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Central European Institute of Technology

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Use of AFM for mechanical mapping of nanostructured surfaces

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EUROPEAN UNION
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2007–13
OP Research and Development for Innovation

Content

- Equipment – AFM, biosensors
- BioAFM – imaging, stiffness mapping, adhesion studies
- Samples, analysis
- Ongoing projects, cooperation, examples

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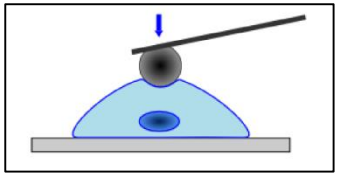
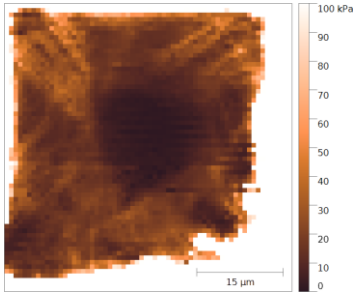
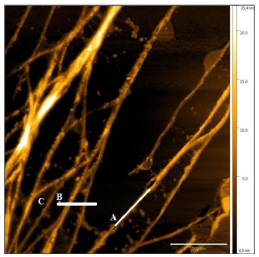
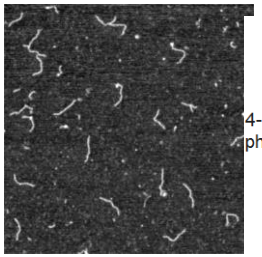
AFM microscopy / spectroscopy



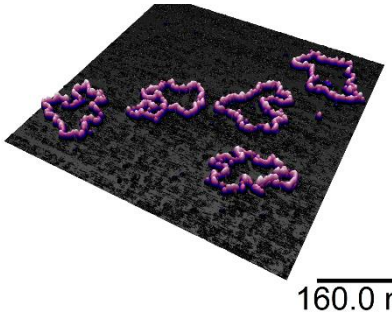
Stiffness mapping (nanoindentation)

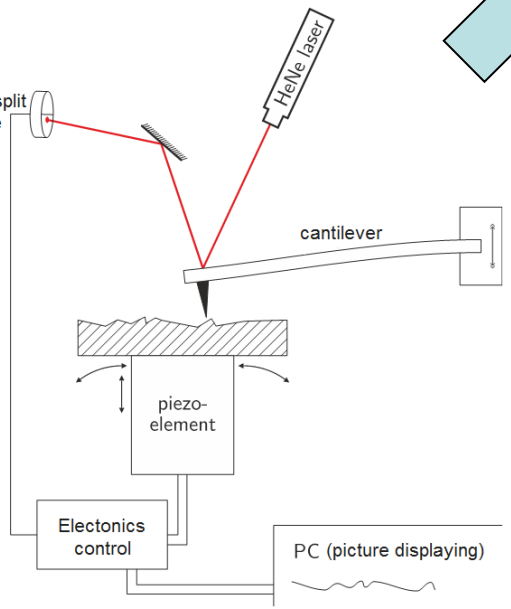


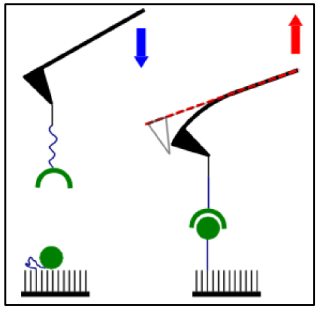




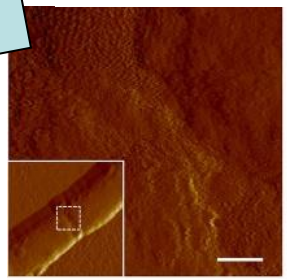
imaging

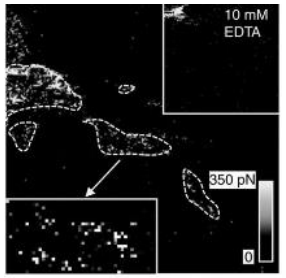






Affinity interaction





http://physics.mff.cuni.cz/kfpp/povrchy/method/afm

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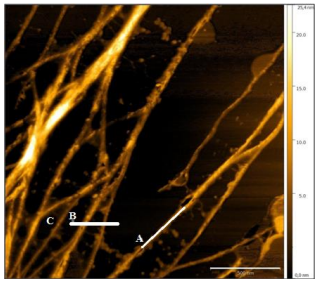
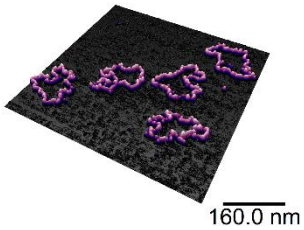
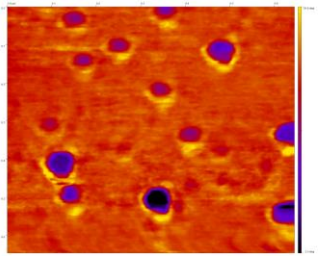
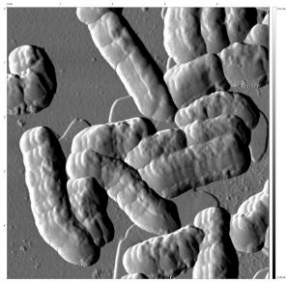
AFM imaging

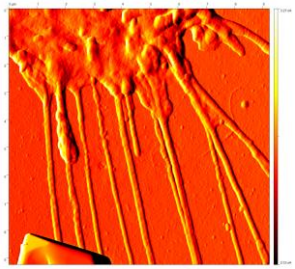
Biosamples

- **DNA – proteins – nanoparticles – liposomes – bacteria – single cells – cell clusters**



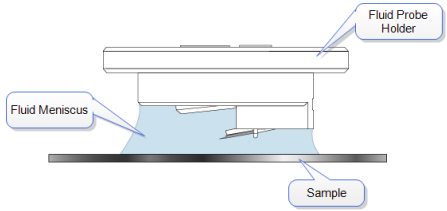




Working environment

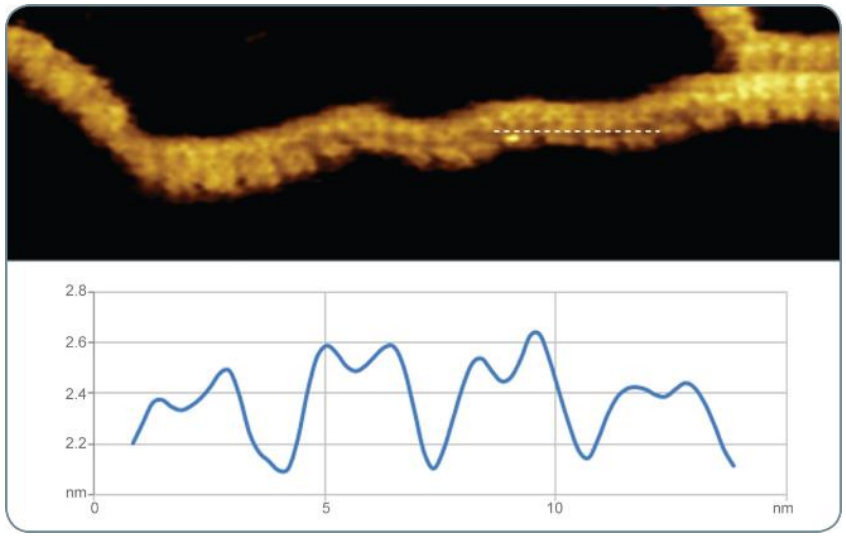
- **Lab atmosphere**
- **Liquid environment (drop/Petri dish)**
- **Elevated temperatures (37 ºC)**

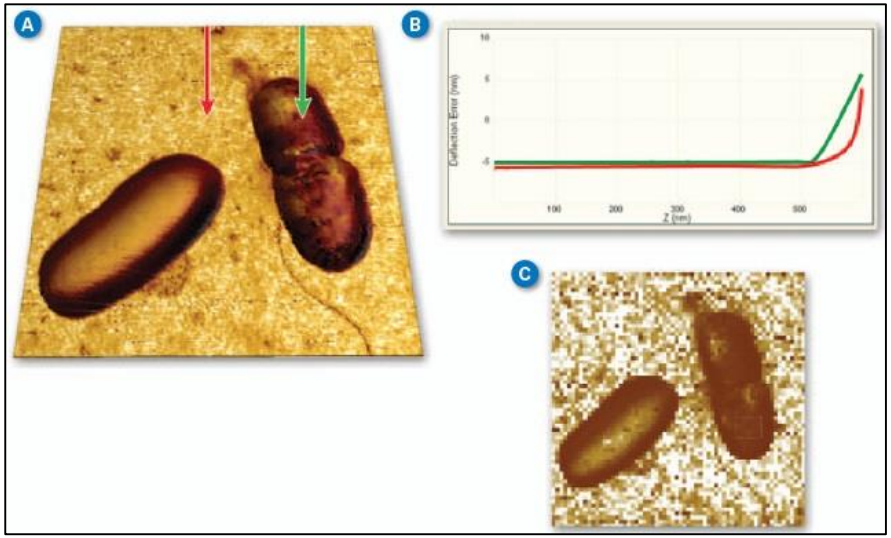



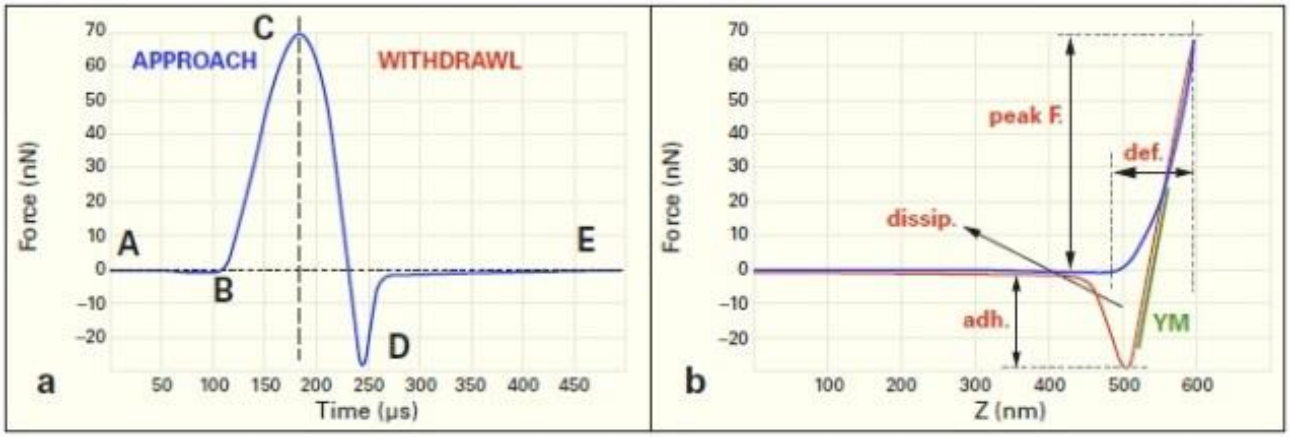
(AFM images: V. Hornakova, S. Solny, J. Pribyl. Photos by: nanophys.kth.se, anowerk.com)

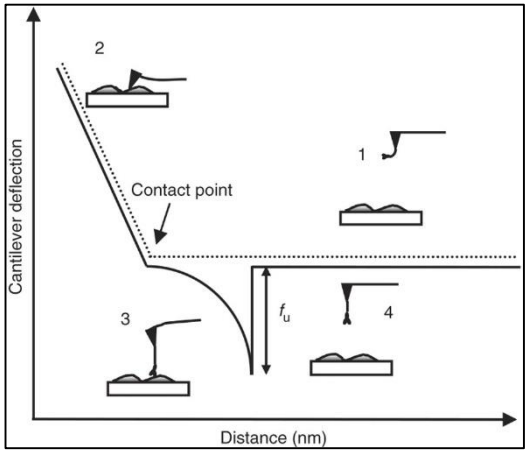
Hybrid modes: QI, QNM

→ Topography&Mechanical properties

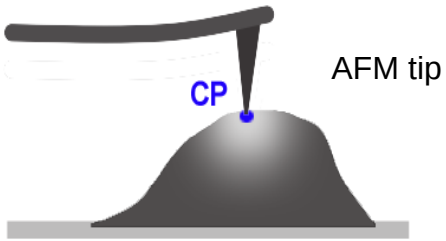


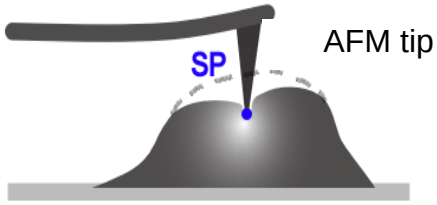


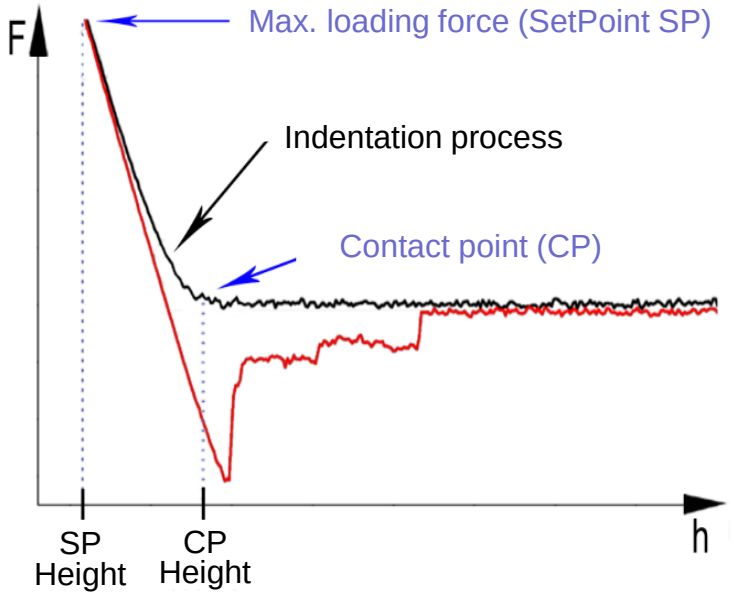


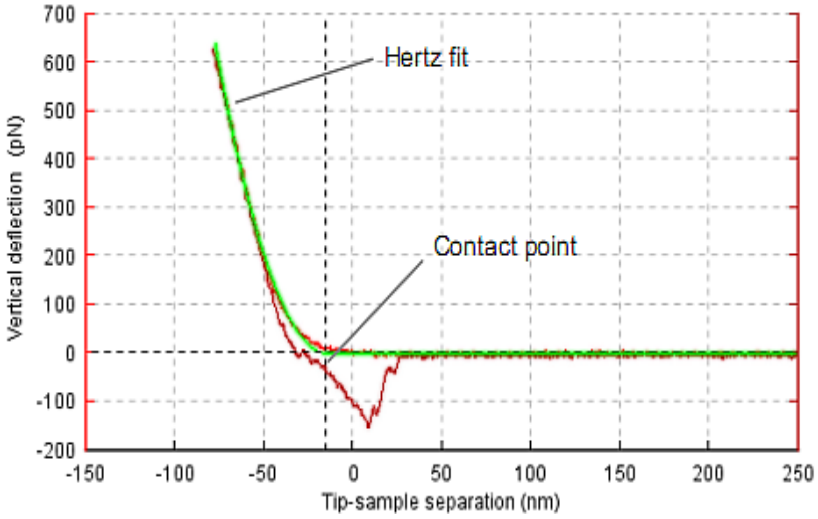


Force spectroscopy (nanoindentation studies)









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

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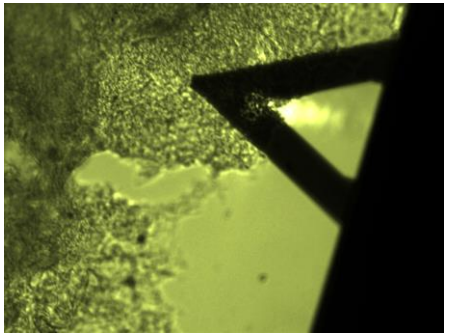
dECM

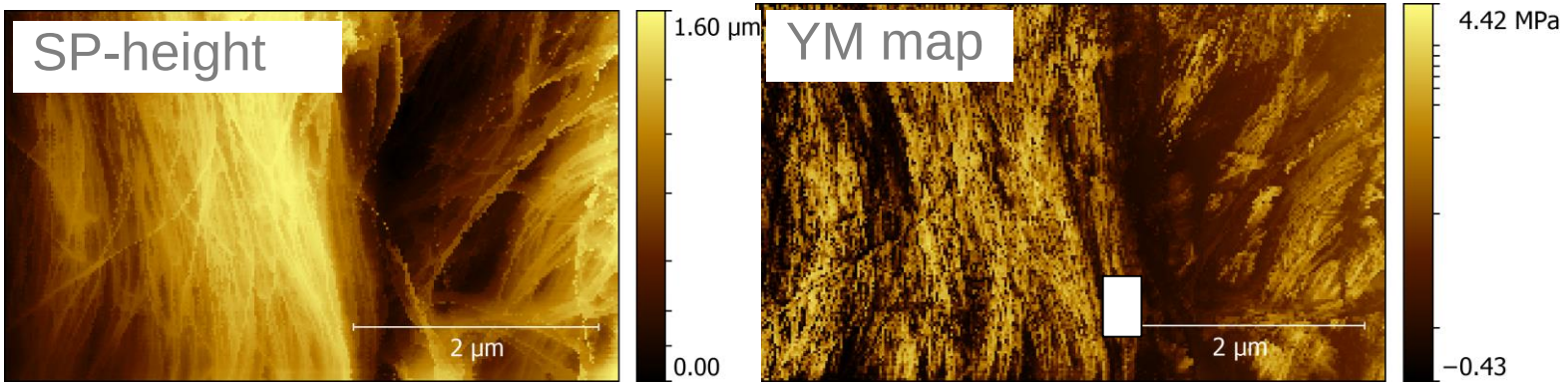
AFM cantilever above the dECM (optical view)

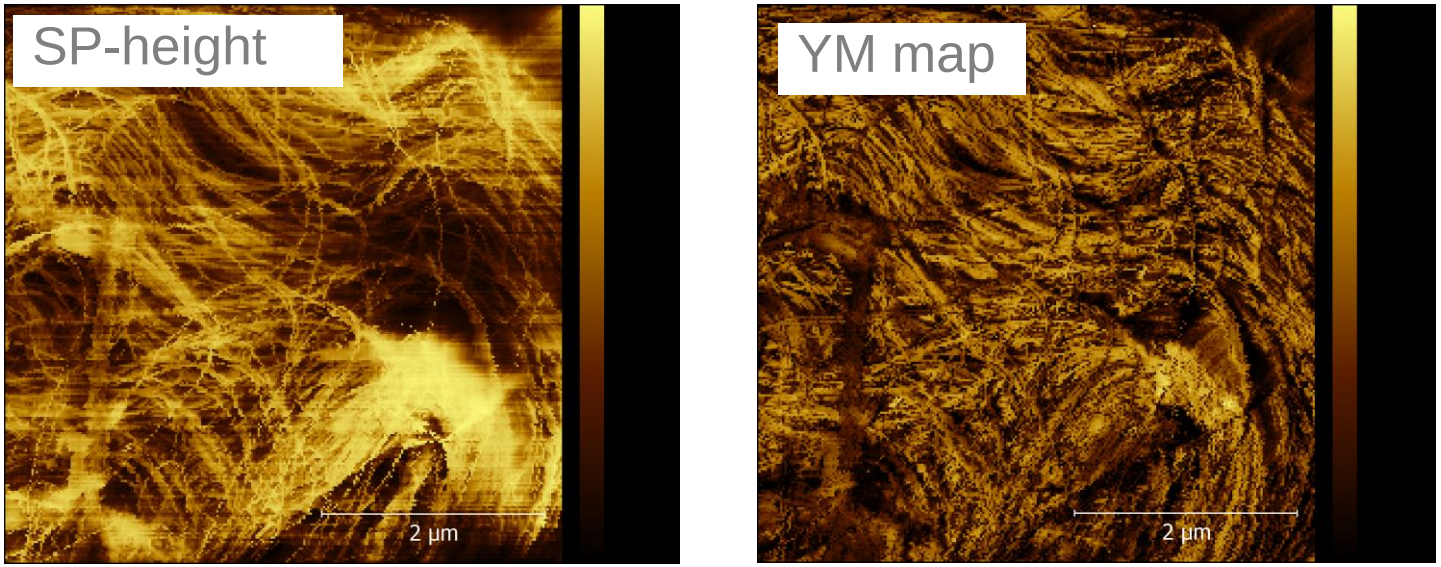


→ **For tissue engineering**

ForceMapping as imaging method

- High attraction forces
- Soft material (macroscopic view)





Samples by group of Gancarlo Forte, ICRC, Brno
AFM operator: Guido Caluori

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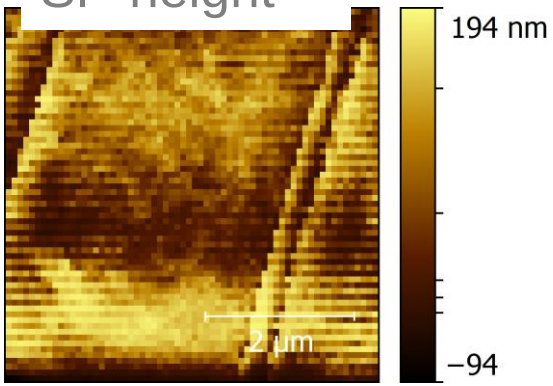
Chromatin-protein interaction

→ **For tissue engineering**

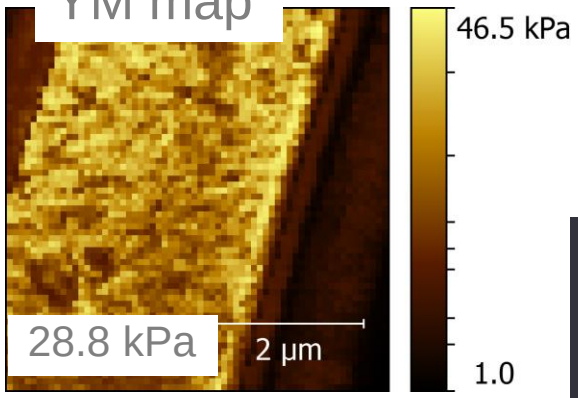
ForceMapping as imaging method

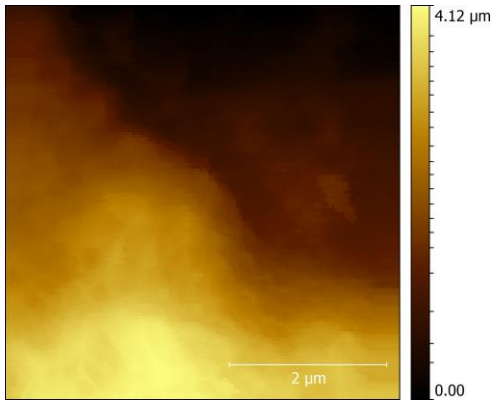
- Collapsive material
- Soft material (macroscopic view)

