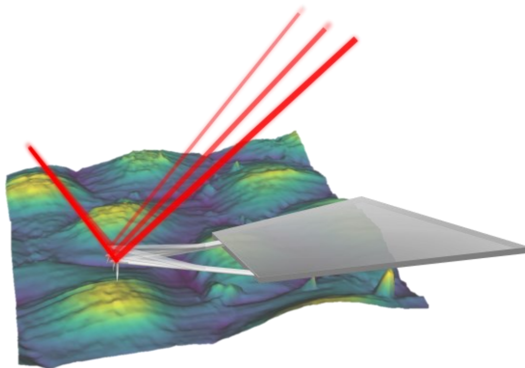
CEITEC NANOBIO

Summer workshop on BioAFM microscopy 2023

6th – 8th of September 2023
Brno, Czech Republic

Book of Abstracts



MŠMT

CIISB
Czech Infrastructure for Integrative Structural Biology

mtm.eu měřicí technika morava

Welcome address

Dear workshop participants,

After a forced break caused by the pandemic, we are happy to meet you again at the Summer BioAFM Workshop. We hope that the workshop will introduce this technique to absolute beginners, but that advanced and experienced users will also find the appropriate information. Meeting with other scientists and application engineers on a theoretical level and during practical demonstrations could certainly be beneficial for everyone.
Let this year's event become the basis for a regular event that hopefully will not be interrupted by any negative event.

We cordially look forward to meeting you all.
On behalf of the organization team,

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

MŠMT
CIISB
Czech Infrastructure for Integrative Structural Biology
mtm.eu měřicí technika morava

Programme

Wednesday, September 6th

8:30 – 9:00	Registration, coffee (E35, atrium)
9:00 – 9:15	**Workshop opening**, J. Nováček (Deputy director for research infrastructure) (E35, 211)
9:00 – 9:45	**Basics of AFM**, L. Pařízek, MTM (E35, 211)
9:45 – 10:30	**Advanced measuring modes,** L. Pařízek, MTM (E35, 211)
10:30 – 11:00	Coffee break (E35, 211)
11:00 – 12:00	**Sample preparation**, J. Přibyl, CEITEC MU (E35, 211)
12:00 – 13:00	Lunch (E35, atrium)
13:00 – 13:45	**AFM data processing**, Š. Klimovič, CEITEC MU (E35, 211)
13:45 – 14:30	**Integration of AFM with other techniques**, A. Dulebo, Bruker (E35, 211)
14:30 – 15:00	Coffee break CEITEC MU (E35, 211)
15:00 – 16:45	• **AFM probes**, A. Dulebo (Bruker) • **SPR, AFM - a study of living cells for the diagnosis of oncological diseases,** M. Domšicová (IMPG, Bratislava) • **Potential of label-free techniques with AFM measurements,** D. Hadraba (IP CAS, Praha) • **My experience with Atomic Force Microscopy**, A. Tripepi (CEITEC MU) • **Enhanced cell growth by thin ALD coatings on TiO_2 nanotubes,** K. Baishya (CEITEC BUT) • **Exploring bacterial topography with atomic force microscopy**, M. Khýrová (CEITEC BUT)

	• **Summarization of various AFM data improvement methods and their limitations,** D. Kabanov (CEITEC MU)
16:45 – 16:50	• Coffee break CEITEC MU (E35, 211)
16:50 – 17:30	• **Presentation of Cellular Imaging CF,** M. Ešner (CETEC MU) • **Introducing Josef Dadok National NMR Centre,** R. Fiala (CEITEC MU) • **Biomolecular Interactions and Crystallography CF,** J. Houser (CEITEC MU) • **Cry o-electron Microscopy and Tomography CF,** K. Škubník (CETEC MU)
17:30 – 18:00	**Tour de labs** (University Campus, CF labs)

Thursday, September 7th

8:30 – 9:00	Getting together, coffee (E35, 211)
9:00 – 9:45	**Company presentation**, J. Horák, MTM (E35, 211)
9:45 – 10:30	**Core facility Nanobiotechnology**, J. Přibyl, CEITEC MU (E35, 211)
10:30 – 11:00	Coffee break (E35, atrium)
11:00 – 12:00	**Splitting into groups, introduction** (E35, CF labs)
12:00 – 13:00	Lunch (E35, atrium)
13:00 – 17:30	**Afternoon practical session** (E35, CF labs) • **Bruker Biosoft** – Mechanical testing of hydrogels (E35, 2S017) • **Bruker Multimode 8** – Single-molecule imaging (E35, 2S011) • **Bruker Fastscan** – Membrane testing (E35, 2S014) • **JPK Nanowizard 4XP** - Cellular biomechanics (E35, 2S015)
19:00 – 22:30	**Workshop dinner** – Restaurant Baroko (Orlí 17, Brno)

