



Central European Institute of Technology
BRNO | CZECH REPUBLIC

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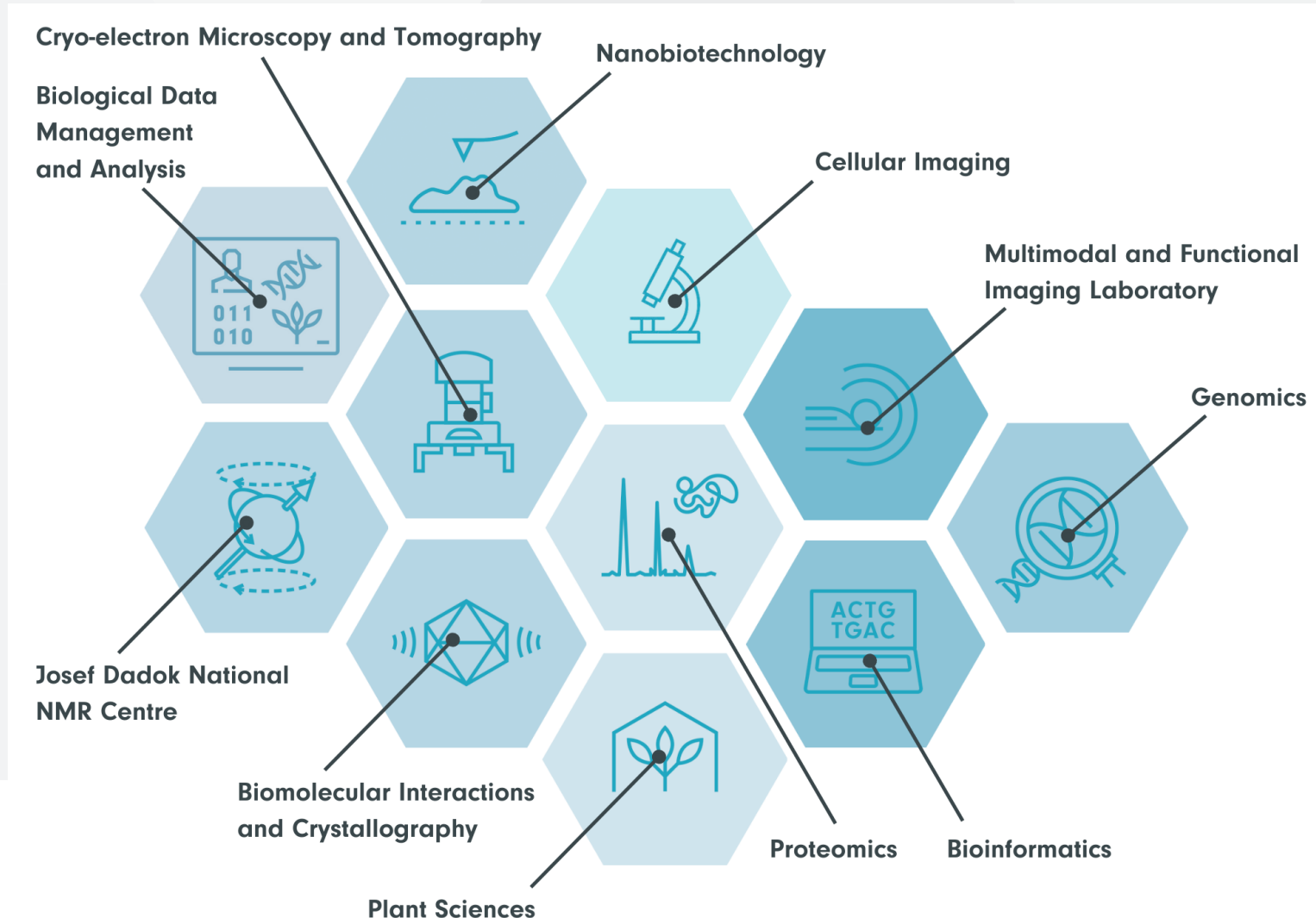
Core Facility Nanobiotechnology

The logo for Masaryk University (MUNI) is displayed in white, uppercase, sans-serif font. The letters are widely spaced and positioned in the upper right area of the slide, partially overlapping the image of the building.

MUNI

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

Core Facilities at CEITEC Masaryk University



Core facilities are **TECHNOLOGICAL UNITS** equipped with the **FIRST-RATE EQUIPMENT** available to scientists from a range of fields.

The aim is to provide scientists with **INFRASTRUCTURE** allowing for them to effectively carry out scientific projects without the necessity to buy their own **INSTRUMENTS**.

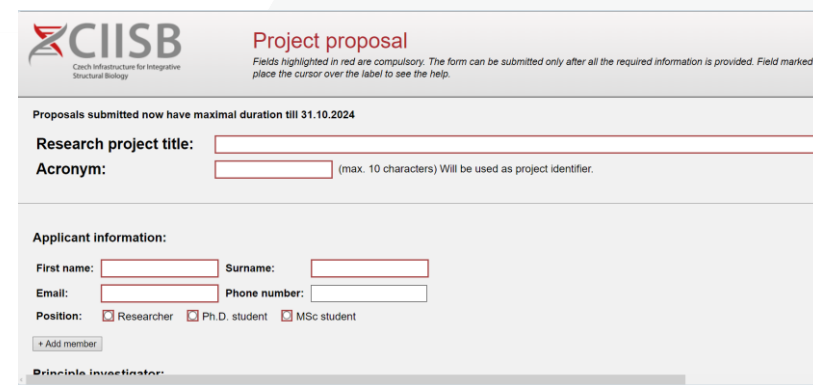
Basic rules

- **OPEN ACCESS** principle.
- Reservation of **INSTRUMENT TIME** or **COMPLETE SERVICE** (plus consultations and training from **CF EXPERTS**)
- Reservation of the instrument/service **BOOKING SYSTEM**
- **NOT FOR FREE** – low prices thanks to CIISB infrastructural project
- <https://www.ceitec.eu/core-facilities/>



The screenshot shows the CEITEC booking system interface. At the top, there are navigation tabs: "Plánovací tabulka", "Seznam rezervací", "Požadavky", and "Infrastruktura". Below this, there is a header "Vybírejte službu pro požadavek" and a sub-header "Other". The main content area lists several services with their descriptions:

- Biomolecules - imaging**: Zobrazení biomolekul (proteiny, DNA, makromolekuly) a jejich komplexů. Standardní podklad – sídla (mica), lze použít i jiné – HOPG, křemík, kovové elektrody, atp. Metody: poklepnový 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 kontaktní mikroskopii (vhodný typ nejprve konzultujte s námi). Fixované (např. PFA) buňky na sklo. Metody – kontaktní mód, QI, PF-QNM, Force Volume. Post-processing a export dat. Možná kombinace s optickou mikroskopií (BF, fluorescence, kontaktní 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 kontaktní 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 mikroskopií (BF, fluorescence, kontaktní 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 senzorů. Možnost dvoukanalových měření, vysoká citlivost, nízký šum. SW Autolab Nova pro analýzu dat.
- SPR biosensor**: Dvoukanalový průtokový SPR (biosenzor využívající metody rezonance povrchového plasmonu. Sledování a charakterizace optických vlastností tenkých vrstev a jejich změn v reálném čase – v kapalině i nauceho. 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**: Zobrazení nano-objektů (nanočástice, nanotrubky, nanodrátky, atp.) a jejich komplexů Standardní podklad – sídla (mica), lze použít i jiné – HOPG, křemík, kovové elektrody, atp. Metody: poklepnový režim, PF-QNM, QI, Force Volume. Vyhodnocení a export dat.



The screenshot shows the CIISB Project proposal form. At the top, there is the CIISB logo and the text "Project proposal". Below this, there is a note: "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." The form contains several sections:

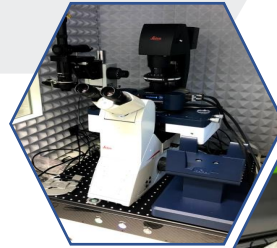
- Proposals submitted now have maximal duration till 31.10.2024**
- Research project title:** [Red highlighted input field]
- Acronym:** [Red highlighted input field] (max. 10 characters) Will be used as project identifier.
- Applicant information:**
 - First name:** [Red highlighted input field]
 - Surname:** [Red highlighted input field]
 - Email:** [Red highlighted input field]
 - Phone number:** [Red highlighted input field]
 - Position:** Researcher Ph.D. student MSc student
 -
- Principal investigator:** [Red highlighted input field]

Core Facility Nanobiotechnology

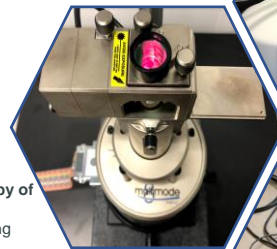
Imaging, mechanical mapping, Raman microscopy



Atomic Force Microscopy of biosamples
Combined with fluorescence microscopy



Atomic Force Microscopy of biosamples
High-resolution imaging



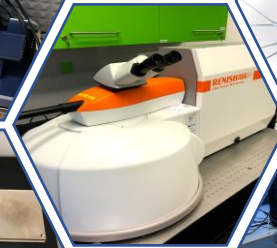
Multielectrode Array (MEA)
Cellular electrophysiology



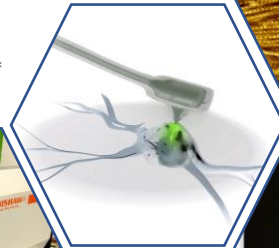
NanoIndenter
Indentation of soft samples



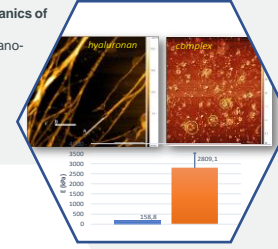
Raman microscopy
Chemical mapping of surface



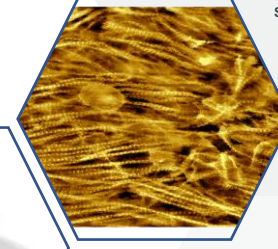
FluidFM
AFM based microfluidics



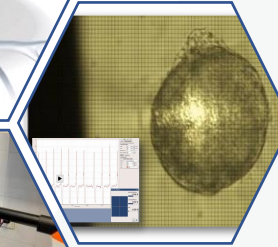
Structure and biomechanics of biomolecules
(AFM imaging and nano-indentation)



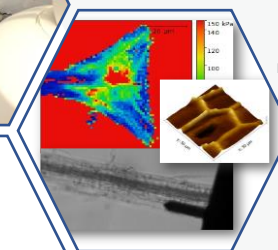
Structure of biomolecules
Under native conditions



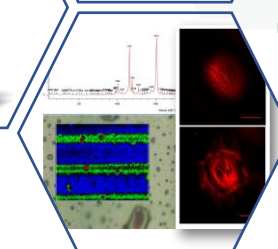
Biomechanics of contractile cells
Toxicology and cell development



Stiffness mapping of cells and tissues
Nanoindentation under semi-physiology



Fluorescence and Raman microscopy
Structural and chemical characterization



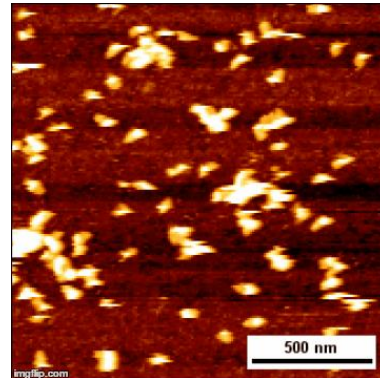
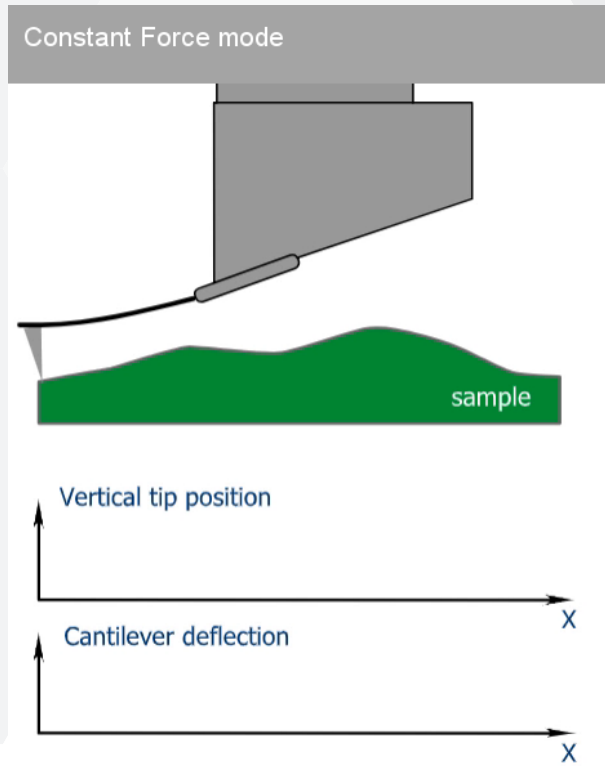
Core Facility NanoBiotechnology

