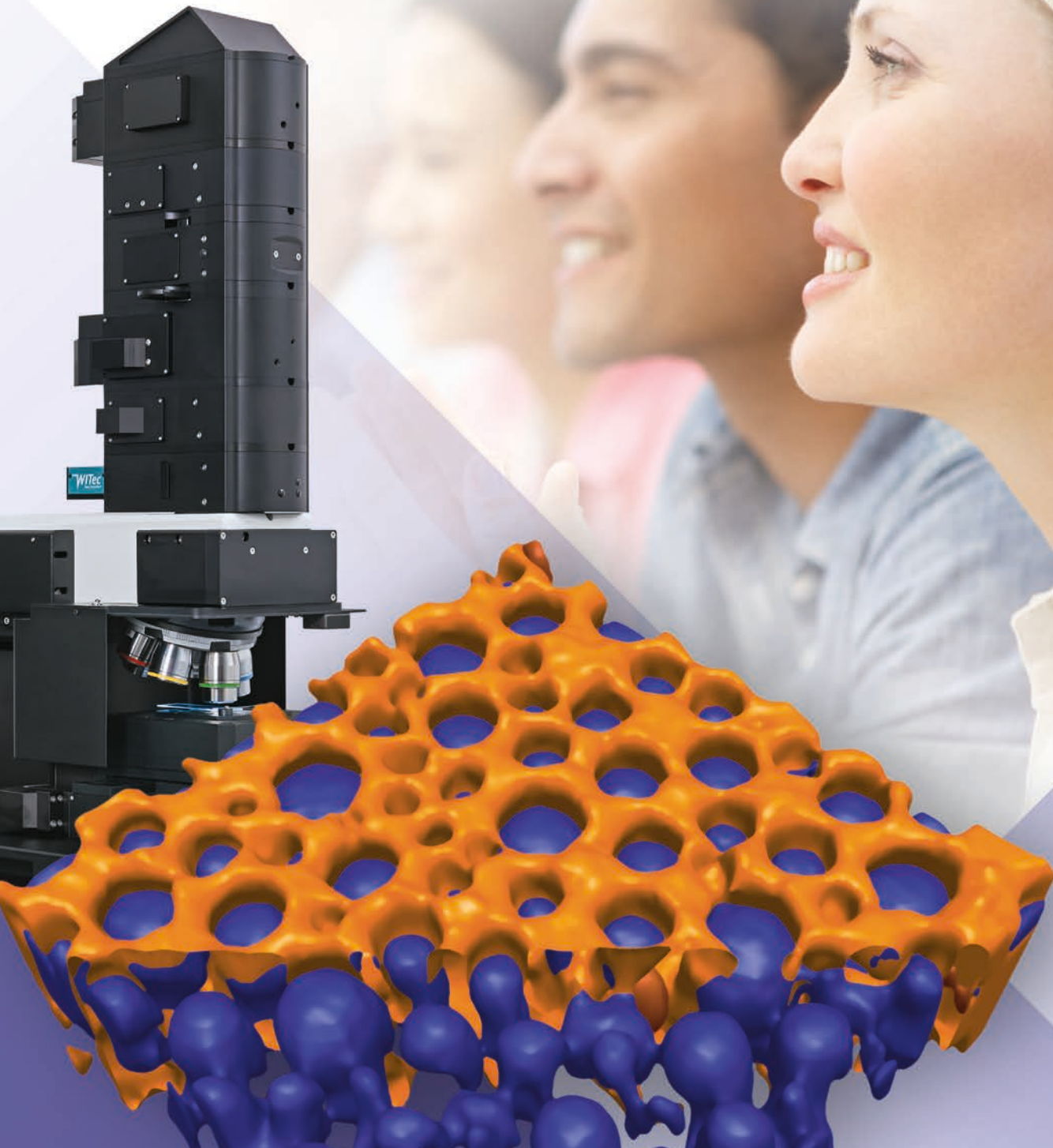


19th Confocal Raman Imaging Symposium

September 25 - 27, 2023
Ulm, Germany



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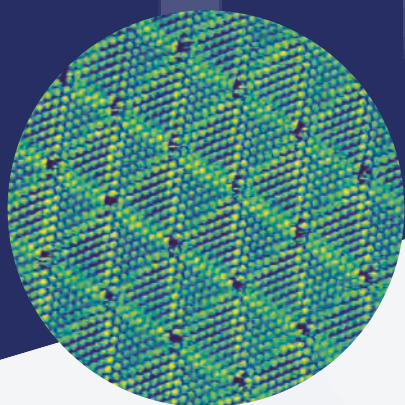
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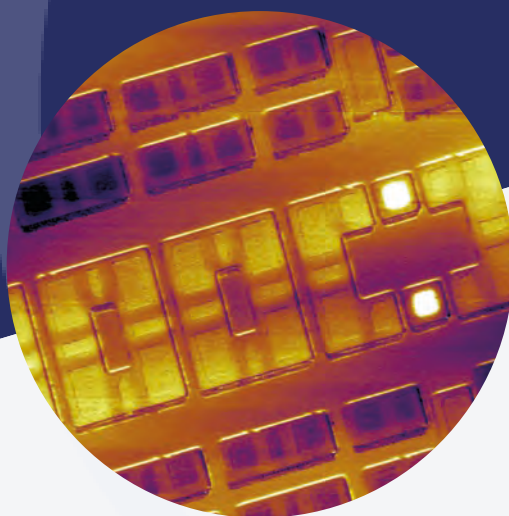
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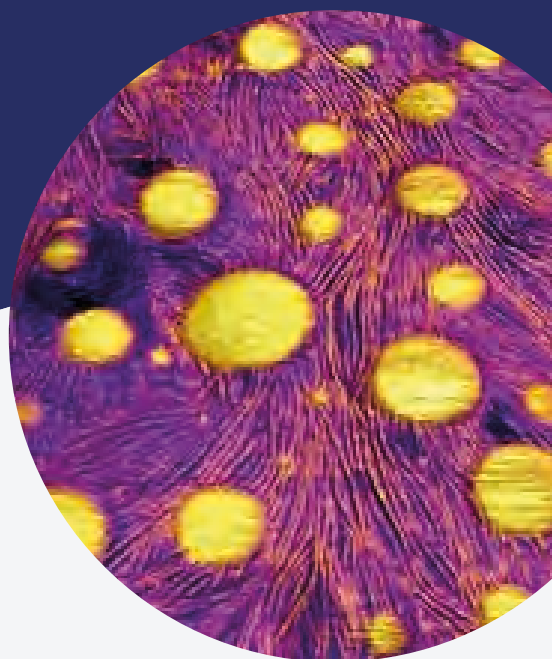
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Conductive AFM (CAFM) image of twisted trilayer graphene on hBN, revealing moiré and super moiré patterns



Surface potential of an SRAM sample using Kelvin Probe Force Microscopy (KPFM)



Viscoelastic modulus mapping of a polystyrene (PS) / polycaprolactone (PCL) polymer thin film



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WITec Paper Award

The annual WITec Paper Award recognizes exceptional scientific papers published in peer-reviewed journals that include results acquired with a WITec microscope. Scientists from all over the world are invited to submit research that appeared (in print or online) during the current year.

The article must be in English and the use of a WITec microscope should be clearly documented in the article itself or its supporting information. Publications co-authored with an employee of the Oxford Instruments Group are not eligible for a WITec Paper Award.

Any co-author can submit a paper and their participation will automatically enter their name in a prize draw. The prizes are ten WITec backpacks with RFID protection and the draw will take place shortly after the submission deadline. Submitting multiple publications per year is allowed and increases the chance of winning in the prize draw.

Publications from the current year can be submitted until January 31st of next year.

A WITec jury will evaluate the submitted papers in terms of scientific relevance, data quality and the level of instrument feature utilization to determine the winners. The first author(s) of the three winning publications will receive Amazon™ gift cards as prizes: 500 EUR, 300 EUR and 200 EUR for the Gold, Silver and Bronze Awards, respectively.

HOW TO CONTRIBUTE:

- 01** Send your paper(s) in PDF format to papers@witec.de. Include the supplementary information (if applicable) and your full contact information.
- 02** Receive a WITec backpack with RFID protection if you're one of the 10 winners of the prize draw.
- 03** Await the results of the WITec Paper Award competition.

For more information about the WITec Paper Award including a list of previous winners, please visit:
<https://Raman.oxinst.com/paper-award>.

WITec Headquarters

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info@WITec.de . www.WITec.de



Welcome

It is our great pleasure to welcome you to the 19th Confocal Raman Imaging Symposium.

Over the years, this conference has become highly regarded among Raman microscopists for the variety and scientific quality of the results presented from its stage, and its atmosphere conducive to discussion that creates a real sense of community. The Symposium's poster sessions, talks and equipment demonstrations reflect where Raman technology and applications are today, and the knowledge exchanged between attendees, speakers and organizers provides a basis for future developments and collaborations.

For the 19th Confocal Raman Imaging Symposium, we're again honored to host renowned speakers to share their expertise and insight. The first day will feature a thorough introduction to Raman spectroscopy and microscopy by Prof. Sebastian Schlücker, before the keynote lecture is delivered by Prof. Maike Windbergs with a focus on Raman imaging in biomedical research. During the evening lecture, Dr. Martina Griebner will present a striking investigation of an incomparable historical treasure: the Imperial Crown of the Holy Roman Empire.

The second day's talks will illustrate the use of Raman microscopy in diverse fields of application, ranging from the study of battery and low-dimensional materials through pharmaceutical research and food science to analyses of microplastics and astromaterials. The posters on display will further exemplify the possibilities of the technique and the creativity of the scientists driving it forward.

Our application specialists look forward to welcoming you to WITec headquarters on the third conference day for interactive equipment demonstrations in our laboratories. A mixture of presentations and hands-on sessions will showcase our latest hardware and software advances while offering opportunities for individual discussions.

We wish you a memorable and rewarding time in Ulm, with many inspiring discussions during the three days of the 19th Confocal Raman Imaging Symposium.

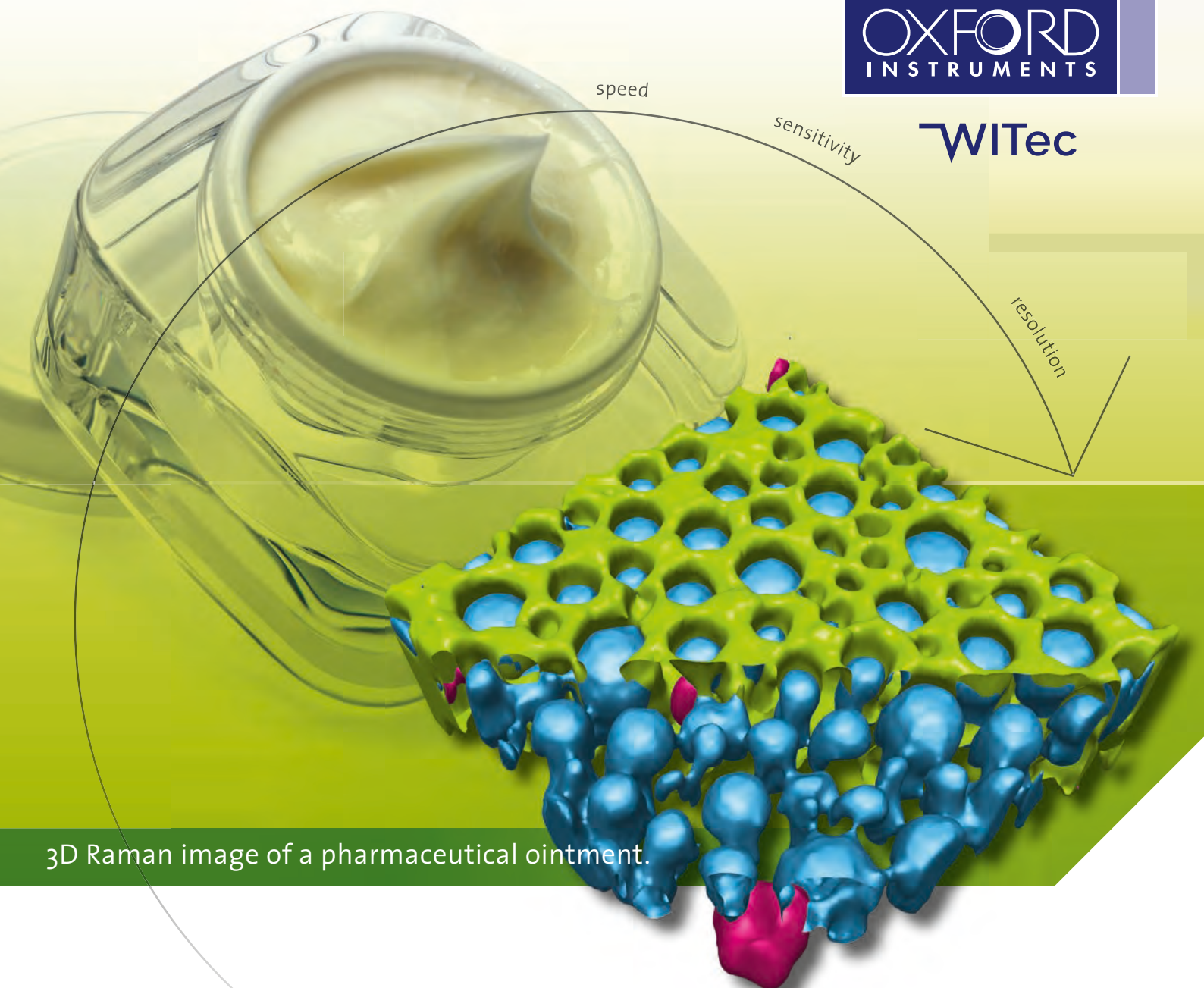
Sincerely,

Dr. Joachim Koenen and Dr. Olaf Hollricher

Dr. Joachim Koenen (left)
Dr. Olaf Hollricher (right)
—
Founders and Managing
Directors of WITec



General Information



3D Raman image of a pharmaceutical ointment.

3D Raman Imaging

Turn ideas into **discoveries**

Let your discoveries lead the scientific future. WITec's confocal 3D Raman microscopes enable cutting-edge chemical imaging, Raman-based particle analysis and correlative integration with SEM, AFM, SNOM or optical profilometry. Discuss your ideas with us at info@witec.de.



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www.witec.de

General Information

Locations

See enclosed city map and directions for details (pp. 11 - 13).

Conference talks and poster sessions (September 25 - 26)

Stadthaus Ulm, Münsterplatz 50, 89073 Ulm

Directly southwest of the Ulm Minster (see p. 11)

Conference dinner (September 26, 7:00 p.m.)

Restaurant "Zum Haberkfelder, Ulmer Wirtshaus", Neue Straße 56, 89073 Ulm

Ca. 300 m from the Stadthaus (see p. 11)

Equipment demonstrations (September 27)

WITec Headquarters, Lise-Meitner-Str. 6, 89081 Ulm

Streetcar stop "Science Park II" nearby, parking spaces available (see pp. 12 - 13)

Meals

Lunch and dinner on September 26 as well as lunch on September 27 will be provided by WITec.

WLAN

WLAN will be available throughout the conference.

Participants will receive individual access codes for the WLAN in the Stadthaus.

In the WITec Headquarters building, you can access the WLAN for guests with the following details.

WLAN name: Pegasus

WLAN password: Goldwing4all!

Contact the organizers

The event is organized by WITec GmbH, Lise-Meitner-Str. 6, 89081 Ulm, Germany.

E-Mail: events@WITec.de, Phone: +49 (0) 731 140 70 0

Ulm & Neu-Ulm

The Twin Cities on the Danube

Middle ages meets modernity

Ulm's citizens are an audacious people; they commissioned star architect Richard Meier to design the dazzling white and geometric Stadthaus, located adjacent to the revered and ornate Ulm Minster. Modern buildings, including the Weishaupt museum and the glass-walled pyramidal central library, surround the medieval town hall.

Ulm is also home to a truly unique superstar, the "Löwenmensch" (lion man). It was made in a cave near Ulm some 32,000 years ago from the tooth of a young mammoth. "Löwenmensch" is to date the oldest figurative art ever found.

Other famous residents of Ulm were Albert Einstein, born in the town, and resistance fighters Hans and Sophie Scholl, who were executed in 1943 in Munich.

Founded around 850 and long renowned as a free imperial city, Ulm has many sites of historical interest. The best known is the gothic Minster, the tallest church in the world. Begun in 1377, it was built for 20,000 people, then twice the population of the city. Building costs were paid completely by Ulm's citizens. Another nearby attraction is the historical town hall with its lavishly painted, early Renaissance facade. The ornamental astronomical clock was installed around 1520.

From here to the southwest extends the fishermen's and tanners' quarter with its half-timbered houses, which dates back to the Middle Ages. This district includes, at and over the river Blau, the Leaning House. Nearby is the Oath House. From 854 to the 14th century it was the King's Palace in Ulm, then it was used as a commercial center. Today it's the home of the local history museum. It plays a central role in an important Ulm tradition: Every year on "Schwörmontag", a local public holiday in July, the Mayor of Ulm reaffirms the historical oath of the town's constitution.



Ulm Minster and Stadthaus

© Karin Holtricher, WITec GmbH

Neu-Ulm and its Danube island were separated from Ulm in the aftermath of the Napoleonic Wars. Since then, Ulm has belonged to Baden-Württemberg, Neu-Ulm to Bavaria.

Both images below:

© Ulm/Neu-Ulm Touristik GmbH - Anna Beyrer (CCO)



View of the Ulm Minster and old town on the river Danube



The lion man in the Ulm Museum

Ulm City Map

Symposium Locations 2023



Streetcar stop
"Theater"
Line 2 to
Science Park II

Main Train
Station

Streetcar stop
"Main Station"
Line 2 to
Science Park II

Dinner at restaurant
"Zum Haberfelder, Ulmer Wirtshaus"
Neue Straße 56, 89073 Ulm
September 26, 7.00 p.m.

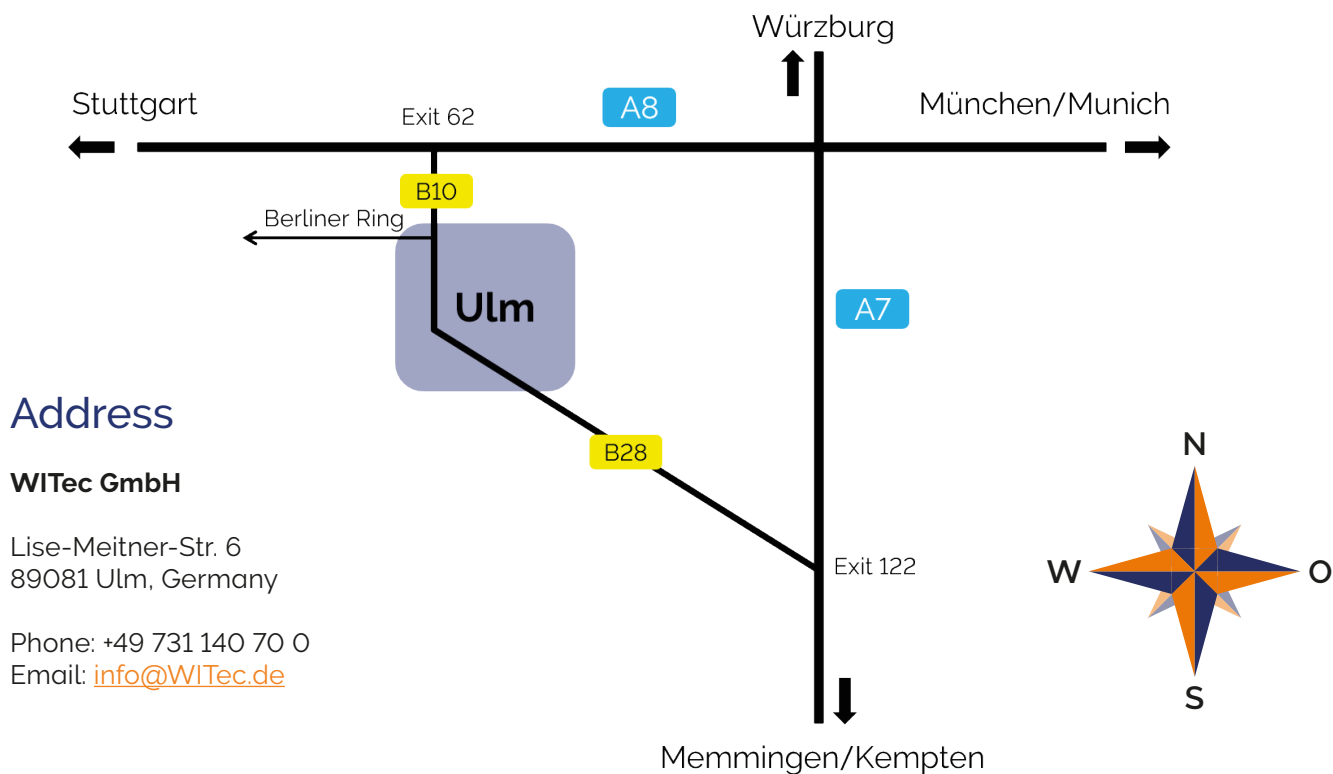
Conference location
Stadthaus
Münsterplatz 50, 89073 Ulm
September 26 and 27

Map © Ulm/Neu-Ulm Touristik GmbH- IKONS Intermedia Concepts.

