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Nanobiotechnology

Scanning Probe Microscopies

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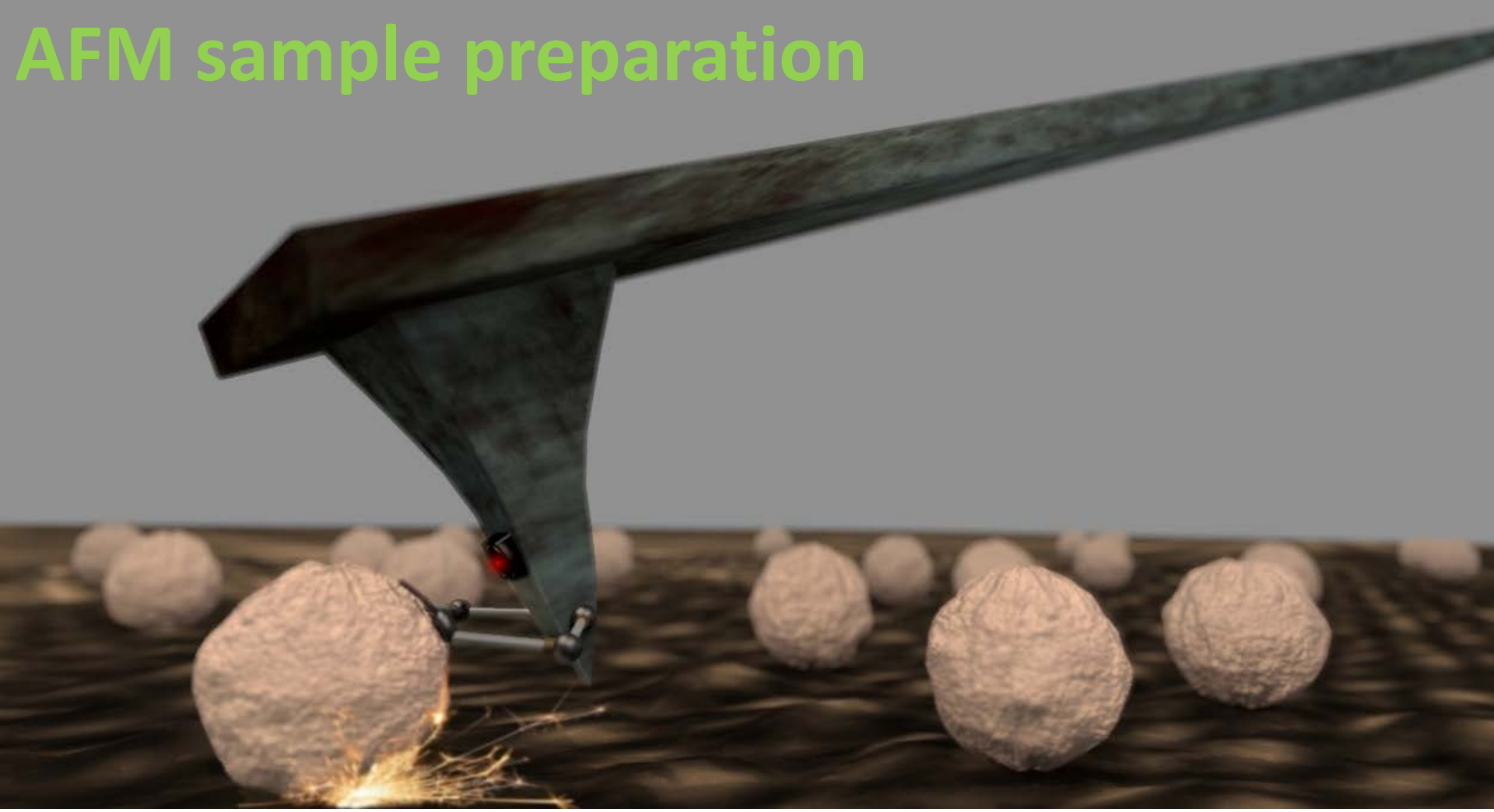
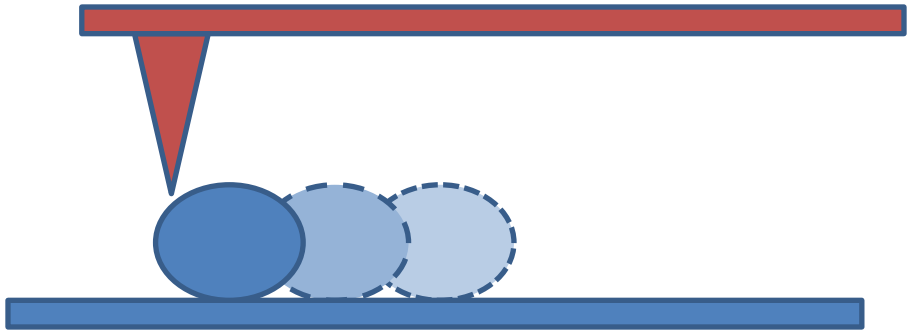
2007–13
OP Research and Development for Innovation

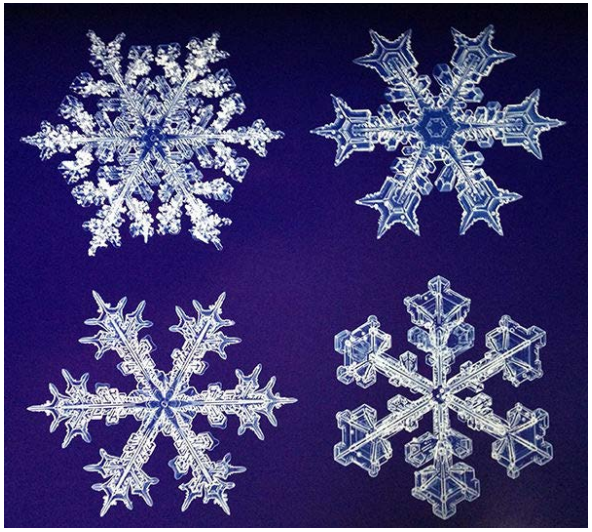
Sample preparation for AFM

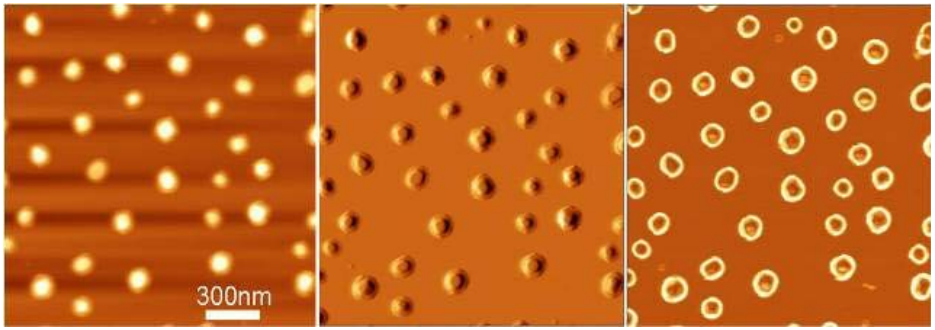
AFM sample preparation

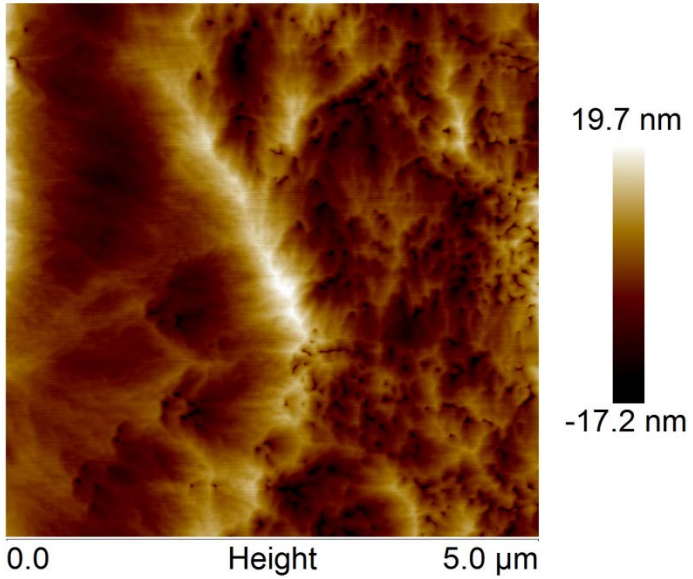




Concentration – surface density



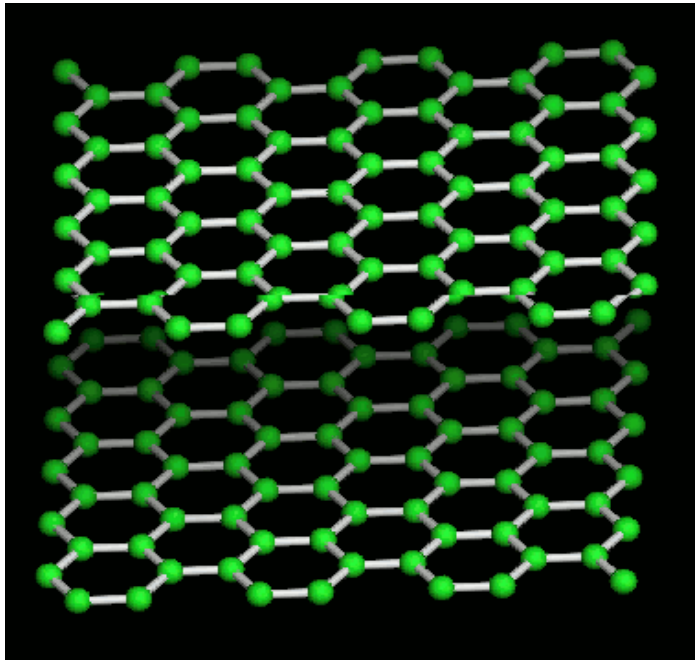




Substrates for preparation of AFM samples

Atomically flat surfaces

1. **HOPG** Highly Ordered Pyrolytic Graphite

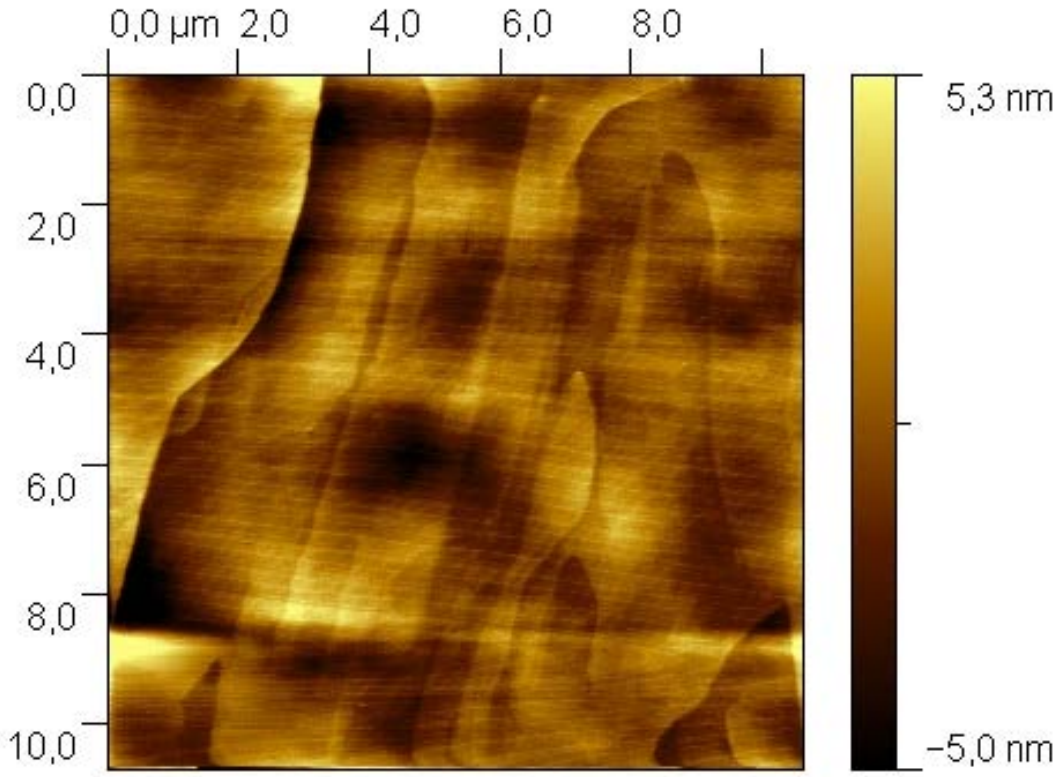


- Kish's graphite, waste in steel production
- Hexagonal planar structure
- C-C bond142 pm, layer-layer distance335 pm
- Conductive, highly hydrophobic
- Planar structure
- Synthetic form of graphite, high chemical purity
- Traditionally – substrate for SEM, STM i AFM (→ **conductivity**)
- **Immobilization** – spontaneous adsorption (→ **hydrophobicity**)

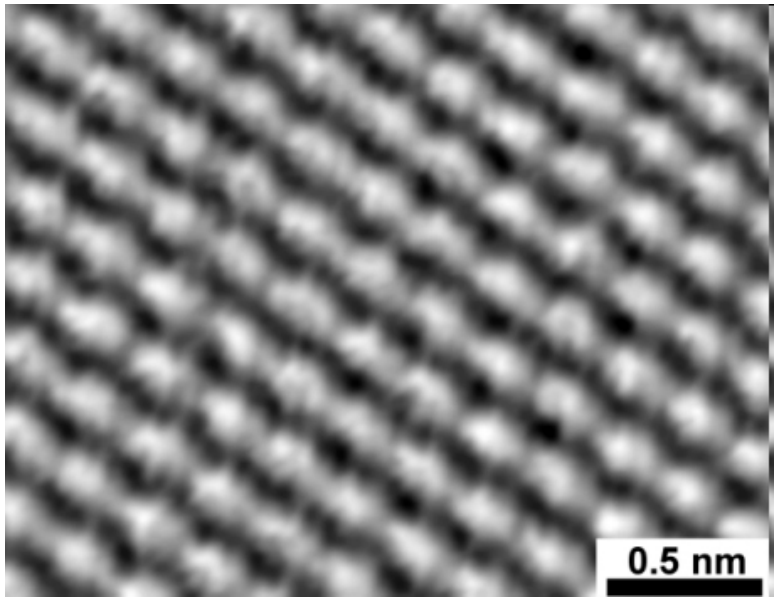
Atomically flat surfaces

1. **HOPG** Highly Ordered Pyrolytic Graphite

Large areas
visible layers



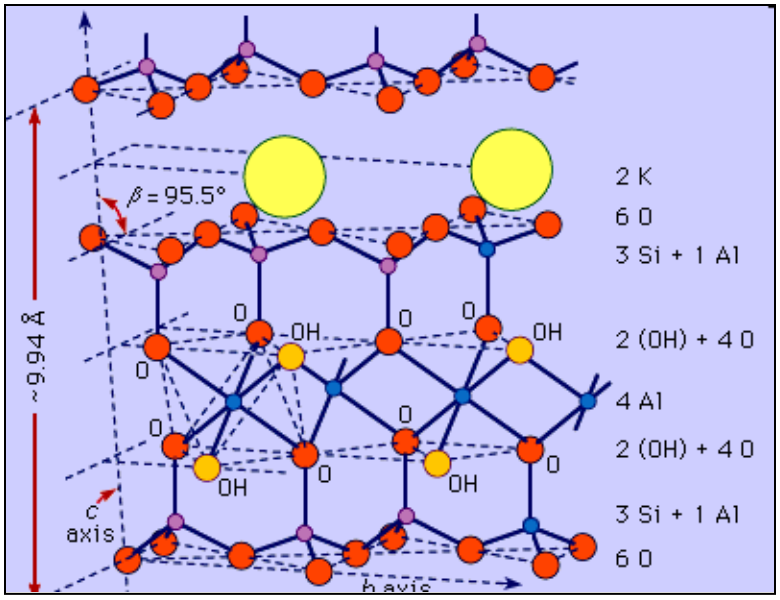
Small areas
atomically flat



Atomically flat surfaces

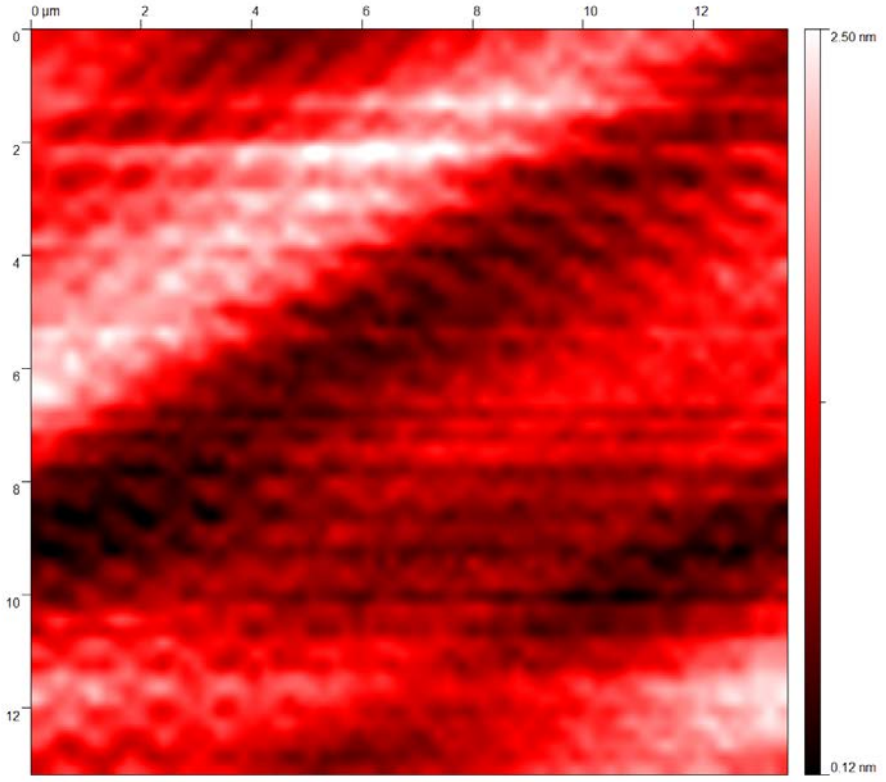
2. Mica (muscovite)

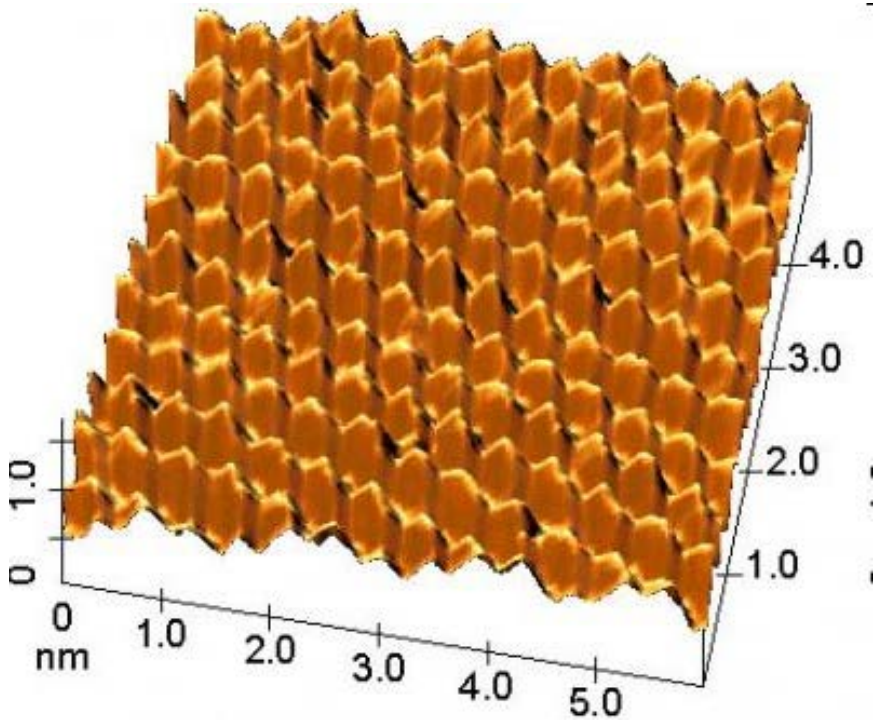
- „Cat's silver", muscovite acc. to city of Moscow
- Chem. structure: $K_2O \cdot Al_2O_3 \cdot SiO_2$
- Hydrophilic surface
- Easy to be modified by chemical synthesis
- Immobilization by **chemical bonding** as well as **ionic interaction**
- pKa ~ 3, physiological pH → negative surface charge
- Mica = silicate, hydrated SiO_2 (~ Si-OH) from the chemical point of view



Atomically flat surfaces

2. Mica (muscovite)





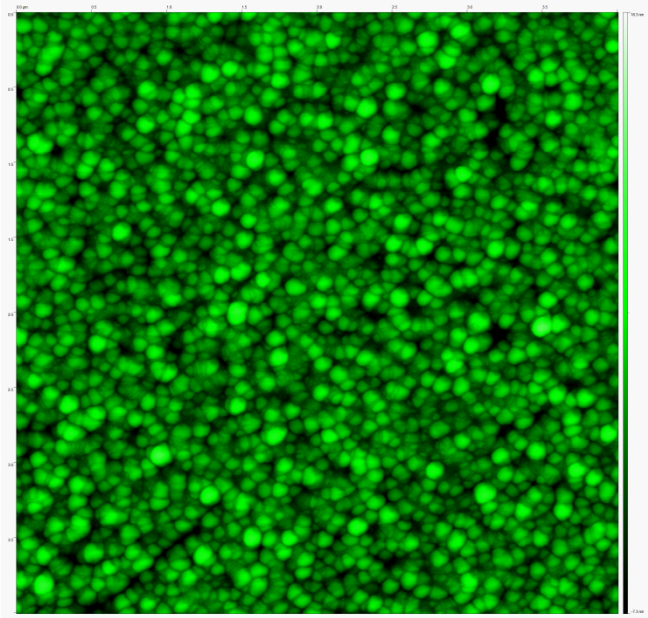
Extremely flat on small and larger areas

