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We warmly thank the following sponsors for their financial and logistic support.

PhotoThermal ScienTec, for sponsoring the “Cheese & Wine” session. The Cancéropôle Est, for its contribution to the student prizes.

The CNRS GDR ImaBio, for supporting the participation of Sébastien BONHOMMEAU as an invited speaker.

The IOS Press Biomedical Spectroscopy and Imaging, for supporting the participation of Erik GOORMAGHTIGH as an invited speaker.

We also thank the Reims City Hall for hosting the welcome reception and the Departments of Medicine & Pharmacy of URCA for providing facilities to host the conference.

INTRODUCTION

Organizers' welcome note

Dear ECSBM participants;

We are very delighted to welcome you in Reims, the City of Coronations and of Champagne, for the 19th edition of the ECSBM. In 1986, the first edition was held at this same place, but despite less technological progresses, it was certainly easier to organize without the sanitary constraints that we have known over these last years. So, we thank you very sincerely to have made it here to participate to ECSBM 2022. The scientific program mainly focuses on optical spectroscopy more and more at the expense of other spectrometric techniques such as mass spectrometry or NMR, that have their own dedicated conferences. Nevertheless, our program offers a quite rich diversity of approaches. We hope this will be an excellent opportunity for you to have rich scientific interactions and friendly exchanges during both the scientific part and the social events. Thank you again for your participation and we are convinced that you will make this conference a successful one.

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)
2007	Paris (France)
2009	Palermo (Italy)
2011	Coimbra (Portugal)
2013	Oxford (United Kingdom)
2015	Bochum (Germany)
2017	Amsterdam (the Netherlands)
2019	Dublin (Ireland)

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PROGRAMME

Monday 29th August 2022

8h00-8h45	Registration
8h45-9h00	Welcome
9h00-10h15	**BIOANALYTICAL APPLICATIONS (Part 1)** **Chair: Marek PROCHÁZKA**
9h00	**Invited speaker: Erik GOORMAGHTIGH** Infrared imaging of protein microarrays for high throughput characterization
9h30	**Neil HUNT** Detection of Paracetamol Binding to Albumin in Blood Serum using 2D-IR Spectroscopy
9h45	**Ali ASSI*** Effects of solar radiations on Stratum Corneum hydration: Protective role of skin surface lipids and solar filters efficacity
10h00	**Maria Paula MARQUES** Profiling of Human Burned Bones by Vibrational Spectroscopy: Forensic and Archaeological Applications
10h15-10h45	Coffee break
10h45-12h15	**BIOANALYTICAL APPLICATIONS (Part 2)** **Chair: Neil HUNT**
10h45	**Cécile LEVASSEUR-GARCIA** Near Infrared Spectroscopy and Food Quality
11h00	**Alžbeta KUIŽOVÁ*** Drop coating deposition Raman spectroscopy of contaminants
11h15	**Nils Kristian AFSETH** FTIR spectroscopy as a future bioprocess monitoring tool – Characterisation of hydrolysed proteins and peptides
11h30	**Omar DIB** Chemometrics Tools for the non-Targeted Research of Microorganisms in Food Samples by Raman spectroscopy
11h45	**Ning YANG** Online monitoring of fermentation bioreactor based on Raman spectroscopy
12h00	**Katarzyna DZIEDZIC-KOCUREK** Avian vs human erythrocytes – preliminary spectroscopic studies
12h15-13h45	Lunch
13h45-15h00	**MOLECULAR SPECTROSCOPY 1** **Chair: Sophie LECOMTE**
13h45	**Invited speaker: Susan QUINN** Time-Resolved Infrared Study of Adenine photo-oxidation by an Intercalated Chromium Polypyridyl Complex
14h15	**Maxim BOKOV*** Rearrangement of intracellular crystalline guanine as an adaptation for various illumination levels

14h30	**Igor SAZANOVICH** Conformational relaxation and molecular oxygen rebinding in alpha and beta subunits within valency hybrids of human hemoglobin
14h45	**Janne IHALAINEN** The structural effect between the output module and chromophore binding domain is a two-way street via the hairpin extension
15h00-15h30	Coffee break
15h30-17h15	**BIOMEDICAL APPLICATIONS 1** **Chair: Malgorzata BARANSKA**
15h30	**Invited speaker: Hugh BYRNE** Biomedical Applications of Vibrational Spectroscopy: the continued drive towards Clinical Translation
16h00	**Antoine HUGUENIN** Candida albicans outbreak in a Maternity Unit: Epidemiologic study by FTIR spectroscopy, MALDI-TOF spectrometry and microsatellite analysis of strains from neonates
16h15	**Uraib SHARAHA** Rapid determination of Proteus mirabilis susceptibility to antibiotics using infrared spectroscopy in tandem with random forest
16h30	**Anna NOWAKOWSKA** On the way to clinical diagnostics: can Raman spectroscopy detect leukemic cells?
16h45	**Murali Krishna CHILAKAPATI** Raman and FTIR spectroscopic stratification of saliva of healthy, habitués and oral cancer subjects
17h00	**Lila LOVERGNE** Infrared spectral biomarkers of neurodegenerative diseases
18h30	**Welcome reception, City Hall** **Talk by Daniel Cordier,** **University of Reims Champagne-Ardenne:** The icy moons of the solar system : Titan and its family

Tuesday 30th August 2022

8h45-10h15		**ISOTOPIC LABELLING** **Chair: Maria Paula MARQUES**
	8h45	**Invited speaker: Howbeer MUHAMADALI** Spectroscopic probing of bacterial metabolism at single-cell level
	9h15	**Christophe SANDT** Do Galleria mellonella larvae metabolize polyethylene? An FTIR microspectroscopy study using isotopoic labelling
	9h30	**Giulia GIUBERTONI** Strong isotope-effect on the conformation and self-assembly of collagen
	9h45	**Malgorzata BARANSKA** Labelling endothelial cells with Raman probes improves the specificity and sensitivity of their imaging
	10h00	**Stephan PISSOT*** Rapid antibiotic susceptibility testing using FTIR spectroscopy and deuterium isotope probing
10h15-10h45		**INDUSTRIAL SPONSORS** **Chair : Ali TFAYLI**
	10h15	**ENDRESSHAUSER -KAISER**
	10h25	**METROHM**
	10h35	**OPTON LASER INTERNATIONAL**
10h45-11h15		Coffee break
11h15-12h45		**ENHANCEMENT TECHNIQUES** **Chair: Igor CHOURPA**
	11h15	**Invited speaker: Alois BONIFACIO** Label-free SERS spectra of biological samples: characteristics and potential significance for clinical applications
	11h45	**Petr PRAUS** Magnetron-Sputtered Polytetrafluoroethylene-Stabilized Silver Nano-island Surface for Surface-Enhanced Fluorescence
	12h00	**Bruno TORRE** Hot Electron Nanoscopy and spectroscopy (HENs): from probe design to real applications
	12h15	**Petra HELLWIG** Plasmonic nanostructures for SEIRAS on membrane proteins
	12h30	**Marek PROCHÁZKA** Study of the compactness and permeability of the polymer brushes by surface-enhanced Raman spectroscopy
12h45-14h15		Lunch
14h15-15h30		**BIOMEDICAL APPLICATIONS 2** **Chair: Hugh BYRNE**
	14h15	**Invited speaker: Francesca PALOMBO** Probing tissue biomechanics with chemical specificity in Brillouin-Raman microscopy

14h45	**Ewelina BIK*** Drug induced phospholipidosis in endothelial cells studied by Raman imaging
15h00	**Nicolas GOFFIN*** Characterization of cancer-associated adipocytes by Raman spectroscopy
15h15	**Karolina ŠIŠKOVÁ** Bimetallic Au-Fe(III) nanocomposites for multimodal imaging
15h30-16h00	**INDUSTRIAL SPONSORS** **Chair : Ali TFAYLI**
15h30	**OPTOPRIM**
15h40	**PERKINELMER**
15h50	**PHOTOTHERMAL SCIENTEC**
16h00-16h30	Coffee break
16h30-17h30	**MOLECULAR SPECTROSCOPY 2** **Chair: Susan QUINN**
16h30	**Invited speaker: Sébastien BONHOMMEAU** Tip-enhanced Raman spectroscopy for nanoscale chemical and structural characterization of biomolecules
17h00	**Monica MARINI** Background-free DNA-protein interactions: structural insights by Raman spectroscopy
17h15	**Stepan JILEK*** Raman optical activity as a potent tool for studies of mononucleotide G-quadruplexes
17h30-18h15	**FLASH PRESENTATIONS** **Chair: Francesca PALOMBO and Agnieszka BANAS**
	Almar AL ASSAAD* Monitoring of the accumulation of Squalene-Gemcitabine nanomedicine within single living breast cancer cell by Raman imaging
	Ohood ALSHAREEF* Single Cell FTIR Imaging with Novel ZnS Hemispheres for Studying Phospholipidosis in Live Macrophages
	Karolina AUGUSTYNIAK* Early detection of stem cells transformation using FTIR and High-Resolution Raman Imaging
	Uladzislau BLAZHKO SIMIECORR: Mie scatter correction without a prior assumption about the chemical composition of a sample
	Arianna BONIZZI* Identification of a biochemical signature of dysfunctionality by Raman spectroscopy analysis of lipoproteins
	Annalisa CARRETTA* From synthetic identity to biological function of a doxorubicin liposomal formulation

Gary COONEY*
Tip-Enhanced Raman Spectroscopy of Tau fibrils: Measurement and Chemometric Analysis

Markéta FOUSKOVÁ
Raman Spectroscopy in the Early Diagnosis of Colorectal Cancer

Francesca GASPARIN*
Live-cell Mid-infrared Optoacoustic Microscopy and Spectroscopy for Longitudinal Metabolic Monitoring

Philip GASSE*
Two-dimensional infrared spectroscopy of carbohydrates with site-specific reporter groups

Pooja GIRISH*
Spectral tissue imaging for ex-vivo cancer diagnosis and survey

Julien GUILLARD*
FTIR spectral imaging analysis of cirrhosis development in two murine models

Mahmoud HULEIHEL
Infrared Spectroscopy in Tandem with Machine Learning for Simultaneous Rapid Identification of Bacteria Isolated Directly from Patients' Urine Samples and Determination of Their Susceptibility to Antibiotics

Maria KRAJAČIĆ*
Artificial Neural Network and Support Vector Machine Regression for Forensic Age Determination Using Raman Spectra of Teeth

Chen LIU*
Raman-based Detection of Antibiotics in Pharmaceutical Formulations and Biological Matrices

Shibarjun MANDAL*
Bacteria localization in hematogenous osteomyelitis using fluorescence and Raman imaging

Nathan MEYER*
Detection of Aβ 1-42 aggregates by RT-FAST: toward a new tool for the diagnostic of Alzheimer's disease

Pierre NIZET*
Assessment of Ovarian Tumor Growth in Wild-Type and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry

Imane OUDAHMANE*
Vibrational spectroscopy applied on biofluids: infrared spectroscopy for bladder cancer diagnosis using urine samples

Ayyoub RAYYAD*
Analytical quality control of therapeutic mAbs preparations by Raman spectroscopy

José Javier RUIZ*
Identification and biochemical characterization of breast cancer cells resistant to neoadjuvant treatment by Raman Spectroscopy

Oliva SALDANHA
Calcium induced vesicular interactions studied with ATR- FTIR spectroscopy

František ŠANDA
Lineshape analysis of 2D spectra for fifth order spectroscopies: exciton transport, annihilation and spectral diffusion dynamics

Till STENSITZKI
High-throuput 2D-IR spectroscopy using the HARE chip

Laurence VAN GULICK
Effects of obesity on the structural organization and mechanical properties of type I collagen

Clara WATTIEZ*
Determining the influence of H/D exchange on IR spectroscopy and vibrational dynamics of polypeptide secondary structures

Martina ZANGARI*
The role played by protein-asbestos fiber interaction in asbestos pathogenicity

18h15-20h45 **POSTER SESSION – WINE AND CHEESE**

1. **Samar ADAWI***
   Using FTIR-ATR spectroscopic method to monitor the development of fungi in plants and bread
2. **Almar AL ASSAAD***
   Monitoring of the accumulation of Squalene-Gemcitabine nanomedicine within single living breast cancer cell by Raman imaging
3. **Ohood ALSHAREEF***
   Single Cell FTIR Imaging with Novel ZnS Hemispheres for Studying Phospholipidosis in Live Macrophages
4. **Ali ASSAF**
   Monitoring of algal production in photobioreactors by Raman spectroscopy and chemometrics
5. **Karolina AUGUSTYNIAK***
   Early detection of stem cells transformation using FTIR and High-Resolution Raman Imaging
6. **Luís BATISTA DE CARVALHO**
   Who's who? Discrimination of Breast Cell Lines by FTIR Microspectroscopy
7. **Vladimír BAUMRUK**
   Absolute configuration determination of promising new drug for Parkinson's disease via Raman optical activity
8. **Lucie BEDNÁROVÁ**
   Structural Investigation of α/γ-Hybrid Peptide Oligomers
9. **Uladzislau BLAZHKO**
   SIMIECORR: Mie scatter correction without a prior assumption about the chemical composition of a sample
10. **Arianna BONIZZI***
    Identification of a biochemical signature of dysfunctionality by Raman spectroscopy analysis of lipoproteins
11. **Radek BURA***
    Isotopic labeling of microalgae: Raman study

12. **Annalisa CARRETTA***
From synthetic identity to biological function of a doxorubicin liposomal formulation
13. **Murali Krishna CHILAKAPATI**
Raman isotope probing (RIsP) for identifying antimicrobial resistance
14. **Murali Krishna CHILAKAPATI**
Raman Spectroscopy based metabolomics for bioprocess monitoring
15. **Murali Krishna CHILAKAPATI**
Raman Spectroscopy Analysis of Plasma of Diabetes Patients without and with Retinopathy, Nephropathy, and Neuropathy
16. **Gary COONEY***
Tip-Enhanced Raman Spectroscopy of Tau fibrils: Measurement and Chemometric Analysis
17. **Mohammed ESSENDOUBI**
Raman Micro-Spectroscopy for skin and hair cosmetics testing
18. **Markéta FOUSKOVÁ**
Raman Spectroscopy in the Early Diagnosis of Colorectal Cancer
19. **Francesca GASPARIN***
Live-cell Mid-infrared Optoacoustic Microscopy and Spectroscopy for Longitudinal Metabolic Monitoring
20. **Philip GASSE***
Two-dimensional infrared spectroscopy of carbohydrates with site-specific reporter groups
21. **Pooja GIRISH***
Spectral tissue imaging for ex-vivo cancer diagnosis and survey
22. **Giulia GIUBERTONI**
In situ identification of secondary structures in unpurified Bombyx mori silk fibrils using polarized two-dimensional infrared spectroscopy
23. **Cyril GOBINET**
Supervised learning of infrared spectral images for the diagnosis of different types of breast cancer
24. **Julien GUILLARD***
FTIR spectral imaging analysis of cirrhosis development in two murine models
25. **Petra HELLWIG**
Vibrational spectroscopies and microscopies: a tool to study and identify neurodegenerative diseases
26. **Mahmoud HULEIHEL**
Infrared Spectroscopy in Tandem with Machine Learning for Simultaneous Rapid Identification of Bacteria Isolated Directly from Patients' Urine Samples and Determination of Their Susceptibility to Antibiotics
27. **Seydou KANE**
Reduction of acquisition time in Fourier transform infrared spectroscopy by deep learning

28. **Hichem KICHOU***
Analytical performance of Raman spectroscopy for quantification of active ingredients in Human stratum corneum

29. **Eva KOČIŠOVÁ**
Surface-enhanced Raman spectroscopy of biologically important molecules on V_2O_5 nanoparticle films

30. **Maria KRAJAČIĆ***
Artificial Neural Network and Support Vector Machine Regression for Forensic Age Determination Using Raman Spectra of Teeth

31. **Martin KRÁL***
Infrared s-SNOM imaging of surface adhesive polydopamine layers formed on various substrates

32. **Kateřina KRÁLOVÁ**
Combining Vibrational Spectroscopy, Metabolomics and Proteomics – Comprehensive Analysis of Blood Plasma for Clinical Diagnostics

33. **Lenka KREPSOVÁ**
Photoswitching of Triptycene-Based Molecular Machines Followed by Raman Spectroscopy

34. **Chen LIU***
Raman-based Detection of Antibiotics in Pharmaceutical Formulations and Biological Matrices

35. **Vanessa LOBOGNON***
Characterization of Bone-Implant Interface after Osseodensification by Infrared Imaging: Development of an Experimental Model

36. **Shane MAGUIRE***
ATR-FTIR spectroscopy of calcium-dependent lipid-binding proteins

37. **Shibarjun MANDAL***
Bacteria localization in hematogenous osteomyelitis using fluorescence and Raman imaging

38. **Lorenz MATTES**
Dual-comb-IR-spectroscopy to study temperature-jump dynamics of polyQ model peptides

39. **Aidan MEADE**
Detection of radiosensitive subpopulations ex-vivo via Raman microspectroscopy of lymphocytes

40. **Nathan MEYER***
Detection of Aβ 1-42 aggregates by RT-FAST: toward a new tool for the diagnostic of Alzheimer's disease

41. **Pierre NIZET***
Assessment of Ovarian Tumor Growth in Wild-Type and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry

42. **Jonas OSHAUG PEDERSEN**
Controllable deposition of gold nanoparticles using a one-step centrifugation process and its application for SERS

43. **Imane OUDAHMANE***
Vibrational spectroscopy applied on biofluids: infrared spectroscopy for bladder cancer diagnosis using urine samples

44. **Pierre PRADA**
Identification of circulating biomarkers of Crohn's disease and spondyloarthritis using FTIR spectroscopy

45. **Ayyoub RAYYAD***
Analytical quality control of therapeutic mAbs preparations by Raman spectroscopy

46. **Ayyoub RAYYAD***
Confocal Raman microspectroscopy as a tool to access the quality of chicken egg

47. **José Javier RUIZ***
Identification and biochemical characterization of breast cancer cells resistant to neoadjuvant treatment by Raman Spectroscopy

48. **Oliva SALDANHA***
Calcium induced vesicular interactions studied with ATR- FTIR spectroscopy

49. **František ŠANDA**
Lineshape analysis of 2D spectra for fifth order spectroscopies: exciton transport, annihilation and spectral diffusion dynamics

50. **Christophe SANDT**
Heterogeneity of human hair medulla lipids, studied by synchrotron μFTIR and OPTIR microspectroscopy

51. **Igor SAZANOVICH**
ULTRA at Central Laser Facility

52. **Ramona SCHLESINGER**
The Photoreaction of the Proton-Pumping Rhodopsin 1 from the Maize Pathogen Basidiomycete Ustilago maydis

53. **Karlis SHVIRKSTS**
Radiation-induced continuous effect on the secondary structure of keratin studied by FTIR spectroscopy

54. **Till STENSITZKI**
High-throuput 2D-IR spectroscopy using the HARE chip

55. **Paul STRITT***
Resolving lipid dynamics in the photocycle of bacteriorhodopsin by mid-IR quantum cascade laser spectroscopy

56. **Daniela TÄUBER**
Comparative investigation of fibrillar actin using Nano IR spectroscopic and fluorescence microscopy imaging

57. **Laurence VAN GULICK**
Effects of obesity on the structural organization and mechanical properties of type I collagen

58. **Vincent VAN HEMELRYCK**
A new convenient tool to analyse protein glycosylation based on FT-IR spectroscopy

59. **Elise VINCENT**
FTIR and biochemical characterisation of glycosaminoglycans (GAGs) content in ovarian cancer cells

60. **Jehan WAEYTENS***
Characterization of secondary structure of protein by infrared nanospectroscopy

61. **Clara WATTIEZ***
Determining the influence of H/D exchange on IR spectroscopy and vibrational dynamics of polypeptide secondary structures

62. **Martina ZANGARI***
The role played by protein-asbestos fiber interaction in asbestos pathogenicity

Wednesday 31th August 2022

8h45-10h15		**CHEMOMETRIC ADVANCES (Part 1)** **Chair: Achim KOHLER**
	8h45	**Invited speaker: Thomas BOCKLITZ** Photonic Data Science: Data pipelines for the analysis of vibrational spectral data using machine learning and chemometrics
	9h15	**Krzysztof BANAS** Open Science paradigm for spectroscopic analysis of biological molecules: from experiment design to data evaluation and reporting
	9h30	**Aidan MEADE** Digital deparaffinisation of Paraffin-Embedded Breast Cancer FTIR Spectra Using an Autoencoder Approach
	9h45	**Ahmad SALMAN** Fast diagnosis of the etiology of oncology patients' infections by monitoring their immune system response to infection: Infrared spectroscopy of leucocytes
	10h00	**Warda BOUTEGRABET** Unsupervised feature selection by a genetic algorithm for mid-infrared spectral data
10h15-10h45		Coffee break
10h45-11h45		**CHEMOMETRIC ADVANCES (Part 2)** **Chair: Thomas BOCKLITZ**
	10h45	**Invited speaker: Achim KOHLER** Understanding and modelling of scattering and absorption phenomena in infrared microscopy
	11h15	**Eirik MAGNUSSEN** Deep Learning-enabled Inference of Distribution of Molecular Absorption of Biological Cells from FT-IR Spectra
	11h30	**Jean-Emmanuel CLEMENT** Gaussian Weighted Background Correction for Raman images
11h45-12h30		**INDUSTRIAL SPONSORS** **Chair : Sébastien BONHOMMEAU**
	11h45	**BRUKER**
	11h55	**OXFORD INSTRUMENTS - WITec**
	12h05	**THERMOFISHER SCIENTIFIC**
	12h15	**HORIBA SCIENTIFIC**
12h30-13h00		Lunch bag
13h00-17h30		Social programme: guided tour of the champagne vineyard and champagne tasting at Hautvillers
19h30-23h00		Conference dinner in the Caveau Castelnau champagne house

Thursday 1st September 2022

9h00-10h15		**NANOSCALE ANALYSIS (Part 1)** **Chair: Joachim HEBERLE**
	9h00	**Agnieszka BANAS** The recent technical development and applications performed at ISMI beamline at Singapore Synchrotron Light Source
	9h30	**Margaux PETAY*** Chemical speciation of breast microcalcifications in cancerous tissues: from a micrometer to a nanometer scale description
	9h45	**Sebastien BALME** Single Nanopore to investigate protein assembly
	10h00	**Daniela TÄUBER** Nano-infrared spectroscopic imaging (NanIRim): Promises and Challenges for Application in Biophotonics
10h15-10h45		Coffee break
10h45-11h30		**NANOSCALE ANALYSIS (Part 2)** **Chair: Ariane DENISET-BESSAU**
	10h45	**Mustafa KANSIZ** Enhanced Tri-modal Optical-Photothermal Infrared (O-PTIR) Spectroscopy – Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)
	11h00	**Miguel PLEITEZ** Functional live-cell mid-infrared microscopy and spectroscopy by optoacoustic and optothermal detection
	11h15	**Joachim HEBERLE** Infrared Nanoscopy and Tomography of Intracellular Structures
11h30-12h30		Closing conference and student prizes

ORAL SESSIONS

Monday 29th August 2022
9h00-12h15
Bioanalytical applications

Infrared imaging of protein microarrays for high throughput characterization

Joëlle De Meutter, Erik Goormaghtigh

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Context and Objective: In the field of protein research in general and the pharmaceutical industry in particular, it is now necessary to perform measurements of the secondary structure of proteins on many samples simultaneously, for instance to evaluate the action of multiple environmental conditions.

Methods: We propose a new approach to evaluate the secondary structure of proteins on a very large scale (approximately 2000 to 4000 samples / cm^2), by combining infrared imaging and 2D printing of protein microarrays.[1,2] In view of the large amount of data, in a first step, methods for automating the extraction of spectra of interest from microarray infrared images and for automating the processing of the spectra were developed.

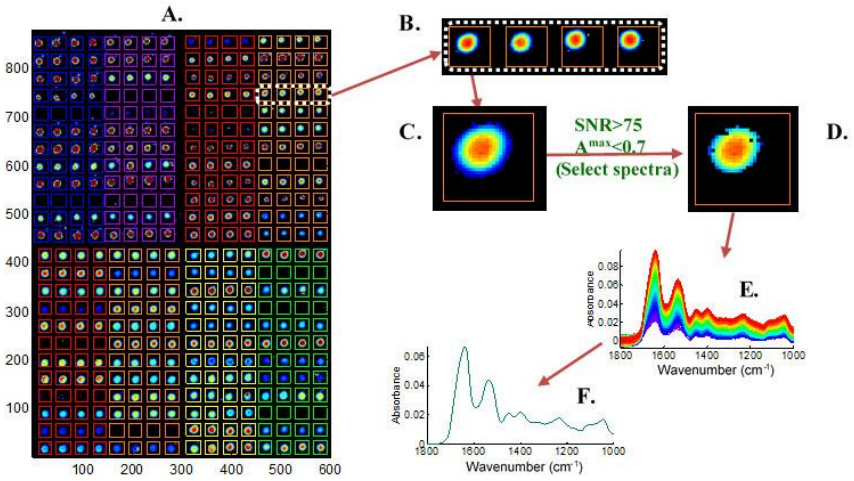

Illustration of the FTIR analysis of a 384 spot protein microarrays (A). The image contains 528,000 full FTIR spectra. Each spot contains ca 1 ng protein. Protein spots are identified (B,C), spectra filtered (D), extracted (E), and averaged (F).

A protein bank consisting of 92 commercially available proteins, the structure of which was well characterized by Xray crystallography, was established for this purpose.[3]

Results and Conclusions: After development of predictive models for secondary structure determination and validation of the protein microarray approach,[4] we attempted to further optimize the models by considering different approaches such as different secondary structure definitions,[5] partial deuteration[6] or subtraction of amino acid side chain contributions.[7] Improvements turned out to be modest for each of the parameters. Dealing with non-native

structure induced by mild denaturation conditions was another challenge as such structures were not present in the reference protein library. Multivariate curve resolution-alternating least squares (MCR-ALS) was used to model a new spectral component appearing in the protein set subjected to denaturing conditions. It represented a spectroscopic marker of aggregation and could allow a semi-quantitative evaluation of the aggregation.[8] While the assessment of secondary structure was well established, tertiary structure and stability are also critical. Hydrogen / deuterium exchange (HDX) is a potential approach for studying the structure and dynamics of proteins.[9,10] A device that allowed to follow the HDX exchange kinetics simultaneously on the entire microarray was built and HDX kinetics could be monitored for all proteins.

Perspectives: FTIR imaging of microarray opens the door to high throughput analysis of protein structure and glycosylation.[11] It also opens the door to high throughput analysis of biological fluids such as urine, blood and serum.

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(2) De Meutter, J.; Derfoufi, M. K.; Goormaghtigh, E. Analysis of Protein Microarrays by FTIR Imaging. *Biomed. Spectrosc. Imaging* **2016**, *5*, 145–154.

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Detection of Paracetamol Binding to Albumin in Blood Serum using 2D-IR Spectroscopy

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Context and Objective: Binding of drugs to blood serum proteins can influence both therapeutic efficacy and toxicity. The ability to measure the concentrations of protein-bound drug molecules quickly and with limited sample preparation could therefore have considerable benefits in biomedical and pharmaceutical applications. Vibrational spectroscopies provide data quickly but are hampered by complex, overlapping protein amide I band profiles and water absorption. Ultrafast two-dimensional infrared (2D-IR) spectroscopy has been shown to suppress signals due to H_2O and to be able to study the protein composition of blood serum. Here we assess the ability of 2D-IR to measure drug binding to serum proteins *in vivo*.

Methods: Ultrafast 2D-IR spectra of pooled equine serum samples spiked with paracetamol at concentrations ranging between 7 µM -1.8 mM were obtained. These samples span the normal clinical range for paracetamol from trace amounts to levels associated with potential hepatotoxicity. Principal Component Analysis was used to identify spectral changes to serum 2D-IR spectra caused by paracetamol addition.

Results and Conclusions: We show that 2D-IR spectroscopy can achieve rapid detection and quantification of paracetamol binding to serum albumin in blood serum at physiologically-relevant levels with no additional sample processing. By measuring changes to the amide I band of serum albumin caused by structural and dynamic impacts of paracetamol binding we show that drug concentrations as low as 7 µM can be detected and that the availability of albumin for paracetamol binding is less than 20% in serum samples, allowing identification of paracetamol levels consistent with a patient overdose.