Friday, September 8th

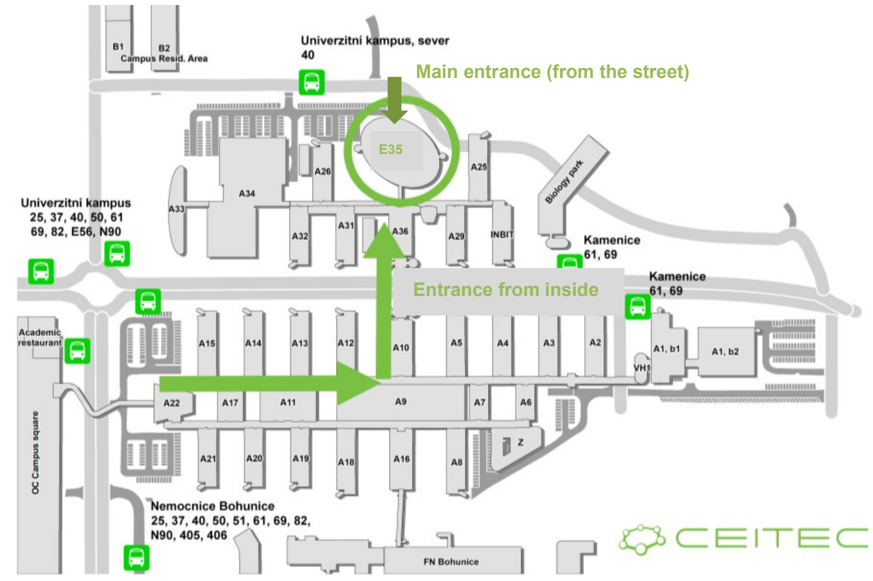
8:30 – 9:00	Getting together, coffee (E35, atrium)
9:00 – 12:00	**Practical session** (E35, CF labs) • Bring your own sample • Hands-on practice
12:00 – 13:00	Lunch (E35, atrium)
13:00 – 13:30	Closing remarks (E35, atrium)

CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

CEITEC NANOBIO

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**.



CIISB
Czech Infrastructure for Integrative Structural Biology

měřicí technika morava

Abstracts of lectures

Basics of Atomic force microscopy (L. Pařízek)	7
Advanced measuring modes (L. Pařízek)	8
Sample preparation (J. Přibyl)	9
AFM data processing (J. Přibyl)	10
Integration of AFM with other techniques (A. Dulebo)	11
AFM probes (A. Dulebo)	13
SPR, AFM - a study of living cells for the diagnosis of oncological diseases (M. Domšicová)	14
Potential of label-free techniques with AFM measurements (D. Hadraba)	16
My experience with Atomic Force Microscopy (A. Tripepi)	17
Enhanced Cell Growth By Thin ALD Coatings On TiO2 Nanotubes (K. Baishya)	18
Summarization of various AFM data improvement methods and their limitations (D. Kabanov)	22
Presentation of Cellular Imaging Core Facility (M. Ešner)	23
Introducing Josef Dadok National NMR Centre (R. Fiala)	24
Biomolecular Interactions and Crystallography Core Facility (J. Houser)	26
Cryo-electron Microscopy and Tomography Core Facility (K. Škubník)	28

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

Basics of Atomic force microscopy

Ladislav Pařízek*

Měřící technika Morava, Babická 619 Zastávka 664 84, Czech republic
*Corresponding Author: ladislav.parizek@mt-m.eu

In the realm of nanoscale exploration, Atomic Force Microscopy (AFM) stands as a remarkable tool that has redefined our ability to visualize and manipulate matter at unprecedented resolutions. This talk delves into the fundamentals of AFM, providing a comprehensive overview that spans its historical inception, technological underpinnings, and two basic operational modes.
Following the trail from the initial invention of STM by Gerd Binnig and Heinrich Rohrer, through the subsequent development of AFM by Binning, Quate, and Gerber in 1986, we arrive at today's more intricate and specialized systems which are able to scan living cells, bacteria or biomolecules under natural condition.
However, it remains crucial to comprehend the underlying working principles – such as the mechanics of piezoelectric elements, the working principle of a feedback loop and the essentiality of the probe for topographic detection. This entails summarizing the operational mechanics of the two fundamental measurement modes, namely contact and tapping, while elucidating their respective benefits and disadvantages.

Keywords: Atomic Force Microscopy, Contact mode, Tapping mode

Advanced measuring modes

Ladislav Pařízek*

Měřící technika Morava, Babická 619 Zastávka 664 84, Czech republic
*Corresponding Author: ladislav.parizek@mt-m.eu

Embarking on an enthralling expedition that transcends the bedrock of Atomic Force Microscopy (AFM) fundamentals, this presentation unveils an array of avant-garde measuring modes, fundamentally reshaping our capabilities in delving into the nanoscale domain.
We'll explore techniques like force curves, force volume, and force mapping, which help us learn about the properties of materials by looking at how they interact and respond to forces at the tiniest level at the nanoscale level. The groundbreaking PeakForce Quantitative Nanomechanical Mapping (PF QNM) and Quantitative Imaging (QI) techniques revolutionize our ability look into material stiffness, adhesion, and energy dissipation. These modalities, in synergy with the innovative Cytosurge approach, even open the portals to nanoscale fluid dynamics and manipulation, heralding a new era for AFM.
The presentation culminates with a high-speed AFM showcase, where real-time observations of dynamic processes at the nanoscale become reality. The convergence of these pioneering techniques propels AFM beyond the realm of mere imaging, elevating it to a platform for exhaustive nanoscale characterization.

