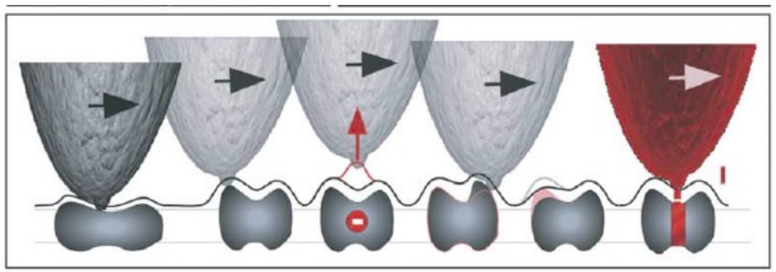
Elettra Sincrotrone Trieste

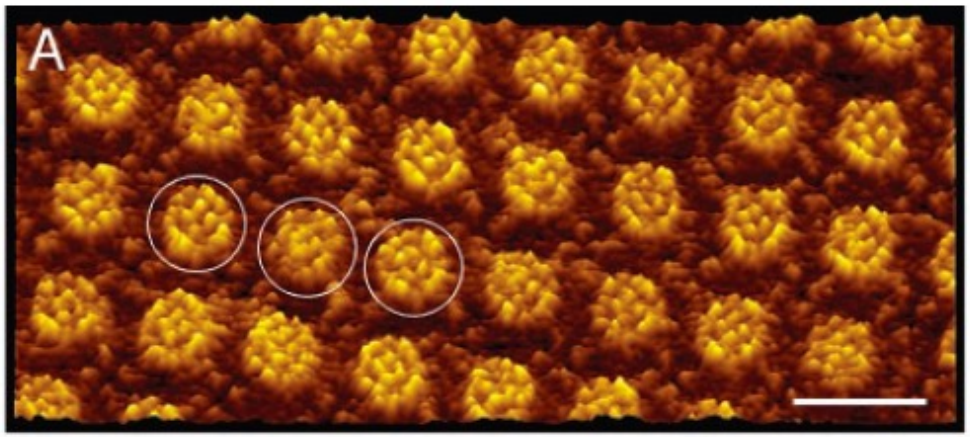
ATOMIC FORCE MICROSCOPY
Imaging in Biology

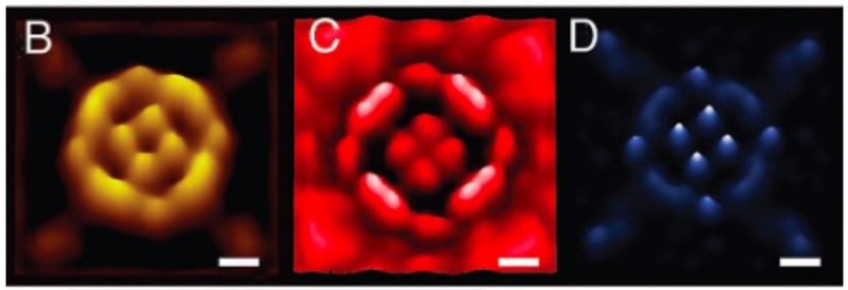
Sources:
Fundamentals of Scanning Probe Microscopy, V. L. Mironov
NanoHub.org

Nanotechnologies in Biology: AFM

The importance of the development of Atomic Force Microscopy in biology is comparable to that of EM and Optical Microscopy.
Its major advantage is that it can produce **high-resolution topographic images of biomolecules/cells** in aqueous and physiologically relevant environments without the need of staining or labeling.
High-resolution AFM has been applied to the imaging of bacterial membrane proteins, deriving the free energy landscape for domains within single protein molecules.



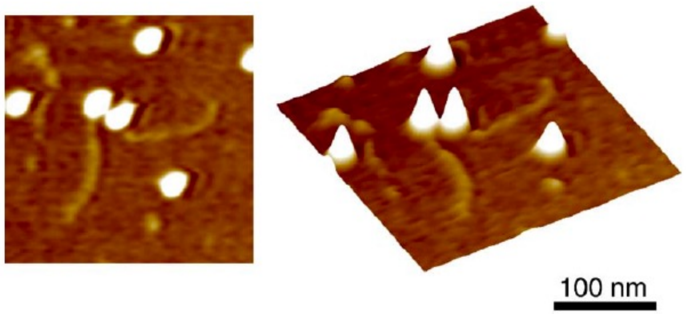


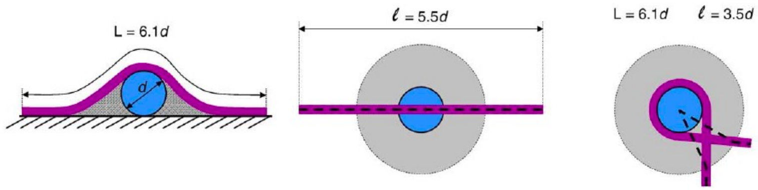


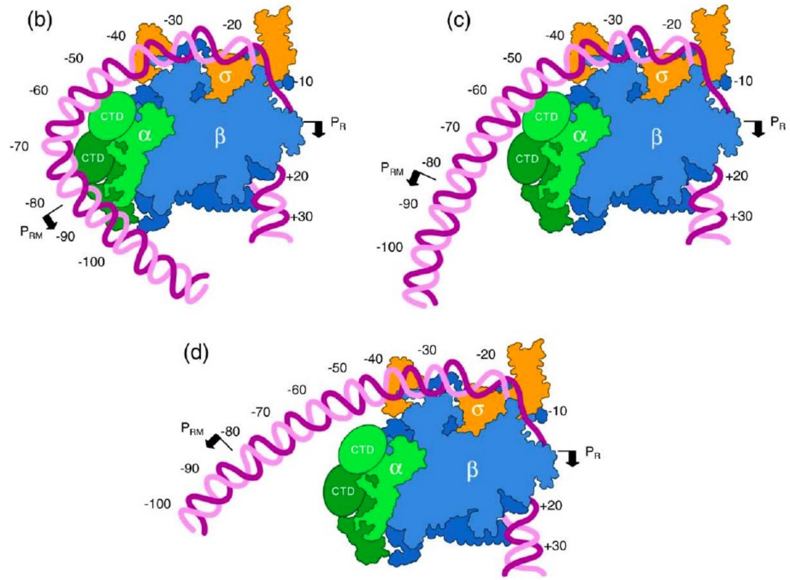
S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

AFM Imaging

Also, **high-resolution AFM imaging** has been recently employed to study topological details of DNA/RNA – enzymes interaction. Here is an example of the upstream interaction of Escherichia coli RNA polymerase (RNAP) in an open promoter complex (RPo) formed at the PR and PRM promoters of bacteriophage λ.



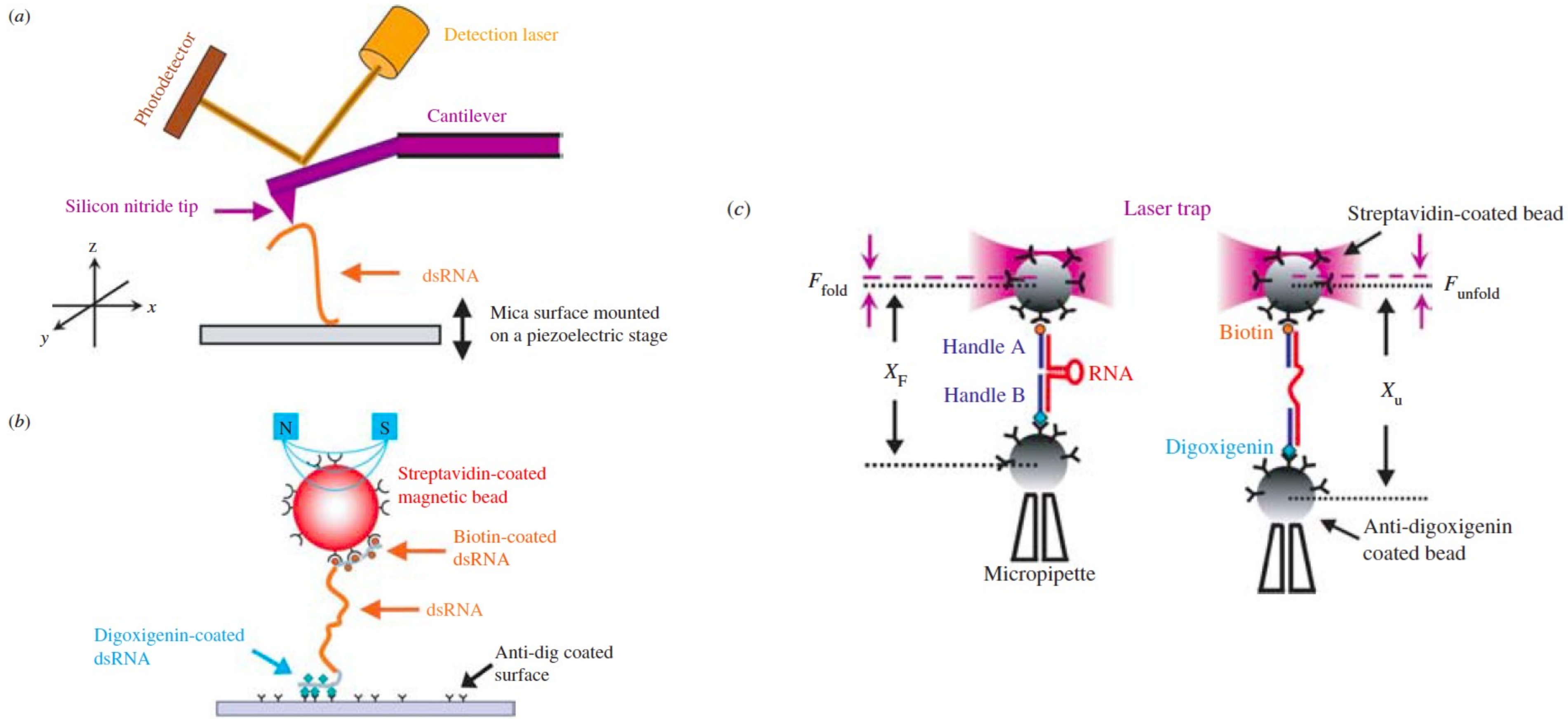




I. Mangiarotti, S. Cellai, W. Ross, C. Bustamante, C. Rivetti, *L. Mol. Biol.* **385**, 748 (2009)

Single Molecule Detection

Force spectroscopy techniques (AFM, optical tweezers) exert and/or quantify forces to allow manipulation and characterization of the mechanical properties, functional state, conformations and interactions of biological systems to molecular resolution.



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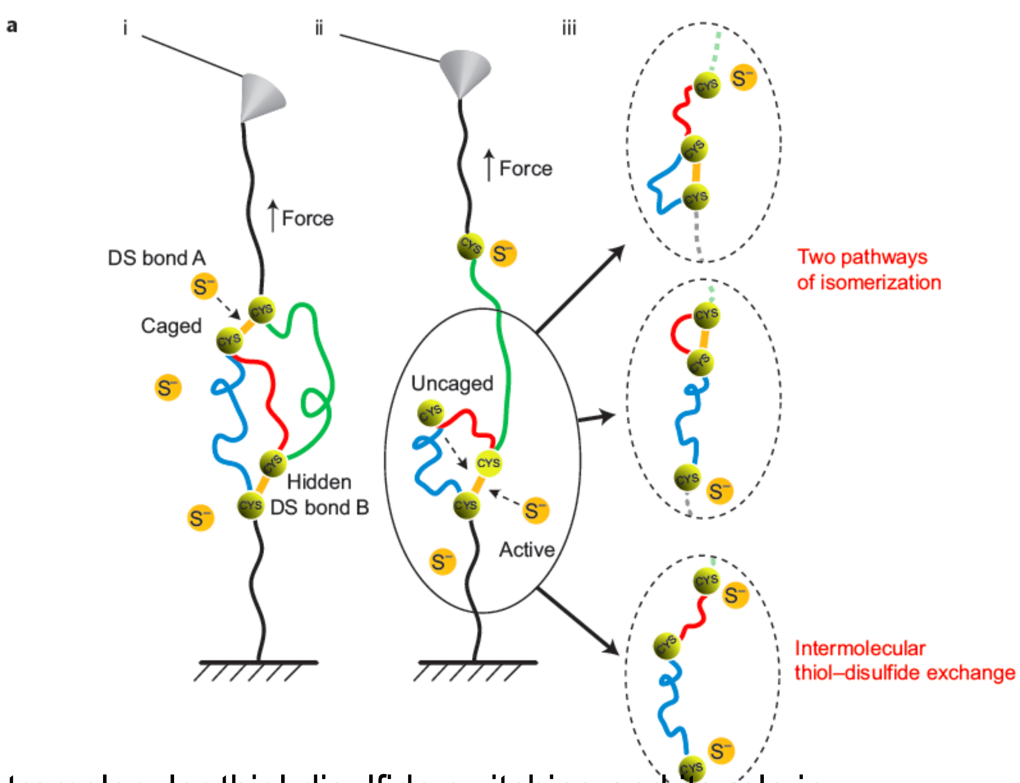
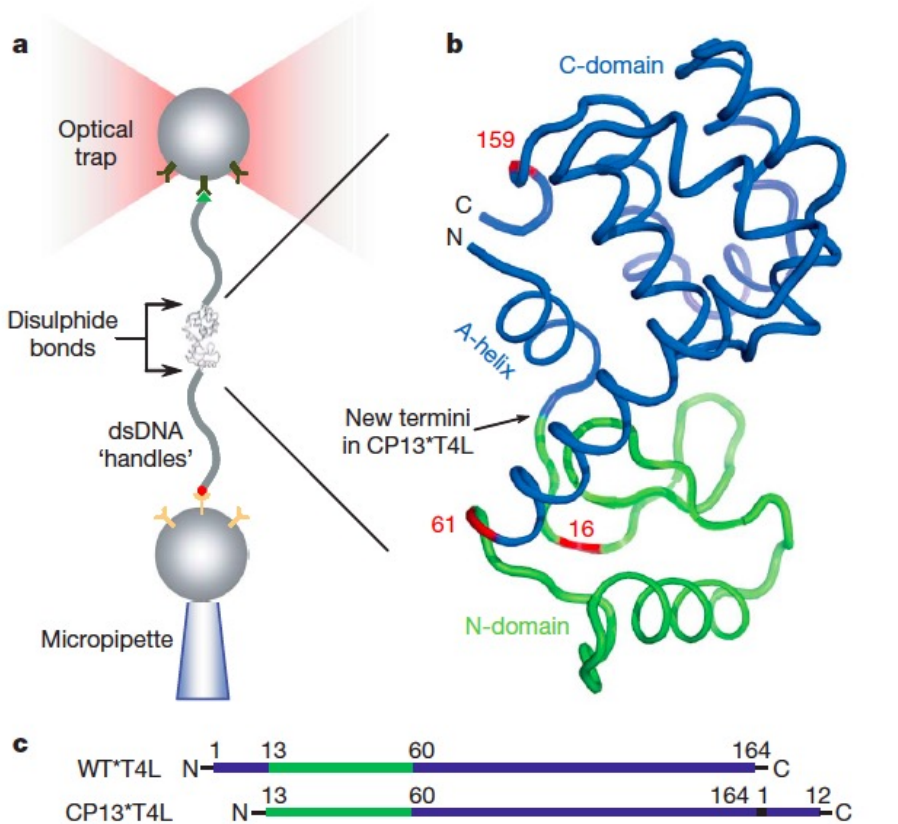
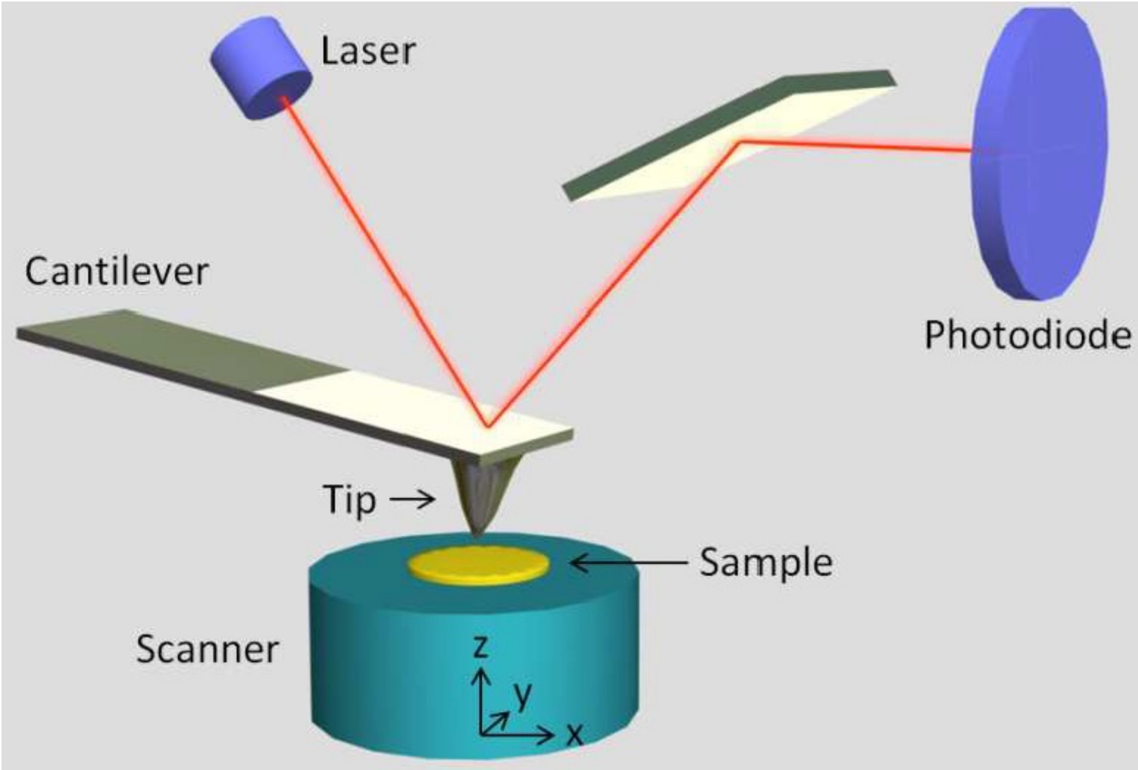


Intramolecular thiol-disulfide switching and its role in protein folding (J. Fernandez)



Folding cooperativity and chain topology (C. Bustamante)

Atomic Force Microscopy



Unique characteristics:

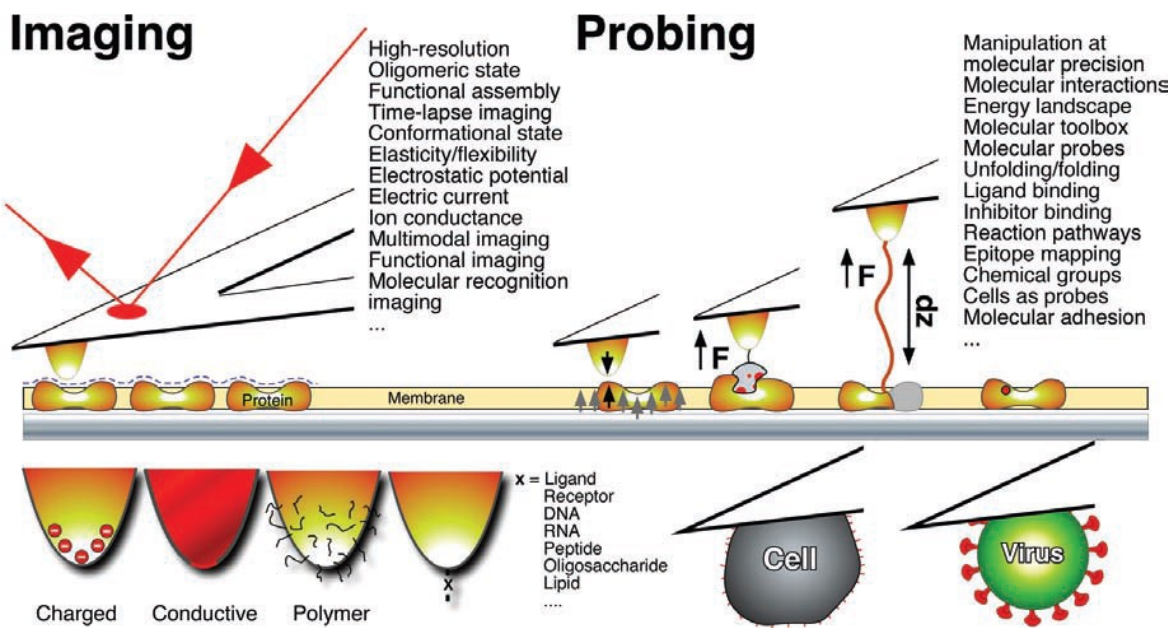
1. built-in atomic scale sensitivity
2. precise motion control technology
3. fabrication technology (nanolithography)

AFM does not rely on EM radiation to create an image.
It is a mechanical imaging instrument that derives the 3-D profile (topography) and the physical properties of a surface by measuring the INTERACTION FORCES with a scanning, nanometer sized probe.

Atomic Force Microscopy

High-resolution AFM has been applied to the imaging of bacterial membrane proteins, deriving the free energy landscape for domains within single protein molecules.

AFM is complementary to X-ray and electron crystallography.



Imaging resolution in cell membranes: 10 nm

Imaging resolution in supported cell membranes: better than 1 nm (no fixing, labeling, Staining, room T, buffer solution)

S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

VOLUME 56, NUMBER 9 PHYSICAL REVIEW LETTERS 3 MARCH 1986

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)

Control the tip-substrate force!

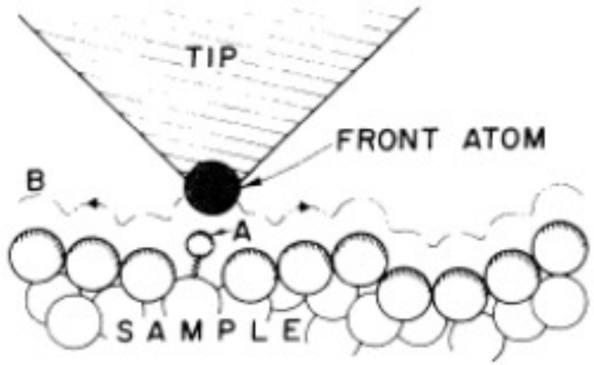
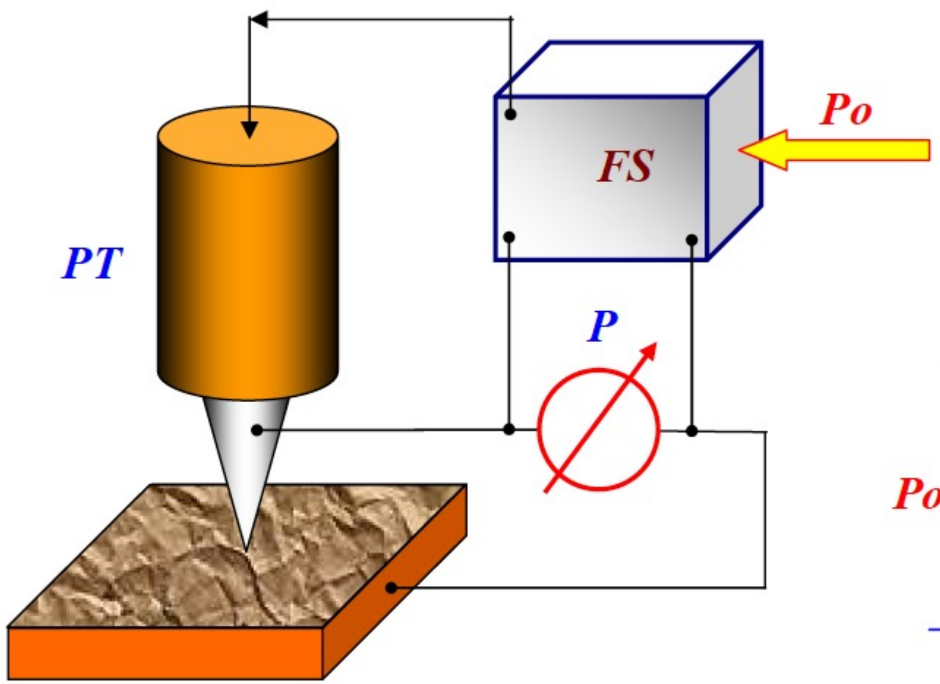


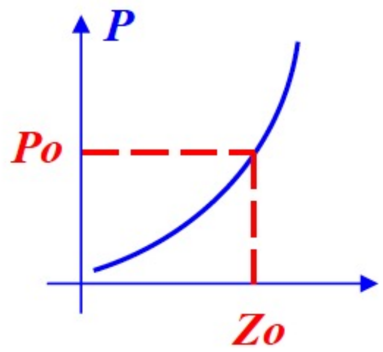
FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM 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.

Scanning Probe Microscopes (AFM, STM..)

1981: Scanning Tunneling Microscope (STM, Binning and Rohrer)
1986: Nobel Prize in Physics
1986: Atomic Force Microscopy introduced (Binning, Quate, Gerber)

Both use feedback loop to keep a set point (tunneling current, force) constant





Unique characteristics:

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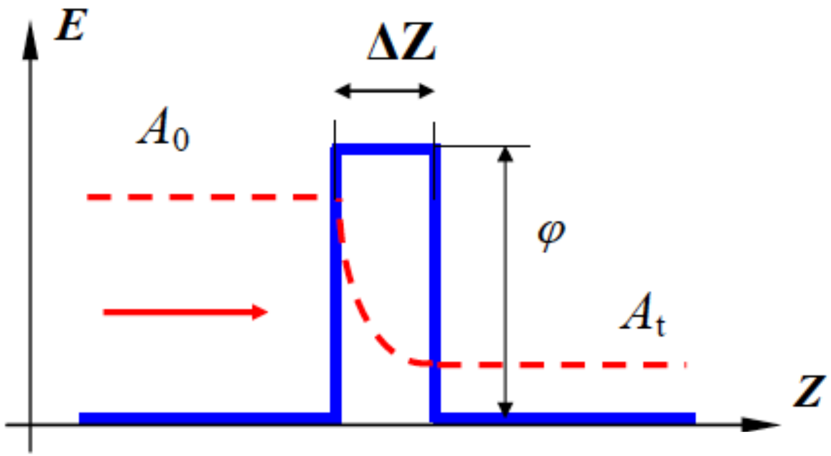
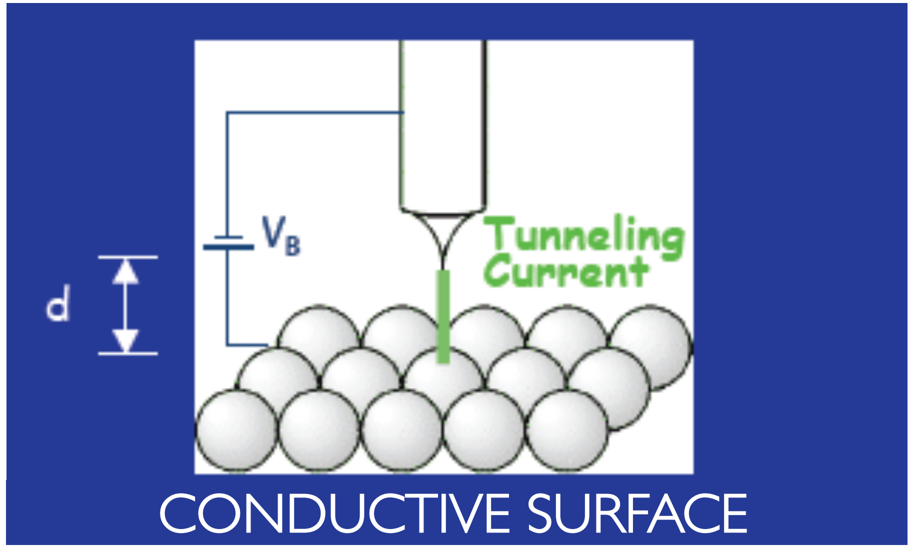
Scanning Tunneling Microscopy





$$\varphi^* = \frac{1}{2}(\varphi_T + \varphi_S).$$

average work function ϕ *

$$W = \frac{|A_t|^2}{|A_0|^2} \cong e^{-k\Delta Z},$$

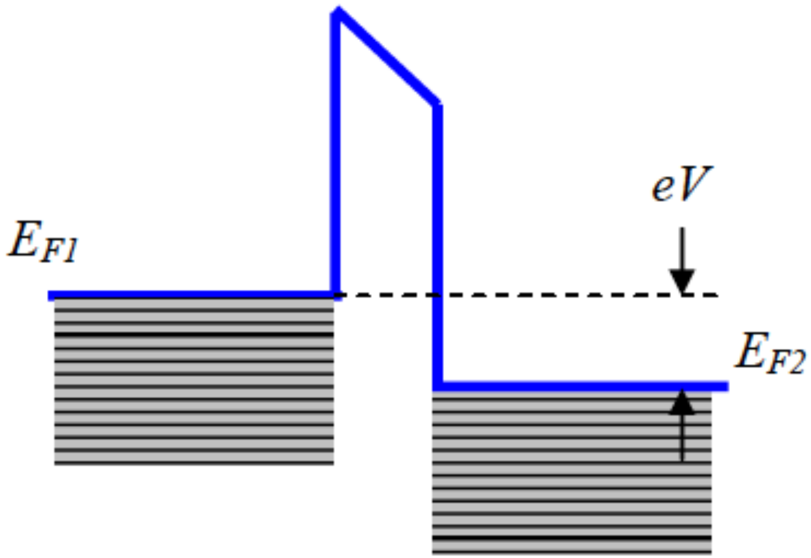
probability of electron tunneling
(transmission coefficient)
A = amplitude of electron wave function

$$k = \frac{4\pi\sqrt{2m\varphi^*}}{h},$$

attenuation coefficient in metals

Scanning Tunneling Microscopy

If a potential difference V is applied to the tunnel contact, a tunneling current appears.



$$j_t = j_0(V)e^{-\frac{4\pi}{h}\sqrt{2m\varphi^*}\Delta Z}$$

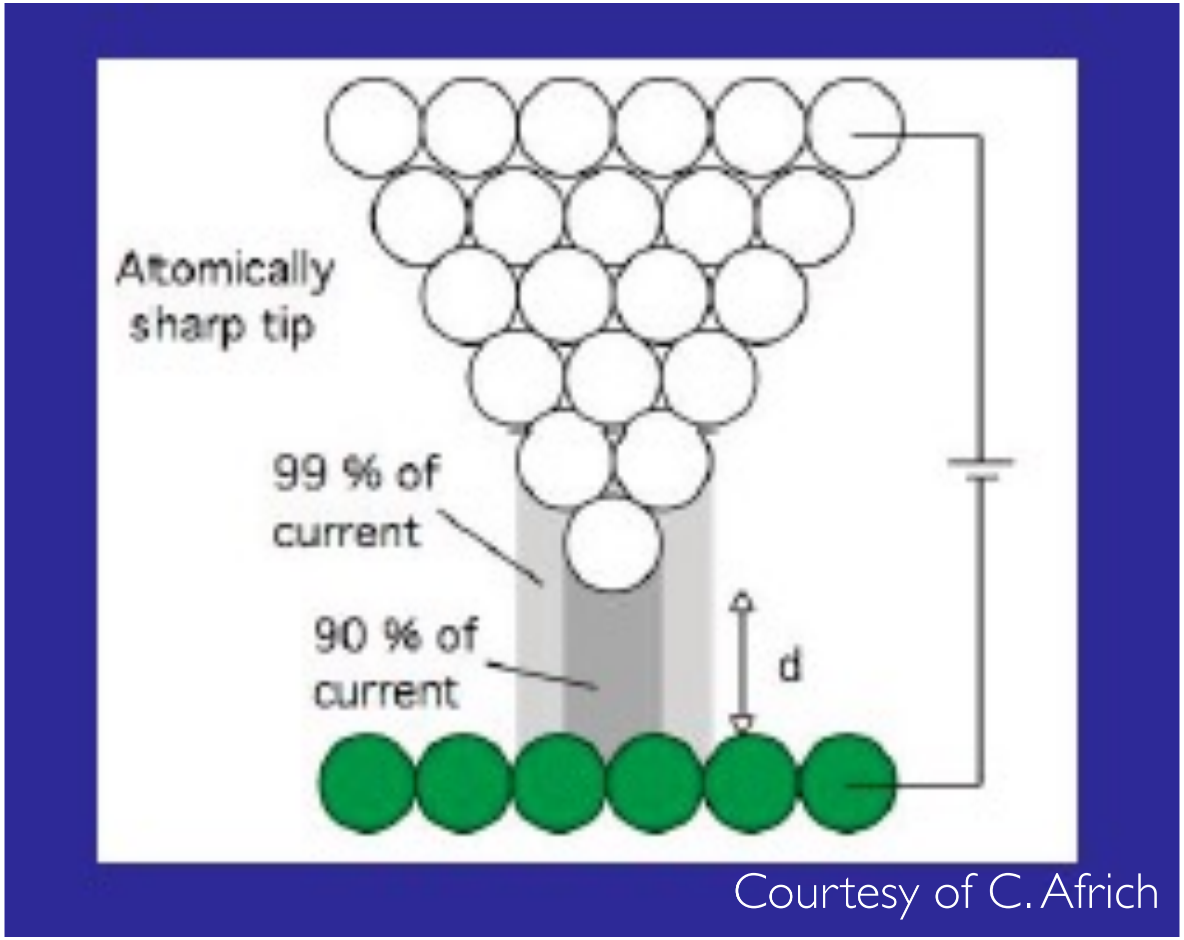
For typical values of the work function

$\phi \sim 4$ eV

the attenuation coefficient k is about 2 Å^{-1}

when ΔZ changes of about 1 Å, the current value varies of one order of magnitude!