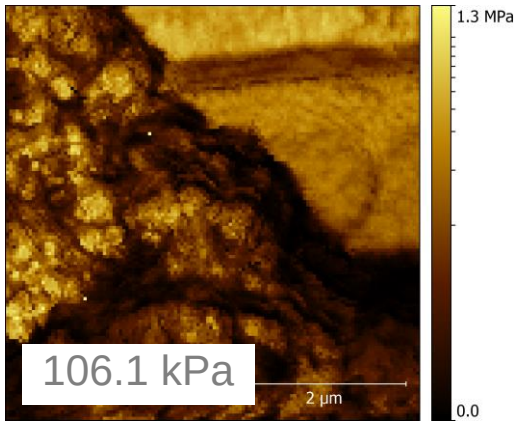
SP-height

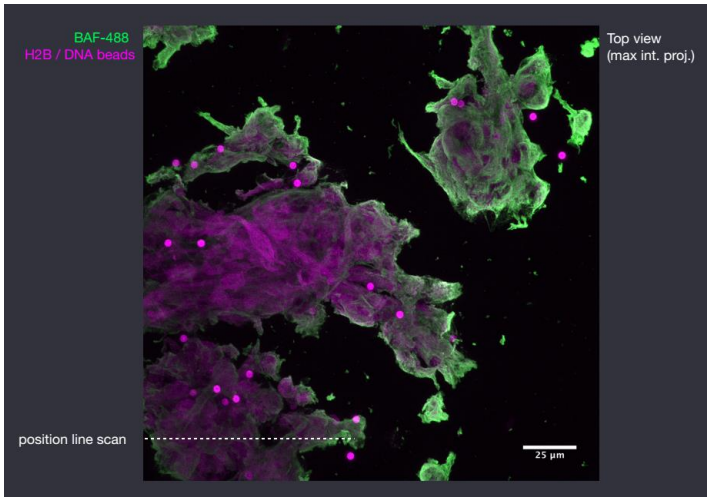


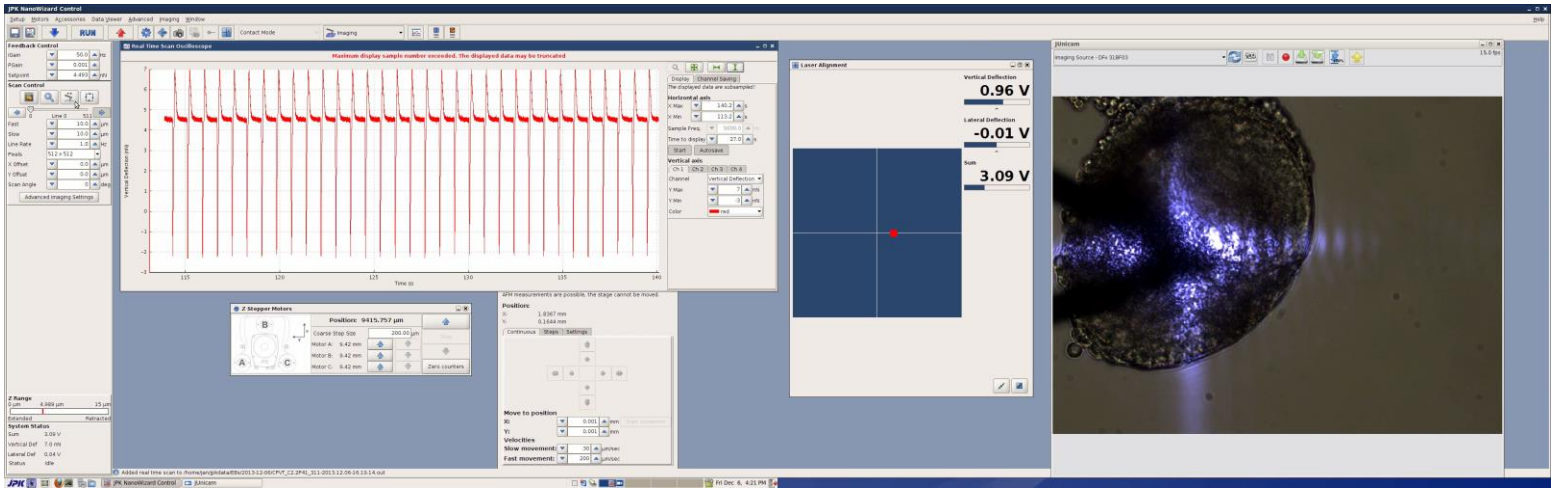
YM map



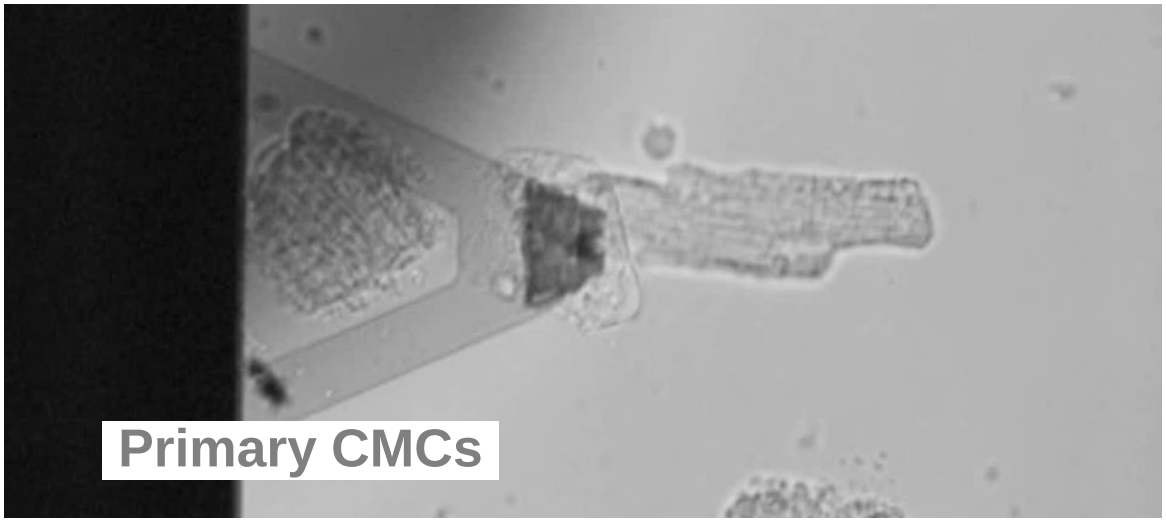








EBs



Primary CMCs

Pesl M, Pribyl J, et al. 2016 Atomic force microscopy combined with human pluripotent stem cell derived cardiomyocytes for biomechanical sensing *Biosensors and Bioelectronics* **85** 751–7
Pesl M, Pribyl J, et al. 2016 Phenotypic assays for analyses of pluripotent stem cell–derived cardiomyocytes J Mol Recognit n/a-n/a
Pesl M, Acimovic I, Pribyl J, et al. 2014 Forced aggregation and defined factors allow highly uniform-sized embryoid bodies and functional cardiomyocytes from human embryonic and induced pluripotent stem cells Heart Vessels 29 834–46

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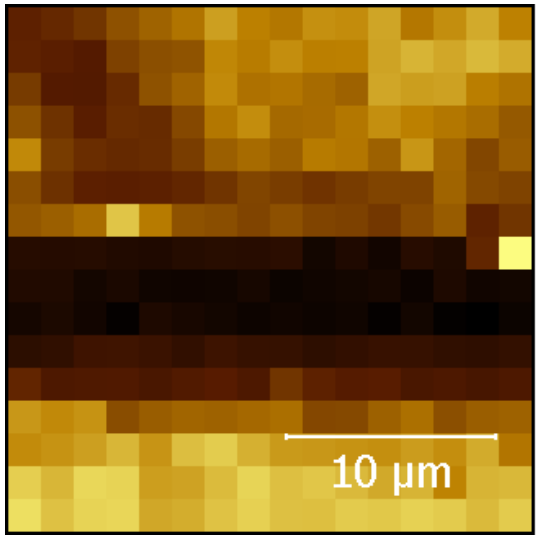
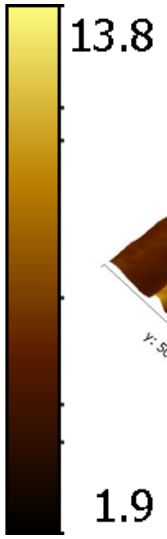
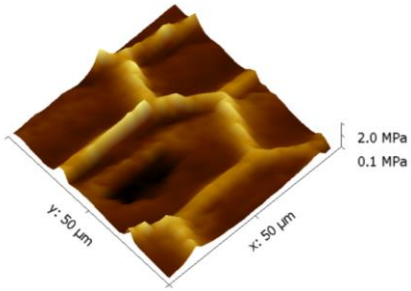
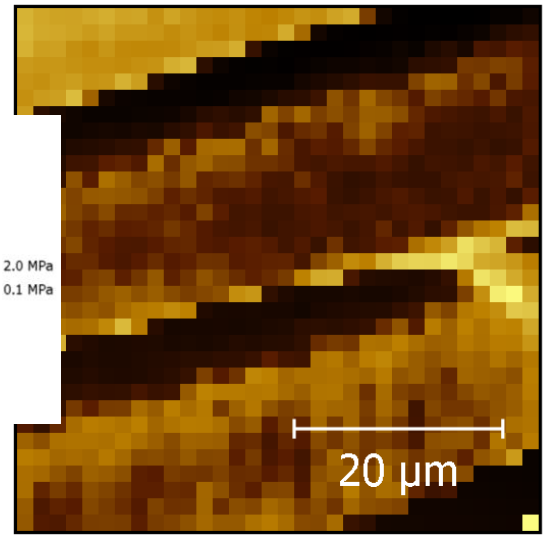
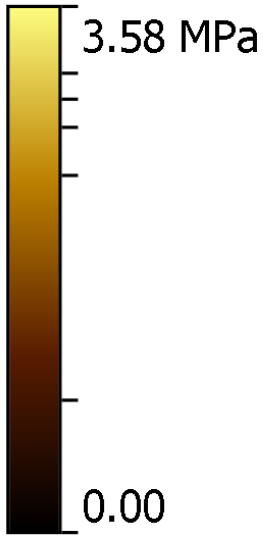






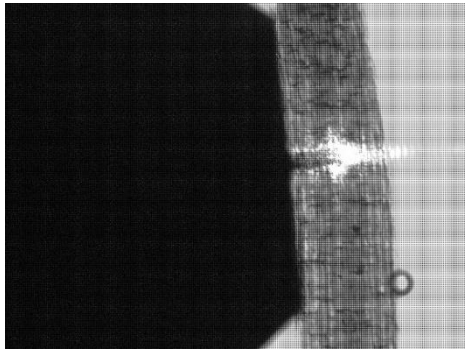




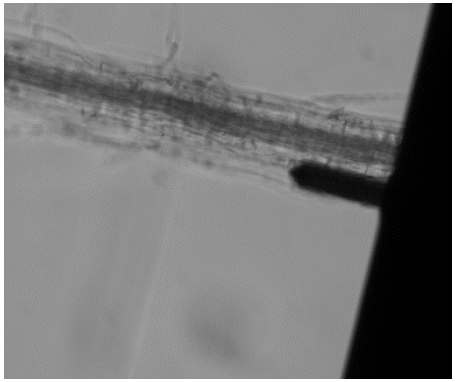
Water – YM 7.09 MPa

Manitol YM 0.69 MPa

Plant samples under AFM spectroscopy investigation



Hypocotyl and root parts



by Marçal Gallemí

Eva Benkova Lab

& Jan Hejatko Lab

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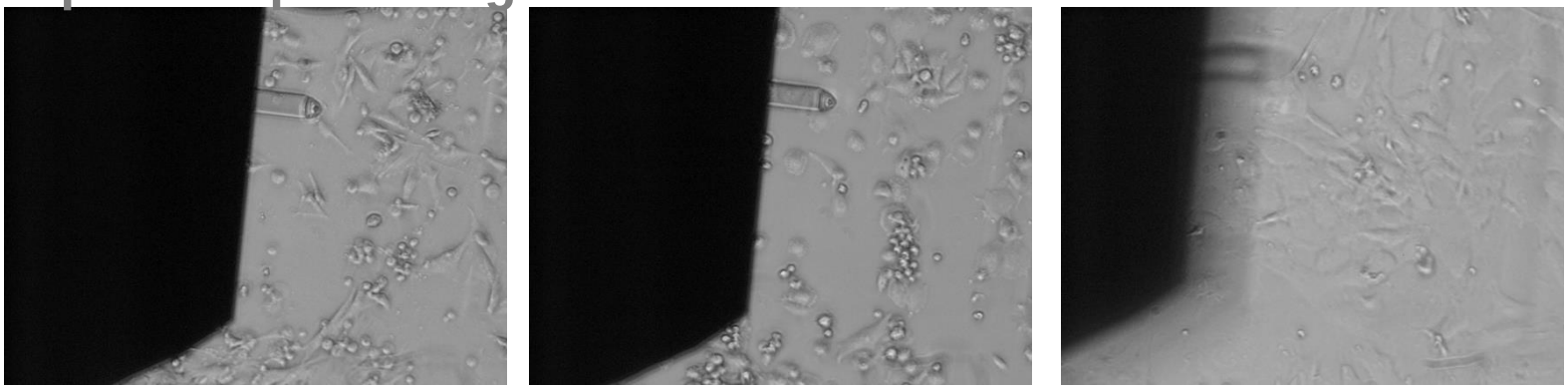
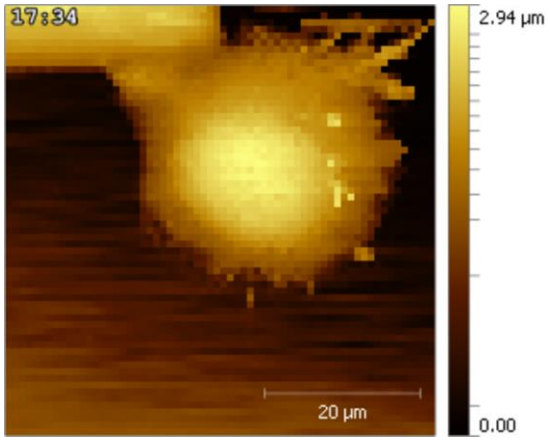
Top view optical imgs

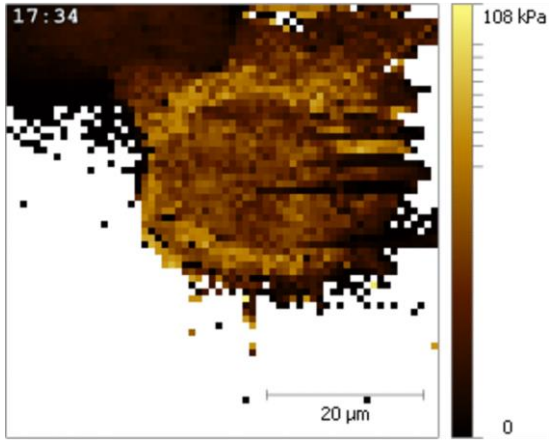


Fibroblasts thawing process

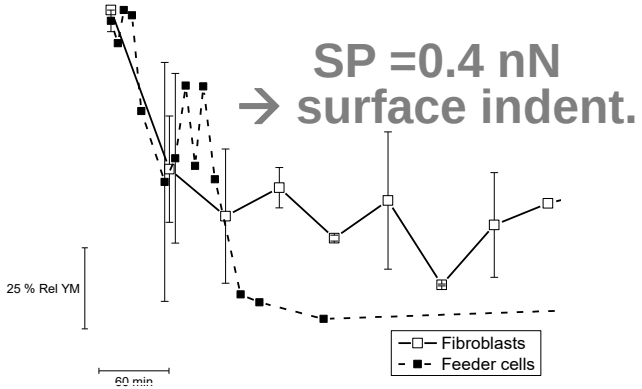
model case to study IVF related thawing

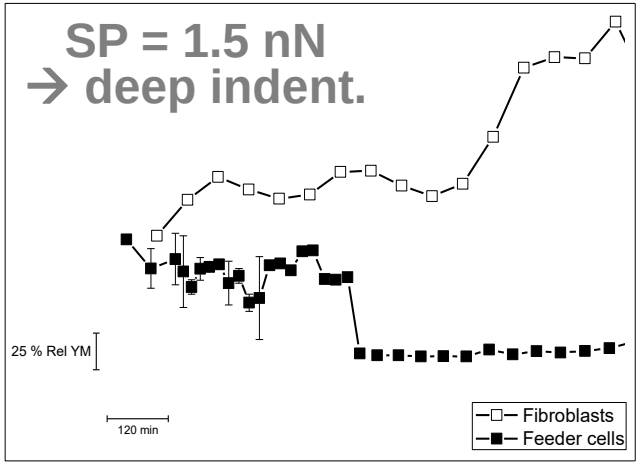


SP height profile
(12 hours)



YM profile
(12 hours)





Together with I. Kratochvilova (Institute of Physics ASCR)

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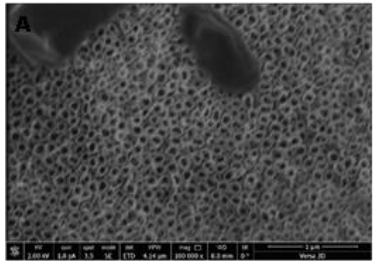
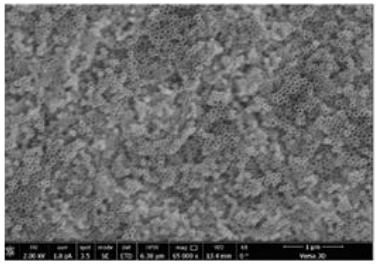
Titanium nanotubes