Effects of solar radiations on Stratum Corneum hydration: Protective role of skin surface lipids and solar filters efficacity

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Context and Objective:
Skin is the largest organ of the body and plays several physiological functions such as a protective barrier against water loss and environmental stresses including solar radiations. Skin surface lipids (SSL) film is a mixture of sebum and keratinocyte membrane lipids. It covers the surface of the stratum corneum (SC) and thus participates to its barrier function. SSLs are composed of a complex of free fatty acids (FFAs) and other lipids that come from sebaceous secretion such as triglycerides, wax esters, and squalene, or from desquamation such as free and esterified cholesterols with FFAs. The aim of this study was to firstly evaluate the effect of solar radiations on the SC hydration and to explore the role of skin surface lipids in the protection of water homeostasis in the SC against solar radiations. Then, we evaluate the limits of the protection capacity of skin surface lipids, and we explore the protective action of sun filters on both SSLs composition and the water hydration and dehydration kinetics in the SC.

Methods:
In this study, we proposed a novel *ex vivo* approach to evaluate the impact of solar radiations on the SC. It is based on the hydration and the dehydration kinetics using Raman spectroscopy by calculating the vOH/vCH ratio to monitor the relative water content in the whole SC during the drying process. The composition of SSLs was analyzed using HT-GC/MS.

Results and Conclusions:
Our results showed an alteration in the water caption and homeostasis within the SC after solar radiations. In addition, we showed that SSLs confer a protection of the water uptake mechanism and water loss process in the SC and suggested that the protective role of SSLs against solar radiations works by absorbing a part of the energy of the solar radiations, either directly on the surface of the SC or on a slide (not in contact with the SC). This was confirmed by the decrease of Squalene, polyunsaturated fatty acids, and Cholesterol after irradiating the SSLs and analyzing it in HT-GC/MS. Meanwhile, this protective function may be limited and is dependent to the distribution of SSLs over the body surface. Thus, in the second part of this study, we showed the complementary protection that can be offered by the solar filters with the natural protection provided by skin surface lipids. This significant protection highlights the importance of using sunscreens to preserve the skin barrier integrity and the SSLs equilibrium.

Profiling of Human Burned Bones by Vibrational Spectroscopy: Forensic and Archaeological Applications

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Context and Objective: Human burned bones are often the only remains found in forensic scenarios (*e.g.* from terrorist attacks or fires) and archaeological settings, from which the bioanthropologists aim to identify victims or obtain information on past populations. This study applied complementary optical and neutron-based vibrational spectroscopy to the study of human bones burned under controlled conditions. The results obtained should contribute to a more accurate identification of skeletal remains subject to unknown heating events.

Methods: Modern human bones were burned in a wide range of temperatures (400 to 1000 ºC), under aerobic and anaerobic conditions, and were analysed by infrared (FTIR-ATR), Raman and inelastic neutron scattering (INS) spectroscopies. Additionally, archaeological skeletal samples from the *Hypogeum of the Garlands* (Grottaferrata, Lazio, Italy) were studied, based on the data previously obtained for the modern bones (taken as references).

Results and Conclusions: Heat-induced effects on human bone were found to vary depending on the burning conditions, regarding temperature and oxygen availability. Decomposition of the organic components (lipids and proteins) took place at *ca.* 550 ºC (aerobic) or *ca.* 800 ºC (anaerobic). INS allowed the concomitant observation of the hydroxyl libration ($OH_{libration}$), hydroxyl stretching (ν(OH)) and ($OH_{libration}$+ν(OH)) combination modes, leading to an unambiguous assignment of these INS features to bioapatite. The OH_{lib}, ν(OH) and $\nu_4(PO_4^{3-})$ bands were identified as spectral biomarkers, which displayed clear quantitative relationships with temperature, revealing heat-induced changes in bone´s H-bonding pattern during the burning process. These results are expected to enable a better understanding of the effect of heat on bone´s constituents in distinct environmental settings, thus contributing to a more accurate characterisation of forensic[1-6] and archaeological[7-9] human skeletal remains.

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Near Infrared Spectroscopy and Food Quality

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Food quality refers to its nutritional, but also sanitary, organoleptic or technological properties.

This quality is important all along the agri-food chain. For example, for cereals, quality is expressed in terms of yield and resistance to diseases and pests for the farmer, and in terms of organoleptic, nutritional, and sanitary for the consumer. Processors have requirements to optimize their transformation processes because each use implies a specific quality. They want standardized and normalized raw materials, whose nutritional, sanitary, or technological characteristics are well defined and identified.

Some quality criteria are regulated, while others are listed in contractual specifications. Commercial contracts are thus superimposed on regulations to allow raw materials to be oriented towards a specific use.

Food quality analyses are generally carried out by internal laboratories, or by independent accredited organizations. These classical analyses are, for the most part, performed by wet chemical methods. They prove to be very reliable but are destructive, often long to implement, and expensive in terms of chemical reagents and manpower. The development of alternative techniques, based on the physical properties of the components, is therefore the subject of numerous studies.

One of the techniques that is gaining ground because of its ease of use and its ability to handle a wide range of applications is near infrared spectroscopy.

This technology is already used routinely in many applications. This presentation proposes to explore the potential of this tool for the determination of food quality.

Drop coating deposition Raman spectroscopy of contaminants

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Context and Objective: Contaminants such as pesticides for controlling pests and weeds (herbicides bentazone and picloram, fungicide thiram) or food additives (melamine) can be both life and environment threatening. Pesticides use in agricultural areas can result in the unintended release into groundwater contamination. It is of high importance to detect these substances at biologically and environmentally relevant concentrations by fast, simple and highly sensitive methods.

Methods: Raman spectroscopy is a powerful analytical tool providing unique information about the vibrational motion of molecules. However, the conventional Raman spectroscopy approach to solutions is generally limited to highly concentrated samples. To study solutions or suspensions in low concentration (~µM) and small volumes (~2 µl), a special method, drop coating deposition Raman spectroscopy (DCDR), was introduced. DCDR technique is based on the deposition of a small droplet of liquid sample on the hydrophobic substrate where subsequent evaporation of solvent leads to the preconcentration of analyte into a dried pattern from which Raman spectra can be acquired. Compared to conventional Raman spectra from the solution, the sensitivity of DCDR can be significantly improved, often by several magnitudes.

Results and Conclusions: Raman spectra of selected contaminant solutions were acquired by DCDR technique employing the commercial hydrophobic substrate with an attempt to detect low concentrations [1]. The Raman fingerprint spectral bands of studied contaminants were distinguished at relevant concentrations and assigned to specific molecular vibrations. For the food additive, the real sample was prepared, where the commercial powder infant formula was contaminated by melamine and successfully detected by DCDR.

Reference(s): [1] Kuižová et al., Spectrochim. Acta A, 262 (2021) 120109

Acknowledgement: Support by grant 290120 from the Grant Agency of Charles University.

FTIR spectroscopy as a future bioprocess monitoring tool – Characterisation of hydrolysed proteins and peptides

Nils Kristian Afseth[1], Ulrike Böcker[1], Kenneth Kristoffersen[1], Bijay Kafle[1], Ingrid Måge[1], and Sileshi Gizachew Wubshet[1]

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Context and Objective: Enzymatic protein hydrolysis (EPH) is hailed as a central element in the circular bioeconomy mobilization of the food industry. During EPH, proteins from food processing by-products are digested and solubilized by the catalytic action of proteases. The resulting protein ingredients have a range of potential applications, from feed to food ingredients, nutraceuticals, and even pharmaceuticals. FTIR spectroscopy is a powerful tool that has been used for protein characterization for decades. In recent years, we have been developing FTIR as a potential tool for characterization of hydrolysed proteins and peptides. Our long-term goal is the development of an industrial bioprocess monitoring tool for characterization of hydrolysed proteins and peptides based on dry-film FTIR spectroscopy.

Methods: Hydrolysed proteins from a range of different sources using a range of different enzymes were produced in the laboratory and in industrial processes. All hydrolysed proteins were analysed using dry-film FTIR spectroscopy, as well as Size-Exclusion Chromatography, degree of hydrolysis and collagen contents measurements.

Results and Conclusions: When proteins are broken down by the action of proteases, the protein structure changes along with the increasing number of C- and N-terminal ends. All of these changes are readily available in an FTIR spectrum. We have seen that these changes can be quantified and linked to changes in the overall sizes of the hydrolysed proteins and peptides. Additionally, the FTIR spectra reveal information on raw material characteristics and the action of the enzymes, meaning that the FTIR fingerprints themselves potentially can be used for process optimization and control. Since collagens have unique structural characteristics that is also maintained after partly degradation, we have also been able to make good quantitative models for the collagen contents in hydrolysed proteins. We are currently developing and testing a dry-film FTIR prototype system that in the near future will be taken into industrial environments for product characterization purposes.

Chemometrics Tools for the non-Targeted Research of Microorganisms in Food Samples by Raman spectroscopy

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Context and Objective: Food safety is the foundation of trust for food stakeholders. Contamination, particularly biological contamination, at food processing plants threatens this foundation, impacting consumer health and causing economic losses. A rapid, effective, and noninvasive method for detecting bacteria is, therefore, a necessity for the food industry. Here, we propose using Raman micro-spectroscopy with advanced statistical tools to detect and differentiate several types of bacteria, thus avoiding the complexity of traditional culture-based detection methods.

Methods: Fifty-two bacterial strains of 39 different genera were analyzed using Raman spectroscopy. As a result, about 2,563 Raman spectra were generated and integrated into the database. This huge amount of spectral data was analyzed using several chemometric tools, including principal component analysis (PCA), factorial discriminant analysis (FDA) k-nearest neighbors' algorithm (KNN), and convolutional neural network (CNN).

Conclusion: Multivariate data analysis proved the rapidity of the developed method and its ability to distinguish several strains. FDA models showed mediocre performances, whereas KNN models allowed good bacterial classification for most of the analyzed strains (average correct classification 90–95%). CNN achieved a higher classification accuracy, of 97%, compared with other models. Combining Raman spectroscopy with chemometric tools makes it a robust bacterial assessment method that is simple, rapid, and efficient.

Online monitoring of fermentation bioreactor based on Raman spectroscopy

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In recent years, real-time Raman monitoring models for bioreactors primarily depended on collecting the Raman database of fermentation samples and quantitative results by High-performance liquid chromatography (HPLC). Therefore, the obtained chemometrics models had limited application and were very susceptible to fluctuations in experimental conditions. This study aims to develop a more robust online monitoring model based on the Raman spectra of the pure standard substances involved in the fermentation process and introduce the bio-matrix variation, with the prospect of extending the model to industrial applications.

Firstly, a Raman spectra database of standard solutions was obtained using a Kaiser Raman RXN2 993 nm. Secondly, a quantitative regression model for glucose and ethanol has been developed by principal compounds analysis (PCA) and partial least squares (PLS). The limits of detection (LOD) and the Root mean square error of prediction (RMSEP) results were determined. Then, the impact of concentration has been investigated, along with the effect of one compound on the other one has been determined using different concentration differences.

The results indicated that: 1) the Raman acquisition parameters have been tuned to get a good signal-to-noise ratio (SNR) and satisfy the dynamic variation of the alcohol fermentation process; 2) PCA analysis results showed that the first two PCs could well represent the ethanol and glucose, and a convenient linear correlation has been obtained; 3) for the investigation of modeling by PLS, decreasing the concentration range of the training set will lose part of the accuracy but improve the LOD of the model at the same time; 4) When mixing the two compounds together gradually in parallel concentration, Ethanol has a significant impact on the Glucose at high concentrations, while Glucose doesn't. In cross-concentration, the impact of the two substances with higher differences on the prediction results was not significant.

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2. Lourenco N, et al. Bioreactor monitoring with spectroscopy and chemometrics: A review. Analytical and bioanalytical chemistry. 2012;404:1211-1237.

Avian vs human erythrocytes – preliminary spectroscopic studies

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Context and Objective: the energy demand of vertebrates varies depending on the level of activity - during exercise it can even reach a value many times higher than the value measured at rest. Such different levels of energy demand are strictly dependent on aerobic metabolism. The oxygen necessary for these processes cannot be stored but must be supplied continuously from the bloodstream, where the key role is played by erythrocytes. It is not clear how oxygen transport itself is regulated in avian erythrocytes, where the O_2 carrier is iron ion embedded in the haemoglobin molecule. As a first step, a method for studying haemoglobin oxidation in zebra finches erythrocytes has been developed and will be used to understand the role of the system responsible for oxygen transport.

Methods: we used 3 types of materials: methaemoglobin lyophylizates (Sigma-Aldrich), human RBCs (erythrocytes' concentrates, blood bank), zebra finches' *(Taeniopyga guttata)* erythrocytes (fresh whole avian blood). Following analytical physical and chemical methods have been applied: biochemical blood analysis, UV/Vis and Mössbauer (MS) spectroscopies (for MS low temperature (5K) and external magnetic field (8T)), ESEM microscopy, Optical Photothermal Infrared (O-PTIR) spectroscopy.

Results and Conclusions: at first, we managed to obtain MS results on the blood samples with its natural iron abundance (its native form). Typically, such measurements are performed on samples enriched in ^{57}Fe isotope. The use of external magnetic field (8T) enabled to determine both Fe valence and spin state within the porphyrin ring. The answer to that question has remained doubtful since many years. O- PTIR has been used to visualize the overall chemical information within single RBC with sub-micron resolution. Such detailed analysis highlighted first of all the presence of the major biocomponents. From the evolutionary perspective, these studies will allow a better understanding of the delivery of 0_2 during active and rest phases among avian. These studies have been performed within the 2019/03/X/NZ1/01631 project.

Reference(s): A. Bury et al., J. Exp. Biol. (2019) 222, jeb 193227; J. Niedojadło et al., J. Avian Biol. (2018) 49 (3), jav-01596; A. A. Kamnev et al., Mössbauer Spectroscopy in Biological and Biomedical Research, in Mössbauer Spectroscopy, eds. V. K. Sharma et al. (2013), John Wiley & Sons, Inc.

Monday 29th August 2022
13h45-15h00
Molecular spectroscopy 1

Time-Resolved Infrared Study of Adenine photo-oxidation by an Intercalated Chromium Polypyridyl Complex

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DNA interactions with metal polypyridyl complexes are of great interest due to their ability to (a) trigger photoinduced processes in DNA for photodynamic therapies, and (b) to signal the presence of DNA for imaging and diagnostic applications. Previously we have shown that time-resolved infrared (TRIR) is a powerful technique to study these systems.[1-3] The objective of this work is to understand the dynamics involved in direct photo-oxidation by earth abundant chromium complexes as potent therapeutic alternatives to Type II phototherapies. We have used TRIR to understand the excited state dynamics associated with photo-oxidation by intercalating chromium dppz (dipyridophenazine) polypyridyl complexes bound to DNA.[4] Our experiments identified a characteristic transient band associated with sensitized oxidation of adenine. Critically, our results show that while the long-lived metal centered excited state can diffuse in solution to photo-oxidize adenine and guanine, DNA photo-oxidation by the intercalated complex, which locates the dppz in contact with the target purines, is dominated by a dppz ligand centered excited state. These results have implications for future phototherapeutics and photocatalysis.

Reference(s):

1. Chem. Sci., 2020,11, 8600.
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Rearrangement of intracellular crystalline guanine as an adaptation for various illumination levels

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Context and Objective: It is well known that biogenic guanine crystals are widely used by various animals to manipulate light. Because of extremely high refraction index and plate-like arrangement, multiple arrays of guanine crystals can act as diffuse scatterers, broad- and narrowband reflectors, tunable photonic crystals, and image-forming mirrors. Crystalline inclusions composed of guanine or related purines were recently identified also in different phylogenetically unrelated photosynthetic microalgae. In the case of dinoflagellate *Amphidinium carterae* they were shown to serve as a long-term, high capacity store of nitrogen [1]. However, nitrogen storage does not exclude other roles, and light manipulation was already speculated for photosynthetic eukaryotes.

Methods: A confocal Raman microscopy was used for visualization of crystalline guanine within intact cells of *A. carterae* cultivated under low light (5 μmol(photons)·m^{-2}·s^{-1}) and high light (200 μmol(photons)·m^{-2}·s^{-1}) conditions.

Results and Conclusions: We show that location of guanine crystals in *A. carterae* depends on the intensity of illumination. When the cells are cultivated under continuous supply of inorganic nitrogen but exposed to low light intensity, their adaptation consists of increasing amount of plastids and synthesis of guanine crystals situated behind the plastids, closer to the center of the cell. Acting as photonic mirrors or diffusers for redirecting untrapped photons back to plastids, guanine crystals might increase efficiency of photosynthesis. In the case of high light intensity, extensive layers of guanine crystals are organized between the cell wall and plastids, shielding plastids from the excessive illumination.

This work was supported by the Grant Agency of the Charles University (No. 361121).

Reference:

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Conformational relaxation and molecular oxygen rebinding in alpha and beta subunits within valency hybrids of human hemoglobin

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Context and Objective: Human hemoglobin (Hb) is a tetramer consisting of two α and two β subunits. Each subunit contains one identical ferrous heme group which can reversibly bind one ligand molecule (e.g. O_2). At present, there is no definitive agreement on how ligand-induced conformational changes influence individual ligand binding properties of the α and β subunits in the different conformational forms of tetrameric Hb. In the present study we attempted to answer that question.

Methods: The oxy-cyanomet valency hybrids, $\alpha_2(Fe^{2+}\text{-}O_2)\beta_2(Fe^{3+}\text{-}CN)$ and $\alpha_2(Fe^{3+}\text{-}CN)\beta_2(Fe^{2+}\text{-}O_2)$, were used as models for the oxygenated Hb. Only the ferrous subunits within these hybrids reversibly bind O_2, while the ferric subunits do not. As the chemical bond between the ferrous heme iron and O_2 is photosensitive, time-resolved laser spectroscopy in the visible (Soret) region was used. Time-resolved measurements were performed on ULTRA apparatus.

Results and Conclusions: A kinetic model for the geminate O_2 rebinding in the ferrous hemoglobin subunits, ligand migration between the primary and secondary docking site(s), and nonexponential tertiary relaxation within the oxygenated quaternary structure, was introduced and discussed[a]. Significant functional non-equivalence of the α and β subunits in both the geminate O_2 rebinding and concomitant structural relaxation was revealed. The conformational relaxation following the O_2 photodissociation in the α and β subunits was found to decrease the rate constant for the geminate O_2 rebinding, this effect being more than one order of magnitude greater for the β subunits than for the α subunits. We observed the modulation of the O_2 rebinding within Hb by the intrinsic heme reactivity through a change in proximal constraints upon the relaxation of the tertiary structure on a picosecond to microsecond time scale.

Reference(s): [a]Lepeshkevich et al., Chem. Sci., 2021, 12, p. 7033.

The structural effect between the output module and chromophore binding domain is a two-way street via the hairpin extension

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Context and Objective: Signal transduction processes are frequently described as unidirectional reactions starting with ligand binding or co-factor activation and eventually affecting different biological activities. In red light sensing phytochromes the signal propagation "starts" with the isomerization of the bilin chromophore and "ends" in the regulation of diverse output modules. Along that route, several functional elements have been shown to influence the light sensing properties. One central element is the hairpin extension of the PHY domain, also referred to as the tongue. The effect of the output module on functional properties of the tongue and vice versa can differ substantially in these complex multidomain proteins.

Methods: In our study, we use bacteriophytochrome from *Deinococcus radiodurans* and studied the relation between the dynamics of the tongue and the output module by means of so-called pH-jump experiments, which reveal the solvent accessibility of the chromophore [1,2], FTIR spectroscopy [2], and ultra-fast transient IR-spectroscopy.

Results and Conclusions: We show that the composition of the output module has a strong impact on the stability of the hairpin extension. This reflects even to the excited state kinetics of the chromophore. Our study highlights the complexity of how conformational properties of the hairpin extension act as a bidirectional link between the chromophore binding site and functional properties of diverse output modules.

References: Rumfeldt, J.A., Kurttila, M., Takala, H., Ihalainen, J.A., 2021. The hairpin extension controls solvent access to the chromophore binding pocket n a bacterial phytochrome: a UVvis absorption spectroscopy study. Phoochem. Photobiol. Sci 20, 1173–1181.

Kurttila, M., Etzl, S. Rumfeldt, J.A., Takala, H., Galler, N., Winkler, A., and Ihalainen, J.A., 2022. Submitted.

Monday 29th August 2022
15h30-17h15
Biomedical applications 1

Biomedical Applications of Vibrational Spectroscopy: the continued drive towards Clinical Translation.

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The presentation will review the current status of the development of infrared and Raman spectroscopies for biomedical applications, towards the aspirations of clinical translation for improved patient diagnosis and prognosis. It is informed in particular by the proceedings of the 12th International Conference on Clinical Spectroscopy, held in Dublin, Ireland, in June 2022, and thus will consider developments under the thematic headings of Clinical Translation, In vivo, Ex vivo and Theranostic/therapeutic monitoring applications, Data Science and Emerging Technologies. Promising trends of increasing numbers of clinical based studies, start up companies for exploitation of emerging technologies and adaptation of established techniques to the clinical workflow are noted and discussed, as well as the increasing adoption of ever more advanced methods of Artificial Intelligence and Deep Learning for data am imaging processing and refinement. The presentation will attempt to provide perspectives on the continued drive towards Clinical Translation over the coming years.

Candida albicans outbreak in a Maternity Unit: Epidemiologic study by FTIR spectroscopy, MALDI-TOF spectrometry and microsatellite analysis of strains from neonates

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Context and Objective:

This study demonstrates the application of two phenotypic methods: Fourier Transform Infrared (FTIR) spectroscopy and Matrix-Assisted Laser desorption/ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) as epidemiological investigation tools in a cluster of oral candidiasis of neonates in a maternity unit.

Methods:

Candida albicans strains isolated from oropharyngeal swabs of 8 neonates, 2 *C. albicans* adult strains (used as reference) and one closely related species strain, *C. dubliniensis,* were analyzed by FTIR spectroscopy, MALDI-TOF MS and Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) using microsatellite markers analysis. Typing of strains was done by classifying FTIR spectra using hierarchical cluster analysis method.

Results and Conclusions:

Our results show three distinct clusters corresponding respectively to the 8 strains from the neonates, the 2 adult reference strains and to the *C. dubliniensis* strain. These results were confirmed by MLVA reference method. However, MALDI-TOF MS failed to reliably differentiate neonates strains from reference strains. This study is the first to demonstrate that a nosocomial transmission of *C. albicans* can be evidenced by FTIR spectroscopy. This new approach constitutes a rapid and simple way to type *C. albicans* strains. This technique can be proposed as a first step during epidemiological investigations and such a procedure could contribute to accelerate the implementation of control measures in a clinical setting.

Rapid determination of Proteus mirabilis susceptibility to antibiotics using infrared spectroscopy in tandem with random forest

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Context and Objective: Bacterial infections cause an array of serious illnesses; these infections are generally treated with antibiotics. The currently used methods in medical centers for detecting bacterial antibiotic susceptibility consume a 48-72-hour period, leading to uncontrolled and overprescribed use of antibiotics. As a result, many bacterial species have acquired resistance to almost all commonly available antibiotics, resulting in difficult-to-treat infections.

Thus, there is an urgent need to develop efficient methods for accurate and rapid determination of bacterial susceptibility to specific antibiotics. The combination of machine learning and Fourier Transform Infrared (FTIR) spectroscopy has generated a promising diagnostic approach in medicine and biology.

The study's main goal is to examine the potential of FTIR spectroscopy to determine the susceptibility of Proteus mirabilis (*P. mirabilis*), a pathogen involved in various human infections, to a specific range of antibiotics, within about 20 minutes after the first culture.

Methods: We measured 489 different *P. mirabilis* isolates using FTIR spectroscopy and then analyzed them by Random Forest (RF).

Results and Conclusions: A classification success rate of ~84% was achieved in differentiating between the resistant and sensitive isolates based on their susceptibility to different antibiotics.

On the way to clinical diagnostics: can Raman spectroscopy detect leukemic cells?

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Context and Objective: Leukemia is a broad term that describes hematologic malignancies, which is an extremely heterogeneous group [1, 2]. The goal of our studies was to optimize measurement conditions using Raman imaging for the reliable classification of molecular subtypes of hematologic cancers and the identification of spectral markers of leukemia.

Methods: Both normal leukocytes and leukemic cells were imaged using the WITec Alpha 300 confocal Raman system. The collected spectra were analyzed with the use of cluster analysis, principal component analysis, and partial least squares discriminant analysis.

Results and Conclusions: Our results show that the preparation of the sample influences the Raman profiles of leukemic cells. However, Raman imaging combined especially with supervised chemometric analysis is an efficient tool that enables differentiation between the studied subtypes of lymphocytes [2], as well as the identification of leukemia cells that carry different genetic mutations in an automated way [1]. The main differences between malignant and normal cells lies in the intensity of the characteristic bands for nucleic acids and lipids [1].

Acknowledgements The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

References:

[1] Leszczenko P, Cancers (2021); 13(21), 5483,

[2] Borek-Dorosz A, Journal of Advanced Research (2022); *in press*

Raman and FTIR spectroscopic Stratification of saliva of healthy, habitués and oral cancer subjects.

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Context and Objective: Minimally invasive cancer detection using bio-fluids is been actively followed due topractical limitations of in vivo methods. Saliva is one such clinically informative bio-fluid that offers advantage of easy and multiple sample collection. Despite its potential in cancer diagnostics, saliva analysis is challenging due to its heterogeneous composition. Recently, there has been an increase in saliva exploration by Raman spectroscoy [1-5]. In current study unstimulated morning saliva sample of Control (C-16), Habitues (HT-21) and Tumour (T-26) were examined by both Raman and IR Spectroscopy.

Methods: Raman spectra of air dried 20 µl of sample, on a calcium fluoride (CaF2) window, were recorded by Raman microscope WITec alpha300RS (WITec GmbH, Ulm, Germany, 532 nm, 50X, 20 mW, 5s and 10 accumulations). Infrared spectra were collected using Spectrum 2 (Perkin-Elmer, USA, 12 scan, resolution 4 cm-1) on air dried thin film, on CaF2. Pre-processed Raman and IR, spectra were analysed by principal component analysis (PCA) and PCA-based Linear Discriminant Analysis (PC-LDA).

Results and Conclusions: Multivariate analysis of Raman spectral data revealed 91 % correct classification of C group, 52 % classification of HT group and 77% classification of T group. On the other hand, analysis of IR showed 31% correct classification in C group, 80% in HT group and 48% in T group. Findings of this preliminary study suggest, Raman spectroscopy provides better stratification of control and tumour groups than IR.

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Infrared spectral biomarkers of neurodegenerative diseases

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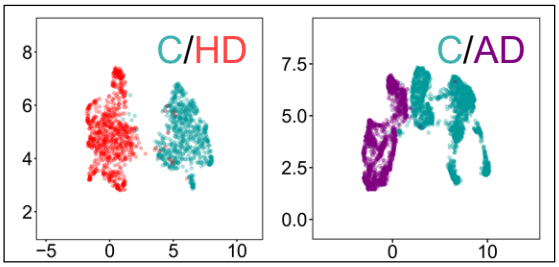
Context and Objective: Although some neurodegenerative diseases can be identified by behavioral characteristics relatively late in disease progression, we currently lack methods to predict who has developed disease before the onset of symptoms, when onset will occur, or the outcome of therapeutics. New biomarkers are needed.

Methods: We have developed a general-use tool based on the Fourier Transform Infrared (FTIR) spectromicroscopy technique to predict disease status from cell spectral images. Our strategy was (1) to develop a robust algorithm model using a stable mouse system of Huntington's disease (HD) with little biological variation, and (2) to test the algorithm with more variable human HD or Alzheimer's (AD) disease fibroblast samples, which were used as brain cell surrogates.



Spectral phenotyping can identify neurodegenerative disease classes from patient and healthy control fibroblasts.

Results and Conclusions: Spectral phenotyping was not only successful in disease classification in the absence of overt pathology in the mouse model, but also predicted neurodegenerative disease class in HD and AD patients using fibroblasts as surrogates for brain cells while individual signature for each patient was also retained. Fibroblasts have different functions and do not report on the biology of brain cells. However, skin cells share the same genotype with brain cells and undergo chemical changes that track with a disease as biomarkers. We will expand our preliminary analysis of disease prediction using human HD and AD fibroblasts as surrogate cells to predict disease status in larger patient cohorts.