Scanning Tunneling Microscopy



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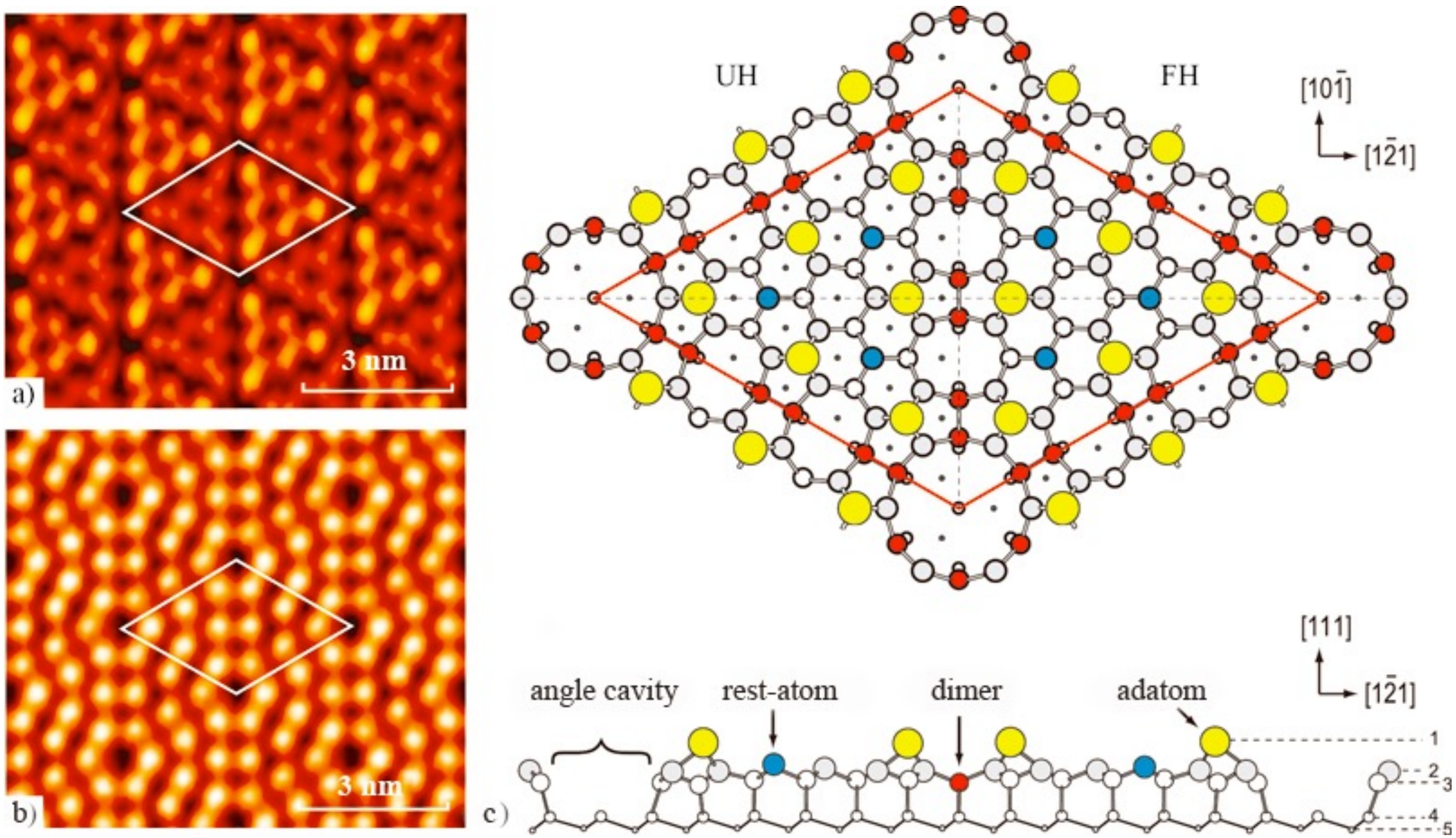
Scanning Tunneling Microscopy

VOLUME 50, NUMBER 2 PHYSICAL REVIEW LETTERS 10 JANUARY 1983

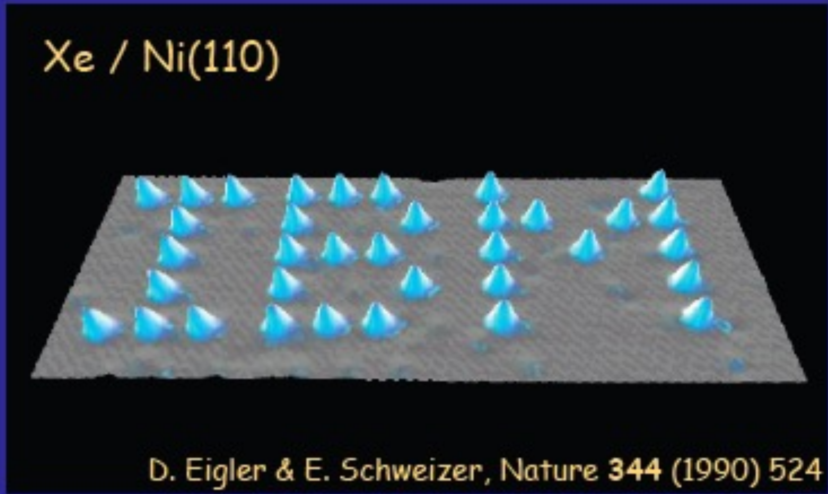
7 × 7 Reconstruction on Si(111) Resolved in Real Space

G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel
IBM Zurich Research Laboratory, 8803 Rüschlikon-ZH, Switzerland
(Received 17 November 1982)

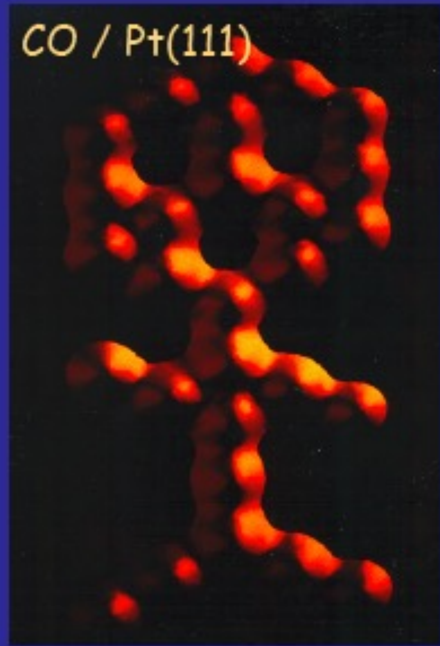


Surf. Sci. 1985. V. 164. P. 367. 7x7 rec. reduces dangling bonds from 49 to 19

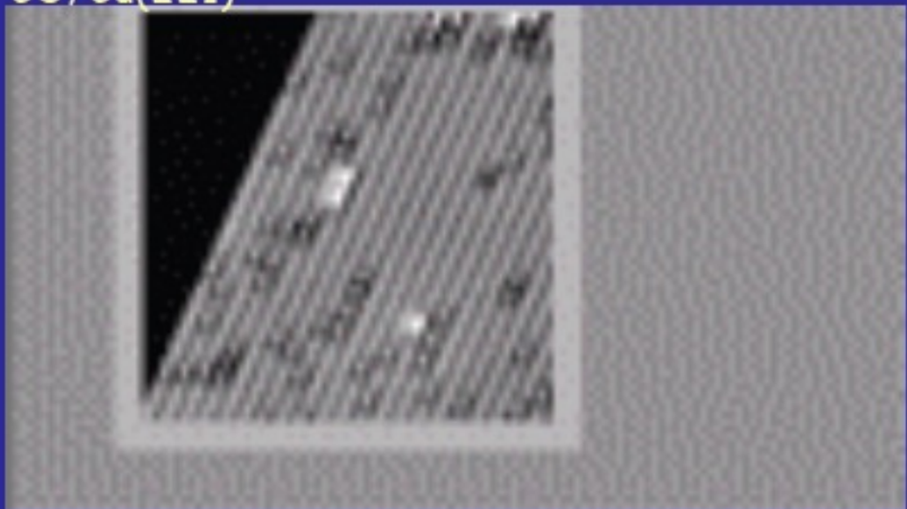
Manipulation by STM



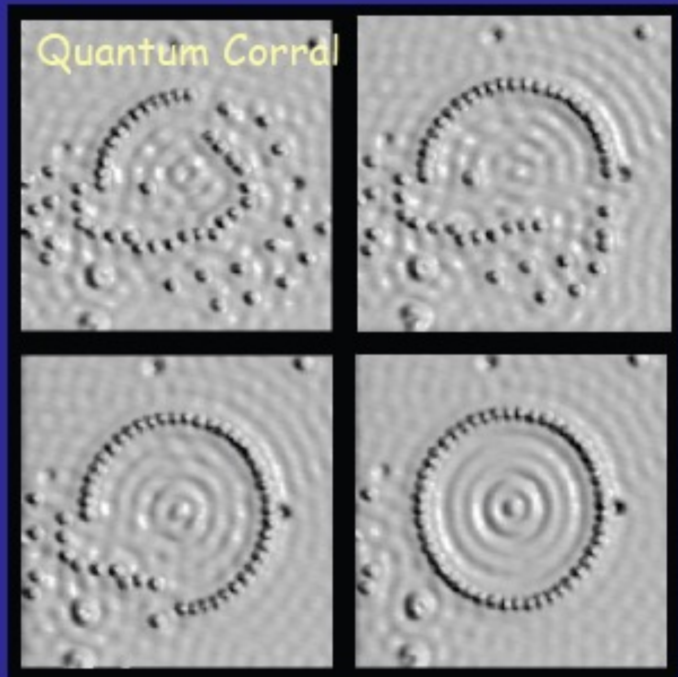
D. Eigler & E. Schweizer, Nature **344** (1990) 524



CO/Cu(221)



G.Meyer *et al*, Single Mol. **1** (2000) 1
http://www.physik.fu-berlin.de/~ag-rieder/LT-STM2/



Many materials of interest do not conduct electricity. Is it possible to use scanning probe to study them?

Even at the First International STM Conference in July 1986, there was discussion about how to extend STM techniques to non-conducting materials.

Overcoming Limitation of a Conducting Substrate: the Atomic Force Microscope

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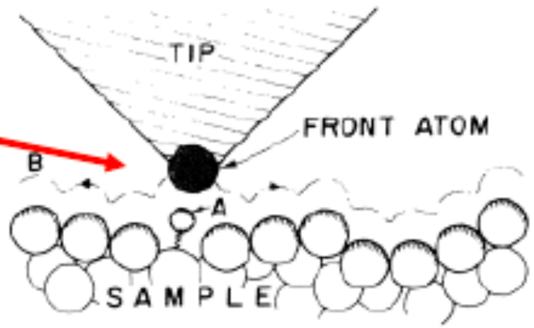

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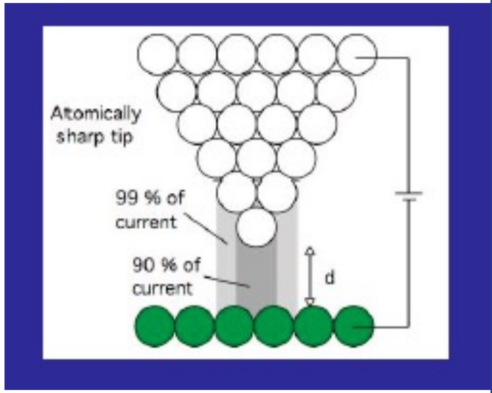
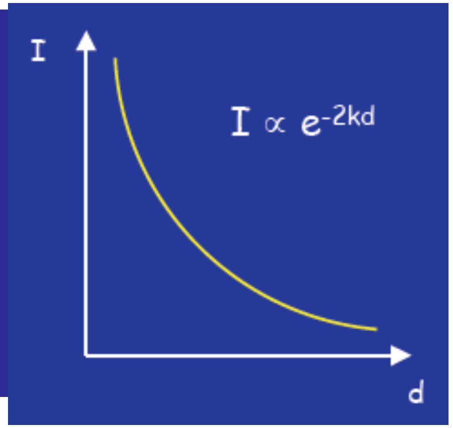
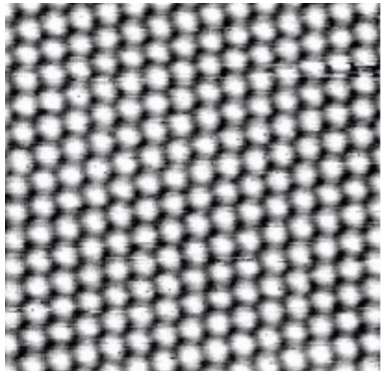
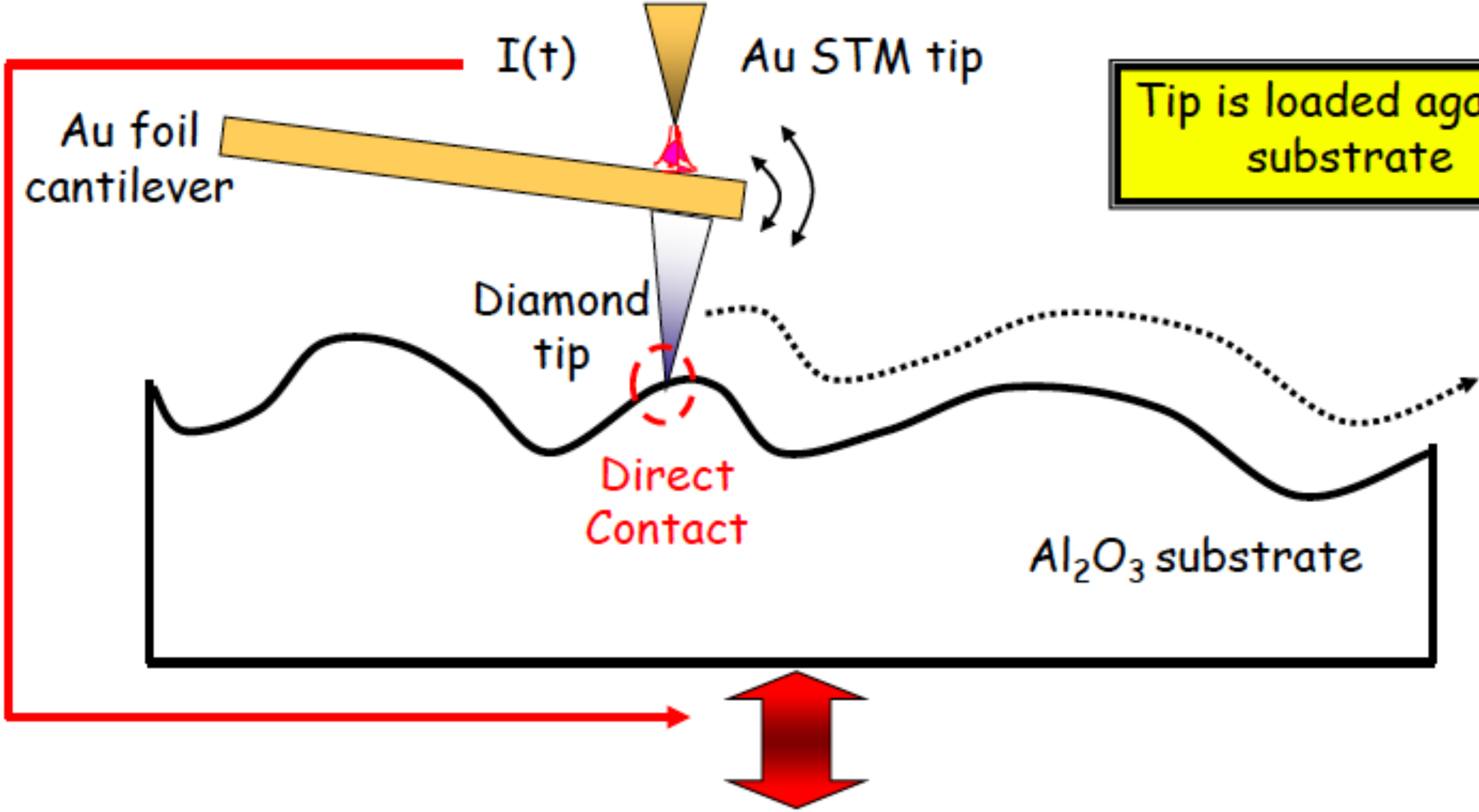
Key Idea: use sensitivity of STM to measure the rise and fall of a tip mounted on a cantilever when rastered across an insulating substrate.



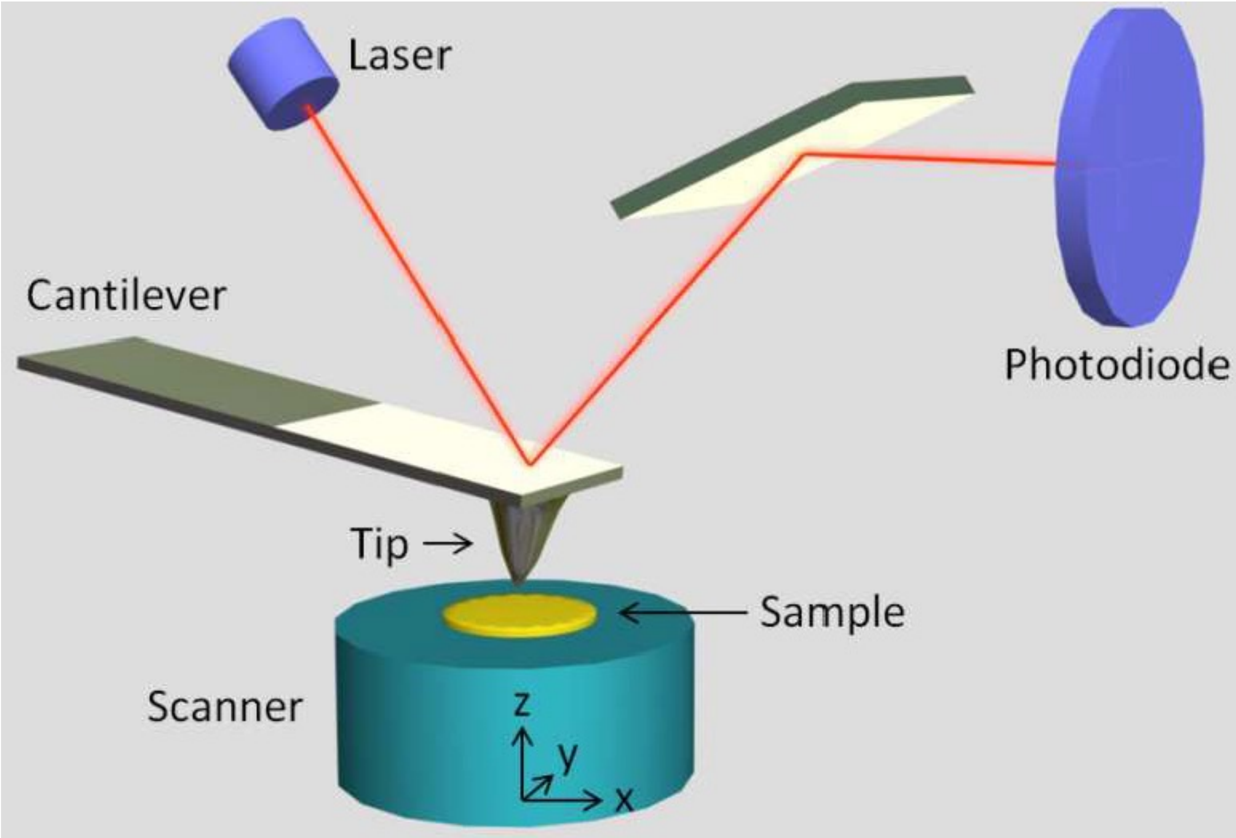






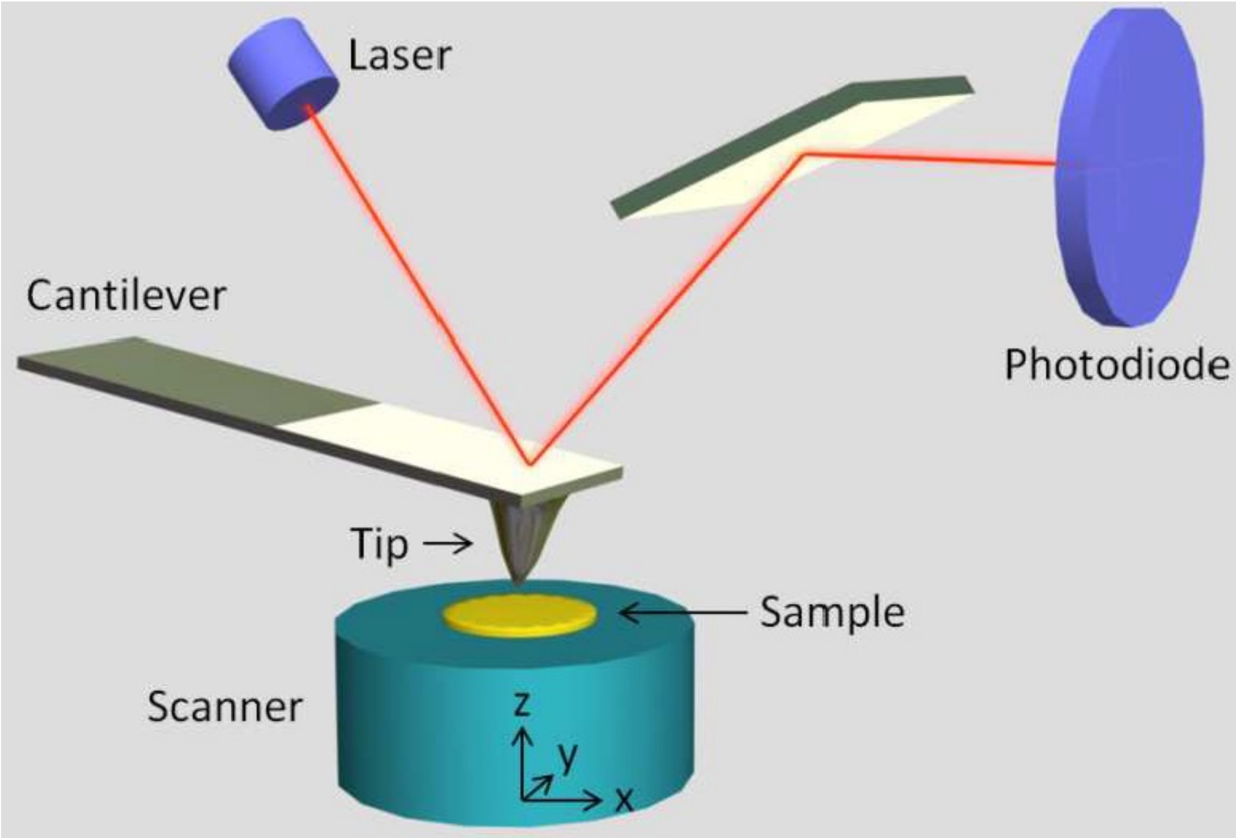
Tip is loaded against substrate

Atomic Force Microscopy



AFM is a mechanical imaging instrument that derives the 3-D profile (topography) and the physical properties of a surface by measuring the INTERACTION FORCES with a scanning, nanometer sized probe.

Atomic Force Microscopy



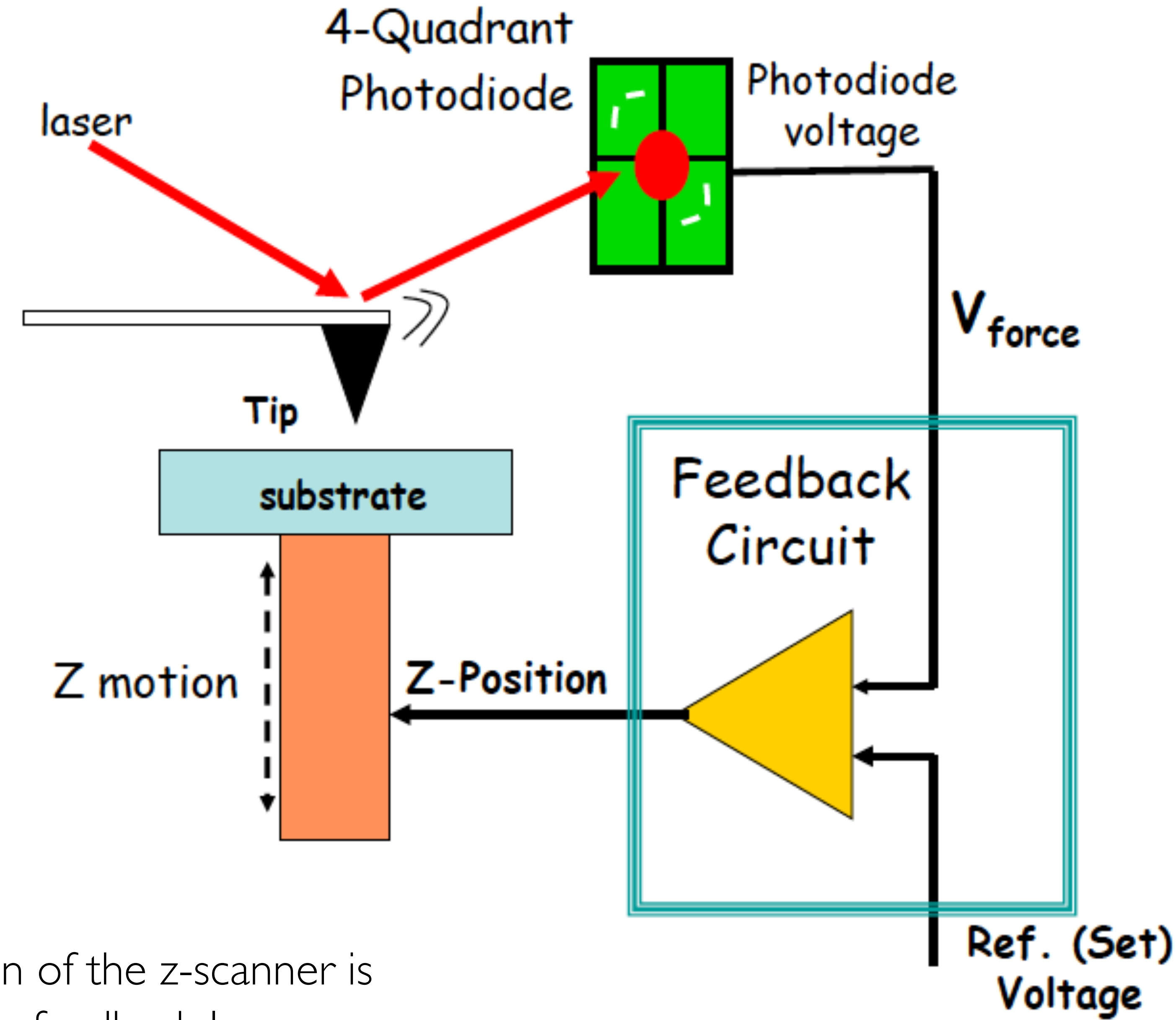
Topographic image of the sample obtained by:

•plotting the deflection of the cantilever versus scanner x,y position (seldom);

•plotting the height position of the translation stage versus versus scanner x,y position.

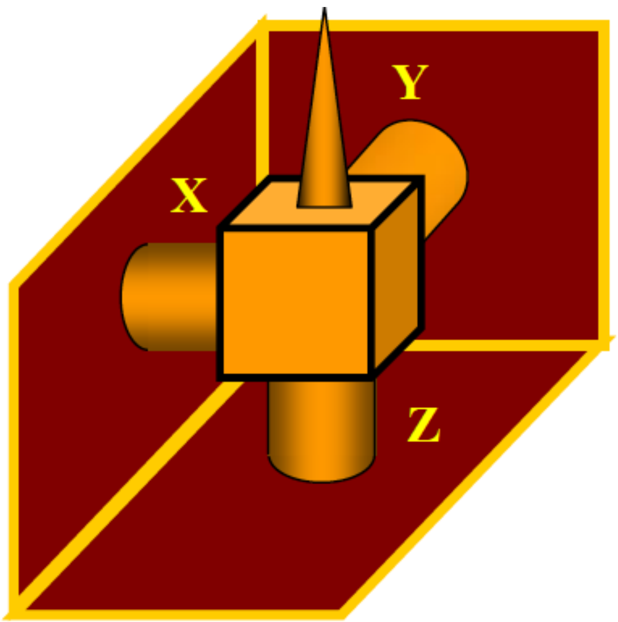
Image contrast arises because the force between the tip and sample is a function of both tip–sample separation and the material properties of tip and sample.

Maintaining a constant force



Height position of the z-scanner is controlled by a feedback loop

Key element of the feedback system: piezoscanner

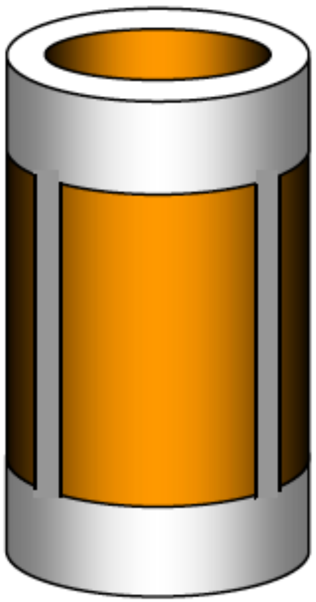


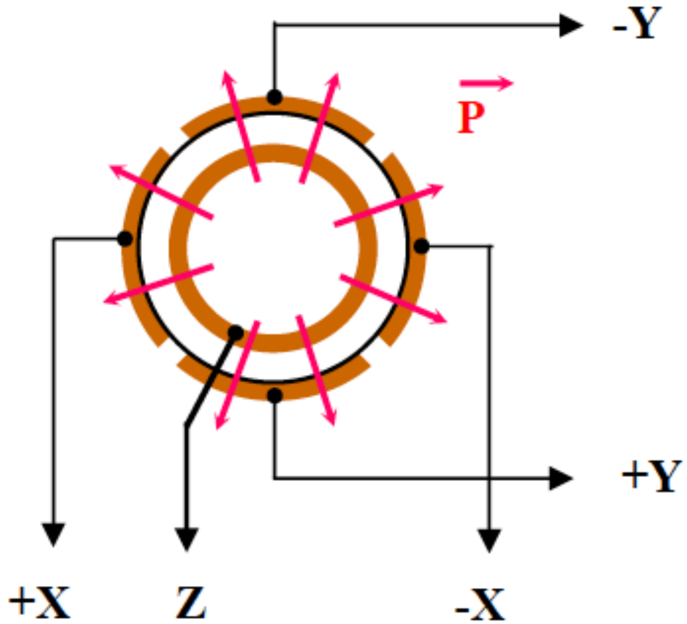
Tripods : strongly asymmetic

Single tube scanner

The polarization vector (ceramic) is radially directed

The external electrode is divided by cylinder generatrixes into four sections: 3D scanning





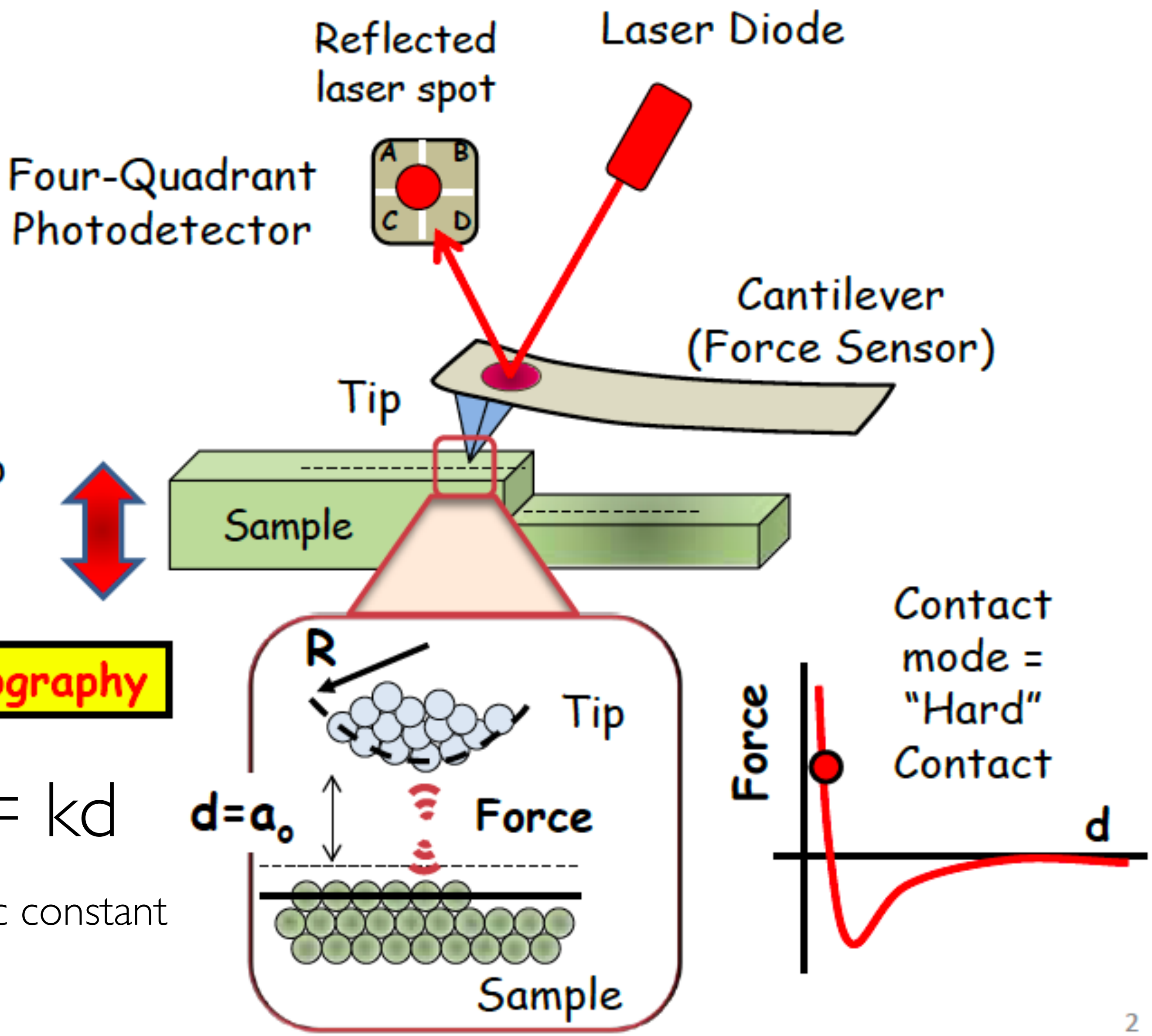
The Purpose of a Microscope is to Obtain an Image

Move sample to keep deflection of cantilever constant

Sample Motion = Topography

$$F = kd$$

K= cantilever elastic constant

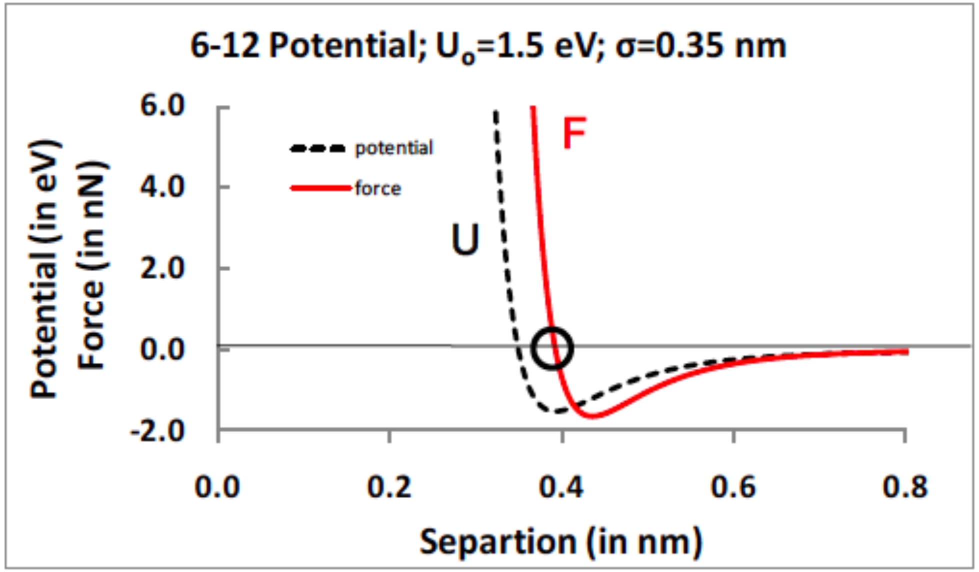


Intermolecular interactions probed by AFM

- Simple *ad hoc* model that tries to couple dispersion forces and Pauli repulsion.

$$U(r) = 4U_o * \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^{6} \right]$$

- U_o is depth of potential, σ is value at which $U_o(r=\sigma)=0$
- F = -dU(r)/dr
- While attractive part follows that from the general dispersion relation, the repulsive part is *adhoc*.



Tip-Substrate Interactions

Electrostatic,magnetic

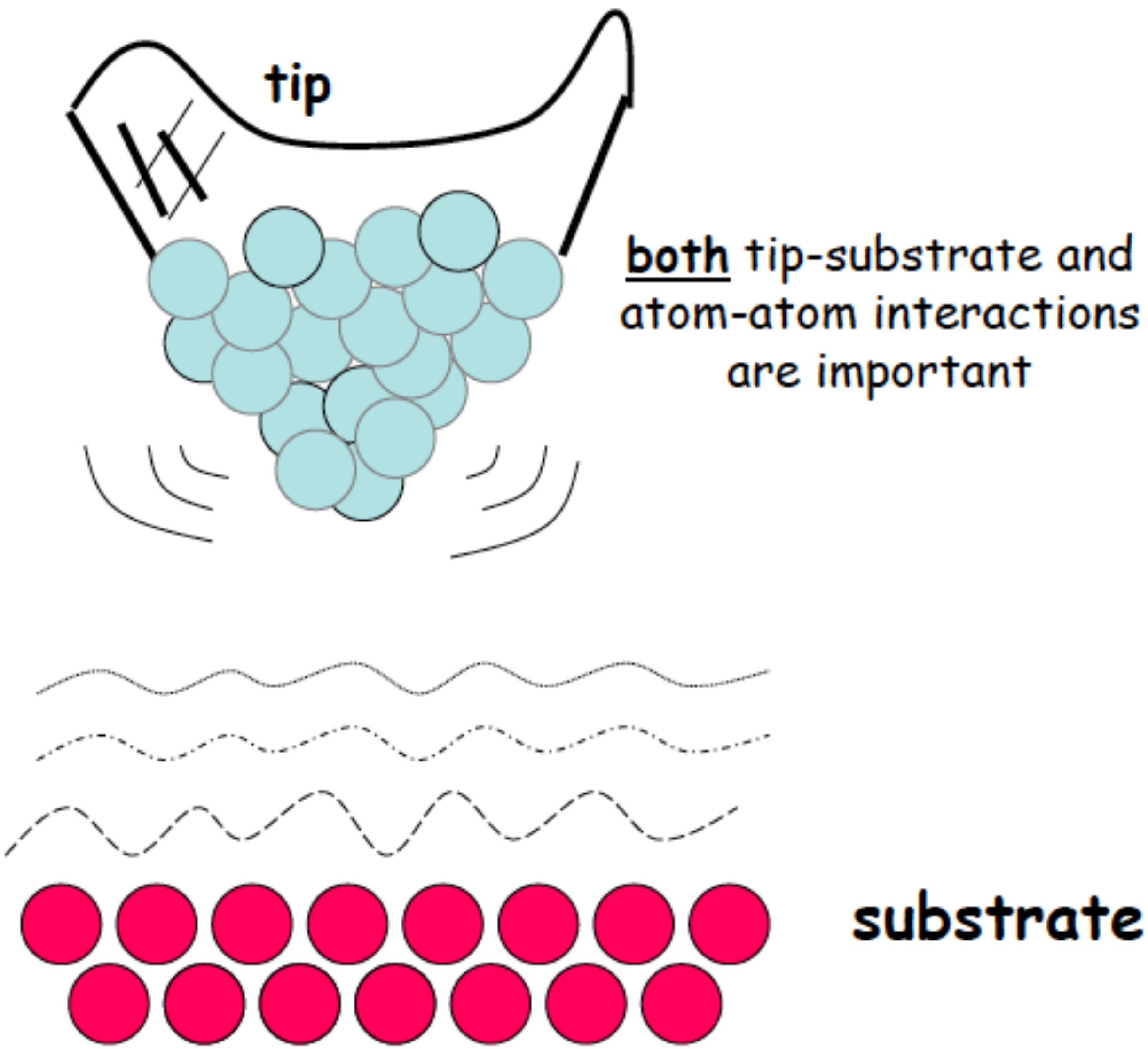
van der Waals (1873)

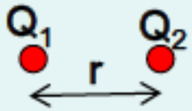
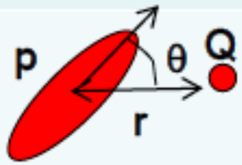
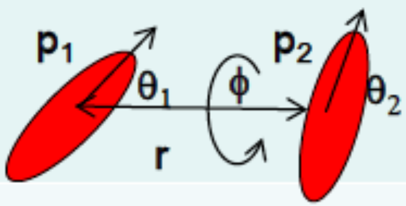
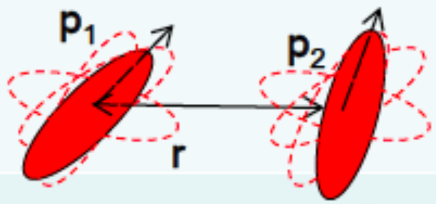
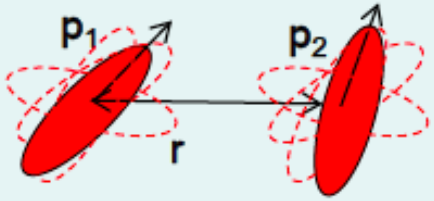
Capillary

Chemical:
(chemisorbed,physisorbed)

Friction

Deformation (indentation)



	Type of interaction
	Ion-ion electrostatic $U(r)=\frac{Q_1Q_2}{4\pi\varepsilon\varepsilon_0 r}$
	Dipole-charge electrostatic $U(r)=-\frac{Qp\cos(\theta)}{4\pi\varepsilon\varepsilon_0 r^2}$
	Dipole-dipole electrostatic $U(r)=-\frac{p_1p_2\left[2\cos(\theta_1)\cos(\theta_2)-\sin(\theta_1)\sin(\theta_2)\cos(\phi)\right]}{4\pi\varepsilon\varepsilon_0 r^3}$
	Angle-averaged electrostatic (Keesom force) $U_{Keesom}(r)=-\frac{p_1^2p_2^2}{3(4\pi\varepsilon\varepsilon_0)^2k_BT}\frac{1}{r^6}$
	Angle-averaged induced polarization force (Debye force) $U_{Debye}(r)=-\frac{p_1^2\alpha_{02}+p_2^2\alpha_{01}}{(4\pi\varepsilon_0\varepsilon)^2}\frac{1}{r^6}$
	Dispersion forces act between any two molecules or atoms (London force) $U_{London}(r)=-\frac{3}{2}\frac{\alpha_{01}\alpha_{02}}{(4\pi\varepsilon_0\varepsilon)^2}\frac{(I_1)(I_2)}{I_1+I_2}\frac{1}{r^6}$

