Book of Abstracts

Multimodal Microscopy Workshop
Explore Cutting-Edge Imaging and Analysis Techniques
2025

10th – 12th September 2025
Brno, Czech Republic



© 2025 Masaryk University
ISBN 978-80-280-0759-1

Welcome address

It is with great pleasure that we welcome you to the 2025 Multimodal Microscopy Workshop. We are proud to see its continued growth — both in scope and in the number of participants joining us each year.
This year's workshop marks a significant milestone, not only in terms of attendance but also in the diversity of its program. We are excited to present a diverse range of cutting-edge microscopic techniques, including atomic force microscopy, Raman spectroscopy, and fluorescence confocal imaging, all of which are available in one place.
Equally important is the synergy between academic research and industrial innovation that defines this year's event.
Whether you are here to share your latest findings, explore new instrumentation, or forge new collaborations, we hope this workshop provides a stimulating and rewarding experience.

Thank you for being part of this vibrant community.
I would also like to thank the members of our laboratory for their help in making this event possible. Thanks also for going to the CEITEC MU Events Department.

On behalf of the organizing team,

Jan Přibyl, Ph.D. | **CEITEC MU**
Head of Core Facility Nanobiotechnology

Programme

Wednesday, 10th September (E35, atrium)

8:30 – 9:00	Registration, coffee (E35, atrium)
9:00 – 9:15	*Workshop opening* – **Microscopy and Its Importance in the Brno Region** (*J. Neuman, Brnoregion microscopy, Brno*)
9:15 – 9:45	**Structure, mechanical properties, and chemical composition of biosamples** AFM, optical, and Raman microscopy (*J. Přibyl, CEITEC MU, Brno*)
9:45 – 10:00	*Company presentation* – **From Ångström to Zoology – Measuring Every Detail** (*J. Horák, Měřicí technika Morava s.r.o., Czech Republic*)
10:00 – 10:30	Coffee break (E35, atrium)
10:30 – 11:15	*Invited lecture* – **Enhanced Atomic Force Microscopy by optical imaging techniques** (*D. Hadraba, Bioimaging Facility - CZBI, Institute of Physiology, Prague*)
11:15 – 11:45	*Company presentation* – **Micro Meets Nano: Integration of Atomic Force Microscopy and Optical Techniques** (*A. Dulebo, JPK BioAFM, Bruker Nano GmbH, Berlin, Germany*)
11:45 – 12:00	**Controlled Release of Insoluble and Unstable Flavonoids from Magnetic Nanocarriers by External Magnetic Fields** (*S. Šegota, IRB, Zagreb, Croatia)*
12:00 – 13:30	Lunch (E35, atrium)

13:30 – 14:00	*Company presentation* – **Pioneering the Future of Microscopy: ZEISS Innovations in Light Microscopy** (*T. Cicerone, Carl Zeiss N.V., Belgium*)
Section 1	**Visualization of a new type of samples** (15 min each) (E35, atrium)
14:00 – 15:00	• **Imaging Extracellular Vesicles: From Light Microscopy to Cryo-EM** (*V. Pospíchalová, Faculty of Science, MU, Brno*) • **New Immobilization Techniques** (*Š. Vrana, CEITEC MU, Brno*) • **Multimodal atomic force and optical microscopy reveal mechanical properties of multi-phase biomolecular condensates** (*J. Cibulka, Faculty of Science, MU, Brno*) • **Advanced Imaging of Lipid-Based Nanoparticles for Drug Delivery** (*M. Malásková, E. Maraková, CEITEC BUT, Brno*)
15:00 – 15:30	*Company presentation* – **Leica Microsystems Product Portfolio** (*V. Koláčková, Specion, s.r.o., Prague*)
15:30 – 16:00	Coffee break (E35, atrium)
16:00 – 16:30	*Company presentation* – **Smart experiments, fast analysis: TI2-E and NIS Elements powered by AI** (*J. Karas, Nikon ČR, Czech Republic*)
Section 2	**Fire talks** (E35, atrium) (5 min each)
16:30 – 17:30	• **Bacterial biofilm with piezoelectric properties** *(M. Khýrová, Faculty of Chemistry, Brno University of Technology, Brno)* • **Manufacturing of CAD/CAM crowns with and without a 3D printed model in terms of marginal fit** *(M. Al-Akkad, V. Prokupek, Faculty*

	of Medicine and Dentistry, Palacky University Olomouc, Olomouc) • **Vizualization of bacterial biofilm using AFM** *(B. Yöney, Faculty of Science, MU, Brno)* • **Measuring of SEM charging directly via AFM** (*J. Hruška, CEITEC MU, Brno*) • **Fitting correlated data in AFM force distance curves** *(A. Ch. Campbell, Primary Nanometrology and Technical Length, Czech Metrology Institute, Brno)* • **High-Resolution Hyperspectral Microscopy for Efficient Analysis of Biological Systems** (*O. Vaculík, VUT, Brno*) • **Molecular imprinting as a solution for environmental problems** (*E. Birgusová, Mendel University in Brno, Brno*)

Thursday, 11th September

8:30 – 9:00	Getting together, coffee (E35, atrium)
9:00 – 9:45	*Company presentation* – **Live Demonstration of Optical Photothermal Infrared (O-PTIR) - Multimodal submicron IR, Simultaneous Raman and Fluorescence microscopy** *(Miriam Unger, Photothermal Spectroscopy Corp., Santa Barbara CA, USA)*
Section 3	**Emerging Approaches for Biomolecular Analysis Using AFM/Raman microscopy** (15 min each) (E35, atrium)

mt měřicí technika morava
NenoVision
ZE telight Seeing beyond
Nikon
SpeciOn

9:45 – 10:15	• **Use of Localized Atomic Force Microscopy to improve imaging resolution** *(B. Papulová, CEITEC MU, Brno)* • **Antimicrobial peptides - Bilayers and AFM** *(R. Obořilová, CEITEC MU, Brno)*
10:15 – 10:45	Coffee break (E35, atrium)
10:45 – 11:15	*Company presentation* – **Telight LiveCodim – Universal super-resolution platform** *(M. Krijt, Telight s.r.o., Czech Republic)*
11:15 – 11:30	• **Alkaptonuria, homogentisate mapping by Raman microscopy** *(Barbora Lišková, Faculty of Science, MU, Brno)*
11:30 – 12:00	*Company presentation* – **Correlative AFM-in-SEM analysis** *(V. Hegrová, NenoVision s.r.o., Czech Republic)*
12:00– 12:15	• **3D electromechanical cell biosensors for drug and disease screening** *(M. Pešl, Faculty of Medicine, MU, Brno)*
12:15 – 13:30	Lunch (E35, atrium)
Section 4	**Afternoon practical session - Getting together** (E35, atrium)
13:30 – 17:30	• **Dimension FastScan (Bruker)** – Bilayer rupture test by AFM spectroscopy (E35, 2S014) • **Raman microscopy (Renishaw)** – Microplastic detection (E35, 2S014) • **JPK Nanowizard 4XP AFM** – CellWizard Stage – Smart cell imaging (E35, 2S012) • **JPK Nanowizard 3 AFM** – Correlative imaging (E35, 2S012) • **LiteScope AFM-in-SEM (NenoVision)** – Topography imaging (E35, 1S118)

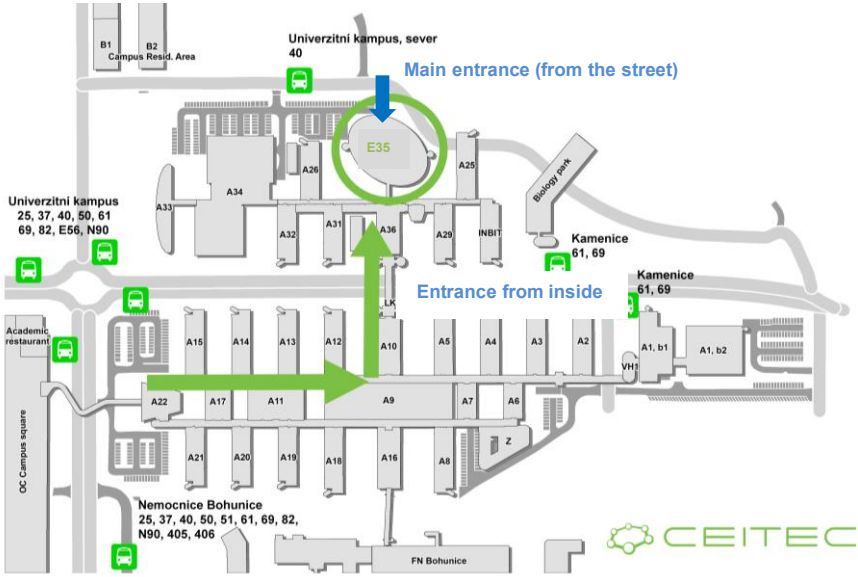
	• **Commercial demonstration – Zeiss, Nikon, Specion** (E35, atrium)
19:00 – 22:30	**Workshop dinner** (E35, atrium)

Friday, 12th September

9:00 – 9:30	Getting together, coffee (E35, atrium)
9:30 – 12:30	**Practical session** (E35, CF labs) • Bring your own sample • Hands-on practice
11:30 – 12:00	Coffee break (E35, atrium)
12:00 – 12:30	Closing remarks (E35, atrium)

How to reach us

The workshop venue is the CEITEC of Masaryk University (**CEITEC MU**), located at **Kamenice 5**, in building **E35** (original A35), on the university campus in **Brno-Bohunice**.



