Multimodal Microscopy Workshop: Probing the Triad of Structure, Mechanics, and Chemistry in Biological Systems 2024

10th – 12th of June 2024
Brno, Czech Republic

Book of Abstracts

MASARYK UNIVERSITY

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Welcome address

Dear Colleagues and Participants,

We are pleased to welcome you to the annually organized workshop focused on atomic force microscopy combined with other microscopies.

Our program is designed to cover a broad spectrum of topics, including cutting-edge developments in structural analysis, mechanical characterization, and chemical mapping of biological specimens. Through a series of engaging presentations, hands-on sessions, and interactive discussions, we aim to foster a collaborative environment where ideas can be exchanged, and innovative solutions can emerge.

We hope you find the event informative and inspiring.
Welcome, and enjoy the workshop!

On behalf of the organization team,

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

Programme

Monday, June 10th

8:30 – 9:00	Registration, coffee (E35, atrium)
9:00 – 9:15	**Workshop opening**, (E35, Atrium)
9:15 – 9:40	**Company presentation,** J. Horák, MTM (E35, Atrium**)**
9:40 – 10:10	**AFM introduction and probe selection guide,** L. Pařízek, MTM (E35, Atrium)
10:10 – 10:30	Coffee break (E35, Atrium)
10:30 – 11:30	**Invited talk: Biomechanics and biorheology – their relation to a cell diseased state**, Małgorzata Lekka, Department of Biophysical Microstructures, Polish Academy of Sciences, Krakow, Poland (E35, Atrium)
11:30 – 12:30	Lunch (E35, atrium)
12:30 – 12:50	**Mechanics by AFM**, A. Dulebo, Bruker (E35, Atrium)
12:50 – 13:10	**AFM with other techniques**, A. Dulebo, Bruker (E35, Atrium)
13:10 – 13:30	**High-speed AFM**, A. Dulebo, Bruker (E35, Atrium)
13:30 – 14:00	**AFM data processing**, Š. Klimovič, CEITEC MU (E35, Atrium)
14:00 – 14:30	Coffee break CEITEC MU (E35, Atrium)
14:30 – 15:00	**Core Facility Nanobiotechnology**, J. Přibyl, CEITEC MU (E35, Atrium)
15:00 – 17:00	**Sample / User stories** (E35, Atrium) **Microphysiological Analysis of Cardiomyocyte Function in 3D Organoids,** Martin Pešl, Masaryk University and ICRC-FNUSA, Brno, Czech Republic.

	Correlative analysis of biomechanical properties and morphology on connective tissue and significance for fascia, Daniel Hadraba, Institute of Physiology, CAS, Prague, Czech Republic. **Detection of oncomarkers by AFM,** Michaela Domšicová, CBs SAS, Bratislava, Slovakia. **Characterization of surface and mechanical properties of microbial cells,** Markéta Khýrová, Faculty of Chemistry, Brno University of Technology and Institute of Scientific Instruments, Brno, Czech Republic. **Investigation of Mechanisms Underlying Stiffness Change in Prostate Cancer Cells Using AFM Computational Simulations,** Lucie Jadrna, Brno University of Technology, Brno, Czech Republic. **Characterization of Bacillus cereus extracellular vesicles reveals insights into their versatile landscape and plasticity,** Astrid Laimer-Digruber, University of Veterinary Medicine, Vienna, Austria

Tuesday, June 11th

8:30 – 9:00	Getting together, coffee (E35, Atrium)
9:00 – 9:30	**Specific approaches in microscopy samples preparation**, J. Přibyl, CEITEC MU (E35, Atrium)
9:30 – 10:30	**How to prepare biological samples for AFM measurements**, Małgorzata Lekka, Department of

	Biophysical Microstructures, Polish Academy of Sciences, Krakow, Poland (E35, Atrium)
10:30 – 10:45	Coffee break (E35, Atrium)
10:45 – 11:00	**Cytotoxicity of nanoparticles released from titanium implants,** Jakub Hruška, CEITEC MU and Faculty of Medicine, Masaryk University, Brno, Czech Republic (E35, Atrium)
11:00 – 11:30	**Radka Martínková**, Delong Instruments and Brno region Microscopy Community (E35, Atrium)
11:30 – 12:00	**Quick demonstration of instruments**
12:00 – 13:00	Lunch (E35, atrium)
13:00 – 17:30	**Afternoon practical session** (E35, CF labs) • **Bruker TS77 and Biosoft** – Mechanical testing of hard biological samples (E35, 2S015) • **Bruker JPK NanoRacer HS-AFM** – Observing molecular dynamics using AFM (E35, 2S012) • **Bruker Fastscan** – Membrane testing (E35, 2S014) • **JPK Nanowizard 3 + Olympus FluoView 1200** - Combination of AFM with confocal fluorescence – application on cells (E35, 2S012) • **Renishaw InVia Raman** microscope – combination of optical and Raman microscopy – study of microplastic contamination (E35, 2S014)
19:00 – 22:30	**Workshop dinner**

