



CEITEC

Central European Institute of Technology
BRNO | CZECH REPUBLIC

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Nanobiotechnology Core Facility

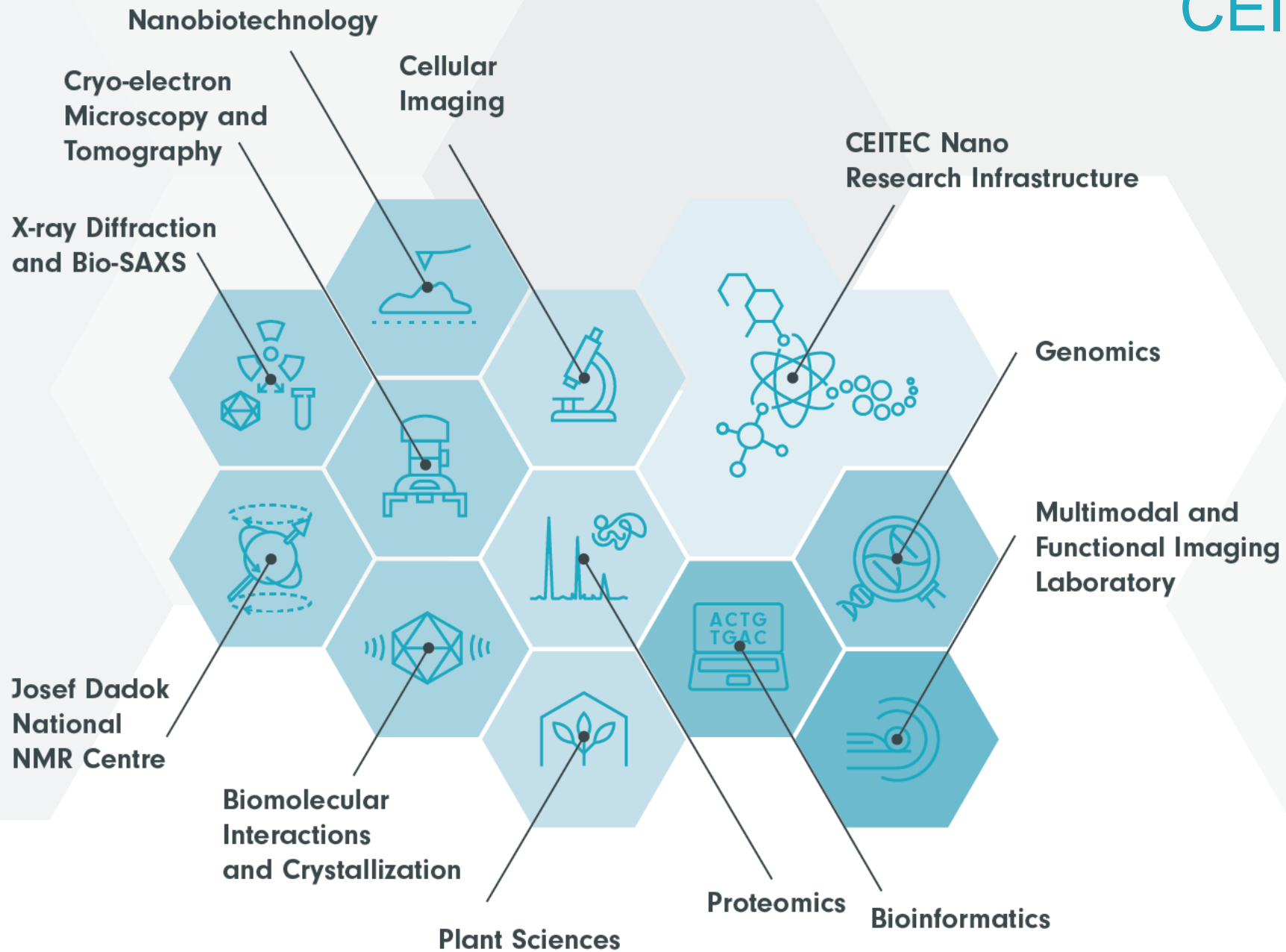
Summer workshop on BioAFM
microscopy 2023

MUNI



CEITEC consortium

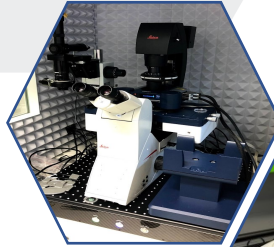
Core Facilities



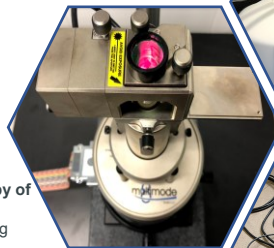
Core Facility Nanobiotechnology

Imaging, mechanical mapping, Raman microscopy

Atomic Force Microscopy of biosamples
Combined with fluorescence microscopy



Atomic Force Microscopy of biosamples
High-resolution imaging



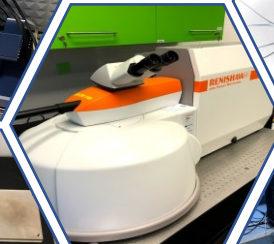
Multielectrode Array (MEA)
Cellular electrophysiology



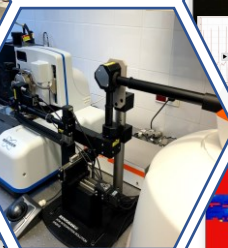
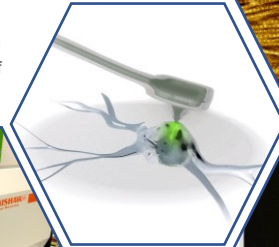
NanoIndenter
Indentation of soft samples



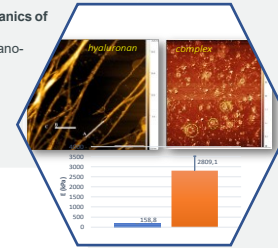
Raman microscopy
Chemical mapping of surface



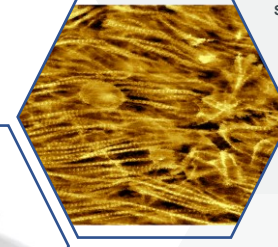
FluidFM
AFM based microfluidics



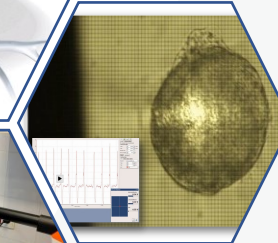
Structure and biomechanics of biomolecules
(AFM imaging and nano-indentation)



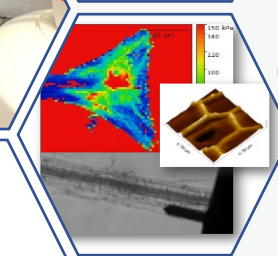
Structure of biomolecules
Under native conditions



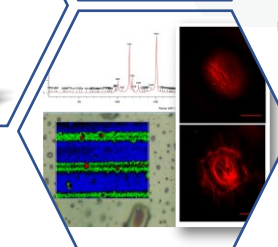
Biomechanics of contractile cells
Toxicology and cell development



Stiffness mapping of cells and tissues
Nanoindentation under semi-physiology



Fluorescence and Raman microscopy
Structural and chemical characterization



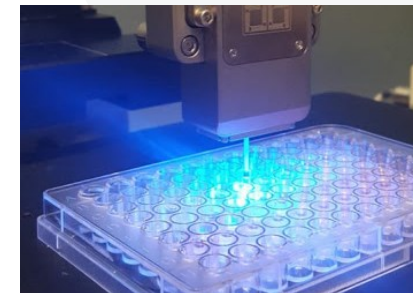
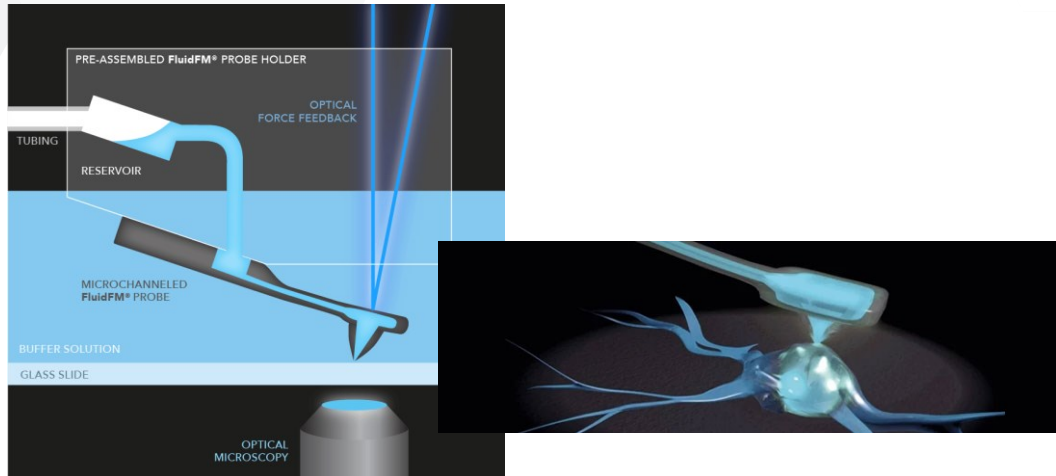
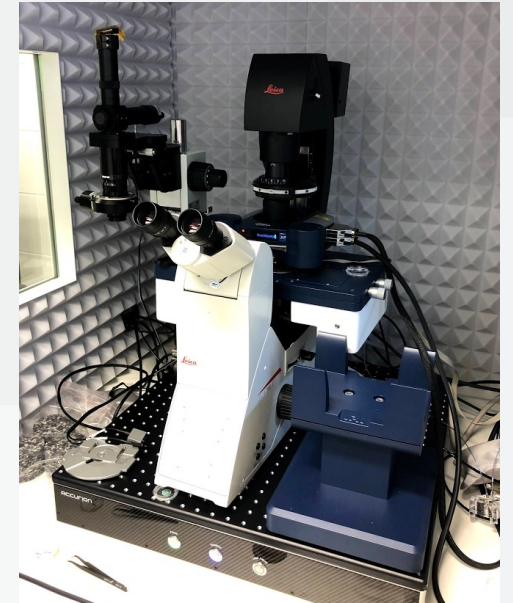
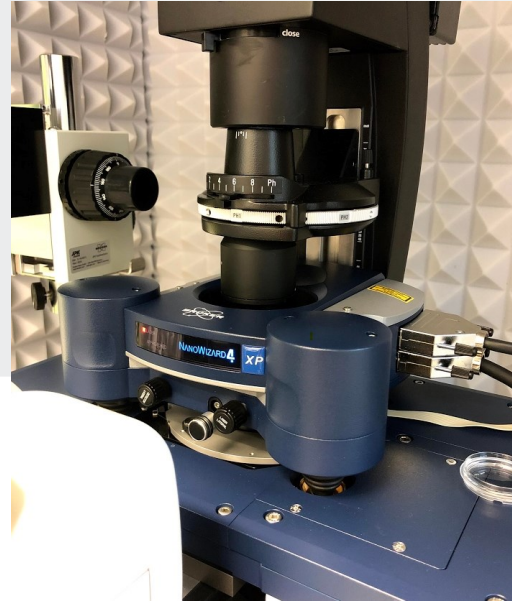
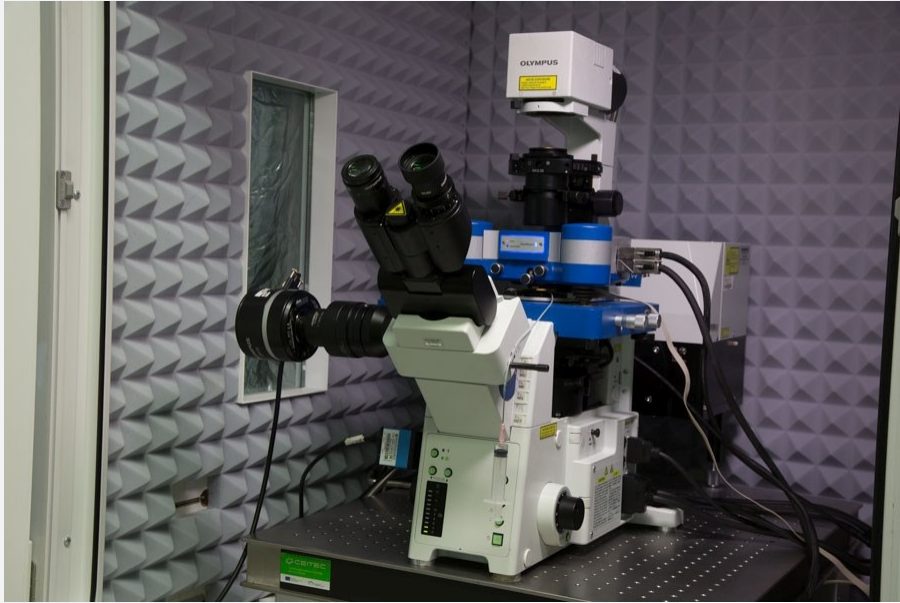
CF NanoBio Introduction

- Established 2013
- CF helps researchers (structural biologists, biochemists, and chemists) better understand the **complex cellular processes**
- **Nanometer-scale imaging** - single **biomolecules** and **nano-objects** and their complexes with biomolecules.
- Monitoring **biological samples** in their **native state** and their physiological environments
- Strong role of **biomechanics** and **chemical composition** of single biomolecules, nano-objects, living cells, and
- CF technology - **AFM** probe microscopy, **Optical microscopy**, **Raman** microscopy, **Multielectrode array**, **Biosensor platforms**, **Fluid AFM**, **NanoIndentors**
- **Strategy** - to conduct **cutting-edge research**, **competitiveness**, recruitment and retaining of strong internal and external users, and competitiveness for external research funding
- **Mission** - service and maintenance of exceptional and **expensive equipment** to offer a **cost-effective** and maximally productive environment
- **Vision** - **excellence** in everything we do, **communication** with users - consistent flow of information.

