AFM - Atomic Force Microscopy

basics and applications

Dan COJOC

cojoc@iom.cnr.it

Consiglio Nazionale delle Ricerche
Istituto Officina dei Materiali - Materials Foundry
www.iom.cnr.it

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Outline

1. Why Atomic Force Microscopy ?

2. AFM – working principle

3. Force vs tip-substrate distance

4. AFM - operation modes:
   - imaging or force spectroscopy
   - static and dynamic

5. Examples of AFM imaging in biology / life sciences

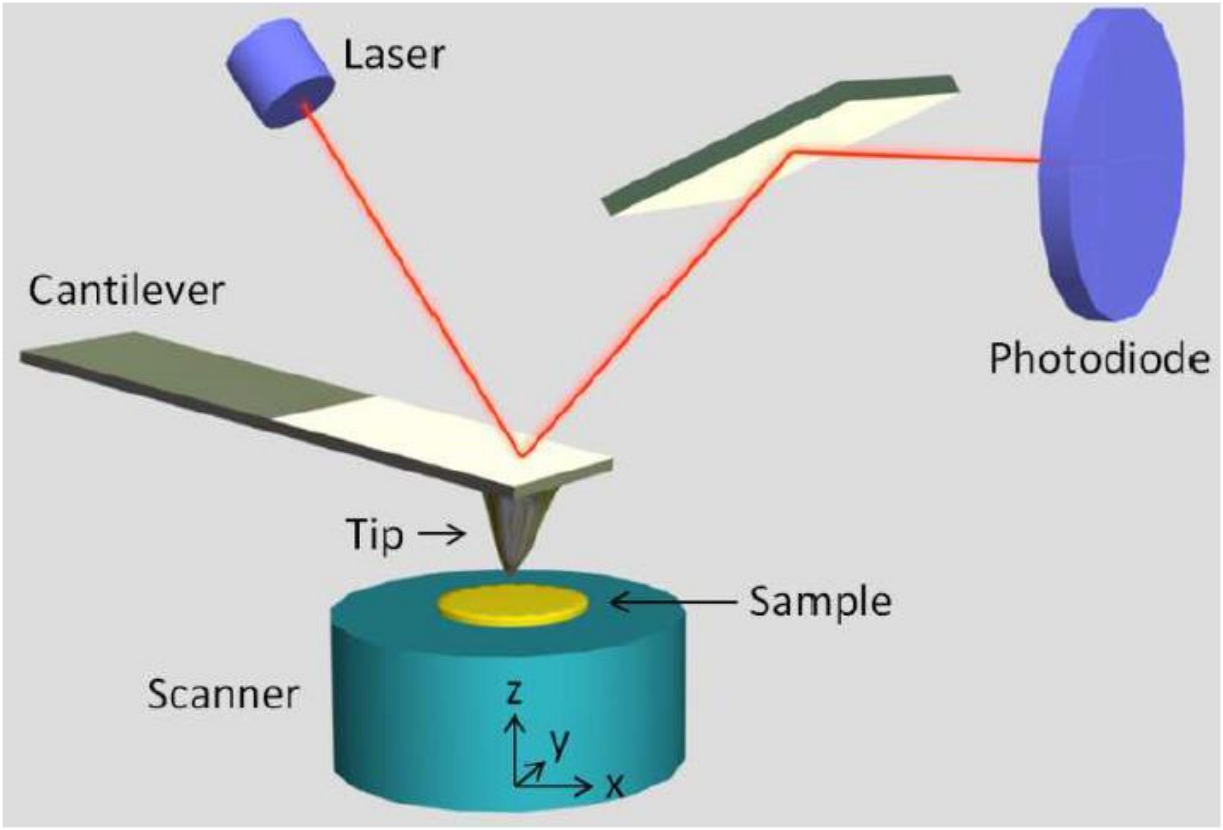
Acknowledgments:

Loredana Casalis (Elettra) and Marco Lazzarino (CNR-IOM)
for slides used from their lectures in the past years

Why Atomic Force Microscopy (AFM)?

- Atomic force microscopy (AFM) is a powerful technique that enables the imaging with nanometer resolution of almost any type of surface, including polymers, ceramics, composites, glass and biological samples.
- AFM is used to measure and localize many different forces, including adhesion strength, magnetic forces and mechanical properties.
- The newest generation of bio-AFMs combine ease of use and integration with live-cell epifluorescence or more advanced optical microscopies.
- AFM is becoming a prevalent tool in cell biology and biomedical studies, especially those focusing on the mechanical properties of cells and tissues.
- High-resolution AFM has been applied to image bacterial membrane proteins, study topological details of DNA/RNA – enzymes interaction
- AFM allows also to measure interaction forces in biological systems with molecular resolution

Atomic Force Microscope (AFM)



The AFM consists of a sharp tip which is attached to a cantilever.

The tip moves in response to tip–surface interactions, and this movement is measured by focusing a laser beam with a photodiode.

AFM – working principle (how the image is obtained)

The Atomic Force Microscopy (AFM) is a Scanning Probe Microscopy (SPM), introduced by Binning, Quate and Gerber in 1986 as a combination of the principles of Scanning Tunneling Microscopy (STM) and the Stylus Profilometer (SP)

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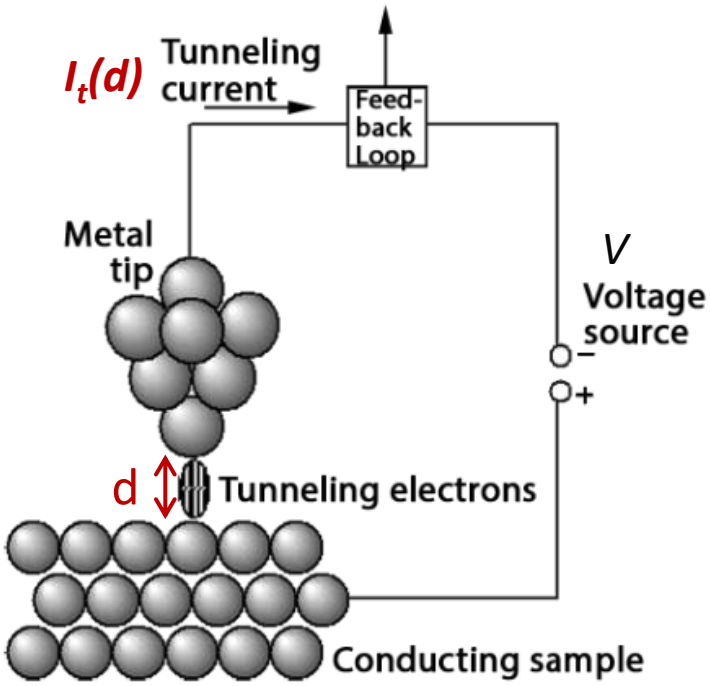
Atomic Force Microscope

G. Binnig[a] and C. F. Quate[b]
Edward L. Ginzton Laboratory, Stanford University, Stanford, California 94305
and
Ch. Gerber[c]
IBM San Jose Research Laboratory, San Jose, California 95193
(Received 5 December 1985)

The scanning tunneling microscope is proposed as a method to measure forces as small as 10^{-18} N. As one application for this concept, we introduce a new type of microscope capable of investigating surfaces of insulators on an atomic scale. The atomic force microscope is a combination of the principles of the scanning tunneling microscope and the stylus profilometer. It incorporates a probe that does not damage the surface. Our preliminary results *in air* demonstrate a lateral resolution of 30 Å and a vertical resolution less than 1 Å.

The Nobel Prize in Physics 1986 was awarded one half to Ernst Ruska "for his fundamental work in electron optics, and for the design of the first electron microscope",
the other half jointly to Gerd Binnig and Heinrich Rohrer "for their design of the scanning tunneling microscope"

Scanning Transmission Microscopy (STM), how it works ?



STM consists of a very sharp metallic tip/probe that is moved very close (<nm) to the sample surface (conductive), inducing the interaction between the superficial atoms of the metallic tip and of the conducting sample.

The electrons move from sample to tip generating the (tunneling) current ***$I_t(d)$***, which decays exponentially with the distance ***d*** between tip and sample:

$$I_t(d) \propto I_0 e^{-d/z_0}$$

z_0 – constant

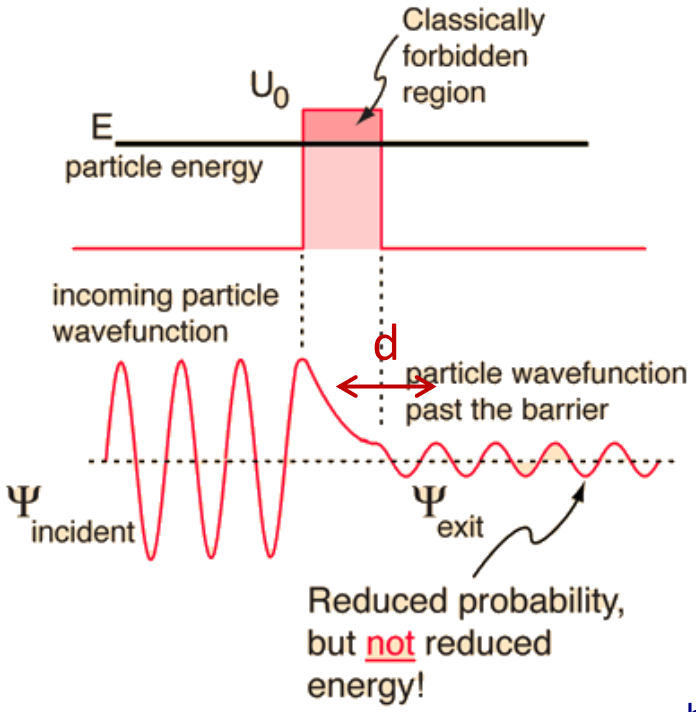
STM is very sensitive with distance

e.g. if z_0=1 nm, for d= 1 nm, ***$I_t(1) = I_0/e$*** e= 2.71828

STM is based on the quantum tunneling.