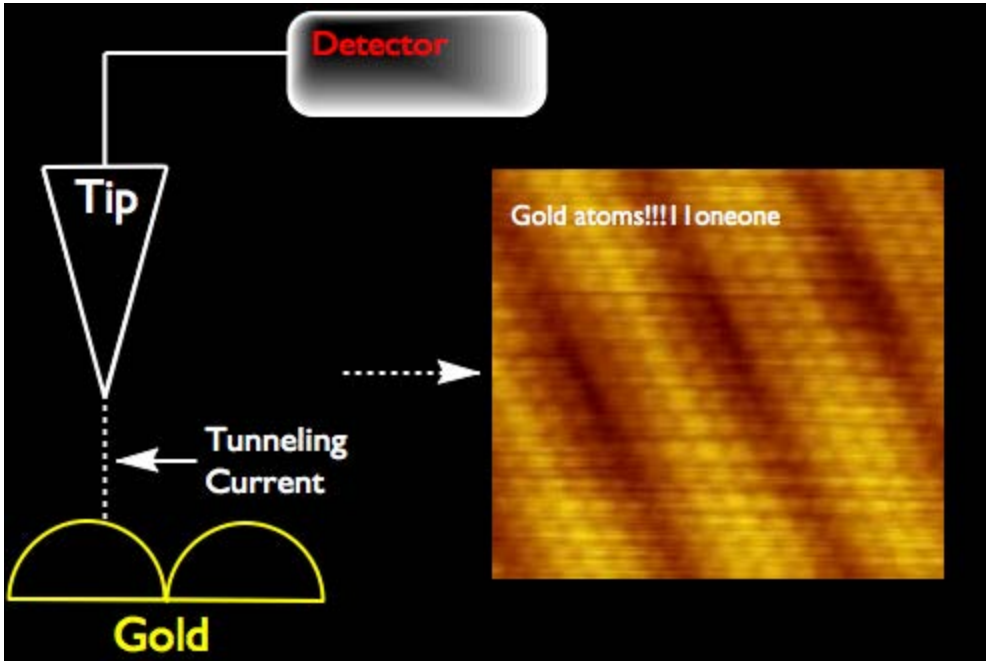
Other surfaces

3. Gold

- Inert metal
- Traditionally in (bio)electrochemistry (i.e. biosensors) - electrodes
- Conductive - STM + AFM
- Hydrophobic: spontaneous non-selective adsorption of molecules (proteins, DNA, …)
- Specific chemical binding of thiols (-SH) – organic molecules + cysteine
- Prepared usually by evaporation
- Adhesion layer for operation in liquids (Al/Cr/Ti)

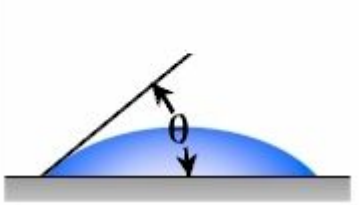
Sputtered gold layer
image by tapping mode AFM



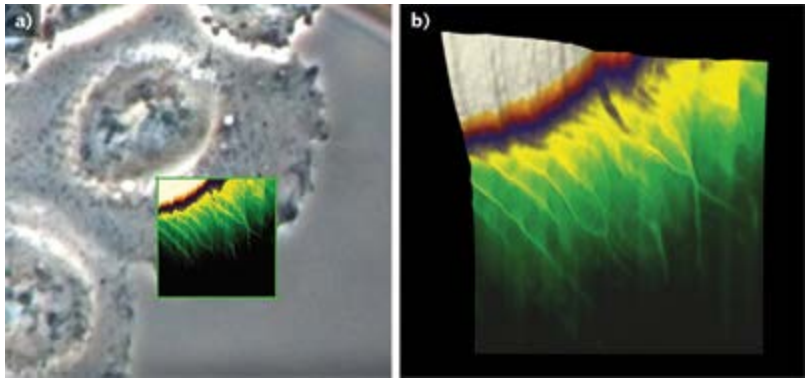


Other surfaces

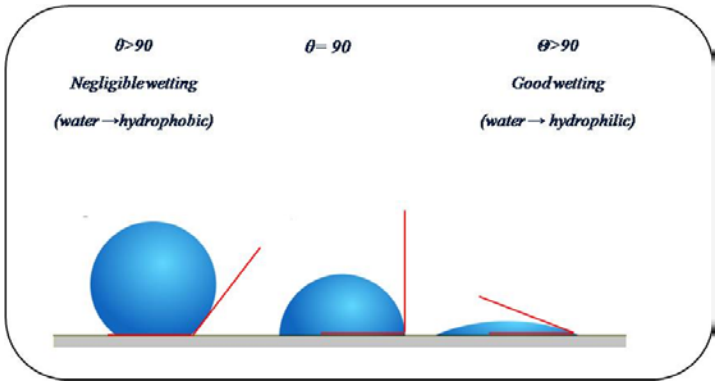
4. Glass



- Amorphous noncrystalline structure
- Lab glass composition: 75% SiO_2 plus Na_2O, CaO, borate and minor additives
- Si-OH → from chemical point of view
- Less hydrophilic comparing to mica
- Roughness much higher comparing to mica (production by pressing)
- **Not** suitable for **individual molecules** imaging with AFM
- Typically used together with optical microscopy – cell compartments, whole **cells**

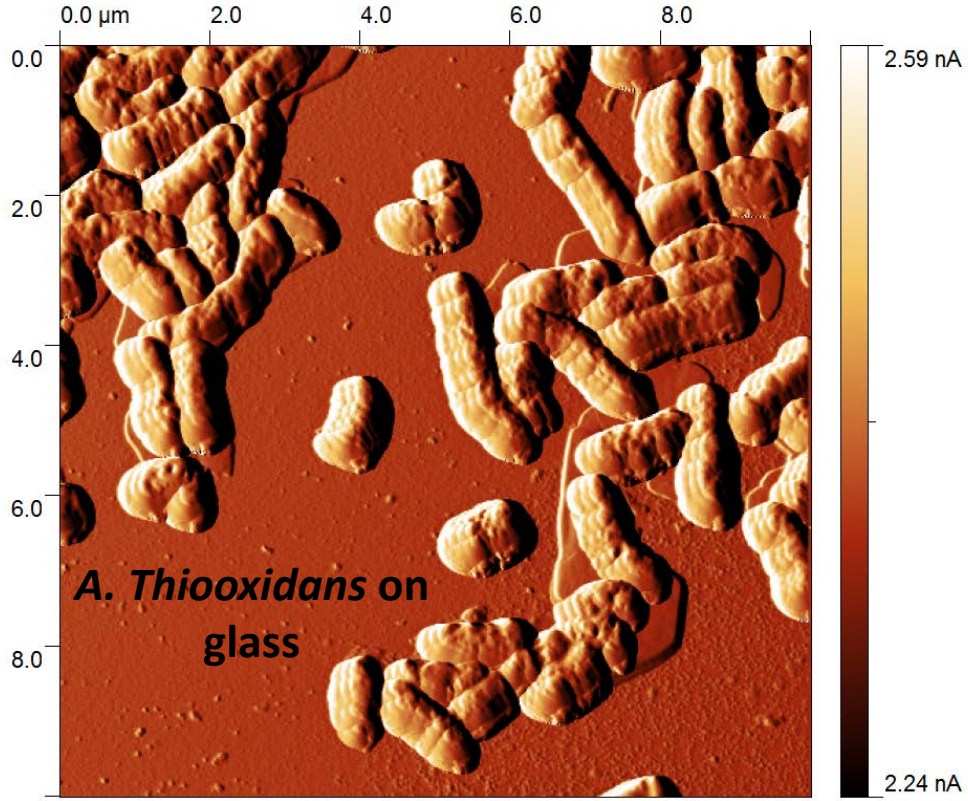


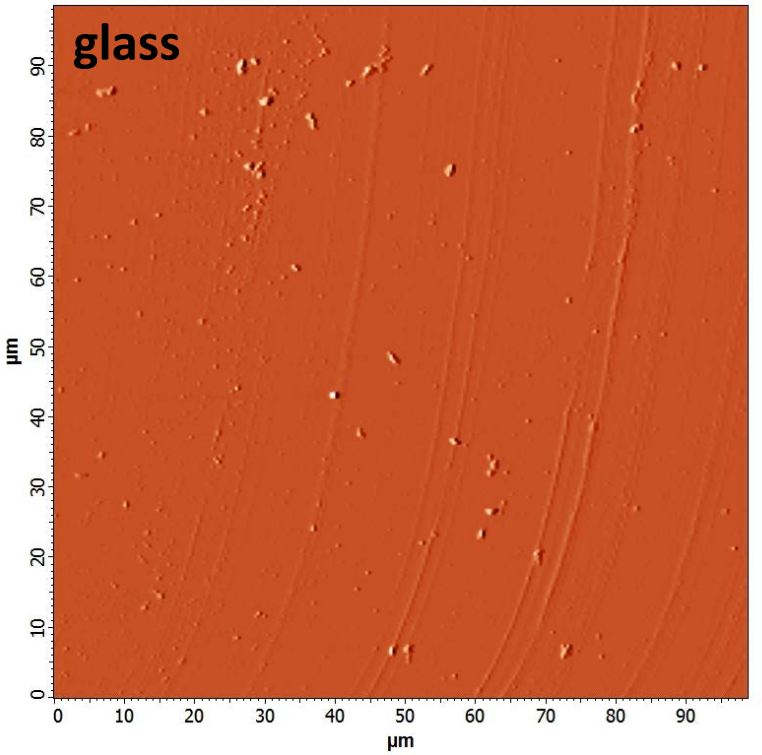
AFM – optical image overlap

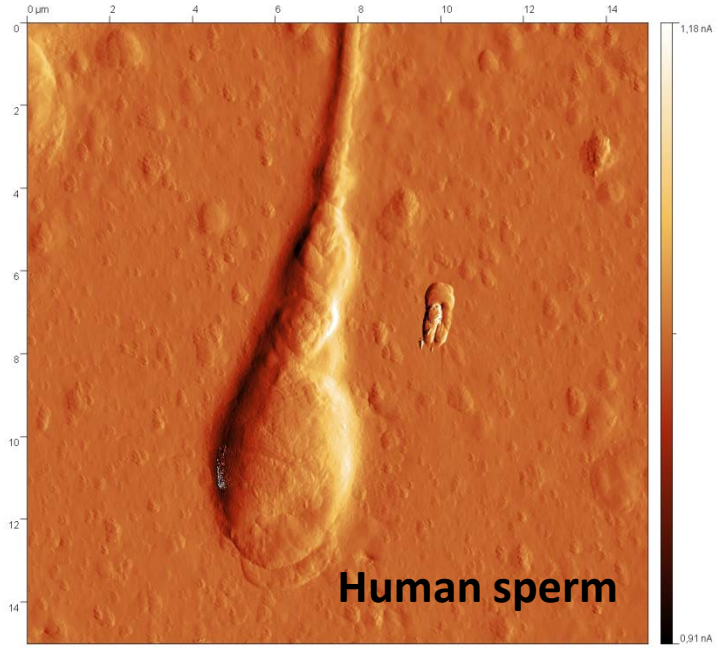


Whole cells on glass

under AFM

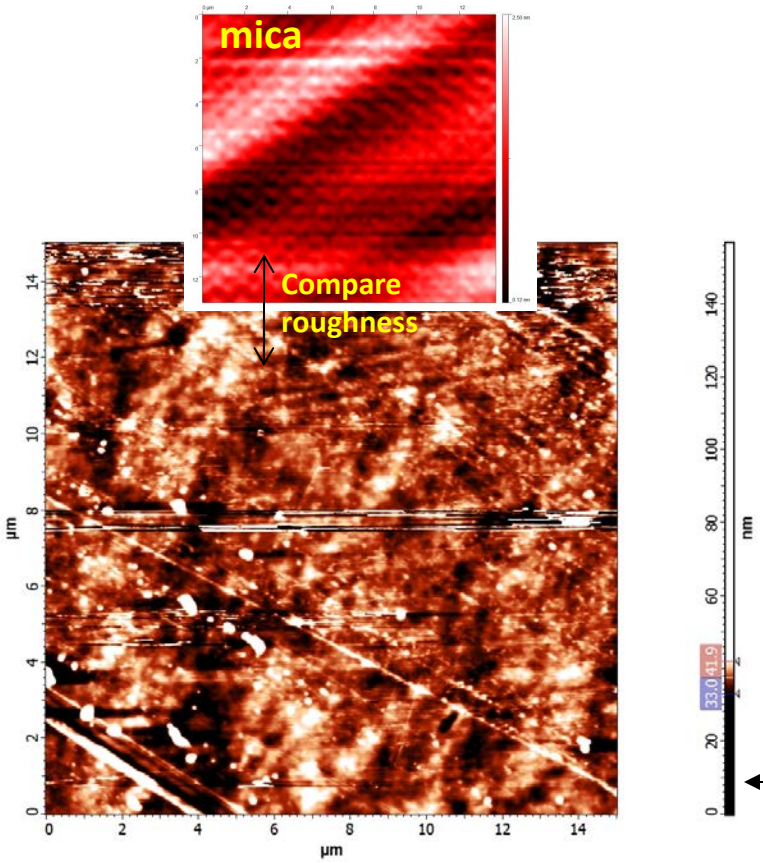


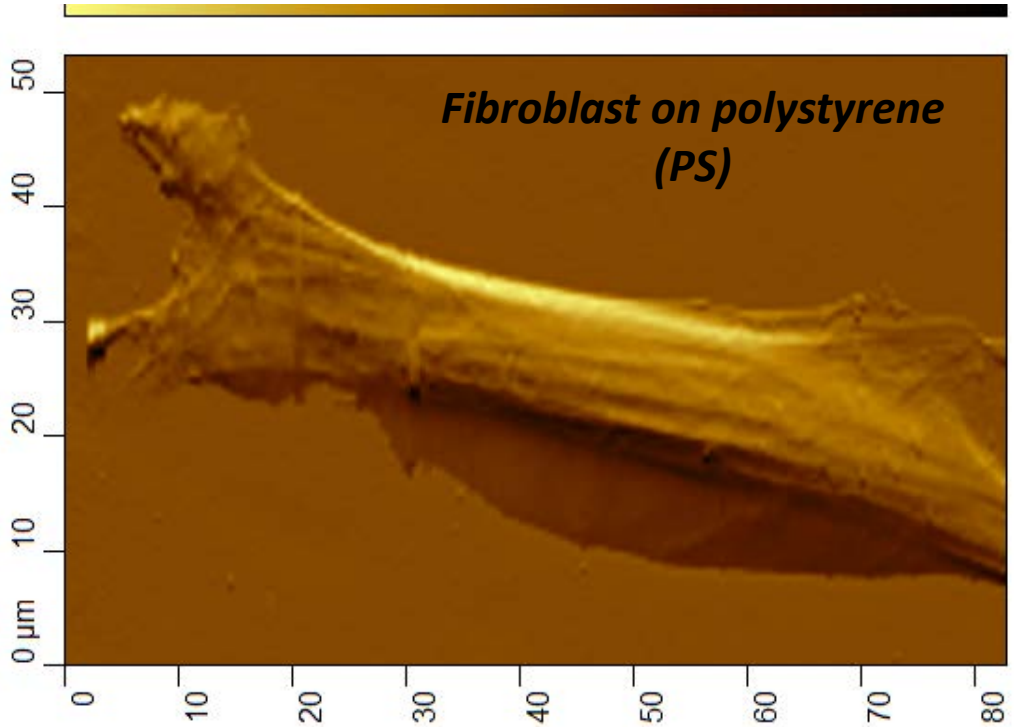




Other surfaces

5. Plastic materials





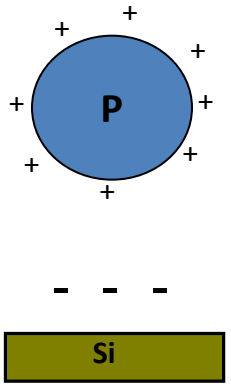
Non-modified polystyrene (PS)

- Most of lab supplies made of plastic (**PP, PE, PS**)
- **No functional groups** to be used in covalent binding
- **PS – hydrophobic** → spontaneous non-specific adsorption of proteins → usually as underlying support (i.e. for cell attachment)

Immobilization procedures

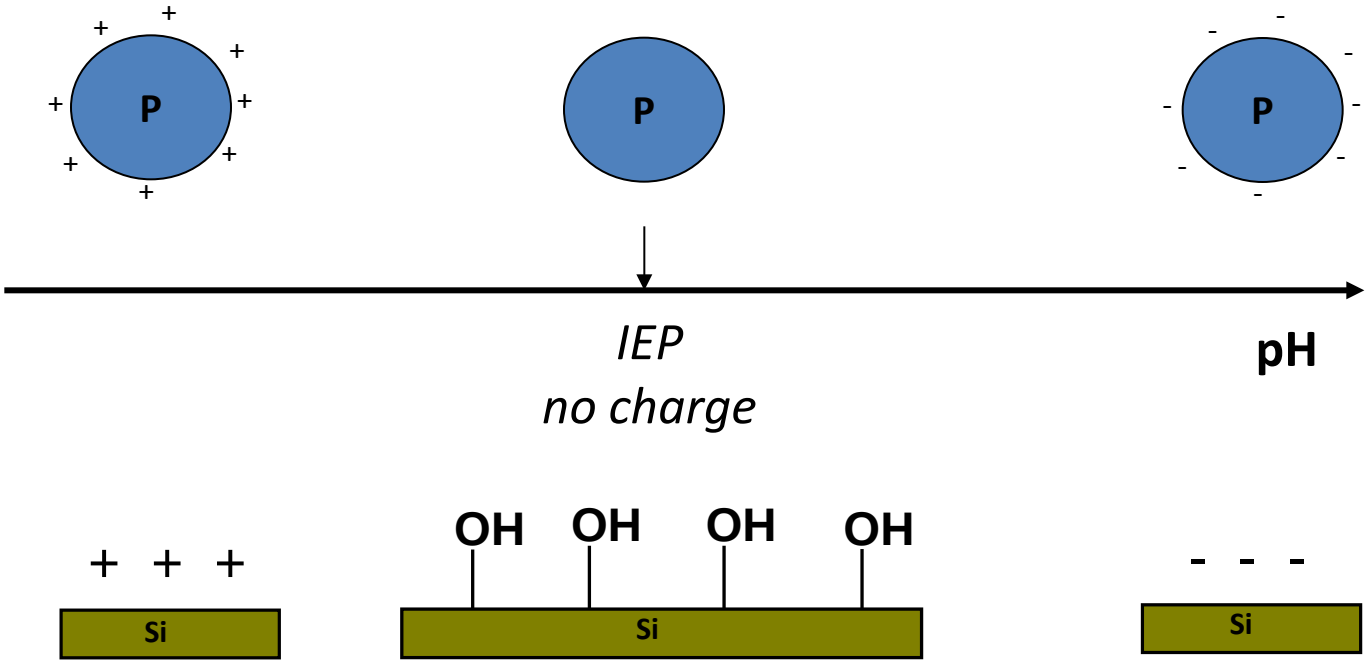
1. Proteins

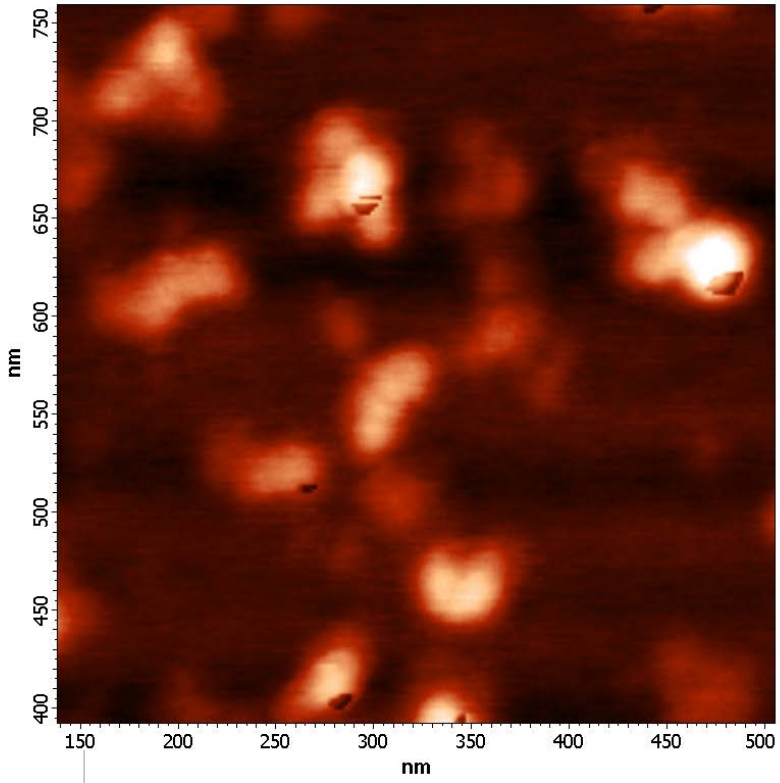
Surface: **mica or HOPG** (extremely flat)



Protein: **charge** is given by IEP + pH

Immobilization on mica: **pKa (mica) < pH < IEP**

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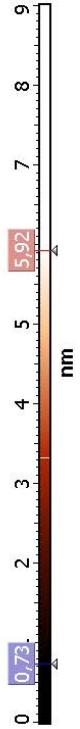
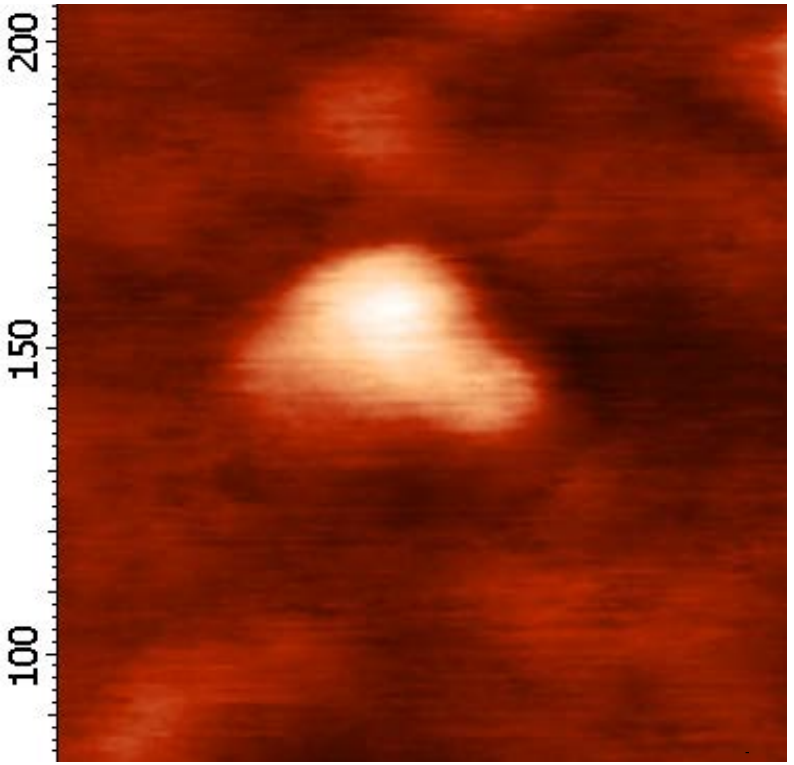
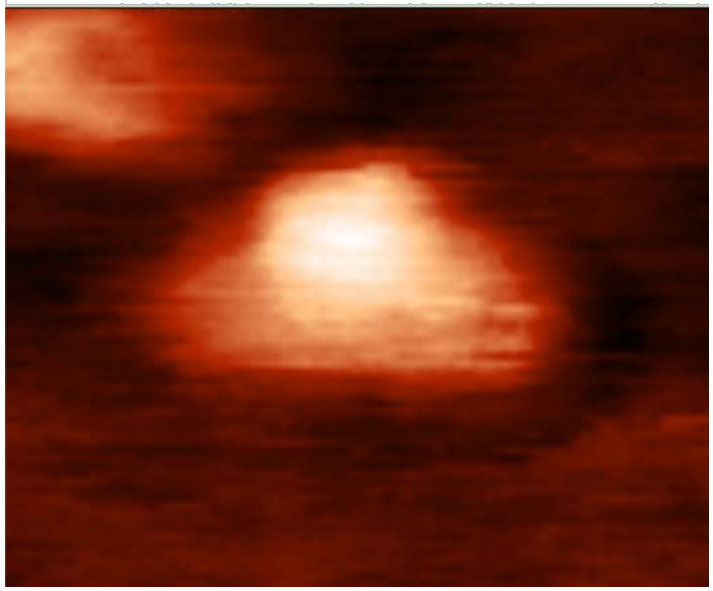
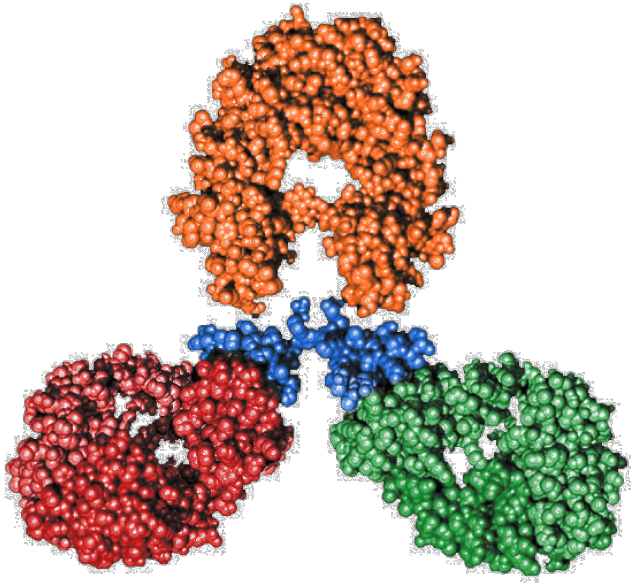
700
650
600
550
500
450
400
150
200
250
300
350
400
450
500
nm


