BioAFM Summer School

Introduction

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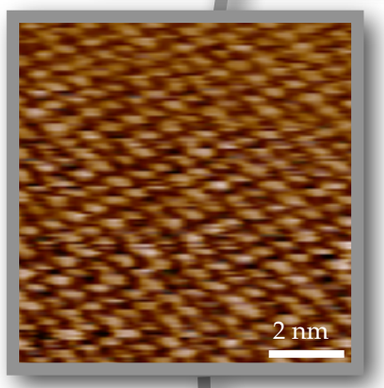
“3M” ...

Nanobiotechnológiai és In Vivo Képalkotó Központ
Semmelweis
NIVIC
Nanobiotechnology and In Vivo Imaging Center

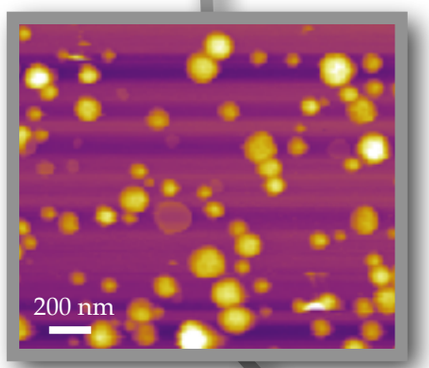
…”molecule - mouse - man”

AFM
(AR MFP1D,
MFP3D,
Cypher)

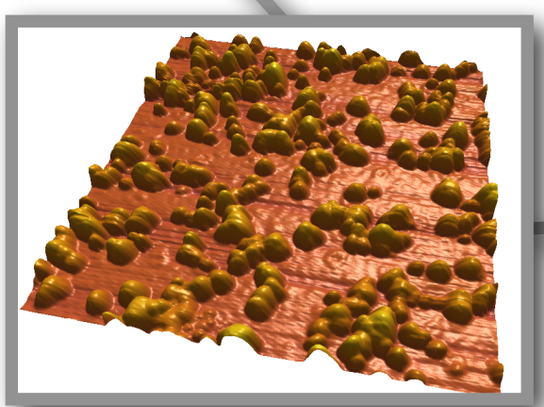
optical
tweezers



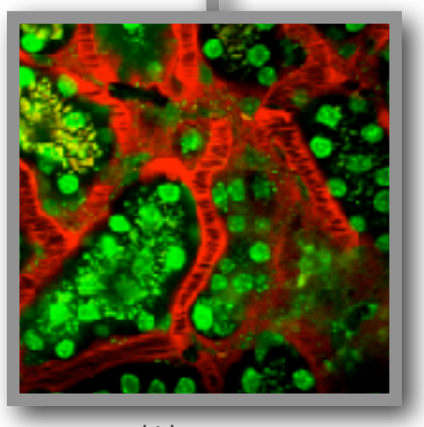
atoms on mica



liposomes

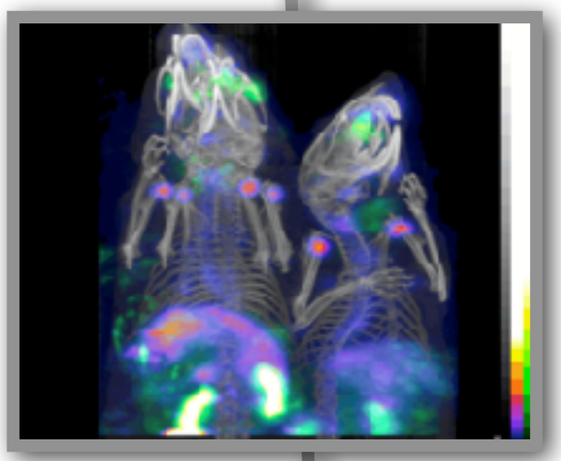


multi-
photon
microscopy

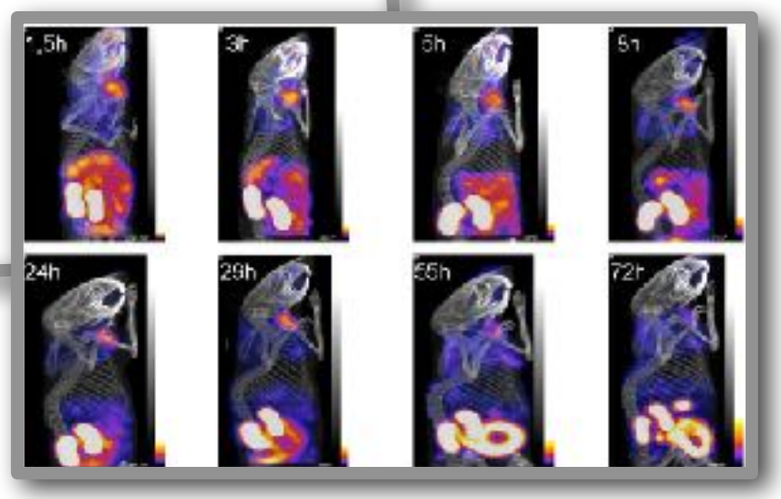


kidney cortex

nanoSPECT/CT



^{99m}Tc-DTPA: BBB - blue/red)
^{201}Tl-DDC: perfusion - green



Prussian-blue multi-modal contrast
nanoparticles

nanoPET/MRI

EKG-gated ^{18}FDG PET/MRI

School outline

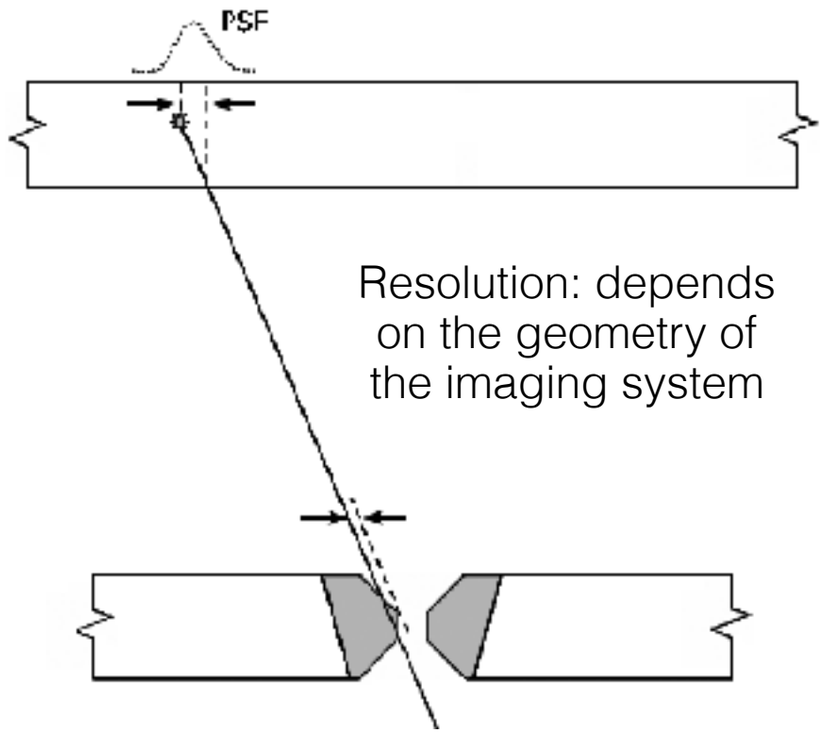
1. Introductory talks (6 July, 2026 morning)
   Miklós Kellermayer: AFM foundations
   Zsolt Mártonfalvi: Force spectroscopy
   Bálint Kiss: AFM applications and trends
2. Practical I (6 July, 2026 afternoon)
   Dóra Haluszka: AR MFP3D, sample preparation, blood vessel imaging
3. Practical II (7 July, 2026 morning)
   Ádám Zolcsák: AR Cypher, SLB imaging
4. Practical III (7 July, 2026 afternoon)
   Bálint Kiss: Nanosurf DriveFM, protein and virus AFM
5. Summary, Q&A, feeedback, certificate ceremony (7 July, 2026 afternoon)

Introduction

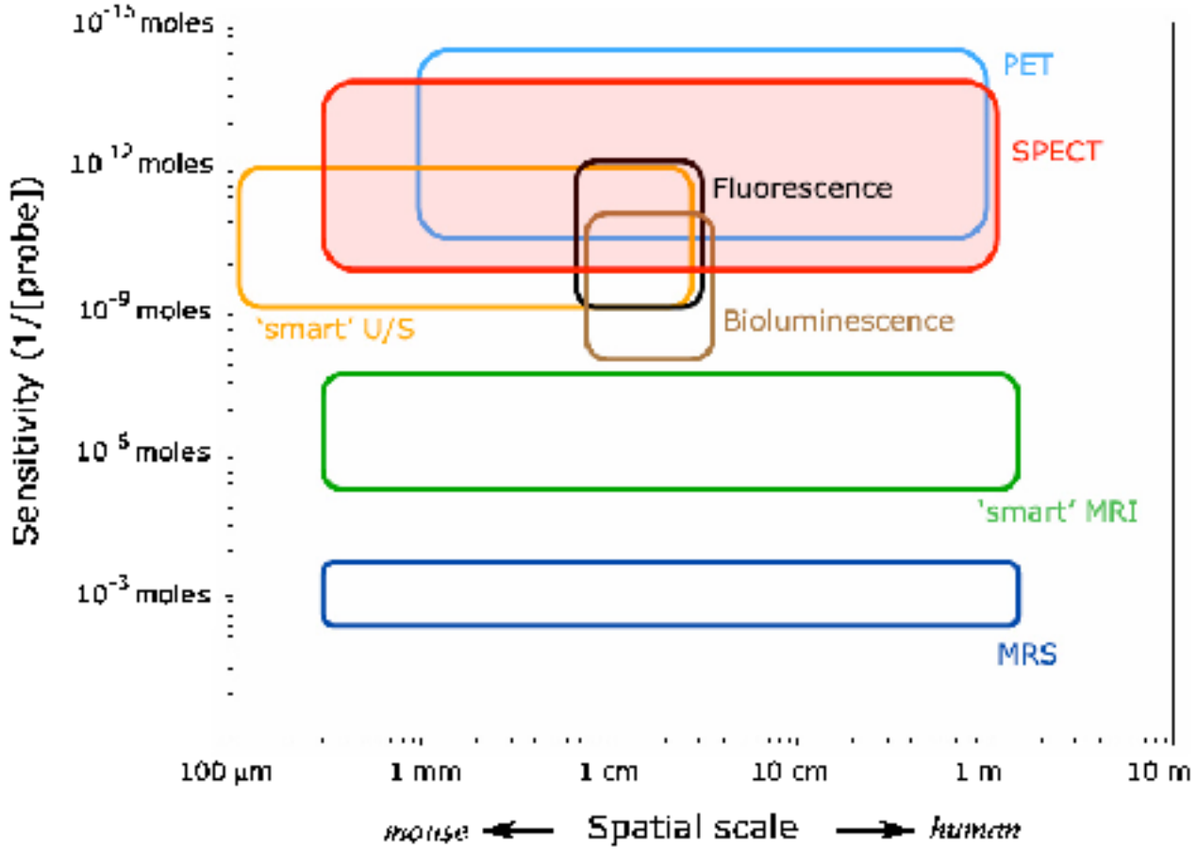
1. History
2. Theory
   atomic interactions, imaging modes, resolution
3. Engineering
   scanning, cantilever deflection and oscillation
4. Practice
   cantilevers and tips, surfaces, sample preparation, analysis, errors
5. Future
   correlative and multimodal imaging, increasing speed, pros and cons
6. Examples

Seeing is believing: spatial resolution problem

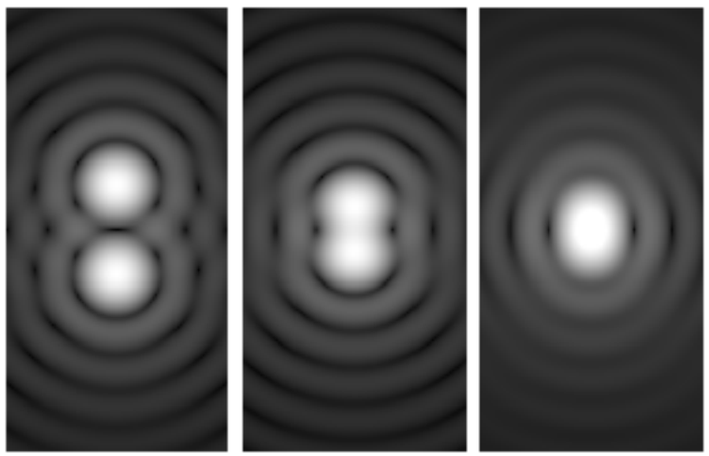
Tomographic imaging



Resolution: depends on the geometry of the imaging system

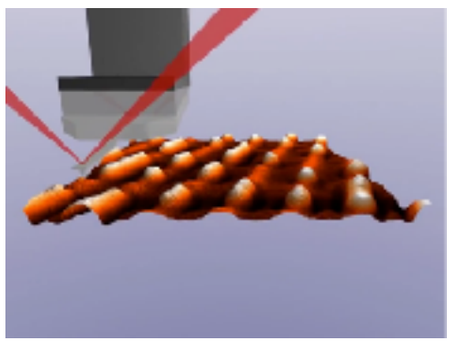


Light microscopic imaging

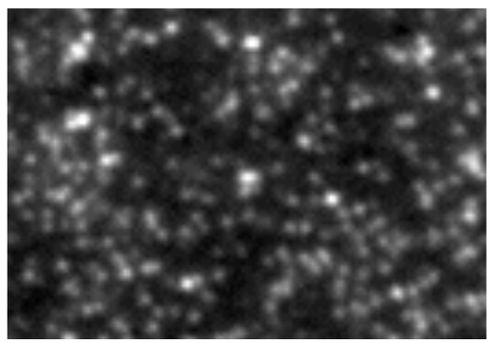


Resolution: diffraction limited, depends on wavelength

But!

