WITec
focus innovations

15th Confocal Raman Imaging Symposium

International Conference for
Chemical Characterization & Imaging

September 24-26, 2018
Ulm, Germany

Welcome

Would you like to learn more about analyzing structures and chemical properties in the sub-micron range, which is essential for today's materials sciences? You now have the opportunity.

With these words we announced our first WITec Raman Symposium – which we then modestly called a Raman workshop. The idea was to establish a unique network for scientists all over the world that use Raman imaging and to strengthen the communication between researchers and industry. From the beginning, fields as diverse as semiconductors, cell biology and pharmaceutical devices were represented, reflecting the immense range of this powerful technique.

We started out with five speakers and about 20 participants visiting our earlier headquarters. The workshop evolved into an annual conference. Now in 2018, for our 15th Confocal Raman Imaging Symposium we'll welcome 15 speakers and about 100 participants, the capacity of our current headquarters.

Over the years we've had many presenters and attendees who are well known throughout the Raman community. The popularity of the event shows that WITec has not only expanded its customer base, but that we've been successful in establishing a truly international Raman network. We believe, that the strength of this Raman Symposium lies in the mixture of scientific lectures on state-of-the-art experimental methods combined with demonstrations of the latest technology and our most recent developments.

We hope you'll enjoy your days with WITec in Ulm.

Olaf Hollricher	Joachim Koenen
Managing Director Research & Development	Managing Director


WITec

Invited Talks

Confocal Raman Microscopy for Life Science Applications – Case Studies

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In 2016, the Faculties of Chemistry and Physics of the University of Vienna decided to acquire a fully equipped Raman Microscope (WITec alpha 300), combined with AFM and SNOM that should provide a versatile range of applications in life science. It is hosted in the Faculty Center for Nano Structure Research.

Although there was a high level of experience in IR spectroscopy, Raman microscopy was a new field for all users. Moreover, many different applications resulting from research fields of two faculties of life science caused many new different strategies of measurements and data evaluation.

In the present work, some examples of applications of Raman Spectroscopy in life science are shown. Moreover, simple and more complex problems arising from very different questions that should be answered by Raman measurements are reported. Further multivariate data analysis, leaving WITec standard software, will be presented in brief.

The following research projects investigated by various research groups of the University of Vienna will be introduced:

- Characterization of bacteria imprinted polymers with Raman microscopy (Fig. 1)
- Time resolved measurements of polymerization processes
- Deep profiles of colors in colored hairs
- Penetration monitoring of drugs and additives into skin
- Characterization of reduced graphene oxide in graphene coating
- Vanadium based misfit layer compounds

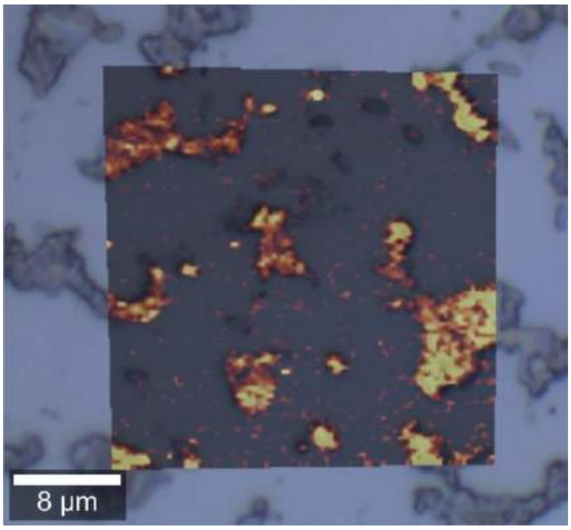

Fig. 1: Molecular imprinting prepares a polymer to function as a sensor for the molecules used in the imprinting process. The surface of such a molecular imprinted polymer (MIP) was analyzed using Raman spectroscopy. The overlay of white light image of MIP with Raman images shows a possible differentiation between E. coli, imprints (holes) and the polymer. Moreover, different bacteria can be distinguished using multivariate data analysis of Raman images (from Felix Thier, master thesis in prep.).

Mapping with Raman spectroscopy down to submicron length scales

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We have used Raman microscopy to examine a number of heterogeneous systems. These include investigating drug dissolution from nanofibers, interstratification in serpentinite minerals, fossils in amber. If we focus on the serpentinite example, these minerals have a tetrahedral silicate sheet below a Mg-rich octahedral sheet. Due to the misfit between the tetrahedral and octahedral sheets, there can be slight deviations in composition or nanostructures resulting in several forms. The serpentine form lizardite is enriched in Al or Fe to accommodate the misfit while the forms chrysotile and antigorite adopt fibrous and modulated sheet-like nanostructures. It is these slight differences in nanostructure that allow discrimination between these three main serpentine forms; additionally, which form is thermodynamically favoured depends on the temperature and pressure conditions during subduction.The very subtle differences in structure make it difficult to differentiate these forms as they have similar optical properties and very similar XRPD signals. Vibrational spectroscopy can be more effective at detecting subtle bonding changes but infrared spectroscopy is compromised by anomalous dispersion effects. Raman spectroscopy does not suffer from these issues and can unambiguously detect the different forms. We have used Raman microscopy to examine serpentinite fault rocks which have been subjected to sufficient forces to produce the different forms. Maps examining thin sections of these samples show the presence of all three forms. Careful analysis of some of the samples reveal interstratification of lizardite and chrysotile with layers ranging from below the spatial resolution (370 nm) to several micrometres in width (Fig 1).

This method can now be used to examine the rheological and thermal history of samples contains serpentinite-bearing minerals.

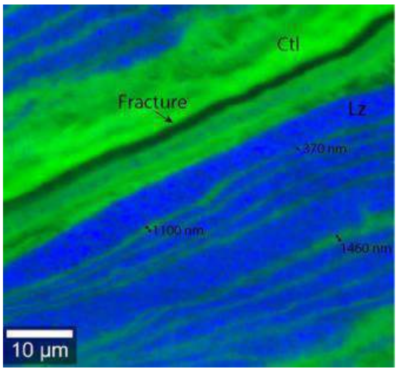

Fig. 1: Raman map showing interstratification of lizardite (blue) and chrysotile (green); two forms of serpentinite.

Advanced Nanocarbon Materials for Solar Energy Conversion Schemes

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Carbon is the key to many technological applications that have become indispensable in our daily life. Altering the periodic binding motifs in networks of sp^3-, sp^2-, and sp-hybridized C-atoms is the conceptual starting point for a wide palette of carbon allotropes. To this end, the past two decades have served as a test-bed for measuring the physico-chemical properties of low-dimensional carbon with the advent of fullerenes (0D), followed in chronological order by carbon nanotubes (1D), carbon nanohorns, and, most recently, by graphene (2D). These species are now poised for use in catalysis.

Expanding global needs for energy have led to a significant effort to develop alternatives to fossil fuels. While alternative sources for energy are already in use, they comprise a small percentage of the energy demands needed to carry us through the 21st century. No single source will solve the global needs, but the development of photocatalysis has vast potential as a point-of-use power source.

I report on our efforts regarding a unifying strategy to use the unprecedented charge transfer chemistry of 0D fullerenes, the ballistic conductance of 1D carbon nanotubes, and the high mobility of charge carriers in 2D graphene, together in a groundbreaking approach to solving a far-reaching challenge, that is, the efficient use of the abundant light energy around us. For example, hybrid materials based on nanocarbons and metaloxides are the ideal design for realizing breakthroughs in high photon conversion efficiencies suitable for the catalysis of water. Major emphasis will be on the use of Raman techniques as a complement to transient absorption measurements.

Confocal Raman Microscopy: Instrumentation, Resolution, Configurations and Correlative Techniques

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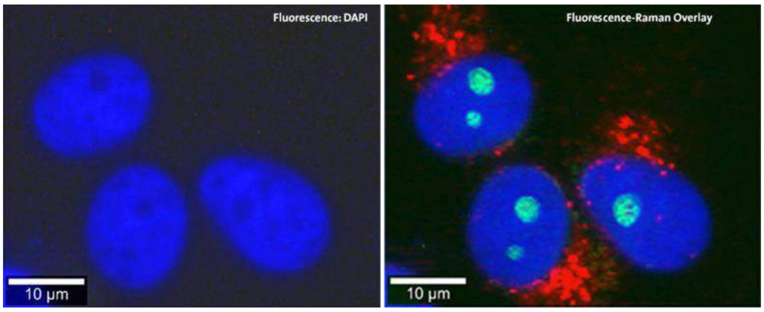
Confocal Raman microscopy is an indispensable tool for the analysis of of chemical species and their spatial distribution either on surfaces or in small 3D volumes.

As the name states, two techniques are combined in one instrument. The confocal microscope provides diffraction limited spatial information, while Raman spectroscopy reveals the chemical composition of the sample. By acquiring a complete Raman spectrum at every image pixel, the chemical information can be linked to the spatial distribution in the sample volume, resulting in nondestructuve imaging of chemical properties without specialized sample preparation. Differences in chemical composition appear in the Raman image, although they are completely invisible in the optical image.

Aim of this contribution is to highlight the instrumental requirements for a high throughput, high resolution confocal Raman microscope. Several new developments and their field of application will be presented.