CF NanoBio - Equipment

JPK NanoWizard 3 and 4 with extended scanning range

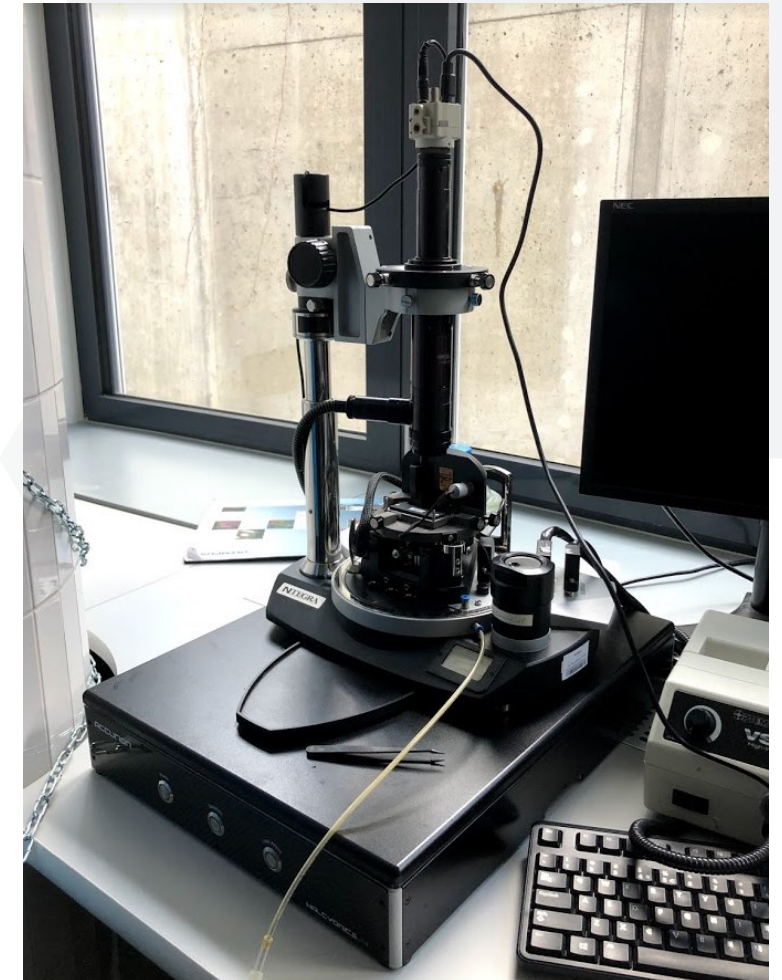
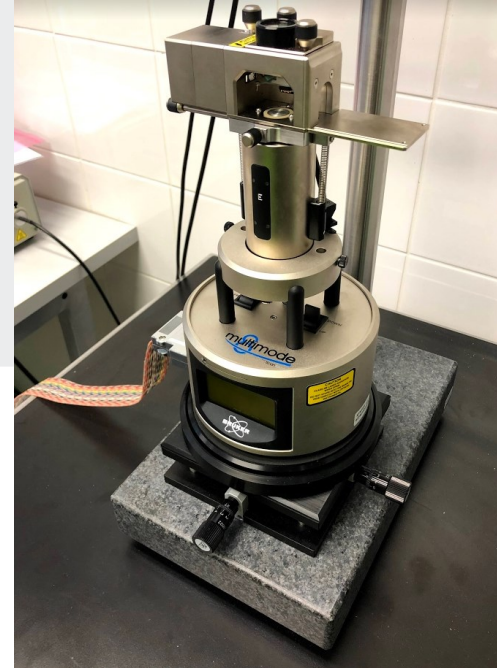
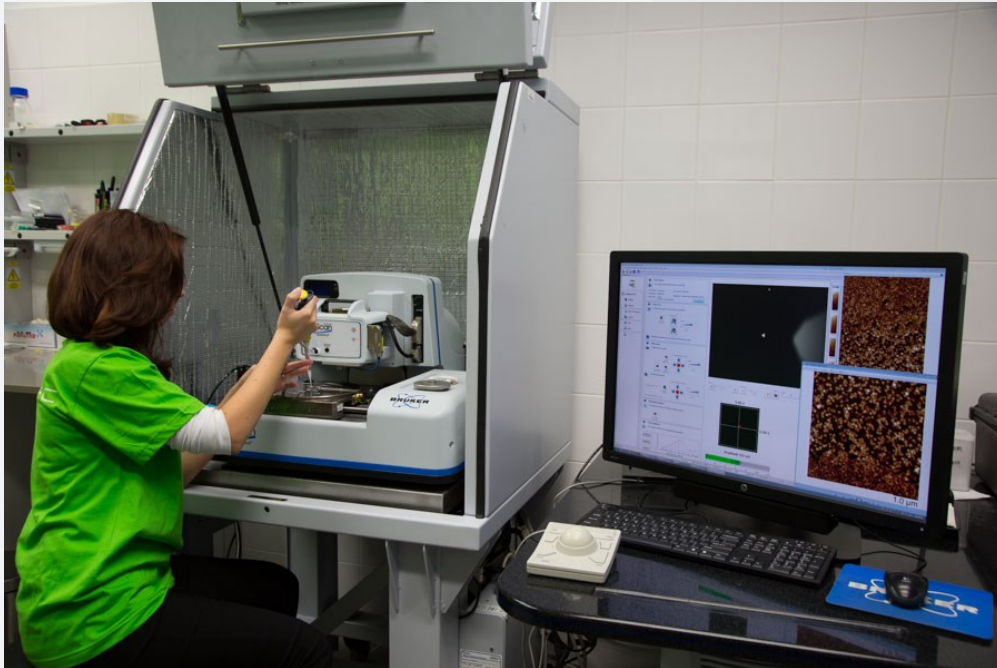
BioAFM – living cells and tissues



+ CytoSurge FluidFM module

+ Biosoft NanoIndenter

BioAFM – molecules, nanoobjects, molecular complexes



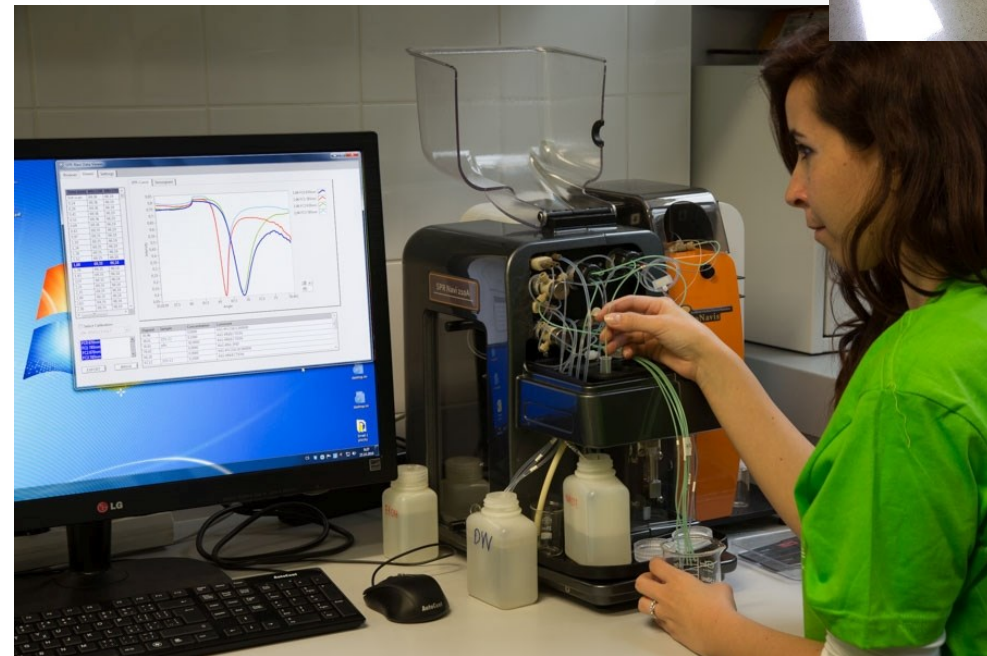
**Bruker Dimension Icon FastScan and MultiMode 8HR
NTMDT Ntgra Vita**

Raman microscopy, SPR affinity biosensor, Upconverting particles UCNP reader

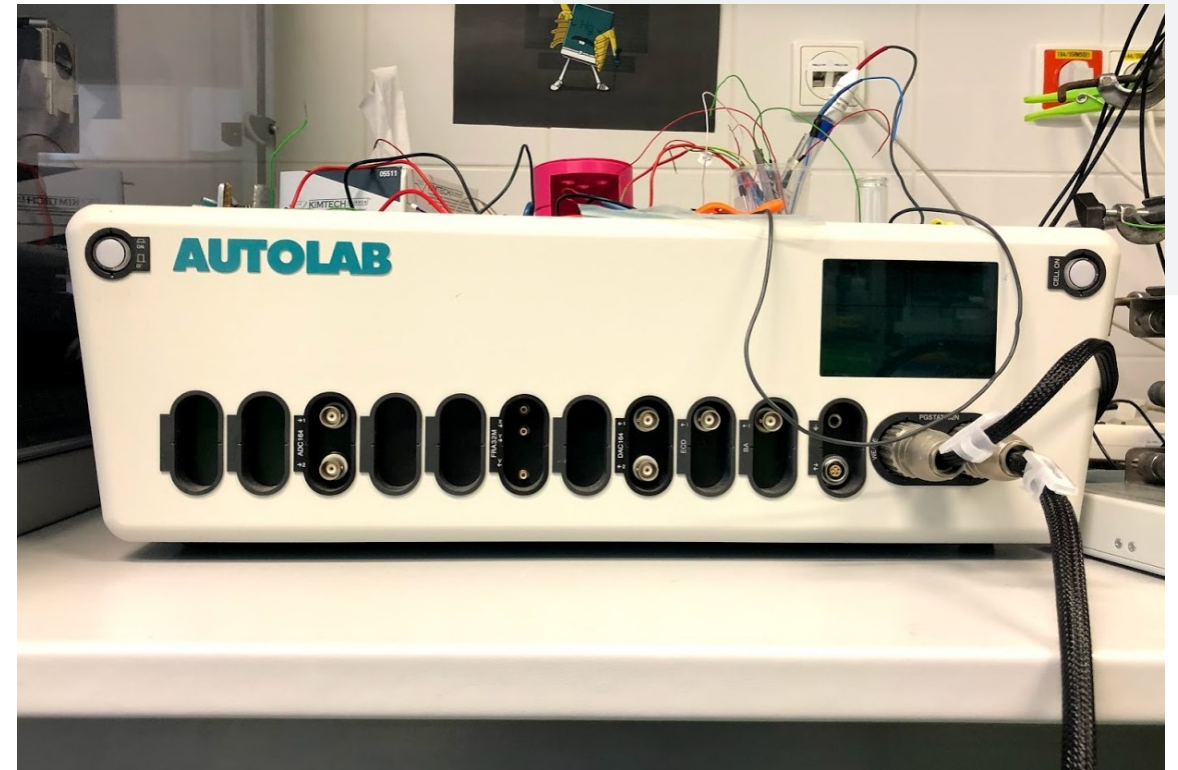
Renishaw InVia Raman microscope

Bionavis SPR biosensor device

Labrox UPCON reader



**Autolab Modular potentiostat
MultiChannel MEA2100Lite**



CF NanoBio

Technology and Expertise

Technology and Expertise

List of services

1. **Cells – mechanical properties**
2. **Cells - imaging**
3. **Biomolecules - imaging**
4. **Nano-objects imaging**
5. **Raman-AFM combined microscopy**
6. **Raman microscopy**
7. **Electrochemical measurements**
8. **Nanodeposition system**
9. **SPR biosensor**
10. **Scanning of upconversion luminescence**
11. **Multielectrode array recording of cellular potential**

FULL SERVICE / MEASUREMENT only / DATA PROCESSING only

CF NanoBio

Administrative background

CIISB Project applications, 2021

- **CIISB projects** submitted to <https://stigmator.ceitec.muni.cz>)
 - Total number **26 projects**
 - **7** from **CEITEC MU**
 - **5** from **CEITEC BUT**
 - **2** from **foreign institutions.**
- **Cell mechanobiology 10**
 - **Tissue mechanobiology 4**
 - **Cellular electrophysiology 1**
 - **Raman microscopy mapping 3**
 - **Biomolecules imaging 5**
 - **Nano-objects imaging 5**
 - **Some projects combined**

CIISB project



Project proposal

Fields highlighted in red are compulsory. The form can be submitted only after all the required information is provided. Field marked with * place the cursor over the label to see the help.

Proposals submitted now have maximal duration till 31.10.2024

Research project title:

Acronym:

(max. 10 characters) Will be used as project identifier.

Applicant information:

First name: Surname:

Email: Phone number:

Position: Researcher Ph.D. student MSc student

Principle investigator:

- 10% of costs covered by users
- Project submission and reviewing
- Acknowledgment.

Project management						
Jan Příbyl, jan.pribyl@ceitec.muni.cz, CF Nanobio						
PID	Acronym	Principal investigator	Proposal	Technical feasibility	Peer-review	Project status
230096C	Cancer	Michal Masarik	View PDF	■ CF Nanobio View PDF	not required	Service in progress Finish service
230091C	Mechanobio	Jifi Navrátil	View PDF	■ CF Nanobio View PDF	not required	Service in progress Finish service
230087C	pep-mem	Robert Vacha	View PDF	■ CF BIC ■ CF CryoEM ■ CF Nanobio View PDF ■ CF Prot	not required	Service in progress Finish service
230086C	DivIVA	Imrich Barák	View PDF	■ CF CryoEM ■ CF Nanobio View PDF	not required	Service in progress Finish service
230085C	RAD51FIL	Lumir Krejci	View PDF	■ CF CryoEM ■ CF Nanobio View PDF	not required	Service in progress Finish service
230078C	Stau	Peter Josef Lukavsky	View PDF	■ CF Nanobio View PDF	not required	Service in progress Finish service

Impacted Publications

- The main objective of the Core Facility - help them produce scientific results publishable in the impacted journals
- **2019 – 2020: 33 papers** with the Acknowledgement to CF Nanobio
- Complete list in the Report SI
- Selected papers:

1. RAUDENSKÁ, Martina, Monika KRATOCHVÍLOVÁ, Tomáš VIČAR, Jaromír GUMULEC, Jan BALVAN, Hana POLANSKÁ, Jan PŘIBYL a Michal MASAŘÍK. Cisplatin enhances cell stiffness and decreases invasiveness rate in prostate cancer cells by actin accumulation. *Scientific reports*, London: NATURE PUBLISHING GROUP, 2019, roč. 9, č. 1660, s. 1-11. ISSN 2045-2322. doi:10.1038/s41598-018-38199-7.
2. CALUORI, Guido, Jan PŘIBYL, Martin PEŠL, Šárka JELÍNKOVÁ, Vladimír ROTREKL, Petr SKLÁDAL a Roberto RAITERI. Non-invasive electromechanical cell-based biosensors for improved investigation of 3D cardiac models. *Biosensors & bioelectronics : the international journal for the professional involved with research, technology and applications of biosensors and related devices*, Elsevier Science, 2019, roč. 124, JAN 15 2019, s. 129-135. ISSN 0956-5663. doi:10.1016/j.bios.2018.10.021.
3. Cernochova, P.; Blahova, L.; Medalova, J.; Necas, D.; Michlicek, M.; Kaushik, P.; Pribyl, J.; Bartosikova, J.; Manakhov, A.; Bacakova, L.; Zajickova, L., Cell type specific adhesion to surfaces functionalised by amine plasma polymers. *Sci Rep* 2020, 10 (1), 14.
4. Laidou, S.; Alanis-Lobato, G.; Pribyl, J.; Rasko, T.; Tichy, B.; Mikulasek, K.; Tsagiopoulou, M.; Oppelt, J.; Kastrinaki, G.; Lefaki, M.; Singh, M.; Zink, A.; Chondrogianni, N.; Psomopoulos, F.; Prigione, A.; Ivics, Z.; Pospisilova, S.; Skladal, P.; Izsvak, Z.; Andrade-Navarro, M. A.; Petrakis, S., Nuclear inclusions of pathogenic ataxin-1 induce oxidative stress and perturb the protein synthesis machinery. *Redox Biology* 2020, 32.
5. 9. SADZAK, A., J. MRAVLJAK, N. MALTAR-STRMECKI, Z. ARSOV, G. BARANOVIC, I. ERCEG, M. KRIECHBAUM, V. STRASSER, Jan PŘIBYL a S. SEGOTA. The Structural Integrity of the Model Lipid Membrane during Induced Lipid Peroxidation: The Role of Flavonols in the Inhibition of Lipid Peroxidation. *Antioxidants*. Basel: MDPI, 2020, roč. 9, č. 5, s. 430-457. ISSN 2076-3921. doi:10.3390/antiox9050430.