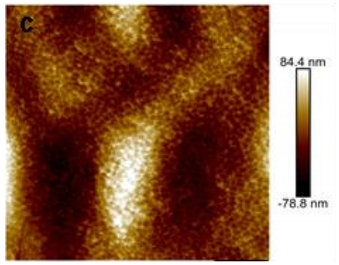
Osteoblast adhesion compatibility

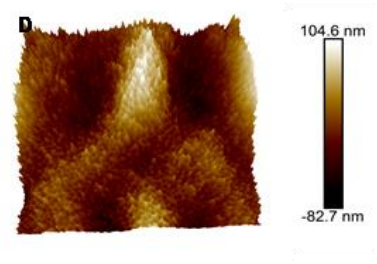
Aqueous phase

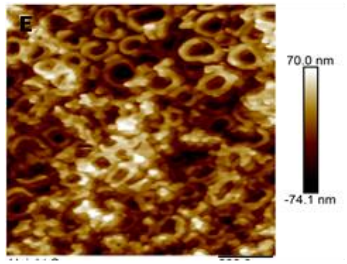
Organic phase

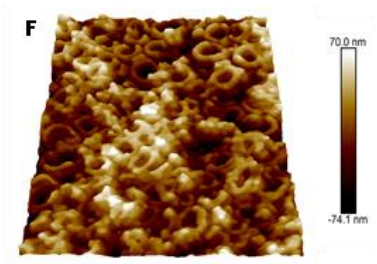


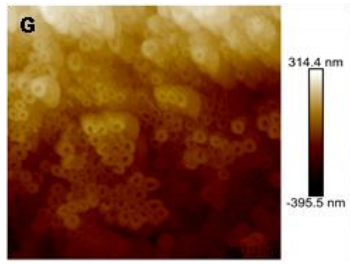


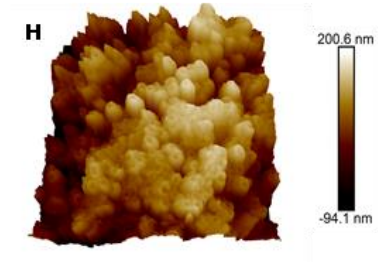




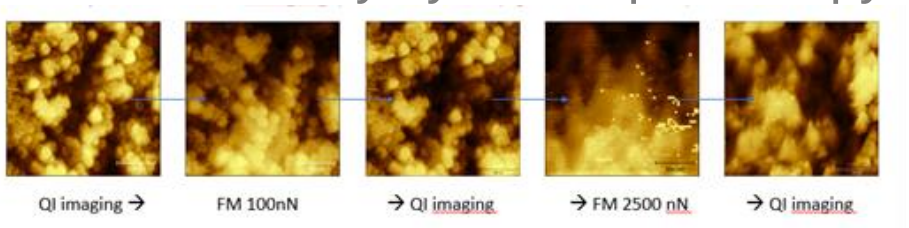


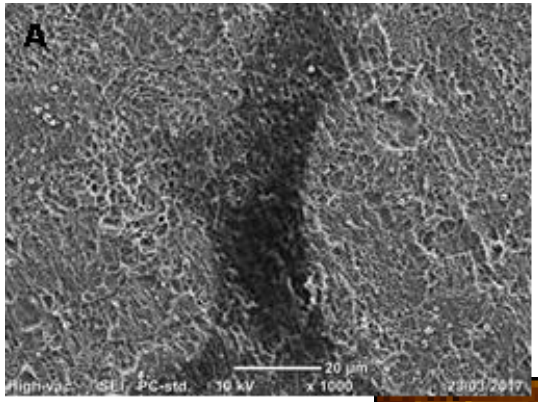




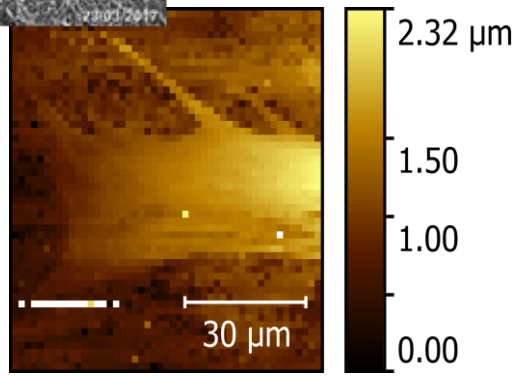


TiNT stability by force spectrscopy





SEM – fixed cell



Nanoindentation – living osteoblast

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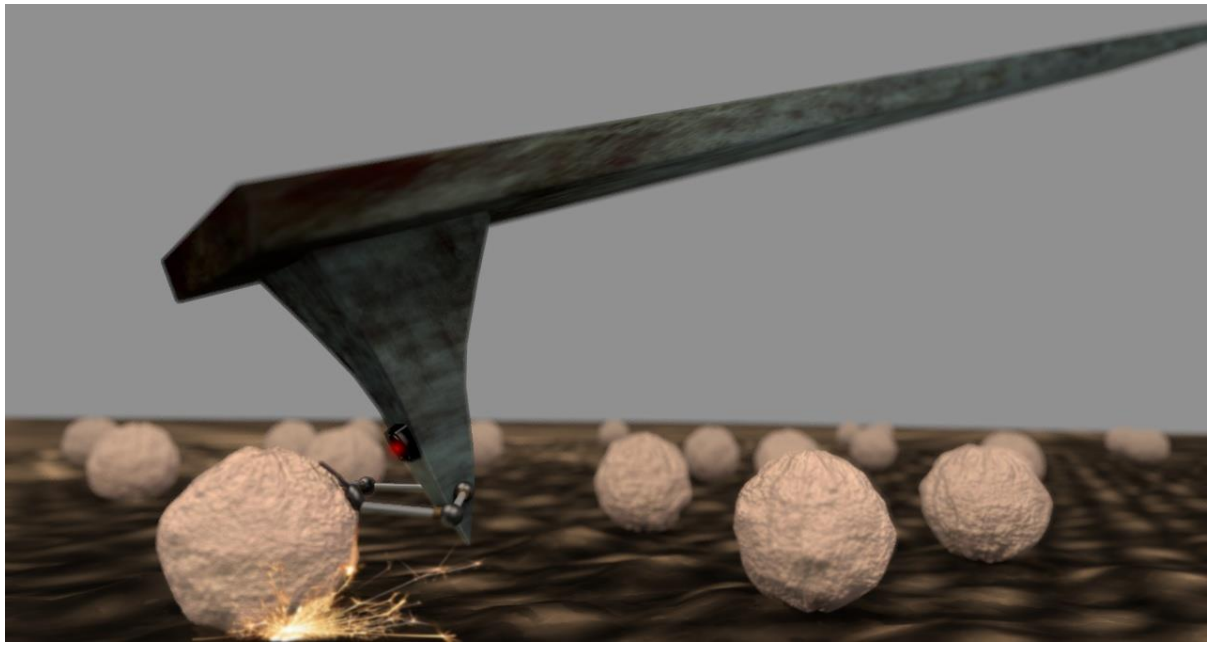
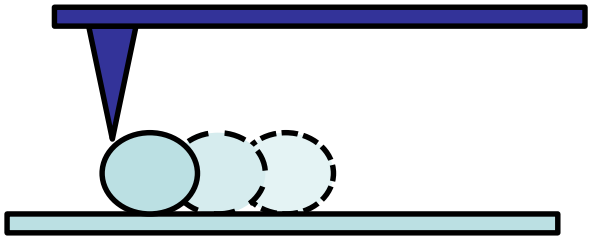
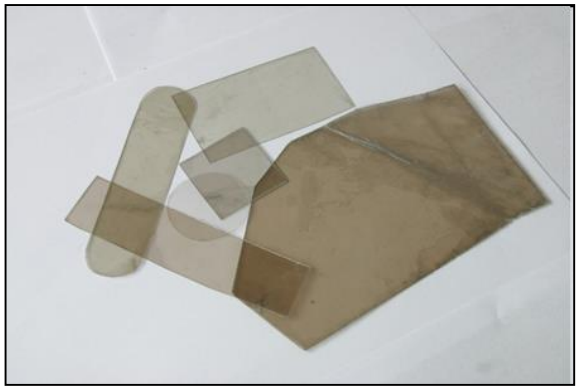
Improved Method for Surface Immobilization of DNA Molecules Used in AFM Single Molecule Imaging 1-(3-Aminopropyl)silatrane (APS)

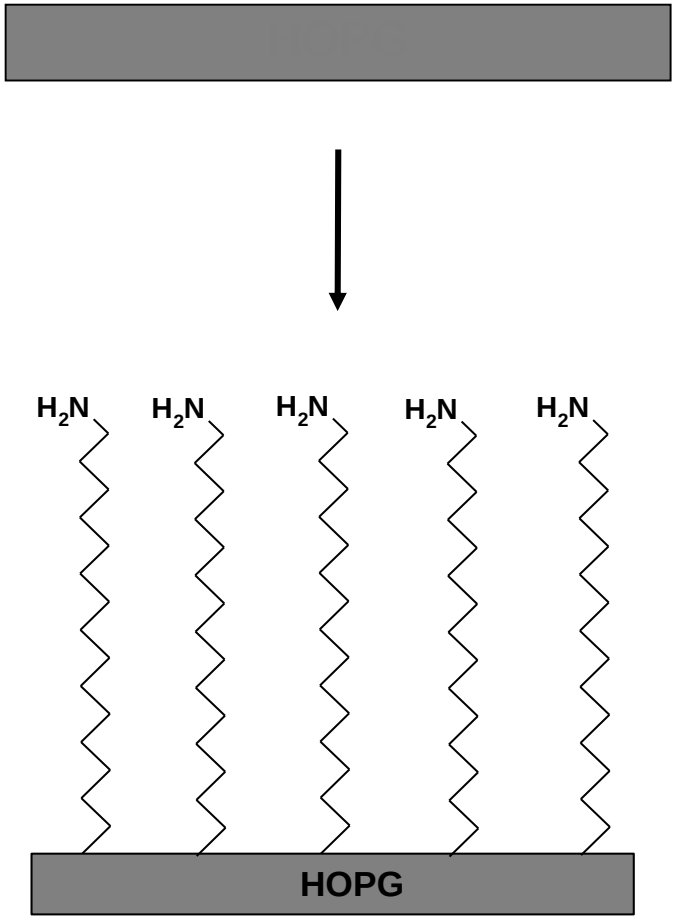




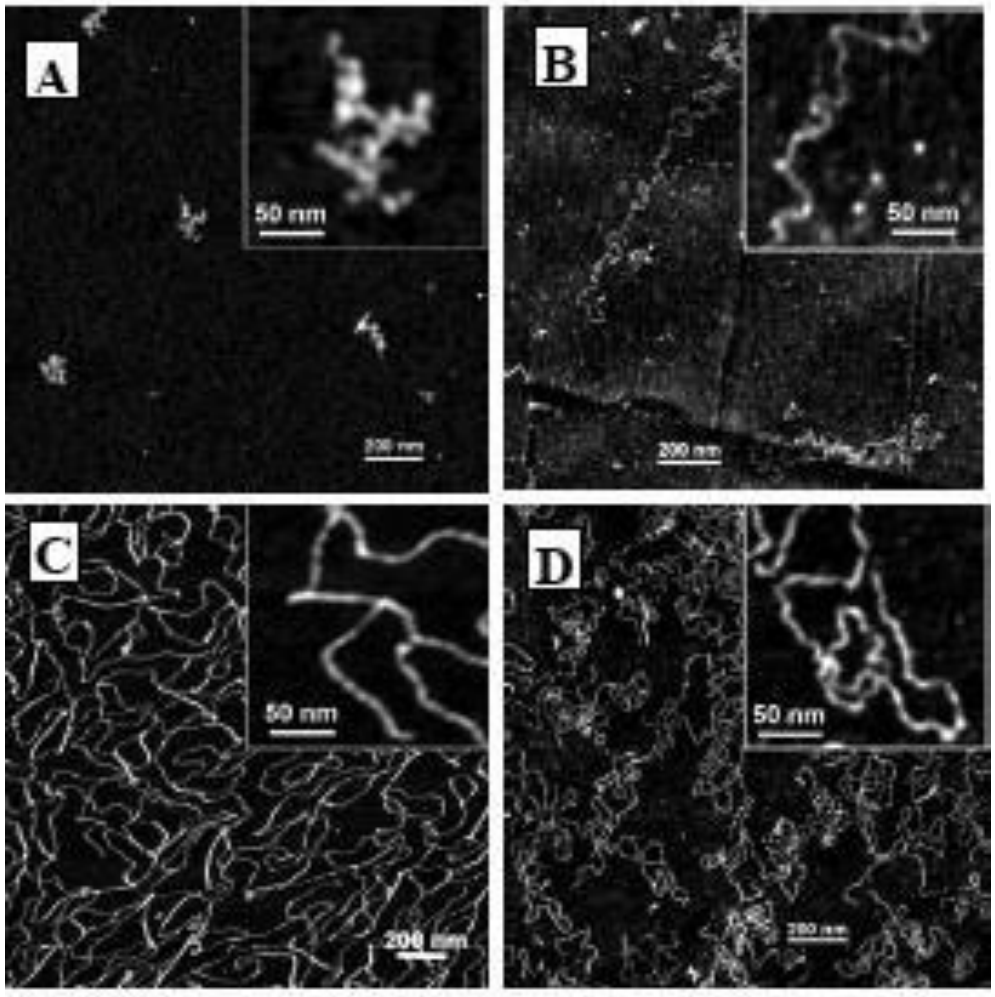


- Chem. structure: $K_2O \cdot Al_2O_3 \cdot SiO_2$
- Hydrophilic surface
- Easy to be modified by chemical synthesis
- pKa ~ 3, physiological pH → negative surface charge
- Mica = silicate, hydrated SiO_2 (~ Si-OH)

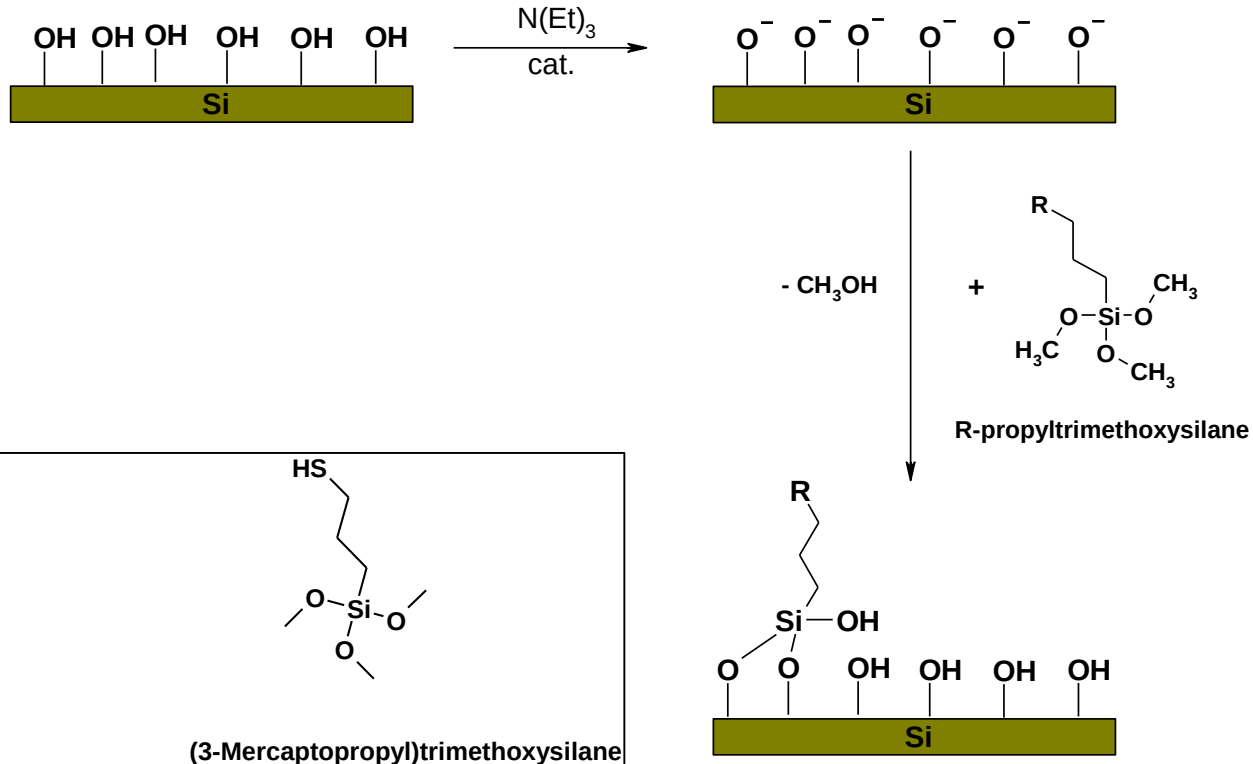
DNA on graphite (HOPG)



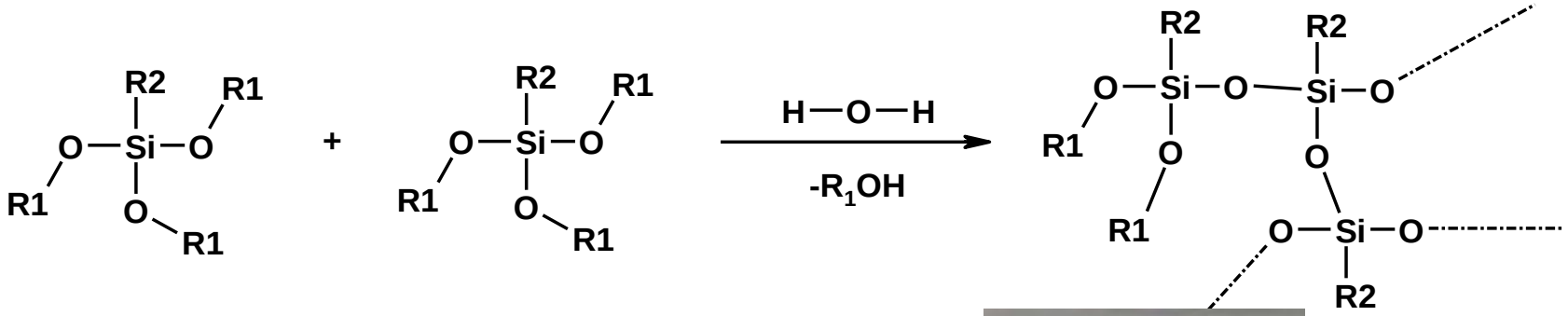
- Low roughness
- High hydrophobicity



Examples of **alkylsiloxanes**
Silanization process



Silanization process, practical complications



Silanization
Hydrophobization

- Especially with **APTES** during liquid silanization
- Even vapors of water can cause this effect
- Fixation for **optical** microscopy – **expected** factor
- In contrary – in fixation for **AFM** – very **disturbing**
- Solution:
  - silanization in **vapours** under **vacuum** (i.e. in desiccators)
  - **mono**alkoxysilanes – can not polymerize

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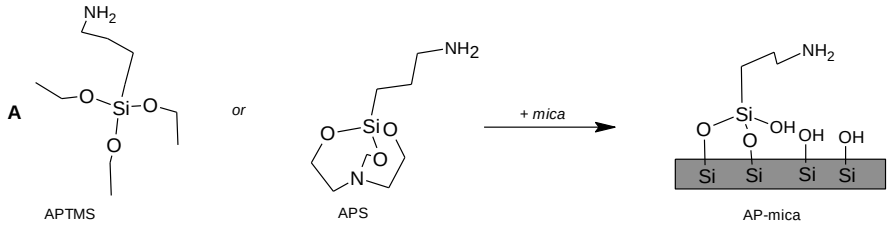
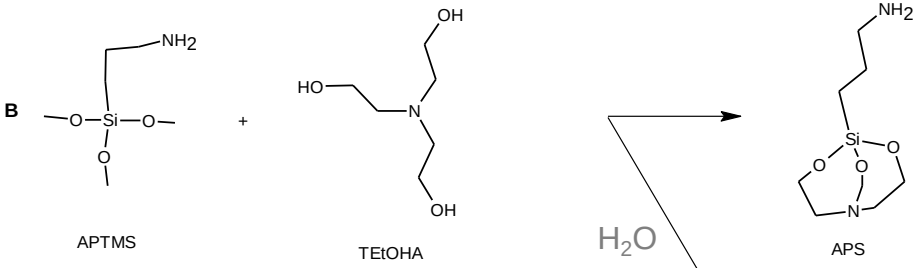
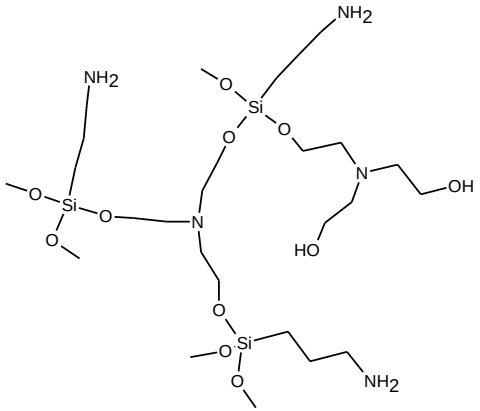
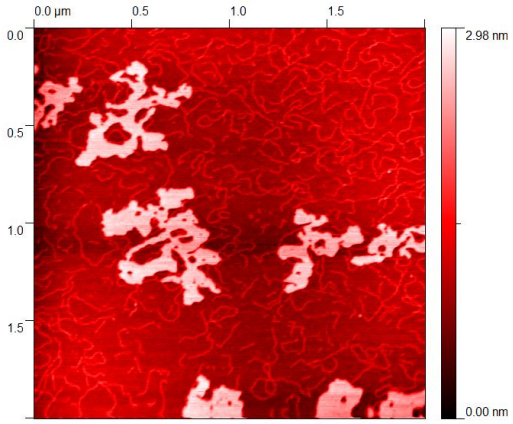
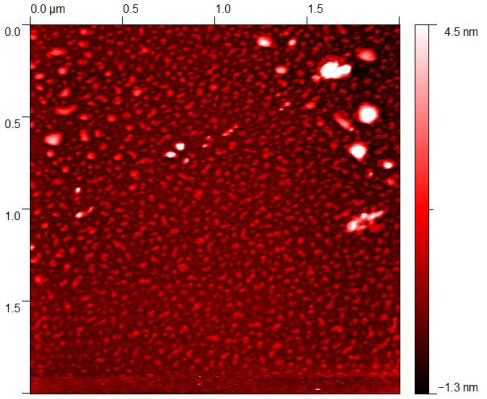
Reactivity of **aminosiloxanes**