Content

Structure, mechanical properties, and chemical composition of biosamples (J. Přibyl)	**10**
From Ångström to Zoology – Measuring Every Detail (J. Horák)	**12**
Enhanced atomic force microscopy by optical imaging techniques (D. Hadraba)	**14**
Micro Meets Nano: Integration of Atomic Force Microscopy and Optical Techniques (A. Dulebo)	**16**
Controlled Release of Insoluble and Unstable Flavonoids from Magnetic Nanocarriers by External Magnetic Fields (S. Šegota)	**18**
Pioneering the Future of Microscopy: ZEISS Innovations in Light Microscopy (T. Cicerone)	**20**
Imaging Extracellular Vesicles: From Light Microscopy to Cryo-EM (V. Pospíchalová)	**22**
New Immobilization Techniques (Š. Vrana)	**24**
Multimodal atomic force and optical microscopy reveal mechanical properties of multi-phase biomolecular condensates (J. Cibulka)	**26**
Advanced Imaging of Lipid-Based Nanoparticles for Drug Delivery (M. Malásková, E. Maraková)	**28**
Leica Microsystems Product Portfolio (V. Koláčková)	**30**
Smart experiments, fast analysis: TI2-E and NIS Elements powered by AI (J. Karas)	**32**
Characterization of bacterial biofilm with piezoelectric properties (M. Khýrová)	**33**

Manufacturing of CAD/CAM crowns with and without a 3D printed model in terms of marginal fit (M. Al-Akkad, V. Prokupek)	**35**
Pathogen-on-a-Chip: Impedance-Based Detection of Biofilm Formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* (B. Yöney)	**38**
Measuring of SEM charging directly via AFM (J. Hruška)	**40**
Fitting correlated data in AFM force distance curves (A. Ch. Campbell)	**41**
Molecular imprinting as a solution for environmental problems (E. Birgusová)	**42**
High-Resolution Hyperspectral Microscopy for Efficient Analysis of Biological Systems (O. Vaculík)	**44**
Live Demonstration of Optical Photothermal Infrared (O-PTIR) - Multimodal submicron IR, Simultaneous Raman and Fluorescence microscopy (M. Unger)	**46**
Use of Localized Atomic Force Microscopy to improve imaging resolution (B. Papulová)	**48**
AFM Spectroscopy for the Study of Lipid Bilayer Stability and Morphology (R. Obořilová)	**50**
Telight LiveCodim – Universal super-resolution platform (M. Krijt)	**52**
Raman Fingerprinting of Metabolite Accumulation in Biological Samples (B. Lišková)	**54**
In-situ Correlative AFM-in-SEM Microscopy for Biological Nanostructures (V. Hegrova)	**55**
3D electromechanical cell biosensors for drug and disease screening (M. Pešl)	**57**

Structure, mechanical properties, and chemical composition of biosamples

Jan Přibyl[1,*], Radka Obořilová[1], Šimon Vrana[1], Jakub Máčala[1], Jakub Hruška[1]

[1] *Core Facility Nanobiotechnology CEITEC MU Masaryk University, Brno, Czech Republic*

*Corresponding Author E-mail: jan.pribyl@ceitec.muni.cz

Atomic Force Microscopy (AFM) enables high-resolution imaging, nanomechanical mapping, and single-molecule force spectroscopy under near-physiological conditions. Our facility specializes in AFM applications to diverse biological samples, enhanced by correlative techniques including Raman and fluorescence microscopy, polarization microscopy, and microelectrode arrays (MEA). This multimodal approach allows simultaneous acquisition of mechanical, structural, and chemical data, enhancing analysis of complex biological systems.

We apply these methods across a range of studies, including stiffness mapping of living cells, mechanobiology of cardiac and hepatic tissues, and chemical imaging of amyloid deposits in Alzheimer's disease. Key research highlights include elucidation of protein fibrillization, assessment of nanoparticle effects on cell functions, and investigation of liver fibrosis dynamics. This platform is also highly suitable for structural and mechanical studies of liposomes and extracellular vesicles.

By fostering interdisciplinary collaboration and offering open-access services to academic and industry users, we provide comprehensive workflows, training, and data processing support. Our integrated strategy exemplifies the power of AFM-based multimodal microscopy to probe the interplay between structure, mechanics, and chemistry at multiple biological scales. The Core Facility Nanobiotechnology at CEITEC Masaryk University plays a vital role in advancing bioscience research by providing access to state-of-the-art instrumentation and expert consultation, making it an excellent partner for the characterization of extracellular vesicles and complex biological samples.

The authors acknowledge funding from CIISB, Instruct-CZ Centre,

supported by MEYS CR grant numbers LM2023042 (CF Nanobiotechnology) and LUC24105. European Regional Development Fund-Project No. CZ.02.01.01/00/23_015/0008175. Masaryk University is acknowledged for its support by the interdisciplinary fund MUNI/G/1125/2022.

From Ångström to Zoology – Measuring Every Detail

Jakub Horák[1,*]

[1]Měřicí technika Morava s.r.o., Babická 619, 664 84 Zastávka, Czech Republic

*Corresponding Author E-mail: jakub.horak@mt-m.eu

Presentation reflects the comprehensive scope of Měřicí technika Morava's portfolio of advanced laboratory instruments. Modern research requires tools that can deliver both extreme precision at the atomic scale and versatile solutions for complex biological systems. This presentation provides a concise overview of our measurement technologies with a special focus on applications in the life sciences.

From high-resolution microscopy and spectroscopic tools capable of resolving structures from the ångström level to imaging and analytical systems supporting cell biology, molecular diagnostics, and ecological research, our solutions enable scientists to address questions across the entire spectrum of biology. By combining accuracy, reliability, and user-friendly operation, our instruments help transform data into meaningful insights, whether in molecular biology, medical research, material research or environmental monitoring.

With this presentation, we invite you to explore how cutting-edge measurement technology can empower discovery, accelerate workflows, and expand the frontiers of knowledge in biosciences.



Figure: *NanoWizard bioAFM with CellWizard stage for an automated, AI-guided, multi-chamber, in vitro imaging combined with nanomechanical analysis.*

Keywords: Atomic force microscopy; BioAFM, O-PTIR, Biomechanic

Enhanced atomic force microscopy by optical imaging techniques

Hadraba Daniel[1,*]

[1]*Bioimaging Facility - CZBI, Institute of Physiology, Czech Academy of Sciences, Videnska 1083, Prague, Czech Republic*

*Corresponding Author E-mail: daniel.hadraba@fgu.cas.cz

Atomic force microscopy (AFM) has long been recognized as a powerful tool for nanoscale surface characterization, providing unparalleled information on topography, mechanics, and local physical interactions. Yet, AFM alone cannot fully capture the diverse structural, functional, and dynamic properties of biological and material systems.

Combining AFM with light microscopy offers a pathway toward a more holistic multimodal imaging strategy. The quick overview will focus on spatial and temporal requirements and introduce the basic principle of microscopy techniques as well as the more advanced ones such as super-resolution microscopy, nonlinear microscopy methods including second- and third-harmonic generation (SHG, THG), and coherent anti-Stokes Raman scattering (CARS), which provide intrinsic contrast based on molecular vibrations and structural organization. Special emphasis will be placed on the complementarity of these approaches with AFM—highlighting how optical methods can probe temporal dynamics, molecular composition, and 3D context, while AFM provides nanoscale mechanical and topographical readouts.

Key themes will include:

- Strengths and limitations of light microscopy compared to AFM in terms of spatial and temporal resolution.
- How nonlinear and super-resolution techniques expand the accessible imaging scales beyond conventional fluorescence microscopy.
- Strategies to combine AFM with optical modalities for experiments that bridge mechanical, structural, and functional

data.

