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Central European Institute of Technology

BRNO | CZECH REPUBLIC

MUNI

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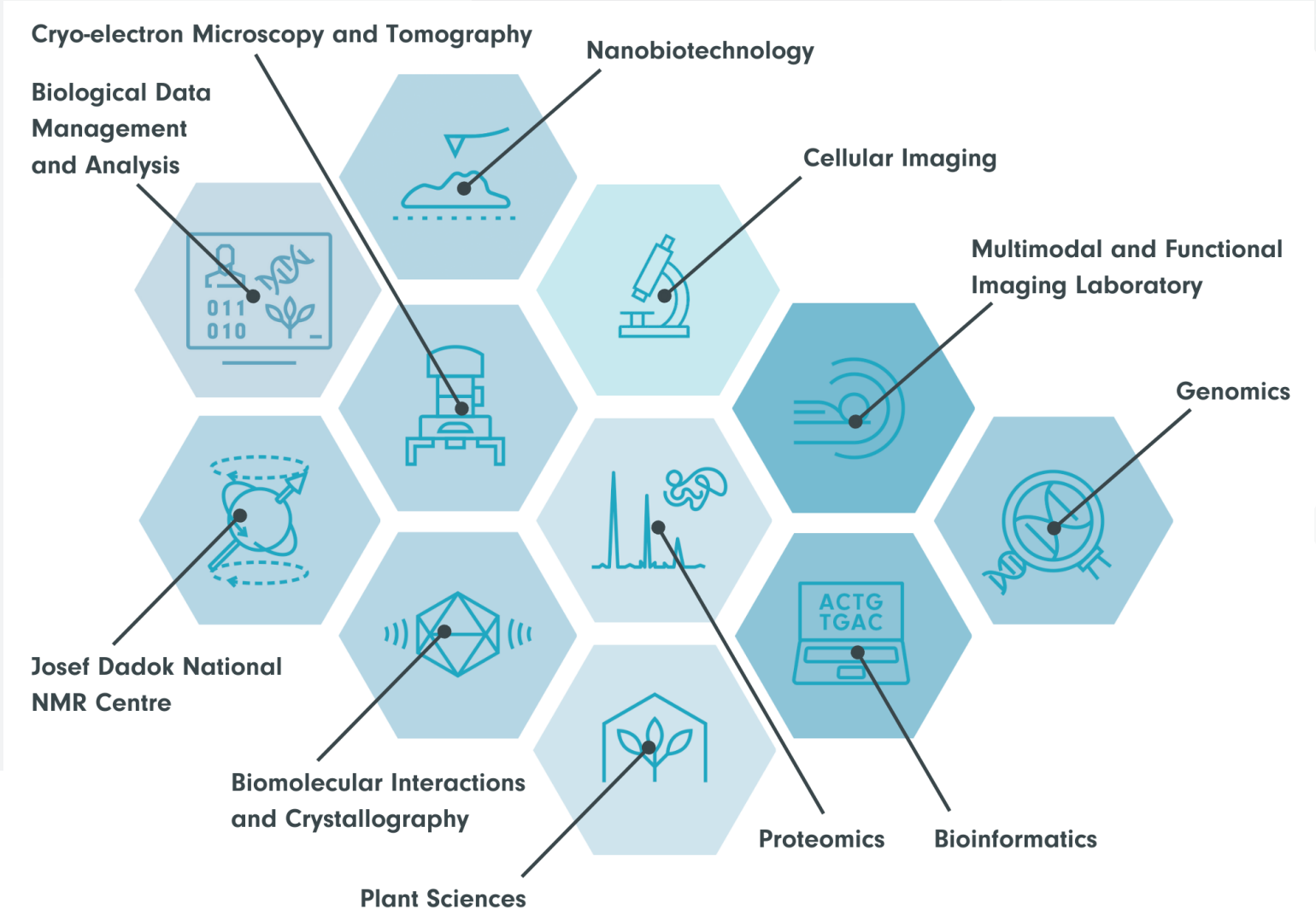
Core Facility Nanobiotechnology

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

Core Facilities at CEITEC Masaryk University

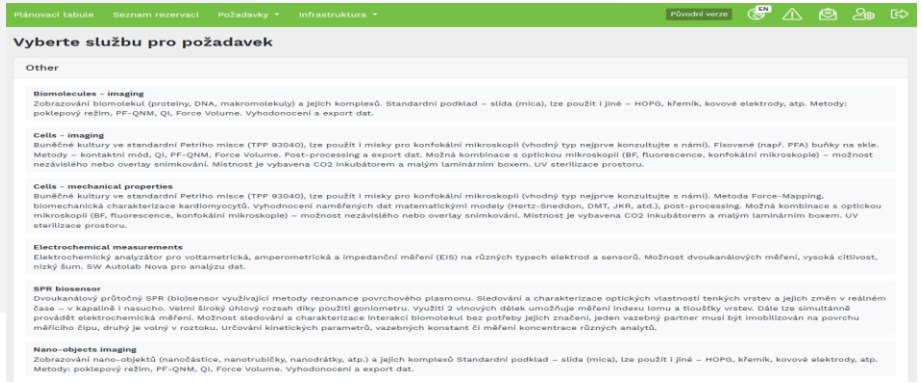


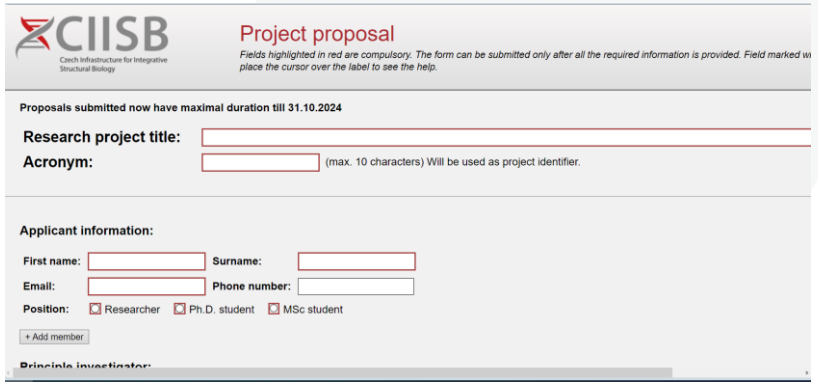
Core facilities are TECHNOLOGICAL UNITs equipped with the FIRST-RATE EQUIPMENT available to scientists from a range of fields.

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/

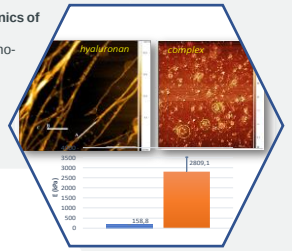




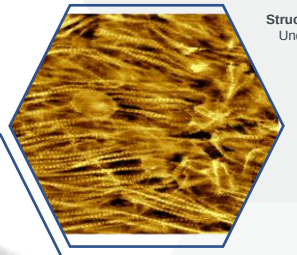
Core Facility Nanobiotechnology

Imaging, mechanical mapping, Raman microscopy

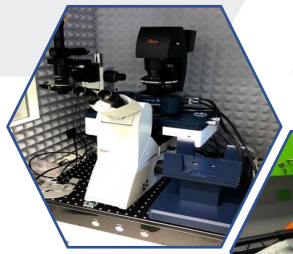
Structure and biomechanics of biomolecules
(AFM imaging and nano-indentation]



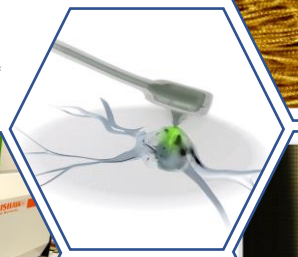
Structure of biomolecules
Under native conditions



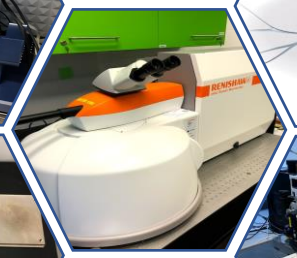
Atomic Force Microscopy of biosamples
Combined with fluorescence microscopy



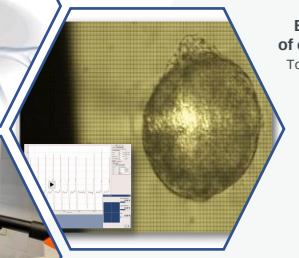
FluidFM
AFM based microfluidics



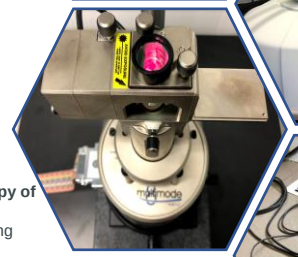
Raman microscopy
Chemical mapping of surface

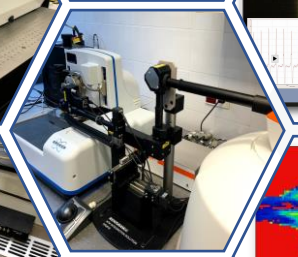


Biomechanics of contractile cells
Toxicology and cell development

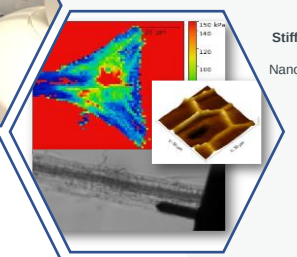


Atomic Force Microscopy of biosamples
High-resolution imaging





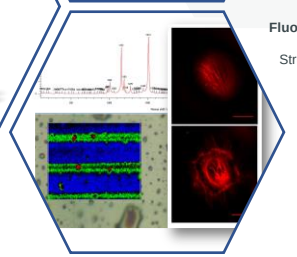
Stiffness mapping of cells and tissues
Nanoindentation under semi-physiology



Multielectrode Array (MEA)
Cellular electrophysiology

NanoIndenter
Indentation of soft samples

Fluorescence and Raman microscopy
Structural and chemical characterization



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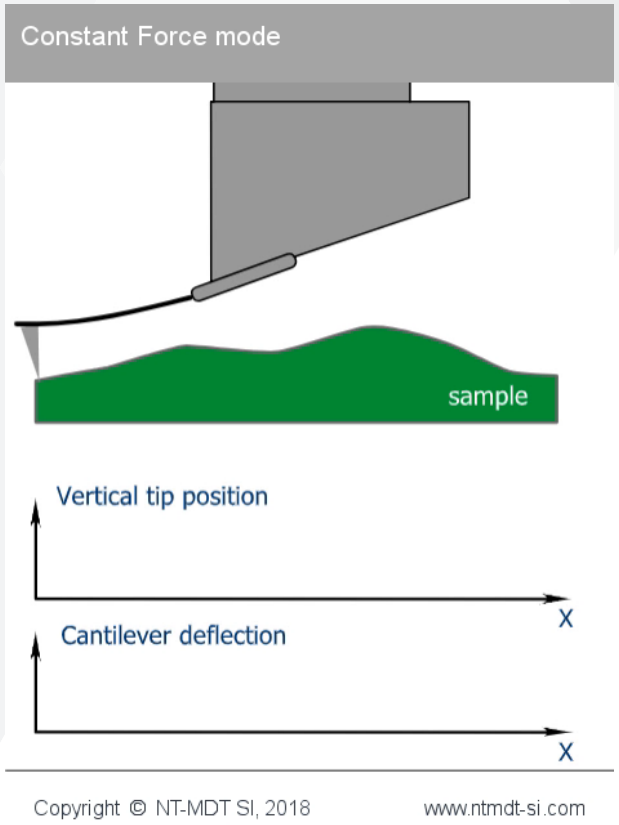
Core Facility NanoBiotechnology

