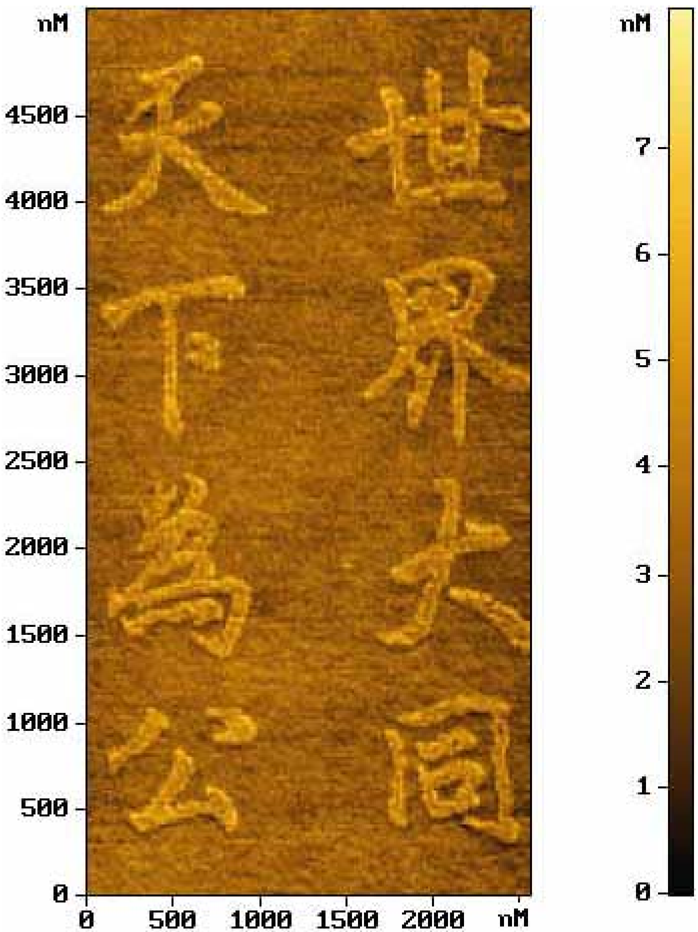
Detection and Analysis of Macromolecules
by
Atomic Force Microscopy (AFM)



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Oliver I. Wagner, PhD
Assistant Professor

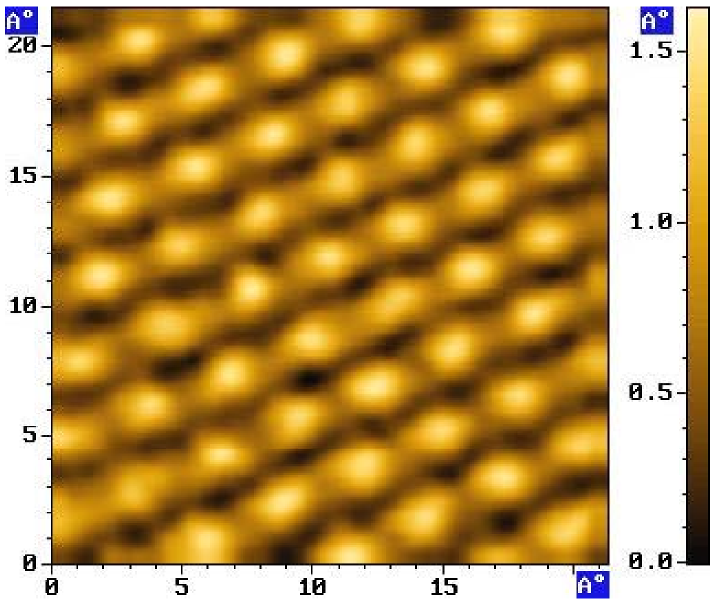
National Tsing Hua University

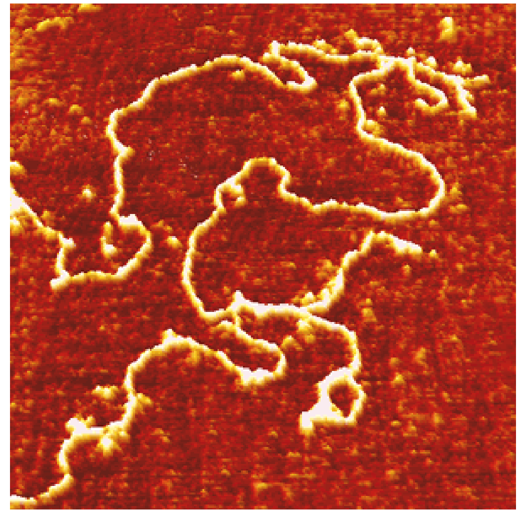
Institute of Molecular and Cellular Biology &
Department of Life Science

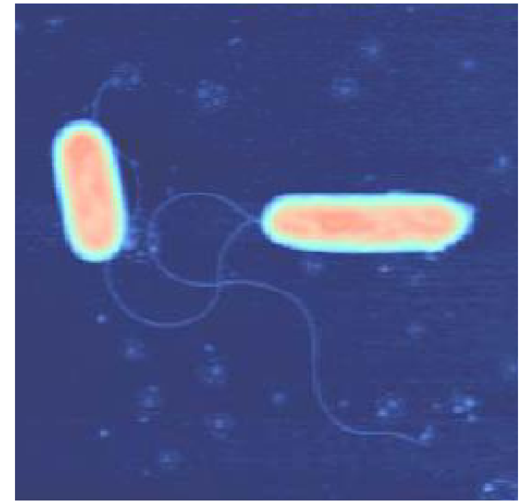
Content

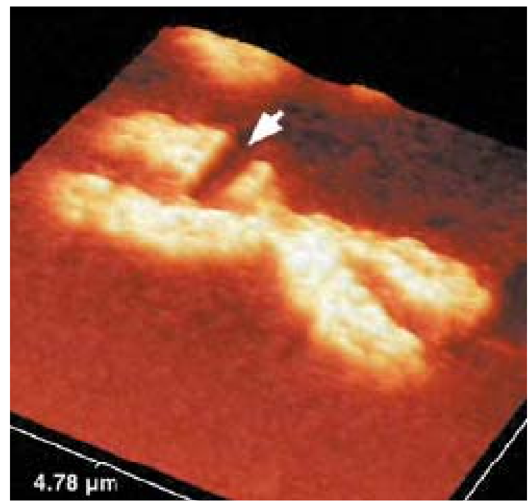
2:00-4:00PM

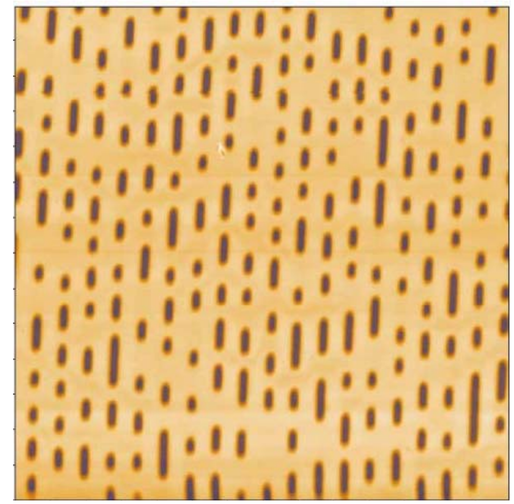
1) What will we learn:







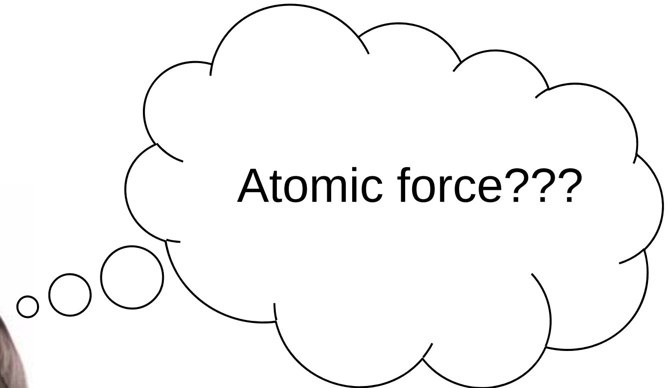




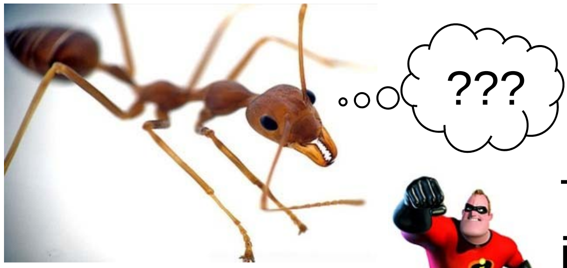
4:00-5:00PM

2) Hands on! We are going to have a demo of an AFM experiment at the Department of Science and Engineering!

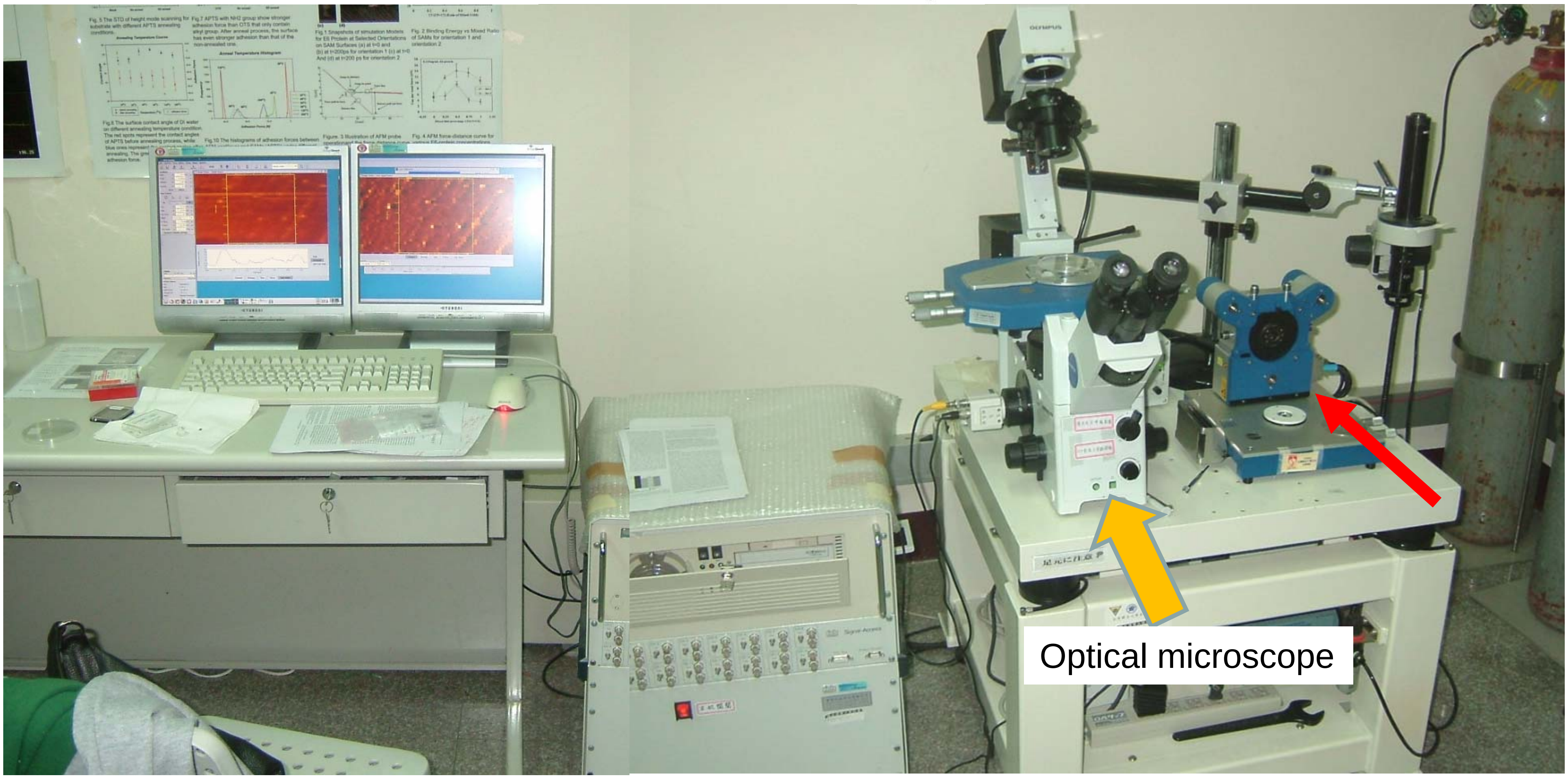
Atomic force microscope



Good news:
No atomic plant connected!



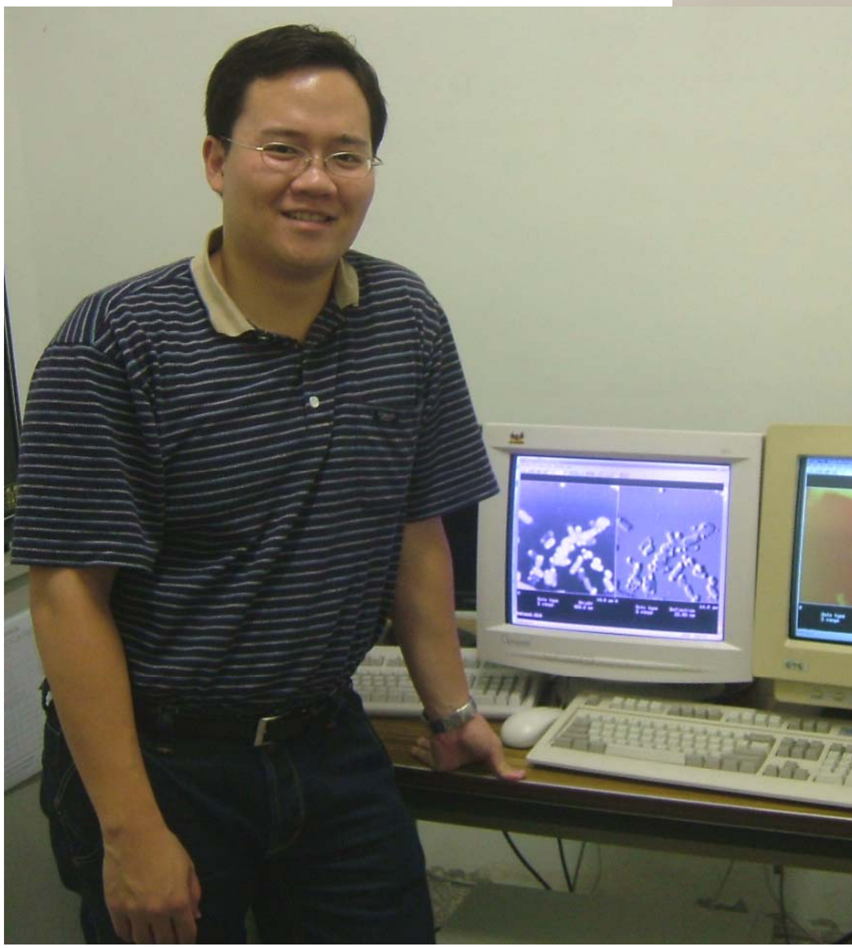
The forces used for AFM imaging are in the "atomic range"

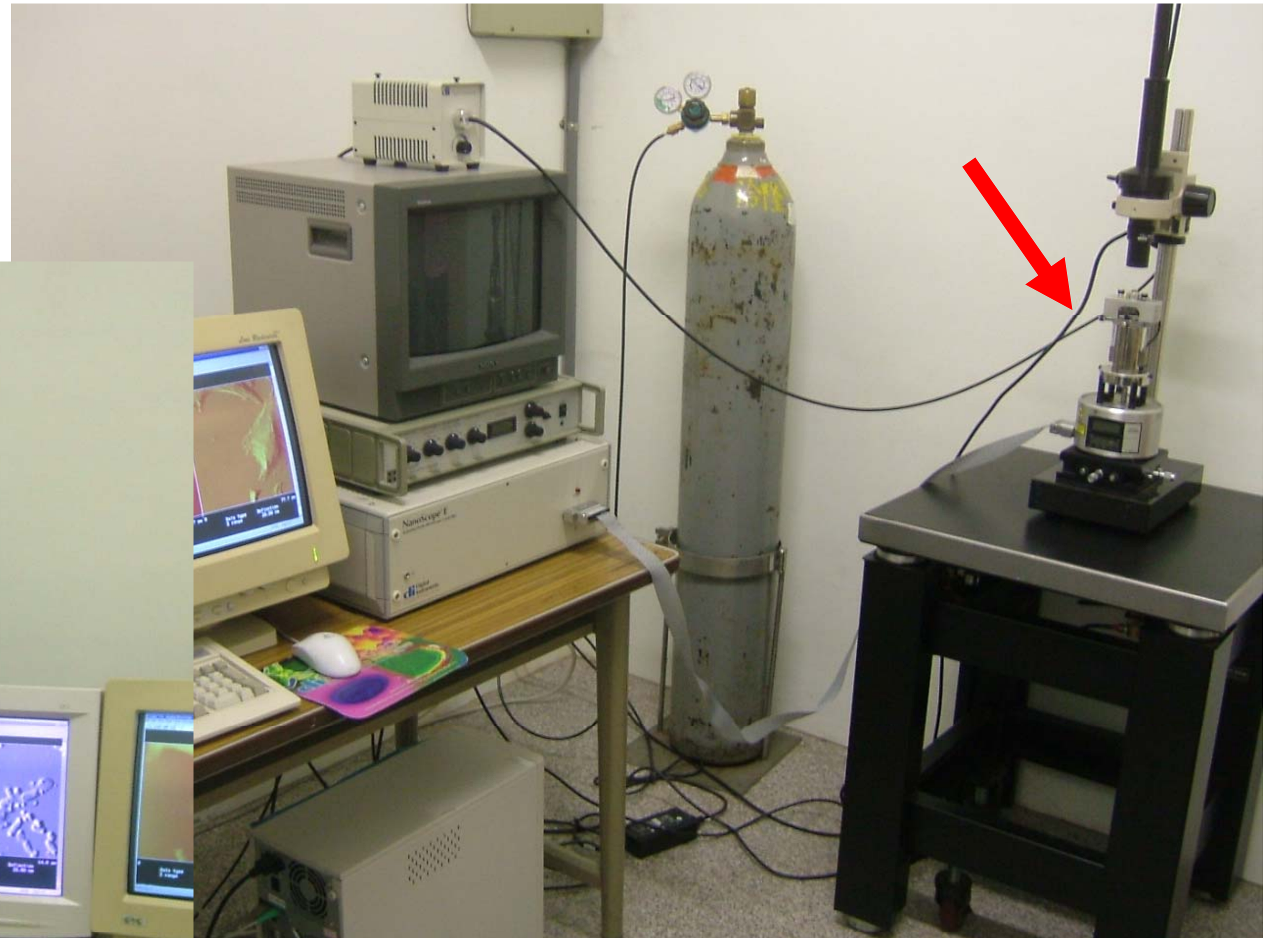


JPK AFM Department of Science and Engineering at NTHU

Two AFM will be introduced today: JPK and Multimode (Veeco)

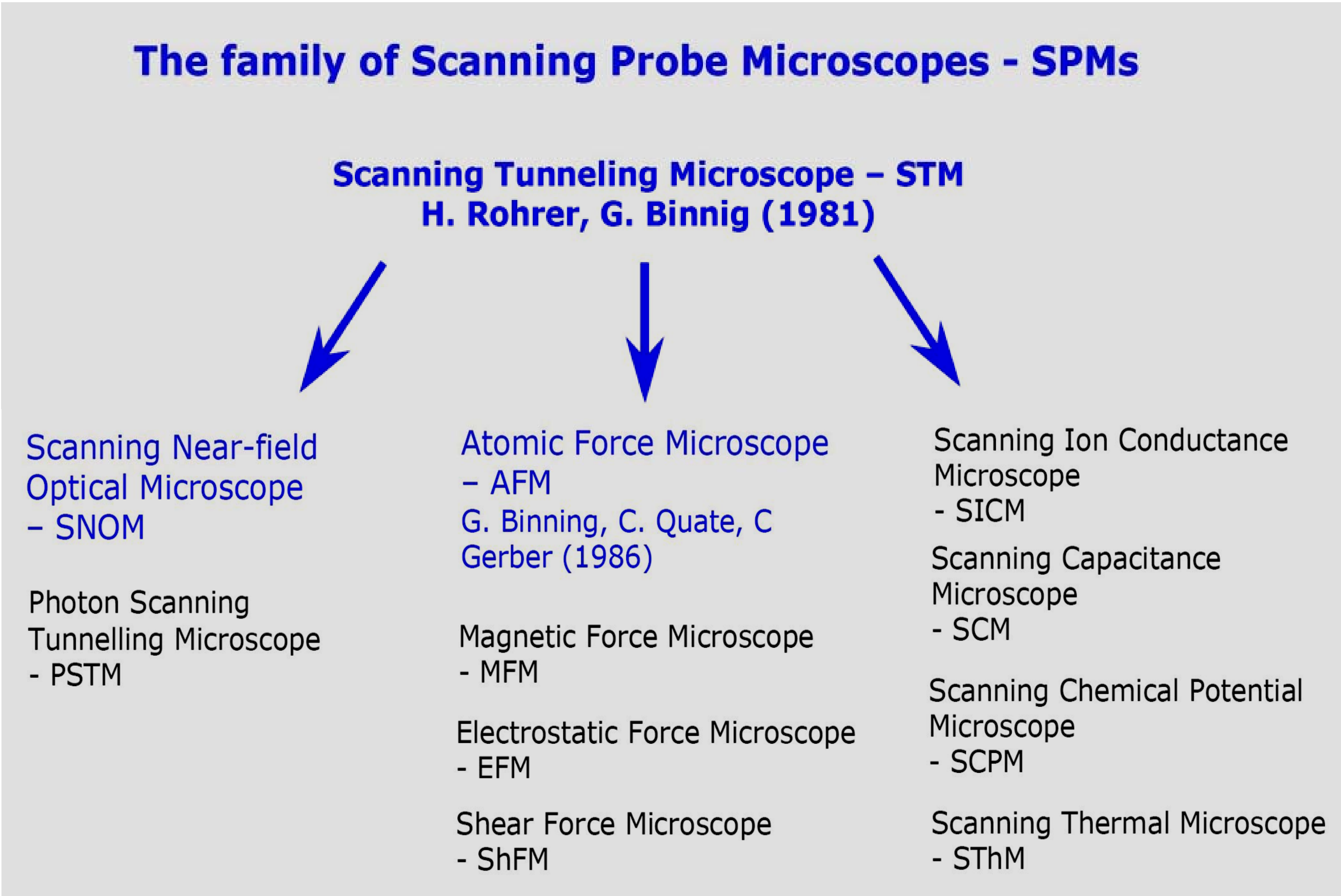
佐民 Joe-Ming





Multimode AFM at the Department of Science and Engineering at NTHU

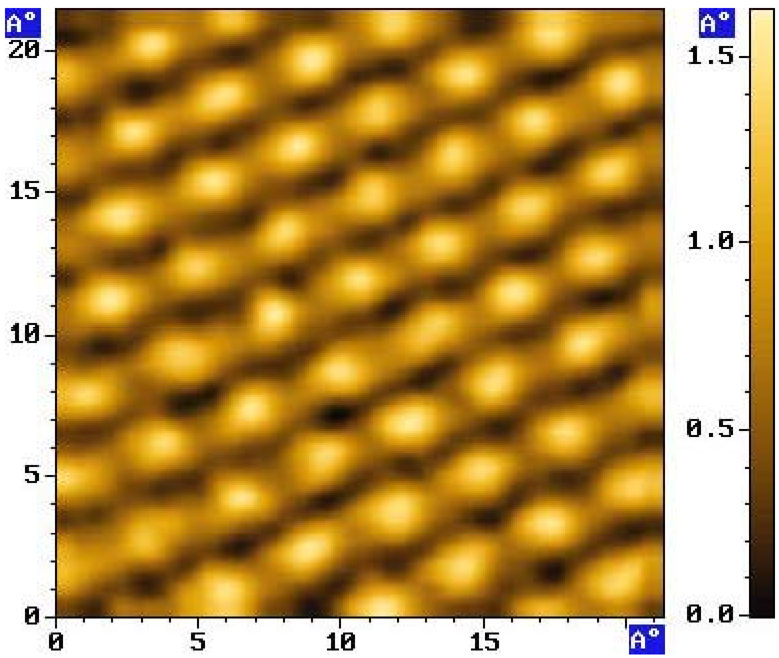
AFM is a modified SPM (Scanning Probe Microscope)

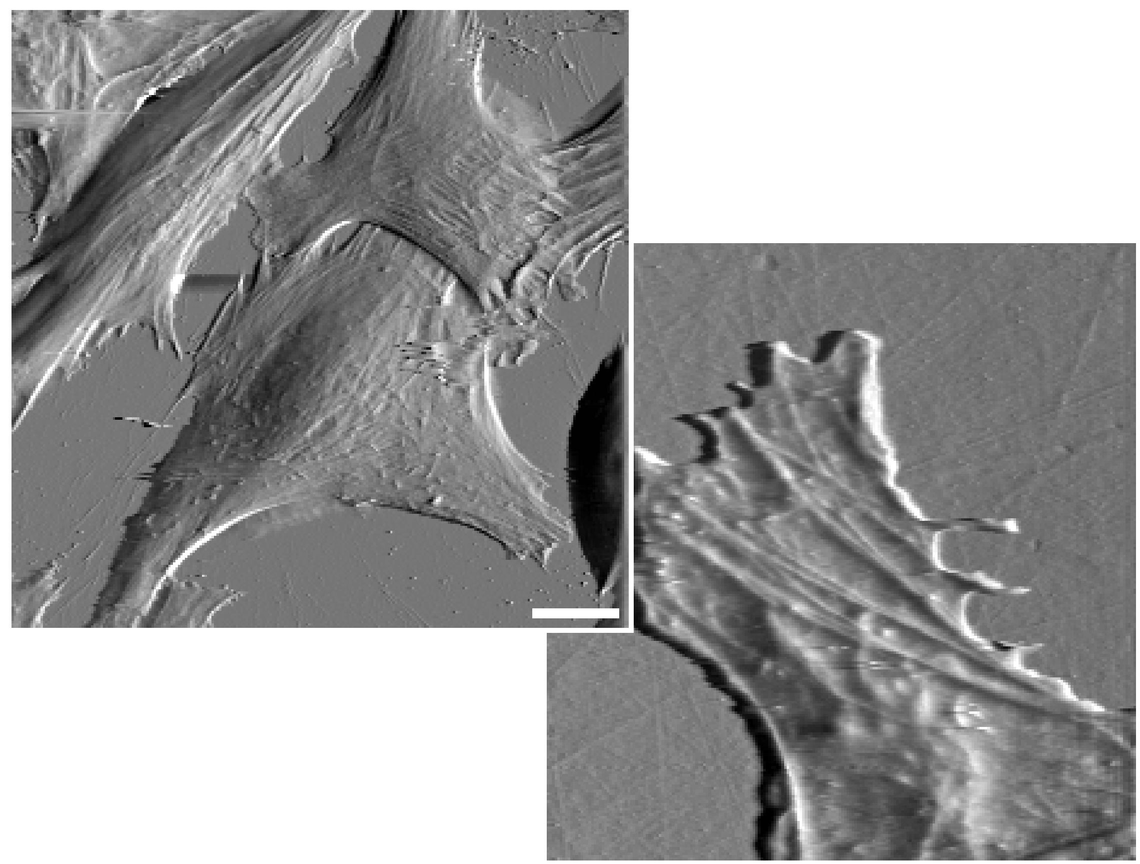


1980s first scanning probe microscope (SPM) presented the first **atomic-scale** image of a surface

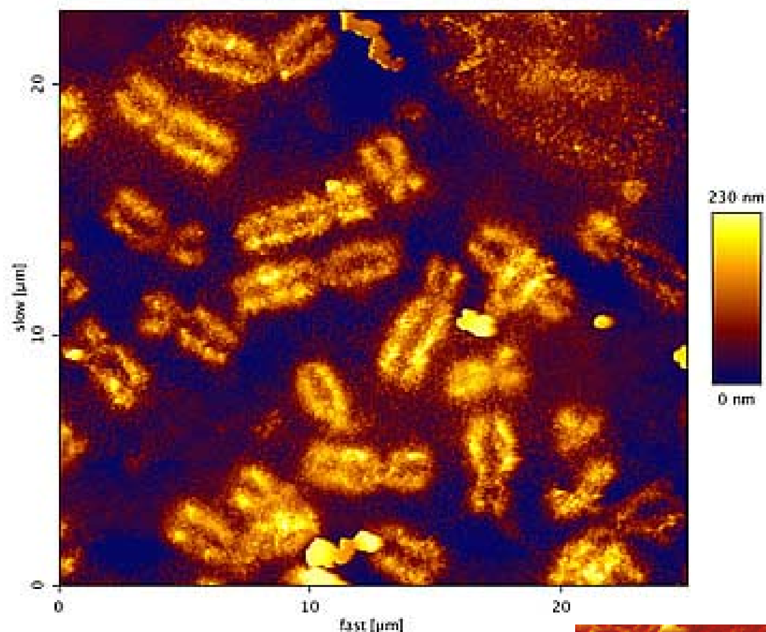
Nowadays SPMs are routinely used in science:

- surface science, material science, life science etc. (basic science)
- routinely checking the roughness of surfaces etc. (industry)
- impressive 3D dimensional imaging from Angstrom graphite atoms to µm-protrusions on a surface of a living cell



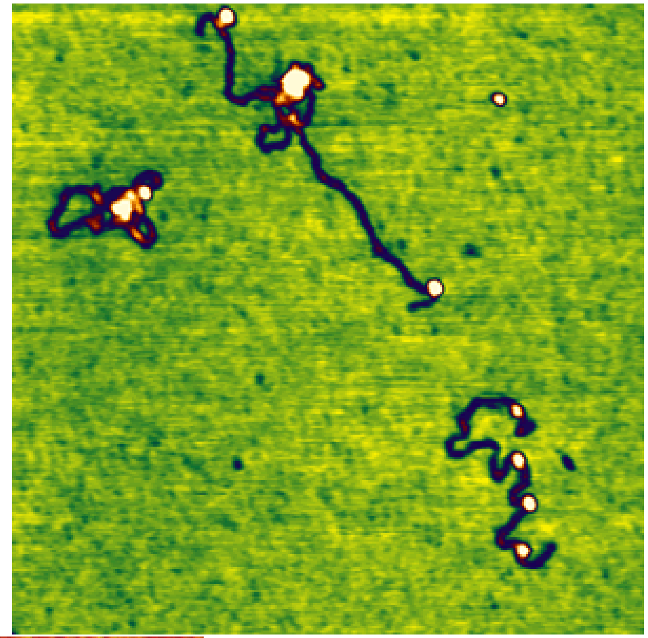


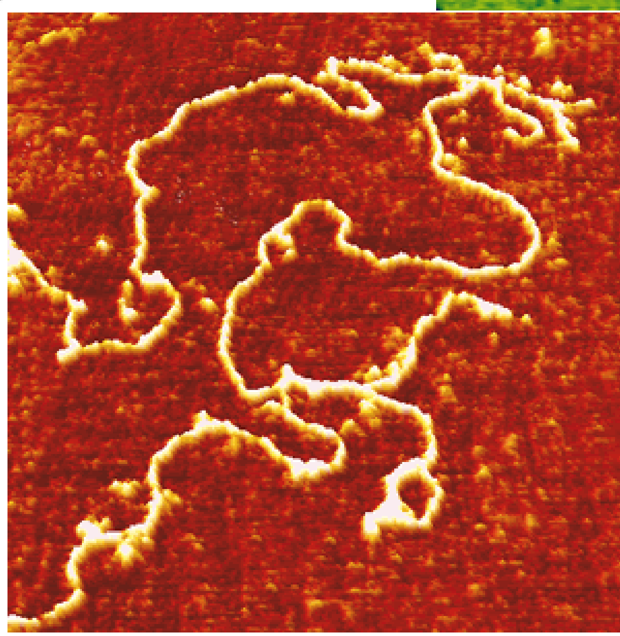
Chromosomes



DNA-nucleosome complexes

x/y = 1.17 µm * 1.17 µm; z-range = 0 - 2 nm



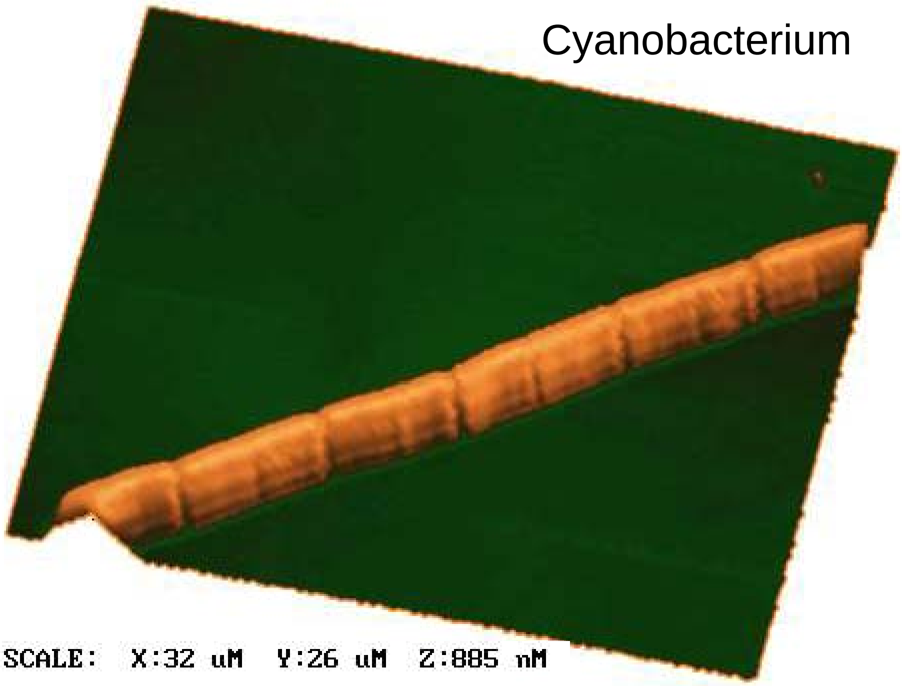


DNA molecules

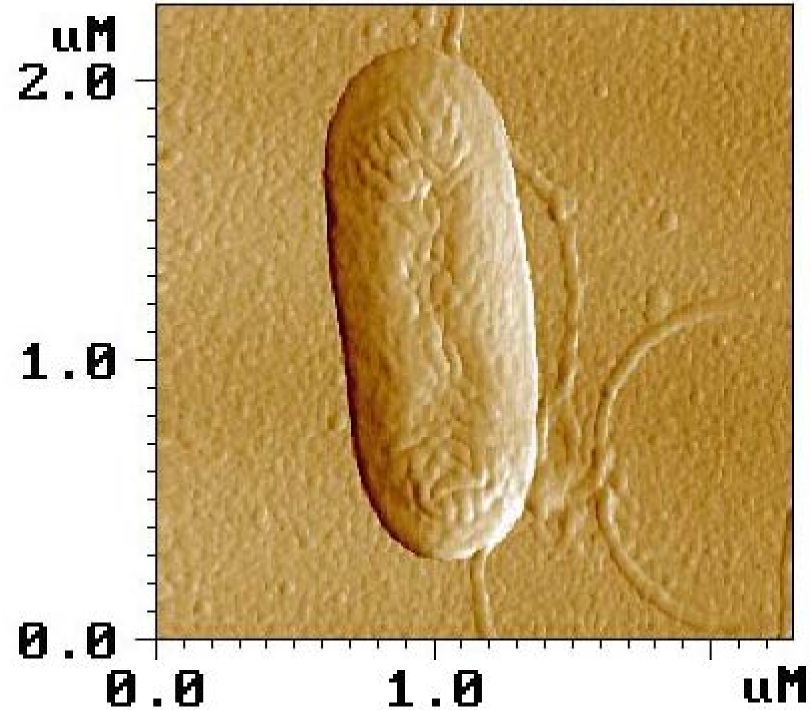
x/y = 540 nm * 540 nm

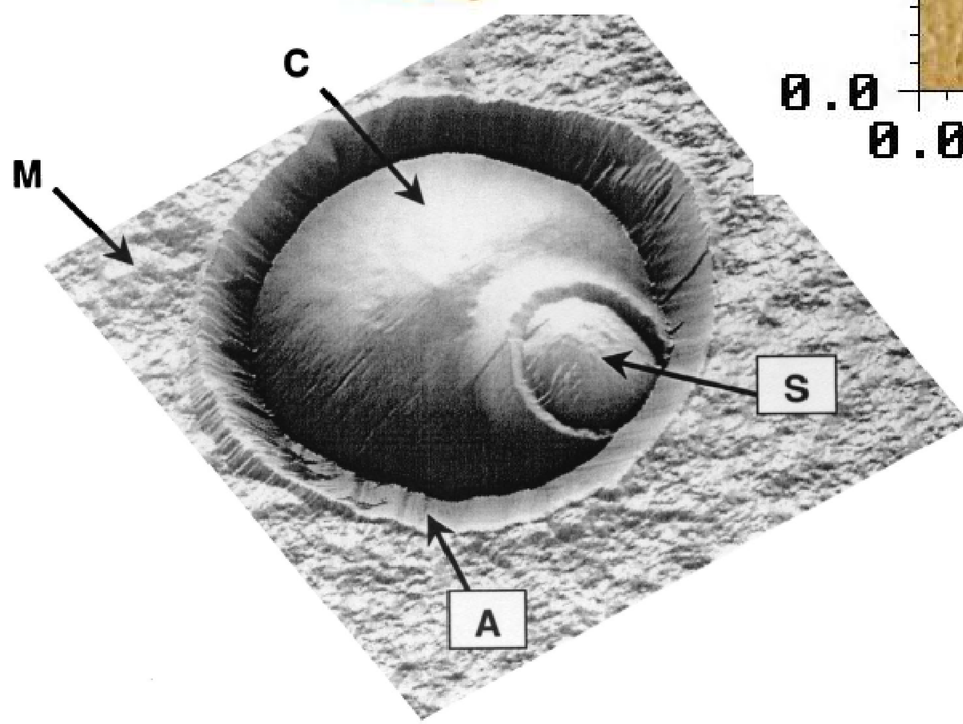
z-range 0 - 40 nm

Cyanobacterium



Pseudomonas bacteria





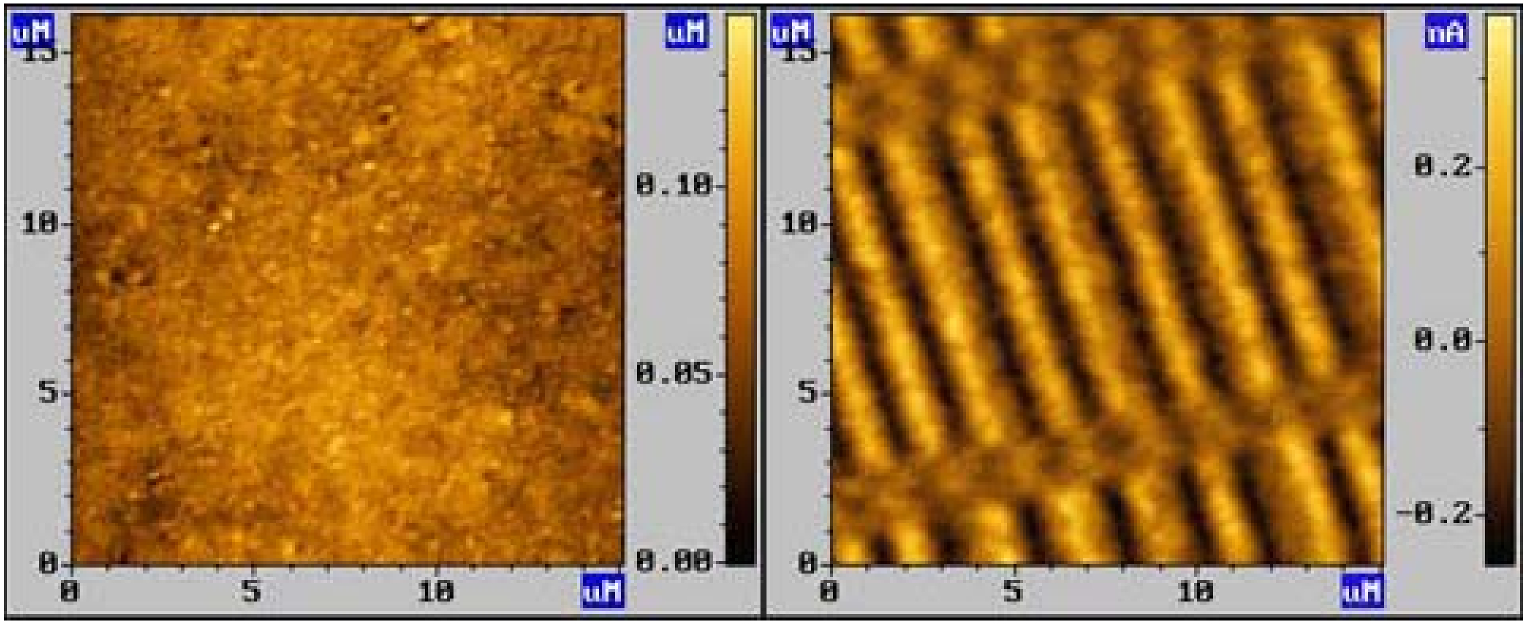
Budding yeast

SPM is an imaging tool placed between optical and electron microscopes

Imaging and measuring **physical properties** of the sample at the same time:

- surface conductivity
- static charge distribution
- localized friction
- **magnetic fields**
- elastic moduli

Single magnetic bits on a hard-disk

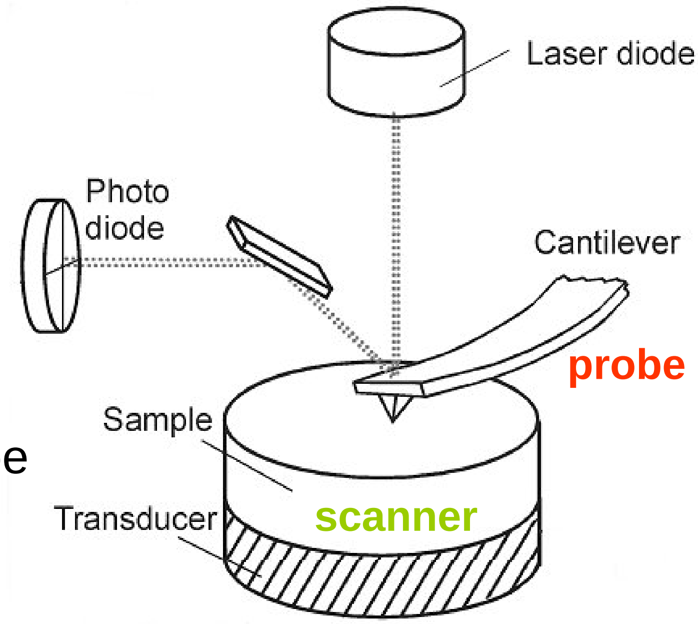


Left: AFM Image of hard disk surface topography; Right: MFM image of hard disk tracks.