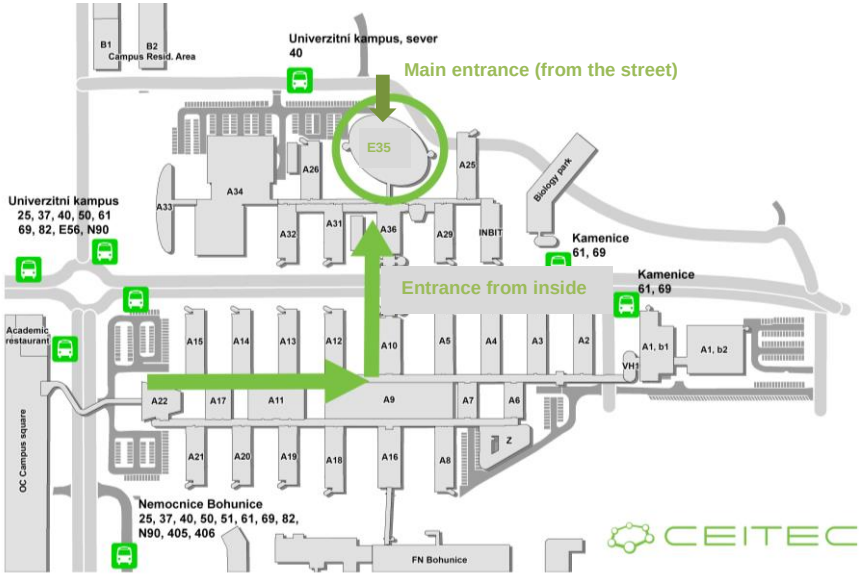
Wednesday, September 12th

8:30 – 9:00	Getting together, coffee (E35, atrium)
9:00 – 12:00	**Practical session** (E35, CF labs) • Bring your own sample • Hands-on practice
12:00 – 13:00	Lunch (E35, atrium)

13:00 – 13:30	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** (formaly A35), on the university campus in **Brno-Bohunice**.



Abstracts of lectures

AFM introduction and probe selection guide (L. Pařízek)

Invited talk: Biomechanics and biorheology – their relation to a cell diseased state (M. Lekka)

Atomic Force Microscopy: mechanics, high-speed, and integration with other techniques (A. Dulebo)

AFM with other techniques (A. Dulebo)

AFM probes (A. Dulebo)

AFM data processing (Š. Klimovič)

Core Facility Nanobiotechnology (J. Přibyl)

Microphysiological Analysis of Cardiomyocyte Function in 3D Organoids (M. Pešl)

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Detection of oncomarkers by AFM (M. Domšicová)

Characterization of surface and mechanical properties of microbial cells (M. Khýrová)

Investigation of Mechanisms Underlying Stiffness Change in Prostate Cancer Cells Using AFM Computational Simulations (L. Jadrna)

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How to prepare biological samples for AFM measurements (M. Lekka)

Cytotoxicity of nanoparticles released from titanium implants (J. Hruška)

AFM introduction and probe selection guide

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Embarking on an enlightening journey through the realm of Atomic Force Microscopy (AFM), this presentation unveils the foundational principles of AFM, its pivotal role in probing mechanical properties at the nanoscale, and the importance of probe selection for accurate measurements.
Beginning with an introduction to AFM, we'll explore its origins, technological advancements, and its transformative impact on nanoscale research. From its humble beginnings to its current state-of-the-art capabilities, AFM has revolutionized our ability to visualize and manipulate matter with unprecedented precision.
Moreover, we'll discuss the critical role of probe selection in AFM experiments. Choosing the right probe is essential for achieving accurate and reliable measurements. We'll provide a comprehensive guide for probe selection, highlighting considerations such as tip geometry, stiffness, and material compatibility. Additionally, we'll explore the specialized FluidFM probes, which offer unique capabilities for nanoscale fluid dynamics and manipulation, further expanding the horizons of AFM applications.
In conclusion, this presentation showcases how AFM empowers researchers to explore the nanoscale world with unparalleled precision, offering insights into mechanical properties and material behavior that were once unimaginable. By understanding the fundamentals of AFM and employing proper probe selection strategies, we can unlock new opportunities for scientific discovery and technological innovation.

Keywords: Atomic Force Microscopy, AFM probes, cytosurge, FluidFM

Biomechanics and biorheology – their relation to a cell diseased state

Małgorzata Lekka[1]

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Cell mechanics and biorheology are fundamental for understanding biological processes linked with developing various pathologies, including cancer. Such methods as atomic force microscopy (AFM) allow the quantification of local viscoelastic properties of cells, which serves as a biomarker of disease-related changes. However, the relativeness of these measurements frequently hinders the consistent and effective characterization of (visco)mechanical cell phenotype. Microscopically, the cell mechanics is governed by the internal cytoskeleton, consisting of three primary components: actin filaments, microtubules, and intermediate filaments, along with the nucleus and a multilayered membrane structure. The fibrous arrangement of the cytoskeleton exerts diverse levels of influence on cell stiffness at different cellular locations. Mechanical biomarkers are emerging as significant indicators in disease pathology, often facilitating early diagnosis. Although indentation-based mechanics is widely accepted and well-established in characterizing pathological alterations of cell mechanics, microrheological measurements offer a complementary approach to derive mechanomarkers, promising for implementing rheological measurements in diagnostics and drug efficacy studies. Advancements in instrumentation and measurement methodology facilitate their transition into clinical applications (the work was financed by the NCN project OPUS project no. UMO-2021/43/B/NZ4/01133).