User Training

- 2019 – 2021: **6 workshops**
- **Over 200 participants**
- Workshop content shared **online** – youtube, Data Storage

Workshop title	Date	Main objectives	No of participants
Atomic Force Microscopy (AFM) for Bio Applications	April 16-17, 2019	Combined characterization of biosamples by AFM, practical applications, hands-on session	20
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Introduction to Raman microscope Renishaw inVia	June 23 rd , 2020	Introducing the Raman microscope Renishaw inVia, User samples characterization	12
(Bio) Atomic Force Microscopy (bioAFM), Basic Course	October 1 st , 2020	Basics of AFM, Sample preparation techniques, Data processing	80
Introduction to JPK NanoWizard 4 AFM microscope	May 4 th , 2021	Introduction to a new JPK NanoWizard AFM system combined with CytoSurge and NanoIndenter module	10



Cytoplasmic live-cell biopsies for the temporal profiling of single-cells

Go beyond in single cell manipulation

Gentle and accurate single-cell injection and cytoplasmic biopsies

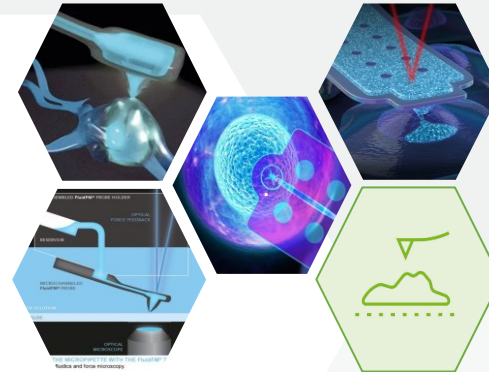
Fluidic force microscopy, or FluidFM is a biophysical technique for conducting **single-cell biopsies**. This innovative approach enables the extraction of a part of the cytoplasm from individual living cells while preserving their viability.

These cytoplasmic biopsies can be used for subsequent highly-sensitive, low-input **RNA-seq analysis** to characterize **single cells multiple times** throughout their lifetime.

Moreover, the **FluidFM Nanosyringes** extend their utility by facilitating the **targeted introduction** of various molecular components into cells, including **RNA, DNA**, proteins, and even molecular complexes such as **CRISPR/Cas9 RNPs**.

This functionality streamlines the transfection processes for plasmids and transcription factors and enables entire cell line engineering workflows.

By exploring the capabilities of **FluidFM** in this seminar, we seek to uncover its **potential implications** for advancing the comprehension of intricate cellular processes, thus fostering new dimensions in **cellular analysis** and **molecular investigation**.



Core Facility
Nanobiotechnology
CEITEC MU, Masaryk university
Kamenice 5 , Brno, Czech
Republic

jan.pribyl@ceitec.muni.cz
Web: www.ceitec.eu/nanobio

Register
[here](#)



CYTOSURGE®

FluidFM®

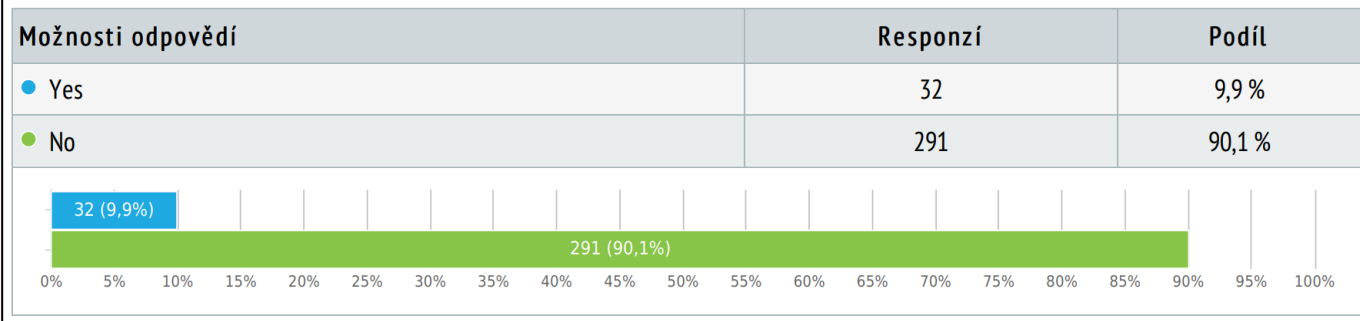
October 18th
2023

University Campus Bohunice
Brno, Czech Republic
building E35, room 145

User Survey

173. Have you used Nanobiotechnology (Nanobio) core facility?

Výběr z možností, zodpovězeno 323x, nezodpovězeno 0x



176. Do you have any comments about service quality?

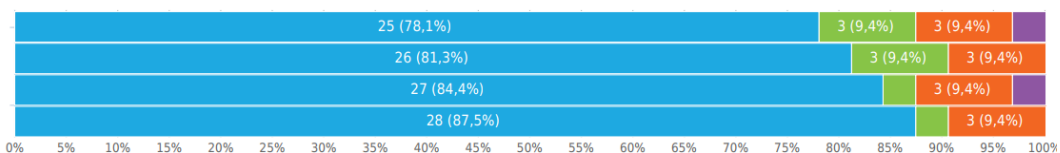
Textová odpověď, zodpovězeno 13x, nezodpovězeno 310x

- CF personel is underestimated
- Everything ok.
- OK
- excellent
- No
- I especially welcome help with adjustment of measurement parameters
- Staff is always willing to help, experts in their fields.
- prompt measurement but until now no results processed
- Dr Pribyl has excellent knowledge not only in AFM area
- Excellent service, facility is willing to help with anything needed. Always available for your questions.
- Jan Pribyl was a great help during our intense measurements on AFM NanoWizard 3.
- (2x) no

175. Service quality: How satisfied are you with service quality at Nanobio?

Matice výběru z možností, zodpovězeno 32x, nezodpovězeno 291x

Odpověď	● 1	● 2	● 3	● 4	● 5	● Not applicable/can not answer
Ease of access (clear presentation of services, prices and access modalities)	25 (78,1%)	3 (9,4%)	0	0	3 (9,4%)	1 (3,1%)
Project specific support (core facility is willing and able to provide detailed guidance and additional support on top of the standard services)	26 (81,3%)	3 (9,4%)	0	0	3 (9,4%)	0
Expertise of the core facility staff	27 (84,4%)	1 (3,1%)	0	0	3 (9,4%)	1 (3,1%)
Professional and friendly service (I feel welcome when contacting core facility staff, I get quick and competent answers to my questions and requests)	28 (87,5%)	1 (3,1%)	0	0	3 (9,4%)	0



- N/A
- 20 samples per month
- Possibly some software for volumetric analysis
- pink gloves
- Nanoparticle characterization, stability studies - DLS, zeta-potential. On a regular basis..
- no idea
- Nap room, so that the short stay of AFM measurement would allow more data measured by the visitor, into very late hours and with short periods of sleep
- -
- no

Technology offers for industrial partners

Cooperation with industrial partners (<http://industry.ceitec.cz/>), Daniela Tršová manages this topic.

Bio-AFM microscopy imaging and biomechanical studies

AFM microscopy (structure and mechanical properties) of bio-samples (biomolecules, cells, tissues) under semi-physiological conditions (37 °C, liquid media).

- **Raman microscopy of biosamples**

Raman mapping of biosamples (molecular complexes, cells, tissues) with high resolution (~ 500 nm)

- **Drug testing on cardiac cells**

Biomechanical (bioAFM) and electrical field potential signal as a response to drug exposition. Human stem cells and/or primary animal cells can be used. *! Coordination with Vladimir Rotrekl RG – essential!!!*






- **Tunable hydrogel system**

The new system of stable and Robust biocompatible hydrogel system with tunable mechanical properties.

! Coordination with Vladimir Rotrekl RG – essential!!!

Booking system

Under development for last 2 years..

Plánovací tabule Seznam rezervací Požadavky ▾ Infrastruktura ▾ Původní verze     

Vyberte službu pro požadavek

Other

Biomolecules - imaging
Zobrazování biomolekul (proteiny, DNA, makromolekuly) a jejich komplexů. Standardní podklad – slída (mica), lze použít i jiné – HOPG, křemík, kovové elektrody, atp. Metody: pokleповý režim, PF-QNM, QI, Force Volume. Vyhodnocení a export dat.

Cells - imaging
Buněčné kultury ve standardní Petriho misce (TPP 93040), lze použít i misky pro konfokální mikroskopii (vhodný typ nejprve konzultujte s námi). Fixované (např. PFA) buňky na skle. Metody – kontaktní mód, QI, PF-QNM, Force Volume. Post-processing a export dat. Možná kombinace s optickou mikroskopii (BF, fluorescence, konfokální mikroskopie) – možnost nezávislého nebo overlay snímkování. Místnost je vybavena CO2 inkubátorem a malým laminárním boxem. UV sterilizace prostoru.

Cells - mechanical properties
Buněčné kultury ve standardní Petriho misce (TPP 93040), lze použít i misky pro konfokální mikroskopii (vhodný typ nejprve konzultujte s námi). Metoda Force-Mapping, biomechanická charakterizace kardiomyocytů. Vyhodnocení naměřených dat matematickými modely (Hertz-Sneddon, DMT, JKR, atd.), post-processing. Možná kombinace s optickou mikroskopii (BF, fluorescence, konfokální mikroskopie) – možnost nezávislého nebo overlay snímkování. Místnost je vybavena CO2 inkubátorem a malým laminárním boxem. UV sterilizace prostoru.

Electrochemical measurements
Elektrochemický analyzátor pro voltametrická, amperometrická a impedanční měření (EIS) na různých typech elektrod a sensorů. Možnost dvoukanálových měření, vysoká citlivost, nízký šum. SW Autolab Nova pro analýzu dat.

SPR biosensor
Dvoukanálový průtočný SPR (bio)sensor využívající metody rezonance povrchového plasmonu. Sledování a charakterizace optických vlastností tenkých vrstev a jejich změn v reálném čase – v kapalině i nasucho. Velmi široký úhlový rozsah díky použití goniometru. Využití 2 vlnových délek umožňuje měření indexu lomu a tloušťky vrstev. Dále lze simultánně provádět elektrochemická měření. Možnost sledování a charakterizace interakcí biomolekul bez potřeby jejich značení, jeden vazebný partner musí být imobilizován na povrchu měřicího čipu, druhý je volný v roztoku. Určování kinetických parametrů, vazebných konstant či měření koncentrace různých analytů.

Nano-objects imaging
Zobrazování nano-objektů (nanočástice, nanotrubičky, nanodrátky, atp.) a jejich komplexů Standardní podklad – slída (mica), lze použít i jiné – HOPG, křemík, kovové elektrody, atp. Metody: pokleповý režim, PF-QNM, QI, Force Volume. Vyhodnocení a export dat.