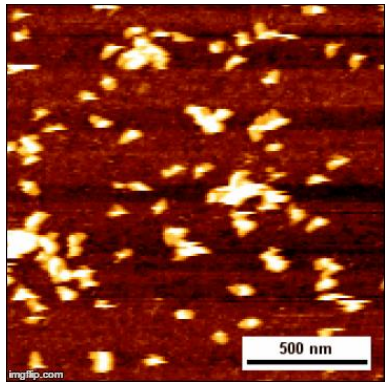
Techniques and Equipment

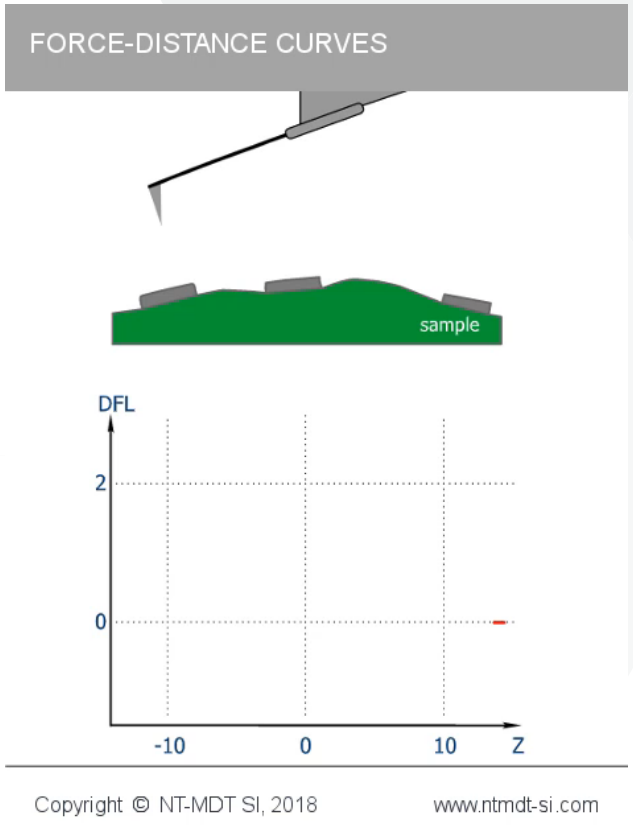
Atomic Force Microscopy

AFM (Atomic Force Microscope)

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

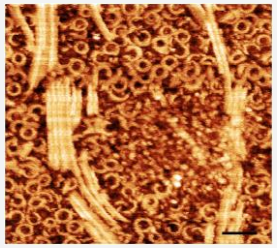


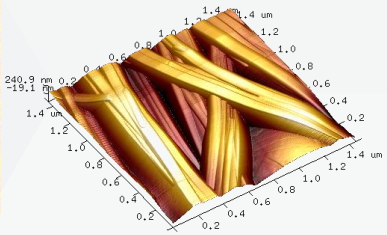




Bio Atomic Force Microscopy (BioAFM)

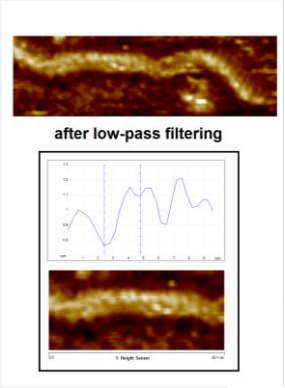
Non-denstructive modes

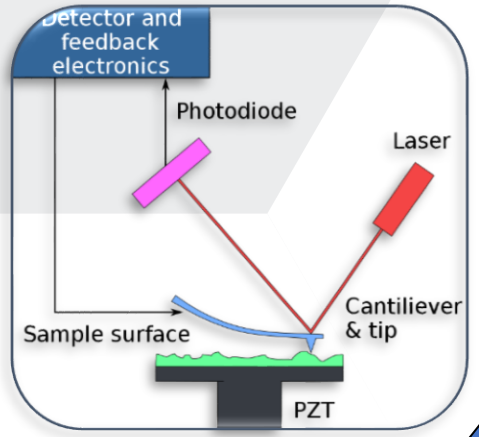




IMAGING

Proteins, DNA, Nanoobjects

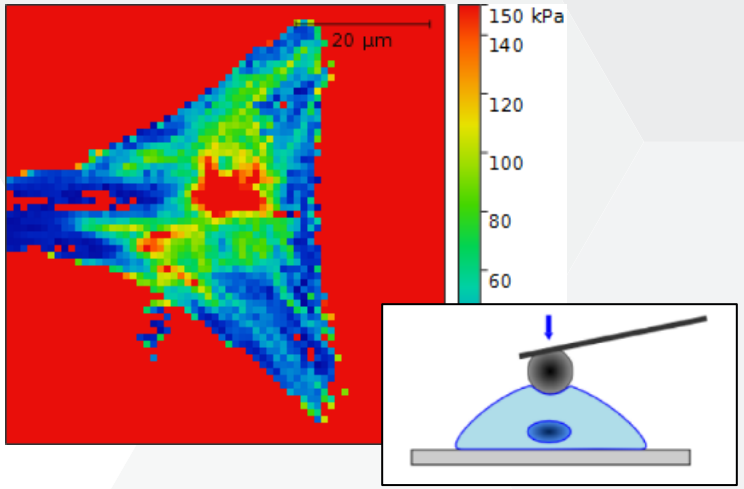




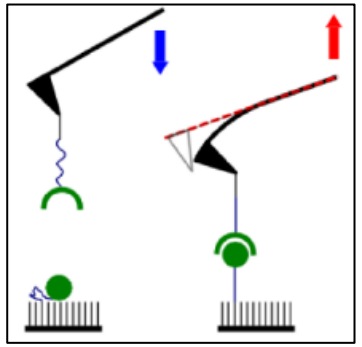
by OverloardQ

NANOINDENTATION

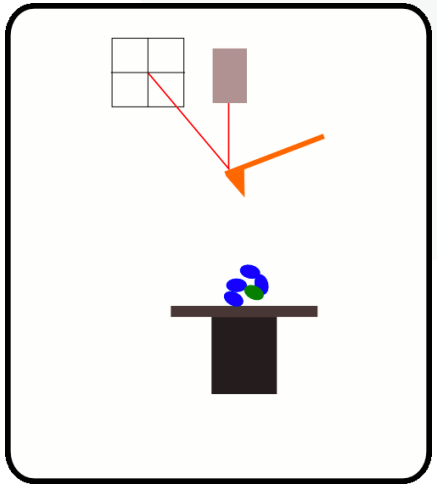
Stiffness mapping

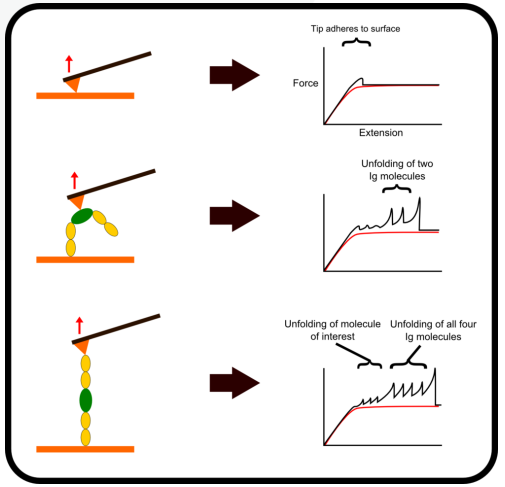


AFFINITY INTERACTION



Single-molecule force spectroscope (SMFS)





http://biomechanicalregulation-lab.org/smfs

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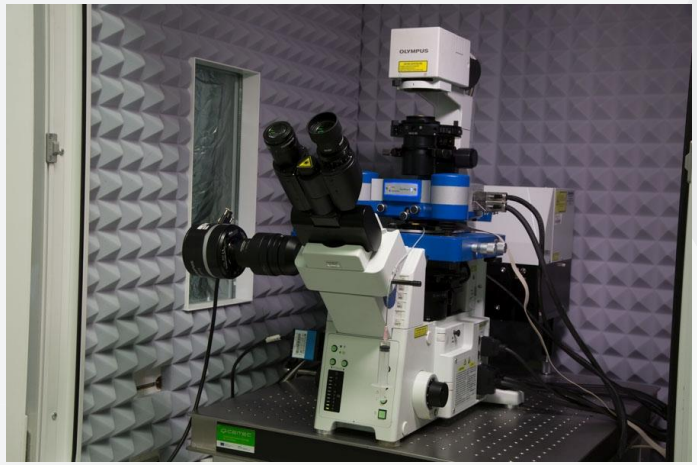
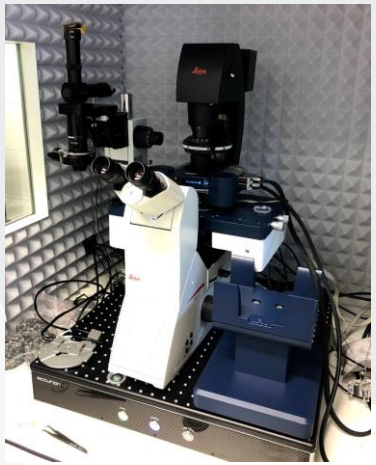
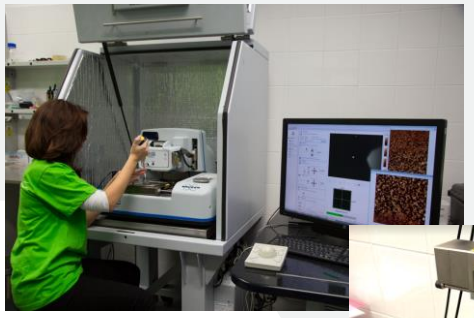
JPK NanoWizard 3 and 4 with extended scanning range

BioAFM – living cells and tissues

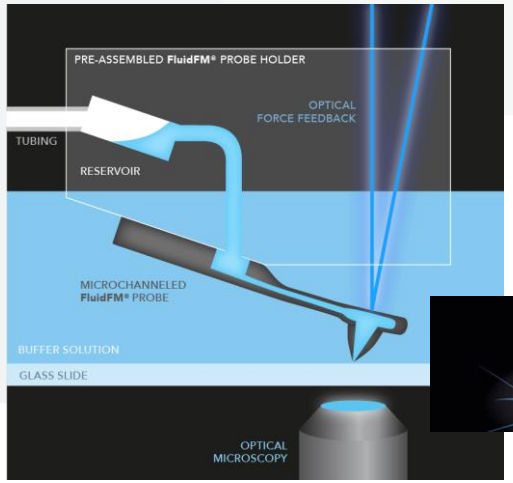




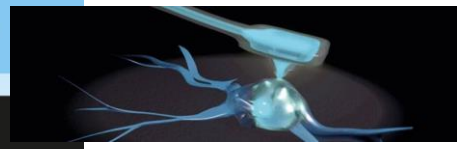


BioAFM – molecules, nanoobjects, molecular complexes

Bruker Dimension Icon FastScan and MultiMode 8HR
NTMDT Ntgra Vita

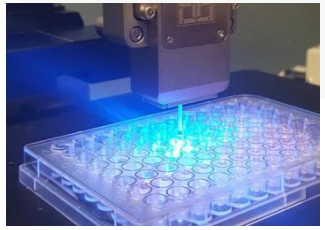


Nanoaspiration, nanodelivery (single cell), nano3D printing



Cell/organoid electrophysiology

Mechanobiological studies on tissues, hydrogels, etc.