Top: Confocal Raman microscopy - alpha300 R*i* for inverted Raman microscopy

Bottom: Correlative fluorescence (DAPI) – Raman microscopy of cells. Left: fluorescence of DAPI, marking the nuclei. Right: fluorescence-Raman overlay. Red = endoplasmic reticula, green = nucleoli



Shifted Excitation Raman Difference Spectroscopy - A Powerful Tool for Microscopy and in-situ Applications

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Shifted excitation Raman difference spectroscopy (SERDS) has been successfully demonstrated in confocal Raman microscopic investigations and various in-situ applications, such as food safety control, medical diagnostics and precision agriculture.

For SERDS two slightly shifted excitation lines generate two slightly shifted Raman spectra. A subsequent subtraction of both spectra separates the Raman signals from disturbing background interferences such as laser induced fluorescence and ambient light.

In this contribution, SERDS investigations using confocal Raman microscopy and in-situ experiments outside the lab will be presented. In order to realize these applications, at the Ferdinand-Braun-Institut customized diode lasers as excitation sources were developed. Miniaturized hybrid devices cover the spectral range between 457 nm and 830 nm whereas monolithic devices were manufactured for the range between 671 nm and 785 nm. The spectral distance for the SERDS excitation, typically 10 cm^{-1} is realized by the design of the DBR gratings and can be adjusted using an implemented heater element close to the gratings. The excitation power up to 1 W can be adjusted by the injection current with respect to the sample under study. For SERDS an alternating operation between both excitation lines with modulation frequencies up to 1 kHz is realized.

For Raman microscopy the laser light is coupled into a polarization maintaining single-mode optical fiber which is connected to a WITec confocal Raman microscope. Moreover, in our institute, portable SERDS sensor systems were realized for in-situ measurements. These instruments will be also described.

SERDS experiments in various application fields will be presented. The results demonstrate SERDS as a powerful and easy-to-use spectroscopic tool allowing microscopic as well as in-situ Raman investigations of samples which are often not accessible due to the disturbing backgrounds. A fast alternating operation between both laser lines allows short measurement times down to the millisecond range which enables rapid and undisturbed Raman investigations.

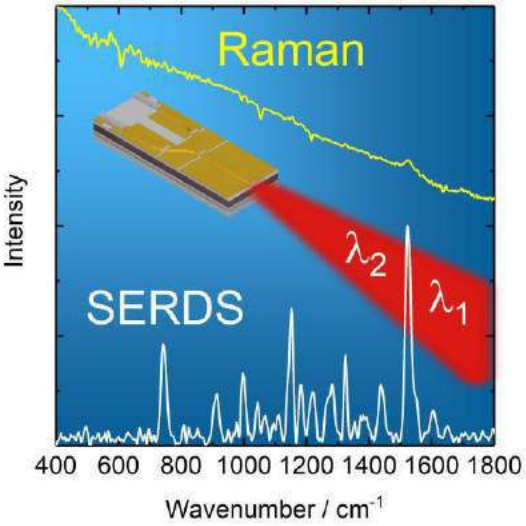

Fig. 1: Dual-wavelength diode laser at 785 nm for SERDS, Raman and SERDS spectrum of green apple leaf.

Correlative Raman Microscopy: Techniques and Applications

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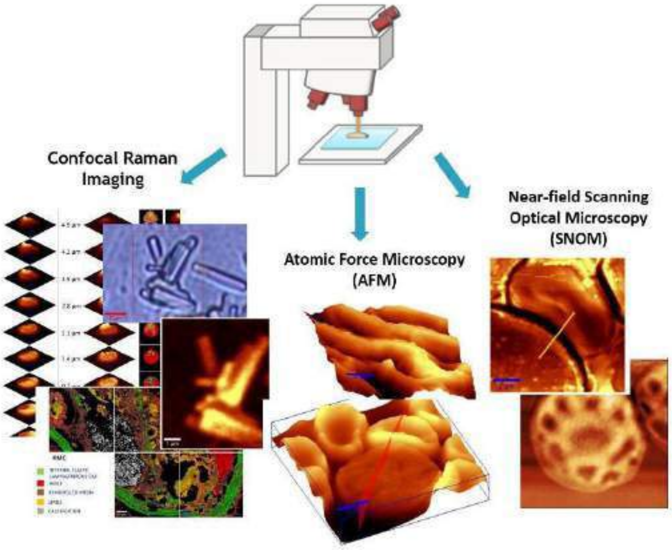
This lecture is devoted to the presentation of a complementary approach to the analysis of cells and tissues based on Raman Spectroscopy (RS) supported by other complementary techniques, such as atomic force microscopy (AFM), scanning near-field optical microscopy (SNOM) or IR spectroscopy. RS is a non-invasive and non-destructive method which allows for the detection and assessment of the distribution of various biomolecules inside a measured material. This makes it extremely useful in biomedical studies in combination with other techniques, and its application in this field is the main topic of this lecture.



The first part of the lecture will be focused on the application of correlative Raman microscopy to tissue studies, which allows for the analysis of their chemical composition, i.e. main components but also specific compounds, and their changes upon the development of different lifestyle diseases[1,2]. We may include here studies on the tissues obtained from the vascular wall, brain, lungs and their changes due to atherosclerosis or cancer progression[1-5].

The second part of the lecture will be focused on detection of the biochemical alterations and their respective distribution in a single cell or even at the subcellular level. Additionally, as RS has long been applied to monitor the molecular dynamics of different metalloproteins, part of the lecture will be focused on *in vitro* studies of functional human red blood cells (RBCs)[6,7]. Examples of the detection, analysis and visualization of 2D and 3D distributions of heme in both cells and tissues will be presented, including a high–resolution Raman imaging of a single cell[3,6-8].

ACKNOWLADGEMENT

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Multiscale Raman Imaging and Spectroscopy of High Energy Density Battery Materials and Interfaces

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Electrochemical transport across lithium-ion composite electrodes is highly heterogeneous and non-uniform. The origins of such inhomogenities are both the intrinsic nature of the electrode active material as well as extrinsic factors such as composition (binder and conductive diluents) and processing conditions. The talk will provide an overview of recent work on micro-Raman spectroscopy and imaging to obtain chemical and morphological information on pristine and cycled lithium-ion battery electrodes. Topics to be presented include micro-Raman imaging of electrochemically cycled, high-voltage, high-capacity lithium-ion cathodes to understand the structure-electrochemical property correlation and the evolving chemical heterogeneity.[1-2] Based on the metal-oxygen vibrational modes of transition metal oxide cathodes, Raman spectroscopy has been used to measure the local spectroscopic state of charge (SoC) of electrodes.[1] The variation of this local spectroscopic SoC is sensitive to local lithium content, defects, and structural transformation. In-situ and ex-situ confocal Raman imaging has been applied to multi-lithium cathode systems Li_2MnO_3[2] and $Li_2Cu_{0.5}Ni_{0.5}O_2$[3] to understand formation of secondary phases and structural transitions during delithiation and its correlation with oxidative stability. Finally, the talk will detail recent progress on the application of Raman imaging in silicon anodes[4] and ongoing work on Surface Enhanced Raman (SERS) and Tip Enhanced Raman Spectroscopy (TERS) applied to battery electrolytes and anodes[5].

Acknowledgement: This Research is supported by the Assistant Secretary for Energy Efficiency and Renewable Energy, Office of Vehicle Technologies, U.S. Department of Energy.

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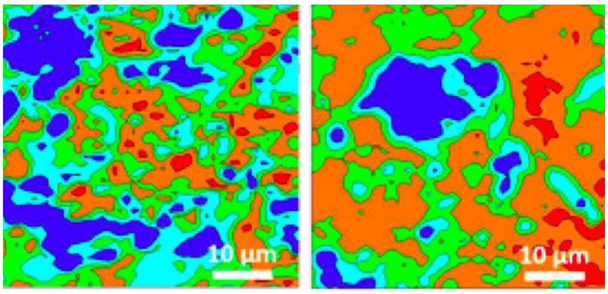

Fig. 1: Micro-Raman imaging of silicon-carbon composite electrodes for lithium-ion batteries

Micro-Raman Imaging of Sedimentary Mineral Assemblages to Elucidate Biosignatures since the Eoarchean

Dominic Papineau
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The origin of life on Earth is a topic intertwined with the search for biosignatures in the oldest sedimentary rocks, which only go back to perhaps 4,280 million years ago (Ga) in the Nuvvuagittuq Supracrustal Belt (NSB). It is therefore necessary to search for chemical biological signatures of microbial lifeforms, which in turn can inform both the emergence of life and chemical pathways in prebiotic environments. These may occur in mineral assemblages characteristic of mineralised, decomposed biomass in some of the oldest sedimentary rocks on Earth. Last year, we discovered the oldest microfossils in jasper concretions from a banded iron formation (BIF) in the NSB. Using micro-Raman imaging, we also documented a number of mineral assemblages that support a biological origin for the filamentous and tubular microfossils. Raman spectroscopy has enabled the following observations: 1) graphite with a weak D1-peak around 1340 cm^{-1} and a strong and narrow G-peak around 1580 cm^{-1} occurs in apatite and carbonate, 2) poorly crystalline graphite (PCG) occurs in association with apatite, phyllosilicate, and haematite and with a strong and narrow D1-peak around 1345 cm^{-1} and a strong and narrow G-peak around 1575 cm^{-1}, 3) graphitic carbons are associated with chlorite bridges between outsized magnetite crystals, 4) PCG and graphite occur as coatings and inclusions in apatite and calcite inside microscopic rosettes and millimetric granules and outsized laths, and 5) a third type of organic carbon has a strong and broad D1-peak around 1355 cm^{-1} and a strong and broad G-peak around 1595 cm^{-1} and occurs with concentrically-layered pyrite and haematite rosettes. Broader analyses in younger Precambrian sedimentary rocks show that these crystalline-to-disordered features and minerals associations of organic matter are common and generally consistent with metamorphic grades. This uniformitarian perspective demonstrates that there are filamentous microfossils of Fe-oxidizing bacteria in younger jasper and concretions from throughout the rock record.