Directions to WITec, Ulm



WITec



Address

WITec GmbH

Lise-Meitner-Str. 6
89081 Ulm, Germany

Phone: +49 731 140 70 0

Email: info@WITec.de

By car

On motorway A7 from the South (Kempten/Memmingen)

At interchange Hittistetten take exit Ulm/Neu-Ulm/Senden (Exit 122) onto B28. Drive past Neu-Ulm, continue along the B10 through Ulm, up the hill. Take exit Langenau/Wissenschaftsstadt/Kliniken/Eselsberg. Keep left - you are now on the Berliner Ring ...

On motorway A7 from the North (Würzburg)

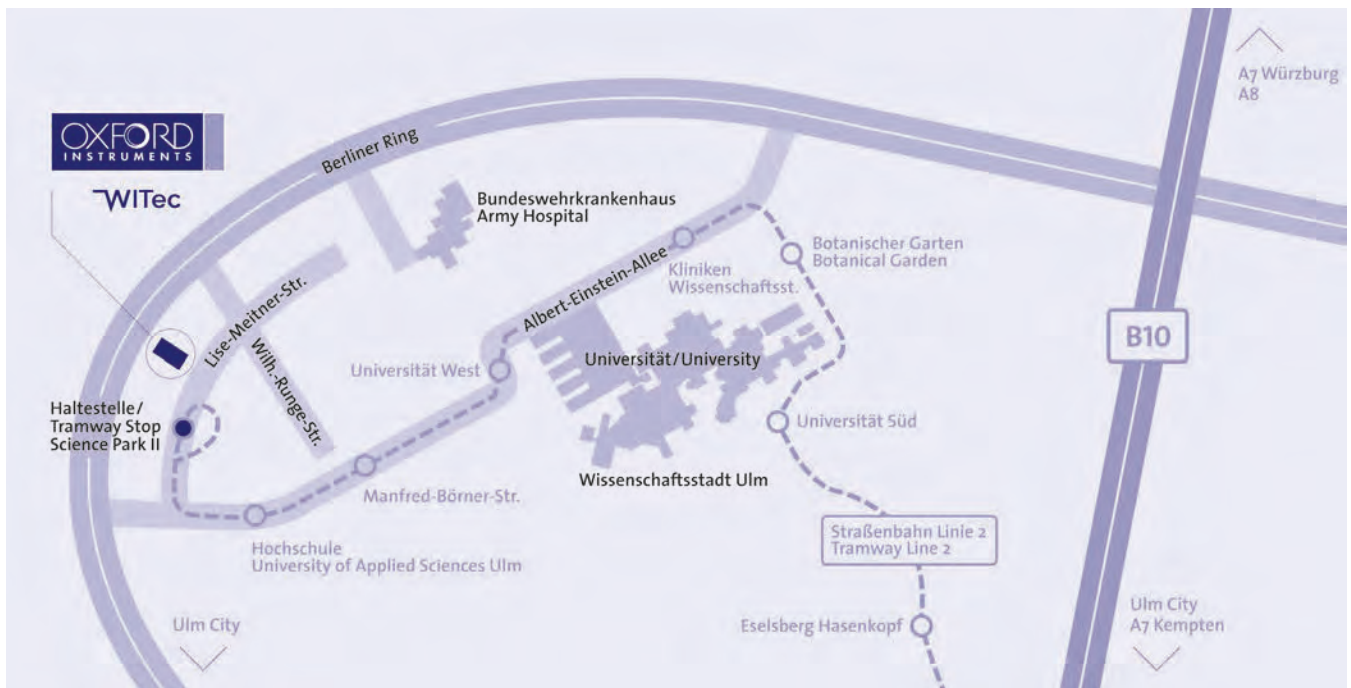
At interchange Ulm/Elchingen merge onto A8 towards Stuttgart. Take Exit 62 Ulm-West onto the B10 towards Ulm/Friedrichshafen. After about 4 km take exit Blaustein/Wissenschaftsstadt to get onto the Berliner Ring ...

On motorway A8 from East (München) and from West (Stuttgart)

Take Exit 62 Ulm-West onto the B10 towards Ulm/Friedrichshafen. After about 4 km take exit Blaustein/Wissenschaftsstadt to get onto the Berliner Ring ...

... continued

When you have reached the Berliner Ring, turn left at the second light onto Wilhelm-Runge-Straße/ Science Park II. Then turn right at the next crossing onto Lise-Meitner-Straße. After 200 meters you will have reached your destination, WITec Headquarters. Parking places are in front of the building or ca. 150 m down the street next to the tram station.



From airports by trains

Frankfurt (280 km)

Take the train from the airport to Ulm Hbf (Ulm main station). Takes 2 - 2.5 hrs.

Stuttgart (80 km)

Take the S-Bahn (local train) S2 (towards Schorndorf) or S3 (towards Backnang), platform 2 and exit at Stuttgart Hbf (main station). From there, take a train to Ulm Hbf (Ulm main station). Takes 1.5 - 2 hrs.

München/Munich (160 km)

Take the S-Bahn (local train) S8 (towards Herrsching) to München-Pasing or the S-Bahn S1 (towards München Leuchtenbergring) to München Hbf (main station). From there change to a train to Ulm Hbf (main station). Takes 2 - 2.5 hrs.

Memmingen (60 km)

Take the bus line 810 or line 2 to Memmingen Bahnhof/ZOB. Then take a train to Ulm Hbf (main station).

Ulm main station

From Ulm Hauptbahnhof (Hbf, main station), take the streetcar/tramway (Straßenbahn) Line 2 towards Science Park II. Get off at the final stop (Science Park II) and walk uphill, along Lise-Meitner-Straße. After about 150 meters you will find our building on your left hand side.

Taxi Ulm: Phone +49 (0) 731 - 66 0 66, www.ulm-taxi.de

Conference Program 2023

Monday, September 25

Stadthaus Ulm, Münsterplatz 50, Ulm

14:00 – 14:30		<i>Registration & Coffee</i>
14:30 – 14:45		Welcome
14:45 – 15:45	Sebastian Schlücker	The principles of Raman spectroscopy and its application in microscopy
15:45 – 16:00		<i>Coffee</i>
16:00 – 16:45	Thomas Dieing	3D confocal Raman imaging: Instrumentation, performance and correlative techniques
16:45 – 17:30	Maike Windbergs	Raman microscopy in biomedical research – From single cells to advanced drug delivery systems
17:30 – 19:15		<i>Poster session & Get-together with snacks and beverages</i>
19:15 – 20:00	Martina Grießer	Evening lecture: The splendor of the Imperial Crown of the Holy Roman Empire - Illuminating the gemstones by photoluminescence and Raman spectroscopy

Tuesday, September 26

Stadthaus Ulm, Münsterplatz 50, Ulm

08:45 – 09:00		<i>Coffee</i>
	Session I – Advanced Materials Analysis	
09:00 – 09:30	Xiujuan Zhuang	Application of optical microscopy in the study of low dimensional semiconductor spectroscopy
09:30 – 10:00	Stephen Wu	Process induced strain engineering in 2D materials and heterostructures
10:00 – 10:30	Florian Klein	Confocal Raman microscopy in battery materials development
10:30 – 11:00		<i>Coffee</i>
	Session II – Geo- and Environmental Science	
11:00 – 11:30	Natalia Ivleva	Raman microspectroscopy for analysis of (plastic) microparticles: Applicability and limitations
11:30 – 12:00	Mehmet Yesiltas	Micro-Raman investigation of astromaterials
12:00 – 13:00		<i>Lunch & Poster session (continued)</i>
	Session III – Pharma, Food and Life Science	
13:00 – 13:30	Ricardo Faccio	Raman imaging as a relevant tool in pharmaceutical and biological sciences
13:30 – 14:00	Klaus Wormuth Fanny Gaston	Characterization of particulate matter in biopharmaceutical manufacturing using Raman microscopy
14:00 – 14:30		<i>Coffee</i>
14:30 – 15:00	Laura Gómez-Mascaraque	Applications of confocal Raman imaging to study the microstructure and nutritional quality of food
15:00 – 15:30	Jean-François Masson	Machine learning driven SERS optophysiology
15:30 – 16:00		<i>Coffee</i>

Tuesday, September 26 – continued

Session IV – Contributed Presentations		
16:00 – 16:20	Robin van der Meijden	Combined Raman and electron microscopy reveals that bone collagen matrix guides mineral orientation during <i>in vitro</i> mineralization
16:20 – 16:40	Patrycja Leszczenko	Raman microscopy for the diagnosis of B- and T-cell acute lymphoblastic leukemia
16:40 – 17:00	Arnold Gucsik	The macromolecular carbonaceous phase of the Kaba meteorite: A Raman spectroscopy case study
17:00 – 17:20	Moshawe J. Madito	A correlation of the Fermi level shift and the electrochemical performance: Raman spectroscopy analysis
19:00		<i>Conference dinner & Poster Award ceremony</i>

Wednesday, September 27

WITec Headquarters, Lise-Meitner-Str. 6, Ulm

09:00 – 09:15	Welcome	
09:15 – 10:00	Equipment demonstration	Confocal Raman imaging microscopy: The alpha300 microscope series
10:00 – 10:15		<i>Coffee Break</i>
10:15 – 11:00	Software demonstration	WITec Software Suite SIX
11:00 – 11:30	WITec house tour	Guided tour through WITec headquarters
11:30 – 13:00		<i>Lunch</i>
13:00 – 16:00	Equipment demonstrations Choose which Demo Stations to visit, and in what order – Limited seating: first arrived, first admitted	20 minutes per station: <ul style="list-style-type: none"> • Open forum with alpha300 R for individual Q&A • TrueSurface – Topographic Raman imaging • alpha300 <i>apryon</i> and TrueOrigin – Measurement automation and correlation • alpha300 <i>Ri</i> – Inverted Raman and fluorescence microscopy • alphaCART – Mobile Raman microspectroscopy • RISE – Correlative Raman-SEM
16:00		<i>Wrap-up & Coffee</i>

Please note our policy regarding photography and recording of symposium presentation images.



1. Attendees are not permitted to take photos or videos of speakers' slides, posters or demonstration instruments unless the speakers/poster presenters give their explicit permission.
2. We will take pictures during talks and the poster sessions and we'll also take a group photo of all participants that we will use for press activities. We will not ask for written consent. Please inform us (i.e. during registration) if you do not want to appear in our photos.

Invited Speakers 2023

Thomas Dieing

Dr. Thomas Dieing is Product Manager for the WITec alpha300 product line and its accessories. He obtained his PhD from La Trobe University, Melbourne, Australia in 2005 investigating the MBE growth of nitrogen containing III/V semiconductors. In 2006 he joined WITec's application team and became Director of Applications and Support. In his role as Product Manager since 2019 he is responsible for all activities related to the product development process.

Ricardo Faccio

Prof. Faccio is Full Professor of Physics and Head of Department at Facultad de Química, Universidad de la República, Uruguay. His research focus includes Materials Science, nanotechnology and carbon nanomaterials with a strong focus on spectroscopic methods and a hybrid theoretical-experimental approach. Most recently he has been focused, both experimentally and theoretically, in the study of nanomaterials for solar cells applications and lithium-ion batteries applications. Recently, he has made contributions in biological and pharmaceutical sciences applying confocal Raman imaging methodologies.

Fanny Gaston

Dr. Fanny Gaston is Manager of the Particulates Laboratory at Sartorius. She has a Ph.D. in Analytical Chemistry from the University of Marseille, and years of experience in the application of Raman spectroscopy to the characterization of polymeric materials. She leads the development of laboratory methods that apply Raman and infrared microscopy to count, size and chemically identify particles.

Laura Gómez-Mascaraque

Dr. Laura G. Gómez-Mascaraque is a Research Officer leading the work on Food Microstructure at Teagasc Food Research Centre Moorepark (Ireland). Her research interests include the application of alternative proteins and carbohydrates to develop novel food structures, and the use of advanced microscopy and spectroscopy techniques to study the structure and quality of food.

Martina Grieser

Dr. Martina Grieser is Head of the Conservation Science Department at the Kunsthistorisches Museum Vienna. She deals mainly with investigations of the painting techniques and alterations of old master paintings and polychrome sculptures, the identification of corrosion products on different objects (i.e. metals, glass, enamel), the development of new conservation treatments, and the preventive conservation measures within the museum's collections. Since 1999 she is a lecturer at the University of Applied Arts Vienna, Institute of Conservation and Restoration.

Natalia Ivleva

PD Dr. Natalia Ivleva is Head of the Raman and SEM Group at the Institute of Hydrochemistry and Chair of Analytical Chemistry and Water Chemistry at the Technical University of Munich, Germany. Her research interests focus on applications of Raman microspectroscopy, surface-enhanced Raman scattering and stable-isotope Raman microspectroscopy for the nondestructive chemical 2D- & 3D-analysis of various environmental matrices/pollutants, ranging from biofilms and microorganisms through (sub)micro- and nanoplastic to engineered (magnetic) nanoparticles.

Jean-François Masson

Jean-François Masson is a professor of chemistry at the University of Montreal in Canada. His research focuses on the development of optical sensors using nanoplasmonics for the detection of biomolecules in complex samples. The sensors rely on the combination of plasmonic nanomaterials, surface chemistry, Raman spectroscopy and advanced data processing methods such as chemometrics and machine learning.

Florian Klein

Florian Klein is a research scientist at the Center for Solar Energy and Hydrogen Research Baden-Württemberg (ZSW). His research focus is on the development and upscaling of more sustainable and cost-efficient Co-free high energy materials for Li-Ion Batteries into kg-scale as well as on a deeper understanding of the structural relationship between the homogeneity of the particle microstructure and the electrochemical performance of the materials.

Sebastian Schlücker

As Professor of Physical Chemistry at the University of Duisburg-Essen (Germany), his research interests include the design, synthesis and bioanalytical applications of SERS nanoparticle probes as well as the development and application of laser spectroscopic techniques in biophysical chemistry.

Maike Windbergs

Maike Windbergs is a full professor for Pharmaceutical Technology and the director of the Institute for Pharmaceutical Technology and Biopharmaceutics at Goethe University, Frankfurt am Main, Germany. Her research interests are focused on novel drug delivery approaches and their effective translation into clinics. She uses Raman microscopy to investigate human cells and tissues as well as drug delivery systems and their interactions with biological environments.

Klaus Wormuth

Dr. Klaus Wormuth is Principal Scientist at Sartorius, a company-wide expert on the measurement and prevention of particulate matter in single-use systems applied in biopharmaceutical manufacturing. His specific interests are in microscopic/spectroscopic methods for particle counting, sizing and chemical identification. In addition, he leads standards development task groups within the ASTM E55 committee on the manufacture of pharmaceutical products.

Stephen Wu

Stephen Wu is Assistant Professor at the Department of Electrical and Computer Engineering at University of Rochester. His research interests involve merging the world of quantum materials science with nanoscale electronic device engineering for the advancement of electronics technology beyond Moore's law. For the characterization of novel 2D electronic systems he uses Raman imaging and correlative analysis techniques.

Mehmet Yesiltas

Mehmet Yesiltas is an associate professor in the Department of Space Sciences at Kırklareli University. His research interests revolve around laboratory investigation of extraterrestrial samples, especially their organic and mineral content. He uses confocal 2D and 3D Raman imaging and spectroscopy to detect and characterize extraterrestrial organic matter and minerals in meteorites in order to understand their formation and evolution in space.

Xiujuan Zhuang

Xiujuan Zhuang is a professor at the College of Semiconductor (College of Integrated Circuits) in Hunan University, China. Her research area is low dimensional semiconductor optics and optoelectronic devices.

Abstracts:

Invited Talks

A-Z by presenter name

3D Confocal Raman Imaging: Instrumentation, Performance and Correlative Techniques

Thomas Dieing

WITec GmbH, Ulm, Germany

Confocal Raman microscopy is an indispensable tool for analyzing a sample's chemical composition. In confocal Raman imaging, a complete Raman spectrum is recorded at each pixel of an image. The obtained chemical information is then color coded in the image to visualize the components' spatial distribution. A high-quality confocal Raman microscope enables nondestructive imaging of chemical properties on surfaces or within 3D volumes at diffraction-limited resolution (Fig. 1). Raman imaging can also be integrated with complementary techniques for more comprehensive sample analyses.

This presentation will highlight the technical requirements of a high-throughput, high-resolution confocal Raman microscope. The benefits of fully automated systems will be explained, which simplify operation and can be controlled remotely for measurements in enclosures. An overview of correlative Raman imaging approaches will also be given. By equipping a Raman microscope with an optical profilometer (TrueSurface microscopy), Raman images can be recorded on roughly textured, curved or inclined surfaces and topographic Raman images can be generated (Fig. 2). The presentation will also explain how Raman microscopes can be integrated with atomic force or scanning electron microscopes in order to analyze chemical properties and surface structure at the same sample area and correlate the information. Finally, it will be shown how microparticle analyses can profit from the combination of an automated Raman microscope and particle analysis algorithms, as particles in a sample can be characterized according to size and shape and chemically identified. Application examples from various fields will illustrate these cutting-edge techniques.

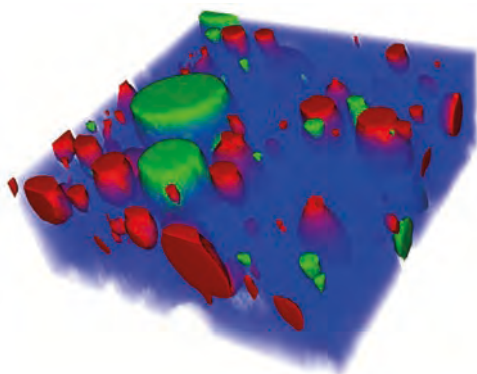


Fig. 1: 3D confocal Raman image of a moisturizing hand cream. The emulsion consists of a water phase (blue) and two oil phases with different moisturizing ingredients dissolved in them (red and green). Scan volume 40x40x15 μ m³.



Fig. 2: Topographic Raman image of a pharmaceutical tablet, revealing the distribution of the tablet's ingredients on the curved sample surface and in the approximately 500 μ m-deep groove: two different APIs (red and blue), adjuvant (orange) and excipients (green and yellow). Scan area 7x7mm².

Raman imaging as a relevant tool in pharmaceutical and biological sciences

Ricardo Faccio

Área Física. DETEMA, Facultad de Química, Universidad de la República, Montevideo, Uruguay

Raman spectroscopy is a powerful tool for the characterization of drugs, drug formulations, and their distribution in cells and microorganisms. It can be used to identify the components of a drug, to study their interactions, and to assess their distribution and stability. In this presentation, we will review four recent studies that have used Confocal Raman Microscopy to advance drug development, with a focus on anthelmintics, antitrypanosomal drugs, and glioblastoma.

In the first case, Raman spectroscopy was used to characterize self-dispersible nanocrystals (SDNCs) of the novel anthelmintic drug, Valero-fenbendazole (VAL-FBZ). The SDNCs were found to be highly homogeneous and to have a significantly improved dissolution rate compared to the parent drug. This suggests that SDNCs could be a promising formulation for VAL-FBZ, and could lead to improved efficacy and safety of the drug. [1]

In the second study, Raman spectroscopy was used to study the pharmacokinetic behavior of SDNCs of the anthelmintic drug, fenbendazole (FBZ). The study found that SDNCs resulted in significantly higher plasma concentrations of FBZ than the parent drug. This suggests that SDNCs could be a promising way to improve the bioavailability of FBZ, and could lead to improved efficacy of the drug. [2]

In the third study, Raman spectroscopy was used to characterize a new class of anti-trypanosomal drugs based on Re(I) tricarbonyl compounds. The compounds were found to have potent activity against *Trypanosoma cruzi*, the parasite that causes Chagas' disease. Raman spectroscopy was also used to study its colocalization in the parasite, to advance the understanding of the mechanism of action of the compounds, and to identify potential targets for further drug development. [3]

In the fourth study, Raman spectroscopy was used to characterize a new lead compound for the treatment of glioblastoma (GBM). The compound was found to have potent in vitro and in vivo antitumor activity. Raman spectroscopy was also used to study the cellular localization of the compound and to identify potential mechanisms of action. [4]

These four studies demonstrate the potentialities of Confocal Raman Microscopy as a tool for drug development. Raman spectroscopy can be used to identify new drug candidates, study their mechanisms of action, and assess their stability. This makes Raman spectroscopy an essential tool for the development of new and improved drugs.

References

- [1] M.E. Melian, A. Paredes, B. Munguia, M. Colobbio, J.C. Ramos, R. Teixeira, E. Manta, S. Palma, R. Faccio, L. Dominguez, *AAPS Pharm. Sci. Tech.*, 21 (2020) 237.
- [2] M.E. Melian, M. Ibarra, L. Ceballos, A.J. Paredes, B. Munguía, R. Faccio, S. Palma, L.I. Álvarez, L. Domínguez, *Res. Vet. Sci.*, 142 (2022) 110-116.
- [3] M. Soba, G. Scalese, F. Casuriaga, N. Pérez, N. Veiga, G.A. Echeverría, O.E. Piro, R. Faccio, L. Pérez-Díaz, G. Gasser, I. Machado, D. Gambino, *Dalton Transactions*, 52 (2023) 1623-1641.
- [4] C. Alamón, B. Dávila, M.F. García, S. Nievas, M.A. Dagrosa, S. Thorp, M. Kovacs, E. Trias, R. Faccio, M. Gabay, N. Zeineh, A. Weizman, F. Teixidor, C. Viñas, M. Gavish, H. Cerecetto, M. Couto, *Mol. Pharm.*, 20 (2023) 2702-2713.

