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MUNI

Advanced biophysical methods for characterization of biosamples

Atomic Force Microscopy of biosamples

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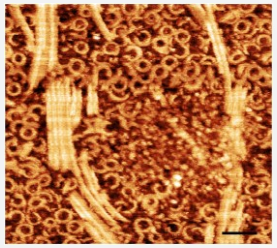
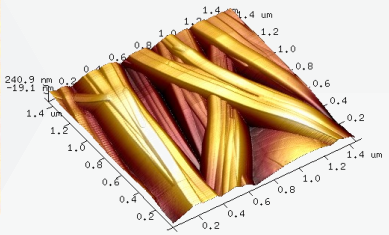
Content

- **AFM, BioAFM technology**
- **AFM operation modes: Imaging, Indentation, …**
- **Examples**
- **Core Facility NanoBiotechnology**

Introduction

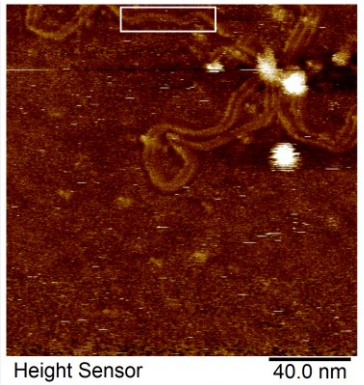
Bio Atomic Force Microscopy (BioAFM)

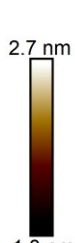


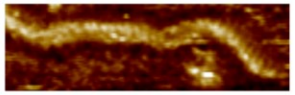


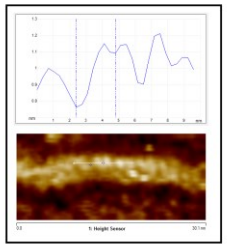
IMAGING

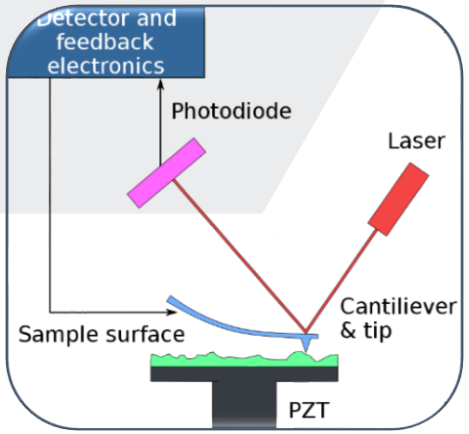
Proteins, DNA, Nanoobjects







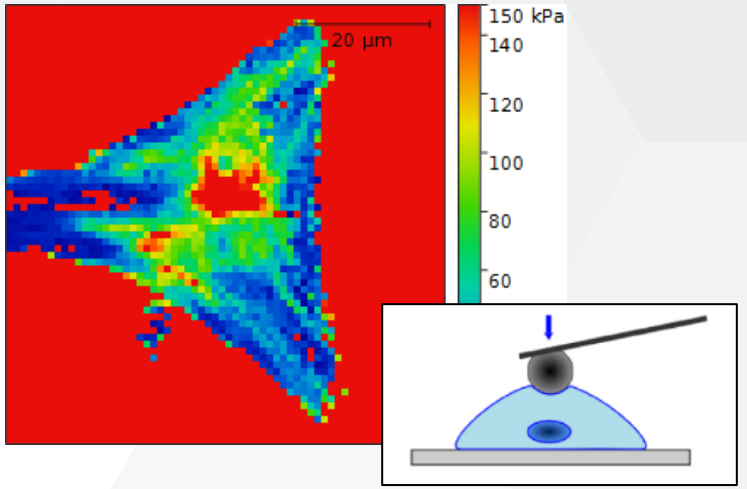




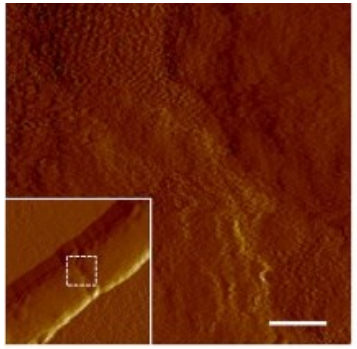
by OverloardQ

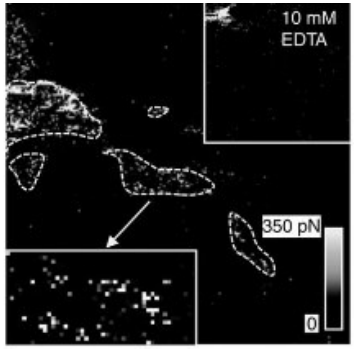
NANOINDENTATION

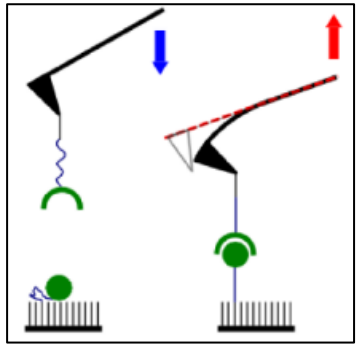
Stiffness mapping



AFFINITY INTERACTION

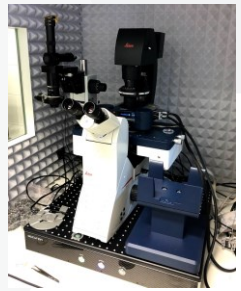




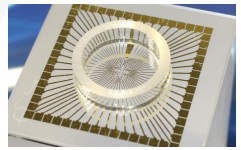


Combination with other techniques

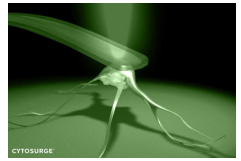
- *Optical (fluorescence) microscopy*



- *Multielectrode Array (MEA)*



- *FluidFM*



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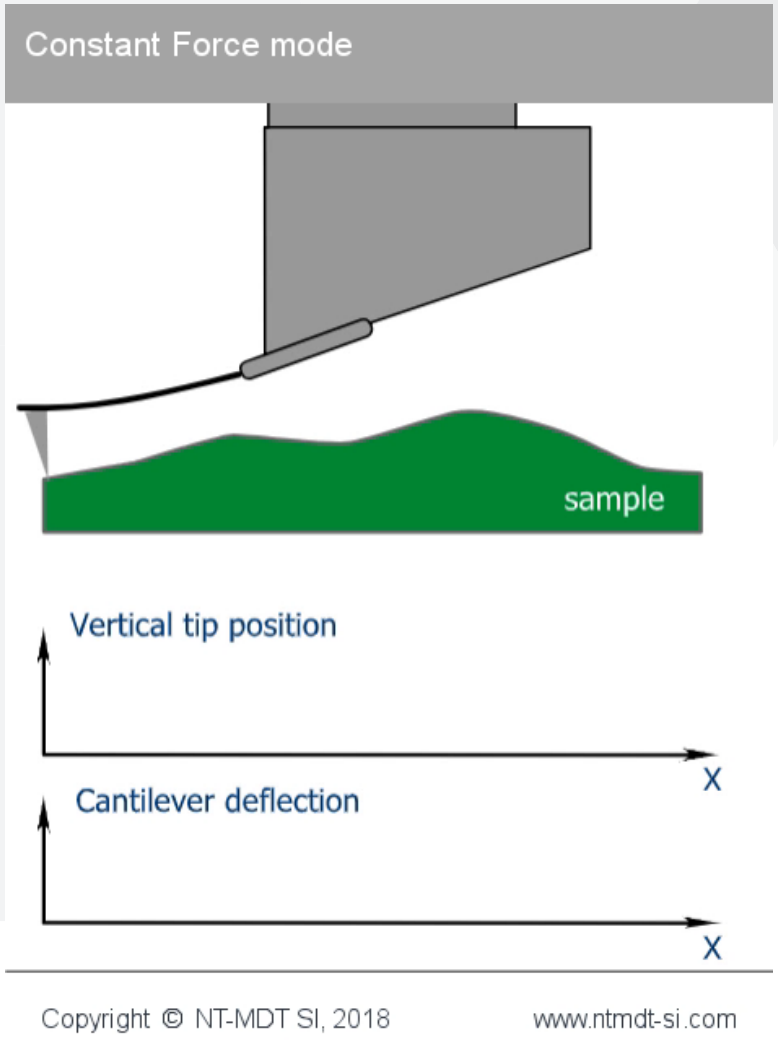
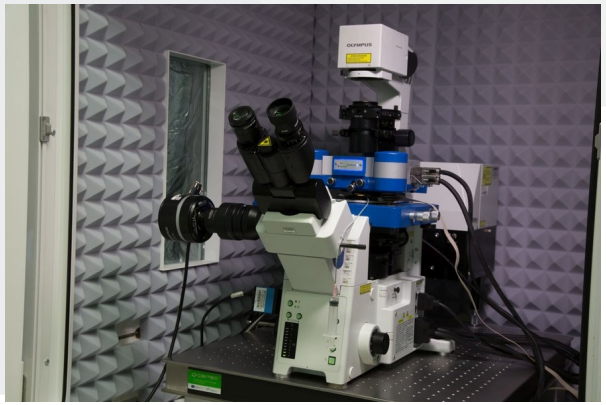
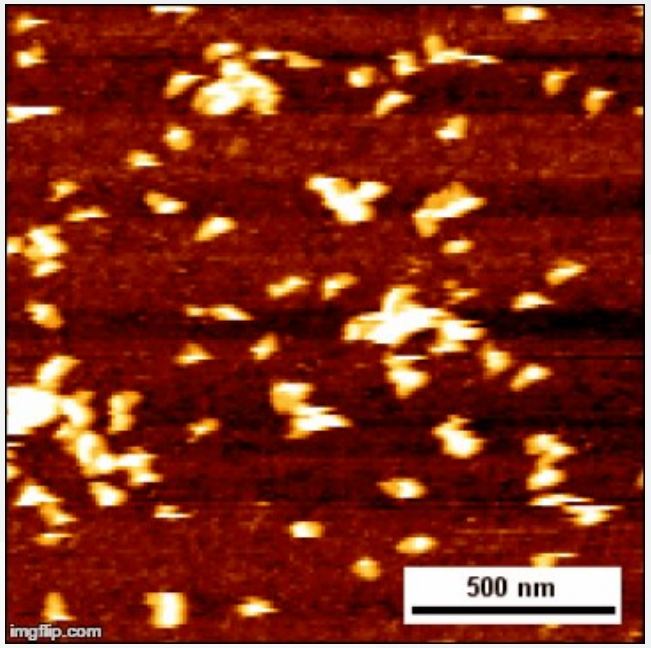
Jak funguje AFM



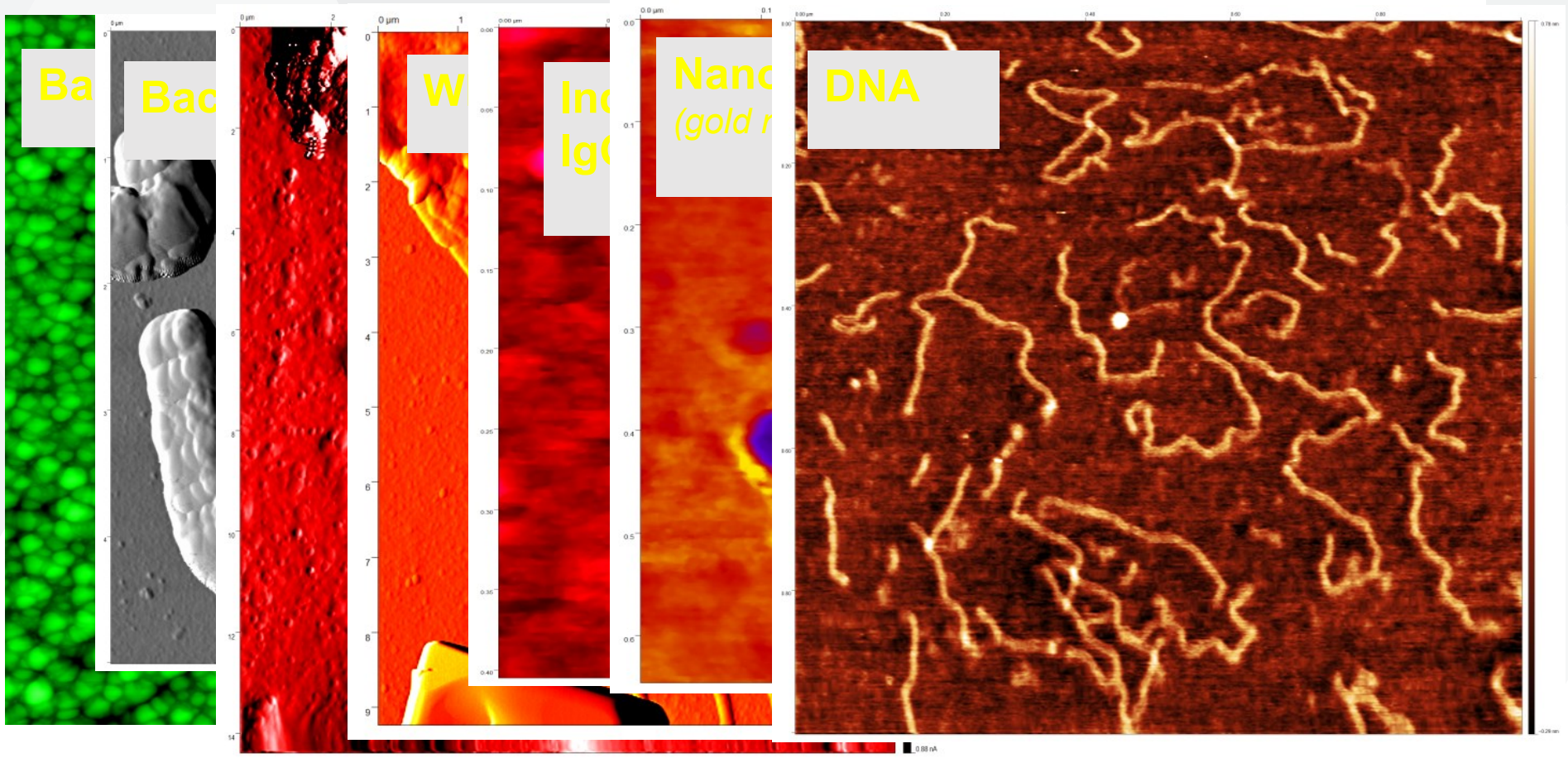




AFM (Atomic Force Microscope)

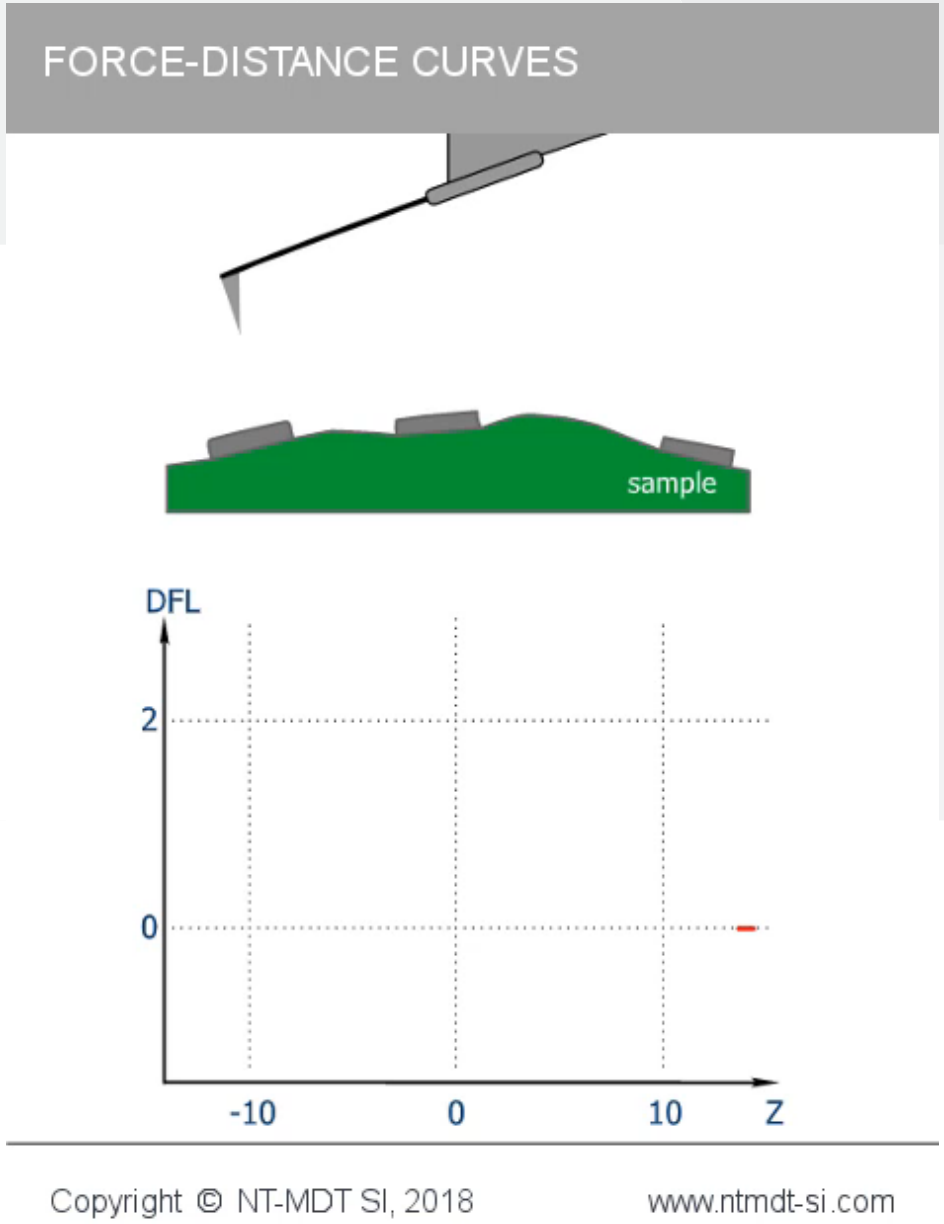
- Z rodiny tzv. Probe microscopies = skenující próbou
- Posun hrotu nad vzorkem = topografie povrchu
- Další vlastnosti vzorku (tuhost, adhezivita) z analýzy signálu

AFM visualization of biomolecules and bioobjects



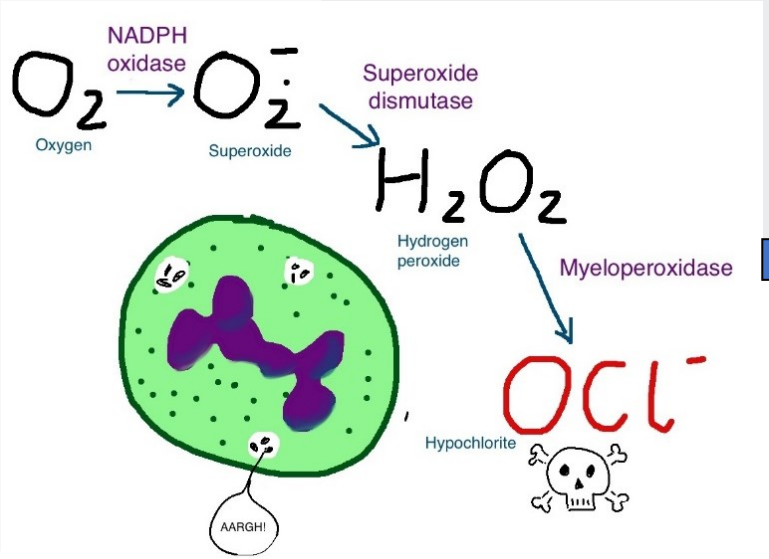
AFM indentation

- **AFM cantilever = nanosensor**
- **Force-distance curve (FDC) – describes interaction/deformation**
- **FDC = Young's modulus, adhesivity, rigidity, etc.**



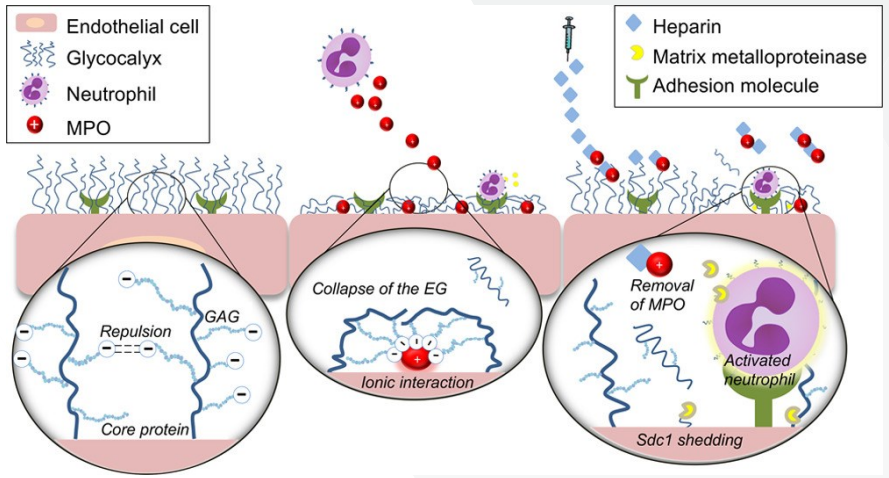
Imaging is not enough…

Hyaluronan-myeloperoxidase complex



myeloperoxidase

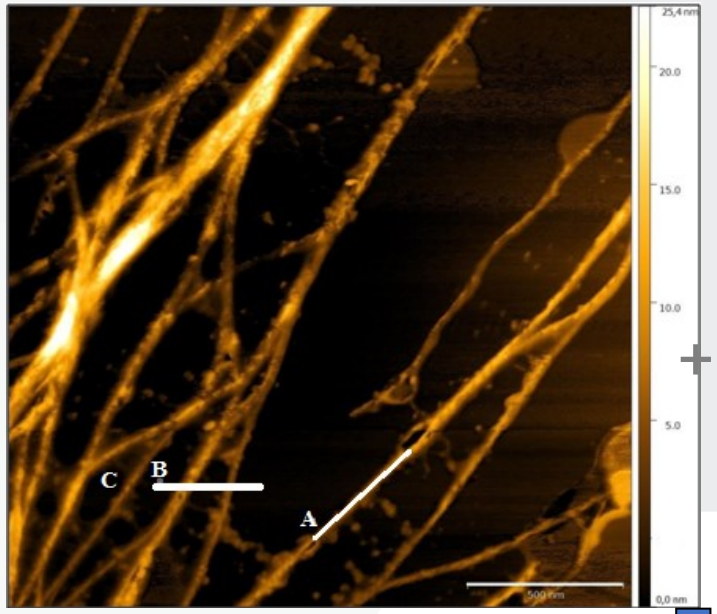
MPO (Myeloperoxidase) Reduces Endothelial Glycocalyx Thickness Dependent on Its Cationic Charge



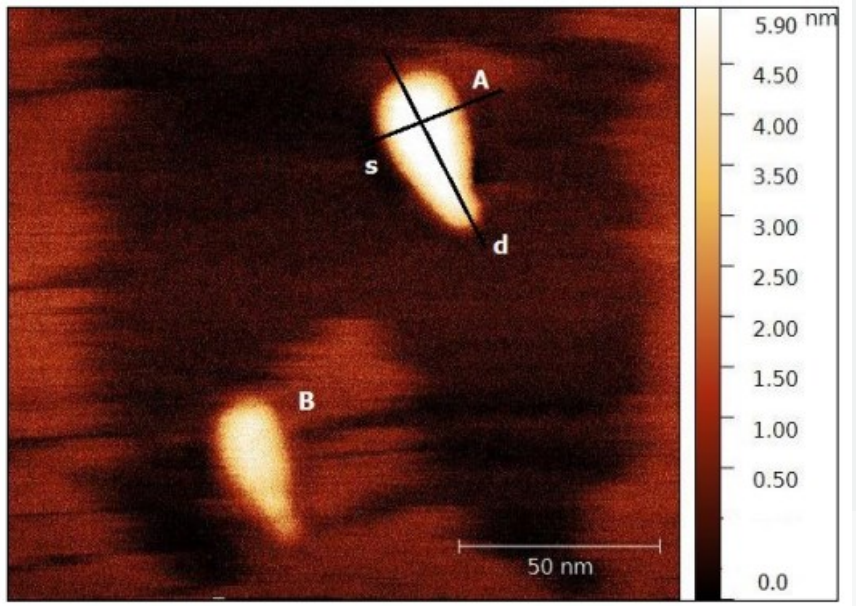
Kashish Manchanda, et al. Arteriosclerosis, Thrombosis, and Vascular Biology. 2018;38:1859–1867

Study by Stepan Solny

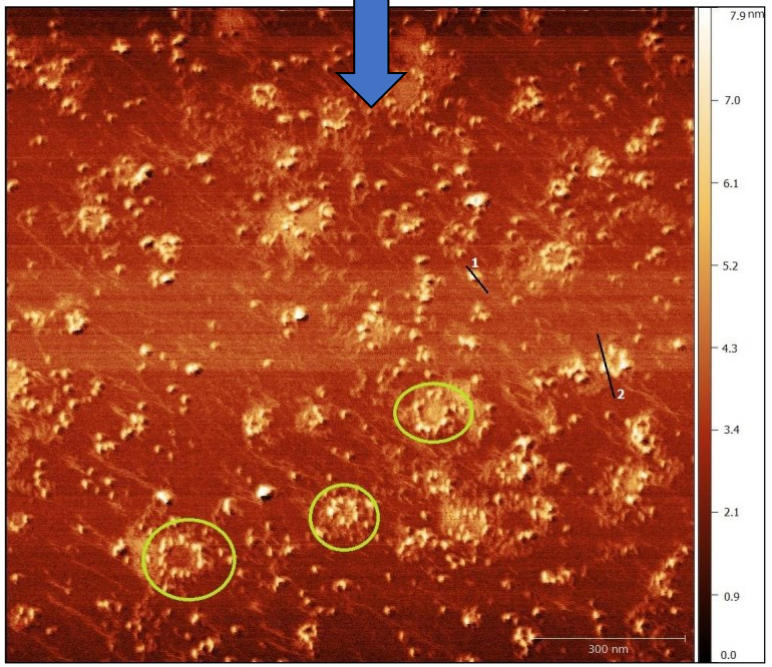
Imaging



hyaluronan



myeloperoxidase



Molecular complex

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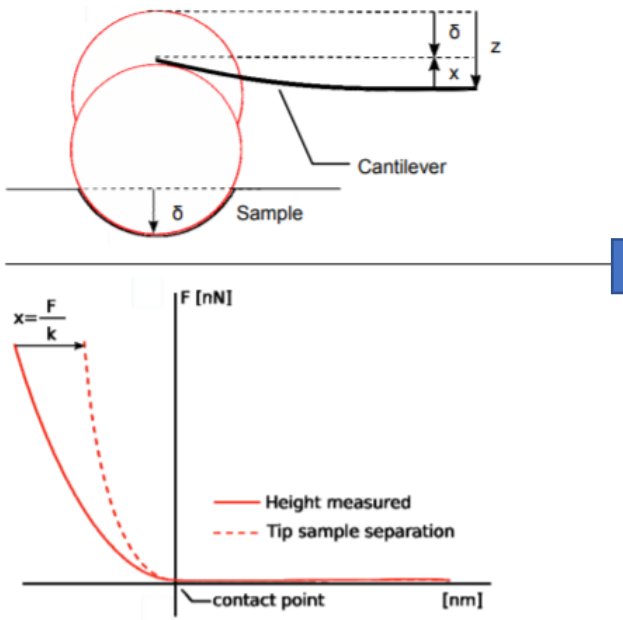
FDC analysis
Young's modulus