The crystallinities of graphitic carbons indicate variable metamorphic effects on indigenous organic matter and mineral associations of diagenetic origin that influence the structure of the organic matter. Upcoming work is now targeted at the daunting task of identifying the molecular functional groups, heteroatoms, and macromolecular fragments in these graphitic carbons that represent an ancient organic soup of most likely biological origin. Micro-Raman imaging is also used to test the null hypothesis by searching for organic matter associated with apatite and carbonate in carbonatite, serpentinite, peridotite, and hyaloclastite, to distinguish biological from non-biological organic matter. Besides understanding the earliest microbial ecosystem on Earth, the ultimate goal of this work is to pave the way for the micro-Raman and correlated microscopy analysis of returned martian specimens.

Confocal Raman Imaging for Biomedicine

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As an analytical technique sensitive to the specific composition of biological and engineered systems in a non-destructive nature, Raman spectroscopic techniques have proven highly valuable tools for biomedicine. Analysis of the numerous tissues and disease states, engineered constructs and drug delivery systems used as therapeutic interventions allows for better characterization of disease history, status, prognosis, and therapeutic response. Confocal Raman spectroscopy has been extensively applied by the Stevens group in order to address numerous challenges facing biomedical research in an effort to improve our understanding of native tissues to better model them as tissue replacements. We have further applied these methods to analyze the composition and organization of engineered tissues to assess their complex structure, architecture, and biochemical composition. Utilizing these same methods for cellular systems, we have been able to study subcellular features in a label-free manner across a variety of substrates that impact cellular fate. Another approach based on confocal Raman techniques allows us to study the heterogeneity of nanoparticles within a sample to obtain a more complete understanding of complex processes such as loading, functionalization, and dynamic interactions for a variety of novel drug delivery vehicles. Through the continued development and novel application of confocal Raman microspectroscopic methods, we are able to gain a more complete understanding of the composition and organization of a wide variety of samples, both natural and synthetic, that have direct implications for and great potential to impact biomedicine.

Valley Polarization Trions and Magnetoresistance in MoS_2/YIG Heterostructures

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Manipulation of spin degree of freedom (DOF) of electrons is the fundamental to spintronic and valleytronic devices. Two-dimensional transition metal dichalcogenides (2-D TMDCs) exhibit an emerging valley pseudospin. This valley polarization gives a DOF for spintronic and valleytronic devices. Recently, magnetic exchange interactions between graphene and magnetic insulator Yttrium Iron Garnet (YIG) have been exploited. However, the physics of 2-D TMDCs with YIG have not been shown before. Here we demonstrate strong many-body effects in a heterostructure geometry comprising MoS_2 monolayer and YIG. The electron doping density is up to ~10^{13} cm^{-2}, resulting in a large splitting of ~40 meV between trion and exciton. The trions exhibit a high circular polarization of ~80% under optical pumping by circularly polarized light at ~1.96 eV; it is confirmed experimentally that both phonon scattering and electron-hole exchange interaction to contribute to the valley depolarization with temperature; importantly, a magnetoresistance (MR) behavior in MoS_2 monolayer were observed, and a giant MR ratio of ~30% is achieved. Our experimental results confirm that the giant MR behaviors are attributed to the interfacial spin accumulation due to YIG substrates. Our work provides an insight into spin manipulation in a heterostructure of monolayer materials and magnetic substrates.

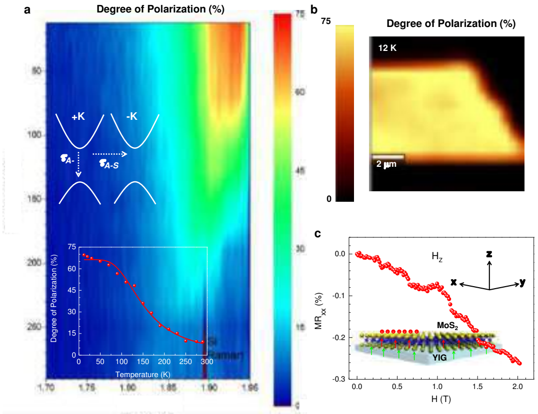

Fig 1: (a) Degree of circular polarization *η* as a function of temperature in the range of 1.70-1.96 eV. The insets represent the temperature dependence of *η* of trion and the spin relaxation. (b) The two-dimensional image of degree of circular polarization at 12 K, (c) Magnetoresistance behaviors of MoS_2 monolayer on YIG and SiO_2/Si substrates. [1] Peng, B.*; Li, Q.; Li. J; Deng, L., *et al. ACS Nano* 2017, *11*, 12257-12265

The Principle of Raman Spectroscopy and its Application in Microscopy

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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.

Finally, quiz questions allow the participants to test their knowledge anonymously in an interactive format (feedback and discussion).

Confocal Raman Imaging Spectroscopy in the steel industry – analytical topics along the steel production process

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Confocal Raman microspectroscopy is currently used in various scientific and industrial areas and allows a characterization and measurement of a great variety of inorganic and organic substances. Steelmaking in an integrated steel mill is a complex process with a lot of feedstocks, intermediates and surface-finished end products. Therefore Raman-microspectroscopy represents a suitable tool for a wide variety of questions along these process lines. For this reason, confocal Raman-microspectroscopy is receiving an increasing attention at thyssenkrupp Steel Europe.

This presentation gives an insight view into the applications of confocal Raman-spectroscopy at thyssenkrupp Steel and the daily business including this analytical method in an integrated steel mill. Therefore some of the analytical questions that have emerged in recent years and have been solved using confocal Raman microspectroscopy, are presented as examples. These analyses include e.g. routine analyses, identification/characterization of unknown substances and spectroscopic imaging. In addition the repetitive challenges using this analytical method in an industrial environment are described and future issues that can be solved with Raman-spectroscopy are presented.

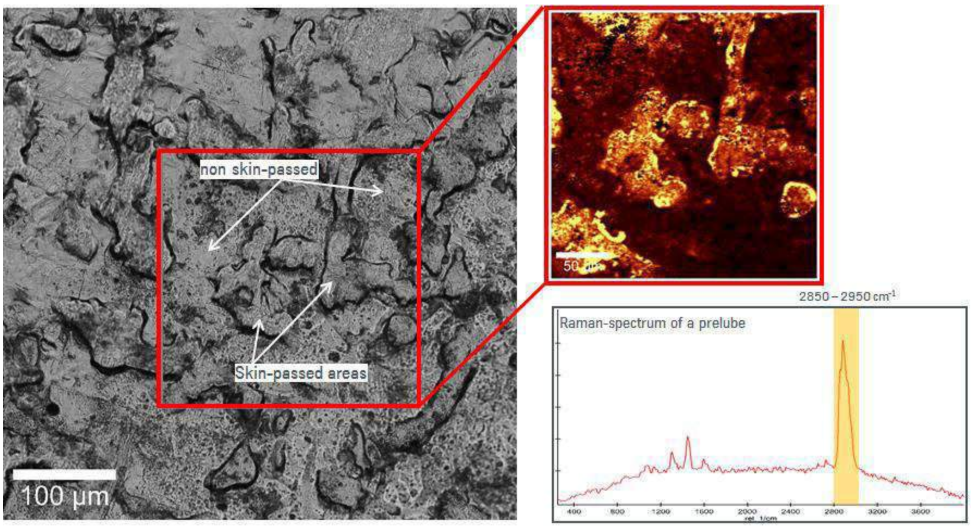

Fig. 1: False-colour image showing the distribution of a lubricant on a skin-passed hot-dip galvanized steel surface. The lubricant is mainly found in the skin-passed areas on the surface.

RISE, the confocal Raman mapping in SEM : a very promising approach for environmental asbestos issues

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In the field of geomaterials, the combination of composition and cristallographic or structural information is frequently required to identify a mineral. The combination of a -SEM-EDS (scannning electron microscope, equiped with energy dispersive X-ray spectroscopy) coupled to confocal Raman-in-SEM imaging in a unique system (RISE) is an efficient method for identifying the mineral nature of inorganic natural or anthropic materials. Then, the RISE microscope can provide a complete mineralogical description of mineral samples.

Asbestos consists of natural mineral fibers crystallized in a specific way with specific properties including flexibility, high tensile strength, resistance to heat and chemical degradation. The term asbestos refers to six different minerals. Asbestos is considered a Category 1 human carcinogen, as inhalation of asbestos fibers causes respiratory diseases, in particular asbestosis, lung cancers and malignant mesothelioma. Due to the pathogenicity, their use has been banned in most countries around the world. The ban applies to natural materials, which are quarried and used for public works projects.