Techniques and Equipment

Atomic Force Microscopy

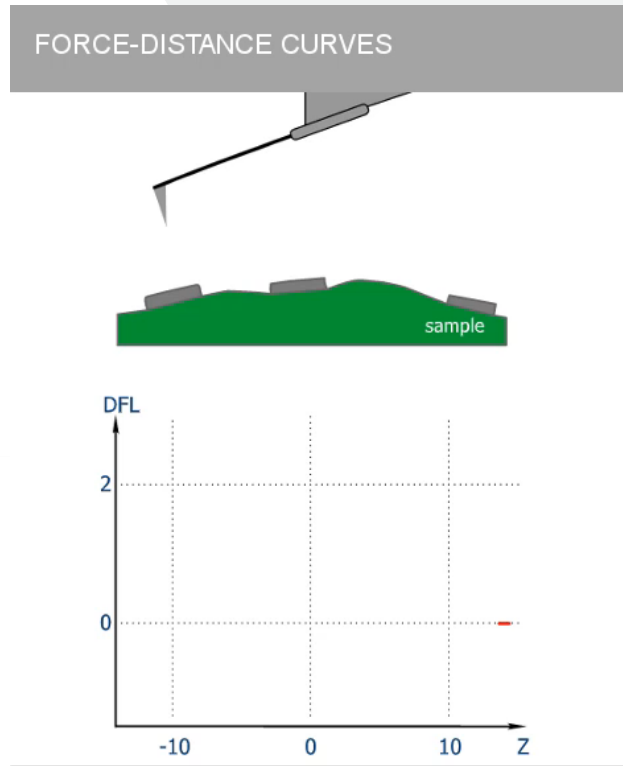
AFM (Atomic Force Microscope)

- SPM= Scanning Probe Microscopy
- Topography, but much more information...



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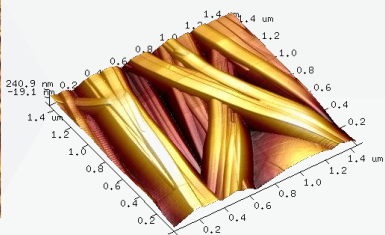
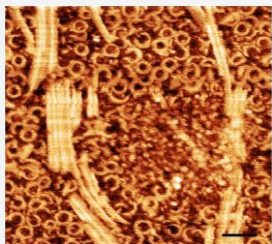


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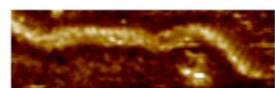
Bio Atomic Force Microscopy (BioAFM)

Non-destructive modes

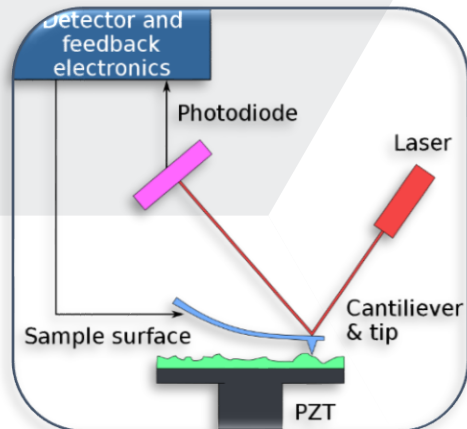
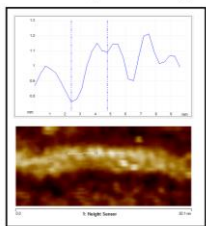


IMAGING

Proteins, DNA, Nanoobjects



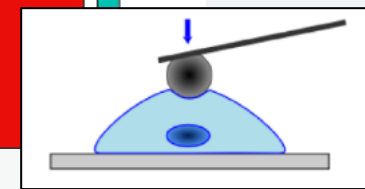
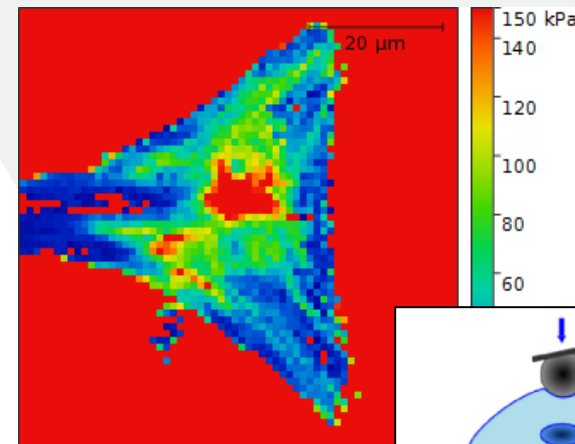
after low-pass filtering



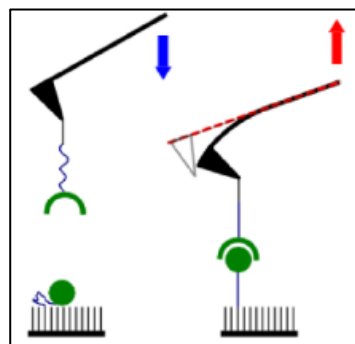
by OverloadQ

NANOINDENTATION

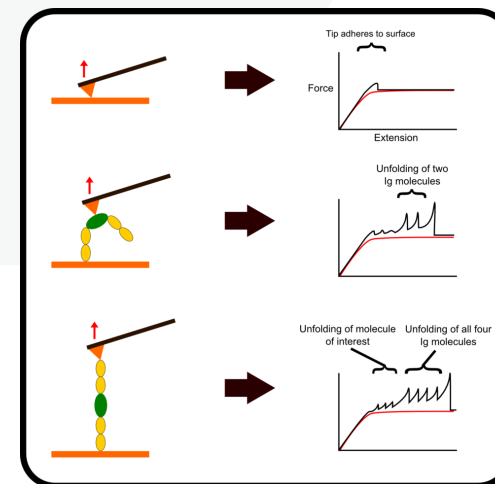
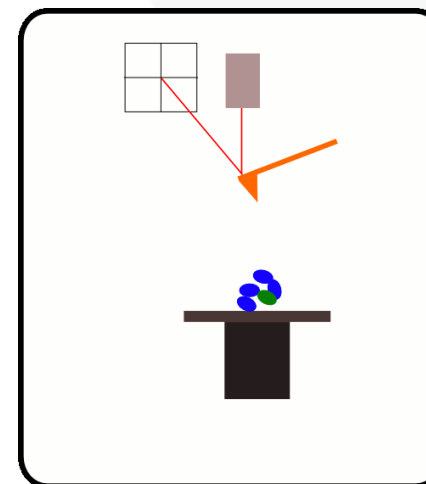
Stiffness mapping



AFFINITY INTERACTION

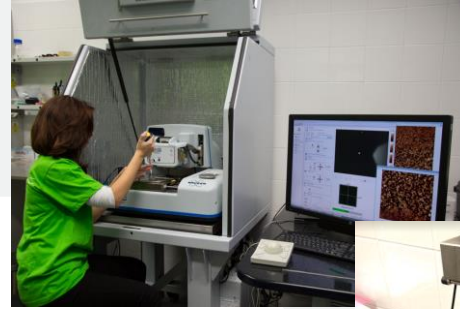
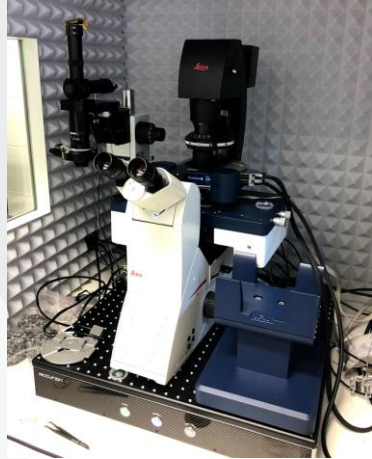
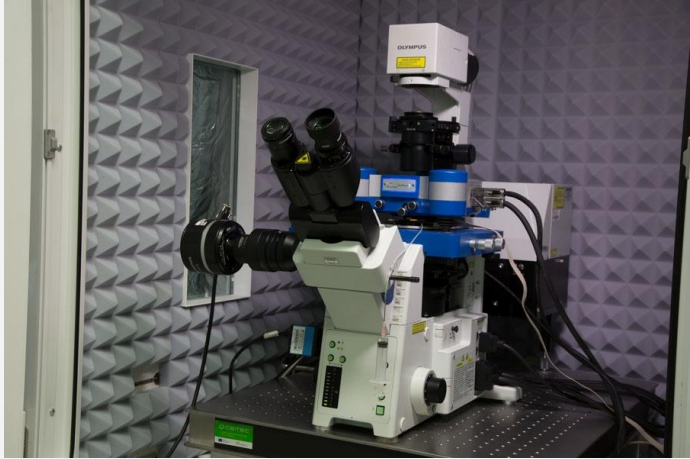


Single-molecule force spectroscopy (SMFS)



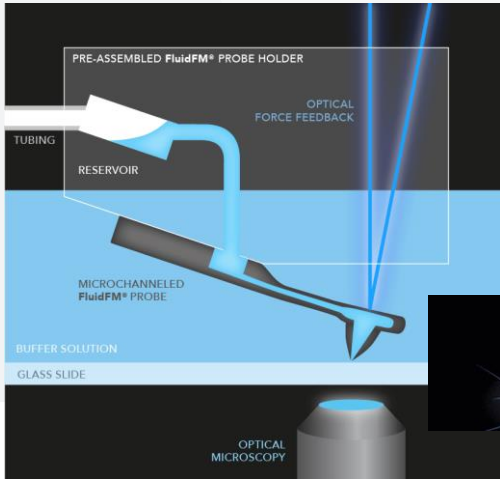
JPK NanoWizard 3 and 4 with extended scanning range

BioAFM – living cells and tissues



BioAFM – molecules,
nanoobjects, molecular
complexes

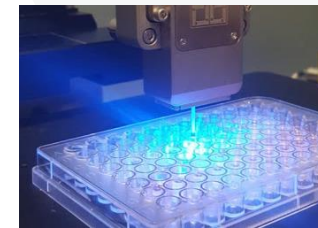
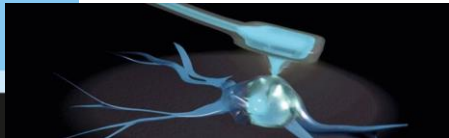
Bruker Dimension
Icon FastScan and
MultiMode 8HR
NTMDT Ntgra Vita



Nanoaspiration, nanodelivery
(single cell), nano3D printing

Cell/organoid
electrophysiology

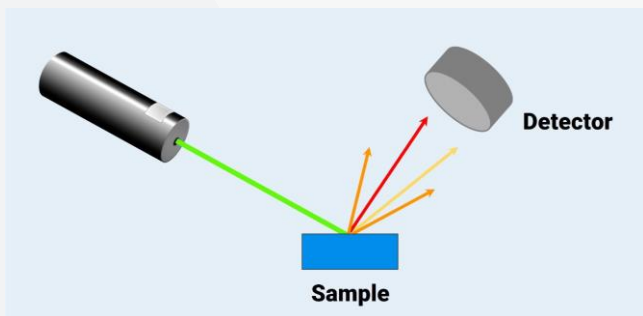
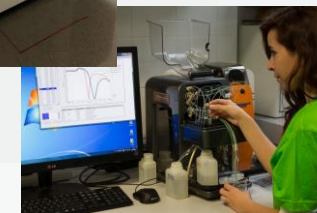
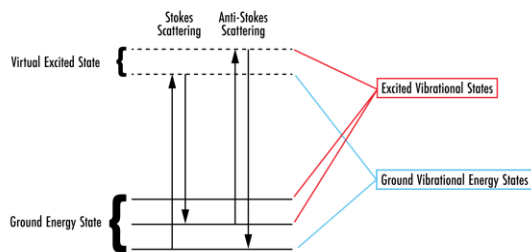
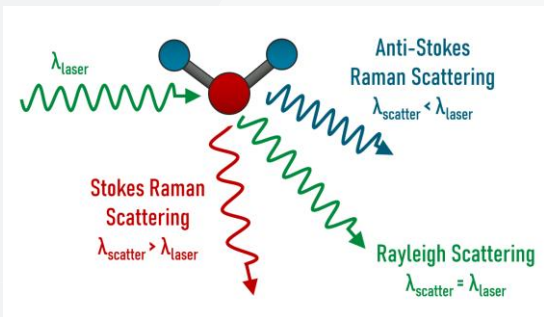
Mechanobiological studies on
tissues, hydrogels, etc.