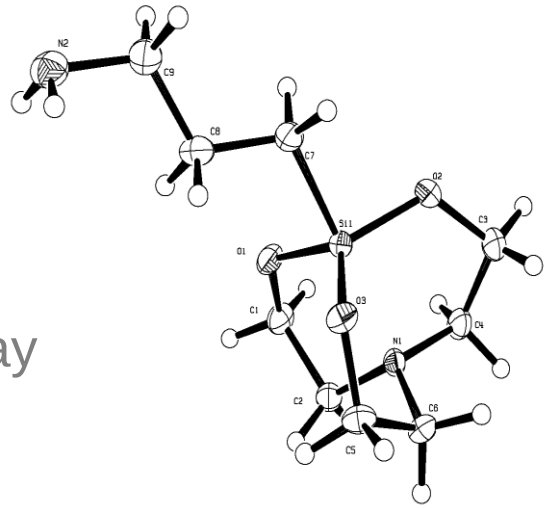


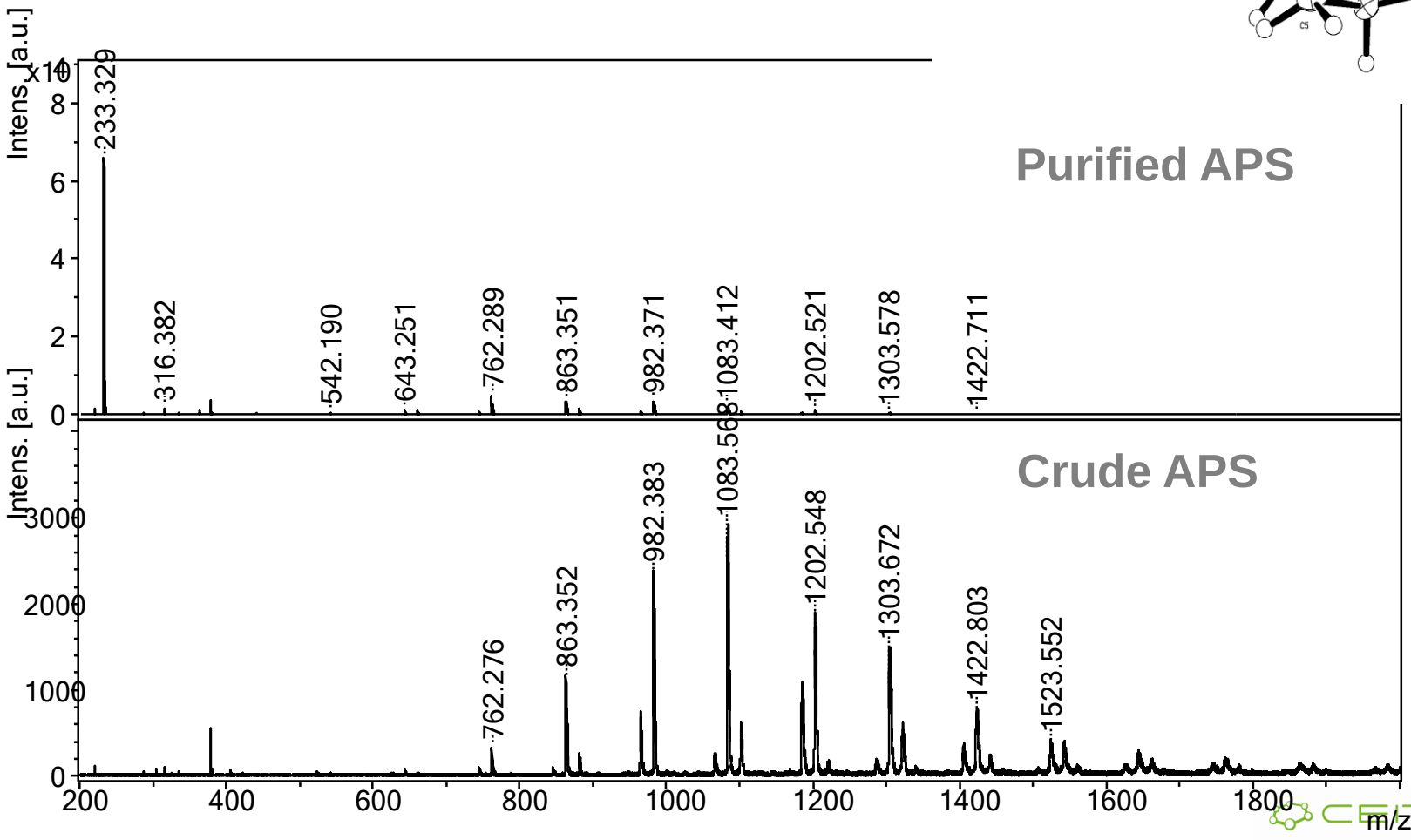


Self-polymerization

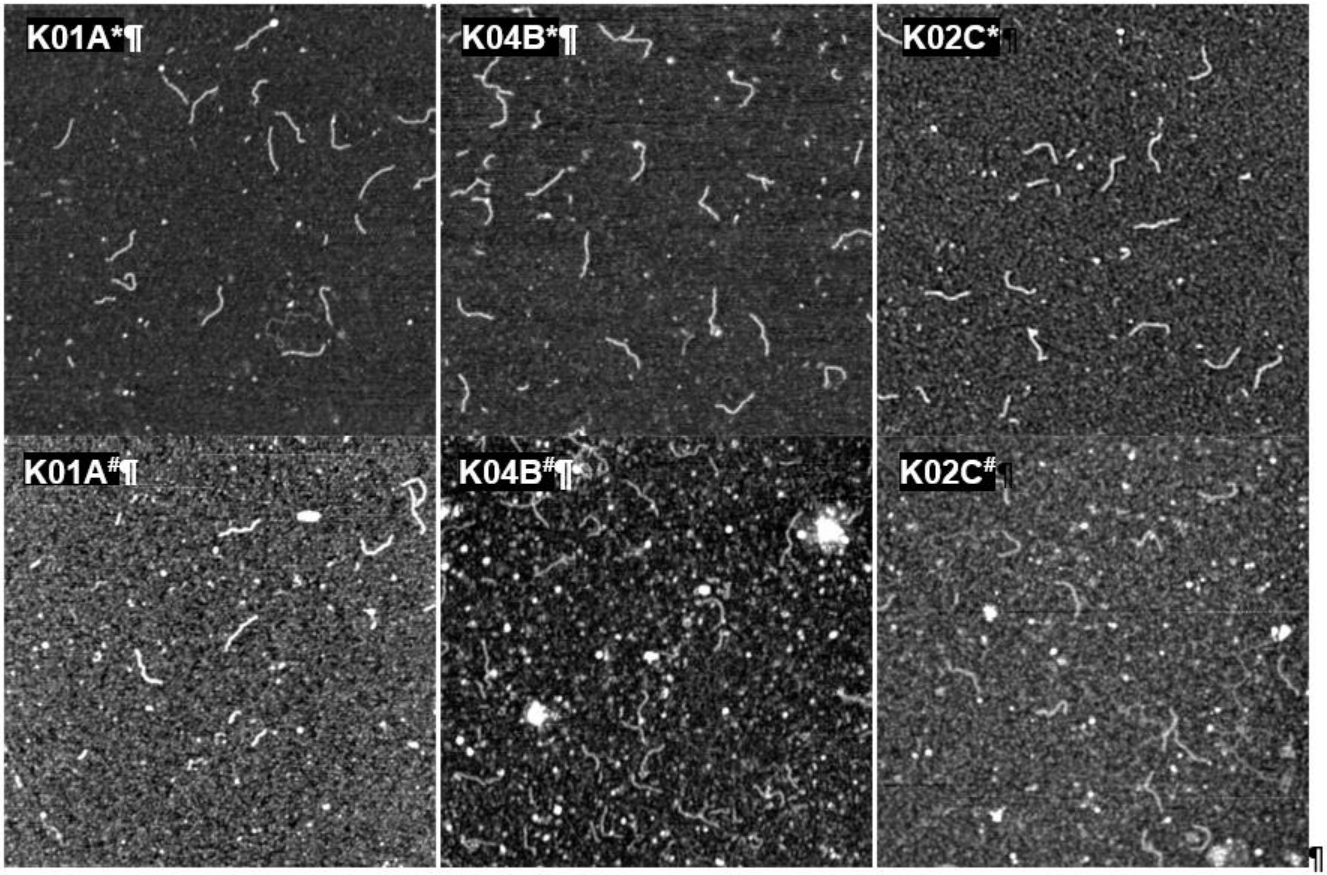
APS purification

- Reacts with many solid phases
- Purification by solvent extraction and crystallization
- Structure analysis by MS-ESi, MALDI-TOF and X-Ray





APS use for DNA imaging



Purified APS

Crude APS

- APS aquatic solution stable in water
- One step, short time applicability
- Low roughess of mica surfaces modified with purified APS

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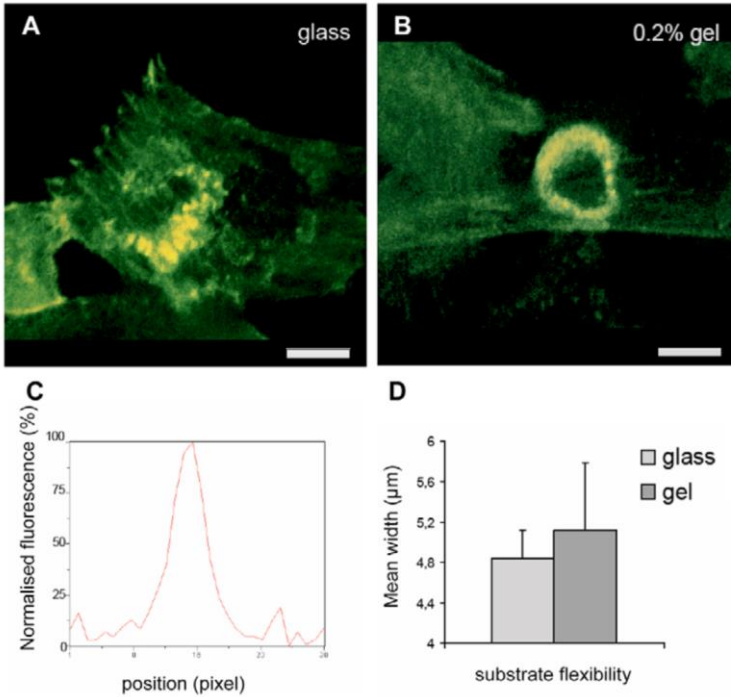
Effect of substrate stiffness on mechanical and morphological properties of fibroblasts

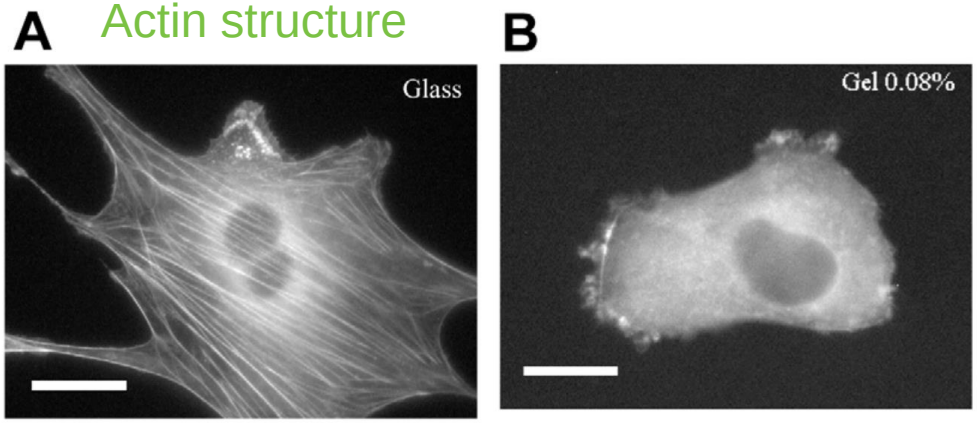
Extracellular matrix rigidity – plays a role:

- Locomotion
- Groth control
- Differenciation
- Phagocytosis
- Etc.

Cell height



Actin structure

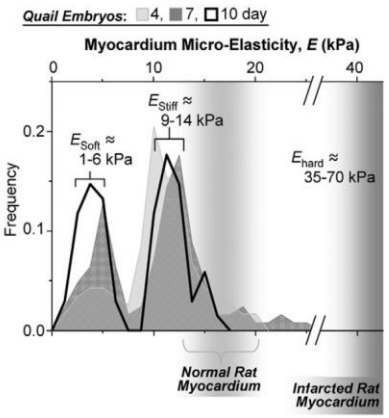


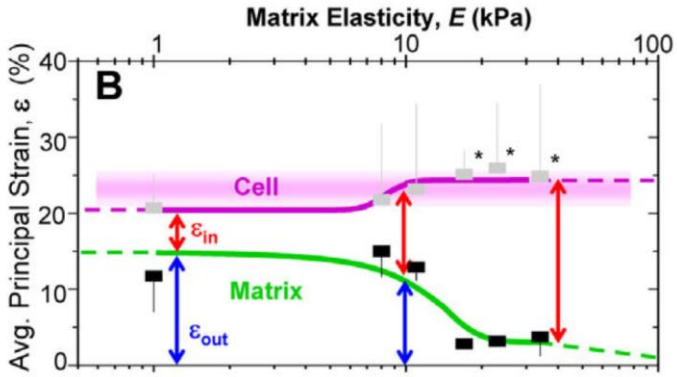
O. Collin, et al. “Spatiotemporal dynamics of actin-rich adhesion microdomains: influence of substrate flexibility,” *J. Cell. Sci.*, vol. 119, 1914–1925, 2006.

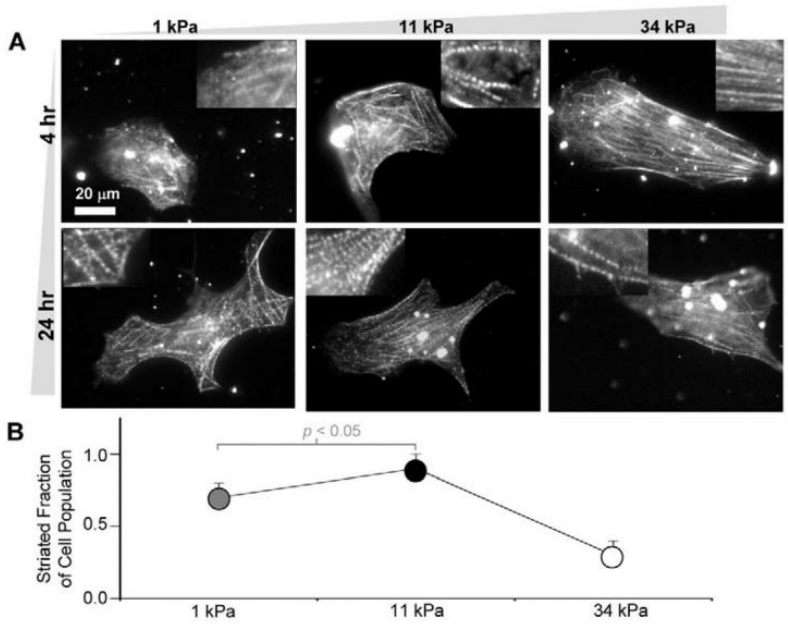
Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating

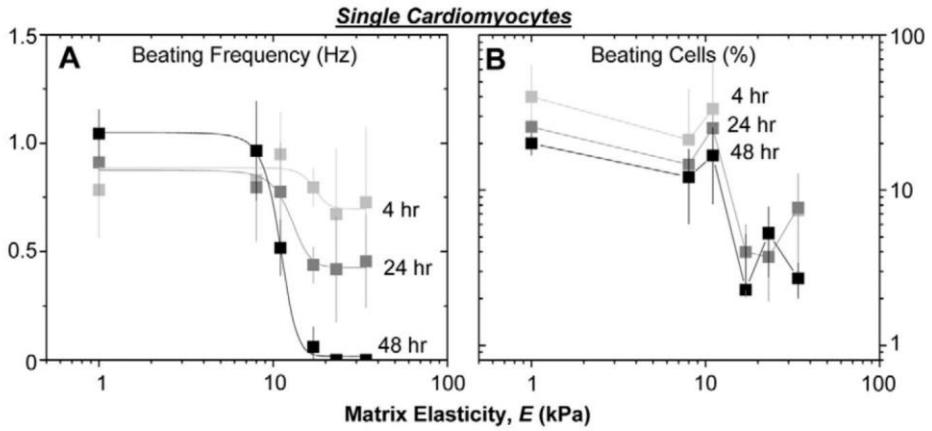
Polyacrylamide polymer

Acrylamide plus bisacrylamide crosslinking with different ratio







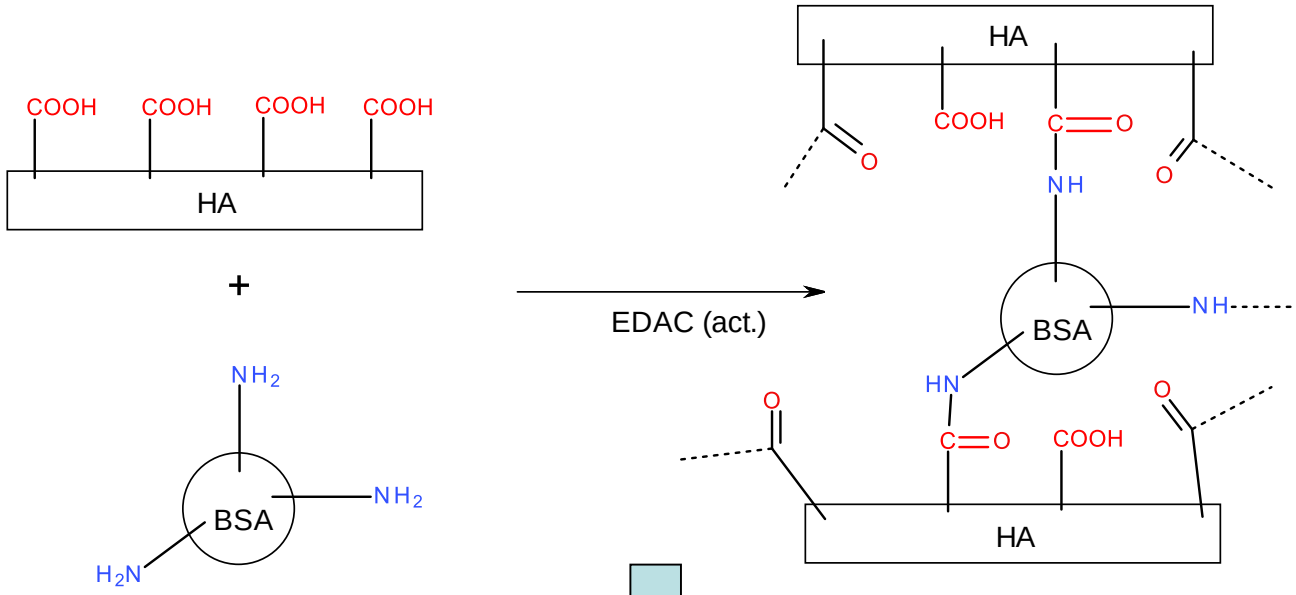


A.J. Engler, et al. “Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating” *J Cell Sci. 2008, 121, 3794.*

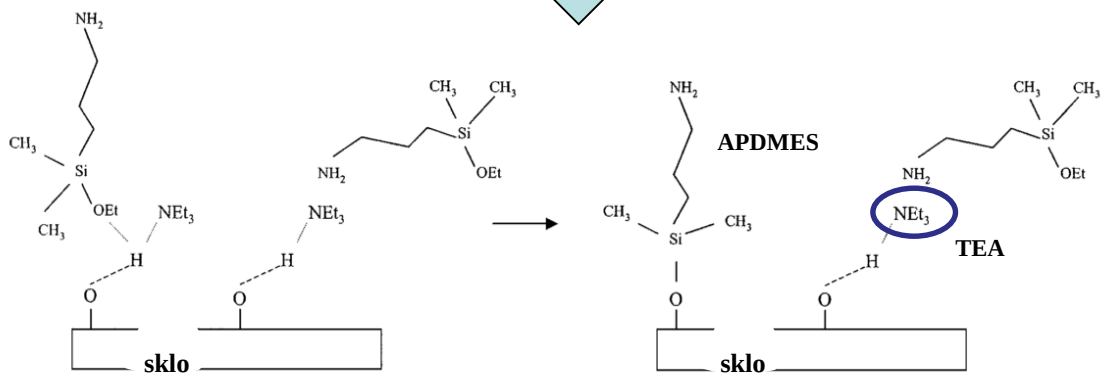
Protein crosslinking

2 types of gel:

GHB = hyaluronan - BSA
GHBG = hyaluronan – BSA - gelatin



In-situ covalent immobilization



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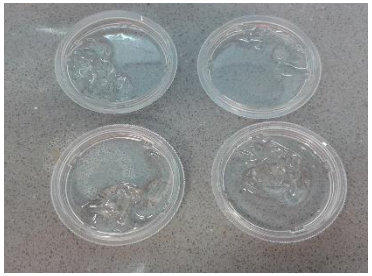
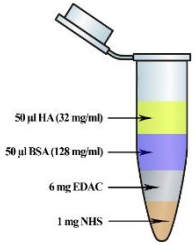
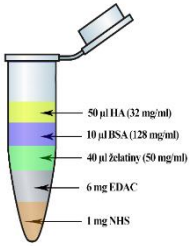
Practical aspects

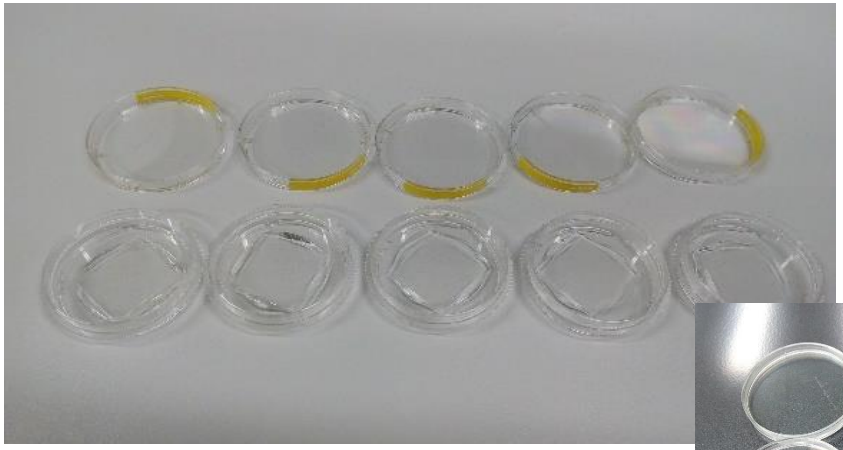


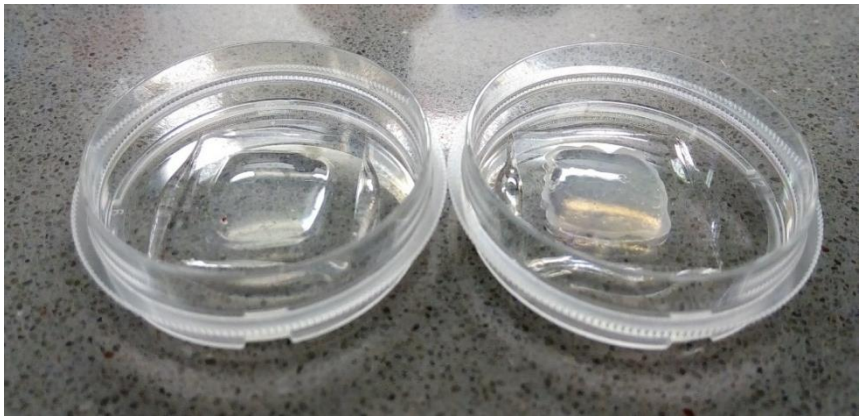
PAA (polyacrylamide) **gel** – poorly adherent to the dish, instable and very sticky in aquatic solutions





GHB and GHBG gels
immobilized on microscopic slides





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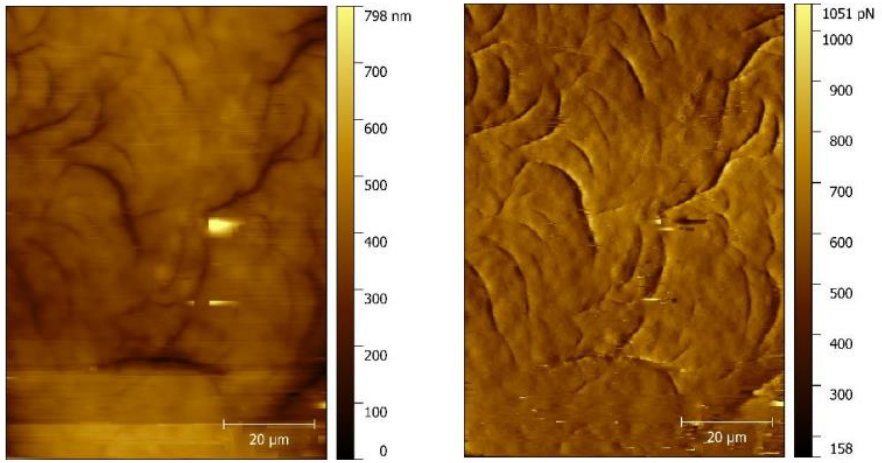
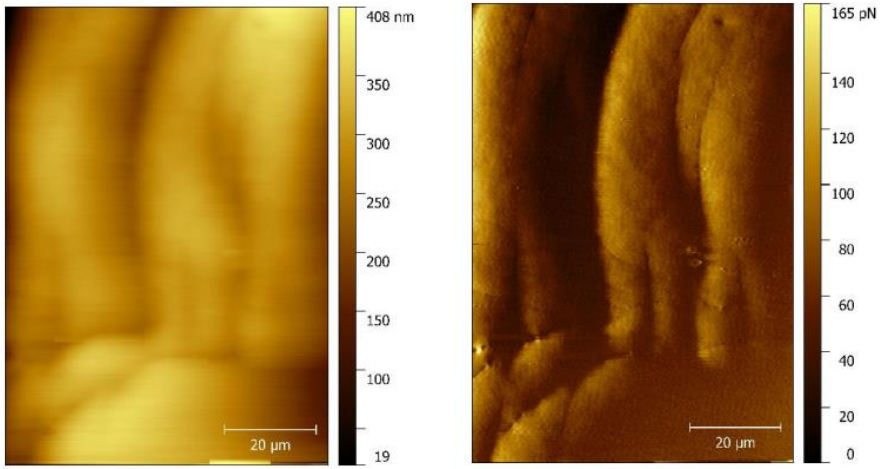
Surface roughness



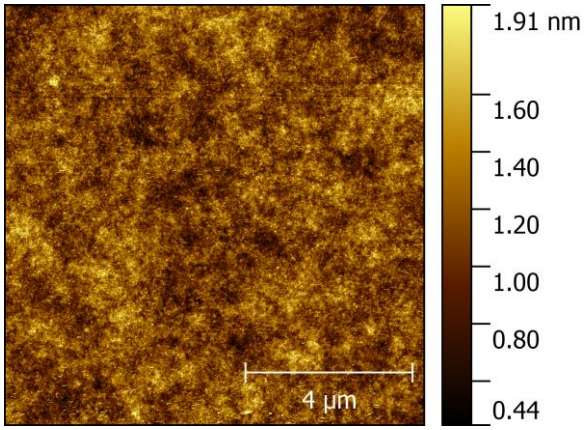
GHB gel

Rms = 57.2 nm (aver. rough.)