Quantum tunneling or tunneling refers to the quantum mechanical phenomenon where a particle tunnels through a barrier that it classically could not surmount.

Quantum Tunneling

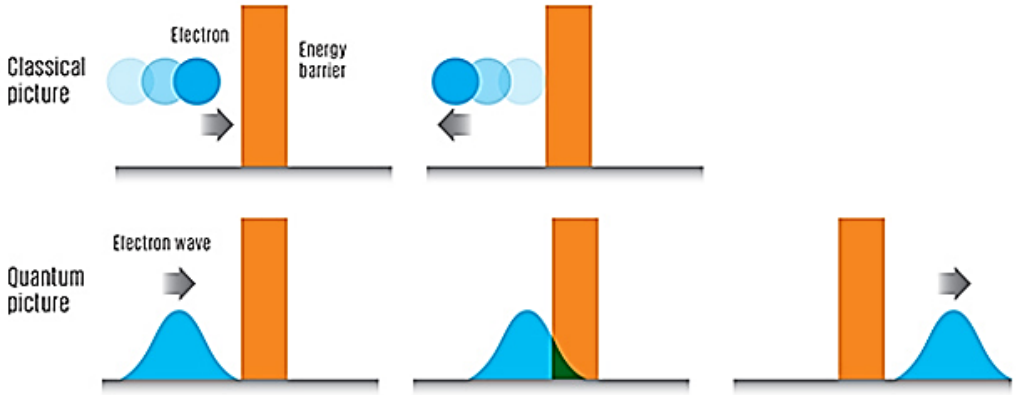


According to classical physics, a particle of energy E less than the height U_0 of a barrier could not penetrate - the region inside the barrier is classically forbidden.

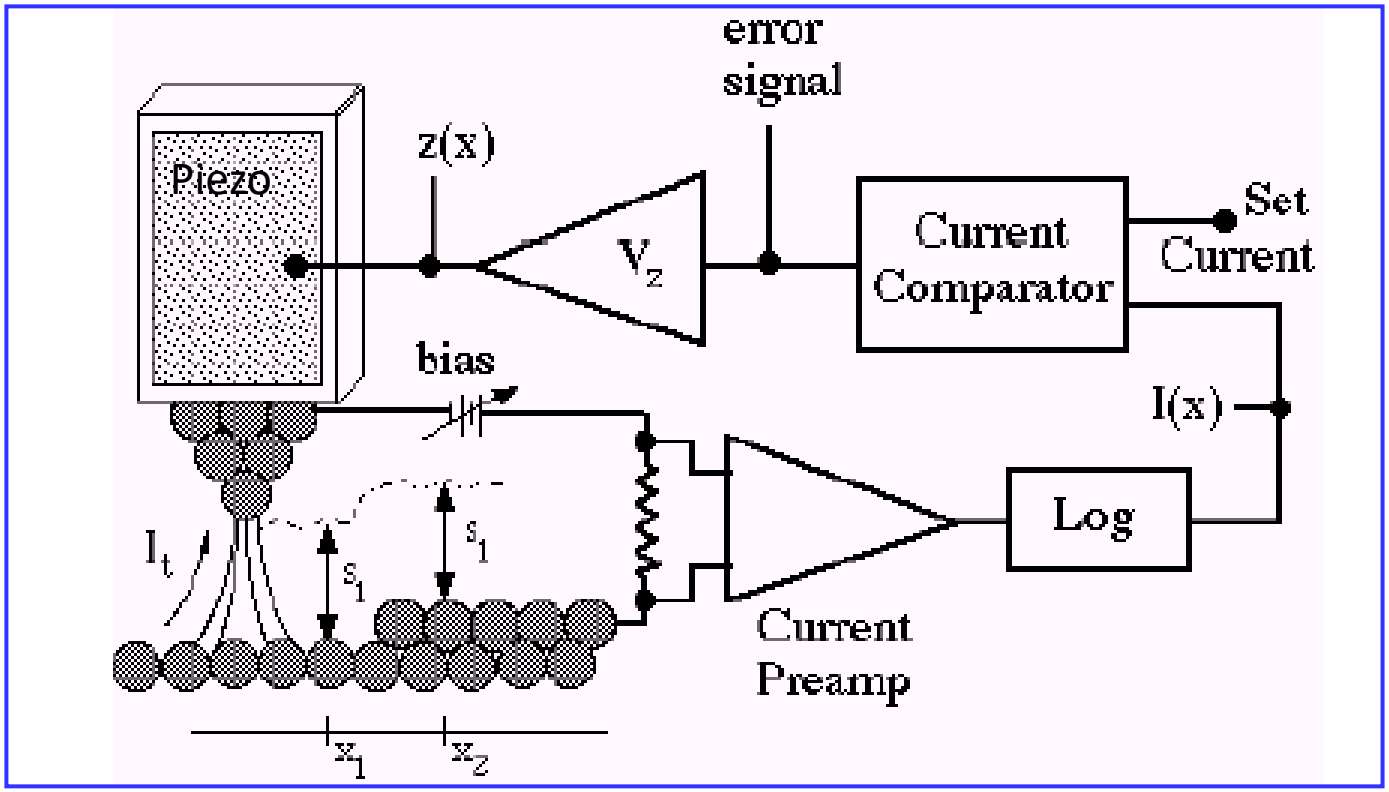
But the wavefunction associated with a free particle must be continuous at the barrier and will show an exponential decay inside the barrier.

The wavefunction must also be continuous on the far side of the barrier, so there is a finite probability that the particle will tunnel through the barrier.

http://hyperphysics.phy-astr.gsu.edu/hbase/quantum/barr.html



Scanning Transmission Microscopy – setup

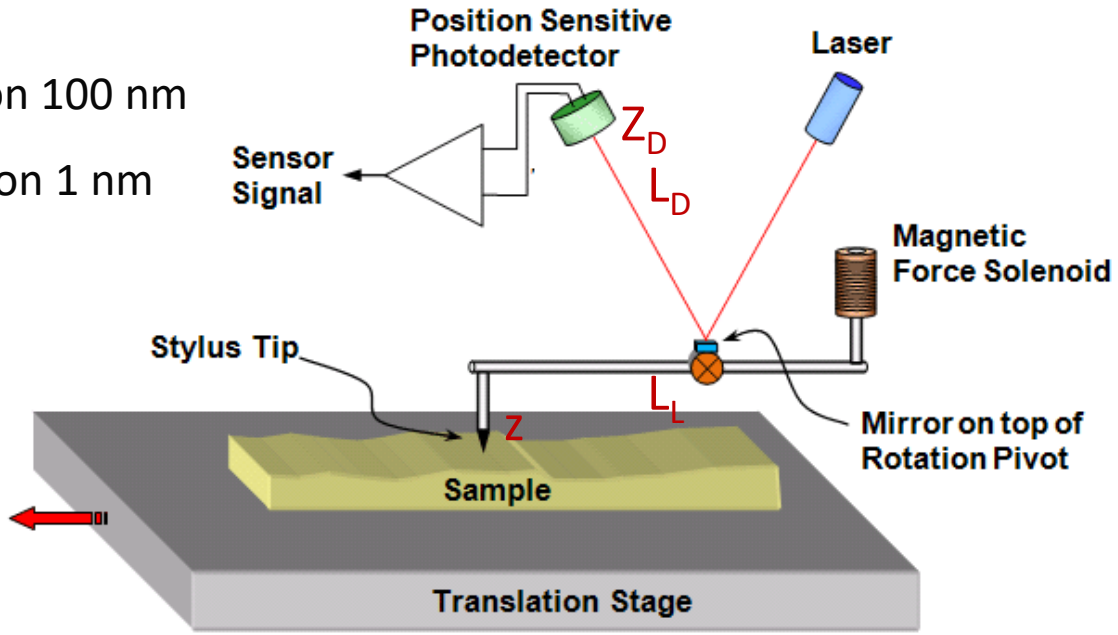


- Tunneling currents about 1nA, tip to sample separation < 1 nm
- Feedback → piezo → constant current
- Ultra high vacuum to keep sample and tip clean,

Stylus Profilometer

Lateral resolution 100 nm

Vertical resolution 1 nm



- The tip (about 1um radius) is in contact with the sample and it is deflected according to the sample height; loading force from 10^{-2} to 10^{-5} N

- The vertical deflection z of the tip is detected through the deflection Z_D of the laser beam on a position sensitive detector, with an <u>amplification</u> $A = Z_D / z = L_D / L_L$

AFM = STM + SP principles (Binning, Quate and Gerber, PhysRevLett 1986)