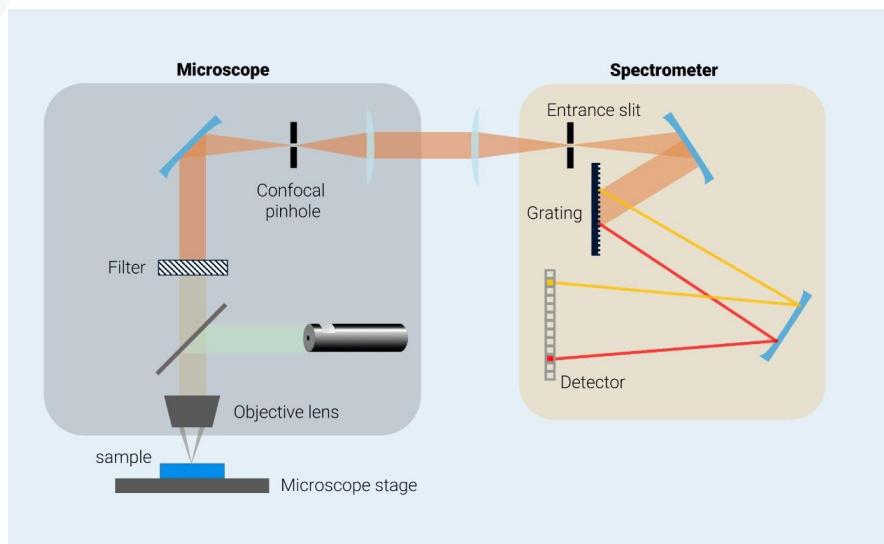
+ CytoSurge FluidFM module
+ MultiElectrode Array (MEA)

+ Biosoft NanoIndenter

Raman microscopy, UPCON readers

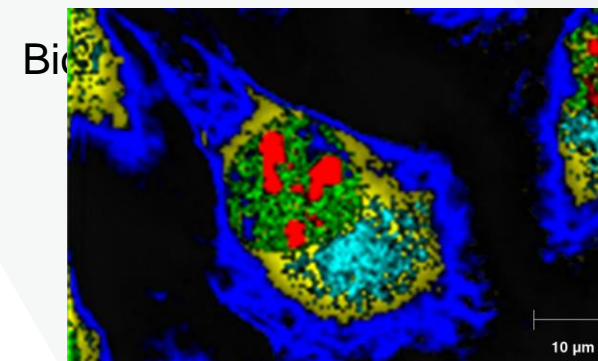


Raman spectroscopy



Raman microscopy

Renishaw InVia Raman microscope



Raman image of human osteosarcoma (bone cancer) cells.

Core Facility NanoBiotechnology

Techniques and Applications

Technology and Expertise

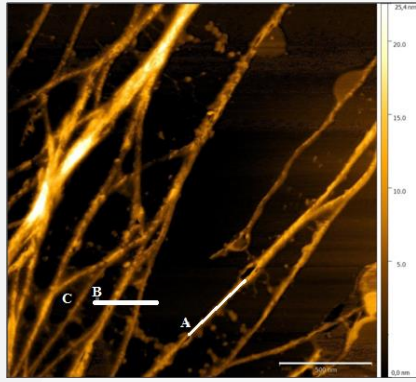
List of services

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

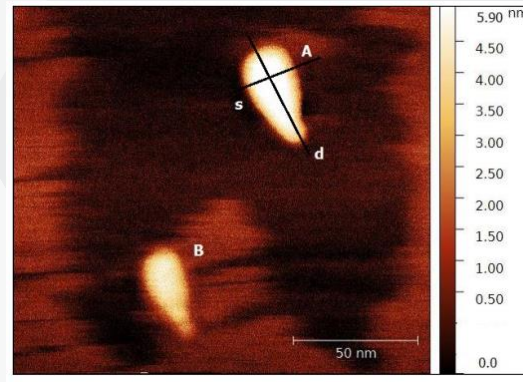
Typical applications

Biomolecules – imaging and biomolecules

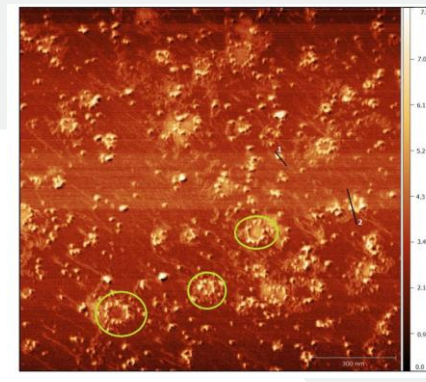


hyaluronan

+

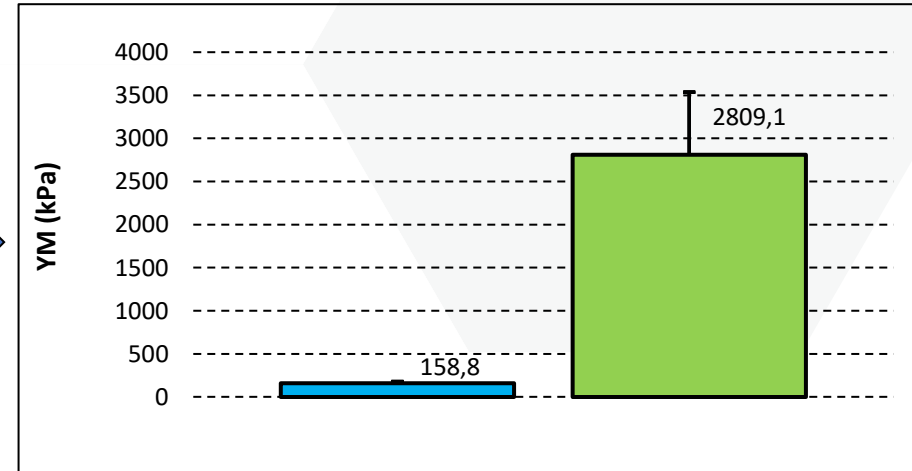
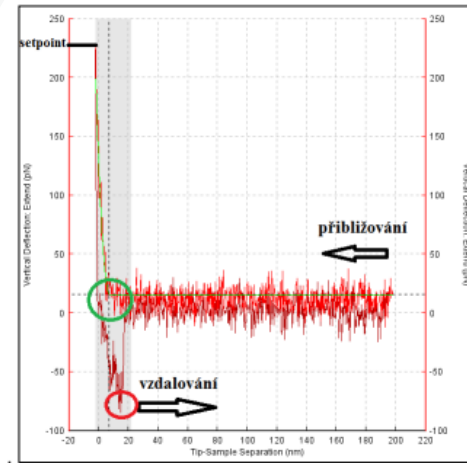
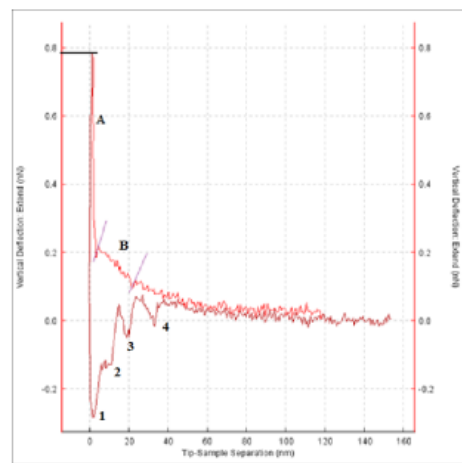


myeloperoxidase



Molecular complex

FD curve recording and analysis

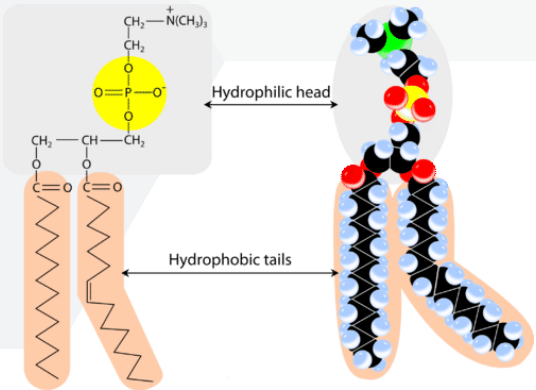
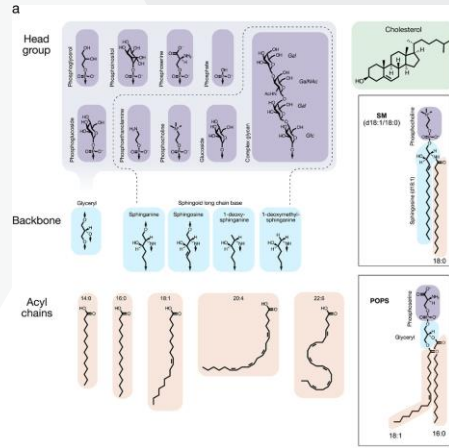
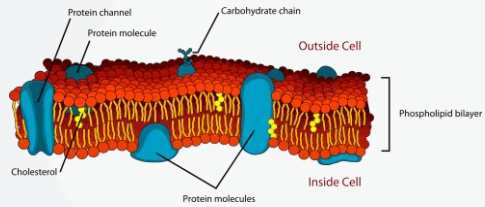


