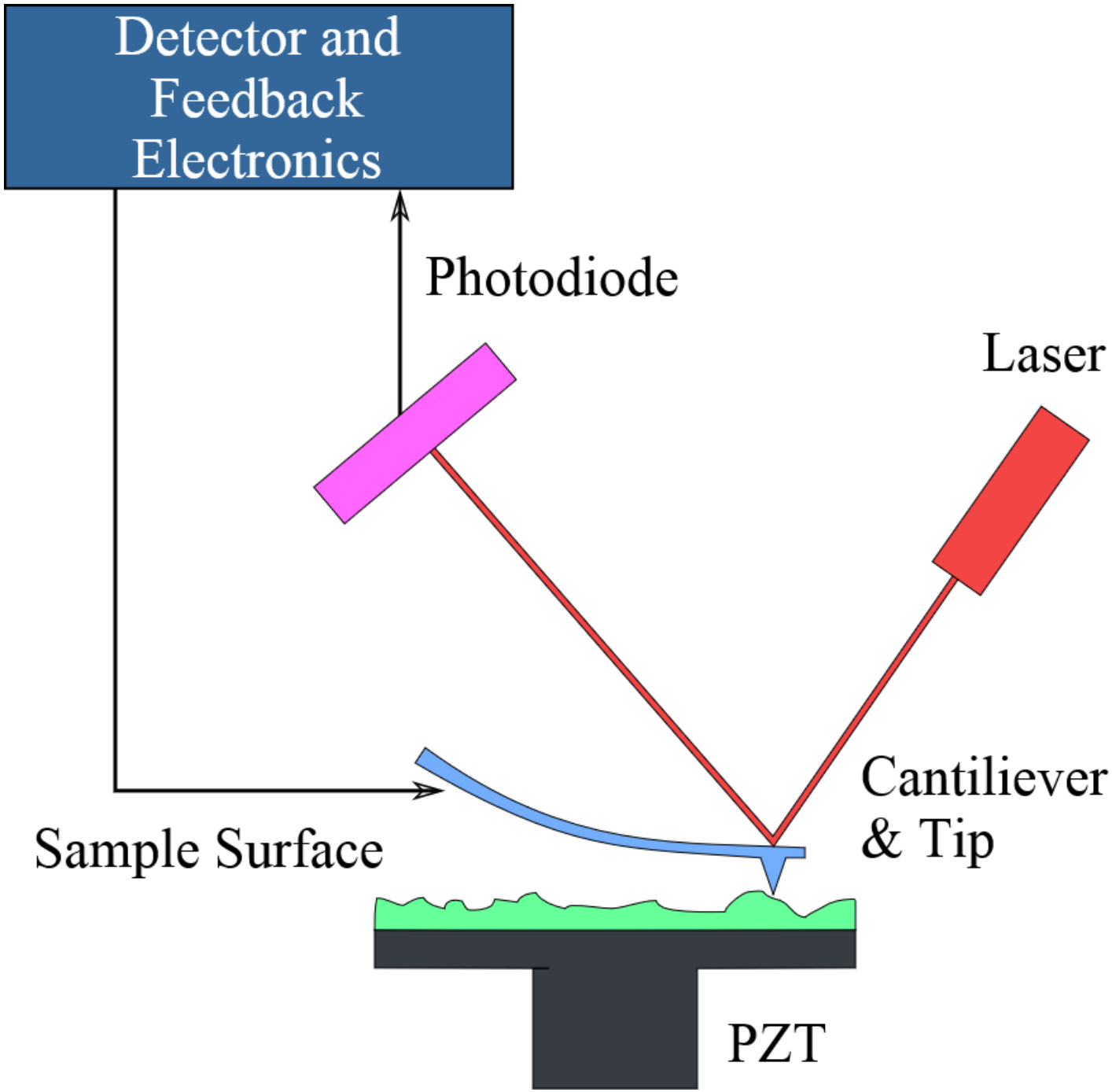
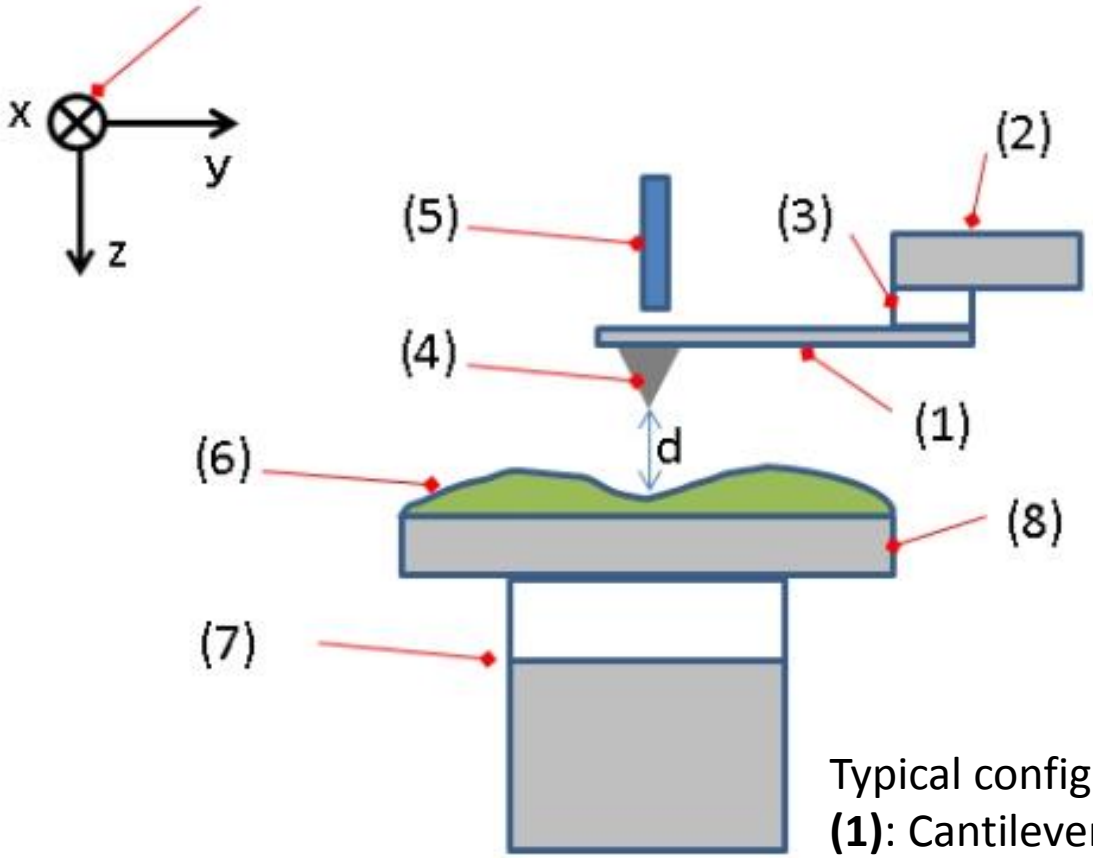
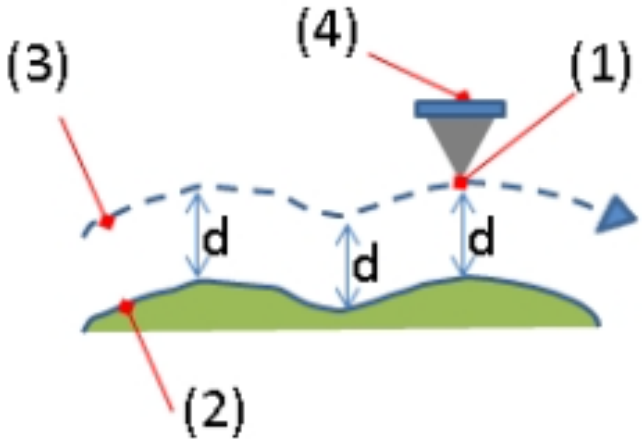
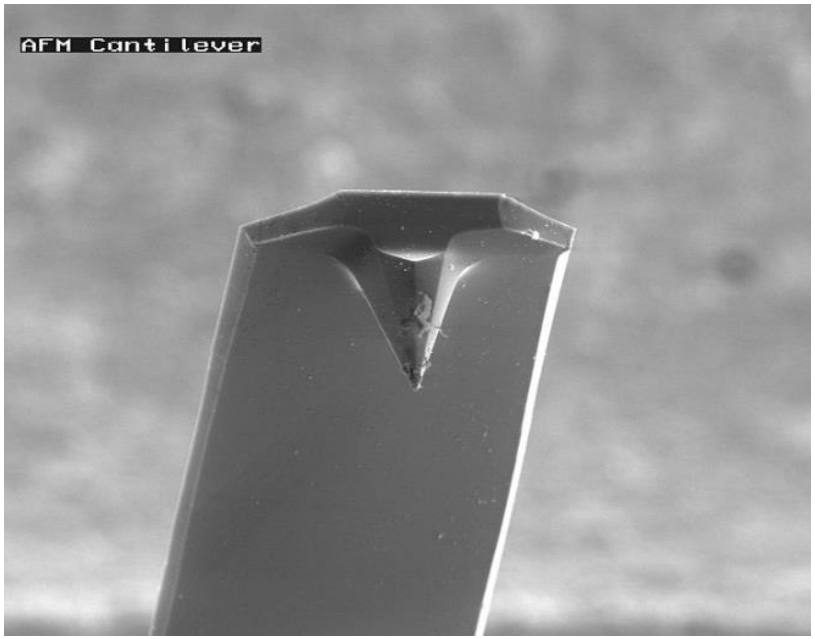
AFM ATOMIC FORCE MICROSCOPY