How does it work?

Two important components:
• the **probe**
• the **scanner**

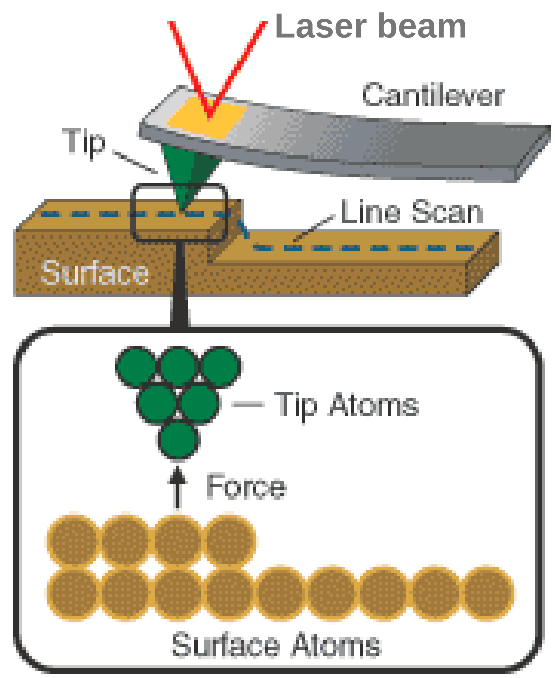
• the **probe** examines the sample's surface properties
• the **scanner** controls the precise position of the probe in x-y-z directions (3D imaging possible)



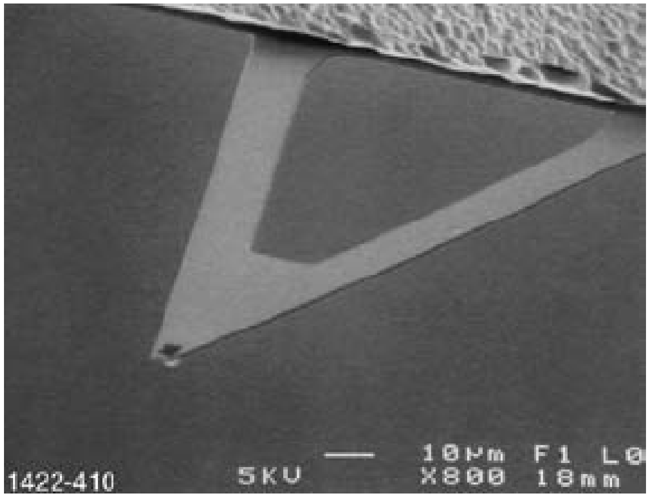
The probe
When two materials are brought very close together, interactions **at the atomic level** occur
- These interactions are the basis for SPMs
- SPM probe can sense these interactions

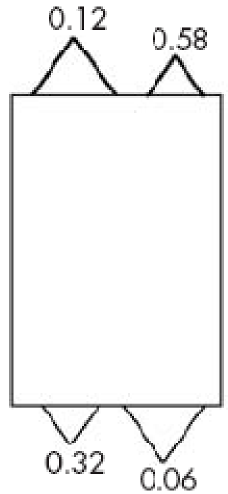
⇒ Magnitude of this interaction varies as a function of the probe-sample distance
⇒ **Mapping** of the **sample's topography** possible by scanning over the samples surface

=> How to scan the probe so precisely?



AFM probes

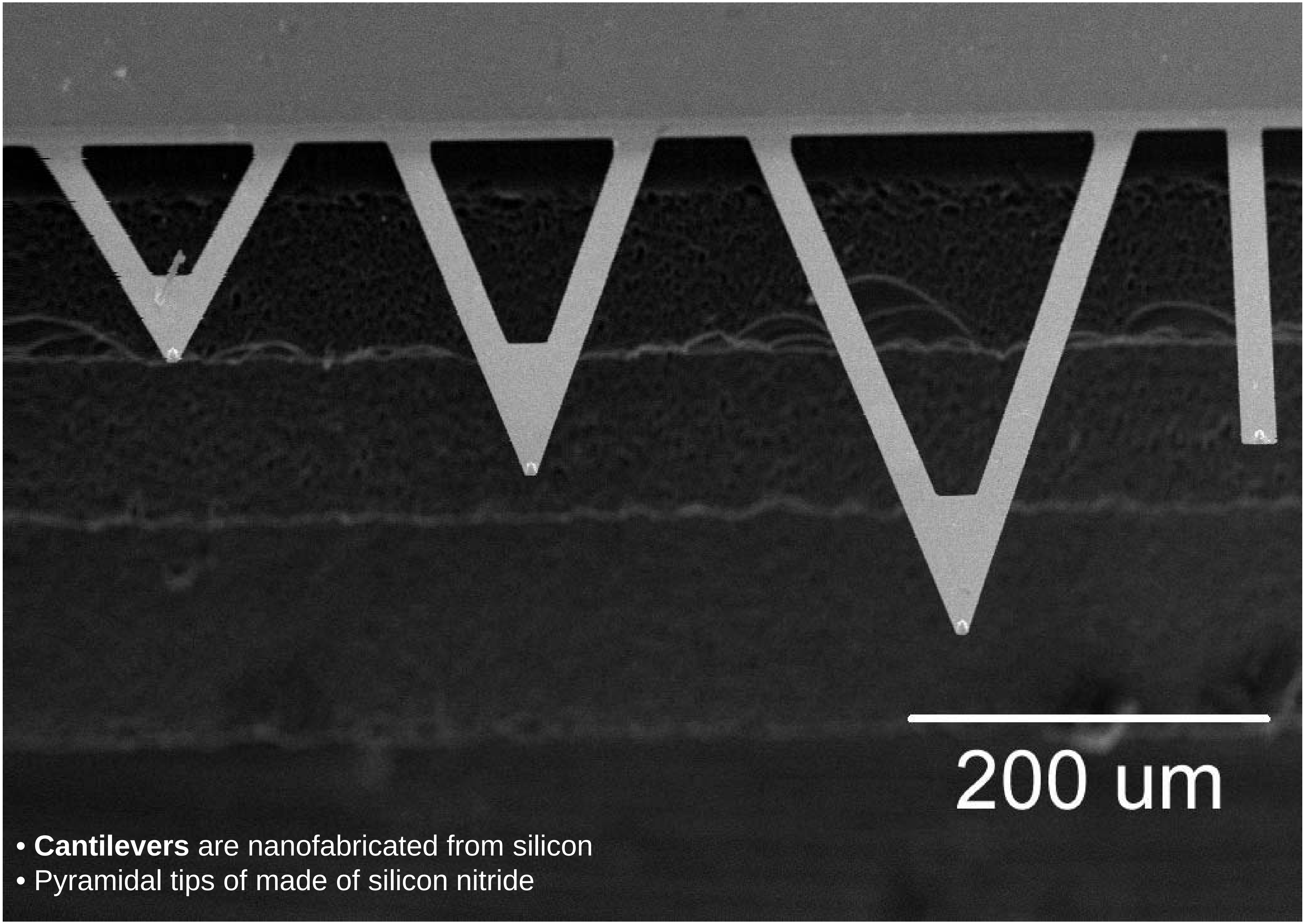




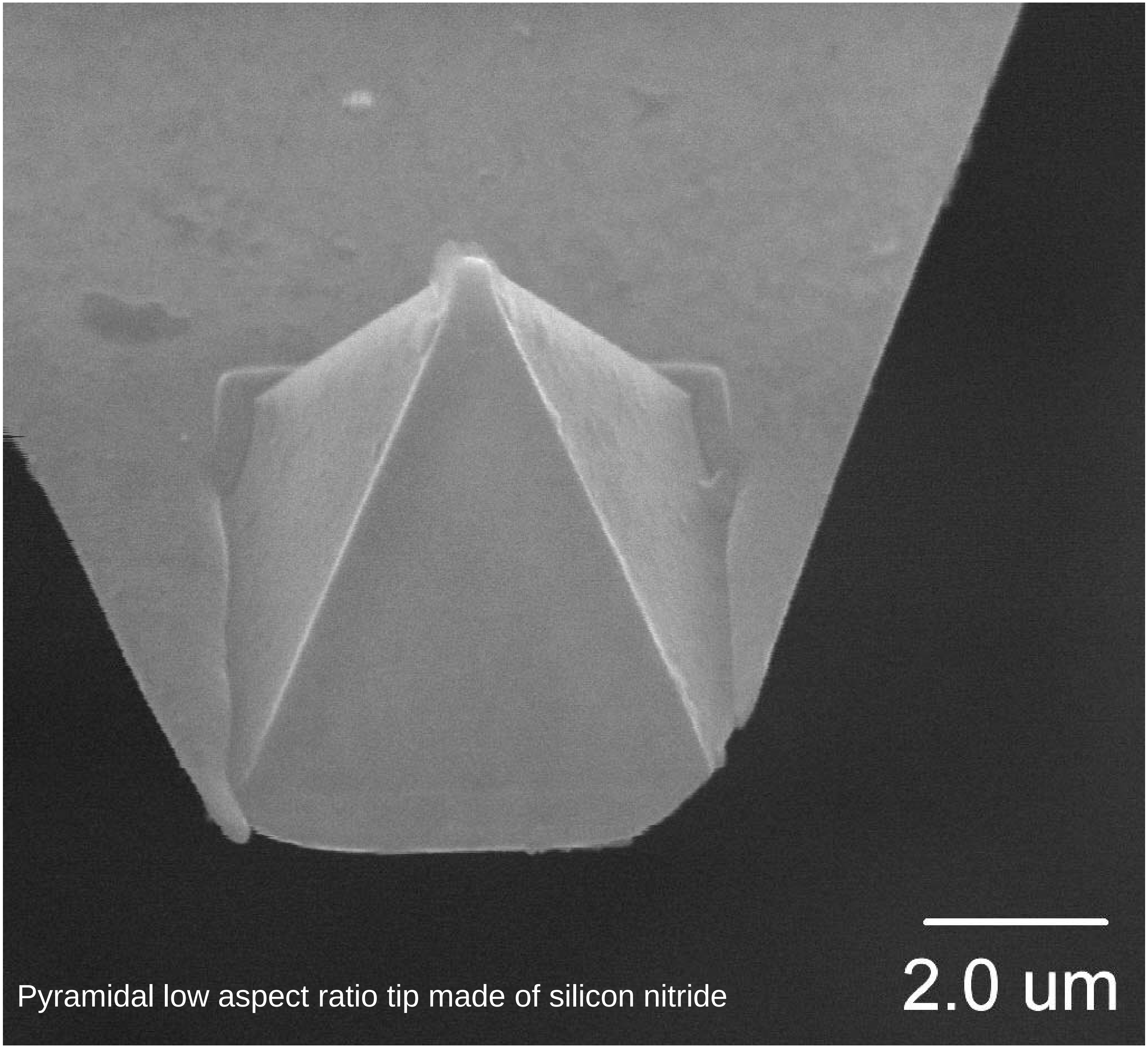
Silicon nitride probes have a sharp tip that, conversely, still does not hurt the sample

Spring constant (k)	0.58, 0.32, 0.12, 0.06 N/m*
Nominal tip radius of curvature	20–60nm
Cantilever lengths	100 and 200µm
Cantilever configuration	V-shaped
Reflective coating	Gold
Sidewall angles	35° on all four sides

Ultralever tip (high-aspect ratio tip made from silicon and carbon but also more invasive)



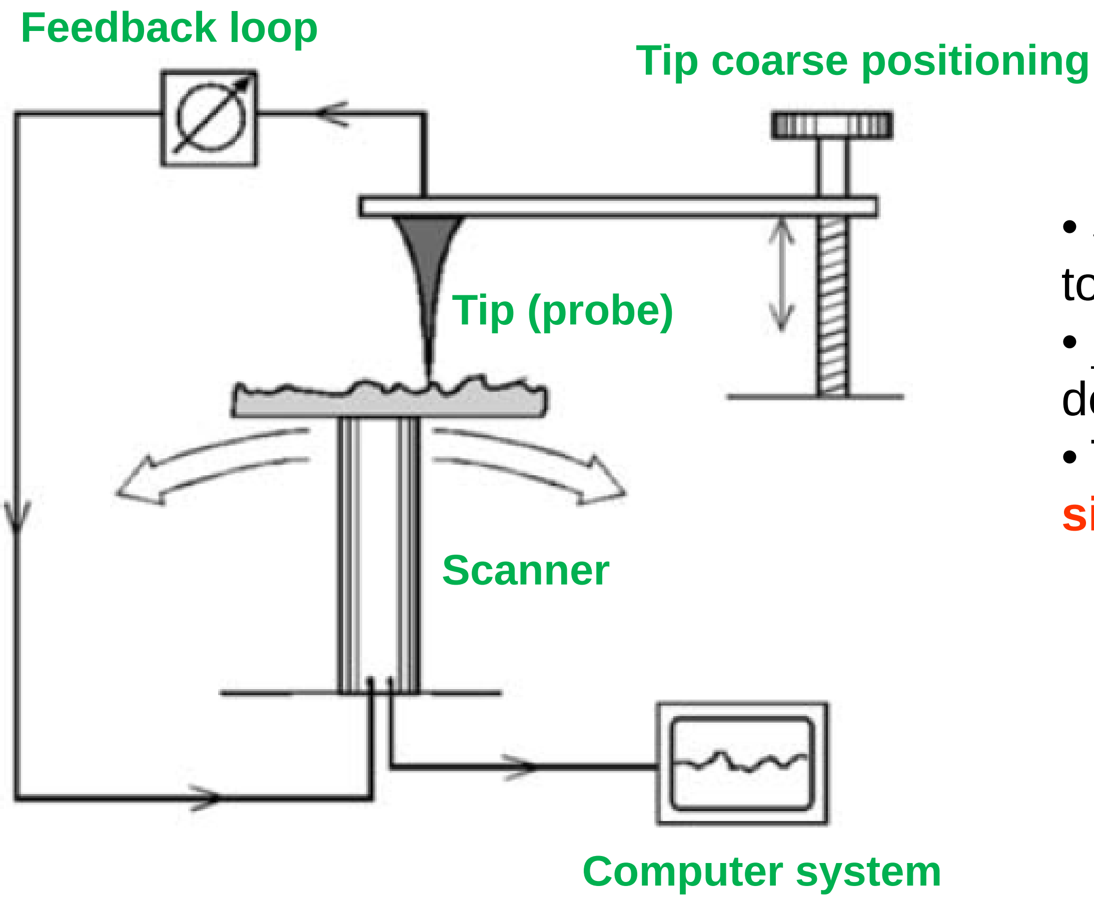
- **Cantilevers** are nanofabricated from silicon
- Pyramidal tips of made of silicon nitride



Pyramidal low aspect ratio tip made of silicon nitride

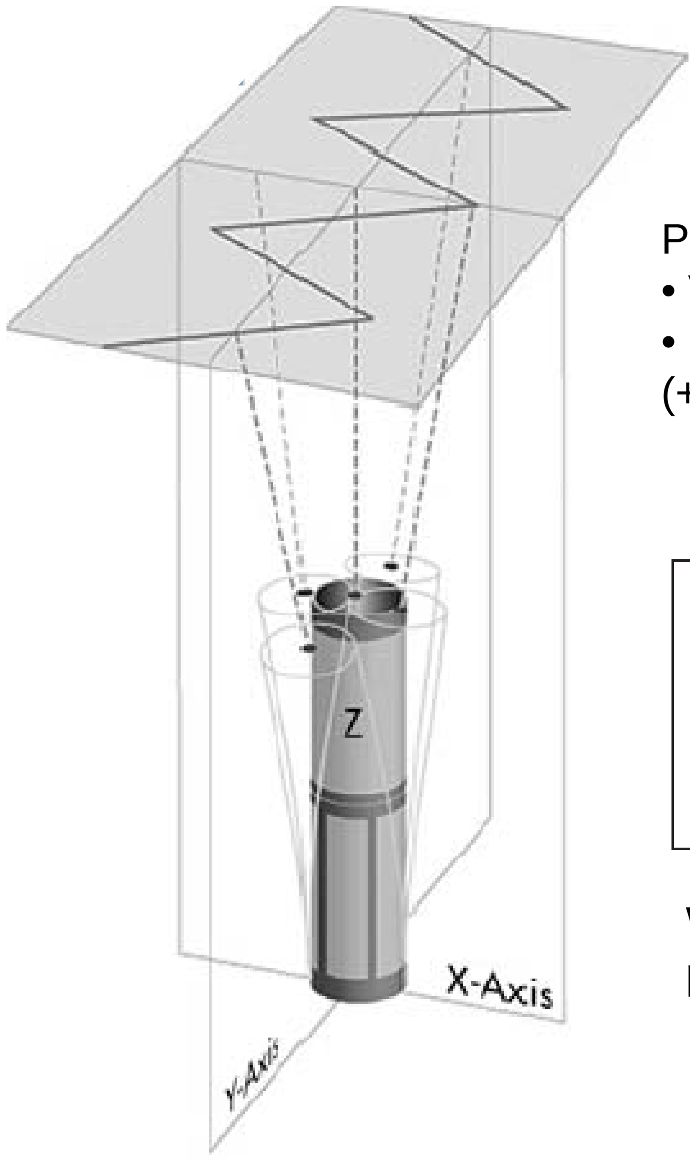
The scanner

- Made of a piezoelectric ceramic: changes its geometry when a voltage is applied
- Can bend, expand and contract in a precise controlled manner

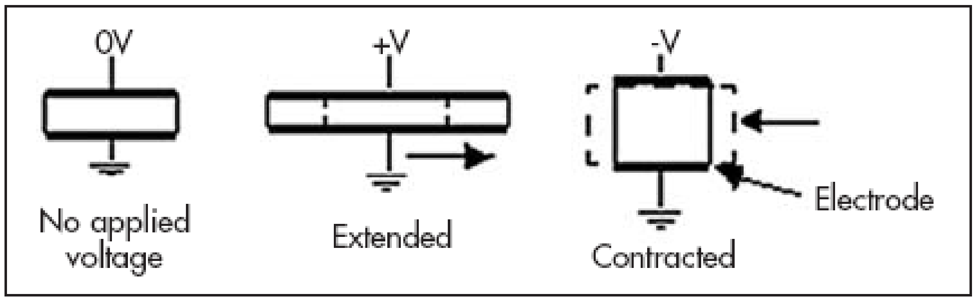


- Scanner moves probe close to the surface of the sample
- Probe then produces a signal depending on its interaction strength
- This signal is named the **detector signal**

The scanner



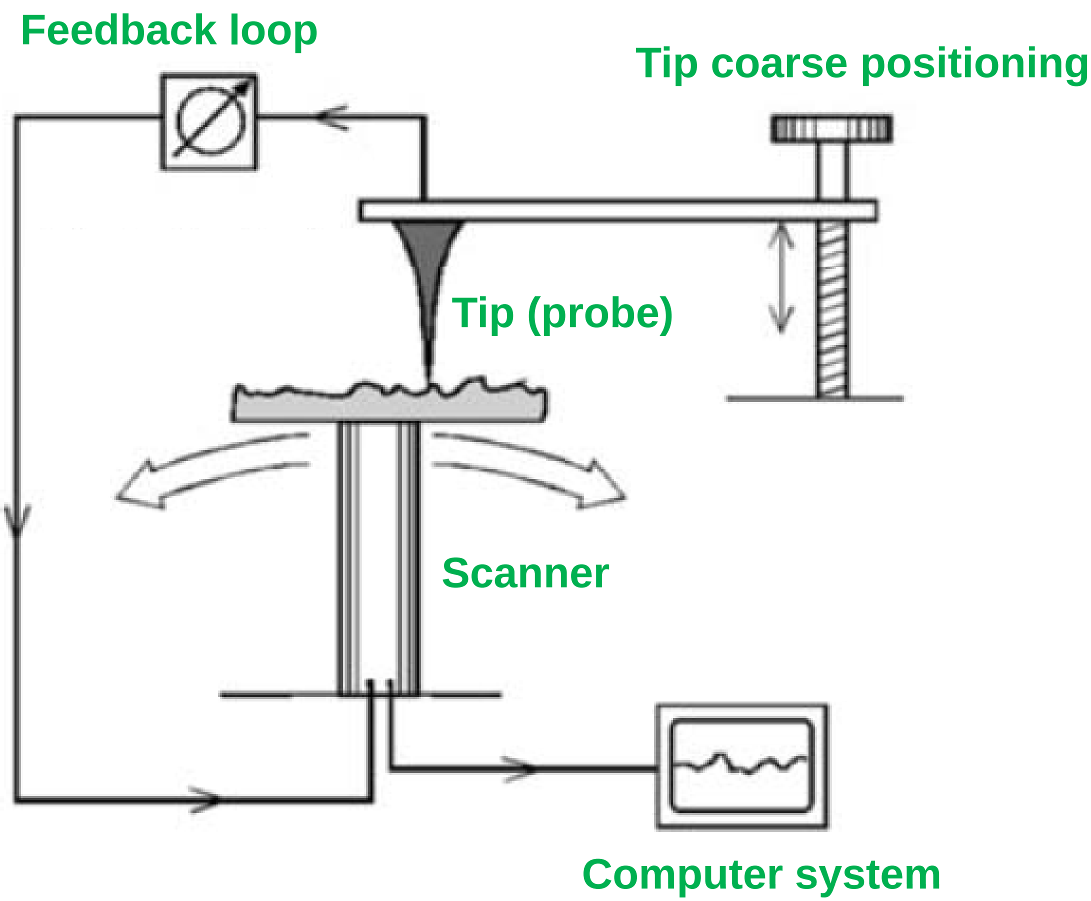
Piezo-movement during a scan:
• Voltage applied to the X- and Y-axis produces the pattern
• Maximum scan size is about 100 µm (1024 data points/line) (+220V to -220V)



Whether the piezo contracts or expands depends on the polarity of the voltage applied

SPM feedback loop

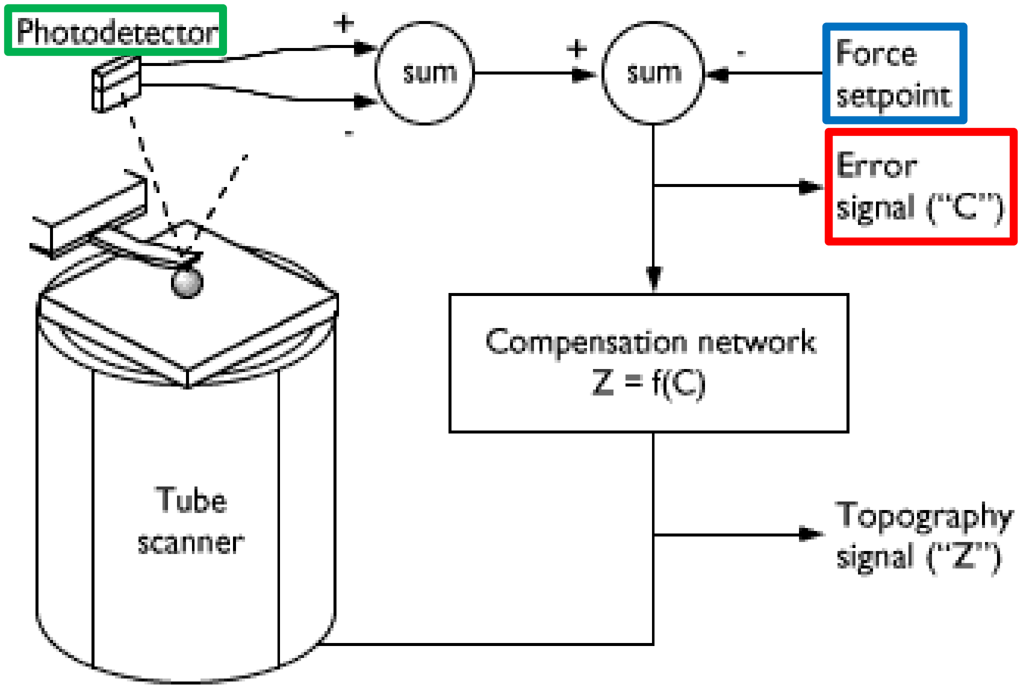
After the probe produces the first detector signal a reference value is created: the **setpoint** value

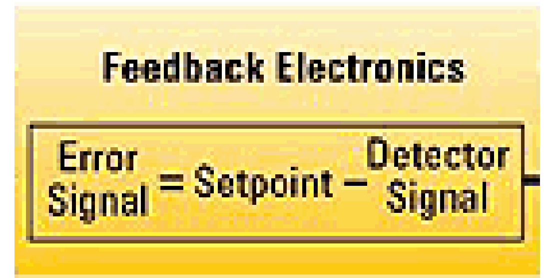


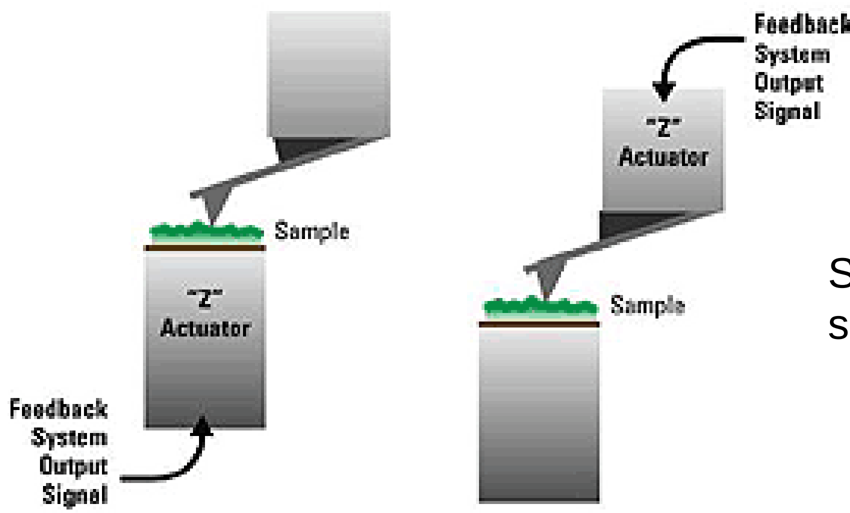
- Scanning only starts when the detector signal is equal to the setpoint
- When detector signal changes during scanning (e.g. height changes of the sample) then the difference between the detector signal and setpoint is called the **error signal**

The **error signal** is the raw data used to generate an image of the surface

SPM feedback loop







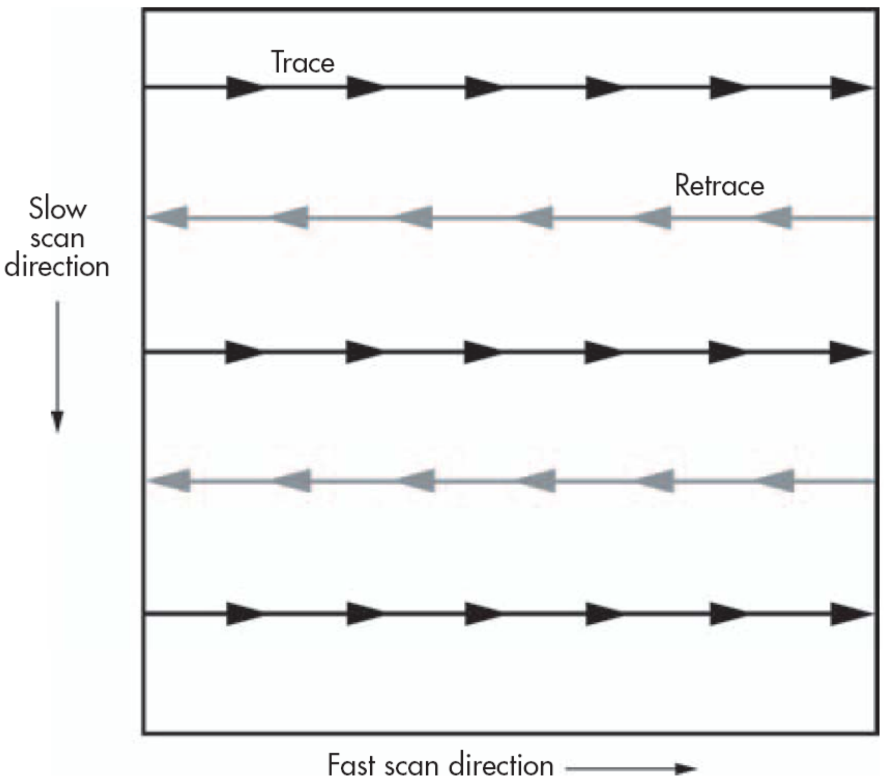
Sample-height-controlled Configuration

Probe-height-controlled Configuration

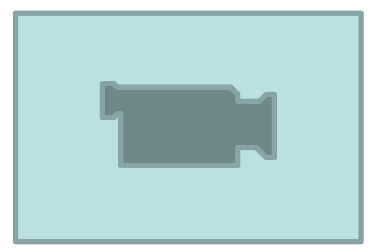
Scanner or ("Z" actuator) controls sample-height or probe height

Trace and Retrace

The scanner moves the probe over the surface in a precise, defined pattern



- Data can be collected as the probe moves from left to right (“trace”) and from right to left (“retrace”)
- Ability to collect data in both directions can be very useful in <u>ruling out artifacts</u>

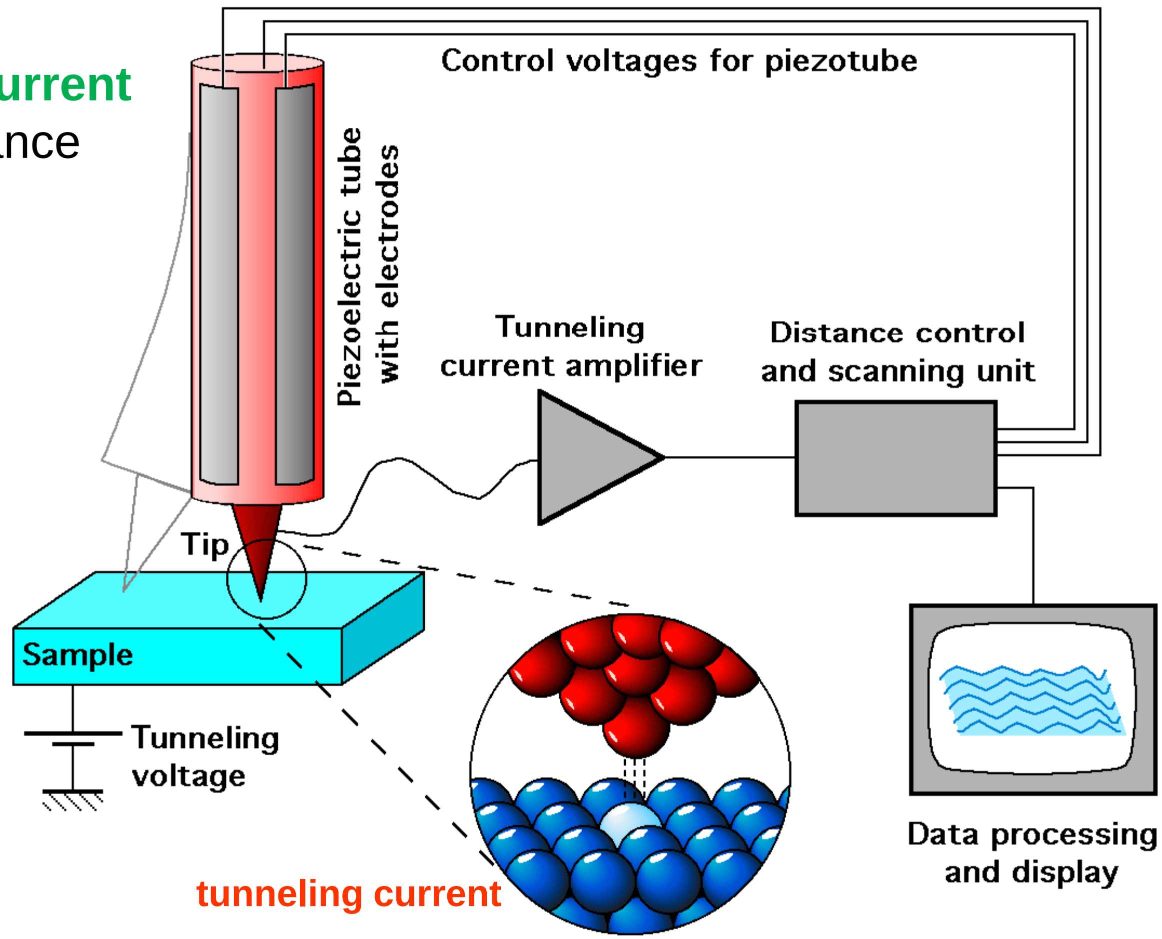


Scanning Tunneling Microcopy (STM)

STM measures the topography of a surface using a **tunneling current**
=> the tunneling current depends on the separation between the probe tip and the sample surface

- The probe is a conducting sharp tip (platinum-iridium or tungsten)
- A voltage is applied between the tip and the sample
- When the tip is brought close enough to the sample, **electrons begin to tunnel through the gap**
- The resulting **tunneling current** changes with tip-sample distance

⇒ **detector signal**



Scanning Tunneling Microcopy (STM)

STM is typically performed on conductive or semiconductive surfaces

Common applications:

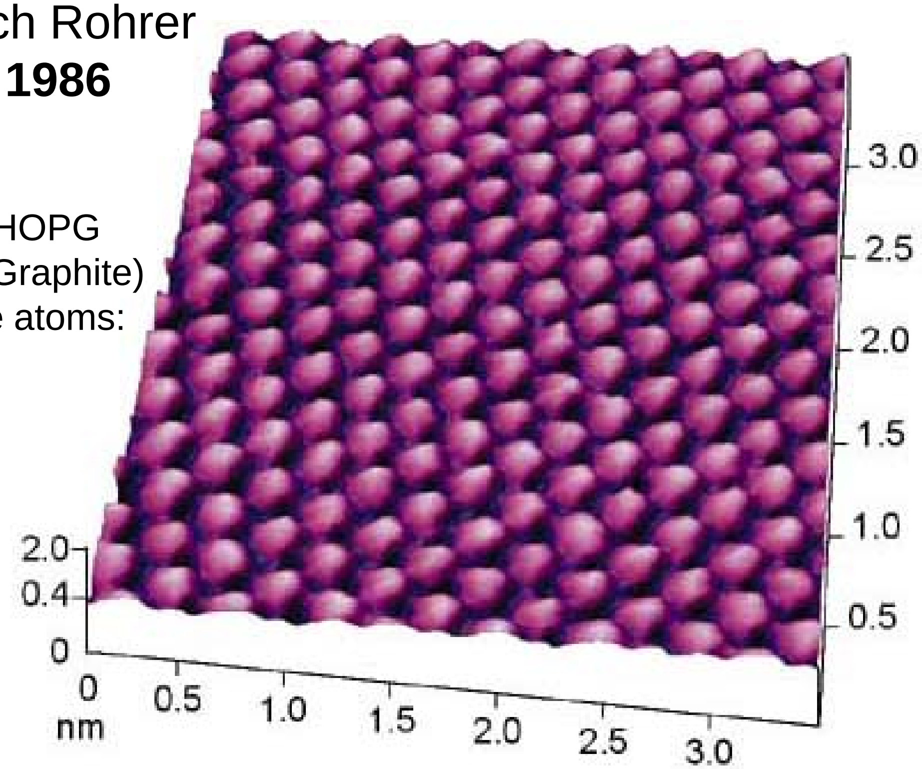
- Atomic resolution imaging
- Electrochemical STM
- Scanning Tunneling Spectroscopy (STS)
- Low current imaging of poorly conductive samples (low-current STM)

➢ Ancestor of all SPMs

➢ Invented 1981 at IBM Zürich. First instrument to generate images of surfaces with atomic resolution

➢ The inventors, Gerd Binning and Heinrich Rohrer were awarded the **Nobel Prize in Physics 1986**

STM atomic resolution on HOPG
(Highly Oriented Pyrolytic Graphite)
⇒ distance between single atoms:
0.25 nm or 2.5 Angstrom

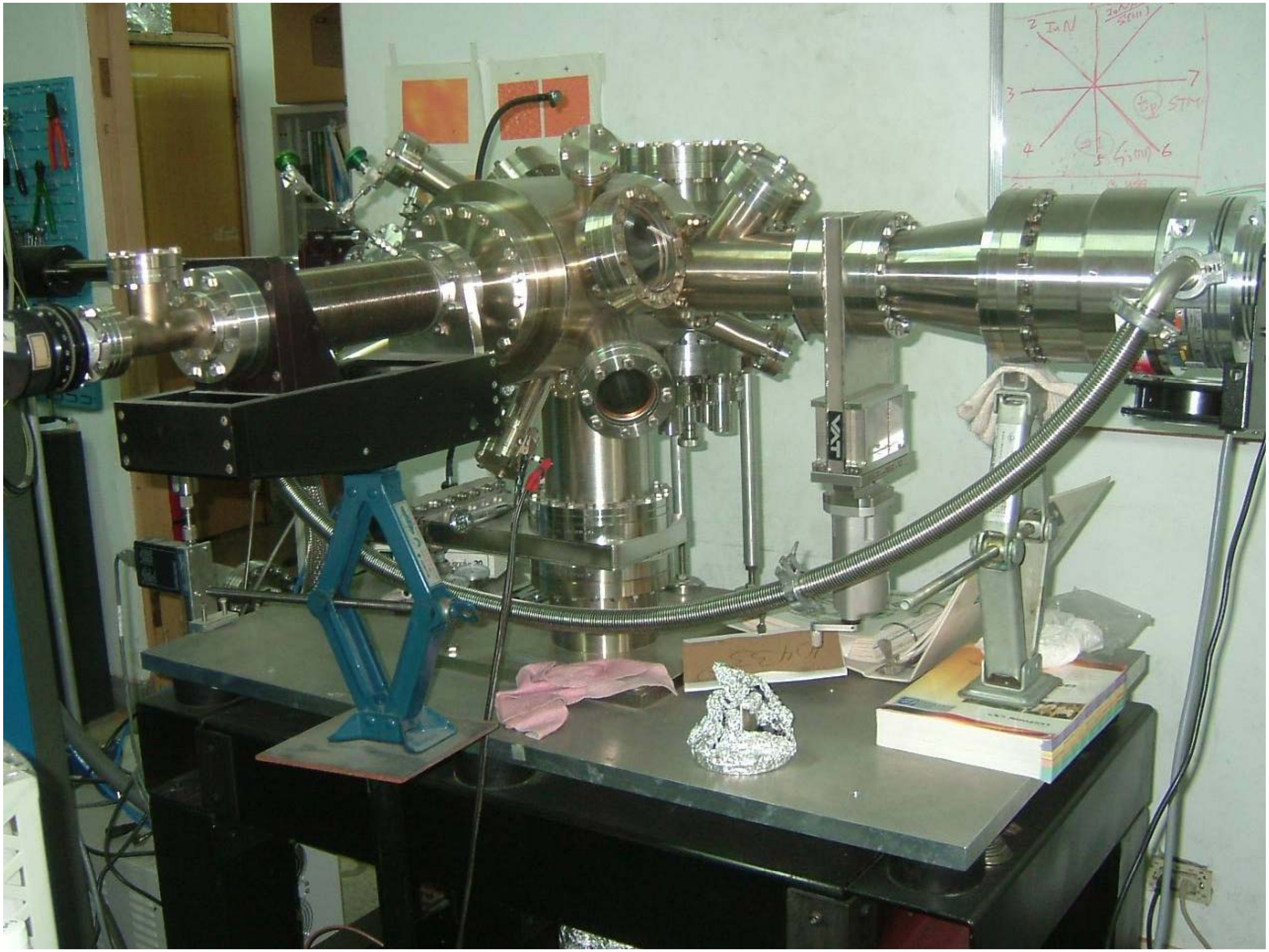


Scanning Tunneling Microcopy (STM)

