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17th Confocal Raman Imaging Symposium

International Conference for
Chemical Characterization & Imaging

September 27th – October 1st, 2021

Greetings from Ulm

In the announcement of the Virtual Raman Imaging Poster Summit in 2020, we expressed our hope to welcome you again in person for the 17th Confocal Raman Imaging Symposium. As 2021 unfolded, however, it became clear that continued planning uncertainties and travel complications would necessitate a reimagining of the event. The Virtual Poster Summit had been enthusiastically received by the international Raman imaging community and so we decided to adopt and expand the online format for the Symposium. This new variation of the conference was also met with a thoroughly positive response from contributors and participants.

Five oral presentations by renowned scientists and 55 posters from around the world and across the spectrum of research fields were on display. They covered a vast range of applications, including life sciences and biomedicine, microplastics analysis and advanced materials characterization. We received more than 500 registrations for the event – about twice as many as for our previous online forum. More than 80 researchers signed up to take part in the live equipment demonstration sessions that showed our Raman technology in action, including automated particle analysis and topographic Raman imaging. The outstanding level of engagement, in attendance and discussions, was a clear indication of how much the international Raman imaging community values this opportunity to reconnect with each other and be updated on the latest developments in methodology and hardware.

The 17th Confocal Raman Imaging Symposium pivoted successfully to the virtual format, and given current circumstances, it was the best forum available for maintaining the sense of community that has developed since the conference's inception. Nonetheless, we very much look forward to welcoming you personally, here in Ulm, when it's possible to do so. Our expanded headquarters facility is in its final phase of its construction and fitting out, with the new conference lounge and demo area almost ready for hosting the exchange of ideas that has long defined the event.

We thank everyone for their contributions and participation in this year's conference. We can hardly wait to see what advances and discoveries you make during the next year. The 18th Confocal Raman Imaging Symposium will take place from September 26th through the 28th, 2022.

Olaf Hollricher and Joachim Koenen
Managing Directors at WITec GmbH

Abstracts

Featured Speaker Abstracts

p. 5

Poster Abstracts by Topic

Advanced Materials Analysis

p. 11

Environmental and Geo Science

p. 31

Life Sciences, Biomedical and Pharma Research p. 41

SUBMIT UNTIL JANUARY 31

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Featured Speaker Abstracts

Featured Speakers

A-034

The Principles of Raman Spectroscopy and its Application in Microscopy

S. Schlücker

Universität Duisburg-Essen, Physikalische Chemie, Essen, Germany

This lecture gives an introduction into the principles of Raman spectroscopy and its applications in microscopy.
First, both classical and quantum mechanical descriptions of the Raman effect are discussed. The latter (perturbation theory, Kramers-Heisenberg-Dirac dispersion formula) then serves as a starting point for introducing the concept of resonance Raman scattering (RRS). Several examples of RR (from diatomics to proteins) highlight the advantages of this Raman technique.
In addition to the Raman effect, also fundamentals of molecular vibrations and their symmetry (basic group theory) are covered by using the water molecule as an example.
We then make the transition to Raman microscopy, starting with the invention of the first Raman "microprobe" in the 1970s. Also other specialized Raman techniques such as surface-enhanced Raman scattering (SERS) and coherent-anti-Stokes Raman scattering (CARS) microscopy are briefly introduced and their specific advantages over conventional Raman spectroscopy are highlighted.

Featured Speakers

A-046

Ex-vivo skin penetration analysis by confocal Raman microspectroscopy

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Universität Tübingen, Pharmazeutische Technologie, Tübingen, Germany

Confocal Raman microscopy (CRM) is increasingly used in the development of dermal dosage forms. Applications of CRM in this field include the characterization of formulations at early development stages as well as for quality control during shelf life. Furthermore, the tracking of ingredients (pharmaceutical actives or excipients) within the skin is gaining increasing attention. Conventionally, ex vivo skin permeation or penetration experiments are conducted to determine the skin absorption of pharmaceutical or cosmetical actives. These experiments are elaborate and time consuming as samples need to be withdrawn at multiple time points and/or the skin needs to be segmented, extracted and the samples analysed subsequently by HPLC. CRM offers the advantage of non destructive and lable free detection of an active inside the skin with no or minimal sample preparation beforehand [1]. Further, the distribution of the active with relation to the microstructure of the skin can be evaluated [2]. Recently, we also developed a skin incubation cell to be used under the Raman microscope to allow for in-situ analysis of active penetration [3,4]. In the course of the experiments, a model formulation containing procaine HCl was used as procaine can be detected reliably by CRM. The impact of penetration enhancers was investigated.

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[4] https://doi.org/10.3390/pharmaceutics13010067

Featured Speakers

A-073

3D Raman imaging of the oldest methanogens, Barberton greenstone belt, South Africa

B. Cavalazzi[1, 2], L. Lemelle[3], A. Simionovici[4], S. L. Cady[5], M. J. Russell[6], E. Bailo[7], R. Canteri[8], E. Enrico[9], A. Manceau[4], A. Maris[1], M. Salomé[10], E. Thomassot[11], N. Bouden[11], R. Tucoulou[10], A. Hofmann[2]

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While Earth may have been a habitable planet long before the Paleoarchean Era (3.6 to 3.2 billion years ago), as rocks of this age retain a diversity of fossil evidence for life, the complexity of life's cellular constituents, range of metabolic pathways, the niches it occupied locally, and global diversity of its habitats remain largely unknown.
Here, we present the discovery of exceptionally well-preserved, ~3.42-billion-year-old putative filamentous microfossils that inhabited a paleo-subseafloor hydrothermal vein system of the Barberton greenstone belt in South Africa. 3D confocal Raman imaging in combination with *in situ* morphological and chemical characteristics as investigated over a range of scales, and the paleohabitat reconstruction point out to biological origin of these ancient filaments that can be considered the oldest methane-cycling microorganisms and the oldest direct evidence for subsurface microorganisms. This finding expands the frontiers of early Earth habitability.

Acknowledgments

This research received support from the Europlanet 2024 RI, which has received funding from the European Union's Horizon 2020 research innovation programme grant no. 871149.

Featured Speakers

A-076

Correlative imaging of transition metal dichalcogenides

U. Schmidt, J. Englert, O. Hollricher, T. Dieing

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Transition metal dichalcogenides (TMDs) belong to a new class of single or few-layer nanomaterials with great potential for optoelectronic device applications. The electronic, optical and thermal properties of TMDs differ significantly from those of their respective bulk precursors and can be tuned by varying characteristics such as the number of layers, chemical composition, crystal symmetry, strain and growth defects. For a comprehensive analysis of TMDs, it is beneficial to combine several microscopy techniques in order to obtain information about every one of these properties.
This contribution presents a comprehensive study of tungsten disulfide (WS_2) crystals using various imaging techniques, including Raman and photoluminescence (PL) imaging, atomic force microscopy (AFM), scanning electron microscopy (SEM) and second harmonic generation (SHG) microscopy. Using correlative systems, the same sample area can be imaged with several techniques and the complementary information can be linked. Thus, TMDs can be characterized in terms of crystal symmetry, defects, strain, morphology and topography. Different growth mechanisms can be distinguished and edge effects can be investigated.

Featured Speakers

A-143

Three-dimensional exploration to heterogeneous materials with confocal Raman spectroscopy and beyond

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University of Central Florida, Physics Department and NanoScience Technology Center, Orlando, United States

Exploring the volume of heterogeneous materials with microscale spatial resolution is important to monitor local changes responsible for loss of performance or failure of macroscale systems such as jet engines. The multi-layered structures used in thermal barrier coatings (TBCs) to control temperature gradients of jet engines are highly susceptible to failure after infiltration of sands and volcanic ashes present in the atmosphere, due to the high temperatures reached in the engine. Molten calcium, magnesium, and alumino-silicates (CMAS) is known to infiltrate the TBCs, leading to stiffness increase and phase destabilization. Here, we discuss how confocal Raman spectroscopy can be used to probe the extent of thermochemical degradation in TBCs non-destructively. Hyperspectral maps are used to reveal the distribution of monoclinic phase volume fraction (mPVF) formed in the columnar structures of a model yttria-stabilized zirconia (YSZ) top-coat because of CMAS ingression. They also show that phase destabilization varies from the outer layer of the columns to their core, which retain their original tetragonal phase, and that exposing the TBCs to CMAS for 10h at 1250^{o}C increases the regions of the top-coat undergoing thermochemical changes. This ability to quantitatively and non-destructively characterize degradation of CMAS-infiltrated TBCs is expected to improve quality control on existing engines, and to identify better degradation-resistant coatings. However, the latter may require to monitor processes and properties at the nanoscale such as at interfaces, which is not achievable with confocal Raman spectroscopy alone. We will summarize some potential avenues to reach nanoscale subsurface imaging in view of advancing functional materials research.

Poster Abstracts by Topic

Advanced Materials Analysis

Advanced Materials Analysis

A-025

Imaging of the physically unclonable surfaces via SERS

M. Sakir, N. Torun, I. Torun, M. Kalay, M. S. Onses

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It is favorable to physically unclonable surfaces whith multi-layered security levels for anti-counterfeiting applications. For this, stochastic processes, in which deterministic production methods are adopted, are generally used. Here, the dewetting instabilities of a polymer film in nano-scale were used to generate physically unclonable surfaces. The randomness in the nature of the dewetting process creates a favorable platform for physically unclonable surfaces. The dewetting of poly(2-vinyl pyridine) (P2VP) on polystyrene brushes induced with thermal annealing enables the fabrication of random patterns on the surface. The patterns that are separated at a microscopic length scale can be verified via the reflection of visible light with an optical authentication system. In the second step, citrate-stabilized Au nanoparticles were immobilized on P2VP patterns by utilizing the electrostatic interaction between them. This allowed the patterns that could not be physically unclonable to acquire plasmonic properties. Au nanoparticles through localized surface plasmons cause a strong surface-enhanced Raman scattering (SERS). With the use of probe molecules, we have a molecular vibration-based security level. The patterns of P2VP that we have obtained with a stochastic and deterministic approach provide us with a double-layered security label. The physically unclonable surfaces such as these can be easily used as anti-counterfeiting security labels on almost any product that affects our lives, such as banknotes, passports, valuable papers, jewelry, electronic device, and medicines.

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Advanced Materials Analysis

A-037

Raman Analysis of Nanocluster carbon using Near Field Scanning Microscope (NSOM)

M. P. Nirupama, B S Satyanarayana, O.S Panwar

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The nanocarbon thin film and its many facets can be grown using a cathodic arc system by varying deposition parameters. One of the interesting and novel materials of interest in the research work is Nanocluster Carbon (NC) thin films. It is a unique mixed phased material, containing sp^2 and sp^3 carbon bonds. To understand the feasibility of nanocluster carbon thin films using a Cathodic arc system for space-related applications, samples are grown under various process parameters and characterized for their various properties. The type of characterization method adopted is based on the kind of information required from the thin film. In the research work, several characterization methods are used to study the information about the nanocluster carbon thin films for selecting the best suitable film for the potential application in FEEP electrical propulsion system.
Near Field Scanning Microscope (NSOM) was used to carry out the Raman analysis of the nanocluster carbon and wavelength of 532nm excitation laser used for Raman analysis. Using this instrument it is possible to acquire high resolution and high-speed images. A single spectrum from the sample can be acquired in 100milliseconds. The shift in the energy of the excitation laser light, which is due to inelastic scattering occurring in the sample is defined in the Raman spectrum. The vibrations within the molecules due to inelastic scattering result in vibrations of chemical bonds. Different materials possess different atoms and different chemical bonds, hence it is easy to identify the molecules using its Raman spectrum. Every material will exhibit a unique Raman spectrum. The initial Raman data indicated that all the control can be achieved at room temperature. Hence the next study was focused on incorporating nitrogen for enhanced conductivity and clustering, the experiments again were carried out at room temperature. From the Raman spectrum it is possible to identify the material is crystalline or nano crystalline or amorphous, the cluster size, type of bonding: sp^2 or π bond (graphite-like) or sp^3 or σ bond (diamond-like) and sp^2/sp^3 ratios etc.

Advanced Materials Analysis

A-044

Raman measurements of trialkoxysilyl-based protective coatings against corrosion

A. K. Surca, M. Rodošek

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Protective coatings against corrosion represent a huge issue since the estimations of financial damage from corrosion are over 4 % of global GDP [1]. Different materials have been examined and an important role among them have sol-gel coatings. In order to prepare compact and thick sol-gel protective coatings, the processes of hydrolysis and condensation should be precisely designed and monitored. Various trialkoxysilanes are used as precursors since their alkoxy groups can highly crosslink via the formation of –M-O-M- bridges. The efficiency of coatings can be tested using different electrochemical measurements, for example, potentiodynamic polarization. The processes that occur on the surface of the protective coatings during such forced electrochemical treatment can be followed using vibrational spectroscopy: either near grazing incidence angle reflection-absorption (NGIA IR) or Raman spectroscopy [2]. While the first gives information on the average behavior of the sample, the Raman spectra are either measured as single spectra at one site with increasing potential or as images after the application of the potential.
Accordingly, Raman spectroscopy with a laser excitation wavelength of 532 nm was used to examine sol-gel coatings deposited on aluminum alloy AA 2024 during electrochemical treatment. The *ex-situ* approach was used to study the coatings prepared from (3-glycidoxypropyl)trimethoxysilane and (3-aminopropyl)trimethoxysilane combined with hydrophobic poly(dimethylsiloxane). In a second step, a custom-made *in situ* Raman cell was developed and used for spectroelectrochemical measurements [3]. In this round Teflon cell, the Pt grid is used as a counter electrode and an Ag/AgCl/KCl_{sat} as a reference electrode. The various trialkoxysilyl-based coatings are deposited on AA 2024 and tested in 0.5 M NaCl electrolyte. Raman imaging was used to follow the formation of pits.

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Advanced Materials Analysis

A-045

Raman measurements of vanadium oxide electrochromic films

A. K. Surca, G. Dražić, M. Mihelčič

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Vanadium(V) oxide prepared in a thin film form expresses electrochromic characteristics. When a classical sol-gel route is used for its preparation the temperatures above 300 °C are demanded. This prevents the deposition of crystalline V_2O_5 films on polymeric substrates [1]. Alternatively, V_2O_5 powder can be prepared, milled, and then incorporated in an appropriate dispersant for the deposition of films on foils and their curing at 150 °C [2].
The electrochromic effect is a consequence of the intercalation/deintercalation of small cations into the layered structure of V_2O_5 from electrolyte. Such changes can be followed using Raman spectroscopy. Our study was performed using a WITec alpha 300 confocal Raman spectrometer and the laser excitation wavelength of 532 nm. The spectra of initial V_2O_5 films show the characteristic vanadyl in-phase stretching mode in c-direction (996 cm^{-1}) and other modes of O_A, O_B, and O_C atoms, as has already been reported by Baddour-Hadjean et al. [3], [4], [5]. We chronocoulometrically discharged/charged films in 1 M $LiClO_4$ in propylene carbonate to a certain potential and the spectra were measured *ex-situ*. It was noted that two modes appeared in the vanadyl stretching region of the colored film while the intensity of the bands between 800-300 cm^{-1} decreased. However, the initial modes re-appear in the spectrum of bleached V_2O_5 film. In the case of the low-temperature V-oxide film, the bands re-appear but remain much broader after bleaching than in its initial spectrum. All the changes that happened in the spectra of colored and bleached states will be described and commented on. As well, the behavior of low-temperature V-oxide films with regard to the crystalline V_2O_5 films will be discussed.

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Advanced Materials Analysis

A-053

Surface-enhanced Raman scattering on Au nanoparticle-graphene-Au nanoparticle hybrid structures

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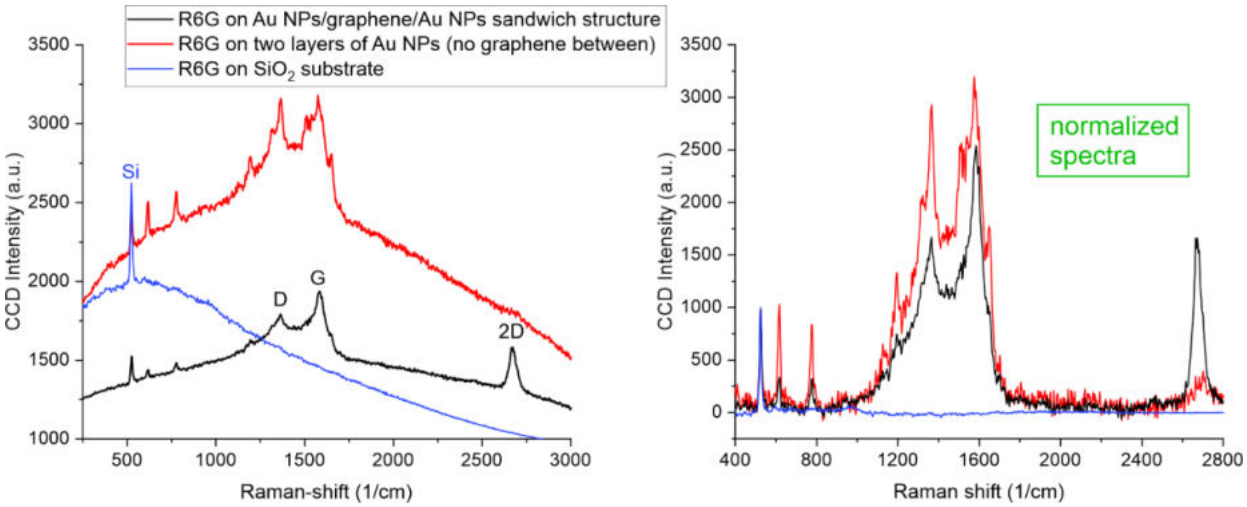
The synthesis of graphene-plasmonic metal nanoparticle hybrid materials has been of great interest towards the development of advanced substrates for molecular sensing based on surface-enhanced Raman scattering (SERS). Previously, we investigated the structure [1] and vapour sensing properties [2] of graphene-covered Au nanoparticles. Here, we report a Raman spectroscopy study of a Au-graphene-Au structure, prepared by sandwiching graphene between two Au nanoparticle layers. We measured peak intensity maps using confocal Raman imaging. The Raman response of graphene was significantly enhanced in this hybrid structure. Exposing the sandwich structure to a dilute solution (10^{-7} M) of rhodamine (R6G), the graphene layer quenched the fluorescent background of R6G, while the Raman peaks were less enhanced (see Figure) compared to a substrate with only closely-spaced Au nanoparticles. We attribute this finding to a significant concealment of "hot spots" by the graphene layer.