Applications of confocal Raman imaging to study the microstructure and nutritional quality of food

L. G. Gómez-Mascaraque

Teagasc Food Research Centre, Moorepark, Co. Cork, Ireland

The techno-functional and organoleptic properties of food products, and therefore their processability and the overall consumer experience, strongly depend on their (micro)structure, which also has an impact on food stability and even the bioavailability of nutrients. Hence, understanding how the different components are organized and interact within the food matrix is essential for a rational food product design, and can also help in process troubleshooting.

Confocal Raman microscopy can provide compositional and microstructural information of specimens simultaneously with minimal sample preparation, being a powerful imaging technique to study the microstructure of materials in a number of fields. However, its applications in food research are still limited due to the particularities of food samples. Some of the challenges of imaging food with this technique include 1) the compositional and conformational complexity of food components, with overlapping Raman spectra; 2) the poor Raman scattering intensity of some food macronutrients, such as proteins or carbohydrates; 3) the sensitivity of many food samples to local heating due to their organic nature. Thus, imaging methods need to be specifically developed for each targeted food product.

At the National Food Imaging Centre in Teagasc Moorepark (Ireland), we have used confocal Raman microscopy to study the microstructure and nutritional quality of food products such as protein gels, oleogels and various dairy products. This technique allowed mapping the distribution of protein, fat, carbohydrates and other minor components in the samples when using multivariate approaches for data analysis. They also allowed distinguishing solid from liquid fat and studying melting transitions, as well as discriminating samples with different levels of unsaturated lipids. Confocal Raman microscopy also provided new insights on interactions taking place between different ingredients in food formulations. In combination with other microscopy techniques, Confocal Raman microscopy is expected to become an essential tool in the characterization of food microstructure, allowing the identification of components that are not easily identified by other microscopy techniques, like certain micronutrients present in low concentrations in food.

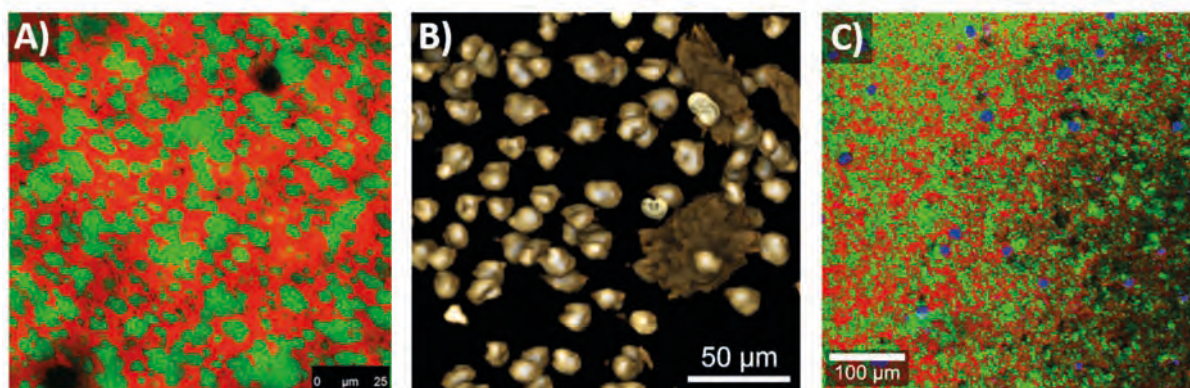


Fig. 1: Cheddar cheese. A) Fat and protein observed by CLSM; B) salt crystals observed by X-ray computed microtomography; C) Fat, protein and salt crystals observed by confocal Raman microscopy. Adapted from Lourenco et al. (2022)

Raman microspectroscopy for analysis of (plastic) microparticles: Applicability and limitations

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Microplastics (MPs, synthetic polymer fragments in the size range of 1 μm – 1 mm, doi.org/10.1021/acs.est.8b05297) are found in the environment all around the globe (incl. water, soil, atmosphere, and biota) as well as in drinking water and food, thus raising concerns about their impacts on the environment and human health. To adequately address these issues, reliable information on the ambient concentrations of MPs in the entire size range is required. Here, Raman microspectroscopy (RM) is a suitable tool for the chemical identification and quantification of MPs down to 1 μm (doi.org/10.1021/acs.chemrev.1c00178). To ensure that the results are reliable and representative, a (very) high number of particles/fibers has to be analyzed, especially for small MPs. Hence, automation of RM analysis becomes essential. Therefore, we developed *TUM-ParticleTyper* – open-source program which enables the automated detection, quantification, and morphological characterization of (plastic) fragments in dark field images of optical microscopy, followed by the automated RM-based identification of MPs and non-plastic fragments (doi.org/10.1371/journal.pone.02347). This approach has been already successfully applied for the characterization of reference MP particles and real samples, where up to 7000 particles/fibers down to 10 μm , randomly selected on the entire filter were analyzed ([doi:10.3389/fchem.2020.00169](https://doi.org/10.3389/fchem.2020.00169), doi.org/10.1016/j.watres.2022.118549).

Since the number of particles/fibers increases with decreasing the fragment size, it becomes nearly impossible to detect all particles down to 1 μm on the entire filter. Therefore, we developed novel alternative strategy – *random window subsampling*, where the automated acquisition of optical image and localization of (MP) fragments are followed by RM measurements from window to window. We also introduced a *bootstrap* method, to provide an error quantification with confidence intervals from the available window data (doi.org/10.1007/s00216-021-03326-3). Finally, we implemented in *TUM-ParticleTyper 2* new RM measurement algorithm that calculates confidence intervals *on-the-fly* during the analysis and automatically stops, if an appropriate number of fragments is identified (doi.org/10.1007/s00216-023-04712-9). Additionally, we implemented advanced image processing for better recognition and morphological characterization of (plastic) microparticles and fibers, including automated *adaptive de-agglomeration* and improved shape classification.

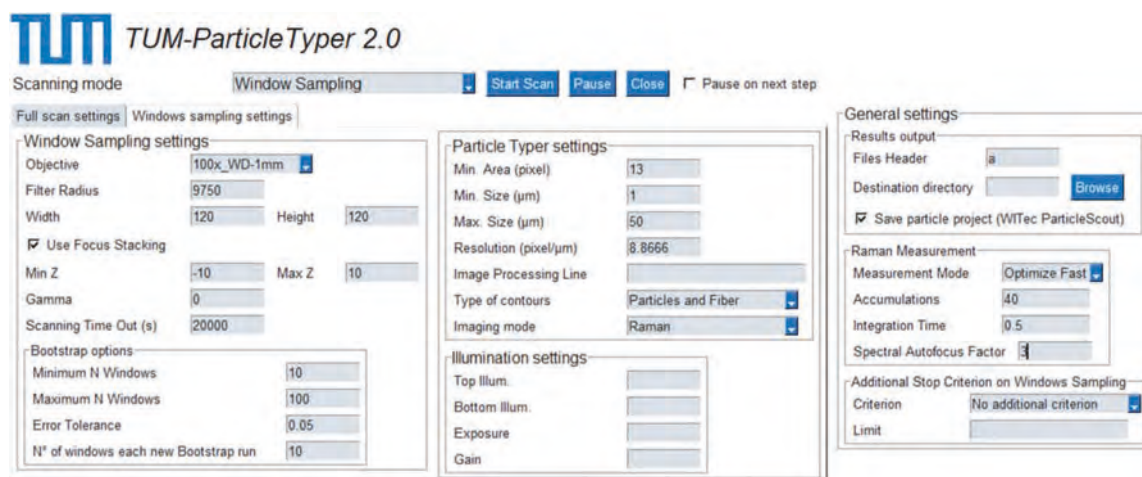


Fig. 1: Graphical user interface of the open-source software *TUM-ParticleTyper 2* (doi.org/10.1007/s00216-023-04712-9)

Confocal Raman microscopy in battery materials development

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Li-ion batteries are an essential part of our high-tech dominated daily life due to their long cycle lives and high energy densities. To further increase performance, reduce costs and environmental footprint, further improvement of active components such as anode and cathode materials is necessary. However, the development of cathode materials (positive electrode) is a very complex process with multiples of parameters to adjust in order to gain the best properties for each application. Such development is only possible with extensive chemical and structural analysis to gain deeper understanding of the materials. Beside well-known methods such as elemental analysis, electron microscopy or X-ray diffraction, Raman spectroscopy can be used to provide local structure information that is not accessible with other methods.

In this presentation, an overview on different fields of application of confocal Raman microscopy in battery materials development will be given. In addition to the structural identification of different types of active materials, our focus will be on the characterization and Raman-based understanding of Co-free cathode materials. In this context, correlative SEM-Raman microscopy will be demonstrated as a very strong method to combine local morphological information (electron microscope image) with elemental distribution (energy dispersive X-ray spectroscopy), and structural information from vibrational spectra (Raman microscopy).

Machine learning driven SERS optophysiology

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We exploit the concept of SERS optophysiology, which combines a SERS nanosensor on the tip of a pulled fiber to provide spatially and temporally specific molecular information near or inside biological material. To accomplish this, a SERS nanofiber decorated with a dense and well dispersed array of Au NP has been developed for the measurement of neurotransmitters and other metabolites in proximity of cells. The nanosensors are thus highly compatible with current physiology experiments also relying on similar nanosensors based on electrochemistry and electrophysiology. The nanosensor can be mounted on a micromanipulator or a modified cantilever holder on an AFM-SERS microscope and placed in proximity of cells or in tissue. Fluorescence imaging can be added to the microscope to visualize cells and tissues and position the optophysiology nanosensor. Specifically, we will show that the SERS optophysiology nanosensor can measure a panel of metabolites near cells in a single experiment.

SERS and Raman spectroscopy yield large data sets with information-rich spectra. Classical linear methods are limited, especially for SERS spectra of single molecules, where the spectra are highly dependent on the orientation of molecules on surfaces and for large data sets. Methods from data sciences are increasingly used to classify spectra into categories and predict SERS spectra for new data based on trained algorithms. The SERS spectra of neurotransmitters or other metabolites were identified with a barcoding data processing method, processed with TensorFlow using a convolutional neural network architecture. This machine-learning driven data processing significantly improved the positive assignment rates for a series of metabolites and allows for complex measurements of the cell's biochemistry. In addition to these untargeted SERS nanosensors, we also designed molecularly specific sensors to measure pH, H_2O_2 and heavy metals inside cells using the same nanosensor architecture. This suite of SERS nanosensor will open the door to survey molecular changes in proximity of healthy and diseased cells.

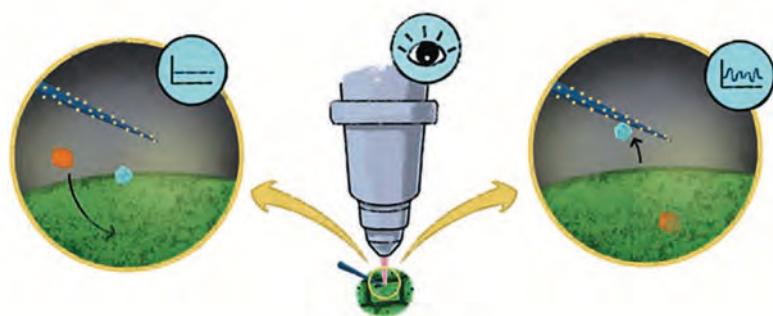


Fig. 1: Concept of SERS optophysiology

The Principles of Raman Spectroscopy and its Application in Microscopy

Sebastian Schlücker

University of Duisburg-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.

Raman microscopy in biomedical research – From single cells to advanced drug delivery systems

Prof. Dr. Maike Windbergs

Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt am Main, Germany

Confocal Raman microscopy recently gained a lot of attention in the field of biomedical research due to advantages like chemical selectivity and the option for three-dimensional non-invasive measurements. For elucidating disease mechanisms, the targets structures under investigation are often unknown, so the use of specific dyes is impossible, while in drug delivery bulky labels modify the physicochemical properties of the drug or carrier systems and falsify the results.

The talk will give an overview about the potential of label-free confocal Raman microscopy for different applications in biomedical research. While differentiation processes on a single cell level can be monitored, Raman microscopy also allows for novel insights into growth and secretion patterns of three-dimensional cell cultures, like organoids. For ex vivo tissue samples, disease progression can be investigated, as successfully shown for Alzheimer's disease in brain sections, as well as regeneration like wound healing. During the development of novel therapeutics, the distribution of the drug in the carrier systems is of high importance. In this context, the talk will highlight several case studies, in which Raman microscopy allowed for visualization of drug distribution and release from carrier system.

Finally, data will be presented on interaction studies of drug delivery systems and with human cells and tissues.

Further, advantages and limitations of Raman microscopy for such investigations will be discussed and a future outlook will be given.

Characterization of particulate matter in biopharmaceutical manufacturing using Raman microscopy

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The recent pandemic situation required rapid scale-up of biopharmaceutical manufacturing processes, which resulted in more than 13 billion of doses of COVID-19 vaccines administered worldwide. The ready-to-use and easily scalable nature of single-use bioprocessing equipment (single-use systems) enabled biopharmaceutical manufacturers to meet this enormous vaccine demand.

Single-use systems (SUS) range from small bioprocessing containers (“bags”) to highly complex 2000 L stirred tank reactor systems. SUS find increasing application closer to the final drug product, in final-filling and in the aseptic manufacturing of cell and gene therapies. In these critical process applications, particulate matter attached to the surfaces of SUS may potentially release and contaminate final drug products. SUS are manufactured in controlled clean room environments, however, cutting and welding processes along with manual assembly processes are potential sources for particulate matter.

A quality risk management approach drives measures to reduce the *probability of occurrence* of particulate matter and motivates development of test methods to increase the *detectability* of particulate matter. In this presentation we describe in detail methods to count, size and rapidly chemically identify particles extracted from SUS based upon automated membrane light microscopy and confocal Raman microscopy. The rapid chemical identification achievable using Raman microscopy allows a comprehensive assessment of particle sources, which facilitates actions to reduce risks from particulate matter in the manufacturing of biopharmaceuticals.

Process induced strain engineering in 2D materials and heterostructures

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Strain engineering in electronics has been widely utilized over the last 20 years to enhance carrier mobility in most standard Silicon-based transistor fabrication processes. Therefore, every piece of electronics created today relies on nanometer scale control of tensile or compressive strain at the individual transistor level to engineer for faster, smaller, and more energy efficient electronic devices. This level of control is achieved through process-induced strain engineering techniques, which are derived from the nanofabrication process itself. They are simple, reliable, can be applied device-to-device, and are highly scalable down to the nanometer scale. In this talk, I will introduce our group's work in exploring how process-induced strain engineering translates to the world of 2D materials, and how this may be applied to engineer novel electronic devices and control quantum materials properties within this exciting new class of electronic materials. Control over the strain degree-of-freedom in 2D materials opens new pathways for exploration in engineered quantum materials and devices, since the level of control over strain in weakly-bonded 2D systems can go far beyond strain-engineering in conventional 3D-bonded materials. A brief historical overview of the use of Raman spectroscopy to understand strain effects in Si-based electronics in the past (1990s-era) will be used to introduce our group's ongoing work Raman spectroscopic studies of strained 2D materials in the present. I will also introduce a few ongoing projects in our group which utilize strain to engineer the properties of materials: 2D straintronic phase-change transistors/memristors, moiré superlattice engineering with strain in twisted bilayer 2D heterostructures, and strain-controllable edge state superconductivity in 2D topological Weyl semimetals.

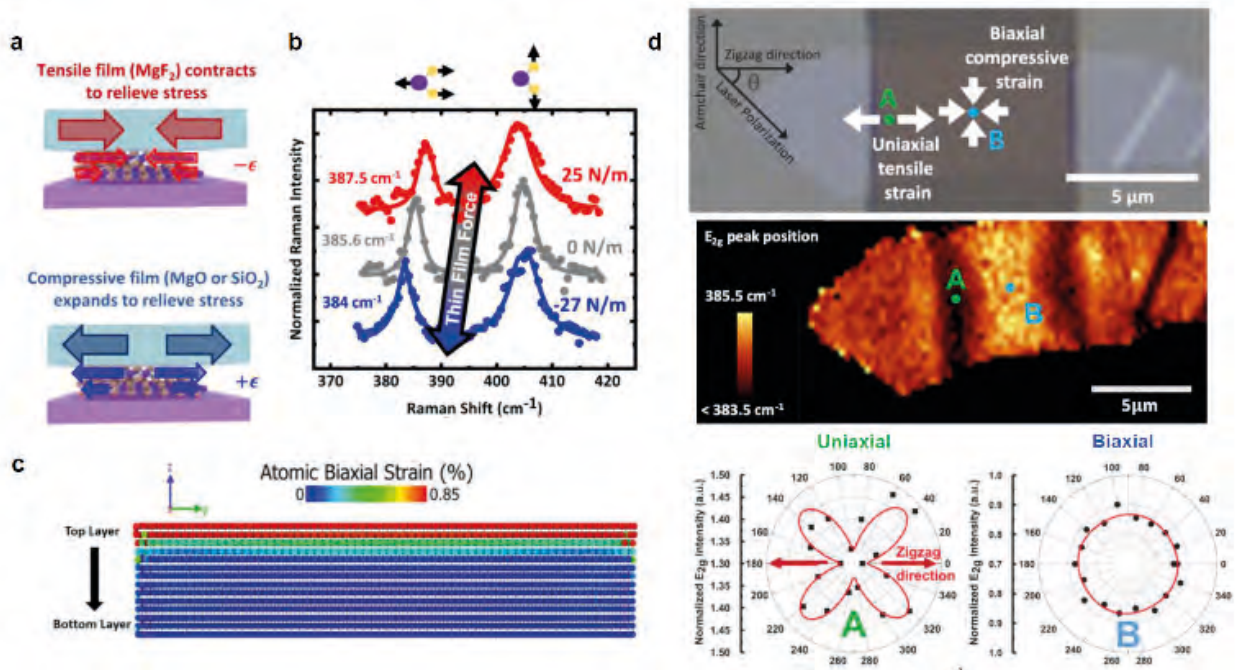


Fig. 1: (a) Tensile and compressive thin film strain engineering. (b) Raman spectroscopic analysis of 2D strain transfer with thin film stressors. (c) Full multiscale simulation of layer-by-layer strain. (d) Demonstration of uniaxial and biaxial strain engineering on 2H-MoS₂.

Micro-Raman Investigation of Astromaterials

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Extraterrestrial materials provide scientists a unique window into the early solar system. Some of the extraterrestrial samples, such as primitive meteorites and returned asteroid samples, still preserve the earliest history of our solar system because they contain primitive materials such as minerals and organic materials that did not undergo harsh planetary formation processes. These astromaterials are enriched in minerals, water, and organic matter, thus have significant potential for providing us the clues to the origin and evolution of the Solar System. Carbon-rich extraterrestrial materials are particularly of interest as they may have contributed to the origin and evolution of life on Earth. Therefore, some of the extraterrestrial organic matter is of astrobiological importance.

Raman spectroscopy allows non-destructive chemical characterization of a variety of materials including astromaterials at sub-micron spatial scales. It is highly sensitive to molecular functional groups including carbon-rich structures, it can also be used as a geothermometer to shed light on the thermal history of planetary materials. As such, it is a widely used analytical technique in meteoritics and planetary science. In this talk, results of our Raman spectroscopic and imaging investigations on meteorites, micrometeorites, and returned asteroid particles will be presented. A particular emphasis will be on our confocal Raman spectroscopic investigations of the samples of the carbonaceous asteroid (162173) Ryugu returned to Earth in 2020 by the Hayabusa2 spacecraft.

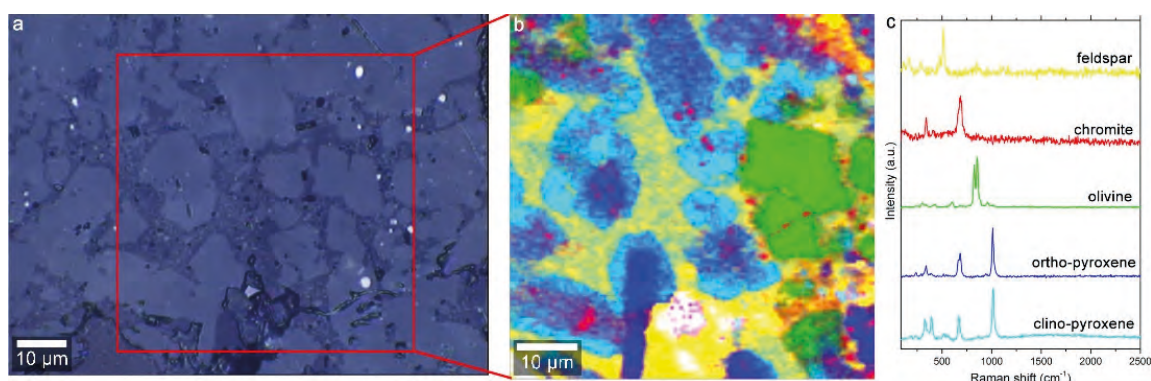


Fig. 1: Raman imaging of an ordinary chondrite consisting of 14400 Raman spectra. Data were collected using a WITec alpha300 R confocal Raman imaging system equipped with a 600 g/mm grating, 532 nm Nd:YAG laser, and a 50x objective (NA = 0.8). Colors in the middle panel correspond to the spectra given in the right panel.