Prof. Dr. Gwo / Department of Physics at NTHU

(UHV = ultra high vacuum)

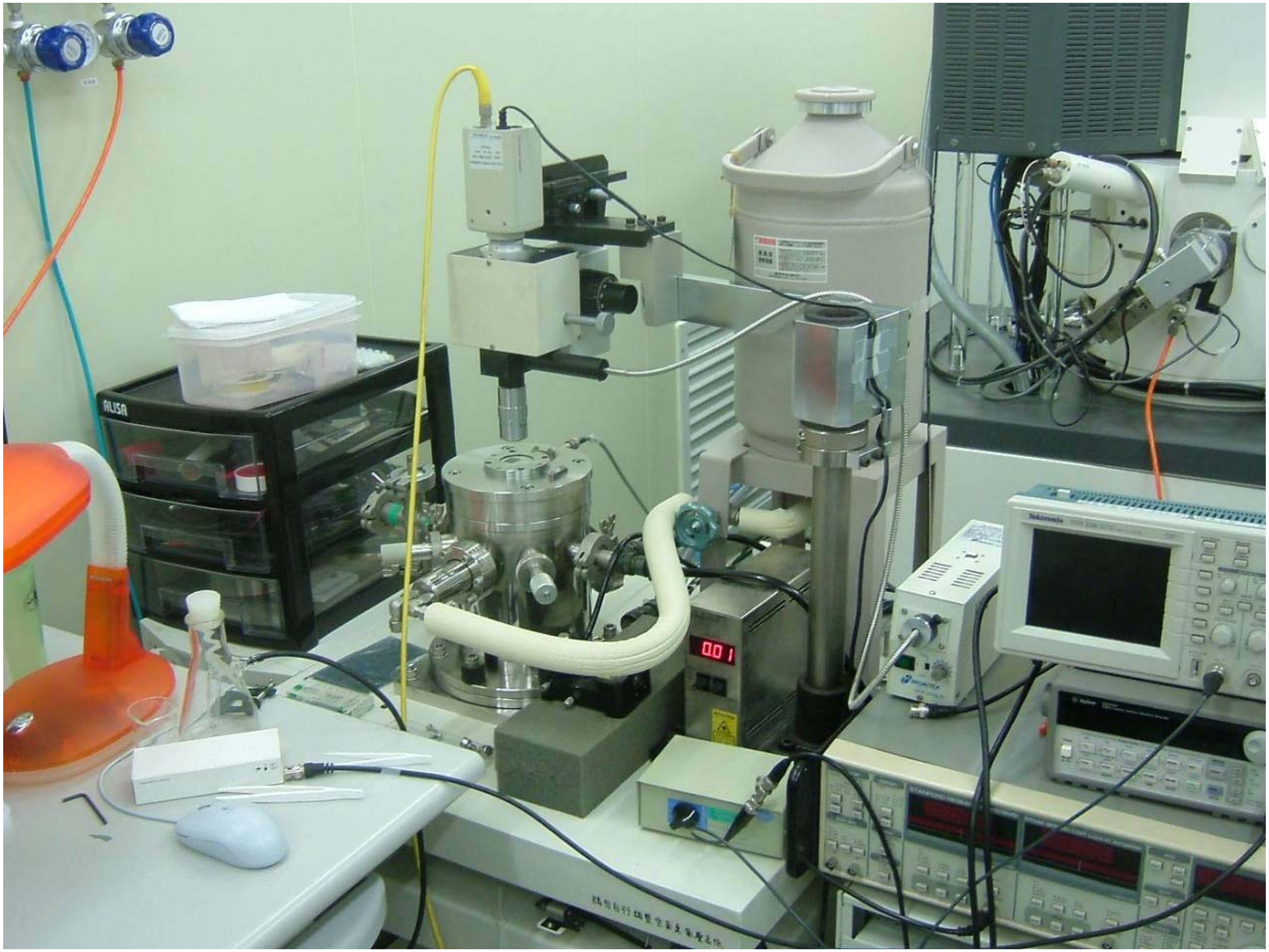
Scanning Tunneling Microcopy (STM)



Prof. Dr. Gwo / Department of Physics at NTHU

(UHV = ultra high vacuum)

Surface Force Microscopy (SFM)



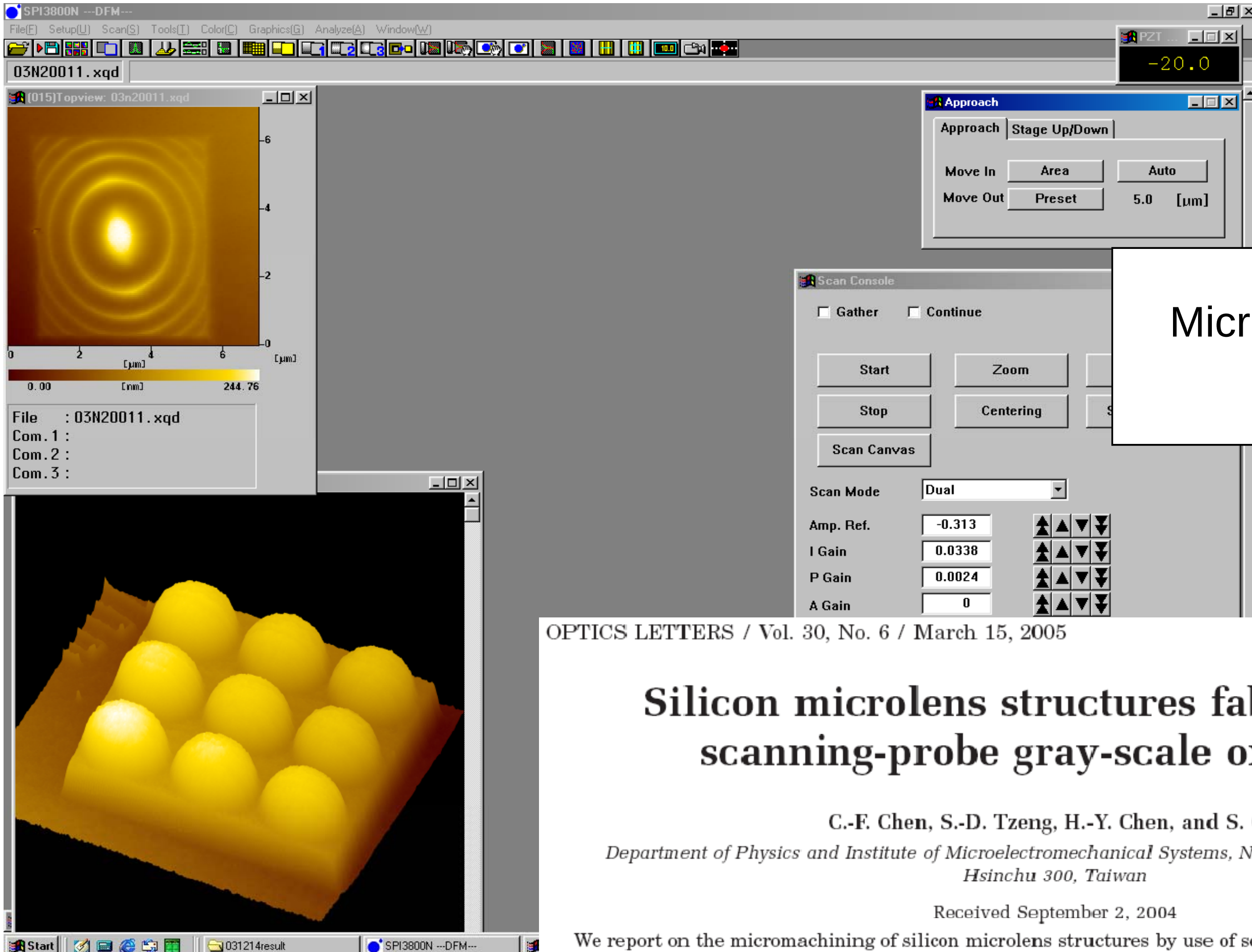
Prof. Dr. Gwo Department of Physics at NTHU

Surface Force Microscopy (SFM)

airlock/clean room

Mr. Chen Chi Fan from Dr. Gwo's lab

Surface Force Microscopy (SFM)



Micromachining of silicon microlenses

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Silicon microlens structures fabricated by scanning-probe gray-scale oxidation

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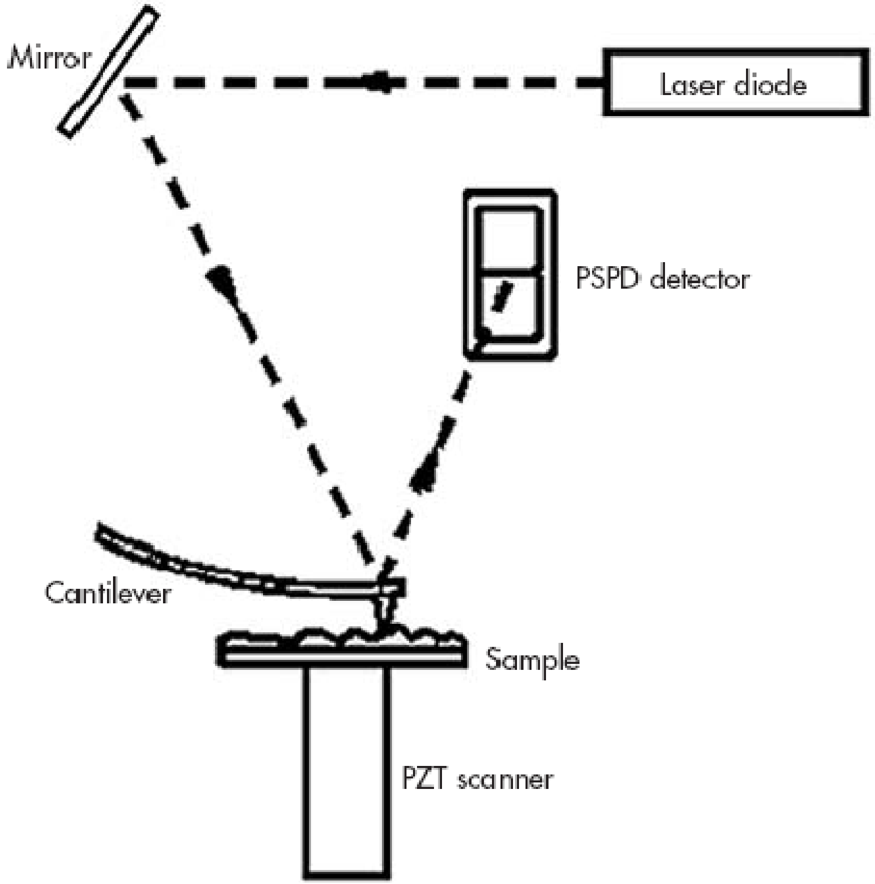
Received September 2, 2004

We report on the micromachining of silicon microlens structures by use of scanning-probe gray-scale anodic oxidation along with dry anisotropic etching. Convex, concave, and arbitrarily shaped silicon microlenses with diameters as small as 2 μm are demonstrated. We also confirm the high fidelity of pattern transfer between the probe-induced oxides and the etched silicon microlens structures. Besides the flexibility, the important features of scanning-probe gray-scale anodic oxidation are small pixel size and pitch (of the order of tens of nanometers), an unlimited number of gray-scale levels, and the possibility of creating arbitrarily designed microlens structures with exquisite precision and resolution. With this approach, refractive, diffractive, and hybrid microlens arrays can be developed to create innovative optical components.

OCIS codes: 220.3630, 350.3950, 230.4000, 220.3740.

Atomic force microscopy (AFM)

- AFM was developed from the basis of STM and is now the most popular SPM
- Probe is a sharp (silicon nitride) tip of about 10 nm in diameter
- The tip is glued on a so called cantilever (5 µm in height and 100-500 µm long)
- Forces between the tip and the sample can **bend or deflect the cantilever**
- A **laser beam is focused on the tip** and during scanning the detector measures the cantilevers deflection



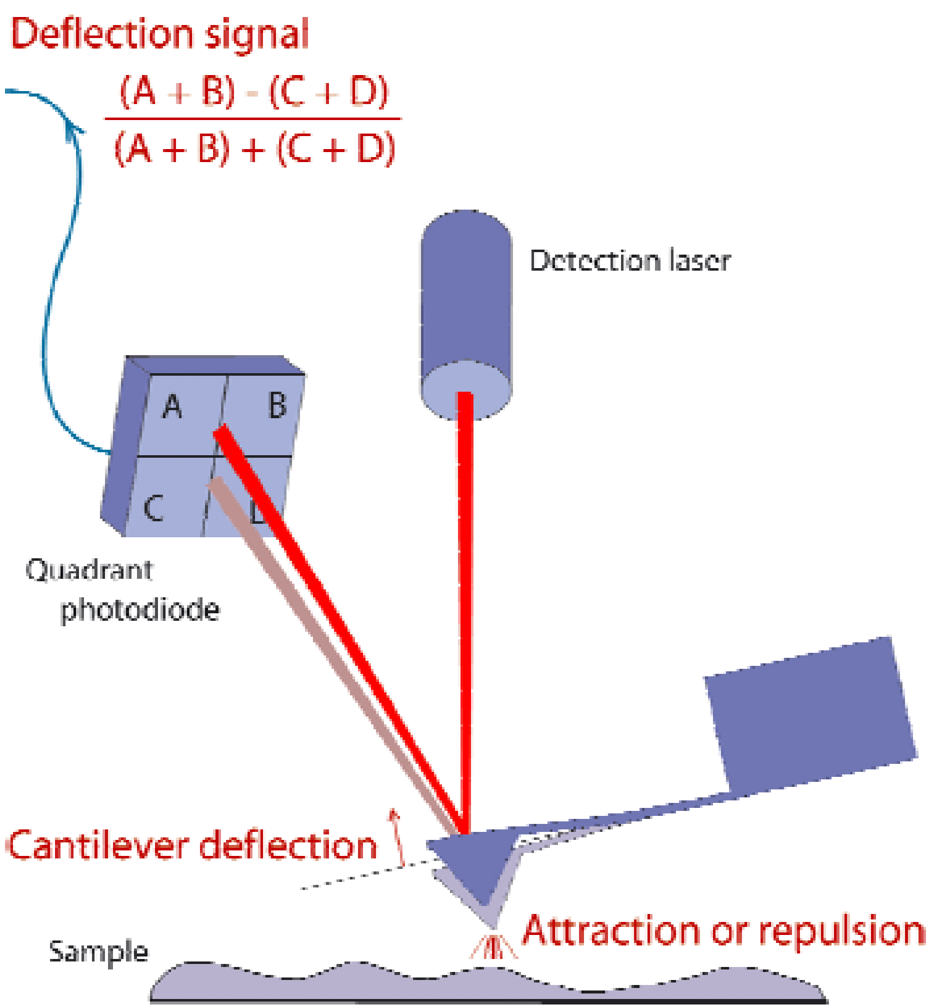
- A computer generates a surface topography from the cantilever deflection
- High signal amplification is gained, because of the ratio

$$\frac{\text{path length cantilever} \leftrightarrow \text{detector}}{\text{length cantilever}}$$

- Due to this large amplification the system can detect **topography changes in the range of 1 nm**

PSPD = position-sensitive photo-detector

The quadrant photodiode is an important feature of the AFM



- Movement of laser spot on photodiode **produces electrical signal** in each quadrants
- Differences in laser intensities between the top two segments and the bottom two segments reveals the **up and down motion of the tip**
- Differences in laser intensities between the left and right pairs of segments reveals the **lateral or twisting motion** of the tip
- The signals from the four quadrants of the detector are **converted to a 3D topography image** or the spectrum of the interaction force between the tip and surface

AFM imaging modes

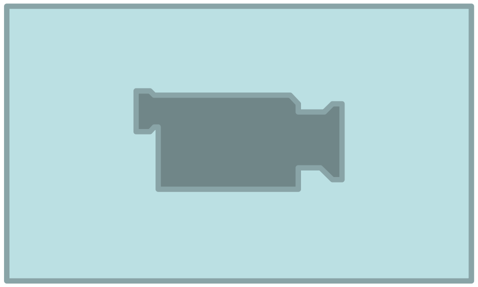
5 main modes:

- **Tapping mode**
- **Contact-mode**
- Non-contact
- Torsional resonance
- **MAC mode**

<u>Tapping mode</u>:

• Most commonly used mode: tip is oscillating and lightly tapped on the surface

• Cantilever's oscillation amplitude changes with sample surface topography

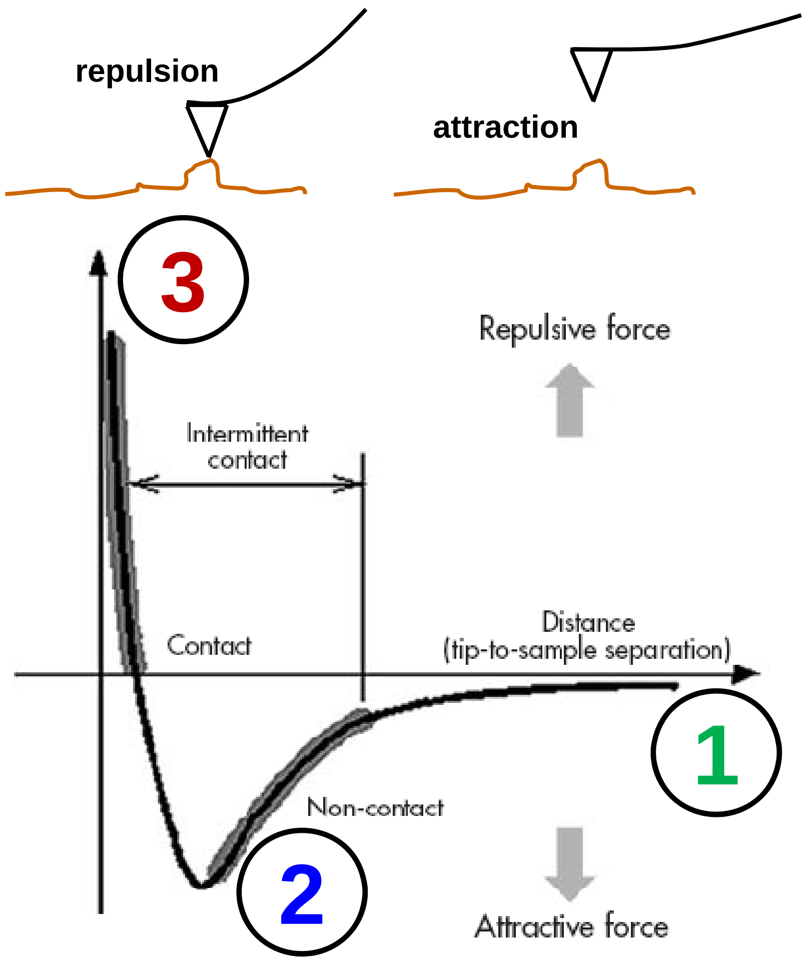


Advantage: less damage to the sample (good for imaging sensitive material as cells and tissues)

Disadvantage: reduced resolution compared to contact mode

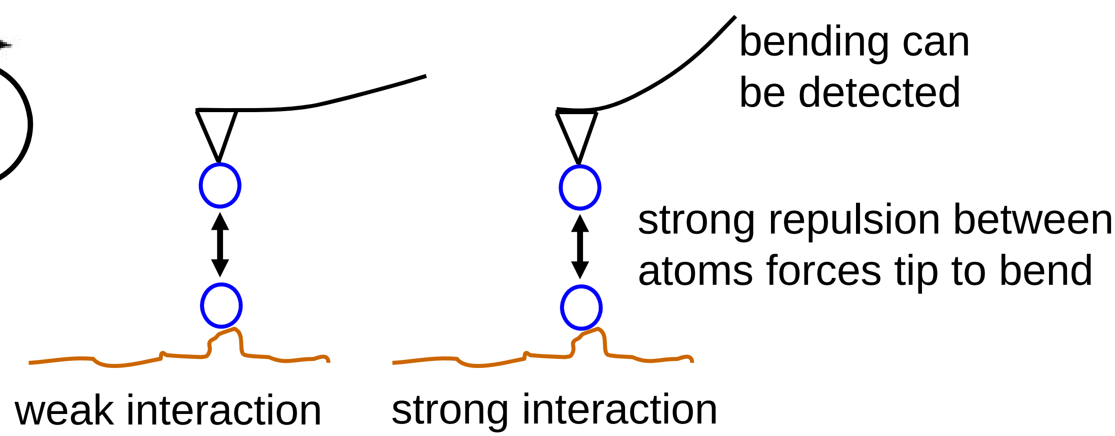
AFM imaging modes

<u>Contact-mode</u>: permanent contact between the tip and the surface (better resolution, more invasive)

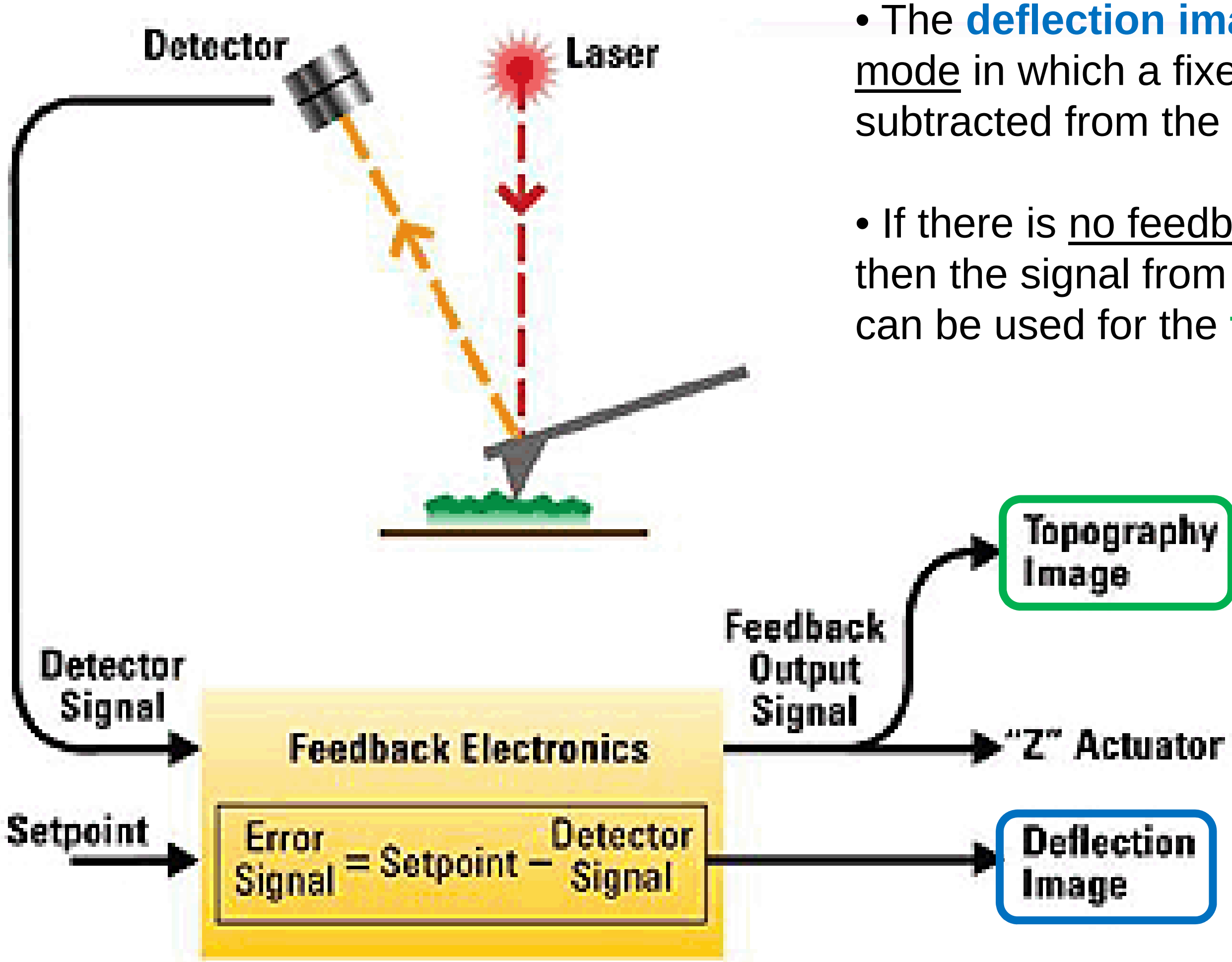


1) **Approach**: neither attractive nor repulsive forces
2) Close distance (**non-contact**): increasing weak interaction between tip and sample atoms (van der Waals forces)
3) **Contact**: increasing repulsive forces => **atoms** are so close together that they **repel each other**

During increasing contact with the surface the <u>cantilever bends</u> rather than that the tip-atoms are forced closer to the sample-atoms



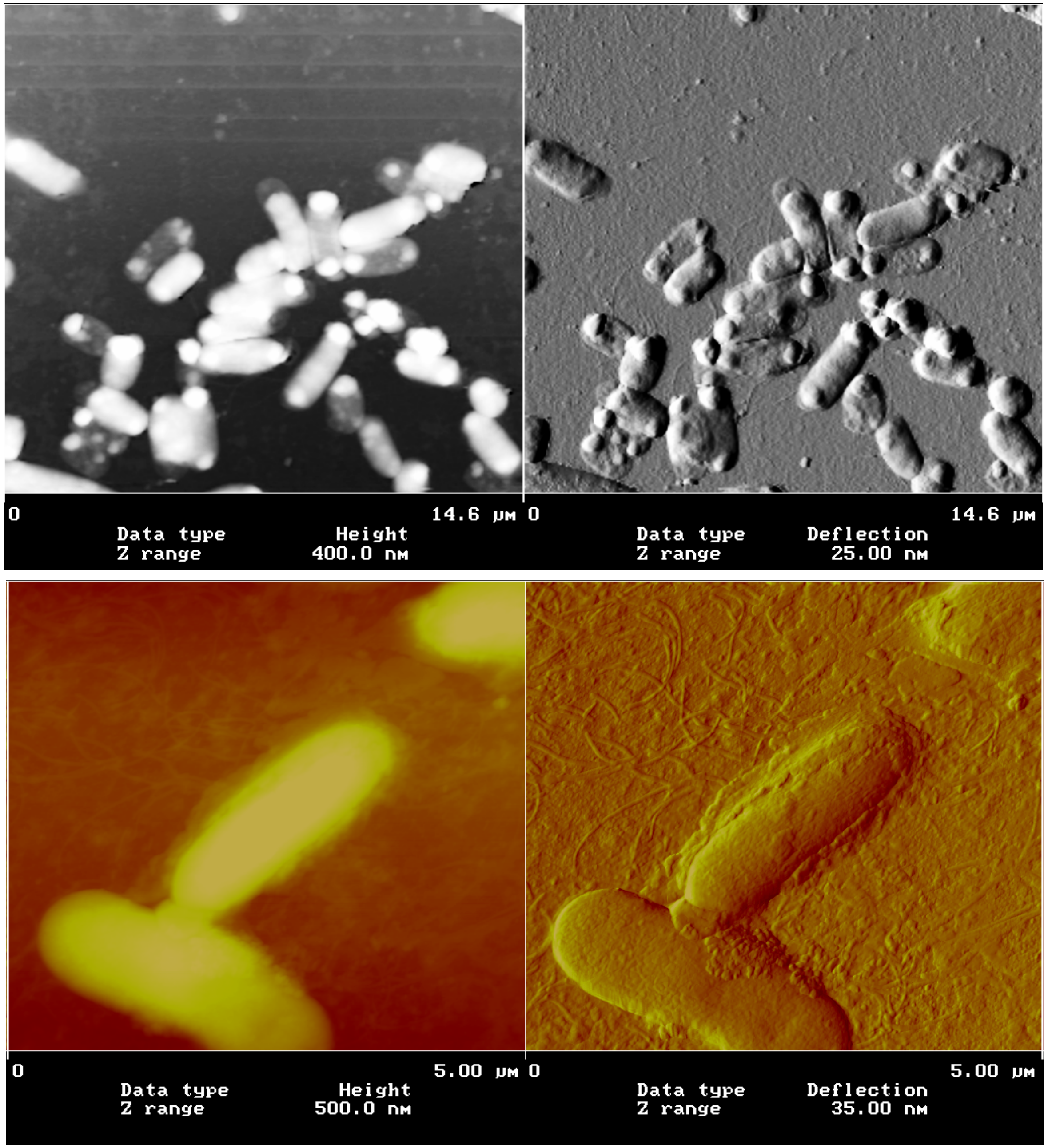
Difference between height/topography and error/deflection mode



- The **deflection image** results from the error-mode in which a fixed setpoint value is subtracted from the detector signal
- If there is no feedback on the error-signal then the signal from the **feedback output** can be used for the **topography image**

Topography (height) image

Deflection (error) image



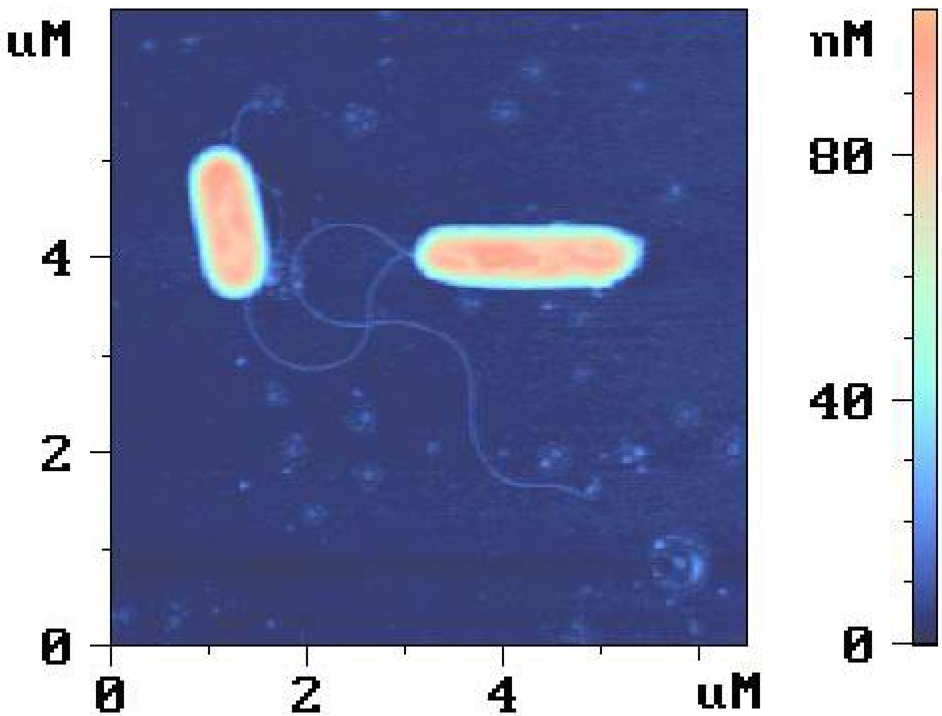
Zoom

Zoom

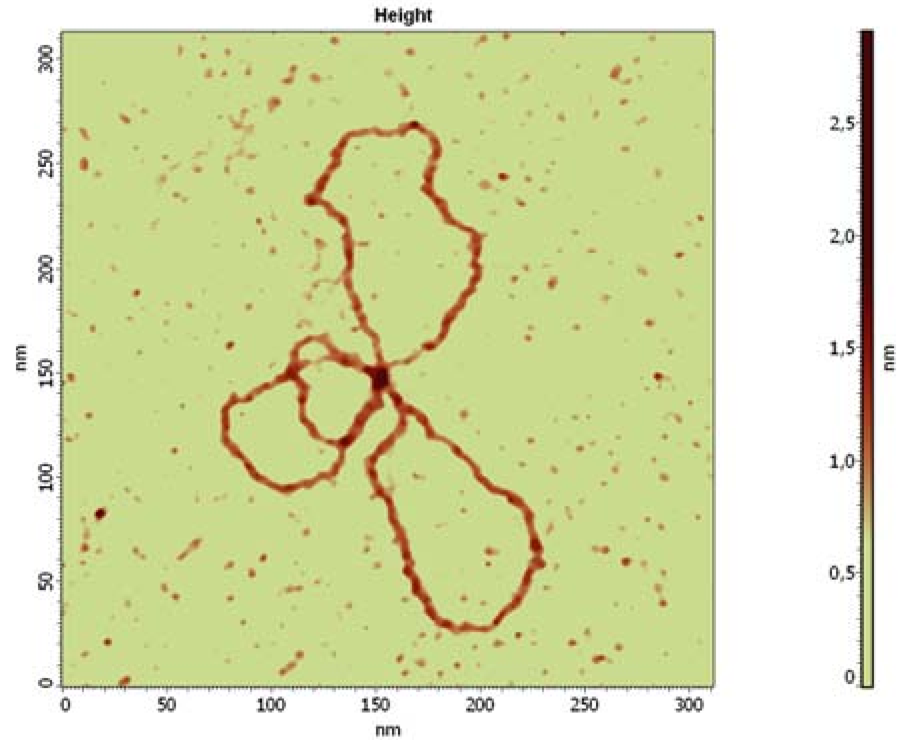
E-coli bacteria

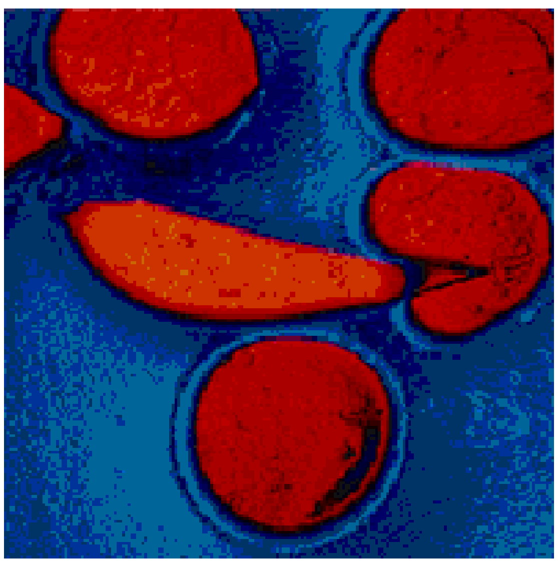
DI/Veeco AFM image by Gary

Pseudomonas bacteria

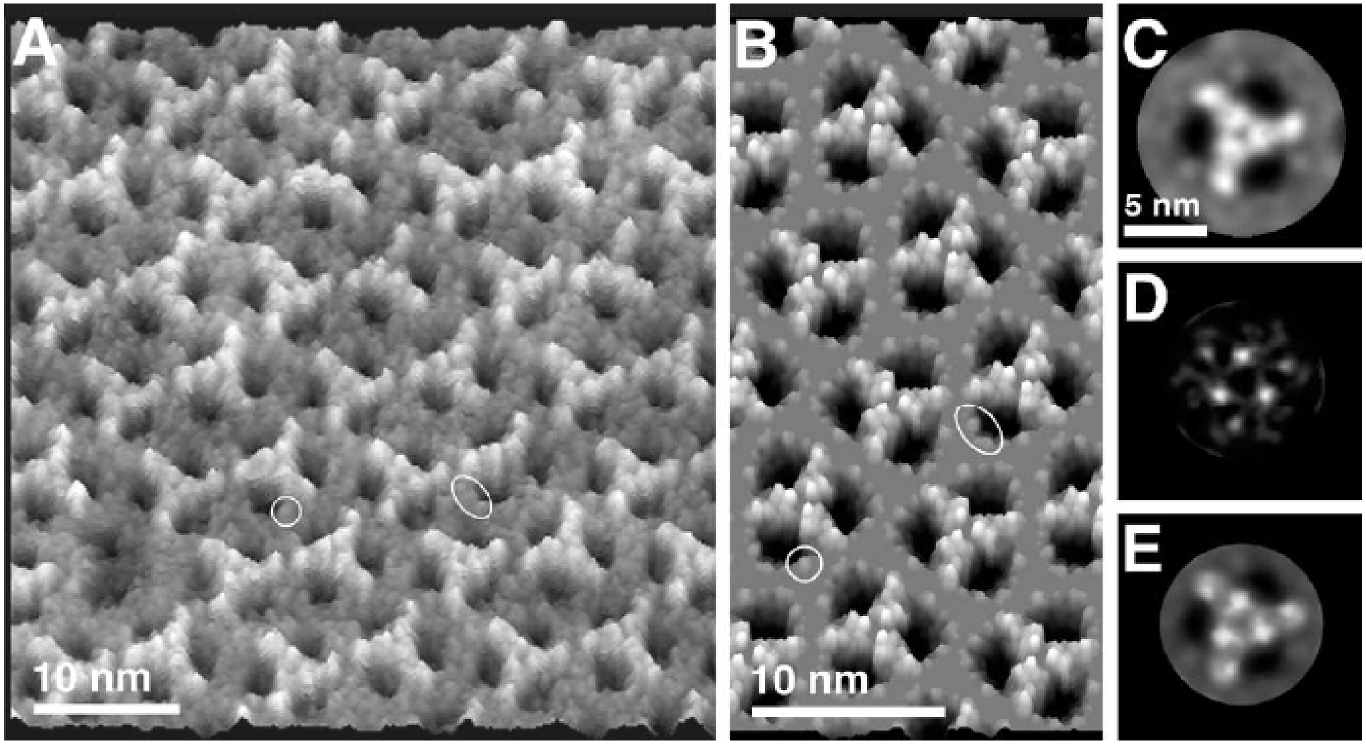


Circular plasmid DNA (pEGFP, 3.4 kb)

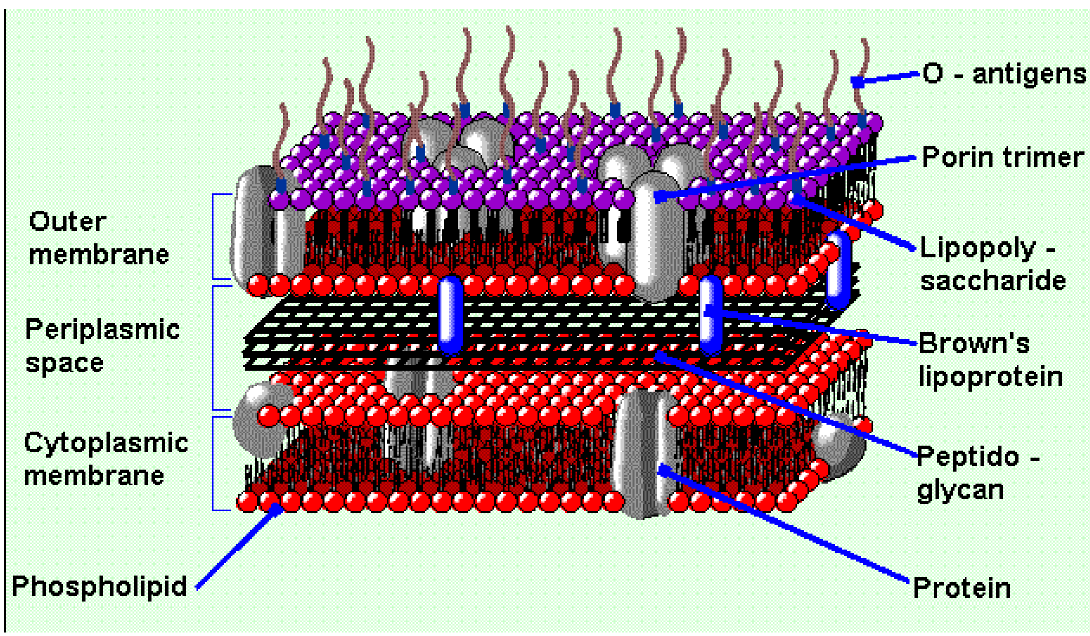




Normal- and sickle red blood cells
(the rigid sickle-cell indented the softer red
blood cell)



