder cancer

H-O.12

Title: *Exploring the potential for Deep Raman Spectroscopy for non-invasive sex determination of chicken eggs*

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Abstract:
In order to meet the demand for consumption eggs, billions of specially bred layer chickens are hatched every year. Serving no purpose in the industry, over 372 million one-day old male chickens are culled every year in Europe. Current, accurate (>95%) commercial in-ovo sexing techniques are unfit for sexing before day 9 of incubation (E9) and their invasive nature imposes a risk for bacterial infection. With upcoming new legislation aiming to outlaw the culling of chicken embryos after E7, there is a need for non-invasive early in-ovo chicken sex determination methods. In recent years, fluorescence and Raman spectroscopy were demonstrated as promising techniques for the retrieval of sex-related biomarkers from embryonic blood for early and accurate in-ovo sexing.[1,2] However, the high optical scattering of the eggshell has proven a yet insurmountable challenge in the application of these techniques in a non-invasive manner. Seeking to overcome this issue, this work explores the application of spatially offset-, transmission and time-resolved Raman Spectroscopy (Deep Raman Spectroscopy, DRS) techniques for the non-invasive retrieval of sex-related biomarkers from embryonic tissues. To estimate the impact of the large sample volume inherent to DRS on the retrieval of key biomarkers, the presence, distribution, and discriminative value of hemoglobin, protoporphyrin IX, and nucleic acids in-vivo were determined in different embryonic tissues at various developmental stages using backscatter Raman spectroscopy. Different instrumental configurations were selected to study the limited spatial specificity and volumetric averaging inherent to DRS to evaluate the retrieval of the targeted biomarkers. The weak contributions of these biomarkers highlights the anticipated challenges and limitations of DRS for subsurface analysis in extremely turbid media. Based on these findings, various strategies to improve the suitability of DRS for non-invasive in-ovo sexing are proposed.

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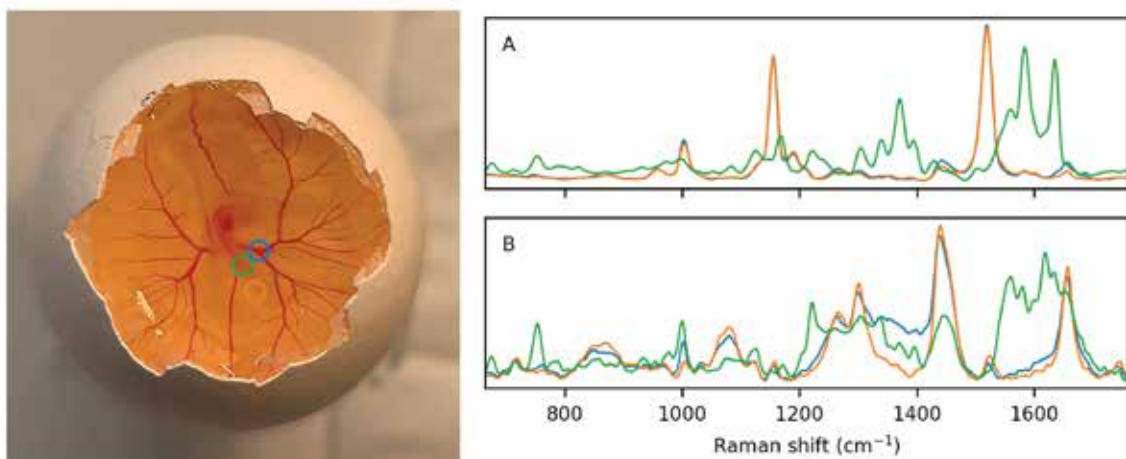
Figure captions:
Raman spectra obtained from different (extra-)embryonic tissues at 532 (A) and 785 (B) nm excitation on the 4th day of incubation, all showing hemoglobin's porphyrin breathing vibration at 750 cm-1.

Keywords: Biophotonics, Deep Raman Spectroscopy

H-O.13

Title: *Raman-based evaluation of in vitro myeloid precursor differentiation toward macrophages*

Author: Adriana Adamczyk[1], Anna Nowakowska[1], Justyna Jakubowska[2], Katarzyna Majzner[1], Malgorzata Baranska[1]

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Abstract:
Haematopoiesis is a complex process characterised by the unique metabolic activity, physiology, and morphology of precursors to well-functioning mature blood cells. The maturation process occurs in the bone marrow; therefore, the isolation of blast cells is an invasive method. Most studies concerning the influence of drugs on cell maturation, metabolic activity, etc., are performed using in vitro models, e.g., HL-60, U937. They are able to differentiate toward monocytes, and macrophages under different chemical stimuli [1].

The method supplying good spatial resolution is Raman spectroscopy (RS), which allows non-destructive identification of the cellular composition. Here, we focus on the molecular characterisation of the induced differentiation process toward monocytes and macrophages, which plays a crucial role during inflammation. The RS results obtained for, among all, PMA-treated U937 cells indicated changes in the phenotype and biochemical profile associated with disruption of subcellular compartments and their biological state.

Despite many advantages of the label-free approach in studies on biochemical changes at subcellular level, the concept of specific molecular Raman probes (RP) is gaining importance. RPs exhibit bands in the spectral region of 2800-1800 cm^{-1} due to the presence of triple bonds or deuterium in their molecular structure together with a selective group, targeting the RP molecule to appropriate organelle [2].

The application of lipid-specific falcarinol and MitoBADY, which accumulate according to mitochondrial membrane potential, allowed a better indication of changes related to lipid droplets and mitochondria upon induced differentiation. We showed that RS is a useful tool for studying biochemical changes, following induced differentiation. Moreover, our results show that the application of RP can improve the sensitivity and specificity in classification, which can be translated to nonlinear techniques such as stimulated Raman scattering.

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Keywords: induced differentiation, macrophages, Raman probe

H-O.14

Title: *Brillouin and Raman micro-spectroscopy to characterise human bone and cartilage: from healthy phenotype to biomedical applications in osteoarthritis and bone infections.*

Author: Martina Alunni Cardinali[1], Sara Stefani[1], Marco Govoni[2], Dante Dallari[2], Leonardo Vivarelli[2], Matilde Tschon[3], Silvia Brogini[3], Alessandra Maso[4], Elisa Storni[4], Francesca Valenti[5], Melania Maglio[3], Maurizio Mattarelli6, Alessandra Anna Passeri[6], Silvia Caponi[7], Assunta Morresi[1], Paola Sassi[1], Daniele Fioretto[6]

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Abstract:
Musculoskeletal tissues have a complex structure that is well designed to ensure their exceptional mechanical performance. Human bone tissue is characterized by a hierarchical architecture with multiple levels of organization, while articular cartilage is shaped to form a bearing layer that prevents friction between the bones in the joint. Their ability to both resist and adapt to the mechanical and chemical stresses is highly dependent on maintaining a correct arrangement of all their components already at the microscale. In several common degenerative diseases, such as osteoarthritis (OA), the tissue structure is destroyed by inflammatory processes. This results in a rearrangement of the overall structure of the organ, leading to a complete loss of function. Similarly, in different pathologies, the invasion of musculoskeletal tissue by common hospital bacteria such as *Staphylococcus* aureus leads to progressive tissue deterioration and the need for subsequent surgery. In both cases, the use of minimally invasive techniques to probe the lesions can become a valuable resource for the surgeon and limit the resection to really damaged areas only. This would have a significant benefit for the patient's recovery.
Brillouin and Raman micro-spectroscopy is a scattering technique that allows the simultaneous assessment of tissue mechanical and chemical properties with micrometric resolution1. It is non-destructive, non-contact and does not require labelling, thus having the potential for future in vivo applications. It has been successfully used for single cell studies and whole tissue description in both physiological and pathological conditions. Here we discuss the results obtained by our group in characterizing human musculoskeletal tissue and the first attempt to use this technique in combination with machine learning to determine the extent of OA and to localize Staphylococcus aureus damage on the human.

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Keywords: Raman Spectroscopy, Brillouin Spectroscopy, Bone

H-O.15

Title: *Fourier Transform Infrared Microspectroscopy identifies single cancer cells in blood. A feasibility study towards liquid biopsy.*

Author: Lewis M. Dowling[1], Paul Roach[2], Eirik A. Magnussen[3], Achim Kohler[3], Srinivas Pillai[4], Daniel G. Van Pittius[4], Ibraheem Yousef[5], Josep Sulé-Suso[1]

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Abstract:
The management of cancer patients has markedly improved with the advent of personalised medicine where treatments are given based on tumour antigen expression amongst other. Within this remit, liquid biopsies will no doubt improve this personalised cancer management. Identifying circulating tumour cells (CTCs) in blood allows a better assessment for tumour screening, staging, response to treatment and follow up. However, methods to identify/capture these CTCs using cancer cells' antigen expression or their physical properties are not robust enough. Thus, a methodology that can identify these CTCs in blood regardless of the type of tumour is highly needed.

Fourier Transform Infrared (FTIR) microspectroscopy, which can separate cells based on their biochemical composition, could be such technique. In this feasibility study, we studied lung cancer cells (squamous cell carcinoma and adenocarcinoma) mixed with peripheral blood mononuclear cells (PBMC). Glass coverslips widely available in pathology departments were used as substrates. They allow to obtain spectral information down to 1350 cm^{-1} as we have previously described[1-3]. Moreover, these cheap substrates allow further histopathological cell analysis (staining, immunohistochemistry,...) after FTIR spectra are obtained. The data obtained shows, for the first time, that FTIR microspectroscopy together with Random Forest classifier is able to identify a single lung cancer cell in blood. This separation was easier when the region of the IR spectra containing lipids and the amide A (2700 to 3500 cm^{-1}) was used. Hence, FTIR microspectroscopy could become another tool to be used in liquid biopsies for the identification of CTCs, and in the personalised management of cancer.

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Keywords: FTIRmicrospectroscopy, circulatingtumourcells, lungcancer, peripheralbloodmononuclearcells

H-O.16

Title: *Raman spectroscopy in the biochemical characterisation of THP-1 leukemic cells modified to overexpress mutated FLT3 receptor.*

Author: Sylwia Orzechowska[1], Paulina Laskowska[2], Aleksandra Borek-Dorosz[1], Anna Maria Nowakowska[1], Wiktoria Korona[1], Marcin Szydłowski[2], M. Zasowska[2], Piotr Juszczyński[2], Małgorzata Barańska[3], Piotr Mrówka[4], Katarzyna Majzner[1]

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Abstract:
Acute myeloid leukemia (AML) is a heterogeneous disease that develops from progenitors of the myeloid lineage. Treatment of patients with AML is influenced by the presence of specific structural aberrations in malignant cells. FLT3 mutations have been found in approximately 30% of adult AML, making it the most frequent genetic alterations in AML. The FLT3 gene encodes a tyrosine kinase receptor that regulates proliferation and differentiation of during haematopoiesis. An internal tandem duplication the most common FLT3 mutation (FLT3 /ITD) in AML is associated with a poor prognosis [1-2].
The objective of our study was to identify metabolic changes associated with FLT3 mutations in an *in vitro* model of leukemic cells overexpressing the FLT3-ITD mutant using Raman imaging combined with multivariate statistical analysis.
Raman imaging is a promising tool that allows not only the biochemical characterisation of cells and the identification of molecular and metabolic changes due to mutation within cells, but also allows the differentiation of leukemia subtypes in clinical samples [3]. Therefore, the objective of the study was the molecular characterisation of THP-1 cells with a mutation in the FLT3 gene. Parental THP-1 cells expressing the wild-type (WT) sequence of the FLT3 gene served as control samples. Control and FLT3 mutated transgenic cells were imaged using the WITec Alpha 300 confocal Raman system.
The Raman-based distinction between cells with wild-type FLT3 and FLT3/ITD was obtained by multivariate chemometric analysis. The results indicate that the Raman spectra of the studied groups of cells are showing differences in the lipids and hemoproteins content. Differences in the biochemical profile of THP-1 cells caused by FLT3 mutations are subtle, but significant. It shows the potential of Raman spectroscopy for the detection and study of leukemia driver mutations.

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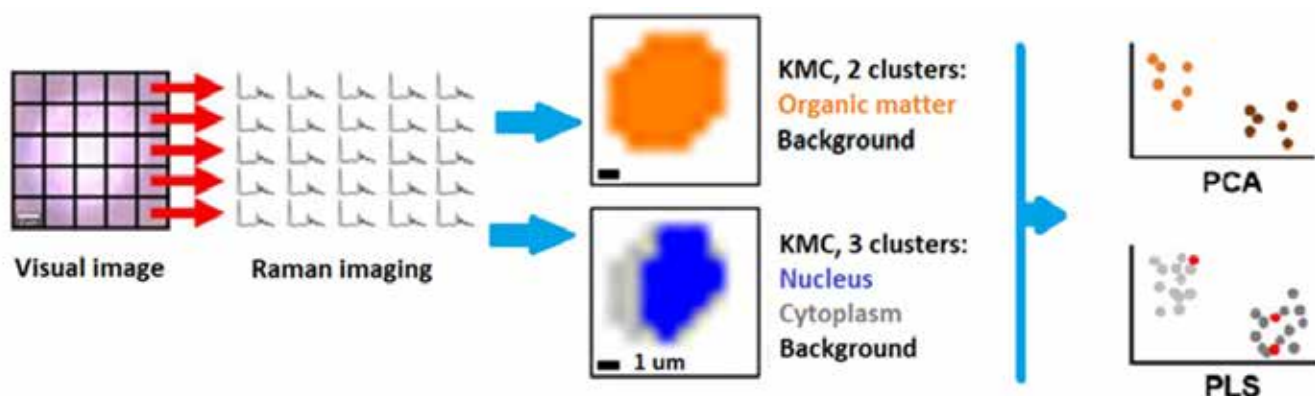
Figure captions:
Scheme of Raman imaging and multivariate analysis of THP-1 cells with and without FLT3/ITD.

Keywords: Raman imaging, acute myeloid leukemia,

H-O.17

Title: *Identification of Chemical Modifications of Myocardium in Heart-Failure with Preserved Ejection Fraction*

Author: Leonardo Pioppi[1], Reza Parvan[2], Alan Samrend[2], Gustavo Jose Justo Da Silva[2], Marco Paolantoni[1], Alessandro Cataliotti[2], Paola Sassi[1]

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Abstract:
In recent years, the spectroscopic investigation of biological samples has mostly focused on the diagnosis of several types of cancers and neurological disorders[1], but other pathologies, such as cardiovascular ones which affect the lives of millions of people in the world, need attention. Vibrational spectroscopy can be a valuable tool to monitor the markers of cardiovascular diseases[2]. Heart Failure with Preserved Ejection Fraction (HFpEF) is a major clinical challenge that is associated with a markedly high risk of death. Its development is often the result of the contribution of severe comorbidities such as obesity, diabetes, and hypertension[3]. The challenging diagnosis of HFpEF is often belated and the treatment of this condition remains largely unsuccessful, with a five-year survival rate of 43% after a first diagnosis. In addition, the wide plethora of comorbidities associated with HFpEF makes the disease's progression mechanism unknown. In this work, we perform an ex-vivo analysis of the cardiac ventricles of rats to detect biochemical alterations due to the progression of HFpEF and its related comorbidities. Spectroscopic markers (lipids, carbohydrates, and glutamate bands) were recognized as due to obesity and diabetes. Besides, abnormal collagen cross-linking and a decrease in tryptophan content were observed and related to the stiffening of ventricles and to the inflammatory state which is a favourable condition for HFpEF. By the analyses of tissues, FTIR and Raman techniques were shown to be highly sensitive and selective in detecting changes in the biochemistry of the heart caused by the complex HFpEF clinical picture.

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Keywords: Vibrational spectroscopy, cardiovascular diseases, biodiagnostic

H-O.18

Title: *Application of Raman spectroscopy to examine tattoo pigments in tissues*

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Abstract:
We present the use of Raman spectroscopy for analyzing tattoo pigments in tissue. As tattoos have become more popular, it's important to understand their safety and composition to avoid complications or to react appropriately when they occur. This study aims to examine the pigments non-invasively and directly in the skin by using Raman spectroscopy. The results of such research can be valuable in the treatment of complications after a tattoo (allergies, other skin reactions), and tattoo removal, as well as in the assessment of lesions in the tattoo area.
In the first step, we measured pigments with a Raman spectroscopy setup, then we designed and produced highly specialized phantoms mimicking tattooed skin. We've done similar research in the past, but with tattoo inks. Currently, the studies are at the next stage in which we take into account that once the tattoo has healed, only the pigment remains, and this pigment should be present in the skin model in order to reflect the conditions of measuring on real patients. The research challenge was that the chemical composition of a healed tattoo needed to be accurately replicated in order to create an accurate skin model for measuring purposes. We produced phantoms with various optical parameters, and with different chemical compositions. Moreover, we designed and produced skin phantoms mimicking skin lesions, and lesions in clear as well as tattooed skin.
All samples were characterized, and then measured with the use of a specially modified Raman spectrometer. Results indicate that Raman spectroscopy is a promising method for the analysis of tattoo pigments in tissue.

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Keywords: Raman spectroscopy, skin phantoms, tattoo

H-O.19

Title: *Raman analysis of breast microcalcifications, correlation with pathology*

Author: <u>Carlo Morasso</u>[1], Renzo Vanna[2], Francesca Piccotti[1], Marta Truffi[1], Sara Albasini[1], Thomas Huthwelker[3], Laura Villani[1], Oliver Bunk[3], Cinzia Giannini[4], Fabio Corsi[5]

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Abstract:
Microcalcifications (MCs) are small calcium deposits frequently found during mammography and often difficult to classify. To date, their importance as a potential marker of breast cancer is known, but the origin of MCs and their connection with the presence of malignant breast lesions remains unclear [1]. This study aimed to investigate the mineral composition and structural features of MCs present in breast tissue using Raman spectroscopy (RS) and examine their correlation with breast lesions' pathology. Breast tissue samples were analysed using a RS mapping approach for a total of 822 MCs from 107 patients. The chemical composition of MCs was automatically extracted for each MCs using an automated ad-hoc algorithm [3]. Results of the study showed that hydroxyapatite (HAp) was the most abundant mineral in all diagnostic categories, followed by whitlockite (Whit), a particular form of Magnesium substitute of calcium phosphate. Whit was found almost exclusively in a subset of benign MCs [3]. By looking at the spatial distribution of Whit in benign samples was found to be disposed at the edge of the HAp core of MCs. Conversely, in malignant lesions, Whit deposits were extremely scarce and mainly isolated and not close to other minerals. The crystallinity of MCs was measured by RS looking at the full width at half maximum of the phosphate peak of HAp at 960 cm-1, with malignant MCs found to have a higher crystallinity than benign MCs. Additionally, our study found that the HAp core of MCs presenting Whit had a lower degree of crystallinity than those without Whit, which may be useful in determining the nature of MCs in future studies. Our findings suggest that Whit may be a marker of benignity in MCs, providing valuable information for improving the diagnosis and treatment of breast lesions. Specifically, the presence of Whit detected by RS could support the hypothesis that certain MCs are benign and do not require further invasive procedures, such as a biopsy.

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3. R. Vanna, C. Morasso, B. Marcinnò, F. Piccotti, et al., Raman Spectroscopy Reveals That Biochemical Composition of Breast Microcalcifications Correlates with Histopathologic Features. Cancer Res, 80 (2020) 1762-1772. doi: 10.1158/0008-5472.CAN-19-3204.

Figure captions:
Spatial distribution of Whit within benign MCs. Bright field (A,C) and false colour Raman maps (B,D) (HAp = green; Whit = red) of two representative benign (B2) MCs

Keywords: Raman spectroscopy, Breast Cancer, Microcalcifications

H-O.20
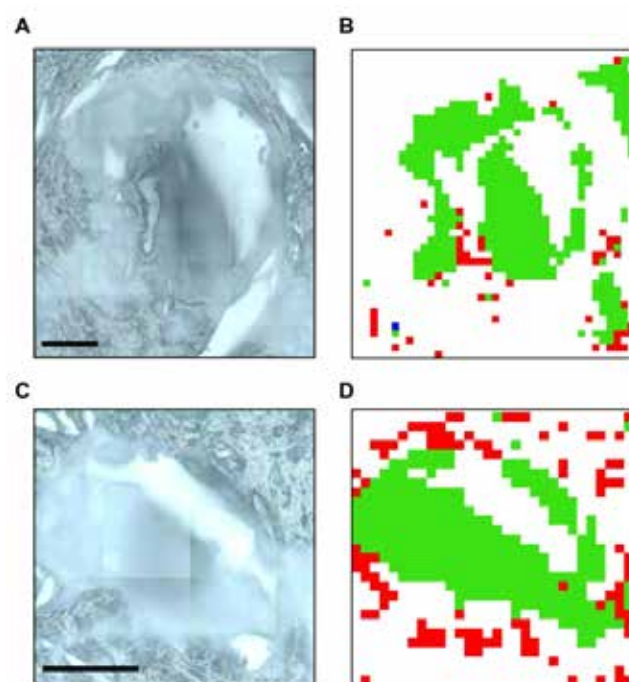
Title: *Pre-clinical characterization of Osteopetrosis in Mice Models by Raman microspectroscopy*

Author: Marco Ventura[1], Alejandro De La Cadena[1], Morteza Behrouzitabar[2], Maria Lucia Schiavone[3], Federico Vernuccio[2], Giulio Cerullo[2], Cristina Sobacchi[3], Dario Polli[2], Renzo Vanna[1]

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[2]Politecnico di Milano
[3]IRCCS Humanitas Researc Hospital

Abstract:
Background: osteopetrosis is a genetic pathology normally diagnosed during first months of life, characterized by increased bone density caused by abnormalities in osteoclast development and activity, and resulting in bone resorption failure, deformities, and bone fractures. At least eleven different osteopetrosis subtypes are known, associated with different inheritance modalities, prognosis and severity. Osteopetrosis diagnosis and subtyping require complex diagnostic protocols, including bone biopsy, which is an invasive and painful procedure, especially for very young patients. The perspective of using laser light to study this pathology in a non-invasive way is therefore very promising, and can be an opportunity, first, to better understand the etiopathology of osteopetrosis and, second, to potentially allow in vivo diagnosis and disease monitoring.
Methods: homozygous oc/oc mice, reproducing the phenotype of osteopetrosis, were compared with C57BL/6J mice. Bone samples, including femur, skull and vertebrae were selected. Single point Raman spectra and hyperspectral images were acquired using a home-built Raman microspectrometer equipped with a 660nm laser and a 60Xw objective.
Results: the results show that one of the main differences between healthy and osteopetrosis bone is hydroxyapatite crystallinity, which is significantly higher (reduced phosphate band width) in osteopetrosis. A significantly lower mineral-to-matrix ratio has been also detected in pathological samples, if compared to the normal ones. Hyperspectral images of bone slices also show an increased bone density. Both evidences indicate alteration at morphological and mineral level, underlining a bone growth retardation condition, and these data cannot be easily retrieved using standard tools.
Conclusions: this study suggests that Raman spectroscopy can be an adequate tool for the direct and non-invasive study of osteopetrosis, opening new research and diagnostic perspectives.

References:
1. M. Unal, R. Ahmed, A. Mahadevan-Jansen, J.S. Nyman, Compositional assessment of bone by raman spectroscopy. Analyst, 146(24):7464–7490, 2021.
2. C. Sobacchi, A. Schulz, F. Coxon, Anna Villa, M.H. Helfrich, Osteopetrosis: genetics, treatment and new insights into osteoclast function. Nature Reviews Endocrinology, 9(9):522–536, 2013.

Figure captions:
Comparison between hyperspectral Raman images of osteopetrosis (OP) and healthy control (HC) mice vertebra slices after performing multivariate curve resolution (MCR) analysis.

Keywords: Raman, osteopetrosis, diagnosis, hydroxyapatite, bone

H-O.21
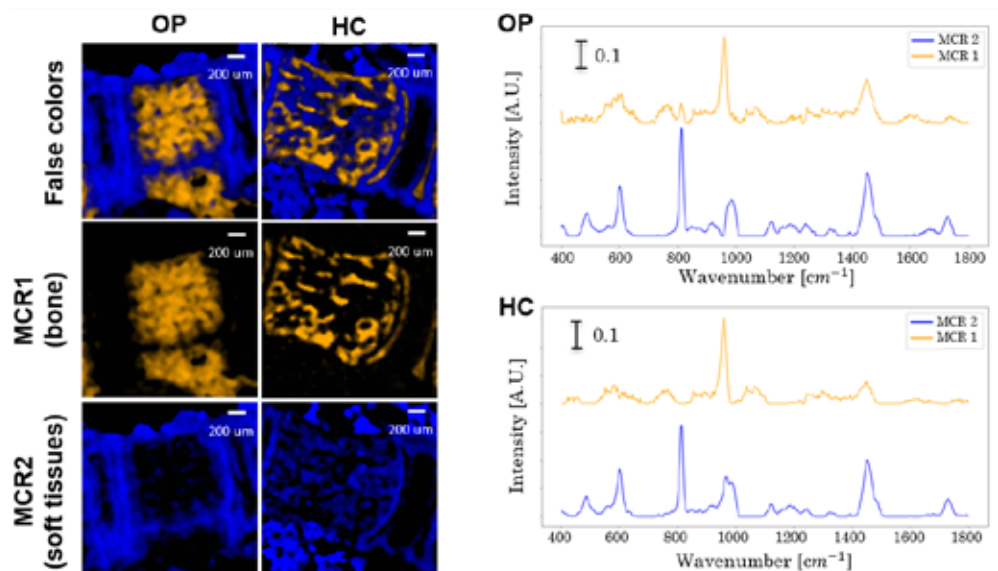
Title: *Study on the effects of cryoconservation on human platelets*

Author: Diana E. Bedolla[1], Gaia Gavioli[2], Agnese Razzoli[2], Eleonora Quartieri[3], Barbara Iotti[3], Pamela Berni[3], Giovanni Birarda[4], Lisa Vaccari[4], Davide Schiroli[3], Chiara Marraccini[3], Roberto Baricchi[3], Lucia Merolle[3]

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Abstract:
Fresh platelets (PLTs) have a short shelf life thus excluding the possibility to store large supplies and difficulty for storage. Frozen PLTs can be used as an alternative, allowing a longer time conservation. However, their clinical applications for prophylactic purposes are still under evaluation. In this study, cryoconserved (cryo)-PLTs were compared to fresh-PLTs as a standard reference with the aim to assess its possible use on onco-haematological patients. Briefly, fresh components were reconstituted in plasma, analysed within 5 days from collection, whereas cryo-PLTs were thawed after 6 months, and analysed at 1, 3 and 6 hours after reconstitution in PLTs additive solution. Cryo-PLTs displayed a reduced viability and clotting capacity, which were also accompanied by a glycoprotein pattern modification and massive extracellular vesicles (EVs) shedding. Macromolecular changes induced by cryopreservation on membrane lipids and protein modifications were revealed by FTIR spectroscopy evidenced by the increase of the membrane stiffness (CH2/CH3 ratio) and loss of protein content (protein/lipid ratio) which occur irreversibly 3 h after thawing. In addition, cryopreservation impairs protein folding and membrane phospholipids structure with significant increase of peroxidation process occurring over time after thawing. Spectra were also acquired during freezing-thawing cycles on PLTs, and these latter were measured with nano-FTIR highlighting morpho-chemical variations after the freezing cycles.
The data demonstrated that cryopreservation affects membranes' functional characteristics, integrity and clotting capacity. Our results allow concluding that the time after thawing is crucial and significantly affects platelets functional characteristics and integrity.

Keywords: platelets, blood, cryoconservation, freezing, time-series

H-O.22

Title: *Fighting peripheral nervous system tumors-hyperspectral imaging as a novel approach to monitor the therapeutic efficacy of cannabidiol*

Author: Karolina Chrabąszcz[1], Katarzyna Pogoda[1], Klaudia Suchy[1], Agnieszka Panek[1], Czesława Paluszkiewicz[1], Wojciech M. Kwiatek[1]

[1]Institute of Nuclear Physics, Polish Academy of Science, Krakow, Poland

Abstract:
When peripheral nervous system tumors (PNS) occupy large blood vessels, spinal nerves, or more than one peripheral nerve, stereotactic radiosurgery is recommended. This approach consists of one or more (<5 fractions) of high doses of ionizing radiation (~5-16 Gy). However, it often causes complications such as dizziness, and damage to the adjacent nerves, including the auditory nerve, or leads to tumor malignancy. Therefore, it is important to look for alternative methods of treatment to effectively reduce the volume of the tumor while maintaining the normal function of the surrounding cells. 1,2
Recently, cannabidiol (CBD) has been gaining popularity for its proven anticancer, anti-inflammatory, neuroprotective, and antioxidant effects. CBD affects many types of cancer, inhibiting their growth and migration of tumor-forming cells. So far CBD was investigated as a substance supporting the treatment of malignant tumors of the central nervous system. However, its use in the therapy of tumors of the PNS has not yet been investigated.3,4
The presented studies are the first steps towards the use of imaging techniques such as Raman (RS) and infrared (IR) spectroscopies, as well as the combination of IR with AFM microscopy (AFM-IR) to monitor biochemical changes induced by CBD in cancer and control (normal) cells of PNS (Fig 1. Presents the experimental scheme). It is important to answer the question of whether CBD selectively increases the toxicity of ionizing radiation (X-ray) in cancer cells and simultaneously displays a protective effect on non-cancerous cells. Cyto- (MTS) and genotoxic (COMET) assays allow to establish the effective concentration of CBD and X-ray dose. Spectroscopic imaging of both irradiated cell lines indicates information about biochemical changes (e.g. lipid saturation, transformations in the order of protein structure, and carbohydrate metabolism).

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3. S. Atalay et. al Antioxidative and anti-Inflammatory properties of cannabidiol, (2020) Antioxidants, 9, 21.
4. T. Huang et. al Cannabidiol inhibits human glioma by induction of lethal mitophagy through activating TRPV4, (2021) Autophagy, 17(11):3592-3606.
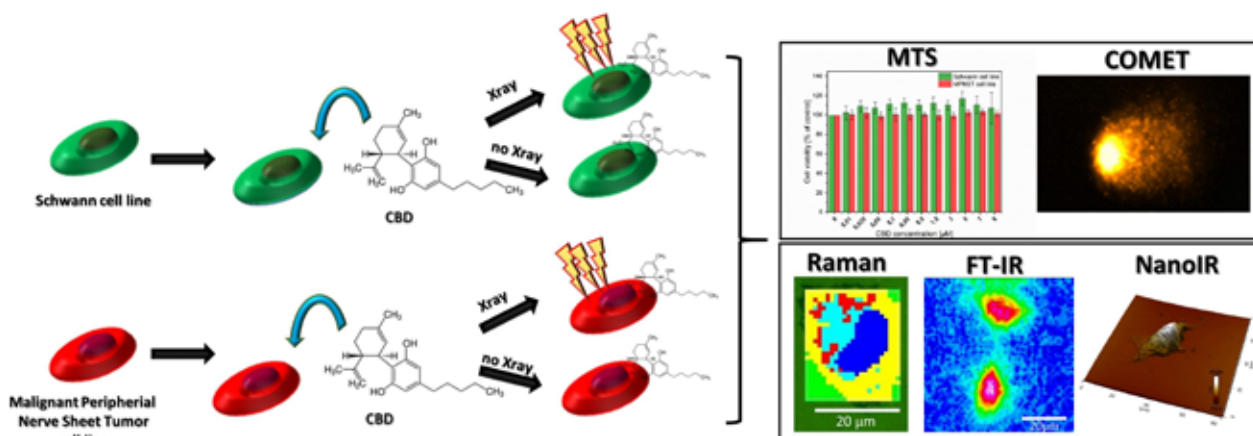
Figure captions:
Figure 1. The experimental scheme.

Keywords: hyperspectral imaging, anti-cancer therapy, cannabidiol

H-O.23

Title: *Infrared tissue analysis of Hirschsprung's disease*

Author: Cymoril Combescot[1], Anne Durlach[2], Valérie Untereiner[3], Francesco Laconi[2], Olivier Piot[1]

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[2]Reims University Hospital
[3]University of Reims Champagne Ardenne, Cellular and Tissular Imaging Platform

Abstract:

Hirschsprung's disease is a congenital disease with an incidence of 1/5000 birth, defined by an absence of ganglion cells in the colon causing an absence of intestinal peristalsis leading to constipation, bowel obstruction, or enterocolitis which can be deadly. The only treatment is surgical resection of the colon, which is usually done on newborns during their first months of life (1). During the operation, it is necessary to search by *extempore* examination of histological sections for the presence of ganglion cells to confirm where the pathological area ends. However, this verification has two major limitations. The first one is that it is time-consuming: 30 mins by verification while the anesthesia is still ongoing. The second is the difficulty to distinguish the transitional area from the pathological and healthy areas since it may present ganglion cells but those being unfunctional, which can lead to a re-operation later (1, 2). This is where vibrational spectroscopy is being considered as a candidate method to confirm diagnosis in real time and to help surgeons to delineate the pathological region, directly in the operating room. The objective of this preliminary study is to check two hypotheses that could allow the future development of an automated diagnostic method:

The first one is based on the search in the tissue for characteristic elements, with specific spectral signatures, such as ganglion cells for healthy tissue or hypertrophic cholinergic innervation for pathological tissue. (3)

The second relies on the capability to identify spectral markers, distinguishing between healthy and pathological tissues by focusing on neighboring tissue structures such as in particular the muscular.

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(2) Vickery JM, Shehata BM, Chang EP, Husain AN. Reoperation for Hirschsprung Disease: Two cases of Vanishing Ganglion Cells and Review of the Literature. Pediatr Dev Pathol. 2023 Jan-Feb;26(1):77-85.

(3) Braczynski, A.K., Gfroerer, S., Beschorner, R. et al. Cholinergic innervation and ganglion cell distribution in Hirschsprung's disease. BMC Pediatr 20, 399 (2020).
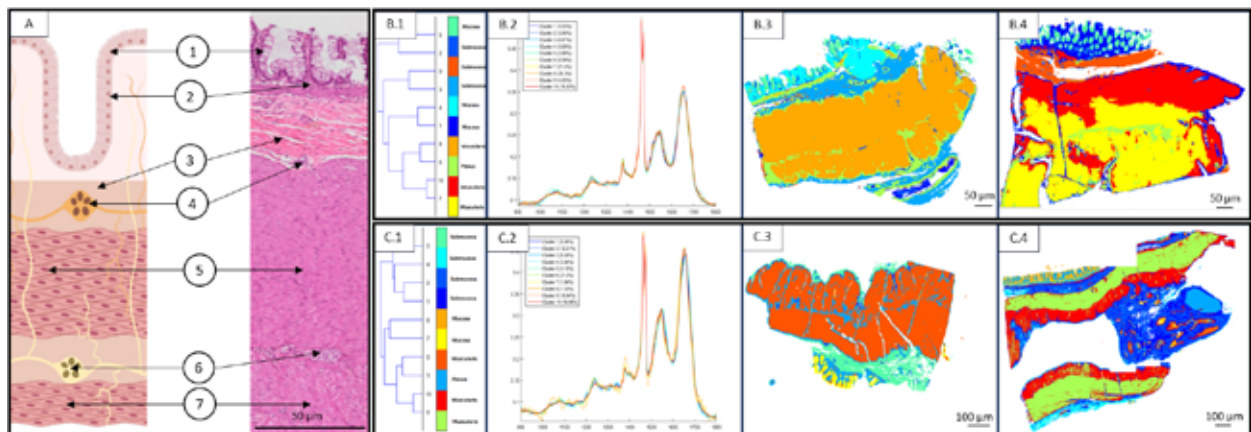
Figure captions:
Analysis of FTIR spectral images of healthy and pathological samples from two patients, using K-means clustering

Keywords: Hirschsprung, aganglionosis, IR spectral imaging,

H-O.24

Title: *Infrared spectral biomarkers of neurodegenerative diseases*

Author: <u>Lila Lovergne</u>[1], Dhruba Ghosh[2], Renaud Schuck[1], Aris Polyzos[1], Michael Martin[3], Edward Barnard[4], James Brown[5], Cynthia McMurray[1]

[1]Lawrence Berkeley National Laboratory/ Division of Molecular Biophysics and Integrated Bioimaging
[2]Lawrence Berkeley National Laboratory/ Department of Statistics
[3]Lawrence Berkeley National Laboratory/ Advanced Light Source
[4]Lawrence Berkeley National Laboratory/ Molecular Foundry
[5]Lawrence Berkeley National Laboratory/ Department of Statistics, and Division of Environmental Genomics and Systems Biology

Abstract:
Although some neurodegenerative diseases can be identified by behavioral characteristics relatively late in disease progression, we currently lack reliable methods to diagnose patients. Therapies are also urgently needed, but we have no biomarker to determine when to start treatment and we lack approaches to predict the onset of disease or the efficacy of therapeutics. The development of reliable and affordable FTIR biomarker tests for neurodegenerative diseases, as an early biomarker for disease risk, has the potential to revolutionize how we approach diagnosis.
We have developed a general-use tool based on the Fourier Transform Infrared (FTIR) spectromicroscopy technique to predict disease status from cell spectral images. Our strategy was (1) to develop a robust algorithm model using brain cells of a stable mouse system of Huntington's disease (HD) with little biological variation, and (2) to test the algorithm with more variable human HD, Alzheimer's (AD) and Parkinson's (PD) disease fibroblast samples, which were used as brain cell surrogates. Spectral phenotyping was not only successful in disease classification in the absence of overt pathology in the mouse model, but also identified neurodegenerative disease classes of HD, AD and PD patient fibroblasts when compared to healthy controls while individual signature for each patient or control was also retained. Fibroblasts have different functions and do not report on the biology of brain cells. However, skin cells share the same genotype with brain cells and undergo chemical changes that track with a disease as biomarkers. We will expand our preliminary analysis using human surrogate cells to predict disease status in larger patient cohorts.

References:
L. Lovergne, D. Ghosh, R. Schuck, A.A. Polyzos, A.D. Chen, M.C. Martin, E.S. Barnard, J.B. Brown, C.T. McMurray. An infrared spectral biomarker accurately predicts neurodegenerative disease in the absence of overt symptoms. Sci. Reports. 11 (2021) 15598.
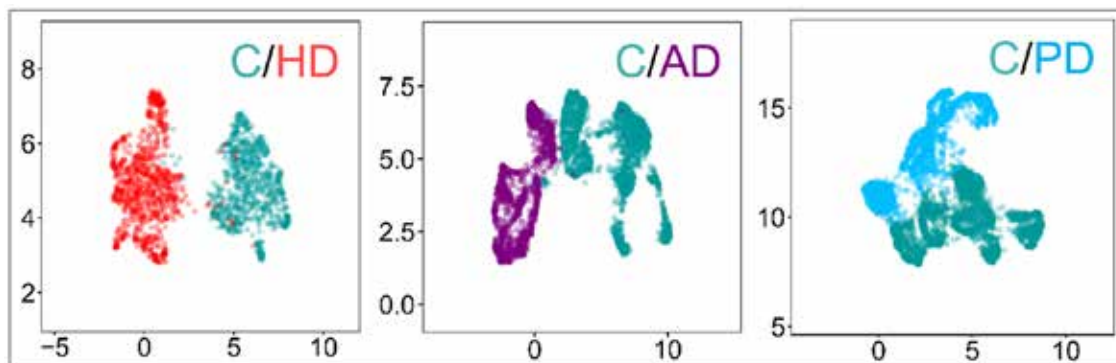
Figure captions:
UMAP analysis showing that Huntington's (HD), Alzheimer's (AD), Parkinson's (PD) patient and healthy control (C) fibroblasts are distinguished by spectral phenotyping.

Keywords: Infrared imaging, neurodegeneration, diagnosis, fibroblasts

H-O.25

Title: *Multimodal spectroscopic imaging of cervical cancer cells exposed to the adaptogenic drug*

Author: <u>Ewa Pięta</u>[1], Katarzyna Pogoda[1], Klaudia Suchy[1], Karolina Chrabąszcz[1], Czesława Paluszkiewicz[1], Wojciech Kwiatek[1]

[1]Institute of Nuclear Physics Polish Academy of Sciences, PL-31342 Krakow, Poland

Abstract:
Cervical cancer is women's fourth most common cancer worldwide, with over 600,000 new cases in 2020 [1]. These alarming reports prompted the dynamic development of new therapeutic paths in the treatment of cervical cancer. Nevertheless, there is still no fully effective solution, and the prolonged survival of patients pays special attention to the side effects of chemo-therapeutics on non-cancerous cells. Therefore, in recent years, substances classified as adaptogens have been identified as promising sources of drugs in the prevention and treatment of cancer due to their ability to attack multiple molecular targets [2]. This investigation considers the anti-tumor activity of withaferin A, an adaptogenic substance, on HeLa cervical carcinoma cells [3]. The research was carried out using high-definition vibrational spectroscopy methods, i.e. Fourier-transform infrared spectroscopy (FT-IR), Raman spectroscopy (RS), and atomic force microscopy-based infrared spectroscopy (AFM-IR) imaging at the single-cell level. Both the effect of different concentrations of withaferin A and the extension of the incubation time (24h, 48h, and 72h) with the adaptogen were taken into account. The spectroscopic measurements were preceded by the MTS cell viability assay and fluorescence staining, which confirmed the enormous potential of withaferin A in inhibiting the proliferation of cervical cancer cells *in vitro* and allowed the selection of experimental conditions for spectroscopic studies.
These studies revealed that high-definition vibrational spectroscopy imaging techniques are very sensitive and useful for assessing biochemical alterations occurring at the single-cell level. It has been shown that with the increase in adaptogen concentration, the share of spectral signals from nucleic acids, proteins, carbohydrates and lipids changes, and withaferin A inhibits the growth of HeLa cells in a dose-dependent manner at IC_{50} concentrations <0.5 µM.

References:
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3. E. Pięta, et al. Adaptogenic activity of withaferin A on human cervical carcinoma cells using high-definition vibrational spectroscopic imaging, BBA – Molecular Basis of Disease 1869 (2023) 166615.
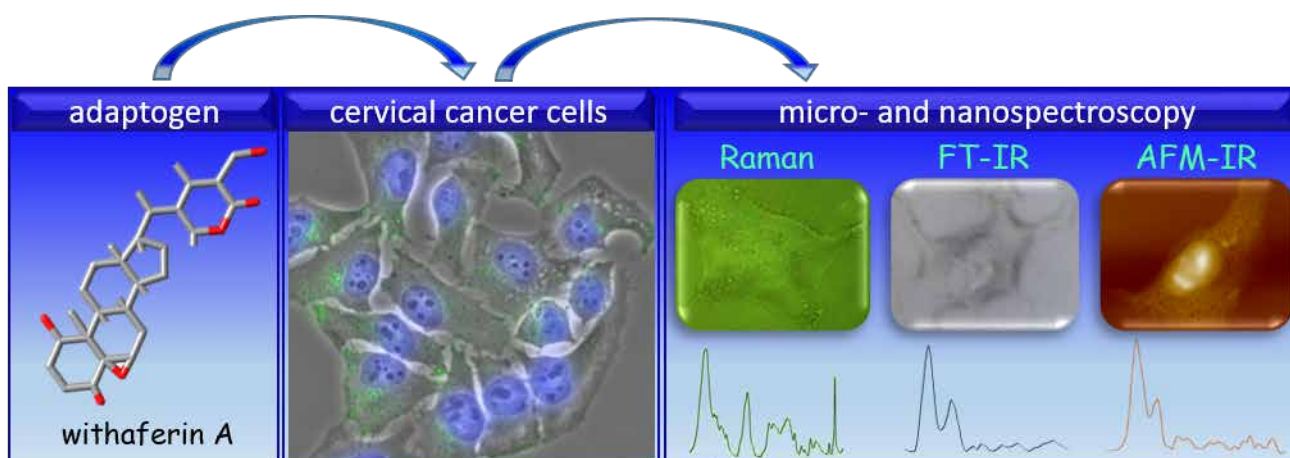
Figure captions:
Schematic representation of the performed experiment.

Keywords: adaptogen, HeLa cervical cancer cells,

H-O.26

Title: *FTIR imaging of kidney tissues to diagnose hypertensive organ damage and pharmacological treatment*

Author: <u>Paola Sassi</u>[1], Leonardo Pioppi[1], Niki Tombolesi[1], Reza Parvan[2], Gustavo Da Silva[2], Raffaele Altara[3], Marco Paolantoni[1], Assunta Morresi[1], Alessandro Cataliotti[2]

1University of Perugia
2University of Oslo
3Maastricht University

Abstract:
The kidneys are one of the main end organs targeted by hypertensive disease.[1] Although the central role of the kidneys in the regulation of high blood pressure has been long recognized, the detailed mechanisms behind the pathophysiology of renal damage in hypertension remain a matter of investigation.[2] Early renal biochemical alterations due to salt-induced hypertension in Dahl/salt-sensitive rats were monitored by Fourier-Transform Infrared (FTIR) micro-imaging. Furthermore, FTIR was used to investigate the effects of proANP$_{31-67}$, a linear fragment of pro-atrial natriuretic peptide, on the renal tissue of hypertensive rats. Different hypertension-induced alterations were detected in the renal parenchyma and blood vessels by the combination of FTIR imaging and principal component analysis on specific spectral regions. Changes in amino acids and protein contents observed in renal blood vessels were independent of altered lipid, carbohydrate, and glycoprotein contents in the renal parenchyma. FTIR micro-imaging was found to be a reliable tool for monitoring the remarkable heterogeneity of kidney tissue and its hypertension-induced alterations.[3] In addition, FTIR detected a significant reduction in these hypertension-induced alterations in the kidneys of proANP$_{31-67}$-treated rats, further indicating the high sensitivity of this imaging modality and the beneficial effects of this novel medication on the kidneys.

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Keywords: IR spectroscopy, kidney disease, hypertension

H-O.27

Title: *SERS analysis of urine for prostate cancer detection*

Author: Nicolae Leopold[1], Stefania D. Iancu[1], Andrei Stefancu[1], Vlad Moisoiu[1], Teodora Telecan[2], Iulia Andras[2], Nicolae Crisan[2]

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[2]Urology Department, Iuliu Hatieganu University of Medicine and Pharmacy

Abstract:
The use of surface-enhanced Raman scattering (SERS) in liquid biopsies is an emerging clinical approach for screening and medical diagnosis support. SERS enables the detection of biofluid metabolites that adsorb to metal nanoparticles. Purine metabolites show a high affinity for metal surfaces [1-3], giving rise to intense peaks in the SERS spectra of serum, urine and saliva [3]. Urine has gained attention for SERS, due to its easy accessibility, presence of end-stage metabolites, and its characteristic of being a protein-free biofluid.
In this study, urine samples from patients with prostate cancer were analyzed alongside control urine samples from patients with benign prostatic hyperplasia. The unsupervised principal component analysis (PCA) revealed a grouping tendency based on PC2, which was dominated by the SERS fingerprint of uric acid (see Figure).
The scores of the PCs, which significantly differentiate prostate cancer and control groups, were used as input features for classifying prostate cancer and benign prostatic hyperplasia patients using machine learning algorithms. A sensitivity of over 80% for the detection of prostate cancer was achieved.
The use of portable Raman instruments in clinical spectroscopy research, and their application in detecting multiple diseases, advances the translation of the Raman technique into the clinical setting.

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3. V. Moisoiu et al. SERS liquid biopsy: An emerging tool for medical diagnosis. Colloids Surf. B 208 (2021) 112064.
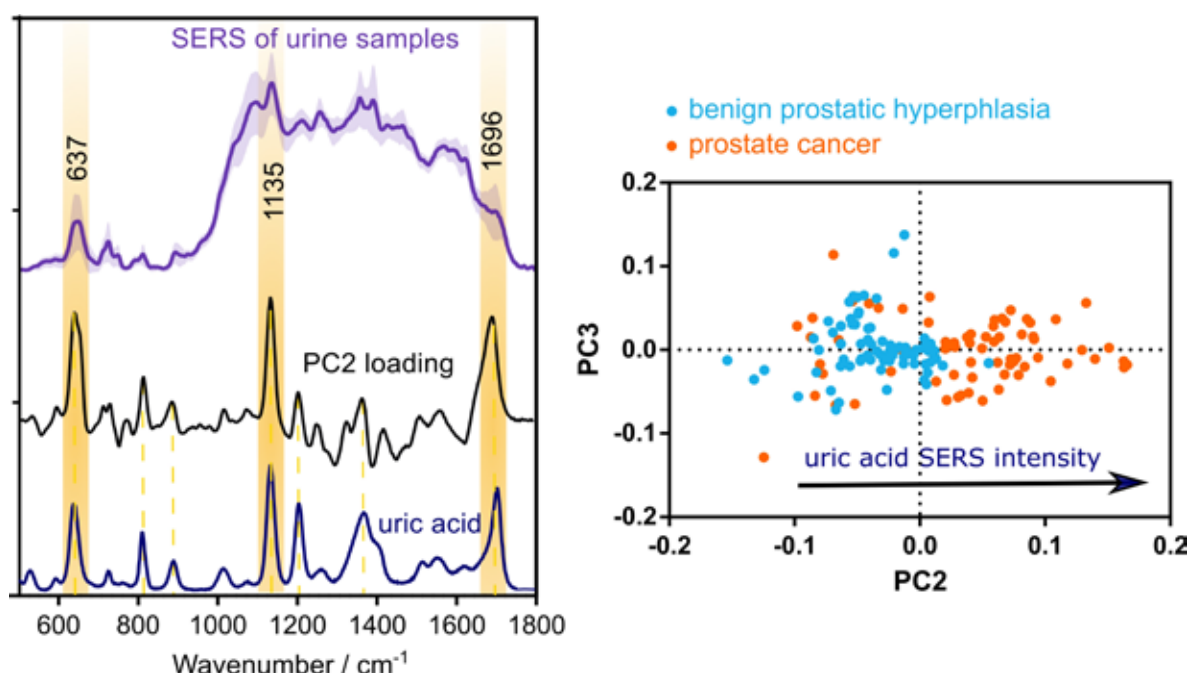
Figure captions:
Similarity of PC2 loading plot with uric acid SERS spectrum. The grouping of most prostate cancer samples in the positive region of PC2 in the PCA score plot indicates higher SERS intensity of uric.

Keywords: SERS, urine, prostate cancer

H-O.28

Title: *Vibrational spectroscopy for differential diagnosis of patients with rheumatoid and psoriatic arthritis*

Author: Sylwester Mazurek[1], Izabela Kokot[2], Agnieszka Piwowar[2], Renata Sokolik[2], Monika Kacperczyk[2], Kamil Rodak[2], Roman Szostak[1], Lucyna Korman[2], Ewa Kratz[2]

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Abstract:

Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are chronic inflammatory diseases in which both innate and adaptive responses of the immune system are induced. RA is an autoimmune joint disease that leads to progressive symmetrical arthritis and destruction of cartilage and bone, while PsA is characterized as a chronic inflammatory arthropathy, often accompanied by cutaneous psoriasis. Both chronic inflammatory arthritis have a complex signaling pathways. Knowledge about the participation of individual immunological elements is crucial to introduce effective targeted therapies [1,2].

Despite the differences in clinical presentation, radiographic findings, comorbidities and pathogenesis, there is still a great need for fast and accurate diagnosis of RA and PsA, in order to quickly implement treatment and plan individual therapeutic strategies. The aim of our research was to evaluate the potential application of vibrational spectroscopy to diagnose patients with RA and PsA, and in particular to identify spectral and biochemical markers differentiating not only patients with RA or PsA from healthy individuals but also allowing for reliable differentiation of both these groups. Additionally, we determined a specific panel of cytokine/chemokine parameters for each of the three studied groups.

Partial least squares discriminant analysis (PLS-DA) models were developed based on serum cytokine/chemokine biomarkers and FTIR ATR, NIR and Raman spectra of lyophilized samples. Hybrid models combining the biochemical and spectral data were also constructed. The iPLS algorithm was applied to select the most significant variables allowing differentiation of the tree studied groups of donors. It occurred that hybrid models were characterized by the best discriminating ability, with the overall accuracy exceeding 90%. This correlates with the results of our previous studies on the classification of endometriosis [3].

References:

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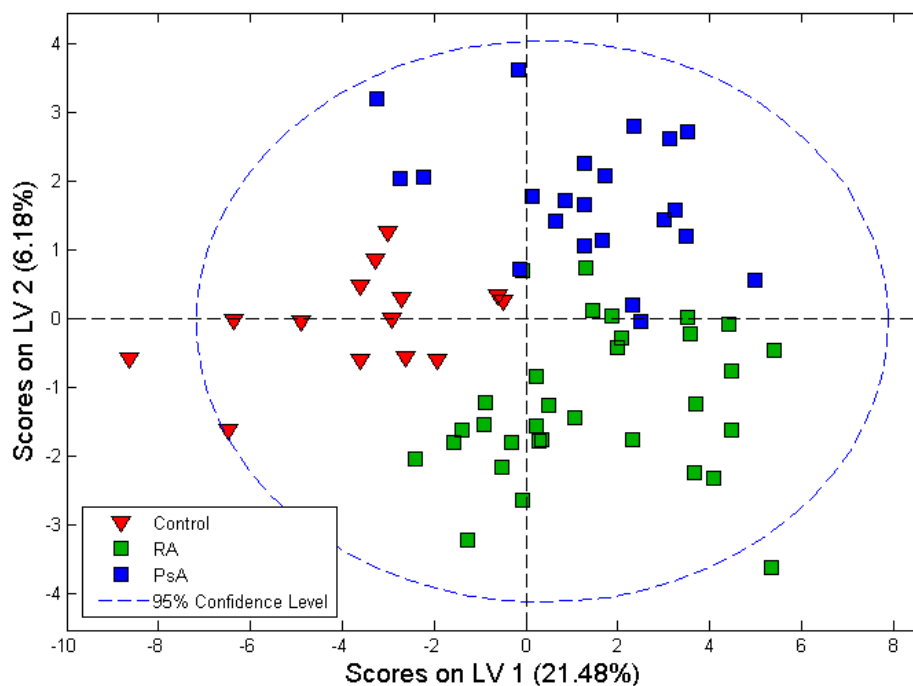
Figure captions:
Scores plots of PLS-DA hybrid model based on selected biochemical parameters and ranges of FTIR ATR spectra.
H-O28 Mazurek

Keywords: arthritis, spectroscopy, non-invasive diagnostics, PLS-DA

H-O.29

Title: *Infrared spectroscopy for rapid and objective diagnosis of the etiology of infection as bacterial or viral using a simple peripheral blood test.*

Author: Ahmad Salman[1], Uraib Sharaha[2], Guy Beck[3], Yotam D. Eshel[3], Gal Cohen-Logasi[4], Adam H. Agbaria[5], Itshak Lapidot[6], Joesph Kapelushnik[3], Mahmoud Huleihel[2], Shaul Mordechai[5]

[1]SCE-Sami Shamoon College of Engineering/ Department of Physics
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[3]Soroka University Medical Center/Department of Hematology and Oncology, Saban Pediatric Medical Center
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Abstract:
For appropriate treatment of patients with infectious diseases, it is crucial to diagnose the etiology of infection as viral or bacterial. Bacterial and viral infections have historically been one of the major factors in human morbidity and mortality and they share almost the same symptoms, although the immune system reacts differently to each pathogen. There is no objective etiology-diagnosis technique for infections that are inaccessible, and the current techniques for accessible infections are time-consuming (2-4 days). Thus, physicians routinely and subjectively diagnose the infection's etiology based on symptoms, their experience, and medical interventions, and then begin unneeded antibiotic therapy. Hence, to determine the etiology of infections for both accessible and inaccessible infections it is crucial to develop new sensitive and rapid techniques.
Our hypothesis [1, 2] states that the varied immune responses to viral or bacterial infections lead to both molecular and other changes in white blood cells (WBC). In this study, the responses of the immune system due to the type of infection were monitored by infrared spectroscopy to evaluate the possibility of mid-infrared spectroscopy for rapid and accurate diagnosis of the etiology of infection.
Peripheral blood samples from patients under 18 years old were collected and separated into their components. The infrared absorption spectra of WBC were acquired, preprocessed, and analyzed using machine-learning algorithms to determine the etiology of the infection as bacterial or viral. Our results indicate that one hour after the collection of the blood samples, it is possible to identify the etiology of the infection as being bacterial or viral with >94% sensitivity and >90% specificity for both accessible and inaccessible infections. Moreover, our approach showed that >23% of the doctors' subjective assessments of the origins of inaccessible infections were found inaccurate.

References:
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2. A. H. Agbaria et al, Diagnosis of inaccessible infections using infrared microscopy of white blood cells and machine learning algorithms. Analyst 145 (2020) 6955-6967.

I-I.1

Title: *Two-trace two-dimensional (2T2D) FTIR correlation spectra applied as input data in classification and discriminant analysis*

Author: Bogumiła Kupcewicz[1]

[1]Nicolaus Copernicus University, Faculty of Pharmacy

Abstract:

The analysis of spectroscopic data to obtain qualitative or quantitative insight is a common task in the natural, medical, and pharmaceutical sciences. Two-trace two-dimensional (2T2D) correlation spectroscopy (COS) was introduced by Noda in 2018 [1], and developed in recent years [2,3]. This is a new concept where the 2D correlation spectrum is generated from only a pair of spectra (sample and reference) instead of a series of dynamic spectra in classical 2D-COS. 2T2D-COS can effectively solve the spectral overlap and improve distinguishing the weak signals, reducing the analysis error caused by baseline and noise. Two-dimensional spectra can be applied as input data in chemometric techniques, which are crucial for mining the most valuable data and building efficient models. Traditional multivariate methods (PLS-DA and support vector machines - SVM) can utilize correlation spectra calculated as synchronous, asynchronous, and integrated spectra or 2T2D slices. Moreover, deep learning algorithms, such as ResNet, can be used to model 2T2D spectra as spectral images. It is also essential that the choice of an appropriate reference spectrum significantly influences the obtained results and can help extract useful information from spectra.

The study aimed to show the possibility of improving results in the chemometric models by using 2T2D correlation spectra to analyze synthetic mixtures of compounds and complex natural products. 2T2D correlation ATR-FTIR spectra combined with multivariate methods are successfully employed to detect adulteration of herbal products [4], drugs, and foods. Combining two-trace two-dimensional (2T2D) correlation spectra and multidimensional chemometrics is a promising tool that can provide better classification and discrimination analysis results. In addition, the field of application of 2T2D correlation spectroscopy is constantly expanding, and new methods emerge, which will continue to develop and improve.

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Keywords: 2T2D correlation spectroscopy, FTIR, chemometrics

I-I.2

Title: *In silico experimentation to guide optimization and experimental design in clinical spectroscopy.*

Author: <u>David Perez-Guaita</u>[1], Victor Navarro-Esteve[1], Jaume Bejar-Grimalt[1], Angel Sanchez-Illana[1], Hugh J. Byrne[2]

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Abstract:
Research in clinical spectroscopy generally involves experimentation with well characterised clinical samples. This is a major limiting factor in the investigation process, requiring the allocation of resources to handle ethical approval and reference data from clinical partners as well as specialised infrastructure such as biosafety and culture laboratories to obtain a significant number of clinical samples and biological replicates. Furthermore, due to the multivariate nature of clinical spectroscopy, spectra are normally treated using machine learning and data mining techniques. In those cases, the designing of experimental conditions (e.g. the required number of samples to provide enough statistical power in a diagnostic model or the temporal resolution in pharmacokinetic studies) as well as the interpretation of the results becomes a challenge, as it cannot be performed with traditional univariate methodologies.
To overcome this, here we discuss the use of in silico experimentation, which involves the simulation of the sample spectra from the combinations of its constituents to model different biological processes of interest, such as changes in spectral markers of a specific disease or the evolution of metabolic cellular processes. Simulated spectra can be used then in a wide range of applications, including i) to optimise sample preprocessing methods, ii) to predict the number of samples needed to obtain reliable values of sensitivity and specificity in a diagnostic methodology and iii) to evaluate the optimal value of measurement timepoints in pharmacokinetic studies. We provide different examples of experiments with simulated spectra which can guide the real experimentation and assist in the interpretation of results, enhancing the significance and value of real samples when those ones are scarce. We also discuss some limitations, such as the difficulties encountered when modeling non-linear effects and the challenge of simulating complex biological samples.

Keywords: INFRARED, CLINICAL, IN-SILICO EXPERMIENTATION, CHEMOMETRICS

I-I.3

Title: *Sparse Wavelength Sampling in Mid-Infrared Spectroscopy*

Author: Valeria Tafintseva[1], Miriam Aledda[1], Boris Zimmermann[1], Nageshvar Patel[1], Volha Shapaval[1], Achim Kohler[1]

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Abstract:

The emergence of Fourier Transform Infrared (FT-IR) spectrometry in the 1950s gave rise to vast amount of spectroscopic data. The data obtained from conventional FT-IR instruments consist of broadband spectra comprising thousands of highly collinear variables. Modern IR technologies, such as quantum cascade lasers (QCLs) and light-emitting diodes (LEDs), generate sparse IR spectral data – data with a limited number of wavenumber channels. Given the growing demand for photonics-based solutions in various industries, there is a need for data analysis techniques. This can be a challenge, as only a few spectral variables are available for analysis.

This study focuses on selecting the best machine learning methods for the analysis of the sparse data: both preprocessing and modelling. We compare Partial Least Squares Regression (PLSR), Random Forest (RF) and Multiple Linear Regression (MLR) methods to find the optimal modelling algorithm. We test different pre-processing approaches such as model-based pre-processing like Multiplicative Signal Correction (MSC), baseline corrections and peak normalization as well as raw spectra where appropriate. We propose an approach for improving modeling outcomes by increasing the number of spectral wavelengths in a way that has direct practical implications for the architecture of photonics-based devices. The approach improves dramatically the performance of the models even for the very limited number of spectral wavelengths used for modelling – three to five.

Both regression and classification problems are considered in the study utilizing different datasets. To simulate photonics data, the sparse data were obtained by reducing broadband FTIR spectra comprising several thousand spectral variables into datasets comprising only few (three to nine) spectral variables which were optimized for the task. The results of the modelling are compared for the sparse spectral data, for selected wavelength regions and the full broadband spectra.

Keywords: sparse spectra, QCL, machine learning

Title: *Decoupling of morphological and chemical information in µFTIR spectra using deep learning*

Author: <u>Uladzislau Blazhko</u>[1], Eirik Magnussen[1], Johanne Solheim[1], Simona Dzurendova[1], Volha Shapaval[1], Achim Kohler[1]

[1]Norwegian University of Life Sciences

Abstract:
Infrared measurements of microscopic samples often contain both chemical and morphological information arising from absorption and Mie scattering phenomena, respectively. Previous studies have shown that it is possible to decouple chemical and morphological information for diverse samples assuming a spherical shape. In these studies, the search for a pure chemical absorbance spectrum was initiated by a reference spectrum that is close to the solution of the search. As the search for the solution starts close to the reference spectrum and the solution is expected to lie in the vicinity of the reference spectrum, we consider this approach as *conditioned*. In our study, we explore to what extent chemical conditioning can be weakened or even dropped.

To solve the inverse problem numerically, we have trained deep neural networks (DNNs) on a large number (>106) of spectra that were simulated using the exact Mie solutions. We compare the case where simulated spectra were chemically conditioned, i.e. assuming an underlying restricted space of chemical variability, and the case where the chemical composition of the samples was arbitrary. The established neural networks were validated on real-world spectra representing various kinds of chemistry and morphology.

We showed that chemically independent DNNs could decouple chemical information for different types of samples. While chemically conditioned DNNs were more accurate, especially in case of low signal-to-noise ratio, they struggled to predict features outside the given chemical variability. Thus, decoupling can be done without any knowledge of underlying chemical composition of a sample, eliminating the necessity for a reference spectrum and avoiding solutions that are biased by a reference spectrum.

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Keywords: infrared-microspectroscopy, deep-learning, mie-scattering,inverse-scattering-problem

I-O.2

Title: *Investigation of the bread aging process by handheld NIR spectroscopy in tandem with 2D-COS and MCR-ALS analyses*

Author: Marina De Géa Neves[1], Isao Noda[2], Heinz Wilhelm Siesler[1]

[1]Department of Physical Chemistry, University Duisburg-Essen
[2]Department of Materials Science and Engineering, University of Delaware

Abstract:
The investigations for this presentation were carried out using the technique of handheld near-infrared (NIR) spectroscopy for time-dependent diffuse-reflection measurements of the cut face of a fresh baguette. The critical structural factors in the staling process of bread are the crystallization of amylopectin in starch and the decrease of water content by evaporation and diffusion from core to crust. To monitor the synchronicity and sequence of these changes, Two-Dimensional Correlation Spectroscopy (2D-COS) was applied. Due to the significant variation of spectral changes at different stages of the aging process, the spectra were split into two sets of 0-6/6-48 hr. 2D-COS analysis allowed differentiating the detailed sequence of the following structural events for these time intervals: crystallization of amylopectin/evaporation of weakly and strongly hydrogen-bonded water/reorganization of starch OH-functionalities. Furthermore, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) was used to investigate the changes in the spectra profile as a function of aging time.

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M. De Gea Neves, I. Noda, and H. W. Siesler; Investigation of bread staling by handheld NIR spectroscopy in tandem with 2D-COS and MCR-ALS analysis; Microchemical Journal; 190; 2023, 108578.
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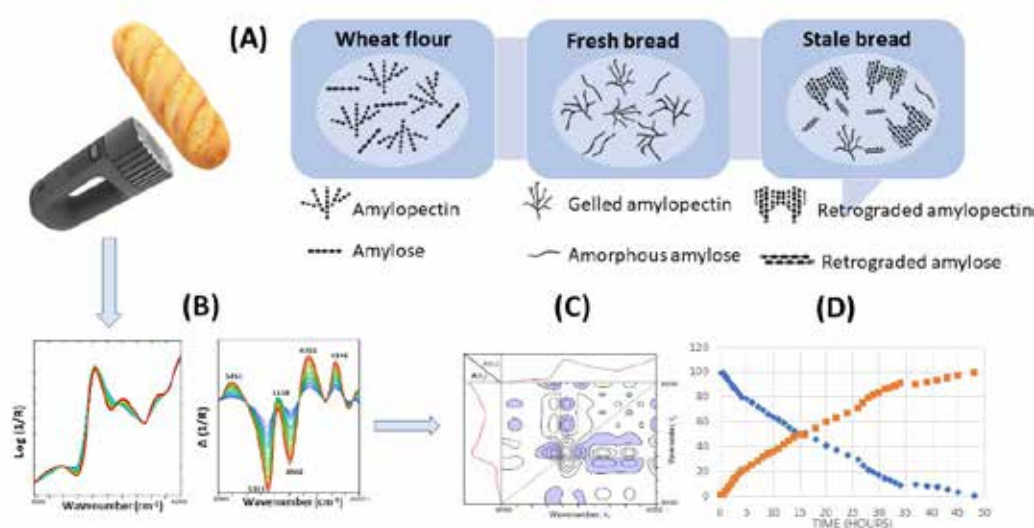
Figure captions:
(A) Handheld NIR measurement set-up; (B) overlaid/evolving difference spectra (0 – 48hr);
(C) 2D-COS asynchronous map; (D) MCR-ALS concentration profiles.

Keywords: handheld NIR, bread staling, 2D-COS/MCR-ALS

I-O.3

Title: *Can we follow the metabolism of single leukemic cells using Raman spectroscopy?*

Author: <u>Anna M. Nowakowska</u>[1], Aleksandra Borek-Dorosz[1], Patrycja Dawiec[2], Patrycja Leszczenko[2], Adriana Adamczyk[2], Kacper Siakala[1], Justyna Jakubowska[3], Marta Zabczynska[3], Agata Pastorczak[3], Kinga Ostrowska[3], Wojciech Mlynarski[3], Malgorzata Baranska[4], Katarzyna Majzner[1]

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[2]Jagiellonian University in Krakow, Faculty of Chemistry, Department of Chemical Physics, Krakow, Poland; Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland
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[4]Jagiellonian University in Krakow, Faculty of Chemistry, Department of Chemical Physics, Krakow, Poland; Jagiellonian University in Krakow, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland

Abstract:
Leukemia is one of the blood malignancies estimated to cause the death of 83.000 people in the next 20 years[1]. Dysfunction in leukemia cells leads to increased rate of proliferation that involves uptake of massive amounts of energy and the high demand for building blocks. Alterations in cellular metabolism are closely linked to the cellular growth and enable malignant cells to survive in a constantly changing microenvironment. Therefore, targeting different metabolic checkpoints appears to be a promising diagnostic issue. The purpose of the study was to identify key metabolic changes associated with leukemogenesis using Raman imaging and multivariate statistical analysis.

In the study, both normal lymphocytes and leukemia cells (with different gene mutations) were measured using the WITec Alpha 300 confocal Raman system and a home-built stimulated Raman spectroscopic setup. Within the study group we used leukemia cell lines and malignant cells derived from patients. Furthermore, to better understand the lipid metabolism of leukemic cells, the uptake of deuterated fatty acids was examined. The collected Raman spectra were analyzed using cluster analysis, principal component analysis, and partial least squares discriminant analysis.

Obtained results showed that Raman imaging combined with a supervised chemometric approach, is an effective tool enabling differentiation between normal[2] and leukemic cells[3], carrying different genetic aberrations in a walk-away automated convenience. Furthermore, the developed method specifies a panel of Raman markers for the recognition of molecular changes related to alterations in the metabolism of different leukemia subtypes.

Our research indicated that lipids and nucleic acids undergo the most prominent changes during carcinogenesis. The use of deuterated fatty acids allowed us to trace their uptake, accumulation, and metabolism in single leukemic cells, and to analyze not only the molecular background but also the phenotype.

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Keywords: Raman spectroscopy, leukemia, cellular metabolism, deuterated fatty acids, chemometrics

I-O.4

Title: *Advancing cancer stem cell detection through line illumination Raman microscope and hydrogel substrate.*

Author: <u>Jean-Emmanuel Clément</u>[1], Zannatul Ferdous[1], Thomas Bocklitz[2], Katsumasa Fujita[3], Jian Ping Gong[1], Shinya Tanaka[1], Tamiki Komatsuzaki[1]

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[2]University of Bayreuth
[3]Osaka University

Abstract:
Cancer stem cells (CSCs) are a crucial target for developing effective cancer treatments but identifying and characterizing them remains a major challenge in cancer research. Recently, a phenomenon called the Hydrogel Activated Reprogramming Phenomenon was discovered in which differentiated cancer cells cultured on hydrogels exhibit stem-like characteristics [1]. The combination of hydrogel-based cell culture systems with Raman microscopy, a label-free imaging technique with rich spectral fingerprints, and chemometrics methods could potentially enable more accurate and comprehensive identification and characterization of CSCs across different cancer types. Specifically, the line-illumination Raman microscope, a high-speed Raman microscope, has received considerable attention for visualizing the distribution of various molecules within live single cells [2-3]. However, to enable subsequent chemometric analysis that classify different types of cells based on spectral and/or spatial information, it is necessary to develop data standardization techniques that minimizes experimental artifacts, including substrate heterogeneity and laser intensity variations in Raman images. In this contribution, we detail a preprocessing data workflow that minimizes experimental artifacts arising from hydrogels and the line-scanning Raman microscope. Briefly, the first step of our method concerns image segmentation of Raman images into two distinct groups: single cells and background region (hydrogels zone without cells), via the use of advanced hyperspectral image denoising framework. Second, we reduce each single pixels of a Raman image belonging to single cells by a local weighted gaussian average background estimated in the pure hydrogel zone, whose arameter are optimized with a bayesian optimization framework. We show that this correction allows to reduce the spectral variability between different type of substrates and different biological replicate experiments.

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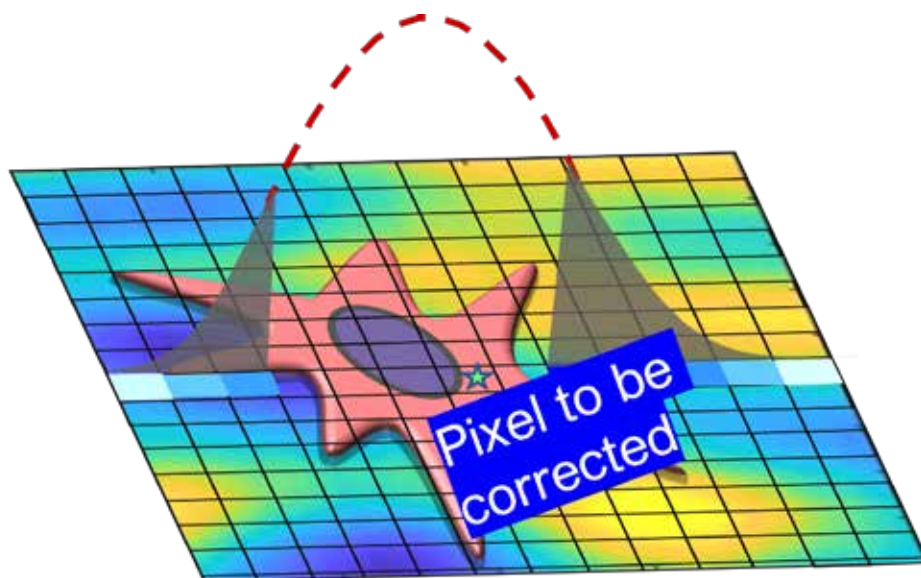
Figure captions:
Local background correction of cell pixels using a Gaussian weighted approach.

Keywords: Hydrogel, Raman spectroscopy, Local Background

I-O.5

Title: *Discrimination between chemoresistant and chemosensitive ovarian cancer cells with confocal Raman microscopy*

Author: Elina Harju[1], Teemu Tomberg[1], Sara Fraser-Miller[2], Jukka Saarinen[1], Kathleen J. Sircombe[3], Sarah Hook[3], Keith C. Gordon[2], Clare J. Strachan[1]

[1]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki
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[3]School of Pharmacy, University of Otago

Abstract:

Chemotherapy is a cornerstone of cancer treatment. Chemoresistance, either intrinsic or acquired, is a substantial cause of treatment failure and ultimately patient death. One form of cancer in which chemoresistance is common and a significant contributor to mortality is ovarian cancer[1]. Current clinical practice lacks tools to predict patient-specific chemoresistance in ovarian cancer and thus personally optimise chemotherapy selection prior to treatment. In this project, the potential of Raman spectroscopy for detection of chemoresistance in ovarian cancer is investigated. At this stage of the project, the specific aim was to differentiate between chemosensitive and chemoresistant ovarian cancer cell lines.

Raman microscopic line maps were collected from two cell lines, the chemosensitive TYK-nu ovarian cancer cell line and its more chemoresistant TYK-nu-CPR subline, after chemical fixation and using a confocal Raman microscope (alpha 300R+, WiTec, Ulm, Germany) with 532 nm excitation. Two thirds of the samples were randomly selected to create support vector machine (SVM) classification models with the remaining third of samples used as the test set. Preliminary results show that when the average spectrum from each line map was used, the test set classification accuracy for cell type was 86 %, suggesting this is a promising avenue to explore in the future for differentiating between chemosensitive and chemoresistant ovarian cancer cells. The classification of data from localized parts of the cell were also explored.

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Acknowledgments:
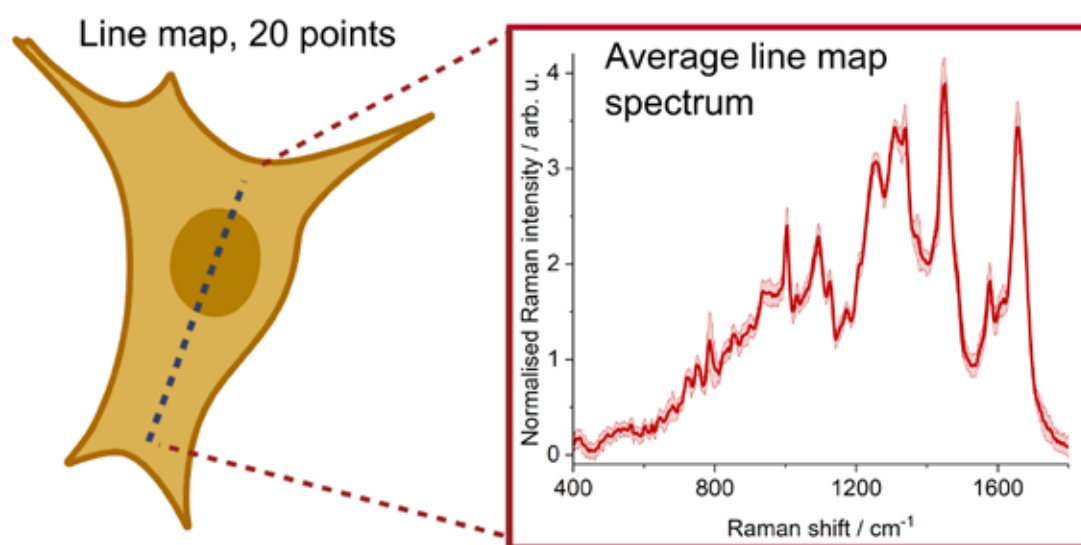
Figure captions:
Average line map spectra were used for discriminating chemoresistant TYK-nu-CPR cells from chemosensitive TYK-nu cells (created with BioRender.com)

Keywords: Confocal Raman microscopy, ovarian cancer, chemoresistance, chemometrics

I-O.6

Title: *Can we diagnose the KMT2A leukemia subtype with Raman microscopy?*

Author: Patrycja Leszczenko[1], Anna M. Nowakowska[1], Justyna Jakubowska[2], Agata Pastorczak[2], Marta Zabczynska[2], Wojciech Mlynarski[2], Malgorzata Baranska[1], Kinga Ostrowska[2], Katarzyna Majzner[1]

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Abstract:
B-cell progenitor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood neoplasm accounting for over 20% of all malignancies in children. BCP-ALL consists of a wide variety of subtypes with extremely diverse clinical and biological outcomes. ALL cells might harbor structural genomic alterations, which are assigned as prone to be more chemoresistant and therefore generate relapses of the primary disease. Among these genomic lesions, the prominent place belongs to chromosomal translocations of *KMT2A* (formerly MLL). The *KMT2A* rearranged BCP-ALL (*KMT2A*-r BCP-ALL) are cytogenetically heterogeneous and characterized by hyperleukocytosis, aggressive behavior with early relapse, and a relatively high incidence of central nervous system (CNS) involvement. Additionally, *KMT2A*-r can also be found in acute myeloid leukemia (AML).
In this study, we used Raman imaging and extensive chemometric analysis (k-means cluster analysis, principal component analysis, partial least squares discriminant analysis) to provide a comprehensive biochemical characterization of ALL and AML leukemic cells carrying *KMT2A*-r.
Our results revealed that KMT2A-r BCP-ALL differs significantly from other studied molecular subtypes of BCP-ALL or *KMT2A*-r AML. The Raman profile of these cells exhibits numerous spectroscopic differences reflecting the peculiar nature of this subtype (e.g., the Raman bands at 1187 and 1130 cm-1 are of lower intensity compared to the spectra of the BCR-ABL1, *TEL-AML1*, and *TCF3-PBX1* subtypes). Furthermore, analysis of cellular and subcellular morphology can be used as an effective marker for the identification of ALL cells with *KMT2A* gene rearrangement. Importantly, we developed a Raman-based method for the automatic identification of aldehyde-fixed *KMT2A*-r BCP-ALL cells which can be applied in diagnostic practice. It is a fast and label-free screening method, currently patent pending.

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Figure captions:
Average Raman spectra with the standard deviation of 0.5% GA fixed blasts: KMT2A-r AML, KMT2A-r BCP-ALL, TEL-AML1, TCF3-PBX1, and BCR-ABL1.