Experiment +

FDC recording



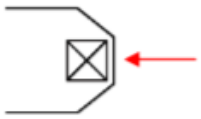
Tip (indenter) definition

Four-sided pyramid

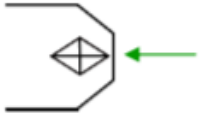
$$F = \frac{E}{1-\nu^2}\frac{\tan\alpha}{\sqrt{2}}\delta^2$$

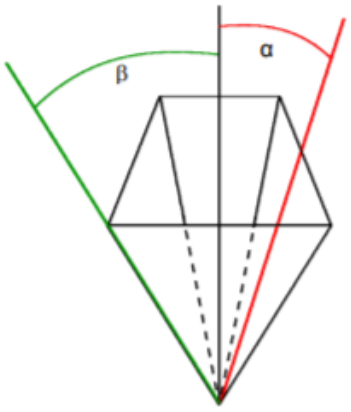
$$a = \frac{\tan\alpha}{\sqrt{2}}\delta$$

α = face angle, usually given for Si_3N_4-cantilevers

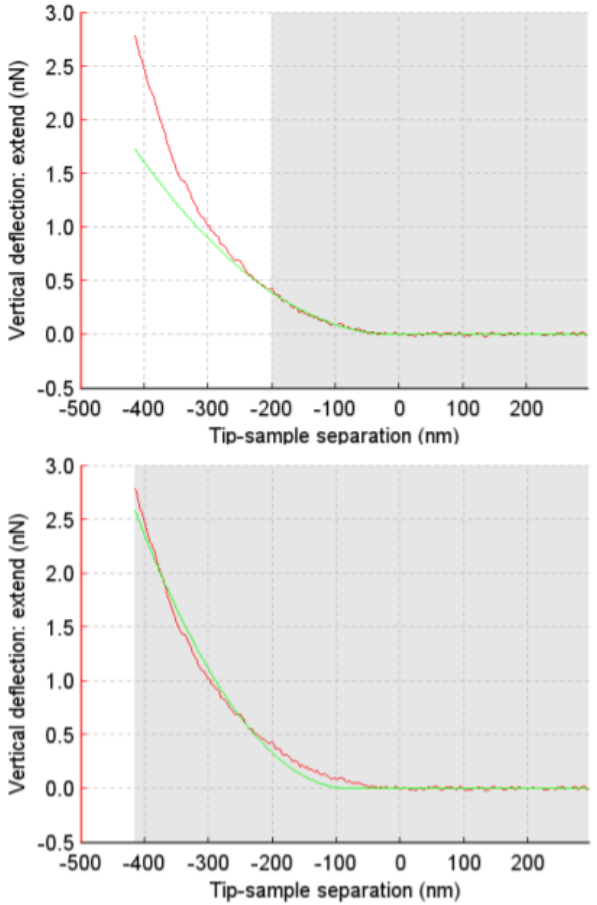


β = edge angle, usually given for Si-cantilevers



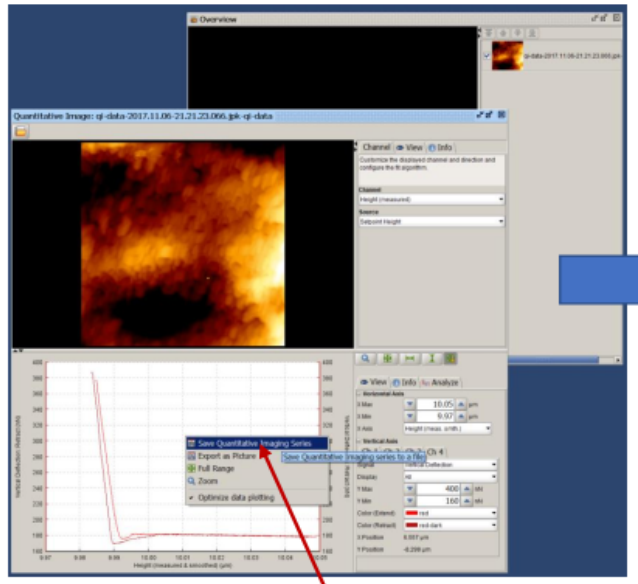


FDC fit

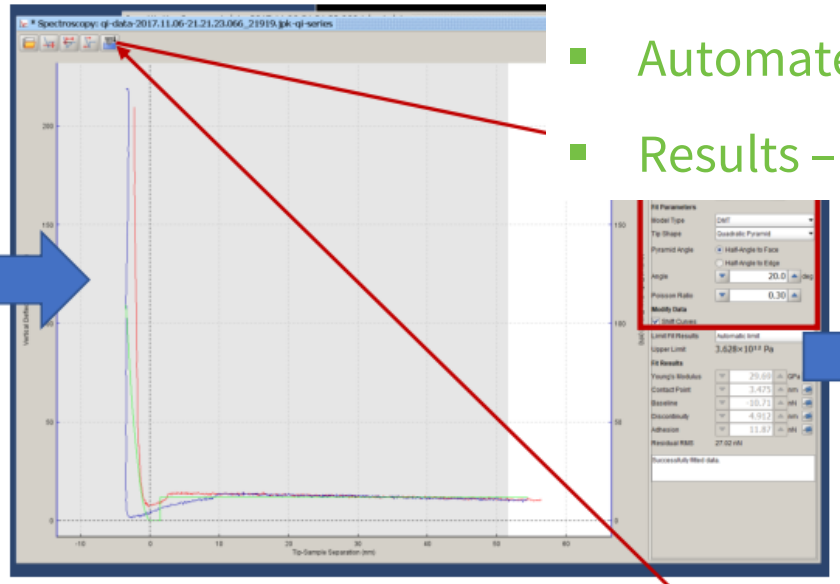


FDC automatic analysis
Young's modulus

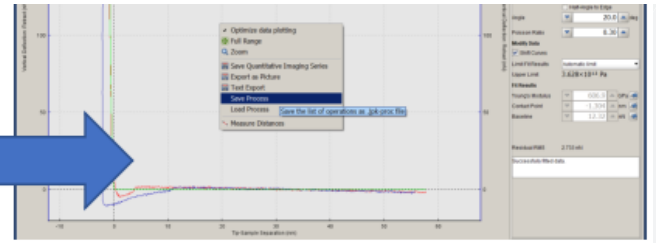
- Force-Distance curves maps
- Typically, 32x32, 64x64 and more
- Automated FDC processing
- Results – stiffness maps, adhesivity maps, …



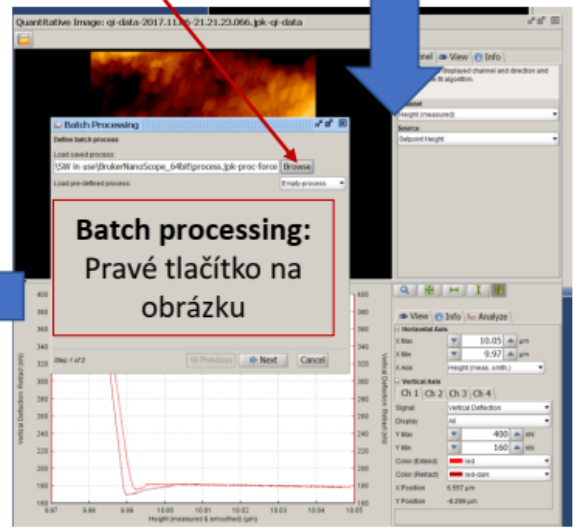
Vybrat pěknou křivku - uložit (pravé tlačítko na křivce)

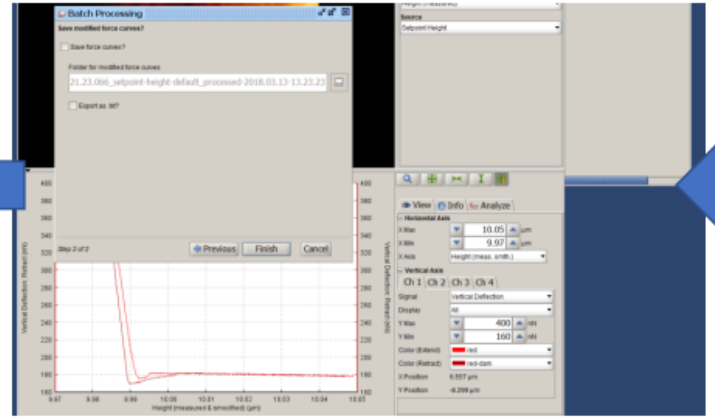


Křivku otevřít – nadefinovat požadovaný proces



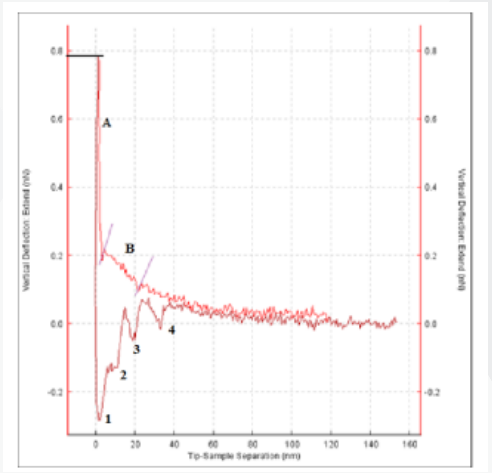
Uložit např. jako:
process.**jpk-proc-force**

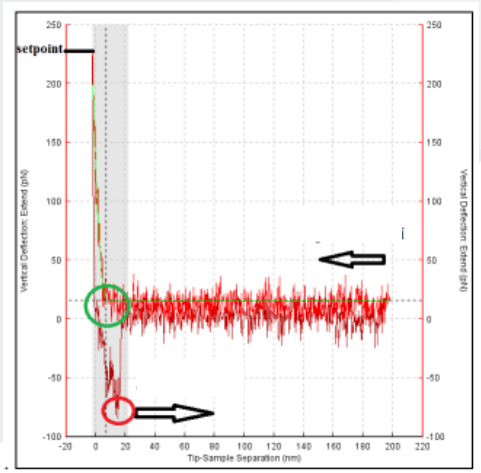




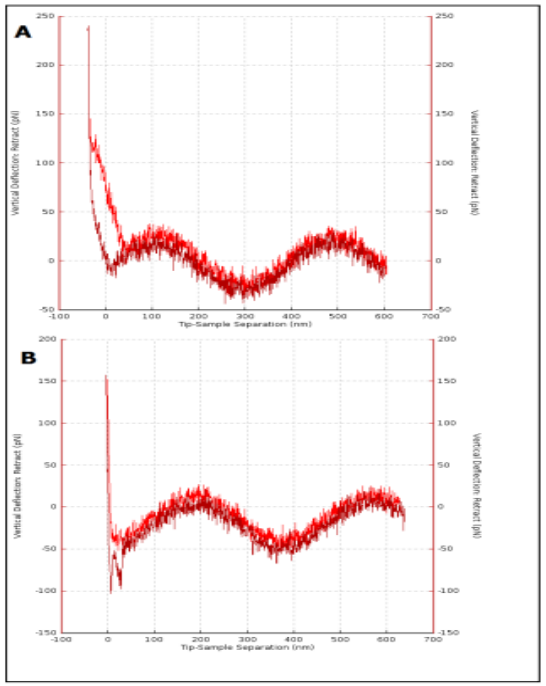
… další strana

FD curve recording and analysis

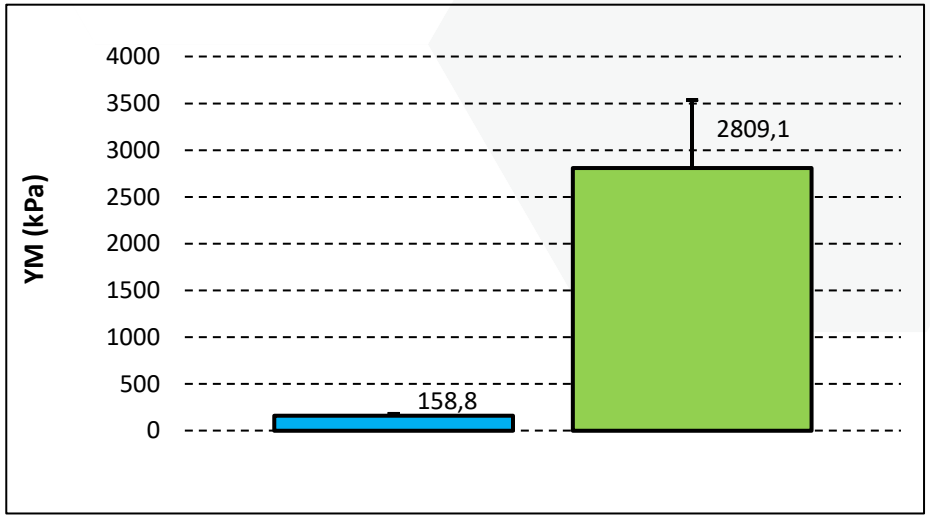




Not too strong!



HA-MPO complex is 20x stiffer comparing to HA



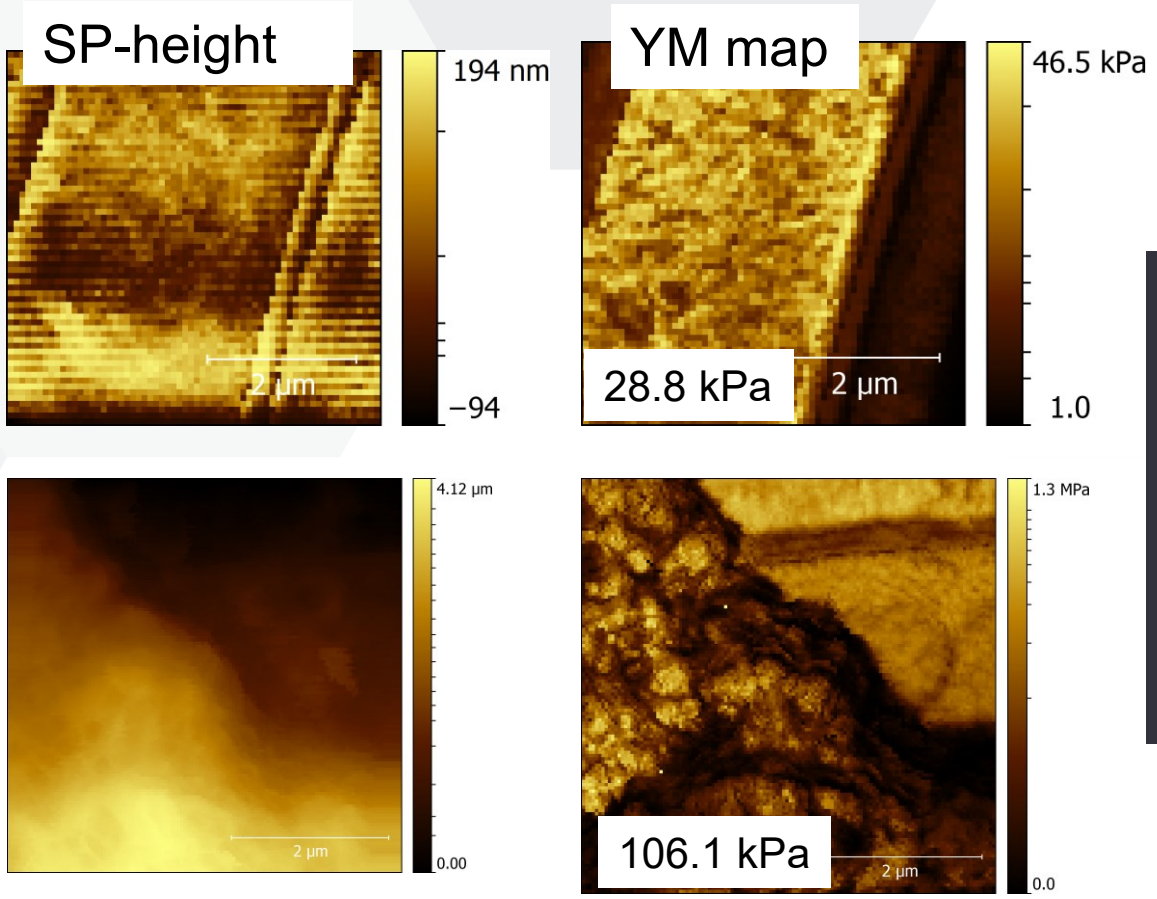
Select the proper curve

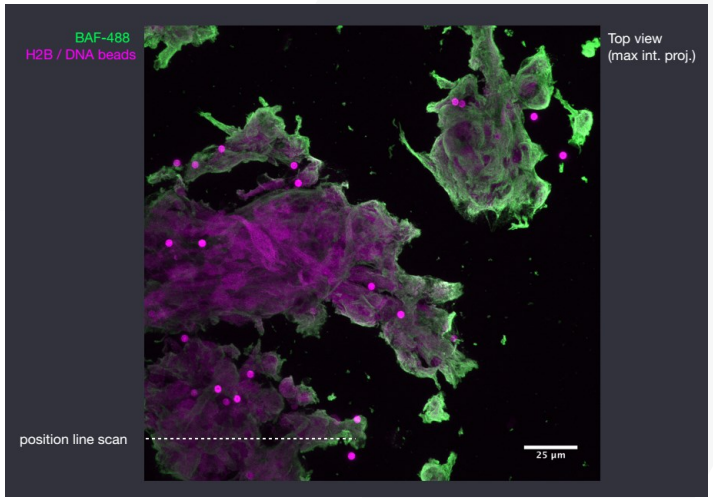
Chromatin-protein interaction

→ **For tissue engineering**

ForceMapping as imaging method

- Collapsive material
- Soft material (macroscopic view)





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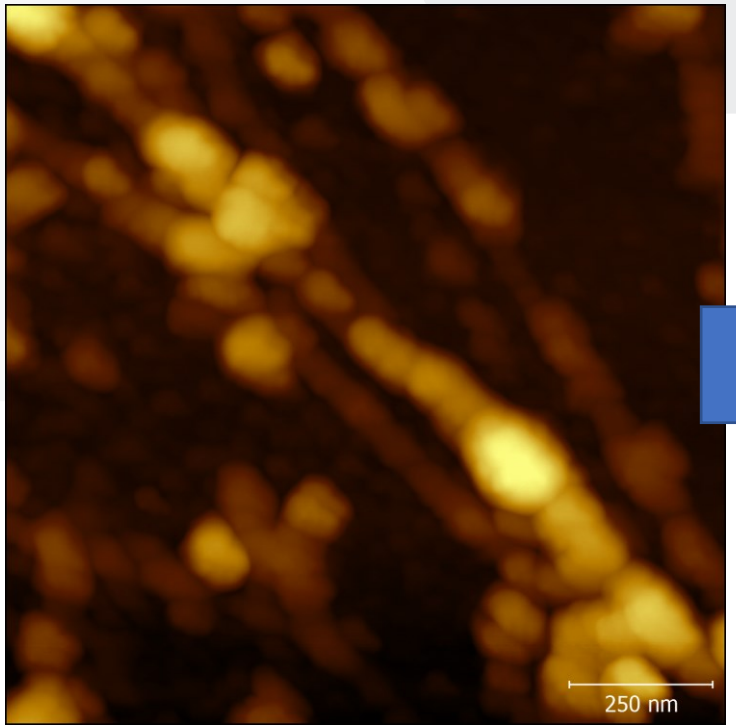
Samples by group of Matthias Nowak, IST, Vienna

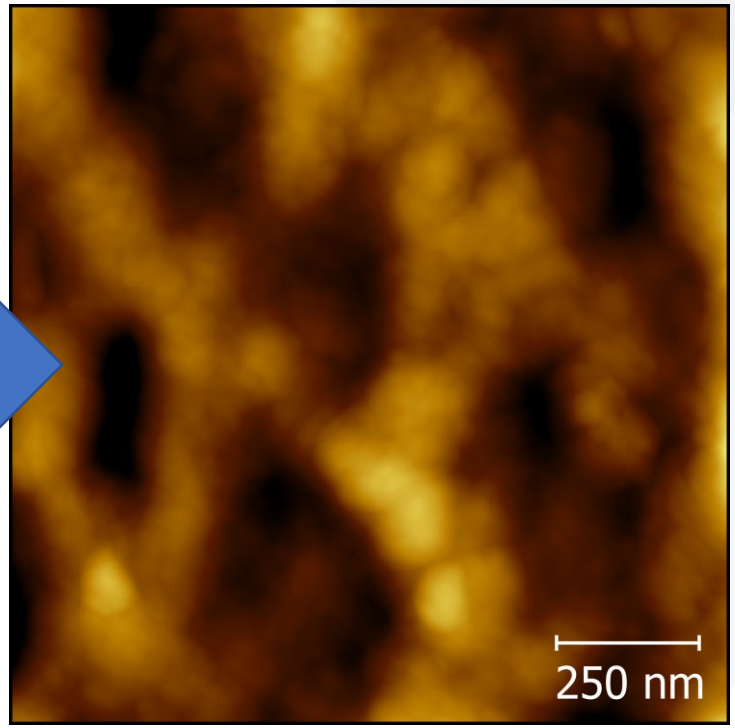
SUMMARY

Chromatin only
Chains composed of ellipsoids



+ BAF

Chromatin-BAF
Granular structure, grains composed of fibers (?) (~17nm)



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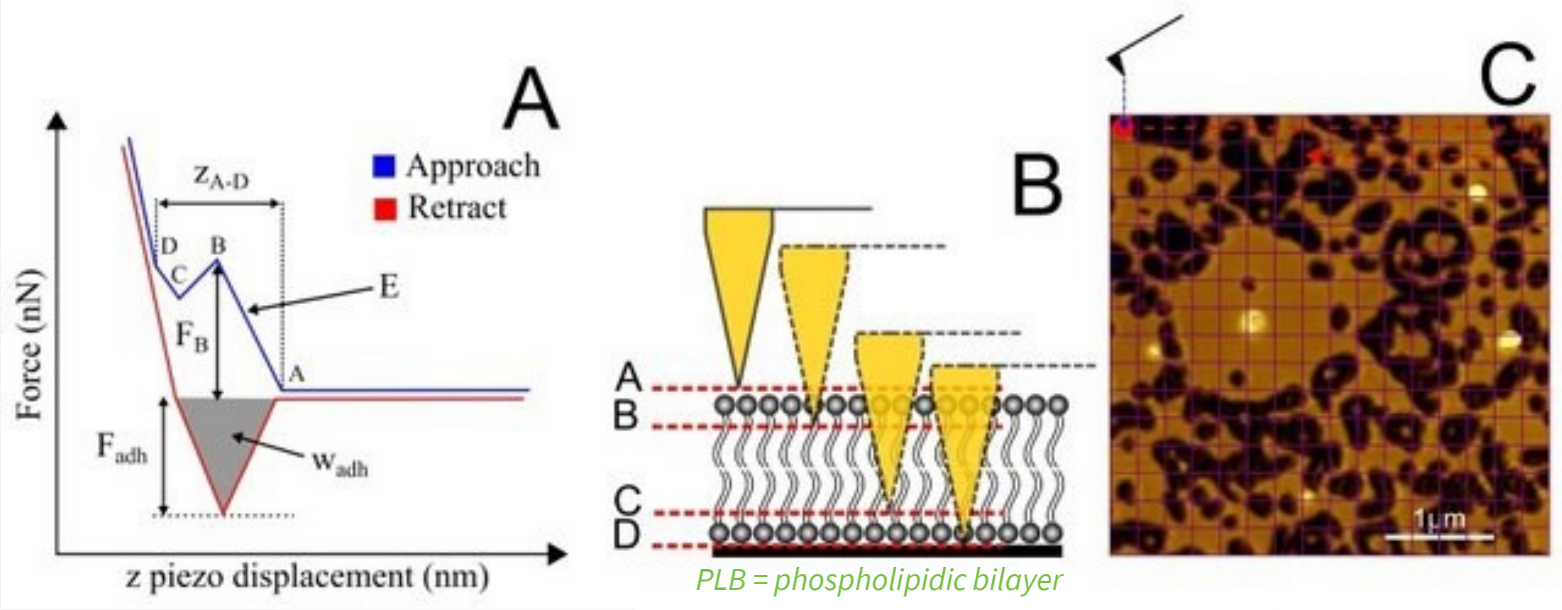
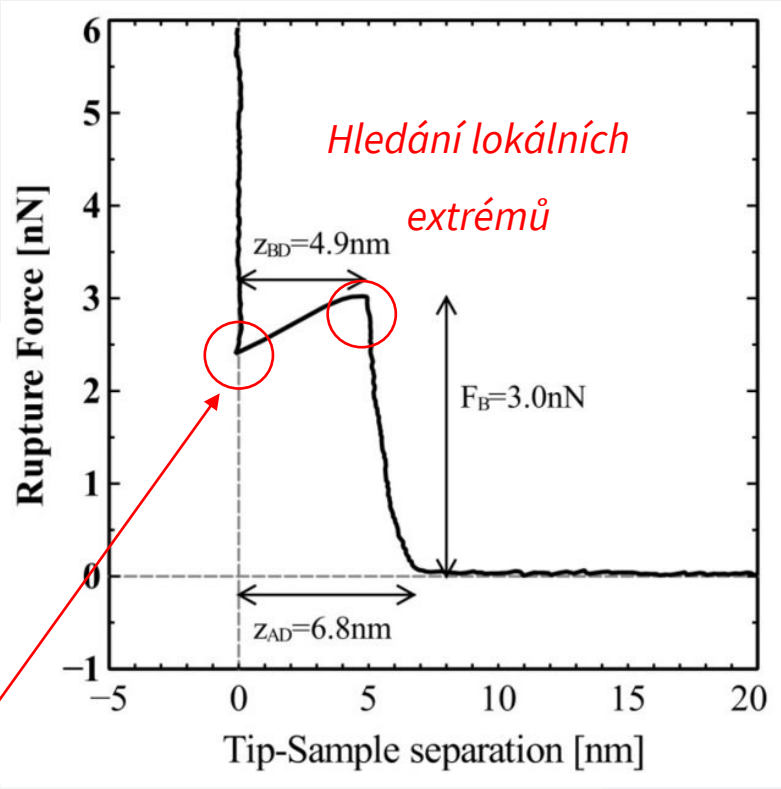
AFM as destructive sensor