Data Sharing

- Medium Storage of MU – complicated for external users
- OneDrive – limited space to 1 TB
- IT manager missing

711025-Core Facility Nanobiotechnology	ADR	10.03.2021	17:34:28
711025-Core Facility Nanobiotechnology-BIOLOGY	ADR	04.02.2020	14:32:55
711025-Core Facility Nanobiotechnology-CF_Internal	ADR	16.04.2021	14:08:04
711025-Core Facility Nanobiotechnology-Guides	ADR	04.04.2021	21:07:03
711025-Core Facility Nanobiotechnology-Workshops	ADR	18.12.2020	13:10:26
<hr/>			
_MACHINES_backups	ADR	04.12.2020	10:06:32
_Guides	ADR	04.04.2021	21:07:03
_Software	ADR	17.03.2021	13:03:19
_Workshops	ADR	18.12.2020	13:10:26
A-beta	ADR	19.02.2021	18:01:57
Andrej Besse	ADR	23.03.2020	12:45:00
BIOLOGY	ADR	04.02.2020	14:32:55
BOUCHAL	ADR	21.12.2019	17:34:19

Sharing of

- *Data*
- *Workshop content*
- *Software*
- *Guides*

Impacted Publications

- The main objective of the Core Facility - help them produce scientific results publishable in the impacted journals
 - **15 – 20 impacted publications per year (CF acknowledgement)**
-

Grants received

Project name	Provided by	Period
CIISB Large infrastructure project	MEYS	2016 - 2022
OP VVV – CIISB4Health	MEYS	2017 - 2021
OP VVV – UP CIISB	MEYS	2020 - 2022
Long-life education	MU	2021
RIAT-CZ	Interreg	2016 - 2020

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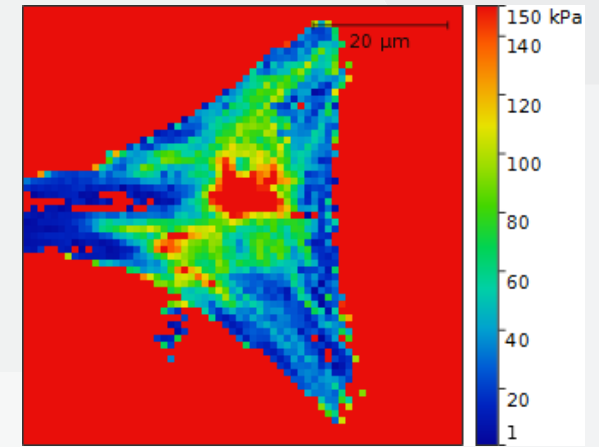
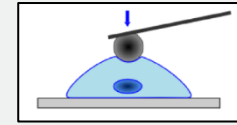
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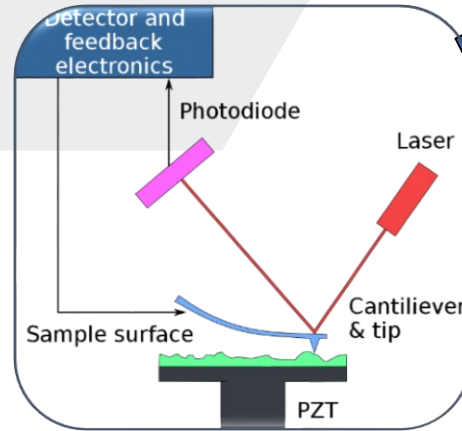
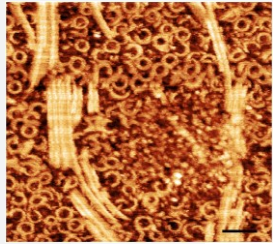
CF NanoBio - Applications

AFM microscopy / spectroscopy

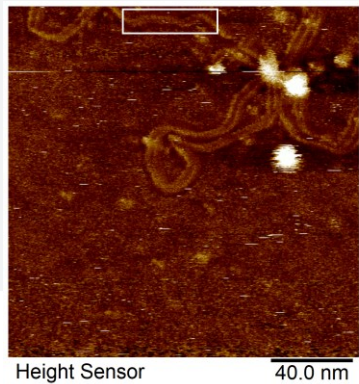
Stiffness mapping (nanoindentation)



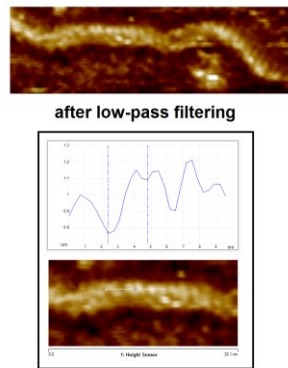
imaging



Protein, DNA molecules
Nanoobjects



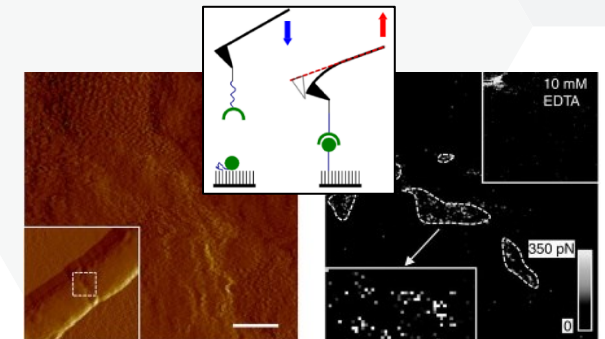
2.7 nm
-1.8 nm



+ FluidFM

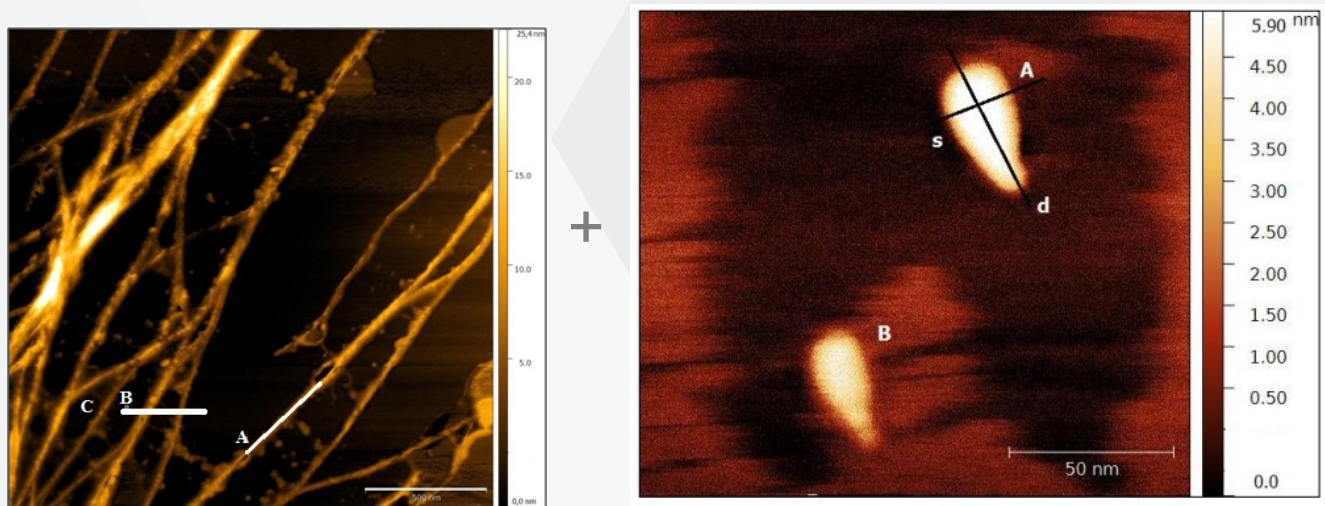
+ NanoIndenter

Affinity interaction



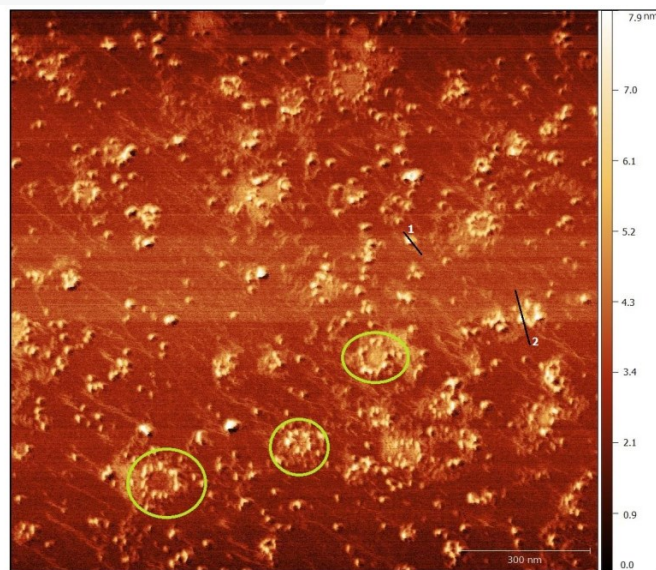
AFM schematics by
OverloadQ

Molecular imaging and biomechanics



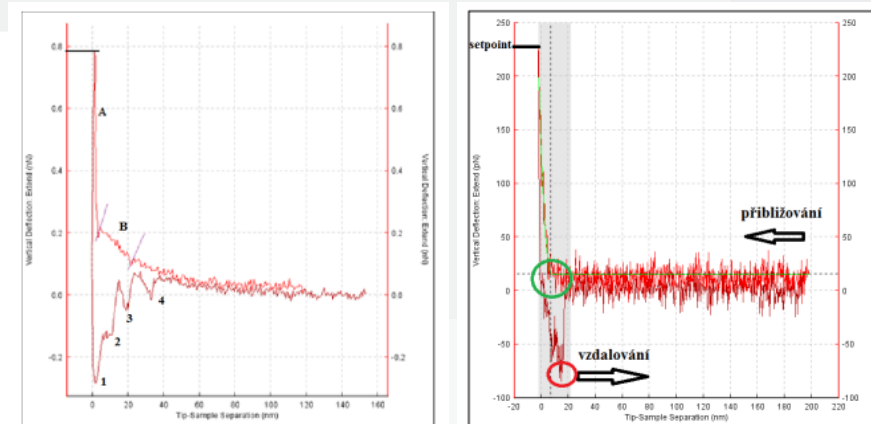
hyaluronan

myeloperoxidase

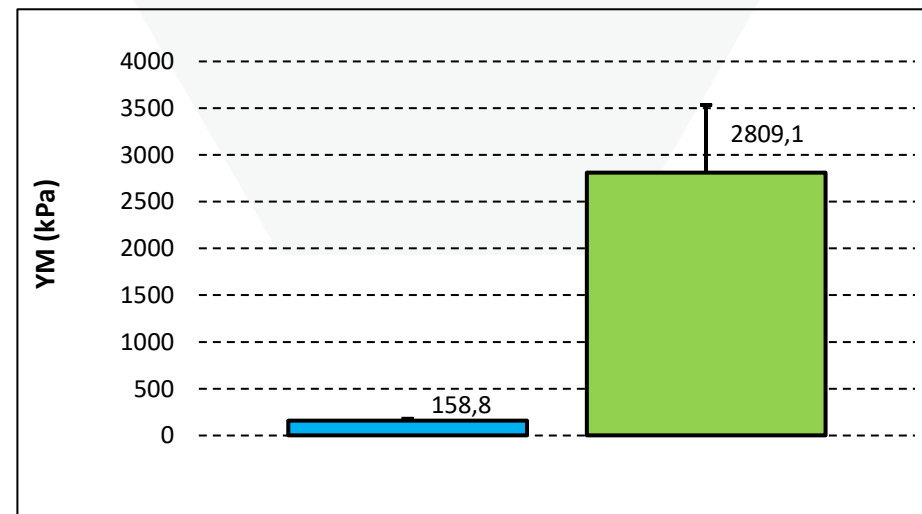


Structure of the complex

FD-curve analysis



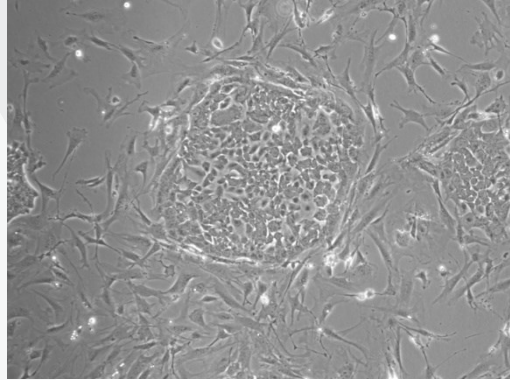
HA-MPO complex is 20x stiffer comparing to HA



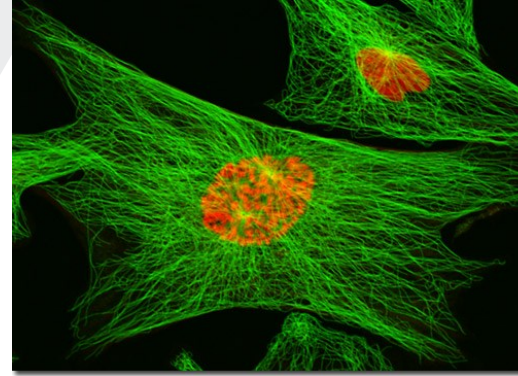
Nanomechanical mapping of living cells

Motivation

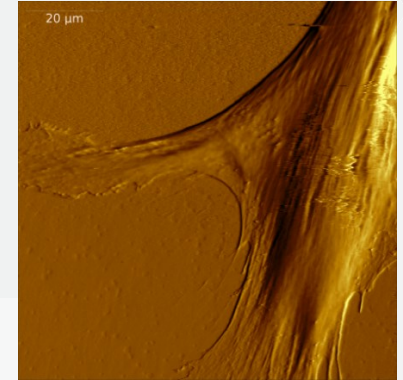
Optical microscopy



Confocal microscopy



AFM



Young's modulus mapping



Motivation

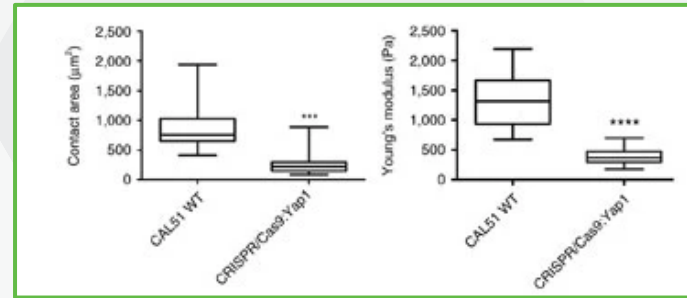
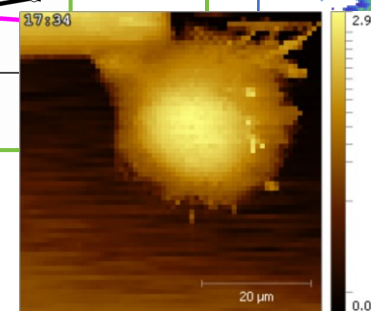
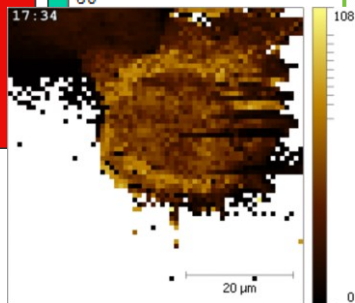
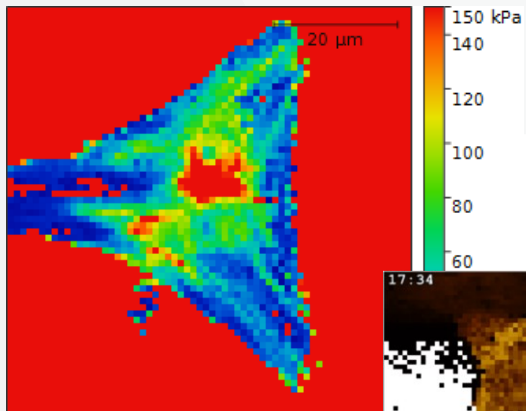
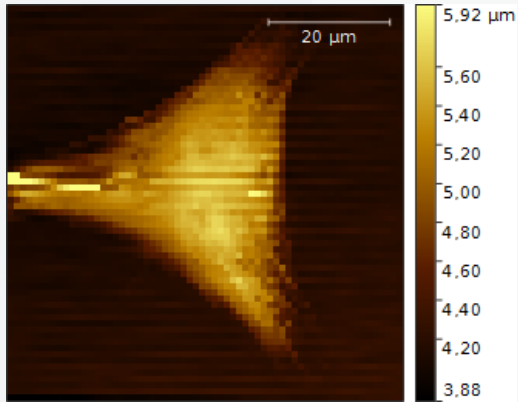
Why to quantify elasticity of (living) objects?