AFM can be applied also to insulators, not only to conductive materials

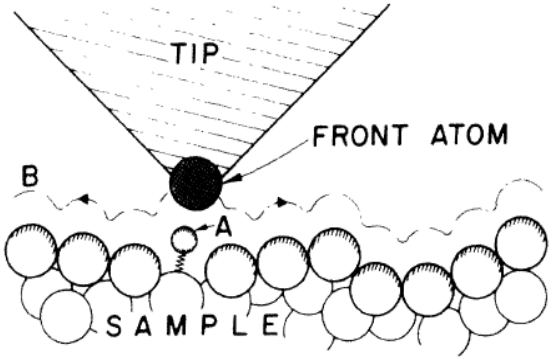
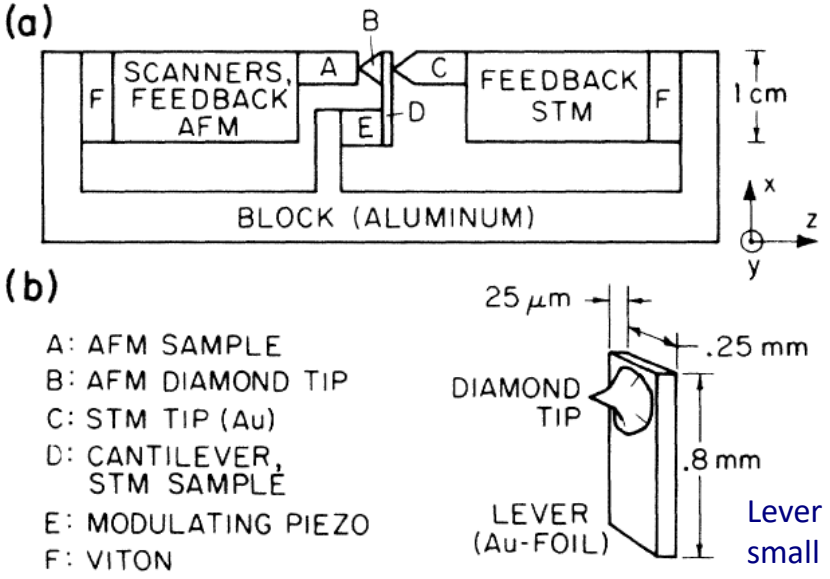


FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM itself may probe forces when a periodic force on the adatom *A* varies its position in the gap and modulates the tunneling current in the STM. The force can come from an ac voltage on the tip, or from an externally applied magnetic field for adatoms with a magnetic moment.



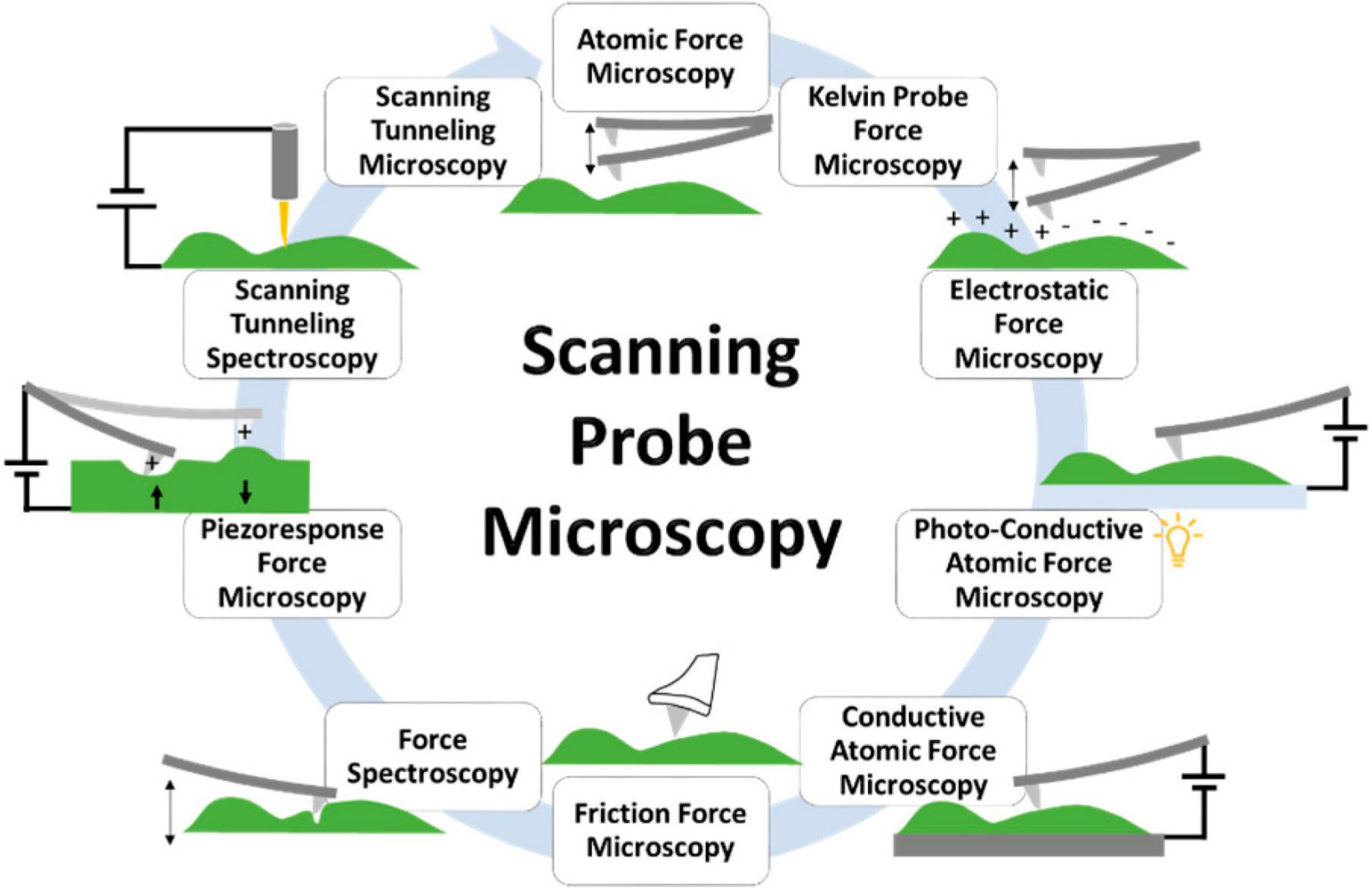
Lever with very small mass

FIG. 2. Experimental setup. The lever is not to scale in (a). Its dimensions are given in (b). The STM and AFM piezoelectric drives are facing each other, sandwiching the diamond tip that is glued to the lever.

The force required to move the lever through measurable distances (10^{-4} A) can be as small as 10^{-18} N

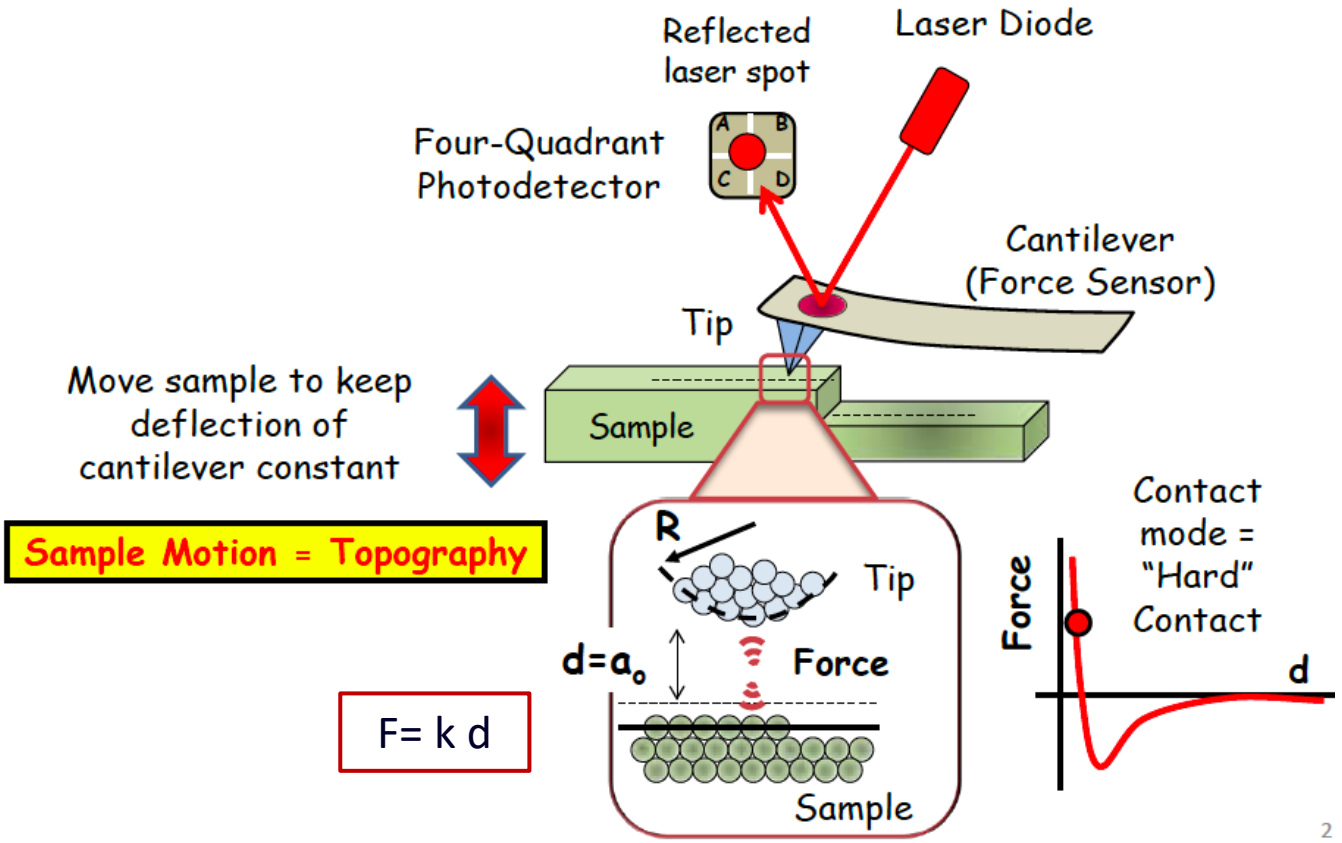
Key Idea:

use the sensitivity of STM to measure the displacement of a tip mounted on a cantilever when rastered across an insulating substrate



https://doi.org/10.3390/cryst7070216

Atomic Force Microscope (AFM)



The topographic image of the sample is obtained by plotting the height position of the translation stage or the deflection of the cantilever versus the scanning position x-y

The image contrast arises because the force between the tip and sample is a function of both tip–sample separation and the material properties of tip and sample.