Keywords: atomic force microscopy; microrheology; cell mechanics; viscoelasticity;

Atomic Force Microscopy: mechanics, high-speed, and integration with other techniques

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Over the last three decades atomic force microscopy (AFM) has become an indispensable tool for characterisation of samples from single molecules to complex living systems, featuring cells and tissues, while simultaneously being able to correlate topography and mechanics at near native/physiological imaging conditions. In turn, the combination with advanced/customised optics leverages the advantages of immunolabelling techniques for truly correlative microscopy. Specifically, the use of a tip-scanning AFM, as compared to a sample-scanning system, enables simultaneous high-resolution correlation experiments with advanced optical techniques.

In three talks we will cover latest AFM-based nanomechanical mapping modes (QI, PeakForce QNM, PeakForce QI, CellMech package), and their application to various samples from single-molecules to living cells and tissues. We will further introduce the concept of automated large area multiparametric characterization of densely packed cell layers and highly corrugated tissue samples.

We will also introduce the most recent high-speed tip-scanning atomic force microscopy (AFM) developments that enable kilohertz linerate imaging and visualization of molecular dynamics by enabling temporal resolution on the sub-100-milisecond scale. We will highlight several examples in which high-speed tip-scanning AFM were applied for studying of structural transitions and biomolecular dynamics in samples, such as triangular DNA origamis and amyloid fibrils. We will also cover novel data analysis technique called localization AFM (LAFM), which utilizes the potential of high-speed data acquisition of high-speed AFM to beat current resolution limit down to 0.1 nanometre.

The third talk will be dedicated to integration of the AFM with other techniques, like various types of microscopies (fluorescence, confocal, super-resolution), chemical identification methods (Raman, IR-spectroscopy) as well as microfluidics (FluidFM).

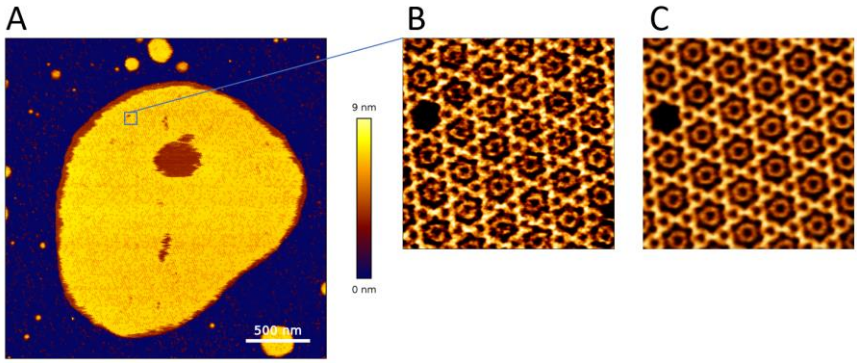

***Figure**: 2D Crystaline P6 Arrangement of Annexin V protein trimers inside lipid bilayer. (A) lipid patch with Annexin V proteins inside, captured with 20 lines/s rate; (B) zoom-in region of 100x100nm in size, captured with 50 lines/s rate; (C) averaged image of 15 consecutive frames.*

Keywords: AFM; High-Speed AFM; PeakForce QNM; nanomechanics.

AFM with other techniques

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One of the key strengths of Atomic Force Microscopy (AFM) lies in its successful integration with various complementary techniques, notably various microscopies and spectroscopy methods. Given AFM's primary function as a surface characterization technique, the combination between optical microscopy and AFM proves particularly natural, especially for biological applications. Optical microscopy provides insights into large-scale structure, inner composition, and specific content of biological objects, which align seamlessly with AFM's capabilities.

In the realm of optical microscopy, AFM has been successfully combined with a range of techniques including brightfield (e.g., DIC, phase-contrast), fluorescent (e.g., epi-fluorescence, confocal), and super-resolution (e.g., STED, PALM, FLIM, TIRF) microscopy. Remarkably, some of these techniques can operate not only sequentially on the same sample area but even simultaneously during AFM scanning.

However, optical microscopy is not the sole technique that can be integrated with AFM. Raman spectroscopy, a potent analytical technique for probing molecular vibrational modes, offers insights into molecular structure, chemical composition, and bonding interactions within a sample. Combining Raman with AFM can substantially enhance spatial resolution and sensitivity of the first. The so called Tip-Enhanced Raman Spectroscopy (TERS), allows researchers to obtain detailed information about molecular vibrational modes at the nanoscale, surpassing the diffraction limit of traditional Raman microscopy.

Recently, the merger of AFM with infrared (IR) spectroscopy has resulted in AFM-based nano-IR (Atomic Force Microscopy-based nanoscale Infrared Spectroscopy), granting access to high-resolution infrared spectra and mapping. This integration employs a specialized AFM tip with a tunable laser source, illuminating the sample surface with infrared light at precise frequencies. Upon interaction with the sample, the infrared light prompts molecular absorption, leading to localized heating and subsequent thermal expansion. The expansion triggers a mechanical response detected by the AFM's cantilever, producing a signal directly related to the sample's nanoscale infrared absorption.