9
8
7
5,92
5
4
3
2
0,73
0
nm

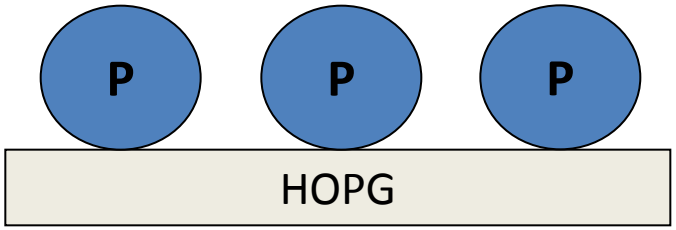

200
150
100

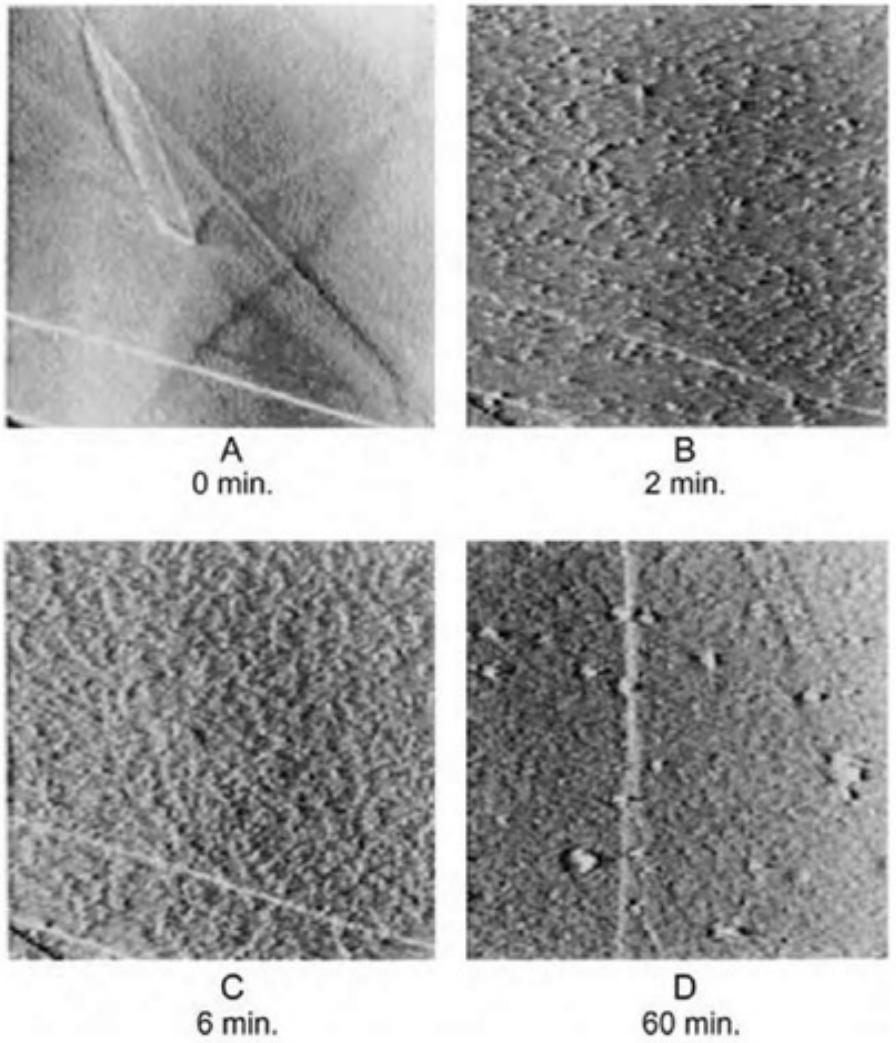




Protein immobilization on HOPG

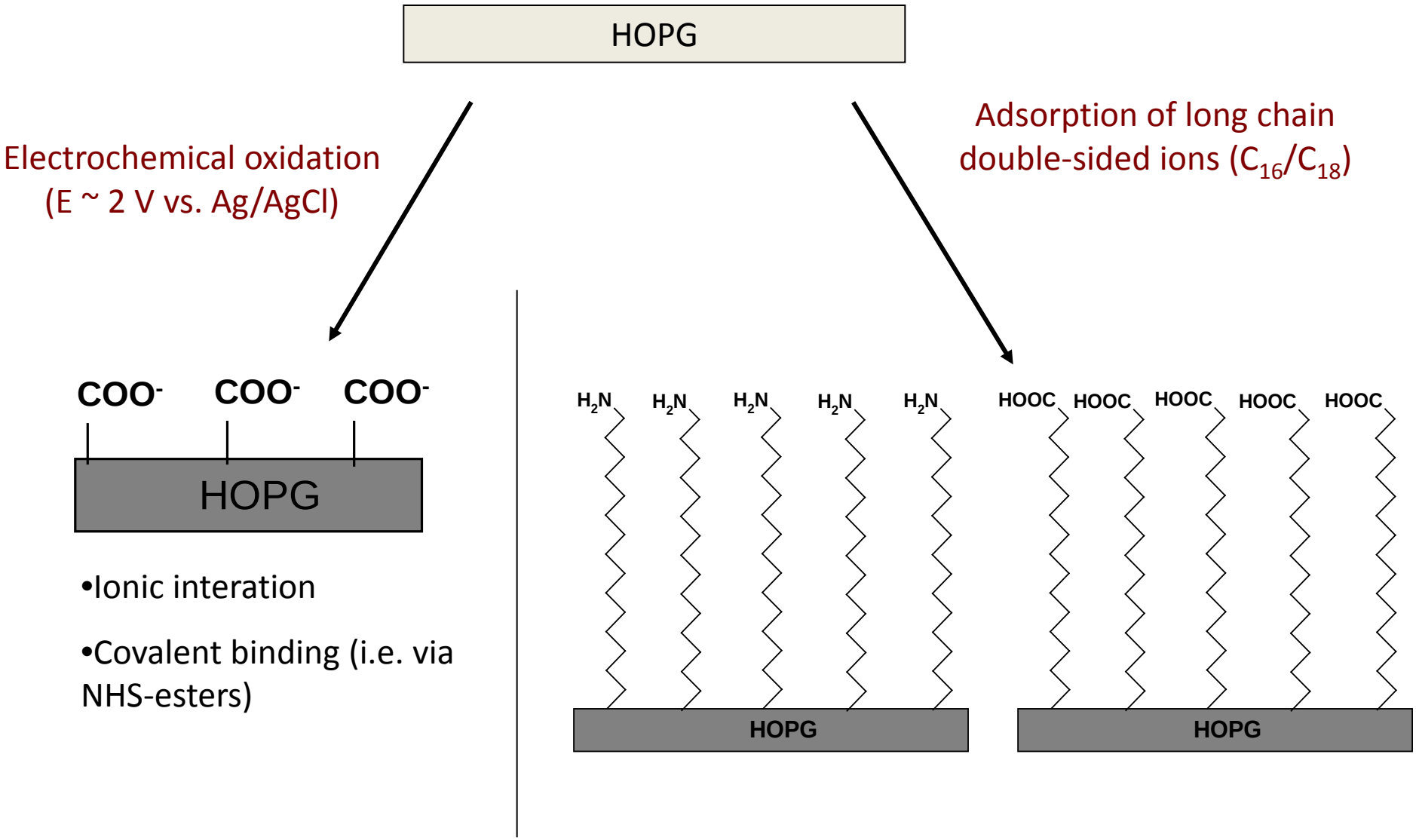
A. **Spontaneous** (non-specific) **adsorption** of protein → hydrophobic surface (best results at zero charge *pH = IEP)*





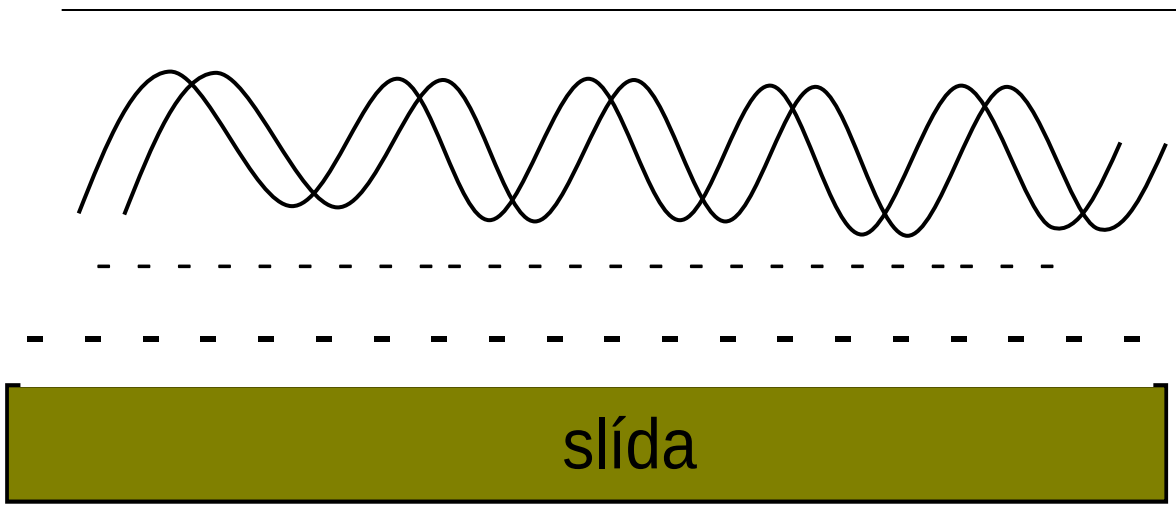
Lysozyme molecules on HOPG

B. **Ionic** (specific) **binding** of molecules → creation of charge/chem. groups on HOPG surface



2. DNA

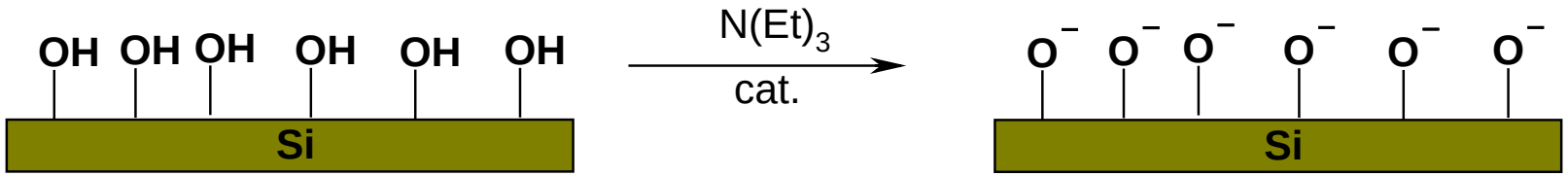
Surface: **mica or HOPG** (extremely flat)



Immobilization problem:

DNA (sugar-phosphate bone) as well as **mica – negative charge** under physiological pH

→ surface introduction of **positive charge**



Silanization

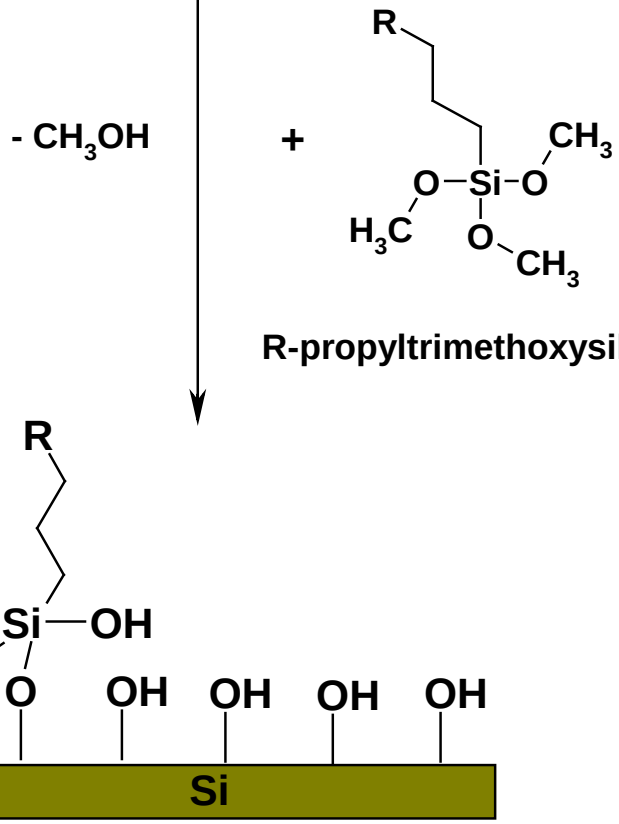
= chemical (covalent) modification of mica surface

- Aim: **introduction** of **functional group**

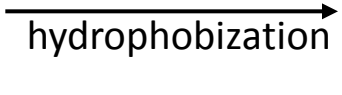
- Applicable also for: glass, quartz, silicon, titanium, …

- Strong basis catalysis

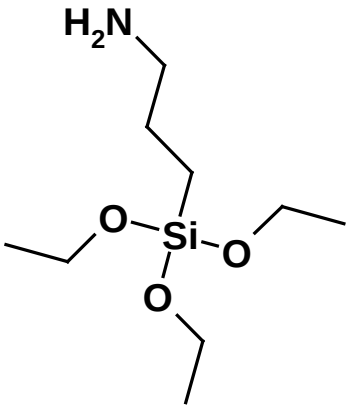
- Procedure can monitored by water contact angle measurement



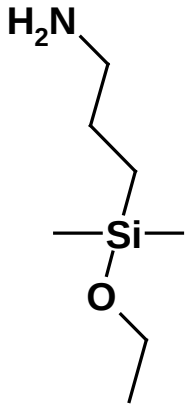
A. DNA on mica



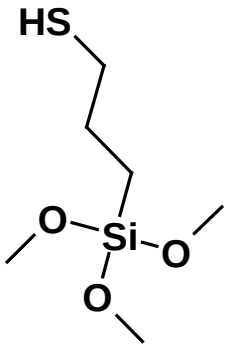
Examples of **alkoxysiloxanes**



(3-Aminopropyl)trimethoxysilane
APTES



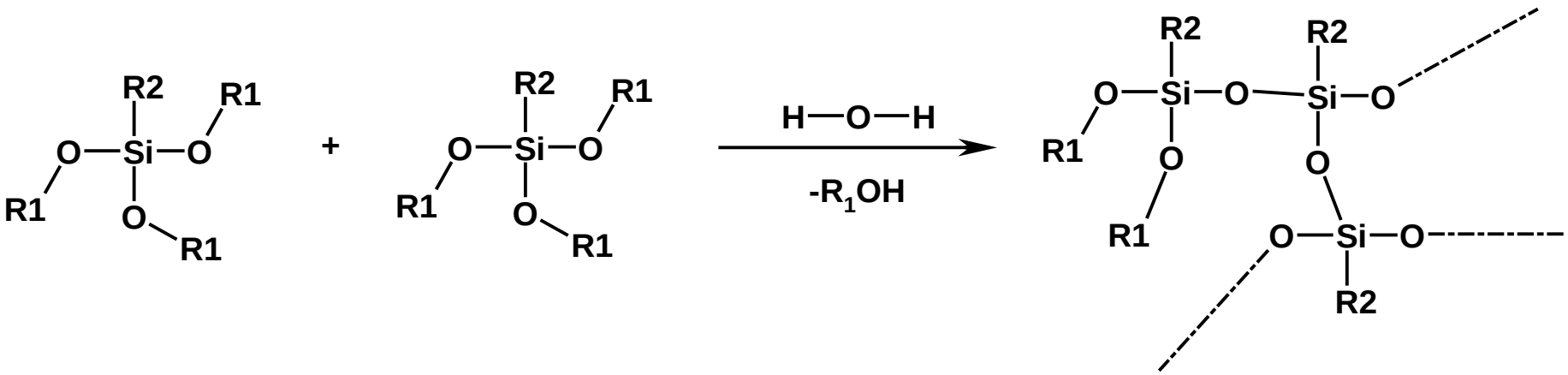
3-(Ethoxydimethylsilyl)propylamine
APDMES