Tuesday 30th August 2022
8h45-10h15
Isotopic labelling

Spectroscopic probing of bacterial metabolism at single-cell level

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Microbes are considered the earliest life forms, and continue to play an integral part in sustaining life on the planet. Whilst some of these microorganisms are harmful, a large number of them are beneficial to humans, plants, animals, and the ecosystem as a whole. Despite recent advances in molecular biology, providing fast detection and identification of bacteria, most microorganisms (>99%) remain uncultured under laboratory conditions, which holds back our understanding of the functional roles of many bacterial communities. During the past two decades application of molecular microspectroscopy techniques, in particular Raman microspectroscopy, combined with stable isotope probing (SIP) strategies has provided the opportunity to bypass the culturing steps, allowing to probe bacteria at single cell level. This talk will provide an overview on some of our recent studies that have successfully combined SIP with various spectroscopy techniques to demonstrate the wide range of applications of this strategy for better understanding of bacteria in terms of metabolic function, and cellular response to various stress conditions.

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Do *Galleria mellonella* larvae metabolize polyethylene? An FTIR microspectroscopy study using isotopoic labelling.

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Context and Objective: Environmental pollution by non-biodegradable polyethylene (PE) plastics is of major concern, thus organisms capable of bio-degrading PE are required. The larvae of the Greater Wax Moth, *Galleria mellonella* (Gm), were identified as a potential candidate to digest PE[1]. In this study[2], we tested whether PE was metabolized by Gm larvae and could be bioassimilated in their tissues using various FTIR microspectroscopy (µFTIR) techniques[3] and isotopic labelling.

Methods: Gm larvae were produced on site in the insectarium at INRAE Micalis Institute and fed with various diets of beeswax, pollen and/or PE. We tested the implication of the larval gut microbiota using conventional and axenic reared larvae. We used deuterium labelling to detect the bio-assimilation of food: larvae were fed either with deuterated oil ($C_{16}D_{34}$) or deuterated PE (PED_4). ATR-FTIR, µFTIR imaging and synchrotron µFTIR were used to seek for the presence of C-D bonds in larva cryosections and the biodegradation of PE films.

Results and Conclusions: our study showed that neither beeswax nor PE alone favour the growth of young larvae. We found deuterated lipids in tissues of larvae fed 24 h or 72 h with 1 to 2 mg of $C_{16}D_{34}$ oil. No bio-assimilation was detected in the tissues of larvae fed with 1 to 5 mg of PED_4 for 72 h or 19 days, but micron sized PE particles were found in the larval digestive tract. We evidenced weak bio-degradation of PE films in contact for 24 hours with the dissected gut of conventional larvae; and in the PED_4 particles from excreted larval frass. Our study confirms that Gm larvae can bio-degrade PE but cannot necessarily metabolize it.

Reference(s): [1] Bombelli, et al, Curr. Biol. 27 (2017) R292.

[2] Réjasse, Jet al.Environ Sci. & Technol. 56 (2022) 525.

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Strong isotope-effect on the conformation and self-assembly of collagen

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Context and Objective: Collagen comprises 30% of the protein in our body, ensuring the mechanical integrity of all connective tissue. Cells produce collagen as monomers that self-assemble hierarchically into rope-like fibrils. Despite its biological importance, our understanding of collagen self-assembly is still very limited. To investigate this pro-cess, we apply a wide range of spectroscopic and imaging methods.

Recently, we discovered that there is a very strong H/D-isotope effect on colla-gen self-assembly: not only the kinetics, but even the protein conformation and the structure of the fibril network are very different in H2O, D2O, and their mixtures. We believe such isotope effects, probably related to the stronger hydrogen-bonding in D2O, are a generic phenomenon that should be taken into account by everyone studying proteins in D2O instead of H2O.

Methods: We dissolve type-I collagen powder in HDO/D2O/H2O with different H/D ratios at concentrations ranging from 0.1 to 5 mg/ml, and use conventional and two-dimensional IR spectroscopy to study the protein conformation in the monomers and fibrils, turbidity measure-ments to track the self-assembly kinetics, and confocal microscopy and rheology to study the morphology and elastic properties of the final fibril network.

Results and Conclusions: We find that in D2O collagen monomers preferentially adopt an α-helix conformation instead of the PPII conformation adopted in H2O, and that the conforma-tional change during fibrillation is much less in D2O than in H2O, where the PPII content in-creases at the expense of α-helix. These structural differences are correlated to a three-fold faster self-assembly in D2O as compared to H2O, and to a final fibril network that is softer and has a larger mesh-size with respect to the one formed in H2O and HDO. Since H/D-isotope exchange likely enhances hydrophobic effects, these results indicate that hydrophobicity plays a major role in the stability of collagen at all hierarchical levels, and is probably the main driving force of the self-assembly. Our results demonstrate that researchers should be extremely care-ful in using D2O as a solvent when studying protein structure and dynamics, since both can be strongly isotope-dependent.

Labelling endothelial cells with Raman probes improves the specificity and sensitivity of their imaging

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Context and Objective: Multiple diseases are at some point associated with altered endothelial function. Biochemical changes of endothelium can be linked to various cellular organelles, including mitochondria, endoplasmic reticulum, nucleus, so organelle-specific insight is needed for better understanding of endothelial pathobiology.

Methods: Raman imaging is considered as a method of choice for subcellular analysis, however its specificity and sensitivity is limited, as well as a speed of the measurement. The latter can be improved by applying Stimulated Raman Spectroscopy in reference to fluorescence microscopy. But by labelling cells with the specific Raman tags a better insight into cells can be achieved.

Results and Conclusions: The innovations in SRS microscopy is presented, on the background of which the layout and performance of our homemade setup is discussed. It is built from commercially available elements enabling for imaging of the molecular structure of single cells over the spectral range of 800 – 3600 cm^{-1}. Labelled Raman and SRS imaging, combining chemical specificity with microscopic resolution, proved to be useful in detecting e.g. mitochondrial activity and newly synthesized DNA. Edu-labelling occured a way of following endothelial cell proliferation, whereas MitoBADY probe was used to detect changes in mitochondrial membrane potential.

Acknowledgement

This study was supported through LogicLab ITN, funded by the European Union's Horizon 2020 Research and Innovation Programme under Marie Sklodowska-Curie Grant Agreement 813920 and by a grant from the National Science Center Poland (NCN) (OPUS15 no. UMO-2018/29/B/ST4/00335 to MB).

Reference(s):

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Rapid antibiotic susceptibility testing using FTIR spectroscopy and deuterium isotope probing

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Context and Objective: Antibiotic resistance is a rising public health concern around the world. To tackle it efficiently, fast and reliable diagnostic methods are urgently needed. The current culture-based methods for Antibiotic Susceptibility Testing (AST) are subject to long incubation times. By probing the metabolic status of bacteria simultaneously to the exposure to antibiotic stress, AST time-to result might be significantly shortened.

Methods: Deuterium Isotope Probing (DIP) was assessed through Fourier Transform InfraRed (FTIR) transmission spectroscopy, so as to investigate the impact of antibiotic stress on metabolism. Bacteria were exposed to a given antibiotic concentration in a deuterated Mueller-Hinton broth, with a view to estimating the metabolic status as, in the presence of heavy water, carbon-deuterium (C-D) bounds are created through enzymatic reactions. A metabolically active bacterium processes deuterons from its environment, creating large amounts of labelled lipids and proteins, *i.e.* containing C-D bounds. On the opposite, a weakened bacterium will produce C-D bounds in smaller quantities, resulting in a lowered absorbance of the corresponding IR absorption peak. By observing the bacterial metabolic changes at different antibiotic concentrations, the antibiotic susceptibility may be determined. For each strain, the absorbance of dried bacterial suspensions at 2140cm^{-1} (C-D) is compared with the absorbance at 2129cm^{-1} (C-H , used as a reference) after two hours of incubation in a nutrient medium with 50% v/v heavy water.

Results and Conclusions: A significant drop in C-D absorption occurs when a bacterial strain is exposed to an antibiotic concentration above its minimal inhibitory concentration (MIC). This suggests a promising new way of testing for antibiotic sensibility in less than two hours, either with a FTIR spectrometer or through infrared multispectral imaging.

Tuesday 30th August 2022
11h15-12h45
Enhancement techniques

Label-free SERS spectra of biological samples: characteristics and potential significance for clinical applications.

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Biological samples are rich in clinically relevant information, but their biochemical complexity is also posing several challenges for analytical spectroscopy. In label-free Surface Enhanced Raman Scattering (SERS) spectroscopy, bands are due to the vibrations of the analytes of interest directly adsorbed on the nanostructured gold or silver substrates. When applied to chemically complex mixtures such as biological samples (e.g. biofluids, tissues, cells, etc.), label-free SERS methods yields spectra whose bands, in principle, might be due to a broad variety of biomolecules.

After presenting some methodological issues, this contribution will discuss the common characteristics of label-free SERS spectra of different biological samples obtained on various substrates, arguing that, in spite of biochemical complexity, in most cases just a handful of metabolites are responsible for the bands observed across different samples. Some reasons will be given to support this interpretation, along with some considerations on how this information could be put to use in developing effective clinical applications based on label-free SERS. Limitations and opportunities of this point of view on label-free SERS will be then outlined at the end of the talk.

Magnetron-Sputtered Polytetrafluoroethylene-Stabilized Silver Nano-island Surface for Surface-Enhanced Fluorescence

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Context and Objective: We studied the surface-enhanced fluorescence (SEF) intensity and lifetime dependence of riboflavin (vitamin B2) as a model biomolecule adsorbed on a spacer-modified Ag substrate with respect to the thickness of the spacer. The substrates were prepared as Ag nano-islands deposited onto magnetron-sputtered polytetrafluoroethylene (ms-PTFE). The spacer was formed by the ms-PTFE layer with the thickness ranging from ~5 to 25 nm.

Methods: The riboflavin dissolved in dimethylsulfoxide (DMSO) at a low (10 μM) concentration forms, at the ms-PTFE surface, a homogeneous layer of adsorbed molecules corresponding to a monomolecular layer. The microspectroscopic measurements of the adsorbed layer were performed using a confocal microspectrofluorimeter adapted for phase-modulation time-resolved fluorescence measurements to obtain the SEF spectra and to determine the fluorescence lifetimes. An optical configuration of a laser sheet passing the sample layer from the side was also used for fluorescence lifetime imaging.

Results and Conclusions: The riboflavin dissolved in DMSO or water at a low concentration forms a homogeneous layer of adsorbed molecules that corresponds to a monomolecular layer. Time-resolved fluorescence measurements carried out through a sessile DMSO droplet for the Ag nano-island surfaces covered by ms-PTFE films of various thicknesses have shown the SEF for thicknesses of 5 nm and higher, while for the bare Ag nano-islands, the generally expected non radiative quenching was confirmed. Time-resolved fluorescence determines the enhanced fluorescence quantum yield due to the shortening of the radiative decay in the vicinity of the plasmonic surface. We found the 5 nm ms-PTFE layer possessing the largest (estimated 4×) fluorescence enhancement, the quantum yield was increased 2.3×. Fluorescence lifetime imaging in an optical laser sheet configuration allows observing the average lifetime from a wider area at a specific height above the spacer surface. These measurements from side revealed for aqueous riboflavin solution the stability and very good reproducibility of lifetimes but the shortening was less significant.

Reference: Šubr, M. et al., *Nanomaterials* 2020, 10, 773.

Hot Electron Nanoscopy and spectroscopy (HENs): from probe design to real applications

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Context and Objective: Hot Electron Nanonscopy and spectroscopy (HENs) is a recently developed Atomic Force Microscopy based technique exploiting the emission of monochromatic photons and unbiased energetic electrons in a nanometer area at the tip apex after plasmon decay. The technique has is capable to inject energized electrons (or holes) to states at energies of few eV from (or below) the Fermi level of the sample, i.e through Schottky junction in a semiconductor or to the LUMO and LUMO+n (HOMO, HOMO-m) states of a molecule. Contextually, Photons are emitted from the tip due to the same plasmon decay with high yeald. Photons and Hot electrons are used to perform nano-Raman and electronic spectroscopy on states far from the Fermi level at zero bias with nanometer resolution, allowing the investigation of structural and electronic properties.

Methods. We designed a custom setup and fabricated micro/nano-structured AFM probes to efficiently convert a well characterized laser excitation source, focused on an AFM probe, into Surface Plasmon Polaritons [1], to guide them to the apex of the AFM tip and promote their decay into energized electrons and photons [2,3], obtaining a very high efficiency. This experiment was also aided by theoretical calculations and numerical simulations to exploit a tradeoff between fabrication complexity and the final efficiency for different designs [4].

Results and Conclusions. In recent years we have used HENs on different AFM setups on several inorganic semiconductors, proofing an extremely high efficiency high spatial resolution [1-3]. More recently, we proved this technique capable to detect and image edge states in 2D MoS_2, not visible to other electrical AFM modes [4]. Lately we developed the first application to p-doped organic semiconductor, broadening the range of applicability to a new class of materials where conductance is dominated by holes.

However, we highlight that the technique has unique features suited to investigation at single molecule level :1) its high efficiency in photons and electrons generation allows correlating electronic and Raman Studies on molecules with extremely low cross section ;2) charges can be directly injected on HOMO and LUMO or even higher states, usually inaccessible to more traditional conductance test ;3) All responses are obtained with nanometer resolution.

Additionally our estimation show enough signal enhancement to add orbital momentum sensitivity to our setup, allowing the direct investigation of molecular local chirality, thus opening to direct investigation to investigations on proteins, DNA or drugs.

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Plasmonic nanostructures for SEIRAS on membrane proteins

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Context and Objective: Plasmonic nanostructures, including nanoparticles, nanoantennas (rods and bowtie) are excellent subtracted for the in-situ investigation of the reaction large membrane proteins at picomolar concentration.[1,2] Here we compare different types of substrates and investigate the influence on the spectral properties. [2-3]

Methods: The different gold nanostructures have been deposited by means of chemical deposition, or lithography on silicon substrates. The geometry of the nanoantennas was calculated with the help of FDTD methods.[4] The nanostructures have then been characterized by microscopies and the immobilization followed by IR spectroscopies and microscopies. The membrane proteins studied are sugar and lactose transporters described before. [1, 3]

Results and Conclusions: The membrane proteins studied, have been found to be stable and functional on the different substrates when immobilized via a His-Tag. The effect of the local electric field on the spectral properties was studied and rod type nanoantennas have been found to strongly influence the shape of the infrared signals. This effect was avoided in bow type antennas. Finally different reactions of the transport proteins have been successfully characterized.

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Study of the compactness and permeability of the polymer brushes by surface-enhanced Raman spectroscopy

Marek PROCHÁZKA[1], Monika SPASOVOVÁ[1,2], Josef ŠTĚPÁNEK[1], Ondřej KYLIÁN[3], Hana VAISOCHEROVÁ-LÍSALOVÁ[2]

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Context and Objective: The objective is to provide new fundamental discoveries on molecular mechanisms of bio-fouling to functionalizable polymer brushes on gold surfaces used as a platform for biosensing techniques.

Methods: Zwitterionic carboxybetaine (CB)-based polymer brushes (PBs) and recently developed random copolymers combining CB and non-ionic moieties of hydroxypropyl methacrylamide in different concentrations were prepared according to ref. 1. We used the original method enabling us to investigate the permeability of PBs with respect to small molecules. It is based on rafting the polymer brush to a custom-made surface-enhanced Raman scattering (SERS) substrate prepared by magnetron sputtering of Ag nanoislands on Au surface. By checking SERS spectra of the reporter molecule penetrating through the brush we are able to gain information about the PB permeability.

Results and Conclusions: After deposition of about 1μL droplet of a low concentrated solution of reporter molecule (methylene blue-MB), a spectroscopic scan via a confocal Raman microscope reveals clearly distinct points exhibiting SERS signal of MB penetrated to the vicinity of the SERS-active layer. Based on series of verification measurements, optimal measurement conditions (wavelength and power of excitation, acquisition time) were found and the lateral resolution was determined. MB reporter molecule provides an intensive signal, exhibits high stability, and allows distinguishing whether the MB molecule is in a direct contact with the SERS-active substrate or only situated in its proximity. The series of SERS spectral maps of MB measured from Ag nanoislands/brush substrate were treated by factor analysis. The results allow us to compare the permeability of different polymer brushes.

Reference: 1. Víšová, I *et al*. *Macromol Biosci* **2020**, *20*, e1900351.

Acknowledgment: Support by grant 21-19779S from the Czech Science Foundation.

Tuesday 30th August 2022
14h15-15h30
Biomedical applications 2

Probing tissue biomechanics with chemical specificity in Brillouin-Raman microscopy

Authors list with the name of presenting author underlined:

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Mechanical properties of cells and tissues are key to their biological function, and impairments can lead to disease. Degenerative conditions such as corneal ectasia, osteoarthritis, and cardiovascular diseases, which are the leading cause of death globally, all involve mechanical impairment of tissues.

In biomechanics and mechanobiology, the realization that mechanical properties are key to biological function has led to an impetus in new techniques capable of imaging non-invasively, at depth and with increasing spatial resolution.

Here we present Brillouin Light Scattering (BLS) spectroscopy as an emerging method in biomedical sciences, providing a contrast based on propagation of acoustic waves at high frequency (GHz) generated spontaneously by thermal effect. BLS gives access to the longitudinal modulus and viscosity on a microscale, opening the way to a myriad of applications including diagnosis of pathology.[1] The combination with Raman spectroscopy enhances the chemical specificity of the method, thus correlating mechanical and molecular properties of a sample in a label- and contact-free manner.[2]

References:

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(2) F. Scarponi et al. *Phys. Rev. X*, 7, 031015 (2017)

Drug induced phospholipidosis in endothelial cells studied by Raman imaging

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Context and Objective: In this work, the effect of cationic amphiphilic drugs (CADs), such as fluoxetine, amiodarone, clozapine, and risperidone, on human microvascular endothelial cells (HMEC-1) was studied. The aim was to find spectroscopic markers of drug induced phospholipidosis (DIPL), as well as to understand relationship between lysosomotropism, DIPL, activation of autophagy, and decreased cell viability caused by those drugs in endothelium.

Methods: A combined methodology of label-free Raman imaging and fluorescence staining, was used for tracking cellular lipid accumulations upon incubation with CADs. Spectroscopic comparison of DIPL with autophagy in endothelium, was supported with Ratiometric Raman imaging of lipid abundance (lipid/(lipid+protein) ratio)[1].

Results and Conclusions: We demonstrated that CADs in nontoxic concentration induced the formation of lipid accumulation related to DIPL. Raman-based signatures of phospholipidosis and autophagy in endothelium are distinct and suggesting different mechanisms involved in CADs-induced phospholipidosis and autophagy activation. Robust lipid signal in cytoplasm features by effect size calculated based on lipid/(lipid+protein) ratio > 1, together with an increased content cholesterol esters, choline-containing phospholipids, and fatty acids at a low unsaturation level in the perinuclear region represent a reliable spectroscopic profile of DIPL in endothelium[1].

Reference(s):

1 E. Bik, J. Orleanska, L. Mateuszuk, M. Baranska, K. Majzner and S. Chlopicki, *Biochim. Biophys. Acta - Mol. Cell Res.*, 2022, **1869**, 119186.

Acknowlegement:

This work was financed by National Science Centre (Preludium, UMO 2019/35/N/ST4/03896 to EB). EB acknowledges the fellowship with the project no. POWR.03.02.00-00-I013/16.

Characterization of cancer-associated adipocytes by Raman spectroscopy.

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Context and Objective: Adipocytes are the major cellular components comprising the breast cancer microenvironment. In the early stage of breast cancer, cancer cells locally infiltrate the nearby adipose tissue, which results in the activation of adjacent adipocytes into cancer-associated adipocytes (CAAs). Consequently, it gives advantages to breast cancer cells in terms of survival, growth, and metastasis. Compared to normal adipocytes, CAAs are mainly characterized by a decrease in size, lipid content, adipocyte differentiation markers, and an increase in adipokines and inflammatory factors. We propose to use Raman spectroscopy to identify new biomarkers representative of the status of CAAs.

Methods: To capture transition between adipocyte and CAAs, a 2D in vitro co-culture model, providing a contact area between adipocytes (differentiated 3T3-L1 cells) and breast cancer cells (MDA-MB-231, Claudin-low subtype) was developed. However, cell heterogeneity is a major drawback of this cell culture model. For example, each kinetic time is marked by the coexistence of immature (fibroblast-like) and mature (adipocyte-like) 3T3-L1 cells non-equally differentiated. On the other hand, the adipocyte/CAAs transition induced by breast tumor cells varies from one adipocyte to another. To address this issue, we adapted algorithmic tools, recently implemented for single cell transcriptomic analysis, to vibrational Raman spectral data. These tools, known as trajectory inference or pseudotemporal ordering, aim to reconstruct evolving pathways from different cell states, coexisting simultaneously in a cell population. Our research focused on the use of Partition-based graph abstraction (PAGA) algorithm combined to uniform manifold approximation and projection (UMAP) to decipher adipocyte cells heterogeneity and highlight CAAs population.

Results: We show how Raman spectroscopy can be used in association with TI approach to visualize and resolve the cell heterogeneity of this cell model. More specifically, the results support the evidence of diverse differentiated adipocyte subtypes and a specific CAAs subpopulation. This new approach will pave the way for a better comprehension of cell heterogeneity and may reveal new molecular states and subpopulation-specific responses to external perturbations.

Bimetallic Au-Fe(III) nanocomposites for multimodal imaging

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Context and Objective: This work is devoted to the development of a novel type of bimetallic Au-Fe(III) nanocomposites possessing multimodal imaging properties. While gold in the form of nanoclusters (AuNCs) is known for intrinsic fluorescent properties as demonstrated by us recently (1, 2), Fe(III) in superparamagnetic state can be exploited in magnetic resonance imaging (MRI). These bimetallic Au-Fe(III) nanocomposites were generated in bovine serum albumin (BSA) which served as reducing and simultaneously as capping agent for the as-formed Au-Fe(III) nanocomposites.

Methods: Optimal conditions (including reactants ratios, medium composition, type of heating, time of maturing, purification, storage) of the preparation of bimetallic Au-Fe(III) nanocomposites with brilliant fluorescence and significant contrasts in MRI are found out. Characterization is performed by steady-state fluorescence and absorption spectroscopies, dynamic light scattering, zeta potential measurements, circular dichroism, scanning transmission electron microscopy and energy dispersive spectroscopy. The presence of superparamagnetic Fe(III) in these bimetallic nanocomposites was unequivocally determined by Mőssbauer spectroscopy (including measurements at 5K, 5T). These samples were also assessed by MR relaxivity (1.5T, 37°C) and imaging at 4.7T.

Results and Conclusions: It is evidenced that the presence of superparamagnetic Fe(III) does not cancel fluorescent properties of AuNCs in BSA. Simultaneously, relaxivity values of the bimetallic Au-Fe(III) nanocomposites are approaching the values of commercial MRI contrast agents which, on the contrary, does not provide any fluorescence signal.

Acknowledgement: Financial support by GACR (project no. 19-03207S) and Internal Grant Agency of Palacký University (IGA_PrF_2021_003 and IGA_PrF_2022_003) is thanked.

Reference(s):

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Tuesday 30th August 2022
16h30-17h30
Molecular spectroscopy 2

Tip-enhanced Raman spectroscopy for nanoscale chemical and structural characterization of biomolecules

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Tip-enhanced Raman spectroscopy (TERS) has emerged as a powerful technique for chemical and structural characterization with nanoscale (and even sub-nanoscale) spatial resolution. TERS combines the chemical specificity of Raman spectroscopy and the high spatial lateral resolution of scanning probe microscopies (such as AFM or STM). Several TERS configurations have been proposed for the description of biomolecules and biomaterials, either in bottom-, top- or side-illumination geometries. They allowed TERS signatures of nucleic acids, proteins/peptides, lipid membranes, viruses and cells to be unraveled [1].

Here, we present scientific and technical achievements in TERS, and especially those realized at the University of Bordeaux. After a short introduction describing the TER scattering phenomenon, a first part will explain advantages and drawbacks of several TERS configurations, provide a brief overview of the manufacturing of AFM-TERS optical nanosources (the named TERS tips) and indicate the most frequent issues during TERS experiments [1,2]. A second part will discuss important works on nucleic acids and proteins with a special emphasis on the composition and structure analysis of amyloid fibrils to better understand their formation and activity in the frame of neurodegenerative diseases [3-5]. Finally, future challenges for TERS applications in biology will be presented.

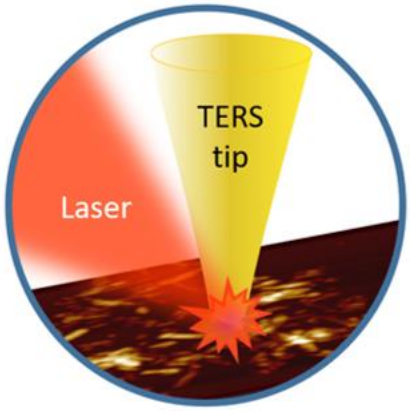

Figure 1. Side-illumination TERS configuration [1]

References:

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Background-free DNA-protein interactions: structural insights by Raman spectroscopy

Monica Marini[1], Bruno Torre[1], Marco Allione[1], Andrea Giugni[2], Tania Limongi[1], Masateru Takahashi[3], Fahad Rashid[3], Samir M. Hamdan[3], Candido F. Pirri[1], Enzo di Fabrizio[1]

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Context and Objective: The interaction of proteins with DNA have a pivotal role in genome integrity and nucleic acids repair regulation. A key protein in DNA repair pathways is Rad51. Here we combined biological preparation, superhydrophobic surfaces (SHS), and Raman spectroscopy for the structural study of the Rad51-DNA interactions in 2 cases: (i) a physiological-like state where Rad51 recognizes ATP and then gets in contact with ssDNA and (ii) a pathological-like condition where Rad51 recognizes ssDNA before the administration of ATP, leading to genomic disorders and tumours[1]. The role of ATP has been analyzed by comparing the two systems with a control sample of Rad51-ssDNA without ATP.

Methods: Rad51[1] was incubated 30 mins at 37°C[2] with ssDNA (50kb) in physiological buffer, $Mg^{2+,}$ and ATP. 5µl of the solution was then pipetted on an SHS[2,3] until complete dehydration to obtain filaments suspended on the pillars of the SHS. Suspension has been verified by SEM and Raman studies done with a confocal Raman system (WiTec, Germany), solid-state 532nm laser (~5 mW), Newton CCD (-80°C, Andor), a 100x objective (Zeiss, 0.9 NA). Spectra have been re-centered and background removed (range 600-1800cm^{-1}) as reported in[4].

Results and Conclusions: Rad51-DNA filaments are self-organized, aligned, and suspended over the SHS, offering (i) background-free experimental conditions (ii) filaments purified with a single deposition step due to self-sieving effect[4]. Spectral response in physiological and pathological states showed remarkable differences, based on fine rearrangements and stiffness rather than structural disruptions. Raman shifts analysis indicates (i) Rad51 recognizes and binds ssDNA through loops in every condition but (ii) with higher efficiency and order in physiological state; (iii) heterogeneous DNA forms are detected in pathological

conditions resulting in inefficient system. Quantitative analysis indicates an evolution of pathological samples towards systems lacking ATP. Amide I features (α-helices, disordered structures, β-sheets) showed double peaks with area ratio confirming the transition of pathological systems. Our strategy is well suited to localize, distinguish and describe the interactions between biomolecules. The disordered structures in the free-standing filaments were detailed in their pristine environment, tremendous advantage for proteins where is still unclear the role of flexible regions due to NMR/X-Ray structures discrepancies[5].

Reference(s): 1 Shin, D. S. *et al.* 2003, *EMBO J.* **22**, 4566–4576.

2 Marini, M. *et al.* 2017, *Nanoscale* **9**.

3 Stassi, S. *et al.* 2019, *Nat. Commun.* **10**, 1690.

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5 Uversky, V. N. *et al.* 2021, *Biophys. Chem.* **279**, 106694.

Raman optical activity as a potent tool for studies of mononucleotide G-quadruplexes

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Context and Objective: The tendency of guanosine-5´-monophosphate (5´-GMP) to self-associate at higher concentrations into regular higher-order arrangements – G-quartets and further to G-quadruplexes (G4s) – is known for 60 years[1], therefore it has been thoroughly studied and has significant possible applications both in pharmaceutics and nanotechnology. These associates hold together through a combination of several contributions: Hoogsteen base pairing, base stacking, hydrophobic interaction, cation coordination, and H-bond network among phosphates and ribose hydroxyls of different 5´-GMP. The latter effect has a major impact on the stability of the associate (as we show further) but has not been sufficiently described yet.

