20th European Conference on Spectroscopy of Biological Molecules

1-5 September 2024, Lodz, Poland

https://ecsbm2024.p.lodz.pl/

ABSTRACT BOOK

Organized by
Lodz University of Technology, Faculty of Chemistry,
Institute of Applied Radiation Chemistry,
Laboratory of Laser Molecular Spectroscopy, Lodz, Poland

Welcome to ECSBM 2024

It is our pleasure and pride to host the 20th European Conference on the Spectroscopy of Biological Molecules (20th ECSBM) in Lodz, Poland. The conference is organized by Lodz University of Technology, The Faculty of Chemistry on September 1-5, 2024.

The conference is dedicated to advances in biological, medical, biomedical and related areas of molecular spectroscopy, its applications, statistical and chemometric analysis methods and is an excellent opportunity to discuss and exchange cutting-edge knowledge and ideas in molecular spectroscopy in a broad sense. We have organized 8 oral sessions. During each session plenary and invited talks will be presented. Additionally, a poster session is scheduled. We do hope that you will find the scientific programme of 20th ECSBM interesting and that contact with spectroscopists from other countries will be a rewarding experience for all the participants and will contribute to stimulating academic development, establishing new cooperation and broadening horizons.

The Organizers are very grateful for the contributions of all participants, sponsors and exhibitors.

Welcome to Lodz!

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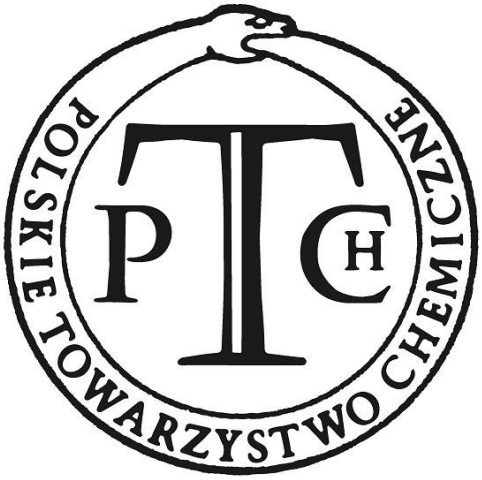

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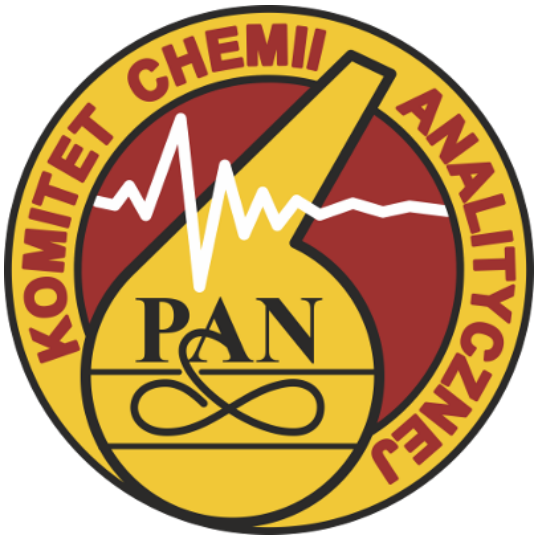

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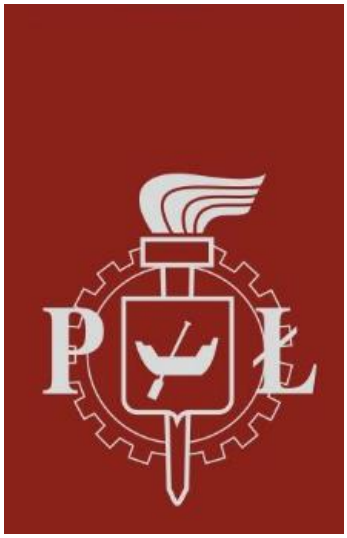

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About ECSBM

The European Conference on the Spectroscopy of Biological Molecules (ECSBM) was founded in 1985 and is held every two years in a different European country. The meetings are attended by researchers using traditional spectroscopic techniques (Raman, IR, UV-Vis, fluorescence, NMR, EPR), as well as those who work on imaging techniques applicable to the study of biomolecules, cells, or tissues. Recently, particular attention has been devoted to the applications of biomolecular spectroscopy in the fields of biomedical imaging, anticancer research, drug characterization for pharmaceutical applications, drug delivery and nano-biotechnology. Researchers not only from all countries of Europe attend the conference, but also from numerous countries from all over the world.

For its qualified attendance and for the broadness of scientific topics covered, ECSBM has become during the last years a leading conference located at the crossroad of three fundamental scientific fields: Physics, Chemistry and Biology.

The greatest specialists in the field participate in the conference, sharing their achievements, discoveries, and experience. Thanks to the cultivated tradition of organizing conferences and the involvement of the recognized research community, the conference quickly gained prestige and recognition in the world of science.

Previous Conferences

1985 - Reims, France

1987 - Freiburg, Germany

1989 - Rimini, Italy

1991 - York, United Kingdom

1993 - Loutraki, Greece

1995 - Lille, France

1997 - Madrid, Spain

1999 - Enschede, the Netherlands

2001 - Prague, Czech Republic

2003 - Szeged, Hungary

2005 - Aschaffenburg, Germany

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Sessions of Conference

(A) Molecular Spectroscopy

(B) Chemometric Advances

(C) Enhanced Techniques

(D) Biomedical Applications

(E) Isotopic Labelling

(F) Bioanalytical Applications

(G) Nanoscale Analysis

(H) Non-Linear Optics and Time-Resolved Spectroscopy

Venue

20th ECSBM will take place in the **Faculty of Chemistry Lodz University of Technology in Lodz (Poland)**, located at **the Campus of Lodz University of Technology in the 'Alchemium – Magic of Tomorrow' building (in the hall of Prof. Tadeusz Paryjczak) on Żeromskiego Street 114, Lodz, Poland.**

Welcome Cocktail

The Welcome Cocktail will take place in the foyer of the hall of Prof. Tadeusz Paryjczak in the Alchemium building on Żeromskiego Street 114, Lodz, Poland.

Conference Dinner

The Conference Dinner will take place in the Orientarium ZOO Lodz, Konstantynowska Street 8/10, Lodz, Poland.

Schedule of Conference

Day 1 – Sunday, September 1st, 2024	
15^{00}-19^{00}	Registration
17^{00}-19^{00}	Welcome Cocktail

Day 2 – Monday, September 2nd, 2024	
8^{00}-8^{45}	Registration
9^{00}-9^{15}	Welcome and Opening Ceremony
Bioanalytical Applications I (F) 9^{15}-10^{25} Chair of the session: M. Barańska	
9^{15}-9^{45}	Plenary Speaker: **J. Popp**, Friedrich Schiller University Jena, Germany, *"Raman spectroscopy for biomedical and life-science analysis"*
9^{45}-10^{05}	Invited Speaker: **K. Małek**, Jagiellonian University, Poland, *"Insights into intercellular interactions by FTIR and Raman spectroscopy imaging"*
10^{05}-10^{25}	Invited Speaker: **A. Sroka-Bartnicka**, Medical Univeristy of Lublin, Poland, *"Multimodal spectral imaging FTIR, Raman vs DESI and MALDI mass spectrometry imaging in diabetic lipid changes of rat kidney"*
10^{25}-10^{30}	Group photo
10^{30}-11^{10}	Coffee break
Sponsor session I 11^{10}-11^{40} Chair of the session: K. Beton-Mysur	
11^{10}-11^{25}	Speaker: **S. Paul**, Attocube Systems AG, *"In-situ infrared nanoscopy - investigating dynamic soft matter systems on the nanoscale in their native liquid environment"*
11^{25}-11^{40}	Speaker: **I. Iermak**, Oxford Instruments WITec, *"New Perspectives in 3D Raman Imaging for Life Sciences"*
Bioanalytical Applications II (F) 11^{40}-13^{10} Chair of the session: M. Marques	
11^{40}-11^{55}	Speaker: **M. Kashif**, FOCAS Research Institute, Technological University, Ireland, *"Pesticide Residual Analysis of Lambda cyhalothrin by Surface Enhanced Raman Spectroscopy, Comparison of Silver Nanodendrites and Silver Nanosphere Substrates"*
11^{55}-12^{10}	Speaker: **K. Chrabąszcz**, Institute of Nuclear Physics PAS, Poland, *"Obtaining radiation effects on biomolecules-FT-IR and AFM-IR cellular investigations"*

12^{10}-12^{25}	Speaker: **E. Kočišová**, Charles University, Czech Republic, *"Detection and segregation of biological molecules by droplet deposition method in Raman spectroscopy"*
12^{25}-12^{40}	Speaker: **P. Hańczyc**, University of Warsaw, Poland, *"Laser spectroscopy in studies on biomolecules linked with genetic and neurodegenerative diseases"*
12^{40}-12^{55}	Speaker: **N. Patil**, Technological University Dublin, Ireland, *"Targeted kinetic Raman microspectroscopy of extracellular medium to elucidate glycolysis pathway kinetics"*
12^{55}-13^{10}	Speaker: **A. Pragnąca**, Jagiellonian University, Poland, *"An effect of mercury toxicity and oxygen depletion on the main cellular components of the blood-brain barrier. FTIR and Raman microscopy study"*
13^{10}-13^{15}	Information about poster session and city tour
13^{15}-14^{30}	Lunch
Nanoscale Analysis (G) 14^{30}-16^{40} Chair of the session: A. Sroka-Bartnicka	
14^{30}-15^{00}	Plenary Speaker: **S. Ruggeri**, Wageningen University, The Netherlands, *"Nano-chemical Imaging and Spectroscopy of Biomolecular Processes"*
15^{00}-15^{20}	Invited Speaker: **M. Kamp**, Utrecht University, The Netherlands, *"Raman micro-spectroscopy reveals the spatial distribution of fumarate in cells and tissues"*
15^{20}-15^{40}	Invited Speaker: **K. Koynov**, Max Planck Institute for Polymer Research, Germany, *"Fluorescence correlation spectroscopy: monitoring the mobility of (bio)molecules and nanoparticles at the nanoscale"*
15^{40}-15^{55}	Speaker: **M. Dulski**, University of Silesia, Poland, *"Empowering Environmental Sustainability Functionalized SBA-15 as a Cutting-Edge Solution for Effective Metal Uptake"*
15^{55}-16^{10}	Speaker: **B. Torre**, INRiM, Italy, *"Adiabatic Nanofocusing in Raman spectroscopy and Hot Electron Nanoscopy (HENs): principles and applications"*
16^{10}-16^{25}	Speaker: **N. Piergies**, Institute of Nuclear Physics PAS, Poland, *"AFM-SEIRA nanospectroscopy for the molecular orientation studies"*

16^{25}-16^{40}	Speaker: **R. Stiufiuc**, TRANSCEND Research Center, Romania, *"Chiral separations of pharmaceutical compound by means of SERS: elucidating the role of molecular nanoscale interactions in this process"*
16^{40}-17^{15}	Coffee break
16^{40}-18^{00}	Poster session

Day 3 – Tuesday, September 3rd, 2024	
8^{00}-11^{30}	CITY TOUR
11^{30}-11^{50}	Coffee break
Biomedical Applications I (D) 11^{50}-13^{30} Chair of the session: K. Małek	
11^{50}-12^{20}	Plenary Speaker: **K. Majzner**, Jagiellonian University, Poland, *"From leukemia treatment to endothelial health: Raman spectroscopy in evaluating drug cytotoxicity and chemoresistance"*
12^{20}-12^{40}	Invited Speaker: **M. Marques**, University of Coimbra, Portugal, *"New Routes for Cancer Treatment Tackled by Neutrons"*
12^{40}-13^{00}	Invited Speaker: **M. Marini**, INRiM, Italy, *"From DNA-protein repair mechanism to epigenetics: studies by Raman spectroscopy"*
13^{00}-13^{15}	Speaker: **K. Dziedzic-Kocurek**, Jagiellonian University, Poland, *"Revealing the features of the single-cell nucleated avian and non-nucleated mammalian erythrocytes with the use of O-PTIR technique"*
13^{15}-13^{30}	Speaker: **J. Depciuch**, Medical Univeristy of Lublin, Poland, *"Common FTIR marker of prostate cancer in urine and tissues correlated with PSA level"*
Sponsor session II 13^{30}-14^{05} Chair of the session: J. Surmacki	
13^{30}-13^{50}	Speaker: **A. Sozańska**, Renishaw
13^{50}-14^{05}	PIK Instruments
14^{05}-15^{15}	Lunch
Biomedical Applications II (D) 15^{15}-16^{55} Chair of the session: M. Marini	
15^{15}-15^{35}	Invited Speaker: **P. Gardner**, University of Manchester, The United Kingdom, *"Separating pussycats and tigers: Risk stratification of prostate cancer using infrared imaging"*

15^{35}-15^{55}	Invited Speaker: **H. Sato**, Kwansei Gakuin University, Japan, *"Fat analysis in live cell and tissue with Raman spectroscopy"*
15^{55}-16^{10}	Speaker: **A. Salman**, SCE-Sami Shamoon College of Engineering, Israel, *"Rapid Identification of Viral and Bacterial Infections, and Assessment of Antibiotic Efficacy in Bacteremia Patients through a Simple Peripheral Blood Analysis: Expert System Employing Infrared Microscopy of White Blood"*
16^{10}-16^{25}	Speaker: **O. Piot**, University of Reims Champagne-Ardenne, France, *"Processing of Raman spectra by trajectory inference: applications to the study of adipocyte differentiation and to the characterization of cancer-associated adipocytes"*
16^{25}-16^{40}	Speaker: **K. Pogoda**, Institute of Nuclear Physics PAS, Poland, *"Revealing nanoscale properties of plasma membrane vesicles using the combination of spectroscopic modalities"*
16^{40}-16^{55}	Speaker: **H. El Tahech**, Université de Reims Champagne-Ardenne, France, *"Supervised learning of infrared spectral images for the differential diagnosis of subtypes of breast cancer"*
Isotopic Labelling (E) 16^{55} – 18^{00} Chair of the session: O. Piot	
16^{55}-17^{25}	Plenary Speaker: **P. Paneth**, Lodz University of Technology, Poland, *"Applications of isotope-ratio mass spectrometry"*
17^{25}-17^{45}	Invited Speaker: **A. Barth**, Stockholm University, Sweden, *"Isotope-edited Fourier-transform and nanoscale infrared spectroscopy for studying amyloid-β aggregation"*
17^{45}-18^{00}	Speaker: **W. Korona**, Jagiellonian University, Poland, *"Library of Spectral Reporters of Metabolic Pathways: Metabolomics based on Multiplex Cell Labeling"*

Day 4 – Wednesday, September 4th, 2024	
Molecular Spectroscopy (A) 9^{00}-11^{45} Chair of the session: P. Gardner	
09^{00}-09^{30}	Plenary Speaker: **K. Gerwert**, Ruhr University Bochum, Germany, *"Misfolding of Biomarkers Indicates Alzheimer's and Parkinson's Disease in Early Stages by the Immuno-Infrared Sensor (iRS)"*
09^{30}-09^{50}	Invited Speaker: **N. Hunt**, University of York, The United Kingdom, *"Advances in 2D-IR spectroscopy: From protein structural libraries towards clinical diagnostics"*
09^{50}-10^{10}	Invited Speaker: **M. Wolszczak**, Lodz University of Technology, Poland, *"Influence of ionizing radiation on human serum albumin solutions"*
10^{10}-10^{30}	Invited Speaker: **T. Andruniów**, Wrocław Univ. of Science and Technology, Poland, *"Tuning two-photon absorption properties in fluorescent proteins"*
10^{30}-10^{45}	Speaker: **Z. Mirveis**, Technological University Dublin, Ireland, *"Off-Line monitoring of extracellular media by ATR-FTIR Spectroscopy to explore metabolic pathway kinetics"*
10^{45}-11^{00}	Speaker: **B. Gieroba**, Medical Univeristy of Lublin, Poland, *"Spectral assessment of chitosan curdlan matrices with drugs in the context of potential biomedical applications"*
11^{00}-11^{15}	Speaker: **P. Meyvish**, Ghent University, Belgium, *"Monitoring the transformation of resistant biomolecules to geomolecules: a micropaleontological perspective and applications"*
11^{15}-11^{30}	Speaker: **S. Zahra**, Jagiellonian University, Poland, *"A Novel Approach to Assess Chemical Composition and Ultraviolet Light-Stress Effects on Japanese Knotweed: A Vibrational Spectroscopy Study"*
11^{30}-11^{45}	Speaker: **J. Rode**, Institute of Nuclear Chemistry and Technology, Poland, *"Influence of conformational freedom, substituents, and solvents on chiroptical spectra"*
11^{45}-12^{15}	Coffee break

Chemometric Advances (B) 12^{15}-14^{10} Chair of the session: J. Kneipp	
12^{15}-12^{45}	Plenary Speaker: **T. Bocklitz**, University of Jena, Germany, *"Advances in chemometrics by incorporating deep learning techniques and data science methods in the data analysis workflow of bio-spectral data"*
12^{45}-13^{05}	Invited Speaker: **M. Keating**, Technological University Dublin, Ireland, "Seeding multivariate algorithms, a data augmentation approach which can enhance analytical performance"
13^{05}-13^{25}	Invited Speaker: **K. Cieślik - Boczula**, University of Wrocław, Poland, *"Supported by chemometric analysis FTIR studies of the structure of protein-lipid membrane systems"*
13^{25}-13^{40}	Speaker: **S. Mostafapour**, Friedrich-Schiller-Universität Jena, Germany, *"Siamese Neural Networks for Clinically Relevant Bacteria Classification based on Raman spectroscopy"*
13^{40}-13^{55}	Speaker: **A. Adamczyk**, Jagiellonian University, Poland, *"Assessment of erythroid differentiation and heme biosynthesis in red blood cells by spontaneous coherent Raman microscopy"*
13^{55}-14^{10}	Speaker: **S. Kane**, Université de Reims Champagne-Ardenne, France, *"Usefulness of Data Simulation for Training Deep Learning Denoising Algorithms in Infrared Spectral Histology"*
14^{10}-15^{25}	Lunch
Biomedical Applications III (D) 15^{25}-16^{55} Chairs of the session: A. Salman and K. Majzner	
15^{25}-15^{40}	Speaker: **K. Stawoski**, Jagiellonian University, Poland, *"Single-cell Raman studies of the effect of ruxolitinib on JAK-mutated B-cell acute lymphoblastic leukemia cell lines"*
15^{40}-15^{55}	Speaker: **B. Zupančič**, National Institute of Chemistry, Slovenia, *"Detection of diabetes-related macromolecular changes in human tissues using vibrational spectroscopy"*
15^{55}-16^{10}	Speaker: **K. Siąkała**, Jagiellonian University, Poland, "*Raman probes-a spectroscopic approach to study cell metabolism via organelle-specific compounds active in the 'silent region'*"

16^{10}-16^{25}	Speaker: **K. Jarczewska**, Lodz University of Technology, Poland, *"The impact of xanthophylls on brain and breast cancer development"*
16^{25}-16^{40}	Speaker: **K. Beton-Mysur**, Lodz University of Technology, Poland, "*Studies on the Effect of Statin Supplementation on Human Colon Cells Using Raman Imaging and AFM"*
16^{40}-16^{55}	Speaker: **P. Dawiec**, Jagiellonian University, Poland, *"Spectroscopic profiling of therapeutic responses of B-cell acute lymphoblastic leukaemia to tyrosine kinase inhibitors"*
19^{30}	Meeting on the TUL campus and departure to Orientarium ZOO Lodz
20^{00}-23^{00}	CONFERENCE DINNER
23^{00}	Return by bus to the TUL campus

Day 5 – Thursday, September 5th, 2024	
Enhanced Techniques (C) 9^{00}-11^{00} Chair of the session: S. Quinn	
09^{00}-09^{30}	Plenary Speaker: **J. Kneipp**, Humboldt-Universität zu Berlin, Germany, *"Vibrational spectra in enhanced local fields: Possibilities for probing biomolecules and cells"*
09^{30}-09^{50}	Invited Speaker: **A. Kamińska-Michota**, Institute of Physical Chemistry PAS, Poland, *"SERS-based method for detecting and identifying pathogenic microorganisms - bacteria and viruses"*
09^{50}-10^{10}	Invited Speaker: **S. Aştilean**, Babes-Bolyai University, Cluj-Napoca, Romania, *"Plasmonic-Based Nanoplatforms for Enhanced Biosensing and Bioimaging"*
10^{10}-10^{30}	Invited Speaker: **S. Kogikoski Jr.**, University of Potsdam, Germany, *"Recent Advancements in DNA Origami Plasmonic Nanoantennas for Advanced Single-Molecule Surface Enhanced Raman Spectroscopy"*
10^{30}-10^{45}	Speaker: **T. Wróbel**, Jagiellonian University, Poland, *"Imaging of Three-dimentional Molecular Orientation Vibrational Microspectroscopies"*
10^{45}-11^{00}	Speaker: **M. Prochazka**, Charles University, Czech Republic, *"Surface-enhanced Raman spectroscopy on non-plasmonic vanadium pentoxide and vanadium pentoxide/an nanostructures"*

11^{00}-11^{30}	Coffee break
Non-Linear Optics and Time-Resolved Spectroscopy (H) 11^{30}-13^{20} Chair of the session: M. Wolszczak	
11^{30}-12^{00}	Plenary Speaker: **S. Quinn,** University College Dublin, Ireland, *"Good Vibrations for DNA Reporting: Exploiting the Excited States of Ruthenium Polypyridyl infrared Probes"*
12^{00}-12^{20}	Invited Speaker: **G. Angulo Nunez**, Institute of Physical Chemistry, PAS, Poland, *"Photoinduced reactions in complex condensate media"*
12^{20}-12^{35}	Speaker: **M. Pastorczak**, Institute of Physical Chemistry, PAS, Poland, *"Stimulated Raman techniques applied to biological systems"*
12^{35}-12^{50}	Speaker: **A. Jarota**, Lodz University of Technology, Poland, *"On the Relaxation Dynamics of Diarylethene Photochromic Switches"*
12^{50}-13^{05}	Speaker: **J. Firlej**, Jagiellonian University, Poland, *"Spontaneous and coherent Raman scattering integration for optical biosensing improvement"*
13^{05}-13^{20}	Speaker: **O. Krysheva**, Stockholm University, Sweden, *"Unravelling the structure of Aβ42 oligomers in membrane-mimetic environments"*
13^{45}-14^{00}	Student prizes and closing ceremony
14^{00}-15^{00}	Lunch

The Organizing Committee is grateful to all who kindly sponsored 20th European Conference on Spectroscopy of Biological Molecules

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Plenary Lectures

Plenary 1 - A - Molecular Spectroscopy

Misfolding of biomarkers indicates Alzheimer's and Parkinson's disease in early stages by the immuno-infrared sensor (iRS)

K. Gerwert[1*]

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We conducted discovery and validation studies to determine the potential of protein misfolding as biomarker for Alzheimer´s (AD) and Parkinson´s disease (PD). For this purpose, we developed a new platform technology, the so-called immuno-infrared sensor (iRS). The iRS technology relies on the extraction of protein biomarkers out of complex fluids, such as CSF and Plasma by antibodies [1]. It is crucial that all possible conformational forms of the target analytes are detected. For this purpose, we developed a highly inert, antibody-coated functionalized ATR surface. The frequency downshift of the secondary structure sensitive Amide I band as read out indicates the disease.

For the Alzheimer studies, we investigated the misfolding of amyloid-beta (Aβ) in different stages of this continuum disease ranging from symptom-free, subjective cognitive declined and mild cognitive impaired, up to clinical AD. Plasma Aβ misfolding predicts the risk of AD diagnosis up to 14 years in advance in a symptom-free stage with an AUC of 0.85 [2]. In a further cohort, consisting of patients with subjective cognitive decline, the risk could be predicted up to six years in advance and was further improved by combination with the Aβ42/Aβ40 ratio [3] to an AUC of 0.99. In a recent study, misfolding of Aβ indicated the risk of AD up to 17 years before clinical onset in plasma [4] in symptom-free stages with an AUC of 0.82.

For Parkinson´s disease we analyzed 134 CSF samples from patients with idiopathic and atypical parkinsonian disorders as well as controls from five different medical centers. Alpha-synuclein misfolding as biomarker was determined by the immuno-infrared-sensor (iRS) [1,5].
In the discovery study, we achieved an AUC of 0.92 for the discrimination of PD and non-neurodegenerative controls. In a validation study, we performed a single-center study with another cohort. Here we achieved an AUC of 0.88 for the discrimination of alpha-synuclein misfolding positive versus negative cases. The discriminative power could be further increased up to 0.94 by subdividing individuals into groups with high (diseased), intermediate, and low (non-diseased) misfolding, like a traffic light scheme.

For the first time, we present a CSF biomarker which can directly distinguish synucleinopathies like PD and MSA from atypical syndromes and other controls. This allows a precise stratification of early-stage-individuals for targeted therapeutic interventions, such as L-Dopa treatment.

This work on fluid biomarkers is complemented by infrared microspectroscopy of brain tissue. In the field of neurodegenerative diseases, we have examined the brains of Alzheimer's and Parkinson´s patients [6,7].

In addition to neurodegeneration, we also use IR-Imaging for the diagnosis of cancer. In a study with 547 colorectal cancer patients, we have shown that the microsatellite instability of tumors can be determined on unstained tissue thin sections with an AUC of 0.9 [8]. This automated and label-free differential cancer diagnosis is made possible by combining IR microspectroscopy with artificial intelligence (deep learning).

[1] A. Nabers, et al., J Biophotonics 2016;9(3):224–34.
[2] A. Nabers, et al., EMBO Mol Med 2018;10(5).
[3] J. Stockmann, et al., Alzheimer's research & therapy 2020;12(1):1–13.
[4] L. Beyer, et al., Alzheimers Dement. 2022 Jul 19. doi: 10.1002/alz.12745.
[5] A. Nabers, et al., Anal Chem 2016;88(5):2755–62.
[6] D. Röhr, et al., Acta neuropathologica communications 2020; 8: 222.
[7] S.H. Shahmoradian, et al., Nat Neurosci. 2019 Jul;22(7):1099-1109. doi: 10.1038/s41593-019-0423-2.
[8] K. Gerwert, S. Schörner et al., Eur J Cancer, 2023, 182, 122-131

Plenary 2 - B - Chemometric Advances

Advances in chemometrics by incorporating deep learning techniques and data science methods in the data analysis workflow of bio-spectral data

***T. Bocklitz*[1,2*]**

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Photonic measurement techniques, including vibrational spectroscopic techniques such as Raman-spectroscopy, IR-spectroscopy, and their variants, are increasingly used in scientific fields, such as life science, medicine and environmental science. This increased usage is linked to the method's inherent advantages like non-destructive measurements, but it is also triggered by the improvements of both measurement methods and setups, as well as the development of data science methods and data infrastructures. The latter two sets of methods allow the extraction of information and knowledge from the measured photonic data. Thereby, the high-level information depends on the task and the sample, e.g. predicting properties of the sample like tissue types, disease states, or concentrations of constituents.
To link photonic data such as Raman spectra, data of nonlinear Raman variants or IR spectra to high-level information a sequence of computational steps including experimental design, sample size planning, data pre-treatment, data pre-processing, chemometric and machine learning-based data modeling, and model transfer methods are required. These steps are typically combined in a data pipeline to photonic data and to extract reliable high-level information.
This presentatuon provides an overview of advances in chemometrics by incorporating deep learning techniques and data science methods. As a starting point, we will present our studies aiming at a standardized data analysis pipeline for biomedical Raman spectra and multi-contrast image data [1]. We will highlight the pitfalls, challenges, and opportunities in constructing this data pipeline and show how transfer learning or model transfer can be implemented to enable more generalizable models in the small sample size scenario [2,3]. Furthermore, we will present methods for inverse modeling of measurement processes and data improvement that allow for standardized data [4, 5]. Finally, follow-up studies of a European interlaboratory comparison [6] of Raman spectra will be presented, aiming at the comparability of Raman spectra between instruments and laboratories.

Acknowledgements
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Plenary 3 - C - Enhanced Techniques

Vibrational spectra in enhanced local fields: Possibilities for probing biomolecules and cells

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Enhanced local optical fields provide unique opportunities for molecular spectroscopy, for the manipulation of chemical reactions, and for the characterization of complex biological systems. Vibrational spectra benefit greatly from an enhancement by high local fields, either through an enhancement of excitation and emitted light in a Raman process or an improved absorption of infrared radiation.
Surface-enhanced Raman scattering (SERS) is probably the most-used enhanced vibrational approach, as it can result in very high enhancement levels. This talk will highlight the implications and possibilities offered by the highly localized and selective probing that are hallmarks of SERS in the context of biospectroscopy.[1] Specifically, it will discuss SERS data from proteins, lipids, and nucleic acids that are found in cells, and the observation of spectra from these compounds in their native environment.
The enhancement by local optical fields is even stronger when more than one field is involved in the vibronic excitation. This is the case in the spontaneous two-photon excited process of hyper Raman scattering (HRS). Surface-enhanced HRS (SEHRS) gives information complementary to SERS, and can also be combined with other multi-photon microscopic and spectroscopic tools.[2] In this way, molecular and morphofunctional information can be combined. As an example, combined applications in probing of cells and biomolecules using SERS, SEHRS, and second-harmonic generation (SHG) will be shown. The talk will also discuss the possibilities and limitations arising from the high confinement of the enhanced local fields for probing of biomolecules. As it enables the collection of spectral signals from nanoscale volumes, probing of small structures, especially in biological samples is feasible, in experiments with SERS, SEHRS, and nano-infrared spectra collected in scanning-probe near field microscopes.

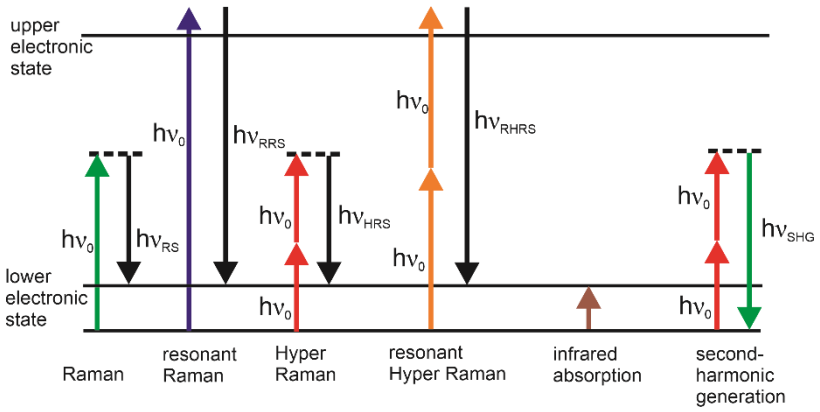

Figure 1. *Different processes that can be used for vibrational probing or highlighting of biomolecules and that benefit from enhanced optical fields*

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Plenary 4 - D - Biomedical Applications

From leukemia treatment to endothelial health: Raman spectroscopy in evaluating drug cytotoxicity and chemoresistance

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Leukemia, a blood cancer originating from genetic abnormalities in progenitor cells, requires subtype-specific treatment guided by predictive markers. Accurate treatment is essential for patient recovery. However, the formidable challenge in leukemia therapy is chemoresistance, which can lead to treatment failure and disease relapse. Chemoresistance in leukemia therapy is a significant challenge, but identifying specific vulnerabilities in leukemia cells allows targeted treatments to be developed.

Although targeted therapies are precise against cancer cells, they can also affect normal cells, including endothelial cells (ECs), which are crucial for the vascular system's health. Endothelial dysfunction during leukemia treatment can lead to cardiovascular complications such as hypertension, thrombosis, and atherosclerosis. Some chemotherapeutic agents, including anthracyclines, inhibitors of mitosis, and tyrosine kinase inhibitors (TKI), have been associated with disruption of endothelial function. Therefore, comprehensive studies are necessary to assess the broader effects of chemotherapy and develop strategies that minimize adverse vascular effects.

Integrating Raman imaging into cell-drug studies enables the simultaneous detection of cellular components and their spatial distribution. The combination of chemical specificity and the ability to monitor changes in cellular constituents makes RS a valuable technique in cell biology and pharmacology. RS enables metabolic profiling, tracking changes, and identifying drug response/resistance signatures, which is challenging with other methods.

We used the WITec Alpha 300 Raman system to image leukemia and normal endothelial cell lines. Chemometric methods were then used to identify the molecular fingerprint of changes induced by chemotherapeutic drugs in the cells studied. In our study, we evaluated the impact of various drugs, including doxorubicin (DOX), TKIs (imatinib, dasatinib), and vincristine (VCR), on the biochemical profile of sensitive and resistant leukemia cells and ECs.

Our study revealed that all investigated drugs decreased the nucleic acid content in the ECs. Additionally, an increase in the lipid content was found to be related to the development of inflammation in the ECs. The results also showed changes in the cytochrome signal for DOX—and TKI-treated ECs, indicating mitochondrial damage. In the case of VCR, the protein content in the cytoplasmic region increased, which may reflect the mobilization of cells to form the karyokinetic spindle and the arrest of mitosis. Nucleic acid content and lipid profile changes were observed for TKI-sensitive cells (BV-173 cell line).

Ongoing research and advancements in understanding the molecular mechanisms of drug resistance will continue to shape the development of targeted treatment strategies, making them more precise and effective while minimizing adverse effects on normal cells. Insight into in vitro endothelial dysfunction caused by leukemia drugs is pivotal for shaping safer and more effective therapeutic strategies in clinical settings.

Acknowledgments
This work was supported by the „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project, which is carried out within the Team-Net program of the Foundation for Polish Science, co-financed by the EU (POIR.04.04.00-00-16ED/18-00), and by a grant from the National Science Centre Poland (UMO-2016/21/D/ST4/00870 to KM).

Plenary 5 - E - Isotopic Labelling

Applications of isotope-ratio mass spectrometry

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I'm going to start the presentation with a brief discussion of isotope effects and the main sources of the resulting isotopic fractionation. This will be followed by presenting basic technical differences between "routine" and isotope-ratio mass spectrometry and the methodology of sample preparations. Finally, some examples of basic studies of isotope effects on enzymatic systems, as well as our recent involvement in studies of isotopic fractionation in cancerous cells will be presented.

Plenary 6 - F - Bioanalytical Applications

Raman spectroscopy for biomedical and life-science analysis

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In addition to fluorescence microscopy, both linear and non-linear Raman technologies have made significant strides in life sciences and biomedical research. These methods increasingly complement established imaging techniques such as fluorescence microscopy. Raman spectroscopy and microscopy offer insights into the morphochemistry of living organisms, providing access to the molecular origins of functional and structural changes. Notably, Raman spectroscopy requires minimal sample preparation, allowing for the use of samples in their natural state. Moreover, unlike fluorescence microscopy, Raman microscopy does not necessitate external labeling.

While Raman spectroscopy boasts unparalleled specificity and versatility, its sensitivity is limited, hindering the detection of molecules present in very low concentrations. However, this drawback can be addressed through specialized techniques such as resonance Raman spectroscopy, surface-enhanced Raman scattering (SERS), or non-linear phenomena like coherent anti-Stokes Raman scattering (CARS) or stimulated Raman scattering (SRS).

In this discussion, we underscore the significant potential of Raman methodologies in biological and biomedical analysis. We demonstrate that Raman spectroscopy enables non-invasive, label-free morphochemical characterization across a wide range of biological samples, including prokaryotic and eukaryotic cells, fungi, biofilms, tissue sections, and whole organs. The presented examples focus on three main applications:

I. Microbial analysis, particularly the rapid detection of pathogens, assessment of antibiotic resistance, and understanding host responses.
II. Intraoperative tumor characterization, utilizing spectral histopathology to aid in tumor identification.
III. Visualization of metabolic, defense, or chemical communication processes in cells and plant tissues through Raman spectroscopy (also in combination with a triple bond tag concept).

Acknowledgements
Financial support of the EU, the "Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft", the "Thüringer Aufbaubank", the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl-Zeiss Foundation are greatly acknowledged.

Plenary 7 - G - Nanoscale Analysis

Nano-chemical Imaging and Spectroscopy of Biomolecular Processes

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Biological processes rely on a wide class of biomolecular and macromolecular machines that have nanoscale physical dimensions and whose function emerges from a correlation between their chemical and structural properties. A fundamental objective of modern analytical methods is the comprehension of how physical-chemical properties and heterogeneity of single biomolecules underlie their role in cellular function and disease. While innovative nanoscale imaging methods have been developed to characterise biomolecules, imaging microscopies are to the most part chemically blind; thus, hampering the characterisation of inhomogeneous and complex systems.
The introduction of photothermal infrared nanospectroscopy (AFM-IR) has revolutionized the field of nano-chemical analysis in a wide-open range of fields, including biological, material and polymer sciences. Here, we will present an overview of our latest development and application of AFM-IR in combination with advanced spectroscopic analysis and chemometrics, as a real breakthrough for the analysis of heterogeneous (bio-)molecular systems and materials down to the single molecule level.
To illustrate our path towards single-molecule AFM-IR, we first show the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power, and short pulse ORS-nanoIR. [1] This approach enables the accurate determination of the secondary structure elements of single proteins and amyloids in the amide band I region. We will then showcase the application of this unprecedented single molecule sensitivity to: i) unravel molecular structure and interactions of protein and organic molecules [2]; ii) origin of chirality in click chemistry polymers [3]; iii) detect nano-plastics in drinking water [4]. Finally, we illustrate the application of this sensitivity to probe the surface and structural properties of functional materials, such as artificial model membranes [5], functional protein self-assemblies, and perovskites-amino acids hybrid materials [7].
Overall, our aim is to expand the capabilities of analytical nanoscience to shed light on the structure-activity relationship of biomolecules and functional materials design.

Keywords
Nano-Imaging; Spectroscopy; Single-Molecule; Atomic Force Microscopy; Supramolecular Assembly.

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Plenary 8 - H - Non-Linear Optics and Time-Resolved Spectroscopy

Good Vibrations for DNA Reporting: Exploiting the Excited States of Ruthenium Polypyridyl Infrared Probes

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Photoinduced processes triggered by DNA bound metal polypyridyl complexes can be exploited for applications in photodynamic therapy and DNA imaging and diagnostics. We have extensively studied the excited state dynamics of these processes for intercalating ruthenium dppz (dipyridophenazine) polypyridyl complexes bound to DNA.[1-3] While the phenanthroline (phen) light-switch complexes can signal the presence of DNA, the tetraazaphenanthrene (TAP) complexes can cause photodamage by participate in direct one-electron photo-oxidation of guanine, which is sensitive to the local DNA environment and the binding orientation. In addition, the use of osmium can extend the optical window into the NIR.[4,5] In this talk I will share some recent results on DNA binding polypyridyl systems and show time-resolved methods, including time-resolved infrared (TRIR) complemented by structural and computational studies, can be used to identify the binding site of photoactive metal complexes in solution, and to monitor sensitized DNA photo-oxidation. In particular, I will highlight the use of metal complexes modified with infrared nitrile probes as powerful probes of excited state processes in DNA systems. I will describe how the nitrile containing Ru(II)polypyridyl complex $[Ru(phen)_2(11,12\text{-dCN-dppz})]^{2+}$ (**1**) acts as a sensitive infrared probe of its hydrogen bonding solution environment, see Figure 1, and how this can be applied to discriminate binding to different G-quadruplex (G4) structures.[6] Finally, I will share some recent results to show how such probes allow sensitive tracking of photoredox processes.