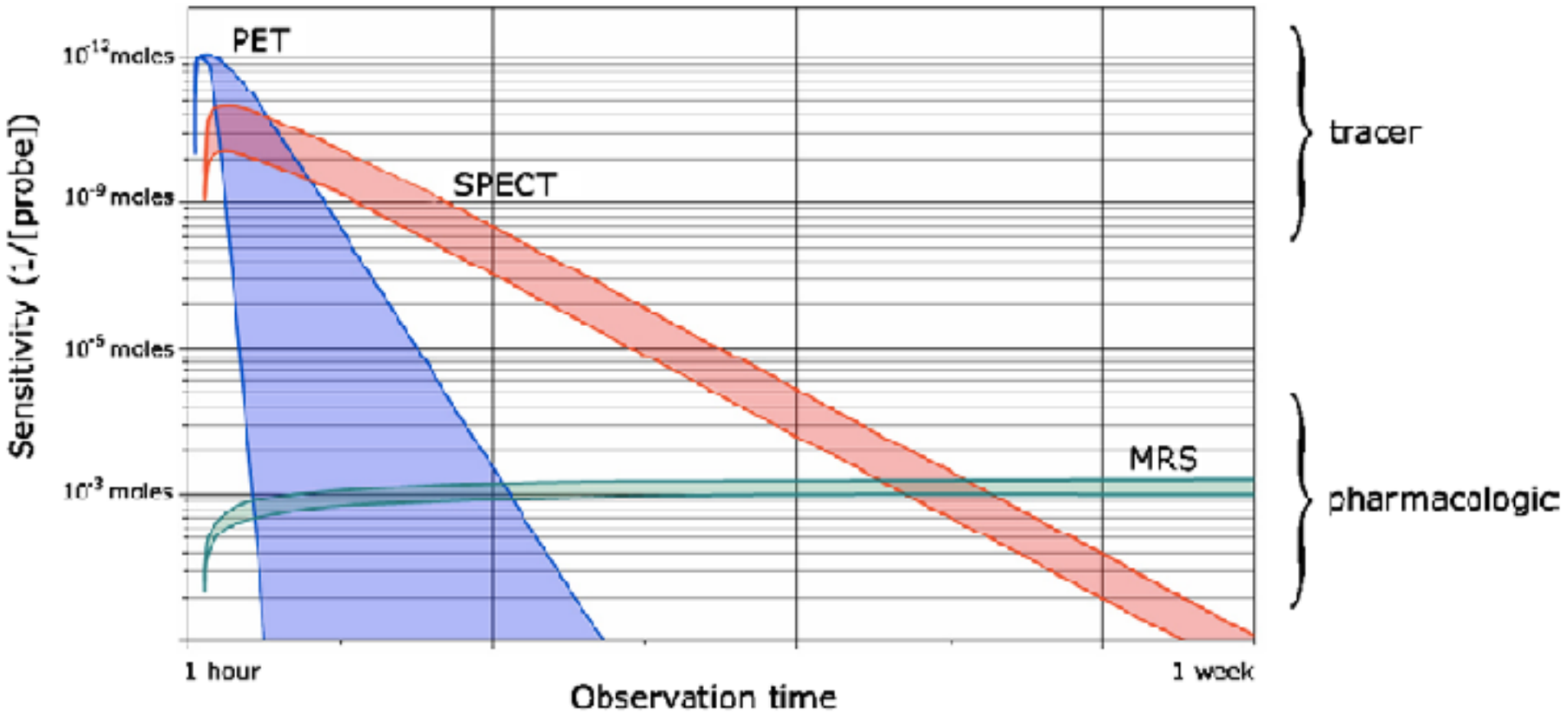


Scanning probe microscopy

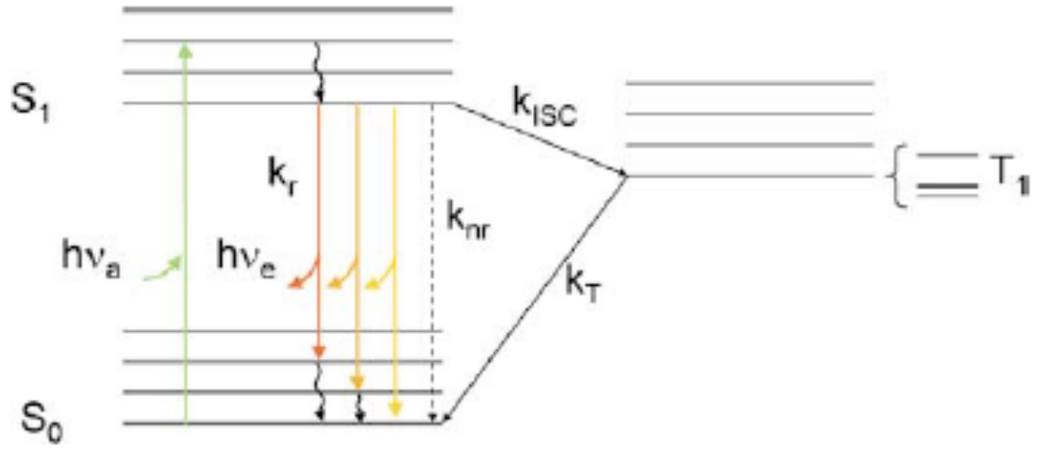


Fluorescence
(PGK - Alexa 488)

Time problem



But!



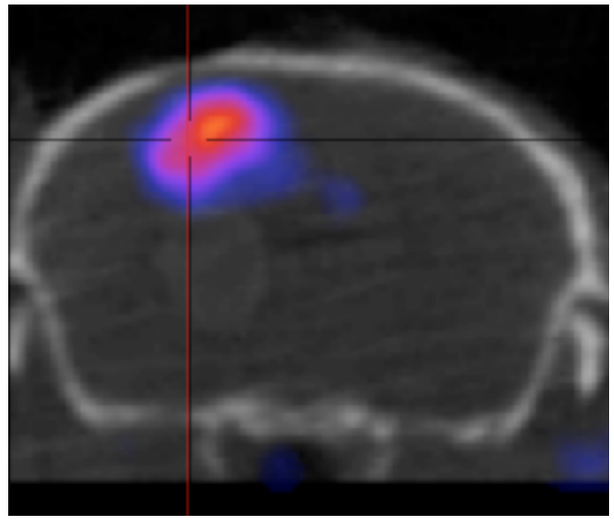
Fluorescence - nanoseconds

Field problem

- Chemical “field”

  concentration

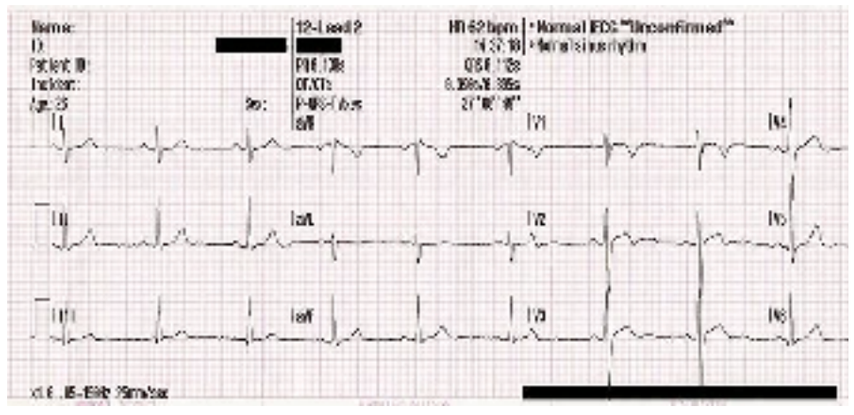
  [functional information; ideally, discrete signal can be detected only if a certain function occurs (e.g., interaction, binding, enzyme reaction, etc.)]

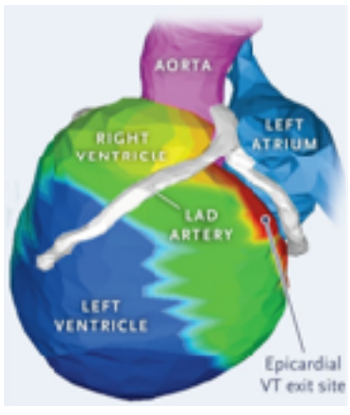


- Electric field

  voltage,

  voltage-vector changes

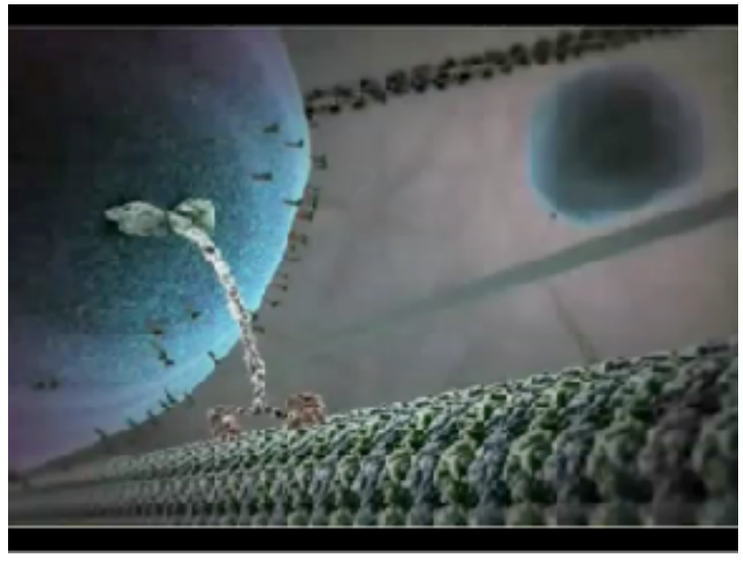




- Mechanical “field”

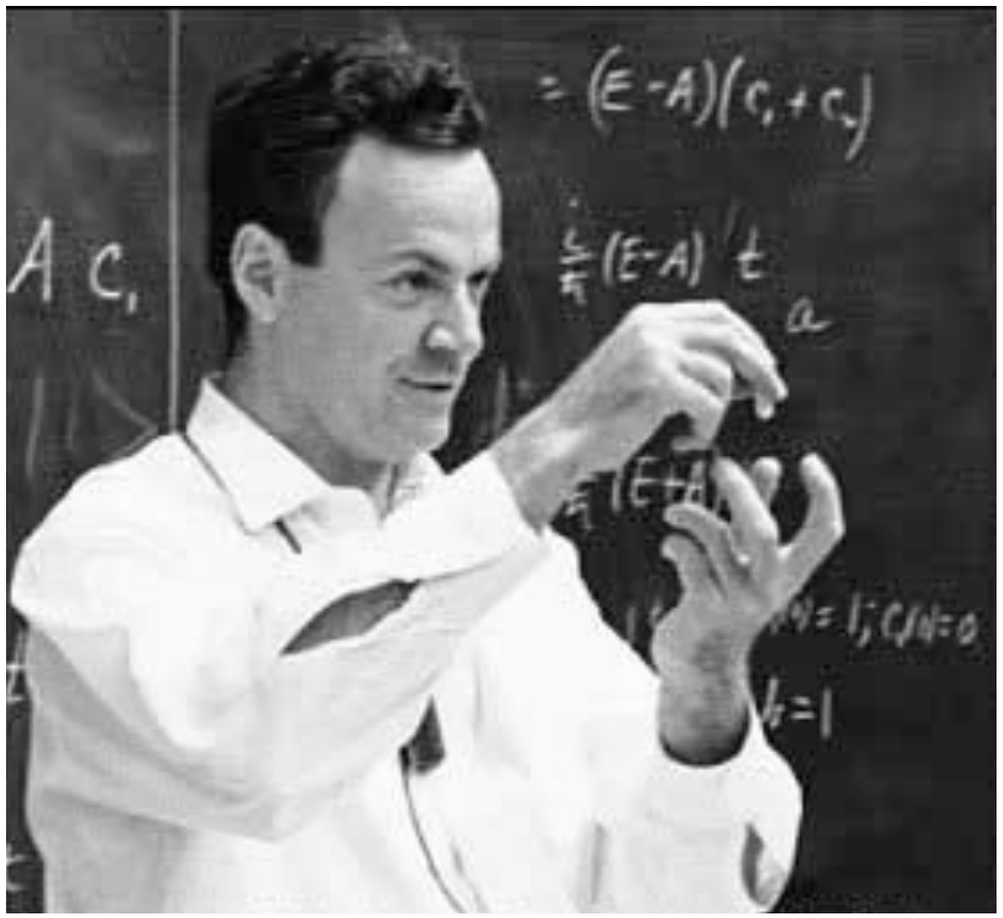
  elasticity, force generation

  neglected parameter of biology

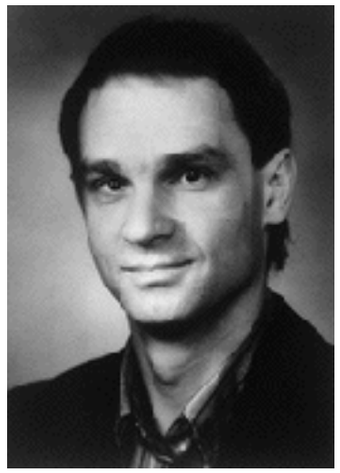


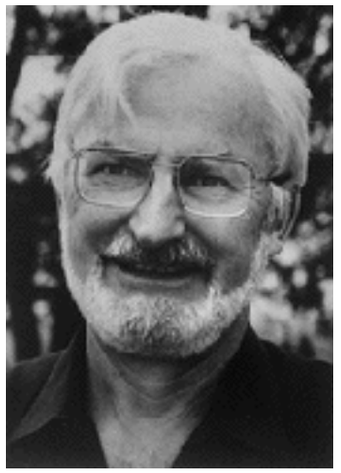
Scanning Probe Microscopy (SPM)