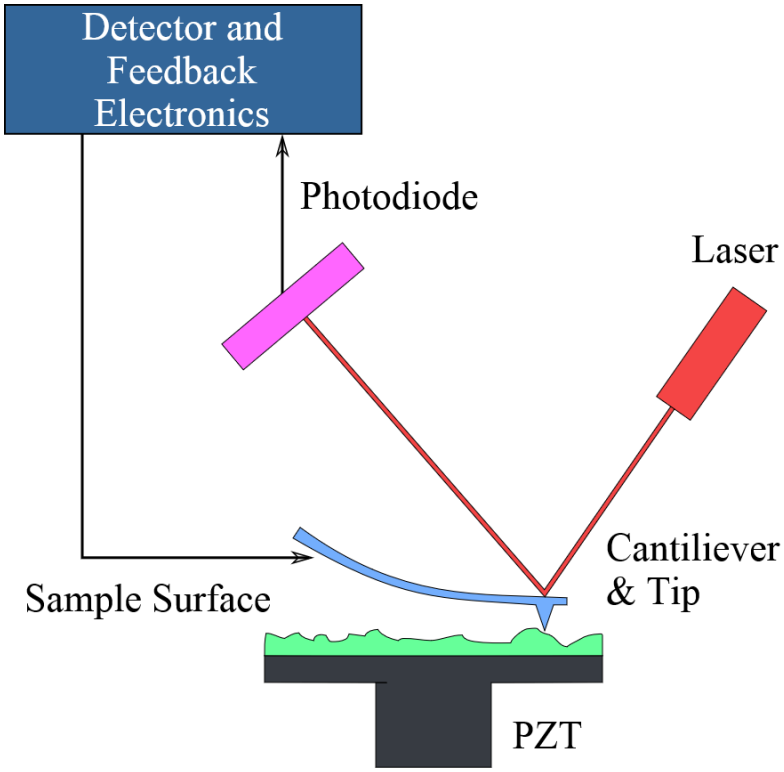

Detector and
Feedback
Electronics
Photodiode
Laser
Cantiliever
& Tip
Sample Surface
PZT



Typical configuration of an AFM.
(1): Cantilever, **(2)**: Support for cantilever, **(3)**: Piezoelectric element(to oscillate cantilever at its eigen frequency.), **(4)**: Tip (Fixed to open end of a cantilever, acts as the probe), **(5)**: Detector of deflection and motion of the cantilever, **(6)**: Sample to be measured by AFM, **(7)**: xyz drive, (moves sample (6) and stage (8) in x, y, and z directions with respect to a tip apex (4)), and **(8)**: Stage