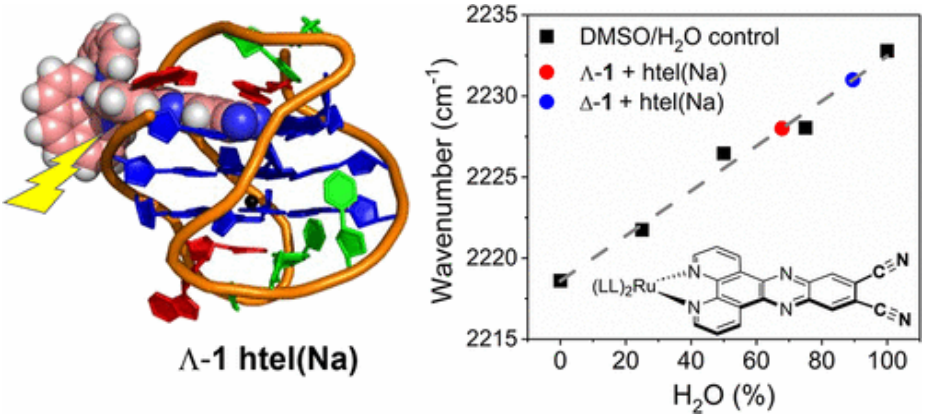

Figure 1. *Left: Molecular model of binding interaction of* ***1*** *to sodium stabilized quadruplex DNA. Right: Correlation of the position of nitrile transient for Λ-1 and Δ-1 bound to G4 structure to the Hydrogen bonding nature of the solution environment.*

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Abstracts
Invited & Oral

I.1 - A - Molecular Spectroscopy

Tuning two-photon absorption properties in fluorescent proteins

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Since the early 1990s Fluorescent proteins (FPs) have been widely used in the study of complex biological systems at the molecular level. However, there is a growing interest in designing biomarkers with new spectral features, especially improved two-photon absorption properties.

In this contribution we investigate one- and two-photon absorption (1PA and 2PA) spectra for green and yellow FPs differing in amino acid sequence using quantum mechanical (QM) and mixed quantum mechanics/classical mechanics (QM/MM and QM/MM MD) approaches. In particular, we aim to reveal the role of specific electrostatic interactions and hydrogen-bonds between the chromophore and its protein environment in 2PA enhancement.

Our results highlight key factors that should be considered when rationally designing fluorescent proteins for two-photon applications. [1-6].

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I.2 - A - Molecular Spectroscopy

Advances in 2D-IR spectroscopy: From protein structural libraries towards clinical diagnostics

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Ultrafast 2D-IR spectroscopy has shown considerable promise for the label-free analysis of the protein amide I band, leading to 2D-spectral fingerprints of protein samples that report sensitively on secondary structure, ligand binding and molecular and solvation dynamics. [1] A particular strength of 2D-IR is its ability to overcome the need for H/D isotopic exchange of the solvent, which enhances its utility for measuring proteins under near-physiological conditions.[2]
Here, we report the combination of label-free amide I 2D-IR spectroscopy with rapid 2D-IR data acquisition enabled by high pulse repetition-rate laser systems to construct a 2D-IR protein spectral library of some 7000 2D-IR spectra from more than 35 proteins with secondary structures that span a wide range of motifs in H_2O-based solutions. Using this library as a database for the training and development of machine learning models provides the ability to determine the amide I spectral signatures of specific secondary structure elements in H_2O, leading to quantification of protein structure and dynamics in an unknown sample (Fig. 1).
In a second application of this 2D-IR protein library, the use of 2D-IR spectral fingerprints as a means to quantify protein concentrations within human blood serum samples is reported. Specifically, we show that the 2D-IR signature of a serum sample is sensitive to changes in concentration of a number of the major protein constituents. As protein compositional changes are known to respond sensitively to the health of the patient, this provides a basis for 2D-IR applications in biomedical diagnostics. To this end, we report progress towards using 2D-IR spectroscopy for the measurement and differentiation of protein amide I signatures of serum samples obtained from patients undergoing treatment for melanoma.

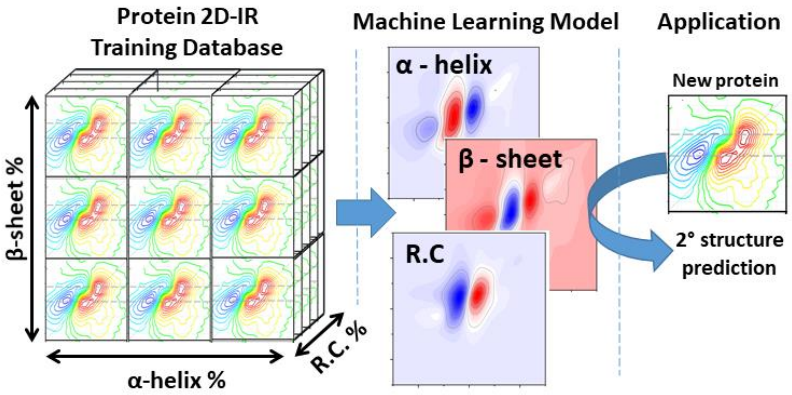

Figure 1. *Diagram showing use of protein 2D-IR library for amide I-based protein structural analysis in H_2O-rich solutions and biofluids.*

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I.3 - A - Molecular Spectroscopy

Influence of ionizing radiation on human serum albumin solutions

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The main goal of this presentation is to demonstrate the usefulness of the pulse radiolysis technique in the study of protein redox processes. The scavenging of hydrated electron (e_{aq}^-) by molecule of human serum albumin (HSA) in solution under neutral pH was studied [1]. The rate constant of reaction e_{aq}^- with HSA is diffusion controlled (1.1×10^{10} $dm^3\ mol^{-1}$). The transient absorption spectrum recorded during pulse radiolysis of HSA solution under reductive conditions shows maximum at 420 nm. The comparison of transient absorption spectra and the reactivity of e_{aq}^- with amino acids or HSA suggest that electron attachment to disulfide bond. As a result some the disulfide bonds are broken. Recombination of sulfur–centered radicals play a crucial role in generating of HSA nanoparticles, which are stabilized by intermolecular disulfide bonds. The process of creating disulfide bridges under the influence of ionizing radiation is a promising method for the synthesis of biocompatible protein nanostructures for medical applications. The conducted research showed that the effectiveness of HSA aggregation depends on two main factors: dose rate (number of electron pulses per unit time) and the temperature of the irradiated solution. The scavenger molecules (ligands) embedded in a hydrophobic domain at Sudlow's site 1 are very well protected against e_{aq}^- attack [1]. Unlike site 1, the entrance to site 2 is exposed to solvent and the ligands in this domain are readily reduced by e_{aq}^- . The use of spectroscopic techniques has shown that HSA undergoes reversible self-aggregation through protein–protein interactions [2]. The HSA oxidation process related to the transfer of one electron was investigated by pulse radiolysis and photochemical methods. It has been shown that the irradiation of HSA solutions under oxidative stress conditions results in the formation of stable protein aggregates different from self-aggregates. Dityrosine (DT) produced by the intermolecular recombination of protein tyrosine radicals as a result of radiolysis of an aqueous solution of the protein is the main cause of HSA aggregation by cross-linking. Analysis of the oxidation process of HSA confirmed that the reaction of mild oxidants ($Br_2^{\bullet -}$, $N_3^{\bullet -}$, $SO_4^{\bullet -}$) with albumin leads to the formation of covalent bonds between tyrosine residues. In the case of •OH radicals and partly, $Cl_2^{\bullet -}$, species other than DT are formed. In the analysis of the formation of protein aggregates, intramolecular electron transfer from the tyrosine moiety to Trp• radical should be taken into account [3].

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O.1 - A - Molecular Spectroscopy

Spectral assessment of chitosan and curdlan matrices with drugs in the context of potential biomedical applications

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Curdlan is a water-insoluble linear beta-1,3-glucan, a high-molecular-weight polymer of glucose produced by *Alcaligenes faecalis* var. *myxogenes* [1]. In turn, chitosan s a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) obtained by the partial or complete alkaline deacetylation of chitin [2]. They both have garnered much interest due to its properties and possible applications. These polysaccharides exhibit excellent bioavailability, biodegradability, and non-toxicity. Mentioned properties make them very useful in various types of applications in multiple fields like drug carrier, food packaging, dietary supplement, chelating agent, pharmaceutical and biomaterial purposes, etc [3]. This research aims to examine the physico-chemical properties of curdlan and chitosan matrices with different concentrations and molecular weight loaded with ibuprofen and gentamicin, respectively, demonstrating their role in advancing the field of localized, and accelerated or prolonged/sustained drug delivery systems. Molecular arrangement and physicochemical properties were determined before and after drug release from matrices with vibrational spectroscopic techniques - Fourier transform infrared FT-IR, and Raman spectroscopy. The development of applications based in vibrational spectroscopy enables the rapid and simple non-destructive measurement, without the need of special sample preparation [4]. It was proven that ibuprofen (Fig. 1.) and gentamycin was successfully incorporated into all studied polysaccharide matrices. Furthermore, it has been shown that both chitosan and curdlan have the likely application as an effective drug carrier, potentially improving their pharmacokinetic and pharmacodynamic parameters.

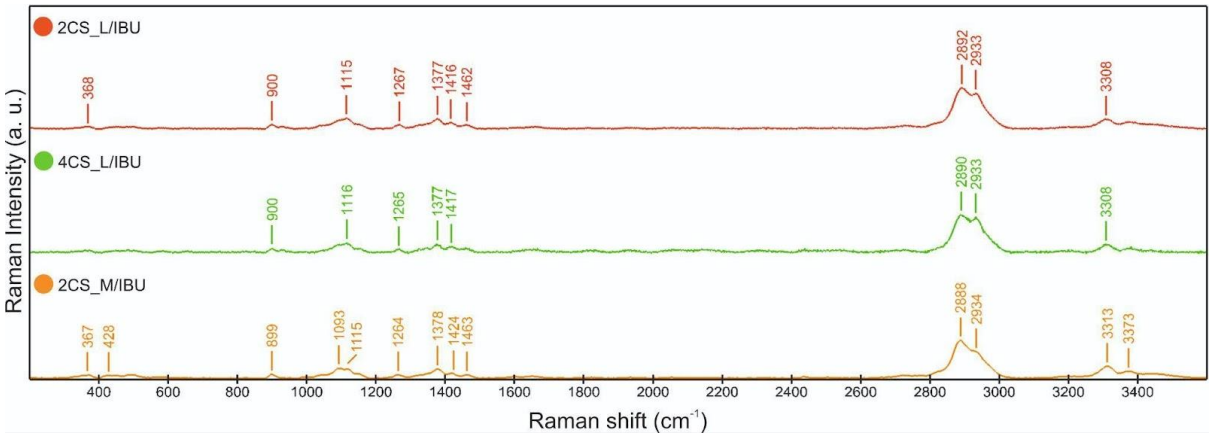

Figure 1. *Raman spectra of ibuprofen-enriched chitosan polymers.*

To conclude, spectroscopic methods are versatile tools in pharmaceutical and biopharmaceutical sciences, with a wide spectrum of applications ranging from characterization of drug formulations in both qualitative and quantitative analyses to investigation of interactions between drugs and (semi)synthetic, and native macromolecules [5].

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O.2 - A - Molecular Spectroscopy

Monitoring the transformation of resistant biomolecules to geomolecules: a micropaleontological perspective and applications

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Some aquatic microorganisms are able to synthesize exceptionally resistant, structural, organic biomacromolecules that can be preserved in sediments and sedimentary rocks, contributing to a rich microfossil record. With transported terrestrial organic matter included, common microfossil groups encompass dinoflagellate resting cysts, phycoma of green algae, and spores and pollen, which are composed of dinosporin, algaenan, and sporopollenin, respectively. When these distinct compounds become buried under layers of sediments, they gradually transform from biomolecules to geomolecules during diagenesis. More specifically, at elevated temperatures and pressures, the original molecular components such as proteins, lipids and sugars are transformed into stable heterocyclic polymers that can retain information on the original tissue composition, as well as phylogenetic relationships [1]. This realization led to the recent re-invigoration of the field of molecular paleobiology in which researchers integrate morphological and molecular analyses to infer the phylogenetic relationships (e.g., macroevolution), physiology and even the metabolism of extinct organisms [2], often by combining modern and fossil samples. To date, most of these efforts have been directed towards macroscopic organisms, often consumers in the food chain, leaving an obvious knowledge gap with regard to the molecular taphonomy of typically microscopic remains of primary producers. For the present study, a broad selection (>650 specimens) of largely primary producer-related microfossils composed of a variety of dinosporins, algaenans and sporopollenins were characterized using several spectroscopic methods (mainly infrared spectroscopy). This was done for a wide range of samples from different geological time intervals, some up to ~240 myr old. Furthermore, morpho-molecular changes occurring in young microfossils were monitored during a process of artificial thermal maturation, allowing for a direct comparison with the natural fossils.
Similar molecular transformations were observed in naturally and artificially matured microfossils, highlighting the utility of diagenesis simulations, and revealing that dinosporins, algaenans and sporopollenins can still be chemically distinguished, even after being rendered into heterocyclic polymers. This is especially promising in the scope of assigning biological affinities to organic-walled microfossils that are morphologically unclassifiable (i.e., 'acritarchs'). Some acritarchs predate confirmed dinoflagellate resting cysts, green algae, pollen or spores. Their compositional attribution to a known group could lead to new insights related to the early macroevolution of some major groups of primary producers that exist(ed) on Earth. Furthermore, dinosporin compositional variability in modern dinoflagellate cysts is likely linked to specific aquatic depositional environments [3]. Here, a similar variability was also observed in naturally and artificially matured cysts, which demonstrates that possible environmental signals can survive diagenesis and might hold future applications as paleoenvironmental proxies. Additionally, the results suggest that different dinosporin types evolved at different times and that they have specific phylogenetic occurrences in certain dinoflagellate families. Finally, a highly resistant natural biopolymer like sporopollenin finds use in biomedical applications [4], especially after elucidation of its molecular structure [5]. Here, a ^{13}C solid-state nuclear magnetic resonance spectrum is presented, collected—for the first time—from dinosporin occurring in a modern dinoflagellate cyst taxon, bringing us closer to a holistic characterization of another potentially useful inert biopolymer.

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O.3 - A - Molecular Spectroscopy

Off-Line monitoring of extracellular media by ATR-FTIR Spectroscopy to explore metabolic pathway kinetics

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This study investigates the application of FTIR spectroscopy, enhanced by Attenuated Total Reflection (ATR), for monitoring kinetics in metabolic pathways such as glycolysis and glutaminolysis. FTIR spectroscopy, which measures molecular composition by detecting infrared light absorption, offers a unique molecular fingerprint based on absorption frequencies of functional groups and bonds. This technique allows for the precise quantification of extracellular glucose, glutamine, and lactate, elucidating the effects of glutamine on glycolytic flux.

We used ATR-FTIR to analyze varying concentrations of these metabolites, observing a linear correlation between peak absorbances and concentrations, particularly for glutamine which showed a nearly perfect correlation (R^2 = 0.99967). Characteristic stretching vibrations in O-H and C-H, and C=O groups at higher concentrations were noted in the infrared spectra. Additionally, PLSR analysis across these metabolites confirmed high accuracy in quantifying concentrations, with the glutamine model capturing 99.9949% of variance with two latent variables (LVs), and similarly robust results for glucose and lactate. These findings demonstrate that ATR-FTIR spectroscopy, coupled with PLSR analysis, can effectively quantify and monitor key metabolites, offering insights into their dynamic interplays in disease states like cancer and potential therapeutic targets.

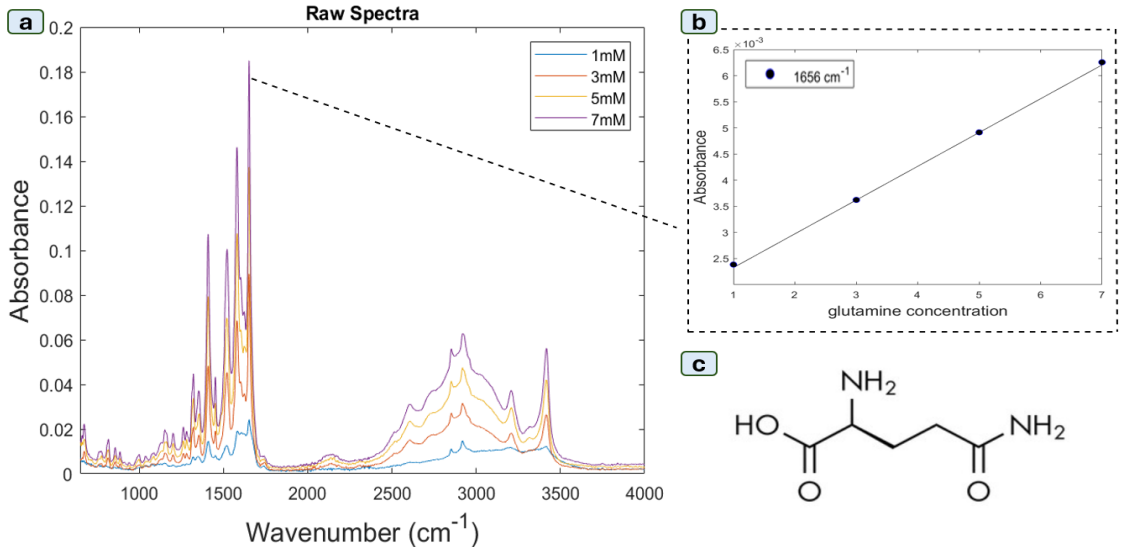

Figure 1: *Raw Infrared Spectrum for Varying Glutamine Concentrations in 650-4000 cm-1 Wavenumber Region (a), Absorbance vs. Concentration for Peak at 1656 cm-1. (b), molecular structure of glutamine (c).*

O.4 - A - Molecular Spectroscopy

Influence of conformational freedom, substituents, and solvents on chiroptical spectra

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Enantiomers and their interaction with other chiral entities may give rise to distinct physiological and/or toxicological responses. The use of thalidomide (TD), marketed in the 1950s, demonstrated the importance of chirality in biological action: while (*R*)-TD was an effective analgesic, the (*S*)-TD one had a teratogenic effect, causing over 10,000 birth defects. On the other hand, atenolol *β*-blocker drug to treat hypertension and cardiovascular disorders is manufactured in a racemic form, while only its (*S*)-enantiomer shows selective *β*1-blocking activity. We have recently designed voltammetric enantiosensors to determine of thalidomide [1] and atenolol [2] enantiomers in the blood plasma. The electroactive materials for both sensors were newly synthesized chiral naphthalene diimide (NDI) derivatives. NDI moiety (Fig. 1) is an important core for modern low molecular-weight organic semiconductors and other applications. In our INCT group, we characterize the chiral compounds using *i.a.* spectroscopy techniques. The standard optical methods (UV-vis, IR, and Raman) are insensitive to chirality. However, chiroptical methods such as electronic circular dichroism (ECD), vibrational circular dichroism (VCD), or Raman optical activity (ROA), exhibit different absorption/ scattering coefficients for the left- and right-circularly polarized light. As a result, the spectra of the enantiomers are mutual mirror images [3]. These methods are very sensitive to conformational flexibility and molecular surroundings that influence the band position, its intensity, and even sign. Moreover, they are especially useful in the assignment of absolute configuration, conformational analysis, and studying intermolecular interactions when supported by quantum mechanical calculations. Still, several problems arise when the chiroptical spectra are modeled. First, reproducing the spectra of a flexible molecule requires a proper evaluation of its conformational space. There are compounds of which different conformers exhibit almost mirror image spectra [4,5]. Hence, the proper estimation of the conformer population is crucial for the correct interpretation of the chiroptical spectra [6]. Second, solvents interacting with molecules that form hydrogen bonds can change the stability of the conformations and thus the final chiroptical ECD spectrum (Fig. 1), which is usually believed to be only slightly solvent-dependent [7,8]. Third, also the substituent bulkiness matters. This presentation shows experimental VCD or ECD spectra of some chiral NDIs and sulfonamides. The performed DFT calculations allowed us to interpret the observed changes upon conformations, substituents, and solvents.

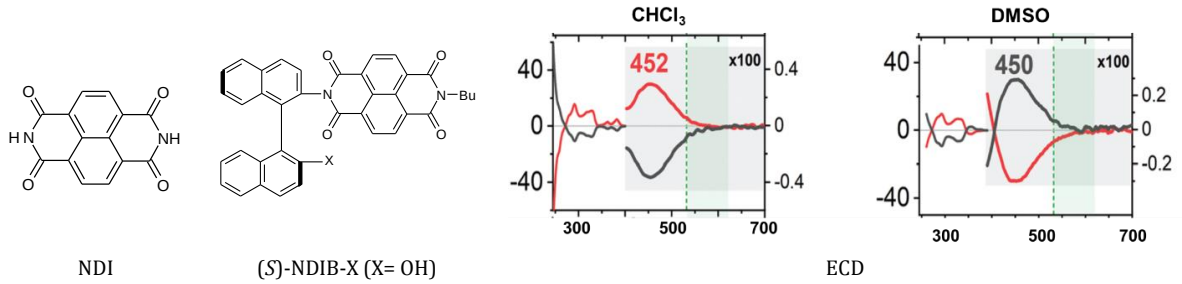

Figure 1. *Structure of NDI and (S)-NDIB-X. ECD spectra of (R)-NDIB–OH (black) and (S)-NDIB–OH (red) in chloroform and DMSO.*

Acknowledgment

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O.5 - A - Molecular Spectroscopy

A Novel Approach to Assess Chemical Composition and Ultraviolet Light-Stress Effects on Japanese Knotweed: A Vibrational Spectroscopy Study

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Understanding the impact of UV light stress on plant physiology is crucial for managing invasive species such as dwarf Japanese knotweed (*Reynoutria japonica* Houtt.) [1,2]. So far, this effect has been examined regarding metabolic alternations in the plant, particularly those affecting the photosynthetic system and the synthesis of secondary metabolites. This study elucidates the potential of vibrational spectroscopy techniques, including attenuated total internal reflection-Fourier transform infrared (ATR-FTIR) spectroscopy as well as Raman and FTIR spectroscopy imaging to identify the significant alterations in the biochemical composition of Japanese knotweed exposure to UV light. These alterations primarily involve changes in carbohydrates, proteins, lipids, and other metabolites. No labels and extraction procedures are required. We also focus our attention on comparing the spectral information gathered from the homogenized tissues and cross-sections of roots, shoots, and leaves.

Japanese knotweed plants were cultivated under controlled conditions and then exposed to UV light in the regions of UV-A, UV-B, and UV-C for 8 h, 3.5 h, and 25 min., respectively, and compared with the untreated group. Hierarchical Cluster analysis (HCA) of the ATR-FTIR spectra of the homogenized samples revealed the segregation of the experimental groups according to the expected effects of the UV light regions, e.g. the UV-A stressed group was similar to the control. This significant variance in the chemical composition due to the UV light stress was further verified by the analysis of the variance (ANOVA) of the selected marker bands assigned to lipid classes, proteins, carbohydrates, and phenolic compounds. We noted significant differences in the plant physiology between the tissues. FT-IR and Raman imaging precisely identify the deposition and distribution of these key biochemical compounds within the tissue cross-section of the plant.

By demonstrating the efficacy of molecular vibrational techniques in assessing UV-stress effects on plant physiology, this study paves the way for identifying and monitoring stress responses in invasive plant species. The investigation of the biochemical composition of Japanese knotweed (*Reynoutria japonica* Houtt.) not only deepens our understanding of this invasive species but also sheds light on the invasive tendencies exhibited by other closely related invasive plant species, underscoring the significance of this research.

Keywords: ATR-FTIR spectroscopy, Raman and FT-IR spectroscopy imaging, UV stress, Japanese knotweed, invasive species, plant physiology, chemometric analysis.

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I.1 - B - Chemometric Advances

Supported by chemometric analysis FTIR studies of the structure of protein-lipid membrane systems

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It is commonly known that mutual interactions in lipid membrane systems modified by proteins/peptides or organic compounds with biological activity play 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 reorganization in secondary structures of proteins/peptides as well as phase separation, alterations in Van der Waals and hydrophobic interactions between hydrocarbon lipid chains in lipid membranes [2,3]. By using chemometric methods such as PCA, MCR-ALS, 2D COS and mowing window 2D COS, an increase in quality of abstained spectroscopic results and a rise in structural information about multicomponent biomolecular systems under study will be shown.

Acknowledgments:
This work was supported by National Science Centre, Poland, project OPUS 22 with decision number UMO-2021/43/B/NZ9/00345.

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I.2 - B - Chemometric Advances

Seeding multivariate algorithms, a data augmentation approach which can enhance analytical performance.

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Seeding spectral datasets augmenting the data matrix with either the full spectra or selected spectral features of them in order to bias the multivariate analysis towards the solution of interest. Such seeding of the dataset can have a profound effect on the endpoint of the analysis. Taking Principal Components Analysis as the first example. seeding with known spectral profiles greatly increases the ability of the algorithm to differentiate two distinct data subsets.

Other examples of where seeding may be applied includes datasets for Multivariate Curve Resolution – Alternating Least Squares analysis (MCR-ALS). In the example presented, adding pure components to the dataset improves the ability of the algorithm to both model the kinetics of time series data and extract the component spectra more accurately than the unseeded dataset.

Multiblock Partial Least Squares Regression (PLSR) can also be seeded, such that the second block in the dataset is used to guide the first test block in the dataset. In the example shown, simulated spectra of the peroxidation of oleic acid are regressed in combination with a block of individual pure spectral components of oleic acid. Improvements in model linearity and prediction error are achieved, compared to standard PLSR.

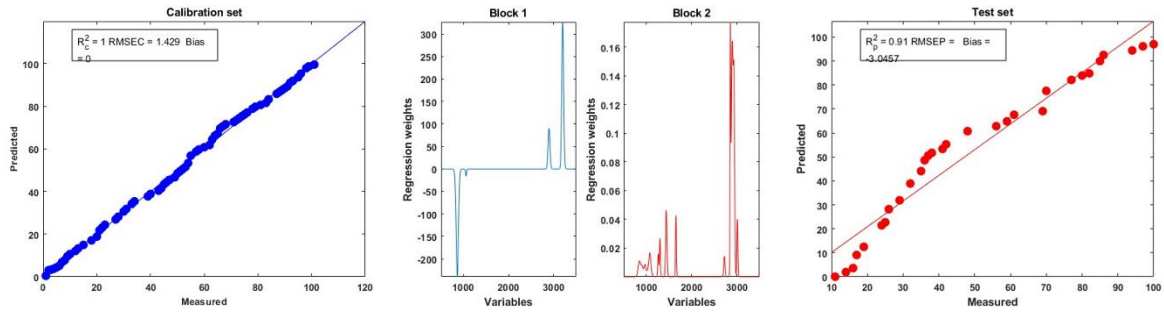

Figure 1. *Seeded Multiblock PLSR for Oleic acid. Showing Calibration data, regression coefficients and test data .*

O.1 - B - Chemometric Advances

Assessment of erythroid differentiation and heme biosynthesis in red blood cells by spontaneous and coherent Raman microscopy

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Erythroid differentiation is a tightly regulated multistep process that starts in bone marrow from haematopoietic stem cells *via* various precursors and culminates in mature red blood cells (RBCs). Any disruption of this process influences tissue oxygenation and other blood functions, including immunity, resulting in e.g. leukaemia. Analysis of the erythroid differentiation process allows accessing changes in morphology and phenotype, extending to the level of individual organelles, to identify key players (such as molecular changes or signalling pathways) involved in the differentiation process and discover new approaches to induce differentiation [1]. This comprehensive understanding sheds light on the fundamental mechanisms governing erythropoiesis and unveils potential targets for therapeutic intervention.
Considering the invasive procedure and the short lifespan of cells isolated from peripheral blood and bone marrow, the K562 cell line serves as a valuable model for following erythropoiesis *in vitro*. Among a broad toolkit of techniques, including microscopy, flow cytometry, transcriptomics, and proteomics, Raman spectroscopy holds great promise for studying erythroid differentiation by providing detailed molecular and structural information at the cellular and subcellular levels.
Herein, we present results of Raman microscopy, used as the method of choice for label-free, non-destructive and molecular-specific identification of the subcellular changes in the composition of erythroid precursors and RBCs in doxorubicin-induced K652 cells *in vitro*. Raman signatures of heme (1555, 1608 cm^{-1}) and unsaturated lipids (3015, 1660 cm-1) were proved to vary during the studied process (Figure 1). Considering the crucial role of mitochondria in iron metabolism and heme biosynthesis [2], we introduced MitoBADY for labelling and imaging mitochondria. MitoBADY accumulates according to the mitochondrial membrane potential and, due to the presence of -C≡C- groups in its molecular structure, can be easily detected at 2220 cm^{-1} [3]. MitoBADY as a Raman tag enhanced the sensitivity and specificity of Raman spectroscopy, providing insight into mitochondrial changes. Moreover, identified Raman markers of differentiation were verified with a nonlinear technique, Stimulated Raman Scattering, allowing for rapid classification of erythroblasts and red blood cells in the studied *in vitro* model.

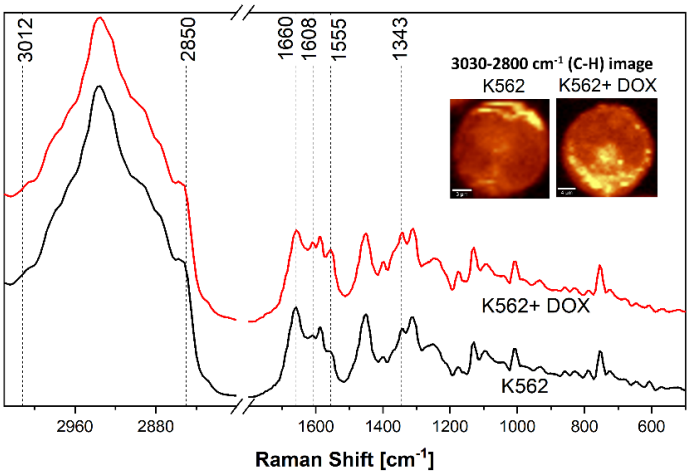

Figure 1. *Mean Raman spectra of erythroid precursors (K562) and doxorubicin treated cells (K562+DOX). 2800-3030 cm^{-1} images of C-H stretching.*

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O.2 - B - Chemometric Advances

Usefulness of Data Simulation for Training Deep Learning Denoising Algorithms in Infrared Spectral Histology

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Introduction:
In the field of spectral imaging, optimizing acquisition time is a critical challenge, especially for clinical applications. Our initial work focused on developing deep learning-based solutions to accelerate this process, thereby enabling faster, more efficient diagnostic applications compatible with clinical routines. However, practical implementation of these solutions is often hindered by the need for large amounts of real data, which can limit crucial problem-solving capabilities such as noise reduction, essential for ensuring image quality. Moreover, extending these methods to other vibrational spectroscopy techniques naturally raises the need for flexible, generalizable solutions. To address these challenges, we explored data simulation in this study. By simulating spectra in a controlled manner, we aimed to overcome limitations related to real data availability while broadening the application horizons of these technological advancements. Thus, this study aims to explore and demonstrate how data simulation can not only overcome obstacles related to data quantity but also extend the applicability of these techniques to a wider range of vibrational spectroscopy methods.

Materials and Methods:
To faithfully replicate the complex nuances of real spectral data, we developed specialized functions for generating pure spectra modeled as weighted sums of Lorentzian, Gaussian, and Voigt peaks [1], incorporating baseline shapes and complexities. These functions were designed to accurately capture essential spectral features, including peak central positions, full-width at half-maximum, and peak amplitudes. To recreate realistic experimental conditions, various types of noise were systematically introduced into our datasets. We explored Gaussian noise, both additive and multiplicative, Poisson noise, and combinations thereof. These experiments perturbed our datasets, creating spectrum pairs suitable for learning. The objective was to denoise spectra using deep learning.
To assess the effectiveness of our approach, the following steps were implemented: (i) A Residual UNet model, based on 1D convolutional layers, was trained on infrared spectral images acquired from paraffin-embedded human renal graft sections. (ii) The same architecture was subsequently trained on our simulated spectra. (iii) Both models were then evaluated on a separate set of real test data not used in the training of the model in step (i). Various metrics commonly used in the literature, such as RMSE (Root Mean Square Error), SNR (Signal to Noise Ratio) product, and SSIM (Structural Similarity Index Measure) [2], were implemented to compare the two models and assess the impact of data simulation on denoising algorithm training.

Results and Discussion:
In this study, four experiments were conducted using simulated datasets with different characteristics to train and test denoising models on real data. Dataset 1 consisted of spectra simulated with fixed paraffin bands and amide bands, demonstrating good reproduction of the main bands on test data but with observed smoothing effects on other parts. Dataset 2 included spectra simulated with randomly selected numbers and varied band widths, showing satisfactory reconstruction of wide bands but encountering difficulties with paraffin bands. Dataset 3 featured spectra simulated with broad and narrow bands, revealing significant challenges in denoising. Finally, Dataset 4 comprised spectra simulated with constant paraffin bands and varied protein bands, offering overall satisfactory reconstruction and the best performance, with results comparable to real data.

Conclusion:
The results highlighted that the various configurations of simulated spectra significantly influenced model performance, with varying success depending on the data characteristics used. This approach demonstrated that training on simulated data not only yields robust reconstructions but also saves time compared to acquiring and using real data. These findings underscore the effectiveness of data simulation as a tool for the rapid and efficient development of spectral imaging denoising techniques, thereby opening avenues for practical deployment in applications.

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O.3 - B - Chemometric Advances

Siamese Neural Networks for Clinically Relevant Bacteria Classification based on Raman Spectroscopy

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Bacteria analysis is critical for applications ranging from food safety monitoring to disease prevention and environmental monitoring, but traditional culture-based methods are slow and labor intensive. Improved techniques such as PCR and immunological assays offer greater specificity. However, they are limited in field testing and require expensive reagents. Therefore, alternative methods are needed to improve testing efficiency. Raman spectroscopy is emerging as a promising solution, offering rapid, sensitive, and non-destructive bacterial detection by providing chemical fingerprints of sample molecules. However, the interpretation of Raman data is challenging and requires the use of chemometric techniques for analysis. Classical machine learning methods such as LDA, SVM, and RF facilitate accurate identification and discrimination of bacteria.
Spectral preprocessing is a critical step in classical machine learning methods for vibrational spectroscopy analysis, but it presents challenges due to varying noise characteristics and the potential for introducing errors. To address these issues, deep learning techniques have gained traction in recent years, offering advantages such as handling large datasets and achieving superior performance. Among these techniques, convolutional neural networks (CNNs) have proven to be particularly effective for spectral matching, extracting fingerprint features that lead to higher classification accuracy. However, CNNs require extensive training data and retraining when databases or training sets are modified, resulting in impractical computational costs. To overcome these limitations, Siamese neural networks (SNNs) have been proposed. Unlike CNNs, Siamese neural networks determine the similarity or dissimilarity between pairs of data points and are trained to generate a similarity score or distance measure for each input pair, making them more flexible and efficient, especially in cases where data is limited.
In this study, we investigated the performance of these classical machine learning and deep learning methods on a Raman data set containing six bacteria species grown in nine independent biological replicates (9 batches), totaling 5420 individual bacteria spectra. We use two batches of cross-validation to evaluate the stability of the results. In every cross-validation fold, the test set contains two batches, and the rest is used as a training and validation set. (70% training and 30% validation). We examined the data set by PLS-DA, PCA-RF, PCA-LDA, PCA-SVM, Shallow CNN, Deeper CNN, SNN model1 and SNN model2. The models achieved test accuracies of 78.58%, 79.15%, 79.48%, 80.51%, 82.80%, 84.13%, 82.65% and 83.61% respectively. These results show that CNNs and SNNs outperform the classical machine learning methods and that SNN performs as well as a "normal" CNN, while offering the additional advantages mentioned above. Therefore, SNNs represent a promising approach for spectral analysis of biological data sets.

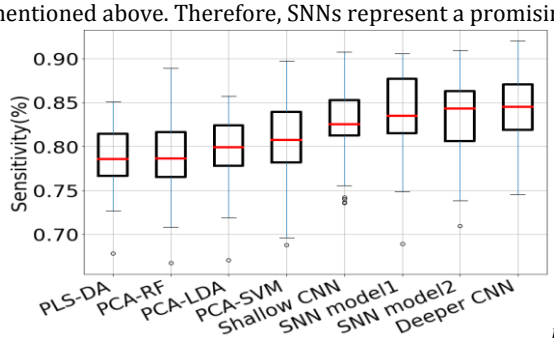

Figure1: The sensitivity of different models in classification of bacteria species. The CNNs and SNNs outperform the classical machine learning methods and that SNN performs as well as a "normal" CNN.

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I.1 - C - Enhanced Techniques

Plasmonic-Based Nanoplatforms for Enhaced Biosensing and Bioimaging

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Due to their unique optical properties determined by the excitation of surface plasmons, which lead to electromagnetic field enhancement, heat generation, and hot electron generation, plasmonic nanoparticles have garnered enormous scientific interest in various fields, ranging from medicine and life sciences to information and energy technology, as well as environmental and food safety monitoring. These specific optical properties, combined with the ease of surface modification, enable plasmonic nanoparticles to be integrated in versatile nanoplatforms with improved biocompatibility and multiple functionalities towards biomedical applications.

In this talk, we present an overview on plasmonic-based nanoplatforms and their various applications in surface-enhanced Raman scattering (SERS) sensing, plasmon-enhanced scattering, cell imaging and plasmon-based nanotherapy, we developed in recent years in our laboratory. Our first fabrication method involves the chemical synthesis of gold or silver nanoparticles with controlled size and shape (rods, prisms, bipyramids) exhibiting well-defined localized surface resonance (LSPR) across the visible to near-infrared (NIR), along with desired biocompatibility and specific functionality. The second route focuses on fabrication of solid SERS substrates and hybrid nanoplatforms composed of multiple components, including graphene oxide (GO), biopolymer and plasmonic nanoparticles. Plasmonic responses of as-fabricated nanostructures are investigated and correlated with their nanometer-scale morphology, surface topography, and composition by experimental (SEM, TEM, DLS) and theoretical (FDTD) methods. Several classes of as-fabricated plasmonic and hybrid nanoplatforms were utilized as versatile optical nanoprobes for spectroscopic investigation of single cells *via* SERS, localized surface plasmon resonant scattering (LSPR-S), and steady-state and fluorescent lifetime imaging (FLIM). Scanning confocal Raman/SERS microscopy combined with dark-field and Re-scan fluorescence microscopy were used to record relevant intracellular information as nanoparticle localization, local chemical interaction, and intracellular pH mapping. In addition to bioimaging applications, our research group has implemented several "proofs of concept" for plasmon-enhanced photothermal therapy (PTT), plasmon-enhanced photodynamic therapy (PE-PDT) and controlled delivery of chemotherapeutic drugs [1-2].

Recently, we utilized graphene oxide-gold nanoparticle platforms (GO-AuNPs) as SERS substrates to investigate DNA detection and hybridization. We employed as model for hybridization a repetitive sequence of adenine nucleotides (polyadenine or poly(A)) with varying lengths (5, 10, 15, and 20 bases) and a complementary poly(thymine) (polyT) sequence. Firstly, we investigated SERS detection of poly(A)-SH which features a thiol group (-SH) at its 5' end, allowing direct attachment to the gold surface via a thiol bond and Poly(A)-NH2 which possesses an amine group (-NH2) at its 5' end, enabling attachment to the GO functional groups through an amide bond. In both cases, the main modification we observed in SERS spectra is a decrease in the peak height at 732 cm^{-1}, attributed to the adenine ring breathing vibration, as the number of adenine bases decreased. The decrease in peak height, however, differs between the two types of linkages, which can be attributed to specific locations of adenine molecules at nanoparticle surface and hot-spots in-between-nanoparticles. Further investigations are currently underway to refine our understanding of hybridization of Poly A with complementary Poly T sequences.