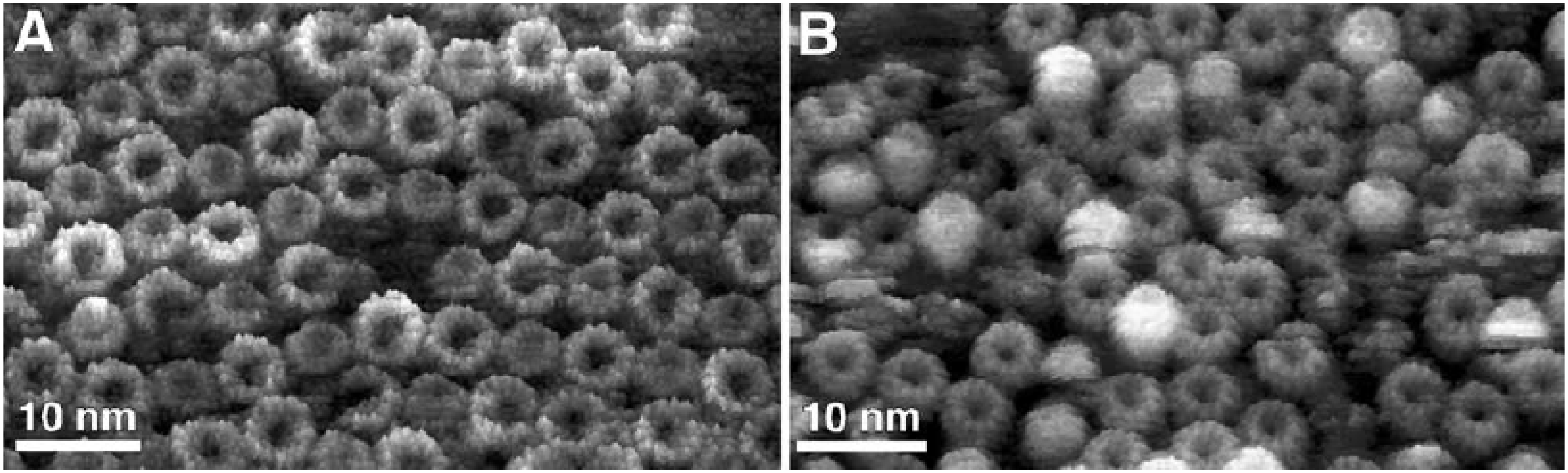
OmpF porin structure of 0.3 nm resolution



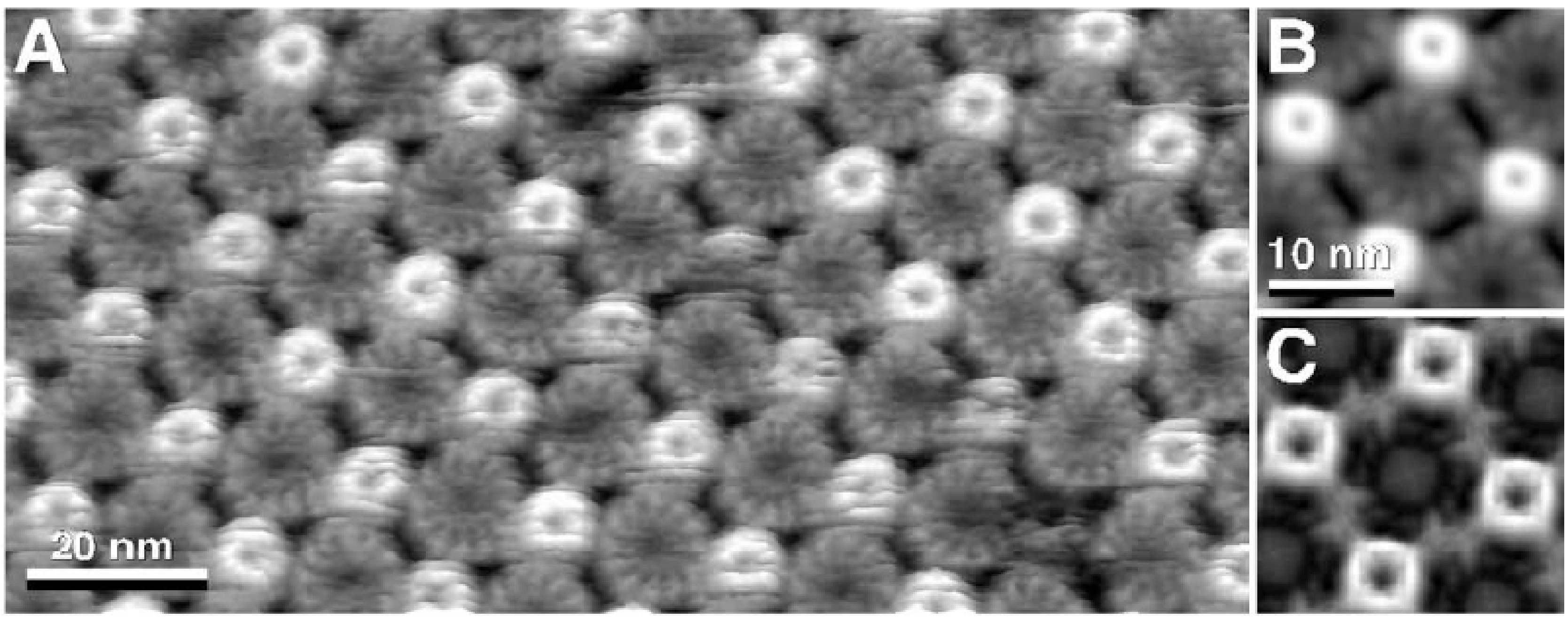
- The Gram-negative envelope of *E. coli* ompF porin is an integral membrane protein located in the outer membrane
- It is a transport channel for small molecules

F_0F_1-ATP synthases from *I. tartaricus* with 11 subunits

F_0F_1-ATP synthases from *spinach* with 14 subunits

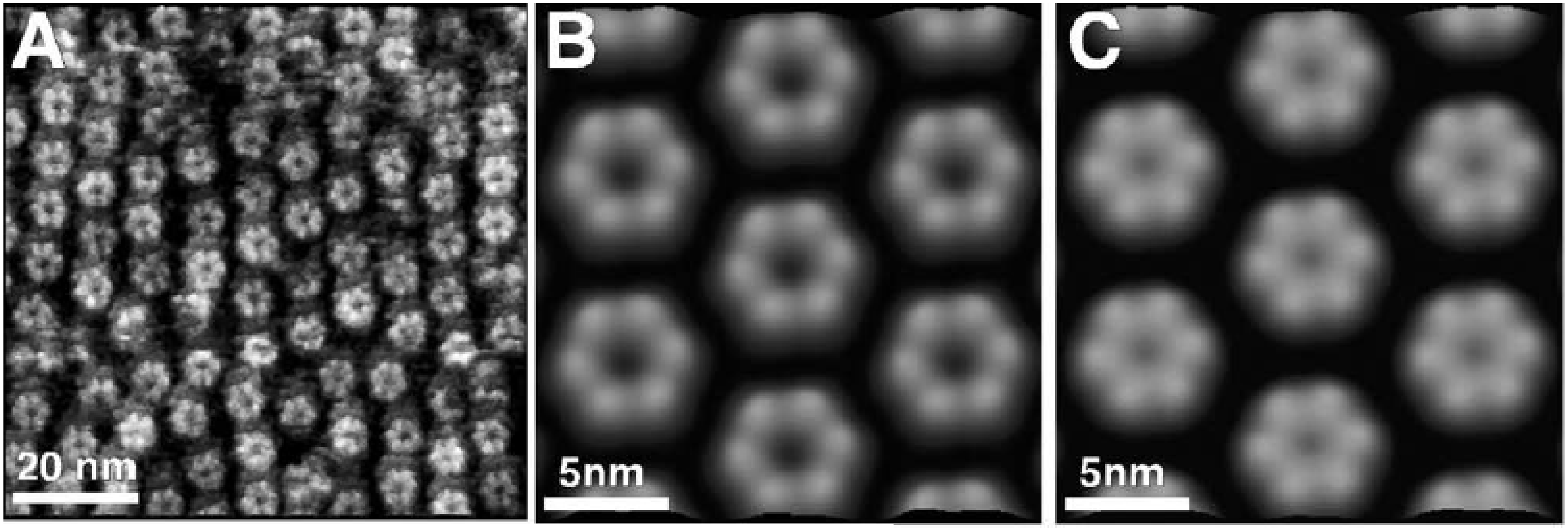


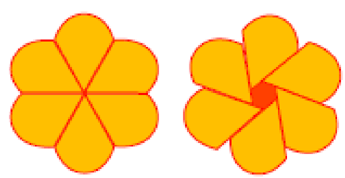
Rotary rotors from bacteriophages

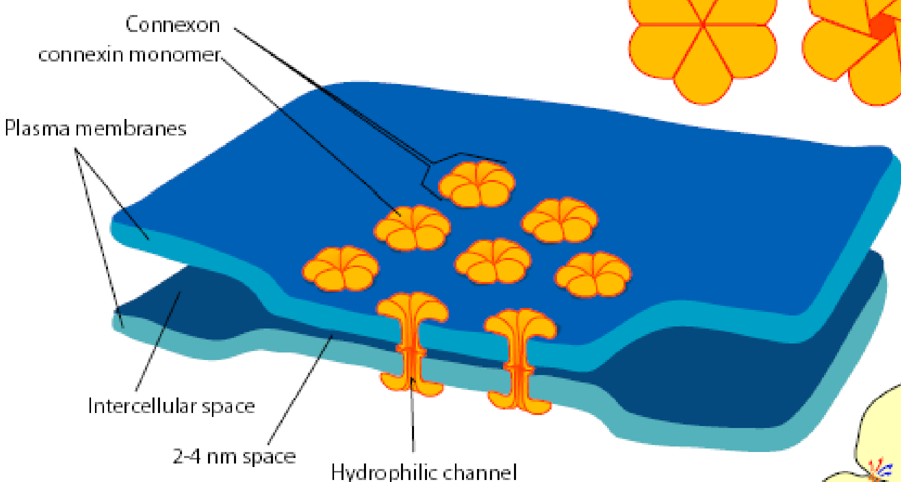


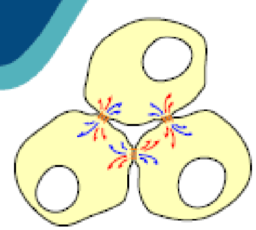
Ca2+ induced conformational changes in connexins

0.5 mM Ca^{2+}



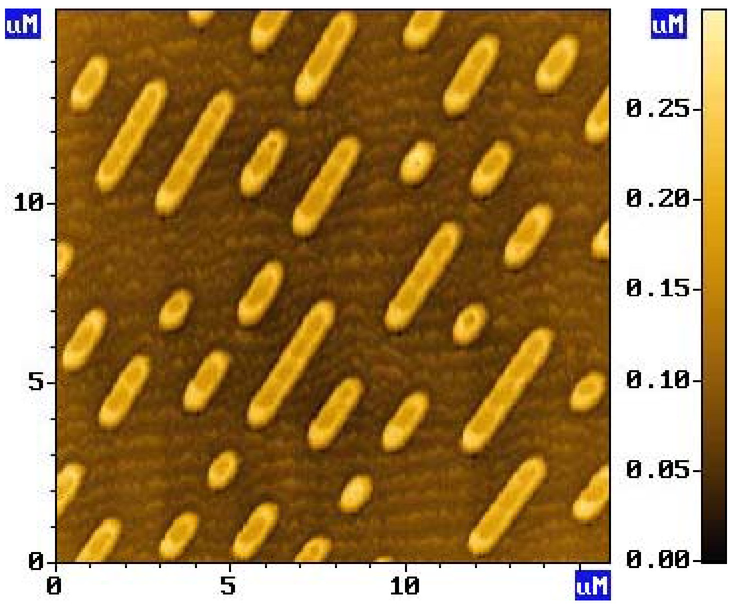


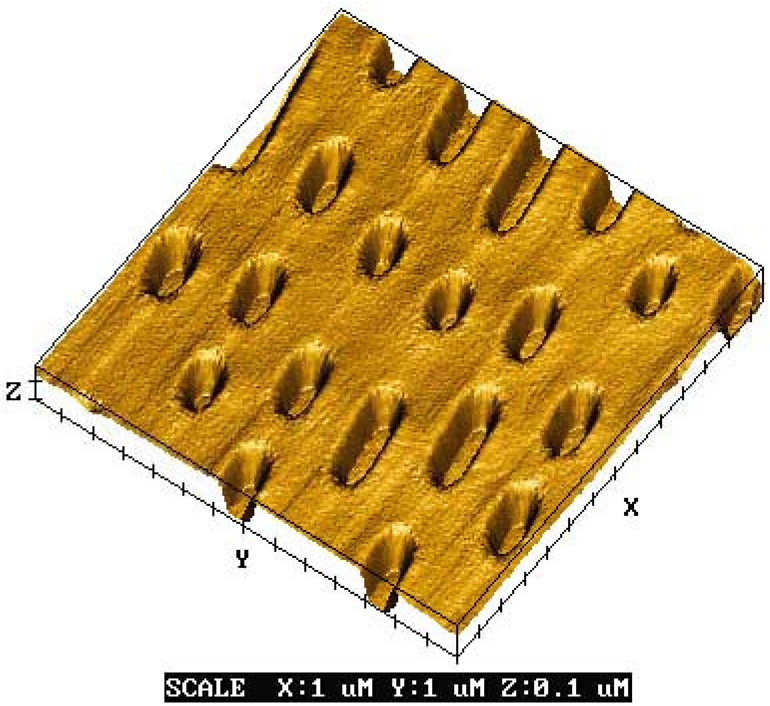




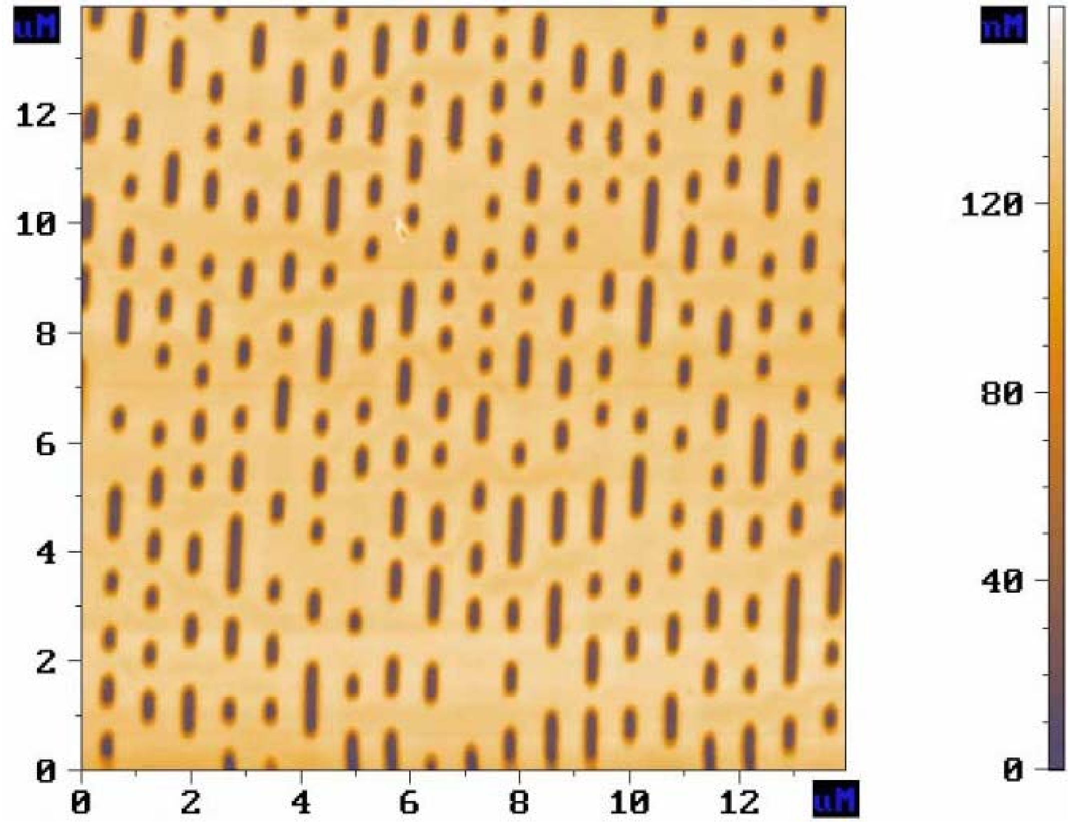
connexin is a major gap-junction protein

CD Disk

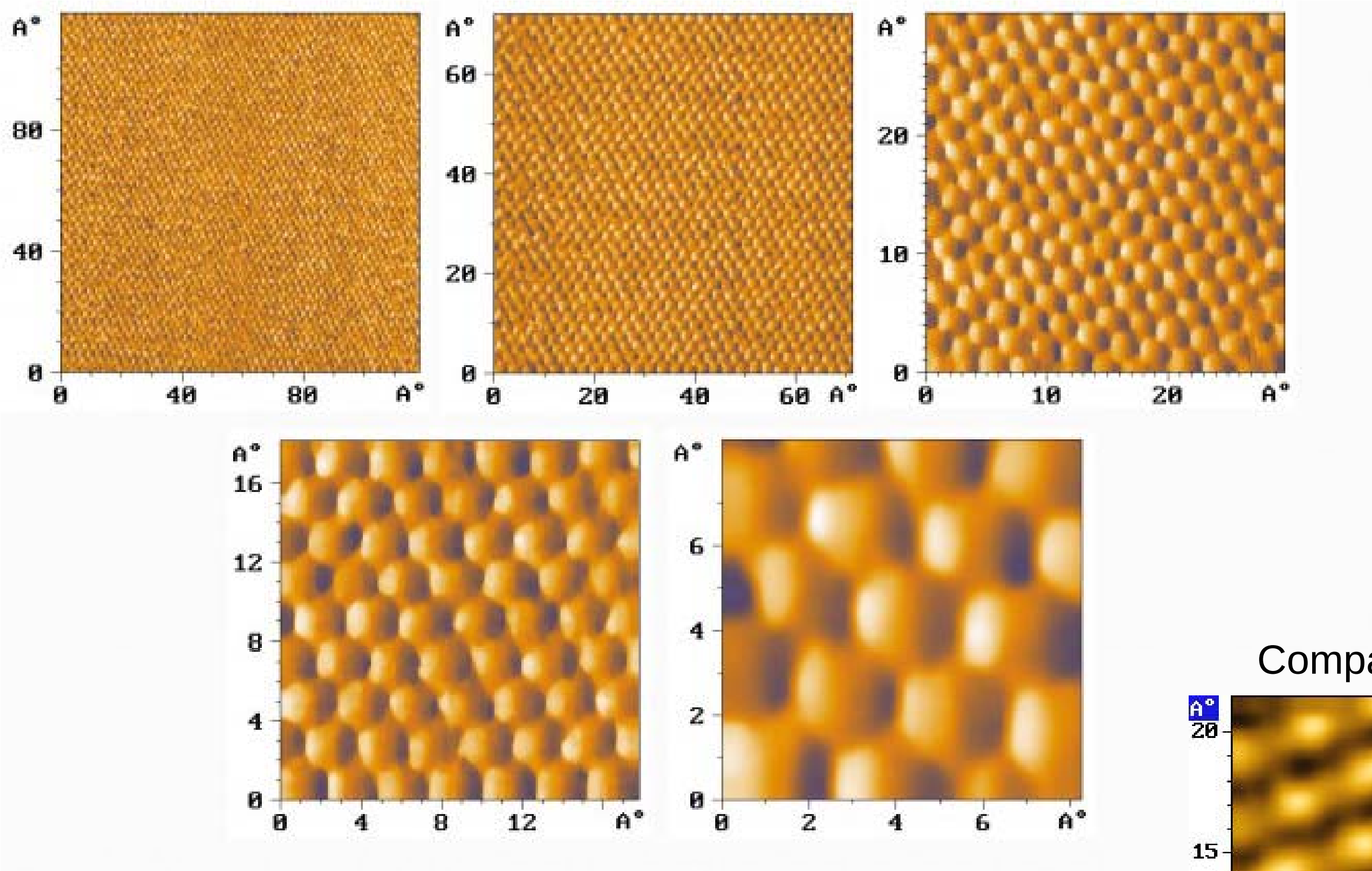




DVD Disk

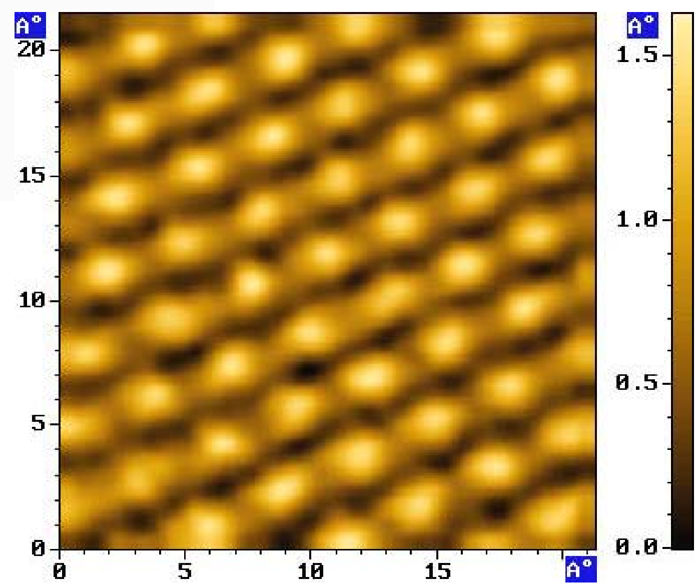


AFM providing atomic resolution



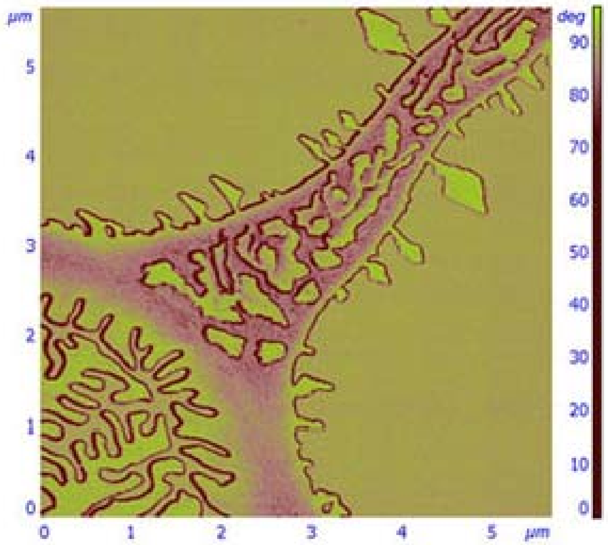
Highly Oriented Pyrolytic Graphite with atomic resolution

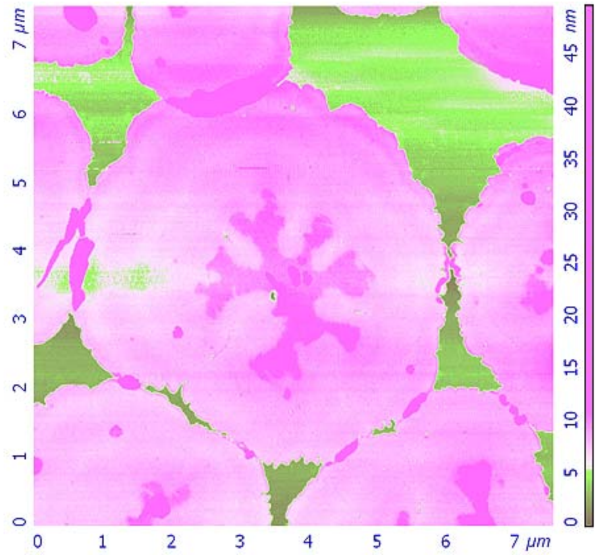
Comparable to STM!

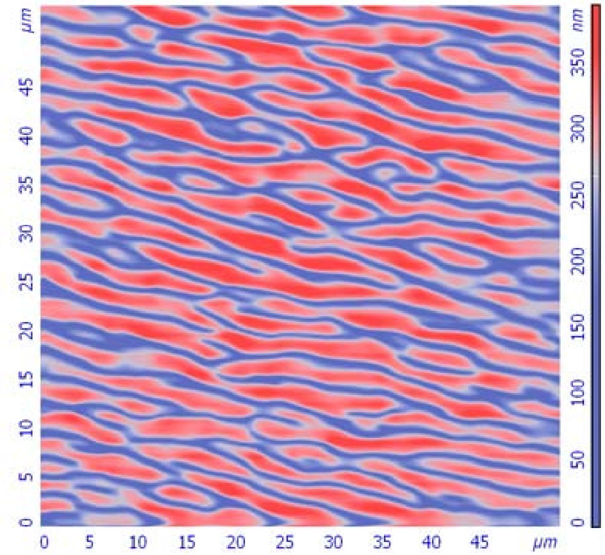


Thin films

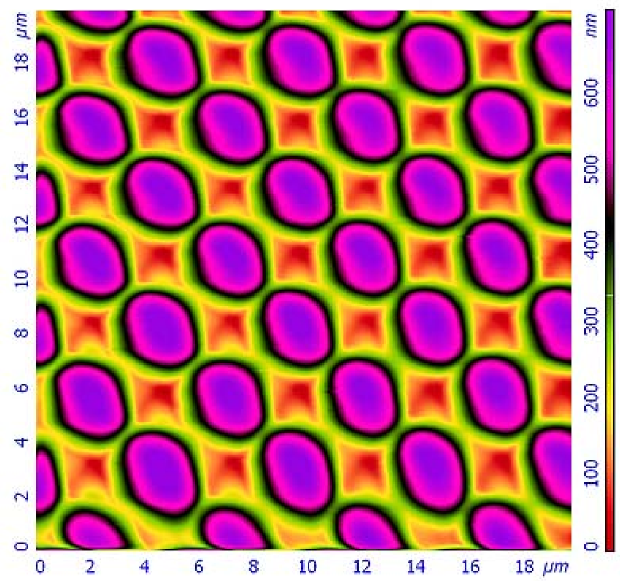
Polycaprolactone

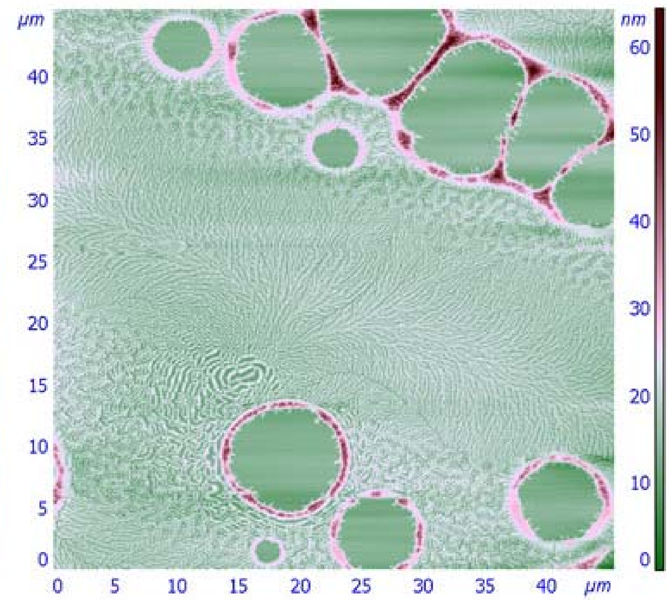






Polymethylmetacrylate



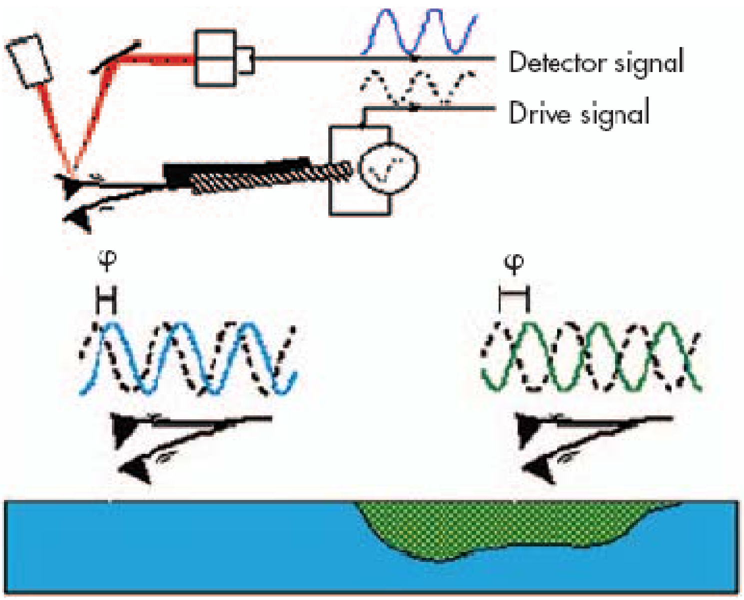


So many secondary AFM imaging modes!

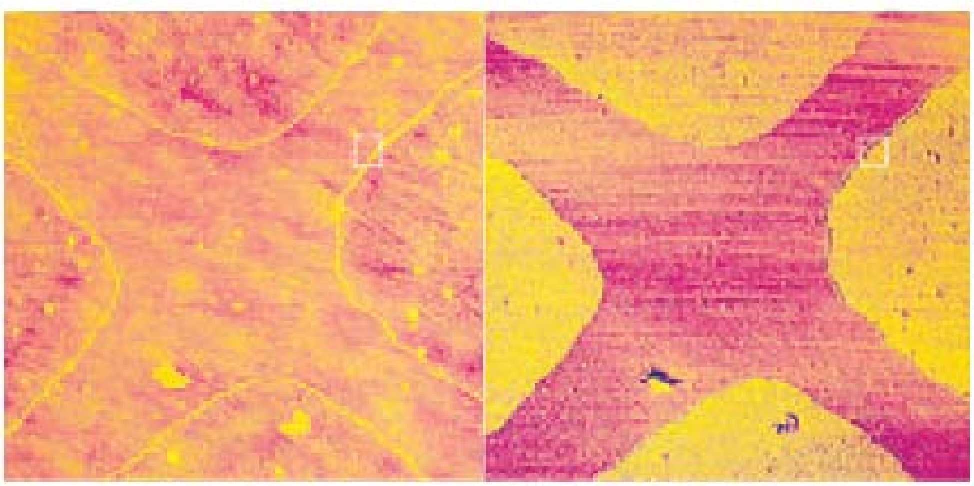
- Lateral Force Microscopy (LFM)
- **Phase Imaging**
- **Magnetic Force Imaging (MFM)**
- Conductive AFM (CAFM)
- Tunneling AFM (TUNA)
- Electric Force Microscopy (EFM)
- Surface Potential Imaging (SP)
- **Force Modulation Imaging**
- Scanning Capacitance Microscopy (SCM)
- Scanning Spreading Resistance Microscopy (SSRM)
- Scanning Thermal Microscopy (SthM)

Secondary AFM imaging modes

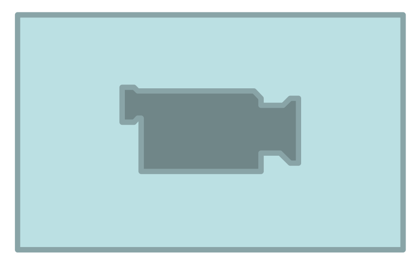
Phase Imaging



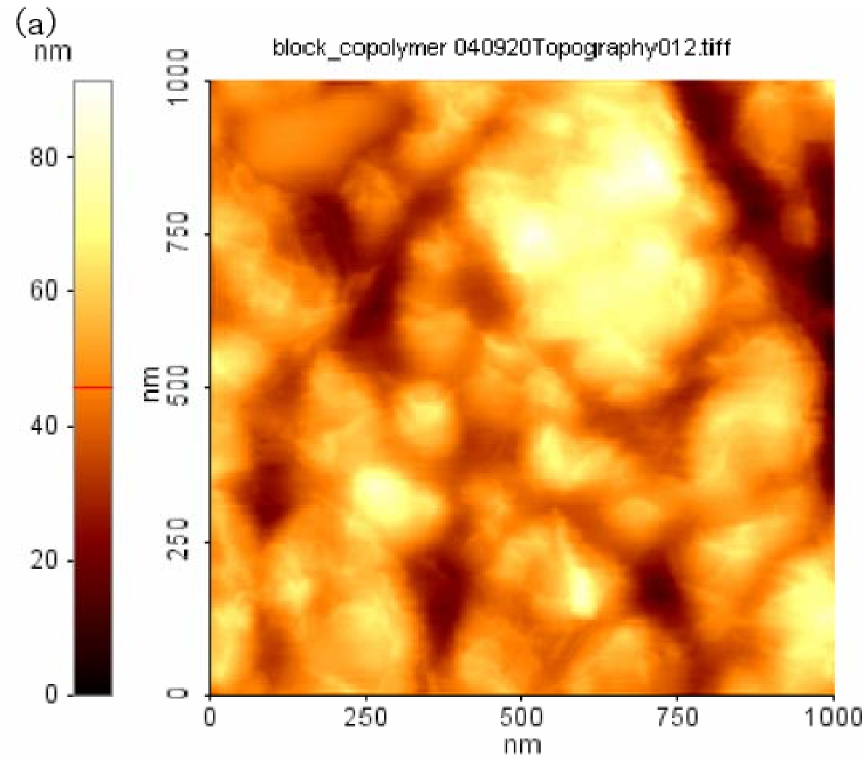
- In tapping mode the oscillation of the tip can be separated into a phase and amplitude
- Changes in tip-oscillation due to changes in surface properties causes detectable **phaseshifts**
- Detection in **sample-composition**: adhesion, friction, viscoelasticity (stiffness/softness), electric and magnetic variations



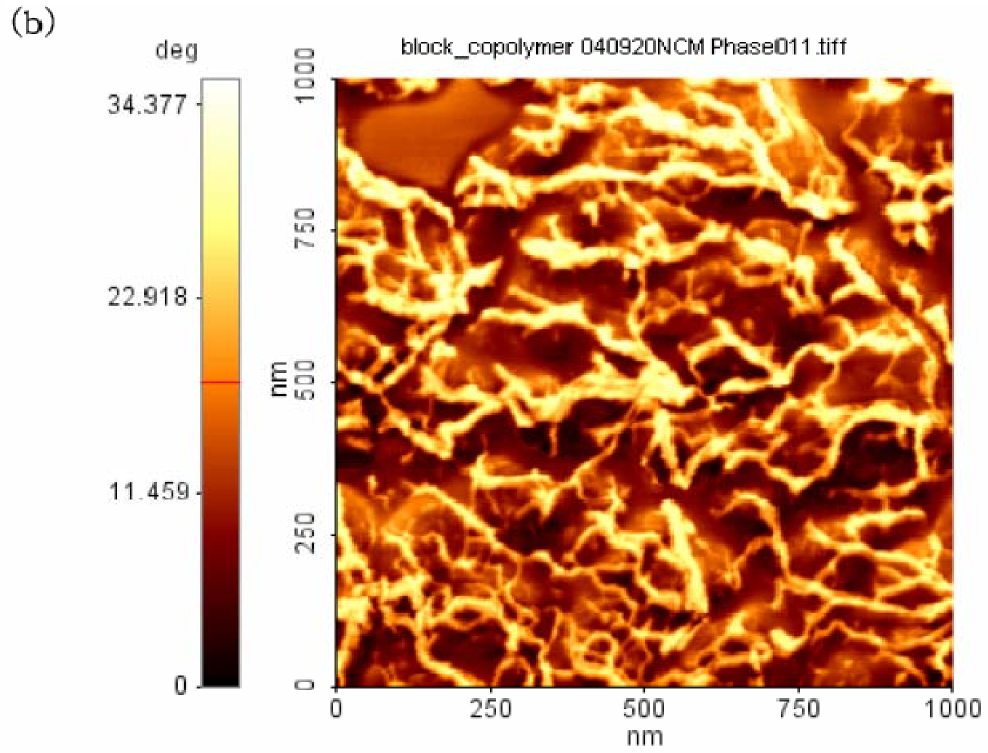
Silicone hydrogel manipulated with a higher hydrophobicity in the cross-like area