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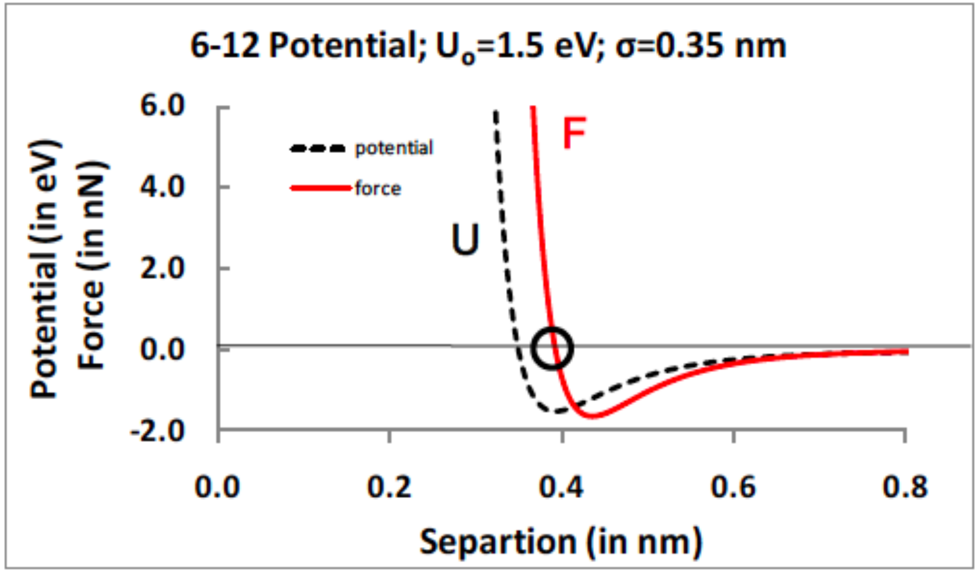

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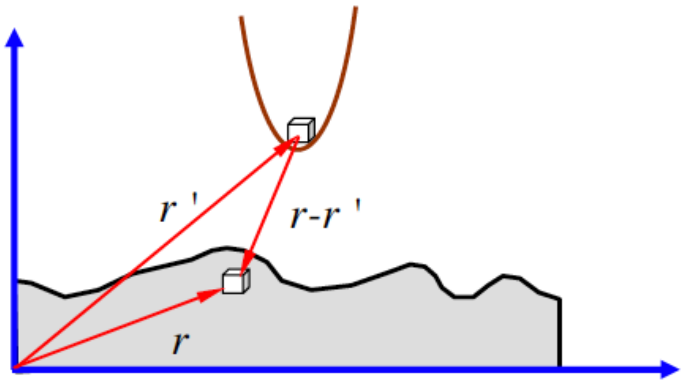
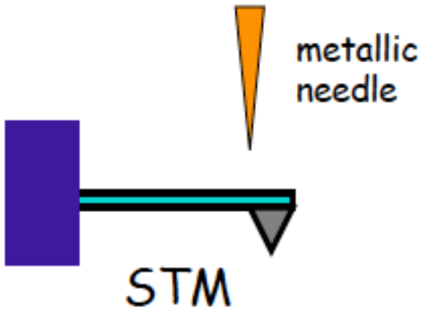


Fig. 61. How to calculate the energy of interaction between tip and sample atoms

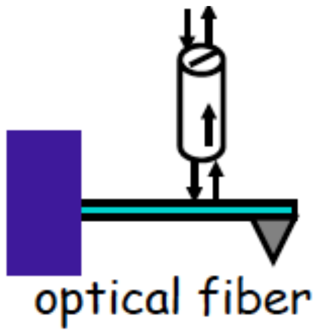
$$W_{PS} = \iint_{V_P V_S} U_{LD}(r - r')\, n_P(r')\, n_S(r)\, dV\, dV'$$

$n_{p,s}$ (r’,r) are the densities of atoms in tip and sample

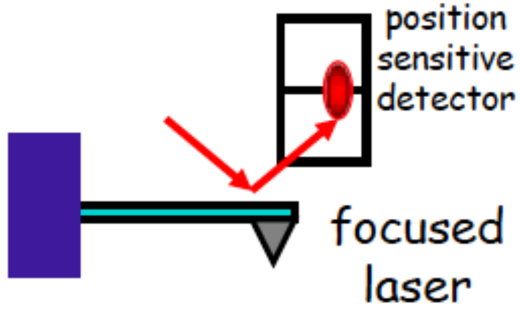
AFM: the deflection detection system



Binnig *et al.*, Phys. Rev. Lett. **56** (1986)

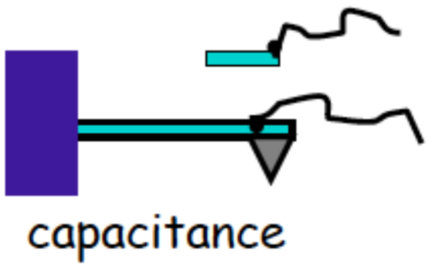


Martin *et al.*, J. App. Phys. **95** (1987)

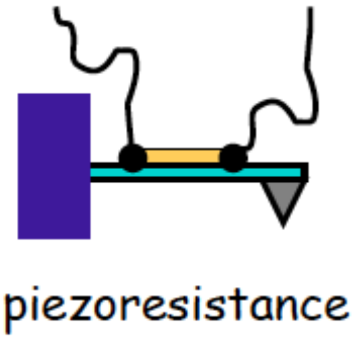


Marti *et al.*, J. Microscopy, **152** (1986)

G. Meyer and N. Amer, Appl. Phys. Lett. **53** (1988)



Göddenhenrich *et al.*, J. Vac. Sci. Technol. **A8** (1990)



Tortonese *et al.*, App. Phys. Lett. **62** (1993)

AFM: the deflection detection system

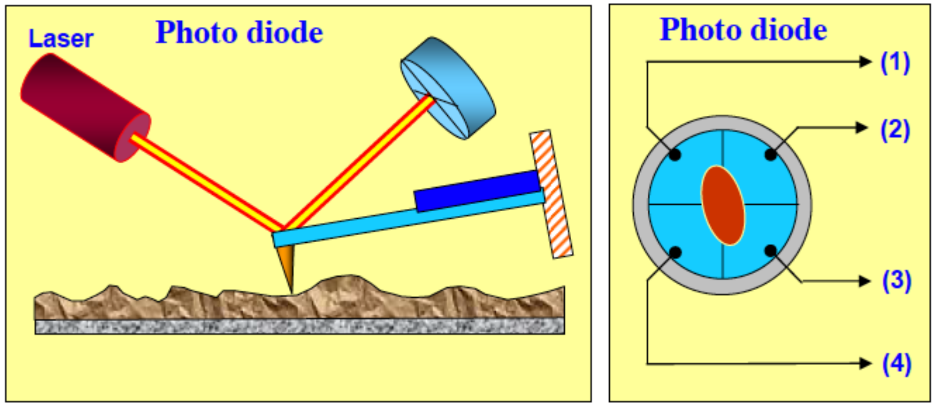


Fig. 62. Schematic description of the optical system to detect the cantilever bending

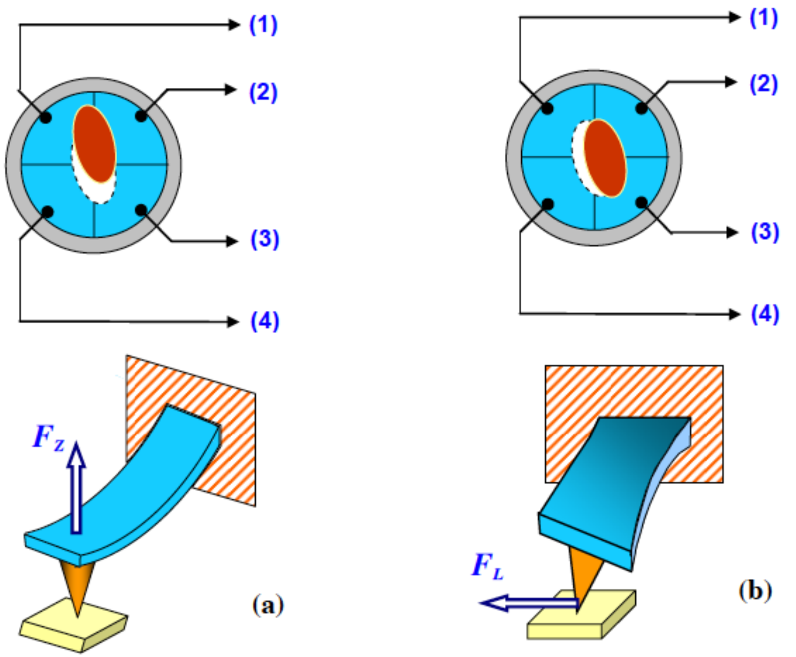


Fig. 63. Relation between the types of the cantilever bending deformations (bottom) and the change of the spot position on the split photodiode (top)

Normal forces:

$$\Delta I_z = (\Delta I_1 + \Delta I_2) - (\Delta I_3 + \Delta I_4)$$

Lateral forces:

$$\Delta I_L = (\Delta I_1 + \Delta I_4) - (\Delta I_2 + \Delta I_3)$$

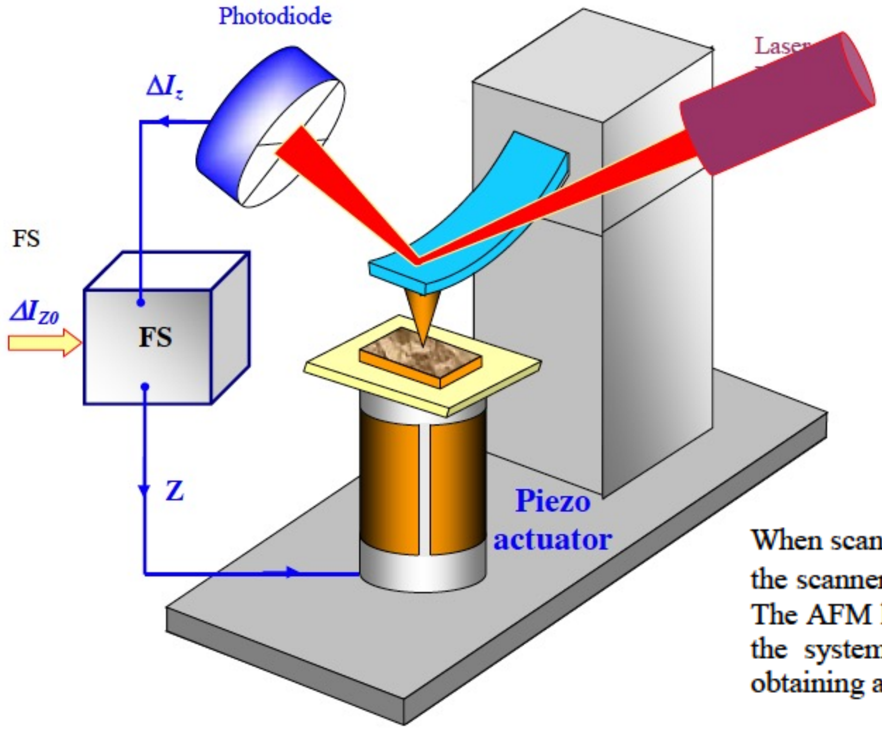
With ΔI_Z used as input to the feedback loop

The feedback system (FS) keeps ΔI_Z = const with th help of a piezoelectric transducer (scanner), which controls the tip-sample distance in order to make the bending ΔZ equal to the value ΔZ_0 preset by the operator.

AFM: the deflection detection system

ΔI_Z is used as input to the feedback loop

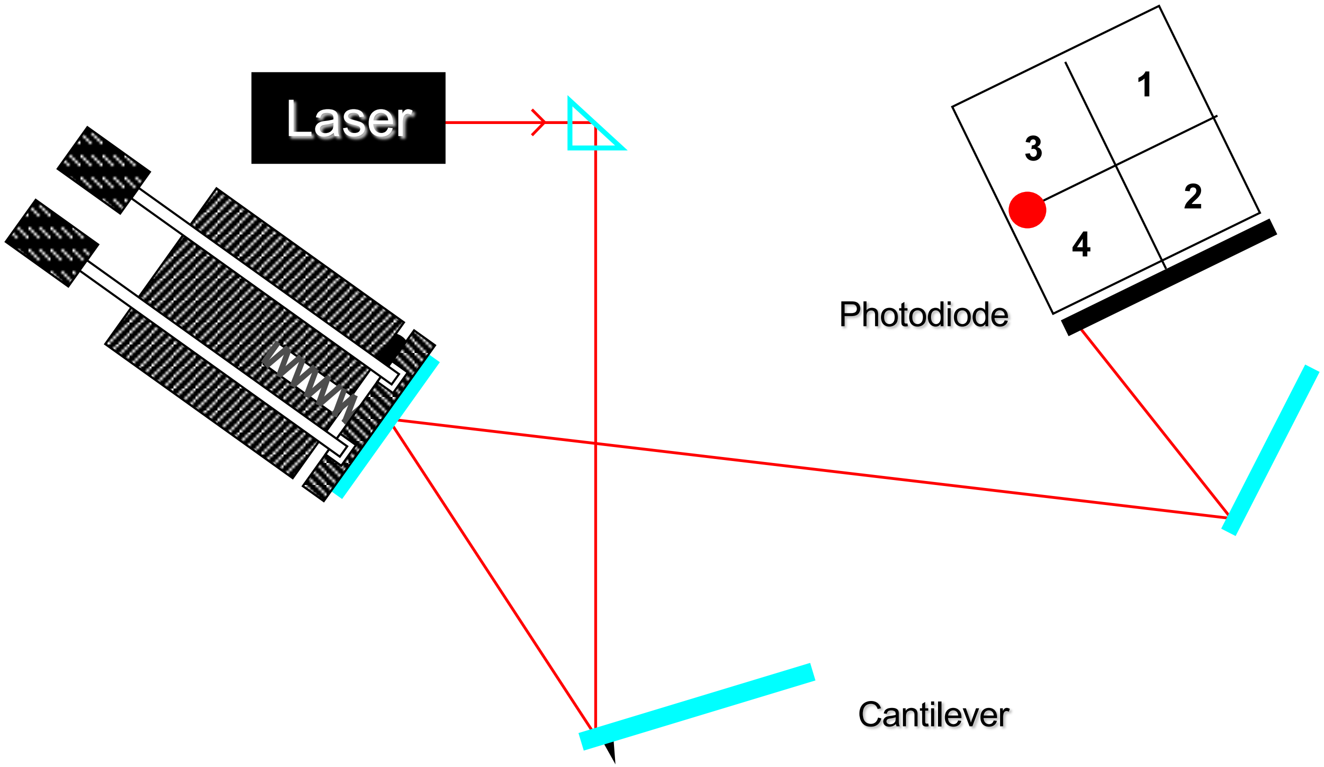
The feedback system (FS) keeps ΔI_Z = const with the help of a piezoelectric transducer (scanner), which controls the tip-sample distance in order to make the bending ΔZ equal to the value ΔZ_0 preset by the operator.

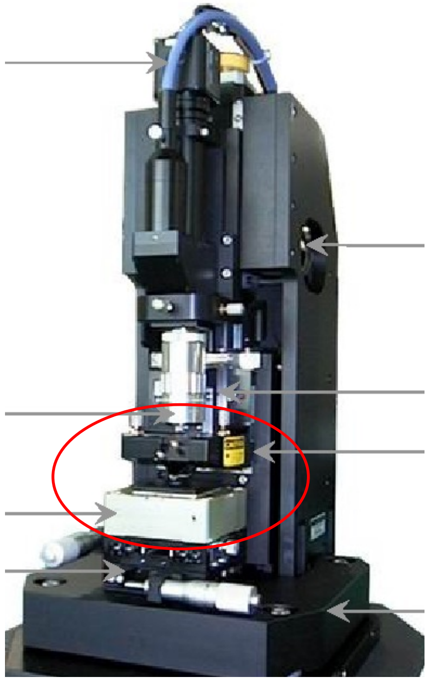


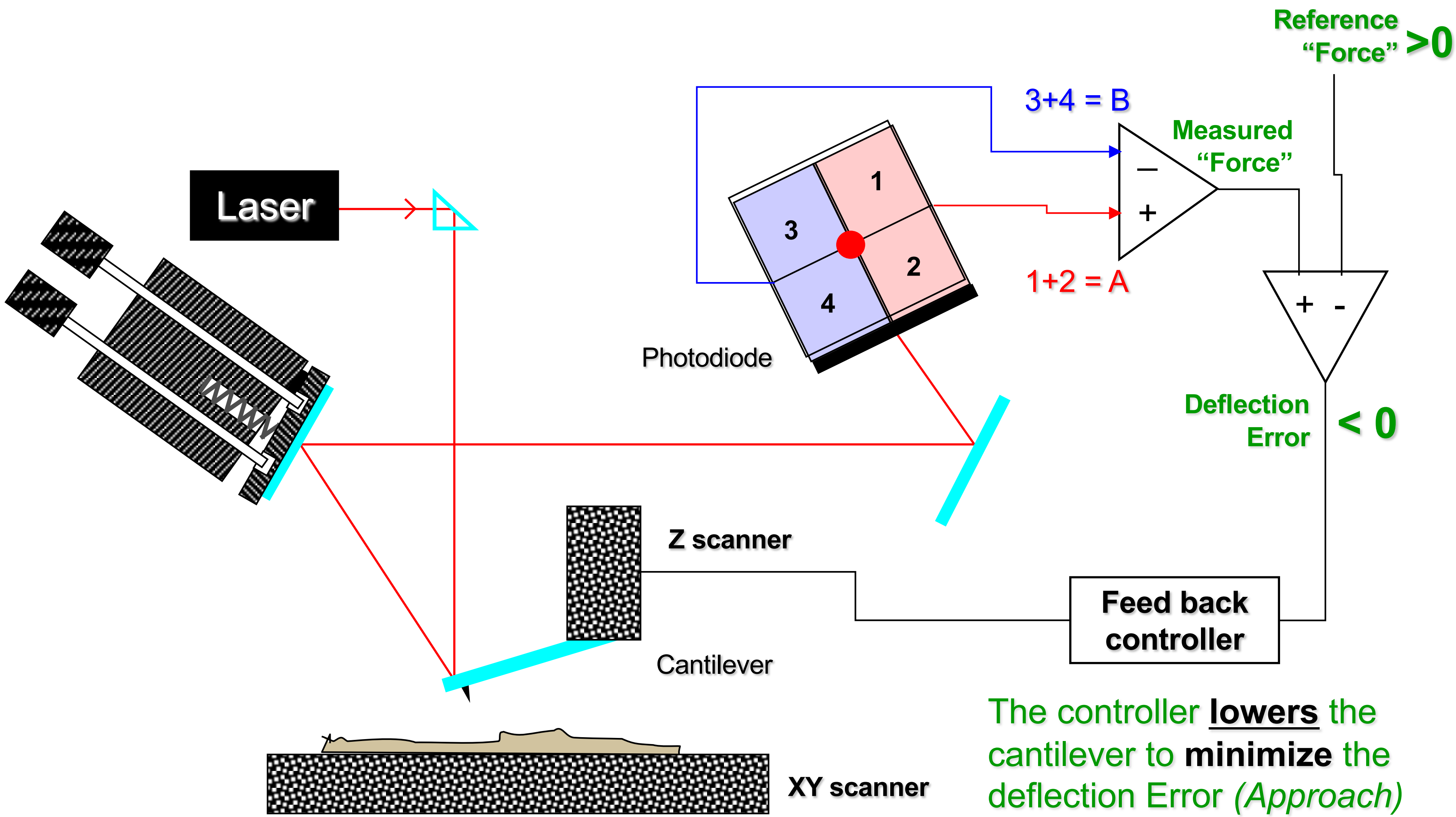
When scanning a sample in a $\Delta Z = const$ mode the tip moves along the surface, thus the voltage on the scanner Z-electrode is recorded in the computer memory as a surface topography $Z = f(x, y)$. The AFM lateral resolution is defined by the radius of curvature of the tip and by the sensitivity of the system in detecting the cantilever deviations. Currently the AFM are designed to allow obtaining atomic resolution.

Fig. 64. Simplified scheme of the feedback in an optical lever detection AFM

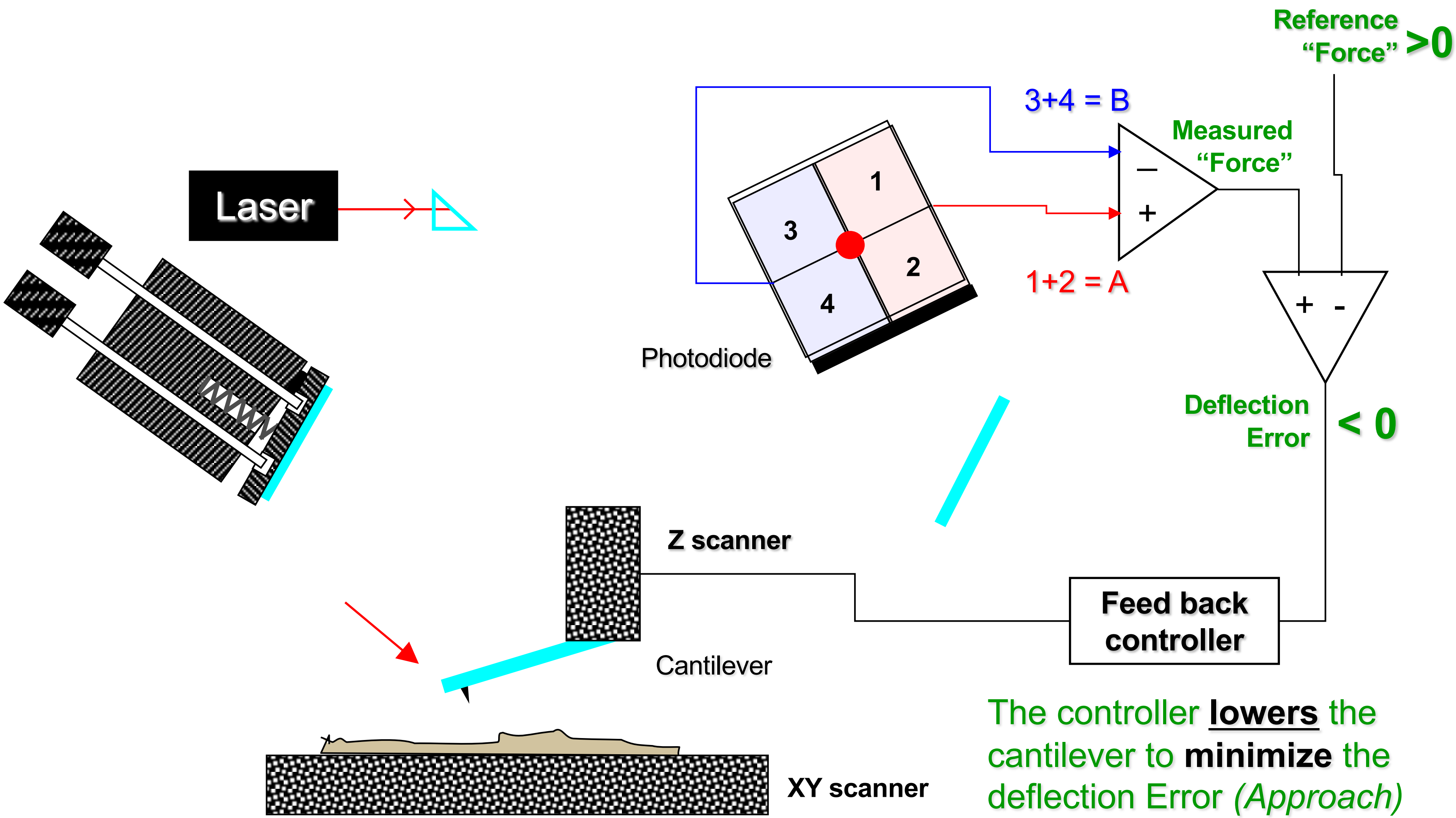
Atomic **F**orce **M**icroscope: *PSIA XE-100 Park system*





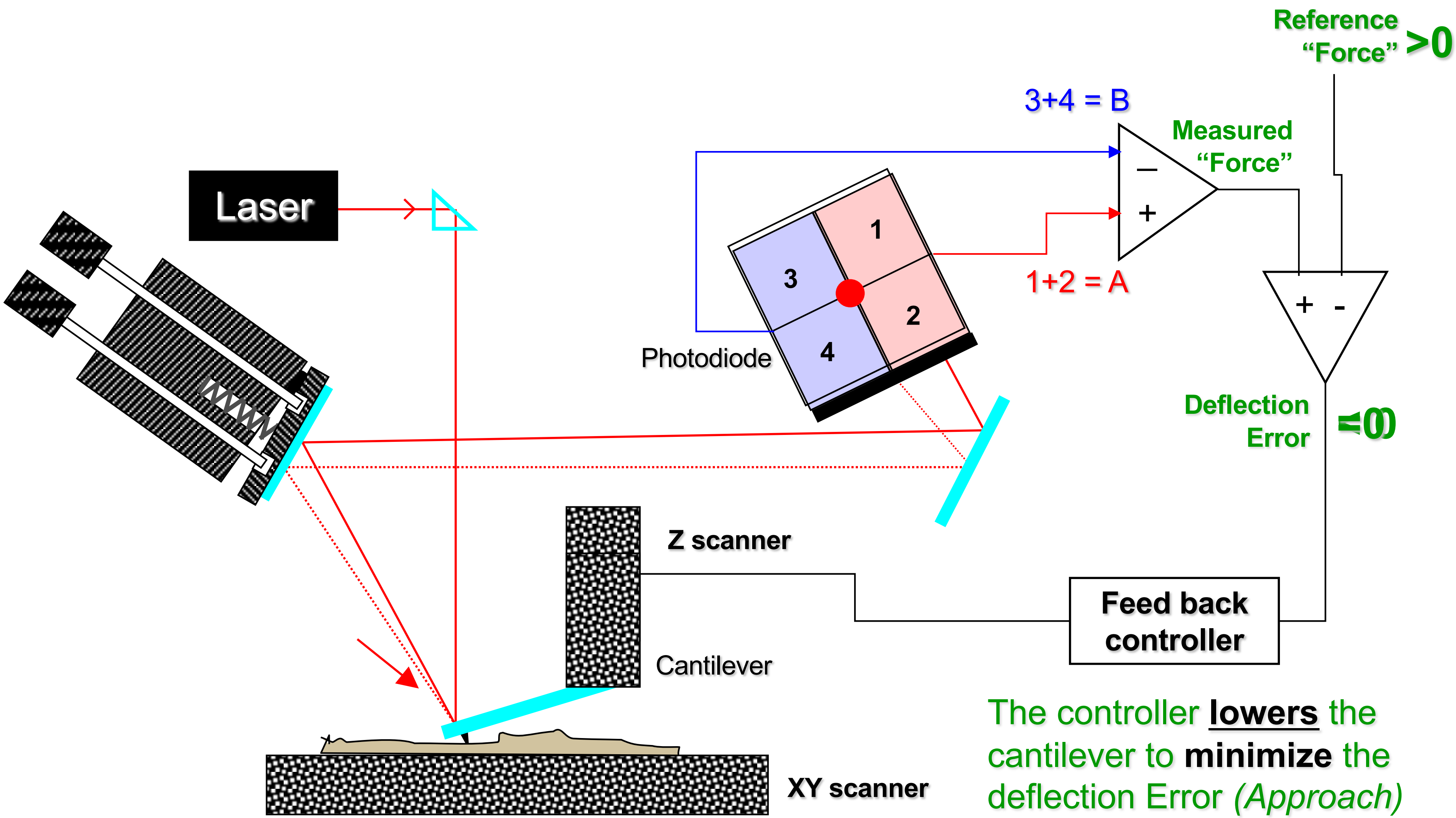

Reference
"Force" >0
3+4 = B
Measured
"Force"
Laser
1
3
2
4
1+2 = A
+
-
Photodiode
Deflection
Error
< 0
Z scanner
Feed back
controller
Cantilever
XY scanner
The controller **lowers** the cantilever to **minimize** the deflection Error *(Approach)*

Force Measurement



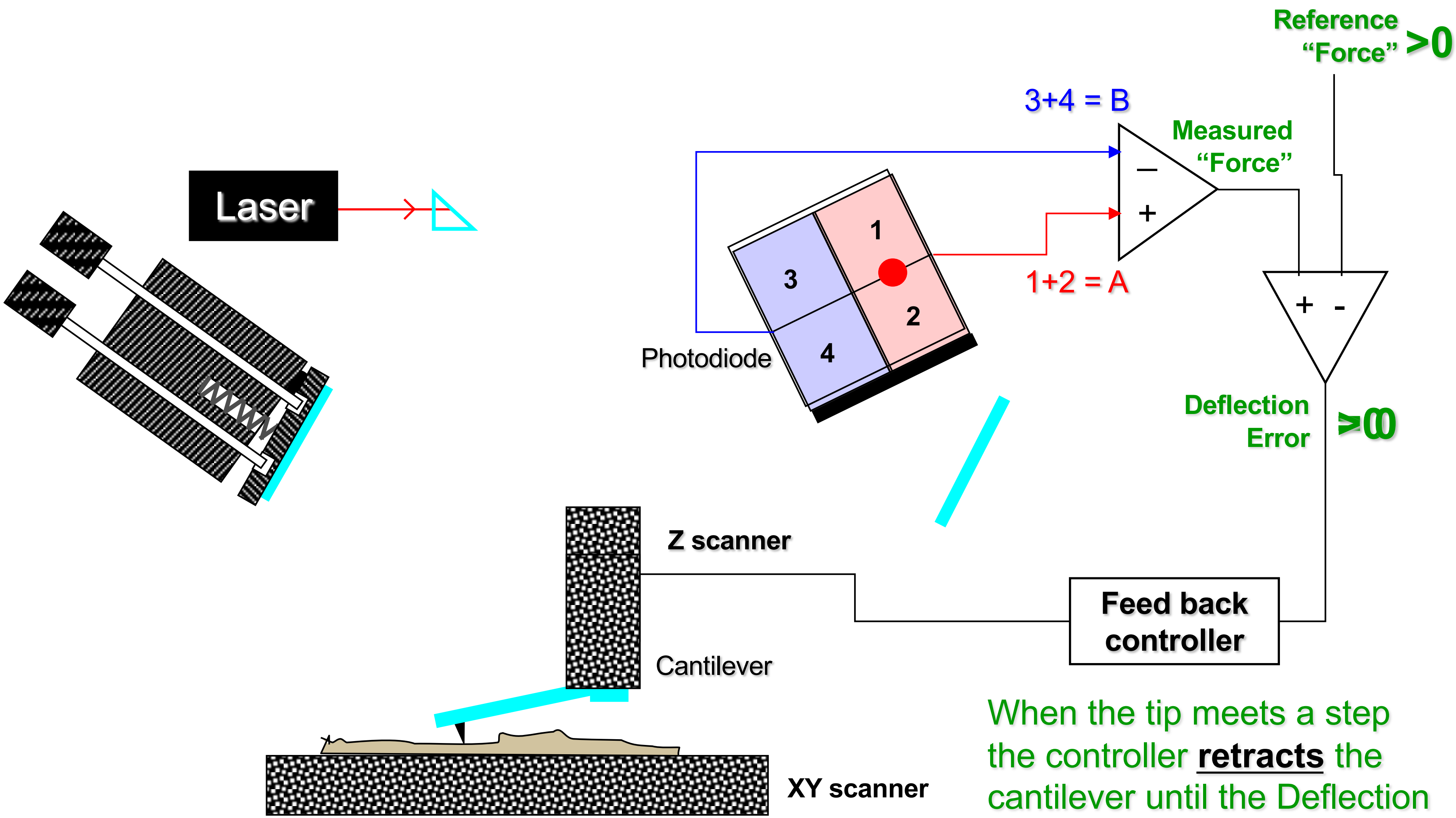
The controller **lowers** the cantilever to **minimize** the deflection Error *(Approach)*

Force Measurement



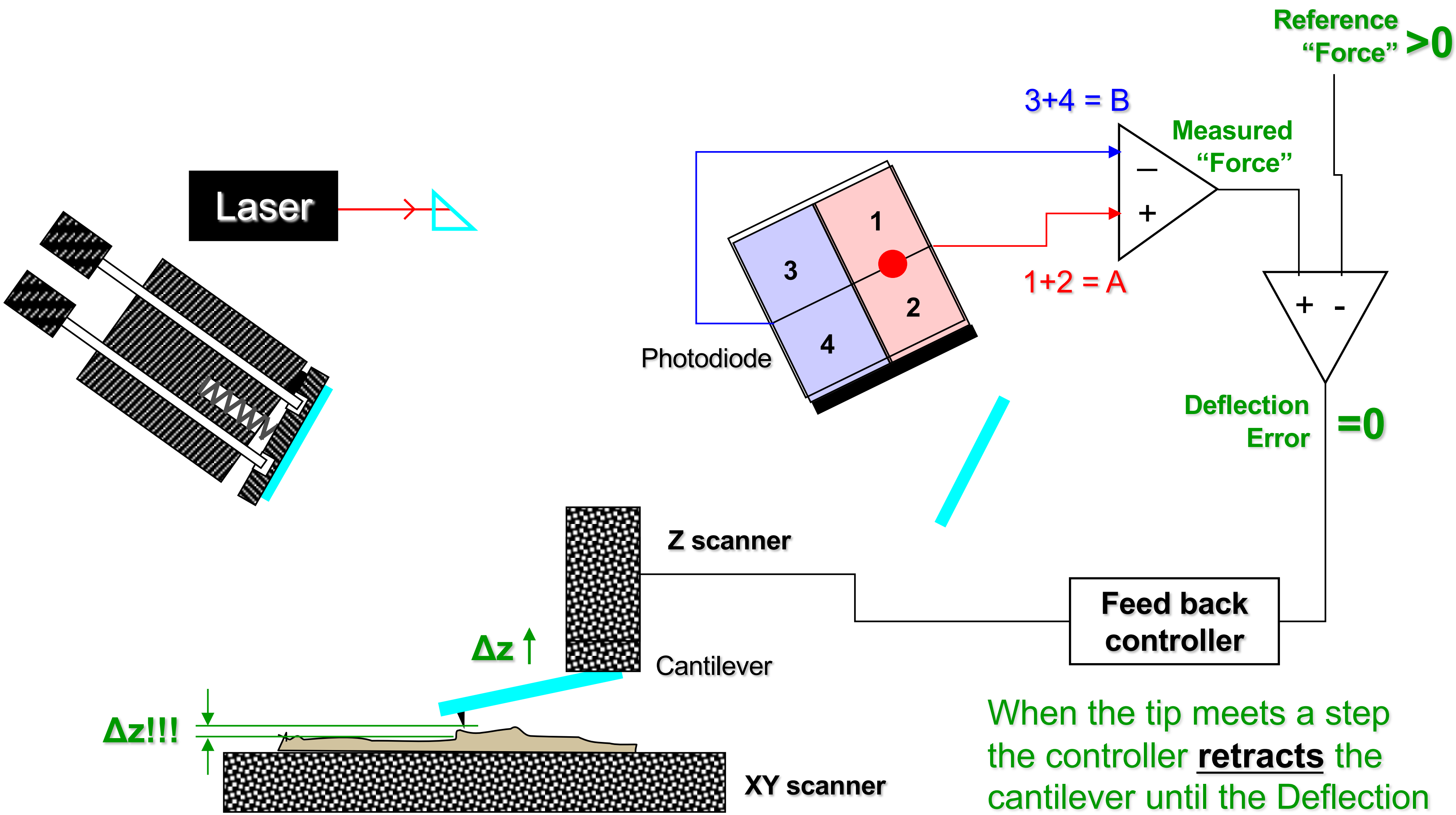
The controller **lowers** the cantilever to **minimize** the deflection Error *(Approach)*

Scanning Microscopy in Contact Mode



When the tip meets a step the controller **retracts** the cantilever until the Deflection Error is **again zero**

Scanning Microscopy in Contact Mode



When the tip meets a step the controller **retracts** the cantilever until the Deflection Error is **again zero**

The **Movement** of the **z-scanner** is recorded as the **Topographic Signal**

AFM probes

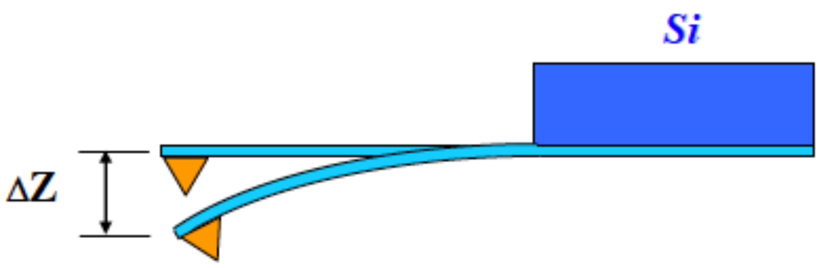


Fig. 65. Schematic picture of the AFM probe

The curvature radius of AFM tip apex is of the order of 1 ÷ 50 nanometers, depending on the type and on the technology of manufacturing.

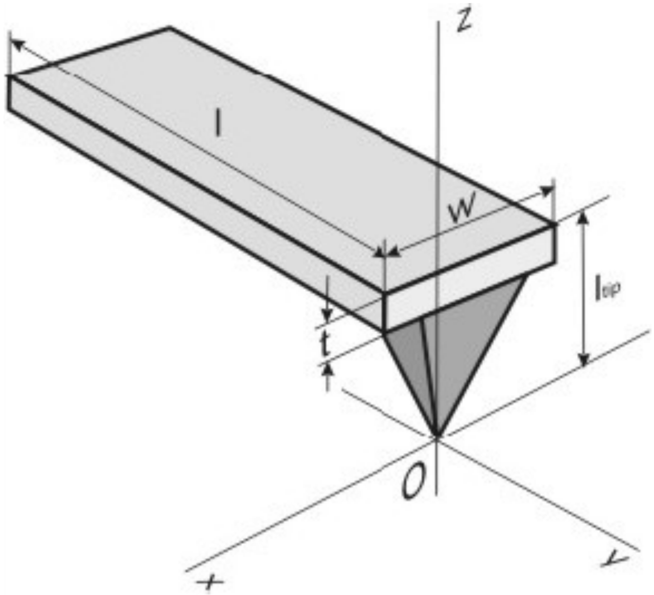
The angle near the tip apex is 10 ÷ 20°.