Cell Membrane

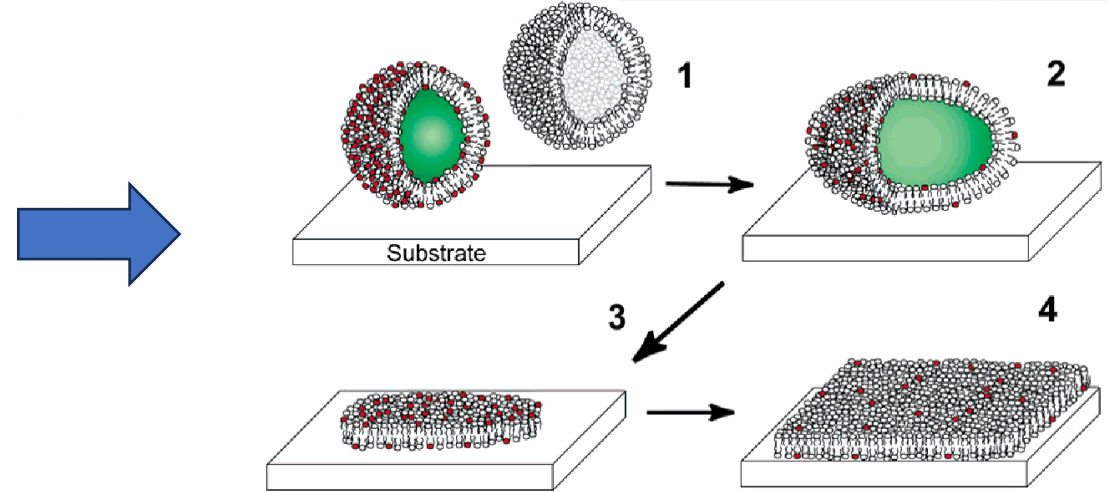
Chemical composition

Synthetic membrane

SLB = Supporting Lipid Bilayer



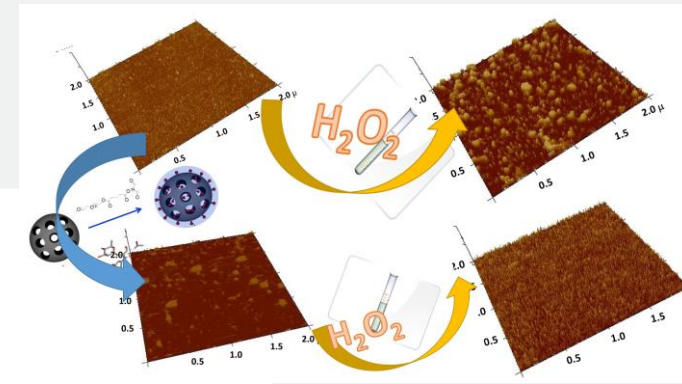
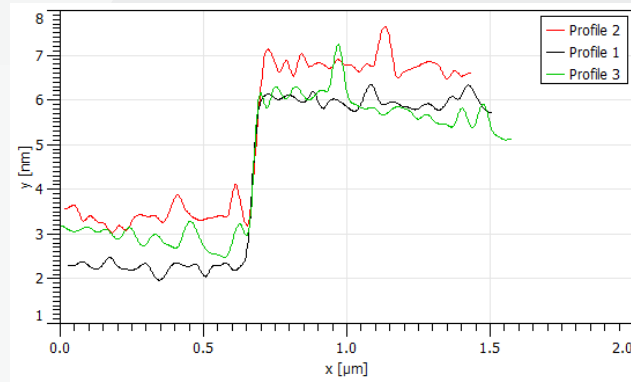
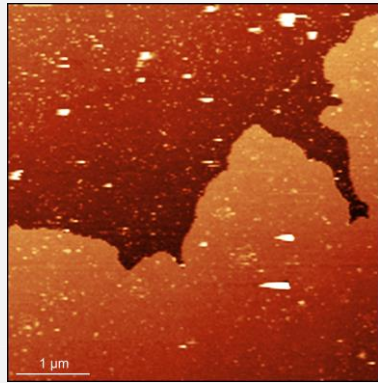
Fusion of vesicles (SUVs) at the surface



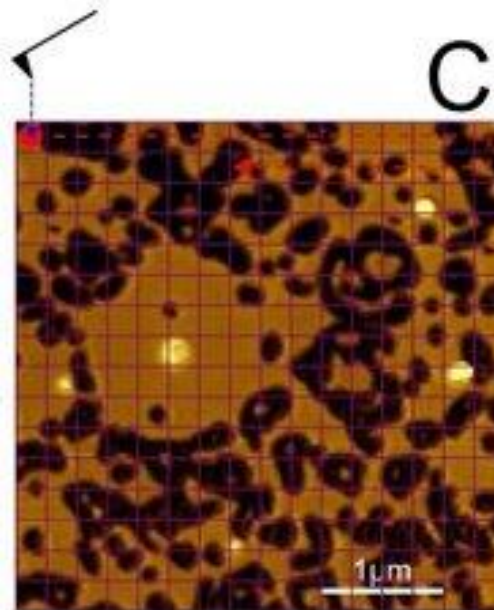
Langmuir 2004 Dec 21;20(26):11600-6
 Bioessays 2021 May;43(5):e2100021

Islands of bilayers

Non-compact / disrupted SLBs



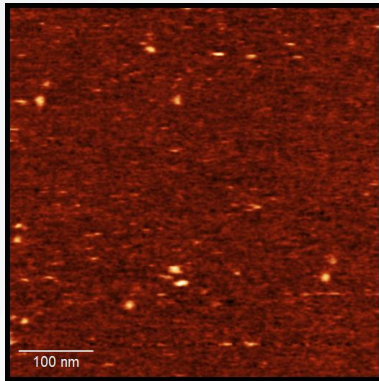
Oxidative stress



Pores

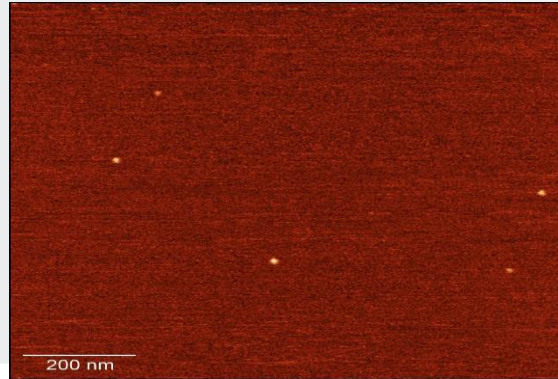
Peptide disruption of SLBs

Compact bilayers

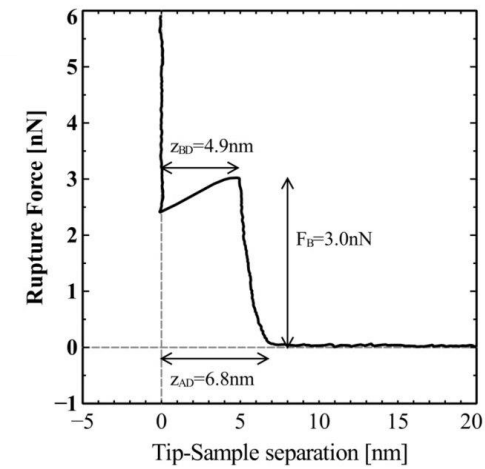
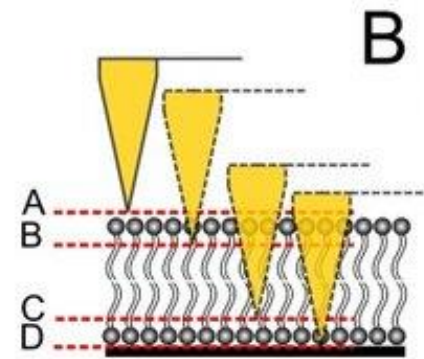
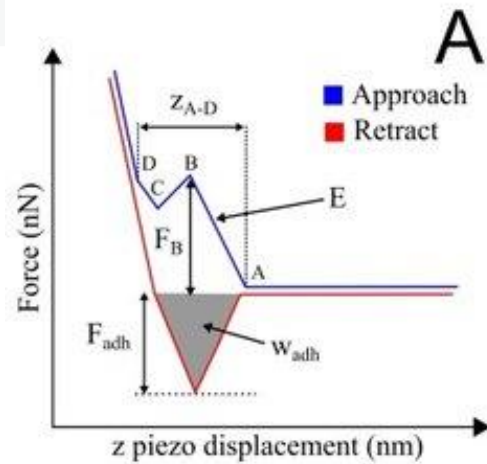
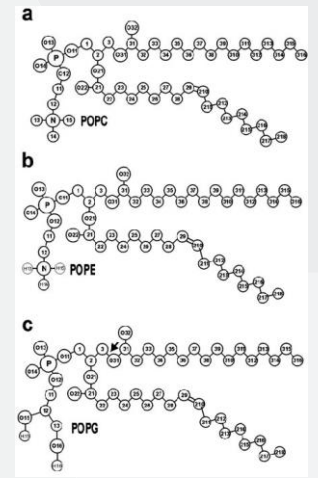
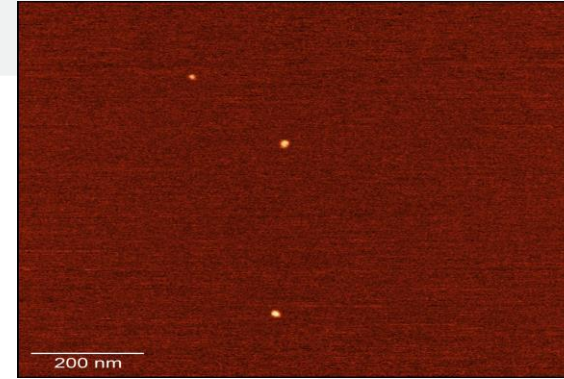


Mica

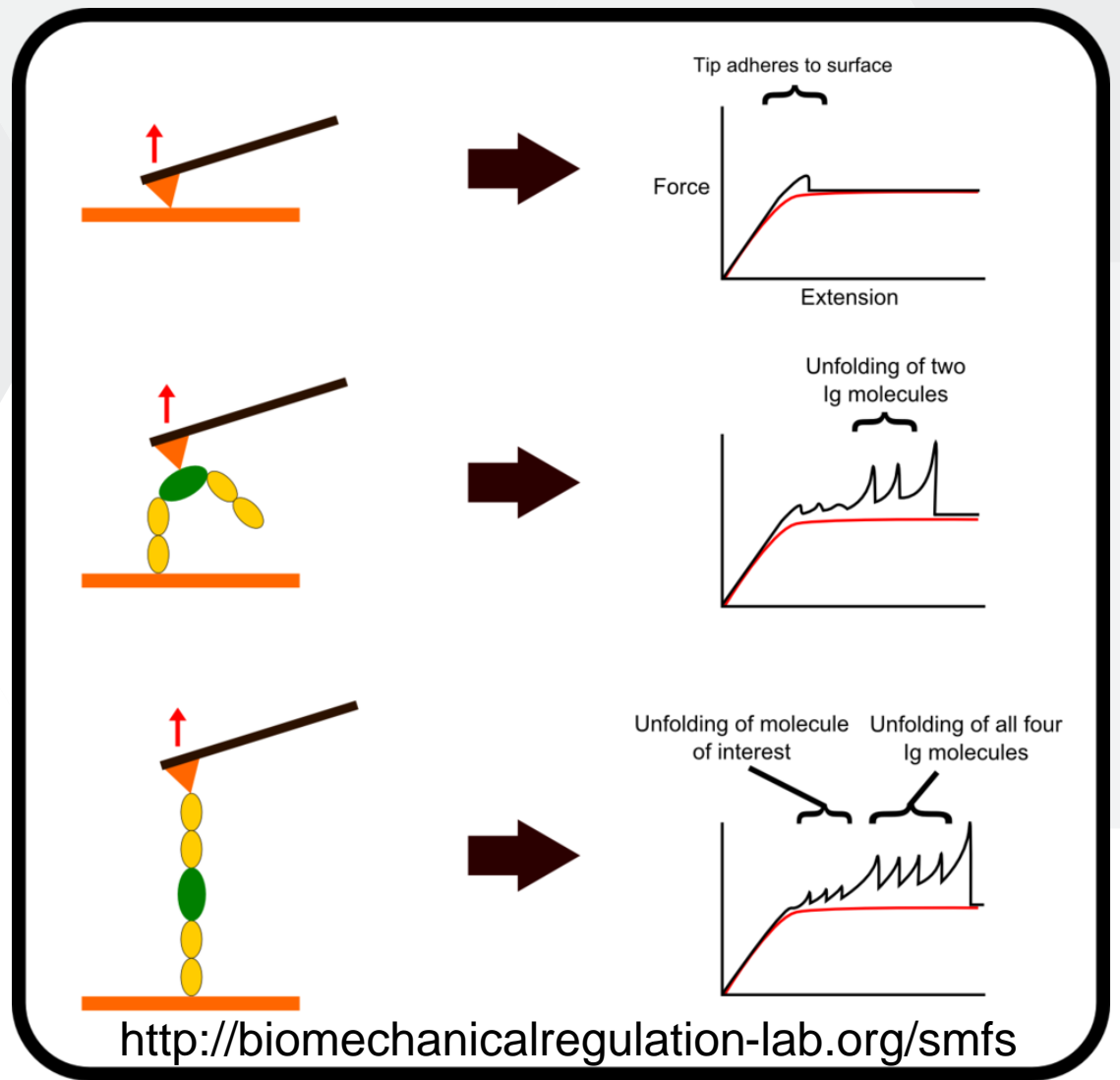
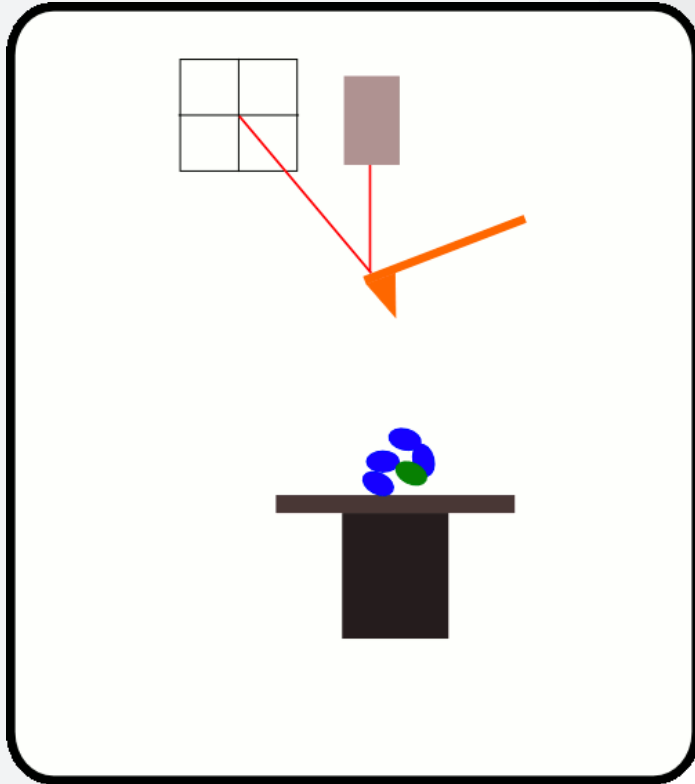
Phosphatidylethanolamine-Phosphatidylglycerol Bilayer