The combinations discussed above represent the most common and extensively utilized combinations of AFM with established techniques. However, AFM's versatility extends further and integrations with other tools and techniques such as electron microscopy, tensile stages, FluidFM, and external magnetic fields, to name a few. Notably, this presentation will explore some of the distinctive integrations that highlight AFM's potential.

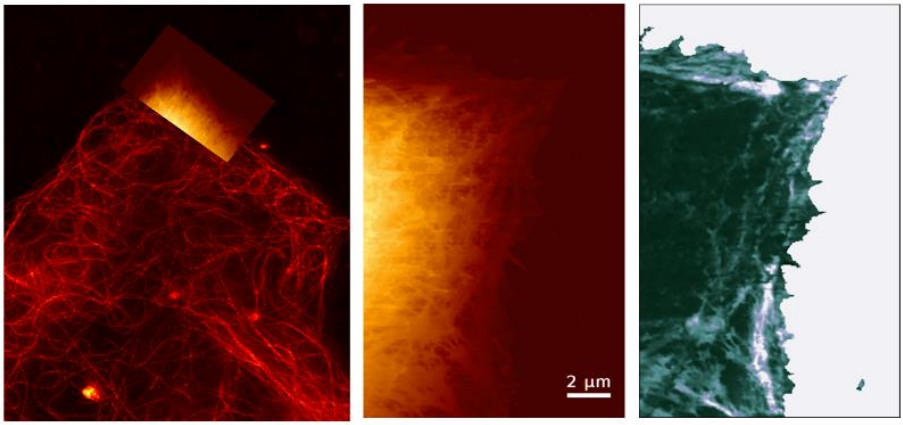

***Figure**: AFM & STED on living human lung cancer cells (A549). **Left:** STED image of microtubules labelled with silicon rhodamine overlayed with AFM topography. **Mid:** AFM topography image at 240 pN imaging force (height range 3.5 µm). **Right:** Corresponding Young's modulus image (Z range 100 kPa).*

Keywords: AFM; super-resolution; microscopy; nanoscale.

AFM data processing

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AFM has emerged as a powerful tool for imaging and probing materials at the atomic level, enabling unprecedented insights into surface topography, mechanical properties, and interactions. This presentation delves into the intricate realm of AFM data processing, elucidating key steps and methodologies. Beginning with raw data acquisition, the presentation navigates through image analysis techniques encompassing roughness analysis, feature extraction, and quantitative measurements of surface parameters. Next, advanced methods such as force spectroscopy and nanomechanical mapping (Figure 1[1]) are discussed to illuminate their role in uncovering local mechanical properties. The presentation will also show data processing insights of other techniques provided by CF Nanobiotechnology, such as single-molecule force spectroscopy studies or rheology measurements by BioSoft indenter. Furthermore, the presentation emphasizes the role of data visualization in translating complex AFM datasets into meaningful insights. Various software tools and algorithms designed for data interpretation and visualization are highlighted. By elucidating these complex processes, the presentation highlights the pivotal role of AFM data processing in advancing life sciences research and materials characterization.

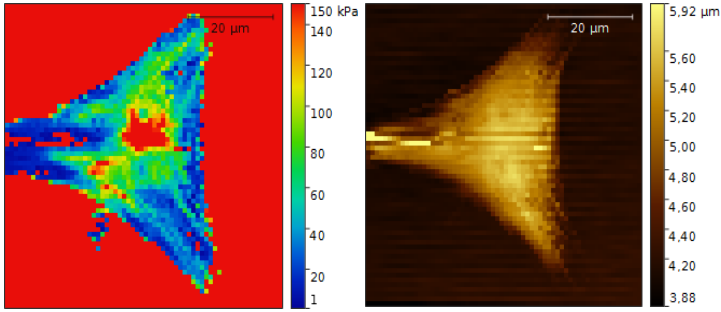

Figure 1 Force mapping of single cell.

Keywords: AFM, mechanical properties, force spectroscopy, data processing

[1] NARDONE, Giorgia, Jorge Oliver-De La CRUZ, Jan VRBSKY, Cecilia MARTINI, Jan PRIBYL, et al., 2017. YAP regulates cell mechanics by controlling focal adhesion assembly. Nature Communications [online]. **8**, ncomms15321.

Core Facility Nanobiotechnology

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Nanobio CF helps researchers (structural biologists, biochemists, and chemists) better understand the complex cellular processes, obtain nanometer-scale images of single biomolecules, and characterize nano-objects and their complexes with biomolecules. The instruments available within the CF allow monitoring of biological samples in their native state and their physiological environments, thus allowing a better understanding of the behavior of complex biological systems. However, not only the structural properties can be studied, but also the biomechanics and chemical composition of single biomolecules, nano-objects, living cells, and tissues can be studied by either individual use or in-situ combination of instruments available in our labs (AFM probe microscopy, optical microscopy, Raman microscopy, multielectrode array). Biosensor platforms support the obtained data by high-throughput analysis of biomolecular interaction.