This work was supported by the project BioPlasmonics funded by European Union–Next generation EU and Romanian Government, under National Recovery and Resilience Plan for Romania, contract no760037/23.05.2023, cod PNRR-C9-I8-CF-199/28.11.2023.

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I.2 - C - Enhanced Techniques

SERS-based method for detecting and identifying pathogenic microorganisms - bacteria and viruses

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Surface-enhanced Raman spectroscopy provides a unique vibrational signature of the scattered molecules. SERS as an ultrasensitive, label-free and non-destructive technique, reveals 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, including viruses including SARS-CoV-2 [1-3]. The developed SERS-based sensors challenge the current standard method of bacterial detection and identification in terms of sensitivity, selectivity, cost, and time of analysis. The direct SERS analysis of bacteria (even a single bacteria cell) is performed directly from Perspective 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 [4-5]. The long-term incubation of bacteria was eliminated, and the total analysis, including the numerical analysis of recorded SERS data, did not exceed 15 minutes. Coupling of plasmonic nanostructures with microfluidic systems ensures miniaturization of the developed methods for their further applications. This invention 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 centers), food industry and environmental protection.

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I.3 - C - Enhanced Techniques

Recent Advancements in DNA Origami Plasmonic Nanoantennas for Advanced Single-Molecule Surface Enhanced Raman Spectroscopy

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DNA origami nanostructures, with their unique ability to arrange both plasmonic nanoparticles and receptors for analyte molecules with nanometer precision, hold great potential in providing optimized substrates for surface-enhanced Raman scattering (SERS). In recent years, we have demonstrated the detection of few- and single-molecule SERS in different nanoparticle arrangements. We have created a dedicated DNA origami nanoantenna, which was also used to study chemical changes in hemin and to detect single proteins. Very recently, we have further optimized the plasmonic nanoantennas by comparing the SERS performance of dimers of different nanoparticle species from spherical Au and Ag nanoparticles to anisotropic gold nanoflowers and combinations thereof. We could show that a combination of Au nanoflower and Ag nanosphere allows for a broadband SERS excitation and improved single-molecule detection.

In the present contribution recent single-molecule SERS results are presented focusing on the following aspects: i) We explore the influence of the orientation of molecules within the hot spot of DNA origami-assembled nanoparticle dimers on the single-molecules SERS spectra. ii) Single proteins are investigated in detail using SERS. Using cytochrome c as an example we demonstrate the occurrence and analysis of the amide III band as a structural marker band in single-molecule SERS spectra[5]. Additionally, we studied the enzymatic activity of horseradish peroxidase (HRP) on the single-molecule level[6].

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O.1 - C - Enhanced Techniques

Surface-enhanced Raman spectroscopy on non-plasmonic vanadium pentoxide and vanadium pentoxide/Au nanostructures

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The metal oxides (MeO) represent promising non-plasmonic materials for surface-enhanced Raman scattering (SERS) spectroscopy [1]. Since in this case, charge transfer (CT) is the main enhancing mechanism, MeO provide lower signal enhancement as compared to plasmonic metals. On the other hand, the main advantages of MeO-based SERS substrates over plasmonic ones include good signal uniformity, stability, spectral reproducibility, and low local heating upon laser irradiation.

We developed a new approach for the synthesis of vanadium pentoxide (V_2O_5) nanostructural film and nanoparticles (NPs) using magnetron-based gas aggregation sources of vanadium NPs and thermally induced transformation of deposited vanadium NP films into V_2O_5 ones [2]. The Au nanoislands, selected as a model plasmonic material that exhibits SERS activity due to the electromagnetic (EM) mechanism, were deposited either on Si substrates or Si substrates precoated with smooth V_2O_5 or V_2O_5-NP films. The Au deposition was performed in a separate high-vacuum deposition chamber. SEM image of V_2O_5-NPs/Au film is shown in Figure 1, left.

For the SERS experiment, the solution of the analyte (methylene blue – MB) was dropped onto the SERS substrate and dried in the air. Then the SERS spectra were measured from dried spots using 632.8 nm excitation. We compared the SERS activity of Au nanoislands, V_2O_5-NPs without Au, and smooth V_2O_5 and V_2O_5-NPs decorated with Au. A dramatic increase in the MB signal was observed for both V_2O_5-NPs/Au and V_2O_5-film/Au substrates. The results for V_2O_5-NPs are shown in Figure 1, where the SERS band of MB at about 1630 cm^{-1} that corresponds to the C-C ring stretching is plotted for all three substrates and the same concentration of MB (5×10^{-6} M). We suggest that the signal enhancement in the case of V_2O_5 nanostructure with Au is most likely connected with the highly nanostructured character of this substrate that facilitates the formation of "hot spots" between individual Au nanostructures and consequently EM enhancement. This approach, therefore, could be the combination of plasmonic and non-plasmonic materials.

The study explores the potential of plasma-assisted synthesis of non-plasmonic MeO/metal plasmonic nanostructures as a method to enhance SERS using rare metals while concurrently preserving the signal stability typical of non-plasmonic materials.

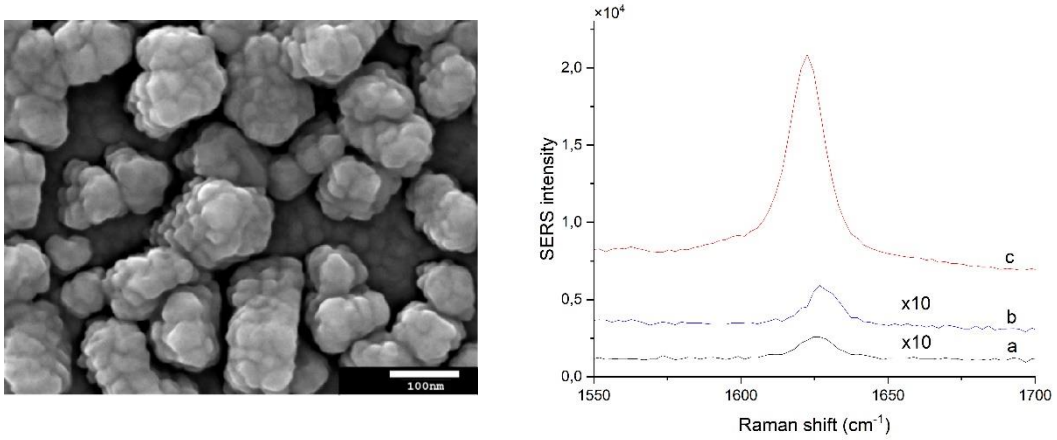

Figure 1. *Left: SEM image of V_2O_5-NPs/Au. Right: SERS band of MB at 1630 cm^{-1} obtained from Au nanoislands on Si (a), V_2O_5-NPs (b) and V_2O_5-NPs/Au (c).*

The financial support by the Czech Science Foundation (22-16667S) is acknowledged.

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O.2 - C - Enhanced Techniques

Imaging of Three-dimensional Molecular Orientation Vibrational Microspectroscopies

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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. 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].

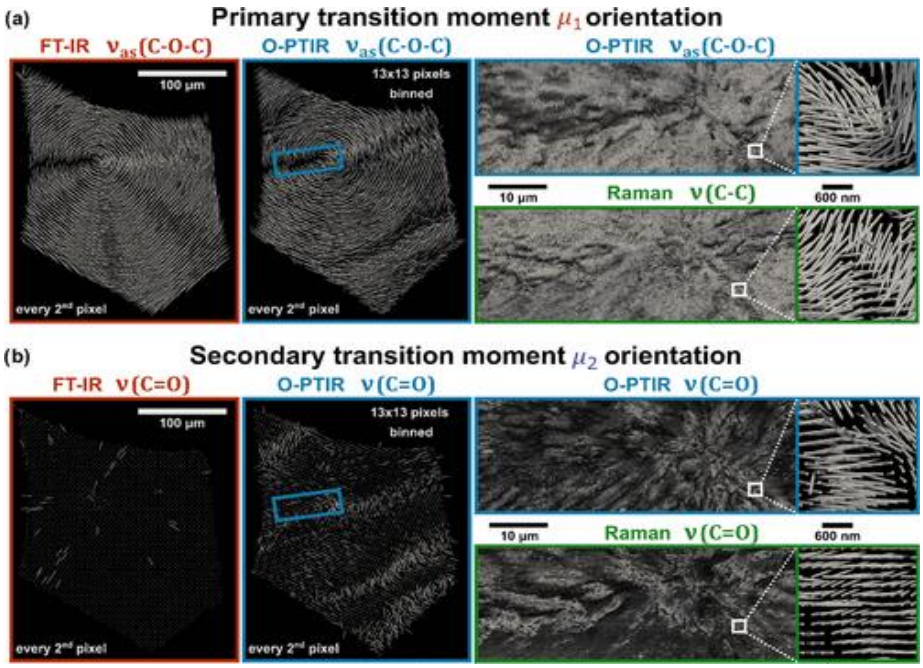

Figure 1. *4P-3D orientation results. Visualization of the primary (a) and secondary (b) transition moment orientation calculated for FT-IR, O-PTIR, and Raman. From [5].*

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 [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 2022 [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 very promising, we proved that this method can be easily applied not only to FT-IR but also to O-PTIR and Raman imaging. We now extend the applications to more complex biological systems and polymeric systems almost completely amorphic. 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.

Grant No. 2018/31/D/ST4/01833; Project No. MRPO.05.01.00-12-013/15

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I.1 - D - Biomedical Applications

Separating pussycats and tigers: Risk stratification of prostate cancer using infrared imaging

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The identification of high-risk non-metastatic prostate cancers is extremely challenging. This means that most of these patients are put into the low risk group and the most common treatment option is to go onto active surveillance. This involves the clinicians keeping a watch on your condition (say every 6 months) without any aggressive intervention. However, it is well known that a small number of men in this situation suddenly develop aggressive prostate cancer (tigers) and have a survival rate similar to those men who present with metastatic disease. The identification of these cases is beyond current standard pathology practice. In this study, we have measured ~1400 archived FFPE tissue cores from 183 patents using infrared spectroscopic imaging. From this data, we use various machine learning approaches to identify the key tissue components and then, using outcome data we have been able to stratify the tissue. Importantly, in the low risk non-metastatic group we have identified a high-risk sub-population who go on to develop aggressive disease. These results have important implications for clinical practice since this could alter current treatment options for these patients.

I.2 - D - Biomedical Applications

From DNA-protein repair mechanism to epigenetics: studies by Raman spectroscopy

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The correct interactions between ssDNA molecules and the repair protein Rad51 are at the basis of an efficient homologous DNA recombination (HR) pathway. Alterations in the DNA-protein filament can lead to severe diseases[1] therefore a detailed description of the DNA-protein binding mechanism is of fundamental importance but still missing. In this work we provided a detailed study of physiological and non-physiological-like states of the nucleoprotein. A droplet of the biomolecules solution is deposited over a micropatterned superhydrophobic surface (SHS). Free-standing, self-sieved, oriented nucleoproteins extended fibers are suspended on the SHS at the end of the dehydration process and have been characterized by Raman spectroscopy[2,3]. We described the structural properties of Rad51 and ssDNA under interaction and, for the first time, the fundamental role of the non-crystallizable features of Rad51. Fine rearrangements on stiffness in the range of 1 meV strongly affect DNA bases orientation, homology search, and pathologies outbreak.

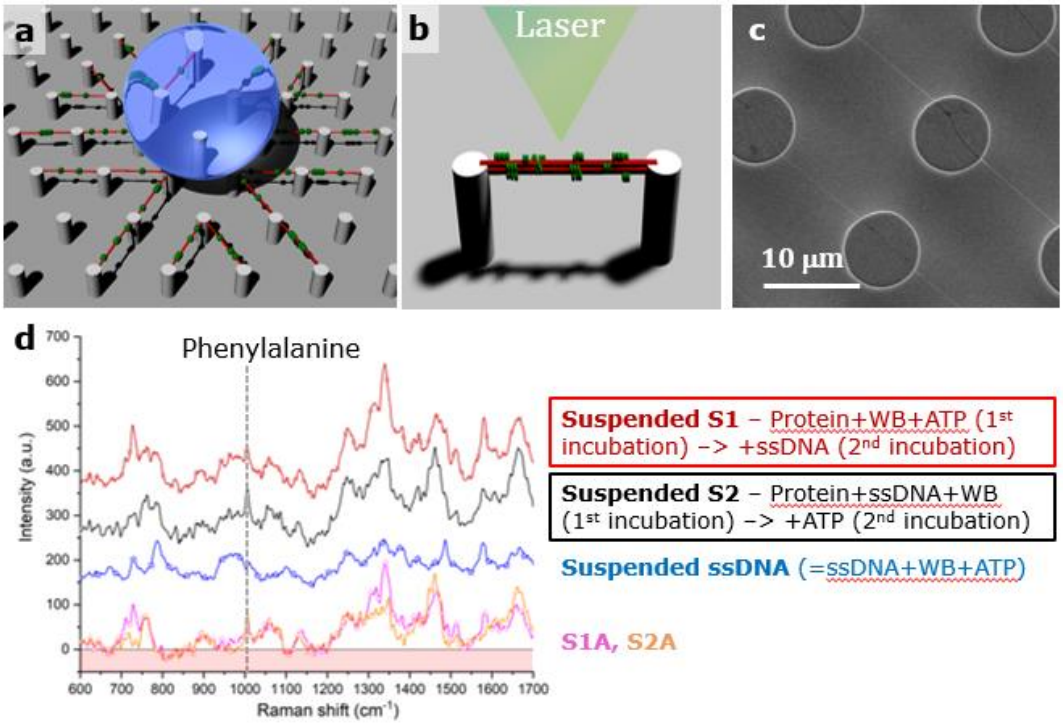

Figure 1. Figure 1: *Sketch of a) suspension processes on a SHS and b) spectroscopic analysis of c) suspended ssDNA-Rad51 fibers. d) The Raman spectra differences S1A and S2A show the variations in the DNA-Rad51 recognition event upon introduction of ATP in different moments of the preparation.*

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I.3 - D - Biomedical Applications

New Routes for Cancer Treatment Tackled by Neutrons

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Normal-to-cancer transition (NTC) is still an ill-understood process, closely associated to cellular biomechanical properties. These are strongly dependent on intracellular water´s structural and dynamical profiles, which play a fundamental role in cellular function. Improved chemotherapeutic strategies are an urgent clinical need, since cancer is still the second leading cause of death worldwide, with an expected rising incidence. Metal-based drugs developed upon the discovery of cisplatin (cis-(NH3)2PtCl2) have aimed at coupling an enhanced efficacy to decreased acquired resistance and harmful side effects. These metallodrugs encompass Pt- and Pd-complexes with more than one metal centre, extensively studied by the authors in the last decade [13], which trigger a selective DNA damage – through metal coordination to the purine bases or via electrostatic interaction with the phosphate groups [4,5].
Inelastic and quasi-elastic neutron scattering techniques (INS and QENS), combined with Raman and Fourier Transform Infrared (FTIR, including with synchrotron radiation) spectroscopies, are currently reported to deliver a comprehensive set of data, at the conformational and dynamic levels, on: (i) NTC transformation [6]; (ii) activity of newly developed Pt/Pd-anticancer agents (on DNA, glutathione, proteins, cellular metabolism and intracellular water) [7-9]. Variations in the dynamical profile of intracellular water were unveiled for malignant cells/tissues as compared to healthy ones. In addition, clearly distinct effects were revealed for Pt- vs Pd-agents regarding their impact on either the cellular cytoplasm or hydration water in cancer cells, as well as concerning their specific interactions with biomolecules. This is a pioneer study on the impact of cisplatin-like chemotherapeutic agents on vital cellular components, which is key for a thorough understanding of their molecular basis of cytotoxicity.

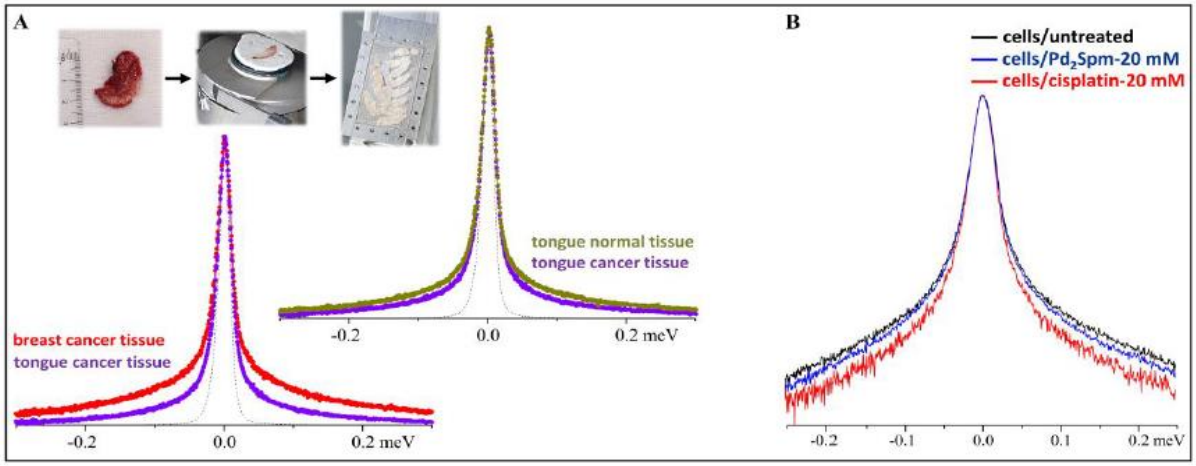

Figure 1. *QENS profiles (normalised to maximum peak intensity): (A) human breast and tongue tissues, cancer vs non-cancer (310 K, all Q values). (B) human cancer breast cells (MDA-MB_231), with and without drug (298 K, at Q=1.079 Å-1, log yy´scale).*

These results are expected to foster the development of improved anticancer drugs – displaying high specificity and optimised efficacy. Ideally, these are aimed to act simultaneously on more than one site (multitarget approach), intracellular water being suggested as a potential pharmacological target. Advanced chemotherapeutic strategies such as these will contribute to a better prognosis and quality of life of cancer patients.

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I.4 - D - Biomedical Applications

Fat analysis in live cell and tissue with Raman spectroscopy

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Obesity is an important medical issue. A symptom of fat accumulation, especially in the liver in the absence of excessive alcohol consumption, is referred to as nonalcoholic fatty liver disease (NAFLD). The accumulated fat induces hepatic cell death and inflammation in the liver. In contrast, adipocytes are able to hold much fat. The reaction of hepatocytes is different from that of adipocytes. What is the difference come from? Previous reports indicated that saturated fatty acids induced apoptosis in hepatic cells. To solve the problem, it is necessary to analyze fatty chain compositions in the cells and tissues.

The purpose of the present study is to apply Raman spectroscopy for quantitative analysis of fatty chain compositions in their fat accumulations in live cells and tissues. As the fats are hydrophobic, they often generate fat droplet in the cell, in which their concentration is almost 100 %. It indicates that it is able to obtain strong Raman signals, if one can make the focus of Raman measurement exactly in the fat droplets. In contrast, there is a drawback for analysis of fatty chains with Raman spectroscopy. As Fig. shows, the Raman spectra of fatty chains are similar very much. Especially, the fatty acids having the same number of double bonds resemble quite closely. It was one of the reasons to make quantitative analysis difficult for Raman spectroscopy.
We have developed a ball lens top hollow optical fiber Raman probe (BHRP) with long working distance, about 400 μm

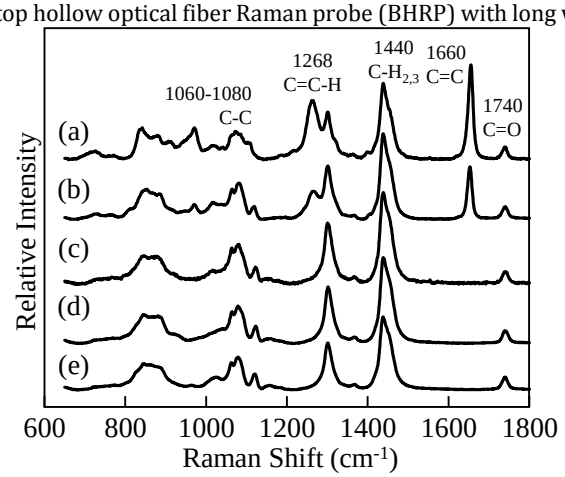

Figure. *Raman spectra of linoleic (a; 18:2), M-oleic (b; 18:1), stearic (c; 18:0), palmitic (d; 16:0) and myristic (e; 14:0) methyl esters.*

and applied analysis of subcutaneous fat in live hamster.[1] Since the hamster has sebaceous gland, it has fats near the skin. The hamsters were treated with diets with high concentration of linoleic and decanoic chains. The BHRP has successfully obtained Raman spectra of subcutaneous fat in the totally noninvasive manner. The resected fat tissues were analyzed with Raman spectroscopy and gas chromatography (GC) as well to make analytical models for linoleic and decanoic chains. The models were applied to the subcutaneous Raman spectra and provided highly accurate concentrations of the fatty chains in the tissues. The result suggested that adipose cells accumulate linoleic chains with high rate when the feeding include linoleic chains, but not for decanoic chains.

The reactions of liver cells to different fatty acids have been studied with Raman spectroscopy.[2] A liver model cell, HepG2, was cultured excess concentrations of palmitic (PA), stearic (SA), oleic (OA) and linoleic acids (LA). Previous studies pointed out that saturated fatty acids induce cell death of HepG2 cells. However, our result showed that not only with PA and SA but also with LA induced apoptosis to HepG2 cells. The Raman monitoring with the survived cells suggested that the cells uptake LA quickly but induced cell death after that.

The results demonstrate that Raman spectroscopy has high viability for noninvasive analysis of fatty chain composition in fat tissues. We work on to improve accuracy in the quantitative analysis of fatty chains with different chain length and to develop a longer WD of BHRP, to apply Raman spectroscopy for human fat analysis.

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O.1 - D - Biomedical Applications

Studies on the Effect of Statin Supplementation on Human Colon Cells Using Raman Imaging and AFM

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One of the most important areas of medical science is oncology, which is responsible for both the diagnostics and treatment of cancer diseases. Over the years, there has been an intensive development of cancer diagnostics and treatment. This research shows the comparison of normal (CCD-18Co) and cancerous (CaCo-2) cell lines of the human gastrointestinal tract on the basis of nanomechanical and biochemical properties in order to obtain information on the cancer biomarkers useful in oncological diagnostics. The studies included the effect of the statins: mevastatin, lovastatin and simvastatin and their influence on biochemical and nanomechanical changes of cells properties using Raman imaging and AFM techniques. In the presented study the innovative combination of biochemical and nanomechanical characterization of human colon cells: normal, cancer and cancer upon statin supplementation by using Raman spectroscopy and imaging and AFM techniques including the influence of statins type will be discussed.

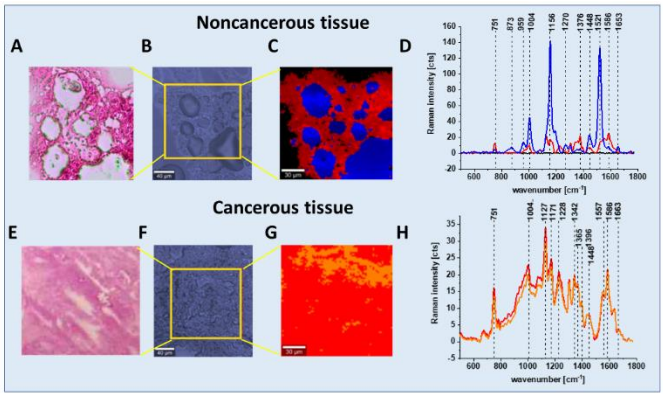

Figure 1. *The histological image (A, E), microscopy image (B, F), Raman image (C, G), and the average Raman spectra (D, H) typical for noncancerous and cancerous human colon tissues respectively. The white bar in the pictures, in the lower left corner, is the inner scale which is 40 μm (A, B, E, F) and 30 μm (C, G).*

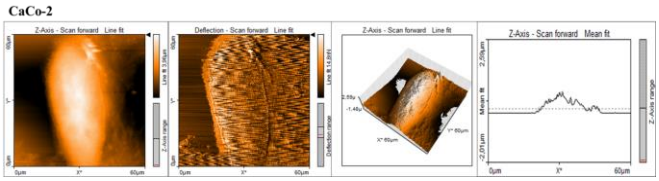

Figure 2. *AFM topography maps of CaCo-2 with deflection maps, 3D topography, and curves related to the topography measurements for forward and backward traces. The scale bar equals to 60μm is the same for all cell images, topography 3D and deflection maps.*

One of the main goals of our study was to determine the statistically significant differences between normal and cancer human colon cells including cancer cells supplemented by mevastatin, simvastatin and lovastatin based on vibrational features of them. We investigated systematically how the Raman imaging and Raman spectroscopy methods respond to *in vitro* normal and cancer human cells without and upon the supplementation by statins. We presented a valuable, fast and costless method for the cells structures visualization and the cells virtual staining, that adds the biochemical information given by the Raman intensity to the pseudo-colour images. These label-free images with high spatial resolution enable a direct analysis of all human colon cells substructures, which can help the tracking of biochemistry changes typical for cancerogenesis and can help the analysis of anti-cancer treatment.

Based on the Raman data obtained for normal, cancer and supplemented by statins cancer cells we compared the vibrational futures of human colon cells using the average spectra calculated for cells as a whole and Raman band intensities ratios calculated for the main building blocks of biological samples: proteins, nucleic acids and lipids.

Based on our research we proved that accumulating evidence suggests that long-term use of lipophilic statins may also affect the overall incidence of cancer or the incidence of certain types of cancer. Moreover, statins may increase the sensitivity to chemotherapy and influence clinical outcomes in patients who have already been diagnosed with cancer.

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O.2 - D - Biomedical Applications

Spectroscopic profiling of therapeutic responses of B-cell acute lymphoblastic leukaemia to tyrosine kinase inhibitors

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In the pathogenesis of B-cell leukaemias, deregulation of the activity of enzymes called tyrosine kinases (TKs) are found to be important. Tyrosine kinases are enzymes that regulate various cellular processes by adding phosphate groups to tyrosine residues on target proteins, thereby altering their function. In B-cell leukemias, particularly chronic myeloid leukemia (CML) and some types of acute lymphoblastic leukemia (ALL), a specific tyrosine kinase called BCR-ABL is often implicated.

BCR-ABL is a fusion protein resulting from a chromosomal translocation between the Abelson (ABL) gene on chromosome 9 and the breakpoint cluster region (BCR) gene on chromosome 22, leading to the presence of *Philadelphia* chromosome (*Ph*). This fusion protein exhibits constitutive tyrosine kinase activity, which drives uncontrolled cell proliferation, inhibits apoptosis, and disrupts normal cellular signalling pathways. A way to inhibit such processes is through tyrosine kinase inhibitors (TKIs), like imatinib (IM) and dasatinib (DAS).

Assessing the drug-cell interaction is essential for understanding treatment efficacy and the development of drug resistance. Cancer cells are notorious for their ability to evolve and adapt, often acquiring resistance mechanisms during therapy that allow them to survive and proliferate despite treatment. Since cancer cells can bypass the effects of targeted drugs, they may activate alternative signalling and metabolic pathways by upregulating compensatory pathways or activating parallel signalling cascades.

Addressing the issue of drug resistance in cancer cells is crucial to the development of biomarker-guided therapy. Identifying predictive biomarkers that can indicate response to therapy or the development of resistance allows for personalised treatment approaches. Moreover, monitoring of treatment response through imaging techniques, biomarker analysis, and other methods can help identify resistance early and modify treatment strategies accordingly. Herein, a strategy utilising vibrational spectroscopy, such as Raman and infrared absorption spectroscopies, was proposed as an approach that can be indispensable in this scenario. Thus, employing an approach focused on molecular profiling of TKI-stimulated cells enables a comprehensive assessment of the changes generated by molecularly targeted drugs at the morphological, metabolic, and chemical levels. This study aims to use label-free spectroscopic imaging to assess the biochemical and metabolic status of B-cell ALL in response to TKIs.

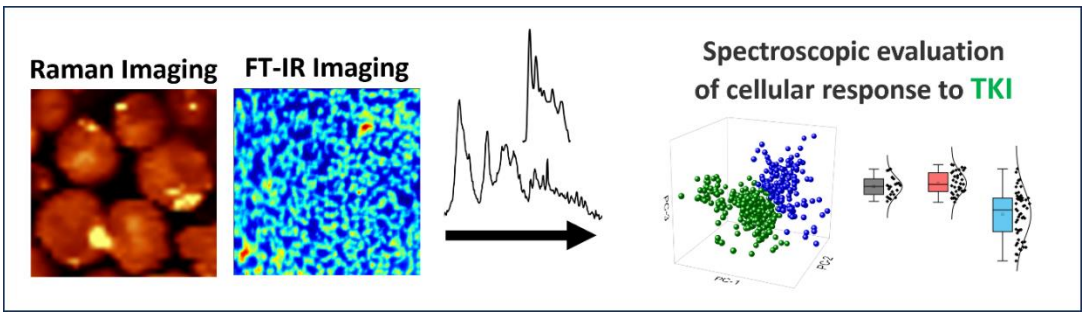

Figure 1. *Schematic representation of the purpose of the study based on oscillatory spectroscopy methods to assess the effect of tyrosine kinase inhibitors on leukaemic cells.*

To identify spectroscopic markers of TKI-induced molecular response, a BV-173 cell line was utilised as an *in vitro* model of B- ALL carrying the BCR-ABL1 rearrangement, which is a potential therapeutic target for selected drugs. In contrast, the TANOUE cell line, a potentially TKI-resistant cell, was selected as the control group. The cells were incubated with IM and DAS, then fixed and imaged using confocal Raman and FT-IR imaging. Chemometric analyses (principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA)) were performed on the single-cell average spectra of leukaemic cells. Integration analyses of selected characteristic bands of the most important biological components were performed to evaluate TKI-induced morphological changes in sensitive and resistant cells. The study successfully captures cellular response differences between sensitive and resistant cells to TKIs. The chemometric analysis has revealed significant changes in the lipid and protein composition of BV-173 cells as a result of the TKIs. Our findings demonstrates that Raman and FTIR imaging are a valuable tools for monitoring leukemia cells' response to targeted therapies, providing valuable insights at both, molecular (spectra) and morphological (images) levels. These insights can support studies on new drug development and monitoring of personalized treatment.

O.3 - D - Biomedical Applications

Common FTIR marker of prostate cancer in urine and tissues correlated with PSA level

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Fourier transform infrared spectroscopy (FTIR) is a vibrational spectroscopy technique allowing to obtain, after exciting vibrations of functional groups, the chemical composition of the tested material. Consequently, by comparing two substances with each other, chemical differences between them can be detected. Therefore, FTIR was used to investigate chemical differences in prostate tissues and urine caused by prostate cancer and to correlate these data with medical biomarker of prostate cancer - Prostate Specific Antigen (PSA). In FTIR spectra of prostate tissues collected from non-healthy patients, more peaks originating from phospholipids, amide and lipid vibrations was detected in comparison with FTIR spectra of control prostate tissues. While in spectra obtained for urine, higher absorbances in the range from 900 cm^{-1} to 1200 cm^{-1}, as well as for vibrations of NH groups were noticed in no-control group of patients. Moreover, Principal Component Analysis (PCA) showed that it is possible to differentiate two types of tissues using FTIR range corresponding from (i) phospholipids and amides as well as from (ii) lipids vibrations. However, for urine, differentiation was not visible. Machine learning methods showed that higher number of mean importance relevant wavenumbers was obtained for fingerprint (800 cm^{-1} – 1800 cm^{-1}) range than for lipids one (2800 cm^{-1} - 3000 cm^{-1}) in both types of analyzed biological materials. Continuing, also values of the area under curve (AUC), accuracy, F1, precision and recall were higher for fingerprint range than for second one for urine and tissues. However, values of all these parameters in both analyzed ranges were higher than 0.85. Consequently, FTIR spectroscopy detected cancer prostate tissues as well as urine with high possibility. Proposed FTIR marker of prostate cancer in tissues (wavenumbers at 1685 cm^{-1} and 3001 cm^{-1}) was not correlated with PSA, but correlated with pGleason, cGleason and ISUP, while wavenumbers, which were candidates of prostate cancer from urine (1614 cm^{-1} and 2972 cm^{-1}) correlated with PSA, cGleason, group of risk and MRI PIRADS. The obtained results suggested that FTIR spectroscopy reflected medical characterization of prostate cancer.

O.4 - D - Biomedical Applications

Revealing the features of the single-cell nucleated avian and non-nucleated mammalian erythrocytes with the use of O-PTIR technique

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Non-nucleated mammalian red blood cells (RBCs) are characterized through a high degree of deformability, and typically also with aggregability, both major determinants of whole blood viscosity [1,2]. Such deformability seems hardly present in nucleated RBCs of birds, while aggregability is even absent [3]. Existing hypotheses regarding the evolution of enucleated erythrocytes in mammals fail to explain why and how birds retained cell organelles in their erythrocytes and we have no clear suport for hypotheses why enuclation has evolved [4]. We consider the application of new physicochemical methods for comparison of nucleated and non-nucleated RBCs will allow us to inject new visons that will help to understand prerequisites for blood flow and circulation in general, and the enucleation of mammalian red blood cells in particular.

FTIR microspectroscopy probes intrinsic molecular vibrational frequencies of bonds between molecules present in a sample. Since vibrational frequencies depend on the characteristic parameters of molecular structures, they can provide valuable information on cellular biochemical changes through relative quantification of lipids, proteins, and carbohydrates. As has already been described, vibrational spectroscopy and microspectroscopy reveal the fingerprint of proteins which are dominant constituents in all cells and tissues, and are easily observed by infrared microspectroscopy [5, 6]. On the other side vibrational spectroscopy also enables precise DNA spectroscopical analysis [7]. However, it is worth emphasizing that there are shortcomings associated with FTIR, which may prevent its use in single cell-based studies. One of the most critical measurement parameters in FTIR microspectroscopy is the spatial resolution restricted by the so-called diffraction limit. In all IR systems, we actually obtain average information from biochemically heterogeneous cells, a fact that severely limits intra-cellular imaging. The last decade has seen impressive progress in the development of new techniques based on the interpretation of vibrational frequencies of bonds present in molecules. Optical Photothermal Infrared (O-PTIR) spectroscopy is one of the novel and highly versatile techniques offering submicron resolution for IR imaging and spectroscopy. This method is also label-free, requires no special sample preparation steps, and operates in an easy-to-use far-field mode (non-contact). O-PTIR revealing the molecular distribution of the cell constituents is needed to provide fundamental understanding on precise red blood cell shape, deformation and ultimately movement through vessels.
Determination of the differences in the molecular distribution of mammalian and avian erythrocytes applying the same methodology is needed to understand the morphological differences that enable us to deduce functional differences in elasticity of the cells and viscosity of the whole blood, ultimately will help to understand and formulate hypotheses on the enucleation of mammalian erythrocytes.

A pilot O-PTIR measurements were applied to provide a detailed view of the nature of an inner structure of avian RBCs (zebra finches *Taeniopygia guttata*) compared do the non-nucleated mammal ones (bank voles *Clethrionomys glareolus*). The results could provide valuable biochemical information about the molecular distribution within the individual mammalian and avian erythrocytes, what could be a source of crucial information for studying metabolic processes, predicting deformability and modes of blood circulation. This aspect is of a great importance providing novel understanding and hypotheses for our understanding of the enucleation of mammalian erythrocytes [4].

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O.5 - D - Biomedical Applications

Supervised learning of infrared spectral images for the differential diagnosis of subtypes of breast cancer

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Introduction: Breast cancer is the most diagnosed cancer in women and the leading cause of cancer-related deaths among the female population. Therefore, early diagnosis of breast cancer is crucial to improve patient care and limit its progression. The aim of this study is to explore the potential of infrared spectral imaging and machine learning for the early diagnosis of breast cancers, with a particular focus on the differentiation of two of its subtypes, i.e. Human Epidermal Growth Factor Receptor 2 (HER2+) and Triple-Negative Breast Cancer (TNBC) given that they are the most aggressive and associated with the poorest prognosis.

Materials and Methods: Infrared spectral images were acquired at a spatial resolution of 6.25 µm/pixel, within the spectral range of 900 to 1800 cm^{-1}, from 8 µm serial tissue sections from 33 cancer patients (18 HER2+ and 15 TNBC) and 14 benign patients who underwent curative surgery without chemotherapy.
In a first part, the spectral images were corrected from the contributions of water vapor and carbon dioxide, then denoised by a Savitzky-Golay filter, and finally preprocessed by Extended Multiplicative Signal Correction (EMSC) to neutralize baseline effects and lipid contributions by normalizing all spectra around a reference spectrum, typically the average tissue spectrum.
In a second part, the tissue regions in each image were automatically selected using the clustering algorithm Kmeans. Then, recent and popular supervised learning algorithms, i.e. deep learning and LightGBM, were trained and optimised first to differentiate benign versus cancer samples (8 benign and 11 cancer patients in the train set, and 4 benign and 22 cancer patients in the test set), then to differentiate the HER2+ and TNBC subtypes among the cancer samples (11 HER2+ and 9 TNBC patients in the train set, and 7 HER2+ and 6 TNBC patients in the test set).

Results and Discussion: For each classification task, 10 different models were optimized from a different random distribution of patients in the training and validation sets. For model optimization, cross-validation was used by calculating the number of accurately classified patients for each parameter combination. Specifically, for a given patient in the validation set, the model's prediction is calculated, and the patient is assigned to the class where the majority of its spectra (pixels) are predicted. The test set remains fixed and unchanged for all 10 models. In order to evaluate the performance of the models and in order to exploit the diversity of these 10 models, ensemble learning by majority voting was implemented in this work in order to classify the patients in the test set. A patient is classified into the class that is most frequently predicted across the 10 models.
This methodology was tested for classifying benign and cancer patients, as well as for distinguishing between TNBC and HER2+.
For the classification between benign and cancer patients, a sensitivity of 100% and a specificity of 100% were achieved on the test set, proving the efficiency of infrared spectral imaging combined to recent supervised classification methods for the diagnosis of breast cancer from suspect benign samples.
For the TNBC versus HER2+ classification, HER2+ test samples were correctly identified in 86% of cases and TNBC test samples in 83%, proving the ability of infrared spectral imaging to exploit the tiny spectral differences between these two breast cancer subtypes.
Whatever the used supervised classification algorithm, i.e. deep learning or LightGBM, both gave the same results on the test set. However, deep learning being much more time-consuming, LightGBM seems to be a better choice for this kind of application.

Conclusions: The combination of infrared spectral imaging and recent and powerfull supervised classification algorithm is able to automatically identify cancer samples from benign ones, and more interestingly to differentiate HER2+ from TNBC samples. In a future work, this methodology will be extended to the identification of another breast cancer subtype, named luminal tumor. However, this more complex problem will require the construction of a more consequent dataset, by the acquisition of numerous spectral images or by implementing data augmentation strategies.