Tip-Substrate Interactions

Electrostatic,magnetic

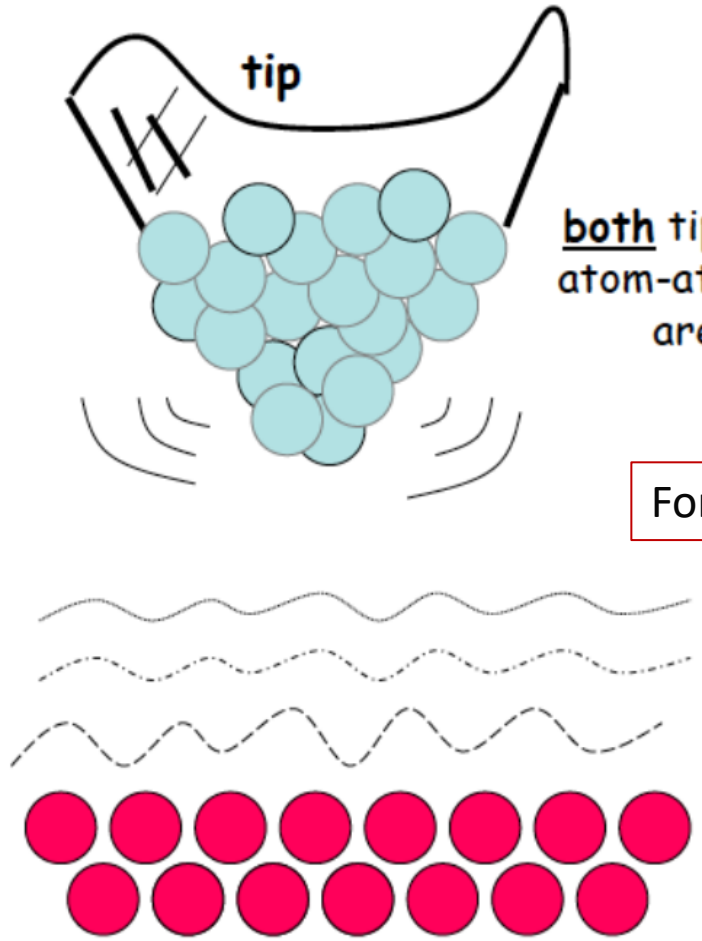
van der Waals (1873)

Capillary

Chemical:
(chemisorbed,physisorbed)

Friction

Deformation (indentation)



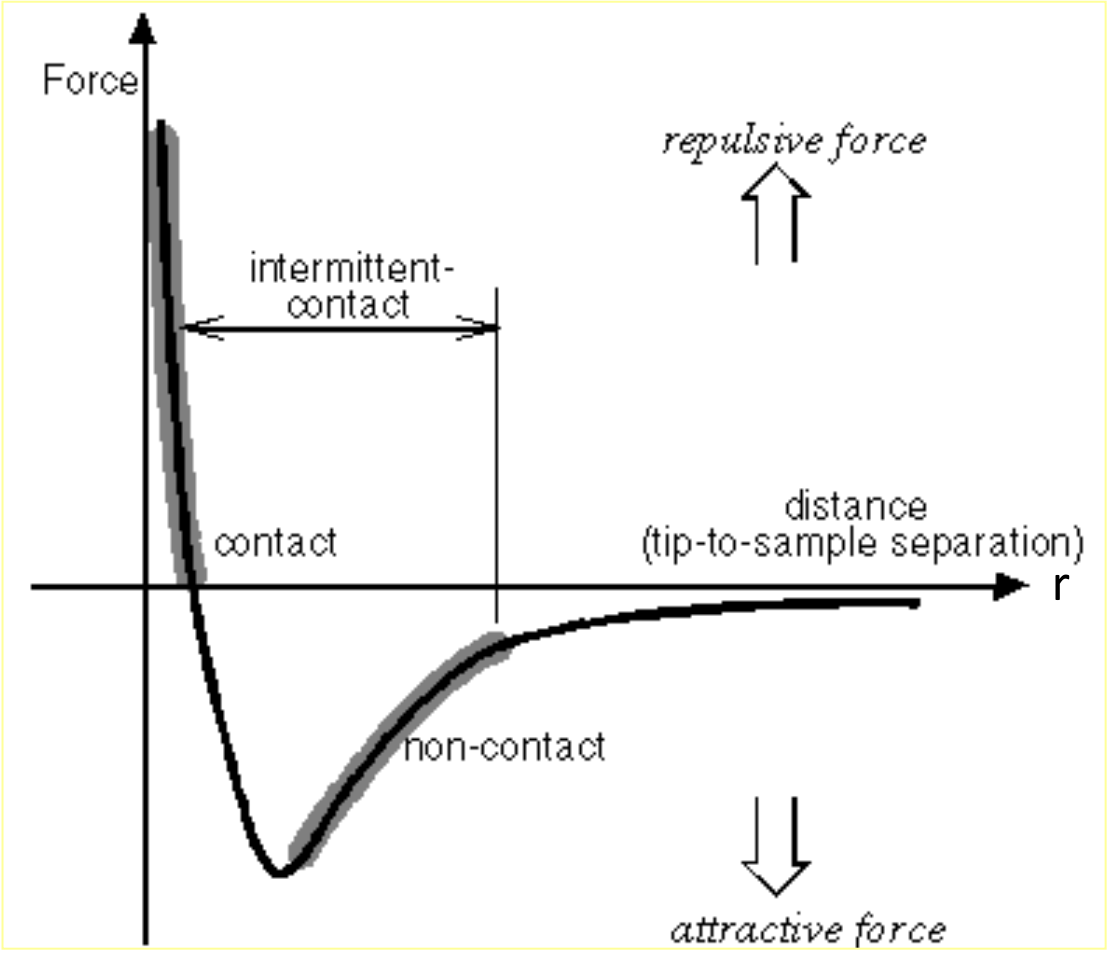
both tip-substrate and atom-atom interactions are important

Forces:1 pN – 10 nN

Force vs tip-substrate distance

Simplified model

the only forces that govern the tip-sample interaction are van der Walls forces described by the Lennard-Jones potential:



$$V_{LJ} = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$$

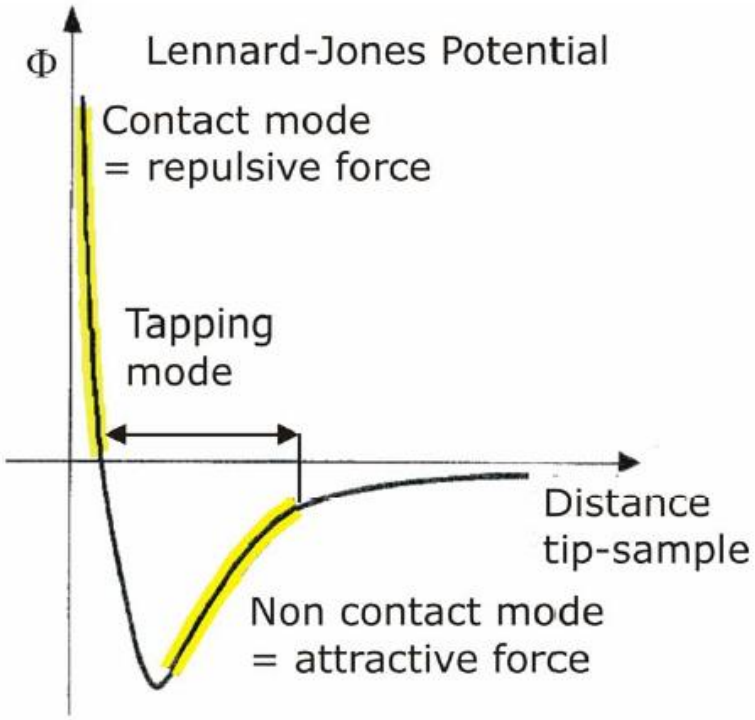
σ , ε – material parameters.