Left: Average Raman spectra of rhodamine (R6G) on the Au NPs/graphene/Au NPs sandwich structure (black), on Au NPs without graphene between (red), and on SiO_2 substrate (blue). Right: the same spectra as left, background-subtracted and normalized to the Si (520 cm^{-1}) peak.

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Advanced Materials Analysis

A-054

Degradation of $Li_{3.95}Mn_{0.05}Ti_5O_{12}$ induced by laser radiation with a power of 0.09-4.87 mW

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Raman spectroscopy is often considered as a non-destructive method of studying the structure and the interaction of laser radiation with the object of study is not always given due attention. This is especially noticeable for those publications where this method is auxiliary and is used to confirm the target phase. The object of this study is lithium titanate $Li_4Ti_5O_{12}$ (LTO), which is used as an anode material for lithium batteries. A large number of publications with lithium titanate spectra can be found, but most of these research are conducted by specialists in the field of solid state chemistry and electrochemistry, and therefore the question of the effect of laser radiation on the material under study is usually not considered in these works.
The lack of publications on the topic of LTO degradation under the influence of laser radiation is partly due to the fact that this material has a spinel structure ($Li[Li_{1/6}Ti_{5/6}]_2O_4$) that is resistant to various types of influences. However, pure LTO has an extremely low electronic conductivity and its doping is proposed as a solution to create additional conduction centers in the volume of the material. This paper presents the results of a study of the effects of laser radiation with a wavelength of 633 nm and different power on individual particles $Li_{3.95}Mn_{0.05}Ti_5O_{12}$.
A comparison of the spectra measured at different values of the exciting laser radiation power for different particles allowed us to propose a physicochemical model of the degradation of doped LTO with "order-disorder" phase transitions. In the course of fast processes, the induced phase transition was accompanied by laser ablation of the sample with the reprecipitation of nanoparticles, but with the preservation of the shape of the particle that experienced the phase transition (Fig.1). In addition to degradation processes, the effect of carbon redeposition was demonstrated even for samples that do not contain a conductive carbon coating.

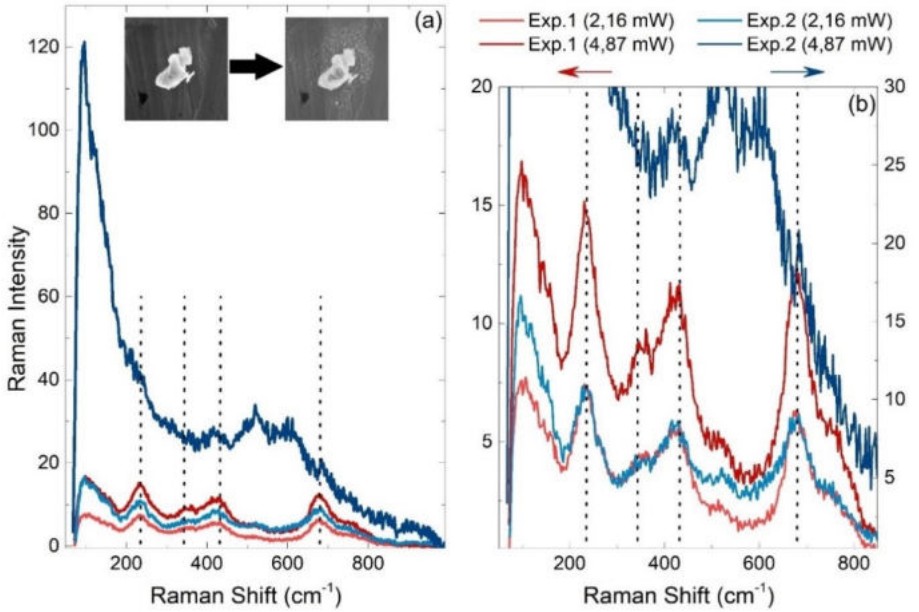

Fig.1. Changes in the Raman spectra and morphology of the particle $Li_{3.95}Mn_{0.05}Ti_5O_{12}$ under the action of laser radiation. The time difference between the first (Exp. 1) and the second (Exp. 2) measurements was about two months. The dotted lines indicate the positions of the characteristic LTO peaks.

Advanced Materials Analysis

A-068

Harmonization of Raman spectroscopy to characterize materials across their life cycle – the CHARISMA Project

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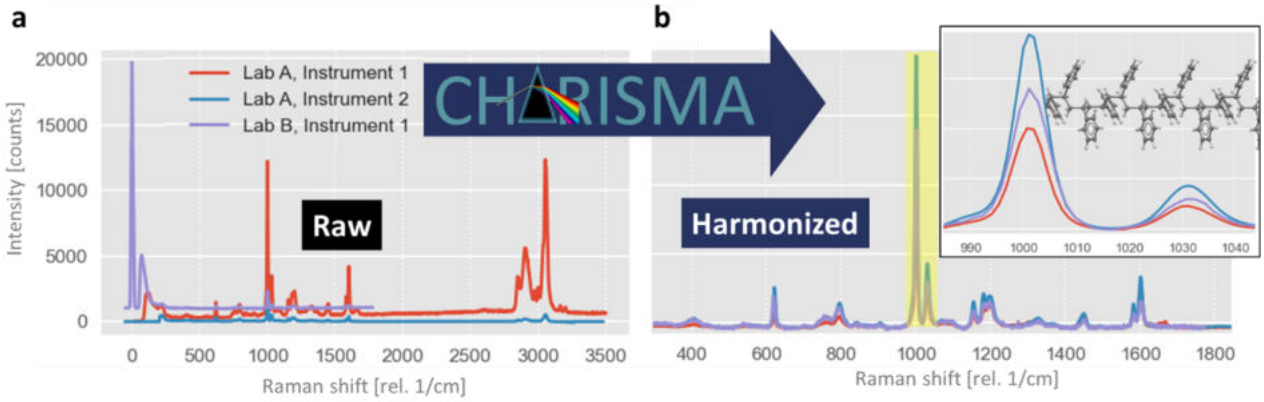
The EU-funded project CHARISMA aims to harmonize and standardize characterization by Raman microscopy and spectroscopy, including hardware, measurement protocols, and in silico methods, enabling end users to share digital spectral data across domains and across the entire life cycle of diverse products. The project will demonstrate the feasibility of its concept in three industry cases. In the long term, it aims to remove existing limitations on cross-validation and data consistency. Thus, it will implement and foster an open innovation environment in existing and developing industries through Raman spectroscopic characterization techniques.
CHARISMA will normalize the harmonization of Raman spectroscopy in the Nanotechnologies, Advanced Materials, Biotechnology, and Advanced Manufacturing and Processing community. It will develop a model to harmonize Raman spectroscopy and will generate a FAIR Raman data repository. Furthermore, CHARISMA aims to harmonize Raman spectra and characterization data and to standardize Raman protocols.
We outline the mission of this multinational research effort and present an overview of methods used, including

- the development and production of universal, robust and readily available calibration standards
- the standardization of measurement procedures
- easy data conversion to a universal and open data format implemented in Python
- a database enabling exchange, collaboration and training of artificial intelligence prediction models for material properties
- teaching of experimental and data analysis techniques to students, researchers and professionals

We introduce industrial and academic use cases and show first results.



CHARISMA will implement harmonization of raw Raman data from various sources and formats (a) through pre-processing and calibration. Harmonized data (b) can be quantitatively compared, grouped, and used for statistical model development. The example shows spectra of polystyrene, a common polymer (inset in (b)).

Advanced Materials Analysis

A-077

RISE imaging of various phases of SiC in sintered silicon-carbide ceramics

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Silicon carbide (SiC) is a high-performance ceramic made of non-oxide powders that can be manufactured to fulfill requirements for a wide range of applications. Ceramics made of SiC play a key role as materials for high temperature applications due to their resistance against corrosion, heat and wear. Furthermore, due to its wider band gap and resistance to radiation damage and electrical breakdown, it is also an attractive material for use in the fabrication of microelectronic and optoelectronic devices. The properties of such ceramics strongly depend on the microstructure and grain growth of SiC polymorphs. This contribution presents a study of recrystallized silicon carbide (RSiC): a pure silicon carbide material with 11-15% open porosity.
This study uses Raman imaging and scanning electron (RISE) microscopy and 3D Raman imaging to investigate RSiC ceramics. RISE microscopy combines all features of a stand-alone SEM and a research-grade confocal Raman imaging microscope within one instrument. The sample remains within the vacuum chamber for both measurement modes, allowing for the correlation of chemical and structural information from the same sample area. Low kV SEM images show the ceramic's surface structure and correlative RISE imaging shows the distribution of different SiC polytypes, revealing unique substructures (Fig. 1). Raman depth scans and 3D Raman images also visualize small inclusions of different phases in the analyzed grains.

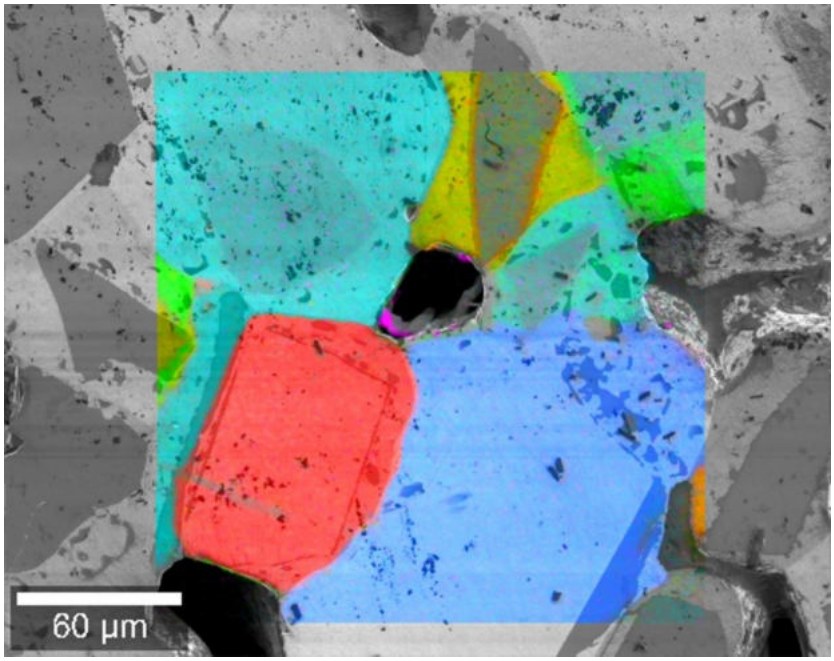

Figure 1: Correlative Raman-SEM (RISE) image of recrystallized silicon carbide (RSiC) ceramics. The SEM image shows the structure, while the Raman image reveals the distribution of various SiC polymorphs.

Advanced Materials Analysis

A-079

A Raman spectroscopy Study of Corrosion Inhibition of copper by Mentha Spicata oil in 1M Nitric Acid solution

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Raman spectroscopy is playing an increasingly important role in the study of corrosion due to its remarkable advantages such as easy sampling [1]. It is a non-destructive technique that is well suited for the in situ characterization of various oxides involved in the corrosion [2, 3, 4].
In the present study we have used a HORIBA LABRAM HR Raman spectrometer to characterize the corrosion products formed on the surface of copper during the study of its corrosion inhibition by Mentha Spicata oil in 1 M HNO_3 solution. We also investigated the effect of adding barium chloride using the weight-loss method with a variable solution temperature and various inhibitor concentrations.
It was found that the inhibition efficiency increases with an increase in concentration of inhibitors and temperature. The effect of temperature on the corrosion behavior with the addition of optimal concentration of $BaCl_2$was studied in the temperature range 298 -328 K. The adsorption of the inhibitors on the copper surface is in agreement with Frumkin and Langmuir adsorption isotherm. On the bases of thermodynamic adsorption parameters, it can be interpreted that the adsorption of this inhibitor on the copper surface takes place through both chemical and physical adsorption.
Raman investigations and mapping revealed that the inhibition of copper corrosion is achieved by strong adsorption of inhibitors molecules onto the copper surface, preventing it from being corroded.

Key words: Raman spectroscopy, corrosion, copper, green inhibitor, Mentha Spicata oil.

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Advanced Materials Analysis

A-081

Transformation of Nanodiamonds to Onion-like Carbons by Ambient Electrospray Deposition

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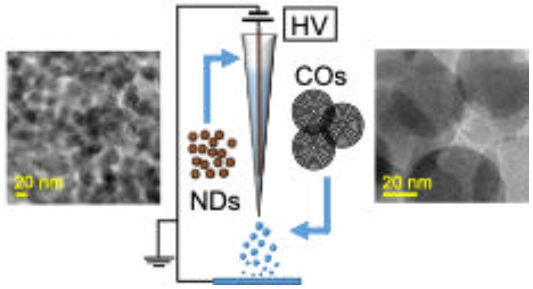
Onion-like carbons (OLCs) are a class of fullerene-like circular nanoallotropes of carbon, typically synthesized from nanodiamond (ND) via thermal annealing, plasma spraying, and laser ablation. These methods require high temperature, high vacuum, or inert gas. Here, we report an ambient electrospray deposition (AESD) process to transform NDs (11 ± 1 nm in size) into OLCs (50 ± 13 nm in size) in water. Transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), Raman spectroscopy, and X-ray photoelectron spectroscopy (XPS) were used for the characterization of NDs and OLCs. High-resolution TEM images showed an increased interplanar spacing from ND (0.23 nm) to OLC (0.39 nm). Raman spectra showed a shift in the ND peak from 1336 cm^{-1} to D band at 1349 cm^{-1}, and XPS quantitatively estimated an increase in the graphitization ratio (sp^2/sp^3) from 0.95 to 3.16 after AESD. Comparison of electrospray with sonic spray confirmed that such a transformation required an external voltage as well. AESD was also performed for NDs dispersed in ethanol and acetonitrile, which showed a solvent-dependent transformation.



Advanced Materials Analysis

A-087

The graphene edge method for accurate dimensional measurement of the confocal volumes of Raman microscopes

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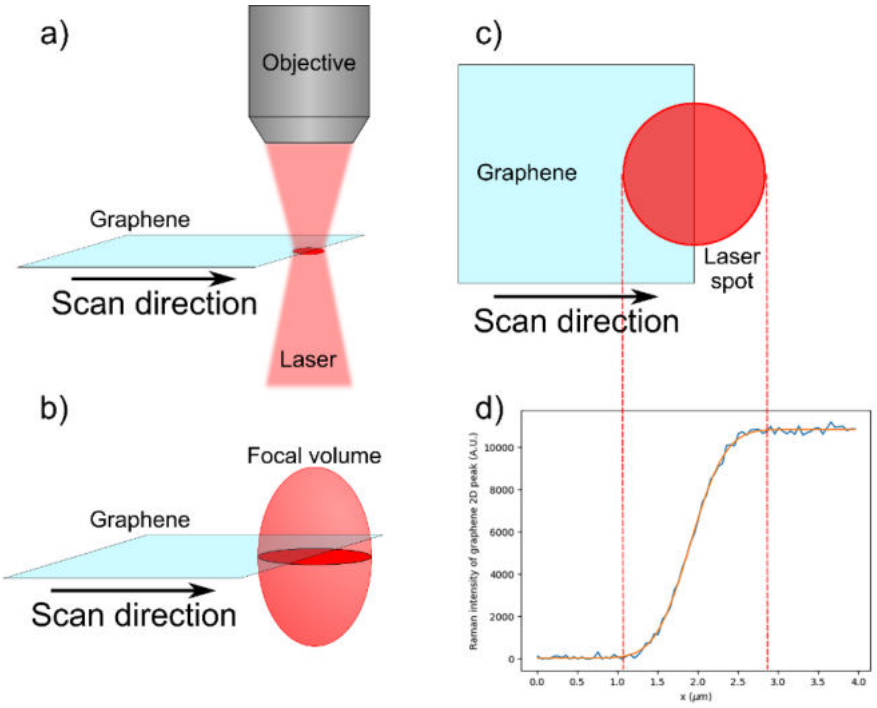
Istituto Nazionale di Ricerca Metrologica (INRiM), Quantum Metrology and Nanotechnology Department, Turin, Italy

Raman microspectroscopy is a vibrational analysis technique for chemical characterization of samples; the employment of visible light focused by a (usually confocal) microscope allows spatial resolutions below 1 µm. As of now, Raman is seldom employed for relative quantification and it is primarily a qualitative analysis tool in industry and research, while absolute quantification is not yet traceable to the International System of Units (SI). One of the causes is the lack of standard procedures and reference materials for the accurate measurement of the dimensions of Raman microscopes focal volumes, which influences calibration and uncertainties both for length and amount of substance estimations.
In this work, we propose the use of a graphene sample with a straight edge as a standard material to measure the three dimensions of confocal volumes of three Raman microscopy setups with imaging. The high Raman cross section of graphene, along with its chemical and mechanical stability and its atomic thickness, makes it an ideal Raman spatial probe. By scanning the surface of the graphene layer and its straight edge in different directions, Raman intensity profiles are collected in order to assess the shape and size of the volume and beam waist near the focus point. In addition to this, actual projections of the volume on planes parallel to the optical axis (“side views” of the volume) can be obtained by combining multiple planar scans. The proper metrological dimensional measurement of the focal volumes and beam waists of Raman microscopes with measurement uncertainties is a much sought-after goal, and this novel method could be a step towards it.



Graphene edge method for the lateral profile measurement of the beam waist of a top illumination/collection confocal Raman microscope. a) Side view. b) Same view with focal volume explicitly shown. c) Top view. d) Example measurement data of a profile (blue line) and least-squares functional regression (orange line).

References:

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Advanced Materials Analysis

A-090

Chemical strategies to fabricate textile-based composites for SERS and Raman imaging applications

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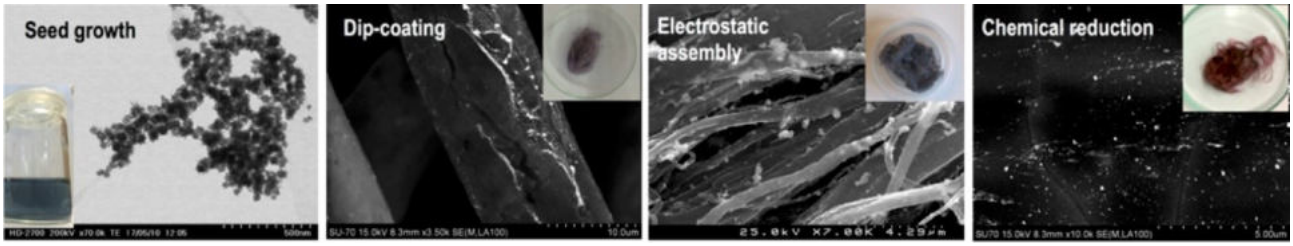
Optical methods based on surface-enhanced Raman scattering (SERS) spectroscopy and Raman imaging are among the techniques most used in recent years to detect vestigial amounts of organic compounds of environmental interest [1], [2]. The rapid development of SERS has been in line with the scientific advances in nanofabrication and Raman instrumentation, such as confocal Raman microscopy (CRM). In particular, CRM can offer high-resolution images with short measurement times in the analysis of nanoscale materials. On the other hand, textile-based nanocomposites containing metal nanoparticles (*e.g.* Au, Ag) have gained increased attention as a new class of malleable, flexible and low-cost SERS substrates to be used as analytical platforms.