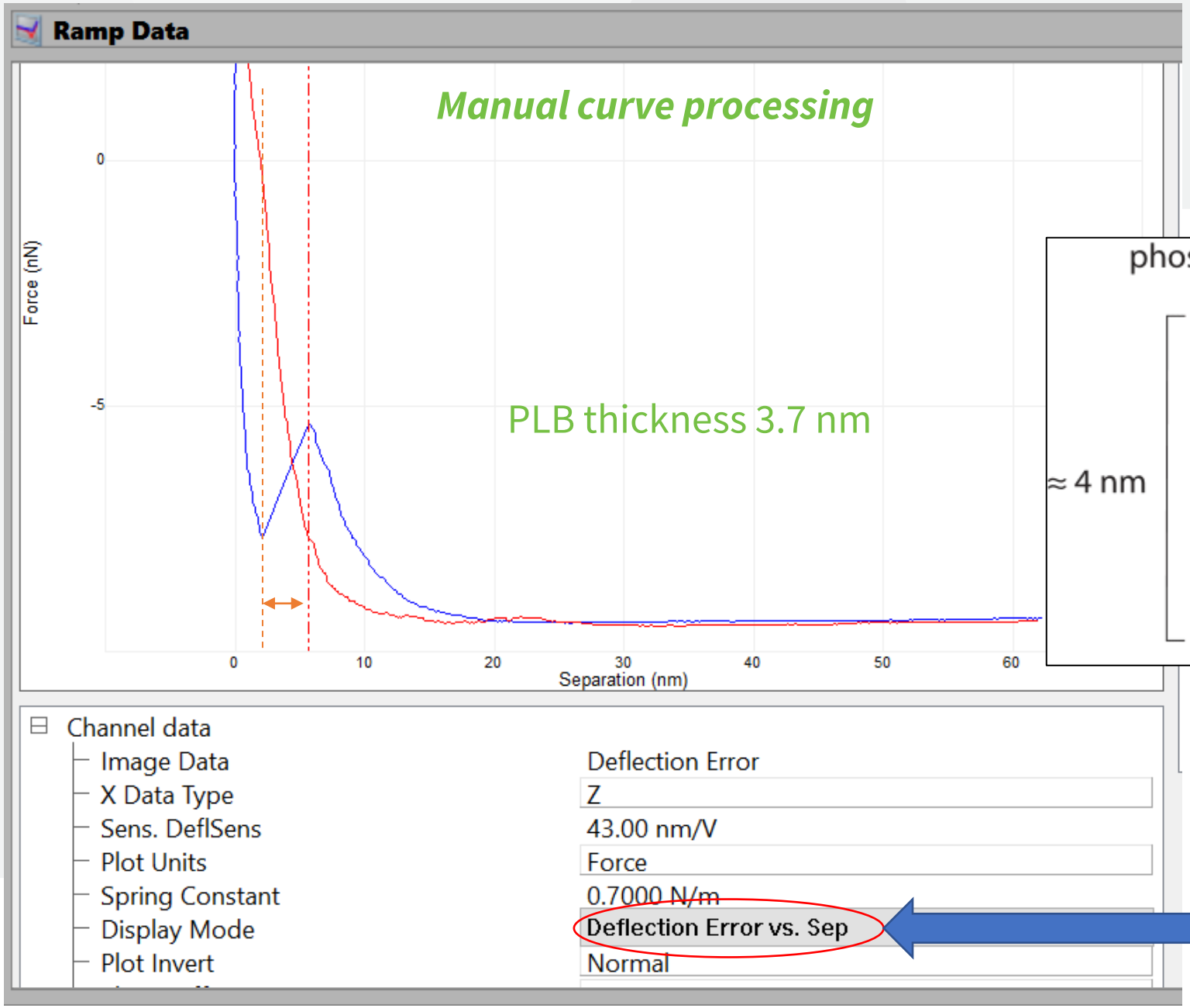
FDC, Rupture events

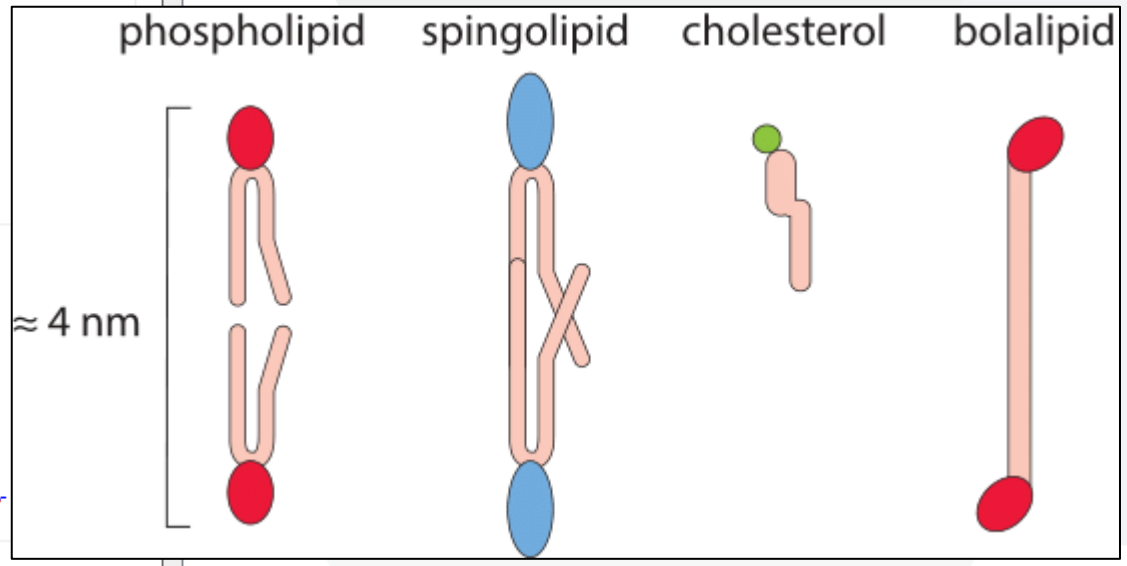


PLB = phospholipidic bilayer



- PLB = PhosphoLipidic Bilayer (= cell membrane *in-vitro*)
- Force application = bilayer rupter (=rupture event)
- *Z* = PLB thickness
- *F* = PLB strength
- Difficult automatic analysis





Separation signal

Roi.mca

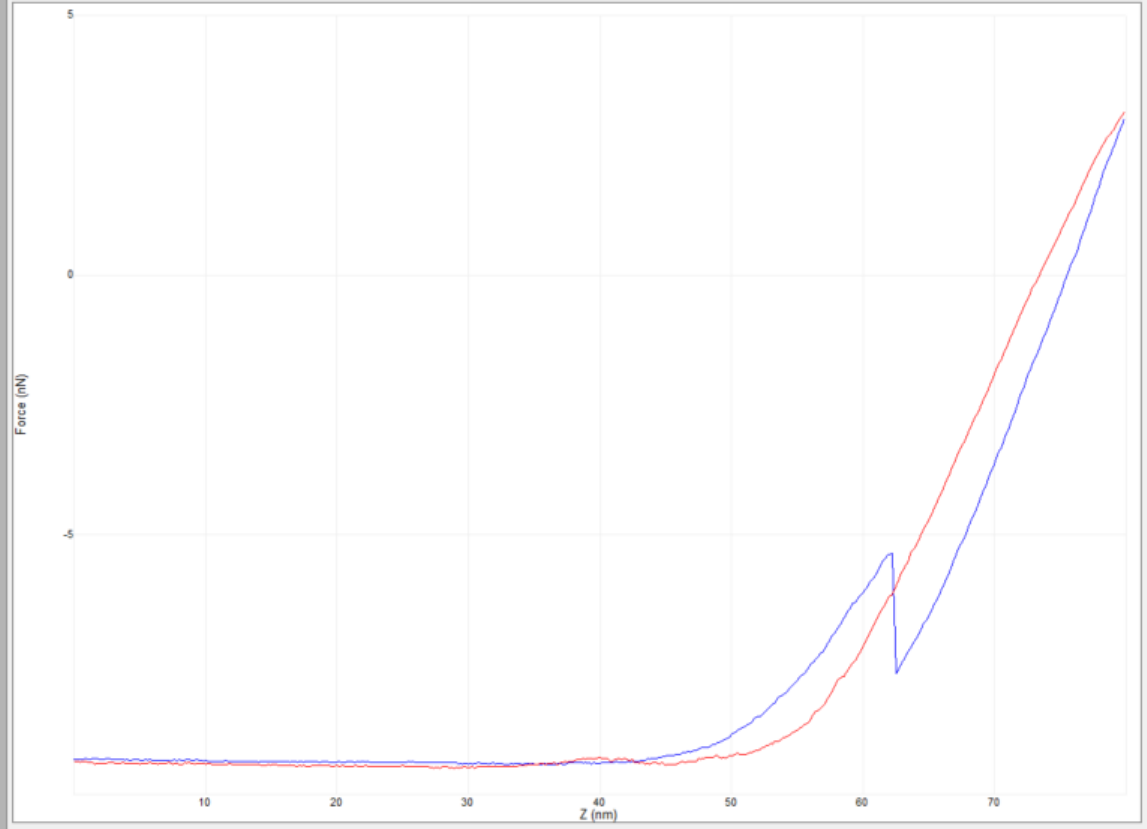
cr00012.0....spm

Ramp Data

Deflection Error

Height Sensor

Deflection Error

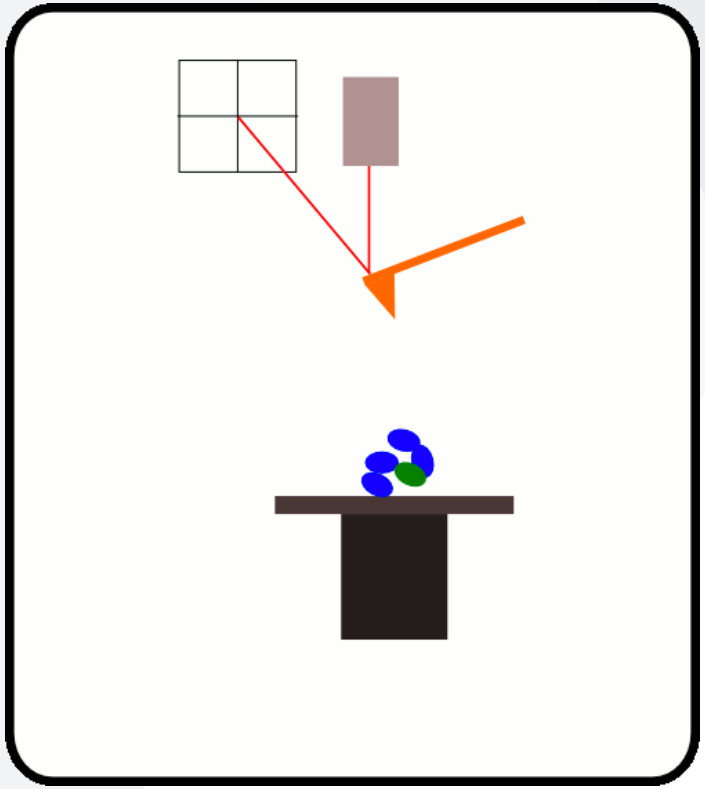


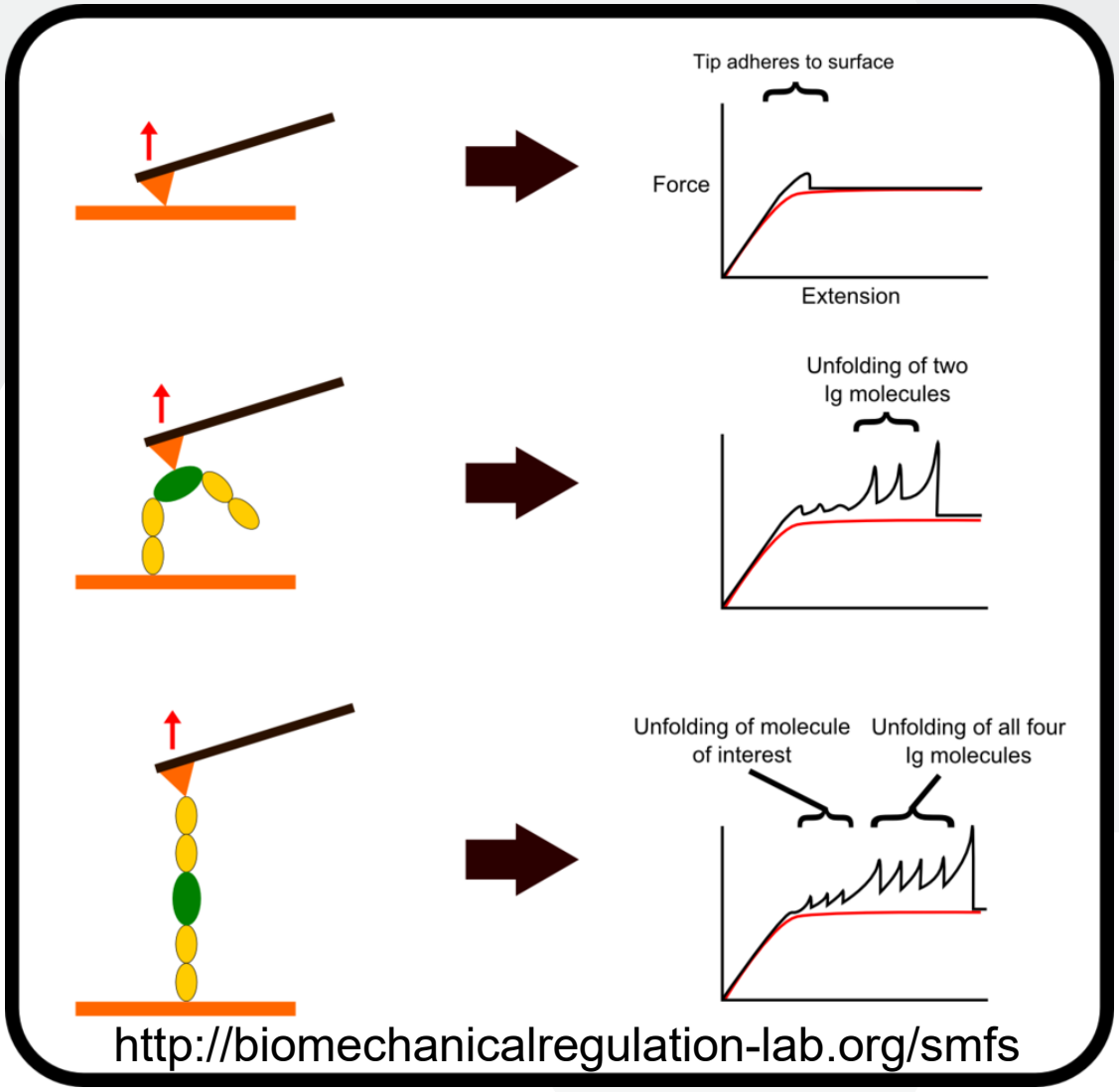
- Marker Pair 1
  - X0 0.000 nm
  - X1 0.000 nm
  - Y0 0.000 nN
  - Y1 0.000 nN
  - X1 - X0 0.000 nm
  - Y1 - Y0 0.000 nN
  - dY/dX
  - dX/dY
  - Least Squares Equation
  - Root Mean Square 0.000 nN
  - Average Deviation 0.000 nN
- Marker Pair 2
  - X2 0.000 nm
  - X3 0.000 nm
  - Y2 0.000 nN
  - Y3 0.000 nN
  - X3 - X2 0.000 nm
  - Y3 - Y2 0.000 nN
  - dY/dX
  - dX/dY
  - Least Squares Equation
  - Root Mean Square 0.000 nN
  - Average Deviation 0.000 nN

- Channel data

Image Data	Deflection Error
X Data Type	Z
Sens. DeflSens	43.00 nm/V
Plot Units	Force
Spring Constant	0.7000 N/m
Display Mode	Deflection Error vs. Ch2:
Plot Invert	Normal
Phase Offset	0.00 ms
Fit Type	None
Active Curve	Retract

Single-molecule force spectroscope (SMFS)

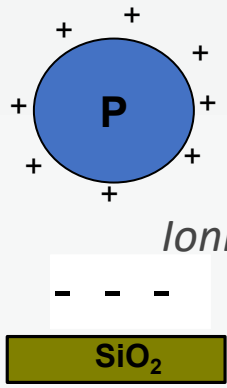




Force dependency of biochemical reactions measured by single-molecule force-clamp spectroscopy
Ionel Popa Pallav Kosuri Jorge Alegre-Cebollada Sergi Garcia-Manyes Julio M. Fernandez
Nature Protocols June, 2013

Protein immobilization

- **Glass**



- **Gold:**

- Cysteine (SH-) tags

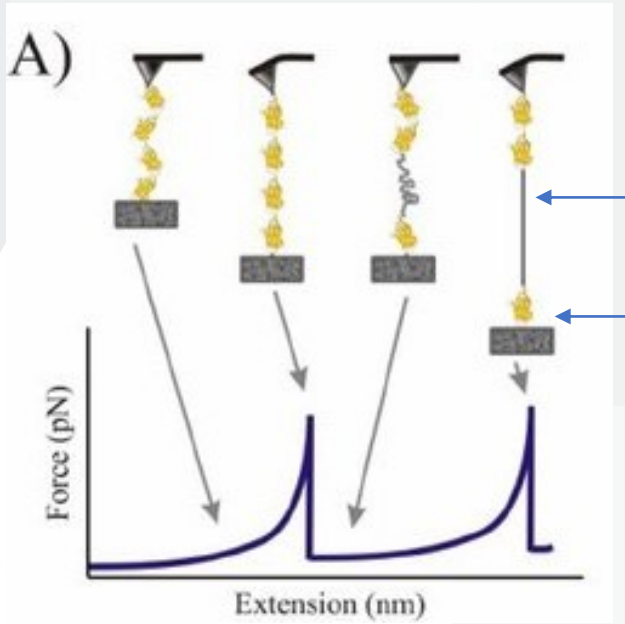
- F ~ 1 nN → Ok for most of the unfolding forces

- **Ni-NTA**

- F ~ 50 pN

- OK for spectrin, ankyrin or C2 domains

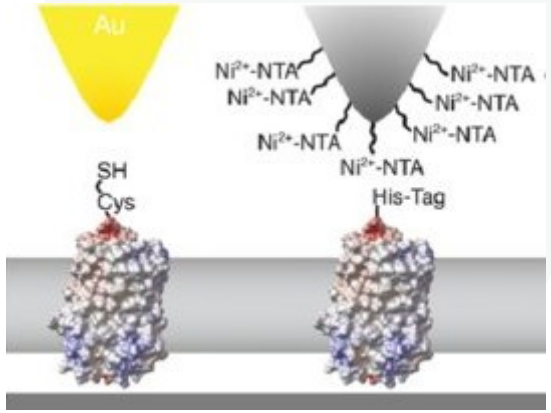
- NOT sufficient e.g., for Ig-domains



Adhesion force > Unfolding force

Probe (cantilever and tip) selection

- **Gold-coated tip** *(few types on the market)*
- **Very soft** *(k < 0.01 N/m)*
- **Silicon Nitride material** *(typical for operations in liquid)*
- **Smaller is better** *(lower noise in liquid)*



Probe (cantilever and tip) selection

- **Olympus Biolever** and **Bruker MCLT-Au** – *most of publications*
- **Discontinued** ☹

OLYMPUS Micro Cantilevers

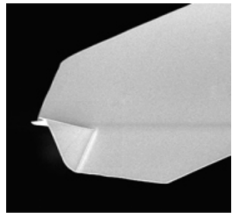
Home | Product features | Measurement example | P

Home > Product Line > BioLever

Silicon cantilever for AC mode

BioLever Discontinued

Cantilevers for Biological Sample Measurements In Water



Both side Gold coating

BL-RC150VB-HW
(210 chips/box, Strip Type)

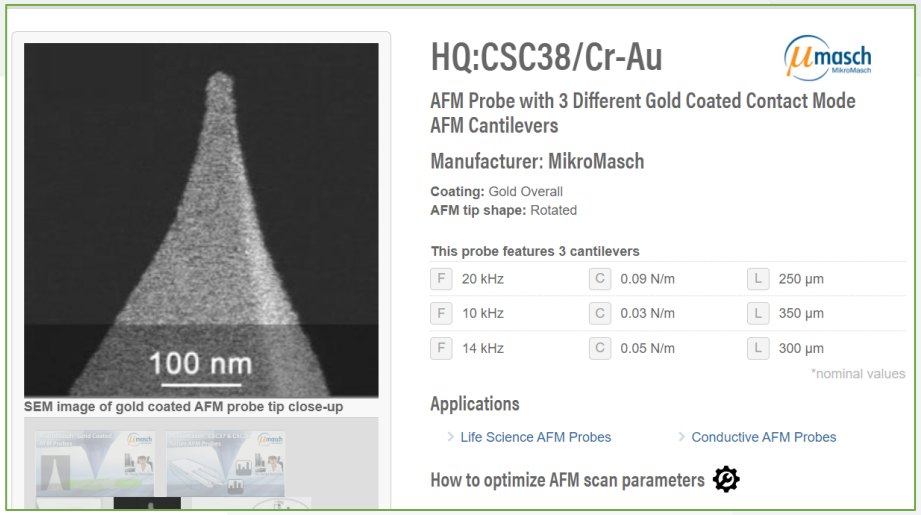
BL-RC150VB-C1
(24 chips/box, Pre-separated chips)

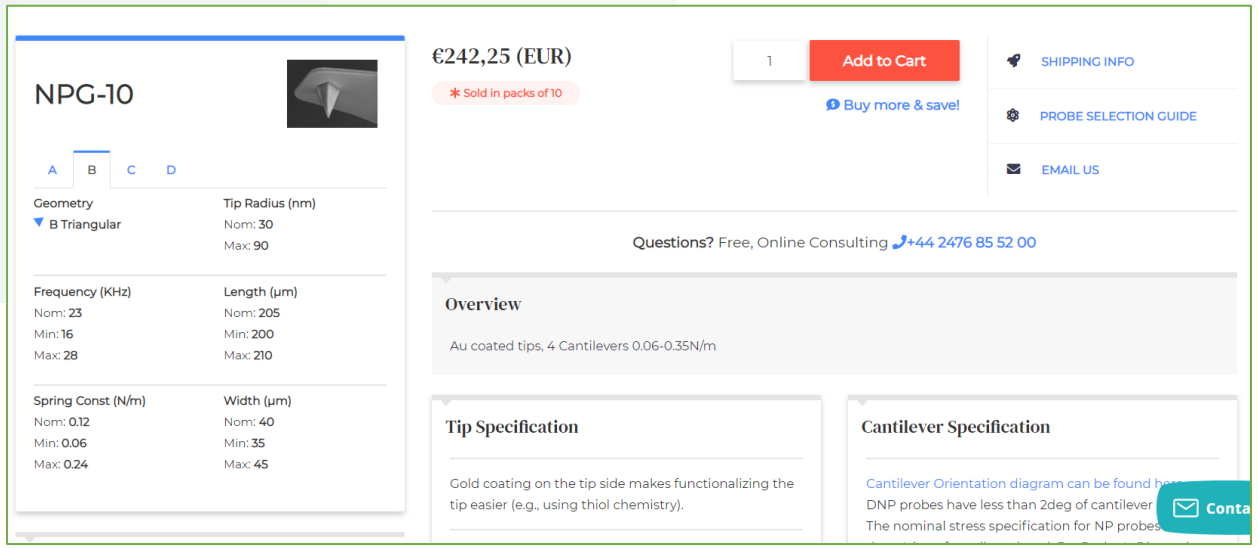
Material	
Probe apex	Silicon Nitride
Lever	Silicon Nitride
Coating Metal	
Probe side	Gold
Reflex side	Gold

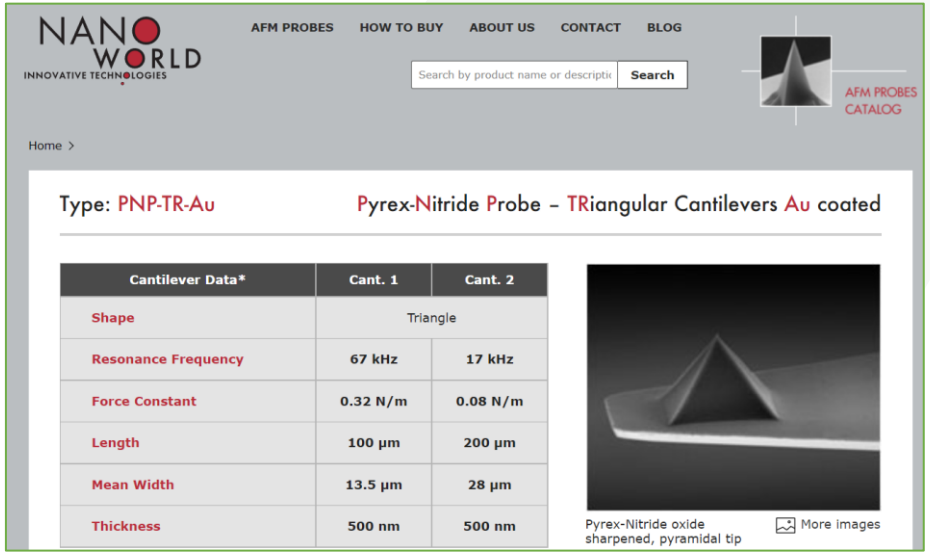
BL-RC150VB-HW, BL-RC150VB-C1

Probe (cantilever and tip) selection

- ***New cantilever types***
- ***Ordered – to be tested***

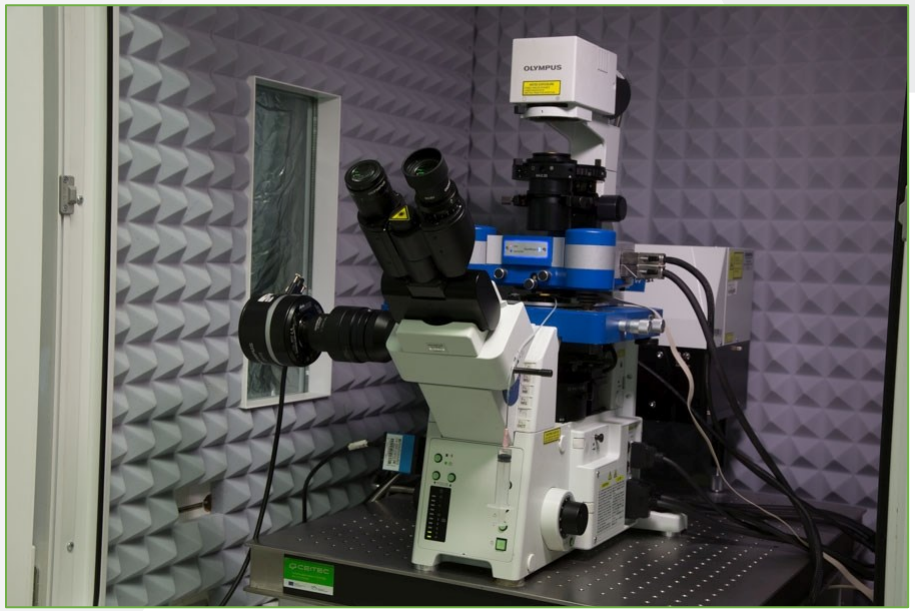






Experimental settings

JPK NanoWizard 3



ATOMIC FORCE MICROSCOPE

ForceRobot® 300

Automated Force Spectroscope

Label-free, single molecule technique
Measure forces between and within molecules. Characterize molecular and receptor-ligand interactions and protein (un)folding events. Target individual molecules.

ForceRobot measurement head

Fully automated

200,000 Force curves per day

Powerful software for efficient data collection, processing, and evaluation. Automated laser and detector alignment. Standardized auto-calibration procedures.