GHBG gel

Rms = 39.7 nm (aver. rough.)

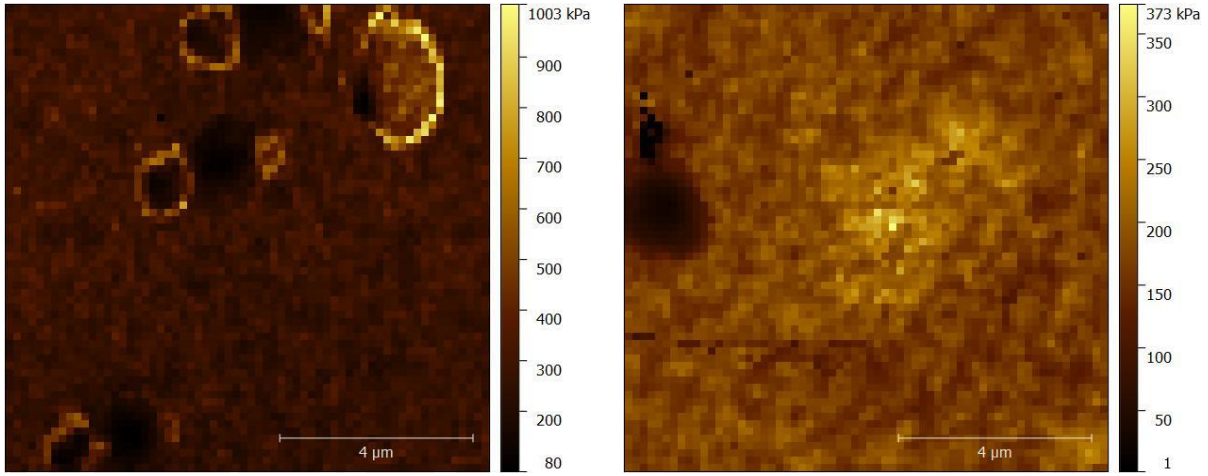


Microscopic glass

Rms (Sq): 228.8 pm

Surface stiffness

RT **GHB gel** **37ºC**



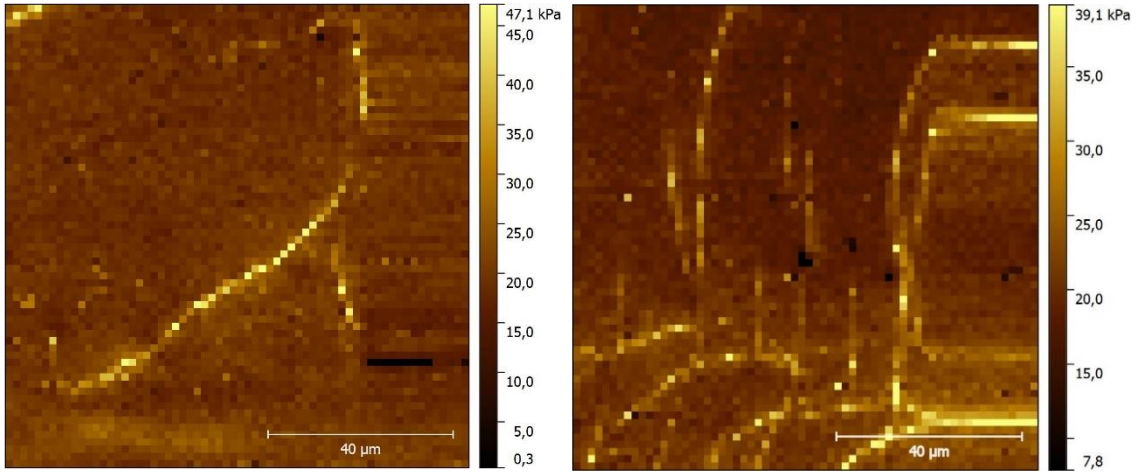
E_{aver}

285.2 kPa 135.9 kPa

Glass

E_{aver} ~ 70 MPa

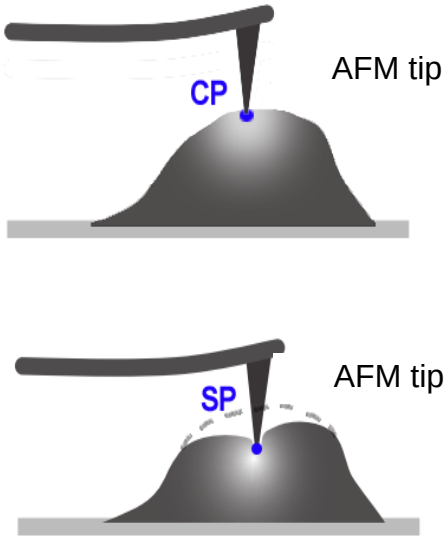
RT **GHBZ gel** **37ºC**

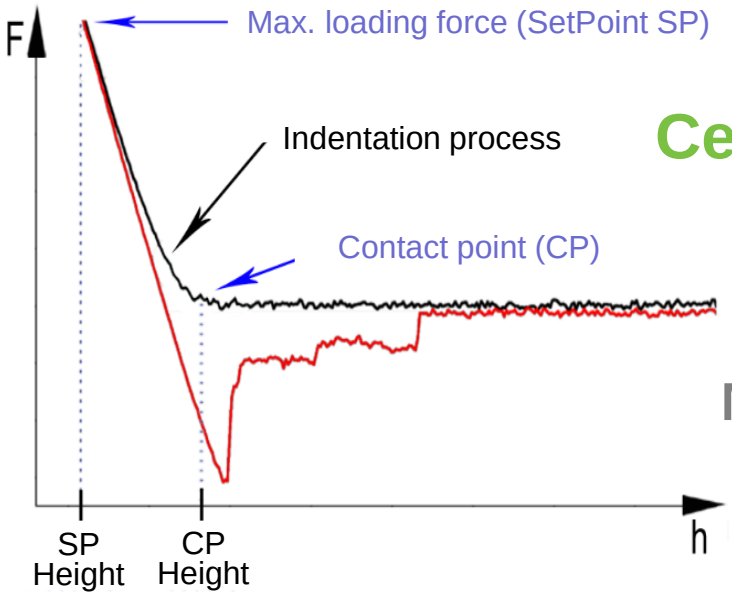


E_{aver}

20.2 kPa 19.9 kPa

CEITEC

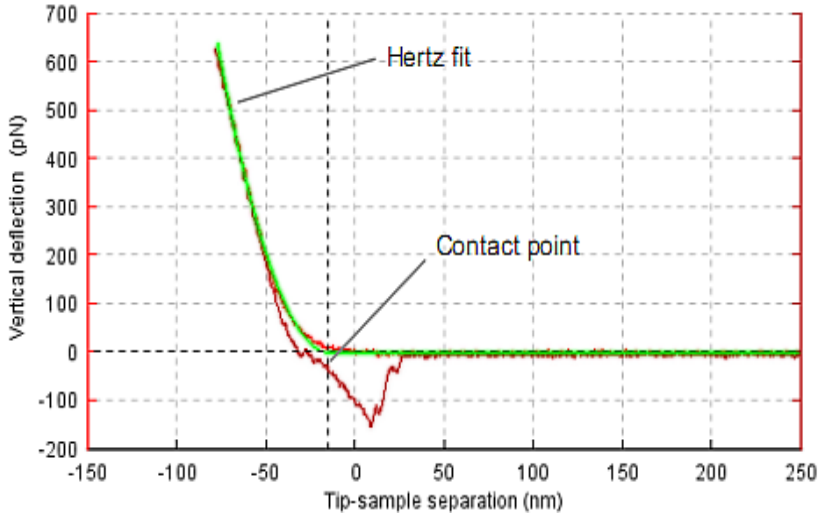


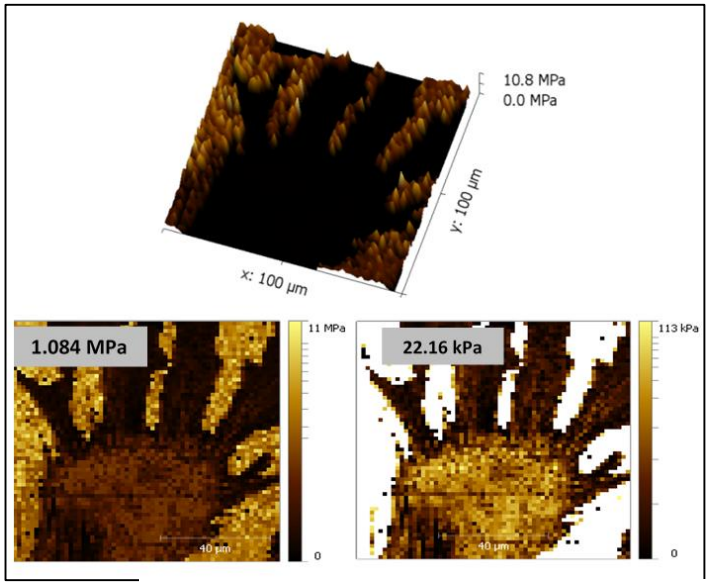


Cell Stiffness by AFM nanoindentation

Mouse fibroblast cells

GHB and GHBG gels
immobilized on microscopic slides





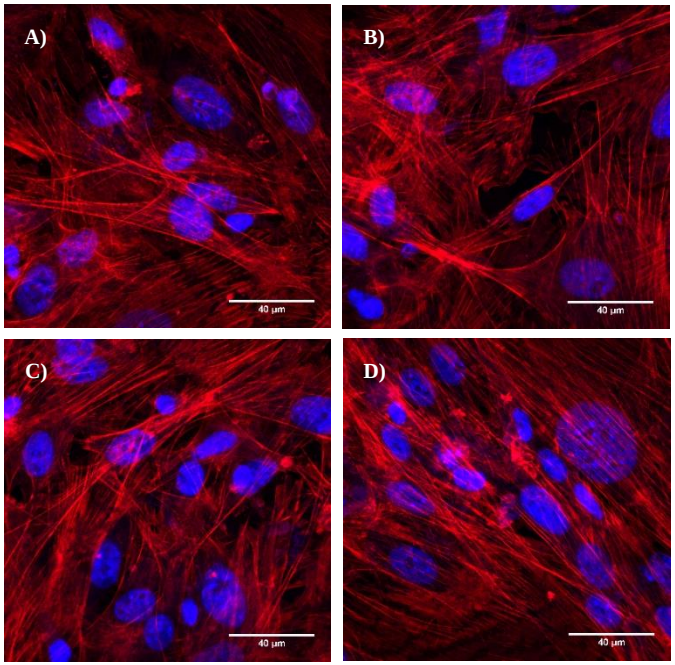
Mouse fibroblast on glass

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

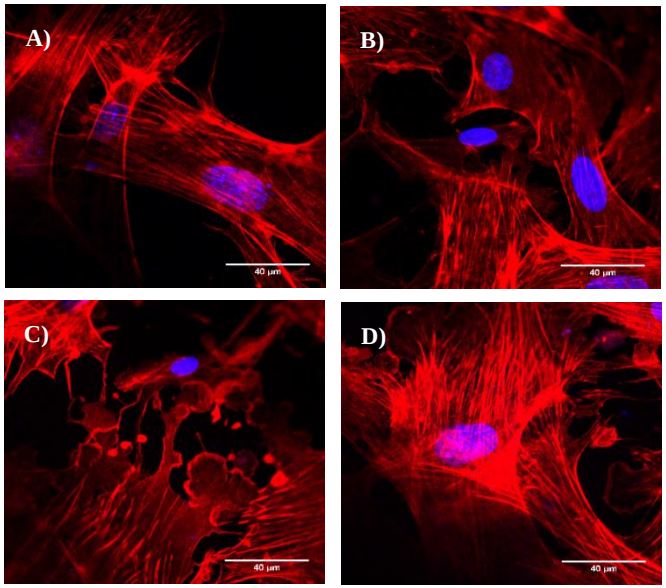
Cell morphology - substrate stiffness

Confocal microscopy

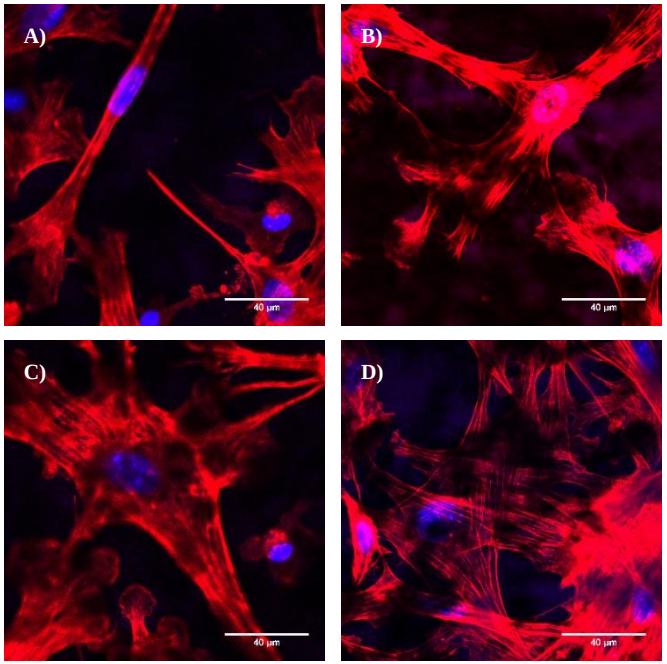
- DAPI nucleus staining
- Actin staining by Phalloidin



Glass 70 MPa



GHBG 19.9 kPa



GHB 135.9 kPa

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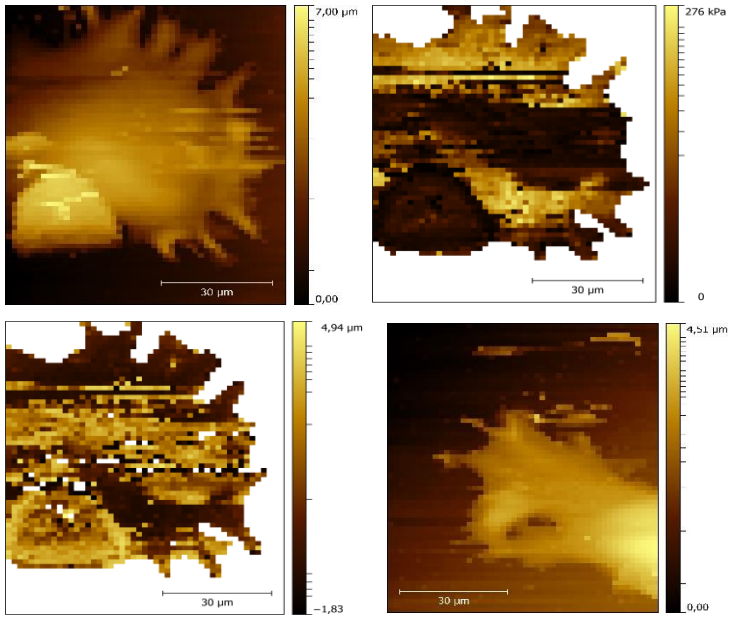
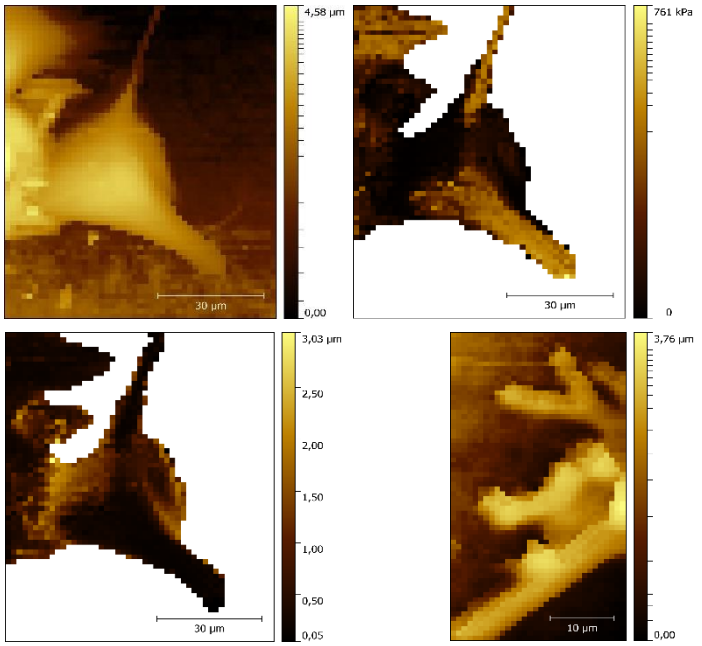
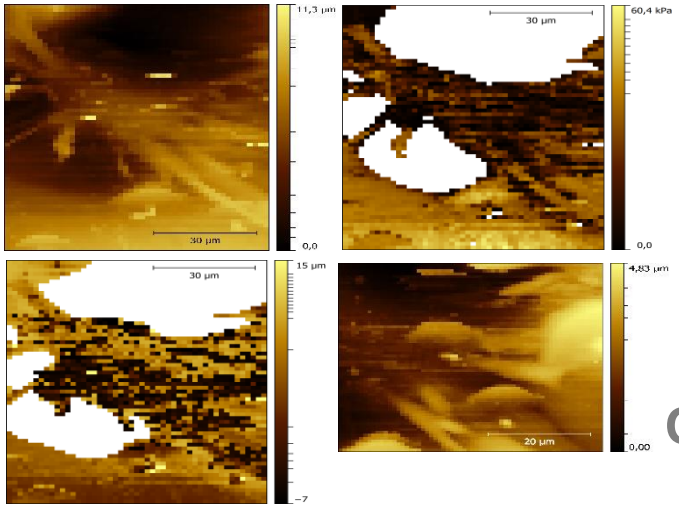
Effect of substrate stiffness on filopodia / lamellipodia structure



Glass 70 MPa



GHB 135.9 kPa



GHBG 19.9 kPa

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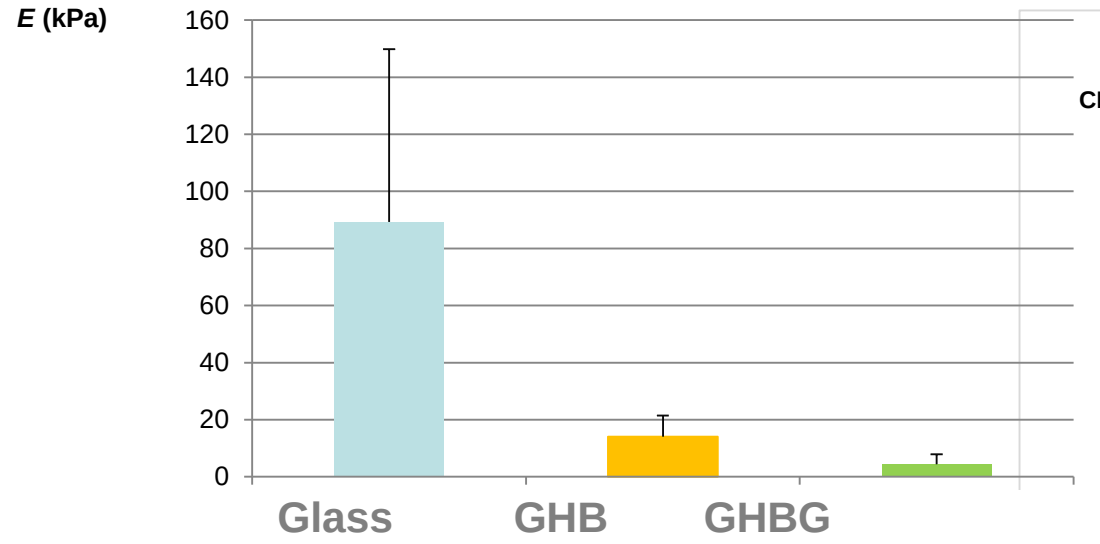
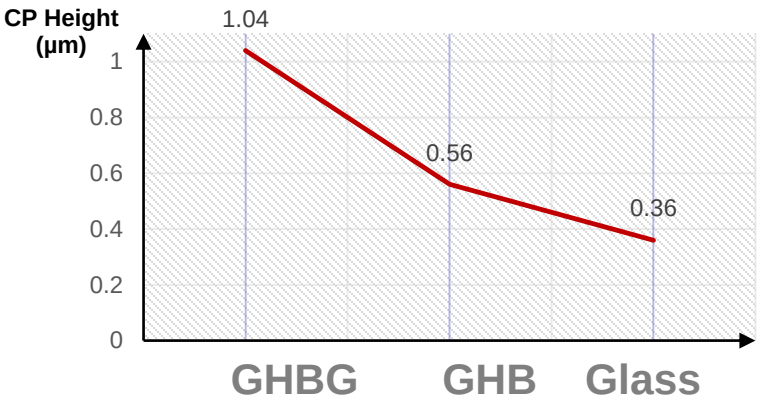
Substrate stiffness vs. cell stiffness and height





Gels based on crosslinked proteins and hyaluronan

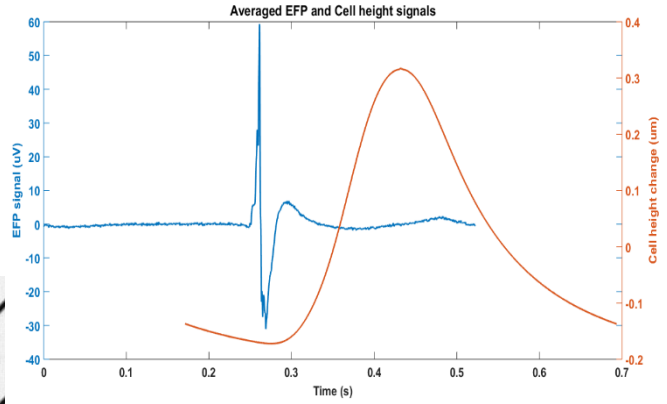
- Mechanical properties similar to tissues
- Biocompatibility, non-toxic for cells
- Keeps adhesivity and mechanical properties (long term)
- Transparent – compatible with optical microscopies
- Adhesive for cells
- Outlook: application for cardiomyocytes (single cells, EBs)

Samples

5. Combination with other methods

- **AFM+MEA (microelectrode array)**

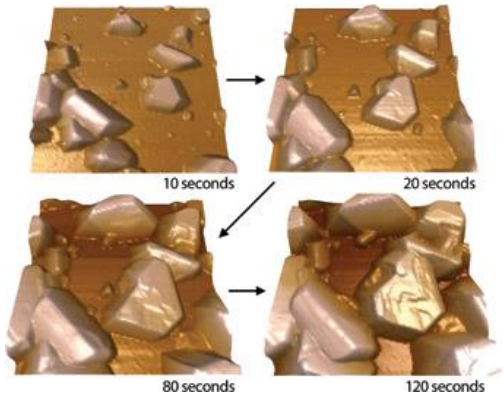
→ **Mechanical&electrical prop. of CMCs**



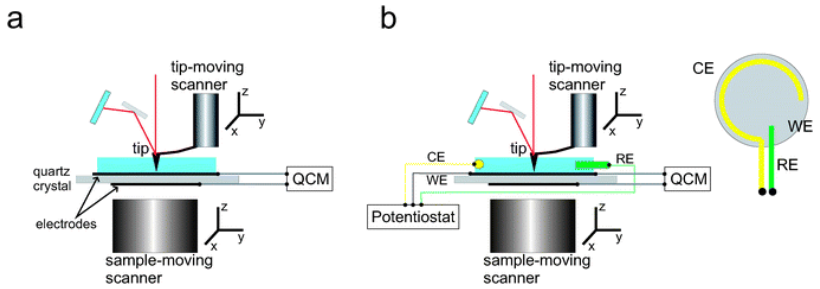
Img & graph by Guido Caluori

- **AFM+electrochemistry (*in-situ*)**

→ **Combined study of electrochem. processes**



asylumresearch.com



Nanoscale, 2009, 1, 40-49

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Jorge Olivier De La Cruz

IST Austria

Marcal Gallemi
Eva Benkova

Institute of Physics

Irena Kratochvilova
Martin Golan

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

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Thank you for your attention!