Methods: We studied 5´-GMP associates using Raman spectroscopy and its chirally sensitive variant Raman optical activity (ROA) which benefits from the inherent nucleotide chirality. Data were analyzed by the multivariate factor analysis. Usage of Raman spectroscopy enabled simple aqueous solution measurements and studies of highly concentrated samples. At the same time, we can easily change the external conditions (e.g., temperature, pH, and ions) which affects the higher-order structure, stability, and dynamics of these structures.

Results and Conclusions: We obtained well-resolved Raman and ROA spectra of 5´-GMP moieties over the full range of fundamental molecular vibrations (~50–4500 cm^{-1}) and found ROA to be much more sensitive to the formation of G4s than Raman scattering. In ROA, agglomeration is reflected by sharpening and double intensity increase of vibrational features in the fingerprint region and striking over the magnitude signal increase in the terahertz region (50–200 cm^{-1}) reflecting the higher-order arrangement of the observed systems in the presence of different stabilizing cations (Na, K, etc.). Moreover, we observed a major loss in G4s stability when ribose was changed for 2´-deoxyribose in 5´-GMP, pointing to the crucial role of H-bond networking.

Reference(s):

[1] M. Gellert, M. N. Lipsett, D. R. Davies, *P.N.A.S.,* **1962**, *48*, 2013.

Wednesday 31th August 2022
8h45-11h45
Chemometric advances

Photonic Data Science: Data pipelines for the analysis of vibrational spectral data using machine learning and chemometrics

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Photonic measurement techniques including vibrational spectroscopy are increasingly utilized in various disciplines such as life science and medicine. This increased utilization is linked to an improvement of the measurement methods and setups, but also to a development of data science methods and computational infrastructures. With these photonic data science methods, the detection and extraction of high-level information from subtle differences in (bio-medical) vibrational spectra are feasible. Thereby, the high-level information depends on the task and the sample. For example, the prediction of tissue types, disease states or properties of the samples like concentrations of constituents might represent such high-level information.

Vibrational spectroscopic techniques such as Raman spectroscopy and IR spectroscopy feature several advantages, e.g., they can be used as non-destructive fingerprinting techniques. To utilize the full potential of these vibrational fingerprints, the whole data life cycle of the spectroscopic data from its generation, via the data modelling and to the archiving is important and must be studied in a holistic way. Especially, the experimental design, the sample size planning, the data pre-treatment, the data pre-processing, chemometric and machine learning based data modelling, model transfer methods and transfer learning are important. All procedures are sequentially combined in a data pipeline, which standardizes the vibrational data and extracts reliable high-level information.

Herein, our recent studies with the aim to construct a standardized data analysis pipeline for bio-medical Raman spectra [1] will be presented and the comparability of Raman spectra between instruments and labs, which was tested within a European ring trial [2], will be discussed. Beside that the possibility of using deep learning for advanced correction procedures of vibrational spectroscopic data will be outlined [3,4].

Reference(s): [1] S. Guo, Nature protocols 2021, 16, 5426; [2] S. Guo, Analytical Chemistry 2020, 92, 15745; [3] S. Guo, Analyst 2020, 145, 5213; [4] R. Houhou, Optics Express 2020, 28, 21002.

Open Science paradigm for spectroscopic analysis of biological molecules: from experiment design to data evaluation and reporting

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Context and Objective: Open Science is the movement to make scientific research and its dissemination accessible to all levels of society [1]. It is transparent and accessible knowledge that is developed and shared through collaborative networks [2]. It includes practices such as publishing in the open access journals, encouraging young scientists to practice open-notebook science, emphasizing the importance of reproducibility, broad dissemination and engagement in science and generally making it easier to publish, access and communicate scientific results. In this contribution implications of Open Science requirements for spectroscopic experiments will be discussed on the example of stratum corneum characterization by means of infrared spectro-microscopy.

Methods: Practical approach of experimental design and considerations for the reproducibility will be covered. Implementation of spectral data processing (baseline correction, normalization and outlier removal), statistical analysis (dimension reduction, clustering and classification), visualization and reporting using R Environment - open source platform will be presented.

Results and Conclusions: R Platform available for many contemporary operating systems (Windows, Mac OS and popular Linux distributions) seems to be adequate solution for spectral data obtained for stratum corneum; that is the perfect example in the Open Science context. Code-driven solutions allows for transparency and repeatability of the evaluation. Implementation of multivariate analysis, machine learning techniques and various visualization options is easy and straightforward. As the open source infrastructure R Platform allows for seamless extension to new, created by the User, algorithms.

Reference(s):

1. Ke Gong (2022) Open science: The science paradigm of the new era Cultures of Science. 5(1) 3-9 doi:10.1177/20966083221091867
2. Lonni Besançon et al. (2020) Open Science Saves Lives: Lessons from the COVID-19 Pandemic. BMC Medical Research Methodology. 21 (1): 117. doi:10.1186/s12874-021-01304-y

Digital deparaffinisation of Paraffin-Embedded Breast Cancer FTIR Spectra Using an Autoencoder Approach

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Context and Objective

Fourier Transform Infrared (FTIR) microspectroscopy can provide the basis for development of objective systems for biochemical recognition and non-invasive spatial characterisation in histopathology. This approach critically relies upon chemical or digital dewaxation of the sample to remove the contaminating spectral features of paraffin. With current digital dewaxation approaches chemical deparaffinization is often necessary, which is time consuming and requires costly chemical reagents. This work piloted a novel approach using deep learning to remove signatures of paraffin wax with reference to chemically dewaxed tissue.

Methods

In this study, an approach to digital dewaxation using a deep-learning autoencoder, which is developed on breast cancer samples in which spectral measurements are performed pre and post chemical dewaxation for network training. This network was then applied to an external validation set incorporating tissue samples with no dewaxation. Additional functionality of the network included the ability to remove the resonant scattering background in addition to the removal of spectral noise.

Results and Conclusions

The quality of the deep-learning autoencoder was evaluated in terms of its improvement in classification of images from breast cancer tissues, which were subdivided on treatment class (Liminal A, Luminal B and HER2). Superior classification of tumour subtype was observed for digitally dewaxed and processed images when compared to that achieved with images from samples without dewaxation (chemical opr otherwise). This demonstrates the capability of advanced processing approaches towards chemical imaging histopathology.

Fast diagnosis of the etiology of oncology patients' infections by monitoring their immune system response to infection: Infrared spectroscopy of leucocytes

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Context and Objective: For centuries, infectious diseases have been among the leading causes of morbidity, disability and mortality that presented a growing challenge to health security and human progress. A reliable detection of the causative agent of infection is not possible based on clinical measures since most pathogens can cause a wide spectrum of clinical syndromes in humans. Therefore, physicians usually encounter difficulties in diagnosing the infection as viral or bacterial infections based on symptoms. Moreover, the classical methods of culturing for bacterial infections and RT-PCR for viral infections are time consuming (>48h) and are limited for accessible infections. This intricate issue perplexes doctors and researchers since it has serious repercussions. Many studies have proven that FTIR can detect and monitor the minute molecular and biochemical changes in biological samples. Our hypothesis is that FTIR spectroscopy is capable to detect minor changes in the WBC cells resulting from the different immune system response for viral/bacterial infections [1,2].

Methods: Leucocytes were separated from the blood samples and measured by FTIR microscopy The obtained spectra were analyzed using different machine learning methods.

Results and Conclusions: Based on our approach [1,2], it is possible to differentiate between controls and infections (combined bacterial and viral) with 95% accuracy, enabling the diagnosis of the etiology of accessible infections as bacterial or viral with >94% sensitivity and > 90% specificity within one hour after the collection of the blood sample.

Reference(s): 1. Biophotonics. 2020;13(2):e201900215. doi: 10.1002/jbio.201900215. 2. Analyst. 2020, 26;145(21):6955-6967. doi: 10.1039/d0an00752h.

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Unsupervised feature selection by a genetic algorithm for mid-infrared spectral data

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Context and Objective: Infrared spectroscopy has been proved as an efficient versatile biophotonic technology. Its efficiency when applied to complex biomedical problems is highly dependent on chemometrics used to analyze the highly multidimensional information composing an infrared spectrum. In particular, feature selection is usually applied in a supervised context in order to identify important discriminant wavenumbers. The aim of this study is to propose a new unsupervised method of variable selection based on genetic algorithm (GA) combined with clustering and validity indices.

Methods: Our GA starts with the random initialization of a population where each chromosome is composed of a subset of wavenumbers. Then, this population evolves by applying successive operators such as chromosome evaluation, parent selection, parent crossover and mutation. Numerous repetitions of these steps ensure the convergence of the algorithm to a near-optimal solution. The particularity of our approach is that for the chromosome evaluation step, we defined a new fitness function based on the measure of a validity index computed from a clustering partition. To demonstrate the effectiveness of our method, its impact on clustering performance was analyzed by calculating the accuracy before and after variable selection on an artificial spectral image and three increasingly complex real public IR datasets: i) a DRIFT-MIR dataset of lyophilized coffees, ii) a ATR-FTIR dataset of fresh meats, iii) a ATR-FTIR dataset of fresh and archived fungal spores.

Results and Conclusions: Due to lack of place, only results obtained on the DRIFT-MIR spectra acquired on lyophilized coffees are described here. Our GA identified the difference between the two coffees species by selecting two spectral bands, 1660-1670 cm^{-1} and 1690-1715 cm^{-1}, attributed to caffeine and chlorogenic acid. KMeans accuracy increased from 89.58% without variable selection to 99.14% with variable selection. Same results were obtained on the simulated dataset and on the two ATR-FTIR public datasets.

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Understanding and modelling of scattering and absorption phenomena in infrared microscopy

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Understanding and modelling of scattering and absorption phenomena in infrared microscopy of cells has been a central research interest in our group during the last two decades. Biological cells such as pollen, fungi, algae, and human cells are strongly absorbing and scattering objects in the mid-infrared region of the electromagnetic spectrum. The most prominent scattering features are Mie wiggles and ripples [1]. While wiggles are slowly oscillating interference features, ripples are highly oscillating patterns that are due to whispering gallery resonances. Whispering gallery resonances are caused by standing waves at frequencies where an integer number of nodes fits into a spherical scatterer. Both wiggles and ripples are hampering the interpretation of the absorption features of the measured spectra and the spectroscopist is at a loss to conclude if the measured signal is due to radiation that is lost because of absorption or because of scattering.

A deeper understanding of the effect of different morphological features of microscopic samples on the scattering features in infrared microscopy absorbance spectra and the interaction of the sample with the microscopic slides used for infrared spectroscopy has been aspired by our group [2].

Based on a deeper understanding of the scattering and absorption features, we have developed a framework for retrieving pure absorbance spectra from highly scatter-distorted infrared spectra of cells and tissues by incorporating Mie theory and other electromagnetic models in a multivariate modelling framework [1, 3-5]. The Mie Extinction Extended Multiplicative Signal Correction (ME-EMSC) algorithm is the state-of-the-art pre-processing technique and can recover pure absorbance spectra from highly scatter distorted spectra [6]. While the ME-EMSC algorithm is computationally expensive, and the correction of large infrared images could require hours of computations, a deep convolutional Descatter Autoencoder trained on ME-EMSC corrected spectra can be used for correction of hyperspectral infrared images of cells and tissues [7]. The speed advantage of the Descattering Autoencoder allows to preprocess images in near real-time, and thereby making it feasible to use such images in medical applications. Recently, we introduced Mie diffraction tomography that allows 3D reconstruction of refractive index distributions in infrared microspectroscopy.

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Deep Learning-enabled Inference of Distribution of Molecular Absorption of Biological Cells from FT-IR Spectra

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Context and Objective: Infrared microspectroscopy is a valuable and much used tool for studying biological cells and tissue, as it delivers information about the chemical composition of the studied samples encoded in the molecular absorption as well as scattering information depending on the morphology and optical properties. Using focal plane array (FPA) detectors one can easily get the 2D distribution of chemical composition as hyperspectral maps. However, obtaining the 3D distribution is not easily achieved, and although it is possible to realize this experimentally through IR spectro-microtomography, the process is very time-consuming and technically demanding. We suggest exploiting the scattering-contributions to measured IR spectra and solving the inverse scatter problem (ISP) to perform diffraction microtomography, which would allow volumetric 3D chemical imaging using conventional IR spectrometers.

ethods: We employ a deep convolutional neural network (DCNN) to solve the full-wave ISP and recover the 3D distribution of molecular absorption and optical properties from measured IR spectra of radially symmetric microobjects. We train the DCCN on simulated data, where we first sample molecular absorption spectra from a PCA decomposition of measured pure spectra coming from several different data-sets. Thereafter, we solve Maxwell's equations and simulate the electromagnetic scattering on billions of different samples with different molecular composition, morphology and optical properties, where we take into account that radiation is collected over a numerical aperture at the detector. Our model is trained to recover the 3D distribution of the complex refractive index from single measured scatter-distorted spectra.

Results and Conclusions: We corroborate our approach on measured spectra of fungal and algal cells, oak and juniper pollen and on PMMA spheres. We confirm that the model predicts the morphology of the samples accurately, as well as making predictions for chemical compositions of the cell wall and interior which are in good alignment with literature. We demonstrate that our model works well on two-layered spheres, but the approach is fairly general and can be extended to systems where the spherical symmetry is broken. However, the simulation of the ground truth is more time-consuming as it requires advanced numerical solutions of Maxwell's equations for such systems.

Gaussian Weighted Background Correction for Raman images

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Context and Objective: In the chemometric workflow, restoring Raman data free from experimental variations is an essential part that contributes to reliable data analysis. In this contribution we propose an extension of the spectral preprocessing described in [1] for single cell Raman images contaminated with spatial variations such as substrate heterogeneity, non-inhomogeneous illumination, or others.

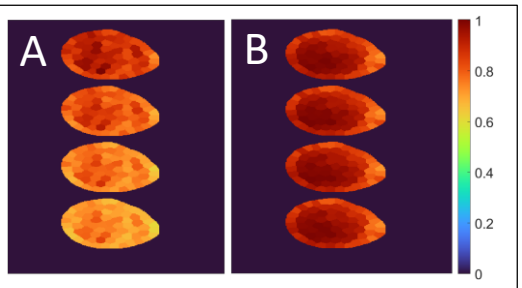

Figure 1 . Lipid distribution of a sythetic cell placed at different locations. A) Raman spectra are preprocessed with a standard protocol sheme B) with our method.

Methods: A Raman image $X \in \mathbb{R}^{m \times n \times B}$ (m, n, B the spatial height, width, and spectral dimension) is first denoised by using a recent spatio-spectral denoising scheme. A 2D skewness map $S \in \mathbb{R}^{m \times n}$ is estimated from the restored Raman image. S is clustered into several group with the k-means algorithm to detect cell regions from background regions. Each single pixel $s \in \mathbb{R}^{B}$ of each single cell is reduced by a weighted average background $b \in \mathbb{R}^{B}$ whose best weights are estimated by using Bayesian optimization.

Results and Conclusions: Fig1 exhibits the lipid distribution of a synthetic biological cell placed at different locations in the space domain, when preprocessed with standard preprocessing workflow (Fig1.A) and preprocessed with our method (Fig1.B). The Raman image is initially contaminated with unwanted space variations. We demonstrated that our scheme removes spatial contamination (here a non-homogeneous illumination profile) linked to experimental factors. This method can be applied to preprocess single cell Raman images collected with complex substrates such as hydrogels.

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Thursday 1st September 2022
9h00-11h30
Nanoscale analysis

The recent technical development and applications performed at ISMI beamline at Singapore Synchrotron Light Source

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ISMI (Infrared Spectro Microscopy) beamline is one of the end-stations available at the Singapore Synchrotron Light Source (SSLS). It is known that the use of synchrotron radiation as a source of infrared radiation brings additional tangible benefits. Synchrotron radiation is hundreds of times brighter than the thermal radiation of a Globar lamp (calcium carbide) used as a laboratory source of infrared radiation (mainly for the mid-infrared region: mid-IR). In addition, infrared radiation obtained at synchrotrons is emitted from a point source into a narrow solid angle and can be easily focused to dimensions of 3-10 μm; thus, spectra measured have a high signal-to-noise ratio. It should also be mentioned that this radiation is harmless even for living cells - during the measurement, cells temperature increases slightly, no more than 0.5 ^{0}C.

FTIR (micro)-spectroscopy has been used in an infinite number of applications at ISMI beamline. However, it is necessary to remember that the diffraction limit of mid-IR wavelengths (2.5-25 μm) constrains the lateral resolution of this method. Therefore, only the chemical and structural information of the objects with limited sizes (usually greater than 10 μm) can be revealed. The amount of light absorbed from the IR beam entering the detector from minute samples is small, leading to noisy spectra.

In this talk, the latest technical development and applications performed at ISMI beamline will be presented. Following case studies: characterization of physical/chemical changes in peptide-assisted regeneration of enamel crystals, studies of rare form of malaria, studies of drug induced changes inside P. falciparum infected RBCs, effect of glycerol and oleic acid treatment on stratum corneum, were chosen to present all benefits of newly emerging O-PTIR (Optical- Photothermal Infrared) and AFM-IR spectroscopies for the analysis of demanding samples.

Chemical speciation of breast microcalcifications in cancerous tissues: from a micrometer to a nanometer scale description

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Context and Objective: Breast microcalcifications (BMCs) are calcium-based mineral deposits within the breast tissue. Their presence on mammograms helps the clinician, as they can be considered early signs of breast cancer. As of now, two types of BMC are acknowledged in the medical field based on their composition: calcifications of type I made of calcium oxalate dihydrate and type II made of calcium phosphate apatite. However, two other types of BMC have been observed in breast tissue, which are BMCs made of whitlockite and amorphous carbonated calcium phosphate. A few studies based on vibrational spectroscopies (infrared (IR) and Raman) outlined a correlation between BMCs chemistry and breast cancer severity, and especially their carbonate content [1,2]. Our project aims to provide an insightful description of BMCs' chemistry to better understand their relationship with cancer. In that regard, we established a multiscale approach for the morphological and physical-chemical characterization of BMCs inside human breast biopsies. Using scanning electron microscopy (SEM), we localize BMC and describe their morphological features. Then, their chemical speciation is determined: i) at the micrometric scale using infrared microspectroscopy (µ-FTIR) and ii) at the nanoscale using IR nanospectroscopy technique AFM-IR.

Methods: BMCs were investigated in patients diagnosed with either *in-situ* or invasive carcinoma, as well as mastopathies (benign microcalcifications used as control). 4 to 8 µm thick slices of the paraffin-embedded biopsies were placed on Low-E microscope slides (substrate compatible with both SEM analysis and IR measurements; MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis). Paraffin was removed using xylene, and microcalcifications chemical analyses were conducted by µ-FTIR and AFM-IR. µ-FTIR measurements were performed using Lumos IR microscope from Bruker, and AFM-IR measurements were done, in both contact and tapping mode, using NanoIR2 from Bruker nano.

Results and Conclusions: Our analyses outline that the current description of BMCs is incomplete. Thanks to the high spatial resolution (up to a few nm) of AFM-IR [3], we outlined the presence of chemical heterogeneities within BMCs at the nanometric level that have not been reported yet. Indeed, our results suggest that BMCs might be composed of, not one, but a mix of the chemical phases previously mentioned: heterogeneities within BMCs themselves that can't be described by µFTIR. These findings highlight the potential of AFM-IR to investigate BMCs directly in breast tissue resections, to describe their fine structure, and to have a better understanding of the nucleation processes implied during the mineralization processes. This nanoscale chemical speciation opens new opportunities to explore the early stages of the formation of calcification and to understand their part in the pathology.

References: [1] Baker et al., *J. Cancer,* 2010. [2] Haka et al., *Cancer Res*., 2002. [3] A. Dazzi et al., *Chem. Rev.,* 2017.

Single Nanopore to investigate protein assembly

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Context and Objective: Amyloid-type protein self-assembly is involved in age-related pathologies including Alzheimer's and Parkinson's diseases. In order to understand the mechanism of the aggregation as well as develop innovative strategies for early diagnosis, proposing cutting-edge approach allowing continuous detection and characterization of protein aggregates at single molecule level are crucial. To this end, single nanopores are an exciting class of sensors that allow label-free detection of single protein assemblies [1]. Our work aims (i) to develop a nanopore sensor showing a good correlation between the aggregate size and the signal generated by its translocation and (ii) to provide the limit of geometrical model for the amyloid size analysis.

Methods: To conduct our work, we synthesis a series of amyloid standard using Aβ peptides and α-synuclein characterized by TEM. Their sensing was done by RPS using nanopipettes with diameter about 24 nm+/4 nm [2]. The detection strategy is based on the resistive pulse sensing that consists in applying a constant voltage through a nanopore and recording the current perturbation induced by the passage of the protein aggregate [3]. These current perturbations (amplitude, time) are dependent on the properties of the object (size charge etc.).

Results and Conclusions: The analysis of the current blockade amplitude of amyloid Aβ and α-synuclein standard show a good correlation with geometrical model assuming a conical shape of the nanopore and cylindrical shape for the amyloid. Interestingly, upper 50-75 nm length the most discriminant parameter is the diameter of the fibre and not its length. This means that such nanopore is suitable to discriminate for small aggregates and protofibrils. Then further data analysis using machine learning was done to improve the discrimination of small aggregate.

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Nano-infrared spectroscopic imaging (NanIRim): Promises and Challenges for Application in Biophotonics

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Context and Objective: The Life Sciences will ultimately benefit from the ability to achieve chemical information at subcellular and single molecule level. Recently, a number of nano IR spectroscopic imaging (NanIRim) methods have been developed, which overcome the limitation caused by long IR wavelengths. Such methods are based on recent technological advances and integration of other detection schemes, for example the use of visual wavelengths or scanning force microscopy.

Several NanIRim methods have been established in Materials Science. An increasing number of applications in Life Sciences using NanIRim methods has been reported. While components in Materials Science usually can be easily discriminated by separate IR absorption bands, the situation is far more complex in biomaterials. High spatial resolution leads to a considerable reduction of the number of chemical bonds contributing to the signal. Thus, important chemical variations have to be identified above a rather heterogeneous background. The comparably slow scanning force microscopy further enhances such issues by yielding much less data per time, which limits the amount of available data within an experiment considerably.

Methods: We applied mid-IR photo-induced force microscopy (PiF-IR) using a VistaScope (Molecular Vista, US) and tapping atomic force microscopy infrared spectroscopy (AFM-IR), using a neaspec system (Attocube, Germany) to a variety of materials ranging from organic monolayers on various substrates and biopolymer compositions[1] to single bacteria and human retina. Comparative measurements were achieved using optical photothermal IR spectroscopy (OPTIR) on a mIRage (Photothermal Spectroscopy Corporation, Santa Barbara, US) and conventional FTIR (model 670, Agilent, US).

Results and Conclusions: In a recent study we investigated interference effects in layered systems comparing experimental and calculated FTIR spectra of polymer films on different substrates to PiF-IR spectra and to OPTIR spectra in collaboration with other groups at the Leibniz-IPHT, Jena. We find similar effects in FTIR and OPTIR spectra, while PiF-IR spectra differ from their corresponding FTIR spectra due to the high surface sensitivity of the method.[2]

PiF-IR enables hyperspectral imaging at a fascinating spatial resolution of about 5 nm. However, a drawback are the small data sets of about 1000 single spectra. We applied PIF-IR to well-known interactions of antibiotics with Bacillus *subtilis*. To meet the challenge of finding

the local interactions in hyperspectral images of single bacteria, we developed an advanced cluster analysis. Our findings are very promising for successful applications of PiF-IR to the investigation of local variations in the surface areas of cells and tissues. Such visualization of molecular interactions at the single cell level will boost our understanding of interactions in the Life Sciences.

Acknowledgement: Funding by the German Research Foundation: DFG Ta1049/2

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Enhanced Tri-modal Optical-Photothermal Infrared (O-PTIR) Spectroscopy – Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)

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Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a cutting edge vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopic tools, providing submicron simultaneous IR+Raman microscopy, in non-contact mode with high sensitivity. The ability to collect, for the first-time submicron IR spectroscopic data in an optical microscope has enabled new research outcomes across a range of application fields, such as life sciences (cells, tissues, bacteria), polymers, cultural heritage and microplastics.

A new modality, "counter-propagating" has been engineered to provide for enhanced IR (and Raman) spatial resolution and sensitivity, through decoupling the need for a reflective objective. The IR pump beam can now be directed to the sample via the underside, thus allowing the collection objective for the visible probe (and Raman excitation beam) to be a high-NA refractive objective. This improves spatial resolution to ~300nm for both IR and Raman, whilst improving sensitivity, image quality and facilitating immersion objective studies.

To further integrate vibrational spectroscopic tools into life science workflows, we coupled widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy. Rather than, or in addition to the visible image, the fluorescence image can now be used to guide the user to the region of interest, thus combining the well-established specificity of fluorescence imaging with the broad macromolecular profiling capabilities of IR spectroscopy

Several life sciences examples from bacteria, cells and tissues will be provided to demonstrate these new capabilities and how they can enable new experiments and research findings.

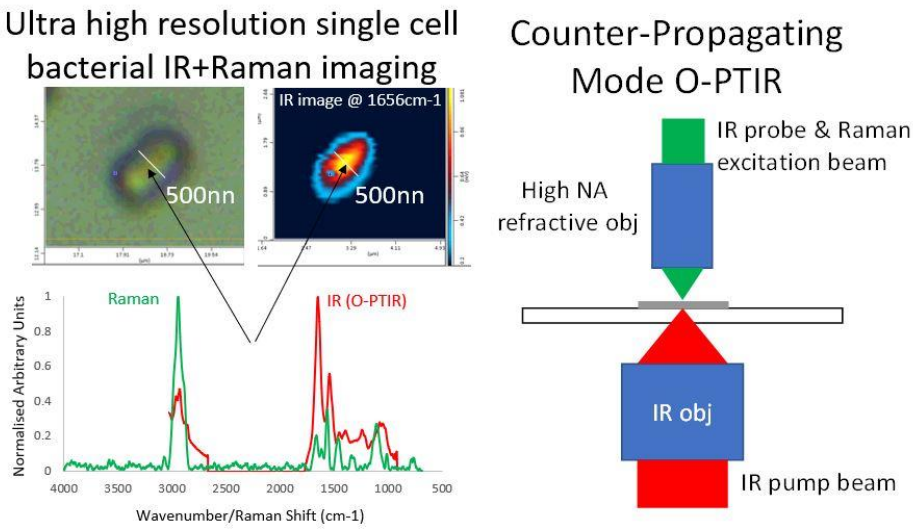

Fig 1. Left: Single E.Coli cell imaged in counter-propagating mode with 50nm pixel/step size and submicron simultaneous IR+Raman spectra from centre of bacterial cell. Right: Schematic of counter-propagating layout.

Functional live-cell mid-infrared microscopy and spectroscopy by optoacoustic and optothermal detection

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Context and Objective: Mid-infrared (mid-IR) excitation and optoacoustic/optothermal (OA/OT) sensing are excellent examples of perfectly complementary technologies. Mid-IR absorption excites molecular-specific vibrational-transitions that are de-excited in the form of heat, generating OA and OT signals which intensity primarily depends on efficient heat deposition. The combination of mid-IR excitation and OA/OT detection is highly sensitive and we apply it for longitudinal assessment of biomolecular composition in living cells and tissues without the need of exogenous labels; i.e., for label-free metabolic imaging.

Methods: We present two different approaches for label-free metabolic imaging: **1)** Mid-infraRed Optoacoustic Microscopy (MiROM) and **2)** Wide-field Optothermal Mid-infrared Microscopy (WOMiM). MiROM uses tightly focused optical excitation with coaxially focused ultrasound detection and Chemical-contrast imaging is obtained by raster scanning the sample along the focal plane; simultaneously acquiring OA signals produced at specific molecular vibrations excited by pulsed mid-IR radiation.[1] WOMiM is a wide-field chemical-contrast imaging method using pump-probe detection of OT signals by optical phase change due to mid-IR excitation.[2] Chemical-contrast imaging is obtained by the difference in Phase Contrast microscopy images of the sample with and without mid-IR illumination.