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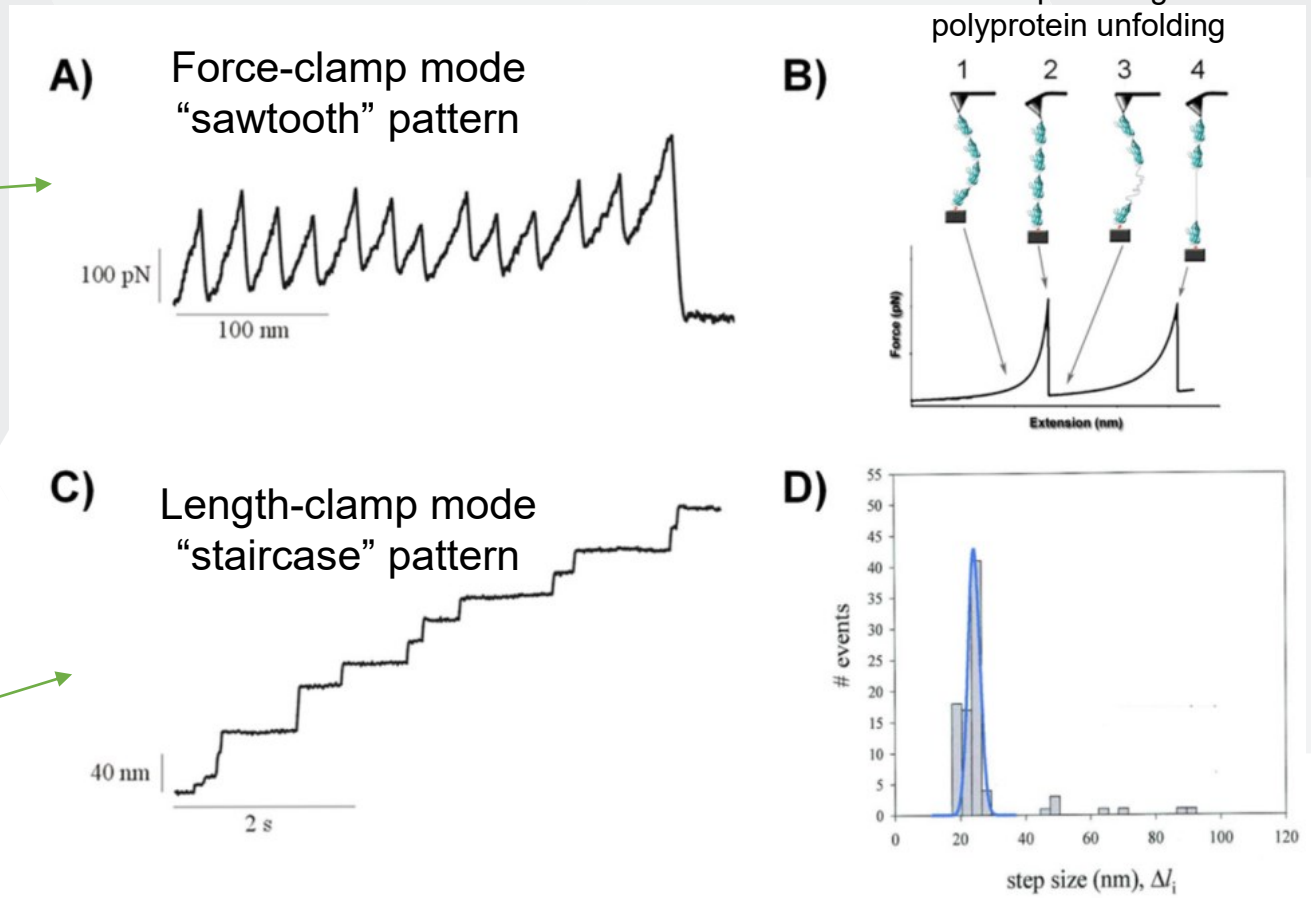
Experimental settings

Modes of operation

- ***Force-clamp mode***
  - ***Protein folding/unfolding; Mechanisms of enzyme catalysis***
  - ***Force measurement with high speed of feedback gain – 0.1 to 5 milliseconds***
  - ***Typical settings: F = 50-500 pN, pulling speed 1 µm/s***

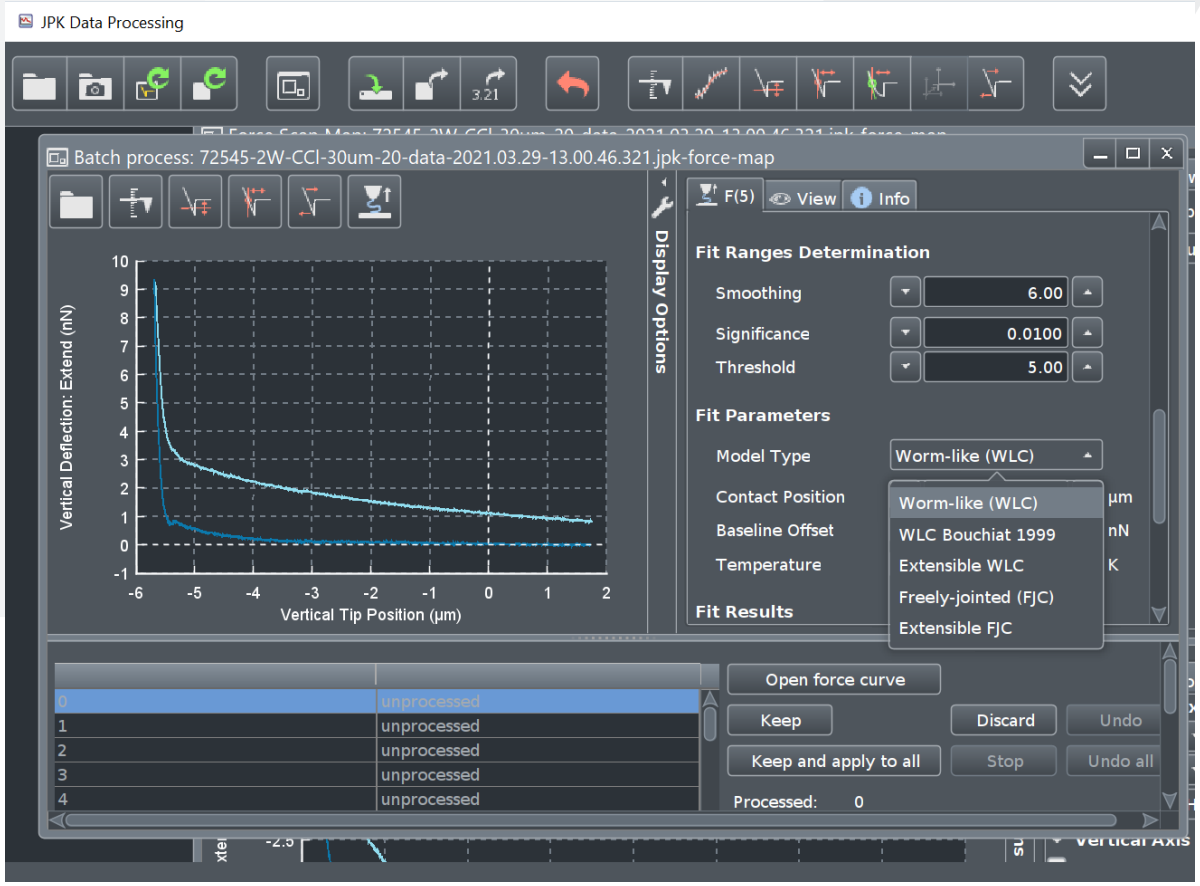
- ***Length-clamp mode***
  - ***Difficult interpretation***
  - ***For multidomain proteins (native multi-domain proteins (such as titin, tenascin or spectrin)***
  - ***Typical settings: F = 50-500 pN, pulling speed 1 µm/s, no feedback driving***

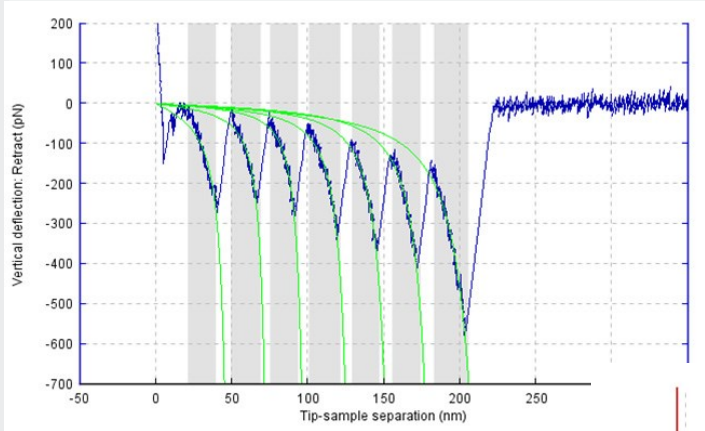


Data processing

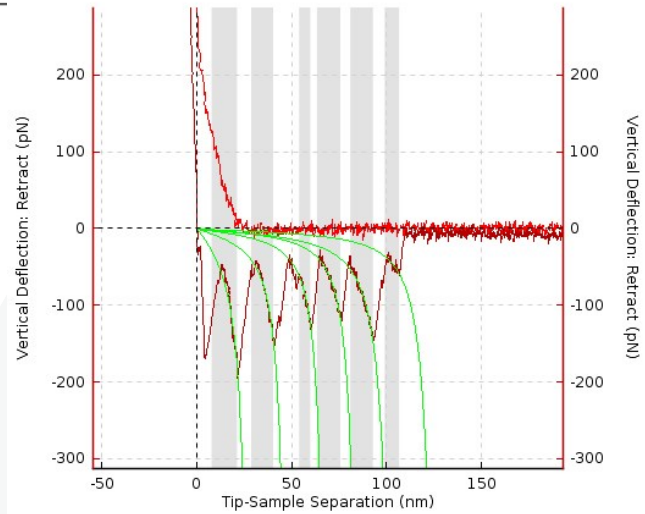
JPK Data Processing software

- ***Predefined functions***
- ***Batch processing of curves***
- ***Data sorting***

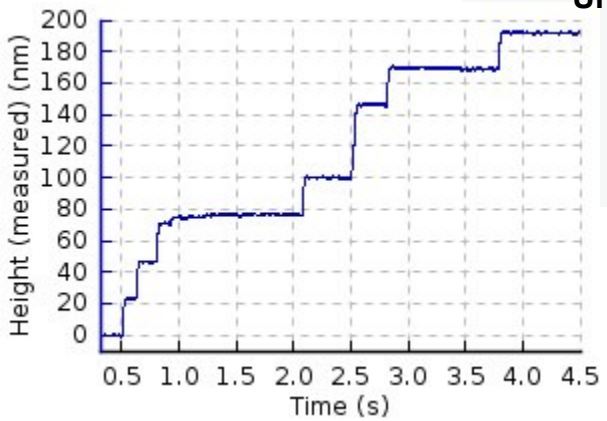




Fitting of force-extension profile with worm-like fit



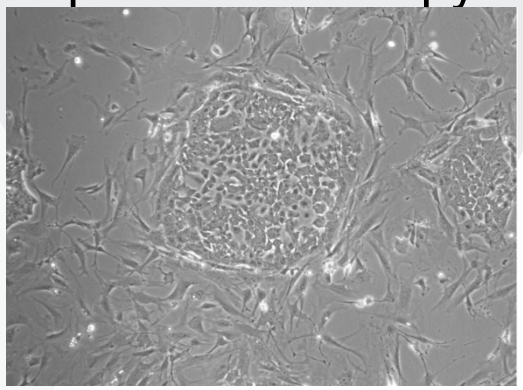
Unfolding of artificial polyprotein GB1



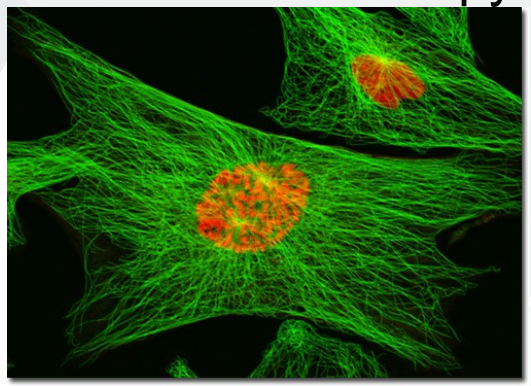
Unfolding of individual titin domain

Nanomechanical mapping of living cells

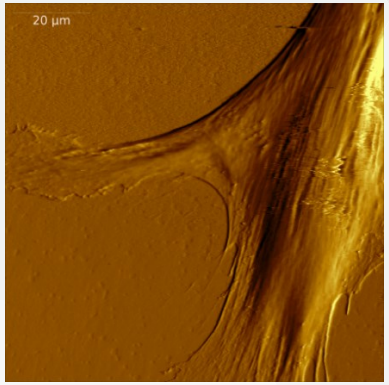
Optical microscopy

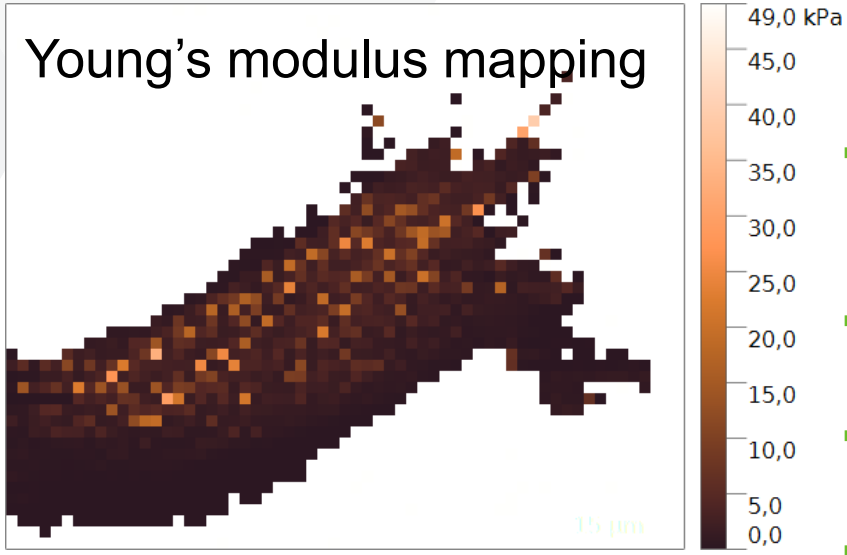


Confocal microscopy



AFM



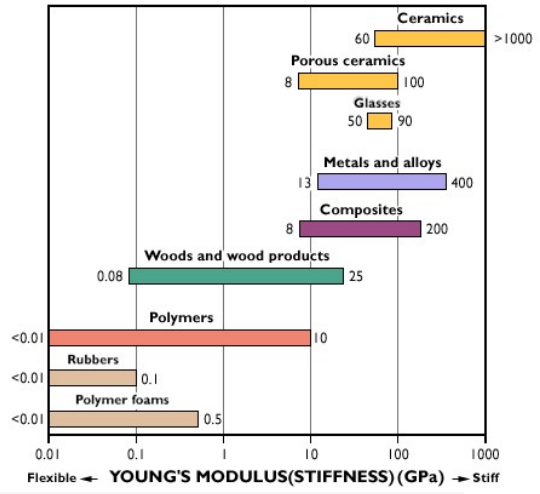


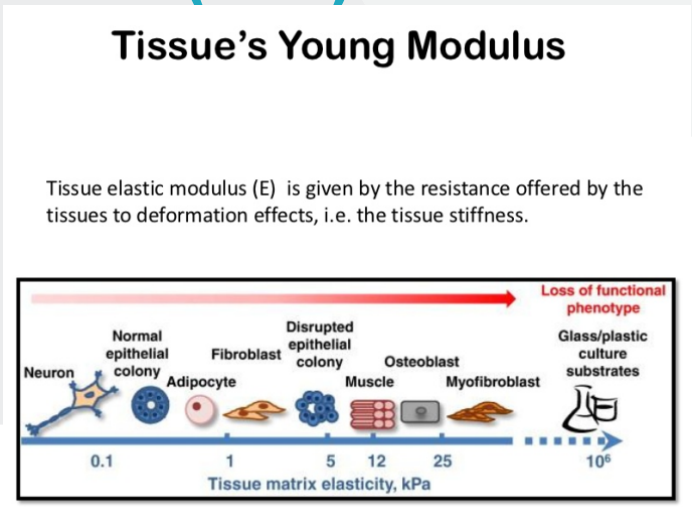
Motivation

Why to quantify elasticity of (living) objects?

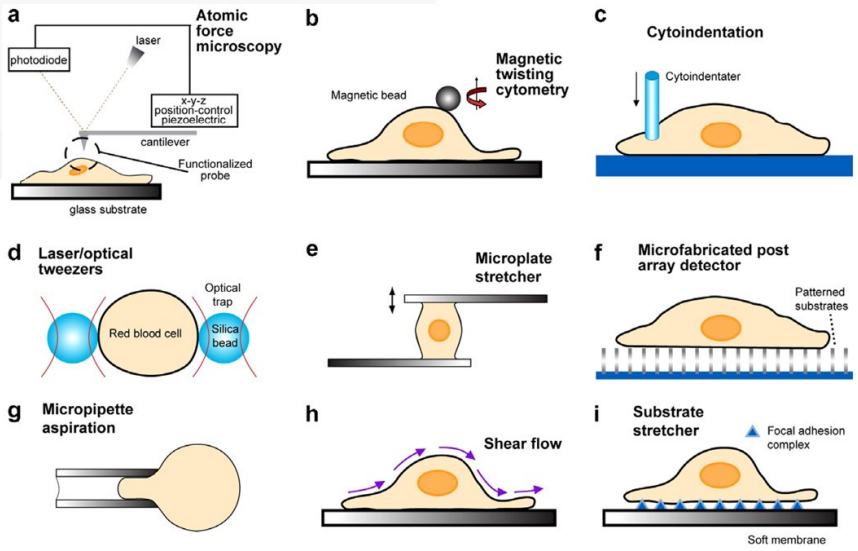
- **Stiffness** (Young's modulus) **mapping**
  → stiffness = basic parameter of any material
- **Elasticity-phenotype** relation ship
- **Mechanobiological** characterization
- **Driving of instrument** properties (QNM, QI)

Young's modulus (YM) of materials





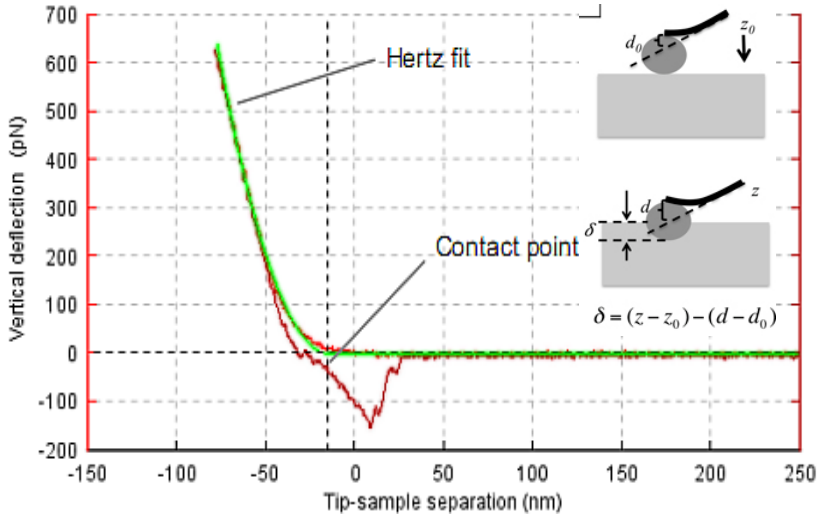
www-materials.eng.cam.ac.uk/



+ Holographic Q-phase microscope

Acta Biomater. 2007 Jul; 3(4): 413–438.

Methods for YM measurement



J. Vis. Exp. (76), e50497, doi:10.3791/50497 (2013).

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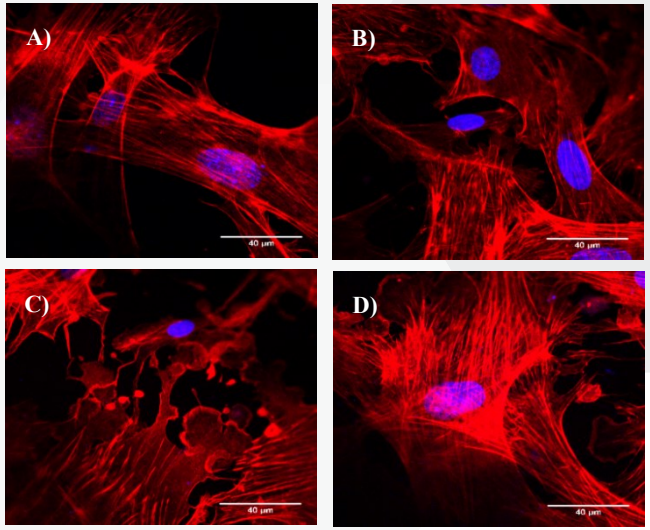
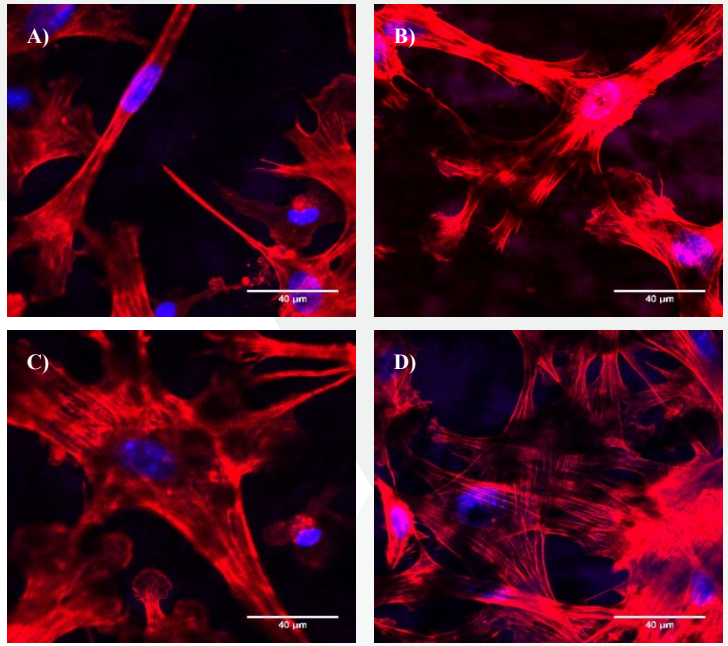
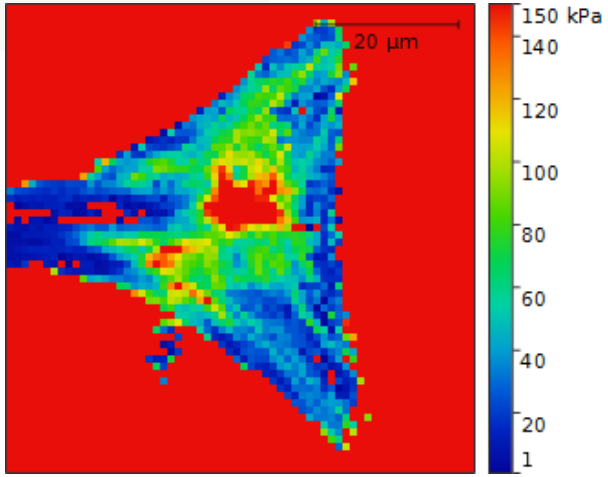
Confocal vs. AFM

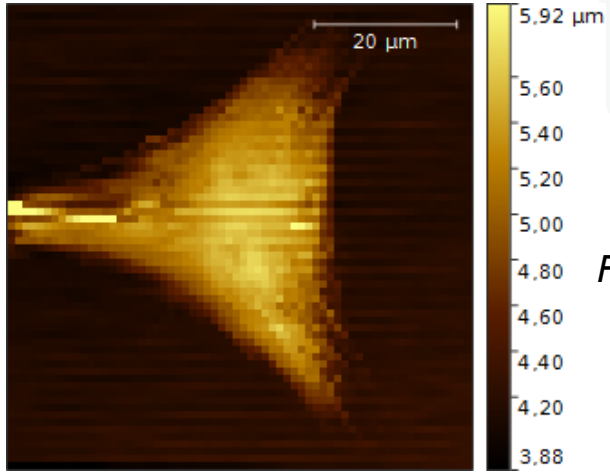


Confocal microscopy

- DAPI nucleus staining
- Actin staining by Phalloidin







Force Mapping on single cells

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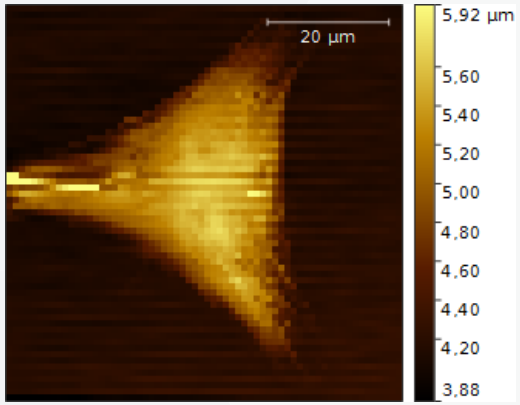
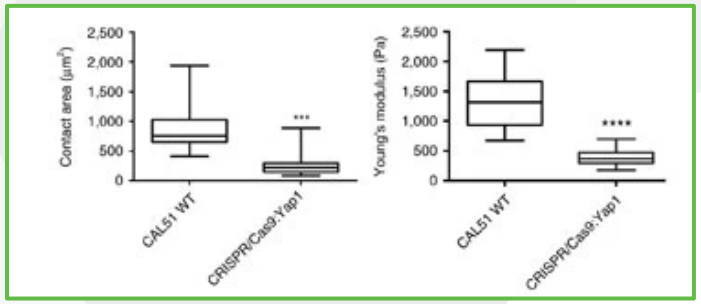
NARDONE, Giorgia, Jorge Oliver-De La CRUZ, Jan VRBSKY, Cecilia MARTINI, Jan PRIBYL, et al., 2017. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nature Communications* [online]. **8**, ncomms15321.