Keywords: Raman microscopy, leukemia, KMT2A, chemometrics

I-O.7
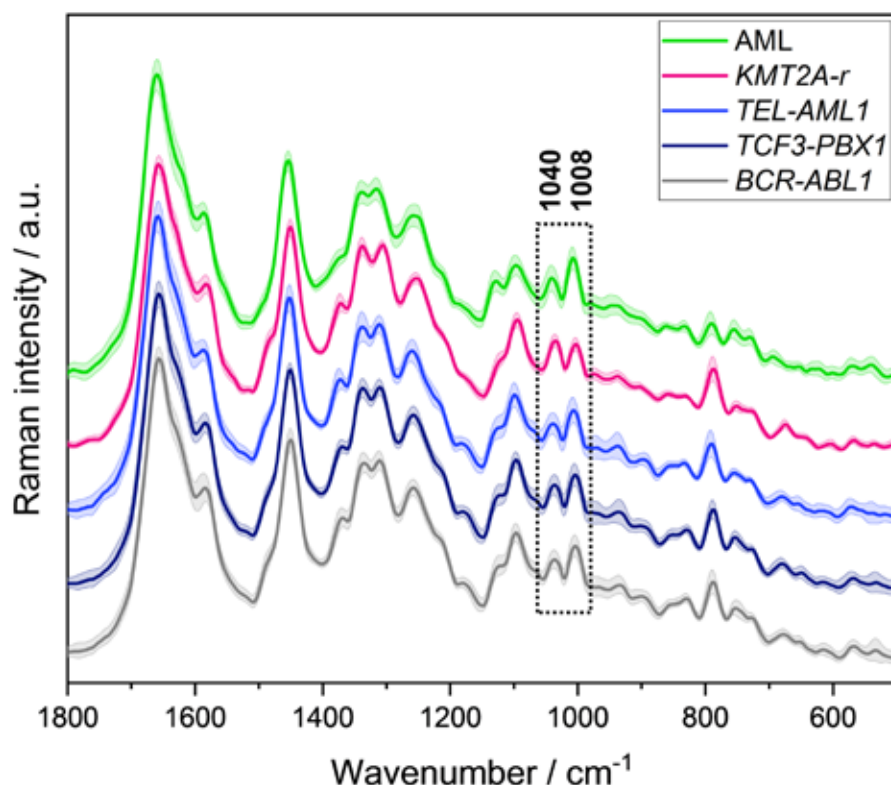
Title: *Pretreatment routines in analysis of Raman spectra recorded in different excitation wavelength and its effect on classification models*

Author: Sara Mostafapour[1], Thomas Dörfer[2], Ralf Henke[2], Petra Rösch[2], Jürgen Popp[1], Thomas Bocklitz[3]

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[3]1.Leibniz Institute of Photonic Technology, Jena, Germany/2. Institute of Physical Chemistry and Abbe Centre of Photonics, Friedrich Schiller University of Jena, Jena, Germany/3. Institute of Computer Science, Faculty of Mathematics, Physics & Computer Sc

Abstract:
The workflow for Raman spectral analysis begins with the pretreatment and preprocessing of spectral data [1,2]. Hence, before qualitative and quantitative analysis is performed, data pretreatment is considered as a crucial step. However, there are no clear rules to decide when to use a specific pretreatment technique [3], if a single technique could be enough and, if not, which techniques to combine and how (e.g., in what order). Herein, different combinations of pretreatment methods are investigated to show the effect of each step in minimizing the differences between Raman spectra of the same sample in different experimental conditions, namely excitation wavelengths. Method combinations contain 1) Interpolation and preprocessing that has four parts, spike removal, smoothing, background correction and normalization (IP+PP) 2) wavenumber calibration, interpolation and preprocessing (WN+IP+PP) 3) wavenumber and intensity calibration, interpolation and preprocessing (WN+INT+IP+PP) and 4) wavenumber and intensity calibration, interpolation and preprocessing and ω^4 correction (WN+INT+IP+PP+ ω^4 correction). This process is evaluated for six substances. The spectral differences between Raman spectra recorded with three excitation wavelengths (532nm, 633nm and 830nm) are evaluated and the influence on a classification model is tested.
The result shows that the choice of an optimal pretreatment method or combination of methods strongly influence the analysis results, but is far from straightforward, since it depends on the characteristics of the data set and the goal of the data analysis.

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Keywords: Raman spectroscopy, Data pre-treatment, Chemometric

I-O.8

Title: *Infrared molecular fingerprinting for multi-phenotyping of human health and disease*

Author: Kepesidis V. Kosmas[1], Marinus Huber[2], Liudmila Voronina[1], Tarek Eissa[1], Frank Fleischmann[1], Cristina Leonardo[1], Jacqueline Hermann[1], Ina Koch[3], Thomas Kolben[4], Gerald Schulz[5], Friedrich Jokisch[5], Juergen Behr[6], Nadia Harbeck[4], Maximilian Reiser[7], Christian Stief[5], Ferenc Krausz[1], Mihaela Zigman[1]

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[2]Max Planck Institute of Quantum Optics (MPQ)
[3]Asklepios Biobank for Lung Diseases, Department of Thoracic Surgery, Member of the German Center for Lung Research, DZL, Asklepios Fachkliniken München-Gauting
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[5]University Hospital of the Ludwig Maximilians University Munich (LMU), Department of Urology
[6]University Hospital of the Ludwig Maximilians University Munich (LMU), Department of Internal Medicine V
[7]University Hospital of the Ludwig Maximilians University Munich (LMU), Department of Clinical Radiology

Abstract:
Recent advances in optical spectroscopy have opened new prospects in probing of living systems at molecular level. Our objective is to advance and evaluate vibrational spectroscopy - as an analytical platform - to probe human systemic biofluids and evaluate the feasibility of infrared fingerprinting for high-throughput in vitro biomedical diagnostics. Specifically, we evaluate its feasibility for analysis of human blood serum and plasma for clinical diagnostics.
Combining vibrational fingerprinting of liquid blood plasma and serum, integrating machine learning data analyses, the outcome of experimental[1,2,3], *in silico*[4], and conceptual findings from several populational studies will be discussed: On a smaller scale we reveal a high within-person stability of infrared molecular fingerprints in longitudinal settings[1], foundational for any health screening application. In a study of several thousands of individuals, we provide evidence that four cancer entities leave their signatures in the spectral information of plasma and serum, the efficiency of disease detection correlates with the stage of the cancer progression[2], highlighting possible applications forging primary cancer diagnostics. Yet, blood-based vibrational fingerprints are not able to detect only very severe health phenotypes, like cancer. Examining a large-scale general population, we find that infrared spectra carry the capacity to also capture common chronic aberrations in health. Intriguingly, we further uncover their capacity to further distinguish between different cancers, as well as between diverse multimorbidities within a single measurement - opening the possibility to screen for diseases as well as for enhanced risk assessments. The critical discussion will focus on blood-based infrared molecular fingerprinting as a possible multi-phenotyping platform that can provide explainable healthcare analytics.

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Keywords: infrared spectroscopy, health profiling, phenotype

I-O.9

Title: *Spatially offset low frequency Raman spectroscopy for discriminating microcalcifications immersed and under varying depths of paraffin wax*

Author: Mitchell Chalmers[1], Sara Miller[1], Teemu Tomberg[2], Keith Gordon[1]

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Abstract:
In clinic screening for early stages of breast cancer is desirable for improving clinical outcomes.[1] Microcalcifications are considered a robust proxy of breast cancer1, whose identification is crucial to distinguish benign and malignant lesions. Raman spectroscopy and its use in spatially offset2 and transmission[3] modes have shown promise for in clinic, in vivo implementation but is not yet at an accuracy level for clinical uptake. Low frequency Raman spectroscopy is particularly sensitive to the solid state form of materials[4] and may provide additional information to aid characterization of buried calcifications. Here we explore the potential for including low frequency Raman spectroscopy with an established spatial defocusing method[2] for improving the accuracy of identifying buried model calcifications.
Calcium oxalate (CaOx) and partially carbonate substituted hydroxyapatite in amorphous (HAp-Amo) and crystalline (HAp-Cry) forms were used as our model calcifications and were mounted in wax. Wax blocks of varied thicknesses (0 to 10.2 mm) were placed above these for varied tissue thicknesses. Defocusing was applied by offsetting the sample 0, 2 and 5 mm along the beam axis using a panzer mount and samples were measured in triplicate at each offset with a 785 nm excitation LFR setup.[4]
The spectra were collected with data from each offset analysed in SIMCA using PLS-DA after preprocessing, generating three models. The microcalcifications were successfully identified and classified in the 2 and 5 mm offset models with test set classification accuracies of 0.83 and 0.75 respectively and 0.68 for the 0 mm offset. Further analysis was performed on the offsets with the data split into low (-340 to 340 cm^{-1}), mid (800 to 1800 cm^{-1}) and combined spectral regions using leave one third out cross validation. Test set classification accuracies for the 2 mm offset were 0.93, 0.89 and 0.91 for the low, mid and combined spectra respectively.

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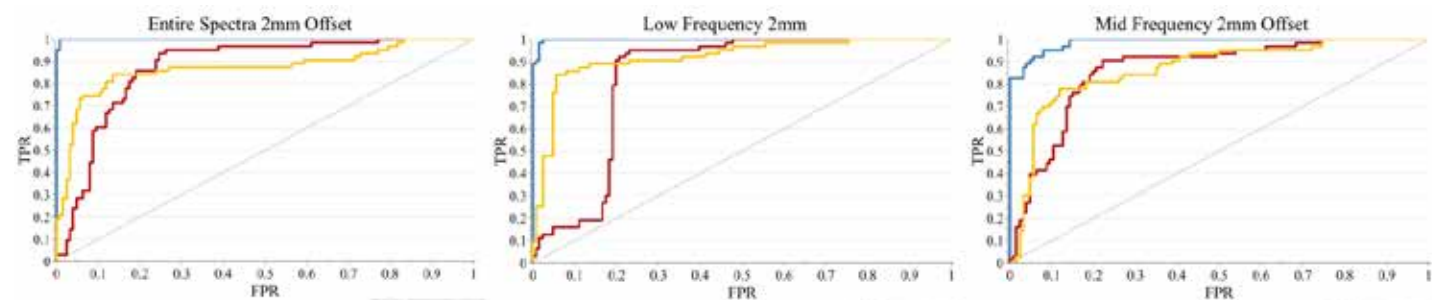
Figure captions:
Split frequency spectra ROC curves of the 2mm Offset data modelled using PLS-DA with leave one third out cross validation. Blue – Calcium oxalate, Yellow, Red - Hydroxyapatite Crystalline, Amorphous.

Keywords: SOLFRS, microcalficaitions, PLSDA

I-O.10

Title: *The data exploring expedition. A practical outline to processing and investigation of experimental spectra with the selected methods of chemometric data modeling*

Author: Andrzej J. Kałka[1], Andrzej M. Turek[1]

[1]Jagiellonian University in Cracow, Faculty of Chemistry

Abstract:
Interpretation of the spectral signal resulting from performed measurements frequently forms a centerpiece of the conducted spectroscopic research. This complex procedure, that not rarely consists of several stages, may be however facilitated thanks to advances in a brand new computer-based domain of chemistry, known under the name of chemometrics. In principle, the latter focuses on practical utilization of mathematical and statistical calculus for recovery and processing of the information carried by the experimentally collected data [1]. Although it is often associated with multivariate regression techniques [2] (such as e.g. Principal Component Regression, PCR), the scope of the chemometrics extends far beyond purely quantitative purposes [1] and covers, among others, preprocessing of the signal [3], exploration of spectral datasets or modeling and decomposition of (multicomponent) spectra [2,4]. However, according to the Authors' observations, the aforementioned aspects of data analysis in case of vibrational spectroscopy are still rather uncommonly addressed with the outlined methodology. Accordingly, during the presentation several potent approaches, that may allow to facilitate and to some extent also automate analysis of recorded spectra, shall be demonstrated together with a user-friendly commentary on their practical implementation and performance. A particular focus will be put on how well-recognized Singular Value Decomposition (SVD) algorithm may be used to enhance widely defined quality of digital signal and to extract valuable information initially "hidden" in the processed dataset, including spectra of individual components out of which the studied sample is made up [4].

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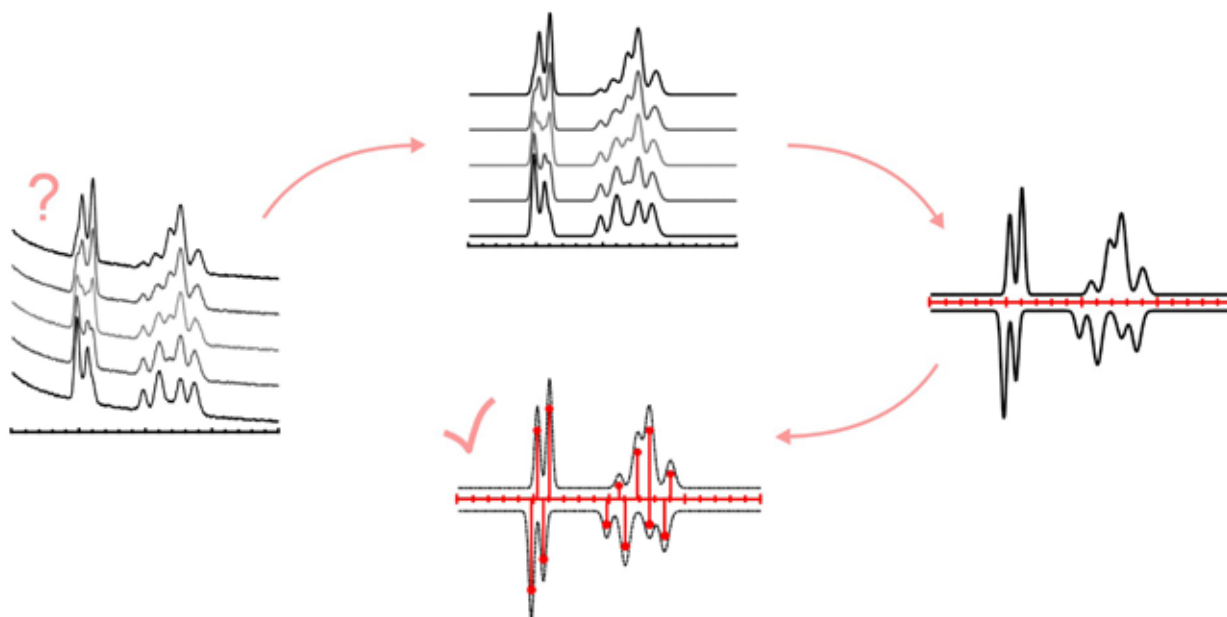
Figure captions:
Schematic depiction of multicomponent spectra processing cycle. At each consecutive step, the procedure may be facilitated through application of dedicated chemometric algorithms.

Keywords: Signal processing, Data modeling, Factor

I-O.11

Title: *RamApp: a modern web-based toolbox for the processing and analysis of hyperspectral imaging data*

Author: Elia Broggio[1], Andrea Masella[1], Giulia De Poli[1], Manuela Bazzarelli[1], Dario Polli[2], Matteo Bregonzio[1], Renzo Vanna[3]

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Abstract:
The task of processing and analysing hyperspectral imaging data in various fields, such as biomedical research and material science, currently requires the writing of custom software or the use of specialised commercial tools. However, these options often present various challenges, as they need programming expertise, are difficult to use, are expensive, or are a combination thereof. As a result, the ability of many researchers to readily perform various analyses is reduced, effectively limiting research output and hindering collaboration. This is often further constrained by the limited computing resources available. RamApp (ramapp.io) is a free web-based application aiming to be intuitive, interactive and easy to use, allowing researchers from various backgrounds to efficiently process, explore and analyse hyperspectral imaging data. Thanks to its web-based design, it can be accessed from any modern browser and operating system without requiring local installation or local computing resources, and new features and bug fixes are readily available to users without any action on their part.
By reading popular open and commercial file formats, RamApp fosters data interoperability and provides a tool capable of supporting users of both commercial and custom-built instruments. Raw and processed data, as well as high-quality images, can be easily exported for further downstream analysis and publication.
RamApp offers several spectral and spatial preprocessing methods and algorithms, as well as a variety of analysis and visualisation features for hyperspectral data, ranging from cropping, denoising, substrate identification and correction, clustering, spectral unmixing (MCR, N-FINDR), and the creation of masks and intensity maps. Although RamApp is currently optimised for Raman data (spontaneous and coherent), its main features make it suitable for other hyperspectral data as well.
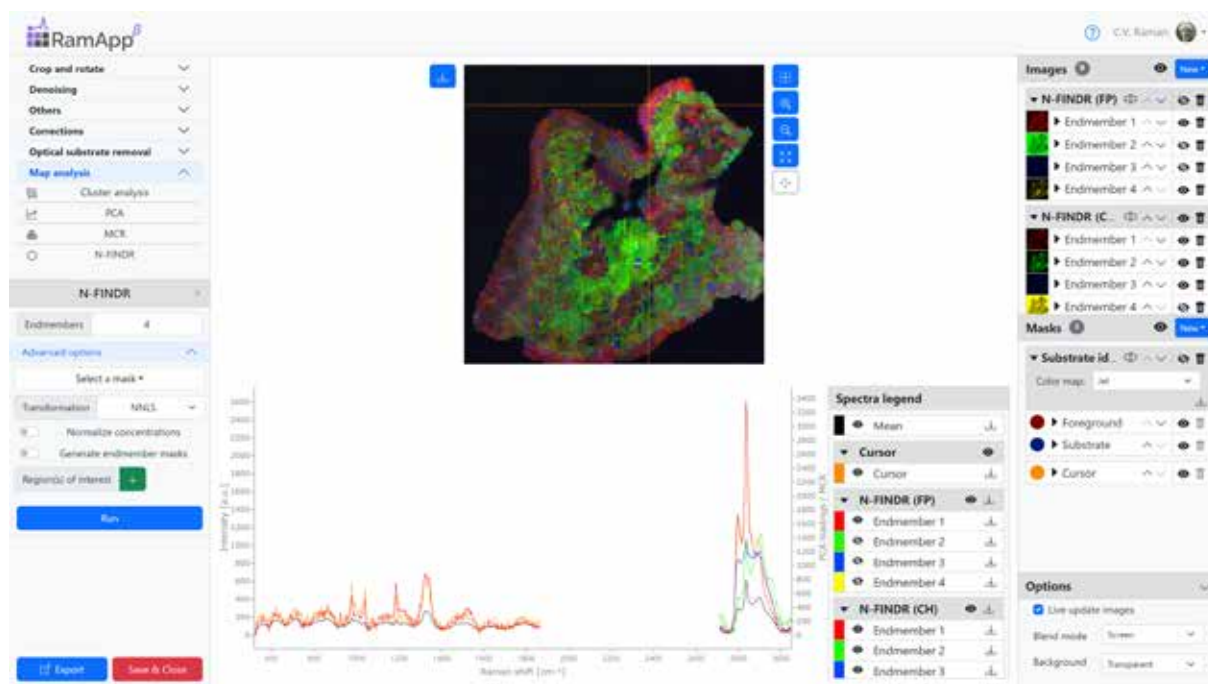
Figure captions:
Example of RamApp usage, where the aim was to detect paraffin contamination (highlighted in red) in a 6x6mm slice of FFPE tumour tissue deposited on glass.

Keywords: software, hyperspectral, imaging, Raman, chemometrics

I-O.12

Title: *Tensor decomposition assisted super-resolution in polarized Raman microscopy*

Author: Andrii Kutsyk[1], Oleksii Ilchenko[1], Yurii Pilhun[2], Jens Wenzel Andreasen[1]

[1]Technical University of Denmark
[2]Lightnovo ApS

Abstract:
Polarized Raman microscopy is a valuable tool for non-destructive 2D and 3D orientation mapping of polycrystalline materials [1]. Due to mixed contribution of polarized Raman signals from neighboring grains actual Raman orientation mapping resolution is higher than the diffraction limit. Hence, super-resolution image restoration techniques could be used for the enhancement of the spatial resolution.
Chemometric assisted super-resolution image restoration has been proven a valuable tool for increasing of spatial resolution of image and chemical mapping [2,3]. The basic idea is to use chemometric techniques to decrease data dimensionality and perform super-resolution restoration on chemical images. Polarization resolved maps can be considered as trilinear data and a parallel factor method such as PARAFAC can be used for decomposition [3,4].
We used different blind and model-based PARAFAC and PARALIND [3] decomposition techniques with further super-resolution image restoration to enhance spatial resolution. The model-based approach takes into account explicit expression of Raman intensity, and it allows simultaneous crystallite orientation determinations. Some PARAFAC loadings for single-phase samples may be linear dependent and PARALIND decomposition should be used instead [3]. The proposed approaches were tested for both synthetic data and polarization resolved maps of polycrystalline silicon.

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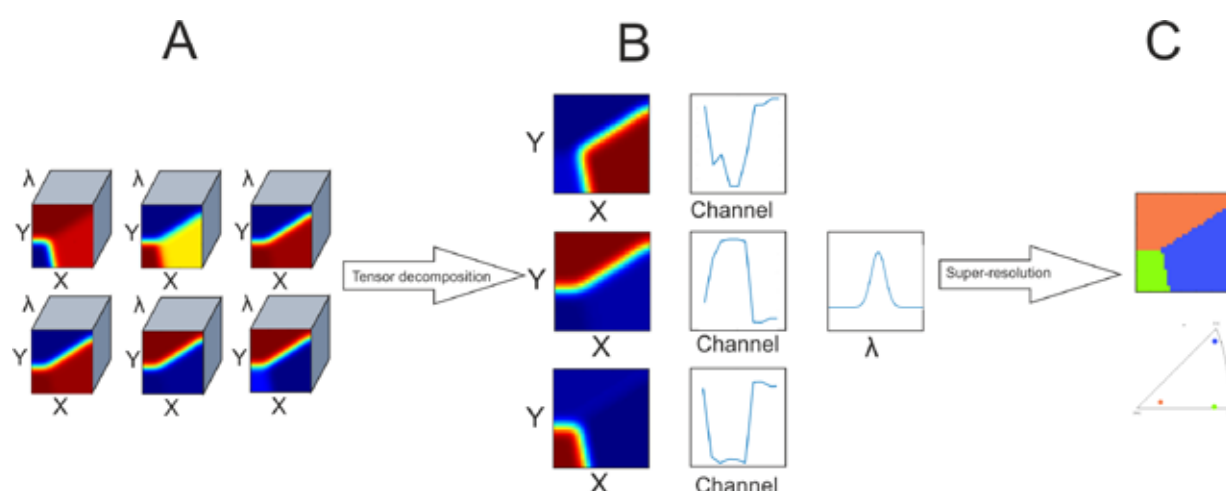
Figure captions:
Figure 1. Schematic representation of the analysis workflow. (A) data collected at different polarization setups; (B) decomposition results; (C) super-resolved grains orientation distribution.

Keywords: Polarized Raman microscopy, super-resolution, tensor

I-O.13

Title: *Extensive Evaluation of Machine Learning Models and Data Preprocessings for Raman Modeling in Bioprocessing*

Author: Michaela Poth[1], Gordon Magill[2], Alois Filgertshofer[1], Oliver Popp[1], Tobias Großkopf[1]

[1]Therapeutic Modalities, Roche Innovation Center Munich, Bioprocess Research, Roche Pharma Research and Early Development
[2]Cell Culture Development and Bioprocess, Genentech Inc.

Abstract:
In recent years, Raman spectroscopy has become a very promising tool for real-time mammalian cell culture monitoring of key analytes. It has potential for gathering process information online without sample preparation in a nondestructive and noninvasive way. In addition, its limited interaction with water and the development of immersion probes make it attractive for direct monitoring and control of bioprocesses. However, major challenges are associated with this promising technology in aqueous bioprocessing matrixes such as a strong background fluorescence, which is typically addressed by computational preprocessing of the raw Raman spectra.
In this study, we present an extensive combinatorial assessment of various machine learning algorithms with numerous popular preprocessing methods on the performance and robustness of Raman based real-time predictions of key analytes. We show that preprocessing methods have a large influence on algorithmic performance. Furthermore, there is a large variance across the various combinations of preprocessing steps and tested machine learning regression algorithms. We demonstrate that neural networks and random forest regression show very good performance and robustness across different, bioprocess relevant analytes. They significantly outperform partial least squares regression, the most widely used regression algorithm in the field. Overall, this extensive study provides a sound basis for building robust, next-generation models for monitoring analytes in real-time based on Raman spectroscopy.

Keywords: PAT, MachineLearning, bioprocess, Raman, preprocessing

I-O.14

Title: *Pre-Processing and Unsupervised Unmixing of Hyperspectral Raman Datasets with RamanLIGHT*

Author: Robert W. Schmidt[1], Sander Woutersen[2], Freek Ariese[1]

[1]Vrije Universiteit Amsterdam
[2]University of Amsterdam

Abstract:
Raman spectroscopy is an established analysis method for the identification and classification of mixtures of chemical compounds in a broad range of samples. Raman microspectroscopy makes it possible to acquire spectra locally within a sample and by adding a movable xy stage one can obtain a 2D Raman image that can visualise the dissociation, aggregation, and correlation of compounds in the sample. However, in the case of unknown samples, finding the specific Raman spectra of the compounds will be difficult and one cannot manually compare tens of thousands of spectra in the data set to find unique spectra. Especially challenging datasets like Alzheimer's disease brain tissue feature only two additional peaks and one slightly shifted peak [2].
For exploratory research, it is important to quickly determine the pure components in a sample and their spatial location without being biased. Therefore, we created a user-friendly, easy to use and freely available MATLAB app that makes it possible for everyone to unmix pure spectra with unsupervised unmixing algorithms, and reveal the spatial abundancies in a Raman image without a deep understanding of the coding language and signal processing[1].
Additionally, the app contains a collection of pre-processing methods for smoothing, baseline correction, and rescaling of hyperspectral Raman mapping data.
We demonstrate the capabilities of the RamanLIGHT app by applying it to hyperspectral Raman images of pharmaceutical, biomedical, and polymer samples.

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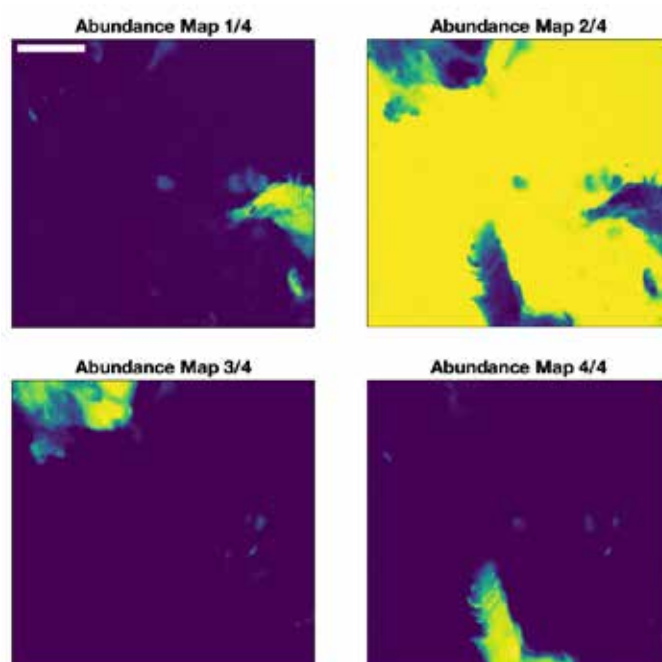
Figure captions:
Abundance maps of three types of plastic particles fixed in a silicone polymer matrix. Map 1: PET, map 2: the matrix, map 3: polypropylene (PP) 3, and map 4: polystyrene (PS). Scale bar 200 μm.

Keywords: Raman, hyperspectral, unsupervised, multivariate

I-O.15

Title: *Long short-term memory and Transformer in Classification and Correction of ATR distorted spectrum*

Author: Rui Cheng[1], Johannes Kiefer[1]

[1]University of Bremen

Abstract:
Attenuated total reflection (ATR) infrared spectroscopy is commonly used for the analysis of a large variety of materials including complicated mixtures of high-refractive index materials and organic solvents. Such samples frequently result in distorted spectra that are difficult to interpret. The traditional method for correcting a distorted spectrum uses the Kramer-Kronig (KK) transform, inverse fast Fourier transformation (IFFT) and fast Fourier transformation (IFFT) [1]. This procedure is challenging as it requires detailed knowledge about mathematics and physics as well as the optical properties of the involved materials. Therefore it is desirable to develop methods that can deal with such spectral distortions automatically.
Deep learning [2], especially neural networks, is very promising in this regard. In the present work, we first obtained artificially distorted spectra by using IFFT and FFT, and then successfully trained the corresponding model by using LSTM [3, 4] and transformer [5] as the learning method to distinguish the distorted and normal experimental spectra, and to finally return the correction of the distorted spectrum.

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Keywords: ATR distorted spectrum, LSTM, Transformer

I-O.16

Title: *Classifying Cheddar cheese based on maturity level and manufacturer using vibrational spectroscopy and chemometrics.*

Author: Gerson R. Dewantier[1], Peter J. Torley[1], Ewan W. Blanch[1]

[1]RMIT University

Abstract:
Cheddar cheese is a well-appreciated fermented dairy product, commercially available in an extended range of maturity levels. Ripening is an essential step of the cheesemaking process, where complex and interconnected biochemical reactions produce high-flavoured compounds. Control of the ripening process is crucial for the industry, and soon regulatory agencies may request proof of ageing. The way to assess the extent of cheese ripening is through traditional methods, which are time-consuming and need dangerous chemicals. Vibrational Spectroscopies like Fourier-transformed Infrared Spectroscopy (FTIR) and Raman can collect much molecular information on complex food matrixes. Combined with chemometrics, they can identify small changes in the composition or condition of complex foods like cheeses. Due to their specificity, each technique highlighted different molecular characteristics, making their combination into an analytical platform desirable. In this work, we combined FTIR and Raman with PCA to investigate the effects of ageing in commercial cheddar cheeses. Changes in the amide I and II bands were the main spectral characteristic responsible for classifying commercially available Cheddar cheese based on the ripening time and manufacturer using FTIR. Lipids bands were changes in Raman spectra.
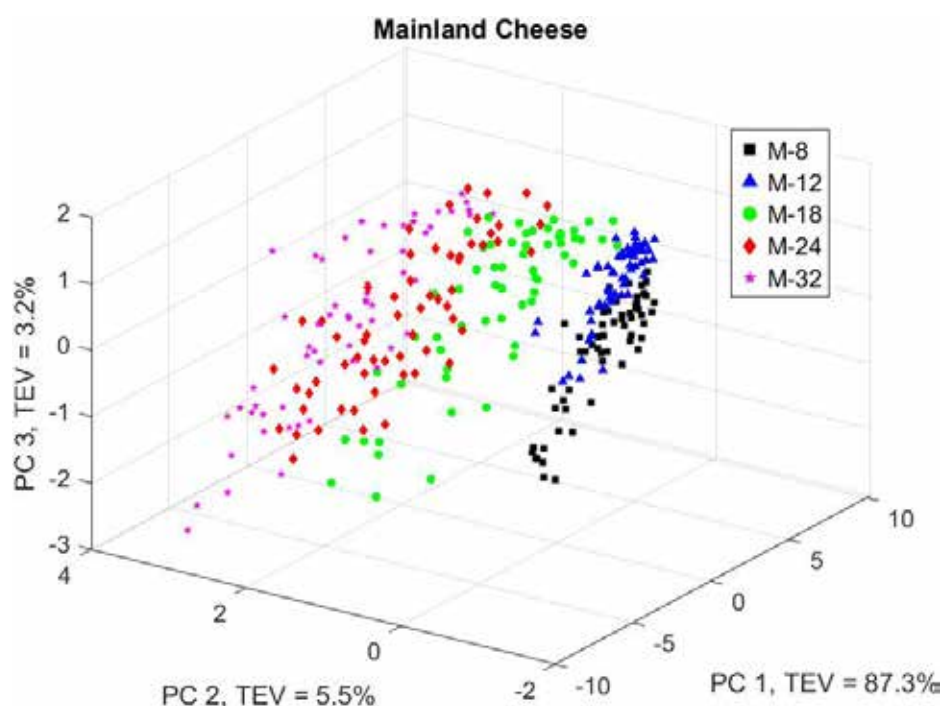
Figure captions:
Scores plot of Mainland Cheddar cheeses from FTIR spectra.

Keywords: Raman, FTIR, Cheddar Cheese, Ripening

I-O.17

Title: *Characterization of root tissue development associated with lodging tendency in tef using Raman micro-spectroscopyscopy*

Author: Sabrina Diehn[1], Noa Kirby[1], Shiran Ben-Zeev[1], Yehoshua Saranga[1], Rivka Elbaum[1]

[1]The Robert H Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem

Abstract:

Tef (*Eragrostis tef* (*Zucc.*) *Trotter*) is the most important staple crop in Ethiopia and Eritrea. Moreover, its grains are gluten-free and protein rich, so it is considered as a "super-food". Adapting tef to modern farm practices is challenging due to its high lodging susceptibility that can cause significant crop losses. Lodging describes the displacement of roots (root lodging) or fractures in the culm (stem lodging) forcing the plant to bend or fall from its vertical position. Lodging is facilitated by various abiotic and biotic factors and often occurs in overpopulated fields.

In tef lodging is most likely caused by root lodging[1], so here we focused on crown roots, aiming to understand the structural-microscopic properties underlining tef tolerance/susceptibility to lodging. We thus analyzed plants 5 and 10 weeks old after emergence. Root cross sections from tef genotypes showing diverse levels of lodging susceptibility[2] were characterized by scanning electron microscopy (SEM) and Raman micro- spectroscopy. Additionally, we compare the development of the endodermis in young and mature tissue combining Raman micro-spectroscopy and univariate/ multivariate analyses. Our results indicate that lodging susceptible genotypes exhibited early tissue maturation, including developed aerenchyma, intensive lignification, and lignin with high levels of crosslinks. In order to reduce lodging in tef and facilitate mechanical harvest, we suggest a genetic selection with focus on slow maturation of lignin in crown roots.

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Keywords: Raman micro-spectroscopy, cell wall, PCA

I-O.18

Title: *Plasmonic surface enhanced infrared spectroscopy aided with artificial intelligence for structural protein biomarker based neurodegenerative disease detection*

Author: Deepthy Kavungal[1], Pedro Magalhães[2], Senthil Kumar[2], Rajasekhar Kolla[2], Hilal Lashuel[2], Hatice Altug[1]

[1]Institute of Bioengineering, EPFL
[2]Brain Mind Institute, EPFL

Abstract:

Parkinson's Disease is the fastest growing neurodegenerative disorder, and its early diagnosis is challenging owing to the lack of tools in detecting the pre-clinical biomarkers. The conformational misfolding of disordered alpha-synuclein (aSyn) monomers into beta-sheet enriched pathological oligomeric and fibrillary aggregates is a key event in the disease progression. We introduce an immunoassay coupled plasmonic nanorod metasurface sensor based on Surface Enhanced Infrared Absorption Spectroscopy (ImmunoSEIRA) which detects these structural biomarkers with clinically relevant specificity in real time and discerns the distinct conformational species using their unique absorption signatures. We augmented the sensor with Deep Neural Network enabling quantitative differentiation of pathological species which is unprecedented. The sensor can retrieve the absorbance fingerprint in the presence of human cerebrospinal fluid and is capable of multiplexing for simultaneously monitoring multiple pathology-associated biomarkers. Thus, our sensor is a promising candidate for the early diagnosis of PD in humans with parallel application in monitoring disease progression and drug engagement research.

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Acknowledgments:

Figure captions:
(A) ASyn misfolding (B-E) ImmunoSEIRA based detection (F) Structural differentiation of aSyn species (G) DNN-ImmunoSEIRA for accurate quantitative prediction of different aggregates in a mixed sample

Keywords: infrared spectroscopy, artificial intelligence, nanoplasmonics, neurodegenerative diseases, metasurfaces
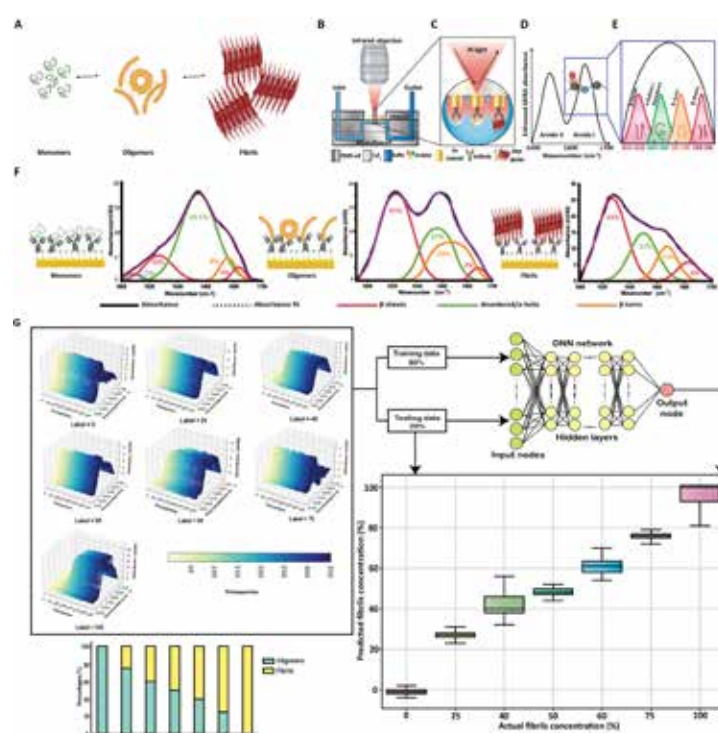
Title: *The use of NIR spectroscopy for the analysis of Fumonisin B1 (FB1)*

Author: Anja Laubscher[1], Paul J. Williams[1], Lindy J. Rose[1]

[1]Stellenbosch University

Abstract:
Maize is a staple food for many South Africans, therefore its contamination with Fumonisin B1 (FB1), a mycotoxin classified as a group 2B human carcinogen, must be closely monitored.[1] Although the South African Department of Health has legislated maximum levels for this toxin at 4 ppm, effective analysis methods are required to ensure adherence to these levels.[2] Near Infrared (NIR) spectroscopy is a rapid, accurate and non-destructive method which has successfully been used for the analysis of FB1 in maize in various studies.[3,4] However, most of these studies used indirect measures which are related to FB1, such as fungal count and the changes the colour, vitreousness, and nutritional content of maize kernels, to predict its content. Due to the focus on indirect detection, the absorbance bands of FB1 in maize kernels have not yet been identified. Therefore, the purpose of this study was to investigate the absorbance bands of FB1. The spectra of 100 ppm FB1 solutions, constituted in methanol, as well as methanol-only samples were recorded in the 1000 – 2500 nm region. Multiplicative scatter correction was applied to the spectra and a partial least squares discriminant analysis (PLS-DA) model was computed. Important wavelengths were selected based on the variable importance in projection (VIP) scores and selectivity ratio (SR) values. A new PLS-DA model was computed with 454 chosen wavelengths and the regression vector of this model was investigated to further identify and remove irrelevant wavelengths. The final model was computed with 150 wavelengths and 9 latent variables and obtained a classification accuracy of 100% for both the calibration and external validation sets. Potential FB1 bands were identified from the regression vector of the final model at 1446 nm, 1453 nm, 1891 nm, 2036 nm, 2046 nm, 2148 nm, 2224 nm, 2262 nm and 2273 nm. This study was therefore able to identify potential FB1 bands at the ppm level using a creative chemometric approach.

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Acknowledgments:

Keywords: Near Infrared Spectroscopy, fumonisin B1

I-O.20

Title: *A multivariate surface-enhanced infrared absorption (SEIRA) method based on quantum dots and universal attenuated total reflectance (UATR) accessory for atrazine determination*

Author: Felipe Trindade[1], Izabel Souza Sobrinha[1], Giovannia Pereira[1], Claudete Pereira[1]

[1]Universidade Federal de Pernambuco

Abstract:
Alternative analytical methods for the chromatographic[1] determination of herbicides like atrazine (ATZ) in samples as water, soil and plants is a challenge. Surface-Enhanced Infrared absorption (SEIRA) analysis appears as a sensible, fast, with little or no sample preparation[2]. This work proposes a multivariate SEIRA method based on silver selenide quantum dots (QDs), stabilized with mercaptopropionic acid (Ag_2Se/MPA) and a universal attenuated total reflectance (UATR) accessory for atrazine determination is proposed. This UATR accessory has only one internal reflection, which is a challenge for SEIRA studies as the signal intensification obtained by the technique is directly proportional to the area of electromagnetic radiation interaction with the sample. The Ag_2Se/MPA QDs were one-pot synthesized in an aqueous medium, to minimize environmental damage. SEIRA spectra were evaluated with the experimental enhancement factor (EF), which was calculated comparing an ATZ peak at determined wavelength with and without Ag_2Se/MPA. Enhancements up to 15 times were found at ATZ 150 µg/mL. In addition, a novel multivariate approach, named 'Multivariate Enhancement Factor' (MEF) based on Principal Component Analysis (PCA) was used to determine enhancement by employing the interpoint distances between each pair of samples (i.e., with and without Ag2Se–MPA) along the PC1 and PC2 axes and Euclidian distance[3]. For this, all spectra were preprocessed via a baseline offset correction and mean centered. Results obtained in this work were very promising, suggesting a new methodology to determine ATZ and other herbicides, using only a single one-reflection ATR accessory. In addition, MEF is a more realistic way to quantify the enhancement effect across the entire spectrum rather than at a single wavelength, which is more suitable for optimizing the experimental conditions in surface-enhanced studies.

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Figure captions:
Infrared spectra from ATZ 15 mg/mL (red), ATZ SEIRA using Ag2Se/MPA as substrate (blue), Ag2Se/MPA substrate using itself as background, and ethanol solvent (grey).

Keywords: SEIRA, atraz

I-O.21
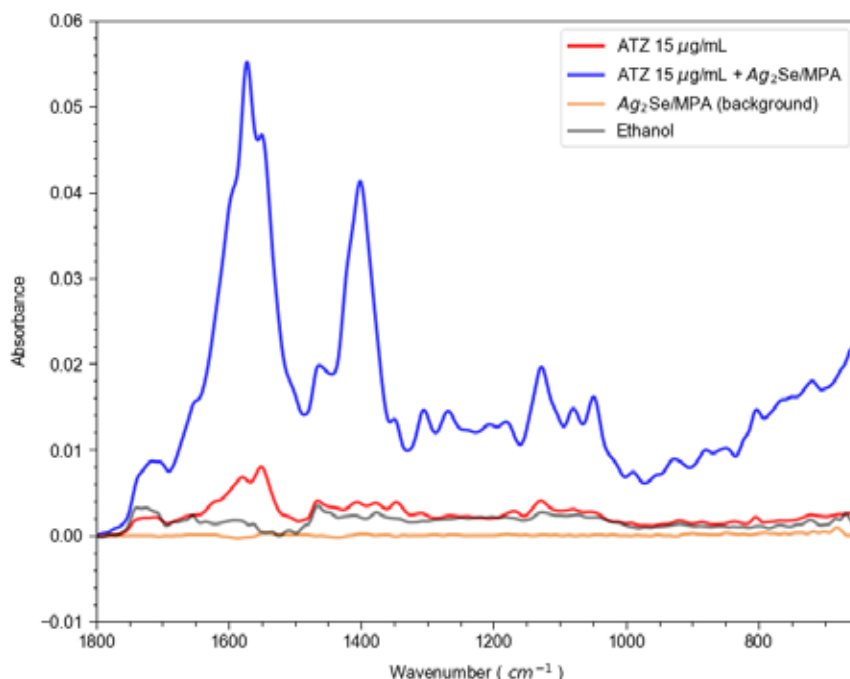
Title: *Green Pharmaceutical Quality Control via Infrared Spectroscopy*

Author: Silke Lehner[1], Mona Tawab[2], Holger Latsch[2], Sandra Ganß[2], Boris Mizaikoff[3], Robert Stach[1]

[1]Hahn-Schickard
[2]Zentrallaboratorium-Deutscher Apotheker
[3]Hahn-Schickard;

Thanks to the Zentrallaboratorium Deutscher Apotheker e.V. for the support with measurements.

Abstract:
Current gold standard techniques in pharmaceutical quality control, e.g. liquid chromatographic methods produce a high amount of organic solvent wastes. Hence, they do not meet the common goals of sustainable analytical chemistry. The demand for green analysis techniques is steadily increasing as exemplified by the European 'green deal agreement' aiming at zero pollution for a toxicant-free environment.[1,2] Herein, a versatile green solution using photonic techniques is demonstrated based on infrared attenuated total reflection (IR-ATR) spectroscopy for pharmaceutical quality control. Besides improved analysis times and only minimal sample preparation organic waste is reduced essentially to zero. Combined with adaptable and self-learning chemometric data evaluation strategies active substances in pharmaceuticals can be identified and quantified even within complex matrices such as gels or pastes containing additional stabilizers, preservatives, etc.[3]
In the present case study, IR-ATR spectroscopy has been adapted to quantify dexamethason in carmellose gel matrices and validated via high performance liquid chromatography (HPLC).
The suitability of this highly promising analytical alternative is currently demonstrated beyond a proof-of-principle will be discussed in this presentation highlighting results from an ongoing ring trial involving pharmacies across Germany with hundreds of samples analyzed and validated.

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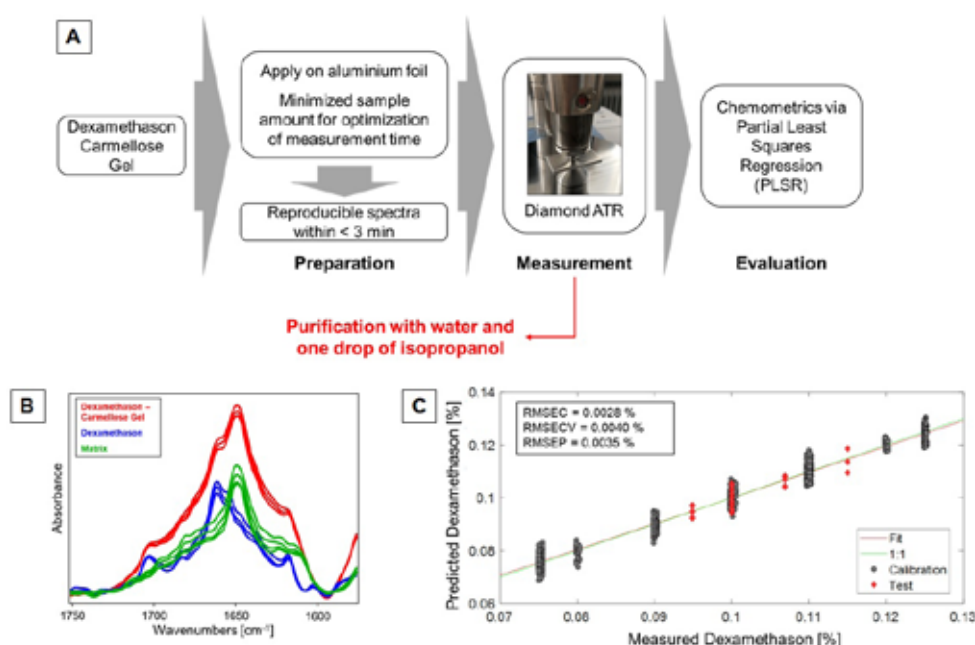
Figure captions:
(A) Measurement and analysis of active substance. (B) Zoom in of IR-ATR spectra of gel and its ingredients. (C) Multivariate validation of active substance with calibration and validation data.

Keywords: IR-ATR, Green Chemistry, Chemometry, PLS-R

Title: *Influence of Infrared Imaging measurement modes on breast tissue recognition and cancer diagnosis*

Author: <u>Danuta Liberda</u>[1], Tomasz P. Wróbel[2]

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Abstract:
Fourier Transform Infrared Imaging (FTIR) combined with machine learning has been successfully applied for different tissue recognition and cancer diagnosis. The important factor that may determine successful classification is pixel size resulting from the chosen objective magnification. In the case of breast tissue, classification results based on data measured with pixel sizes of 1.1 μm (High Definition) and 6.25 μm (Standard Definition) were compared by Mittal et al. [1]. It was shown that even using data measured with a 6.25 μm pixel size model was able to recognize malignant and benign tissues with AUC (Area under the ROC curve) above 0.95. Measurement time is an important factor to make the method closer to the clinical application. Therefore, in our research, we decided to apply a 3.5x magnification objective giving a projected pixel size of 11.4 μm that allows us to measure broader sample areas in a shorter time. The second factor that influences a method's large-scale application capabilities is measurement cost. Sample carries – low-e slides applied in transflection mode are inexpensive in comparison to transmission mode. However, additional spectra distortions (i.e. interference) are visible in measured spectra [2]. Accordingly in this research, we measured two breast tissue microarrays (TMAs) with 272 patients in two measurement modes: transmission and transflection. Data were preprocessed: denoised with the Minimum Noise Fraction method, baseline corrected, transformed into metrics, and normalized to Amide I band. Finally four-class (cancer, necrosis, fibers, benign) Random Forest model was created. Accuracy on the tissue level was close to 0.8 for both measurement modes. On the patient level, AUC values were very high - above 0.95. Therefore, we can state that models created in both measurement modes have the potential for biopsy fast cancer screening.

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Keywords: Infrared imaging, cancer detection, chemometrics

Title: *Infrared Diffraction Microtomography of Biological Samples by Solving the Inverse Scatter Problem*

Author: <u>Eirik Almklov Magnussen</u>[1], Boris Zimmermann[1], Uladzislau Blazkho[1], Simona Dzurendova[1], Benjamin Dupuy-Galet[1], Dana Byrtusova[1], Florian Muthreich[2], Valeria Tafintseva[1], Kristian Hovde Liland[1], Volha Shapaval[1], Achim Kohler[1]

[1]Norwegian University of Life Sciences
[2]University of Bergen

Abstract:
Infrared microspectroscopy is a valuable and much used tool for studying biological cells and tissue, as it delivers information about the chemical composition of the samples being studied encoded in the molecular absorption as well as scattering information depending on the morphology and optical properties of the sample. Using focal plane array (FPA) detectors one can easily get the 2D distribution of chemical composition as hyperspectral maps. However, obtaining the 3D distribution is not easily achieved, and although it is possible to realize this experimentally through IR spectro-microtomography, the process is very time-consuming and technically demanding. We suggest exploiting the scatter contributions to measured IR spectra and solving the inverse scatter problem (ISP) to perform diffraction microtomography, which would allow volumetric chemical imaging using conventional IR spectrometers. We employ a deep convolutional neural network (DCNN) to solve the full-wave ISP and recover the 3D distribution of molecular absorption and optical properties from measured IR spectra of radially symmetric microobjects. We train the DCCN on simulated data, where we first simulate molecular absorption spectra from a PCA decomposition of measured pure spectra coming from several different data-sets. Thereafter, we solve Maxwell's equations and simulate the electromagnetic scattering on billions of different samples with different molecular composition. Our model is trained to recover the 3D distribution of the complex refractive index from the single measured scatter-distorted spectrum.
We demonstrate that our model works well on two-layered spheres. The approach is fairly general and can be extended to systems where the spherical symmetry is broken. However, the simulation of the ground truth is more time-consuming as it requires advanced numerical solutions of Maxwell's equations for such systems.

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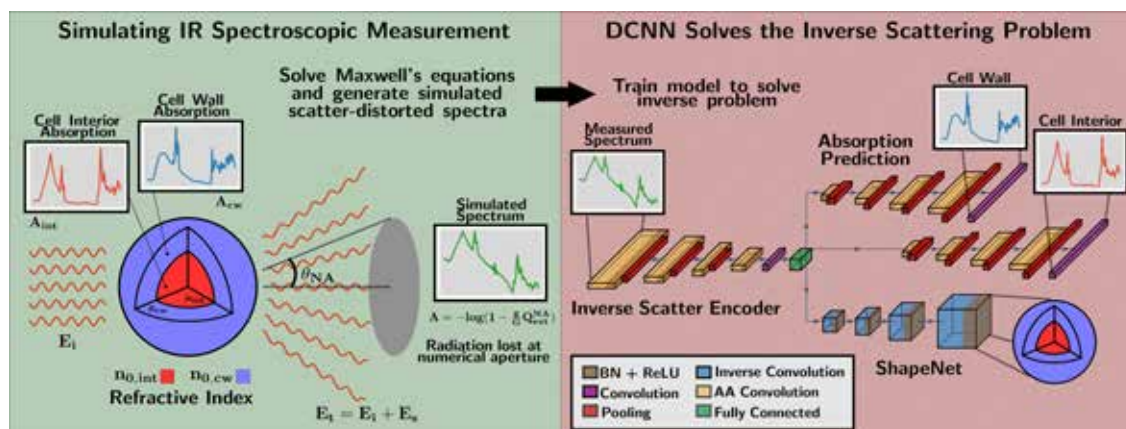
Figure captions:
Illustration of the approach to training the model on simulated data to recover the absorption in the cell interior and wall, and the morphology and optical properties from measured IR spectra.

Keywords: Deep learning, IR spectroscopy

Title: *Raman Optical Activity: Simulations Outside and In Resonance*

Author: Petr Bour[1]

[1]Institute of Organic Chemistry and Biochemistry

Abstract:
Simulations of Raman and Raman optical activity (ROA) spectra provide a useful link between the spectral shapes and molecular structure. If the computations are reasonably accurate, one can directly decompose the experimental spectra into calculated subspectra of the conformers and from that obtain conformer populations in studied samples.[1] We applied the decomposition for a set of model nucleodites, where the simulations were challenging because of molecular polarity and flexibility.[2] To deal with a large number of molecular dynamics snapshots that have to be averaged for a reasonable agreement with experiment, we adapted the decomposition algorithm so that the methodology in the final effect provided not only the conformer populations, but a section of the potential energy surface (Figure).

For absorbing ("resonating") samples the Raman and ROA signal and thus the sensitivity of the method can be in principle increased, but currently they are many challenges in this direction. Experimentally, the effect of electronic circular dichroism and polarized Raman scattering must be separated from resonance ROA.[3] Computational procedures exists[4]; 5 but so far only for a few examples the power of the technique has been documented, revealing in principle also the electronic structure.[6]

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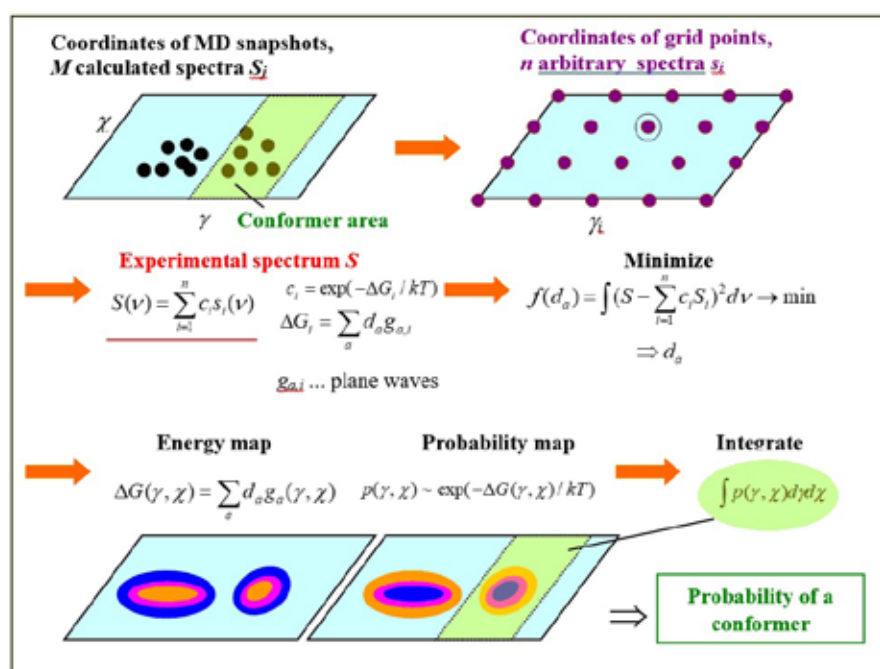
Figure captions:
Figure A workflow of a decomposition of an experimental spectrum into simulated ones, providing a potential energy surface.

Keywords: Raman optical activity, nucleotides, complexes,

J-I.2

Title: *CHIROPTICAL SPECTRA: WHEN CALCULATIONS MEET THE EXPERIMENT*

Author: Joanna E. Rode[1]

[1]Institute of Nuclear Chemistry and Technology, Dorodna 16

Abstract:

In chiroptical methods such as electronic circular dichroism (ECD), vibrational circular dichroism (VCD), or Raman optical activity (ROA), the absorption/scattering coefficients of the left- and right-circularly polarized light are different. As a result, the spectra of the enantiomers are mutual mirror images.[1] The methods are especially useful for the unambiguous determination of the absolute configuration of chiral molecules. However, they are also sensitive to the molecular surrounding that influences the band position, its intensity, and even sign. Therefore, these methods are used in physico-chemical studies.

Still, several problems arise when the chiroptical spectra are modelled. First, reproducing the spectra of a flexible molecule requires a proper evaluation of its conformational space. There are compounds which different conformers exhibit almost mirror image spectra.[2,3] Hence, the proper estimation of the conformer population is crucial for the correct interpretation of the chiroptical spectra. Second, solvent interacting with molecules forming hydrogen bonds can change the stability of the conformations and thus the final chiroptical spectrum, even ECD (Figure), which is usually believed to be only slightly solvent dependent.

In this presentation, we show experimental VCD and ECD spectra, supported by DFT calculations to interpret the observed changes upon substituents and solvents.[4]

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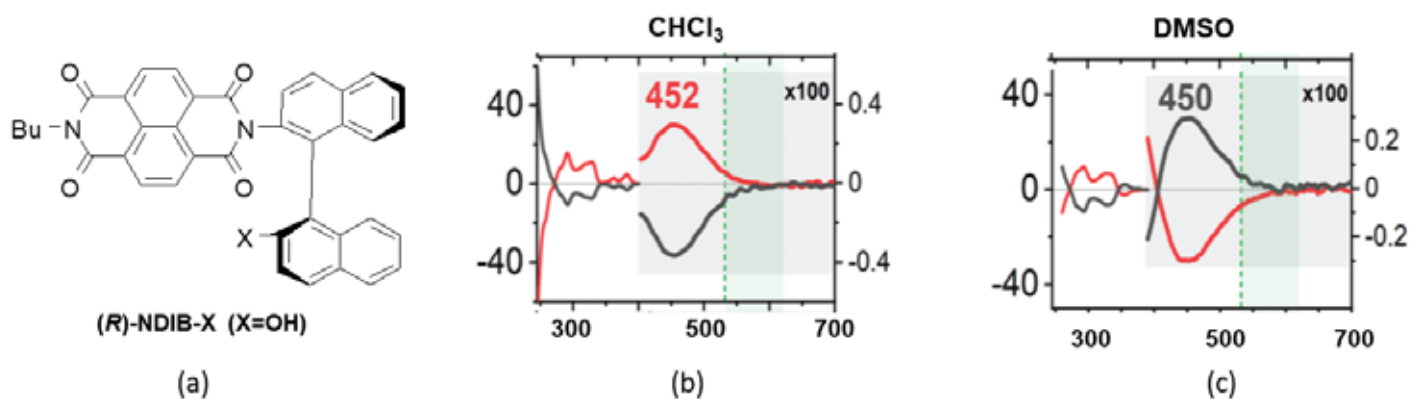
Figure captions:
Structure (a) and ECD spectra of (R)-NDIB–OH (black) and (S)-NDIB–OH (red) in chloroform (b) and DMSO (c).

Keywords: VCD, ECD, calculations, conformation, solvent

J-O.1

Title: *A study of synchrotron-based UV-resonance Raman spectra of N-acetylamino saccharides – In combination with their ATR-far ultraviolet spectroscopy study*

Author: Kousuke Hashimoto[1], Fatima Matroodi[2], Mariagrazia Tortra[2], Barbara Rossi[2], Yusuke Morisawa[3], Yukihiro Ozaki[1], Hidetoshi Sato[1]

[1]School of Biological and Environmental Sciences, Kwansei Gakuin University
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[3]School of Science and Engineering, Kindai University

Abstract:
Ultraviolet resonance Raman spectroscopy (UVRR) has so far been used for the structural analysis of proteins. Still, if the excitation light is extended to shorter wavelengths, it can be used to study the structure of sugars and lipids. In the present state, FUV spectroscopy rarely has been studied on sugars and lipids. Still, the complementary relationship between FUV spectroscopy and UVRR allows us to effectively promote the investigation of the relationship between electronic state and molecular structure. For example, FUV spectral data to determine the excitation wavelength of UVRR. On the other hand, it is expected that the UVRR excitation wavelength dependence will provide helpful information for analysis, such as attribution to the detailed structure of the bands in the FUV spectrum. We have developed the FUV spectra for bio-molecules using the attenuate total reflection technique1 and interpretation of the FUV spectra using quantum chemical calculation.2 In the present study, FUV spectra and UVRR were measured for N-acetylamino sugars, as seen in the sugar chains. In this presentation, we consider the assignment of FUV spectra based on quantum chemical calculations and discuss the excitation wavelength dependence of the UVRR spectra of N-acetylamino sugars.

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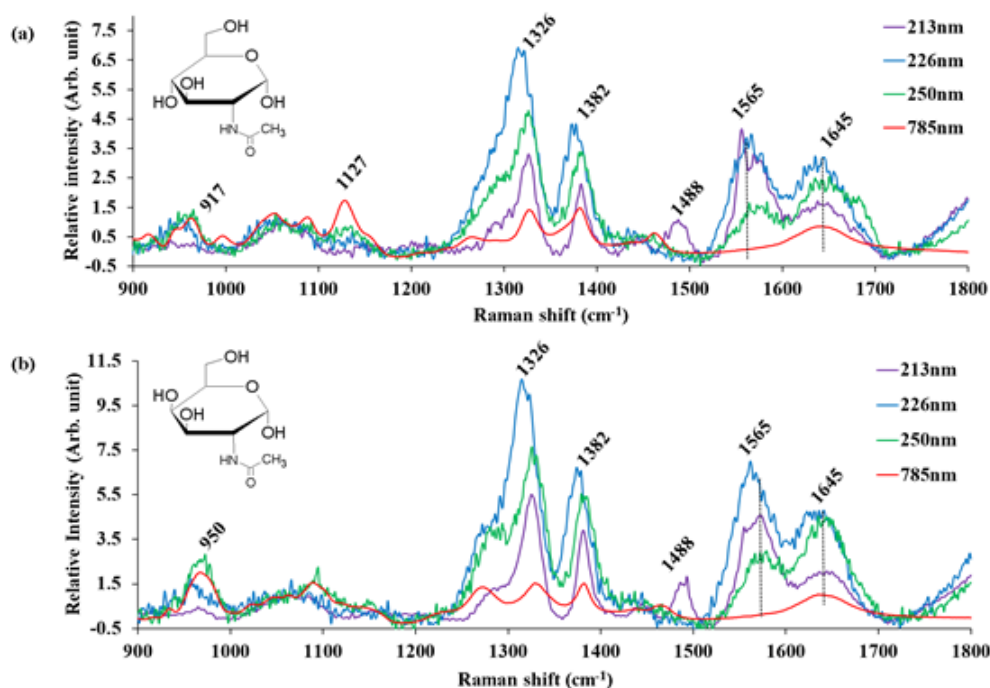
Figure captions:
Raman spectra of (a) GlcNAc and (b) GalNAc aqueous solutions excited with 213, 226, 250, and 785 nm.

Keywords: N-acetylglucosamine, N-acetylgalactosamine, FUV spectroscopy, Ultraviolet Resonance Raman Spectroscopy

J-O.2

Title: *Vibrational Circular Dichroism of Chiral Crystals: The Interplay of Symmetry and Chirality*

Author: Sascha Jähnigen[1], Anne Zehnacker[2], Rodolphe Vuilleumier[1]

[1]Ecole Normale Supérieure
[2]Institut des Sciences Moléculaires d'Orsay, Université Paris-Saclay

Abstract:
Chiroptical spectroscopy provides an increasingly important, cost-effective alternative for the study of chiral substances in the solid state. In recent years, vibrational circular dichroism (VCD) has come into focus as a very sensitive probe of molecular conformation and environment. It has been applied to a wide range of molecules including natural products, host-guest systems, proteins, nanoparticles, or catalysts, as well as the formation of chiral phases from achiral subunits. VCD differs from electronic circular dichroism in that it relates directly to vibrational transitions in the supramolecular chiral framework, such as functional groups connected by covalent or non-covalent interactions. Therefore, the identification of enantiomorphisms in the crystal structure – at constant IR absorption – is a key feature of solid-state VCD, which can be used to distinguish polymorphic forms.[1–4]

Accurate calculations are required to interpret VCD spectra, which also requires the magnetic response of the electrons to the vibrational transition. This can be done in the realms of perturbation theory and has become a standard feature of many quantum chemical codes. However, the calculation of the VCD of solids has long been considered infeasible due to the periodicity imposed by the crystal structure.

In this contribution, we show that it is possible to formulate VCD with an explicit account for periodicity, reconnecting the theoretical model to the physical system.[4,5] This allows us to distinguish between contributions originating from molecular chirality and from chiral crystal packing. Our calculations of several crystalline systems find that while IR absorption hardly depends on the symmetry of the space group, the situation is different for VCD, where completely new non-local patterns emerge: even for achiral space group, a single proper symmetry operation has a large impact on the VCD spectrum, which reflects the supramolecular chirality of the crystal.[1,2]

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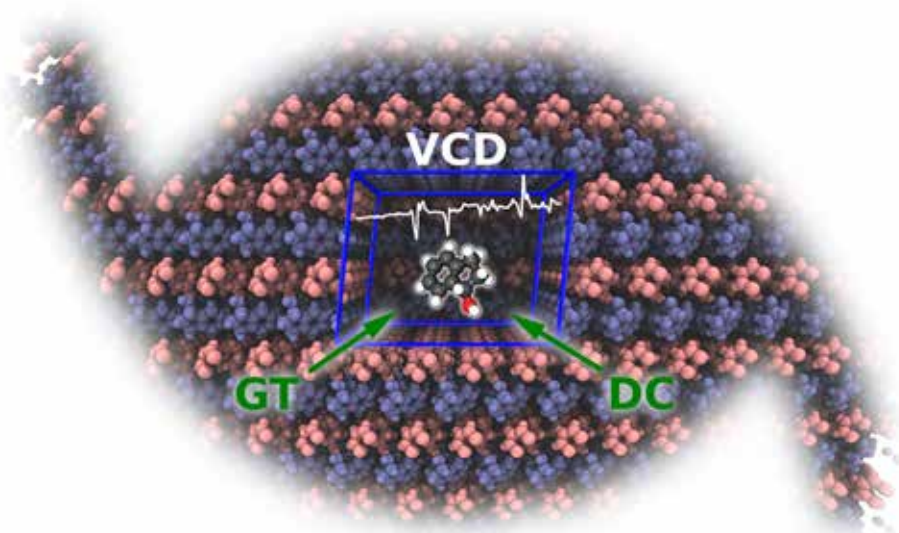
Figure captions:
Solid-state VCD is closely linked to crystal symmetry: A twofold screw axis already generates important non-local VCD patterns that appear as two terms: direct coupling (DC) and gauge transport (GT).

Keywords: VCD, Solid-State, Crystal, Chirality, DFT

J-O.3

Title: *Infrared spectrum, Barrier heights and Density Functional Theory calculations of N-(n-Butyl)-N'-[(p-Chloro phenoxy) acetyl] Urea*

Author: J Sunil[1], Kanugula Srishailam[1], B Venkatram Reddy[2], G Ramana Rao[2]

[1]SR University
[2]Kakatiya University

Abstract:
N-(n-Butyl)-N'-[(p-Chloro phenoxy) acetyl] Urea (BPU) was characterized, by recording its Fourier transform infrared spectrum in the range 4000-400 cm-1. Geometry optimization was made using DFT and B3LYP exchange correlation functional in conjunction with 6-311++G(d,p) basis set. The barrier heights were calculated around 10 flexible bonds (C-O, O-C, C-C, C-N, N-C, N-C, C-C, C-C, and C-C; see figure). They were compared with closely related systems. General valence force field, vibrational fundamental frequencies, infrared potential energy distribution (PED), nonlinear optical (NLO) behaviour, frontier molecular orbital parameters (FMO) and natural bonding orbital (NBO) characteristics were evaluated at same level of theory. Electronic transitions in UV-Visible spectrum were obtained with the help of time-dependent density functional theory (TD-DFT) and values of absorption maxima (λmax) were calculated. A comparison of observed and computed parameters was made wherever possible. They show close agreement. We used eigenvectors and PED to make an unambiguous vibrational assignment of all fundamental frequencies for the first time. The compound is good for NLO applications, which is substantiated by NBO analysis. The calculations established the presence of bifurcated intramolecular hydrogen bond in this molecule.
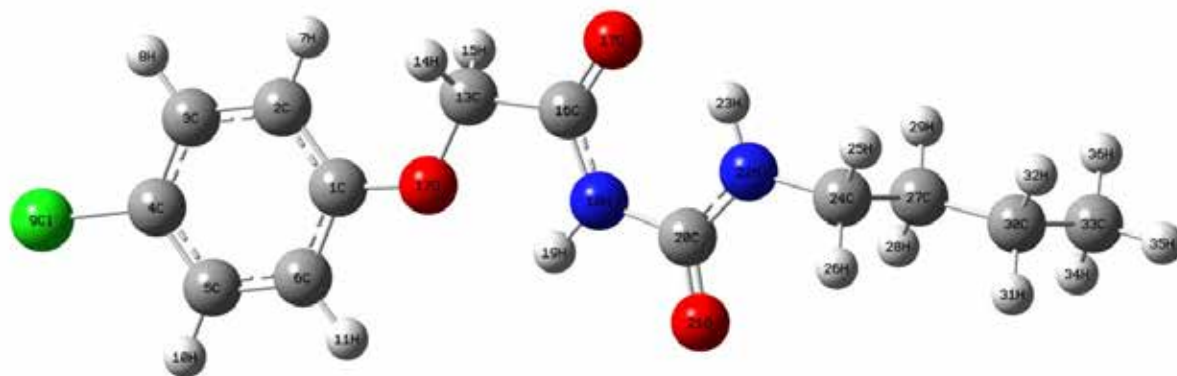
Figure captions:
Optimized molecular structure of BPU (EBPU = -3416.587×103 kJ mol-1), showing numbering of atoms and bifurcated intramolecular hydrogen bond.

Keywords: N-Butyl Urea, Vibrational Spectra, Barrier

Title: *Quantitative evaluation of IR and corresponding VCD spectra*

Author: <u>Thomas Mayerhöfer</u>[1], Ankit Singh[1], Jer-shing Huang[1], Christoph Krafft[1], Juergen Popp[1]

[1]Leibniz Institute of Photonic Technology

Abstract:
Contemporary literature is unable to explain the infrared and vibrational circular dichroism (VCD) spectra of chiral molecules from a classical perspective. As a result, the primary usage of VCD spectra is to determine the absolute configuration of a chiral molecule through comparison of experimental data and quantum mechanical calculations. Classical treatments of IR and VCD spectra, however, can provide insight into spectra, such as the fact that the area of all bands of wavenumber-normalized absorbance above the zero must be the same as the area below zero. In addition, sophisticated band fitting, i.e., dispersion analysis based on wave optics and dispersion theory can be applied, which was extended by Born and Kuhn to include chiral substances. Dispersion analysis uses pairs of coupled oscillators to quantitatively describe the dielectric function and the chiral admittance functions that make up IR and VCD spectra. Oscillator strength, damping, oscillator position, vertical distance between coupled oscillators, and the coupling constant are the five parameters used to model the dielectric functions and chiral admittance functions of α-Pinene and Propylene oxide. In most cases, an oscillator model using two coupled oscillators is good enough to reach a good correspondence between experimental and simulated data, although sometimes a model involving three coupled oscillators may be necessary. Conjugated oscillators can cause bands with different signs, and the areas of these bands are equal. This helps to identify conjugated pairs, and the coupling can also impact the conventional IR spectra of chiral compounds by shifting peaks and transferring oscillator strengths.

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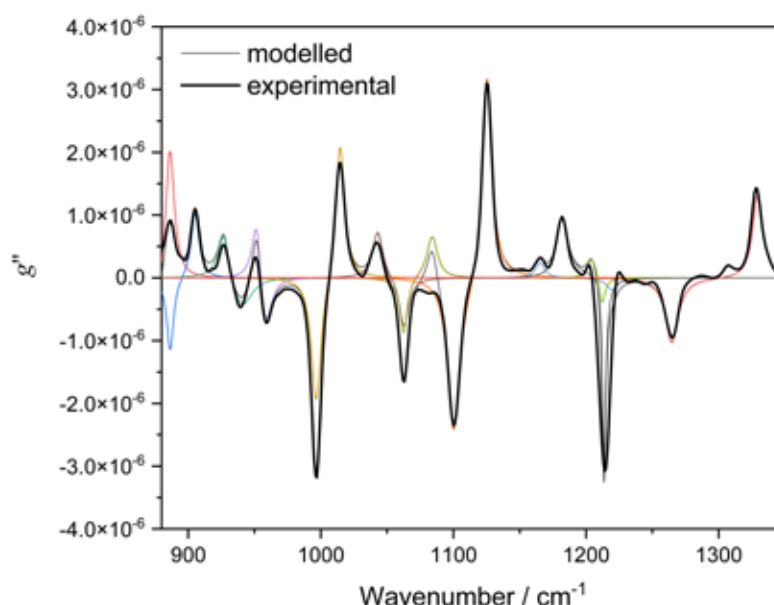
Figure captions:
Experimental chirality admittance and fitted bands using the Born-Kuhn oscillator model for (+) -Pinene.

Keywords: Vibrational circular dichroism, band fitting

J-O.5

Title: *Computing Raman and Raman optical activity spectra for molecules under resonance*

Author: James Cheeseman[1]

[1]Gaussian, Inc.

Abstract:
One approach to extend the computation of Raman and Raman optical activity (ROA) spectra to molecules under resonance is the finite-lifetime method in which an imaginary empirical damping parameter, corresponding to an effective inverse lifetime of the excited states, is added to the incident frequency. This method has been useful in distinguishing between resonance ROA and eCP-Raman, a combination of electronic circular dichroism and circularly polarized Raman [1]. The finite-lifetime method includes near-resonance contributions, but is missing the excited state vibrational contributions for states in strong resonance. Vibronic methods [2], which include the vibrational levels of the ground and excited states, are useful under strong resonance conditions but typically do not recover the near-resonance contributions. A method which combines these two approaches, to obtain both the near- and in- resonance contributions, is proposed.

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Keywords: Resonance Raman, ROA, eCP-Raman, DFT

J-O.6

Title: *Yes we can! Calculational study of Human Serum Transferrin distinguishes between resonance Raman optical activity and circularly polarized Raman*

Author: Jonathan Bogaerts[1], James Cheeseman[2], Wouter Herrebout[1], Christian Johannessen[1]

[1]University of Antwerp
[2]Gaussian Inc.

Abstract:
Human Serum Transferrin (HST) is an intriguing protein, both from a structural and spectroscopic point of view. As an iron transporter, HST exist naturally in an apo form, but also as holo-HST, ligating iron atoms with natural amino acids and a bidentate, making the protein an unusual non-heme containing ferrous metallo-protein. The absence or presence of iron in the protein has previously been shown to switch HST between conventional Raman scattering and resonance Raman when going from the apo to the holo-form. Equally, resonance Raman optical activity (ROA) of the holo-form was reported, in contrast to the non-resonant ROA of the apo protein [1]. Recently, a distinguishment between resonance ROA and circularly polarized Raman (eCP-Raman) was reported, thus asking the relevant question: "Can one measure resonance ROA?" [2] In this contribution, we answer that question with a resounding "YES!". (TD)-DFT level calculations of the iron binding site of HST in the holo and apo form have been performed, including resonance ROA calculations, and were found to support the previous empirical assignment of resonance ROA bands. Furthermore, the contribution of eCP-Raman to the resonance ROA spectrum of holo-HST has been estimated and found to be all but negligible, showing the in the case of HST, resonance ROA is the main contributor to the experimental spectrum.

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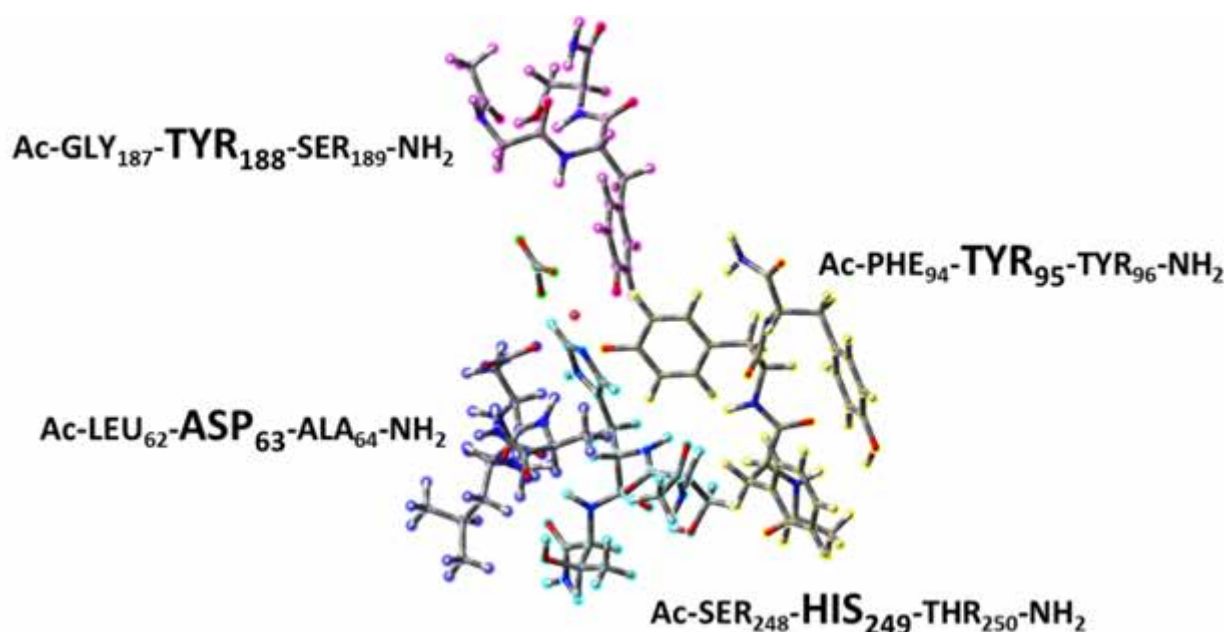
Figure captions:
Model of holo-HST. High spin Fe(III). Sextet dianion.

Keywords: RROA, Transferrin, DFT, ECD

J-O.7

Title: *Simulation of vibrational spectroscopies in various environments*

Author: <u>Vincent Liegeois</u>[1]

[1]NISM, UNamur

Abstract:
Vibrational signatures are sensitive to the surroundings of the target system. In this paper, we are particularly interested in surface enhanced vibrational spectroscopies (SEVS) and in vibrational optical activity (VOA) techniques. Both have their advantages and their difficulties regarding their simulation using ab initio methods. For instance, SEVS are based on the very large response of probed vibrational modes when an applied electromagnetic radiation is resonant with the nanostructured plasmonic system and are applied to single-molecule detection, mapping of molecules and surface defects, or time-resolved analysis of chemical reactions. But, first-principles (1stP) calculations remain very demanding in terms of computational time, which limits their use to systems of relatively small size. This proscribes their use for simulating the plasmon resonant response of nanostructures, with typically more than one million active electrons. VOA techniques can distinguish between enantiomers and are also very sensitive to the environment of the system such as the solvent or guest encapsulation. While their simulation has become routinely, their still exists many challenges as pointed out by Nafie [1] ("properly describe weak intermolecular interactions, solvent effects") and Barone [2] ("the analysis of the conformational potential energy surface (PES) for flexible systems").

In this paper, we propose different strategies to overcome these issues. For SEVS, we investigate second principles (2ndP) methods, which target mesoscale systems while keeping 1stP accuracy predictive power [3,4]. 2ndP approaches aim at finding an effective way to reproduce 1stP data while avoiding the full treatment of the electronic system. For VOA, we simulate the Raman Optical Activity signatures of Cryptophane derivatives, cage systems which encapsulates guest particles such as Xe atom, and compare the spectrum with experimental one recorded by our collaborator at the University of Bordeaux.

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Keywords: VOA,SEVS,DFT,Computational Chemistry

J-O.8

Title: *Anharmonicity of amide bands in NIR region – overtones, combinations, structural fingerprint of peptides*

Author: <u>Justyna Grabska</u>[1], Krzysztof B. Bec[1], Christian W. Huck[1]

[1]University of Innsbruck

Abstract:

Near-infrared (NIR) spectroscopy is a powerful analytical technique widely used in academia and industry [1]. Despite its success, the complex nature of NIR spectra has presented challenges in interpreting the underlying molecular vibrations and relationships between spectra and physical background. Unlike the cases of mid-infrared (MIR) and Raman techniques, structural fingerprinting by NIR spectroscopy is much less straightforward. While chemometrics has been successful in providing analytical performance, it has not been able to explain the origins of spectral variability only by itself. This creates an opportunity for NIR spectroscopy to form a practical synergy with computational chemistry [2].