The interaction force F of a tip with the surface can be estimated from the Hooke law:

$$F = k \cdot \Delta Z$$

where k is the cantilever elastic constant; ΔZ is the tip displacement corresponding to the bending produced by the interaction with the surface. The k values vary in the range $10^{-3} \div 10$ N/m depending on the cantilever material and geometry.

AFM probes



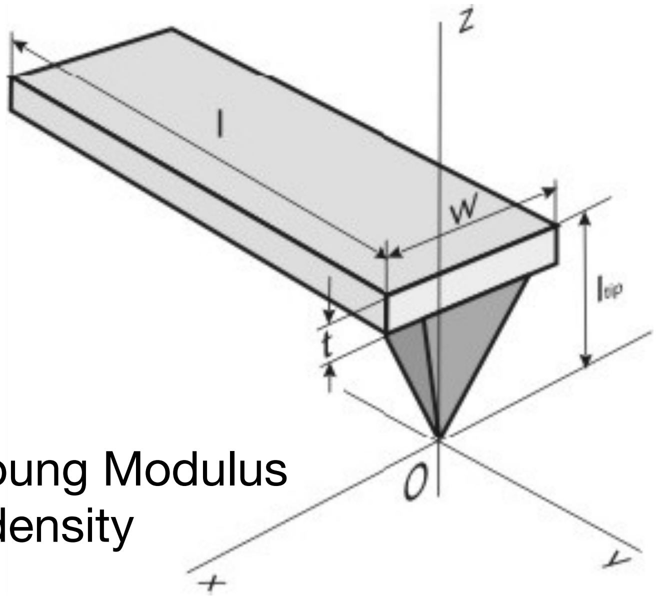
E= Young Modulus
ρ = density

$$k_c = \frac{F}{Z_c} = \frac{Ewt_c^3}{4L^3}.$$

A good cantilever should have a high sensitivity. High sensitivity in Zc is achieved with low spring constants or low ratio tc/L.

Typical E value: 1.5×10^{11} N m^{-2} in silicon nitride

AFM probes



E= Young Modulus

ρ = density

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} = \frac{1}{4\pi}\frac{t}{l^2}\sqrt{\frac{E}{\rho}}$$

External vibrations, such as vibrations of the building, the table, or noise, which are usually in the low frequency regime, are less transmitted to the cantilever when its frequency is high ---- t/l^2 should then be high!

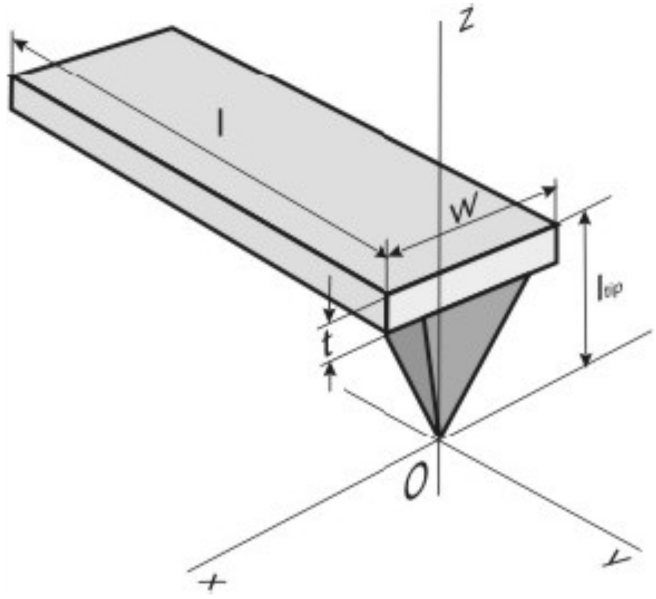
A high resonance frequency is also important to be able to scan fast ---- the resonance frequency limits the time resolution

$$k_c = \frac{F}{Z_c} = \frac{Ewt_c^3}{4L^3}.$$

A good cantilever should have a high sensitivity. High sensitivity in Zc is achieved with low spring constants or low ratio tc/L.

Typical E value: 1.5×10^{11} N m^{-2} in silicon nitride

AFM probes



$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} = \frac{1}{4\pi}\frac{t}{l^2}\sqrt{\frac{E}{\rho}}$$

Hence, the optimal design of a cantilever is a compromise between different factors. Depending on the application the appropriate dimensions and materials are chosen. Cantilevers for AFM AC modes are usually V shaped to increase their lateral stiffness.

They are typically L = 100–200 μm long, each arm is about W=40 μm wide and tc = 0.5–1 μm thick. Typical resonance frequencies are 20–200 kHz in air.
Cantilever for fast imaging are shorter L = 10 μm, thin tc = 0.2–0.3 μm and have resonances of 2 MHz

$$k_c = \frac{F}{Z_c} = \frac{Ewt_c^3}{4L^3}.$$

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} = \frac{1}{4\pi}\frac{t}{l^2}\sqrt{\frac{E}{\rho}}$$

AFM probes

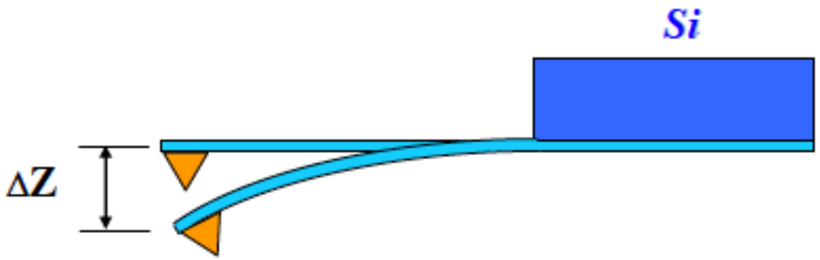


Fig. 65. Schematic picture of the AFM probe

The curvature radius of AFM tip apex is of the order of 1 ÷ 50 nanometers, depending on the type and on the technology of manufacturing.

The angle near the tip apex is 10 ÷ 20°.

The cantilever resonant frequency is important during AFM operation in oscillating modes. Self frequencies of cantilever oscillations are determined by the following formula:

$$\omega_{ri} = \frac{\lambda_i}{l^2}\sqrt{\frac{EJ}{\rho S}}$$

l cantilever length; **E** Young's modulus;
J inertia moment of the cantilever cross-section;
ρ the material density; **S** the cross section;
$\boldsymbol{\lambda_i}$ a numerical coefficient (1÷100), depending on oscillations mode.

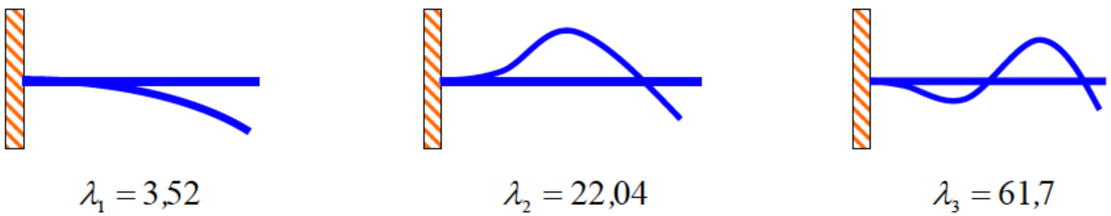
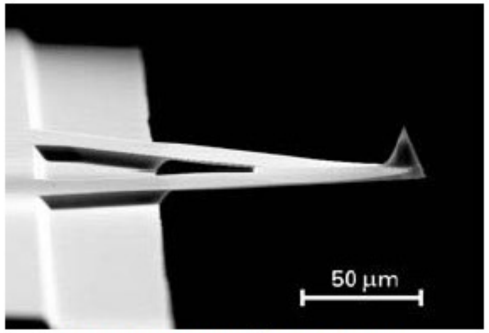


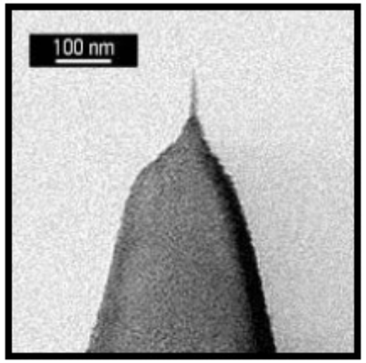
Fig. 66. Main cantilever oscillations modes

Frequencies are usually in the 10÷1000 kHz range. The quality factor Q of cantilevers mainly depends on the media in which they operate. Typical values of Q in vacuum are $10^3 - 10^4$. In air drops to 300 – 500, in a liquid it falls down to 10 – 100.

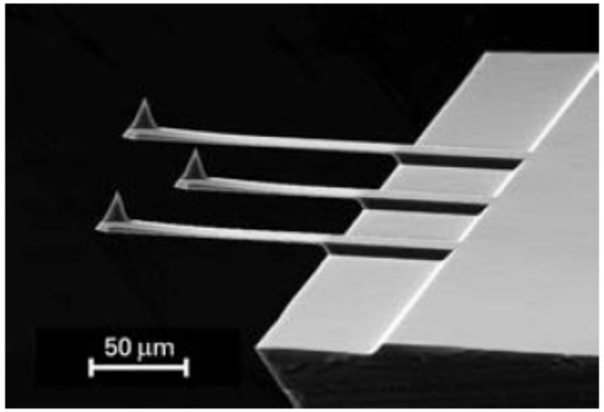
AFM probes



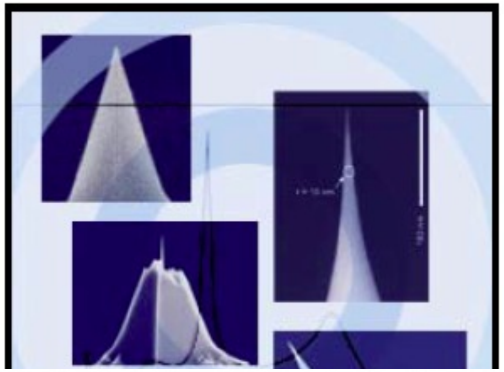
www.spmtips.com



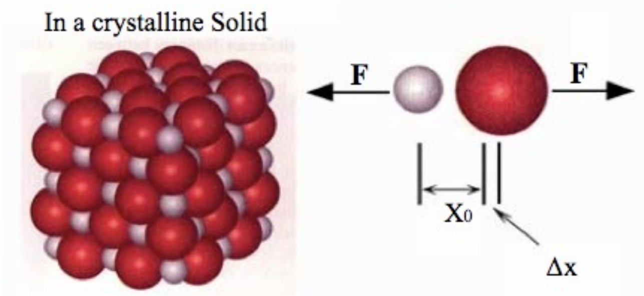
µmasch



www.spmtips.com

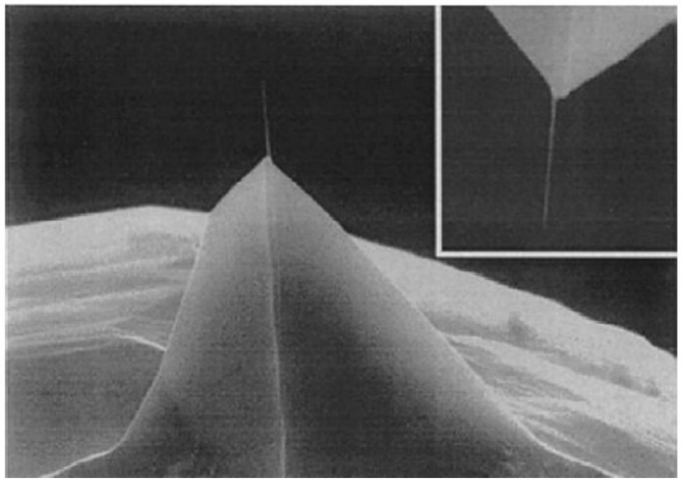


Typical use	k (N/m)	f_o (kHz)
Non-contact	10-100	100-300
Intermittent contact	1-10	20-100
Contact	0.1-1	1-50



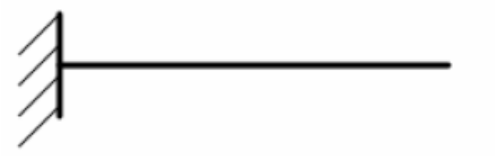
$F = k\Delta x$

$k \sim 10$ N/m


CNT tip

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

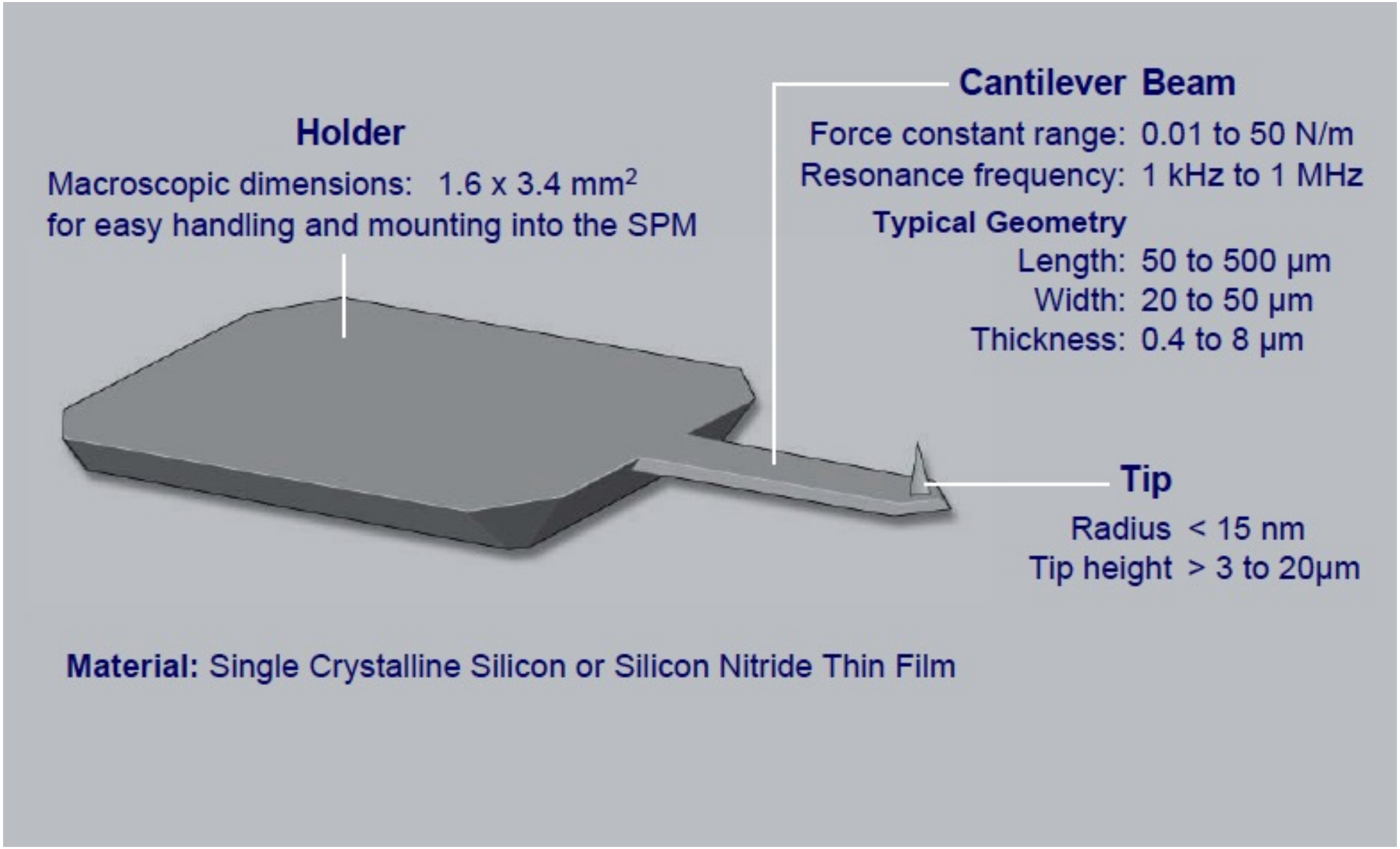


$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4 \rho}}$$

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

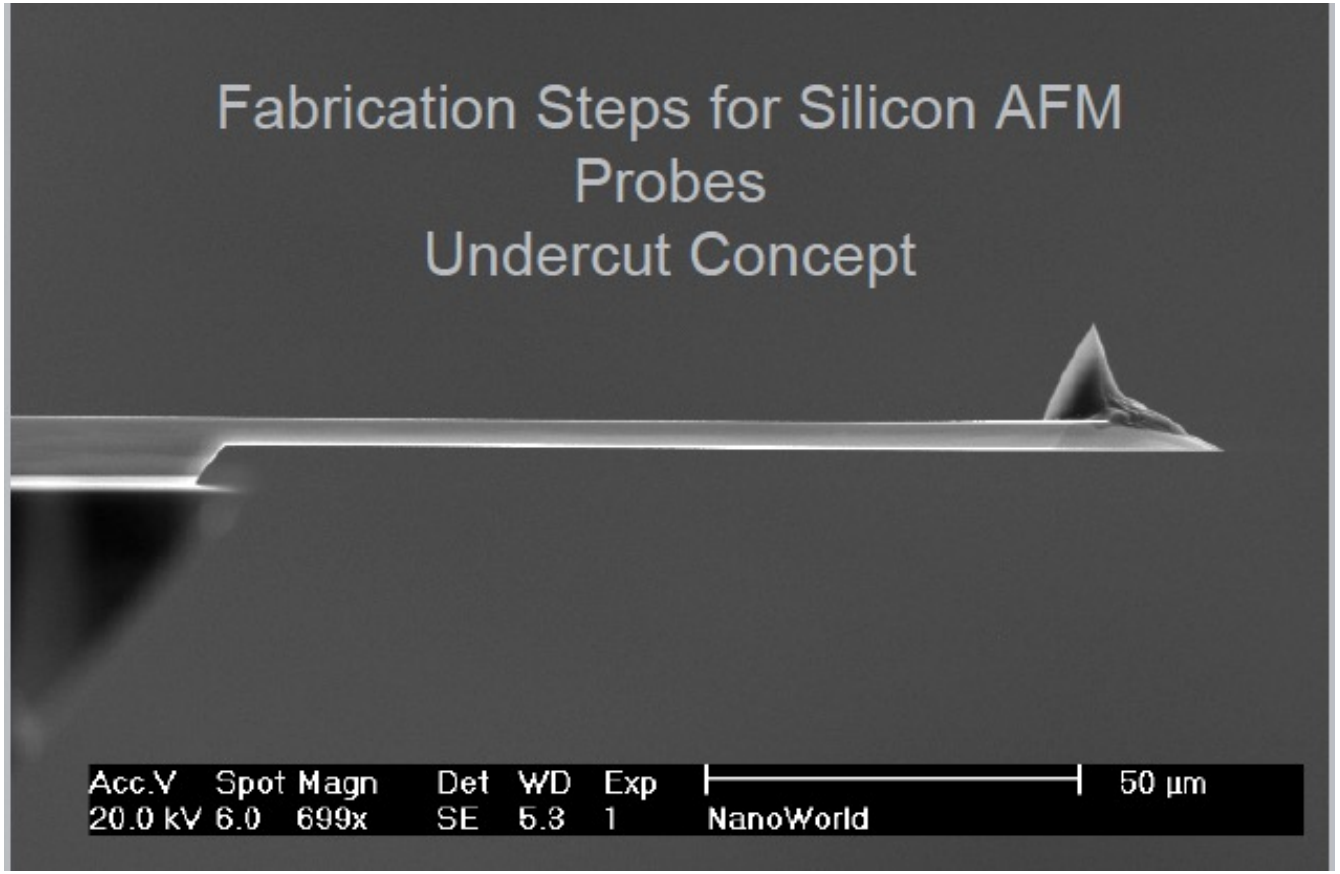
make cantilevers short to increase ω_0 and thinner to restore k

AFM probes



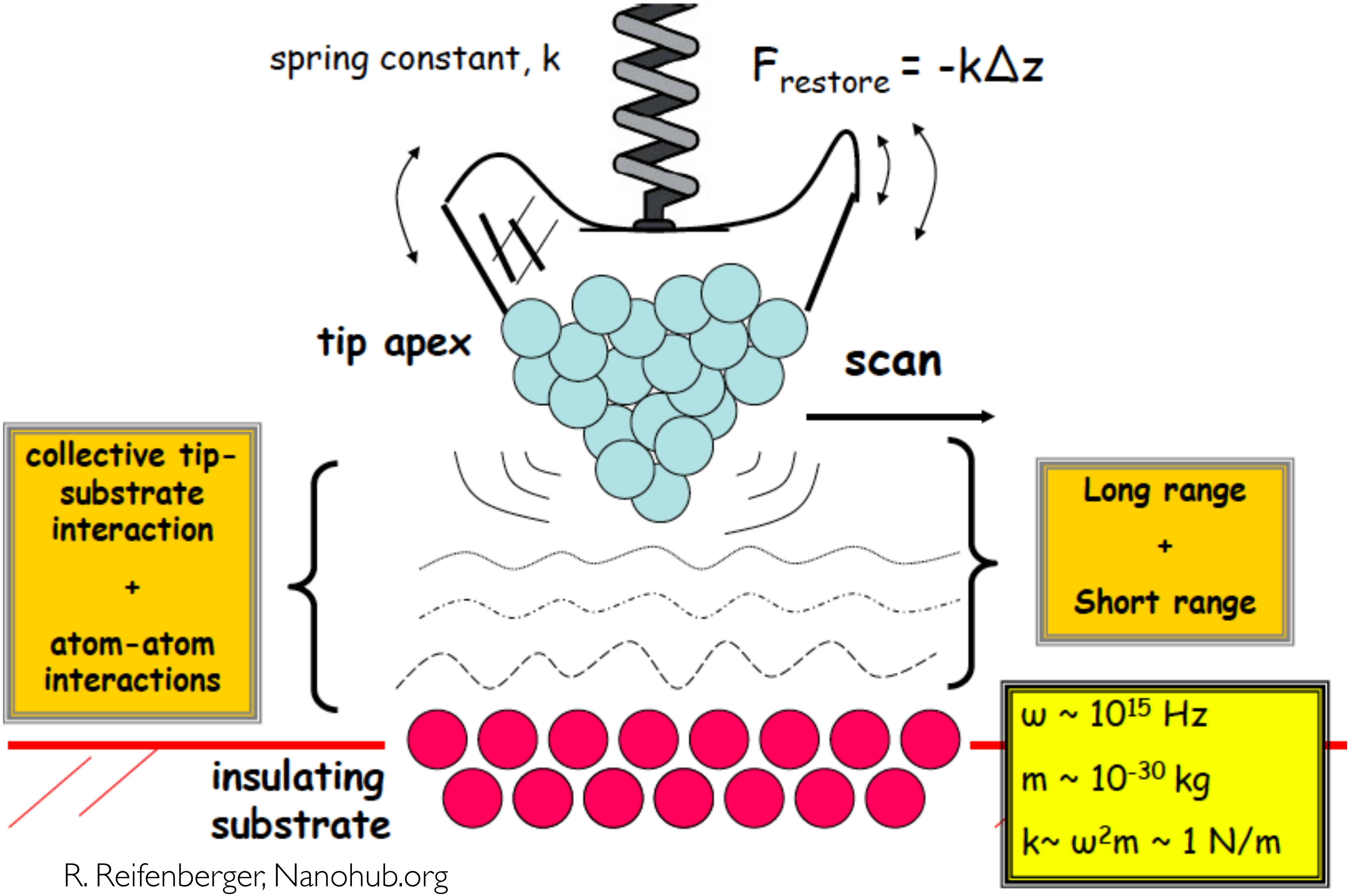
Holder
Macroscopic dimensions: 1.6 x 3.4 mm²
for easy handling and mounting into the SPM
Cantilever Beam
Force constant range: 0.01 to 50 N/m
Resonance frequency: 1 kHz to 1 MHz
Typical Geometry
Length: 50 to 500 μm
Width: 20 to 50 μm
Thickness: 0.4 to 8 μm
Tip
Radius < 15 nm
Tip height > 3 to 20μm
Material: Single Crystalline Silicon or Silicon Nitride Thin Film

AFM probes



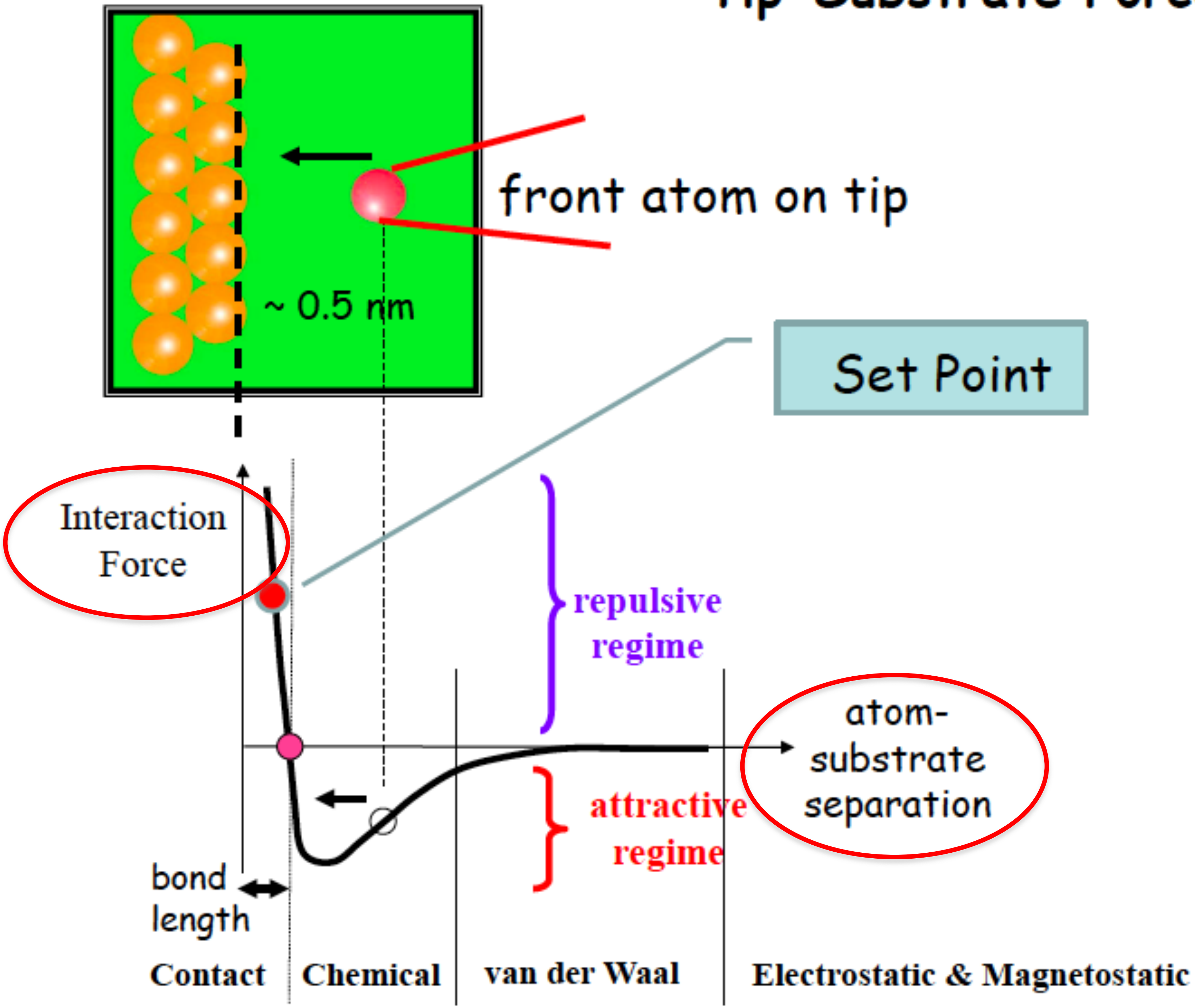
SEE NANO WORLD SLIDES

What controls the atomic force?



R. Reifenberger, Nanohub.org

Tip-Substrate Forces



AFM imaging modes

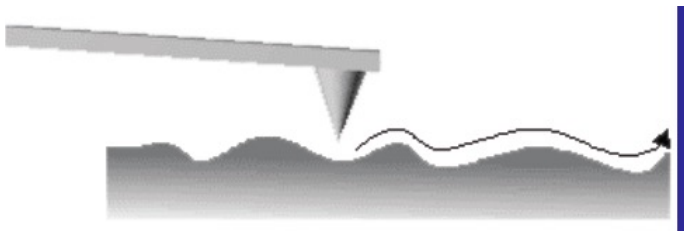
Contact Mode:

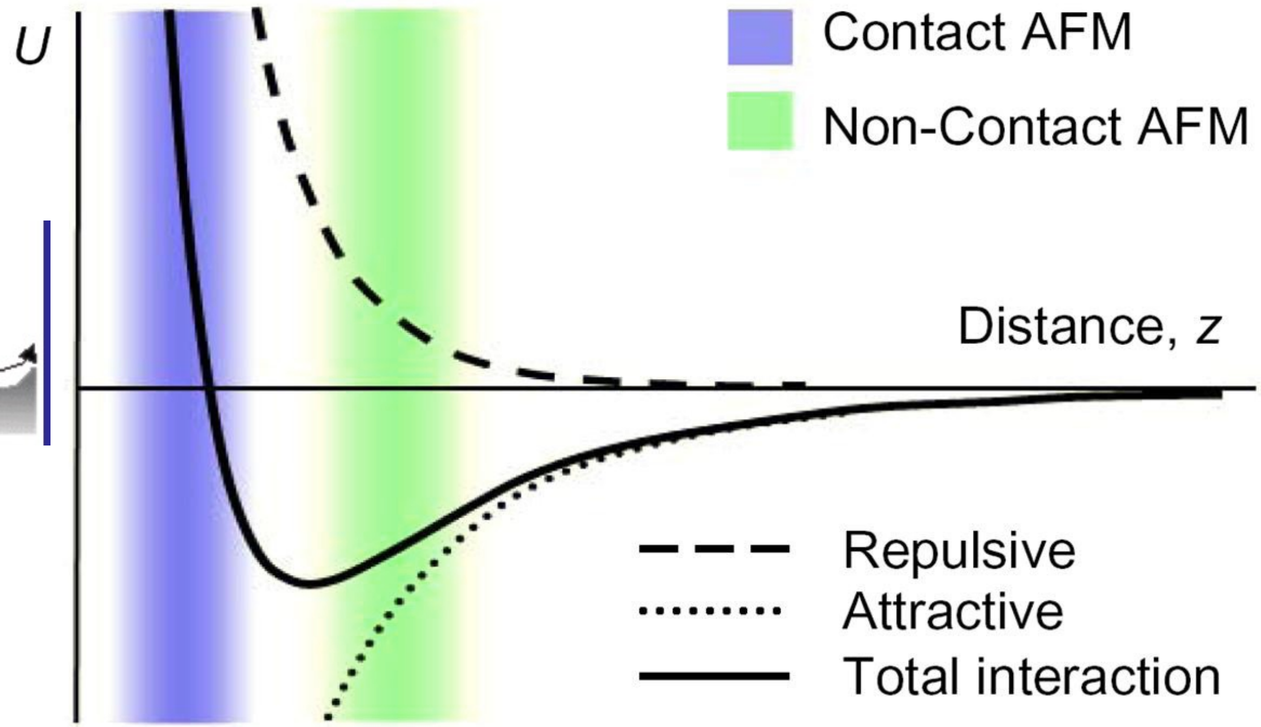
d < 5 Å

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

Atomic resolution

Problems: frictional forces, capillary fo





Non-Contact Mode:

d = 10÷100 Å

Actractive forces - ~10^{-12} N

Soft, elestic materials

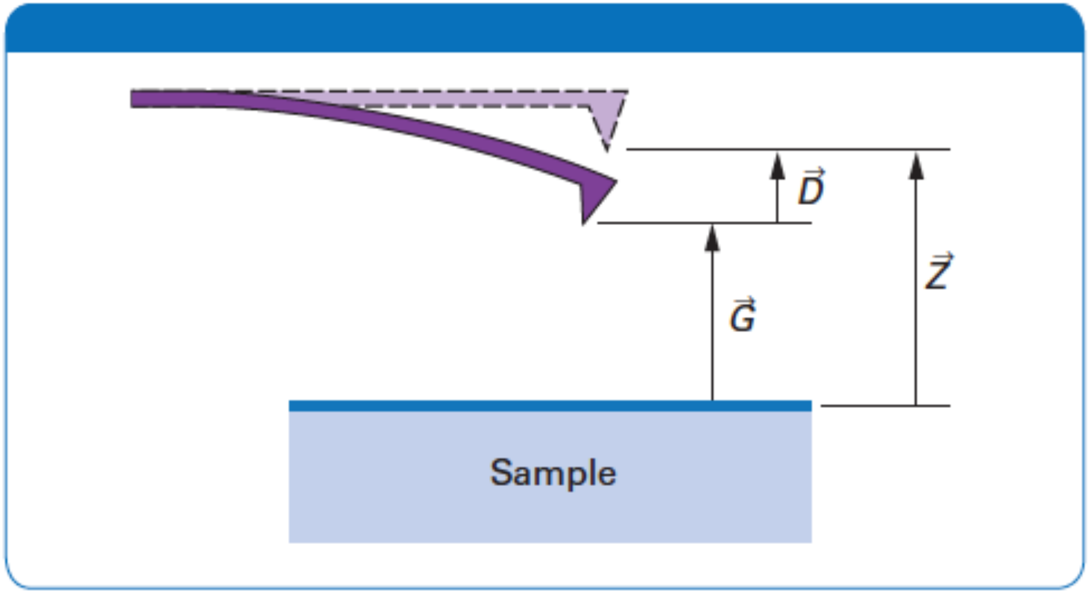
Tapping Mode:

d = 5÷20 Å

Intermitting contact

Big scanning areas, no friction

AFM contact imaging mode



tip-sample gap G = Z-D.

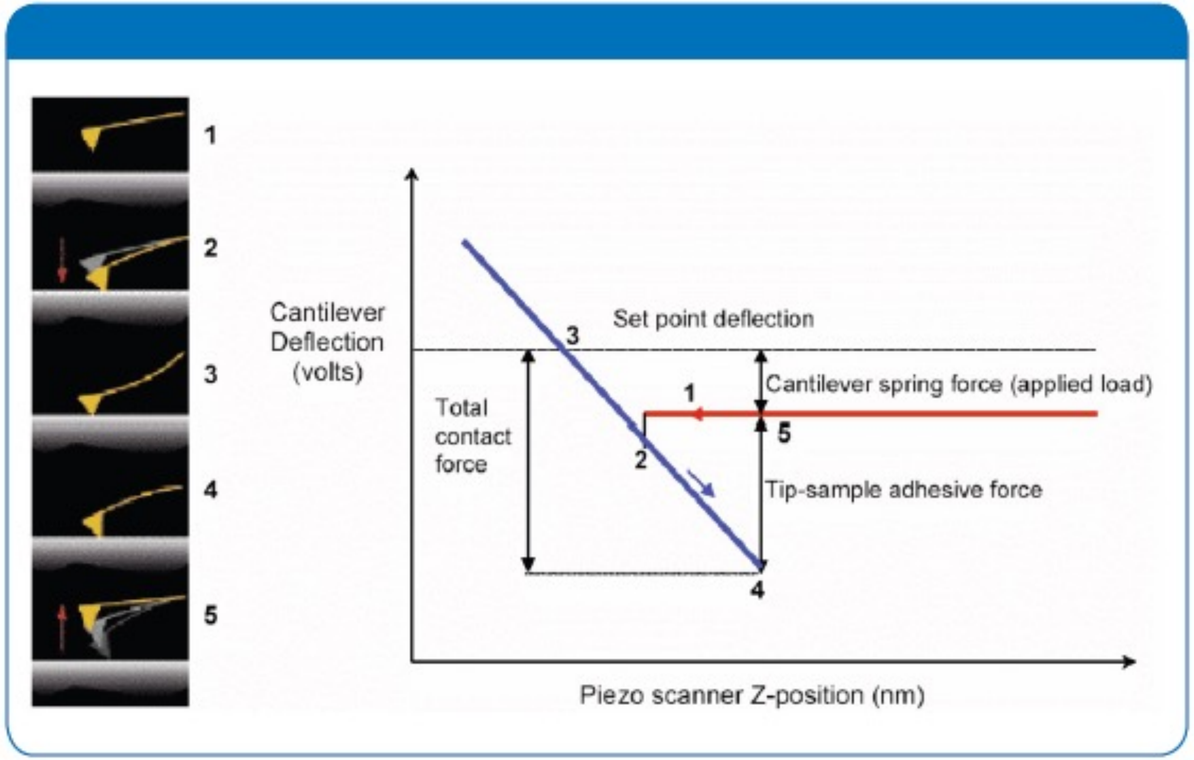
D deflection of the free end of the lever due to tip-sample interactions.

G directly proportional to the applied force action on the cantilever.

With that setup, **the lever motion becomes proportional to the movement of the laser beam on the split photodetector amplified by B = 3s/l,** with s being the distance between the cantilever and photodetector, and l the cantilever length.

By increasing the distance, the spot size of the beam on the detector also increases, in turn making the actual sensitivity of the system independent of l and proportional to 1/s

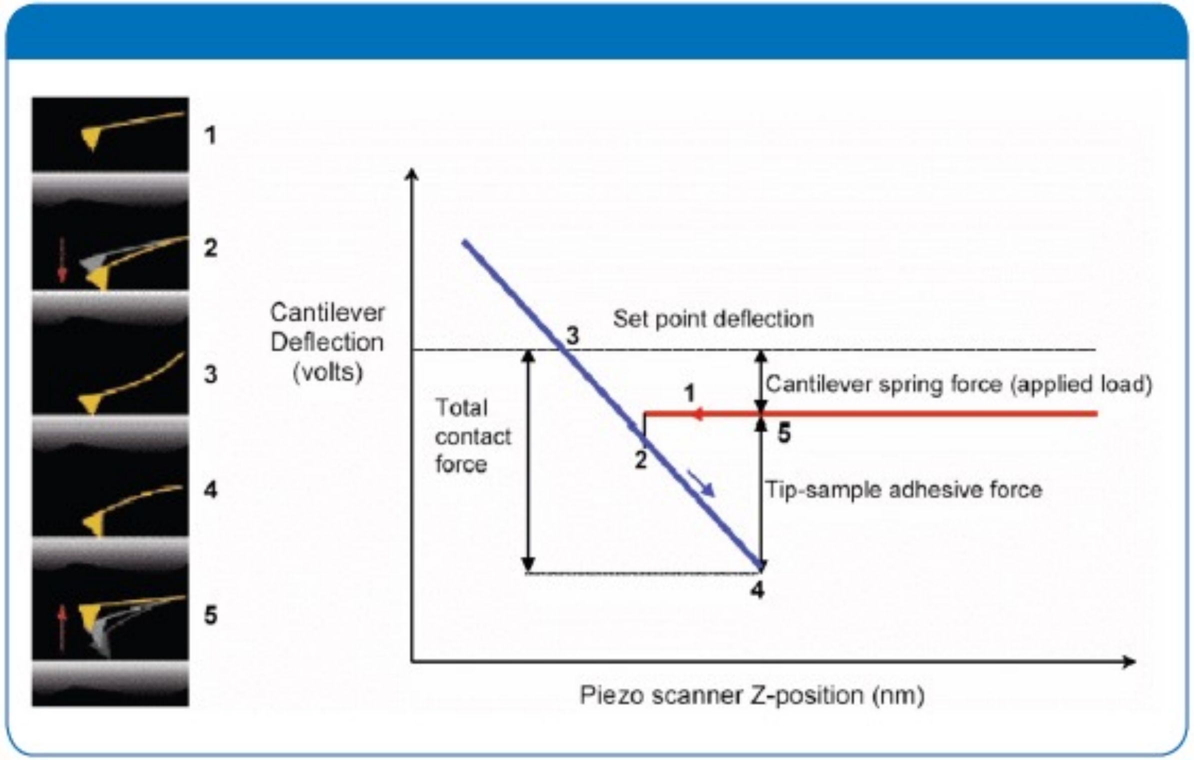
AFM in contact: force distance curve



A basic AFM operation, which helps explain contact mode, is **the force-distance curve**. No feedback in z!!!!