+ CytoSurge FluidFM module
+ MultiElectroda Array (MEA)

+ Biosoft NanoIndenter

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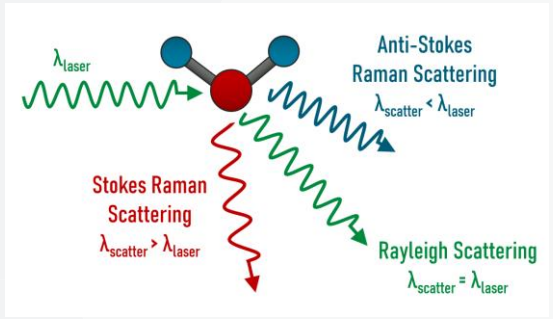
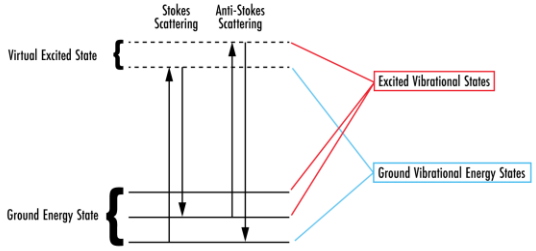
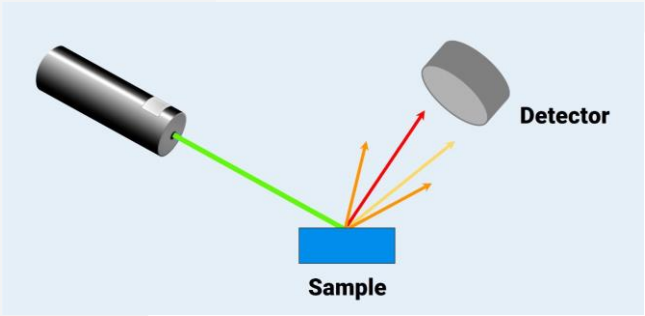
Raman microscopy, UPCON readers

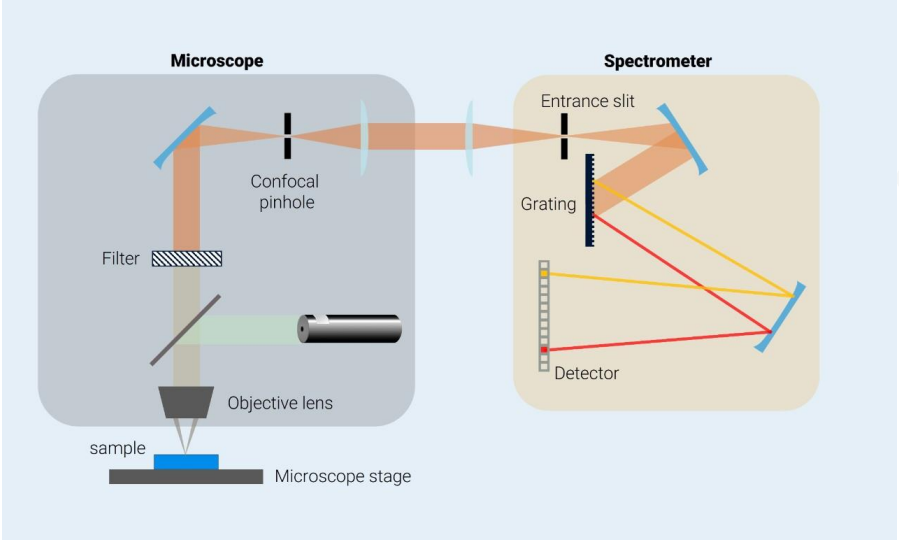




Renishaw InVia Raman microscope

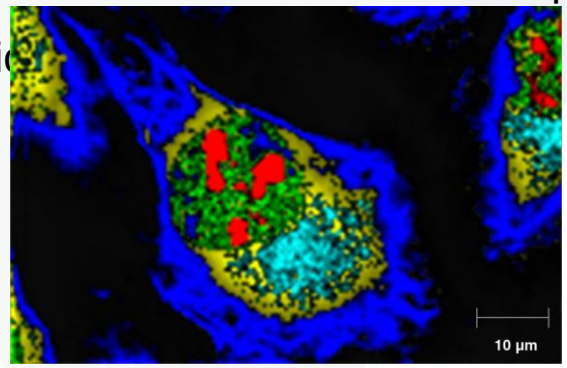


Raman spectroscopy



Raman microscopy

Bi



Raman image of human osteosarcoma (bone cancer) cells.

Core Facility NanoBiotechnology

Techniques and Applications

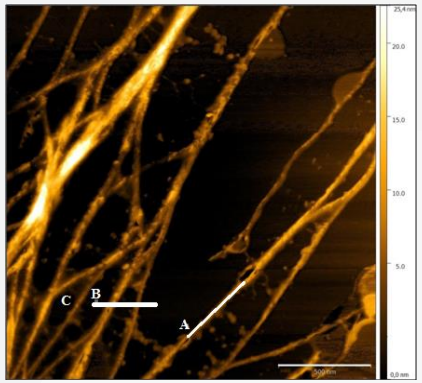
Technology and Expertise
List of services

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

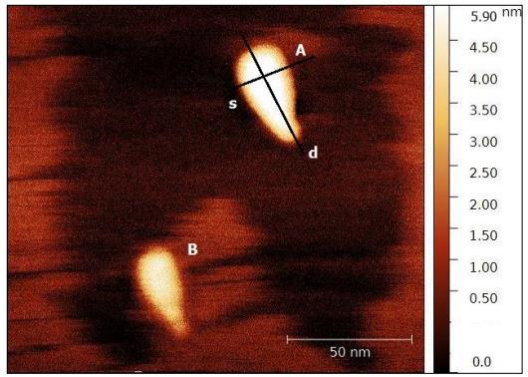
Typical applications

Biomolecules – imaging and biomolecules

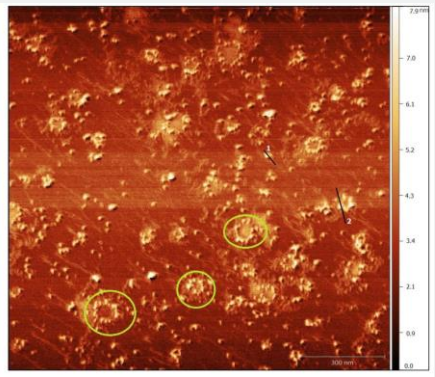


hyaluronan

+

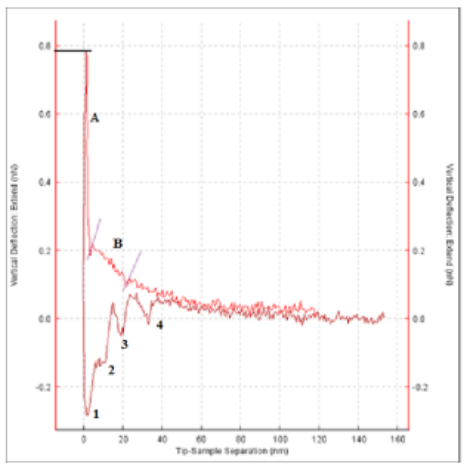


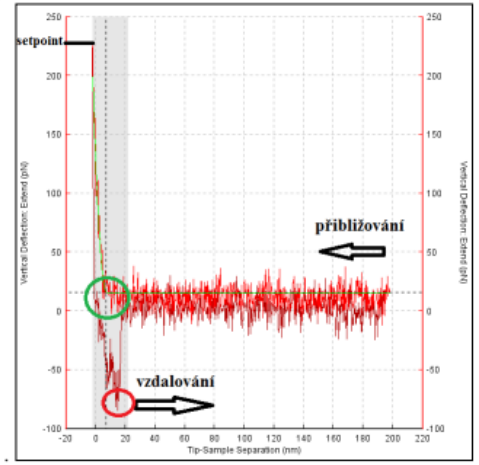
myeloperoxidase

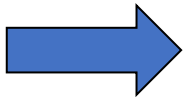


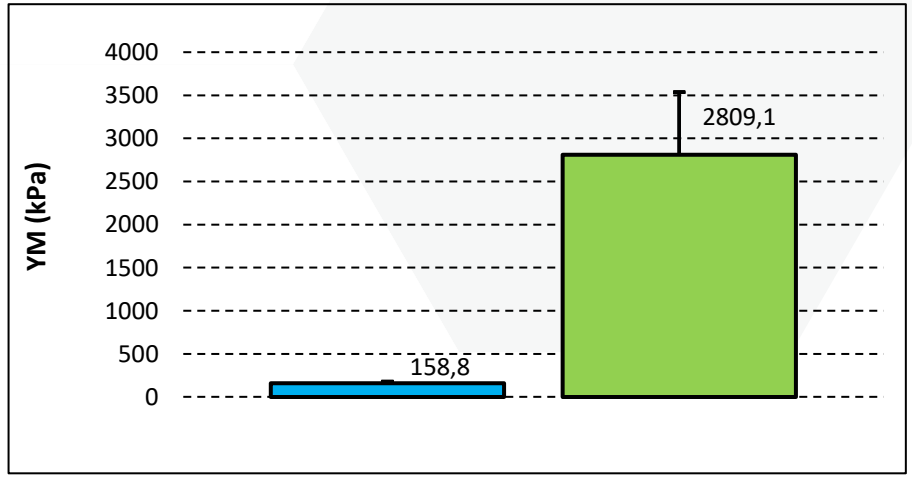
Molecular complex

FD curve recording and analysis









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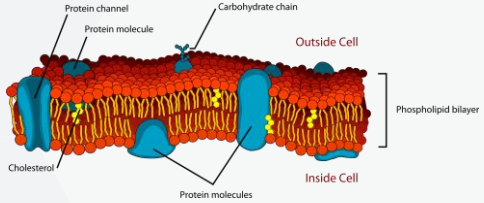
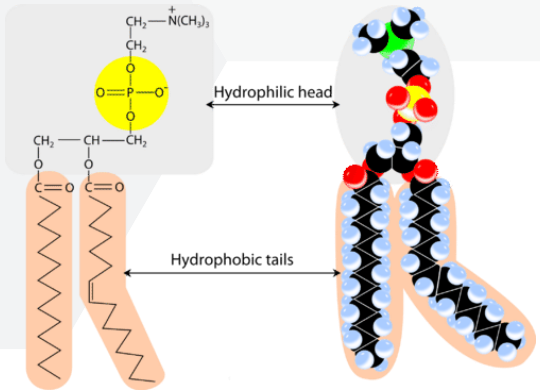
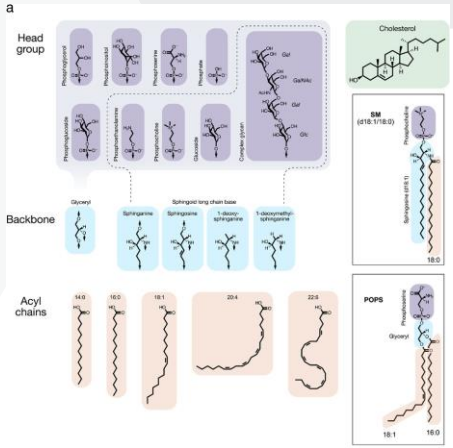
Cell Membrane

Chemical composition



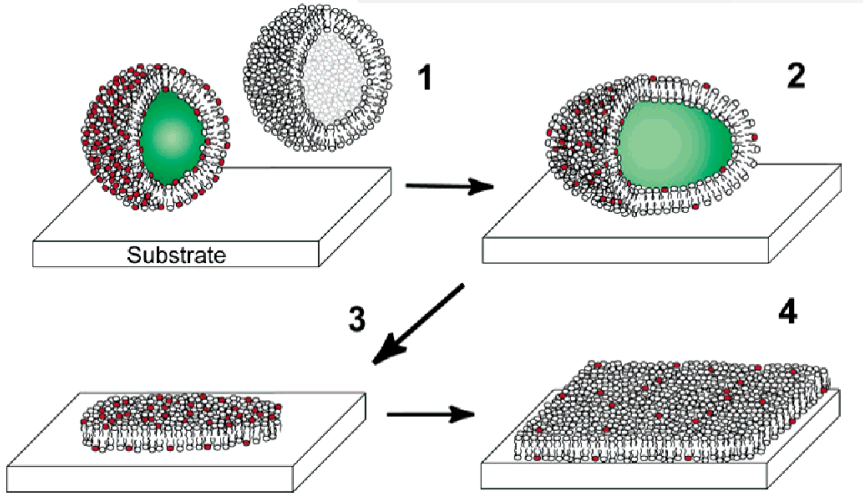




Synthetic membrane

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

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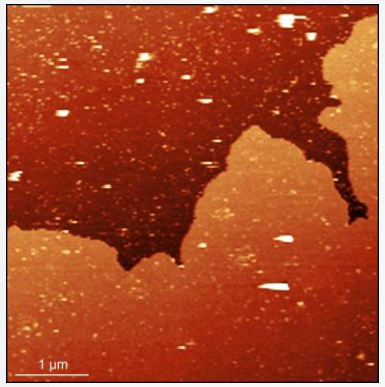
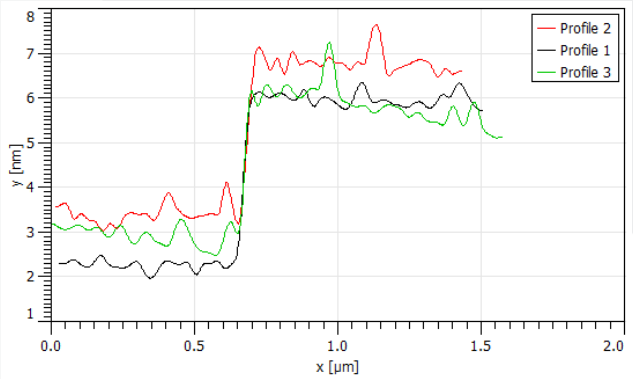
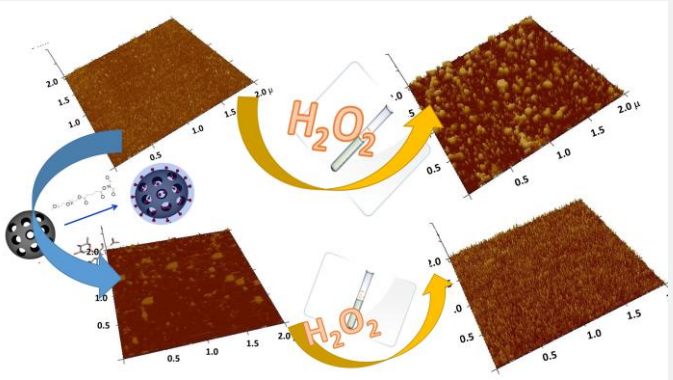
Islands of bilayers