Phase imaging of block copolymer

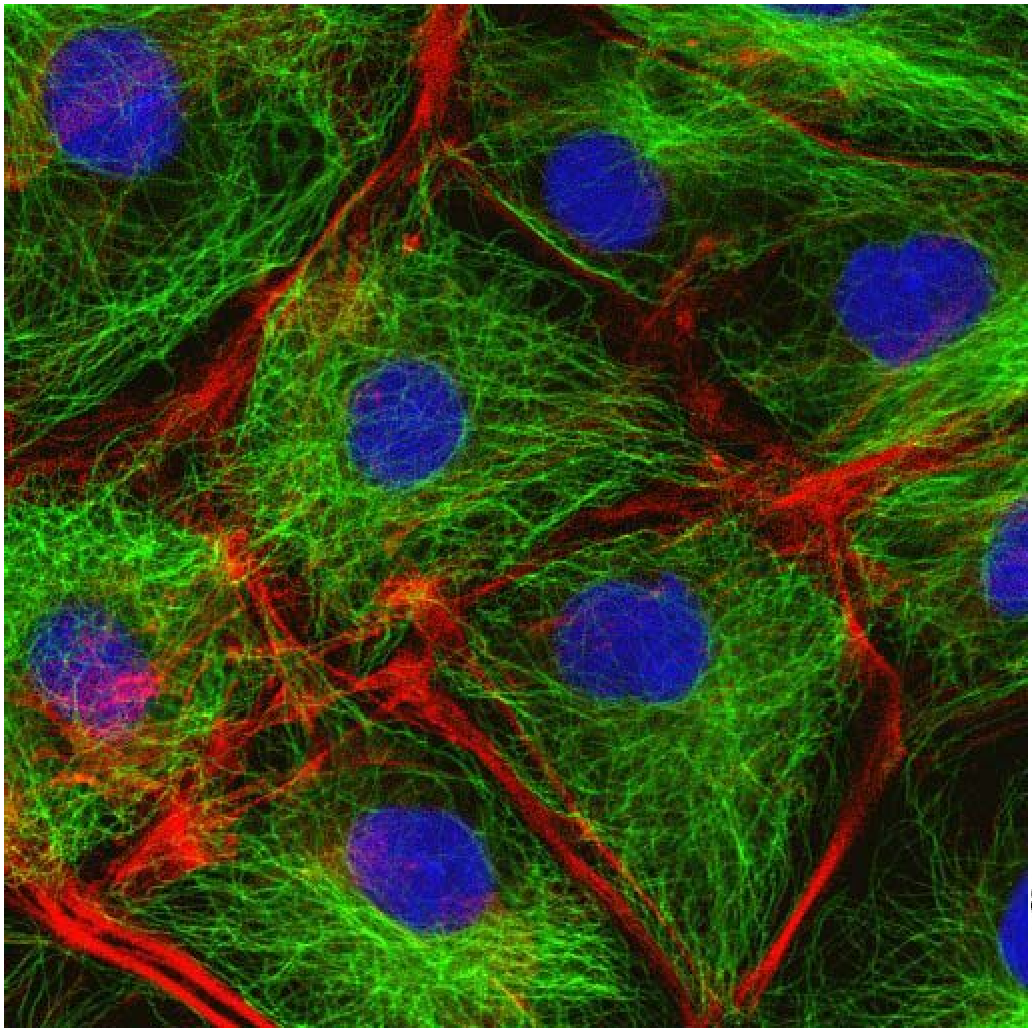


Topography image

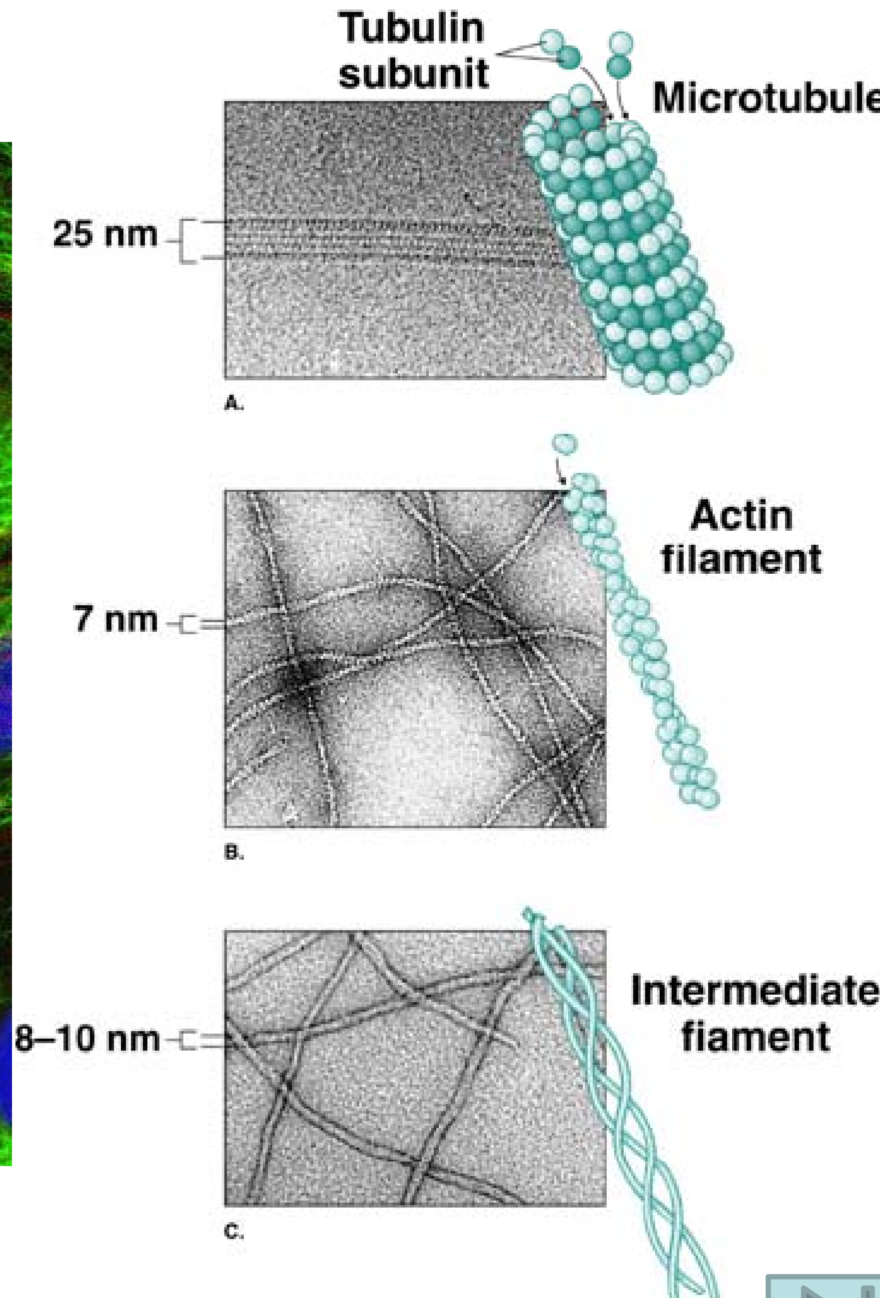


Phase image

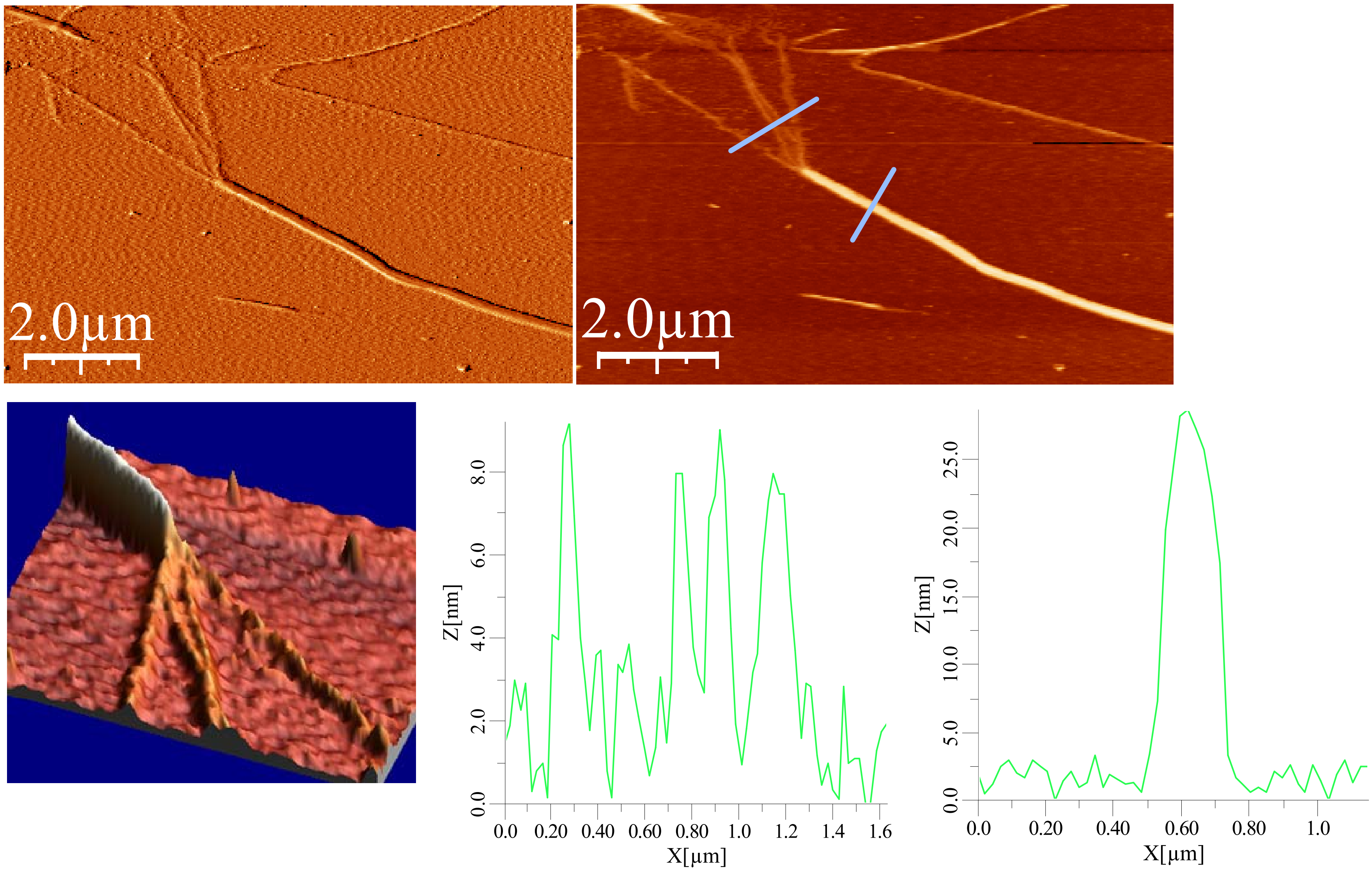
Analysis of cellular components

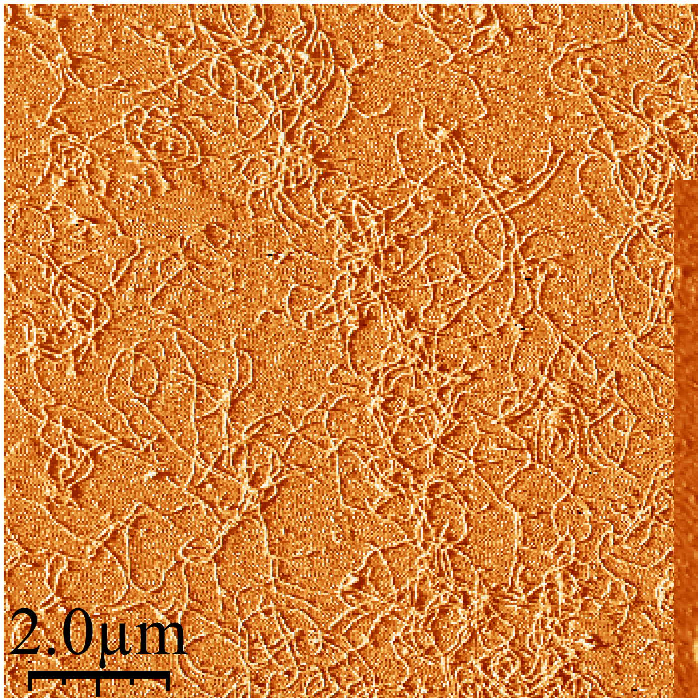


DNA
Microtubules
Actin stress fibers

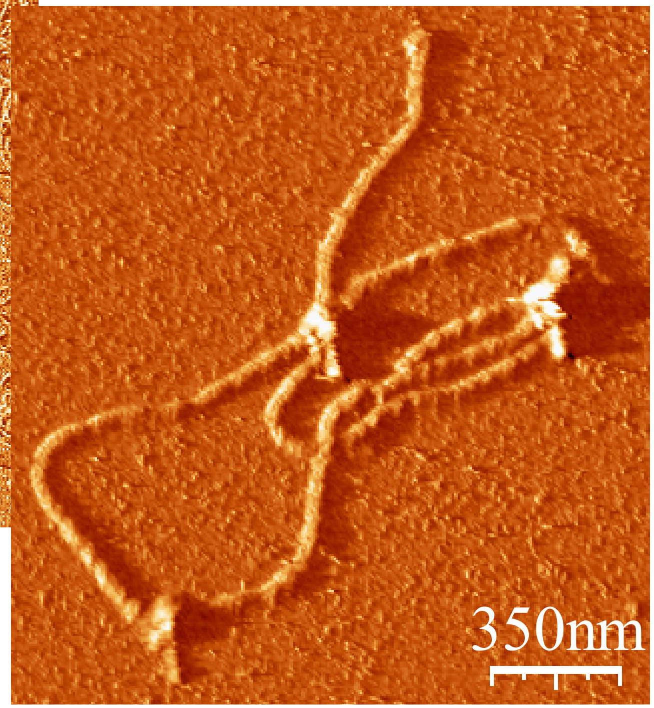


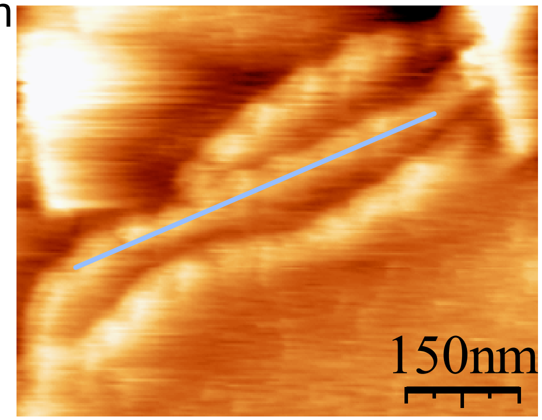
Tapping mode on isolated microtubules in physiological buffer

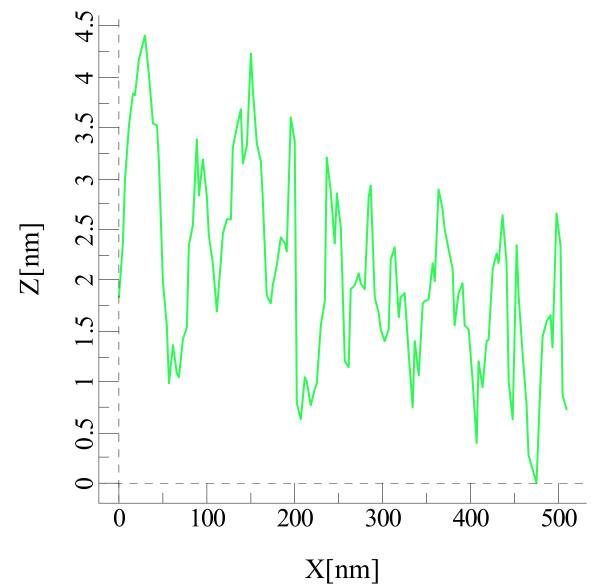


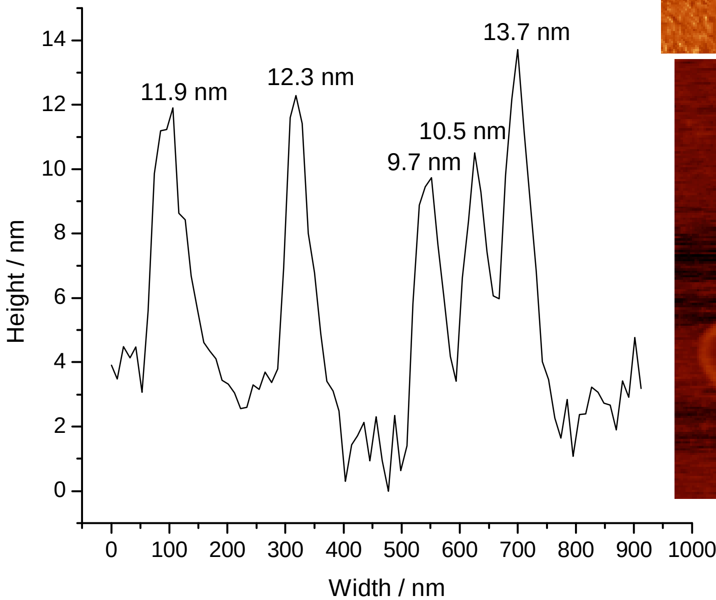


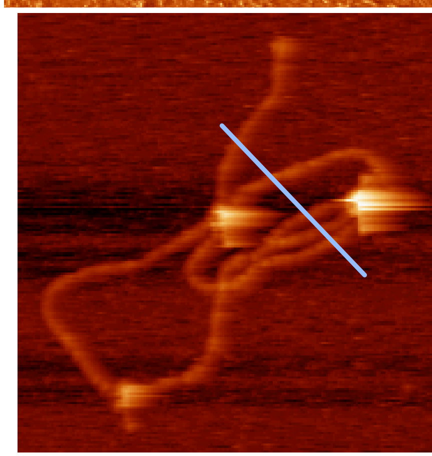
Neurofilaments from bovine brain









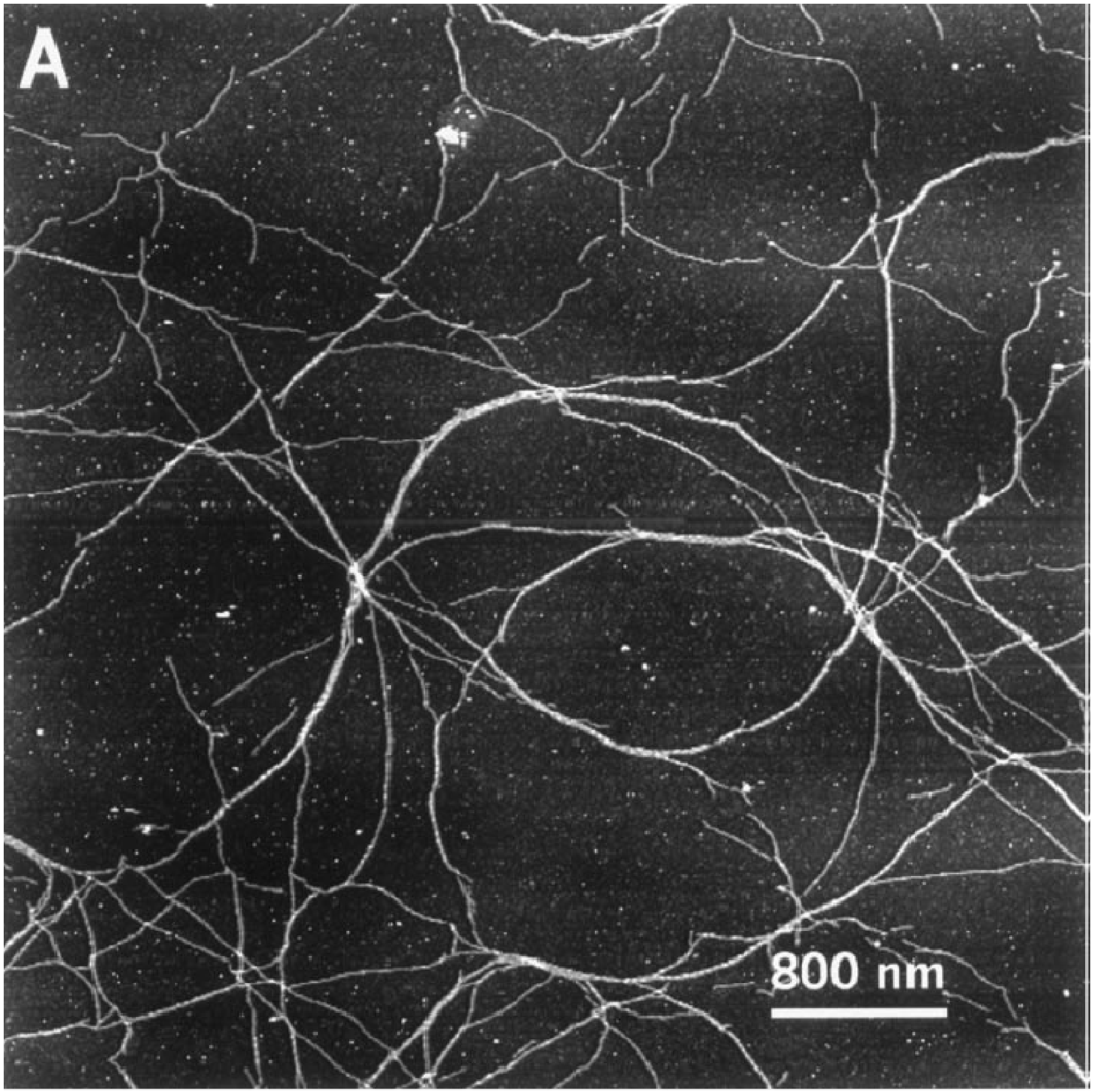


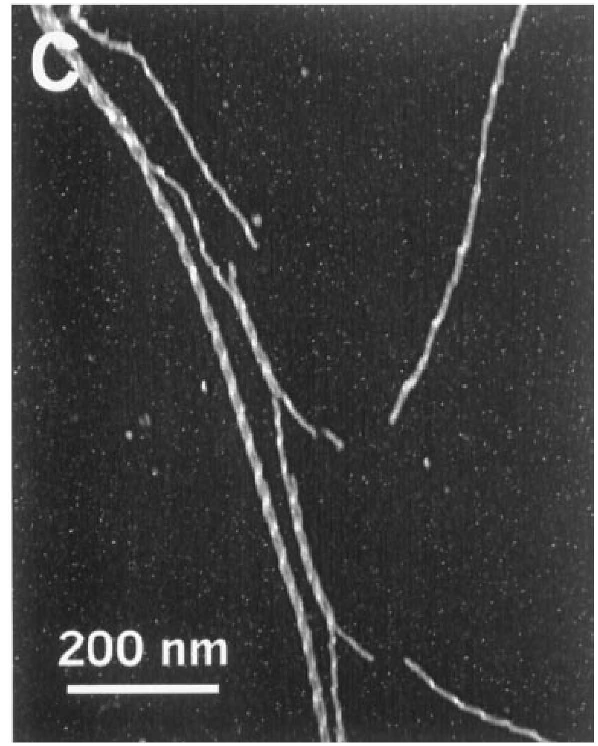
Periodicity between positive peaks:
54.9 nm +/- 10 nm
From 37 measurements: 52.7 nm +/- 8.1

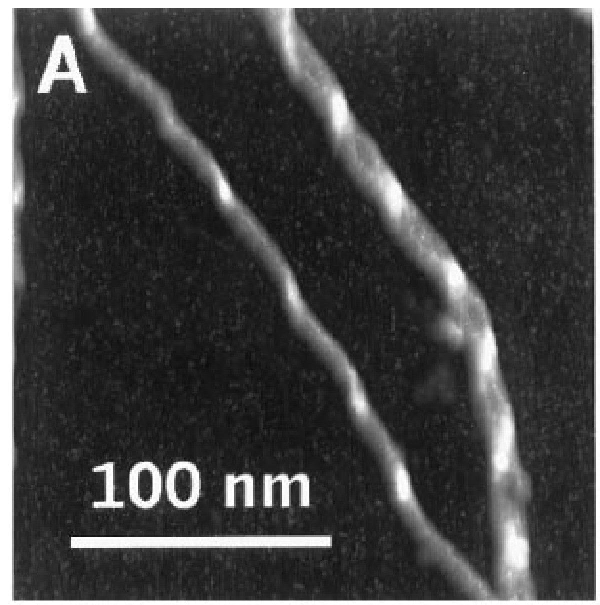
Cryo-AFM increases resolution

AFM head is operated in liquid nitrogen and operated at -180°C

Single and twisted f-actin filaments

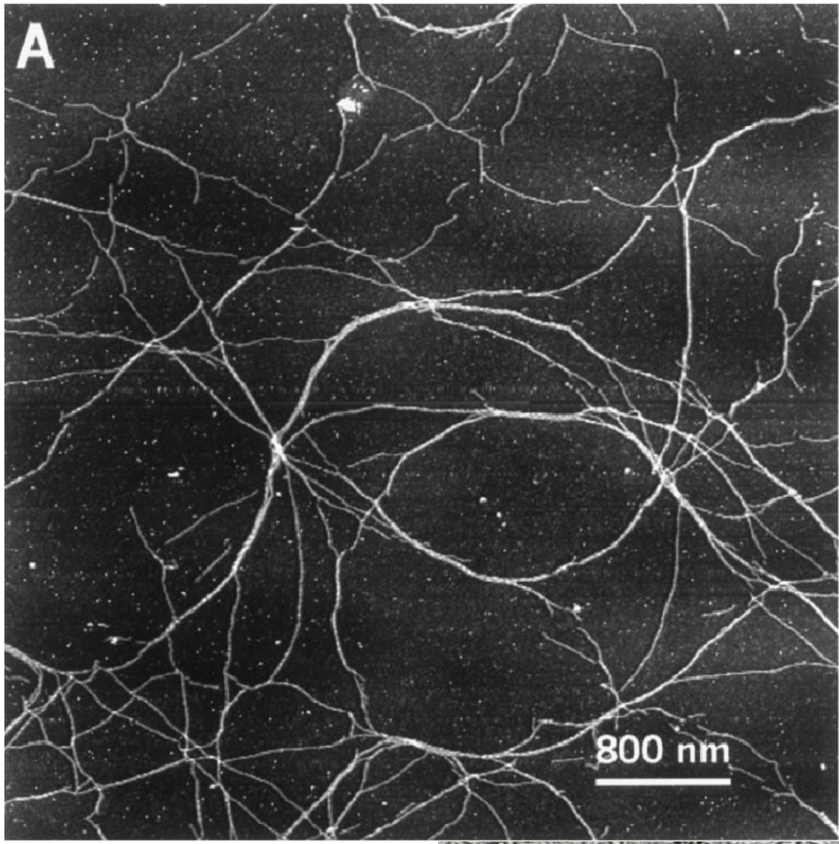




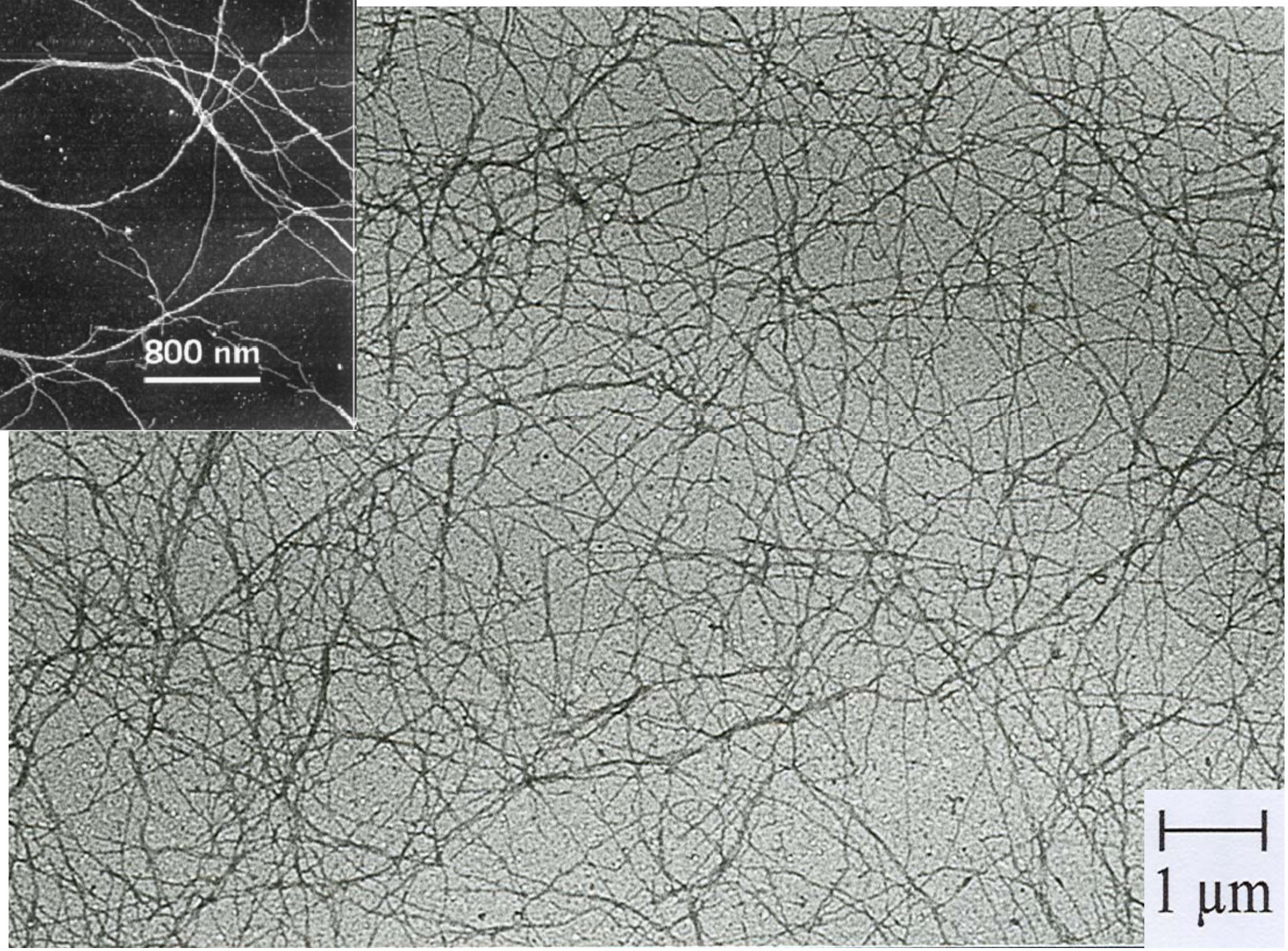


AFM resolution comparable
with electron microscopy

TEM

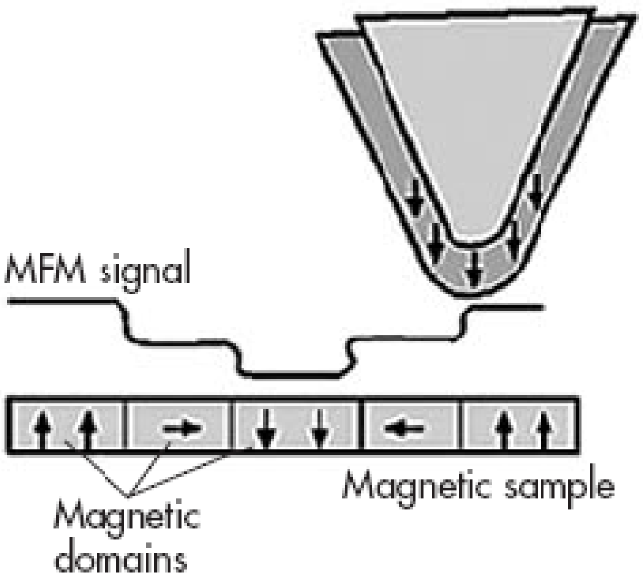


AFM



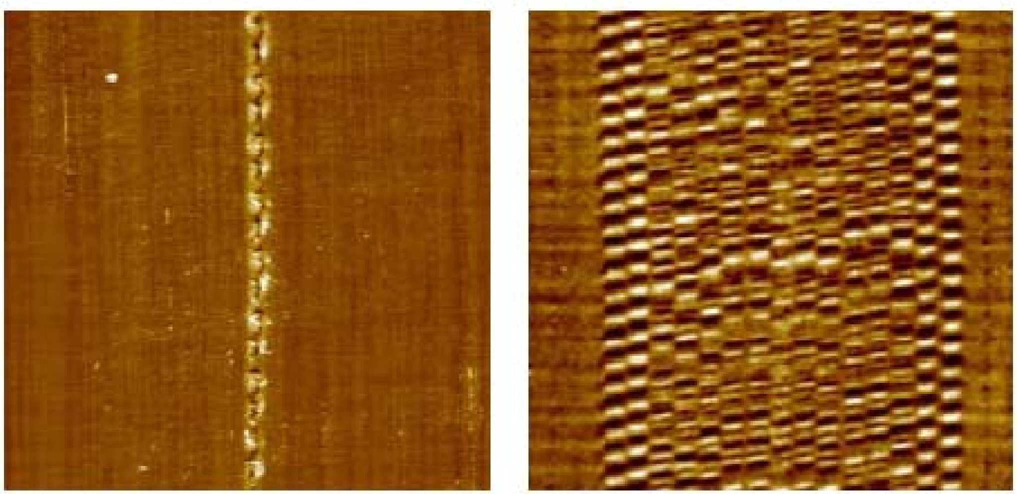
Secondary AFM imaging modes

Magnetic Force Microscopy (MFM)

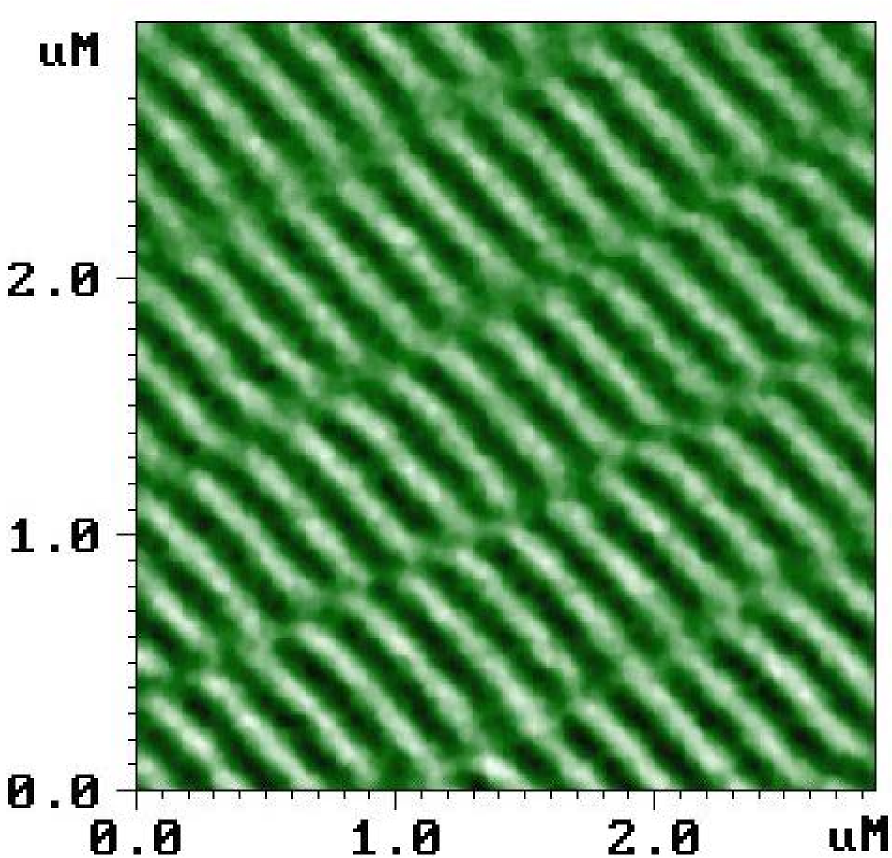


- Maps magnetic gradients
- Tip is coated with a ferromagnetic thin film
- Depending on the magnetic field properties of the sample, the tip-sample separation changes frequency and amplitude of the tip

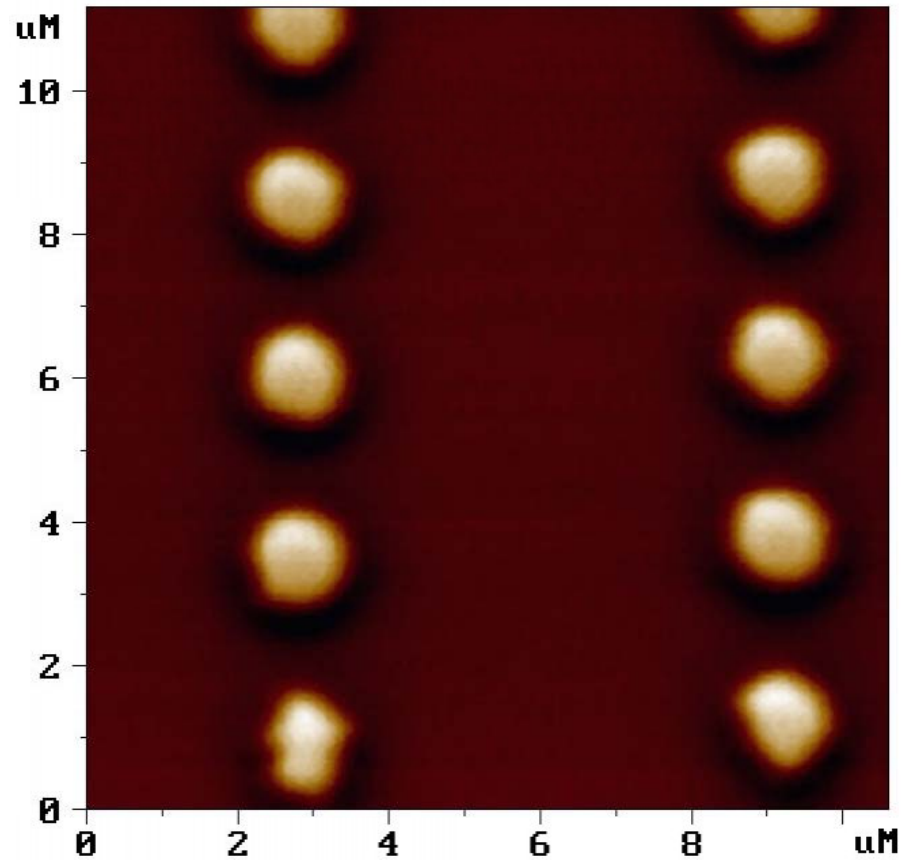
Hard Disk (HDD)



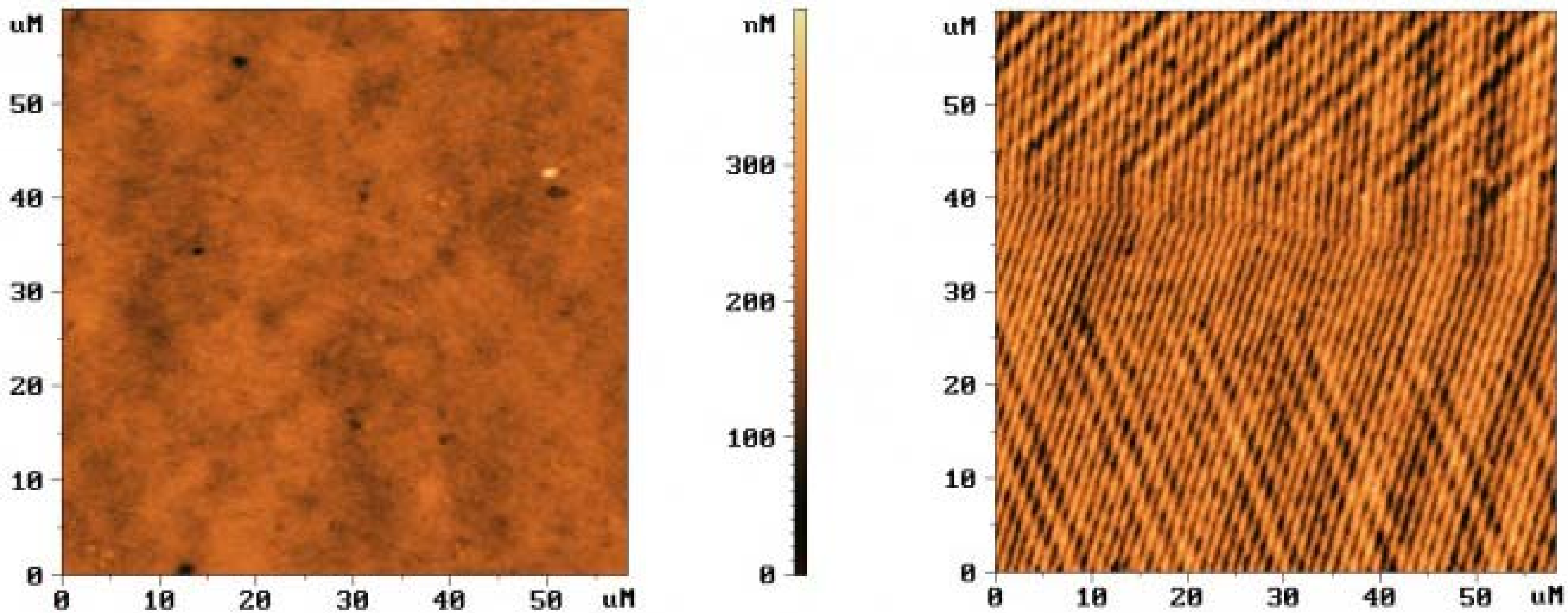
Left: Topography
Right: MFM image => single magnetic bits visible



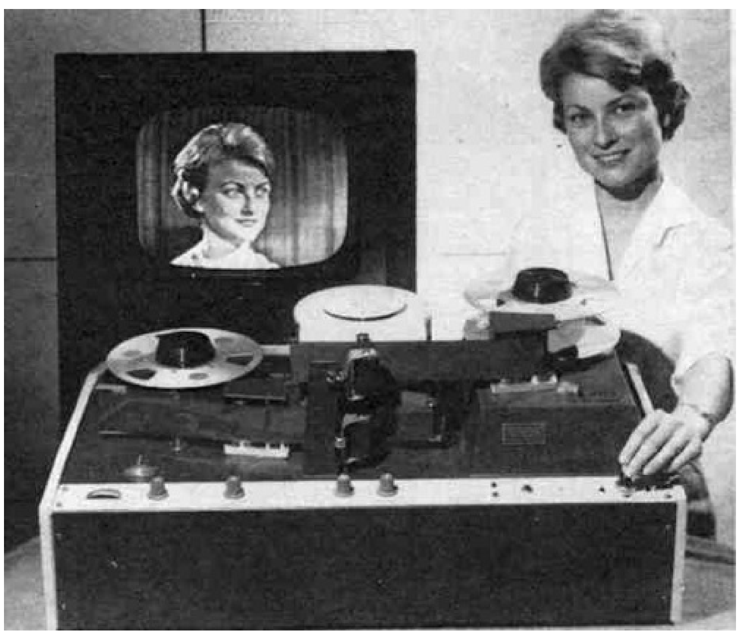
High density Floppy disk (HDD)
(distance between bits = 200 nm)



Magneto-Optical Disk
(showing single magnetic bits)



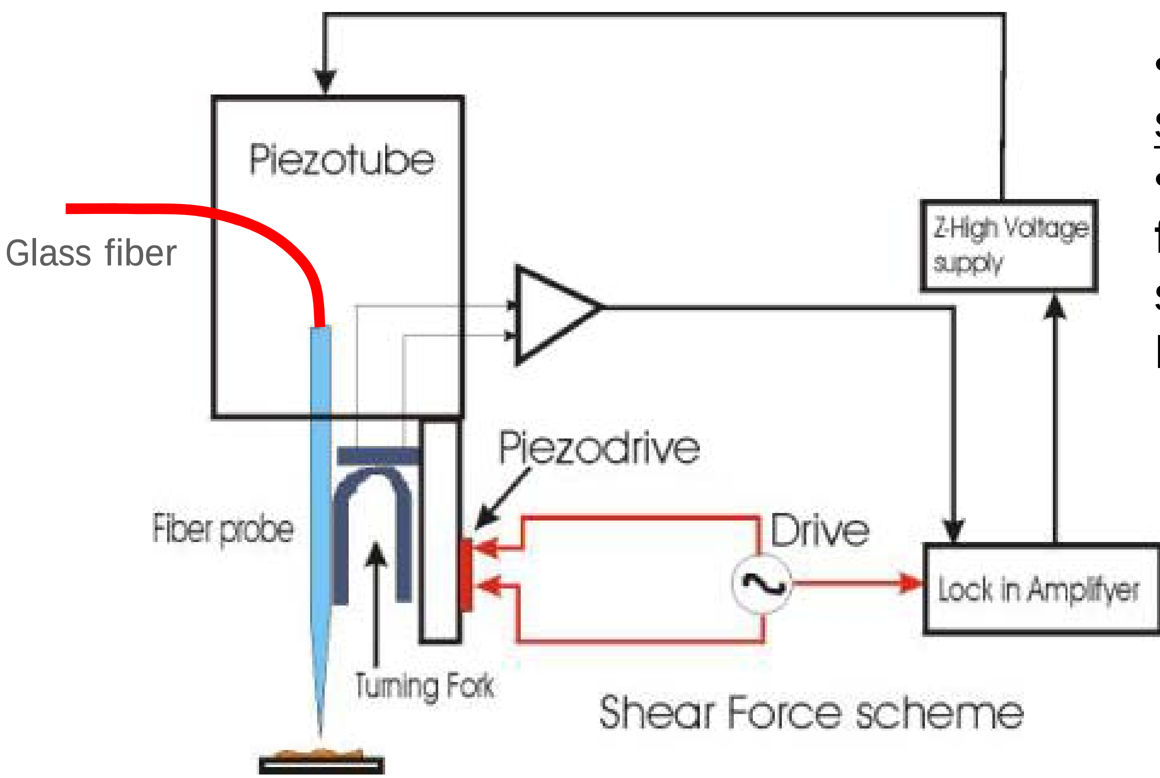
Video-tape: Left - topography, right - magnetic image (imaged at the same time)