Not only the individual services (Biomolecules – imaging, Cells – imaging, Cells - mechanical properties, Electrochemical measurements, Measurement of upconversion luminescence, Multielectrode array (MEA), Nano-objects imaging, Raman microscopy, Raman-AFM combined microscopy, SPR biosensor) and equipment (see Figure 1) will be presented, but also an insight into the practical operation of the shared laboratory - booking, check-in, check-out, and other practical details.

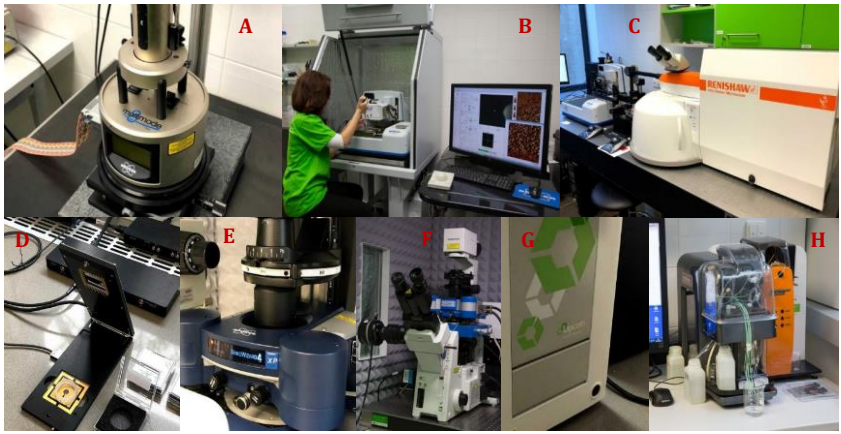

***Figure 1**: Core Facility Equipment, photos of the most used devices: **A** – Bruker MultiMode 8HR AFM, **B** – Bruker Dimension FastScan Bio AFM, **C** – Renishaw InVia Raman microscope combined with Bruker Dimension Icon AFM, **D** - MultiChannel Systems MEA2100-Lite cellular electrophysiological platform, **E** – JPK NanoWizard 4XP combined CytoSurge FluiFM, **F** - JPK NanoWizard 3, **G** – Labrox Upcon reader, **H** - SPR module with goniometer Navi™ (SPR Navi 210A).*

Keywords: Atomic Force Microscopy, Raman Microscopy, Nanoindentation, Biosamples

Acknowledgment: We acknowledge CF Nanobiotechnology of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

Microphysiological Analysis of Cardiomyocyte Function in 3D Organoids

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Human cardiomyocytes differentiated from pluripotent stem cell lines in 3D organoids allow non-invasive and long-term functional analysis of the organ-on-a-chip type. The functional phenotype can be described by a set of three methods: mechanical response mediated by the cantilever of the atomic force microscope (AFM), cellular electrophysiology by means of the microelectrode field (MEA), and calcium homeostasis by means of Ca imaging. This electrical/nanomechanical combination provides a real-time link of electrophysiology to the contracting force of cardiomyocytes. In various settings, the methods allow testing of new and known drugs, including combinations. For example, in pulmonary bronchodilators, it was thus possible to describe their relationship to cardiac arrhythmias. Testing is then possible in cells without known mutations as well as in lines of specific hereditary diseases (e.g., Duchenne muscular dystrophy, or catecholaminergic polymorphic ventricular tachycardia). The methodology, as well as clinical relevance, will be presented together with a new model of dual biosensor, for the first time allowing the study of conduction aspects of arrhythmia.

The work was supported by AZV grant NU20-06-00156 and by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) – Funded by the European Union – Next Generation EU.

Keywords: Atomic force microscopy; Beat rate; Cardiomyocytes; Contraction force

Correlative analysis of biomechanical properties and morphology on connective tissue and significance for fascia

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Multimodal correlative analysis provides additional insight into biological problems. The biological systems are frequently heterogeneous and therefore only AFM or tensile test information, especially about the mechanical properties, can be misleading or insufficient for treating the condition. Therefore, we propose to perform correlative measurements on intact tissue where multiple methods are introduced and evaluated to understand better the underlying nature of material mechanical characteristics in the diabetes study of fascia. For such a purpose, we apply the methods of second and third harmonic generation microscopy (SHG, THG), coherent anti-stokes Raman spectroscopy (CARS), fluorescence lifetime imaging (FLIM) together with biaxial tensile test and AFM microscopy.