O.6 - D - Biomedical Applications

The impact of xanthophylls on brain and breast cancer development

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Cancer is one of the most common diseases that causes death to 10 million people each year according to WHO. [1] This disease needs a balance between enzymes, which control the number of carotenoids and their metabolites. Every imbalance might cause a change in cancer development. These imbalances can be monitored by using Raman imaging. Raman techniques are non-invasive for the cell and allow tracking the metabolism of various compounds, as well as carotenoids. [2] Carotenoids are a group of more than 700 compounds that can be divided into hydrocarbon carotenes and xanthophylls. Xanthophylls are compounds that contain oxygen atoms. They are found in plants such as saffron and algae. Xanthophylls are metabolized into retinoids in the human body. [3] The metabolism of xanthophylls as carotenoids is extremely important for humans, as these compounds exhibit valuable properties. [4] Xanthophylls are a large group of chemical compounds. In our study, we investigated the effects of crocin and fucoxanthin on brain and breast cells, lutein on brain cells and crocetin on breast cells. Our research concerns the diagnostic potential of xanthophylls in brain and breast cancer cells. The main aim of the research is to understand cancer mechanisms related to the supplementation of crocin, fucoxanthin, lutein and crocetin by Raman imaging in normal physiology and pathophysiological states. Cells were supplemented with selected compounds at different concentrations and incubated for 24 or 48 hours. We used Raman imaging to determine the qualitative and quantitative composition of the cells. The data were analyzed using statistical methods such as ANOVA or PLS-DA test. We observed that the compounds from the xanthophyll group show effects on cell metabolism. Xanthophylls change the amount of lipids and proteins and alter the redox state of cytochrome *c*. These changes may be related to the process of apoptosis and thus to the regulation of cancer development. The Raman imaging and spectra of brain and breast cancer cells are presented in Figure 1.

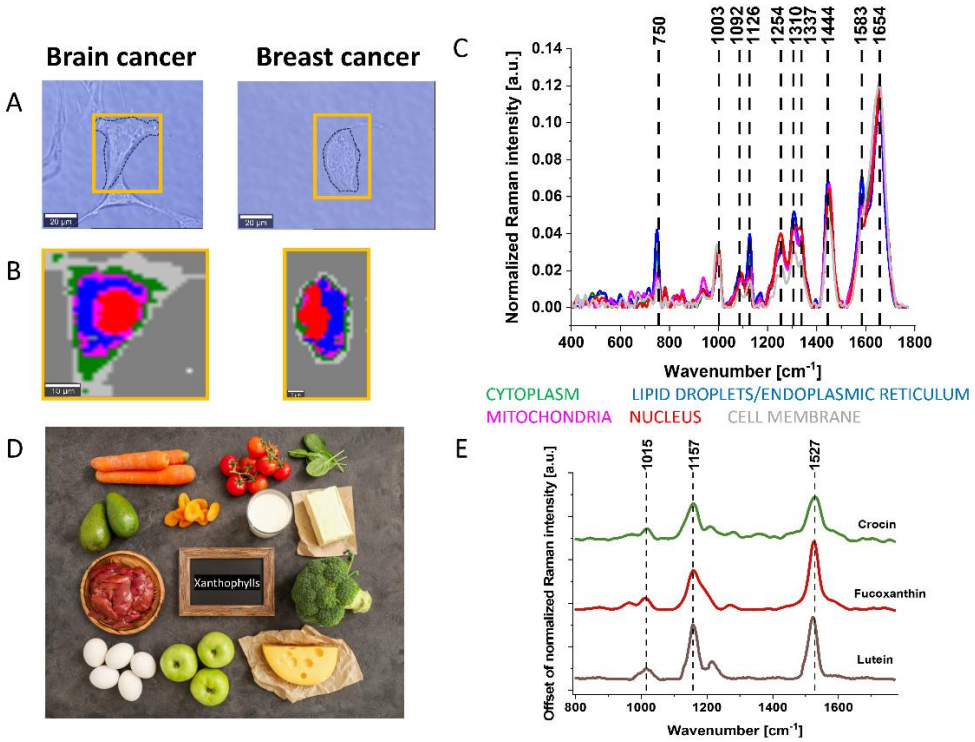

Figure 1. *The microscope image of the brain and breast cells (A) and corresponding Raman images (B), representative normalized Raman spectra of each cell's organelles (C), carotenoids sources (D) and Raman spectra of crocin, fucoxanthin and lutein (E).*

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O.7 - D - Biomedical Applications

Processing of Raman spectra by trajectory inference: applications to the study of adipocyte differentiation and to the characterization of cancer-associated adipocytes

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Cell heterogeneity is a crucial parameter for understanding the complexity of numerous biomedical issues. Trajectory inference-based approaches are recent tools developed for single-cell transcriptomics (scRNA-seq) data analysis. They aim to reconstruct evolving pathways from the variety of cell states that coexist simultaneously in a cell population.

We adapted trajectory inference to Raman spectra of cells, especially adipocytes. Partition-based graph abstraction algorithm (PAGA) combined with the Uniform Manifold Approximation and Projection (UMAP), was first applied on a Raman-like simulated dataset and on a Raman dataset collected during the differentiation process of 3T3-L1 cells into adipocytes. The objective of this first application was to illustrate the interest of trajectory inference for resolving dynamic and evolving processes [1]. The calculation of a pseudo-time permitted to highlight the cellular heterogeneity of the differentiation process by performing an artificial synchronization of the cells on the basis of their Raman profile.

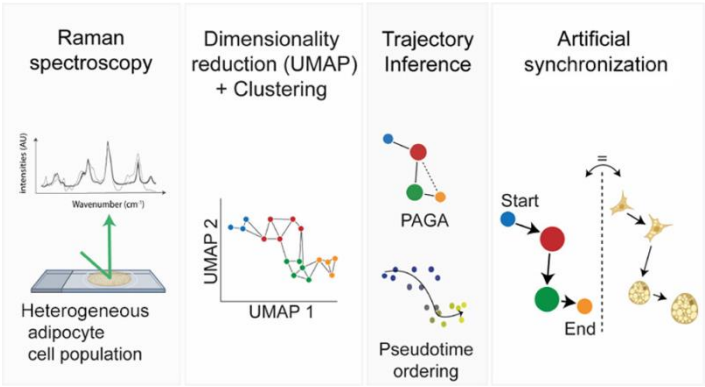

Figure 1. *Schematic representation of the workflow implemented for trajectory inference processing of adipocytes Raman data. Taken from [1]*

In a second step, this method was used to investigate the interactions between 3T3-L1 differentiated adipocytes and MDA-MB-231 breast cancer cells in a 2D co-culture model. This model permits to mimic cancer-associated adipocytes, known to be involved in the cancer progression and therapy resistance. Our experiments revealed the existence of subpopulations of adipocytes and the molecular changes associated with CAA phenotype.

Trajectory inference are interesting tools to exploit spectral data of biological systems by deciphering the inherent heterogeneity of the samples and by unraveling dynamics of the biological processes.

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O.8 - D - Biomedical Applications

Revealing nanoscale properties of plasma membrane vesicles using the combination of spectroscopic modalities

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Cell-derived plasma membrane vesicles can be considered as the most physiologically relevant model systems to study native membrane composition and architecture [1]. Abnormal cell membrane structure accompanies neoplastic transformation and subsequently is a hallmark of tumor cells. Alterations in membrane composition can lead to significant changes in its biophysical properties, what supports higher resistance to anticancer treatment [2]. For now, there is no specific biophysical or biochemical profile for cancer cell membranes, mostly due to limited approaches for both nanoscale and spatial characterization of such thin and flexible layers.

Here we propose the use of atomic force microscopy working in the force spectroscopy mode to analyze nanoscale mechanical properties of normal (microglia) and cancerous (glioblastoma) cell lines (Fig. 1A). For the first time, we will reveal that the mechanical properties of the plasma membrane vesicles (Fig. 1B) resemble properties of the cells they origin (Fig. 1C). Further, we will describe the differences in vesicles biomolecular composition based on FT-IR data and PCA analysis. Finally, we will show that the combination of atomic force microscopy and IR spectroscopy for native plasma membrane studies unveils extreme local heterogeneity of the plasma membranes that could not be otherwise captured (Fig. 1D).

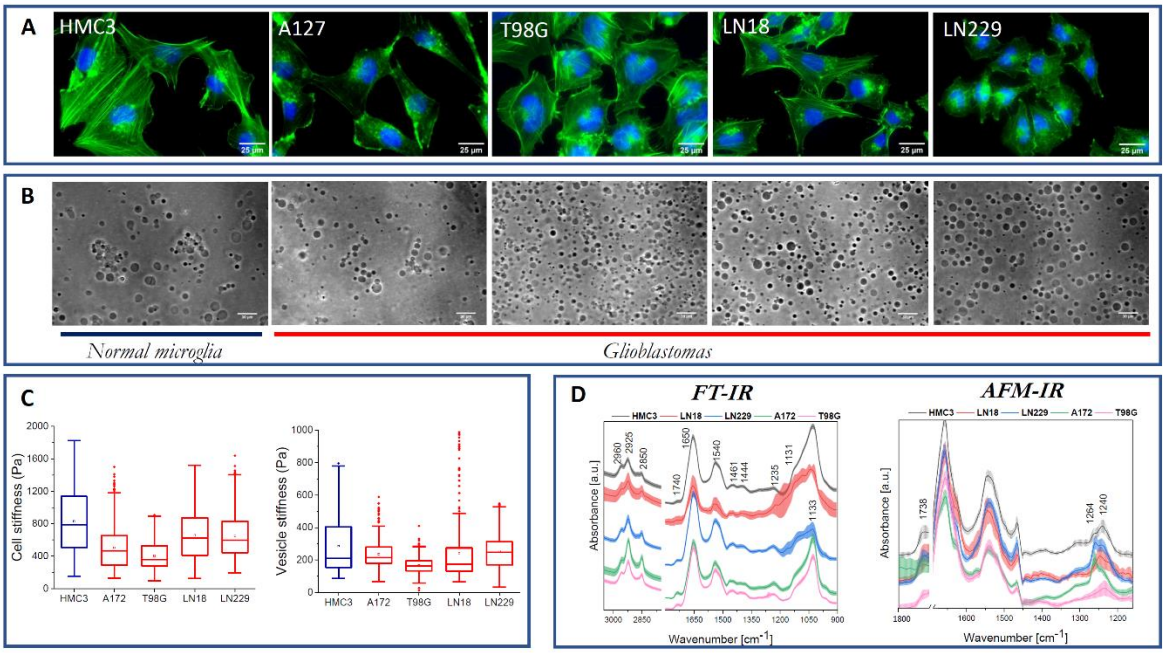

Figure 1. *(A) Fluorescence images of normal microglia (HMC3) and glioblastoma cell lines (A172, T98G, LN18, LN229). (B) Optical images of the membrane vesicles isolated from live cells. (C) The mechanical properties of cells and isolated vesicles measured using atomic force microscopy. (D) Averaged FT-IR and AFM-IR absorption spectra of the vesicles isolated from microglia and glioblastoma cells (shading denotes standard deviation).*

Acknowledgments

This work was supported by the National Science Centre of Poland under research project no UMO-2021/42/E/ST4/00407.

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O.9 - D - Biomedical Applications

Rapid Identification of Viral and Bacterial Infections, and Assessment of Antibiotic Efficacy in Bacteremia Patients through a Simple Peripheral Blood Analysis: Expert System Employing Infrared Microscopy of White Blood

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Across history, infectious diseases have remained a constant challenge, impacting both health security and societal advancement by significantly increasing rates of morbidity, disability, and mortality. The shared symptoms of bacterial and viral infections complicate the precise diagnosis of these illnesses. Bacteremia is a grave concern, especially for oncology patients with weakened immune defenses. Bacteremia represents a grave peril, with the potential to induce severe health complications, including organ infections and life-threatening conditions such as sepsis. Swift and accurate diagnosis, followed by timely intervention, is paramount to curtail the propagation of infection and alleviate ensuing risks. This enables promptly administering specific antibiotics to effectively counteract the invading pathogens. Precise identification of the underlying cause of infectious diseases is essential for successful patient management. The varied clinical presentations of infections pose challenges for conventional diagnostic approaches to reliably identify the causative agent. Present diagnostic techniques, particularly for inaccessible infections, are time-intensive, prompting physicians to resort to subjective evaluations and sometimes unnecessary antibiotic prescriptions.

Over the past decade, immune checkpoint inhibitors (ICIs) have emerged as a prevalent cancer treatment option [1]. Recent research underscores the pivotal role of the gut microbiome in influencing cancer responses to immunotherapy, highlighting concerns about the detrimental effects of antibiotics on treatment outcomes [2]. Dr. Pinato's study emphasizes the need to reassess the timing of antibiotic use in cancer patients, particularly those undergoing ICIs within NHS treatment protocols [3]. The urgent need for rapid bacterial infection diagnosis underscores the importance of cautious antibiotic prescribing practices, given the complexities in distinguishing between bacterial and viral infections [4,5].

Our hypothesis suggests that distinct immune responses to infections prompt molecular changes in white blood cells (WBCs). We employed mid-infrared spectroscopy for swift and precise diagnosis. Blood samples from individuals under 18 were taken, WBCs were isolated, and infrared spectroscopy was used for examination. Machine-learning algorithms then differentiated between bacterial and viral infections based on infrared absorption patterns of WBCs. Additionally, we evaluated the effectiveness of antibiotic treatment in febrile pediatric oncology patients with bacteremia [9].

Our research indicates that our approach yielded a sensitivity of over 94% and a specificity of more than 90% in differentiating between bacterial and viral infections within just one hour of sample collection, applicable to both accessible and inaccessible cases. Notably, our method identified over 23% inaccuracies in the physicians' subjective assessments of inaccessible infections. Preliminary results suggest that the integration of infrared spectroscopy and machine learning offers a promising avenue for monitoring antibiotic therapy effectiveness, with subsequent outcomes demonstrating an accuracy exceeding 87%. This innovative strategy facilitates the rapid detection of treatment failure, indirectly contributing to the mitigation of antibiotic resistance progression.

In summary, our investigation illuminates the profound potential of infrared spectroscopy and machine learning in revolutionizing the swift and precise diagnosis of infectious diseases. This breakthrough paves a bold new path toward the creation of highly effective and precisely targeted treatment strategies.

Acknowledgment: This research is supported by the ISRAEL SCIENCE FOUNDATION (grant No. 1087/20).

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O.10 - D - Biomedical Applications

Raman probes – a spectroscopic approach to study cell metabolism *via* organelle-specific compounds active in the 'silent region'

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Raman microscopy is known to be a versatile tool that is used in a wide range of applications. One of them is the exploitation of the chemical biology of various systems, from the complex tissues to the elemental living units, i.e. cells. Investigation of biosystems can be conducted in two ways. The first and most common one does not require the application of any external labelling compounds; thus, it is called a label-free manner. The second way depends on the usage of Raman probes (Rp, also called Raman tags or reporters). These compounds permeate through the cellular membrane and, due to the presence of appropriate chemical moieties, bind to the specific organelle or enter the particular metabolic pathways. However, from the diagnostic point of view, the most important feature of Rp is the presence of characteristic moieties that are active in the Raman silent region (1800 cm^{-1} – 2800 cm^{-1}). The scheme presented in Figure 1 shows the most common chemical groups present in Rp used for the examination of biological systems, which exhibit characteristic bands in the silent region.

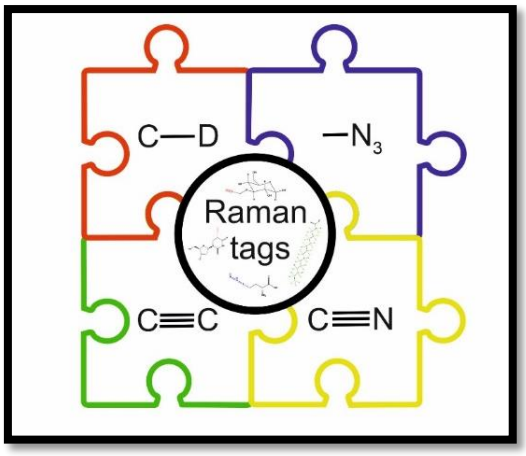

Figure 1. *Chemical moieties of Raman probes used to investigate cellular metabolism*

The main aim of this work is to demonstrate the applicability of Rp in the examination of cell activity and to present the new possibilities and development potential of this approach. We performed Raman imaging of different *in vitro* models, including cancer cells incubated with various Rp, chosen to track different metabolic pathways, mainly related to energy maintenance in cells. We used deuterium-substituted fatty acids (such as palmitic or oleic acid) to track lipid metabolism, and alkylated glucose (3-OPG) to track glucose metabolism. We performed single cell imaging using a WITec Alpha 300 microscope (WITec GmbH, Ulm, Germany) microscope equipped with an air-cooled laser with an emission wavelength of 532 nm. To visualize the incorporation of Rp into subcellular compartments, we utilized machine learning methods, such as K-means analysis.

Combining the specificity of binding or entering metabolic pathways with spectral activity makes Raman probes powerful and prominent tools for examining the metabolic activity of cells. Our study correlated the uptake of deuterated analogues of fatty acids with lipid metabolism. In turn, 3-OPG provided information on glucose metabolism in cancer cells [1].

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O.11 - D - Biomedical Applications

Single-cell Raman studies of the effect of ruxolitinib on JAK-mutated B-cell acute lymphoblastic leukemia cell lines

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Leukemia is a clonal blood malignancy hallmarked by transformed hematopoietic progenitor cells and scattered infiltration of bone marrow. The most common type of childhood blood malignancy is acute lymphoblastic leukemia (ALL) derived from B- and T-lymphoid progenitors. B-cell precursor ALL (BCP-ALL) constitutes 85% of total ALL cases and is classified into several molecular subtypes, characterized by peculiar and unique gene expression profile and various biological and clinical outcomes.[1] Some B-ALL subtypes are characterized by mutations in genes that encode components of the JAK/STAT signaling pathway, which regulates many essential cellular processes, such as hematopoiesis, apoptosis, and cell proliferation in response to extracellular factors, e.g. cytokines. Mutations lead to overactivation of JAK kinases, which phosphorylate STAT proteins responsible for activating transcription factors in the nucleus.[2] Thus, selective inhibitors of the JAK1/2 such as ruxolitinib (RUX) seem to be promising support for the treatment of ALL with JAK-STAT alterations. A method that shows a promising prospect in tracing the effect of anticancer drugs, such as RUX, on neoplastic cells is Raman spectroscopy. This non-destructive and label-free molecular technique has already been applied to reveal biochemical differences between leukemic and healthy cells.[3]

The aim of this study was to find spectroscopic features of RUX activity towards two B-ALL cell lines hallmarked with point mutations resulting in constantly active JAK2-STAT signaling pathway: MHH-CALL4 (JAK2 I682F mutation) and MUTZ-5 (JAK2 R683G mutation). As control, cell line without a mutation in JAK2 was used (SEM). The single-cell Raman spectra were analyzed using machine learning methods. Dimension reduction using PCA (Principal Component Analysis) and t-SNE (t-distributed Stochastic Neighbor Embedding) revealed grouping between treated and untreated cell lines carrying JAK2 mutation. Deeper classification with O-PLS-DA (Orthogonal Partial Least Squares Discriminant Analysis) confirmed significant differences between MUTZ5 and MHH-CALL-4 cells depending on whether they were exposed to RUX or not. Detected spectral markers indicating JAK2 inhibition under the pressure of RUX were DNA bands in the 780-800 cm^{-1} range. The analysis also confirmed no effect of Ruxolitinib on control cell line (SEM).

This work was supported by the “Label-free and rapid optical imaging, detection and sorting of leukemia cells” project and is carried out within the Team-Net program (POIR.04.04.00-00-16ED/18-00) of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

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O.12 - D - Biomedical Applications

Detection of diabetes-related macromolecular changes in human tissues using vibrational spectroscopy

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A complex framework of human health and disease relies more and more on biomedical research conducted as a combination of novel alternative methods, models and technologies. In the last 15 years, the beneficial role of some alternative approaches has been confirmed in basic and clinical studies on cancer, diabetes, cardiovascular diseases, Alzheimer's disease, etc. It is crucial to identify the types of alternative approaches, the potential and conditions for their use in biomedical research, and to analyze their strengths, weaknesses and limitations [1]. With this in mind, our work aims to investigate the extent to which infrared spectroscopy can reveal diabetic changes in the macromolecular composition of certain human tissues as an alternative to the gold standard of histochemistry.

Samples of four skeletal muscles and three fasciae were prepared from the tissues harvested during standard autopsies of diabetic and non-diabetic (control) male individuals. The ATR-FTIR spectra of the samples were measured at room temperature and pre-processed for further chemometric analyses using the MCR-ALS decomposition method to obtain spectral components and the corresponding weights, which served as the main parameters for the assessment of macromolecular composition [2]. The information obtained from the spectral components allowed the identification of various macromolecular constituents (Figure 1) and their proportion in the overall composition of the tissue.

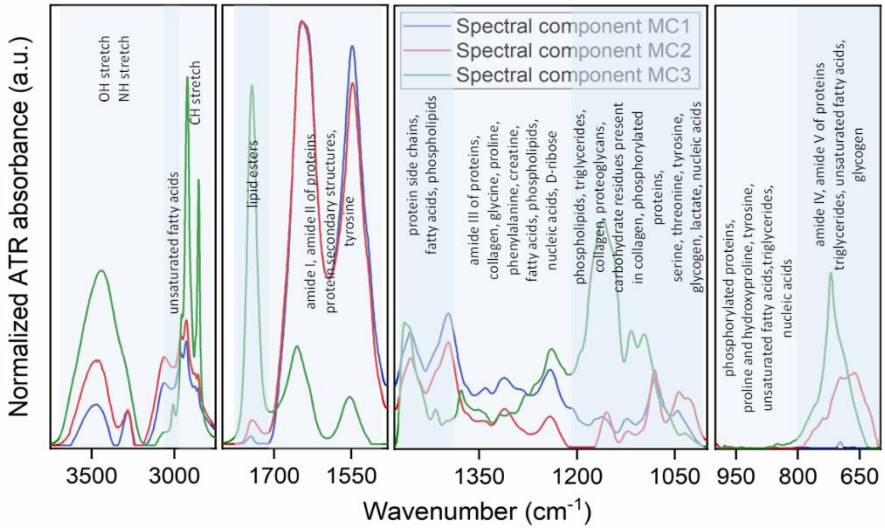

Figure 1. *Spectral components obtained by MCR-ALS decomposition with tentative assignment of vibrations bands*

On this basis, we analyzed how the macromolecular composition of the diabetic tissues evolved in comparison to the non-diabetic control tissues and gained a comprehensive insight into the trends of macromolecular modifications caused by diabetes. We have demonstrated the ability of infrared spectroscopy-based methodology to efficiently identify and quantify different macromolecular species that compose the tissue from a single analyzed sample in a single experiment. This work paves the way for the successful transfer of this methodology to large-scale studies.

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I.1 - E - Bioanalytical Applications

Isotope-edited Fourier-transform and nanoscale infrared spectroscopy for studying amyloid-β aggregation

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Two aspects of our work are presented: (i) a study of the local secondary structure and of inter-residue contacts in amyloid-β (Aβ) peptide oligomers and (ii) a scattering-type scanning near-field optical microscopy (s-SNOM) study demonstrating that peptides can be distinguished by isotope labeling in nanoscale images.
(i) Aβ oligomers are thought to be one of the causes of Alzheimer's disease but are difficult to study with common structural biology methods. Therefore we use a combination of Fourier transform infrared spectroscopy, site-specific labeling, and spectrum calculations to study the structures of 3 types of oligomers [1]. Our detergent-free oligomers (~110 kDa) incorporate V18, F20, A30 and I32 in β-sheets, F20 is close to A30 of the same monomer and to I32 of an adjacent monomer. In contrast, only A30 and I32 reside in a β-sheet in our two SDS-stabilized oligomers (~20 and ~60 kDa) and no proximity between different residues could be detected. The results clearly show that the local structure at V18, F20, A30, and I32 is different for different oligomers.
(ii) Interactions between molecules are fundamental in biology. They occur also between amyloidogenic polypeptides that are associated with different amyloid diseases, which makes it important to study their properties in mixed samples. However, addressing such research questions with imaging techniques is hindered by the problem to distinguish different polypeptides without adding artificial probes for detection, which might modify their properties and interactions. Here we show that ^{13}C, ^{15}N-labeling can be used to distinguish peptides in nanoscale images of their infrared absorption, even when they have similar secondary structure [2]. Using s-SNOM, we studied different aggregation states of Aβ (see Figure 1) and its interaction with an inhibitory, cell-penetrating peptide (NCAM1-PrP). Labeled and unlabeled peptides could be discriminated by comparing images of the optical phase taken at wavenumbers characteristic for either the labeled or the unlabeled peptide. NCAM1-PrP seems to be able to dissolve or associate with existing Aβ fibrils because "naked" Aβ fibrils are not detected after mixing.

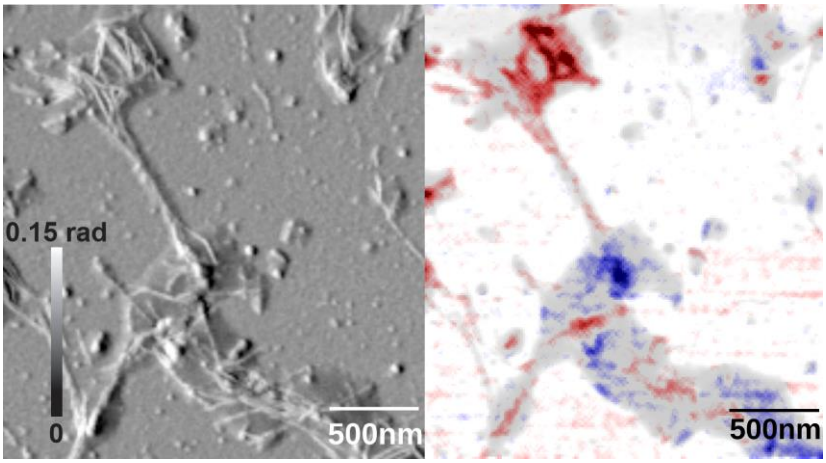

Figure 1. *Isotope-edited infrared nanospectroscopy reveals the distribution of chemically very similar peptides. Protofibrils of the 42-residue variant Aβ42 were unlabeled, whereas fibrils of the 40-residue variant Aβ40 were ^{13}C, ^{15}N-labeled. Left: mechanical phase image from atomic force microscopy, right: difference image of the infrared absorption at wavenumbers characteristic for either labeled or unlabeled peptide. Red areas contain predominantly the labeled Aβ40, which often forms fibrils. Blue areas consist mostly of the unlabeled Aβ42, which forms amorphous and small globular aggregates.*

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O.1 - E - Bioanalytical Applications

Library of Spectral Reporters of Metabolic Pathways: Metabolomics based on Multiplex Cell Labeling

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The comprehensive understanding of cellular metabolism is a cornerstone for medical research, paving the way for enhanced insights into the development of various diseases and leading to more effective treatment strategies[1]. Among numerous studies, spectroscopic techniques such as IR, Raman, and Coherent Raman Microscopy have emerged as versatile tools for detecting subtle changes in molecular composition, offering sensitivity at the macromolecular level [2,3]. The specificity of detection can be additionally enhanced by utilizing the Raman probes (Rp), i.e. chemical compounds with the specific tags based on isotope, alkyne, or azide group (C-D, C≡C, N_3), which show a characteristic signal in the spectral "silent" region (1800-2800 cm^{-1}). Due to specialized binding to molecules, the Rps can be metabolically incorporated into cellular active pathways and reflect changes in cellular metabolism under various physiological and pathological conditions. We present here the Metabolic Pathway Reporters Libraries and an overview of prospective Rps in terms of their use in studying the metabolic activity of cells, i.e. determining lipid (palm-15-yne), glucose (d_7-glucose), mitochondria (mitoBADY) and nucleus (EdU). Characteristic Rps band and the possibility of being used in multiplex have been demonstrated. We show **multiplex labeling**, utilizing more than one Rp to track multiple metabolic pathways, effectively avoiding overlap of Rp bands in the silent region of the Raman/IR spectra. This multiplexed approach opens the possibility of simultaneously tracking several metabolic pathways in different organelles, thus enabling the tracking of cellular metabolism. This also show the applicability of probe-oriented approach with the use of various spectroscopic methods, proving the complementarity and comprehensiveness of advanced cell imaging techniques in a labeled manner.

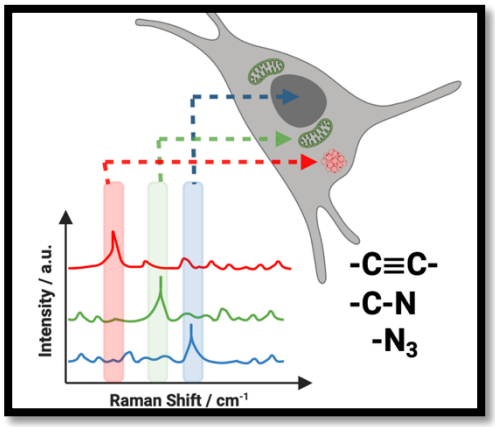

Figure 1. *Multiplex imaging to track several pathways of cellular metabolism.*

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I.1 - F - Bioanalytical Applications

Insights into intercellular interactions by FTIR and Raman spectroscopy imaging

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FTIR and Raman (RS) spectroscopies have been well-assed tools for investigating various biological specimens for several years due to their sensitivity to changes in biochemical composition and molecular structures. This has opened new strategies for probing cells and tissues, overcoming the limits of conventional techniques used in biomedical research, e.g. the recognition of external effects on the cellular functionality or the localization of abnormal cells in tissue. In this presentation, we summarize our FTIR and Raman-based methodology for following the intercellular interactions between cells to identify predictors of such contacts. We have established IR/RS parameters for clinical recognition of ischemic stroke, cancer invasiveness, and metastasis and those suitable for monitoring cell differentiation and cell therapies [1-5]. FTIR and RS spectroscopies combined with the imaging mode combine the ability to record molecular composition with the visualization of its spatial diversity. It is well-known that they deliver complementary information, and this feature is invaluable for biomedical applications. RS is sensitive to lipids, aromatic amino acids, nucleotides, and hemoproteins, while FTIR is more specific for secondary structures of proteins, esterified lipids, nucleic acids, and carbohydrates. FTIR spectroscopy imaging allows the measurement of a large sample area in a few minutes, whereas RS microscopy shows the spatial distribution and chemical composition of cellular compartments. It allows for enormous possibilities to investigate cells at various levels of their organization and in different environments and next for developing prediction models. We have shown that FTIR spectroscopy imaging how the phenotype of the cancer cells undergoes molecular transformations due to metastasis from the origin site and growth of the tumor. We developed an interesting approach to investigate the intercellular interactions between the progenitor cells used in cell therapies with the target of those therapies that are difficult to identify by conventional biochemical assays. This approach was successfully employed by us in monitoring stem cell differentiation and observing interactions in the blood-brain barrier.

ACKNOWLEDGMENTS
KM gratefully acknowledges the support from the National Science Center—Poland (OPUS 21, grant No. 2021/41/B/ST4/02000).

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I.2 - F - Bioanalytical Applications

Multimodal spectral imaging FTIR, Raman vs DESI and MALDI mass spectrometry imaging in diabetic lipid changes of rat kidney

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Diabetes is a major global health challenge, estimated to affect over 537 million adults, with a remarkable 80% of whom reside in low- and middle-income countries (LMICs). Type 2 Diabetes Mellitus (T2DM) accounts for over 90% of diabetes mellitus cases. This disorder results from two crucial factors: reduced insulin sensitivity in peripheral tissues and defective insulin secretion by pancreatic β-cells.
T2DM significantly impacts the kidneys and can lead to a condition known as diabetic kidney disease or diabetic nephropathy. This condition is one of the most common complications of diabetes and a leading cause of chronic kidney disease and kidney failure. It is mainly caused by elevated blood sugar level, which damages the blood vessels located in the kidneys. Due to the commonness of this condition the novel biomarkers for the early detection of kidney disease are needed. The aim of this study was therefore to find the changes in the kidneys caused by T2DM. Those changes might serve as the early biomarkers of kidney disease in the future.
Methods:
Fourier-transformed infrared (FTIR) spectroscopic imaging, Raman spectroscopic imaging, desorption electrospray ionization (DESI) mass spectrometric imaging and matrix-assisted laser desorption/ionization (MALDI) mass spectrometric imaging were used for the identification of the metabolic changes in the kidney tissues. FTIR spectroscopic imaging samples were subjected to AdaBoost classification with a decistion tree classifier estimator. The classification was validated using leave-two-samples-out-cross-validation. The wavenumbers that impacted the classification the most were extracted to obtain the potential biomarkers of early kidney disease. Raman spectroscopic imaging, DESI mass spectrometric imaging and MALDI mass spectrometric imaging data were used for the observation of the distribution changes of the metabolites (lipids in case of mass spectrometry).
Results:
AdaBoost classification of the FTIR data showed the mean F_1 score of ~0.75. The wavenumbers that impacted the classification the most were at 1026, 1184, and 1701 cm^{-1} These bands might be assigned to C-O stretching of carbohydrates, PO_2^- asymmetric stretching of phospholipids and C=O stretching of carboxylic acids and esters (lipids). Raman spectroscopic imaging showed differences in the distribution and concentration of lipids. DESI mass spectrometric imaging and MALDI mass spectrometric imaging showed change in the distruibution of selected lipids species, especially phosphatidylcholines, sphingimyealins, phosphatidylinositol and lyso-phospatidylcholine
Conclusions:
In this study we were able to find the impact of T2DM on the rat kidneys. The metabolic changes are mainly on the lipid concentration and distribution on those organs. Further studies are needed to obtain potential biomarkers for early detection of diabetes-associated kiney disease

Funding:
This work has been achieved as part of a grant from the National Science Centre, project SONATA "Synergy of chemical imaging methods in diabetic model" (project UMO-2020/39/D/ST4/01604) and within statutory activity of Medical University of Lublin (DS 642 project).

O.1 - F - Bioanalytical Applications

Obtaining radiation effects on biomolecules – FT-IR and AFM-IR cellular investigations

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Radiotherapy is one of the most common approaches for cancer treatment, especially in the case of peripheral nervous system tumors. As it requires exposure to high doses of ionizing radiation, it is important to look for substances that support efficient reduction of the tumor volume with simultaneous prevention of the surrounding non-cancerous cells. Cannabidiol (CBD), which exhibits both anticancer and neuroprotective properties, was applied as a potential modulator of radiological response, however its influence on cells undergoing irradiation remains elusive. Here we have applied high-resolution optical spectroscopy techniques to capture biomolecules associated with cannabidiol`s shielding of normal and damaging of cancerous cells upon X-ray exposure.

Presented studies compare the capabilities of the conventional FT-IR global screening and detailed nanoscale AFM-IR in monitoring of selected biomolecules accumulation and distribution upon radiotherapy and cannabidiol treatment. FTIR analysis, which offers ~1µm spatial resolution, provided overall semi-quantitative information about the alterations in DNA, cholesteryl esters and phospholipids content, but was not sensitive to local conformational changes. At the same time, AFM-IR spectra determine not only the variations in the ratio of biomolecules, but also in their conformation caused by applied therapeutic scheme. Besides, AFM-IR evaluation provided information about the cell morphology and chemical distribution of cholesteryl esters with 40 nm spatial resolution (Fig. 1). Based on the obtained results we propose label-free and fast analytical method engaging optical spectroscopy to assess the mechanism of normal and cancerous cells susceptibility to ionizing radiation when pretreated with CBD.

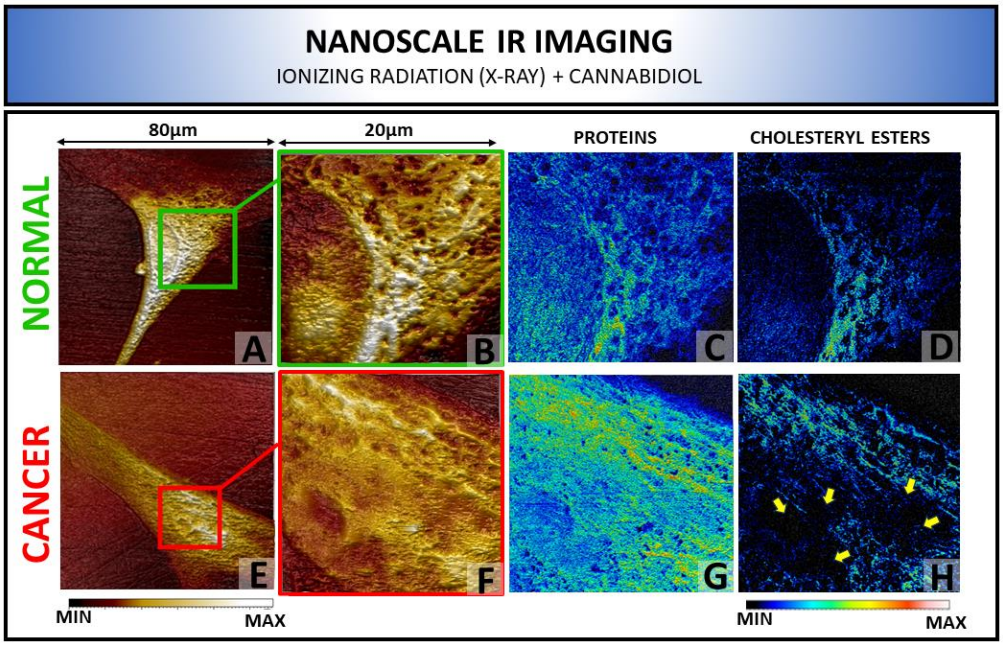

Figure 1. *The comparison of AFM-IR topography images [A, B, E, F] and intensity maps for the proteins (1650 cm^{-1} band)[C, G] and cholesteryl esters (1740 cm^{-1} band) [D, H] distribution for normal [Schwann cell line, top panel] and cancer cell [Malignant Peripheral Nerve Sheath Tumor cell line, bottom panel] affected with CBD and 10 Gy X-ray exposure dose.*

Acknowledgments

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O.2 - F - Bioanalytical Applications

Laser spectroscopy in studies on biomolecules linked with genetic and neurodegenerative diseases

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To successfully treat genetic and neurodegenerative diseases there is an emerging need for the early detection of disease signs long before the cognitive symptoms occur[1].
Among the specific DNA cavities there are a few of high biological significance, including i-motifs and G quadruplexes (G4). Their genetic relevance was found in the expression of tumor cells, gene regulation, or cell division.
In the case of Alzheimer's disease, the main perpetrators are believed to be small mobile protein aggregate forms called amyloid oligomers. It indicates that degenerative processes occur at the sub-cellular level during the protein misfolding.
A common and widespread method to detect specific biomolecular structures is fluorescence. For that purpose, biomolecules are stained with organic fluorophores. However, fluorescence has limitations, for example in detecting transient states of early-stage protein aggregates or local distortion of DNA helix.
To boost the fluorescence sensitivity one can amplify light in the process of stimulated emission[2]. Lasing in fluorophore-stained amyloid proteins and DNA G-quadruplexes has orders higher detection sensitivity than standard fluorescence, thus it helps to reveal critical molecular structures involved in disease development [3,4].
Lasing was detected in different states of protein aggregation. The appearance of lasing thresholds were used for recognition of various DNA structures. Finally, light amplification was examined in the recombinant proteins seeded with human cerebrospinal fluid (CSF) as well as strongly scattering tissue doped with amyloids[4]. By monitoring laser emission a remarkable recognition sensitivity to biomolecules linked with diseases can be achieved. Thus, in contrast to fluorescence, lasing can be used to detect and differentiate specific biomolecular structures and evaluate the risk levels of genetic and neurodegenerative diseases in potential patients before the clinical symptoms occur so that patients can receive rapid information about their health condition and have a better outcome in the therapy.