Here the cantilever is brought from a location above the surface but within the range of the z-piezo (Z < Z piezorange) toward the surface until the tip contacts the surface. Any further movement of the z-piezo toward the sample surface will result in an upward deflection of the lever and/or sample deformation. The z-scanner position is commonly generated by a triangular waveform applied to the z-piezo.

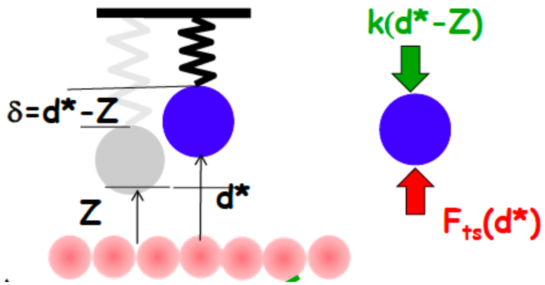
AFM in contact: force distance curve



Force curves in themselves can reveal a variety of sample properties, such as adhesion and compliance

Points 2 and 4 describe two important occurrences in a loading curve. These are the points where the tip-sample interaction force is not balanced by the restoring force of the cantilever, i.e., dF/dx > k at point 2.

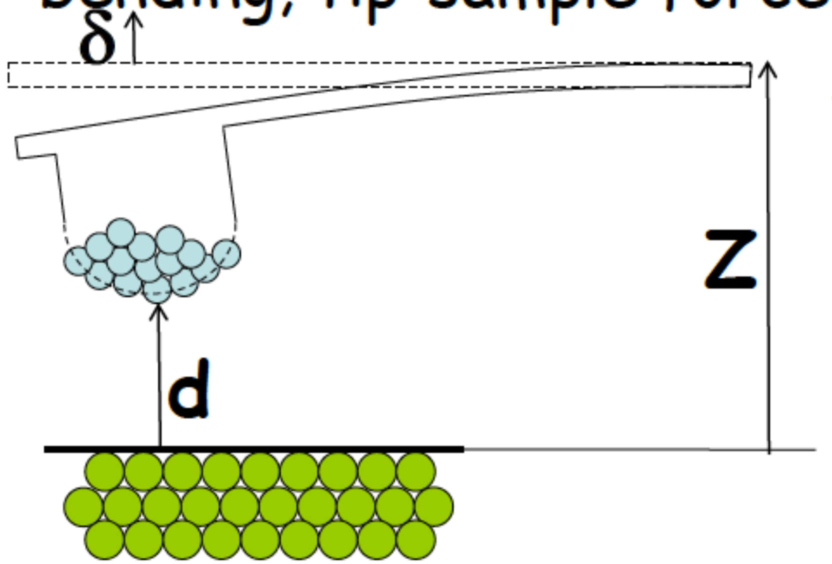
Setpoint denotes the deflection value used for the z-feedback.



Arvind Raman
Mechanical Engineering
Birck Nanotechnology Center

Force-distance curves

- Z is the Z-piezo displacement, δ is the cantilever bending, tip-sample force is $F_{ts}=k_{cant}\delta$



AFM's measure F_{ts} vs. Z !!

- How to convert force-displacement (F vs Z) to force distance (F vs d) and vice versa?
- Collect F-Z data and for every F value, evaluate d= Z+δ, to within an arbitrary constant

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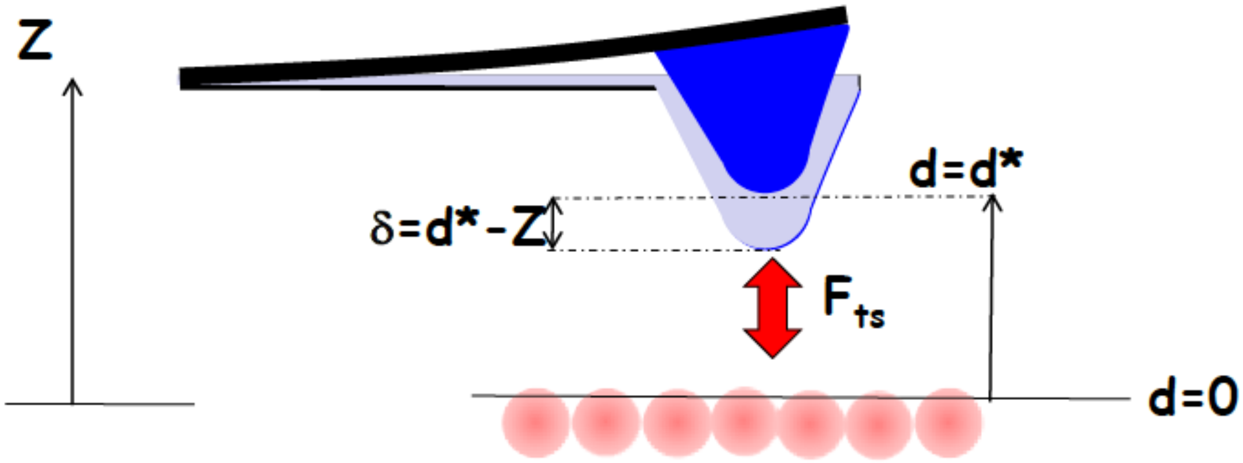
Birck Nanotechnology Center

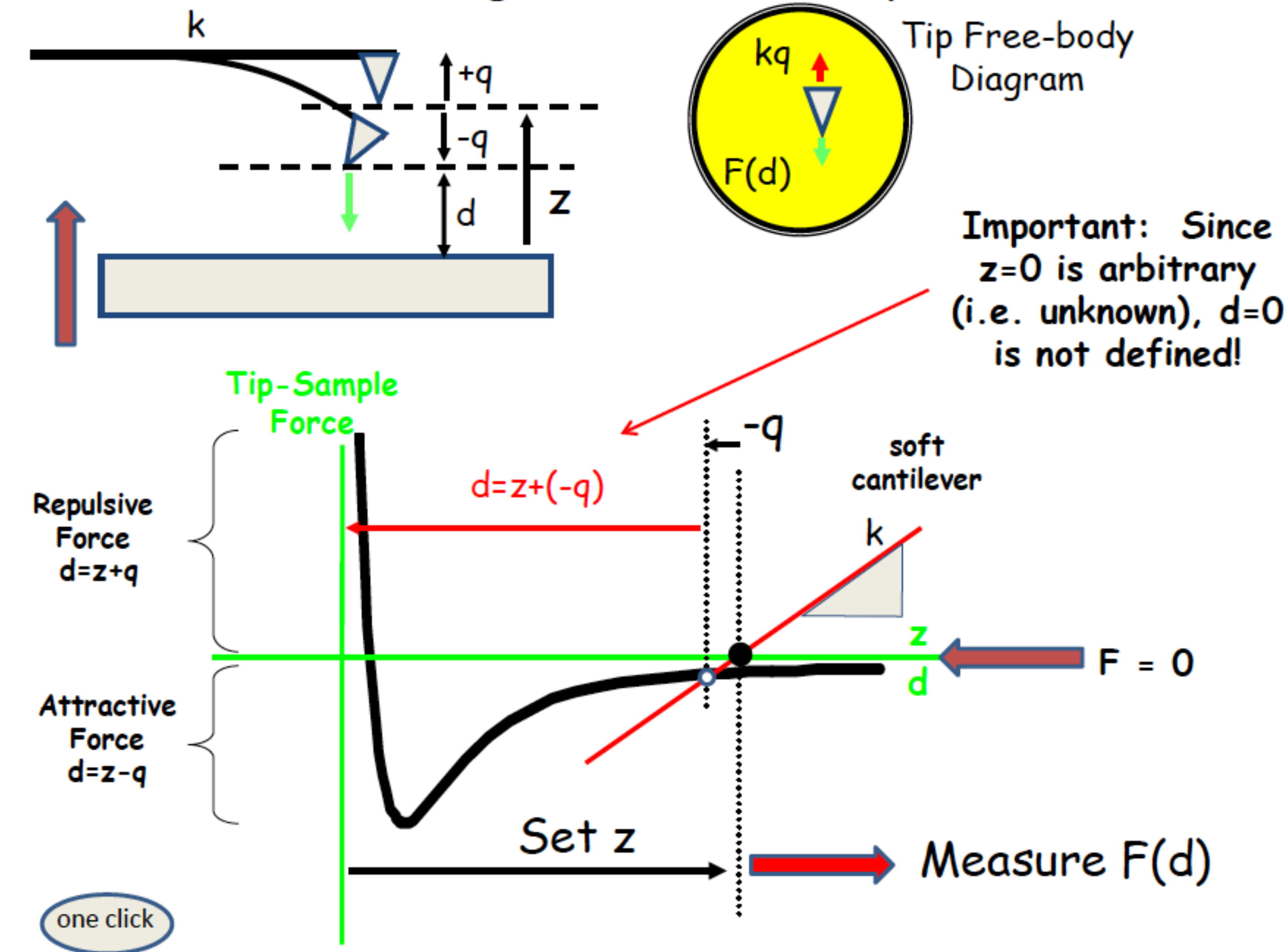
Force-distance curves

equilibrium positions



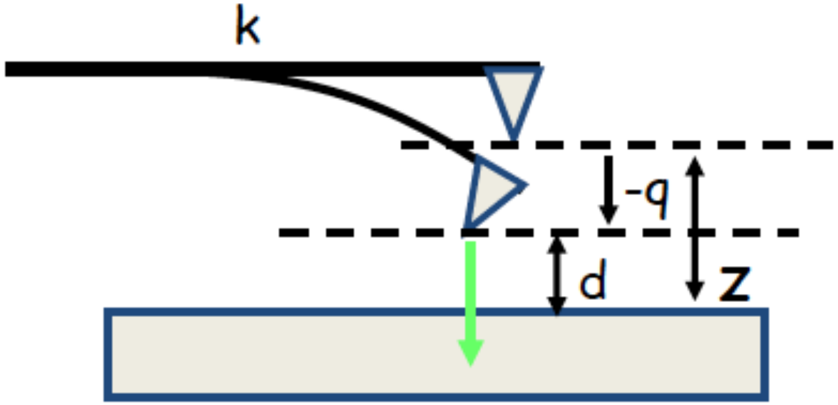
- How do d* and δ change as Z is reduced during approach and then retracted?
- Note that technically $\delta = d^* - Z - Tip\,height$ but tip height is basically an arbitrary constant

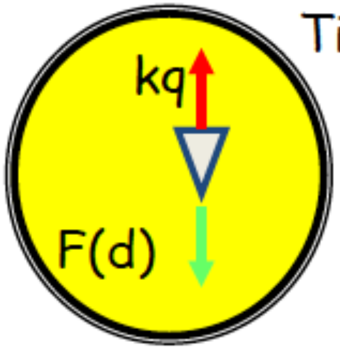
Measuring Force vs. z-displacement



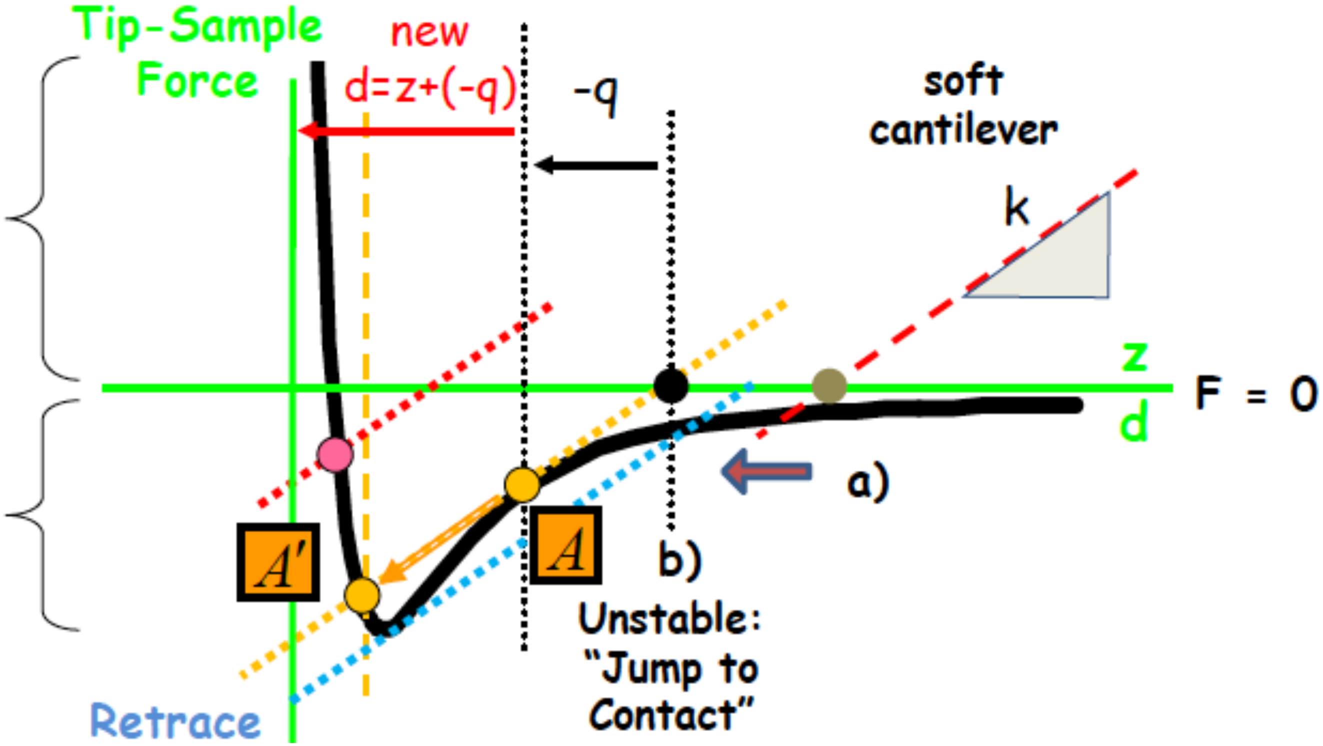
P1_Wk4_L2

Jump to Contact



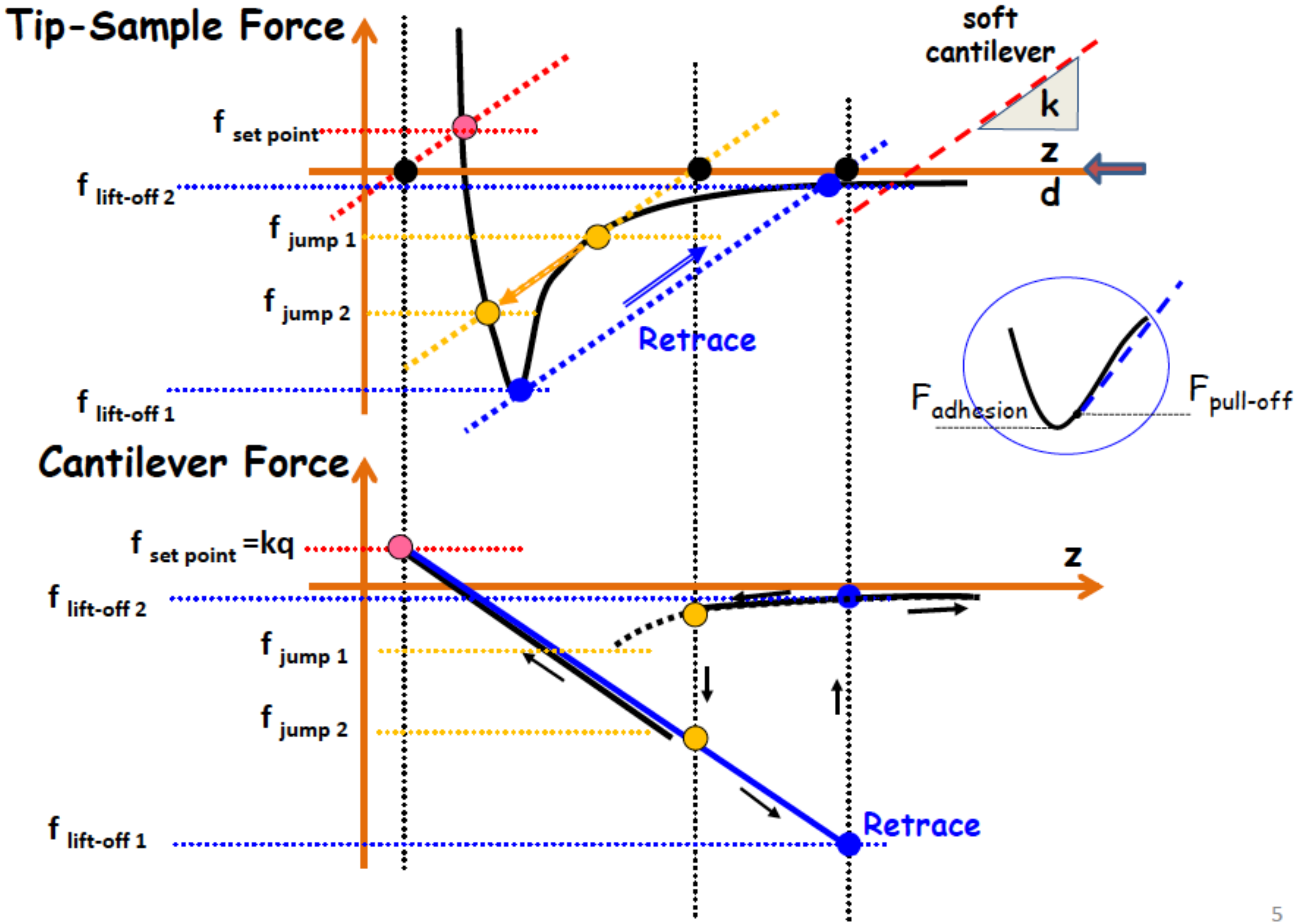


If the force gradient is larger than the effective elastic constant, the cantilever becomes unstable and "jumps" onto the surface.



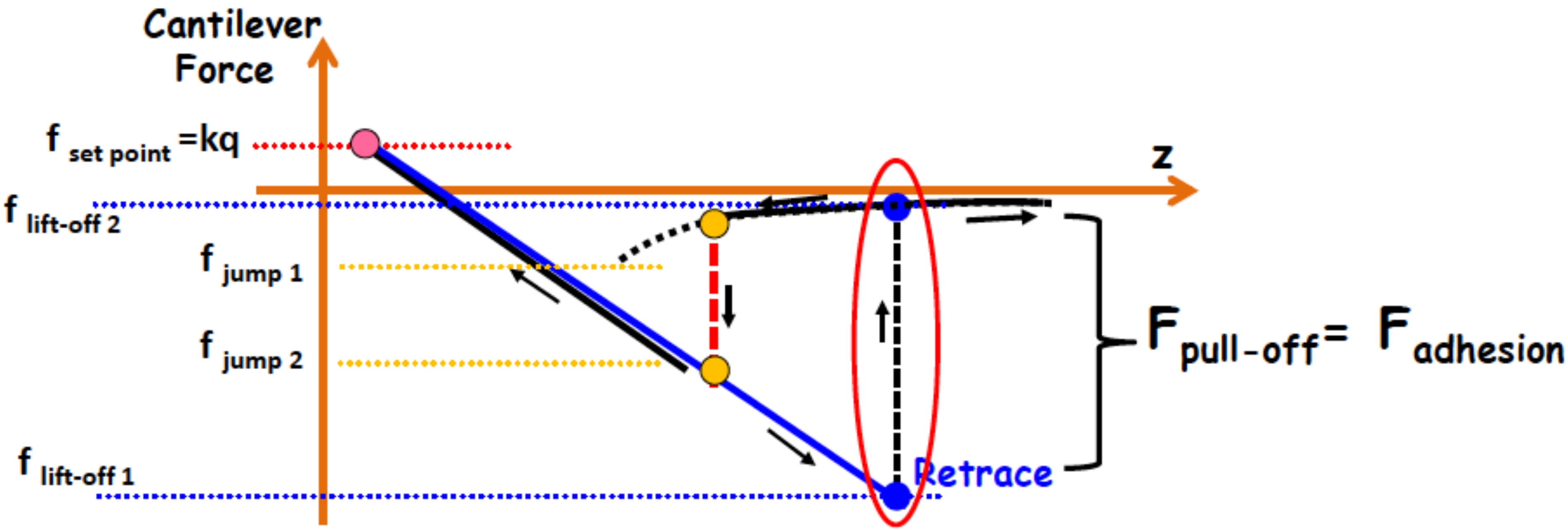
- With soft cantilevers (small k) it is not possible to measure entire 'd' range

Force vs. Separation Curve



P1_Wk4_L2

The pull off force feature



$$F_{pull-off} = f_{lift-off\,2} - f_{lift-off\,1}$$

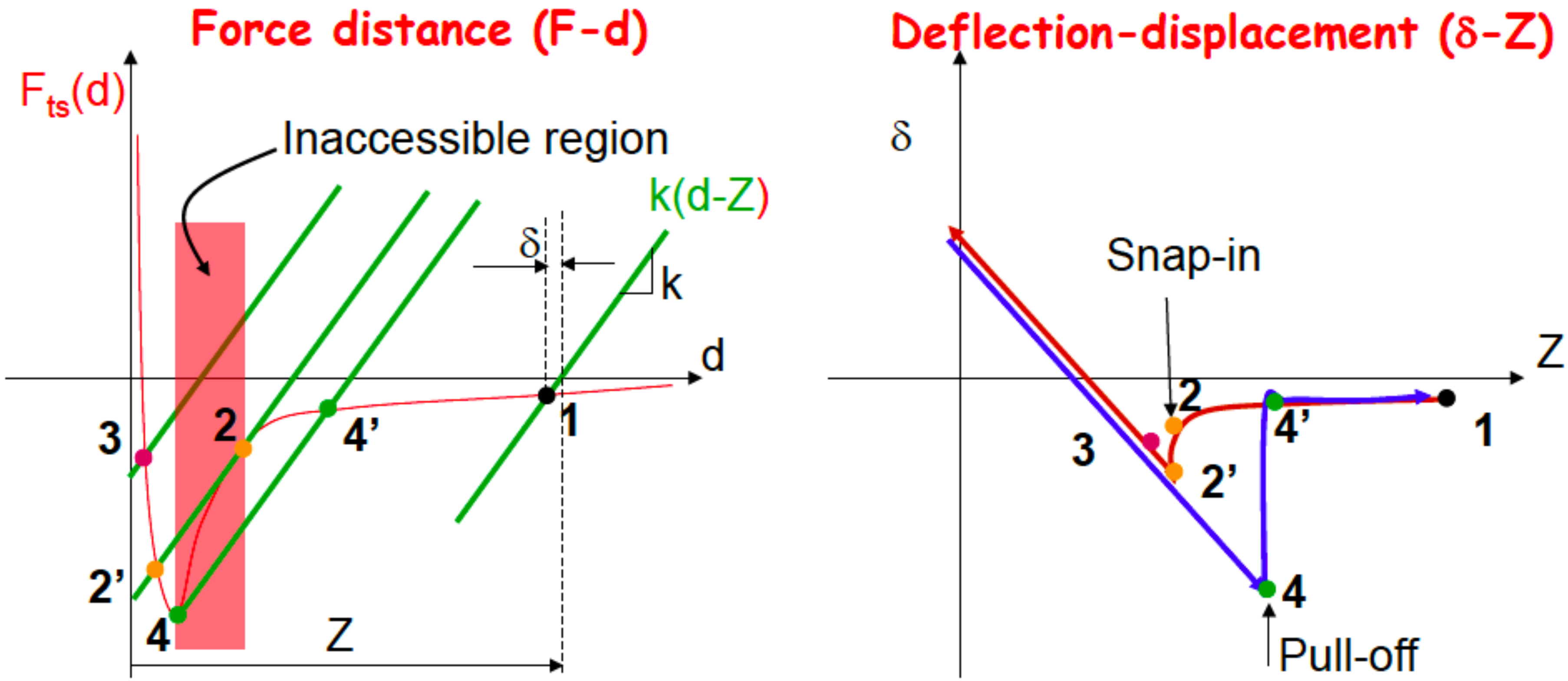
$$F_{adhesion} = F_{electrostatic} + F_{vdW} + F_{capillary} + F_{chemcial} + \ldots$$

1. To understand $F_{pull-off}$, the tip-sample deformation, adhesion and contact area are required
2. **If** vdW forces dominate, then , e.g, the DMT model predicts

$$F_{adhesion}^{DMT} = 2\pi \mathrm{R}_{tip} \mathrm{W}_{132}$$

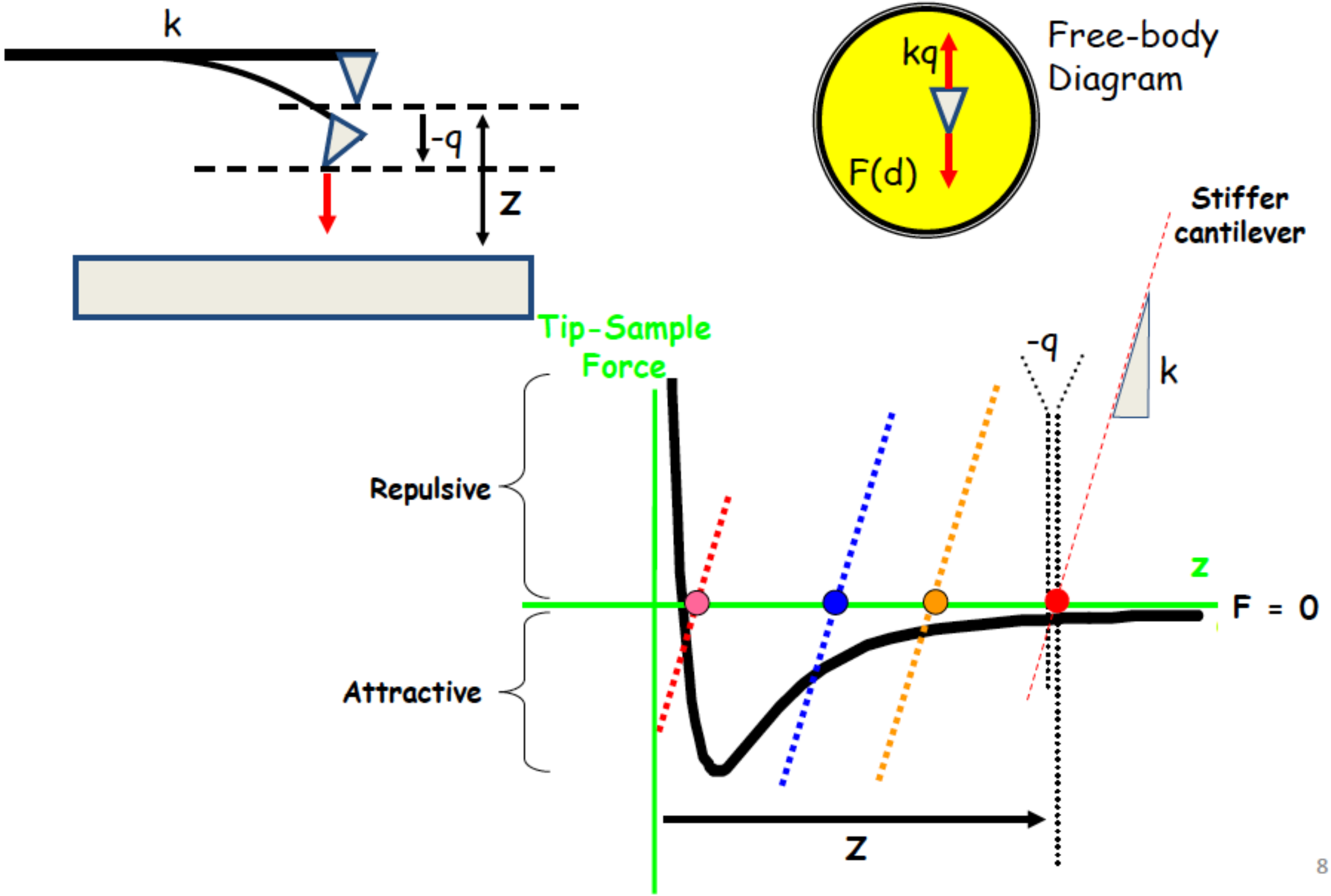
Force-distance curves

F-d F-z conversion

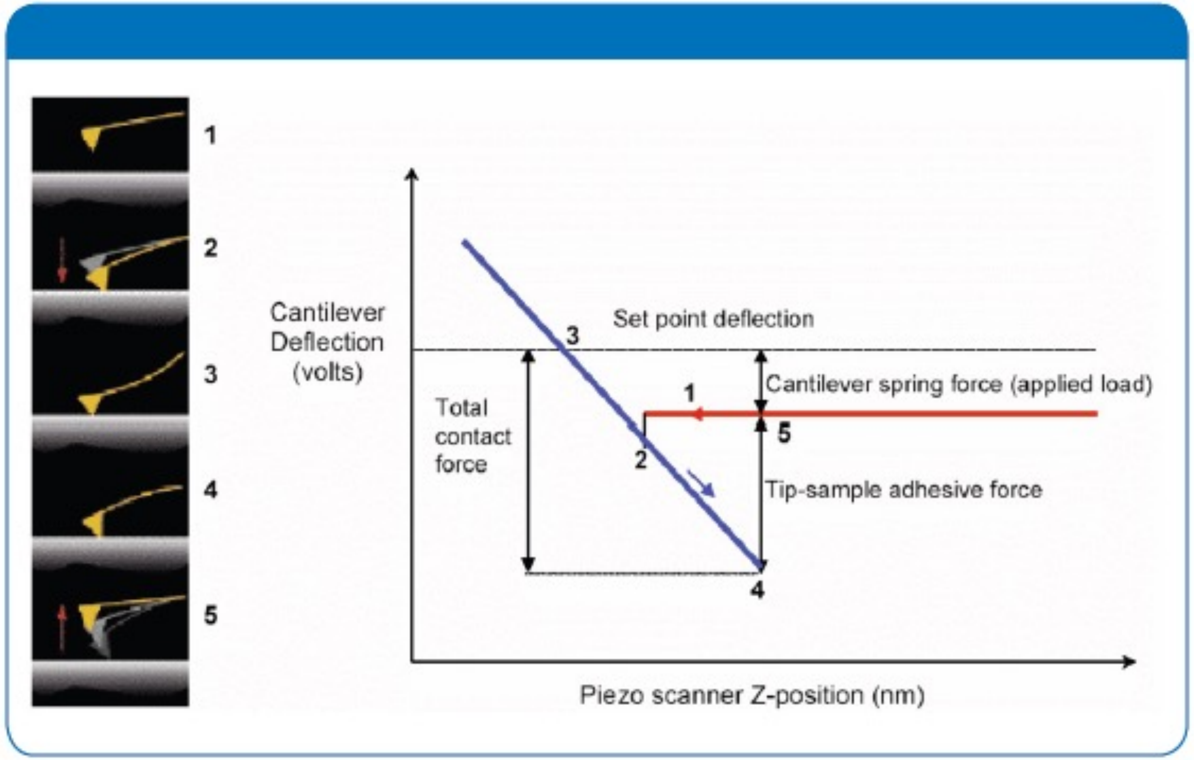


- Note that hysteresis occurs in the δ-Z curve between approach and retraction even though $F_{ts}(d)$ in conservative

Stiffer Cantilever



AFM in contact: force distance curve

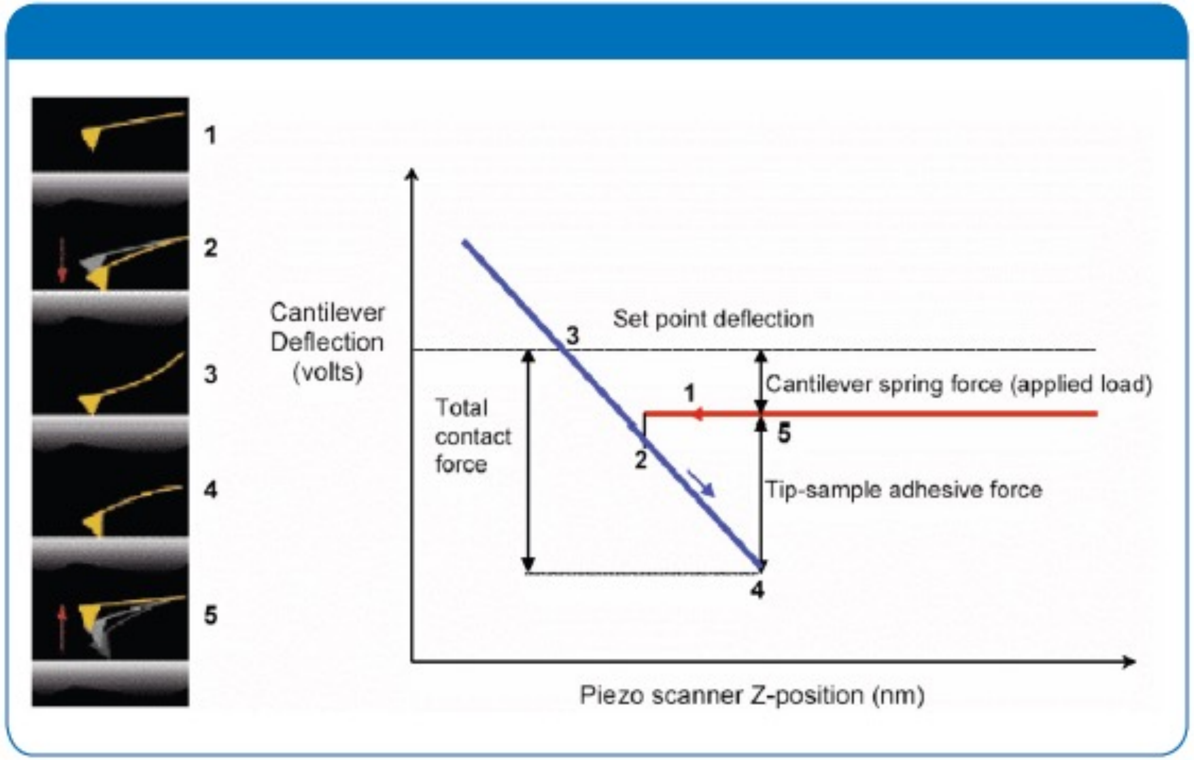


Force curves in themselves can reveal a variety of sample properties, such as adhesion and compliance

Contact mode imaging is carried by simply keeping the setpoint (point 3 in the force curve diagram) constant while raster scanning the tip and sample relative to each other. The movement of the z-piezo then becomes the sample topography that is plotted as a function of xy.

The feedback loop has to be fast enough to allow the z-piezo to respond to changes in sample topography but slow enough to avoid oscillations of the system

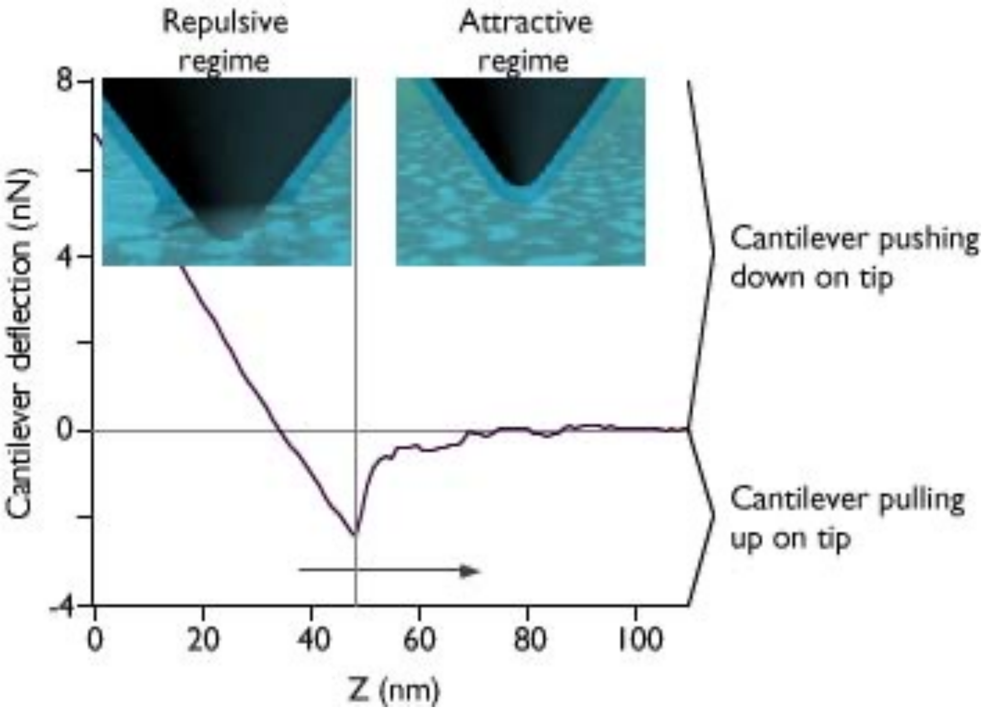
AFM in contact: force distance curve



Force curves in themselves can reveal a variety of sample properties, such as adhesion and compliance

Even though reasonably easy to operate, contact mode has the inherent drawback that lateral force exerted on the sample can be quite high. This can result in sample damage or the movement of relatively loosely attached objects. A solution to that problem was to oscillate the cantilever during imaging, which led to intermittent contact Imaging (or tapping mode).