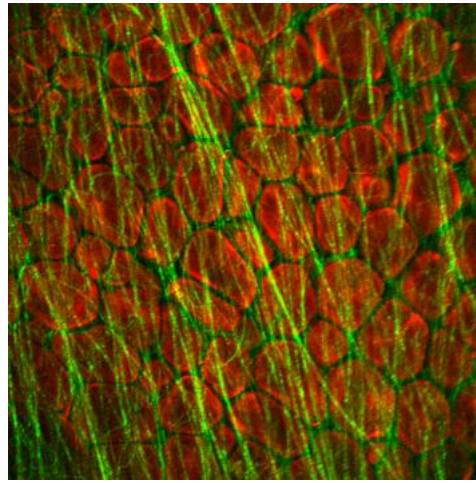


***Figure**: Label-free image acquisition visualizing the adipose tissue with elastin fibres. Courtesy of IPHYS BIF, Dr. David Vondrasek. The IPHYS BIF facility is supported by MEYS CR (Large RI Project LM2023050 Czech-BioImaging).*

Keywords: Optical microscopy, Light microscopy, Correlative microscopy

mt měřicí technika morava
NenoVision
ZEISS Seeing beyond
telight
Nikon
SpeciOn

Micro Meets Nano: Integration of Atomic Force Microscopy and Optical Techniques

Alexander Dulebo[1,*]

[1]JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

* Corresponding Author E-mail: alexander.dulebo@bruker.com

Over the past three decades, atomic force microscopy (AFM) has become a cornerstone in the characterization of biological and material samples, from single molecules to living cells and tissues. Its unique ability to correlate topography and mechanics under near-physiological conditions makes it indispensable for probing nanoscale structure–function relationships. When integrated with optical methods, AFM expands its scope, enabling correlative microscopy that leverages fluorescence labelling, confocal imaging, and super-resolution techniques. In particular, tip-scanning AFM platforms allow seamless, high-resolution correlation with optics, surpassing the limitations of sample-scanning systems.

Recent advances in AFM-based nanomechanical mapping modes – such as Quantitative Imaging (QI), PeakForce QNM, PeakForce QI, and the CellMech package – offer powerful tools to interrogate mechanical and structural properties with high sensitivity. These approaches have been applied to diverse systems, from single biomolecules to dense cell layers and tissues, enabling automated, large-area, multiparametric characterization. Correlative AFM – optical strategies are especially valuable for studying complex processes such as immune responses and tissue remodelling.

A significant step forward is the CellWizard Stage, a next-generation accessory for the Bruker BioAFM platform. Combining precision engineering with artificial intelligence (AI), it autonomously identifies features of interest, navigates multi-compartment environments, and performs reproducible, high-accuracy measurements. By coupling automated AFM scanning with advanced optical imaging, it delivers multiscale, multiparametric analysis in vitro, spanning from nanoscale interactions to mesoscale tissue structures. The system's machine-learning

capabilities support both pre-trained and user-trained models, making it adaptable for diverse applications.
By merging AFM with advanced optics and AI-driven automation, researchers gain an unprecedented ability to explore biological systems across scales. This integrated approach provides deeper insights, higher throughput, and enhanced reproducibility – marking a pivotal step toward intelligent, fully correlative microscopy.

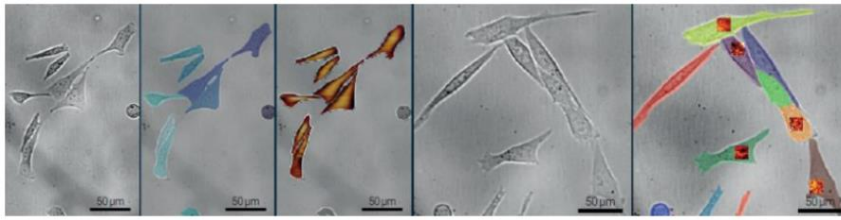


Figure: *AI-driven segmentation of optical phase contrast images of living 3T3 cells, combined with successive automated AFM scan region selection and measurement using SmartMapping (left three images) of the entire cells, and QI Advanced Young's modulus imaging of the cell nuclei (right image).*

Keywords: Atomic Force Microscopy (AFM); Correlative Microscopy; Artificial Intelligence (AI); Nanomechanical Mapping

Controlled Release of Insoluble and Unstable Flavonoids from Magnetic Nanocarriers by External Magnetic Fields

Suzana Šegota[1,*], Lucija Mandić[1]

[1] *Ruđer Bošković Institute, Division of Physical Chemistry, 10000 Zagreb, Croatia*

*Corresponding Authors E-mail: ssegota@irb.hr

Flavonoids have considerable therapeutic potential due to their anti-inflammatory, anticancer and antioxidant properties, which are attributable to their polyphenolic structure. However, limited bioavailability and absorption in the body, poor solubility in water and instability under physiological conditions reduce their efficacy as biologically active substances. This study showed the potential of magnetite nanocarriers functionalized with polyethylene glycol (PEG). Flavonoids, including quercetin, myricetin and myricitrin were encapsulated into the magnetite nanocarriers. The in vitro kinetics of flavonoid release from the magnetite nanocarriers was precisely controlled (see Figure) by application of both external permanent and oscillating magnetic fields. The results demonstrate the successful development of a highly efficient and versatile nanocarrier that can simultaneously encapsulate both hydrophobic and hydrophilic flavonoids. Furthermore, the controlled release of flavonoids from magnetite nanocarriers can be achieved by controlling the external oscillating and permanent magnetic field. This also opens up possibilities for advanced magnetically controlled drug delivery systems and represents a significant advance in nanotechnology-assisted therapy.

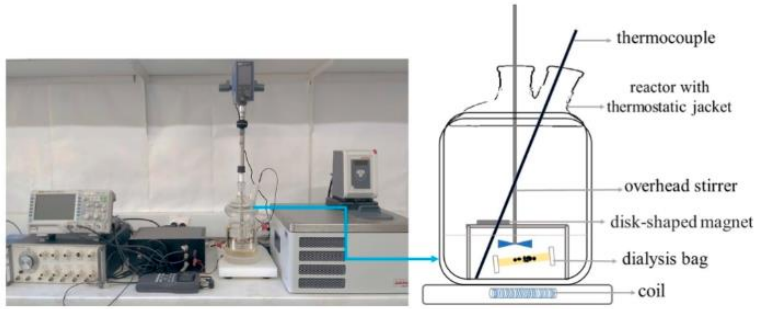


***Figure**: Experimental setup of the release kinetics and schematic illustration of a reactor.*

Keywords: Magnetite nanocarrier; Flavonoids; Drug Release; Magnetic Field

Pioneering the Future of Microscopy: ZEISS Innovations in Light Microscopy

Tudor Cicerone[1,*]

[1]*RMS, Carl Zeiss N.V.- S.A. Aile ouest, Ikaroslaan 49, 1930 Zaventem, Belgium*

*Corresponding Author E-mail: cicerone.tudor@zeiss.com

Microscopy is evolving at a remarkable pace, and ZEISS continues to lead by developing technologies that set new standards in light microscopy. This presentation will offer an overview of the ZEISS portfolio, with a focus on automation, lightsheet, confocal, and lattice SIM microscopy, and how these innovations are driving scientific discovery.

We will start with ZEISS automation solutions, designed to streamline workflows and increase productivity in the lab. By reducing manual steps and integrating seamlessly into existing infrastructures, these systems enhance precision and efficiency, enabling researchers to dedicate more time to data analysis and interpretation.

The second part will highlight ZEISS lightsheet microscopy. This revolutionary imaging method offers high-resolution, 3D views of biological specimens with minimal phototoxicity, making it particularly powerful for live-specimen and long-term imaging. We will explore unique system features and showcase examples where lightsheet microscopy has accelerated research.

We will then turn to ZEISS confocal microscopy, a cornerstone of modern imaging. Confocal systems provide precise optical sectioning and contrast for detailed analysis of complex samples, supporting applications from basic biology to clinical diagnostics. Key innovations, including advanced detectors and software tools, will be discussed in the context of how they enable deeper insight into cellular and molecular processes.

Finally, we will present ZEISS lattice structured illumination microscopy (SIM), which breaks through the resolution limits of conventional light microscopy. Combining speed, resolution, and ease of use, lattice SIM allows researchers to visualize subcellular structures with unprecedented clarity. Principles and impactful applications of lattice SIM will be illustrated.

Throughout the talk, we will emphasize how these complementary modalities are integrated within the ZEISS ecosystem to deliver comprehensive imaging solutions. Together, they represent ZEISS's ongoing commitment to expand the possibilities of light microscopy and empower researchers to explore new scientific frontiers.
Join us for a fast-paced overview of the ZEISS light microscopy portfolio and discover how these innovations are redefining microscopy for the future.

Keywords: Lightsheet; Confocal microscopy; Lattice SIM; Light microscopy

Imaging Extracellular Vesicles: From Light Microscopy to Cryo-EM

Vendula Pospíchalová[1,*], Stanislava Sladeček[1], Šárka Hrachovinová[1], Daniel Pinkas[2]

[1] *Department of Experimental Biology, Faculty of Science - Masaryk University, Kotlářská 2, 61137, Brno, Czech Republic*
[2] *Cryo-Electron Microscopy and Tomography Core Facility, Central European Institute of Technology - Masaryk University, Kamenice 5, 62500, Brno, Czech Republic*

*Corresponding Author E-mail: pospich@sci.muni.cz

Extracellular vesicles (EVs) are nanoscale, membrane-bound particles secreted by cells that mediate intercellular communication and hold great promise in diagnostics and therapeutics. Their small size and heterogeneity, however, make them challenging to study. A broad spectrum of imaging techniques provides complementary insights into EV biology. AFM enables label-free morphometry and nanomechanical profiling, while fluorescence microscopy—ranging from diffraction-limited to super-resolution methods—offers information on EV release, uptake, and molecular composition. Among electron microscopy approaches, cryo-EM uniquely preserves native morphology and allows clear visualization of the EV double lipid bilayer, enabling discrimination from single-membrane lipoproteins. Complementary methods such as nanoparticle tracking analysis and dynamic light scattering provide ensemble-level size and concentration estimates but require careful standardization. This talk will introduce EVs to the imaging community, outline the strengths and limitations of different methods, and highlight the unique role of cryo-EM in advancing EV research.