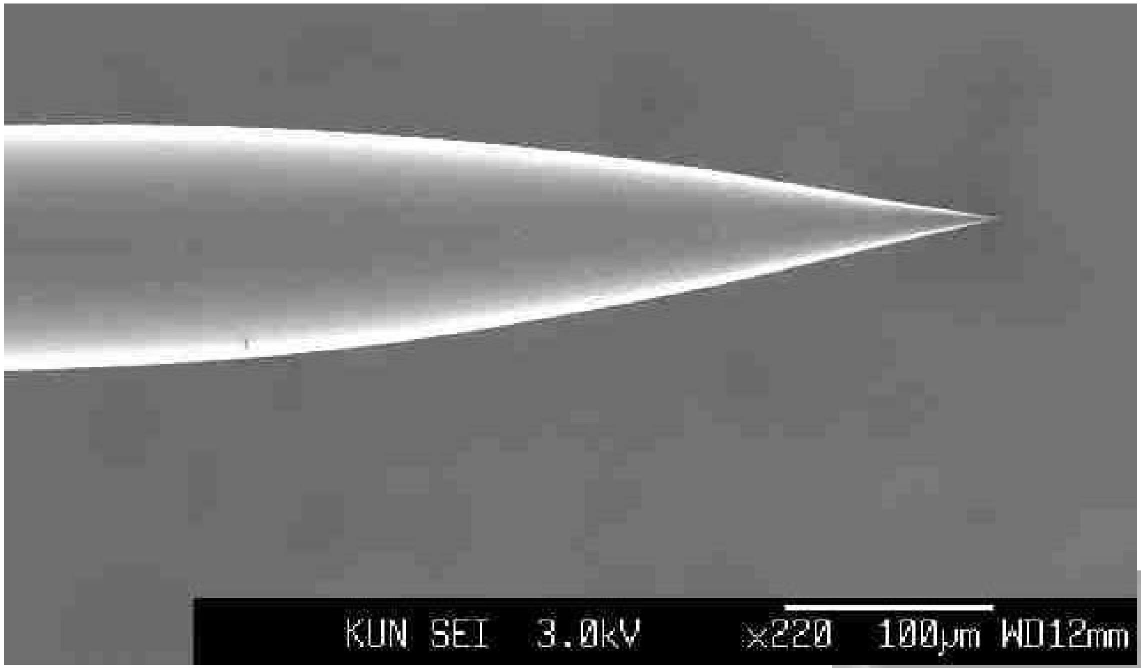
Scanning Near-field Optical Microscopy (SNOM)

- SNOM is an **optical microscope** based on AFM techniques
- The tip consists of a sharpened optical glass fiber
- The light passes thru an **aperture in the nanometer range**

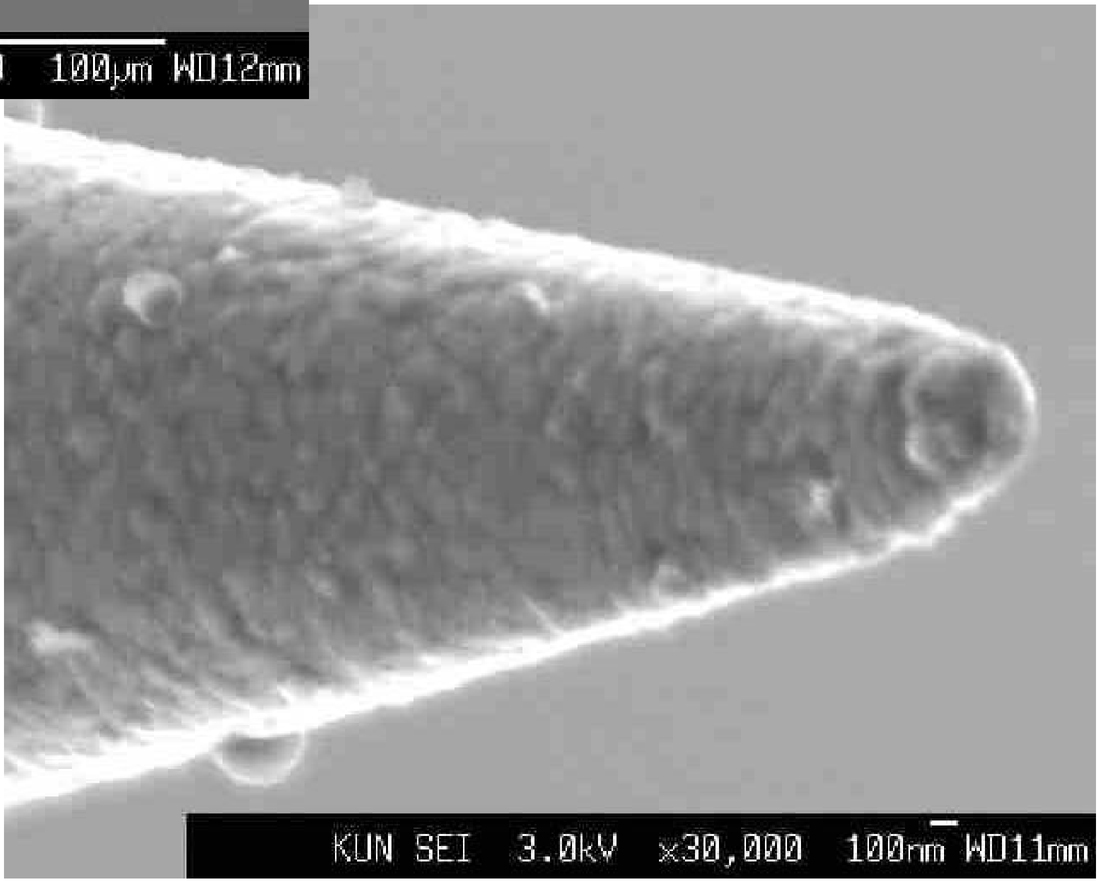
⇒ work far beyond the diffraction limit = near field
(⇔ far field = regular optical microscope)



- A **tuning fork** regulates the tip-sample distance during scanning
- The fork also **detects shear-forces** between the tip and the sample (also called Shear Force Microscopy)

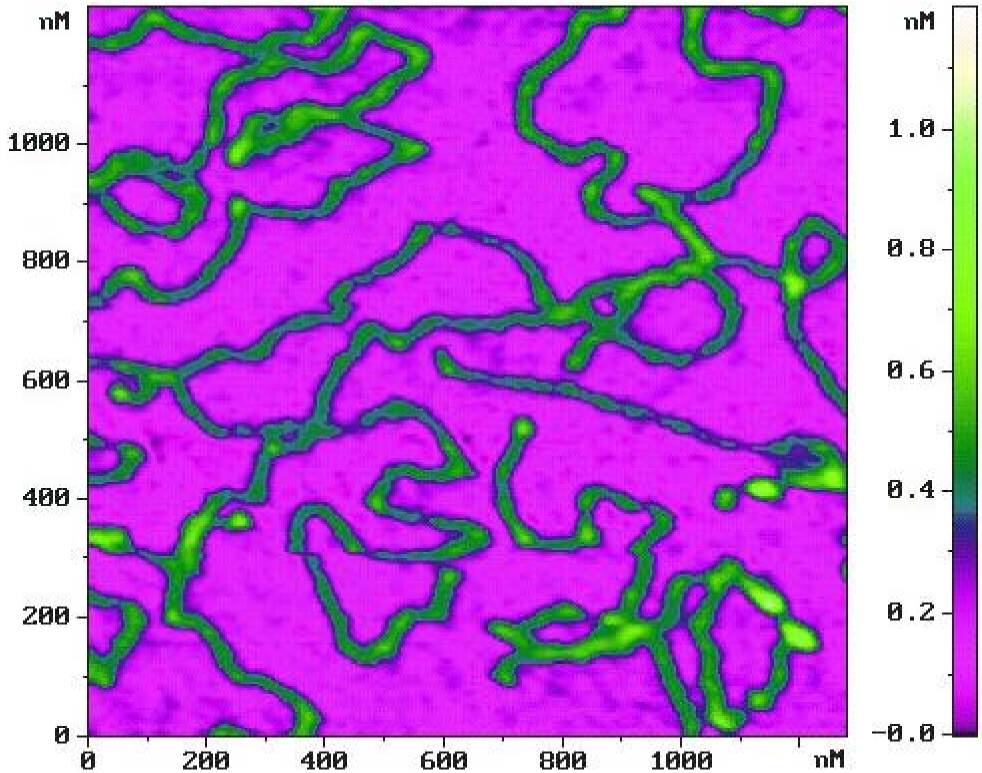


80 nm opening (aperture)
of the glass fiber
("tunneling of photons")



SNOM

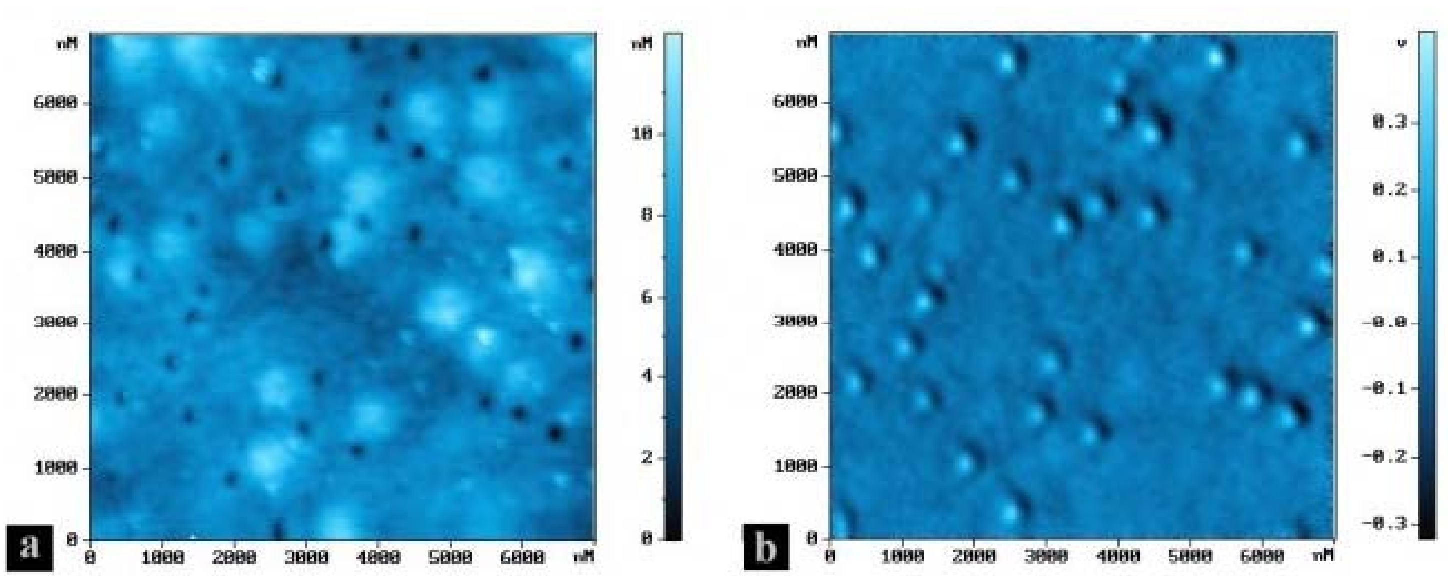
DNA shear-force image (false colored)



Linearized DNA plasmid

SNOM

Quantum Dots



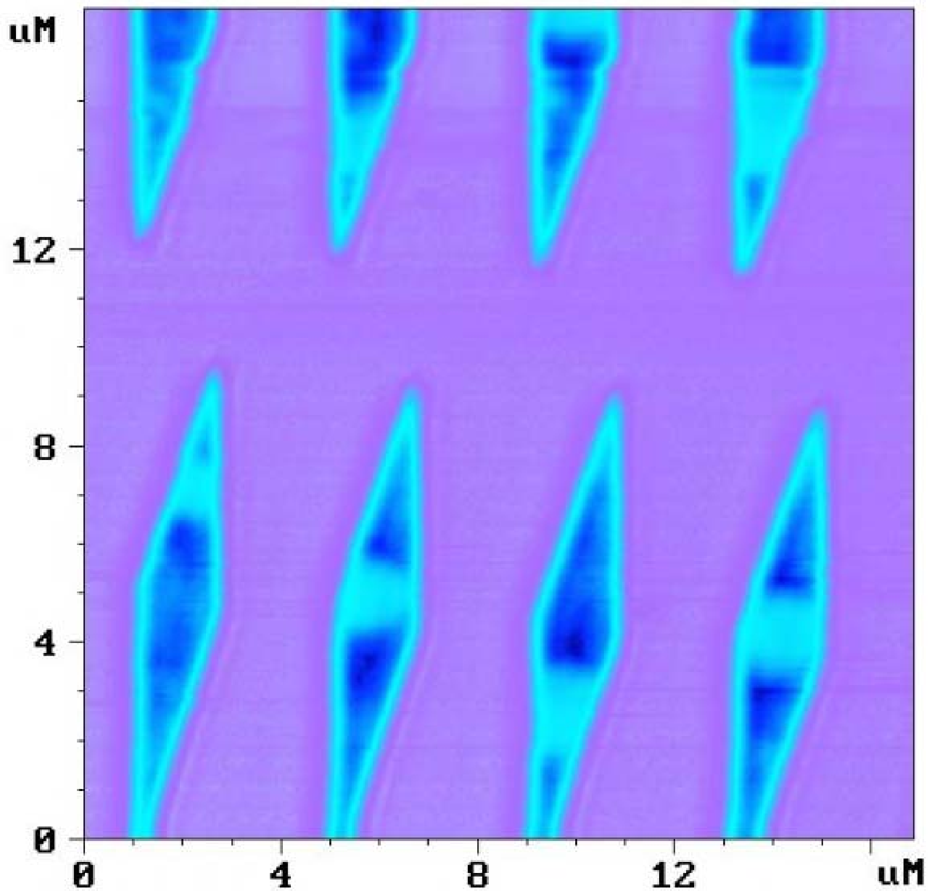
a) shear force (topography)

b) reflection image

- Quantum dots are semiconductor nanocrystals (2-10 nm)
- After excitation they can emit light in different colors
- The larger the dot, the redder the fluorescence

SNOM

Optical grating (mesh) in transmission mode



Non-imaging Modes

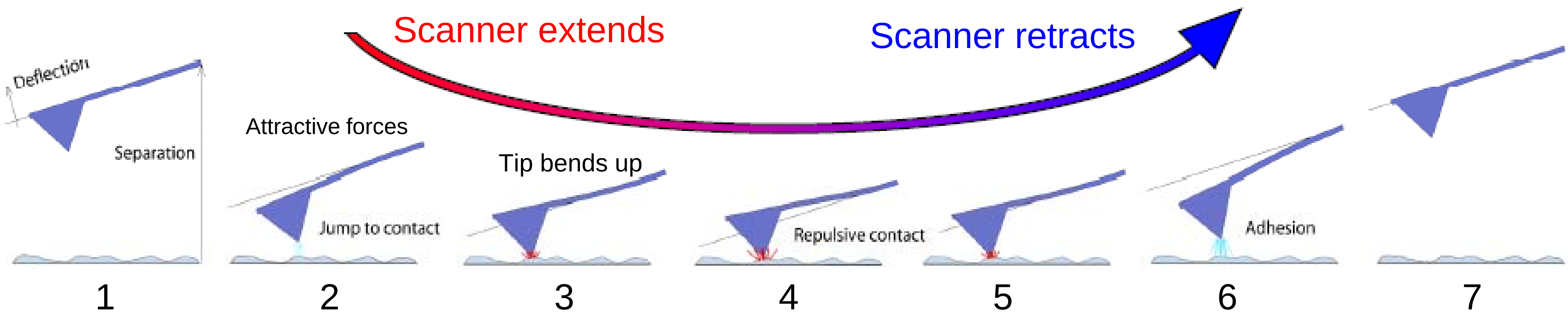
- ➢ Scanning Tunneling Spectroscopy (STS)
- ➢ Force Spectroscopy (“Force Pulling”) and Force Volume
- ➢ Nanolithography

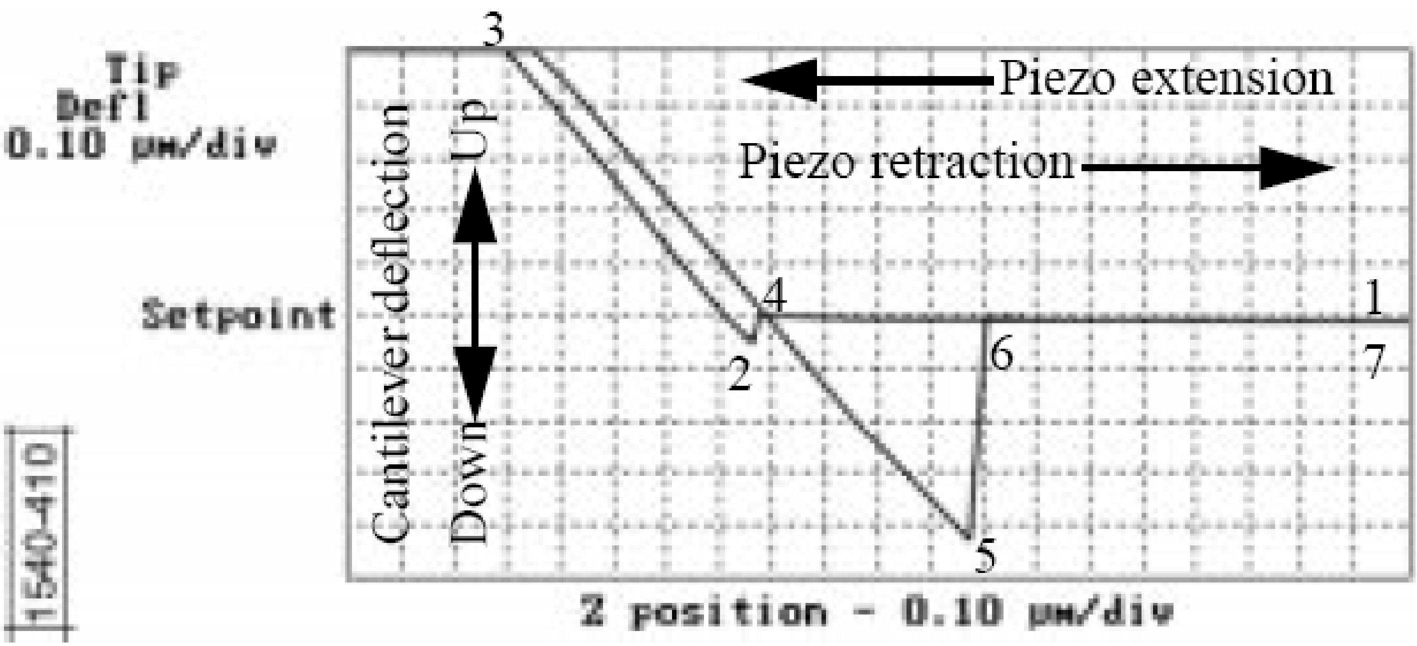
Here, images are not taken as the scanner usually only measures the material properties of the surface: **SPM spectroscopy**

Force Spectroscopy

- **Force-distance curves** are produced = force on the cantilever during its interaction with the surface
- Analyze: adhesion properties and elastic properties of the material
- Protein-unfolding: strength of chemical bonds within a macromolecule

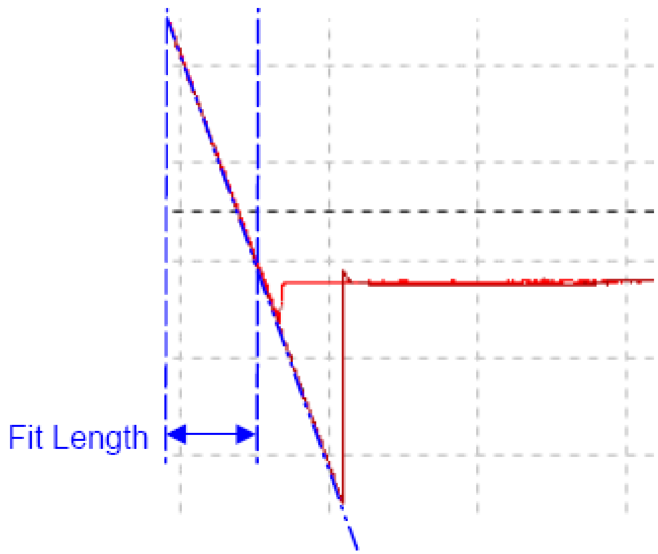
Mechanics properties of surface as revealed by force-distance curves



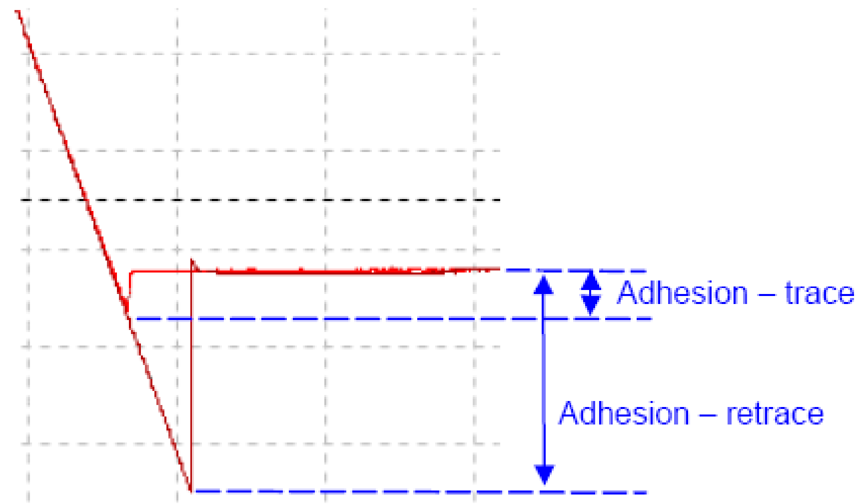


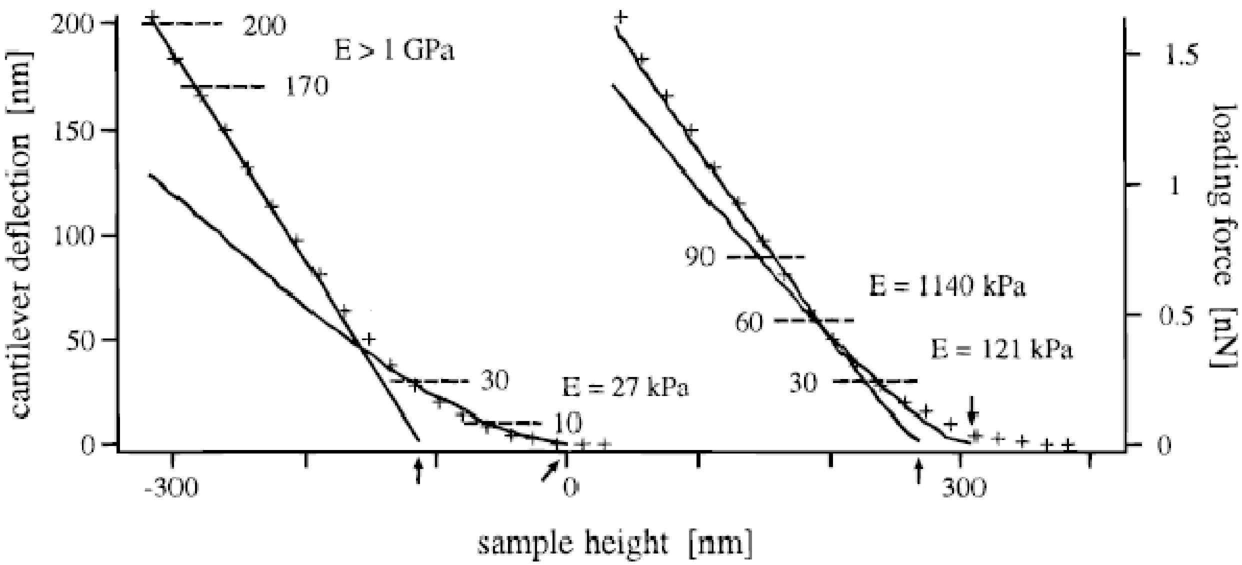
Calculation of elasticity and adhesion

Determining **elastic** properties



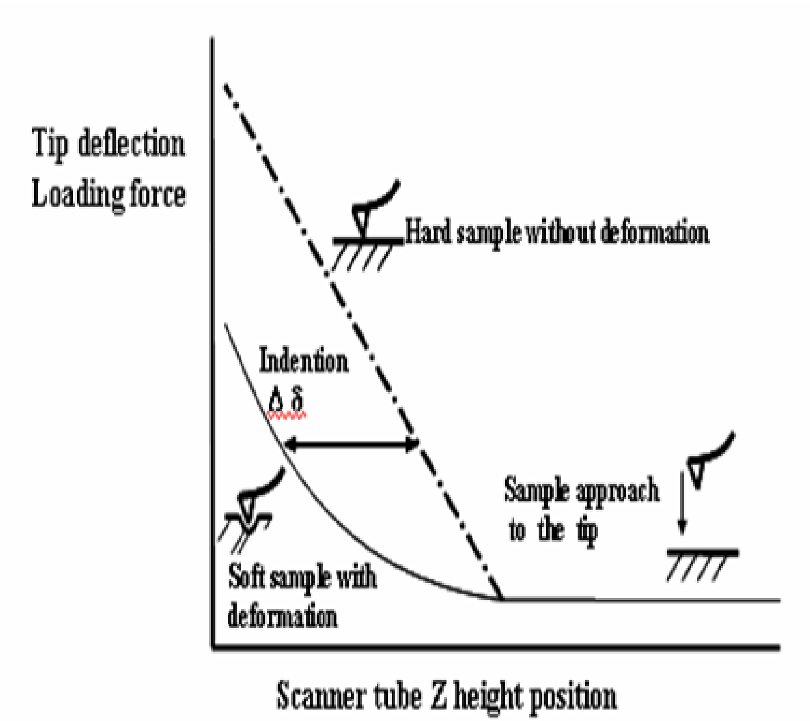
Determining **adhesion** properties

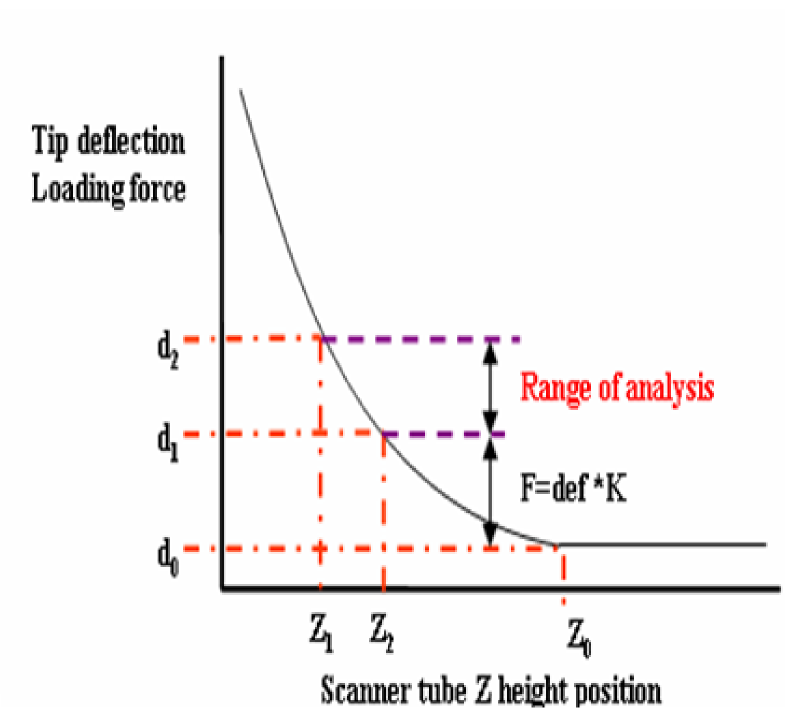




From the slope of the fitted contact curve we can calculate the Young's modulus

Calculations are based on Hooke's law and the Hertz model



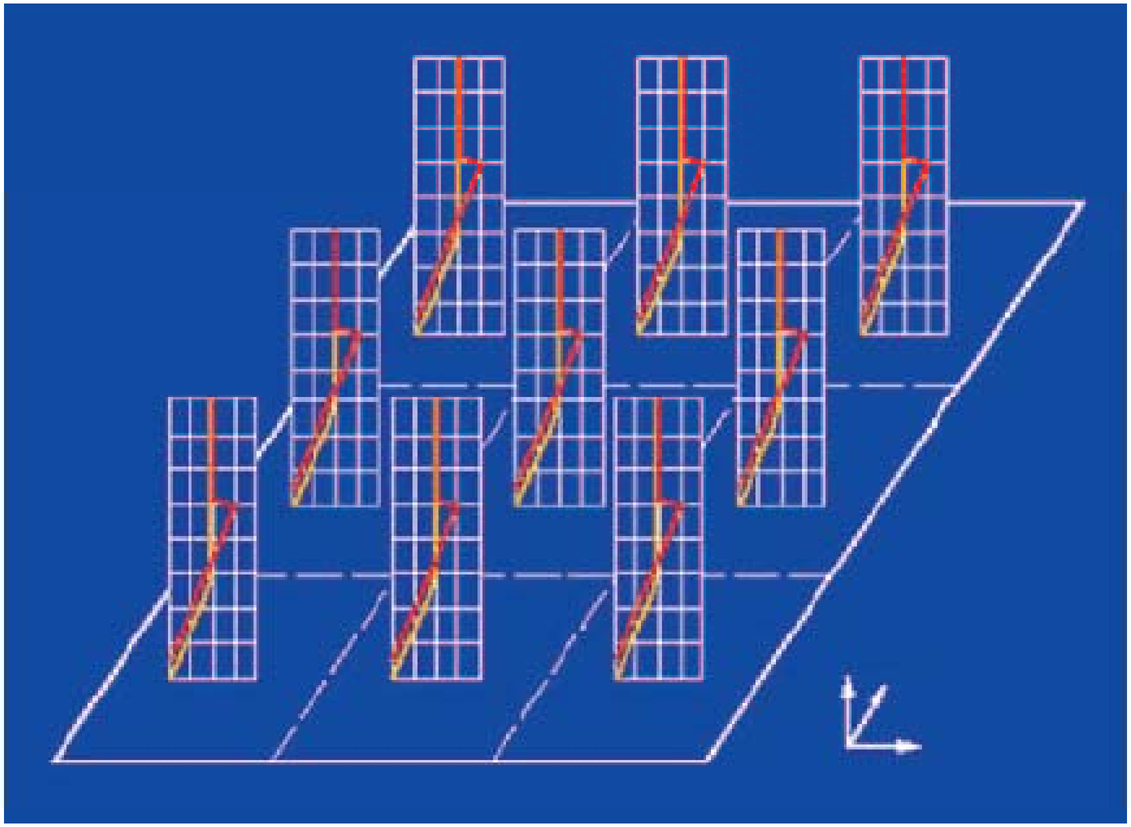


Hooke's law: $F = k \times d = k \times (z - \delta)$
Combined with **Hertz model**: $F = (2/\pi) \times [E/(1-\nu^2)] \times \delta^2 \times \tan(\alpha)$

E = Youngs modulus
ν = Poissons's ratio (shear force) = 0.5
δ = Indentation depth
k = Spring constant
F = Applied force
d = Cantilever deflection
α = Half opening angle of the indenting cone

$$z - z_0 = d - d_0 + \sqrt{\frac{k(d - d_0)}{(2/\pi)[E(1 - \nu^2)] \tan(\alpha)}}$$

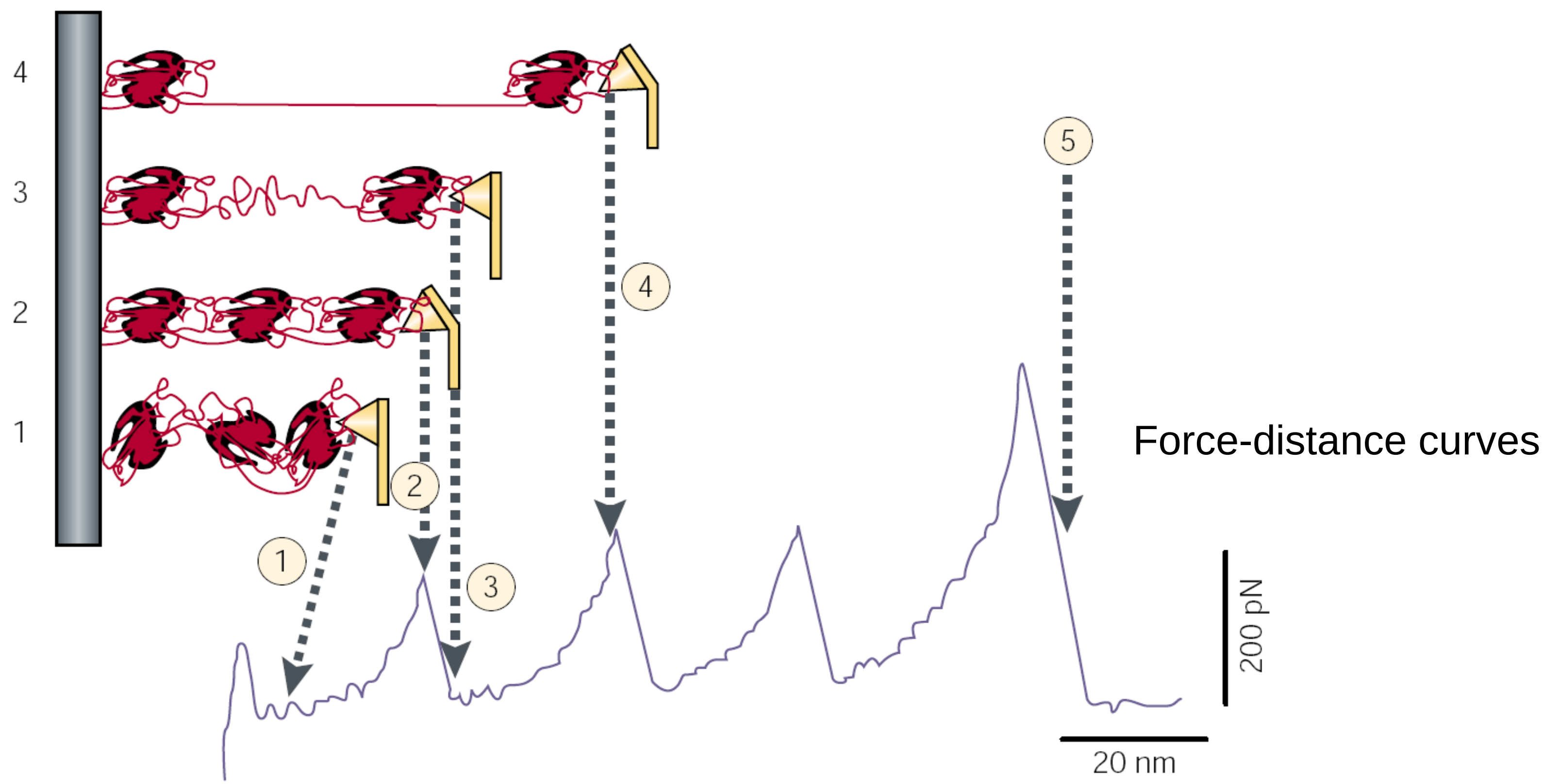
Force volume and force mapping



Collecting many force curves on an surface area allows for generating a map of elastic properties of a material at each point in 2D (force map) or 3D (force volume)

Advanced Force Spectroscopy

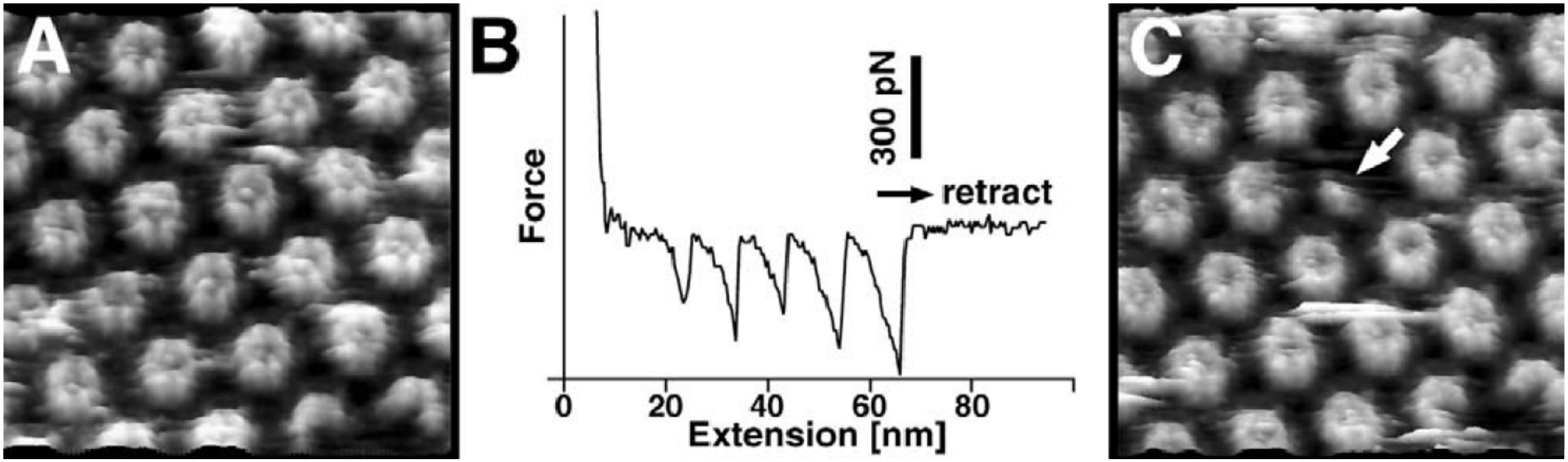
• **Protein unfolding**: AFM tip grabs the end of a protein (attached to a surface)
=> **protein unfolds in its several domains**
• Resulting **force-distance curve** shows a series of **snap-back points** each representing the breaking of a chemical bond



Domain unfolding of repeating immunoglobulin-like domains

Domain unfolding made visible

Unzipping the bacterial S-layer protein (membrane pore)

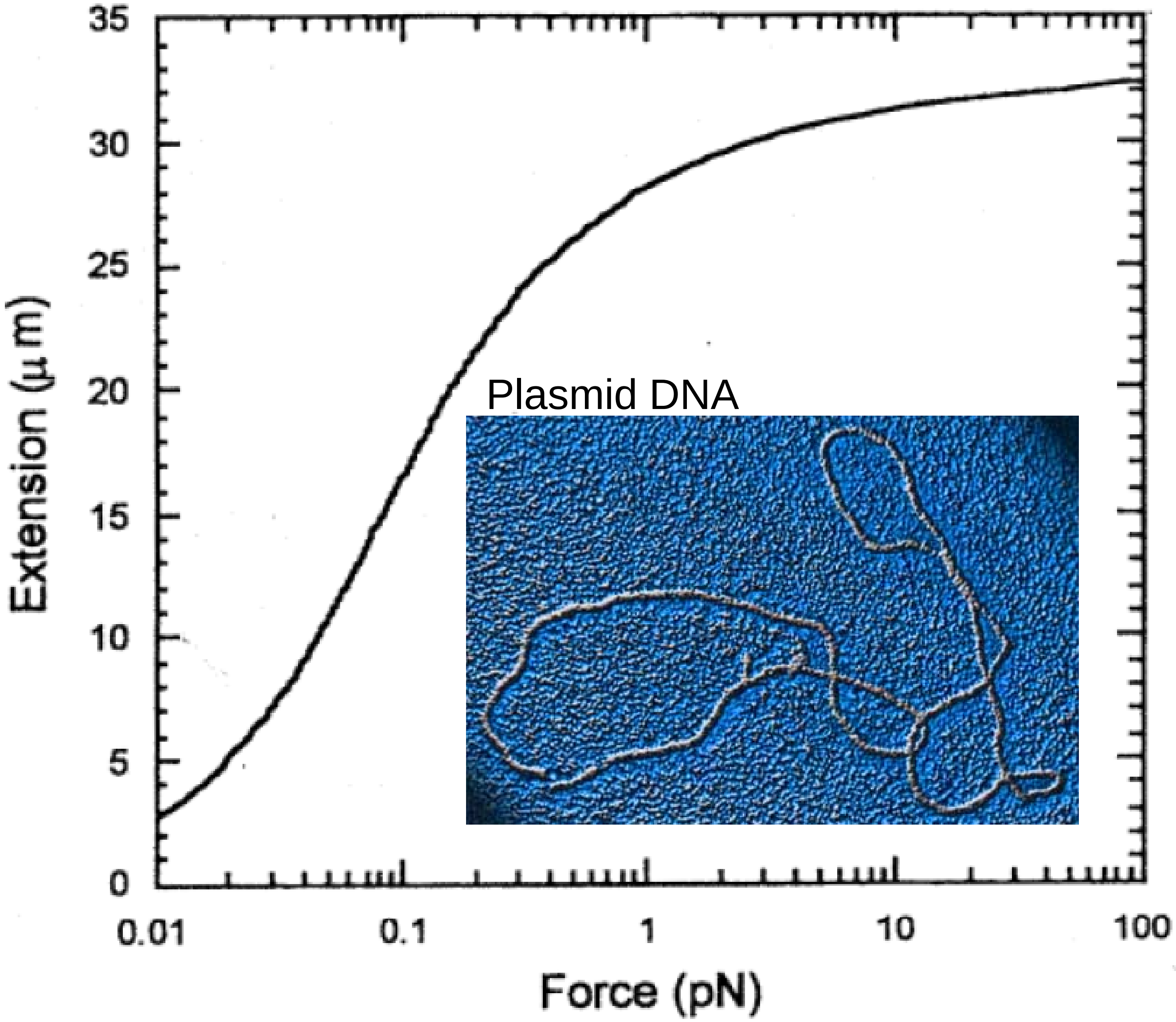


S-layer protein is a hexamer
(18 nm between hexamers)