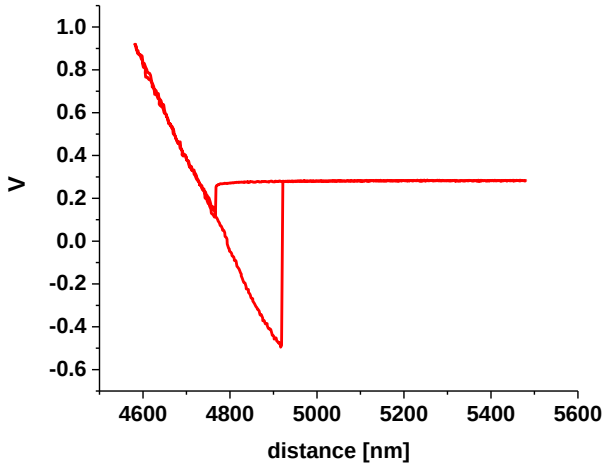

(3)
(4)
(1)
d
d
d
(2)

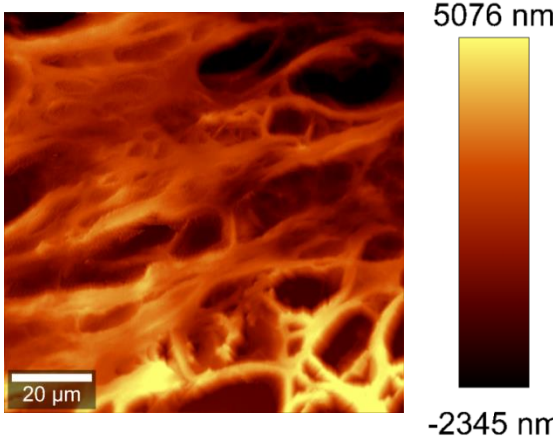

AFM Cantilever

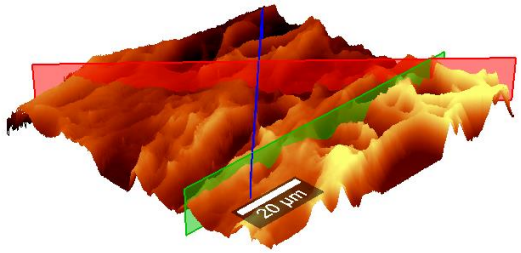

Detector and
Feedback
Electronics
Photodiode
Laser
Cantiliever
& Tip
Sample Surface
PZT

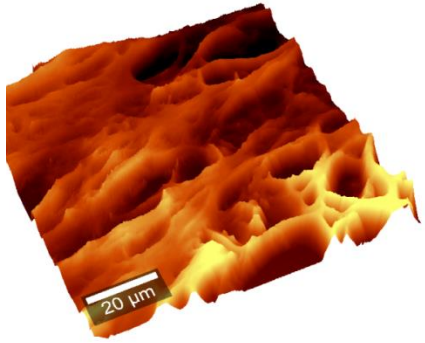
Abilities:

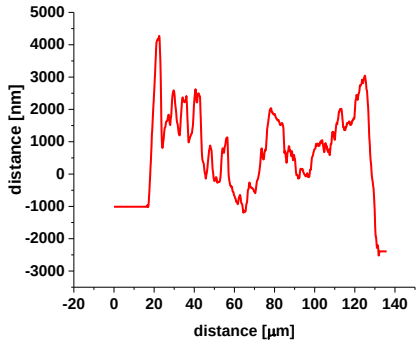
force measurement
imaging
and manipulation