Keywords: Extracellular vesicles; Cryogenic electron microscopy; Atomic force microscopy; Nanoparticle tracking analysis

mtm měřicí technika morava
NenoVision
ZEISS Seeing beyond
telight
Nikon
SpeciOn

New Immobilization Techniques

Šimon Vrana[1,*], Radka Obořilová[1,2], Jakub Máčala[1], and Jan Přibyl[1]

[1]*CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

*Corresponding Author E-mail: simon.klimovic@ceitec.muni.cz

Atomic force microscopy (AFM) provides unique insights into the structure, mechanics, and interactions of biological samples at nanometer resolution. A critical prerequisite for reliable AFM measurements is the effective immobilization of macromolecules and cells on substrates or cantilevers, ensuring both stability and preservation of native properties. This presentation will first summarize conventional immobilization strategies, including physical adsorption, chemical cross-linking, and adhesive proteins coatings, highlighting their strengths and limitations. Building on this, the presentation will then introduce more new immobilization approaches designed to improve measurement reproducibility. These include the use of specialized cellular adhesives such as Cell-Tak, which enable robust yet gentle anchoring of non-adhesive cell types, as well as streptavidin-coated spherical cantilevers for specific and high-affinity attachment of biotinylated proteins. By comparing these methods, I will illustrate how tailored immobilization strategies expand the experimental toolkit of AFM, facilitating more accurate and biologically relevant characterization of macromolecules and living systems.

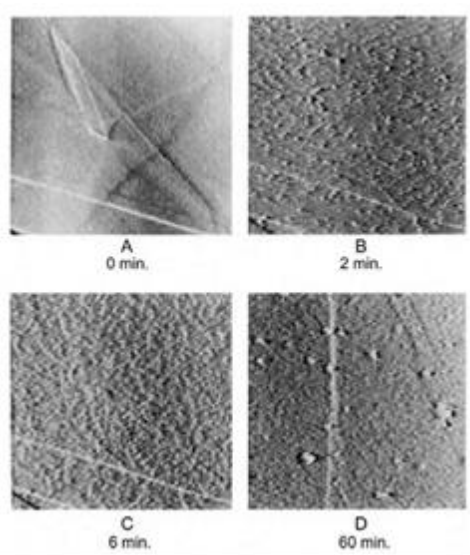


***Figure**: Spontaneous (non-specific) adsorption of proteins on HOPG surface.*

Keywords: Immobilization strategies, AFM samples, Imaging

Multimodal atomic force and optical microscopy reveal mechanical properties of multi-phase biomolecular condensates

Jakub Cibulka[1], Katerina Krejci[1], Simon Vrana[2], Jan Pribyl[2], Chiara Merigliano[3], Irene Chiolo[3], Lumir Krejci[1,*]

[1]*Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5/A7, Brno 62500, Czech Republic*
[2]*Central European Institute for Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*
[3]*Department of Biological Sciences, Molecular and Computational Biology Section, University of Southern California, Los Angeles, CA 90089, USA*

*Corresponding Author E-mail: lkrejci@chemi.muni.cz

Biomolecular condensates originate through phase separation of proteins and nucleic acids to reversibly organize cellular processes into defined compartments. Their function often relies on a complex architecture with multiple mutually non-miscible phases, each of them permissible for a distinct set of molecules and biochemical reactions. Recently, we have reported that double-stranded DNA breaks occurring within a condensed heterochromatin are protected from aberrant recombination by condensates of NUP98 protein, until they relocate to the nuclear periphery for safe repair. *In vitro*, NUP98 forms two-phase condensates with heterochromatin protein HP1 and we hypothesize that their mutual immiscibility creates capillary forces that drive the exclusion of NUP98 condensates to the surface of heterochromatin domain. Here we employed atomic force microscopy (AFM) combined with optical imaging to probe the mechanical properties of both NUP98 and HP1 phases. Our data shows that NUP98 condensates are significantly more rigid compared to the HP1, correlating with our fluorescence microscopy observations. Importantly, our experimental approach can be extended to relate the condensate mechanical properties to their miscibility, permeability for relevant factors and other characteristics.

Keywords: Atomic force microscopy; AFM; Phase separation; Biomolecular condensates

Advanced Imaging of Lipid-Based Nanoparticles for Drug Delivery

Magdalena Malaskova[1], Ester Marakova[1], Michaela Vojnikova[1,2], Šimon Vrana[3], Zbynek Heger[1,*]

[1]*Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic*
[2]*Central European Institute of Technology, Brno University of Technology, Brno, 612 00, Czech Republic*
[3]*Central European Institute of Technology, Masaryk University, Kamenice 5, CZ-625 00 Brno, Czech Republic*

*Corresponding Author E-mail: zbynek.heger@mendelu.cz

Atomic force microscopy (AFM) has emerged as a powerful tool for characterisation of lipid-based nanoparticles (LBNPs), offering unique insight into their morphology and surface properties. In this work, AFM was used for advanced physicochemical characterisation of three distinct classes of LBNPs, all of which are currently under development for therapeutic and theranostic purposes: a) gadolinium-containing theranostic liposomes (TNPs) and ellipticine-loaded theranostic liposomes (Elli@TNPs), b) solid lipid nanoparticles (SLNs), and c) bio-inspired LBNPs for delivery of functional cytochrome c as a pro-apoptotic protein cargo (CYT LNPs) with anionic lipid 1,2-dioleoyl-sn-glycero-3-phosphate DOPA (CYT EM LNPs) and with anionic lipid cardiolipin (CYT CL-LNPs). High-resolution AFM imaging enabled us to visualise the morphology of each nanoparticle type either in endosome-like buffer (pH 6) or in near-physiological conditions (pH 7.4). TNPs and Elli@TNPs displayed a uniform spherical morphology with diameters consistent with dynamic light scattering (DLS) data, while demonstrating fusion of nanoparticles at pH 6 in later time points of measurements. This is attributable to the incorporation of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), a pH sensitive lipid into the phospholipid bilayer. The phospholipid bilayer of nanoparticles demonstrated significant rigidity, which is related to the lipid composition, since they contain a significant amount of cholesterol (30 mol. %) and gadolinium-

containing lipid Gd.DOTA.DSA (33 mol. %). Moreover, Elli@TNPs exhibited higher rigidity compared to TNPs, which can be attributed to ellipticine encapsulated in a phospholipid bilayer.
AFM analysis of the standard SLN formulation (Compritol 888 ATO, Span 80, Pluronic F127) in PBS (pH 7.4) confirmed spherical morphology with uniform distribution. Young's modulus measurements demonstrated the robustness and durability of SLNs, with morphology and structural stability maintained over time.
For the CYT LNPs, AFM provided clear evidence of morphological changes under acidic, endosome-like conditions, where vesicles showed partial deformation and fusion events indicative of destabilisation, a prerequisite for efficient intracellular release of cytochrome c. Additionally, AFM-based force spectroscopy was used to probe the mechanical stability of the LNPs. These measurements highlighted significant differences in elasticity and rigidity between CYT EM-LNPs and CYT CL-LNPs, as well as apparent stabilisation of the CYT CL-LNPs after the interaction with cytochrome c.
Together, our results establish AFM as a necessary technique for the proper biomechanical understanding and morphological characterisation of LNPs in different environments. By employing AFM, we were able to gain new knowledge regarding morphology changes, mechanical properties, and environmental responsiveness, which enables us to provide a comprehensive study of the characteristics of LNPs in biomedical applications and predict behaviour *in vitro* and *in vivo*.