Force:

$$F = dV/dr$$

Basic AFM modi

- Imaging mode
  - contact mode
  - non contact mode
  - intermittent / tapping mode
- Force-distance mode
  - force spectroscopy
  - combined imaging & force spectroscopy



AFM imaging modes

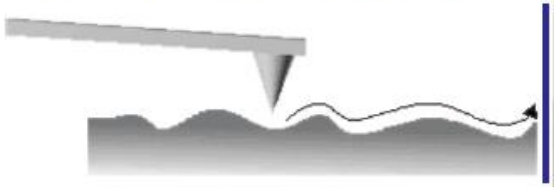
Contact Mode:

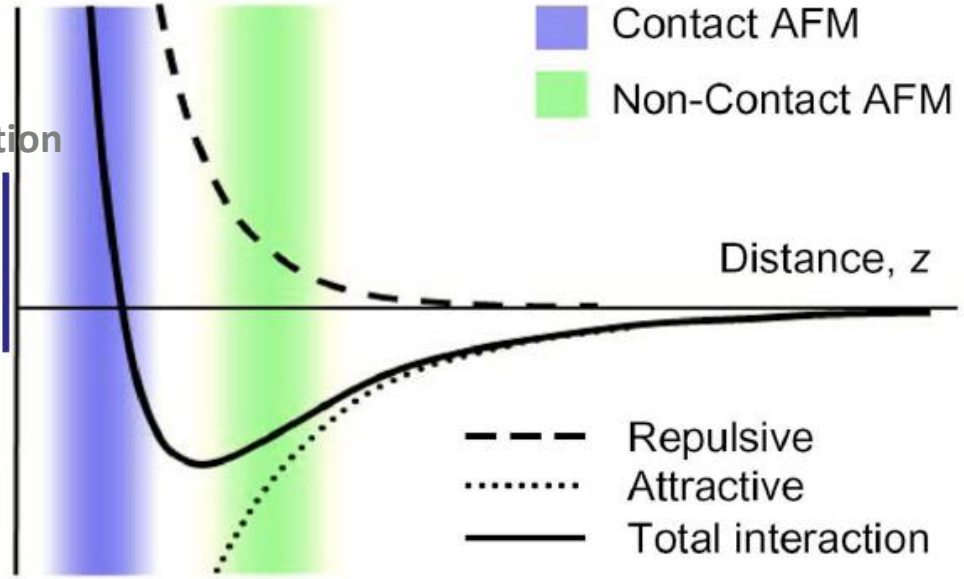
d < 5 Å

e--e- repulsive forces- 10^{-9}-10^{-6} N

Atomic resolution

Problems: frictional forces, capillary friction





Non-Contact Mode:

d = 10÷100 Å

Actractive forces - ~10^{-12} N

Soft, elestic materials

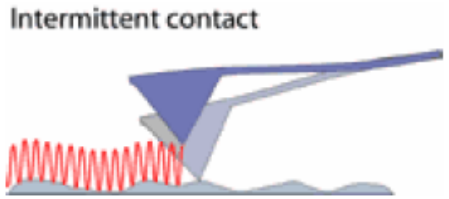
Tapping Mode:

d = 5÷20 Å

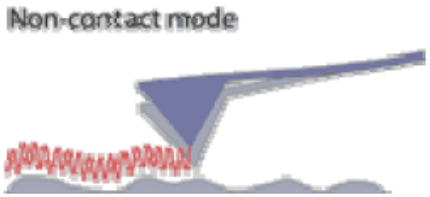
Intermitting contact

Big scanning areas, no friction

- 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

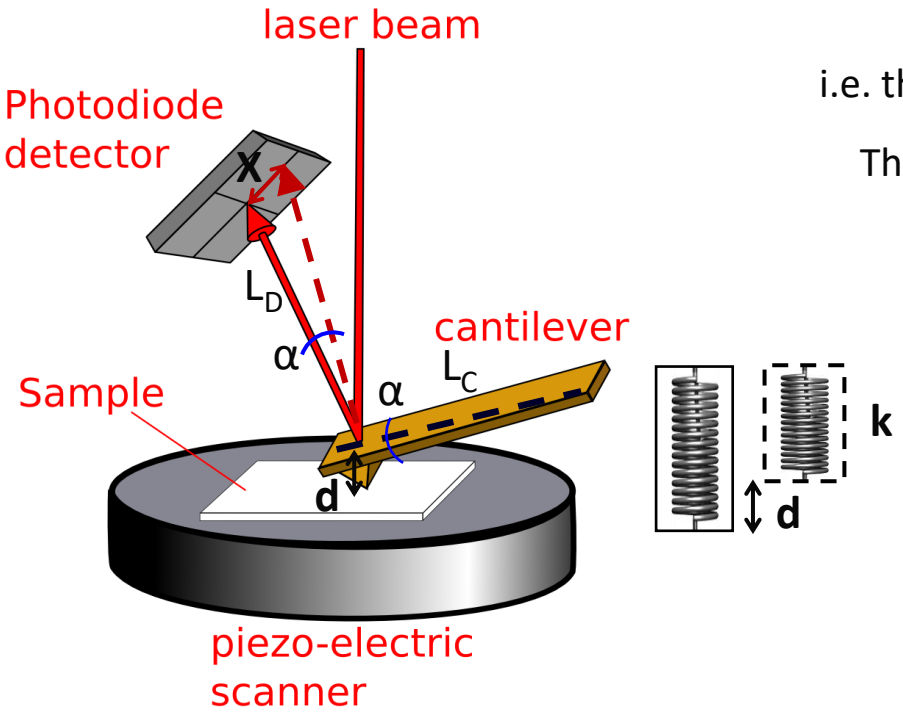


$$\omega = \sqrt{\frac{k}{m_{eff}}}$$

detect: amplitude
phase
deflection

Force measurement using the cantilever deformation / displacement

Measuring the tip/probe displacement



$$\alpha \approx \frac{X}{L_D} = \frac{d}{L_C} \longrightarrow X = \frac{L_D}{L_C} d$$

$$\boxed{A = \frac{L_D}{L_C}}$$ Amplification factor A ~ 500 !

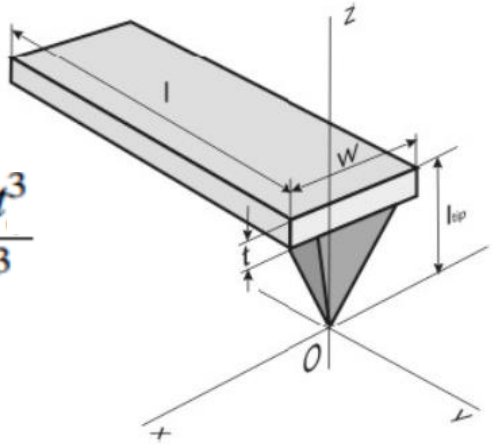
Cantilever behaves like a spring

$$\boxed{F = k\,d}$$

i.e. the force F is proportional to the deformation d

The spring constant k is defined by the cantilever geometry and material

$$k = \frac{Ewt^3}{4L^3}$$

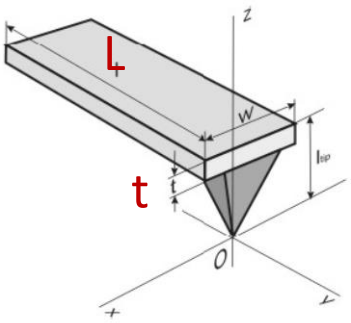


Example: cantilever of Silicon Nitride Si_3N_4
E - Young modulus , E= 1.5 10^{11} N/m^2
t= 1 µm, w= 40 µm , L= 100 µm

k = 1.5 N/m= 1.5 nN/nm

It means a deformation of 1 nm for every nN of force increment !

AFM probes



$$F = k\,d \qquad k = \frac{F}{d} = \frac{Ewt^3}{4l^3}$$

A better sensibility means a smaller k,
which is obtained by a small ratio t / L

small cantilevers are faster

	l (µm)	w (µm)	t (µm)	ω_0 (kHz)	k (N/m)	
rc800	200	20	0.8	3	0.05	8 s
bl150	60	30	0.18	8	0.03	3 s
ac40	38	16	0.2	25	0.1	1 s
ac10	9	2	0.13	500	0.1	50 ms

Typical use	k (N/m)	ω_0 (kHz)
Non-contact	10-100	100-300
Intermittent contact	1-10	20-100
Contact	0.1-1	1-50

The resonance frequency of the cantilever ω_0:

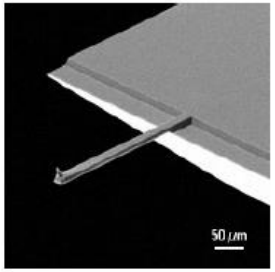
$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4\rho}}$$

m ~ 10^{-10} kg

Short cantilevers to increase ω_0 and thinner to restore k

Microfabricated AFM cantilevers

silicon nitride cantilevers



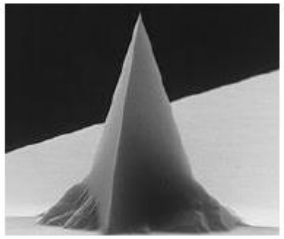
silicon cantilevers

diamond tip

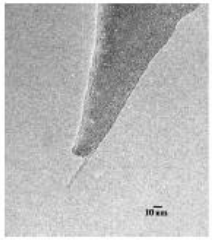
Typical cantilevers:
1µm thick, 100s µm long
k_{spring} ~ 0.01 ... 20N/m
f_{res} ~ 4 ... 400kHz
r_{tip} ~ 1 ... 20nm
reflective backside coating:
- better signal

spring constant:
force resolution
resonance frequency
tip radius:
lateral resolution
tip aspect ratio:
"depth" resolution