Dream tool of the nanoworld

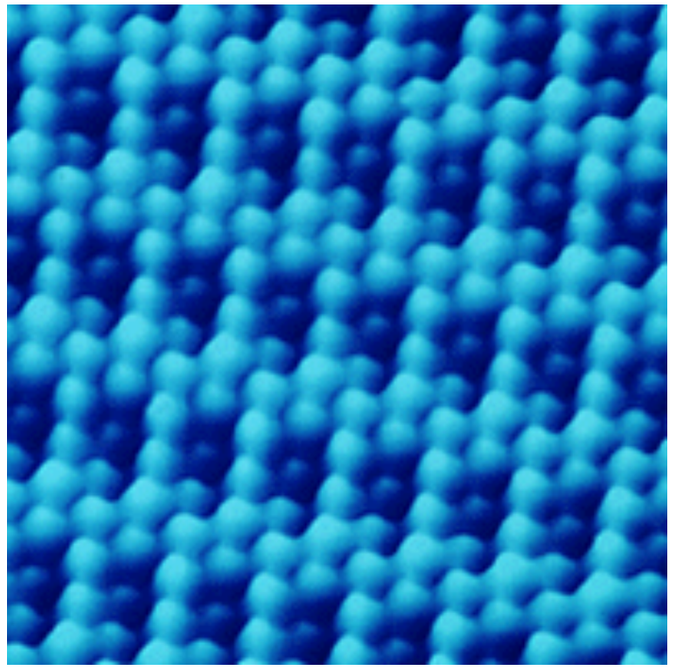


Richard P. Feynman:
“There is plenty of room at the bottom”
29 December, 1959





Gerd Binnig Heinrich Rohrer



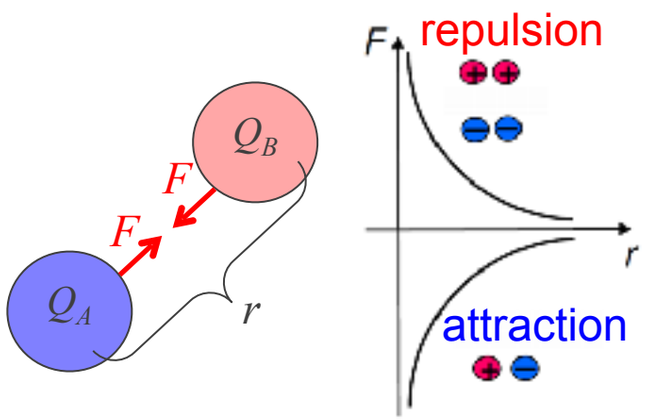
Oxygen atoms on a rhodium crystal

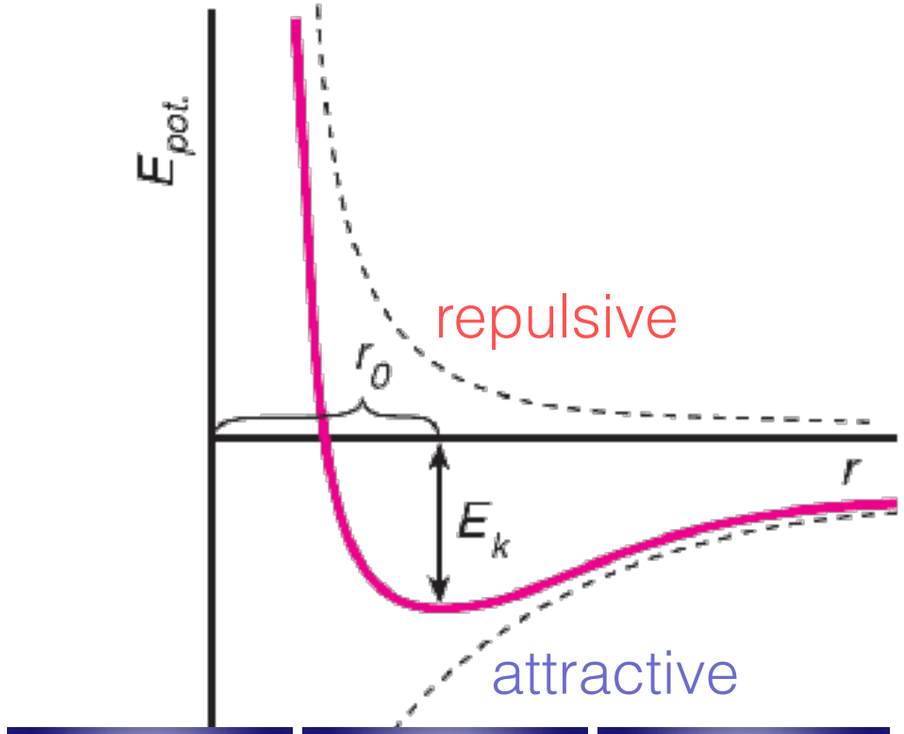
Dimension of the nanoworld:
1 nanometer

Interactions in nature

Interaction type	Binding particle	Range (m)	Relative strength
gravitation	every particle	infinite (~1/r^2)	10^{-40}
electrostatic (Coulomb)	charged particles	infinite (~1/r^2)	10^{-2}
strong nuclear	nucleons	10^{-15}	1
weak nuclear	every particle	10^{-18}	10^{-13}

Coulomb-interaction





$$E_{pot} = E_{attraction} + E_{repulsion}$$

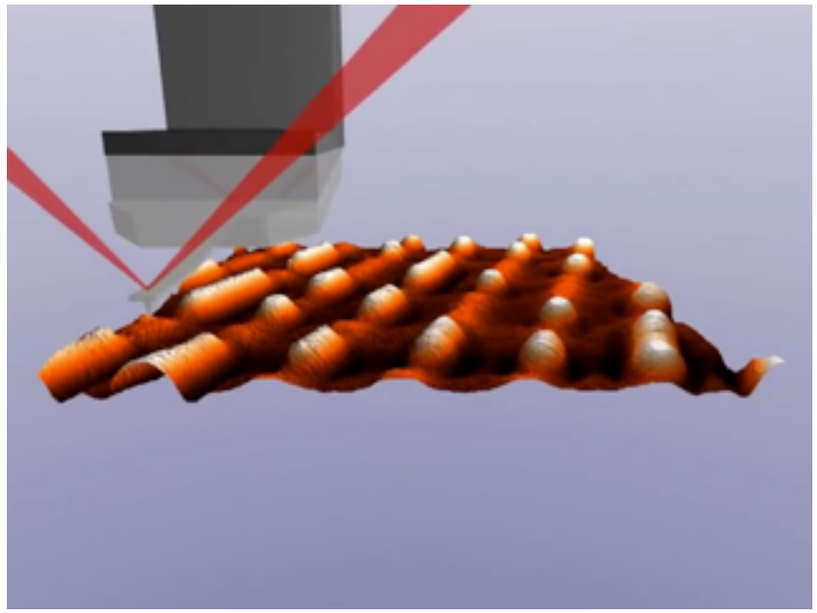
$$E_{pot} = -\frac{A}{r^n} + \frac{B}{r^m}$$

A, B: constants that depend on the interaction and the atoms
n (attraction) < m (repulsion)

r_0: binding distance

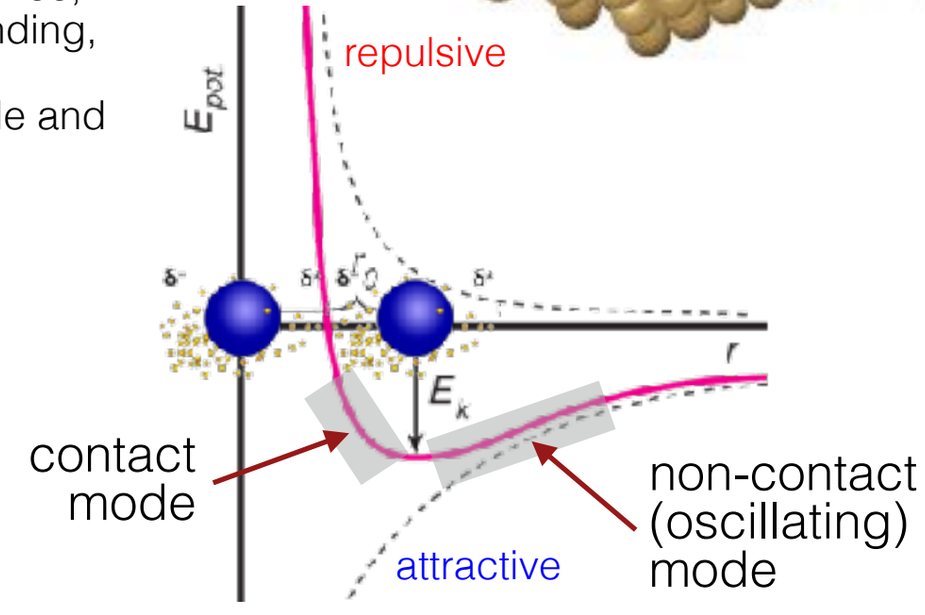
E_k: binding energy

Principles of SPM operation

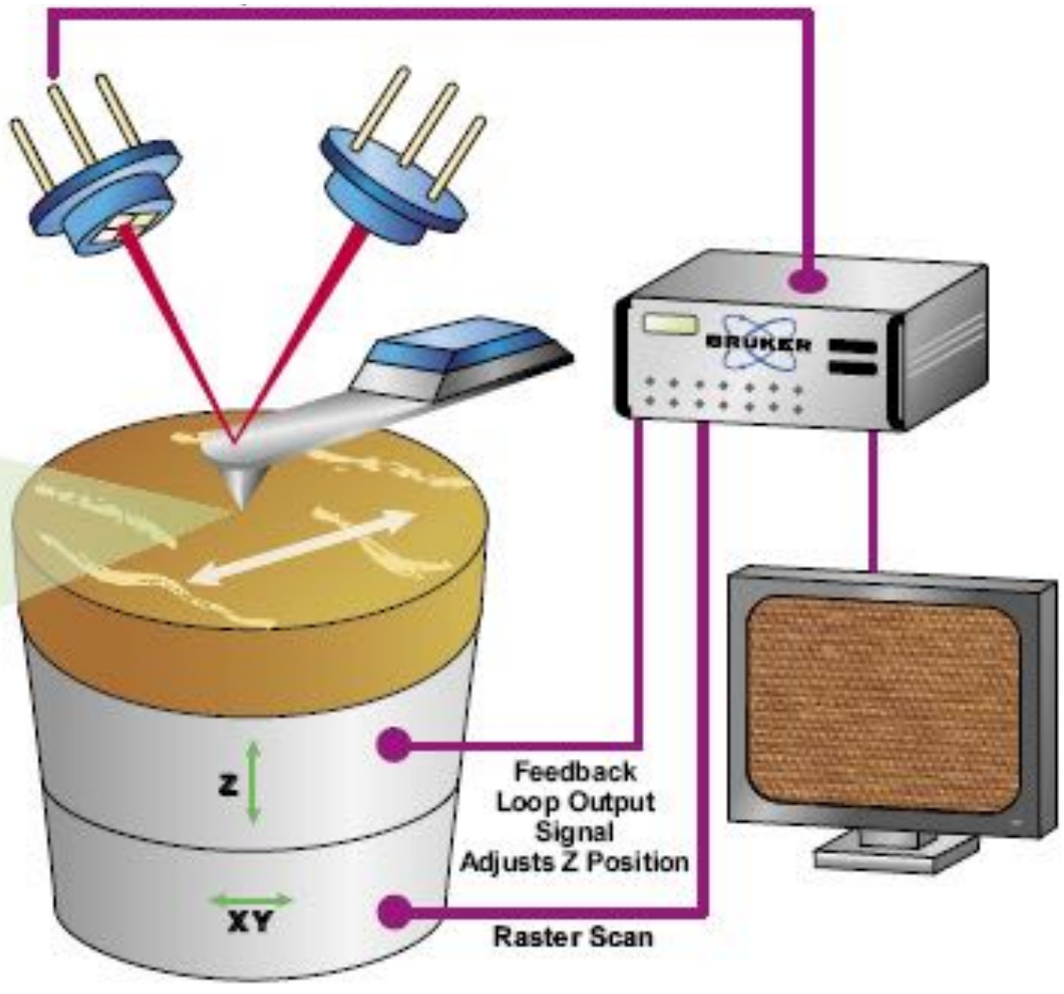


1. Van der Waals interactions between the atoms of the tip and the sample

N.B.: Bias (e.g., voltage, magnetic field, temperature difference, complex ligand binding, etc.) can be set up between the sample and the tip, providing additional contrast mechanisms.



2. Deflection of a flexible cantilever is measured with a laser beam reflected from its back side

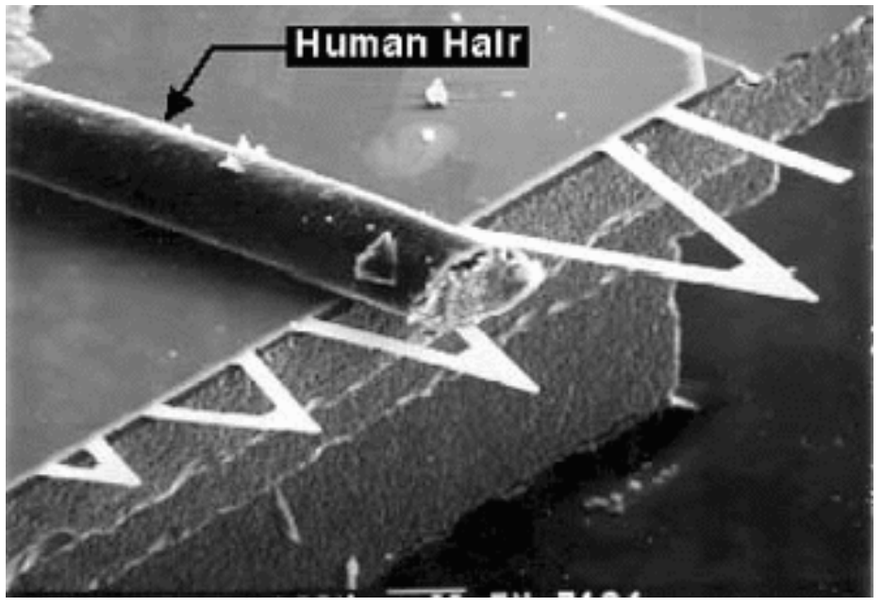


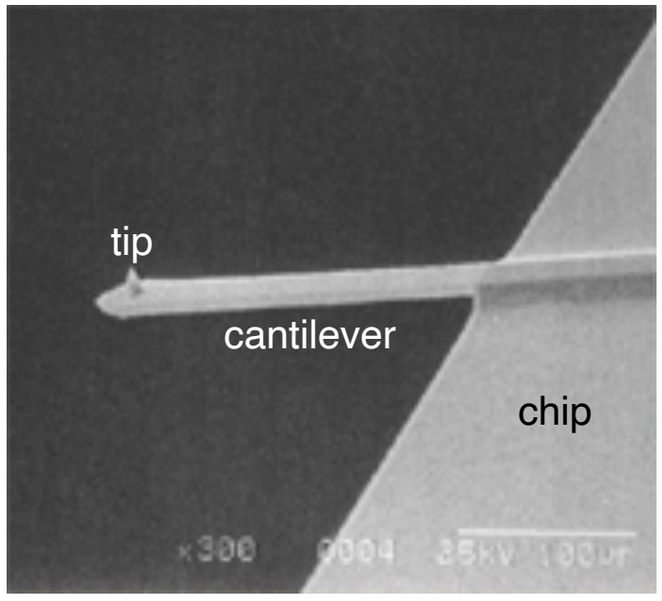
3. The sample and the tip are moved relative to each other in the XY plane (raster scan) and the Z direction

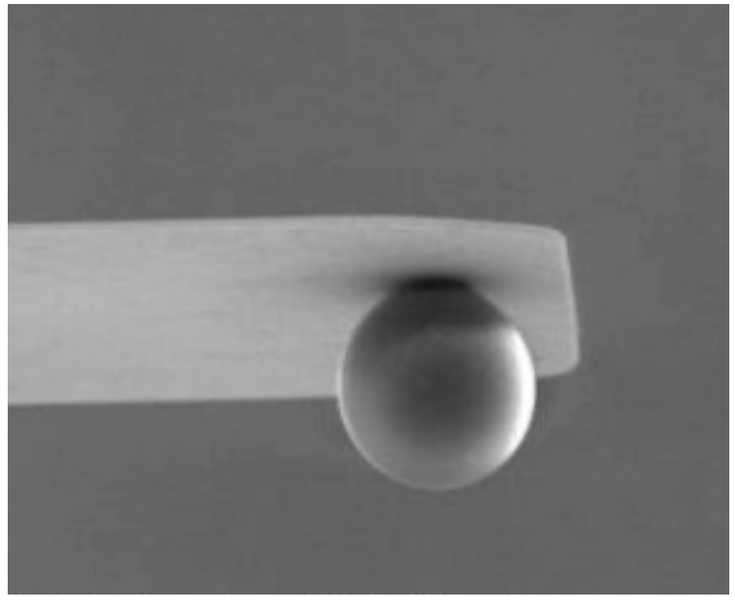
4. The vertical position (Z-axis) direction is adjusted based on a feedback loop