Our interest in this field led us to explore both methods to develop SERS nanostructured substrates to detect organic pollutants in water [3], [4], [5]. This communication provides an overview of our research on developing easy-handled SERS substrates based on textile fibres for analytical detection. Chemical strategies employed for the coating of textile fibres with metal nanoparticles will be described. Illustrative examples of SERS applications and their evaluation using Raman imaging will also be provided, along with perspectives of development in chemical detection applied to real contexts.



Distinct chemical strategies to prepare textiles-based nanocomposites

Acknowledgements: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate cofinanced by FEDER under the PT2020 Partnership Agreement. Sara Fateixa acknowledges the costs resulting from the FCT hirings funded by national funds (OE), through FCT-Fundação para a Ciência e aTecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

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Advanced Materials Analysis

A-097

The penetration depth of micro-Raman spectroscopy for particulate $LiFePO_4$

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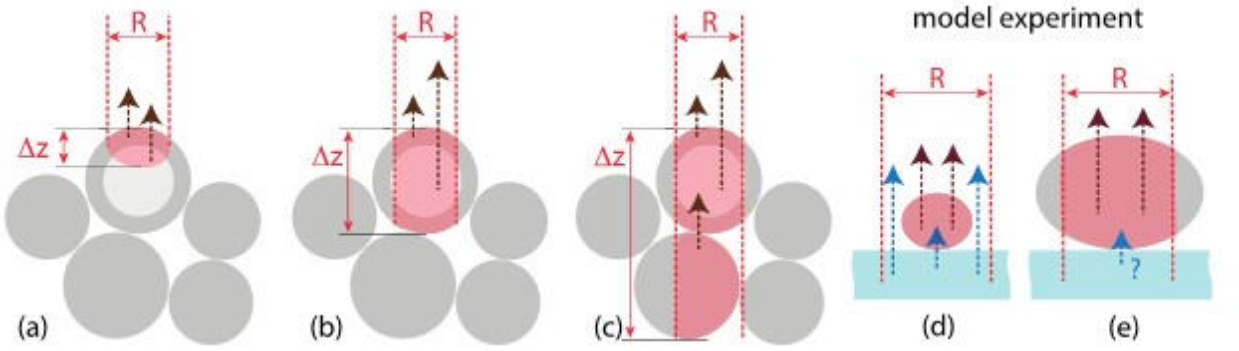
During the last decade, the lithium battery industry is thriving due to steady demand from automobile and energy industries. This maturing phase strongly needs production scale growth and cell price decreasing. Both trends increase the role of quality control tools, especially ones, suitable for industrial applications. Raman spectroscopy (RS) is one of the most promising candidates for industrial quality control tool due to a relatively low acquisition times, inexpensive equipment, and ability to be built-in production lines. But there is still a gap between chemical and physical aspects of materials characterization.
To correctly interpret Raman measurements, it is important to understand the area from which we receive the signal. For popular electrode material lithium iron phosphate (LFP) it is still unclear: "The penetration depth inside $LiFePO_4$ is unknown, but it should be small" [1].
Mostly, LFP can be found in lithium-ion batteries of electric vehicles and in stationary energy storage solutions. As well as other electrode materials, it is usually produced as a micron/submicron-sized powder. To estimate the penetration depth, we used the model experiment: the single LFP particles were measured on a Raman-active silicon substrate. The ratio of the intensities of Si 521 cm^{-1} band to LFP 951 cm^{-1} band was analyzed in dependence of the estimated size of LFP particles (from 0.37 to 7.7 μm). We found that the optical properties can vary from particle to particle and that the penetration depth of the laser beam could exceed several microns.
From one hand, LFP is a transparent material; but from the other hand, the pinhole in the confocal microscopes is supposed to cut-off the inputs from the out-of-focus volume. We discuss this contradiction in terms “excitation” and “response” localizing.
In summary, the observed variation of local probing depths is explained by spatial heterogeneity of defects/impurities concentration, Mie scattering, blocking properties of pores, and surface plasmon resonance. So, in practice, the measured spectra of LFP can contain both superficial and bulk response from a single layer or several layers of particles.



A schematic illustration of possible μRS probing depths for a micro-scale powder system (a)-(c) and a single particle on RS-active substrate (d) and (e).

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Advanced Materials Analysis

A-114

Advances in cryogenic Raman microscopy

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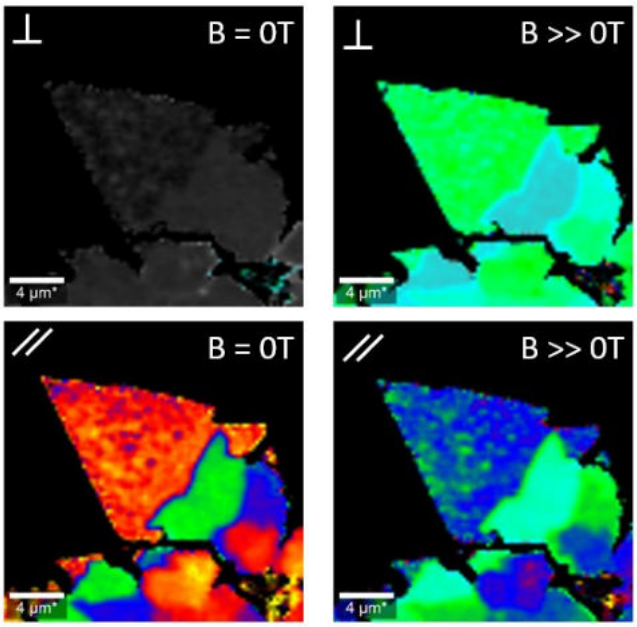
In the last decade, cryogenic Raman spectroscopy in high magnetic fields has become an indispensable tool for studying various novel materials, in particular for researchers focused on phase transitions or emergent properties of low-dimensional materials with application potential in electronics or sensorics. A recent surge in the number of published cryogenic Raman studies is dominated by research on transition metal dichalcogenides and van der Waals heterostructures. In order to meet evolving market requirements, attocube systems and WITec – world leaders in their respective specialties of cryogenic scanning probe microscopy and Raman spectroscopic imaging – teamed up to develop cryoRaman - the cutting-edge solution for cryogenic Raman spectroscopy.
cryoRaman offers a tremendously wide range of experimental possibilities while maintaining a user-friendly mode of operation. In the attoDRY2100 cryostat the sample base temperature is <1.8K and is variable up to 300K. Within this range of temperatures, Raman and photoluminescence peaks can be observed, and their temperature-dependent shifts and changes are of great interest in the study of phase transitions. High resolution Raman maps with a lateral resolution of <400nm not only allow for material characterization, but also for selectively identifying regions of interest. Raman peaks down to 10 rel. 1/cm are conveniently detected and polarization control offers many possibilities in the field of spin-valley physics. This is complemented with unidirectional and vector magnet options. Moreover, cryoRaman offers the sharpest depth resolution on the market (2µm), cryogenic objectives with highest NA (0.82), lowest z-vibrations (1nm peak to peak), most accurate power control (0.1 mW), highest spectral resolution (< 0.8 rel. 1/cm) and highest data acquisition speed enabled by the highest sensitivity (<1ms per spectrum).
Here we exemplify the capabilities of cryoRaman [1] by proof-of-principle measurements dependent on temperature, magnetic field and polarization.



Intensity ratio of two characteristic Raman signals of MoS_2 recorded at 2K for different magnetic field strengths and polarization states

References:

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Advanced Materials Analysis

A-116

Analyse of fructose, glucose and sucrose by Raman spectroscopy

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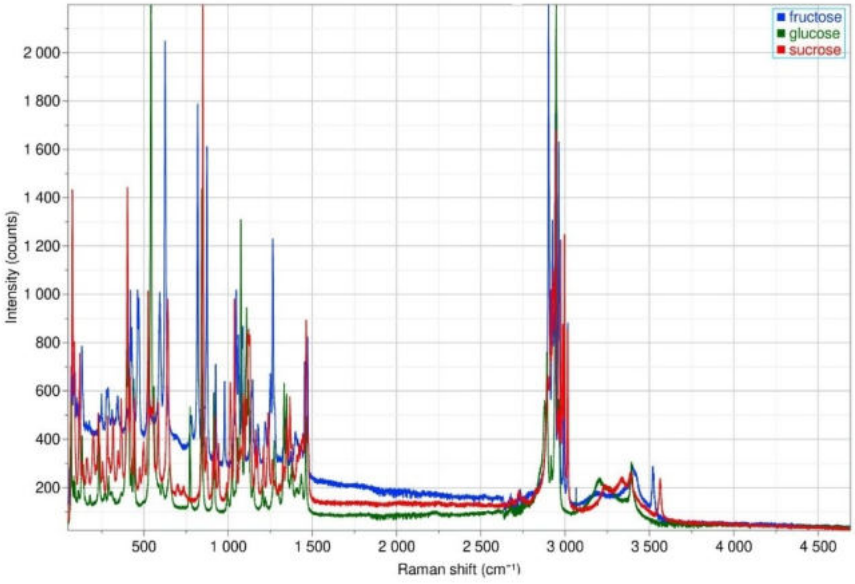
Raman spectroscopy is a useful tool for differentiating sugars and detection of adulteration in natural products [1]. Raman spectroscopy was used to detect the level of simple sugars belonging to the carbohydrate family, which are glucose, fructose and sucrose [2]. We have focused on these three sugars because they are used in the cheating of natural substances, the most famous of which is cheating in honey. Due to the high content of monosaccharides on the honey spectrum and to distinguish the individual contribution of fructose, glucose and sucrose in a honey spectrum, we collected their Raman spectra. The main objective of using a Raman spectroscopy is to compare the characteristic bands between fructose, glucose and sucrose. The wavelength was fixed at 633 nm to attenuate the fluorescence effects, the analysis results show that are some similarities between the three sugar spectra, but there is a difference in intensity, which we have made it possible to easily know the type of sugar, for example, at 825 cm^{-1} the characteristic peak of the C-O-C function is more intense in sucrose than fructose. Otherwise, in glucose the signal of this vibration is less intense.



In this image we saw the difference between the three spectra of fructose, sucrose, glucose by Raman spectroscopy analysis.

Key words: Raman Spectroscopy, Fructose, Glucose, Sucrose, Analyse

References:

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Advanced Materials Analysis

A-122

Development and characterization of nano pyramid arrays as substrates for surface enhanced Raman spectroscopy (SERS)

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Question: Raman spectroscopy is a versatile technique for characterization of biological and material samples by measuring their vibrational states [Langer2020]. A critical drawback is the very low cross section for excitation of Raman scattered light. The Raman signal can be enhanced by using metallic nanostructures. This work addresses the preparation of surface enhanced Raman spectroscopy (SERS) platforms based on nanopyramids on flexible substrates to allow for detection of low abundance analytes [Linn2009].
Methods: Nanosphere lithography was performed with 520 nm diameter polystyrene nanospheres (PN) that were spin-coated onto a (100) silicon wafer. The PNs were then etched with an oxygen plasma to achieve diameters of 300-350 nm, and a chromium etch mask was evaporated. The removal of the PNs left behind a silicon wafer with a chromium layer with nanocavities. A potassium hydroxide solution was used to etch inverted pyramids, then the chromium etch mask was removed. The resulting structure served as the negative mold for the gold nanopyramid arrays. The mold was functionalized with a F_{13}TCS anti-sticking layer, and a 50 nm thin film of gold was evaporated. The silicon mold with the functionalized gold film was pressed onto an elastomeric PDMS patch. The mold could then be stripped off, leaving behind the continuous gold film with the hollow nanopyramid arrays. This could then be used for SERS measurements with 4-Mercaptobenzoic acid (4-MBA). Three different concentrations (2 mM, 100 µM and 50 µM) were chosen to test the SERS sensitivity of the nanopyramid arrays [Simo2021].
Results: The template stripping of the continuous gold film with hexagonal nanopyramid arrays reproducibly transfered to the PDMS while maintaining the pyramidal shape with average baselengths of 400 nm and tip radii of 14 nm. SERS data was acquired with a confocal Raman spectrometer (Renishaw inVia) equipped with a HeNe Laser (633nm). Raman spectra of the 4-MBA were taken on the areas structured with nanopyramids and compared to spectra taken on a continuous gold film on the same sample. Raman maps were acquired over larger areas. The 4-MBA ring breathing mode signal from each grid point was collected and averaged to calculate the enhancement factor. The spectra and maps showed a clear enhancement of about three orders of magnitude on the structured nanopyramid areas compared to the flat gold.

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Advanced Materials Analysis

A-141

The influence of gold nanoparticles supported on TiO_2 nanomaterials in Raman scattering spectroscopy

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In this work, gold nanoparticles loaded prepared mesoporous TiO_2 materials are studied by Raman scattering spectroscopy which is a powerful tool giving information on the crystallinity, phase composition and oxygen vacancy concentrations of nanomaterial [1]. Figure 1 depicts Raman spectra of both pure and gold modified mesoporous Titania catalysts. For all samples, Raman active modes are typical of anatase phase of TiO_2. The Raman lines at 144, 198, 397, 516 and 638 cm^{-1} are assigned to the Eg,(1) , Eg,(2), B1g,1, combination of A1g and B1g(2), and Eg,(3) modes of the TiO_2 anatase phase, respectively [2]. The intensity of Raman spectrum of Titania is stronger than the one of modified Titania with gold nanoparticles at different loadings. As shown in figure 1, the presence of gold species on the Titania surface reduced the intensity of the strongest Eg(1) mode at 144 cm^{-1}. This result indicates that the presence of gold nanoparticles affects the surface properties of Titania. This phenomena was confirmed by other studies in the relevant literature [1, 2]. This statement signifies that Raman bands of Titania was modified after incorporation of gold nanoparticles with a slightly modification of the Raman bands position.

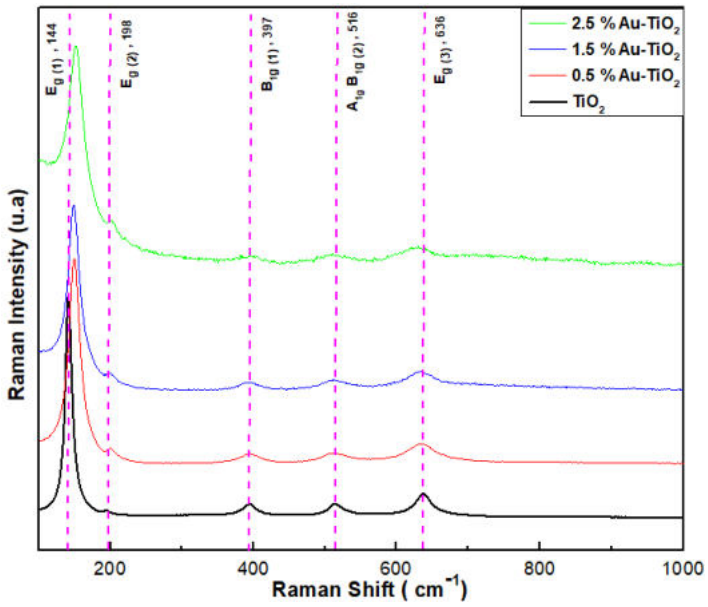

Figure 1. RAMAN spectra of elaborated materials

Reference

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Advanced Materials Analysis

A-142

Raman Spectroscopic characterization of ultrathin $FeTe_2$ Nanocrystals

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$FeTe_2$ nanocrystals are of high interest for application in the new generation of hybrid energy storage devices. In this study, $FeTe_2$ Nanocrystals was synthesized and characterized using WiTec Alpha Raman microscope. Based on characteristic Raman peaks, synthesized nanocrystals was confirmed as $FeTe_2$ crystals. 2D Raman mapping mode are used to identify the size, shape and homogeneity of the nanocrystal. Shape-and size-dependent Raman spectra were observed and discussed.

Advanced Materials Analysis

A-144

In-situ High Temperature Raman spectroscopy for Probing Oxygen Vacancies in undoped and 20 mol% Samaria Doped Ceria Solid Solution

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Doped cerium oxide has been widely used as solid electrolyte for solid oxide fuel cell operating in the temperature range of 673-1073K. The structural properties such as lattice distortion and oxygen defects in the pure CeO_{2-d} and 20 mol% Sm doped ceria ($Ce_{0.8}Sm_{0.2}O_{1.90}$) were studied by in-situ high temperature Raman microscope in the temperature range of 673-1073K. The results clearly show Raman active lattice oscillation at 465 cm^{-1} which is a symmetrical breathing mode of oxygen anions in the fluorite structure called 'F2g' mode [Ref1] (Fig. 1 a&b). The partial reduction behavior of pure ceria observed at temperature >573K was evidenced from the shifting of the F2g mode to lower wave numbers (465-447 cm^{-1}).The phonon frequency revealed that the compressed Ce–O bonds are stiffer which result in the increase of the F2g oscillation frequency. An additional peak at 597 cm^{-1} attributed to a non-degenerate longitudinal optical mode is caused by 'intrinsic oxygen defects' associated with the CeO_8 complex (Fig. 1a). The trivalent Sm^{3+} dopant ceria has introduced 'extrinsic oxygen defects' which gave rise to an additional peak at 557 cm^{-1} in SDC20 (Fig.1b). The oxygen vacancy concentration ($VO\cdot\cdot$) was evaluated by spatial correlation model [Ref2]. The SDC20 shows increased $VO\cdot\cdot$ of 3.21×10^{22} cm^{-1} at 973K which is doubled the value of pure ceria under the ideal conditions. The relative intensity of F2g peak intensities of both the samples were mapped (Fig.1 c-f) to understand the defect distributions. It is noticed that the intrinsic defects in CeO_2 are more active and uniform in the selected grains. The SDC20 shows periodic arrangement of the extrinsic oxygen vacancies with less intense and intrinsic defects were resolved in higher temperature range of 673-1073K (Fig. 1b, e & f). This behavior indicates the deviation of fluorite symmetry at local bonding environment (Ce-O and Sm-O) [Ref3].