Keywords: Nanomedicine; Solid-lipid nanoparticles; Theranostics; Drug delivery

Leica Microsystems Product Portfolio

Veronika Koláčková[1,*]

[1]Specion, s.r.o., Květnového vítězství 332/31, 149 00, Praha 4, Czech Republic

*Corresponding Author E-mail: kolackova@specion.cz

Specion, s.r.o. is a Czech company specializing in the distribution and integration of advanced technologies in the fields of life science research, biochemistry, pharmaceutical research, industrial applications, and materials development. With a strong focus on high-quality instruments, professional consulting, and customer-oriented support, Specion helps research institutions, universities, hospitals, and industrial laboratories achieve precision and reliability in their scientific and diagnostic workflows. Specion is also a Gold Authorized Partner of Leica Microsystems, a global leader in the development and production of high-precision optical instruments. Leica Microsystems is renowned for its expertise in microscopy, imaging systems, and sample preparation technologies. The portfolio of Leica Microsystems includes light and stereo microscopes, digital imaging solutions, surgical microscopes, and electron microscopy sample preparation systems. With a strong focus on ergonomics, user-friendly design, and digital integration, these instruments not only deliver superior optical performance but also enhance productivity and comfort in everyday laboratory work. During our hands-on sessions you will have the unique opportunity to try out the Leica Mica automated microscope. This system combines widefield and confocal microscopy in one platform with full automation for faster, easier workflows. With FluoSync technology, it captures up to four fluorescence channels at once, while the built-in live-cell incubator supports long-term imaging under controlled conditions. Integrated AI-powered analysis further simplifies interpretation and improves reproducibility. Another microscope you will have the opportunity to try is the Leica Mateo. This system offers multimodal imaging with fluorescence, brightfield, and phase contrast in one device. AI-powered automation streamlines tasks like cell counting and confluency measurement, while dual built-in

cameras enable seamless capture without hardware changes. Its compact, user-friendly design saves space and ensures accessibility, and full lab-safety compatibility makes it ideal for BSL-1/2 environments.



***Figure**: Leica Mica is an AI-powered fluorescence imaging platform developed by Leica Microsystems. It is often described as a Microhub because it combines multiple imaging technologies and workflows into a single, compact system.*

Keywords: Specion, Leica Microsystems, Leica Mica, Leica Mateo

Smart experiments, fast analysis: TI2-E and NIS Elements powered by AI

Jakub Karas[1,*]

[1]Nikon ČR, Czech Republic

*Corresponding Author E-mail: Jakub.Karas@nikon.com

The ECLIPSE Ti2 inverted microscope delivers an unparalleled 25mm field of view (FOV) that revolutionizes the way you see. With this incredible FOV, the Ti2 maximizes the sensor area of large-format CMOS cameras without making compromises and significantly improves data throughput. The Ti2's exceptionally stable, drift-free platform is designed to meet the demands of super-resolution imaging while its unique hardware-triggering capabilities enhance even the most challenging, high-speed imaging applications. Furthermore, the Ti2's unique, intelligent functions guide users through imaging workflows by gathering data from internal sensors, eliminating the possibility of user errors. In addition, the status of each sensor is automatically recorded during acquisition, providing quality control for imaging experiments and enhancing data reproducibility.
In combination with Nikon's powerful acquisition and analysis software, NIS-Elements, the Ti2 is a total innovation in imaging.

Keywords: ECLIPSE Ti2, Super-resolution imaging, NIS-Elements

Characterization of bacterial film with piezoelectric properties

Markéta Khýrová[1,2*], Jakub Vejrosta[2,3], Mojmír Šerý[2], Tomáš Plichta[2] and Petr Sedláček[1]

[1]*Faculty of Chemistry, Brno University of Technology, Purkynova 118, Brno, 612 00, Czech Republic*
[2] *Institute of Scientific Instruments, Czech Academy of Sciences, Kralovopolska 147, Brno, 612 00, Czech Republic*
[3]*Faculty of Mechanical Engineering, Brno University of Technology, Technicka 2896, Brno, 616 69, Czech Republic*

*Corresponding Author E-mail: marketa.khyrova@vut.cz

The field of study dealing with piezoelectric biomaterials has evolved significantly during recent years, particularly regarding their use in the medicine. Therefore, there is a requirement for them to be non-toxic. Polyhydroxyalkanoates (PHA), particularly polyhydroxybutyrate (PHB), are promising biomaterials for regenerative medicine due to their biocompatibility, biodegradability and piezoelectric properties [1]. The main advantage of these materials is that they can be produced by many bacterial strains from different waste sources. In this study, we have focused on detailed characterization of bacterial film formed by *Cupriavidus necator* H16. This bacterial strain was selected due to the ability to produce up to 80% PHB of the dry cell weight. The aim was to optimize the precise cell orientation and the degree of PHB biocrystallization to enhance piezoelectric response of prepared material. For this purpose, microstructured surfaces were prepared using various photoresists. Moreover, standing surface acoustic wave (SSAW) was used for the precise cell orientation. Biocrystallization of PHB can be induced by stress factors including temperature or osmotic stress.

A comprehensive analysis of the prepared films was performed using multimodal imaging techniques. Confocal microscopy provided an overview of the material's organization and the distribution of the cellular mass. Atomic force microscopy (AFM) was used to measure the layer thickness, surface morphology and precise orientation of bacterial cells on

the microstructured substrates. In addition, the degree of biocrystallization was measured using Fourier transform infrared spectroscopy (FTIR). This combination of techniques enabled us to correlate microstructural orientation with the overall architecture and behaviour of the bacterial film.
These findings support the potential of PHB as a functional biomaterial for applications in tissue engineering and biosensing applications.

Keywords: Atomic force microscopy, Bacterial orientation, Confocal microscopy, Standing surface acoustic wave

[1] FUKADA, Eiichi a Yutaka ANDO. Piezoelectric properties of poly-β-hydroxybutyrate and copolymers of β-hydroxybutyrate and β-hydroxyvalerate. *International Journal of Biological Macromolecules*, **8**(6), 361-366, (1986)

Manufacturing of CAD/CAM crowns with and without a 3D printed model in terms of marginal fit

Marwan Al-Akkad[1], Bedirhan Savas Yigit[2], Iva Voborna[3], **Vaclav Prokupek**[4], Oluwafunmibi Fasanya[5], Radek Mounajjed[1*]

[1]*Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic. D.C.M clinic, Hradec Kralove, Czech Republic*
[2]*Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic*
[3]*Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic*
[4]*Department of Experimental Physics, Faculty of Science, Palacky University Olomouc, Olomouc, Czech Republic*
[5]*Department of Statistic, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, Nebraska, USA*

*Corresponding Author E-mail: Mounajjed@me.com

Marginal fit is a critical factor in the long-term success of dental crowns, directly affecting their mechanical stability and biological compatibility. A marginal misfit can lead to microleakage, bacterial infiltration, plaque retention, and ultimately, restoration failure. Precise measurement of marginal gaps is therefore essential—not only for evaluating the quality of crown fabrication techniques but also for guiding improvements in clinical protocols. Measuring the marginal fit requires a multidisciplinary approach, bringing together clinical dentistry and experimental biophysics to ensure both relevance and methodological rigor. The unpublished results from a research study conducted at Palacky University Olomouc, which compares two groups of dental crowns fabricated by Computer-Aided-Design/Computer-Aided-Manufacturing (CAD/CAM): one group subjected to marginal adaptation procedures on a printed 3D model, while the other group was subjected to adaptation without a model. Using high-resolution digital microscopy, we quantify differences in marginal fit and assess the impact of adaptation techniques on the precision of crown seating. The study employs a VHX-5000

microscope (Keyence, Osaka, Japan) equipped with a standard VH-Z20R zoom lens. The findings contribute to a better understanding of how crown design and processing affect clinical quality. The presentation is delivered by two researchers currently pursuing their PhDs—a dentist and a biophysicist/nanotechnologist—each contributing expertise from their respective fields. The dentist introduces the clinical rationale, explaining why marginal fit matters, what must be measured, and how these measurements relate to treatment outcomes. The biophysicist follows with a detailed discussion on how the measurements are performed. This part also addresses practical challenges and some tips and tricks to overcome them. By combining clinical insight with technical precision, this presentation demonstrates how a collaborative, cross-disciplinary approach can address complex questions in dental materials research and lead to more effective, evidence-based restorative practices. Acknowledgement: Palacky University supported this work with the grant IGA_LF_2024_009. Figure 1: High-resolution digital optical microscopy (VHX-5000; Keyence, Osaka, Japan) at the Department of Experimental Physics at the Faculty of Science at Palacký University Olomouc showing one of the measurements. Figure 2: Six micrographs of the same field with magnification x20, x30, x100, x150, x200, respectively, showing the marginal misfit at the mesio-palatal measurement point of one of the crowns.

mt.eu měřicí technika morava
NenoVision
ZE telight Seeing beyond
Nikon
SpeciOn

Keywords: CAD/CAM, 3D printing, Dentistry, Prosthodontics, Dental Marginal Adaptation, Dental Crowns, Digital microscope

Pathogen-on-a-Chip: Impedance-Based Detection of Biofilm Formation of *Staphylococcus aureus* and *Staphylococcus epidermidis*

Bengisu Yöney[1,*], Radka Obořilová[1,2], Karel Lacina[1,2], Petr Skládal[1]

[1]*Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

*Corresponding Author E-mail: bengisuyoney@mail.muni.cz

Bacterial biofilms are complex microbial communities that adhere to surfaces and contribute to the pathogenesis of chronic infections. Therefore, it is crucial to detect biofilm-associated infections as their treatment becomes more complicated. Herein, we describe a label-free electrochemical impedance spectroscopy (EIS) method for detecting biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Printed circuit board-based biamperometric gold electrodes were modified with poly-L-lysine to enhance bacterial attachment to the sensor surface. Formation and inhibition of formation of biofilms were evaluated based on changes in solution resistance (R_s) and charge transfer resistance (R_{ct}). The R_{ct} value increased from ~ 30 kΩ for the control group to ~ 120 kΩ for *S. epidermidis* biofilm and ~ 90 kΩ for *S. aureus* biofilm. Electrodes were stained with safranin and examined using optical microscopy. Biofilm-forming electrodes absorbed the dye, whereas control samples did not show staining. Atomic force microscopy (AFM) was employed to visualize the biofilm on the electrode surface and to assess surface roughness and cross-sectional topography. The roughness parameters indicate that *S. aureus* forms a rougher biofilm than *S. epidermidis*, while *S. epidermidis* produces more compact biofilm and a higher amount of extracellular polymeric matrix. These findings suggest that the optimized EIS-based method effectively monitors changes related to biofilms and serves as a promising tool for evaluation of new anti-biofilm agents, such as antibiotics, phages or antibodies.