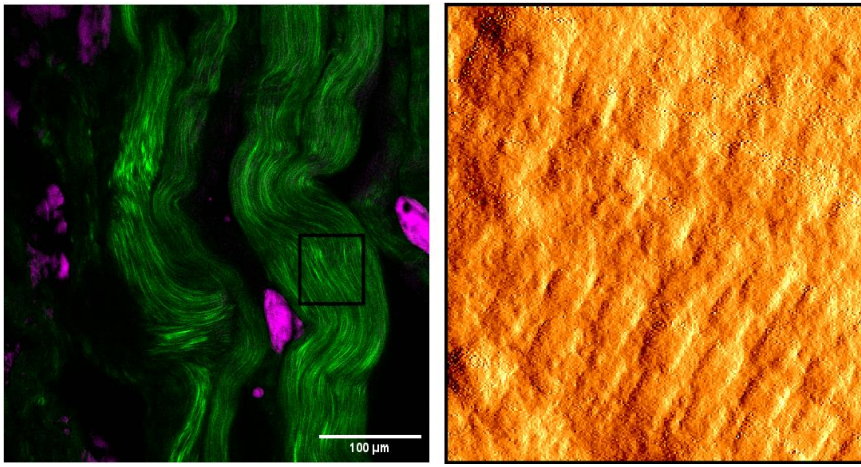

***Figure**: Left) The combination of SHG (green) and CARS (magenta) microscopy on fascia sections. Right) The AFM image of the scanned fascia area.* The sections are measured in collagenous areas using AFM and then several random positions are imaged for material composition, such as fat, collagen or level of cross-linking.

Acknowledgement: The work was supported by the GACR (Project No. 22-02756K) and ARIS (Project No. N3-0256 and P3-0043).

Keywords: Label-free microscopy; Force spectroscopy; AFM; biomechanics, Connective tissue.

Detection of oncomarkers by AFM

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Cancer remains a leading cause of global mortality, demanding novel diagnostic strategies. Despite targeted research efforts, overall oncological patient survival rates have not seen significant improvement in recent years. Early detection is a basis of effective cancer management. Therefore, the development of simple, highly sensitive diagnostic methods is a clinical priority.

The performance of digital devices employed in biomedical research continues to improve, facilitating the advancement of scientific knowledge. One such advancement is the atomic force microscopy (AFM), which offers high-resolution analysis of a diverse range of samples, including cancer cells. This technology, coupled with the latest research on oncological diseases and breakthroughs in biotechnology, opens up exciting possibilities for investigating with the use of aptamers.

Aptamers are single-stranded DNA or RNA oligonucleotides or peptides that can be specifically designed for the recognition of biomarkers on cell surfaces. Aptamers are biomolecules, that exhibit exceptionally high affinity for their target molecules, thanks to their unique ability to fold into intricate secondary and tertiary structures in solution. These structures harbor a dedicated binding site to specifically recognize selected molecules, such as oncomarkers – proteins associated with cancer [1].

In this study, we employed single-molecule force spectroscopy (SMFS) with AFM to quantify, in real-time, the interaction forces between aptamers and cell surface receptors. This approach allowed for determination of certain kinetic parameters for both specific and non-specific interactions. The interaction was confirmed visually using fluorescently labelled aptamers by confocal microscopy. Understanding the complex mechanisms by which aptamers interact with cell receptors at molecular level, is crucial for developing sensitive and specific diagnostic tools for early cancer detection.

This work demonstrates the use of AFM-based SMFS for comprehensively characterizing aptamer-cell binding kinetics, paving the way for the design of novel oncological diagnostics.

[1] Adachi T, Nakamura Y. Aptamers: A Review of Their Chemical Properties and Modifications for Therapeutic Application. Molecules. 2019 Nov 21;24(23):4229. doi: 10.3390/molecules24234229.

Acknowledgment: This work was funded by the Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences, grant numbers VEGA 2/0160/21 and VEGA 1/0157/24

Keywords: Aptamer; Oncomarker detection; SMFS; kinetics.

Characterization of surface and mechanical properties of microbial cells

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The mechanical properties of prokaryotic organisms have attracted much attention in recent years. In this study, the bacterial strains *Cupriavidus necator* and *Rhodospirillum rubrum* have been selected for their ability to accumulate intracellular substances called polyhydroxyalkanoates (PHAs) as energy storage or protection against various stress conditions (pH, ionic strength, etc.) [1]. In addition, these bacterial strains exist in PHA-producing variants and non-producing mutants. The PHAs are biopolymers unique because of their biodegradability and biocompatibility, making them potential replacements for petrochemical plastics. Mechanical properties of living bacteria producing biopolymers could help us understand as much as possible about these bacteria to increase the production of PHAs at a lower cost. For this purpose, the cells were analysed *in vivo* using atomic force microscopy (AFM) and microcompression tests. During all experiments, the mentioned bacteria were measured in a liquid medium under physiological conditions after being immobilized on a glass substrate using poly-L-lysine. JPK NanoWizard 4 was used together with MLCT – A or MLCT-SPH-1UM-DC – A tips to perform AFM measurements using the QI™ mode. Microcompression tests were performed using Hysitron BioSoft in displacement control mode with flat end tips (R_c 20 and 50 µm). The data from AFM and microcompression tests were analyzed according to the Hertzian and Overbeck models, respectively, to obtain Young's modulus of individual cells [2]. The topographic maps and measured force-distance curves from AFM showed that PHA-accumulating bacteria had greater heights and elastic modulus than non-accumulating strains. In addition, the microcompression data revealed 10× higher Young's modulus than measured by AFM. PHA-producing bacteria were found to have a higher Young's modulus than their non-producing mutants. This study highlights the mechanical diversity

among bacterial strains and the potential for optimising PHA production, contributing to more sustainable biopolymer production.