- **Stiffness** (Young's modulus) mapping
→ stiffness = basic parameter of any material
- **Elasticity-phenotype** relation ship
- **Mechanobiological** characterization
- **Driving of instrument** properties (QNM, QI)

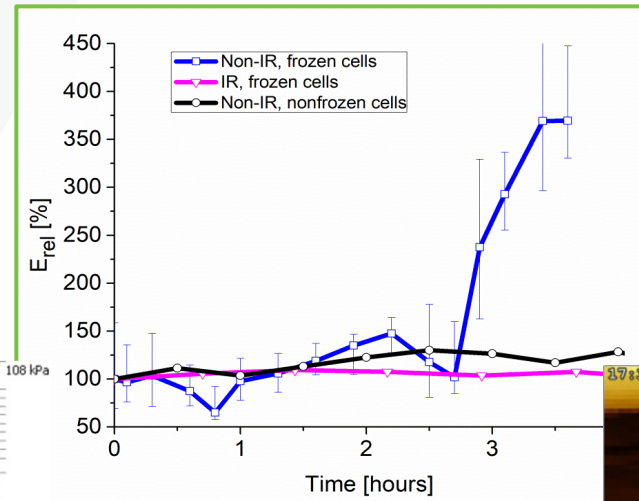
Cellular nanomechanics

By means of AFM

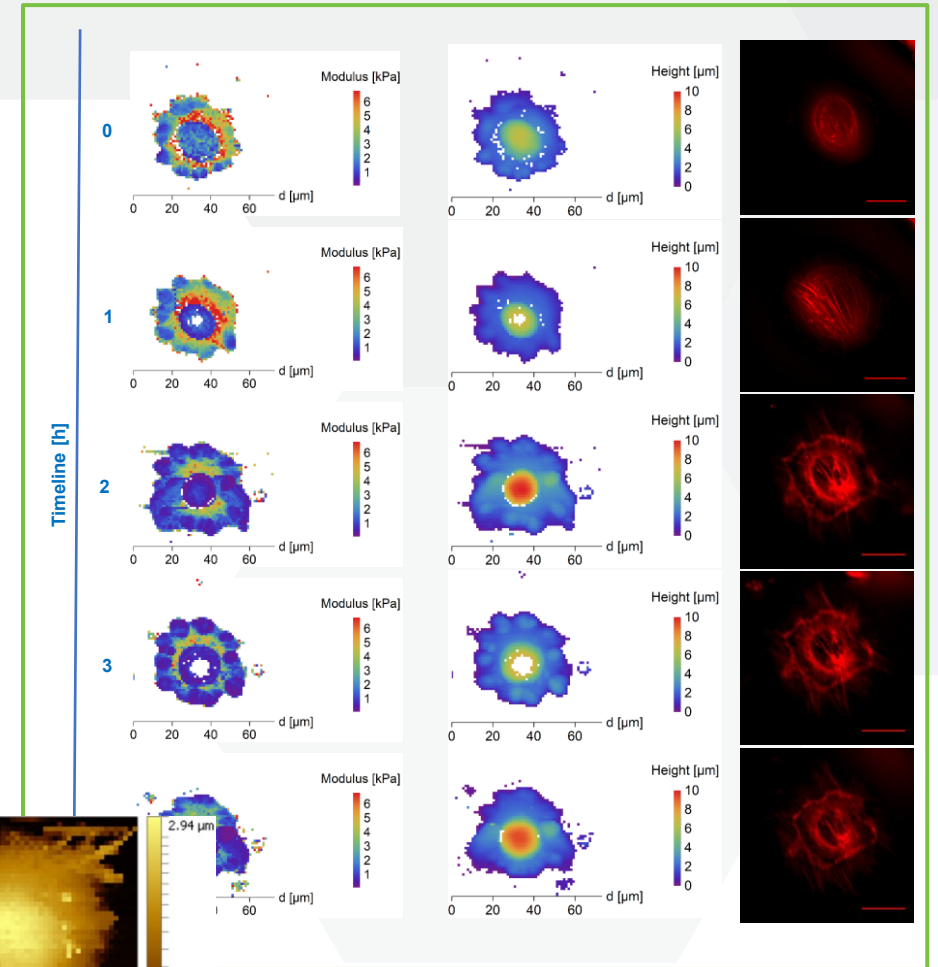
AFM mapping - correlation with fluorescence microscopy



Evaluation - statistics

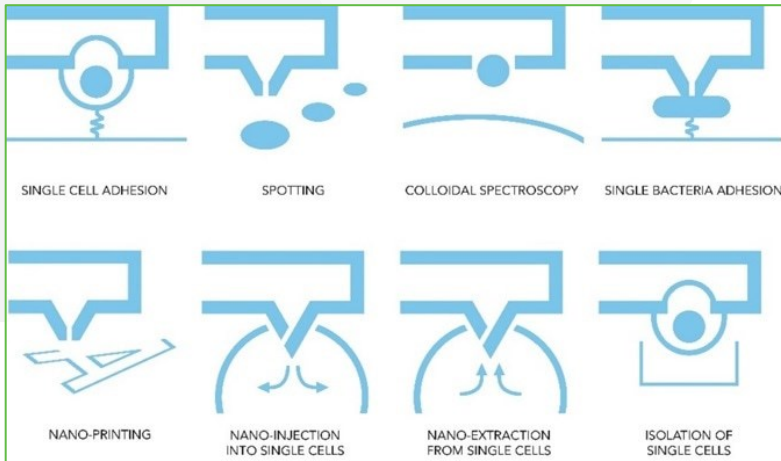
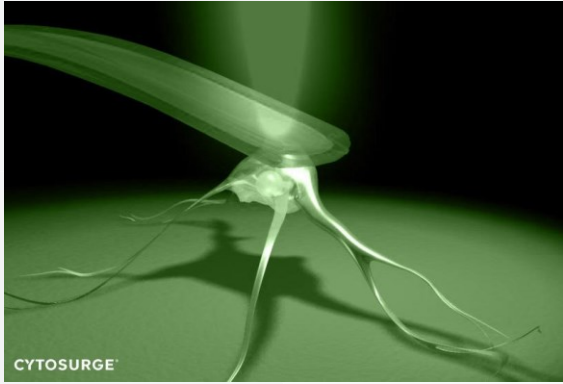


Time-lapsed biomechanics

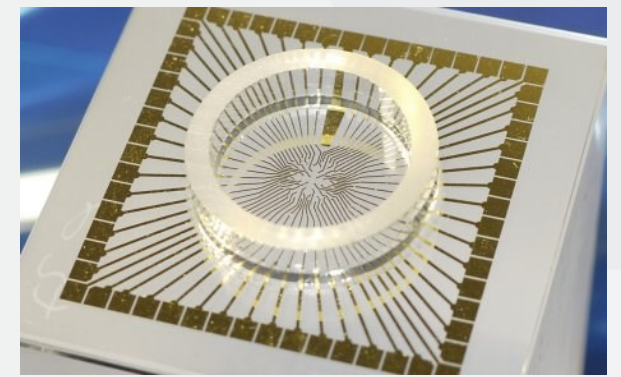
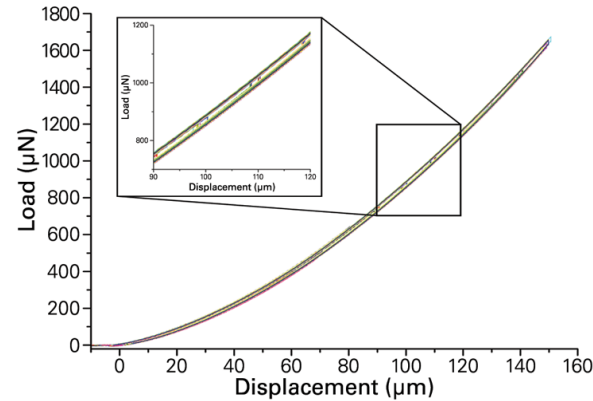
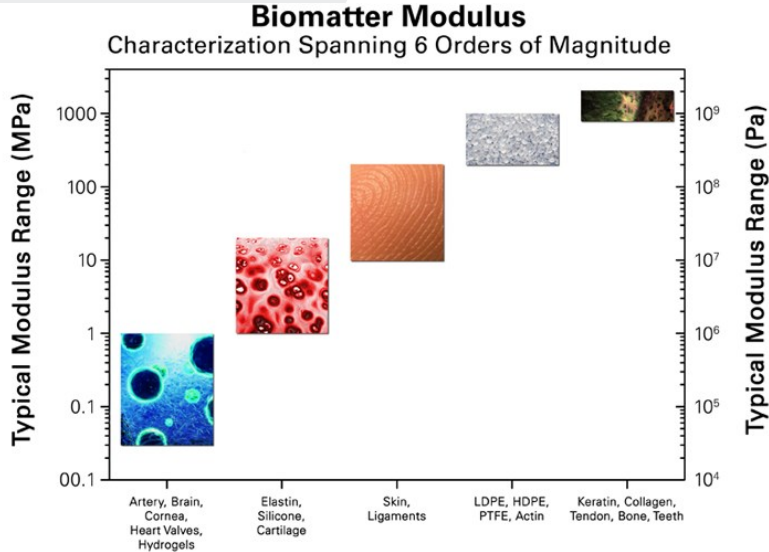


Combination with other techniques

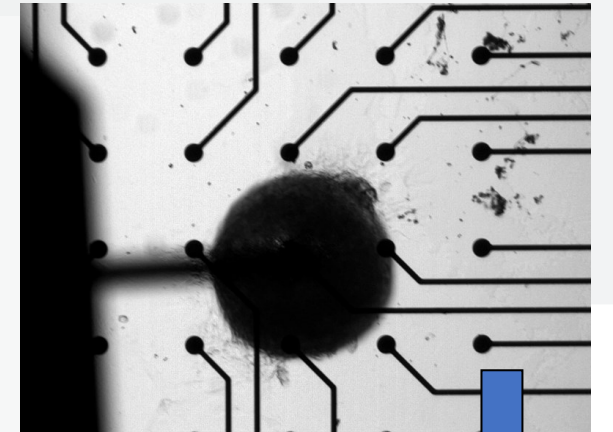
CytoSurge Fluid FM module



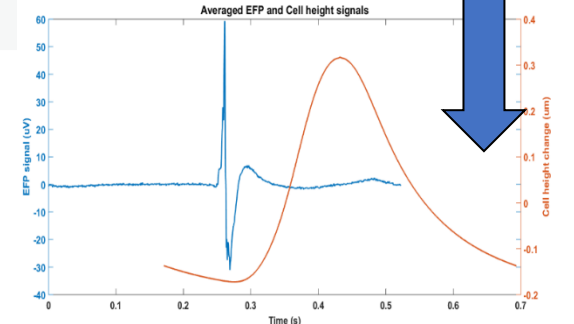
NanoIndentation Single point indentation curves