The study would further help in understanding the local structural changes of Sm^{3+} ions in ceria such as oxygen vacancies ordering or association effects under the influence of temperature.

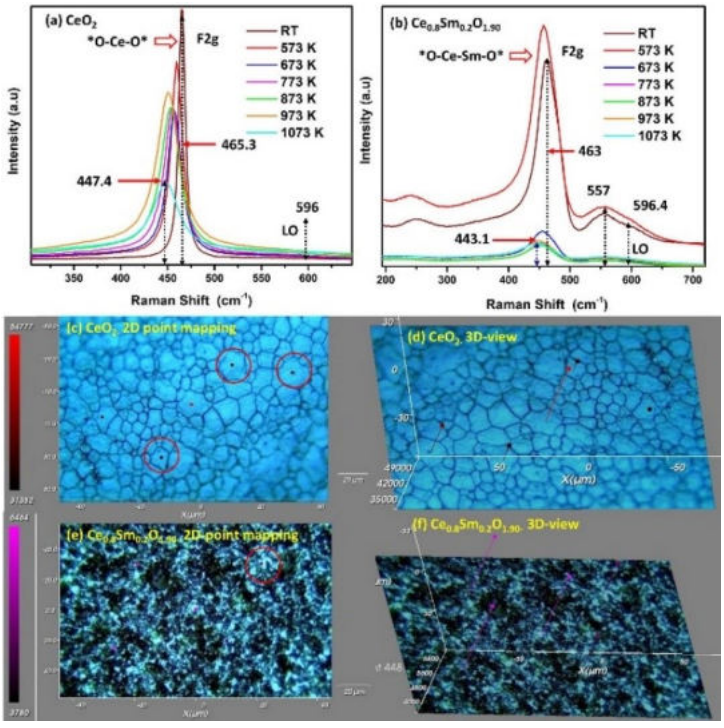

Fig. 1 (a) Raman spectra of CeO_2, (b) SDC20 in the temperature range 573-1073 K, (c) 2D Raman mapped image of CeO_2 acquired at the band intensity 465 cm^{-1} at 1073 K, (d) 3D Raman mapped image of (c), (e) 2D Raman mapped image of SDC20 acquired at the band intensity 463 cm^{-1} at 1073 K, (f) 3D Raman mapped image of (e).

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Poster Abstracts by Topic

Environmental and Geo Science

Environmental and Geoscience

A-056

Screening of microplastics in indoor settled dust using confocal micro–Raman spectroscopy

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Plastic products are widely used in indoor environments. The action of mechanical stressors on these products in indoor spaces could lead to their degradation. This could result in the release of micro-sized particles (<5mm) known as microplastics. They can act as both sources and sink for various hazardous contaminants. The human exposure pathway to these particles includes inhalation, ingestion, and dermal contact. The available literature has highlighted their ability to induce deleterious effects on human health [2]. Thus, in recent decades amplified attention has been given to their detection and quantification in indoor spaces. Indoor dust can be a potential sink for various types of microplastics [1], [2]. The data regarding their presence in indoor spaces in the Indian subcontinent is rather limited. To fill this gap, the present study is aimed at the identification of different types of microplastics in indoor settled dust samples collected from residential settlements in a metropolitan area (Greater Cochin) located in the southwestern part of India. The samples were analyzed using confocal micro - Raman spectroscope equipped with a 532 nm laser. We detected the presence of different types of microplastics such as polystyrene, polypropylene, polyvinyl chloride, and polyamide (figure 1). The concentration of microplastics in the samples ranged from 4.6 – 9.5 particles/g. The dominant type of microplastics was polypropylene.

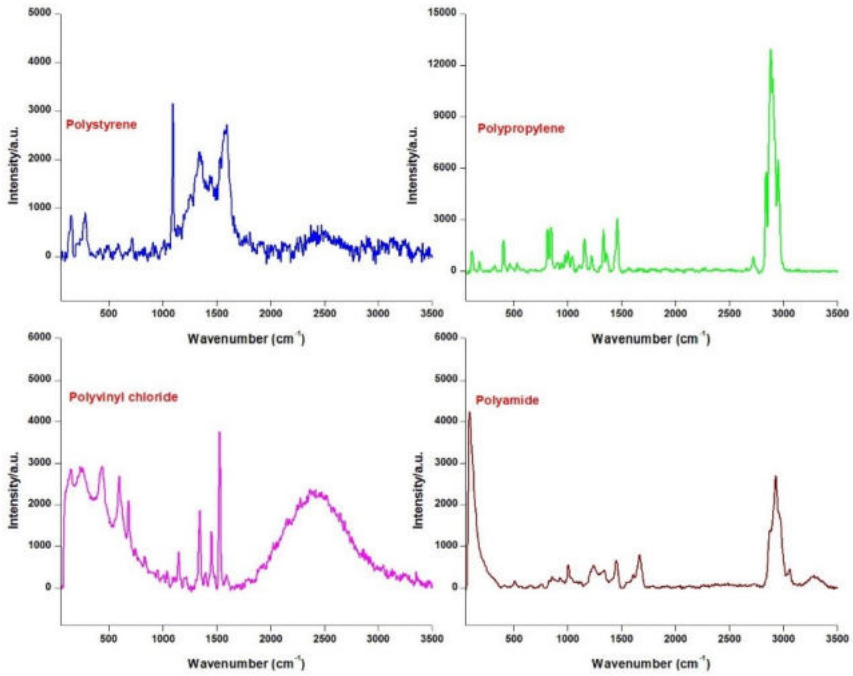

Figure 1: Raman spectra of identified microplastics in the dust

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Environmental and Geoscience

A-095

Detection of Various Nanoplastics via Gold Nanoparticle-Based Surface Enhanced Raman Spectroscopy (SERS) Substrates

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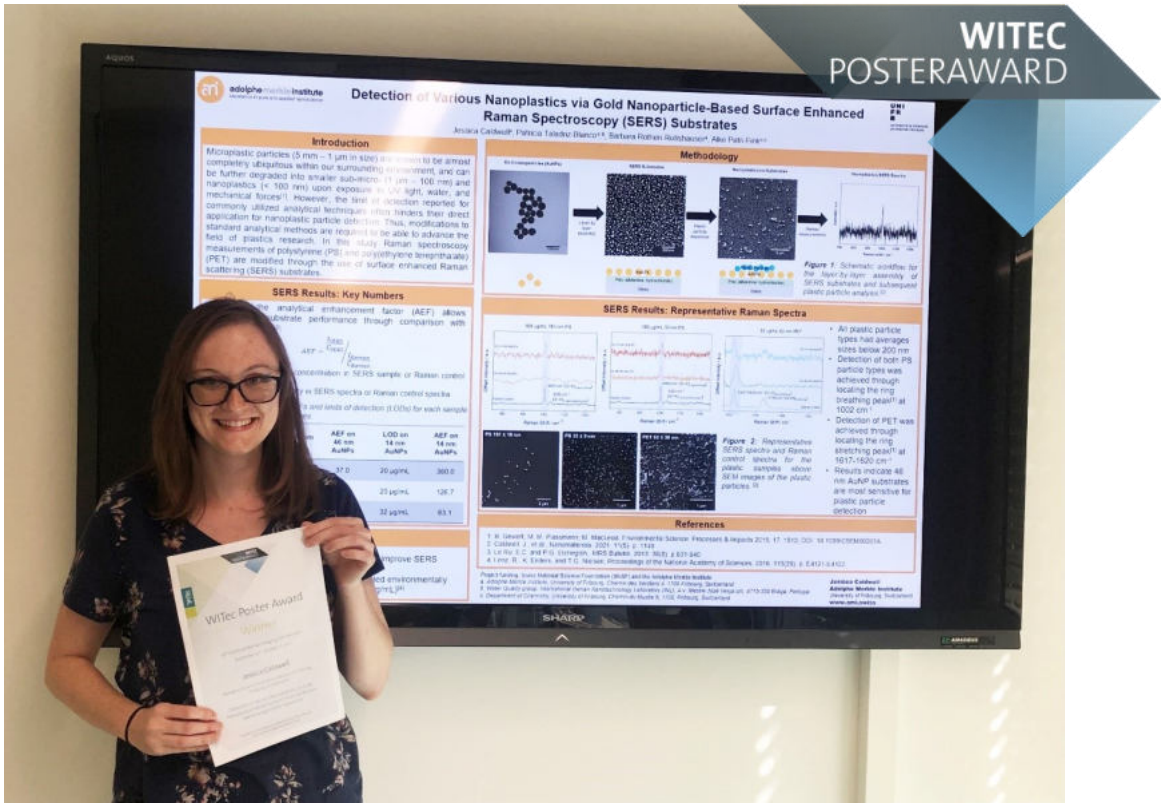
Plastics are reported to be nearly ubiquitous within our surrounding environment. However, to date relatively few methods exist for the reliable detection of nanoplastic particles released into food products or the environment; particularly when the plastic particle concentrations are on the order of micro- or nanograms. This work aims to address this knowledge gap in the field through the use of SERS substrates composed of gold nanoparticles (AuNPs) with various sizes to improve the Raman scattering signal of nanoplastic particle samples. As a proof of concept, the substrates were used to analyze spherical polystyrene (PS) particles with average sizes of 161 nm or 33 nm and milled poly(ethylene terephthalate) (PET) particles with an average size of 62 nm. The limits of detection (LODs) and analytical enhancement factors (AEFs) that could be achieved for the plastic particles were highly dependent on the SERS substrate utilized; with the size of the AuNPs the substrates are composed of impacting the final results obtained.



Environmental and Geoscience

A-099

Online-coupled FFF-Raman approach for the simultaneous separation and detection of nanoplastics and inorganic particles

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With the rising awareness of micro- and nanoplastic pollution in the environment, also the interest in different approaches for the detection of these particles is increasing. While there are several techniques available for the microplastic size range that can provide concentration, size and material characterization, for the nanoplastic size range only recently a technique was developed that could provide this information within a single measurement: An online-coupled field flow fractionation (FFF)-UV-multiangle light scattering (MALS)-Raman approach. It could be shown that this technique allows for particle detection in a size range that is not accessible to common optical microscopy methods. The FFF system is used to separate particles in the size range of 100 nm to 5 µm and the MALS detector can provide the corresponding size information. In this work asymmetric flow FFF (AF4) and centrifugal FFF (CF3) are used to achieve particle separation for different mixtures of synthetic particles. A custom designed flow cell enables the chemical characterization via Raman microspectroscopy in an online-coupled mode to the FFF systems. Using the optical forces (optical tweezers) of the Raman laser, particles are trapped in the focus of the laser beam long enough for spectral acquisition. In the field of nanoplastics this setup has been validated for polystyrene (PS), polyethylene (PE) and polymethylmethacrylate (PMMA) so far. Inorganic particles (SiO_2, TiO_2, Fe_xO_y) can in some cases not only be detected, but also their polymorphs might be distinguished with this technique. Especially for environmental samples there is a great need for the differentiation between nanoplastics and other inorganic particles. Thus, the latest results on the simultaneous separation and characterization of both nanoplastics and inorganic particles will also be presented.

Environmental and Geoscience

A-118

High precision CO_2 densimetry using Raman spectroscopy and a Fluid Density Calibration Apparatus

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Fluid and melt inclusions rich in CO_2 are useful to constrain the P-T-X conditions of fluids that form ore deposits and are our best tool to estimate the pressures of inclusions in volcanic systems. When appropriately calibrated, Raman spectroscopy can determine the density of CO_2 captured in inclusions for most sample sizes (> 1 µm) at great precision. However, discrepancies among Raman-based published CO_2 densimeters are significant and can be related to different calibration procedures, hardware, and lack of measurements for densities between 0.2 and 0.7 g/mL. Here, we structurally re-designed a Fluid Density Calibration Apparatus (FDCA) first described by Lin et al. (2007, GCA). Particularly, we incorporated high accuracy temperature measurements made directly inside the FDCA that significantly reduce the error for critical region measurements. Our new highly precise calibration equations applied to a set of melt inclusions from a Pico do Fogo eruption from Cabo Verde show that the total percent uncertainty in calculated CO_2 contents of bubbles derived from our densimeter are always below 5% except for inclusions with densities in the most sensitive part of the critical region (~7%). We freely provide all the new diagrams, designs and detailed operational procedures with the goal of providing the community a new high-precision and high-accuracy FDCA for Raman spectroscopy. This study has been published as DeVitre et al. (2021, Chem. Geology).

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Environmental and Geoscience

A-121

Raman imaging of polyphase inclusions in K-bearing clinopyroxene from the Kokchetav UHPM rocks

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Findings of Kokchetavite (hexagonal polymorphs of $KAlSi_3O_8$) and K-cymrite ($KAlSi_3O_8*H_2O$) by Raman imaging of the polyphase inclusions in K-bearing clinopyroxene from the Kokchetav UHPM rocks were reported by Mikhno et al. (2013) [1]. These two K-bearing species are important petrological indicators because their stability fields are controlled by H_2O and K_2O activity in fluid/melt. In this study, the confocal Raman images for several inclusions were collected by acquiring 2D arrays of complete Raman spectra from a defined sample area with an Apyron at IGM SB RAS (Novosibirsk). From each area (15 µm x 15 µm) an array of 10000 complete Raman spectra was acquired. The system was equipped with two lasers with an excitation wavelength of 488 nm and 633 nm and a Zeiss 100x (NA j 0.9) air objective resulting in a diffraction-limited laser spot diameter of 360 nm. A system that uses fiber optic cables (e.g. WITec) as the confocal pinhole and for light transmission to the detector is capable of very high-resolution imaging with good fluorescence-suppression capability. The spectrometer was equipped with two gratings, a 300 g/mm (BLZ = 500) and a 1800 g/mm. The used spectroscopic configuration allows the acquisition of complete Raman spectra over the spectral range of 4000 wavenumbers using a CCD camera with 1600 pixels. This leads to a spectral resolution of 2.5 wavenumbers with an accuracy of 0.1 cm^{-1}. The integration time for each spectrum from the spectral array was 1 or 5 s. The thousands of Raman spectra were evaluated using k-means cluster analysis (Dieing & Ibach, 2010) [2]. In this data evaluation method, the acquired Raman spectra are grouped into most similar spectra, and images are generated, which display the distribution of the selected components, their various phases, and/or their strain state. After that additionally, we collected individual Raman spectra of the daughter phases using the grating 1800 g/mm. Both gratings were automatically calibrated using the Ar-Hg lamp. In the present study, we compare the obtained results and discuss the potential problem with the identification of daughter phases within the polyphase inclusions. The study was supported by RSF 18-17-00186.

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Environmental and Geoscience

A-130

Exploring the potential of Raman microspectroscopy for the analysis of microbial degradation of microplastics

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Biodegradation of microplastics in the aquatic environment is not studied sufficiently to predict the fate of microplastics – synthetic polymer particles with a diameter of up to 1 mm [1]. Mainly indirect methods like mass loss of the polymer or formation of CO_2 and other metabolites were often used to determine biodegradation of plastics. Zumstein et al. directly tracked carbon from synthetic polymers in soils into microbial biomass, using Nanoscale secondary ion mass spectrometry (NanoSIMS) [2]. In this work, Raman microspectroscopy (RM) will be applied with the aim to directly monitor the carbon flow from the polymers into the biomass on a single cell level in water samples and thereby detect phenotypical heterogeneity within the microbial community. Therefore, the bacteria *Sphingomonas koreensis*, which was isolated from an environmental aquatic sample, was chosen for the first study due to its potential to biodegradate Polylactide acid (PLA) particles. It contains carotenoids in various cell compartments, which can be used for resonance Raman due to its chromophoric system. This way, Raman spectra can be obtained in only 2 s with a laser power of 1 mW at the sample. Quantitative ^{13}C labeling of carotenoids was previously shown by the group of Huang [3].
Since isotopically labeled polymers are either too expensive or not commercially available, a reverse labeling approach is chosen. The bacterial cells are initially labeled with ^{13}C-glucose, which results in a red-shift of carotenoid signals, which can be linearly assigned to the according ^{13}C-content of the substrate. Once complete ^{13}C -labeling in the bacteria is ensured with resonance RM, bacteria are transferred to minimal medium without carbon source and aged PLA microplastic particles [4] are added to the suspension. If the bacteria can metabolize the polymer, the carotenoid signals should be shifted back to the initial wavenumber, which then directly links the carbon from the polymer into the microbial biomass on a single cell level. D_2O will be added to the reverse labeling, which is known to show microbial activity as H_2O takes part in all known biosynthesis lipid pathways [5]. The ν_2 band of the carotenoids looks promising for the combined labeling approach as its peak position depends on the $^{12}C/^{13}C$ ratio and its intensity on the D_2O uptake.

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Environmental and Geoscience

A-134

Aiming at reliable microplastics identification in the < 10 µm size range using WITec ParticleScout

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The oral uptake of microplastics by the customer is considered as certain [Paul2020]. However, a holistic risk assessment of micro- and nanoplastics (MP and NP) is still not possible. A main contributor to that is the lack of suitable and validated analytical methods to perform a reliable exposure assessment, which includes the quantification of MP and eventually NP in food products.
In our poster, we present ongoing work using a combination of optical particle detection and Raman microspectroscopy as provided by the WITec ParticleScout software to establish a reliable MP identification and quantification protocol with 1 µm as the lower particle size limit. We employ bottled mineral water as a model sample matrix and discuss, amongst others, workflow optimizations regarding the sample positioning, the z-coordinate determination and the choice of parameters for the Raman measurement.