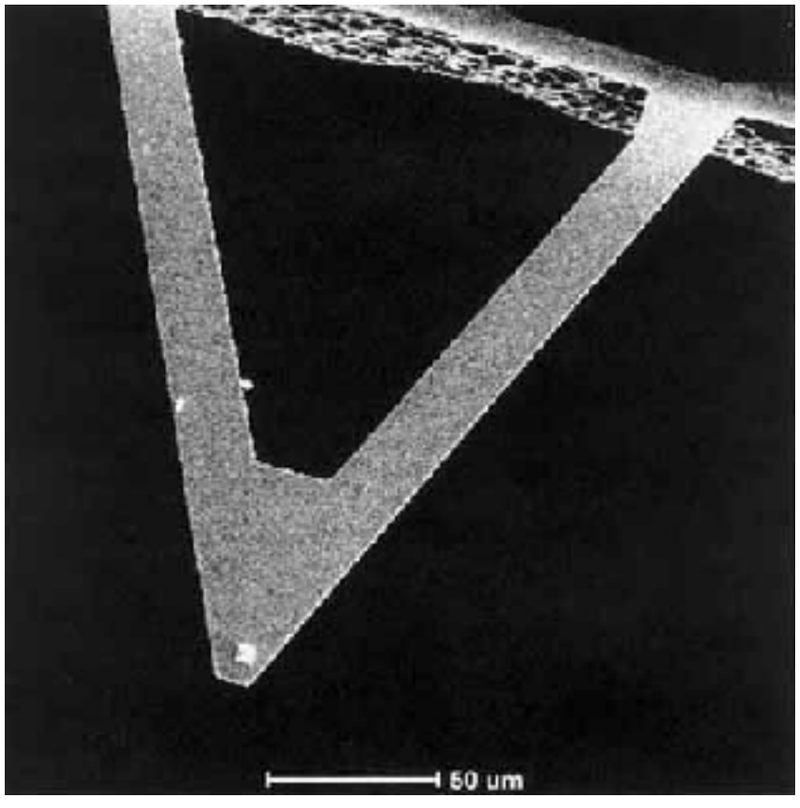
AFM cantilevers and tips

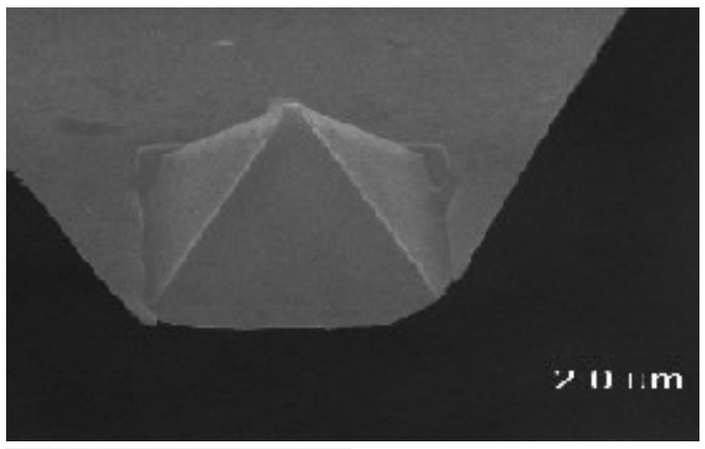
Material: silicon-nitride, silicon, coating: Au, Al
Tip: usually the same material as the cantilever; diamond, carbon nanotube, polystyrene bead
Resolving power of AFM depends on the tip's radius of curvature

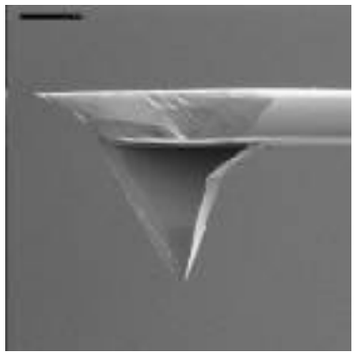


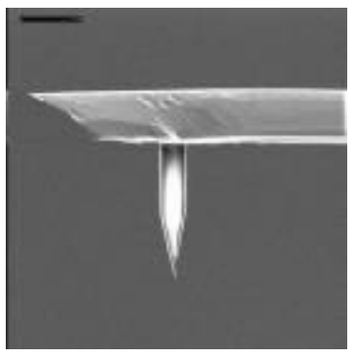


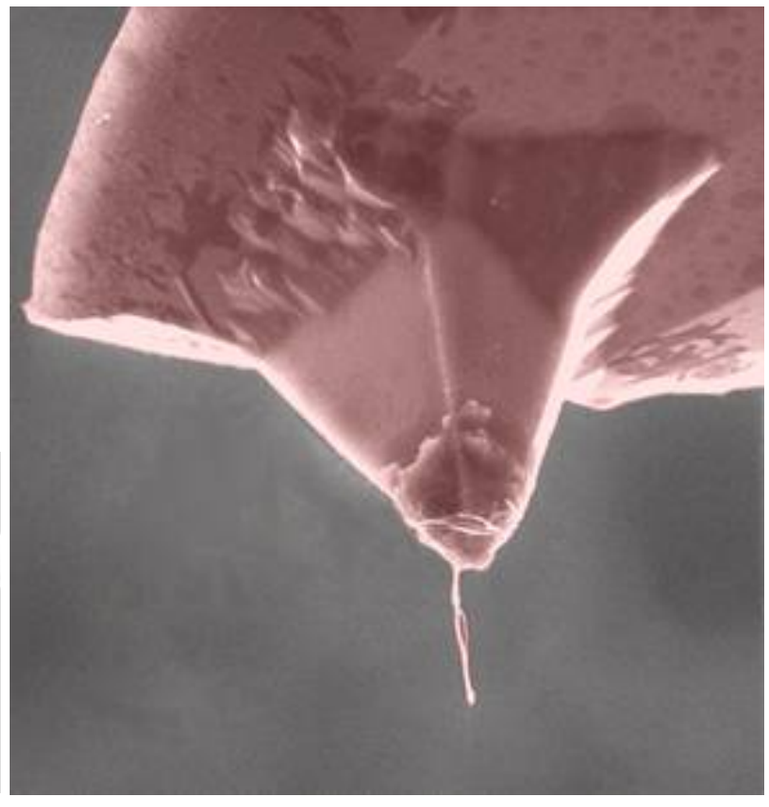






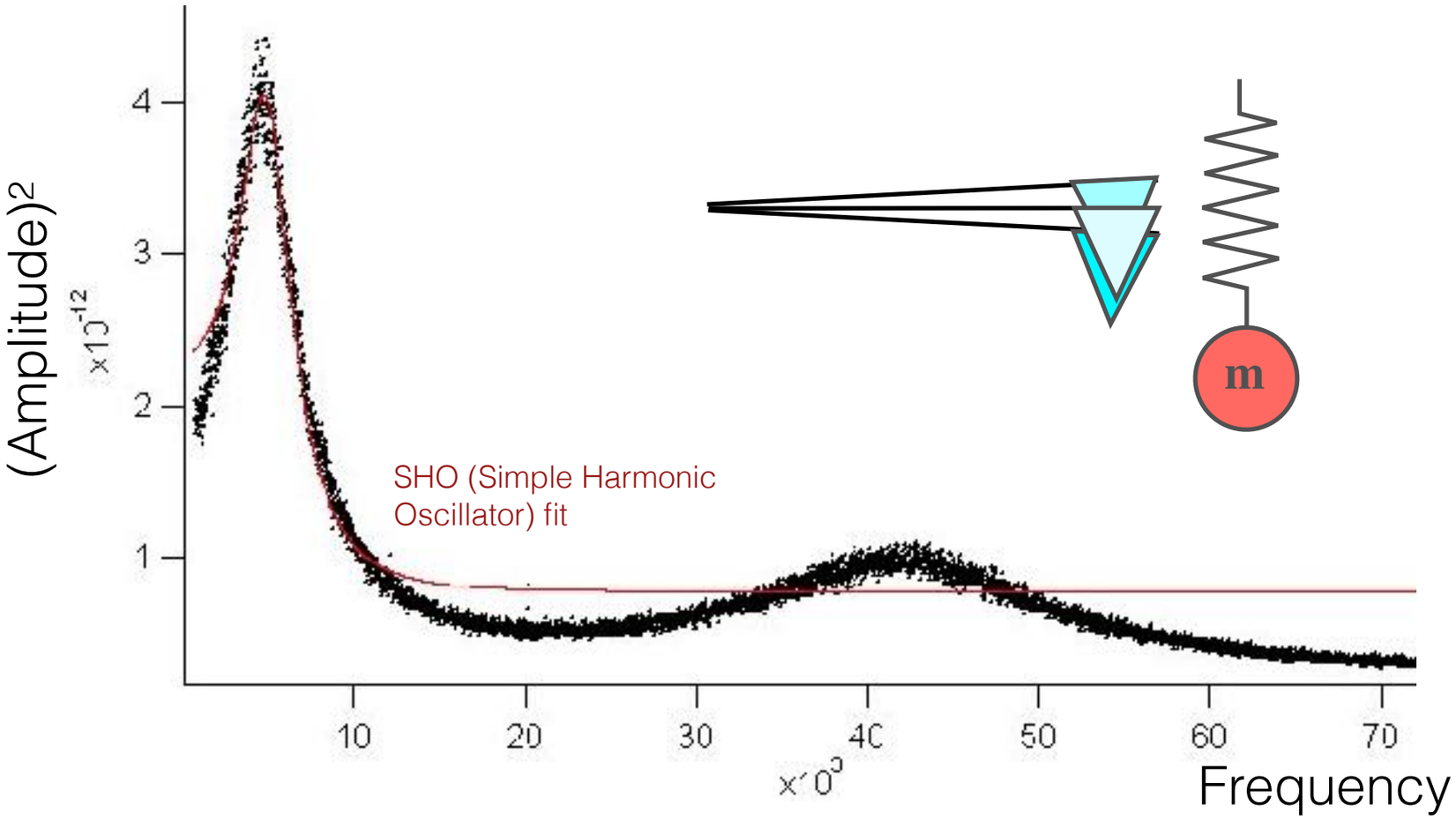






Calibrating the cantilever

Thermal tune: finds resonance frequency and stiffness
Frequency sweep: finds resonance frequency and harmonics



$$\kappa = m\omega^2$$

$$\frac{1}{2}\kappa\langle x^2\rangle = \frac{1}{2}k_BT$$

κ = stiffness
m = mass
ω ~ resonance frequency
x = deflection

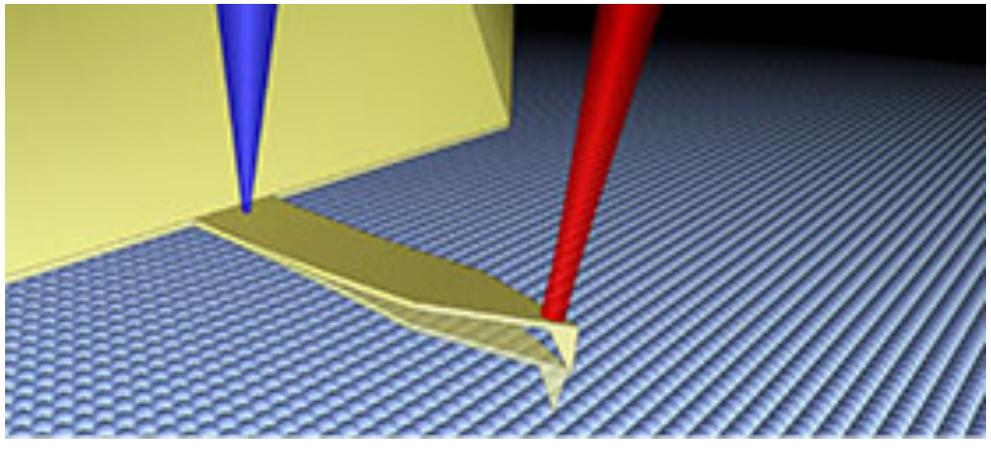
AFM cantilever stiffness ~ 10-1000 pN/nm

Oscillating the cantilever

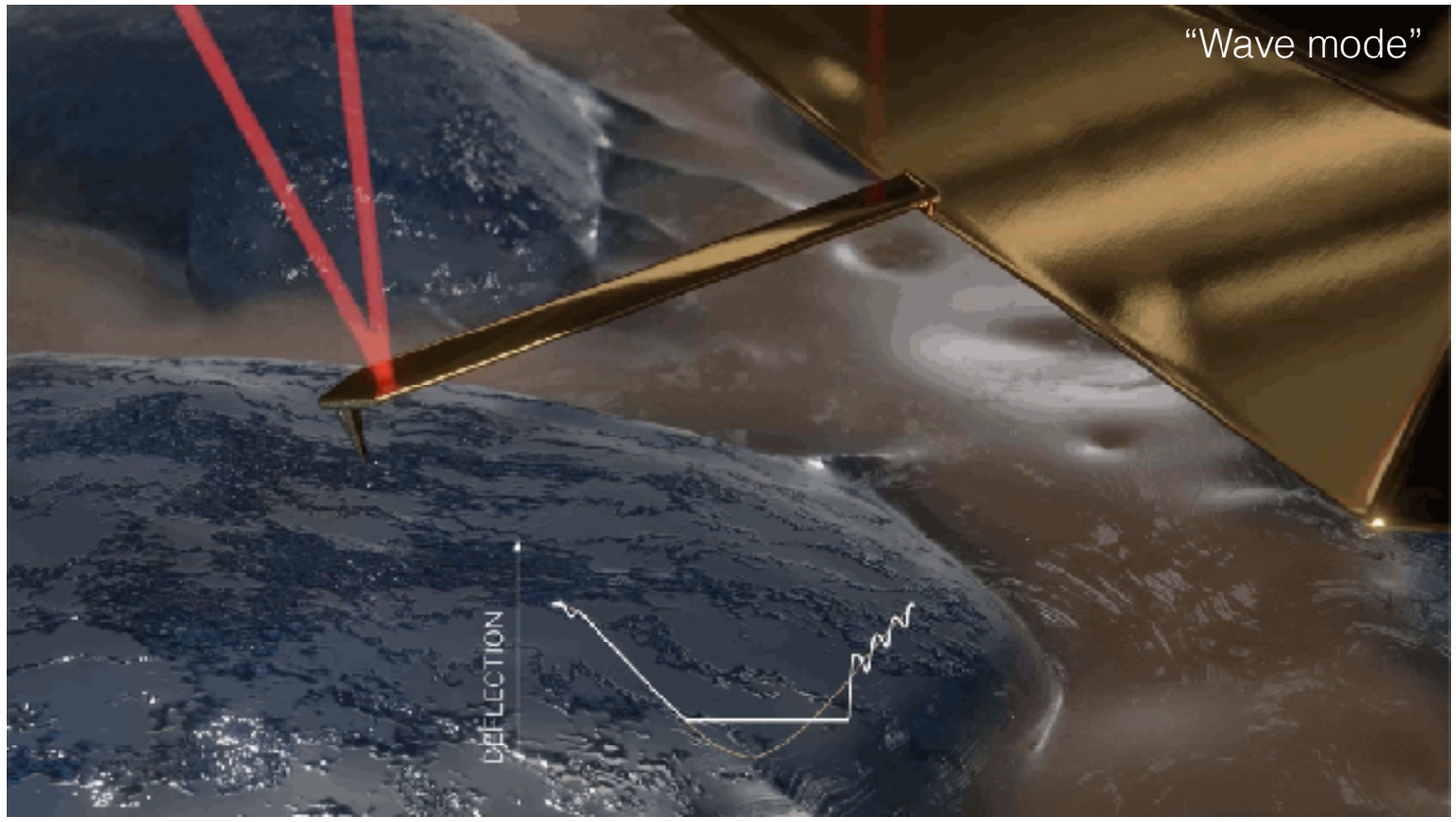
1. Piezo actuator
Problem: PZT far away from cantilever tip