Results and Conclusions: By MiROM, we are able to monitor lipid, protein, and carbohydrate dynamics down to the single-cell level with a lateral resolution of ~5 µm. For instance, in living adipocytes, we were able to observe the spatio-temporal distribution of carbohydrates used for triglyceride formation during adipogenesis. While with WOMIM, we achieved chemical-contrast in Triglyceride drops for field-of-views up to 180 µm in diameter, at imaging speeds up to 1 ms/frame.

The unique features resulting from the combination of mid-IR excitation with OA/OT sensing have lead to the development of positive-contrast label-free metabolic imaging of living-cells. This presentation will discuss the basic principles of mid-IR spectroscopy and detection as well

as the most recent developments on mid-IR OA and OT sensing, focusing on label-free live-cell molecular microscopy for longitudinal metabolic research.

References:

1-Pleitez, M. A.; Khan, A. A.; Soldà, A.; Chmyrov, A.; Reber, J.; Gasparin, F.; Seeger, M. R.; Schätz, B.; Herzig, S.; Scheideler, M., Nat. Biotechnol. 2020, **38**, 293-296

2-Yuan, T.; Pleitez, M. A.; Gasparin, F.; Ntziachristos, V., Anal. Chem. 2021, **93**, 15323-15330

Infrared Nanoscopy and Tomography of Intracellular Structures

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Context and Objective: Although techniques such as fluorescence-based super-resolution imaging or confocal microscopy simultaneously gather both morphological and chemical data, these techniques often rely on the use of localized and chemically specific markers.

Methods: To eliminate this flaw, we have developed a method of examining cellular cross sections using the imaging power of scattering-type scanning near-field optical microscopy and Fourier-transform infrared spectroscopy at a spatial resolution far beyond the diffraction limit. Herewith, nanoscale surface and volumetric chemical imaging is performed using the intrinsic contrast generated by the characteristic absorption of mid-infrared radiation by the covalent bonds.

Results and Conclusions: We employ infrared nanoscopy to study the subcellular structures of eukaryotic (*Chlamydomonas reinhardtii*) and prokaryotic (*Escherichia coli*) species, revealing chemically distinct regions within each cell such as the microtubular structure of the flagellum. Serial 100 nm-thick cellular cross-sections were compiled into a tomogram yielding a three-dimensional infrared image of subcellular structure distribution at 20 nm resolution. The presented methodology is able to image biological samples complementing current fluorescence nanoscopy but at less interference due to the low energy of infrared radiation and the absence of labeling.

Reference: Kanevche K, Burr DJ, Nürnberg DJ, Hass PK, Elsaesser A & Heberle J (2021). *Infrared nanoscopy and tomography of intracellular structures.* Commun. Biol. 4: 1341. doi.org/10.1038/s42003-021-02876-7.

POSTER SESSION

Tuesday 30th August 2022

18h15-20h45

Using FTIR-ATR spectroscopic method to monitor the development of fungi in plants and bread

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Context and Objective: — Plants diseases are one of the biggest concerns of farmers around the world that causes by Phytopathogenic of fungi. The loss of food has significant social, environmental, economic consequences and losses amounting to billions. Early detection of these pathogens can be highly important for effective treatment and limiting the spread of fungal pathogens. Classical methods are time consuming, non-specific, and expensive. Therefore, a quick, sensitive, and cost-effective method for determining the presence of the infectious phytopathogens highly required. Infrared spectroscopy is considered as a rapid and sensitive technique which can detect minor molecular changes in cells. The main objective of our study to evolve a computerized expert system, based on an infrared spectroscopy method and to examine its potential for the detection and diagnosis of various fungal pathogens at an early stage.

Methods: We grew tow mold Fungus, Aspergillus and Rhizopus, in SDA plates. After isolating the pure fungus, we pollute the bread that used as a model in the study and measured by infrared spectroscopy in following days to test the potential of the infrared method to monitor fungus at early stages of its growth before it starts being visible to the eye. The obtained spectra were manipulated and analyzed by Machine learning algorithms in order to identify the differences between uninfected bread and infected bread at an early stage.

Results and Conclusions: Our analysis revealed that, it is possible to differentiate between tow fungi species Aspergillus and Rhizopus with 100% accuracy. Moreover, FTIR-ATR with machine learning can detect the infection in the bread after the first day with 95% accuracy. Therefore FTIR-ATR is considered as a powerful and rapid method for detection the infected bread and determination the type of infection.

Monitoring of the accumulation of Squalene-Gemcitabine nanomedicine within single living breast cancer cell by Raman imaging.

Almar AL ASSAAD[1], Didier DESMAËLE[2], Emilie FOSSIER[1], Laurence VAN GULICK[1], Florian SLIMANO[1], Sylvain DUKIC[1], Hamid MORJANI[1], Abdelilah BELJEBBAR[1]

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Context and Objective: Gemcitabine (Gem) is one of the most clinically active drugs in the treatment of cancer. However, following treatment, most of the treated patients present recurrent disease due to the short half-life of the drug and several mechanisms of resistance developed by tumor cells. Gem squalenoylation strategy has permitted significant advances against such processes, allowing the emergence of new treatments with better specificity and efficiency. In such a strategy, the drug is bound to squalene (SQ), the natural precursor of the cholesterol's biosynthesis, leading to a SQGem complex. Thus, as cholesterol, the SQGem complex seems to be associated to low density plasma lipoproteins (LDL).

In this work, we have investigated the distribution of Gem and SQGem ^{2}H (deuterated) at the cellular level by Raman microspectro-imaging to elucidate the mechanisms that allow higher cellular incorporation of SQGem in tumor cells. Due to the weak signal of the Raman signature of SQGem, the deuteration strategy was used to make its detection possible at the cellular level. Indeed, the deuterated form of SQGem presents a significant Raman signal at around 2200 cm^{-1} in a so-called "silent" spectral region (1800-2800 cm^{-1}).

Methods: Deuterated SQGem nanoparticles (NPs) were synthesized by nanoprecipitation of the organic solution of SQGem NPs in water and subsequent solvent evaporation. The in vitro cytotoxicity of SQGem NPs and free Gem was performed on human breast cancer cell lines MDA-MB-231 and MCF-7 cells using the MTT test. The IC50 values were measured for Gem and SQGem NPs on these two cell lines. Raman Images were measured on single living cells untreated and treated with different concentrations of Gem and SQGem ^{2}H NPs.

Results and Conclusions: Raman spectral maps were reconstructed using: i) specific bands of the deuterated drug (2000-2200 cm^{-1}) to follow its spatial distribution on the cellular level, ii) relative bands of different cellular components (proteins, lipids, nucleic acids) to identify biochemical changes associated to drug treatment. However, cells treated with SQGem ^{2}H NPs exhibited a deuterium signal localized in the cytoplasm of MDA-MB-231 and MCF-7 cells. Furthermore, the accumulation of SQGem NPs was more important in MDA-MB-231 cells than the MCF-7. This difference in drug accumulation is probably due to a lower expression level of LDL receptors in MCF-7 cells than MDA-MB-231 cells.

Our results demonstrated the potential of Raman microspectroscopy to monitor, through quantitative measurements, the distribution of SQGem NPs using deuterated strategy. In addition, we have identified the changes molecular composition in cell compartments induced by gem and SQGem treatments.

Single Cell FTIR Imaging with Novel ZnS Hemispheres for Studying Phospholipidosis in Live Macrophages

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Context and Objective: Alveolar macrophage responses are served as a critical parameter in inhaled drug toxicology studies. However, morphology (vacuolated or foamy cytoplasm) observation alone provides limited understanding of the foamy macrophage (FM) response. It cannot distinguish between an adverse or adaptive response to a stimulus. In this study, FTIR imaging is used to study the effect of amiodarone, a cationic amphiphilic drug, which was used as a model to induce adverse type of FM with lipid accumulation (phospholipidosis), and compared with fluticasone, which is a marketed corticosteroid used for inhalation therapies that is not known to cause FM but can induce lipid accumulation**. This study aimed** to study phospholipidosis using a novel hemispherical optical substrate with FTIR microscope which allowing to maintain cells viability and achieve subcellular FTIR image in transmission mode.

Methods: Alveolar macrophages-like cells (J774A.1) were exposed to 10 µM solutions of either amiodarone or fluticasone in cell culture medium for 24 h and 48 h. During FTIR imaging, the live cell was maintained in CO_2-independent cell culture media, seeded on the ZnS hemisphere and then sandwiched between two ZnS hemispheres with a 6 µm spacer.

Results and Conclusions: Principal component analysis revealed separation of the single live cell spectra between untreated and treated groups at CH stretching bands region which indicates an increase in lipids. PC1 loading for the 24 and 48 h (variability 88, 92% respectively) highlighted the major bands that contribute to the separation include the CH_3 and CH_2 asymmetrical stretching and CH_2 symmetrical stretching in addition to olefinic (C=CH-) groups stretching. In the fingerprint region, the ester carbonyl band from triacylglyceride also shows a significant contribution to PC1 (variability 56, 64%). This study demonstrates the ability of the ZnS hemisphere to preserve cell viability during analysis and to produce a high-enough FTIR spectral quality of single living cells to detect the small differences in effects on the cell induced by amiodarone and fluticasone.

Monitoring of algal production in photobioreactors by Raman spectroscopy and chemometrics

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Raman spectroscopy offers a promising technique for rapid and non-destructive analysis in several domains: medical, pharmaceutical, food, and bioprocess. The main objective is simplifying the analysis process and reducing the investigation time of samples. The analysis does not require an extensive sample preparation and the molecular composition can be obtained without labelling or the use of any chemical products.

The present study shows an example of application of Raman spectroscopy to monitor the algal production. In fact, microalgae have a considerable interest due to its bio compounds that are used in health supplements, cosmetics, and biofuels. The high demand on this photosynthetic microorganism promotes its bioproduction on a large scale resulting in a need to optimize the bioprocess monitoring. Our study shows the different possibilities to use Raman spectroscopy for in line control of algal bioproduction [1]. The experiments were done on Parachlorela kessleri in different production conditions to evaluate the ability of this technique to monitor the physiology of cells in a complex environment. The statistical exploration of data shows good classification efficiency for each physiological growth phases of algal cells. Regardless of the advantages offered by this technique, many challenges remain for its application in real conditions. Indeed, several factors affect the spectral profile of biological elements and Raman data are subject to considerable variability. The cells can be permanently in move inducing problems of irregular scattering of Raman photons and the reproducibility of measurement. The development of data processing software could provide a considerable help in the resolution of the problems of characterization and to extract the useful signature from the global signal and the recent technological development could propose portable devices having an acceptable price to help this technique to become widely used.

Keywords: microlagae; monitoring; Raman spectroscopy; chemometrics

Reference(s): [1] Lieutaud, C., Assaf, A., Gonçalves, O., Wielgosz-Collin, G., & Thouand, G. (2019). Fast non-invasive monitoring of microalgal physiological stage in photobioreactors through Raman spectroscopy. Algal Research, 42, 101595.

Early detection of stem cells transformation using FTIR and High-Resolution Raman Imaging

***Karolina AUGUSTYNIAK*[1], *Hubert LATKA*[1], *Monika LESNIAK*[2], *Jacek Z. KUBIAK*[2], *Robert ZDANOWSKI*[2], *Kamilla MALEK*[1]**

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Context and Objective: In order to be able to track such a complex process as cells transformation, multiple markers, commonly for immunostainings, must be not only used but also appropriately selected to follow the subsequent steps of process. However, with this method, the information about biochemical changes is not provided [1]. Therefore, vibrational imaging techniques, widely used in the research of cells and tissues, can complete the characterization of the transformation process and indicate its early steps.

Methods: Primary MSCs isolated from adipose tissue of C57BL6 mice were used for the study. The differentiation process towards adipocytes was observed from 6 h up to 14 days. Raman spectra were collected with a Witec Alpha 300 spectrometer while FT-IR images of cellular films was recorded using an Agilent 670-IR spectrometer coupled with a 620-IR microscope and a Focal Plane Array (FPA) detector. Statistical and chemometric methods were used for data analysis.

Results and Conclusions: The determination of changes in spectral markers of lipids in the differentiation process showed alternations in their content, saturation and acyl chain shortening. Additionally, the transformation of secondary structure of proteins and nucleic acids was also observed. These features discriminated the early and late phases of the transformation process and indicated that after 2 days cells exhibit a unique composition among all investigated time points. With chemometric methods such as HCA and PCA, the special biochemical character of Day 2 was confirmed. Noteworthy, just with the reference methods – Oil Red staining and immunofluorescence, the information about the content alternations cannot be obtained.

This research was fund by the Ministry of Science and Higher Education, PL (Diamond Grant, No. DI2018 018048).

Reference(s): [1] D. J. Maltman et al., Neurochem Int, 2011, 59, 347–356.

Who's who? Discrimination of Breast Cell Lines by FTIR Microspectroscopy

Inês P. Santos[1,†], Clara B. Martins[1,†], Luís A. E. Batista de Carvalho[1], Maria P. M. Marques[1,2], and Ana L. M. Batista de Carvalho[1]

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Despite all efforts, cancer is still a growing health problem worldwide, metastatic breast carcinoma being the second most lethal cancer among women [1]. Discriminating between malignant breast cell lines is of primary importance, as being able to identify the different subtypes of breast cancer in a rapid and accurate way will enable more effective treatment options [2,3].

The aim of this work was to use FTIR microspectroscopy to unveil specific and sensitive molecular biomarkers in human triple-negative breast cancer (TNBC) and non-triple negative (non-TNBC), assessing whether this technique is able to detect even more subtle differences than those between malignant and non-malignant cells [4]. Four human breast cell lines – TNBC (MDA-MB-231, MDA-MB-468, HCC-1143) and non-TNBC (MCF-7) were measured by FTIR microspectroscopy in the mid-IR interval (400-4000 cm^{-1}), in transmission mode. For both the fingerprint and high wavenumber spectral regions, data was statistically analyzed by Principal Component Analysis (PCA) allowing to obtain a good discrimination between the different sets of samples.

The vibrational modes found to play a more significant role in the discrimination between TNBC and non-TNBC cell lines were ascribed to DNA and protein vibrational signatures, which were found to predominate in the former while the signals from lipids appeared to be prevalent in non-TNBC cells. When comparing different TNBC subtypes (mesenchymal vs. basal-like 1) a good discrimination was obtained, mainly based on DNA and lipid contributions.

A particular FTIR signature was obtained or each cell line under study, evidencing the occurrence of specific spectral biomarkers allowing the detection of different types of human breast carcinoma. This constitutes a paramount advance in cancer diagnosis, particularly regarding a type of malignancy with high morbidity and mortality.

References:

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[2] Lehmann, B.D. *et al*., Identification of Human Triple-Negative Breast Cancer Subtypes an Preclinical Models for Selection of Targeted Therapies. *J Clin Invest* (2011); **121**, 2750.
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[4] Batista de Carvalho, A.L.M. *et al*., Chemotherapeutic Response to Cisplatin-like Drugs in Human Breast Cancer Cells Probed by Vibrational Microspectroscopy. *Farad Discuss* (2016); **187**, 273.

Absolute configuration determination of promising new drug for Parkinson's disease *via* Raman optical activity

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Context and Objective: L-3,4-dihyroxyphenylalanine (L-DOPA, levodopa) is a gold standard treatment for Parkinson's disease. Lately, it has been found that some of its deuterated analogs exhibit higher potency in the treatment; thus, they could replace L-DOPA. In some cases, the selective deuteration ends in the creation of a new chiral center, as i. e. In the case of the double deuterated derivative of L-DOPA (α,β-D2-L-DOPA). Although different enantiomers (and diastereomers) of chemicals are known to have different biological effects, the chirality at carbon C_β of this compound has not yet been addressed.

Methods: We have studied L-DOPA and three of its deuterated derivatives by the means of vibrational and chiroptical spectroscopy (Raman, Raman optical activity (ROA), IR, and VCD). The quantum mechanical simulations based on the density functional theory enabled the detailed assignment of experimental spectral features to individual vibrational modes of the L-DOPA.

Results and Conclusions: We obtained high-quality ROA and Raman spectra of L-DOPA and its deuterated analogs in the entire range of fundamental molecular vibrations (75 – 4000 cm^{-1}). We also obtained IR and VCD spectra but the measurement of the latter has been very difficult due to the low DOPA molar absorptivity. Based on the combination of experiments and simulations we addressed spectral changes associated with deuteration at various carbon atoms as well as with the deuteration at easily exchangeable positions (amine, hydroxyl, and carboxyl groups). Finally, we reliably determined the absolute configuration of double deuterated α,β-D2-L-DOPA sample as (S-α, S-β). The latter result has direct practical application in clinical research and pharmaceutics.

Structural Investigation of α/γ-Hybrid Peptide Oligomers

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Peptide-like oligomers are among the most widely investigated foldamers because of their large structural diversity and functionalities, mostly provided by using non-natural amino acids, resulting in potential applications in biomaterials, drug-delivery systems, and catalysis. Several fundamental foldamer types employing non-natural amino acids have been investigated based on their basic building blocks, prominently homooligomers assembled from α-, β-, or γ- amino acids or hybrid oligomers constructed by combination of (αβ)-, (βγ)-, or (αγ)-dipeptide units. In our study peptide-like foldamers consisting of alternating α,β,γ-triamino acids 3-amino-4-(aminomethyl)-2-methylpyrrolidine-3-carboxylate (AAMP) and natural amino acids glycine and alanine were synthesized and their secondary structure was determined. We demonstrate that both non-amide bond forming amino groups present in AAMP contribute to secondary structure formation in α,γ-oligopeptides and induce new folding patterns. The relatively stable secondary structures of all oligomers containing AAMP units are supported by both ECD and VCD spectroscopies. VCD investigations point to a handedness of the studied foldamers. Thus, in a more general context, extra functional groups with hydrogen bonding capabilities may be considered as a step towards encoding new folding propensities in peptides and thus open new directions in engineering of bio-inspired materials.

SIMIECORR: Mie scatter correction without a prior assumption about the chemical composition of a sample

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Context and Objective: Objects in the micrometer range are highly scattering in the mid-infrared region. It was shown that highly non-linear Mie scattering effects can heavily distort spectra of human tissues and cells. Current Mie scattering correction methods require prior knowledge about underlying chemical composition of the sample in terms of a reference spectrum. In this paper, the main objective is to present a method for Mie scattering correction that does not require prior knowledge or assumptions about the chemistry of the sample.

Methods: Two deep convolutional neural networks (DCNN) were trained on two simulated data sets for retrieval of pure absorbance spectra from highly scatter-distorted spectra. Pure absorbance spectra for the first dataset were simulated using random Lorentz-Gaussian profiles (peaks) and therefore did not require a priori assumption about the chemical composition of the samples, while for the second data set spectra were simulated using a data set of pure absorbance spectra of filamentous fungi. For both data sets we simulated Mie scatter distorted spectra according to Mie theory. The DCNNs were validated with infrared microspectroscopic (i) single element data of PMMA spheres and lung cancer cells; (ii) imaging data of filamentous fungi.

Results and Conclusions: The chemically independent neural network corrects Mie scattering in scatter-distorted spectra well for most of sample spectra validated. Corrected spectra have a high correlation value with the state-of-the-art method [1]. The chemically informed Neural Network works very well for the fungal data set as a priori chemical knowledge about the fungi was used for the training. The presented results demonstrate that the DCNN for Mie scatter correction can be trained without any assumptions about the chemical composition of the samples. If spectra contain a high level of noise, chemical conditioning of the DCNN is required, otherwise DCNN are at a loss of differentiating between noise and sharp peaks.

Reference(s):

1. Solheim, Johanne H., et al. "An open-source code for Mie extinction extended multiplicative signal correction for infrared microscopy spectra of cells and tissues." Journal of biophotonics 12.8 (2019): e201800415.

Identification of a biochemical signature of dysfunctionality by Raman spectroscopy analysis of lipoproteins

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Context and Objective: High-Density Lipoprotein-cholesterol (HDL-C) is as an important protective factor against cardiovascular disease, but its beneficial effects don't depend solely on its concentration. HDL (High-Density Lipoproteins) possess several atheroprotective function associated to the anti-oxidant, anti-inflammatory, anti-thrombotic properties as well as to the ability to support endothelial physiology. In pathological conditions both the structural composition and function of HDL may be significantly altered, converting these anti-atherogenic particles into dysfunctional ones[1]. The quantitative measurement of cholesterol levels is not indicative of the risk associated with these alterations, the quality of the HDL appears to be a better biomarker than the quantity of HDL-C. In this context, Raman spectroscopy (RS) represents an innovative technique that allows the analysis of biological samples without any sample preparation. Moreover, this approach permits obtaining simultaneous information about both the biomolecules composing lipoproteins and the relative amount. The purpose of our study is thus to characterize the biochemical composition of HDL in dyslipidemic subjects by RS, to identify a signature of dysfunctionality.

Methods: HDL were extracted from human plasma of 37 subjects (18 healthy controls [HC]; 19 dyslipidemic subjects [DS]) by ultracentrifugation in discontinuous KBr density gradient. Spectral differences were evaluated between the two different groups by RS to identify a dysfunctional signature.

Results and Conclusions: Compared with HC, the DS Raman spectra were characterized by less intense peaks corresponding to cholesterol (700 cm-1), carotenoids (1155 and 1525 cm-1) amino acids (1355 cm-1) and unsaturated lipids (1270 cm-1) highlighting an alteration of the structural composition of dysfunctional HDL. Our preliminary results, show that RS could be a viable approach for a qualitative biochemical characterization of HDL in pathological contexts such as dyslipidemia to identify a dysfunctional signature to frame individual patient risk.

1 *Bonizzi et al*., HDL dysfunctionality: clinical relevance of quality rather than quantity, Biomedicines, 2021.

Isotopic labeling of microalgae: Raman study

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Context and Objective: Autotrophic microalgae can produce a number of substances such as polysaccharides, lipids, proteins, carotenoids, polyphosphates, or crystalline purines directly from inorganic sources. Different microalgal species are used for the economic production of high-value products. Due to their autotrophic nature, microalgae can biosynthesize complex isotopically labeled biomolecules from simple isotopically labeled inorganic substances. Analysis of the chemical composition of microalgae by means of chemical-analytical methods is relatively complex, time-consuming, and laborious. Confocal Raman microscopy (CRM) represents practical optical method by which the biosynthesis of isotopically labeled compounds can be monitored *in situ*, i.e. directly within intact cells.

Methods: The CRM combining confocal optical microscopy with Raman spectroscopy enables fast and non-destructive analysis of the chemical composition of substances in the investigated objects, including the effect of isotopic labeling. The chemical composition of the investigated objects is reflected by their Raman spectra, in the case of Raman mapping of microscopic objects by their chemical maps. In this work, two specific cases of isotopic labeling were studied, namely the effect of heavy water (deuteration) and ^{15}N-enrichment of biomolecules in living cells of the marine dinoflagellate *Amphidinium carterae*.

Results and Conclusions: The main result was the detection of variously deuterated and 15^{N}-enriched forms of crystalline guanine depending on the ratio of D_2O/H_2O and $^{15}N/^{14}N$-labelled nitrates in the culture medium and the determination of the mean degree isotopic labeling by comparison of the biosynthetized guanine with synthetically prepared isotopic forms of guanine. An interesting and valuable discovery, which was not originally the intention of this work, was the finding of unexpected persistence against the dissolution of nanocrystalline guanine even in highly diluted solutions.

This work was supported by the Grant Agency of the Charles University (No. 158222).

From synthetic identity to biological function of a doxorubicin liposomal formulation

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Context and Objective: Encapsulated Doxorubicin (DOX®) proved superior performance with respect to molecular Doxorubicin (DOX) in clinical application. However its in-cuvette "synthetic identity" is still limitedly understood, due to the lack of analytical tools that can quantitatively dissect the molecular organization of the drug within the intact liposomal formulation. The form in which the drug is administered and reaches the tumor cells can affect its therapeutic efficacy. For instance, non-monomeric and non-isolated DOX molecules cannot interact with DNA with the same efficacy of monomeric DOX.

Methods: The supramolecular organization of Doxorubicin is investigated within the standard Doxoves®, a formulation of 85 nm-diameter PEGylated liposomes loaded with Doxorubicin through remote-loading mechanism. Exploiting visible light and Doxorubicin intrinsic fluorescence, with phasor approach to fluorescence lifetime imaging (phasor-FLIM) is possible to map each pixel onto the so called "phasor" plot. In such graphical representation the polar coordinates are derived by the Fourier transform of the fluorescence decay in time at the angular repetition frequency of the measurement. When a pixel contains a combination of two (or more) distinct lifetime decays its position depends on the contribution of each species in terms of actual molar fraction and brightness (given by the product of quantum yield and molar absorption coefficient).

Results and Conclusions: The phasor-FLIM signature of DOX® is resolved into the contribution of three co-existing fluorescent species (each with its characteristic mono-exponential lifetime): crystallized DOX (0.2 ns), free DOX (1.0 ns) and DOX bound to the liposomal membrane (4.5 ns). The exact molar fractions of the three species are determined by combining phasor-FLIM with quantitative absorption/fluorescence spectroscopy on pure standards. With this label-free procedure we quantified that most of the drug in DOX® is in crystallized form (~98%). Next step will be the study of supramolecular organization of Doxorubicin after interactions with cells.

Reference: P. Tentori, et al., Fluorescence lifetime microscopy unveils the supramolecular organization of liposomal Doxorubicin, Nanoscale, 2022, 14, 8901-8905, DOI: 10.1039/D2NR00311B.

Raman isotope probing (RIsP) for identifying antimicrobial resistance

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Context and Objective: Microbial infections are one of the major causes of death in both developed and developing countries. In addition to putting a large burden on the healthcare system, these are also known to be detrimental to human life. Colistin is considered as the preferred treatment option for infections caused by multidrug-resistant Gram-negative microbes such as *Enterobacter* species, *Acinetobacter* species, and *Pseudomonas aeruginosa*. Resistance to this drug is rare but reported in the literature. Existing approaches to diagnose these resistant species include culturing of the bacterial samples and determining its susceptibility to Colistin. However, the process is slow and can take many days even in the advanced labs. The primary aim of the present work is to develop an interdisciplinary approach based upon heavy water labelling and Raman spectroscopy to identify Colistin resistant microbes.

Methods: The pure bacterial cultures are treated with heavy water (deuterium oxide) and spectra were acquired using a Raman micro spectrometer. Spectral preprocessing and multivariate analysis using PCA was performed using MATLAB based in-house codes.

Results and Conclusions: The spectral features suggest incorporation of deuterium (D) in metabolically active bacteria. Successful delineation between sensitive and resistant bacterial species have been achieved. A regression based framework is being developed by tying of the spectral pattern with corresponding antibiotic dose. A novel application of Raman spectroscopy for identification of Colistin resistance through heavy water incorporation will be developed upon the completion of the work. In addition, the findings of the study could also serve as a foundation for developing a method to prevent the spread of Colistin resistance that could also be helpful in improving the treatment of multidrug-resistant Gram-negative bacterial infection.

Raman Spectroscopy based metabolomics for bioprocess monitoring

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Context and Objective: Different kind of bioprocesses mediated by microorganisms play a vital role in the maintenance of life on earth. However, linking bacteria with a specific metabolic process is not feasible. It is of a big concern to track and study microbial metabolites in an efficient and non-destructive way. Raman spectroscopy combined with stable isotope probes such as carbon-13, and deuterium has been a breakthrough in the qualitative and quantitative study of biomolecules and metabolomes in-vivo. Raman Spectroscopy in microbiology offers a stain-free and non-destructive analytical tool to assess the biochemical composition of microorganisms. In this work, we are trying to find a Raman spectroscopy combined with stable isotope probing based bio sensing techniques to monitor the changes in the microbial metabolome.

Methods: We have used normal glucose and C-13 labeled glucose as the only carbon source in the medium to identify C-13 incorporation in *E. coli* biomass in a time-dependent manner. A comparative study of labeled and control *E. coli* culture have been performed. The spectra from pure E. Coli cultures treated with C-13 labelled glucose were acquired using a Raman micro-spectrometer. Spectral preprocessing and multivariate analysis using PCA was performed using MATLAB based in-house codes.

Results and Conclusions: Findings suggest a clearly visible bio-fingerprint shifts in Raman spectra of major biomolecules such as nucleic acids, phenylalanine, tyrosine, and amide I amide III. Kanamycin, a protein synthesis inhibitor was used to monitor the effect of antibiotics on the shifted peak intensity at different time points to study the sensitivity of Raman spectroscopy to study the metabolic changes. This work will help us to further understand and develop a Raman-based efficient biosensor for monitoring the different metabolites *in-vivo*.