Keywords: Atomic Force Microscopy, PeakForce Quantitative nanomechanical mapping (PF QNM), Quatitative Imaging (QI), force curve, force mapping, cytosurge, high speed AFM

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřící technika morava

Sample preparation

Jan Přibyl*

CF Nanobiotechnology, CEITEC MU, Masaryk University, Kamenice 5/E35, CZ-62500 Brno, Czech Republic,
*Corresponding Author: jan.pribyl@ceitec.muni.cz

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 illustrates 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.

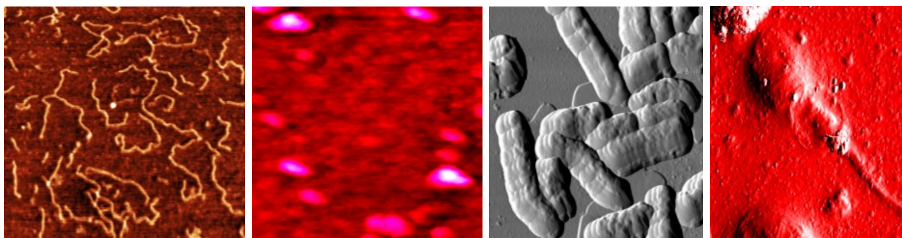


***Figure 1**: Imaging of various biosamples with the use of AFM microscope – from left to right - |DNA (177 bp fragments), IgG immunoglobulin single molecules, bacterial cells (Thiobacillus ferrooxidans), and human sperm cells.*

Keywords: Atomic Force Microscopy, Biomolecules, Immobilization, Cells

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).

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mtm.eu měřicí technika morava

AFM data processing

Šimon Klimovič*

CF Nanobiotechnology, CEITEC MU, Masaryk University, Kamenice 5/E35, CZ-62500 Brno, Czech Republic,
*Corresponding Author: simon.klimovic@ceitec.muni.cz

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.

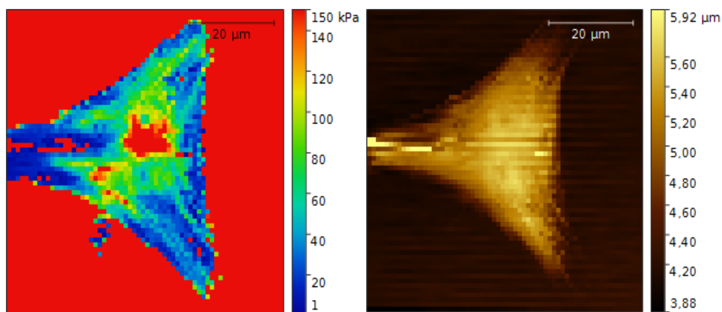


Figure 1 Force mapping of single cell.

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.

Integration of AFM with other techniques

Alexander Dulebo

JPK BioAFM, Bruker Nano Surfaces, Am Studio 2D, 12489 Berlin, Germany
alexander.dulebo@bruker.com

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.

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

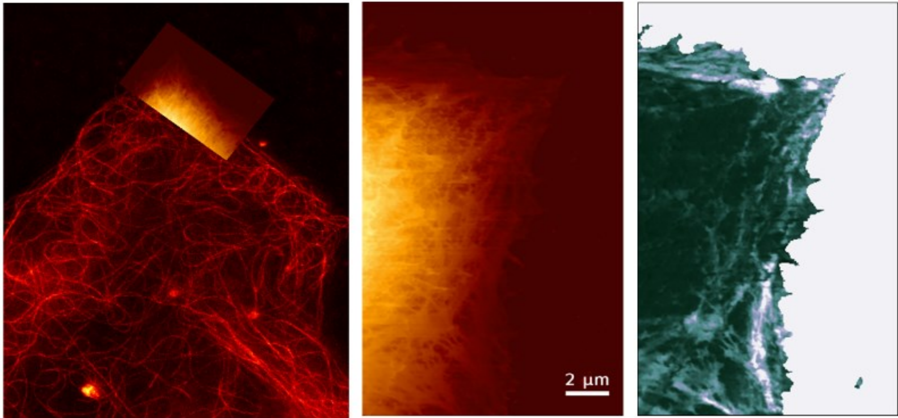


***Figure**: AFM & STED on living human lung cancer cells (A549). **Left:** STED image of microtubules labelled with silicon rhodamine overlayed with AFM topography. **Mid:** AFM topography image at 240 pN imaging force (height range 3.5 µm). **Right:** Corresponding Young's modulus image (Z range 100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

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

AFM probes

Alexander Dulebo

JPK BioAFM, Bruker Nano Surfaces, Am Studio 2D, 12489 Berlin, Germany
alexander.dulebo@bruker.com

AFM probes play an indispensable role in facilitating high-resolution imaging and precise force measurements at the nanoscale. Regardless of the quality or cost of the AFM instrument, the accuracy of acquired images and data hinges upon the suitability of the chosen AFM probe.
This presentation offers a comprehensive overview of Atomic Force Microscopy (AFM) probes. It dives into the details of their manufacturing process, underscoring the paramount significance of precision and thorough quality control to ensure consistent performance. The discussion will examine critical parameters including cantilever geometry, spring constant, and tip radius, shedding light on their profound influence on imaging precision and force sensitivity.
Furthermore, the talk will uncover the art of selecting appropriate AFM probes tailored for specific applications, spanning from material characterization to biological studies. A nuanced consideration of variables such as sample characteristics, intended application, and imaging modes will guide this exploration.
The presentation also encompasses the concept of AFM probe reusability and effective cleaning techniques that prevent contamination and maintain the consistency of results. This comprehensive investigation of this vital AFM consumable equips researchers and AFM users with a practical grasp of AFM probes, ensuring skilled selection for a diverse array of scientific inquiries.