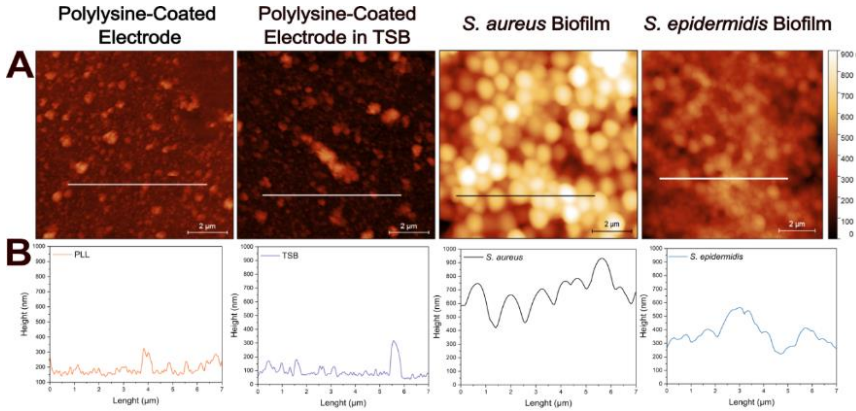


Figure: *A) Height sensor images of: PLL-coated bare electrode, PLL-coated electrode incubated in TSB, PLL-coated electrode with S. aureus biofilm, and PLL-coated electrode with S. epidermidis biofilm. (B) Representative height profiles corresponding to each condition along the marked 7 µm lines. Scale bar: 2 µm.*

Keywords: Microbial biofilm; Electrochemical impedance spectroscopy; Atomic force microscopy; Disposable gold PCB electrode

Measuring of SEM charging directly via AFM

Jakub Hruška[1,2*], Jan Přibyl[1] and Jiří Nováček[1,2]

[1]*Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

*Corresponding Author E-mail: **Jakub.Hruska@ceitec.muni.cz**

Charging is a persistent challenge in scanning electron microscopy (SEM), often degrading image quality and limiting resolution. While its presence is easily recognised in the SEM image. To mitigate the effect, one must be able to measure/characterise it. Measuring charging at the nanoscale is critical for the development and validation of new resins or coatings with enhanced charge-dissipation properties. Here, we propose the application of atomic force microscopy (AFM) in electrostatic force microscopy (EFM) mode for semiquantitative analysis of surface charge accumulation. This approach enables direct correlation between charge distribution and SEM imaging artefacts, offering valuable insights for the optimisation of material design and sample preparation strategies in electron microscopy.

Keywords: Charge measurement, EFM, SEM

Fitting correlated data in AFM force distance curves

Anna Charvátová Campbell[1,*], Radek Šlesinger[1]

[1]*Department of Primary Nanometrology and Technical Length, Czech Metrology Institute, Okružní 31, 638 00 Brno, Czech Republic*

*Corresponding Author E-mail: acharvatovacampbell@cmi.gov.cz

Data fitting is essential in modern metrology, but ordinary least squares regression struggles with complex models, high uncertainty in independent variables, and correlations between data (e.g., from reference samples or sensor crosstalk). Generalized least squares can address these issues. We present the Optimum Estimate of Function Parameters by Iterated Linearization (OEFPIL) algorithm, which accommodates diverse functions and general covariance matrices. Its use is demonstrated on AFM force–distance curves to extract mechanical properties such as Young's modulus and adhesion. OEFPIL provides uncertainties consistent with Monte Carlo simulations, but more efficiently.

Keywords: AFM, Force distance curve, Data fitting

Molecular imprinting as a solution for environmental problems

Eliška Birgusová[1,*], Milada Vodová[1], Lukáš Richtera[1], Peter A. Lieberzeit[2], Jan Přibyl[3]

[1]*Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1665/1, 613 00 Brno, Czech Republic*
[2]*University of Vienna, Faculty for Chemistry, Department of Analytical Chemistry, Waehringer Strasse 38, A-1090 Vienna, Austria*
[3]*CEITEC, Masaryk University, Brno, Czech Republic*

*Corresponding Author E-mail: eliska.birgusova@mendelu.cz

Molecular imprinting has emerged as a powerful and versatile technique to create synthetic recognition elements with high selectivity and stability. Traditionally, molecularly imprinted polymers (MIPs) have been designed for small molecules such as hormones, pesticides, and pharmaceutical residues, offering a low-cost and robust alternative to natural receptors. Their capacity to mimic biological recognition processes while maintaining superior physical and chemical stability makes them highly attractive for environmental monitoring. However, environmental challenges today extend beyond dissolved organic pollutants. The increasing prevalence of larger and more complex contaminants—including engineered nanomaterials, nanoplastics, microplastics, and even microorganisms—demands innovative approaches that expand the scope of imprinting technology.

By bridging molecular imprinting with electrochemical technologies, this approach offers not only highly selective detection but also the potential for simultaneous remediation. Imprinted materials can serve as sorbents for pollutant removal, followed by regeneration and reuse, thereby extending their lifecycle and reducing overall environmental burden. This dual functionality—detection and mitigation—highlights molecular imprinting as more than an analytical tool; it positions it as a sustainable strategy for environmental management.

Coupled with electrochemical sensors, imprinted systems can deliver sensitive, selective, and reusable tools that meet the growing demand for

real-time, on-site, and sustainable environmental monitoring. This lecture will provide an overview of recent advances, demonstrate examples of innovative applications, and outline future directions in which molecular imprinting can contribute to solving urgent environmental problems.

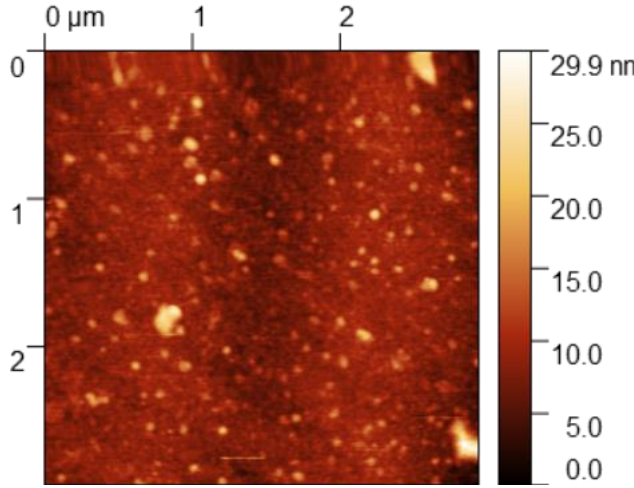


Figure 1. Nanoparticles of AgO on the surface of the sensor with a polymer layer after water application.

Keywords: Molecular imprinting, AFM, Environmental chemistry, Nanoplastics, Nanomaterials, Sensor

High-Resolution Hyperspectral Microscopy for Efficient Analysis of Biological Systems

Ondřej Vaculík[1,2*], Mojmír Šerý[2]

[1] *Institute of Physical Engineering, Faculty of Mechanical Engineering, Brno University of Technology, Czech Republic*
[2] *Department of Microphotonics, Institute of Scientific Instruments of the Czech Academy of Science, Czech Republic*

*Corresponding Author E-mail: Ondrej.Vaculik1@vut.cz

Hyperspectral (HS) imaging, a non-destructive spatial-spectral analysis technique based on imaging spectroscopy, has become increasingly popular in various fields in recent years. HS images provide detailed spectral responses for each pixel of the image and, therefore, are rich in information related to the structure and composition of the observed object. The effective use of HS data requires an understanding of the origins and limitations of the HS measurement system and specialised processing approaches to interpret the collected data.

A custom HS microscope workstation was developed in collaboration between the Institute of Scientific Instruments and PSI (Photon Systems Instruments) spol. s r.o. as part of my PhD studies. This HS microscope platform is equipped with a high-resolution HS camera delivering sub-nanometre spectral resolution with 150 nm spectral bandwidth tunable from 450 to 900 nm. The HS microscope setup is highly modular and is equipped with positioners for automated measurement. My work also incorporates HS data analysis, showcasing the potential of HS microscopy for biological applications in algae research. A model organism of different marine cyanobacteria strains was measured using our HS microscope platform, and the HS data were processed with the goal of spectral unmixing and mixing ratio estimation based on similar spectral responses. To aid in the processing of the large HS dataset, machine learning and chemometric techniques were used to reduce the dimensionality of the HS data. The high-resolution spectral imaging capabilities of this system have the potential to enhance our understanding

of biological systems, enable new discoveries, and allow for more precise material identification or automated labelling of microorganisms.