2. Magnetic actuator
Problem: Requires special cantilevers

3. Photothermal excitation



"Blue drive"



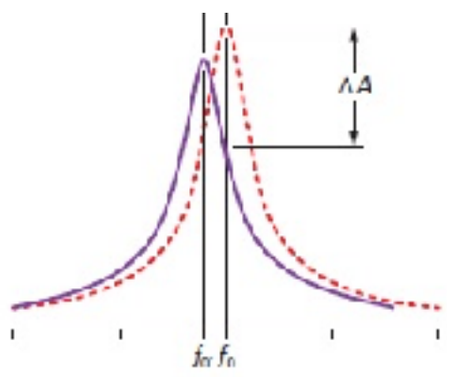
Basic non-contact image modalities

Tapping mode, oscillating mode, AC-mode, dynamic mode, etc.

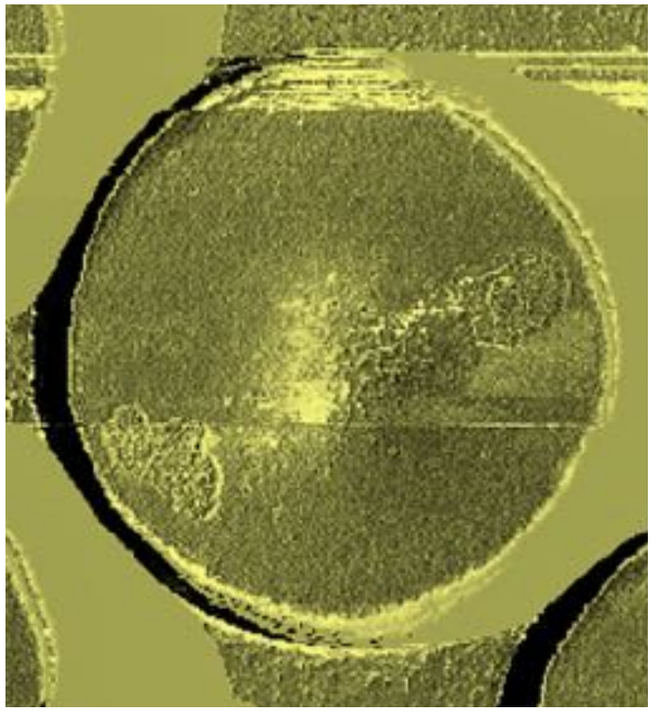
Height contrast

Actual topographical height of the sample at the specific XY coordinates

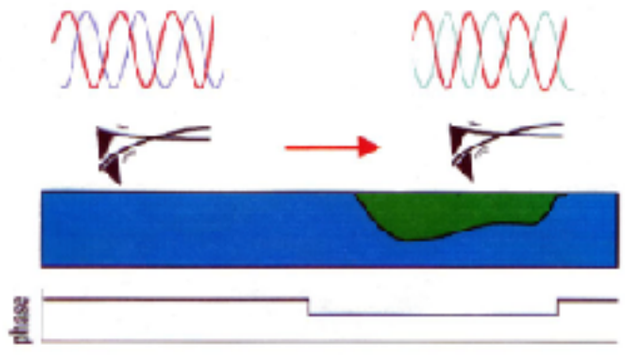
Amplitude contrast



detuning of the resonance peak due to local interactions



Phase contrast

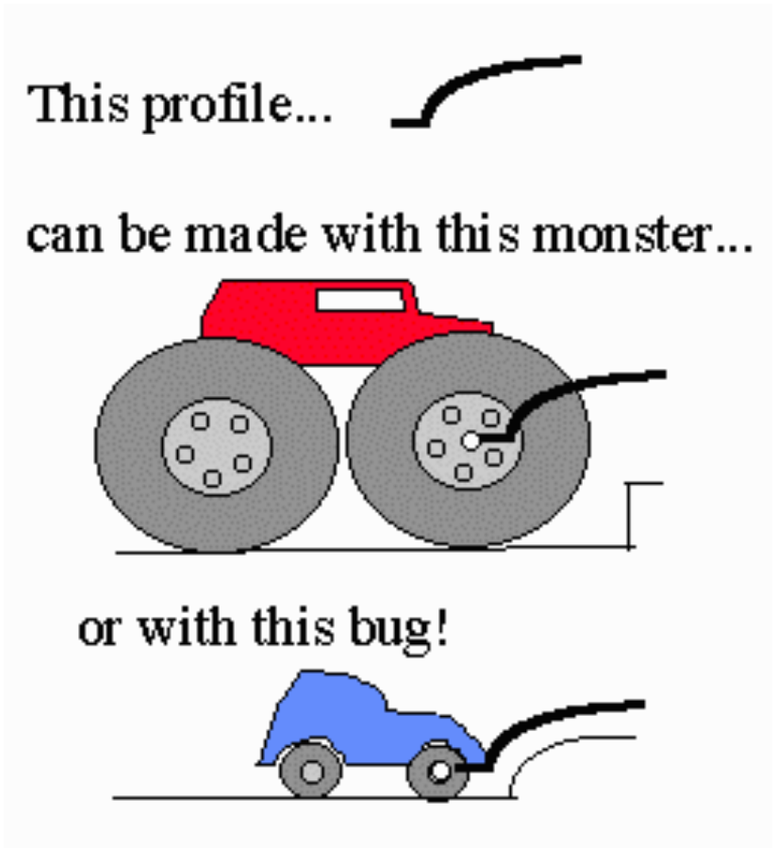


phase difference between driving and detected signals

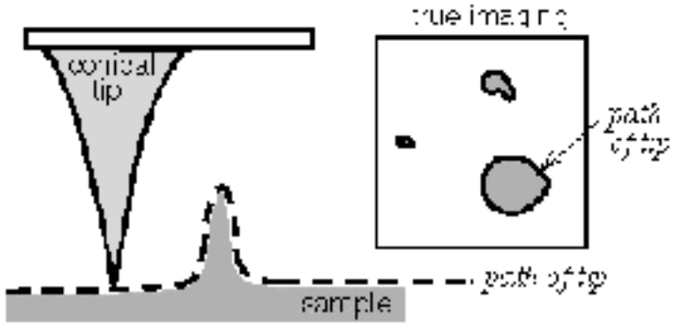
Imaging errors

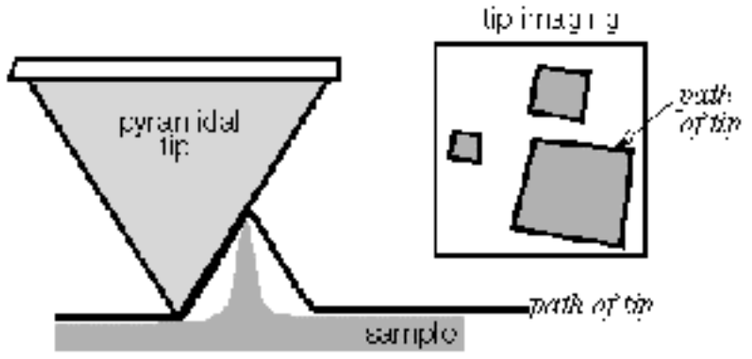
IMPORTANT:

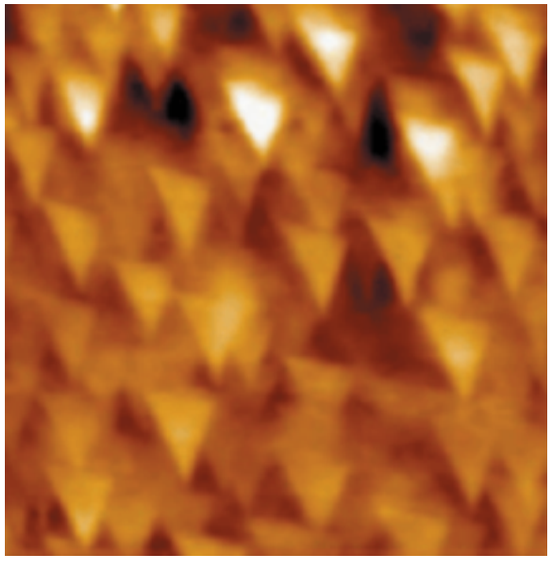
The AFM image is a convolution between the sample surface (base function) and the cantilever (transfer function)



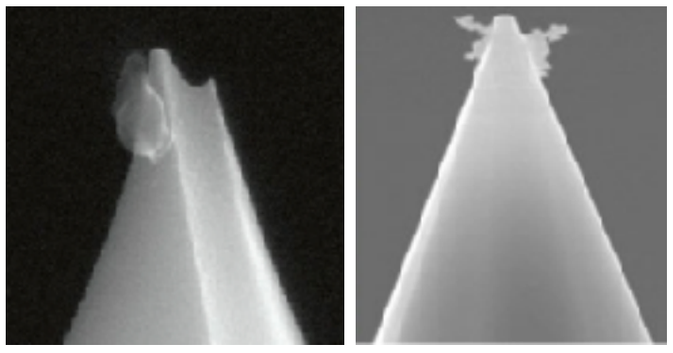
"Sample scanning"