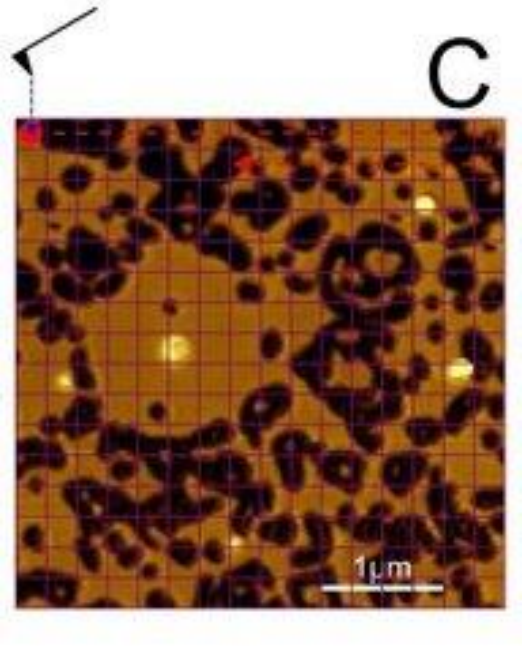
Non-compact / disrupted SLBs







Oxidative stress



Pores

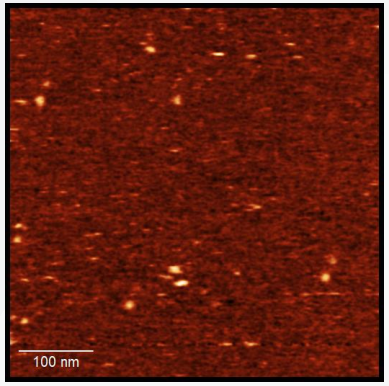
Peptide disruption of SLBs

Journal of Lipid Research Volume 64, 10, 2023, 100430

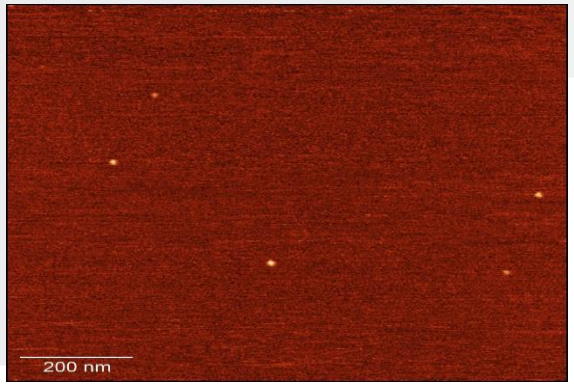
Int. J. Mol. Sci. **2019**, *20*(11), 2709

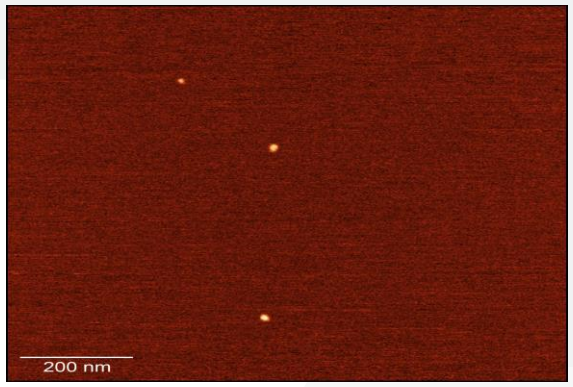
Compact bilayers

Phosphatidylethanolamine-Phosphatidylglycerol Bilayer

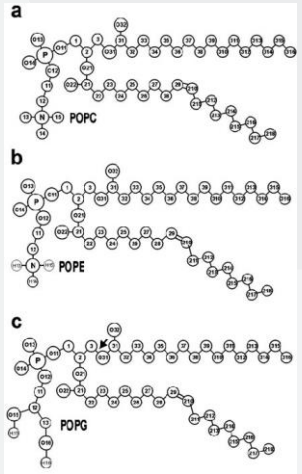


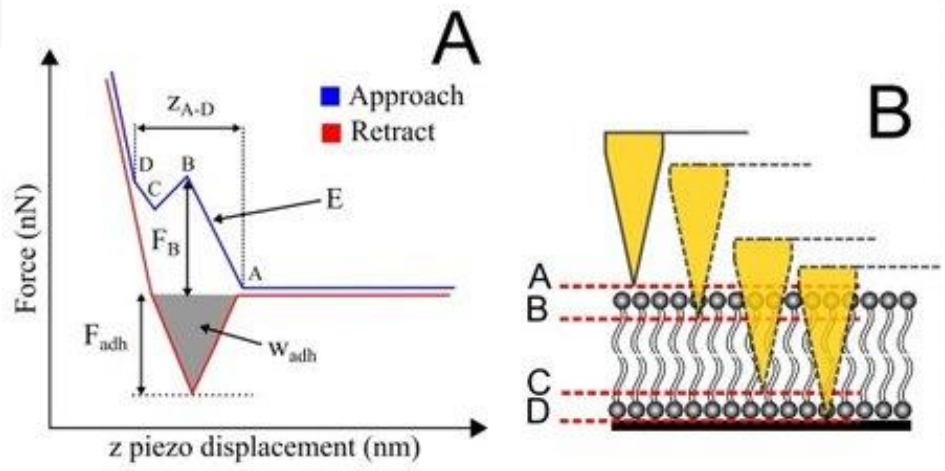
Mica

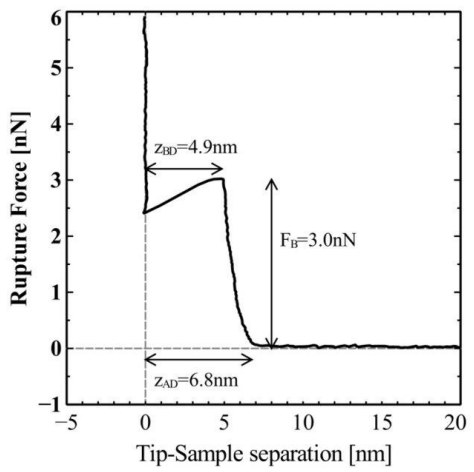




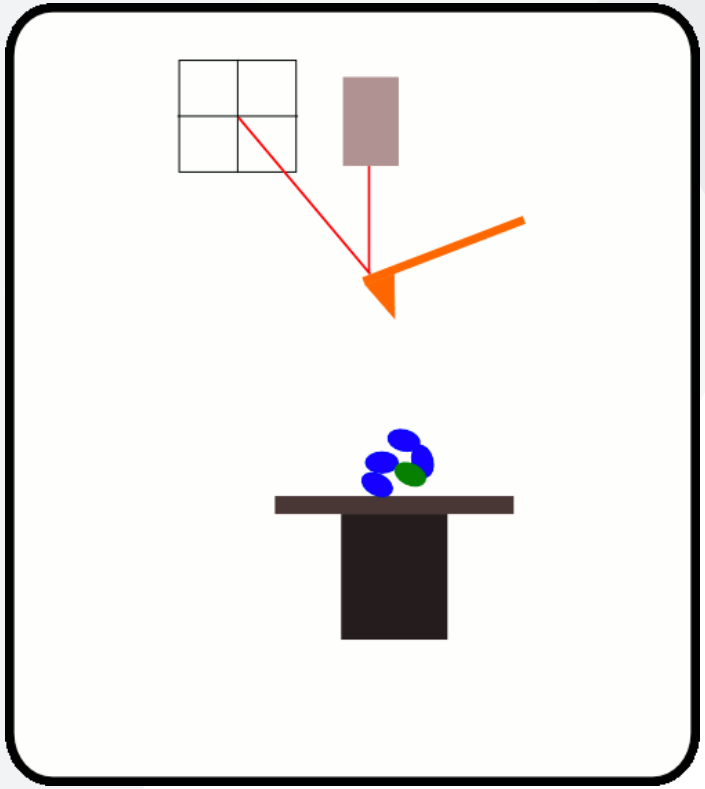
POPC / PCPG bilayer

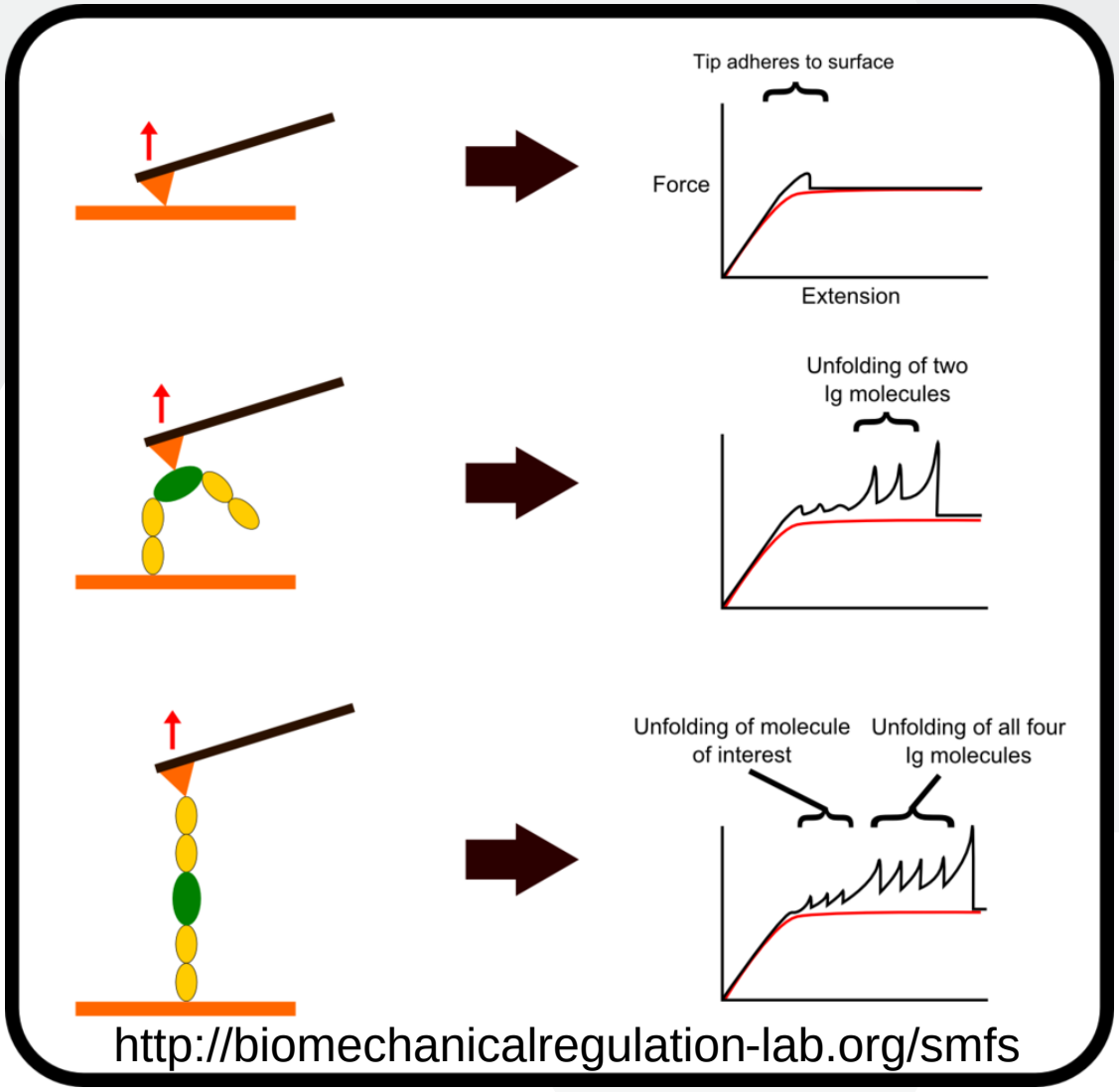






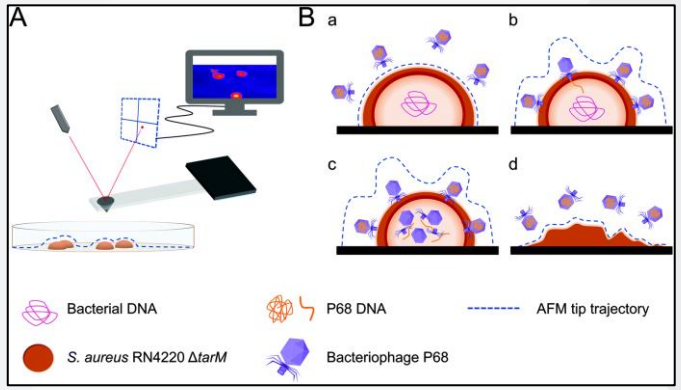
Single-molecule force spectroscope (SMFS)