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Environmental and Geoscience

A-135

Characterization of Biofilms used in Microbial Fuel Cells with Raman- and Scanning Electron Microscopy-based Techniques

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It is estimated that 80% of the world's wastewater from industry and private households is discharged into water bodies without a pre-treatment. In developing countries, this leads to a reduction in water quality and thus in the standard of living [1]. The discharge of industrial wastewater in industrial countries, e.g., Germany is regulated by law and serves to minimise environmental pollution. This work focuses on the wastewater treatment in the brewing industry. In this sector, microbiological treatments as a four-stage anaerobic process including the steps of hydrolysis, acidogenesis, acetogenesis and methanogenesis have been established for the degradation of organic compounds in wastewater [2]. A disadvantage of this wastewater method is the high energy requirement of up to 2 kWh/m^3. In addition, this treatment produces greenhouse gases (CO_2 and N_2O), which contribute to the environmental pollution [3]. Here, the renewable energy approach offers an alternative to the conventional wastewater treatment by integrating microbial fuel cells (MFC) into the wastewater treatment process.
To optimise the efficiency of MFCs for real wastewater samples, different strategies are used to develop a stable biofilm with a high degradation rate of the organic wastewater load. One of these strategies fall into the field of microbiology, using wastewater sludge as inoculum for the targeted adaptation of the microbial consortium to real water conditions and the formation of an electroactive biofilm. The project focuses on analysing the biofilm using imaging methods such as scanning electron microscopy (SEM) and Raman microspectroscopy (RM) to understand the mechanisms of electricity production [4]. The use of RM enables the spatial *in situ* analysis of chemical components of a biofilm during the stages of the primary cell attachment, biofilm formation and the maturation of biofilms. These include polysaccharides, inorganic compounds, and cellular components, such as the protein cytochrome *c* or carotenoids, which are particularly responsible for the conduction of electrons in an electroactive biofilm. SEM completes the information obtained by RM with the statement on the three-dimensional structure of biofilms. Altogether should help to get a more detailed information on the biofilm composition and structure, and to improve the performance of MFCs.

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Environmental and Geoscience

A-137

Confocal Raman imaging for detection and quantification of graphene oxide in seawater during bioaccumulation tests

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Since its first successful synthesis and characterization attempt in early 2000s [1], graphene oxide (GOx) has been transformed into a very important material of the 21st century. Thanks to its unique electronic, mechanical and chemical properties, its applications can be found nowadays in many aspects of modern life [2]. The fast growing and strong demand for GOx-based industrial products, is followed by an increased possibility of their direct interaction with human and environment, hence rendering necessary safety precautionary actions to understand the impact of the use of nanomaterials in daily consumer goods. Moreover, this growth of production promotes an increase of GOx release into the aquatic environment with some studies pointing out that the environmentally relevant concentration of GOx could reach 1 mg/L [3]. Therefore, the identification and quantification of GOx is the first step to evaluate the environmental risk. Raman spectroscopy is a well-established technique for characterizing GOx, which has the unique signals G band and D band [4]. Here, we design an analytical approach to accurately determine the concentration of GOx dispersed in seawater exposed to mussels by Raman imaging. This approach involves the preconcentration of GOx by filtering 60 mL of seawater using a lab-made PMMA chamber and subsequently their detection on the nylon membrane by mapping of Raman peaks' intensity [5] at 1350 cm^{-1} (D band) and 1585 cm^{-1} (G band) into an area of 1000 x 1000 µm. This analytical method presents a linear range of 0.01 to 0.1 mg/L, being able to detect concentration in the ng/L range. This method allows the determination of GOx concentration in seawater during a bioaccumulation assay on mussels. Moreover, this approach could also be adapted for other types of carbon-based nanomaterials (e.g. carbon nanotubes, carbon dots) or water samples (e.g. freshwater).

Acknowledgements: This research was funded by H2020 project SbD4Nano (862195).

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Poster Abstracts by Topic

Life Sciences, Biomedical and Pharma Research

Life Sciences, Biomedical and Pharma Research

A-027

Raman spectroscopy and machine learning for prediction and characterization of embryonic stem cells during iPS differentiation and reprogramming

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A number of investigations has addressed the use of label-free spontaneous Raman spectroscopy to monitor the differentiation process in stem cells as an alternative to destructive or invasive methods. In this study, using label-free imaging Raman spectroscopy combined with intelligent algorithms, we analysed the reprograming process in an isogenic mouse cell line and compared reprogrammed cells with their original embryonic stem cells (ES) and differentiated cells. Neural network, regression models, and ratiometric analyses were used to discriminate the cell states and extract several important biomarkers specific to differentiation or reprogramming. After appropriate processing, the spectral signatures of single living cells were used as an input of neural network model. Results show a high accuracy in predicting the ES cells, neuronal cells, and cells after 10 or 20 days of reprogramming (with sensitivity and specificity scores of 92% or more). By contrast, an intermediate cell state (5 days of reprogramming) exhibited a lower predictive accuracy (66%) which reveal an heterogenous population of metabolic signatures. Linear regression models and ratiometric analyses confirmed this result and allowed us to extract several spectral wavelengths to characterize and predict the different states. Interestingly, our results make a strong case for substantial differences between stem cells (ES) and cells undergoing reprogramming, notably through the contribution of the lipid band at 1445 cm^{-1} which is associated to synthesis of fatty acids. This difference was seen even in cells after 20 days of reprogramming which are positive for pluripotency markers (Nanog, Oct4, Sox2 and SSEA-1). Finally, we also showed that the laser does not damage living cells, underlying the technical advantage of our method.

Life Sciences, Biomedical and Pharma Research

A-031

An injectable SERS based plasmonic sensor for continuous remote cancer progression in patients

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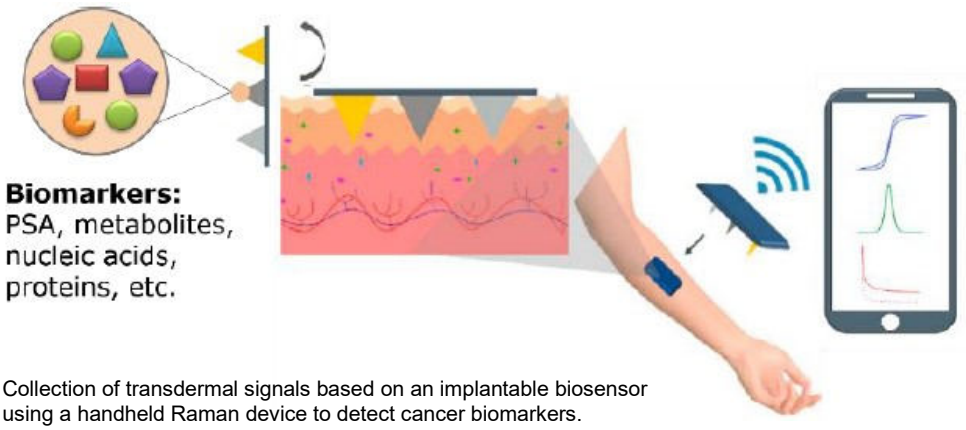
Remote patient monitoring (RPM) of cancer diseases can be potentially used to increase current predictive rates, while contributing for a more cost-effective and accessible diagnosis and treatment. Patients at high risk of cancer recurrence would constitute an ideal population for such improved cancer monitoring tools. These novel tools should have the ability to remotely monitor patient data, which should be used to detect disease onset or progression [1].

Herein, we propose the development of a remote nanobiosensor for high-risk profile cancer patients, to be injected in the dermis layer of the skin. To develop this nanobiosensor, gold nanostars (GNSs) were selected as the plasmonic material to be embedded in hydrogels that will serve as the biocompatible matrix to support the GNSs. These gold nanoparticles, were characterised morphologically, optically and spectroscopically (TEM, UV-Vis-NIR and Raman) [2].

This hybrid nanobiosensor will be injected subcutaneously, and the biomolecules in the interstitial fluid (ISF) will diffuse through the sensor and subsequently detected by means of surface-enhanced Raman scattering (SERS) spectroscopy. The main function of SERS will be to obtain the fingerprint of the cancer biomarkers in close vicinity to the GNSs [3], [4].

In previous results, it was shown that hydrogels with more concentration of GNSs associated to the Raman Reporter 4-MBA, had a higher signal than those with a lower concentration of gold, as expected. Moreover, we performed the SERS analysis of individual components of ISF and of whole artificial ISF to first have a library of the expected Raman signals [5].



Collection of transdermal signals based on an implantable biosensor using a handheld Raman device to detect cancer biomarkers.

The next steps are to detect tumor biomolecules by SERS, from clinical samples (serum, plasma, cellular supernatants, etc.) that will be used in the future, to train a machine learning system for the classification of prostate cancer patients with the intradermal implants.

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Life Sciences, Biomedical and Pharma Research

A-038

Regulatory role of Rac1 in lipid droplets formation, and nanostructural changes induced by TNF in vascular endothelium in the isolated murine aorta

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Endothelial inflammation is recognized as a critical condition in the development of cardiovascular disorders. Changes in the biochemical (formation of lipid droplets determined Raman and fluorescence imaging), and nanostructural properties (AFM and SEM imaging) of TNF-activated endothelial cells (ECs) in the isolated blood vessels were ascribed to the development of vascular inflammation, as evidenced by overexpression by ICAM-1, but the mechanical insight linking these parameters to each other was missing.
Using the combination of techniques: Raman spectroscopy, fluorescence imaging, atomic force (AFM), and scanning electron microscopy (SEM) we demonstrated that formation of lipid droplets (LDs), and nanostructural properties were Rac1-dependant and partially reversible by the inhibition of Rac1. Raman imaging uncovered that the reservoir of LDs included mainly LDs rich in highly unsaturated lipids and negligible content of cholesterols and phospholipids. Furthermore, it was possible to distinguish LDs localized in endothelium and smooth muscle cells (SMCs).
In conclusion, this work demonstrated that Rac1 activation is the mechanism of crosslinking biochemical, and nanostructural alterations of TNF-induced endothelial cell inflammation. In particular, we revealed a significant role of Rac1 in the regulation of the formation of high-unsaturated LDs. Our results suggest Rac1 as a central pathway in the regulation of biochemical, and nanostructural aspects of vascular inflammation.

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Life Sciences, Biomedical and Pharma Research

A-039

Inside the cell therapy of neurodegenerative diseases – spectroscopic investigation of intercellular interactions of endothelial progenitor and brain endothelial cells

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Alzheimer's disease (AD) is a neurodegenerative disease that is mainly manifested by progressive cognitive dysfunction. It accounts for the major proportion (65–70%) of dementia in the elder population. However, there is no effective drug to block the development of AD. As impairment of the blood-brain barrier (BBB) leads to abnormal amyloid-β (Aβ) transport, which is crucial in the pathogenesis of AD, the repair of BBB is a novel approach in the AD treatment [1].
The potential of migration and interaction of endothelial progenitor cells (EPCs) with brain endothelium indicated their role as carriers for drug (ex. therapeutic protein encoded inside the cell) [2]. However, the conventionally used imaging techniques are not specific enough to investigate these interactions. Therefore, the aim of this research is to show the great potential of FTIR and Raman spectroscopic imaging as techniques assessing biochemical characterization of early endothelial progenitor cells (EPCs) and brain endothelial cells (BECs).
The experiment was based on the cultures of immortalized early endothelial progenitor cell line established from aorta–gonad–mesonephros (AGM) of murine embryo [3] and brain endothelial cells. After their deposition on CaF_2 windows, the cells were examined with high resolution Raman Confocal Imaging. The research was completed with Fourier-Transform Infrared Imaging. Spectroscopic methods assisted with a chemometric analysis enabled the establishment of the characteristic spectral biomarkers of the investigated cultures. k-Means cluster analysis of Raman images of single cells discriminated EPCs as cell-culture with enhanced presence of lipid droplets with saturated fatty acids and an increased content of cytochromes in endoplasmic reticulum. Fast FTIR imaging confirmed these observations. Principal component analysis on Raman and FTIR spectra clearly separated both cultures with variance of ca. 30%. Next, the mixture of investigated cells was examined. The comparative and PCA analysis of the co-cultured cells indicated intercellular interactions between BECs and EPCs.

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Life Sciences, Biomedical and Pharma Research

A-040

Fungi identification by Surface Enhanced Raman Spectroscopy with non-conventional nanoparticles

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We present results on the use of Surface Enhanced Raman Spectroscopy (SERS) to fungi (*Candida albicans*, and *Fusarium ssp*) identification. In this preliminary study, nanoparticles (NP) of diverse elements such as Ag, Au, and “non-conventional” [1], [2] Co, Sb, Sn, Ni, and Bi were synthesized via laser ablation and used as SERS substrates. Fungi identification has relied almost exclusively on conventional culture-based methods that are laborious and time-consuming. The introduction of molecular, DNA-based identification techniques − which involves PCR or its variations − provide increased analysis reliability, reducing turnaround times. As an alternative, optical vibrational methods, particularly SERS is highly sensitive, fast, cost-effective, and may provide chemical specificity for the identification of biological molecules and microorganisms [3], [4], emerging as a promising tool to both characterize and differentiate fungal species. The nanoparticles used in this study were characterized by UV-Vis optical absorption, dynamic light scattering (DLS), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The Raman/SERS spectra and images were obtained using a confocal Raman Alpha 300R microscope from WiTec working in the backscattering mode (532 nm, 10 mW). Each clinical isolate was cultured on Sabouraud dextrose agar and aqueous suspensions were produced right before the measurements. SERS activity was demonstrated by mixing microliter drops of both NP colloids and fungi suspensions onto microscope slides, and Raman spectra (100 averages) were taken for 0.5 seconds. Our results using TEM and Raman show images NPs on fungi surface with clear hotspot identification, in addition to indicate that non-conventional NP lower background Raman fluorescence signals, compared with Au and Ag SERS spectra, allowing for better sample analysis in SERS mode, even in an aqueous environment. The characteristic peaks in the spectral window from 600 cm^{-1} to 1800 cm^{-1} permit the characterization of *Candida albicans* and *Fusarium ssp* and represent the first step in fungi SERS identification using non-conventional NP. This study offers an alternative for conventional fungal diagnostics with promising application perspectives in fast cost-effective and label-free diagnostic tests for fungal pathogen detection and identification.

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Life Sciences, Biomedical and Pharma Research

A-041

Biochemical characterisation of lipid droplets induced by lipopolysaccharides and tumor necrosis factor-α during endothelial inflammation studied using Raman spectroscopy

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Endothelium covers the innermost layer of blood and lymphatic vessels and fulfils many functions maintaining cardiovascular homeostasis, for example regulates the vessel diameter, takes part in the immune system functioning and regulates the endothelial inflammation.[1]
The endothelial inflammation is known to have a huge contribution to the development of life-style diseases as atherosclerosis, diabetes type 2 or cancers. The inflammation can be induced by many substances such as lipopolysaccharides (LPS), and tumor necrosis factor-α (TNF-α). They are both proinflammatory factors, but they have different origin and signaling pathways: TNF-α is a cytokine, while LPS is a derivative from the cell wall of Gram negative bacteria, and it is an endotoxin.[2]
In the endothelium stimulated with both: LPS or TNF-α, the lipid droplets (LDs) formation was negligible, their content was quickly metabolized as evidenced by the effect of atglistatin (Atgl). In the presence of atglistatin, LPS or TNF-α induced the LDs formation was studied by Raman spectroscopy to gain information about their chemical composition and level of unsaturation, and by fluorescence microscopy to localize their distribution in the tissues. The results uncovered that the LDs stimulated by LPS or TNF-α in the presence of atglistatin were found in the isolated murine aorta in large numbers, they have high level of unsaturation and negligible content of cholesterols and phospholipids.
In summary, in this work we analyze the changes in the biochemical composition of the LDs in LPS or TNF-α induced endothelial cells inflammation. Although the pathway of endothelial activation by LPS or TNF-α were different, occurrence, heterogeneity of biochemical composition, and size of LDs formed in ECs within *en face* aorta stimulated by LPS+Atgl, TNF-α+Atgl were similar in both cases.

This work was supported by the National Science Centre, Poland, SONATINA1 No.: DEC-2017/24/C/ST4/00075.

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Life Sciences, Biomedical and Pharma Research

A-057

Confocal Raman imaging of hydrogel coated microfiber scaffolds

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Microfiber scaffolds emerged as favorable matrices for many biomedical applications such as drug-delivery systems and implants. Suitable polymers for this approach have to exhibit appropriate characteristics to allow for cytocompatibility, biodegradability, and mechanical strength. Poly(ε-caprolactone) (PCL) features the aforementioned properties but is also rather hydrophobic hampering its biomedical use. To combat this major disadvantage, the polymer system can be coated with a hydrogel leading to improved surface hydrophilicity. In this study, a PCL microfiber scaffold is fabricated by melt electrowriting and subsequently coated with the hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA). However, analytical characterization of such multi-component systems is challenging concerning the differentiation of individual components. As an integration of dyes might modify the physicochemical behavior of the polymers and labeling after manufacturing in three-dimensional scaffolds is challenging, we strived to implement confocal Raman microscopy for the evaluation of component distribution in polymer systems with multiple components. Raman imaging was used for spatially resolved polymer identification based on chemical moieties. Raman spectra revealed a unique band at 1111 cm^{-1} indicating characteristic backbone stretching of aliphatic chains [ν(C–C)] in PCL. Also, a specific peak at 829 cm^{-1} was detected and is attributed to the symmetrical stretching vibration [ν(C–O–C)] of ester groups in PHEMA. The corresponding overlayed false-color image allowed for the differentiation of PCL fibers and polymerized hydrogel spanning the meshes of the scaffold. In addition, single PCL strands within the coating were visualized, displaying defects that could not be identified with light microscopy. A three-dimensional specimen model was reconstructed based on Raman data of z-stacks depicting hydrogel coating spanning the PCL meshes in a “hammock-like” manner. Confocal Raman microscopy successfully visualized the PCL scaffold and confirmed the presence of the hydrophilic PHEMA coating. The spatially resolved visualization of the coating distribution was pivotal to achieve a detailed insight into the polymerization process, where the surface-initiated polymerization is confounded by the expected, still undesired bulk polymerization. These findings highlight Raman imaging as a powerful tool for the chemical selective analysis of multicomponent polymer systems.

Life Sciences, Biomedical and Pharma Research

A-071

Feasibility of integrated high-wavenumber Raman imaging and fingerprint Raman spectroscopy for fast margin assessment in breast cancer surgery

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Intraoperative assessment of surgical margins remains one of the main challenges in cancer surgery. Raman spectroscopy can detect cancer cells with high accuracy, but it is time-consuming. In this study, we investigated a selective sampling Raman spectroscopy approach, based on high wavenumber (HW) Raman imaging (spectral range 2,500–3,500 cm^{-1}) and fingerprint Raman spectroscopy (spectral range 600–1,800 cm^{-1}), to reduce the overall tissue analysis time while maintaining high diagnostic accuracy. HW Raman mapping was used as a first step to identify the adipose tissue regions based on the C–H stretching bands at 2,700–2,950 cm^{-1}. As residual tumors are typically found in non-adipose tissue, an algorithm was developed to allocate sampling points for fingerprint Raman spectroscopy at locations corresponding to low intensity in the HW-Raman maps. Preliminary results show that HW-Raman imaging based on a 671 nm laser is effective and fast for mapping of adipose tissue in breast resections, with typical imaging times of 2 min for tissue areas as large as 2 × 2 cm^2 areas. Albeit the remaining high fluorescence background in the fingerprint region prevents the use of single 671-nm laser, the HW Raman imaging can be still exploited in combination with 785-nm excitation Raman spectroscopy for identifying residual tumor. Although this study demonstrates the feasibility of this approach, further improvements, such as using single element detectors for HW Raman imaging, are required to increase the analysis speed further towards intraoperative use in the routine clinical setting.