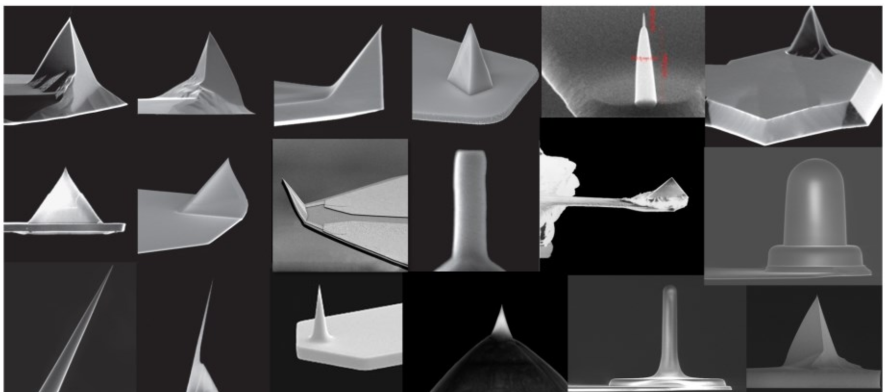


***Figure**: Variety of Bruker AFM probes.*

Keywords: AFM; AFM probe; cantilever; nanoscale.

MŠMT
CIISB
Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

SPR, AFM - a study of living cells for the diagnosis of oncological diseases

Michaela Domšicová[1,*], Alexandra Poturnayová[1], Šimon Klimovič[2,3], Radka Obořilová[2,3,4]

1 Institute of Molecular Physiology and Genetics CBs SAS, Bratislava, Slovakia
2 CEITEC, Masaryk University, Brno, Czechia
3 Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czechia
4 Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czechia
*Corresponding Author: michaela.domsicova@savba.sk

Oncological diseases as a leading cause of death represent a global problem for society. Despite targeted interest, there has been no significant progress in the survival rate of oncology patients in recent years. Early diagnosis is an irreplaceable tool in the fight against cancer, and it is necessary to develop simple, but highly effective methods. Nanotechnology, nanomaterials and nanobiosensors play an important role in clinical science and can be used in the quantification of clinical biomarkers for disease detection.
Biomarkers are defined as functionally important molecules found naturally in cells, tissues or human fluids and are present in abnormal amounts in people with cancer or in a precancerous state. They can be specific to one type of cancer or associated with several types of cancer [1].
Our goal is to highlight the potential of DNA aptamers and their clinical applications in biosensors. Aptamers are single-stranded synthetic oligonucleotides that bind a wide variety of substrates with high affinity and specificity. Detection of oncomarkers using aptasensors can help to capture tumor cells in the initial stages of the disease directly at the point of medical care [2].
In this work, we analyzed the molecular mechanisms of interactions between DNA aptamers and a specific marker of acute myeloid leukemia on the surface of diseased cells. We used the sensitive SPR method, which allows monitoring interactions directly in time, we laid the foundations of these mechanisms in clinical use and designed a DNA aptasensor, sensitive enough for detection. We optimized the modification of the biosensor surface with specific oligonucleotides suitable for SPR flow measurements to maximize the sensitivity of the aptasensor. We verified the specificity by direct comparison between cancer and control lines. Using the monitoring of interactions in different concentrations, we obtained the sensitivity of aptasensor by determining the detection limit.
An innovative approach to the early diagnosis of oncological diseases is the use of AFM, which allows measuring the elasticity of cell membranes. The mechanical properties of cells define their function, mobility, tissue formation and differentiation. Changes in cellular elasticity are a marker for abnormalities and play a role in the pathogenesis of many progressive diseases. For cells as heterogeneous objects, it is possible to describe the elastic properties using the effective modulus of elasticity. Tumor cells differ from healthy ones in the overexpression of tumor-associated markers, which is reflected in the elasticity of the cell membrane [3]. Therefore, determining the modulus of elasticity can significantly

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt m .eu měřicí technika morava

help in the early diagnosis of oncological diseases.
Changes in cell membrane elasticity were measured using AFM for cell lines and patient samples. We proposed procedures for cell immobilization and obtaining mechanical data using AFM, which lays the foundations for use in clinical diagnostics of oncological diseases.
Acknowledgment: The presented work was supported by projects VEGA 2/0160/21, CIISB and The National Scholarship Program of the Slovak Republic.
[1] Deng, Y. et al. (2022) Biosensor-based assay of exosome biomarker for early diagnosis of cancer. Front. Med. 16, 157–175.
[2] Das, S. et al. (2023) Surface Plasmon Resonance (SPR) Sensor for Cancer Biomarker Detection. Biosensors 13(3), 396–429.
[3] Nguyen, HL. et al. (2022) Elastic moduli of normal and cancer cell membranes revealed by molecular dynamics simulations. Phys. Chem. Chem. Phys. 24(10), 6225–6237.