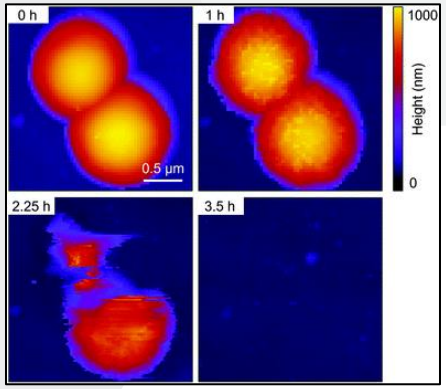


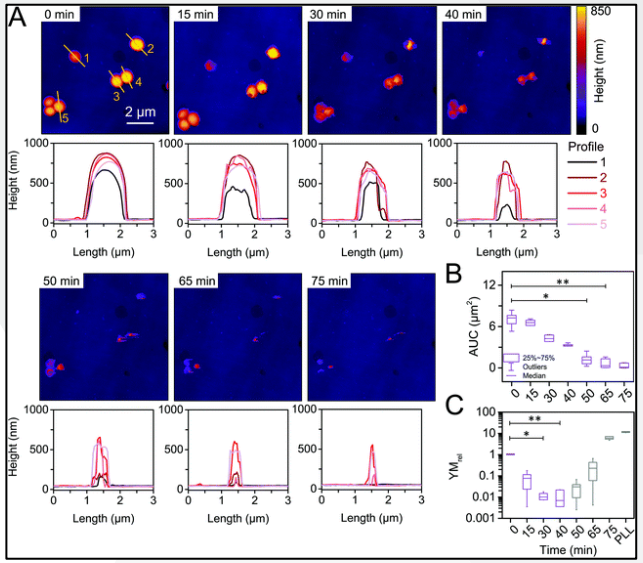


Force dependency of biochemical reactions measured by single-molecule force-clamp spectroscopy
Ionel Popa Pallav Kosuri Jorge Alegre-Cebollada Sergi Garcia-Manyes Julio M. Fernandez
Nature Protocols June, 2013

AFM imaging of bacterial lysis

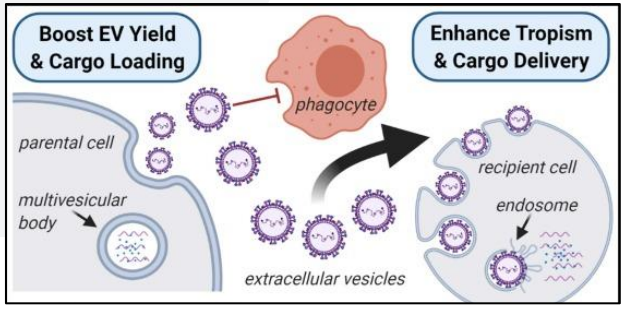


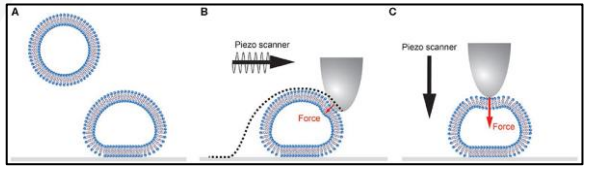


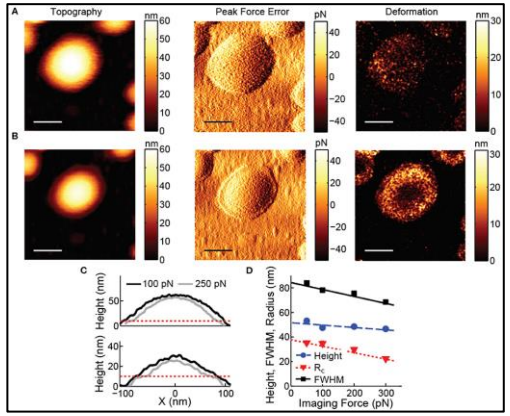


Nanoscale, 2021,**13**, 13538-13549

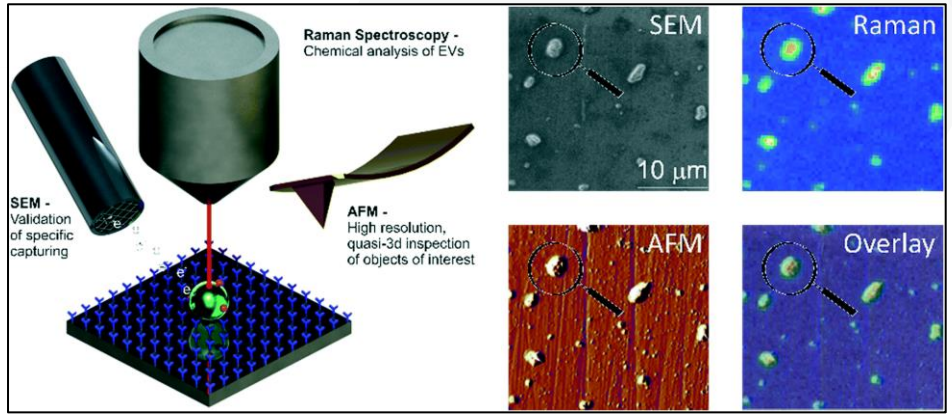
Extracellular vesicles







Mechanical characterization

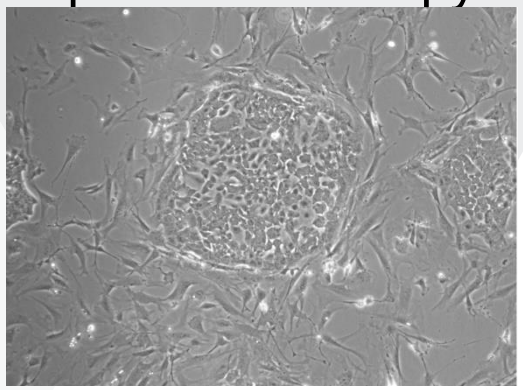


Multimodal characterization

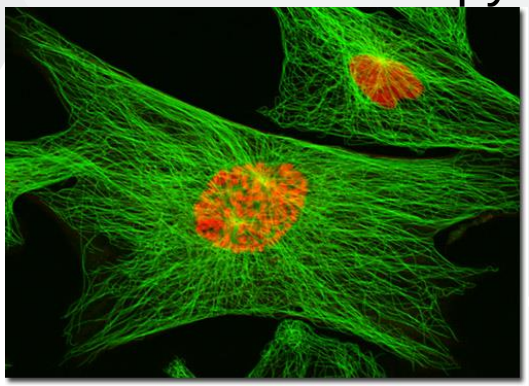
Front. Mol. Biosci., 21 July 2020

Nanomechanical mapping of living cells

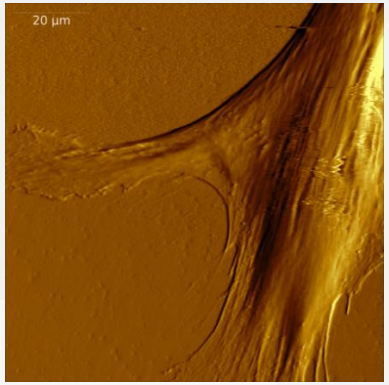
Optical microscopy

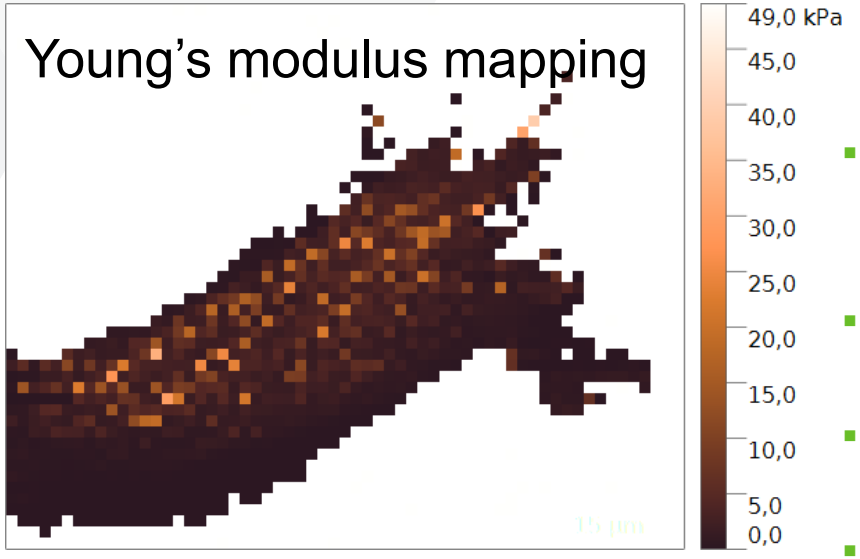


Confocal microscopy



AFM





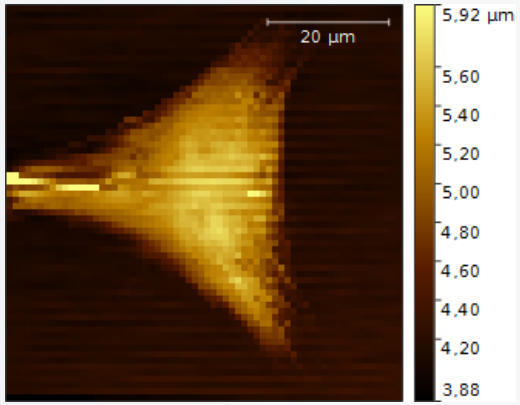
Motivation

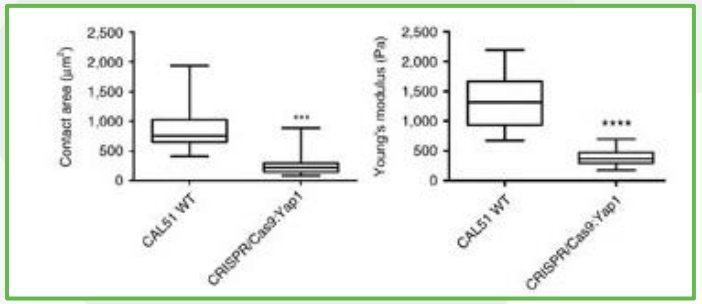
Why to quantify elasticity of (living) objects?