Life Sciences, Biomedical and Pharma Research

A-075

In Vivo Biomolecular Imaging of Zebrafish Embryos using Confocal Raman Spectroscopy

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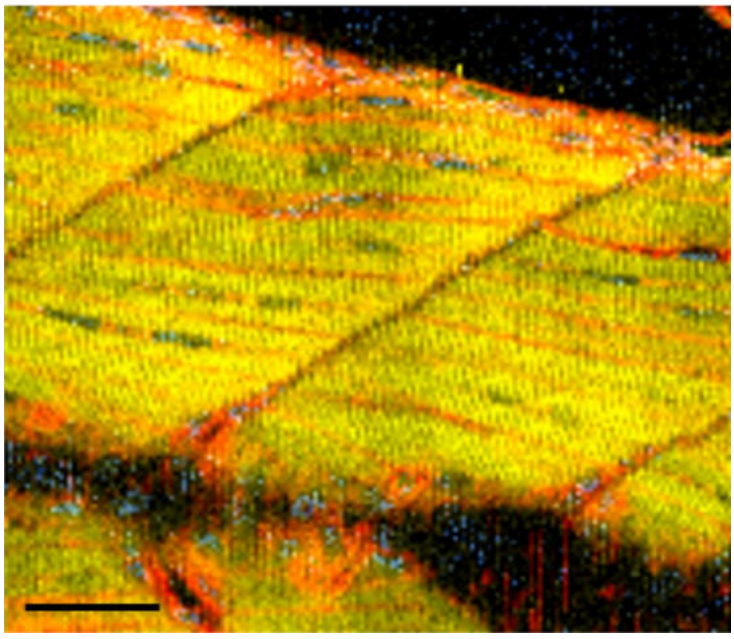
Zebrafish embryos are widely used for microscopic analysis of complex biological processes, typically using fluorescence microscopy. Here, we demonstrate that confocal Raman spectroscopic imaging can be used as a complementary approach for biomolecular imaging and analysis of zebrafish embryos. Raman spectroscopic imaging can be used to directly obtain hyperspectral datasets without the use of labeling and can be used in combination with multivariate component analysis to visualize biomolecular features in biological samples [1]. We outline a workflow of sample preparation, imaging, and analysis and validate this method by collecting three-dimensional biomolecular images of whole zebrafish embryos and resolving fine anatomical features at subcellular spatial resolution. We also apply confocal Raman spectroscopic imaging for the biomolecular profiling and discrimination of wild-type and ΔRD1 mutant mycobacteria in a zebrafish embryo model of tuberculosis. Finally, we demonstrate the use of confocal Raman spectroscopic imaging for in vivo temporal monitoring of the wound response in living zebrafish embryos. Overall, confocal Raman spectroscopic imaging constitutes a new imaging modality for zebrafish research, enabling the first comprehensive biomolecular analysis in fully intact and living zebrafish embryos [2].



High-resolution confocal Raman spectroscopic image of zebrafish embryo muscle tissue. collagen-rich regions at 918 ± 20 cm^{-1} (yellow), DNA-rich regions at 789 ± 10 cm^{-1} (blue), lipid-rich regions at 2850 ± 10 cm^{-1} (red) Scale bar: 50 µm

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Life Sciences, Biomedical and Pharma Research

A-080

Matrix microstructure and chemically-selective visualization of components distribution for estimation of drug release mechanism in transdermal delivery systems

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Silicone-based pressure sensitive adhesives (PSA) are considered as an alternative for polyacrylate PSA drug carriers due to thoroughly investigated biocompatibility. However, the potential use of silicone type of PSA is still limited mainly by the insufficiently evaluated drug-polymer interactions and as a result, the diffusivity of the matrices crucial for the prediction of drug release. In consequence, it can be discouraging for formulating transdermal delivery systems (TDS) with the silicone PSA, especially on the early stage of the pharmaceutical dosage form development. Although number of research with standard straightforward formulating approach relying on *in vitro* permeation tests and basic physicochemical state evaluation have been reported, little was done for detailed visualisation of the TDS and its microstructure.
In order to gain comprehensive understanding of the observed drug release rates from silicone based TDS, imaging approaches – scanning electron microscope and Raman microscopy were implemented in the presented study. Silicone type PSA (BioPSA 4502, DowCorning) was used as an adhesive matrix for the TDS. Silicone oil (SO) or propylene glycol (PG) were added to examine the possibility of modification of the diffusive properties of the silicone PSA for a model drug – indomethacin (non-steroidal uninflammatory drug). Surface of the patches was evaluated with Raman microscopy. Detailed maps of indomethacin distribution on the surface of the silicone TDS were obtained before and after the *in vitro* release test (PhEur paddle over disc protocol). In order to determine excipients-depended (SO or PG) alterations, not only further Raman mapping was conducted, but also PSA microstructure imaging by SEM was implemented.
Based on these results, cause of decrease in the release rate of indomethacin from silicone TDS in presence of PG was identified. Spectroscopy-based analysis correlated with SEM imaging proved to be a powerful tool for an innovative approach of the analysis of transdermal delivery systems on the early stage of pharmaceutical dosage form development.

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Life Sciences, Biomedical and Pharma Research

A-082

Development of a Tri-Functional Nanoprobe for Background-Free SERS Detection of Sialic Acid on Cell Surface

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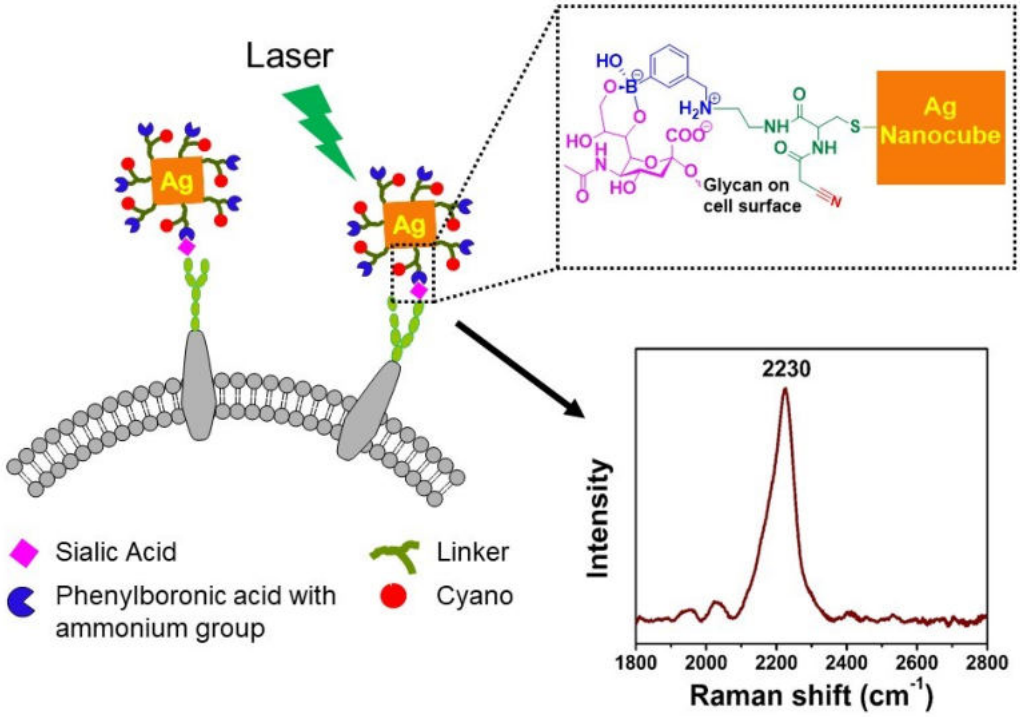
Sialic acid on the cells surface plays important roles in numerous physiological and pathological processes. Therefore, the sensitive and reproducible detection of sialic acid is crucial for diagnosis and therapy in many diseases. In this work, we design and synthesize a tri-functional nanoprobe as a sensitive and straightforward surface-enhanced Raman spectroscopy (SERS) nanoprobe, containing reporter conjugated silver nanocubes, for sialoglycan detection on cell surface. The reporter provides three key functionalities that make it ideal for sialic acid detection. First, we employed two recognition groups, phenylboronic acid and an ammonium group, that enhance sialic acid recognition and capture efficiency. Second, we used cyano as the Raman reporter because its vibrational molecules emit in the cellular Raman silent region. Finally, thiol acted as an anchoring agent to conjugate the reporter to silver nanocubes to provide SERS enhancement and protect silver nanocubes from oxidation. Our molecular nanoprobe design demonstrated the ability to detect sialic acid on the cell surface with high sensitivity and selectivity, opening up new routes to cellular diagnostics.



Life Sciences, Biomedical and Pharma Research

A-083

Raman microscopy in monitoring adipogenesis – investigation of primary adipocytes vs mature preadipocytes

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As a multifunctional organ, despite the physiological diversity and distant anatomical residues, the adipose tissue (AT) is a consistent pool of various cell types. Among typically mature, fully developed adipocytes and other fractions, e.g. immunocompetent macrophages or endothelial progenitors, there is a self-renewing line represented by a section of fibroblastic adipose-derived stromal/stem cells (ASCs). Growing interest in better understanding of malfunctioned mechanisms correlated with obesity shed more light on the regulatory properties of ASCs and emerged from them preadipocytes [1], particularly in the context of proper biogenesis of lipid droplets (LDs) responsible for energy storage and cellular metabolism of fatty acids [2]. The importance of well-conducted adipogenesis led to the need for thorough monitoring of cells at different stages of adipocyte growth and increased the demand for more specific insight into the differentiation process under pathophysiological conditions. In fact, there is a rising emphasis on the role of the perivascular adipose tissue (PVAT) in the development of numerous disorders including diabetes, insulin resistance, and cardiovascular diseases such as atherosclerosis [3]. However, no commercial PVAT cell lines are currently available so there appears a niche for representative *in vitro* models of AT suitable for mechanism studies and drug testing.
The purpose of this work was to monitor the development of cell cultures of ASCs isolated from epididymal (eWAT) and interscapular (iBAT) adipose tissue of C57Bl/6 mice, using Raman imaging (WITec Alpha, Ulm, Germany) to compare phenotype and spectral transitions of both types of AT during adipogenesis and *de novo* LDs formation at the selected time points. Obtained data indicate massive changes in the chemical composition corresponding to morphology alteration and progressive decrease in lipid unsaturation degree (I_{1660}/I_{1440}) within the next stages of the cell maturation. Our results outline that Raman microscopy finds useful application as a non-invasive approach and supporting method to biomolecular techniques in the search for an association between shifts in lipidome matter and the state of cellular integrity during structural conversions in evolving AT.

Acknowledgments: The study was supported by the project from the National Science Centre Poland (NCN) (OPUS17, 2019/33/B/ST4/0087).

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Life Sciences, Biomedical and Pharma Research

A-089

Tracking mucus secretion in *in vitro* models of the human intestinal mucosa

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The cultivation of *in vitro* cell culture systems to mimic human tissues and organs has experienced a surge in popularity over the last decades as a promising alternative to animal models. In the field of preclinical testing of novel pharmaceutics, the implementation of *in vitro* models of human biological barriers, like the lung and the intestinal epithelium, holds special importance with respect to resorption and targeted drug delivery studies. Here, the passage of the drug molecule is not only hindered by the cellular barrier but is gravely impeded by the presence of a mucus layer on top of the epithelium. Hence, modelling the mucus layer in these *in vitro* models is crucial and calls for a comprehensive evaluation of its formation, ideally before or during experiments. Since conventional approaches use histology or *in situ* staining approaches, which require fixation of the sample, the use of dyes and are prone to washing away the mucus, confocal Raman microscopy was evaluated as a means of non-invasive visualisation of mucin production and secretion.
In this study, three different *in vitro* models of the human intestine, composed of monocultures of either enterocytes or goblet cells, as well as co-cultures of both cell types, were grown over the course of 21 days. The cultures were analysed regarding the mucus production and secretion, which was found to be minimal in enterocytes and very pronounced in goblet cell cultures, in accordance with literature. Co-cultures were found to increase mucin production over the course of the 21-day cultivation period. The same models were analysed using established approaches, including *in situ* alcian blue staining and histological analysis, which confirmed the results from the Raman-based imaging approach. Further, findings were compared to histological and Raman-based analysis of biopsies of the human intestine, which confirmed high Raman spectral similarity between *ex vivo* and *in vitro* mucins and a physiological amount of mucus within the *in vitro* models.
These findings highlight confocal Raman microscopy as a reliable tool for the non-invasive analysis of mucus secretion in *in vitro* cell culture models of the human intestine with the benefit of preserving the fragile mucus layer as an advantage over conventional analysis approaches. The study presents a promising starting point for future *in situ* analysis of drug-tissue-interactions at the human intestinal mucosa in the context of preclinical studies.

Life Sciences, Biomedical and Pharma Research

A-094

An insight into perivascular adipose tissue growth due to short-term high fat diet by Raman imaging

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In 2020, it was reported that as many as 39 million children under the age of 5 were obese or overweight, almost a million more than the year before (38.2 million) [1]. Obesity is associated with excessive lipid accumulation and leads to a chronic inflammatory state of the adipose tissue. Importantly, adipose tissue may enlarge improperly due to excessive energy intake. The growth mechanism may occur in two ways: hypertrophy (cell size increase) and hyperplasia (cell number increase) [2]. Moreover, the adipose tissue phenotype is local-dependent and leads to different tissue responses to pathological factors. One of the adipose tissues that surround large blood vessels is called aortic perivascular adipose tissue (PVAT) and may have features of brown adipose tissue (BAT) i.e. thoracic aortic PVAT (TA PVAT) and white adipose tissue (WAT) i.e. abdominal aortic PVAT (AA PVAT) [3]. Since PVAT is involved in vascular homeostasis and what is more TA and AA PVAT respond differently to HFD [4] it is extremely important to take into account these two sections separately in cardiometabolic study.
This work aimed to investigate the effect of a short-term high-fat diet (HFD) on various types of adipose tissue using Raman microscopy. The results show that HFD has a significant effect on chemical composition and structural changes in adipose tissue. It has been observed, among others, that HFD causes hypertrophy of TA and AA PVAT, but not interscapular BAT (iBAT) nor epididymal WAT (eWAT). What is more, the unsaturation of lipids decreases in lipid droplets of eWAT what can be associated with the excess accumulation of saturated fat. There is also detected an increase in the number of lipid droplets in iBAT, but without alteration in the degree of unsaturation. First of all, these studies highlight that the response of adipose tissue to HFD depends on its phenotype and localization. Even adipose tissues with a similar phenotype change differently. Additionally, it proves that Raman imaging is a sensitive tool for the examination of adipose tissue from various locations and enables visualization of adipocytes in fresh adipose tissue.

Acknowledgments: The project was supported by the National Science Centre (AK: OPUS 17, 2019/33/B/ST4/00878).

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Life Sciences, Biomedical and Pharma Research

A-096

Raman imaging of cationic amphiphilic drug-induced phospholipidosis in endothelial cells

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Cationic amphiphilic drugs (CADs) of various therapeutics actions, e.g. antipsychotic, antidepressant, antiarrhythmic and antimalarial can contribute to drug-induced phospholipidosis (DIPL) in cells and tissues. Moreover, CADs can decrease cells viability, activate autophagy but little is known about their relationship with DIPL in endothelial cells.

The objective was to develop Raman imaging protocol for label-free spectroscopic detection of drug – induced phospholipidosis (DIPL) in vitro. In view of growing interest in Raman spectroscopy based methodology in pharma research, we present a spectroscopic approach for accurate and sensitive DIPL detection.

Antidepressant fluoxetine, antipsychotic clozapine, and antiarrhythmic amiodarone, from the group of CADs served here as model compounds to induce DIPL in HMEC-1 (human microvascular endothelial cell-1) cell line. We compared spectroscopic markers of DIPL in endothelium defined as an increased content of choline-containing lipids, fatty acids, cholesterol esters and robust increase in the ratio (lipid/(protein + lipid)) with spectral signature of starvation – induced autophagy which manifested only with subtle changes in the lipid profile, proving biochemical changes due to DIPL are distinct from autophagy. Lipid abundance and distribution was estimated with ratiometric Raman imaging based on lipid/(protein + lipid) ratio.

We also demonstrated with fluorescence staining (LysoTracker, LipidTox, LC3B, and JC-1) that lysosomal trapping, phospholipidosis and autophagy activation as a sequence of events is not a direct source of the reduction of endothelial viability caused by fluoxetine and amiodarone but mitochondrial membrane depolarization is related to cytotoxicity. All those events are depended on accumulation of a drugs in acidic lysosome environment, whereas toxicity is attributed to mitochondrial mechanisms. Based on its physicochemical properties antipsychotic risperidone can be considered as a drug with potential to induce DIPL, in fact is devoid of such action, so *in silico* models, to predict phospholipidosis are not always accurate and should be verified *in vitro*.

To sum up with Raman imaging of HMEC-1 cells exposed to non-toxic concentration of CADs we identified spectroscopic markers of phospholipidosis which are distinct from spectroscopic signatures of starvation-induced autophagy.

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Life Sciences, Biomedical and Pharma Research

A-098

The effect of iron oxide nanoparticles in *in vitro* cellular models studied by Raman microspectroscopy

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Raman spectroscopy is a widely used and important analytical method in the assessment of biochemical changes of biological samples. It is popular in many fields of science and particularly in biology, medicine and toxicological research [1,2]. Iron oxide nanoparticles (IONPs) are an attractive class of nanomaterials with excellent magnetic properties and great possibilities of medical applications. However, the mechanisms of toxicity of these nanomaterials are still poorly understood and regular study in this area are necessary [3]. In our study, Raman microspectroscopy was utilized to determine anomalies in the distribution and structure of biomolecules which appear in different cells after the treatment with 5, 10 and 30 nm IONPs with PEG coating. For this purpose mouse macrophages (P388D1) and human glioblastoma multiforme cell line (U87MG) were exposed to the tested nanomaterials in the concentration of 25 µg Fe/ml.
The results of the study showed that the Raman spectra recorded for cells exposed to the tested IONPs, included the additional bands at the wavenumbers 1870-2000 cm^{-1} and 2360 cm^{-1} which presumably origin from the adducts or complex containing Fe. The anomalies observed for the treated cells included also the alterations in the content of organic matter, proteins and lipids.
The Raman spectra of cells were processed with the use of cluster analysis what enabled us to classify them as belonging to the nucleus, cell membrane and cytoplasm. The spectra of particular parts of cells were then subjected to the principal component analysis which confirmed the significance of biomolecular differences between the exposed and normal cells.