MultiElectrode Array Extracellular Cell Potential Cardiac cells and Neurons

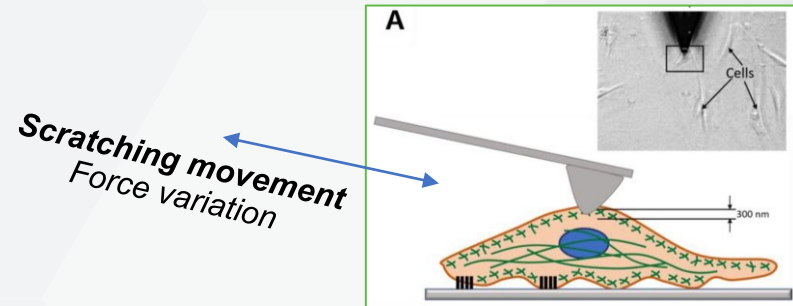


AFM + MEA = electromechanical (de)coupling

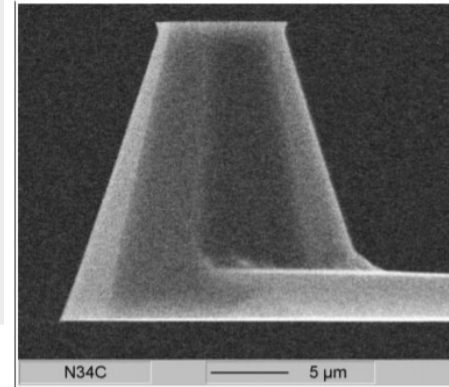


Other applications of AFM-based biomechanics

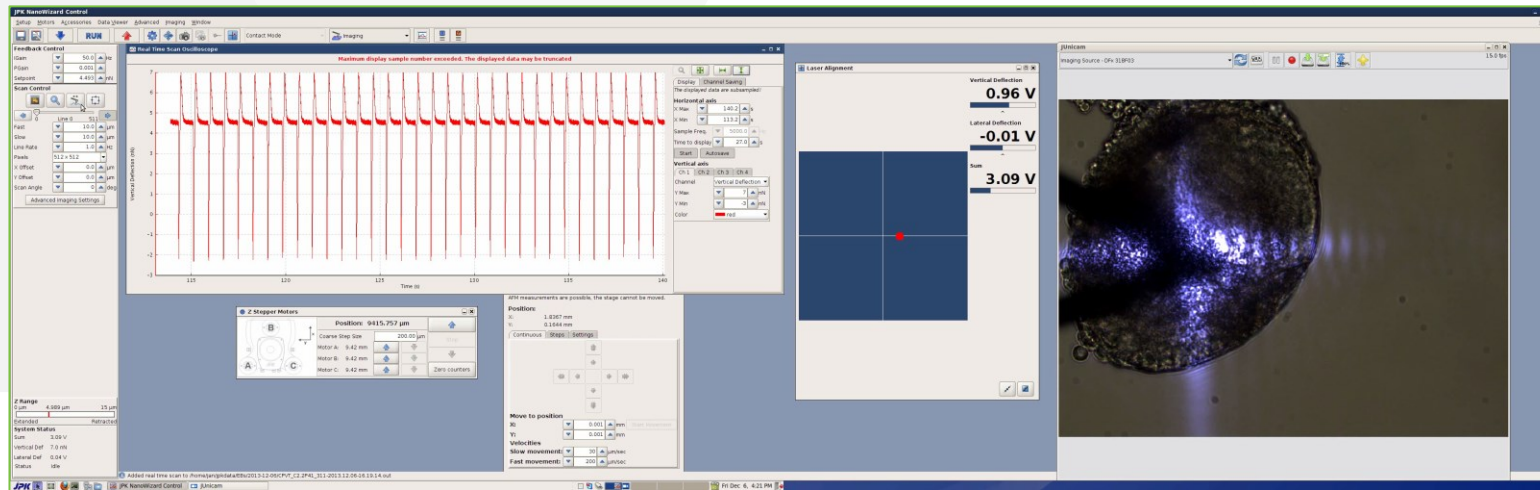
Cell scratching = cell adhesion



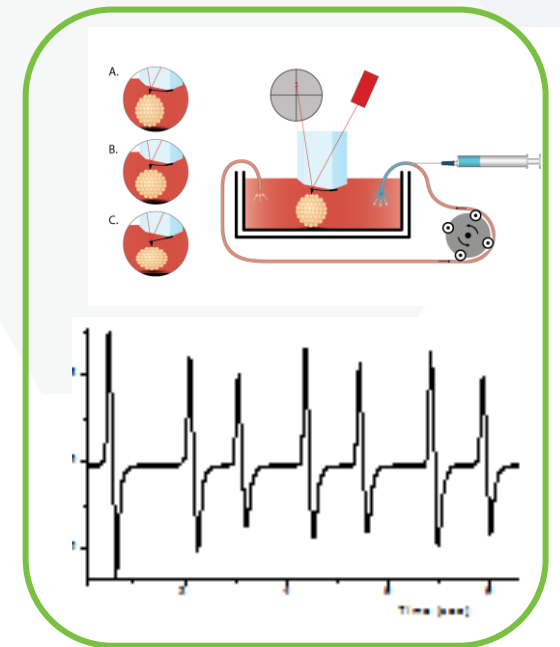
Large Plateau AFM Tips



Cardiac cells biomechanics



Setup scheme

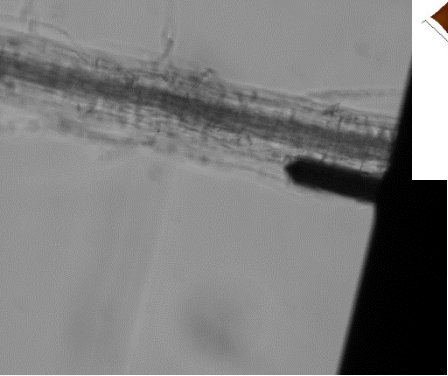
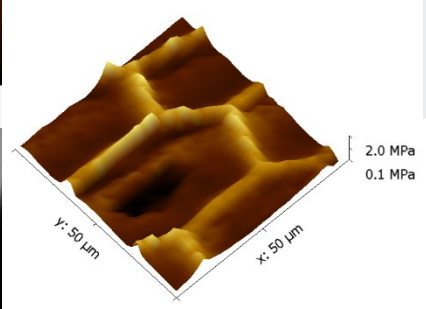
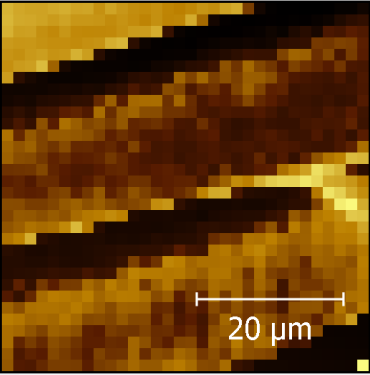


Pesl M, Pribyl J, et al. 2016 *Biosensors and Bioelectronics* **85** 751–7

Pesl M, Pribyl J, et al. 2016 *J Mol Recognit* n/a-n/a

Pesl M, Acimovic I, Pribyl J, et al. 2014 *Heart Vessels* 29 834–46

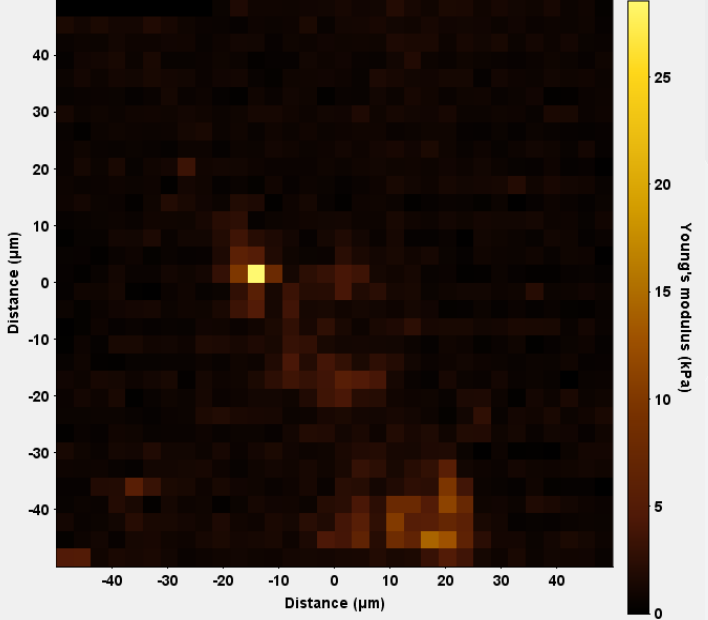
AFM-based biomechanics



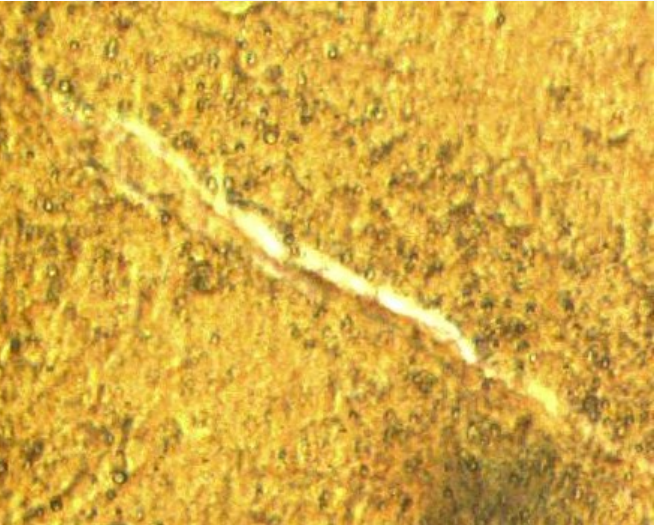
Plant samples
(*hypochoerid*)



by **Marçal Gallemí**
Eva Benkova Lab
& Jan Hejtko Lab



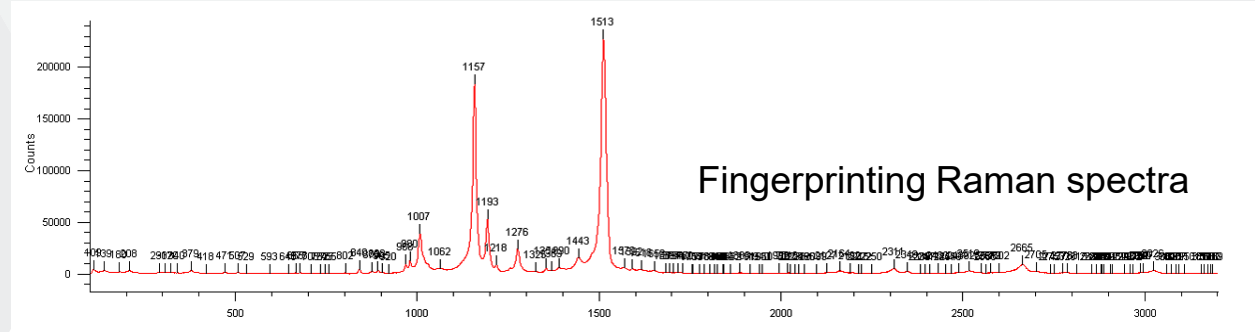
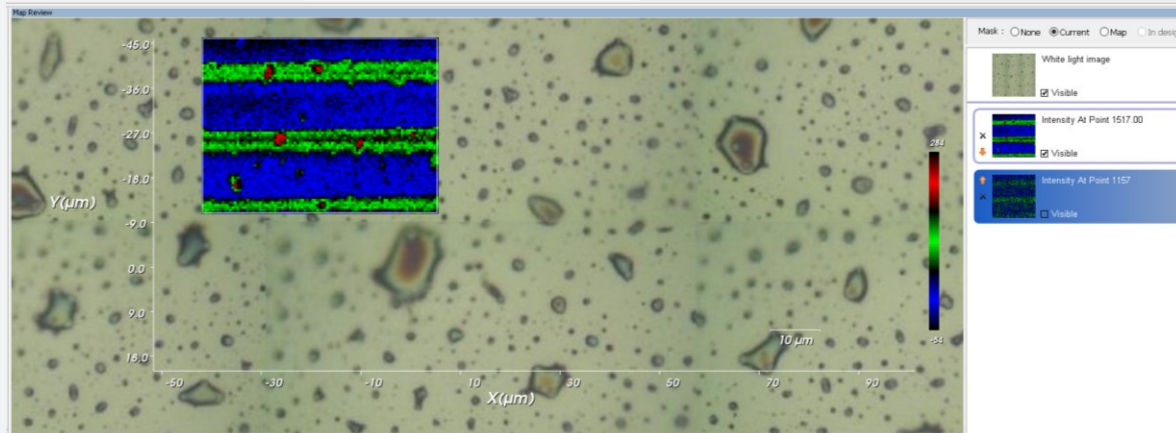
Liver cirrhosis
Correlation of
Collagen fibers by polarized microscopy
AFM nanoindentation



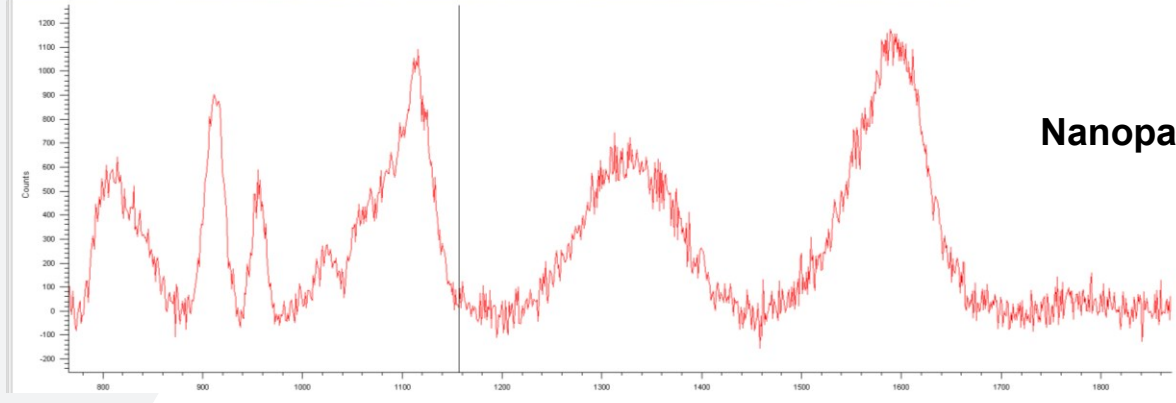
by **Srikant Ojha**
Martin Gregor Lab

Raman microscopy

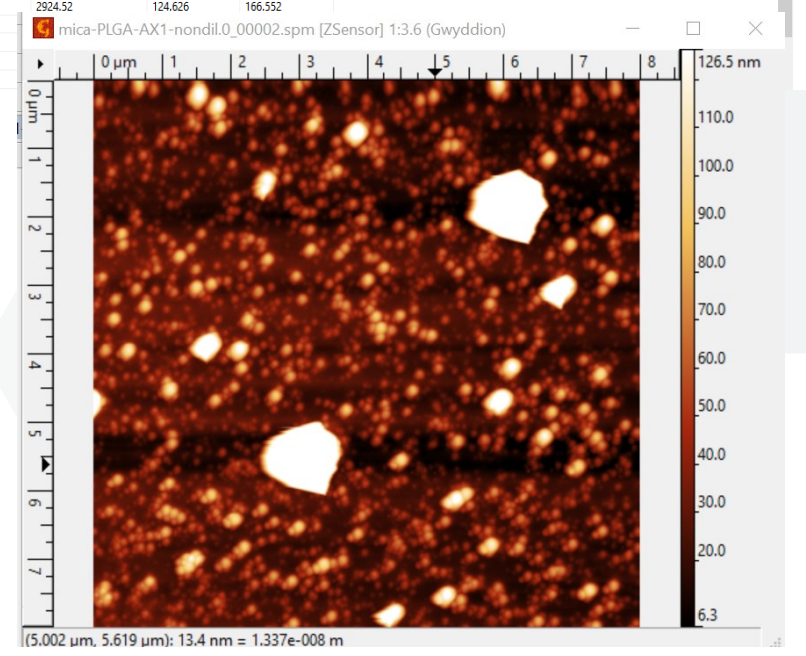
Chemical mapping



Peak no.	Centre	Height	Width	Area	Absolute intensity	Low edge	High edge
1	108.873	3059.03	9.28978	78556.2	4036.61	102.247	123.312
2	139.016	1668.17	14.5499	122921	2924.52	124.626	166.552
3	180.065	804.539	9.00485	55816.4			
4	208.109	1870.6	14.0121	110061			
5	291.483	692.559	7.83973	51650.1			
6	306.84	476.022	6.30329	31306.7			