POPC / PCPG bilayer



Single-molecule force spectroscopy (SMFS)

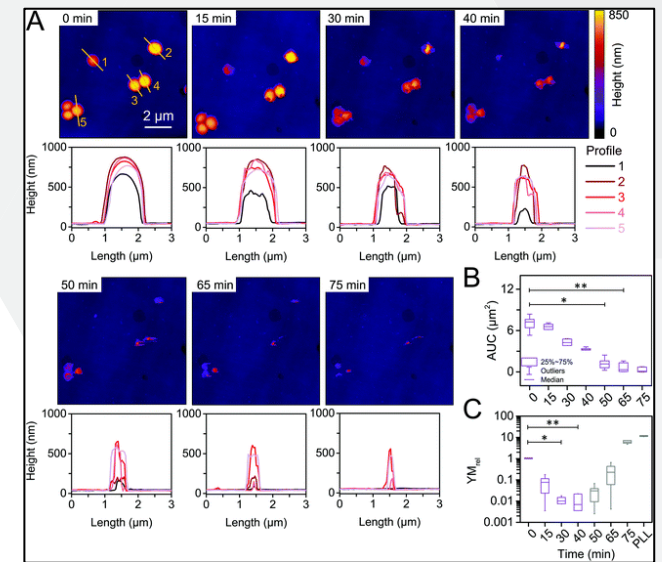
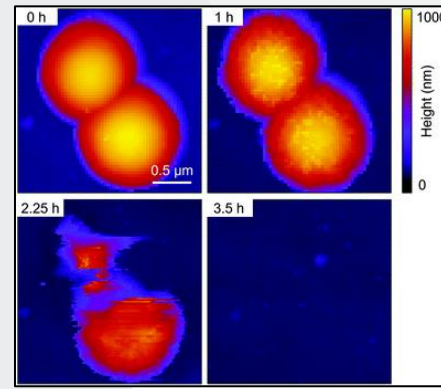
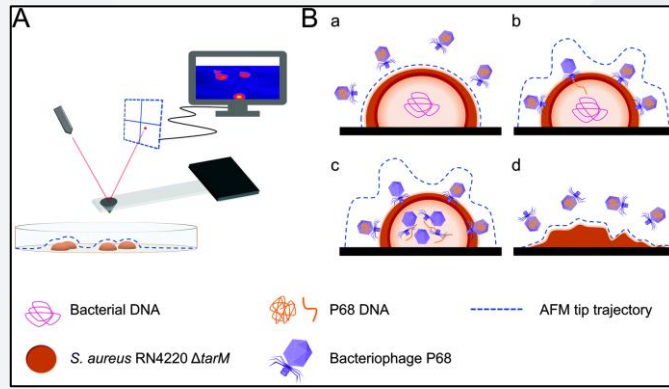


Force dependency of biochemical reactions measured by single-molecule force-clamp spectroscopy

Ionel Popa Pallav Kosuri Jorge Alegre-Cebollada Sergi Garcia-Manyes Julio M. Fernandez

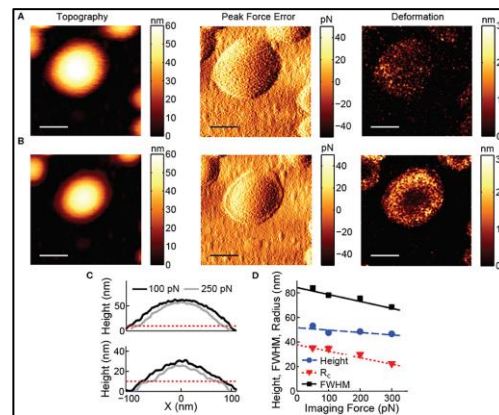
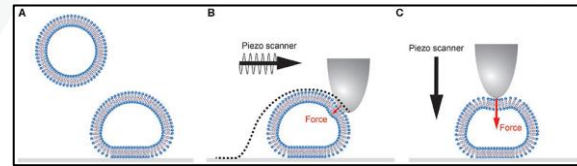
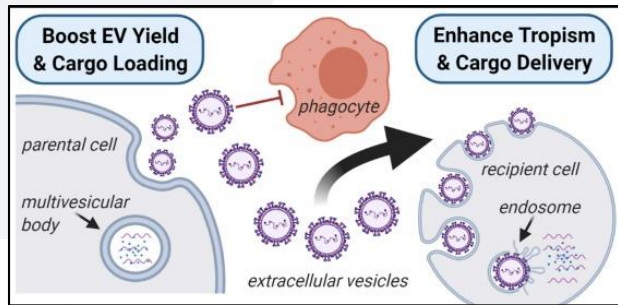
Nature Protocols June, 2013

AFM imaging of bacterial lysis

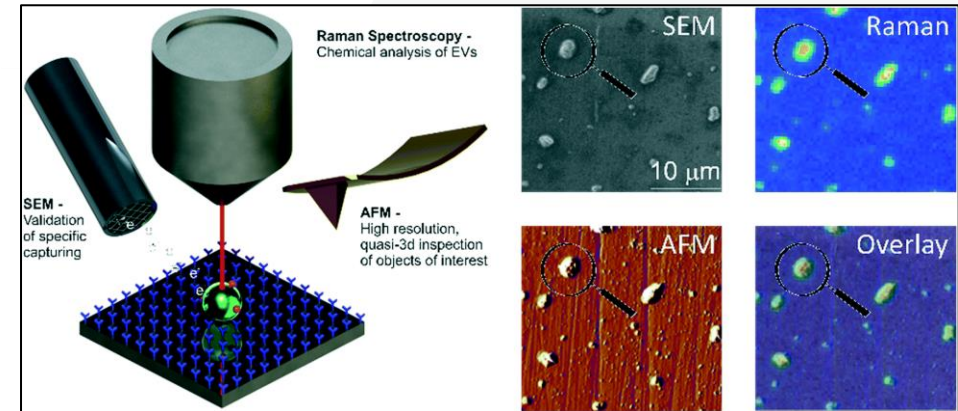


Nanoscale, 2021,13, 13538-13549

Extracellular vesicles



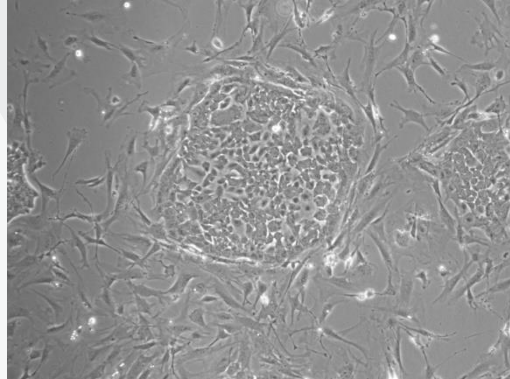
Mechanical characterization



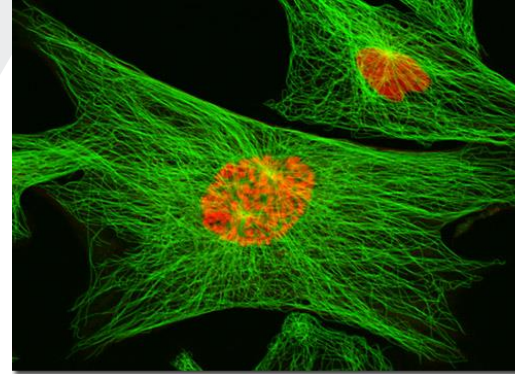
Multimodal characterization

Nanomechanical mapping of living cells

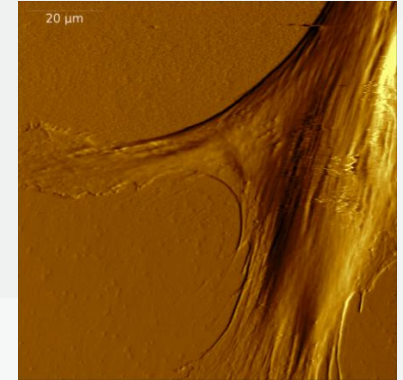
Optical microscopy



Confocal microscopy



AFM



Young's modulus mapping



Motivation

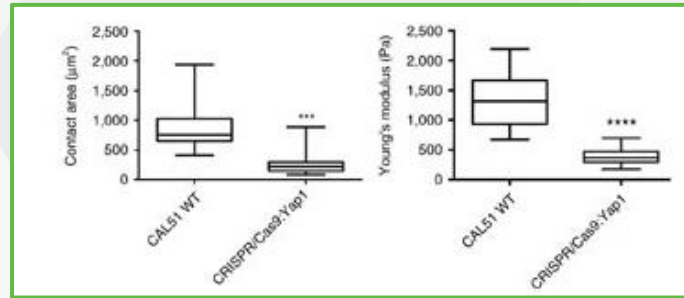
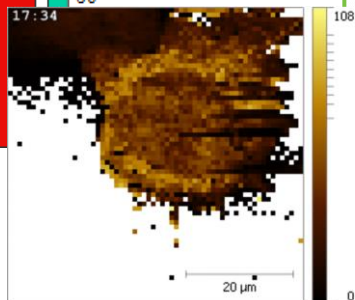
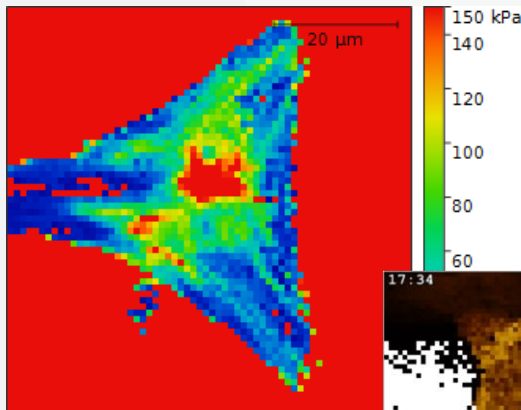
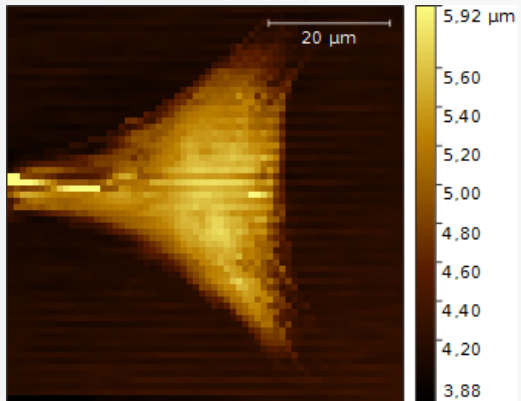
Why to quantify elasticity of (living) objects?