Contact mode AFM

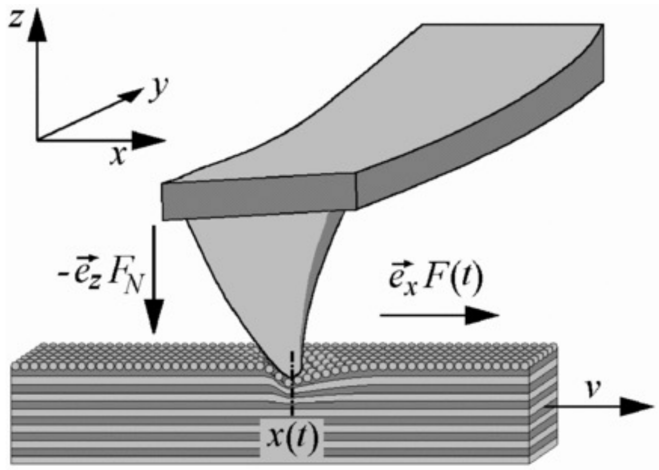


- Useful for scanning non deforming materials
- "Soft" (low force constant) cantilevers are more sensitive
- Applicable for operation in liquids without complication

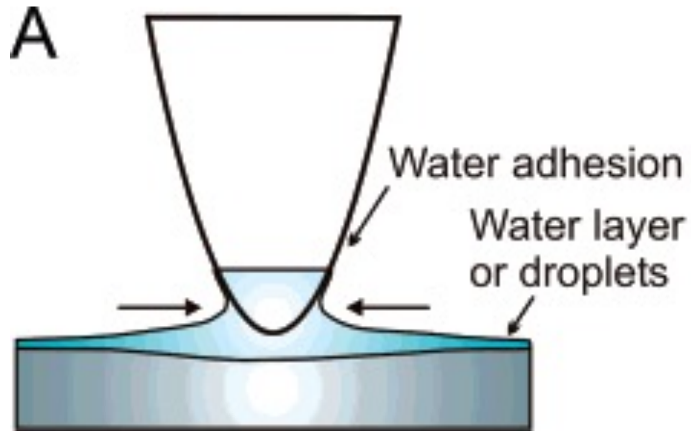
Problems:

1) Frictional forces
2) Water mesiscus in air: adhesion forces
3) Dragging forces, important for biological samples which are usually loosely bound and easily damageable

AFM Contact mode-associated imaging modes

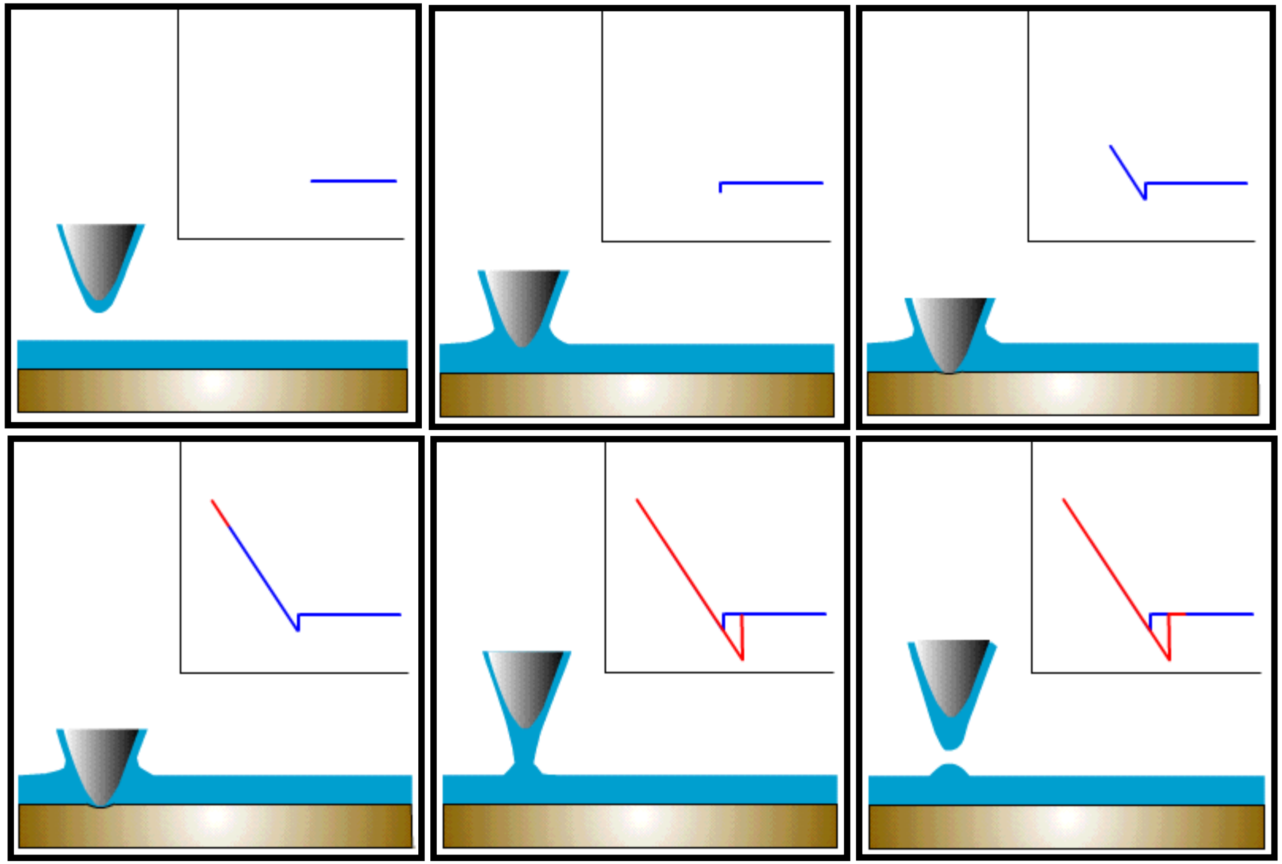


Friction:
The cantilever bends laterally due to a friction force between the tip and the sample surfaces.



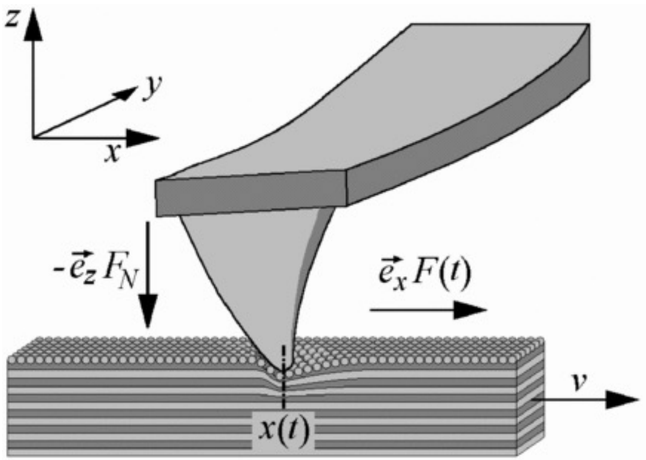
Adhesion:
at ambient conditions, in addition to the intrinsic adhesion between tip and sample, there is another one from the capillary neck condensing between the tip and water meniscus. interference from the humidity.

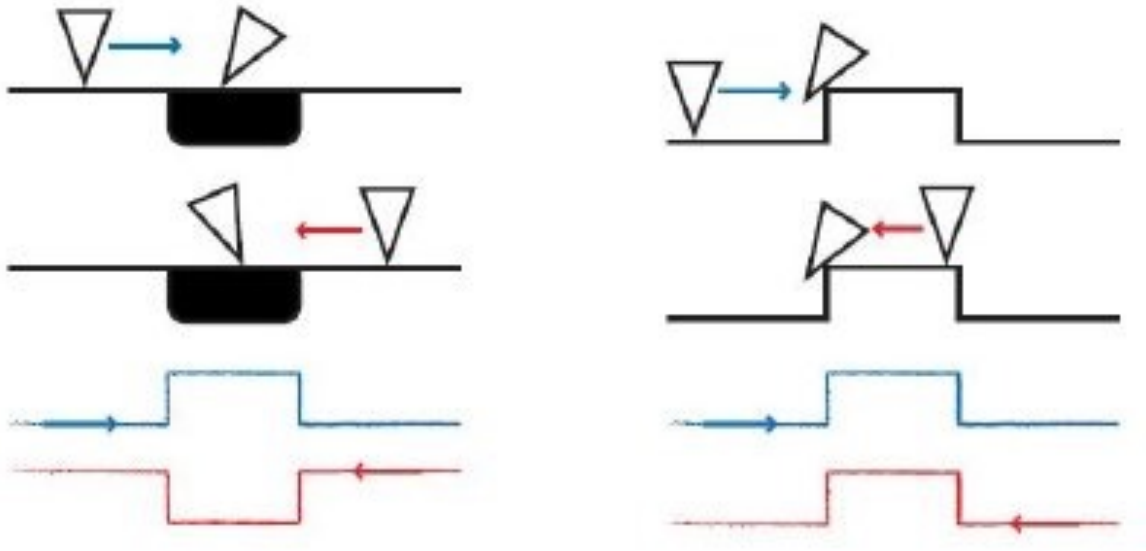
Water meniscus



Lateral Force Microscopy

sample friction





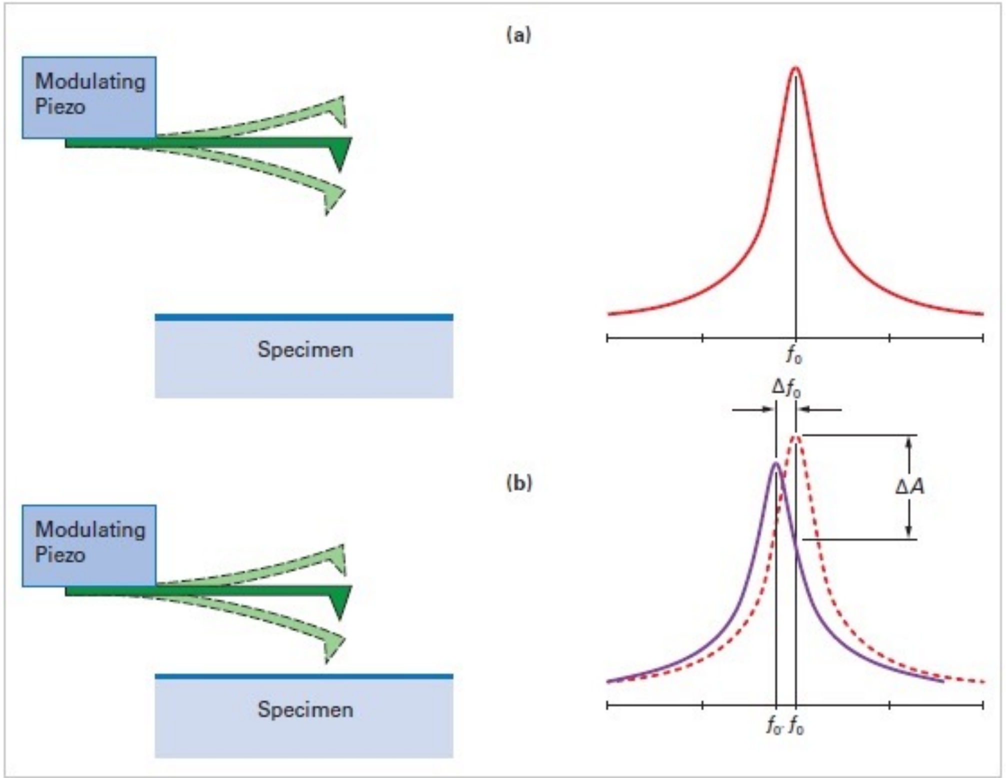
Differences between lateral forces caused by friction and the ones caused by topographic features of the scanned surface. (Left) Mirroring of lateral deflection due to frictional forces. (Right) No mirroring with topographically induced lateral deflection. All forward scan traces are in blue, backward scan traces in red.

Contact mode AFM

However, lateral dissipation can be a huge problem for biological samples:

- damage induced
- molecular dragging (single molecules)

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

AFM intermittent contact imaging mode

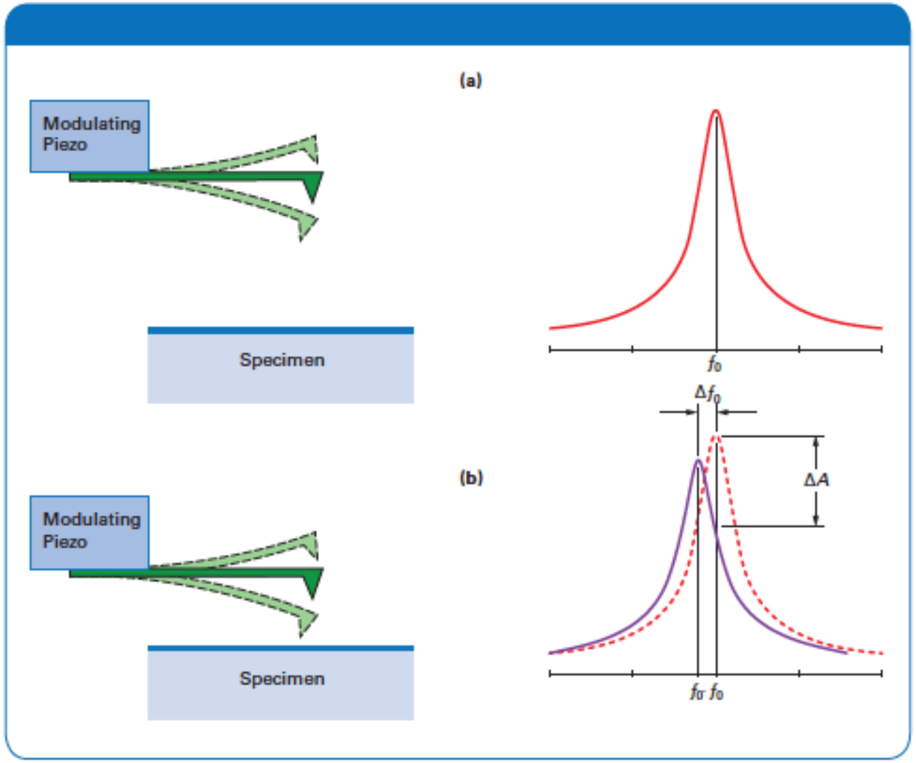
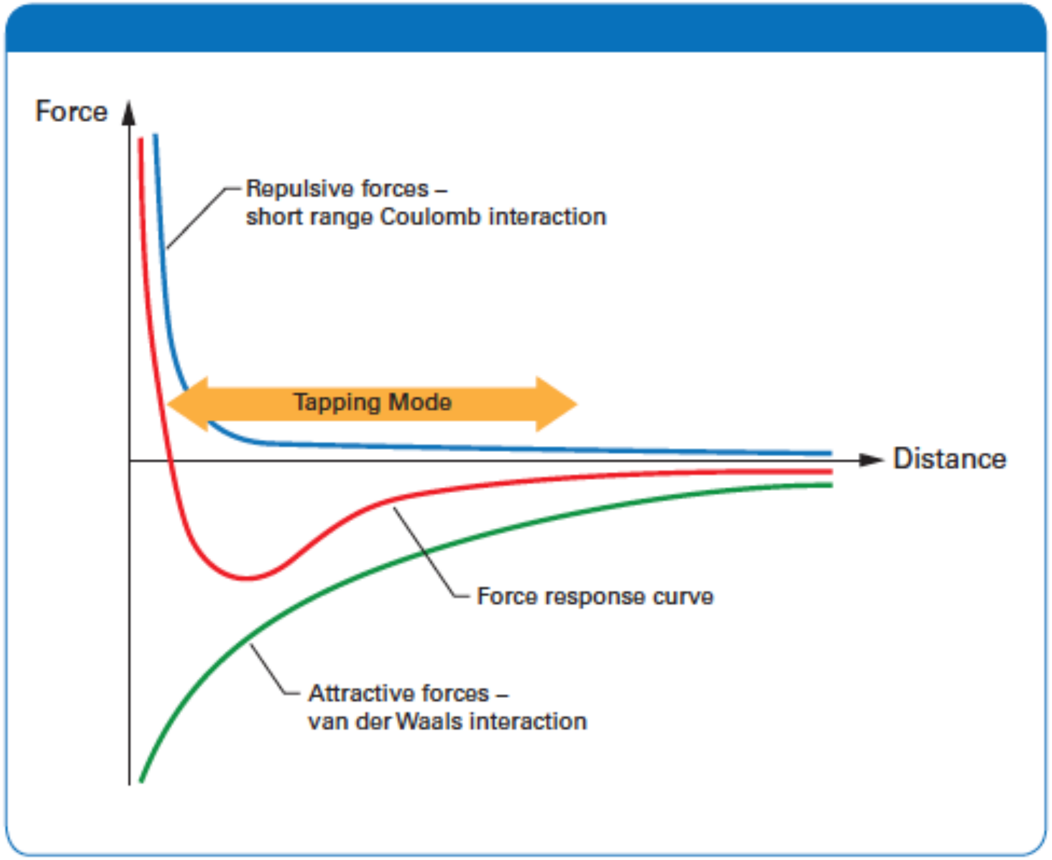


Figure 6. Resonance curve of a TappingMode cantilever above and close to the surface. Note that the resonance shifts to lower frequencies and exhibits a drop in amplitude.

The tip touches the surface only for a short time, thus avoiding the issue of lateral forces and drag across the surface

The cantilever is oscillated at or near its resonance frequency normal to the sample surface. Typical amplitudes of oscillation are in the range of tens of nanometers, and thus very small compared to the cantilever length.

AFM intermittent contact imaging mode



Typical TappingMode operation is carried out using amplitude modulation detection with a lock-in amplifier. This means a frequency close to the cantilever resonance is selected, and the tip-sample spacing is changed to maintain a constant cantilever amplitude without changing the drive frequency. Similar to contact mode, the movement of the z-piezo when plotted as a function of xy becomes our sample topography.

AFM intermittent contact imaging mode

Movement of the cantiliver described as damped harmonic oscillator model:

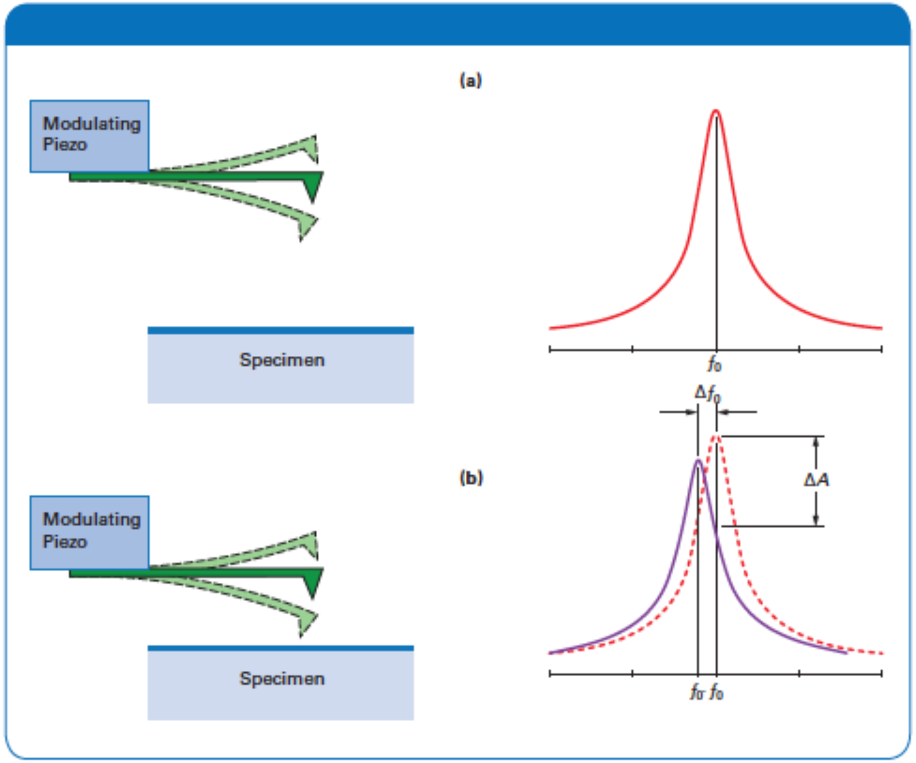


Figure 6. Resonance curve of a TappingMode cantilever above and close to the surface. Note that the resonance shifts to lower frequencies and exhibits a drop in amplitude.

$$m^* \frac{\partial^2 z}{\partial t^2} + b \frac{\partial_z}{\partial t} + k_z = F_0$$

with m* being the effective mass of the cantilever, z the displacement of the lever, b the damping coefficient, k the spring constant, and F_0 the driving force (F_0= k A_0). With the natural frequency:

$$\omega_0 = \sqrt{\frac{k}{m^*}} \quad \text{and the relaxation time} \quad \tau_0 = \frac{m^*}{b}$$

one can write the amplitude of the lever as:

$$A(\omega_e) = \frac{A_0}{\sqrt{\left(1 - \left(\frac{\omega_d}{\omega_0}\right)^2\right) + \frac{1}{(\omega_0 \tau)^2} \left(\frac{\omega_d}{\omega_0}\right)^2}}$$

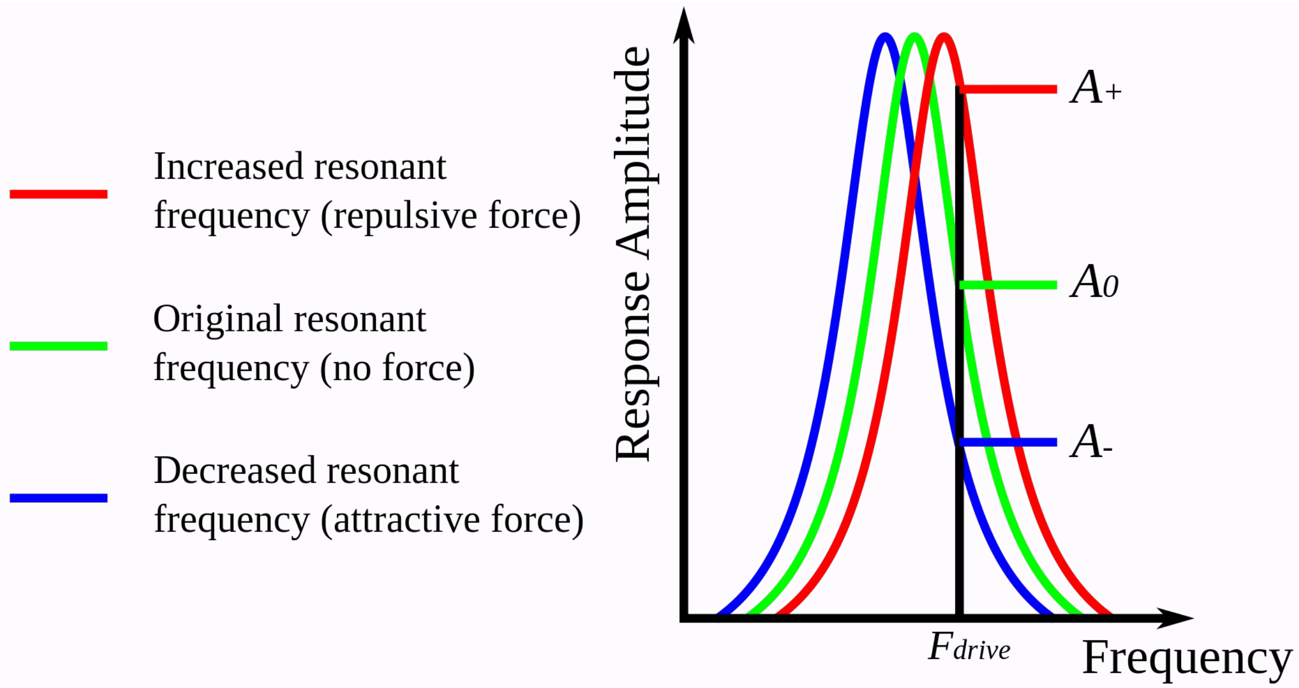
The quality factor "Q" of the system is the ratio of the energy stored in the system divided by the energy loss per cycle. For a lightly damped system, Q= **ω0 τ.** The maximum amplitude at resonance then becomes: Ar = A0 Q.

Dynamic AFM: basics

In intermittent contact mode, also called **tapping mode** or **amplitude modulation (AM)** mode, the cantilever is excited to oscillate at or near its first resonance frequency. The excitation frequency is fixed at a constant value. The oscillating tip interacts with the sample surface at its bottom swing (about 10% of the oscillation cycle), by which the oscillation amplitude is reduced.

Amplitude reduction: dissipation; shift of the frequency due to interaction with the sample

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

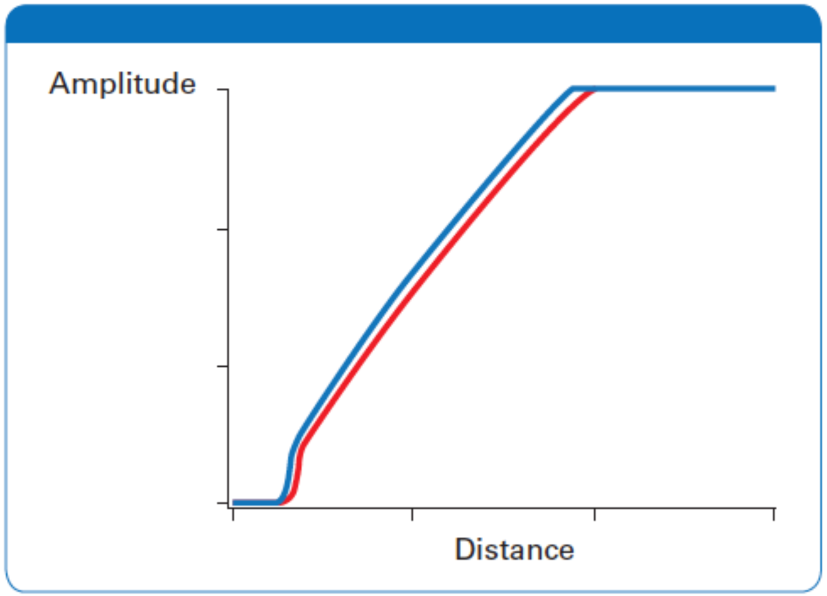


AFM intermittent contact imaging mode

We are not measuring a direct force in TappingMode.

When the probe approaches the sample, it experiences an attractive force and is pulled toward the surface until contact is made. From that point on, the repulsive interaction forces dominate the response. The probe can then be retracted and additional information can be extracted from that trace.
The TappingMode AFM, while experiencing these interactions, does not actually measure this force curve, nor the direct forces between the tip and the sample for that matter.
AFM oscillates back and forth on this curve, interacting without being in direct control of the force, and **reporting only an average response of many interactions though the lock-in amplifier**.

One can certainly measure the reduction of cantilever amplitude as tip and sample approach each other, but it must be understood that **each point on that curve represents an average value and not a single interaction.** This restricts information beyond sample topography.



AFM intermittent contact imaging mode

Then, the adjustment of the feedback system is essential to achieving reliable information from the AFM.
It is easier to control a contact mode scan when compared to a TappingMode scan due to the added complexity of the oscillating system. TappingMode operates at cantilever resonant frequency, where the cantilever dynamics are relatively complicated. The cantilever dynamics can be dramatically changed by changing the amplitude set-point, while the tapping dynamics depend strongly on the sample properties.

TappingMode does however offer the undeniable benefit of lateral force free imaging, which has made it the dominant imaging mode in AFM to date.

Peak force tapping mode

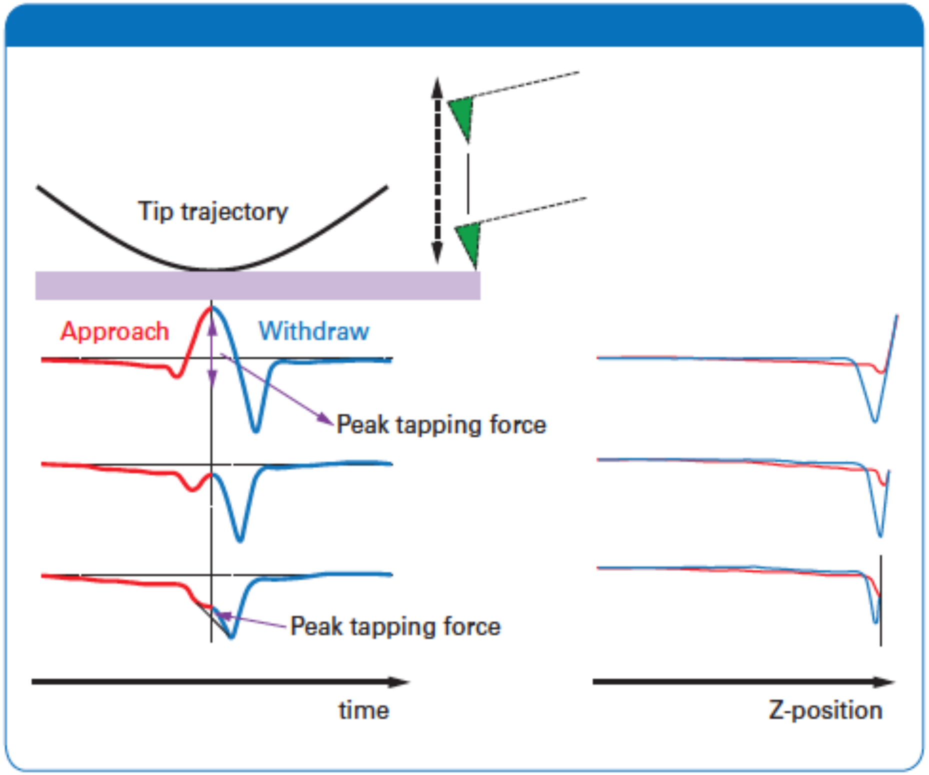


Figure 9. Experimental data of force curves for a cantilever operated in PeakForce Tapping. The lever is driven by a sinusoidal wave and the curves are displayed as force versus time and force versus distance.

PeakForce Tapping operates similarly to TappingMode.

However it operates in a non-resonant mode. The PeakForce Tapping oscillation is performed at frequencies well below the cantilever resonance, thus avoiding the filtering effect and dynamics of a resonating system.

In PeakForce Tapping, we now have an oscillating system that combines the benefits of contact and TappingMode imaging: direct force control and avoidance of damaging lateral forces.

The differences to a conventional force curve are that the z-position is modulated by a sine wave and not a triangular one, thus avoiding unwanted resonances at the turnaround points.

1 micron scan of DNA origami in buffer

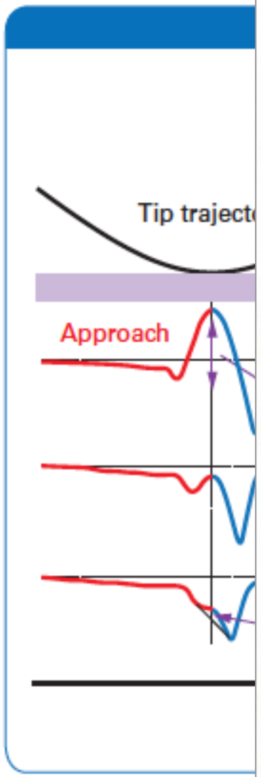
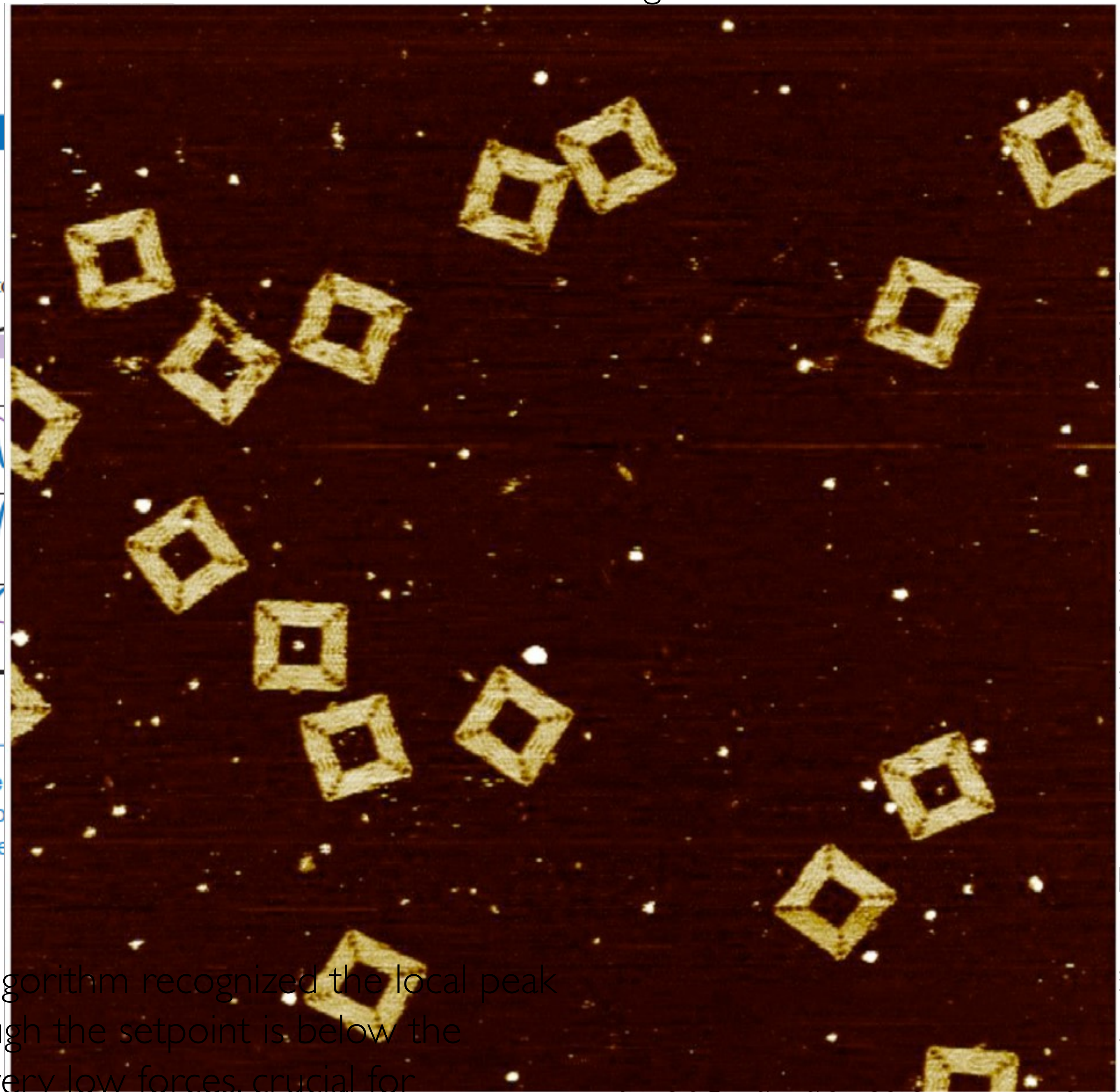


Figure 9. Experime
operated in PeakFo
wave and the curve
versus distance.

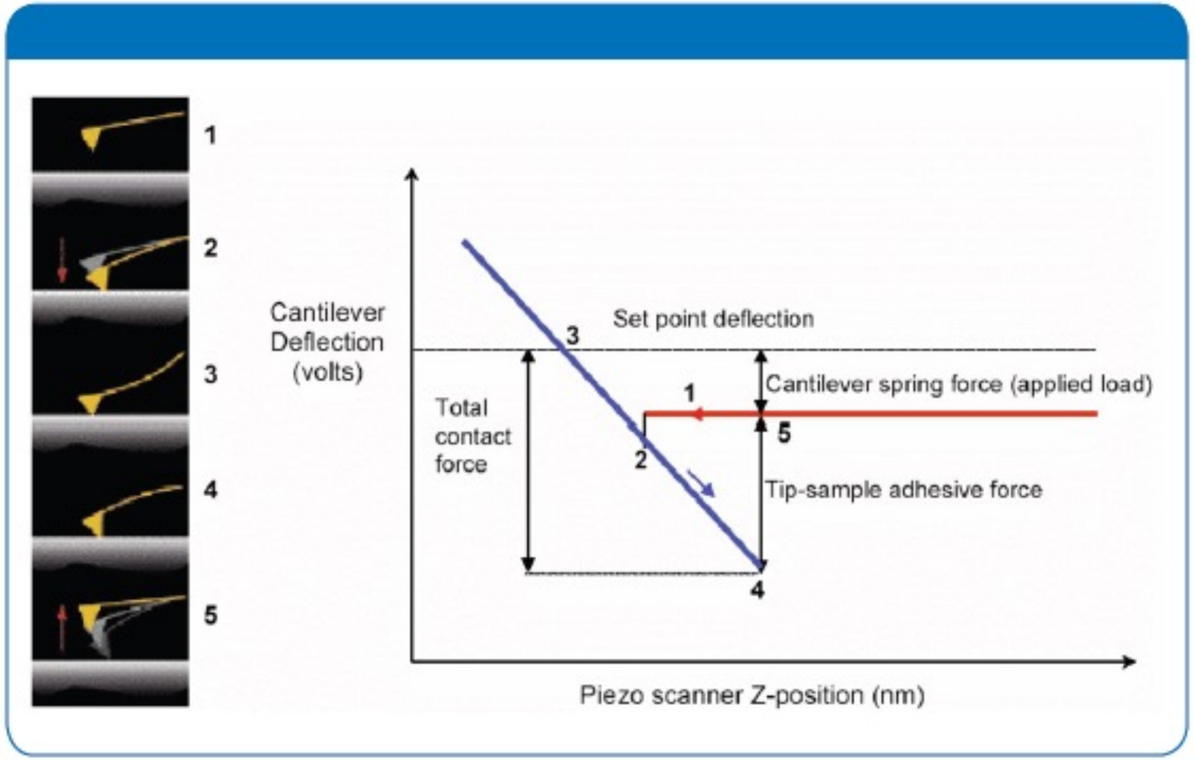


ınd an
curves from
ete the package.

ajectory as it
ed underneath
e) as a function
vely.
ple surface, it
der Waals
ng the
with the
t range
nteraction,
e approaching
unload it goes
, and finally
becomes free.

The feedback algorithm recognized the local peak force even though the setpoint is below the baseline, i.e. at very low forces, crucial for obtaining high-resolution data on soft samples.