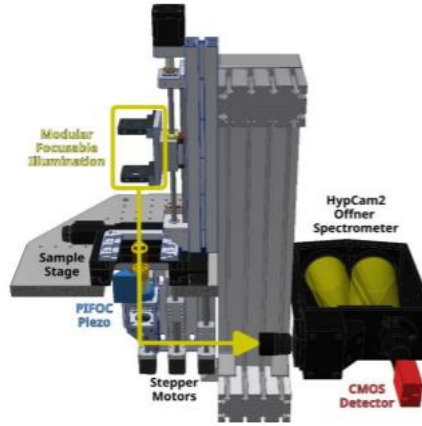


Figure: *Cross section of the CAD model of HS microscope workstation, with key component description and light path denoted by yellow arrow.*

Keywords: Hyperspectral imaging, Machine learning, Spectral unmixing, Dimensionality reduction

Live Demonstration of Optical Photothermal Infrared (O-PTIR)
–
Multimodal submicron IR, Simultaneous Raman and Fluorescence microscopy

Miriam Unger[1,*]

[1]*Photothermal Spectroscopy Corp., Santa Barbara CA USA*

*Corresponding Author E-mail: miriam@photothermal.com

Using Optical Photothermal IR (O-PTIR) spectroscopy, the accepted limitations of traditional IR microscopy are broken, providing for true submicron spatial resolution operating in contact-less reflection mode (no ATR), whilst providing for FTIR transmission/ATR-like data quality. Furthermore, it bridges the gap between conventional IR microspectroscopy and nanoscale IR spectroscopy. Now, in a world first, it also provides **IR+Raman** – simultaneous IR and Raman measurements at the same time, from the same spot and the same resolution.
In recent developments, a new modality, "counter-propagating" has been engineered to provide for enhanced IR spatial resolution and sensitivity, with the use of high NA refractive objectives. This improves spatial resolution to ~300nm for both IR and Raman, whilst also improving sensitivity, image quality and facilitating immersion objective studies in fluid. Further, we demonstrate widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy with very recent advances now using the fluorescence signal as means to deliver widefield IR imaging that also enhances the IR sensitivity, in a mode called Fluorescence Enhanced O-PTIR.
During this live demo, the multimodal capabilities of O-PTIR will be highlighted. Measurements of different samples will be demonstrated.



Figure: mIRage-LS system demonstrated here.

Keywords: Optical Photothermal Infrared; Submicron resolution; Simultaneous IR+Raman

Use of Localized Atomic Force Microscopy to improve imaging resolution

Berta Papulová[1,*], Jan Přibyl[1]

[1]*Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

Corresponding Author E-mail: 533700@mail.muni.cz

Atomic Force Microscopy (AFM) is a type of scanning probe microscopy that obtains information about the structure and mechanical properties of a sample through interactions between the nanoscale probe tip and the surface of a biomolecule. An advantage of AFM is its ability to measure unlabeled molecules under physiological conditions. However, the resolution and accuracy of AFM topography images are influenced by factors such as molecular motion in liquid environments, tip geometry and other imaging artifacts.

Localization Atomic Force Microscopy (LAFM) enhances the resolution of surface structures beyond conventional AFM capabilities when localization algorithms are applied to analyze the spatial fluctuations of topographic features in AFM images. Development of this method was inspired by stochastic optical reconstruction microscopy and photoactivated localization microscopy, achieve super-resolution imaging by accurately identifying and isolating fluorescence signals over multiple frames.

In the presentation, the application of LAFM to biomolecules of fibrillar structure, such as DNA and Tau protein fibrils, measured in a liquid environment will be demonstrated. The image processing was performed using open-source software.

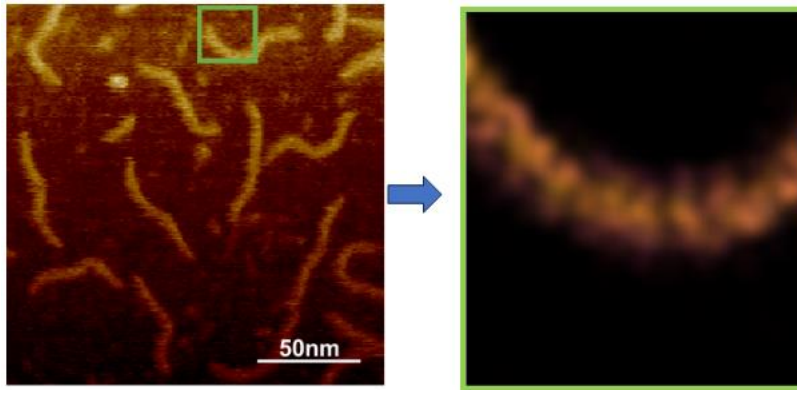


Figure: From raw AFM image of DNA to LAFM map with better resolution

Keywords: Localization Atomic Force Microscopy; Image processing; Tau protein; DNA

AFM Spectroscopy for the Study of Lipid Bilayer Stability and Morphology

Radka Obořilová[1,2,*], Rahul Deb[1], Robert Vácha[1], Jan Přibyl[1]

[1]*CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

*Corresponding Author E-mail: Radka.oborilova@ceitec.muni.cz

Atomic Force Microscopy (AFM) enables the visualization of nano-objects, biomolecules, and cells while simultaneously probing their mechanical properties. Force-distance curves derived from indentation experiments provide insights into the elastic and viscoelastic properties of macromolecules and living cells. For example, supported phospholipid bilayers can be used as mimics of cell membranes. And simulate cellular events *in vitro*. Moreover, puncturing the bilayer by AFM tip with a low bilayer radius creates a characteristic peak in the force-distance curve. Location of this break was called a "rupture event" and provides valuable information about the phospholipid membrane, such as its thickness, fluidity, or composition, etc. AFM can also capture protein-membrane interactions and the effects of specific agents on lipid bilayers or cell membranes, demonstrated here on pore-forming peptides with antimicrobial and anticancer potential.

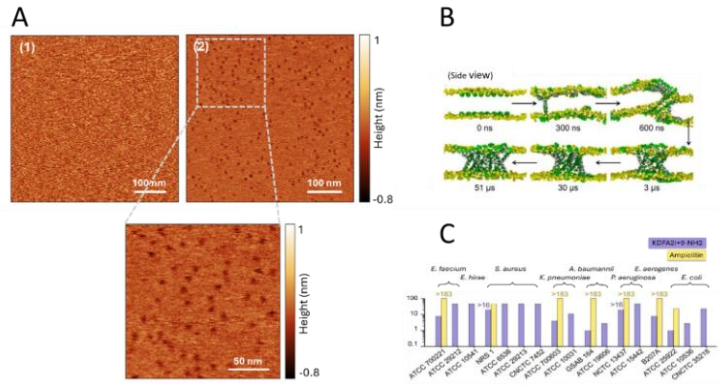


Figure: (A) AFM topographic images of the SLBs composed of POPC:POPG (1:1) lipids, before (1) and after 1 hour incubation with 1 µM KDFA2i+9-NH2 peptides. (B) Snapshots from CG simulations showing spontaneous formation of transmembrane barrel pores (TBPs) by RDFA2 peptides in POPC:POPG membrane (shown with only lipid phosphates for clarity). (C) Antimicrobial activity of the peptide KDFA2i+9-NH2 and the antibiotic ampicillin against ESCAPE.

[1] Deb, R.; Kabelka, I.; Pribyl, J.; Vacha, R. Biophys. J., 122 (3) (2023) 155a.
[2] Sadžak, A.; Mravljak, J.; Maltar-Strmečki, N.; Arsov, Z.; Baranović, G.; Erceg, I.; Kriechbaum, M.; Strasser, V.; Přibyl, J.; Šegota, S., Antioxidants, 9 (5) (2020) 430.

Keywords: AFM; Lipid bilayers; Membrane mechanics; Force-distance curve

The authors acknowledge funding from CIISB, Instruct-CZ Centre, supported by MEYS CR grant numbers LM2023042 (CF Nanobiotechnology) and LUC24105. European Regional Development Fund-Project No. CZ.02.01.01/00/23_015/0008175. Masaryk University is acknowledged for its support by the interdisciplinary fund MUNI/G/1125/2022.