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

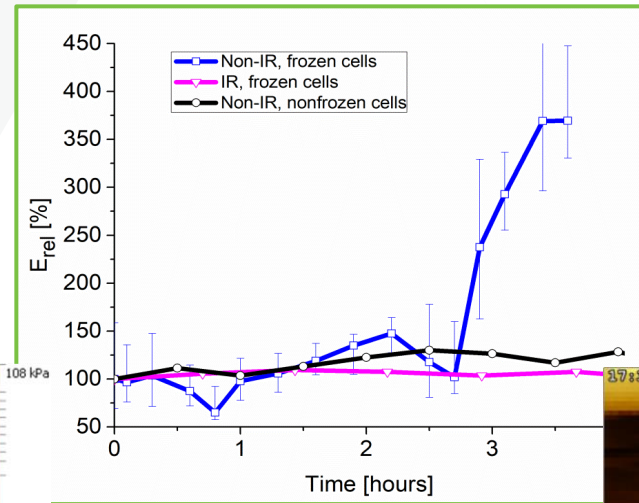
Cellular nanomechanics

By means of AFM

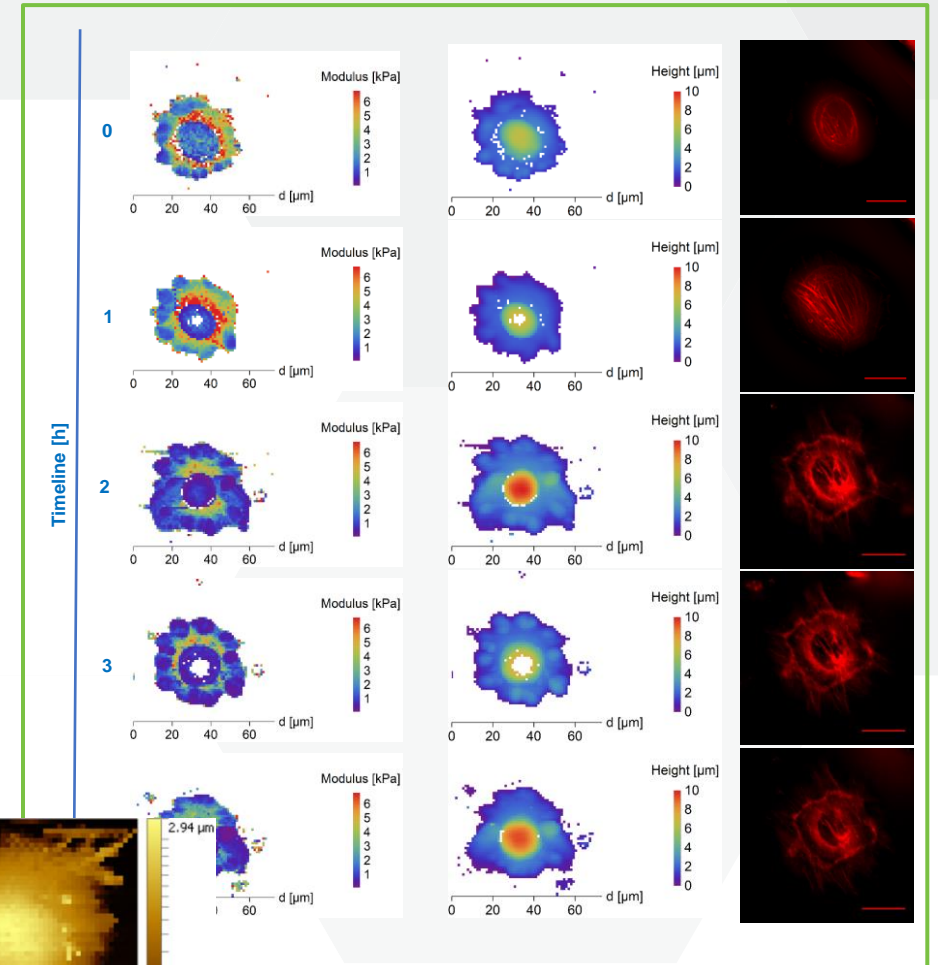
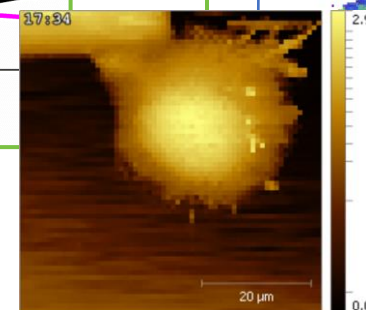
AFM mapping - correlation with fluorescence microscopy



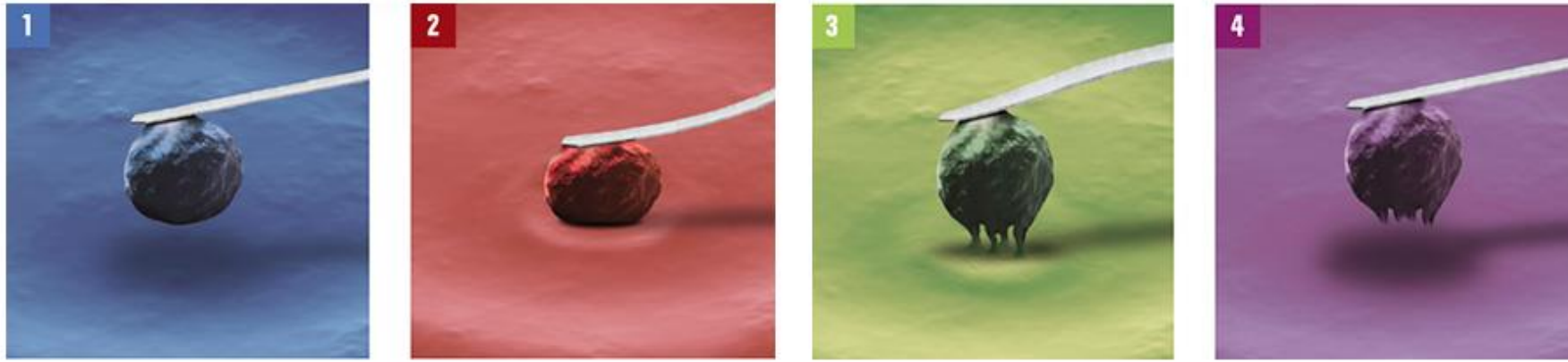
Evaluation - statistics



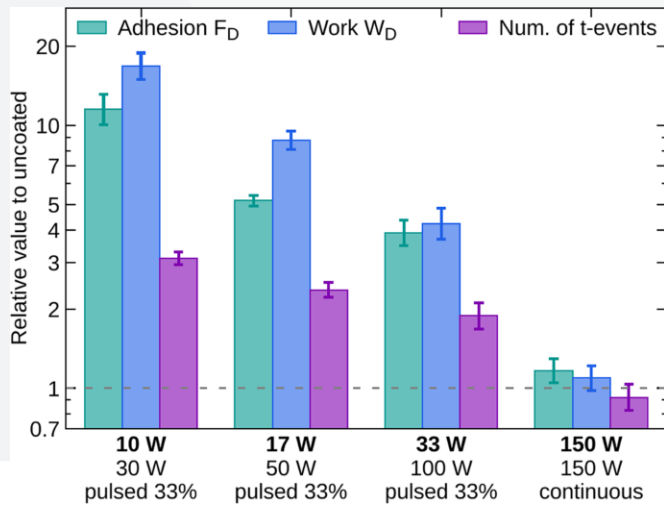
Time-lapsed biomechanics



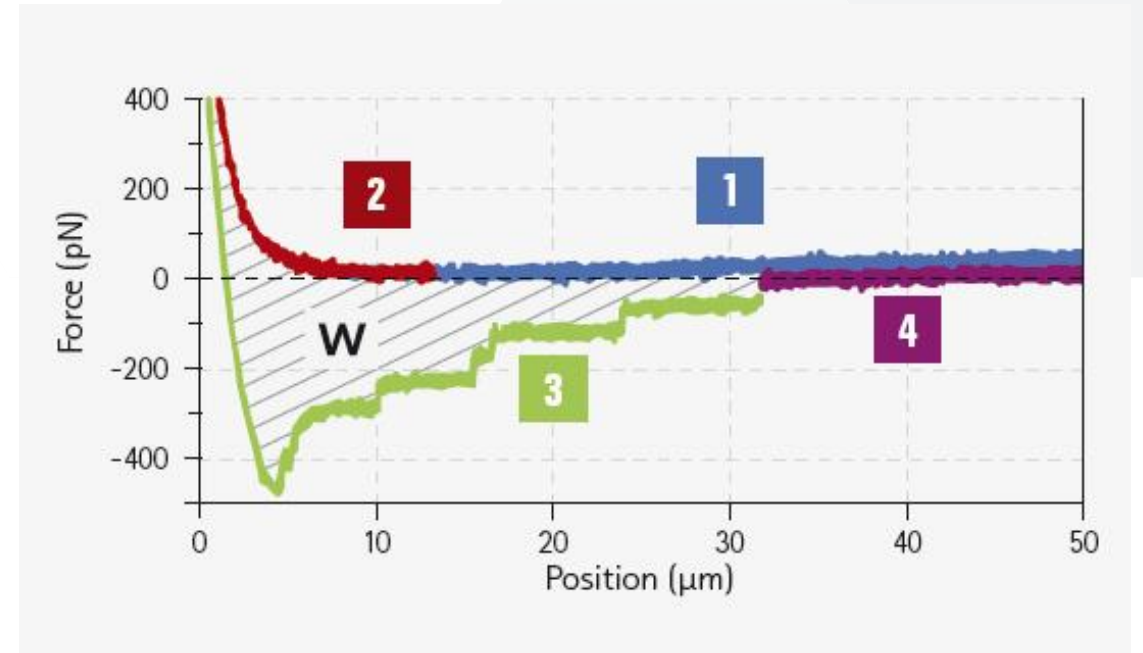
Cell-adhesion experiments



www.jpk.com



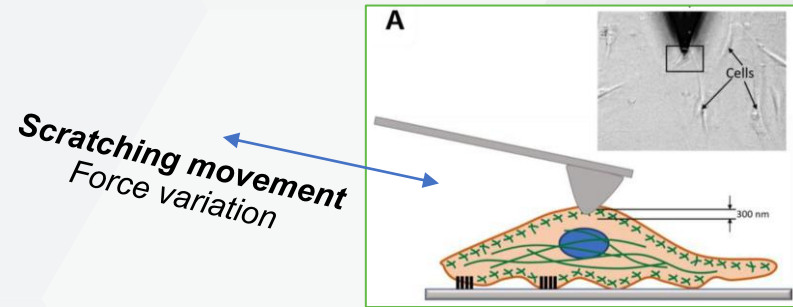
Relative adhesion of cells to plasma modified surfaces