Raman Spectroscopy Analysis of Plasma of Diabetes Patients without and with Retinopathy, Nephropathy, and Neuropathy

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Objectives: Diabetes is now one of the major public health challenges globally[1]. Prolonged diabetes leads to various diabetes microvascular complications (DMCs) like retinopathy, nephropathy, and neuropathy[2,3,4]. Multiple factors are likely to be involved in predisposing diabetic subjects to complications[5]. Early detection or diagnose are very essential in developing strategies to reduce the risk factors for the development of diabetic complications. In this study, we employed Raman Spectroscopy to analyse the plasma samples of diabetes patients without and with diabetic retinopathy (DRe), diabetic nephropathy (DNe), and diabetic neuropathy (DNu) along with the plasma samples of control subjects.

Methods: Raman spectra of plasma collected from 20 healthy volunteers, 20 subjects of diabetes, Diabetic Neuropathy, Diabetic Nephropathy, and Diabetic Retinopathy. were recorded using WITec alpha300R with 532nm, Laser power 30m W, Grating 600 grooves/mm, Acquisition time 5sX10, Objective 50X, and in spectral range – 400 to 4000 cm-1. For every sample 10-12 spectra were recorded from random points. Each of the spectra interpolated in 600-1800 cm^{-1} region. The pre-processed spectra from each serum sample were averaged and used for multivariant analysis.

Results and Conclusions: Spectra suggested sublte but significant difference among control, diabetes and DMC groups which can be attributed to variations in protein bands. Analysis of control and diabetes and dmc groups gave, correct classification 85% (17/20) of and 73 % (58/80), respectively. Analysis among diabetes and dmc indicated 70% (14/20) of Diabetes spectra, 55% (11/20) of Diabetic Nephropathy spectra, 55% (11/20) of Diabetic Neuropathy spectra and 65% (13/20) of Diabetic Retinopathy spectra have been correctly classified. Thus this preliminary study suggest the feasibility of noninvasive and rapid Raman approaches for diabetes and DMC group stratification.

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Tip-Enhanced Raman Spectroscopy of Tau fibrils: Measurement and Chemometric Analysis

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Context and Objective: Tip-enhanced Raman scattering (TERS) spectroscopy has unprecedented potential to provide nanoscale structural and chemical characterization of biomolecules and their interactions to better comprehend molecular mechanisms of biological processes involved in cell activity and disease. However, traditional peak-picking methodologies for TERS spectral processing are inappropriate for complex biological systems due to their time-consuming nature and potential for information omission. Chemometric methods tailored to TERS datasets allow for an improved understanding of investigated systems by mapping spatial distributions of characteristic chemical groups of biomolecules. AFM-TERS with chemometric processing was applied to amyloid Tau fibrils with different cofactors to elucidate their incorporation into the fibril structure.

Methods: Full-length 2N4R Tau fibrils with either heparin sodium or RNA homopolymers as cofactors on ultra-flat template-stripped gold substrates were measured using AFM-TERS in reflection geometry. TERS tips were produced by sputtering 30-100 nm silver onto commercial AFM silicon tips TERS mapping was performed under 633 nm laser excitation, with integration times per spectrum being 5-15 s. TERS maps were processed using LabSpec6 and chemometric methods implemented using MATLAB and PLS_Toolbox.

Results and Conclusions: A chemometric pipeline for TERS signal processing of Tau fibrils is presented. The spectral contributions from the heparin sodium and RNA homopolymer cofactors are used to speculate their incorporation into the fibril structure.

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Raman Micro-Spectroscopy for skin and hair cosmetics testing

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Context and Objective: Confocal Raman microspectroscopy has proved to be a powerful tool with strong potential for hair and skin analyses, and for innovative applications in dermatology and cosmetology fields. This is non-destructive biophotonic technique allows the access to very specific molecular information without any particular labeling “label free” or sample preparation. This technique can be of interest in several applications in cosmetology, as for the characterization of the cutaneous constituents (lipids and proteins conformations), the evaluation of water content and skin hydration, the monitoring of active ingredient permeation in human skin or again the determination of keratin conformation in human hair fiber.

Methods: The referenced work focused on three examples of Raman spectroscopy applications in cosmetology. The first one is about the *in-vivo* evaluation of the hydration of the skin after application of a moisturizing cream. This study was performed by an *in-vivo* confocal Raman probe and the axial (Z) Raman profiles were recorded directly in real-time on volunteers, which make it possible to obtain molecular information from epidermal layers of the skin. In the second study, we have applied Raman micro-imaging to investigate on skin sections (*ex-vivo*) the penetration and distribution of active ingredients. Finally to better understand the molecular effects of hair cosmetic products, confocal Raman analysis was performed directly in the intact human hair fibres.

Results and Conclusions: The results of the *in-vivo* study show a moisturizing effect for the skin treated with the active ingredient compared to the placebo. For the *ex-vivo* study, our results allowed the identification of specific spectroscopic markers that are relevant for active ingredients monitoring in skin layers. Then, by matching these markers and chemometric analysis, we have shown the transepidermal permeation of cosmetic ingredients (1, 2).

Finally, for Raman hair analysis the results show that cosmetic products have a smoothing effect on the human hair fibres by acting on α-helix and β-sheet keratin conformation and on the tertiary structure of keratin (3).

The examples of application in cosmetology demonstrate that Raman spectroscopy presents multiple advantages. It gives direct molecular information, and enables to monitor the changes in the skin and hair fibers induced by the application of cosmetic products. In addition, there is no need for any special preparation and it can be used for *in-vitro*, *ex-vivo* or *in-vivo* investigations at the micrometer scale.

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Raman Spectroscopy in the Early Diagnosis of Colorectal Cancer

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Context and Objective: In times of COVID-19, the use of elective care was significantly limited, as was the conduct of many screening programs, including those for colorectal carcinoma. Consequently, this reduction in preventive care will result in a more frequent diagnosis of advanced-stage colorectal tumors and possibly a 16.6% increase in their mortality in the future [1]. The timely removal of precancerous lesions, adenomatous polyps, before they transformed into a malignant form could have prevented such outcome. Therefore, our approach aims to accelerate the diagnostic procedure for these precancerous and cancerous lesions in their initial stages by introducing in vivo Raman spectroscopy coupled with machine learning methods for data handling in the process. Compared to traditional diagnostic methods, consisting usually of a biopsy followed by a lengthy histopathological analysis, our methodology represents a faster, less invasive, and lower risk alternative with comparable accuracy.

Methods: During routine endoscopic procedures, we acquired Raman spectra of normal and cancerous epithelial linings of the colon and rectum in vivo using a custom-made fiber optic microprobe. Moreover, a variety of polyps of numerous etiologies, degree of dysplasia, and malignant potential were analyzed spectroscopically. The acquired Raman spectra categorized according to their clinical diagnoses were algorithmically preprocessed, enabling the training of multivariate statistical models in order to provide a rapid diagnostic response.

Results and Conclusions: Our advanced methodology allowed us to classify in vivo Raman spectra of colorectal tissue as normal, cancerous, or benign with an overall accuracy of more than 85%, which illustrates the significant potential of this approach in the field of clinical diagnostics.

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Live-cell Mid-infrared Optoacoustic Microscopy and Spectroscopy for Longitudinal Metabolic Monitoring

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Context and Objective: Imaging modalities based on vibrational spectroscopy, such as Coherent Raman Scattering, have high chemical specificity for label-free imaging of biomolecules, but their sensitivity remains in the low mM range—which is inadequate for live-cell imaging of most metabolites. On the other hand, conventional mid-infrared methods have high sensitivity, but are restricted mainly to dry tissued due the strong absorption of water. Although strong water absorption can be partially overcome by synchrotron radiation and thin path-lengths of ≤10μm, this alternative can cause photo-damage and alters cells' morphology and physiology. Moreover, mid-infrared methods are inherently limited by negative contrast detection, wherein only transmitted or reflected photons are detected. In order to address these limitations, we developed Mid-infraRed Optoacoustic Microscopy (MiROM), a label- free, bond-selective, and positive contrast modality, which allows spectral imaging of biomolecules (e.g., lipids, proteins, carbohydrates and nucleic acids) in living cells.

Methods: A broadly tunable pulsed quantum cascade laser in the spectral range of 3.4-11.0 μm (mid-IR) is used for optoacoustic generation. The mid-IR absorption map of the sample is obtained by scanning it along the focal plane simultaneously detecting the optoacoustic signal by a focused ultrasound transducer. The transducer and the reflective objective are coaxially aligned to share the same focal plane where the sample is placed in a custom-made mid-IR dish. Cell medium or water serve as acoustic coupling between the transducer and the sample.

Results and Conclusions: MiROM is a new imaging/spectroscopy technology, which allows label-free detection of biomolecules and dynamic processes directly in living cell (for instance detection of lipids, proteins, and carbohydrates contrast during lipolysis or lipogenesis). In this work, we demonstrate that MiROM yields unique label-free metabolic imaging abilities for a broader range of bioanalytical studies in living cells.

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Two-dimensional infrared spectroscopy of carbohydrates with site-specific reporter groups

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Context and Objective: The work presented here aims to enable two-dimensional infrared spectroscopy of carbohydrates, by using the site-specific reporter group thiocyanate as a vibrational probe. We used a new synthesis method [3] to incorporate thiocyanate into different carbohydrate samples. This reporter group allows using the transparent spectral window of carbohydrates for IR-spectroscopy [4] and provides a high local resolution. Two-dimensional infrared spectroscopy can reveal dynamics on a femto- to picoseconds timescale. The high temporal resolution is of high interest, as events like bond building and breaking appear in that timescale. The investigation of ultrafast structural dynamics of biomolecules with reporter groups and 2D-IR spectroscopy is already in practice for proteins [1] [2], but was net jet done with carbohydrates.

Methods: Nine different thiocyanate-monosaccharides are investigated with FTIR, transient, and two-dimensional infrared spectroscopy.

Results and Conclusions: We show the first 2D-IR spectra of carbohydrate compounds and evaluate the data, specifically aiming for spectral markers of structural differences between carbohydrate samples. Next to structural differences, ring flip dynamics are addressed [5].

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Spectral tissue imaging for ex-vivo cancer diagnosis and survey

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Raman spectroscopy is an inelastic scattering process which has incredible potential in biological sample analysis. This technique is capable of rendering information on the vibrational modes of molecules of interest. Raman spectroscopy is also less time consuming compared to conventional methods of tissue analysis, because the hassle of sample preparation is minimum or not required. The goal of this project is to employ Raman imaging for ex-vivo cancer diagnosis. The initial experiments were carried out to investigate the spontaneous Raman spectrum of breast cancer tissues using the LabRAM HR Evolution, a high resolution spectrometer developed for Raman micro spectroscopy, in order to characterize the differences in lipid content between the invasive front of the tumor and at distance of the tumor. The objective of this first experiments was to determine precisely wavenumbers assigned to lipids subclasses presenting differences of structural organizations. In the latter part of the research work, new samples of breast cancer were analyzed by Raman scattering and mid-infrared absorption imaging. These samples have the particularity of presenting a tumor part as well as a front of tumor invasion on which we focused our analysis. The experiment was divided into two stages:

1) Identification of the regions of interest from the tissue sample and verify the structural integrity of the tissue sections by using IR imaging of the sample. The imaging was carried out using Spotlight™ IR microscope (Perkin Elmer) and the IR images were then pre-processed using Extended Multiplicative Scatter Correction (EMSC) and K-means clustering. The results from the processing indicated for some samples, a poor localization of the lipids in the tissue sections. This calls for an improved condition for sample preparation.
2) Investigation of the tissue sections by using Raman spectroscopy. The second stage of the experiment was carried out simultaneously with sample sections prepared with same parameters as mentioned above. The acquisitions were carried out using XploRA, a compact Raman microscope developed in Horiba. The spectra acquired from the tissue sections from invasive front of the tumor and the tissue from a distance from tumor, by using same parameters, were compared.

The next step is to study tissue samples from patients with different body mass index in order to investigate the impact of obesity in cancer microenvironment and its progression.

Eventually we aim also to carry out stimulated Raman scattering imaging and second harmonic generation imaging on the tissue sample to study the global environment of breast cancer.

In situ identification of secondary structures in unpurified Bombyx mori silk fibrils using polarized two-dimensional infrared spectroscopy

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Context and Objective: The mechanical properties of multi-scale hierarchical biomaterials are dictated by the molecular properties of their building blocks that self-assemble to form ordered hierarchically supramolecular structures. Among the different proteins that act as biomolecular building blocks, silk proteins are among the most extensively studied and have become a model biopolymer system, as a result of their accessibility for research and outstanding mechanical and biocompatible properties. Silk proteins self-associate to form hetero-nano composite and highly hierarchical supramolecular fibrils. These fibrils are composed of ordered nano-crystals embedded in disordered amorphous regions. Although there is a clear understanding of the adopted conformation in the nano-crystals, the molecular arrangement in the amorphous regions is still debated, principally due to the fact that most structure-sensitive techniques identify these regions only by omission, or samples require harsh treatments that can strongly affect and change the protein structure. Here, we apply two-dimensional infrared (2D-IR) spectroscopy to investigate *in situ* the secondary structures present in films produced using unpurified fibroin from *Bombyx mori* silkworms.

Methods: Films were prepared using the native silk feedstock (NSF) from the middle-posterior (MP) sections of silk glands from commercially reared *B. mori* silkworms (four-way poly-hybrid cross of two Japanese and two Chinese strains) in their 5th instar. We use conventional and 2D-IR spectroscopy to identify and analyse the present secondary structures in dry condition and after exposure to high humidity.

Results and Conclusions: We show that we can use 2D-IR to study unpurified samples of silk produced by *Bombyx mori* caterpillars by using thin film samples. We demonstrate that (1) we

can disentangle the 2D-IR spectra of unpurified silkworm films, resolving the presence of definite vibrational bands, and, in case of β-sheet and α-helix,(2) assign the vibrational bands to specific secondary structures and (3) determine their relative change when exposing silkworm film to humidity without further data analysis. We thus prove the potential of 2DIR spectroscopy as a tool to study the molecular architecture and interactions in natural biomaterials in a unique, label-free and not-invasive manner.

Supervised learning of infrared spectral images for the diagnosis of different types of breast cancer

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Context and Objective: Breast cancer is the most frequent cancer and the main cause of cancer-related death among women. However, several breast cancer types exist with a specific therapeutic management. The diagnosis of the breast cancer type is thus of primary importance. The purpose of this study is to investigate how mi-infrared imaging, machine learning, and eventually deep learning can assist in the diagnosis of 4 types of breast cancer, namely human epidermal growth factor receptor 2 positive (HER2+), luminal A (LumA), luminal B (LumB) and triple negative (TN) breast cancers.

Methods: For each cancer type, ten breast tumor biopsies were retained coming from ten different patients who received curative surgery without neoadjuvant chemotherapy or adjuvant radiotherapy. Two consecutive 10 µm thick slices were prepared from each biopsy one for conventional HE histological analysis and one for mid-infrared spectral imaging. The acquired images were corrected from atmospheric water vapor and CO_2 contributions, and preprocessed by Extended Multiplicative Signal Correction. K-Means clustering was applied to detect the various tissue structures. After annotation of this database by an anatomo-pathologist, two different random forest supervised classifiers were trained: i) one to identify tissue structures and ii) one to discriminate between the five forms of breast cancer.

Results and Conclusions: Using a training-validation-test methodology, the different tissue structures of the breast cancer sections were identified with an accuracy of 97%. Using a 8 patients-out cross-validation on the class of tumor cells identified by the previous classifier, the tumor cell type was identified with an accuracy of 65%. This last result will be improved by correcting the highly variable lipid signal and by selecting regions of interest containing more tumor cells.

FTIR spectral imaging analysis of cirrhosis development in two murine models

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Context and Objective: Most chronic liver diseases are characterized by the progressive development of hepatic fibrosis in response to various aggressive agents. The wound healing process of fibrosis is mainly driven by inflammatory reaction to hepatocyte injury and leads to the constitution of cirrhosis. The aim of our study was to analyze the different steps of hepatic abnormalities from healthy to cirrhotic liver using Fourier Transform Infrared (FTIR) spectral histology on two mouse models of cirrhosis to uncover markers of the disease progression.

Methods: The study was performed on murine liver samples from the STAM™ mouse model of metabolic cirrhosis and from the CCl_4-induced cirrhosis mouse model. For the STAM™ model, mice were injected with streptozotocin 2 days post-natal and then fed a high-fat diet causing both diabetes and a metabolic syndrome [1]. Animals were sacrificed at multiple time points mimicking human pathology: 4 weeks (healthy liver), 6 weeks (steatosis), 8 weeks (steatohepatitis), 12 weeks (fibrosis), and 16 weeks (cirrhosis). Concerning the CCl_4 model, 7 weeks old mice received CCl_4 intra-peritoneally twice a week. Mice were sacrificed before CCl_4 injection (T0) and then, after 7, 14, 21, and 28 days of exposure. Liver samples were purchased as paraffin-embedded preparations from SMC Laboratories, Inc (Tokyo). For each time point, several adjacent 3 µm thick sections were obtained. These sections were deposited on glass slides for histological examinations and on calcium fluoride windows for spectral histology. FTIR imaging of liver sections was performed at a projected pixel size of 25 µm × 25 µm. Spectral images were pre-processed with EMSC for digital dewaxing and analyzed with k-means clustering on the 1000-1350 cm^{-1} spectral range.

Results and Conclusions: In both models, histological images underlined the progressive appearance of cirrhosis. Analysis of spectral images by k-means clustering with 2 and 4 clusters differentiated the early and late stages of the pathology *via* characteristic peaks of glycogen (1024, 1081, 1154 cm^{-1}) [2] and nucleic acids (1055, 1124, 1166 cm^{-1}) [3] in the two models. The clusters associated with a higher glycogen and lower nucleic acid absorbances were predominant in the early stages of the disease and gradually decreased up to the stage of cirrhosis. In contrast, the proportion of clusters associated with higher nucleic acid and lower glycogen absorbances gradually increased during the development of the disease and were predominant at the cirrhosis stage. FTIR spectral histology demonstrates that the depletion of glycogen in hepatocytes is not only observed at the final step of cirrhosis as previously reported but is already present at earlier stages. The increase in nucleic acid content in the liver appears also as a progressive pathogenic mechanism during the development of cirrhosis, which may be related to increased hepatocyte proliferation and polyploidy in response to hepatocyte

injury. These findings provide a longitudinal insight into the pathogenesis of cirrhosis regardless of the origin of liver damage.

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Vibrational spectroscopies and microscopies: a tool to study and identify neurodegenerative diseases

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Context and Objective: Neurodegenerative diseases are a major challenge for society. Previously it was shown that infrared (IR) and Raman microscopies can visualize the modification in a diseased tissue and contribute to the understanding of diseases, like for example the aggregation processes in Alzheimer disease. [1] Here we focus on the diagnosis based on IR spectra of sera.

Methods: IR spectra were analyzed in combination with a machine learning algorithm with the aim of distinguishing the IR signatures of sera of a first episode of Neuromyelitis optica spectrum disorder (NMOSD) from those of a first episode of relapsing-remitting (multiple sclerosis) MS, as well as from those of healthy subjects and patients with chronic inflammatory demyelinating polyneuropathy.[2]

Results and Conclusions: Our results showed that NMOSD patients were distinguished from MS patients and healthy subjects with a sensitivity of 100% and a specificity of 100%. We also discuss the distinction between the different NMOSD serostatuses. The coupling of infrared spectroscopy of sera to machine learning is a promising cost-effective, rapid and reliable differential diagnosis tool capable of helping to gain valuable time in patients' treatment

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Infrared Spectroscopy in Tandem with Machine Learning for Simultaneous Rapid Identification of Bacteria Isolated Directly from Patients' Urine Samples and Determination of Their Susceptibility to Antibiotics

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Context and Objective: — Urinary tract infections (UTIs) are considered to be the most common bacterial infections worldwide, which are caused mainly by *Escherichia* (*E.*) *coli* (about 80%), *Klebsiella pneumoniae* (about 10%) and *Pseudomonas aeruginosa* (about 6%). Although antibiotics are considered as the most effective treatment for bacterial infectious diseases, unfortunately, most of the bacteria already have developed resistance to the majority of the commonly available antibiotics. Therefore, it is crucial to identify the infecting bacteria and to determine its susceptibility to antibiotics for prescribing effective treatment. Classical methods are time-consuming, require ~48 hours for determining bacterial susceptibility. Thus, it is highly urgent to develop a new method that can significantly reduce the time required for determining both infecting bacterium at the species level and diagnosing its susceptibility to antibiotics. Many studies have proven that FTIR can detect and monitor the minute molecular and biochemical changes in biological samples. The main goal of this study is to examine the potential of FTIR spectroscopy, in tandem with machine learning algorithms, to identify the infecting bacteria at the species level and to determine *E. coli* susceptibility to different antibiotics directly from patients' urine in about 30 minutes.

Methods: 1600 different *E. coli* isolates were isolated from different patients' urine sample, measured by FTIR and analyzed using different machine learning algorithms like Random Forest, XGBoost and CNN.

Results and Conclusions: Based on our analysis, it is possible to identify the infecting bacteria with 96% accuracy and to determine its susceptibility to different antibiotics with 86% accuracy within **3**0 minutes after the collection of the patient urine sample.

Reduction of acquisition time in Fourier transform infrared spectroscopy by deep learning

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Context and Objective: In Fourier transform infrared (FTIR) spectroscopy applied to biomedical challenges, data quality is of primary importance to achieve clinical objectives. However, different noise sources affect the infrared signal coming from the sample. Generally, the number of scans per pixel is fixed to a high value in order to ensure a high signal to noise ratio. However, higher the number of scans, higher the acquisition time, particularly for the acquisition of IR spectral images with an increase of the acquisition time of several hours which is incompatible with clinical practice. The objective of this work is therefore to use deep learning techniques to efficiently reconstruct an IR spectrum acquired with a long acquisition time from an IR spectrum registered with a short acquisition time.

Materials and Methods: IR spectral images were recorded from 9 formalin-fixed paraffin-embedded tissue sections with a 10 µm thickness coming from biopsies from 9 different renal graft recipients. For each tissue section, the same region of interest was imaged first with 1 scan corresponding to very noisy spectra, and second with 64 scans corresponding to high-quality spectra. To predict high-quality spectra from noisy spectra, we tested two different deep learning architectures described in the literature to denoise data, i.e. autoencoder and ResUNet. We also tested three different configurations for each architecture, i.e. multilayer perceptron, 1D-CNN and 2D-CNN. In a first experiment, these different architectures and configurations were trained on 1-scan spectra in order to predict 64-scan spectra in order to learn to remove real noise. To make the procedure adaptable to different acquisition times and thus to different noise levels, we added Gaussian noise on the spectra of the 64-scan images with a random multiplicative factor in a second experiment.

Results and Conclusions: The results of the different models are promising with accurate denoising and reconstruction of IR spectra. The PMC-based architectures perform better with the amount of data currently available. The perspective of this study is to investigate if deep learning can also improve spectral and spatial resolutions to gain in acquisition time to make the treatment compatible with clinical routine.

Analytical performance of Raman spectroscopy for quantification of active ingredients in Human *stratum corneum*

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Abstract: Confocal Raman Microscopy (CRM) has become a versatile technique that can be applied routinely to monitor skin penetration of active molecules. In the present study, CRM coupled to multivariate analysis (namely PLSR—partial least squares regression) is used for the quantitative measurement of an active ingredient (AI) applied to isolated (*ex vivo*) human *stratum corneum* (SC), using systematically varied doses of resorcinol, as model compound, and the performance is quantified according to key figures of merit defined by regulatory bodies (ICH, FDA, and EMA). A methodology is thus demonstrated to establish the limit of detection (LOD), precision, accuracy, sensitivity (SEN), and selectivity (SEL) of the technique, and the performance according to these key figures of merit is compared to that of similar established methodologies, based on studies available in literature. Principal Components Analysis (PCA) and ratios calculated from the area under the curve (AUC) of characteristic resorcinol and proteins/lipids bands (1400–1500 cm^{-1}) were used to examine the data variability and model linearity, respectively. The AUC results show clearly that the intensities of Raman features in the spectra collected are linearly correlated to resorcinol concentrations in the SC (R^2 = 0.999) despite a heterogeneity in the distribution of the active molecule in the samples. Then, a cross-validated PLSR analysis was applied to perform quantitative analysis in the fingerprint region. The Root Mean Square Error of Cross-Validation (RMSECV) (0.017 mg resorcinol/mg SC), The Root Mean Square of Prediction (RMSEP) (0.015 mg resorcinol/mg SC), and R^2 (0.971) demonstrate the reliability of the linear regression constructed, enabling accurate quantification of resorcinol. Furthermore, the results have enabled the determination, for the first time, of numerical criteria to estimate analytical performances of CRM, including LOD, precision using bias corrected mean square error prediction (BCMSEP), sensitivity, and selectivity, for quantification of the performance of the analytical technique. This is one step further towards demonstrating that Raman spectroscopy complies with international guidelines and to establishing the technique as a reference and approved tool for permeation studies.

Surface-enhanced Raman spectroscopy of biologically important molecules on V_2O_5 nanoparticle films

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Context and Objective: Development of new approaches for the synthesis of vanadium oxide (V_2O_5) nanoparticle films based on gas aggregation sources. Study of the impact of process parameters on properties of produced nanomaterials and their applicability for surface-enhanced Raman scattering (SERS) spectroscopy of biologically important molecules.

Methods: V_2O_5 nanoparticles on a silicon wafer were prepared by a magnetron-based gas aggregation source. Vanadium nanoparticle films were subsequently transformed into vanadium pentoxide ones by annealing in air following the same protocol as in [1]. Solution of studied analyte was dropped to the surface, dried in air and SERS spectra were measured using confocal Raman system.

Results and Conclusions: SERS spectra of biologically important molecules including methylene blue, crystal violet, and triphenylphosphine oxide have been obtained. Spectral detection limits were determined as $5x10^{-8}$ M, $1x10^{-6}$ M, $4x10^{-6}$ M for methylene blue, crystal violet, and triphenylphosphine oxide, respectively. Spectral mapping over the surface proved excellent spectral reproducibility (RSD less than 10%). Enhancement factor about 10^5-10^6 can be explained by charge transfer mechanism.

Reference(s): [1] A. Hanková et al., Surf. Coat Technol. 431 (2022) 128015.

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Artificial Neural Network and Support Vector Machine Regression for Forensic Age Determination Using Raman Spectra of Teeth

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Context and Objective: Age determination is a common procedure in investigating human remains. There are different age determination techniques available today, however, they are either subjective and based on the investigator's experience, not accurate enough, or expensive and time-consuming. Teeth are the most lasting tissue of the human body and are usually found undamaged. Raman spectrometry of teeth offers non-destructive analysis without any previous sample preparation and has clear advantages for forensic purposes.

Methods: For this study, a sample of 71 teeth was used from donors aged between 11 and 76 years. Raman spectra were recorded using FT-Raman accessory with a 1064 nm laser on three distinct spots on the tooth, crown, neck and apex. Spectra were analyzed using principal component regression (PCR), support vector machine (SVM), and artificial neural network (ANN).

Results and Conclusions: This study showed that age determination can be achieved using regression techniques and that the result of classification models depends on both, the recording site on the tooth and the technique used. Models built with ANN and SVM, especially when built with spectra recorded on the apex, showed better results than those obtained using PCR. The coefficient of correlation, R^2, varies with the number of PCs used, which suggests that optimizing the selection of PCs used might improve the final classification accuracy of the model. Difference between male and female teeth occurs regardless of the technique used.

Infrared s-SNOM imaging of surface adhesive polydopamine layers formed on various substrates

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Context and Objective: Polydopamine (PDA) is a common and universal biopolymer for the preparation of protective adhesive layers for biomedical and tribological applications. The main benefits of using PDA for the listed purposes are the high speed and low cost of the deposition process and its ability to form adhesive layers on almost any object. To improve the properties of prepared PDA layers, it is crucial to describe their morphology and physicochemical properties at the nanoscale level. It is expected that these properties somewhat depend on the chosen substrate material and the deposition time of PDA. Our goal was to investigate these hypotheses using chemical imaging with subdiffractional spatial resolution.