$$k_{spring} = \frac{E \cdot w \cdot t^3}{4 \cdot l^3}$$



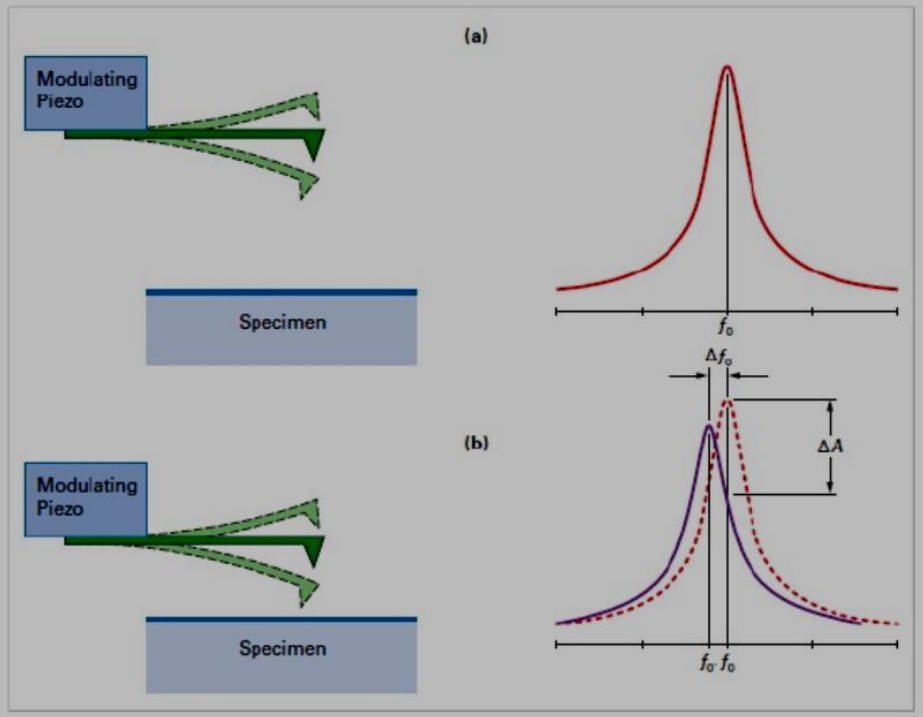
standard tip



nanotube tip

sharpened tip

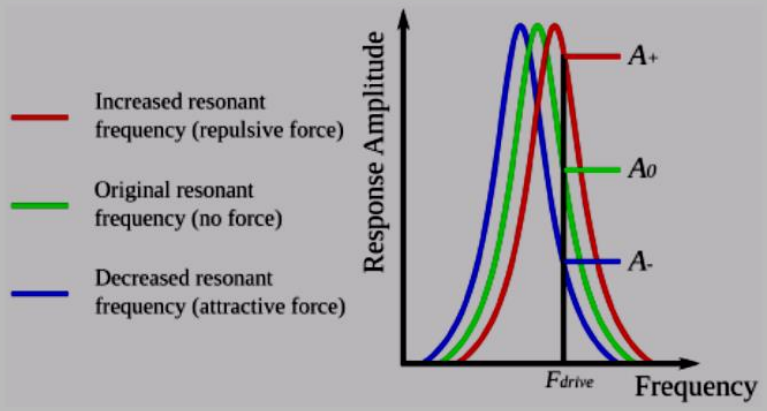
Dynamic AFM: basics



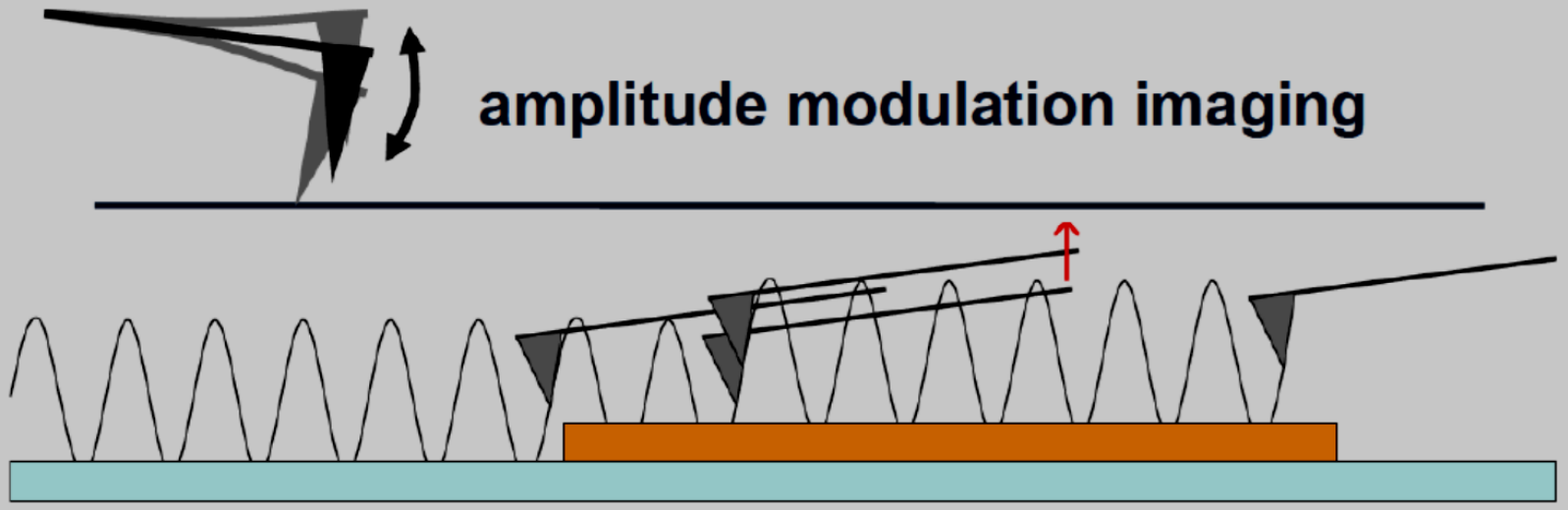
In AC mode AFM, the cantilever is excited into resonance oscillation with a piezoelectric driver.
Change in the interaction causes a shift in the operational frequency and hence a change in the measured amplitude of oscillation.

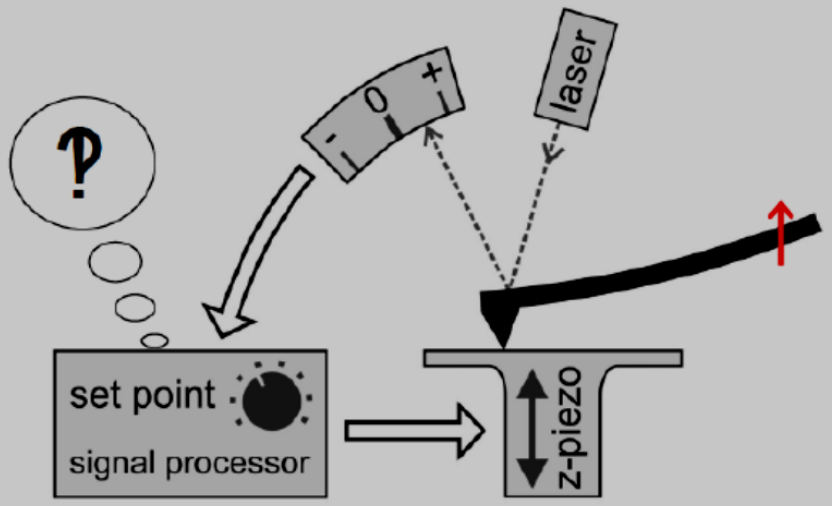
Frequency or amplitude are used as feedback parameter

$$k_{eff} = k - \frac{\partial F_{total}}{\partial z}$$



amplitude modulation imaging





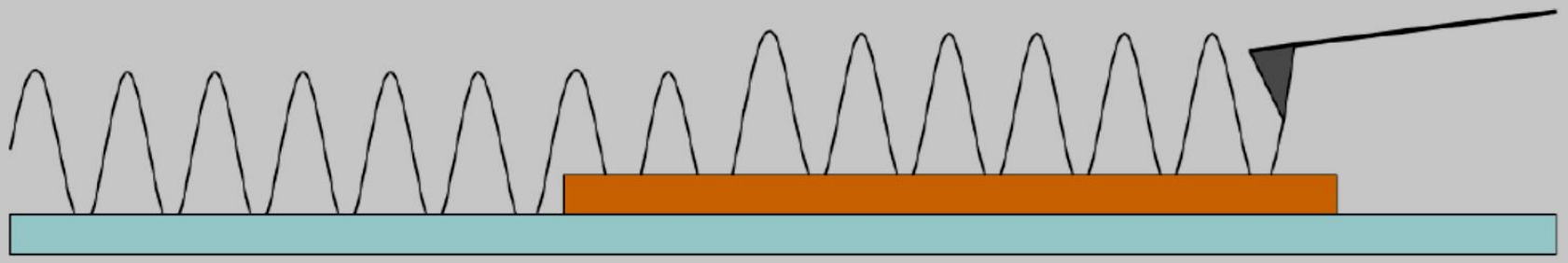
adjust piezo height (z) to keep amplitude constant