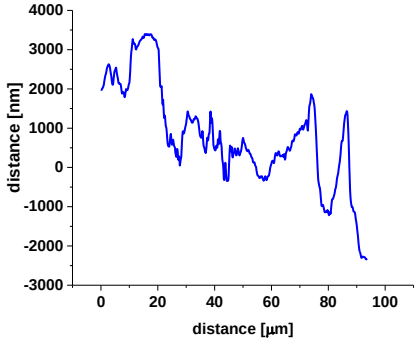


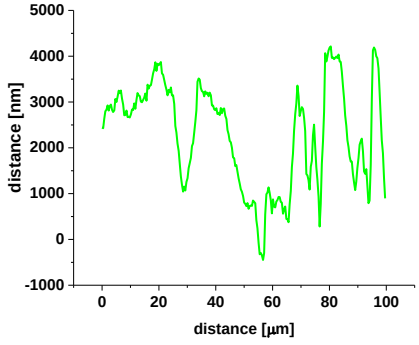






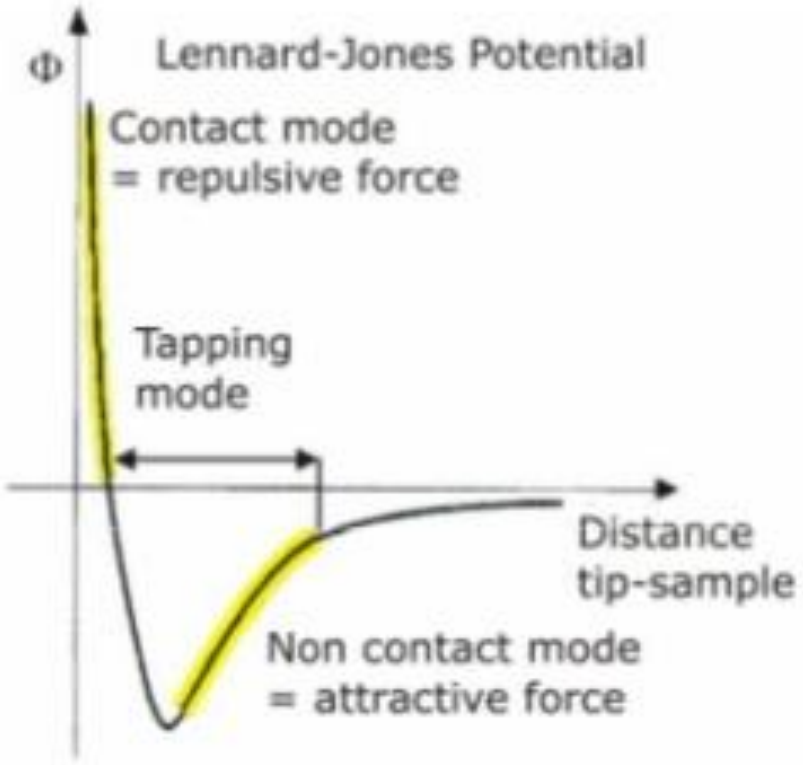


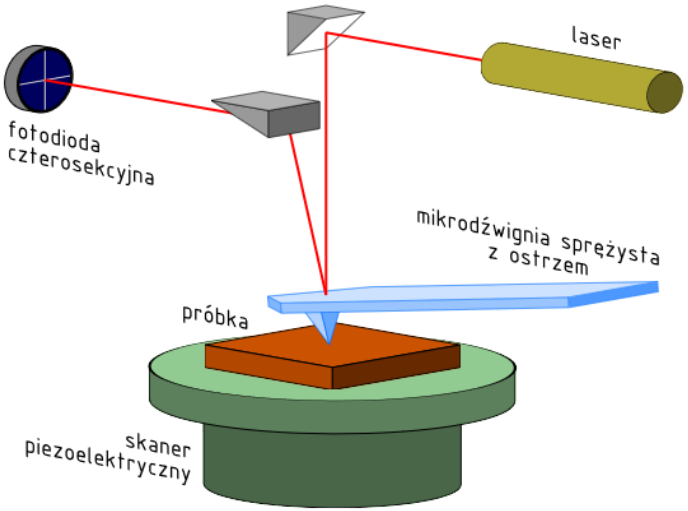




- Imaging mode
  - contact mode
  - non contact mode
  - intermittent / tapping mode

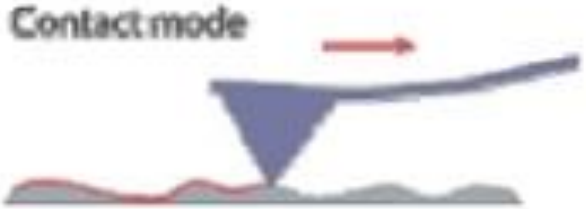
- Force-distance mode
  - force spectroscopy
  - combined imaging & force spectroscopy





- Contact mode:
  - tip in continuous contact with sample
  - preferably used for hard samples
  - imaging in air and liquid
  - high resolution

  detect: deflection



- Force spectroscopy mode:
  - consecutive cycles of tip approach and retract
  - interaction forces between tip and sample are recorded

$F = - k_{spring} \cdot \Delta x$

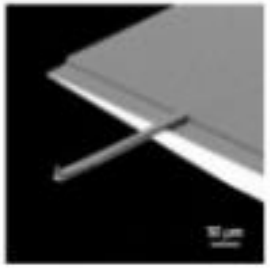
- Intermittent/tapping mode:
  - oscillating cantilever, tip touching surface gently and frequently
  - often used for biological samples
  - imaging in air and liquid
  - good resolution

- Non contact mode:
  - oscillating cantilever, tip not in contact with sample
  - used for soft samples
  - imaging in vacuum
  - distance range 50Å - 150Å

detect: amplitude
phase
deflection

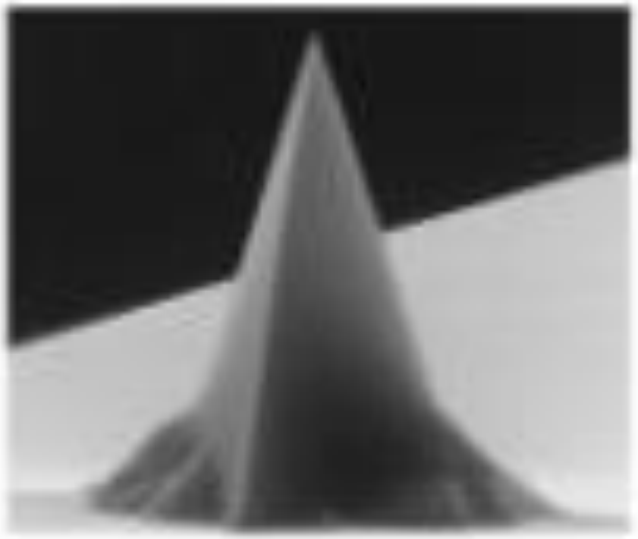
http://www.jpk.com/tutorial/tutorial1.htm