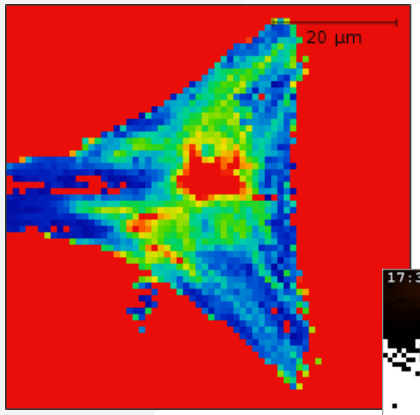
Cellular nanomechanics
By means of AFM

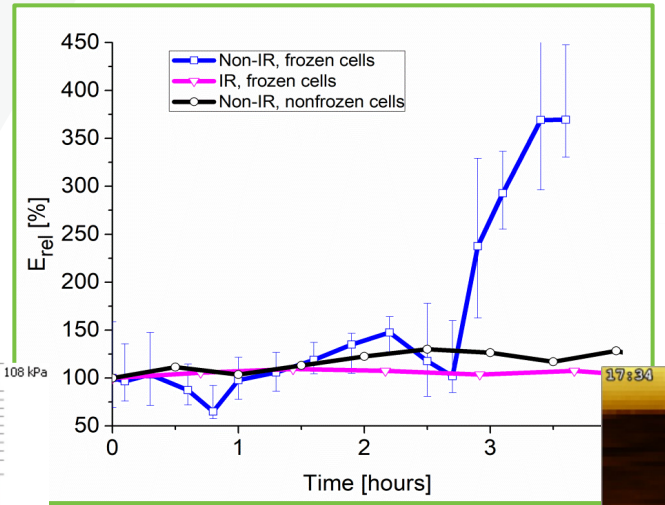
AFM mapping - correlation with fluorescence microscopy



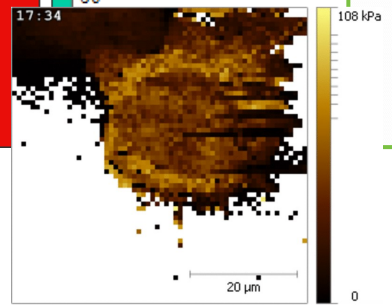


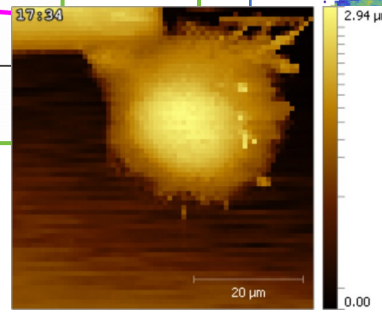
Evalutation - statistics

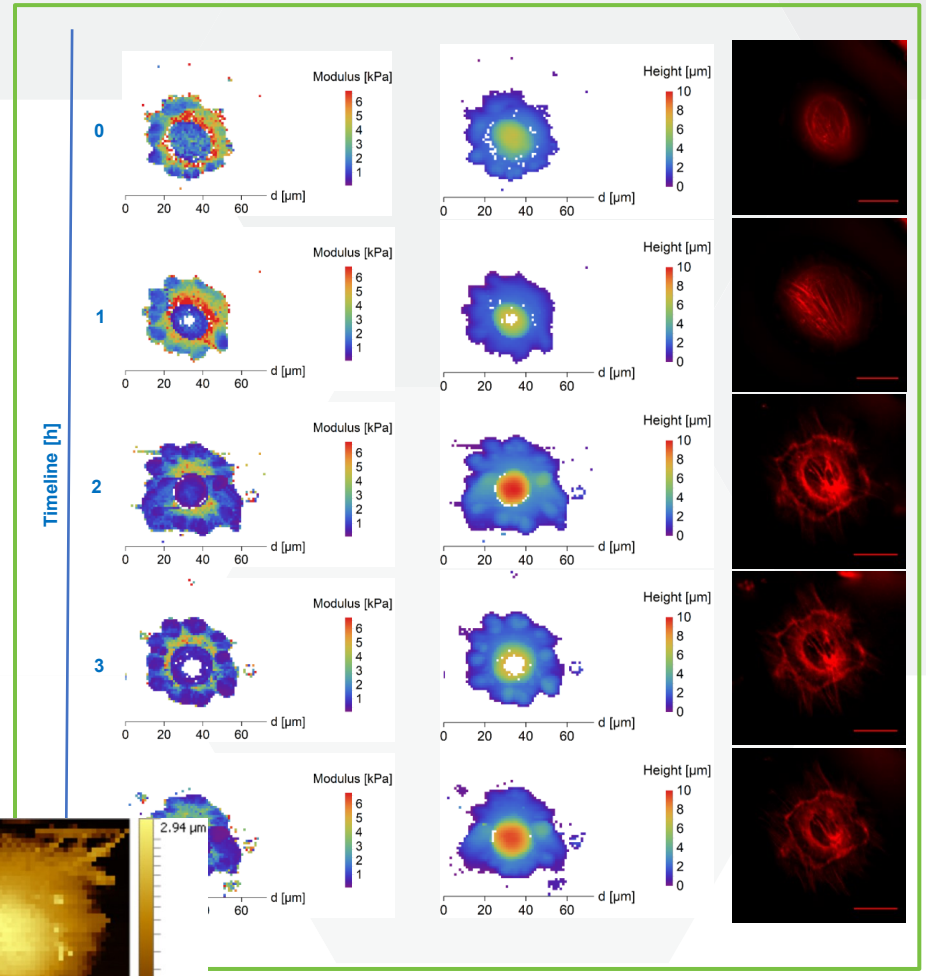




Time-lapsed biomechanics







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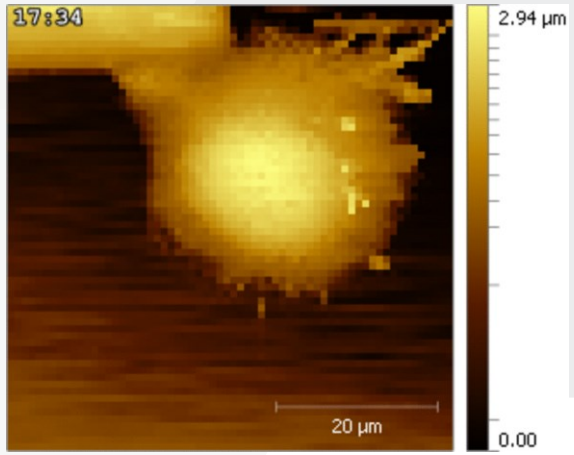
NARDONE, Giorgia, Jorge Oliver-De La CRUZ, Jan VRBSKY, Cecilia MARTINI, Jan PRIBYL, et al., 2017. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nature Communications* [online]. **8**, ncomms15321.

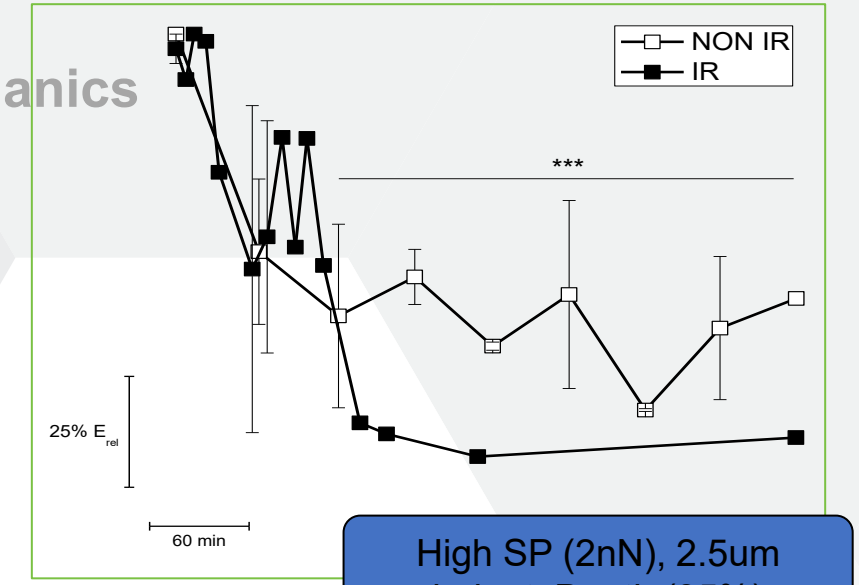
GOLAN, Martin, et al. Front. Physiol., 29 June 2018

Fibroblasts thawing process

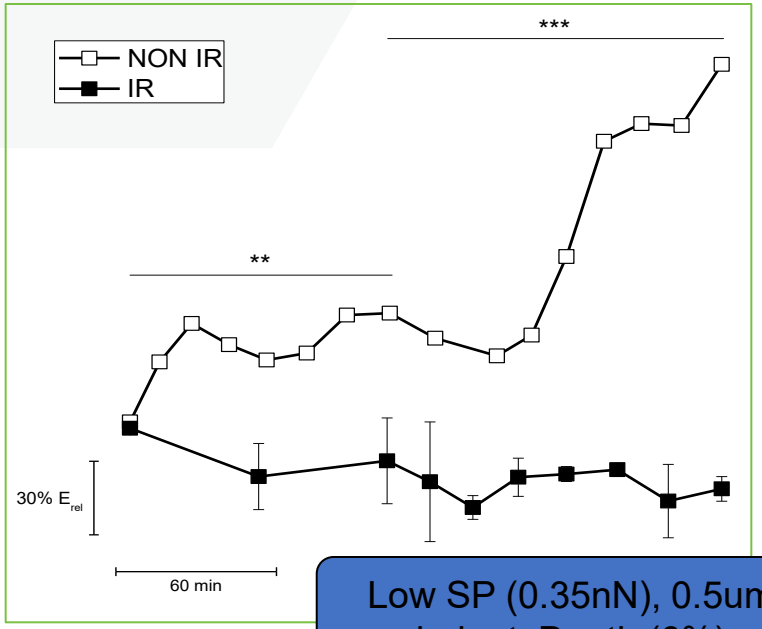
Time-lapsed monitoring of cell biomechanics



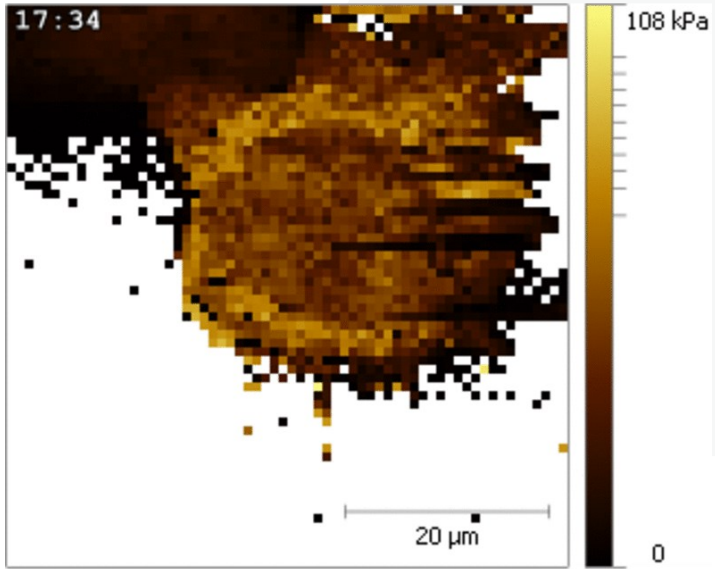
SP height profile *(12 hours)*



High SP (2nN), 2.5um indent. Depth (35%)



Low SP (0.35nN), 0.5um indent. Depth (6%)



YM maps *(12 hours)*

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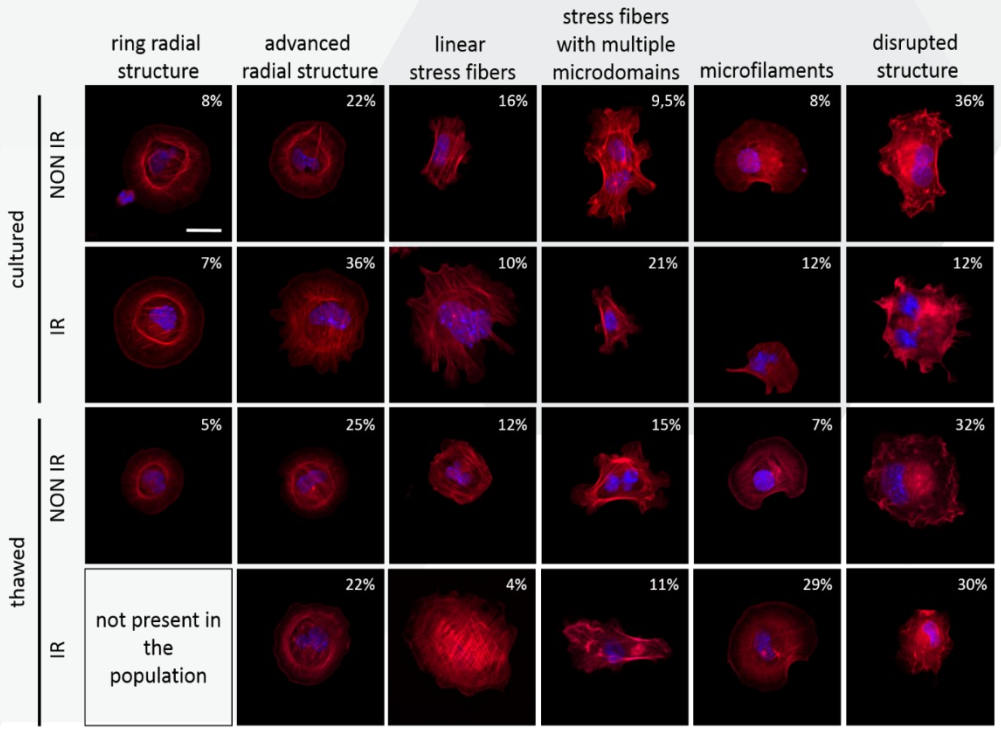
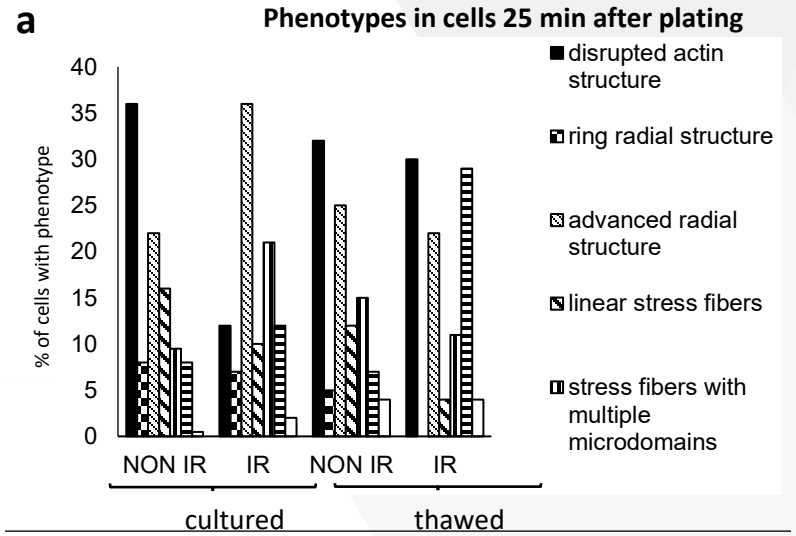
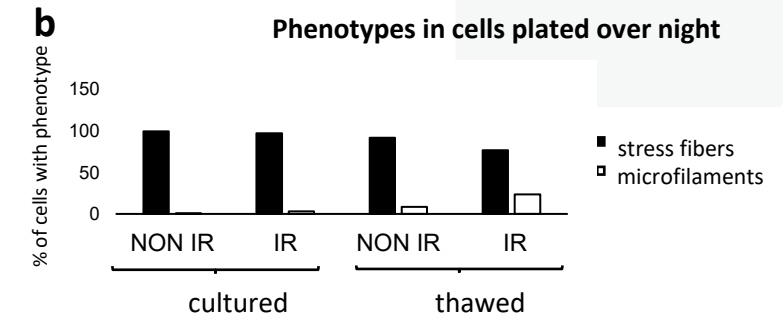
*GOLAN, Martin, et al. IEEE transactions on nanobioscience 2018. **17**(4), 485–497*

Phenotypes in cells 25 min after plating







Cytoskeleton staining
(Phalloidin/DAPI)

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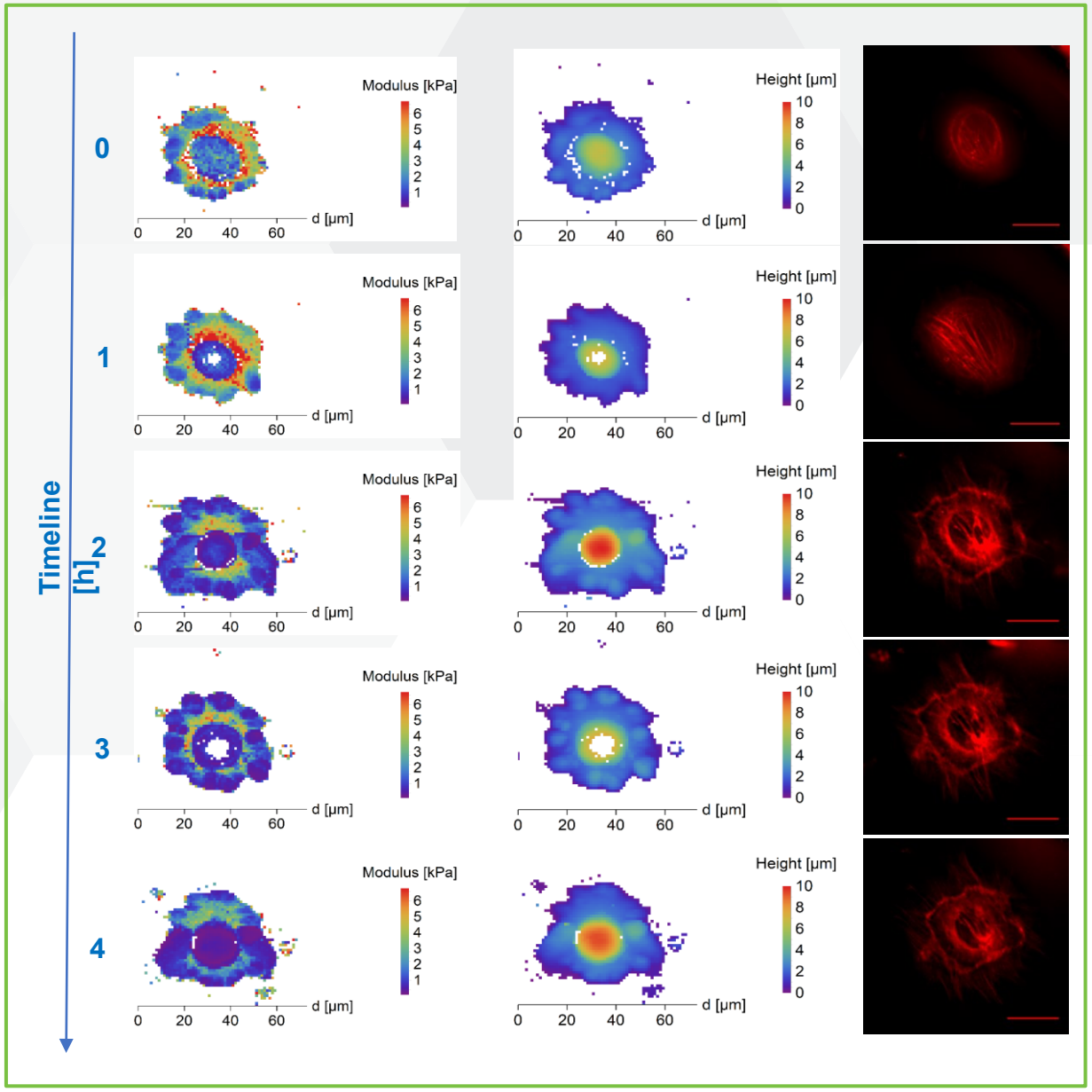
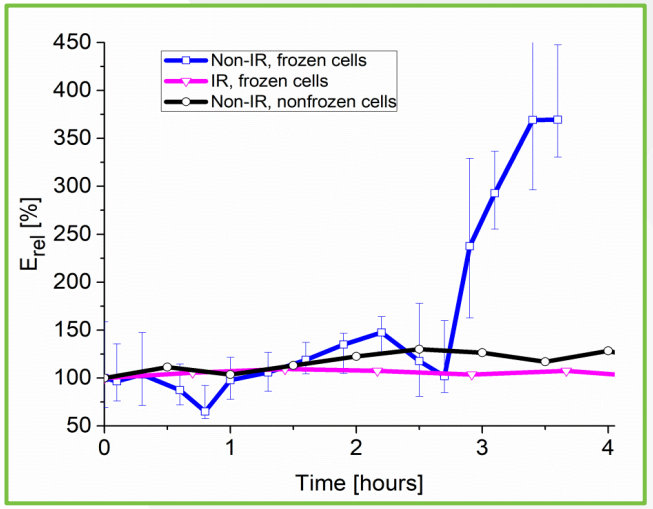
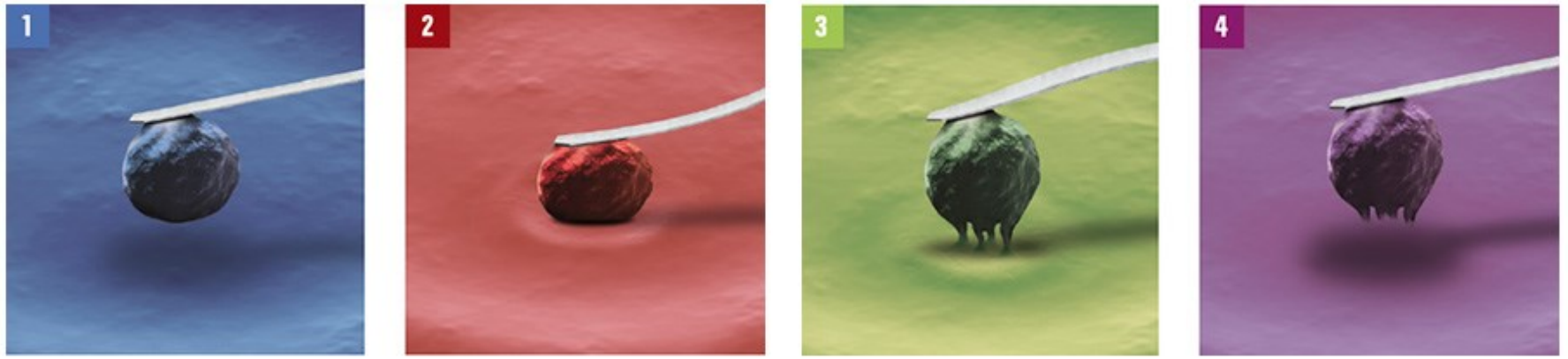




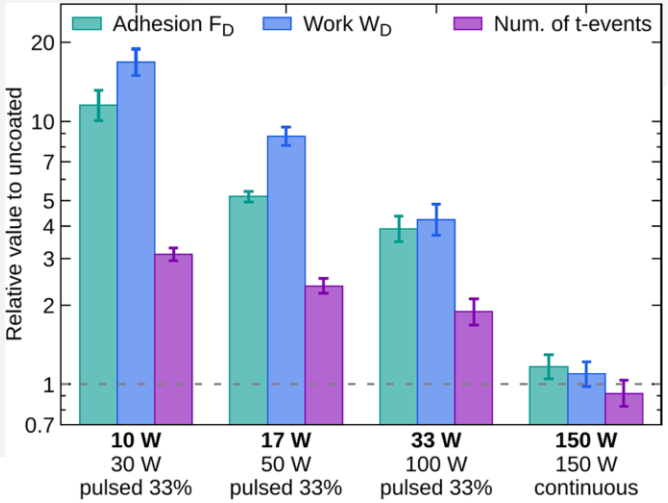
SiR
live actin staining

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

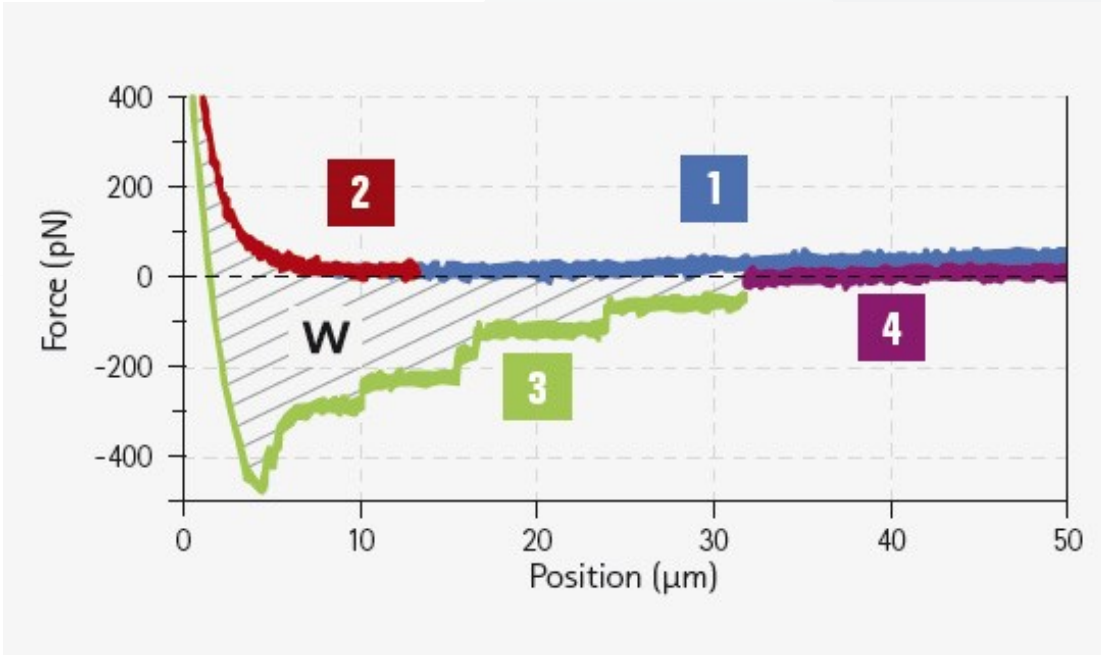
Cell-adhesion experiments



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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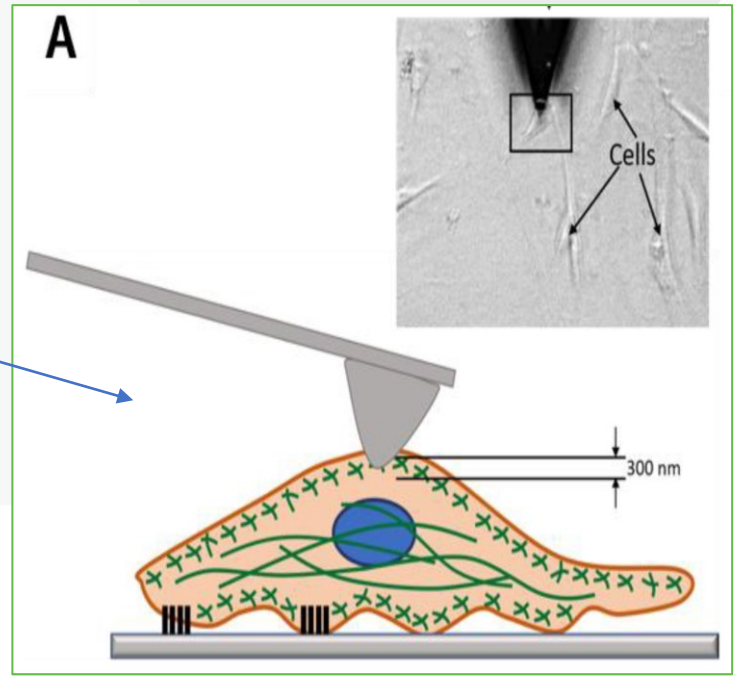
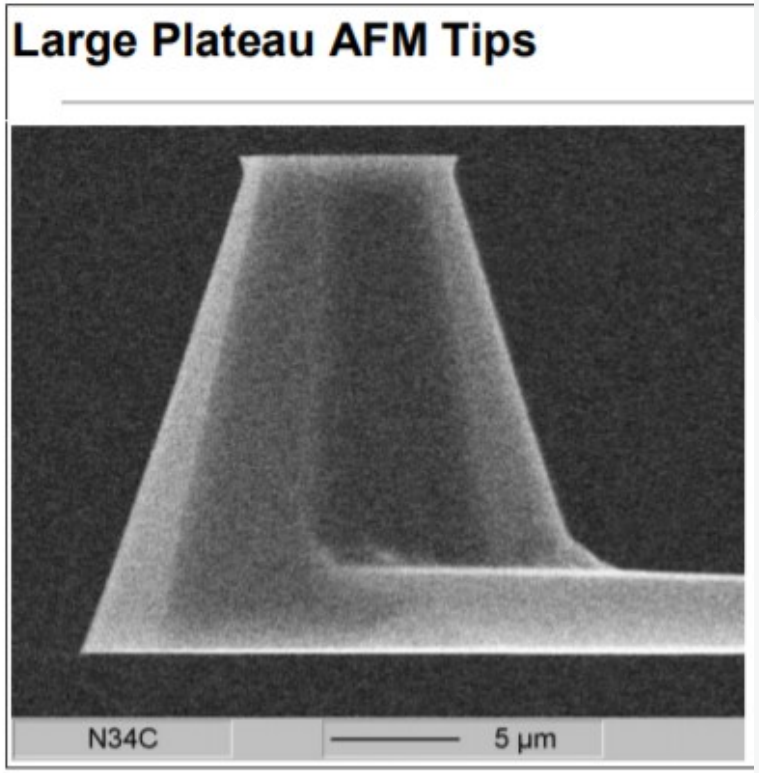
Other applications of AFM-based biomechanics