Keywords: AFM; SPR; aptamer; cancer.

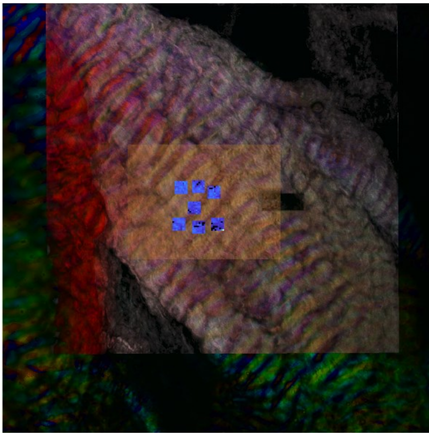
MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

Potential of label-free techniques with AFM measurements

Hadraba Daniel[1*], Vondrášek David[2*]

1. *IPHYS Bioimaging Facility, Institute of Physiology, CAS, Czech Republic*
2. *Faculty of Physical Education and Sport, Charles University, Prague*

*Corresponding Author: daniel.hadraba@fgu.cas.cz; david.vondrasek@fgu.cas.cz



Multimodal analysis provides additional insight into biological problems. The biological systems are frequently heterogeneous and therefore only AFM information, especially about the mechanical properties, can be misleading or insufficient. 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. For such a purpose, we apply the methods of second and third harmonic generation microscopy (SHG, THG), coherent anti-stokes Raman spectroscopy (CARS), polarized light microscopy together with AFM microscopy.

***Figure**: Combination of polarized light microscopy, SHG microscopy, THG microscopy, laser ablation, Bright field image and AFM force maps.*

Acknowledgement: This project was supported by IPHYS BIF – MEYS CR (Large RI Project LM2023050 Czech-BioImaging) and ERDF (Project No. Z.02.1.01/0.0/0.0/18_046/0016045). We also acknowledge CF Nanobiotechnology of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

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

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

My experience with Atomic Force Microscopy

Andrea Tripepi*

Peter Lukavsky RG, Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czechia`
*Corresponding Author: andrea.tripepi@ceitec.muni.cz

AFM provides amazing possibilities to analyse biomolecules like proteins and nucleic acids. After the initial problems, we managed to make pictures of DNA, RNA, proteins, RNA-protein complexes. 1 nM protein samples were heated at 94 °C for 3 minutes, cooled in ice, and loaded onto negatively charged MICA grids. RNA and protein samples were separately heated at 72 °C and cooled on ice. RNA samples were loaded onto positively charged APTES grids, then protein samples were loaded on previously applied RNA samples. These samples were washed 4 times in our GF buffer in order to remove nonspecific particles. We used 37 °C heated grids provided for our analyses, this way allowed us to avoid aggregates and detect several Staufen tertiary structures. We made 500 x 500 nm pictures that we subsequently flattened, filtered and cropped by Gwyddion. Particles of interested were analysed by Gwyddion. We obtained data on particle length, particle section surface by Gwyddion and ImageJ analysis. Minimum particle volumes were obtained by Gwyddion. In general, AFM greatly helped us to obtain data in a fast and reliable way. Together with other techniques like Electron Microscopy, X-Ray crystallography, Small Angle X-Ray Scattering; AFM represents an integrated approach to analyse biomolecules.

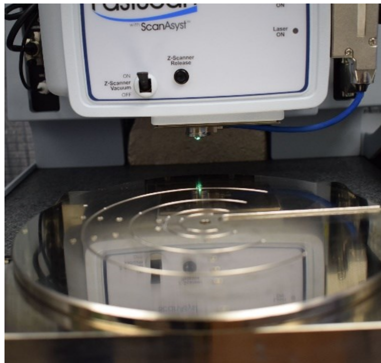


***Figure**: Atomic Force Microscope Bruker Dimension Icon FastScan in the laboratories of Nanobiotechnology Core Facility during preparation for measurements.*

Keywords: AFM; RNA; protein; biomolecular complex

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

CEITEC NANOBIO

Enhanced Cell Growth By Thin ALD Coatings On TiO_2 Nanotubes

Kaushik Baishya[1], Mahnaz Alijani[1], Jan Přibyl[2], Tomáš Roušar[3], and Jan M. Macak[1,4*]

[1] *Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic, Kaushik.Baishya@ceitec.vutbr.cz*

[2] *Central European Institute of Technology, Masarykova University, Studentská 625 00, Bohunice, Czech Republic.*

[3] *Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Nam. Cs. Legii 565, 53002 Pardubice, Czech Republic.*

[4] *Center of Materials and Nanotechnologies, Faculty of Chemical Technology, University of Pardubice, Nam. Cs. Legii 565, 53002 Pardubice, Czech Republic.*

*Corresponding Author E-mail: jan.macak@ceitec.vutbr.cz

Anodized TiO_2 nanotube (TNT) layers have been recognized as excellent biocompatible materials owing to their low cytotoxicity, high stability, antibacterial properties, and wetting ability. A pioneering work demonstrated that TNTs with a diameter of 15 nm are the most suitable for the growth of various cells [1].

Recently, we demonstrated that an ultrathin coating on TNT by suitable oxides (e.g. TiO_2) using Atomic Layer Deposition (ALD) can enhance cell growth and adhesion [2]. These properties make them excellent as final surfaces for medical and dental implants based on Ti alloys.