Nanoparticles loading study

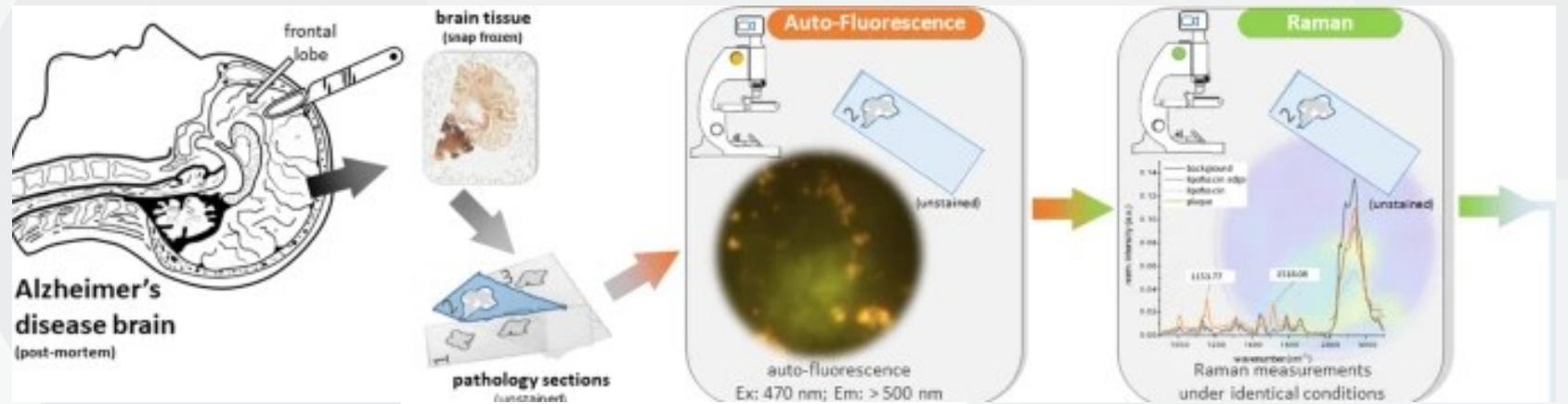


+ combination with AFM topography

Raman microscopy

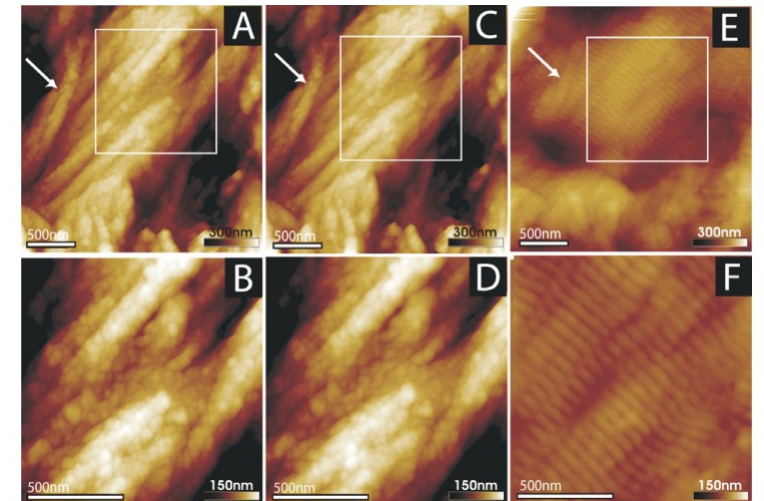
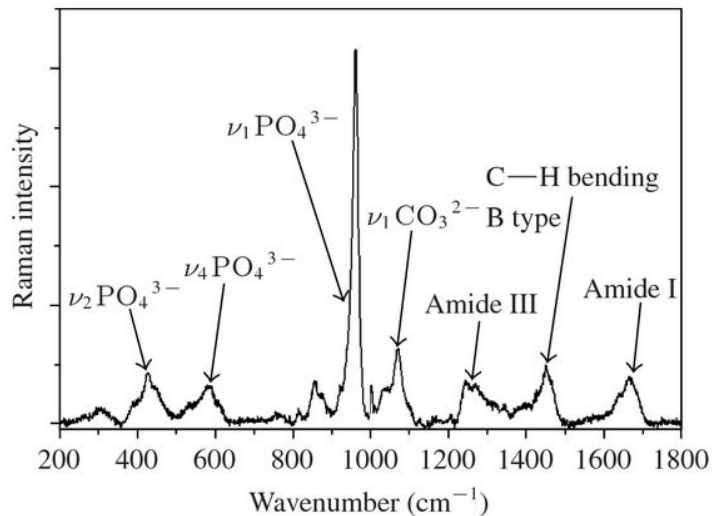
On bio samples

Lochocki, B., Boon, B.D.C., Verheul, S.R. *et al.* Multimodal, label-free fluorescence and Raman imaging of amyloid deposits in snap-frozen Alzheimer's disease human brain tissue. *Commun Biol* **4**, 474 (2021).



Raman imaging of **amyloid** deposits in snap-frozen **Alzheimer's disease** human brain tissue

Calcification level and Collagen Fibers Arrangement in Bone Tissue

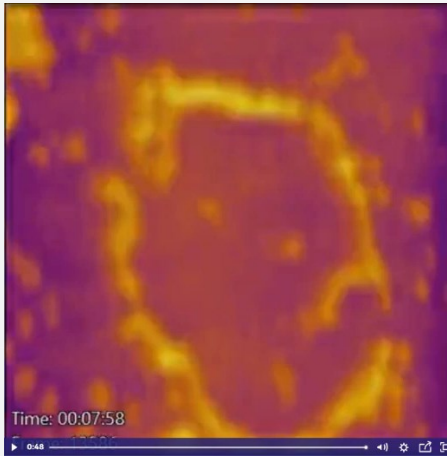


+ combination with AFM topography



Where to go next...?

1. High-Speed (Video-Rate) AFM



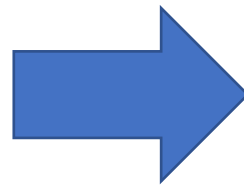
Size: **425 nm**, line scan rate of **1250 lines/second**
160×32 pixels, (**28 fps**). The playback rate 10×
Source: <https://afm.oxinst.com/Video-Rate-AFM-Movies?wvideo=abneb64e3y>

Asylum Cypher VRS1250
Video-Rate High-Speed AFM



Prices from 500 tEUR

Bruker MultiMode 8HR upgrade



The screenshot shows the EPFL website with a navigation menu at the top. The main content area is titled 'Open Hardware' and features a sidebar with links to 'Research', 'Open Hardware', 'Equipment', 'Publications', 'Teaching', 'Join LBNI', 'Funding', 'People', and 'Contact'. The 'Open Hardware' section contains the text: 'We believe that the concept of open science should go beyond publishing papers in open access journals and sharing source code.' Below this, there are two highlighted boxes: one for 'AFM head for small cantilevers, with photothermal drive' and another for 'SPM Controller/Software'. The URL <https://www.epfl.ch/labs/lbni/openhardware/> is displayed at the bottom of the screenshot.

<https://www.epfl.ch/labs/lbni/openhardware/>

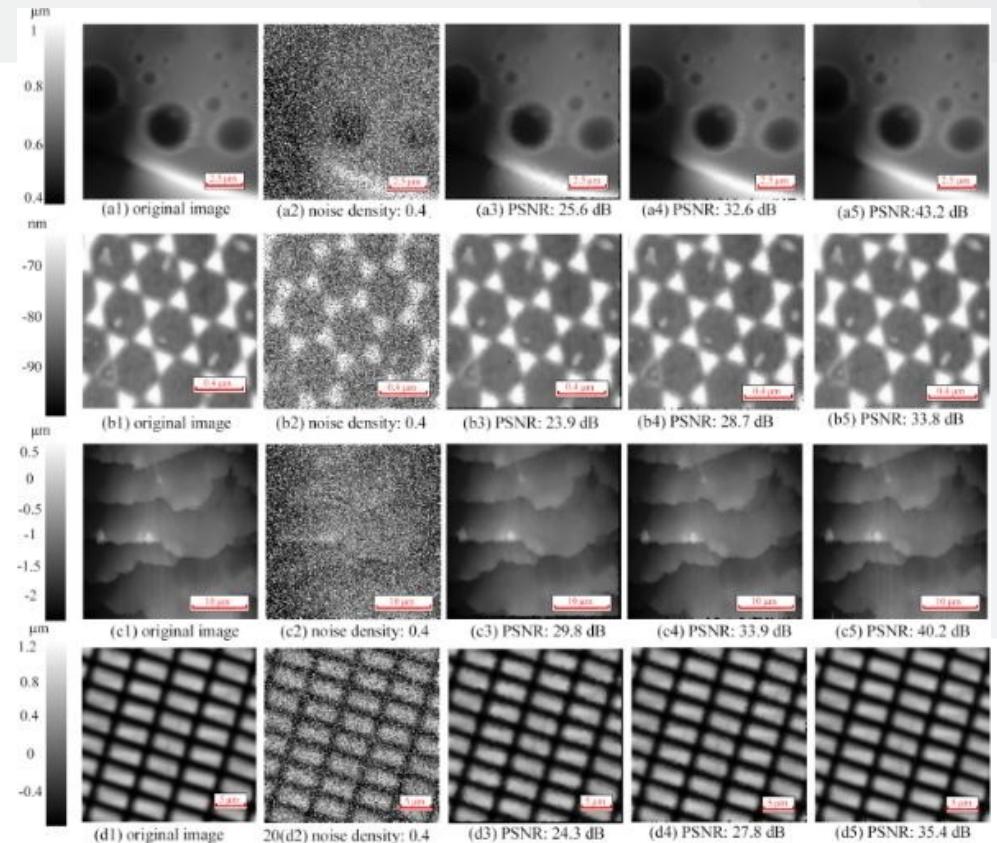
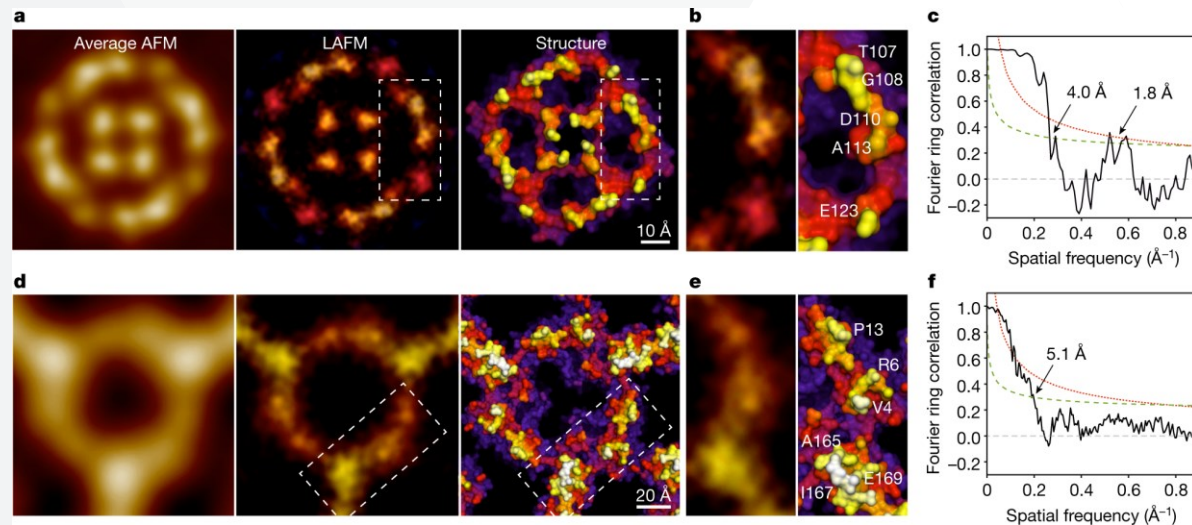
Price 65 tEUR

Advanced data processing

Deconvolution image processing

Denoising with AI:

Deconvolution filters – 50-100 images
input (HS-AFM):



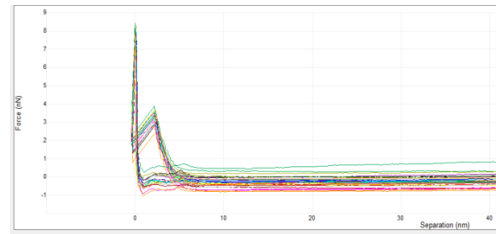
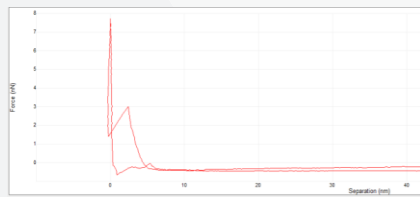
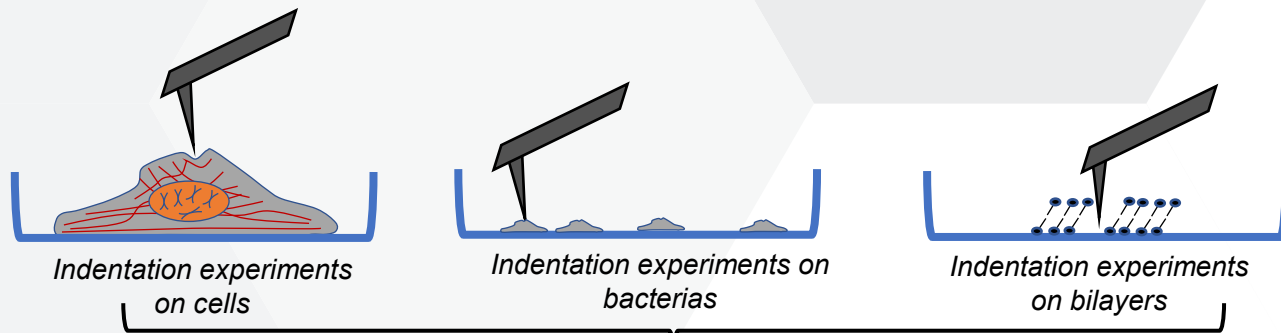
Beilstein J. Nanotechnol. 2019, 10, 2346–2356.

Heath, G.R., Kots, E., Robertson, J.L. *et al.* Nature 594, 385–390 (2021).

Advanced data processing

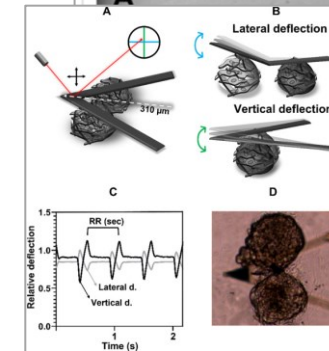
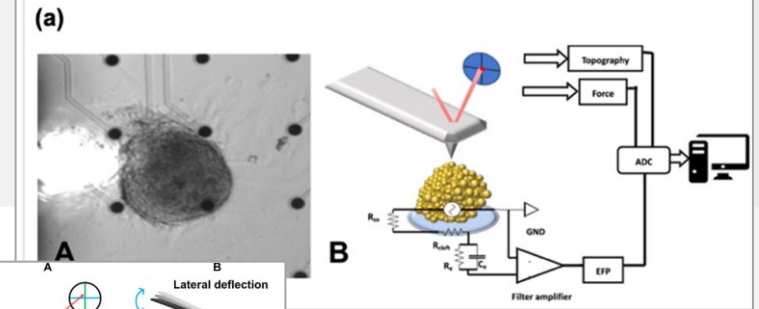
Mechanobiological data processing

Indentation data processing



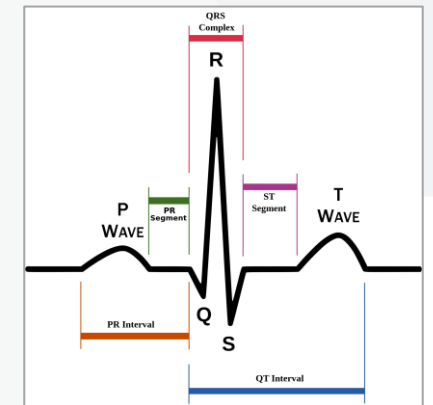
Data processing

Deep Data Correlation (Machine learning, ...)