Methods: For this purpose, we have prepared samples of PDA layers using three different substrates (gold, silicon with natural silicon dioxide layer and nitrogen-doped titanium oxide) and several different times of PDA deposition. The prepared samples were subsequently analyzed using a scattering-type scanning near-field optical microscope utilizing four excitation energies in the mid-infrared region to detect the chemical contrast originating from vibrational modes of selected chemical moieties.

Results and Conclusions: It was found that the polymerization process leads to a formation of a surface confluent PDA layer as well as deposition of some PDA nanoaggregates. The number and size of nanoaggregates were increasing with the deposition time for all studied substrates. The observed optical contrast at energies corresponding to the vibrations of indole units of PDA and quinoid groups of polymerization intermediates also indicated a slightly different composition of the nanoaggregates and the surrounding confluent layer.

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Combining Vibrational Spectroscopy, Metabolomics and Proteomics – Comprehensive Analysis of Blood Plasma for Clinical Diagnostics

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Context and Objective: Blood plasma and serum are the preferred materials in many clinical studies and applications. As blood represents the key medium for molecular transport and is in direct contact with tissues, it may exhibit disruptions in molecular composition under pathological conditions. Moreover, it is collected via a routine and almost non-invasive procedure, which makes it convenient for repeated sampling or screening of the general population. Our goal is to design a complex methodology that could uncover the disease-induced changes in blood plasma on different molecular levels early on. To do so, we combine various sample pre-processing steps and analytical methods, each targeting different components of blood plasma.

Methods: The developed process was primarily tested on the blood plasma samples of patients suffering from hepatocellular carcinoma, patients with liver cirrhosis and healthy controls. To obtain different fractions of the sample, we employed a combination of physical and chemical separation methods. Subsequently, FTIR and Raman vibrational spectroscopies were used together with NMR spectroscopy, LC-ESI-Q-TOF and MALDI-TOF-MS to analyse the low- and high-molecular-weight blood plasma fractions as well as extracted plasma lipids and proteins.

Results and Conclusions: The developed methodology for the analysis of blood plasma using a diverse combination of analytical methods and various pre-processing steps significantly increases the amount of information obtained from the blood plasma sample. The diagnostic power of this approach will be evaluated in the future by analysing a series of blood plasma samples and developing complex statistical models to differentiate the studied diseases.

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Photoswitching of Triptycene-Based Molecular Machines Followed by Raman Spectroscopy

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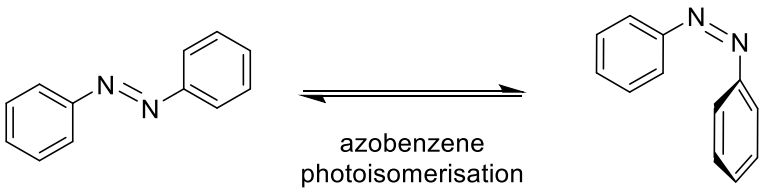
Light-powered molecular machines, particularly once organized into regular 2D structures, are attracting great scientific interest due to their potential applications ranging from information processing, nanoelectromechanical systems, energy, biology, to medicine. The photoswitches are a type of molecules that can change its structural geometry and chemical properties upon irradiation with electromagnetic radiation. For example, photoisomerization of double bonds in the molecule lead to changes in the *cis*- or *trans*- configuration (**Scheme 1**).

Recently we characterized triptycene-based molecular rods prepared using Langmuir−Blodgett (LB) technique on a gold (111) surface. As the next step, photoswitchable molecular machines were added on the surface of these molecular rods. Here we compare their isotropic Raman spectra in solution with anisotropic spectra of LB monolayers as well as their ability to photoswitch in both states. The experimental results were in a good agreement with extensive Density-functional theory (DFT) calculations.



Scheme 1 : Photoisomerisation of azobenzene

Reference(s): E. Kaletová, C. Santos Hurtado, I. Císařová, S. J. Teat, J. Kaleta - ChemPlusChem 87 (4): e202200023 (2022)

Raman-based Detection of Antibiotics in Pharmaceutical Formulations and Biological Matrices

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Context and Objective: Raman spectroscopy is a powerful tool for bioanalytical detection methods due to its molecular specific fingerprint information. The limitation associated with the small Raman cross section can be overcome by the application of powerful plasmonic-active nanostructured sensing surfaces, i.e. by means of surface-enhanced Raman spectroscopy (SERS). Thus, analyte molecules in the µM range or lower can be detected with high specificity even in complex biological matrices.

Methods: For the characterization of the optical parameters of SERS-active nanostructures, UV-VIS absorption spectroscopy is applied. To record the vibrational fingerprint information of the target analytes, Raman as well as SERS spectroscopy is used.

Results and Conclusions: A Raman analytical method was developed to detect the antibiotic ciprofloxacin (CIP) in various pharmaceutical formulations. With low Raman background contribution of excipient and carrier substances, the quantification of CIP occurs directly Raman-based without any sample preparation. A high Raman background of the formulation itself impedes a direct detection of CIP. Therefore, a routine was developed, i.e. sample dilution with water 1:5000 and application of SERS-active nanosensors, to allow for quantification of CIP in complex pharmaceutical formulations. [1] Moreover, as a second example, pyrazinoic acid (POA), the metabolite of the tuberculosis drug pyrazine amide (PZA), is detected in sputum culture supernatants by using specially designed gold nanoparticles coated with Prussian blue as SERS sensor. The results illustrate the potential of the proposed detection scheme for an assessment of the resistance of *M. tuberculosis* in cultures as only sensitive strains allow the metabolism from PZA to POA.

Reference(s): [1] C. Liu, L. Müller-Bötticher, C. Liu, J. Popp, D. Fischer, D. Cialla-May, Raman-based detection of ciprofloxacin and its degradation in pharmaceutical formulations, Talanta (2022) 123719, doi.org/10.1016/j.talanta.2022.123719.

Characterization of Bone-Implant Interface after Osseodensification by Infrared Imaging: Development of an Experimental Model

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Context and Objective: The long-term success of dental implants relies on the quality of the osseointegration and the structural and functional bonds at the bone-implant interface. It can be improved by new surgical techniques such as bone osseodensification. The aim of this study was to use infrared spectroscopy and compare two acquisition modes, transmission and reflection, to simultaneously explore the organic and mineral composition of the bone interface.

Methods: One bone site was prepared with a Densah® kit and received a Bego implant (titanium, Ø 3.75 mm x 10 mm long, Ref 58166). Measurements were performed on a 19 µm thick implanted bone section, on a bone pellet from pig femoral head and also on an hydroxyapatite pellet. The spectral regions were recorded in both transmission and reflection modes on the 4000-650 cm^{-1} spectral range, with a pixel resolution of 6.25 µm, a spectral resolution of 4 cm^{-1} and 128 scans/pixel.

Results and Conclusions: Independently of the acquisition mode, the specific mineral and organic spectral information of the bone matrix could be characterised. The transmission spectra were free from physical artefacts but bone sections need to be thinner. In the reflection mode, it was important to have a proper adhesion of the section to its support in order to obtain a spectrum without fringes. Finally, the resin peaks overlapped with specific bone bands and hindered quantitative measurements. Overcoming these difficulties should make these methods amenable for a better understanding of the osseointegration process.

ATR-FTIR spectroscopy of calcium-dependent lipid-binding proteins

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Context and Objective: Arabidopsis ALG-2-INTERACTING PROTEIN X (ALIX) is a cytoplasmic protein and is responsible for endocytic degradation of ubiquitinated cargos to the late endosomes. However, recently a novel interactor of ALIX, calcium-dependent lipid-binding protein (CaLB) was identified. CaLB is a C2 domain-containing protein that binds in the presence of calcium to phosphatidylinositol 3-phosphate (PI3P) phospholipid, which may occur at the late endosomes and on autophagosomes. The study of CaLB is therefore of significant relevance as seems that it might be involved in autophagy.[2]

Methods: In this study, attenuated total reflection (ATR)-Fourier-transform infrared (FTIR) spectroscopy was used to study protein-membrane interactions.[3] Herein, ATR-FTIR methods provide continuous monitoring of protein-membrane interactions of solid-supported lipid membrane (SSLB) and lipid vesicles of different lipid compositions and sizes.

Results and Conclusions: It was seen that there was an increase in binding of CaLB to a POPC/PI3P 99:1 bilayer with increasing calcium concentration over 2 hours. Furthermore, a lipid-binding specificity of CaLB to POPC/PI3P bilayer in the presence of 20 µM calcium was observed with less binding specificity to a POPC/PI4P bilayer control. This suggests that CaLB binds to specific membranes and that the binding is calcium-dependent.

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Bacteria localization in hematogenous osteomyelitis using fluorescence and Raman imaging

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Context and Objective: Hematogenous osteomyelitis by *S. aureus*, leads to bone deformation and pain if not treated. Knowledge of bacterial localization at different stages of osteomyelitis is important for treatment development, however not well understood yet. The objective of our study is to localize *S. aureus* in *ex vivo* bone tissues of a mouse model, using fluorescence microscopy, and label-free Raman spectroscopy and imaging.

Methods: Using a chronic hematogenous osteomyelitis mouse model [1], deformation in the left pelvis was observed with X-ray. After sacrification, bone isolation, and decalcification, 100 µm thick bone cross-sections were prepared for fluorescence imaging with *S. aureus*-specific antibodies, measured in a confocal laser scanning microscope. For Raman spectroscopic imaging, an adjoining cross-section of similar thickness was used.

Results and Conclusions: Imaging methods revealed the manifold location of *S. aureus* in the bone tissue. An abscess filled with *S. aureus* was detected in the bone marrow in the fluorescence images. Individual bacteria were also found in hard bone and surroundings and were quantified in 3D fluorescence image stacks. In an adjacent tissue slide, bacteria could be visualized in high-quality false-color Raman images without the need for extra staining using spectral unmixing techniques.

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Dual-comb-IR-spectroscopy to study temperature-jump dynamics of polyQ model peptides

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Context and Objective:

Glutamine (Q) rich proteins tend to aggregate and form fibrils. These aggregates can cause neurodegenerative polyQ diseases like Huntington disease. In our research we study the folding dynamics of Trpzip-Q_n peptides with time-resolved IR-spectroscopy at single wavelengths after a nanosecond laser-induced temperature-jump (1). The further development of dual-comb quantum cascade lasers (QCLs) allows measurements with microsecond resolution over a spectral range of 60 cm^{-1} (2). This facilitates the possibility of measuring and analyzing spectral changes in several interesting regions of the spectra at the same time.

Methods:

A Q-switched Ho:YAG laser (IPG Photonics, U.S.A.) at 2090 nm induces a temperature-jump of 5-10 K by exciting the overtone vibration of the solvent D_2O. For single wavelength measurements, a home-built spectrometer with tunable QCLs of a MIRcat-QT laser system (Daylight Solutions Inc. U.S.A.) and an MCT-detector (KMPV11-1-J2, Kolmar Technologies, U.S.A.) are already established. For dual-comb measurements, the beam of an IRis F1 dual-comb-spectrometer (IRsweep, Switzerland) is coupled into the sample cell of the IR-spectrometer.

Results and Conclusions:

With the dual-comb approach, time resolved spectra are available in a short time. We get comprehensive insights into structural dynamics by analyzing characteristic amide I' modes in the same measurement. The impact of individual glutamines on structure and dynamics can be explored.

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DETECTION OF RADIOSENSITIVE SUBPOPULATIONS EX-VIVO VIA RAMAN MICROSPECTROSCOPY OF LYMPHOCYTES

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Context and Objective

Although significant advances in understanding the molecular drivers of acquired and inherited radiosensitivity have occurred in recent decades, a single analytical method which can detect and classify radiosensitivity remains elusive. Raman microspectroscopy has demonstrated capabilities in the objective classification of various diseases, and more recently in the detection and modelling of radiobiological effect. In this study, Raman spectroscopy is presented as a potential tool for the detection of radiosensitivity, and within a mixed cohort of lymphoblastoid cell lines (from suffers of ataxia telangiectasia (N=2), non-Hodgkins lymphoma, and Turner's syndrome (N=2)) and lymphocytes from both healthy controls (N=23), and prostate cancer patients (N=19).

Methods

Spectroscopic measurements were made ex-vivo both before and after exposure to photonic ionizing radiation, in parallel to measurements of DNA damage, DNA damage sensing and intrinsic radiosensitivity. Ground-truth classifications of radiosensitivity were established on the basis of radiation-induced G2 scores.

Results and Conclusions

Support vector machine models developed on the basis of the spectral data were capable of discrimination of radiosensitive populations before and after irradiation, with superior discrimination when spectra were subjected to a non-linear dimensionality reduction (UMAP) as opposed to a linear (PCA) approach. Overall this study suggests that Raman spectroscopy may have potential as a tool for the detection of intrinsic radiosensitivity using liquid biopsies.

Detection of Aβ 1-42 aggregates by RT-FAST : toward a new tool for the diagnostic of Alzheimer's disease

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Context and Objective: The self-assembly of the misfolded Aβ 1-42 peptide is involved in Alzheimer's disease (AD). The oligomeric species formed at an early stage of aggregation are considered as one of the main biomarkers of the disease. Nowadays, no efficient tools are available to detect and size them due to their transient nature and their low concentration (fM) in biofluids such as the cerebro-spinal fluid (CSF). Therefore, our goal is to propose an approach to detect these biomarkers (oligomers) directly in the CSF.

Methods: Recently, we developed an innovative method to detect preformed α-synuclein oligomers called Real-Time Fast Amyloid Seeding and Translocation (RT-FAST)[1]. The principle is based on the properties of preformed oligomers to promote the aggregation of the monomer providing large aggregate faster than the control condition (without oligomers). These newly formed aggregates are detected using a single nanopore by resistive pulse sensing. The latter consists to measure transient ionic current blockages under constant voltage due to the passage of an aggregate. The main interest of the approach is to use a nanopipette as vial allowing accelerating the aggregation (reservoir about 70 µL) and nanopore sensor.

Results and Conclusions: Experimentally, current blockades are observed after 120 minutes of aggregation only for the sample containing the preformed Aβ 1-42 oligomers in the buffer. Further analysis of the current blockade amplitudes allows estimating a volume in the order 10^5 nm^3 using a geometrical model. Interestingly, in presence of CSF same trend is observed. However, the blockades are detected after 240 minutes of aggregation. This can be explained by the fact that the molecules present in CSF slow down the aggregation. In addition, the proteins of CSF decrease the adsorption constant of the Aβ known to accelerate the aggregation.

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Assessment of Ovarian Tumor Growth in Wild-Type and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry

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Context and Objective: Ovarian cancer remains one of the most fatal cancers because of lack of robust screening methods of detection at early stages. Extracellular matrix (ECM) mediates interactions between cancer cells and their microenvironment *via* specific molecules. Lumican, a small leucine rich proteoglycan (SLRP), maintains ECM integrity and inhibits both melanoma primary tumor development and metastatic spreading. The aim of this study was to analyze the effect of lumican on tumor growth of murine ovarian epithelial carcinoma.

Methods: C57BL/6 wild-type mice (n=12) and lumican-deficient mice (n=10) were subcutaneously injected with murine ovarian epithelial carcinoma ID8 cells and sacrificed after 18 days. The ovarian primary tumors were subjected to histological and immunohistochemical staining using anti-lumican, anti-αv integrin, anti-CD31 and anti-cyclin D1 antibodies and further examined by label-free infrared spectral imaging (IRSI), second harmonic generation (SHG) and Picrosirius Red staining.

Results and Conclusions: Analysis of tumor volumes demonstrated an inhibitory effect of endogenous lumican on ovarian tumor growth. The IR tissue images identified different ECM tissue regions of the skin and the ovarian tumor. Moreover, IRSI showed a good correlation of αv integrin immunostaining and collagen organization within the tumor. Our results demonstrate for the first time that lumican inhibits the growth of ovarian cancer mainly by altering collagen organization and distribution.

Controllable deposition of gold nanoparticles using a one-step centrifugation process and its application for SERS

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Context and Objective: The deposition of gold nanoparticles (AuNPs) on silicon is important for the design of sensors and substrates for Surface Enhanced Raman Spectroscopy (SERS) and consistency in the deposition step is critical to ensure the reproducibility of the results. Herein, we report a method for deposition of hydroquinone-synthesized AuNPs (HQ-AuNPs) of various sizes onto silicon wafers by a one-step centrifugation method.

Methods: The protocol consists of centrifugation of an HQ-AuNPs sol in a microcentrifuge tube containing a silicon wafer oriented perpendicularly to the centrifugal field, using a fixed-angle rotor. By varying the ionic strength of the AuNP sol and the concentration of particles, it is possible to achieve variable, homogeneous surface distributions of particles, reaching surface coverages of 28%.

Results and Conclusions: This work reports a simple, reproducible and time-effective strategy for controlled AuNP deposition onto silicon substrates, with applications in SERS. The deposited AuNP films modify the surface reflectance, depending on particle density and aggregation, and display activity as SERS substrates.

Vibrational spectroscopy applied on Biofluids: infrared spectroscopy for Bladder cancer diagnosis using urine samples

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Context and Objective: Diagnosis and monitoring of bladder cancer (BCa) are mainly based on cystoscopy, which is invasive, high-cost and patient discomfort. We attempt to detect BCa non-invasively by a combination of Fourier Transform Infrared Spectroscopy (FTIR) technique with machine learning algorithms using urine as proximal biofluid of tumor site.

Methods: Random urine samples of 50 BCa patients and 53 healthy volunteers were collected from Urology department (Reims University Hospital) and analyzed by high-throughput FTIR. In total 295 spectra were included in the study. After specific chemometric processing, Support Vector Machines (SVM) and Random Forest methods were used to construct models in order to develop a binary classification to distinguish between BCa and normal control group. clinico-biological characteristics of the two groups were also collected.

Results and Conclusions: First, patients with glycosuria upper than 20mM were excluded to avoid a bias in our classification, due to spectral interferences of sugar bands. Then, using total urine, both models had the same specificity (64%), whereas the sensitivity of SVM (75.5%) was better than the Random Forest method (66%). These results can be explained by the existence of high intra and inter variability in urine samples and the lack of sensitivity of FTIR technique. The obtained results are insufficient to include our method in BCa diagnosis strategy but they show that infrared spectroscopy may provide a discriminative information. To improve specificity of our method, we are optimizing the sample preparation with adding a centrifugation step in our sample preparation protocol to separate sediment and supernatant of urine samples. The use of urinary extracellular vesicles will be also investigated.

Identification of circulating biomarkers of Crohn's disease and spondyloarthritis using FTIR spectroscopy

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Context and Objective: Crohn's disease (CD) and spondyloarthritis (SpA) are two inflammatory diseases with common genetic predispositions. CD mainly affects the digestive tract while SpA attacks the joints. They cause many symptoms that complicate the lives of patients. In these diseases, there is a lack of diagnostic markers of certainty because their identification is not easy. While the diagnosis of CD is made by a combination of clinical, biological and endoscopic criteria, the diagnosis of SpA may take several years to be confirmed. Based on the hypothesis that SpA and CD alter the biochemical profile of plasma, the objective of this study was to evaluate the analytical capability of Fourier transform infrared spectroscopy (FTIR) in identifying diagnostic signatures in plasma for these chronic inflammatory diseases, as well as biomarkers associated with both conditions.

Methods: Plasma from 104 patients (control, CD, spondyloarthritis) was collected and analyzed by FTIR. After processing the spectra obtained, EMSC (Extended multiplicative signal correction) and LDA (linear discriminant analysis) were used. We studied the discriminating vibrations in order to identify characteristic markers of these pathologies. The data processing was done using Python.

Results and Conclusions: We demonstrated that it was possible to distinguish CD and SpA from controls with an accuracy of 90% and 76% respectively. We have highlighted intensity differences in the spectral regions corresponding mainly to glycogen and lipids. Our promising results showed that FTIR analysis is efficient to identify plasma biosignatures specific, on the one hand to CD, on the other hand to SpA, and also common to both pathologies. The next step would be to carry out this same study on a larger number of patients in order to validate a possible clinical application.

Analytical quality control of therapeutic mAbs preparations by Raman spectroscopy

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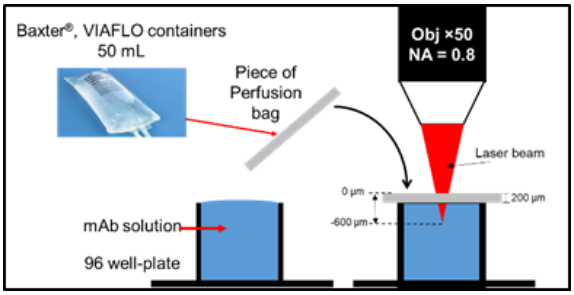

***Figure1**: in situ like experimental setup for CRS*

		Training (SET_01 /SET_02)			Test (SET_03)	
	mAb	RMSECV (g/L)	R^2	LVs	RMSEP (g/L)	R^2
Raman	TRS	0.3338	0.9964	6	0.4490	0.9962
	BVC	0.2578	0.9989	6	0.5147	0.9989
	ATZ	0.4579	0.9968	6	0.4828	0.9948

***Table1**: PLSR results obtained from CRS analysis*

Context and Objective: Centralization of preparation and reconstitution of injectable solutions of anticancer drugs like monoclonal antibodies (mAbs) greatly reduced risks and errors. In order to ensure that patients receive the correct dose prescribed for their treatment, Analytical Quality Control (AQC) has to be performed systematically. Established protocols rely on flow injection analyzer requiring withdrawal of samples from perfusion bags, that potentially generates a risk of sample contamination and of exposing staff members to hazardous compounds. An alternative is to make AQC of mAbs *in situ*, in the perfusion bags, for example by using Confocal Raman spectroscopy (CRS). CRS gives qualitative information of molecules (their structure-specific vibrational signatures) and allows their quantitation. While clinical formulations of mAbs contain high concentrations of various excipients, their spectral signatures are not well documented in the literature.
Here, we investigated the possibility of *in situ* AQC of clinical mAbs formulations by CRS coupled to multivariate analysis protocols, namely to PLSR – Partial Least Square Regression. An *in situ*-like experimental setup (Fig. 1) has been developed to collect CRS data from a range of clinical formulations of three mAbs: Trastuzumab (TRS), Bevacizumab (BVC), and Atezolizumab (ATZ). We evaluated the analytical performances of CRS, namely the precision and the accuracy of quantitative determination, but also the specificity of the analysis performed and the role of excipients in the predictive models constructed.
Methods: Samples were prepared by diluting commercial solutions in 0.9 % NaCl as it done in hospital. Three independent sample sets (identified as SET_01, SET_02 and SET_03) were prepared for each formulation. With each concentration, 400 µL of the solution was used. Typically, 10 spectra per sample were recorded (4 accumulations of 90s each), from different locations on the same depth of 600 µm (below the plastic wall). Data have been pre-processed and analyzed using MATLAB® (Mathworks, USA). Raman spectra were subjected to a Savitzky-Golay smoothing filter (K=6, W=31), followed by a baseline correction (Lieber function, 2nd order polynomial, 10 iterations) and a vector normalization. PLSR analysis was performed using SET_01 and SET_02 for cross validation and SET_03 as the test set.
Results and Conclusions: With PLSR analysis (Table 1), Root Mean Square Error Cross validation, Root Mean Square Error Prediction and R^2 values demonstrated the ability of CRS *in situ* to deliver reliable AQC. However, the regression coefficients highlighted contributions from both proteins and excipients features in the predictive models.

Confocal Raman microspectroscopy as a tool to access the quality of chicken egg

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Context and Objective: The quality of chicken eggs produced for consumption depends on various factors such as the age of the hens and the storage conditions. The present work aimed at investigating molecular mechanisms involved in the variability of egg quality and antibacterial activities using confocal Raman microspectroscopy.

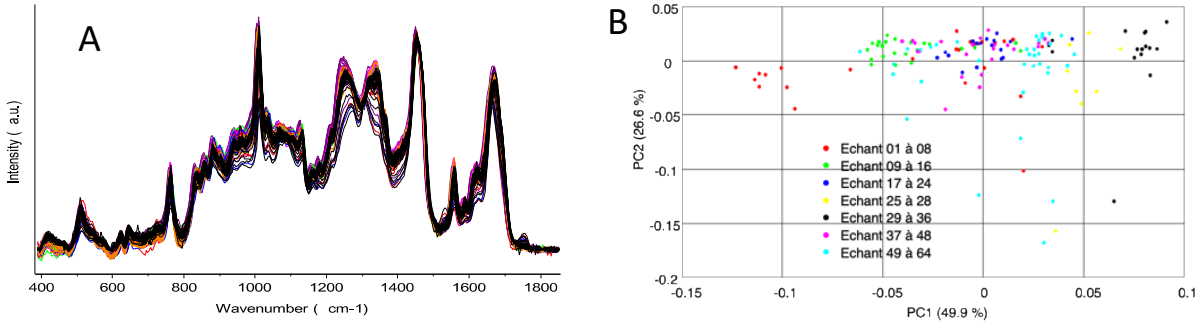

Fig.1. Distinguishing molecular changes of vitelline membranes from Raman spectra. A: Average Raman spectra of 7 groups of samples (64 spectra). B: PCA analysis of the spectral differences between the 7 groups of samples.

Methods: Lohmann Tradition laying hens were set up at the Pôle d'Expérimentation Avicole de Tours (PEAT, INRAE Nouzilly) and reared on the ground for egg production at different times of the laying cycle. The smooth running of the breeding was verified thanks to the regular monitoring of the animals (weight, food consumption, performance and egg quality) throughout the period concerned. The eggs were collected at different ages of hens: 31-34 weeks (peak of lay), 71-74 weeks (end of cycle) and 94-100 weeks (late end of cycle) for the study of the effects of the age of laying hens, and 80-82 weeks for the study of the effects of storing eggs in an atmosphere enriched with CO_2. The eggs produced were stored for 0, 14 and 21 days under controlled conditions, variable in terms of temperature (4 or 20°C), relative humidity (50 or 75-80%) and $C0_2$ (0 or 10%). The samples of vitelline membranes were extracted from fresh eggs (Bregeon et al, 2021), washed in cold water, disposed on CaF_2 substrates and dried at ambient conditions. Raman spectra of the dried vitelline membranes were collected via acquisition of hyperspectral maps (24 x 10 spectra each, 4 maps per sample, 2 maps on each side of the membrane) via an x50 objective of a confocal Raman microspectrometer (LabRam, Horiba Scientific, France) equipped with the 691 nm laser source. The data acquisition was made using a LabSpec software (Horiba Scientific, France). The data have been then pre-processed (baseline correction, vector normalization) and analyzed by PCA (principal component analysis) using MATLAB® (Mathworks, USA).

Results and Conclusions: For each group of samples, selected according to chicken age and storage conditions (duration, temperature and atmosphere composition), Raman spectra (Fig.1) show significant spectral changes. The obtained results confirm the potential of confocal Raman microspectroscopy to reveal the specific features of protein composition/conformation of the vitelline membranes, depending on the egg quality.

Identification and biochemical characterization of breast cancer cells resistant to neoadjuvant treatment by Raman Spectroscopy

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Context and Objective: Breast cancers (BC) of type HER2+ have an aggressive tumor growth and the introduction of neoadjuvant therapy has led to great advances in their treatment. However, up to 40% of HER2+ at a metastatic stage develop resistance and, up to date, the mechanisms underlying such resistance are unknown and there is not a method that predicts which BC will resist specific treatments. Therefore, we propose and test the potential of Raman spectroscopy (RS) to non-invasively stratify resistant and non-resistant cells and obtain relevant chemical information that may unravel their resistance mechanisms [1].

Methods: The enriched HER2+ cell line MDA-MB-453 has been used. We developed HER2-targeted therapies resistant cell lines by continuous drug exposure for 6 months. Living non-resistant wild-type (WT) and resistant cells were measured using RS. Then, the Partial Least Squares - Discriminant Analysis (PLS-DA) method was used to classify cells and the Multivariate Curve Resolution (MCR) algorithm was used to study their chemical content [2].

Results and Conclusions: Excellent classification accuracy was obtained in distinguishing WT from resistant cells. Also, we identified changes in the cellular molecular content between them. Therefore, RS seems a promising objective technique that could potentially help clinicians in their correct treatment selection in clinics due to its non-invasive, relatively fast, and cost-affordable setup.