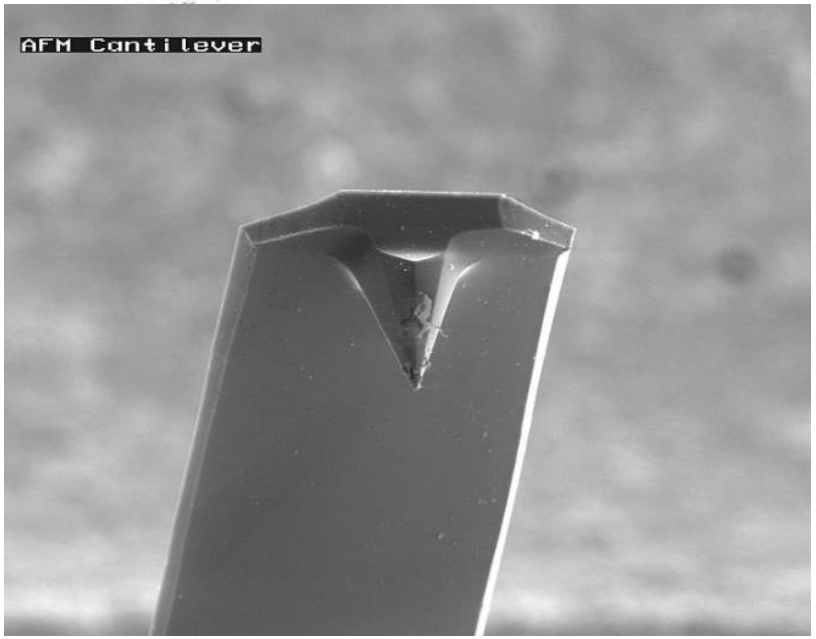
silicon nitride cantilevers



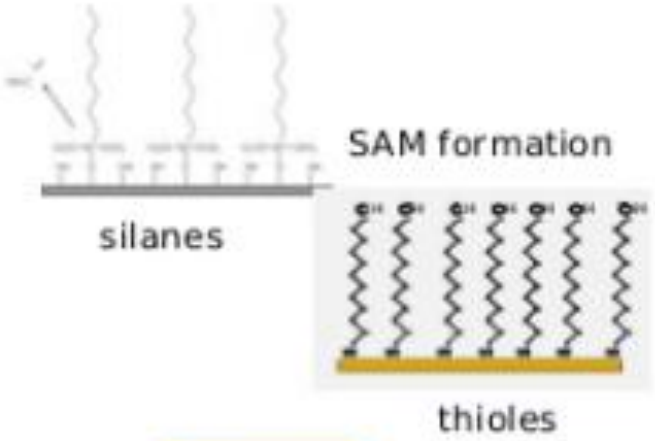
silicon

Typical cantilevers:
1µm thick, 100s µm long
k_{spring} ~ 0.01 ... 20N/m
f_{res} ~ 4 ... 400kHz
r_{tip} ~ 1 ... 20nm
reflective backside coating:
- better signal





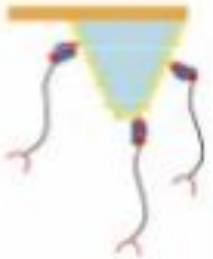
- Surface modification
  self assembling monolayers (SAM)
  - silanes on glass- and Si-surfaces
  - thioles on Au-surfaces



- Tip modification
  - Adsorption of molecules from solution
    e.g. proteins

  - Decrease AFM tip radius with
    attachment of molecules or nanotubes

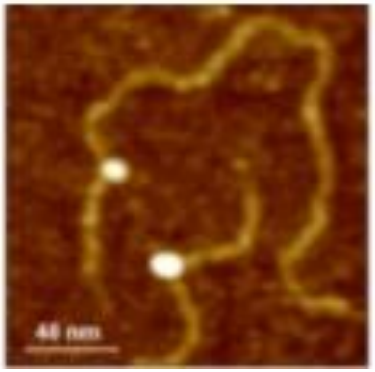
  - Attachment of linker molecules
    e.g. PEG linker for antibodies,
    crosslinker for SH-, NH-groups



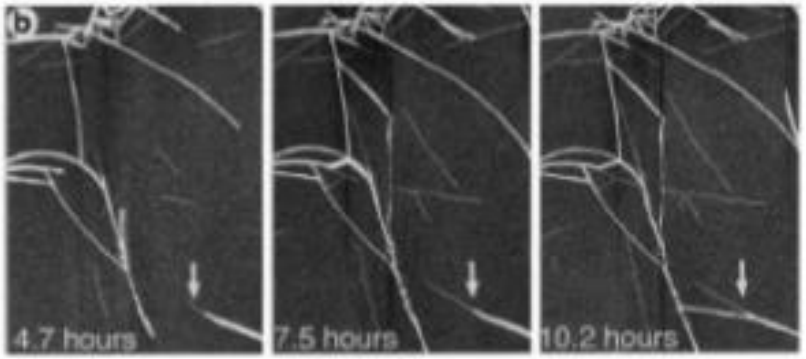
Biomolecular structure

Imaging DNA-protein complexes on aminoterminated mica

DNA plasmids

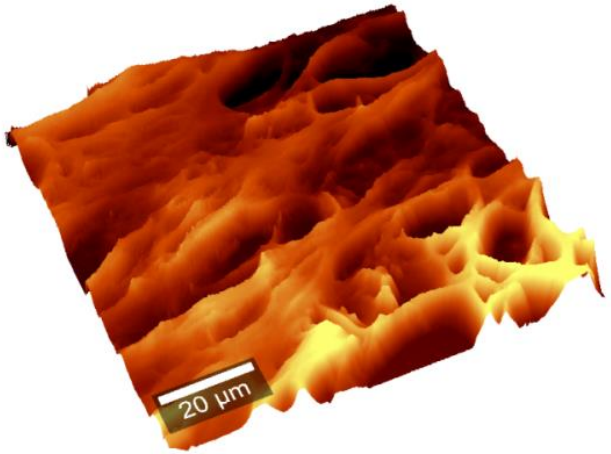


λ-DNA restriction enzyme complex (Hae III restriction endonuclease induces bending at GGCC)