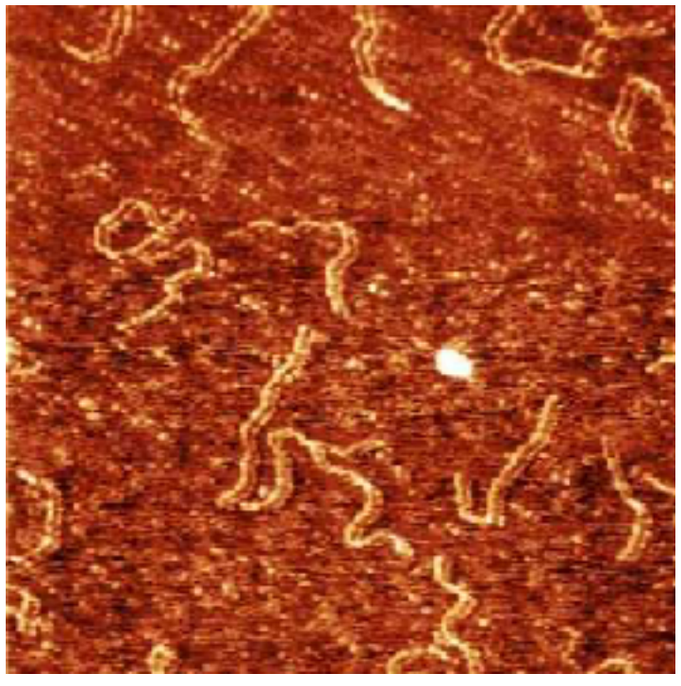




Double tip



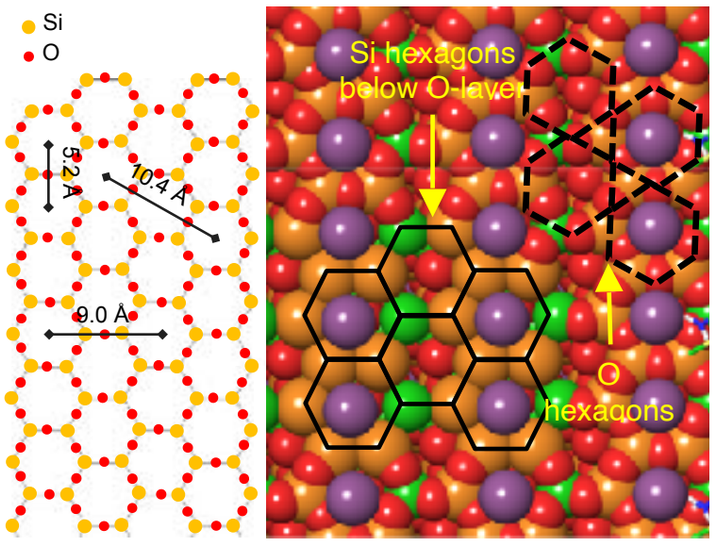
Broken or dirty tip

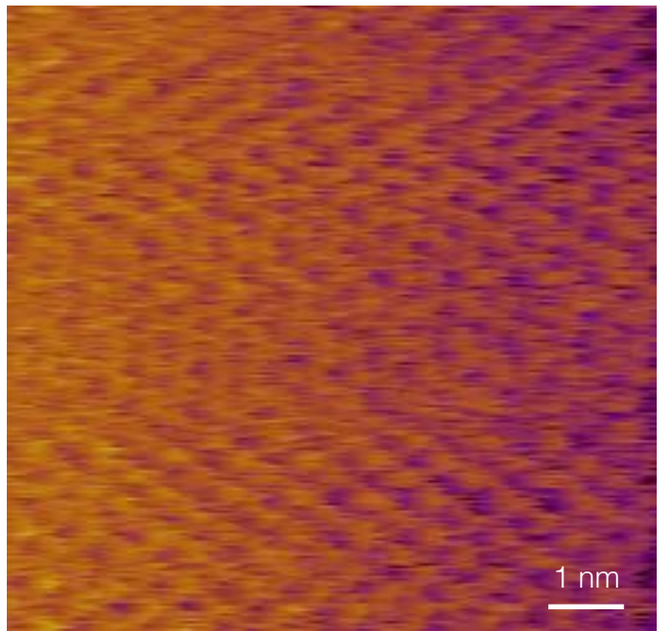


Sample preparation

1. Substrate selection

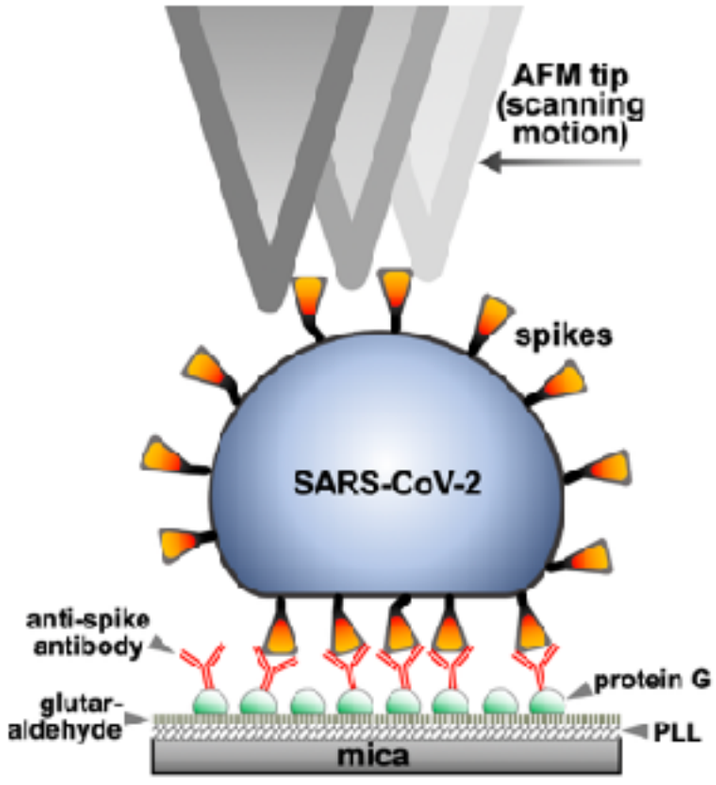
Common substrates:
mica, HOPG (highly oriented pyrolytic graphite), quartz, glass





2. Surface modification (if needed)

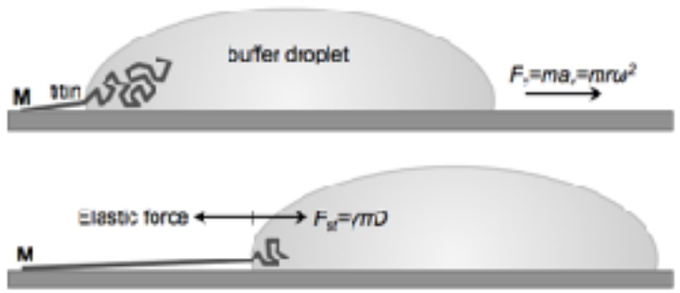
Charge modulation
(divalent positive ions, PLL, plasma treatment)
Cross-linker use
(glutaraldehyde, APTES, antibodies)

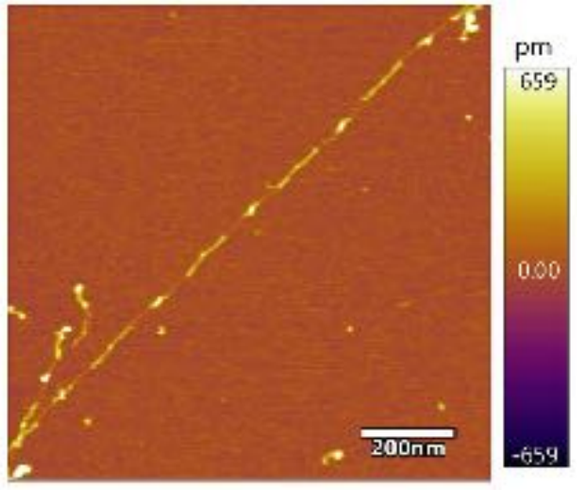


3. Sample application

Non-specific adsorption
Incubation
Cross-linking
Chemical fixation
Drying

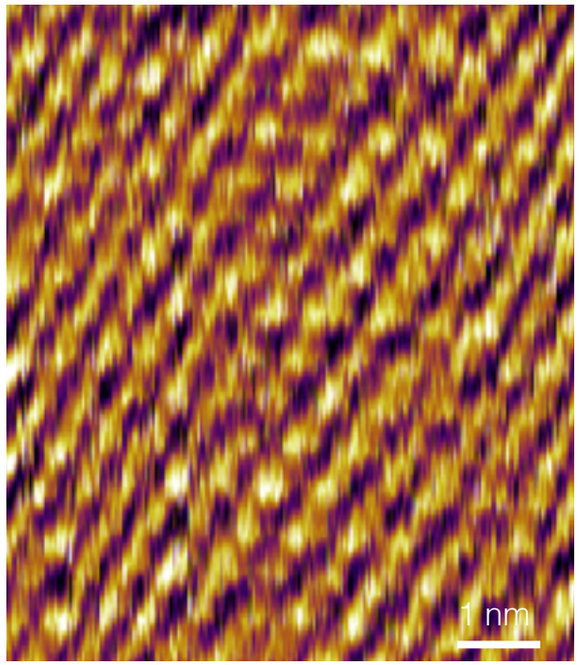
Sample may be mechanically modulated during application: meniscus stretching