The present work investigates and compares the influence of ALD TiO_2 and ZrO_2 coatings on TNT layers and reference Ti foils for the proliferation of fibroblast cells. Ti sheets and anodized TNT layers with a distinct inner diameter of 12 nm, 15 nm, and 100 nm were used as substrates, as they appear to be the most suitable for cell growth in general [2,3,4]. A part of the TNT layers was coated with five ALD cycles of TiO_2 and ZrO_2. An increase in cell density for all materials coated by 5 ALD cycles was observed irrespective of the ALD-coated oxide layers. In the presented work, we investigated the shaping, adhesion, proliferation, and cell density on TNTs coated with ALD TiO_2 and ZrO_2 and the nanotopography. Moreover, the single-cell adhesion of the cells to the TNTs was studied by the bio-atomic force microscopy (bio-AMF) technique [4].

Keywords: Ti sheets, TiO2 nanotube, Anodization, Cell behavior, bio-AFM, ALD.

MŠMT

CIISB Czech Infrastructure for Integrative Structural Biology

mt.eu měřicí technika morava

References:

1. Park, J.; Bauer, S.; Schlegel, K. A.; Neukam, F. W.; von der Mark, K.; Schmuki, P. TiO2 Nanotube Surfaces: 15 Nm-An Optimal Length Scale of Surface Topography for Cell Adhesion and Differentiation. Nanoparticle growth: Small 6/2009, vintage:5 iss:6 pages:666 -671.

2. Motola, M.; Capek, J.; Zazpe, R.; Bacova, J.; Hromadko, L.; Bruckova, L.; Ng, S.; Handl, J.; Spotz, Z.; Knotek, P.; Baishya, K.; Majtnerova, P.; Prikryl, J.; Sopha, H.; Rousar, T.; Macak, J.M. Thin TiO2 Coatings by ALD Enhance the Cell Growth on TiO2 Nanotubular and Flat Substrates. ACS Appl. Bio Mater. 2020, 3, 6447−6456.

3. Baishya, K.; Rousar, T.; Macak, J.M. et al., 2022, [Manuscript in preparation].

4. Baishya, K.; Vrchovecká, K.; Alijani, M.; Pereira, J.R.; Thalluri, S.M.; Goldbergová, M.P.; Pribyl, J.; Macak, J.M. Bio-AFM Study of Human Gingival Fibroblast Behavior on TiO2 Nanotubular Substrates with Thin TiO2 Coatings. [Manuscript submitted for publication].

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

CEITEC NANOBIO

Exploring bacterial topography with atomic force microscopy

Markéta Khýrová, Eva Slaninová

Faculty of Chemistry, Brno University of Technology, Purkynova 646/118, Brno, 612 00, Czechia
Marketa.Khyrova@vut.cz

Atomic force microscopy (AFM) is becoming widely used technology for studying biological samples, since it provides high resolution scans both in atmospheric and liquid environment, where living cells can be measured. This work offers an overview of an investigation employing AFM to study mainly topography of living bacterial cells. In particular, the study is focused on bacteria with the ability to produce intracellular polyesters called polyhydroxyalkanoates (PHAs). It is already known that these polyesters play very important role for the bacterial cells such as source of carbon and energy but also as protectants against various stress conditions (temperature, pH, etc.). Therefore, the AFM technique could be used as a very efficient tool to analyse condition of bacterial cells *in vivo*. In our study, we analysed bacterial strain *Halomonas halophila* (CCM 3662), requiring highly saline substrates and *Rhodospirillum rubrum* (DMS 467). Additionally, the study examined the difference of topography within PHA producing bacterial strain *Cupriavidus necator* H16 (CCM 3726) and its mutant strain *C. necator* PHB^{-4} (DSM 514) without the ability of PHA production.

The initial optimalization of bacterial screening was performed in atmospheric environment using TESPA-V2 tip in QI^{TM} mode, where the bacterial cells were fixed on a glass surface by drying. Subsequently were accomplished measurements in phosphate buffer (PBS) as the liquid medium and natural environment of bacterial cells. For the sufficient fixation, several fixing techniques were tested using Poly(ethyleneimine) (PEI) and Pol-L-lysine (PLL), alongside with evaluation of SNL and MLCT cantilevers where PLL provided best results as fixative agent. Simultaneously, cantilevers MLCT-A and SNL-B with spring constant of approximately 1 N/m were confirmed as reliable and were used for topography scanning. The results showed significant difference in height between bacteria accumulating PHA in comparison to bacteria non-accumulating PHA.