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

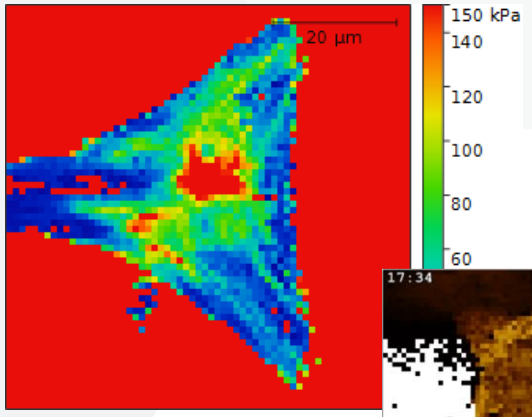
Cellular nanomechanics
By means of AFM

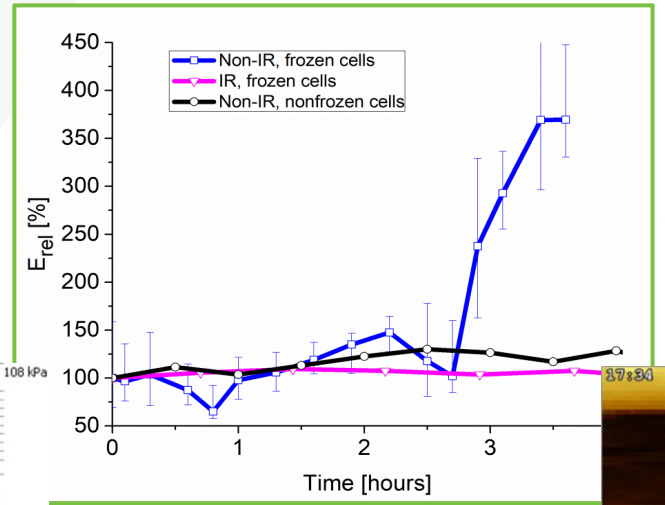
AFM mapping - correlation with fluorescence microscopy



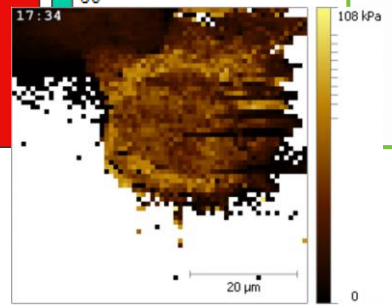


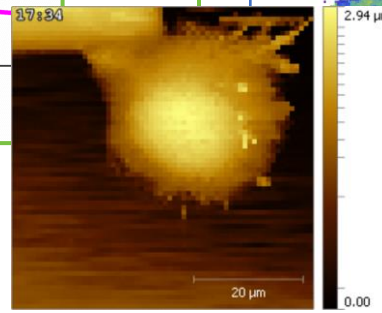
Evalutation - statistics

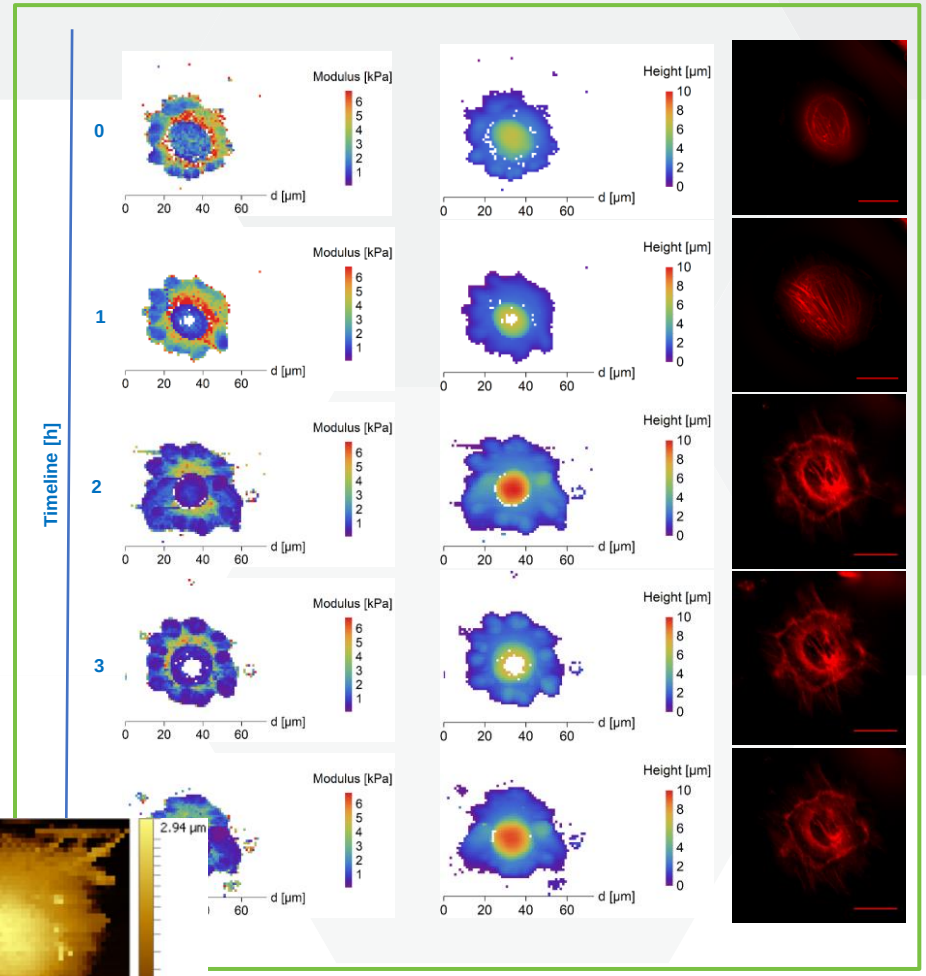




Time-lapsed biomechanics



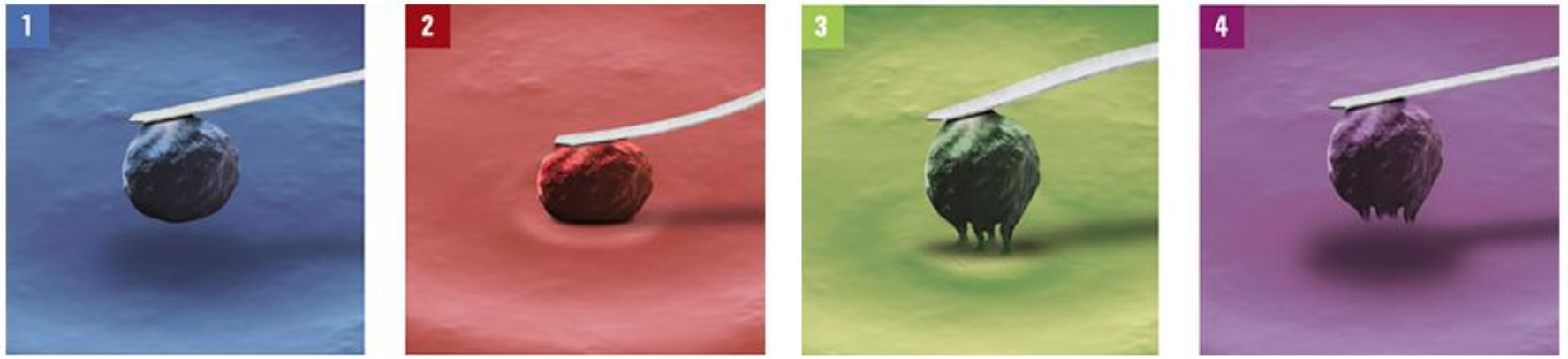




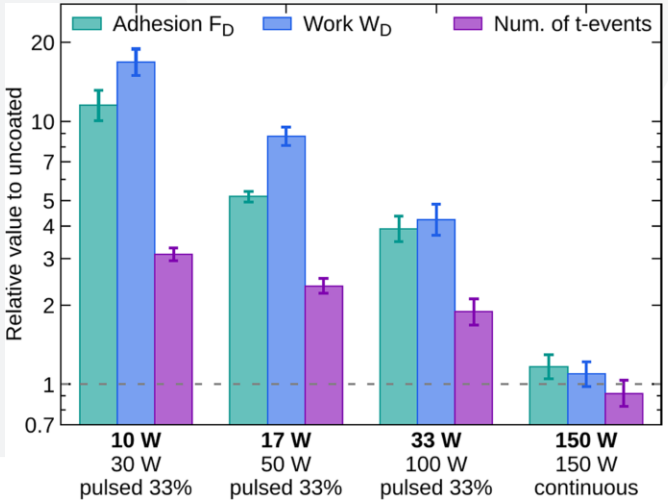
NARDONE, Giorgia, Jorge Oliver-De La CRUZ, Jan VRBSKY, Cecilia MARTINI, Jan PRIBYL, et al., 2017. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nature Communications* [online]. **8**, ncomms15321.