The use of the RISE microscope is a new and efficient method for identifying the mineral nature of sub-micrometer fibers or fibrous bundles included (or not) in a mineral matrix. SEM coupled to EDS provides a qualitative or semi-quantitative composition of the fiber. The crystal structure is determined by confocal Raman spectroscopy (with lateral resolution down to 360 nm). Thus the combination of these two kinds of information allows for accurate identification of both the nature and the morphology of the mineral. The combination of SEM, EDS and confocal Raman imaging in a unique analytical system (RISE) allows precise location of the same area / fiber and identification of its mineral nature. In addition, RISE allows the acquisition of 3D data, which is able to provide morphological information of the mineral distribution in the sample volume and leading to the determination of the aspect ratio, a critical parameter for asbestos (i.e. asbestiform or non-asbestiform). Moreover, this combination of techniques is not destructive. The coupling of SEM-EDS with RISE is a powerful analytical system that simplifies and reinforces existing analytical procedures.

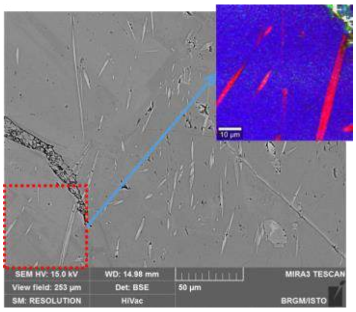

Fig. 1: Identification of sub-micrometric mineral fibers in a mineral matrix by RISE

Contributed Talks

Raman imaging of α-synuclein aggregates in a rat model of Parkinson's disease

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Accumulation of Lewy bodies in neurons of the brain is a hallmark of Parkinson's disease (PD). Lewy bodies consist of abnormal aggregates of misfolded α-Synuclein. The diagnosis of PD is limited to the manifestation of motor symptoms such as tremors rigidity and bradykinesia, since the brain can only be analyzed post-mortem. In patients and animal models phosphorylated α-Synuclein was detected in the gut, hence, raising the hypothesis that early-stage PD could be diagnosed based on colon tissues. Non-invasive marker-free imaging represent an ideal method to potentially detect aggregated pathological α-Synuclein *in vivo*. Raman imaging is a promising technique for the detection of molecular changes such as alterations in protein structures and the formation of aggregates. In this study, Raman microscopy of colon tissues of wild-type and transgenic rat model (BAC-SNCA) from either 7-11 weeks old or 18-19 month old rats was performed. Raman images were generated from the *muscularis mucosae* of rat colon tissue sections using 532 nm laser excitation. Raman images from transgenic and wild-type rats were analyzed using true component analysis (TCA). Spectral components that indicate protein aggregates in transgenic rat colon tissues were extracted. Moreover, the spectral analysis specified an increase of proteins in β-sheet formation in transgenic rat colon compared with wild-type colon. In summary, Raman imaging accomplished to detect α-Synuclein aggregates in colon tissues of a PD rat model. In conclusion, Raman spectroscopy could be potentially very useful to support diagnosis in PD pathology. Further studies using Raman imaging could unravel the formation and progression of these protein aggregates, and support to explore their expansion within neuronal tissues.

Investigation of reaction mechanism in Metal (Li, Mg)/Sulfur batteries by Insitu Raman

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Metal(Li,Mg)-sulfur (S) batteries are considered as one of the promising rechargeable battery technology due to their low cost, ecofriendly and high theoretical energy density. The high theoretical specific capacity (~1672 mAh g^{-1}) of Metal (Li, Mg) /S battery is the result of reversible two electron conversion reactions between sulfur and Li or Mg ($S_8 + 16Li^+ + 16e^- \leftrightarrow 8Li_2S$, $S_8 + 8Mg^{2+} + 16e^- \leftrightarrow 8MgS$) and finally form Li_2S/MgS through a series of intermediate polysulfides. Since the elemental sulfur and the different polysulfide species which are forming as reaction intermediates at different stages of the discharge/charge cycle are strongly Raman active, in-situ Raman analysis of a Metal (Li, Mg) / S cell helps to understand the redox reactions.

In the present work, we investigated the charge/discharge mechanism of a Li/S cell and Mg/S cell by in-situ Raman spectroscopy in glyme-based electrolytes. Raman spectra suggest that S_8 ring is reversibly decomposed and reformed during the cycling of Metal/S cell. The insitu spectra clearly demonstrate the stepwise conversion of solid S_8 to different higher order polysulfides (Li_2S_x, MgS_x, x=8-4) and their co-existence at high discharge potentials. The spectra also show the presence of an intermediate $S_3^{\bullet -}$ species during charge/discharge and its formation can be associated with the disproportionation reactions of polysulfides. In addition, we studied the self-discharge in metal sulfur battery by in-situ Raman spectroscopy.

The present results prove that in-situ Raman spectroscopy is a valuable tool to understand the redox mechanisms of Metal-sulfur (Li, Mg /S) batteries, which is highly dependent on the electrolytes used in the cell.

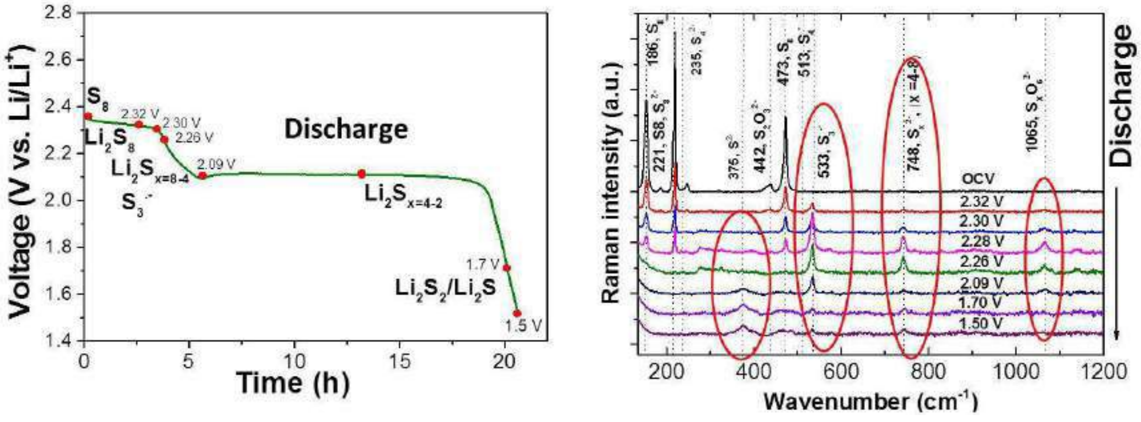

Fig. 1: In situ Raman spectra and associated discharge voltage profile of a Li/S battery

Illuminating the intracellular metabolism of the cancer targeting agent Neratinib by Raman microscopy

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Targeting epidermal growth factor receptors (EGFRs) has emerged as a potent strategy in cancer therapy. EGFRs are overexpressed in many malignancies. They are endogenously activated and can modulate tumor growth. Tyrosine kinase inhibitors, such as Neratinib, are developed to intervene with the EGFR activation mechanism that can eventually suppress the tumor. However, the intracellular metabolic fates of these agents have not yet been fully understood. Here, confocal Raman microscopy was conducted to detect the subcellular uptake and distribution of Neratinib. Cyanide group stretching vibration was used as Raman marker band for mapping the uptake and distribution of Neratinib inside cancer cells. Furthermore, two new metabolites of Neratinib have been detected based on their Raman spectral features. Upon conducting hierarchical cluster analysis (HCA) on the Raman datasets, the Raman spectral pixels of the two metabolites can be illuminated, they were further overlaid with the corresponding fluorescence images of the same cells. The results indicated that these metabolites are accumulating in the lysosomes, and the EGFR receptors are directed toward the endosome-lysosomal pathway for degradation. By the aid of DFT calculations and LC-MS analysis, Raman microscopy has been used to identify the metabolic biochemical structural changes of Neratinib in human breast and lung cancer cells. These findings open a new venue for Raman microscopy as a valuable tool to study drugs pharmacokinetics.

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Poster Abstracts

Raman spectroscopy of some amorphous metal phosphates

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Metal phosphates in both crystalline and vitreous forms are extensively studied for their potential application in heterogeneous catalysis [1], renewable energy [2], biomedical engineering [3], and environmental remediation [4]. Among these phosphates, those belonging to Nasicon family have attracted much attention due to their diverse properties [5]. Many compounds of Nasicon family can be prepared also in the glassy form, they are called Nasiglasses (Na super ionic glass) [6].
Nasiglasses belonging to systems [Na_2O - Li_2O - CaO - TiO_2 - P_2O_5] and [Na_2O - CaO - TiO_2 – GeO_2- P_2O_5] were synthesized and characterized by DSC, DTA, PXRD, Raman spectroscopy, density and ionic conductivity measurements. When Na^+ is gradually replaced by Li^+ in phosphates $Na_xLi_yCa_zTi_t(PO4)3$ some physical properties (density, molar volume, glass transition temperature, and ionic conductivity) vary in a non-linear manner, as a result of the mixed ions effect.
Raman spectroscopy study was carried using a confocal micro-Raman Labram (Horiba/Jobin-Yvon) spectrophotometer with a backscattering mode at room temperature. The Raman spectra shows that the glasses contain short -Ti-O-Ti-O-Ti- chains, and P2O7 (Q1) and PO4 (Q0) groups. The presence of -Ti-O-Ti-O-Ti- chains indicates that the TiO6 octahedra are linked to each other through corners. This proves that when lithium replaces sodium, indicating that Li^+ ions don't modify the glass structure and occupy the network cavities.