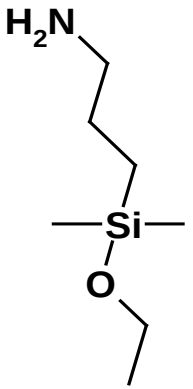
(3-Mercaptopropyl)trimethoxysilane
MPTS

Self-polymerization

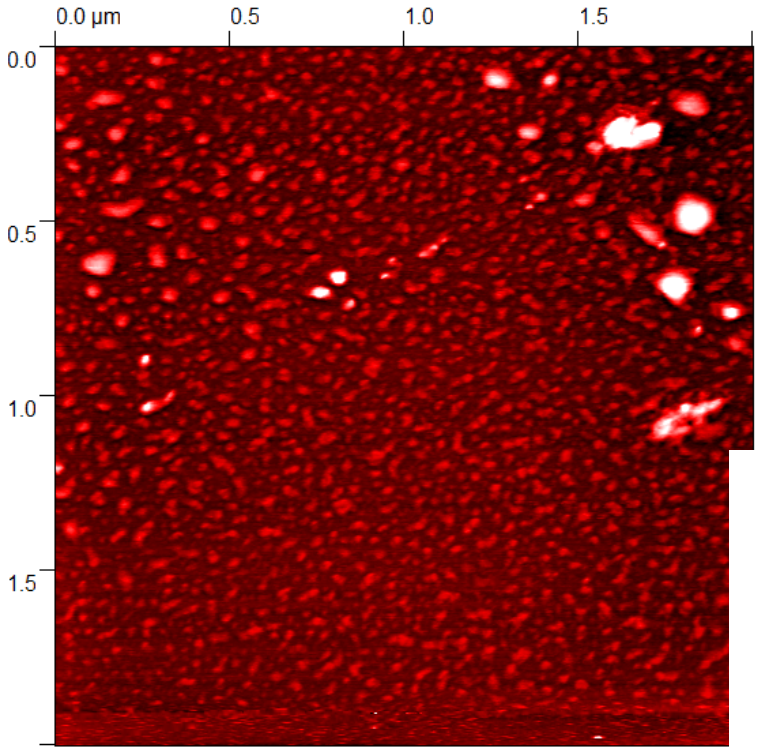
practical complication



- 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

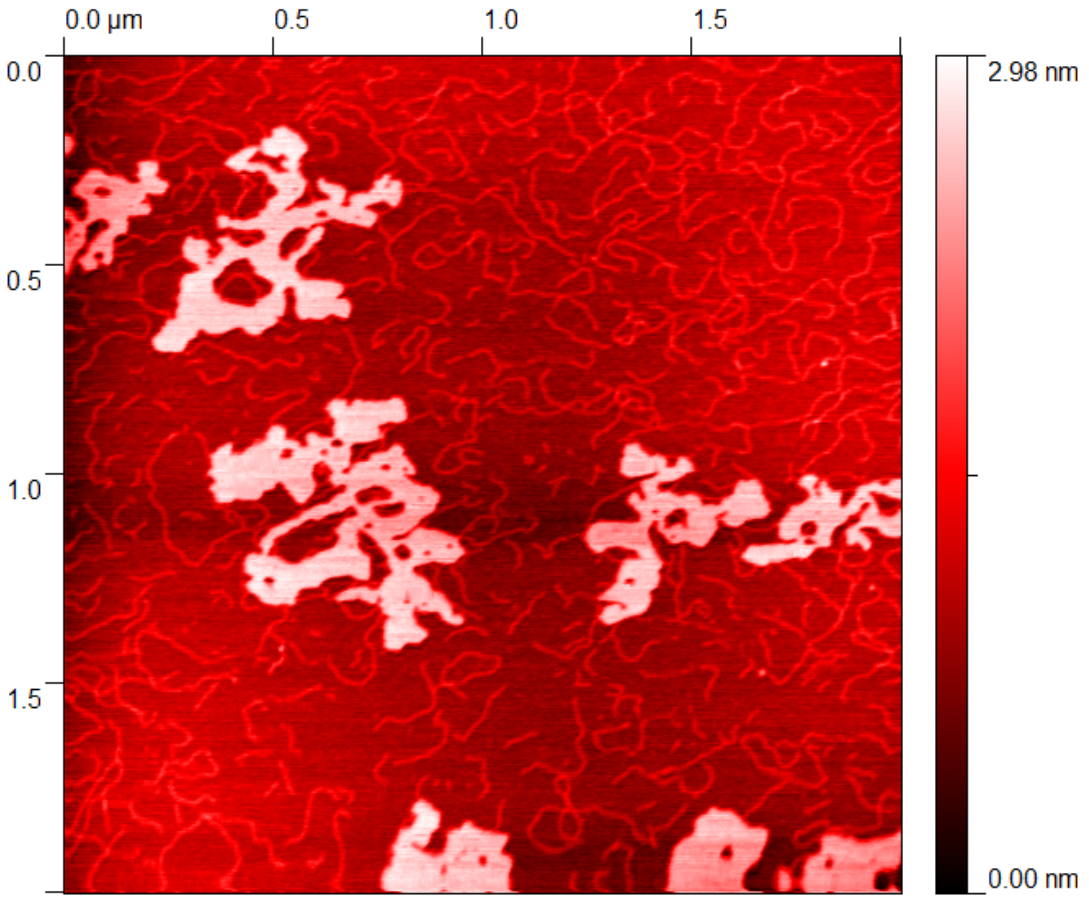


3-(Ethoxydimethylsilyl)propylamine
APDMES



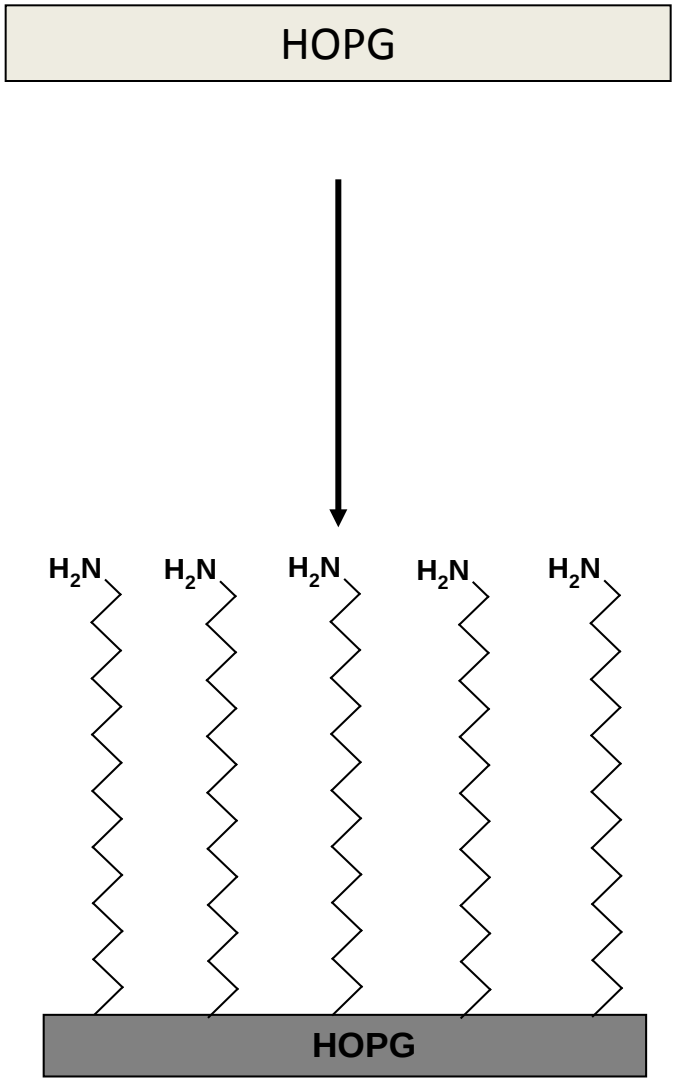
Self-polymerization

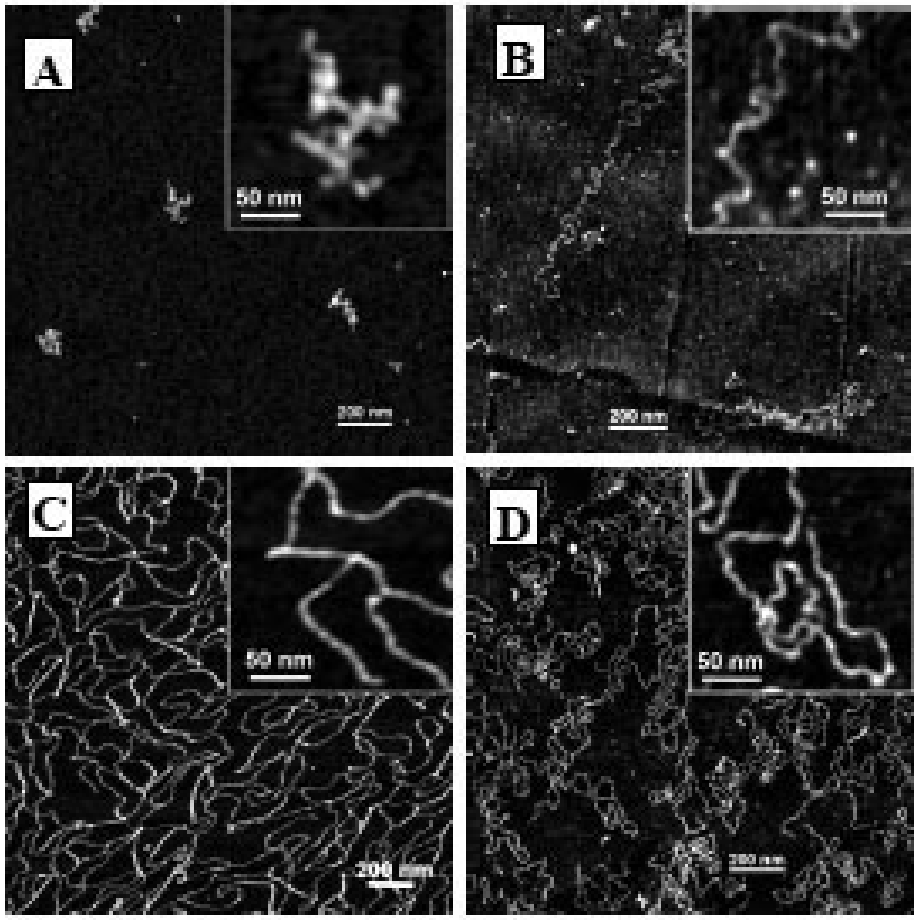
examples



B. DNA on HOPG

Adsorption of long chain double-sided ions (C_{16}/C_{18})

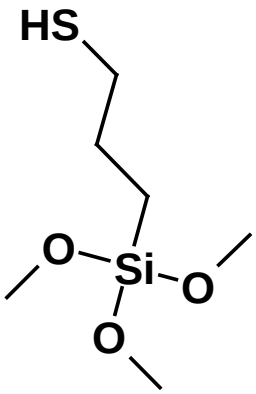




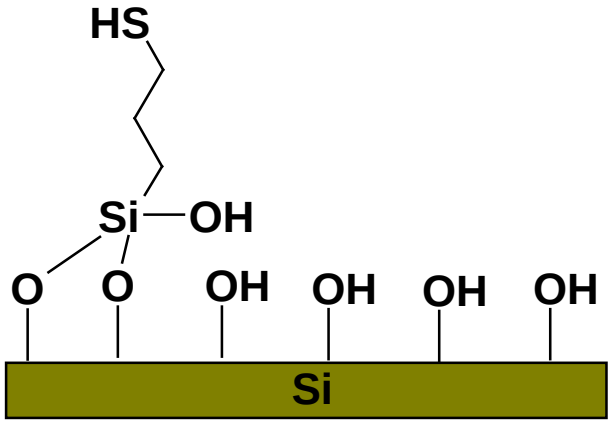
3. Nanoparticles

Substrates for immobilization: **mica** / **HOPG** (smooth surfaces), also gold, glass in selected cases.

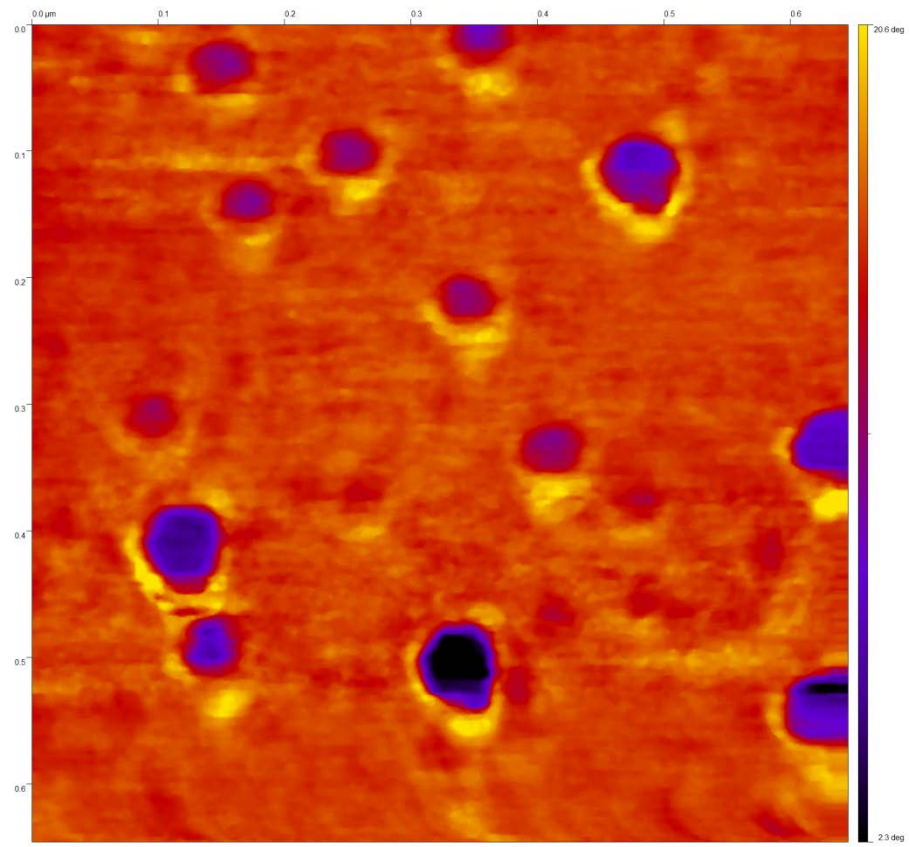
Example: gold nanoparticles (AuNP) mercapto-silanized mica (SH-mica):

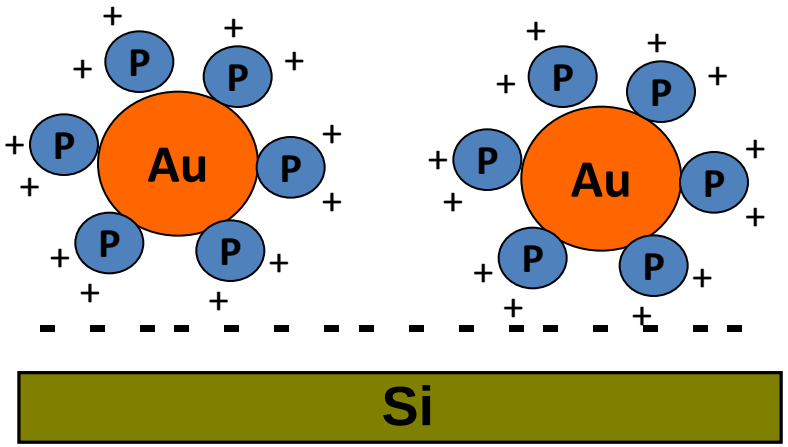


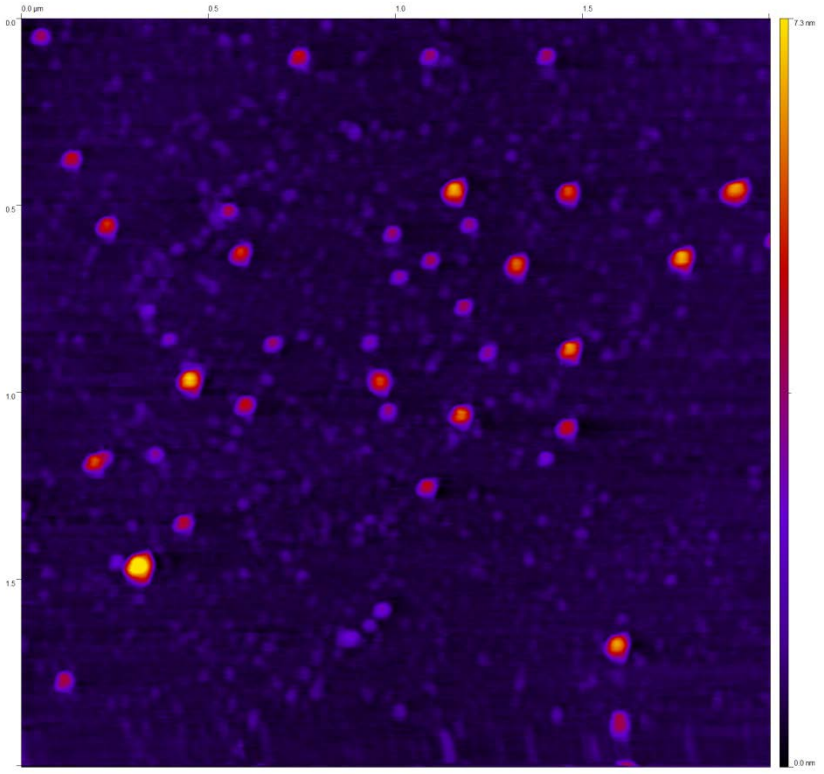
(3-Mercaptopropyl)trimethoxysilane
MPTS



SH-mica



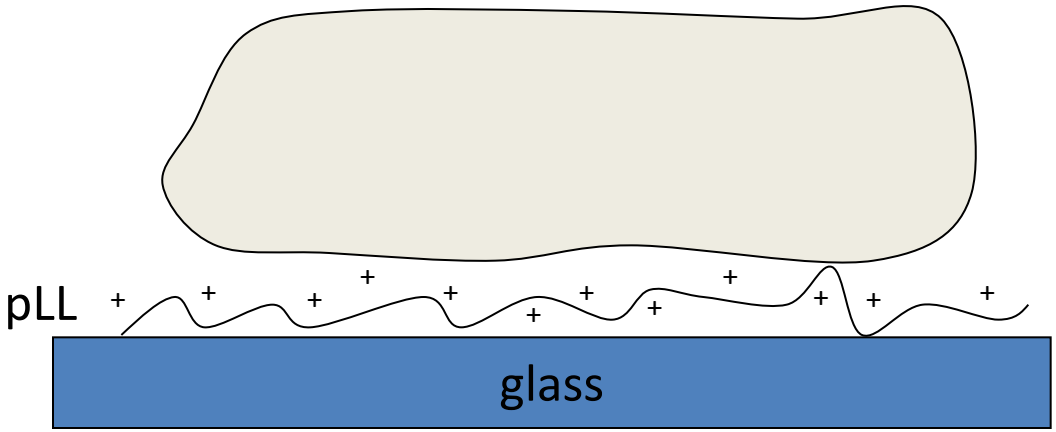


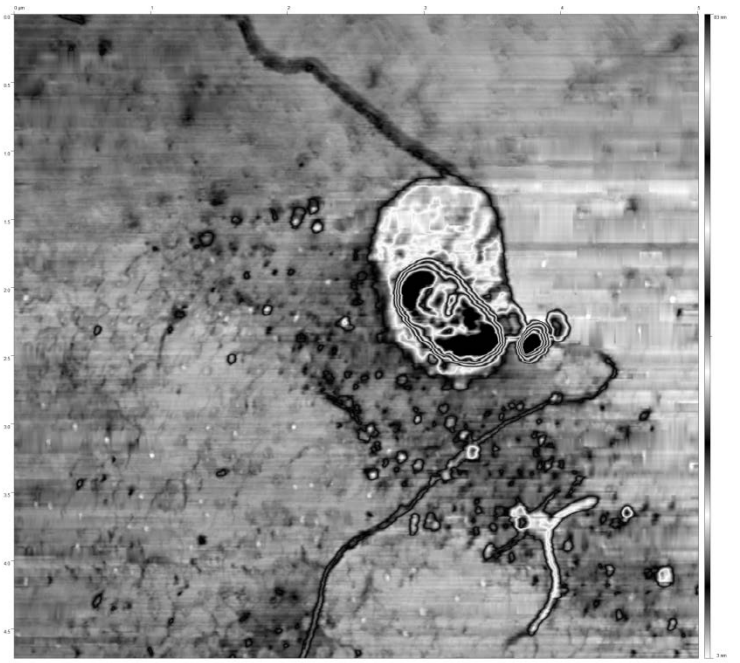


Gold nanoparticles (**AuNP**)
conjugated with **protein** molecules:
protein = immobilization bridge

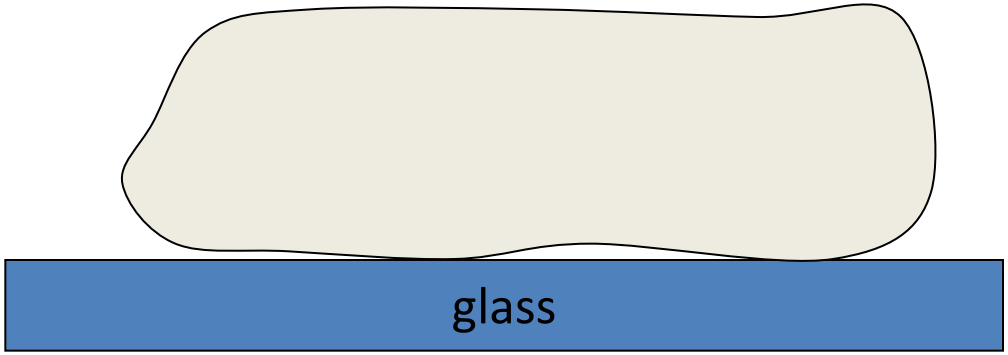
3. Bacteria, spores

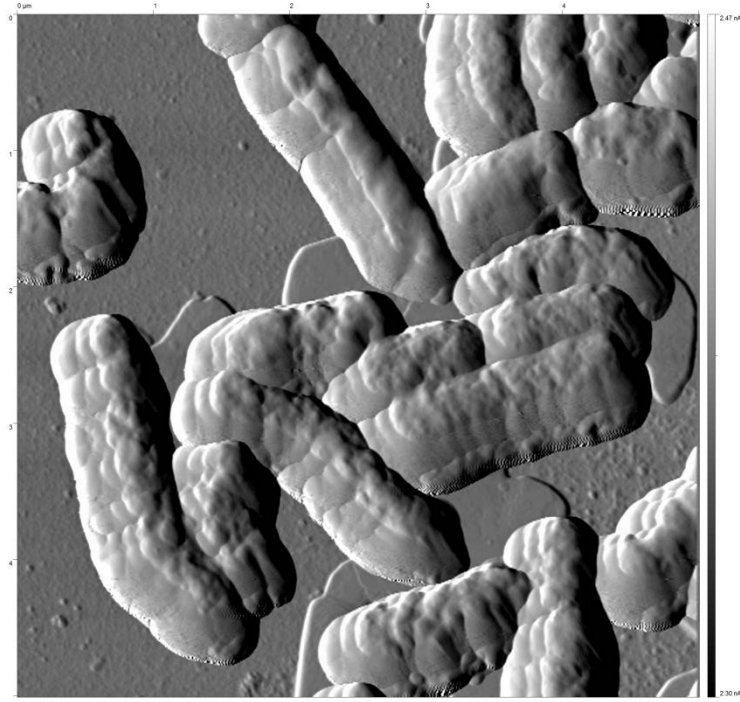
Protein adhesive layer, i.e. pLL
(poly-L-lysine → introducing positive charge)





Standard coating on glass

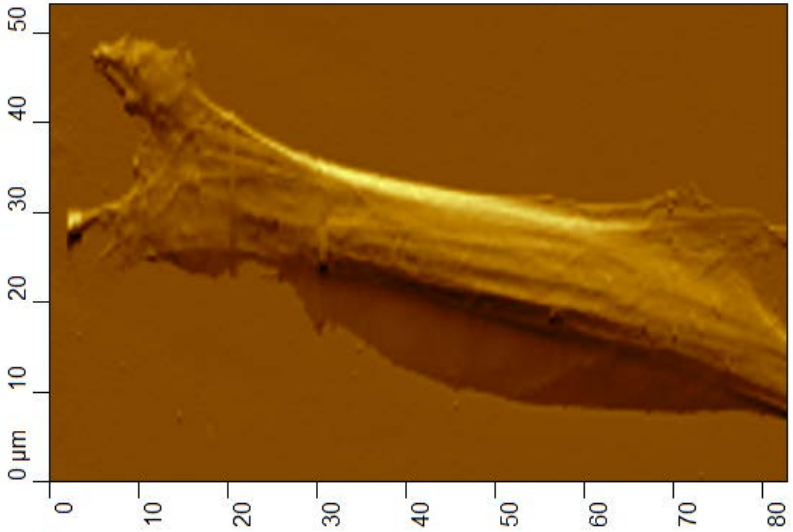


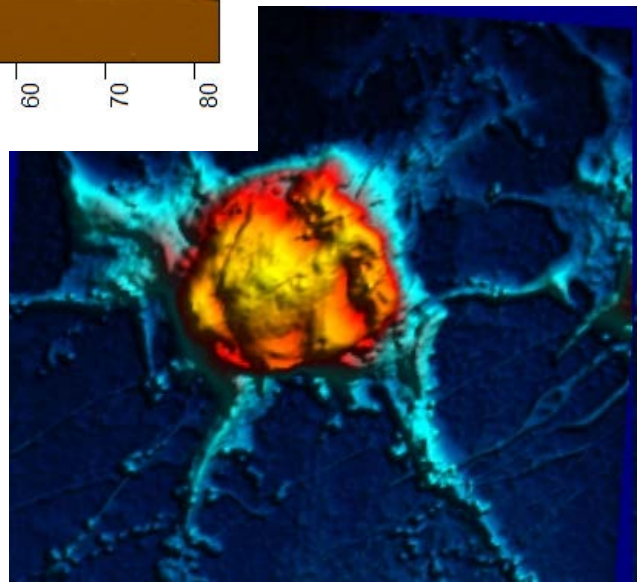


5. Eukaryotic cells

A. Standard culturing on polystyrene dishes

Adhesive protein layers usually takes place (i.e. pLL, RGD adhesion factors, fibronectin, etc.)