[1] Obruca S, Sedlacek P, Krzyzanek V, Mravec F, Hrubanova K, Samek O, Kucera D, Benesova P, Marova I. Accumulation of Poly(3-hydroxybutyrate) Helps Bacterial Cells to Survive Freezing. *PLoS One*. 2016; 11(6), doi: 10.1371/journal.pone.0157778.

[2] A. Overbeck, S. Günther, I. Kampen a A. Kwade. Compression Testing and Modeling of Spherical Cells – Comparison of Yeast and Algae 40(6), 1158-1164, (2017).

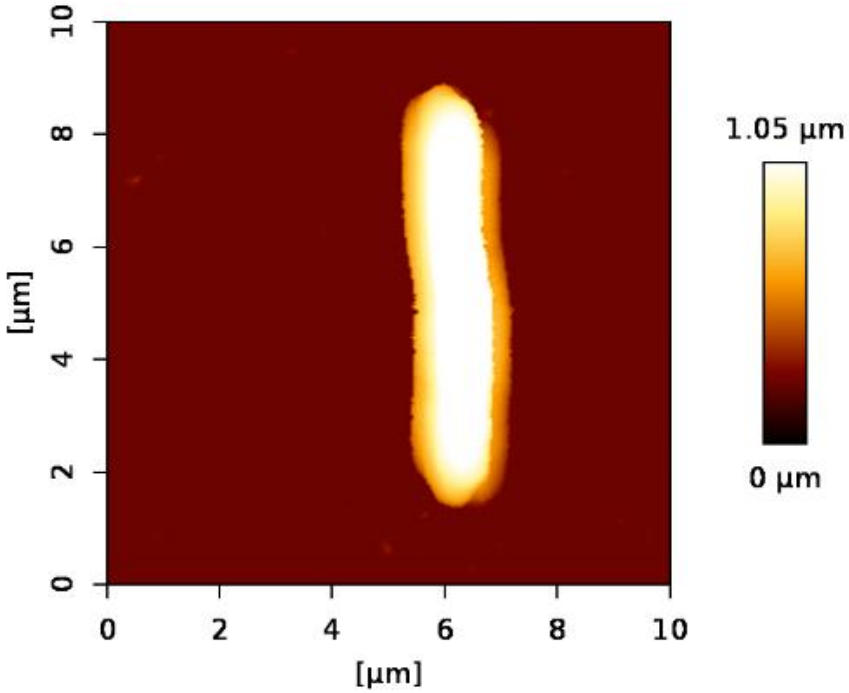

***Figure**: AFM image of Cupriavidus necator H16*

Keywords: Atomic force microscopy, Nanoindentation, Bacteria, Polyhydroxyalkanoates

Investigation of Mechanisms Underlying Stiffness Change in Prostate Cancer Cells Using AFM Computational Simulations

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Cancer mechanics is subjected to a wide variety of alterations during cancer progression among which the stiffness change, oftentimes tied to the level of cancer aggressivity, seems to be the most prominent. This study intends to present initial steps in revealing the mechanisms underlying such stiffness alterations between two prostate cancer cell lines 22Rv1 and PC-3 cells selected as model cell lines. To assess the differences in the cellular stiffness across the cell lines, we subjected the cell lines to Atomic Force Microscopy (AFM) stiffness mapping as it is the most often employed method in stiffness-related studies concentrating on uncovering the mechanisms of stiffness change. Stiffness change is also often a first marker being investigated in comprehensive biomechanical studies. As a significant difference in vimentin and actin content across the cell lines has been uncovered by the protein profile, we suspected to be responsible for a dramatic stiffness change between the investigated cell lines.

Corroborating the experimental findings, we employed a structural Finite Element Model that accounts for the nucleus, cellular membrane, cytoplasm as well as cytoskeletal components: actin cortex and actin bundles to which the actin protein content was attributed, microtubules (tubulin content) and intermediate filaments (vimentin content); further details in Bansod, 2018. Reflecting the changes in the protein content in the computational setup under local AFM loading conditions uncovers, that the considered cytoskeletal structure might not be fully capable of explaining the observed stiffness changes. This is especially valid for vimentin role in the cellular mechanics as the AFM probes mainly the superficial actin cortex and thus, the intermediate filaments (wavy in the unloaded setup) do not become stretched during the AFM load cycle and may not be as prominent in cellular response as many studies assume.

Another underlying mechanism is to be considered for playing a crucial role in the mechanical response. This points to questions about whether the different organization of filaments, different mechanical properties of the nucleus or potentially even some other organelles may play a role in cell stiffness

Keywords: cellular response to mechanical loading, structural cell FEM model, cytoskeleton, cancer cells.

Characterization of *Bacillus cereus* extracellular vesicles reveals insights into their versatile landscape and plasticity

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The pathogen *Bacillus cereus* is a Gram-positive endospore-forming rod causing food-borne diseases. Despite an increasing focus on extracellular vesicles (EVs) from Gram-positive bacteria, the exact mechanisms of EV secretion remain elusive. Recently, we have shown that *B. cereus* secretes biologically active EVs that exert pathogenic effects contingent on their cargo. To advance EV screening platforms and diagnostic tools, it is crucial to comprehensively study their plasticity to understand their role in infection and inflammation.