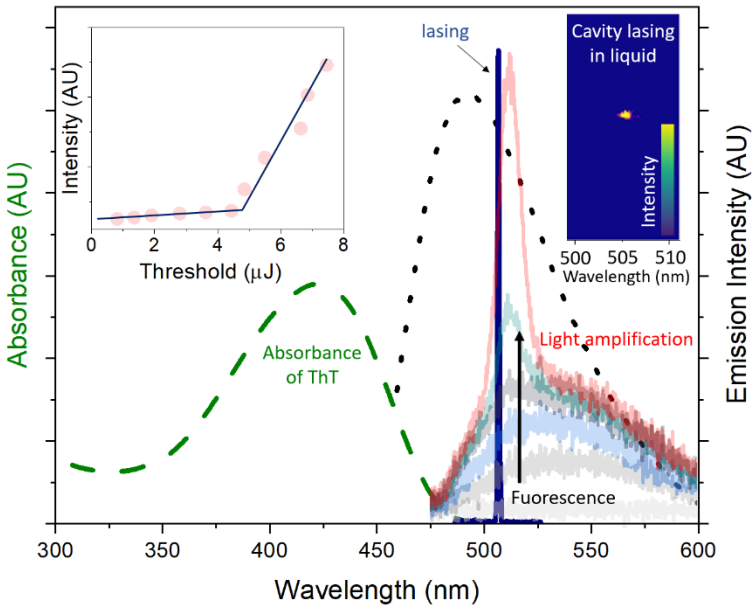

Figure 1. *Absorbance (green dashed), emission (black dotted), light amplification process upon increasing excitation energy (without cavity) and lasing in cavity (dark blue solid) whereby light is enhanced by the multiple mirrors reflections. The left inset shows the rise of emission energy and threshold energy whereby light is amplified. The right insets show lasing readout in the camera. Modified graph taken from ref. [2].*

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O.3 - F - Bioanalytical Applications

Pesticide Residual Analysis of Lambda cyhalothrin by Surface Enhanced Raman Spectroscopy, Comparison of Silver Nanodendrites and Silver Nanosphere Substrates

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Surface-enhanced Raman spectroscopy (SERS) is one of the several modern analytical spectroscopic techniques which are used for qualitative and quantitative detection of variety of chemical and biochemical analytes. Ag NPs offer several-folds enhancement of Raman signals of analyte in SERS.[1] Ag NPs can be synthesized in different morphologies. In this study, the effect of different morphologies of silver nanoparticles (Ag NPs) for SERS analysis of pesticide residue, lambda cyhalothrin was studied using Ag-nanospheres [2] and Ag-nanodendrites substrates.[3]

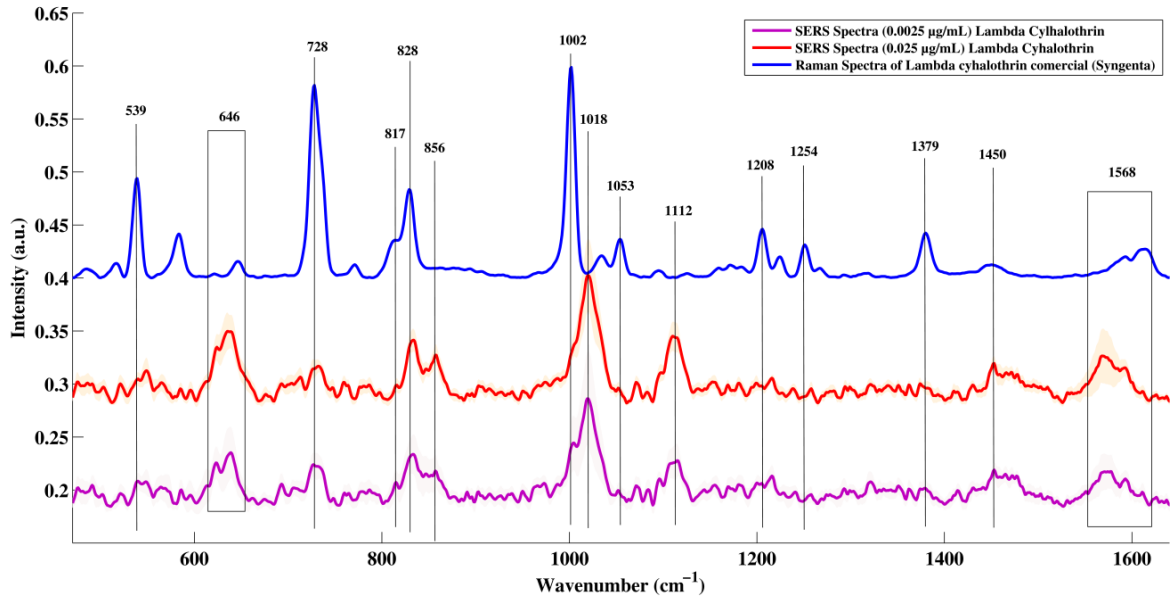

Figure 1. *Mean Raman spectra (Blue) of Lambda Cyhalothrin commercial25g/Lw/v (Syngenta), (Red) SERS spectra of (0.025µg/mL) Lambda Cyhalothrin and (Magenta) SERS spectra of (0.0025 µg/mL) Lambda Cyhalothrin using silver dendrites as substrate.*

Capability of both morphologies of the Ag NPs as SERS substrates in identifying the pesticide molecule and differentiating the trace level concentrations was tested by using our in-house synthesized Ag-nanospheres and Ag-nanodendrites. SERS spectral features were obtained at 0.025 and 0.0025µg/mL concentrations using both morphologies of Ag NPs and compared with the Raman spectral features obtained at commercial concentration of Lambda cyhalothrin for testing the potential at trace level identification of pesticide. Ag-nanodendrites were found more promising for both trace level identification by generating more robust SERS features and for distinction in trace level concentrations of the Lambda cyhalothrin in principal component analysis (PCA).

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O.4 - F - Bioanalytical Applications

Detection and segregation of biological molecules by droplet deposition method in Raman spectroscopy

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The droplet deposition method is based on the liquid droplet deposition of analyte on a suitable (generally hydrophobic) solid surface. When drying, the flow in a liquid sample carries dispersed material to the droplet edge, and it forms coffee-ring or small spot patterns where the analyte is pre-concentrated [1,2]. This approach itself receives considerable attention in sensitive detection using Raman spectroscopy when measuring classical spectra from locations with strong analyte pre-concentration. Moreover, even higher sensitivity can be obtained in combination with surface-enhanced Raman spectroscopy.
This contribution will focus on advanced applications of droplet deposition on biological molecules. Two main advantages will be principally emphasized: the detection sensitivity of various analytically important molecules and the drying-induced segregation of the components from the mixtures (such as body fluids). For example, recently, we reported the sensitive detection of melamine together with the spatial segregation of lipids and carbohydrates from pure infant formula into dried patterns [3]. This was confirmed by Raman spectral mapping from the dried mixture on a hydrophobic commercial microRIM substrate. Lipids tended to accumulate in the coffee-ring, and carbohydrates formed the thin layer in the central part of the ring (Figure 1). The same separation in the dried pattern was also observed for melamine-blended infant formula, where melamine was detected only from the thin central layer together with carbohydrates due to the melamine glycation by lactose. Therefore, we could profit not only from the efficient pre-concentration and drying-induced spatial separation but also from the chemical reaction potential of molecules of interest present in a complex solution or suspension.

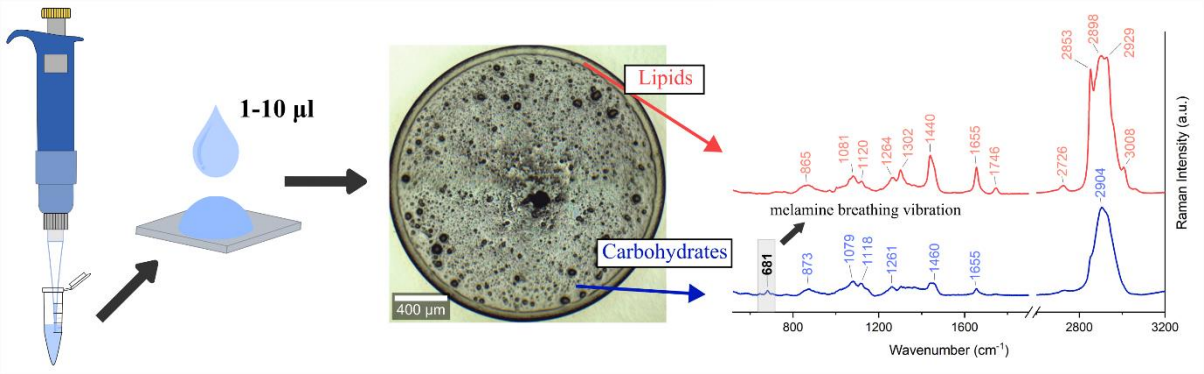

Figure 1. *Example of drying-induced segregation of lipids and carbohydrates from milk infant formula: Deposition of a small volume of analyte in the form of a solution or suspension using a micropipette on a hydrophobic substrate and subsequent drying and Raman spectra acquisition.*

This work was supported by the Charles University (SVV-2023-260716).

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O.5 - F - Bioanalytical Applications

Targeted kinetic Raman microspectroscopy of extracellular medium to elucidate glycolysis pathway kinetics.

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The potential of kinetic Raman microspectroscopy to monitor the glycolysis pathway kinetics as a function of time was explored in this study. The spectroscopy was primarily targeted towards quantification of the extracellular carbon source glucose and the extracellular glycolytic byproduct lactate, to elucidate the glycolysis pathway kinetics. Initially, the limits of detection (LOD; 0.85mM: glucose, 2.8mM: lactic acid) and quantification (LOQ; 2.5mM: glucose, 9.5mM: lactic acid) for the pure individual metabolites in the biological range were elucidated to test the sensitivity of the approach (Figure 1). Further, mammalian cells were cultured in multi-well plates in simple salt medium with glucose as a sole carbon source and kinetic spectra were acquired as a function of time. The spectra were resolved and fitted with a kinetically constrained-model using the multivariate curve resolution- alternating least squares (MCR-ALS) tool to elucidate the pathway kinetics and the rate of change. A demonstration of the MCR resolving complex spectrum of glucose and lactic acid in a mixed sample is shown in Figure 2A and when the varying concentrations were overlaid on a simulated time-scale the ALS showcased the evolution of individual components as a function of time (Figure 2B). The technique is superior to the conventional kinetic assays capable of monitoring the pathway kinetics such that it can monitor the start and the end product, thereby increasing confidence in the analysis. The approach has potential applications for high-content drug screening for drug discovery. Furthermore, the MCR-ALS toolbox can potentially be used for sub-cellular spectroscopic data for elucidating metabolic kinetics form the kinetic spectra of the complex cell soup using spectralomics approach based on a kinetic model optimised by the presented data. Kinetic Raman microspectroscopy showcases potential applications for drug discovery, disease diagnosis, bioprocess monitoring and optimisation, etc. This approach shows potential to overcome the limitations of the present omics approaches limited to a snapshot of cellular metabolism to kinetic high-content insights into the cellular metabolism as a function of time.

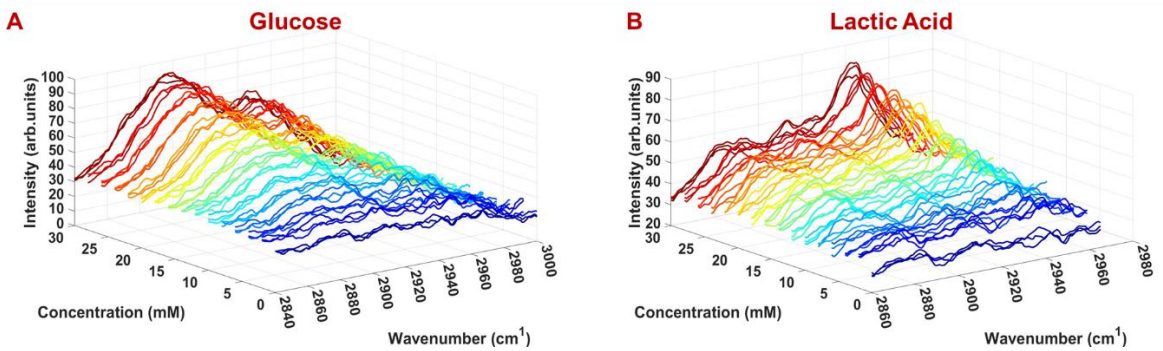

Figure 1. *Varying glucose (A) and lactic acid (B) concentrations in triplicates in the biological range for elucidating the limits of detection and quantification.*

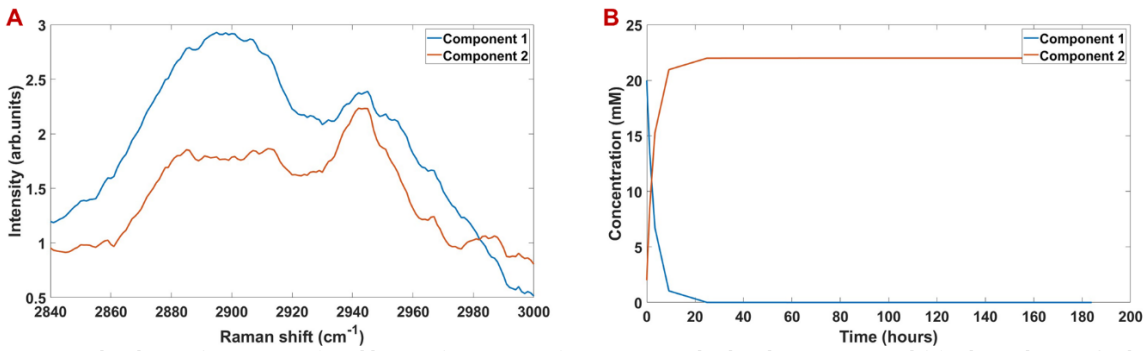

Figure 2. *The glucose (component 1) and lactate (component 2) spectrum resolved in the MCR-ALS tool (A). The evolution of individual components from 'A' on a simulated timescale (B).*

O.6 - F - Bioanalytical Applications

An effect of mercury toxicity and oxygen depletion on the main cellular components of the blood-brain barrier. FTIR and Raman microscopy study

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The blood-brain barrier (BBB) is a selective semipermeable cellular membrane composed of pericytes, astrocytes, and endothelial cells. They maintain and control the permeability and resistance of the BBB to external factors[1]. In this study, we investigated the effect of neurodegenerative factors such as Hg toxicity and oxygen depletion (hypoxia) on human BBB cells to establish the spectral markers unrevealed by conventional in vitro methods (Fig. 1). For this purpose, astrocytes (NHA) and endothelial cells (HMBEC) were cultured in hypoxic conditions (1% O_2) for 24 h or exposed to 20 μM $HgCl_2$ for 24h and next Raman and FTIR spectroscopy imaging was employed. Both factors lead to impairment and dysfunction of the blood-brain barrier and are in vitro models of neurodegeneration[2]. We aimed to discriminate the nuclei and cytoplasm in Raman and IR images and other cellular compartments in high-resolution Raman images. We showed that this approach of molecular imaging and data fusion is a very efficient method for observing subtle molecular changes in cellular metabolism and epigenetic alternation[3]. In general, the hypoxic conditions associated with brain injury induced several molecular modifications at each level of the cellular organization compared to mercury neurotoxicity. The endothelial cells lining brain microvessels were more sensitive to both factors than astrocytes. The most specific spectral markers are the oxidation of lipids associated with mitochondrial activity, the formation of β-sheet conformation in cytoplasm proteins, and the shrinking of chromatin. Our work shows the methodological approach of IR/Raman data analysis for spectroscopic in vitro studies and comparative studies of neurodegenerative effects.

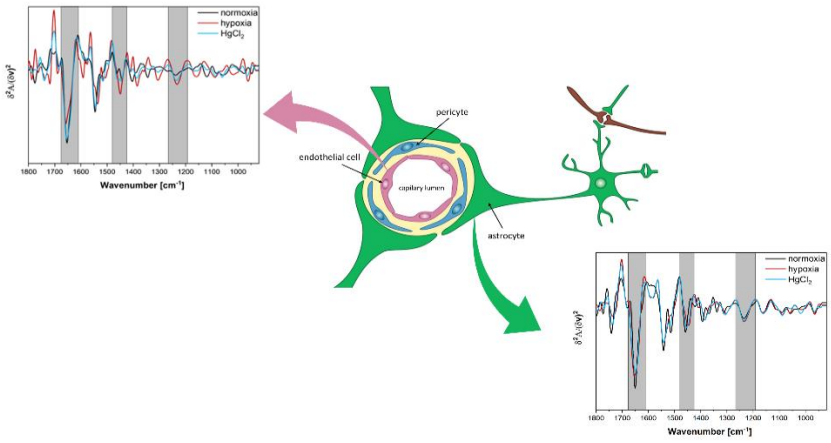

Figure 1. *Exemplary results from the spectroscopic analysis: second derivatives of average FT-IR spectra of the BBB components exposed to neurodegenerative factors.*

ACKNOWLEDGMENTS
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I.1 - G - Nanoscale Analysis

Raman micro-spectroscopy reveals the spatial distribution of fumarate in cells and tissues

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Aberrantly accumulated metabolites elicit intra- and inter-cellular pro-oncogenic cascades, yet current measurement methods require sample disruption and lack spatio-temporal resolution, limiting our ability to fully characterize their function and distribution. In this talk, we discuss how Raman spectroscopy can detect accumulated fumarate in living cells in vivo, and can distinguish between Fumarate hydratase (Fh1)-deficient and Fh1-proficient cells based on fumarate concentration. Moreover, Raman imaging reveals the spatial compartmentalization of fumarate within cellular organelles in Fh1-deficient cells. These results suggest Raman microspectroscopy could be adopted as a valuable tool for small molecule metabolic imaging, enabling in situ non-destructive evaluation of fumarate compartmentalization.

I.2 - G - Nanoscale Analysis

Fluorescence correlation spectroscopy: monitoring the mobility of (bio)molecules and nanoparticles at the nanoscale

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The fluorescence correlation spectroscopy (FCS) is a very sensitive and selective technique for studying the mobility of small fluorescent molecules, (bio)macromolecules or nanoparticles in various environments. FCS is based on monitoring and recording the fluctuations of fluorescence light intensity originating from species diffusing through a very small (< 1 μm³) observation volume, commonly created in confocal microscopy configuration (Figure 1). A correlation analysis of these fluctuations yields information on the diffusion coefficient of the studied species, their concentration and fluorescence brightness. During the last three decades FCS has become a powerful tool in fields ranging from polymer and colloid science [1] to nanocarrier based drug delivery [2].

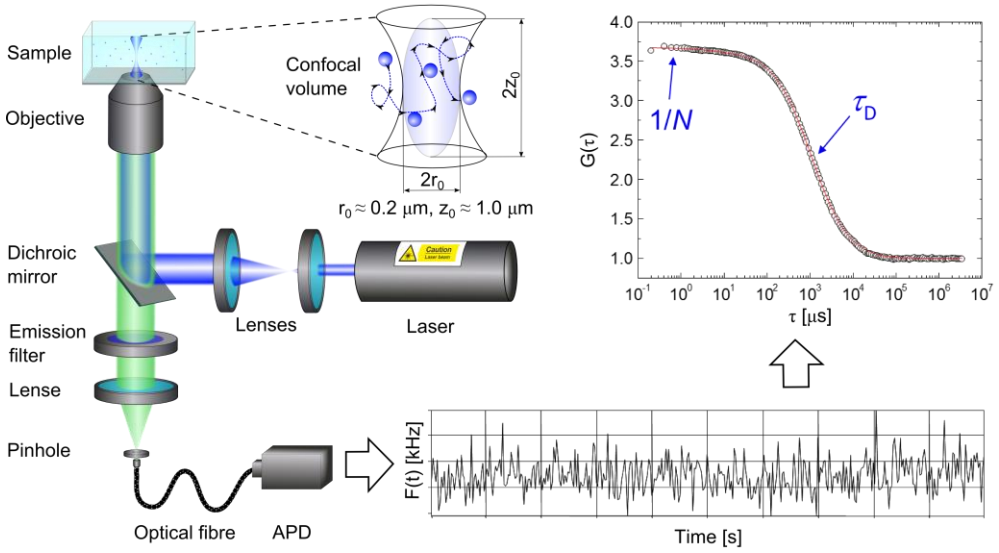

Figure 1. *Schematic representation of a typical confocal FCS setup and it's principle of operation.*

In this talk I will present some recent results of our group on using FCS to study various biomolecules and biological systems. We apply the method in two ways.
First, by monitoring the mobility of fluorescent tracers with known size we obtain information on the local viscosity of the surrounding environment, which may range from a living cell cytosol, to a hydrogel or a fluid interface. For example, we measured the diffusion coefficient of small fluorescent molecules (Alexa 488) in two types of liquid condensates of the fused in sarcoma (FUS) protein. The six-time slower tracer diffusion, measured in one of the condensates suggested a denser, more viscous environment at the molecular scale compared to the other condensate [3].
Second, we use FCS to measure hydrodynamic radius, fluorescence brightness and local concentration of fluorescently labeled (bio)macromolecules (polymers, proteins, RNAs, DNAs) and nanoparticles and thus investigate conformational changes, mutual interactions, aggregation, etc. Such FCS studies are especially useful to characterize drug nanocarriers (NCs) and allow monitoring of the carriers' formation, drug loading efficiency, stability and kinetics of drug release [2]. To reach their target sites, NCs need to circulate in the bloodstream for prolonged periods without aggregation, degradation, or cargo loss. However, it is very difficult to identify and monitor small-sized NCs and their cargo in the dense and highly complex blood environment that is not transparent for visible light. Thus, I will elaborate on two newly developed FCS based methods that allow NCs characterization in samples of whole blood [4,5].

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O.1 - G - Nanoscale Analysis

Empowering Environmental Sustainability Functionalized SBA-15 as a Cutting-Edge Solution for Effective Metal Uptake

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One of the most promising and modern solutions to combat environmental pollution is the development of bio-perforated, mesoporous SBA-15 silica. This material boasts an impressive surface area of approximately 800m2/g. It features structurally amorphous properties, forming uniaxially ordered hexagonal channels with a diameter of 5 nm and a length on the order of micrometers. These channels are uniformly distributed throughout the volume, allowing for high capillary properties. SBA-15 also demonstrates a neutral impact on the environment and living organisms, exhibiting no toxic or irritating effects. Furthermore, its physicochemical properties are easily adjustable through various technological processes, offering great flexibility in terms of chemical modification. This high degree of adaptability allows for the functionalization of SBA-15 with a wide range of functional groups, either on its outer or inner walls, while maintaining precise control over their concentration within the material's volume.
The significant advancement in environmental protection, as proposed by our research team, has been achieved by activating the mesopores using specific functional groups tailored for capturing specific types of metals. This functionalization involves the use of various functional groups, such as propyl-carbonate (for metal-binding I, e.g., silver), propyl-phosphate (for metal-binding II, e.g., copper), and cyclam (1,4,9,11 tetraazacyclodecane), which is capable of chelating metal chlorides like copper, chromium, cobalt, nickel, and more. Importantly, this functionalization process ensures the homogeneous distribution of these functional groups within the silica pores.
The potential of metal ion uptake has been rigorously verified using advanced techniques such as SAXS, Positron-Electron annihilation, BET (Brunauer-Emmett-Teller), and spectroscopy methods like UV-VIS spectroscopy. Time-dependent metal uptake curves have been instrumental in estimating the real-time sorption capacity of functionalized SBA-15. Mechanical studies, including Young's modulus parameters at various force levels (ranging from nN to N), have been conducted on individually prepared SBA-15 pellets to assess their stability and mechanical properties. The combined efforts of functionalizing SBA-15 and evaluating its sorption potential through various methodologies aim to pave the way for developing an entirely new class of materials with unique properties for remediating contaminated environments. This research holds the promise of making a significant and positive impact on preserving our environment and protecting human health.

Acknowledgment
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O.2 - G - Nanoscale Analysis

AFM–SEIRA nanospectroscopy for the molecular orientation studies

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In recent years, atomic force microscopy integrated with infrared spectroscopy (AFM–IR) has been extensively used in many different areas. [1] The application of the AFM tip as a detector of the infrared light absorption gave the possibility to break the diffraction limit and achieve the ultra–high spatial resolution of several dozen nanometers. Such a combination allows for studying the sample's topography together with its chemical composition.

Since IR spectroscopy is known to be sensitive for the surface plasmon resonance effect (SEIRA technique) such phenomenon was also expected for AFM–IR. Application of the metal substrates ensures twofold advantages – *(i)* enhancement of the spectral signal and *(ii)* determination of the molecular adsorption orientation. The latter feature is extremely important in the context of drug delivery systems development based on metal nanostructures.

Fig. 1 A illustrates the conventional AFM–IR and surface–enhanced AFM–IR (AFM–SEIRA) spectra of a small molecule–drug erlotinib before (red) and after (green) its adsorption on the silver nanoparticles (AgNPs) monolayer. The observed differences in the spectral features prove the existence of the AFM–SEIRA effect. The application of the well-known surface selection rules that govern the bands' enhancement in the SEIRA spectra [2] allows for the interpretation of molecular orientation based on the AFM–SEIRA data with ~15 nm spatial resolution. Additionally, AFM topography (Fig. 1B) together with the AFM–SEIRA chemical map (Fig. 1C) visualize the drug/metal monolayer and coupling resonance effect appearing between the neighboring nanoparticles.[3] These studies are the first to provide significant evidence of the surface plasmon effect in AFM–IR and open new possibilities for the characterization of molecular adsorption geometry on the metal nanosurface with spatial resolution previously unattainable for IR. [3-6]

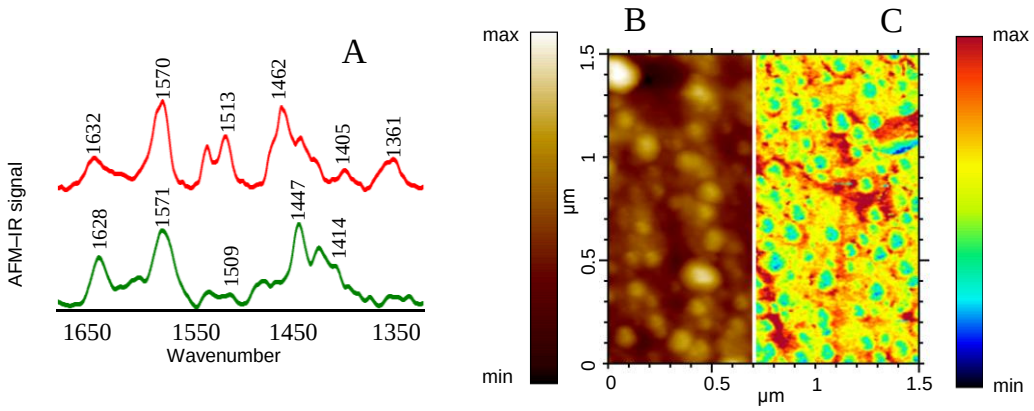

Figure 1. *(A) The AFM–IR and AFM–SEIRA spectra of erlotinib alone and after its adsorption on the AgNPs monolayer (red and green, respectively). (B) Surface topography of the studied nanosystem overlayed with (C) the AFM–SEIRA intensity map collected at 1571 cm^{-1} [ν(CC)$_{Phe}$ band of erlotinib].*

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This work was supported by the National Science Centre Poland (No. 2016/21/D/ST4/02178).

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O.3 - G - Nanoscale Analysis

Chiral separations of pharmaceutical compound by means of SERS: elucidating the role of molecular nanoscale interactions in this process

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The investigation of biofluids and pharmaceutical compounds by means of Raman and (Surface Enhanced) Raman Spectroscopy (SERS) has attracted much interest lately. The specific vibrational "fingerprint" allows getting information about structural properties of different molecules. Even in very complex biological media, SERS analysis of biofluids (*e.g.* blood serum, plasma, saliva, salivary exosomes, etc.) combined with AI assisted analysis of the recorded spectra allowed the development of early detection methods for several types of cancers [1].

Raman/SERS spectroscopy is gaining also popularity in different areas of pharmaceutical industry mainly due to its ability to provide information on the fundamental vibrational bands, also offering a high degree of specificity in analysis [2]. Nevertheless Raman/SERS investigation forms an ideal complement for existing methods of analysis such as nuclear magnetic resonance, mass spectrometry and elemental analysis.

On the other hand, chiral recognition and differentiation in living organisms represents one of the most intriguing natural phenomena, assuring a high-fidelity transfer of molecular information. This phenomenon has a significant role in the pharmaceutical industry, since chirality plays a key role in the development of target drug candidates, being a structural variable parameter that needs complete elucidation. In this context, the subject of chiral purity gained a particular importance in the broad field of biomedical applications.

In this presentation, we will show that by taking advantage of the unique capacities of some molecules (cyclodextrins - CD and cucurbituril -CU) to interact and form intermolecular host-guest complexes with different pharmaceutical compounds (propranolol -PRNL, atenolol - ATNL) their chiral separation was successfully achieved by means of SERS [3]. The quantum chemistry calculations of the nanoscale intermolecular complexes formed with the two host molecules have been used for a proper understanding of the interaction mechanism responsible for this separation. For instance, in the case of CDs it has been observed (experimentally and theoretically) that β-CD (compared with the other two classes of native α and γ CDs) had the best chiral recognition ability for propranolol enantiomers, hence producing the largest difference in the SERS spectra of propranolol enantiomers - native cyclodextrin complexes. A similar analysis has been performed in the case of complexes formed with CU molecules, both by PRNL and ATNL molecules.

The key role played by the plasmonic substrates (size, shape, chemical composition) involved in the SERS based chiral separation will be also discussed.

Acknowledgment: This work was supported by a H2020 grant – ERA-Chair, no 952390.

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O.4 - G - Nanoscale Analysis

Adiabatic Nanofocusing in Raman spectroscopy and Hot Electron Nanoscopy (HENs): principles and applications

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Adiabatic nanofocusing[1] refers the concentration of propagating plasmons (Surface, Plasmon Polaritons, SPP) in a tapered metallic structure (nanocone), allowing for virtually lossless energy transfer from the micrometer to the nanometer scale. The geometrical requirements for application in the visible range allowed the direct exploitation of the phenomenon by incorporating the nanocone at the apex of nano-fabricated device on Atomic Force Microscopy tips [2], with nanofocusing resolution down to 3 nm and unprecedented efficiency, yet maintaining AFM spatial resolution [3]. At the tip apex, SPPs decay into energized electrons and holes (Hot carriers) and photons, to be used to target charge transfer to stated far from the Fermi level and Raman Spectroscopy at the nanometer scale, referred as Hot Electron Nanonscopy and spectroscopy (HENs) [4].The key point of the technique is its capability to inject energized electrons (holes) to states at energies of few eV above (below) the Fermi level of the sample, e.g. through Schottky junction in a semiconductor or to the LUMO and LUMO+n (HOMO, HOMO-m) states of a molecule. Contextually, photons emitted from the tip are used to perform nano-Raman for the structural characterization of the material.

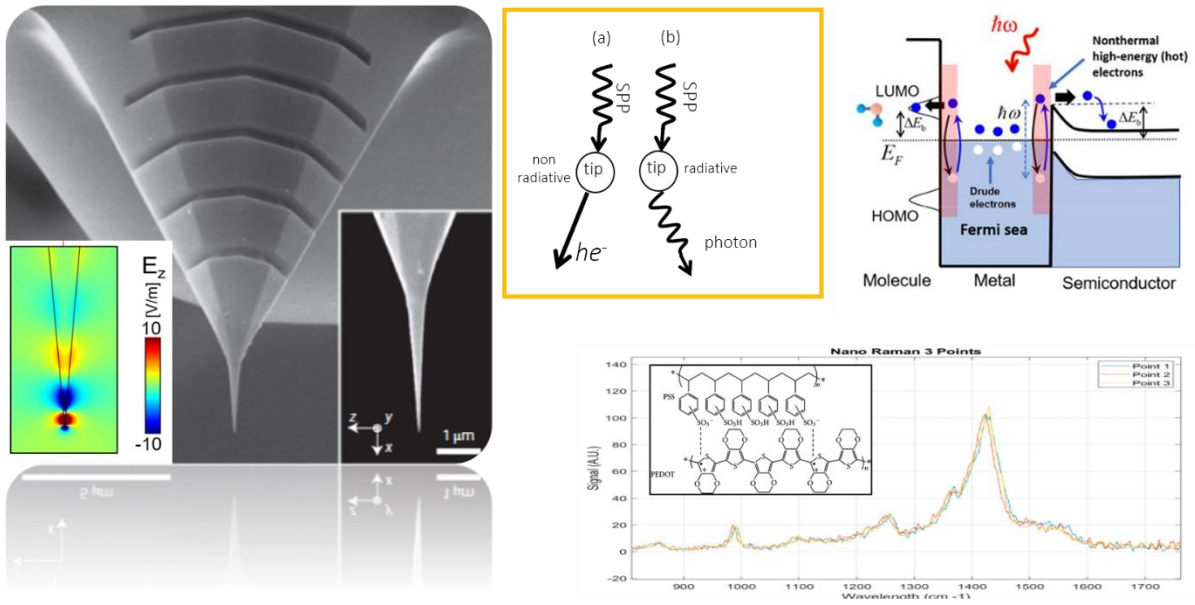

Figure 1 HENS working principles, tip fabrication and plasmonic decay into Hot Carriers and photons, used as probes for electron and Raman analysis

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I.1 - H - Non-Linear Optics and Time-Resolved

Photoinduced reactions in complex condensate media

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Our current understanding of the kinetics of chemical reactions in liquid solution [1] has arrived to a point that enables to study more complex environments. At least what regards electron transfer [2]. This has been accomplished only in the last decades thanks to the synergy between fast photophysical methods and advanced models for the reactivity (extended Marcus) under the influence of molecular diffusion (Encounter Theories). After briefly summarizing the former, we will discuss the peculiarities of media mimicking three important biological environments: the extracellular matrix, the cell membrane and the cytoplasm. We have attempted to perform experiments and rationalize them in the respectively equivalent model systems: hydrogels, nematic liquid crystals [3] and polymer crowded solutions. The current common paradigm is that in comparison with diluted solutions, reactions will always be slower in the former media. This is only partially true and strongly depends on the time scale of observation. In extreme cases, this could lead to enhanced reactions yields and recombination efficiencies.[4]

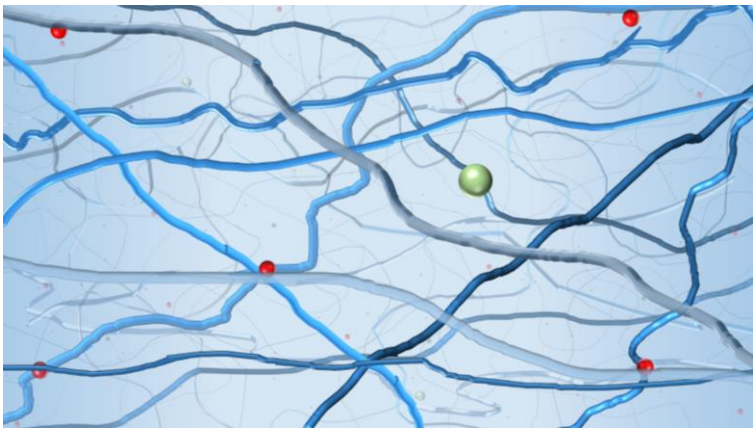

Figure 1. *Pictorial representation of a fluorophore surrounded by quenchers in a hydrogel.*

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O.1 - H - Non-Linear Optics and Time-Resolved

Spontaneous and coherent Raman scattering integration for optical biosensing improvement

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Raman scattering (RS) microscopy, due to its universality and comprehensiveness[1], is one of the fundamental method in biology[2] chemistry[3] or material science[4], however, it has low selectivity, limited measurement speed and fluorescence[1,5] are the crucial issues of this technique. On the other hand, coherent Raman scattering (CRS), with the two major methods of stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS), is rapid and selective, but the complex and expensive equipment and acquisition of single selected wavenumber data makes CRS methods less universal than RS.[6,7]
Therefore, combination of RS comprehensiveness with CRS selectivity and speed is proposed – an attempt to observe a single specimen *via* RS/SRS/CARS without changing the microscope and even a microscope stage.
To present the capabilities and advantages of this multimodal approach, preliminary research was conducted on nonadherent leukocytes. Although mobile and fragile, a set of different images (SRS, CARS, RS, epifluorescence) were obtained from a single group of carotene saturated HL-60 cells. With this series of measurements, the distribution of organelles or various chemical compounds in the examined cells was captured.
Multimodality can significantly improve the complementarity of performed studies, standarize measurement conditions and enhance the efficiency of analysis.

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O.2 - H - Non-Linear Optics and Time-Resolved

On the Relaxation Dynamics of Diarylethene Photochromic Switches

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Photochromic switches are molecules that, when exposed to light, can undergo a reversible reaction, which changes their chemical structure. Each form of the switch has distinct electronic absorption spectra and physicochemical characteristics, such as oxidation-reduction potentials or conductive and luminescent properties. The capability to modulate these properties repeatedly and reversibly has paved the way for a wide range of potential applications.[1,2]

We investigate the photocycloreversion reactions of three diarylethene derivatives, which differ only in the positioning of two sulfur atoms within the cyclopentene rings (Fig. 1). Even though the structural differences are so subtle, their absorption spectra, reaction quantum yields and rates differ substantially. By utilizing femtosecond transient absorption spectroscopy in the UV-Vis and IR spectral ranges as well as quantum chemical dynamics simulations, we explore the connections between the quantum yield, electronic and vibrational relaxation times, and the structural characteristics of the dithienylethene photoswitches. Our findings suggest that the local aromaticity of the central ring in diarylethenes may serve as an indicator of the quantum yield and the cycloreversion rate. Although femtosecond transient absorption results in the UV-Vis range suggest that the cycloreversion is completed within just a few picoseconds, complementary experiments using an IR probe show that nuclear rearrangements required for the formation of a stable photoproduct take considerably longer (12-30 ps) for all three derivatives.