In this presentation, we demonstrate the recent advancements in computational chemistry tools that have enabled the interpretation of NIR spectra of moderately complex molecules with focus on oligopeptides. This study utilizes simulations to identify and reconsider NIR band assignments for peptides. The simulated spectrum accurately reproduces significant NIR peaks yielding new insights and identification of important peptide bands in NIR region. This indicates that conventional NIR assignments for peptides need to be reconsidered as the manifestation of the amide bands in NIR spectrum is significantly less straightforward than it is in the fundamental region. On the example of polyglycine, the contribution of amide A mode has been speculated to appear as a broad absorption structure near 4890 cm-1; however, the peak in fact arises from the combination of amide A and amide II mode. The findings provide detailed insights into NIR band assignments for peptides, contributing to the understanding of the role of anharmonicity in their spectral properties.

References:

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Keywords: anharmonicity, NIR spectroscopy, peptides, DFTJ-O8

J-O.9

Title: *Resonance Raman Optical Activity: how to properly measure, correct and simulate spectra*

Author: <u>Grzegorz Zając</u>[1], Ewa Machalska[2], Katarzyna Gorczowska[3], Josef Kapitán[4], Petr Bouř[5], Malgorzata Baranska[6]

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Abstract:
Raman optical activity (ROA) is a very potent chiroptical tool for biomolecular studies. It can be used to investigate the conformational equilibria and absolute configuration of chiral compounds, and also secondary and tertiary structure of biomacromolecules. The most widely used form of ROA is scattered circular polarization (SCP) that measures the intensity difference in Raman scattering of right- and left-circularly polarized light. Usually, outside resonance, the difference is quite small, 3-4 orders of magnitude weaker than the Raman signal. Long accumulation times, highly concentrated samples and high laser power are needed to measure good quality ROA spectra.
Resonance Raman optical activity (RROA) can be used to study chiral molecules in much lower concentrations, however RROA measurements, spectral analysis, and theoretical calculations are still challenging. Recently it has been reported that natural RROA signals may be altered by circularly polarized Raman scattering combined with electronic circular dichroism (ECD-Raman). Luckily the effect can be now recognized, calculated and subtracted from the measured signal. [1,2] We demonstrate on a few examples (e.g. vitamin B12 analogs, carotenoid aggregates) how to correctly measure RROA, calculate and subtract the ECD-Raman effect. In addition, we performed quantum chemical calculations of RROA on carotenoids, both in monomeric and aggregated forms, using various computational approaches, to investigate their potential and limitations.

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Keywords: Resonance, ROA, ECD-Raman, spectra simulations

Poster Presentations
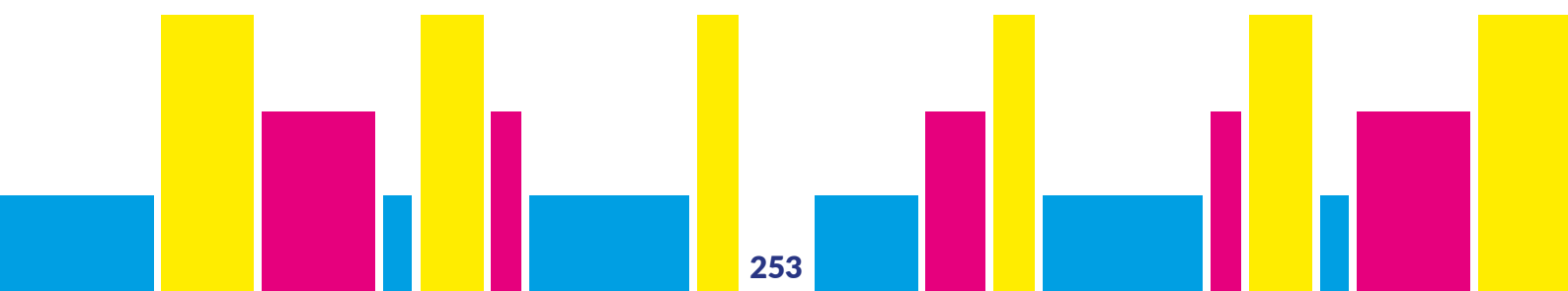
A-P.1

Title: *Super-Resolution Raman Spectroscopy-Applications to Diamond Identification*

Author: Yishai Amiel[1], Prof. Yaakov Tischler[2], Yaakov Mandelbaum[3], Hadass Tischler[3]

[1]Bar-Ilan University
[2]Device Spectroscopy Lab , Bar-Ilan University
[3]Jerusalem College of Technology

Abstract:

In this project, we present a method to perform super-resolved Raman spectroscopy, which improves the resolution of obtained spectra, and allows unambiguous detection of molecules with similar spectra. Experimental investigations were carried out on the spectra of a Natural and Lab-Grown Diamond, where a spectral resolution improvement of at least 2.5X of the original spectrum has been obtained.

A Fabry–Perot (F-P) Etalon filter (Finesse <30, FSR = 2/cm), mounted on an angle-tunable motor, was added to the classical Raman setup in which automatically measuring the spectra for many different states of the F-P filter coupled with decoded experimental results yielded a spectrum of higher resolution having a reduced linewidth in Diamonds spectrum. Therefore, in addition for it being helpful for improving the detection of substances using a low-resolution Raman spectroscopy system, this method can also be a potential method for improving the differentiation of Natural vs. Lab-Grown Diamonds. In addition, this can also be an efficient solution for the improvement of imaging systems with low-resolution sensors. Unlike previous published experiments, which varied the mirror distances (d) or the refractive index (n) of the Etalon, we vary the angle (θ). The rationale and benefits of varying the angle is that the etalon itself is fabricated plane and parallel by design (a set of innovations developed by Light-M), obviating the need to maintain parallelism of the etalon plates if the gap, d, was to be scanned. Also, the motion is only required around one axis, which simplifies both the actuation and metrology.

After optimizing the alignment of the proposed setup, we obtained several sets of repeatable data, for two sorts of diamonds mentioned above. This data was compared to the theoretical model which has led to a significant improvement in resolution and to a differentiation of the two diamonds by their super resolved Raman Spectra.

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4. Dror Malka et al 2019 J. Opt. 21 075801

Figure captions:

Raman Spectroscopy setup with an automated angle-tuned F-P, and the calculated Super-Resolved Raman spectrum of a Natural Diamond sample.

Keywords: Super-Resolution, Diamond Identification, Raman Spectroscopy
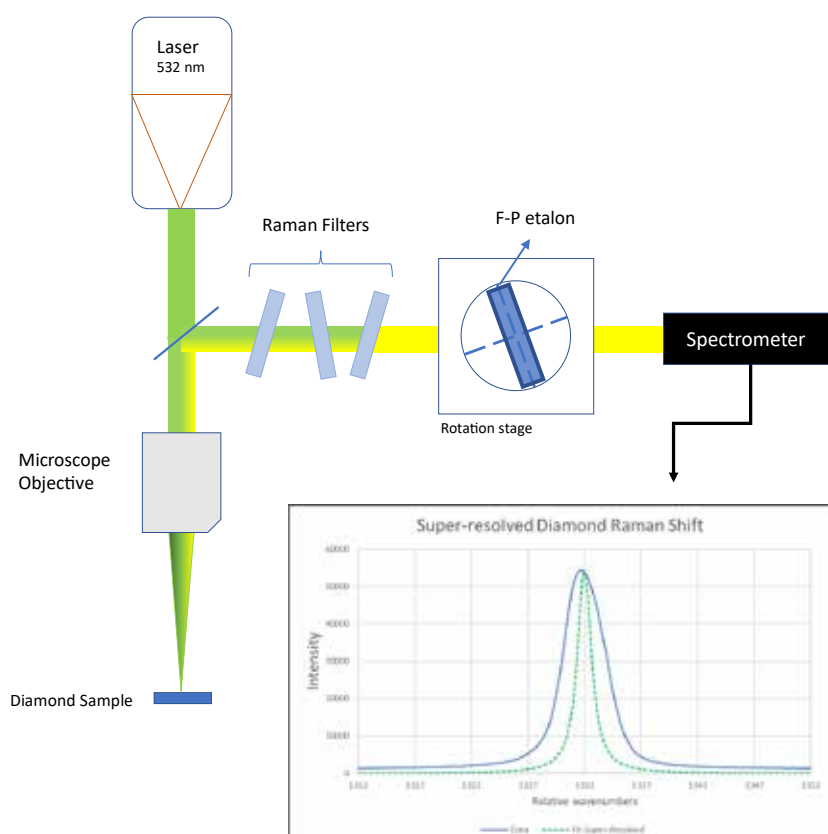
Title: *Operando FTIR analysis of photocatalyst as a reliable testing methodology for CO2 photoreduction in gas phase*

Author: Joudy Dankar[1], Céline Pagis[2], Mickael Rivallan[2], Mohamad El Roz[3]

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[2]IFP Energies nouvelles, Rond-point de l'échangeur de Solaize – BP 3
[3]Laboratoire Catalyse et Spectrochimie, Normandie Université

Abstract:
Determining the link between the surface properties/structure and performance of catalyst requires the use of multi-technique *operando* approach that is capable of probing the system under operating conditions. In this scope, a continuous flow operando gas-phase photoreactor coupled with time resolved FTIR spectroscopy surface analysis and GC and MS gas phase analyses was developed[1] to investigate the photocatalytic reduction of CO_2 in vapor phase.
In this work, we point out the role of carbonaceous residues (C-species) on the raw photocatalyst surface and their contribution during the photocatalytic reduction process. The influence of operating conditions under irradiation and the C-species/H_2O ratio are also highlighted. Further, investigations of acquired IR spectra through a chemometric multivariate curve resolution-alternating least squares (MCR-ALS) approach allowed us to overcome the limitation commonly encountered in IR band assignment (1800-1000 cm^{-1} region), in view of overlapping spectral features in this region[2]. We were able to separate complex raw spectra and assign them to individual chemical species. Beyond revealing the type and nature of three principle carbonaceous species initially on the photocatalyst surface, this work delivers an in-depth understanding of the role of the residual carbon species in catalysis implying an evolutive behavior of the Pt/TiO_2 surface. With our multi-approach analysis, we demonstrate that surface contaminants in the form of carboxylates can participate in surface reactions. On the other hand, temporal analysis of surface species concentration and gaseous evolution with respect to irradiation time reveals important information on reaction mechanisms and points to acetates as likely reaction intermediates to CO_2 photoreduction reaction.

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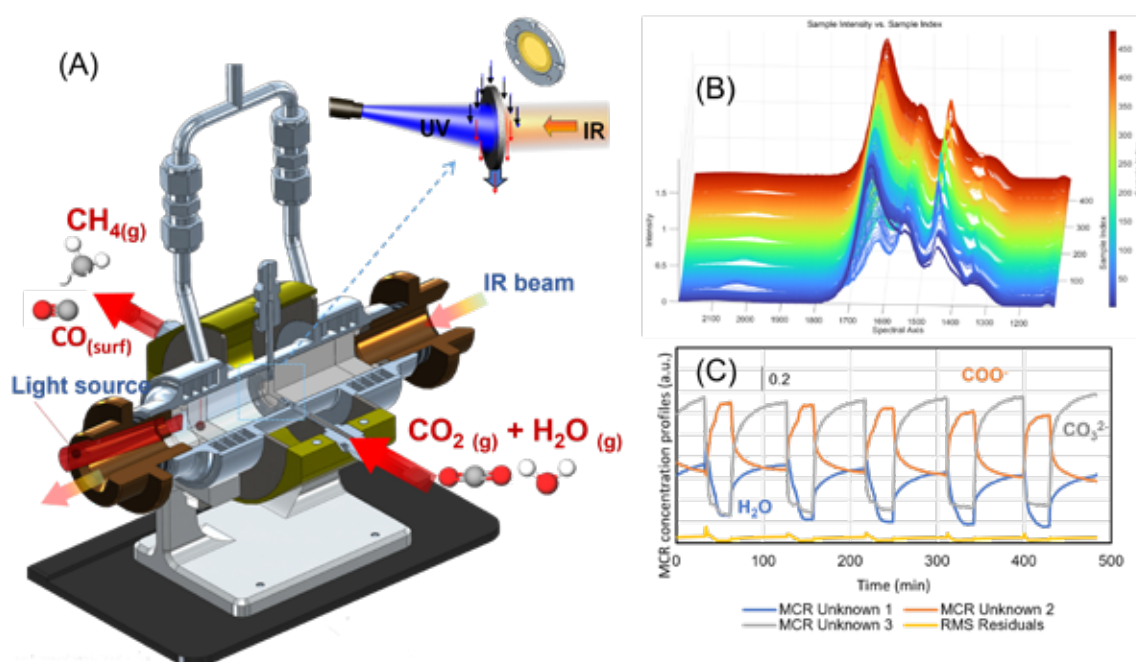
Figure captions:
(A) Scheme of operando photoreactor IR transmission setup, (B) FTIR spectra during cycles of photocatalytic reduction of CO2 with water, and C) corresponding MCR-ALS decomposition of spectra

Keywords: operando, FTIR, chemometrics, photocatalysis, surface

A-P.3

Title: *Investigating NBD-Cl and its derivative NBD-Ceramide in living cells using surface enhanced Raman scattering*

Author: Yiqing Feng[1], Christoph Arenz[1], Janina Kneipp[1]

[1]Humboldt-Universität zu Berlin

Abstract:
4-Chlor-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl) is widely used as a fluorescent dye for proteins, peptides and other biomolecules, based on its sensitive reactions with amino and thiol groups. One of its important derivatives, NBD-ceramide (NBD-CER), has been employed to follow sphingolipid metabolism and localize Golgi apparatus, membrane and vesicles in cellular models. Here, we studied NBD and NBD-ceramide using surface enhanced Raman scattering (SERS), to explore the capacity of SERS to follow cellular changes after NBD-Cl or NBD-CER uptake in living cells. SERS spectra of NBD and NBD-CER are reported with 30 nm citrate-stabilized gold nanoparticles. We then treated macrophage and fibroblast cells with NBD-Cl or NBD-CER after the incubation with gold nanoparticles. As will be shown, the spectra obtained from live cells showed distinct features, indicating the different influences of NBD-Cl and NBD-CER in cells. Spectral differences were observed between the two cell lines as well. The results demonstrate the potential of SERS with gold nanoparticles to probe the intracellular physiological processing of NBD and its derivative lipid in cells and will benefit to study the enzyme function in lipid metabolism in cellular models and other systems.

Keywords: SERS, gold nanoparticles, NBD-ceramide, lipids, cells

A-P.4

Title: *In situ studies for electrocatalytic nitrogen reduction on C2N-type carbon materials using vibrational spectroelectrochemistry*

Author: Linda Feuerstein[1], Martin Oschatz[2]

[1]Chair of Electrochemistry, Technische Universität Dresden
[2]Institute for Technical and Environmental Chemistry, Friedrich-Schiller-Universität Jena

Abstract:

C_2N-type carbon materials are an attractive metal-free electrocatalyst for the nitrogen reduction reaction (NRR) that produces the valuable basic chemical ammonia. However, the nature of the catalytically active sites of the nanoporous and amorphous material remains largely unclear. In situ electrochemical vibrational spectroscopy is a powerful method for gaining mechanistic insights into the processes during electrocatalysis that can be used to tune the catalyst towards higher efficiency and selectivity. By characterising the material using vibrational spectroscopy, information about its chemical bonding and structural motives are derived that might be beneficial for NRR catalysis. Numerous nitrile groups in the carbonaceous material act as vibrational sensor groups that display changes within the structure of the conjugated C_2N-framework when electric potential is applied and higher catalytic activity towards the NRR is achieved.

Keywords: electrocatalysis, nitrogen-rich carbon, in-situ spectroelectrochemistry

A-P.5

Title: *Multimodal Microscopy Characterisation of 2D Materials*

Author: Angela Flack[1], Stuart Thomson[1]

[1]Edinburgh Instruments

Abstract:
Spatial characterisation of transition metal dichalcogenides (TMDCs) is crucial for understanding their optoelectronic properties and optimising film growth conditions. In this work, we present a multimodal microscopy platform combining conventional widefield, Raman, photoluminescence and second harmonic generation imaging for the characterisation of 2D materials. The platform was used to characterise CVD grown WSe_2 crystals. Raman imaging identified the presence of both monolayer and multilayer WSe_2 through a change in the intensity and position of the E^1_{2g} / A_{1g} phonon modes. Photoluminescence imaging confirmed the presence of monolayer WSe_2 with emission at 780 nm and identified two distinct multilayer regions through changes in the photoluminescence wavelength and intensity. Lastly, second harmonic generation imaging under femtosecond laser excitation was used to obtain the relative growth orientation of the three identified domains in the crystal.
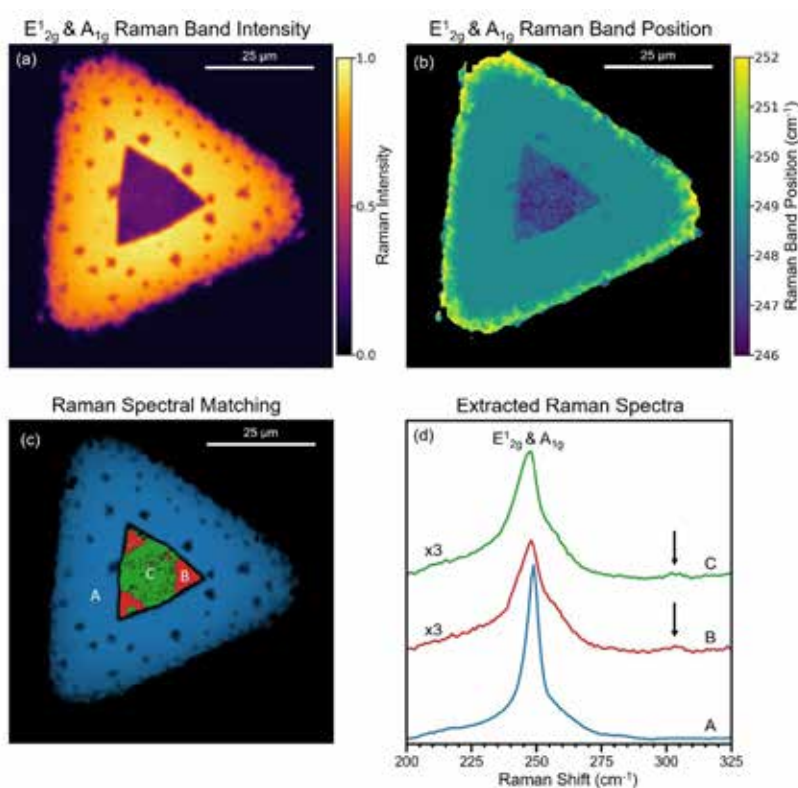
Figure captions:
(a) Intensity of the E12g / A1g Raman band, (b) Peak position of the E12g / A1g Raman band, (c) least squares spectral matching showing three distinct Raman spectral areas, (d) Averaged Raman spectra

Keywords: multimodal microscopy, 2D materials, WSe2

A-P.6

Title: *Spectroscopic studies on the adsorption of neuroleptics belonging to phenothiazine derivatives on gold nanoparticle surfaces*

Author: Patrycja Gnacek[1], Natalia Piergies[2], Oliwia Kowalska[1], Magdalena Oćwieja[1], Piotr Niemiec[3]

[1]Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences
[2]Institute of Nuclear Physics Polish Academy of Sciences
[3]Faculty of Mathematics and Natural Sciences, Department of Chemistry, University of Applied Sciences in Tarnow

Abstract:

Chlorpromazine (CPZ) and fluphenazine (FPZ) are phenothiazine derivatives widely used for the treatment of psychotic diseases. The attention of scientists is focused on these drugs because numerous literature reports have shown that they can influence the progression of neurodegenerative diseases. On the other hand, some evidence has indicated that these phenothiazines are able to inhibit the fibrillization processes of proteins. Similarly, gold nanoparticles (AuNPs) have turned out to be active in the inhibition of fibrillation processes of such proteins as α-synuclein and amyloid-β. For these reasons, our attention was focused on the development of methods that provide possibilities to prepare biologically active conjugates of these neuroleptics with AuNPs. The aim of our study was to determine the adsorption pattern of CPZ and FPZ on AuNP surfaces with the use of modern spectroscopic techniques and to evaluate the stability of formed conjugates.

The conjugates of neuroleptics with AuNPs were prepared via controlled adsorption of the drug on negatively charged AuNPs of an average size of 55 ± 5 nm. The electrokinetic measurements revealed that the surface charge of conjugates was dependent on the concentration of adsorbed drug molecules, pH conditions, and ionic strength. The spatial conformation of the drugs on AuNPs, and the stability of the conjugates dispersed in the solutions of controlled pH, ionic strength, and temperature were determined based on the spectra of surface–enhanced Raman spectroscopy (SERS) and surface–enhanced infrared absorption spectroscopy (SEIRA). Moreover, the theoretical calculations (DFT) was conducted. The results of studies showed that the adsorption of drugs on the negatively charged surfaces of AuNPs was highly effective. At the pH lower than 8.5, the conjugates were positively charged due to the protonation of amine moieties. The desorption of the drug molecules was detected neither at room temperature or at elevated temperatures.

Acknowledgments:

Keywords: surface–enhanced Raman spectroscopy, gold nanoparticles

A-P.7

Title: *Raman Spectroscopy of Anhydrous and Hydrated Calcium Aluminates*

Author: Magdalena Król[1], Włodzimierz Mozgawa[1]

[1]AGH University of Science and Technology

Abstract:
The properties of many materials, including cement based composites, such as strength, permeability to liquid and gas, resistance to frost, and sorption, are largely determined by their structure, which can be analyzed through various methods. However, due to the complexity of cement composite structure, specific research methods are required, with vibrational spectroscopy being a significant approach. Vibrational spectroscopy, including both infrared (IR) and Raman, is especially useful in studying cement chemistry [1, 2].
In this study, four synthetic clinker minerals, reference cementitous composites, and cement slurries kept in an aggressive environment were analyzed using spectroscopic techniques. The results demonstrate the potential of vibrational spectroscopy in exploring cement chemistry. With a high signal-to-noise ratio and distinctive bands for individual phases, this method is effective in qualitatively identifying cement minerals. The mid- and far-infrared range spectra, as well as the Raman spectra, of the primary phases of synthetic clinker provide insights into the types of bonds present in them [3]. The spectral data obtained enable the investigation of the phases present in commercial materials. Raman imaging was also performed to visualize the spatial distribution of cement phases in clinker. Results are in excellent agreement with spectra of reference materials. Additionally, the Raman spectra obtained in this study augment the existing data published in the literature.

References:
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[3] M. Król, A. Koleżyński, P. Florek, P. Jeleń, D. Kozień , W. Mozgawa, J. Mol. Struct. 1255 (2022) 132454.

Keywords: cement minerals, cementitious materials

A-P.8

Title: *Crystallographic Orientation Effects Elucidated by Raman Spectroscopy in Lizardite*

Author: Jeremy Rooney[1], Matthew Tarling[2], Keith Gordon[1], Steven Smith[1], Marianne Negrini[1]
[1]University of Otago
[2]McGill University

Abstract:
Serpentinites are a common rock type that are primarily composed of serpentine minerals ($Mg_3Si_2O_5(OH)_4$). There exist four common forms of serpentine: lizardite, chrysotile, antigorite and polygonal serpentine. Each form has a distinct structure that is a means of accommodating a slight lateral mismatch between the tetrahedral silicate sheet and octahedral magnesium sheet that make up a T-O layer.

Over the course of several Raman measurements of serpentinite thin sections, it was observed that there were significant shifts in the main O-H stretching frequency of lizardite – this was also observed occasionally throughout the literature.[1] While initially attributed to metal cation substitution distorting the lattice, it became apparent from scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) that there was insufficient compositional differences between crystals within most samples to account for such shifts (ca. 14.5 cm$^{-1)}$).

A summary of results from our recent publication are presented whereby it was determined that the O-H peak shifts were dependent on the inclination of lizardite's c-axis relative to the impinging laser.[2] This was determined through Raman measurements on polycrystalline thin sections whose orientation of grains were determined by electron backscatter diffraction (EBSD). Additionally, Raman measurements were taken on a single crystal mounted on a spindle stage that permitted the inclination of the *c-axis* to be altered.

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Keywords: Serpentine, Lizardite, Raman, Anisotropy, Orientation

A-P.9

Title: *Molecular Profiling of Erythrocyte Membrane at the Nano-Scale and at the Single Molecule Level*

Author: <u>Tetiana Stepanenko</u>[1], Katarzyna Bułat[2], Natalia Wilkosz[2], Ewelina Wiercigroch[3], Maciej Roman[4], Tomasz Wróbel[4], Katarzyna Maria Marzec[5]

[1]Jagiellonian University, Doctoral School of Exact and Natural Sciences, National Synchrotron Radiation Centre SOLARIS, Łukasiewicz Research Network
[2]Łukasiewicz Research Network, Krakow Institute of Technology
[3]Jagiellonian Centre of Innovation
[4]Jagiellonian University, National Synchrotron Radiation Centre SOLARIS
[5]AGH University of Science and Technology

Abstract:
Red blood cells (RBCs) are highly specialized cells composed of hemoglobin encased by a delicate membrane. The proper functioning of erythrocytes is critical for the survival of practically all other cells in the organism. The membrane's ability to remain intact and flexible is key to the erythrocytes' functional properties [1]. Pathophysiological changes to the membrane structure and function can occur when RBCs' membranes are exposed to an unfavorable environment. Vibrational spectroscopy techniques have proven useful in determining the biochemical profile of RBCs and the changes that occur in different pathologies [2,3]. This work aims to perform a highly sensitive and label-free biochemical analysis of erythrocyte membranes at the nano-scale using scattering-type scanning near-field optical microscopy (s-SNOM) and at the single-molecule level using Surface Enhanced Raman Spectroscopy (SERS).
SERS is an advanced and non-invasive vibrational spectroscopy technique that has been demonstrated to have tremendous potential as a medical screening tool [4]. In this study, metal nanoparticles (NPs) were employed to achieve a significant enhancement in the SERS signal from erythrocyte membranes, enabling the detection of proteins, phospholipids, and cholesterol. SERS measurements were conducted using both 532 nm (silver NPs) as well as 633 nm (gold NPs) excitation wavelengths. The chemical and mechanical s-SNOM maps of RBCs provided valuable information regarding the secondary structure conformation of proteins within intact cell membranes, the presence of membrane peroxidation, and the distribution of sugar moieties. To perform nanoimaging, an AFM tip was illuminated with a tunable QCL laser. Images were captured for various wavenumbers in the range of 1040 cm^{-1} to 1733 cm^{-1} of selected erythrocytes. Furthermore, an analysis was conducted on the first, second, third, and fourth harmonics of the s-SNOM phase, and the results were presented and compared.

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1. J. E. Smith, "Erythrocyte Membrane: Structure, Function, and Pathophysiology", Vet. Pathol. 2447 1-476 (1987)
2. A.Blat, T.Stepanenko et al., "Spectroscopic Signature of Red Blood Cells in a D-Galactose-Induced Accelerated Aging Model", International Journal of Molecular Sciences, 2021.
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A-P.10

Title: *Influence of SrO and Cs2O on iron-polyphosphate glass network*

Author: <u>Paweł Stoch</u>[1], Malgorzata Ciecińska[1]

[1]AGH University of Science and Technology

Abstract:
Phosphate glasses are materials of versatility with possible applications. They can be used as biomaterials and a part of optoelectronic, and optical devices. High phosphate content glass is characterized by a low water resistance and therefore can be used as materials of controlled solubility in which the parameter can be tailored by chemical composition. Adding iron strongly improves chemical resistance as it can yield superior durability. Thus, iron-phosphate glasses are considered a matrix for the vitrification of nuclear and hazardous waste. During vitrification, waste are melted with glass at relatively high temperatures. In this case, Cs and Sr are problematic due to their volatility. Therefore, the melting temperature and the melting time should be as short as possible. Iron phosphate is characterized by lower melting temperature and viscosity of the melt compared to the traditional "waste glasses", which can lead to temperature and time decrease of the process.

The properties of glasses are strongly dependent on the structural features of the glass network. In the work, Raman and FT-IR spectroscopies are used to describe the structural characteristics of the $30Fe_2O_3$-$70P_2O_5$ glass and influence on the gradual substitution of SrO and Cs_2O up to 50 mol%. In both cases, the glass network is being depolymerized with the increase of the oxide content. The base glass is made up of long phosphate chains that are joined with each other. The estimated average dimensionality of the network is about 2. The depolymerization takes place by shortening the chains and the network dimensionality is also decreasing to 1 for about 30 mol% of SrO and 40 mol% of Cs_2O. The values may be considered as a limit of the oxides concentration in the glass that should ensure its effective immobilization. In the work we also show that careful decomposition of the FT-IR and Raman spectra gives the possibility to estimate the quantities of the specific glass network structural units.

Keywords: phosphate glass, glass network, vibrationalspectroscopy

A-P.11

Title: *The low-lying Rydberg states of Cd_2 molecule – the influence of PMT waveform integration window parameters on the observed spectra*

Author: Tomasz Urbańczyk[1], Joanna Sobczuk[1], Jarosław Koperski[1]

[1]Jagiellonian University, Smoluchowski Institute of Physics

Abstract:

In spectroscopic measurements of van der Waals dimers produced in a supersonic expansion beam which are conducted in our laboratory, the choice of the PMT waveform integration limits has significant impact on the obtained spectra [1]. In particular, the proper choice of the integration window allows detection of signal originating from the excitation to various different electronic energy states. A new, unpublished fragment (transitions to $u' < 40$) of the LIF excitation spectrum recorded using the $^3 1_g(6^3S_1) \leftarrow b^3 0_u^+(5^3P_1)$ transition in Cd_2 molecule [2] will be presented. It will be also shown how the selection of the waveform integration limits can be used to isolate from experimental data of another spectrum associated with transition to the newly observed electronic energy state which is positioned above the $^3 1_g$. Figure 1 c) and d) show two LIF excitation spectra recorded using different a) and b) integration windows, respectively, which were applied at the data analysis phase.

References:

[1] T. Urbańczyk, J. Koperski Mol. Phys. 112 (2014) 2486.
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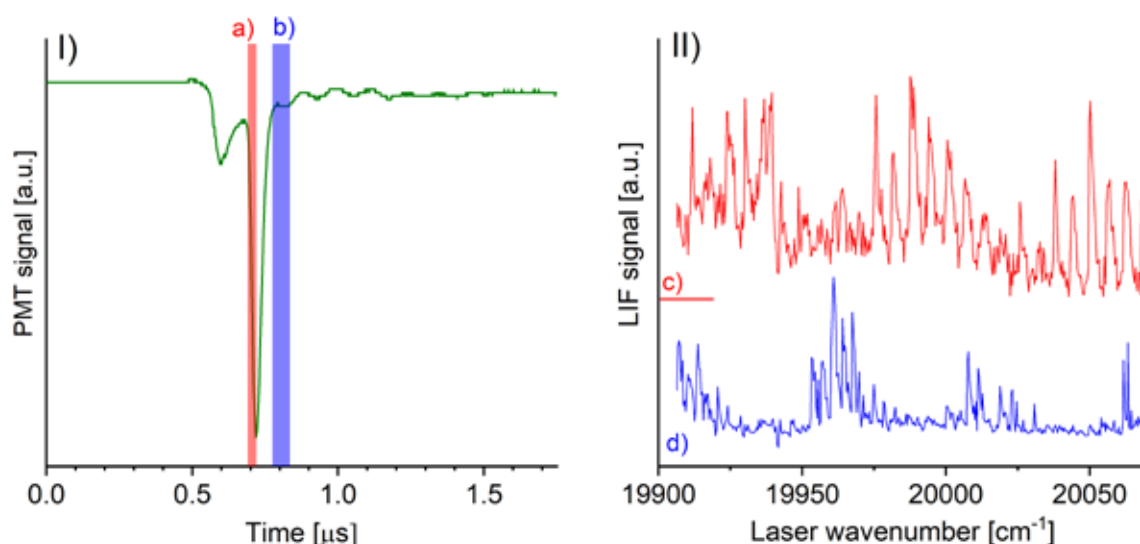
Figure captions:

I) an example of PMT signal with two integration windows indicated: a) red and b) blue rectangles. II) the LIF spectra c) and d) associated with windows shown in a) and b) parts of I), respectively.

Keywords: LIF, Cd_2 dimer, Rydberg states,

A-P.12

Title: *Characterization of new ammonium salts derived from N-allylamines by vibrational spectroscopic techniques*

Author: Anita Wysopal[1], Maria Owińska[1], Magdalena Hasik[1]

[1]AGH University of Science and Technology

Abstract:
Ammonium compounds are cationic substances with positive charge on nitrogen atom. In particular, quaternary ammonium salts, which belong to this group, show many intriguing physical and chemical properties. They are used as phase-transfer catalysts, ionic liquids or electrolytes. They also show germicidal properties and due to this fact are applied as herbicides and antibacterial materials [1]. Quaternary ammonium compounds are also capable of killing viruses, including coronaviruses [2]. Ammonium compounds can be characterized by various methods. Vibrational spectroscopic techniques, such as infrared in the mid infrared range (MIR), Raman or ultraviolet-visible (UV-Vis) are very useful ones. MIR spectra represent molecular vibrations that occur upon absorption of MIR radiation and are commonly used to identify functional groups present in organic compounds [3]. UV-Vis method is based on the absorption of electromagnetic radiation from the ultraviolet and visible regions, which can provide information about the electronic state of a molecule. However, UV-Vis spectra typically have only a few broad absorption maxima that are difficult to link to specific chromophores. As a result, early applications of this method concentrated on identifying and quantifying single-component systems, typically employing colorimetric techniques that are still in use today [4]. In the work, ammonium salts were obtained from N-allylaniline, N-allylcyclo-hexylamine and N-allylpiperidine. They were characterized using IR and UV-VIS spectroscopic techniques as well as by elemental analysis and NMR (1H and 13C) spectroscopy. For comparison, the starting amines were also studied. It is shown that vibrational spectroscopy is a powerful tool that provides complementary information on the investigated compounds to that obtained by other techniques.

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Keywords: Ammonium salts, N-allylaniline, N-allylcyclohexylamine, N-allylpiperidine

Title: *Probing nanoscale polymer anisotropy using plasmonics*

Author: <u>Yuling Xiong</u>[1], Rohit Chikkaraddy[2], Charlie Readman[1], Jeremy Baumberg[1]

[1]University of Cambridge
[2]University of Birmingham

Abstract:
PEDOT has been studied intensively due to its high conductivity, reversible doping, and mechanical flexibility. However, nanoscale characterization of its doping/redox (Fig.1A) remains extremely challenging. Here, we demonstrate an electro-chromic nanoparticle-on-mirror structure (eNPoM)[1], where gold nanoparticles are encapsulated by a thin layer of PEDOT and drop-cast onto a gold mirror. In this geometry (Fig.1B), light is confined to volumes <100 nm^3 forming an optical hotspot with gap size defined by the polymer thickness. The plasmon resonance of this geometry is highly sensitive to the local refractive index and spectrally shifts for changes in polymer charge state (Fig.1C). This redox reaction is spectrally probed in real time by incorporating eNPoM samples in a spectrochemical cell with three electrodes. Combining SERS with in-situ cyclic voltamme-try now resolves the doping mechanism during redox. Surprisingly, different behaviours are observed when varying polymer thickness down to sub-10 nm, both in switching direction of dark-field scattering peaks (Fig.1C) and in SERS spectra. Our results demonstrate how extreme anisotropies and inverted orientations are produced for conducting polymer chains close to the interface, of strong interest for sensing and switching. More generally, it shows a novel viable spectroscopic technique giving detailed information about redox on the few-nm scale, accessing few electron transport at complex interfaces, rele-vant for widespread applications such as wearable devices.

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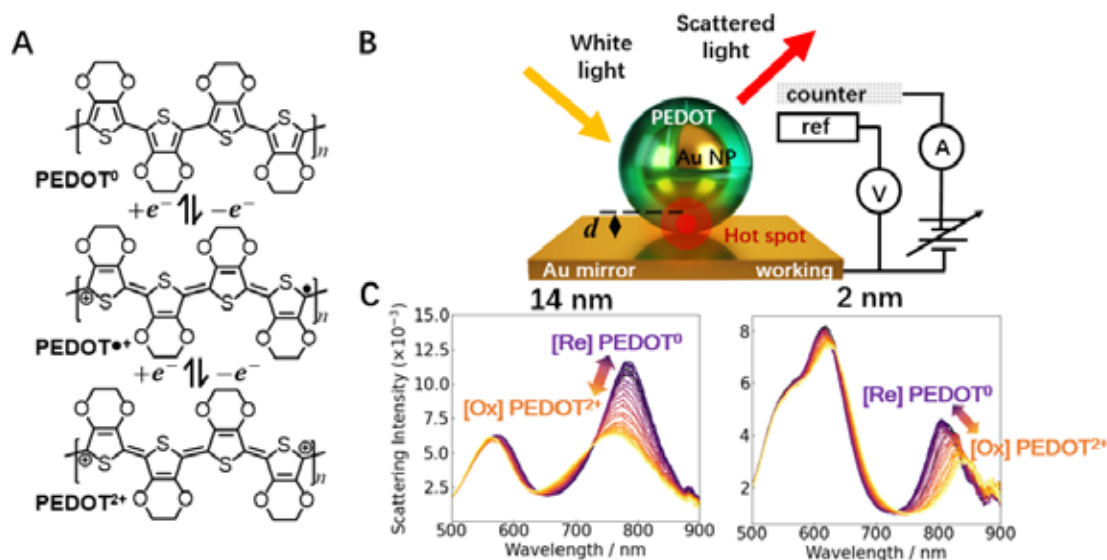
Figure captions:
(A) Redox reactions of PEDOT. (B) eNPoM in spectrochemical cell with Au mirror (working), Pt counter-electrode and Ag/AgCl reference, gap size d. (C) Electrochromic switching of eNPoMs.

Keywords: conjugated polymers, spectro-electrochemistry, anisotropy, plasmonics,SERS

A-P.14

Title: *Organic-inorganic perovskites – chemical mapping and spectroscopy at the nanoscale with AFM-IR*

Author: Ufuk Yilmaz[1], Bernhard Lendl[1], Georg Ramer[1]

[1]TU Wien

Abstract:
Organic-inorganic, hybrid perovskites are considered as highly promising candidates for the next generation of lighting and light harvesting technologies. Perovskite LEDs (PeLEDs) and perovskite photovoltaics (PePV) are cheap, light, flexible, efficient, and easy to process and manufacture. However, current state of the art perovskite devices suffer from rapid degradation through various failure modes (humidity, UV aging, heat). To overcome these issues researchers are adjusting the chemical composition (metal cation, halogen anion, organic cation, matrix components and stabilizers) of perovskite materials. Research into PeLED/PePV has been going on for two decades [1]. Characterization of perovskite devices at the nanoscale is required to understand failure modes and optimize devices. In our current research we study novel perovskite materials by combining scanning probe microscopy with mid-IR spectroscopy (AFM-IR) achieving nanoscale spatial resolution with infrared absorption imaging. Here, a local, short-lived photo-thermal expansion by absorption of infrared light is induced by a pulsed, tunable EC-QCL source. Subsequently, the photo-thermal expansion is measured by the cantilever probe. The oscillation amplitude is directly proportional to the absorption of the sample and thus an absorption spectrum can be generated [2]. Thus, using this nearfield technique allows us to study these materials, even down to a single perovskite crystallite. Using hyperspectral nanoscale chemical imaging we can collect images of the distribution of perovskites and stabilizers and thus provide information required to optimize Pe devices.

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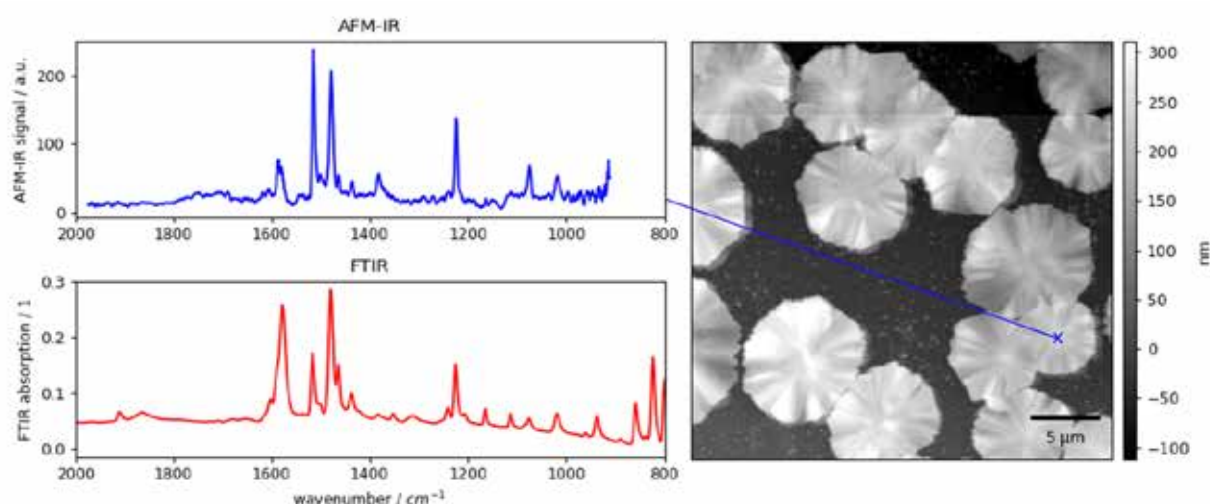
Figure captions:
Fig.1: Bulk, averaged FTIR spectra compared to AFM-IR spectra at nanoscale

Keywords: infrared spectroscopy, nanoscale, nearfield, imaging

A-P.15

Title: *FTIR microscopy and nanoscopy analysis of protein- fiber interaction in asbestos body model assembling.*

Author: <u>Martina Zangari</u>[1], Federica Piccirilli[2], Annalisa Bernareggi[3], Giuliano Zabucchi[3], Lisa Vaccari[2]

[1]Physics Department, Universtity of Trieste
[2]Elettra Sincrotrone
[3]Life Science Department, University of Trieste

Abstract:
Here we present a recent study, performed at SISSI-Bio beamline of Elettra Sincrotrone Trieste [1], exploiting IR microscopy and nanoscopy of protein misfolding caused by asbestos fibers.
In the lungs, the interaction between asbestos fibers with mucopolysaccharides and proteins (e.g. ferritin) [2] determines the formation of asbestos bodies (AB). Understanding this interaction is essential to shed light on the onset of asbestos-related disease and to set up therapeutic approaches for its treatment. The present contribution is based on the development of in vitro AB models (AB-M) to figure out the fiber-protein interaction mechanism and to highlight the protein structural changes following their binding to asbestos. AB-Ms are made with iron storage proteins holo- and apo-ferritin (HoloF, ApoF), and chrysotile fibers (Chry) [3]. AB-Ms are prepared mimicking cytosol and phagosome-like steps in the lung environment, after fiber ingestion, at different incubation times. FTIR microscopy analyses are able to reveal time-depending aggregation of both proteins, but more pronounced for ApoF than HoloF due to the presence of iron in the last one. In addition, infrared s-SNOM spectroscopy and nanoimaging with PsHet detection are fundamental to establish the strong interaction of HoloF-Chry and the role of iron as an active reaction partner. HoloF binds to asbestos fibers intercalating itself into the silicate structure. Instead, ApoF cannot penetrate the structure and binds indeed onto fibers by creating a protein skin around them. These findings suggest that iron may have a key role in the AB-M formation and protein-Chry interaction triggers the misfolding process. In conclusion, these data add new experimental evidence in the AB formation and provide solid bases for better dissecting their pathogenicity.

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Keywords: FTIR microscopy, IR nanoscopy, protein

B-P.1

Title: *Tuning the Electron Transport Chain of a [NiFe] Hydrogenase - Insights from Vibrational Spectroscopy*

Author: <u>Tamanna Manjur Ahamad</u>[1], Jana Schoknecht[1], Ingo Zebger[1], Oliver Lenz[1], Christian Lorent[1]

[1]Institut für Chemie, Technische Universität Berlin

Abstract:
Hydrogenases play a crucial role in the metabolism of many microorganisms being able to reversibly split hydrogen into protons and electrons. Elucidating fundamental processes of microbial energy conversion is essential for promoting biotechnological applications of hydrogen as a clean fuel. Particularly interesting are the oxygen-tolerant [NiFe] hydrogenases capable of H_2/H^+ cycling even under ambient oxygen. To gain a comprehensive understanding of the mechanism, the characterization of the heterobimetallic active site structure, which incorporates biologically unusual CO and CN^- ligands, as well as the electron transport chain, consisting of iron-sulfur clusters is required. Therefore, we apply infrared and more recently, resonance Raman spectroscopy,[1-2] to probe the intra-ligand (CO and CN^-) and metal-ligand vibrations (Ni/Fe-S and Fe-CO/CN) of the active site respectively, as well as the Fe-S modes of the iron sulfur. While standard-type [NiFe] hydrogenases harbor two [4Fe-4S] and one [3Fe-4S] clusters, the regulatory hydrogenase from *Cupriavidus necator* contains a total of three [4Fe-4S] clusters. Using site-directed mutagenesis, vibrational and electron paramagnetic resonance spectroscopy we studied the structural and functional implications of the [4Fe-4S]-to-[3Fe-4S] exchange in the medial position of the electron transport chain.

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Keywords: Resonance Raman, Infrared Spectroscopy, Hydrogenases

B-P.2

Title: *Determination of kinetics of strain-promoted alkyne-azide cycloaddition using infrared spectroscopy*

Author: Matúš Tomčo[1], Anna Kubíčková[1], Svatava Voltrová[1], Lucie Bednárová[1], Petr Beier[1]

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Abstract:

Investigation of biomolecules and their function in native environments is a challenging task. For this purpose, the development of bioorthogonal chemical reactions, i.e. reactions that do not interfere with biological processes, is important. Such reactions should fulfill some criteria such as water compatibility, no side reaction with biomolecules, and fast reaction rate under physiological conditions. (1) One of these reactions is the strain-promoted alkyne-azide cycloaddition (SPAAC). Cyclooctynes and azides are inert to naturally occurring functionalities. They react efficiently forming a stable triazole but with a relatively low reaction rate. (2) To make SPAAC a proper tool for the investigation of biomolecules there is a need to improve its kinetics with a good choice of its reagents. It was already shown that fluorine addition to cyclooctynes increases reaction rates. (3) Our question thus was if the fluorine addition to the azide molecule will have a similar effect.

As organic azides have a strong spectral band in the region of ˜2100 cm^{-1} due to the asymmetric stretching vibration of N=N=N (n_{AS} (N=N=N)), the SPAAC reaction could be followed using infrared spectroscopy, which is also suitable for following fast reactions. Furthermore, this spectral region is spectrally silent for other chemical functionalities, enabling us to monitor the reaction directly in low concentrations and in the aqueous environment. We followed the reactivity of α-fluorinated azides (5 mM) with strained alkyne (bicyclo[6.1.0]non-4yne) (10 mM). The reaction rate for a fluorinated azide was found 0.29 $M^{-1}s^{-1}$ and it was ˜7 times higher compared to its nonfluorinated variant. Thus, fluorinated azides seem to be promising for use in SPAAC chemistry.

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Keywords: infrared spectroscopy, kinetics, alkyne-azide cycloaddion

B-P.3

Title: *Rearrangement of intracellular crystalline guanine as an adaptation for various illumination levels*

Author: Maxim Bokov[1], Matúš Hodoš[1], Radek Bura[1], Jana Pilatova[2], Peter Mojzeš[1]

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[2]Department of Experimental Plant Biology, Faculty of Science, Charles University

Abstract:
The utilization of biogenic guanine crystals by various animals for light manipulation has been well-documented [1]. These crystals possess a high refraction index and plate-like arrangement, making them effective diffuse scatterers, broad- and narrowband reflectors, tunable photonic crystals, and image-forming mirrors [1]. Recently, crystalline inclusions containing guanine or related purines have been found in photosynthetic microalgae [2], including the dinoflagellate *Amphidinium carterae*, where they have been identified as a long-term, high capacity store of nitrogen [2]. However, the role of guanine crystals in light manipulation has also been speculated for photosynthetic eukaryotes [3]. Through the use of confocal Raman microscopy, we have visualized the location of guanine crystals within intact cells of *A. carterae*, demonstrating that their location is dependent on the intensity of illumination. Under conditions of continuous inorganic nitrogen supply and low light intensity (ca 5 μmol(photons) m^{-2} s^{-1}), the cells adapt by increasing the amount of plastids and synthesizing guanine crystals situated behind the plastids, closer to the center of the cell. Acting as photonic mirrors or diffusers, these guanine crystals redirect untrapped photons back to the plastids, potentially increasing photosynthetic efficiency. Conversely, under conditions of high light intensity (ca 200 μmol(photons) m^{-2} s^{-1}), extensive layers of guanine crystals are organized between the cell wall and plastids, shielding plastids from excessive illumination (Figure 1.).

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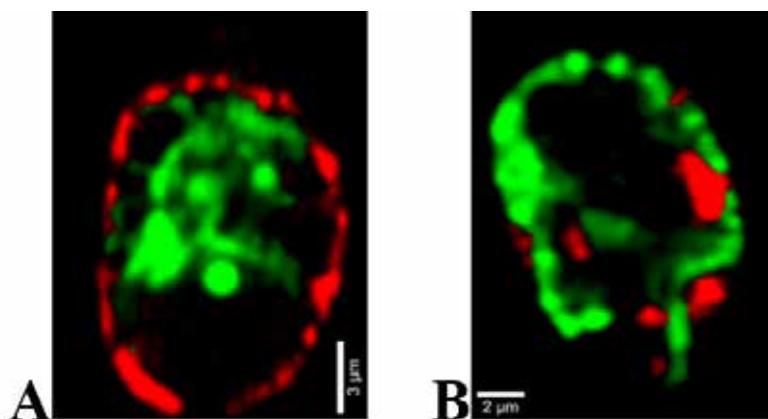
Figure 1. Raman chemical maps of Amphidinium carterae (scale at 3 micrometers). Panel A: guanine crystals (red) are allocated at the periphery and close to the cell membrane and plastids (green) come after. Panel B: Plastids (green) allocated at the periphery and closer to the cell membrane than the guanine crystals (red).

Figure captions:
Figure 1. Raman chemical maps of Amphidinium carterae (scale at 3 micrometers)

Keywords: guanine, algae, amphidinium, raman, crystals

B-P.4

Title: *Spectroscopic characterization of the coproporphyrin ferrochelatase from Corynebacterium diphtheriae*

Author: Andrea Dali[1], Federico Sebastiani[1], Thomas Gabler[2], Giada Zoppi[1], Christian Obinger[2], Paul G. Furtmüller[2], Maurizio Becucci[1], Stefan Hofbauer[3], Giulietta Smulevich[1]

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[3]Department of Chemistry, Institute of Biochemistry, University of Natural Resources and Life Sciences

Abstract:
The coproporphyrin-dependent heme biosynthesis pathway utilized by monoderm Gram-positive bacteria to produce heme b has been discovered in 2015 [1]. In the penultimate step, the coproporphyrin ferrochelatase (CpfC) catalyzes the insertion of ferrous iron into the coproporphyrin III (cpIII), producing iron coproporphyrin III (coproheme). In the final step, the coproheme decarboxylase generates heme b by a two-step decarboxylation of the propionate groups at positions 2 and 4 of coproheme, forming vinyl groups.

Our group has already investigated the CpfC of the firmicute *Listeria monocytogenes* (*Lm*) [2,3]. Here, we characterized the wild-type (WT) CpfC from actinobacterial *Corynebacterium diphtheriae* (*Cd*CpfC) in its apo form, and complexed with the substrate (cpIII) and the product (coproheme) using UV-vis electronic absorption and resonance Raman (RR) spectroscopies. Unlike the *Lm ferrochelatase*, X-ray diffraction studies of the apo *Cd*CpfC reveal that this bacterial *ferrochelatase* contains a [2Fe-2S] cluster. However, the function of this cluster in this protein is not known, as it does not seem to be involved in the iron insertion process. RR spectroscopy allowed us to obtain information about the structure of the cluster as its stretching modes are sensitive to the type, configuration, symmetry, and nature of the ligands [4].

The spectroscopic characterization of the WT *Cd*CpfC complexed with the substrate (cpIII) and the product (coproheme) indicates that the porphyrin ring, inside the active site, is stabilized by several hydrogen-bond interactions established between polar residues and the propionate groups of the porphyrin ring, as previously observed for the WT and selected variants of CpfC from firmicute *Lm* [2,3].

Moreover, the RR spectra of the CO adducts of the WT and selected variants of *Cd*CpfC complexed with coproheme allowed us to monitor the interactions of the distal polar residues with the iron-bound ligand.

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Keywords: Bacterial ferrochelatases, resonance Raman spectroscopy,

B-P.5

Title: *Protein structure investigation via ROA-CPL spectroscopy and Eu(III) probe*

Author: Agnieszka Domagała[1], Grzegorz Zając[2], Małgorzata Barańska[3]

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Abstract:
It has been proven many times that different protein alterations might be responsible for common civilization diseases. Structural changes such as post-translational modifications, polymerization, and aggregation influence pathological states. For example, phosphorylation is connected with cancers, and acetylation, methylation, and glycosylation with cardiovascular and neurodegenerative diseases; dimerization of glutathione (GSH) due to reactive oxygen species (ROS) leads to glutathione disulfide formation (GSSG) and indicating the oxidative stress conditions; aggregated fibers of tau protein and amyloid-β accumulating in brain tissue are associated with Alzheimer's disease (AD).[1,2] Thus, studying such modified proteins is essential for a better understanding of pathogenic processes and can improve their diagnostic and treatment strategies.
In our study, we applied ROA-CPL spectroscopy using europium(III) chloride probe for the structural investigation of selected peptides. It is a promising approach in chiroptical spectroscopy that allows for the simultaneous measurement of circularly polarized luminescence (CPL) and Raman optical activity (ROA), enabling the observation of the CPL signal on the ROA spectrum. SCP-ROA spectrometer equipped in the 532 nm laser line is used here to detect both the Raman scattered and emitted circularly polarized light. Eu(III) compound plays the role of a specific structural information probe. Lanthanide-based probes are famous for their unique luminescent properties and high sensitivity to chemical surroundings. The chiral environment, such as peptides or proteins, can transfer the chirality to the probe, which induces the achiral compound's CPL signals. ROA-CPL spectroscopy using the Eu(III) probe already has some successful applications, like structure and intermolecular interactions investigation of proteins or nucleic acids, and recognition of saccharides with high structural similarity.[3]

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Keywords: ROA, CPL, europium(III) probes, peptides

Title: *Raman Optical Activity is a sensitive tool to detect changes in the structure of biomolecules and supramolecules*

Author: Monika Halat[1], Magdalena Klimek-Chodacka[1], Ewa Oclon[1], Josef Kapitán[2], Rafal Baranski[1], Grzegorz Zajac[3], Valery Andrushchenko[4], Malgorzata Baranska[3]

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Abstract:
Raman Optical Activity (ROA) is a sensitive tool dedicated to studying the 3D structure of important chiral biomolecules – such as proteins in water environment[1]. The possibility of measuring aqueous solutions allows monitoring of structural changes of proteins in their natural surroundings, which is a great advantage of ROA spectroscopy over more popular methods like X-ray crystallography and it also directly reflects fast conformational changes unlike NMR spectroscopy. ROA registers bisignate spectra containing detailed information about protein geometry like secondary and tertiary structure, backbone hydration, side chains conformation, as well as structural features present in unfolded states e.g., the extended PPII helix. Here, we present the ROA study of the Cas9 protein and its ribonucleoprotein (RNP) complex belonging to the CRISPR/Cas system known as "molecular scissors" intended for a precise genome editing, recently rewarded by the Nobel Prize in Chemistry in 2020[2]. Thus, we show that ROA technique can be used to verify the Cas9 ability to bind especially designed gRNA molecule, as well as to identify a successfully formed RNP complex in a non-destructive manner.

Nowadays, ROA spectroscopy is also increasingly used to study the structure of more complex systems like supramolecular aggregates built from biomolecules connected by noncovalent bonds[3]. As an example, we show the ROA studies supported by electronic circular dichroism (ECD) and theoretical calculations on the chirality induction process observed through the aggregation of achiral carotenoid such as canthaxanthin (CAX). Consequently, CAX molecules in water solution of glycosaminoglycans (heparin and hyaluronic acid) built optically active structures exposing chirality at supramolecular level, which has been confirmed by the nonzero signal of ECD and ROA. In addition, collected experimental data were rationalized by the theoretical calculations of several supramolecular models (CAX dimers).

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Keywords: ROA, ECD, CRISPR/Cas, supramolecular aggregates

Title: *Systematic Tuning of Electronic Ground and Excited States in Donor-Acceptor Dyes; Steps Towards Designer Compounds for Modern Technologies.*

Author: Samuel Harris[1]

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Abstract:
Donor-acceptor dyes are an integral part of modern chemical materials exhibiting unique photophysical properties, such as the electronic charge transfer (CT) transition. This displaces electron density throughout the structure of a compound. A behaviour which can elicit desirable properties that are used in many modern technologies including, solar cells,[1] OLEDs, and molecular sensors.[2] One of the most important aspects for these dyes in each application is the energy at which the electronic CT occurs. Hence, predictable tuning of this feature towards desirable wavelengths would be useful for the design and fabrication of future compounds used in these technologies. Often research will compare the discrepancies between different donor or acceptor moieties in efforts towards CT tuning. However, a much more subtle and controlled approach is to alter the substitutions or linkages within these units.[3] Due to the localisation of the HOMO and LUMO frontier molecular orbitals to the donor and acceptor units respectively, adjustments to these groups can vary the energy of these two orbitals and hence the CT transition. Our investigation has looked into the subtle tuning properties of six donor-pi-acceptor dyes containing a carbazole donor and dithienothiophene bridging unit, bonded by meta or para linkages, and three variations of an indane-type acceptor. These dyes indicate the controlled CT tuning which can be achieved by making these small alterations. Additionally, the dyes also exhibited other interesting excited state characteristics, such as dual emission, high quantum yield and molar absorptivity, lending to potential uses in the afore mentioned technologies.

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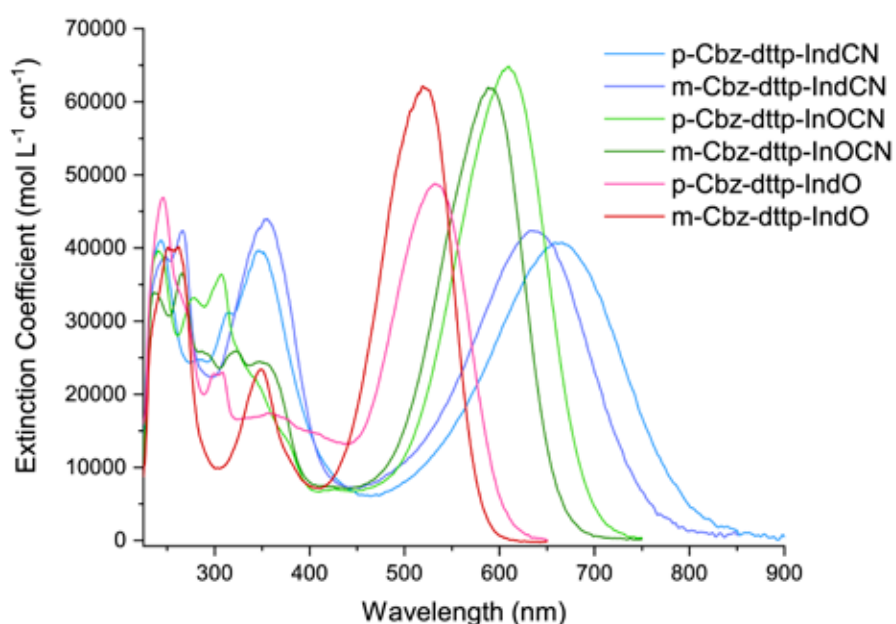
Figure captions:
Electronic absorption spectra for a series of six systematically donor acceptor dyes tuned in DCM.

Keywords: Spectroscopy, DFT, Donor-acceptor dyes

B-P.8

Title: *Secondary Structure Components and Nanoimaging of α-Synuclein Aggregates*

Author: Antonia Intze[1], Raffaella Polito[1], Maria Eleonora Temperini[1], Jakob Rupert[2], Elsa Zacco[3], Gian Gaetano Tartaglia[2], Michele Ortolani[1], Valeria Giliberti[4]

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Abstract:

α-Synuclein (αS) is of wide interest because its aggregation is a hallmark of many synucleinopathies, including Parkinson's disease. RNA affects the protein aggregation in neurological disorders forming protein-RNA assemblies[1] and thus disrupting RNA processing. Here, we are using the FTIR microspectroscopy (micro-FTIR) technique to investigate the in vitro aggregation of αS (Fig. 1a). Specifically, we track the changes of the Amide-I infrared absorption band at wavelengths around 6 µm, which is related to C=O stretching mode of peptide bonds and is sensitive to the different protein secondary structure components (α-helix, parallel and antiparallel β-sheet, turn, and random coil) (see inset of Fig. 1b). As the αS in vitro aggregation proceeds, we observe an increase of the parallel β-sheet component (a spectral marker of amyloid fibril formation).[2,3] The simultaneous atomic force microscopy (AFM) of aggregates at sampling times from 96 hours onwards shows the formation of micrometer-long amyloid fibrils. In parallel, we have also characterized the protein aggregation process with circular dichroism spectroscopy and AFM-assisted infrared spectroscopy (AFM-IR) (data not shown) as a control for micro-FTIR, finding the results consistent with both techniques. The advantage of AFM-IR technique is the simultaneous observation of the morphology and quantification of the secondary structure of individual nanoscale protein aggregates, down to 50 nm diameter and 5 nm thickness. Finally, we have investigated the co-aggregation of αS with RNA and its effect on the in vitro protein aggregation (Fig. 1b), finding statistically significant differences in the fibril morphology and packing density (Fig. 1c-d), in the intensity of the parallel β-sheet component, and in the aggregation timescales (Fig. 1a-b) if compared to the RNA-free samples.

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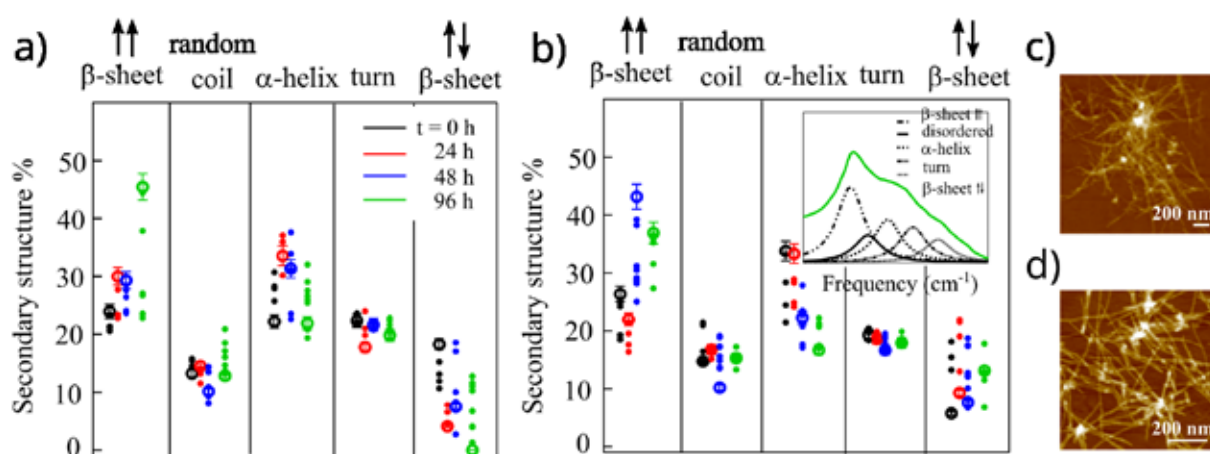
Figure captions:
Percentage of the Amide-I components of αS (a) and αS+RNA (b) from micro-FTIR data. Inset in (b) shows the deconvolution of Amide-I band. AFM topography maps of αS (c) and αS+RNA (d) fibrils at 96 h.

Keywords: α-synuclein, protein aggregation, infrared spectroscopy

B-P.9

Title: *Formation and Behavior of Guanosine-5´-Monophosphate assemblies at low pH: temperature and cation effects*

Author: Štěpán Jílek[1], Josef Kapitán[2], Ivan Barvík[1], Václav Profant[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University
[2]Department of Optics, Faculty of Science, Palacky University Olomouc

Abstract:
Self-assembly of mononucleotides and their oligo- and polymeric forms (nucleic acids) into higher-ordered structures is ubiquitous in living systems. However, Guanosine 5'-monophosphate (5´-GMP) and its derivatives are special among other nucleotides, because at higher concentrations they self-associate into unique structures – first guanine quartets1, which then stack and form G-quadruplexes (G4). G4 are stabilized by a combination of several contributions: Hoogsteen base pairing, base stacking, cation coordination, hydrophobic interaction, and H-bonds between phosphates and hydroxyl groups of neighboring nucleotides. 5´-GMP self-association is pronounced at low pH (~5) resulting in a highly viscous gel. Under these conditions, the planarity of the stacked G-quartets is broken, and G4 forms a continuous helix2 (Fig 1, left).
We studied the process of 5´-GMP agglomeration at low pH (~5) using Raman spectroscopy (Fig 1, right) and its chirally sensitive variant Raman optical activity (ROA)3 which benefits from the inherent nucleotide chirality and in this case also from the helical ordering of G-quartets. Data were analyzed by multivariate factor analysis. The usage of Raman spectroscopy enabled simple aqueous solution measurements (unlike X-ray diffraction) and studies of highly concentrated samples (unlike absorption-based techniques). At the same time, we can easily study the influence of the different external conditions (e.g., temperature and ions) which affects the secondary structure, stability, and dynamics of these assemblies. Other vibrational techniques as IR and VCD were applied for complementarity. The melting and annealing experiments analysis revealed the reversible formation of the G-quadruplex arrangement and identified the pre-melting structural transition in the presence of potassium ions.
The agglomerates were also studied in the solid phase using Raman microspectrometry and X-ray scattering to characterize their higher supramolecular arrangement.

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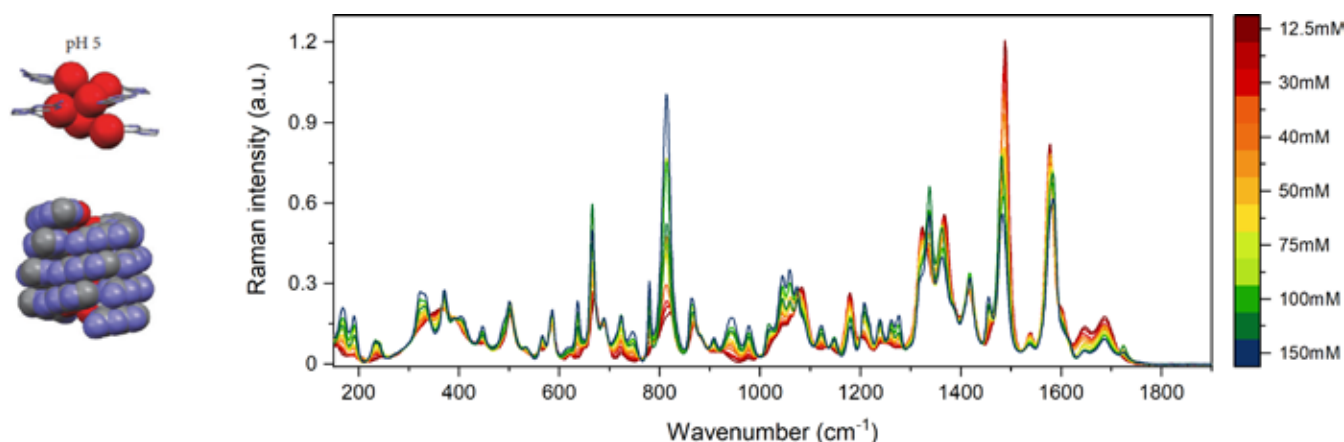
Figure captions:
Structure of mononucleotide G4 adapted from ref. 2 (left). The concentration dependency of 5´-GMP with K+ ions at pH~5 indicates the formation of mononucleotide G4 (right).

Keywords: G-quadruplexes, GMP, hydrogel, Raman, ROA

B-P.10

Title: *Structure of 15N-glycosylanilines and their interaction with galectins*

Author: <u>Jakub Kaminský</u>[1], Nina Habanová[1], Jakub Zýka[1], Radek Pohl[1]

[1]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences
This work was supported by the Czech Science Foundation (project 22-17586S).

Abstract:
Galectins are a subfamily of carbohydrate-binding proteins that specifically bind galactose-containing saccharides, such as lactose. Different types of galectins have been described in humans, differing in tissue expression, cellular localization, specificity for saccharide ligands, etc., and are responsible for a variety of functions, including immune response and pathogen virulence [1]. To fully understand the recognition process of individual sugars by galectin, it is necessary to describe in detail their conformational behavior both in the free state and in the complex with galectin.

This work deals with the structural study of ^{15}N-glycosylanilines derived from lactose, or their analogues. The conformational behavior of free ^{15}N-labelled ligands was described by a combination of Raman optical activity, NMR spectroscopy, and advanced theoretical calculations. This approach has been shown as very efficient in structural studies of saccharides [2, 3]. The labelling of the ligands offers an extra NMR observable J-coupling constants (e.g., J_{NH} and J_{NC}) that are sensitive to conformations [4]. The analysis of the structure and conformation of the ligands bound to Galectin-3 was based on Saturation Transfer Difference NMR (STD NMR). It was found that the conformational preferences of e.g. *N*-lactosylacetanilide in the free and the bound state differ. The experiment was complemented by computer simulation of the interaction of ligands with galectin.

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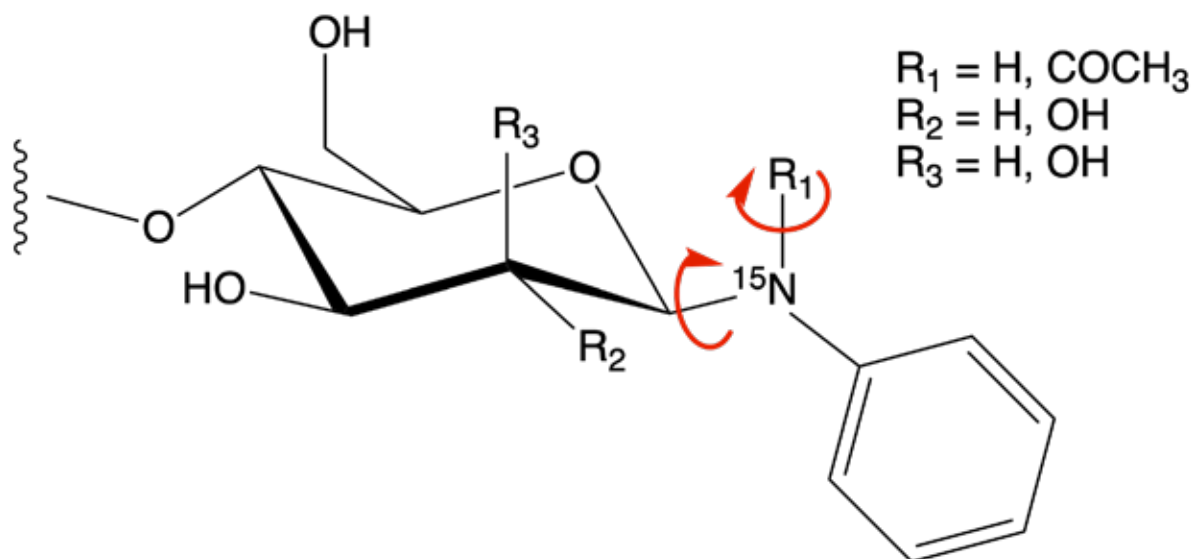
Figure captions:
The studied glycosylanilines and the most important structural parameters considered

Keywords: ROA, NMR, glycosylaniline, conformation

B-P.11

Title: *Characterizing the large subunit of a membrane-bound [NiFe] hydrogenase by combined IR spectroscopic and computational studies*

Author: Chara Karafoulidi-Retsou[1], Jovan Dragelj[1], Sagie Katz[1], Maria Andrea Mroginski[1], Oliver Lenz[1], Giorgio Caserta[1], Ingo Zebger[1]

[1]Technische Universität Berlin

Abstract:
Hydrogenases are ancient metalloenzymes responsible for the cleavage of molecular hydrogen into H+ and e-, exhibiting great potential to support a sustainable H_2-based economy in the future.[1] Here, the large subunit (HoxG) of the membrane-bound hydrogenase (MBH), which harbors the enzyme's bimetal NiFe active site, is studied. CN and CO^- ligate to the Fe ion of the bi-metallic center, thus serving as IR markers for the inherent redox states. While the heterodimeric MBH (HoxGK) is well characterized, studies focusing solely on the large subunit are so far sparse.[2,3] Herein, combined IR spectroscopic and computational studies are used to characterize HoxG both immobilized and in solution. Our data demonstrate that in its as-isolated form, HoxG comprises two Nir-S resting states, exhibiting obvious structural differences compared to the ones detected in the large subunit of a regulatory hydrogenase[4] of the same organism. Further, following the approach of Heidary et al. on HoxGK,[5] HoxG is studied by surface-enhanced IR absorption spectroscopy during and after its immobilization on Au electrodes. The electrodes are coated with biocompatible SAMs of differently charged headgroups. Control of the SAM's protonation degree is assessed as a function of the buffer's pH. Thus, different orientations of the protein are achieved, whereby a minimization of the active site-electrode distance facilitates its electrochemical control. Complementary molecular dynamics simulations provide a deeper insight into the protein orientation, its conformational changes and the immobilized protein's stability. Our studies provide a new understanding of the Nir-S species and their structural differences, depending on the function of the hydrogenase in the cell. Finally, they initiate future applications such as the isolation, immobilization and electrochemical control of enzymes, where the Ni of the active site is replaced by other metals, potentially exhibiting new catalytic activities.

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5. Heidary, N. et al. Orientation-controlled electrocatalytic efficiency of an adsorbed oxygen-tolerant hydrogenase. PLoS One 10, (2015).

Figure captions:
Immobilized large subunit, HoxG of CnMBH on top of SAM functionalized Au-electrode

Keywords: Spectroscopy, Sprectroelectrochemistry, SAMs, Hydrogenases, Immobilization

B-P.12
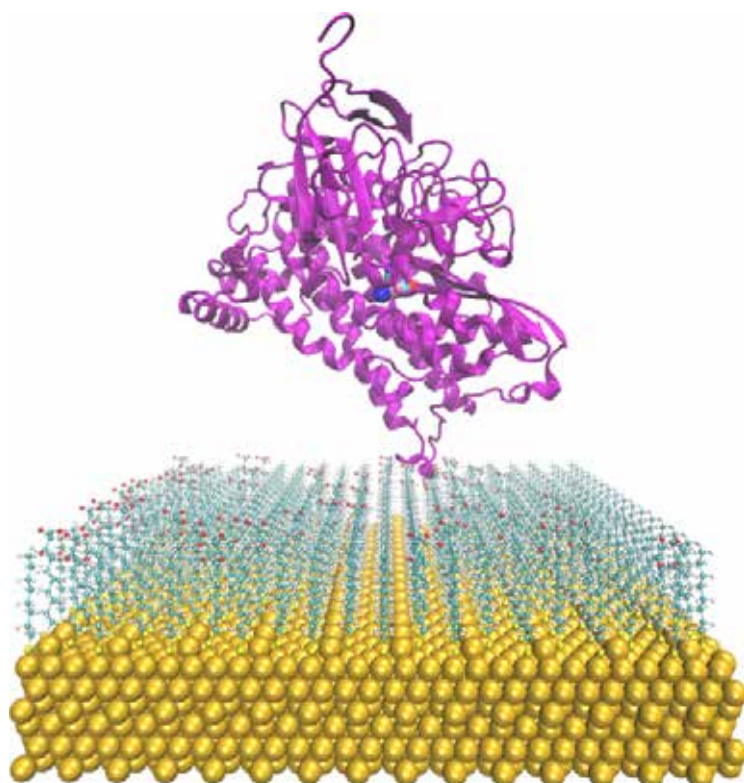
Title: *Molecular structure of spider silk in various conditions of spinning*

Author: Norihisa Katayama[1], Takumi Maeda[1]

[1]Graduate school of Science, Nagoya City University

Abstract:
Spider silk is known as fiber with high strength, stiffness and toughness. Spinning dope of spider is present as liquid crystalline at the spider's body. Therefore, the spider can spin silk with less metabolic cost [1]. Among several different kinds of spider silk, the dragline constituted by fibroins from major ampullate gland is considered as the most functional silk. In the study of structure of spider silk, infrared and Raman spectroscopy have been used in respect of sampled dragline with constant spinning speed [2].
In this study, we obtained samples with controlled spinning speed of dragline of Nephila clavata at range of 0.5–100 mm/s. The study on molecular structure has been applied by microscopic polarized FT-IR spectroscopy.
IR spectra of draglines with spinning speed of 0.5 and 100mm/s are shown in Fig. 1. In the obtained infrared spectra, the shoulder band around 1690 cm-1, which is assigned to antiparallel β-sheet of Amide I band, intensified with increasing of spinning speed in case of 0° polarized measurement. On 90° polarized spectra, the feature at 1630 cm-1 assigned β-sheet appeared. These results indicate that β-sheet structure grew with increasing spinning speed. Consequently, it is revealed that the relationship between the variation of spinning speed of spider dragline and molecular orientation of protein by polarized FT-IR spectroscopy.

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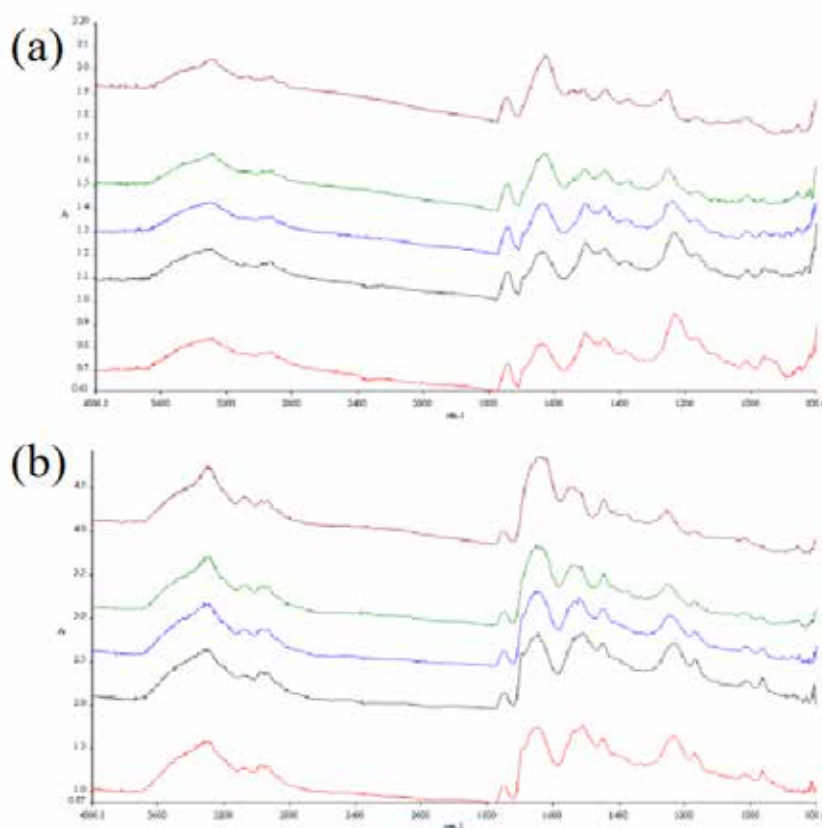
Figure captions:
Fig.1. IR spectra of draglines with spinning speed of (a) 0.5 mm/s and (b) 100mm/s Each line shows the spectrum with polarized angle of 0°, 30°, 45°, 60° and 90° from the bottom to top, respectively.