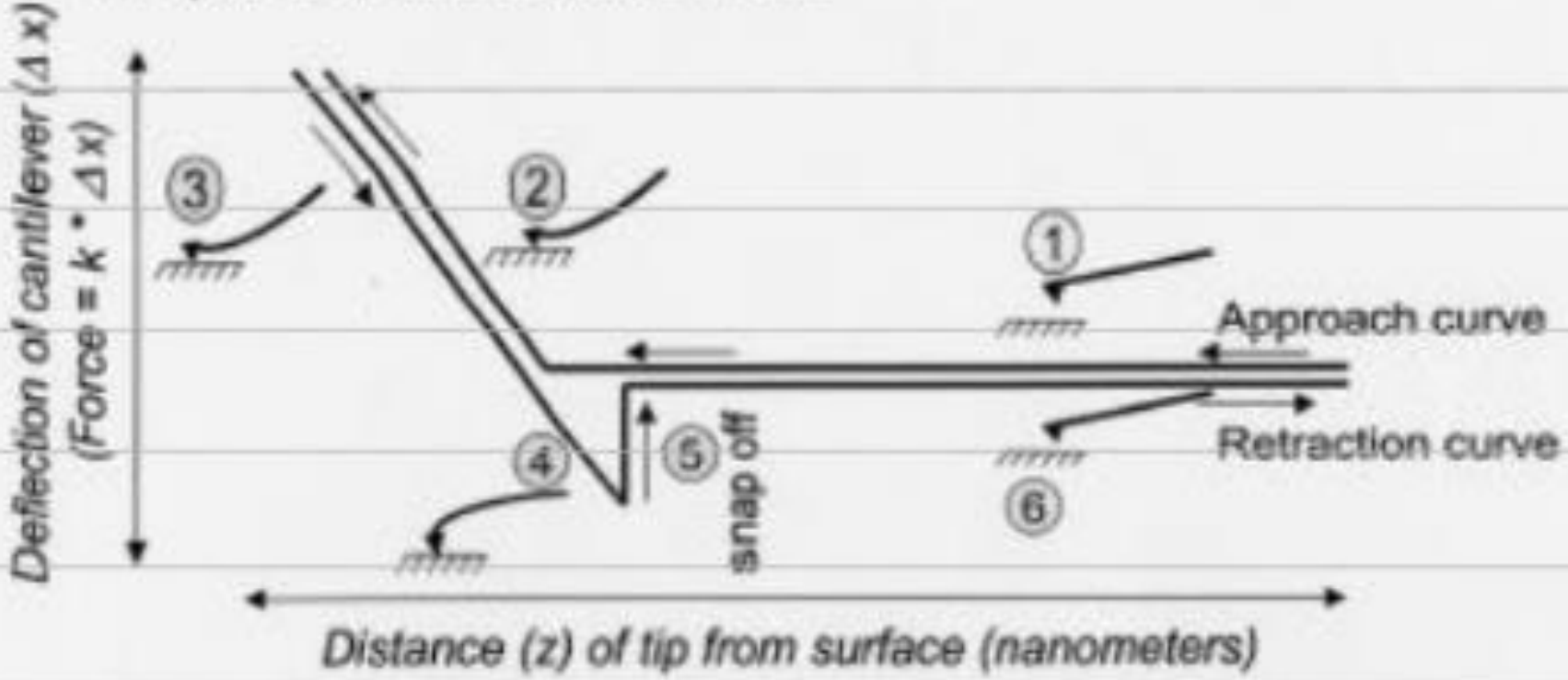


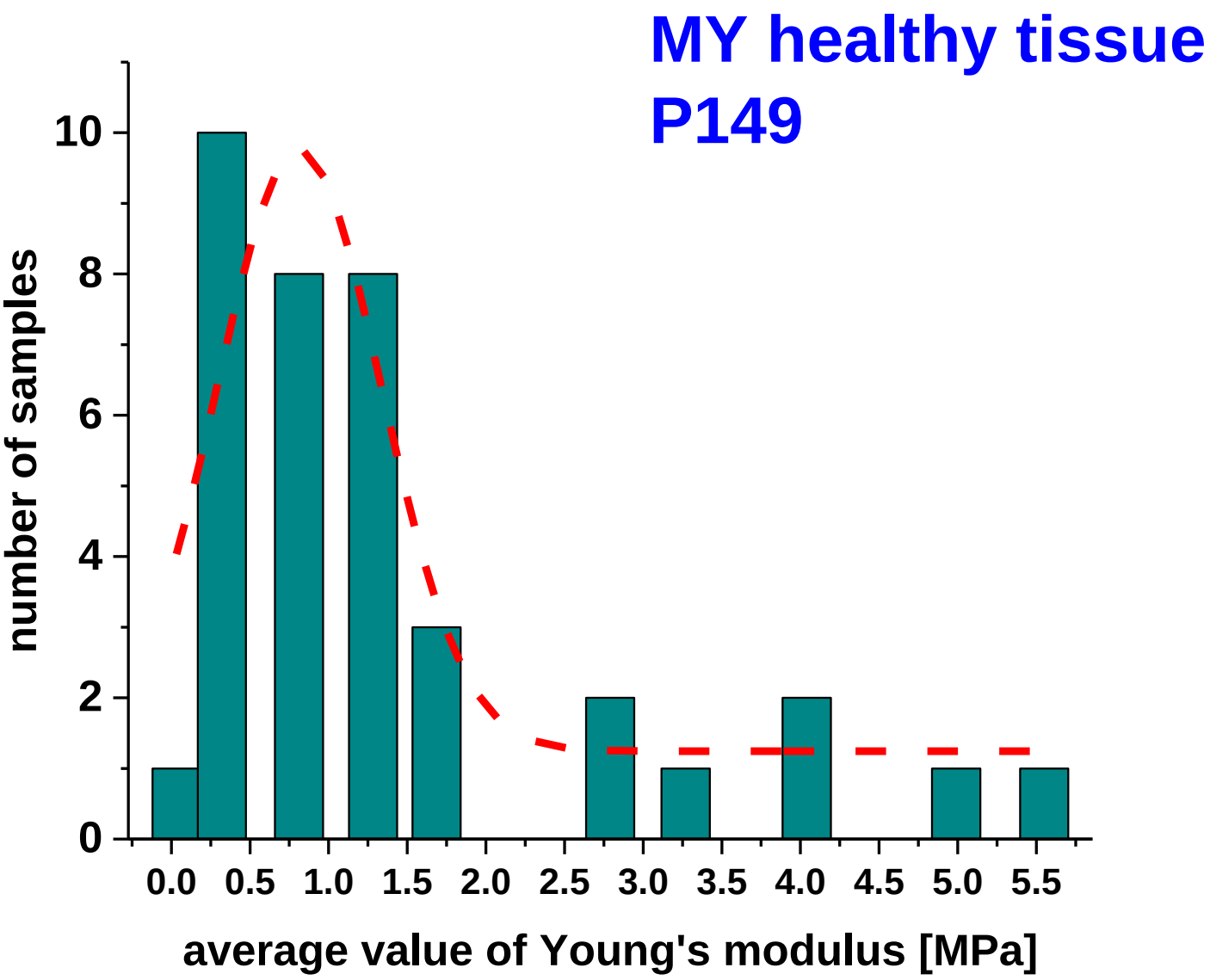
Time-lapse AFM for imaging growth of amyloid fibrils (synthetic human amylin)