Mechanobiological+
electrophysiological data
– cell models

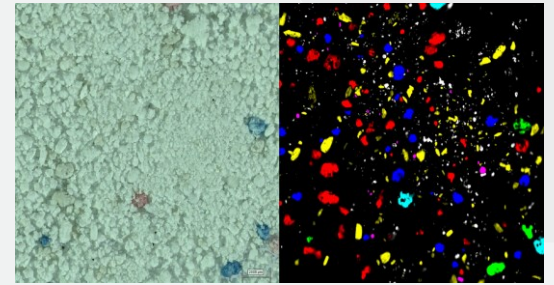
ECG – patient's data



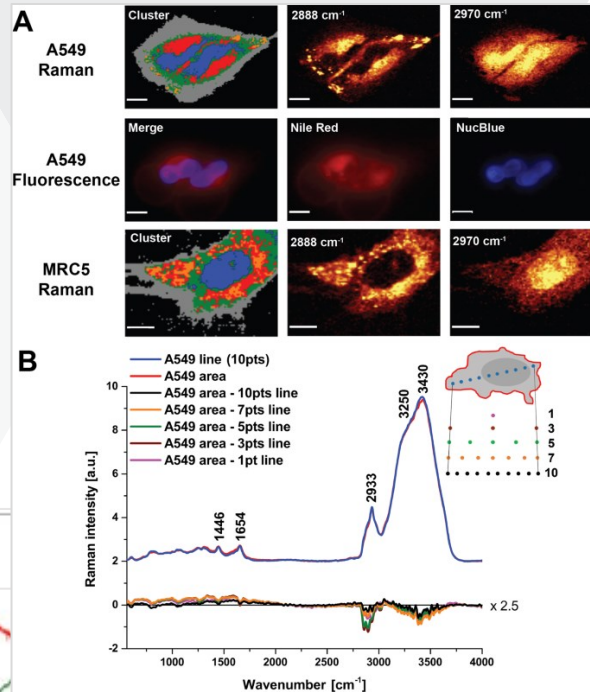
AI/ML – data correlation

2. Raman microscope upgrade

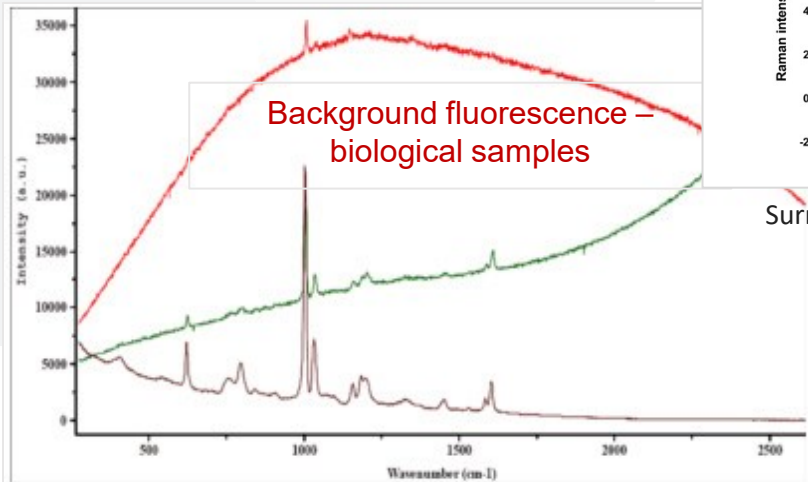
Renishaw InVia Raman microscope



White light and Raman images of powder



Surmacki, J.M., et al. *Sci Rep* **8**, 12604 (2018).



<https://www.azom.com/article.aspx?ArticleID=2950>

Raman part - upgrade

+ laser 785 nm, 100 mW (fluorescence decrease)

+ 1 x 785 nm polarization

+ 1 x 633 nm polarization

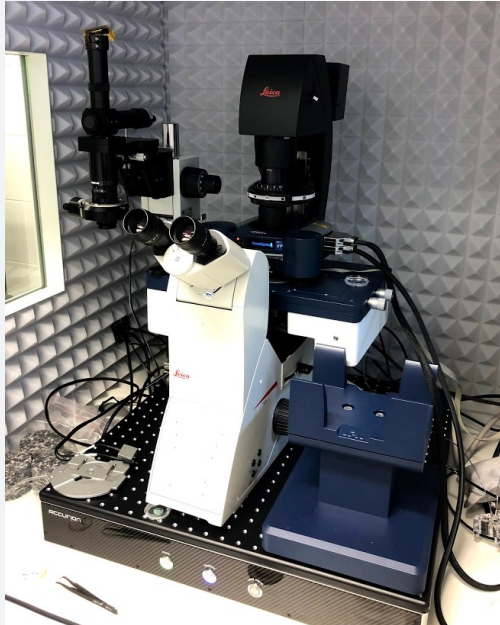
+ Software upgrade to version **WiRE 5.6**

(incl. **particle analysis** and spectral database modules – **microplastics**)

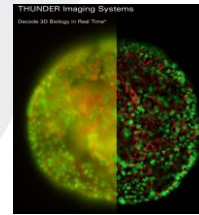
Full integration with AFM

- AFM correlative software
- AFM microscope antivibration solution

3. Leica Microscope Upgrade



Leica DMI8
(combined with JPK NW4XP)



Leica THUNDER Imaging Systems

Easily tackle biologically relevant 3D models with THUNDER Imagers.

They bring you high-speed, multicolor imaging of thin and thick samples with increased temporal resolution in the first attempt itself.

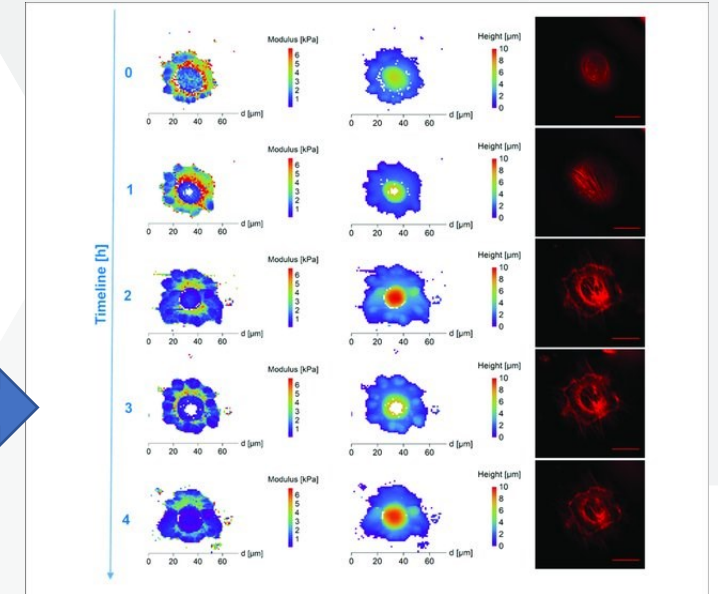
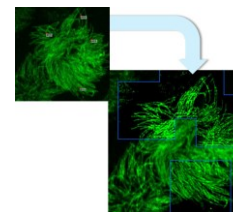
+



Telight LiveCodim

From conventional to super-resolution microscopy

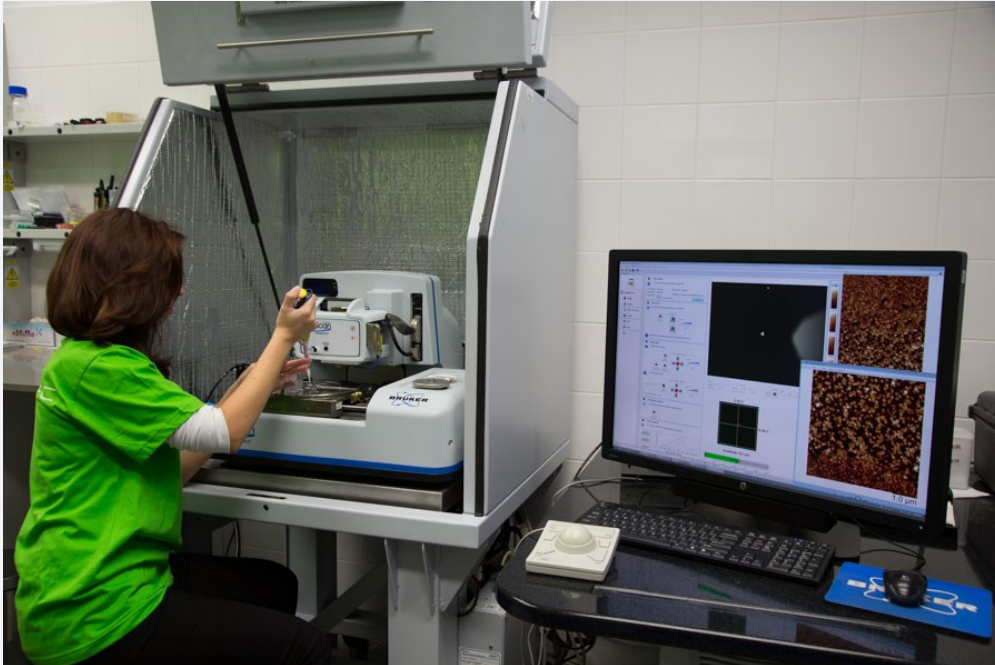
LiveCodim is a universal, super-resolution imaging platform, designed to interface with any standard fluorescence microscope. It is the solution for live imaging with high resolution and low phototoxicity.



AFM – confocal correlation microscopy

Price 120 tEUR

4. Bruker Dimension FastScan Upgrade



In everyday operation from 2014



AFM controller upgrade:

ATOMIC FORCE MICROSCOPY

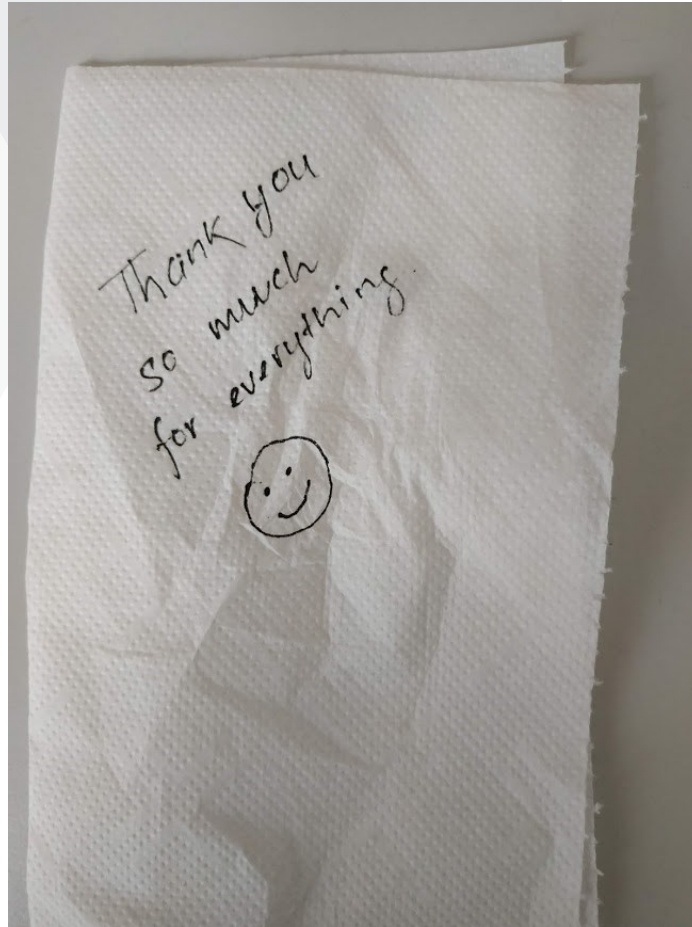
NanoScope 6 Controller

Unmatched power and capabilities for Dimension Icon®, FastScan®, XR, HPI, Pro, and MultiMode® 8HR AFMs



Price 80 tEUR

Let's all the measurements end up with this...



Acknowledgment text - CIISB

- Preferred version: „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.“
- Short version: „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).“



CEITEC

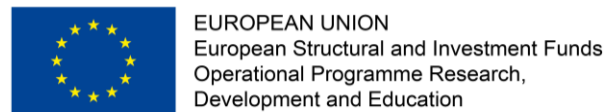


@CEITEC_Brno



Thank you for your attention.

Web: ceitec.eu/nanobio



OP VVV CZ.02.1.01/0.0/0.0/18_046/0015974

Thank you for your attention!

Cytoplasmic live-cell biopsies for the temporal profiling of single-cells

Go beyond in single cell manipulation

Gentle and accurate single-cell injection and cytoplasmic biopsies

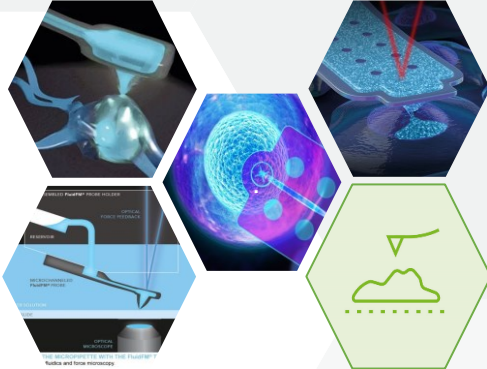
Fluidic force microscopy, or **FluidFM** is a biophysical technique for conducting **single-cell biopsies**. This innovative approach enables the extraction of a part of the cytoplasm from individual living cells while preserving their viability.

These cytoplasmic biopsies can be used for subsequent highly-sensitive, low-input **RNA-seq analysis** to characterize **single cells multiple times** throughout their lifetime.

Moreover, the **FluidFM Nanosyringes** extend their utility by facilitating the **targeted introduction** of various molecular components into cells, including **RNA, DNA**, proteins, and even molecular complexes such as **CRISPR/Cas9 RNPs**.

This functionality streamlines the transfection processes for plasmids and transcription factors and enables entire cell line engineering workflows.

By exploring the capabilities of **FluidFM** in this seminar, we seek to uncover its **potential implications** for advancing the comprehension of intricate cellular processes, thus fostering new dimensions in **cellular analysis** and **molecular investigation**.



Register [here](#)

CYTOSURGE®
FluidFM®

October 18th
2023
University Campus Bohunice
Brno, Czech Republic
building E35, room 145

**Core Facility
Nanobiotechnology**
CEITEC MU, Masaryk university
Kamenice 5, Brno, Czech
Republic

jan.pribyl@ceitec.muni.cz
Web: www.ceitec.eu/nanobio