Keywords: SpiderSilk, β-sheet, Amide, polarizedIR

B-P.13

Title: *Imaging 3D macromolecular orientation by IR microscopy in crystalline-amorphous anisotropic biopolymer film*

Author: Karolina Kosowska[1], Paulina Koziol[1], Tomasz Wrobel[1]

[1]Solaris National Synchrotron Radiation Centre, Jagiellonian University

Abstract:
The structural phase transition taking place in a material can be partially followed by infrared spectroscopy because vibrational modes are sensitive to intermolecular interaction and chain conformation. Our team proved that concurrent analysis of two bands from infrared spectra of perpendicular transition moment orientations proposed by Lee [1] can be applied in practice [2]. In our work, we retrieved the orientation ordering of chains in polycaprolactone spherulite in three dimensions. Xu et al. published similar results for a thin film also made from polycaprolactone [3]. Applying a shear deformation increased the degree of macromolecule organization in the thin film. The method based on the use of four angles of linearly polarized IR light (4P-3D) seems to be very useful in materials engineering, especially for highly oriented materials. Biological materials like tissue exhibit much more complex morphology compared to artificial materials [4] and the aplication of the 4P-3D method requires further development. A more complicated polymer system with anisotropic morphology was prepared by stopping the crystallization of poly(lactic acid). Collected IR data were used to determine the orientation of the transition dipoles. The 4P-3D method allows the exploitation of interesting details of the material's architecture. Figure 1 shows the orientation of polymer chains in the border between spherulites, the edge, and in the amorphous phase. A comparison of two pairs of vectors (Fig. 1 c,d) used for calculation in the same region reveals the difference in sensitivity to changes in macromolecule organization.
Our study opens the way to applications for spatially resolved orientation studies of a wide range of polymeric and biological materials. Obtaining an entirely new kind of information can be used for understanding the relationship between structure and properties.

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Figure captions:
Optical image of PLA film (a). 4P-3D results: borders between spherulites (pair 1088-1041 cm-1, b), spherulites and amorphous phase (pair 1088-1041 cm-1, c), the same region for 1088-1207 cm-1 (d).

Keywords: macromolecules, spherulites, organization, polarization, polymers

B-P.14
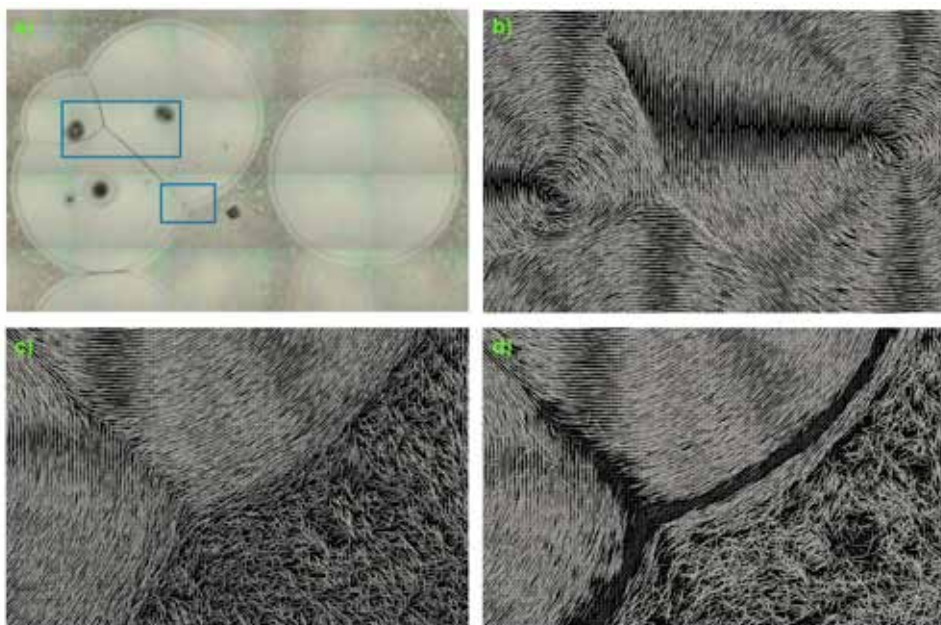
Title: *Factor analysis of time spectral series of SERS active Ag nanoparticles systems*

Author: Jan Kožíšek[1], Ivana Šloufová[1], Miroslav Šlouf[2]

[1]Charles University, Faculty of Science, Department of Physical and Macromolecular Chemistry
[2]Institute of Macromolecular Chemistry, Czech Academy of Sciences

Abstract:
The surface-enhanced Raman scattering (SERS) is an effect of Raman signal enhancement of molecules which are localized on the surface or in the surroundings of the plasmonic nanoparticles or nanostructures. The enhancement is strongly dependent on the selected excitation wavelength, which optimum can be derived from the maximum of surface plasmon extinction (SPE) spectra of studied system[1]. The main aim of this study is demonstration how the information about SPE changes can be obtained by multivariate statistical analysis (factor analysis; FA) from the SERS spectral sets[2].
Shorter excitation wavelengths (445 nm) were used for non-aggregated Ag nanoparticles system. SPE changes were monitored by evolution of water stretching vibration band together with the progress of vibrations in fingerprint region. The FA of water stretching band region provided information about arrangement and disarrangement of water molecules at the Ag nanoparticles surface as well. Longer excitation wavelengths (785 nm) were used for aggregated Ag nanoparticles systems. The FA of these systems showed contribution of the 2nd electromagnetic enhancement to the overall SERS signal.
The aggregating SERS active systems were prepared by adding the aqueous $[Fe(tpyCl)_2SO_4]$ (Fe-tpyCl) solution to Ag hydrosol with or without of ethanol (EtOH) as an internal standard [3]. FA of both sets provided one more subspectrum than was expected. In both cases these subspectra showed evolution of SPE between 785 and 900 nm which corresponds to the range of measurement (0-1600 cm^{-1}) for the excitation wavelength 785 nm (Fig.1). The accord between the time course of orthonormal coefficients and $SPE_{785-900}$ values indicates the contribution of the second electromagnetic enhancement of the SERS signal in the aggregating systems with Fe-tpyCl.

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Figure captions:
System Ag-FetpyCl-2-EtOH – A) 3rd FA subspectrum of low-wavenumber region, B) corresponding Vi3 coefficients, C) time evolution of SPE spectra, D) plot of SPE at 785 and 900 nm, with their difference.

Keywords: Ag nanoparticles, Surface-enhanced Raman scattering,

B-P.15
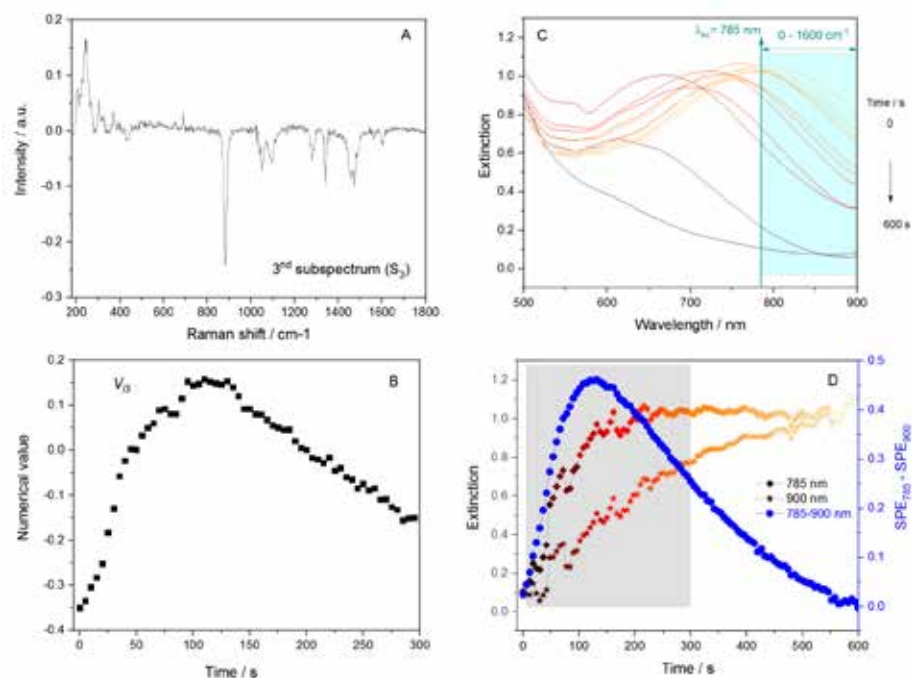
Title: *The revelation of interactions in model myelin with FTIR spectroscopy*

Author: <u>Petra Maleš</u>[1], Danijela Bakarić[1]

[1]Ruđer Bošković Institute/ Croatia

Abstract:
The myelin sheath in the central nervous system is a compact multilamellar membrane system that enables rapid transmission of nerve impulses. [1] Unlike the cellular membranes, myelin has a very high lipid-to-protein mass ratio (roughly 80 %-to-20 %), and high proportions of glycosphingolipids and cholesterol.[2] Along with lipids, which are dominant in myelin composition, the complex structure is maintained by myelin basic protein (MBP), which is presumably crucial for myelin disruption in multiple sclerosis. [1] Many biophysical studies showed that MBP interacts with lipid membranes employing electrostatic and hydrophobic interactions to assemble the proper multilamellar structure of myelin sheath. To gain an insight into the interactions of MBP with model myelin, multilamellar liposomes (MLVs) were constituted from phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM) and cholesterol at molar ratios 21: 16: 3: 5: 33 and 15: 17: 7: 2: 38 in order to mimic myelin at physiological and pathological conditions, respectively. [2] MLVs were studied using calorimetric and different spectroscopic techniques [3], emphasizing FTIR spectroscopy that provided crucial information on the engagement of MBP cationic amino acids in association with model myelin.

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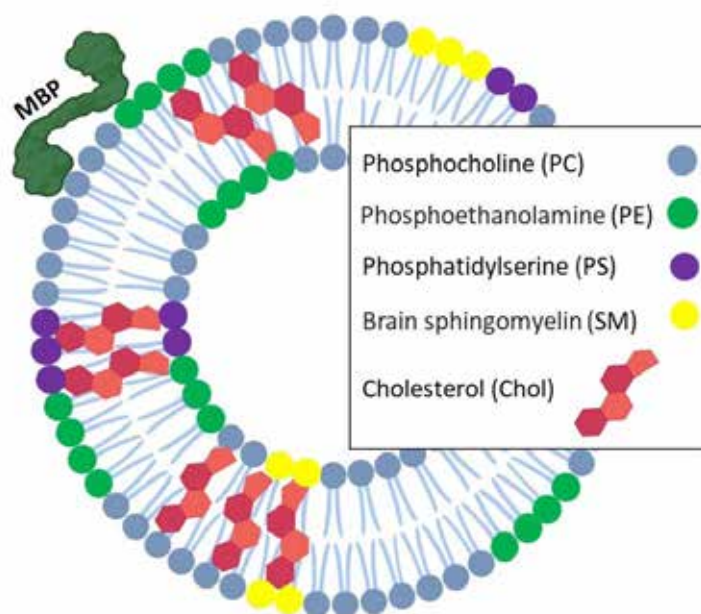
Figure captions:
Adsorption of myelin basic protein on model myelin at physiological conditions.

Keywords: Myelin, FTIR, Lipids, MBP

B-P.16

Title: *Spectral features of Interfacial Water in Imidazolium-based Ionic Liquids/water mixtures: UV Resonance Raman Approach*

Author: <u>Fatima Matroodi</u>[1], Cettina Bottari[1], Barbara Rossi[1], Marco Paolantoni[2], Laszlo Almasy[3], Andrea Mele[4]

[1]Elettra
[2]University of Perugia
[3]Neutronspektroszkópiai Laboratórium
[4]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano

Abstract:
The formation of water-rich nano-domains and heterogeneities in aqueous solutions of Ionic Liquids (IL) at specific hydration regimes has a dramatic impact on the physicochemical properties of these IL. In this work, a set of a mixture of H_2O and D_2O with prototypical imidazolium-based ionic liquids [MIM][HSO$_4$], [BMIM][HSO$_4$] and [BMIM][MeSO$_4$] are studied, in a high diluted regime, by UV resonance Raman spectroscopy to provide insights into the degree of H-bond association of water molecules confined inside the IL nanostructures. Various kinds of interactions of water-water/water-IL and IL-IL interactions lead to significant spectral alterations in the Raman signals associated with the C-H groups of the imidazole ring of IL (3000-3200 cm^{-1}) and in the O-D or O-H stretching profiles of water. The analysis of these bands carried out also by exploiting differential methods, shows two distinct behaviors for the three ILs as a function of the molar fraction of water in IL. The higher frequency C-H doublet (~3170 cm^{-1}) shows roughly the same trend in all samples indicated by blue shift as water increases. But the lower frequency one (3120 cm^{-1}) has a distinct trend and in general, it lies at higher wavenumbers in the case of [MIM][HSO$_4$]. Overall these results provide insights into the properties and the H-bond arrangement of the interfacial water molecules confined in the IL/water mixtures.

References:
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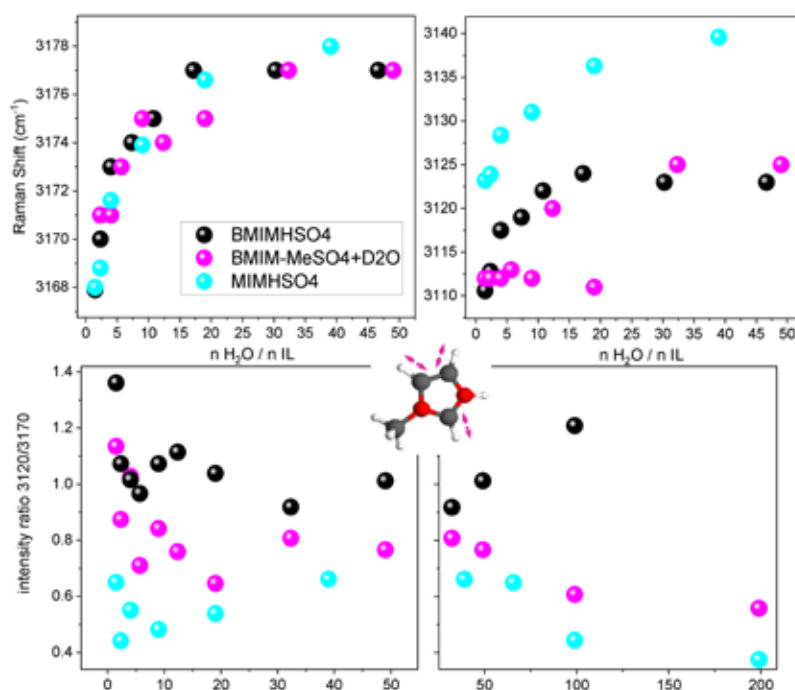
Figure captions:
Wavenumber position and relative intensity of the Raman signal attributed to the C-H doublet of the imidazole ring (3000-3200 cm-1) as a function of water numbers per IL molecule.

Keywords: Ionic Liquid, imidazolium, UVRR, water

B-P.17

Title: *How to properly register Raman optical activity spectra of chiral and light-absorbing biomolecules?*

Author: Katarzyna Pajor[1], Ewa Machalska[2], Josef Kapitán[3], Andrzej Kudelski[4], Malgorzata Baranska[1,2], Grzegorz Zając[2]

[1]Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
[2]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348 Krakow, Poland
[3]Department of Optics, Palacký University Olomouc, 17. listopadu 12, Olomouc, Czech Republic
[4]Faculty of Chemistry, University of Warsaw, Ludwika Pasteura 1, 02-093 Warsaw, Poland

Abstract:
Nowadays, chiroptical methods measuring the different absorption or scattering of left and right circularly polarized (CP) light attract attention because they provide rich structural information about molecules. These techniques include electronic circular dichroism (ECD) and Raman optical activity (ROA) spectroscopy.
A disadvantage of ROA is the weakness of signal. The ROA signals are typically weaker than the un-polarized Raman signals by 3−4 orders of magnitude. This limitation not only makes that the ROA spectra are obscured by spectral artefacts, but also leads to the need to apply highly concentrated solutions and long acquisition time. One of a strategy to increase the ROA signal level is to register ROA in resonance condition when the energy of the excitation laser is in (pre)resonance with one or multiple electronic states of a chiral and colour molecule. Under the resonance regime, it is possible to detect strong mono-signate or, even richer in structural information about molecule, bisignate resonance ROA spectra with ROA/Raman intensity. However, using a resonance effect is not straightforward for ROA spectroscopy. We have reported that true RROA spectra of colour and resonating compounds may be masked by other effects, such as ECD in combination with (CP)Raman (i.e., ECD-Raman effect). An example of the ECD-Raman effect is the recording of a strong signal of achiral solvents in the presence of chiral solutes. Moreover, this effect can be observed also for chiral, light-absorbing solutes. The ECD-Raman effect has probably been ignored in many previously conducted RROA measurements that could result in incorrect interpretation of experimental data [1]. Thus, in the recent study we developed a possibly universal protocol that will enable routinely separating the ECD-Raman signals from the RROA spectrum. The subject of our research were optically active and light-absorbing molecules, such as polyene aggregates and vitamin B12 analogues.

References:
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Keywords:
ECD-Raman effect, ROA, biomolecules, chirality

B-P.18

Title: *Conformational study by IR resonant VUV-MATI mass spectroscopy*

Author: Sung Man Park[1], Chan Ho Kwon[1]

[1]Department of Chemistry and Institute for Molecular Science and Fusion Technology, Kangwon National University

Abstract:
Determination of the conformational structure of a polyatomic molecule is crucial for understanding its chemical and biological activities. However, deciphering the contribution of each conformation in the vibrational spectrum of the molecule is extremely challenging, because the conformers have similar force fields between their atoms. As a result, determining conformational structure still remains a subject of debate. Recent advances in conformer-specific vibrational spectroscopic techniques have made it possible to obtain identifiable vibrational spectra for individual conformers in both neutral and cationic states. By using IR dip VUV-MATI spectroscopy, we can measure the vibrational spectrum of each neutral conformer, whereas the vibrational spectrum of each cationic conformer can be obtained through IR hole-burn VUV-MATI spectroscopy based on the IR dip VUV-MATI spectra. In this poster, we will show the effectiveness of the conformer-specific vibrational spectroscopy in molecules such as ketone and aldehyde, providing insights into their unique conformational structures and chemical properties.

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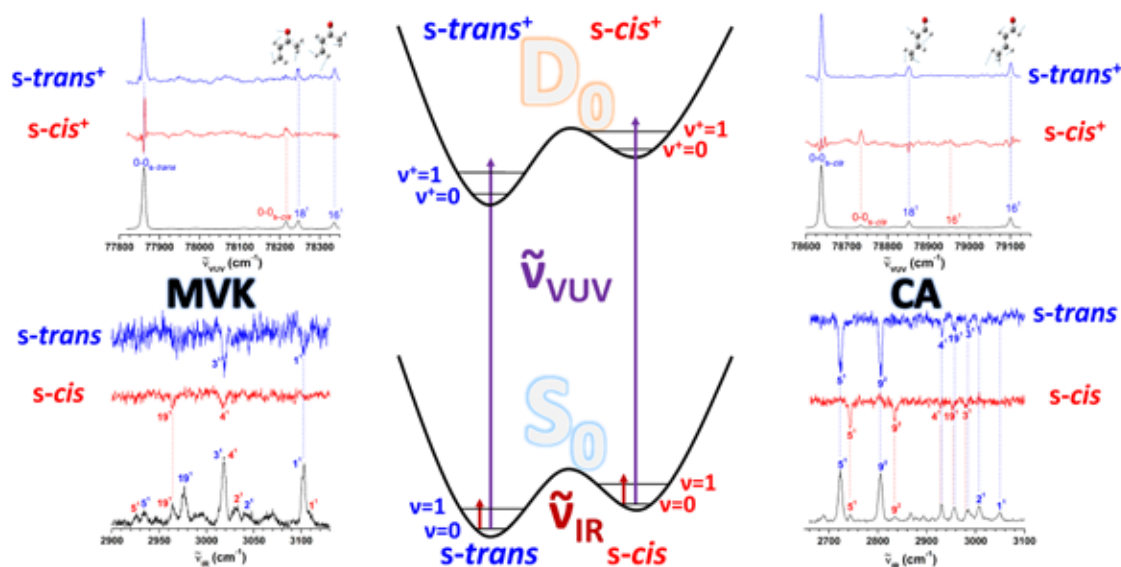
Figure captions:
Fig. Conformer-specific vibrational spectra of individual conformers for C4H6O isomers in the neutral and cationic states using IR resonant VUV-MATI spectroscopy.

Keywords: IR resonant VUV-MATI, conformational stability, crotonaldehyde, MVK, pivaldehyde

B-P.19

Title: *Stereochemical analysis of phosphorus-containing compounds*

Author: Markéta Pazderková[1], Jana Šplíchalová[1], Markéta Tichotová[1], Aneta Ešnerová[1], Lucie Tučková[1], Lucie Bednárová[1], Ondřej Baszczyňski[1], Eliška Procházková[1]

[1]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Abstract:
Structural analysis of organic compounds usually relies on nuclear magnetic resonance (NMR), molecular spectroscopy, and mass spectrometry. The stereochemical assignment is often performed using X-ray diffraction analysis; however, it could sometimes be challenging. For the structural investigation of compounds containing phosphorus – an essential element of many chemical and biological systems, the advanced methods of NMR can be employed. The stereochemistry of compounds with a stereogenic center on the phosphorus atom (known as P-chirogenic molecules) can be advantageously studied by the methods of chiroptical spectroscopy.

We have already demonstrated that NMR spectroscopy utilizing a magnetically active ^{31}P nucleus can provide structural insights into the stereochemistry of phosphorus-containing proline-based compounds (Scheme 1) (1). Here, we compare the NMR results with structural assignment performed using vibrational (VCD) and electronic circular dichroism (ECD) spectroscopy. Particular emphasis is given to the interpretation of VCD results, as they may help us to gain insight into the stereochemistry of the phosphorus chiral center.

References:
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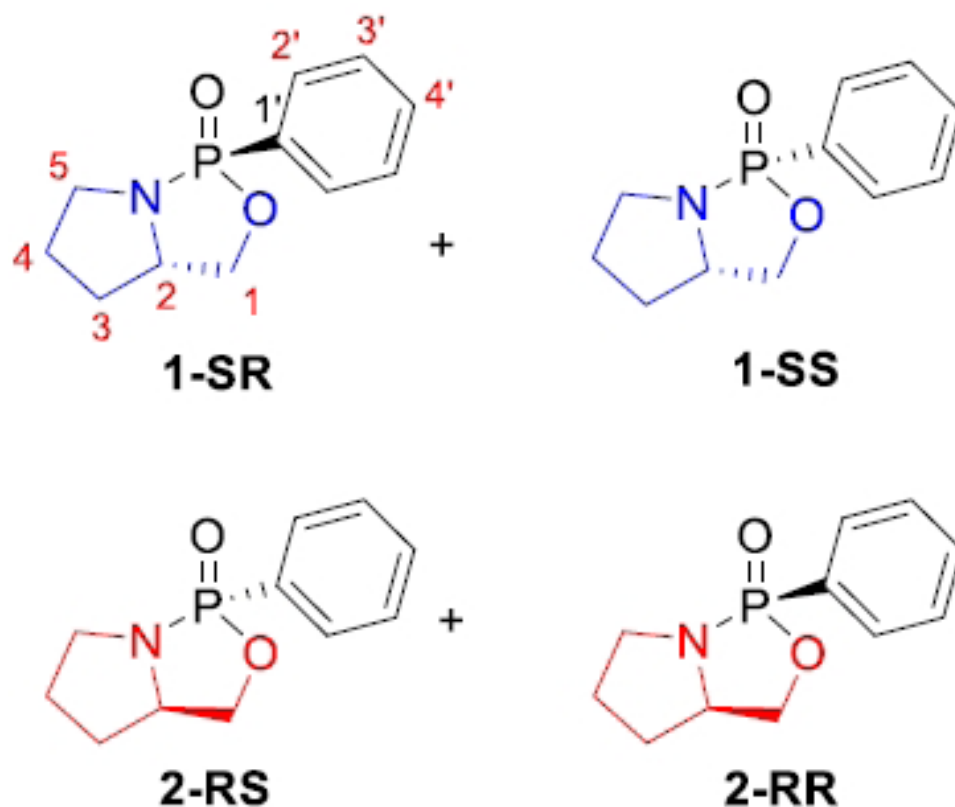
Figure captions:
Scheme 1: Model compounds 1, 2

Keywords: phosphorus-containing compounds, stereochemistry, VCD, ECD

B-P.20

Title: *Temporal Evolution of Single-Molecule Surface-Enhanced Raman Scattering Spectra*

Author: Patryk Pyrcz[1], Sylwester Gawinkowski[1]

[1]Institute of Physical Chemistry Polish Academy of Sciences

Abstract:
Raman spectra provide insights into the molecule's structure and its interaction with the surrounding environment. Although the Raman scattering process is very inefficient, the use of properly fabricated noble metal nanoparticles can enhance the Raman signal by more than ten orders of magnitude. Such a significant enhancement enables the registration of SERS spectra from single molecules [1]. Even though more than twenty years have passed since the first report of single-molecule SERS (SM-SERS) spectra [6], some unexplained experimental results [5] make their interpretation and understanding difficult. These observations include fluctuations in the SM-SERS signal. Here we will present a study of the time evolution of SM-SERS spectra in plasmonic nanocavities. Currently, these fluctuations are attributed to the movement and rotation of molecules within plasmonic nanocavities, atomic reorganization of nanocavity, chemical reaction, desorption, alteration in the molecule's electronic properties, and even decomposition of the molecule. [1-5]

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(4) Weiss, A. et al. Time-Dependent Single-Molecule Raman Scattering as a Probe of Surface Dynamics J. Phys. Chem. B 2001, 105 (49), 12348–12354
(5) Pszona, M. et al. Influence of Bulky Substituents on Single-Molecule SERS Sensitivity J. Chem. Phys. 2022, 156 (1)
(6) Nie, S. et al. Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering Science 1997, 275 (5303), 1102–1106

Keywords: SERS, single molecule, fluctuations, nanocavity

B-P.21

Title: *Protonation of the second coordination sphere in a hangman-type iron porphyrin complex promotes HER: Insights via in-situ Raman spectroelectrochemistry*

Author: Anthony Ramuglia[1], Markus Göbel[1], Vishal Budhija[2], Khoa H. Ly[1], Matthias Schwalbe[3], Inez Weidinger[1]

[1]Technische Universität Dresden
[2]Humboldt-Universität zu Berlin
[3]Utrecht University

Abstract:
Protonation of the second coordination sphere in a hangman-type iron porphyrin complex promotes HER: Insights via in-situ Raman spectroelectrochemistry The iron-based porphyrin complex containing a bispyridine-based hanging unit referred to as Py_2XPFe was immobilized on a modified gold electrode surface and investigated via spectroelectrochemical methods under catalytic conditions for the hydrogen evolution reaction (HER). Immobilization of the Py2XPFe was facilitated using a pyridine-based amine linker molecule, grafted to the electrode surface through electrochemical amine oxidation. The linker molecule allows for effective coordination of the iron porphyrin compound to the electrode surface through axial coordination of the pyridine component to the Fe center. Resonance Raman (RR) spectroelectrochemistry was performed on the immobilized catalyst at increasing cathodic potentials in aqueous pH 7 buffer, facilitating HER while concurrently allowing for the observation of the v_4, v_3 and v_2 porphyrin marker bands which are sensitive to oxidation and spin state changes at the metal center. Surfaced enhanced Raman (SER) spectroelectrochemistry was also conducted on the system so as to observe the bonding nature of the pyridine substituents within the second coordination sphere for HER. RR in conjunction with SER of the Py_2XPFe immobilized at the electrode surface provides spectroscopic evidence at varying potentials into the bonding nature at the Fe center, the surrounding second coordination sphere and their cooperation in promoting catalysis.

References:
1. A. Ramuglia, M. Göbel, V. Budhija, K.H. Ly, M. Schwalbe, Potential induced protonation of the second coordination sphere in a hangman-type iron porphyrin complex promotes HER: Insights via in-situ Raman spectroelectrochemistry. Inog. Chem. (Submitted)

Keywords: Raman spectroelectrochemistry, HER, iron porphyrins

B-P.22

Title: *In-situ Raman studies of the structural changes during the synthesis of a conjugated 2D metal organic framework on the water surface*

Author: Fanny Reichmayr[1]

[1]TU Dresden

Abstract:
Metal organic frameworks (MOFs) have shown promise as materials for gas storage, catalysis and sensing, among others. To achieve optimal performance and develop new materials with improved properties, a comprehensive understanding of the molecular structure and properties of MOFs is required. Vibrational spectroscopy like Raman is a useful measurement technique to study the structures of MOFs. However, the complexity of the crystal structures of MOFs poses a major challenge in the interpretation of vibrational spectra. Here, we report the successful *in-situ* monitoring of the synthesis of a conjugated two-dimensional MOF constructed from Zn-phthalocyanine linkers and Cu-oxo clusters. The synthesis takes place at the water surface and can therefore be monitored with Raman spectroscopy. Structural changes were observed with time resolution using vibrational spectroscopy. The ratio of the intensities of MOF-specific to linker-specific bands allows conclusions to be drawn about the kinetics of the reaction. In addition, the influence of the concentration of the modulator Na-acetate on the reaction could be investigated by adjusting its concentration. This work highlights the potential of Raman spectroscopy as a tool to study MOF synthesis and provides new insights into the molecular details of MOF structures. In the future, the more detailed understandings of MOF syntheses could facilitate the development of MOFs with tailored properties and improved performance for various applications.

Keywords: in-situ, Raman, MOF, synthesis, watersurface

B-P.23

Title: *Conformation of choline-chloride-based deep eutectic solvents and its temperature dependence observed with Raman spectroscopy*

Author: Naoki Sakurai[1], Koich Iwata[1]

[1]Faculty of Science, Gakushuin University

Abstract:

Deep eutectic solvents (DESs) are eutectic mixtures in the liquid form at room temperature. The melting point of reline, one of the well-known DESs, is 12°C[1], although it is a mixture of two solid compounds, choline chloride (ChCl) and urea, with a molar ratio of 1:2. DESs and ionic liquids have some similarities that include large viscosity and small vapor pressure. They are expected as solvents having smaller impacts to the environment. The constituents for DESs tend to be less costly than those for ionic liquids. On the other hand, properties of DESs have not been fully clarified, as compared with those for the ionic liquids. In this study, we record the Raman spectra of some ChCl-based DESs and examine their structures.

We measured the Raman spectra of aqueous solutions of ChCl (ChCl/H_2O) and DESs. The DESs were reline (ChCl/urea), ethaline (ChCl/ethylene glycol), and ChCl/1-methylurea with a molar ratio of 1:2. These DESs were liquid when the Raman spectra were recorded. The excitation wavelength was 632.8 nm. The light scattered from the sample was collected, dispersed by a spectrograph (focal length of 32 cm with a grating of 1800 g/mm), and detected by a liquid-nitrogen-cooled CCD detector.

There are two Raman bands commonly observed at 716 and 767 cm^{-1}. These bands have been assigned to the N-CH3 symmetric stretch vibrations of gauche- and trans-ChCl, respectively[2]. ChCl in the sample has gauche- and trans-conformers, while ChCl in the solid powder has only the gauche-form. We calculate the relative area intensities of the Raman bands with the least squares fitting analysis. The gauche- and trans-form Raman bands are fitted by a Voigt and Lorentz function, respectively. The result indicates that there are more *gauche*-ChCl molecules in the DESs than in ChCl/H_2O. The gauche-rich structure of ChCl in solid is possibly maintained partially in the DESs as nanodomains.

References:

1. A.P. Abbott, G. Capper, D.L. Davies, K.R. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, Chem. Commun. 1 (2003) 70–71.
2. H. Akutsu, Direct Determination by Raman Scattering of the Conformation of the Choline Group in Phospholipid Bilayers, Biochemistry 20 (1981), 7359-7366.

Keywords: Deep eutectic solvents, Raman spectroscopy

B-P.24

Title: *Characterization of sofosbuvir polymorphs using polarized Raman microscopy*

Author: Věra Schrenková[1], Adam Sklenář[1], Josef Kapitán[2]

[1]Institute of Organic Chemistry and Biochemistry
[2]Palacký University

Abstract:
Most drugs are produced in a solid state, but these are often poorly soluble and therefore have low bioavailability. One way to tackle this problem is to improve its pharmacokinetics by preparing different polymorphs, cocrystals, solvates, or salts of the drug. In pharmaceutic production, it is desirable to monitor such crystalline forms by efficient analytical tools. In this work, we investigate the potential of linearly and circularly polarized Raman microscopy for discrimination of three polymorphs of sofosbuvir, an antiviral drug used to treat hepatitis C[1]. The polarized Raman spectroscopies provide additional information on the symmetry of vibrational modes by changing the polarization of incident and scattered light. To this end, Raman spectra at parallel and perpendicular orientations of linearly polarized light were obtained using a modified Witec Raman microscope equipped with two half-wave plates. Using two quarter-wave plates, we recorded Raman spectra in corotating and contrarotating circular polarization. Furthermore, the resulting spectra include a large signal of low-frequency vibrations close to the laser line, which often reflect the intermolecular interactions and packing in the polymorphs[2]. The three polymorphs of sofosbuvir already gave different unpolarized Raman spectra, but the polarization measurements made the distinction more reliable.

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$$DOC(i) = \frac{I_R^i - I_L^i}{I_R^i + I_L^i}$$
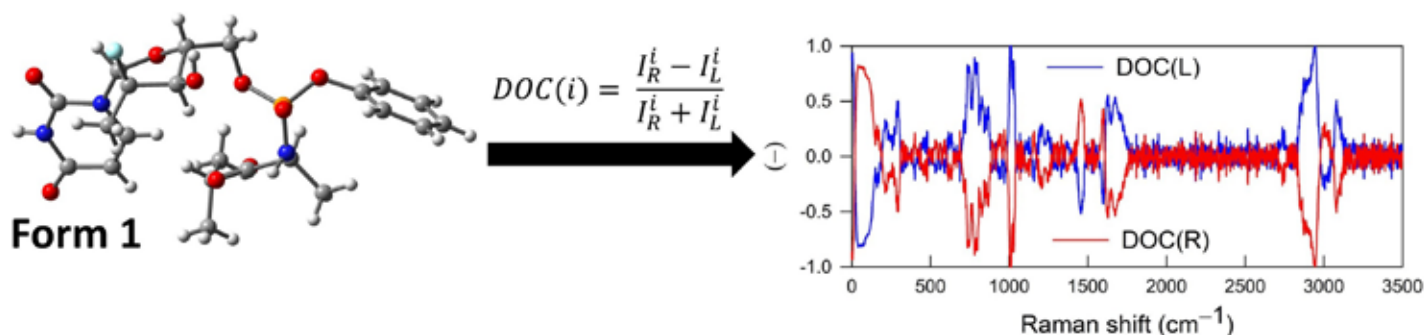
Figure captions:
Structure of sofosbuvir form 1 and degree of circularity (DOC) obtained from the Raman spectra recorded in corotating and and contrarotating circular polarization.

Keywords: Raman microscopy, polymorphs, polarization

B-P.25

Title: *Surface-enhanced Raman Scattering for probing the molecular structure of MutS protein*

Author: <u>Sara Seweryn</u>[1], Yiqing Feng[2], Dawid Lupa[1], Lars Dannenberg[3], Ewelina Lipiec[4], Janina Kneipp[5]

[1]1Instytut Fizyki im. M. Smoluchowskiego, Uniwersytet Jagielloński, ul. Łojasiewicza 11, 30 348 Kraków
[2]2Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany
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[5]5Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany

Abstract:
The MutS protein is involved in the Mismatch Repair (MMR) process, induced by polymerase misincorporation errors upon DNA replication or an incorrect process of DNA damage repair. The methodological and instrumental limitations make it difficult to investigate the intermolecular interactions between damaged DNA and repair proteins. MutS binding to DNA has been observed already by scanning electron microscopy (SEM) and atomic force microscopy, however, both methods are chemically blind and did not allow investigation of DNA chemical structure and its conformation.
In this presentation, we report on studies that involve the optimization of surface enhanced Raman scattering (SERS) for effective probing of the molecular structure and composition of MutS constitute. We used citrate-stabilized gold nanoparticles obtained in a chemical reduction process. Several important steps in optimization of the SERS experiments for efficient protein measurements will be discussed, specifically SERS probing of the MutS molecular structure and the protein-gold nanoparticle interaction based on the presence of specific signals in the SERS spectra in an approach that was introduced previously [1]. Selectivity in SERS probing, based on changed interaction upon changes in ionic strength of buffers and protein concentration, as well as other sources of variation in the spectra will be discussed. Furthermore, we applied a high resolution Atomic Force Microscopy (AFM) for direct visualization of the protein structure. These studies provide a solid basis for further research concerning direct monitoring of molecular changes induced by the interaction between repair proteins and the damaged DNA. The comprehensive comparison of the SERS spectra and AFM measurements will be demonstrated.

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1. Szekeres, G.P.; Montes-Bayón, M.; Bettmer, J.; Kneipp, J., Fragmentation of proteins in the corona of gold nanoparticles as observed in live cell surface-enhanced Raman scattering. Analytical Chemistry, 2020, 92, 8553-8540

Keywords: SERS, MutS protein, DNA repair

B-P.26

Title: *Vibrational circular dichroism reveals structural differences in pharmaceutically relevant solids*

Author: Adam Sklenar[1]

[1]IOCB Prague

Abstract:

Most medicaments are manufactured in solid forms[1] and various analytical methods are utilized to characterize them. Detailed knowledge of the specific structural form is necessary to control the required pharmacokinetic and pharmacodynamic properties of the product.

We present vibrational circular dichroism (VCD) investigations of multicomponent pharmaceutical solids. Distribution of drugs in the solid state, e.g., in salts and cocrystals, is a convenient way to tackle their low solubility. Structure characterization of these forms is sometimes quite challenging. We prepared a cocrystal of the anti-inflammatory drug naproxen with alanine, by liquid-assisted grinding, and recorded its IR and VCD spectra. We show that IR and especially VCD can well distinguish the pure active pharmaceutical ingredient (API) and its cocrystal with a coformer, as well as an API/coformer mixture from the cocrystal. VCD is also sensitive to minor changes in conformation and can monitor weak non-covalent interactions in the solid phase[2]. Theoretical calculations of vibrational properties provided deeper insight into the relationship between spectra and structures. The O-H bond of the naproxen carboxyl group is prolonged in the cocrystal due to the presence of alanine in the proximity which has consequences for the spectral shapes. The studied system can therefore be considered as an intermediate between cocrystal and salt.

Our results suggest that solid-state VCD represents a useful tool for identifying crystal structures and studying non-covalent interactions in the solid state, with a potential for supramolecular chemistry or pharmaceutical development.

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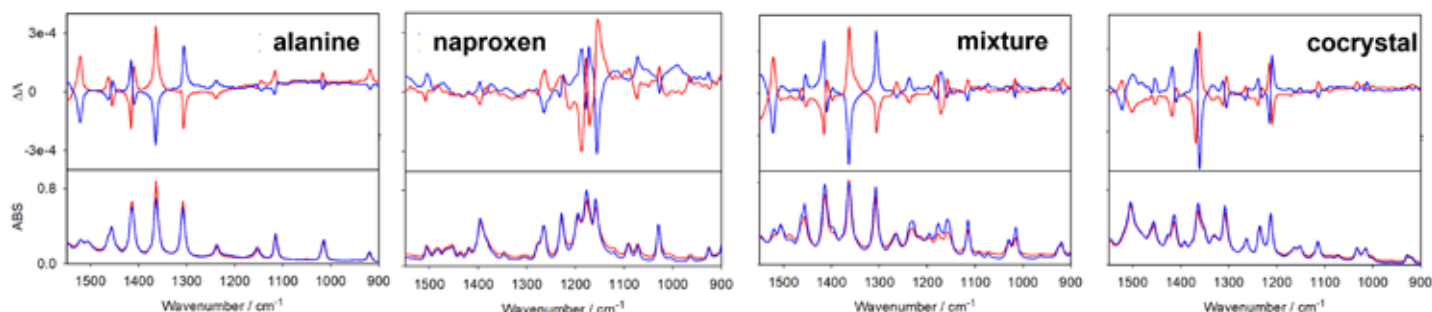
Figure captions:
Solid-state VCD spectra of L- and D-alanine, R- and S-naproxen, mixtures of S naproxen with L-alanine and R-naproxen with D-alanine, and cocrystals of S naproxen/L-alanine and R-naproxen/D-alanine.

Keywords: solid-state vibrational circular dichroism, cocrystal

Title: *The double-well lowest-lying Rydberg state in CdAr resolved*

Author: <u>Joanna Sobczuk</u>[1], Tomasz Urbańczyk[1], Jarosław Koperski[1]

[1]Smoluchowski Institute of Physics, Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University

Abstract:
The study of highly excited molecular states opens the way to fascinating research owing to the possibility to answer fundamental questions about interatomic interactions, non-trivial behavior of molecules in such states, and emission to lower-lying electronic states, to which are not accessible from the ground state. Recently, a shape of the double-well $E^3\Sigma_1^+(6^3S_1)$ molecular state in CdAr has been concluded based on experimental data [1]. The shape of the $E^3\Sigma_1^+{}_{in}$ deeper well have been obtained from the $E^3\Sigma_1^+{}_{in}$, $\upsilon'\leftarrow A^3\Pi_0^+(5^3P_1)$, $\upsilon''=6$ vibrational spectra using inverted perturbative approach (IPA) method [2], whereas the shallow $E^3\Sigma_1^+{}_{out}$ well has been investigated based on vibrational $E^3\Sigma_1^+$, $\upsilon'\leftarrow B^3\Sigma_1^+(5^3P_1)$, $\upsilon''=1\text{-}4$ spectra. The most significant achievement, though, is resolving a shape of the potential barrier based on interference free$\leftarrow$bound $E^3\Sigma_1^+\leftarrow B^3\Sigma_1^+$, $\upsilon''=0\text{-}4$ spectra for the first time.

References:
[1] J. Sobczuk, T. Urbańczyk, J. Koperski, The lowest-lying Rydberg state of CdAr van der Waals complex: the improved characterization of the interatomic potential, Spetrochim. Acta A, 282 (2022) 121655
[2] T. Urbańczyk, M. Krośnicki, A. Kędziorski, J. Koperski, The E3Σ1+ +(63S1) ← A3Π0+(53P1) transition in CdAr revisited: The spectrum and new analysis of the E3Σ1+ Rydberg state interatomic potential, Spectrochim. Acta A, 196 (2018), 58

Keywords: laser spectroscopy,Rydberg state,van der Waals

B-P.28

Title: *UV resonance Raman of serum albumins*

Author: <u>Cecilia Spedalieri</u>[1], Janina Kneipp[1]

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Abstract:
Serum albumin is the most abundant protein in vertebrate blood and plays an important role as carrier. Human serum albumin (HSA) and bovine serum albumin (BSA) have a sequence overlap of approximately 75.6%,[1] yet very different interactions and thermodynamic properties.[2] Resonant excitation in the UV has been shown to be very useful in the Raman spectroscopic characterization of proteins, microorganisms and eukaryotic cells. Excitation with wavelengths above 200 nm emphasizes the contributions in the spectra by the aromatic amino acid side chains. In serum albumins, different aromatic residues are located in or near binding sites for drugs or other molecules.
In this work, we discuss UVRR spectra of bovine serum albumin (BSA) and human serum albumin (HSA) measured with 220 nm excitation wavelength, with the aim of assigning the contributions of different aromatic amino acid side chains to the protein spectral signature in a wide range of frequencies.[3] The comparison of the fingerprint region of the protein spectra with solutions containing these amino acids in a concentration ratio that corresponds to those in the proteins reveals a weaker contribution by the amide I and amide III modes to the spectra of both proteins. In addition, the regions of overtone and combination bands in the wavenumber range from 2900-5100 cm^{-1} are differently pronounced in the spectra of the proteins. The spectra in the region above 1900 cm^{-1} was found to be rich in bands of phenylalanine and tyrosine, and our assignments of spectral features to combinations comprising fundamentals and overtones were supported by experiments with amino acid mixtures that contained deuterated tyrosine. Making use of the overtones and combination bands in the Raman spectrum of the proteins could help better understand structural characteristics of these biomolecules, and provide information that is complementary to NIR absorption spectroscopy of the proteins.

References:
[1]. K.A. Majorek, P.J. Porebski, A. Dayal, M.D. Zimmerman, K. Jablonska, A.J. Stewart, M. Chruszcz, W. Minor, Structural and immunologic characterization of bovine, horse and rabbit serum albumins, Mol Immunol, 52 (2012) 174-182.
[2]. G.P. Szekeres, J. Kneipp, Different binding sites of serum albumins in the protein corona of gold nanoparticles, Analyst 143 (2018) 6061-6068.
[3]. C. Spedalieri, J. Plaickner, E. Speiser, N. Esser, J. Kneipp, submitted.

Keywords: UV-resonance-Raman, serum-albumins, tryptophan, tyrosine, phenylalanine

B-P.29

Title: *Formation of vitamin D3 observed by picosecond time-resolved Raman spectroscopy*

Author: Risa Suzuki[1], Tsukasa Tokita[1], Koichi Iwata[1]

[1]Faculty of Science, Gakushuin University

Abstract:
When irradiated with UV light, 7-dehydrocholesterol (DHC) undergoes a photochemical reaction that results in the ring-opening of its cholesterol B ring. A sigmatropic rearrangement then follows that produces Vitamin D3. The reaction time of the ring-opening in the initial reaction step has been reported as 1 to 2 ps [1] and that for the sigmatropic rearrangement is 91 hours in 25°C solution [2]. The initial DHC ring-opening reaction is assumed to be a Woodward-Hoffmann type pericycle reaction. However, to the best of our knowledge, the proposed reaction mechanism has not been fully supported by experimental results. Vitamin D3 plays important roles in physiological processes, including the immune system reactions and bone formation. Deficiency of vitamin D3 causes various diseases. It is therefore important to know the molecular structure of DHC after UV irradiation. In this study, we attempt to elucidate the vitamin D3 formation mechanism by picosecond time-resolved Raman spectroscopy, which is an effective tool when discussing the structure of short-lived reaction intermediates. Picosecond time-resolved Raman spectra of photoexcited DHC in acetonitrile are shown in Figure. A band at about 1550 cm^{-1} attributed to a C=C stretch vibration of the cyclic structure of DHC was successfully observed. In the steady-state Raman spectrum of DHC, a C=C stretch vibration is observed at 1601 cm^{-1}. By the UV irradiation, the frequency of the C=C stretch vibration has shifted from 1601 to about 1550 cm^{-1}, which suggests the decrease in the bond order. Contrary to the expectation that a strong Raman band would be observed around 1-2 ps during the ring-opening reaction, a strong Raman band was observed even after 100 ps. We intend to elucidate this chemical reaction by examining the results from time-resolved Raman spectroscopy.

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2. M.F. Holick, X.Q. Tian, M. Allen, Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic animals, Proc. Natl. Acad. Sci. USA. 92 (1995) 3124-3126.
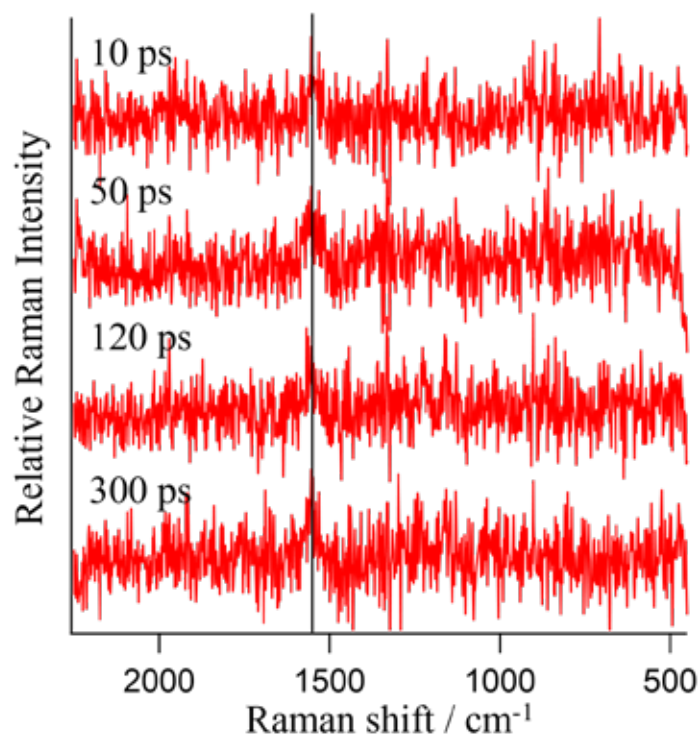
Figure captions:
Picosecond time-resolved Raman spectra of photoexcited DHC in acetonitrile.

Keywords: Time-resolved Raman, 7-dehydrocholesterol, vitamin D3

B-P.30

Title: *Structural Determinants of the Catalytic Nia-L Intermediate of [NiFe]-Hydrogenase*

Author: <u>Armel Franklin Tadjoung Waffo</u>[1], Christian Lorent[1], Sagie Katz[1], Janna Schoknecht[1], Oliver Lenz[1], Ingo Zebger[1], Giorgio Caserta[1]

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Abstract:

[NiFe]-hydrogenases catalyze the reversible splitting of H_2 molecules into $2H^+$ and 2e-.[1] The mechanism of H_2 activation occurs at the bimetallic active site composed of Ni and Fe. The latter of which is *i.a.* coordinated by inorganic CO and CN^- ligands. Several catalytic intermediates have been characterized on the basis of their characteristic IR signature.[2] Among them, the involvement of Nia-L species in the catalytic cycle is still controversially discussed, as these states comprise several sub-forms making their structural elucidation challenging.[3] Another obstacle lies in the identification of amino acid residues involved in H_2 activation as well as their role in stabilizing such intermediary states. In this work, the regulatory [NiFe]-hydrogenase from *Cupriavidus necator* (CnRH) was used as model enzyme to elucidate the structural basis of two hitherto elusive Ni_aL intermediates utilizing cryogenic infrared spectroscopy (Fig. 1).[3] Illumination experiments at cryogenic temperatures have enabled the acquisition of high-quality IR difference spectra providing new information on the course of the reaction. These observations could be validated by means of EPR spectroscopy. Additionally, the protonation state and H-bonding network in the first and second coordination spheres of the active site was analysed, including a proton-accepting glutamate and a Ni-bound cysteine residue. More specifically, yet unknown conformational changes of amino acid residues, e.g. of a highly conserved Arginine, were shown to be involved in the formation of these states in IR measurements. This study reveals the importance of the protein scaffold in fine-tuning proton and electron dynamics in [NiFe]-hydrogenases.

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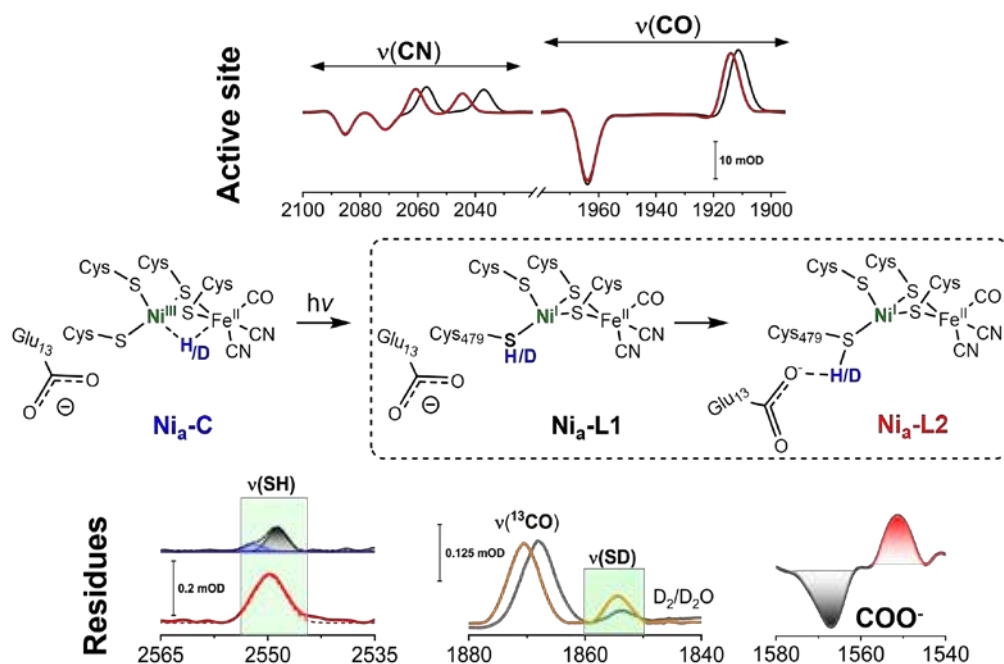
Figure captions:
Fig1. Schematic representation of the sequential reaction Nia-C to Nia-L1 to Nia-L2 with characteristic IR signature of the active site (top) and the involved amino acid residues (bottom).

Keywords: Hydrogenases, Catalytic cycle, Cyo-IR Spectroscopy

C-P.1

Title: *Development of high-Throughput Raman imaging to investigate the efficacy of Doxifluridine Squalenoyl nanomedicine on single breast cancer cells*

Author: Cherine Alaouta[1]

[1]Université de Reims Champagne-Ardenne

Abstract:
Doxifluridine (5-deoxy 5-fluorouridine; DXF) is a fluoropyrimidine derivative first synthesized by Cook[1]. It cannot be phosphorylated and assimilated into nucleic acid unless it is first metabolically converted into 5-fluorouracil (5FU) by the action of uridine phosphorylase[2].

Although not yet FDA-approved, DXF shows promise, but its delivery and targeting to tumors remains a challenge. One strategy to improve drug delivery and target specificity is the use of biocompatible nanoparticles. "Squalenoylation" nanotechnology involves covalently binding the drug to squalene, a natural triterpene, creating nanoparticles that can self-assemble and target tumors[3,4]. This study involved the synthesis of new SQ-doxifluridine (SQ-DXF) nanoparticles using a nanoprecipitation method. The physicochemical properties of the bioconjugate were then characterized using dynamic light scattering (DLS) and UV absorbance spectroscopy. The antitumor activity of DXF and SQ-DXF NPs was evaluated on MCF-7 and MDA-MB-231 cell lines, which differ in their LDL receptor expression levels[5]. The IC50 value of SQ-DXF NPs was found to be 12-fold and 23-fold lower than DXF in MDA-MB-231 and MCF-7 cells, respectively, indicating higher cytotoxicity of the NPs compared to the free drug.

We have then investigated the effects of DXF and SQ-DXF NPs on living MDA-MB-231 and MCF-7 cell lines using Raman imaging approach. Our results revealed changes in the subcellular localization of biomolecules such as proteins, lipids and nucleic acids between untreated cells and treated with DXF and SQ-DXF. Raman data were correlated with the cytotoxic effect of these drugs. This approach will provide valuable and objective information to predict treatment response based on specific spectral signatures in different cellular compartments.

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Keywords: Raman, Spectroscopy, Doxifluridine, Nanoparticles, BreastCancer

C-P.2

Title: *The impact of the resonant Raman effect on the detection of single molecules*

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Abstract:
It has been a quarter of a century since the first single-molecule surface-enhanced Raman scattering (SM-SERS) spectra were reported.[1,2] However, despite SERS' potential to detect a broad range of chemicals on the single-molecule level, only a relatively limited number of chemicals with high Raman cross-sections have been reported thus far. An examination of SM-SERS literature reveals that for the majority of chemicals, the resonant Raman effect is essential to accomplish single-molecule detection. In SERS, the enhancement of the Raman signal is a product of electromagnetic and chemical effects. By adjusting the excitation frequency to match the electronic transition of the substance, the overall signal enhancement can be further increased through the resonant Raman effect.

In this study, we explored how adjusting the excitation wavelength in SERS affects the detection of single molecules in porphycene and its derivatives. Previous research reported SM-SERS spectra of porphycene using 633 nm excitation, but detecting single molecules in other porphycene derivatives was more challenging.[3] We investigated the impact of fine-tuning the excitation wavelength to the resonance Raman conditions on SM-SERS detectability. Our findings show that the resonance enhancement is strongest for excitations close to the first electronic transition.[4] However, the energy of this resonance is redshifted for molecules deposited on nanoparticles. The overall signal was approximately two orders of magnitude stronger than for excitations at 633 nm. These could significantly enhance single-molecule detection. Moving the excitation away from the first electronic resonance led to a notable decrease in the number of detected single-molecule events. Our findings stress the importance of the resonant Raman effect for single-molecule detection using SERS. Moreover, adjusting the excitation wavelength can enhance detectability, essential for SERS' analytical applications.

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Figure captions:
Fig. 1: Normalized resonance enhancement profile of CY5Pc as a function of excitation frequency (blue) and its absorption in ethanolic solution (red).
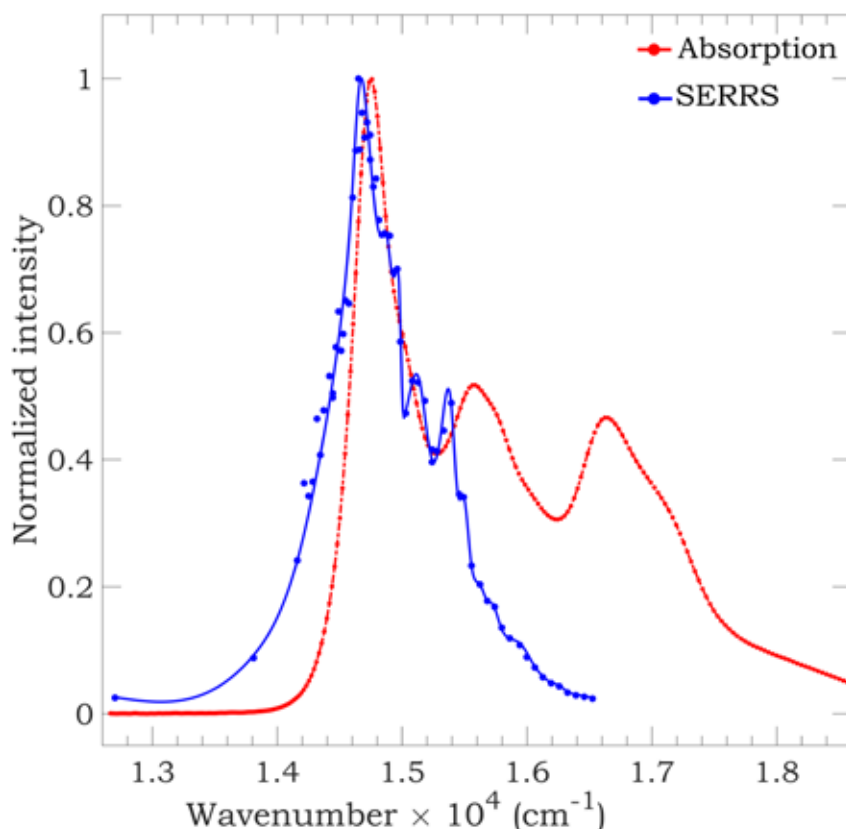
Keywords: SERS, Resonant, single-molecule detectability

C-P.3

Title: *Surface-enhanced Raman scattering (SERS) of biomolecules – Can the variations tell a story?*

Author: Shrobona Banerjee[1], Lars Dannenberg[1], Janina Kneipp[1]

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Abstract:
A wealth of information is contained within the surface-enhanced Raman scattering (SERS) spectra of biomolecules, most of which is yet to be deciphered accurately. This is because biomolecules, especially macromolecules such as proteins and nucleic acids, have varied conformations and interaction with plasmonic nanostructures. The interaction of the molecule with the plasmonic substrate is dependent on environment properties such as pH, ionic strength, local electromagnetic fields and their gradients, and concentration to name a few. In addition, the nanostructures display structural heterogeneity; they may interact with the molecule of interest differently, partially or not at all depending on their structure, morphology and organisation. At the same time, efficiency of SERS varies due to varying plasmonic conditions, e.g., caused by different shapes, sizes, and materials. All of these issues lead to SERS spectra often displaying apparently non-specific variation.
Therefore, it is an important aim understand the causes of such variation and their corresponding effects on the information contained in the Raman spectra of the molecules. In this work, we tried to address this by investigating the behaviour of different plasmonic substrates and biomolecules in conjunction with each other, under varying physical and chemical conditions. We will discuss spectral variation in the interaction of biomolecules, specifically proteins, with different plasmonic structures. Thereby we can understand them better and obtain more information about their structure, composition and interaction.

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C-P.4

Title: *Optimising gold nanorods: accounting for the unique surface chemistry of CTAB-coated gold nanorods when labelling for SERS applications*

Author: Ioana Blein-Dezayes[1], Francesca Palombo[1], Nick Stone[1]

[1]University of Exeter

Abstract:
Our project aims to create an all-in-one platform to diagnose and treat cancer using gold nanoparticles. Their localized surface plasmon resonance can enhance the Raman signal of molecules adsorbed on their surface (Raman reporter). This property, combined with targeting functions, such as monoclonal antibodies, should enable us to retrieve a highly intensified signal from the Raman reporter, giving the localization of the nanoparticles targeting the cancer cells at depth in tissues, using Raman spectroscopy. Gold nanorods (AuNRs) are good candidates as, unlike gold nanospheres, they have the additional benefit of being very efficient at converting light energy into heat, making them valuable candidates to develop targeted photothermal therapy as well.[1,2]

This work focuses on how we optimised the functionalisation of AuNRs with a Raman reporter by first priming the AuNRs surface via centrifugation to remove the surfactant CTAB. While CTAB is necessary for the silver assisted seed-mediated growth method for AuNRs synthesis, as it plays a role both in the anisotropic growth of the rods and in preventing aggregation, during and post synthesis, it is also a long molecule that hinders the gold surface.[3] By centrifuging the AuNRs before labelling with our Raman reporter, the gold surface is exposed, increasing Raman reporter coverage and leading to a SERS signal on average 3 times better.

Ongoing work includes investigating the relationship between the AuNRs' aspect ratio and their efficacy for photothermal therapy applications as well as their enhancement of the SERS signal.

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Keywords: Gold nanorods, SERS, Raman reporter

C-P.5

Title: *TERS in research of Langmuir-Blodgett lipid monolayer*

Author: <u>Michał Czaja</u>[1], Sara Seweryn[1], Katarzyna Skirlińska-Nosek[1], Anna Chachaj-Brekiesz[2], Dawid Lupa[1], Anita Wnętrzak[2], Ewelina Lipiec[1]

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Abstract:
Tip-enhanced Raman spectroscopy (TERS) is a modern analytical technique combining a spatial resolution of scanning probe microscopy (SPM) and the chemical selectivity of vibrational spectroscopy. In this technique, laser radiation illuminates a metallic probe of SPM, leading to a strong localization of electromagnetic field, due to the excitation of localized surface plasmons (LSPRs) at the tip apex. Excited plasmons result in the enhancement of optical signal in the near-field providing single-molecule sensitivity. This makes TERS a perfect tool for studying nanoscale objects, especially highly inhomogeneous systems, such as lipid membranes [1].

In the presented research, we performed a nanoscale chemical characterization of lipid monolayers deposited using the Langmuir–Blodgett method [2]. The studied film was prepared from a mixture of DPPC, DPPE, and cardiolipin. The prepared monolayer was characterized by high-resolution atomic force microscopy (AFM) in tapping mode with phase contrast providing observation of different lipid fractions. To obtain information about the chemical composition of the observed domains we performed a TER mapping. Spectra collected from the lipid blend were compared with Raman spectra of individual lipids. To reduce the dimensionality of the obtained hyperspectral images, we applied multivariate data analysis, such as principal component analysis (PCA), and hierarchical cluster analysis (HCA). This approach allowed us to extract crucial information from collected data in particular exploring nano-spectroscopic spectral markers of selected lipids and providing their spatial distribution in the investigated monolayer.

Performed research shows that TERS nanospectroscopic approach is an efficient tool in studies of model lipid layers formed from various components. The obtained results show that the TERS approach provides new insights for future research in such fields as pharmacology, toxicology, and nanomedicine.

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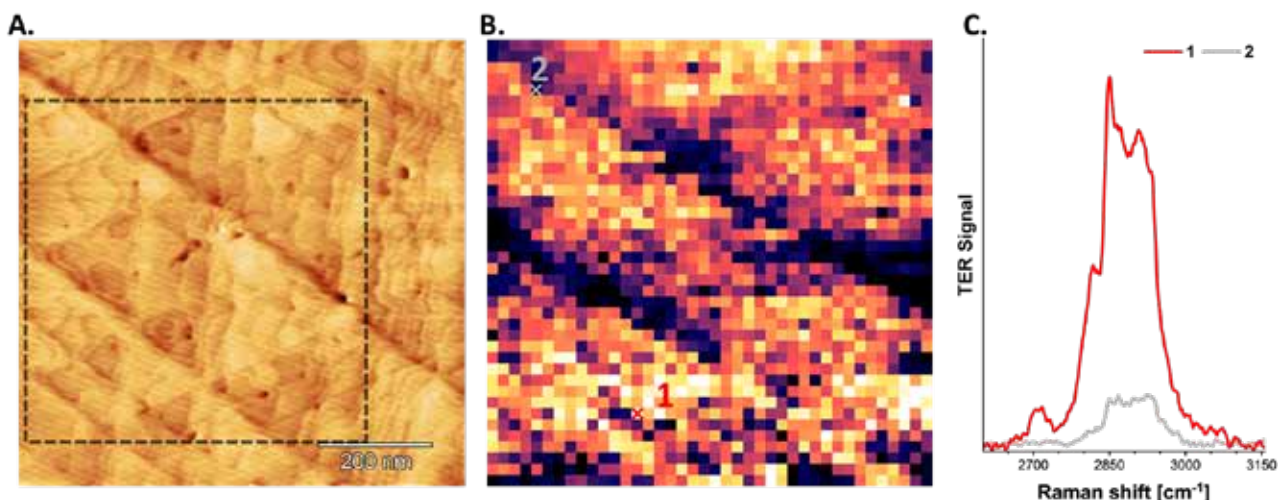
Figure captions:
(A) STM topography of CL/DPPC/DPPE monolayer on gold substrate with (B) TER map collected from area marked on the panel A. (C) Two representative spectra collected from marked pixel.

Keywords: TERS, lipid monolayer, nano-optics, nanospectroscopy

Title: *Optical properties and SERS analysis of quasi-3D plasmonic nanostructures fabricated by colloidal lithography*

Author: Ioana Marica[1], Veronica Zani[2], Raffaella Signorini[2], Roberto Pilot[3], Simona Cîntă Pânzaru[4], Cosmin Farcău[5]

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Abstract:
After almost half-century from its discovery in 1974, surface-enhanced Raman spectroscopy (SERS) is still increasingly applied technique not only in biomedical, environmental, pharmaceutical, forensic and other fields requiring trace detection of certain molecular species, but also in probing widely developed nanofabricated substrates for complex bio-nanosensing strategies. Although substantial advancement is achieved both theoretically and experimentally, SERS is still scarcely validated method to be included in currently regulated protocols for medical theragnostic or environmental control. In efforts to validate such approaches, here we present a new class of nanostructured SERS substrates with good uniformity, reproducibility, low costs and high enhancement. Their fabrication comprises colloidal self-assembly, plasma etching, and metal film deposition by e-beam evaporation. A compact and highly uniform monolayer of 460 nm polystyrene (PS) nanospheres was self-assembled on a PS plate substrate, followed by plasma etching and deposition of 150 nm thick gold films. The morphology of both the spheres and the substrate were modified by etching, thus leading to a quasi-3D gold-PS surface. The method offers good-enough reproducibility and good control over the area deposited, allowing to prepare SERS substrates up to several cm2. A detailed characterization of the SERS enhancement of the nanostructured substrates was carried out by the Wavelength-scanned Surface-enhanced Raman spectroscopy (WS-SERS) in the NIR-IR spectral range. The SERS enhancement profile was evaluated by using benzenethiol as a Raman reporter, and correlated with the optical response of the nanostructured film, analyzed by reflectance measurements. The applicability of these nanostructured substrates has been explored for the detection of nanoplastics, an emerging pollutant of global concern.
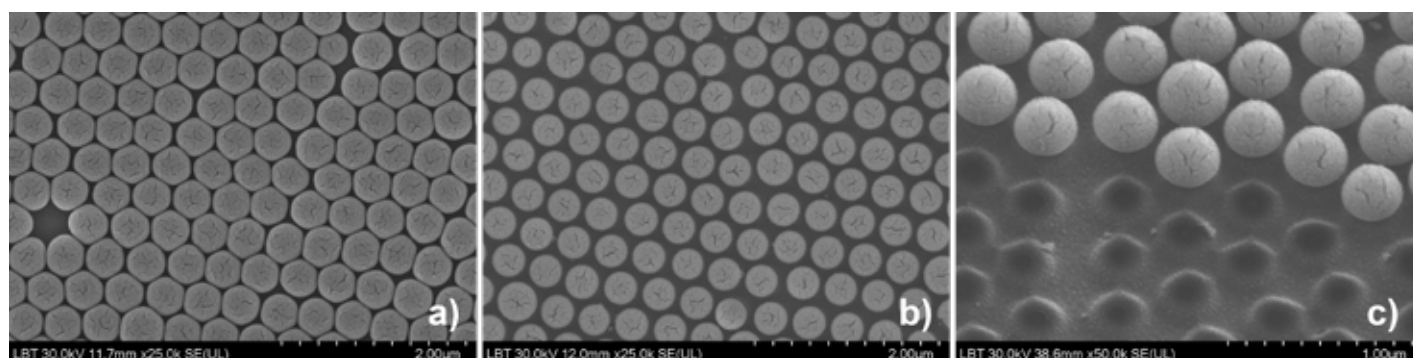
Figure captions:
SEM images of an unetched (a) and etched (b) nanostructured substrates, and angled view of an etched substrate (c).

Keywords: WS-SERS, nanostructured plasmonic substrates, self-assembly, nanoplastic

C-P.7

Title: *Factors affecting the blinking and bleaching of single-molecule SERS spectra*

Author: Thanyada Sukmanee[1], Sylwester Gawinkowski[1]

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Abstract:

Single-molecule surface-enhanced Raman scattering (SM-SERS) spectroscopy represents an ultrasensitive analytical technique with immense potential across various fields, such as chemistry, biology, and materials science. For instance, SM-SERS has been employed in the investigation of porphycenes [1,2]. Nonetheless, a primary concern in SM-SERS observation relates to spectral fluctuations, which include the total intensity of the spectrum, relative intensities, band shifting, and the limited lifetime of the SM-SERS signal. These depend on various factors, such as temperature, excitation intensity, and molecular orientation [3]. These fluctuations are attributed to the properties of the molecule and the hotspot. For example, the blinking dynamic often occurs during measurement due to the diffusion of the molecule into and out of the hotspots. In addition, SM-SERS intensity and spectral position fluctuate over time. Therefore, it is challenging to identify the structural orientation, as molecular decomposition and photobleaching can occur [4]. To overcome this challenge, multiparameter studies are required to obtain more subtle spectral changes in the SM-SERS spectra. Furthermore, reducing the temperature of the sample can minimize the undesired influence of the photo-induced thermal motion of SM and prevent molecular movement during the integration time.

To fully harness the potential of SM-SERS, it is essential to understand and optimize the sources and nature of those fluctuations, as well as the factors influencing the lifetime or survival time of a single molecule at a hotspot. In this study, we present the results of examining spectral changes and molecular dynamics through the time evolution of the SM-SERS signal, accompanied by a statistical analysis of variations in vibrational modes over the course of the detection period.

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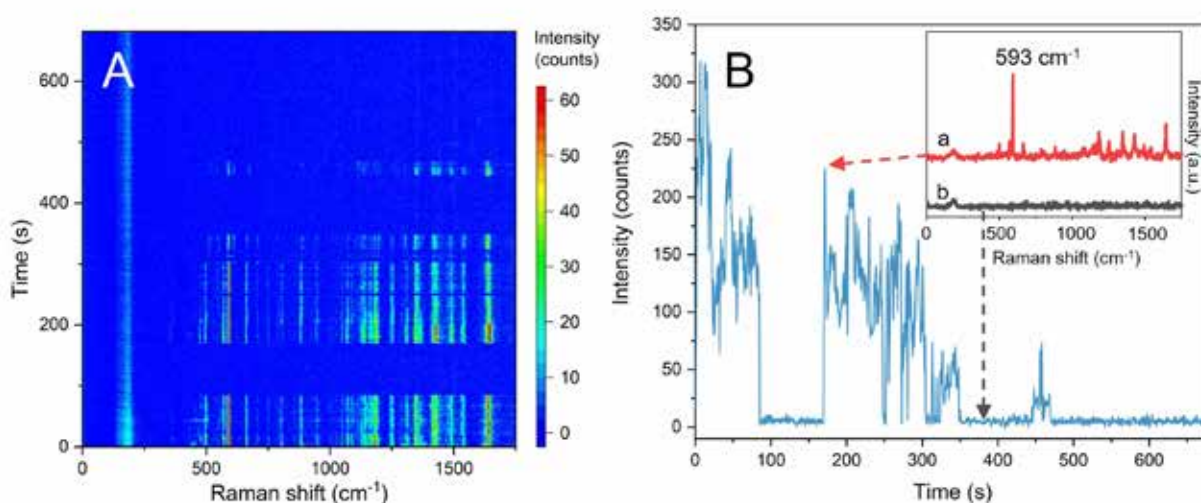
Figure captions:

Temporal evolution of Nile blue SM-SERS spectra(A) and time evolution of the integral intensity of the 593 cm−1 peak(B) with 0.5 s exposure time. The inset shows the spectra at specified time points.

Keywords: SERS, single-molecule, intensity fluctuations, molecular dynamics, photobleaching

C-P.8

Title: *Structural changes of bleomycin-treated chromosomes*

Author: Marta Urbańska[1], Kamila Sofińska[1], Michał Czaja[1], Krzysztof Szymoński[2], Katarzyna Skirlińska-Nosek[1], Sara Seweryn[1], Dawid Lupa[1], Marek Szymoński[1], Ewelina Lipiec[1]

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Abstract:
Chromosomes are intranuclear structures that are made of tightly packed DNA and histone proteins. Their primary function is to carry genetic information. Naturally, DNA is susceptible to numerous types of damage. If unrepaired or repaired incorrectly, they can lead to mutations and chromosomal aberrations or even cell death [1]. Here, we studied chromosomes isolated from HeLa cells under influence of anticancer drug – bleomycin. This genotoxic agent leads to single- and double-strand breaks of DNA double helix. To assess occurring molecular and morphological changes, we used two complementary techniques. Atomic force microscopy enabled the observation of chromosomal morphology alterations, followed by Raman microspectroscopy to detect chemical changes in the chromatin [2]. We focused on alterations at the level of the single chromosome. Spectra corresponding to chromosome I/chromatin were extracted using convolutional neural network (CNN), which were further analysed with the principal component analysis (PCA) algorithm. Such a complex approach revealed several changes in the chromatin of individual chromosomes I upon treatment with various concentrations of bleomycin, including: DNA conformational transitions, alterations in methylation level and increased protein expression.

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Raman hyperspectral map of chromosomes
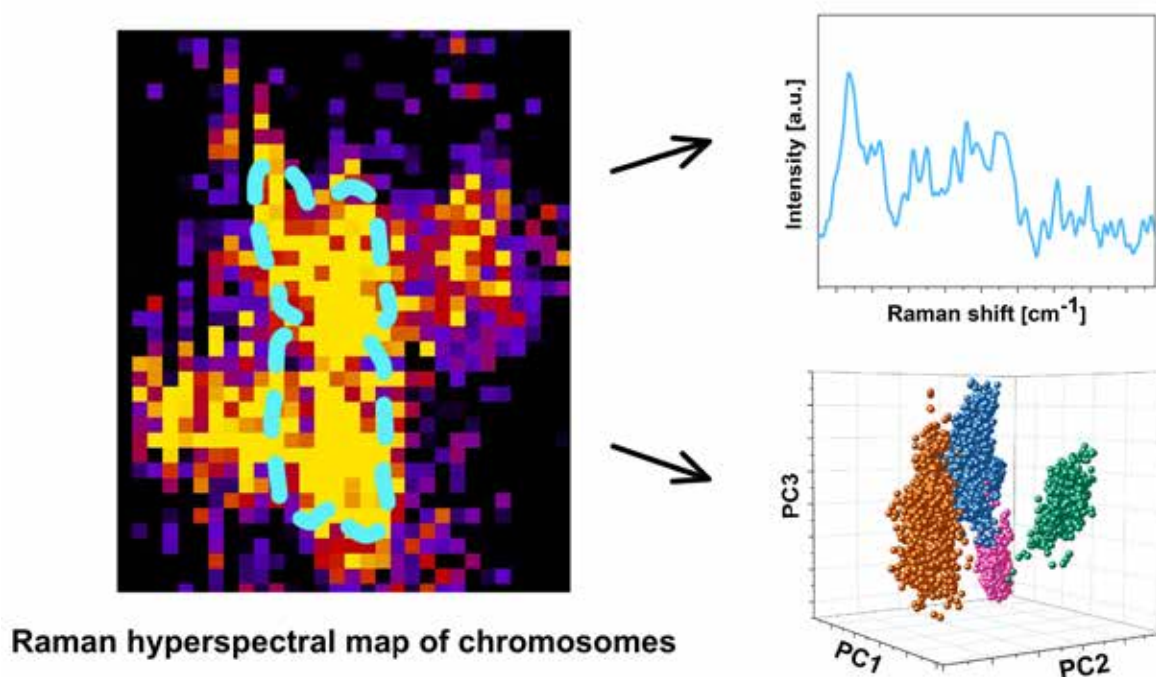
Figure captions:
Spectra acquired from single chromosomes were extracted and analysed using principal component analysis (PCA).

Keywords: bleomycin, chromosomes, Raman spectroscopy, AFM

C-P.11

Title: *A new approach in the SERS blinking analysis*

Author: Beata Wrzosek[1], Yasutaka Kitahama[2], Yukihiro Ozaki[3]
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Abstract:
The SERS blinking phenomena was analyzed by the power low analysis only on spherical nanoparticles[1,2] until this study[3]. An attempt to analyze this phenomenon on anisotropic nanoparticles forced a revision of the equations describing the probability of the dark state distribution against the duration time and the addition of the β parameter as the one controlling the nature of the truncation of the dark state plot. As a consequence, two new types of SERS blinking treatments of truncated power low analysis were created – β self-fitting and multitruncation. They allowed to identify several types of systems in which the SERS blinking was recorded. A thorough analysis of the power low analysis parameters α_{on}, α_{off}, τ and β dependencies, together with the imaging of these dependencies with the optical trapping energy well features: depth, thickness and periodicity, enabled to match these systems to the individual types of hot spots.
The analysis of one of those hot spots allowed additionally to observe the influence of remaining on the surface after the nanoparticles synthesis cyclodextrin molecules on the dark state parameters. In other cases, analysis suggested the formation of an artificial periodicity of the optical trapping energy well by the vicinity of the hot spots of varying strength.
The results of analyzes using the multitruncation fitting also showed the distinction of complex hot spot systems and the ability to switch between them during a single blinking cycle.
The both new analysis treatments allowed not only to reveal the anisotropic nature of the system, but motivated to revise the definition of the time required to overcome the energy barrier from a dark to bright state and the energy barrier itself.