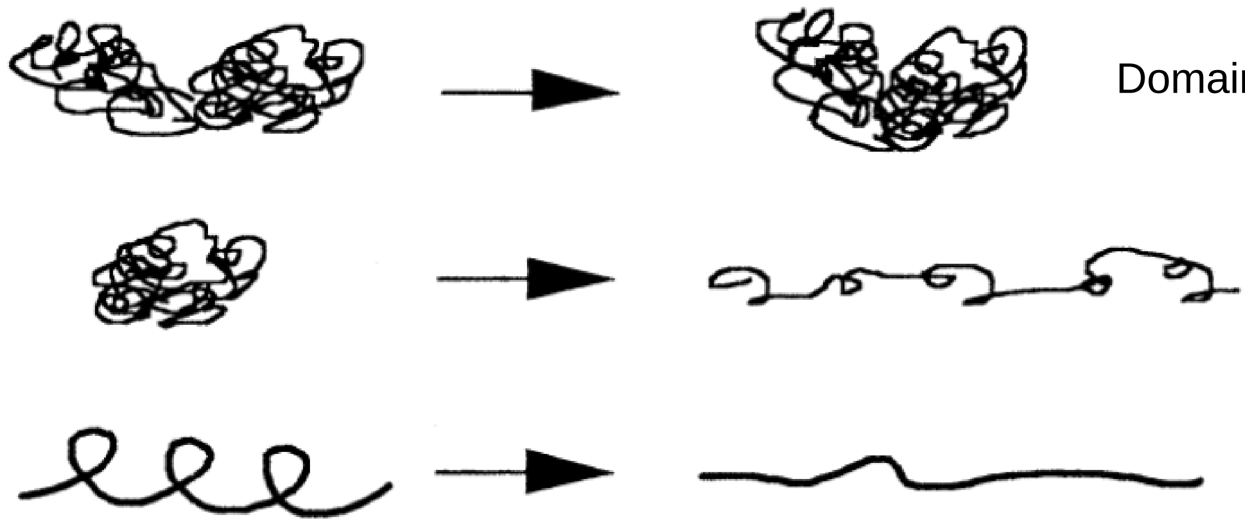
Five domains were unfolded

One domain is left

DNA stretching

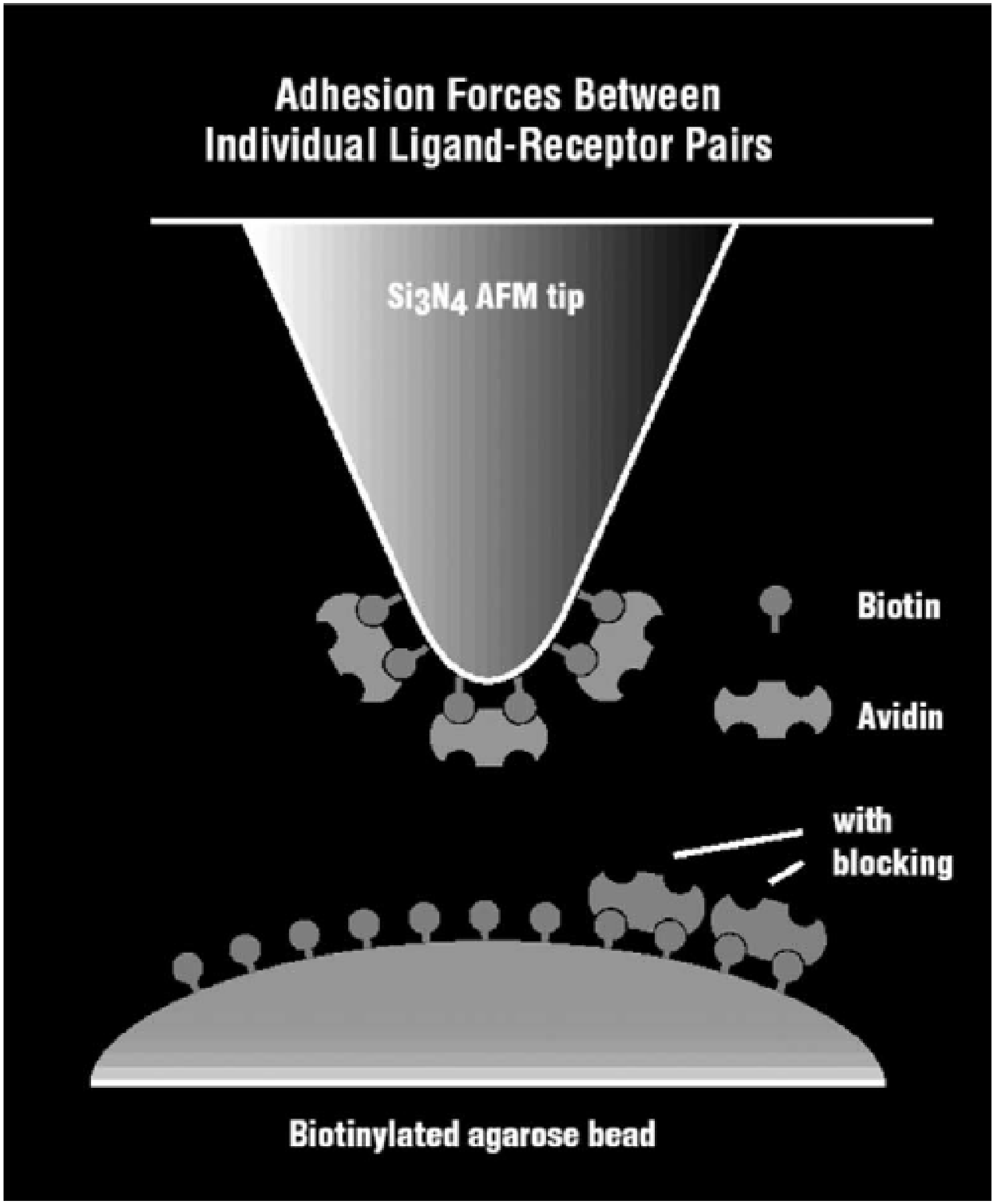


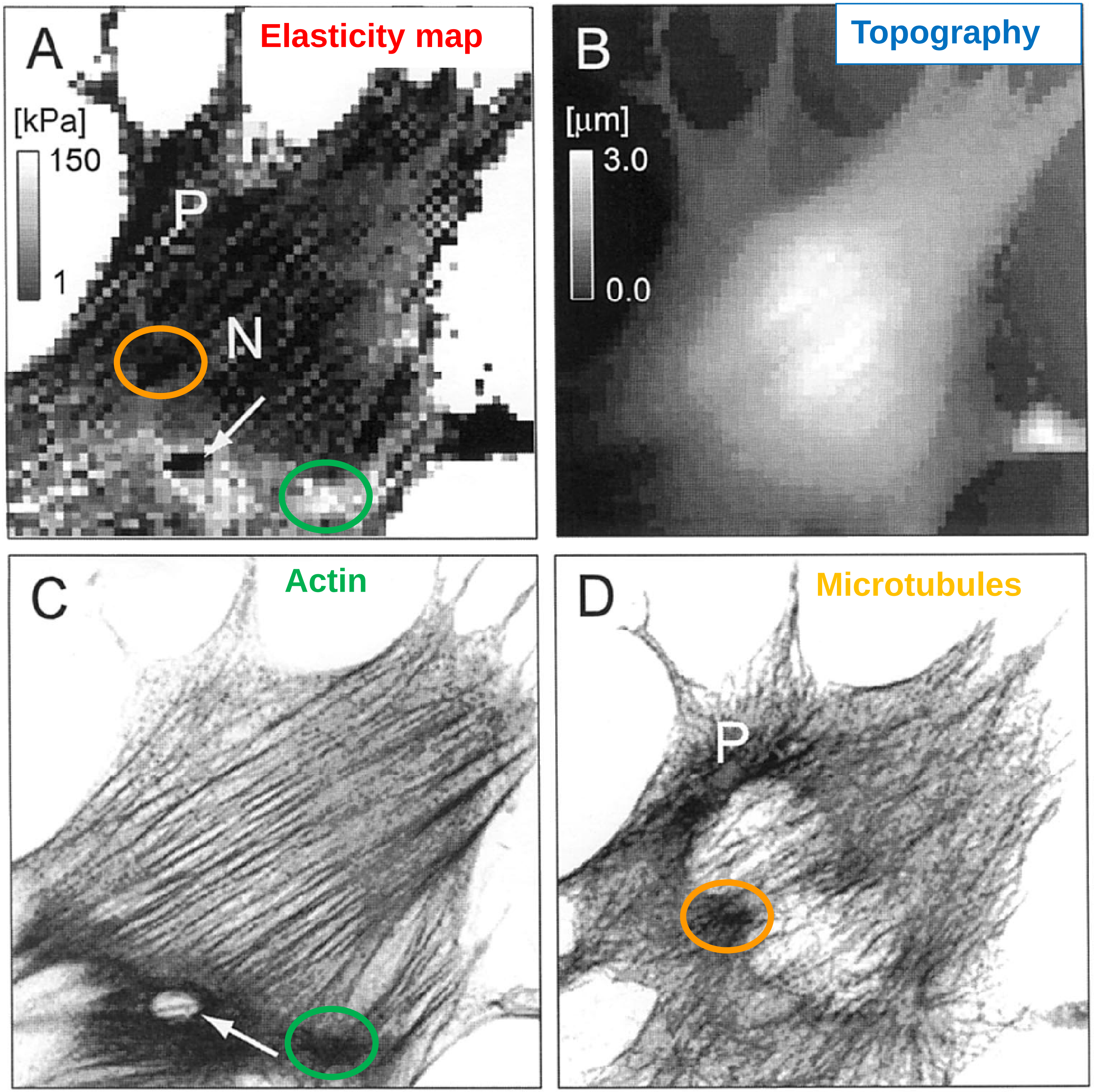
Modes of protein deformation and forces required for stretching



Size (nm)	Force (pN)	Bond energy (pN · nm)
α-helix (~1.7)	Twist DNA (~0.1)	van der Waals attraction (~0.7)
β-sheet (~2.0)	Stretch DNA (~5.0)	Hydrogen bond (~7.0)
Domains (~2–10)	Motor molecules (~5–15)	Ionic bond (~21)
Whole protein (~5–200)	Domain unfolding (~100)	Covalent bond (~630)

Zhu et al., 2000, Annu Rev Biomed Eng


Adhesion Forces Between
Individual Ligand-Receptor Pairs
Si3N4 AFM tip
Biotin
Avidin
with
blocking
Biotinylated agarose bead



Elasticity Map:

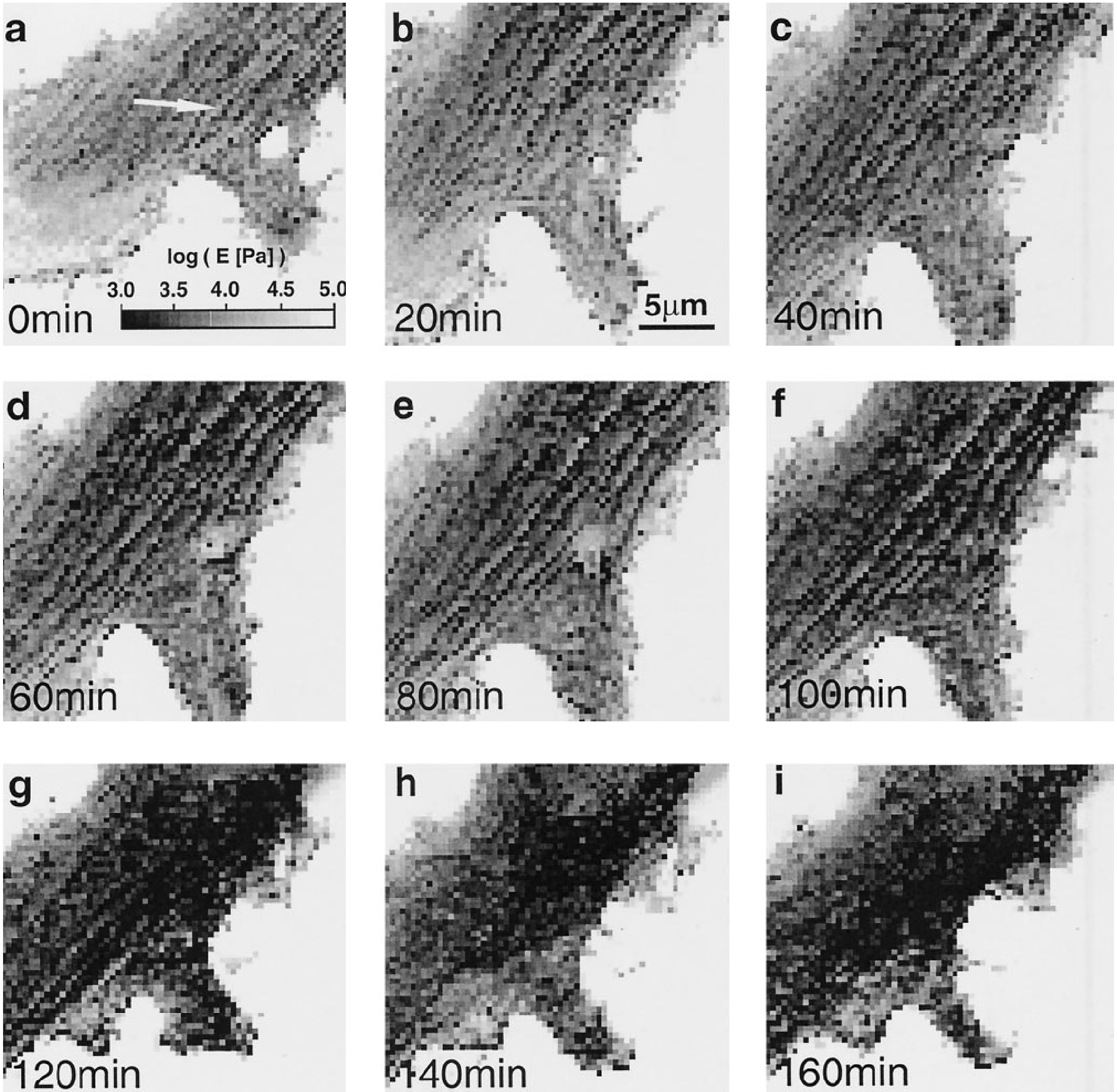
White = Stiff
Black = Soft

Nucleus surprisingly soft (arrow A and C)

Occurrences of dense F-actin surprisingly **stiff** (A, C)

Occurrences of dense microtubules surprisingly **soft** (A, D)

Living fibroblast



The drug “cyto-chalasin” cuts actin filaments => the cell becomes softer

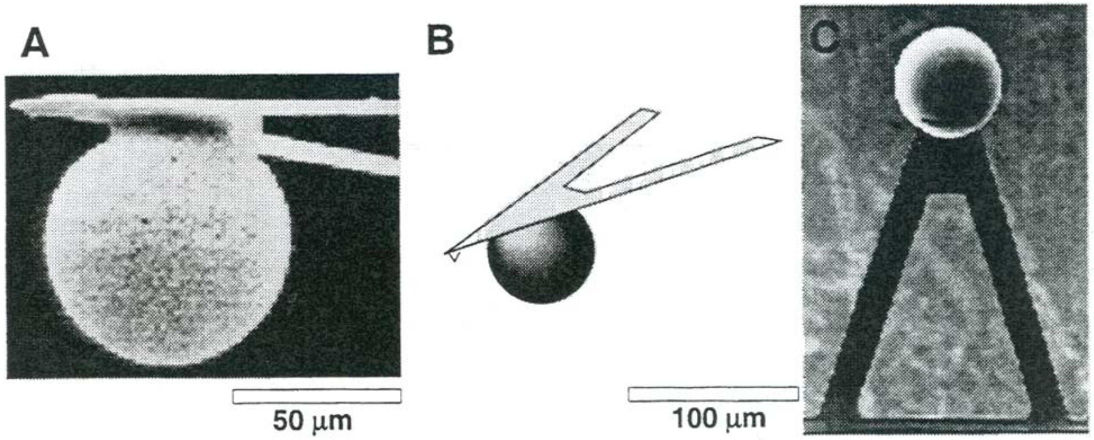
Cell type	Elastic modulus (kPa)	Method
Rat aortic smooth muscle	1.5–11	Elongation between plates
Endothelial	1.5–5.6	AFM
Aortic endothelial Normal/ cholesterol depleted	0.32/0.54	Microaspiration
Endothelial	0.5 cytoplasm 5 nucleus	Uniaxial compression
Inner hair cell	0.3	AFM
Outer hair cell	2–3.7	AFM
Cardiac myocytes	35–42	AFM
Fibroblast	0.6–1.6	AFM
Fibroblast	1–10 (differential stretch modulus)	Uniaxial stretching/compression
Bovine articular chondrocytes	1.1–8	Creep cytoindentation apparatus
Chondrocytes, Endothelial	0.5	Microaspiration
Neutrophils passive/activated	0.38/0.8	AFM
C2C12 myoblasts	2	Cell loading device (global compression)
Alveolar epithelial	0.1–0.2	Magnetic twisting cytometry
Epithelial normal/cancerous	10–13/0.4 – 1.4	AFM
Osteoblast	1–2	AFM
Fibroblasts Normal/transformed	0.22/0.19; 0.42–0.48/1.0	Optical stretcher
Melanoma	0.3–2.0 frequency dependent	Magnetic twisting rheometry
Kidney epithelial Cell cortex Cell interior	0.16 0.04	Magnetic twisting rheometry Tracer diffusion
3T3 fibroblast before/after shear flow	0.015/ 0.06	Tracer diffusion
C2-7 myogenic	0.66	Uniaxial stretching rheometer

Heart cells have more actin and stress fibers

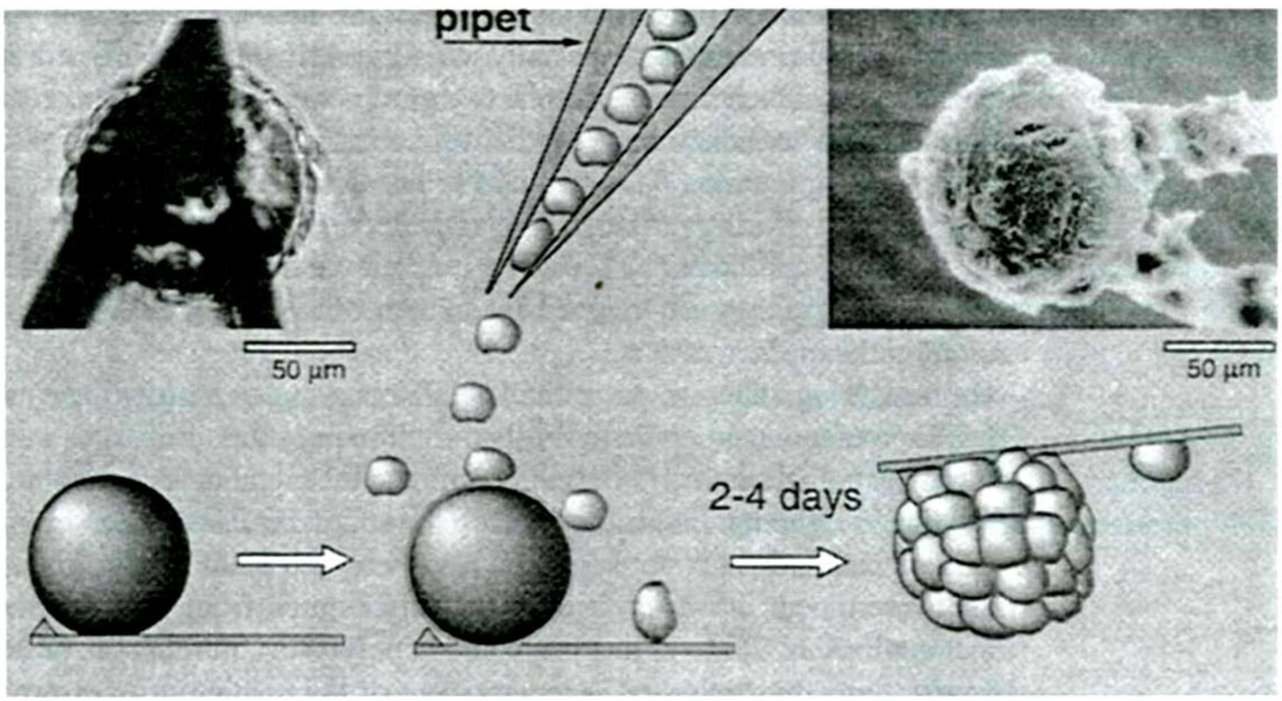
Cancer cells are less elastic

Janmey et al., 2007, Annu Rev Biomed Eng

Cell adhesion measured by force spectroscopy

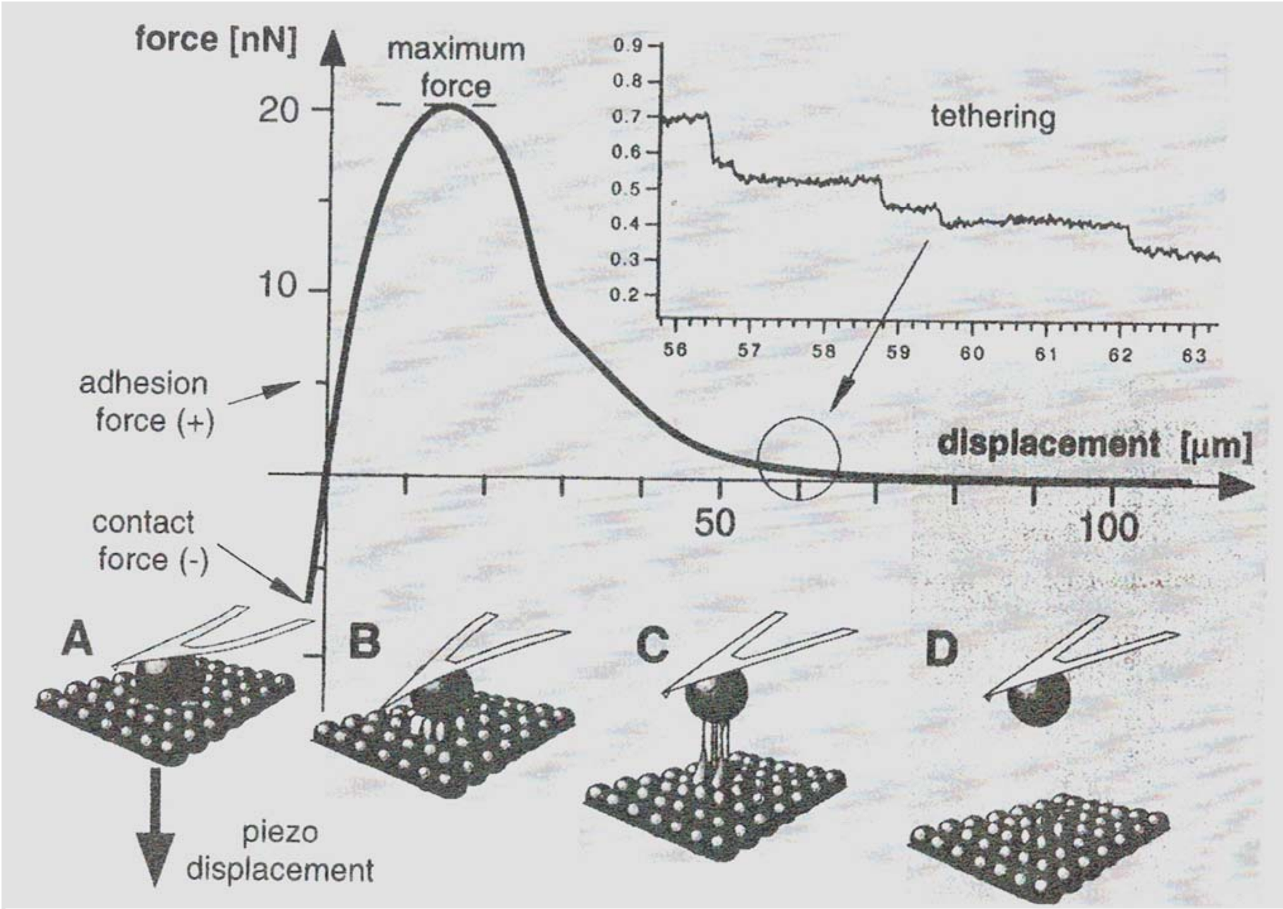


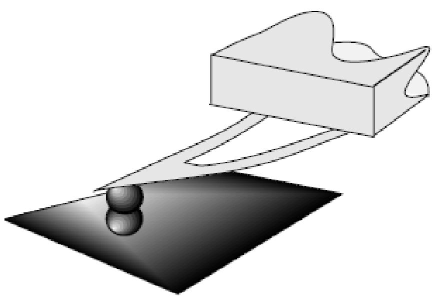
Attaching glass or plastic spheres (60 µm) to a cantilever



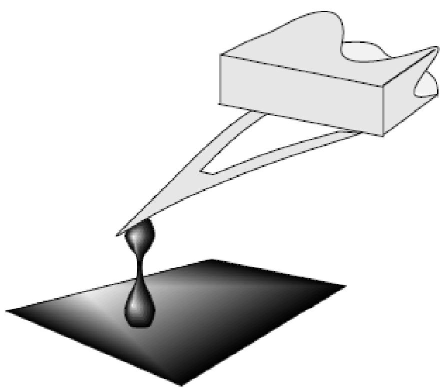
Growing cells on the spheres

Cell adhesion measured by force spectroscopy

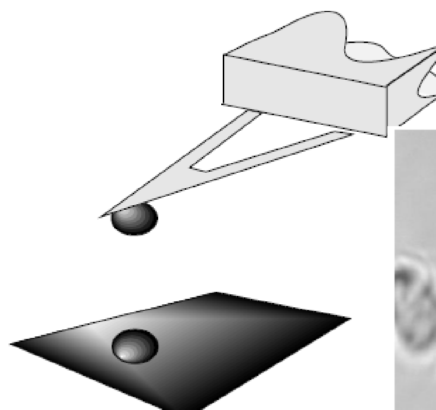




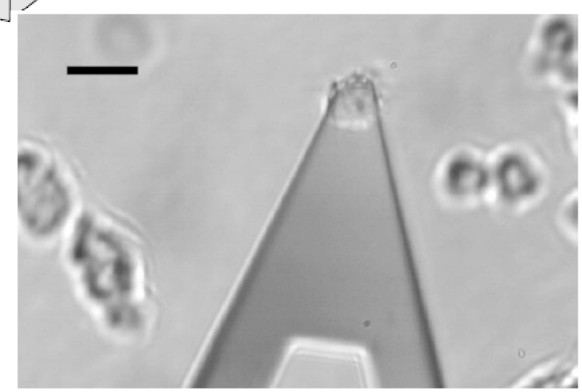
Contact formation



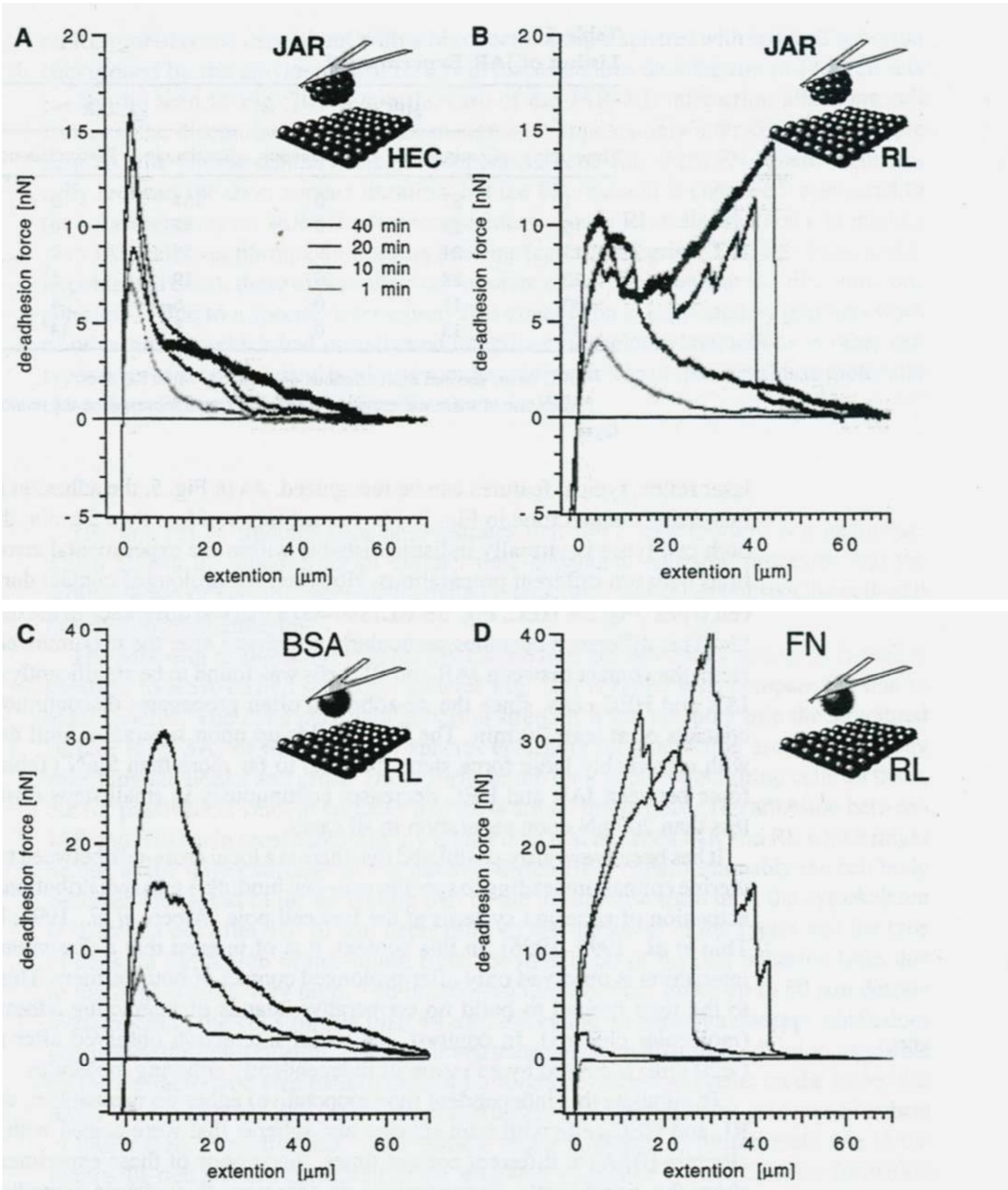
Adhesion



Bond rupture



Cell adhesion measured by force spectroscopy



Adhesion between three types of epithelial cells are measured:
JAR, RL and HEC cells.

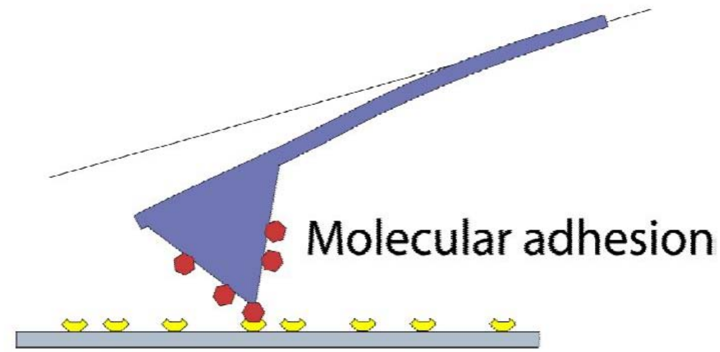
It is well known that JAR and HEC do not form cell-cell contacts, but JAR and RL.

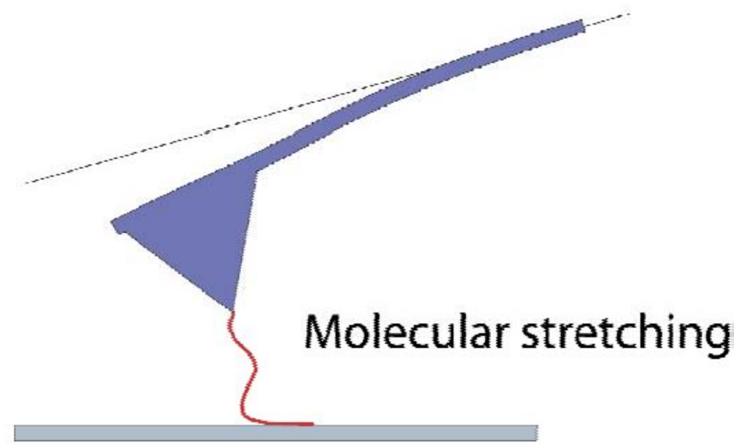
As a control, BSA and the extracellular matrix protein fibronectin (which connects cells to different kinds of matrices)

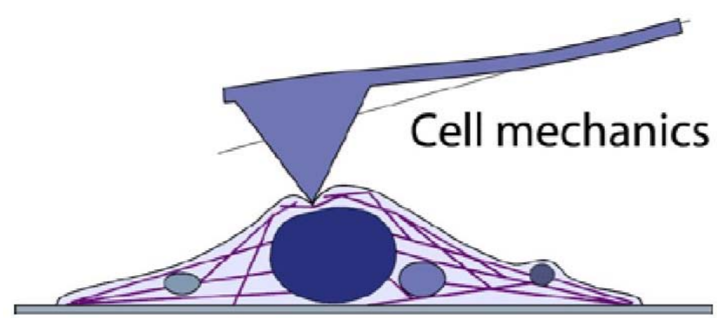
- JAR = placenta cancer cell line
- RL = human B lymphoma cells
- HEK = human embryonic kidney cell line

Summary biological applications

- ✓ Imaging of biomolecules in their native, physiological environment
- ✓ Study unfolding of proteins
- ✓ Force measurements on cells
- ✓ Antibody-antigen binding studies
- ✓ Ligand-receptor binding studies
- ✓ Binding forces of complimentary DNA strands
- ✓ Study surface frictional forces
- ✓ Ion channel localization
- ✓ … and more

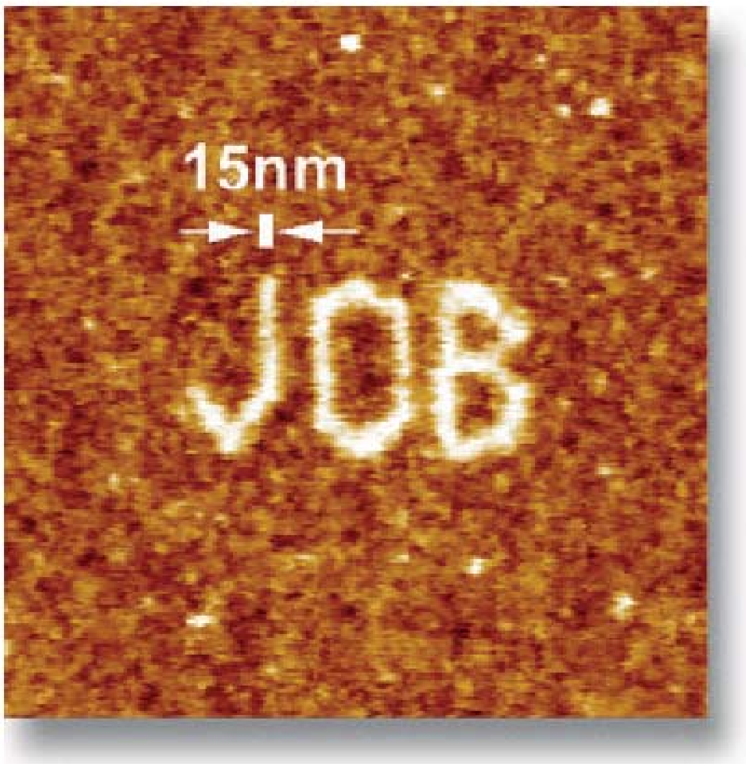


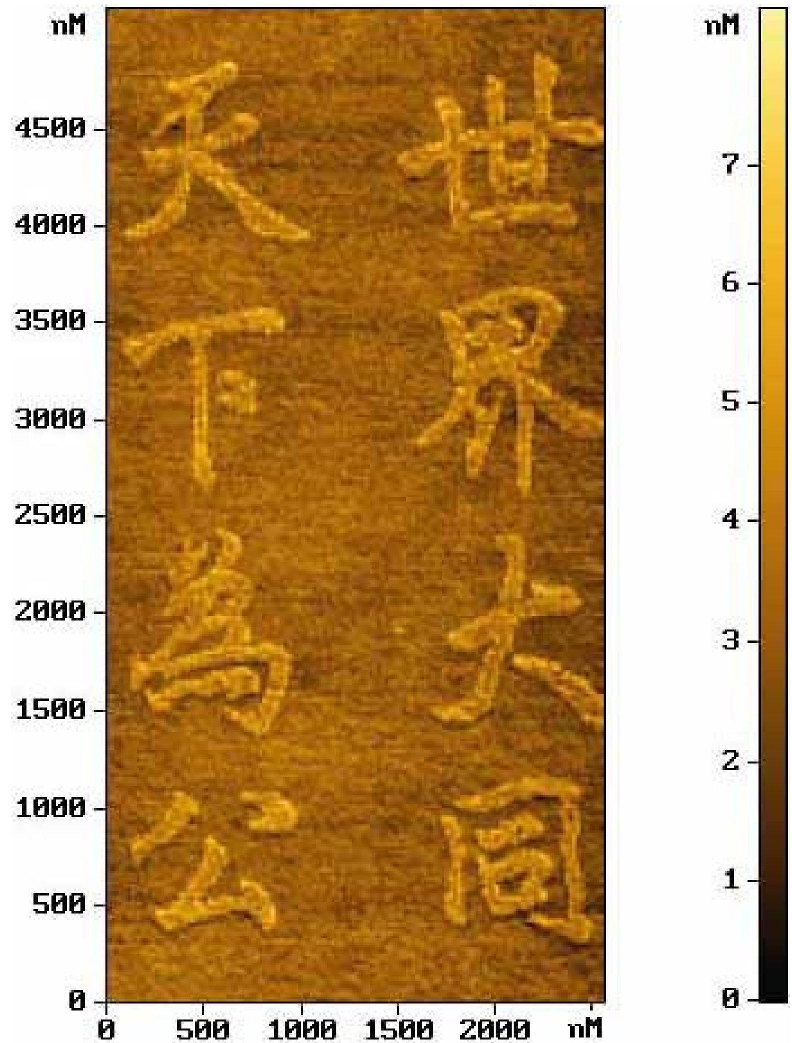




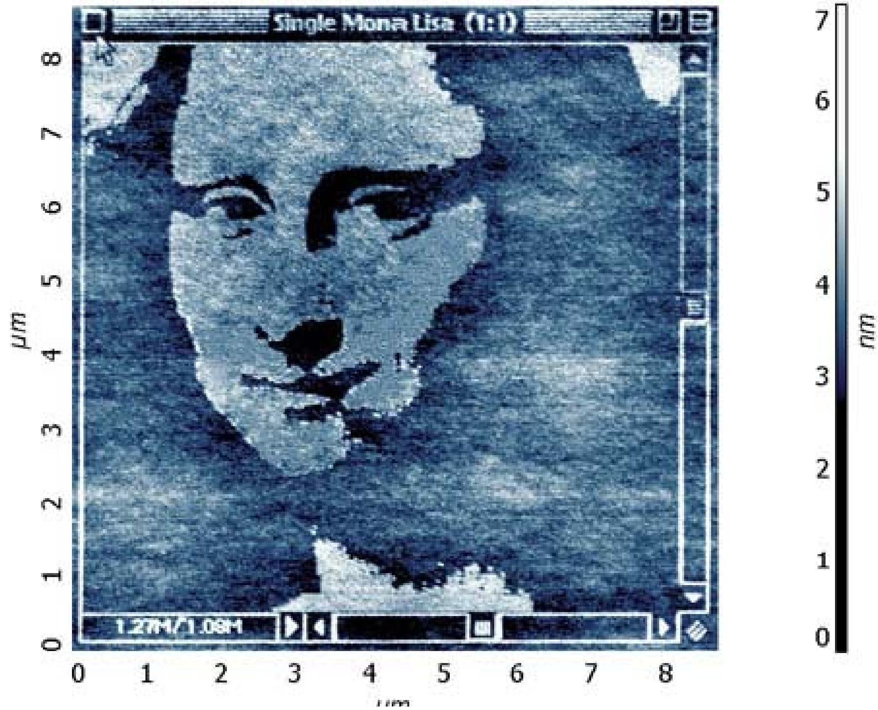
Nanolithography

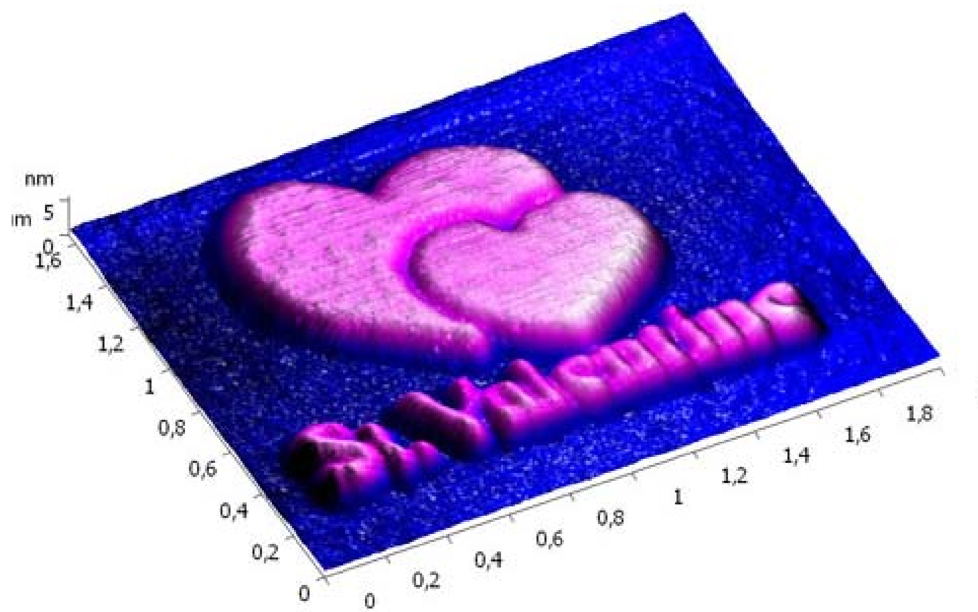
Draw a nanometer-scale pattern on a sample using a SPM tip by applying excessive force to a surface (or local electric fields to oxidize the surface)