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Life Sciences, Biomedical and Pharma Research

A-101

Assembly and improvement of a Raman fiber-optic probe for remote dental applications

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Tooth enamel is the most highly mineralized and hardest tissue in the human body, which covers and protects the anatomic crown of the tooth. Currently, in clinical practice the caries detection is still mostly limited to conventional visual and visual-tactile techniques such as sharp explorers and dental radiographs. Raman technique, a form of vibrational spectroscopy, is presently considered a viable optical method for biomedical applications mainly due to its uniqueness and precise results, non-destructive and non-invasive modality to analyse and provide detailed information about molecular composition, chemical structure, phase and polymorphy, crystallinity and as well as molecular interactions. This technique has shown to be appropriate for the characterization of dental tissues, from caries detection to the evaluation of demineralization caused by acidic external agents. The sensitivity of this spectroscopic technique for alterations in the symmetric stretching band of phosphate ions in the hydroxyapatite matrix could be used as a powerful tool for early diagnostics, even earlier the typical signs of demineralization are detected with conventional methods. This evaluation is successfully accomplished through spectral parameters such as Raman band position, the full width at half maximum as well as depolarization ratio (ρ_{959}) and polarization anisotropy (A_{959}). This work aims to develop the optical layout and assemble of a fiber-optic probe for remote Raman measurements, which must contain the following components: focussing and collimating lenses, band-pass filter (laser transmitting filter), dichroic mirror (in our case the incident beam and collected signal light share a common path, a dichroic 45° beam splitter transmits the laser light through the optics to the sample while efficiently reflecting the returning Raman-shifted signal light in direction to collection fiber), notch filter since it is required an excellent filtering, essential for blocking the very intense laser light while still allowing high transmission with enhanced isolation of the slightly wavelength-shifted Raman scattered signal.

Keywords: Raman spectroscopy, tooth enamel, Raman signal, fiber-optic probe

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Life Sciences, Biomedical and Pharma Research

A-108

Raman microscopy in the study of changes in glycogen and lipid metabolism occurring in cervical epithelial cells in the process of oncogenesis

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Introduction HPV infection is estimated to cause 99% of cervical cancer cases. The literature reports that oncogenic proteins of the virus, incl. E6 and E7 can cause changes in glucose and lipid metabolism. Raman microscopy is ideal for studying changes in the content of organic compounds in cells, thanks to its freedom from labels and unbiasedness.
Materials and Methods The subject of the research were cervical swabs obtained from 96 women, aged 19-85 years. Based on the pathomorphological evaluation, the cells were divided into the following groups: N (normal cells), LSIL (low grade squamous intraepithelial lesion), HSIL (high grade squamous intraepithelial lesion), SCC (squamous cell cancer). Based on the HPV infection test, the cells were finally classified into 8 groups. The cells were subjected to Raman microscopy (WITec Alpha 300) and the results processed using the Opus and WITec programs. Cells were also subjected to molecular tests for the degree of methylation of the sterol regulatory element-binding protein 1 (*SREBF1*) gene, as well as for the amount of mitochondrial DNA in the cell.
Results The analysis of the results obtained thanks to Raman microscopy showed that HPV-infected cells, whose nucleus diameter exceeds 10 μm, show an atypical profile of glycogen metabolism (in identical groups of advancement of pathological changes, the glycogen level was statistically significantly lower in cells infected with HPV), while cells with a diameter of cell nuclei below 10 μm do not show this tendency. Moreover, the lipid level in the cytoplasm inversely correlates with the level of glycogen in the cytoplasm – it is the highest in SCC cells and the lowest in LSIL cells. Additionally, lipid unsaturation in lipid droplets is lowest in SCC cells and highest in LSIL cells. The results of molecular studies showed that the degree of methylation of the *SREBF1* gene is the highest in the LSIL group and the lowest in the SCC group, and that the mitochondrial DNA copy number is the highest in the HSIL/HPV+ and SCC/HPV+ groups, and the lowest in the LSIL group.
Conclusions Single cell analysis using Raman microscopy combined with molecular methods enabled better understanding of neoplasia development in cervical epithelial cells. Observed changes in glycogen and lipid metabolism may be related to the action of oncogenic proteins E6 and E7. Application of Raman microscopy may contribute to the development of an automated HPV test in the future.

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Life Sciences, Biomedical and Pharma Research

A-109

Biochemical characterization and discrimination of B-type acute lymphoblastic leukemia (B-ALL) by Raman imaging

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Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. B-type ALL (B-ALL) originates from immature B cell precursors. The ALL subtype, characterized by the detected fusion gene, influences the prognosis and the effectiveness of the therapy [1]. Currently used diagnostic methods are expensive and time-consuming. Therefore, seeking for novel diagnostic methods of blood malignancies is still unflagging need within oncology prospect. Raman spectroscopy (RS) is a rapid technique providing spatial resolution at the subcellular level, thereby supplying information about the biochemical composition of the sample. RS due to many advantages might be considered as a potential and valuable diagnostic method over the next years [2],[3].
In our studies, we used Raman imaging supported with chemometric analysis, in order to effectively distinguish healthy B cells from their leukemic counterparts. Samples of three subtypes of B-ALL (*BCR-ABL1*, *TCF3-PBX1*, *TEL-AML1*), were isolated from the bone marrow of diagnosed patients, whereas B cells were derived from whole peripheral blood of healthy donors. Samples were measured by RS with excitation at 532 nm and 633 nm, resulting in a total number of spectra above 153 600. Raman images of single cells were analysed using *k*-means cluster analysis (KMCA). Principal component analysis (PCA) allowed to reveal the subtle differences between the spectra of studied cells. We discovered that the Raman spectra of healthy B cells were heavily influenced by signals attributed to nucleic acids and proteins. Partial least squares (PLS) regression was used to establish an algorithm to differentiate spectra of healthy B lymphocytes from leukemic cells. The method delivered prosperous results with high accuracy. However, designing the algorithm to distinguish spectra of all studied subtypes of B-ALL was not possible. Despite difficulties, we managed to obtain discrimination between *BCR-ABL1* and *TCF3-PBX1* samples, proving that spectral determination of molecular subtypes of B-ALL is possible. Presented results demonstrate the potential of RS in combination with chemometric analysis in the diagnosis of leukemia in clinical practice.

Acknowledges: 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.

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Life Sciences, Biomedical and Pharma Research

A-111

Accumulation of lipids as a marker of T cell activation revealed by label-free Raman spectroscopy

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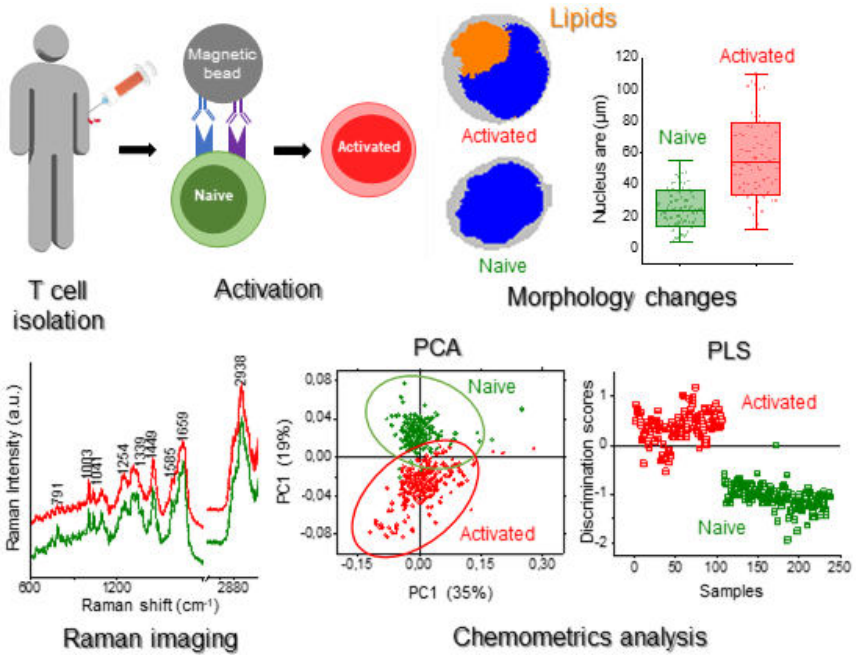
T cells are one of the most important white blood cells of the immune system and play a central role in the adaptive immune response. T cells are originated from pluripotential hematopoietic stem cells [1]. In order to become fully functional effector cells, the activation requires specific antigens, binding to receptors presented in T-cell (TCR). Another requirement is the co-stimulating interaction of other surface proteins and cytokines. Activating signal leads to a cascade of intracellular events resulting in a production of subtype-specific effector proteins and acceleration of proliferation [2]. A deeper understanding of biochemical changes followed by T cells activation will allow the improvement of immunotherapy and CAR-T treatment. Therefore, the goal of the studies was the identification of molecular changes triggered by the activation of T cells by means of Raman imaging.

In our studies, we applied label-free Raman imaging for molecular characterization and discrimination of naive and activated T cells. We have defined spectral biomarkers characterizing the activation process, such as increased concentration of lipids in the cell cytoplasm, changes in the nucleus morphology and chromatin condensation and decrease of carotenoids accumulation. These molecular changes were clearly evidenced in the Raman spectra collected from naïve and activated T lymphocytes and proved by multivariate analysis, which was carried out (Fig. 1). The reliable recognition of activated T cells based on the Raman spectra was possible after a detailed analysis of the average spectra and application of principal components analysis (PCA) and partial least squares discrimination analysis (PLS-DA) methods.



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Life Sciences, Biomedical and Pharma Research

A-112

Raman analysis of the modified silver nanoparticles during their synthesis

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Intro. Silver nanoparticles (Ag NPs) are used in the surface-enhanced Raman scattering (SERS) to increase Raman signal and to gain more specific information from the objects studied in comparison to the regular Raman spectroscopy. Ag NPs are used as an efficient tool for SERS analysis of living cells in particular bacteria [1].
In this study, the alkyne-modified Ag NPs have been elaborated for specific detection and characterization of the active cells within a cell community via “click reaction”-based coupling and subsequent SERS. The aim of the study was to check synthesized alkyne-modified Ag NPs via Raman spectroscopy.
Methods. Ag NPs were synthesized according to Leopold and Lendl, 2003 [2]. The alkyne modifying compound *5-(1,2-dithiolan-3-yl)-N-(prop-2-ynyl) pentanamide* was synthesized according to Shi et al 2013 [3], and checked via nuclear magnetic resonance, Orbitrap mass spectrometry and Raman spectroscopy. The alkyne modifying compound, Ag NPs, the mixture of these two substances and the target alkyne-modified Ag NPs were analyzed by Raman spectroscopy. The samples were dropped on the aluminum coated slides and dried. Raman analysis was performed with *alpha 300 R* (WITec GMBH) using 532 nm frequency doubled Nd:YAG laser, 100× objective (NA = 0.9), laser power 0.3 – 1 mW, integration time 1 second, 10 accumulations. Obtained data were analyzed using WITec Suite FIVE software package.
Results. Binding of the alkyne modifying compound to the Ag NPs is accompanied with a cleavage of S-S in the dithiolane cycle of the alkyne compound and formation of Ag-S bounds. It is followed by decrease and shift of the Raman peak intensity at 502 cm^{-1} corresponding to S-S stretching in the target compound [4]. Ag-S bounds are reflected in the Raman band at 240 cm^{-1}, alkyne groups are observed as the Raman band at 2121 cm^{-1} in both alkyne modifying and target compounds as well.
Conclusion. Raman analysis confirmed the cleavage in the dithiolane cycle of the alkyne modifying compound *5-(1,2-dithiolan-3-yl)-N-(prop-2-ynyl)* and its binding to Ag NP. This rapid, minimally invasive, sensitive approach is efficient to confirm synthesis of the target compound.

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Life Sciences, Biomedical and Pharma Research

A-113

Molecular Raman Probes-based detection of promieloblastic cells biochemical state

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Raman Spectroscopy is recognized as a label-free method that brings multiple advantages for the characterization of biological samples. Biochemical processes, also linked to pathology development, are associated with dysfunctions of cellular organelles. Marker bands of biocomponents such as DNA, phospholipids, and cytochrome C characterize the biochemical state of crucial cellular compartments: nucleus, endoplasmic reticulum, and mitochondria, respectively. However, the lack of marker bands of some cellular structures or difficulties in the detection outlines the need to develop molecular Raman probes. The combination of selective targeting moiety and a reporting part containing triple bonds or deuterium substitution and displaying signal in 1800-2800 cm^{-1} range enable simultaneous detection of multiple probes without bands overlapping with a compound of cellular origin. Application of Raman probes for recently developing non-linear Raman-based techniques, benefiting in signal increase, could provide an innovative alternative to existing diagnostic methods [1].
The ultimate goal of the study was to evaluate drug-induced granulocytic differentiation of promyelocytic cells towards neutrophile-like cells using the HL-60 cell line. Changes associated with mitochondria state were followed by Raman imaging using MitoBADY probe (Fig.1), displaying band at 2220 cm^{-1}- that accumulates in negatively charged mitochondria membrane, thanks to the positive charge of the compound. We proved that MitoBADY is mitochondria specific probe. Even low concentrations of MitoBADY (100 nM) and low incubation time (15 min) are enough to characterize mitochondria distribution in cells.

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.

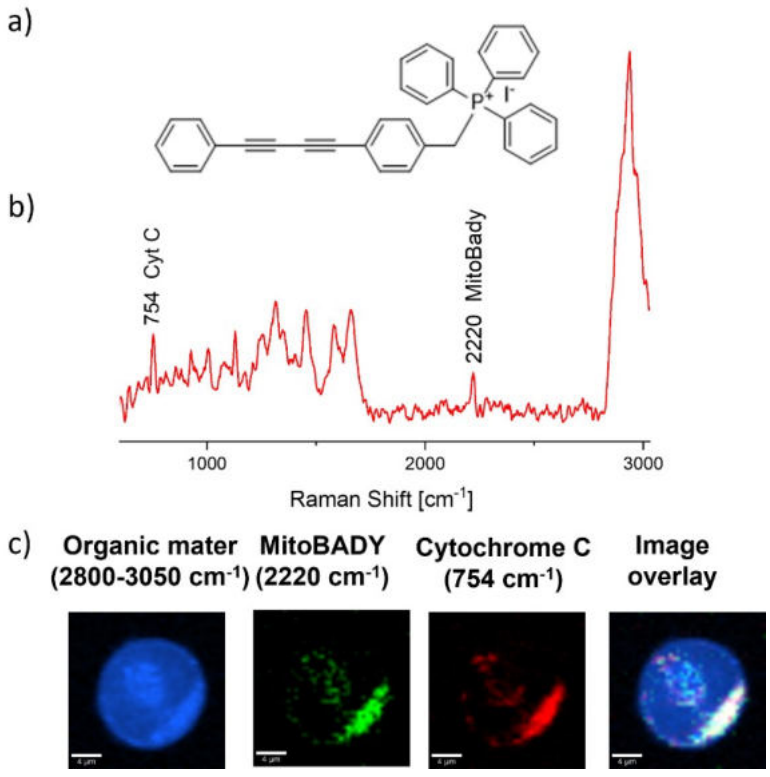

Fig.1 a) Chemical structure of MitoBADY, b) point spectra of mitochondria and c) Raman images of a HL-60 cell incubated for 15 min with 100 nM of MitoBADY (int. time 0.05 s, step size 0.5 µm).

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Life Sciences, Biomedical and Pharma Research

A-117

Assessment of *initial carious lesions* by Raman spectroscopy

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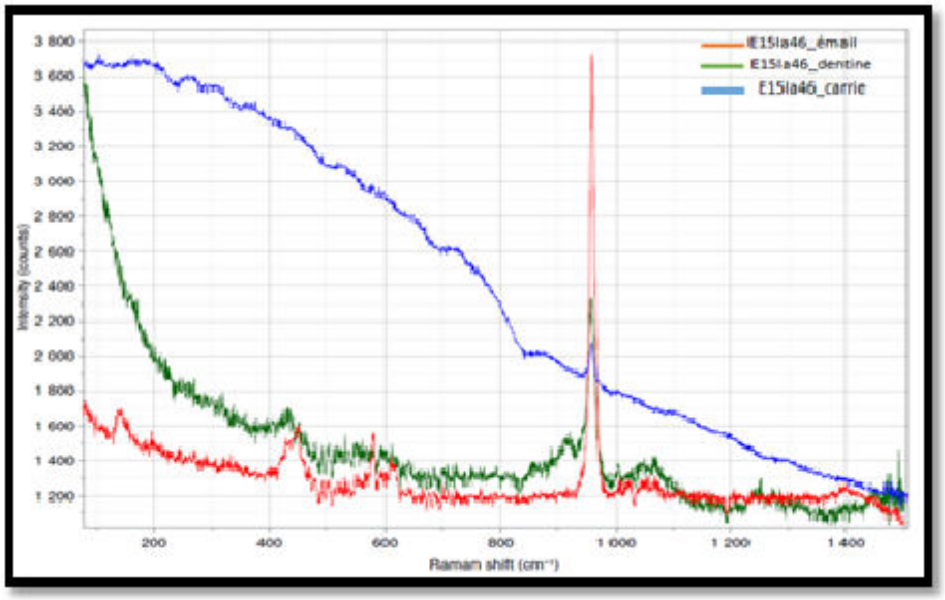
The current dentistry methods for detection of caries in enamel and dentin are unable to detect carious lesions at a very early stage. Several studies on Raman spectroscopy have shown advantageous results in diagnosing early dental caries [1]. The dental caries is characterized by demineralization of inorganic substance (hydroxyapatite crystals) and destruction of organic substance (collagen matter). Raman spectroscopy helps to detect hydroxyapatite dissolution (Ca_{10} $(PO4)_6(OH)_2$). In this study we present Raman spectroscopy as tool for the detection of the initial carious lesions, this technique offers many possibilities in the analysis and imaging of dental tissues and materials.
The objective of this work was to estimate the efficiency of Raman spectroscopy in determining specific changes linked to the breakdown of enamel and dentin. Raman spectroscopy analysis detected the carious lesion of the tooth in early stage (stage 1), the results indicate a strong signal intensity of phosphate ions $(PO_4)^{-3}$ at 960 cm^{-1} for healthy enamel and dentin. On the other hand, we notice a very low intensity of the same signal $(PO_4)^{-3}$ at 960 cm^{-1} around the enamel at initial stage.