Application of Optical Microscopy in the Study of Low Dimensional Semiconductor Spectroscopy

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In the past few decades, a number of low-dimensional semiconductor optoelectronic materials such as quantum dots, nanowires, and two-dimensional layered materials, have attracted much attention and have applied in integrated opto-electronics devices. Among them, in the case of material or devices with sizes in the order of hundreds of nanometers to microns, far-field optical microscopy has the advantages of being more efficient and convenient than the near-field technology. At the same time, with the continuous exploration of the carrier motion recombination mechanism on low-dimensional semiconductor materials, it is hoped that more powerful research methods, such as photocurrent detection, temperature control, time resolved spectroscopy, can be grafted onto the optical microscope. Therefore, in the past decade, we set up several optical microscope systems with different functions, including a scanning photocurrent mapping system, a temporal-spatial resolved microscope with temperature control, and an optical-magnetic microscope. In these systems, the time resolution can reach the order of 100fs, the temperature control can be as low as 10K, and the magnetic field can reach up to 9T while maintaining the optical resolution at diffraction limit. Based on these innovated experimental systems, we carried out extensive and in-depth research on optical absorption and emission properties, carrier dynamics, and photoelectric properties on series of low-dimensional opto-electronic semiconductors. It is believable that research-grade microscopes are developing in the direction of functional diversification and modularity, and there are still valuable technologies to be incorporated on the platforms of optical microscopy.

Abstracts:

Contributed Talks

A-Z by presenter name

The macromolecular carbonaceous phase of the Kaba Meteorite: A Raman Spectroscopy case study

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In this study, a Micro-Raman spectrometer was used to identify the crystallinity of the Macromolecular Carbon (MMC) of the Kaba meteorite. The purpose of the study above is to understand more about the formation mechanism of the carbonaceous matrix of the Kaba meteorite.

Kaba belongs to the oxidized subgroup of the CV3 carbonaceous chondrite class, which fell in Hungary (close to the village of Kaba) in 1857 [1,2]. It is one of the most primitive, least-metamorphosed carbonaceous chondrites [1,2]. Therefore, the Kaba meteorite provides a good opportunity for Raman spectroscopical studies.

A thin section was produced, in which the sample was mounted in non-radiative three component epoxy material. It was polished in a silicon colloidal solution to avoid any carbon-related pollution. The analysis was performed using Ratatoskr, a customized WITec alpha300 Raman imaging spectrometer in the Hlidskjalf Raman Laboratory at NASA Johnson Space Center.

The selected area (LAS01) shows macromolecular carbon (LAS 01). Pyroxene, olivine, and gehlenite were also identified. Lighter colors indicate MMC with a more ordered crystalline structure than darker colors (Fig. 1). The most parsimonious explanation for this is MMC that has seen elevated temperatures relative to the darker-colored MMC. The MMC borders some chondrules and the gehlenite is more ordered than matrix MMC, as is some in discrete assemblages in the matrix. Raman mapping of the Kaba meteorite identified that the Macromolecular Carbon (MMC) is not homogeneous in its carbonaceous matrix, which might be due to the thermal metamorphism. However, some further studies must be applied to clarify the crystalline background of the MMC.

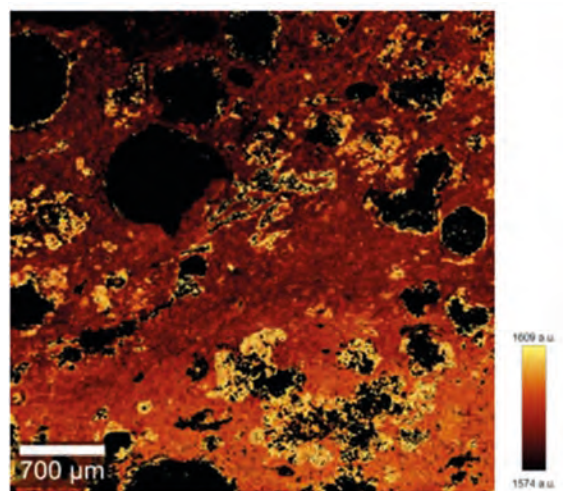


Fig. 1. MMC G band position image, showing position of the G band derived from a Lorentzian fit. ("a.u" on the scale bar is arbitrary units, but the correct units are wavenumbers (cm⁻¹).

Acknowledgement:

Authors are grateful for Dr Mihály Nagy (University of Debrecen, Harka) and Bence Gucsik (Harka, Hungary) for providing the Kaba sample's thin section.

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- [2] Keller, L. P. and Buseck, P. R. (1990) GCA 54, 2113–2120.

Raman Microscopy for the diagnosis of B- and T-cell acute lymphoblastic leukemia

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B and T cells arise from a common progenitor of lymphopoiesis in the bone marrow, however, disturbances in the process of hematopoiesis and the abnormal development of blood cells lead to the expansion of leukemias. There are two main types: B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL). They may carry several genetic mutations, classified by cytogenetic changes of the blasts, with many more B-ALL subtypes than T-ALL known. The identified subtype determines the selection of appropriate therapy, its effectiveness, and the general outcome for the patient. Standard protocols currently used to diagnose leukemias require multidisciplinary and experienced laboratory staff and involve considerable instrumentation/material costs. For this reason, the implementation of novel approaches such as Raman spectroscopy, into the diagnostic of blood malignancies is essential.

In this study, we investigate the biochemical profile of B-ALL and T-ALL blasts and their normal counterparts, B and T cells. We aim to develop algorithms able to sort cells based on their Raman spectra, as healthy or leukemic cells, respectively, to learn the algorithm distinguishing B-ALL from T-ALL blasts. The aforementioned models were established for the spectra collected from single cells of diagnosed patients and healthy donors using a 532 nm and 633 nm lasers. The morphological analysis of Raman images revealed significant changes in the metabolism of the blasts, *inter alia*, and differences in carotenoid accumulation. We also investigated the influence of sample handling on the biochemical composition of the samples. Moreover, we examined two subtypes of B-ALL, *TEL-AML1*, and hyperdiploidy, with the sage of Raman spectroscopy, fluorescence, and biological staining. We studied the influence of incubation with three anticancer drugs routinely used in leukemic regiments, namely methotrexate, doxorubicin, and vincristine on cancer cells' metabolism, and also traced the mitochondrial function by implementing a Raman reporter, MitoBADY.

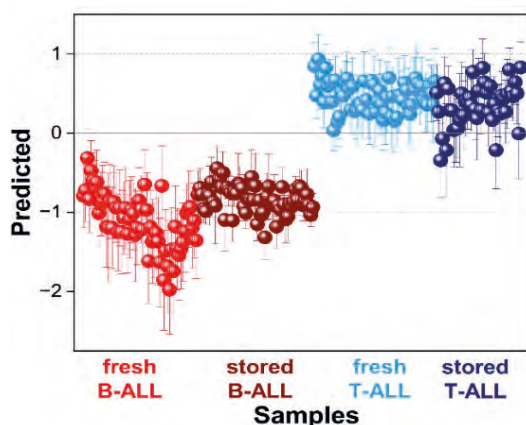


Fig. 1: Prediction results for the test dataset of an PLS-DA algorithm distinguishing Raman spectra of B-ALL and T-ALL.

This work was supported by the Team-Net program (POIR.04.04.00-00-16ED/18-00) of the Foundation for Polish Science and by the "Excellence Initiative – Research University" program at the Jagiellonian University.

A correlation of the Fermi level shift and the electrochemical performance: Raman spectroscopy analysis

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A high structurally stable graphene-manganese phosphate (graphene-Mn₃P₂O₈) composite with excellent cycling stability was prepared by the facile hydrothermal method. The correlation between the high electrochemical performance of graphene-Mn₃P₂O₈ composite and the graphene Fermi level shift was investigated using Raman spectroscopy by monitoring the disorder in the sp²-network of the composite graphene arising from the heterogeneous atoms doping during hydrothermal synthesis. The response of the Raman signatures of graphene to the chemical doping effect correlated to the electronic band structure in the vicinity of the Dirac point showed an upshift in the graphene Fermi level with an average value of about 190 meV, indicating that the composite graphene is n-type doped. This was confirmed by X-ray photoelectron spectroscopy data which showed a relatively high concentration of electron-donating heterogeneous atoms in the composite graphene. The electrochemical analysis confirmed that the n-type doped composite graphene has enhanced the electrical conductivity of the Mn₃P₂O₈ electrode and decreased the potential barriers between the electrode surface and electrolyte HOMO (highest occupied molecular orbital) for enhanced interfacial charge transfer between the electrode surface and the electrolyte.

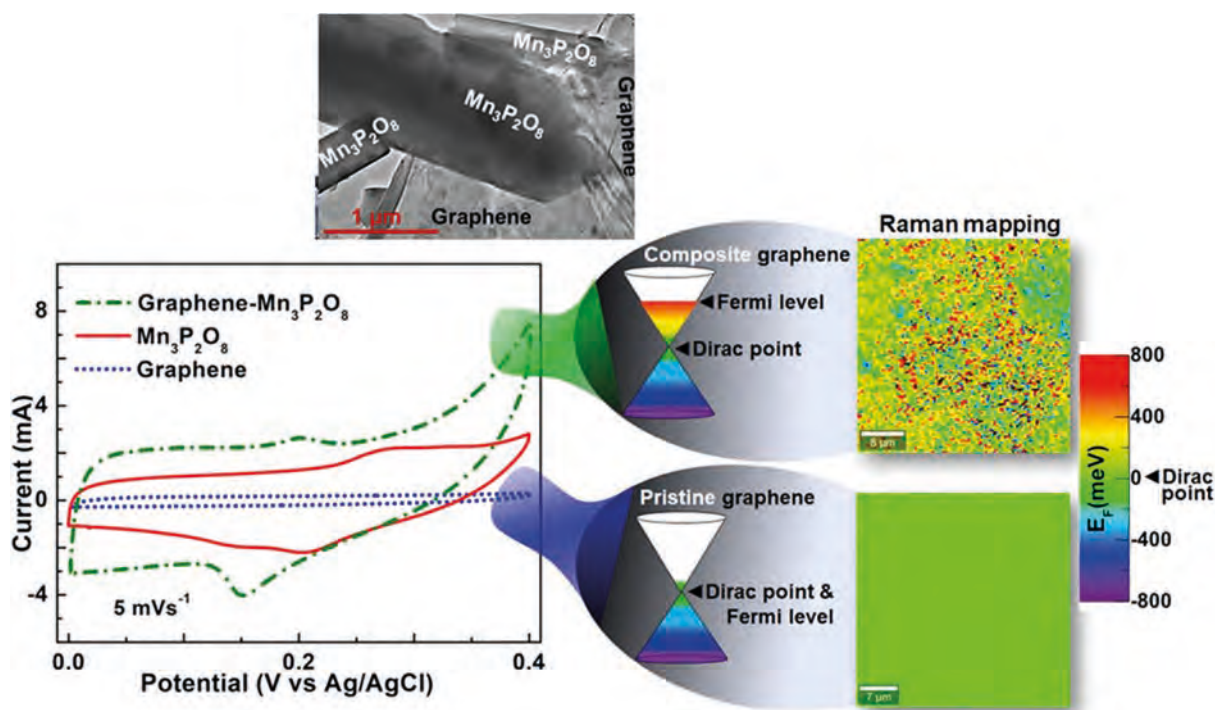


Fig. 1: Micrograph (top image). Cyclic voltammetry curves of pristine graphene, Mn₃P₂O₈ and graphene-Mn₃P₂O₈ composite electrodes obtained at a scan rate of 5 mV s⁻¹. Schematic diagrams of the cone energy band structure of graphene demonstrating the shift in the Fermi energy level for composite graphene. Raman mapping showing Fermi level shift with respect to Dirac point for composite graphene.

Combined Raman and Electron Microscopy reveals that bone collagen matrix guides mineral orientation during *in vitro* mineralization

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Bone is a highly organized hybrid material containing mineral platelets embedded in an organized matrix of collagen fibrils. The long axes of the mineral platelets are aligned with the long axes of the collagen molecules within the fibrils that exhibit a plywood-like arrangement. However, whether this organization and alignment of organic and mineral phases is controlled by the cells (biological control) or if it is dictated by the structure of the produced collagen matrix (physiochemical control) is still unknown.

Here we study the organization of the mineral phase created *in vitro* using a biomimetic remineralization strategy. *In-situ* polarized Raman microspectroscopy allows us to follow the progression of the mineralization and identify the ultrastructure of the forming matrix. This shows that when we mineralize a collagen scaffold of demineralized bone tissue, the organization of the formed mineral phase is indistinguishable from that of natural bone. Combined with electron imaging (TEM, electron diffraction and SEM) we show that the resulting material displays the bone morphological and structural characteristics from the nanometer up to the multimicron scale.

Detailed analysis of the spectra shows that differences in the maturation of the mineral occur during the remineralization process, depending on the location of the analysis. Spectral deconvolution allowed us to identify a difference in the concentration of GAGs at these specific locations, indicating that GAGs play an important role in regulating the infiltration and maturation of mineral precursor in the collagen matrix.

Our *in-situ* Raman analysis allowed us to follow the mineralization process with high spectroscopic and spatial detail. This revealed the development of the structure and chemistry during the process which was independently confirmed using different microscopic methods. The application of *in-situ* Raman microscopy with detailed data analysis in this *in vitro* model showed for the first time that the development and organization of the mineral phase in the organic bone matrix only requires mineral-matrix interactions and no cellular activity.

Abstracts:

Posters

A-Z by presenter name

Applications of Raman Spectroscopy in Glass Science

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Raman spectroscopy is a powerful tool to investigate minerals, gases, glasses, and disordered materials in all in-situ conditions. It is widely utilized to identify the presence, and recognize the morphology of organic and inorganic materials; distinguish different compounds within those materials; determine the oxidation state of planetary elements; and study volatiles and gaseous inclusions within minerals and glasses.

Raman spectroscopy has important scientific applications in academic research and in industrial process properties in glass. The glass industry has several problems regarding the presence of impurities and structural changes in the glass network. Most impurities and defects are on a micrometric scale and present crystalline nature. The presence of these defects can also affect both the optical and mechanical properties of the glass product. In this way, studies must be conducted to identify the sources of defects and to understand the chemistry correlated with their formation. The primary defects in the glass that detract from its appearance are bubbles, stones (inclusions), and organic contamination on the glass surface. Numerous Raman spectroscopic studies focused on structural aspects of the non-crystalline glass network have been carried out for various glass systems upon alterations of glass compositions, thermal histories, mechanical stresses, or surface treatments. Similar investigations have shown that Raman measurements provide information for glass structures induced by corrosion.

This study represents an overview of applications on glass and the use of Raman spectroscopy to monitor structural changes in the glass network and identify glass problems (nucleation/growth and crystallization processes, chemical/physical properties, corrosion mechanisms on glass surfaces).

Probing the Influence of Passivating Layers during Olivine Weathering using Confocal Raman Microscopy

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Carbon Capture and Storage (CCS) technologies are crucial to reduce CO₂ levels in the atmosphere, and mineralization offers secure CO₂ conversion. Olivine is a prime candidate for CO₂ mineralization due to its water-driven chemical weathering, yielding silica and carbonate products. Yet, its dissolution is reportedly hindered by a silica-rich passivation layer, which would hamper the reaction speed at the mineral-electrolyte boundary. The nature of this passivation layer is still somewhat elusive, as its chemical composition and spatial features are still not agreed upon in literature.

To understand the chemicophysical properties of the alteration layer, we employed Confocal Raman Microscopy (CRM) to investigate the olivine-aqueous solution interface, using forsterite (Fo₉₀, [Mg, Fe]₂SiO₄) samples. The development of an alteration layer during olivine dissolution under sulphuric and hydrochloric acid was monitored across different pH levels, showing how the formation of a thick passivating alteration layer depends on the presence of specific ions in the aqueous solution. Through CRM, we traced changes in chemical composition, spatial characteristics, and phase migration within the alteration layer, whose Raman spectra fit the properties of a blend of silica and iron oxides (hematite, magnetite). Additionally, we assessed the pH evolution near the solid-liquid interface by analyzing sulphate ion speciation using Raman intensity peak ratios.

A comprehensive grasp of interphase formation during dissolution holds the potential to yield valuable insights into olivine weathering kinetics, thereby paving the way for enhanced olivine-based CCS strategies.

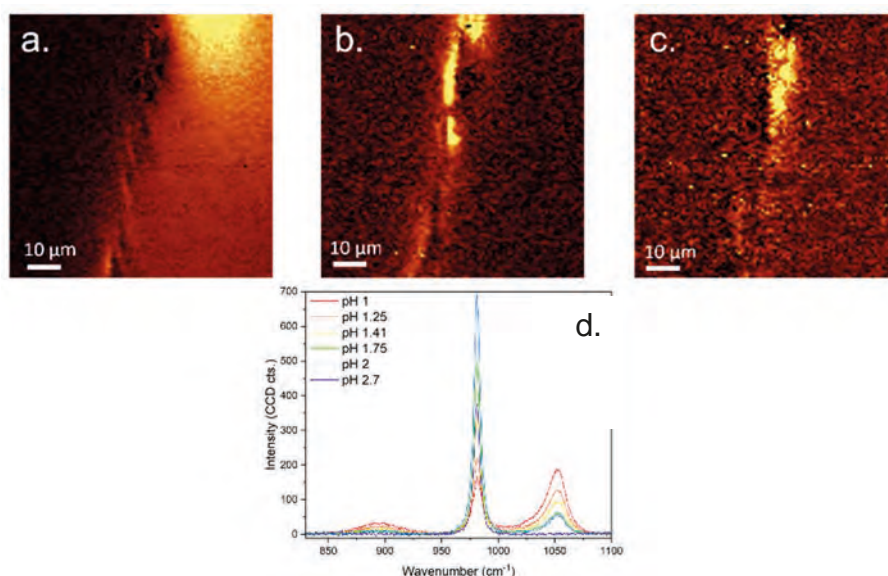


Fig. 1: a. CRM map of an olivine pebble dissolved in H₂SO₄ for 28 days at 60 °C, the scan is obtained by integrating the 856 cm⁻¹ peak of olivine, corresponding to the symmetric stretching vibration of the silicate tetrahedra; b. Integration of the peak at 390 cm⁻¹, attributed to a magnetite-rich alteration layer; c. Integration of the peak at 1350 cm⁻¹, attributed to a hematite-rich alteration layer; d. Variation of the Raman spectrum of H₂SO₄ at different pH. The sulphate-bisulphate peak ratio can aid in keeping track of the aqueous solution pH during the reaction.

Quantification of the crystal chemistry of phyllosilicates by Raman spectroscopy: serpentine, talc, and biotite

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Hydrous layered silicates, such as serpentine (nominally ${}^M\text{Mg}_3{}^T\text{Si}_2\text{O}_5{}^X(\text{OH})_4$), talc (nominally ${}^M\text{Mg}_3{}^T\text{Si}_4\text{O}_{10}{}^X(\text{OH})_2$), and biotite (nominally ${}^A\text{K}^M(\text{Mg}_{3-x}\text{Fe}_x{}^{2+})^T(\text{AlSi}_3)\text{O}_{10}{}^X(\text{OH},\text{F})_2$), are important rock-forming minerals that are stable under a wide range of geological conditions. The importance of these phyllosilicates can be extended beyond the Geosciences, as they are typical mineral components of cultural-heritage objects, for instance of clay tablets, cylinder seals, engraved gems, decorative ceramics, and earth pigments. Thus, the precise mineral identification and determination of the crystallochemical composition within each single mineral constituent will advance the understanding of mineral formation, petrogenesis, and metamorphic reactions in Geosciences as well as promote provenance studies in cultural heritage.

Raman spectroscopy has the potential to probe non-destructively both phase and chemical compositions in petrological thin sections and crude rock samples as well as in cultural-heritage objects, for which sampling is highly undesirable due to their uniqueness. Consequently, there is a continuously growing interest among the geoscientific and archeologic community in the dependence of atomic dynamics on structure and chemistry of complex silicate minerals, from both theoretical and practical aspects. Here, we report the established quantitative correlation trends between the crystallochemical composition, separately calculated by wavelength-dispersive electron microprobe analysis (WD-EMPA), and the Raman parameters of a series of talc and serpentine minerals, as well as of biotites, covering the whole biotite solid-solution series between annite ($\text{KFe}_3{}^{2+}\text{AlSi}_3\text{O}_{10}(\text{OH})_2$) and phlogopite ($\text{KMg}_3\text{AlSi}_3\text{O}_{10}(\text{OH}_2)$). We demonstrate that the Raman signals of the framework ($15\text{--}1215\text{ cm}^{-1}$) and OH-stretching ($3500\text{--}3800\text{ cm}^{-1}$) vibrations, i.e. peak position ω , full width at half maximum (FWHM) and integrated intensity I , can be used as an accurate methodology to quantify non-destructively the major (Mg, Fe, Si, Al, K) and minor (Ti) elements of the crystal structure of biotites. Similarly, we show that the M-site Mg and (Fe+Mn) contents in talc can be quantified by using the Raman scattering of the OH-stretching modes with a precision as good as that of EMPA, while FWHM of the framework vibrations at ~ 130 , 230 , and 380 cm^{-1} can be used to estimate ${}^M\text{Mg}$ and ${}^M\text{Fe}^{2+}$ amounts with relative errors of $\sim 5\%$ in serpentine minerals.

Acknowledgements

The research for this study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2176 'Understanding Written Artefacts: Material, Interaction and Transmission in Manuscript Cultures', project no. 390893796. The research was conducted within the scope of the Centre for the Study of Manuscript Cultures (CSMC) at Universität Hamburg. We thank Stefanie Heidrich and Peter Stutz, Universität Hamburg, to help with WD-EMPA measurements and sample preparation. We are very grateful to the Mineralogical Museum, Hamburg for kindly providing the talc crystals.