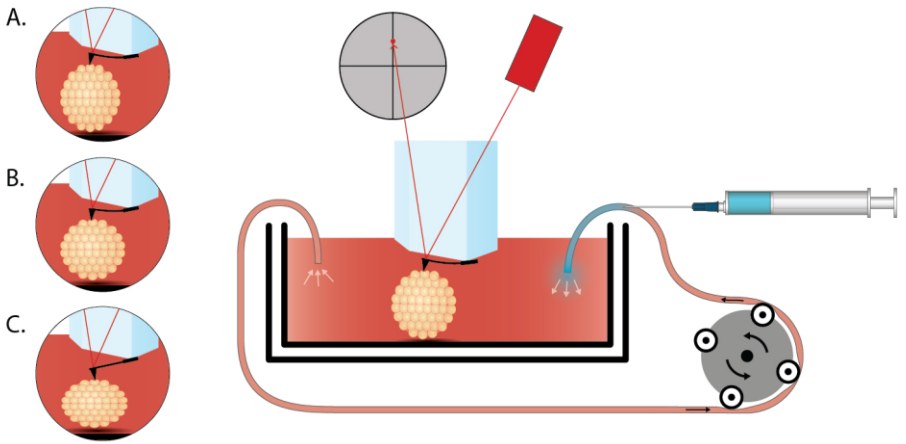
Cell scratching = cell adhesion

Scratching movement
Force variation

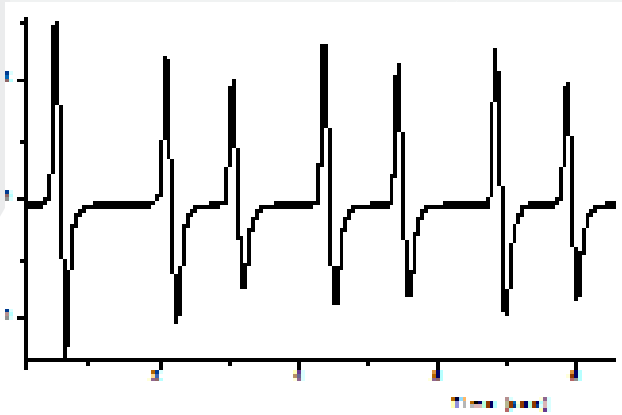




Other applications of AFM-based biomechanics

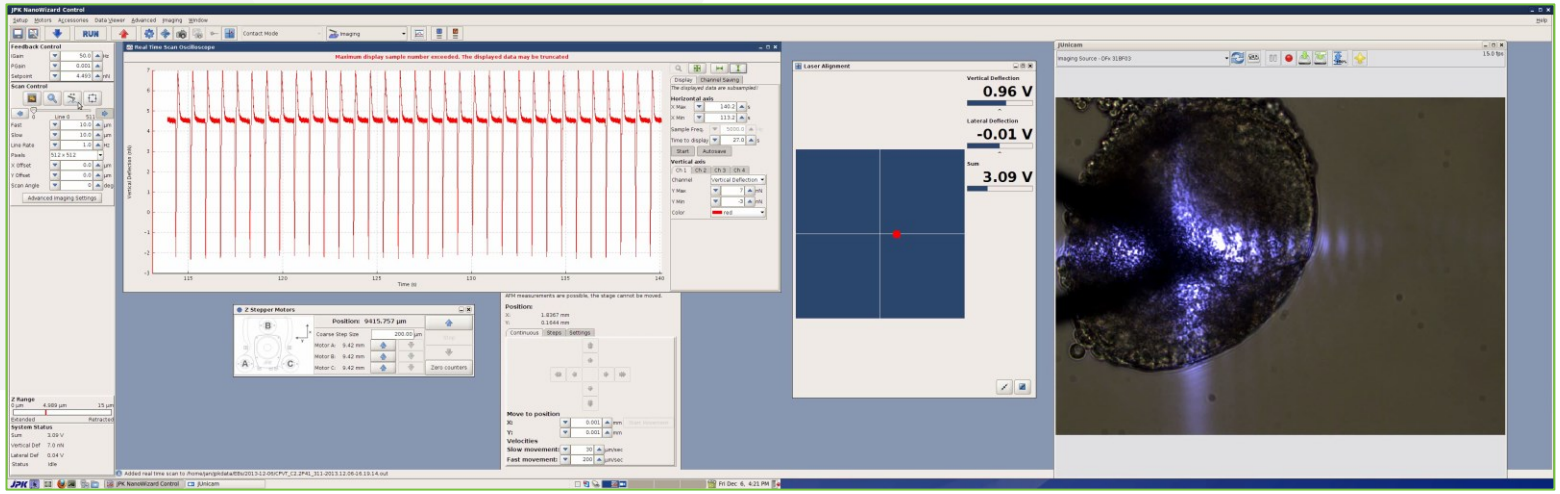


Setup scheme



MCG = mechanocardiogram

Cardiac cells biomechanics



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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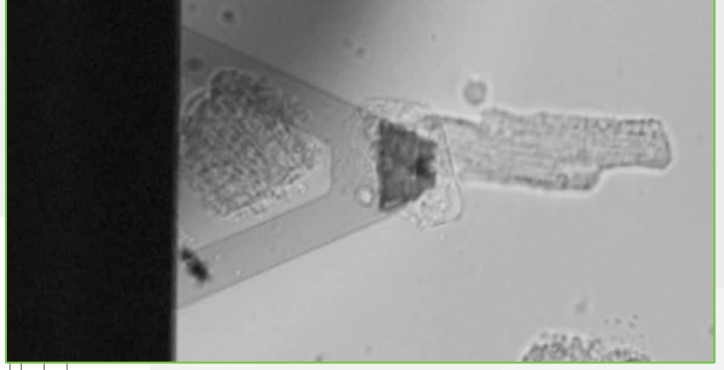
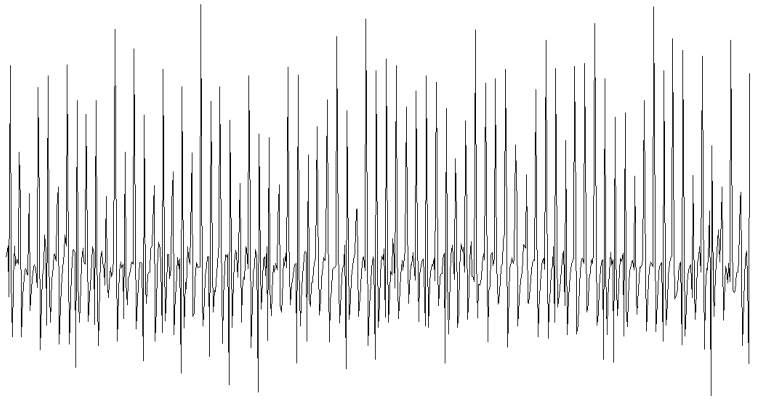
Typical DFL vs. time curves recorded as a result of various drug treatment

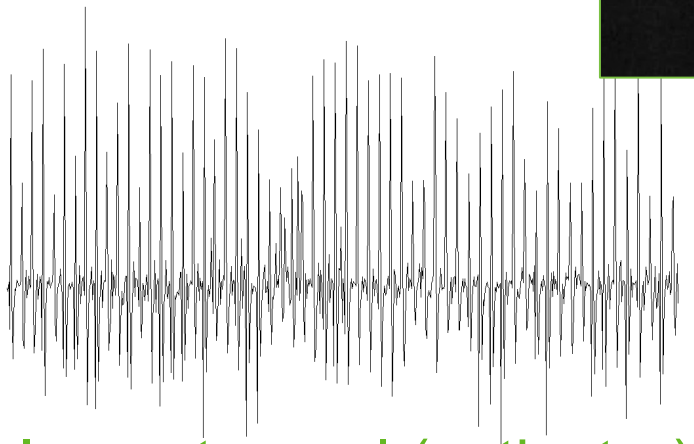


A



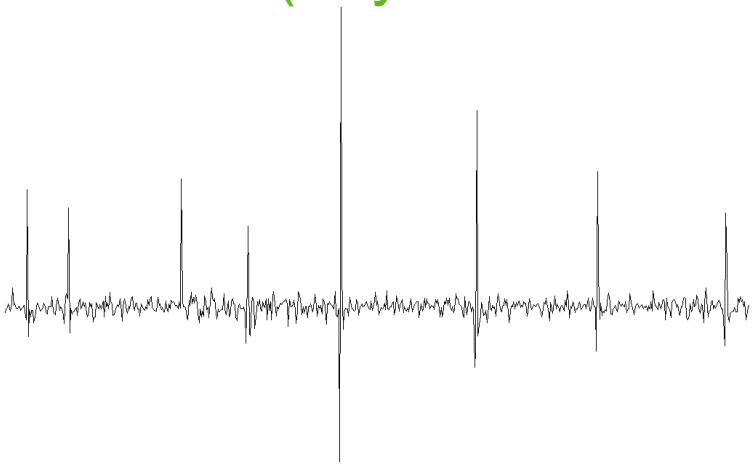
Base line (Thyroid medium)

C



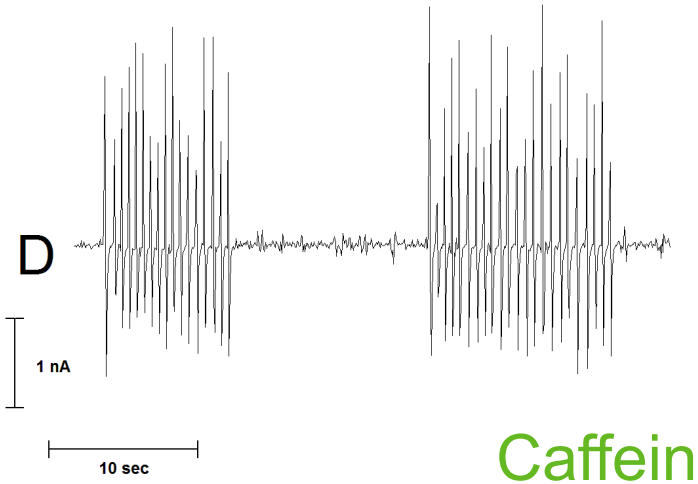
Isoproterenol (activator)

B



Metoprolol (betablocker)

D



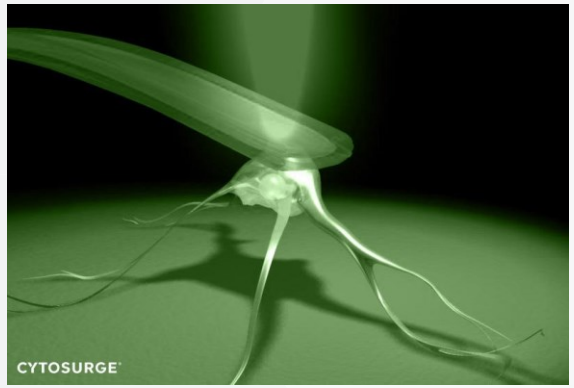
Caffeine

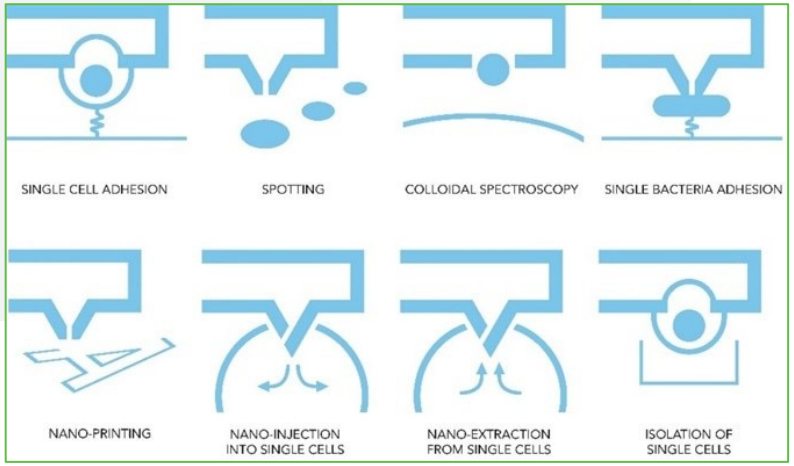
CEITEC

Pesl M, Acimovic I., Pribyl J., et. al. Heart Vessels 2013

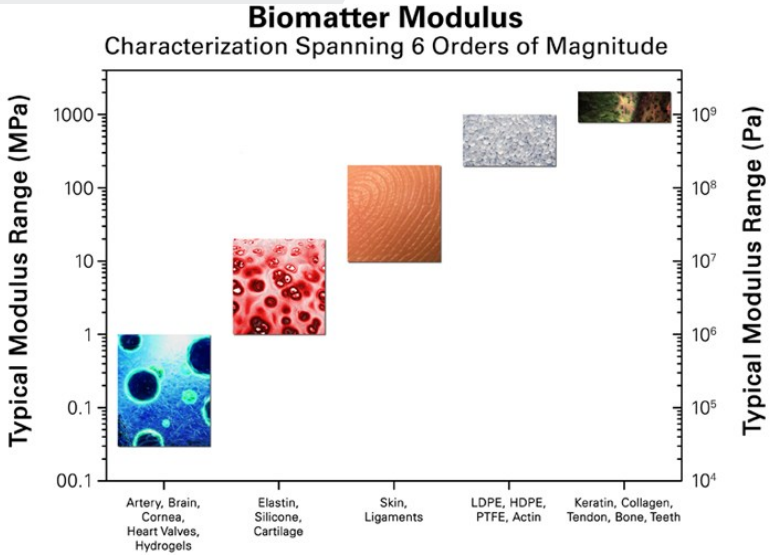
Combination with other techniques

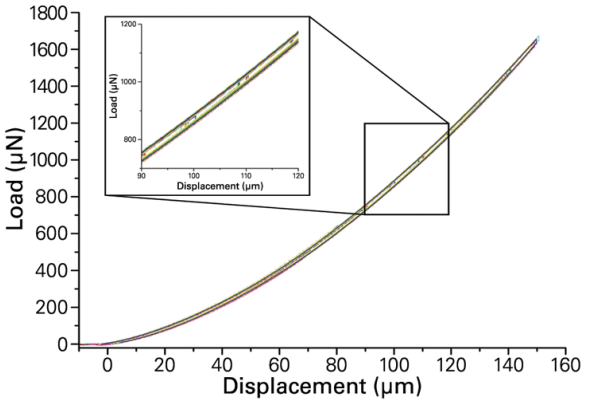
CytoSurge Fluid FM
module

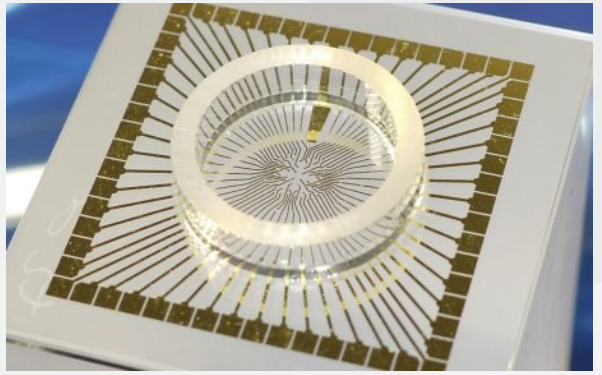




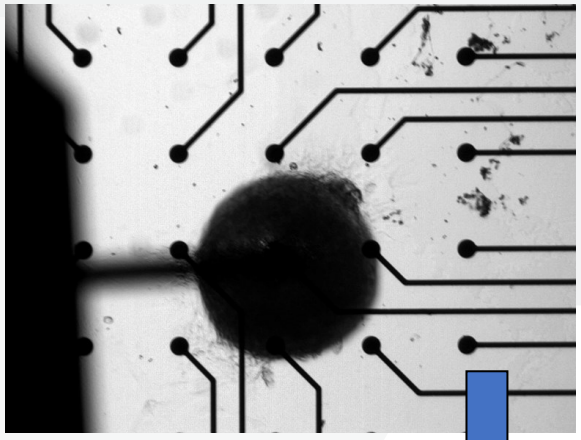
NanoIndentation
Single point indentation curves



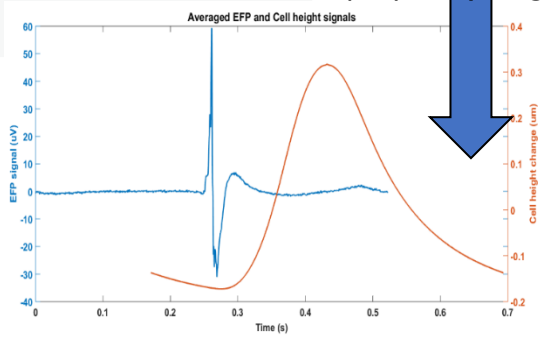




MultiElectrode Array
Extracellular Cell Potential
Cardiac cells and Neurons



AFM + MEA
= electromechanical (de)coupling



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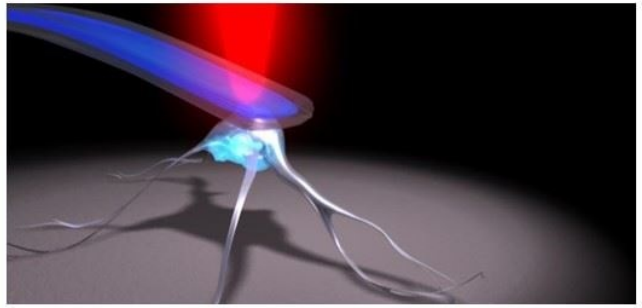
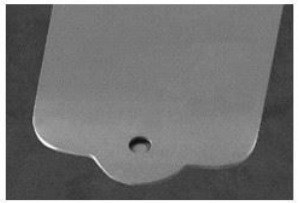
FluidFM – microfluidic force microscopy

FluidFM micropipette



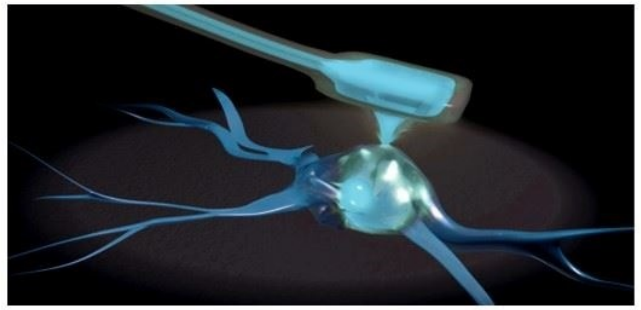


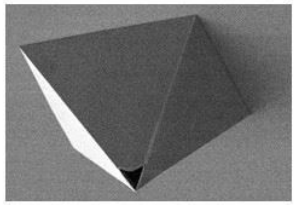
2, 4, 8 µm aperture

Adhesion of single cells

Colloidal spectroscopy

FluidFM nanosyringe



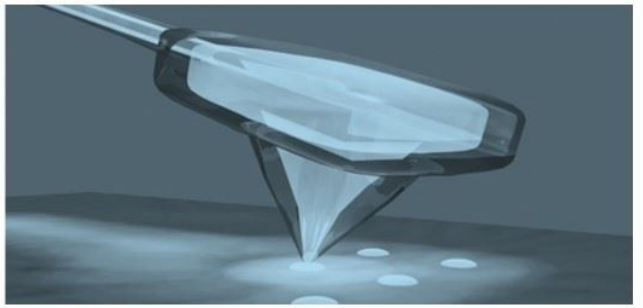


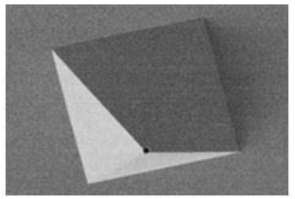
300 - 800 nm aperture

Nano-injection into single cells

Nano-extraction from single cells

FluidFM nanopipette





300 nm aperture

Spotting

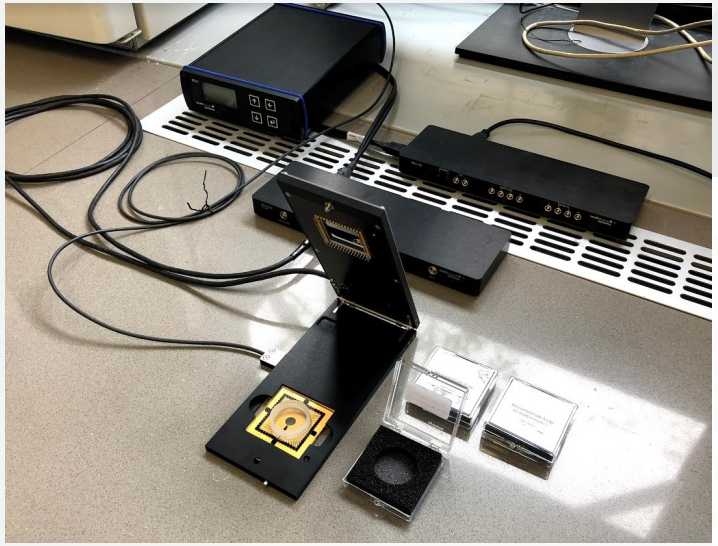
Adhesion of single bacteria

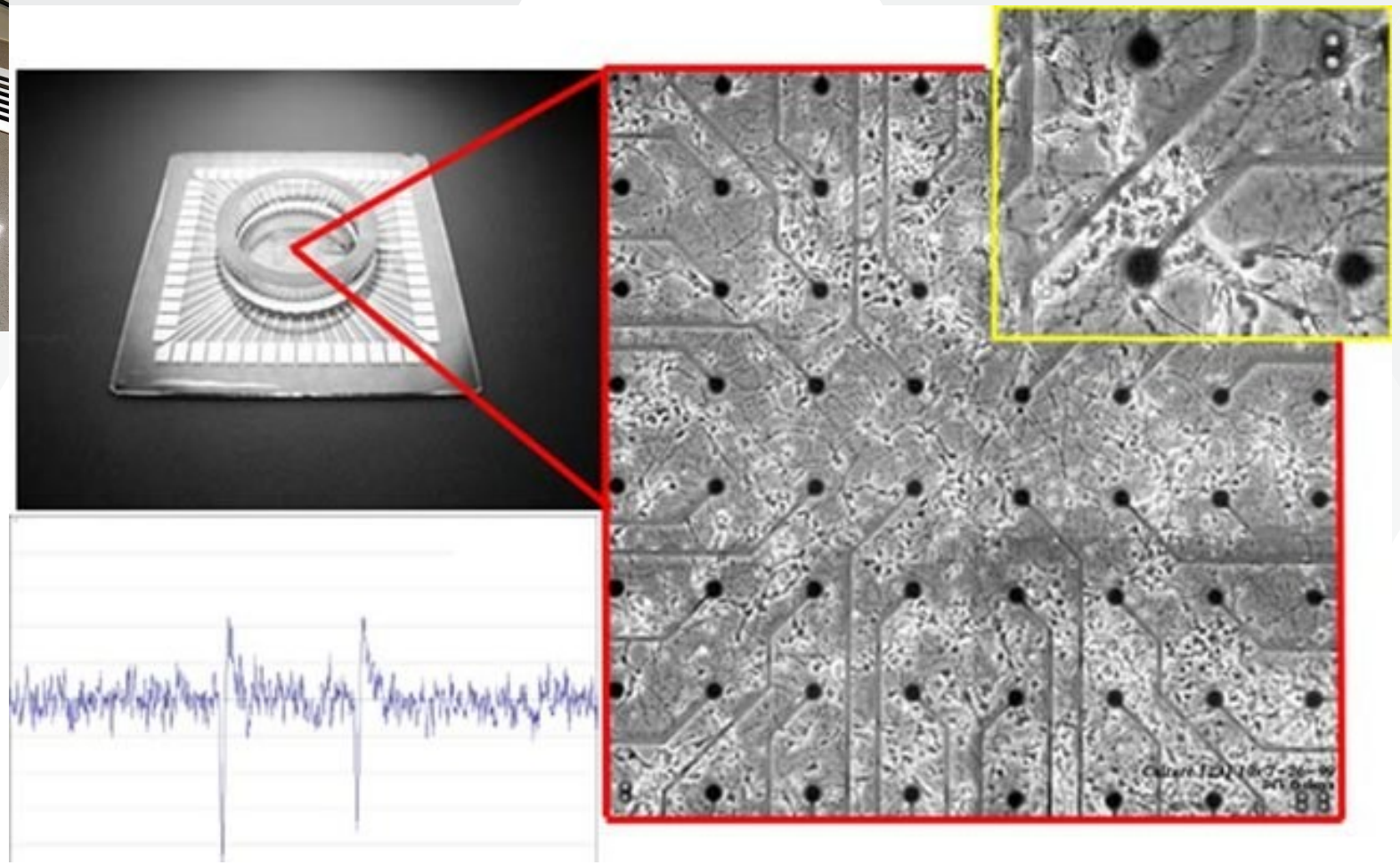
Nano-printing

https://www.news-medical.net

MEA – microelectrode array

cellular electrophysiology

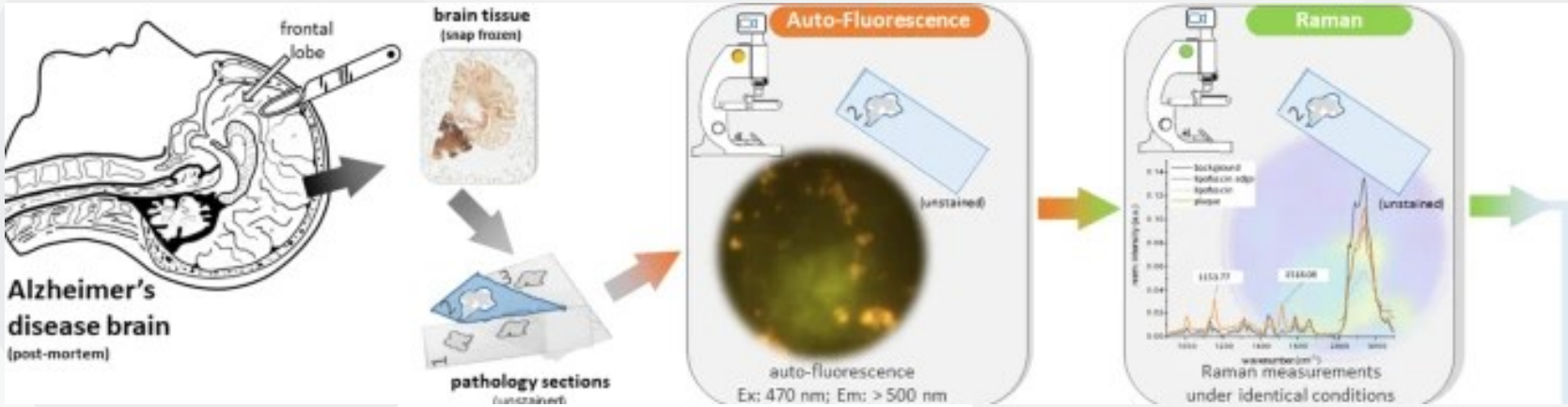




Anal Bioanal Chem (2011) 399:2313–2329

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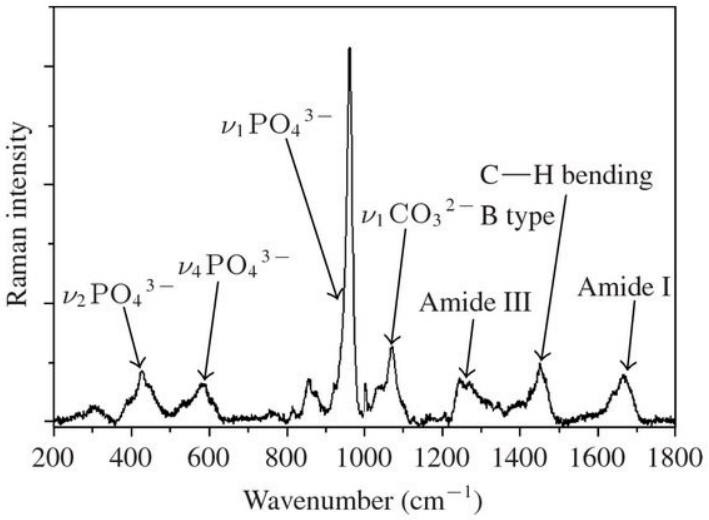
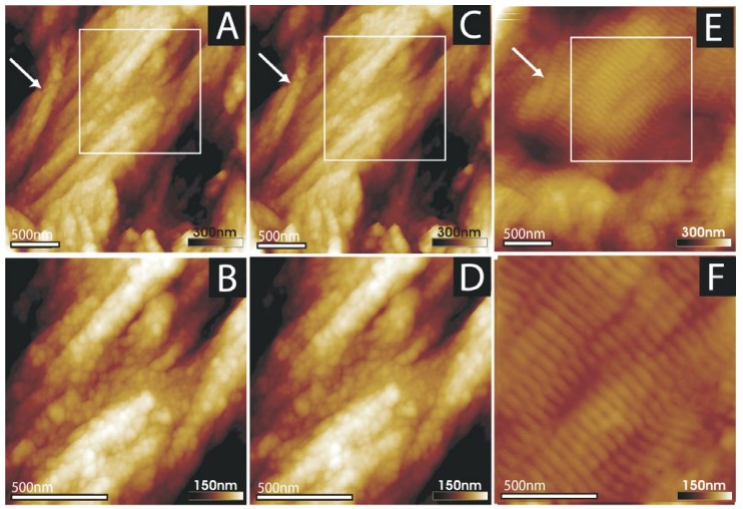
Calcification level and **Collagen Fibers** Arrangement in **Bone Tissue**