Raman of healthy enamel, healthy dentine and carious

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Life Sciences, Biomedical and Pharma Research

A-119

Spectroscopic characterization of IDH1 and IDH2- mutated transgenic HEL cells

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Abnormalities occurring in the genome of the precursors of different blood cells lead to the development of leukemia. Different subtypes of cancer of the blood cells are defined by specific gene alterations influencing phenotype, aggressiveness, and drug resistance of cancer cells. Nowadays, new *in vitro* models are now being sought to enable testing of new anti-cancer drugs and deepening molecular characterization of leukemic cells. Some of the genes that are frequently mutated in leukemias are IDH1 and IDH2 genes, encoding isocitrate dehydrogenase 1 and 2 [1]. Therefore biochemical characterization of transgenic cell lines with IDH1 and IDH2 gene rearrangements is desired in the context of studies of leukemia development. The goal of our study was the identification of metabolic changes associated with IDH1 and IDH2 mutations in HEL cells based *in vitro* model of leukemia using Raman imaging combined with multivariate statistical analysis [2]. We focused on studies of two mutant HEL cell lines related to rearrangements of IDH1 and IDH2 genes, resulting in a replacement of arginine with histidine at the residue 132 (IDH1/R132H) or in a replacement of arginine with glutamine at the residue 140 (IDH2/R140Q). As a result of gene alterations of IDH1 and IDH2 genes, loss of their normal catalytic activity is observed. Indirectly, it leads to abnormal histone and DNA methylation and causes alterations in the differentiation of progenitor cells and neoplasm development. HEL cells with a wild-type (WT) sequence of IDH1 and IDH2 genes served as control samples. Control and IDH-mutated transgenic cells were imaged using the confocal Raman system WITec Alpha 300. Obtained Raman maps were subjected to k-means cluster analysis (KMCA). Obtained mean spectra of cells and cellular components were further analyzed using principal components analysis (PCA) and partial least squares regression (PLS) chemometric methods in order to identify subtle differences of HEL cells carrying different variants of IDH1 and IDH2 genes. Mutated and parental HEL cells primarily differed in the protein and nucleic acids composition.

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Life Sciences, Biomedical and Pharma Research

A-131

Raman Micro-spectroscopic Tissue Map Demonstrates Precision Achievement by Photothermal Therapy (PTT) Mediated *in vivo* Treatment of Cancer

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Question: Photothermal therapy (PTT) inculcates near-infrared (NIR) laser guided local heating effect in presence of plasmonic nanoparticles, a method widely researched worldwide as a therapeutic measure for cancer. However, the nature and extent of cellular/tissue damage by PTT is not well documented till date.
Methods: Herein, we generated comprehensive *in vitro* cellular and *ex vivo* tissue biochemical maps using Raman microscope and classified different subcellular compartments in cells and zones in the PTT treated tumor tissue using *K*-means cluster (KCA) analysis to delineate the PTT associated damage feature and localized effect in preclinical mouse model.
Results: The subcellular compartments i.e. nucleus, cytoplasm and plasma membrane in each different controls and PTT treated cell was distinguished based on Raman bands (at ~1325 cm^{-1} tentatively assigned to nucleic acid; for nucleus, ~1585 cm^{-1} assigned to cytochrome/mitochondria; for cytoplasm ~1740 cm^{-1} ascribed to lipid; for plasma membrane). The subcellular compartments of PTT treated cell not only show dramatic decrease in the intensity of ~1325 cm^{-1}; ~1580 cm^{-1}; and ~1740 cm^{-1} but also show changes in the ~995 cm^{-1} and ~1650 cm^{-1} (tentatively ascribed to proteins), and ~1450 cm^{-1} (ascribed to proteins and lipids) in these subcellular compartments. This clearly indicates that PTT mediated damage features affects the overall cellular bio-macromolecular profile with major changes in nucleic acid, protein, cytochrome/mitochondria and lipids.
The *ex vivo* tissue biochemical map with or without PTT treatment, generated via label-free Raman spectroscopic signatures, classified the tissue in three distinct spectral clusters clusters/zones. The core treated zone showed features of intense nucleic acid (~785 cm^{-1} and 1090 cm^{-1}), protein (1169 cm^{-1}, 1448 cm^{-1} and 1654 cm^{-1}), and cytochrome/mitochondria (1309 cm^{-1} and 1586 cm^{-1}) damage and these features to a lesser extent were also retained in the immediate adjacent areas, but minimal to none in the far zone.
Conclusion: For the first time, by utilizing Raman microspectroscopy as an investigative tool to generate cellular and tissue biochemical maps, PTT associated damage features and the extent of damage in terms of physical distance from the core treated area has provided the much required knowledge on *in vivo* precision achievement of this therapy method.

Life Sciences, Biomedical and Pharma Research

A-139

Fabrication of low-cost SERS substrate using a burnt compact disc

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In this work, we report the fabrication of low cost SERS substrate using a burnt compact disc (CD-ROM). For efficient raman signal enhancement, periodic arrays of micro/nano-structures are required which show plasmonic behavior. This enables high density of 'hotspots' or signal enhancement sites. A burnt CD-ROM contains spiral track or groove winding with depressions ranging from 0.6 microns to 0.8 microns. The typical spacing between these grooves is around 1 micron. The substrate is made of polycarbonate plastic. For fabrication of SERS substrate, we have utilized the submicron sized polycarbonate features of the CD-ROM. Annealing of substrate at 130 ^{o}C for 15 minutes is also done to provide curling to the seemingly straight polycarbonate lines and thus reduce the inter-feature distance. A thin film of gold (10nm) is deposited using PVD on the polycarbonate substrate to realize the SERS substrate. Alternatively, silver nanoparticles in aqueous solution are also synthesized through chemical route for solution based deposition of plasmonic silver nanoparticles onto the polycarbonate substrate. The nanoparticles are characterized using UV-Vis spectrophotometer. The fabricated substrate is characterized using scanning electron microscope (SEM) and a raman spectrometer. Rhodamine-B is used as the test molecule for checking the SERS behavior of the fabricated substrate. The work provides an easy and low-cost solution for fabrication of efficient SERS substrates.

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Life Sciences, Biomedical and Pharma Research

A-147

In situ characterization of different celltypes in bioprinting

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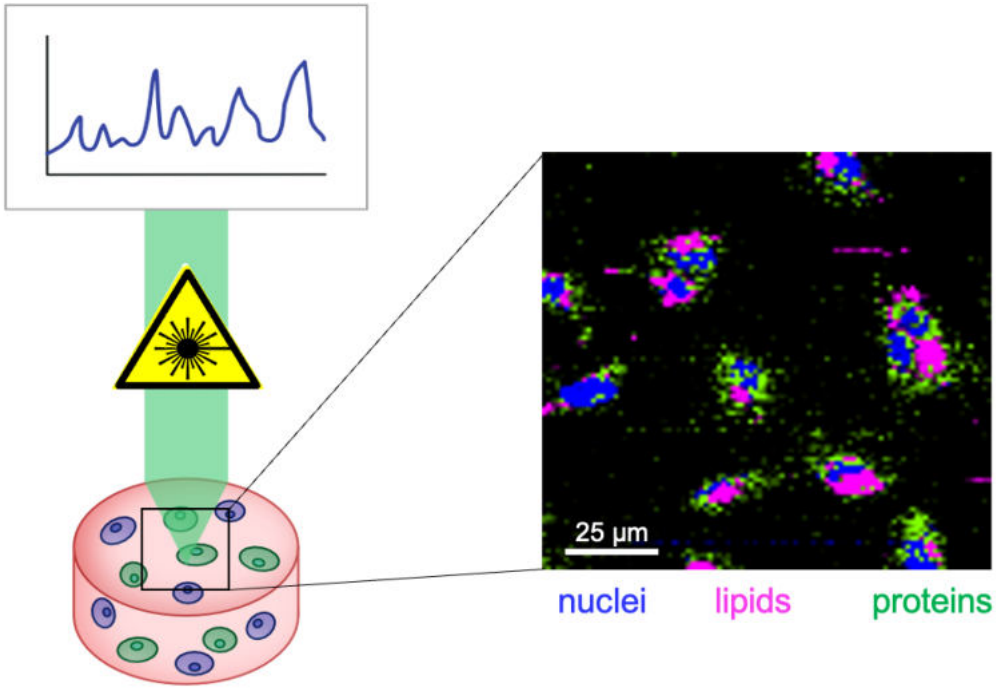
Additive manufacturing using bioinks, comprising cells and biocompatible polymers, is an emerging biofabrication strategy to produce functional tissue models. Despite advancement in building increasingly sophisticated objects, biological and cellular analyses in printed constructs remain challenging and often imply endpoint analyses. Methods that enable non-invasive monitoring of embedded cells in bioprinted constructs would provide further insights on functionality and viability.
Here, we implemented Raman imaging for molecular-sensitive investigations on bioprinted objects. Hyperspectral maps were aquired from bioinks followed by multivariate data analysis (MVA). Different aspects such as culture format (2D, 3D-casted, 3D-printed), cell type (endothelial cells, fibroblasts) and the selection of the biopolymer (alginate/NFC, gelatin, alginate/gelatin) were considered and evaluated. Raman imaging allowed for a marker-independent identification and localization of subcellular components against the surrounding biomaterial background. Furthermore, single-cell analysis of spectral signatures, performed by MVA, demonstrated a discrimination of endothelial cells and fibroblasts and identified cellular features influenced by the bioprinting process.
In summary, Raman imaging was successfully established to evaluate bioinks and access cellular information in 3D culture in situ, representing a promising tool for the quality assessment of bioprinted objects.



In situ Raman imaging was performed on alginate/gelatin bioinks containing endothelial cells and fibroblasts

The work was financially supported by the State Ministry of Baden-Wuerttemberg for Economic Affairs, Labour and Tourism and the BMBF (‘SOP Bioprint’ 13XP5071C).

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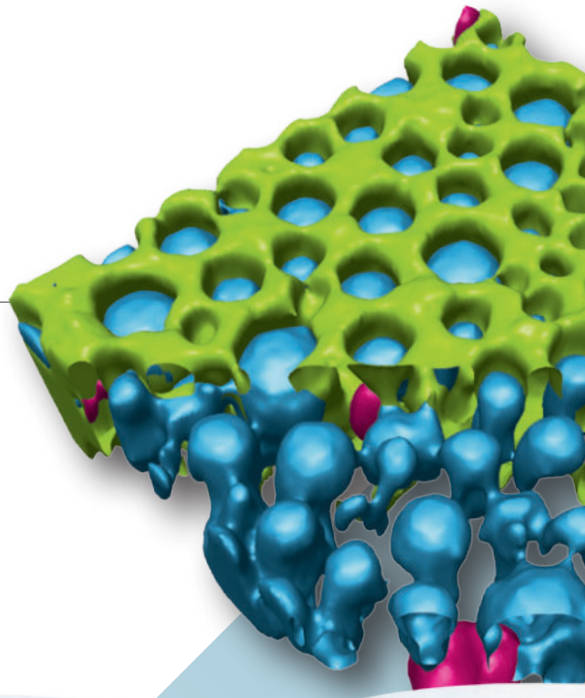

Confocal Raman Microscopy, edited by members of the WITec team and part of the Springer Series in Surface Sciences, has been thoroughly revised and expanded for its 2nd edition. The book provides a comprehensive overview of the fundamentals, practical considerations and real-world applications of Raman microscopy.

With 23 chapters it also includes sub-sections on theory and technology, novel materials, geosciences, life and pharmaceutical sciences, materials science and a historical overview of the Raman effect.

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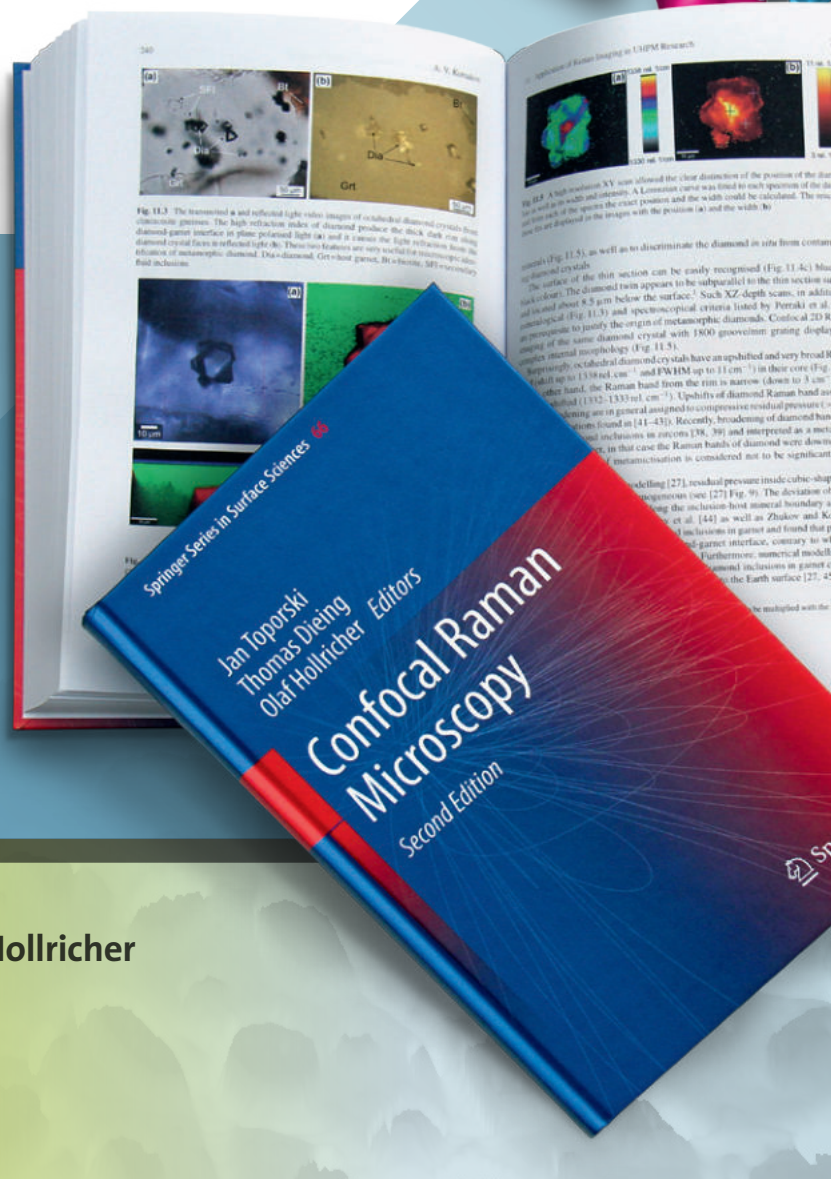

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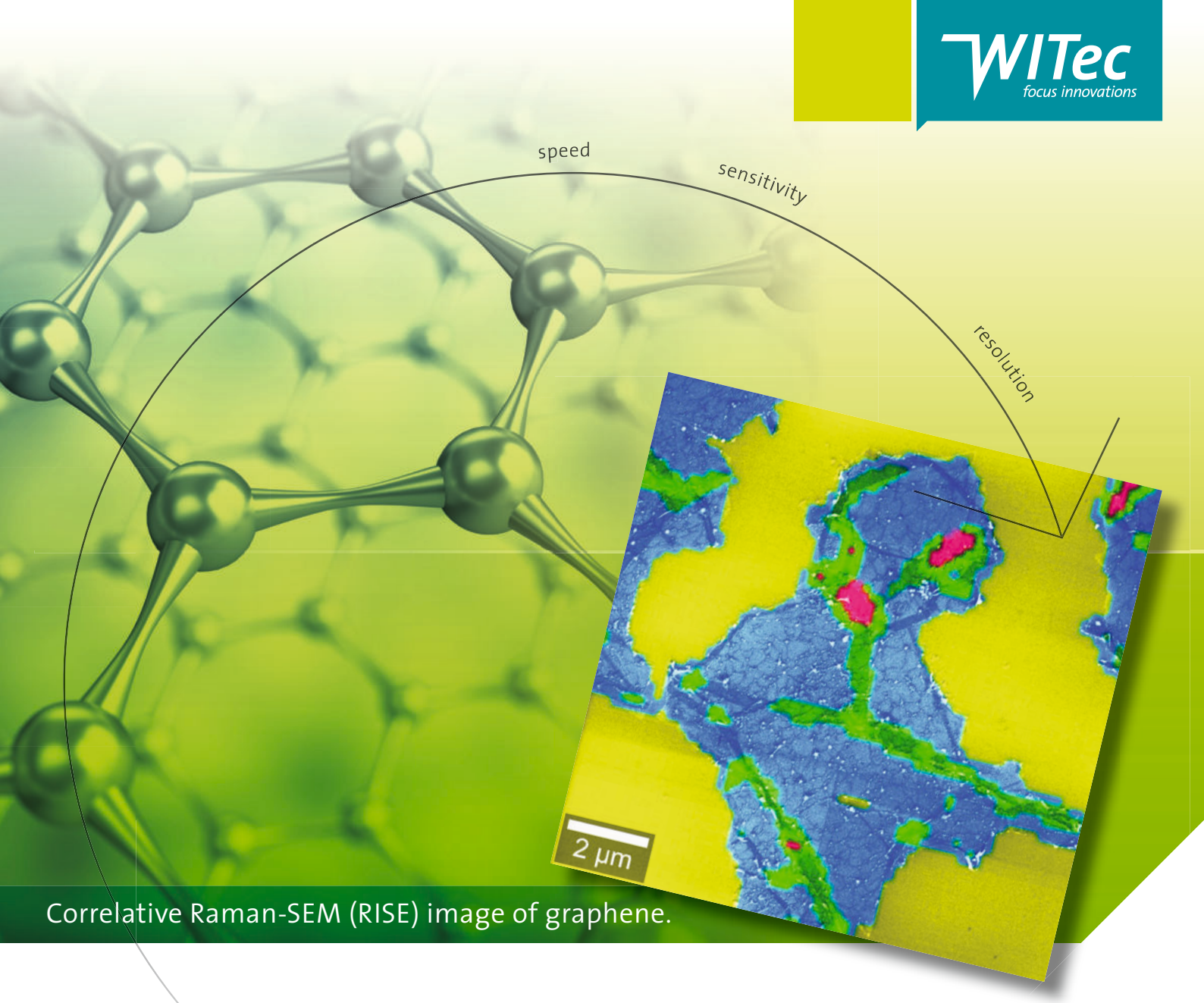

Correlative Raman-SEM (RISE) image of graphene.

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