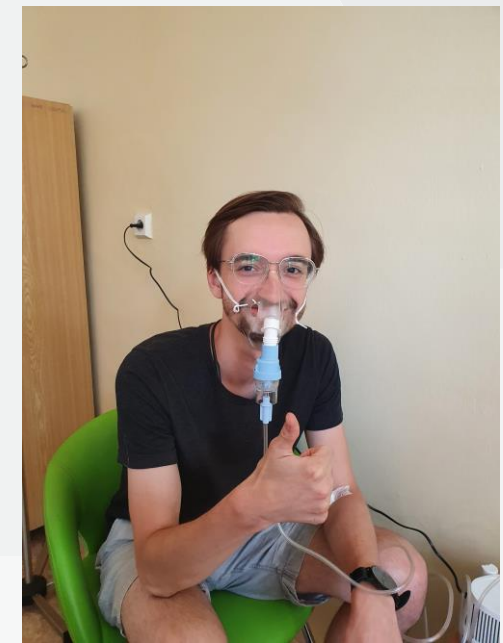
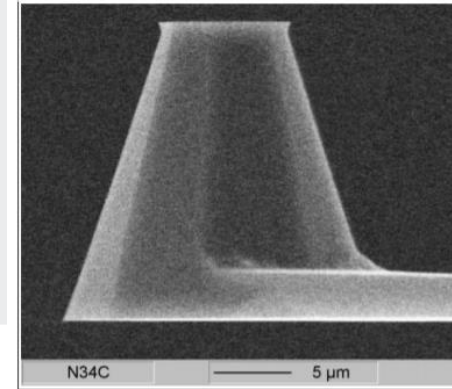
Černochová, P., Blahová, L., Medalová, J. *et al.* Cell type specific adhesion to surfaces functionalised by amine plasma polymers. *Sci Rep* **10**, 9357 (2020). <https://doi.org/10.1038/s41598-020-65889-y>

Other applications of AFM-based biomechanics

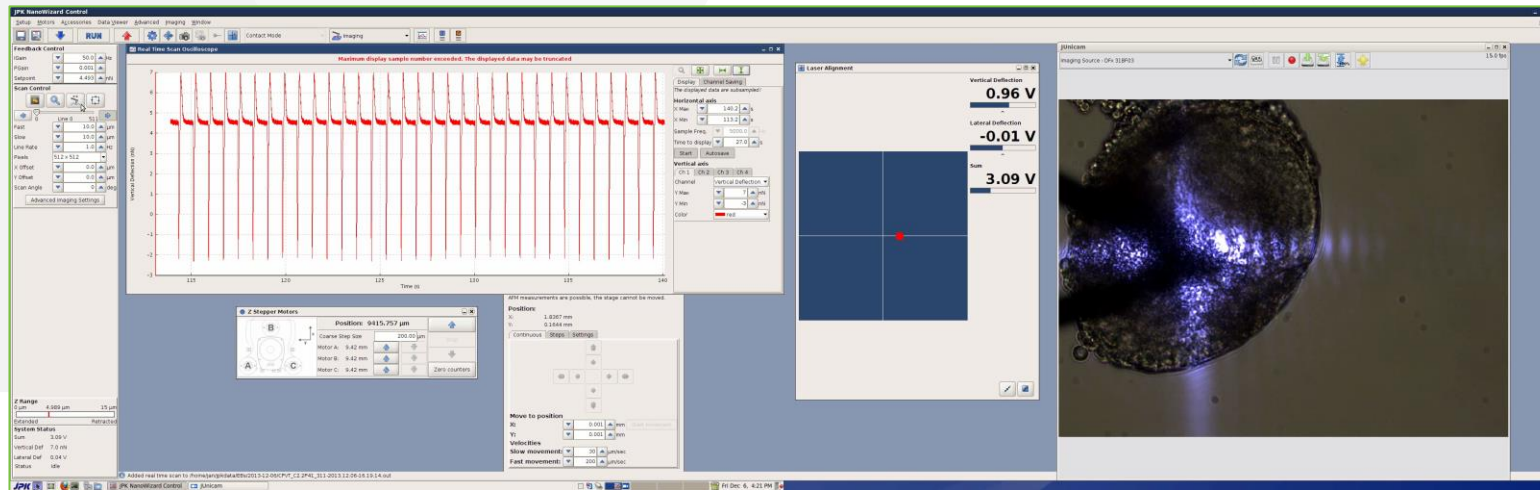
Cell scratching = cell adhesion



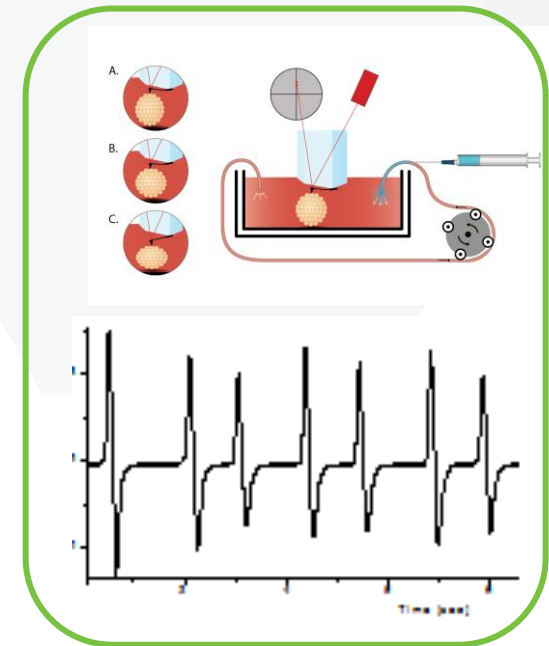
Large Plateau AFM Tips



Cardiac cells biomechanics



Setup scheme



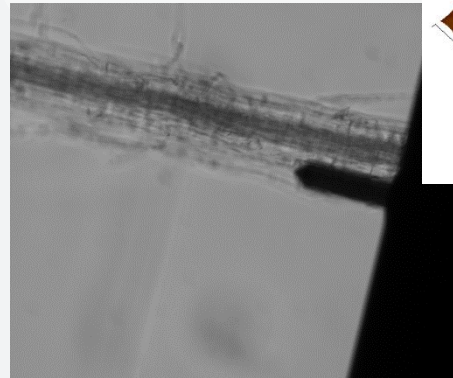
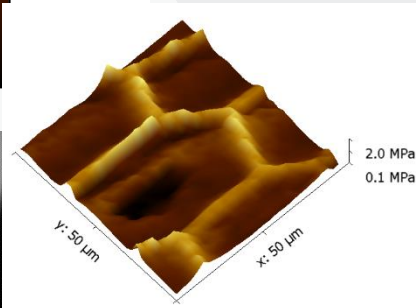
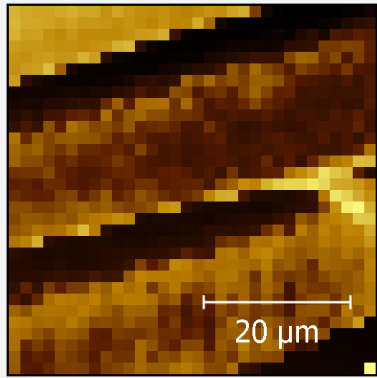
Pesl M, Pribyl J, et al. 2016 *Biosensors and Bioelectronics* **85** 751–7

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

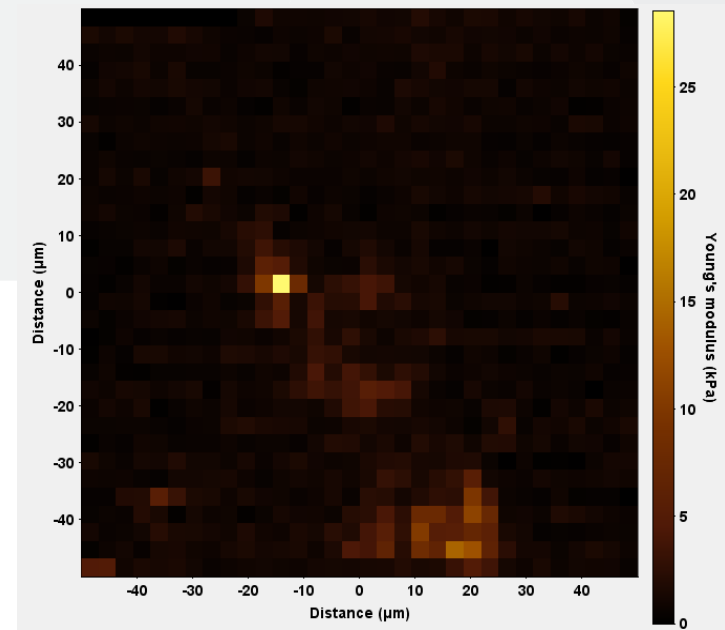
On a tissue level



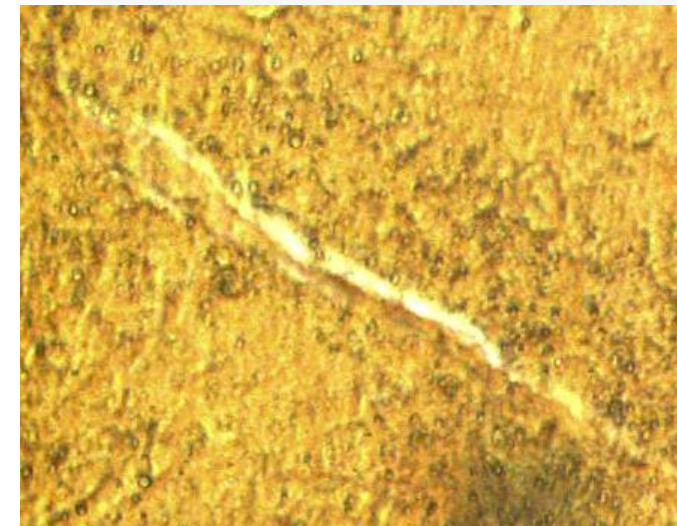
Plant samples
(hypocotyl)



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



Liver cirrhosis
Correlation of
Collagen fibers by polarized microscopy
AFM nanoindentation