GOLAN, Martin, et al. Front. Physiol., 29 June 2018

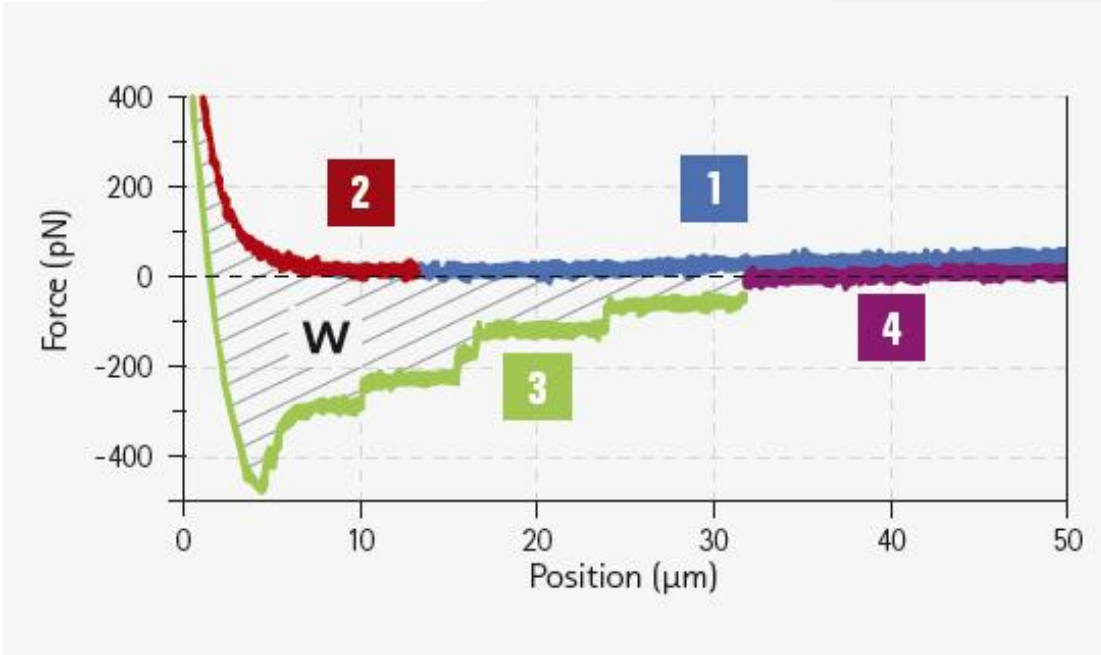
Cell-adhesion experiments



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

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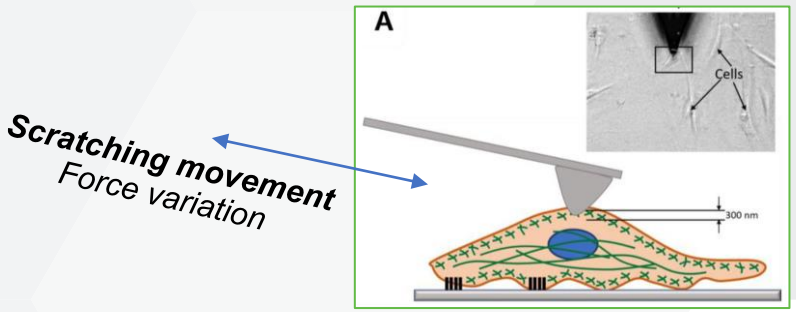
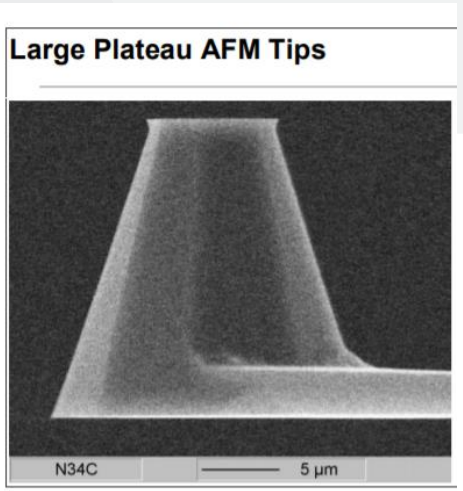
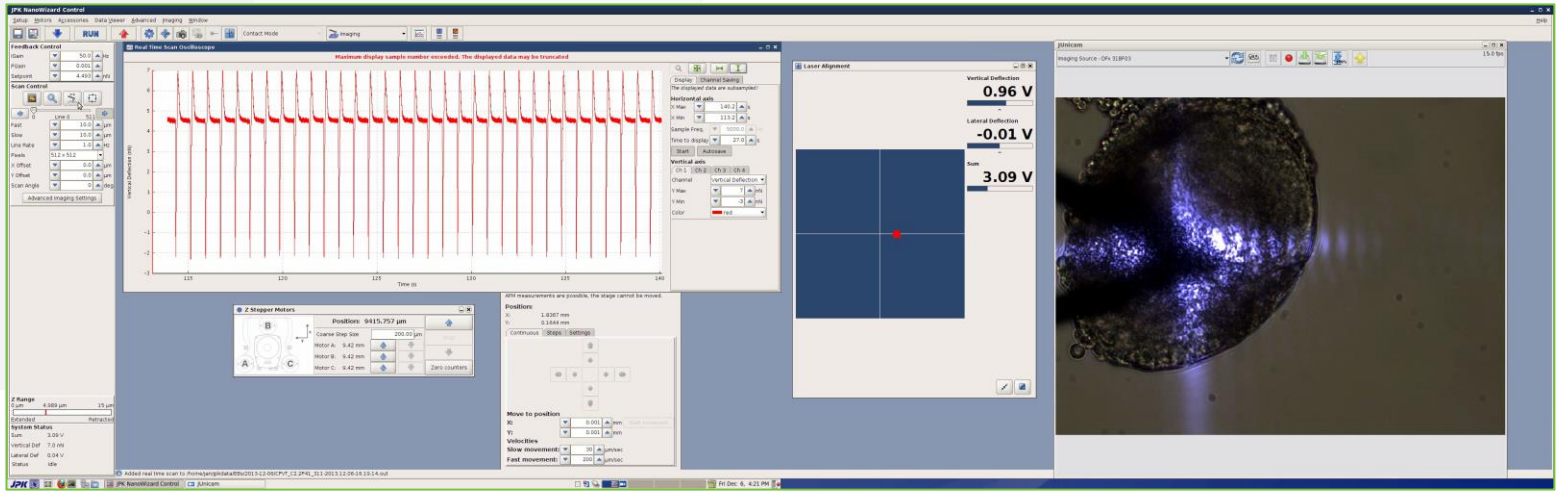
Other applications of AFM-based biomechanics

Cell scratching = cell adhesion

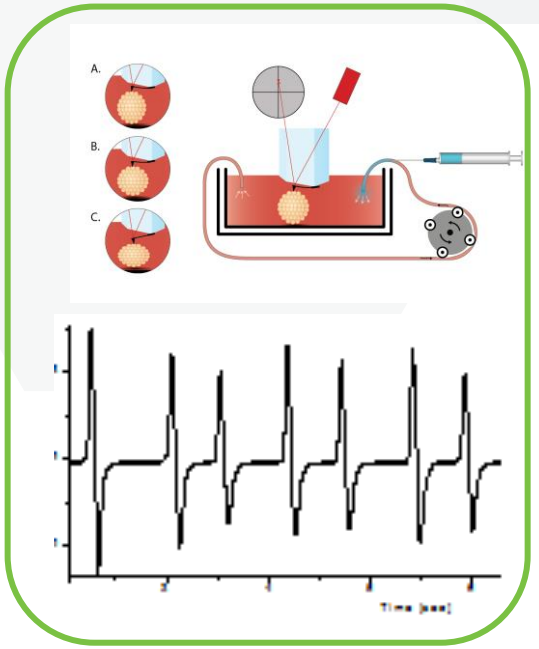




Cardiac cells biomechanics



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

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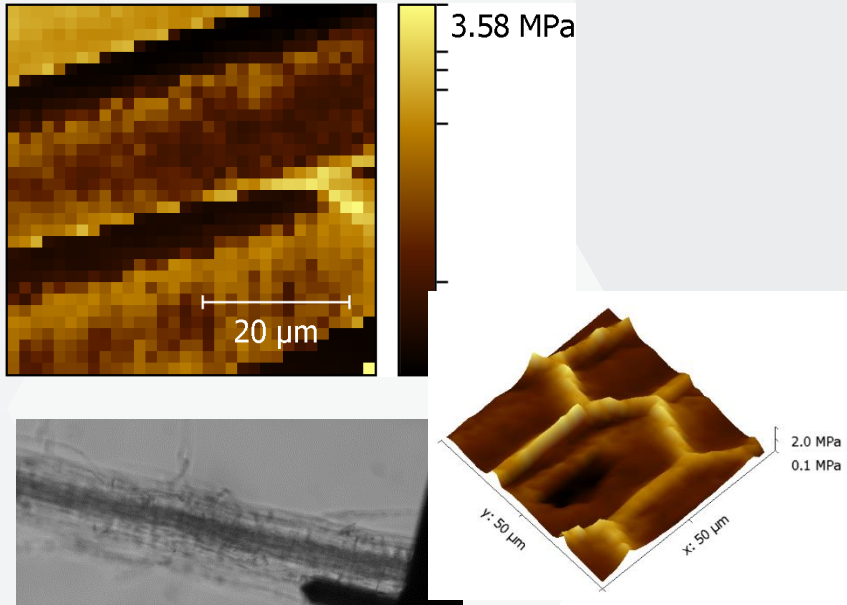
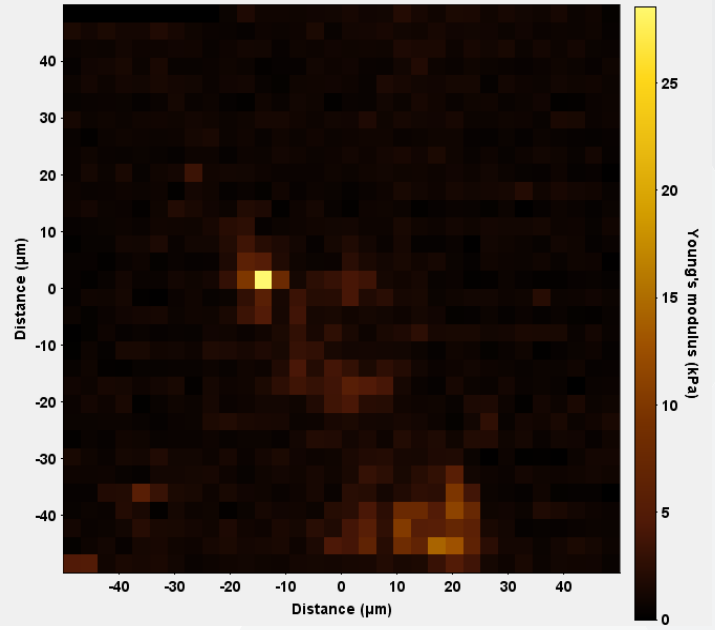
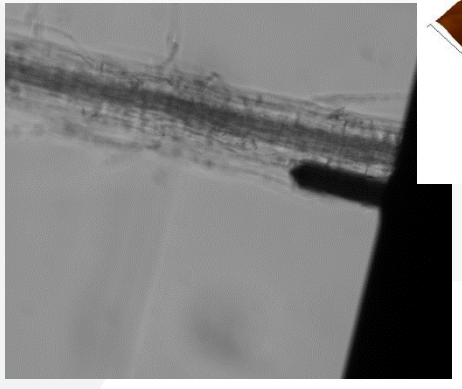
AFM-based biomechanics
On a tissue level





Liver cirrhosis
Correlation of
Collagen fibers by polarized microscopy
AFM nanoindentation

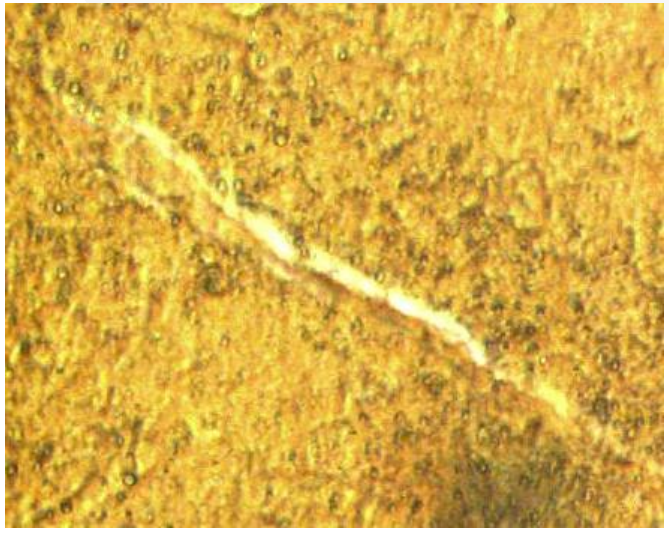


Plant samples
(hypocotyl)

by Marçal Gallemí

Eva Benkova Lab

& Jan Hejatko Lab



by Srikant Ojha

Martin Gregor Lab

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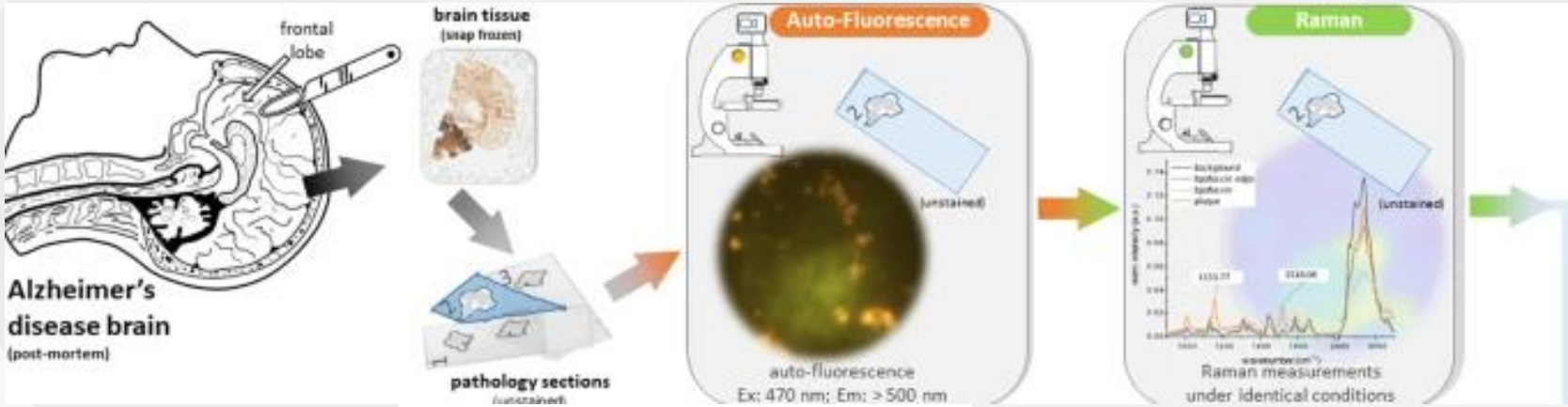
Raman microscopy
On bio samples

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

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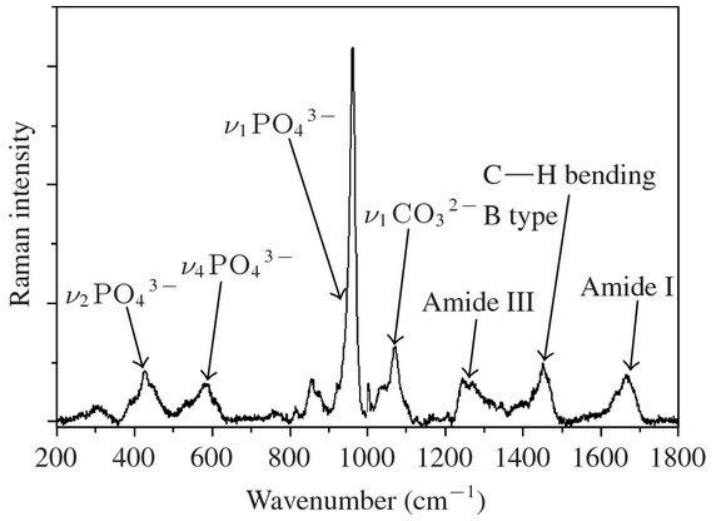
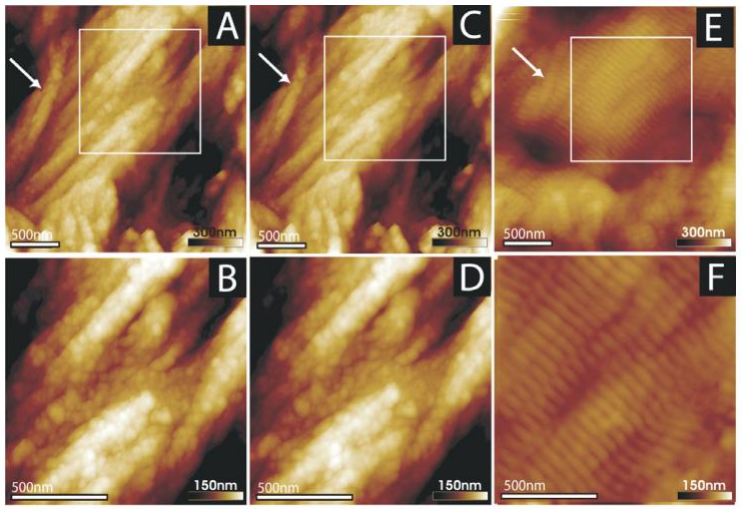
Calcification level and **Collagen Fibers** Arrangement in **Bone Tissue**