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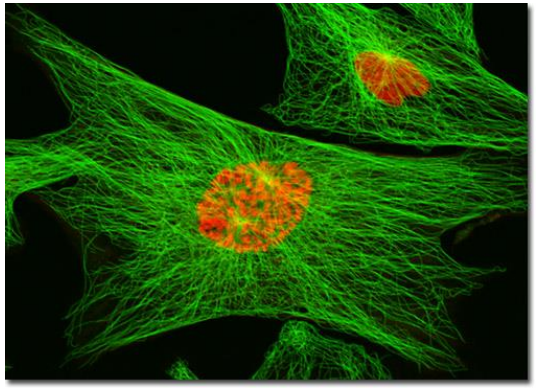
Projects

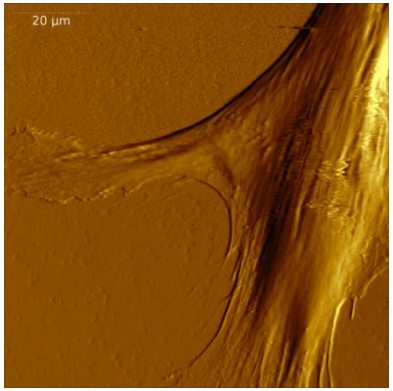
Nanomechanical mapping

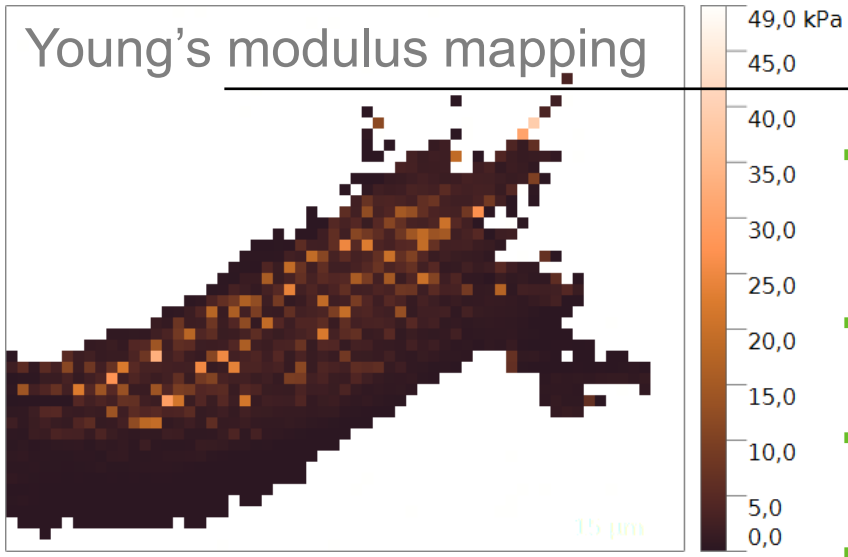
Optical microscopy

Confocal microscopy



AFM



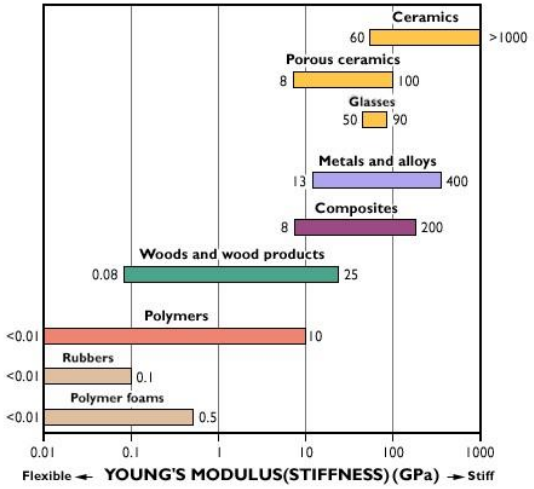


Motivation

Why to quantify elasticity of (living) objects?

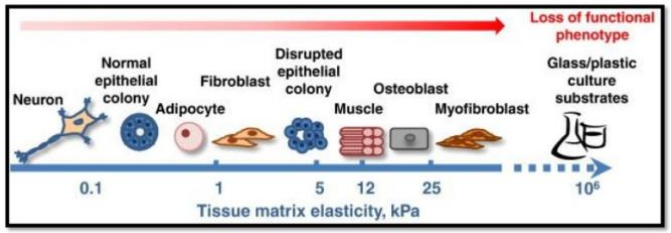
- **Stiffness** (Young's modulus) **mapping**
  → stiffness = basic parameter of any material
- **Elasticity-phenotype** relation ship
- **Mechanobiological** characterization
- **Driving of instrument** properties (QNM, QI)

Young's modulus of materials



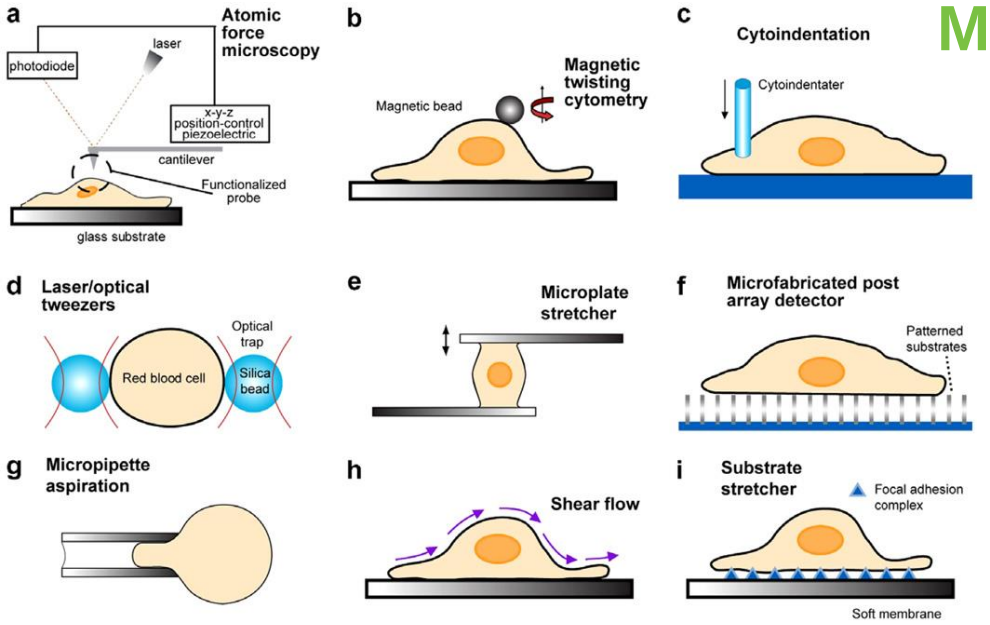
Tissue's Young Modulus

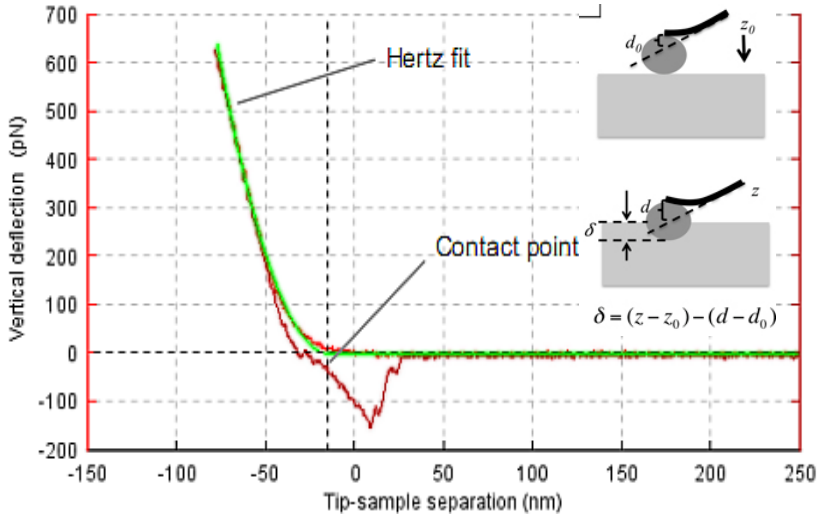
Tissue elastic modulus (E) is given by the resistance offered by the tissues to deformation effects, i.e. the tissue stiffness.



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

Methods for YM measurement





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

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

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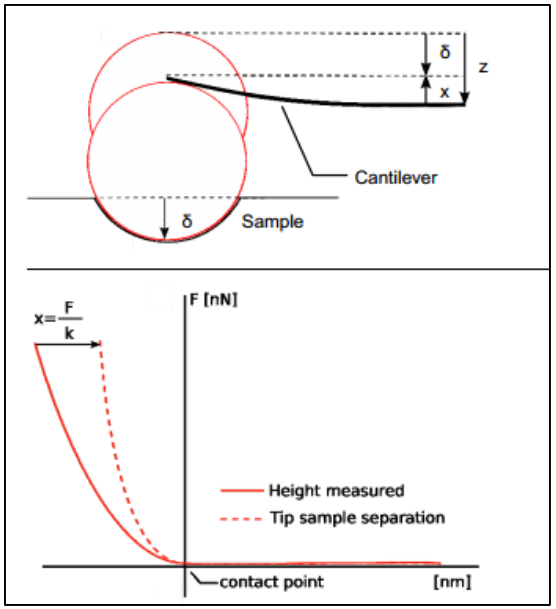
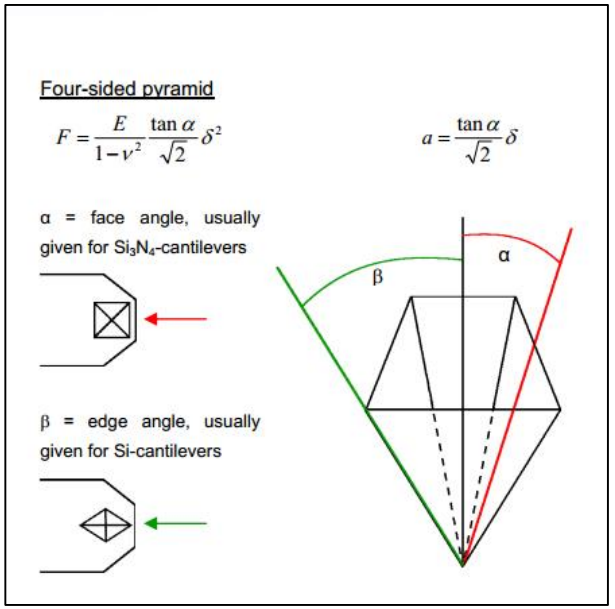
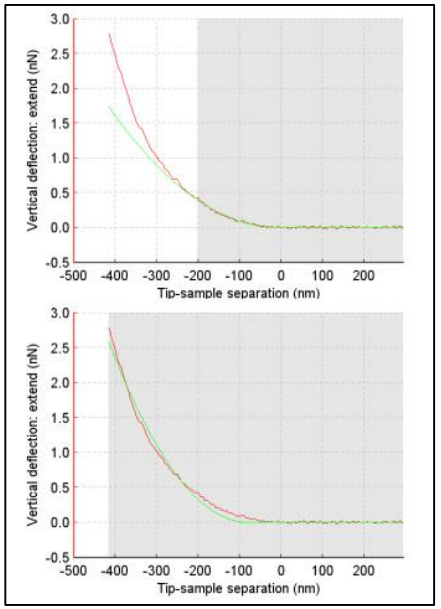
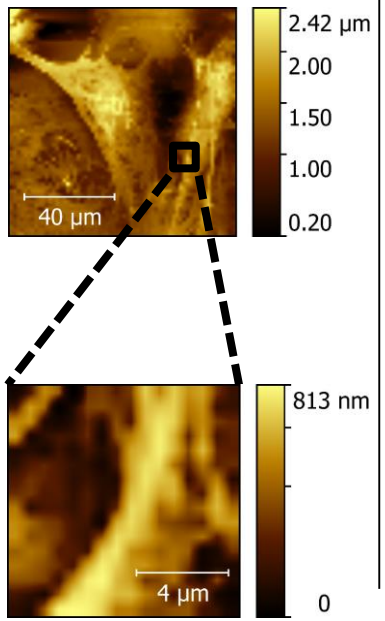
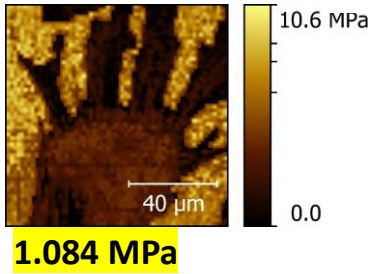
Force distance curve analysis

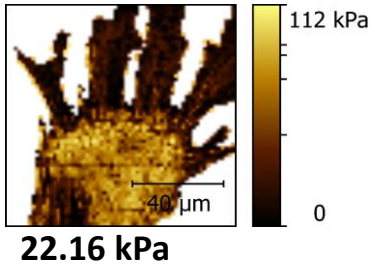


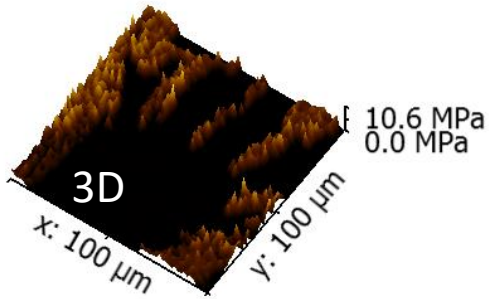








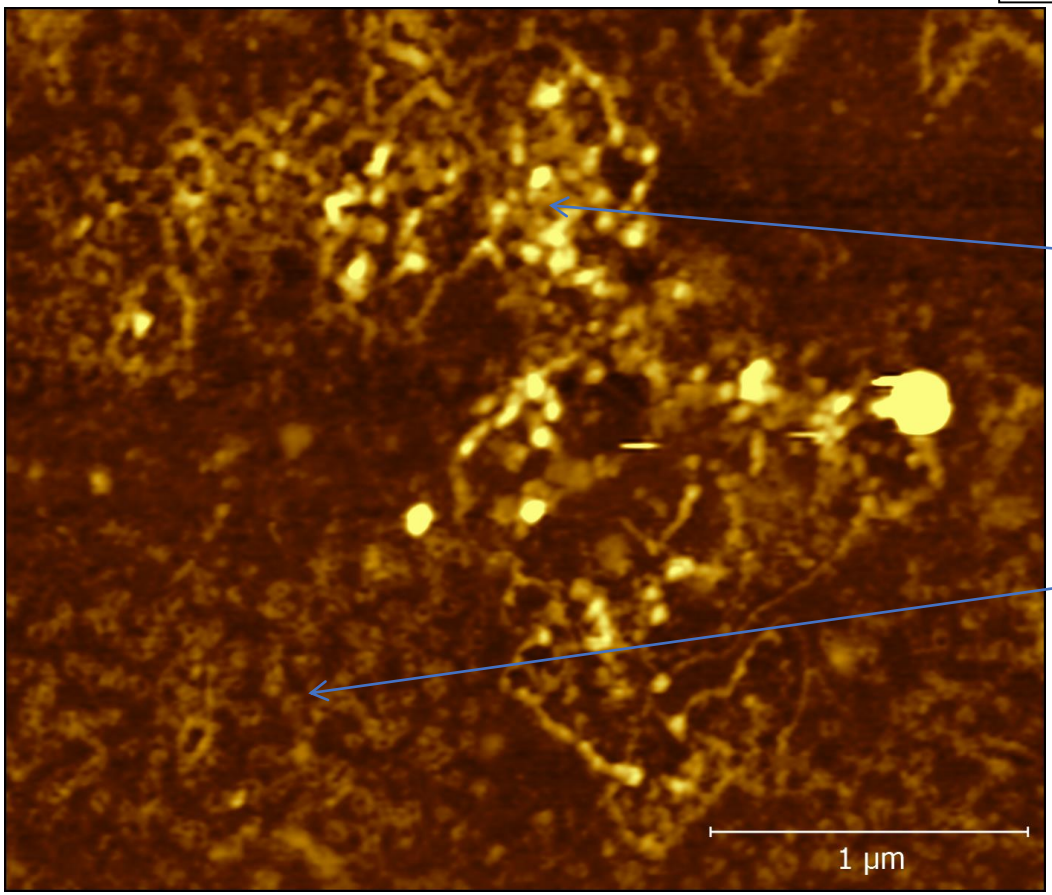


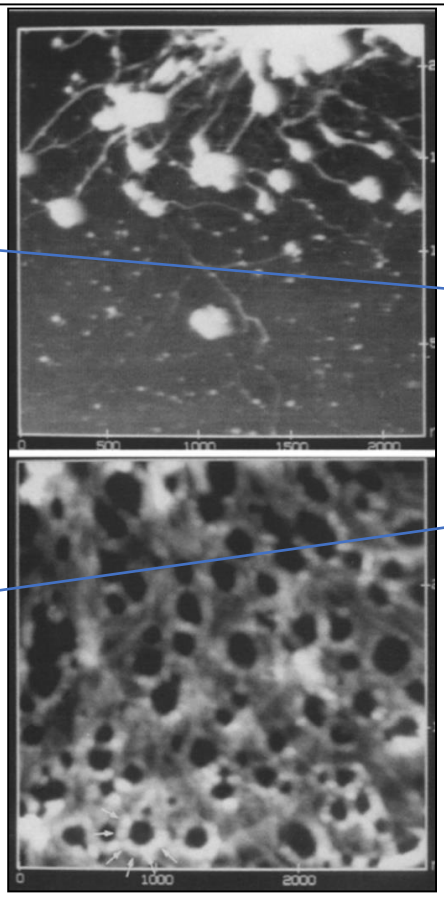


Chromation-BAF complex

on pLL-modified glass

Fig. 5A–C. High resolution AFM images of **A** smooth fibers with interspersed ellipsoid-shaped nodules (the interspersed nodules are 130 nm in width and 15 nm in height), **B** coiled nodular fibers (see *arrows* for example) 100 nm in width and 15 nm in height, and **C** extended smooth fibers the smallest of which are 25 nm in width and 3 nm in height





Similar structures found by Allen et al. **(page 5)**

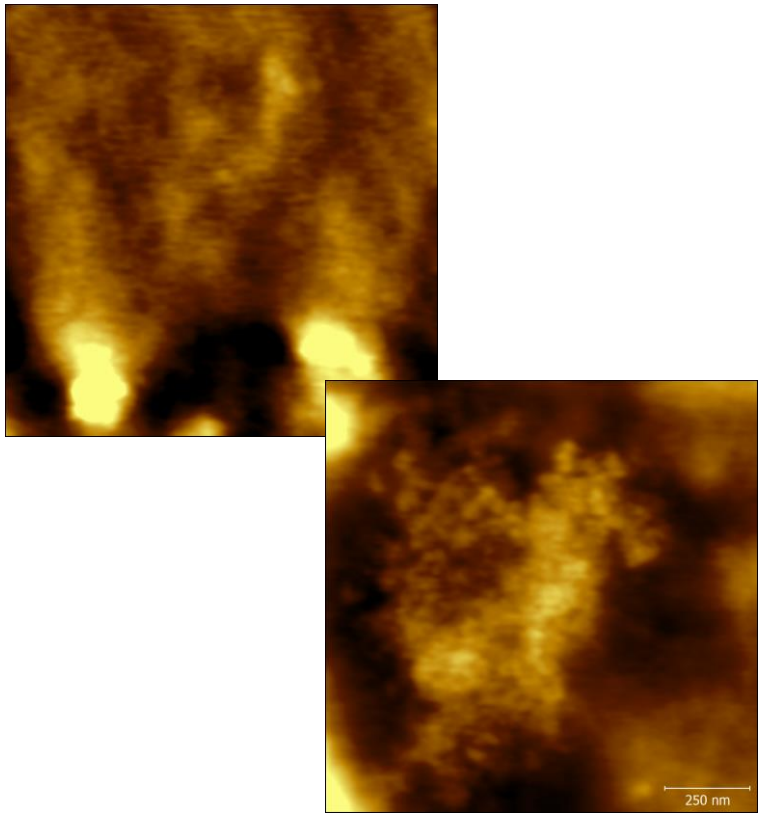
Smooth fibers with interspersed ellipsoids (ellipsoid size **60-90nm**)