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

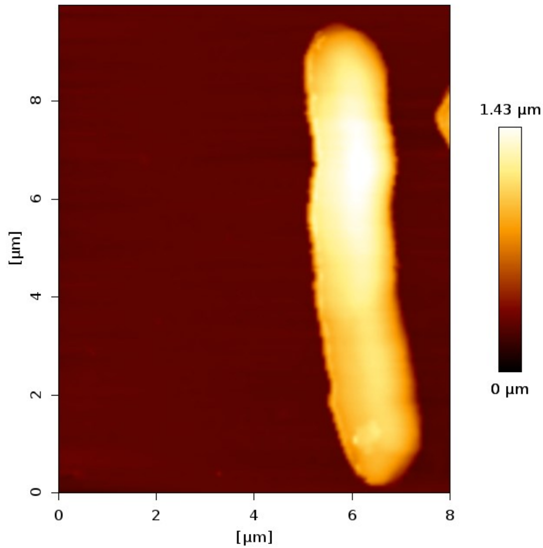


***Figure**: Cupriavidus necator H16 measured in PBS with Atomic Force Microscope JPK Nanowizard 4*

Keywords: Atomic force microscopy, Bacteria, Poly-L-lysine, Polyhydroxyalkanoates

Summarization of various AFM data improvement methods and their limitations

Daniil Kabanov[1,2*], Šimon Klimovič[1,2], Jarmila Mlčoušková[3], Jan Přibyl[2]

[1] Department of Biochemistry, Faculty of Science, [2] CEITEC MU, [3]Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

*Corresponding Author E-mail: 493395@mail.muni.cz

Atomic force microscopy (AFM) methods have been actively developed in recent years. This is also true for various digital methods actively used to solve the natural limitations of various microscopies, including AFM. However, there are not so many conventional methods for processing AFM data, especially when improving the final image or processing non-standard types of digital data, for example, lateral and vertical deflection.

This study focused on summarizing the current methods to improve the final data's information content.

Among the standard methods, the Wiener filter occupies the first place, which makes it possible to achieve image deconvolution. Since this method works primarily on the image and not the original data, there are concerns about possible data loss. This also applies to other methods related to image enhancement with AI and programs to process and enhance images until there is no solution fully optimized for AFM.

Methods of localization microscopy for AFM (LAFM) [1], which allow obtaining an image that is close in its resolution to the molecular structure of an object, are not numerous and are often an adaptation of methods for processing data from confocal microscopes, which brings us to the same problem as in the case of non-conventional filters for AFM images. Since the quality of localization is achieved by using many images, when using LAFM, the drift of stage during scanning of the same object can become a significant problem. In addition, the method is extremely sensitive to the resolution of the initial images, so LAFM is mainly suitable for imaging and improving the resolution of small objects rather than large ones.

[1] Heath GR, Kots E, Robertson JL, Lansky S, Khelashvili G, Weinstein H, Scheuring S. Localization atomic force microscopy. Nature. 2021 Jun;594(7863):385-390. doi: 10.1038/s41586-021-03551-x. Epub 2021 Jun 16. PMID: 34135520; PMCID: PMC8697813.

Keywords: AFM, LAFM, deconvolution, AI

Presentation of Cellular Imaging Core Facility

Milan Ešner*

Cellular Imaging Core Facility, Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czechia`
*Corresponding Author: milan.esner@ceitec.muni.cz

Light microscopy is an indispensable tool in biological research, allowing us to see into the otherwise invisible world of living organisms. Using visible light and specialized staining techniques, we are capable of observing not only the morphology of organelles/cells/tissues but also the dynamic processes taking place within them. Furthermore, with their resolving capabilities, modern light microscopes are approaching electron microscopes' level, which, however, can only observe fixed/frozen specimens. As a result, we can monitor live native specimens with nanometer precision.
The shared facility of Cellular Imaging (CELLIM) serves as a core facility, providing users with access to state-of-the-art equipment and techniques in the field of light microscopy. Our laboratory offers not only standard epifluorescence and confocal microscopes but also advanced super-resolution and lightsheet microscopes. An integral part of our services includes analytical methods for quantifying image data. The shared laboratory is part of the European research infrastructure EuroBioImaging and the national research infrastructure Czech-BioImaging, which is funded by the Ministry of Education, Youth and Sports of the Czech Republic.

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

Introducing Josef Dadok National NMR Centre

Radovan Fiala* and Pavel Kadeřávek

CF NMR, CEITEC, Masaryk University, Kamenice 5/C04, CZ-62500 Brno, Czech Republic

*Corresponding Author: radovan.fiala@ceitec.muni.cz

NMR (Nuclear Magnetic Resonance) spectroscopy is a key technology for research in chemistry, life sciences, and related fields allowing detailed investigation of molecular structure and dynamics at the atomic level, both in solutions and in the solid state. The high-end instrumentation and the team of experienced researchers ensures expert services, user training, and the cost-effective use of resources both for internal and external users. Benefits include access to state-of-the-art high-field NMR instrumentation and support in processing, analysis, and interpretation of the experimental data. Typical applications of NMR include compound identification, structure determination of organic and inorganic compounds in liquids and solids, studies of biomolecular structure, dynamics, and interactions, and quality control such as detection of impurities and solvent residues.

The CF is equipped with NMR spectrometers in the range of proton frequencies from 500 MHz to 950 MHz. Also available is a X-Band (9.75 GHz) EPR spectrometer and a bioreactor for NMR studies of proteins and/or nucleic acids in living mammalian cells. The equipment is suited mainly to the studies of structure, dynamics, and interactions of biomolecules, i.e., proteins, nucleic acids, and carbohydrates. However, the instrumentation is flexible enough to also cover various research needs in material science, organic and inorganic chemistry, biochemistry, biology, and biophysics.

Advantages of NMR

The method provides detailed information at the level of individual atoms. Can study not only structure of molecules, but also intramolecular dynamics and molecular interactions.