Goldsbury et al., J. Mol. Biol. 1999

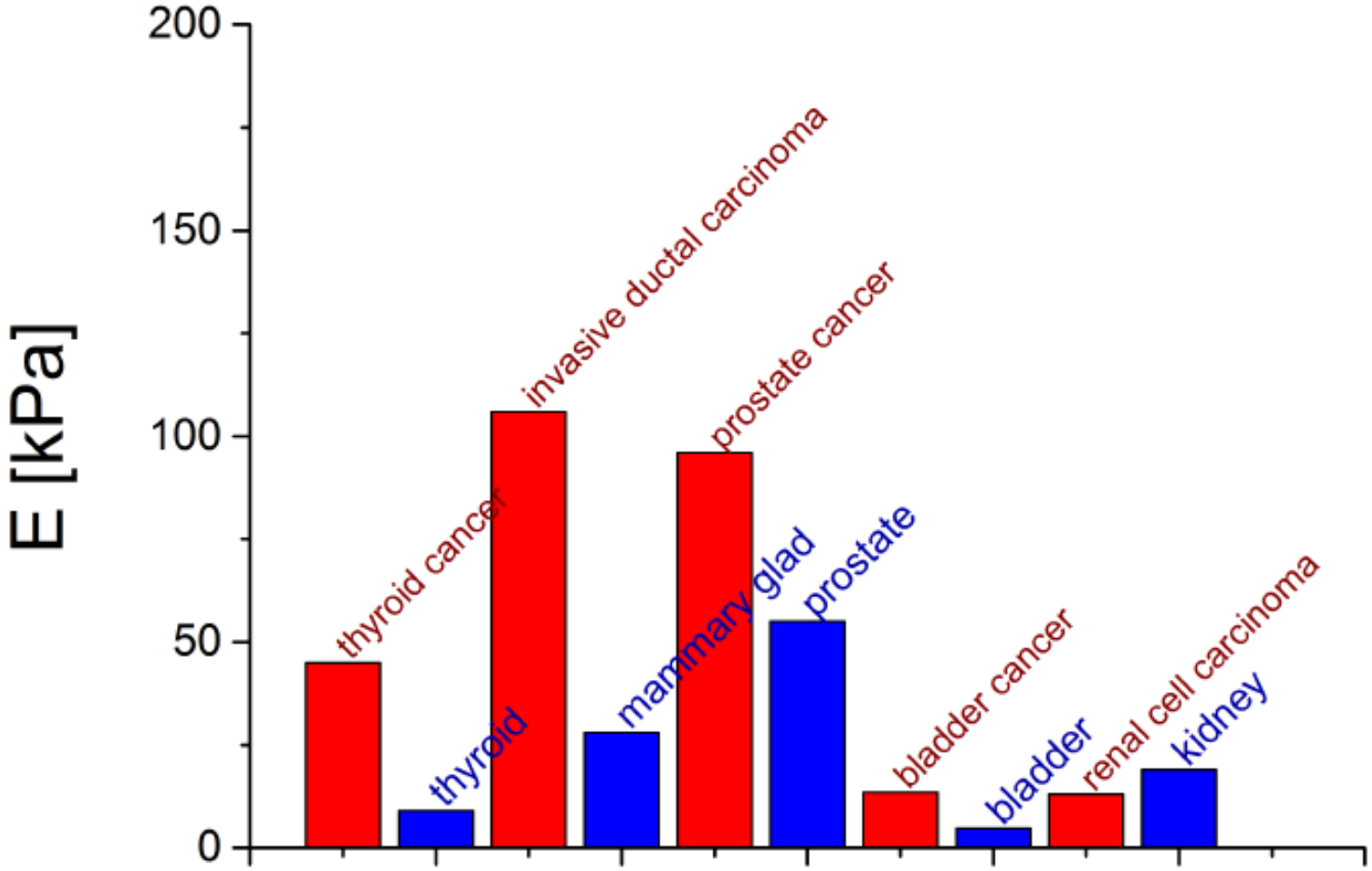


1. AFM tip is not in contact with surface
2. Tip is being pushed into the surface, bending the cantilever
3. Tip is being withdrawn from the surface
4. Tip adheres to sample/surface
5. Tip "snaps off" from surface
6. Tip is not in contact with surface

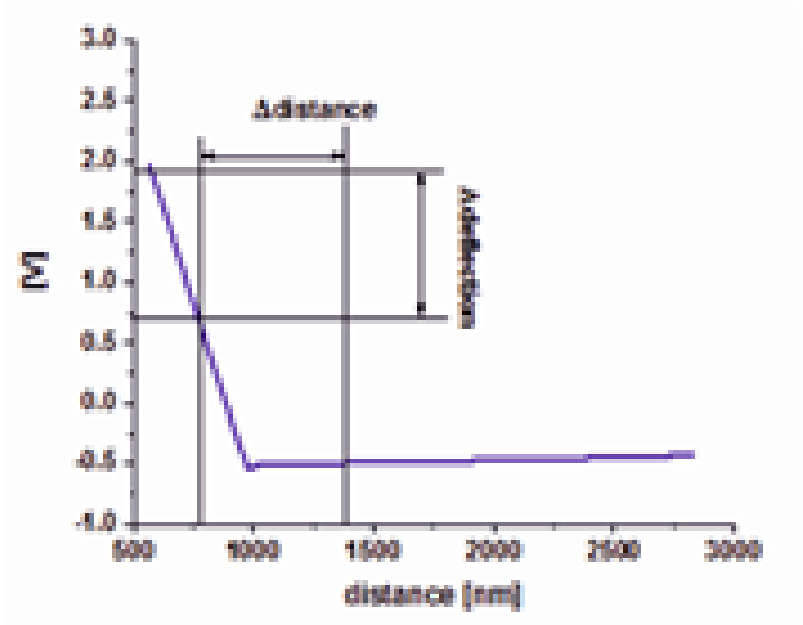


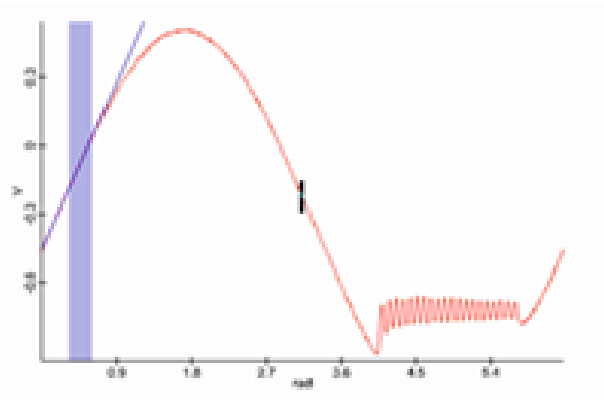

MY healthy tissue
P149
number of samples
0
2
4
6
8
10
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
average value of Young's modulus [MPa]