During lipidomic profiling we identified major shifts in single lipid species and whole lipid classes, therefore we suspect that the biomechanical properties of those EVs will also be changed. In our current project we want to employ AFM to investigate the change of biomechanical properties of EVs, and further relate these changes to functional implications crucial for host-pathogen interactions. Moreover, this study will also aid to understand the influence of culturing conditions on EV secretion and composition, ultimately enhancing our understanding of bacterial EVs and their role during infection.

Keywords: Extracellular vesicles; Bacillus cereuss; AFM; Lipidomics.

Specific approaches in microscopy samples preparation

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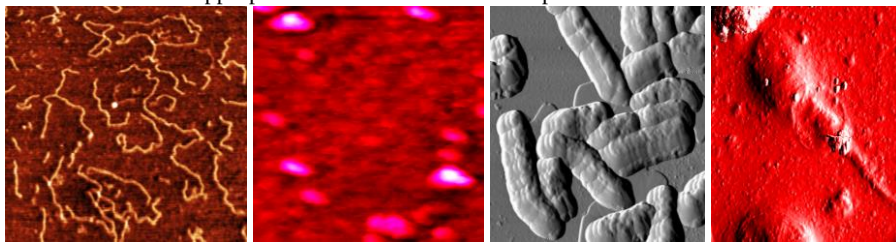
Finding a suitable method for sample preparation is a crucial prerequisite for performing all microscopy techniques; of course, this also applies to AFM microscopy. The presentation will show the specifics of sample preparation for atomic force microscopy compared to other microscopy techniques. Emphasis will be placed on so-called bio-samples, including biomolecules (proteins, phospholipidic bilayers, DNA, RNA, and their complexes), nanoparticles and their conjugates with biomolecules, bacteria, single cells, cell clusters, tissues, tissue sections, and whole organisms, selected examples illustrate this ability below (Figure 1). A strong knowledge of the specific materials (mica, pyrolytic graphite, silicon, gold, glass, plastic) used as a basis for immobilization is essential for such a lecture. This includes a description of chemical composition, stability, modification possibilities, and roughness. Chemical surface modification methods are then required in selected cases for appropriate attachment of bio-samples to individual surfaces. The



individual approaches will then be illustrated with practical examples from bio-sample imaging in our laboratory and examples from the literature.

***Figure 1**: Imaging of various biosamples with the use of AFM microscope – from left to right - |DNA (177 bp fragments), IgG immunoglobulin single molecules, bacterial cells (Thiobacillus ferrooxidans), and human sperm cells.*

Keywords: Atomic Force Microscopy, Biomolecules, Immobilization, Cells

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How to prepare biological samples for AFM measurements

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With the growing applications of atomic force microscopy (AFM) to analyze the mechanical and viscoelastic properties of biological objects such as cells and tissues, significant attention has been directed towards sample preparation methodologies. Numerous efficient protocols for sample preparation have already been documented alongside the obtained results. Among the critical steps is sample immobilization, without which AFM measurements will not be possible to conduct. Another aspect is linked with sample preservation, which allows researchers to overcome limitations associated with using fresh samples or with isolating specific elements such as cell nuclei or conducting tissue decellularization, thereby facilitating the mechanical characterization of the extracellular matrix alone. Despite the large effort dedicated to the preparation of biological samples for AFM measurements, there remains room for further development. With each sample type, a relevant protocol that preserves mechanical and viscoelastic properties needs to be elaborated on. Inadequate protocols can disturb or make AFM measurements impossible or lead to the generation of artifacts in the results (the work was financed by the NCN project OPUS project no. UMO-2021/43/B/NZ4/01133).

Keywords: atomic force microscopy; sample preparation; cell and tissue mechanics;

Cytotoxicity of nanoparticles released from titanium implants

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Titanium and its alloys are extensively used as a material for implants in dentistry and orthopaedics, especially for their stability and durability. However, once implanted, these materials undergo chemical and mechanical wear. Chemical wear is caused by corrosive molecules such as bacterial metabolites, fluorides, etc. On the other hand, mechanical wear results from implant micro-movements, which occur during patient movement. These processes give rise to particles of different sizes, including nanosized particles. Titanium nanoparticles (TiO2) are not bioinert. They can cause an activation of the human immune system via oxidative stress and DNA damage, and even promote chronic diseases of implants such as *peri-implantitis* and *peri-implant mucositis*. This presentation will give the audience insight into the basic cytotoxic mechanisms of TiO2 nanoparticles that occur on a cellular level. Furthermore, experimental data emphasizing nanoparticle stabilization for toxicity studies will be discussed. The final part of this presentation is dedicated to application of atomic force microscopy and Raman spectroscopy in the following toxicity testing.

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"Spot the detail, see the whole.
Vidět detail, poznávat celek."

Surface analysis and imaging

- Atomic force microscopy (AFM, bioAFM, UHV AFM)
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- Surface analysis (XPS, SIMS, etc.)
- Tabletop Scanning Electron Microscopes (SEM)
- 3D optical and mechanical profilometers

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- Plasma assisted depositions and etching – PECVD, RIE, PEALD

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- EPR Instruments

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- Confocal microscopes

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- Nanoindentation and AFM Probes and another accessories
- XRF consumables
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- Fusion machines
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