Key words: Metal phosphates; Glass; PXRD; DTA; DSC; Raman spectroscopy; Ionic conductivity.

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Exploring the Spatial Organization of the Rhizosphere Using Confocal Raman Microscopy

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The rhizosphere as a region of soil adjacent to plant root comprises microbial communities that plays an important role in plant growth. In this project we aim to study the water and nutrient transfer between the plant roots and the associated microorgansims in normal conditions and those affected by drought considering the spatial organization of the rhizosphere. We propose to combine imaging mass-spectroscopic techniques, namely Laser Ablation Inductively Coupled Mass Spectroscopy (LA-ICP-MS), Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS), Fluorescence/Halogen in Situ Hybridization in combination with Stable-Isotope Probing (SIP) and nanoscale SIMS (FISH/HISH-SIP-nanoSIMS), as well as high-resolution imaging scanning electron (SEM) and helium-ion microscopy (HIM). In addition, the possibility of using Confocal Raman Microscopy (CRM) for rhizosphere analysis will be explored by our project consortium. Being a non-destructive molecular-sensitive method with sub-micrometer spatial resolution, CRM is expected to provide information on molecular composition of various rhizosphere compartments and on the function of its associated microorganisms. Using stable–isotope labelling, correlative microscopy and mass spectroscopy our objective is to visualize the in situ function, identity, morphology and spatial distribution of rhizosphere bacteria. The main obstacle for in-situ CRM analysis of rhizosphere samples is a strong auto-fluorescence of soil inorganic materials (SIM) and freshly burrowed pigment containing organic matter that hampers the detection of desired Raman-scattering signals. In our preliminary work, we applied IR laser excitation (785nm) and achieved the reduction of auto-fluorescence by a factor of 10. Using IR excitation instead of visible light may also reduce chemical or physical bleaching. Currently, only few CMR studies on rhizosphere bacteria [1, 2] exist. These involve Nycodenz density gradient cell detachment, cell concentration, co-culturing followed by SIP or/and silver nanoparticles deposition/precipitation for Raman-scattering enhancement. By applying a comprehensive correlative approach we hope to answer key questions regarding the root-microbes-soil particles interactions in the rhizosphere.

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Analytical approach for the identification and quantification of microplastic particles in aquatic samples by a combination of particle analysis with FTIR and Raman microscopy

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The quantitative detection of microplastic particles (MP) in an environmental sample in the size range of 1 µm to 5 mm in a reasonable time represents a challenging mission.

The presentation will describe the procedures to identify and quantify MP in aquatic samples including the work in MP-free cleanrooms, the use of MP-free equipment and clothes, and the use of blank tests in all steps in the lab. The first part is a three-step vacuum filtration to divide the particles in four fractions between 1 µm and 5 mm. After filtration, the particles are on silicon filters which are suitable for FTIR (Bruker Hyperion) and Raman (WITec alpha300) measurements. An optical particle search program determines all particles in shape and dimension and stores their coordinates for the subsequent measurements with FTIR and Raman microscopy. Both methods identify the MP on the filter by their chemical structure using spectral databases. Every particle is assigned via the spectrum to a substance. This can be a polymer, in case of a mixed spectrum a polymer with paint/filler, or no polymer. The fractions above 50 µm are measured mainly by FTIR and the fraction below 50 µm mainly by Raman. However, several samples are measured with both methods, because a combination of both spectroscopic methods delivers a complete analysis of MP.

The advantage of FTIR is a faster measurement and often a better identification of the polymer matrix in colored samples; the disadvantage is the detection limit of 15 µm size. The advantage of Raman is the better detection of additives and paints and the identification of particles up to 1 µm; the disadvantage is the longer measurement time and the appearance of fluorescence in case of not sufficiently cleaned samples. All parameters which influence the measurement and a comparison between FTIR and Raman will be discussed.

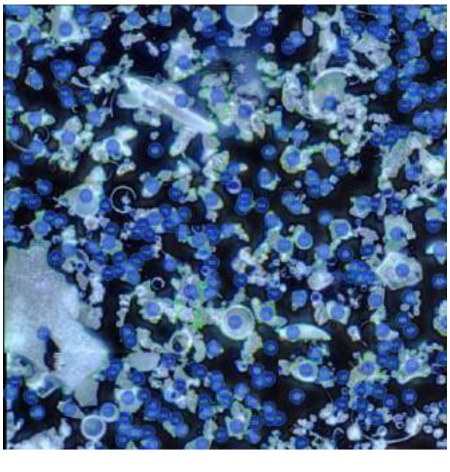

Fig. 1: Sample from a setting sediment sampler, Baltic Sea, Gotland, Sweden; collecting of all floatable particles within 10 days; 1081 particles on filter area of 1 x 1 mm after optical analysis

Early age hydration of monoclinic C_3S studied by Confocal Raman Microscopy

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Portland cement (PC) is the most often used cement type today, and is a key ingredient of concrete. Despite of this, and even after several decades of studies, the exact mechanism of the cement hydration is still subject of debate. Vibrational spectroscopies, like Raman and IR spectroscopy, have proven to render themselves for *in situ* studies of cement hydration, since they do not require any special sample preparation, and allow all the phases taking part in the reaction to be followed.

The principal component of PC is tricalcium silicate, C_3S. Here, we for the first time present Raman microscopy results related to the first stages of C_3S hydration, up to a maximum hydration time of 7 days. The sample also contained a minor fraction of dicalcium silicate, C_2S.

Fig. 1 displays a series of image scans taken from the same spot of a hydrating C_3S sample after different times. The formation of the main product C-S-H (yellow) is clearly visible after around 10 h of hydration. Its formation primarily occurs around C_3S grains (red). The C_2S grains (blue) remain essentially uncovered for several days. The other product of C_3S hydration, portlandite, CH, (cyan), was observed already after a hydration time of 4 h. This difference is surprising, since both products are normally thought to be formed simultaneously. Calorimetric data also suggests that the reaction strongly accelerates after 4 h and decelerates after 14 - 16 h. Since the same difference during the acceleration period can also be found by monitoring the C-S-H and CH peaks in Raman spectra, an intermediate C-S-H phase is expected to be formed first and then transformed to C-S-H. The main difference of these C-S-H phases appears to be the length of the silicate chain. The initially formed C-S-H phase contains dimeric silicate chains, which grow in length over time leading to the later-stage C-S-H. These conclusions are in line with recent detailed solid state NMR data (Pustovgar et al. Nat. Commun. 7 (2016) 10952), and Raman microscopy studies allow further information to be obtained about the spatial distribution of these phases.

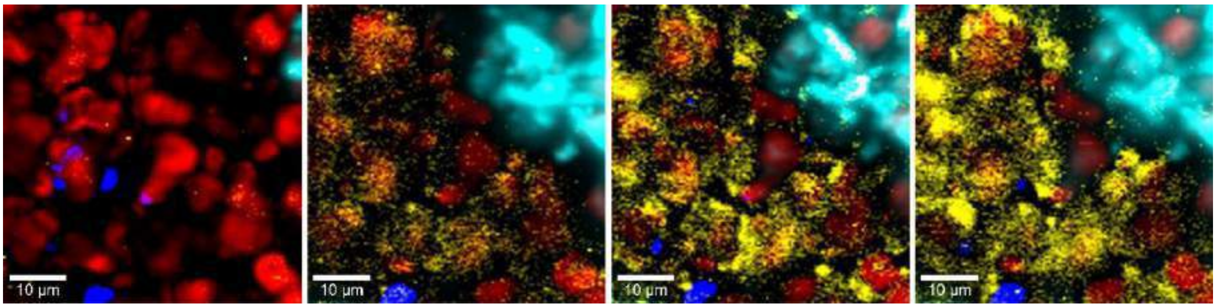

Fig. 1: Raman images of a hydrating m-C_3S sample after 6 h, 12 h, 24 h and 72 h from left to right.

4Pi Raman microscopy as a superresolution method for imaging of single cells and C elegans as multicellular organisms

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The Raman molecular fingerprint of is obtained by illuminating the sample with the laser light and collecting the Raman scattering signals from molecular bonds. By moving the confocal laser spot in roster pattern across the area of interest and collecting the Raman spectrum at each point- the hyperspectral image of this area can be constructed. Applied to cell imaging allows us to visualize the intercellular components without the use of any labels. The majority of the Raman confocal cell images are acquired mainly in lateral plane, because, as in any microscope technique based on light focused through an objective, the axial resolution of conventional Raman confocal microscope is 2-3 times lower than the lateral resolution. We have improved axial resolution by combining the 4π technique with confocal Raman microscope. The 4π effect is achieved by focusing the 2 coherent laser beams on the same spot. The resulting interference between laser beams produce the spot that is sharper then confocal spot leading to increase in resolution.
Here we report the first 4π superresolution hyperspectral Raman axial images of a single mammalian cell. We also show that this imaging technique can be applied to complex multicellular organism such as *C. Elegans.*