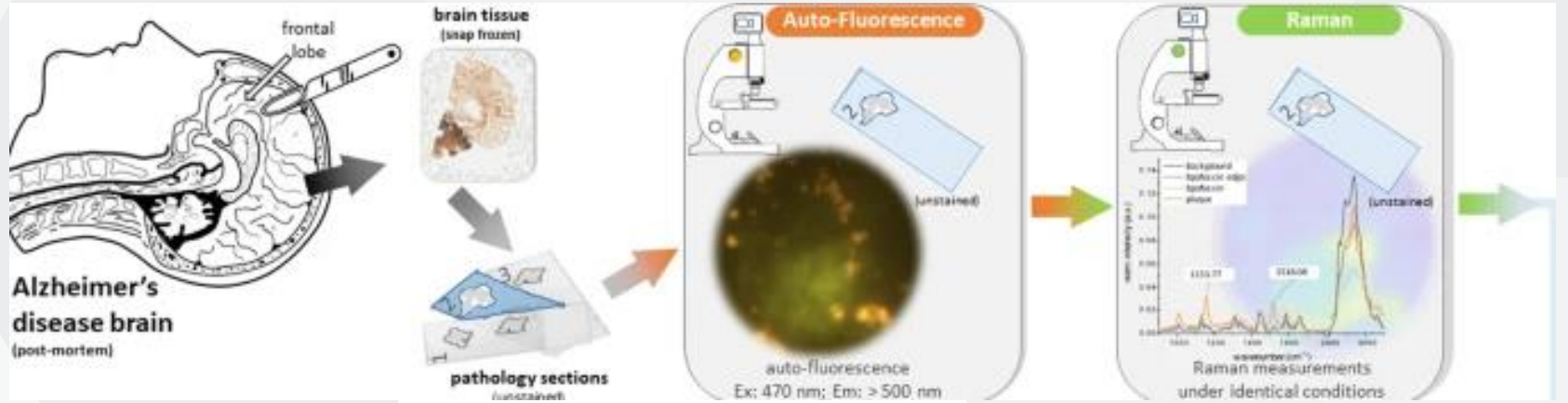


by **Srikant Ojha**
Martin Gregor Lab

Raman microscopy

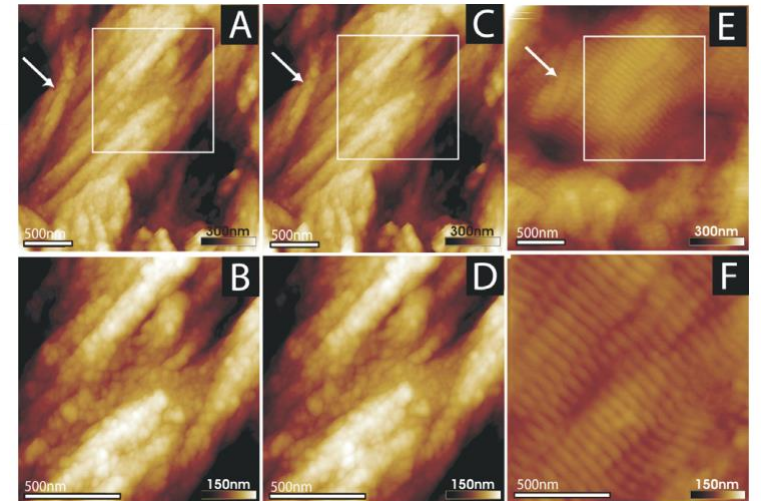
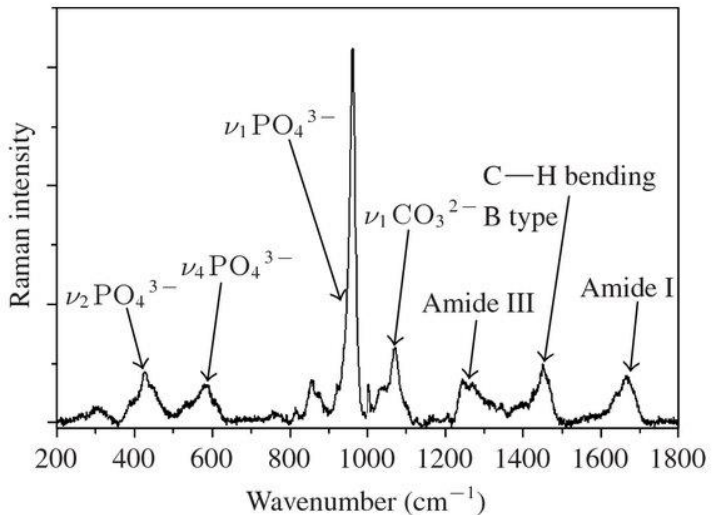
On bio samples

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

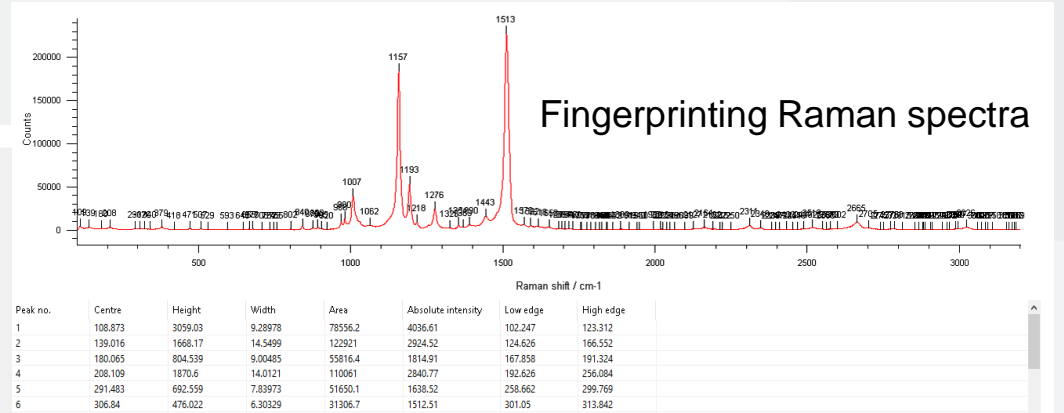
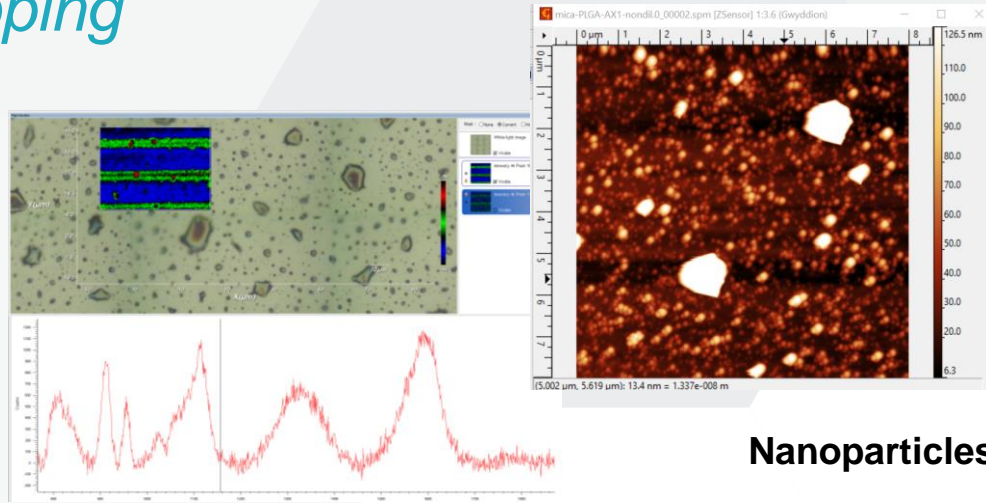
Calcification level and Collagen Fibers Arrangement in Bone Tissue



+ combination with AFM topography

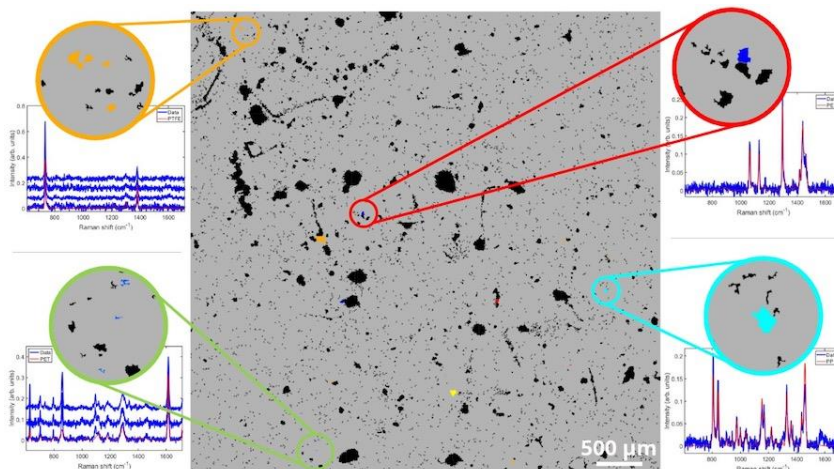
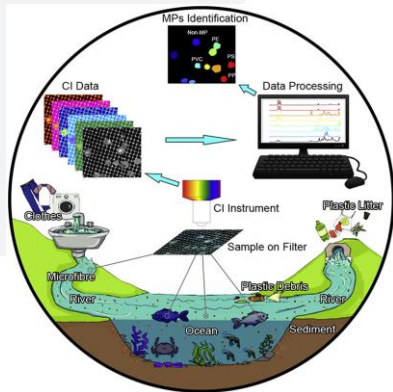


Raman microscopy + AFM Chemical mapping

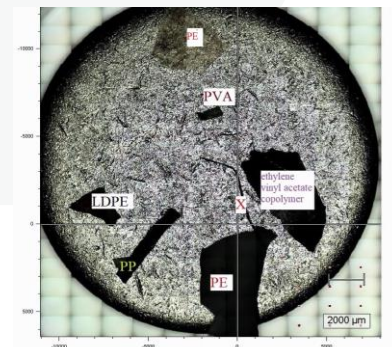


Nanoparticles loading study

+ combination with AFM topography

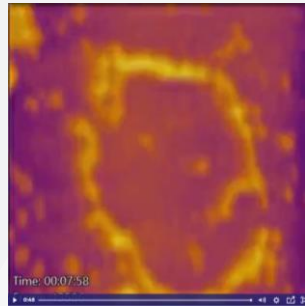


Microplastics identification

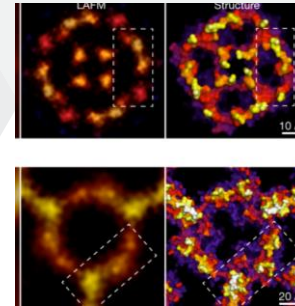


Where to go next...?

1. High-Speed (Video-Rate) AFM

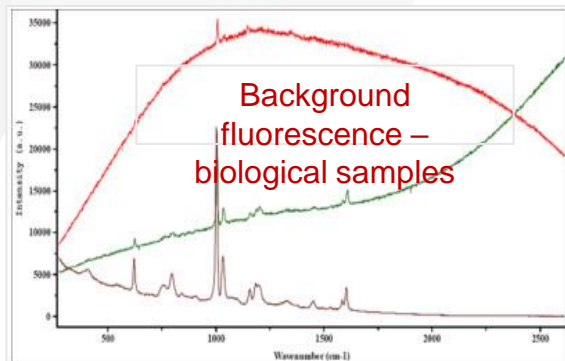


Fast biological processes



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

2. Raman microscope upgrade



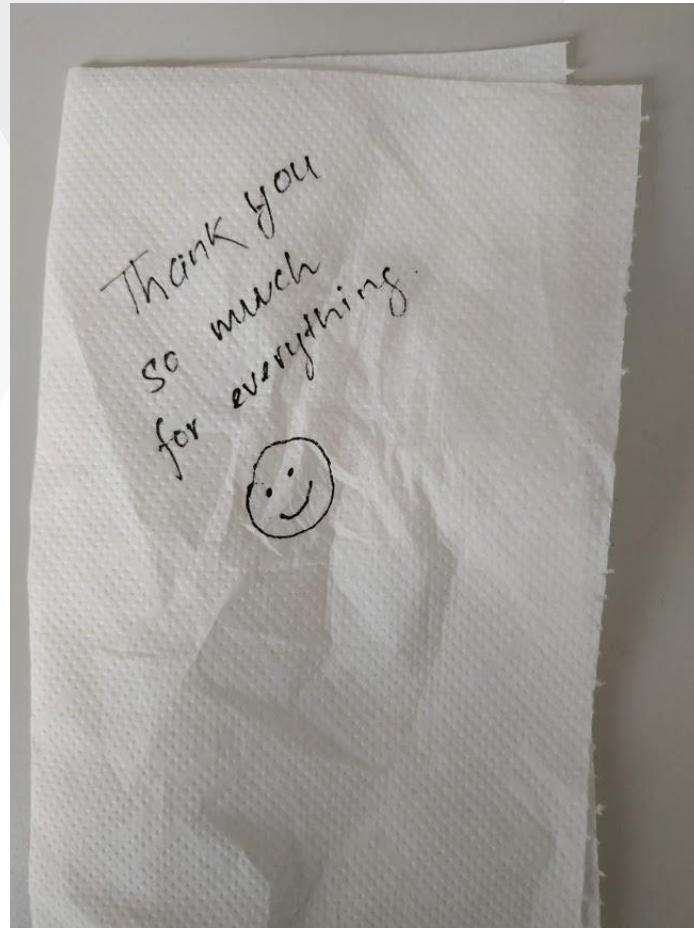
Raman part - upgrade

- + 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

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





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Thank you for your attention.

Web: ceitec.eu/nanobio



(LM2023042)



EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education



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



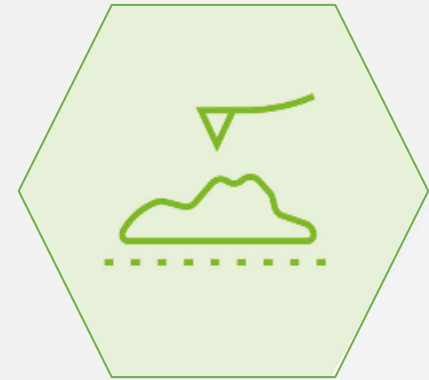
MUNI Grant Agency



Thank you for your attention!

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

*Use of Atomic Force Microscopy, Raman microscopy,
and Fluorescence Microscopy, while emphasizing the
investigation of structure, mechanical properties, and
chemical composition in biological samples*



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building E35, Atrium