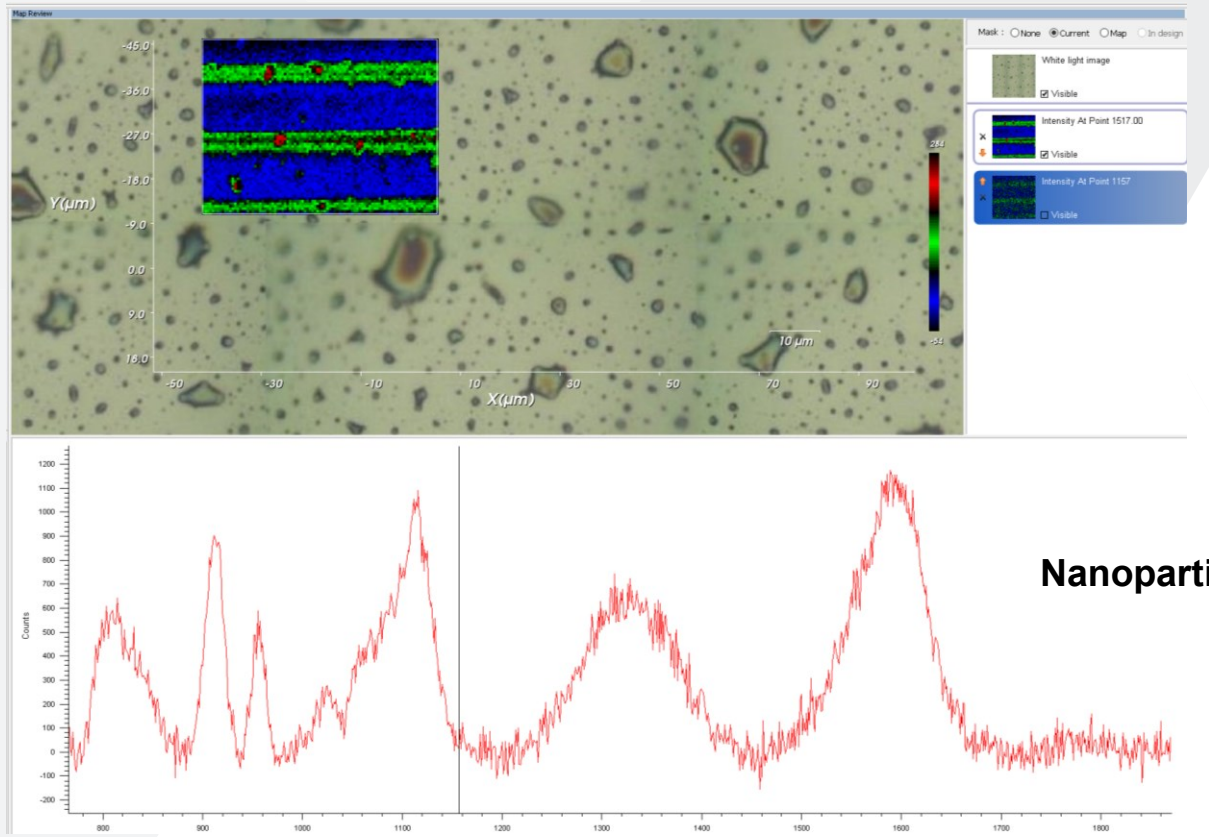


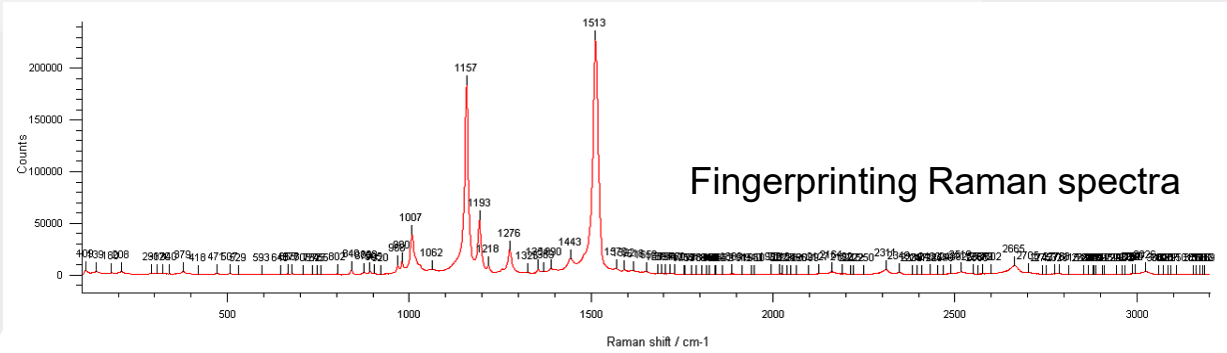


+ combination with AFM topography

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

Raman microscopy
Chemical mapping

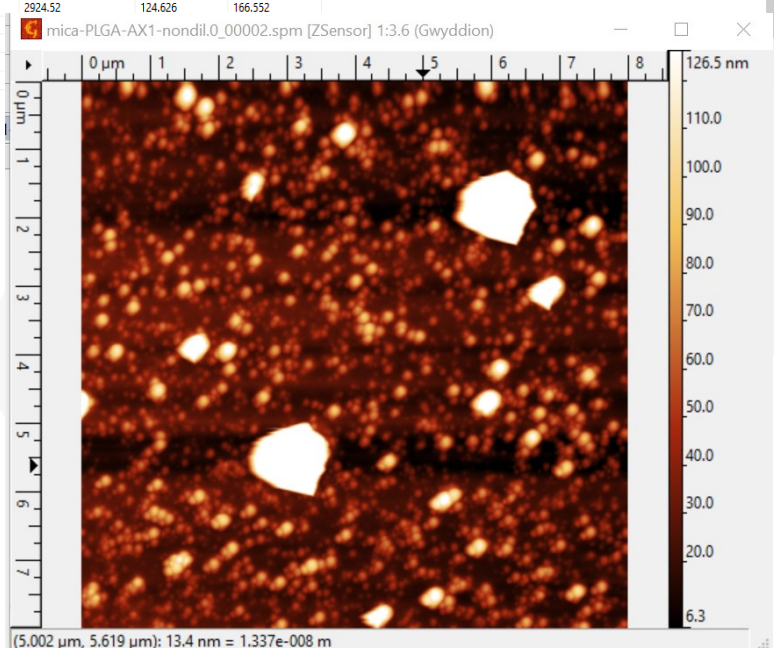




Fingerprinting Raman spectra

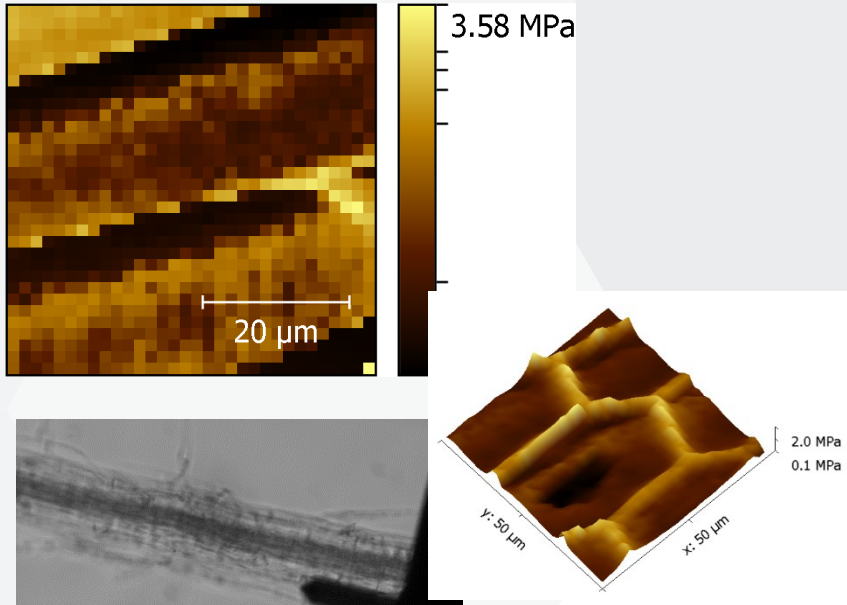
Peak no.	Centre	Height	Width	Area	Absolute intensity	Low edge	High edge
1	108.873	3059.03	9.28978	78556.2	4036.61	102.247	123.312
2	139.016	1668.17	14.5499	122921	2924.52	124.626	166.552
3	180.065	804.539	9.00485	55816.4			
4	208.109	1870.6	14.0121	110061			
5	291.483	692.559	7.83973	51650.1			
6	306.84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

Nanoparticles loading study



+ combination with AFM topography

AFM-based biomechanics
On a tissue level



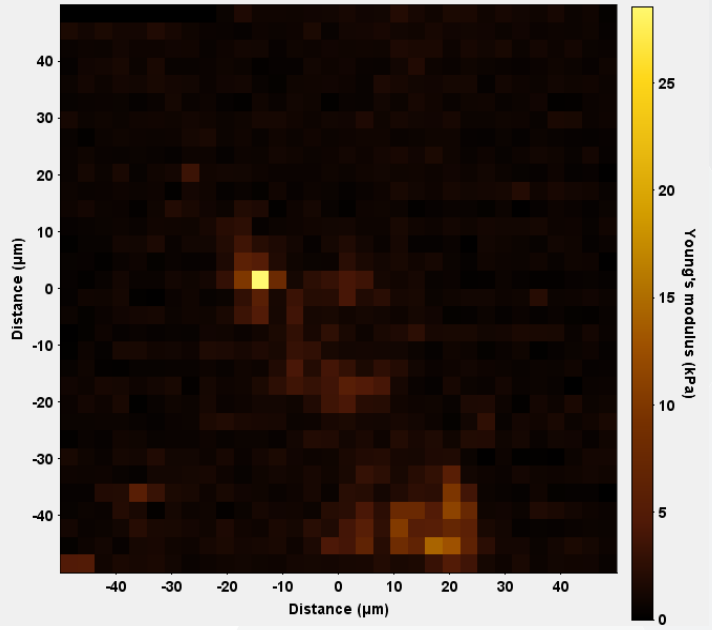
Plant samples

(hypocotyl)

by Marçal Gallemí

Eva Benkova Lab

& Jan Hejatko Lab

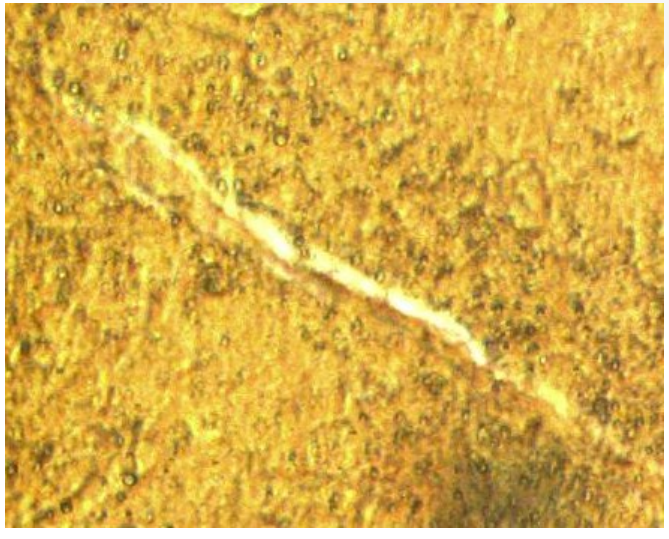


Liver cirrhosis

Correlation of

Collagen fibers by polarized microscopy

AFM nanoindentation



by Srikant Ojha

Martin Gregor Lab

CEITEC

Core Facility NanoBiotechnology

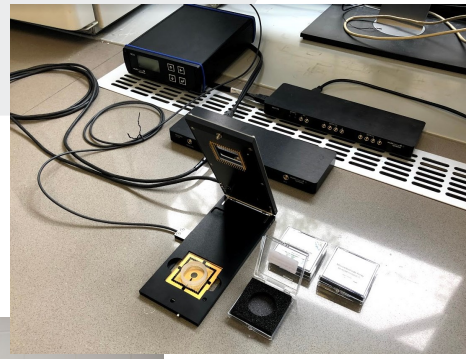
Standard operations of the CF

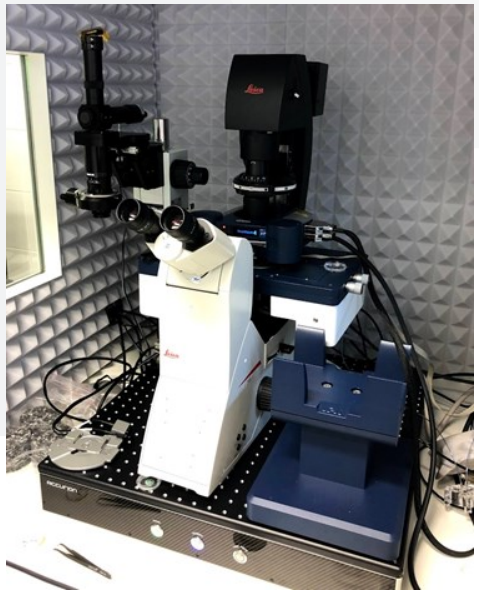
- **BioAFM microscopy** – biomolecules, nano-objects
- **Stiffness mapping** – cells, tissues
- **Combination** of AFM with other techniqeus (BF/fluorescence microscopy)

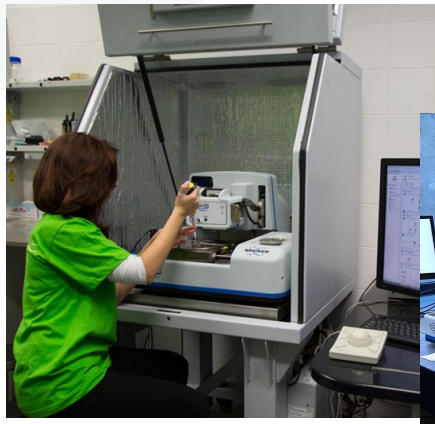
2020/21 Innovations

- **FluidFM** – microfluidic force microscopy
- **Raman** chemical mapping
- **MEA** – micro-electrode array cell electrophysiology

Core Facility NanoBio

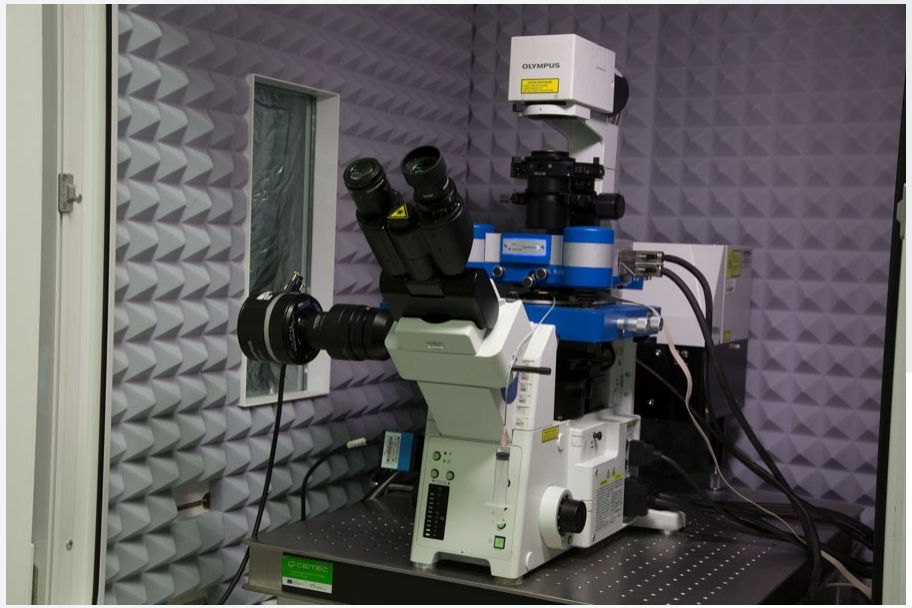


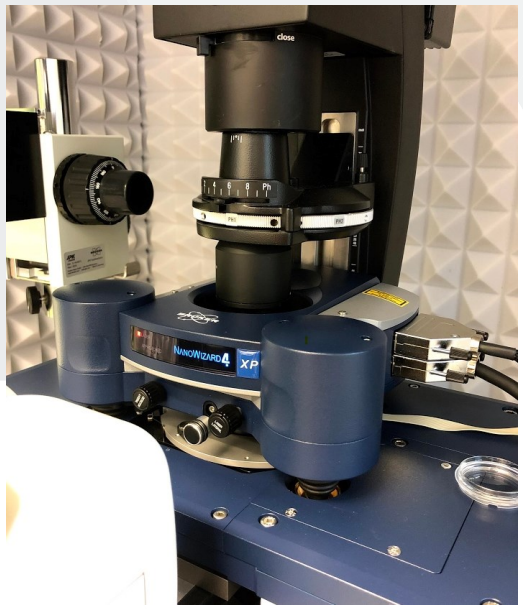


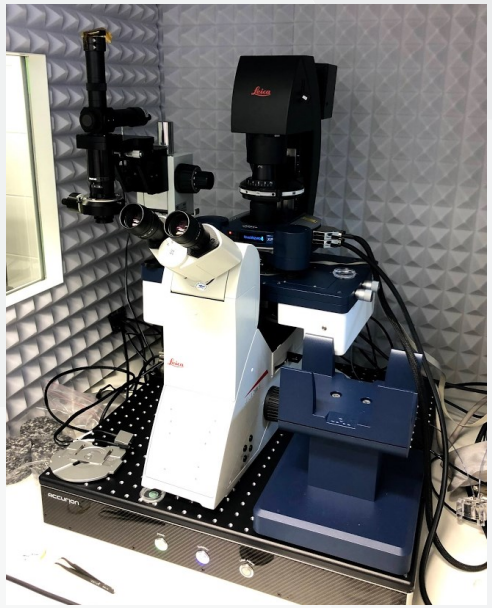


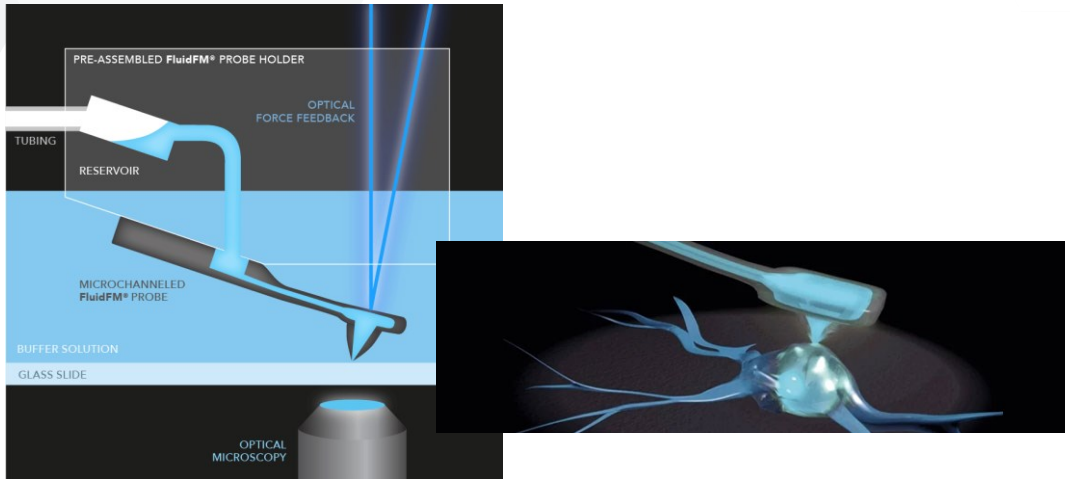

Core Facility
Nanobiotechnology
CIISB
JPK
Instruments

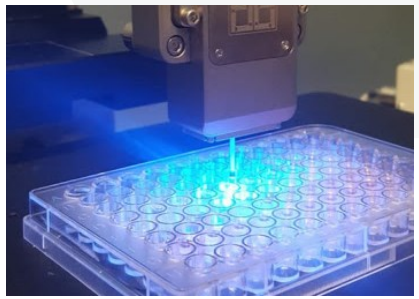
JPK NanoWizard 3 and 4 with extended scanning range











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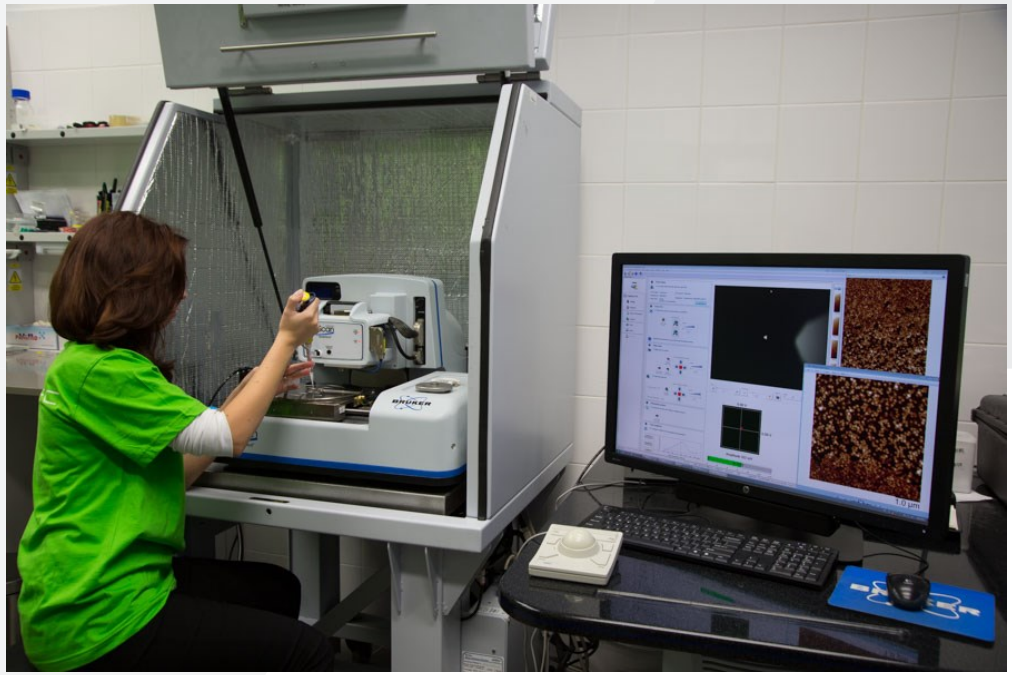
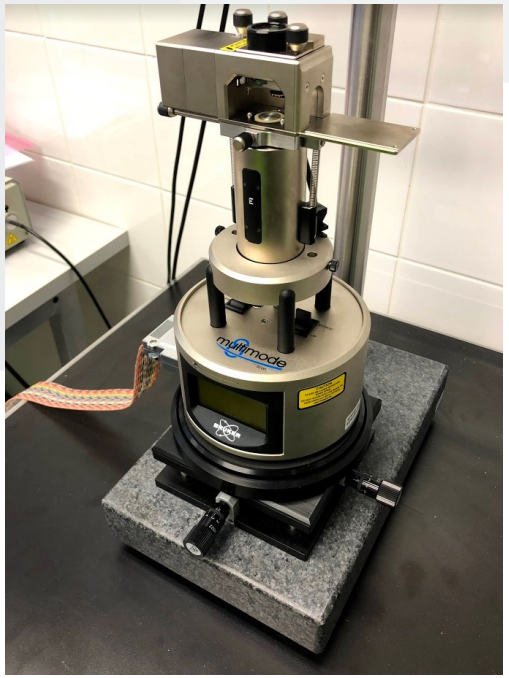
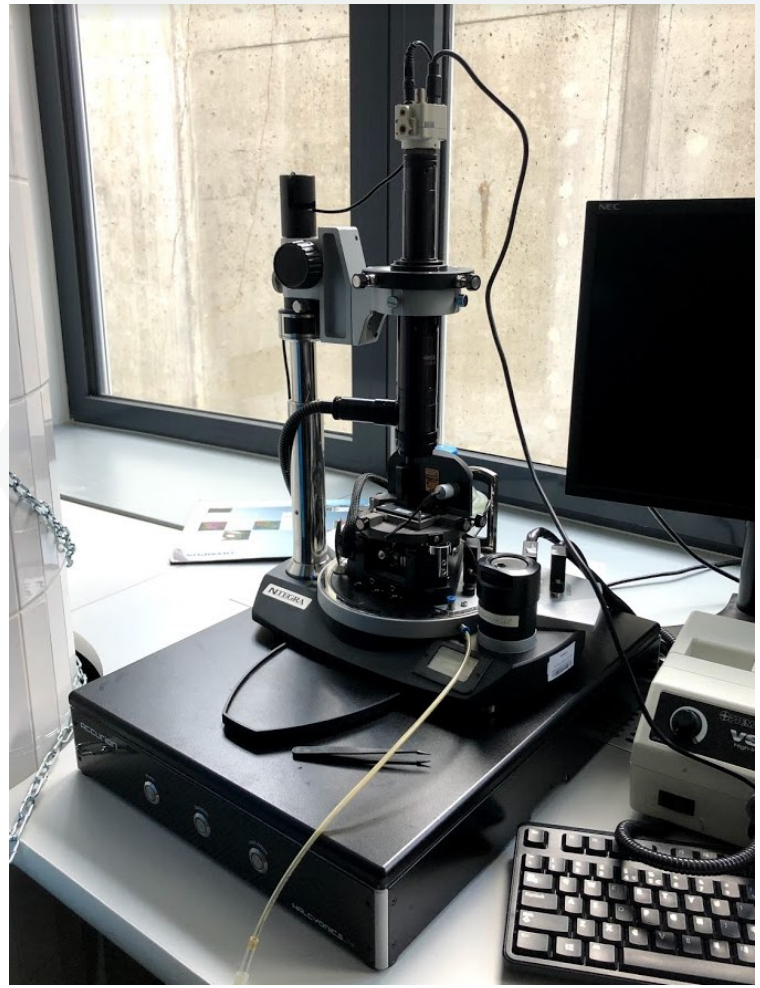
+ CytoSurge FluidFM module