An alternative Raman spectroscopy analysis of laser-reduced graphene oxide

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Since its discovery in 2004 and its Nobel Prize distinction in 2010, graphene has fueled the interest of the scientific community due to its unique and useful properties: mechanical, electrochemical stability, high electron mobility, and optical transparency. In this regard, graphene oxide (GO), the oxidized form of graphene, has also attracted the interest of the community working on these novel 2D materials. In particular, since graphene oxide has several advantages over graphene such as: easy and inexpensive production and water solubility. One of the most significant benefits that GO has over graphene is the possibility to easily control the electrical conductivity, hydrophilicity, and degree of transparency by tuning its level of oxidation. The so-called reduced graphene oxide (rGO) is a derivate of GO that is made more graphene-like after a chemical, thermal, or light irradiation treatment. rGO has inspired a significant number of graphene-based devices including transistors and various types of the sensors. The transformation of GO to rGO by laser annealing is an efficient, easy-to-make and environmentally friendly method to obtain graphene-like materials with arbitrary geometries (patterning). Raman spectroscopy is the tool of choice in the analysis of carbon nanomaterials. In particular, the degree of reduction of rGO by Raman spectroscopy was previously investigated by Claramunt *et al.* They reported that between the D and G bands, there are three other bands, the so-called interbands D*, D', D", that need to be considered in the analysis of the Raman spectra from rGO samples. It was reported the correlation between the oxygen content and the positions of D* and D". The dependency of these bands on the degree of reduction reflected by the oxygen content was verified by X-ray diffraction. However, we found that in many cases such analysis is not sufficient, due to the large uncertainties in the fitting of interbands for the analysis of rGO made by laser reduction. In this work, we report a systematic investigation of laser-reduced rGO patterns by Raman spectroscopy. Using different laser irradiation allowed us to control the degree of reduction in the rGO patterns. The electrical conductivity of the different rGO patterns was evaluated by sensing current atomic microscopy (CSAFM). A further step will be presented correlating the different laser irradiation parameters, degree of reduction, and electrical conductivity. This work opens the door to the fast and accurate investigation of rGO micropatterns made by laser irradiation using an alternative analysis based on Raman spectroscopy.

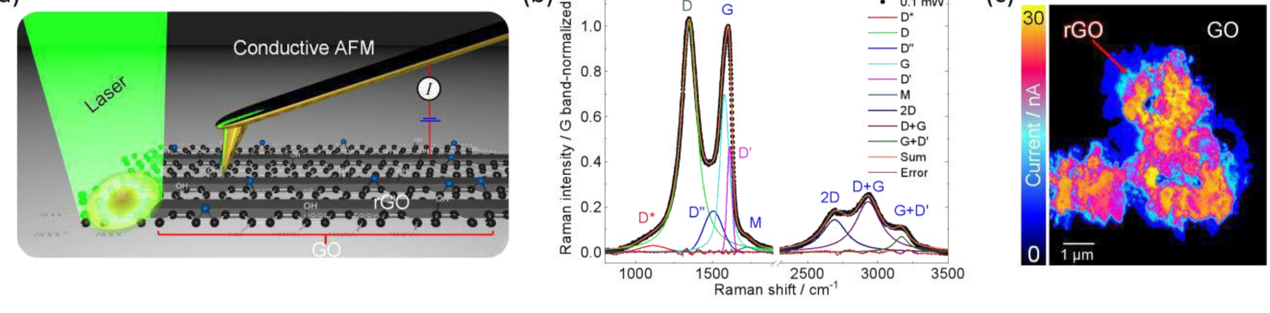

Figure 1: (a) Schematics of the laser patterning reduction of GO. (b) Raman and (c) One CSAFM result on GO film

Plasma-Assisted Synthesis of Oriented Carbon Nanostructures Using Polymer Gels

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Oriented carbon nanostructures have attracted the research interest due to their unique orientation, morphology, open boundaries, electronic properties, large surface area and edge effects[1]. Oriented carbon nanowalls, nanorods, nanoflakes and nanoroses are different vertical carbon nanostructures. Among all the reported synthesis techniques, plasma-assisted deposition techniques are considered as a promising method for the large-scale synthesis of oriented carbon nanostructures. Low-temperature and low-pressure growth conditions are one of the main advantage of plasma-assisted techniques. This study explores an alternative route of plasma-assisted technique for the growth of vertically aligned carbon nanostructures from a carbon precursor. Several studies showed that phenol derived gels can produce carbon nanostructures by carbonization process[2,3]. So that, resorcinol-formaldehyde polymer gels were used as a precursor for a carbon source in this study. A radiofrequency inductively coupled plasma(RFICP) was used for the synthesis process, where a thin layer of polymer gel was cast on a glass substrate and placed in plasma. Argon and hydrogen gas species were used in the surface treatment. Scanning electron microscope (SEM) imaging and Raman spectroscopy were used for the characterization of vertical nanostructures. Moreover, the study explored also the influence of growth time and plasma power on the orientation and morphology of the carbon nanostructures.

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Type 2 diabetes detection based on serum sample Raman spectroscopy

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In this work, we propose to use of the Raman spectroscopy as a new technique for the detection of the type 2 diabetes using blood serum samples. The serum samples were obtained from 15 patients who were clinically diagnosed with type 2 diabetes mellitus and 20 healthy volunteers. The average spectra major differences were peaks found for healthy patients at 661 and 1404 cm^{-1} (glutathione), 714 (polysaccharides), 605 (Phe), 545 cm^{-1} (tryptophan), and the shoulder of amide III at 1230–1282 cm^{-1}, where seem to disappear in the diabetes spectrum. On the contrary, the region that is more highlighted due to that diabetes peaks was 897–955 cm^{-1}. Principal component analysis and linear discriminate analysis were employed for developing discrimination method. The first three principal components provided a classification of the samples from healthy and diabetes patients with high sensitivity and specificity. In addition, when the first principal component was plotted as a function of the Raman shift, it revealed these shifts accounted for the greatest differences between control and diabetes samples, which coincided with the shifts of spectral differences shown by mean spectra. Our results demonstrated that serum sample Raman spectroscopy promises to become a non-invasive support tool of the currently applied techniques for type 2 diabetes detection, decreasing the false-positive cases.

Raman, SEM and EDS Analysis of Coatings on Drug Eluting Stents

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Stents are used to reopen closed or narrowed arteries and veins in humans. To avoid foreign body reactions and to allow optimal acceptance the stent may be coated with dedicated drugs. These drug eluting stents (DES) have the potential to reduce restenosis after angioplasty followed by application of a coronary stent. Drug loading into micro- or nano-pores created on the stent surface could allow defined and sustained release of the drug. To this end we have developed a novel drug eluting stent based on a modified surface. The pores are defined by electrochemically induced pitting corrosion in medical grade steel. These pores are filled during a special coating process with rapamycin [1].

To control and optimize the deposition, we analyzed the quality and distribution of the coating with Scanning Electron Microscopy (SEM), confocal Raman spectroscopy and energy dispersive X-Ray spectroscopy (EDS). Raman shows a clear signature of the drug whereas EDS gives additional information about elemental distribution on the stent surface. The Raman signature of rapamycin shows a prominent line at 1630 1/cm which allows stable mapping of the drug distribution.

To investigate and optimize early states of the drug deposition on the stent surface we plan to use TERS (Tip Enhanced Raman Spectroscopy) in future.

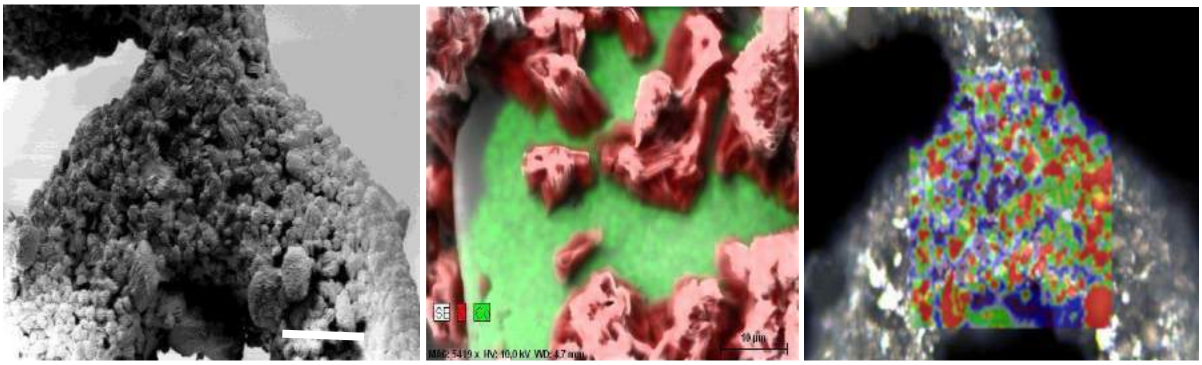

Fig. 1: Stent coated with rapamycin. SEM image (left), EDS cobalt and carbon image of selected area (middle) and confocal Raman image of the rapamycin distribution. Scale bar: 10µm

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Microstructural Properties of ZnO Powder Nanostructures Prepared by Mechanical Alloying

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Introduction

The wide bandgap semiconductors (ZnO) have been studied for several years in a highly competitive international environment given their wide range of applications. In our work we prepared powder nanoparticles mechanically alloyed .

Experimental/theoretical study

ZnO powder nanoparticles mechanically alloyed were doped with iron to investigate their structural and microstructural properties using X-ray diffraction (XRD) , scaning electron microscopy (SEM) [1,2] and differential scanning calorimetry (DSC) for examined pur ZnO and 5% Fe doped ZnO.