Force distance curve for system calibration



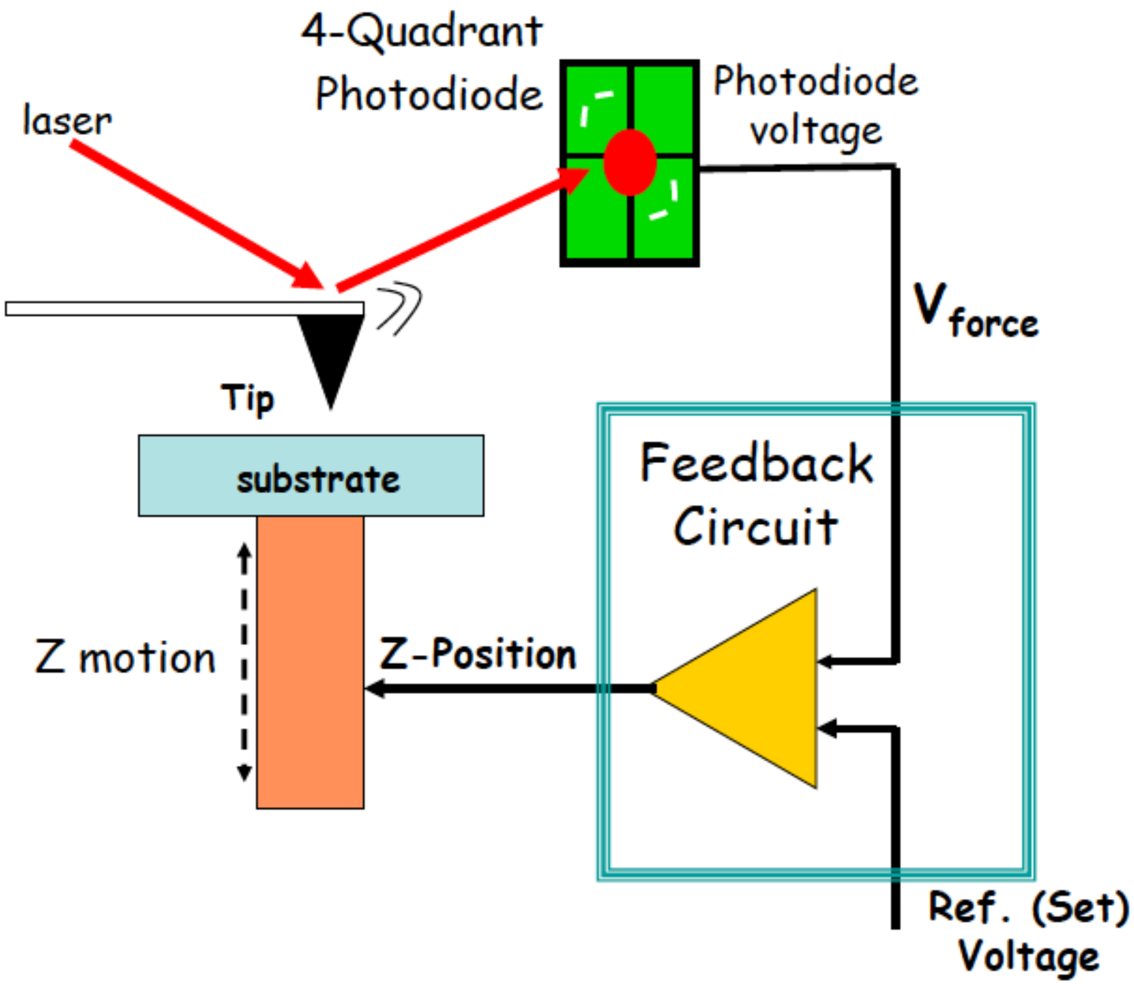
To convert the vertical axis from the photodetector output in volts to units of force, the system must be calibrated.

The first step is to calibrate the photodetector output to the actual cantilever displacement, commonly referred to as "**deflection sensitivity**."

That step is simply carried out on a hard sample with the assumption that the tip-sample gap is zero (G=0). The vertical axis now has the units of length.

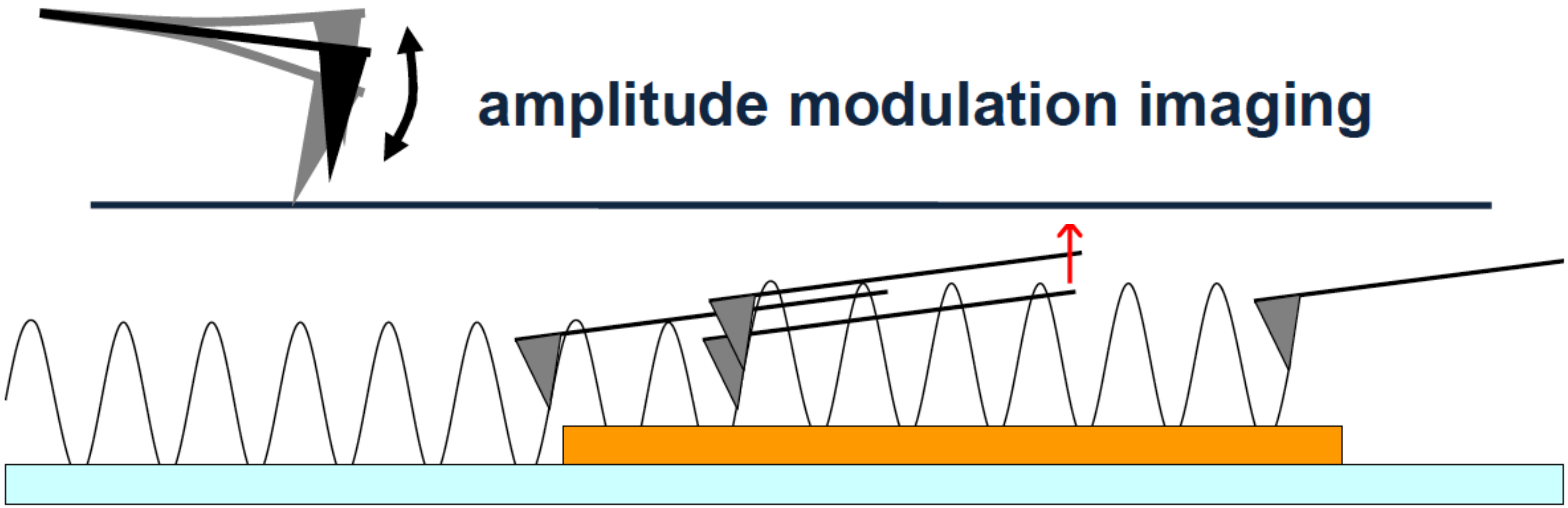
The second step is the determination of the **cantilever spring constant**. For that Force-distance curves are measured,

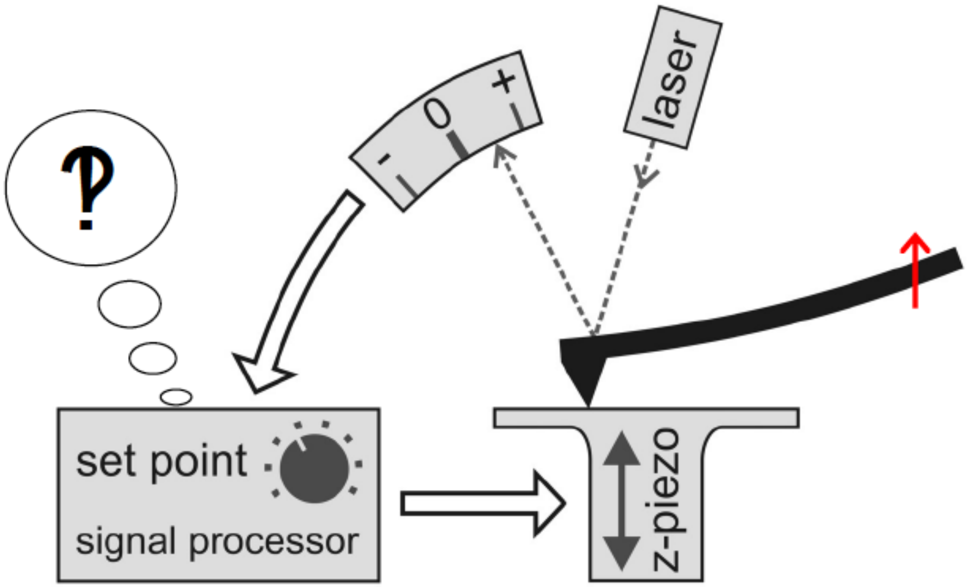
Feedback system requests



feedback loop should be fast enough to allow the z-piezo to respond to changes in sample topography but slow enough to avoid oscillations of the system !

amplitude modulation imaging





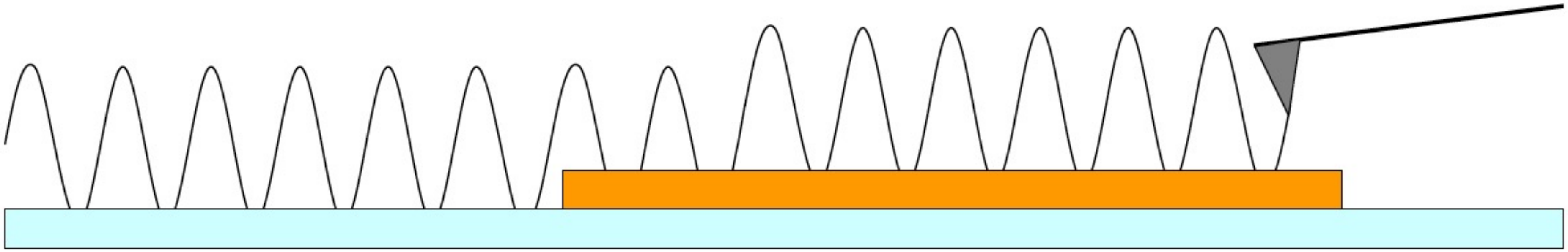
adjust piezo height (z) to keep amplitude constant

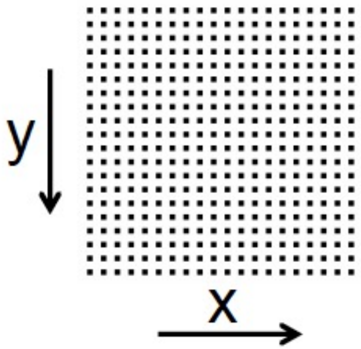
piezo motion gives height info

peak force at height changes

lower lateral/drag forces

Courtesy of I. Schaap



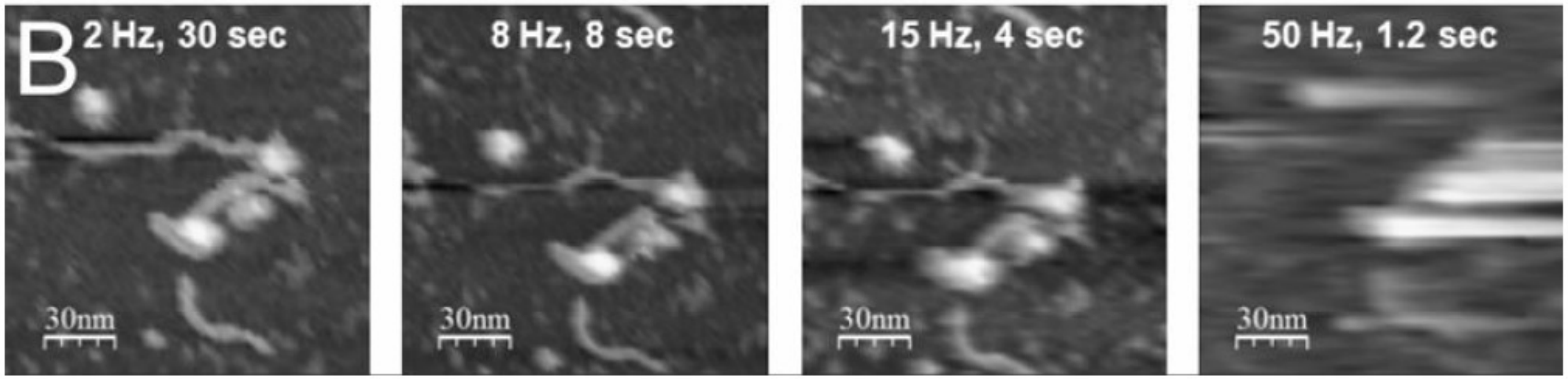


64 x 64 = 4096 px

need a few oscillations per pixel (~5)

fast bio-cantilever ~ 25 kHz

→ 0.04 ms * 5 * 4096 = 1 s

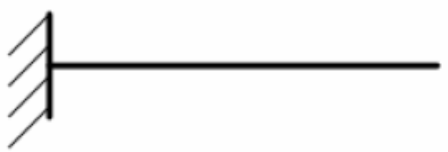


(there are other limiting factors (z-piezo, feed-back loop)

Courtesy of I. Schaap

small cantilevers are faster

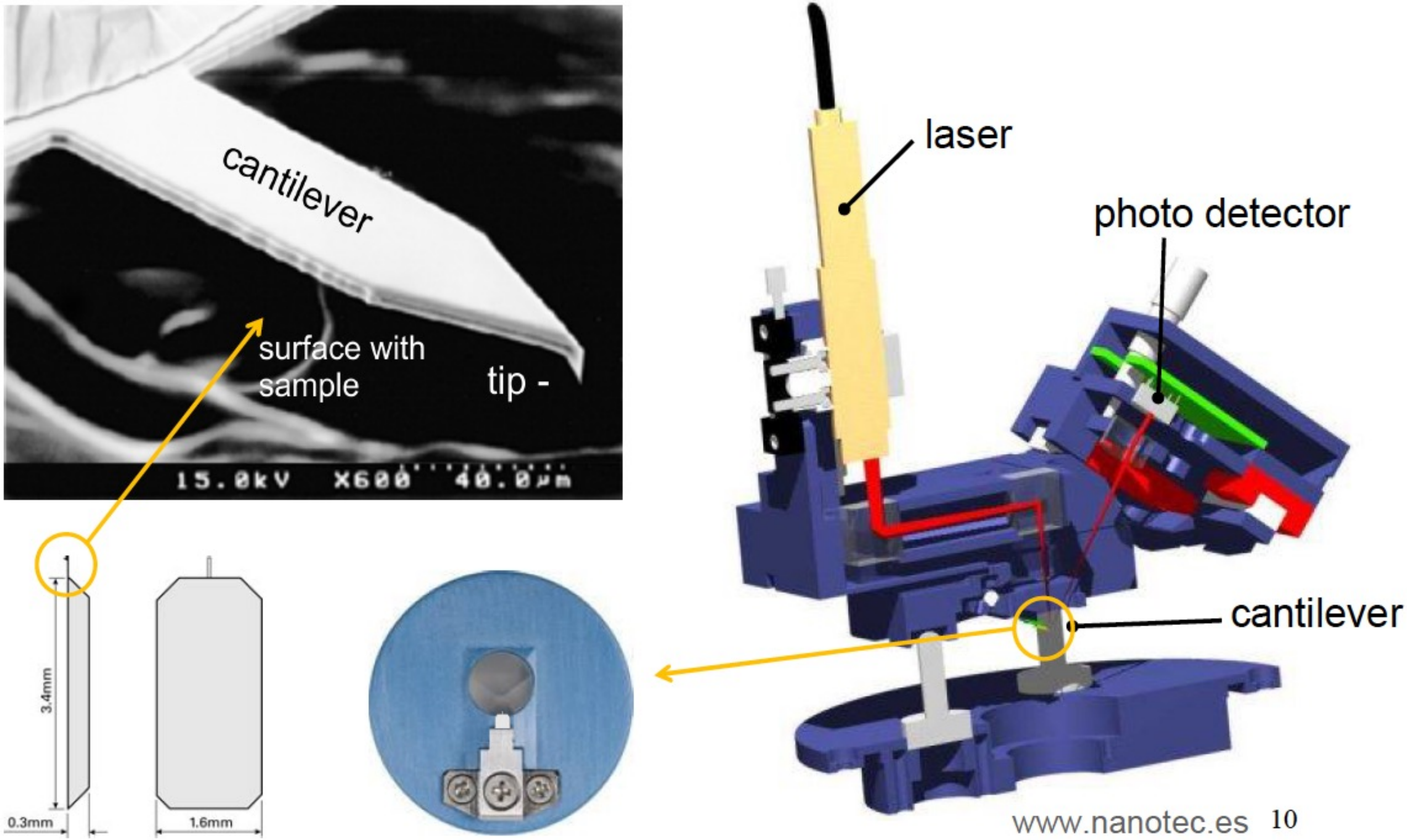
	l (µm)	w (µm)	t (µm)	ω_0 (kHz)	k (N/m)	
rc800	200	20	0.8	3	0.05	8 s
bl150	60	30	0.18	8	0.03	3 s
ac40	38	16	0.2	25	0.1	1 s
ac10	9	2	0.13	500	0.1	50 ms



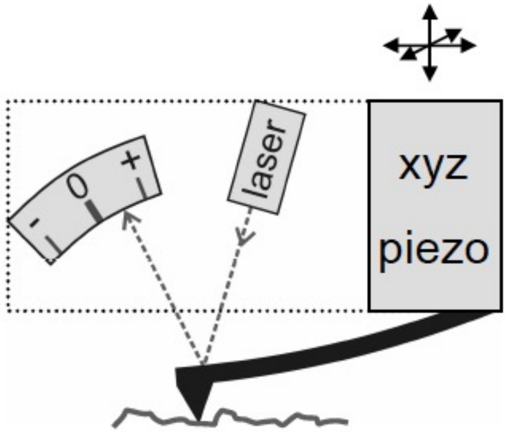
$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4\rho}}$$

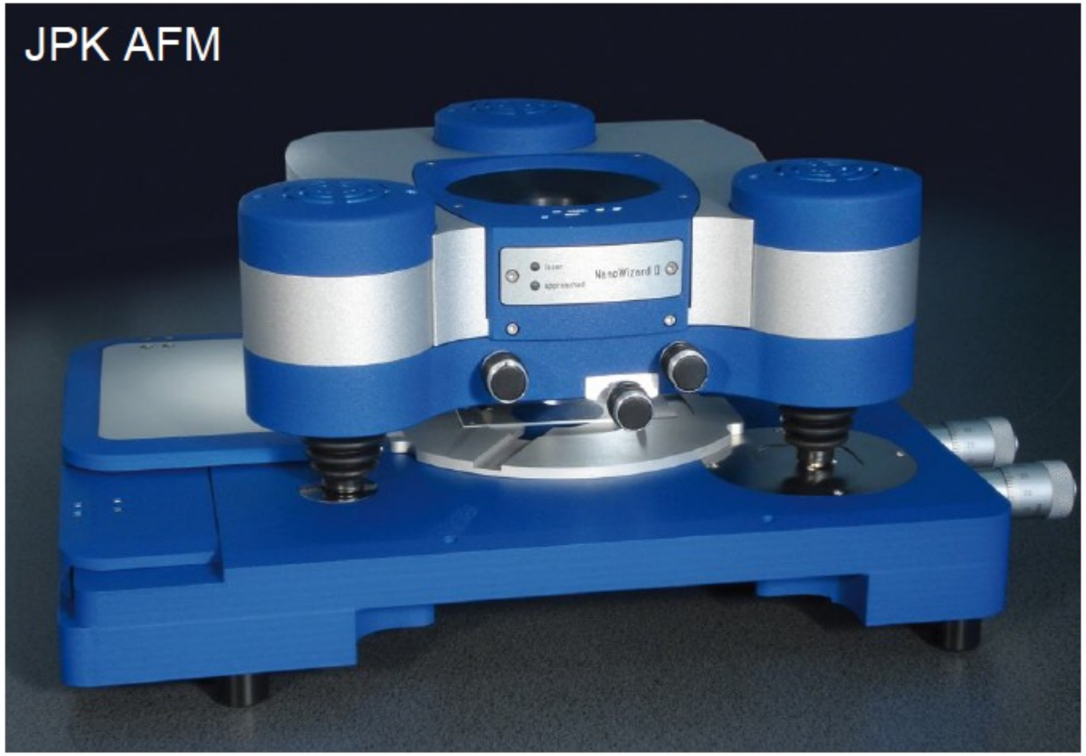
$$k = \frac{F}{d} = \frac{Ewt^3}{4l^3}$$

make cantilevers short to increase ω_0 and thinner to restore k


cantilever
surface with sample
tip -
15.0kV X600 40.0µm
3.4mm
0.3mm
1.6mm
laser
photo detector
cantilever
www.nanotec.es

top xyz scanner



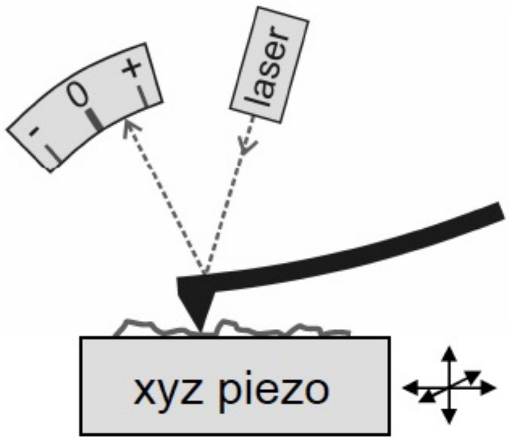


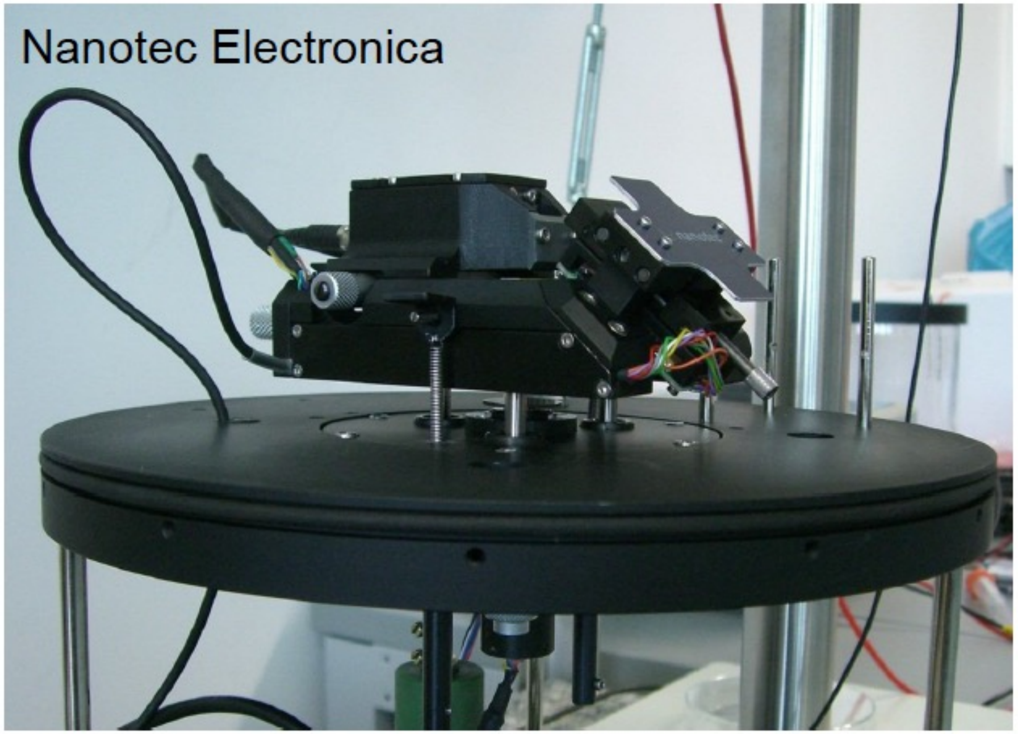
complicated design, not so fast

optical access from below

Courtesy of Iwan Schaap

base xyz scanner





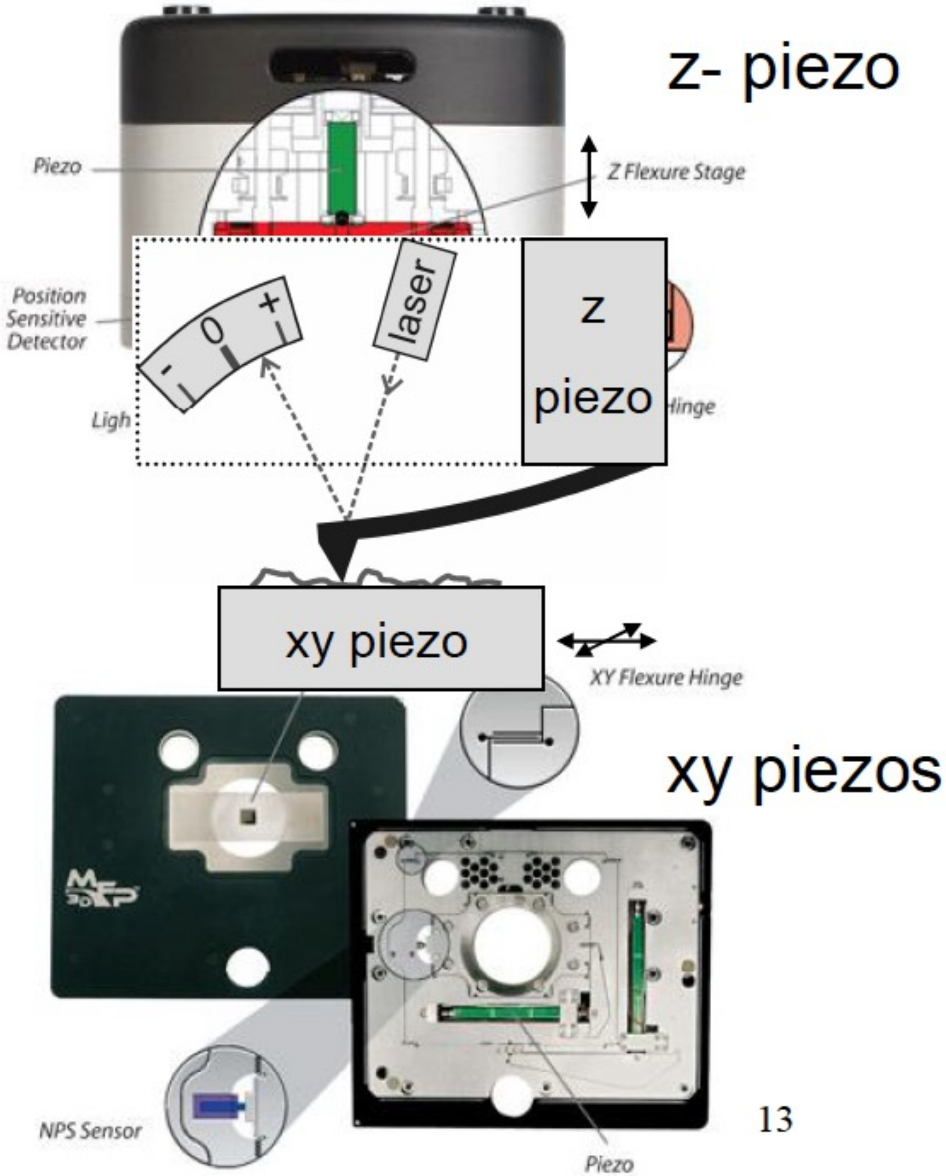
less complex and faster, atomic resolution

limited optical access

Courtesy of Iwan Schaap

separated xy and z scanner

Asylum Research



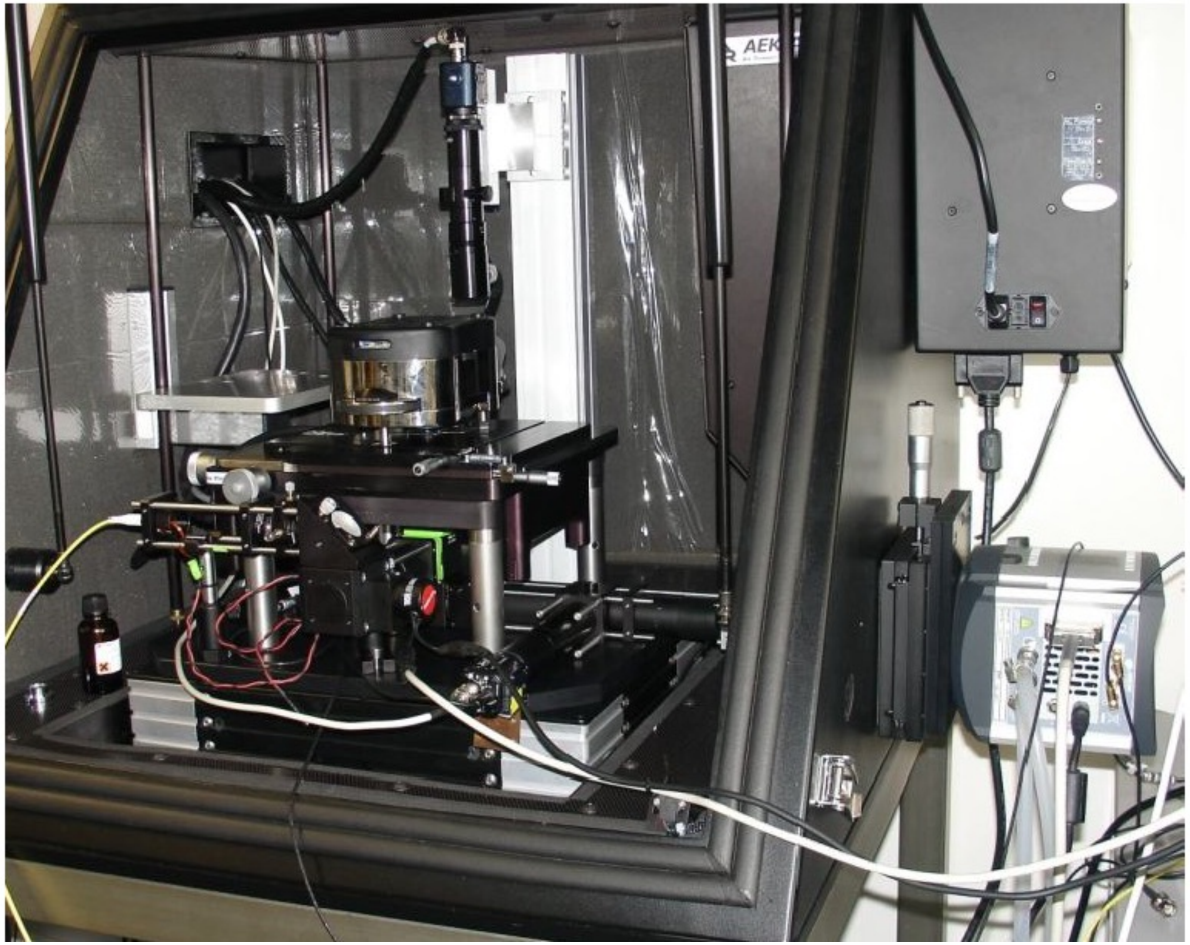
xy and z are mechanically decoupled

optical access from below

not so fast

Courtesy of Iwan Schaap

combining AFM with optical microscopy



camera mechanically and thermally isolated

no resonance body

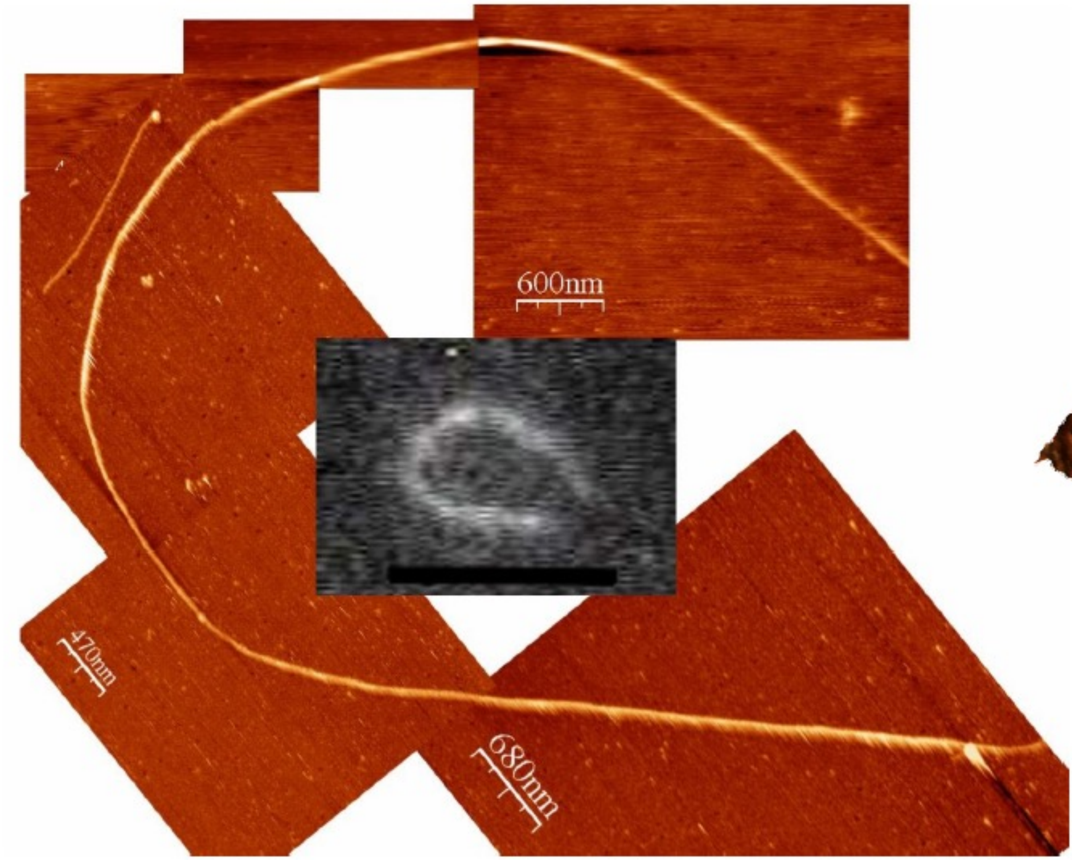
noise z_{RMS}: 0.35 nm

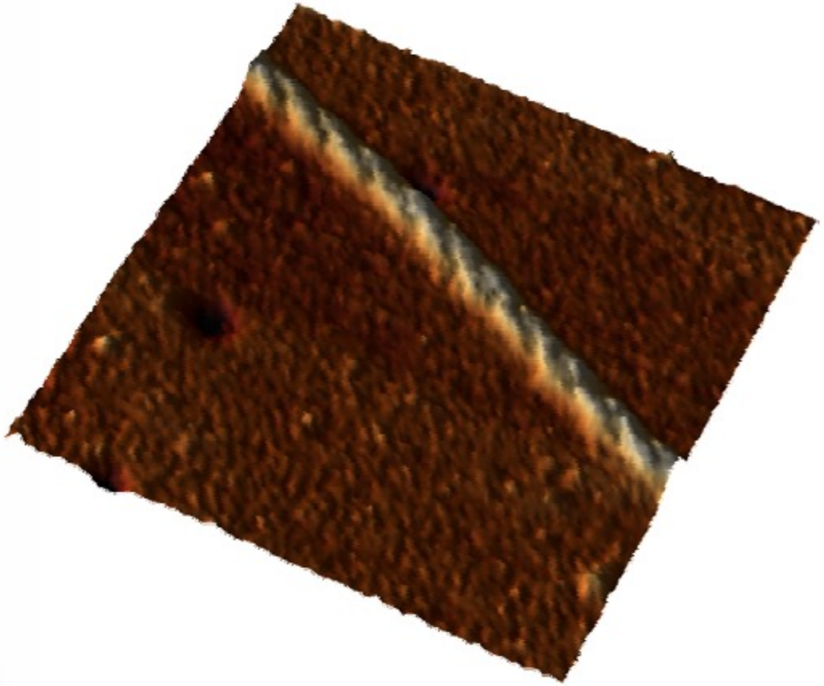
still worse than a simple AFM

noise z_{RMS}: 0.23 nm

Courtesy of Iwan Schaap

combining AFM with optical microscopy



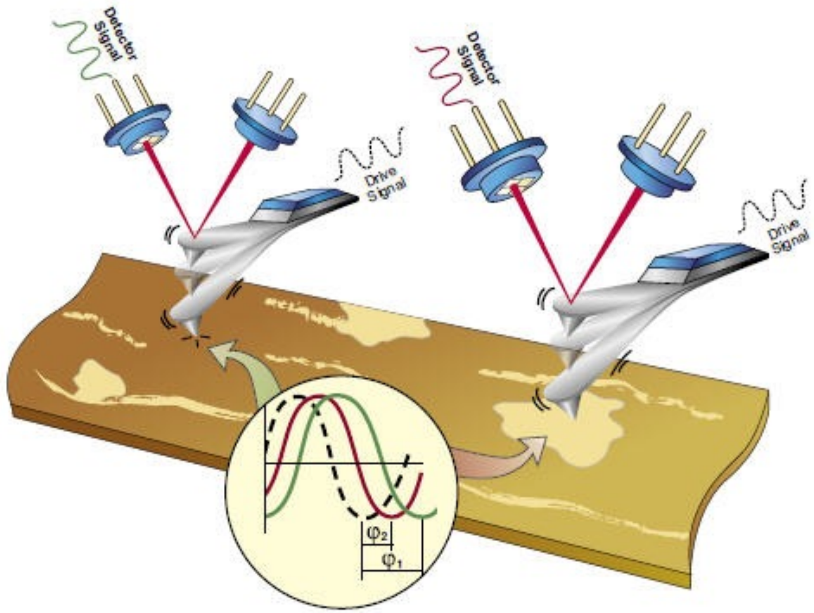


- localization
- identification

Courtesy of Iwan Schaap

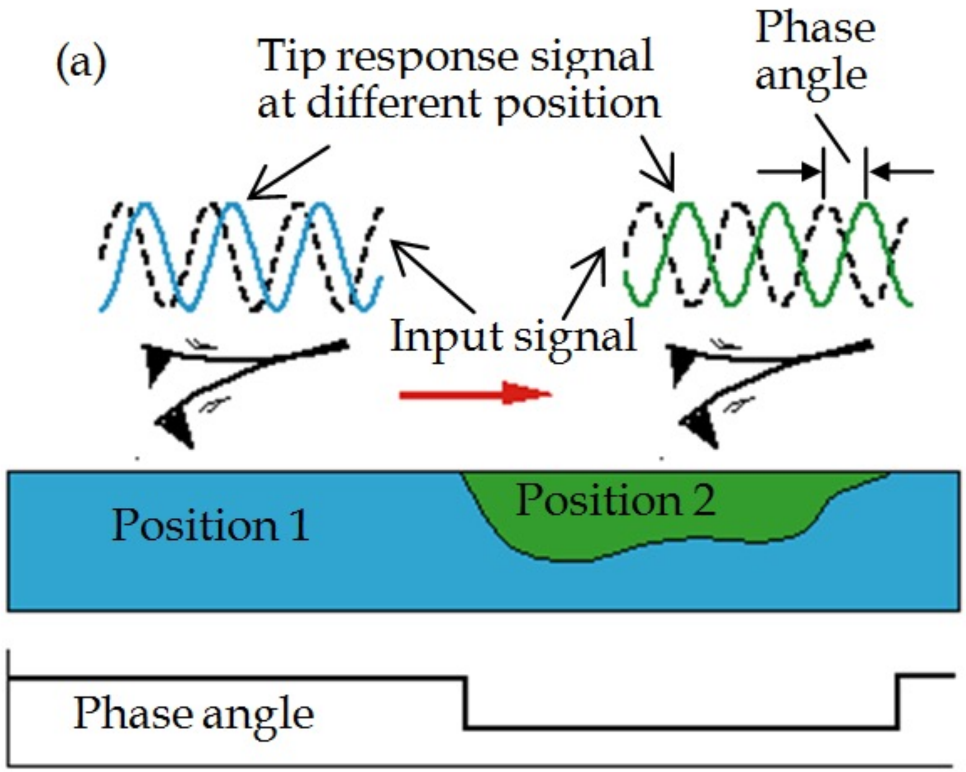
Other AFM Imaging Modes

AC mode: phase imaging



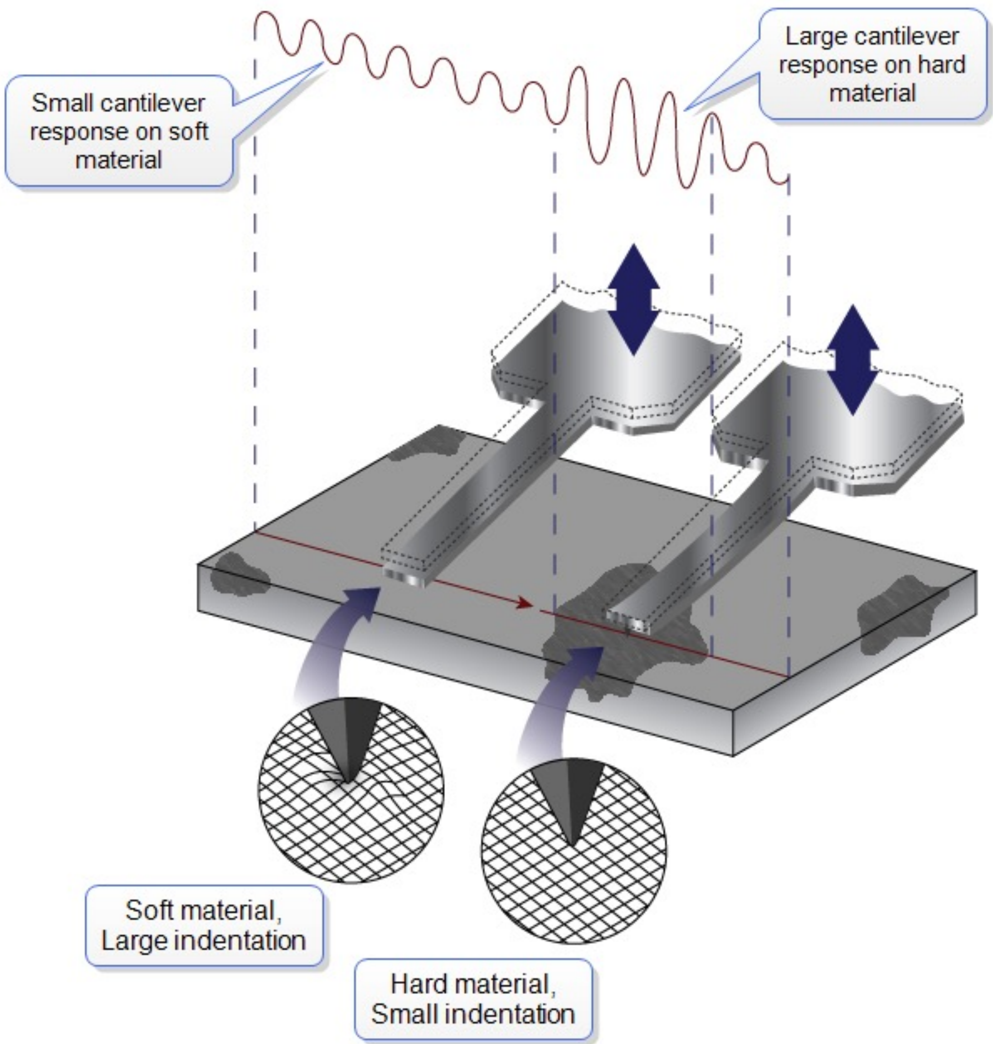
Phase imaging monitors the phase lag between the signal that drives the cantilever to oscillate and the cantilever oscillation output signal. Phase detection images can be produced while an instrument is operating in any vibrating cantilever mode.