+ Biosoft NanoIndenter







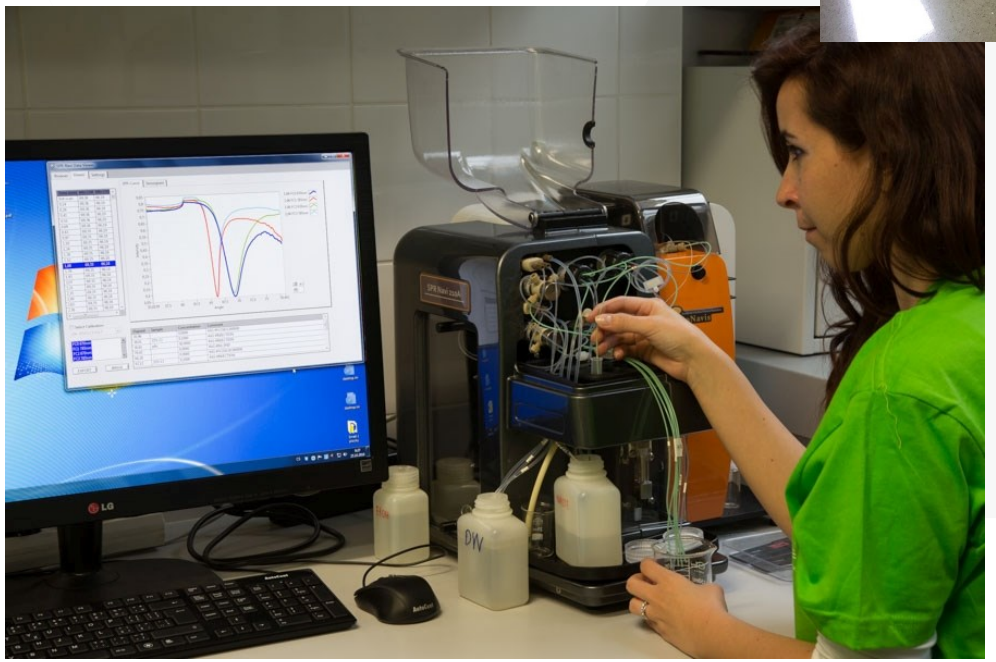
Bruker Dimension Icon FastScan and MultiMode 8HR
NTMDT Ntgra Vita

Raman microscopy, SPR affinity biosensor, Upconverting particles UCNP reader

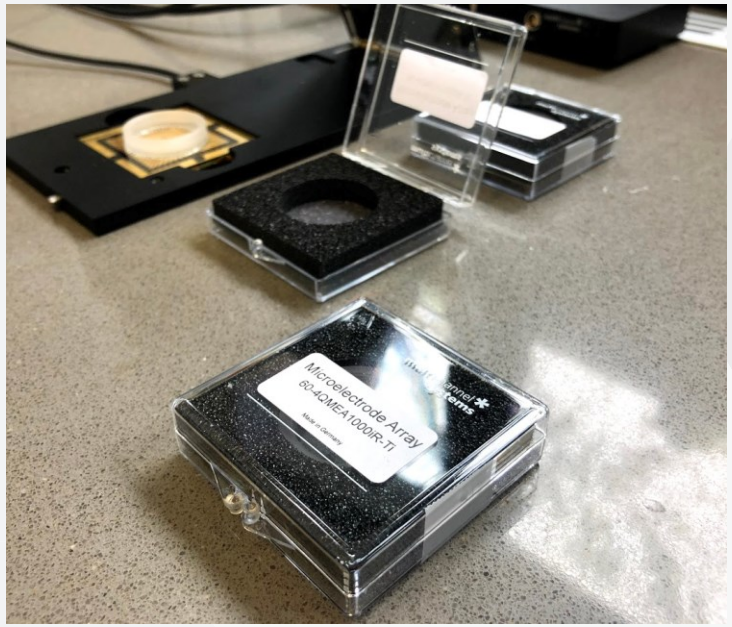
Renishaw InVia Raman microscope

Bionavis SPR biosensor device

Labrox UPCON reader

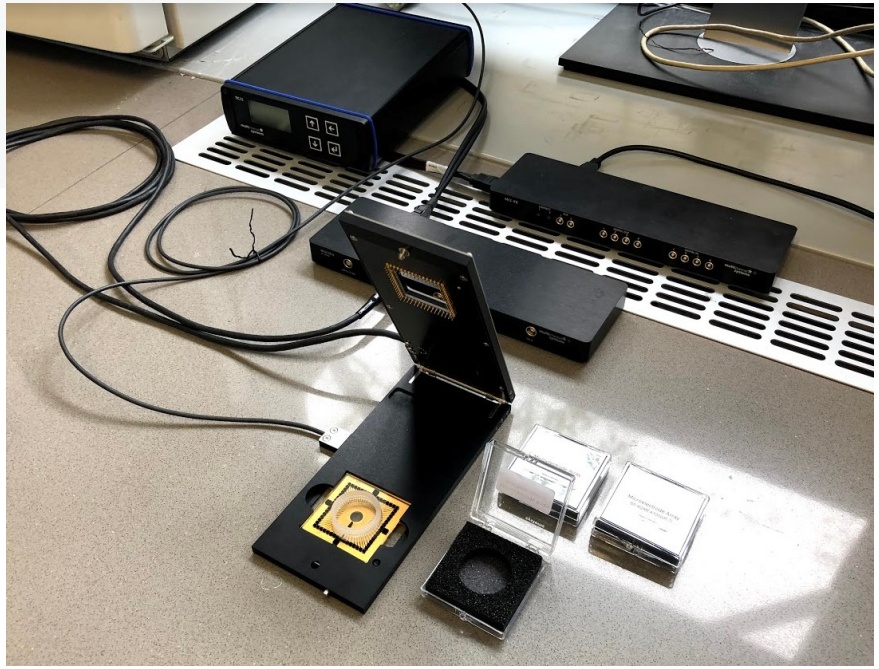


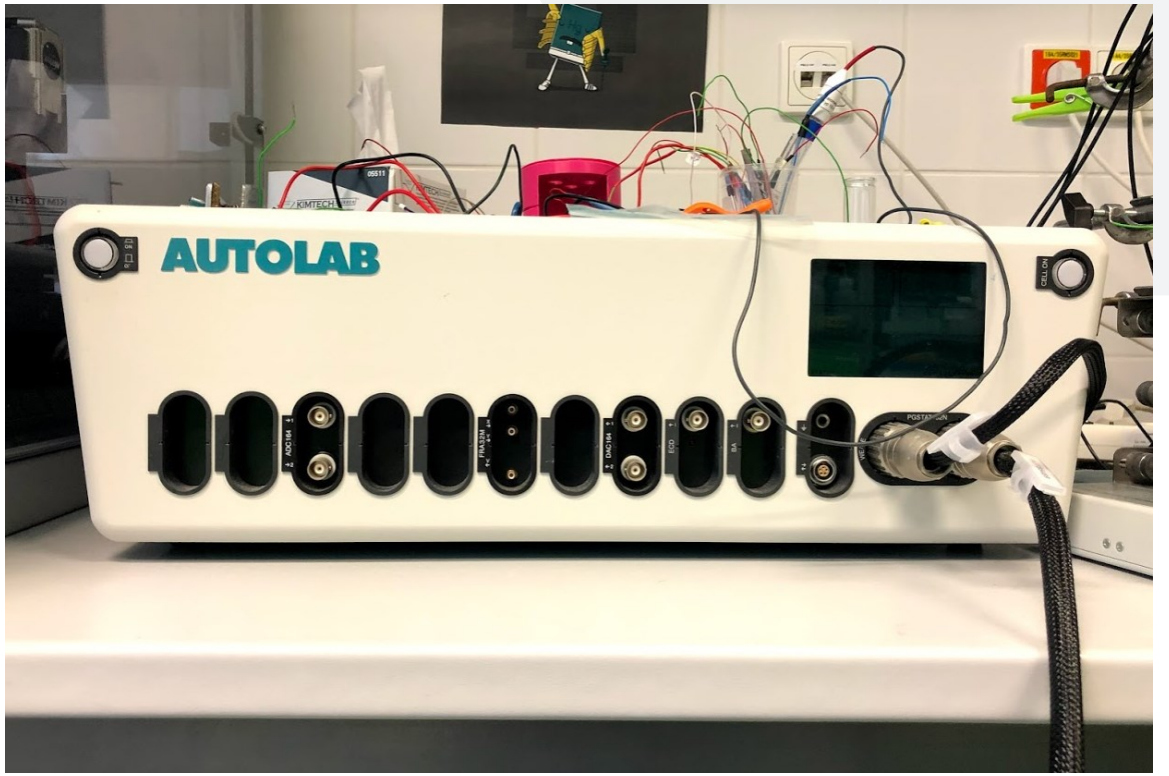
CEITEC



Autolab Modular potentiostat

MultiChannel MEA2100Lite





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

User Training

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

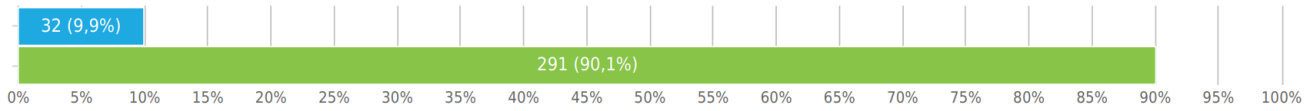
Workshop title	Date	Main objectives	No of participants
Atomic Force Microscopy (AFM) for Bio Applications	April 16-17, 2019	Combined characterization of biosamples by AFM, practical applications, hands-on session	20
Characterization of nanoparticles and proteins by Atomic Force Microscopy	July 30-31, 2019	Characterization of nano-objects and proteins by AFM, practical applications, hands-on session	25
Spring Workshop on BioAFM Microscopy	April 6-8, 2020	Theoretical background and new aspects of bio-AFM microscopy, sample preparation, hands-on session, social event	Canceled
Introduction to Raman microscope Renishaw inVia	June 23rd, 2020	Introducing the Raman microscope Renishaw inVia, User samples characterization	12
(Bio) Atomic Force Microscopy (bioAFM), Basic Course	October 1st, 2020	Basics of AFM, Sample preparation techniques, Data processing	80
Introduction to JPK NanoWizard 4 AFM microscope	May 4th, 2021	Introduction to a new JPK NanoWizard AFM system combined with CytoSurge and NanoIndentor module	10

User Survey

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

Výběr z možností, zodpovězeno 323x, nezodpovězeno 0x

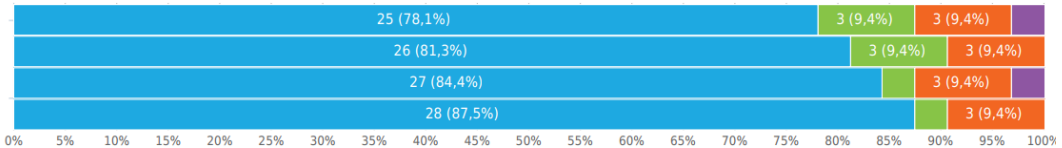
Možnosti odpovědí	Responzí	Podíl
Yes	32	9,9 %
No	291	90,1 %



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



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

- 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 ^{o}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!!!*

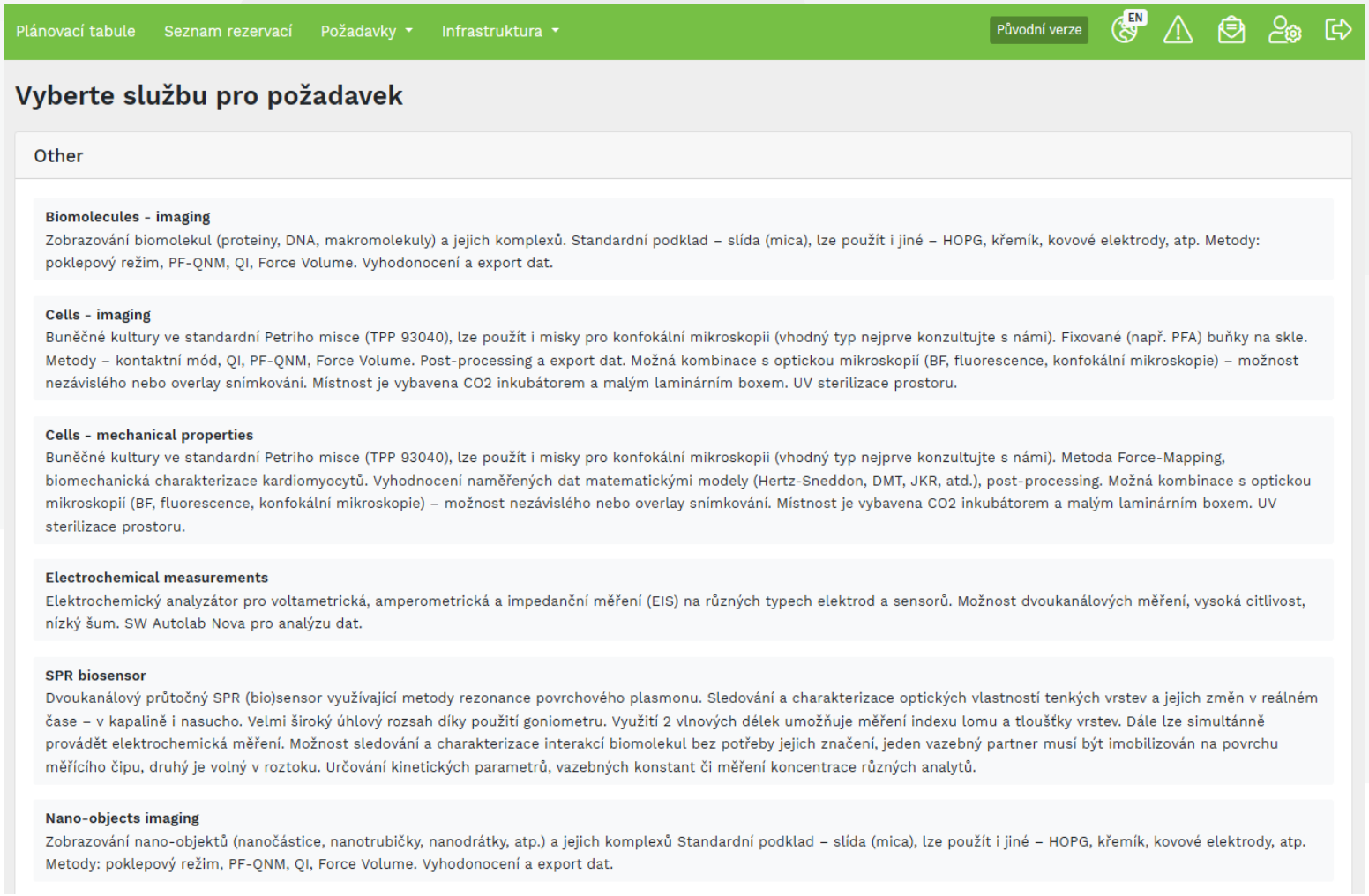
- **Tuneable hydrogel system**

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

  ! Coordination with Vladimir Rotrekl RG – essential!!!

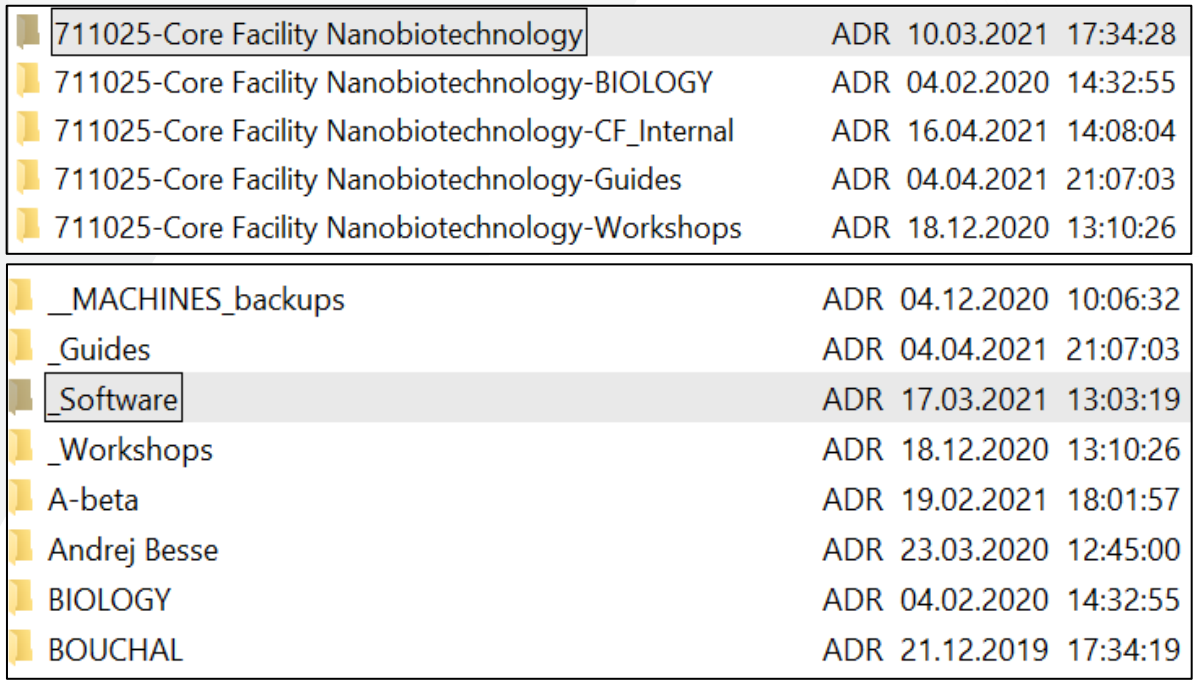
Booking system

Under development for last 2 years..



Data Sharing

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



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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
_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
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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*

CF involvement in the Correlative Microscopy project

Integration of our CF in the project **correlative microscopy** has been proposed in the **Strategic Plan** of the Central European Institute of Technology at Masaryk University for **2021-2028**, section "At the frontiers of technology: Correlative approaches to connect dynamics and structure of living systems."

Correlative AFM-STED microscopy highlight a new and essential aspect and generate a warning: Fluorescence techniques cannot characterize all the products derived from the in vitro aggregation of misfolded proteins. Therefore, the combination of microscopic techniques brings a better understanding of the physiological processes.

Mechanical properties (AFM), calcium levels (fluorescent dyes), and cellular potential (MEA) – **mechano-physical-electrical** coupling

"**Mikroskopie**" (= Microscopy) educational project and in the LLL remote training and propagation project started in the time of the corona crisis.

High-Resolution Correlative Microscopy: Bridging the Gap between Single Molecule Localization Microscopy and Atomic Force Microscopy
P.D. Odermatt et al., *Nano Lett.* 2015, 15, 8, 4896–4904

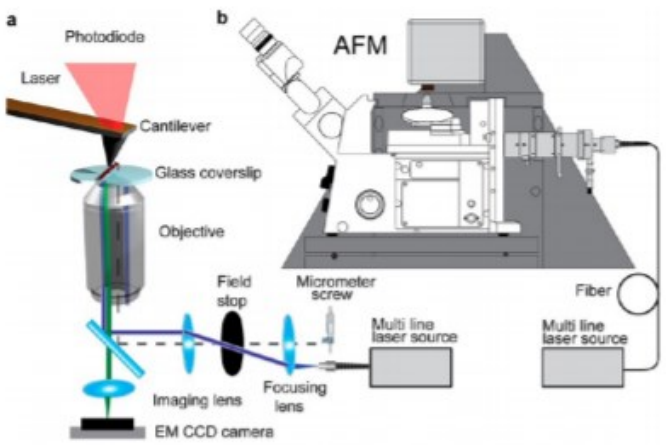


Figure 1. Correlative AFM-SMLM: instrument setup. (a) Schematic

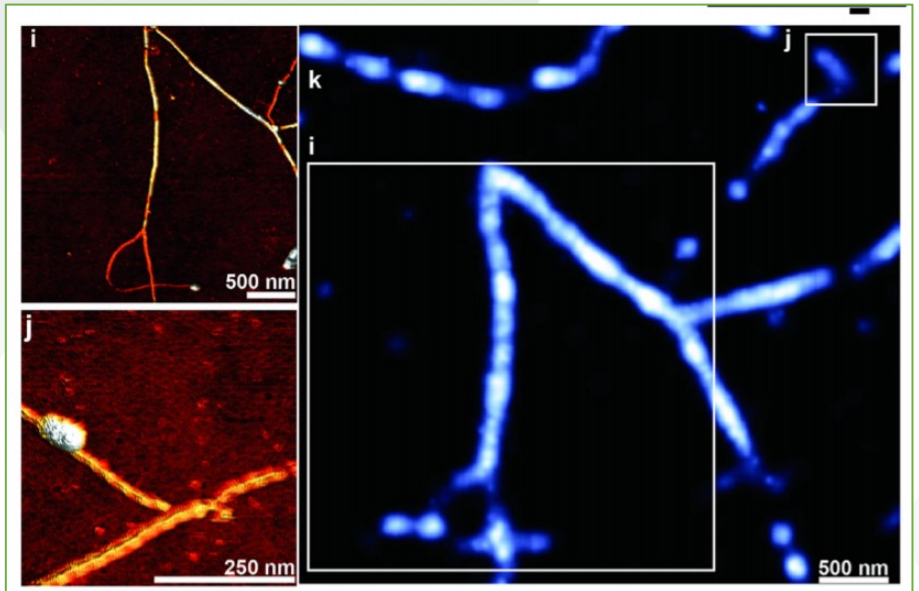


Figure 3. Experimental procedure and representative correlated AFM/dSTORM images. Experimental procedure (a–h): (a) The AFM cantilever is centered in the optical field of view of the camera by translating the inverted microscope in *x*- and *y*-direction. (b) The sample is moved, and a region

F-Actin fibers study

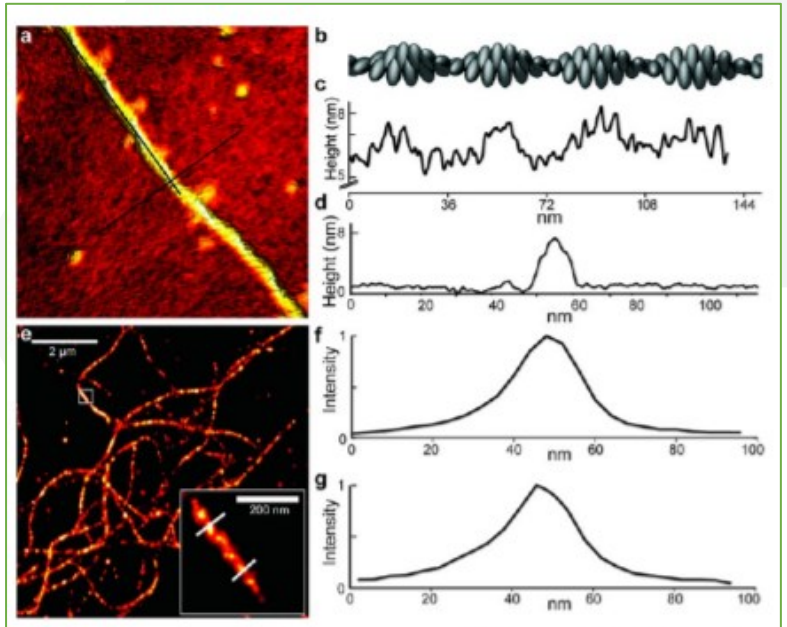
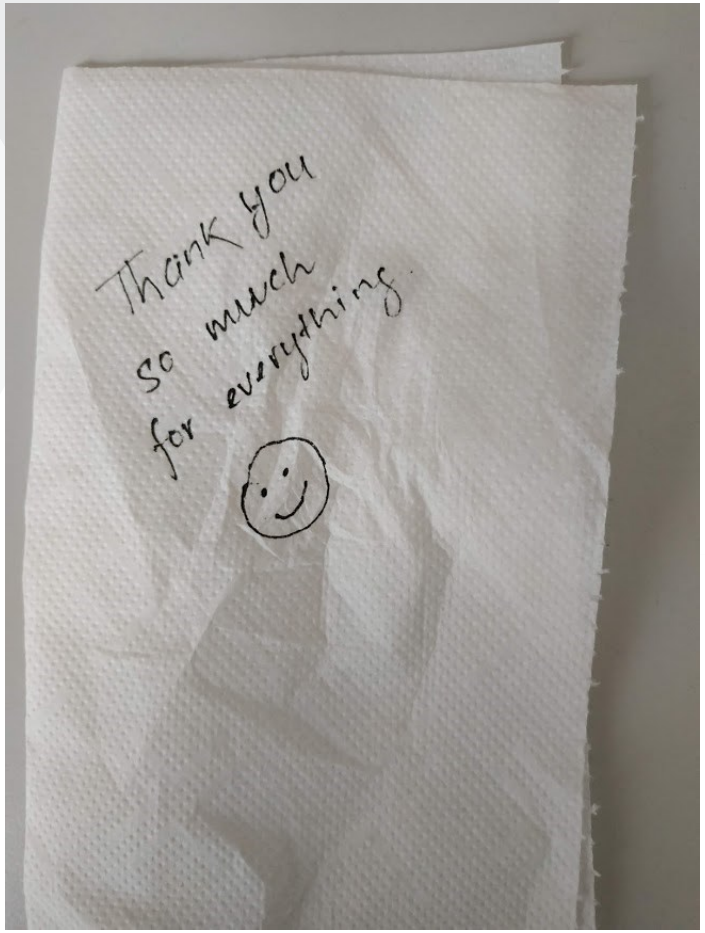


Figure 2. Independent performance of AFM and dSTORM on the combined AFM/SMLM system. (a) AFM image of F-actin deposited

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



Conclusions

- BioAFM microscopy - more than just imaging method
- Easy combination with other methods
- Sample range from molecules to tissue slices
- Mechanobiology - information related to the pathophysiology

Acknowledgment

- Petr Skládal – FS MU
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- E. Benkova, M. Gallemi – IST Wien

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- **Preferred version:** *„CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2018127, 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 (LM2018127).“*

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Thank you for your attention!