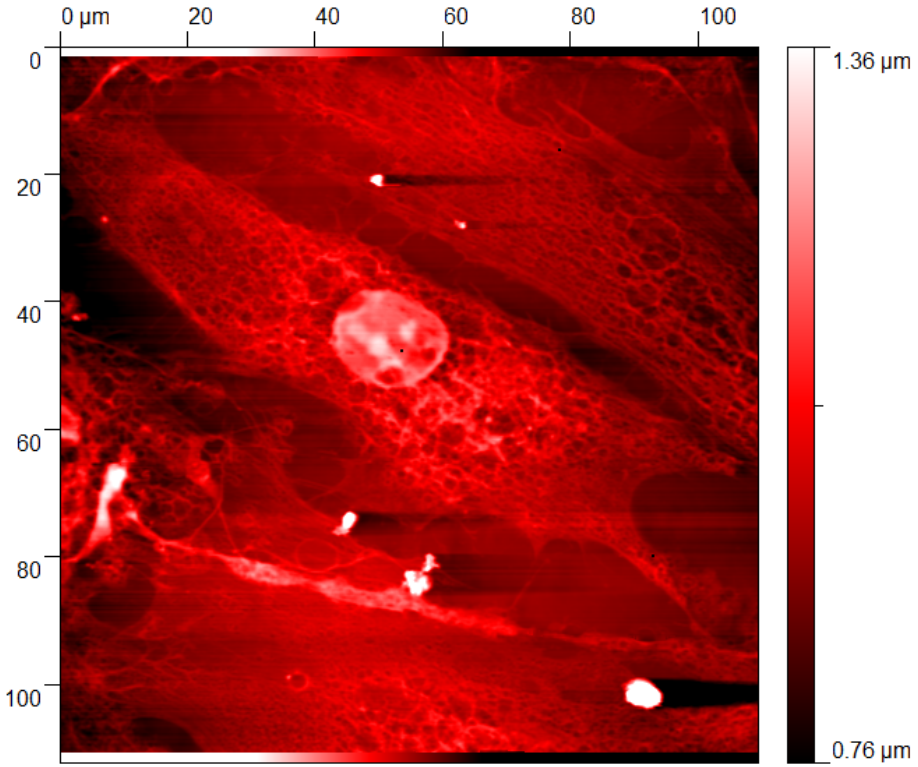


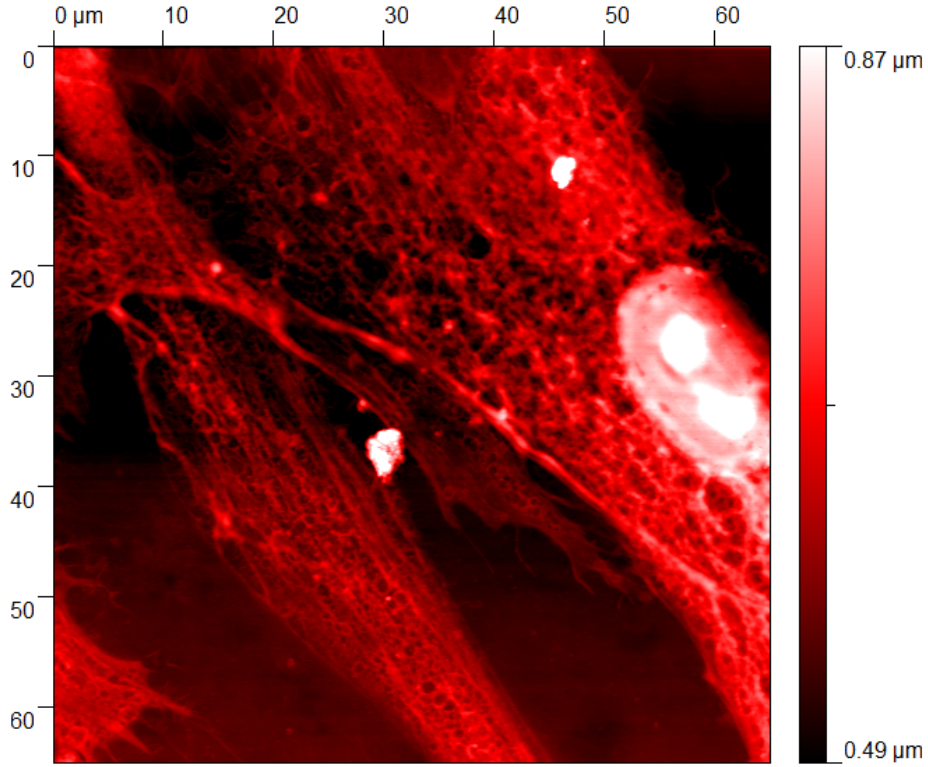
Cell culturing equipment

BioAFM incl. Petri dish **heater** for in-vitro imaging of **cell cultures**

B. Fixation agents

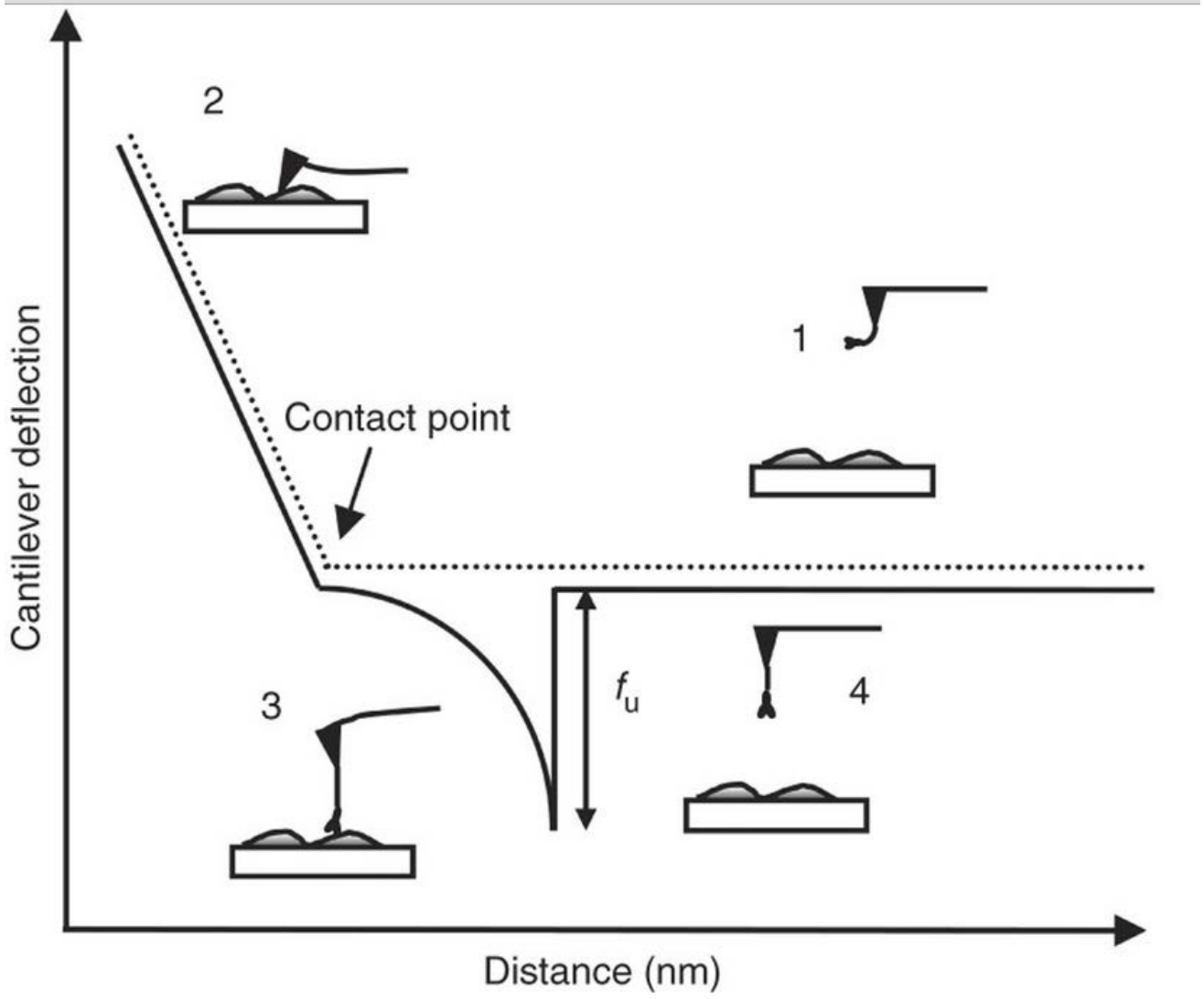
- Adhesion of cells out of incubator (37°C, 5% CO_2) is mostly problematic
- Allows study of cells in long term periods after removal from incubator
- Cell wall destruction
- Example: EtOH, acetic acid, paraformaldehyde, glutardialdehyde



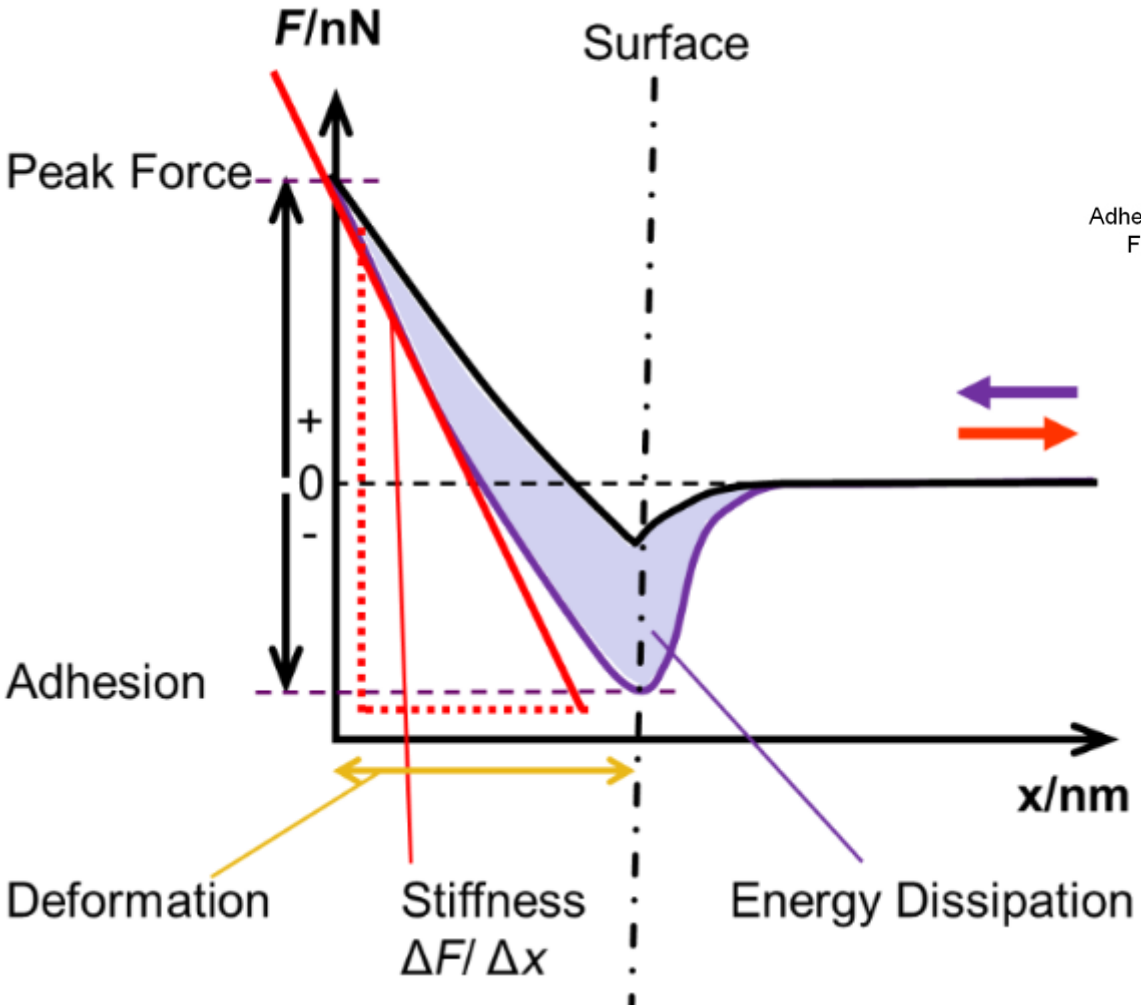


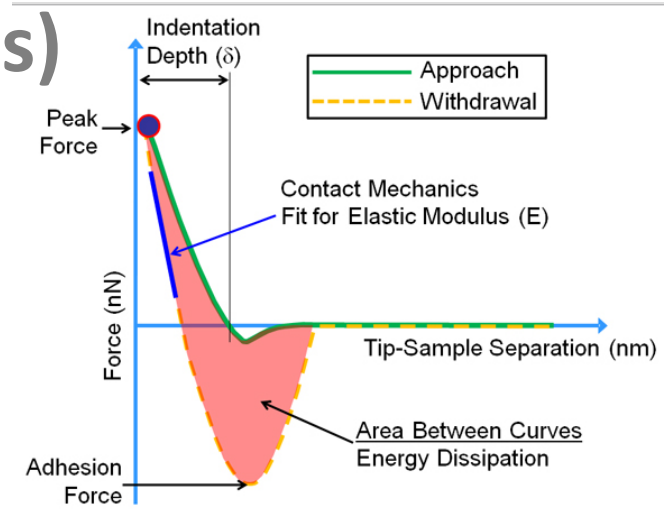
AFM spectroscopy

Force Distance curves (FD curves)



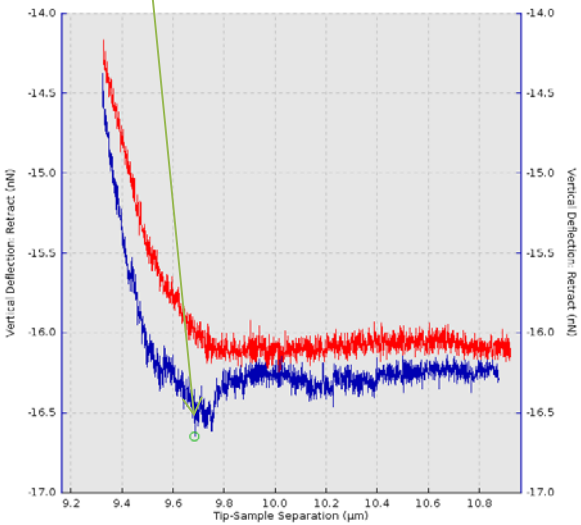
Force Distance curves (FD curves)



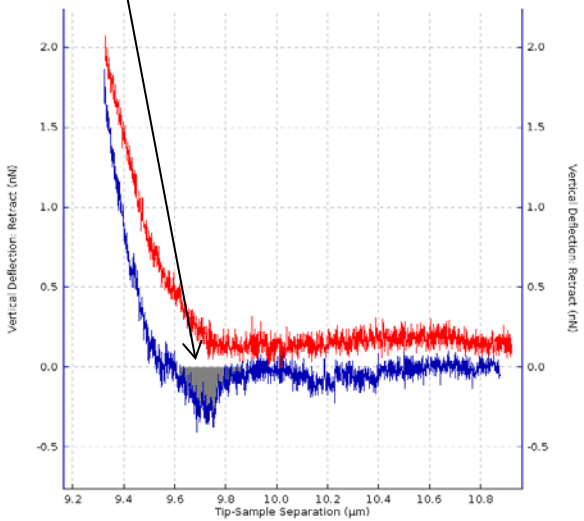


Evaluation of curves containing binding ‘event’

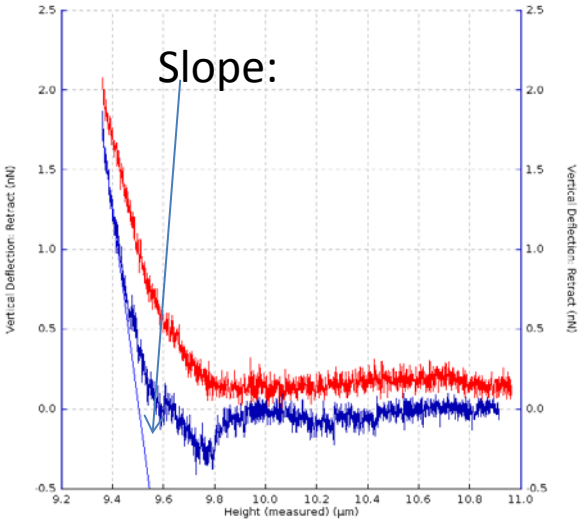
Minimum value:



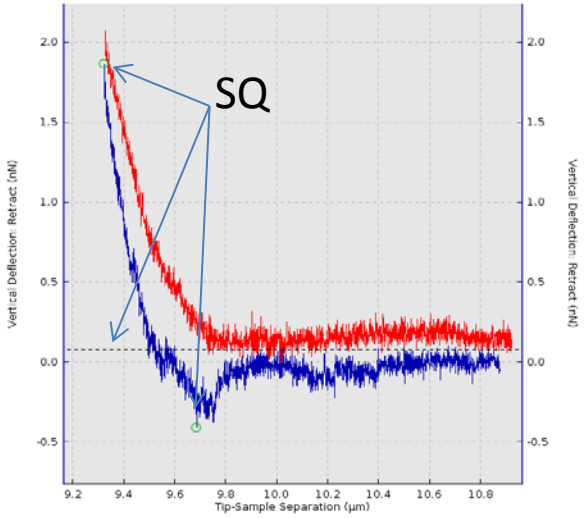
Area under the curve:



Slope:

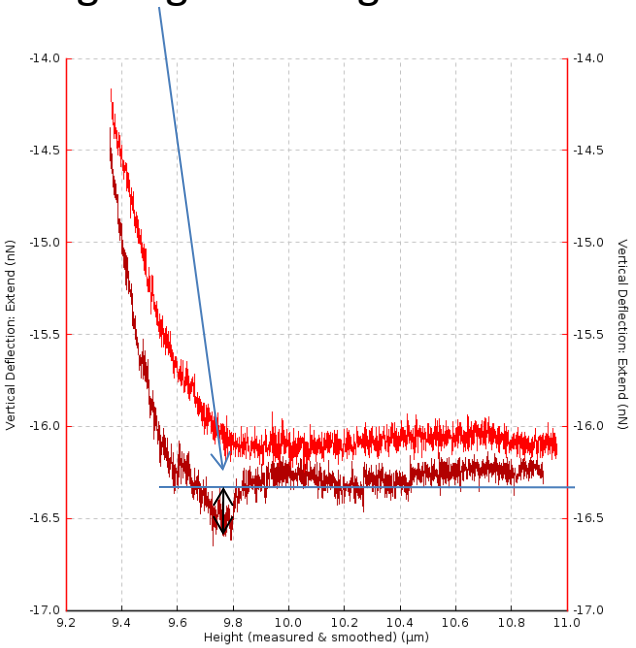


SQ

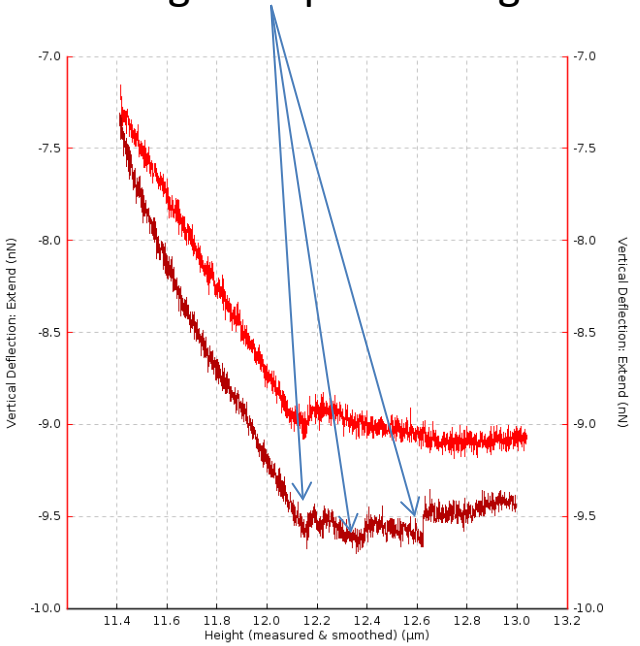


Types of FD curves

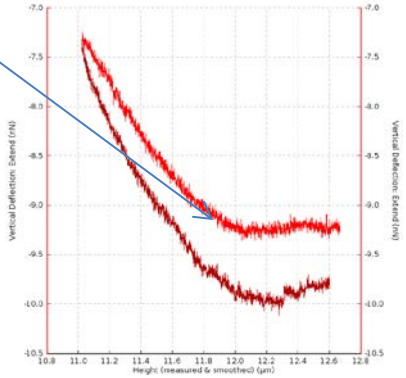
Containing single binding event



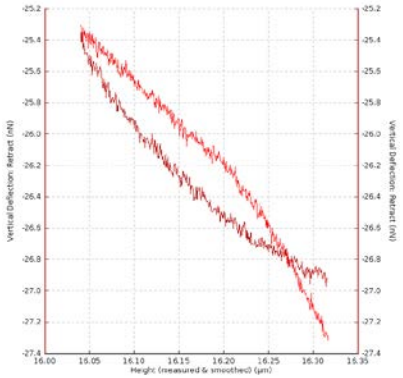
Containing multiple binding events



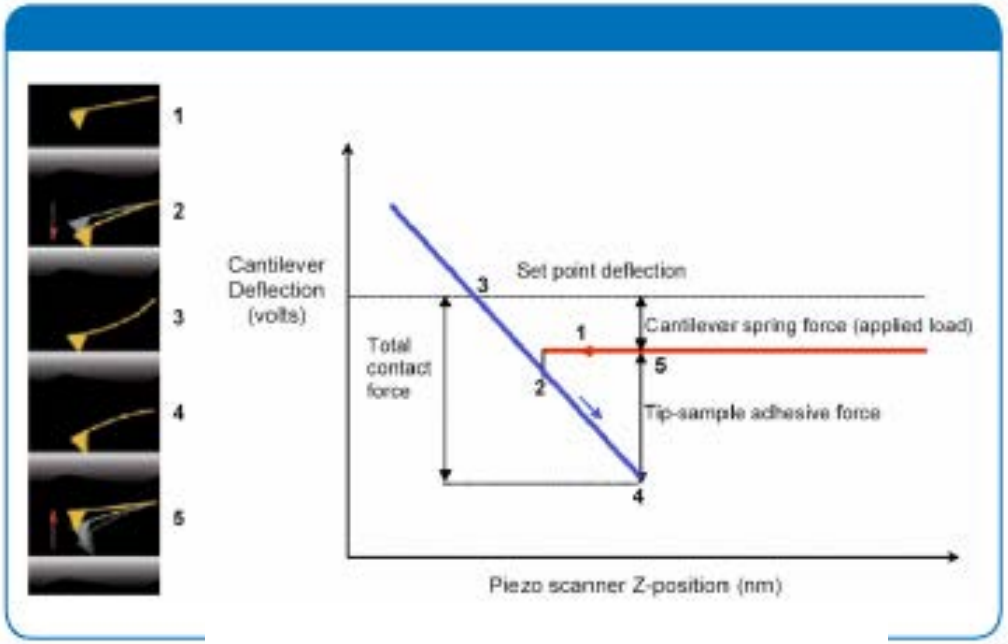
No interaction between tip and surface
(Young's modulus can be determined)