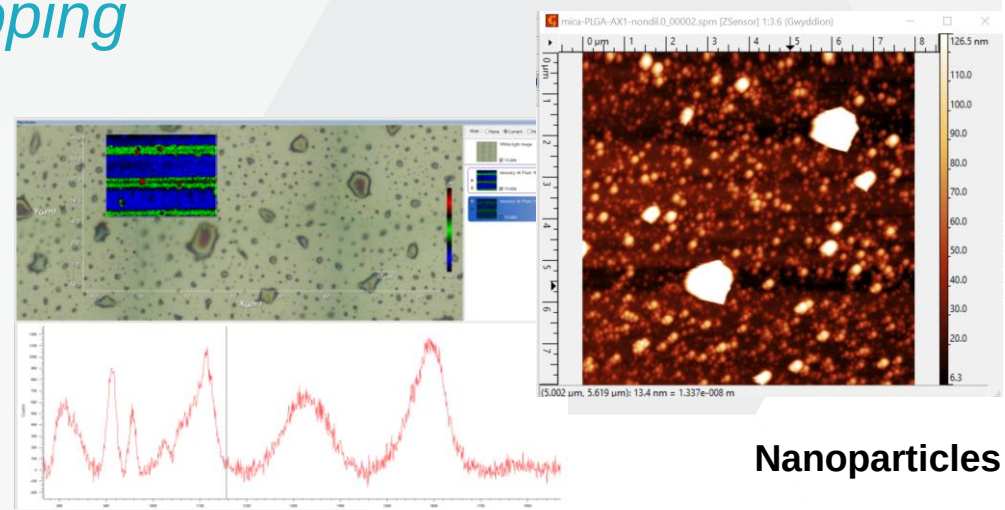


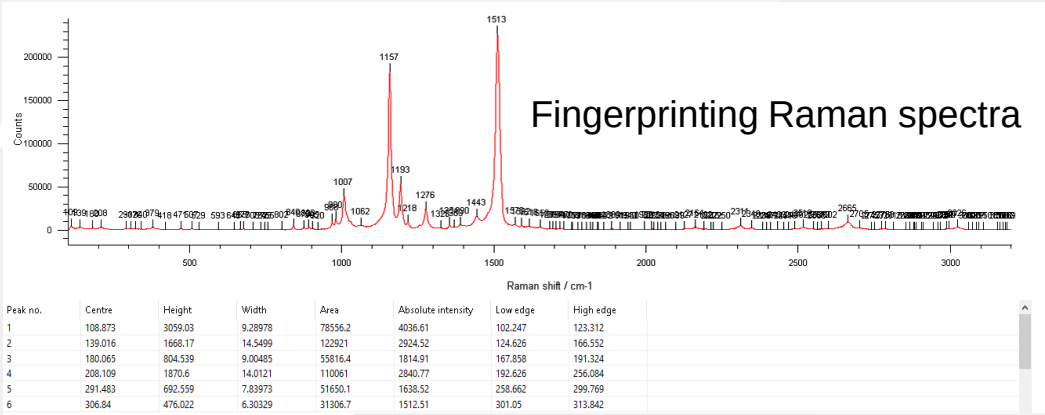
+ combination with AFM topography

Tomasz Buchwald, et al. Spectroscopy 27(2):107-117

Raman microscopy + AFM

Chemical mapping

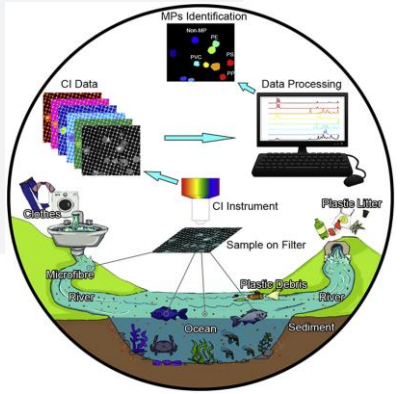


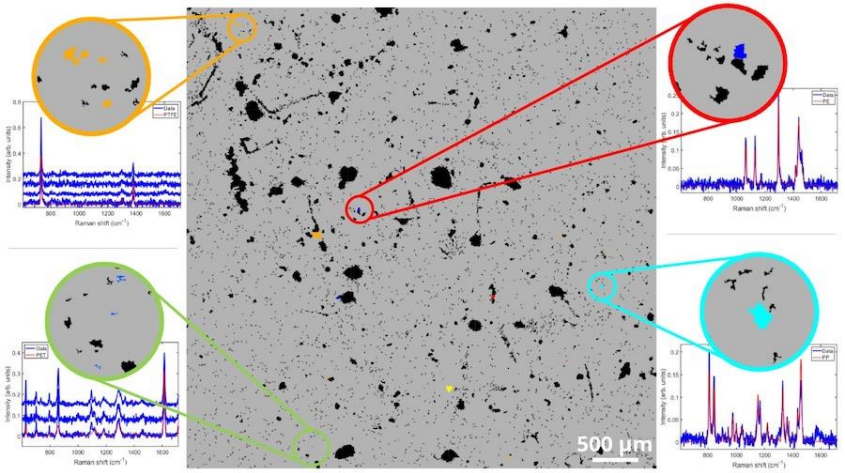


Peak no.	Centre	Height	Width	Area	Absolute intensity	Low edge	High edge
1	108.873	3059.03	9.28978	78556.2	4036.61	102.247	123.312
2	139.016	1668.17	14.5499	122921	2924.52	124.626	166.552
3	180.065	804.539	9.00485	55816.4	1814.91	167.858	191.324
4	208.109	1870.6	14.0121	110061	2840.77	192.626	256.084
5	291.483	692.559	7.83973	51650.1	1638.52	258.662	299.769
6	306.84	476.022	6.30329	31306.7	1512.51	301.05	313.842

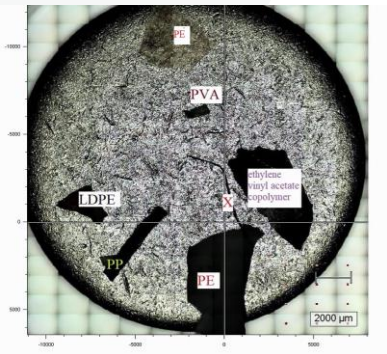
Nanoparticles loading study

+ combination with AFM topography



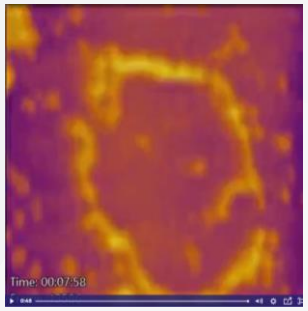


Microplastics identification

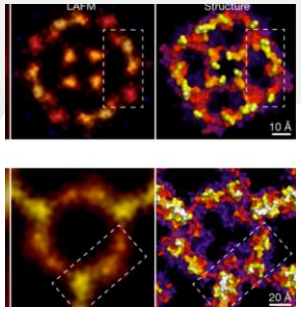


Where to go next...?

1. High-Speed (Video-Rate) AFM

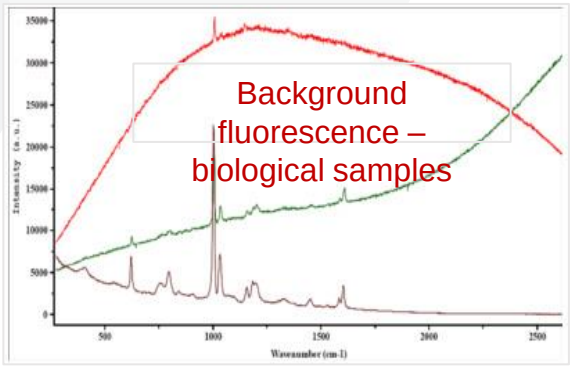


Fast biological processes



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

2. Raman microscope upgrade



Raman part - upgrade

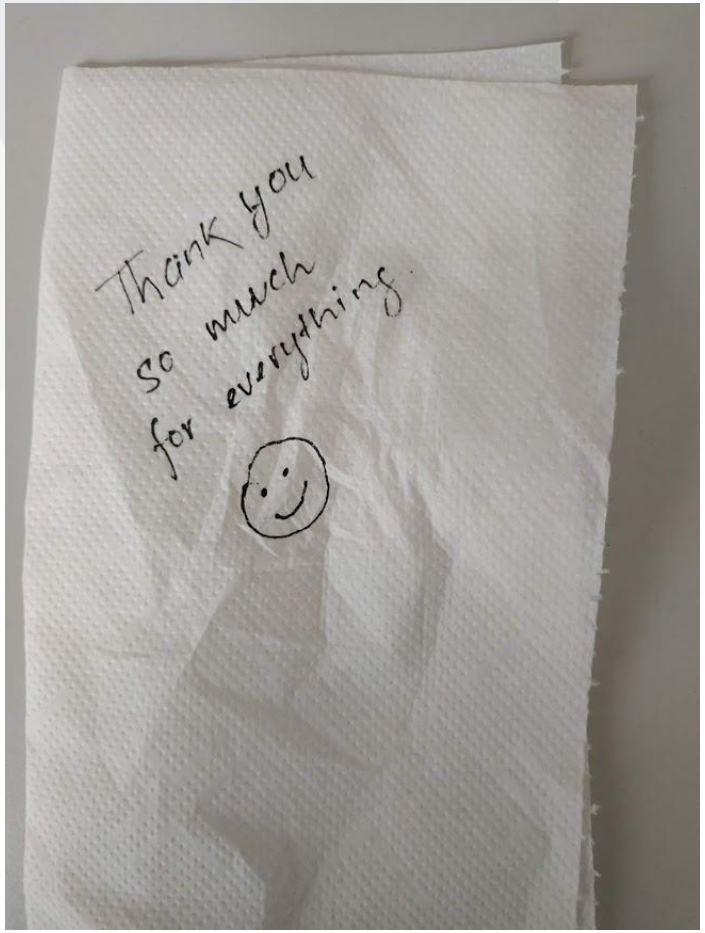
+ **laser 785 nm**, 100 mW (fluorescence decrease)
+ 1 x 785 nm **polarization**
+ 1 x 633 nm polarization
+ Software upgrade to version **WiRE 5.6**
(incl. **particle analysis** and spectral database modules – **microplastics**)

Full integration with AFM

- AFM correlative software
- AFM microscope antivibration solution

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

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



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Thank you for your attention.

Web: ceitec.eu/nanobio

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instruct Integrating Biology

(LM2023042)

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

MINISTRY OF EDUCATION, YOUTH AND SPORTS

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

CarDia
Project reg. number:
LX22NPO5104

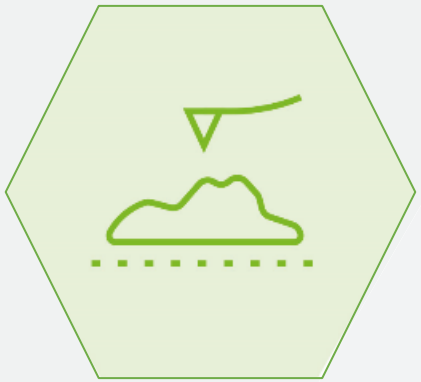
MUNI Grant Agency

JAROSLAV KOČA BRIDGE FUND

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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CIISB Czech Infrastructure for Integrative Structural Biology

MŠMT

May 15-17 2024

University Campus Bohunice
Brno, Czech Republic
*building **E35,** Atrium*

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