Coiled nodular fibers (diameter 60-100nm, fiber width ~ 17nm, not corrected to tip geometry)

However, the complex is spread over the surface, not part of the complete chromatin piece

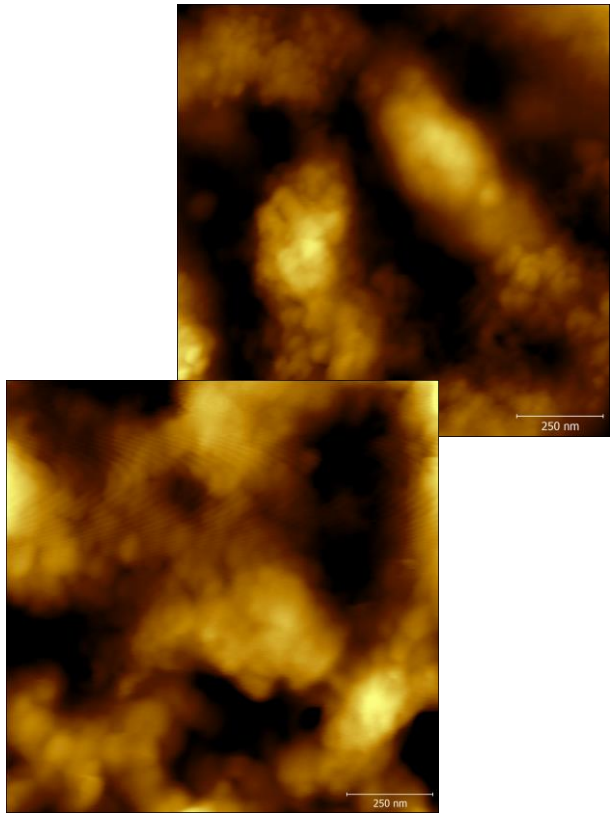
SUMMARY

Chromatin only
Tightly packed chains
(linear / curled)



+ BAF

Chromatin-BAF
Granular structure
(size of grains ~ 50-100nm)



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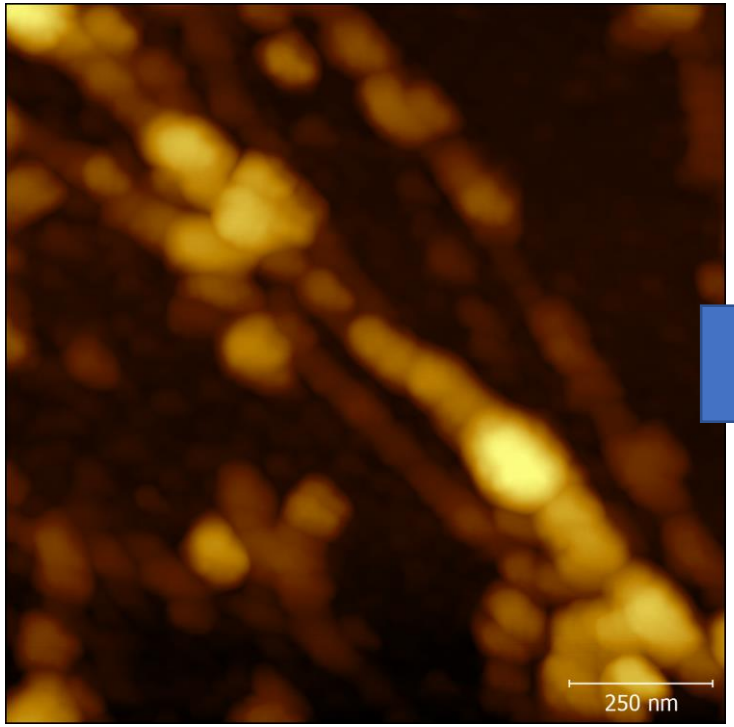
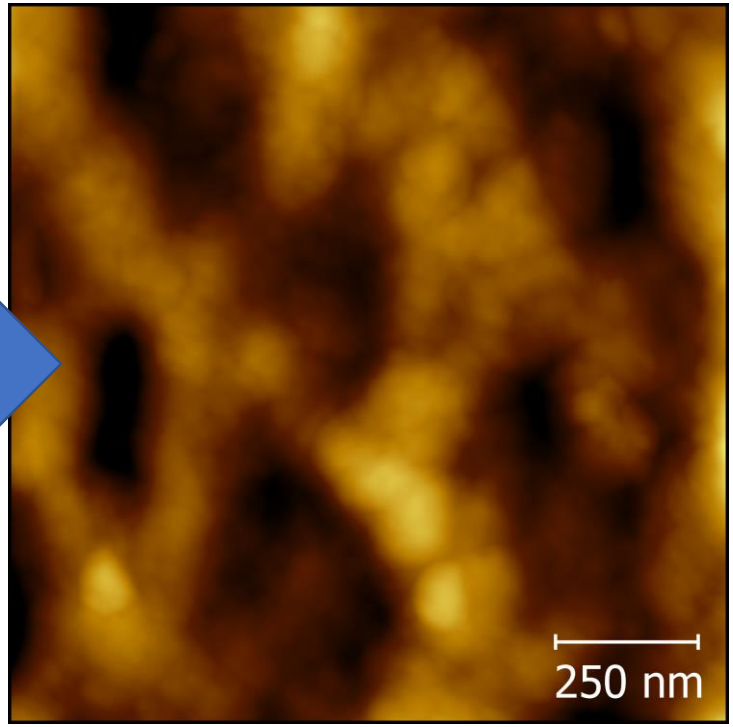
SUMMARY

Chromatin only
Chains composed of ellipsoids

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



+ BAF



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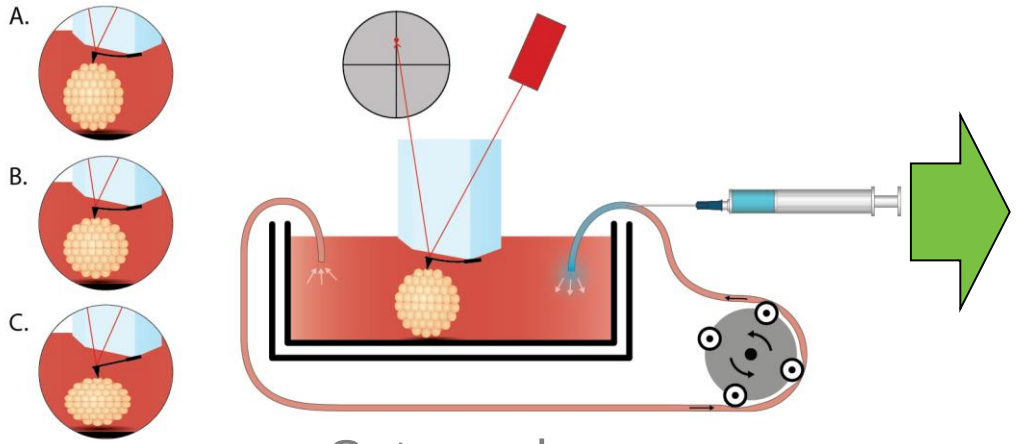
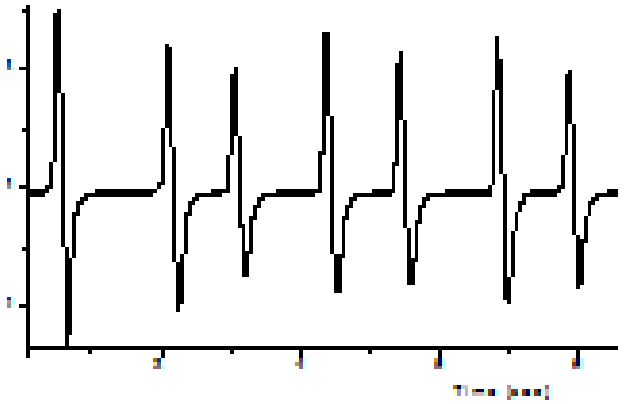
Biomechanical studies on cardiomyocytes

- Primary CMCs
- Embryonic bodies – iPS/HES cardiomyocytes
- Low noise ~ 10pN (~ 230 pm)
- Robust, low comp. requirements
- Possible combination with MEA
- Low throughput

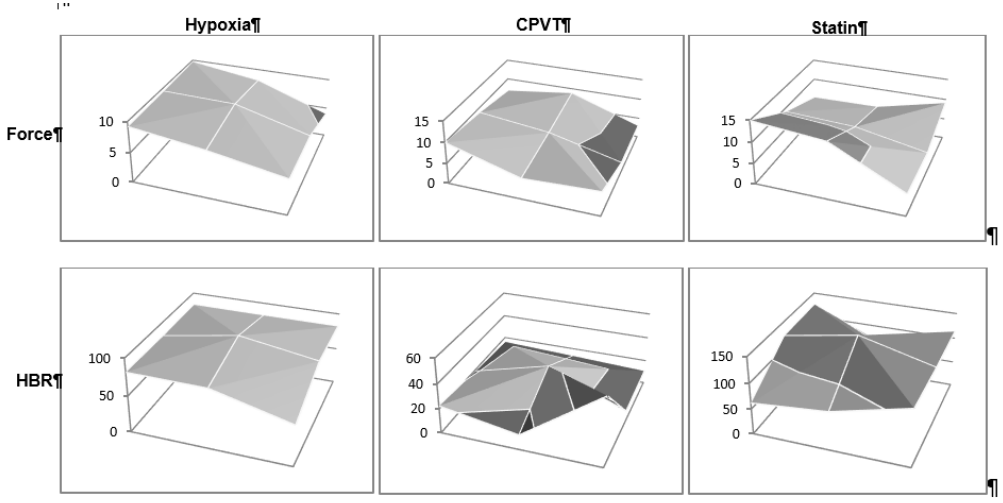


Setup scheme



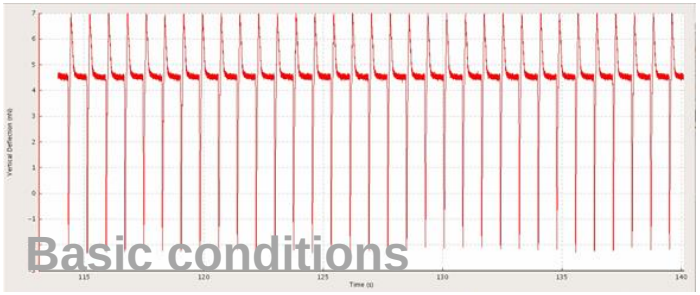
MCG = mechanocardiogram

Mapping of force/beat rate

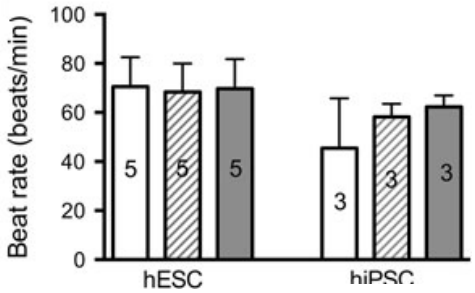


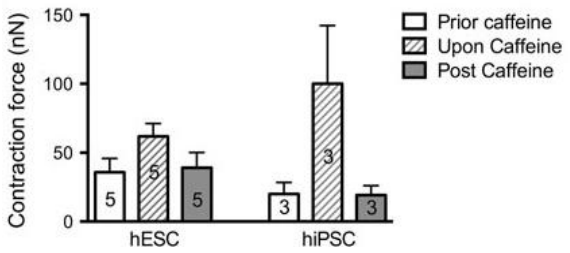
Beta-adrenergic receptors diseases

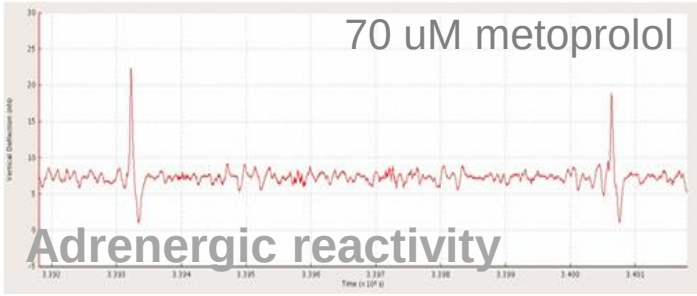
- Duchenne muscular dystrophy
- CPVT

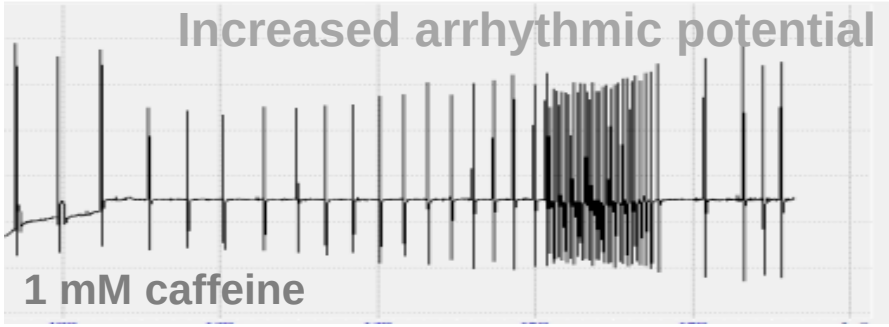


Drug testing studies









In cooperation with V. Rotrekl, M. Pesl, Med Fac, MU

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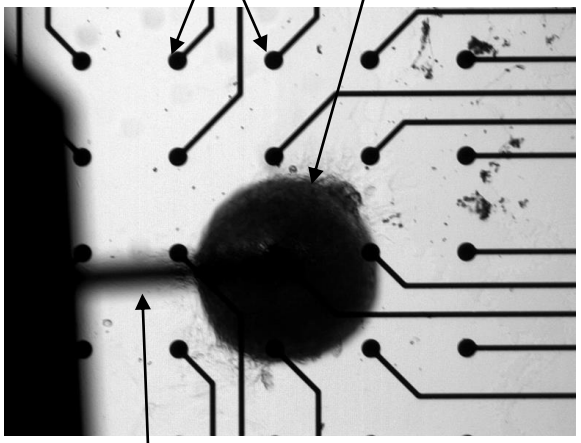
Mechanical & Electrical properties of CMCs

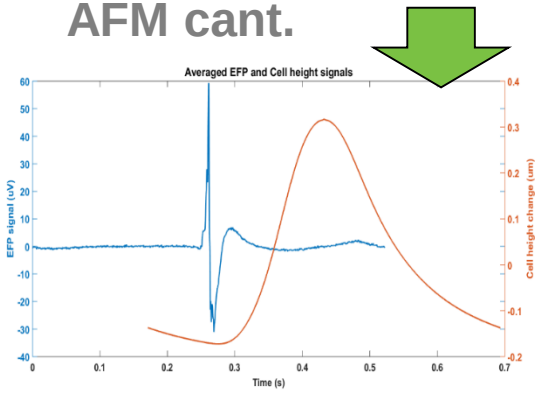
AFM & MEA/conductive tip

MEA field

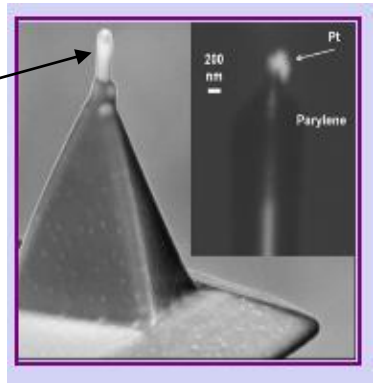
EB cluster

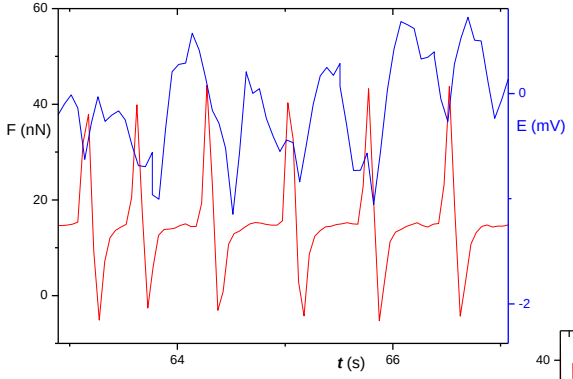


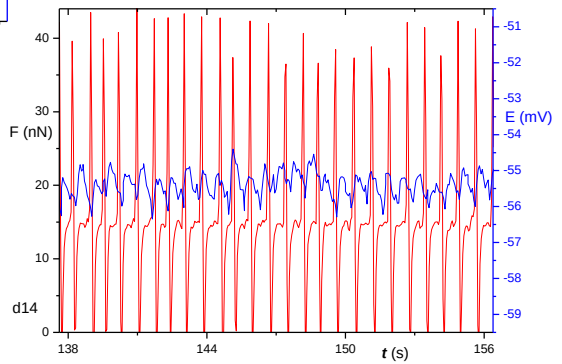
AFM cant.



Exposed End insulated Conductive Tip





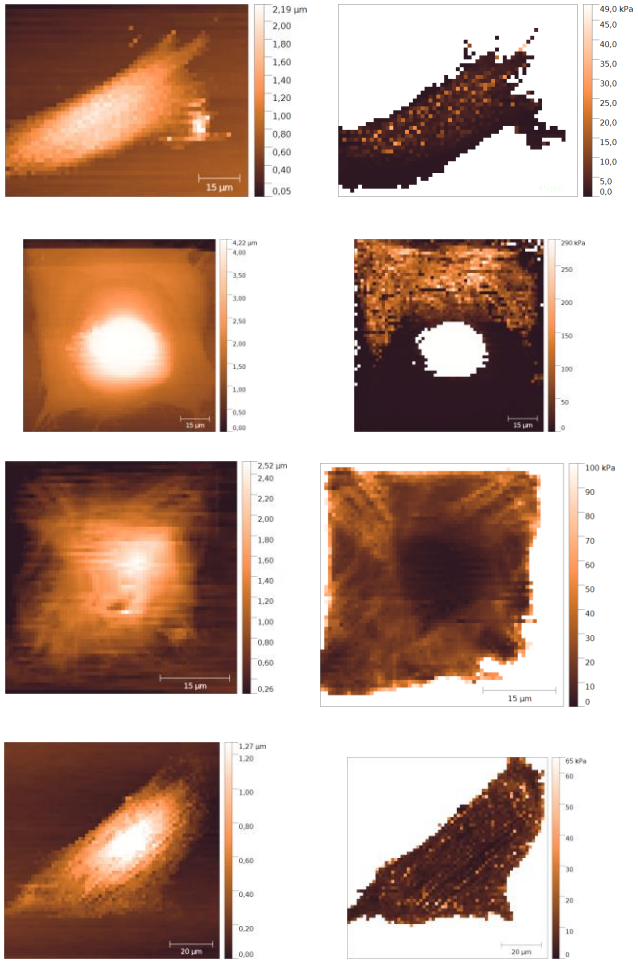


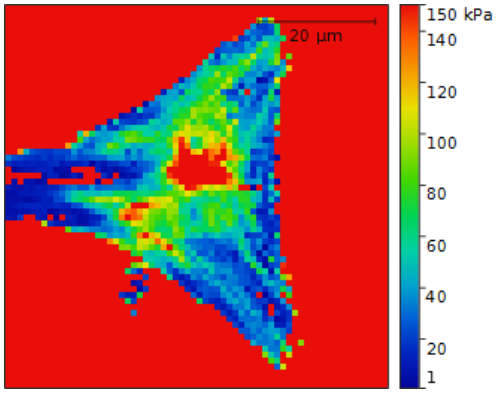
In cooperation with V. Rotrekl, M. Pesl, Med Fac, MU
Instrument interface: R. Raiteri, G. Caluori, Uni Genoa, Italy

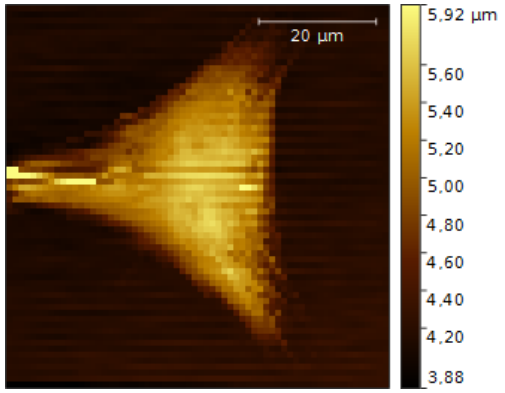
CEITEC

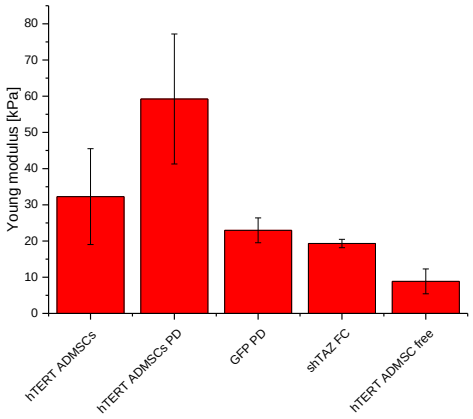
Use of AFM Force Mapping to study Integrin-Focal Adhesion (FA)

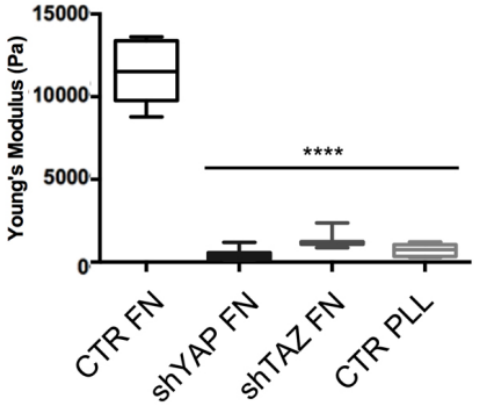
Height Young's mod.