Results and discussion

The ZnO starting pure powder exhibited a hexagonal crystal structure with space group p63mc of ZnO, however with the introduction of 5%Fe in the ZnO milled powder, the hexagonal ZnO phase remained unchanged, whereas the microstructural parameters were subject to significant variations due to the introduction of Fe atoms into the ZnO hexagonal matrix to replace oxygen ones [3]. The size of crystallites and microstrains are found milling time dependent.

Conclusion

This product exhibited a hexagonal crystal structure with space group p63mc of ZnO and with c-axis preferential orientation, however with the introduction of 5 % Fe in the ZnO milled powder, the hexagonal ZnO phase remained unchanged,whereas the microstructural properties were subject to significant variations due to the introduction of Fe atoms into the ZnO hexagonal matrix to replace oxygen ones.

Temperature dependence of the Raman spectra of fish collagen gel

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Modern medicine widely uses exogenous collagen as good material for tissue regeneration, also as natural substrate for cell attachment and proliferation, used to create dressings and to support the treatment of burn wounds or diabetic wounds. Collagen is a safe material that has a high biocompatibility and biodegradability and good cell adhesion. Due to the possibility of transferring Creutzfeld-Jacob disease(*Bovine Spongiform Encephalopathy)* from animals to the human body, the interest in collagen from fish increased.

The collagen we examined comes from the skin of silver carp fish (Hypophtalmichthys molitrix), and was obtained by method of hydration in an aqueous lactic acid solution. Raman spectroscopy was used to study fish collagen gel (FCG) with a commercial micro-Raman spectrometer (inVia Raman microscope; Renishaw) with a 40×long working distance objective (Leica) coupled to a 632.8 nm He Ne laser excitation source (17.8 mW; 8 mW at the sample). Analysis of Raman spectra (Fig.1) allowed to determine the content of amino acids in collagen, including glycine, proline and hydroxyproline. It showed the native nature of the material at 20°C. We also collected Raman spectra during the collagen heating between 20–110°C to investigate process of its denaturation. Raman spectra revealed that the most responsive to temperature changes is tyrosine Fermi doublet connected with lines ~855 cm^{-1} and ~825 cm^{-1} and arising from an interaction between the ring breathing fundamental and the overtone of the C–C–O deformation in the *para*–substituted aromatic ring of tyrosine. Ratio I_{850}/I_{825} changed for the collagen FCG from 0.31 (20°C) to 1.40 (110°C) and exhibited unfolding of protein chains. Raman spectroscopy has been presented as an effective method for testing biopolymers.

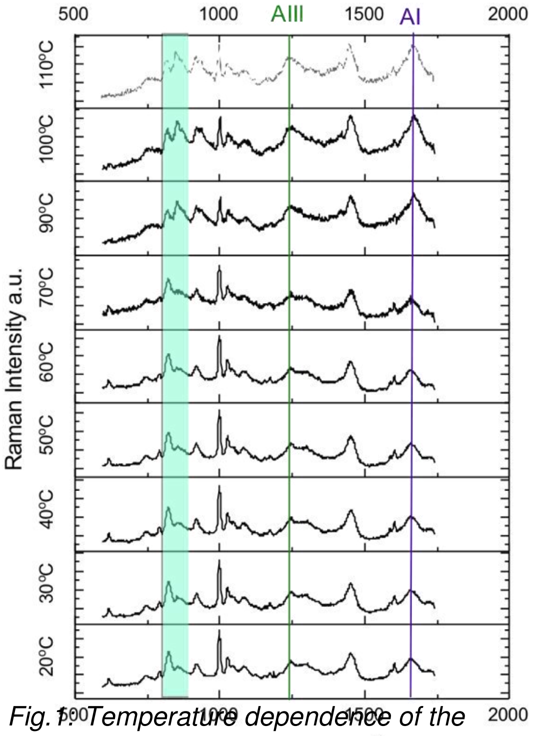

Fig. 1. Temperature dependence of the Raman spectra of fish collagen gel FCG

Application of Raman Imaging to the Analysis of Old Papers

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The knowledge of the exact and detailed chemical composition of old papers can be important and useful for different purposes. It can give information on historic paper making practices and can help paper conservators to evaluate the preservation state of old papers and to find suitable measures for paper conservation or restoration. Furthermore, the chemical analysis of paper can help to reveal forgeries of works of art or of documents.

The complex composition of papers still today presents a great challenge to chemical analysis, especially when the used methods have to be non-destructive and paper components are only present in low concentrations. Raman microscopy gives new possibilities to chemical paper analysis [1,2]. By using the Raman imaging technique, the chemical structure, that means the presence and the distribution of different paper components on the surface or along the cross-section, can be visualized. This includes different fibers, filler pigments, sizing compounds and others. Beside the intentionally added components to the paper, also other substances that came as trace particles with the main components in the paper or were formed during the production process can be detected and identified.

The presentation will demonstrate the application of Raman imaging to paper analysis. Results of measurements on different old papers will be presented.

[1] E. Pigorsch, M. Finger, St. Thiele and E. Brunner, *Appl. Spectrosc.* 67(1) (2013) 59-65

[2] E. Pigorsch, M. Finger, St. Thiele and E. Brunner, Application of Raman Microscopy to Analysis of Paper in Documents and Works of Art, 8th International Conference on the Application of Raman Spectroscopy in Art and Archaeology (RAA), Wroclaw, 2015

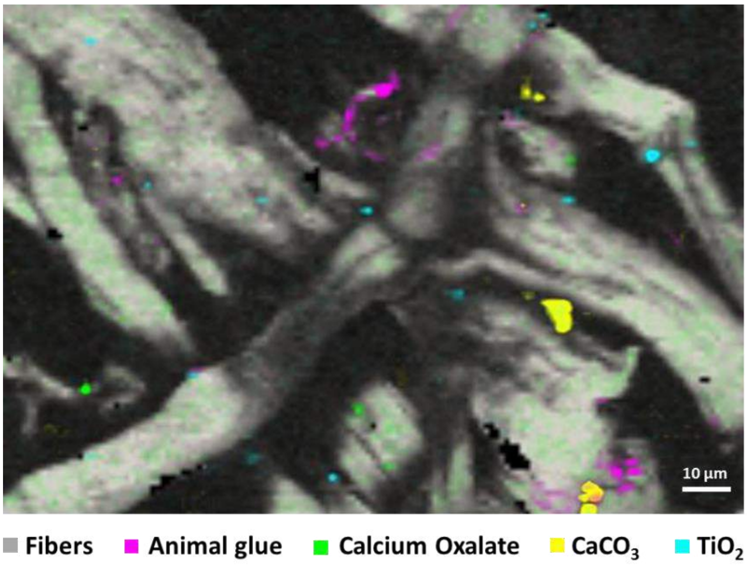

Fig. 1: Raman image of the surface of a 15th century paper

The contribution of bubble-hosted mineral phases to the volatile content of melt inclusions estimated by 3D Raman imaging

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The style, dynamics and intensity of volcanic eruptions are controlled by the volatile content of magmas. Reliable modeling of volcanic processes and magma ascent rates is based on correct assessment of the original volatile budget of magmas. Crystal-hosted melt inclusions (which are droplets of magma entrapped during crystal growth, Fig.1a) record the volatiles contents (e.g. H_2O, CO_2) of the magma at the time of inclusion entrapment and, therefore, represent an important tool for the study of volcanic systems. Glass inclusions often contain micrometer-sized bubbles that form due to cooling and pressure drop during magma ascent and eruption. Bubbles generally contain one or more fluid phases (e.g. H_2O, CO_2); some of them also host solid phases crystallized at the glass-bubble interface, which are mainly carbonates (e.g. calcite, magnesite) and sulfates (e.g. anhydrite) precipitated from the fluid phase (Fig.1b).

Preliminary investigations carried out using Raman spectroscopy indicate that the volume of these solid phases may be important (a few tens vol.% of the bubble). Thus their contribution must be considered for correct estimation of the magma volatile budget, in particular CO_2. High confocal 3D Raman imaging allows us mapping solid phases hosted in bubbles with sub-micron spatial resolution. We present a method to identify the different phases filling inclusion-hosted bubbles and to quantify their volumes by confocal 3D Raman imaging (Fig.1b) using a Renishaw InVia Raman microspectrometer. The method is applied to the study of olivine-hosted melt inclusions erupted from different volcanoes. The original total CO_2 contents of the magmas feeding these volcanoes are quantified after examining the accuracy and the limits of the 3D method.