Useless curve

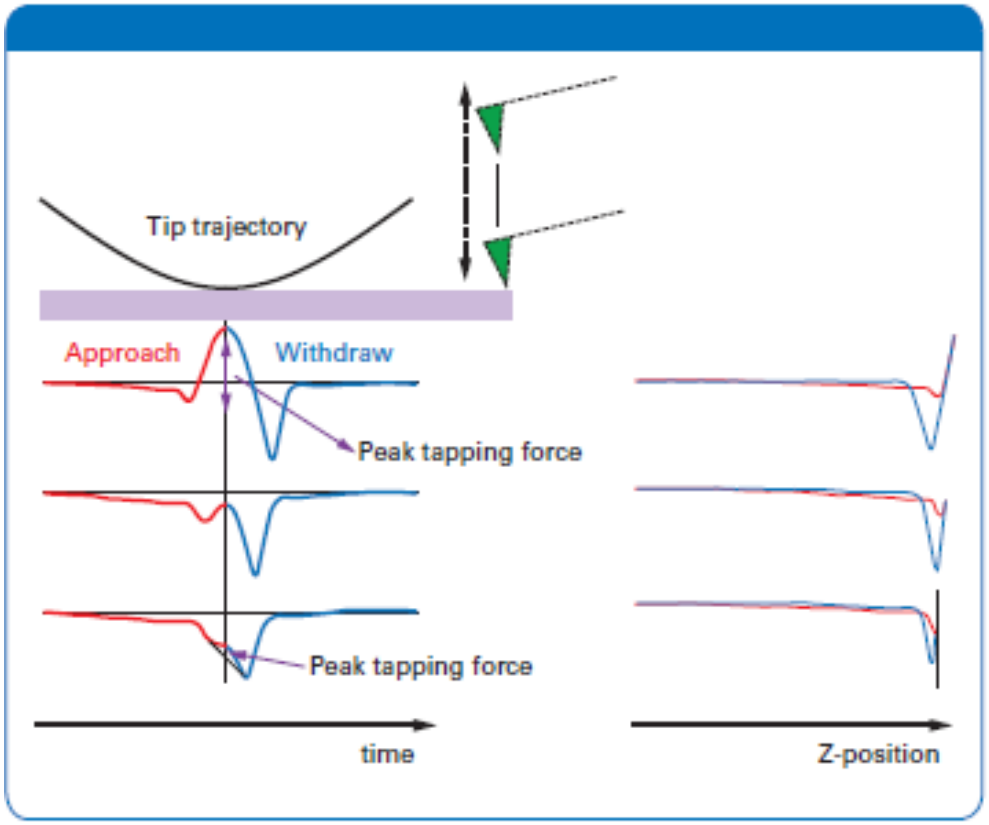


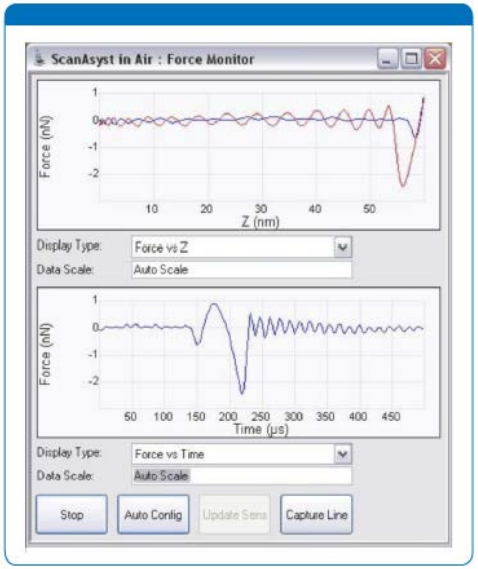
ScanAssyst – automatic AFM

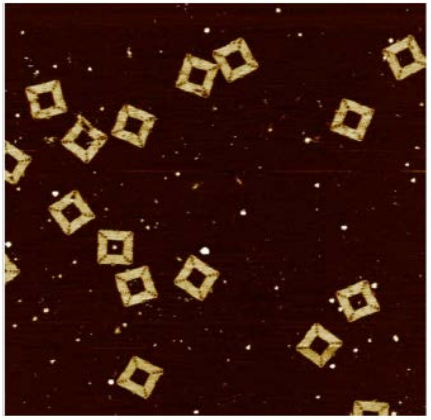


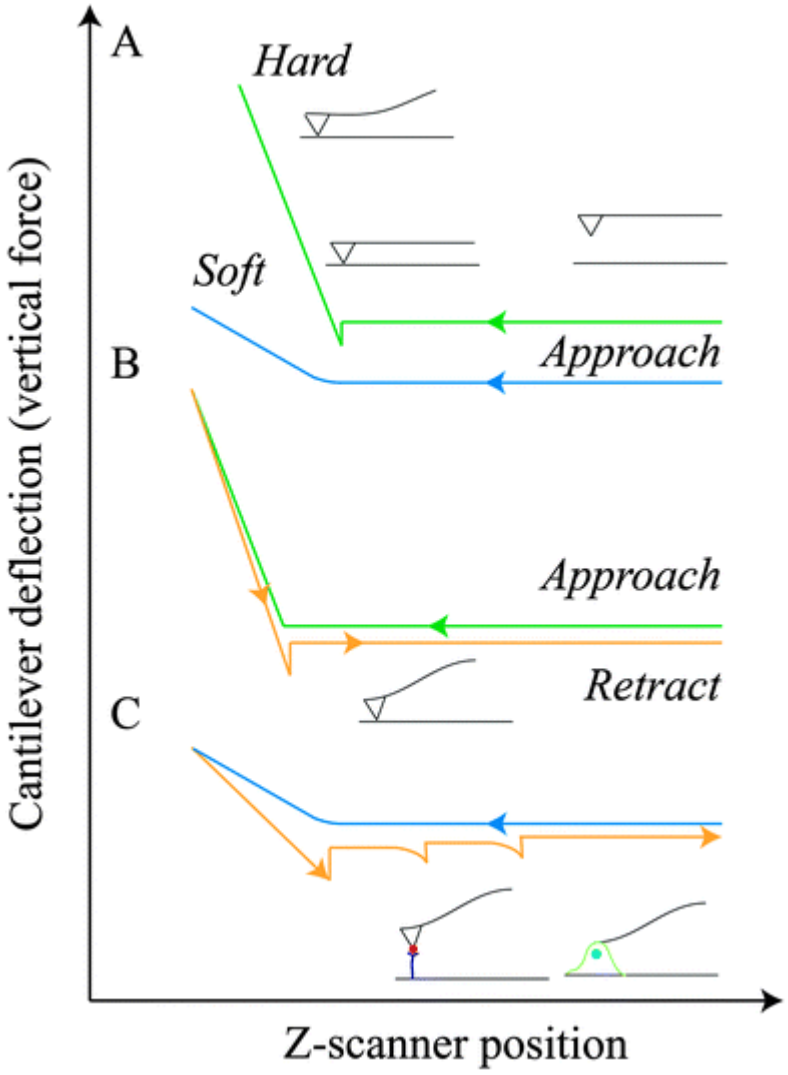
BRUKER

ScanAssyst - principle

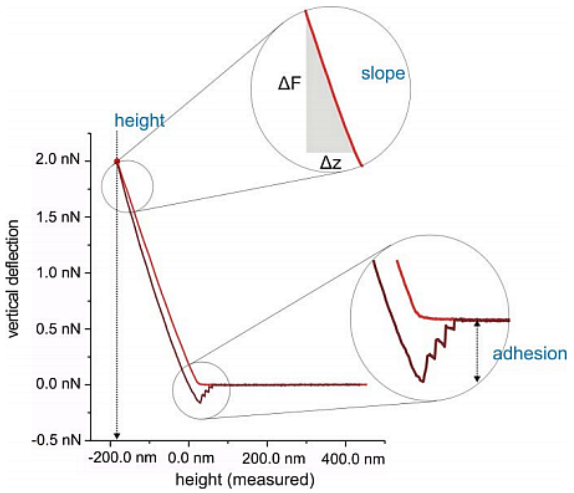




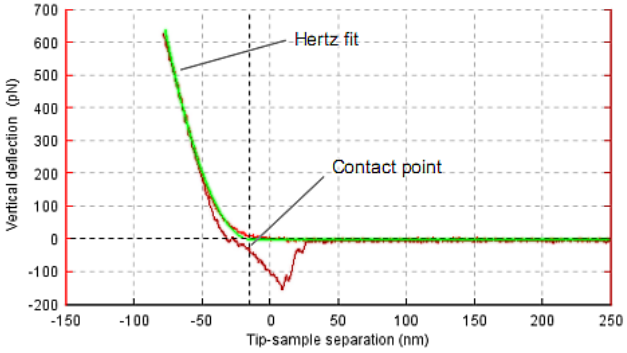


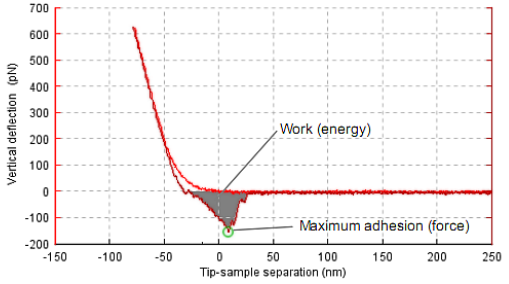


QI mode



Hertzian fit





(A) Typical force–distance curves for hard (green) and soft (blue) materials. (B) Adhesion on a hard surface. (C) Molecule–molecule and cell–surface detachment process with three unbinding events.

Phys. Chem. Chem. Phys., 2015, 17, 2950-2959

QI-imaging examples

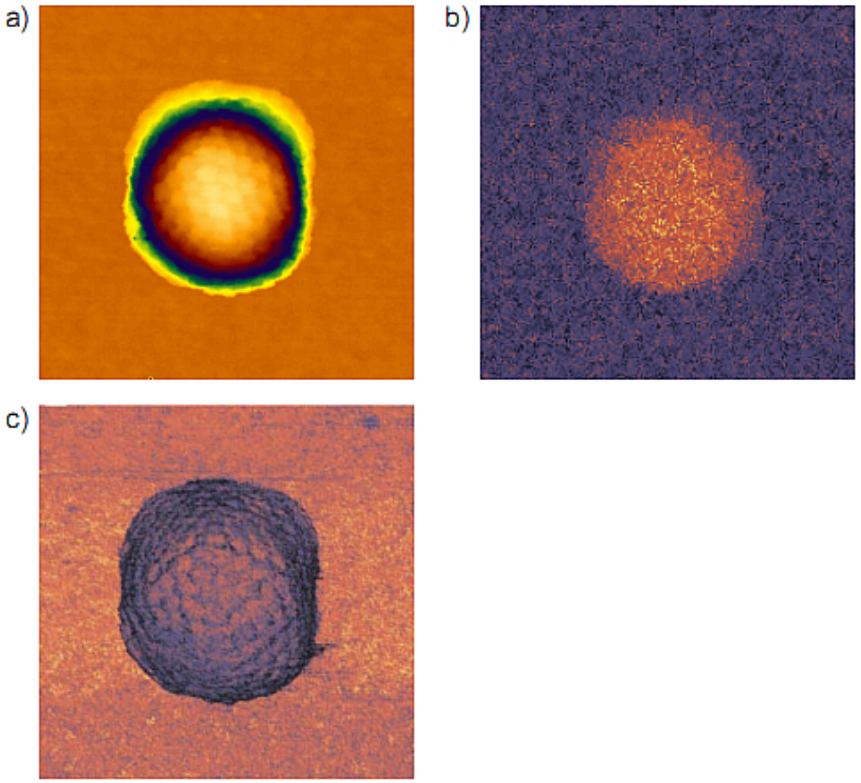


Fig. 10: *Herpes Simplex Virus capsid imaged in liquid, scan size 300 nm x 300 nm. a) Height image (z-range: 100 nm) shows substructure of the virus. b) In the adhesion image it is possible to detect the sticky virus (data range: 200 pN). c) the substructures can be also recognized in the elasticity image.*

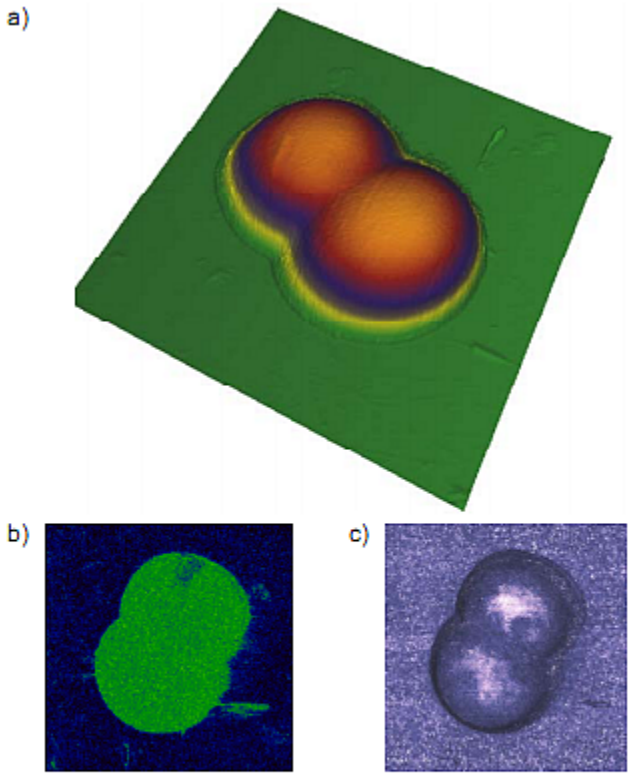
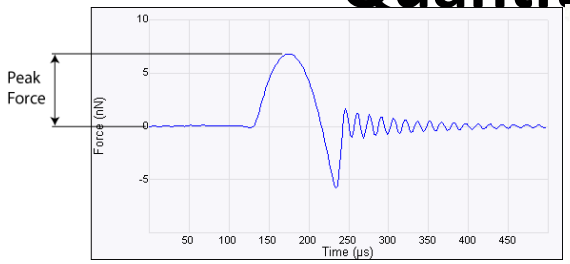
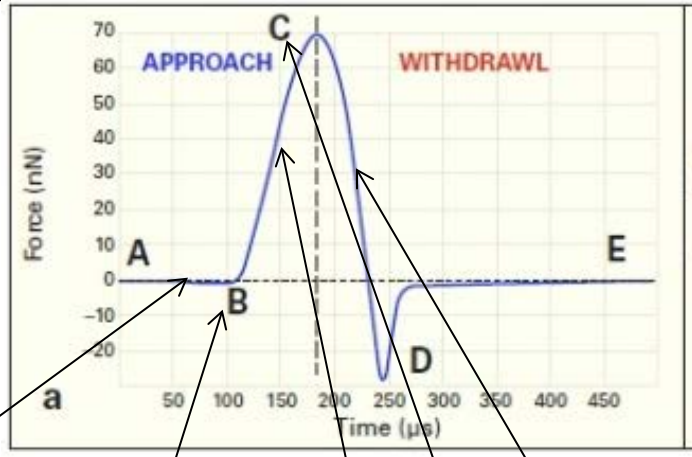


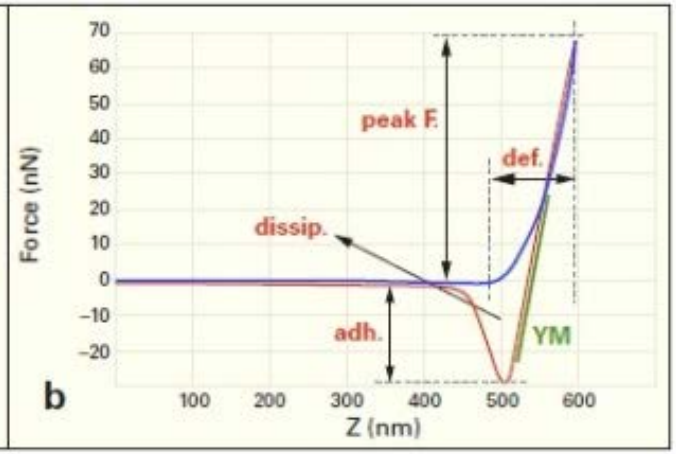
Fig. 6: *Living Cyanobacteria were measured in buffer solution. Scan size 10 µm x 10 µm, z-range 4 µm.*
a) 3D Topography of the Cyanobacteria.
b) Elasticity image (data range: 40 kPa) shows the softness of the bacteria.
c) Adhesion image (data range: 100 pN) illustrates a higher adhesion region on top of the bacteria

JPK supporting info

Quantitative NanoMechanics (QNM)







attractive forces (capillary, VdW, elstat)

negative forces > cantilever' stiffness

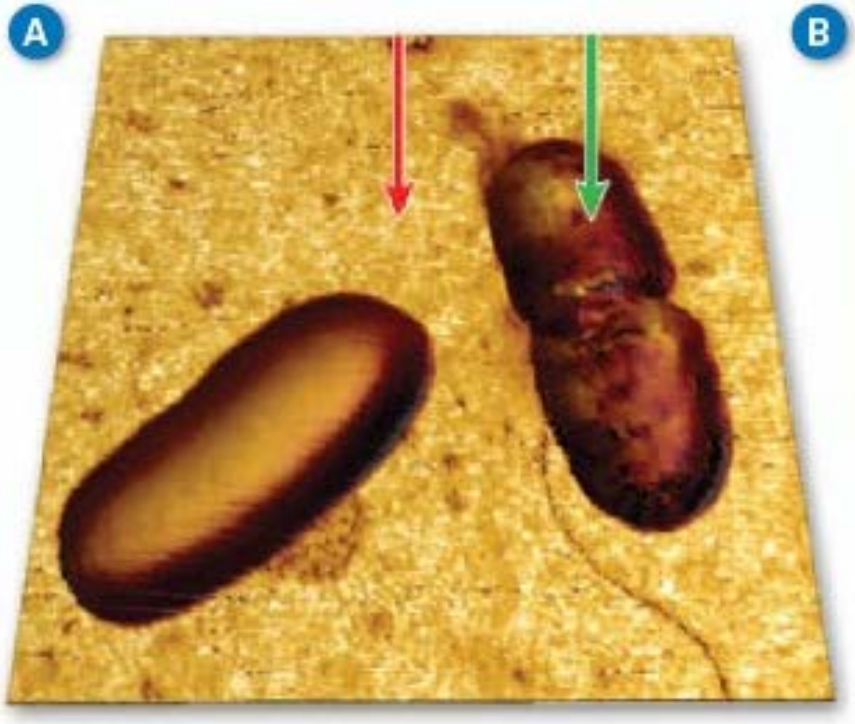
indentation

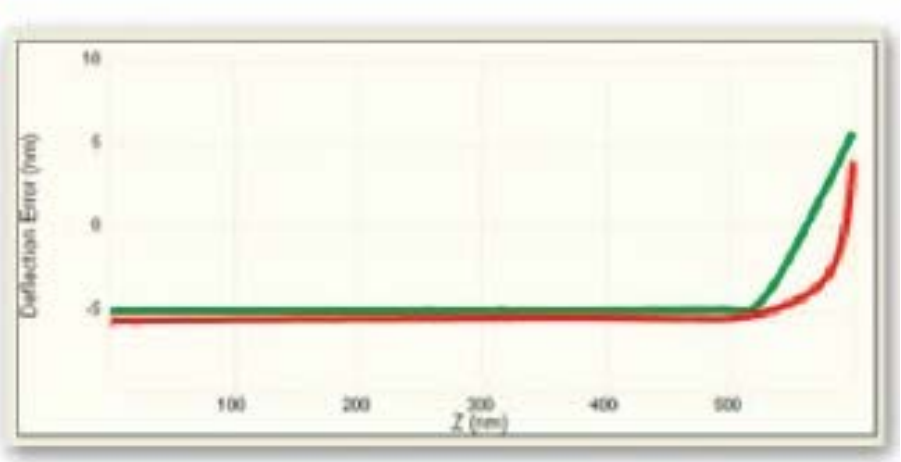
withdrawing

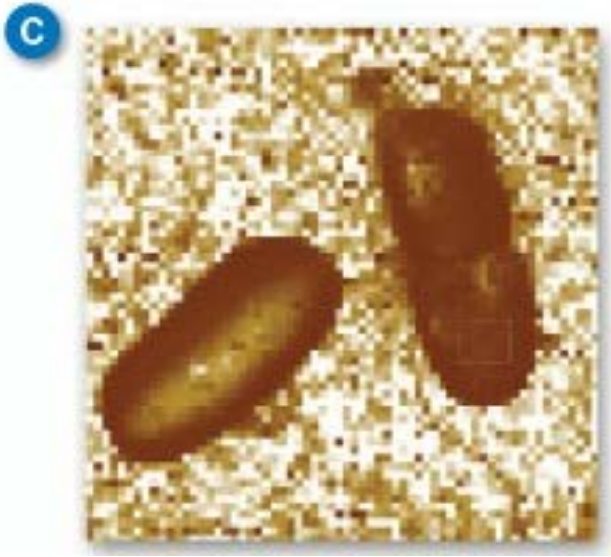
Peak force → feed back control

PeakForce QNM = quantitative nanomechanical information (biological samples without damaging)
Based on **Peak Force Tapping technology** - probe is oscillated (~TappingMode), res. freq 1 - 8 kHz (=sampling rate) depending on the tool).
Difference:
Tapping Mode – const. amplitude,
Peak Force Tapping maximum peak force on the probe (much lower comparing to contact mode – biological samples)

PeakForce QNM on Bacteria



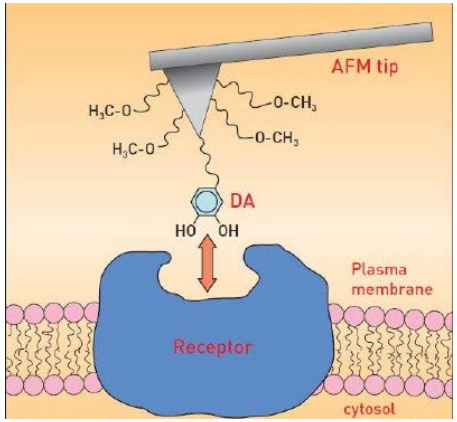


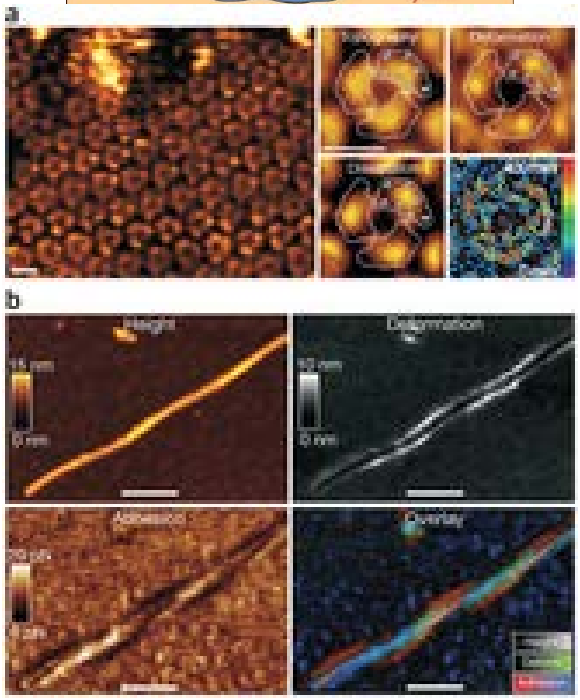


(A) PeakForce QNM (250Hz) Sneddon modulus
(B) PeakForce curves
(C) Force volume Sneddon modulus image of the same bacteria collected at a ramp rate of 2Hz. (Standard DNP-A probe in water with 300nm modulation amplitude, Scan size 5µm.)