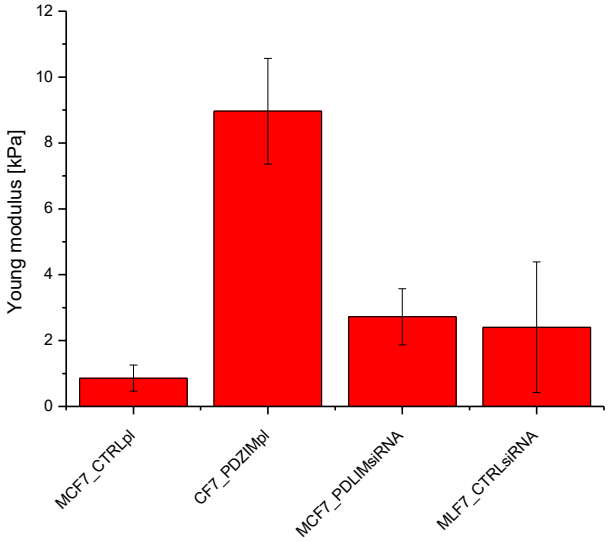
G. Nardone *et al.*, "YAP regulates cell mechanics by controlling focal adhesion assembly," *Nature Communications*, vol. 8, p. 15321, May 2017.

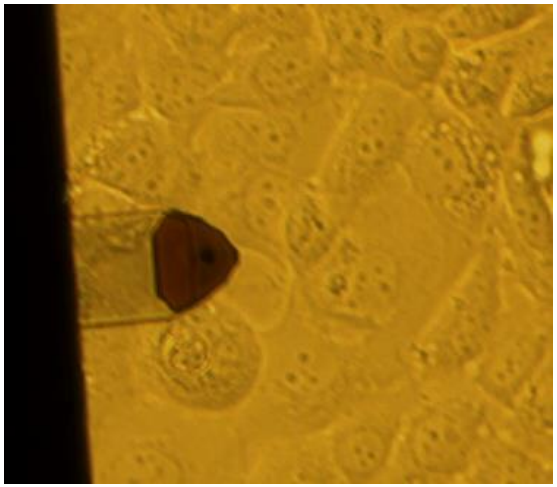
CEITEC

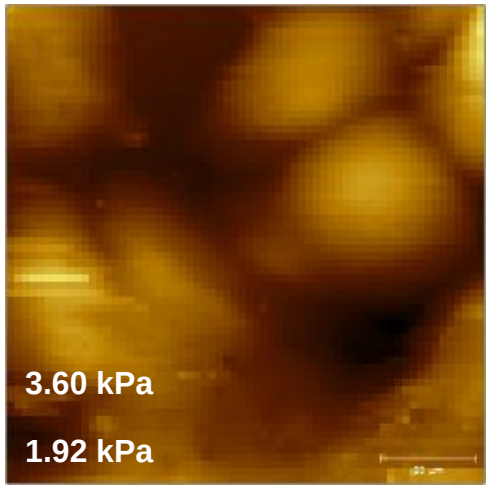
Use of AFM Force Mapping to study cancer cells stiffness

Two independent projects together with:

- *Pavel Bouchal, Biochemistry Dept. MU*
- *Michal Masarik, Med Fac, MU*







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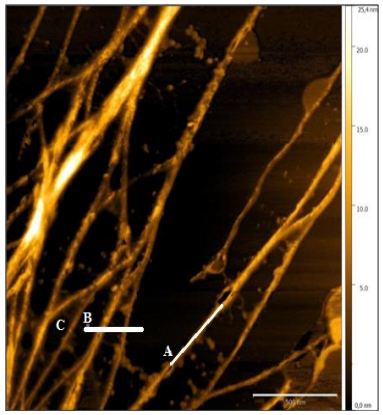
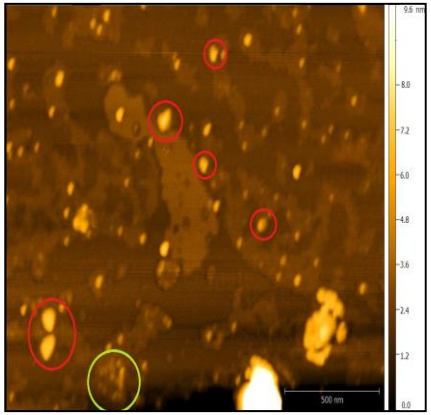
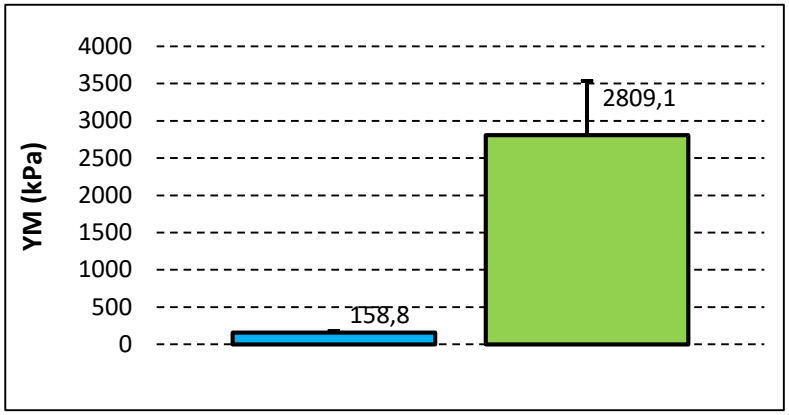
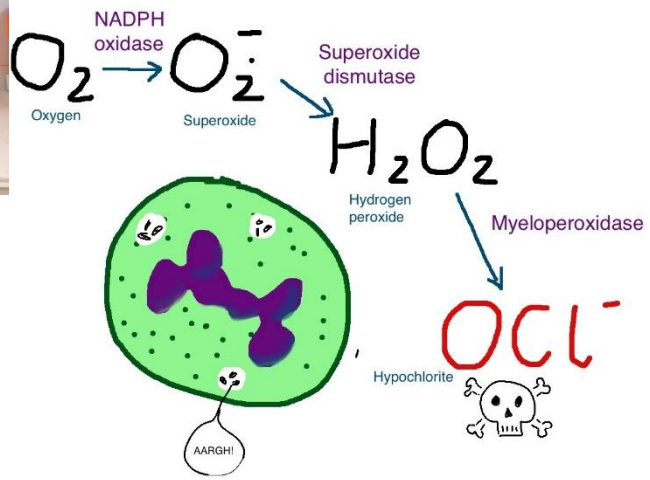


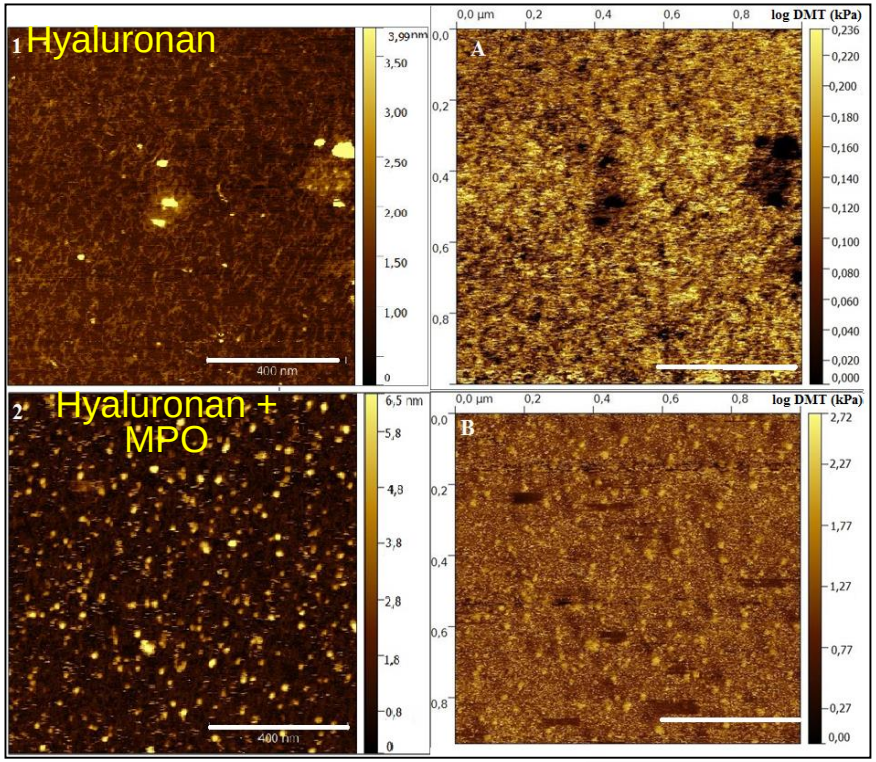




Hyaluronan - myeloperoxidase

structure and mechanical properties



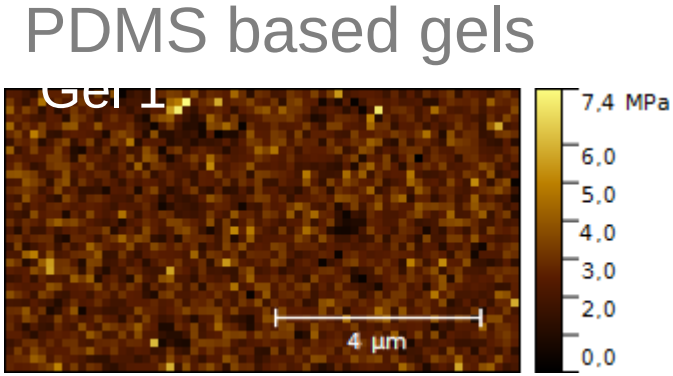


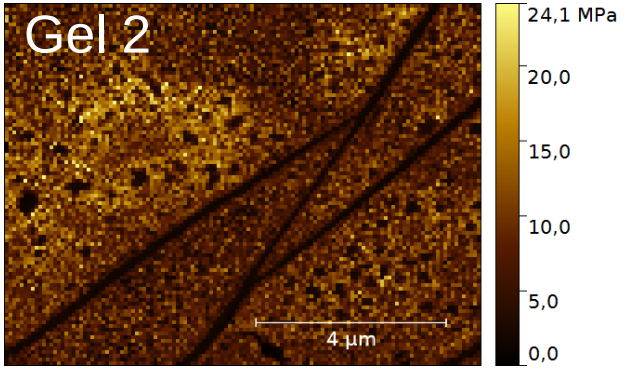
https://wardroundstuff.com/tag/neutrophils/

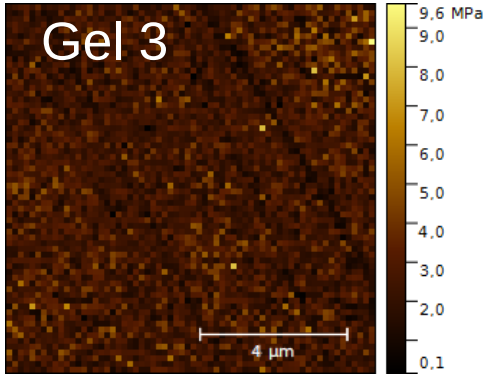
AFM measurements by Stepan Solny CEITEC

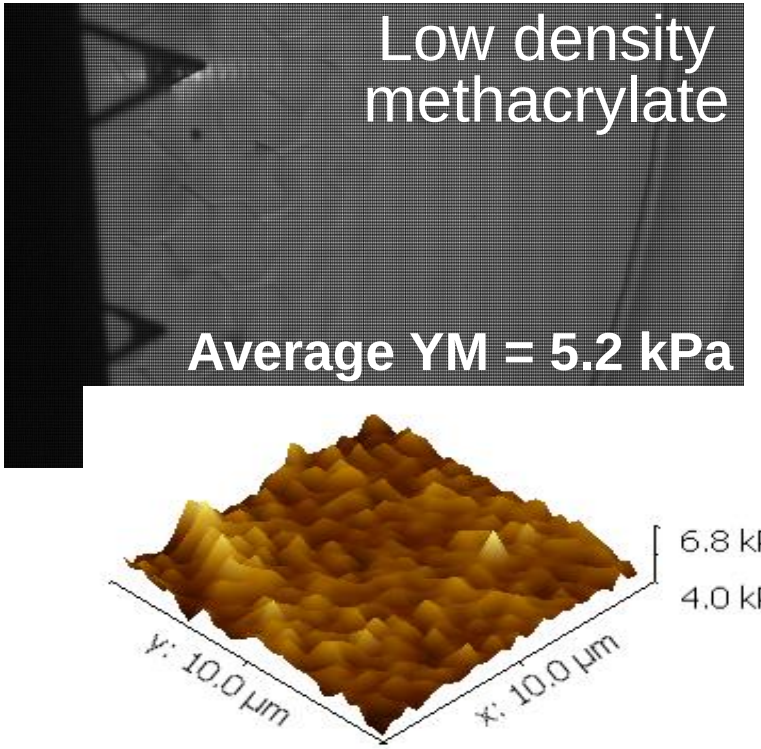
Flexible surfaces (gels)
as support for single CMs

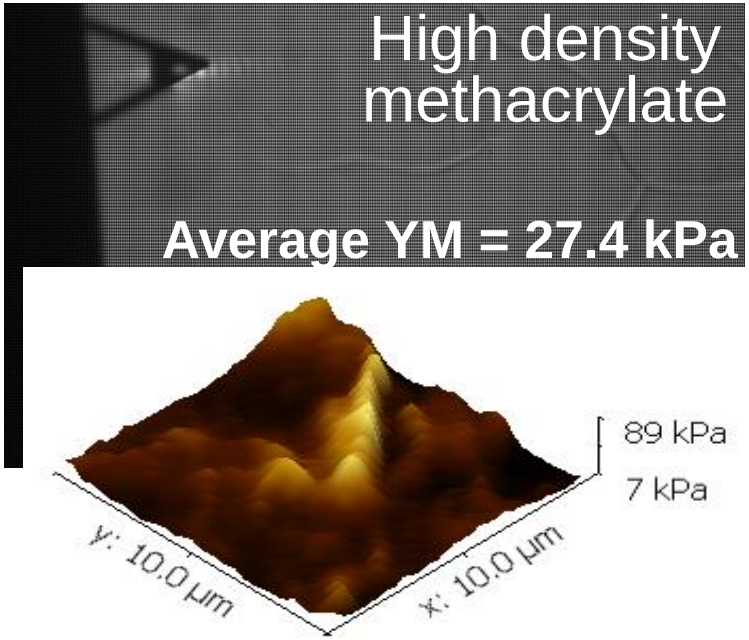
PDMS based gels











*With **Vladimir Vinarsky, Giorgia Nardone, Giancarlo Forte (ICRC, FNUSA, Brno)***

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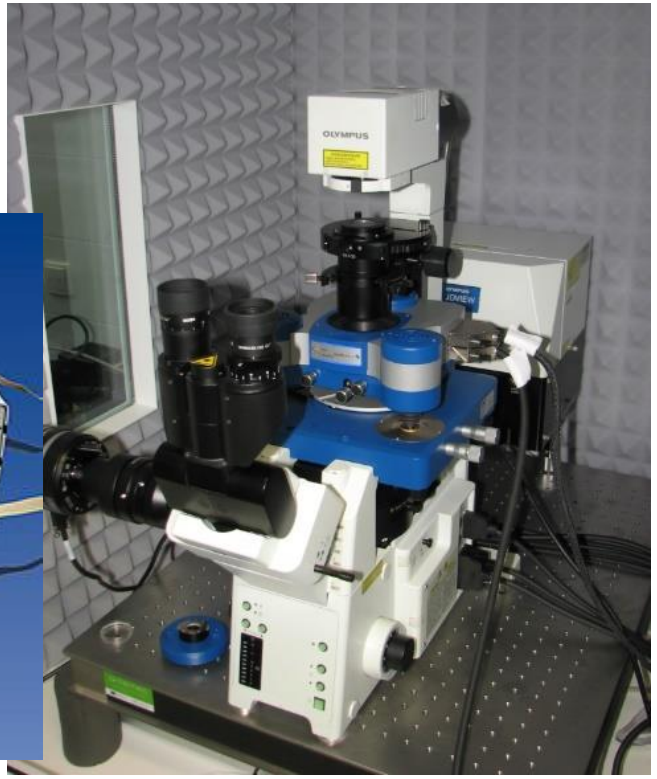
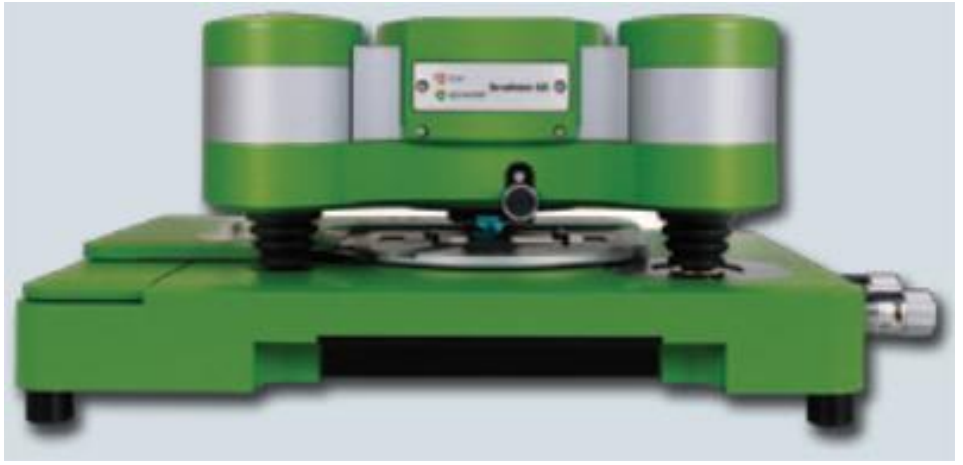
Equipment

1. BioAFM microscopes

- **JPK NanoWizard3**, **ForceRobot300** mounted on the confocal fluor. mic.
  - Contact/Tapping imaging in liquid
  - QI, ForceMapping in liquid (elev. temp.)

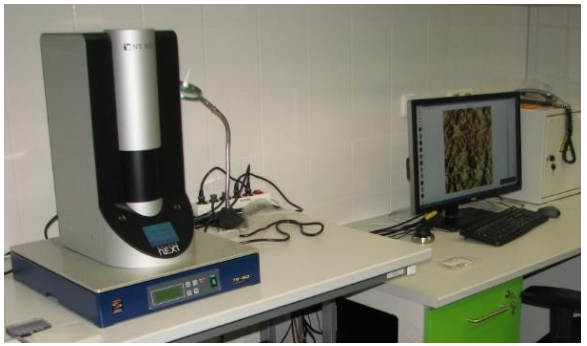


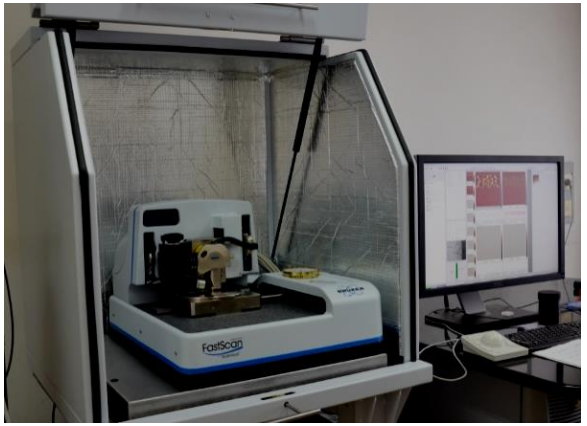
Petri dish heater

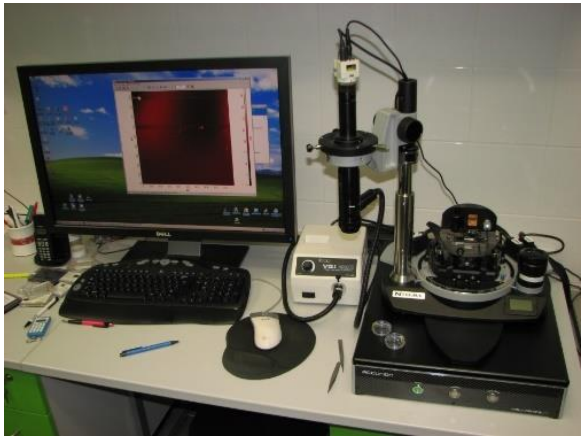


- **NT-MDT Solver NEXT**
  Ntegra Vita / Solaris
  - Contact/Tapping imaging
  - fully automated
  - education
  - *in-situ* elchem cell

- **BrukerNano** Dimension **FastScan** Bio
  - Contact/Tapping imaging in liquid
  - up to 1 image/sec
  - ScanAsyst
  - QNM/ForceMapping

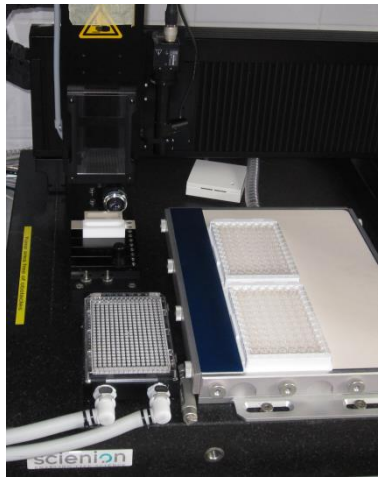






(Images by: Petr Skladal, nanowerk.com)

Equipment



2. Microdeposition of liquids

- **Scienion sciFlex** Arrayers S1 and S3
  - deposition and immobilization of biomolecules
- **InnoScan 1100**
  - 2D fluorescence imaging (0.5 um resolution)

3. SPR biosensor

- **Bionavis 220A**
  - 4-channel SPR for real time kinetics of interaction

4. Supporting services

- **Immobilization/conjugation** of biomolecules
- **ELISA** (Biotek Synergy 2)
- **QCM** biosensors
- **Electrochemistry** (Autolab)

(Images by: Petr Skladal, bionavis.com, trendbio.com.au, innopsys.com)

Conclusions

BioAFM allows:

- Visualize objects (biomolecules to cells) in under near physiological conditions
- Mapping of Young's modulus of immobilized biosamples
- Time lapsed changes of mechanical properties

Future outlook

- BioAFM instrumentation improvement – CO2 chamber, improved in-situ sterility, etc.
- Optical part improvement (objectives, cameras) → overlay imaging
- Tissue related experiments (i.e. heart valves)