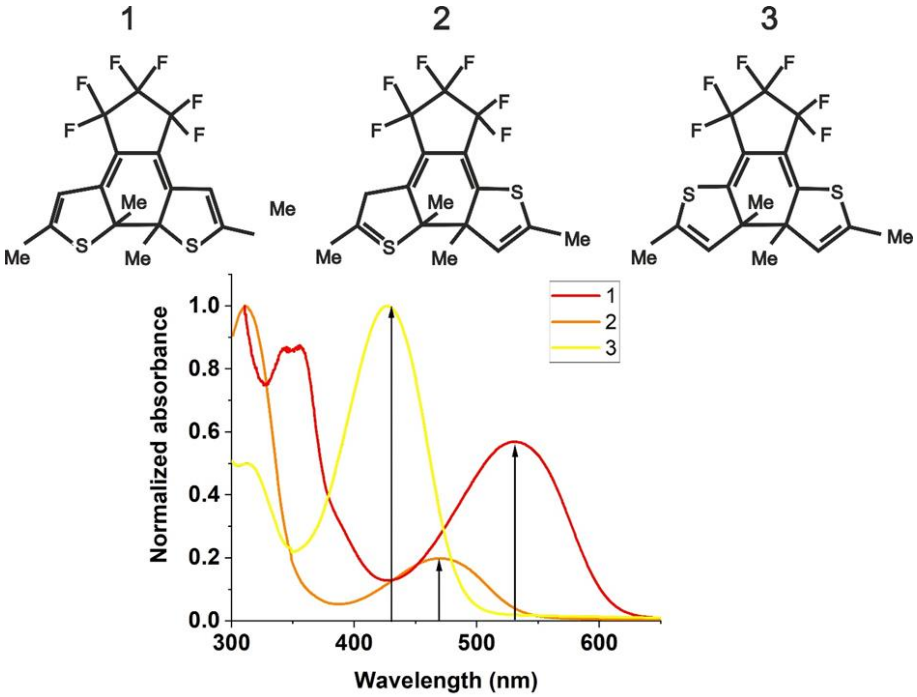

Figure 1. *Molecular structures of studied diarylethene derivatives and their absorption spectra.*

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O.3 - H - Non-Linear Optics and Time-Resolved

Unravelling the structure of Aβ42 oligomers in membrane-mimetic environments

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In the context of Alzheimer's disease, soluble amyloid-β 42 (Aβ42) oligomers are the most cytotoxic species among all types of Aβ. The aim of our study is to understand the aggregation of Aβ in membrane-like environments. Here, we used FTIR spectroscopy in the transmission mode with temperature control, allowing us to monitor the aggregation process by the changes of absorption in the amide I region.

Our previous studies were focused on Aβ oligomerization in the presence of the detergent SDS [1], but in the present work we have used a model closer to the cell membrane: large unilamellar vesicles composed of lipids with different charges. These membrane models were mixed with Aβ at certain lipid-to-peptide molar ratios. Tuning these parameters allowed us to determine the threshold of Aβ structural changes induced by lipids. Several *in vitro* studies demonstrated high affinity binding of Aβ specifically to anionic lipid vesicles, however it is still debated.

We also employed site-specifically ^{13}C-labelled peptides to test whether the local secondary structure of specific residues changes upon binding of Aβ to membranes. This approach is unique for studying lipid-peptide interaction, and our results allow us to study lipid-induced structural changes with residue-level resolution.

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O.4 - H - Non-Linear Optics and Time-Resolved

Stimulated Raman techniques applied to biological systems

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Stimulated Raman spectroscopy (SRS) provides, in principle, the same information about the molecular vibrations in chemical or biological systems as the spontaneous Raman. However, thanks to the nonlinear interaction of two laser pulses with a matter, the SRS signal is around 10^5 times stronger than the spontaneous Raman. That advantage facilitates collecting an intense SRS signal in a very short time, giving access to additional information in various SRS-based techniques.
In this talk, I will present the biological applications of several SRS-based techniques developed or just applied at the Laser Centre, Institute of Physical Chemistry PAS. The SRS is commonly used to study a system's vibrational evolution after the electronic excitation of a chromophore with an ultrashort optical pulse from the visible range. This technique, femtosecond stimulated Raman scattering (FSRS), hence gives us insight into structural dynamics during the electronic relaxation process with high temporal (<50 fs) and spectral (<10 cm^{-1}) resolution with the multiplex advantage. [1]
I will present the potentials of our FSRS setup on the studies of the reversibly photoswitchable fluorescence protein Padron [2], which, after irradiation by 504 nm light, switches from a "dark form" to the fluorescent "bright form" and returns to the dark form by absorbing light at around 396 nm. Structural reorganization after the switching in both directions was followed by FSRS.
In another SRS-based method, to study intermolecular interactions in a system in the electronic ground state, we replaced the visible excitations with the pump pulse from the mid-infrared range, after which the spreading of vibrational energy in the system can be followed in time with the SRS probe. The thus developed method, which we named femtosecond infrared pump–stimulated Raman probe spectroscopy (fs-IR-SRS), gives information similar to the fs-IR pump-probe spectroscopy (fs-IR). I will show the advantage of this method over conventional fs-IR due to the unique possibility of SRS probing all molecular vibrations in a single shot (multiplex advantage) and lack of limitations related to high IR absorption of water. [3]
The SRS's very short acquisition time may also be used in the rapid imaging of biological systems such as tissue or single cells. In the final part of my presentation, I will show applications of SRS microscopy to studies of leukemia cell lines. I will also present some challenges related to merging SRS microscopy with microfluidic devices dedicated to delivering and sorting of leukemia cells.

These works were supported by the Polish National Science Center (NCN) with the project no. UMO-2015/17/B/ST4/04035 and by the TEAM-NET program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund (Grant No. POIR.04.04.00-00-16ED/18-00).

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Poster Presentations

P.1 - A - Molecular Spectroscopy

Analysis of different cell subtypes integrating data fusion techniques

Kazi Sultana Farhana Azam[1,2], *Oleg Rybchykov*[1,2], *Anuradha Ramoji*[1,2], *Tanveer Ahmed Shaik*[1], *Christoph Krafft*[1], *Arnica Karuna Kaiping*[2], *Oana-Maria Thoma*[3], *Sarah Lemire*[3], *Tobias Meyer-Zedler*[3], *Michael Schmitt*[2], *Iwan Schie*[1,4], *Maximilian Waldner*[3], *Jürgen Popp*[1,2], *Thomas W. Bocklitz*[1,2]

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Integrating different data sources through data fusion is a crucial technique that improves classification accuracy and reliability [1]. We investigated data fusion schemes designed specifically for multiclass cell subtype grouping in mice models. Employing five different datasets obtained from Raman imaging, fluorescence lifetime imaging (FLIM), multimodal images (CARS, TPEF, SHG), optical photothermal infrared (O-PTIR) spectra, and high-throughput (HT) Raman spectra, we analyzed the cooperative combination of various modalities.
Our research focuses on improving classification performance through feature fusion, specifically integrating vibrational spectra obtained with different platform. In our study, both spectra-to-spectra and spectra-to-image data are analyzed. We performed combined analysis of the data obtained by Raman imaging with O-PTIR, O-PTIR with HT Raman, and Raman imaging with HT Raman spectra. This fusion process enables us to extract features from the combined spectral datasets. As an example, a schematic diagram of the data analysis strategy for Raman imaging and HT-Raman data is shown in Figure 1. Furthermore, using a pre-trained deep learning model, intracellular features are extracted from multimodal images to select relevant feature vectors for cell area segmentation prior to classification. These features are then fused with the spectral data to enhance classification accuracy following local-level data fusion.We applied nested cross-validation [2] in the analysis (all spectra-to-spectra combination) and when evaluating the performance of the model in the case of spectra-to-image data fusion, we utilized nested group k-fold cross-validation. LDA, PCA-LDA, and PLS are the three models used in the investigations, and they achieved balanced accuracy between 50% and 93% for different techniques used either alone or in different combination. In subsequent steps, we will explore more data fusion strategies.

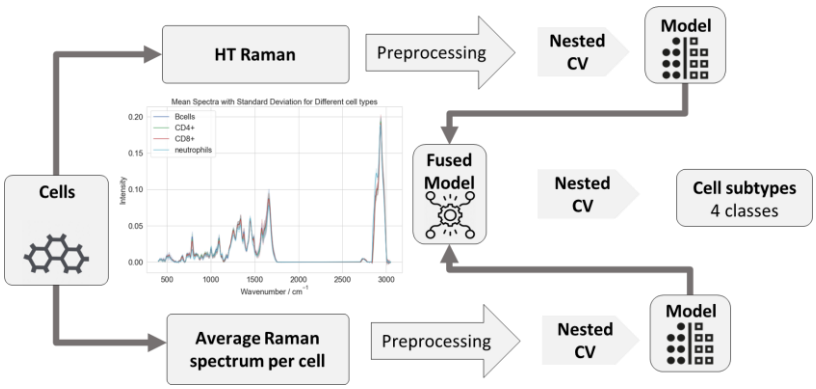

Figure 1. *A workflow to integrate Raman spectroscopy and HT Raman spectral data for cell subtype analysis. Presented here is the workflow for a single combination (and there are more other combinations).*

Acknowledgments
Financial support from the EU, the TMWWDG, the TAB, the BMBF, the DFG, the Carl-Zeiss Foundation, and the Leibniz Association is greatly acknowledged. This work is supported by the BMBF, funding program Photonics Research Germany (13N15715 (LPI-BT4-FSU) and 13N15710 (LPI-BT3-FSU)) and is integrated into the Leibniz Center for Photonics in Infection Research (LPI). The LPI initiated by Leibniz-IPHT, Leibniz-HKI, Friedrich Schiller University Jena, and Jena University Hospital is part of the BMBF national roadmap for research infrastructures.

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P.2 - A - Molecular Spectroscopy

Resonance Raman polarized measurements of heme protein standards

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Heme proteins are essential to maintain a healthy body through oxygen transport, contributing to cell death processes and immunity mechanisms which they owe to the presence of heme moiety within their structure. The heme group is a ring-shaped molecule, in which the interaction between the iron-bound ligand and heme distal amino acid residues plays a vital role in carrying out distinct redox reactions and signal triggering [1]. Thanks to strong electronic transitions in the visible and near UV the resonance Raman (RR) spectroscopy is a particularly sensitive tool in monitoring even subtle alterations in structural and electronic properties of the heme group [2]. Using the excitation source of spectral region laying within the maximum of Soret absorption band for hemes (390 - 430 nm) allows to probe the environment of the heme's active site, i.e. type of porphyrin, oxidation state of the central iron, distortion of heme geometry, interactions with the porphyrin substituents and the axial ligands [3]. Polarized Raman measurements can provide additional information hidden within a Raman spectrum, such as the orientation of molecules in an organized environment. Such an approach is usually applied in studies of polymers, crystal lattices or liquid crystal, however, in the case of heme protein measurements, it can be applied to characterize molecule orientation within tissue samples, e.g., in the aorta, where it can facilitate differentiation of various heme species. Herein, we aimed to distinguish heme proteins such as cytoglobin found in smooth muscle cells, hemoglobin in erythrocytes, cytochrome c in mitochondria and neuroglobin in neurons.

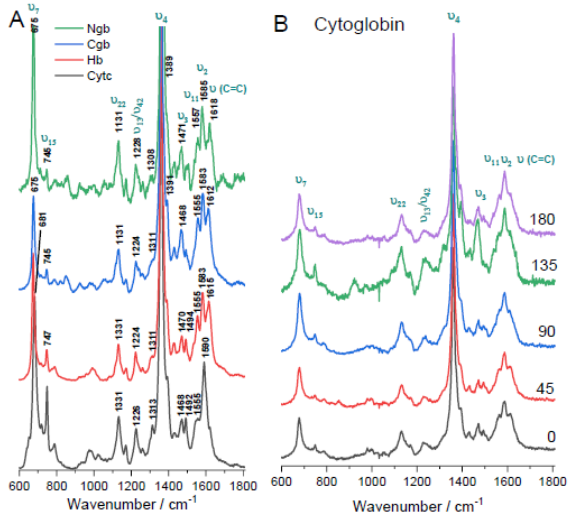

Figure 1. *A: Resonance Raman spectra of four heme protein standards (cytochrome c, hemoglobin, cytoglobin and neuroglobin); B: polarized resonance Raman spectra of cytoglobin with changing polarization angle of 45°. The RR measurements were performed on WITec confocal CRM alpha 300 Raman microscope using 405 nm excitation wavelength and 500 µW – 1 mW laser power.*

Presented here results reveal visible anisotropic response of some vibrational modes in Raman spectra after polarization measurements of four most important heme proteins. Depending on the type of heme protein, different vibrational modes change their intensities upon specific polarization angles. Most significant changes were noticed in higher polarization angles over 90°, which proves that the conventional approach of measuring only parallel or perpendicular polarization components of Raman scattered light is insufficient, and the measurements within the polarization spectrum from 0° to 180° can provide valuable structural information and point out differences between heme proteins. The applied polarized RR methodology allowed us to select some vibrational modes as specific "markes" for distinguishing Raman spectra of structurally similar heme proteins.

Acknowledgements:
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P.3 - A - Molecular Spectroscopy

Study of the effect of sample preparation method on the quality of spectra obtained by FTIR spectroscopy using selected human tissues as an example

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FTIR method meet increasingly interest as it can be a supporting tool to obtain objective diagnosis of some diseases [1]. However, the basic problem is to obtain a reliable model that discriminates pathological changes. For this purpose, it is necessary to collect data from a large population of samples. The most popular and accessible are formalin-fixed, paraffin embedded tissue type (FFPE). Unfortunately absorption bands of paraffin interfere with tissue-specific bands. In order to eliminate the impact of paraffin on the tissue spectra it is necessary to dewax the samples [2].

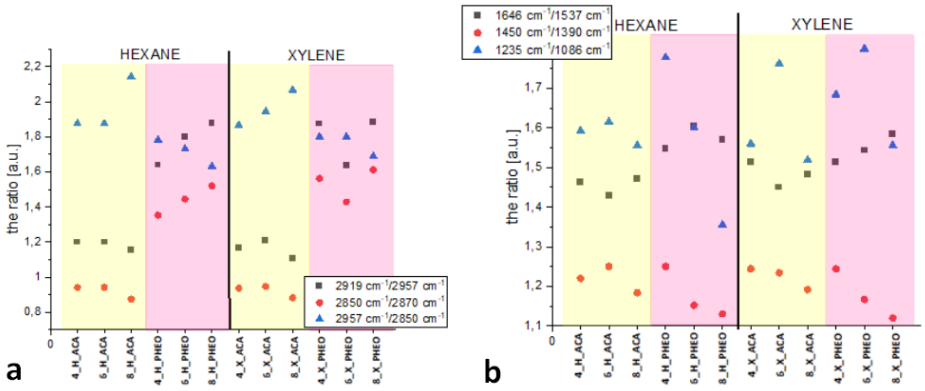

Figure 1. *The ratios of peak heights calculated for ACA and Pheo samples of different thickness and treated with two solvents.*

Nevertheless this process depends on many factors. Their understanding can help in analyzing the results obtained from dewaxed samples. The influence of dewaxing process on quality of FTIR spectra of archival samples was studied. There were two types of samples: adrenocortical adenoma (ACA) and pheochromocytoma (Pheo) which are lipid and protein rich respectively [3,4]. Two solvent type were used: xylene and hexane, and their ability to remove paraffin from tissues were investigated. Additionally the impact of sample thickness for effectiveness of dewaxing process was investigated. It has been observed that the shape of spectra after removing paraffin differ from those collected for "fresh" no fixed samples. This effect was particularly clear for Pheo samples. What is more for ACA and Pheo samples treated with hexane the absorption values behaved in more monotonically way with increasing thickness than for xylene treated ones. This may indicate that for adrenal samples hexane is better deparaffinization agent. It was also noticed that the peak high ratios of some bands changed depending on the thickness what means that the biocomposition might be affected by the dewaxing process.

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P.4 - A - Molecular Spectroscopy

Study of the biomolecular composition of skeletal muscle fibers affected by different types of myopathy using by FTIR spectroscopy

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Myopathies are diverse group of muscle disease. This is a sizable group of sickness with diverse grounds, various course and prognosis, but their common trair is a weakness of muscles. Myopathy can be divided because of the underlying background: inherited – which are separately classified as dystrophy, and acquired diseases. The lack of effective therapies for this disease results in a large amount of research done in this direction.
The samples designed to the biomolecular analysis were prepared and diagnosed at the Department of Pathomorfology of Collegium Medicum Jagiellonian University. The tissue material came from a surgical biopsies and has been prepared by shock freezing in liquid C3H8. For each specimen two adjacent tissue slices were cut into 8 micrometers on the cryo-microtome and placed on the microscope slide and Silicon Nitride Window (membrane thickness: 200 nm, window size: 2x2 mm), respectively. For the measurements two types of FTIR spectrometer were used – first the global sample scanning were performed with a FTIR spectrometer (at AGH WFiIS, Nicolet iN10 MX) and for the detailed study of muscle endomysium the SR-FTIR were used (at the beamline B22 of DIAMOND Light Source, Hyperion 3000).
The data obtained by mentioned methods allows to determine the molecular composition of the studied muscle tissue samples. On the base of multivariate discriminant analysis it was possible to separate analyzed groups as has been showed on Figure 1.

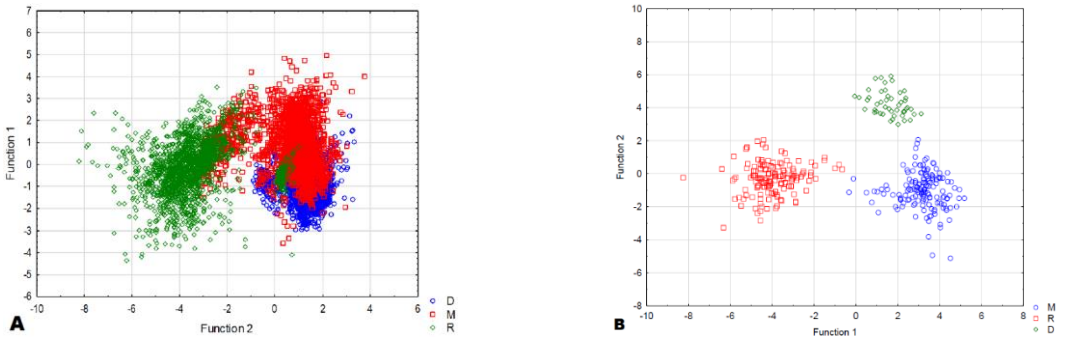

Figure 1. *A) Graphical representation of configurations of discriminant function divided into groups represented by points in the discriminant variable system for: A) selected areas from the samples, B) biomolecular endomysium analysis, M - myopathy, D – dystrophy, R – reference groups*

Molecular analysis of muscle samples showed differences in their composition between the fibers involved in the pathological changes and those classified as a reference group on the basis of medical diagnosis. FTIR spectroscopy seems to be an useful method in the diagnosis of myopathic diseases, specifically for the detection of relatively small pathological changes.

Acknowledgements:
This work was supported by the Polish Ministry of Science and Higher Education and its grants for Scientific Research. In addition research conducted by Paula Kasprzyk has been partly supported by the EU Project POWR.03.02.00-00-I004/16). The synchrotron measurements were conducted as part of the proposal SM2037 on the Beamline B22 Diamond Light Source

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P.5 - A - Molecular Spectroscopy

Structure and spectroscopy of $Re(CO)_3$ complexes with 8-hydroxyquinolines and their antimicrobial and anticancer activity

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Antimicrobial Resistance (AMR) is "one of the biggest threats to global health, food security, and development today" [1]. On the other hand, notwithstanding advances in treatment methods, oncological diseases remain a worldwide leading cause of death [2]. Strangely enough, metal complexes are frequently overlooked as potential antibacterial and/or anticancer (non-platinum) drugs. This prompted us to search for metalloantibiotics to fight against antimicrobial resistance problems in the group of Re complexes and/or simultaneously for attractive non-Pt anticancer agents. This presentation reports on 12 tricarbonyl Re(I) complexes with three 8-hydroxyquinolines bidentate ligands and four monodentate diazole molecules [3] (Fig. 1a). Crystal structures of the complexes were determined by the scXRD method (Fig. 1b), characterized by analytical (EA) and spectroscopic techniques (FT-IR, NMR, and UV-Vis, Fig. 1c), supported by DFT and TD-DFT calculations. Of the Re(I) complexes, only $[Re(CO)_3(MeQ)Him]$ demonstrated significantly 4-fold better action against gram-negative *Pseudomonas aeruginosa* than the free MeHQ ligand. The cytotoxicity was estimated using several cell lines, but only HQ and ClHQ ligands and $[Re(CO)_3(Q)Hdmpz]$ complex had good selectivity toward the MCF-7 breast cancer cell line. The human acute promyelocytic leukemia HL-60 cells were sensitive to all complexes (IC_{50} = 1.5–14 μM). Still, pure HQ and ClHQ ligands were slightly more sensitive than the complexes.

Monitoring of the complexes can be performed based on observation of the lowest energy transitions involving the promotion of an electron from the HOMO to the LUMO state, which is ligand-centered (LC) π → π* transition (Fig. 1d). Metal-to-ligand (Re → bidentate ligand) and ligand-to-ligand (CO → bidentate ligand) charge transfer dominate the other lowest energy transitions: HOMO-1 → LUMO and HOMO-2 → LUMO.

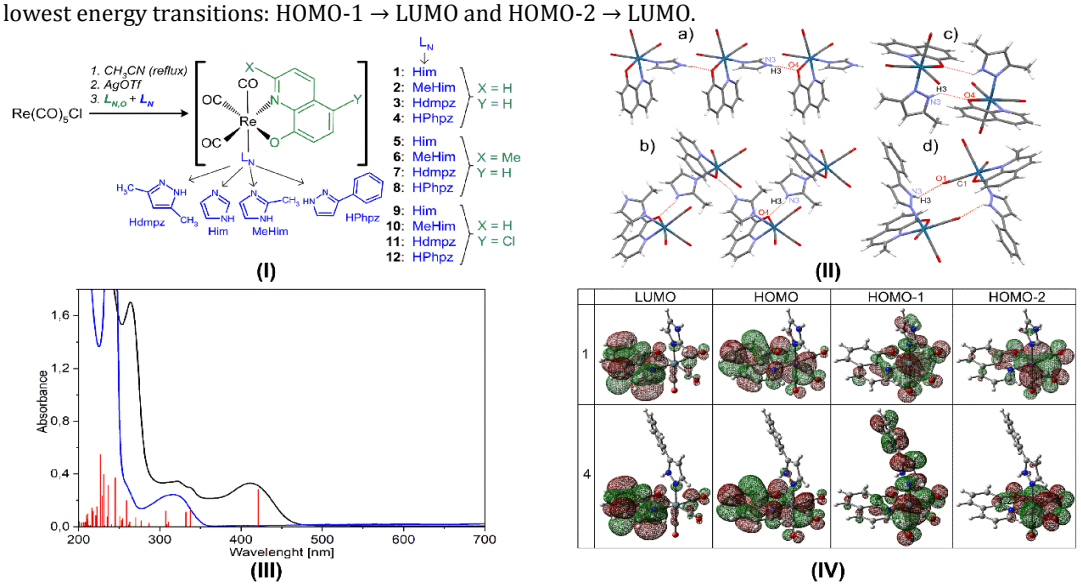

Figure 1. *(I) Synthesis of the rhenium(I) tricarbonyl complexes* ***1****-****12****. (II) Various molecular arrangements in the crystal structure of Re(I) complexes: a) linear chain (****1****); b) zigzag chain (****2****); c) dimer with N–H···O(Q) bonds (****3****); d) dimer with N–H···O(O=C) bonds (****8****). (III) The experimental (black) and simulated (red) UV-Vis spectra of [Re(CO)3(Q)Him] (****1****) and 8-hydroxyquinoline (blue); (IV) Orbital contours of the lowest energy transitions for complexes* ***1*** *and* ***4****.*

Acknowledgment
The National Science Centre of Poland supported this work with Grant No. 2022/47/B/NZ7/02765. Świerk Computing Centre is acknowledged for the generous allotment of computing time.

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P.6 - A - Molecular Spectroscopy

Structure and spectroscopy of a novel polymorph of 5α-dihydrotestosterone

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5α-Dihydrotestosterone (5α-DHT) is a chiral steroid biomolecule that plays the role of a male sex hormone [1] responsible for, i.a., the development of male genitalia or facial, body and pubic hair growth. The steroid participates in the pathogenesis of prostate hyperplasia and cancer. Medicinally, 5α-DHT is utilized for the treatment of male hypogonadism.

While working on various issues related to the quantitative chirality of androgen steroids [2], their chiroptical spectroscopy and their detection via chiral sensors, we were surprised to note that the Cambridge Structural Database [3] contains no proper solid-state structure for 5α-DHT. The database includes 1) the HANDRO entry, which contains the structure of 5α-DHT monohydrate [4], 2) the ZZZJEQ entry, which has only elementary cell data for 'allodihydrotestosterone' [5], and 3) the HYDRAN entry, which despite being labelled as '17β-Hydroxy-androstan-3-one' (which would suggest 5α-DHT), provides coordinates for the enantiomer *ent*-5α-DHT [6].

Given the importance of 5α-DHT as a biomolecule, we solved the structure of a commercial sample of 5α-DHT by single-crystal X-ray crystallography. Our sample has different elementary cell parameters than any 5α-DHT crystal reported previously which leads to the conclusion that it represents a different polymorph.

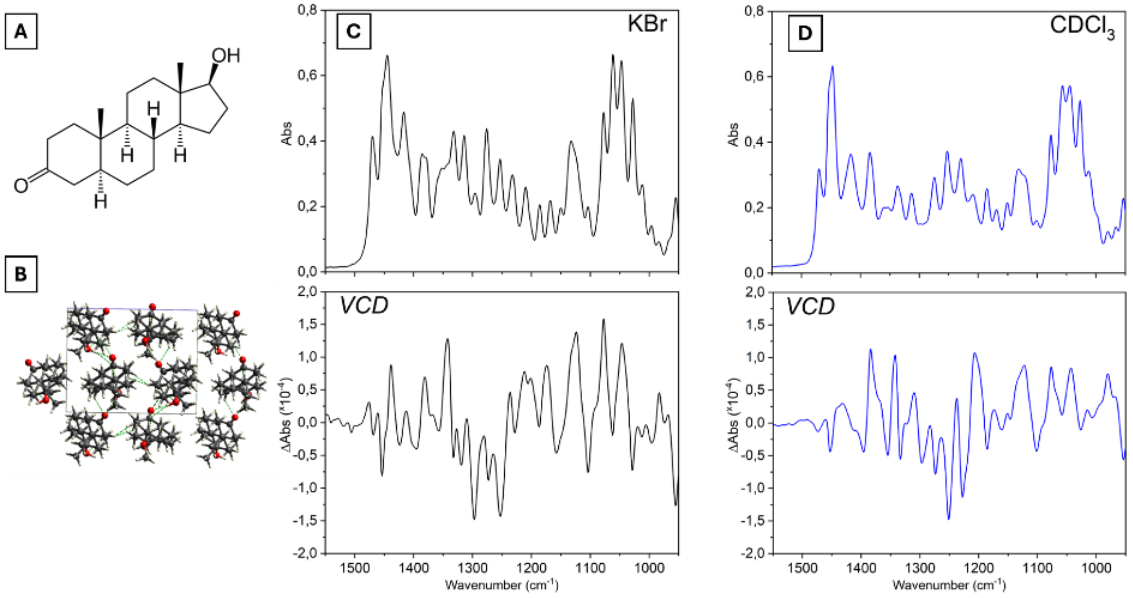

Figure 1. *5α-DHT A) molecular formula, B) crystal structure; IR and VCD spectra C) in the solid state, D) in $CDCl_3$.*

In the poster, we first discuss the intermolecular interactions in the crystal structure. This discussion is supported by Hirshfeld surface analyses [7] and calculations of the intermolecular interaction energies in the crystal according to the CE-B3LYP model [8]. Second, we present the infrared (IR) and vibrational circular dichroism (VCD) spectra of the title steroid measured in solid-state and $CDCl_3$. The environment-specific spectra differences are discussed. This is followed by a comprehensive evaluation of the quantum-chemical calculation method that best reproduces both IR and VCD spectra. Finally, we simulated the IR and VCD spectra of other isomers of 5α-DHT (the molecule has seven asymmetric carbon atoms) and provided spectral characteristics that might help distinguish the isomers.

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P.7 - A - Molecular Spectroscopy

Effect of selected phenolic acids on the structure of gluten proteins (gliadins and glutenins) studied with application of FT-IR spectroscopy

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Gliadins and glutenins are components of the wheat gluten, which is a continuous, viscoelastic network formed within dough during dough mixing process. Glutenin polymers are made up of high and low molecular weight subunits that are attached to each other via disulphide bonds, whereas gliadins interact with glutenin polymers via hydrogen bonding and non-covalent hydrophobic interactions. Structure of gluten proteins directly affect quality of the bread dough as well as wheat bread. Addition of polyphenols to the bread dough changes its mechanical properties and hence disturb structure of the gluten network as well as particular gluten proteins.

The aim of the studies was to determine changes in the secondary structure of gliadins and glutenins extracted from the phenolic acid-supplemented model bread dough. The studied phenolic acids belong to the hydroxycinnamic and hydroxybenzoic acids. Each of these compounds differs in structure and contains different functional groups at the aromatic ring. The model what dough supplemented with phenolic acids were prepared according to Kłosok et al. [1]. The gliadins were extracted from powdered gluten using method of [2]. Briefly, gluten samples were dissolved in 70% ethanol and stirred for 4h. Next, it was centrifuged and the supernatant containing gliadins was collected. The ethanol-insoluble fraction represented glutenins. Both proteins fraction were freeze-dried and studied with application of Fourier transform infrared spectroscopy (FT-IR). Changes in the secondary structure of the gluten proteins were analyzed by calculation of difference spectra in the amide I and amide III bands according to Nawrocka et al. [3].

The obtained results indicate that incorporation of phenolic acids into wheat dough alters the structural conformation of gliadins and glutenins. The type of structural changes observed is affected by the concentration and structure of the phenolic acid. The interactions gliadins-phenolic acids lead to more complex structural changes comparing with interactions glutenins-phenolic acids. Generally, these interactions involve β-structures that form disordered structures in both gluten proteins. Additionally, the results indicate that phenolic acids affect the hydrogen bonding pattern in the gluten network during dough mixing process and interact non-covalently with these proteins [4]. These results may have an impact on the allergenic properties of gluten, particularly in relation to reduction of the β-turn content. β-turns are regarded as one of the factors responsible for gluten allergenicity because it contains amino acid sequences containing proline. During mixing process of non-supplemented wheat dough most of the β-turns are not involved in interchain interactions.

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P.8 - A - Molecular Spectroscopy

Chiroptical studies of lanthanide complexes with chiral alanine

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The peculiarity of energy levels of lanthanides (Ln) renders their complexes' physical and chemical properties unique and valuable also in medical therapy and diagnostics. Indeed, Gd complexes are used in medical magnetic resonance imaging (MRI). Some other Ln are among the best MRI [1,2] and X-ray tomography contrast agents [3]. Various Ln complexes are used as spectroscopic (bio)sensors [1-5]. The Protein Data Bank comprises a few hundred biomacromolecules that coordinate lanthanide ions, among which many different types of proteins [6]. The interactions of Ln ions with proteins are expected to resemble those occurring in their complexes with basic bio-ligands such as amino acids. This prompted us to study a series of Ln (III) complexes (but radioactive Pm) with *L*- and *D*-alanine (Ala) enantiomers [7]. The synthesized $[Ln(H_2O)_4(Ala)_2]_2^{6+}$ complexes crystallize at 100 K as two types of dimers (Fig. 1). Next, we studied the complexes using two vibrational optical activity (VOA) spectroscopies, i.e., vibrational circular dichroism (VCD) and Raman optical activity (ROA). The VCD and ROA chiroptical methods exhibit different absorption and scattering coefficients for the left- and right-circularly polarized light, respectively. This makes them sensitive to chirality, and as a result, the spectra of the enantiomers are mutual mirror images [8]. Together with VCD and ROA, their classical counterparts, IR and Raman, are measured. The studied complexes are water-soluble, which is particularly challenging for VCD. Therefore, most of the measurements (but ROA) were performed for solid-state samples. Several IR band positions and intensities reveal correlations with the Ln–O1 distances, exemplifying the Ln contraction effect. Moreover, the positions of the two ν(C=O) VCD bands correlate with the number of 4*f* Ln electrons, revealing the Ln contraction effect observed for the first time in the VCD spectra. On the other hand, solid-state Raman spectra of $[Ln(H_2O)_4(Ala)_2]_2^{6+}$ complexes change significantly with the excitation line and lanthanide ion and some of them are perturbed by the fluorescence (Fig. 1). Moreover, for some complexes the ROA effect (532 nm excitation line) is accompanied by the circularly polarized luminescence (CPL) spectra. The CPL signal intensity was exceptionally high for the Eu^{3+} and Sm^{3+} complexes because their excitation energies are close to the 532 nm excitation line.

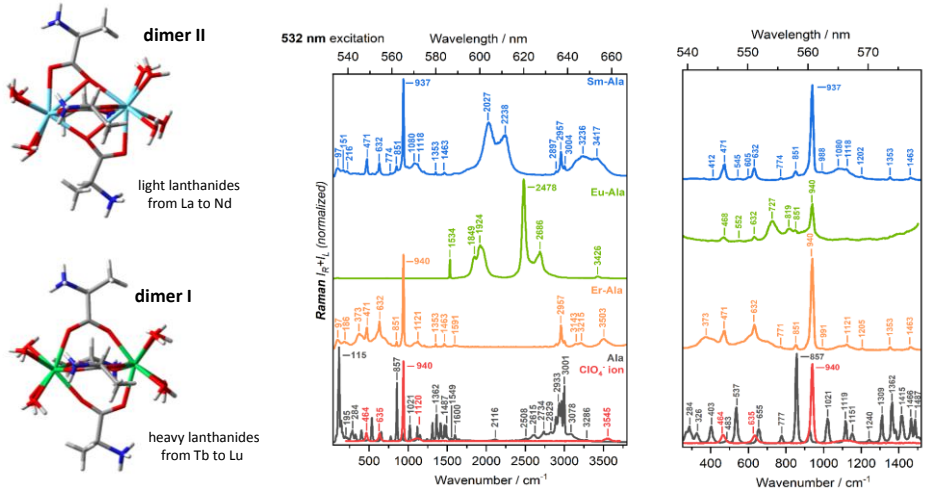

Figure 1. *Molecular structures of $[Ln(H_2O)_4(L\text{-}Ala)_2]_2^{6+}$ complexes measured at 100 K and solid-state Raman spectra with (Sm and Eu-D-Ala) and without fluorescence (Er-D-Ala) registered at 532 nm excitation line.*

Acknowledgment
This work was financed by statutory activity subsidy from the Polish Ministry of Science and Higher Education for INCT. The computational grant from the Świerk Computing Centre is gratefully acknowledged.

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P.9 - A - Molecular Spectroscopy

Optimizing Plant Tissue Preparation for the Advance Spectroscopic Imaging

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The chemical composition of the cross sections of the plant can be better understood by optimizing the method of cutting and preserving to acquire the best vibrational spectroscopic spectral data with high-resolution. To preserve the native state of the sample is essential for comprehensive study of the plant samples by the vibrational spectroscopic techniques **[1][2]**.

In order to achieve the optimal morphology of the cross sections of the root, stalk, and leaf, we employ a variety of strategies. The 15 micron diameter of each cross section is well-suited for the acquisition of spectral data by both Raman and FT-IR spectroscopy, which will facilitate a more comprehensive comprehension of the biochemistry and physiology of the plant parts. Additionally, the substrate (CaF2: exhibits weak signals during spectral acquisition) for implantation and the structural retention of the cross sections are taken into account when selecting the appropriate cross sectioning medium (freeze water medium). The results of the spectral data acquisition indicate that the optimization of the sample preparation is crucial for the characterization of metabolites in the sample, the deposition of chemical compounds, and a more comprehensive understanding of the morphology of the root, branch, and leave cross section.

This investigation contributes to the implementation of a standardized sample preparation protocol for this type of sample, which is combined with advanced vibrational spectroscopic techniques to estimate its biochemical composition. This proves the potential of FT-IR and RS spectroscopy in the field of plant science.

Keywords: Sample Preparation, Raman and FT-IR spectroscopy imaging, Japanese knotweed, Plant Science, Metabolites

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P.1 - B - Chemometric Advances

Reduction of Acquisition Time in FTIR Spectroscopy via Spectral Super-Resolution by Deep Learning

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Introduction:
This study builds upon our ongoing research aimed at reducing acquisition time in infrared spectral imaging which is crucial particularly in clinical applications. In a previous work, we have investigated the use of deep learning techniques to accelerate this process by optimizing the number of scans, a significant limiting factor. We developed models trained on both real and simulated data, providing promising solutions to reconstruct spectral images acquired with a higher number of scans from IR images acquired with a single scan.
In this study, we specifically address spectral resolution as another major challenge in IR acquisition. Our goal is to explore optimal neural network architectures to precisely enhance the spectral resolution of FTIR images. This involves the meticulous reconstruction of infrared spectra from low-resolution data to high-resolution spectra while preserving complex and essential biochemical information.

Materials and Methods:
FFPE biopsies from fourteen renal graft recipients were sectioned into 10 µm slices and mounted on CaF_2 windows for infrared analysis. FTIR images were acquired using a Spectrum Spotlight 400 system coupled with a Spectrum One spectrometer, employing spectral resolutions of 2 cm^{-1} and 16 cm^{-1} per sample. Spectral images were background-corrected and reduced to the 900-1800 cm^{-1} spectral range, with atmospheric correction applied to eliminate atmospheric absorptions.
The architecture of the proposed model is based on ResUNet designed specifically for the spectral super-resolution of hyperspectral image acquired on biological tissues. This model utilizes a convolutional neural network (CNN) to reconstruct high-resolution spectral images from low-resolution inputs. To capture the complex spectral characteristics of biological tissues, we explored three main configurations: 1D-CNN for spectral processing, 2D-CNN for spatial aspects, and 3D-CNN for combined spatial and spectral features. We evaluated and compared the performance of these models against conventional methods from a spectral perspective, as well as their impact on classification using K-means clustering applied on low spectral resolution images, high spectral images and reconstructed images, employing similarity metrics drawn from the literature.

Results and Discussion:
This study demonstrated the effectiveness of our models using 1D-CNN, 2D-CNN, and 3D-CNN configurations for the spectral super-resolution of FTIR images of biological tissues. The results showed that these models could accurately reconstruct infrared spectra, surpassing conventional methods. Integrating spatial and spectral aspects in the models significantly enhanced their ability to faithfully reproduce the complex spectral details of biological samples.
Evaluation using metrics revealed that these models were well-tuned to minimize reconstruction errors while maintaining high similarity with reference spectra. Additionally, applying K-Means clustering confirmed the models' ability to preserve critical biomolecular information, such as paraffin and amide bands, across different spectral resolutions. Mainly, the 2D-CNN and 3D-CNN models exhibit the best performance for our application.

Conclusion:
In conclusion, this study introduced promising methods for the spectral super-resolution of FTIR images, highlighting the importance of convolutional neural network-based approaches in improving the quality and precision of infrared spectroscopic images. The SRSResUNet models, with their various CNN architectures, demonstrated their effectiveness in preserving essential spectral details while significantly reducing acquisition time. Utilizing these models can make clinical and diagnostic applications faster and more accurate. These advances underscore the significant impact of deep learning approaches in infrared spectroscopy, promising continuous improvements in advanced imaging techniques for biology and medicine.