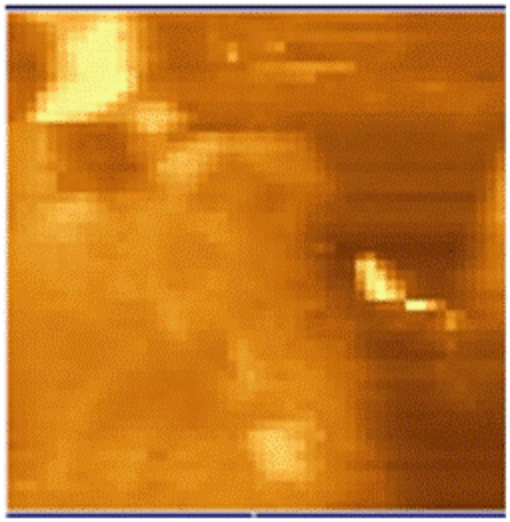
AFM force mapping

Examples

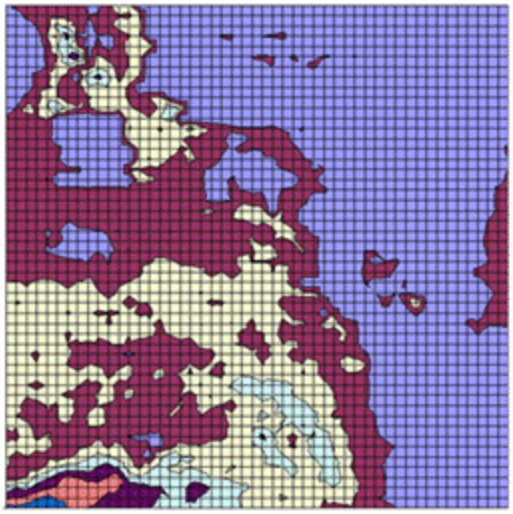




Mapping receptors on living cells under physiological conditions



Topography (100 x 100 µm)

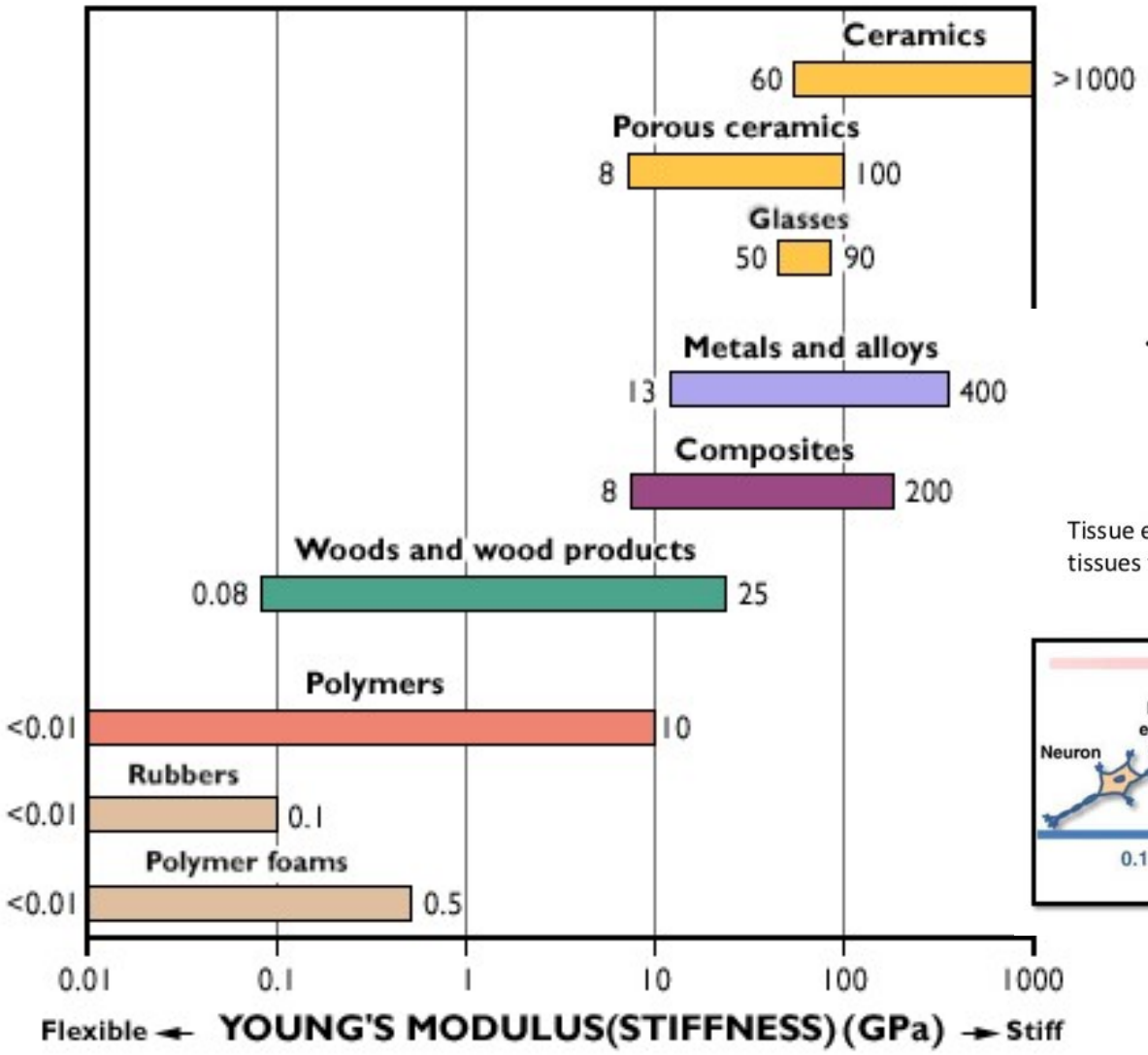


Adhesion (100 x 100 µm)

Material properties mapping by AFM

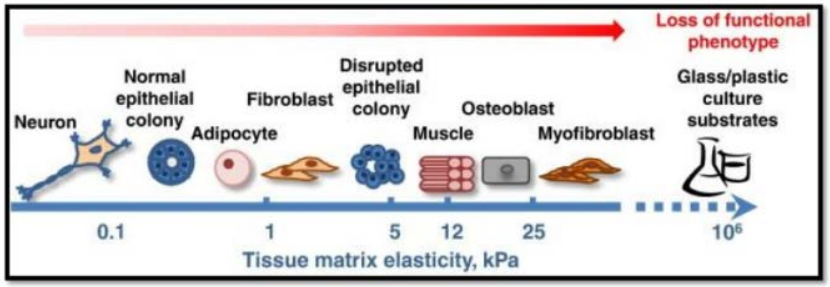
Young's modulus mapping

Young's modulus of materials



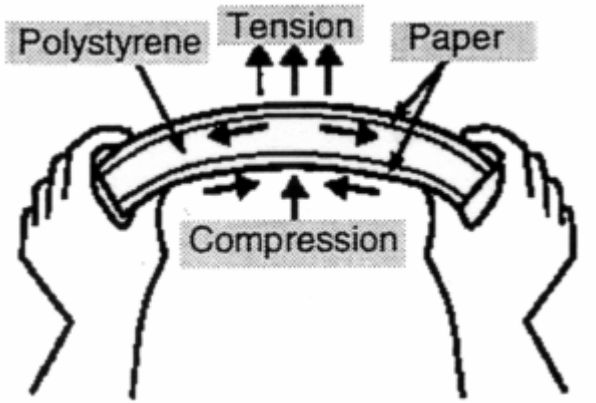
Tissue's Young Modulus

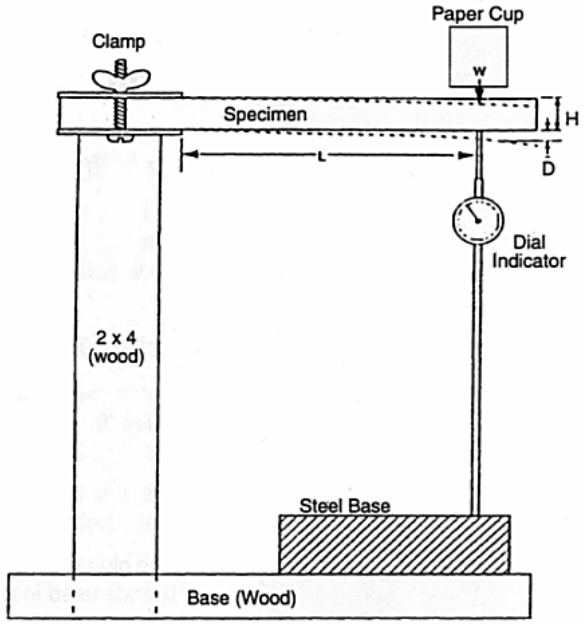
Tissue elastic modulus (E) is given by the resistance offered by the tissues to deformation effects, i.e. the tissue stiffness.



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

Methods for YM measurement



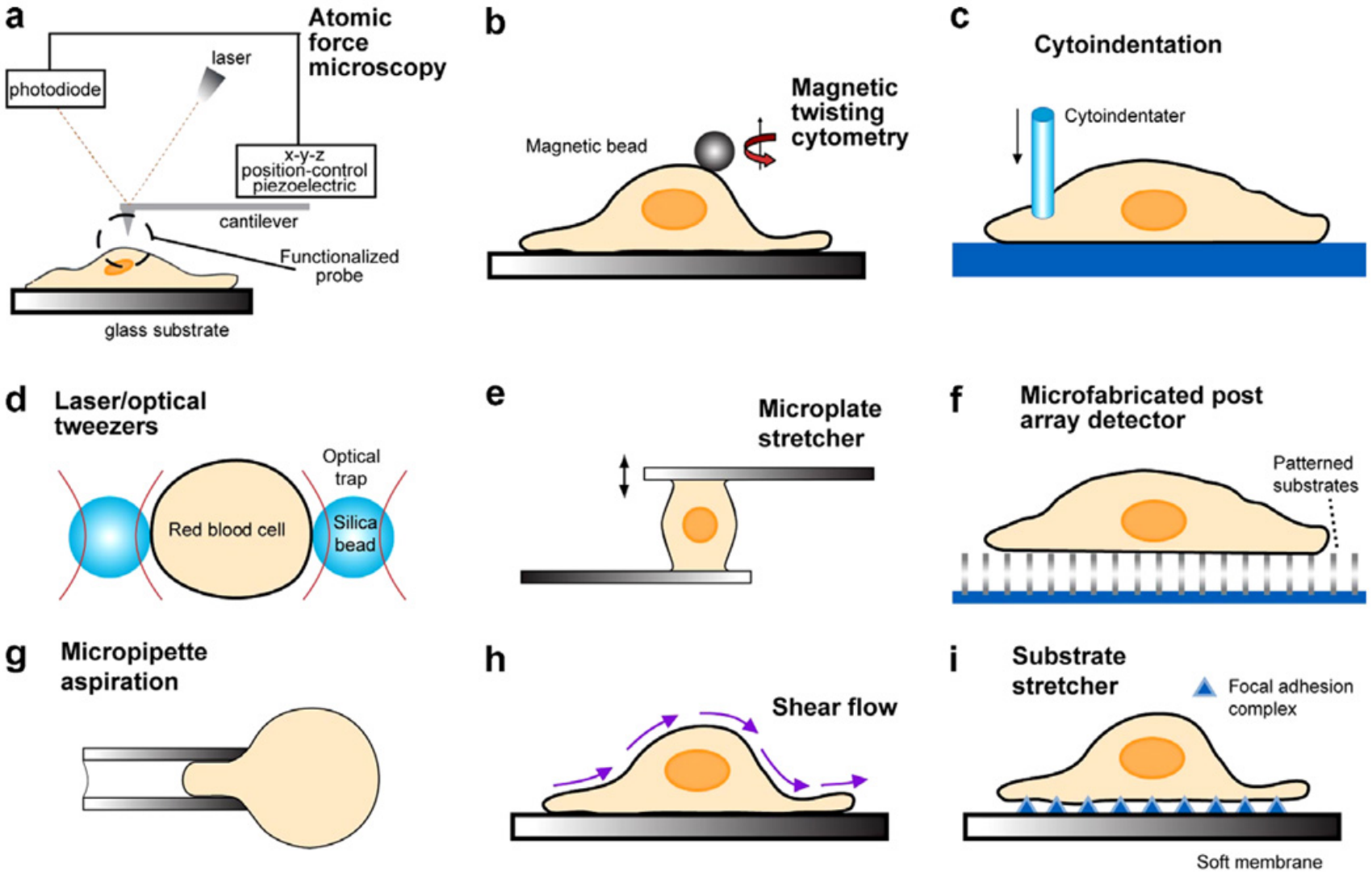


Olympus 38DL PLUS

Measure the longitudinal and shear wave sound velocity of the test piece using the appropriate transducers and instrument setup.

Reference: Battelle PNNL MST Handbook, U.S. Department of Energy, Pacific Northwest Laboratory

Cell Young's modulus - methods



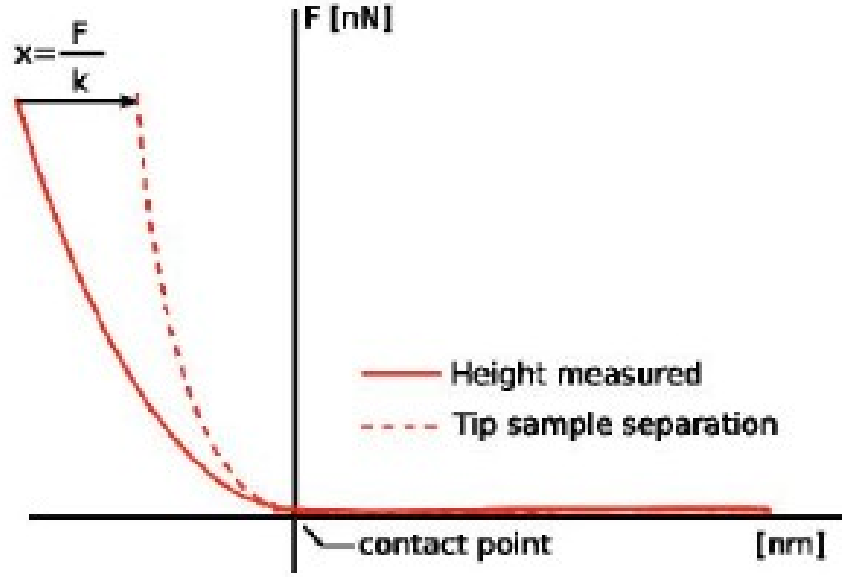
Acta Biomater. 2007 Jul; 3(4): 413–438.

Hertzian fit

Measured curves were fitted to following function:

$$F(\delta) = \frac{4}{3}\frac{E}{(1-\nu^2)}\sqrt{R}\,\delta^{3/2}.$$

where *F* is force, ***E* is Young modulus**, α – face angle, δ – tip-sample separation, ν – Poisson ratio:



Tip-sample separation = correction of measured curve (height) for cantilever bending

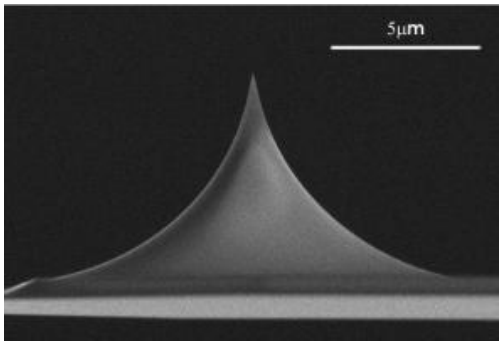
Parabolic tip shape

Parabolic

$$F = \frac{4\sqrt{R_C}}{3}\frac{E}{1-\nu^2}\delta^{3/2}$$

$$a = \sqrt{R_C\delta}$$

R_c = radius of tip curvature



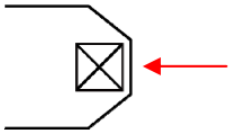
Four sided pyramid

Four-sided pyramid

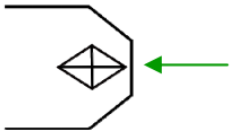
$$F = \frac{E}{1-\nu^2}\frac{\tan\alpha}{\sqrt{2}}\delta^2$$

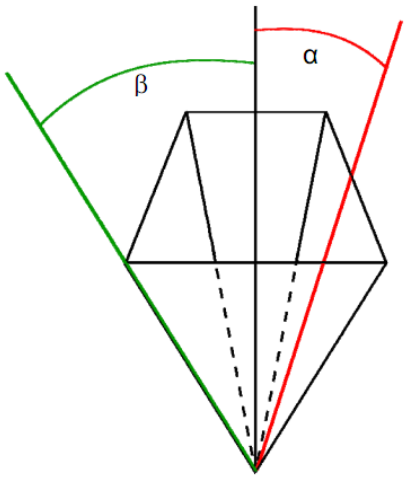
$$a = \frac{\tan\alpha}{\sqrt{2}}\delta$$

α = face angle, usually given for Si_3N_4-cantilevers



β = edge angle, usually given for Si-cantilevers





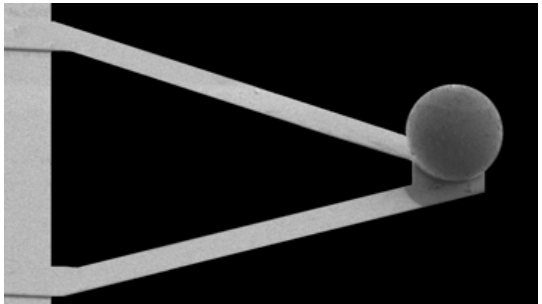
Spherical tip

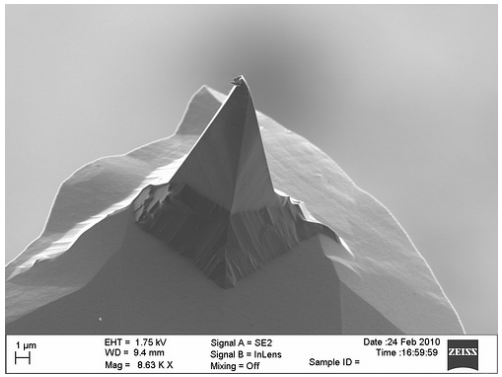
Spherical

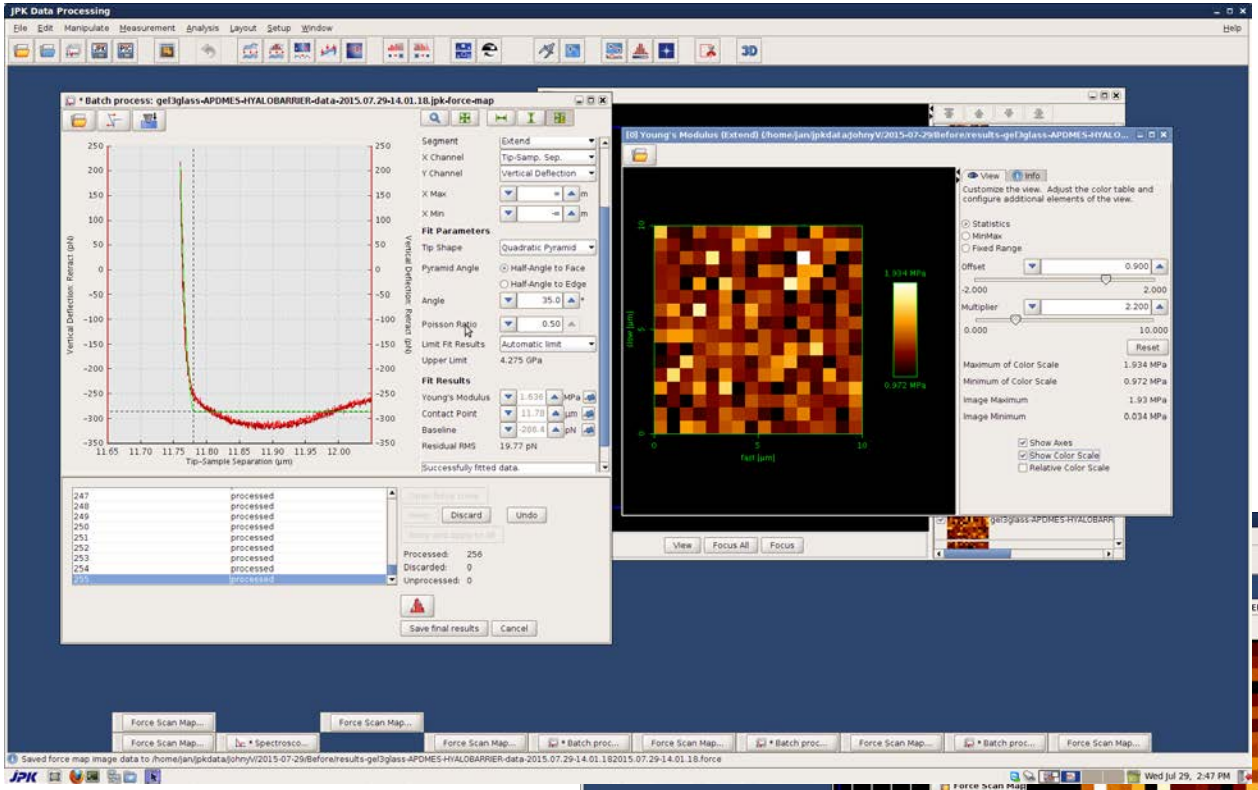
$$F = \frac{E}{1-\nu^2}\left[\frac{a^2+R^2}{2}\ln\frac{R+a}{R-a} - aR\right]$$