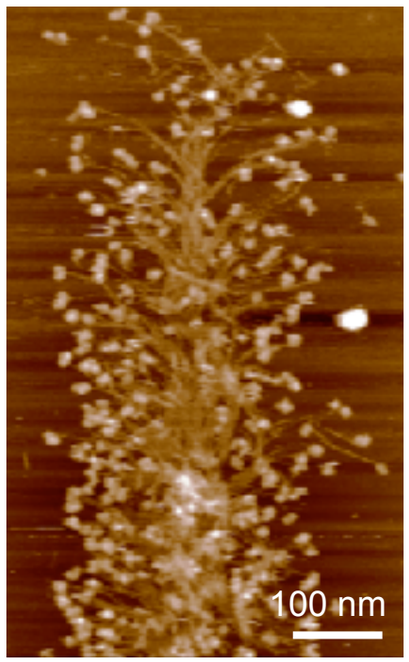


von Willebrand factor multimer

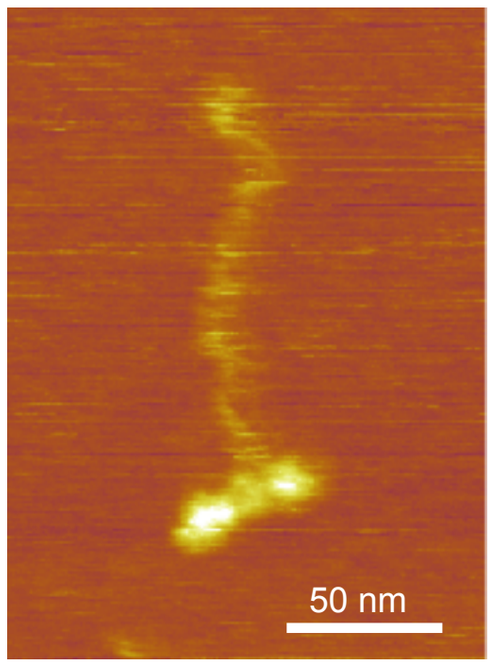
AFM image gallery



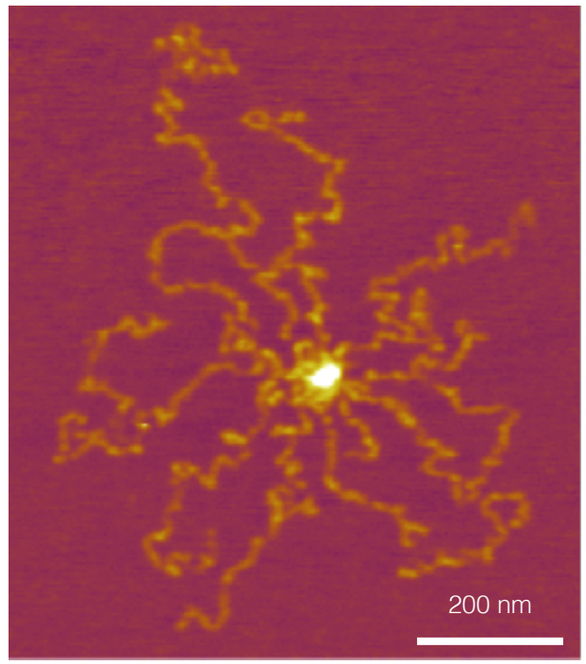
Atomic structure of mica



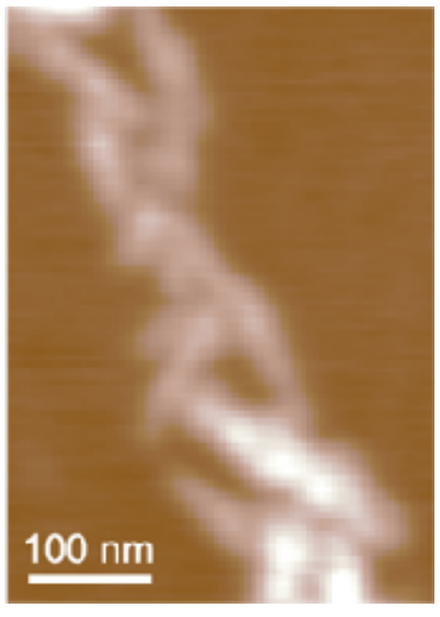
Myosin filament



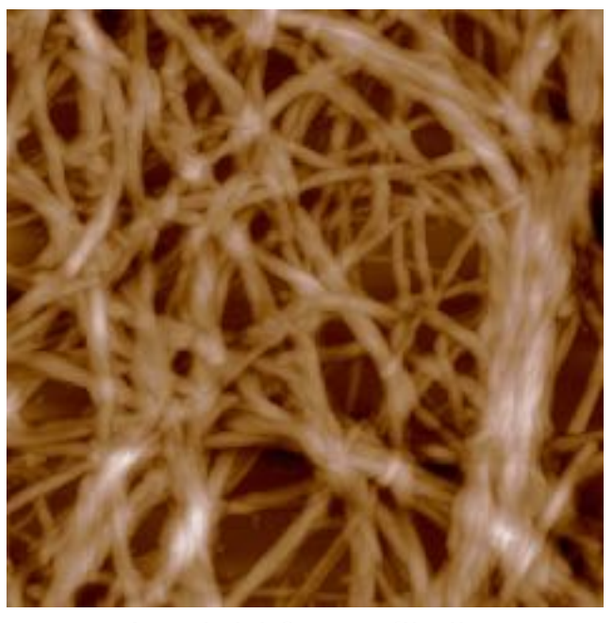
Myosin molecule



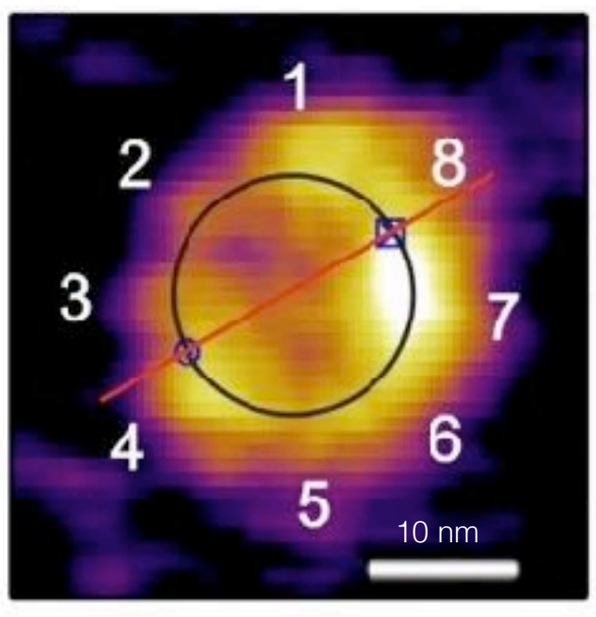
Titin oligomer



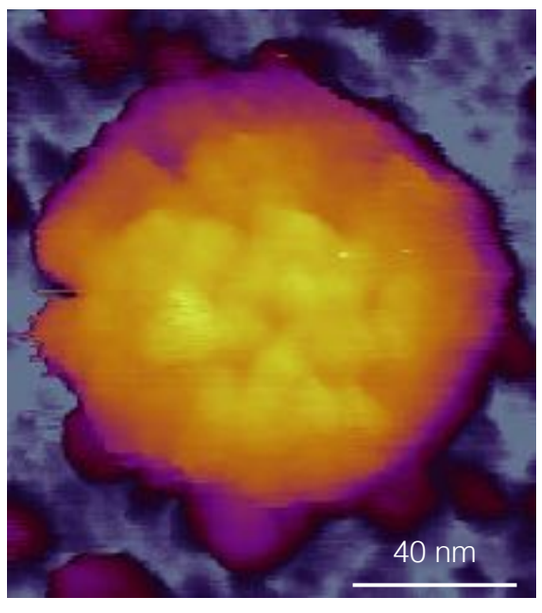
Desmin filament



Amyloid ß1-40 fibrils



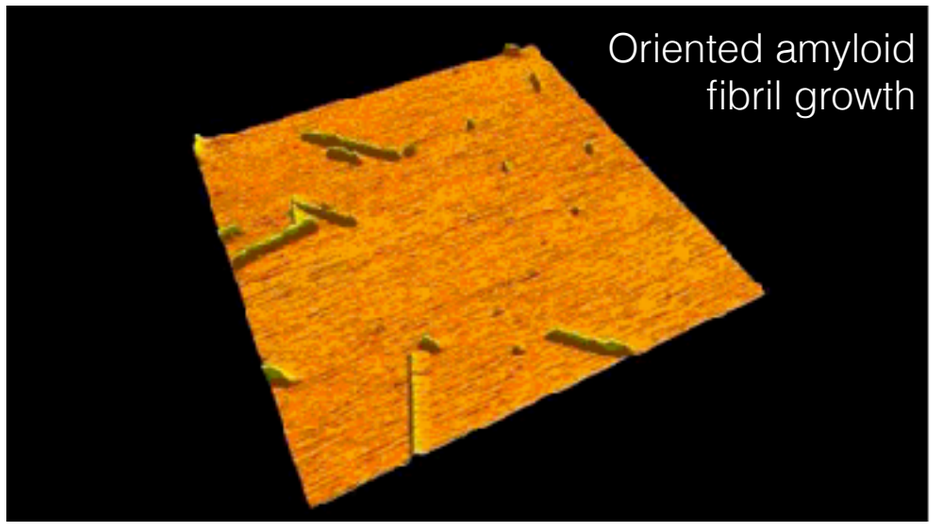
TTR ring oligomer

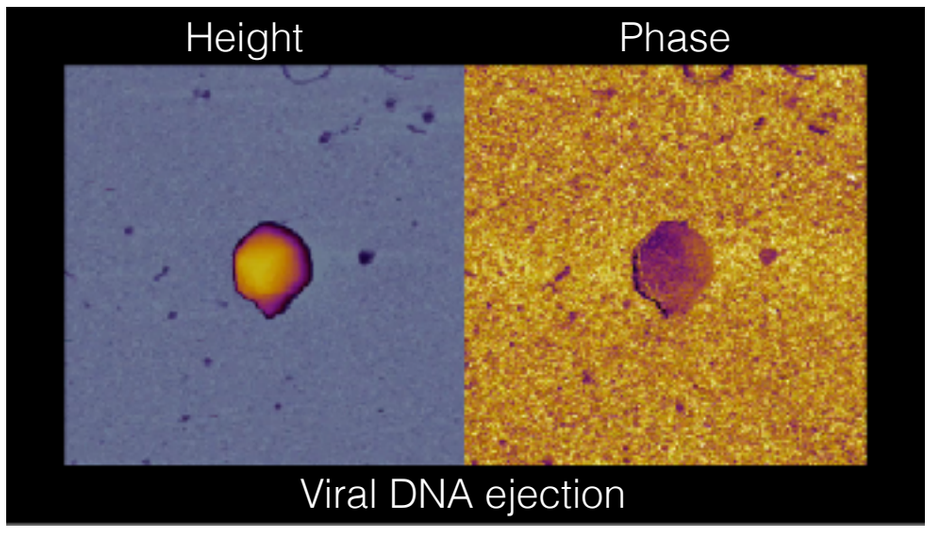


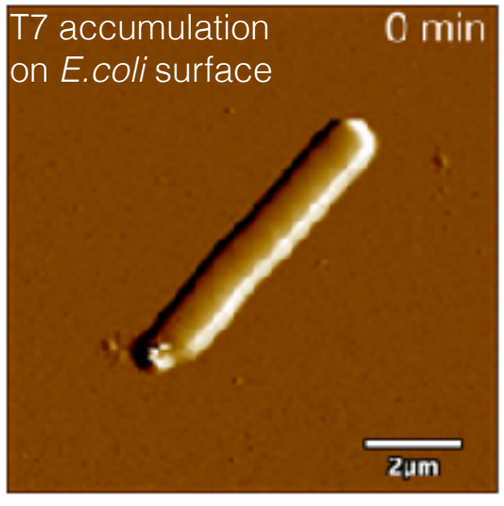
SARS-CoV-2

AFM is much more than static imaging

Time-resolved (*in situ*) AFM

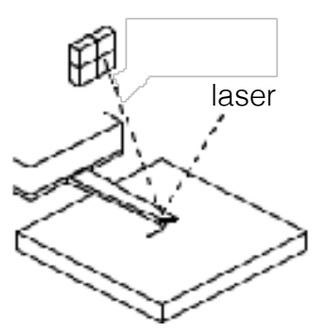


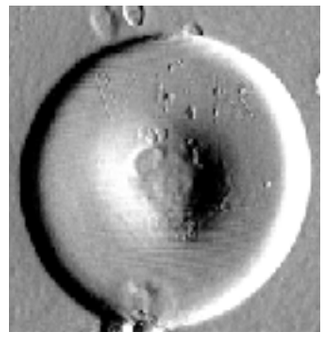




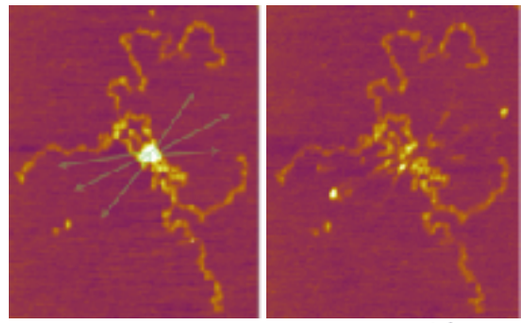
Mechanics: local perturbation with force

1. Nanolithography
(lithos: stone)



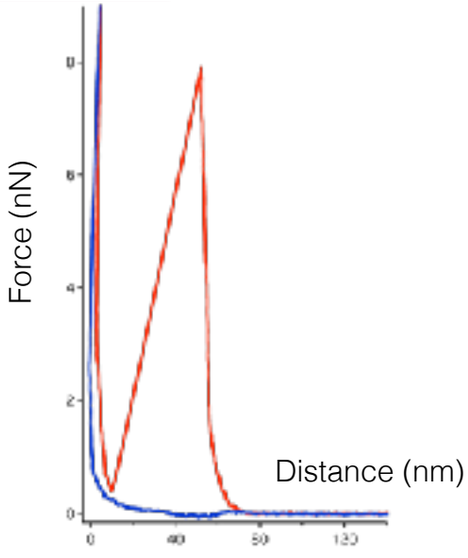


2. Nanosurgery,
nanodissection

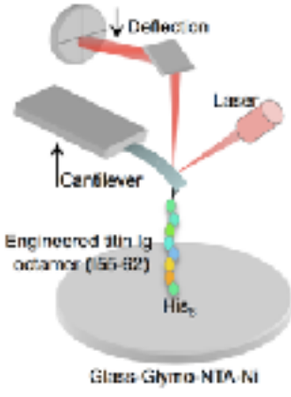


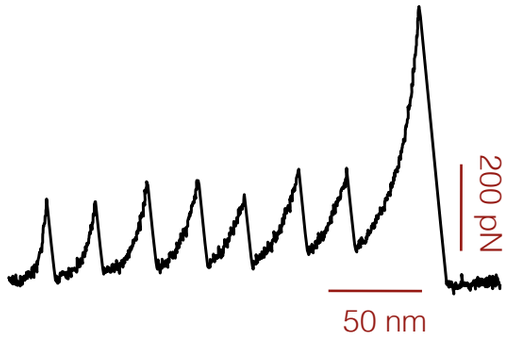
Titin molecule
nanosurgery

3. Nanoindentation



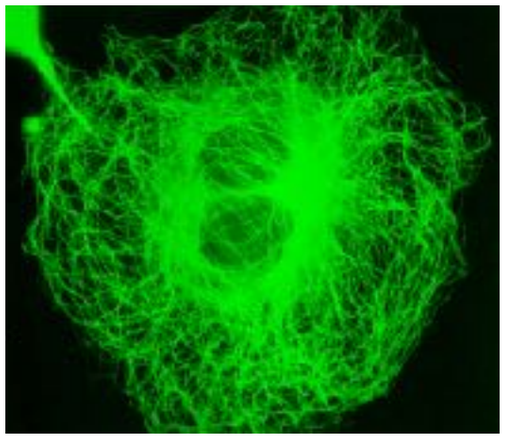
4. Force spectroscopy,
dynamic force
spectroscopy