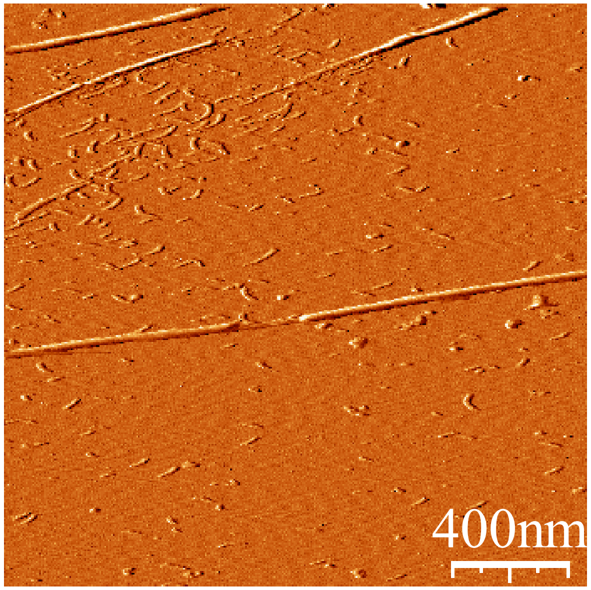


Nanolithography

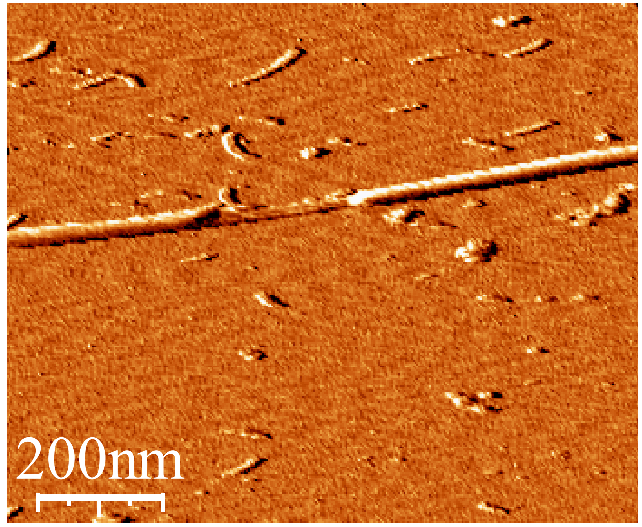




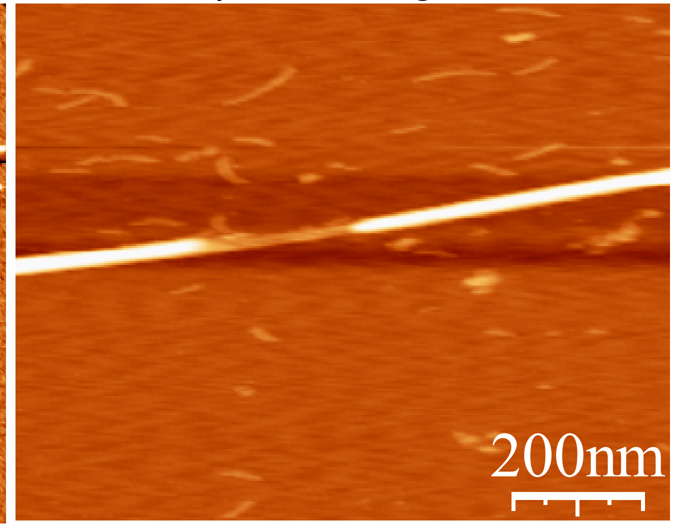
Nano-dissection



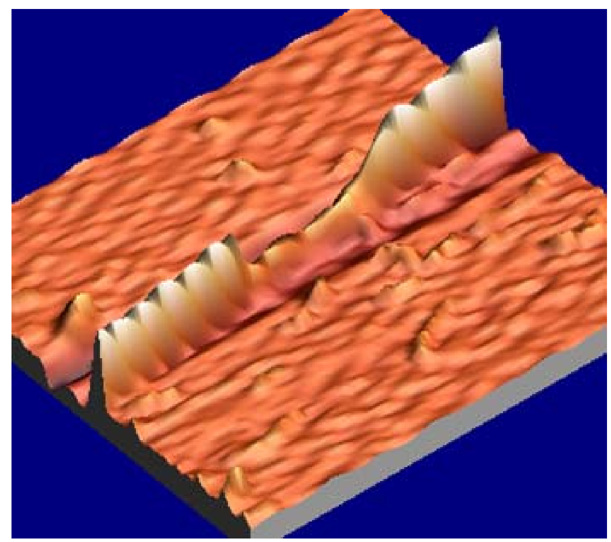
amplitude image



phase image

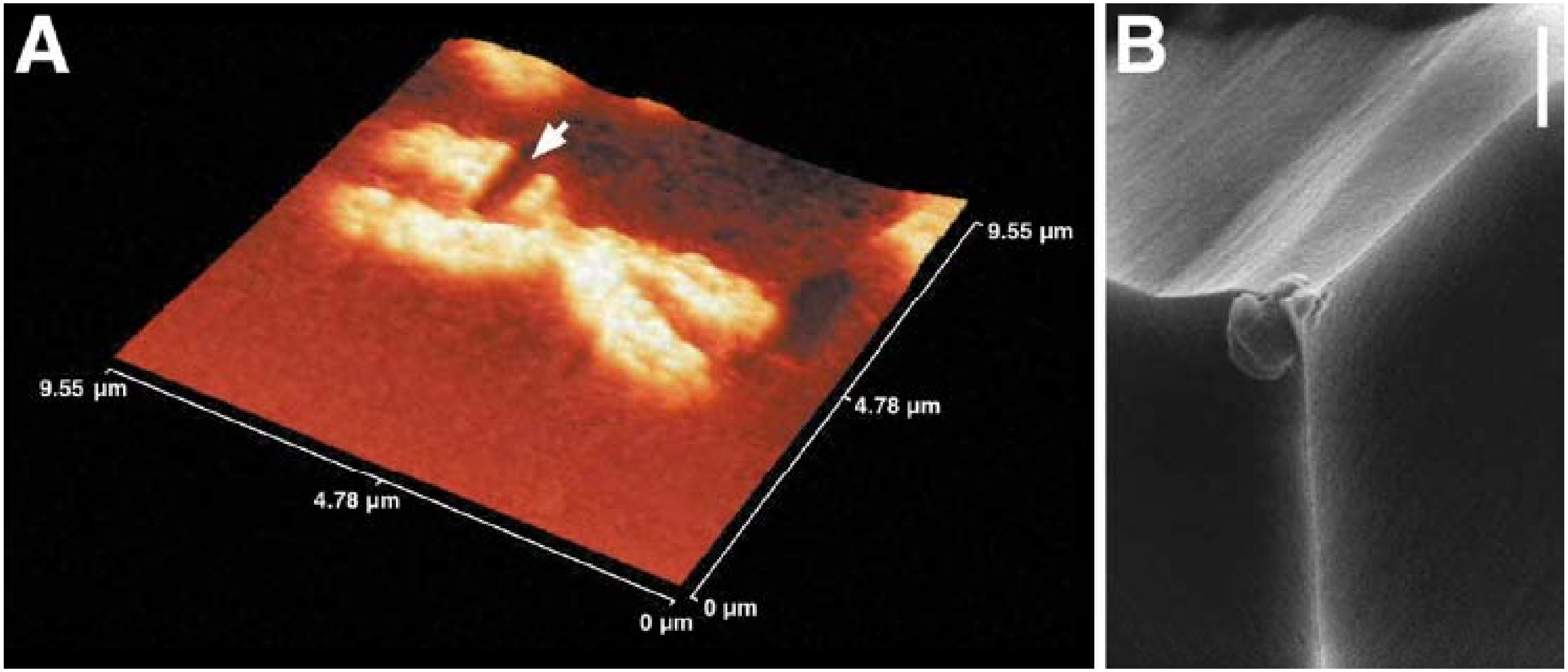


Single microtubule in buffer dissected by AFM tip



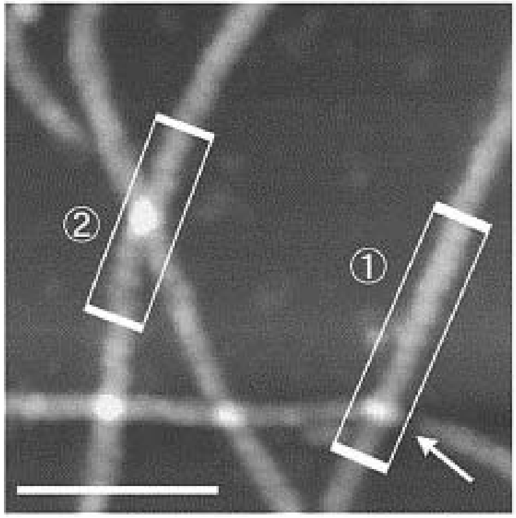
3 D image

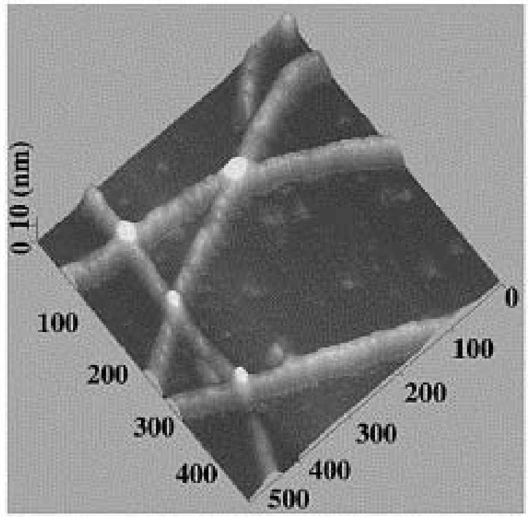
Nano-dissection



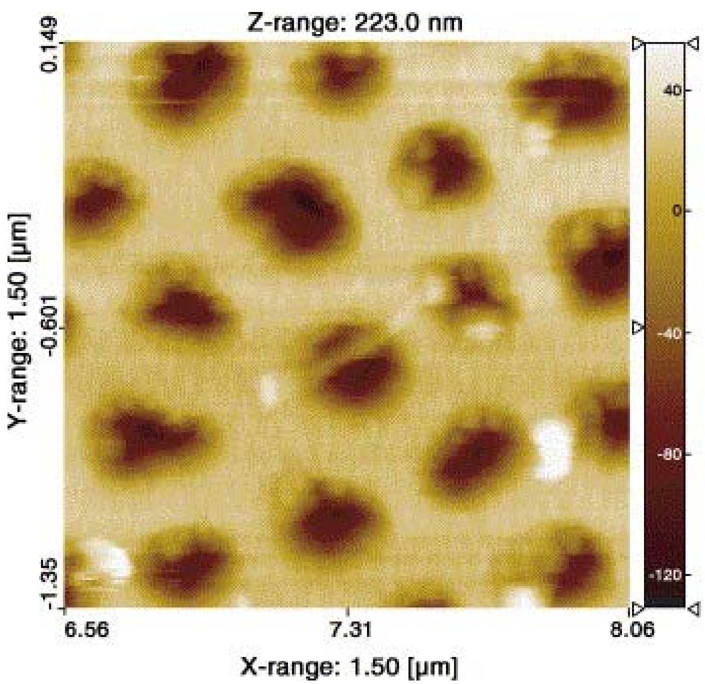
- DNA extraction from a human Chromosome
- SEM image of the tip show the piece of DNA

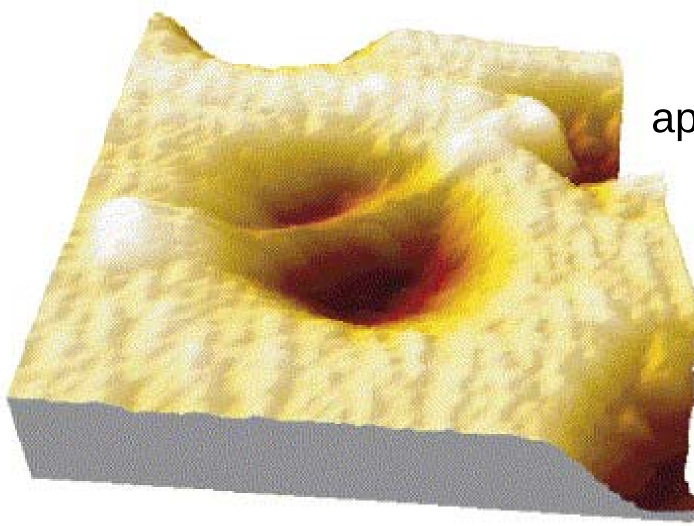
Nano-indentation





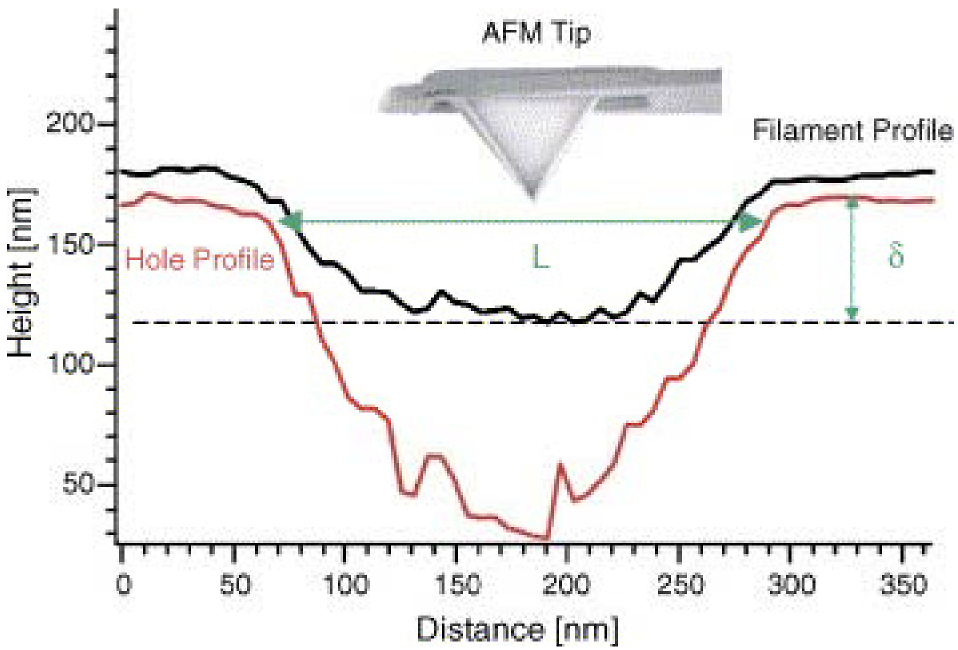
- Measuring bending properties of intermediate filaments
- Tip elastically deforms single filaments hanging over a porous membrane

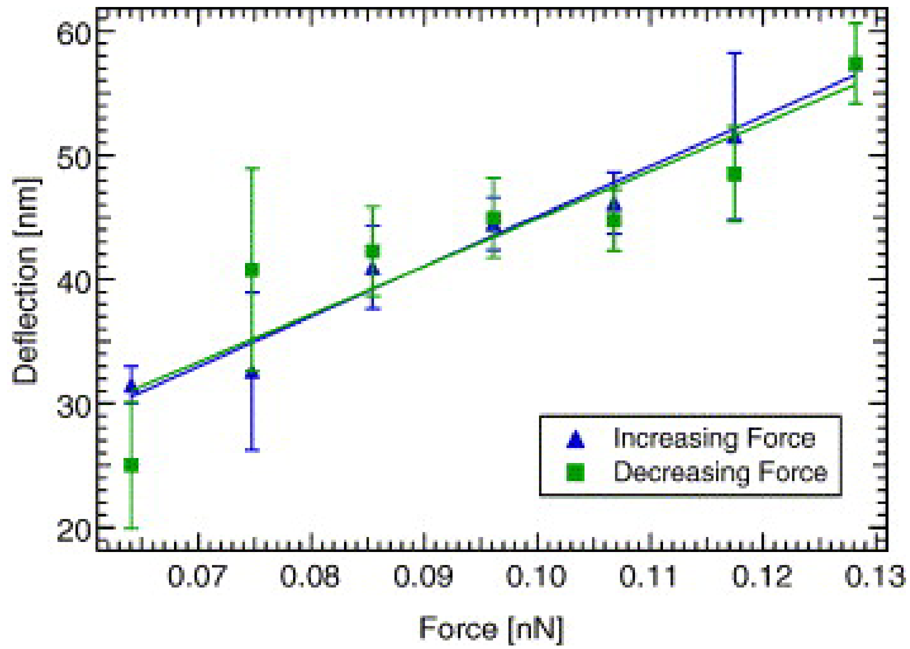




applied force 0.11 nN

Nano-indentation

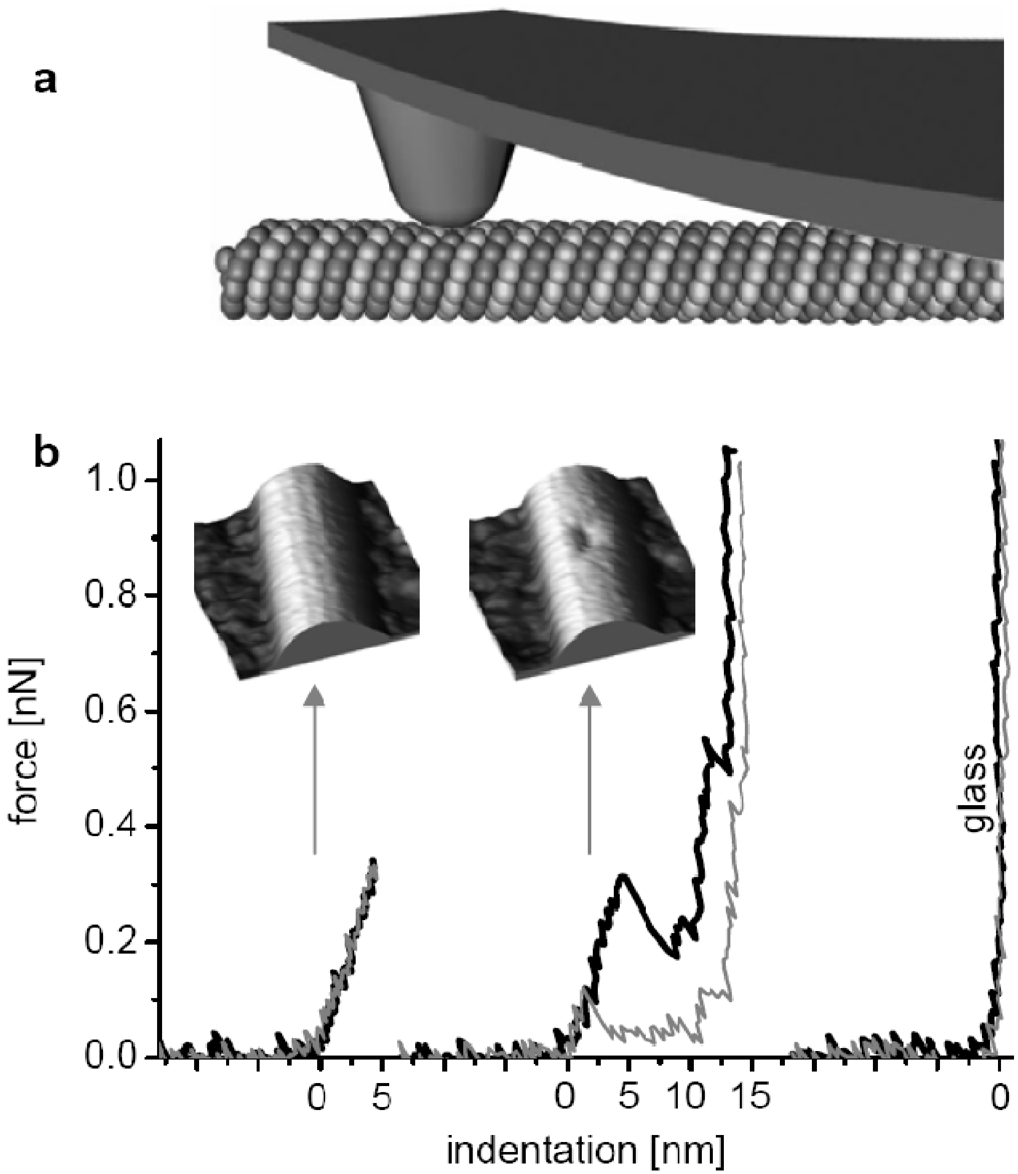




- AFM tip pushes the IF into the hole
- From the height difference between the IF's lowest point (L) and the substrate around the hole, the **deflection** can be **calculated**

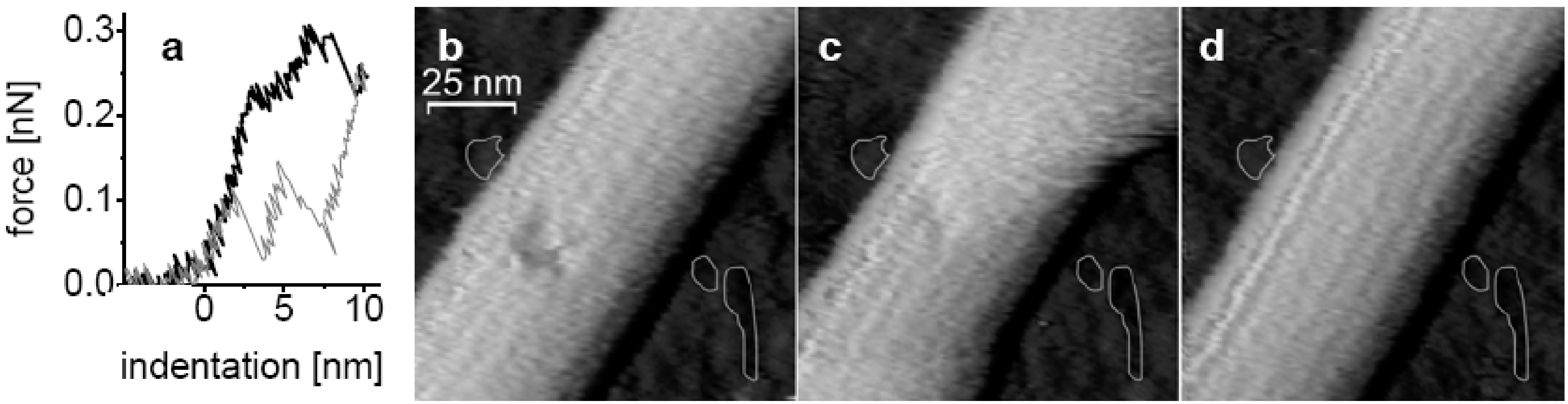
- **Deflection** of one IF as a function of the applied **force**
- $E_{Bending}$ = 300 MPa determined from the slope of the linear fit
- Graph shows that the **filament is elastic** (i.e. it returns to its original position after the force is decreased)

Nano-indentation

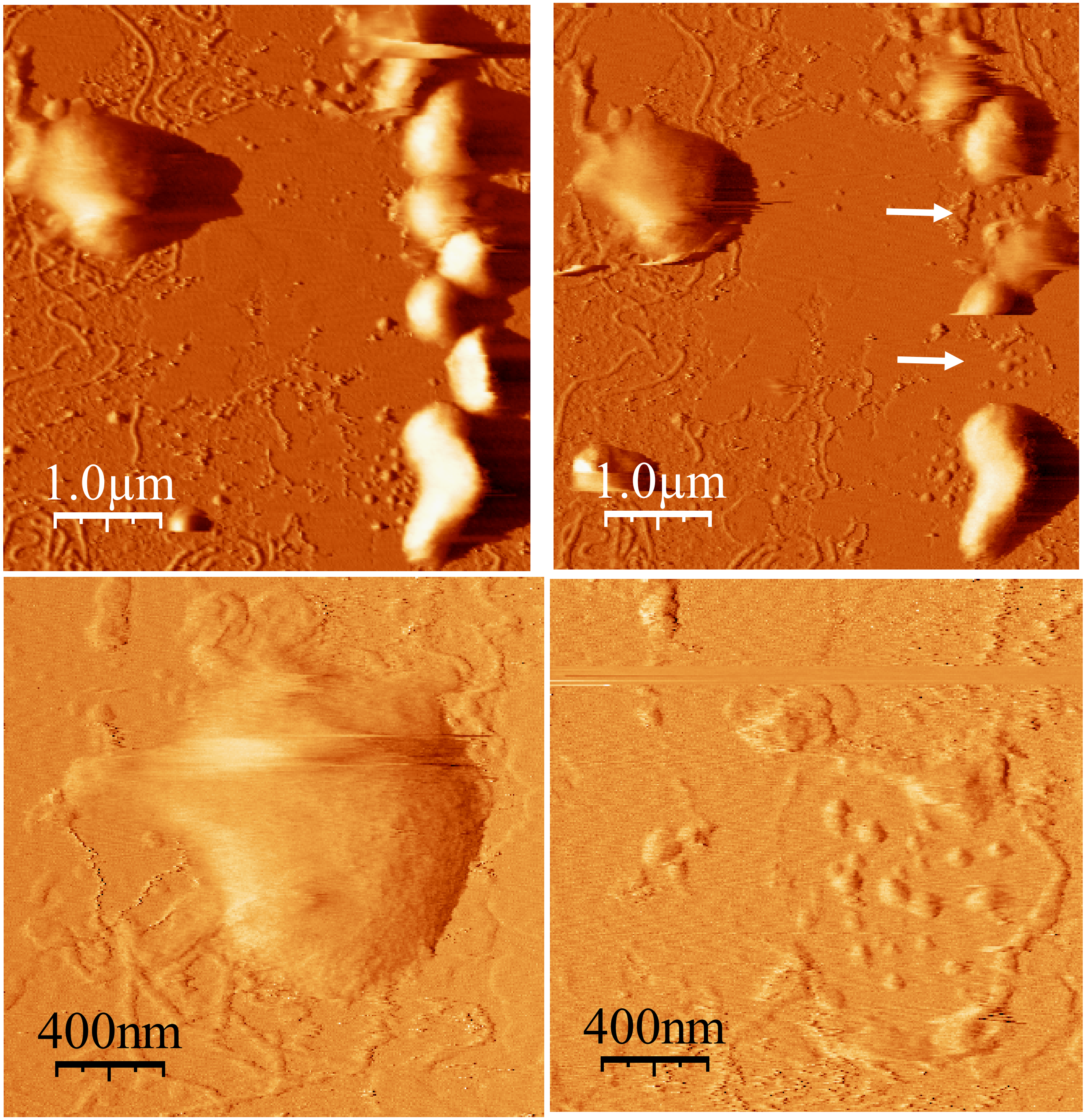


- Measuring **mechanical properties of single microtubules** by lateral indentation with the AFM
- Indentations up to ~ 3.6 nm resulted in an **linear elastic response**, and indentations were reversible
- Higher forces caused substantial damage to the microtubules, which either led to depolymerization or, occasionally, to slowly reannealing holes in the microtubule wall

Nano-manipulation (nano-indentation)

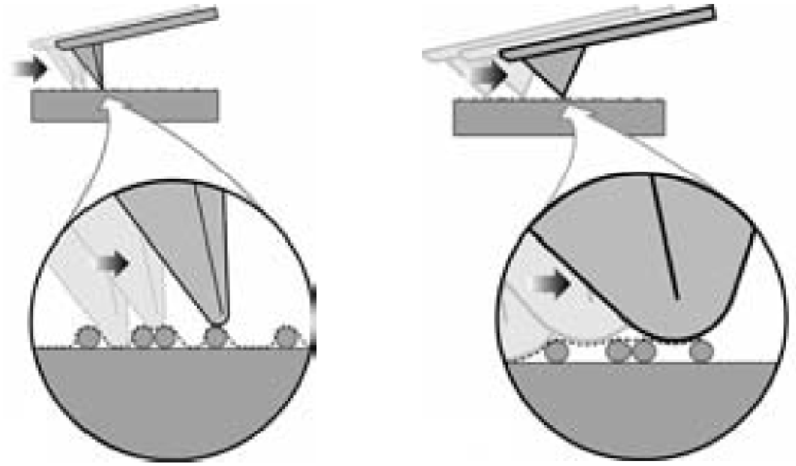


"Self-healing" of microtubules: Higher forces by the tip can cause microtubule damage; however, these holes can undergo a slow reannealing process

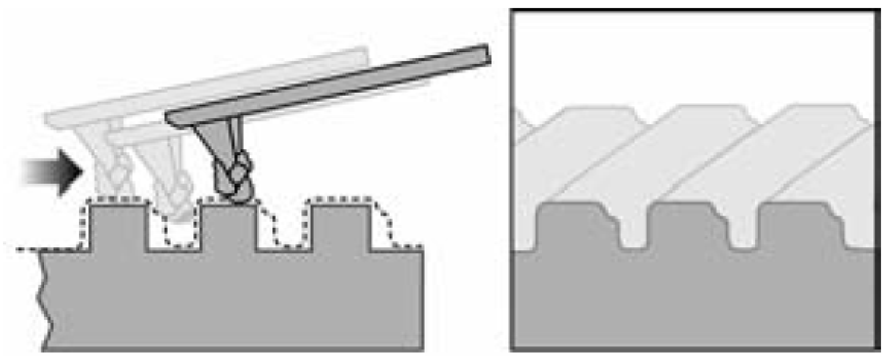


Nano-Manipulation

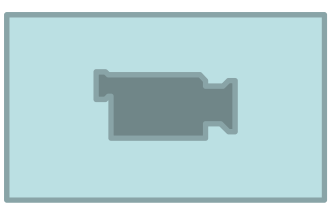
AFM troubles: tip problems



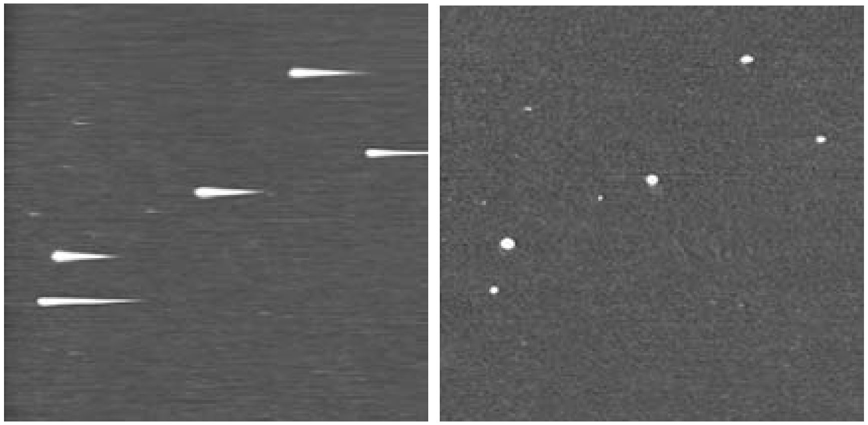
- Left: spheres scanned with a **sharp tip**
- Right: spheres scanned with a **dull tip**



Accumulation of debris on the end of the tip can also dull the tip and result in image distortion

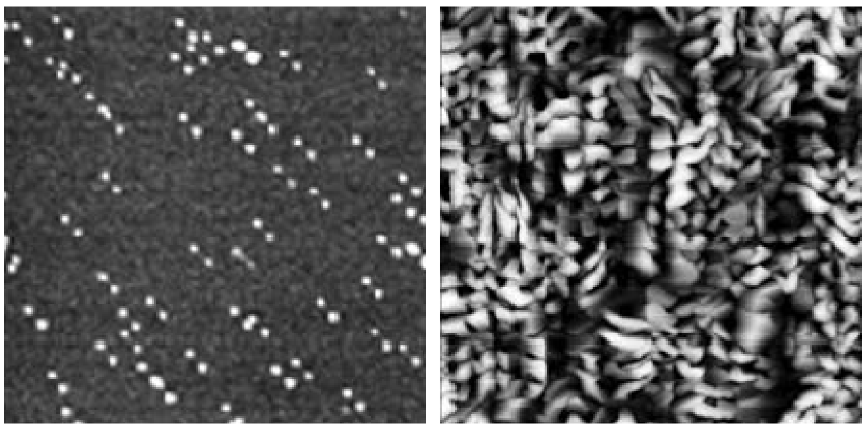


AFM troubles: image artifacts

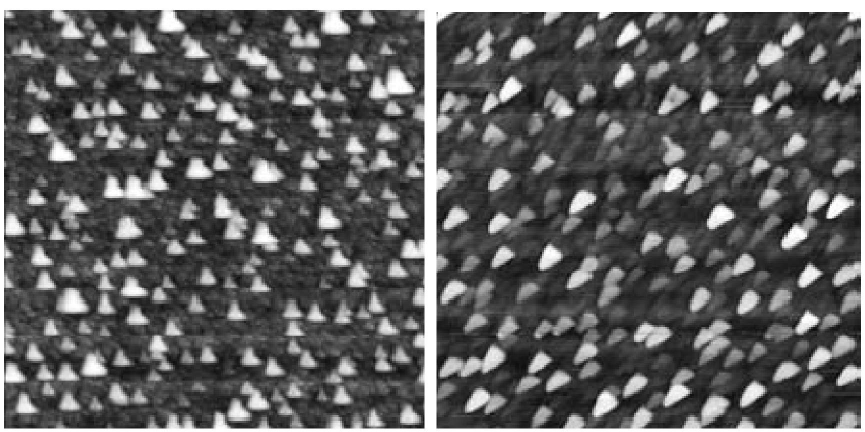


• Left: scanning parameters are not adjusted properly => the tip does not trace down the back-side of the image

• Right: scanner voltage, gains and scan rate was properly adjusted

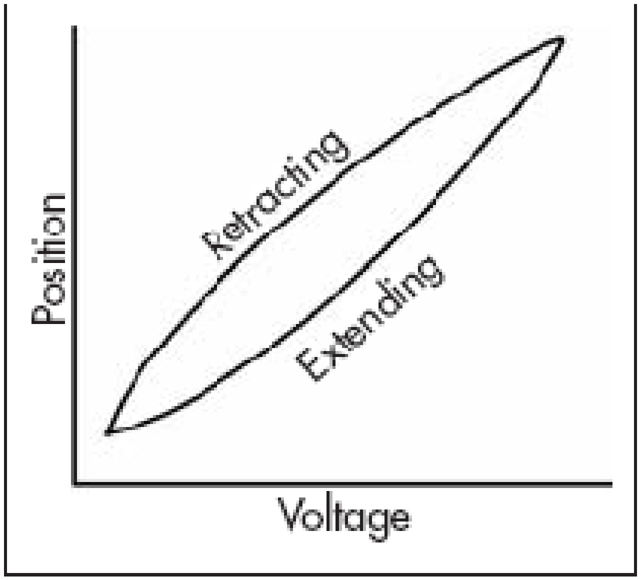


Double tips: due to production errors a tip might have double end points which are in contact with the sample while imaging

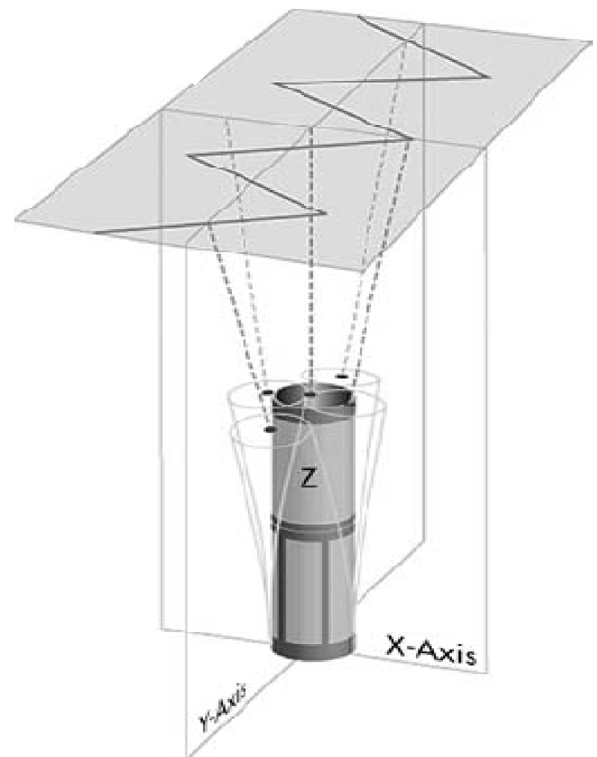


Worn out tip or tip with attached debris: Features in the image have all the same Shape what is really being imaged is the debris, not the morphology of the surface

AFM troubles: scanner hysteresis

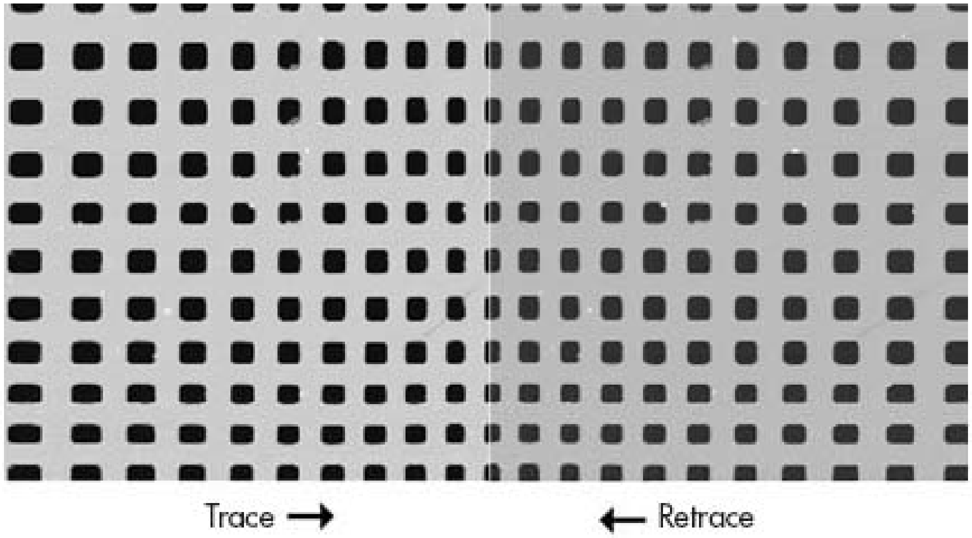


• Because of differences in the material properties and dimensions of each piezoelectric element, **each scanner responds differently** to an applied voltage
=> sensitivity of each piezo differs
• Sensitivity is non-linear: the piezo is more sensitive at the end of a scan line than at the beginning



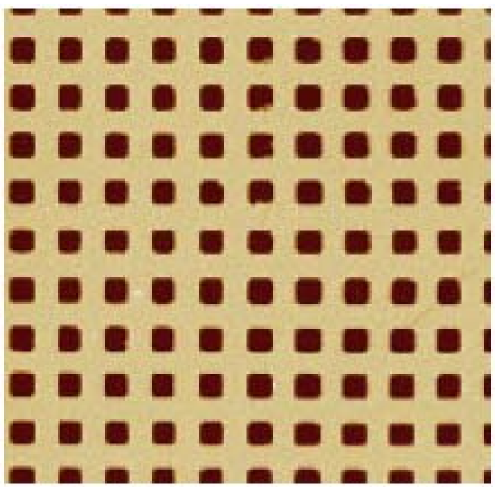
AFM troubles: scanner hysteresis

100 μm x100 μm / each square is 10 μm with a depth of 200 nm



Without proper calibration, hysteresis can cause distortion of the image

Hysteresis can also occur during aging of the scanner



Calibration grid after scanner calibration routine

Thank you for your attention.
Bye bye…