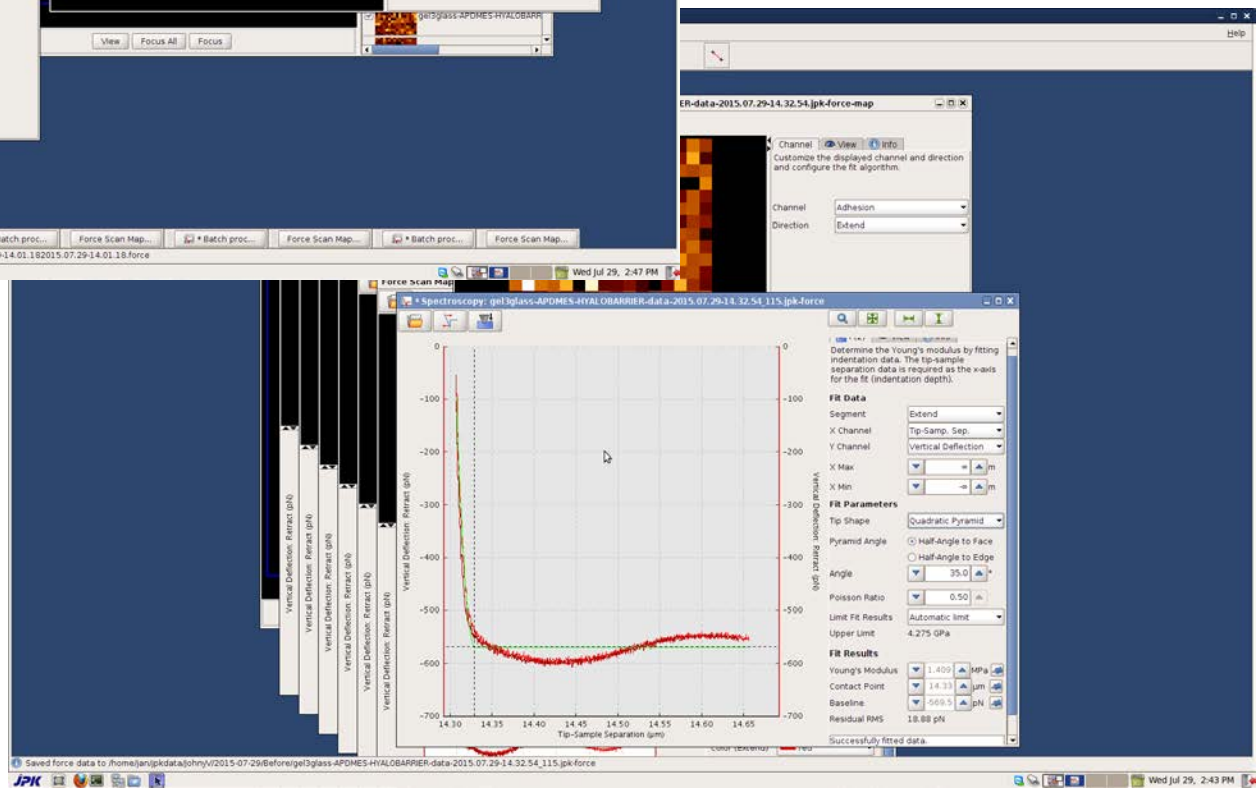
$$\delta = \frac{a}{2}\ln\frac{R+a}{R-a}$$

R = radius of the sphere



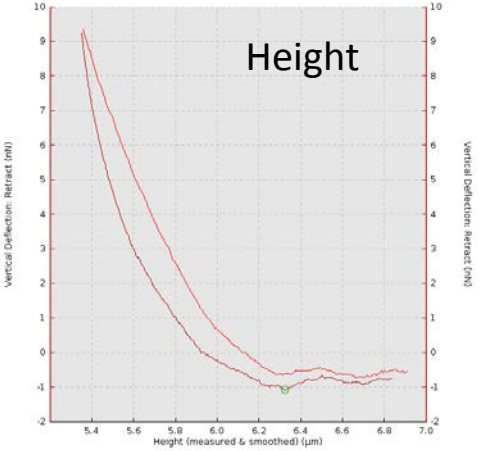


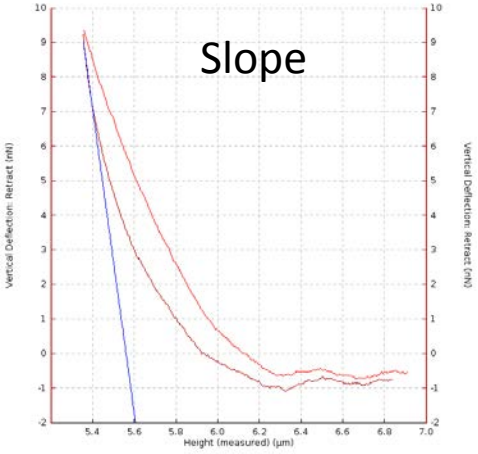


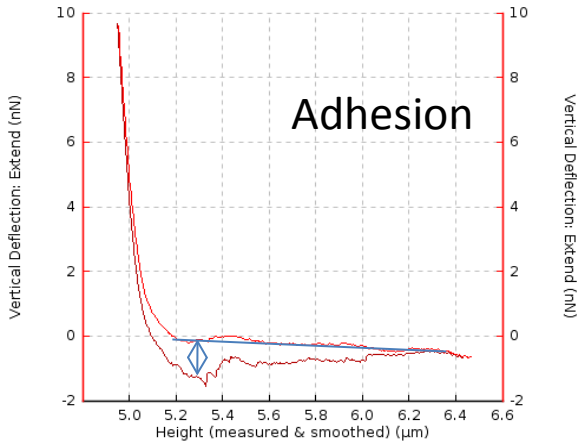


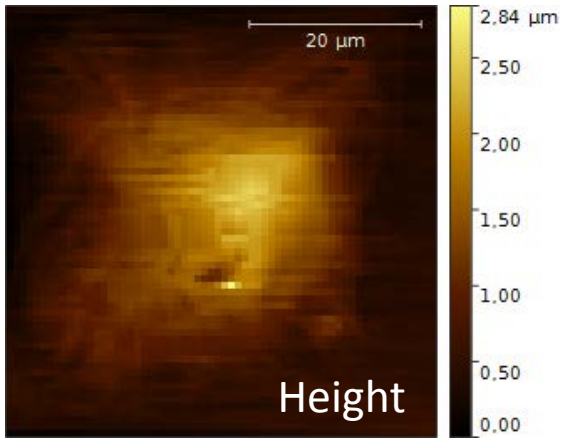
Examples of Force-distance curves and Force Maps evaluation

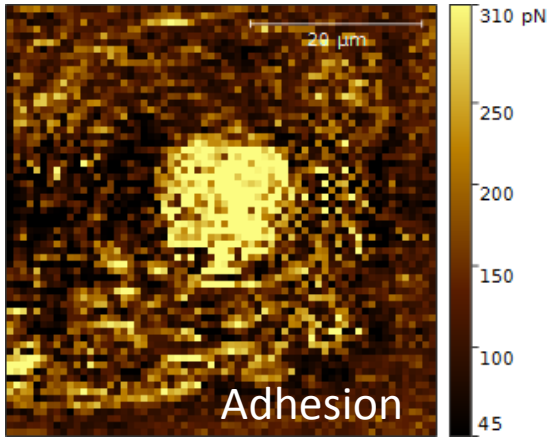
Force-distance curves

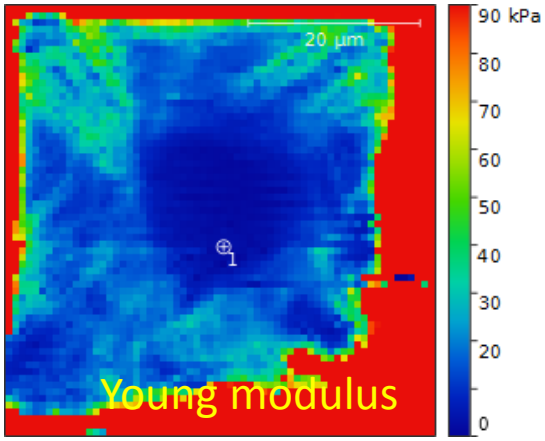






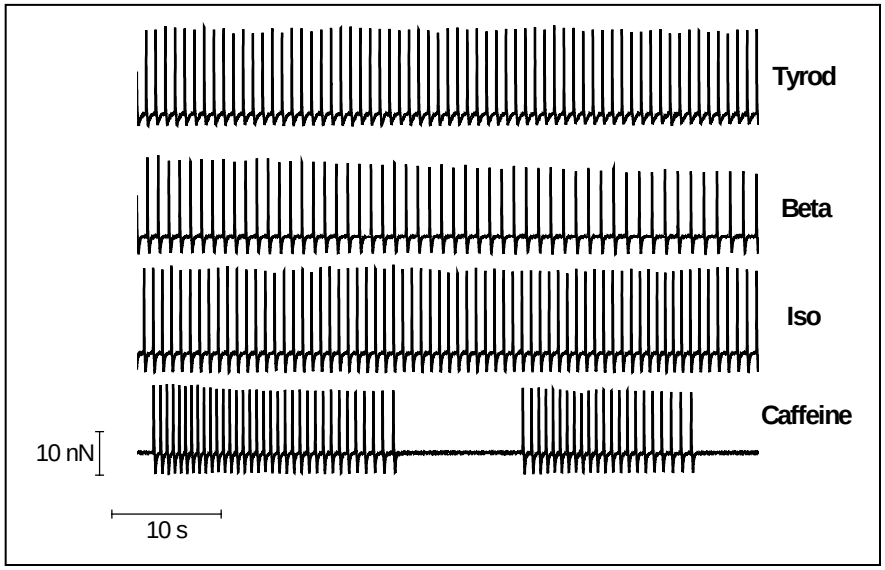


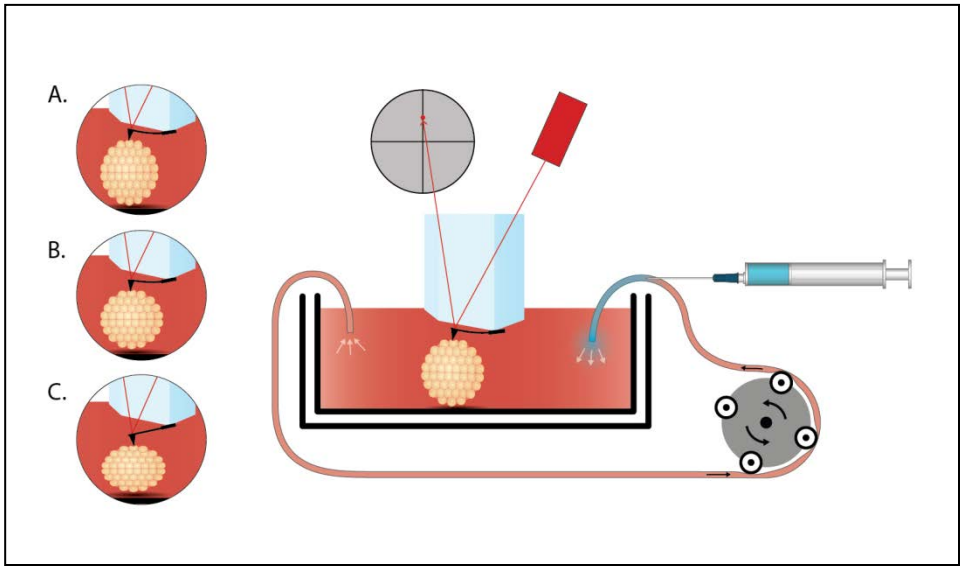


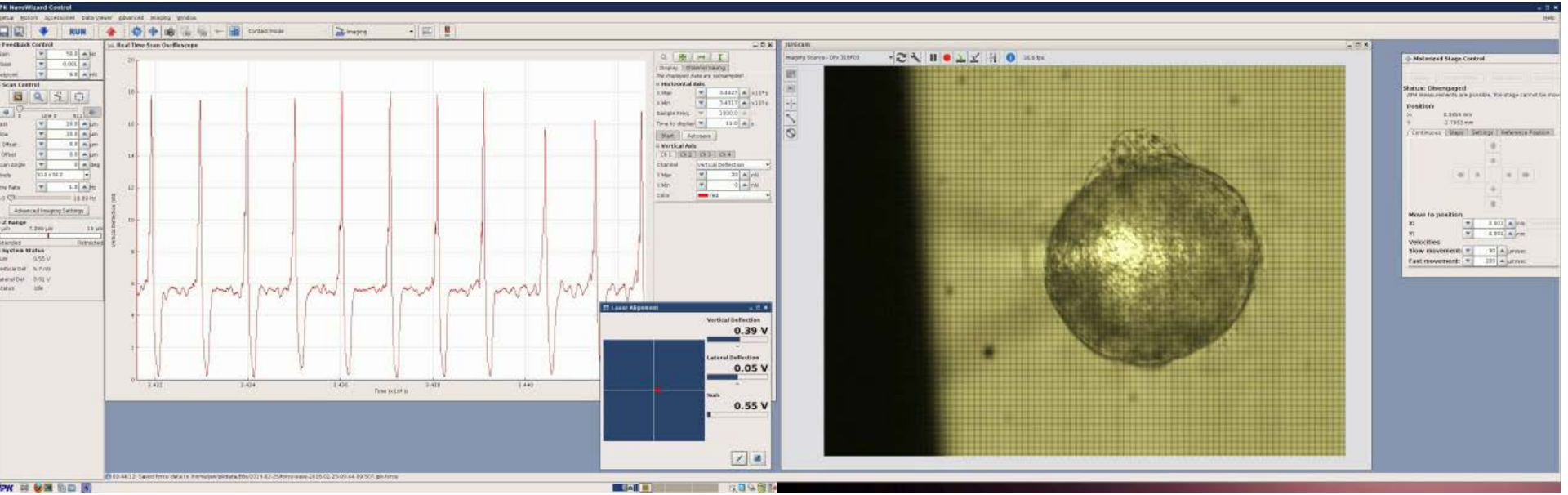


With **Giancarlo Forte, ICRC**

AFM in biomechanical characterization of cardiomyocytes





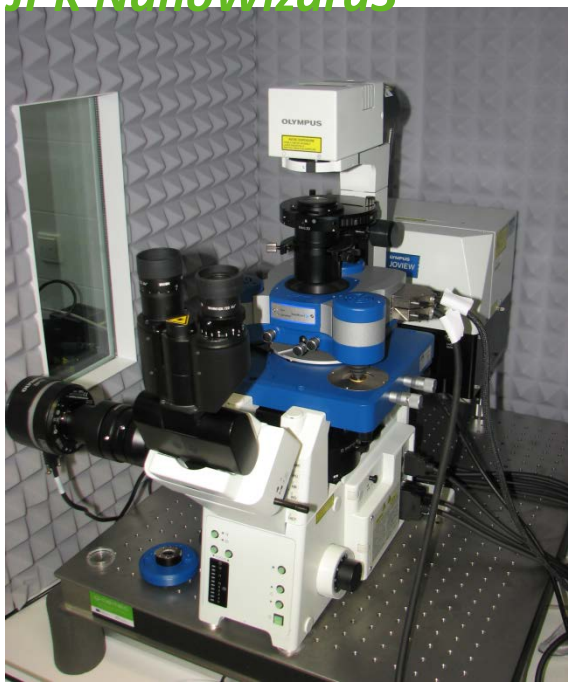


AFM CoreFacility

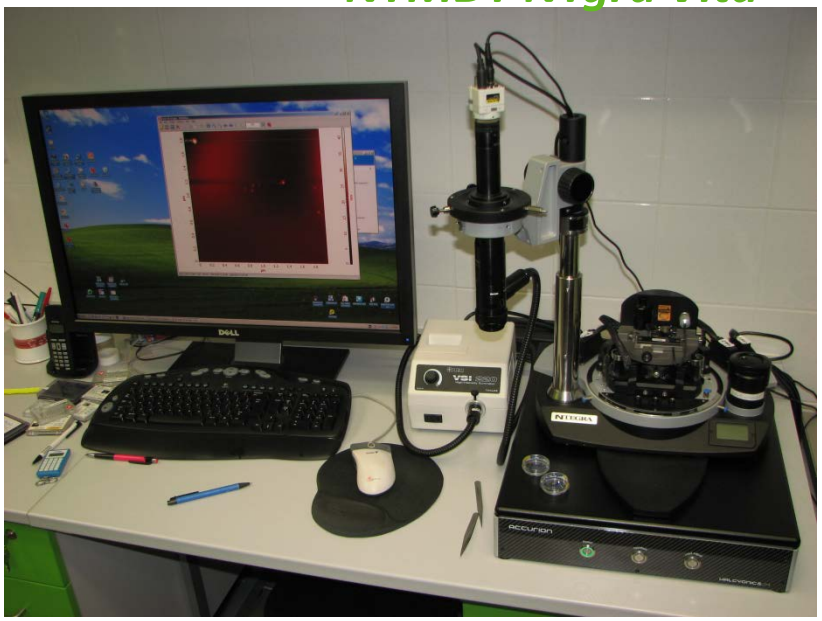
CEITEC MU

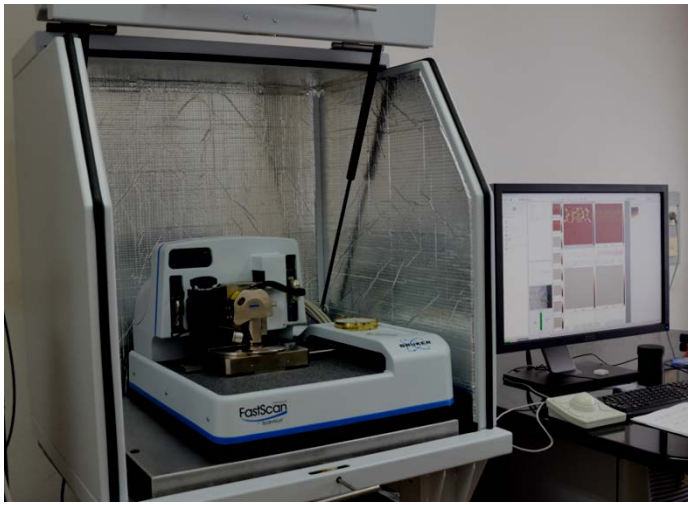
CEITEC AFM CoreFacility

JPK NanoWizard3

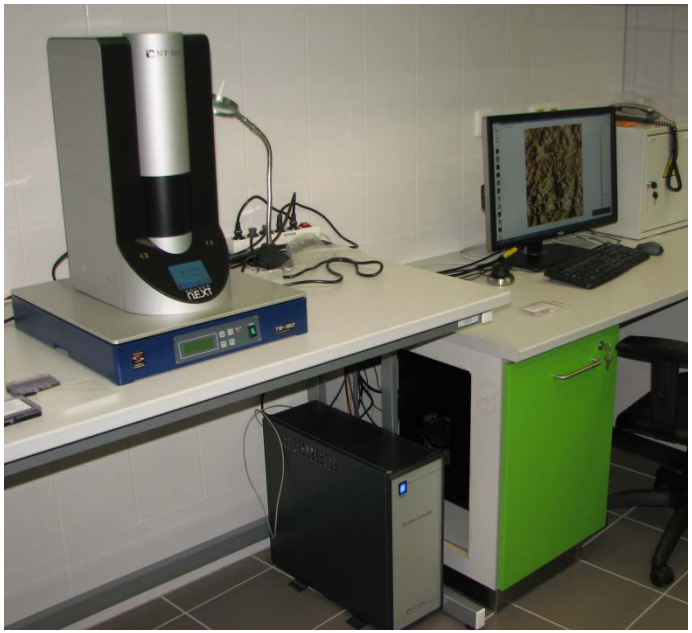


NTMDT NTgra Vita



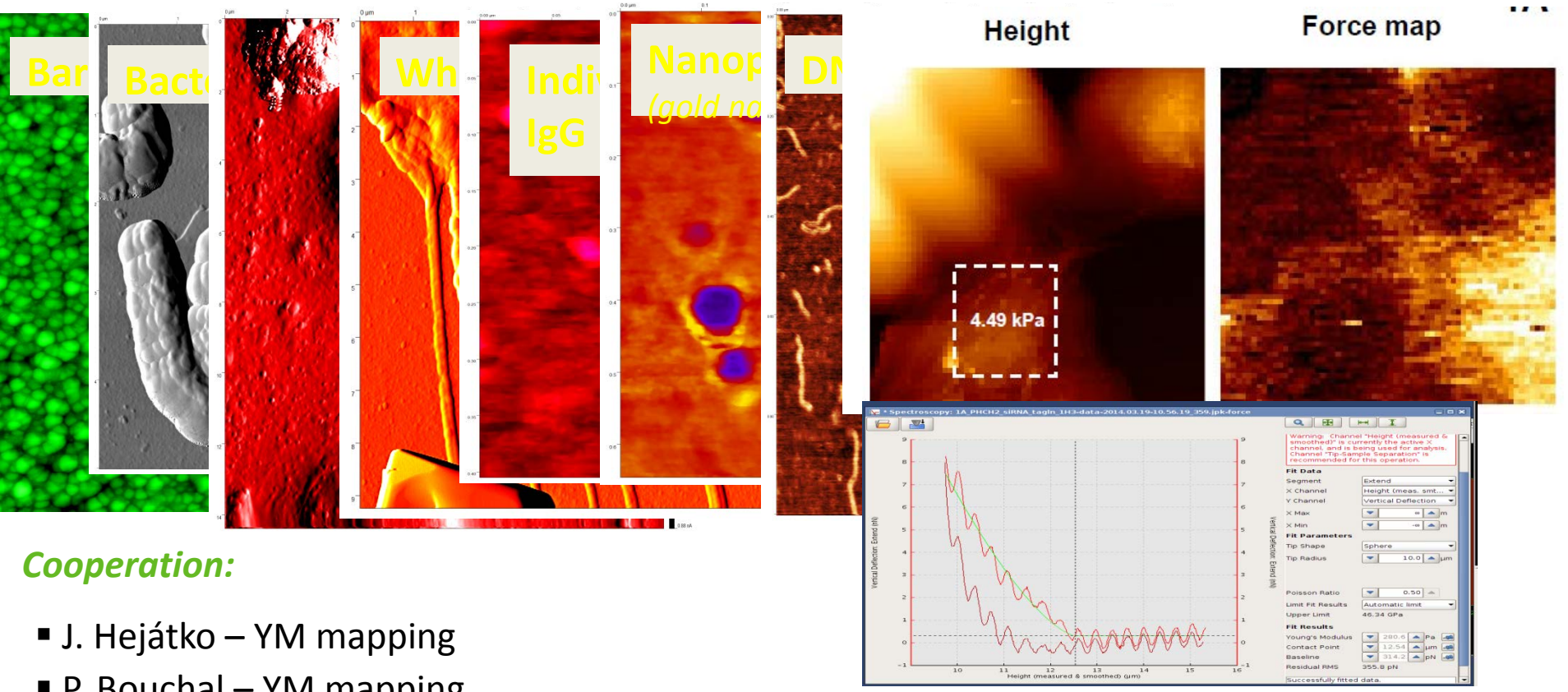


Bruker FastScan Bio



NTMDT Solver Next

AFM visualization of biomolecules and bioobjects



Cooperation:

- J. Hejátko – YM mapping
- P. Bouchal – YM mapping
- J. Paleček - DNA
- M. Pešl, V. Rotrekl CMCs
- J. Sládková – CMCs

- A. Meli - CMC
- M. Kalbáčová – TiO2 NT
- H. Kolářová - DNA
- I. Crha - sperms

Thank you for your attention!