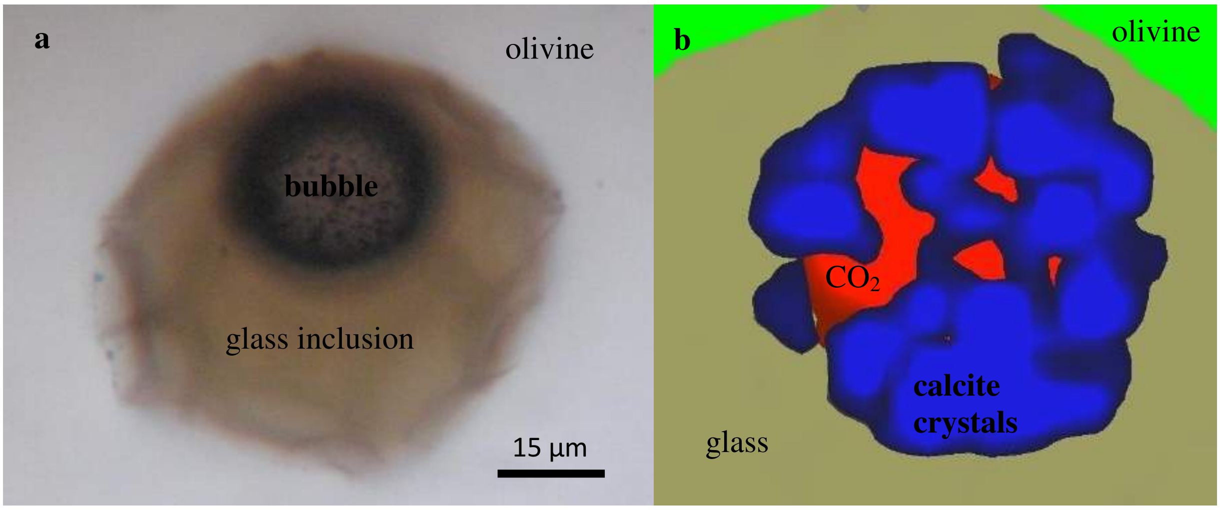

Fig. 1: (a) magmatic inclusion from the Ardèche volcanic province (France); (b) Raman imaging of the inclusion-hosted bubble containing carbonates and CO_2.

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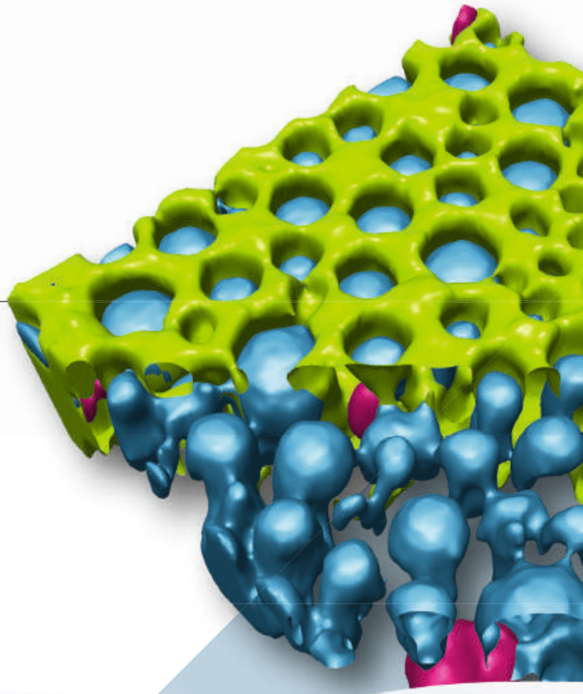

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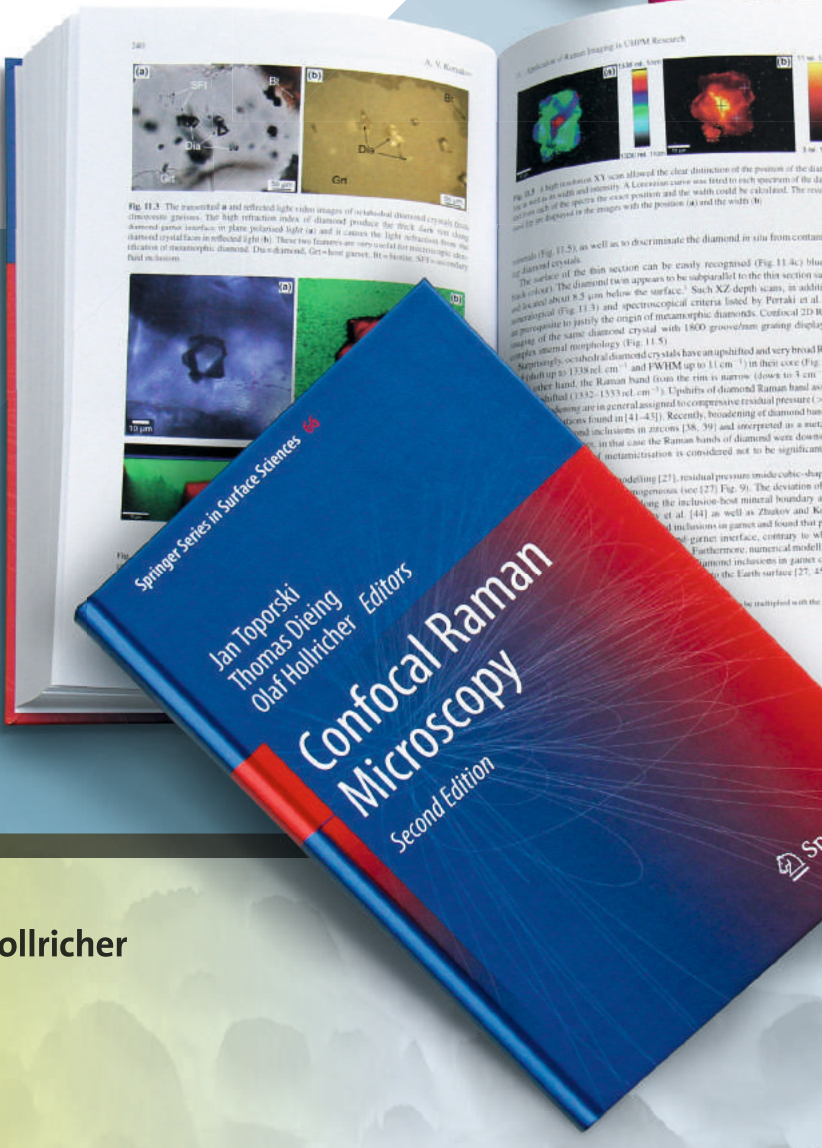

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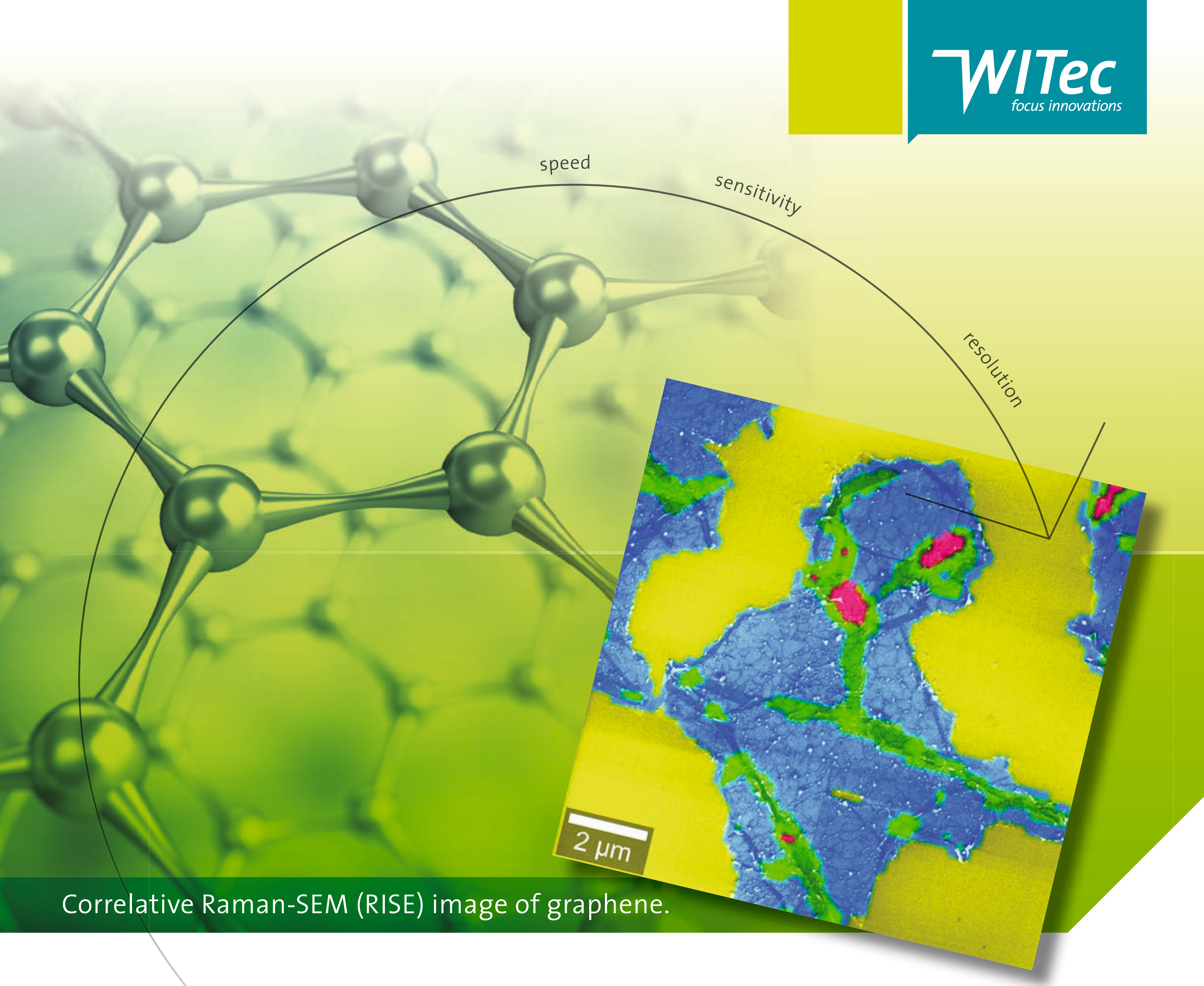
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