Raman-FISH of uncultivated virocells: a preliminary study

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Viruses are considered the most abundant entities in ecosystems worldwide. Viromics has illuminated the diversity of uncultivated prokaryotic viruses in environmental samples. Recently, virus-targeted direct-gene fluorescence *in situ* hybridization (virusFISH) coupled to 16S rRNA gene FISH enabled the identification of prokaryotic virocells – *i.e.*, bacteria or archaea undergoing active infection processes in environmental samples [1]. However, currently available FISH-based techniques fail to link genomic information to biochemical changes. To overcome this caveat, Raman-virusFISH uses virusFISH to label virocells and consequently deploys Raman microspectroscopy to detect biochemical changes taking place during infection processes. Previously, we demonstrated that Raman microspectroscopy distinguishes infected and non-infected bacterial cultures and detects virocells via comparison of nucleic acid/protein ratios [2]. Leveraging this knowledge, in this study, we successfully differentiated non-infected cells and virocells in the same subsurface groundwater sample and characterized changes in nucleic acid/protein ratios via Raman-virusFISH. Interestingly, an unexpected increase of protein-assigned peak intensity of viral particles within virocells suggests that this technique can further elucidate virion composition in infected cells. Multivariate biochemical fingerprinting of virocells provided as-yet inaccessible insights into the infection dynamics of subsurface microbiomes with pivotal roles in global carbon biogeochemical cycling. Raman-virusFISH-informed Raman-SEM will ultimately permit nanometer-resolution, label-free biochemical characterization of environmental virocells.

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Failure analysis by combining Raman and X-ray fluorescence spectroscopy

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Investigations after an incident that occurred during heat treatment in a vacuum-furnace at temperatures above 1200°C are presented. Components made of graphite and of aluminum oxide turned an undesirable grey color. The task was to identify the material of this coating to determine the causal reaction. It was also important to find out as quickly as possible whether there was any risk of toxic substances being released to the furnace operators. By combining the two analytical methods of X-ray fluorescence analysis and Raman spectroscopy, it was possible to quickly determine the compound of which the coating was formed.

Using X-ray fluorescence spectroscopy, a method for qualitatively and quantitatively determining the elemental composition of a sample, the element molybdenum was found to be an essential component of the coating. Since the furnace was heated with molybdenum, it had to be molybdenum that was vaporized by the heating. Raman spectra of the coating were then recorded to identify the molybdenum-containing compound. Several candidates were obvious, because carbon and oxygen were available as reaction partners: Molybdenum carbide Mo_2C , molybdenum hexacarbonyl $\text{Mo}(\text{CO})_6$, and the molybdenum oxides MoO_2 and MoO_3 . In the end, MoO_2 was identified, an oxide that was not classified as harmful to health, but only as an irritant. It was now possible to act specifically on the furnace run to prevent precipitation.

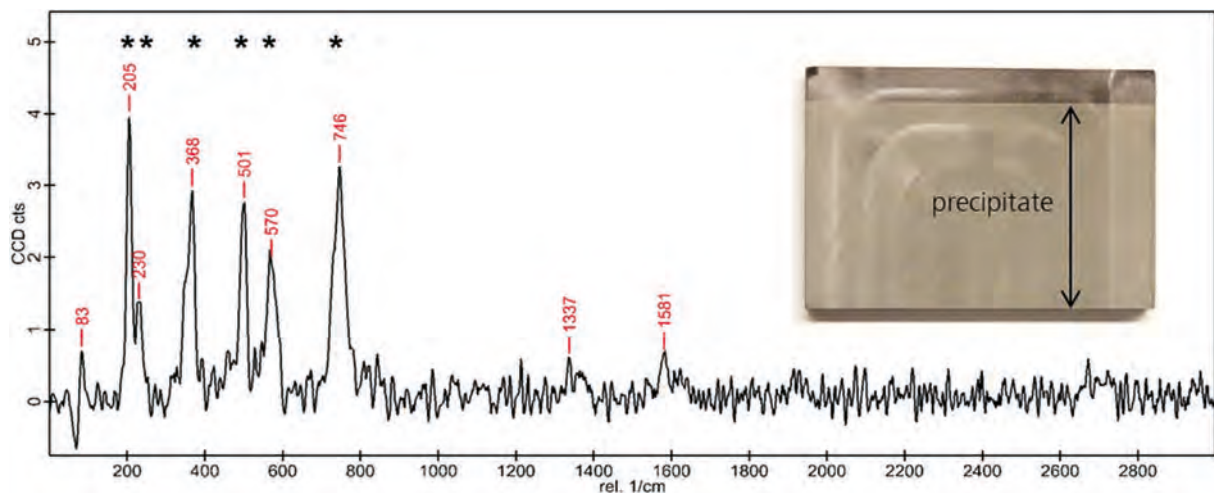


Fig. 1: Damage analysis with Raman spectroscopy. Grey precipitate on graphite plate (small photo) could be identified: MoO_2 (bands are marked with *).

Characterization of complex electrospun matrices through hyperspectral Raman imaging

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Electrospinning is a versatile, widespread and well scalable [1] technique for the preparation of micro- and nanofibers. Electrospun fibrous materials are important tools in biomedicine, based on established applications for tissue engineering, implants, drug delivery and more [2].

In the framework of the EU-MSCA-EJD NanoReMeDi.eu, we expand this technique to pure (non-polymeric) small peptides and peptidomimetics, for long regarded as unsuitable substrates [3]. Beyond biochemical relevance and ability to self-assemble [4], we modify these candidates by selecting the non-natural amino acid(s) [5] and the chain sequence, so finely tuning physical [6] and biological properties. On these new ways to further applications [7], we are currently exploring hybrid matrices that combine hard polymer fibers and soft peptidomimetics.

Electrospinning is governed by complex electrohydrodynamics, so the fibers we produce can exhibit high local variations of their properties. Raman spectroscopy, in combination with confocal microscopy, is a preparation-free and nondestructive tool for the local characterization of a single fiber. It allows simultaneous mapping (or hyperspectral imaging) of both spectral and spatial features of our peptide fibers. This is crucial when we analyze our hybrid fiber matrices.

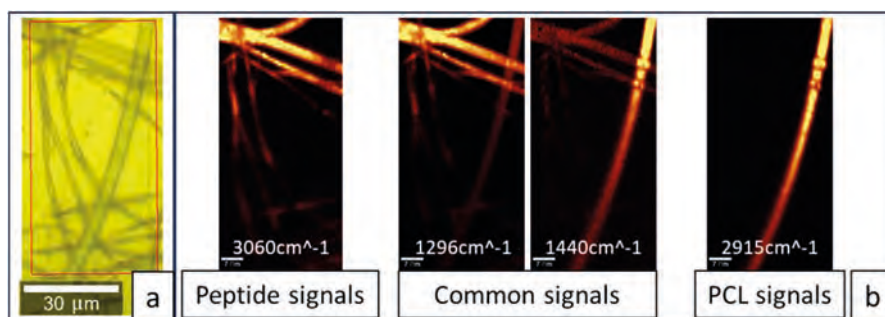


Fig. 1: Hyperspectral characterization of electrospun fiber matrices made of electrospun peptidomimetic and 3D electro-printed polycaprolactone (PCL). *a)* optical image (x100) of electrospun together with 3D electro-printed fibers (indistinguishable). *b)* Raman hyperspectral images, each pixel with a complete Raman spectrum, distinguish the fibers. The images are produced by selecting bands of the Raman spectra, e.g. 2915 cm^{-1} for C-H stretching vibrations. Each band has a specific signal/noise ratio; the intensities are shown in false color.

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How do binary sessile droplets evaporate? The case of water/glycerin

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Evaporation of sessile multi component droplets is ubiquitous in many everyday processes, industry, and nature. Applications include printing, cooling, and coating. During droplet evaporation, thermal and/or surface tension gradients can occur. These can induce convection resulting in concentration gradients. Recently, Diddens et al. were able to simulate concentration maps in FEM simulations. However, experimental evidence for proposed concentration fields is still sought for.

Due to its nondestructive, marker free character Raman spectroscopy is especially well suited to characterize concentrations in binary droplets. Via a confocal setup a lateral focus is accomplished. Measured mixture spectra were correlated to component concentrations via the classical least square method. Here, mixture spectra are fitted by a linear combination of pure substance spectra. Resulting perfectors directly correlate to the components concentrations. Peak width and position can vary depending on composition. This is compensated by iteratively parametrizing each peak by a gaussian fit (modified least squares) allowing a compensation of this effect.

We use Raman and NMR spectroscopy for concentration measurements of 4.2 μl 10 w% glycerol, 90 w% water droplets. NMR provides full droplet concentration maps resolving up to 100 μm close to the 3-phase contact line. However, due to its calibration, observation times are limited and only low resolution (33x33 μm^2) is reached. To increase resolution (2x14 μm^2), elongate observation times and verify results vertical concentration gradients were measured additionally by Raman spectroscopy. Measurement results of both methods agree in their errors. Increasing water contents close to the droplet surface and at the contact line were found.

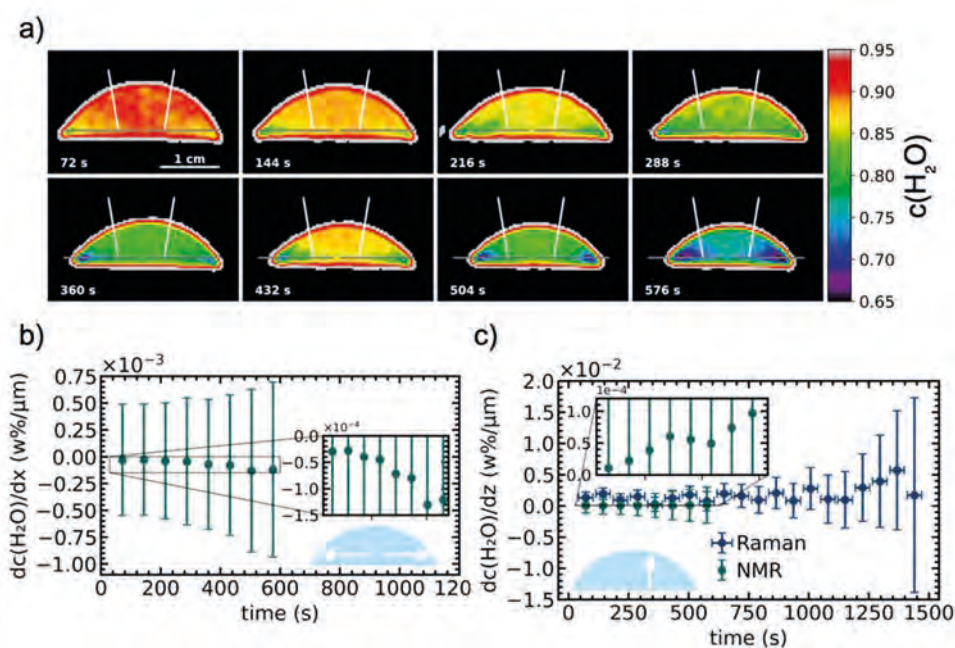


Fig. 1 a) NMR concentration maps of glycerol/water droplets. b) Concentration gradients in horizontal direction as marked in green in a). c) Concentration gradients in vertical direction as marked in white in a).

SERS imaging applied to nanostructured platforms for water quality monitoring

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Water is an essential resource to sustain the existence of all life forms. Water pollution has reached worrying levels, posing serious risks to human health and ecosystems. To effectively address this issue, it is crucial to focus on water remediation technologies but also prioritize preventive measures such as the implementation of water quality monitoring protocols. [1]

In recent years, spectroscopic techniques utilizing surface-enhanced Raman scattering (SERS) have emerged as a powerful analytical technique for detecting vestigial concentrations of poisoning compounds. The advancements in science have significantly enhanced SERS methods through notable progress in instrumentation in conjunction with the advancement of synthetic strategies able to manipulate materials at the nanoscale. Notably, the continuous evolution of Raman instruments, especially in Raman imaging, has revolutionized the understanding and utilization of nanocomposites. [2]

Here, we will present an overview of our research on the development of flexible and easy-handled SERS substrates based on textile fibres [3,4], paper [5,6] and 2D materials [6] for water quality monitoring. Chemical strategies employed for fabricating such flexible plasmonic-based nanocomposites will be described in detail. Illustrative examples of SERS applications and their evaluation using Raman imaging will also be provided, along with perspectives on development in water quality monitoring applied to real-world contexts.

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Acknowledgements:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). The financial support of TEMA by the projects UIDB/00481/2020 and UIDP/00481/2020 from Fundação para a Ciência e Tecnologia (FCT), as well as from CENTRO-01-0145-FEDER-022083—Centro Portugal Regional Operational Programme (Centro 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund. G.G., N.C.T.M. and S.F. thank the FCT for the Programme Stimulus of Scientific Employment—Individual Support (CEECIND/01913/2017) and research contract REF-069-88-ARH-2018, respectively.

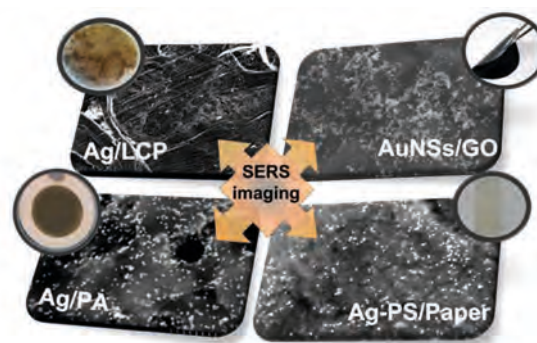


Fig. 1: Transmission electron microscopy images of flexible and highly active SERS platforms based on liquid crystal polymer (LCP), polyamide (PA), graphene oxide (GO) and paper to detect water pollutants using SERS imaging. Inset: Digital photographs of each SERS substrate.

Tracking of cancer cell proliferation through secretome analysis using SERS

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One of the main reasons cancer continues to be one of the highest causes of death among the industrialized world [1] is that the progression of the disease tends to define the efficacy of subsequent treatments. At its later stages, the disease is considered too advanced to the point that multiple tumors would have already been formed, also known as metastasis, accounting almost for 90 % of all cancer-related deaths [2]. As cancer cells start to grow independently and expressing their own phenotypes, stochastic processes begin to dictate the evolution of specific cancer cell populations, thus making treatments much less effective with the passage of time [3]. One promising analytical tool that allows for early-stage monitoring due to its high sensitivity is surface-enhanced Raman scattering (SERS) spectroscopy [4]. SERS combined with AI has the potential to identify patterns and fingerprints of extracellular metabolites produced by cancer cells [5].

This project explores the potential of this analytical technique for monitoring the evolution of cancer cells by capturing and measuring SERS patterns of the secreted molecules at single cell level in disease settings. For this, we are developing a sensing strategy that combines SERS sensor and microdroplets to enable single-cell metabolomics real time analysis during their proliferation.

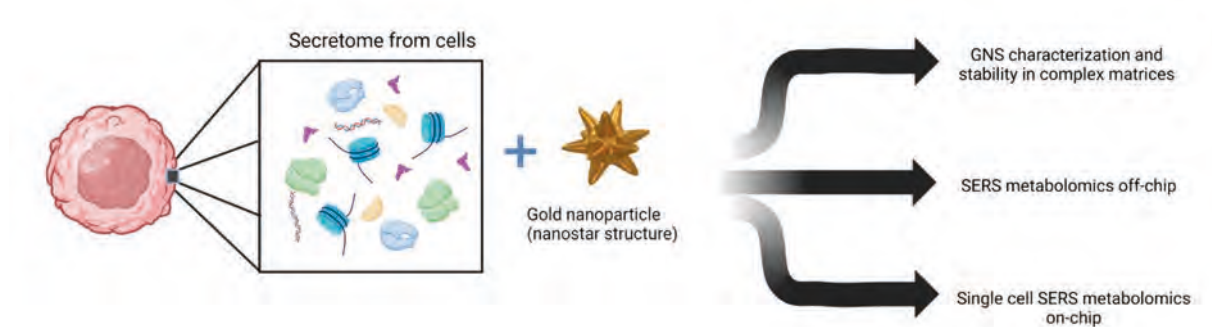


Fig. 1: Illustration of the objectives for the project, regarding the combination of secretomes obtained from cells and gold nanostars for cancer cell studies.

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High Temperature Raman Imaging – Applications in Refractory Research

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The high temperature properties of ceramic refractory materials are strongly influenced by their microstructure and mineralogical composition. Therefore, it is important to understand the reactions (e.g. corrosion, formation or decomposition of phases) that take place during their production and their use. In situ analysis is the key to obtain this knowledge, to study metastable phases, or to avoid misinterpretation of properties. However, to date, there is no analytical tool available to the refractory industry that allows the in situ investigation of mineralogical composition and ceramic microstructure at high temperatures.

High Temperature Raman Imaging can be a solution for this. Former studies demonstrated that solid-solid phase transitions can be investigated in situ at high temperatures with temporal and spatial resolution, providing new insights into the reactions of multi-component systems. Thermodynamic and kinetic phenomena can be studied without the need to quench a test-piece prior to phase analysis. High Temperature Raman Imaging has been used to study the sintering process in the CaO-Al₂O₃-O₂ system. Three application examples are presented showing (i) the formation temperature and formation time of calcium aluminate phases, (ii) the estimation of a semi-quantitative phase content and (iii) the concurrence formation and coexistence of polymorphs. Understanding the temperature and time of calcium aluminate phase formation is crucial for developing refractory materials for specific requirements. This understanding helps to avoid the formation of undesired phases and facilitates the formation of targeted phases.

Another example for the use of High Temperature Raman Imaging is the isothermal sintering process of a mixture of calcite and kaolinite at six temperatures, ranging from 800 °C to 1100 °C. In each experiment, 24 hyperspectral Raman images were obtained over a 48-hour dwell time. A comparable ex situ experiment would have required a series of six experiments, each consisting of 144 individual experiments with a total holding time of more than 600 h. In situ measurements are a time and energy-efficient way of studying sintering processes. Furthermore, in situ experiments offer a high degree of consistency, as measurements are performed on the same test-piece. To obtain the relative phase proportions from each Raman spectrum (i.e., pixel of the image), a classical least squares (CLS) fitting procedure was used, with self-obtained reference spectra. Hyperspectral Raman images were created by false-colouring each pixel of the image according to the main component in the spectrum. The data enables the determination of the proportion of each phase (e.g. gehlenite) within an imaged area as a function of time, providing kinetic information.

The third example shows how polymorphs form concurrently in direct contact with each other. No other in situ technique allows spatial resolution and differentiation between polymorphs simultaneously. The same applies to the formation of metastable phases, which cannot be detected by conventional quench experiments.

The examples demonstrate that High Temperature Raman Imaging offers novel opportunities for the investigation of mineralogical and structural changes in (industrial) high temperature processes. Hence, it also enables targeted product development.

Electrochemical Surface Oxidation Enhanced Raman scattering. A new perspective on different materials for Raman signal enhancement.

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Raman signal enhancement is crucial for the analysis of molecules in low concentrated samples. Since its discovery, Surface Enhanced Raman Scattering (SERS), has been the most widely used effect to improve the Raman signal [1]. These substrates commonly imply the presence of plasmonic metallic nanostructures; however, there are different substrates able to amplify the Raman signal, such as semiconductors or dielectric nanomaterials [2]. A recent strategy for the amplification of the Raman signal was published in 2018, called Electrochemical Surface Oxidation Enhanced Raman Scattering (EC-SOERS) [3]. This effect provides an increase in the Raman signal during the electrochemical oxidation of a silver electrode, under specific electrolytic conditions.

The EC-SOERS phenomenon relies on the interaction of molecules with silver cations adsorbed on the semiconductor/dielectric silver halide nanocrystals generated on the surface of the electrode. To shed more light on this process, the electrochemical oxidation of silver microwires is studied with dark-field microscopy (DFM) under different electrolyte media, including typical conditions for EC-SOERS. The coupling of DFM with electrochemistry allows monitoring of redox processes and transformations of nanoparticles [4]. We used this technique to understand the in-situ generation of EC-SOERS/EC-SERS substrates and further characterized the nanoparticles formed in this process with scanning transmission electron microscopy (STEM) and X-ray photoelectron spectroscopy (XPS).

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Combining Raman spectroscopy and reverse Carbon stable isotope probing for monitoring microbial proteome dynamics in situ

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The microbial proteome is dynamic and non-uniform, as it varies according to cell type. Dynamics of the global proteome are of high importance as they effect the synthesis of metabolites. To analyse proteins and metabolites, highly sensitive and efficient techniques such as liquid chromatography/gas chromatography mass spectrometry, nuclear magnetic resonance are applied. However, these techniques require extraction of proteins from their physiological environment. Therefore, there is a need of new non-invasive and non-destructive approaches. Raman spectroscopy coupled with Carbon stable isotope probing is the new method; however, due to the less abundance of Carbon 13, this method is not efficient for the large culture volumes requirement. In our study we have used a new methodology for studying proteome dynamics by combining Raman spectroscopy with reverse carbon stable isotope probing. Due to reverse labelling, we were able to observe the visible blue shift of protein representative bands i.e. amide I: 1624 to 1661 cm^{-1} and Phenylalanine: 967 to 1003 cm^{-1} . Further, to validate the Proteome band, we have done the inhibition assay using Gentamycin, a known protein synthesis inhibitor. Furthermore, the Carbon Deuterium peak was monitored to track metabolic activity. Our findings explore the potential of Raman spectroscopy in monitoring proteome dynamics in situ.