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Acknowledgments:

$$\log_{10} P_{off}(t) = \log_{10}[A_{off} t^{\alpha_{off}} \exp(-(t/\tau)^{\beta})]$$

$$\log_{10} P_{off}(t) = \log_{10}[A_{off} t^{\alpha_{off}} \{\exp(-(t/\tau_1)^{\beta}) + \exp(-(t/\tau_2)^{\gamma})\ldots$$
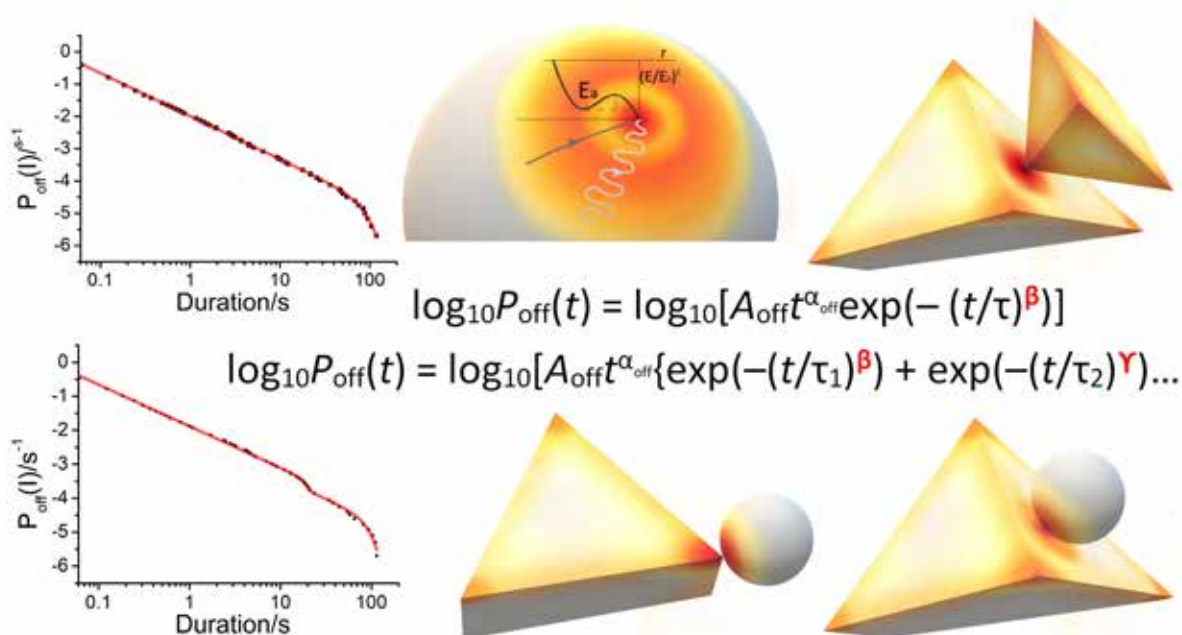
Figure captions:
Exemplary plots and equations of the dark state, nanometer-ordered periodic EM field distribution around the junction of NP dimer and possible hot spot arrangements involving anisotropic NP.

Keywords: SERS blinking, power low analysis

D-P.1

Title: *Evaluation of SERS substrates through average and high fluctuation regimes*

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[2]Metrology Research Centre, National Research Council Canada, Ottawa, ON, Canada
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Abstract:
SERS is a well-known approach for boosting the number of Raman scattered photons by up to 10^10 times. This is accomplished by exciting surface plasmon polaritons (SPPs) at the surface of metal nanoparticles/nanostructures, resulting in highly concentrated local electrical field hotspots (HS) beyond the diffraction limit. Inside a strong HS, a probe molecule generates a SERS signal that may be measured instrumentally. Despite the fact that the enhancement factor (EF) is frequently used to assess SERS substrates, its wide variety of definitions, experimental and theoretical approaches, and lack of a temporal and spatial resolution makes it a poor predictor of substrate performance.
The goal of this research is to develop a more comprehensive set of standards for assessing and contrasting SERS substrates based on the temporal and spatial fluctuations of the SERS signals. While analyzing signals in the average SERS (Av-SERS) domain can give information about the HSs' collective behavior, the high fluctuation SERS (HF-SERS) regime, where the signal is generated by a smaller number of molecules, can reveal information on the characteristics of a particular HS.
Two commercial Au-SERS substrates (A and B) are compared in this study using a variety of criteria. New evaluation parameters are used to measure hotspot strength, density, and uniformity. This study also provides a thorough framework for comparing the substrates and evaluating their effectiveness on a variety of criteria (EF).
The project also involves work on scaling performance and obtaining models to predict the unknown physical characteristics of substrates. These tasks are done in order to introduce the "ideal SERS-substrate" concept and simulate the SERS substrate signal. In various time scales, the simulation reproduces the output SERS signal (microsecond scale for confocal data and seconds for Raman microscope).

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Keywords: SERS substrate, SERS, Metrology

D-P.2

Title: *Dual-tag paradigm in SERS analysis for removal of antibiotics and dyes from waste water treated with biogenic carbonate powder nanoparticles*

Author: Ilirjana Bajama[1], Geza Lazar[1], Fran Nekvapil[1], Tudor Tamas[2], Lucian Barbu-Tudoran[3], Simona Cinta Pinzaru[4]

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[3]Electron Microscopy Centre, Babeș-Bolyai University, Clinicilor 5-7, 400006 Cluj-Napoca, Romania, Advanced Research and Technology Center for Alternative Energy, National Institute for Research and Development of Isotopic and Molecular Technologies, Dona
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Abstract:
The porous biogenic carbonate from crustaceans is a highly ordered 3D nanostructure of pores and channels, ideal for efficient adsorption of solutions. Consequently, powdered material could be turned in formulations for developing new and efficient drug carriers for slow release[1], improved veterinary pharmaceutical formulation[1,2,3] with beneficial calcium and antioxidants supply2 or sustainable and ecological biostimulants4 for soil amendments and improved crop yield with resistance to abiotic stress. In such applications SERS techniques was successfully employed for quantitative analyses, to evaluate the slow released substances from loaded biogenic material in pellet or tablet formulations when exposed to water. In a new formulation of doxycycline hyclate (DOXY) loaded in biogenic carbonate powder, the drug released from tablet showed an increased SERS signal characteristic of drug along six hours of consecutive tests of water solution where tablets were suspended. Moreover, the SERS of DOXY is concentration dependent, higher bands developing at 1333 and 1578 cm^{-1}, when concentration increased in the time of release, indication the re-orientation of the doxycycline adsorbed on the surface of AgNPs2. We exploited here this SERS feature to probe the extended application of SERS for waste waters effective control, potentially comprising dual pollutants such as antibiotic and dyes. The SERS feature of wastewater comprising methylene blue (MB) with strong SERS marker at 1623 cm^{-1} and DOXY above discussed is exploited here to probe the applicability of biogenic powders originated from crustaceans as effective adsorbent of dual compounds from waste waters. Understanding SERS dependencies in such approach proven 1) an innovative approach to extend the environmental applicability of biogenic waste materials; 2) to empower SERS as an effective tool for waste waters control; and 3) to recover the spilled dyes and antibiotics in porous biogenic material.

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Figure captions:
Fig. 1. Graphical design of the concept of empowering SERS as effective tool for waste water management and remediation using biogenic carbonate powders.

Keywords:
Raman, SERS, Doxycycline hyclate, Biogenic carbonate, Methylen blue

D-P.3
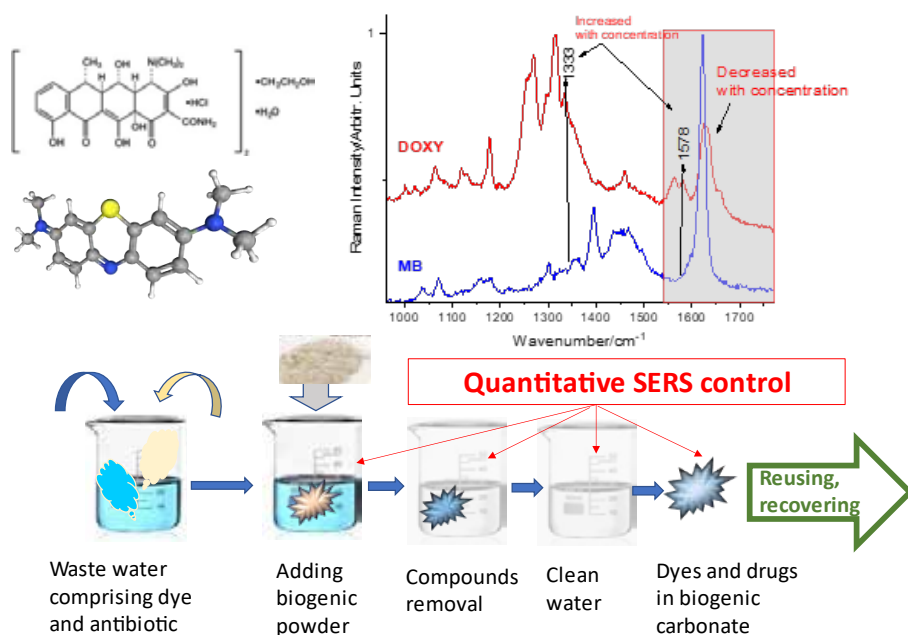
Title: *AFM-based non-gap mode tip-enhanced Raman spectroscopy (TERS)*

Author: <u>Yifan Bao</u>[1], Sen Yan[2], Jun Wang[2], Mengyuan Zhu[1], Xiang Wang[3], Bin Ren[3]

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Abstract:
Tip-enhanced Raman spectroscopy (TERS), which combines the scanning probe microscopy with plasmon-enhanced Raman spectroscopy, can simultaneously obtain the topography as well as the chemical information of the sample with a nanoscale spatial resolution. This powerful technique has been applied to the study of single molecules[1], materials[2] and catalysis. In most of TERS studies, Au, Ag or Cu substrate is used to couple with the TERS tip (so called gap-mode TERS) to obtain a higher sensitivity and a higher spatial resolution. However, this gap-mode TERS limited the TERS study to the thin samples on the Au (Ag or Cu) substrate. In recent years, many researchers have focused on the fabrication of TERS tips with higher enhancement to achieve TERS study of any sample on any substrate (non-gap mode TERS)[3]. In this work, we combined focused ion beam (FIB) nano-fabrication and electrochemical deposition to fabricate non-gap mode TERS active AFM-TERS tips. A groove structure was introduced by FIB milling to the commercial Si AFM tips, then Au layer was deposited by electrochemical deposition. The activity of the TERS tip could be modulated by the distance between the groove structure and the tip apex. With this groove-AFM-TERS tip, around 6 times enhancement of the Raman signal was obtained for WSe_2 on SiO_2. We believe the successful fabrication of non-gap mode TERS tips can help the development of TERS and broaden its applications.

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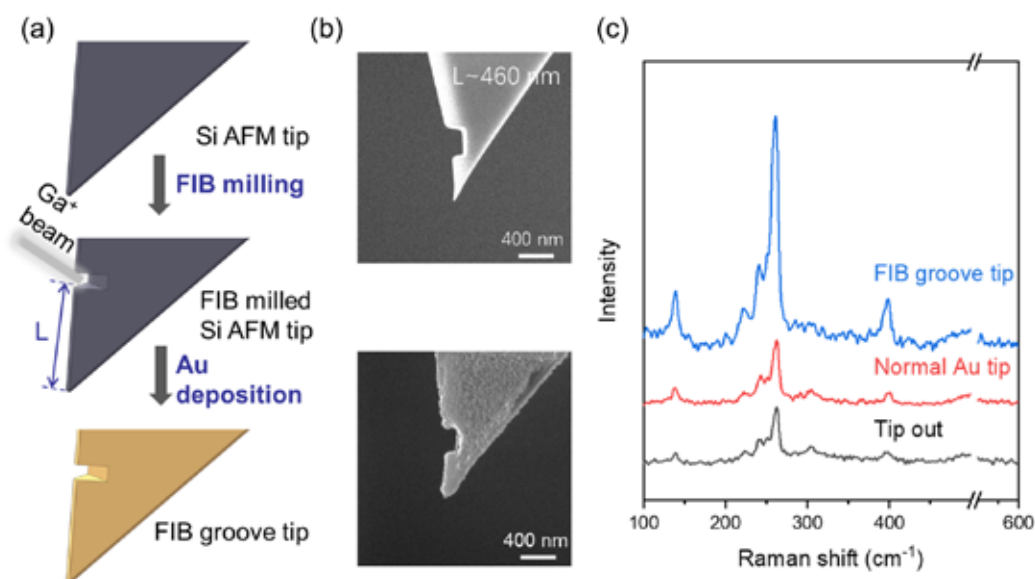
Figure captions:
(a) Schematic illustration of the fabrication process of FIB groove-AFM-TERS tip. (b) SEM images of the FIB milled AFM tip. (c) TERS performance of the TERS tips.

Keywords: Tip-enhanced Raman spectroscopy (TERS), TERS

D-P.4

Title: *Application of Resonance Raman Spectroscopy for label-free differentiation of ferrous and ferric cytochrome c*

Author: Amanda Bartkowiak[1], Elzbieta Szczesny-Malysiak[1], Stefan Chlopicki[1], Jakub Dybas[1]

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Abstract:
Nitric oxide (NO) is a highly reactive signaling molecule, involved in vascular homeostasis[1]. The bioavailability of NO can be controlled by heme proteins, such as cytochrome c (Cc) present inside the mitochondria of endothelial cells (ECs), which build the vessel walls. Depending on the oxidation state, ferric (Fe^{III}) hemes permit NO diffusion and signaling, while ferrous (Fe^{II}) hemes scavenge and shut off the vasodilatory properties of NO[2,3]. Up to date, different methods were employed to study ferrous-NO interaction, however, no technique was sensitive enough to deliver information on ferric-NO. In this work, we present an innovative approach based on rR imaging with 405 nm excitation wavelength – which will ensure strong resonance enhancement of the recorded Raman signal and will provide simultaneous spatial characterization and differentiation of ferrous and ferric hemes. The rR imaging methodology was applied to observe ferrous to ferric Cc transition in four ECs lines with subsequent differentiation between ferrous and ferric species using chemometric methods. The ECs were treated for 1h with calcium ionophore A23187 to stimulate NO release, which caused oxidation of Cc either by NO bounding or through peroxynitrate oxidation pathway. In turn, the increase in ferric Cc was abolished by L-NAME addition, which blocked NO production. Fig. 1 demonstrates an increase in the amount of Fe^{III} after addition of ionophore. On the other hand, a decrease in ratio of Fe^{III} to the total amount of Fe was seen after the addition of NO inhibiting agent (L-NAME) in a dose-dependent manner.
In summary, the applied 405 nm excitation wavelength was proved to be successful in distinguishing between ferrous and ferric hemes within single ECs. The rR studies proved to be useful in detecting subtle molecular alterations in the amount of ferric part within the Cc, directly revealing strong interaction of the NO molecules with the heme active site.

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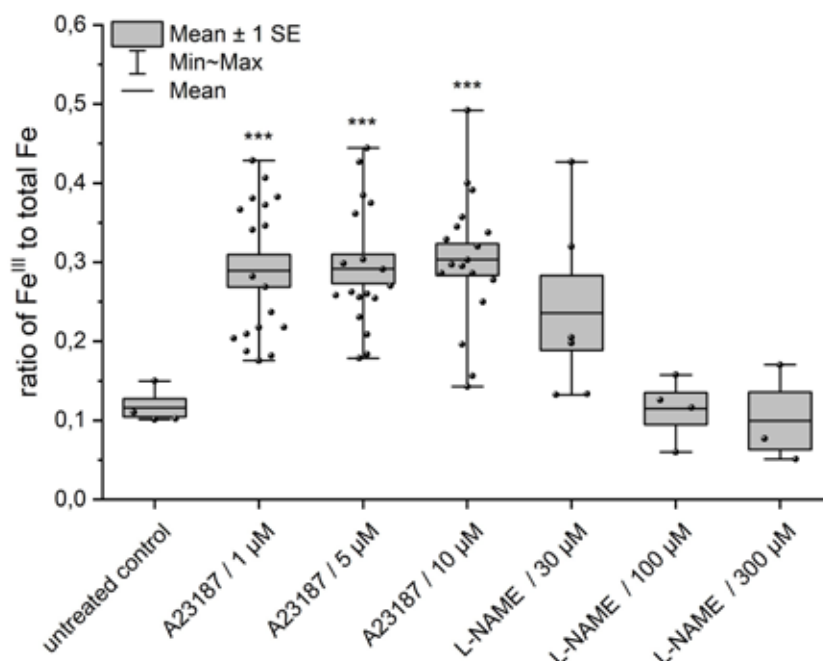
Figure captions:
Fig. 1. Ratio of ferric Cc (FeIII) to total Fe-heme found inside Cc in HAECs detected by rR imaging with 405 nm excitation wavelength.

Keywords: ferric hemes, nitric oxide, endothelium

D-P.5

Title: *Novel nanoplasmonic gas sensor operated based on plant respiration*

Author: Yun Sik Choi[1], Won Ki Son[1], Dae Hong Jeong[1]

[1]Seoul national university

Abstract:
Demands on efficient gas sensors have been uprising from industrial, medical and environmental monitoring field. Especially, detecting trace amount of hazardous gas considered as key challenge to establish environmental safety and public health[1]. Nowadays, electronic gas sensors(electric nose) and chemically specific gas sensors(chemical nose) are in use. Gas sensors for above usages need to be capable of detecting low-concentration gas with high conveniency, but lack of sensitivity and complex instrumental structure limit their usage on practical gas sensor[2]. Moreover, those sensors possess fundamental limitation in that essentially accompany separate configurations for gas collection and concentration. Herein, to overcome above problems and build effective gas sensor for trace amount of analytes, we suggest plant-based nanobionic gas sensor which is capable of detecting gas as well as analyte collection&concentration.
After surface modifications with polymer which stabilize nanoparticle and specifically capture targeted molecule, plasmonic NIR-nanoprobe was injected into plant leaf through by infiltrating through stomata. During the breathing process of plant taking place, analyte gas comes in and emitted out along with other gases. While other gases without specific interaction on nanoprobe exhaled from plant, targeted gas molecule was attracted by polymer(or nanoprobe itself) and concentrated at the surface of nanoprobe. After capturing of targeted gas, by using plasmonic feature of NIR-nanoprobe, SERS signals of analyte was obtained through Raman mapping.
Our plant-based nanobionic gas sensor enables sub-ppb level detection and rapid detection within few minute for certain gaseous analytes. By varying the surface modification strategy, our group proved practical usage of such sensor on detecting gaseous nicotine and plant communication molecule.

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Keywords: Gas sensor, Nanoplasmonic, Plant-based sensor

D-P.6

Title: *Monitoring plasmon-mediated chemical reactions on immobilized noble metal nanoparticles*

Author: <u>Lars Dannenberg</u>[1], Janina Kneipp[1]

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Abstract:
Noble metallic nanostructures can efficiently catalyze chemical reactions by the decay of localized surface plasmon resonances (LSPR), which are generating charge carriers and simultaneously provide high local fields for surface-enhanced Raman scattering (SERS) investigations. The choice of plasmonic structures for a plasmon-assisted reaction and to characterize them precisely plays an important role in elucidating the physical processes and the chemical mechanisms. Well-known reactions, such as the conversion of 4-aminothiophenol to 4,4′-dimercaptoazobenzene, demonstrate the potential of plasmonic catalysis.[1] The disadvantage of nanoparticle solutions is that the spatial resolution of the reaction is not accessible because the particles move in and out of the focal volume. Moreover, the stability of the particle suspension decreases in the presence of molecules and reaction products. In addition to the temporal resolution that can be examined with vibrational spectroscopy in a droplet, also spatial information can be obtained by in spectroscopic experiments with plasmonic nanoparticles immobilized on a functionalized surface.[2] The interaction between a functional group on a solid substrate and the nanoparticles keep them attached to the surface. In addition, a well-defined and characterized surface with immobilized particles can lead to more reproducible results compared to measurements in solution. We present our results on how the particles can be immobilized and on a plasmon-supported reaction that we characterize with temporal and spatial resolution. The immobilization of the nanoparticles in combination with well-chosen plasmon-assisted reactions can lead to new and efficiently fabricated materials.

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[1] Z. Zhang, V. Merk, A. Hermanns, W. E. S. Unger, J. Kneipp, ACS Catalysis 2017, 7, 7803-7809.
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Keywords: Photocatalysis, Immobilization, SERS, hot-electrons, spatial-resolution

D-P.7

Title: *Defects in polymer multilayer films: a new way to investigate based on Raman microscopy*

Author: Céline Eypert[1], Thibault Brulé[1], Ludivine Fromentoux[1]

[1]Horiba France SAS

Abstract:
Polymer multilayer films are presents everywhere in our world. Thus we can found them in food packaging, on car coatings, in phone protection films, among many other applications. But their characteristics are questioning as soon as a defect is present. Unfortunately, it's not easy to locate this defect, and consequently chemically characterize and identify it.
Confocal Raman microscopy is a perfect candidate for such issue, combining the high spatial resolution of optical microscopy with the chemical identification through the spectral characterization. Moreover, we demonstrate in this paper how the QScan patented-technology is a great improvement for defect investigation applying this tool on the analysis of a defect in a multilayer polymer film.
This combination makes our Raman confocal microscope the ideal solution for non-destructive highly resolved characterization of the defect realizing a very fast survey mapping of the sample.
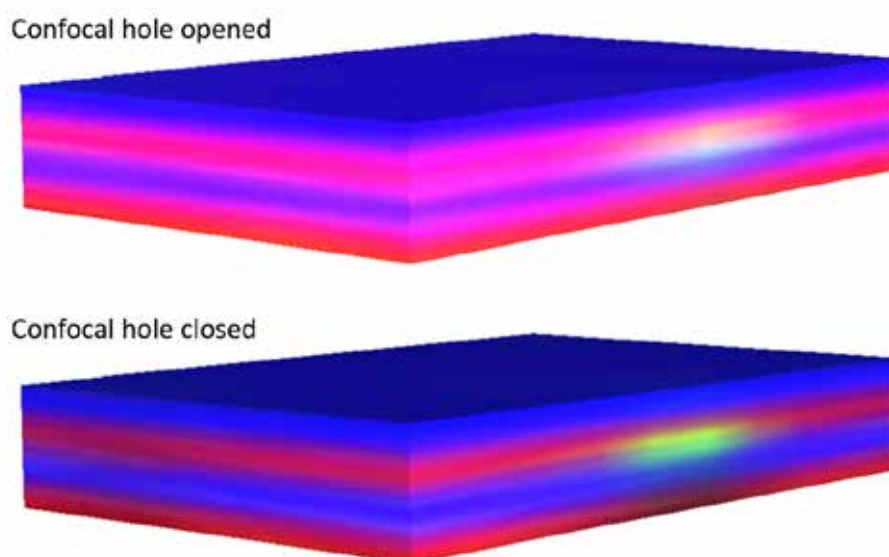
Figure captions:
Figure 1: 3D Raman map of an feature in a multilayer polymer sample. Blue: Plastic tape. Red: Glue. Green: Feature. Dimension: 500x500x100μm.

Keywords: Polymers, Multilayers, Defect, confocal, imaging

D-P.8

Title: *Effect of laser power on SERS Stokes and anti-Stokes intensities*

Author: Sahar Gholami Milani[1], Filomeno Soares De Aguiar Junior[2], Michele L. de Souza[3], Sanker Timsina[1], Rogerio De Sousa[1], Alexanre Brolo[2]

[1]Department of Physics and Astronomy, University of Victoria, Victoria, CA
[2]Department of Chemistry, University of Victoria, Victoria, CA
[3]1Department of Chemistry, University of Victoria, Victoria, CA 2Universidade Federal Fluminense, Rio de Janeiro, Brazil

Abstract:
Raman Spectroscopy is a technique used in the structural investigation of molecules and materials. Raman scatting measures the interaction between the electromagnetic field and molecular vibrations[1]. Depending on the initial vibration quantum state of the molecule, the process can be classified as Stokes (S) and anti-Stokes (aS) scattering [2]. Raman scattering has a low cross-section, but there are processes that increase the Raman efficiency. Surface-enhanced Raman scattering (SERS) is one of those approaches, since it significantly improves the Raman efficiency making even the detection of single molecules possible [3,4].
It has been demonstrated that Raman scattering supports nonclassical Stokes/anti-Stokes (SaS) photon pair production [6,7]. However, the photon yield of the SaS effect is very low, but that can be increased by taking advantage of the SERS effect. In this case, the SERS substrate acts as a nonlinear medium to generate correlated SaS pair production. Two incoming photons must interact simultaneously with the molecule to create two paired photons that are correlated in both time and energy, as a result, the aS photon's probability varies quadratically with the input power.
In this work, we analyze the effect of the incident power on the strength of the Stokes and anti-Stokes SERS scattering to gain insights into the SaS production mechanism. In the experiments, a self-assembling monolayer of Ag NPs mixed with a molecular probe are used as a SERS substrate [8].
Figure 1 shows a representative result of the area under the 1080 SERS peak plotted over the laser power. It indicates a linear dependence of the Stokes intensity with the laser power, whole the anti-Stokes intensity shows a quadratic behavior, supporting the generation of SaS pairs [9] . The results indicate that the aS signal demonstrates a nonlinear behavior after a certain power implying the contribution of the SaS process.

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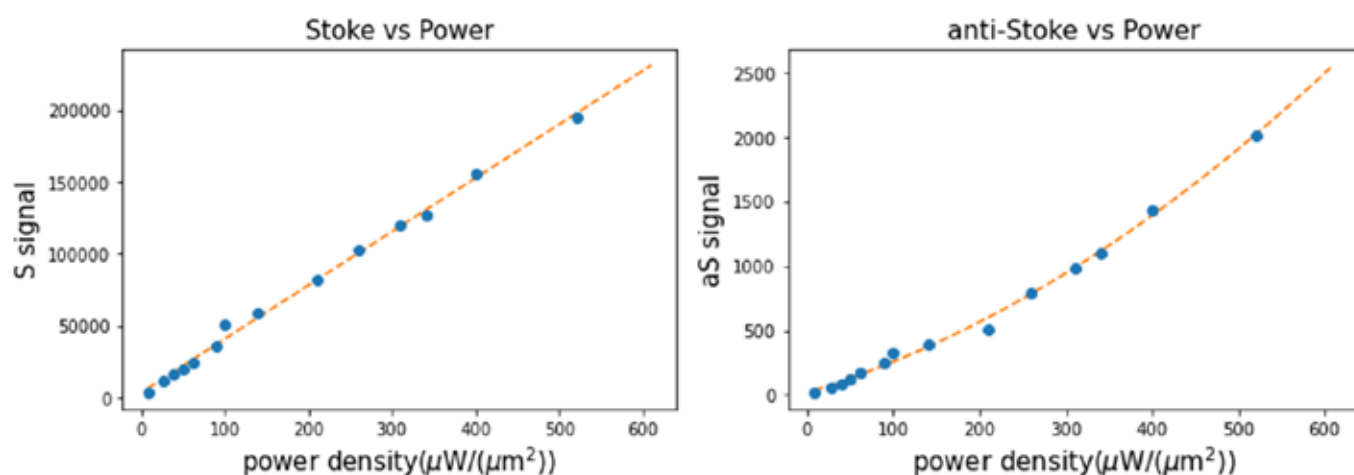
Figure captions:
Figure1. Power dependency of S and aS SERS signal

Keywords: Power dependence in SERS

D-P.9

Title: *Charge Transfer in π-Conjugated Organic Semiconductors Enhanced by Molecular Orbital Delocalization*

Author: Shuang Guo[1], Yeonju Park[1], Eungyeong Park[1], Sila Jin[1], Lei Chen[2], Young Mee Jung[1]

[1]Kangwon National University
[2]Jilin Normal University

Abstract:
π-Conjugated organic semiconductors are promising materials for surface-enhanced Raman scattering (SERS) active substrates based on the tunability levels of electronic structures, molecular orbitals, and solid-state stacking modes. In this study, we investigate the effects of the temperature-mediated structure transitions of PEDOT molecules in poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) films on the interactions between the substrates and probe molecules, thereby affecting the SERS activities of p-conjugated organic semiconductors. The results of absorption spectroscopy and density functional theory calculations show that this effect occurs mainly due to the molecular energy level and the delocalization of the electron distribution in the orbital caused by the molecular structure transition, effectively promoting the charge transfer between the semiconductor and the probe molecule. We study the influences of the delocalization of electrons in molecular orbitals on the SERS activity of substrates for the first time; additionally, we provide some guiding significance for the development of highly sensitive SERS substrates.

Keywords: π-conjugated organic semiconductor, molecular structure

D-P.10

Title: *Optical characterization of polycrystalline silver halide materials by infrared spectroscopy – long- and short-term water adsorption in extruded fibers*

Author: H. Michael Heise[1], Sven Delbeck[1]

[1]SOUTH-WESTPHALIA UNIVERSITY OF APPLIED SCIENCES

Abstract:

Infrared (IR) optical fibers are transmitting radiation with wavelengths greater than approximately 2 μm. Such wave-guides are needed for IR-spectroscopy with remote sensing applications. Besides this, there is an increasing need also for flexible fiber delivery systems for transmitting CO_2 laser radiation in surgical applications. A variety of infrared transparent materials and fibers are available, which include heavy metal fluoride glass and polycrystalline fibers as well as hollow waveguides. While none of these fibers have physical properties even approaching those of conventional silica fibers, they are, neverthe-less, useful in lengths of up to 3 m for a variety of IR sensor and power delivery applications.

Advances in the quality of silver halide fibers and their extrusion with different cross-sections enabled the construction of sensor probe heads suited for process monitoring with a fast sample measurement within the wavelength region from 3 μm to 20 μm [1, 2]. The polycrystalline silver halide material, as provided by several suppliers, is water insoluble and shows also favourable ductile properties [3]. The different materials were characterized by scanning electron microscopy. Over a period of several years, we monitored the water adsorption in core-only fibers of different lengths as stored under ambient labora-tory atmosphere by Fourier Transform spectroscopy. Band positions from stretching and bending modes of water have been compared with band wavenumbers of solid, liquid, vapour-phase and matrix-isolated water. Especially, an additional absorp-tion band below the deformation fundamental vibrational band at 1400 cm^{-1} is evident, which can be uniquely assigned to water, since deuterated water isotopes show band shifts due to the mass differences accordingly, but still needs a theoretical framework for assignment and interpretation.

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Keywords: silverhalide fibers, water adsorption, assignment

D-P.11

Title: *Designing Ag/MXene Nanocomposites for SERS Study*

Author: Zhi Yang[1], Shuang Guo[2], Lei Chen[1], <u>Young Mee Jung</u>[2]

[1]Jilin Normal University
[2]Kangwon National University

Abstract:
MXene and its derivatives, as a new two-dimensional (2D) material, have great application potential in the field of surface-enhanced Raman scattering (SERS). Here, we used a simple self-reduction strategy to modify silver nanoparticles (Ag NPs) on the surface of ultra-thin 2D MXene nanosheets, which can prevent the oxidation of Ag NPs. SERS signals are enhanced by the synergistic effect of chemical and electromagnetic mechanisms between Ag NPs and MXene. This study shows that Ag NPs/MXene substrate combines the characteristics of both Ag NPs and MXene, and Ag NPs are anchored on MXene nanosheets to effectively enhance SERS signal. A new experimental strategy has been developed to promote the controllable synthesis of noble metal nanoparticles on MXene, providing ideas to further improve the practical application of SERS detection function.

Keywords: MXene, nanoparticle, SERS, two-dimensional material

D-P.12

Title: *Atomic-scale dynamics in plasmonic hotspots: fast SERS of picocavities*

Author: Paul Kerner[1], Bart De Nijs[1], Marlous Kamp[2], Jeremy J Baumberg[1]

[1]Cavendish Laboratory, University of Cambridge
[2]Van 't Hoff Laboratory of Physical and Colloid Chemistry, Utrecht University

Abstract:
In this project we focus on fast dynamics of "picocavities" – adatoms on plasmonic metal surfaces that form subnanometric surface-enhanced Raman scattering (SERS) hotspots[1,2]. They are often seen in gold nanoconstructs such as nanoparticle-on-mirror (NPoM)[3], where they manifest as new fleeting SERS spectral lines, owing to light enhancement in a volume overlapping a single Raman-active molecule with temporarily altered vibrational modes.
Picocavity dynamics have so far been extensively probed mainly at over 100 ms timescales[4,5]. This has resulted in limited understanding of the single atom/molecule dynamics at play, but there is potential to uncover more. The project aims to extend the timescale of the studied dynamics by approaching the limits of light detection instrumentation. Time-resolved SERS measurements are performed with a fast detector to study Raman intensity fluctuations and extremely short events, as well as infer adatom-molecule dynamics from the statistics. NPoM derivative superefficient plasmonic nanoarchitectures for Raman kinetics (SPARKs)[6] are used. While 100s of picocavity events have been recorded at fast timescales, the project is in the stage of experimental and analysis method development. The main experimental challenges involve producing reproducible samples that provide sufficiently large amounts of SERS signal for fast detection and pushing the limits of the available detector systems for best signal-to-noise ratios. The analysis must overcome the inevitable noise limitations and extract the interesting single atom dynamic information from the large time-series datasets.
This work would provide a look into ambient atomic-scale dynamics via visible light, usually relegated to the invasive electron beam techniques and extreme environments. It would also be a stepping stone towards applications like sensing on a single molecule level, probing and control of chemical reaction and catalytic mechanisms and atomic-scale microelectronics.

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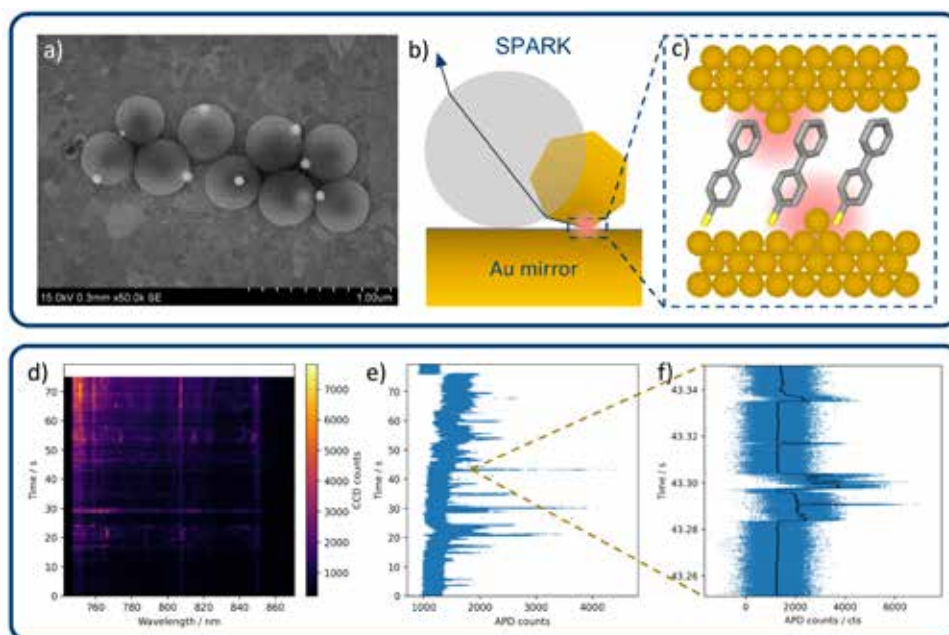
Figure captions:
a) SEM of a SPARK cluster. b) SPARK schematic. c) Nanogap with biphenyl-4-thiol and picocavities. d) Typical SERS timescan of a SPARK. e) Corresponding fast SERS trace. f) Zoom in on a bright event.

Keywords: SERS, picocavities, time-resolved, dynamics, atomic-scale

D-P.13

Title: *SERS detection of shikonin by 'Dyeing' it on gold nanofilm*

Author: Kyunghun Kim[1], <u>Sungjun Kwak</u>[1], Dae Hong Jeong[1]

[1]Seoul National University

Abstract:
We developed a spectroscopic detection method of target molecules by combining surface-enhanced Raman spectroscopy (SERS) and a dyeing method. Mimicking the surface of cellulose fiber, gold nanofilm (AuNF) surface was modified with hydroxyl group, and then treated following a dyeing process to form a mordant-mediated dye ligand complex on the AuNF. Then, the colloidal gold nanoparticles (AuNPs) were dropped and dried to make SERS hot-spots, where shikonins were located at the interstitial space between AuNP and AuNF. The SERS enhancement factor (EF) of 4-mercaptobenzoic acid (4-MBA) between AuNP and AuNF was calculated to be 4.4×10^7. The SERS heat map of 'dyed AuNP-on-AuNF' substrate showed that shikonins were well bound on AuNF surface in the same way as the dyeing of dyes on cellulosic fibers. As such, the signal-to-noise ratio (SNR) of SERS was 2.5 times higher than a conventional substrate by salt-induced metal NP aggregates. This method could be applied to various molecules that can be chelated to metal ion, not just dyes. Furthermore, since the method is similar to IMAC (immobilized metal affinity chromatography), there is the possibility that proteins can also be captured and their SERS signals can be identified.

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red dyes of Japanese origin alkannin and shikonin. Spectrochim Acta A Mol Biomol Spectrosc, 2022.265: p. 120382.

Keywords: Shikonin, Metal-coordinated bond, SERS, NanoParticle-on-Mirror

D-P.14

Title: *Spectroscopic analysis of GPE using two types of biodegradable PHBHx polymer with different crystallinity*

Author: Sujin Lee[1], Yeonju Park[2], Isao Noda[3], Young Mee Jung[1]

[1]Department of Chemistry, Institute for Molecular Science and Fusion Technology, Kangwon National University
[2]Kangwon Radiation Convergence Research Support Center, Kangwon National University
[3]Department of Materials Science and Engineering, University of Delaware

Abstract:
As the range of use of batteries is expanded, various problems that occur due to the use of a large amount of batteries need to be solved. Among them, environmental pollution caused by waste batteries, leakage of liquid electrolytes, and low safety are issues that are very close to our real life.
 In this study, a biodegradable poly(hydroxybutyrate-*co*-hydroxyhexanoate) (PHBHx) gel polymer electrolyte (GPE) was prepared to solve the problems of environmental pollution and liquid electrolytes. PHBHx is an eco-friendly polymer capable of biodegradation in soil and seawater, and since the liquid electrolyte is encapsulated in GPE [1], the liquid electrolyte does not leak well and is safe. Two types of PHBHx with different crystallinity were used in the preparation of GPE, and their chemical properties at the molecular level were investigated by Raman and IR spectroscopies. Their electrical properties were also examined by impedance spectroscopy. Details of the results will be discussed in this presentation.

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Keywords: Gel polymer electrolyte, Biodegradable polymer, Lithium-ion battery, Raman, IR

Title: *Chiral-Induced Surface-Enhanced Raman Optical Activity on Nanoparticle-on-Mirror Substrate*

Author: <u>Sung Gun Lee</u>[1], Dae Hong Jeong[1]

[1]Seoul National University

Abstract:
Raman Optical Activity (ROA) has emerged as a valuable tool for characterizing stereostructural changes in both organic and inorganic compounds, offering complementary steric information to traditional spectroscopic methods. In particular, ROA has garnered attention as a chiral-selective method for analyzing biomolecules such as nucleic acids, and peptides, overcoming the limitations of conventional spectroscopy. However, due to the inherently low cross-section of ROA, which is three to five orders of magnitude lower than typical Raman, achieving sufficient signal sensitivity remains a challenge. To address this issue, efforts have been made to measure ROA signals using SERS, which utilizes plasmonic materials to enhance the Raman signal of analytes via the electric field concentrated around nanoparticles (SEROA). However, biomolecules typically have low Raman cross-sections and poor signal stability at high laser intensity and long measuring times, limiting the utility of SEROA. Recently, chiral-induced ROA has been proposed as a breakthrough, leveraging the "sergeants-and-soldiers" principle to induce chirality in a trace chiral compound, enabling the measurement of chirality with shorter measuring times and lower laser intensities[1].
This study demonstrates the possibility of a nanoparticle-on-mirror (NPoM) substrate and measurement configuration for ROA, which isolates hotspots from particle aggregation. To confirm the reliability of the NPoM configuration for ROA, chirality was induced in a self-assembled monolayer of 4-mercaptopyridine on a gold nanofilm surface.

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Keywords: SERS, ROA, NPoM

D-P.16

Title: *Effects of polystyrene nanoplastics exposure on in vivo-grown Stevia rebaudiana plants*

Author: <u>Loredana Florina Leopold</u>[1], Florina Violeta Scurtu[2], Doina Clapa[2], Nicolae Leopold[3], Stefania Dana Iancu[3], Floricuța Ranga[1], Sonia Socaci[1], Cristina Coman[1]

[1]Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine
[2]Life Sciences Institute, University of Agricultural Sciences and Veterinary Medicine
[3]Faculty of Physics, Babeș-Bolyai University

Abstract:
The aim of this work is the determination of toxicity levels of polystyrene nanoparticles NPs for *Stevia rebaudiana* plant, as well the development of a rapid (in order of minutes) and cost-effective methodology, based on Raman hyperspectral imaging (HSI) for the direct detection and quantification of NP accumulation in plant tissue.
Our research is focused on potential risks associated to polystyrene NPs exposure to plants. We have assessed the accumulation of polystyrene NPs in *Stevia rebaudiana* grown in vitro, and its impact on plant growth, morphology and on a series of plant metabolites (chlorophylls and carotenoids). The plants were exposed to polystyrene NPs (26 nm) at concentrations up to 250 mg/L.
A positive impact on *Stevia rebaudiana* in the presence of low concentrations of polystyrene NPs was observed. The NPs stimulated photosynthesis at low concentration (<10 mg/L). However, at 100 mg/L concentration a clear suppression in photosynthesis was observed. The root morphology and plant length are adversely affected by NPs.
The *Stevia rebaudiana* metabolites were quantified using High-Performance Liquid Chromatography (HPLC). The concentrations of chlorophyll, lutein, zeaxanthin, and beta-carotene showed the same trend upon exposure to increased polystyrene NP levels. Their concentrations increased up to the 250 mg/L NPs dose, more exactly, the concentration of all the investigated analytes increased, compared to controls.
HSI provides beside the optical image of micro-objects a spectral (Vis-NIR extinction or Raman), in each pixel of the map, at a limit of 0.5 μm spatial resolution. The impact of NPs on the plant length and root as well as plant weight can be assigned to a stress response of the plant.
The major economic impact of the developed methodology for imaging, lies in its applicability for screening food matrices at large scale, in order to detect contaminations such as polystyrene NPs, ensuring thus an important tool for food safety and security.

References:
Coman V., et al., Plant Physiology and Biochemistry, 2023, Volume 197, April 2023, 107634

Keywords: nanoplastics, vivo-grown Stevia rebaudiana

Title: *Investigation of the Kaolin-Water Interaction Using Vibrational Spectroscopy*

Author: Sofie Mika[1]

[1]University of Vienna

Abstract:
When water is in contact with other substances, its chemical and physical properties at the interface can deviate from those of bulk water. Interfaces between water and clay, as present in soils, are of high interest for environmental and geophysical processes. [1] Permanently frozen soils are called permafrost soils and are found in cold regions of our planet. In such systems containing water and clay below the melting point of water, a layer of water which is still liquid between ice and soil can be found in experiments and simulations. [2] This so-called pre-melting layer is formed, as the hydrogen bond network of water at the interface is disturbed leading to interface-induced phase transitions. [1] The characteristics of this layer, i.e. is this water more ice or liquid-like, are still under debate. To answer this question, we aim to investigate the vibrational energy dynamics of this pre-melting layer with spectroscopy. As a first step, we investigated with infrared spectroscopy the interaction between water and kaolin by varying the amount of water in contact with the clay. Moreover, we compare the water results with alcohols of varying chain lengths to get further information considering the effect of molecule size on the interaction.

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Keywords: Kaolin-water interaction, vibrational spectroscopy

D-P.18

Title: *The complex electronic structures and properties displayed by charged metal-molecule systems in SERS*

Author: Daniel Aranda[1], Francisco García González[2], Francisco José Avila Ferrer[2], Juan Soto[2], Isabel López-Tocón[2], Juan Carlos Otero[2]

[1]Instituto de Ciencia Molecular (ICMol), Universidad de Valencia
[2]Universidad de Málaga

Abstract:
SERS of cyanine adsorbed on a silver electrode shows two regions which are selected by the voltage and characterized by the differentiated response of the wavenumbers of the v(CN) stretching band. The combination between the experimental SERS and theoretical DFT calculations has allowed for relating the two regions to chemisorbed (C-hybrid, region A) and physisorbed (P-hybrid, region B) surface complexes (see Figure), where cyanide is bonded through the carbon on top of a single silver atom of the surface. The electrode potential selects one or another type of chemisorbed or physisorbed surface complex, which are of different nature having a differentiated response to the applied voltage. Electric potentials tune smoothly the wavenumbers, bond energies, and injected charges of the P-hybrid at more negative potentials than PZC, but the very strong C-hybrid prevents significant changes of these properties at positive excesses of charge.[1] Moreover, DFT calculations on these charged metal-molecule hybrids predict a complex dependence of the energy of the metal-molecule charge transfer states on the chemical nature of the system and, especially, on the effect of applied potentials giving huge or zero energy gain[2,3] depending on the strength of the surface complex, i.e., on the existence of C- or P-hybrids in systems showing a dual electronic structure such as charged molecules bonded to charged metals.
The existence of the dual electronic structure of metal-molecule interfaces might require reinterpreting experiments that are usually discussed by resorting to, for instance, the reorientation of the adsorbate, the formation of complexes with different stoichiometries, the existence of nonequivalent local sites on the surface, or to instrumental artifacts. Moreover, this dual behavior also determines the properties and responses of technological devices where metal-molecule interfaces are involved.

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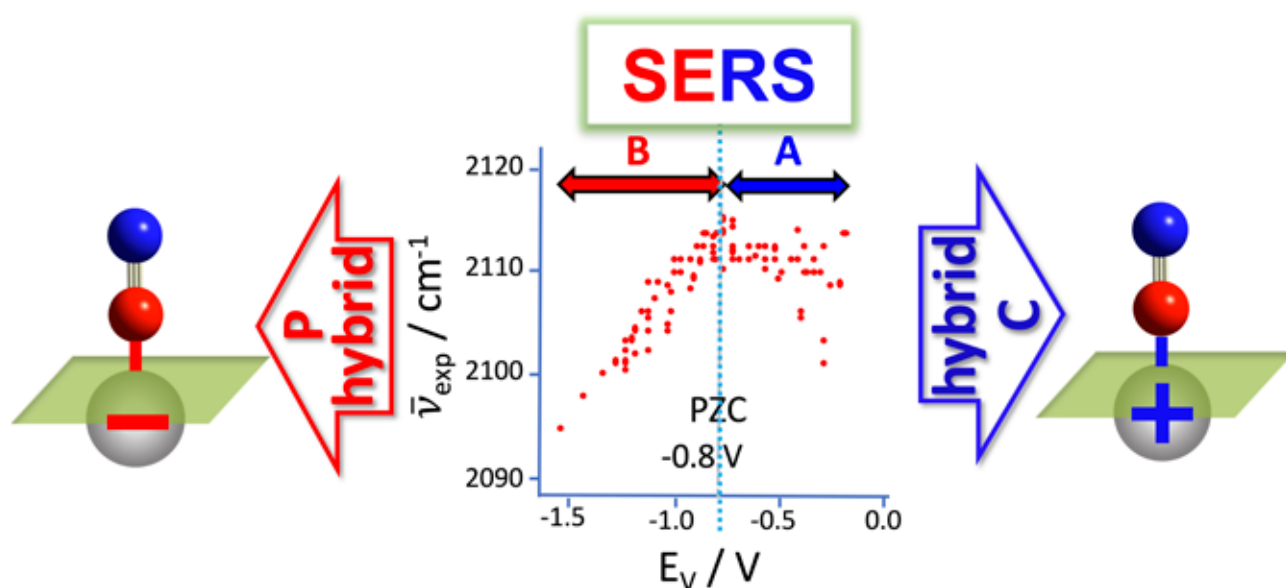
Figure captions:
Effect of the electrode potential on the SERS wavenumbers of cyanide adsorbed on a silver electrode

Keywords: SERS, charge transfer, electronic structure, DFT

D-P.19

Title: *AFM-IR Nanoscale Spectroscopy and Imaging of Intact Cells and their Surface.*

Author: Luca Quaroni[1]

[1]Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Jagiellonian University

Abstract:
I use atomic force microscopy infrared (AFM-IR) with pulsed excitation to perform infrared spectroscopy and imaging measurements on intact eukaryotic cells. I demonstrate the possibility of obtaining images and spectra from the nucleus and some organelles with high spatial resolution, better than the diffraction limit. [1]
I compare the performance of the measurement under resonant and non-resonant conditions. When working under non-resonant conditions, contrast in single wavelength images shows a rich fine structure that is dominated by the mechanical interactions of the tip with the cell surface, not by infrared absorption. In contrast, when working under resonant conditions, it is possible to control the surface selectivity of the measurement by adjusting the structure of laser pulses. I suggest that this effect is at least partially related to the frequency dependence of the penetration depth of thermal waves. [2] Higher pulse frequency allows collecting spectra and images from the surface layer of the cell. Single wavelength resonant images at high frequency show surface structures and domains that range in size from about 20 nm to 1 µm. Although spectral intensity still indicates a dependence from the thermoelastic properties of the surface, resonant spectra of the cell surface are qualitatively comparable to far-field IR spectra. [3]

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3. Quaroni, L., Imaging and spectroscopy of domains of the cellular membrane by photothermal-induced resonance. Analyst, 145 (2020) 5940.

Keywords: AFM-IR, Infrared, Cell Surface

D-P.20

Title: *Hyperspectral angular pattern of the SERS and dark field scatterings of gold nanorods*

Author: Sepehr Razi[1], Abdolvahab Amirsalari[1], Silwester Gawinkowski[1]

[1]Institute of Physical Chemistry, Polish Academy of Sciences

Abstract:

Nanophotonics explores light behavior at the nanometer scale and the interactions between nanometer-sized objects and light. Metallic nanoparticles, known for their unique properties, are among the most frequently studied subjects in this field. The excitation of plasmon resonances in these nanoparticles enables light to be focused at nanometer scales and amplifies light-matter interactions with nearby objects, such as molecules. Consequently, this leads to multiple orders of enhancement in optical signals, allowing for single-molecule level sensing, even when primarily utilizing inefficient processes like Raman scattering [1,2]. Although the surface-enhanced Raman scattering (SERS) process mechanism is largely understood, certain key factors still require further explanation and more comprehensive study.

Comparing angular SERS patterns with dark field (DF) scattering patterns can offer new insights into the scattering process's intricacies. In this study, we present the findings of our investigation into the angular distribution of scattering by isolated and aggregated gold nanoparticles functionalized with dye molecules. We employed a defocused hyper-spectral SERS and mapping technique, which had previously been successfully used in fluorescence studies [3]. Measurements were carried out using a custom-built microspectroscopy setup. This approach yields detailed information on the directionality of scattering for each Raman band, allowing for correlation with plasmonic DF images. A thorough analysis of these images facilitates the determination of the position and orientation of both nanoparticles and molecules. Additionally, it reveals the differences between the directionality patterns emitted from aggregated and single nanoparticles (Fig.1). Data processing algorithms are also employed to evaluate the information from the experimental data.

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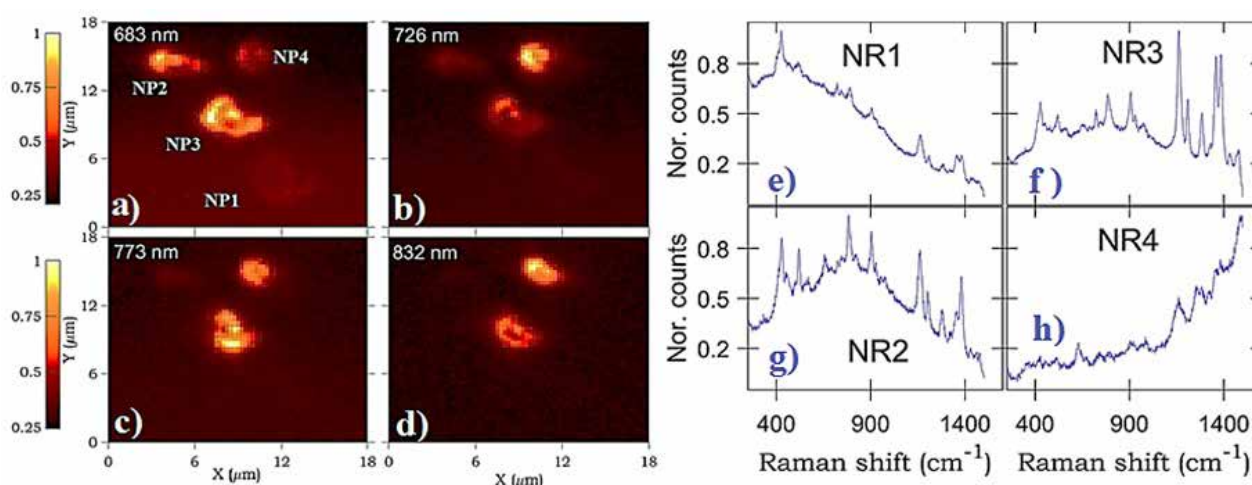
Figure captions:

The defocused dark field scattering patterns of nanorods at different wavelengths (a-d) and normalized SERS spectra registered on each object (e-h).

Keywords: SERS, Raman, Dark field, Hyperspectral, Angularpattern

D-P.21

Title: *Investigation of the effect of DMSO on ß-lactoglobulin fibrillation using AFM-based opto-mechanical techniques*

Author: Divanshu Sharma[1], Tanja Deckert-Gaudig[2], Volker Deckert[2]

[1]Leibniz institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany;Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller University, Helmholtzweg 4, 07743 Jena, Germany
[2]Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany;Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller University, Helmholtzweg 4, 07743 Jena, Germany
Deutsche Forschungsgemeinschaft – project number: 448666227

Abstract:
Several neurodegenerative diseases share a distinct feature: in both cases, aggregation and structural misfolding of proteins, so-called amyloids, are suspected to be involved. The aggregation of native proteins into amyloidogenic structures includes the formation of filamentous structures with a high content of ß-sheet conformation[1]. Since this is a complex but not yet fully understood process, it is not only necessary to analyse the chemical structure of the species involved but to develop strategies to prevent protein fibrillation. It has been reported that dimethyl sulfoxide (DMSO) successfully suppresses the formation of insulin amyloid fibrils[2]. In the present project, the inhibition capabilities of DMSO on ß-lactoglobulin fibrillation were studied. In the first step, the morphology of the species formed during protein incubation in presence of DMSO was characterized using atomic force microscopy (AFM). Figure 1 shows the AFM topographies of ß-lactoglobulin aggregates grown from fibrillation without (A) and with DMSO (B). It will be demonstrated that the higher the DMSO content, the fewer amyloid fibrils and more random aggregates were formed. Information about the nano-mechanical properties of the structures were obtained from force-distance curve AFM measurements. Since such data do not contain information about the chemical structure, nano-FTIR (Fourier transform infrared) spectroscopy was used to characterize the secondary structure by specifically probing the spectral regions of the amide bands (1500 – 1700 cm^{-1}). Nano-FTIR has been previously applied to the characterization of protein fibrils and individual protein complexes[3,4]. The presented approach, in which the combination of AFM and nano-FTIR spectroscopy allows detailed characterization of the morphology and conformation of ß-lactoglobulin aggregates, can be applied to all samples in which mechanical and chemical characterization are targeted.

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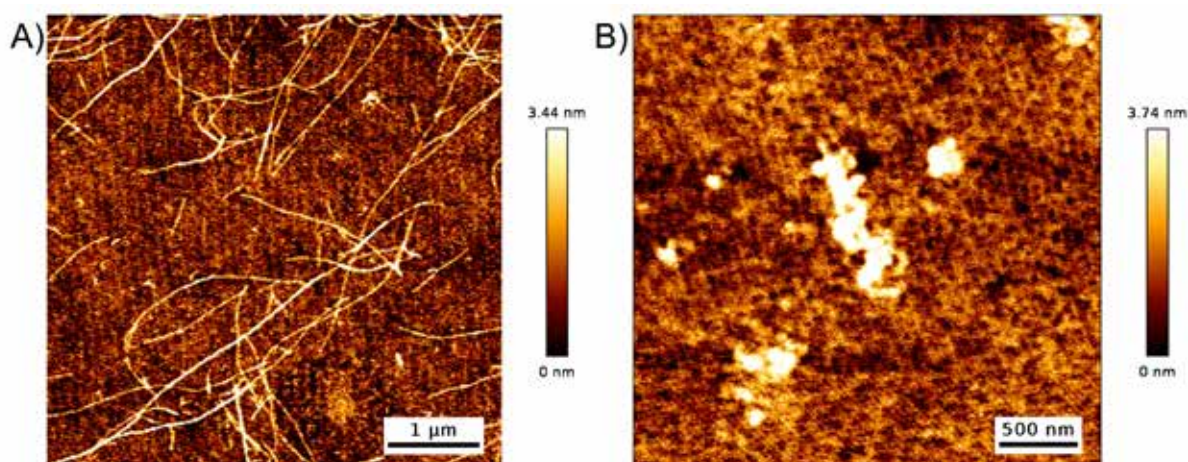
Figure captions:
Figure 1 AFM topography of (A) ß-lactoglobulin fibrils grown in a pH 2 solution at 80°C for 20 h (B) ß-lactoglobulin aggregates formed in presence of 100 µL DMSO in a pH 2 solution, at 80°C for 20 h.

Keywords: AFM, nano-FTIR, amyloid fibrils, protein aggregation

Title: *On demand cost-effective fabrication of AgNP-TERS tips with advanced mechanical stability by means of Microwave Processing*

Author: Xinyue Wang[1], Christiane Höppener[2], Hichem Nasri[3], Aisha Adebola Womiloju[3], Stephanie Höppener[4], Volker Deckert[2]

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[2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena, Helmholtzweg 4, 07743 Jena, Germany; Leibniz Institute of Photonic Technology Jena, Member of Leibniz Health Technologies, Albert-Einstein-Straße 9, 07745 Je
[3]Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstraße 10, 07743 Jena, Germany
[4]Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstraße 10, 07743 Jena, Germany; Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7, 07743 Jena, Germany

Abstract:
Tip-Enhanced Raman Spectroscopy (TERS) provides nanoscale information on the surface structure, chemical composition, and morphology with high spatial resolution, chemical specificity, and detailed information on the local environment and chemical reactions. TERS relies on the use of a metallic tip as a nanoscale antenna for signal enhancement in close proximity to the sample surface. While TERS has demonstrated immense potential, it also poses challenges such as the need for specialized tips, complex tip-sample interactions, tip contamination and the potential for signal interference.
Regarding that TERS tips are delicate and susceptible to damage or contamination, there is a high demand for easy, fast and cost-efficient TERS tip fabrication procedures. In light of these challenges, a microwave-assisted (MW) procedure established for fabrication of silver nanoparticle (Ag-NP) SERS substrates, which provide extraordinarily high mechanical stability even in liquid environments, was recently adopted to generate Ag-NP decorated TERS tips.[1] Briefly, commercial Si cantilever tips are coated with a thin layer of Ag salt, which is reduced by controlled evaporation of EtOH by MW heating. The resulting TERS tips yield a dense layer of Ag-NPs with a diameter of 20-40 nm. TERS performance and the mechanical stability is tested in a series of experiments, including SEM to confirm a high reproducibility of the metal coating as well as the sharpness of the TERS tips, and to demonstrate a good stability in water. The TERS activity of the tips was investigated with a monolayer of 4-NTP on Au nanoflakes, which showed longer tip lifetime and even stronger enhancement under ambient conditions compared to Ag-NP decorated tips fabricated by PVD. These preliminary results provide the prospect for more reliable and stable liquid TERS investigations, which currently are limited by a fast degradation of TERS tips, i.e., AgNP peeling off rapid due to low adhesion.

References:
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Acknowledgments:

Figure captions:
A) Schematic illustration of microwave (MW) based TERS tip fabrication; B) SEM image of a MW TERS Tip; C) Comparison of TERS spectra of MW tips in tip up and tip down state .

Keywords: TERS, silver nanoparticles, stability, microwave

D-P.23
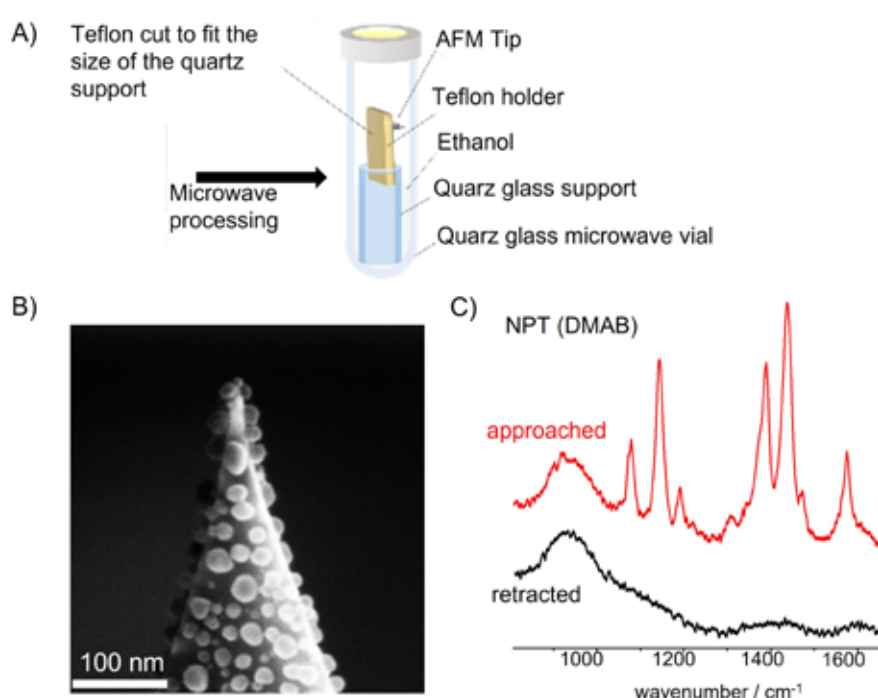
Title: *Photo-induced enhanced Raman spectroscopy on thin Ag-TiO2 nanoplatforms: a study of mechanisms and influence of visible light.*

Author: Adrian Warzybok[1], Łukasz Pięta[1], Kamilla Malek[1]

[1]Department of Chemical Physics, Faculty Of Chemistry, Jagiellonian University

Abstract:

In the era of today's world advances, plasmonic materials are becoming more and more popular among many scientific disciplines. One of the most important discoveries regarding these materials was their use to enhance a Raman scattering signal, thus inventing surface-enhanced Raman spectroscopy (SERS). Hybrid material consists of noble metal nanoparticles (NPs) with plasmonic properties and semiconductors such as TiO_2, WO_3, or ZnO.[1] They open many new possibilities for their utilization in chemical sensing and material science. It has recently been discovered that irradiation of such hybrid materials with UV light results in a significant amplification of the recorded Raman signal in relation to SERS. Thus, a new branch of SERS – photo-induced enhanced Raman spectroscopy (PIERS) was invented. So far, no consensus has been reached on the exact explanation for the origin of the PIERS amplification.[2]

This study allowed us to shed some light on the mechanisms that the reinforcement is subject to, as well as to produce and test a series of nanoplatforms based on combinations of silver nanoparticles and thin layers of titanium oxide in various forms. Synthesized substrates were characterized by localized surface plasmon resonance (LSPR) in the UV and Vis regions of 300 to 450 nm. Time-resolved UV-Vis and Raman measurements made it possible to determine how the migration of electrons in nanoplatforms affects the magnitude of the obtained PIERS gain and the rate of its decay (Fig. 1). The use of thin layers of titanium oxide allowed us to obtain a photoinduced signal up to 20 times higher compared to SERS and relate our observations to mechanisms such as hot electron injection (HEI) and plasmon resonance energy transfer (PRET).[3]

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Figure captions:

Time measurements of the LSPR position for series of four samples: before irradiation with UV light, after irradiation for 0-144h, after irradiation with Vis light for 144-220h.

Keywords:

SERS, PIERS, Nanoparticles, TiO2, Plasmons
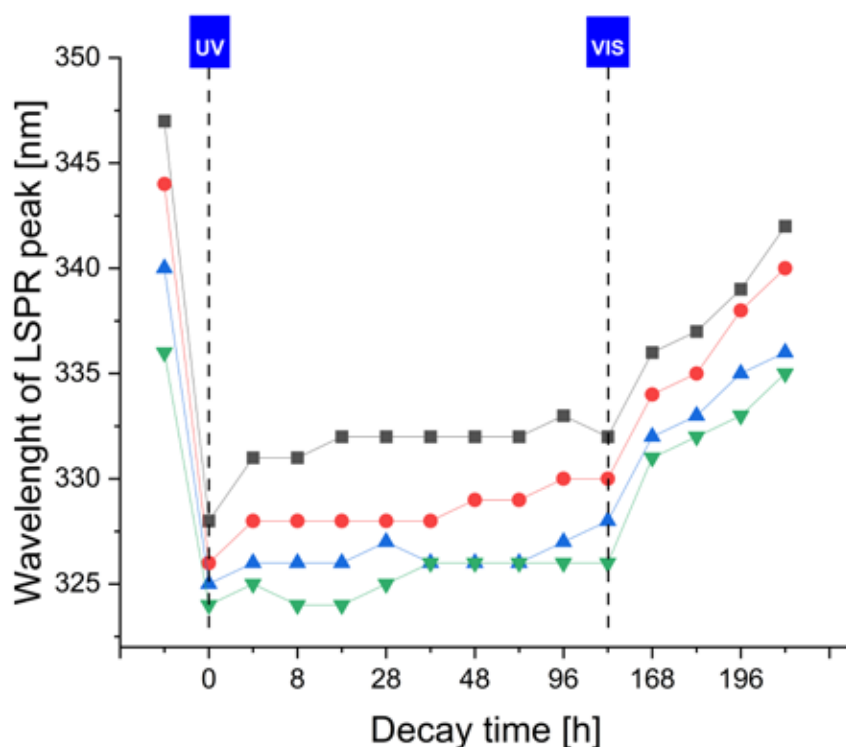
D-P.24

Title: *Understanding Structure, Interference, and Absorption effects in Vibrational SFS Experiments*

Author: Li Zhang[1], Saranya Pullanchery[1], Sergey Kulik[1], Sylvie Roke[1]

[1]Laboratory for Fundamental BioPhotonics, Institute of Bioengineering (IBI), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL)

Abstract:
Ordering of molecular dipoles is crucial in determining an interface's chemical and physical properties. Vibrational sum frequency scattering (SFS) spectroscopy is a unique interface-selective tool to measure the interfacial vibrational structure of sub-micron to micron scale objects dispersed in liquid media and extract information about the ordering of molecular groups at the surface. However, spectral acquisition becomes more challenging when the droplets are dispersed in the absorptive bulk medium. The effect of infrared absorption by bulk water was theoretically described recently[1] that it drastically modifies the SF response from interfacial water stretch modes of objects dispersed in water. In addition to IR absorption, the intensity of SF response is also highly sensitive to the changes in interfacial molecular structure and the constructive or destructive interference between vibrational modes carrying different phases. However, how these two effects manifest in the SFS measurement in an absorptive medium has not been understood. Herein we investigate the SFS response from the neutral surfactant span 80 on the surface of oil-in-water and water-in-oil nanodroplets. Both structure and interference modify the SFS response from oil-in-water droplets, whereas IR absorption acts as an additional contributor to the SFS response from water-in-oil droplets. We show that the spectral changes resulting from a combination of infrared absorption and interference cannot be deconvoluted in a trivial manner because span 80 acquires entirely different molecular structures at water-in-oil and oil-in-water interfaces.

References:
1. Kulik, S.; Pullanchery, S.; Roke, S. Vibrational Sum Frequency Scattering in Absorptive Media: A Theoretical Case Study of Nano-objects in Water. J. Phys. Chem. C 2020, 124, 23078-23085.
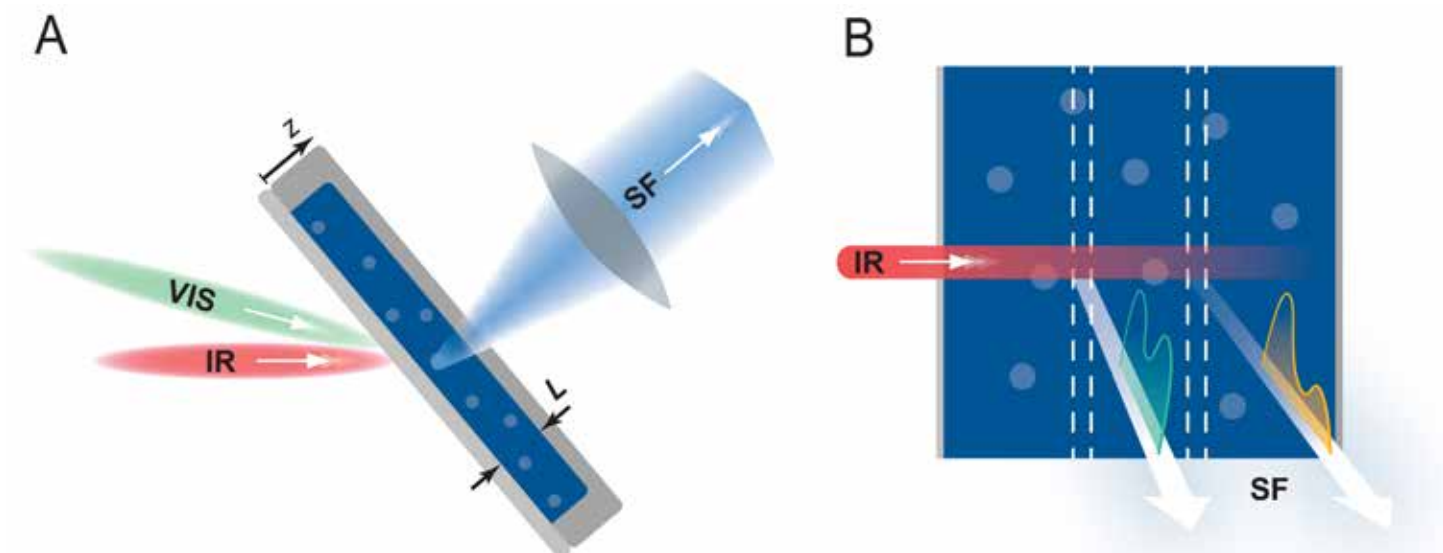
Figure captions:
(A) Illustration of vibrational SFS experiment, and (B) Illustration of linear IR absorption in an SFS experiment. Figure adapted from Ref. 1.

Keywords: Sum frequency scattering, IR absorption

D-P.25

Title: *In-situ nanoscale characterization of CO on Pt/Au bimetallic surface by electrochemical tip-enhanced Raman spectroscopy (EC-TERS)*

Author: Mengyuan Zhu[1], Yifan Bao[1], Maofeng Cao[2], Xiaojiao Zhao[2], Xiang Wang[3], Bin Ren[3]

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Abstract:
The interaction between CO and metallic surfaces is a key topic in electrocatalysis process since CO is an important intermediate as well as product in many electrocatalytic reactions[1,2]. A deep understanding of the interaction between CO and metallic surface is believed to help the design of catalysts with high activity and stability. However, as there is only monolayer or even sub-monolayer CO adsorbed on the surface, which requires a characterization technique with a high sensitivity. Moreover, the interaction between CO and metallic surface is both site-dependent and potential-dependent, which requires characterization technique to be able to work in-situ and with nanometer spatial resolution in order to obtain the information from the CO adlayers. Therefore, to date, it is still a great challenge to in-situ characterize the interaction between CO and metallic surfaces with a nanoscale spatial resolution. In this work, we developed scanning tunneling microscopy (STM)-based top-illumination electrochemical tip-enhanced Raman spectroscopy (EC-TERS) with a high sensitivity and a spatial resolution better than 10 nm. Based on this technique, we in-situ characterized interactions between CO adlayers on different sites of the Pt/Au bimetallic surfaces at different potentials and tracked the dynamic changes of the CO adlayers during the CO oxidation process. These results may help us better understand the interactions between CO and metallic surfaces and their dynamics during the electrocatalytic processes, which in turn help the design of electrocatalysts with better performance.

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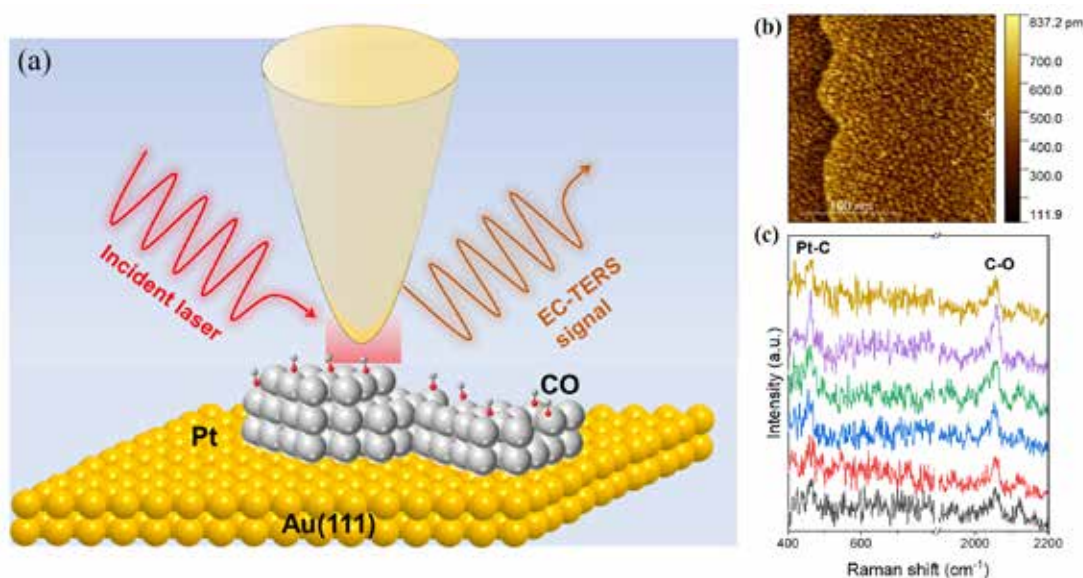
Figure captions:
(a) Schematic illustration of EC-STM-TERS characterization of CO on Pt/Au bimetallic surface. (b) STM image of the Pt islands on Au(111). (c) EC-TERS spectra of CO on different sites on Pt island.

Keywords: Electrochemical tip-enhanced Raman spectroscopy (EC-TERS),

D-P.26

Title: *Highly ordered nanostars-based solid substrate for ultrasensitive SERS*

Topic: (H) biodiagnostic spectroscopy, (D) vibrational spectroscopy of surfaces and interfaces

Author: Alexandre Chícharo[1], Alexandra Teixeira[1], Maria Relvas[1], Marta Aranda-Palomer[1], Jérôme Borme[1], Lorena Diéguez[1], Sara Abalde-Cela[1]

[1]INL – International Iberian Nanotechnology Laboratory

Abstract:
Surface Enhanced Raman Spectroscopy (SERS) is a highly promising analytical tool for non-destructive molecular analysis in fields like medical diagnostics, environmental monitoring, and food safety [1]. It enables molecule detection near metal nanostructures upon laser irradiation, eliminating labelling approaches. Chemical methods traditionally produce metallic nanoparticles with diverse morphologies (nanospheres, nanorods, nanocubes). Recent advancements have led to sharper morphologies like nanoflowers and nanostars [1,2], creating localized hot spots for enhanced signal intensity. Hot spots can also occur at gaps between nanoparticles in nanoarrays [2], but controlling them effectively remains a challenge, hindering SERS technology progress.

Top-down approaches with micro- and nanofabricated substrates create surfaces with well-ordered arrays of metallic nanostructures, allowing precise control of interparticle distances, resulting in predictable enhancement factors, signal homogeneity, and a unique focal plane for analysis. This study developed a high-density SERS array using electron-beam lithography, comprising gold nanodisks with varying diameters (20-650 nm) and interparticle distances (200-700 nm). Ultrasensitive SERS detection was achieved by transforming nanodisks into nanostars with multiple hot spots at their tips, generating highly intense SERS signals. SERS efficiency was evaluated using the Raman Reporter 1-Naphthalenethiol (1NAT), and the substrate detected tryptophan, a tumor-expressed metabolite, as a proof-of-concept. Metabolites play critical roles in cellular physiology, including communication, receptor activation, and energy cycles. Thus, these SERS substrates have the potential in uncovering metabolic processes associated with cancer development [3], intercellular communication, and microenvironmental conditions promoting cancer replication.

References:
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[3] Pavlova N.N. et al., Cell Metab. 23, 27-47 (2016).

Keywords:
SERS, SERS Substrate, Nanofabrication, Biosensors,

E-P.1

Title: *Exploring the Vibrational Structure of [FeFe] Hydrogenases*

Author: <u>Cornelius Bernitzky</u>[1], Yvonne Rippers[1], Solomon Wrathall[2], Barbara Procacci[2], James Birrell[3], Gregory Greetham[4], Neil Hunt[2], Marius Horch[1]

[1]Freie Universitaet Berlin
[2]University of York
[3]University of Essex
[4]Rutherford Appleton Laboratory

Abstract:
Hydrogenases are metalloenzymes that catalyze the cleavage and evolution of dihydrogen, an ideally clean fuel. Due to the presence of CO and CN− ligands at their active sites, IR spectroscopy represents an ideal technique for studying these enzymes. To understand structural details, dynamic properties, and the impact of the protein matrix on the active site, IR_{pump}-IR_{probe} and 2D-IR spectroscopies were successfully used for the characterization of [NiFe] hydrogenases and the disentanglement of complex mixtures of redox-structural states.[1-3] In [FeFe] hydrogenases, three CO and two CN− ligands, as opposed to one CO and two CN− in [NiFe] hydrogenases, are distributed across two iron ions, and one CO ligand is bound in a bridging position between them (Fig. 1A). A protonatable 2-azapropane-2,3-dithiolate ligand and a redox-active [4Fe4S] cluster covalently attached to the [FeFe] site further complicate the active-site structure. This situation gives rise to a complex ligand-stretch vibrational manifold and, thus, a plethora of signals in the 2D-IR spectra (Fig. 1B). Using a stabilized state of the [FeFe] hydrogenase from *D. desulfuricans* as a model system, we have obtained initial insights into these aspects. Our 2D-IR data indicate that the vibrational structure of [FeFe] hydrogenases differs considerably from their [NiFe] counterparts, both in terms of individual bond potentials and the vibrational coupling between different ligand stretching modes. Guided by second-order vibrational perturbation theory, we find that both aspects are highly sensitive to molecular factors that affect bond polarity and charge distribution at the [FeFe] active site. Together with unusual intensity patterns in vibrational ladder climbing, these findings indicate that 2D-IR spectroscopy can provide detailed insights into otherwise inaccessible aspect of molecular bonding in [FeFe] hydrogenases and other complex (and catalytically relevant) polynuclear metal carbonyl compounds.

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Figure captions:
Figure 1: Active site (A) and 2D-IR spectrum (B) of [FeFe] hydrogenase in the Hinact state

Keywords: 2D-IR, vibrational-perturbation-theory, hydrogenases, metalloenzymes, metal-carbonyles
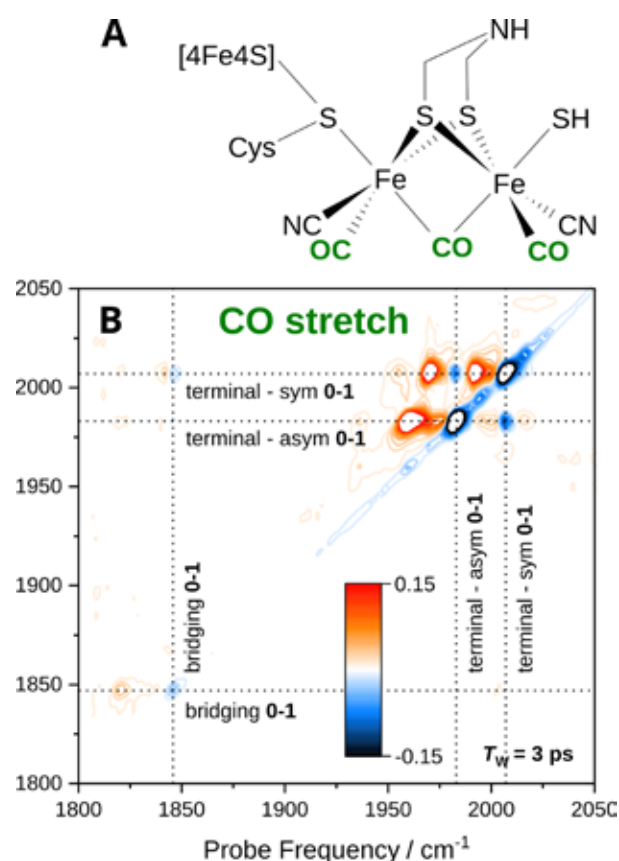
E-P.2

Title: *pH induced poly (methacrylic acid) / poly (ethylene glycol) restructuring on the molecular level*

Author: <u>Mirela Encheva</u>[1], Robert T. Woodward[1], Emina Muratspahic[1], Ellen H. G. Backus[1]

[1]University of Vienna

Abstract:
Polymeric emulsifiers based on a poly (methacrylic acid) (PMAA) backbone and poly (ethylene glycol) methyl ether methacrylate (PEGMA) grafts have a strong pH-triggered interaction present at acidic pH and are absent at alkaline conditions. This interaction was attributed to hydrogen bonds between the acid group of PMAA and the ether oxygens in PEGMA [1][2]. Although the obtained results can be explained with the hydrogen bonding theory, an experimental proof of the polymeric molecular behavior is still missing.
To answer this question, we used sum frequency generation (SFG) spectroscopy, as it measures the vibrational spectra of interfaces. Since the intensity of the vibrational bands in an SFG spectrum relates to the number of molecules and their orientation, this technique becomes an optimal tool for recognizing changes in the polymer structure. In our experiments, we compared the spectra of the PMAA/PEGMA polymer deposited on top of a water surface under acidic (pH 3) and basic (pH 11) conditions. We found that the surface vibrations of the PMAA/PEGMA polymer at pH 11 are mainly produced by the PEGMA component, while at pH 3 the vibrations mainly originate from the PMAA part. Moreover, spectra acquired in the carbonyl region show a shift in the C=O band when comparing the PMAA/PEGMA polymer and a reference sample only containing PMAA. This proves the presence of an additional interaction occurring in the responsive polymer that is absent in the PMAA case.