Telight LiveCodim – Universal super-resolution platform

Matyas Krijt[1,*], Barbora Buryskova[2], Caroline Perone Cane[3]

[1] *Sales Department, Telight s.r.o., Skrobarenska 485/14, 617 00 Brno, Czech Republic*
[2] *Application Department, Telight s.r.o., Skrobarenska 485/14, 617 00 Brno, Czech Republic*
[3] *Application Department, 174 Quai de Jemmapes 75010 Paris, France*

*Corresponding Author E-mail: *matyas.krijt@telight.eu*

LiveCodim by Telight is a universal super-resolution imaging platform that seamlessly integrates into any inverted microscope and empowers multimodal imaging by combining widefield, confocal, and super-resolution modalities within a single system. Based on conical diffraction technology, LiveCodim achieves lateral resolution down to ~90 nm while maintaining exceptionally low phototoxicity and negligible bleaching, making it highly suitable also for live-cell studies. The system supports up to 4 fluorescence channels for multicolor imaging, enables Z-stack acquisition, and operates with user-friendly software interface enabeling autonomous processing. Its versatility allows researchers to investigate a broad spectrum of applications without compromising on sample viability. By offering multimodal performance in a retrofit design, LiveCodim extends the capabilities of standard microscopy platforms, providing an accessible and flexible solutions from conventional to super-resolution microscopy.



Figure: *Overview of multimodal patented devices produced by TELIGHT – 3-in-1 super-resolution imaging platform LiveCodim and holographic quantitative microscope Q-Phase.*

Keywords: Super-resolution module; Confocal; Conical Diffraction Microscopy; Phototoxicity

Raman Fingerprinting of Metabolite Accumulation in Biological Samples

Barbora Lišková, Andrea Benčeková, Petra Lišková, Martina Vágnerová, Petra Čermáková and Peter Fabian

*Corresponding Author E-mail: *fabian@sci.muni.cz*

Alkaptonuria (AKU) is a rare metabolic disorder in which homogentisic acid (HGA) accumulates in body fluids and connective tissues, leading to progressive tissue darkening. We established a zebrafish model that reproduces hallmark human AKU symptoms, including visible bone pigmentation. To identify the chemical nature of this pigment, we applied Raman microscopy (RM), a label-free technique that generates molecular fingerprints through characteristic light scattering. RM spectra from AKU zebrafish bones and paraffin sections matched the HGA reference, which confirms metabolite accumulation and aligns with our analyses of human AKU samples. In preliminary experiments, we also used RM to track how standard drug treatment reduces HGA levels in our zebrafish model, suggesting that this approach could be extended to monitor therapy in patients. Finally, by applying RM to paraffin sections, we resolved local metabolic signatures in specific fibroblast subpopulations. Together, these results demonstrate how RM directly links metabolism to pathology and therapy in rare disease models.

In-situ Correlative AFM-in-SEM Microscopy for Biological Nanostructures

Veronika Hegrova[1,*] Linnea Gustaffson[2], Zuza David[3], Radek Dao[1], Jan Neuman[1]

[1]NenoVision s.r.o, Purkyňova 127, 612 00 Brno-Medlánky, Czech Republic
[2]Division of Micro and Nanosystems, KTH Royal Institute of Technology, Malvinas väg 10, Stockholm, 114 28 Sweden
[3]Department of Chemical Engineering, UCT Prague, Technicka 5, 166 28 Prague 6, Czech Republic, Technicka 5, 166 28 Prague 6, Czech Republic.

*Corresponding Author E-mail: veronika.hegrova@nenovision.com

The study of biological materials and soft-matter systems requires methods that provide nanoscale structural and functional insights under in-situ conditions. Key challenges include the ability to localize ultra-fine features, probe mechanical and surface properties, and correlate multiple signals at the same site without damaging the sample. To address these challenges, novel correlative approaches and workflows are being developed.

One such approach is AFM-in-SEM, where Atomic Force Microscopy (AFM) is integrated inside a Scanning Electron Microscope (SEM) using the LiteScope system. This solution enables non-destructive mapping of nanoscale topography, mechanical, and surface properties directly inside the SEM. It combines the structural contrast of SEM with the high-resolution metrology of AFM, while providing quantitative 3D information and avoiding contamination or environmental changes. A unique workflow of multidimensional correlative imaging (CPEM) further allows simultaneous characterization of topography, electrical and mechanical responses, and chemical or structural features, all at the same location.

This capability was applied across diverse biological and pharmaceutical systems. In spider silk nanowires, targeted navigation enabled by the electron beam guided AFM probes for site-specific bending experiments

and quantitative analysis of elastic and plastic behavior. In mesoporous pharmaceutical carriers, correlative imaging revealed increased surface roughness and localized recrystallization in API-loaded particles, demonstrating the role of drug binding both inside pores and on particle surfaces. In polymer systems, AFM phase imaging combined with mechanical spectroscopy identified distinct material phases, quantified local stiffness, and highlighted changes induced by electron beam irradiation, uncovering mechanical heterogeneity.
In summary, AFM-in-SEM with LiteScope provides a powerful and versatile platform for nanoscale characterization in life sciences. By integrating targeted navigation, spectroscopy, and multidimensional imaging, it enables a deeper understanding of the relationship between structure and function, supporting advances in biomedical and pharmaceutical research.

Keywords: AFM-in-SEM, Correlative microscopy, Nanostructures, In-situ characterization

3D electromechanical cell biosensors for drug and disease screening

Martin Pešl [1, 2, 3], Šimon Vrana[4], Deborah Beckerová[1,2], Markéta Bebarová[5], Vladimír Rotrekl[1,2], Jan Přibyl[1, 4]

[1] *Department of Biology, Faculty of Medicine, Masaryk University, Brno, Cz*
[2] *International Clinical Research Center, St. Anne's University Hospital, Brno, Cz*
[3] *First Department of Internal Medicine – Cardioangiology, Faculty of Medicine, Masaryk University*
St. Anne's University Hospital, Brno, Czech Republic.
[4] *Nanobiotechnology, CEITEC MU, Masaryk University, Brno, Cz*
[5] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.*

*Corresponding Author E-mail: pesl@fnusa.cz

Human cardiomyocytes derived from pluripotent stem cell lines in 3D organoids provide a non-invasive and long-term "organ-on-a-chip" platform for functional analysis. By integrating microelectrode array electrophysiology, atomic force microscopy–based contractility, and calcium imaging, this approach links electrical activity with mechanical force in real time. The system enables testing of novel and established drugs, including combinations, and has revealed, for instance, the pro-arrhythmic potential of pulmonary bronchodilators. Applications extend to both wild-type cells and disease-specific lines, such as Duchenne muscular dystrophy or catecholaminergic polymorphic ventricular tachycardia. We present this methodology together with its clinical relevance and introduce a new model for translational research.

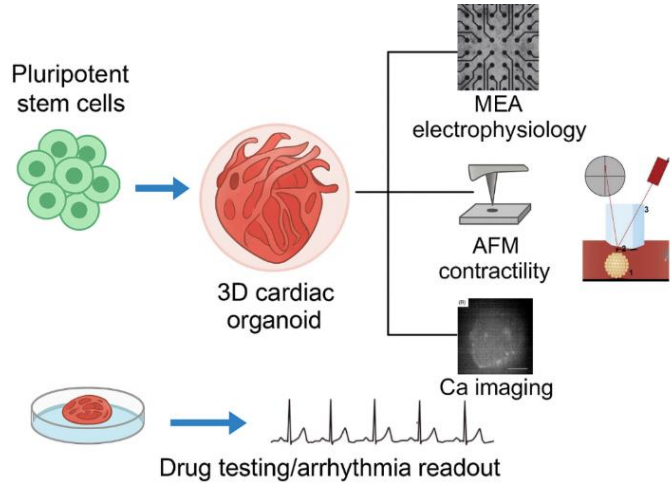


***Figure**: Atomic Force Microscope (AFM) can distinguish arrythmia in 3D cardiac organoid, Microelectrode Arry (MEA) and Calcium (Ca) imaging can facilitate the readout*

Keywords: Atomic force microscopy; Stem Cell Derived Cardiomyocytes; Drug cardiotoxicity screening, Arrhythmia.

The research was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, No. LX22NPO5104) - Funded by the European Union – Next Generation) and by the European Union's Horizon European Innovation Council Transition Open No. 101214165 (CResPulse).

Acknowledgment

Many thanks to the staff of the PR department and Event department, especially to Eliška Janíčková, Tereza Kučerová and Kristýna Šárková. Thanks also go to MTM (Měřicí technika Morava) for their help in organizing the workshop.

Grants support:
The authors acknowledge funding from CIISB, Instruct-CZ Centre, supported by MEYS CR grant numbers LM2023042 (CF Nanobiotechnology) and LUC24105. European Regional Development Fund-Project No. CZ.02.01.01/00/23_015/0008175. Masaryk University is acknowledged for its support by the interdisciplinary fund MUNI/G/1125/2022.

Title: Multimodal Microscopy Workshop 2025

Editors: Jan Přibyl, Radka Obořilová, Šimon Vrana, Jakub Máčala, Tereza Lichnovská, Jakub Hruška

Issued by: Masarykova univerzita, Žerotínovo nám. 617/9, 601 77 Brno

Edition: 1st Edition, 2025

Number of pages: 63

ISBN 978-80-280-0759-1