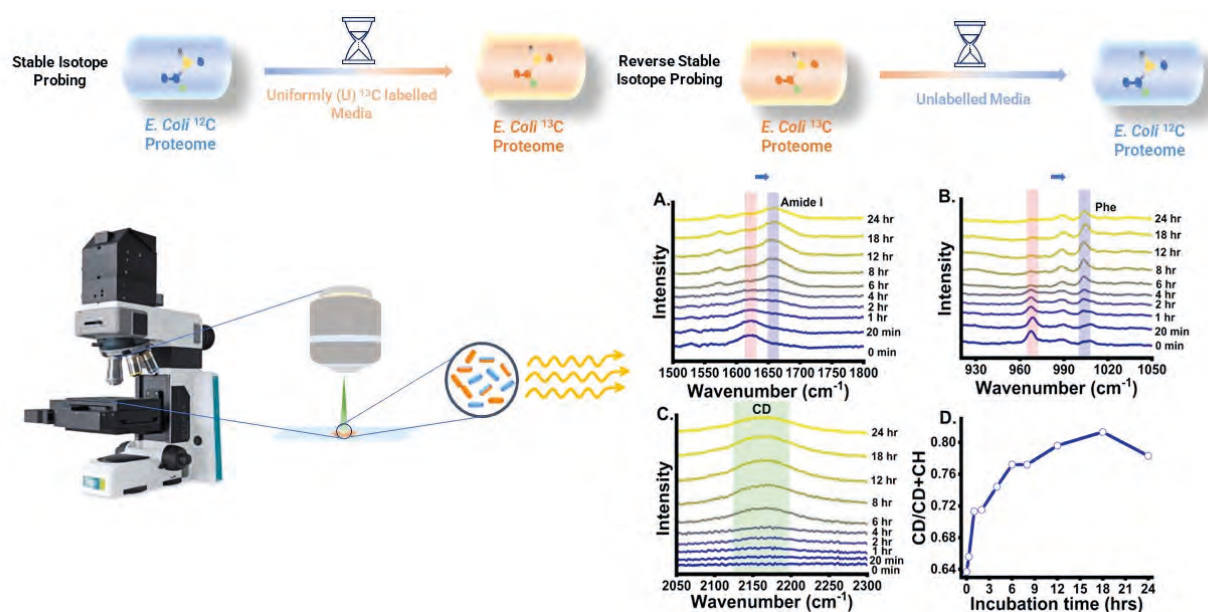


Fig. 1: In situ monitoring of microbial proteome dynamics and metabolic activity in *E. coli* with respect to the incubation timepoints targeting proteome representative band of peptide bond (amide I) and amino acid (Phenylalanine).

Reference:

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Vibrational Spectroscopy Imaging of animal tissues in type two diabetes model

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Type 2 diabetes mellitus is considered one of the most prevalent diseases worldwide. By 2019 more than 460 million people were affected with T2DM and it is estimated that over 1 million causes of death per year are attributed to diabetes. The patients are often diagnosed at late stage of the disease with already developed complications, including dyslipidemia [1,2]. **The aim of this work is to assess metabolomic changes, with emphasis on lipidomics, occurring in type 2 diabetes animal model and correlate the results between different imaging methods.**

The measurements were performed on rat liver tissues from type 2 diabetic model with FTIR Imaging, using Hyperion 3000 spectroscopic microscope coupled with Vertex 80v spectrometer (Bruker Optics, Ettlingen, Germany); Raman Imaging, using Raman Imaging Microscope alpha300 R (WITec, Ulm, Germany) and MALDI MS, using Synapt Q2 (Waters, Wilmslow, Great Britain). The data was analyzed using WITec Project software, Matlab software and Python programming language.

Preliminary data, obtained by MALDI MS technique for the analysis of lipids in homogenates of liver and serum samples, featured significant changes in quantity of specific lipids between control and diabetic group. Most distinct changes occurred in phospholipids, ceramides and triacylglycerols, with increased levels in T2DM group. Both Raman and FTIR Imaging enabled to observe changes in total lipid content and distribution. In the T2DM group, the concentration of lipids is higher than in the control group and they are accumulated in wider areas. Moreover, further analysis of Raman spectra was done to measure the ratio of unsaturated to saturated fatty acid content as a reliable marker of the total degree of lipid unsaturation.

High abundance of lipids within organs has the potential to induce alterations in organ physiology and metabolism, ultimately precipitating conditions such as nephropathy, retinopathy, or neuropathy. Employing multiple analytical techniques enhances the precision and reliability of findings, providing a more comprehensive view on changes in lipidomic profile.

Acknowledgments:

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

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Microplastics toxicity *in vitro* studies - subcellular identification of particles with the use of Raman spectroscopy

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Environmental pollution with plastics is currently one of the leading environmental problems. Particularly dangerous are the decomposition products of these materials in the form of microplastics. Because of their size, they can easily get into water and then into living organisms [1-3]. Microplastics can enter the body in three ways: by inhalation, with food and by transdermal absorption as a result of using plastic objects or directly from the environment [1-3]. When they appear in the blood, they can affect macrophages - the cells involved in the immune response which react to micro-environmental signals by polarization [1]. Functional polarization of macrophages occurs under physiological as well as pathological stages and is considered as an important determinant of development or regression of disease [1].

The main objective of this study was to assess the effect of selected microplastics on human phagocytic cells, which are involved in the immune response. To achieve this goal, we used macrophages M1, which were exposed to the low (0.01 g/l) and high (0.1 g/l) dose of polystyrene microparticles (round shape, diameter of 5 µm). Raman microspectroscopy was used to identify microplastics in cells and to evaluate potential changes in the accumulation and distribution of biomolecules induced by them. For this purpose, a WITec alpha300 R system was used. The cells were subjected to raster scanning with laser beam with the step of 0.5 µm and then the chemical mapping was performed. Moreover, the depth profiles of the samples were recorded, which enabled to confirm the presence of microplastics inside the cells.

This study confirmed that Raman microspectroscopy is useful for the detection and identification of microplastics. This technique allowed not only to determine the cellular structures in which polystyrene particles accumulate, but also examine changes in the accumulation and distribution of biomolecules such as proteins, lipids and organic matter in macrophages resulting from exposure to these particles. It has been also verified that microplastics are phagocytized and internalized by macrophages and this process is dose-dependent. This research sheds new light on the *in vitro* toxicity of the tested particles, and are the basis for further regular research in this area.

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Multimodal Analysis of Concrete and Cementitious Materials

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Concrete is the most used structural material in the world and unfortunately this also means the production of concrete generates a significant carbon footprint. A key research focus is to improve this environmental impact by investigating alternative approaches for concrete and cementitious materials. However, this can only be achieved if the new materials possess the same or better strength and durability of conventional concrete. An important component within the matrix is the hydrated calcium silicate phases (C-S-H) which contributes greatly to the overall strength of the bulk material.

By analyzing the internal chemistries of these alternative concrete matrices, a better understanding of these important physical properties can be obtained. Current analytical methods such as XRD require changes to the natural state though grinding which can lead to misrepresentation. Correlative EDS (Energy Dispersive X-Ray Spectroscopy) and RISE (Raman Imaging and Scanning Electron) analysis can characterize the mixture of calcium silicate phases to assess the degree of hydration that has occurred in a non-destructive manner. Additionally, information on aggregate spacing and distribution is obtained which also contributes to the properties of the bulk. The result is a comprehensive overview of the elemental and molecular components of the set concrete, demonstrating that RISE-EDS analysis offers significant potential for non-destructive analysis of such materials.

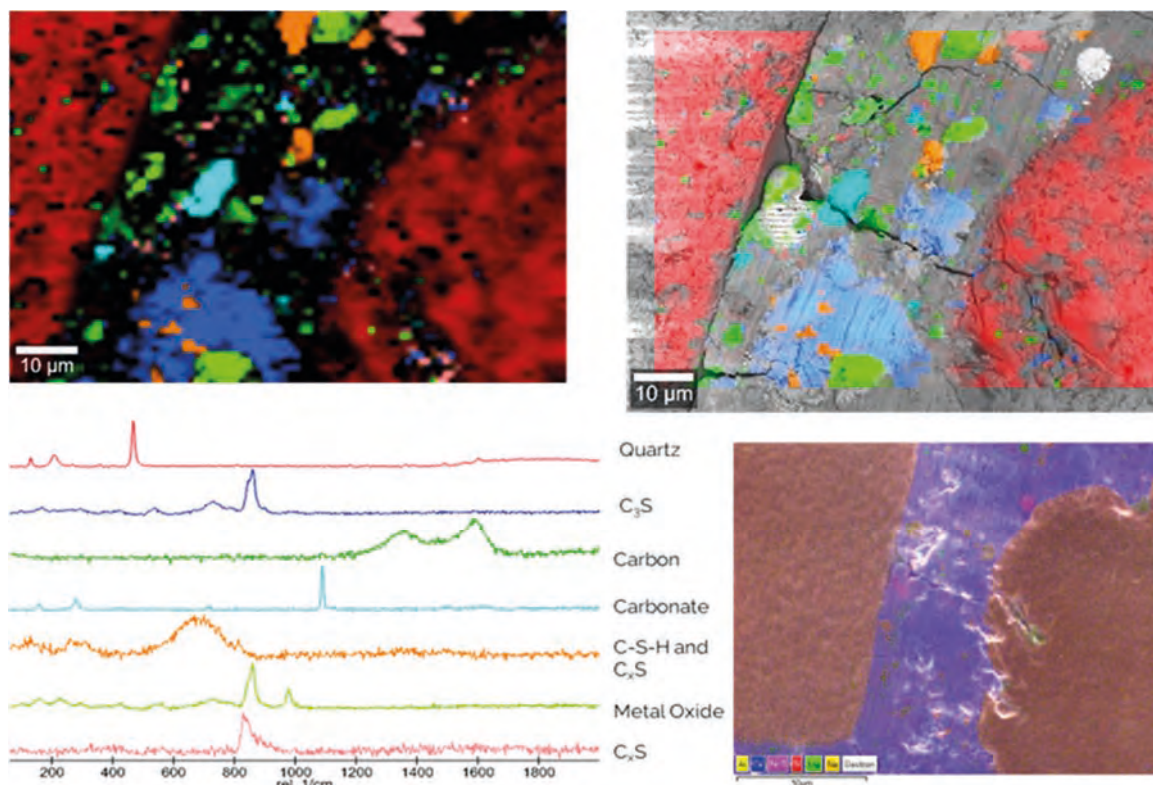


Fig. 1: Clockwise from top left, A Raman map of a concrete sample, the Raman map overlaid on a backscattered electron image, the EDS map of the same region and the components obtained using TrueComponent Analysis.

Inkjet-printed SERS sensors for water quality monitoring

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Water pollution with pesticides, dyes, pharmaceuticals and other contaminants of emerging concern has become a serious worldwide problem. The presence of these compounds in water, even in low concentrations, poses severe risks to the environment, human health, and animal well-being. Monitoring pollutant levels in water is an essential tool for understanding, managing, and mitigating water pollution. It provides valuable information for early detection of the pollution, assessing risks to the environment and human health, implementing effective management strategies, and ensuring regulatory compliance. In the past years, surface-enhanced Raman spectroscopy (SERS) has been recognized as a powerful tool in water quality monitoring because it allows the detection of several water contaminants at trace levels and with minimal sample treatment [1-3]. Developing materials that can act as highly sensitive substrates in SERS is crucial. In this context, hydrophobic SERS substrates are attracting significant attention because in certain conditions they allow the analyte molecules to concentrate in a smaller area of the sensor, thereby increasing its sensitivity [1,2].

In this communication we will present our recent research on the development of hydrophobic paper-based substrates for SERS detection of water contaminants. To produce the hydrophobic substrates two different approaches were followed. One approach consisted in treating office paper with a coating of a biopolymer (chitosan or trimethyl chitosan) followed by printing onto the paper an ink composed of Ag nanoparticles (NPs) [2]. We demonstrate that the biopolymer coatings not only reduce the rugosity and porosity of the paper but also confer hydrophobicity to the paper. By this method, hydrophobic paper substrates with high sensitivity for SERS detection of crystal violet (CV) and ciprofloxacin were obtained. In another approach, inks containing AgNPs and polystyrene beads emulsions were prepared and printed on office paper to obtain hydrophobic substrates in one step [1]. SERS performance of the substrates was optimized by varying the relative amounts of polymer/metal colloidal nanoparticles on the ink formulation and the number of printing layers. These substrates proved to be efficient SERS platforms for detecting trace levels of thiram in water. The detection limits achieved for thiram, CV and ciprofloxacin, using the two types of hydrophobic substrates, were 10^{-9} M, 10^{-7} M and 2×10^{-5} M, respectively. This low SERS detection limits could be achieved using Raman imaging coupled with SERS.

Acknowledgements:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES(PIDDAC). N. C.T. Martins and S. Fateixa are funded by National funds (OE), through FCT-Fundação para a Ciência e Tecnologia, 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 the law 57/2017, of July 19.

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Near Field Scanning Optical Measurement of a Monolayer Self-Assembled Maskless Polystyrene Spheres

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One of the simplest and most affordable fabrication processes for growing a periodic particle array (PPA) with features at the nanometer scale is nanosphere lithography (NSL). It provides a high-throughput, economical self-assembly nanofabrication method that can create a wide range of ordered nanoparticle arrays and nanostructures on any given substrate. The goal of the work is to create nanocarbon field emitter arrays by synthesizing metal nanoparticle arrays using the nanosphere lithography technique. The Near Field Scanning Optical Microscope (NSOM) (ALPHA 100RAS) is used for NSOM and Atomic Force Microscopy (AFM) measurements.

In this study, Polystyrene spheres (PS) with diameters ranging from 100 nm to 500 nm were used to create a monolayer self-assembling mask. An ultrathin layer of Nickel (Ni) was deposited by DC magnetron sputtering and after removing the spheres by ultrasonic agitation, periodic arrays of Ni were obtained. The developed patterns were considered for depositing nanocluster carbon at room temperature using cathodic arc under various optimized deposition parameters. It is suggested that these self-assembled mask-less nanoparticle arrays can be used to make nanocarbon emitter arrays. These nanocarbon emitter arrays are being further investigated for their field emission properties and potential applications in vacuum nanoelectronics devices.

Near Field Scanning Optical image of Polystyrene nanospheres in $0.2\ \mu\text{m}$ area is shown in Figure 1(a) and the 3D NSOM images of the array of highly hexagonally packed nanoparticles are shown in the Figure 1(b).

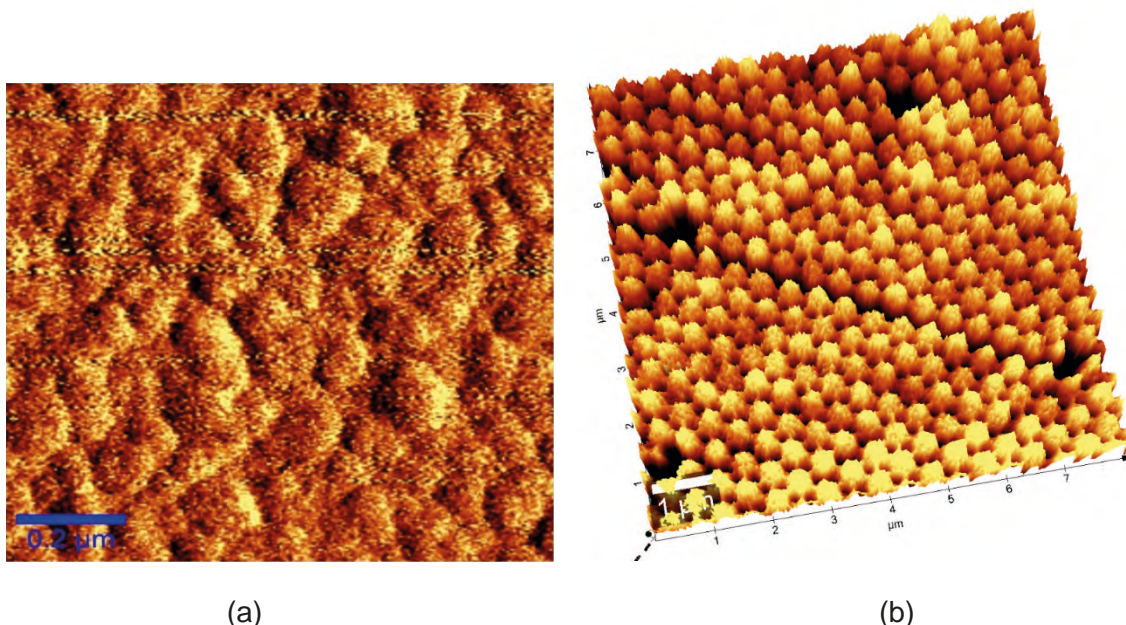


Fig. 1: (a) Near Field Scanning Optical image of Polystyrene nanospheres in $0.2\ \mu\text{m}$ area.
(b): 3D image of the array of highly hexagonally packed nanoparticles.

Validation for Rapid and In Situ Diagnosis of Relevant Fibrotic Disorders using Raman spectroscopy

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During Fibrosis, healthy tissue is replaced by an excessive accumulation of fibrous connective tissue (extracellular matrix) and can lead to permanent scarring and organ malfunction. As collagen is the main component of connective tissue, increased collagen accumulation is an indicator of fibrotic lesions. Currently, the clinical evaluation of fibrosis mainly relies on the evaluation of tissue biopsies by gold-standard histological analyses (H&E and Masson's trichrome staining) or imaging techniques such as MRI and CT. In this poster we will describe our project using Raman spectroscopy and imaging on unprocessed, unfixed tissue to enable classification between healthy and fibrotic regions.

Starting off with an ovine model we designed a method for comparison between unfixed healthy and samples with myocardial infarct. In that study we identified specific spectral changes that were assigned to collagen and merging this with brightfield imaging we could produce heat map images from tissue sections showing accumulation of fibrotic-infarcted zones. Comparison with Masson's Trichrome histological staining of consecutive slides validated this characterization. This work showed a clear distinction in the Raman spectra could be made between healthy and fibrotic tissue [1]. The procedure was used to investigate the influence of Hydrogels in post-ischemic Extra cellular matrix remodelling.

More recently, we have expanded this methodology for the clinical diagnosis of relevant fibrotic disorders in human tissue and have demonstrated that the technique can be used to assess samples for ischemic heart disease and pulmonary fibrosis.

The overall objective of this work is to demonstrate that Raman spectroscopy can be used to streamline biopsy diagnosis of human fibrotic disorders. Future work will expand the range of disorders tested to include liver cirrhosis and kidney disease.

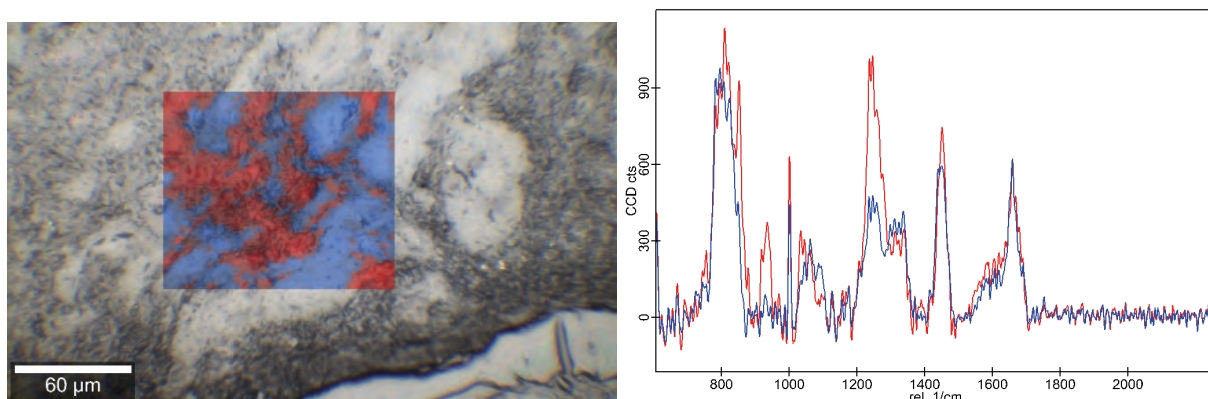


Fig. 1: Dual component Raman Image generated at the 1246cm^{-1} collagen region overlaid on brightfield Human heart sections showing clear distinction between Healthy and Infarct tissue. Associated average spectra, blue healthy, red fibrotic.

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2D Raman mapping of filament cross-sections

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Melt-spun fibers made from a variety of materials are used in a variety of technical textiles. Depending on the applications, such fibers have to meet high demands regarding physical and chemical properties. The cross-sectional microstructure (crystallinity, molecular orientation, phase distribution) is of particular interest for technical textiles, since it affects surface characteristics and mechanical properties of the fibers. It is, however, very challenging to obtain such 2D structural information across polymer filament cross-sections with diameters below e.g. 100 μm .