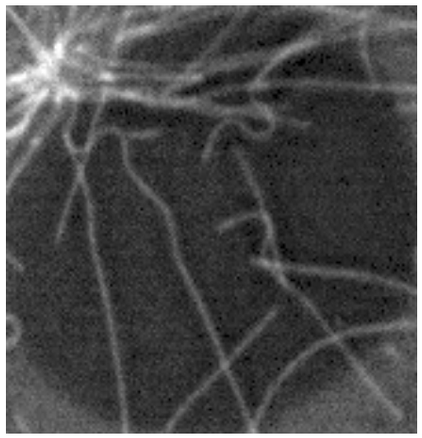


Single-molecule biophysics

1. Individuals (spatial and temporal trajectories) may be identified in a crowd

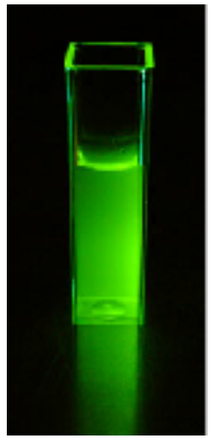


Ensemble - microtubular system

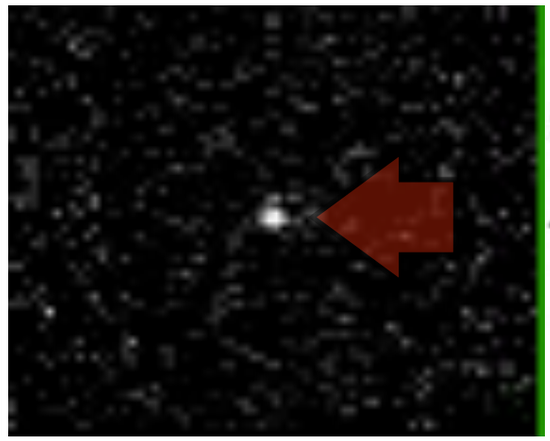


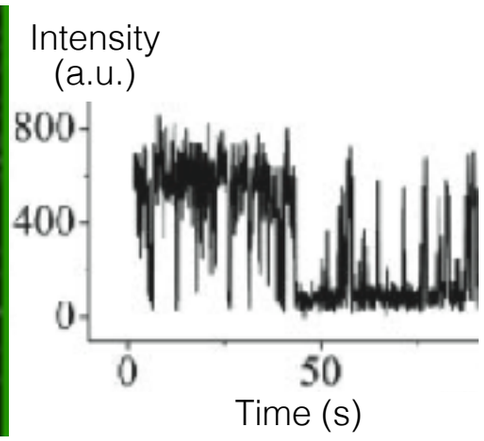
Single microtubues - treadmilling

2. Stochastic processes may be uncovered



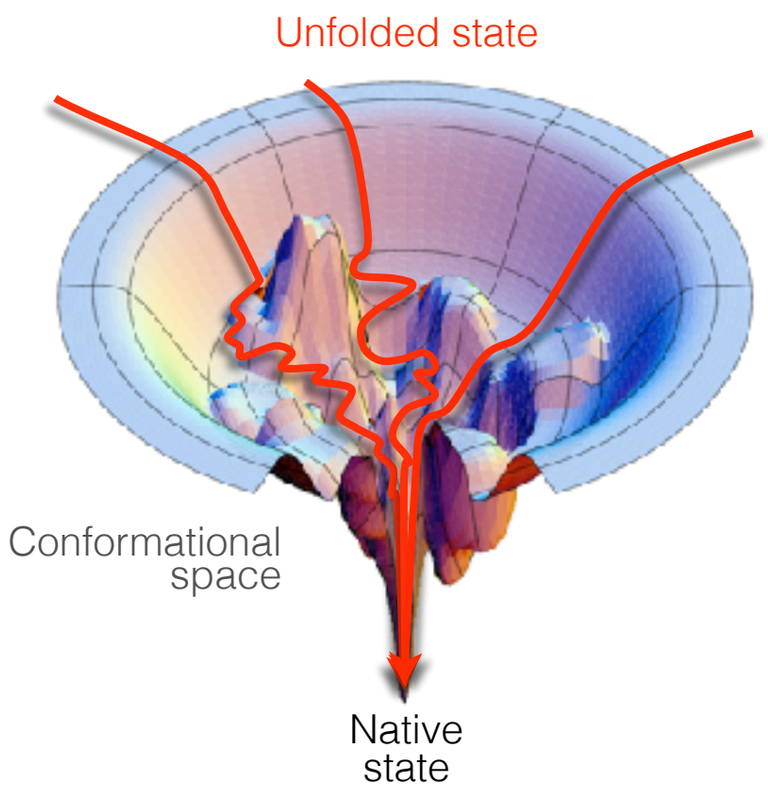
Ensemble - intensity



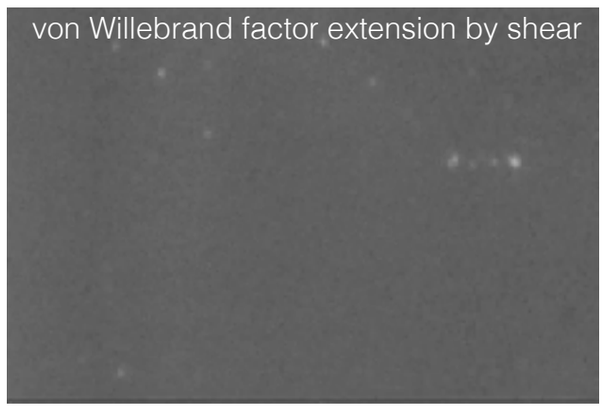


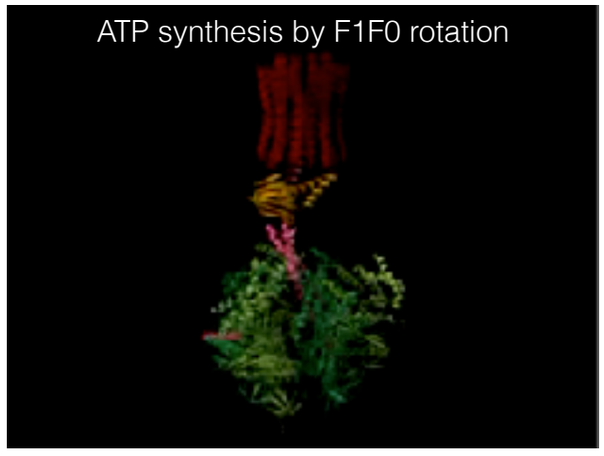
Single quantum dot - blinking

3. Parallel-pathway events may be identified



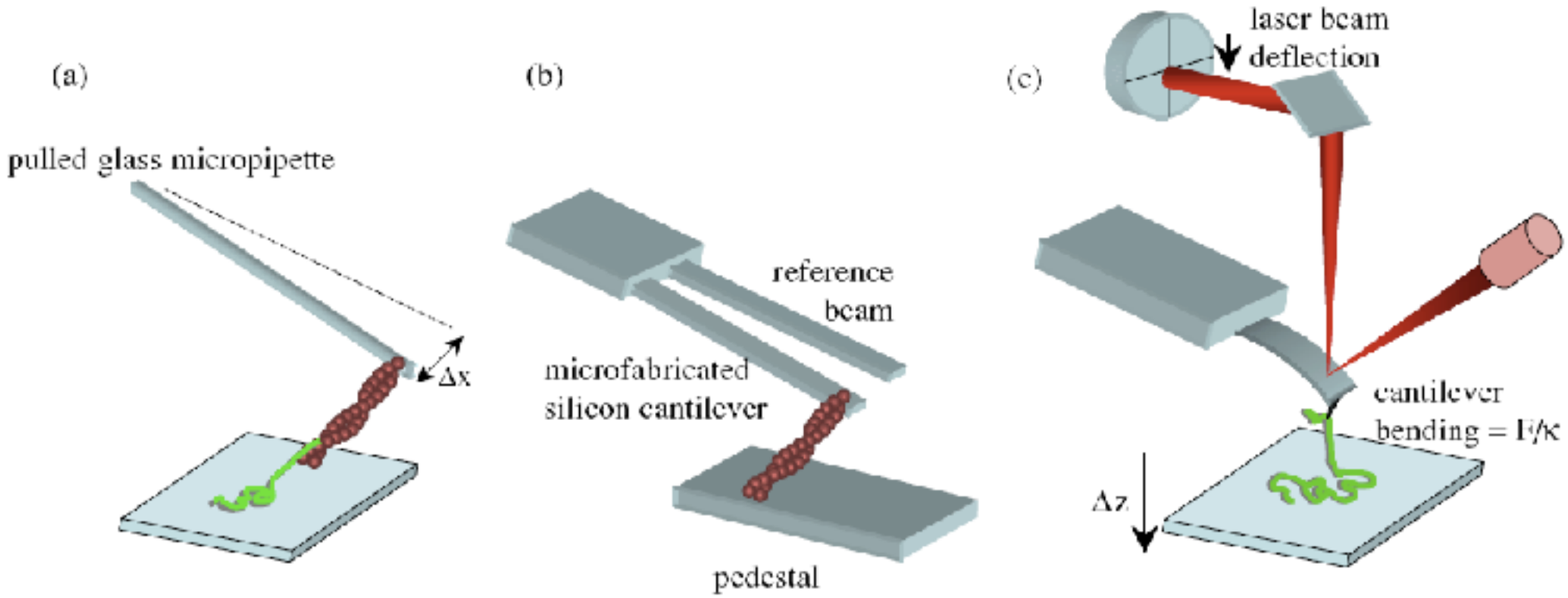
4. Mechanics may be characterized



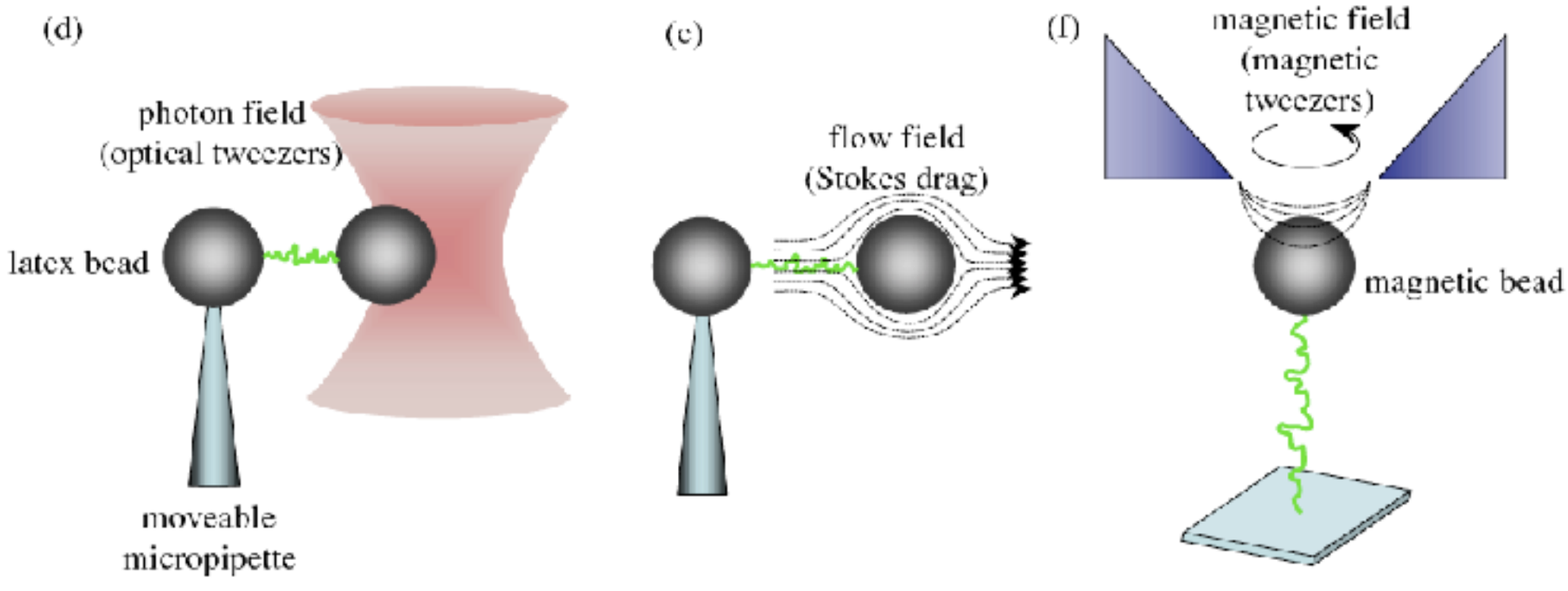


AFM is one of the single-molecule manipulation methods

Cantilever methods

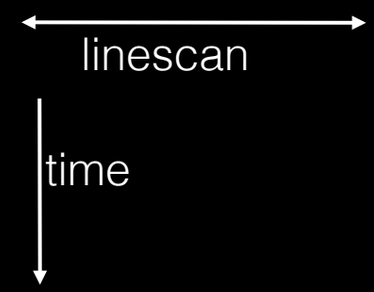


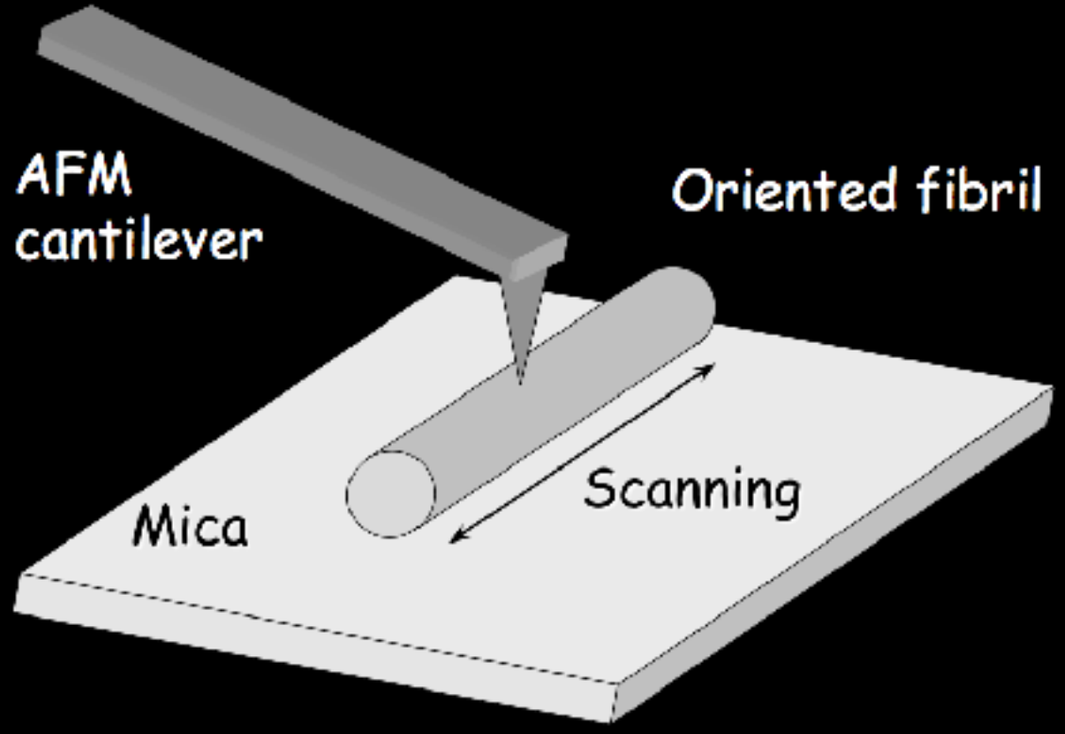
Field-based methods



AFM development

Improving time resolution – Scanning force kymography



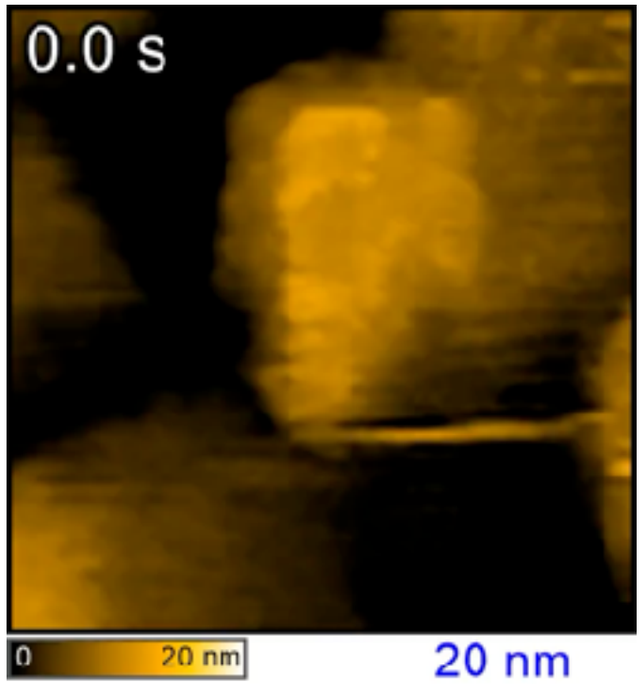


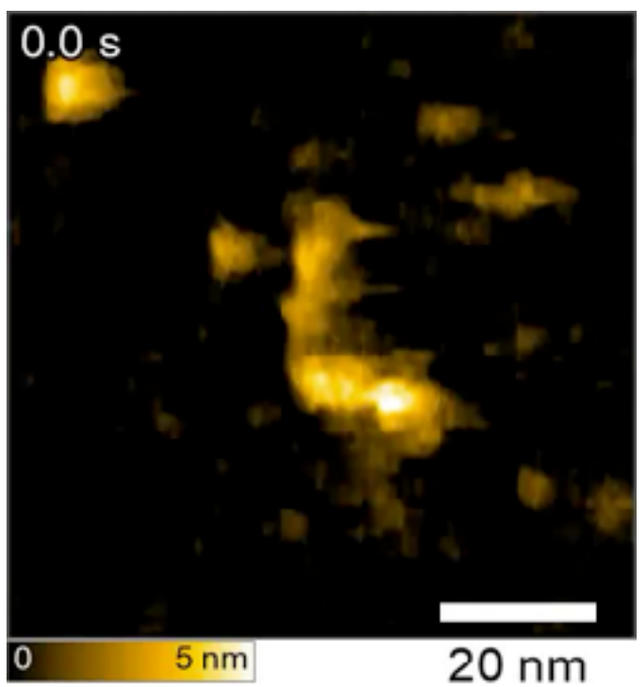
spatial resolution: 1 nm

temporal resolution: ~100 ms

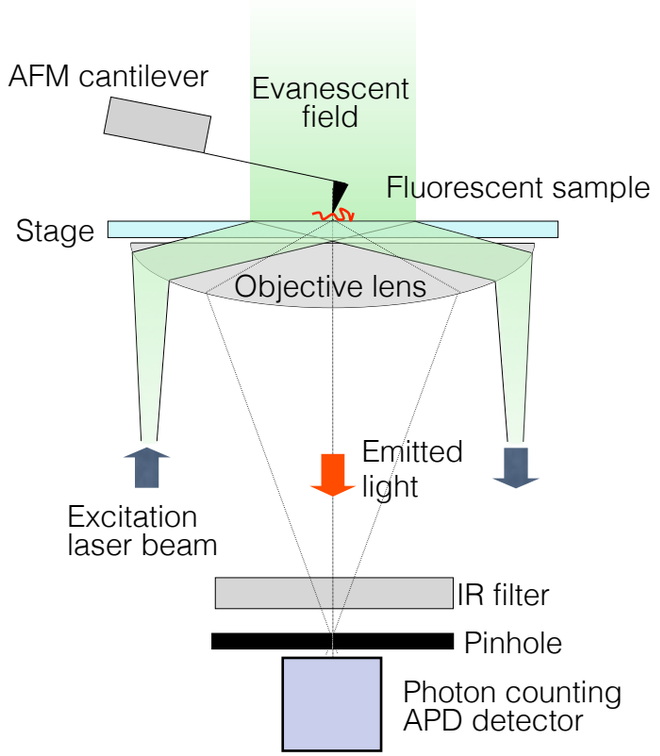
AFM development

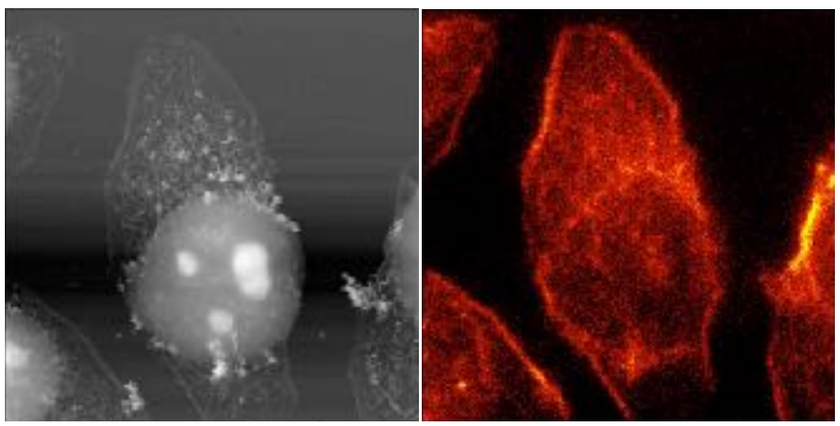
Improving time-resolution: High-speed (HS) AFM

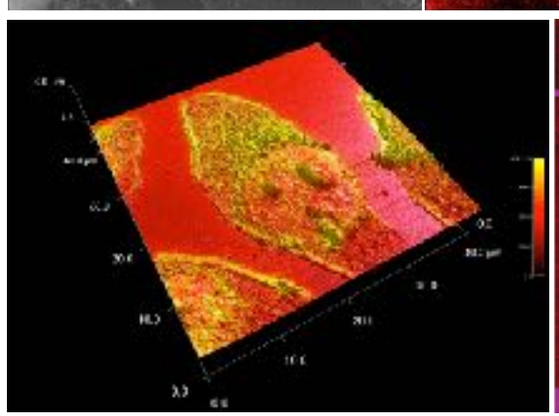




Correlative, multimodal imaging







AFM pros and cons

Pros

Easy sample preparation
Quasi-native conditions
Single-molecule/single-particle method
Multi-layered information
Mechanical manipulation
…

Cons

Substrate adherence
Slow image collection
Surface information
Difficulty of correlating with other HR structural methods
…

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