References:
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2. R.T. Woodward, J.V.M. Weaver, The role of responsive branched copolymer composition in controlling pH-triggered aggregation of "engineered" emulsion droplets: towards selective droplet assembly, Polymer Chemistry, 2 (2011), 403-410

Keywords: Sum frequency generation spectroscopy, pH

E-P.3

Title: *Dye-enhanced stimulated Raman scattering microscopy*

Author: <u>Jakub Firlej</u>[1], Krzysztof Brzozowski[1], Anna Pieczara[2], Małgorzata Barańska[1]

[1]Jagiellonian University
[2]Jagiellonian Centre for Experimental Therapeutics

Abstract:
Raman scattering is mainly associated with label-free imaging of biological samples [1] but as HO. Hamaguchi presented [2], presence of specific solutes may significantly increase solvents hyper-Raman (HR) signal. Phenomenon called "near-field effect" or "molecular near-field antenna effect" is described thoroughly theoretically and experimentally for HR but as conducted pilot studies present, this intriguing phenomenon occurs also at stimulated Raman scattering (SRS) spectroscopy. This sudden discovery can change common understanding of SRS microscopy as only label-free method of imaging.
Initial research was carried out at Faculty of Chemistry Jagiellonian University SRS system constructed by Raman Imaging Group [1]. From profusion of light absorbing molecules, (pre)resonance stains such as ATTO series dyes, IR-783, Malachite Green and Trypan Blue were chosen for studies. As intensity enhancement probe molecule and simultaneously a solvent for examined dyes was dimethyl sulfoxide (DMSO). Research was based on comparing stimulated Raman loss (SRL) values at 2766 cm^{-1} and 3100 cm^{-1} where DMSO does not absorb energy to SRL signal at 2916 cm^{-1} and 3001 cm^{-1} where DMSO absorption bands are situated.

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2. R Shimada , H Kano, HO Hamaguchi, Intensity enhancement and selective detection of proximate solvent molecules by molecular near-field effect in resonance hyper-Raman scattering, J Chem Phys., 129 (2008), 024505

Keywords: Stimulated_Raman, Raman_Microscopy, Antenna_Effect

E-P.4

Title: *Electronic Structure of para-Cyanophenol at the Air-Aqueous Interface from Vibrational Sum Frequency Generation Spectroscopy*

Author: Aruna Kumarasiri[1], Peter Yang[1], Dennis Hore[1]

[1]University of Victoria

Abstract:

Using vibrational sum frequency generation (SFG) spectroscopy, we investigated the optical properties of para-cyanophenol at the air-aqueous interface. Without making any assumptions about the orientational distribution of the molecules, we experimentally demonstrated that neither pure cyanophenol nor pure water are a reasonable approximation for the interfacial refractive index. Furthermore, the interfacial refractive index determined from experimental data combined with results from simulations lies just above the common assumption that the interfacial refractive index should be close to the average of the adjacent air and water refractive indexes.[1] Moreover, we demonstrate that the bulk media hyperpolarizability ratio differs from that of the interface based on spontaneous Raman scattering experiments combined with SFG measurements in three different polarization schemes.[2] Our results show that the surface hyperpolarizability ratio is within the range of 0.11–0.15, whereas the bulk value is 0.082 ± 0.003. This indicates that the polarizability of the cyano group changes less along the direction of the C-N bond when the molecules are adsorbed on the surface than within the bulk aqueous phase of the same molecules.[3]

References:

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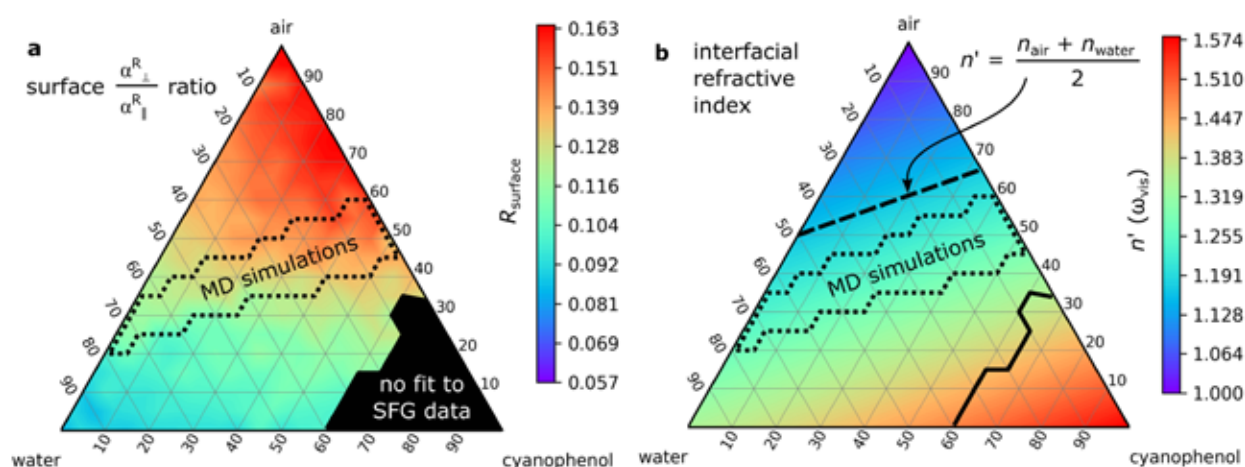
Figure captions:

(a) Hyperpolarizability ratio and (b) Interfacial refractive indices that arise from the surface volume fraction mixing model.

Keywords: SFG, Raman, interfaces

Title: *Determining the Surface Charge Density of Aqueous Interfaces Using Angle Variation Sum-frequency Generation Spectroscopy*

Author: Md Mosfeq Uddin[1], Dennis Hore[1]

[1]University of Victoria

Abstract:
Recently, nonlinear optics has been recognized as an emerging technique for quantifying the surface charge at aqueous interfaces and characterizing the associated water structure within the electrical double layer.[1-5] For surfaces such as minerals that ionize upon contact with water, the surface charge density can be understood from the corresponding equilibrium constants.[6] However for materials with no ionizable groups, such as hydrophobic polymers, the origin of the surface charge is more elusive. Using the second-order nonlinear optical technique of vibrational sum-frequency generation (SFG) spectroscopy, we probe the polymer-aqueous interface at various ionic strengths. The spectral data alone is not sufficient to solve for the surface potential using the Grahame equation. To address this, we propose a method that scans the angle of incidence over a region that spans the critical angles, and thereby modulates the SFG coherence length. We will illustrate that this method can be used to separate the contributions that govern the signal generated in the electrical double layer, and thereby extract the surface charge density.

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Keywords: SFG, surface charge, silica, water

E-P.6

Title: *Using Simulations to Determine Interfacial Hyperpolarizabilities and Orientational Distributions of Organic Molecules at the Air–Water Interface*

Author: Peter Yang[1], Dennis Hore[1]

[1]University of Victoria

Abstract:
When using vibrational sum frequency generation spectroscopy, properties such as the hyperpolarizability and the orientational distribution are of interest. Unfortunately, one of the main challenges in this nonlinear vibrational spectroscopy is having little to no prior knowledge of either quantity; these properties are critically required in order to analyze experimental data. In this work, we use a variety of electronic structure and molecular dynamics simulations in order to elucidate electronic and orientational properties. We leverage ab-initio molecular dynamics simulations to selectively probe electronic properties at the air-water interface. These simulations are used to accurately describe the unique non-isotropic electrical environment at the interfacial layer. In the same vein, we can use classical molecular dynamics to compute orientational properties over a longer trajectory. Our results indicate that the surface density exhibits minimal dependence on the orientation distribution of adsorbed organic molecules. Our computational methods can be expanded to a wide variety of vibrational modes which will enable a deeper level of analysis of experimental nonlinear vibrational spectra.

References:
1. X. Zhuang, P. B. Miranda, D. Kim, Y. R. Shen, Mapping molecular orientation and conformation at interfaces by surface nonlinear optics, Phys. Rev. B. 59 (1999) 12632–12640.
2. M. Thomas, M. Brehm, R. Fligg, P. Vöhringerb, B. Kirchner, Computing vibrational spectra from ab initio molecular dynamics, Phys. Chem. Chem. Phys. 15 (2013) 6608-6622.
3. H.F. Wang, W. Gan, R. Lu, Y. Rao, B.H. Wu, Quantitative spectral and orientational analysis in surface sum frequency generation vibrational spectroscopy (SFG-VS), Int. Rev. Phys. Chem. 24 (2005) 191–256.

Keywords: SFG, molecular dynamics, interfaces

F-P.1

Title: *Low Frequency Raman microscopy for API polymorphisms analysis*

Author: Thibault Brulé[1], Céline Eypert[1]

[1]Horiba France SAS

Abstract:
Since the physical state can affect the pharmaceutical behavior of drug substances, it is important to know what controls crystallization, solid state reactions, phase stability, and solubility. There are numerous methods that have been used to measure the solid state composition of pharmaceuticals; these include X-ray diffraction, optical microscopy, thermal analysis, dissolution testing, particle size analysis, NMR, and infrared (IR) spectroscopy. Raman spectroscopy is a now a validated technique in this industry as a very powerful characterization technique.

Indeed, Raman spectroscopy can provide qualitative and quantitative information of the polymorphy, with 1 µm spatial resolution when necessary. The new generation in Raman technology provides many advantages over the other techniques. Thus, it is a non-destructive analysis, samples can even be examined in transparent glass or plastic containers. Microscopic samples as small as 1µm can be easily characterized, and finally little or no sample preparation is required. Moreover, polymorphic and pseudo-polymorphic phases in microscopic samples can be mapped. This last point is important as the pelletizing can create pressure-induced polymorphic transformation.

By definition, the differences between two polymorphic phases is in the crystal modes, which can be characterize on the low Raman frequencies region. That increases the difficulty for the discrimination of the phases. Thanks to the standard Super Low Frequency standard module available on LabRAM SoleilTM, it becomes easy to reach 30 cm-1 frequency, and so to characterize polymorphisms without additional options. This provides so low frequency spectra with no intensity compromise.

In this presentation, we present an example of polymorphisms characterization by Raman microscopy using the Super Low Frequency module.
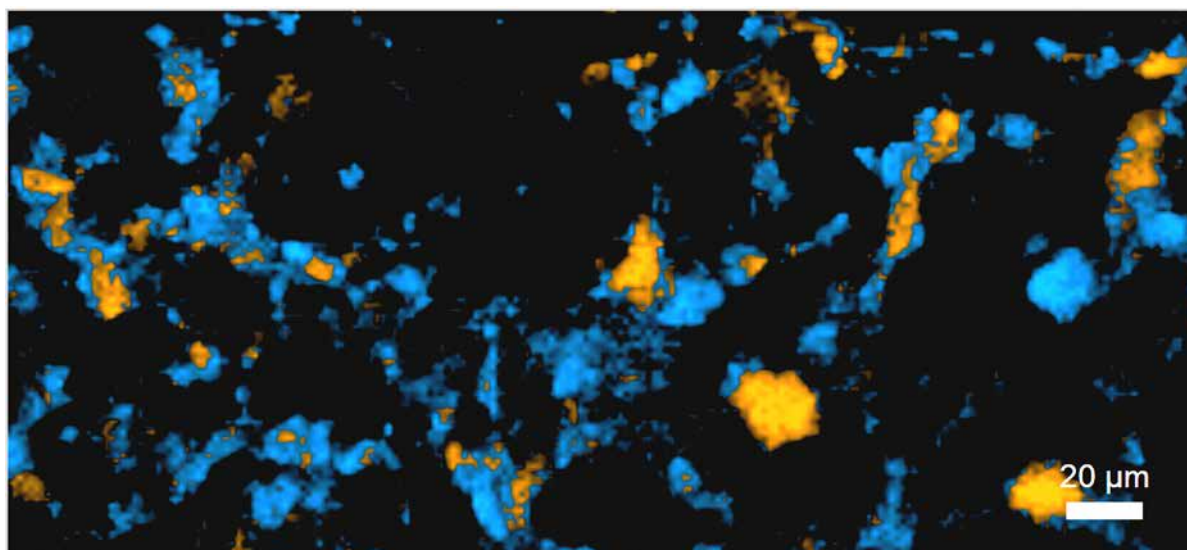
Figure captions:
Figure 1: Carbamazepine distribution in tablet (blue: Form I, orange: Form III, black: excipients)

Keywords: Super low Frequencies, Raman, API

F-P.2

Title: *Portable infrared solution for on-site mycotoxin screening in cereals*

Author: Antoni Femenias Llaneras[1], Polina Fomina[1], Valeria Tafintseva[2], Stephan Freitag[3], Anouk Bosmann[4], Miriam Aledda[2], Francesco Simone Ruggeri[4], Gert Ij. Salentijn[4], Rudolf Krska[3], Achim Kohler[2], Boris Mizaikoff[1]

[1]Ulm University
[2]Norwegian University of Life Sciences
[3]University of Natural Resources and Life Sciences
[4]Wageningen University and Research

Abstract:
Several fungal species produce natural contaminations in the field, mainly toxigenic secondary products known as mycotoxins. Deoxynivalenol (DON) is a well-known mycotoxin with high incidence in cereals which poses an exposure health risk and causes post-harvest waste. Therefore, DON contamination must be controlled from the early stages of food production. Infrared analysis appeared to be a fast, cost-effective, and portable approach for on-site contaminant analysis in food. In this study, a compact attenuated total reflectance spectrometer equipped with a pyroelectric detector array has been used for indirect DON analysis in wheat via extracts analysis. Ethanol-water (30:70) solvent system was selected to extract DON from wheat before extract evaporation on the ATR surface. IR spectra obtained were modeled by Partial Least Squares Discriminant Analysis (PLS-DA) which classified wheat contamination according to the EU regulations (1250 µg/kg) with 84.6% accuracy. The device's portability combined with high analytical performance demonstrated the potential on-site field application for early contamination detection.

Keywords: Infrared, Mycotoxins, Portable, Cereals

F-P.3

Title: *SmartSampling: a revolution in Raman imaging*

Author: Jérémy Brites[1], Thibault Brulé[1], Sébastien Laden[1], Ludivine Fromentoux[1]

[1]Horiba France SAS

Abstract:
From its beginning more than 50 years ago, the Raman microscopy went through many stages that have marked its history. Thus, starting from the point spectral acquisition at the micron scale, the capability to acquire Raman images opened the door to more and more applications. Then, the ability to the multichannel detectors to be faster and faster improved the speed of measurement and so increased the performances of such Raman imaging systems with less than a millisecond acquisition time per spectrum. However, such performances are possible only on high Raman scattering samples, reducing the concerned applications.

With our solution, we now offer the ability to all applications to improve their speed of Raman imaging. Based on the video contrast, this patented algorithm segments the map area in regions of interests of different sizes. Consequently, a quick preview based on high quality spectra is obtained in few seconds and then this rough image is improved step by step, detail by detail. Thus, good quality images are obtained after only few minutes when it needs hours or days to be completed in classical point-by-point mapping.

In this poster we detail how this approach will revolutionized the Raman imaging in all application domains, from physical to life sciences.
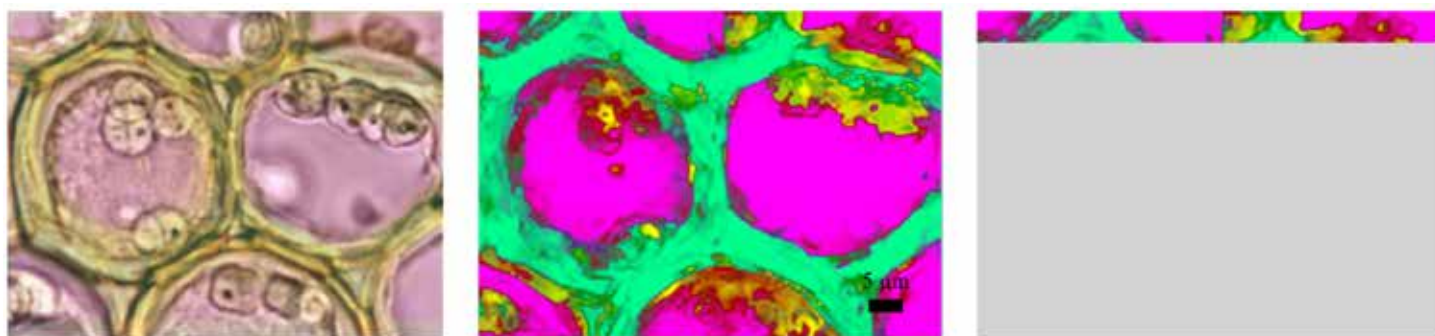
Figure captions:
Figure 1: Covallaria cells. (Left) 14 minutes SmartSampling Raman image. (Right) Equivalent point by point image obtained in 14 minutes.

Keywords: UltraFast, Raman, imaging, algorythm

F-P.4

Title: *Raman Spectroscopic Approaches for Drug Monitoring*

Author: Timea Frosch[1], Juergen Popp[1], Torsten Frosch[2]

[1]Leibniz-Institute of Photonic Technology
[2]Technical University Darmstadt

Abstract:
The combat against infectious diseases is still one of the main challenges in the world. Sepsis is a syndromic response to an infection and is the cause of every fifth death globally [1]. Many of these deaths might be preventable if patients would get the optimal therapy. However, appropriate dosing is challenging, especially in the case of septic patients due to high variations in the pharmacokinetics of the drugs. Therefore, new analytical methods are urgently needed, which can access drug concentrations in body fluids rapidly and at the point-of-care.
Raman spectroscopic methods have proven to be promising to address these problems [2]. Advances in fiber-enhanced Raman spectroscopy for highly sensitive drug monitoring of antibiotics were achieved. By exploiting efficient light guidance in a novel anti-resonant hollow core fiber, the limit of detection of the antibiotic drug cefuroxime was improved by two orders of magnitude [3]. Cross-validation with liquid chromatography on cefuroxime in human urine showed excellent agreement [3]. We analyzed the impact of freezing, centrifugation, and filtration on the concentration of piperacillin in female human urine using deep UV resonance Raman spectroscopy with an excitation wavelength of 244 nm [4]. We also quantified the effect of sex-specific reference matrices on the piperacillin concentration in urine. For female urine samples, a female urine reference pool showed nearly the same results as when using the proband's own urine, whereas using a mixed-sex pool led to much higher deviations. Thus, Raman spectroscopic quantification of antibiotic levels is more accurate when using sex-specific pools as matrix references instead of mixed-sex pools [4].

References:
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Keywords: Raman, Drug Sensing, Antibiotics, Urine

Title: *Chiral sensing for liquid analysis – Synergies between Quantum Cascade Lasers and Vibrational Circular Dichroism*

Author: Daniel-ralph Hermann[1], Georg Ramer[1], Lisa Riedlsperger[1], Bernhard Lendl[1]

[1]TU Wien

Abstract:
Both chiral small molecules and protein derived pharmaceuticals like monoclonal antibodies have been the focus of increased development in the last years, resulting in an increased need for analytics.[1,2] For both categories, their therapeutic effect is intrinsically linked to their structure and indeed their chirality. It is therefore desirable to monitor and control these parameters, the enantiomeric excess for small molecules and the secondary and tertiary structure for proteins, over the production cycle of a drug. Vibrational circular dichroism (VCD) can be used in this regard, as it expands the inherent structure elucidation capabilities of mid-infrared (MIR) spectroscopy by adding chiral sensitivity as additional dimension. Unfortunately, the application of VCD is limited by several factors. The high absorbance of some solvents, like water during protein analytics, restricts the pathlength and therefore the achievable intensity, resulting in high limits of detection. Additionally, this drawback is compounded by VCD being characterized by very low intensity ($\sim 10^{-5}$ compared to classical absorbance), necessitating long acquisition times (hours) to achieve acceptable noise levels.[2]
We now present a dedicated VCD setup, utilizing a quantum cascade laser (QCL), as a viable solution to the discussed challenges. With the high brilliance of the QCL longer pathlengths, resulting in stronger signals, are possible. Our implementation of balanced detection for VCD measurements achieves a considerable reduction of laser noise, providing further advantages. We employed these advances for high time resolution studies of small molecules at a time scale of 3 minutes per measurement and low concentration measurements of the amide I band of proteins in water at 18 minutes of spectral acquisition.

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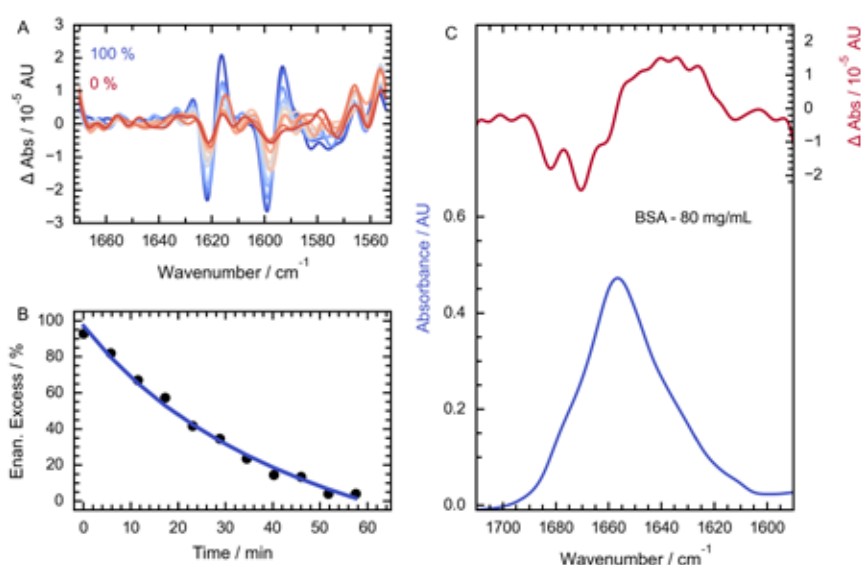
Figure captions:
VCD spectra of 1,1′-Bi-2-naphthol in CHCl3 (A) and the corresponding enantiomeric excess over time (B) as well as bovine serum albumin VCD and MIR spectra in water (C) at 18 minutes measurement time

Keywords: Vibrational Circular Dichroism, Quantum Cascade

F-P.6

Title: *Exploring potential of reverse Raman Stable Isotope Probing and 2D correlation spectroscopy in monitoring metabolic pathway dynamics in situ.*

Author: Jiro Karlo[1], Aryan Gupta[1], Surya Pratap Singh[1]

[1]Indian Institute of Technology Dharwad

Abstract:
Metabolites are highly dynamic in nature. In situ monitoring of metabolite dynamics i.e., it's de novo synthesis and turnover in an efficient and non-destructive way is a challenge. Gold standard techniques such as LC/GC-MS, NMR are highly efficient but are not feasible for in situ dynamic study due to its destructive and/or invasive nature whereas Raman spectroscopy is non-invasive, and non-destructive approach. The Raman stable isotope probes (RSIP) have been extensively studied in the past for studying biomolecules. However, it comes with cost, unreported peaks, and limited abundancy. In our study we have combined reverse RSIP along with 2D correlation spectroscopy (2D COS) for qualitative, and quasi-quantitative study of shikimate pathway in situ. Shikimate pathway is the connecting link between glycolysis and aromatic amino acid biosynthesis: Phenylalanine, Tyrosine and Tryptophan. It is directly involved in protein metabolism and act as precursor of many other metabolites such as aspartame, vitamin E. Our finding suggests a clear blue shift of phenylalanine peak due to the reverse isotopic effect confirming the 12C incorporation in the nascent phenylalanine enabling us to track the synthesis of phenylalanine that we assigned as the qualitative marker for shikimate pathway and the ratio-metric study of this shift as the quasi-quantitative marker for the turnover dynamics. Further we have done the inhibition assay using glyphosate to validate the peak from phenylalanine. Furthermore, to confirm the inhibitory effect on the phenylalanine peak is due to alteration on shikimate pathway only, we have bypassed it by adding aromatic amino acid exogenously. We have also done a shift-free de novo biosynthesis qualitative study. Mapping correlation between bands of dynamic spectra was done using 2D COS with time as perturbation. Successful application of this work can provide an alternate/adjunct tool for temporal monitoring of metabolome level changes.

References:
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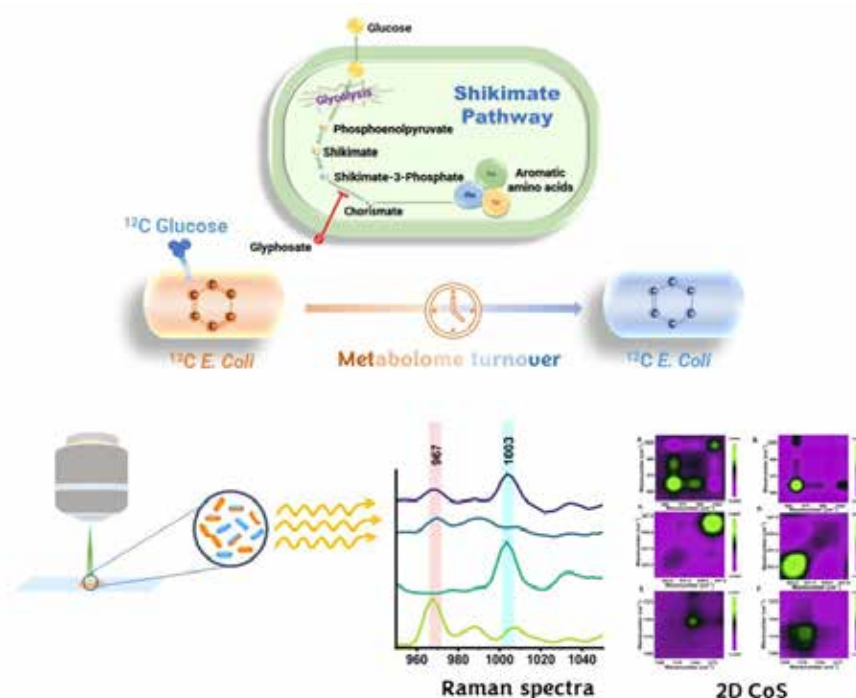
Figure captions:
Figure: In situ monitoring of metabolic shikimate pathway dynamics in E. coli using Raman reverse stable isotope probing and 2D synchronous correlation spectroscopy.

Keywords:
Raman Spectroscopy, metabolite dynamics, reverse stable isotope probing, metabolomics, aromatic amino acid

F-P.7

Title: *Handheld Raman spectrometer with advanced background and transmittance correction for in vivo skin measurements*

Author: Yurii Pilhun[1], Oleksii Ilchenko[1], Andrii Kutsyk[1]

[1]Lightnovo ApS

Abstract:
Nowadays handheld Raman spectrometers are widely used for identification of unknown substances and raw materials control. But small size, attractive price and ease of operation make handheld devices very attractive candidate for applications in different fields, such as health care, medical and biological studies. Naturally, more complex research tasks impose additional requirements for device parameters and resulting spectrum quality. Here we present miniaturized dual-range Raman spectrometer, in which we attempt to overcome challenges of building a small device with high performance and high-sensitivity. Our solution includes in-build reference sample inside the device, which is used for calibration on each acquisition and allowing to maintain perfect wavenumber precision and to compensate on laser power variation during measurements. Additionally, we implemented advanced inherent background subtraction and transmission profile compensations algorithms in our device. Background correction allows to compensate on Raman response originating from intermediate optics of the device itself, but not from the sample. Transmission correction makes spectral response uniform and minimizes differences in spectra measured by different devices. Negative effect of variation of the device sensitivity over wavenumbers is most prominent on highly-fluorescent samples, such as skin and most other biological samples. Non-uniformity manifests itself as variation of background, which easily can be confused with a broad peak or may hide important features of the sample. We correct for non-uniformity of sensitivity by storing calibration profile in each device, and applying it to all measurements. In figure you can see raw and corrected spectra of human skin. We believe that presented technology packaged in a small device could be successfully applied for various in vivo applications including skin disease diagnostics, wound bacteria identification, etc.

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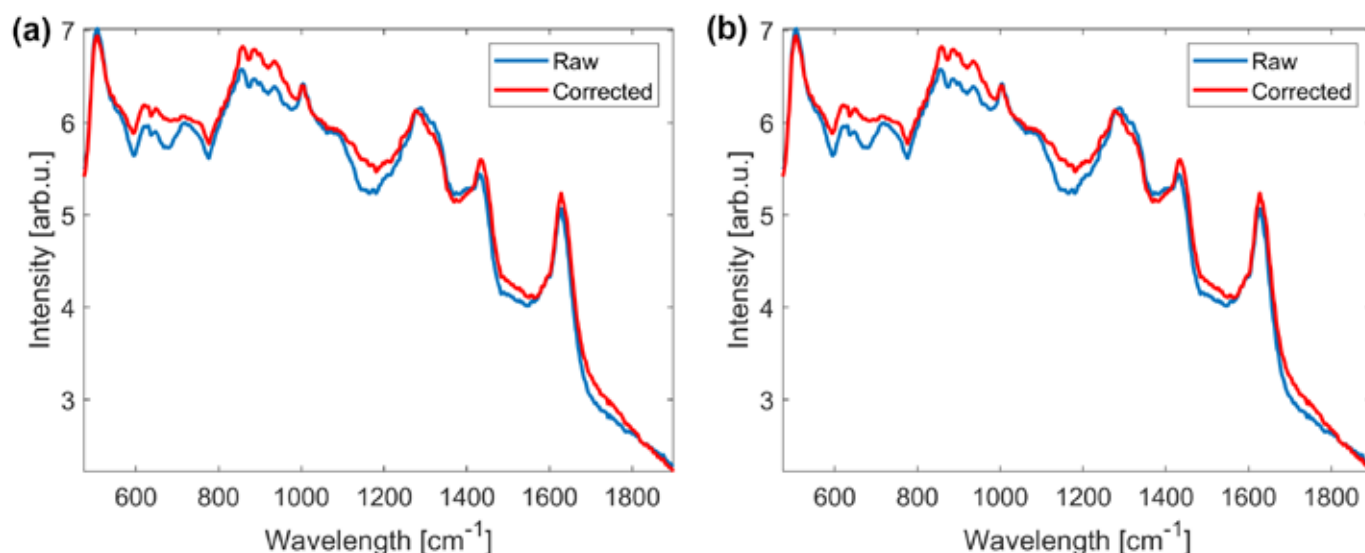
Figure captions:
Figure 1. Raw (blue) versus transmittance corrected (red) Raman spectra of human skin in fingerprint region from 785nm laser (a) and high-wavenumber region from 660nm laser (b).

Keywords: Raman, transmittance correction, real-time calibration

F-P.8

Title: *Optical Cavity Design for I-QEPAS for Isotope Analysis*

Author: <u>Savda Sam</u>[1], Johannes P. Waclawek[2], Harald Moser[2], Liam O'Faolain[3], Bernhard Lendl[1]

[1]Technische Universität Wien, Vienna, Austria
[2]Competence Center CHASE GmbH, Vienna, Austria
[3]Munster Technological University, Cork, Ireland

Abstract:
Although methods such as accelerator mass spectroscopy have been commonly used for isotope ratios analysis, the demand for high sensitivity, high speed, and compact system for on-field analysis has brought an intense interest to optical techniques of detection. Intracavity-Quartz-Enhanced Photoacoustic Spectroscopy (I-QEPAS) is chosen for this work. Since QEPAS can reach lowest detection limit, limited by the thermal noise of the quartz tuning fork [1], enhancing the intracavity power can significantly improve the signal and therefore the sensitivity. The standard two mirrors cavity can provide optical build-up with less complexity, but it has limitations when employed in spectroscopy including the need of an optical isolator at Mid-IR, and of a small focal point at fix position while tuning the cavity length to cover a wide range of the absorption line. To get rid of these limitations, for the proposed I-QEPAS, a bow-tie cavity composed by two curves and two flat mirrors with near-normal incident folding angle will be realized. Locking mechanism will be developed also to make the system compatible with 2f wavelength modulation to minimize the effect of coherent background noise [2]. The bow-tie cavity was primarily studied with ABCD matrix theory to get stability condition that depends on geometry parameter of the cavity. COMSOL simulation with Ray Optics module was done after and the result shows estimated maximum angle error tolerance (the different between the incident beam and the tilt angle of the mirrors) of single micro radian, and the and the beam waist change of larger than 100 um for 1 um error of the curve mirrors position along optical axis. With the estimated power enhancement factor of few hundreds, parts-per trillion detection limits can be reached [3].

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Keywords: Bow-Tie RingCavity, PhaseLocking, PhotoacousticSpectroscopy

F-P.9

Title: *High-Throughput Raman System for Rapid Microplastic Characterization*

Author: Shiwani Shiwani[1], Jürgen Popp[1], Christoph Krafft[1], Iwan W. Schie[2]

[1]Leibniz Institute of Photonic Technology
[2]Department for Medical Engineering and Biotechnology, University of Applied Sciences

Abstract:
The surge in plastic manufacturing over the past half-century has led to an increased prevalence of microplastics (MPs) in nearly every environment, from oceans and lakes to soils, beaches, and even human blood [1]. Given their ubiquity, there is an urgent need for efficient analytical techniques for the identification of MPs.

Addressing this, we have developed a high-throughput Raman system (HTS-RS) for rapidly assessing the presence and types of microplastics, even at low concentrations, on various substrates. By optimizing the optical design parameters, we were able to maximize information throughput while maintaining the highest possible optical resolution. This has resulted in a system with a large field-of-view, while maintaining high optical resolution, enabling quick MP detection and proving particularly beneficial for samples with low MP density. The implementation of our system is illustrated in Fig.1, which depicts a schematic of the HTS-RS setup. The system is designed to quickly capture high-resolution images with a size of 3.2x2.2 mm² at approximately 2 μm spatial resolution, facilitating the rapid detection of MPs. Our system can handle different types, shapes, and size of microplastics, bridging micro and macro-level, by integrating several automatic detection techniques. Fig.1 provides an example of the system's capabilities, presenting the spectra of different microplastic particles detected on a filter substrate. For particle measurement, we used an acquisition time of 0.5 s Raman scan time per particle. An inset showcases an image of the CaF2 with MPs on it.

Our approach offers a repeatable and time-efficient strategy for the detailed morphological and chemical characterization of microplastics, enabling their quick detection and identification.

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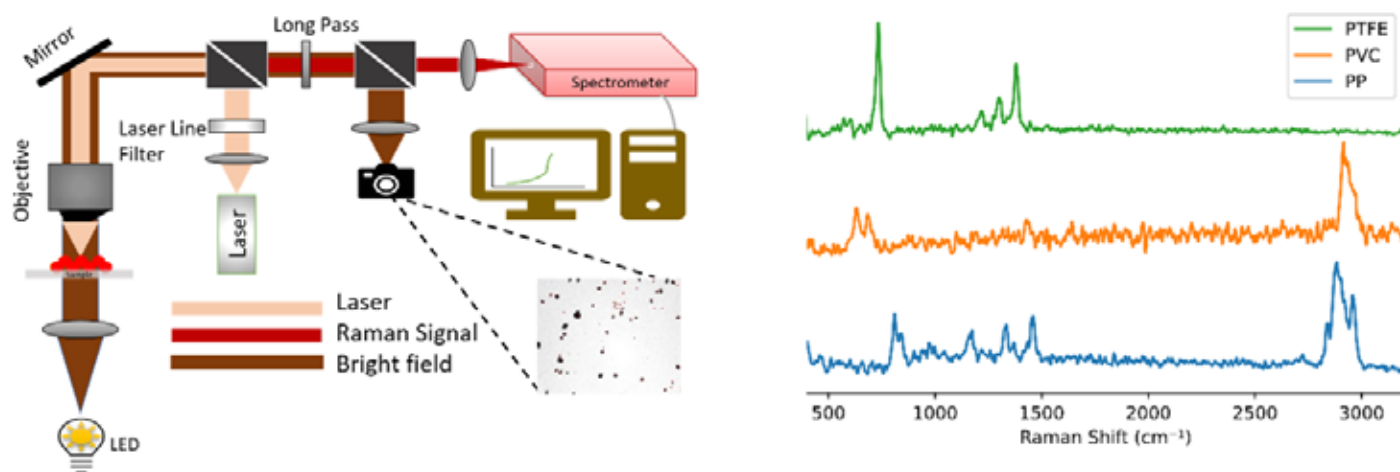
Figure captions:
Fig. 1. Schematic of HTS-RS system for MP detection. Inset shows CaF2 slide with MPs. Raman spectra of detected MPs is shown on the right side.

Keywords: microplastics, Raman, microscopy, high-throughput screening

F-P.10

Title: *Quantification of the Hydrocarbon Content in Water using a Dual DFB-QCL Setup*

Author: <u>Dominik Wacht</u>[1], Christoph Puls[2], Bernhard Lendl[1]

[1]TU Wien
[2]QuantaRed Technologies

Abstract:
Analyzing the hydrocarbon content in water is necessary for both process control and reporting to regulatory authorities. One of the standard reference method is based on the absorption of infrared radiation in the region of 1370 cm^{-1} to 1380 cm^{-1} using a quantum cascade laser after the extraction of the hydrocarbons in water with cyclohexane. Prior to the analysis, water is removed in a two step process, as it would cause a baseline shift resulting in overestimating the amount of dissolved hydrocarbons. This interference can be mitigated when using a dual quantum cascade laser based setup, where a second laser is used for a baseline correction. This allows the application as an online sensing platform when combined with a continuous extraction system.

A setup consisting of two distributed feedback quantum cascade lasers (DFB-QCLs), a polarizer as beam combiner, a temperature-controlled flow cell with a thickness of 1.6 mm and a pyrodetector were used. The lasers emit at 1378 cm^{-1} and 1400 cm^{-1}, where the latter was used for the baseline correction. Solutions of oil dissolved in cyclohexane with unknown water contents were injected into the flow cell and the intensities of the transmitted infrared radiation were measured.

The prepared solutions were both measured using the single and the dual DFB-QCL setup. Similar limit of detections (LODs) are calculated for both measurement schemes. However, when evaluating the goodness of fit according to ISO 8466-1:2021, the dual DFB-QCL setup clearly shows the superior performance. With the dual DFB-QCL approach, the residuals can be considerably decreased by applying the baseline correction with the second laser if water or other interfering species are present in the extracted solution. Furthermore, the use of a second laser circumtvents the water removal process allowing the application of this platform in an online sensing scheme when combined with a continuous extraction system.

References:
[1] ASTM D7678-17, 2022, "Standard Test Method for Total Oil and Grease (TOG) and Total Petroleum Hydrocarbons (TPH) in Water and Wastewater with Solvent Extraction using Mid-IR Laser Spectroscopy", ASTM International, West Conshohocken, PA, 2022, DOI: 10.1520/D7678-17,
[2] ISO 8466-1:2021 Water Quality - Calibration And Evaluation Of Analytical Methods – Part 1: Linear Calibration Function, Geneva, Switzerland

Figure captions:
Comparison of the performance of the obtained calibrations when measuring with the single and the dual DFB-QCL setup.
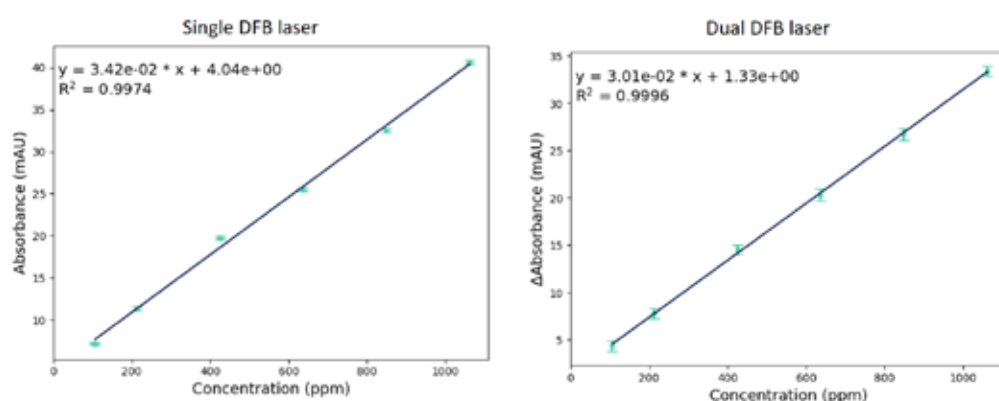
Keywords: Sensing, Process Analytics, Spectroscopy, QCL

Goodness of fit parameters ISO 8466-1:2021

Residual standard deviation
$$s_y = \sqrt{\frac{\sum_i^N (y_i - \hat{y}_i)^2}{N-2}}$$

Standard deviation of the method
$$s_{xo} = \frac{s_y}{slope}$$

Coefficient of variation of the method
$$V_{xo} = \frac{s_{xo}}{\bar{x}} * 100\%$$

Parameter	Single DFB setup	Dual DFB setup
s_y (mAU)	7.30e-1	2.58e-1
s_{xo} (ppm)	21.3	7.5
V_{xo} (%)	4	1

→ lower values indicate a better fit

→ the dual DFB setup clearly outperforms the single DFB setup

G-P.1

Title: *Assessment of the effects of Kuwait's high temperatures and humidity on whole blood stains stability on fabric using ATR-FTIR spectroscopy*

Author: Meshari Al-Qalfas[1], Shaima Al-Fodry[1], Mohamed O. Amin[1], Entesar Al-Hetlani[1]

[1]Kuwait University

Abstract:
Blood is one of the most important pieces of evidence found at crime scenes, which strongly suggests a violent crime has been committed. Additionally, knowledge about the time that bloodshed occurred has important implications in criminal investigations. This, however, can be highly influenced by some environmental conditions, such as light, temperature, and humidity. Kuwait, a country in the Middle East, is characterized by very hot weather during the summer, particularly during the months of June, July, and August, where temperature can reach up to over 50°C [1]. Therefore, the aim of this study is to investigate the effects of Kuwait's weather on traces of whole blood deposited on fabric surfaces utilizing attenuated total reflectance Fourier infrared (ATR-FTIR) spectroscopy as a non-destructive and confirmatory tool. For this purpose, 20 µl of blood samples were obtained from two male and two female donors and deposited on cotton (natural substrate) and modal (semi-synthetic substrate) fabrics. These samples were placed outdoors in a homemade glass box with holes to allow aeration of the sample and direct exposure to sunlight. Similarly, control experiments were carried out by the same donors on the same surfaces and were placed on a bench exposed to light and under air conditioning. Initial assessment of the samples using ATR-FTIR spectroscopy showed common spectroscopic signature of the whole blood with the main two bands of blood on both substrates – amide I and II positioned at ~1640 cm^{-1} and ~1535 cm^{-1}, respectively – with decreased intensity over the first two weeks [2]. Thus, with the proven track record of ATR-FTIR spectroscopy in forensic analysis, the stability of whole blood stains on different fabrics can be assessed as well as age estimation after exposure to extreme environmental conditions.

References:
1. Al-Kandari, N.M., et al. (2016) Journal of Pharmaceutical Sciences and Research, 7 (7). pp. 2852-2873
2. Sharma C.P., et.al. (2023) Forensic Science International: Animals and Environments 3 100060
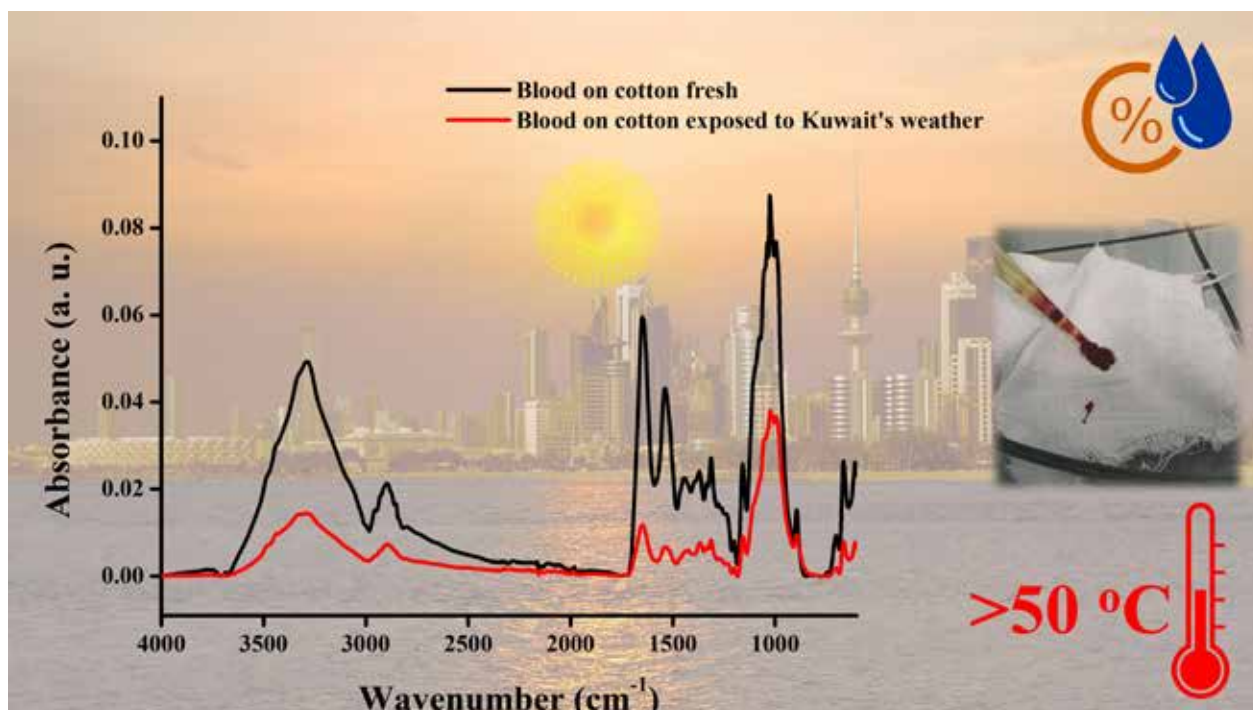
Figure captions:
Schematic illustration of the effect of Kuwait's weather on the stability of blood stains on fabric

Keywords: Forensics, Blood, ATR-FTIR, Kuwait

G-P.2

Title: *Raman Microscopy: A Versatile Analytical Tool for Art Authentication and Forensic Analysis*

Author: Matthew Berry[1], Angela Flack[1]

[1]Edinburgh Instruments

Abstract:

Raman microscopy is a powerful analytical technique that has become increasingly important in the fields of art and forensics. In the art industry, Raman microscopy can be used to analyze pigments in paintings and inks, providing valuable information on their composition and origin. This can help authenticate paintings, detect forgeries, and determine the age of a painting. One of the main advantages of Raman microscopy is that it is non-destructive, meaning that it can analyze samples without damaging them. This is particularly important in the art industry, where preserving the integrity of the artwork is paramount. Raman microscopy can also provide extremely detailed information on the pigments used in a painting, Figure 1. This level of detail can reveal important information about the painting's history, including any previous restorations or touch-ups.

In the forensic industry, Raman microscopy has a wide range of applications. For example, it can be used to analyze trace amounts of explosives or drugs, as each substance has a unique Raman spectrum. Raman microscopy can also be used to analyze ink samples, which can help detect forgeries or link a suspect to a particular document. The technique is particularly useful for analyzing samples that are too small for other analytical methods to be effective.

Overall, Raman microscopy is a versatile and powerful analytical tool that has a wide range of applications in the art and forensic industries. Its ability to provide non-destructive, detailed information on samples makes it an invaluable tool for authentication and analysis purposes. This poster provides a comprehensive overview of the use of Raman microscopy in both the art and forensic industries, highlighting its versatility and effectiveness as an analytical tool.
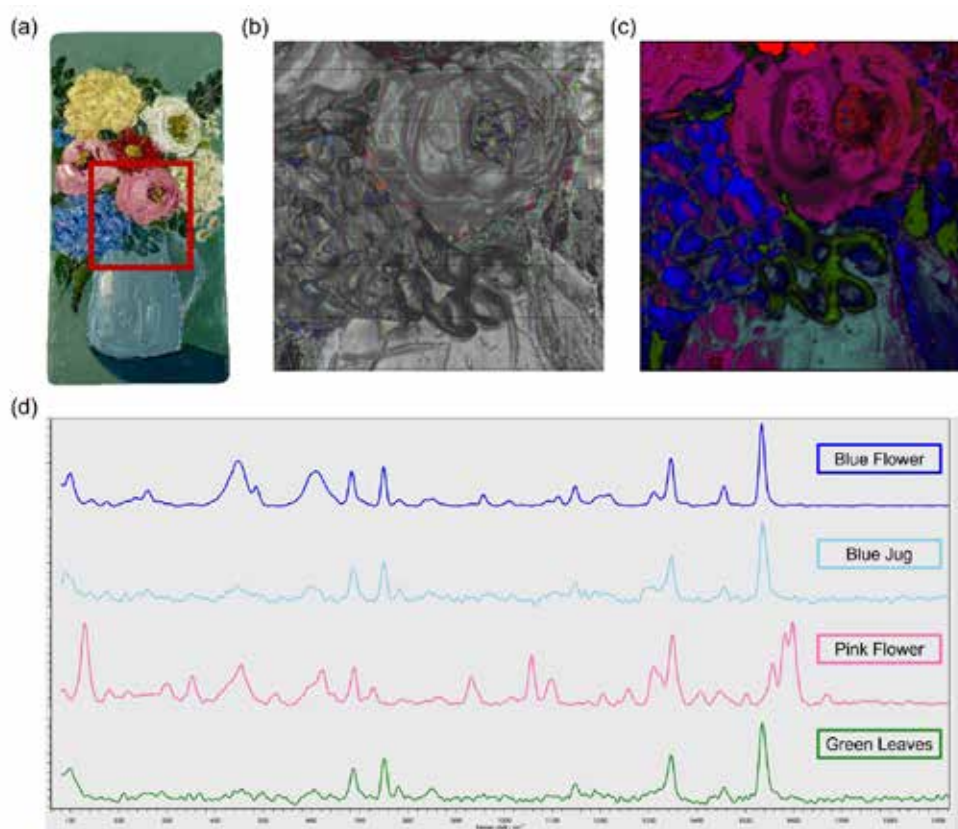
Figure captions:

Figure 1: (a) Painting sample (b) white light image of the area to be Raman mapped (c) false coloured Raman map (d) Raman spectra from each colour region of the painting

Keywords: Raman, art authentication, drug detection

G-P.3

Title: *Cell metabolite quantification using the Dxcover infrared platform*

Author: Loren Christie[1]

[1]University of Strathclyde/Dxcover Ltd

Abstract:

Introduction: Monitoring cell growth in a bioreactor by quantifying cell metabolites such as glucose and lactic acid is vital during production as it allows changes to be made in-situ. Infrared spectroscopy can be utilised in this field either online, at-line or offline, it can rapidly provide operators with real-time information about the cell growth process. As cells grow they consume glucose, reducing the levels within the bioreactor, they concurrently produce lactic acid. It is possible to monitor the health of a batch by quantifying these metabolites as they should be negatively correlated to one another. An insufficient amount of glucose can hinder cell growth and reduce batch yields, conversely an increase in lactic acid can raise the pH of a batch which can cause a toxic environment for cell lines.

Aim: To quantify the concentrations of cellular metabolites within cell media using the Dxcover infrared platform and translating into an at-line process analytical technology (PAT).

Methods: Three concentration profiles were investigated ranging from 200 to 10mg/ml; glucose and lactic acid singularly and finally a combination of both. Three Dxcover sample slides were used per concentration with 3μL of each sample being deposited onto the three sample wells. The slides were analysed using the Dxcover Autosampler coupled to a Perkin Elmer Spectrum Two FTIR spectrometer.

Results: For the binary quantifications, a PLS regression model was used to create a calibration curve to plot the observed vs the predicted concentration. This achieved an $R2 = 0.989$ for glucose and 0.999 for lactic acid. To test the model unknown concentrations were prepared, then predicted. A multiclass PLS regression model was then applied to simultaneously predict the concentrations of both glucose and lactic acid.

Conclusions: he Dxcover platform has the ability to characterise and quantify cell metabolites within cell media to allow for real-time batch monitoring.

G-P.4

Title: *Towards investigation of chiral nature of crude oil – Spectroscopic studies of optically active "molecular fossils"*

Author: Katarzyna Chruszcz-Lipska[1]

[1]AGH University of Science and Technology

Abstract:
The close relationship between chirality and the life originates from the fact that in nature the only source of the optically active substances are living orgaFwanisms. The presence of optically active "molecular fossils", *i.e.* organic compounds, whose structure is so stable that has undergone all the stages of formulation of crude oil and remained unchanged, is one of the evidences of its biogenic origin.

In the literature there are studies devoted to analyzing of crude oil and its individual fractions by using chirooptical spectroscopy techniques like ECD (electronic circular dichroism) and ORD (optical rotatory dispersion). Making use of vibrational chirooptical methods is unknown so far in the literature approach to test crude oil and the optically active "molecular fossils" present in it. In this work, various types of crude oil and their fractions were studied by using VCD (vibrational circular dichroism) spectroscopy for the first time. The obtained VCD spectra are characteristic of each crude oil. Their analysis may provide a new approach to the study of crude oil and its optically active biomarkers. The stability of selected "molecular fossils" makes them likely to be useful, e.g. to identify sources of petroleum pollution in the environment.

G-P.5

Title: *Raman spectroscopy paired with supervised statistical data analysis for honey authenticity control*

Author: <u>Maria David</u>[1], Ariana Raluca Hategan[1], Camelia Berghian-Grosan[1], Dana Alina Magdas[1]

[1]National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donat Street, 400293

Abstract:
The development of recognition models for honey differentiation represents a continuous interest among researchers. Rare varieties of monofloral honey represent one of the most falsified commodities, because of the high demand that is corroborated with the limited availability. Due to this, the false declaration of botanical origin exists and must be discouraged by the implementation of reliable tools for honey authenticity control [1-3]. In this regard, Raman spectroscopy proved to be an effective method, due to being easy-to-use and having a rapid response time. In this study, the Raman spectra of 106 authentic Romanian honey samples harvested during 2020 and 2021, having five floral sources, were collected. In order to develop new powerful classification models capable to differentiate among the botanical origins and harvesting years, partial least squares discriminant analysis (PLS-DA) was applied as supervised chemometric tool. For the model accuracy improvement a data preprocessing step was included. This consisted in the application of several scaling and derivative based transformations followed by a data reduction step. Using this approach, the differentiation models with respect to both honey variety and harvesting year had an accuracy of 97%.
Moreover, based on the variable selection step it was possible to identify the spectral points that have the highest differentiation power. The markers based on which the best botanical classification was performed were attributed to the different concentrations in carbohydrates and amino acids from various honey types. As for the harvesting year classification, the carbohydrates, whose concentration decrease in time, with storage, proved to have the highest discrimination power.
Raman spectroscopy in corroboration with supervised analytical methods proved to be an effective tool in developing new classification models for honey botanical variety and harvesting year authentication.

References:
1. D.A Magdas, F. Guyon, R. Puscas, A. Vigouroux, L. Gaillard, A. Dehelean, I. Feher, G. Cristea, Applications of emerging stable isotopes and elemental markers for geographical and varietal recognition of Romanian and French honeys. Food Chem. 334 (2021) 127599
2. M. David, A.R. Hategan, C. Berghian-Grosan, D.A Magdas, The Development of Honey Recognition Models Based on the Association between ATR-IR Spectroscopy and Advanced Statistical Tools. Int. J. Mol. Sci. 23 (2022) 9977
3. O. Anjos, A.j.A Santos, V. Paixao, L.M Estevinho, Physicochemical characterization of Lavandula spp honey with FT-Raman spectroscopy. Talanta 178 (2018) 43–48

Figure captions:
Figure 1. Stacked FT-Raman characteristic spectra of honey samples.

Keywords: Honey, Raman spectroscopy, PLS-DA, differentiation

Figure 1. Stacked FT-Raman characteristic spectra of honey samples.

G-P.6
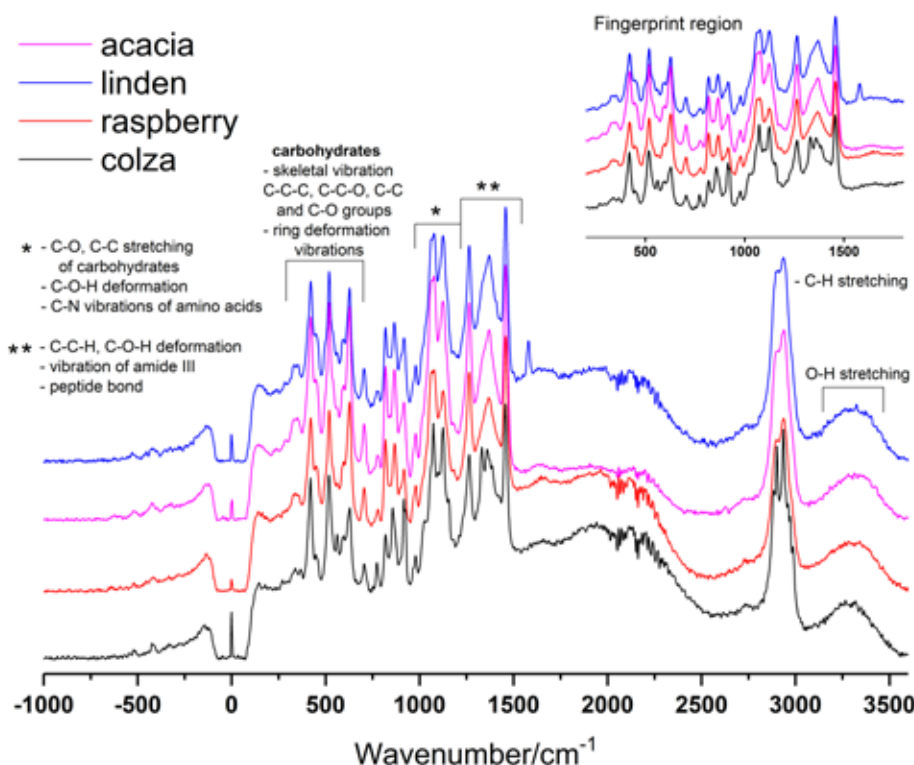
Title: *A comprehensive structural analysis of new antimalarial derivatives using chiroptical and vibrational spectoscopy*

Author: Kristyna Dobsikova[1], Eva Novotna[1], Michal Kohout[1], Vladimir Setnicka[1]

[1]University of Chemistry and Technology

Abstract:
One of the most effective and affordable drug used in prevention and as a standard drug in the chemoprophylaxis and treatment of malaria is *rac-erythro*-mefloquine hydrochloride, commercialized as Lariam® [1]. This molecule is known as a chiral and their stereochemistry not only affects antimalarial activity [2, 3] but also leads to different adverse effects of the respective enantiomers [4]. In recent years, it has been shown that the efficacy of most antimalarials is compromised by the emergence of *Plasmodium* species resistant to available antimalarials [5]. Resistance has been reported with almost all available antimalarials, has reinforced the urgent need to develop new antimalarials against resistant strains.
In this study, we have developed a chiral liquid chromatography method using a polysaccharide chiral stationary phase to separate the corresponding enantiomers of all prepared antimalarial candidates. We utilized molecular spectroscopic methods including chiroptical (electronic circular dichroism and vibrational circular dichroism) and non-polarizable (infrared and ultraviolet absorption) spectroscopies. In combination with density functional theory calculations, we have obtained stable conformers of selected enantiomers in solution and their relative abundances, which we used to simulate their spectra. The experimental and calculated data have been used to elucidate the 3D structure of the enantiomerically pure compounds and assign the absolute configuration of all prepared compounds.

References:
1. Shepherd R. D., Fletcher A.: PCT Int. Appl. WO 9839003 (1998).
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3. Bergqvist Y., Alkabbani J., Pettersson C., Huynh N. H.: J. Chromatogr., Biomed. Appl. 620 (1993) 217–224.
4. Croft A. M. J., World M. J.: Lancet 347 (1996) 326.
5. Patel P., Bharti P. K., Bansal D., Ali N. A., Raman R. K., Mohapatra P. K., Sehgal R., Mahanta J., Sultan A. A., Singh N.: Sci. Rep. 7, (2017) 16690.

Keywords: chiral separation, chiroptical spectroscopy, antimalarials

G-P.7

Title: *Maximizing efficiency in the chemical recovery of the pulp and paper industry through spectroscopic monitoring*

Author: Miranda Eisenköck[1], Barbara Weiß[1], Anna Katharina Schwaiger[2], Wolfgang Fuchs[2], Bernhard Lendl[3], Michael Harasek[4], Martin Kraft[1], Karin Wieland[1]

[1]Competence Center CHASE GmbH
[2]Sappi Europe | Gratkorn Mill
[3]Institute of Chemical Technologies and Analytics | TU Wien
[4]Institute of Chemical, Environmental and Bioscience Engineering | TU Wien

Abstract:

The recovery of chemicals after the extraction process of cellulose from wood in the pulp and paper industry is a key step towards circular process streams including ecological and economic benefits. The main chemical ingredient of the "cooking liquor" used here in the solubilization is $Mg(HSO_3)_2$. After the cooking step the residual material is incinerated and SO_2 is recovered from the hot flue gas in a cascade of Venturi scrubbers. Reiterated interactions of the hot gas with fresh $Mg(OH)_2$ slurry lead to the reformation of the magnesium bisulfite cooking liquor via a 2-step reaction process. Due to harsh conditions (temperatures > 60 °C, pH 4-7, high ionic strength) and complex interactions between gas and liquid phase, the formation of insoluble salts causes clogged pipes and unscheduled downtimes.

A key step in achieving higher recovery efficiency is a deepened understanding of the chemical interplay occurring in the Venturi scrubbers. Raman spectroscopy is used as a non-destructive, in-situ process monitoring tool. In combination with multivariate regression models, the spectral data is translated into critical process-relevant parameters. Continuous monitoring of variables such as free SO_2, total SO_2, sulfate and monosulfite based on the Raman spectral fingerprint allows tight process control of the interaction between the SO_2 gas and $Mg(OH)_2$ slurry.

We demonstrate the efficient use of Raman spectroscopy as a valuable tool in the chemical recovery of the pulp and paper industry. We establish and optimize a multivariate regression model based on several hundred at-line reference spectra to predict multiple target variables. Based on our approach, critical process parameters can be determined within seconds. Effects on the chemical system due to a change in the process control strategy may be corrected or averted by early countermeasures. Hence, long downtimes and loss of valuable chemicals due to unwanted precipitation are reduced.

Acknowledgments:

Figure captions:

Fig. 1 Raman spectra collected along the Venturi chain are translated into critical process-relevant parameters via a multivariate model.

Keywords: PAT, chemical recovery, Raman spectroscopy

G-P.8
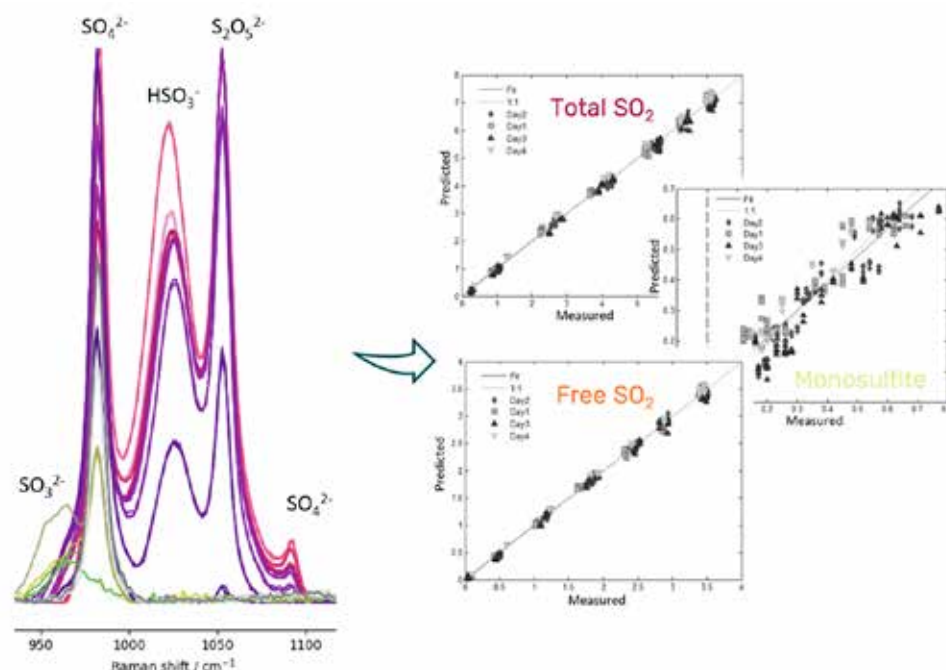
Title: *A new sensitive multi-analyte VOC sensor based on an integrated optics waveguide coated with a functionalised mesoporous sensing layer and QCL-IR spectrometry*

Author: Felix Frank[1], Mattias Verstuyft[2], Bettina Baumgartner[3], Nuria Teigell Beneitez[2], Dries Van Thourhout[2], Bernhard Lendl[1]

[1]TU Wien
[2]Ghent University-imec
[3]Utrecht University

Abstract:
Quantum cascade laser (QCL) based infrared spectroscopy is a highly effective method for detecting single and multiple analytes in the gas phase and has gained widespread use in industrial process analytical technology (PAT) in recent years. Most currently applied sensing schemes are utilising the photothermal and photoacoustic interaction of gases with infrared radiation and are capable of reaching limits of detection in the ppb range. [1] However, the use of single wavelength distributed feedback lasers make multi-analyte detection systems increasingly complex and bulky. Here, broadband laser-based evanescent field sensing comes into play. It shares advantages with established laser-based systems utilising the inherently high intensity but covers broad spectral ranges using external cavity QCLs
In this work, this was achieved by an innovative germanium-on-silicon integrated optics waveguide platform with integrated micro lenses [2] for efficient back-side optical interfacing for the tuneable laser spectrometer. The waveguide chip was coated with a highly sensitive and selective mesoporous silica coating, increasing both the signal by adsorptive enhancement, while eliminating water vapour interferences at the same time. The coating was tuned to selectively enrich multicomponent BTX (benzene, toluene, and xylene) contaminant mixtures from the air, showcased by its high sensitivity and selectivity. Multivariate curve resolution-alternating least squares (MCR-ALS) and other least square fitting methods were used to deconvolute the resulting spectra, showing sub-ppmv limits of detection and enrichment factors of up to 10,000. Finally, a use-case simulation for the continuous monitoring of BTX in a PAT environment was performed, showing the high potential of the technique.

References:
1. H. Moser, W. Pölz, J.P. Waclawek, et al. Implementation of a quantum cascade laser-based gas sensor prototype for sub-ppmv H2S measurements in a petrochemical process gas stream. Anal Bioanal Chem 409 (2017) 729–739.
2. N. Teigell Benéitez, B. Baumgartner, et al. Mid-IR sensing platform for trace analysis in aqueous solutions based on a germanium-on-silicon waveguide chip with a mesoporous silica coating for analyte enrichment, Opt. Express 28 (2020) 27013-27027

Figure captions:
Schematic depiction of the sensing concept (left), deconvoluted spectra of the analytes in the gas phase (top right) and the adsorbed state (bottom right).

Keywords: VOC_sensing, functional_materials, chemometrics, PAT, novel_photonic_technologies

Pure gas spectra in FTIR

Adsorbed gas spectra in QCL spectrometer

G-P.9
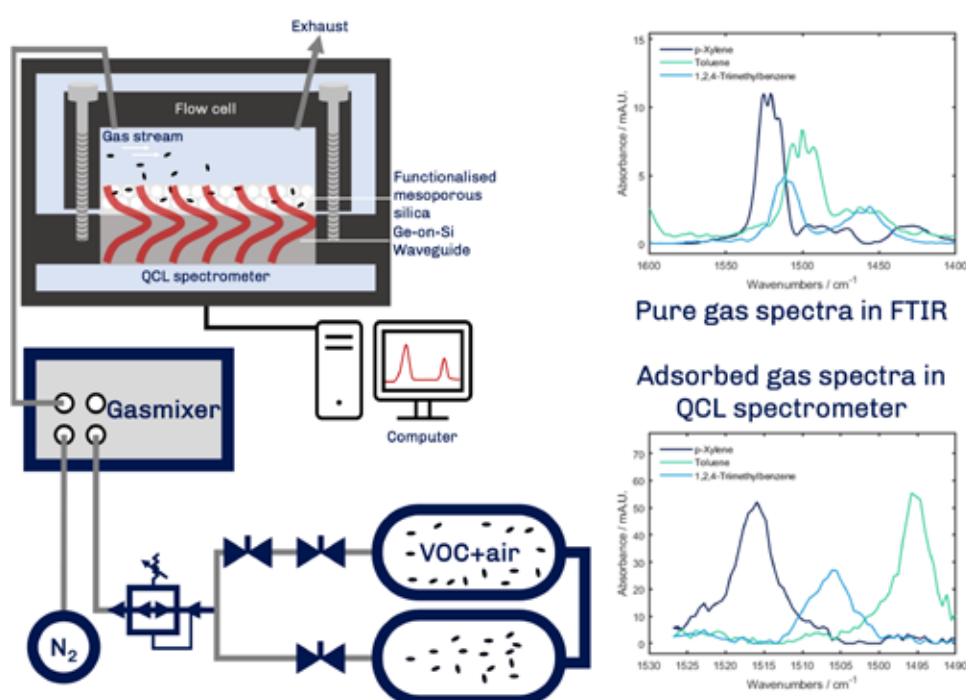
Title: *SERS detection of the herbicide glyphosate*

Author: Francisca Fuenzalida Sandoval[1], Santiago Sanchez-Cortes[2], Pavol Miškovský[3], <u>Zuzana Jurašeková</u>[1]

[1]Department of Biophysics, Faculty of Science, P. J. Šafárik University, Jesenná 5, 040 01
[2]Institute of the Structure of Matter, IEM-CSIC, Serrano 121, 28006
[3]Technology and Innovation Park, P. J. Šafárik University, Trieda SNP 1, 040 11 Košice, Slovakia; SAFTRA photonics, s.r.o., Moldavská cesta 51, 040 11 Košice, Slovakia

Abstract:
Glyphosate (glyph) is a broad-spectrum systemic herbicide used for weed control in agricultural production fields. In the 20th century, the use of glyph increased dramatically due to the introduction of pesticide-resistant crops [1]. Toxicological studies indicate that chronic exposure to this herbicide is potentially carcinogenic and can induce different diseases [2,3]. Glyphosate is currently approved in the EU until 15 December 2023 [4]. In this context, on-site identification and quantification of chemicals such as glyph are essential to promote food safety, human health, national security risk assessment, and disease diagnosis.
Raman spectroscopy (RS), and especially Surface-enhanced Raman spectroscopy (SERS), seems to be a very suitable analytical technique providing 'on-site' quick screening of samples without the need for the sample pre-treatment [5]. SERS can detect and distinguish individual pollutants even in mixtures and at low concentrations [6]. In addition, it is a technique that can detect a wide range of chemicals in the desired ppb-ppm concentration range.
Nevertheless, glyphosate SERS spectra show rather weak signals leading to not so low limit of detections by a direct method [5,7]. The reason is its high polarity, which seriously hinders the approach of the molecule to the surface of metallic nanostructures. Jan et al. proposed a promising indirect method for the SERS detection of the molecule of glyph [8]. We have tested and optimized the method and in the present work, we also show that an appropriate modification of the chemical structure of the glyph molecule can result in a compound that demonstrates a higher affinity for the metal surface and, in consequence, an increased sensitivity of the glyph SERS analysis leading to a limit of detection of 1 ppb.

References:
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Keywords: Environment, glyphosate, SERS (Surface-enhanced Raman)

G-P.10

Title: *Drop coating deposition Raman spectroscopy (DCDRS) as a tool for rapid determination and identification of contaminants and food additives*

Author: Alžbeta Kuižová[1], Václav Profant[1], Marek Procházka[1], Eva Kočišová[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University

Abstract:
Raman spectroscopy proved itself to be a powerful tool providing unique fingerprint molecular information. However, the conventional Raman spectroscopy from solutions is limited to highly concentrated samples. To overcome this disadvantage and to study solutions in low concentrations (~µM) and small volumes (~2 µl), a special method – drop coating deposition Raman spectroscopy (DCDRS) – was introduced [1].
The DCDRS is a simple and rapid method requiring minimal sample preparation. It is based on the deposition of a small droplet of liquid sample on the hydrophobic substrate where subsequent evaporation of solvent leads to the preconcentration of the analyte into dried patterns from which Raman spectra can be acquired. Compared to conventional Raman spectra from the solution, the sensitivity of DCDRS can be significantly improved, often by several magnitudes. DCDRS was already employed for the detection and identification of selected molecules and molecular mixtures [2,3].
Here we measured DCDRS spectra on environmental contaminants – thiram, bentazon and picloram – in aqueous solutions in environmentally relevant concentrations [4]. The Raman fingerprint spectral bands of studied contaminants were distinguished and assigned to specific molecular vibrations. For milk infant formula, an illegal additive, melamine, was intentionally added to milk and subsequently detected and identified. As for the powder infant formula itself, we found out that during the evaporation process, spatial distribution and separation of milk's main constituents (fats, mainly oleic acid and carbohydrates, such as lactose) in the dried deposit pattern occurred. The clear-cut detection of melamine in the dried deposit of contaminated milk was done because of no overlapping Raman bands in milk spectra with the main melamine spectral feature – the breathing vibration at 681 cm-1.

References:
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Keywords: DCDRS, contaminant, milk infant formula, melamine

G-P.11

Title: *Practical application of surface-enhanced Raman spectroscopy in the detection of hazardous materials*

Author: Malwina Liszewska[1], Bartosz Bartosewicz[1], Bogusław Budner[1], Łukasz Gutowski[1], Aleksandra Figat[1], Bartłomiej Jankiewicz[1]

[1]Institute of Optoelectronics, Military University of Technology

Abstract:
Surface-enhanced Raman scattering (SERS) has demonstrated to be a vibrant field of research that is constantly growing in scope and applicability. Rapid, reliable detection of hazardous materials is critical for security and defense applications. The potential advantages of SERS include augmentation of currently deployed Raman instrumentation with greater sensitivity and specificity to sense hazardous materials. SERS could improve the detection, identification, and monitoring capabilities of first responders and military personnel in decontamination verification, post-blast forensics, low-level vapor detection, and trace surface detection for hazardous materials.

The presented research was carried out as part of the NATO Research Task Group SET-RTG-292 "Enhanced Raman Scattering For Defense Applications". The main goal of our studies was to test whether it is possible to detect and identify trace amounts of chemical warfare agents (CWA) simulants using handheld, portable, and laboratory Raman instruments with 785 nm laser excitation. In our studies, we used two SERS substrates, a commercial from Thermo Scientific and a substrate made at the Institute of High-Pressure Physics of the Polish Academy of Sciences. Our studies have shown that portable Raman systems are suitable for SERS measurements of trace amounts of CWA simulants with similar limits of detection. In addition, the possibility of using SERS to detect traces of hazardous materials in the field using NATO sampling and identification procedures was tested.

Keywords: SERS, trace detection, hazardous materials

G-P.12

Title: *Trace detection and evaluation of the presence in situ of Cylindrospermopsin cyanotoxin in environmental waters from Cojocna Transylvania, Romania*

Author: Csilla Molnar[1], Teodora Diana Drigla[2], Simona Cinta Pinzaru[3]

[1]National Institute for Research and Development of Isotopic and Molecular Technologies
[2]Institute for Research, Development and Innovation in Applied Natural Sciences, Babes-Bolyai University
[3]Biomolecular Physics Department, Babeş–Bolyai University

Abstract:
Relying on our previous work [1, 2] on cyanotoxins detection and quantification in aqueous solutions using Raman spectroscopy techniques, theoretical DFT calculations, and plasmonic nanoparticles for SERS trace detection, here we prospect the capability of the SERS technique for field applications, regarding the cyanotoxins monitoring in several eutrophics inland water bodies from Transylvania, Romania). In addition, spectroscopic analyses of two local salt lakes in Cojocna, Romania, were prospected, since their halophilic microbial community revealed, among abundant Dunaliella salina, several filamentous cyanobacteria which potentially could bloom and release toxins when nutrients level increases along with the increasing temperature in spring-summer season. Surface and 1m depth lake waters from hypersaline lakes of Cojocna, Romania, have been investigated in raw form, using SERS techniques. These surface water bodies are particularly important for popular balneotherapy in the area. The observed SERS bands obtained with AgNPs and raw water droplets (Fig. 1) in the winter months monitoring experiment, revealed reproducible SERS signal of adsorbed beta-carotene [3], which originated from the cyanobacteria interaction with AgNPs. The higher the cyanobacteria density, the higher the SERS signal. The results provide deeper insight into the SERS potential for translation to environmental waters monitoring programs in conjunction with our developed aquatic toxins Raman database.

References:
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Figure captions:
Figure1. SERS spectra of the raw water samples from the two Cojocna lakes, collected from the surface and the -1 m depth

Keywords:
Cylindrospermopsin, environmental waters, Raman, SERS

G-P.13
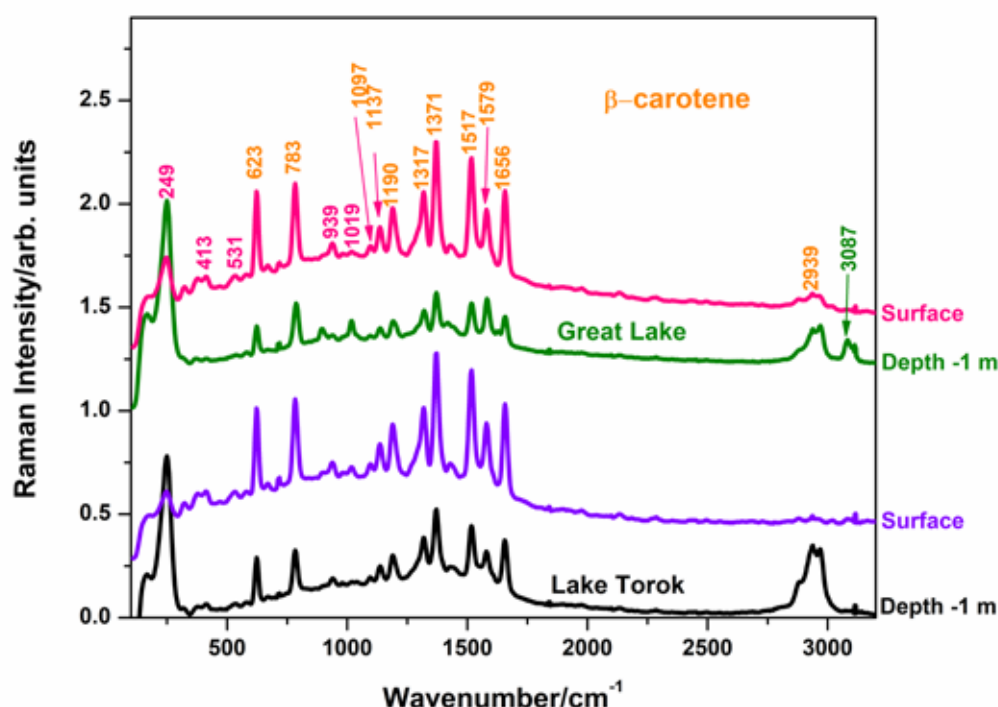
Title: *PoC QUANTIFICATION AND PROFILING OF URINE CELLS BY INTEGRATING CYTOCENTRIFUGATION AND IR MESUREMENTS ON THE SAME SUBSTRATE*

Author: Víctor Navarro-Esteve[1], Birgit Felderer[2], Guillermo Quintás[3], Julia Kuligowski[2], Bayden Wood[4], David Pérez-Guaita[1]

[1]Department of Analytical Chemistry, University of Valencia
[2]Neonatal Research Group, Health Research Institute La Fe
[3]Health and Biomedicine, Leitat Technological Center
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Abstract:
The presence of cells in urine and in particular White Blood Cells (WBCs) is often associated with Urinary Tract Infections (UTIs) and many other diseases. Hence, the development of cost effective Point-of-Care (PoC) diagnostic test could prove to be a valuable tool for non-invasive screening. Fourier transform infrared (FTIR) spectroscopy has the potential to identify and quantify cells in urine. However, urine is a complex matrix and the quantification of components such as cells by compact IR spectrophotometers can be hindered by the presence of highly concentrated biomolecules such as urea or creatinine. The use of separation procedures can assist identifying and quantifying cells but reduces the PoC capabilities of the technology. Here we propose coupling cytocentrifugation with FTIR spectroscopy for the isolation and quantification of cells in urine using FTIR spectroscopy and our recently developed device, the ATR-Spin[1], which enables cytocentrifugation directly onto an Attenuated Total Reflectance (ATR) crystal or transflection substrate. Urine was spiked with monocytes and measured both in ATR and transflection modes using a silicon ATR substrate and a Keveley low-E slide™, respectively. Spectra of the cyto-centrifugated fraction clearly showed typical features of cells for both modalities. A univariate calibration line was prepared and the FTIR signal was proven to be linear ($R^2=0.98$) in the 8×10^3-2×10^5 cells/ml range, thus demonstrating the detection of cells at pathological numbers (pyuria, i.e. $>1 \times 10^4$ WBC/ml). Transflection FTIR yielded better results in terms of linearity and clinical applicability. Furthermore, the quantification of monocytes in urine in the presence of other cell types such as T-lymphocytes was modeled using Partial Least Squares (PLS) regression (RMSECV=4×10^4 cells/ml). The results indicate a strong potential for a fast, simple, versatile, and cost-effective quantification technique for profiling cells in urine.