A high-resolution Raman mapping method has been developed in order to obtain 2D information about structural anisotropies (crystallinity, molecular alignment) in thin filament cross-sections (e.g. diameters between 27 μm and 90 μm). Cross-sections of melt-spun, hot-drawn poly (ethylene terephthalate) (PET) filaments and bicomponent core-sheath PET-polyamide 6 (PA6) filaments have been scanned through a laser beam (spatial resolution $<1 \mu\text{m}$). Raman spectra were analyzed with a specifically developed peak fitting method to obtain Raman maps, e.g., mapped peak height ratios across the face of the fibers. These maps reveal microscopic interconnected networks of crystalline strands within a low crystalline matrix (Fig.1). Radial gradients in PET crystallinity, as well as average and surface crystallinities, were determined. Additionally, we present preliminary results of the microstructure analysis (molecular orientation, amorphous, crystalline and mesophase domains) of P3HB fibers using polarized high-resolution Raman spectroscopy. The presented Raman mapping method to visualize variations in the microstructure of such fine filament cross-sections, and the findings thereof, open a new pathway to better understand how fiber processing parameters affect radial fiber structures.

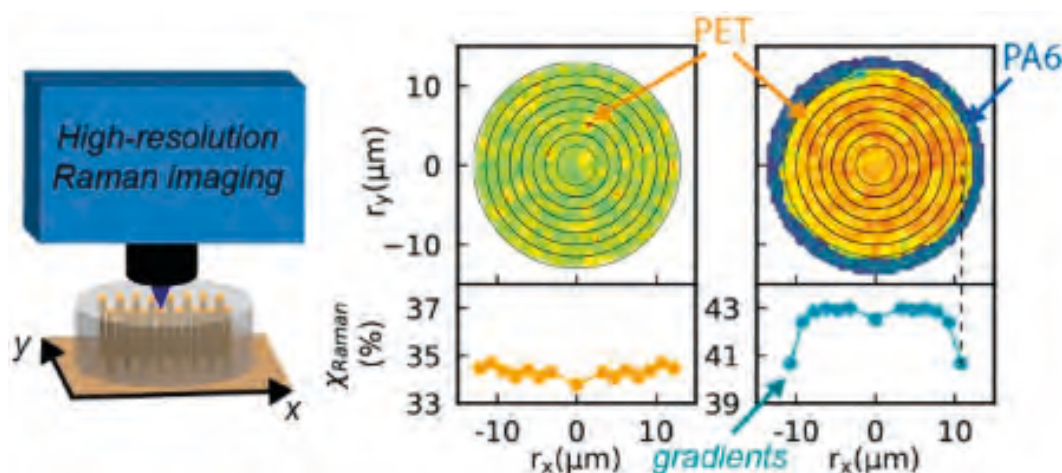


Fig. 1: High-resolution Raman mapping of fiber cross-sections.

Polyethylene upcycling: characterization of unsaturation by Raman and X-ray photoelectron spectroscopy

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Currently, only about 10-30% of the most commonly used polymers (polyethylene, polyesters, polycarbonates and polyurethanes) are being properly recycled. This is mostly done by mechanical methods with limited number of recycling cycles possible and with a loss of material properties leading to low-value plastic products. Thus, new routes that enable high-quality recycling are imperative towards a circular economy. Empa is developing a chemical method to catalytically break down polyolefins to value-added functional monomers, which can be used as raw material for the production of polyesters. A key step in this chemical process is the introduction of unsaturation via C=C double bond formation along the inert polymer chains.

In this work, we have investigated high-density polyethylene (HDPE). Raman spectroscopy was suitable to investigate the C=C stretching vibrations centered on ca. 1600 cm^{-1} (see Fig. 1a). By evaluating the band area ratio of the C=C stretching to the CH₂ bending, an estimation of the unsaturation in the order of 10% was obtained. The sample was further studied with X-ray photoelectron spectroscopy (XPS), which can quantify the unsaturation in more detail by the determination of the sp²/sp³ ratio for the carbon atoms. Thereby, both the C 1s photoelectron peak as well as the X-ray excited carbon KLL Auger peak (see Fig. 1b) have been used to evaluate the hybridization fractions of carbon electrons, resulting in 17.3% of sp². Hence, about every sixth bond in HDPE was unsaturated.

In summary, Raman and XPS spectroscopy have been used to confirm that C=C double bonds were successfully formed into the inert HDPE polymer backbone. This will enable to break down HDPE in later steps (see Fig. 1c) in an energy-efficient and controllable manner to valuable functional raw materials again, which can be used to synthesize engineering plastics, adhesives, coatings and plasticizers.

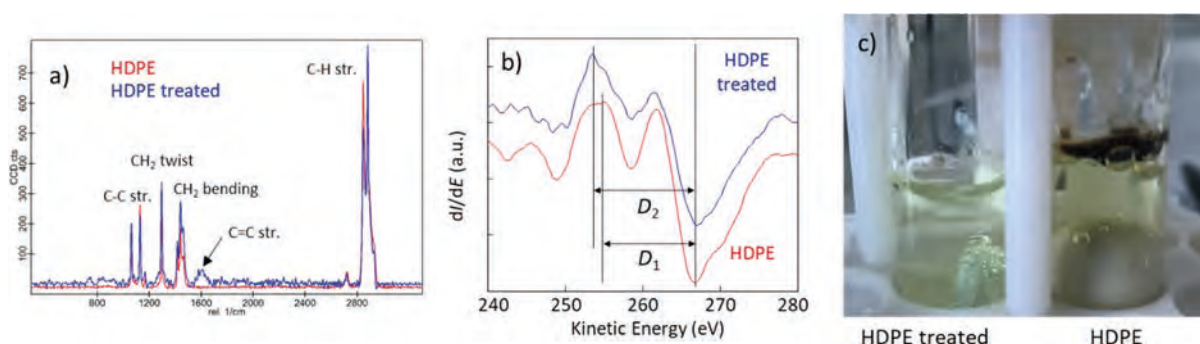


Fig. 1: a) Raman spectroscopy confirming the formation of double bonds for treated HDPE. b) First derivative of the X-ray excited carbon KLL Auger spectra with different spectral widths for HDPE and treated HDPE. c) Post-degradation comparison of raw HDPE and treated HDPE in water solutions.

Hydrogen Evolution Reaction (HER) Performance and Stability of (2D) Platinum Telluride Model Catalyst Materials Probed by Raman Spectroscopy

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Recently, noble metal chalcogenide (NMC) materials such as PtSe₂, PdSe₂, or PtTe₂ have attracted great attention as promising hydrogen evolution reaction (HER) catalysts [1–4]. The intrinsic catalytic properties of bulk NMC materials strongly depend on the edge sites, vacancies, and defects. Thus, 2D layered NMC catalysts serve as an electrocatalyst model system to provide fundamental insights into the structure – composition – activity relationships towards HER. As a promising example, 2D layered MoS₂ has been intensively studied in terms of the influences of layer numbers [5], edge sites [6], or defect doping [7] on the HER performance. However, the activity and stability of 2D layered NMCs as HER catalysts has been rarely reported so far.

In this work, layered 2D platinum telluride (2D-PtTe₂) films were synthesized on highly ordered pyrolytic graphite (HOPG) substrate using molecular beam epitaxy method. Scanning tunneling microscopy (STM), X-ray photoelectron spectroscopy (XPS), and Raman spectroscopy were employed to confirm the phase purity and uniformity of these samples [8, 9]. The electrochemical performance towards the HER was investigated in a custom-built hydrodynamic electrochemical flow cell. Our electrochemical protocol includes open circuit potential (OCP) and linear sweep voltammetry (LSV) measurements for HER activity determination in different electrolyte solutions like in phosphate buffered NaClO₄ (pH=7) and in HClO₄ (pH=1). After each electrochemical measurement, the phase purity and quantitative amount of 2D-PtTe₂ were determined using Raman mapping. Very importantly, no additional phases and only minimal changes in the peak area ratio of Raman bands of 2D-PtTe₂ to HOPG were observed, signifying their high stability under these electrochemical conditions. Furthermore, Raman mappings over multiple days in air also confirmed their high stability against oxidation.

Altogether, we provide fundamental information about the catalytic HER properties and chemical stability of well-defined 2D-PtTe₂ in different electrochemical environments (pH, kind of electrolyte, etc.). In the near future, the influences of step edge functionalization, vacancy doping and surface termination with different Pt-Te phases [8] on the HER activity will be evaluated to develop a relationship between structure – composition – activity.

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Spectroscopic imaging in assessment of biomaterials and mesenchymal stem cells cultured on the hydroxyapatite-based bone scaffolds

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The increasing number of accidents, injuries and bone tumours along with developments in medical sciences result in growing demand for bone substitute materials. Nowadays the challenge for the biomedical engineering technologies is to design bone substitute materials without providing animal tests during pilot experiments. The new approach which allows to reduce the cost of experiments, shorten analysis time of the tested materials and significantly minimize the need for animal testing is needed.

In this project the modern advanced spectroscopic methods - FT-IR, and Raman spectroscopic imaging will be used to assess bone formation markers produced by stem cells cultured directly on the surface of ceramic-based biomaterial. This approach allows to detect structural changes of the organic and inorganic phase of designed biomaterials in vitro. These experiments will determine the bioactivity and biocompatibility of the modified biomaterial without the need for performing animal testing.

The characteristic of pure components, including 1,3- β -D-glucan, hydroxyapatite and obtained biomaterials was performed [1]. Two types of stem cells, adipose-derived stem cells (ADSCs) and bone marrow-derived stem cells (BMDSCs), were cultured on composite surface. The methodology used allowed for the simultaneous assessment of the ceramic part, organic component structures and products of cellular activity, including the extracellular matrix, with particular emphasis on collagen I [2]. Changes in crystallinity and deposition of hydroxyapatite crystals as well as maturation and mineralization of ECM were demonstrated by comparing the process in the presence of differentiated adipose tissue and bone marrow cells.

Acknowledgment

Financial was provided by the Foundation for Polish Science within Reintegration grant no. (POWROT/2017-4/14) POIR.04.04.00-00-4398/17-00 and DS42.

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Confocal Raman Spatial Imaging of Rhombohedral and Tetragonal Structural Coexisting Phases in MPB composition

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Morphotropic Phase Boundary (MPB) composition of binary piezoelectric ceramics exhibits enhanced electromechanical properties such as piezoelectric constant, dielectric constant, electromechanical coupling coefficients. $(1-x)\text{PZrO}_3 - x\text{PbTiO}_3$, $(1-x)\text{Na}_{0.5}\text{Bi}_{0.5}\text{TiO}_3 - x\text{BaTiO}_3$, and $(1-x)\text{Na}_{0.5}\text{Bi}_{0.5}\text{TiO}_3 - x\text{K}_{0.5}\text{Bi}_{0.5}\text{TiO}_3$ are binary systems that have MPB which separates the coexisting rhombohedral and tetragonal structural phases. Close to MPB compositions, also reported to have monoclinic phases from high resolution synchrotron XRD data. The signature of the MPB composition in the XRD patterns is the splitting of (111) and (002) peaks which implies that there are rhombohedral and tetragonal unit cells in the bulk of the sample. Using HREM images, fringe pattern corresponding to both the unit cells can be seen in the real space lattice at nanoscale while SAD shows the 2D reciprocal lattice from data collected from small selected area. It is curious to know, how the spatial contour of the coexisting structural phases exists over the surface of bulk of the sample. Confocal Raman 2D images provide the spatial mapping of the rhombohedral and tetragonal phases in the intermediate length scale, *i.e.* micron scale. Analysis of the 2D images yields phase fraction, complementary to that obtained from Rietveld refinements, poling induced phase fraction changes, polarization measurements hint the domain patterns.

$(1-x)\text{Na}_{0.5}\text{Bi}_{0.5}\text{TiO}_3 - x\text{BaTiO}_3$ composition with $x=0.065$ yields co-existence of rhombohedral and tetragonal structural phases as evidenced in the Fig. 1(b) and (c). The confocal Raman image in Fig. 1(d) is a representative 2D image that depicts the spatial distribution of the two phases in micron scale. Poled and polarization measurement of the 2D images will be discussed.

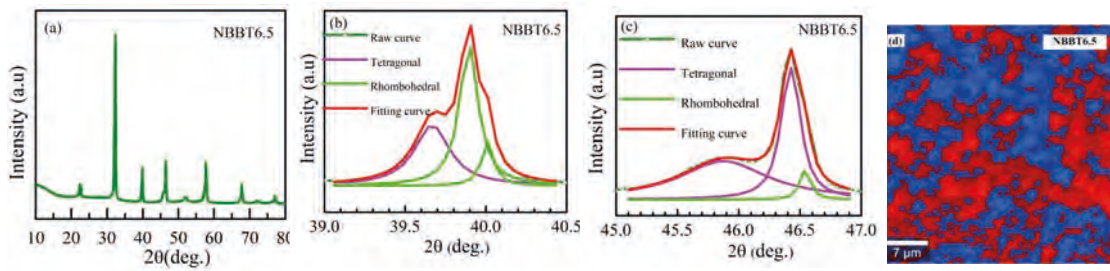


Fig. 1: (a) XRD pattern of NBT-BT MPB ceramics (b) Extended view of (111) (c) (002) peaks and (d) confocal Raman 2D image.

Whitepaper: Charging Ahead – Raman-SEM Analyses of Li-Ion Batteries

Charging Ahead: Raman-SEM Analyses of Li-Ion Batteries

Correlative Structural and Chemical Characterization of Energy Storage Materials

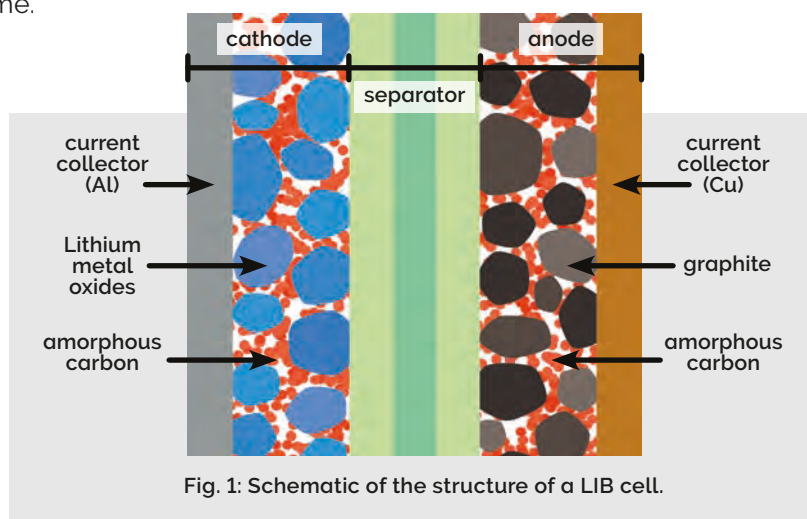
Ute Schmidt, Karin Hollricher, Jan Englert, Damon Strom, Eleni Vomhof, WITec GmbH

The development of high-performance, long-lived, and low-cost lithium-ion batteries (LIBs) requires a thorough understanding of the relationships between their composition, structure, and performance properties. A RISE microscope combines Raman imaging and scanning electron microscopy (SEM) in one, integrated device that can acquire information on chemical composition and surface structure from the same sample location. Energy dispersive X-ray spectroscopy (EDS) provides elemental analysis that can be seamlessly integrated within a RISE instrument. The correlation of results from these three analytical techniques enables the comprehensive characterization of important anode, cathode, and separator properties in battery cells.

Rechargeable batteries provide the power for many electronic devices such as cell phones, tablets and laptops, as well as the electric vehicles that are quickly growing in popularity. Since Alessandro Volta invented the voltaic pile, the first electric battery, around 1800, research on electrochemical cells has advanced continuously, leading to the development of many different energy storage designs, among which LIBs are currently the cutting-edge technology [1, 2]. For their development, the 2019 Nobel Prize in Chemistry was awarded to John B. Goodenough, M. Stanley Whittingham, and Akira Yoshino.

Due to the high demand for effective energy storage, industrial researchers are currently striving to optimize LIB properties in terms of charging speed, energy density and lifetime.

Significant improvements have resulted, for example, from the introduction of new cathode materials and from replacing liquid electrolytes by solid materials [1, 2]. The structure of a LIB cell is shown schematically in Fig. 1. The anode is usually made of graphite and amorphous carbon, while polymers or ceramics are often used for the separator between the two electrodes. Cathode materials used in commercial LIBs include LiCoO_2 (LCO), LiMn_2O_4 (LMO), $\text{LiNi}_{1-x-y}\text{Co}_x\text{Al}_y\text{O}_2$ (NCA), $\text{LiNi}_{1-x-y}\text{Co}_x\text{Mn}_y\text{O}_2$ (NCM/NMC) and LiFePO_4 (LFP). Combinations of different active materials are also used. To reduce cost and environmental impact, the development of batteries with little or no cobalt is being pursued by battery researchers [2-4].



Info Box 1: Raman Imaging

Raman spectroscopy

The Raman effect is based on inelastic light scattering. The interaction of a molecule with photons causes vibrations of its chemical bonds, leading to specific energy shifts in the scattered light that are visible in a Raman spectrum. The Raman spectrum of a material is as distinct as a human's fingerprint, and it can therefore be used to positively identify each component in a sample. In addition, Raman spectra provide information about other material properties, such as crystallinity, crystal symmetry and orientation, and strain.

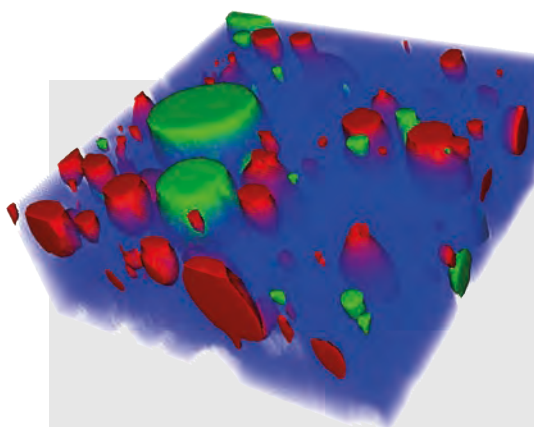


Fig. 2: The 3D Raman image shows the spatial distribution of a hand cream's components ($40 \times 40 \times 15 \mu\text{m}^3$). The oil-in-water emulsion consists of a water phase (blue) and two oil phases with different moisturizing ingredients dissolved in them (red and green).

Confocal Raman microscopy

With the combination of a confocal microscope and a spectrometer, a complete Raman spectrum can be acquired at each image pixel. The resulting Raman image shows the spatial distribution of materials and chemical properties. 3D images can be generated from 2D Raman images acquired at successive focal planes (Fig. 2). The resolution achievable with a confocal microscope is below 300 nm laterally and below 900 nm axially when using an excitation wavelength of 532 nm [6]. Spectrometers with high sensitivity and throughput allow for integration times of less than one millisecond per spectrum and thus for short image acquisition times.

Fully automated Raman microscopes can be controlled remotely, allowing operation in enclosures such as gloveboxes (Fig. 3). This offers great benefits for battery research, semiconductor development, the automotive industry, and other fields that require sample measurements carried out within nitrogen, argon, or other controlled environments [7].

Correlative Raman microscopes combine Raman spectroscopy and complementary analytical techniques in one instrument, so that images of the same sample area can be generated using several methods. By overlaying these images, the chemical information from Raman measurements can be combined with, for example, the surface structure from scanning electron or atomic force microscopy.

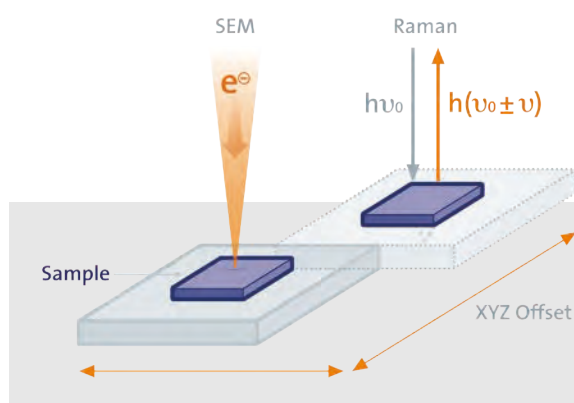


Fig. 3: WITec alpha300 *apyrion* fully automated Raman microscope in a glovebox by MBRAUN (Garching, Germany).

Info Box 2: RISE Microscopy

The principle

Raman Imaging and Scanning Electron (RISE) microscopy combines Raman and SEM in one instrument [8-10]. An automated positioning system transfers the sample between the Raman and SEM/EDS measurement positions inside the vacuum chamber with an accuracy of several micrometers (Fig. 4), therefore enabling the correlation of elemental, molecular, and structural information from precisely the same sample location (Fig. 5-8).



The advantages

The combination of Raman microscopy, SEM and EDS is an ideal correlative approach for many experimental challenges. SEM uses the interaction of electrons with the investigated material to produce high-resolution images of the sample structure. The same electron beam can be used for EDS to reveal the spatial distribution of elements in the sample. Raman microscopy provides information about chemical bonds. Compounds can be identified and polymorphs of a substance can be distinguished based on their Raman spectra. The integration of all three techniques in a RISE microscope thus delivers a truly comprehensive sample characterization.

Fig. 4: The principle of RISE microscopy.

The sample is automatically transferred between the two measurement positions within the vacuum chamber so that Raman, SEM and EDS images can be acquired from the same location.

For the effective development and optimization of long-lived, powerful, and affordable LIBs, the relationships between the structure, chemical composition and performance characteristics of batteries must be investigated. Raman imaging microscopy (see Info Box 1) can acquire and visualize chemical information from the internal components of LIB cells, such as their molecular structure, grain boundaries, solid-electrolyte interphase (SEI) formation, and decomposition processes at the electrodes [3-5]. Lithium-containing compounds can be identified by their Raman spectra and polymorphs such as graphite and amorphous carbon can be distinguished.