The main limitation of NMR is its low sensitivity. You need large quantities of sample (typically 0.5 ml at millimolar concentration). Detailed protein studies usually require samples enriched with ^{15}N and ^{13}C, and measurements can be long (days). NMR equipment is expensive to purchase and run and data analysis can be laborious.

The Core Facility is part of Czech national centre of European Research Infrastructure Consortium INSTRUCT ERIC under the Czech Infrastructure for Integrative Structural Biology (CIISB) funded by the Ministry of Education, Youth and Sports of the Czech Republic. Foreign users can also benefit from access through iNEXT-Discovery project. The facility participates in the Remote NMR (R-NMR) consortium. The centre also offers training enabling non-specialists to develop the necessary skills.

MŠMT
CIISB
Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

***Figure**: 950 MHz NMR spectrometer (left), EPR spectrometer (middle), NMR tube with cells connected to bioreactor (right).*

Keywords: NMR spectroscopy, molecular structure, dynamics, interactions.

MŠMT
CIISB
Czech Infrastructure for Integrative Structural Biology
mt m .eu měřicí technika morava

Biomolecular Interactions and Crystallography Core Facility

Josef Houser*

CF BIC, CEITEC MU, Masaryk University, Kamenice 5/C04, CZ-62500 Brno, Czech Republic,
*Corresponding Author: josef.houser@ceitec.cz

The core facility provides services leading to the biophysical and structural characterization of biomolecules and to the study of (bio)molecular interactions. It is equipped with the instrumentation to perform a complete X-ray crystallography experiment of biomolecules and their complexes, basic characterization of physical properties of the molecules (analytical ultracentrifugation, analytical size-exclusion chromatography, dynamic light scattering, small-angle X-ray scattering, circular dichroism spectroscopy, differential scanning calorimetry, differential scanning fluorimetry), and to study thermodynamics and/or kinetics of interactions (isothermal titration calorimetry, surface plasmon resonance, bio-layer interferometry, microscale thermophoresis, analytical ultracentrifugation).

While most of our instruments is being operated by users themselves, we also offer a number of services including sample characteristization (AUC, SEC-LS, protein sample quality check), calorimetric services (ITC, DSC) and a whole range of crystallography approaches. In addition, we offer instrument-specific trainings and organize workshops for broad scientific community.

More details on our Core Facility as well as information about the latest events can be found on our website bic.ceitec.cz.

***Figure 1**: The portfolio of BIC Core Facility consists of more than 30 individual instruments.*

Keywords: biophysics, crystallography, sample characterization, interactions

Acknowledgment: Measurement at CF BIC of CIISB, Instruct-CZ Centre, is 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).

MŠMT
CIISB
Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

Cryo-electron Microscopy and Tomography Core Facility

Karel Škubník*

Cryo-electron Microscopy and Tomography Core Facility, Masaryk University, Kamenice 5/C04, CZ-62500 Brno, Czech Republic,
*Corresponding Author: karel.skubnik@ceitec.cz

Cryo-electron microscopy and tomography core facility (CEMCOF) is an open-access user facility operated by CEITEC, Masaryk University (Brno, Czech Republic). The facility is available to users from both academia and industry and provides access to state-of-the-art electron microscopy instrumentation for structural and cellular biology research. CEMCOF manages and operates three transmission electron cryo-microscopes, two FIB-SEM microscopes, a wide-field cryo-fluorescence microscope, and a variety of instruments for sample preparation. We are dedicated to provide access to high-end cryo-electron microscopes, sample preparation services, data collection and analysis, training and education on the use of cryo-EM technology, collaboration on research projects, structural studies of macromolecules in situ, sample screening, and support for software and computational analysis.

MŠMT
CIISB Czech Infrastructure for Integrative Structural Biology
mt.eu měřicí technika morava

Acknowledgment

Many thanks to the staff of the PR department and Event department, especially to Šárka Fajtlová, Eliška Janíčková, Barbora Truksová and Karolína Šupejová.
Thanks also go to MTM (Měřicí technika Morava) for their help in organizing the workshop.

Grants support:

CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974), is gratefully acknowledged for the financial support of the measurements at the CF Nanobiotechnology.

Notes

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

Surface analysis and imaging

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

Thin layer deposition and characterization

- CVD – Chemical Vapor Deposition, ALD
- Ellipsometry and reflectometry
- PVD – Evaporation and sputtering systems, PLD, MBE
- Plasma assisted depositions and etching – PECVD, RIE, PEALD

Magnetic resonance

- NMR Instruments
- EPR Instruments

X-ray diffraction, elemental analysis

- X-ray fluorescence spectrometers
- X-ray single crystal diffractometers
- X-ray fluorescence µ-XRF and TXRF
- X-ray powder diffractometers

Fluorescent microscopy

- Multiphoton microscopes
- Light-Sheet microscopes
- Super-Resolution microscopes
- Confocal microscopes

Laboratory equipment

- Nanoindentation and AFM Probes and another accessories
- XRF consumables
- Mills, presses and sieves
- Fusion machines
- Presses

v ČR a SK zastupujeme firmy:

Měřicí technika Morava, s.r.o **www.mt-m.eu**

Babická 619 | 664 84 Zastávka u Brna Česká republika **info@mt-m.eu** +420 **513 034 408**