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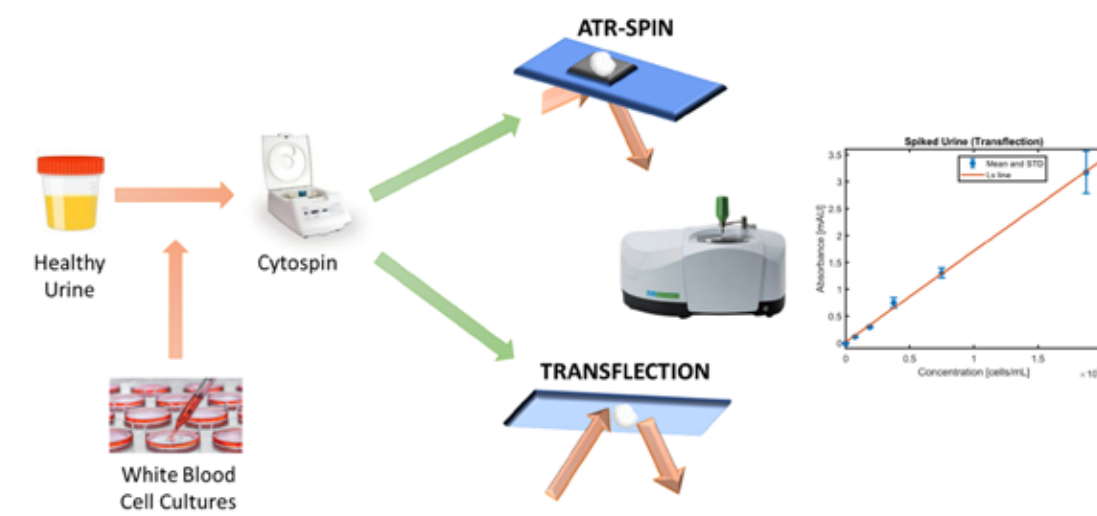
Figure captions:
Conceptual scheme of the methodologies and analysis

Keywords: Infrared-spectroscopy, Point-of-Care, UTIs, Urine, Leukocytes

Title: *Towards the intelligent toilet: SERS sensing of nM-level neurotransmitters with Fe-sensitized self-assembled gold nanoparticle arrays*

Author: Marika Niihori[1], Rakesh Arul[1], Tamas Foldes[2], Charlie Readman[1], David Benjamin-Grys[1], Sarah Sibug-Torres[1], Elle Wyatt[1], Bart De Nijs[3], Edina Rosta[4], Jeremy Baumberg[1]

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[3]University of Cambirdge
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Abstract:
Detection of neurotransmitters from human biofluids such as urine is challenging due to low concentrations (<μM), biofouling/interference and their chemical similarity to other neurotransmitters reducing selectivity. Conventional techniques (LCMS, NMR) are slow, expensive and ineffective methods for precision health. Surface-enhanced Raman Spectroscopy (SERS) is a powerful technique having the ability to address these issues and gives prospects for developing real-time, sensitive, non-invasive and cost-effective sensors. Here, we highlight the exploitation of self-assembled Au nanoparticle (AuNP) aggregates, in both solution and on films, to sense neurotransmitters below physiological levels including dopamine, for the Intelligent Toilet. Initially a simple 'mix-and-measure' protocol is explored by sensitising AuNP aggregates suspended in solution with Fe(III) (Fig.1a), achieving limits of detection (LOD) of 1 nM for dopamine, below clinical concentrations.[1] This method utilises additional enhancements from Fe(III)-dopamine complexation enabling development of fully integrated microfluidic systems. However, the presence of surfactants and stabilisers can interfere with the sensing as well as restricting scope for repeated reuse. Our second approach (Fig.1b) immobilises solution aggregates onto transparent substrates, creating a film of two-dimensional dense-packed arrays of AuNPs, with rigid molecular scaffolds fixing precision nanogap sizes to 0.5-2 nm.[2] The transparent glass substrate allows for efficient access from both sides by fluid and light, allowing repeated sensing in different analytes. By adding Fe(III) sensitisation in these AuNP films, LODs below physiological levels are achieved as well as multiplexed sensing.[3] These systems are highly promising for widespread microfluidic integration enabling a wide range of continuous biofluid monitoring for applications in precision health.

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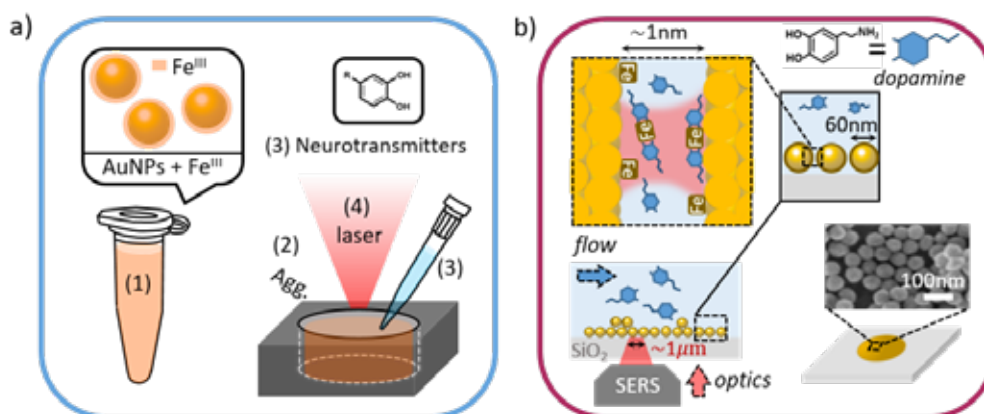
Figure captions:
Fig 1. Schematic of a) solution-based AuNP aggregates sensitised with Fe(III) and b) aggregated AuNPs immobilised onto films. Both have ability to sense neurotransmitters below physiological levels

Keywords: SERS, sensing, neurotransmitters, nanogap, AuNP

G-P.15

Title: *Amorphous quantification of drug compound using Raman spectroscopy and partial least squares regression (PLS)*

Author: Saeideh Ostovar pour[1]

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Abstract:
Solid-state forms transformation of an active pharmaceutical ingredient (API), such as crystalline, amorphous forms can impact its physical, chemical, and mechanical properties. Phase transformation of API can occur during the milling, granulation, and tablet compression process. It is important to monitor and characterise the process-induced disorder early in drug product development. Over the years, various analytical methods have been used to study the solid state of API such as XRPD, TGA, and DSC. However, these methods cannot be implemented during the manufacturing of API to monitor the real-time investigation of the solid state of API.
Raman spectroscopy provides several advantages including enabling real-time measurement, low moisture signal interaction, minimal/no sample preparation, and being non-invasive. In this study, we demonstrate the feasibility of Raman spectroscopy as a sensitive analytical tool with multivariate data analysis (Partial least squares regression (PLS)) to quantify the amount of different solid-state forms present in early-stage APIs monitoring after micritisation. The predicted solid-state forms were further verified with solid-state NMR data.

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Ann W. Newman, Stephen R. Byrn, Solid-state analysis of the active pharmaceutical ingredient in drug products, Drug Discovery Today, 8(2003) 898-905
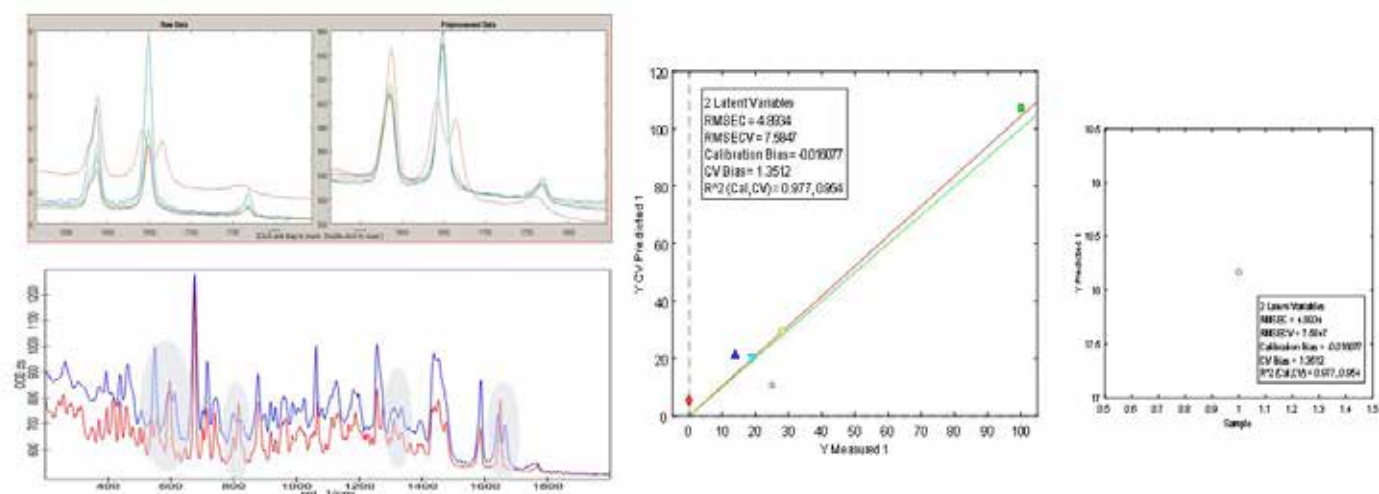
Figure captions:
Figure1: an example of PLS modelling using Calibration data set (left) and test data (right) for amorphous content in micronized sample for DP

Keywords: Solid-state-form, API, Raman spectroscopy, Chemometric

G-P.16

Title: *Bio-chemical assessment of blood cell and PBMC smears using optical photothermal mid-IR spectroscopy for studies of diseases and infections*

Author: Shravan Raghunathan[1], Michael Kiehntopf[2], Susann Piehler[2], Jürgen Popp[3], Christoph Krafft[1]

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Abstract:
Mid infrared spectroscopic methods including FTIR spectroscopy provides molecular "fingerprints" as a result of fundamental vibrational transitions. Optical photothermal mid-IR spectroscopy (O-PTIR) combines infrared absorption of a pulsed quantum cascade laser and refractive index modulation of a visible probe laser to obtain spectral response of biological samples [1]. Advantages of O-PTIR in the context of single cell analysis include sub-micrometer lateral resolution, lack of anomalous scattering effects, compatibility with various substrate materials in reflection and transmission modes, high speed imaging at discrete wavenumbers, and simultaneous collection of IR and Raman spectra [2, 3]. Here, we studied blood cells smeared on glass, and CaF_2 substrates. Based on O-PTIR spectra of single cells, representative bands were selected to separate erythrocytes (red blood cells) from unstained and unstained leukocytes (white blood cells) and to distinguish between different leukocytes. High resolution discrete wavenumber O-PTIR images enabled counting leukocytes among thousands of erythrocytes and determining sizes of cells and cell nuclei. Broad spectral contributions of glass between 1250 and 1000 cm^{-1} were effectively compensated by calculating second derivatives. The perspective is discussed to apply O-PTIR spectroscopy and imaging for cell screening to diagnose infections and other diseases.

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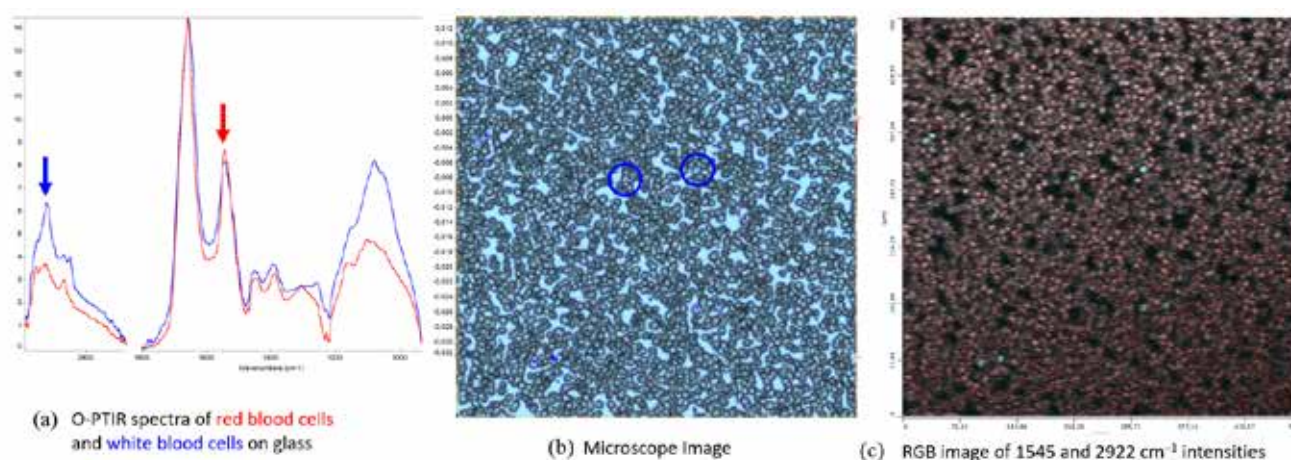
Figure captions:
(a) O-PTIR of red blood cells and white blood cells on glass. (b) Microscope image of the blood smear. (c) RGB overlay of photothermal image at 1545 and 2922 cm-1 intensities

Keywords: Infra-red, spectroscopy, photothermal, cell screening

G-P.17

Title: *Evaluation of different Raman spectrometer for monitoring metabolites in manufacturing of biotherapeutics using at-line multi-attribute Raman spectroscopy (MARS)*

Author: Frederik Schröter[1], Michaela Poth[1], Oliver Popp[1]

[1]Roche Diagnostics GmbH

Abstract:

Since the launch of the Process Analytical Technology (PAT) initiative of the Food and Drug Administration (FDA), innovative technologies for in-process controls have become increasingly important for pharmaceutical industries. The FDA demands a greater control and understanding about the manufacturing processes and its critical parameters to ensure a persistent high product quality. In production of biotherapeutics, concentrations of substrates consumed by the cells and the resulting metabolites display those critical key parameters.

Current offline methods for quantitative metabolomic analysation of cell culture processes are labor-intensive and time-consuming. The demands of the biopharmaceutical industry for a fast, multi-attribute method that delivers results for ten thousands of samples almost in real time, might be addressed by the same analytic technique: high-throughput Raman spectroscopy in combination with multivariate data analysis enables rapid, non-invasive measurements that are easy to handle and can be automated. However, the speed of the measurement of complex biological samples often contradicts a high spectral quality, which drastically reduces the performance of regression models. Although a large number of companies offer high-quality Raman devices, not all are suitable for at-line monitoring of bioprocesses. To evaluate the available instruments of different vendors on their suitability for this application, diverse bioprocess samples with complex background matrices were prepared. Subsequently, a single acquisition method was determined separately for each tested device to allow a proper measurement of all samples. Advanced data handling techniques for preprocessing and modeling enabled highly accurate predictions for more than 30 different metabolites in complex bioprocess samples. Thereby, a comparison of the different devices pronounced the strengths of the single instruments and highlighted the importance of an extensive system evaluation.

Keywords: Raman, PAT, Bioprocess, Modeling, at-line

G-P.18

Title: *Identification and classification of methotrexate and its metabolites in human serum samples using surface-enhanced Raman scattering combined with advanced data analysis*

Author: Gohar Soufi[1], Elodie Dumont[1], Yaman Göksel[1], Roman Slipets[1], Raheel Altaf Rajac[2], Kjeld Schmiegelow[2], Anja Boisen[1], Kinga Zor[1]

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Abstract:
Therapeutic drug monitoring (TDM) is essential in clinical practice and involves the quantification of the administered drugs during therapy. TDM is used because it is important to know the amount of medication to adjust the drug concentration in the patient´s blood within a defined therapeutic range or ensure its clearance to prevent adverse effects. In high-dose methotrexate (MTX) therapy, TDM is vital in administering rescue drugs to prevent MTX toxicity [1,2]. Due to the similar chemical structures of the MTX and its metabolites, analytical techniques often face challenges distinguishing between drugs and their metabolites. The routine methods for TDM of MTX are often based on immunoassays that are not accurate when specific metabolites are present in the sample [3]; in this case, the only option is to use bulky and expensive chromatographic approaches. Therefore, it would be beneficial to develop reliable, cost-efficient, and easy-to-operate assays or sensors for quantifying MTX in the serum samples in the presence of its metabolites. In this work, different machine learning methods, including principal component analysis (PCA), linear discrimination analysis (LDA), and partial least squares discrimination (PLS-DA) analysis were employed to enable us for the first time with SERS, to identify MTX in the presence of its metabolites (7-hydroxy-methotrexate (7-OH MTX) and 2,4-diamino-N(10)-methylpteroic acid (DAMPA)).For the quantification both the univariate and multivariate regression were investigated. Amongst the figures of merit, we found the limit of detection to be 0.15 µM while the limit of quantification was 0.55 µM which has improved five times in compared with previous work [4]. The developed assay based on SERS was used with a tabletop Raman spectrometer that facilitated ease of measurement and data collection. We believe this work is an important step towards enabling affordable, easy-to-use method TDM of MTX at clinics.

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Keywords: SERS, Sensor, Chemometrics, Drug monitoring

G-P.19

Title: *FTIR nano-spectroscopy at SISSI-Bio Beamline: recent insight in the field of Cultural Heritage*

Author: Chiaramaria Stani[1], Giovanni Birarda[2], Giacomo Fiocco[3], Marco Malagodi[3], Giorgia Sciutto[4], Lisa Vaccari[2]

[1]CERIC-ERIC
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Abstract:
Fourier Transform Infrared spectroscopy is one of the most powerful technique applied in the field of Cultural Heritage thanks to its capability to study very small amount of samples providing chemical information on both the organic and inorganic components of complex unknown mixtures [1].
In the last years advanced sub-diffraction limit IR spectroscopies have been developed, opening new scenarios in several research fields spacing from life science and biology to polymers and material sciences. Recently, optical photo thermal IR spectroscopy (O-PTIR), reaching sub-micrometric lateral resolution, has been applied for characterizing precious artworks and their degradation mechanisms [2]. At SISSI-Bio beamline (Elettra Sincrotrone Trieste, Italy), the advantage offered by the infrared scattering-type scanning near-field optical microscopy (IR s-SNOM) to reach spatial resolutions down to tens of nanometers has been exploited for the first time in field of Cultural Heritage for the characterization of complex samples from both a morphological and chemical point view.
Here, the results obtained on two fascinating cases of study will be presented.
The first one represents a detailed investigation combining both micro- and nano-spectroscopy of two of the most valuable violins produced by Antonio Stradivari, the Toscano (1690) and the San Lorenzo (1718) [3]. Thanks to the high lateral resolution achieved, new details on Stradivari's manufacturing technique has been revealed unravelling a widely debated question among the expert of ancient bowed string instruments.
In the second case, the study of zinc white egg and oil painting models at the nanoscale will be presented. The possibility to simultaneously collect both morphological and chemical information gave us the opportunity to observe new peculiarities about the mechanism of growth and crystallization of zinc carboxylates, one of the most studied degradation product in paintings [4].

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Keywords: IR nanospectroscopy, Cultural Heritage, Stradivari

Title: *Vibrational spectroscopy – a valuable PAT tool for crystallization monitoring*

Author: <u>Karin Wieland</u>[1], Thomas Linder[2], Bernhard Sandig[3], Martin Kraft[1], Peter Pöchlauer[3]

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[2]Patheon Austria GmbH & Co KG
[3]Patheon Austria GmbH & Co KG

Abstract:
Crystallization is used in many chemical manufacturing processes to separate, purify, and isolate products from reaction mixtures. Especially when different polymorphic forms may be formed along the way, but typically only one form exhibits the desired physical (e. g. solubility, stability, melting point, etc.) and chemical (chemical reactivity) properties. Hence, the production of the wrong polymorph can have a significant impact on the properties of the final product and can, therefore, affect its suitability for the intended use. Consequently, careful control of the crystallization process is key to ensure formation of the correct polymorph. Vibrational spectroscopy enables real-time analysis of the crystallization process. Together with uni- or multivariate analysis, concentration profiles can be extracted and used for e. g. reaction end-point determination. Raman and FTIR spectroscopy can both be employed for polymorph screening and crystallization progress monitoring but exhibit different advantages and disadvantages; Raman spectroscopy is a weak scattering effect and is typically hampered by fluorescent samples while IR spectroscopy in aqueous environment suffers from strong water absorptions. Both vibrational spectroscopy techniques, however, are affected by temperature changes in terms of band shifts, broadening or intensity. Hence, the effect of temperature needs to be considered in data analysis.
Spectroscopy-based continuous monitoring of crystallization processes is a critical tool for optimizing process performance and ensuring product quality. We show results using a combined approach of Raman and FTIR in-line spectroscopic monitoring during a crystallization process with changing temperature. Along with this multi-sensor approach, valuable process information can be extracted in combination with multivariate data analysis.

Keywords: Crystallization, PAT, vibrational spectroscopy

H-P.1

Title: ***Strong impact of de novo purine biosynthesis on the spectroscopic signature of Staphylococcus aureus revealed by the screening of a gene-defined transposon mutant library***

Author: Emad Adel1, Masoumeh Alinaghi[1], Sabrina Jenull[1], Christian Url[2], Helene Marbach[1], Billi Postlmayr[1], Monika Ehling-Schulz[1], Claus Vogl[1], Guoqing Xia[3], Tom Grunert[1]

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Abstract:
Subtyping of bacterial pathogens is essential for infection control, epidemiological studies and outbreak investigations. IR-spectroscopic fingerprinting has been widely used and allows phenotypic characterization, providing complementary information to molecular genotyping. We successfully applied high-resolution Fourier-Transform infrared (FTIR) spectroscopy to differentiate between *Staphylococcus aureus* strains (Johler et al., 2016). We have identified the expression of surface glycans (Grunert *et al.*, 2013, 2018), but additional factors that discriminate between staphylococcal strains remain unclear. Here, we screened the sequence-defined Nebraska Transposon Mutant Library (NTML), consisting of 1920 S. *aureus* mutants of non-essential genes by FTIR spectroscopy. Wild-type (JE2) and NTML mutants were grown on tryptone soy agar (TSA) at 37°C for 24h, followed by the spectroscopic measurements. We obtained 238 (12.4%) NTML mutants with significant spectral changes in the carbohydrate spectral region (1200 – 800 cm^{-1}) compared to the isogenic wild-type strain JE2. Unsupervised multivariate statistics (incl. HCA, PCA, t-SNE) and functional enrichment analyses were performed to decipher the functional relationship based on each gene-specific, unique FTIR-spectroscopic signature.
Preliminary results indicate that the disruption of genes involved in energy metabolisms, such as *de novo* purine biosynthesis, is responsible for drastic perturbations in the S. *aureus* spectroscopic fingerprint. The computational analysis also allowed functional mapping of genes based on the FTIR spectroscopic signature, providing additional functional clues for genes with hypothetical annotation.
In conclusion, screening of S. *aureus* gene-defined mutants by FTIR spectroscopy reveals first insights into the landscape of regulatory and metabolic genes/pathways that determine the spectroscopic signature and potentially predict new functional associations between genes/proteins.

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Keywords: Staphylococcus aureus, FTIR, subtyping, functional

H-P.2

Title: *Early Diagnosis of Circulating Tumor Cells by Surface-Enhanced Raman Scattering (SERS) Using Fe2O3@SiO2@Au Nanoparticles*

Author: Hae-jin Chung[1], Eungyeong Park[1], Ju Hyun Park[2], Young Mee Jung[1]

[1]Department of Chemistry, Kangwon National University
[2]Department of Biomedical Science, Kangwon National University

Abstract:
Circulating tumor cells (CTCs) detach from primary tumors and spread into the bloodstream to form secondary tumors. Liquid biopsy with CTC detection can be used for early diagnosis of tumors. Surface-enhanced Raman scattering (SERS) is a sensitive, selective, and photostable method that can be used for CTC detection. In this study, $Fe_2O_3@SiO_2@Au$ nanoparticles ($Fe_2O_3@SiO_2@Au$ NPs) were fabricated for SERS detection of CTC. $Fe_2O_3@SiO_2@Au$ NPs have a magnetic core, which can separate cancer cells from a complex system. The properties of $Fe_2O_3@SiO_2@Au$ NPs were analyzed using transmission electron microscopy, energy-dispersive X-ray spectroscopy, UV-Vis absorption spectroscopy, and SERS. The presentation will provide further details on SERS spectra of CTCs.

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Keywords: SERS,CTCs,Magnetic core,Separation,Liquid biopsy

H-P.3

Title: *Therapeutic drug monitoring of methotrexate with surface-enhanced Raman spectroscopy (SERS)*

Author: Elodie Dumont[1], Yaman Göksel[1], Roman Slipets[1], Lasse H. E. Thamdrup[1], Kjeld Schmiegelow[2], Tomas Rindzevicius[1], Kinga Zor[1], Anja Boisen[1]

[1]Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Health Technology, Technical University of Denmark
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Abstract:
Methotrexate (MTX) is a key drug in treating several cancers, such as childhood acute lymphoblastic leukaemia. However, its safe use requires therapeutic drug monitoring (TDM) to follow MTX clearance and thereby avoid delayed elimination toxicity by administering a proper dose of MTX rescue drug. Patients are usually followed until MTX concentration falls below 0.2 µM[1]. Current standard analytical methods for MTX quantification rely on immunoassays or chromatographic techniques, which cannot be afforded by every clinic. This entails delayed response time due to sample shipment to reference labs and has stepped up research on affordable and fast alternative techniques for MTX quantification. Surface-enhanced Raman spectroscopy (SERS) is a promising choice given its short time-to-answer and versatility.
This work presents a dual strategy, based on SERS detection, to monitor MTX levels in serum samples of patients receiving high-dose MTX therapy.
On the one hand, *nanopillar-assisted separation* (NPAS) was applied before SERS detection and enabled MTX quantification in the 5 – 150 µM concentration range[2]. In NPAS, the nanopillars of the SERS substrate function as a sieve or filter, by trapping large molecules such as proteins. As a result, sample handling is kept to a minimum, i.e. serum mixing with an organic solvent.
On the other hand, applying well-defined electric potentials to the SERS substrates resulted in *electrochemically assisted SERS*, which allowed for MTX attraction and pre-concentration on the SERS substrates. Combined with an appropriate sample preparation, namely gel filtration, electrochemically assisted SERS could be used to quantify MTX in the low concentration range (0.5 – 5 µM)[3].
In both cases, the analysis time was short (< 30 min). Moreover, the SERS detection could be conducted on a portable Raman spectrometer, paving the way for point-of-need monitoring of MTX.

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Figure captions:
Illustration of the dual SERS strategy regarding TDM of MTX: NPAS for high MTX concentrations (orange) and electrochemically assisted SERS for low MTX concentrations (green) monitoring.

Keywords: SERS, EC-SERS, methotrexate, quantification, TDM

H-P.4
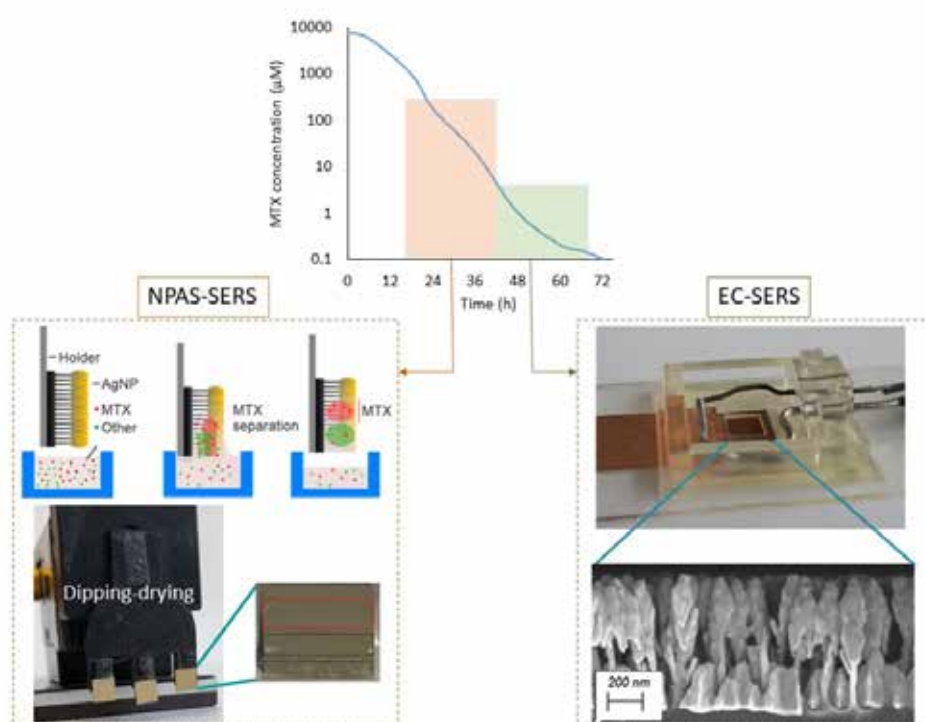
Title: *Metabolic impacts of microplastic exposure in mammalian cells measured via FTIR microspectroscopy*

Author: <u>Helena Friedrich</u>[1], Ka Lung Andrew Chan[1], Stephanie Wright[2], Gianfelice Cinque[3]

[1]King's College London
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[3]Diamond Light Source

Abstract:
Microplastic has attracted much public attention after the early discovery that they are widespread in the marine environment.[1] Since then, microplastics have been found in various environmental reservoirs (ocean, lakes, rivers, soil, sand, atmosphere), in various consumables (fish, honey, beverages), and in human samples (lungs, blood, placenta) raising the question of their cellular fate and potential toxicity to humans. While most research in this area is focused on cell-based cytotoxicity biochemical assays, little is known about how these plastic particles are metabolized in cells and their biological influences at a molecular level.

FTIR microspectroscopy has been established as a label-free and non-destructive tool to study the effect of xenobiotics in cells by providing specific molecular information on the cellular composition. While most FTIR studies are based on fixed or dried cells, we focus on developing a method to measure live cells, hence eliminating the need for fixing or drying, which could introduce artefacts in the measurements. In addition, this method opens the opportunity to observe the kinetics of the biological changes in the cells.[2,3]

In this work, we applied the novel live-cell FTIR technique to investigate the effects of different types of microplastics on a murine lung alveolar macrophage cell model. The FTIR microspectra of the living cells treated with microplastics have shown subtle chemical changes, highlighting the potential of the technique for understanding the chemical and biochemical interactions between these xenobiotics and cells.

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Keywords: microplastics, living cells, FTIR spectroscopy

H-P.5

Title: *Characterization of SARS-CoV-2 Virus-Like Particles by Vibrational Spectroscopy*

Author: Magdalena Giergiel[1], Ankit Dodla[1], Aaron McLean[1], Linda Earnest[2], Julie McAuley[2], Melissa Edeling[2], Dale I. Godfrey[2], Damian F. J. Purcell[2], Jason Roberts[3], Simon Collett[4], Joseph Torresi[2], Bayden R. Wood[5]

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Abstract:
The outbreak of COVID-19 has underscored the urgent need for effective detection, characterization, and treatment of viral infections. Virus-like particles (VLPs) serve as non-infectious counterparts to real viruses and play a vital role in vaccine development. Detailed chemical characterization of VLPs can provide crucial insights into virus structure, function, and evolution. In this study, FTIR and Raman spectra of VLPs were recorded to investigate their chemical composition and evaluate the potential of this technique, in combination with multivariate data analysis methods, for the identification and classification of VLPs.
Accurate chemical characterization is essential to assess the similarity of VLPs to the original virus and to ensure the absence of transcribed genomic material during synthesis. In the development of new vaccines, it is critical to prevent the production of genomic material during VLP synthesis, as it can lead to replication of the infective agent. The sensitivity of vibrational spectroscopy to DNA and RNA makes it an ideal technique to detect the presence of genomic material.
Moreover, the spectral data serve as a valuable resource for comprehensively characterizing the chemical structure of various types of VLPs. FTIR and Raman spectroscopies hold the potential to identify and classify different variants, thereby enabling the development of virus diagnostics based on this technique. By better understanding the structural aspects of VLPs, obtained results may contribute towards establishing quality control measures. Ultimately, this research can facilitate the more efficient and rapid creation of new vaccines and diagnostics in the future.

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Keywords: SARS-CoV-2, Virus-like particles, FTIR spectroscopy,

H-P.6

Title: *Analysis of polymeric nanoparticles for drug delivery using Raman spectroscopy*

Author: Frederike Gladigau[1], Jenny Hemstedt[2], Karl Scheuer[2], Jana Ismail[3], Christian Kretzer[4], Blerina Shkondra[5], Ondrej Stranik[6], Reiner Heintzmann[6], Ulrich S. Schubert[5], Klaus Jandt[5], Stephanie Schubert[7], Oliver Werz[7], Ute Neugebauer[1]

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[7]Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Germany; Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Germany

Abstract:
Raman spectroscopy is an excellent tool to characterize samples on a molecular level without lengthy preparation. In this work we use this method to analyze polymeric nanoparticles that are meant for drug delivery, as it is known, that encapsulation of active substances can improve targeted delivery as well as pharmacokinetics [1].
The parameters that were investigated are crystallinity of nanoparticles, drug distribution and drug release kinetics.
The first point of interest was the crystallinity of samples produced by combining the D- and L-stereoisomers of polylactic acid (PLA) and racemic 3-ethylglycolide (EtGly). This allowed the formation of stereocomplexes with differing crystallinity according to the content of EtGly, which could be precipitated into nanoparticles. The degree of crystallinity was found to correlate with the intensity and position of the Raman band for the C=O stretching region (at around 1760 cm^{-1}). This could be confirmed by Wide Angle X-ray Scattering (WAXS). Using a dielectrophoresis setup allowed measurement directly in suspension [2].
Next, a closer look was taken into the distribution of the active substance TG-201 (a benzimidazole-based 5-Lipoxygenase-activating protein (FLAP) antagonist) encapsulated into nanoparticles made of poly(lactide-co-glycolide) (PLGA) or ethoxy acetalated dextran (Ace-dex). Creating intensity maps by visualizing Raman bands specific to either the drug or the polymers allowed to distinguish between the even distribution seen with Ace-dex and the precipitates of free drug found in PLGA [3]. Lastly, the release kinetics of the antibiotic cefazolin encapsulated into nanoparticles made of PLGA, Ace-dex or polyethylene glycol (PEG) were analyzed. First the extracellular effect bacterial growth was photometrically measured, with the PEG nanoparticles showing the most significant effect. These were then used to perform lysis experiments showing the antimicrobial effect of the drug-loaded nanoparticles inside the cells.

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Keywords: Raman spectroscopy, polymeric nanoparticles

H-P.7

Title: *Lipids balance and lipids peroxidation changes in mice blastocyst after artificial growth arrest measured by Fourier Transform InfraRed spectroscopy*

Author: Hafsa Gulzar[1], Richard Musson[1], Joanna Depciuch[2], Grażyna Ewa Ptak[3]

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[2]"Department of Biochemistry and Molecular Biology, Medical University of Lublin" "Institute of Nuclear Physics, Polish Academy of Science"
[3]Małopolska Centre of Biotechnology, Jagiellonian university
This work was supported by the National Science Centre, Poland (GA no. 2019/35/B/NZ4/03547 and 2021/41/B/NZ3/03507.

Abstract:
Fourier Transform InfraRed (FTIR) spectroscopy is a powerful tool which enables label-free extraction of valuable biochemical information changes from biological samples such as mouse blastocysts[1]. In this study, effect of artificial growth arrest using inhibitors of mTOR (INK128) and C-Myc (FA) on the lipid balance and lipid peroxidation of mice blastocyst is investigated [2,3]. For this purpose, blastocysts of day 4 from female mice were collected and *in vitro* cultured for 24 hours in three groups: Control (without any inhibitor) n=16; INK128 (with mTOR inhibitor) n=14; and INK128+FA (with both mTtor and C-Myc inhibitors) n=7. Later on, FTIR measurements of blastocysts from these three groups were performed and analysed using secondary structure, statistical and principal component analyses (PCA).
We observed various differences in lipids & phospholipids between control and non-control groups. Briefly, a higher peak absorbance around 1750 cm^{-1} depicting increased lipid peroxidation in non-control groups was noticed. Furthermore, changes in the lipids balance were visible as a difference in the ratio of lipids and phospholipids. Also, lower ratios of lipids or phospholipids to C=O bonds were observed in non-control groups. These observations imply lipid/phospholipid peroxidation in non-control groups. Finally, PCA analysis showed different phospholipids fraction between INK128+FA and control group and different CH lipids vibrations in between INK128 and control group. The Pearson correlation test revealed negative correlation between symmetric & asymmetric vibrations of PO^{2-} groups from phospholipids and positive correlation between CH$_2$ & CH$_3$ vibrations from lipids in all analysed groups. In summary, artificial growth arrest of mice blastocyst (inhibition of mTOR & C-Myc) causes significant changes in the lipids fraction with higher lipid peroxidation in non-control groups which indicates its negative effect on the mice blastocyst.

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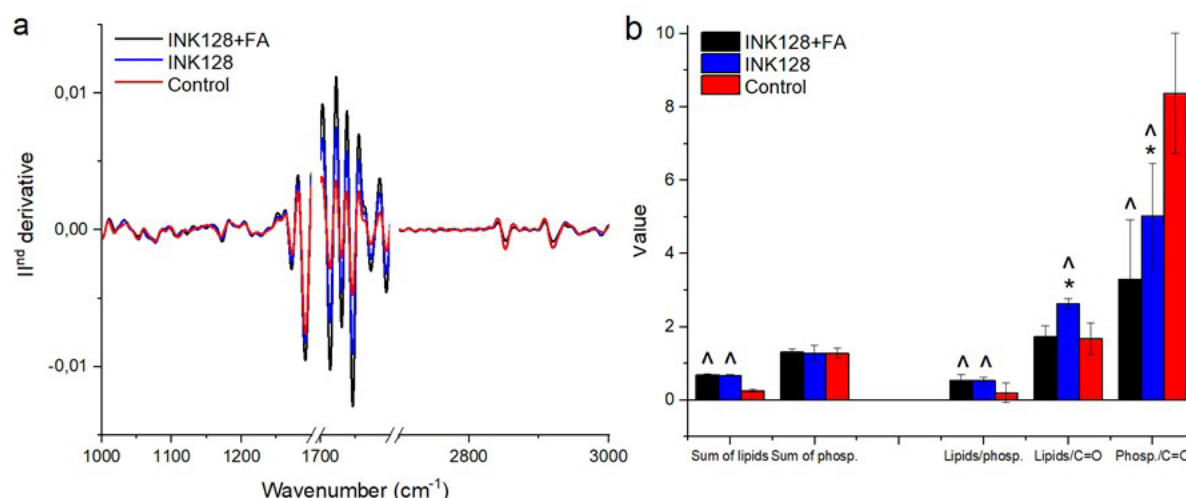
Figure captions:
(a)Second derivative FTIR range. Phospholipid & lipid vibrations in CTR, INK128 and INK128+FA; (b)Sum of lipids & phospholipids signals. ^ significance between CTR, * between INK128, INK128+FA (p<0.05)

Keywords: FTIR; mouse blastocyst; lipids peroxidation

H-P.8

Title: *Early Detection of Pre-cancerous and Cancerous Cells Using Raman Spectroscopy-Based Machine Learning*

Author: Mahmoud Huleihel[1], Uraib Sharaha[1], Daniel Hania[2], Itshak Lapidot[3], Ahmad Salman[2]

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[2]SCE-Sami Shamoon College of Engineering
[3]4Afeka Tel-Aviv Academic College of Engineering

Abstract:
Cancer is the most common fatal disease around the globe, with an annual estimation of 19 million newly-diagnosed patients and about 10 million deaths. Patients with cancer struggle daily with difficult treatments, pain, and financial and social difficulties. Detecting the disease in its early stages is critical for increasing the chances of recovery and reducing the financial burden on the patient and society. Currently used methods for the diagnosis of cancer are time-consuming, producing discomfort and anxiety for patients with significant medical waste. The main goal of this study is to evaluate the potential of Raman spectroscopy-based machine learning for the identification and characterization of precancerous and cancerous cells. As a representative model, the normal mouse fibroblast cells (NFC) of three biological systems represent healthy cells; NIH/3T3 is a fibroblast cell line representing precancerous cells and fully malignant mouse fibroblasts (MBM-T), as cancerous cells were used.

Raman spectra were measured from three different sites of each of the 457 investigated cells and analyzed by principal component analysis (PCA) and linear discriminant analysis (LDA). Our results show that it was possible to classify between the normal and abnormal (precancer and cancer) systems with a success rate of 97.9% and between precancerous and cancerous categories with 82.2% success. Moreover, there is no priority of the measurements' site to the differentiation between the different examined biological systems.

Keywords: Cancer, Raman-spectroscopy, machine-learning, normal-fibroblast, cancerous-cells

H-P.9

Title: *Highly Sensitive Detection of Cancer Biomarker Exosomes Using Au Nanowire Chips*

Author: Sila Jin[1], Ahhyun Woo[2], Jongmin Park[2], Young Mee Jung[2]
[1]Kangwon Radiation Convergence Research Support Center, Kangwon National University
[2]Department of Chemistry, Kangwon National University, Chuncheon 24341, Korea

Abstract:
Exosomes contain DNA, RNA, and proteins of cells and are secreted by all cells in the body through fluids such as blood and urine. It was found that analyzing the genetic and protein information of the cells contained in exosomes in the body fluids of patients with various diseases enables diagnosis of different diseases. However, because exosomes are significantly smaller than cells and the amount of proteins that can be analyzed is much less compared to the protein content of cells, it is difficult to efficiently detect exosomes using conventional methods such as ELISA.

In this study, exosome markers were detected with high sensitivity through surface-enhanced Raman spectroscopy (SERS) using Au nanowire chips. The junctions of the Au nanowires generate stronger hot-spots than flat Au wafers, and the Cassie-Baxter-like structure of Au-crossed nanowires forms a hydrophobic surface, reducing the sample contact area and capturing a greater number of exosomes per unit area. Using this method, four cancer biomarkers, EpCAM, CD24, CD147, and MUC13, were detected in various cell lines. Details of the analysis results will be discussed in this presentation.

References:
Y. Zhang, X.F. Wang, A niche role for cancer exosomes in metastasis, Nat. Cell Biol. 17 (2015) 709-711.

Keywords: Exosome, SERS, biosensor, SERS immunoassay

H-P.10

Title: *Rapid and accurate on-site immunodiagnostics of highly contagious SARS-CoV-2 using a portable SERS-LFA reader*

Author: Younju Joung[1], Kihyun Kim[1], Jaebum Choo[1]

[1]Department of Chemistry, Chung-Ang University

Abstract:
In early 2022, the number of people infected with the highly contagious mutant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), called Omicron, was increasing worldwide. As a result, several countries approved the lateral flow assay (LFA) strip as a diagnostic method for confirming SARS-CoV-2 infection instead of reverse transcription-polymerase chain reaction (RT-PCR), which takes a long time to generate results. However, due to the limitation of detection sensitivity, commercial LFA strips have a high false-negative diagnosis rate for patients with low virus concentrations. Therefore, in this study, we developed a portable surface-enhanced Raman scattering (SERS)-LFA reader based on localized surface plasmon effects to solve the sensitivity problem of commercial LFA strips. We tested 54 clinical samples using this portable SERS-LFA reader, which generated 49 positive and 5 negative results. Out of the 49 positive results, the SERS-LFA system classified only 2 as false negative, while the commercial LFA classified 21 as false negative. This confirmed that the false-negative rate had significantly improved compared to that of commercial LFA strips. We believe that the proposed SERS-LFA system can be utilized as a point-of-care diagnostic system to quickly and accurately determine a virus infection that could spread significantly within a short period of time.

References:
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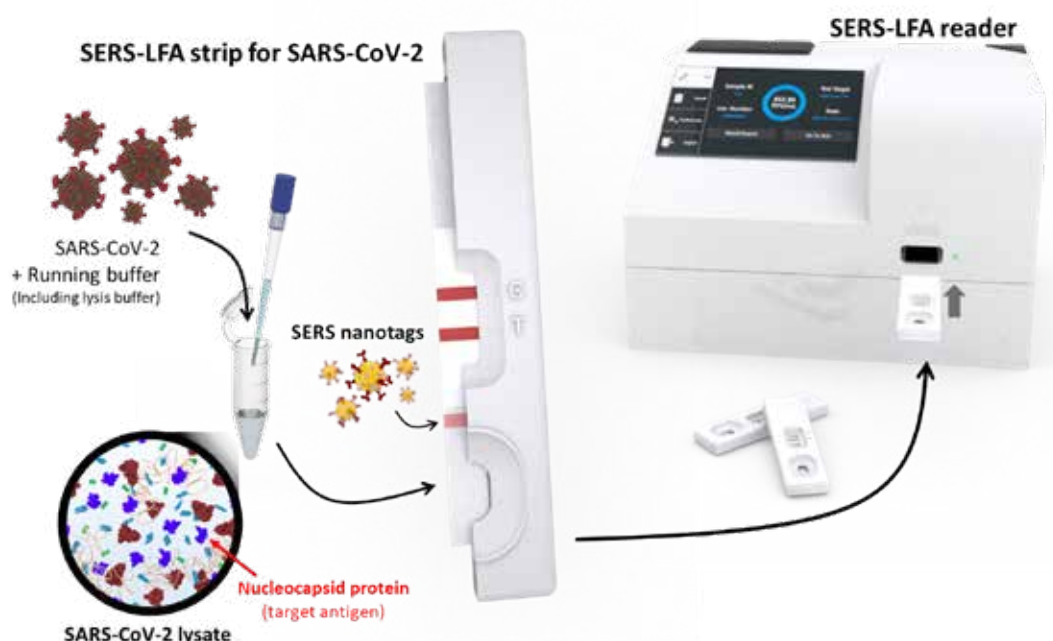
Figure captions:
Illustration of a SERS-LFA system for on-site diagnosis of SARS-CoV-2. The system comprises a SERS-LFA strip and a portable Raman reader for sensitive detection of SARS-CoV-2 nucleocapsid protein.

Keywords: SERS, SERS-LFA, SARS-CoV-2, Portable Raman reader, Immunoassay

H-P.11

Title: *Zebrafish larvae combined Raman and FT-IR spectroscopic imaging – where to start?*

Author: Grzegorz Kalisz[1], Barbara Budzynska[2], Anna Sroka-Bartnicka[1]

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Abstract:
Zebrafish (*Danio rerio*) is a model organism with a well-established reputation in biomedical research. Nowadays, it is widely used as an animal model of several human diseases, genetic and otherwise, as a state-of-play approved translational model[1]. With its advantageous small size, rapid development, genetic tractability, and high prolificity comes optical transparency of larvae, allowing live-imaging and overall microscopic-related approaches favorable[2]. Microspectroscopy combines microscopic imaging with non-destructive, sensitive techniques describing the chemical nature of samples[3]. It requires label-free, minimal sample preparation, however, small constraints may have an effect on experimental procedures in combined Raman and FT-IR measurements[4].
Zebrafish larvae are a promising model for providing structural information on pathologic changes with microspectroscopy. However, sample preparation needs to be considered as it was proven to influence results.
As infrared light is absorbed by water molecules present in biological samples, the dehydrated state is considered suitable for measurements[5]. Formalin-fixed, paraffin-embedded (FFPE) approach routinely used in pathology modifies protein cross-linking, altering spectra in the range of 1500-1700 cm^{-1} and introducing strong paraffin signals to the samples, which can overlap with underlying biology. More challenging technique: cryosectioning is overcoming these issues by snap-freezing a specimen and slicing it prior to placing on microscopic slides[4].
In this work sum up of various zebrafish preparation literature approaches is presented related to techniques used. Since reports do not refer to sample preparation for vibrational spectroscopy, key differences in protocols of related techniques were collected. The application in combined Raman and FT-IR microspectroscopic approaches was considered, assisted with a preliminary evaluation in research.

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Keywords: Raman, FT-IR, zebrafish, animal model

Title: *A SERS-based bacterial sensor using gold nanoparticle-immobilized magnetic beads*

Author: Kihyun Kim[1], Sohyun Park[1], Jaebum Choo[1]

[1]Chung-Ang University

Abstract:
The current gold standard method for the diagnosis of bacterial infection is a culture-based method. However, this method takes at least two days to obtain assay results and usually require additional, complicated steps such as biochemical tests, polymerase chain reaction (PCR), or mass spectrometry to confirm whether the isolated strain is the pathogen of interest [1,2]. To overcome these issues, we developed magnetic beads immobilized with gold nanoparticles (MB-AuNPs) as a surface-enhanced Raman scattering (SERS) substrate. The MB-AuNPs have several advantages, including excellent magnetic properties that make the assay procedures easier and good SERS properties resulting from densely packed AuNPs. By using this MB-AuNPs, we performed a sensitive and rapid SERS-based assay to detect the most common bacterial species causing infection, E. *coli*.

References:
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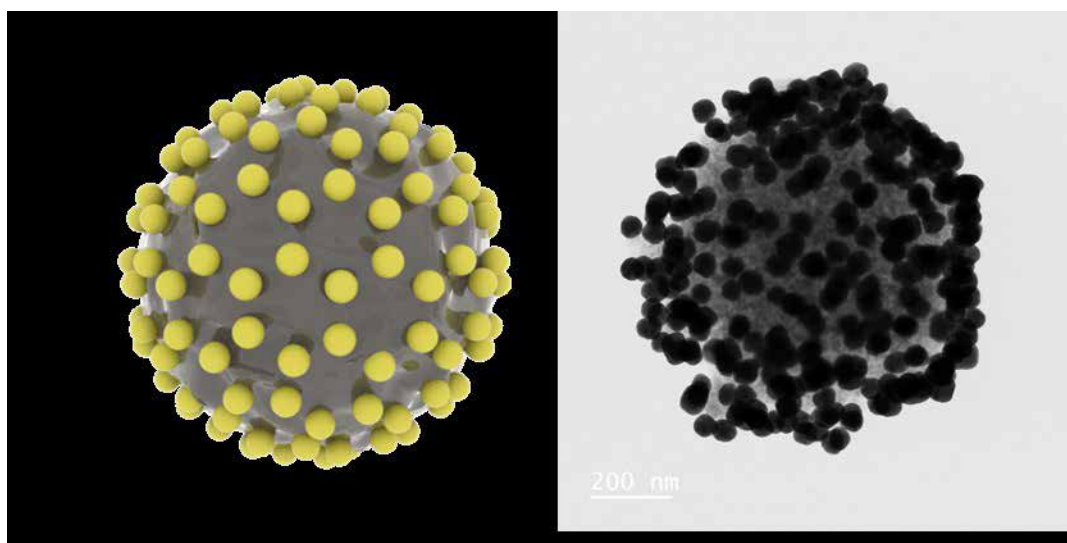
Figure captions:
Graphical and TEM images of a SERS-based bacterial sensor using gold nanoparticle-immobilized magnetic beads (MB-AuNPs).

Keywords: SERS, AuNPs, magnetic-bead, bacteria, diagnosis

H-P.13

Title: *Non-label identification of acute myeloid leukemia with FLT3 gene mutation using Raman spectroscopy.*

Author: Wiktoria Korona[1], Sylwia Orzechowska[1], Paulina Laskowska[2], Aleksandra Borek-Dorosz[1], Małgorzata Zasowska[2], Anna M. Nowakowska[1], Piotr Mrówka[2], Maciej Szydłowski[2], Przemysław Juszczyński[2], Małgorzata Barańska[1], Katarzyna Majzner[1]

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Abstract:
Acute myeloid leukemia (AML) is a heterogeneous group of malignancies characterized by clonal proliferation of progenitor stem cells. The prognosis of AML is influenced by the presence of different molecular aberrations occurring in leukemic cells. Mutations within the FLT3 gene represent one of the most frequently identified genetic alterations in AML cells. The FLT3 gene encodes a tyrosine kinase receptor that plays an important role in the proliferation and differentiation of hematopoietic cells. Mutation in this gene causes constitutive activation of the FLT3 receptor and promotes leukemogenesis. In general, patients with FLT3 mutations tend to have poor prognosis [1]. Raman Imaging enables molecular characterization of malignant cells by identification of biochemical changes in their molecular composition. Therefore, Raman Imaging is considered a promising tool for label-free and non-invasive diagnosis of various leukemia subtypes [2-3].
In the presented study, Raman Imaging followed by chemometric methods has been utilized to distinguish AML cells with FLT3 mutation from normal peripheral blood mononuclear cells (PBMCs), serving as the control group. For this purpose, we developed an *in vitro* model, THP1 AML cell line carrying FLT3-ITD mutation, to investigate molecular composition changes by Raman spectroscopy. Analysis indicated that the spectral differences between leukemic and control cells were due to the Raman bands assigned to nucleic acids and lipids. Our research demonstrates the potential of Raman spectroscopy as an innovative diagnostic tool for characterizing biochemical profiles of leukemic cells.

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Keywords: Raman spectroscopy, chemometrics, acute myeloid

H-P.14

Title: *FTIR imaging and MALDI mass spectrometry study of rat kidney tissues in diabetes type 2 model*

Author: Adrianna Kryska[1], Maryna Khalavka[1], Karolina Kosowska[2], Tomasz Wróbel[2], Leopold Ilag[3], Anna Sroka-Bartnicka[1]

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Abstract:
Type 2 diabetes mellitus is considered one of the most common diseases of XXI century. By 2019 more than 460 million people were affected with T2DM and it's estimated that over 1 million causes of death per year are attributed to diabetes. Due to lack of visible symptoms at the beginning of T2DM, the patients are often diagnosed with already developed complications, including dyslipidemia [1,2]. **The aim of this work is to assess metabolomic changes, with emphasis on lipidomics, occurring in type 2 diabetes animal model.**
The measurements were performed on rat kidney tissues from type 2 diabetic model with **FTIR Imaging and MALDI MS,** using Hyperion 3000 spectroscopic microscope coupled with Vertex 80v spectrometer (Bruker Optics, Ettlingen, Germany) and Synapt Q2 (Waters, Wilmslow, Great Britain). The data was analyzed using CytoSpec, Omnic, Matlab software and Python programming language.
Preliminary experiments were conducted using MALDI MS technique for the analysis of lipids in homogenates of kidney and serum samples. Significant changes in quantity of specific lipids between control an DM group were detected, most distinctly in ceramides and triacylglycerols, having increased levels in T2DM group. Moreover, the Principal Component Analysis showed sufficient separation between DM and control group.
FTIR Imaging enabled to observe changes in total lipid content and distribution. In T2DM group the concentration of lipids is higher than in control group and they are accumulated in wider areas. Statistical analysis of over 1,8mln spectra proved that in diabetic model of kidney cortex, significant changes in phospholipids as well as in overall lipid content occurred.
The excess of lipids in organs can cause changes in organ physiology and metabolism leading to diseases like nephropathy, retinopathy or neuropathy. Implementing more than one analytical technique increases accuracy and credibility of the results and present a more comprehensive view of lipidomic changes.

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Keywords: MALDI-MS, FTIR Imaging, Diabetes, Dyslipidemia

H-P.15

Title: *Raman study for development of a diagnostic method of tongue cancer*

Author: <u>Hiroko Matsuyoshi</u>[1], Yumiko Matsusue[2], Hidetoshi Sato[3]

[1]Tokaigakuen University
[2]Nara Medical University
[3]Kwanseigakuin University

Abstract:
Tongue cancer is the most common malignancy developing in the oral cavity, and resection of tumor is the most important initial step of treatment. Hence, the detection of tongue cancer is critical for its successful therapy. Diagnosis of tongue tumor is based on the histopathological evaluation, which is also applied for determining whether the intraoperative resection margin contains tumor tissues or not. However, it is not easy to correctly evaluate whether a tongue tissue is in a normal or neoplastic condition, using the histological method. Raman spectroscopy is a noninvasive, nondestructive and water-insensitive technique detecting the information of molecular vibration and determining the sample structure. To explore an application possibility of Raman spectroscopy for diagnosis of tongue cancer, we examined the feasibility of this technique for the detection of differences in normal rats and rats with tongue tumor. Tissue degenerative changes detected by Raman spectra were seen time-dependently after 4-NQO, carcinogenic agent, administration and also correlated with the amount of DNA proteins. In addition, these differences in Raman spectra preceded the appearance of histopathological manifestations of neoplastic lesions in the tongue. In conclusion, the Raman technique has a possibility as a noninvasive diagnostic method for detection of tongue tumor.

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Keywords: tongue cancer, Raman spectroscopy

H-P.16

Title: *Raman and Resonance Raman Spectroscopy for Malaria Red Blood Cells Analysis*

Author: <u>Mateusz Migdalski</u>[1], Malwina Birczyńska-Zych[2], Jacek Czepiel[2], Martyna Kucharska[1], Paulina Moskal[1], Grażyna Biesiada[2], Aleksander Garlicki[2], Aleksandra Wesełucha-Birczyńska[1]

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Abstract:
Malaria is a dangerous and potentially fatal disease. An estimated number of people who died from malaria in 2020, is 627,000, 95% of them in the African region [1]. Malaria is a vector-borne disease, parasitic infection transmitted by *Anopheles* mosquitoes. During the intraerythrocytic development phase *Plasmodium* digests and degrades 25-75% of the hemoglobin in an infected erythrocyte [2]. Parasites developed a mechanism for detoxifying heme by converting it to an insoluble, non-reactive crystalline material, the so-called malaria pigment (or hemozoin).

In this paper, the relationship between the Raman spectra of red blood cells of patients diagnosed with malaria *Plasmodium falciparum* hospitalized at the University Hospital in Krakow and healthy volunteers were examined. The spectroscopic tests were carried out using lasers with a wavelength of 442 nm and 785 nm. Using the first of these lasers, for a wavelength of 442 nm, it was possible to observe the phenomenon of resonant Raman scattering. The second laser used allows the observation of the normal phenomenon of light scattering [3].

The use of the principal component analysis (PCA) allowed to recognize changes taking place in infected erythrocytes compared to healthy ones. Peaks characterizing malaria-infected erythrocytes appearing on excitation of the 442 nm laser line show the structure of deoxy-heme. In the case of the excitation line at 785 nm, the significant peaks characterizing the infected blood cells are, apart from the band at 1354 cm^{-1} indicating the structure of deoxy-heme, also the band 1068 cm^{-1} indicating the *gauche* conformation of membrane lipids [4]. The PCA method enabled the reduction and visualization of data, providing information on the similarities and differences in the molecular structure of erythrocytes infected with *P. falciparum* and healthy red blood cells.

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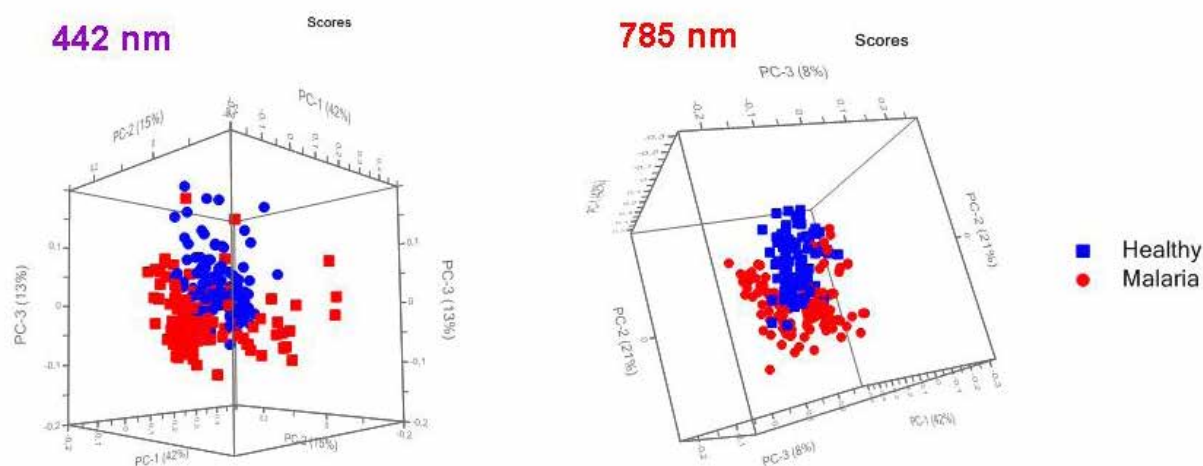
Figure captions:
PCA, PC-1 vs PC-2 vs PC-3 applied to Raman spectra of RBCs patients diagnosed with malaria (red dots) and healthy volunteers (blue dots); 442 nm and 785 nm excitation laser lines, 3200–300 cm−1 range.

Keywords: malaria, RBC, Raman spectroscopy, RRS

H-P.17

Title: *Effect of advanced maternal age and delayed implantation on embryonic DNA and lipids determined by Fourier Transform InfraRed spectroscopy*

Author: Richard Musson[1], Hafsa Gulzar[1], Joanna Depciuch[2], Grażyna Ewa Ptak[1]

[1]Małopolska Centre of Biotechnology, Jagiellonian University
[2]Medical University of Lublin

Abstract:
Fourier Transform InfraRed (FTIR) spectroscopy facilitates the acquisition of biochemical signatures from cellular material, even in small molecules such as DNA[1,2]. Moreover, it is well known that adverse conditions such as advanced age can damage DNA and severely impact embryonic development, although full implications of this are not fully realised[3]. Our previous work has shown that lipids play a crucial role in embryonic diapause, which may benefit the embryo[4]. Therefore, in this study we used FTIR to investigate quantitative and qualitative changes in mouse embryo DNA and lipids in young (CTR), advanced maternal age diapaused (AMA E8) and non-diapaused (AMA E4) embryos. Thus, three groups: CTR (n=5), AMA E4 (n=34) and AMA E8 (n=10) were measured and analysed using secondary structure of FTIR spectra and statistical as well as principal component analyses (PCA).
We observed numerous major differences between CTR and AMA groups, and several differences to suggest improved outcome in diapaused (AMA E8) embryos. Firstly, the lower amide II/I ratio observed in AMA E4 was corrected in AMA E8, resembling CTR samples. This ratio, and a strong peak in the amide II range, is representative of protein secondary structure and points towards higher alpha-helix structure in AMA E4 which is reversed in AMA E8. Another significant alteration observed is the amount of methylated DNA in AMA E4 and AMA E8 which was higher than in CTR embryos, and in AMA E4 the amount of this DNA was lower than in AMA E8. PCA analysis was able to distinguish AMA E4 and AMA E8 using PO_2^- phospholipids vibrations; and AMA E4 and AMA E8 using CH lipids groups. This suggests that advanced age and delayed implantation strongly influence embryonic DNA and lipids. Pearson correlation test showed that in AMA groups correlation between DNA and lipids, DNA and phospholipids and DNA and proteins was found.

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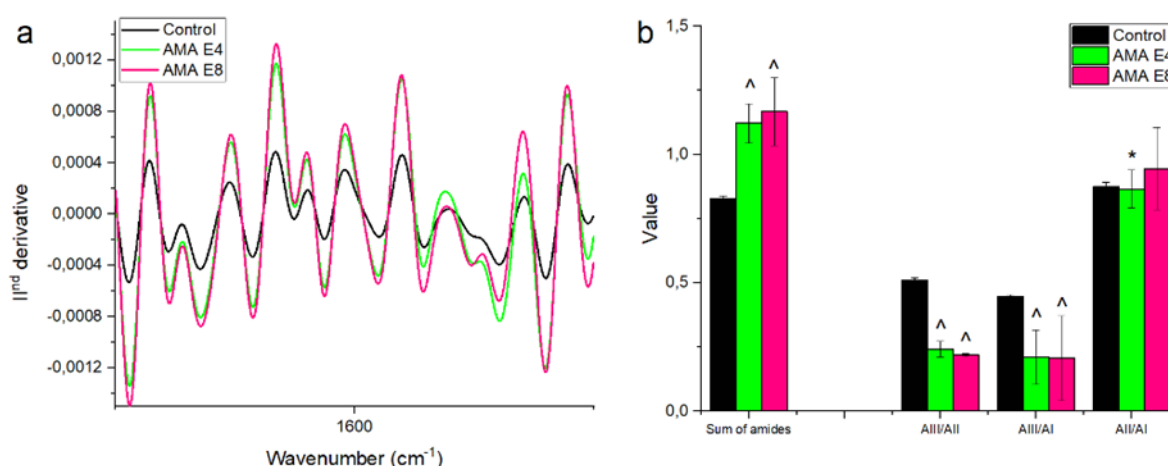
Figure captions:
(a) Second derivative of FTIR range from proteins vibrations in control, AMA E4, and AMA E8; (b) Sum of amides as well as ratio between respectively amides ± SD. Degree of significance is p < 0.05.

Keywords: FTIR, embryo, diapause, DNA, advanced

Title: *SERS-imaging for probing program death ligand-1 immunomarker in real-time tumour progression*

Author: Muhammad[1], Chang-sheng Shao[1], Qing Huang[1]

[1]Hefei Institutes of Physical Science, Chinese Academy of Science

Abstract:

Programmed cell death 1 (PD-1) and its ligand 1 (PD-L1) have been extensively investigated for the critical role in tumor immune evasion and drug resistance, which thus emerge as a unique and promising target for cancer immunotherapy via modulating the balance of T lymphocyte tolerance and immunopathology. State-of-the-art renewal has indicated the multitudinous applications of surface-enhanced Raman spectroscopy (SERS) bioassay for rapid diagnosis and monitoring, yet the feasibility of Au/4-ATP/Ag nanotags as SERS imaging probe for the assessment of dynamic response to nanotags-indicated PD-L1 at clinical level is largely obscure. Therewith, we took advantage of SERS imaging for monitoring the spatio-temporal expression and distribution pattern, and conceptionally verified the feasibility of the high-resolution SERS mapping for the assessment of PD-L1 expression and distribution in different tumor models, which will supply overwhelming new references for PD-L1-related cancer immunotherapy and novel drug development in future.

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Figure captions:

(A) Tumour model and SERS imaging setup. (B) Dynamic SERS imaging of PD-L1 expression and spectra. (C) PD-L1 expression and PCA plots.

Keywords: PD-L1, SERS, tumour imaging, Aptamer

H-P.19
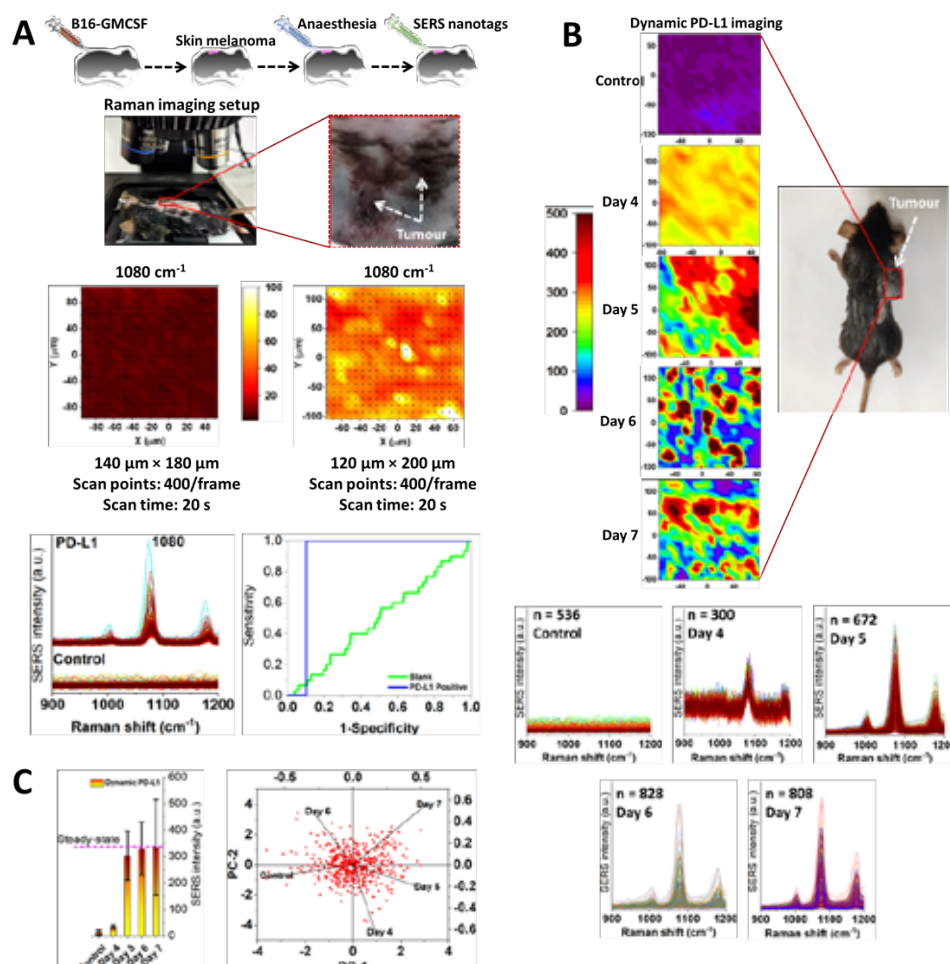
Title: *Surface-Enhanced Raman Spectroscopy for Rapid and Sensitive Identification of RNA Virus Variants with Point Mutations*

Author: Eungyeong Park[1], Yeonju Park[2], Sila Jin[2], Kwang-il Lim[3], Young Mee Jung[1]

[1]Department of Chemistry, Kangwon National University
[2]Kangwon Radiation Convergence Research Support Center, Kangwon National University
[3]Department of Chemical and Biological Engineering, Sookmyung Women's University

Abstract:
RNA viruses are known to rapidly evolve through mutations, which can lead to the emergence of new viral pathogens that may pose a serious threat to public health. Early detection of new viruses and the isolation of infected individuals are critical in curbing the spread of viral infections. In this study, we used probe-free surface-enhanced Raman scattering (SERS) to obtain spectral fingerprints of surface proteins to identify different types of viruses. SERS-based approach can effectively distinguish influenza virus variants with multiple surface protein point mutations. By applying principal component analysis (PCA) to the SERS spectra of viruses, we captured the most important Raman bands that distinguish between the variants of viruses. Our method enables the rapid detection and identification of emerging viruses in real-time without the need for time-consuming and expensive probe development. Our results suggest that the combination of SERS and PCA can be a promising tool for the rapid detection of emerging viruses, improving our ability to identify viral pathogens and implement appropriate control measures.[1]

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Keywords: SERS, Probe-free detection, Influenza virus, Point mutations, PCA

H-P.20

Title: *A SERS-based microfluidic device for sensitive and reproducible detection of SARS-CoV-2*

Author: Sohyun Park[1], Binnam Kang[1], Jaebum Choo[1]

[1]Department of Chemistry, Chung-Ang University

Abstract:
The coronavirus disease 2019 (COVID-19) has been labeled an ongoing pandemic by the World Health Organization (WHO). Real-time quantitative polymerase chain reaction (RT-qPCR) has been considered a gold standard for quantitatively evaluating a target gene. However, it still suffers from the problem of a long detection time. A commercially available lateral flow assay kit can provide results within 30 min, but it has problems in terms of low sensitivity and poor accuracy. To address these issues, we developed a surface-enhanced Raman scattering (SERS)-based immunosensing platform for the rapid and sensitive detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. In this work, we used SERS nanotags, anti-SARS-CoV-2 nucleoprotein antibody-conjugated gold nanoparticles, and magnetic beads to detect SARS-CoV-2 biomarkers. We measured the Raman signals for SARS-CoV-2 magnetic immunocomplexes under flowing conditions. The total analysis time from droplet generation to SERS detection takes less than 10 min because all experimental conditions were controlled inside the exquisitely designed microfluidic channel. In addition, clinical tests were performed on patient samples to evaluate the clinical efficacy of the SERS-based microdroplet sensor. The assay results agreed well with those measured by the RT-PCR method. This novel SERS-based immunosensing platform is expected to be a new point-of-care diagnostic tool for detecting SARS-CoV-2.

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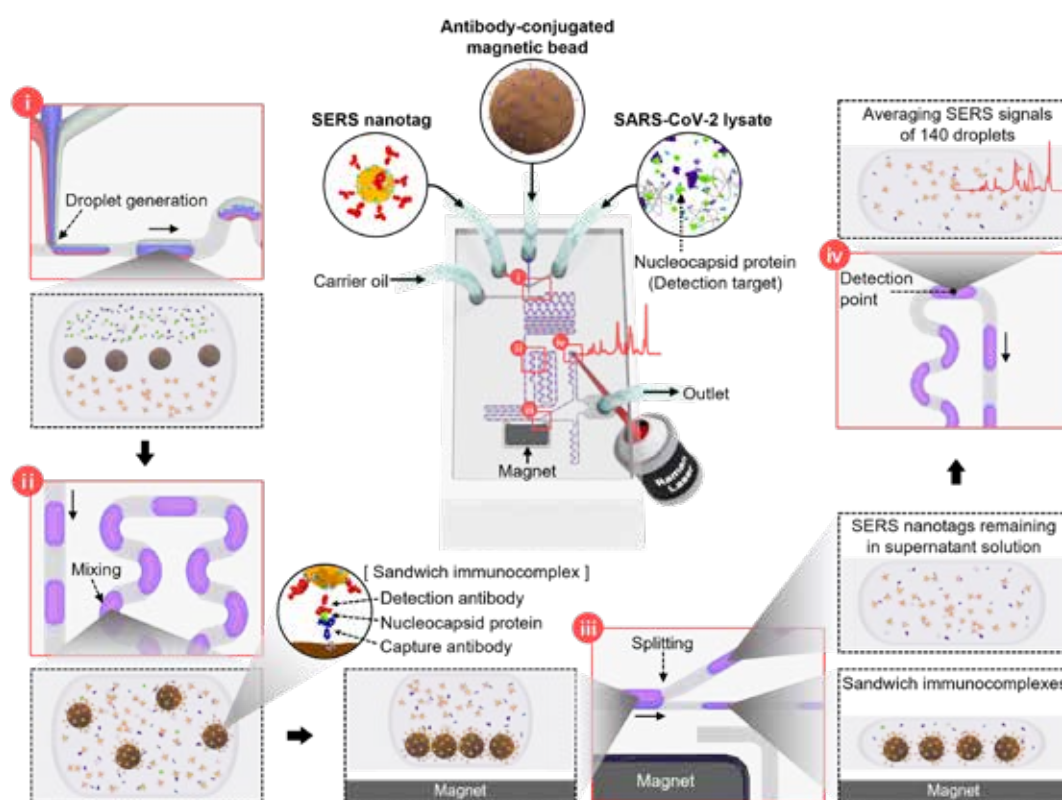
Figure captions:
A schematic design of the microdroplet channel. This microfluidic channel consists of four compartments: droplet generation, droplet mixing, droplet splitting, and optical signal measurements.

Keywords: SERS, microfluidics, SARS-CoV-2, immunoassay, biosensor

H-P.21

Title: *Spectroscopic detection of hypoxic state in the brain endothelium and endothelial progenitor cells*

Author: Aleksandra Pragnąca[1], Anna Antolak[2], Víctor Navarro Esteve[3], Zuzanna Krysiak[4], Joanna Korszun[5], Monika Leśniak[4], Robert Zdanowski[4], Kamilla Małek[2]

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[2]Jagiellonian University, Faculty of Chemistry, Department of Chemical Physics, Raman Imaging Group, Krakow, Poland
[3]Jagiellonian University, Faculty of Chemistry, Department of Chemical Physics, Raman Imaging Group, Krakow, Poland; Department of Analytical Chemistry, University of Valencia, 50 Dr Moliner Street, Research Building, 46100 Burjassot, Valencia, Spain
[4]Military Institute of Medicine National Research Institute, Laboratory of Molecular Oncology and Innovative Therapies, Warsaw, Poland
[5]Military Institute of Medicine National Research Institute, Laboratory of Molecular Oncology and Innovative Therapies, Warsaw, Poland; Bio-Med-Chem Doctoral School of the University of Lodz and Lodz, Institutes of the Polish Academy of Sciences, Universit

Abstract:
Brain endothelial cells are the main structural element of the blood-brain barrier - a physical barrier separating blood vessels from brain tissue[1]. Since they constitute the inner layer of the microvascular network, they are exposed to stress conditions accompanying neurodegenerative processes. As a result of their damage or death, they are replaced by a group of cells in the differentiation stage between stem cells and fully differentiated cells called progenitor cells. Circulating endothelial cell progenitors (ECPs) originating from the bone marrow are also considered to be a powerful tool in the repair of endothelium damage in cell therapies. In this study, we evaluated the effect of oxygen deficiency (hypoxia) in 2D cultures of human brain endothelium and progenitor cells. This is the primary in vitro model reflecting neurological dysfunctions[2].
We aimed to determine biochemical differences between these cells and spectral markers of hypoxia by using Raman and Infrared spectroscopy imaging and the complementarity of both techniques. In particular, IR imaging allows to collection of enormous data sets while Raman images exhibit cellular organelles. Their spectra also highlight the presence of different biomolecules. The experiment was carried out on the cerebral microvascular endothelial cell line (HBEC 5i) and human endothelial progenitor cell line (HEPC-CB.1) in normoxia and hypoxia (1% oxygen in the culture environment). Then, they were examined using Raman, Fourier-Transform Infrared, and fluorescence microscopy. An analysis of spectral data was supported by chemometric analysis (cluster analysis, PCA) to recognize spectral biomarkers. The effect of oxygen deficiency was mainly observed in the cytoplasm and was associated with mitochondrial activity expressed by the signature of cytochromes.