P.1 - D - Biomedical Applications

Uncovering New Chemotherapeutic Methods for Osteosarcoma with Vibrational Microspectroscopy

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Within the next 20 years, it is expected that the number of cancer cases annually would reach 22 million, making it the second leading cause of death worldwide. Osteosarcoma is the most prevalent primary malignant bone cancer with higer incidence in children and young adults [1]. It has a poor prognosis for patients with metastatic or recurrent disease. The administration of a multidrug regimen known as MAP (methotrexate (MTX), doxorubicin (DOX), and cisplatin), increased the survival rates from less than 20% with the standard approach to 65-70%. However, the severe toxicity and deleterious side-effects associated with MAP are a limiting factor. In this regard, the current phase III clinical trial, the European and American Osteosarcoma Study (EURAMOS-1), aims to increase the survival rate of osteosarcoma patients by adjusting MAP concentrations [2]. The advantage of a combined therapy is that it can provide a cytotoxic impact that is either the same or better than what would be possible with each drug used alone. $Pd_3Spd_2Cl_6$ was found to be the most effective complex against the osteosarcoma cell line, MG-63 and it was used against the healthy cell line HOb in order to assess cells' viability.
The bioavailability, biodistribution, metabolic impact, and cellular response to treatment with newly synthesised cisplatin-like compounds (Pd_2SpmCl_4 and $Pd_3Spd_2Cl_6$) alone or in combination according to the MAP regimen against both osteosarcoma (cancer cells), MG-63, and osteoblasts (healthy cells), HOb, cell lines were evaluated using vibrational microspectroscopy, both Raman and FTIR with synchrotron radiation. [3,4]
The IC_{50} values obtained for the MG-63 cell line at 48 hours for cisplatin (12 μM), Pd_2SpmCl_4 (14 μM), and $Pd_3Spd_2Cl_6$ (12 μM) were applied to both cell lines, MG-63 and HOb. The results thus obtained clearly demonstrated a spectral discrimination between the control and the drug-treated cells. As expected, the polynuclear complexes under study presented higher DNA conformation changes from the B-native conformation to the Z-conformation while in the presence of cisplatin the native conformation alterations were to a DNA A-conformation. For both cell lines, the drug combination administration has proven to have a higher impact in proteins when compared to single drug administration. [5]

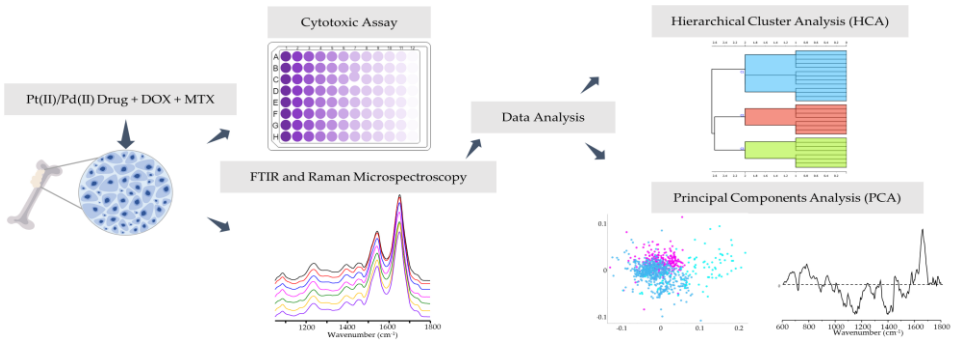

Figure 1. *Graphical procedure of the present work.*

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P.2 - D - Biomedical Applications

Innovative method to track the influence of cholesterol on the development of colon cancer – Raman imaging, fluorescence studies and AFM combined with chemometric analysis

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Colorectal cancer (CRC) is the third most common cancer worldwide. Despite advances in surgery, chemotherapy, and radiotherapy CRC remains the second leading cause of cancer-related deaths in the world. Therefore the social importance of this problem stimulates research aimed at developing new tools for rapid CRC diagnosis and analysis of CRC risk factors. Considering the association between cholesterol level and CRC, we hypothesize that cholesterol spectroscopic and AFM (Atomic Force Microscopy) studies combined with chemometric analysis can be a new, powerful tools used to visualize the cholesterol distribution, estimation of cholesterol content and its influence on the biochemical and nanomechanical properties of colon cells.

Our research presents the analysis of human colon tissues: normal and cancer and human colon single cells normal CCD18-Co and cancer CaCo-2 in physiological state and for CaCo-2 upon mevastatin supplementation.

Based on vibrational features we have shown that: Raman spectroscopy and imaging allow to track cholesterol content in human colon tissues and human colon single cells of both types 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 Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLSDA). The impact of statin on cholesterol content has been studied also by using fluorescence microscopy and AFM.

Based on Raman spectroscopy and imaging study we have proved that human colon normal and cancer tissues are characterized by significant different content of cholesterol. The BA and CA algorithms confirmed independently the higher content of cholesterol in human colon cancer tissues and in single human colon cancer cells.

The presented research is one of the first reports about the use of Raman spectroscopic techniques to cholesterol investigations and the first one about cholesterol investigation using Raman Spectroscopy (RS) on human cells *ex vivo* in a context of colon cancer development.

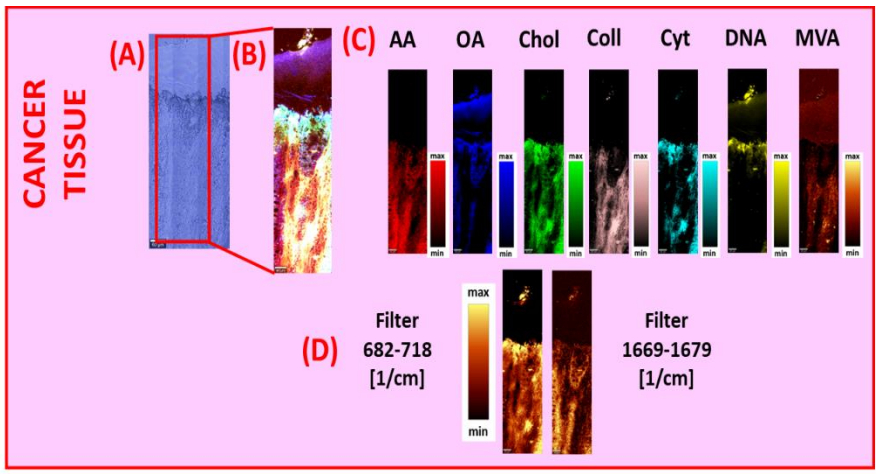

Figure 1. *The microscopy image (A), the Raman image constructed by using BA method (B), distribution of individual chemical components based on BA, results for: arachidonic acid (AA), oleic acid (OA), cholesterol (Chol), collagen (Coll), cytidine (CYT), DNA (DNA), mevalonic acid (MVA) (C) and filters: 682-718 cm^{-1} and 1669-1679 cm^{-1} (D) for ex vivo normal - upper blue panel and cancer- bottom red panel human colon tissues. Scale bar represents 9µm.*

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P.3 - D - Biomedical Applications

Vitamin C and E supplementation and its effect on human gastrointestinal tract tissues and cells: Raman spectroscopy and imaging

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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.

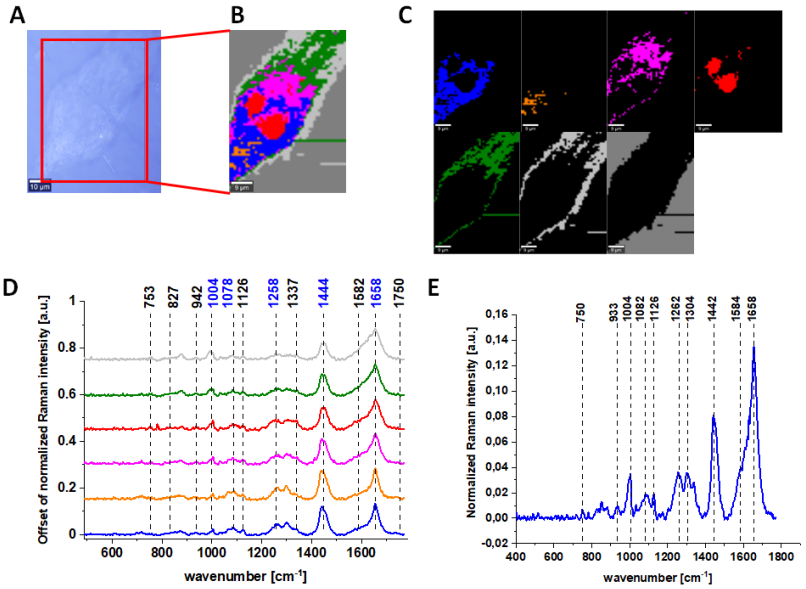

Figure 1. *The microscopy image of human normal gastric CRL-7869 cell (A), Raman image constructed based on Cluster Analysis (CA) method (B), Raman images of all clusters identified by CA assigned to: nucleus (red), mitochondria (magenta), lipid-rich regions (blue, orange), cytoplasm (green), membrane (light grey), and cell environment (dark grey) (C), average Raman spectra typical for all clusters identified by CA in a fingerprint region (D), average Raman spectrum for the whole cell in a fingerprint region (E), cells measured in PBS, excitation wavelength 532 nm.*

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P.4 - D - Biomedical Applications

In vitro spectroscopy assessment of leukaemic cells responses to PARP inhibitors

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PARP inhibitors (PARPi) are a group of drugs that have shown promising results in the treatment of BRCA-mutated ovarian cancer. Their potential in the treatment of certain types of leukaemia, including acute myeloid leukaemia, offers a ray of hope in the battle against cancer. In cases of DNA damage, these inhibitors target the PARP1 enzyme, leading to the accumulation of single-stranded DNA damage and promoting the formation of double-stranded DNA damage.[1]

Vibrational spectroscopy is a technique that enables the tracking of compound distribution in cells, the observation of subtle drug-induced biochemical changes in cells, and the tracking of therapy progression. The proposed research aims to molecularly, metabolically and morphologically (3M) assess the cellular response to PARPi using vibrational spectroscopy techniques: Raman spectroscopy (RS) and Fourier-transform infrared spectroscopy (FT-IR). By identifying spectral biomarkers of PARPi sensitivity and resistance, we set out to provide a non-invasive approach to addressing the acquisition of a resistant phenotype by leukaemic cells.

The HL-60 cell line is known to be resistant to PARP inhibitors such as Olaparib (OB) and Veliparib (VB). [2] The BV-173 cell line, on the other hand, was reported to be significantly sensitive to both drugs, which is manifested by a high percentage of apoptotic cells.[2] Thus, the HL-60 line was used as a model of resistant cells, and the BV-173 line as a model of PARPi-sensitive cells. Leukaemic cells were incubated for 24 h with OB and VB at three different concentrations: OB – 1, 4, and 10 µM; VB – 10, 25, and 50 µM. An approach based on Raman and FT-IR imaging allowed spectroscopic characterisation of tumour cell susceptibility to PARPi based on their single-cell mean spectra. Subsequently, single-parameter analysis and chemometrics enabled the monitoring of PARPi activity.

We have shown that a comprehensive approach based on RS and FT-IR imaging combined with principal component analysis (PCA) was sufficient to capture the different responses of the two cell lines with varying degrees of sensitivity to PARPi (Figure 1). The changes found in the sensitive line (BV-173) are consistent for the two drugs at different concentrations and mainly manifest as alterations in nucleic acids. The response of resistant cells (HL-60) involves a series of changes related to biological components such as proteins and nucleic acids. Our findings indicate that both techniques can track PARPi efflux from a single-cell perspective.

In conclusion, further research is urgently needed to fully comprehend the mechanisms of PARPi action in cancer cells, especially leukaemic cells. This understanding is crucial in our quest to overcome resistance to existing treatment regimens. Vibrational spectroscopy's potential in this regard is a promising avenue that warrants further exploration.

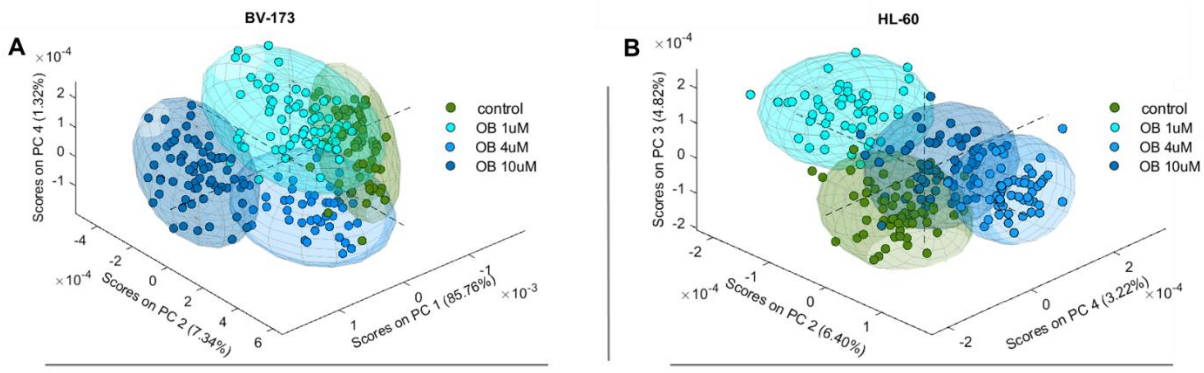

Figure 1. *Results of PCA performed on FT-IR spectra for sensitive BV-173 (A) and resistant HL-60 (B) cell lines.*

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P.5 - D - Biomedical Applications

Analysis of the degree of cartilage tissue degradation of knee joints with use of Raman spectroscopy

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Osteoarthritis is one of the most common causes of disability in the world population. It leads to irreversible changes in the tissue structure of the entire joint. Commercially available diagnostic methods often enable the condition to be diagnosed only at a late stage, where tissue degradation is so severe that endoprosthesis is the only solution. Diagnosis of the first stages of the disease would allow earlier implementation of pharmacological treatment, which can slow down the progression of joint degradation [1-3].
The scientific project our group is working on, covers the development of the OA diagnostic method with the use of Raman spectroscopy, and also, the design and synthesis of new copolymers with a molecular bottlebrush topology and their applications at various levels of advancement of the degenerative disease. The proposed polymers will serve as a lubricant agent on the damaged joint, decreasing friction and preventing further degradation.
The research presented here focuses on determining the relationship between articular cartilage structure and the degree of osteoarthritis development. Using Raman spectroscopy, samples of cartilage tissue taken during endoprosthetic surgery in more than a dozen patients were studied. The research is devoted to verifying the possibility of correlating different spectral parameters with changes in the chemical structure of cartilage with different degrees of degradation. Raman bands characteristic of the hydroxyapatite, chondroitin sulphate and amide III groups were identified, allowing the degrees of mineralization in cartilage tissue and tissue remodeling to be determined, among other things. The results obtained will be used to develop an osteoarthritis severity index [4].
The next stage of the presented research covers *in vivo* experiments with use of portable Raman spectrometer with an endoscopic fiber optic probe, which can be injected inside the tested joint during the arthroscopy procedure.

The research was conducted as a part of the project OPUS UMO-2018/29/B/ST5/02412 and Euronanomed III ENM3/IV/CaPreCon/2021.

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P.6 - D - Biomedical Applications

Changes in astrocytoma cells metabolism after crocin supplementation

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Most of the carotenoids are hydrocarbons, which do not contain any oxygen atoms in their structure, but these carotenoids that contain at least one oxygen atom are called xanthophylls. Crocin is a natural carotenoid from xanthophyll group, extracted from *Crocus sativus*. It is a di-glycosyl polyene ester of crocetin in which D-glucose and/or D-gentiobiose occur as carbohydrate residues. Crocin cannot be absorbed through the human digestive system, thus after the oral administration it is converted into crocetin. [1] Crocin has many valuable properties: antioxidant, antitumour, memory enhancer, antidepressant, and anxiolytic. [2] Plenty of researchers analysed crocin like Soeda, who claims that crocin is a valuable agent to prevent the inhibitory effect of alcohol on impairment of learning and memory and that crocin can inhibit cancer necrosis factor. [3]

Raman spectroscopy has been found as an appropriate tool for carotenoid and cancer cell analysis. This technique provides information about qualitative and quantitative composition of analysed sample. My research involves the supplementation of astrocytoma cells from CRL-1718 line with crocin in two concentrations (10 and 20 μM) and two times of incubation (24 and 48 hours). The analysis of CRL-1718 cells is done using Raman imaging in two spectral ranges: 400-1800 and 2700-3100 cm^{-1}. The main conclusion from this research is that crocin alter cell metabolism by lowering the amount of lipids and increasing the amount of cytochrome *c* in mitochondria, cytoplasm, and lipid droplets. These changes might relate to inducing apoptosis. Figure 1 presents the structural formula of crocin, its Raman spectrum and Raman spectrum of CRL-1718 cell supplemented with crocin.

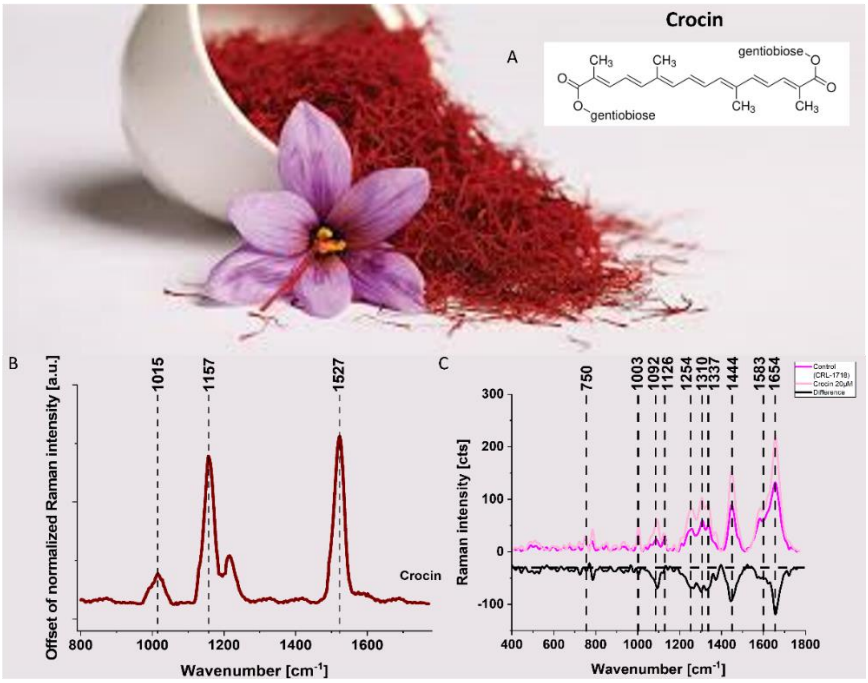

Figure 1. *The structural formula of crocin (A), normalized Raman spectra of 10mM crocin solution in high purity water (B), average Raman spectra of mitochondria in control (unsupplemented) CRL-1718 cells and cells supplemented with 20 μM crocin in the range from 400 to 1800 cm^{-1} (C)*

Funding: FU^{2}N – Fundusz Udoskonalania Umiejętności Młodych Naukowców, Grant: „Zastosowanie spektroskopii Ramana w analizie komórek nowotworowych suplementowanych karotenoidami – określenie wpływu".

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P.7 - D - Biomedical Applications

Should I eat algae? The impact of fucoxanthin on astrocytoma cells

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Xanthophylls are carotenoids containing at least one oxygen atom. One of the representatives of xanthopylls is fucoxanthin, which is present in the chloroplast of brown seaweeds and contains oxygen atoms in ester groups. [1] It has a unique structure containing allenic bond, 9 conjugated double bonds, 5,6-monoepoxide and hydroxyl, epoxy, carbonyl groups. [2] Fucoxanthin is metabolised to fucoxanthinol and amarouciaxanthin A. In the literature, fucoxanthin is presented as an agent in cancer prevention and treatment. There are plenty of fucoxanthin properties: antidiabetic, anti-obesity, anti-inflammatory and anti-mutagenic. [3] Zhang claims that fucoxanthin act against cancer by cell cycle arrest and inducing apoptosis. [2]

Raman spectroscopy and imaging are non-invasive analytical techniques that focus on analyzing the vibrations of individual molecules present in the samples under study. [4] Nowadays, Raman imaging has an increasing number of applications, including in the diagnosis of civilization diseases. In my research work, I use Raman spectroscopy and imaging in the analysis of carotenoids.

In my research, I focused on analyzing model system of brain tumor due to the fact that brain tumors are among the most lethal in adults; however, there is an increase in incidence in children as well. My research hypothesizes that fucoxanthin may affect brain tumor progression by altering metabolism at the cellular level. To asses this, I supplement model astrocytoma cell line – CRL-1718 with fucoxanthin and measure the effect using Raman imaging. The key conclusion from this experiment is that fucoxanthin alters cell metabolism by changing the amount of almost every cell component. Fucoxanthin increases the amount of lipids, proteins and cytochrome c. My research has proven that Raman imaging is an appropriate tool for analysis of fucoxanthin metabolism inside single cell and that fucoxanthin is an interesting compound, which can be used in cancer diagnostics and treatment. Raman data has been presented in Figure 1.

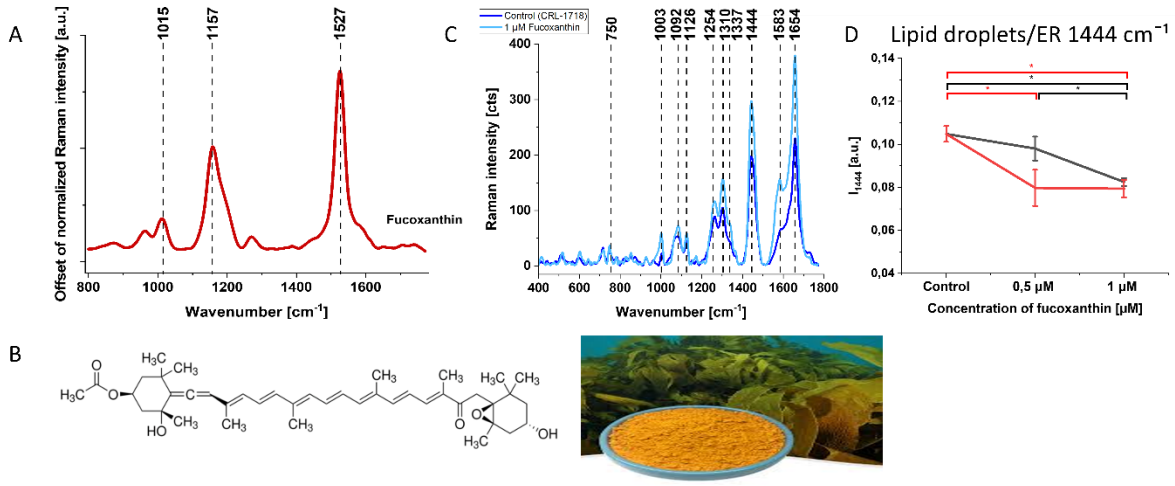

Figure 1. *Normalized Raman spectra of 10mM fucoxanthin solution in DMSO (A), structural formula of fucoxanthin (B), average Raman spectra of lipid droplets in control (unsupplemented) CRL-1718 cells and cells supplemented with 1 µM fucoxanthin in the range from 400 to 1800 cm^{-1} (C), differences in the Raman band at 1444 cm^{-1} peak intensity and standard deviation for lipid droplets/endoplasmic reticulum in CRL-1718 cells supplemented with fucoxanthin: 0.5 and 1 µM after 24 (black) and 48 (red) hours.*

Funding: FU^{2}N – Fundusz Udoskonalania Umiejętności Młodych Naukowców, Grant: „Zastosowanie spektroskopii Ramana w analizie komórek nowotworowych suplementowanych karotenoidami – określenie wpływu".

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P.8 - D - Biomedical Applications

The role of glucose and fructose on lipid droplet metabolism in human normal bronchial and cancer lung cells by Raman spectroscopy

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Fructose is one of the most important monosaccharides in the human diet that the human body needs for proper metabolism. We present an approach to study biochemical changes caused by sugars in human normal bronchial cells (BEpiC) and human cancer lung cells (A549) by Raman spectroscopy and Raman imaging. Results after supplementation of human bronchial and lung cells with fructose are also discussed and compared with results obtained for pure human bronchial and lung cells. Based on Raman techniques we have proved that peaks at 750 cm^{-1}, 1126 cm^{-1}, 1444 cm^{-1}, 1584 cm^{-1} and 2845 cm^{-1} can be treated as biomarkers to monitor fructose changes in cells. Results for fructose have been compared with results for glucose. Raman analysis of the bands at 750 cm^{-1}, 1126 cm^{-1}, 1584 cm^{-1} and 2845 cm^{-1} for pure BEpiC and A549 cells and BEpiC and A549 after supplementation with fructose and glucose are higher after supplementation with fructose in comparison to glucose. The obtained results shed light on the uninvestigated influence of glucose and fructose on lipid droplet metabolism by Raman spectroscopy methods. The results may provide a new opportunity to analyze lipid droplet metabolism caused by two main simple sugars.

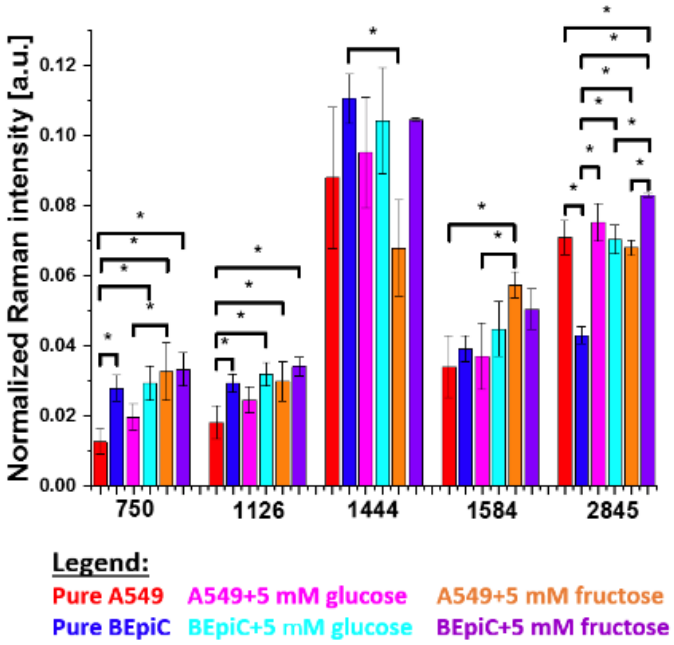

Figure 1. *Bar charts represent normalized Raman intensity for Raman bands for lipid droplets: 750 cm^{-1}, 1126 cm^{-1}, 1444 cm^{-1}, 1584 cm^{-1} and 2845 cm^{-1} for pure A549 cell line (red bar); pure BEpiC cell line (blue bar), A549 cell line supplemented with 5 mM glucose (magenta bar); BEpiC cell line supplemented with 5 mM glucose (turquoise bar), A549 cell line supplemented with 5 mM fructose (orange bar), BEpiC cell line supplemented with 5 mM fructose (purple bar).*

Acknowledgment
This work was supported by the "FU2N-Fund for the Improvement of the Skills of Young Scientists" supporting the scientific excellence of the Lodz University of Technology grant no. W3/2P/2022.

P.9 - D - Biomedical Applications

Chemical changes in lipids in rat kidneys caused by diabetes – correlation of Raman spectroscopy, machine learning, and biochemical data

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease that affects hundreds of millions of people each year [1]. T2DM is a result of a combination of insulin resistance and dysfunction of β cells of pancreatic islets. Insulin resistance occurs when insulin-dependent cells stop responding to insulin and a higher concentration is needed to activate insulin receptors. Once the cells become insulin resistant, glucose in plasma starts to cumulate resulting in hyperglycemia and finally T2DM, which in consequence, can lead to serious complications including diabetic kidney disease [2,3]. In this study, Raman spectroscopy, machine learning algorithms and mass spectrometry analysis were applied to identify differences in the chemical compositions of homogenates collected from the kidneys of rats with diabetes, compared to healthy animals.

Raman measurements were performed by WITec alpha300 R Raman Imaging Microscope (Witec GmbH, Ulm, Germany). C5.0 decision tree (DT), k Nearest Neighbors (kNN), Random Forest (RF) and Support Vector Machine (SVM) algorithms were used to obtain objective classification of Raman results. Moreover, a commercial AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) was used for obtaining biochemical data with Mass Hunter Acquisition B.10.0 (Agilent Technologies, Santa Clara, CA, USA) employed for data acquisition.

Key findings of our research indicate a higher amount of C-H lipid vibrations in the kidneys of T2DM rats compared to the control group. Although Principal Component Analysis (PCA) did not discern between the two groups, lipids metabolic profiling revealed alterations in concentrations of specific glycerophospholipids, indicating potential involvement of lipids in diabetes-induced kidney damage. Moreover, a decision tree analysis pinpointed a particular lipid vibration band as a potential indicator of kidney damage. The correlation between Raman data and lipids profiling results varied between the control and T2DM groups, emphasizing the intricate nature of these relationships. Crucially, our machine learning algorithms achieved an accuracy exceeding 70%, offering robust validation for our findings.

Raman spectroscopy is a promising tool in the detection of changes caused by diabetes in lipids and polysaccharides and in the correlation between those two groups of compounds. The accuracy of the Raman spectroscopy results is higher than 90%. "Raman spectroscopy not only distinguishes between control and diabetic samples but also reveals alterations in the correlation between polysaccharides and lipids in diabetes.

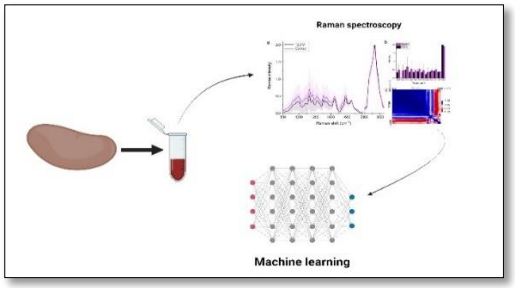

Figure 1. Raman spectroscopy of rat kidney homogenates in conjunction with machine learning algorithms.

This work has been realized as part of a grant from the National Science Centre, project SONATA „Synergy of chemical imaging methods in diabetic model" (project number: UMO-2020/39/D/ST4/01604).

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P.10 - D - Biomedical Applications

Effect of UV radiation on ATR-IR spectra of linoleic acid studied by 2DCOS spectroscopy and moving-window analysis

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The goal of this work was examination of a linoleic acid (LA, cis,cis-9,12-octadecadienoic acid) solution exposed to UV radiation for 24 hours. LA is a stable molecule at ambient conditions, but may be sensitive to electromagnetic radiation. It belongs to essential fatty acids because living organisms cannot synthesize them. Therefore, providing LA to the organism is of great importance for our health. Symptoms of LA deficiency are primarily seen on the skin (i.e. excessive dryness of the skin, hair loss, difficulties in wound healing). Insufficient amount of LA in the body leads to a reduction in the body's resistance. As subsequent changes, hypertension, reduce the number of platelets, or stunted growth can occur [1]. Therefore, it is of great importance to control the concentration and the state of LA in the body.

To monitor the structural changes in LA irradiated by UV radiation, we applied infrared (IR) spectroscopy combined with two-dimensional correlation spectroscopy (2D-COS) and moving window analysis. IR spectroscopy is a powerful tool for the quantitative and qualitative study of biologically important molecules like fatty acids [2,3,4]. The chemometric methods provide additional information that cannot be obtained from classical vibrational spectroscopy [5,6]. Linoleic acid (purity >99%, Sigma Aldrich) was used without further purification. IR spectra were measured on a Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA) using ATR technique (Pike Technologies, USA). In order to investigate the effect of UV radiation, LA solutions were exposed to radiation in the range of 250–600 nm for 24 h. The chemometric analysis was performed by our own software working under MATLAB 2013a [7].

This study shows that ATR-IR spectroscopy joined with chemometric methods is powerful tool for examination of small spectral changes in the biologically important molecules. Spectroscopic analysis of the time-dependent ATR-IR spectra of LA before and after UV irradiation indicates that the extent of spectral changes is significantly higher after an application of UV radiation and it has a complex character. We observed that the absorbance from the C=O group reveals two components slightly shifted in time. During UV exposure within 24 hours occur complex structural variations. At first takes place the *cis*/*trans* isomerization, which accompanies disruption of the C=C double bonds and partial breaking of hydrogen bonds in the cyclic dimers. 2D-COS analysis of time-dependent ATR-IR spectra of LA provides additional information. In the 2D-COS spectra the carbonyl band is resolved into three components suggesting complex character of spectral changes. This indicates the possibility of modifications in the structure of the cyclic dimer. As a side effect of these structural changes, we observed the variations in the orientation of the chains. The characteristic peaks from the methylene groups located at higher wavenumbers were resolved into two components. Analysis of 2D-COS spectra suggests that the breaking of the cyclic dimers and the double C=C bonds are more correlated as compared with the reorientation of the chains. This results from the fact that the first two processes require much higher energy as compared with the reorientation of the side chains. To our best knowledge, this is the first study of long-term effect (24 h) of UV radiation on structure of LA at elevated temperature.

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P.11 - D - Biomedical Applications

The impact of taurine on human brain cancer by confocal Raman imaging

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Extensive research and clinical practices have revealed that taurine has significant physiological impacts, earning its recognition as a natural anti-injury agent. Recent research has extended the scope of taurine's potential benefits to cancer prevention and treatment. Studies suggest that taurine may show anti-cancer properties through mechanisms such as antioxidation, anti-inflammation, and apoptosis induction. These findings have sparked interest in taurine as a supplementary strategy in oncology, aiming to enhance the efficacy of conventional cancer treatments while mitigating their adverse effects. As research continues to unfold, taurine's role in cancer therapy could stand as a promising avenue for integrative medical approaches. Based on this information, we concluded that it would also be worthwhile to test the effect of taurine on astrocytoma. To verify that hypothesis we applied Raman spectroscopy and imaging to analyze astrocytoma cells' responses to taurine supplementation.

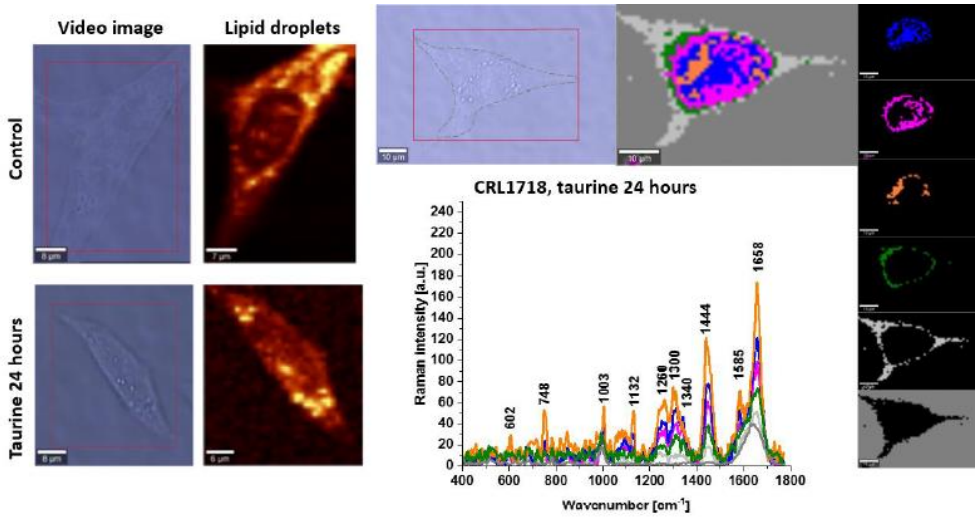

Figure 1. *Raman imaging of astrocytoma supplemented with taurine by 24 hours. Microscopic images of cells before and after supplementation, with Raman maps of lipid droplets*

We will show that taurine might play a crucial role in cancer metabolism by influencing cell growth, energy production, oxidative stress, and the tumor microenvironment. Understanding signaling pathways can lead to new therapeutic approaches in cancer treatment.

P.12 - D - Biomedical Applications

Characterization of pharmacological activity of novel endomorphin-2 analogs by confocal Raman imaging

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Opioid receptors belong to the family of G protein-coupled receptors (GPCRs). Among the three types of opioid receptors (mu, delta and kappa), the mu receptor was identified as the one essential for the pain-relieving effects. However, its activation by opioid analgesic drugs such as morphine is accompanied by serious side effects, such as respiratory depression, constipation, tolerance, and dependence. It was postulated that the therapeutic effect of opioids is associated with the activation of G protein while development of side effects is β-arrestin-dependent. Therefore, one of the new approaches is to develop novel opioid ligands biased to either G protein or β-arrestin in search of the mechanisms useful for the segregation of physiological responses downstream of the receptor and for the development of more effective and safer drugs.

The goal of this study was to characterize the pharmacological activity of a series of linear endomorphin-2 (EM-2) modified by the replacement of Tyr[1] by the Dmt (dimethyl-Tyr), and Pro[2] or Phe[3] with (R)-β2- or (R)-β3-1-Naphthyl alanine, piperidine-2-carboxylic acid or piperidine-3-carboxylic acid, showing bias profile towards activation of G protein/β-arrestin2 pathway. The ability of analogs to interact with mu opioid receptor was tested by tracking changes at the molecular level by Raman spectroscopy and imaging. The antinociceptive activity of compounds and tolerance development potential were tested in the hot-plate test in mice after *i.c.v.* injection. The bead expulsion test was performed to determine the effects of compounds on propulsive motor activity.

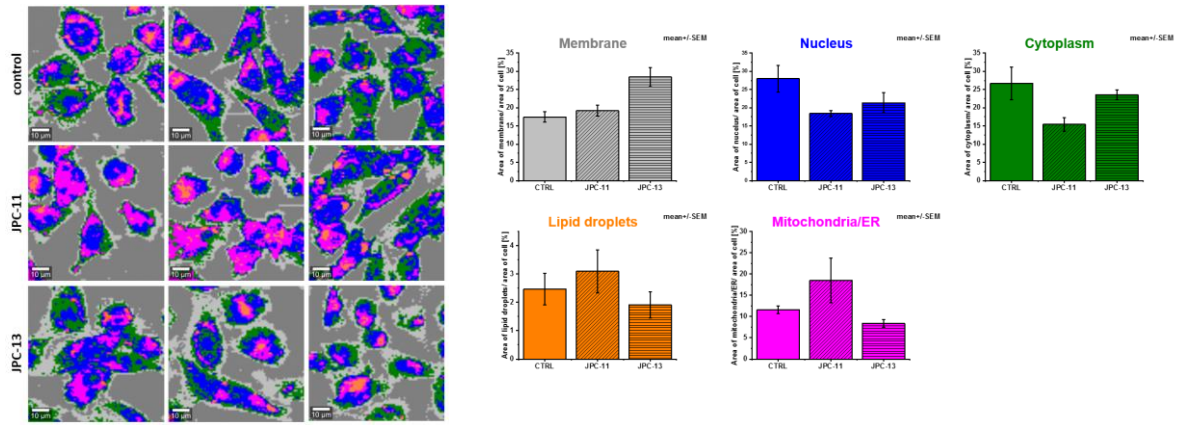

Figure 1. *Raman imaging of CHO cells supplemented with novel opioid ligands by 30 minutes. Raman maps of cells before and after supplementation, with bar plots of the area of different cell organelles per cell area. Raman imaging: 532nm, 10mW, 80x80 μm: 1μm, 0.3sec*

The G protein biased analog (JPC-11) showed a stronger antinociceptive effect in the hot-plate test in comparison to β-arrestin2 biased analog (JPC-13), but also when compared to the parent compound EM-2 which is as a full agonist of both transducers. JPC-11, in opposite to JPC-13, did not evoke significant tolerance development in repeated i.c.v. administration in 5 consecutive days. The JPC-13 induced a strong inhibitory effect on colonic propulsion, at the level of DAMGO. Our Raman data confirmed a potential application of Raman spectroscopy and imaging in distinguishing CHO cells supplemented with novel opioid ligands (JPC-11, JPC-13) based on Raman spectra characteristic for cellular organelles e.g. cell membranes (cell border), nucleus, cytoplasm, mitochondria/ER and lipid droplets.