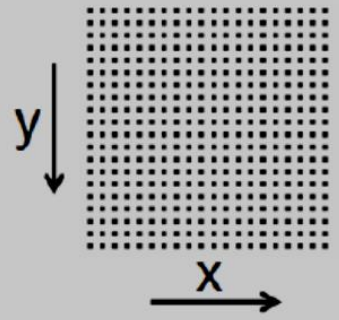
piezo motion gives height info

peak force at height changes

lower lateral/drag forces

Courtesy of J. Schaap



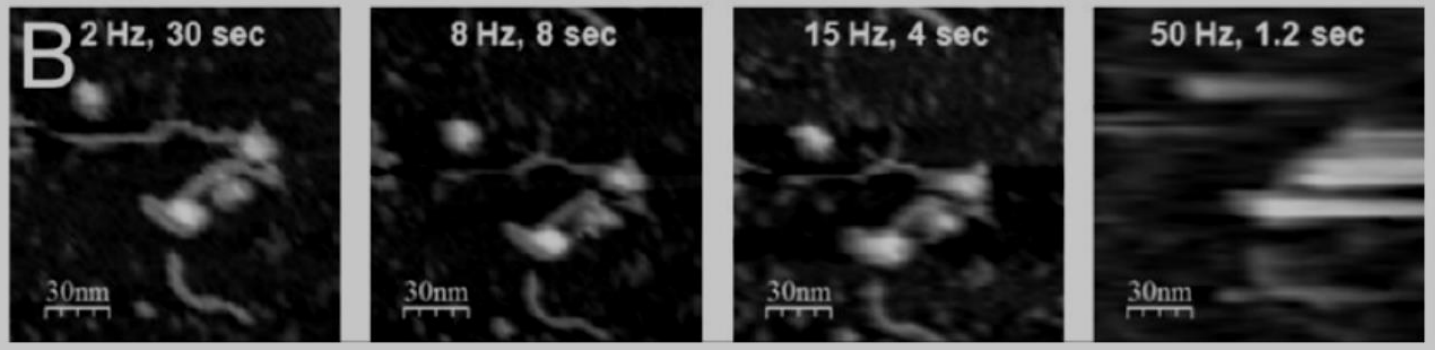


64 x 64 = 4096 px

need a few oscillations per pixel (~5)

fast bio-cantilever ~ 25 kHz

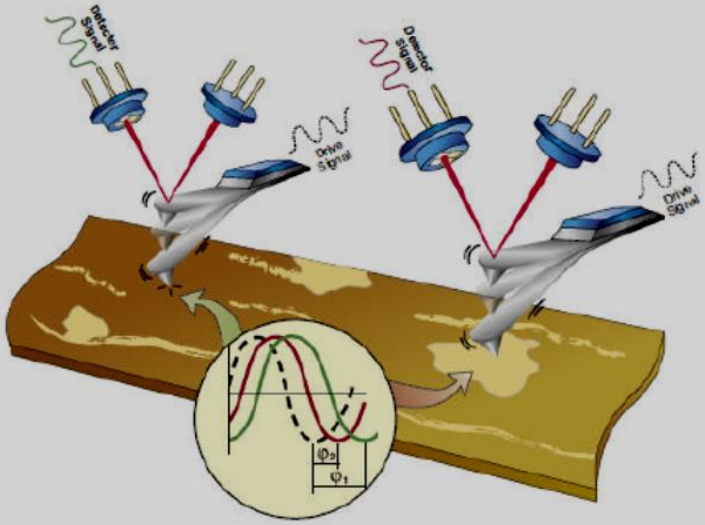
→ 0.04 ms * 5 * 4096 = 1 s



(there are other limiting factors (z-piezo, feed-back loop)

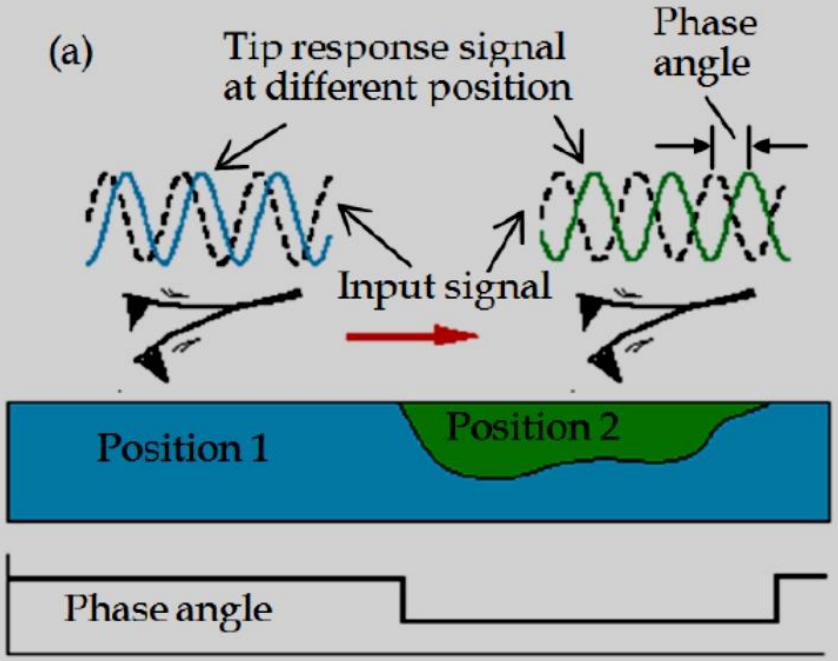
Courtesy of I. Schaap

AC mode: phase imaging



Phase imaging monitors the phase lag between the signal that drives the cantilever to oscillate and the cantilever oscillation output signal. Phase detection images can be produced while an instrument is operating in any vibrating cantilever mode.

Phase imaging is used to map variations in surface properties such as elasticity, adhesion and friction, which all may cause the phase lag. The phase lag is monitored while the topographic image is being taken so that images of topography and material properties can be collected simultaneously -> direct correlation between surface properties and topographies.



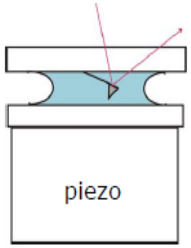
Some examples of AFM imaging in life sciences

Sample preparation

- Suitable substrate
  flat and rigid
  - mica (atomically flat, hydrophilic)
  - SiO_2, glass (nm roughness, hydrophobic)
  - ultraflat gold (stripped gold)

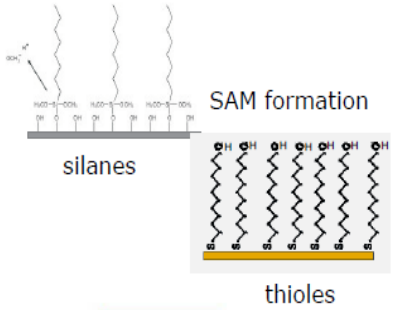
- Immobilisation of sample

- Typical sample size
  - Scanning surface: ~1cm²
  - Scanning tip: ~ Petri dish
  - Liquid sample: 1 ... 100µl

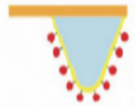


Surface – tip functionalisation

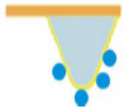
- Surface modification
  self assembling monolayers (SAM)
  - silanes on glass- and Si-surfaces
  - thioles on Au-surfaces



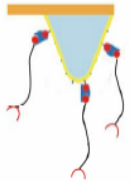
- Tip modification
  - Adsorption of molecules from solution
    e.g. proteins



  - 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



AFM examples of applications
https://www.jpk.com/app-technotes/products_atomic-force-microscopy

Examples of AFM imaging in biology

Cell imaging with AFM

AFM images of cells show a combination of surface and mechanical information, the balance between the two depending on the type of cell sample, and also on the imaging settings and the type of experiments that are performed.

AFM is particularly well suited to studying the membrane surface of the cells.

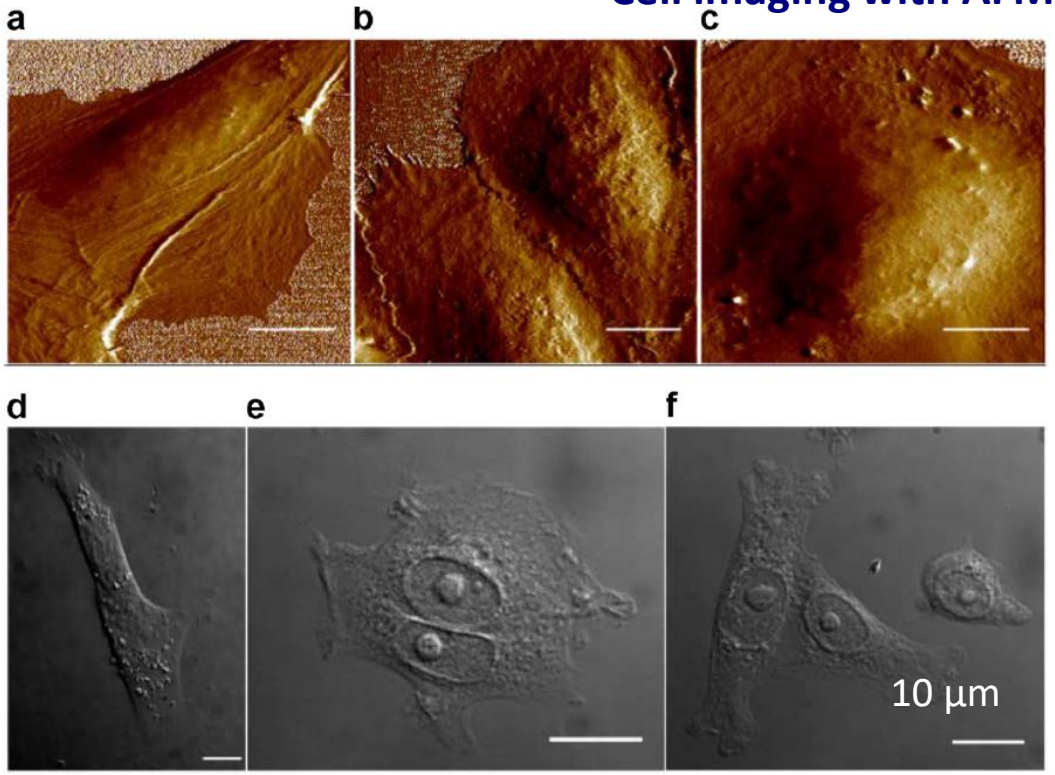
AFM imaging can be performed in buffer or cell culture medium.