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Figure captions:
The idea of the carried out experiments

Keywords:
Ir&Raman imaging, hypoxia, endothelium, progenitors

H-P.22
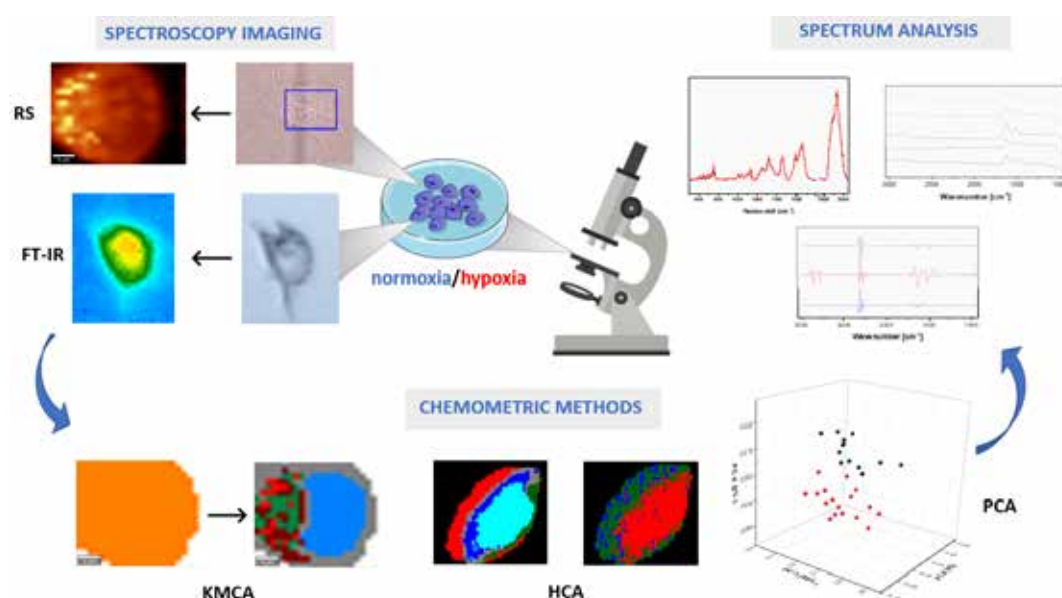
Title: *Effects of antimicrobials on microbial Raman spectra as the first step for detection of antimicrobial resistance*

Author: Ota Samek[1], Katarina Rebrosova[2], Filip Ruzicka[2], Zdenek Pilat[1], Martin Kizovsky[1], Silvie Bernatova[1], Marketa Benesova[1], Adela Mikulova[1], Martin Siler[1], Pavel Zemanek[1]

[1]Institute of Scientific Instruments of the Czech Academy of Sciences
[2]Institutions shared with St. Anne's Faculty Hospital – Faculty of Medicine, Masaryk University

Abstract:
Background: Raman spectroscopy has become a valuable tool in many fields in the last decades, allowing a non-destructive analysis of various samples. Based on inelastic (Raman) scattering of monochromatic light, it provides a fingerprint of chemical bonds present in the sample. This can be subsequently used, among other things, to identify and characterize microbial cells.
Objectives: Our current study aims at the effects of antimicrobial agents on microbial Raman spectra.
Methods: For this pilot study, we acquired Raman spectra from *Candida albicans* ATCC 90028, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228 strains. The staphylococcal strains were exposed to clindamycin, vancomycin, and ceftaroline; the C. *albicans* strain was exposed to amphotericin B, caspofungin, and voriconazole in subinhibitory concentrations. To assess the effect of antimicrobials on Raman fingerprints of microbes, we used the Raman spectrophotometer (Renishaw Invia Raman Spectrometer Renishaw plc., Wotton-under-Edge, UK, laser wavelength: 785 nm, exposure time: 15 s).
Results: The analysis of Raman fingerprints showed the difference between microbes exposed and non-exposed to subinhibitory concentrations of antimicrobials. Therefore, it could be the first step in detecting antimicrobial resistance using Raman spectroscopy in the future.

Keywords: Raman spectroscopy, antimicrobial resistance

H-P.23

Title: *Toward Hepatocellular Carcinoma Diagnostics Utilising Vibrational and Chiroptical Spectroscopy*

Author: <u>Vladimír Setnička</u>[1], Ondřej Vrtělka[1], Kateřina Králová[1], Lucie Habartová[1], Petr Hříbek[2], Petr Urbánek[3]

[1]Department of Analytical Chemistry, University of Chemistry and Technology
[2]Military University Hospital Prague, Department of Medicine 1st Faculty of Medicine Charles University and Military University Hospital Prague; Department of Internal Medicine, University of Defense, Faculty of Military Health Sciences in Hradec Kralove
[3]Military University Hospital Prague, Department of Medicine 1st Faculty of Medicine Charles University and Military University Hospital Prague
This work was supported by the Ministry of Health of the Czech Republic (No. NV19-08-00525) and partially by the Specific university research (No. A2_FCHI_2023_027).

Abstract:

Neoplasms are a major health problem that caused approximately 10 million deaths in 2020 [1], and their incidence steadily rises every year. Even though liver cancer accounted for less than 5% of all cancer cases, the mortality rate made it the third most common cancer-related death worldwide [1]. As opposed to many other cancers, the high-risk group for liver cancer is clearly defined – individuals with cirrhosis. According to the Global Burden of Disease [2], the prevalence of cirrhosis and other liver diseases was up to 1.7 billion cases, and an incidence exceeded 2 million. Up to 90% of cases of hepatocellular carcinoma, the most common type of liver cancer, arise in the background of liver cirrhosis [3]. The fate of patients with hepatocellular carcinoma remains unclear due to poor diagnostic options, especially for the early stages. A liquid biopsy might be the solution to the ongoing problem. Considering that blood is in direct contact with an affected tissue, its altered composition might be revealed by the use of spectroscopic methods. The most suitable option seems to be vibrational spectroscopy, as it represents a fast and effective way to obtain comprehensive information about the sample, but its coupling with chiroptical methods, namely electronic circular dichroism and Raman optical activity, provides additional insight even into the disease-related changes in the conformation of many biomolecules present in blood plasma. Using multivariate statistical approaches, we have identified spectral patterns specific to liver cirrhosis and hepatocellular carcinoma and successfully distinguished samples with an accuracy exceeding 80%, indicating the clinical potential of spectroscopic methods for their inclusion in diagnostics or screening of at-risk individuals.

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Acknowledgments:

Keywords: Blood plasma, diagnostics, hepatocellular carcinoma

H-P.24

Title: *SPECTROSCOPIC ANALYSIS OF THE FATTY ACIDS UPTAKE BY HUMAN LEUKEMIC CELLS AND ACCOMPANYING METABOLIC CHANGES*

Author: Kacper Siąkała[1], Anna M. Nowakowska (*)[1], Aleksandra Borek-Dororsz[1], Patrycja Dawiec[2], Katarzyna Majzner[1], Małgorzata Barańska[3]

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[2]Jagiellonian University in Kraków, Faculty of Chemistry; Jagiellonian University in Kraków, Doctoral School of Exact and Natural Sciences
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Abstract:

The survival mechanisms of cancer cells depend on their continuous adaptation to constantly changing environmental conditions. One of the most pronounced survival mechanisms is intensified β-oxidation of fatty acids (FA) [1]. Therefore, tracking of FA uptake and its metabolism seems to be crucial for understanding the carcinogenesis processes and invasiveness. In our research we focused on two aspects of FA metabolism – tracking of their uptake in real time and following biochemical changes resulting from FA absorption using Raman spectroscopy [2].

Investigated HL-60 cells were incubated with palmitic acid (PA) and deuterated palmitic acid (dPA). Control cells were incubated with BSA (bovine serum albumin). Measurements were made using a confocal microscope coupled with a WITec Alpha3000 R Raman spectrometer. In order to spectroscopically detect FA uptake, chemometric analysis was done, including principal component analysis (PCA).

Spectral profile of HL-60 cells incubated with selected FA in the comparison to the spectral profile of control cells indicated significant increase in lipidity. On the other hand, the control cells were characterized by an increased content of nucleic acids and proteins. In the case of cells incubated with dPA, the analysis of the band at ca. 2100 cm^{-1} allowed us to track its uptake instantaneously and follow the spatial distribution of newly formed lipid droplets in cells.

Raman spectroscopy can be used for the examination of changes in the biochemical profile of cells resulting from the uptake of FA. Furthermore, the usage of deuterated FA allows for the tracking of uptake and formation of lipid droplets in cells because of the occurrence of the C-D marker band, which is not normally present in cell spectra. This approach can be used in the future for real-time tracking of lipid metabolism of cancer cells using both classical and nonlinear spectroscopic techniques, which will help us better understand carcinogenesis processes.

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Keywords: Raman spectroscopy, deuterated fatty acids, uptake, chemometrics, lipid metabolism

H-P.25

Title: *Differentiation and classification of leukemic cells with the use of Raman imaging*

Author: Kacper Stawoski[1], Adriana Adamczyk[2], Anna Nowakowska[1], Justyna Jakubowska[3], Marta Zabczynska[3], Agata Pastorczak[3], Kinga Ostrowska[3], Wojciech Mlynarski[3], Katarzyna Majzner[1], Malgorzata Baranska[4]

[1]Jagiellonian University in Krakow, Faculty of Chemistry, Department of Chemical Physics
[2]1. Jagiellonian University in Krakow, Faculty of Chemistry, Department of Chemical Physics 2.Jagiellonian University in Krakow, Doctoral School of Exact and Natural Sciences
[3]Department of Pediatrics, Oncology and Hematology, Medical University of Lodz
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Abstract:
Leukemia is one of the most common cancers in the developed world and causes hundreds of thousands of deaths every year. The current approach to classifying leukemia is based on the origin of cells which are affected by neoplastic mutations (lymphocytes or myeloid cells) and the rapidity of proliferation. In acute leukemias, malignant cells (called blasts) are immature and incapable of performing their immune system functions[1].
The gold standard and classification of leukemia involves various methods including morphology, cytochemistry, cytogenetics and molecular genetics, and immunophenotyping. These diagnostic methods are required for the stratification of patient treatment and evaluation of treatment outcome[2].
Unfortunately, available diagnostic methods are demanding and time consuming. Raman spectroscopy, a non-destructive, sensitive, and label-free molecular method, emerges as an alternative to the classification of neoplastic cells. Single-cell Raman imaging paired with machine learning statistical analysis methods proved to be successful in characterization of leukemic cells[3]. In this study, we present a diagnostic scheme based on a detailed chemometric analysis of the spectra of normal and neoplastic cells represented by *in vitro* models of acute lymphoblastic leukemia (TANOUE) and myelomonocytic leukemia (MV4;11). In both cases, significant differences in the intensity of bands assigned to nucleic acids and lipids were recognized as markers identifying normal and malignant cells. The next step considered determination of the molecular differences between the two types of acute leukemia. Chemometric analysis of the Raman spectra revealed subtle differences between lymphoblastic and myeloblastic cancer cells.

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Keywords: Raman spectroscopy, leukemia, chemometrics

H-P.26

Title: *Determination of Klebsiella pneumoniae Susceptibility to Antibiotics Using Infrared Spectroscopy-Based Machine Learning*

Author: Manal Suleiman[1], George Abu-Aqil[1], Uraib Sharaha[1], Klaris Riesenberg[2], Itshak Lapidot[3], Mahmoud Huleihel[1], Ahmad Salman[4]

[1]Ben-Gurion University of the Negev
[2]Soroka University Medical Center
[3]Afeka Tel-Aviv Academic College of Engineering
[4]Shamoon College of Engineering

Abstract:

Klebsiella pneumoniae is one of the most aggressive multidrug-resistant bacteria associated with human infections resulting in high morbidity and mortality. Thus, for an effective treatment, it is important to diagnose both the species of infecting bacteria and their susceptibility to antibiotics. Currently used methods for diagnosing bacterial susceptibility to antibiotics are time-consuming (about 24h following the first culture). Thus, there is a clear need for rapid methods to determine bacterial susceptibility to antibiotics. Infrared spectroscopy is a well-known method as sensitive and simple and can detect minor biomolecular changes associated with the initiation of developing abnormalities. The main goal of this study is to evaluate the potential of infrared spectroscopy in tandem with machine learning algorithms to diagnose the susceptibility of *Klebsiella pneumoniae* to antibiotics within approximately 20 minutes following the first culture.

In this study, 1190 *Klebsiella pneumoniae* isolates were obtained from different patients with urinary tract infections and measured by the infrared spectrometer. The recorded spectra were analyzed by Random Forest and XGBoost to determine their susceptibility regarding nine specific antibiotics (amoxicillin, ceftazidime, ceftriaxone, cefuroxime, cefuroxime-axetil, cephalexin, ciprofloxacin, gentamicin, and sulfamethoxazole). Our results confirm that it was possible to classify the isolates based on their susceptibility to specific antibiotic as sensitive or resistant with a success rate range of 80%-85%. These results prove the promising potential of infrared spectroscopy in tandem with machine learning as a powerful diagnostic method for determining *Klebsiella pneumoniae* susceptibility to antibiotics.

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Keywords: UTI, Klebsiella, IR-spectroscopy, machine-learning

H-P.27

Title: *Raman spectroscopic detection of carotenoids in T-cell acute lymphoblastic leukemia*

Author: Szymon Tott[1], Patrycja Dawiec[2], Patrycja Leszczenko[2], Anna Nowakowska[1], Karolina Czuja[1], Justyna Jakubowska[3], Marta Zabczyńska[3], Agata Pastorczak[3], Kinga Ostrowska[3], Wojciech Mlynarski[3], Malgorzata Baranska[4], Katarzyna Majzner[1]

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Abstract:
Raman spectroscopy (RS) is a non-destructive and label-free method that provides detailed information about the chemical composition of a cell and reflecting its physiological state. In RS, nucleic acids, lipids, protein, carbohydrates, and carotenoids can be visualised exclusively on their vibrational.
Carotenoids play mainly a photoprotective, radical quenching, and antioxidant role in the human body. Because of the structure of polyene molecules, they exhibit very strong Raman intensities. It was shown previously that carotenoids, with a dominant contribution of β-carotene, are present exclusively in T cells [1] and some molecular subtypes of B-ALL [3]. Since leukemia is a blood malignancy occurring as a result of genetic abnormalities in progenitor cells [2], the motivation of this work was to investigate whether the presence of carotenoids remains characteristic also in T-ALL blasts.
In this research, we used Raman imaging in order to investigate whether carotenoids can be considered as a marker in the Raman-based detection of T-ALL blasts. T-ALL blasts were obtained from patients who were clinically diagnosed with T-ALL, while normal T cells (control group) were isolated from healthy donors. Cells were measured using a WITec Alpha 300 confocal Raman microscope (Ulm, Germany) equipped with 633-nm excitation wavelengths.
The results confirmed the hypothesis that samples from patients diagnosed with T-ALL and the control group can be discriminated when principal component analysis (PCA) are applied. Analysis of the Raman data revealed that T-ALL blasts reduce carotenoid uptake in comparison to normal, healthy T cells. This would suggest a change in a metabolic pathway dealing with the uptake of carotenoids while undergoing a neoplastic transformation. Further work is required in order to understand the relative contributions of dietary and supplemental carotenoids to overall carotenoid content in T cells, as well as the possible correlation with leukemogenesis.

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Keywords: Raman spectroscopy, leukemia, cancer, chemometrics

H-P.28

Title: *Spectroscopic Liquid Biopsy for Diagnostics of Hepatocellular Carcinoma*

Author: Ondřej Vrtělka[1], Kateřina Králová[1], Markéta Fousková[1], Petr Hříbek[2], Petr Urbánek[3], Vladimír Setnička[1]

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Abstract:

As in many other types of cancer, the lack of suitable early-stage diagnostic options for hepatocellular carcinoma might lead to enormous mortality. With the highest risk factor for hepatocellular carcinoma development being liver cirrhosis of any aetiology, it is crucial to develop a highly accurate diagnostic procedure for monitoring at-risk cirrhotic patients. The progression of pathological conditions from cirrhosis to cancer is associated with the induction of changes in concentration and/or conformation of biomolecules and surrounding tissue, which may be exhibited in the circulating blood. Disruptions in metabolic pathways and alterations of molecular structures might be studied by means of spectroscopic methods. Vibrational spectroscopy provides general information about the sample composition, mainly proteins in the case of blood plasma; hence, we have employed fractionation pre-processing steps to enable overlapped signals to appear. Combining chemical and physical separation approaches, namely lipid extraction using methanol and chloroform and ultrafiltration through 3 kDa molecular weight cut-off filters, allowed us to prepare lipid, protein and low-molecular-weight fractions; thus, increasing the amount of information acquired from a single sample. Subsequent analysis using various spectroscopic methods (Fourier-transform infrared spectroscopy, Raman spectroscopy and surface-enhanced Raman scattering) provided different insights into the prepared blood plasma fractions that could not be gained while analysing the whole blood plasma. Employing machine learning on the accumulated data, we were able to distinguish patients with liver cirrhosis from hepatocellular carcinoma with an accuracy of up to 90%.

Acknowledgments:

Keywords: plasma, fractionation, spectroscopy, cancer, diagnostics

H-P.29

Title: *Raman spectroscopy as a valuable tool in pharmaceutical reverse engineering*

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Abstract:
The development of a new therapeutic product is a long, complex, and expensive process. Usually, it takes years from product design to commercialization. One of the answers to this problem, is a production of generic drugs (after the brand name drug's patent has expired), which is recently one of the most dynamically developing branches of the pharmaceutical industry. The generic drug has exactly the same active ingredients as the reference listed drug, dosage form, and strength, and yields the same therapeutic effects. In the registration process, a producer of a generic drug should provide proof of bioequivalence to the original product, which is examined in clinical in vivo tests. According to ICH M9 guidelines in some cases, bioequivalence can be proved only by using in vitro techniques. Such approach is part of reverse engineering analysis methods, and among the most powerful tools can be found Raman spectroscopy (RS). RS is successfully employed to identify components in a studied product and visualize their spatial distribution of active pharmaceutical ingredients (API) and excipients. Moreover, Raman mapping can deliver information on coating layer thickness, a number of coating layers, and polymorphic forms of API. In order to determine some of these properties, we employed Raman spectroscopy to analyze several available in the Polish market drug products containing omeprazole. Raman mapping-based reverse engineering allowed not only identifies the substances included in studied drugs and visualizes their spatial distribution but also showed differences in the composition and thickness of the coating layer. Additionally, we used scanning electron microscopy (SEM) to provide additional information about the morphology and coating layer structure of drug pellets. In combination, RS and SEM delivered plentiful characteristics on the product design, which could be used in the development of a new generic drug.

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Keywords: reverse engineering, raman mapping, pharamceutics

H-P.30

Title: *Screening Cancer Cell Lines Using Deep-UV Resonance Raman Spectroscopy*

Author: Ahhyun Woo[1], Sila Jin[2], Yeonju Park[2], Jongmin Park[1], Young Mee Jung[1]
[1]Department of Chemistry, Kangwon National University
[2]Kangwon Radiation Convergence Research Support Center, Kangwon National University

Abstract:
Recently, research on early cancer diagnosis and anti-cancer treatment monitoring using Raman spectroscopy, a powerful method for revealing material-specific information in the region of material-specific fingerprint, has been attracting attention. Because exosomes present in body fluids reflect a variety of cellular information, they can be used as a cancer diagnostic method, reducing patient suffering from biopsies. However, it is difficult to obtain Raman spectra of biological samples using visible light lasers because most biological samples emit strong autofluorescence at wavelengths above 260 nm. In this study, a deep-UV laser at 244 nm was used to overcome the problem of autofluorescence of exosomes. Deep-UV Raman spectra of exosomes extracted from various cell lines were collected and subjected to principal component analysis (PCA) analysis. Details of results of analysis will be discussed in this presentation.

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Keywords: Deep-UV resonance Raman spectroscopy, PCA

H-P.31

Title: *Discovery of novel spectral biomarkers for early diagnosis of Lyme Disease*

Author: <u>Jizhou Zhong</u>[1], Ka Lung Andrew Chan[1], Hany Elsheikha[2]

[1]King's College London
[2]University of Nottingham

Abstract:

Lyme Disease has a significant economic and social impact because it is the most common tick-borne infection in the northern hemisphere. An increase in both temperature and humidity, as a result of climate change, is predicted to increase the number of LD patients by more than 20% in the coming decades.[1] The public health risks and rising healthcare costs associated with LD are aggravated by uncertainties surrounding its management, especially in cases of post-treatment Lyme disease syndrome (PTLDS) and "chronic LD".[2] The cost of treating LD in Europe is estimated at €10.1 billion for the acute form and €20.1 billion for the chronic form. In addition to these challenges, the standard methods used for diagnosing LD in practice suffer from many limitations. Existing methods for diagnosis of LD are imprecise and often require days or weeks for test results.

Fourier-transform infrared microspectroscopy is a powerful label-free optical method that has been widely used to interrogate the chemical composition of biological materials.[3] This spectroscopic approach detects the absorbance of IR light due to molecular vibrations, thereby generating a chemical fingerprint that contains (semi)quantitative information on the constituent molecules.[4]

In this study, we used FTIR microspectroscopy and synchrotron-based FTIR microspectroscopy to analyze the chemical changes that are associated with infection of human microglia by B. burgdorferi. Both FTIR microspectroscopic methods showed utility in revealing the chemical alterations in the infected cells. This research will address a major unmet need in the discovery of better diagnostic of LD. The ability to correlate the progression of B. burgdorferi infection to the host cell highly complex biochemical signatures at a single-cell level has never been investigated.

References:

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Keywords: Lyme Disease, FTIR, Synchrotron

H-P.32

Title: *ATR-FTIR of blood serum reveals spectral markers of pancreatic cancer*

Author: <u>Katarzyna Jurkowska</u>[1], Ewelina Lipiec[1], Krzysztof Szymoński[2]

[1]M. Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348
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Abstract:
Cancer is increasingly being diagnosed among patients worldwide, and pancreatic cancer is one of the most aggressive forms of the disease and one of the deadliest. Early detection of this cancer is particularly crucial for a patient's prognosis, as it is often diagnosed at an advanced stage, significantly exacerbating the outlook. Therefore, methods that enable early detection of pancreatic cancer are extremely valuable.

Vibrational spectroscopy, specifically Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)[1-3], has been proposed for this purpose. The aim of the research was to investigate the potential diagnostic capabilities of ATR-FTIR in differentiating between malignant and benign pancreatic masses and other types of cancers. To explore spectral markers of various cancer types chemometric tools were employed.

Fig 1. presents the preliminary results of PCA performed on spectra acquired from non-neoplastic control; pancreatic cancer; gastric cancer; colorectal cancer. Evident separation of spectra along PC-2 is observed (control at the negative side of PC-2, cancerous at positive). The corresponding loading plots demonstrate that bands from proteins (1700-1300 cm-1) and nucleic acids (1090 cm-1, 1230 cm-1) are responsible for the clustering observed in the scores plot.

References:
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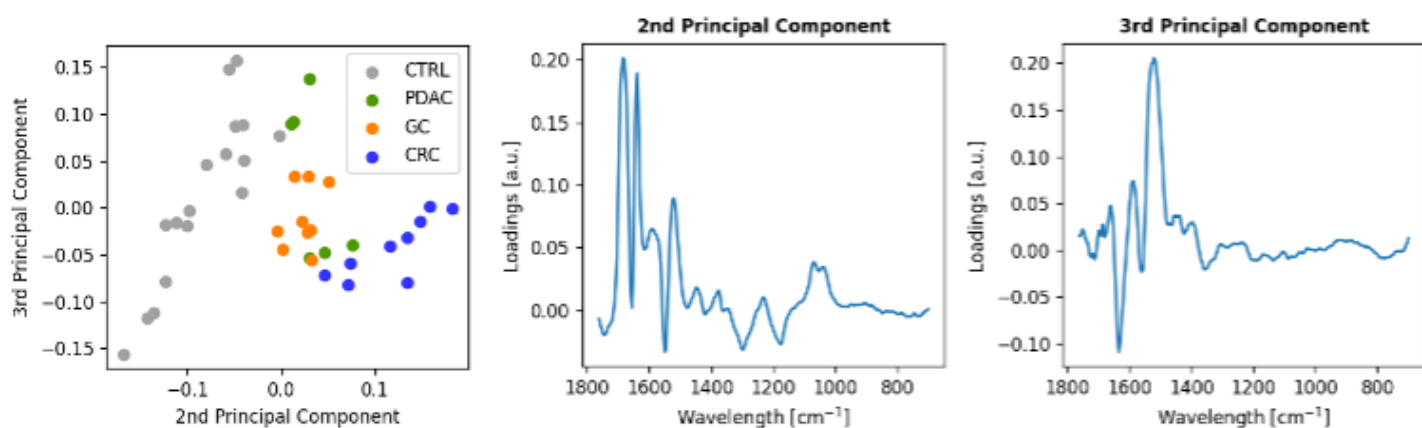
Figure captions:
Preliminary PCA analysis results of ATR-FTIR spectra, demonstrating clustering of samples by cancer type: CTRL-non-neoplastic control, PDAC-pancreatic cancer, GC-gastric cancer, CRC-colorectal cancer.

Keywords:
ATR-FTIR, pancreatic cancer

I-P.1

Title: *Impact of COVID-19 on critically ill patients' – mortality prediction models based on serum FTIR-spectra*

Author: Rúben Araújo[1], Tiago Fonseca[2], Cristiana Von Rekowski[2], Luís Bento[3], Cecília R.C. Calado[2]

[1]Nova Medical School, Comprehensive Health Research Centre, Health & Engineering Lab., ISEL – Instituto Superior de Engenharia de Lisboa
[2]Health & Engineering Lab., ISEL - Instituto Superior de Engenharia de Lisboa
[3]Centro Hospitalar Universitário Lisboa Central, Nova Medical School, Comprehensive Health Research Centre

Abstract:
Due to the relevance of mortality prediction of critically ill patients, it is common practice at intensive care units (ICU) to use physiological scores, e.g., APACHE II. However, these type of scorings don't enable to predict individual patients' outcome, mostly used for comparing groups of patients and ICUs [1]. It is therefore relevant to discover robust biomarkers of mortality prediction at ICU. Since FTIR spectroscopy can capture the whole molecular fingerprint of a system in a very specific and sensitive mode [2,3], in the present work, diverse mortality predictive models of support vector machines (SVM), based on FTIR-spectra of serum of critically ill patients, were developed. Serum samples from 200 patients at an ICU, with half presenting COVID-19 and the other half not presenting this infection, were considered. SVM models were optimized by combining spectral regions with diverse spectra pre-processing methods. A model cross-validation strategy, based on 10 random iterations, with 80% of samples for training and 20% for validation, was implemented. It was possible to develop very good SVM models to predict mortality based only on patients without COVID-19 (AUC=0.90), and a slightly better model was achiever for patients with COVID-19 (AUC=0.93). This difference can result from COVID-19 patients presenting a different metabolic status in relation to non-COVID-19 patients. Indeed, a very good SVM model enabled to discriminate these two populations (AUC=0.83). When considering the mixed population (i.e., with and without COVID-19), a slightly worse predictive SVM model was obtained (AUC=0.88).

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Figure captions:
Fig.1. t-SNE of COVID-19 (red) and non-COVID19 (blue) patients.
Fig.2. t-SNE of deceased (red) and discharged, i.e., survived (blue) patients.

Keywords: FTIR, COVID19, Mortality, ICU, Biomarkers

Fig.1. t-SNE of COVID-19 (red) and non-COVID19 (blue) patients, based on the serum spectra after atmospheric compensation, second derivative (2nd polynomial, 15-point window), and unit vector normalization for the MIR spectra region between 400 - 4000cm^{-1}.

Fig.2. t-SNE of deceased (red) and discharged, i.e., survived (blue) patients, based on the serum spectra after atmospheric compensation, second derivative (2nd polynomial, 15-point window), and unit vector normalization for the MIR spectra region between 400 - 4000cm^{-1}.

I-P.2

Title: *Monitoring of physical effort by infrared spectroscopy of urine composition*

Author: <u>Jaume Béjar Grimalt</u>[1], Ángel Sánchez Illana[1], José Ignacio Priego Quesada[2], David Pérez Guaita[1], Miguel De la Guardia[1], Salvador Garrigues[1]

[1]Department of Analytical Chemistry, Faculty of Chemistry, University of Valencia
[2]GIBD, Department of Physical & Sports Education, Faculty of Physical Activity and Sport Sciences; GIFIME, Department of Physiology, Faculty of Medicine, University of Valencia

Abstract:
Developing new training and recovery methods could be facilitated by comprehending metabolic alterations triggered by intense exercise. One popular way to monitor these changes is the non-invasive analysis of the composition of urine. This work evaluates the use of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) and multivariate analysis as a rapid and cost-effective way to investigate changes in urine composition after intense exercise. The urine spectrum of 21 volunteers (14 going through intense exercise and 7 controls) was measured before and immediately, 2, 5, 9, and 24 h after ran 10 km. Partial least squares- discriminant analysis (PLS-DA) was used to investigate changes in the spectra. Models did not find significant changes in the first hours, but they detected clear changes after the first 11 hours after performing the exercise ($p < 0.05$, Rand t-test permutation testing). In a second step, the spectra of proteins were extracted using ultrafiltration[1] for urines obtained before immediately after, and 11 hours after exercise were measured, finding clear differences between the spectra using principal component analysis (PCA). In conclusion, results indicate that the technique was able to monitor metabolic responses after physical exertion, having found significant changes after 11 hours.

References:
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Figure captions:
Graphical abstract

Keywords: IR spectroscopy, Chemometrics, POC, urine

I-P.3
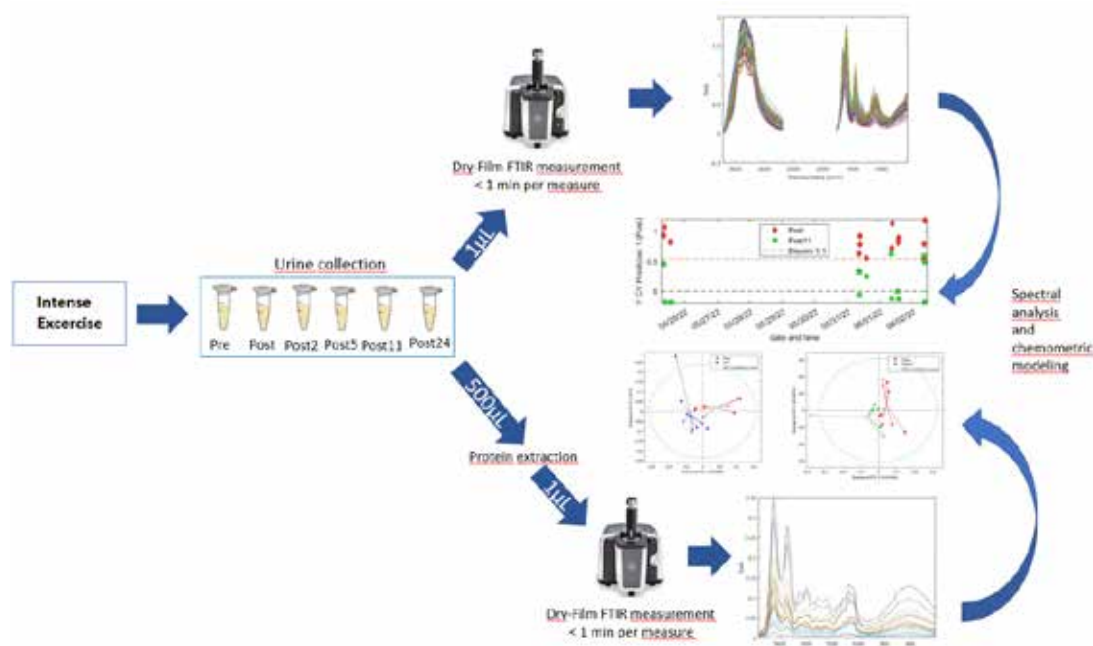
Title: *In silico modeling reveals the prospects and limitations of vibrational fingerprinting for phenotyping biological systems*

Author: Tarek Eissa[1], Kosmas V. Kepesidis[2], Mihaela Zigman[2], Marinus Huber[2]

[1]Ludwig Maximilian University of Munich (LMU), Max Planck Institute of Quantum Optics (MPQ), Technical University of Munich (TUM)
[2]Ludwig Maximilian University of Munich (LMU), Max Planck Institute of Quantum Optics (MPQ)

Abstract:
Vibrational spectroscopy, using infrared or Raman techniques, is a powerful approach that can effectively characterize the chemical composition of molecularly complex samples. Although widely applied in research and practical domains, the prospects and limitations of vibrational fingerprinting are yet to be fully understood. The challenge is that a variety of technical and data acquisition aspects impact the measured spectra. At the same time, the analyzed sample matrix may exhibit an inherent level of sample variability, further challenging the success of the application of interest. To address this knowledge gap, we introduce an *in silico* approach that generates artificial, but realistic, spectra [1]. With the flexibility to fine-tune simulation parameters, our approach offers a versatile and time-efficient means of exploring a range of experimental paradigms *in silico*. We apply machine learning methods to showcase how several, systematically-adjusted, parameters affect the performance of phenotyping biological systems using infrared spectroscopy of blood-based samples (Figure 1). Our results provide foundational insights into the effects of properties such as the noise introduced by the measurement device, the biological variability and chemical complexity of the analyzed samples. Applications of the model which address different clinical questions, such as cancer detection [2] and the prospects of personalized medicine [3], will be presented. The findings will be validated using multiple large-scale experimental studies. Although we focus on infrared spectroscopy of blood-based samples, the concept can be extended to other sample types and molecular fingerprinting techniques. Our work presents a new platform to strengthen our understanding of spectral fingerprinting and expose opportunities to advance its applications.

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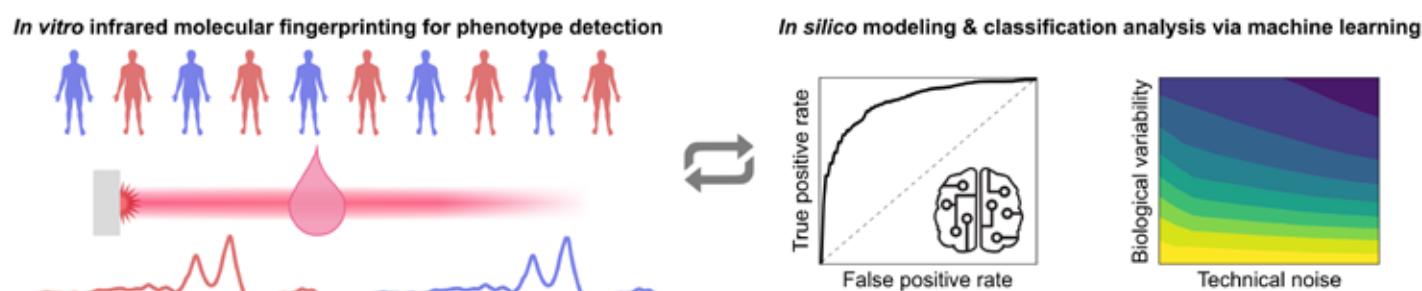
Figure captions:
Molecular fingerprinting of biological substances to detect diseased phenotypes using in vitro and in silico approaches to continuously improve study designs as well as measurement modes.

Keywords: silico, spectroscopy, fingerprinting, disease, classification

I-P.4

Title: *Non-Destructive Assessment of DNA and Glycosaminoglycans Content in Tissue Engineered cartilage using NIR Spectroscopy Coupled with Machine Learning*

Author: Omar Elkadi[1], Florencia Abinzano[2], Ervin Nippolainen[1], Ona González[2], Riccardo Levato[2], Jos Malda[2], Isaac Afara[1]

[1]Department of Technical Physics, University of Eastern Finland
[2]University Medical Center Utrecht, Utrecht University

Abstract:
Background and Aim: Cartilage tissue engineering (TE) is a promising regenerative medicine strategy to address the unmet clinical need for efficient treatment of cartilage injuries. Monitoring changes in cartilage construct properties during culture is critical for optimizing the culture conditions and assessing construct maturity. Glycosaminoglycans (GAGs) to DNA ratio is often used to assess engineered cartilage maturity. However, current methods for this assessment are destructive and impractical for longitudinal monitoring of tissue growth. This study investigates the potential of near-infrared spectroscopy (NIRS) coupled with machine learning (ML) for non-destructive estimation of GAGs and DNA in TE construct. Method: Engineered cartilage constructs (n=36) of cartilage chondroprogenitor cells in norbornene-modified hyaluronic acid scaffolds were incubated for 7 (n=18) or 28 (n=18) days in chondrogenic media supplemented with the growth factors bone morphogenetic protein-9 (n=24) or transforming growth factor-beta1 (n=12). Constructs were subjected to NIRS (3 spectra/sample), followed by reference GAGs and DNA analysis. The spectral data were preprocessed by eMSC and then ML models were developed using 7 different algorithms for predicting the GAGs and DNA contents from the spectra. The predictions were then used to estimate the GAGs/DNA ratio, and the accuracy of predicting the constructs maturity using the estimated ratio was evaluated. Results: AdaBoost-based models were optimal for predicting DNA (R^2=0.74) and GAGs (R^2=0.63). The estimated GAGs/DNA ratios based on the predicted values were able to classify the constructs according to their maturity with an accuracy of 99.07% (CI95%: 94.95-99.98), Sensitivity of 100% (CI95%: 93.40%-100.00%), and Specificity of 98.15% (CI95%: 94.95% to 99.98%). Conclusion: NIR spectroscopy combined with ML can enable non-destructive prediction of engineered cartilage construct maturity via estimating DNA and GAGs content.

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Keywords: Tissue-Engineering, Near-Infrared-Spectroscopy, Machine-Learning, Cartilage

I-P.5

Title: *Weakly Supervised Anomaly Detection Coupled with Fourier Transform Infrared (FT-IR) Spectroscopy for the Identification of Non-normal Tissue*

Author: Dougal Ferguson[1], Alex Henderson[1], Elizabeth McInnes[2], Peter Gardner[1]

[1]The University of Manchester
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Abstract:
The detection and classification of histopathological abnormal tissue constituents using Machine Learning (ML) techniques generally requires example data for each tissue or cell type of interest. This creates problems for studies on tissue that will have few regions of interest, or for those looking to identify and classify diseases of rarity, resulting in inadequate sample sizes from which to build multivariate and ML models. Regarding the impact on vibrational spectroscopy, specifically infrared (IR) spectroscopy, low numbers of samples may result in ineffective modelling of the chemical composition of sample groups, resulting in detection and classification errors. Anomaly detection may be a solution to this problem, enabling users to effectively model tissue constituents considered to represent normal tissue to capture any abnormal tissue and identify instances of non-normal tissue, be it disease or spectral artefacts. This work illustrates how a novel approach using a weakly supervised anomaly detection algorithm paired with IR microscopy can detect non-normal tissue spectra. In addition to incidental interferents such as hair, dust, and tissue scratches, the algorithm can also detect regions of diseased tissue, without the model ever being introduced to instances of these groups, training solely on control data using only the IR spectral fingerprint region. This approach is demonstrated using liver tissue data from an agrochemical exposure mouse study.

Keywords: FTIR, Machine-Learning, histology, mouse liver

I-P.6

Title: *Impact of serum metabolome isolation process on models' prediction of critically ill patients' mortality analyzed by FTIR-spectroscopy*

Author: Tiago Fonseca[1], Rúben Araújo[1], Cristiana Von Rekowski[1], Luís Bento[2], Cecília R.C. Calado[1]

[1]Health & Engineering Lab., ISEL – Instituto Superior de Engenharia de Lisboa
[2]Intensive Care Department, Centro Hospitalar Universitário de Lisboa Central, CHULC

Abstract:
Metabolomics has emerged as a powerful tool in the discovery of new biomarkers for medical diagnosis and prognosis. Metabolomics of biofluids, such as serum, can therefore potentially deliver biomarkers that may be applicable in patients' monitoring [1]. This is especially relevant in the management of critically ill patients. However, there are numerous challenges, including the metabolome isolation process and the subsequent platform applied to analyze it. FTIR-spectroscopy presents diverse advantages for metabolome analysis, since it may be applied in rapid, economic, and high-throughput mode, while enabling to acquire the system's metabolic status with a high sensitivity and specificity [2,3]. In the current project, two extraction protocols of the serum metabolome were evaluated. Both protocols included macromolecules precipitation induced by mixtures of methanol, acetonitrile, and water. Replicas of 5mL extracted serum metabolome, from critically ill patients, were plated in 384 wells-microplates, and after a rapid dehydration step, spectra were acquired between 400 to 4000 cm^{-1}. The impact of the two extraction procedures to isolate the serum metabolome, on reproducibility, based on FTIR-spectra principal component analysis (PCA) was studied. The impact of the two extraction procedures on the performance of predictive models, based on spectra PCA-discriminant analysis of patients' mortality, was also conducted. Serum samples from critically ill patients were obtained according to legal and ethics requirements, including project ethics approval by the Hospital Ethics Committee (Centro Hospitalar Universitário Lisboa Central), and patients' informed consent.

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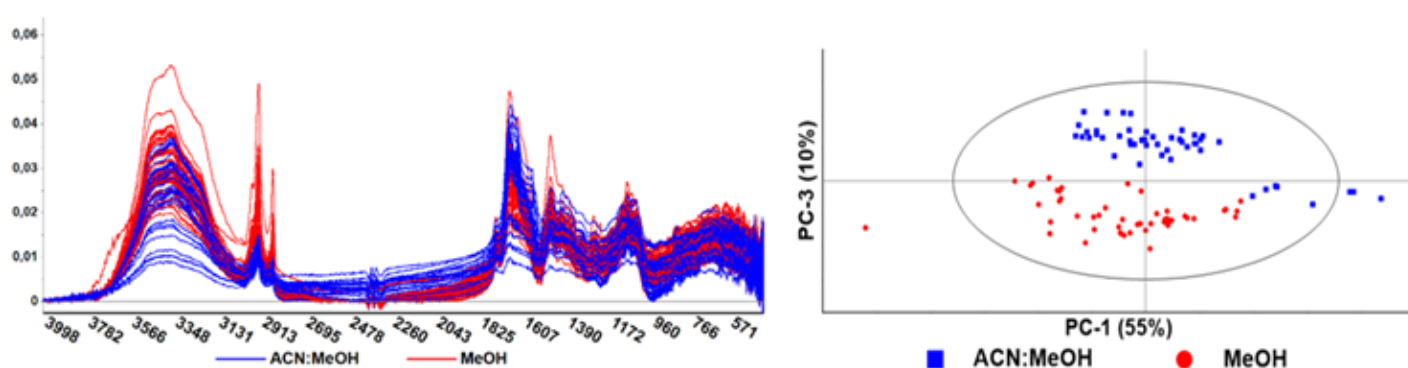
Figure captions:
FTIR-spectra of serum metabolome from critically ill patients and its PCA. Serum metabolome was isolated by macromolecules precipitations by methanol or a mixture of methanol, acetonitrile and water.

Keywords: Metabolome, FTIR, prediction, mortality

I-P.7

Title: *FAIRSpectra: metadata and data encoding, promoting Open Research*

Author: Alex Henderson[1]

[1]University of Manchester

Abstract:

For **Open Research** to develop we need our data to be Findable, Accessible, Interoperable and Reusable. These **FAIR Principles,** described in 2016 [1], are supported by all major funding bodies in Europe, the USA and elsewhere. However, to reach the highest level of accessibility and reusability we need to accurately and precisely detail what our data contains, and to ensure it is readable by other people, and machines.

There are a number of aspects here: we need our metadata (data describing the data) to mean the same thing to all people who choose to examine it. That is, we need a common **Semantics.** We need a **minimum amount of information** to be recorded and stored with the data, so that it is meaningful. We need our files stored in a format that can be **easily read** by other researchers. In order to do this we need a dialog between all vibrational spectroscopy researchers to define what is required, and to develop a common language with which to describe it.

FAIRSpectra is a place where we can come together to **start a conversationin** this area. To develop a common set of terms that **we can all agree upon.** To organise these into reporting requirements, and to build tools and platforms around those.

References:

[1] The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018 (2016). DOI:10.1038/sdata.2016.18

Keywords: Open-Research, Standards, Semantics, File-formats

I-P.8

Title: *Towards IR chemical imaging of tumor metastasis in organ on chip systems*

Author: <u>Nikolaus Hondl</u>[1], Elisabeth Holub[1], Kai-lan Lin[2], Diosángeles Soto Véliz[2], Cecilia Sahlgren[3], Bernhard Lendl[1], Georg Ramer[1]

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[2]Åbo Akademi University, Faculty of Science and Engineering, Cell Biology
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Abstract:
Cancer is a global public health challenge and one of the leading causes of death in most countries. Treatment outcome strongly depends on early detection and treatment, and cancer detection methods are continuously developed. Due to its ability for label free chemical imaging, Mid-IR has been used by multiple groups to identify cancerous tissues and cells [1,2] and to subplant current chemical staining based pathology approaches.
In our ongoing collaboration with oncologists and bio-medical researchers we aim to expand the use of IR to the study of the mechanisms behind cancer metastasis, with the goal to use IR to distinguish healthy, cancerous and metastasizing cells in tumor on a chip systems. While FTIR imaging has been used with great success on tissue section [3], applications in large, water filled channels require a different approach: a mid-IR photothermal (MIP) spectroscopy setup tailored to organ on a chip spectroscopy. Our instrument enables sensitive absorption imaging in presence of several millimeters of water and requires, save for an IR transparent sample carrier, no further adaption to mid-IR spectroscopy.
Here, we present preliminary experiments in using machine learning and infrared spectroscopy to find and distinguish cancerous and healthy cells, as well as comparisons of images taken with conventional FTIR microscopy and our MIP setup. Our findings show that the photothermal spectra compare favourably to the FTIR spectra, thereby opening the way for further research.

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Keywords: Infrared spectroscopy, Nearfield, Chemometrics, Characterization,imaging

Title: *Scattering artifacts correction impact on molecular orientation determination with Polarized Fourier-Transform Infrared Spectroscopy*

Author: Paulina Koziol[1], Karolina Kosowska[1], Tomasz P. Wrobel[1]

[1]SOLARIS National Synchrotron Radiation Centre, Jagiellonian University, Czerwone Maki 98, 30-392 Krakow, Poland

Abstract:
Polarized Fourier-Transform Infrared Spectroscopy (FT-IR) is recently expanding its applications in molecular orientation studies, as a consequence of the Four-Polarization (4P) method development. Determination of the in-plane orientation of collagen in fibrous tissues [1], along with the 3D orientation of molecules in polycaprolactone (PLC) spherulite [2] are some of the latest achievements in this field. However, despite providing exciting results, this research area is still developing and some obstacles need to be addressed. One of them is scattering artifacts, especially predominant in cylindrically shaped samples (like fibers) often investigated in molecular orientation studies. Correction of Mie scattering for spherical samples has many implementations, but scattering from cylindrical samples has only been addressed recently. Here, an Extended Multiplicative Signal Correction based algorithm is presented, with the aim to correct Mie-type scattering for cylindrical samples, including cases with linear polarization of the incident radiation. Due to time constraints, the algorithm has a GPU implementation and is available as an open-source code. Its efficiency is tested using a model PCL fiber sample, with physical properties strongly enhancing scattering effects. The effectiveness of the developed algorithm along with its impact on molecular orientation determination was tested with mentioned PCL fiber along with more complicated systems such as tissue fibers, providing promising results.

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[2] Koziol, P., Kosowska, K., Liberda, D., Borondics, F. & Wrobel, T. P. Super-Resolved 3D Mapping of Molecular Orientation Using Vibrational Techniques. J. Am. Chem. Soc. 144, 14278–14287 (2022)

Keywords: Scattering correction, Polarization, FT-IR, EMSC

Title: *Pre-processing Raman data via deep learning method*

Author: <u>Azadeh Mokari</u>[1], Simone Eiserloh[2], Max Naumann[2], Ute Neugebauer[3], Thomas Bocklitz[4]

[1]Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Strasse 9, 07745 Jena, Germany; 2 Institute of Physical Chemistry (IPC) and Abbe Cente

[2]Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Strasse 9, 07745 Jena, Germany

[3]Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Strasse 9, 07745 Jena, Germany; Institute of Physical Chemistry (IPC) and Abbe Center

[4]Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Strasse 9, 07745 Jena, Germany; Institute of Physical Chemistry (IPC) and Abbe Center

Abstract:

Raman spectroscopy is a type of analytical technique that uses the interaction of light with a sample to provide information about its atomic and molecular properties. However, Raman spectra are frequently overshadowed by inconsistencies in baselines and various sources of noise. These defects and contributions to the Raman data must be rectified before identifying or categorizing the samples. Accordingly, Raman data are processed using AI-based algorithms. To that end, we suggested the use of a deep learning approach as a pre-processing tool for Raman data. As a result, we tested two networks: convolutional denoising autoencoder (CDAE) [1], and U-Net [2]. CDAE and U-Net networks were implemented to test two different pre-processing cases: denoising and denoising with baseline removal. For both cases, the superiority of these methods was evaluated on real and artificial Raman data. In the first case, we aimed to reconstruct high-quality (HQ) Raman spectra that include a background. Therefore, the networks were trained to map between noisy Raman data measured with different integration times, for example, 0.5s as an input and HQ Raman data with 1s as an output. As shown in Figure 1, U-Net/ CDAE network tries to estimate HQ data in experiment data or predict the HQ artificial Raman data. Afterward, in the testing phase, the trained networks are used to predict the HQ data. In the second case, we aimed to reconstruct HQ spectra with baseline removal. In other words, the aim of this case is to remove noise and background in the data at the same time. Therefore, the same noisy Raman data was used as an input and the output was acquired by applying classical pre-processing methods (SG+SNIP on the HQ Raman data). Regarding the evaluation part in Figure 1, U-Net has the capability to remove the noise and baseline simultaneously, while the CDAE is able to remove noise only. In conclusion, the suggested technique outperforms traditional methods in terms of time and error.

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Figure captions:

Figure 1 The reconstruction of HQ spectra using a neural network that maps noisy input signals to longer, cleaner output signals.

Keywords:

Raman spectroscopy, Pre-processing, Deep learning
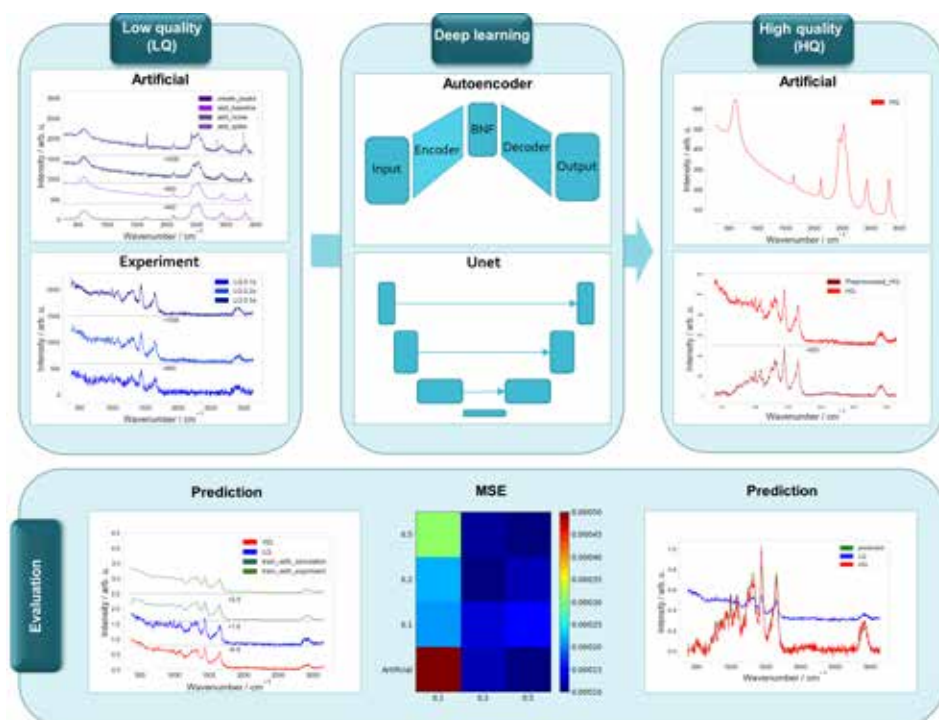
Title: *Optical Photothermal Infrared Spectroscopy for Sub-Micron Analysis of Single PC-3 Prostate Cancer Cells: Hyperspectral and Multispectral Data Congruence and Clustering Challenges*

Author: Buradsakon Pongtippitak[1]

[1]University of Manchester

Abstract:
This study presents an objective examination of single PC-3 prostate cancer cells employing optical photothermal infrared (O-PTIR) spectroscopy. The research demonstrates the obtaining and analysis of sub-micron spatial resolution hyperspectral and multispectral maps of the cell, highlighting key absorption bands linked to biological macromolecules. By applying K-means clustering and the Davies-Bouldin clustering evaluation criterion, the optimal number of clusters for characterizing subcellular features in integrated intensity maps is determined. The results exhibit a notable correlation between the hyperspectral and multispectral data. Nevertheless, differences in K-means clustering patterns emerge when comparing the two datasets, suggesting potential difficulties in data interpretation. These findings enhance the comprehension of single-cell analysis using O-PTIR spectroscopy and offer guidance for subsequent studies on subcellular investigations.

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Keywords: Optical photothermal infrared spectroscopy, Single-cell

I-P.12

Title: *Random Forest Classification of Glycosaminoglycans Based on Cryogenic Gas-Phase Infrared Spectra*

Author: <u>Jerome Riedel</u>[1], Maike Lettow[2], Márkó Grabarics[1], Michael Götze[1], Rebecca L. Miller[3], Geert-jan Boons[4], Gerard Meijer[2], Gert Von Helden[2], Gergo Peter Szekeres[1], Kevin Pagel[1]

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Abstract:
In recent years, glycosaminoglycans (GAGs) have become increasingly important molecules in the pharmaceutical- and biochemical community due to their involvement in a variety of physiological processes ranging from cancer and inflammation to blood coagulation and signal transmission cascades. As highly sulfated linear polysaccharides, their chemical analysis is a complex task due to their inherent backbone diversity, epimerization, and differences in sulfation pattern that often render their identification by mass spectrometric approaches alone impossible. Cryogenic infrared (IR) spectroscopy of mass-selected GAG ions can provide complementary information that allows for the distinction based on their IR spectra and enables the characterization of sulfation- and epimerization patterns. Here, helium nanodroplet action spectroscopy was used to measure the IR spectra of heparan- and chondroitin sulfate (HS/CS) di-, tetra-, and hexasaccharides. The molecular ions are picked up by superfluid helium nanodroplets in a cryogenic ion trap and rapidly cooled down to 0.4 K. By subsequent IR irradiation, vibrational energy is transferred to the helium matrix, resulting in evaporative matrix shrinking and the eventual ejection of the ions, which can be detected as a wavelength-dependent event in a time-of-flight mass analyzer.
The GAG IR spectra were used to develop a novel pipeline to feature-engineer Random Forest models for the classification of structural motifs, such as GAG class (HS/CS) and sulfation (N2, 2S, 4S, and 6S). Prediction accuracies of > 97% were reached, including full characterization of a hexasaccharide. This highlights the exceptional potential of coupling machine learning approaches with cryogenic IR spectroscopy to classify larger GAG oligosaccharides and eventually other biomolecules, such as metabolites.

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Lettow et al, Anal. Bioanal. Chem., 10.1007/s00216-019-02327-7

Keywords: Chemometrics, Random Forest, Gas-Phase Spectroscopy, Glycosaminoglycans, Machine Learning

I-P.13

Title: *Application of artificial intelligence approaches on the infrared spectra of sera of induced EAE multiple sclerosis model in mice*

Author: Krongkarn Sirinukunwattana[1], Gilles Marcou[2], Christian Klein[3], Laurence Meyer[3], Christine Patte-Mensah[3], Youssef EL KHOURY[1], Alexander Varnek[2], Ayikoé Guy Mensah-Nyagan[3], Petra Hellwig[1]

[1]Laboratory of Bioelectrochemistry and Spectroscopy, University of Strasbourg
[2]Laboratory of Chemoinformatics, University of Strasbourg
[3]Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques, INSERM U1119, Fédération de Médecine Translationelle de Strasbourg (FMTS), University of Strasbourg

Abstract:

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). This disease results from the demyelination and inflammation as well as a drop in the levels of allopregnanolone in the CNS of MS patients [1], leading to disability. IFN-β is currently used in the clinic to treat MS patients. It possesses a wide range of anti-inflammatory properties [2]. Yet, the search for new drugs continues and XBD173 is one of the candidates since it helps increasing the level of a neuroprotective steroid allogregnanolone to suppress neuroinflammation [3].

We propose Fourier Transform Infrared Spectroscopy (FTIR) to reveal the effect of the treatments on the examined EAE-mice serum coupled to a random forest machine learning approach [4]. We included in this study healthy (naïve), untreated MS (vehicle), IFN-β-treated and XBD173-treated mice.

Figure 1(A) shows the average second derivative spectra of sera showing the changes in amide I region. Figure 1(B) is obtained from the integral area of the second derivative spectra showing the area ratio of α-helix (~1650 cm^{-1}) and β-sheet (~1633 cm^{-1}). The untreated MS (vehicle) shows a higher amount of β-sheet while treatment with IFN-β leads to lower the amount of β-sheets. The results of the machine learning approach used to better understand the effect of treatment on the spectroscopic signature will be presented.

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Acknowledgments:

Figure captions:

Figure 1(A) Average 2nd derivative spectra of Naïve, Vehicle and MS-treatment and (B) α-helix/β-sheet area ratio

Keywords: Induced EAE multiple sclerosis, Interferon-β,XBD-173, FTIR, Artificial intelligenceapproaches

I-P.14
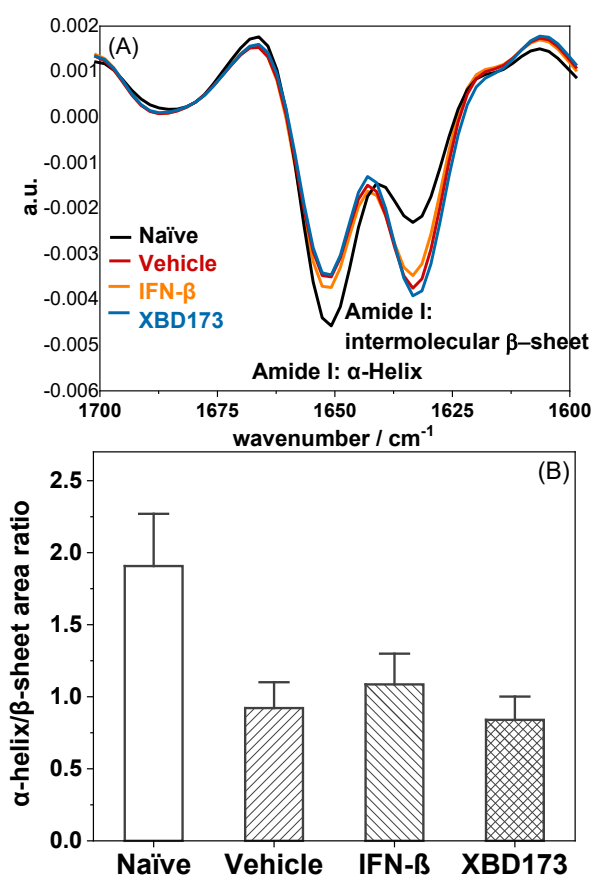
Title: *Non-negative Matrix Factorization (NMF) as a meaningful method for finding the variabilities in global DNA methylation and β-sheet richness among subtypes of pancreatic cancer*

Author: Krzysztof Szymoński[1], Ewelina Lipiec[2], Kamila Sofińska[2], Katarzyna Skirlińska-Nosek[2], Michał Czaja[2], Sara Seweryn[2], Łukasz Chmura[1], Joanna Szpor[3], Dariusz Adamek[1], Marek Szymoński[2], Natalia Wilkosz[2]

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[3]Department of Pathomorphology, University Hospital

Abstract:
The Non-negative Matrix Factorization is a multivariate analysis method that uncovers the collected data's meaningful features. This non-negativity makes the resulting matrices easier to inspect, as NMF's objective is to reduce dimensionality. The overall concept of this method is that the NMF decomposes multivariate data by creating a user-defined number of components in an iterative process. Each feature is a linear combination of the original attribute set. In chemometrics, this useful method presents the chemical compounds of the spectra and allows the production of false-color distribution maps. [1]
The survival of pancreatic cancer patients has not greatly improved even though knowledge about its biology has been rapidly growing in recent decades. To study the biochemical composition of the pancreatic cancer tissues, the Raman hyperspectral mapping combined with advanced multivariate data analysis was held. Three subtypes of pancreatic cancer were examined (AVAC, cPDAC, IPMC) as well as the benign tissue for comparison.
The NMF analysis showed significant differences among cancer tissues that formed a unique fingerprint for each type of pancreatic cancer. Three components associated with a characteristic chemical composition, including intracellular proteins, water, and nucleic acids, that represent the particular region of the studied tissue were calculated. The main findings are the varying content of β-sheet-rich proteins within the pancreatic cancer cells and alterations in the relative DNA methylation level. [2]

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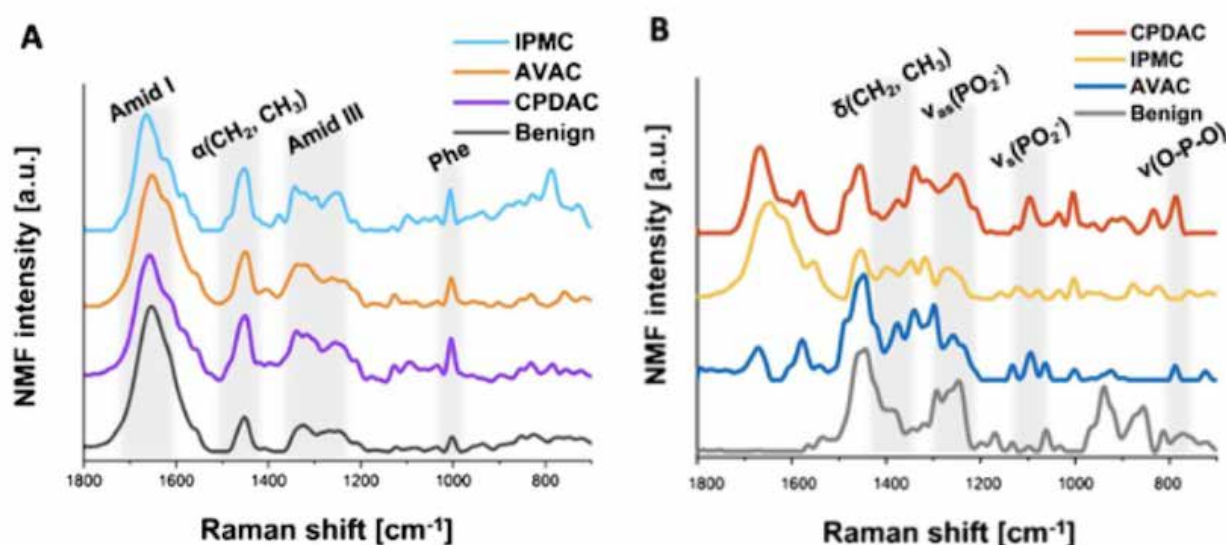
Figure captions:
Comparison plot of spectral marker bands related to proteins (section A) and methylation and DNA conformation (section B) of NMF components of all studied PC types and benign pancreatic duct tissue

Keywords: nmf, pancreatic cancer, multivariate analysis

J-P.1

Title: *Vibrational calculations and SERS activity prevision of hepcidin hormone: contribution for hyperinflammation screening.*

Author: Julian Mateo Rayo Alape[1], Carla Carolina Silva Bandeira[1], Herculano Da Silva Martinho[1]

[1]Federal University of ABC

Abstract:
Evidence had been presented relating to COVID-19 mortality might to viral driven hyperinflammation.The hormone hepcidin, present in saliva is a hyperinflammation marker for COVID-19 and other pathological states. Here, we present density functional theory based on vibrational calculations that showed good agreement with experimental vibrational spectra. The corresponding SERS-activity indicated the α_{zz} component of Raman tensor (Amide vibrations) would present greatest amplifications.

References:
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atomistic simulations of condensed matter systems," Wiley Interdiscip. Rev. Comput. Mol. Sci. 4, 15–25 (2014).

Figure captions:
a) NMR assembly of human hepcidin. b) Peptide hepcidin (sequence DTHFPICIFCCGCCHRSKCGMCCKT) which is usually found in human biofluids. c) The molecular electrostatic potential (MEP) for hepcidin.

Keywords: Hepcidin hormone, computational simulation, Raman, spestroscopy

J-P.2

Title: *Raman Optical Activity signatures of a cage system, Cryptophane–PP–111, and investigation of the solvent and encapsulation effects*

Author: Lou D'haese[1], Nicolas Daugey[2], Vincent Liégeois[1]

[1]University of Namur
[2]University of Bordeaux

Abstract:
Cryptophane derivatives consist in two hemispheres, each made of three benzene rings connected in ortho by three methylene groups. These hemispheres are themselves bound by three $-O(CH_2)_n-O$ linkers to form a cage. The antiarrangement of the linkers makes them chiral (enantiomers PP/MM) thus generating chiroptical properties. Their cavity allows cryptophanes to encapsulate some guest systems. This ability makes them potential biological sensors, as demonstrated for Cryptophane–111 (Cr–111, see Fig. 1) which exhibits a high binding constant for the xenon[1]. The Raman Optical Activity (ROA) signatures of the xenon encapsulation have been experimentally recorded by co-workers at the University of Bordeaux[2]. ROA spectroscopy is a vibrational optical activity (VOA) technique and can study specific interactions since the vibrational signatures are sensitive to the surroundings effects of the target system. Recently, Nafie has identified the future challenges for simulating VOA techniques[3]: to properly describe weak intermolecular interactions, solvent effects, ... Another challenge, as stated by Barone[4], is the analysis of the conformational potential energy surface (PES) for flexible systems. To tackle this issue, the group of Grimme has developed a novel scheme, called ``Conformer-Rotamer Ensemble Sampling Tool'' (CREST), based on a meta-dynamics approach and using a semi-empirical tight-binding level of theory. This tool has already been used in recent articles for sampling the PES of flexible systems before the simulation of their vibrational spectroscopies and seems very promising. In this work, we have tested CREST program for sampling the PES of Cr–111 system, studied the impact of the environment effects on its ROA signatures, and compared our results to the experimental data. Most signatures have been well reproduced by our simulations, proving the efficiency of our methodology. However, in the low frequency region, some improvements can be done.

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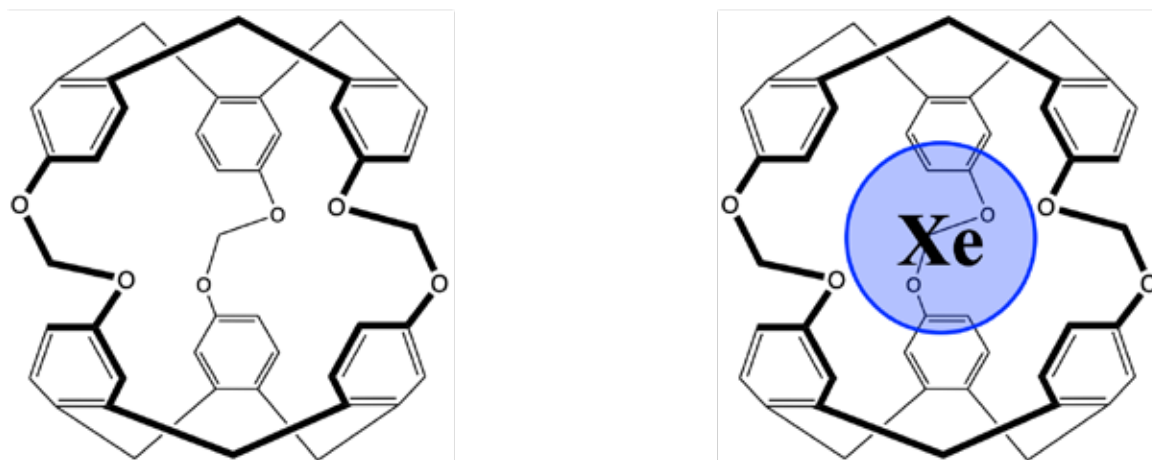
Figure captions:
Fig. 1 – Sketches of Cryptophane–PP–111 (left) and Cryptophane–PP–111 + Xe (right).

Keywords: ROA Spectroscopy, Computational Chemistry, CREST.

J-P.3

Title: *IR/VCD spectroscopic studies on matrix-isolated chiral 1-phenyl-1-propanol*

Author: <u>Corentin Grassin</u>[1], Christian Merten[1]

[1]Ruhr Universität Bochum

Abstract:

In Matrix Isolation (MI), the compound of interest is trapped in an inert gas at cryogenic temperatures[1]. By controlling the host/guest ratio, isolation of monomeric species can be achieved and dimerization, for instance of alcohols or other hydrogen bonding systems, can effectively be suppressed. By using Infrared (IR) spectroscopy to probe the matrices, studies ranging from conformational isomerism[2] to photochemistry[3] or aggregate clustering[4] are possible.

Combining MI with Vibrational Circular Dichroism (VCD), the chiral version of IR spectroscopy[5], broadens scope of possible spectroscopic studies even further. Due to its high conformational sensitivity, MI-VCD could successfully be utilized to study self-aggregation[6], chirality transfer[7], conformational distortions[8] and photoisomerization of chiral photoswitches[9].

In this work, we report the MI-IR/VCD spectra of a chiral alcohol, 1-phenyl-1-propanol, in argon matrix. We show that it is possible to tune the monomer/dimer ratio using different deposition conditions (mostly temperature of the deposition window). While monomeric and aggregated species can be distinguished only based on their OH-stretching vibrations in the IR, the VCD spectra are found to feature characteristic signatures of both species allowing a differentiation in the fingerprint region.

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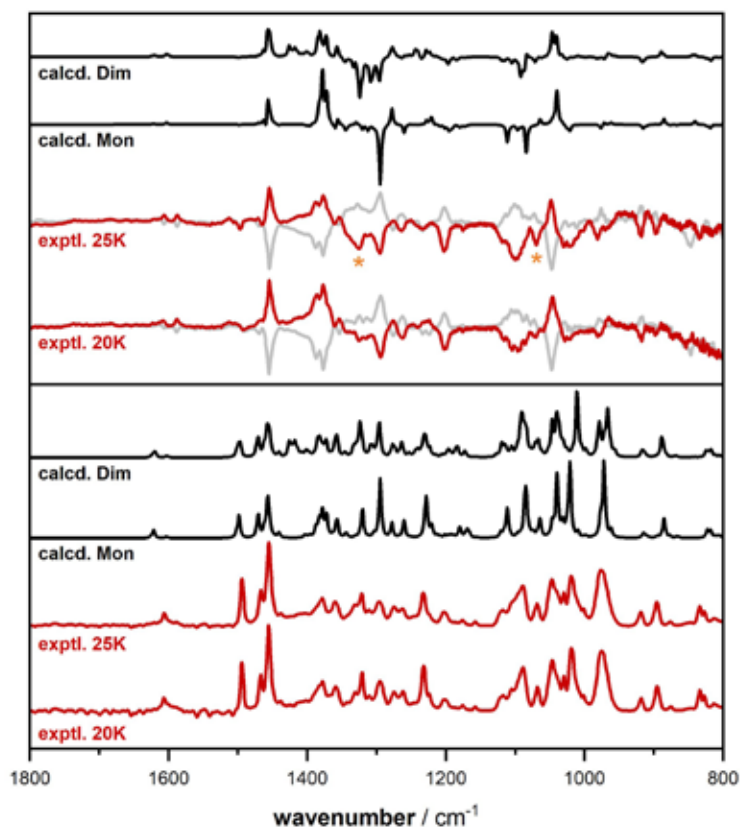
Figure captions:
MI-IR and VCD of R- and S-1-phenyl-1-propanol in Argon compared with DFT calculations.

Keywords: MI, IR, VCD, self-aggregation

J-P.4

Title: *Structure of Histidine-Metal Complexes in Solution Revealed by Raman Optical Activity*

Author: <u>Jana Hudecová</u>[1], Josef Kapitán[1], Martin Dračinský[2], Václav Profant[3], Pavel Michal[1], Petr Bouř[2]

[1]Palacký University
[2]Institute of Organic Chemistry and Biochemistry Academy of Scienc
[3]Charles University

Abstract:
The histidine residue has an exceptional affinity for metals, but structure of its complexes in solution is difficult to study. We have recorded and analyzed Raman and Raman optical activity (ROA) spectra of two histidine forms and histidine zinc and nickel complexes to get an insight into the geometry and broaden the application span of the vibrational optical activity.[1] The link between spectral shapes and geometry was investigated.

The monomeric histidine was found to be quite flexible, many conformers needed to be included to reproduce the experiment by calculations, and ROA bands were relatively weak and broad. The computations suffered from the limited precision of the simulated spectral intensities, nevertheless, they were accurate enough to indicate prevalent forms of the studied compounds in the solutions. For H_3His^+ they indicated a significant drawback of the MD Amber03/TIP3P force field, predicting an unreasonable distribution of the conformers. For H_2His^0 a more even distribution between the ε and δ-tautomers was found by the decomposition of experimental spectra into calculated subspectra than in previous studies.[2]

The combined Raman/ROA/AIMD/DFT methodology also provided important information on the conformer distribution of the metal complexes. The complexation with the metals provided stronger and better resolved ROA bands. An octahedral structure prevailed for the $ZnHis_2$ complex in solution, in contrast to a tetrahedral arrangement in the crystal phase.[3] The solution geometry of $NiHis_2$ is more similar to the octahedral structure found by x-ray.[4] The Raman and ROA structural determinations of metal complexes depend on extensive computations but reveal unique information about the solution geometry that cannot be obtained by other means.

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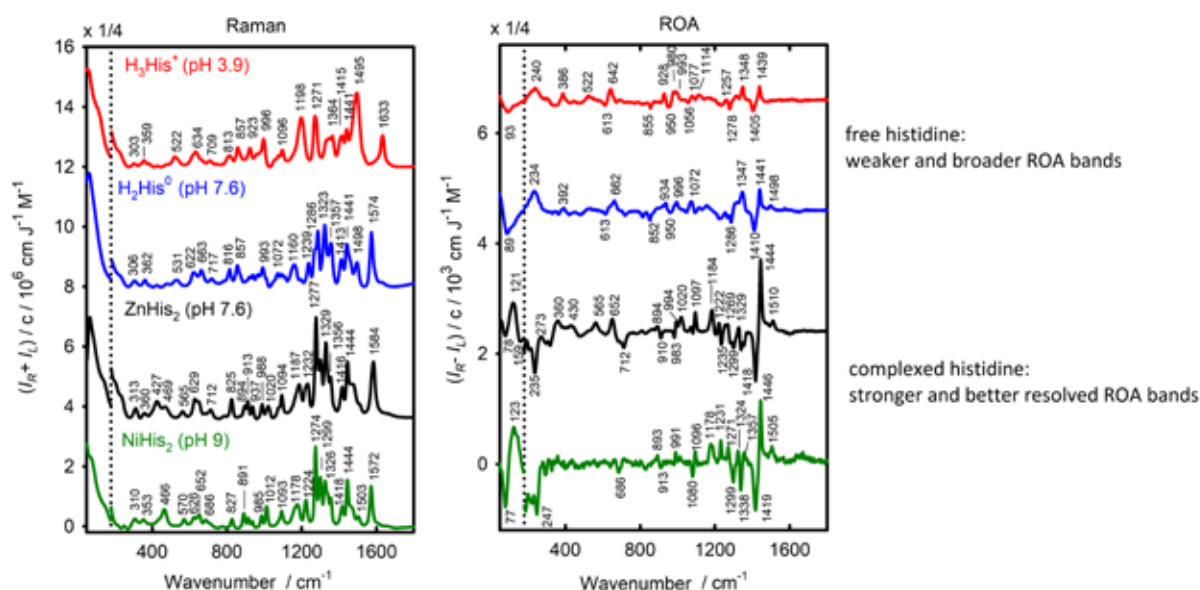
Figure captions:
Experimental Raman and ROA spectra of H3His+, H2His0, and ZnHis2 and NiHis2 complexes, L-enantiomers. The spectra were normalized to the His concentration.

Keywords: histidine, metal complexes, ROA, MD, DFT

J-P.5

Title: *Importance of vibrational circular dichroism in determination of absolute configuration: case of amphetamine derivatives*

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Abstract:
Absolute configuration plays a very important role in biological activity of chiral molecules and many cases of different physiological effect of two respective enantiomers have been described. Determination of absolute configuration is thus an important part of the complete characterization of chiral chemical entity, for instance in description of new pharmaceutical compounds. Another example is amphetamine and its derivatives, where the (S)-enantiomer exhibits much stronger effect on the human body than the (R)-enantiomer [1]. Chiroptical spectroscopy, which is inherently sensitive to the 3D structure of chiral molecules, represents a powerful tool for a detailed study of chiral substances [2]. It can be advantageously combined with the ab initio quantum chemical calculations as they can predict the spectra of specific stereoisomer. The most common chiroptical method is electronic circular dichroism (ECD), which provides fast and relatively cheap analysis. However, ECD spectra usually comprise only few bands of corresponding electronic transitions and their calculations are rather demanding. On the other hand, vibrational circular dichroism (VCD) offers a more detailed analysis of the 3D structure of chiral molecules as vibrations of individual functional groups can be identified in the spectra and VCD is thus considered to be more reliable in the absolute structure determination [3].
In the present work, the model case of amphetamine and its derivatives is presented where VCD proved to be a reliable tool for absolute configuration determination. The stable conformers of the studied compounds were found by the density functional theory calculations and subsequently, the VCD and ECD spectra were calculated and compared to the experiment. VCD spectroscopy enabled a clear determination of absolute configuration, while interpretation of the ECD spectra could have led to incorrect conclusions.

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Keywords: chiroptical spectroscopy, amphetamine, circular dichroism

J-P.6

Title: *Development of Computational Models to Decipher Raman Optical Activity Spectra of G-quadruplexes*

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Abstract:
G-quadruplexes are good models of DNA structures involved in the metabolism of living cells and are frequently adopted by guanine-rich nucleic acids. They play crucial roles in genome functions and stability, the pathogenesis of several neuro-degenerative diseases, and cancer biology. The guanine nucleotide (G) and its derivatives are well known for their property to self-associate into various complexes through H-bonding and stacking interactions. G-quadruplexes are formed at higher concentrations as nanoscale cylindrical structures consisting of G-quartet disks stacked one above the other[1]. These supra-molecular assemblies are also potential candidates for nanotechnology and chemical biology applications.
Raman optical activity (ROA), which measures differential Raman scattering of right and left circularly polarized light, is promising for studying nucleic acid structures and their dynamics because of its sensitivity to subtle changes in geometry[2]. Recently, characteristic Raman and ROA spectral changes upon G-association were observed. A combination of molecular dynamics (MD) and quantum-chemical computational techniques have been used to model and interpret the observed Raman and ROA spectral features of G-quadruplexes under various experimental conditions. The fragment-based cartesian coordinate-based tensor transfer (CCT)[3] method is also employed in the spectra calculations to embrace the enormous size of the G-quadruplexes.

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Keywords: ROA, G-quadruplexes, Molecular Dynamics, DFT

J-P.7

Title: *Study of the Structural Aspects of Glutathione and Glutathione Disulfide using Raman Optical Activity (ROA) Spectroscopy*

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The work conducted was funded by National Science Centre in Poland (Grant No. 2019/35/B/ST4/04161 to GZ)

Abstract:

Glutathione (GSH) is a biologically significant antioxidant that plays a crucial role in maintaining the redox environment of the eukaryotic systems[1]. The oxidized form of GSH turns out to be the glutathione disulfide (GSSG). The ratio between the GSSG and GSH forms reflects the nature of the redox environment in the living systems which is placed in the range of 1: 100 – 1: 1000[2]. The change of the above ratio that happens with the increase of the population GSSG with respect to that of GSH results in the exposure of the living systems to excessive oxidation conditions which in turn points towards the deficiency of the glutathione reductase (GR) and glucose-6-phosphate dehydrogenase entities that are required for the conversion of excessive levels of GSSG back to GSH.

Since the Raman optical activity (ROA) spectroscopy has emerged as a powerful spectroscopic method to identify stereochemical changes of chiral molecules and also due to its capability to register the spectral features of chiral molecules in water[3], it could play a key role in identifying the structural changes possessed by GSH and GSSH.In this study, we have implemented ROA spectroscopy to register the stereo-specific spectral signatures of GSH and GSSG systems in water. The flexible nature of GSH offers a challenge to identify the specific conformational contributions towards the interpretation of experimental Raman, ROA spectra. In order to account for this challenge, we have implemented the CREST[4] (Conformer RotamerEnsemble Sampling Tool) methodology to investigate the conformational spaces of GSH and GSSG. The explicit non-covalent interactions that generate in water are modeled using the non-covalent interaction modes of CREST[5].

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Keywords: ROA, non-covalent interactions, conformational flexibility

J-P.8

Title: *Is the Herzberg-Teller Effect Sufficient for Simulation of One Photon Absorption Spectra?*

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Abstract:
UV-vis absorption and circular dichroism spectra play a significant role in understanding excited-state molecular properties. They developed to routine tools used in a variety of fields, such as pharmacology, chemical analysis, and environmental monitoring. With the advances of instrumentation and broader applications, also spectra simulations face increasing challenges. Theoretical methods fully considering the vibronic effects are replacing simpler procedures. With a better balance of computational cost and accuracy, such simulations are becoming routine.
An important aspect of the vibronic simulations is the treatment of the transition dipole moments. The usual Franck-Condon approximation may not be adequate for weak or forbidden electronic transitions and the first order expansion (Herzberg-Teller, HT) term must be considered. For several organic molecules we show the importance of the HT effects for understanding the spectra. A porphyrine system not only exhibits the HT effects, but also indicates that higher terms should be included as well.

Keywords: Vibrational-resolved, Electronic spectra, Porphrine, Franck-Condon

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