The importance of finding the mu-opioid receptor agonists with safer therapeutic profiles is undeniable. The data obtained in this study provide valuable insights into the pharmacological activity of opioid analogs biased toward G protein or β-arrestin2. Our study can have a significant contribution to the evaluation and understanding of the consequences of biased agonism *in vivo* in terms of therapeutic potential and side effects of opioids.

Acknowledgments

Financial support was obtained from the National Science Centre, Grant "Sonata" No. UMO-2018/31/D/ST5/02489 to JP-C

P.13 - D - Biomedical Applications

Identification of molecular changes in organs of obese rats following transcranial direct current stimulation (tDCS) - studies by FTIR microspectroscopy and chemometrics

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Due to increasing prevalence of obesity new save and non-invasive therapies are search to cope with the epidemic. Transcranial direct current stimulation (tDCS), similar to V-block therapy could be a good alternative for obesity treatment in the future. In our previous studies, we found that tDCS treatment results in reduced food intake and thus decreased weight gain, and furthermore induces significant molecular changes including qualitative and structural properties of lipids and protein secondary structure in brain areas directly involved in appetite regulation [1,2]. However, further studies are required to prove both safety and effectiveness of this innovative technique. Therefore, to boost current knowledge on possible biochemical mechanisms underlying biological action of tDCS we determined the effects of tDCS on structural changes of lipids and proteins in key organs in obese rodents. For this purpose combined approach using Fourier transform infrared micro-spectroscopy and chemometric analysis were applied.

In the experiment male Wistar rats with diet-induced obesity and subsequently treated with tDCS were used. Thin sections of rat organs such as liver, heart, kidney, and muscle were raster-scanned using Nicolet 8700 FTIR Spectrometer coupled with Nicolet Continuum IR Microscope. Additionally, adipose tissue was probed with the use of Horizontal Attenuated Total Reflectance multi bounce technique.

We observed that although tDCS does not induce significant changes in lipid and protein structures in most organs, very interesting results were found for the liver. In this organ, we found reductions in the levels of unsaturated/saturated lipid ratio (LUnsat) and fatty acyl chain length (FACL) relative to non-stimulated obese individuals. Moreover, the best predictive performance of the tDCS-stimulated group was noted for FACL, LUnsat and lipid to protein ratio. It should be added that the liver was one of the main organs in which obesity resulted in an increase in the level of the aforementioned molecular parameters [3]. This, in turn, suggests that tDSC treatment has beneficial effects on obesity-induced changes in the liver.

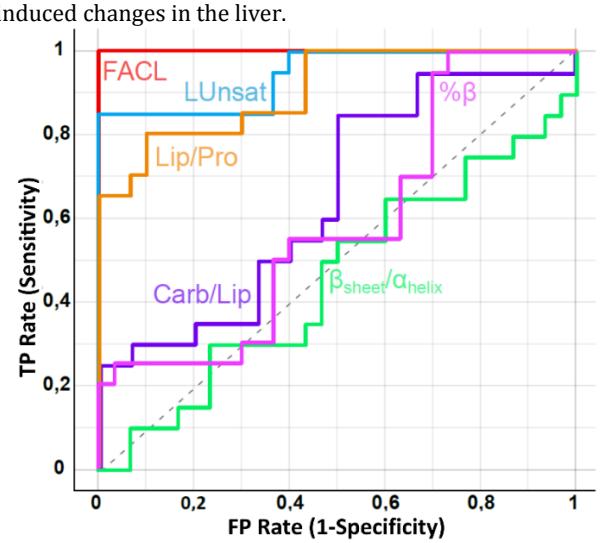

Figure 1. *ROC curves toward predicting tDCS stimulated group based on the molecular parameters in liver (FACL-fatty acyl chain length, LUnsat -lipid unsaturation, Lip/Pro – lipid to protein ratio, Carb/Lip - carbonyl/lipid ratio, %β - β-sheet and β-turn forms fraction from all contributing to amide I forms, $\beta_{sheet}/\alpha_{helix}$ - protein secondary structure ratio; TP - True Positive, FP -False Positive).*

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P.14 - D - Biomedical Applications

Influence of transcranial direct current stimulation (tDCS) on the elemental and molecular composition of brain reward system structures in obese rats

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Obesity is currently a global epidemic problem. Due to multifactorial pathogenesis of obesity, there is no effective and save therapy for excessive adiposity. We have recently proposed transcranial direct current stimulation (tDCS) as an effective experimental technique for body weight gain restriction in rodents [1]. Moreover, we observed altered neurotransmitter activity in the brain regions involved in appetite regulation in obese animals treated with tDCS. It was also found that tDCS treatment induces significant molecular changes in terms of unsaturated lipids and protein secondary structure, accompanied by reduced levels of Na, Cl, K and Ca in brain areas directly involved in appetite control [2]. Motivated by observed changes in brain structures directly related to eating behaviors, we undertook to study the effects of tDCS treatment of obese animals on both molecular and elemental changes also in structures indirectly related to appetite regulation, i.e., the brain reward system.

In the experiment we used male Wistar rats with diet-induced obesity and subsequently treated with tDCS. Thin sections of rat brain containing structures belonging to the reward system were raster-scanned using Nicolet 8700 FTIR Spectrometer coupled with Nicolet Continuum IR Microscope to determine changes in lipid and protein structures. All the samples were raster scanned with 80 µm x 80 µm aperture size, and the step size was 80 µm in both direction. FTIR spectra were acquired in 3900-900 cm^{-1} spectral range. In turn, synchrotron radiation based X-ray fluorescence (SRXRF) was used to determine the elemental levels in the brain structures studied. The SRXRF experiment was carried out at the XRF beamline at Elettra Sincrotrone, Trieste using X-ray beam with an energy equal to 10.5 keV and a beam size of 200 µm × 100 µm (horizontally × vertically). SRXRF analysis was performed for determining the elements related to neural conductivity (Na, Cl, K, Ca, Mg), enzymatic activity (S, Fe, Cu, Zn) and energy metabolism (Mg, P) in the brain areas. FTIR microspectroscopy was used to identify qualitative biochemical information on the lipid structure changes, including lipid saturation/unsaturation, oxidation, hydrocarbon chain length, amount of triglycerides protein secondary structure aberrations in terms of β-sheets and α-helical conformations. To evaluate biochemical changes in brain areas induced by tDCS, statistical and chemometric analysis was included.

We confirmed a significant effect of a brain area on the levels of all the elements examined as well as all the parameters related to lipid structures. In addition, a significant interaction effect was found between the brain structure and the experimental group for all the elements as well as acyl chain length parameter and carbonyl/lipid ratio. We found that, tDCS-treated individuals showed a decrease in Zn levels in the cerebral cortex and an increase in Na and Cu levels in the ventral tegmental area. Regarding molecular parameters, in the substantia nigra area a decrease in the level of lipid-to-protein ratio was observed. The studies showed that in addition to the significant elemental and molecular changes observed in brain structures related to homeostatic appetite regulation [2], tDCS treatment produced significant changes in chemical elements as well as in molecular structure within the brain reward system associated with hedonic feeding behavior.

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[2] A.D. Surowka, A. Ziomber, M. Czyzycki, A. Migliori, K. Kasper, M. Szczerbowska-Boruchowska, Spectrochim Acta A Mol Biomol Spectrosc. 2018, 195,199-209.

P.15 - D - Biomedical Applications

Evaluation of the cartilage tissue degeneration with the use of portable Raman spectrometer

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The loss of synovial fluid and changes in cartilage structure can lead to changes in the mechanism of joint function and in tribological properties. Such disruptions cause degeneration of joint surfaces and the formation of arthritis. This degenerative joint disease is one of the leading causes of disability in the world population. Degradation assessment of cartilage tissue in joints is essential for diagnosing the progression of osteoarthritis. Despite available diagnostic methods such as X-rays and MRI, there are no techniques that can detect the early stages of osteoarthrosis on the market.

Raman spectroscopy is proving to be one of the techniques that allow tissue surface analysis. Quantitative and qualitative analysis, as well as the study of intermolecular interactions, enable the determination of the relationship between the structure of articular cartilage and the degree of degeneration.

Raman spectroscopy allows the analysis of joint tissue providing information on molecular changes caused by the progression of degradation [1]. The scope of the study included the *ex vivo* analysis of human knee cartilage with varying degrees of degradation collected during joint replacement surgery from different patients . Research to date has shown that measurements using Raman spectroscopy can identify damaged tissues.

Moreover, the technique's adaptability may allow its application in a clinical study. With the use of a probe, it may be possible to perform *in vivo* studies. Such an approach would allow the assessment of the degree of cartilage degradation in a joint during an arthroscopy procedure. In this study we presents the preliminary *ex vivo* analysis of human cartilage tissue with the use of fiber-optic Raman probe. Pre-processed data show the potential of Raman spectroscopy applicability in osteoarthritis diagnosis.

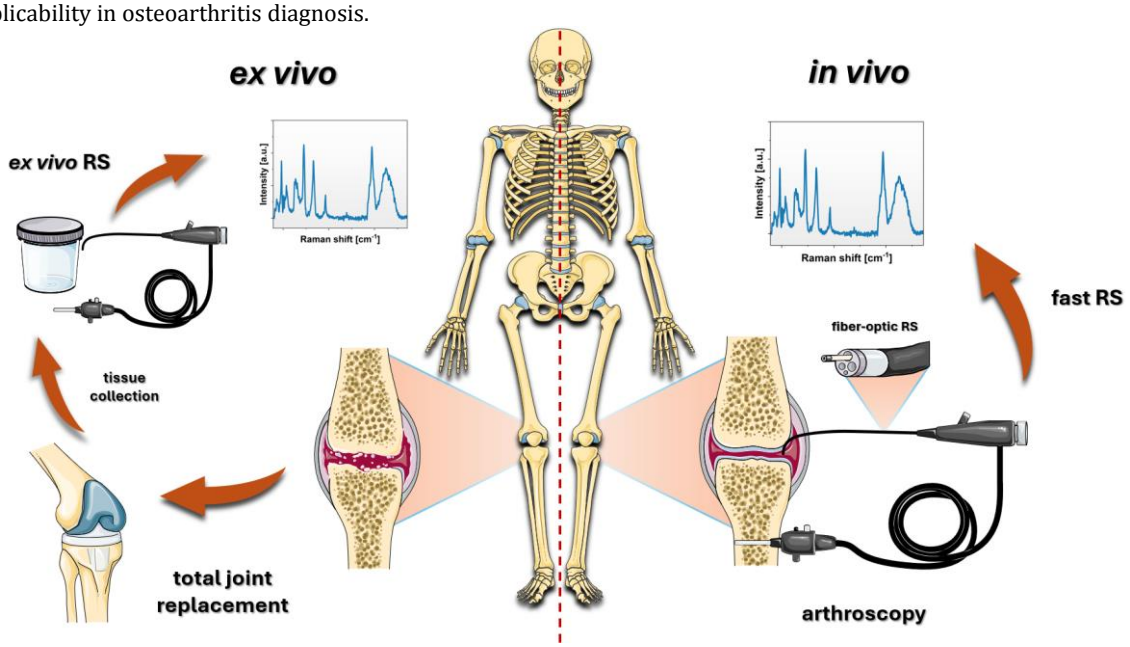

Figure 1. *The scheme of Raman spectroscopy applicability for cartilage tissue evaluation*

The research was conducted as a part of the project OPUS UMO-2018/29/B/ST5/02412 and Euronanomed III ENM3/IV/CaPreCon/2021. This work was conducted while author KT was a PhD Candidate at IDS TUL.

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P.16 - D - Biomedical Applications

Stem Cell Differentiation into Bone
Probing Cellular Events by Vibrational Spectroscopy

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Strategies involving mesenchymal stem cell (MSC) osteogenic differentiation are currently promising tools in regenerative medicine. However, there is a need to further understand the biochemical processes underlying differentiation, in order to optimize their osteogenic commitment. Several intra- and extracellular events, typically occurring over a period of 21 days of osteogenic differentiation, are known to determine osteogenic efficiency – namely protein synthesis and transport to the extracellular matrix (ECM), and mineralization in the ECM (both processes possibly mediated by extracellular vesicles), in tandem with several intracellular metabolic adaptations [1,2]. The use of vibrational spectroscopic techniques with high sensitivity and specificity is known to unveil exquisite detail on subcellular events triggered by different stimuli, *e.g.* carcinogenesis or drug treatment [3,4]. However, such techniques have been underexplored to follow osteogenic differentiation [5]. This work presents, for the first time to our knowledge, a systematic study by complementary vibrational spectroscopy (FTIR and Raman) of human adipose-tissue MSC (hAMSC) across multiple timepoints of osteodifferentiation, with particular focus on biomolecular changes taking place at the intra- and extracellular levels. Along a 21-day period, hAMSC under osteogenic differentiation were compared to the corresponding control samples (absence of osteogenetic stimuli, only cell proliferation occurring). Figure 1 shows the micro-Raman and micro-FTIR spectra of hAMSC after 6 days of differentiation. It is expected that hydroxyapatite features start to appear at the early stages of osteoblastic formation, namely the intense $\nu_1(PO_4)$ band at *ca.* 960 cm^{-1} which is a biomarker of osteogenesis [5]. Additionally, cellular adaptation during osteogenesis will be monitored through changes in membrane fluidity and cytoskeleton/ECM structural characteristics.

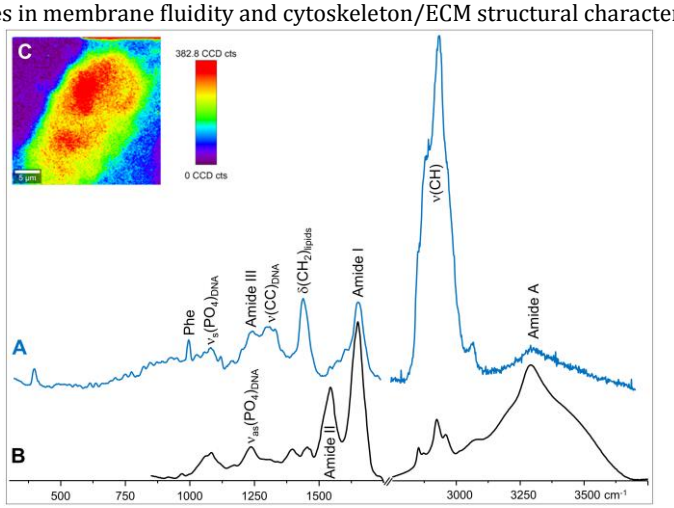

Figure 1. Micro-Raman** (532 nm, 100x objective) **(A) and micro-FTIR** (15x objective) **(B) spectra of hAMSC after 6 days of differentiation, and Raman peak area map (ν_{CH} at 2800-2950 cm^{-1}) of hAMSC before differentiation (C).

Overall, our results demonstrate the promise of subcellular insights of osteogenesis as provided by vibrational spectroscopy in tandem with microscopy. This knowledge should support the definition of informed strategies towards optimization of MSC osteodifferentiation, in biomedical applications.

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P.17 - D - Biomedical Applications

Neurotoxicity model in BBB elements - spectroscopic imaging approach

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Over the past two decades, there has been a significant rise in the percentage of deaths attributed to neurodegenerative disorders[1]. The pathophysiology of neurovascular and degenerative disorders of the central nervous system is directly indicated by malfunction of the blood-brain barrier (BBB), which is a physical barrier, composed of endothelial cells, astrocytes, and pericytes, that regulate the influx of molecules from the bloodstream to the central nervous system (CNS)[2]. Excessive metal accumulation in the body results in neurotoxicity and associated neurological disorders, therefore, among others, mercury (Hg) chemical compounds can be used to model neurotoxic conditions[3]. Astrocytes constitute the primary homeostatic cells in the CNS, which protect neurons against harmful factors, in particular by accumulating heavy metals, which makes them the main target for mercury-induced neurotoxicity.

To identify the spectral markers of neurotoxicity, unrevealed by traditional in vitro techniques, in this work we examined the impact of mercury toxicity on human blood-brain barrier cells. For that purpose, astrocytes (NHA), pericytes (HBVP), and endothelial cells (HMBEC) were exposed to 20 µM $HgCl_2$ for 24 hours. We used Raman and FTIR imaging to observe molecular alternations at the cellular and subcellular levels. The main advantages of this methodology is label-free approach, complementarity of chemical information, and the possibility of identifying unique stress markers. The results of our research indicate significant differences between cells in normoxia and toxicity conditions both at the cellular and subcellular levels. We were able to detect alterations at the whole cell level by FTIR and distinguish cytoplasm, nuclei, and other cellular compartments in high-resolution Raman images. We observed divergent responses of BBB elements to the stress factor. The cellular response to mercuric chloride includes several changes related to biological components such as proteins and nucleic acids. Our work demonstrates the methodological approach to IR/Raman data analysis for comparative investigations of neurotoxicity effects and spectroscopic in vitro studies.

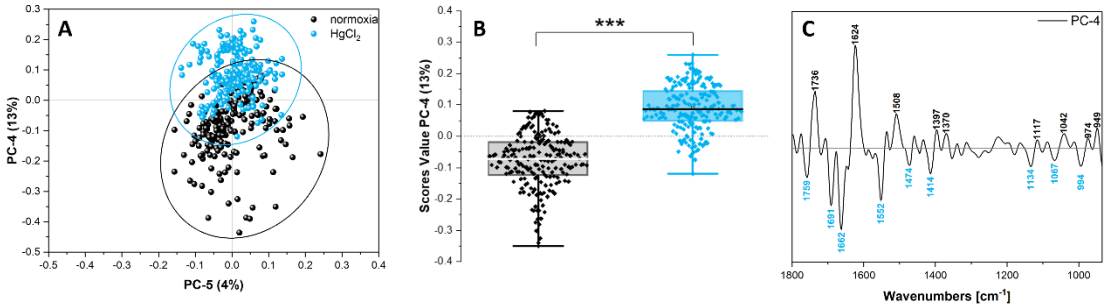

Figure 1. *Principal Component Analysis (PCA) performed on FTIR spectra of the astrocytes under normoxic and mercury toxicity conditions. The scores plots of PC-4 and PC-5 (A), box plot of scores value PC-4 (B), and loadings value PC-4 (C). Each point refers to a single cell.*

ACKNOWLEDGMENTS
The authors acknowledge the financial support from the OPUS 21 project (No. 2021/41/B/ST4/02000) funded by the National Science Center (Poland).

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P.1 - E - Isotopic Labelling

Vibrational Labeling for Enhanced Cell Analysis with Optical Photothermal Infrared (O-PTIR) Technology

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Infrared spectroscopy is a widely used method to examine the molecular states of biomedical samples [1]. Leveraging the full spectral information, high resolution, and video-rate image scanning the Optical Infrared Photothermal (O-PTIR) technique has emerged as a new tool for overcoming the typical limitations of infrared microscopy in terms of cell measurements [2]. Vibrational labeling is a relatively new direction employed in spectroscopic studies for monitoring the biochemical processes with enhanced selectivity by utilizing metabolic-oriented molecules tagged with specific chemical groups (C-D, C≡C, N_3) that produce distinct signals in the cellular spectra [3]. We propose the combination of O-PTIR and vibrational labeling for enhanced metabolism analysis of mammalian cells, i.e. human endothelial aorta endothelial cells (HAEC) labeled with deuterated and azido-tagged fatty acids (oleic and palmitic). The O-PTIR measurements allowed for visualizing the metabolically active sites of the cellular body *via* band-specific image and spectra acquisition. The labeling fashion improved the accuracy of detecting the characteristic signals within the hydrated cells, as the strong absorption of water around 1655 cm^{-1} assigned to amide I obscure the signal from the fingerprint range but has minimal impact on the silent region. Our study is a pioneering first step towards advanced metabolic analysis, demonstrating the potential of this cutting-edge infrared technique.

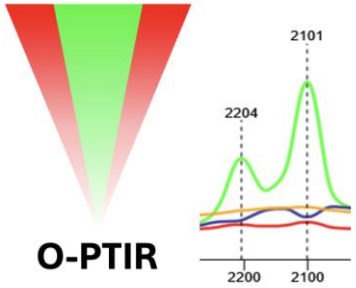

Figure 1. *Detection of Deuterated Fatty Acids Using the O-PTIR Technique.*

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P.1 - F - Bioanalytical Applications

Investigating Diabetes-Induced Alterations in Heart and Liver Lipid Profiles: A Comprehensive Analysis Using ATR-FTIR Spectroscopy, Machine Learning, and Lipid Metabolic Profiling

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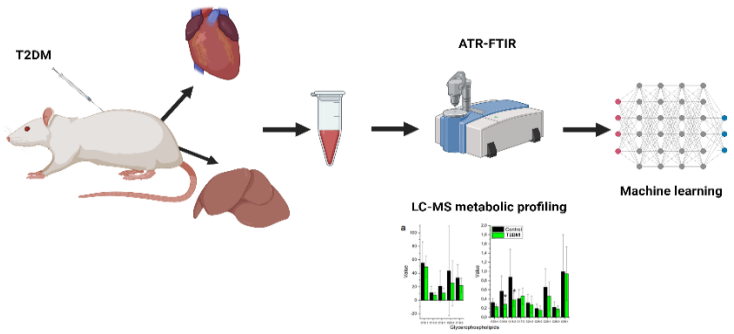

Fig.1 The purpose of the study was presented graphically. ATR-FTIR spectroscopy and LC-MS metabolic profiling were used to analyze rat liver and heart homogenates. The results were combined with machine learning.

Introduction: Diabetes, recognized as a significant health concern by the World Health Organization, leads to debilitating complications affecting vital organs such as the kidneys, cardiovascular system, and digestive system. In 2019, approximately 463 million individuals were affected by diabetes, a figure projected to escalate to 578 million by 2030 and 700 million by 2045. These statistics underscore the pressing demand for integrated, multi-faceted strategies to confront this escalating global health crisis.

Methodology: For this purpose, this study investigates alterations in phospholipid-lipid balance within the heart and liver induced by diabetes. Applying Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC-MS) metabolic profiling, homogenized heart and liver samples from control and type 2 Diabetes mellitus (T2DM) model rats were analyzed.

Results: The study revealed notable differences in phospholipid and lipid profiles between the control and T2DM groups in both heart and liver tissues. Principal Component Analysis (PCA) differentiated control and diabetic liver samples using PC1 and heart samples using PC2. Decision tree analysis highlighted spectral shifts indicative of diabetes in heart and liver tissues, notably in lipid vibration regions. Spectral accuracy was approximately 90% for liver and 100% for heart. Furthermore, lipid metabolic profiling emphasized differences in sphingolipids and glycerophospholipids between diabetic and control heart and liver samples.

Conclusions: The findings suggest that ATR-FTIR spectroscopy, complemented by LC-MS metabolic profiling, holds promise for identifying metabolic changes associated with diabetes mellitus in heart and liver tissues. This approach may offer insights into the pathophysiology of diabetes-related organ damage and aid in the development of diagnostic and therapeutic strategies.

This work has been realized as part of grant from National Science Centre, project SONATA „Synergy of chemical imaging methods in diabetic model" (project number: UMO-2020/39/D/ST4/01604).

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P.1 - G - Nanoscale Analysis

Empowering Environmental Sustainability Functionalized SBA-15 as a Cutting-Edge Solution for Effective Metal Uptake

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One of the most promising and modern solutions to combat environmental pollution is the development of bio-perforated, mesoporous SBA-15 silica. This material boasts an impressive surface area of approximately 800m2/g. It features structurally amorphous properties, forming uniaxially ordered hexagonal channels with a diameter of 5 nm and a length on the order of micrometers. These channels are uniformly distributed throughout the volume, allowing for high capillary properties. SBA-15 also demonstrates a neutral impact on the environment and living organisms, exhibiting no toxic or irritating effects. Furthermore, its physicochemical properties are easily adjustable through various technological processes, offering great flexibility in terms of chemical modification. This high degree of adaptability allows for the functionalization of SBA-15 with a wide range of functional groups, either on its outer or inner walls, while maintaining precise control over their concentration within the material's volume.
The significant advancement in environmental protection, as proposed by our research team, has been achieved by activating the mesopores using specific functional groups tailored for capturing specific types of metals. This functionalization involves the use of various functional groups, such as propyl-carbonate (for metal-binding I, e.g., silver), propyl-phosphate (for metal-binding II, e.g., copper), and cyclam (1,4,9,11 tetraazacyclodecane), which is capable of chelating metal chlorides like copper, chromium, cobalt, nickel, and more. Importantly, this functionalization process ensures the homogeneous distribution of these functional groups within the silica pores.
The potential of metal ion uptake has been rigorously verified using advanced techniques such as SAXS, Positron-Electron annihilation, BET (Brunauer-Emmett-Teller), and spectroscopy methods like UV-VIS spectroscopy. Time-dependent metal uptake curves have been instrumental in estimating the real-time sorption capacity of functionalized SBA-15. Mechanical studies, including Young's modulus parameters at various force levels (ranging from nN to N), have been conducted on individually prepared SBA-15 pellets to assess their stability and mechanical properties. The combined efforts of functionalizing SBA-15 and evaluating its sorption potential through various methodologies aim to pave the way for developing an entirely new class of materials with unique properties for remediating contaminated environments. This research holds the promise of making a significant and positive impact on preserving our environment and protecting human health.

Acknowledgment
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P.2 - G - Nanoscale Analysis

Nanoscale infrared spectroscopy of brain tissue

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Brain is responsible for the control and organization of the nervous system of all vertebrates and most of invertebrate animals. It is the body's largest cluster of neurons and is the most energy-consuming and most specialized organ, responsible for motor control, sensory perception, endocrine regulation, and intelligence development. The healthy brain works quickly and automatically until some brain disease occurs. Typical brain diseases include brain tumors, stroke, genetic brain disorders and degenerative brain diseases like Parkinson's or the more common Alzheimer's disease (AD). Especially the neurodegenerative diseases are a ticking time-bomb for health care systems because they are care-intensive and will soon become much more wide-spread due to increased life expectancy. Therefore, it is highly important to study all unresolved key aspects of brain disorders in order to understand and combat the diseases. The key role is the mapping of the distribution of biomolecules in the brain tissue. The most common techniques for chemical imaging such as Raman and infrared (IR) microscopy or fluorescence have submicron resolution at best and unfortunately cannot resolve the nanoscale biochemical composition.

In this research we used the combination of atomic force microscopy (AFM) and IR spectroscopy called IR nanospectroscopy which has 20 nm resolution and can clearly map peptides and lipids due to their different IR spectra. We chose mouse brain tissue as a model sample to analyze the brain section to set the optimal parameters for our future purpose to study human brain tissues of AD patients. The mouse brain sections were placed on the clear silicon wafer to obtain better optical properties for IR nanospectroscopy. The phase shift obtained from AFM was used for visualization of different parts of the brain tissue because it refers to the mechanical and chemical properties of the sample [1] and subsequently for choosing optimal spots to collect IR nanoscale spectra. Darker areas with smaller phase shifts (compliant domains; probably mitochondria, Golgi apparatus, endoplasmic reticulum, nucleus [1]) were richer on lipids and brighter areas with larger phase shift (rigid domains) were richer on proteins. Therefore, we are able to distinguish different types of the brain tissue by the lipid and protein content (Figure 1). This result can be further used for brain tissues of AD patients e.g. to better distinguish the plaques from the normal tissue.

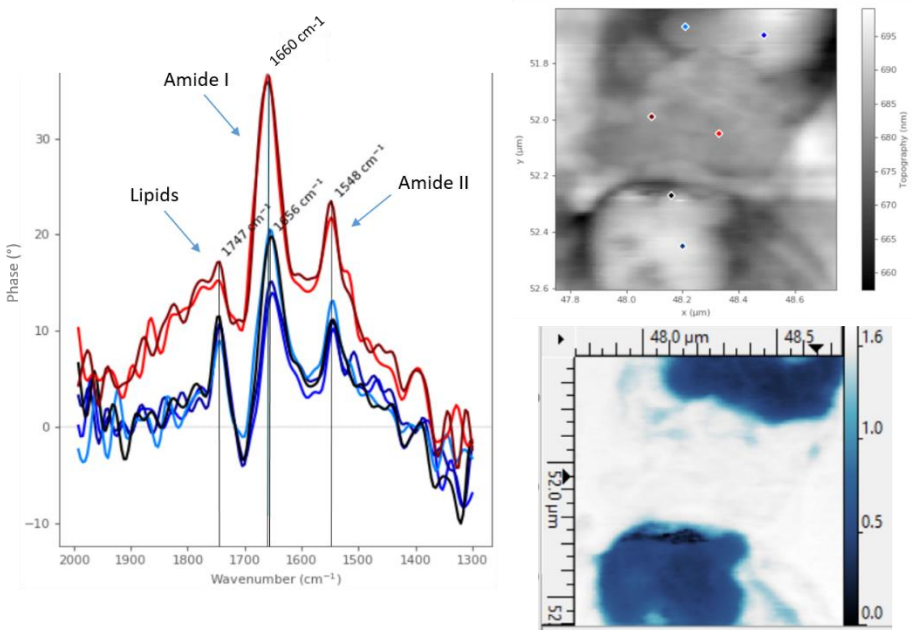

Figure 1. *IR nanoscale spectra from different parts of the brain tissue. The topography image (top left) showed subtle differences, but when using phase shift (bottom left) they became extremely clear. Bluish spectra are collected from dark blue spots, reddish spectra from the light blue spots in phase shift.*

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P.3 - G - Nanoscale Analysis

Aggregation properties of beta-amyloid (Ab) and mutants in presence of iron.

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Alzheimer's disease (AD) is the leading cause of dementia worldwide [1]. One of the peptides involved in AD is the amyloid-beta (Aβ) peptide, whose pathological aggregation is believed to be a central event in the etiology of AD [2]. However, there are still questions regarding the triggering of Aβ's aggregation and the subsequent development of AD. Some hypotheses have been tested, such as the metal ions dyshomeostasis hypothesis [3]. It is now a well-established fact that the onset of AD disturbs the equilibria of physiological metallic ions, named biometals, in the brain such as iron [3, 4]. It is not yet well-understood how various concentrations of iron can influence the relationship existing between the structure, the morphology and the toxicity of Aβ aggregates. Furthermore, while numerous mutations of Aβ have been identified, it is still yet not known how these mutants can influence the aggregation pathways of Aβ.

To further detail and investigate these effects, we propose a biophysical study of Aβ's aggregation, notably using atomic force microscopy coupled to infrared spectroscopy (AFM-IR). AFM-IR combines a nanometric resolution imaging to the analytical power of infrared spectroscopy [5]. It allows for nanoscale recording of infrared spectra, thus breaking the diffraction limit encountered in Fourier-transform infrared spectroscopy (FTIR). Other techniques, such as Thioflavin T (ThT) fluorescence and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) were used to further analyze the behavior of the peptides.

In this work, we study the aggregation properties of Aβ peptide in the presence of iron. We used various biophysical techniques to characterize the aggregated species. We highlight here specific Aβ aggregation properties related to the presence of iron and its concentration. We show specific effects of iron concentration on the type of Aβ aggregates. We also show different behaviors related to the mutations of Aβ.

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P.1 - H - Non-Linear Optics and Time-Resolved

Unravelling the structure of Aβ42 oligomers in membrane-mimetic environments

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In the context of Alzheimer's disease, soluble amyloid-β 42 (Aβ42) oligomers are the most cytotoxic species among all types of Aβ. The aim of our study is to understand the aggregation of Aβ in membrane-like environments. Here, we used FTIR spectroscopy in the transmission mode with temperature control, allowing us to monitor the aggregation process by the changes of absorption in the amide I region.

Our previous studies were focused on Aβ oligomerization in the presence of the detergent SDS [1], but in the present work we have used a model closer to the cell membrane: large unilamellar vesicles composed of lipids with different charges. These membrane models were mixed with Aβ at certain lipid-to-peptide molar ratios. Tuning these parameters allowed us to determine the threshold of Aβ structural changes induced by lipids. Several *in vitro* studies demonstrated high affinity binding of Aβ specifically to anionic lipid vesicles, however it is still debated.

We also employed site-specifically ^{13}C-labelled peptides to test whether the local secondary structure of specific residues changes upon binding of Aβ to membranes. This approach is unique for studying lipid-peptide interaction, and our results allow us to study lipid-induced structural changes with residue-level resolution.

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P.2 - H - Non-Linear Optics and Time-Resolved

Studies of bimolecular reactions kinetics in polymer hydrogels – an environment mimicking the extracellular matrix

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This study aims to describe and enable the prediction of how bimolecular chemical reactions evolve in time in complex environments, such as those found in living cells and extracellular matrices. The restriction of space affects the diffusive movement of the reactants, slowing down the reactions. [1-2] This is known to be the case for long time scales but has not been studied at short time scales, of the order of sub-picosecond to hundreds of nanoseconds.
As a model of an extracellular-like environment, we use polymer (poly(vinyl pyrrolidone) and poly (vinyl methyl ether)) hydrogels crosslinked with the use of electron beam irradiation. This method produces permanent hydrogel networks of different densities without using any additives like crosslinkers, initiators, etc. [3]. Such a straightforward method of synthesis is crucial for the optical methods of study of the bimolecular reactions in a crowded environment; the detection of photoexcited or quenched molecules should not be obscured by the emission of the additives.
As a model reaction, we study the kinetics of photo-triggered electron transfer between a fluorescent dye and an electron donor quencher. This is performed using a dedicated optical setup capable of following fluorescence (FLUPS) and transient absorption (TA) of the reactants in timescales from sub-fs up to sub-µs. More precisely, this allows us to track the dye's fluorescence during the time scale of the reaction (FLUPS) and the evolution of the products (TA).
By comparing the differences between the obtained kinetics in the presence and absence of the hydrogel, we can assess its influence. According to our hypothesis, the presence of the hydrogel should increase the speed of the reaction at short times and significantly decrease the yield of the final products of the reaction due to enhanced recombination.
In cases where a large portion of the reaction happens in time-scales comparable to the duration of this restricted geometry effect, the total reaction yield would deviate from the expected value if simple macroscopic viscosity corrections are made to pure water experiments. We intend to provide a physical model that contains just the sufficient details of the structure of the hydrogel and the reaction able to reproduce the obtained experimental results and extrapolate to uncharted cases in the same manner as already achieved in dilute solutions [4].

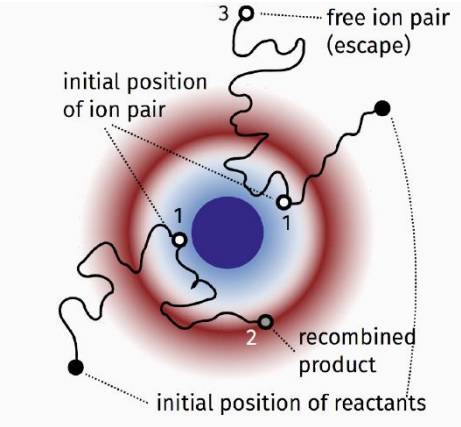

Figure 1. *Pictorial representation of the stages of diffusion-assisted reactions following Smoluchowski's target model. Any obstacles on the way of the reactants, like a polymer, will affect also at short times the kinetics of the reaction.*

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Sponsors Presentations

S.1 – Sponsors Presentations

New Perspectives in 3D Raman Imaging for Life Sciences

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3D confocal Raman imaging is a label-free, powerful, versatile and increasingly common microscopy technique for studies of biological samples. It is capable of quickly identifying components in a sample and visualizing their physical distribution. Correlative microscopy is a hybrid approach that looks at a sample with different microscope technologies, each optimized individually then later linked for a more comprehensive analysis of chemical and structural features.
Raman microscopy is a suitable technique for the studies of cells, tissues, biomolecules such as proteins and nucleic acids, as well as pharmacological formulations and microplastics. In addition, it allows for time-resolved measurements of processes, making it a method with enormous potential for use in life sciences.
This presentation will provide an overview of Raman imaging microscopy, describe recent developments that enable even faster chemical characterization of different biological samples, and offer several vivid examples of its application.

S.2 – Sponsors Presentations

In-situ infrared nanoscopy - investigating dynamic soft matter systems on the nanoscale in their native liquid environment

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Scattering-scanning near-field optical microscopy (s-SNOM) is a state-of-the-art method for nanoscale infrared imaging and spectroscopy far below the diffraction limit of infrared light, commonly reaching resolutions down to 20 nm[1]. The method has been successfully applied in biology to the investigation of a large variety of samples such as cells[2], single proteins[3] and monolayers of lipids[4] in the dried state. However, the measurement of biological samples in their native liquid environment has been extremely challenging due to the tip contaminations, instability of measurements in liquid and attenuation of the infrared light. Here, we showcase an in-situ s-SNOM method that can conduct hour long stable and high-quality measurements on samples in liquid with straightforward s-SNOM optics in reflection mode. The method is based on ultra-thin SiN membranes that permit the near-field of the microscope to penetrate the liquid compartment but protects the s-SNOM tip at the same time from the infrared attenuating liquid water and other contaminations. These capabilities are exemplified on the basis of recent examples of s-SNOM measurements on living bacteria[4], eukaryotic cancer cells[4], photoswitchable liposomes[5] and polymeric particles[1].
We also present as an outlook for new generation s-SNOM measurement on the first integrated microfluidic in-situ s-SNOM systems, which will enable the dynamic exchange of the liquid environment while conducting measurements. This experimental setup will make dynamic in-situ near-field microscopy experiments possible that are based on a change of pH, osmolarity or the injection of chemical or bioactive compounds. We believe that the in-situ s-SNOM method will enable dynamic nanoscale infrared imaging and spectroscopy important for deciphering complex systems related to energy conversion applications, drug delivery systems and cell biology.

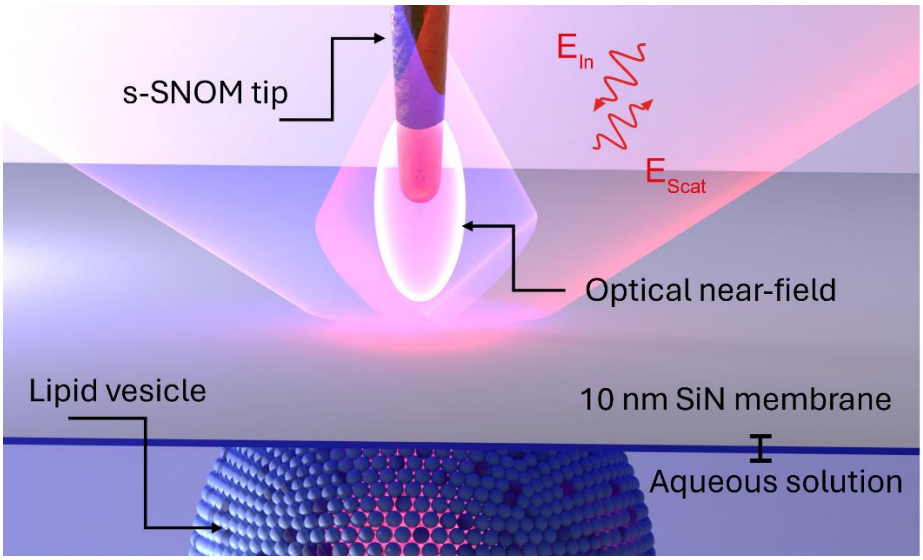

Figure 1. *Sketch of an in-situ near-field microscopy measurement of a photoswitchable lipid nanoparticle. The liquid compartment with the lipid nanoparticle sample in water is separated by an ultra-thin 10 nm SiN membrane from the s-SNOM tip with it's associated near-field. The near-field penetrates the membrane and can be used to image and spectroscopically investigate the sample, while being protected from water contamination.*

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