Lee et al., Yonsei Med. J. (2011)
Krouskop et al. Ultrasonic Imaging, (1998)



NIŻSZY MODUŁ YOUNGA DLA TKANEK PRAWIDLOWYCH

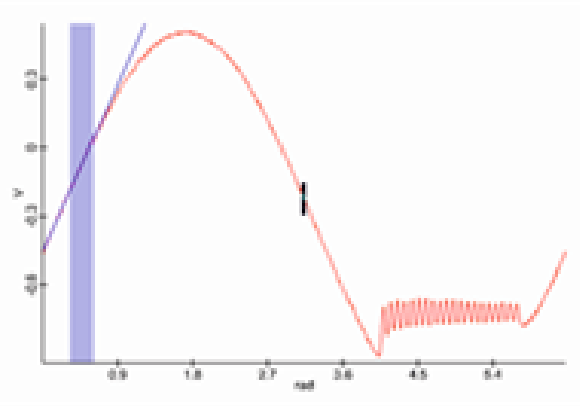




$$Adhesion\ [nN] = k\ [N/m]\ *\ S\ [nm/V]\ *\ V\ adhesion\ [V]$$

$$Sensitivity\ \left[\frac{nm}{V}\right] = \frac{1}{slope}$$

$$Slope\ \left[\frac{V}{nm}\right] = \Delta deflection\ /\Delta distance$$



$$M\ [nm] = U * S * A$$

A – voltage applied to the cantilever

S – sensitivity of the detector

U – modulation factor

Penetration depth ΔZ is calculated using the formula:

$$\Delta Z\ [nm] = M\ * [1 - \cos(\Delta rad)] - a_1 * (\Delta rad) * S$$

$$Stiffness\ [\frac{N}{m}] = \frac{a_1 * (\Delta rad) * k * S}{\Delta Z}$$

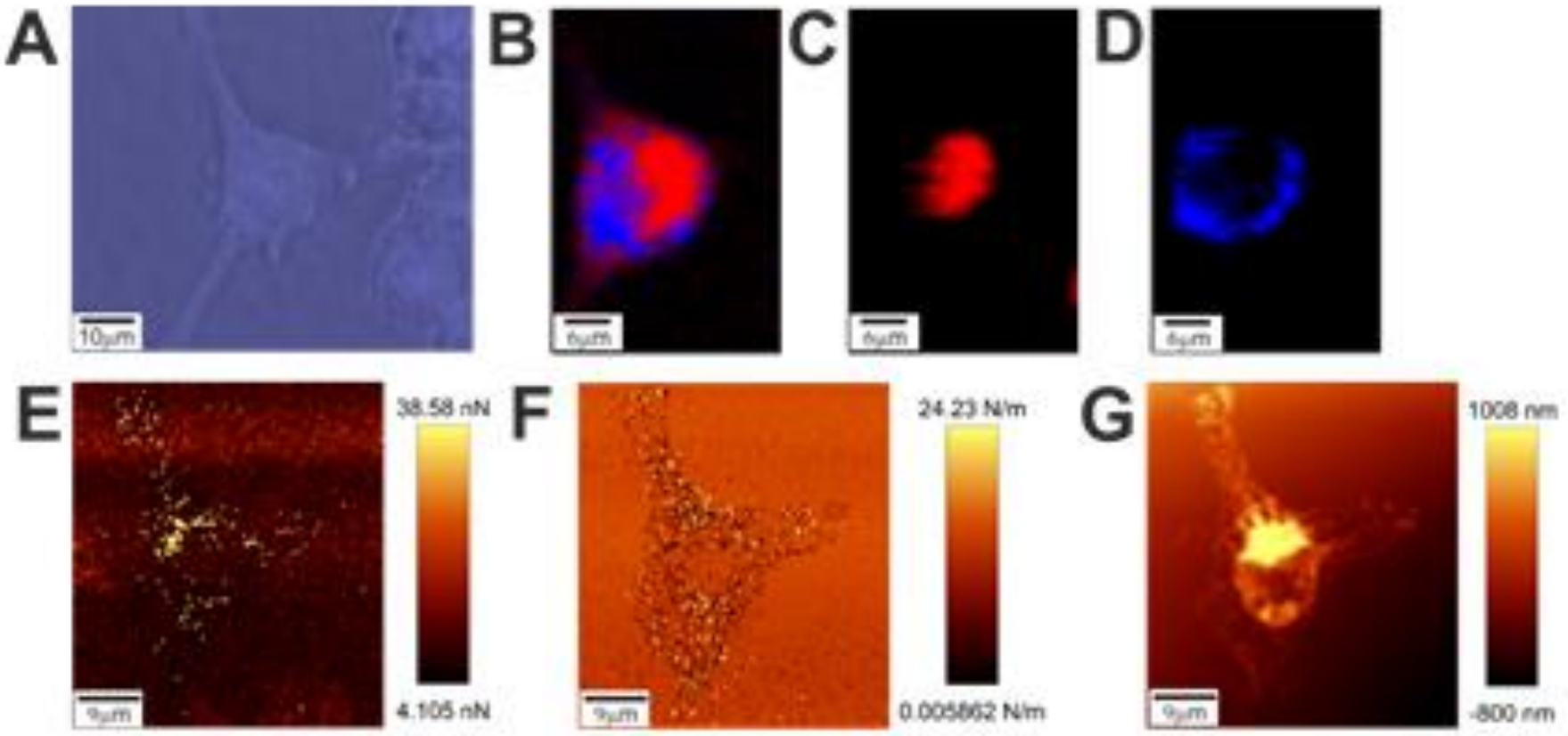


Fig 4. Microscopy image (A), Raman image (red-proteins, blue-lipids) (B), fluorescence images of Hoechst 33342 (C) and Oil Red O (D) of a living U-87 MG cell, adhesion image (E), stiffness image (F) and topography image (G) of air-dried cell.