AFM allows to study interactions between cells and surfaces or cells and other cells, adhesion, force generation by moving cells and dynamic reorganisation of the cytoskeleton

Fuctionalising the tip with bound peptides or antibodies – stimulate cells using specific interactions

Combination AFM – optical microscopy (brightfield, phase imaging, fluorescence, superresolution) very useful to obtain complementary information

Cell imaging with AFM



AFM surface topology images

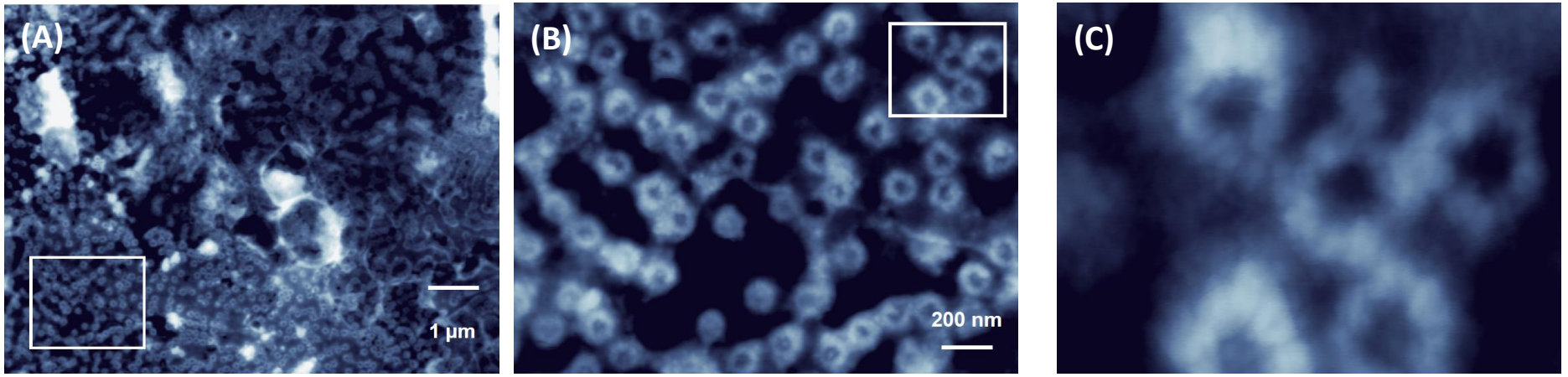
Young's modulus is also measured
See lecture cell mechanics

DIC 90X optical images

Morphology of the breast cancer cells : HBL-100 (a), (d), MCF-7 (b), (e) and MDAMB-231 (c), (f). AFM peak force error channel output was chosen to highlight the fine features in the images. Images were acquired in peak force quantitative nanomechanical mapping mode ScanAsyst / Bruker. 128×128 or 256x256 point PFQNM images were acquired with a scan rate of 0.1–0.2 Hz, a scan size of about 60 µm, tapping amplitude of 750 nm, frequency of 0.25–0.5 kHz and a peak force set point of 1 nN to avoid damaging the cell membranes while scanning. For each cell imaged, multiple information can be obtained: height, peak force, adhesion, deformation, dissipation and Young's modulus.

Coceano et al, Nanotechnology 2016 DOI: 10.1088/0957-4484/27/6/065102

Imaging the nuclear pore complex with AFM mounted on an inverted optical microscope



Nuclear Pore Complex NPC is responsible for transport of various molecules into and out of the cell nucleus.

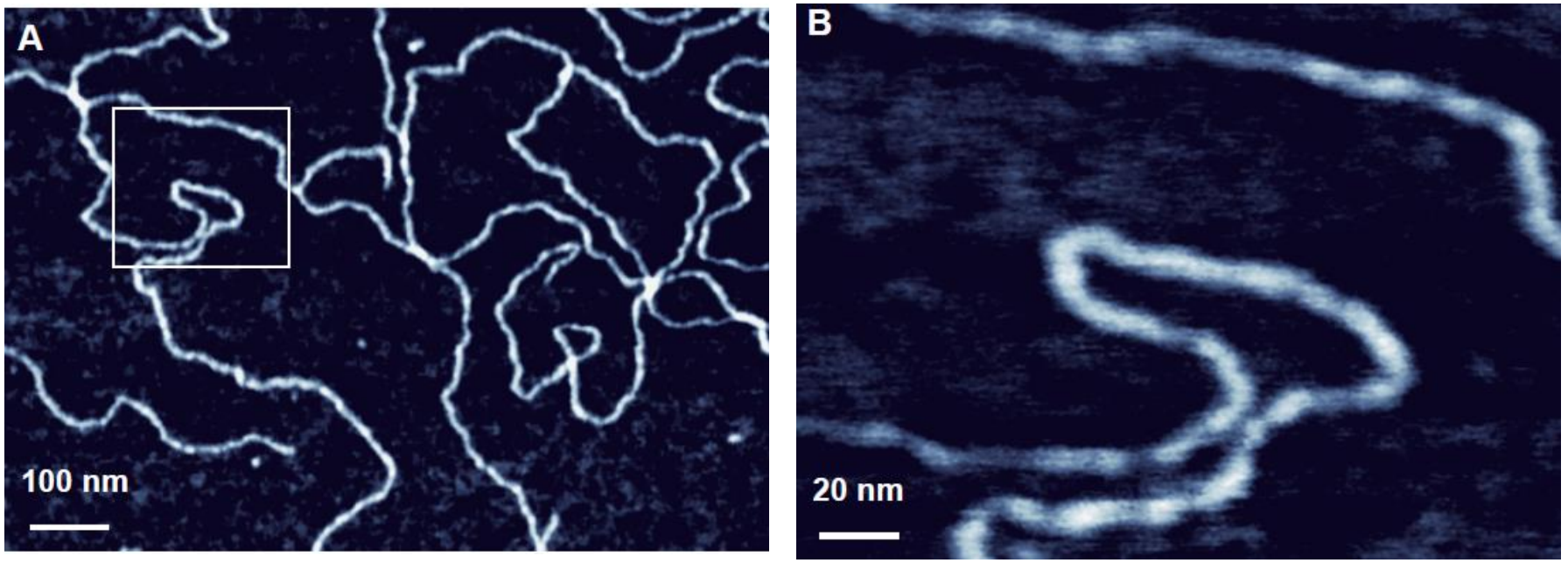
Samples are prepared from whole nuclei (in this case from Xenopus laevis) and hence can be quite heterogeneous, containing contaminating material (debris) for tip (A)

The tip was then positioned over an area with minimal debris and an overview scan acquired (B).

The controlled feedback (capacitively) allows precise selection of an area for a higher resolution scan (C)

https://www.jpk.com/app-technotes/products_atomic-force-microscopy

DNA imaging



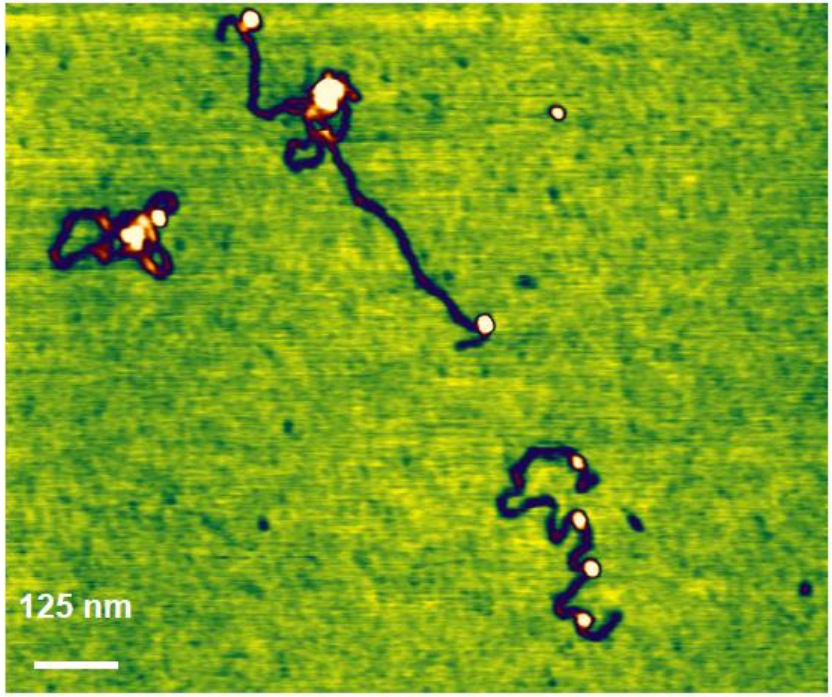
Lambda phage DNA – ac mode in fluid. Colour scale 0-2 nm in Z

Elucidate physical structure of DNA and interaction of with DNA-binding molecules. DNA absorbed to cleaved mica and imaged in buffer.

Lambda phage DNA – ac mode in fluid

Nuclear Pore Complex NPC is responsible for transport of various molecules into and out of the cell nucleus.

DNA imaging



AC mode topograph of DNA-nucleosome complexes. The protein can clearly be distinguished bound along the length of the linearized pGEM plasmid.
Credit: r. Clemens Franz, TUD

Association of DNA with histones to form nucleosomes. This condensing of DNA around the nucleosome core plays a role in the regulation of DNA replication and transcription as the condensed DNA is not accessible to other DNA binding proteins.
In this case the linearized 3kb plasmid pGEM was incubated with nucleosomes (1 mole DNA to 20 moles of histone octamers). The pGEM plasmid has 20 putative nucleosome binding sites, but under incubation conditions nucleosomes did not bind at all 20 binding sites.

- histones are highly basic proteins abundant in lysine and arginine residues that are found in eukaryotic cell nuclei