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Calcium induced vesicular interactions studied with ATR- FTIR spectroscopy

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Context and Objective: Calcium plays an essential role in physiological functions such as membrane protrusion, fusion, foreign body engulfment, cell signaling and motility[1]. When the intra and extra cellular concentration levels are not maintained or fluctuate due to biochemical triggers, a eukaryotic cell can experience cell injury or death[2]. But the singular role of calcium on the membrane is too complex to study in a eukaryotic cell and a better understanding can be gained when stripped down to minimal components.

Methods: Therefore, we generate a minimalistic vesicle model of size ~30-70 µm diameter with electroformation called giant unilamellar vesicles (GUVs) mimicking the cell membrane. The interaction of Ca^{2+} and DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) is studied with attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy at molecular resolution. In order to understand the calcium interactions from within the cell, firstly $CaCl_2$ is encapsulated within the core of the GUVs during production.

Results and Conclusions: Changes occurring in the vibrational modes of the lipid groups show that the Ca^{2+} predominantly interacts with the phosphate head of the lipid molecule and by extension, the alkyl chain undergoes ordering. As the calcium concentration within the GUV increases, IR intensities change indicating dehydration and furthermore that the phosphate head groups accomplish attractive lateral pressure leading to compression. Secondly, the effect of calcium gradient across the membrane is studied by keeping the $CaCl_2$ concentration inside at 500 µM and varying the concentration from 500µM – 10mM outside the GUV. In addition to the effect of compaction from within, vesicle-vesicle interaction occurs as Ca^{2+} ions can bind to the outer leaflets of several vesicles leading to vesicle clustering and it is found that larger calcium gradients induce stronger interactions. We demonstrate that divalent calcium ions cause local and temporal changes to the lipid packing in a biomimetic cell-like model.

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Lineshape analysis of 2D spectra for fifth order spectroscopies: exciton transport, annihilation and spectral diffusion dynamics

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<u>Context and Objective</u>: Fifth order signals (R^5) of coherent nonlinear electronic spectroscopy have been designed to track exciton annihilation dynamics - an essential limit of efficiency for photon harvesting and energy conversion in photosynthetic system. The integrated R^5 signal was shown to report on the annihilation in the idealized case of aligned homo-polymers. As shown in this contribution, analysis of two-dimensional (2D) line shapes allows to extend the analysis to bent structures, and simultaneously track transport and spectral diffusion within the manifold of single and double excited states.

<u>Methods</u>: Experimental: R^5-2D spectroscopy measured on squaraine trimers (as the model of flexible trimer molecular aggregate). Theoretical: Response function theory of nonlinear spectra, (Kubo-Anderson) line-shape theory, quantum master equation description of (Frenkel) exciton transport.

<u>Results and Conclusions:</u> Interplay of annihilation and transport in R^5-2D spectra of model dimers and trimers; role of geometry in integrated R^5 signal [1]. Measurement of R^5-2D spectra on squaraine trimers; identification of peaks representing annihilation dynamics on this model flexible trimer; determination of annihilation rate [2]. Shaping of individual peaks by (bi-exciton) spectral diffusion, characterized by tilt's angle, or center line slopes [3]. The signatures of broad class of bi-exciton dynamics will thus be identified in R^5 -2D line shapes. Implications for characterization of light harvesting systems will be discussed.

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Heterogeneity of human hair medulla lipids, studied by synchrotron µFTIR and OPTIR microspectroscopy.

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Context and Objective: The hair medulla is one of the less studied of the hair compartments due to its small size, fragmented structure and location at the center of the hair fiber. Research to understand its composition have been limited by the restricted array of characterization tools available and by difficulties in extracting its material. The in situ analysis of medullas is an attractive solution to this problem. Due to their small sizes,(<10 µm), high spatial resolution techniques are mandatory to obtain accurate chemical information. Synchrotron Radiation µFTIR (SR-µFTIR) was used to study the chemical composition of 150 individual medullas. Optical photothermal IR (OPTIR) was used to study the spatial heterogeneity of medullas at the submicron scales.

Methods: Thin sections of human hairs were analyzed by SR-µFTIR, Infrared Hyperspectral Imaging (IRHI) and OPTIR. Data were analyzed by difference spectroscopy, spectral histology, and multivariate statistical analysis (PCA, K-means).

Results and Conclusions: While IRHI can be used to measure multiple hairs simultaneously with a high throughput, SR-µFTIR and OPTIR achieve more accurate description of the hair medullas. We developed a protocol to minimize photodamages and photoconversion caused by OPTIR. Thanks to SR-µFTIR, we evidenced the strong, unexpected diversity of the hair medulla spectral signatures, especially their diverse lipid compositions. The cause and biological role of these diverse compositions are currently unknown. We evidenced for the first time the signature of calcium carboxylate and the presence of solid, crystalline lipids in the human hair medulla. While human hair medullas appear mostly homogeneous by SR-µFTIR and IRHI, OPTIR allowed us to reveal their strong spatial heterogeneity at the submicron scale and the presence of micron-sized lipid inclusions. This work demonstrated the usefulness of non-conventional infrared spectroscopy techniques to characterize hair lipids *in situ* and contributed new information on hair lipids.

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ULTRA at Central Laser Facility

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Context and Objective: In this presentation we will give a brief overview of the ULTRA Facility within Central Laser Facility, based at the Rutherford Appleton Laboratories in South Oxfordshire, UK.

ULTRA[a] is multidisciplinary facility dedicated to provide state of the art experimental capabilities to the users from academic community and industry in the areas of life sciences, material sciences, physics, chemistry and others. We specialize in ultrafast time-resolved laser spectroscopy and offer broad range of techniques: Time-Resolved Infrared (TRIR); Visible Transient Absorption (TA); 2-Dimensional Infrared spectroscopy (2DIR); Kerr-gated Raman and Time-Resolved Resonance Raman (TR^3); Femtosecond Stimulated Raman Scattering (FSRS); IR-Raman; Surface Sum-Frequency Generation spectroscopy (SFG). For the 2DIR technique, we can also offer T-jump capability.

Experiments at ULTRA are free of charge at the point of access, and the access is arranged via peer-review process with bids for access twice a year. We typically provide 60 weeks of access per year for academic users (for free of charge access to ULTRA, the PI on the application needs to be based in the UK though).

Find the details how to apply here: https://www.clf.stfc.ac.uk/Pages/Access-to-Octopus-and-Ultra.aspx

Currently ULTRA is running two Ti:Sapphire laser systems and one ytterbium laser system.

In April 2022, we will start a new chapter for ULTRA going through the £17M upgrade project called HiLUX. Within several years HiLUX will boost our capabilities in all the directions: new ytterbium lasers replacing current Ti:Sapphire ones, much higher average power, 10x increase of laser repetition rate, refurbished labs, faster data acquisition, better signal to noise, broader bandwidth and spectral coverage, upgraded sample management etc.

Reference(s): [a] https://www.clf.stfc.ac.uk/Pages/Ultra_Facility.aspx.

The Photoreaction of the Proton-Pumping Rhodopsin 1 from the Maize Pathogen Basidiomycete *Ustilago maydis*

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Context and Objective: Microbial rhodopsins have recently been discovered in pathogenic fungi and have been postulated to be involved in signaling during the course of an infection. Here, we report on the spectroscopic characterization of a light-driven outward proton pump rhodopsin (*Um*Rh1) from the smut pathogen *Ustilago maydis*, the causative agent of tumors in maize plants.

Methods: Site-directed mutagenesis; heterologous expression in yeast *Pichia pastoris*; pumping activity test on whole cells; electrophysiology; Time-resolved UV/Vis Spectroscopy; Fourier-Transform Infrared Spectroscopy; Resonance Raman Spectroscopy;

Results and Conclusions: Time-resolved UV/Vis and vibrational spectroscopy on *Um*Rh1 indicate a pH-dependent photocycle, which is significantly different from the well-known bacteriorhodpsin from archaea (*Hs*BR). We applied site-directed mutagenesis on *Um*Rh1 based on a structural model and sequence alignment, in order to understand this different behavior of proton pumping. A facile pumping activity test was established of *Um*Rh1 expressed in *Pichia pastoris* cells, for probing proton pumping out of the living yeast cells during illumination. We also characterized the impact of the auxin hormone indole-3-acetic acid that was shown to influence the pump activity of *Um*Rh1 on individual photocycle intermediates. We show similarities and distinct differences to *Hs*BR and discuss the putative role of *Um*Rh1 in pathogenesis.

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Radiation-induced continuous effect on the secondary structure of keratin studied by FTIR spectroscopy

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Context and Objective: Sheep wool is a renewable keratin waste and a raw material for composite materials in commercial air purification filtration systems. Modification of the keratin structure may enhance the sorption capacity of sheep wool fibres. In this study we evaluated the irradiation-induced fibre structural changes and their post-exposure sustainability.

Methods: Sheep wool felt (Folder Kids Ltd., Latvia) was irradiated by TBI 4850–150 tote box type gamma irradiator (Scandinavian Clinics Estonia OÜ) to absorbed doses of ~10, 50 and 100 kGy. Control and irradiated fibres were stored for 12 months in dark place, at room temperature, and under normal atmospheric pressure. 10 mg of wool fibres were ground to a fine powder, suspended in 0.8 ml of dH_2O and 5µl of suspension dried on a 384 well silicon microplate. FTIR spectra were recorded on Vertex 70 coupled with HTS-XT (Bruker, Germany) in range of 4000 – 600 cm^{-1}. 2nd derivative spectra were used for further analysis.

Results and Conclusions: Analysis of 2nd derivative spectra reveled changes in keratin secondary structure induced by ionizing radiation. Small increase in absorption band intensities at 1628 cm^{-1} and 1510 cm^{-1} attributed to β-sheets of keratin [1] was observed for all spectra of irradiated samples. After storage for 12 months the increase was even higher, whereas control sample had stayed unchanged. This indicates to radiation induced continuous effect of α-helical structure transformation into more stable β-sheets. The higher content of β-sheets in irradiated samples makes them more viable as starting material for novel bio-based materials useful in industrial formulations and composites.

Acknowledgement. This study was supported by the ERDF project “Development of novel and innovative composite materials with enhanced sorption properties from renewable biological natural resources available in the Republic of Latvia for commercial air purification filtration systems”, 1.1.1.1/20/A/155.

Reference(s): 1. J.M. Cardamone, Journal of Molecular Structure 969 (2010) 97–105.

High-throuput 2D-IR spectroscopy using the HARE chip

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Two-dimensional infrared spectroscopy (2D-IR spectroscopy) is powerful technique adding additional information to already information-rich mid-IR spectra. By revealing a second frequency-axis , the excitation frequency, and adding a time-axis, a 2D-IR spectrum gives us information over vibrational coupling, anharmonicity, vibrational lifetimes, relative orientations and spectral diffusion rates of vibrational bands. Here we present a state-of-the art 2D-IR spectrometer using a fixed target chip [1] to measure different samples in rapid succession.

Our setup is based on a Spitfire Ace (10W, 1 kHz, 40 fs @ 800nm) from which 9W are used to generate high-energy mid-IR femtosecond pulses (up to 70mW) by employing a TOPAS HE module with a NDFG-extension. The mid-IR is directed into an AOM-based 2D-spectrometer (Phasetech) employing a 128x128 pixel MCT. To minimize the required sample-amount and to increase the number of different samples, the HARE chip is employed. The chip (30mm x 30mm) consists of 20736 micro wells, each can be filled differently employing a picolitre dispenser. During measurement, the chip is moved to different sample wells using a xyz-stage, interweaving the different measurements and thereby minimizing systematic errors.

A proof- of-concept measuring the 2D-IR spectra of N-methylacetamide at 20 different pH values is presented and further applications of system are discussed.

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[1] Mehrabi, P., Muller-Werkmeister, H. M., Leimkohl, J.-P., Schikora, H., Ninkovic, J., Krivokuca, S., Andricek, L., Epp, S. W., Sherrell, D., Owen, R.L., Pearson, A.R., Tellkamp, F., Schulz, E. C. & Miller, R.J.D. (2020). J. Synchrotron Rad. 27, 360-370.

Resolving lipid dynamics in the photocycle of bacteriorhodopsin by mid-IR quantum cascade laser spectroscopy

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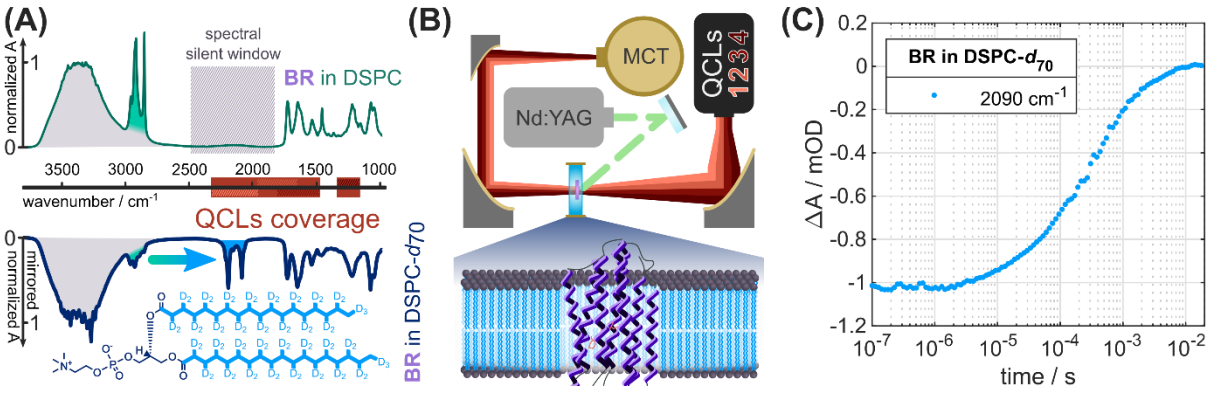
Context and Objective: The function of membrane proteins is highly influenced by their membrane environment. Insights into the impact of different lipid membranes to the photocycle of the light-driven transmembrane proton pump bacteriorhodopsin (BR) were provided by time-resolved Fourier-transform infrared (FTIR) spectroscopy.[1] In the next step of the investigation of photoreceptor-membrane interactions, minor absorbance changes of the lipid membrane dynamics itself must be resolved.



Methods: DSPC proteoliposomes serve as a model environment and by reconstituting BR into liposomes with fully deuterated lipid chains (DSPC-d_{70}), the corresponding bands are frequency-shifted into a spectrally silent window (A). Our home-built quantum cascade lasers (QCLs)-based spectrometer (B) enables time-resolved single wavelength measurements of protein[2] as well as lipid vibrational modes during the BR photocycle.

Results and Conclusions: By similar time constants of the analyzed protonation dynamics of BR in purple membrane, DSPC and DSPC-d_{70}, the suitability as a model environment was confirmed. In comparison to step-scan FTIR, the dynamics of these lipids are resolved by QCL measurements (C). Therefore, direct investigation of lipid modes is enabled. Time constants of the resolved lipid transients and the protein dynamics show correlations.

Reference(s):

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[2] P. Stritt, M. Jawurek, and K. Hauser, Biomed. Spectrosc. Imaging 9, 55 (2020).

Comparative investigation of fibrillar actin using Nano IR spectroscopic and fluorescence microscopy imaging

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Context and Objective: Fibrillar actin is one of the major structural components in cells. Consequently, pathogenic alterations in cell functionality may be revealed by monitoring the re-arrangement of F-actin.[1] However, discriminating protein aggregation in the range below 10 nm is challenging even by high resolution fluorescence microscopy. Two complementary recently developed imaging methods can provide access to local protein organization on a scale below 10 nm: IR spectroscopic photo-induced force microscopy (PiF-IR)[2] and 2D polarization resolved fluorescence microscopy imaging (2DPOLIM).[1] Actin fibrils dispersed on lysinated cover glass were studied using PiF-IR and confocal fluorescence microscopy.

Methods: Fibrillar Actin was prepared from an Actin Binding Protein spin-down assay KIT (Cytoskeleton Inc., France) and stained with DY-490-Phallodoin (Dyomics GmbH, Jena, Germany). Dispersion on lysinated cover glass yields single actin fibers. PiF-IR was conducted on dried samples using a VistaScope (Molecular Vista, US) equipped with 4 QCL chips covering the range from 800 to 1800 cm^{-1}. For confocal fluorescence microscopy the lysinated F-actin was sandwiched between the cover glass and a microscope slide and imaged using a LSM800 (Zeiss AG, Jena, Germany) at 488 nm excitation.

Results and Conclusions: We were able to detect single actin fibers with both confocal fluorescence microscopy and PiF-IR. The actin fibers in the sandwiched samples degraded within a day in agreement to observations from literature. Adding prolong glass antifading mountant (Invitrogen) provided stable fibrils over several weeks.

A single helical actin structure could be observed in an PiF-IR scan image of a dried sample at excitation in the amide I band spectral region. However, in the end of the scan, the fiber disappeared, obviously it was attached to the scanning AFM tip. We further could obtain several single frequency scans from a thicker layer of fibrillar actin. This was confirmed by comparative measurements on plain glass substrate and plain Poly-L-lysine as well as on Buffer solutions.

These first results are very promising for using PiF-IR and 2DPOLIM to discriminate actin (re-)arrangements to monitor pathologic alterations of cells and tissue. Further investigation is planned aiming at increasing the understanding of impacts of infections and tailored therapies.

Acknowledgement: Funding by the German Research Foundation: DFG Ta1049/2

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(2) Cristie-David, A. S.; Chen, J.; Nowak, D. B.; Bondy, A. L.; Sun, K.; Park, S. I.; Banaszak Holl, M. M.; Su, M.; Marsh, E. N. G. Coiled-Coil-Mediated Assembly of an Icosahedral Protein Cage with Extremely High Thermal and Chemical Stability. *Journal of the American Chemical Society* **2019**, *141* (23), 9207–9216. https://doi.org/10.1021/jacs.8b13604.

Effects of obesity on the structural organization and mechanical properties of type I collagen

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Context and Objective: The Western society lifestyle, characterized by an increase in fat diet and a decrease in physical activity, is the main cause of the dramatic increase in the prevalence of obesity. Obesity is often associated with metabolic syndrome and has been shown to significantly increase the risk of developing hypertension, type II diabetes and heart diseases. In addition, obesity can also lead to important disorders related to other diseases such as musculoskeletal disorders, in which the structural organization and mechanical properties of extracellular matrix components are altered. In the present study, we propose to investigate the effects of obesity on the structural organization and mechanical properties of type I collagen, which is the main component of tendons.

Methods: The Wistar rat was used as a model in this study. Animals were subjected or not to high fat dietary regimen. Collagen fascicles were extracted from animals' tail tendons then placed in saline solution (NaCl 0,9%) until use. First, fascicles were analyzed by polarized Raman spectroscopy to characterize the molecular organization of control (CTL) and high fat (HF) rat tail tendon fascicles (RTTFs). For this purpose, anisotropic ratios of Raman bands were used to provide information about the changes in the orientation of collagen fibers. Then a biomechanical device combined with a portable Raman spectrometer was used to simultaneously measure mechanical properties of RTTFs and identify the collagen bonds affected differentially by a mechanical stress in terms of Raman frequency and/or intensity.

Results and Conclusions: Polarized Raman spectroscopy showed that bands related to proline (855 – 920 cm^{-1}), hydroxyproline (875 cm^{-1}), C-C backbone (938 cm^{-1}), amide I (1668 cm^{-1}) and amide III (1242 -1265 cm^{-1}) were highly sensitive to polarization. In fact, the anisotropy degree of Raman bands (855, 875, 938 cm^{-1}) increased from CTL to HF RTTFs, indicating a higher collagen fibers alignment to the fascicle backbone axis in HF tendons, and consequently a higher straightness of collagen fibers with obesity. Investigation of mechanical properties of fascicles showed an increase in yield stress, ultimate tensile strength, and rupture strain in HF RTTFs. Raman spectroscopy was then combined to a tensile stage and used to investigate the variation in the intensity and/or frequency of the main Raman bands of collagen (855, 875, 938, 960 cm^{-1} and amide I) between CTL and HF RTTFs during tensile stress. Data showed that the most relevant frequency shift was observed at 938 cm-1 which is assigned to the C-C backbone. In addition, the intensity of the band at 875 cm^{-1}, corresponding to hydroxyproline increased in HF RTTFs, whereas it was not affected in CTL RTTFs. Our approach sheds a new light on obesity-related changes in molecular properties of collagen upon mechanical stress and could be useful to provide additional translational and relevant information to monitor progression of obesity-related diseases.

A new convenient tool to analyse protein glycosylation based on FT-IR spectroscopy

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Glycosylation is the most common protein post-translational modification (PTM), especially in biopharmaceuticals. It is a critical quality attribute as it impacts product solubility, stability, half-life, pharmacokinetics and pharmacodynamics (PK/PD), bioactivity and safety (e.g., immunogenicity). Yet, current glycan analysis methods involve multiple and lengthy sample preparation steps which can affect the robustness of the analyses. The development of orthogonal, direct and simple method is therefore desirable.

We suggest the use of FT-IR spectroscopy as a suitable and powerful tool to analyse protein glycosylation. Three types of analyses can be realized using this tool: - Comparative study in terms of global glycosylation level [1]; - Comparative study in terms of glycan composition [2]; - Prediction of the monosaccharide content using predictive models based on advanced statistical methods. [3]

The FT-IR-based method to analyse glycosylation offers three key advantages. Firstly, the analysis is performed on intact proteins, which represents a major asset. Indeed, all the existing methods for glycans and monosaccharides analysis involve several preparation steps: glycan release, labelling, separation and hydrolysis for monosaccharide analysis. Secondly, the processing time is extremely short (measurement in maximum 5 minutes and fully automated data analysis). Finally, FTIR spectroscopy can be used as a Multi-Attribute Methodology (MAM). Analysis of other critical parameters for therapeutic proteins (such as the protein structure or protein concentration) can be performed simultaneously.

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FTIR and biochemical characterisation of glycosaminoglycans (GAGs) content in ovarian cancer cells

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Context and Objective: Ovarian cancer remains one of the deadliest cancers due to the lack of robust screening methods for early detection. During tumour progression and metastasis, the extracellular matrix (ECM) is altered and associated with the disorganisation of macromolecules such as collagens and proteoglycans (proteins covalently linked with sulphated GAGs). GAGs are polysaccharides characterized by repeating units and are involved in the sequestration of growth factors regulating tumour growth and progression. In ovarian cancer, a high expression of chondroitin sulphate has been described (Ten Dametol, 2007). Thus, GAGs characterization at the cell level and in their secretome is of great interest. In this study, FTIR spectroscopy was used to characterize ovarian cancer cell lines (synthesizing GAGs) and their secretome.

Methods: Five cell lines (SKOV3, CAOV3, OVCAR3, CHO K1, pgsA 745) as well as their secretome were analysed. Sulphated GAGs content was biochemically assessed using the specific Blyscan® test. In parallel, the secretome was measured using a high throughput FITR unit (INVENIO S, Bruker Optics). In addition, the five cell lines were analysed as single cells using the Spotlight 400 (Perkin Elmer) FTIR microscope.

Results and Conclusions: Blyscan® results showed a difference in the capacity of the cells to secrete sulphated GAGs with the following gradation and after normalising to the protein content: CHO K1 > SKOV3 > OVCAR3 > pgsA 745 > CAOV3. The cell secretome FTIR spectra revealed specific signatures associated to GAGs molecules, in particular the sulphate (1200-1300 cm^{-1}) and polysaccharides (900-1200 cm^{-1}) absorptions. At the single cell level, it was possible to characterise the cell lines with respect to their capacity to synthesise GAG molecules. Altogether, this study shows that biochemical and FTIR data can be correlated.

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Characterization of secondary structure of protein by infrared nanospectroscopy

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Context and Objective: During biological processes, protein conformation can change. A better understanding of those biological processes required a description of these conformational changes. Protein secondary structure is commonly studied by circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR). These spectroscopic techniques are versatile and can monitor those changes; they can be adapted to wide experimental conditions (variation of pH, ligand biding, buffer, metallic ions …) and are non-destructive. In FTIR and in particular in attenuated total reflection (ATR) only a small amount of protein (~100 ng) is needed to obtain good quality spectrum.

ATR-FTIR is very useful for homogeneous sample. An amyloid fiber protein is on the contrary an ensemble of constantly evolving complex structure. ATR-FTIR can only provide an average spectrum even if different species are present. To overcome this limit, optical microscopy can be coupled with FTIR and spectra unfortunately with a resolution of only a few micrometers can be obtain. More recently a technique coupling atomic force microscopy and infrared spectroscopy was developed (AFM-IR). It has a resolution of few nanometers and is therefore able to record spectra of single biomolecule. This technique will improve our knowledge on protein aggregation and other biological processes.

So far, very little is known on the ability of AFM-IR to predict secondary structure of protein. Comparison between FTIR and AFM-IR were reported with good consistency between the two methods, but it was done for a limited number of proteins and using only curve fitting analysis method.

Methods: We study a protein library of 38-well characterized proteins and developed a model of prediction for the secondary structure.

Results and Conclusions: This was done by comparing ascending stepwise linear regression (ASLR) and partial least square (PLS). Even if small differences are observed between FTIR and AFM-IR spectrum, our models of prediction have similar errors for AFM-IR and FTIR around 6 % for α-helix and β-sheet.

Determining the influence of H/D exchange on IR spectroscopy and vibrational dynamics of polypeptide secondary structures

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Context and Objective:

Ultrafast IR spectroscopies have proven to be powerful techniques for investigating the structure and dynamics of proteins. In particular, the amide I band provides information relating to protein secondary structure, but these studies have predominantly been carried out using D2O as the solvent to avoid complications due to spectral overlap with the H2O bending vibrational mode (1650 cm-1). By contrast, it has recently been demonstrated that the water signal is suppressed relative to that of the protein in 2D-IR spectroscopy measurements, making direct observation of protein amide I modes in H2O practicable. However, such results raise fundamental questions about the comparability of measurements of proteins in H2O as opposed to D2O, most notably the impact of solvent exchange on conformational dynamics and vibrational relaxation times. This project focuses on laying the foundations for characterising these differences and interpreting the time-resolved IR and 2D-IR spectra of the building blocks of proteins in H2O, using a polypeptide chain of known, and controllable secondary structure.

Methods:

IR absorption, IRpump-IRprobe, and 2D-IR spectra of polyglutamic acid (PGA, 50-100 kDa and 15-50 kDa) dissolved in H2O and D2O (~100 mg/mL) were recorded. Varying the pH of the sample allowed the secondary structure to be changed from α-helix to random coil.

Results and Conclusions:

Ultrafast 2D-IR spectra of polyglutamic acid in random coil and α-helix conformations show differences in band positions and lineshapes in D2O and H2O, highlighting a clear impact of isotopic exchange. These differences will be discussed alongside solvent-dependent changes in vibrational relaxation timescales observed as a function of secondary structure and solvent environment.

THE ROLE PLAYED BY PROTEIN-ASBESTOS FIBER INTERACTION IN ASBESTOS PATHOGENICITY

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Context and Objective: Respiratory exposure to asbestos fibers is a hazard to human health because their inhalation is associated with severe lung diseases such as asbestosis and pleural mesothelioma. One key process in these diseases onset is the interaction of the fibers with endogenous proteins, that reflects on the asbestos bodies (AB) formation: asbestos fiber *in vivo* coated with proteins (e.g holoferritin)[1]. Our goal is to unravel the mechanism of protein binding to asbestos fibers and their consequent *structural* and functional modifications.

Methods: We set up a protocol to reproduce the AB model *in vitro* composed of *holo*ferritin (holoF) and three different asbestos fibers: chrysotile, amosite and crocidolite, in order to select the best performing model using UV-Vis spectrophotometer and western blotting assay. On the AB model selected, we performed a systematic study with microFTIR, nanoIR and HR-TEM to understand the interaction mechanism between protein and fiber.

Results and Conclusions: UV-Vis spectrophotometry show that holoF interacts with chrysotile, an asbestos fiber that contains only iron traces as contaminant[2], more avidly with respect to the other fiber types, richer in iron. Overall, these results suggest a key role of iron in the formation of ABs, as also proven by HR-TEM microscopy: holoF-chrysotile are characterized by fibers decorated with electron dense structures of about 5 nm in diameter, identified as the mineral core of holoF[2], possibly explaining the strong binding. In addition, microFTIR and nanoIR reveal, both at fiber aggregates and single fiber level respectively, that holoF undergoes a misfolding process upon binding: native holoF has a prevalent α-helix structure, while, in the AB model, a prevalent β-sheet. In conclusion, our data add new and robust experimental evidence for the explanation of the AB formation and therefore provides solid bases for better dissecting their pathogenicity.

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