For even more detailed studies, the combination of different imaging techniques can be especially informative. SEM enables the visualization of electrode structure at very high resolution. EDS integrated within the electron microscope can identify elements and show their spatial

distribution. As Raman imaging yields information about the molecules' chemical bonds, these three techniques are complementary [4]. RISE microscopy (see Info Box 2) combines Raman imaging and SEM in a single instrument that can correlate chemical composition and surface structure information acquired from the same sample location.

In the following examples, new and used LIBs are examined with Raman imaging, SEM, and EDS to characterize the structure and chemical composition of their cathodes, anodes, and separators, and to reveal changes that result from charge cycling. All measurements were performed with a RISE system consisting of a WITec alpha300 confocal Raman microscope and an SEM. The examples highlight the utility of this correlative method for battery research.

Characterization of cathode materials

The following example shows an analysis of the structure and chemical composition of a LIB cell's cathode. The measurements were performed using a RISE microscopy system. For the correlative Raman and SEM measurement, no polishing of the sample was necessary, making it possible to image the compound distribution on the rough surface.

The heterogeneous surface structure of the particles in the cathode can be seen in high resolution in the SEM image (Fig. 5a). The Raman image (Fig. 5b) shows that the cathode is composed of different materials (LCO, NMC,

and amorphous carbon) that could be identified by their Raman spectra (Fig. 5c). The RISE image correlates the surface structure and chemical composition (Fig. 5d). The smooth particles consist of LCO and the rough ones of NMC embedded in amorphous carbon.

For comparison, the sample was polished and analyzed again. The RISE image of the polished cathode clearly shows the distribution of the two different particles (NMC and LCO) and the surrounding amorphous carbon (Fig. 5e).

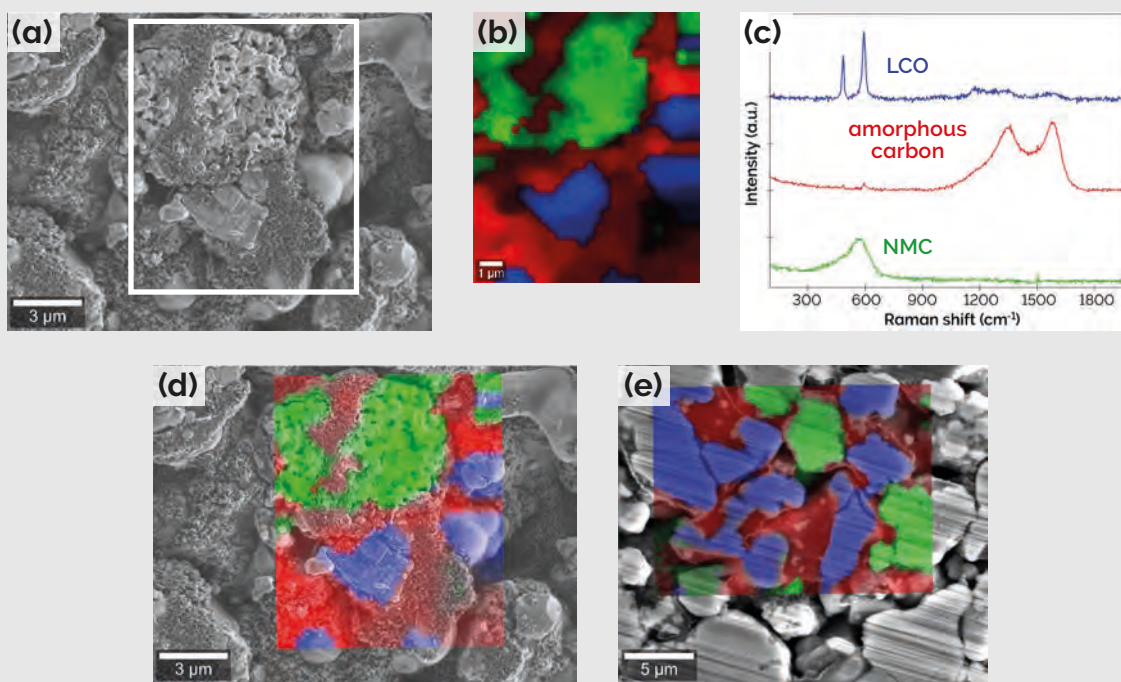


Fig. 5: RISE microscopy investigation of a LIB cathode.

(a) The SEM image of the cathode shows the heterogeneous surface structure of the particles.

(b) The Raman image visualizes the distribution of chemical components in the marked region (white box) in a: amorphous carbon (red), NMC (green), and LCO (blue).

(c) Raman spectra of amorphous carbon (red), NMC (green), and LCO (blue).

(d) The RISE image of the untreated cathode (overlay of a and b) correlates surface structure and material properties.

(e) RISE image of the polished cathode with the same color code as b, c, and d.

Sample courtesy of the University of Southampton, UK.

Influence of fast charging on NMC cathodes

Fast charging of LIBs is very important for electromobility, but it reduces their lifetime. The following measurements investigate local changes of microstructure and composition of a NMC cell, which has suffered a capacity loss of 40% after 400 fast charging cycles. Such reduction in

performance is often a result of inhomogeneous degradation of battery electrodes. Sample cross sections were prepared using a focused ion beam (FIB), which was integrated within the RISE microscope used for the measurements.

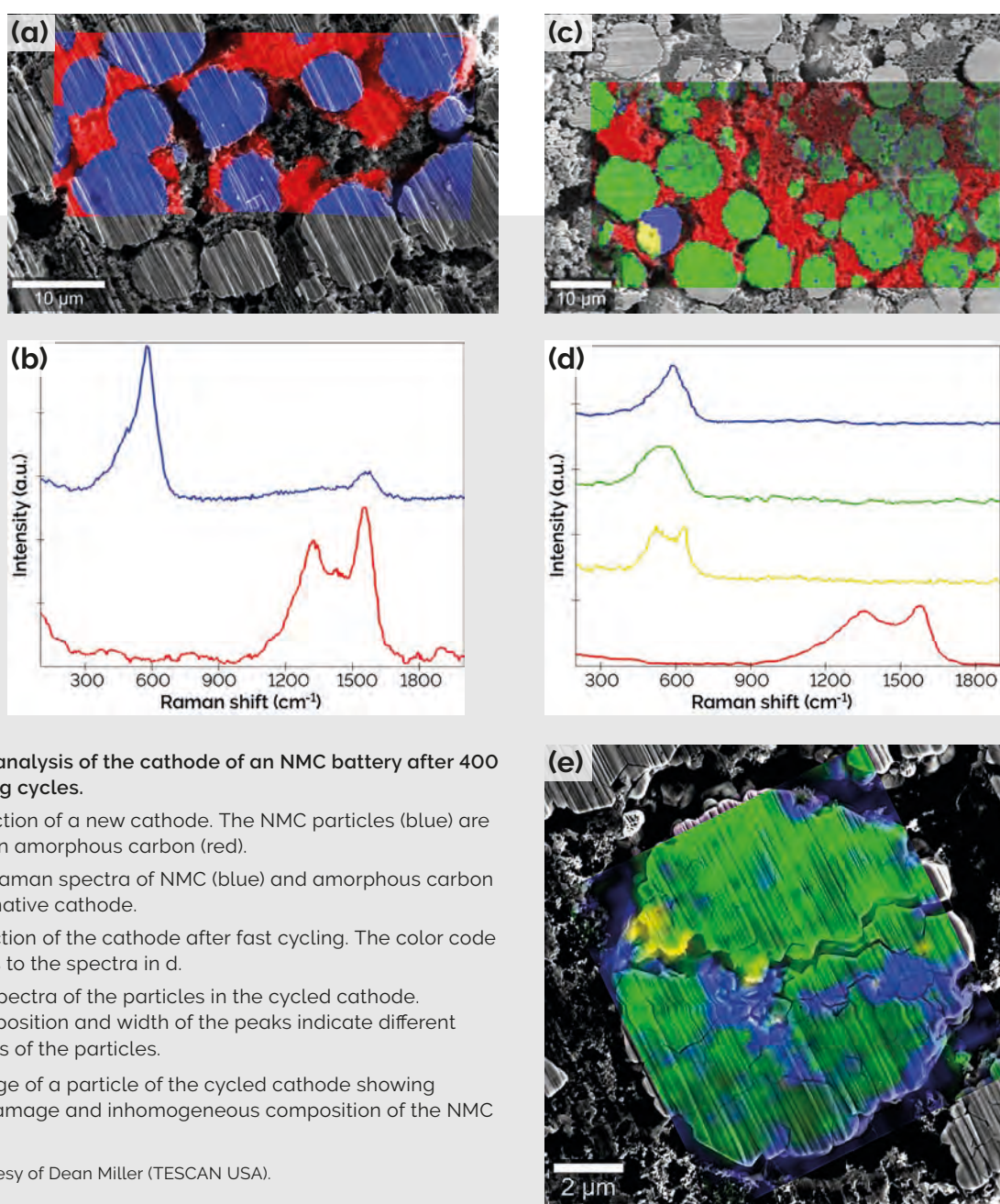


Fig. 6: RISE analysis of the cathode of an NMC battery after 400 fast charging cycles.

(a) Cross section of a new cathode. The NMC particles (blue) are embedded in amorphous carbon (red).

(b) Typical Raman spectra of NMC (blue) and amorphous carbon (red) of the native cathode.

(c) Cross section of the cathode after fast cycling. The color code corresponds to the spectra in d.

(d) Raman spectra of the particles in the cycled cathode. Changes in position and width of the peaks indicate different compositions of the particles.

(e) RISE image of a particle of the cycled cathode showing structural damage and inhomogeneous composition of the NMC material.

Sample courtesy of Dean Miller (TESCAN USA).

Characterization of anode materials

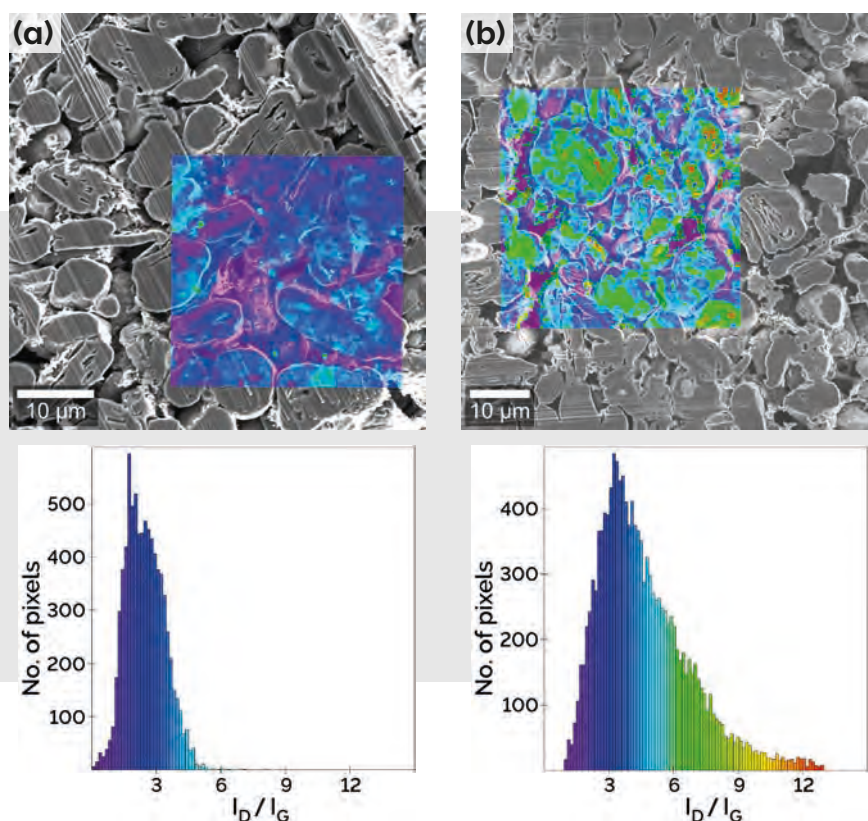
The RISE image of the new charged cathode shows that the cathode particles are composed of homogeneous NMC and embedded in amorphous carbon (Fig. 6a, b). Rapid charge and discharge cycles lead to significant changes in particle composition as indicated by broadening and shifts in the Raman spectra (Fig. 6c, d). Raman imaging can even reveal variations within individual particles (Fig. 6c, e). One particle shows two different spectra, one of which corresponds to the NMC spectrum of the native cathode (blue/yellow particle in Fig. 6c). This indicates that the particle may not have fully participated in the charging and discharging process, possibly due to the crack visible in the SEM image. The RISE image of another particle also shows significant spectral differences within the particle and large cracks (Fig. 6e). Particularly pronounced cracking was observed near the separation membrane (not shown).

Raman spectroscopy can distinguish graphite and amorphous carbon polymorphs in anodes. Additionally, the graphite quality can be investigated. The intensity of the D-band increases with increasing defect density in the graphite crystal. Therefore, the intensity ratio of the D and G bands represents a measure of graphite quality. A RISE microscope was used for the measurements. Each spectrum in the Raman image of a LIB anode was fitted with two Lorentz curves to quantify the area fractions of the D and G bands and their ratio (I_D/I_G) was then color coded in a RISE image (Fig. 7). Comparing a new and a cycled anode reveals that the defect density increases with repeated charging and discharging.

Fig. 7: RISE microscopy measurement of LIB anodes.

RISE images of new (a) and used (b) anodes show structure and material quality. The ratio of D and G bands (I_D/I_G) is color coded in the Raman images. The histograms show the frequency of the I_D/I_G values in the respective image, as well as the color code.

Sample courtesy of Dean Miller (TESCAN USA).



Raman-EDS analysis of anode, cathode, and separator

LIBs of type 18650 (diameter 18 mm, length 65 mm) are found in many portable devices, such as laptops and flashlights, and also in electric vehicles. The following example shows a comparison of two 18650 cells. One was still in its initial state, while the state of health of the other cell was reduced to about 64% after more than 480 charge cycles. Cross sections were prepared in argon gas in a glovebox. For RISE microscopy, the WITec Raman system was coupled to an SEM equipped with an EDS

detector from Oxford Instruments NanoAnalysis. EDS analysis of the new battery shows that the cathode contains two active materials: LMO (Mn-rich regions in Fig. 8a) and NMC (Co/Ni-rich regions). The current collectors are made of copper and aluminum. The separator and anode are not visible in the EDS analysis because polymers and carbon polymorphs are indistinguishable. Raman microscopy provides that information. The anode is composed of graphite and amorphous carbon, while lithium metal oxides

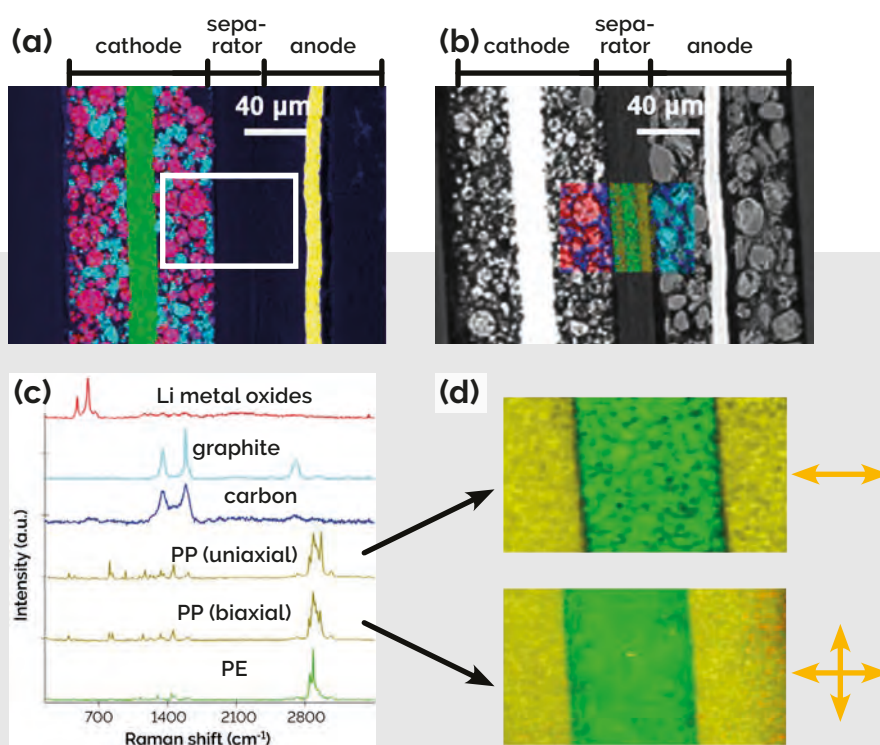


Fig. 8: Correlative EDS and Raman analysis of new and used LIBs.

(a) EDS analysis of a cross section of the new cell. The cathode consists of Co/Ni-rich regions (pink) and Mn-rich regions (cyan). The current collectors are composed of copper (yellow) and aluminum (green). The separator and anode cannot be distinguished.

(b) Overlay of Raman and wide-field image. The anode consists of graphite (cyan) and amorphous carbon (blue), the separator of PP (yellow) and PE (green), and the cathode of lithium metal oxides (red) and amorphous carbon (blue).

(c) Corresponding Raman spectra with the same color code as in b.

(d) Raman images of the separator of the new (top) and used (bottom) battery. Repeated charging causes structural changes in the PP layer.

Sample courtesy of Timo Sörgel and Gerhard Schneider (Institute for Materials Research, Aalen University, Germany).

and amorphous carbon were detected in the cathode (Fig. 8b, c). The separator is composed of a thin layer of polyethylene (PE) between two layers of polypropylene (PP) (Fig. 8b, c). All the compounds were identified by their Raman spectra (Fig. 8c). The two carbon polymorphs and the polymers could be distinguished only by Raman spectroscopy.

Raman analysis also provides information on the influence of charge and discharge cycles on the polymer separator (Fig. 8c, d). While the outer layers of the separator in new batteries consist exclusively of uniaxial PP, the orientation of the polymer chains changes during cycling, so that biaxial PP is detected in the used battery. Such changes in separator composition can significantly affect the performance of LIBs [11, 12].

As already shown in previous examples, SEM images of the cycled cathode reveal that repeated charging and discharging leads to crack formation in the particles (not shown), which can also contribute to the reduction of battery lifetime and performance.

Summary

The combination of confocal Raman microscopy with SEM and EDS is an excellent tool for characterizing the chemical composition and structure of batteries. Structural and chemical changes in the cathode, anode, and separator of used lithium-ion batteries can be visualized. These changes are likely among the reasons for the observed reduction in performance and lifetime of LIB cells.



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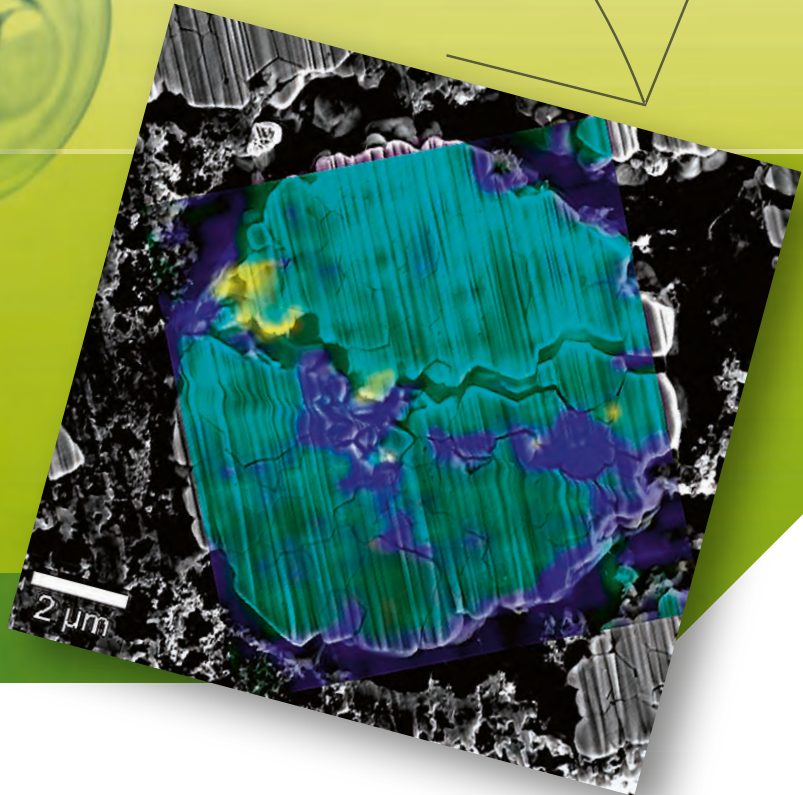


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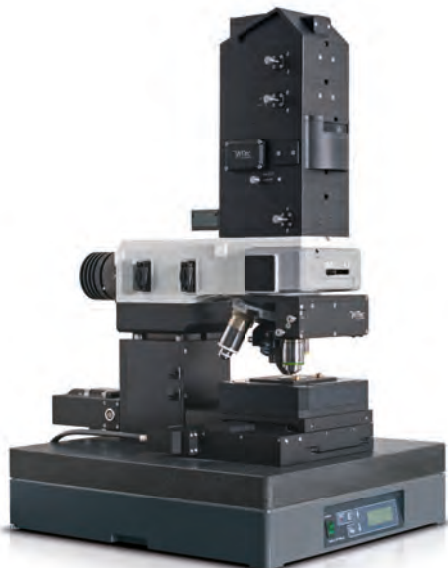
Correlative Raman-SEM (RISE) image
of a lithium-ion battery cathode particle



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