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.



Force Modulation Microscopy

sample elastic properties



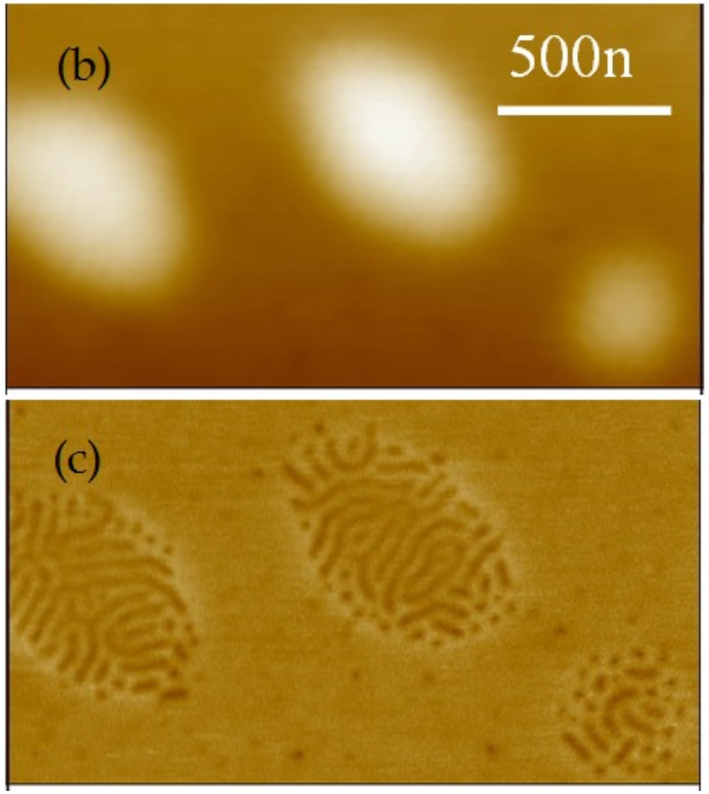
In FMM mode, the tip is scanned in contact with the sample, and the **z** feedback loop maintains a constant cantilever deflection (as for constant-force mode AFM).

- A periodic vertical oscillation signal is applied to either the tip or the sample. The amplitude of cantilever modulation that results from this applied signal varies according to the elastic properties of the sample.

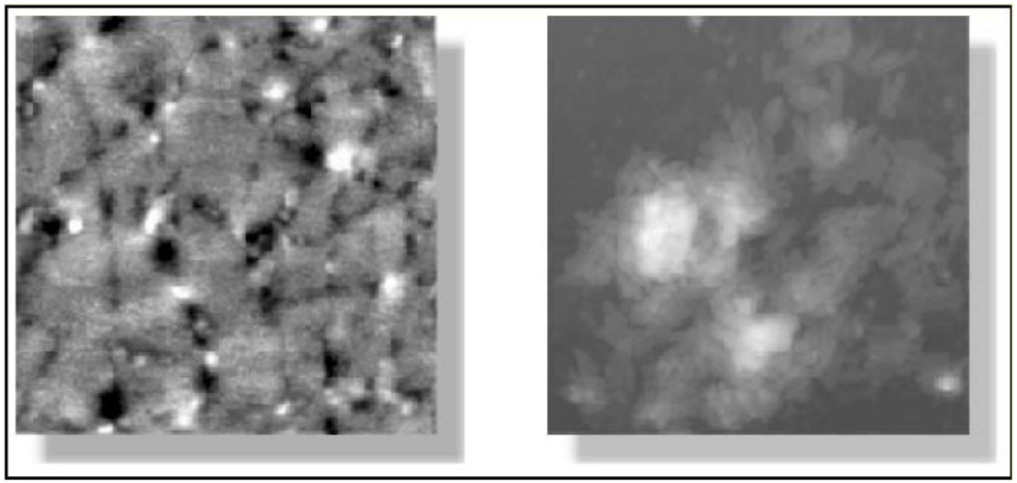
- From the changes in the amplitude of cantilever modulation, the system generates a force modulation image → a map of the sample's elastic properties.

AC mode: phase imaging

In many cases, phase imaging complements lateral force microscopy (LFM), and force modulation microscopy (FMM), often providing additional information more rapidly and with higher resolution.
Phase imaging is as fast and easy to use as Tapping-Mode AFM -> with all its benefits for imaging soft, adhesive, easily damaged or loosely bound samples.



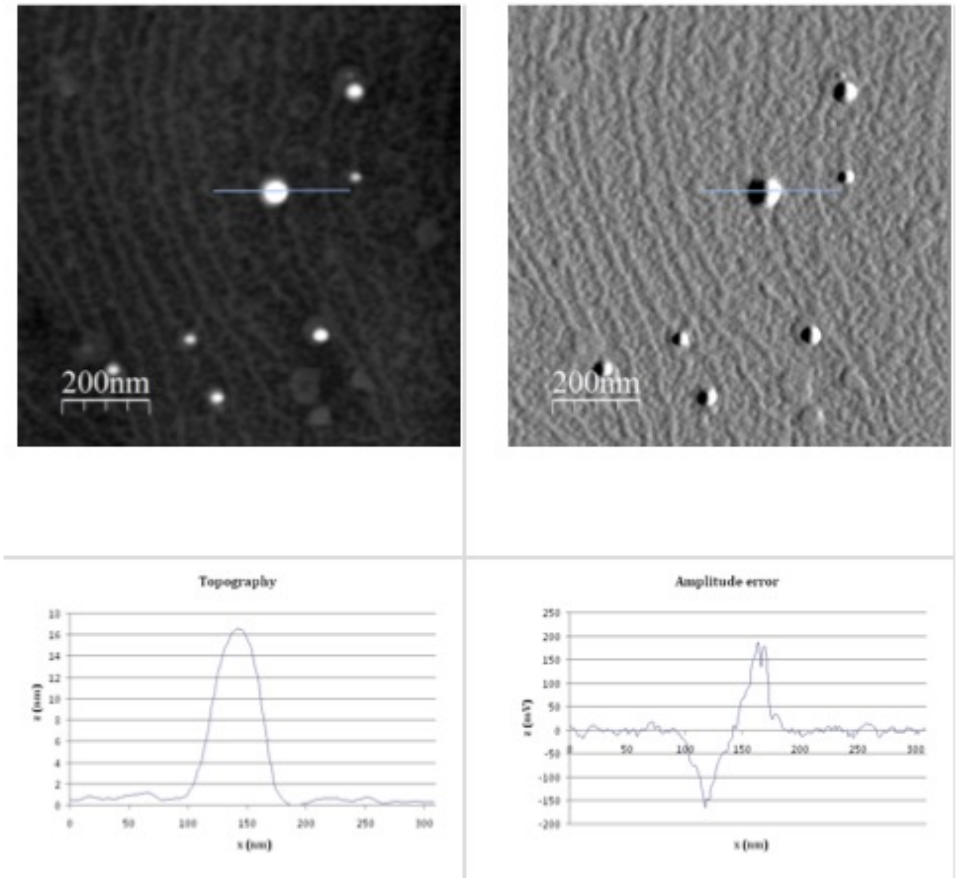
- Identification of contaminants;
- Mapping of different components in composite materials;
- Differentiating regions of high and low surface adhesion or hardness;
- Mapping of electrical and magnetic properties with wide-ranging implications in data storage and semiconductor industries.



Error imaging

In almost all operating modes, a feedback circuit is connected to the deflection sensor and attempts to keep the tip–sample interaction constant by controlling the tip–sample distance. This protects both the tip and the sample. In practice however feedback is never perfect, and there is always some delay between measuring a change from the setpoint and restoring it by adjusting the scanning height.

In tapping mode for example this can be measured by the difference between the instantaneous amplitude of oscillation and the amplitude setpoint. This is known as the amplitude error signal, and highlights changes in surface height.

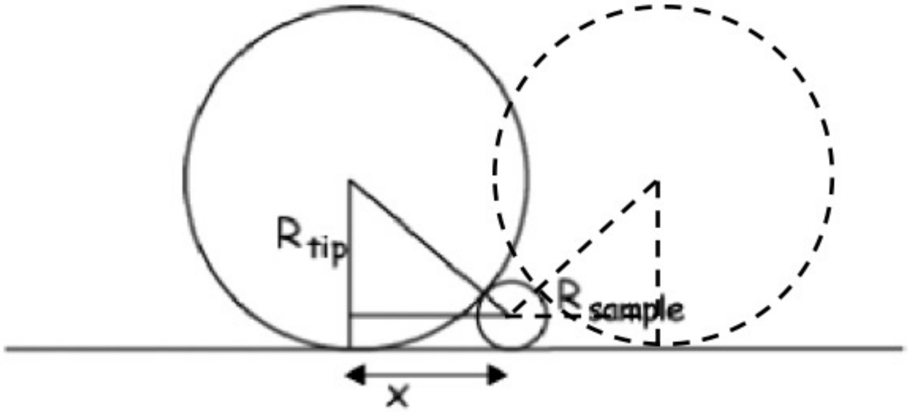


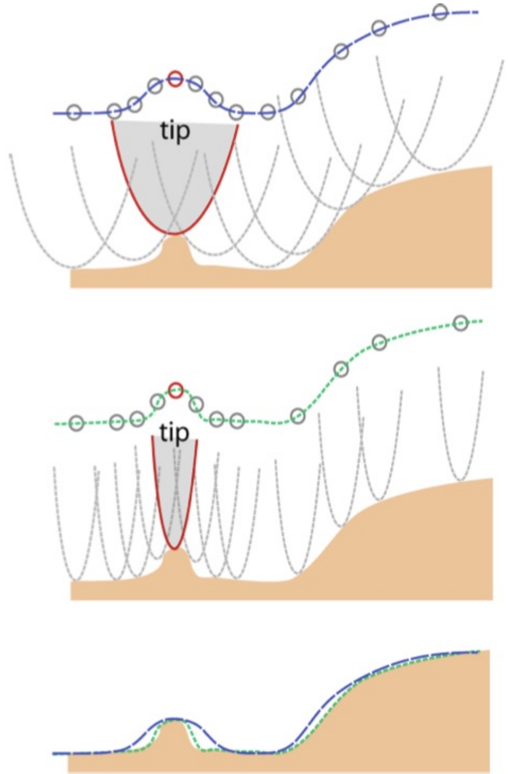
PARAMETERS AFFECTING THE IMAGING:

- *Setpoint*
- *Feedback gains*
- *Scan rate*

Resolution and artifacts

The width w of an object is the convolution between tip and object size





$$x^2 = (R_{tip} + R_{sample})^2 - (R_{tip} - R_{sample})^2$$

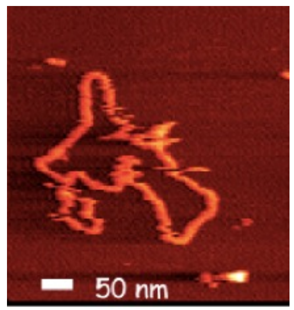
$$x^2 = \not{R_{tip}^2} + 2R_{tip}R_{sample} + \not{R_{sample}^2} - \not{R_{tip}^2} + 2R_{tip}R_{sample} - \not{R_{sample}^2}$$

$$x = 2\sqrt{R_{tip}R_{sample}}$$

$$w = 2x = 4\sqrt{R_{tip}R_{sample}}$$

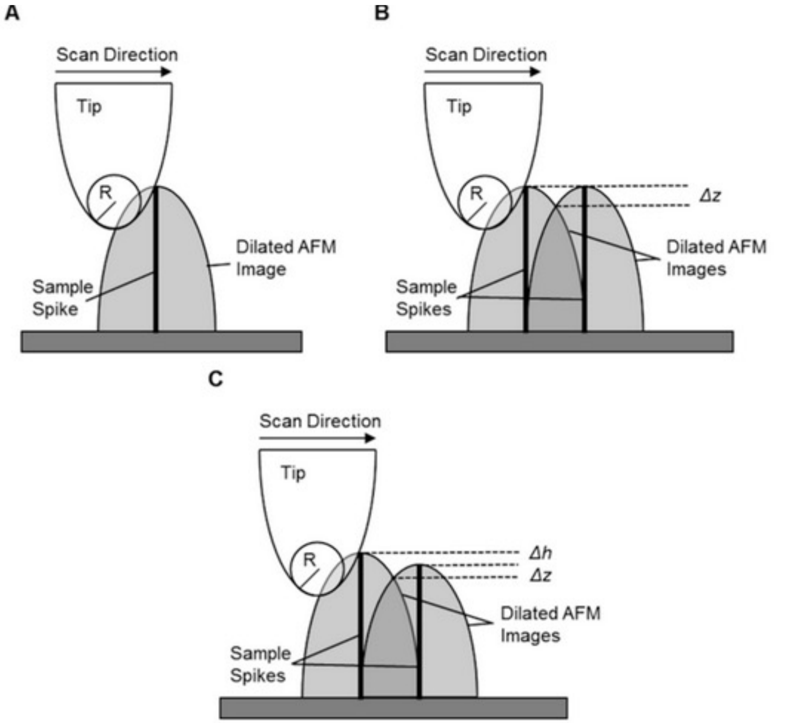
DNA: 2 nm.

tip ~ 20 nm => w = 25 nm
tip ~ 10 nm => w = 18 nm



Resolution and artifacts

Vertical resolution can be defined as a minimum controllable height change and it is dominated by the **noise** in the imaging signal
Lateral resolution is defined as the **minimum separation d** for which the **dimple height Δz is higher than the noise**



$$d = \sqrt{2R}(\sqrt{\Delta z} + \sqrt{\Delta z + \Delta h})$$

Table 1
The ideal lateral resolution d calculated for various combinations of tip radius R and relative height Δh using Eq. (2), with a fixed vertical resolution $\Delta z = 0.02$ nm.

R (nm)	d (nm)			
	$\Delta h = 0$ nm	$\Delta h = 0.20$ nm	$\Delta h = 0.50$ nm	$\Delta h = 1.0$ nm
0.2	0.13	0.39	0.55	0.73
0.5	0.2	0.61	0.86	1.2
1.0	0.28	0.86	1.2	1.6
2.0	0.4	1.2	1.7	2.3
5.0	0.63	1.9	2.7	3.6
10	0.89	2.7	3.9	5.1
20	1.3	3.9	5.5	7.3
50	2.0	6.1	8.6	12

Flatter surface and sharper tips give higher lateral resolution (if sample and tip deformation are negligible).
In case of sample deformation, the surface-tip contact area limits the resolution. The lower the force in contact mode, the higher the resolution.

Resolution and artifacts

Thermal noise in contact mode AFM

$$\Delta z = \sqrt{\frac{4k_B T}{3k}} = \frac{0.074\ \text{nm}}{\sqrt{k}}$$

Thermal noise in AC mode AFM

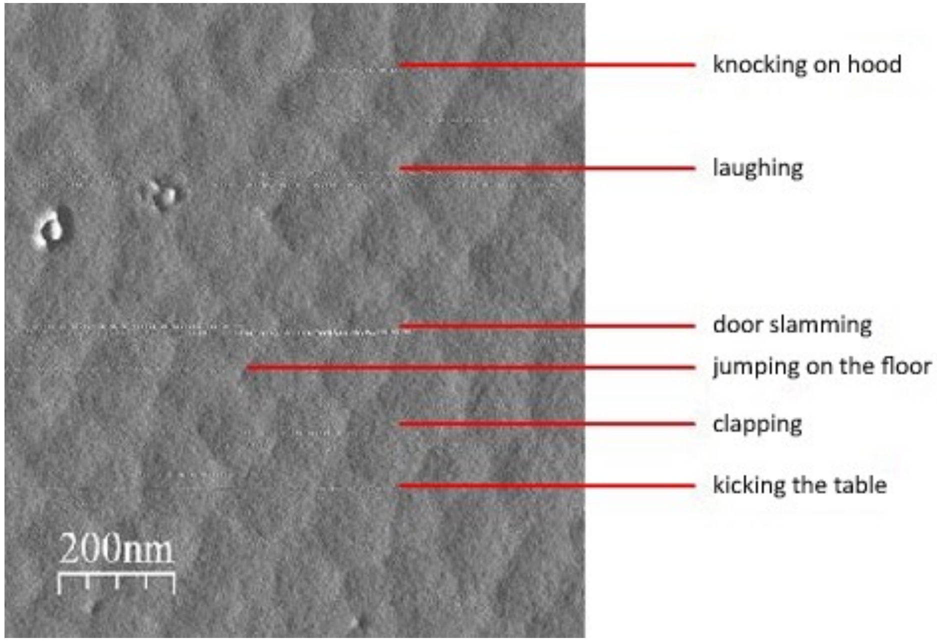
$$\Delta z = \sqrt{\frac{4k_B TQB}{\pi f_0 k}}$$

f_0 = resonant frequency
B = detection bandwidth
K = elastic constant
Q= quality factor,
T = temperature

Cantilever with higher spring constant and resonant frequency has lower thermal noise

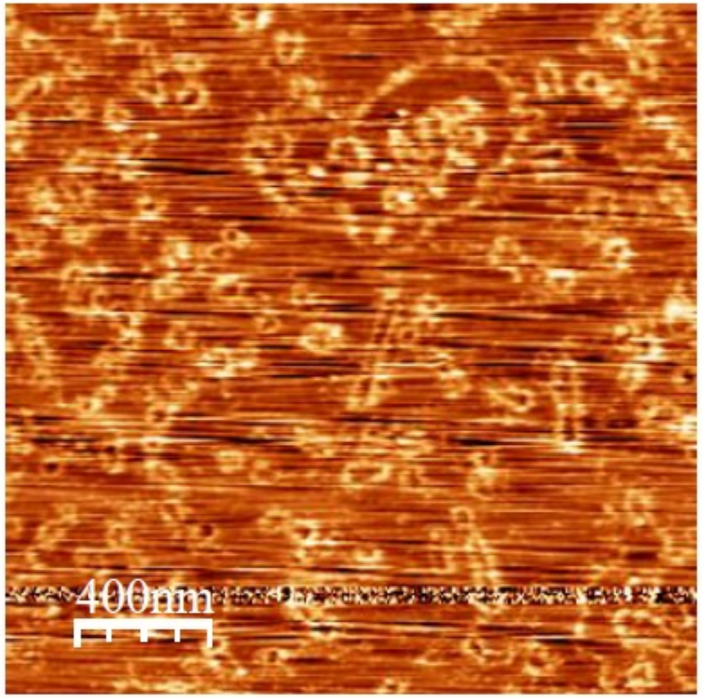
B=1 kHz, f_0= 318 kHz, k=28 N/m, Q=400 give 0.015 nm thermal noise at RT

Resolution and artifacts
Vibrations

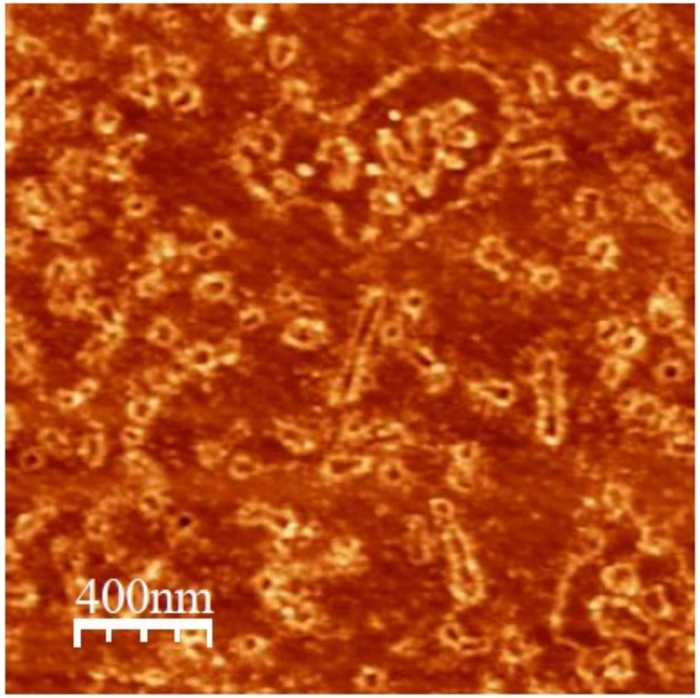


These vibrations may be transmitted through the floor, for example from footsteps or the use of a lift. These can be minimised by the use of a vibrational isolation table, and locating the AFM on a ground floor or below.
Acoustic noise such as people talking can also cause image artefacts, as can drafts of air. An acoustic hood can be used to minimise the effects of both of these.

Resolution and artifacts
Vibrations

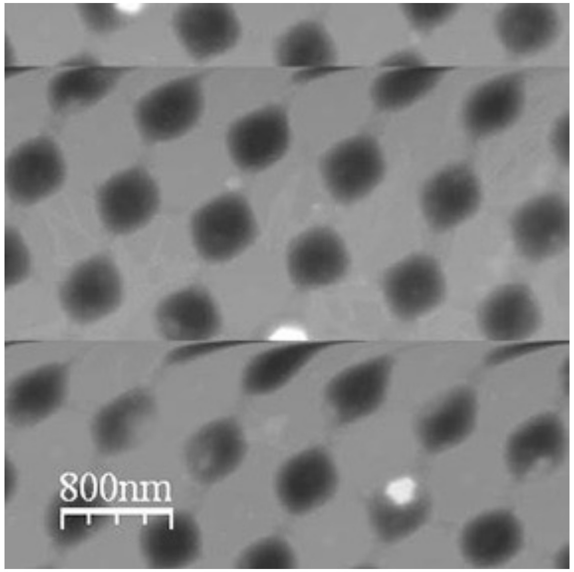


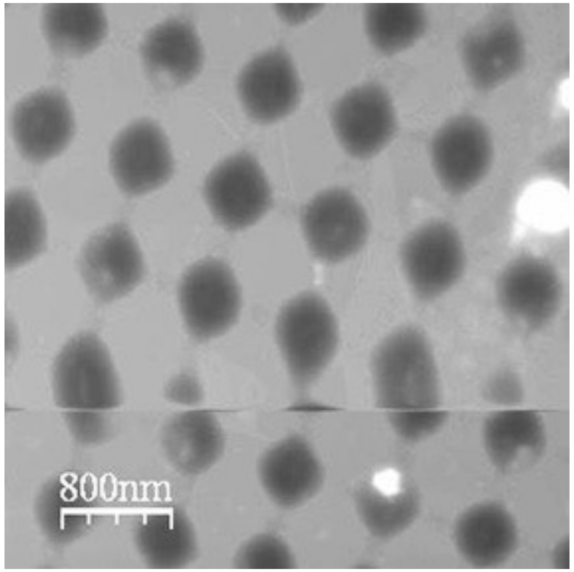
quiet room,
no vibration isolation

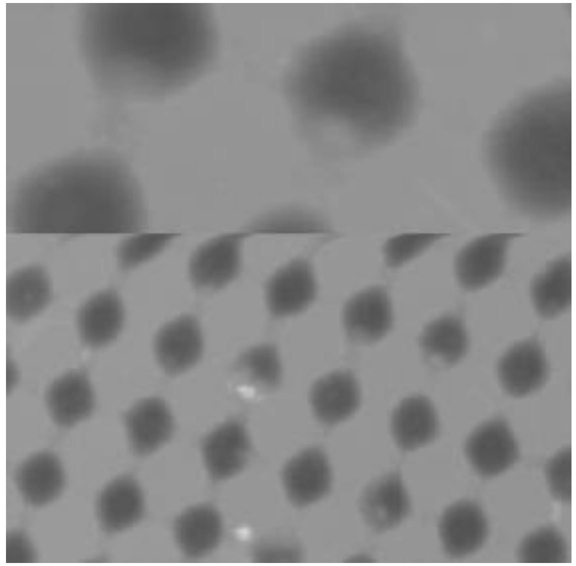


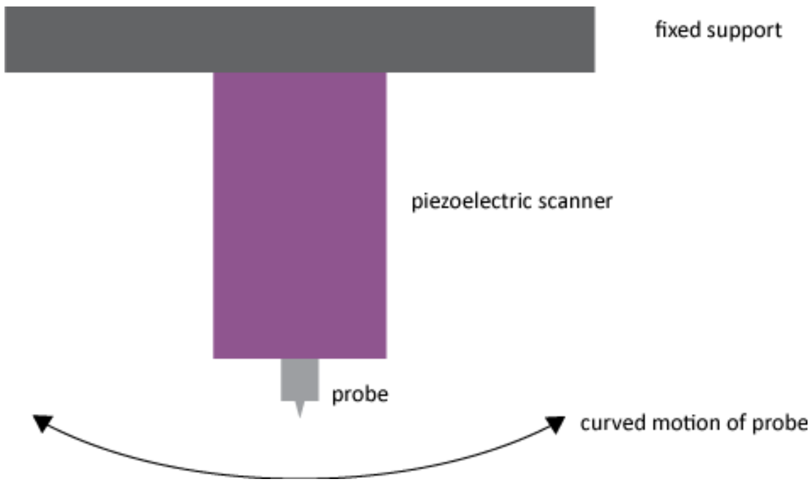
active vibration isolation
resolution is the same,
but the noise is reduced

Resolution and artifacts
Scanner creep





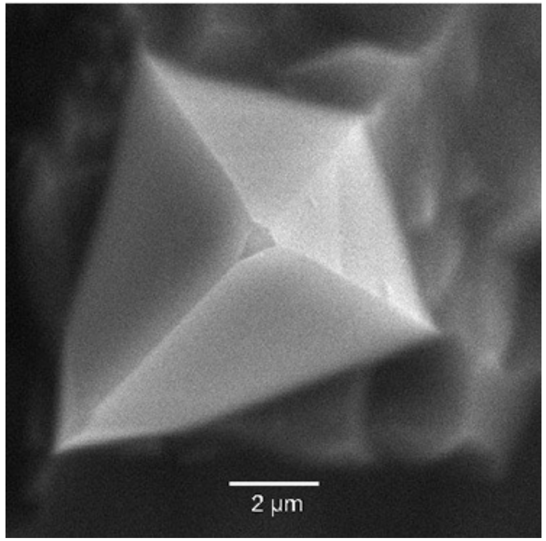


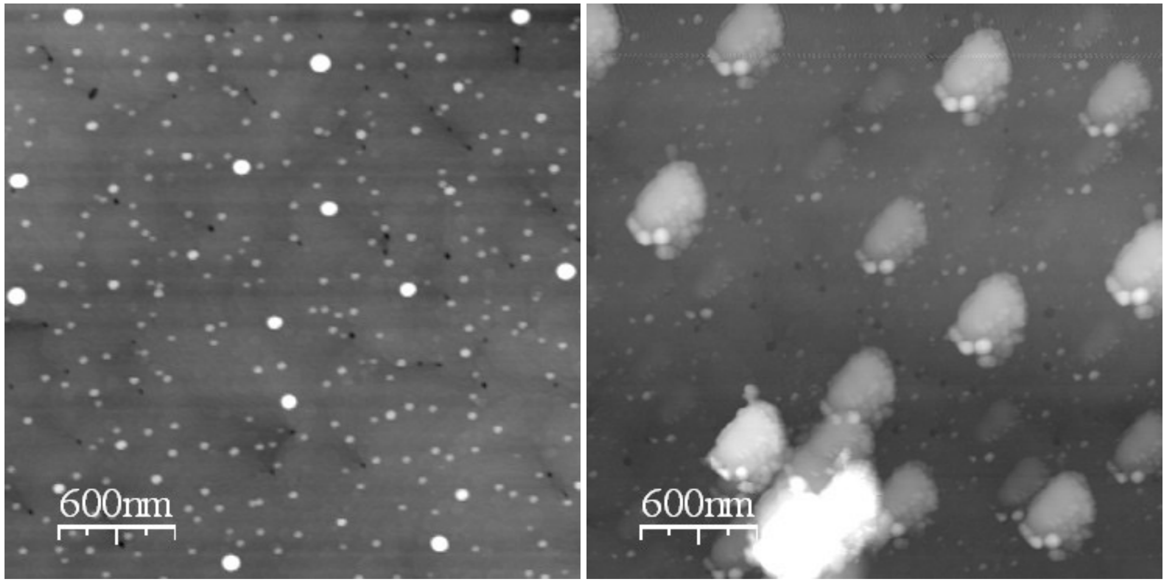


Because of the construction of the piezo-scanner, the tip does not move in a perfectly flat plane. Instead its movement is in a parabolic arc, as shown in the image below. This causes the artefact known as *scanner bow*. Also the scanner and sample planes may not be perfectly parallel, this is known as *tilt*. Both of these artefacts can be removed by using post-processing software.

Resolution and artifacts
Damaged tip

The tip may pick up loose debris from the sample surface. This may be reduced by cleaning the sample with compressed air or N_2 before use. Or the tip can be damaged during scanning, which degrades the images. This may be blunting of the tip, as shown in the SEM image.

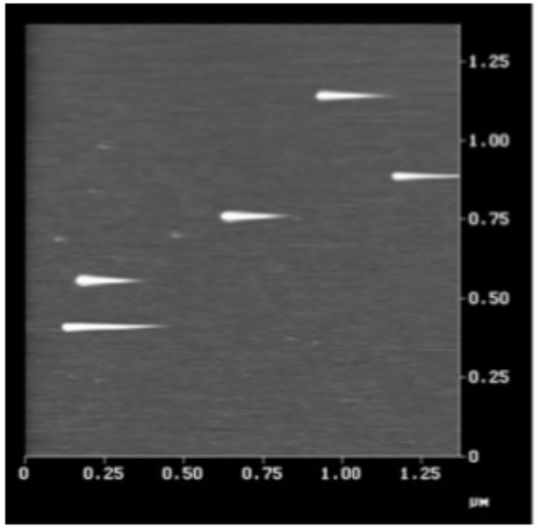


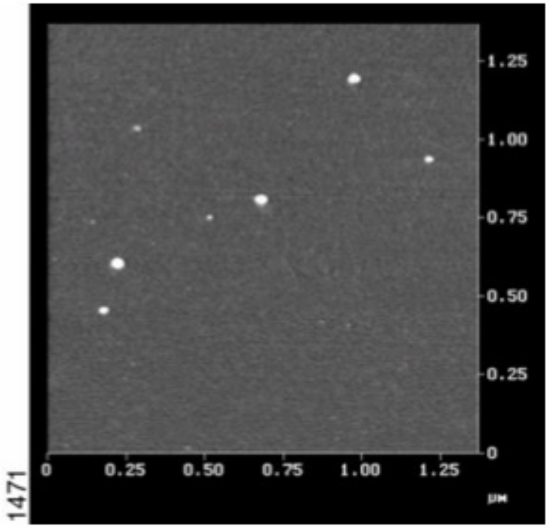


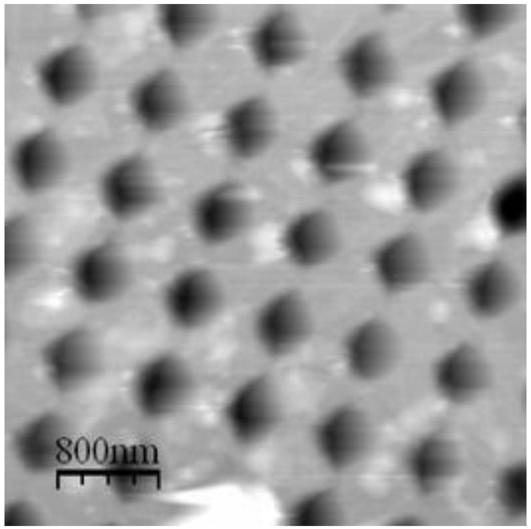
Resolution and artifacts
Feedback artifact

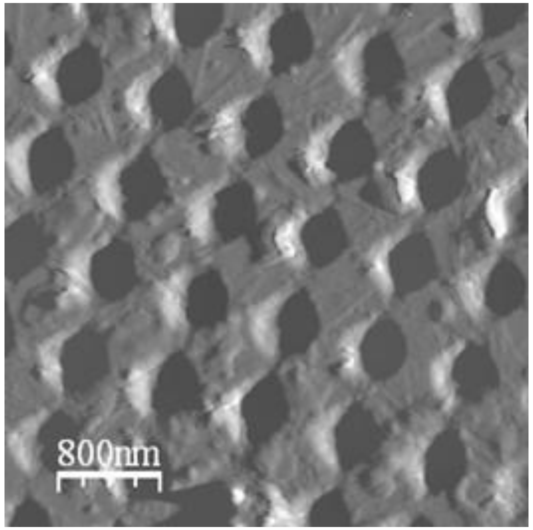
The precise values used for feedback gains will vary between instruments. A good rule of thumb is to increase the gain until excess noise begins to appear, and then reduce it slightly to get good tracking with low noise

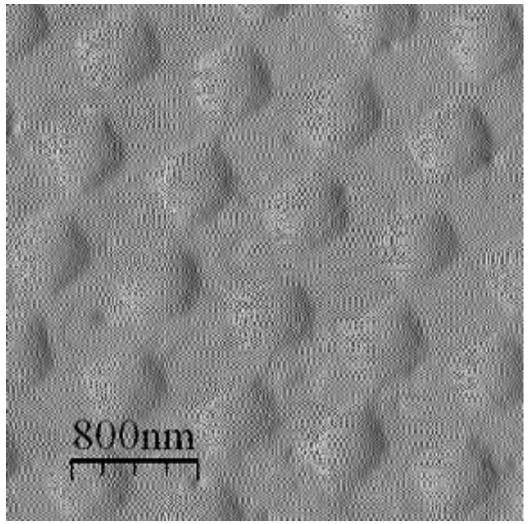
Low gain → Poor tracking
High gain →High frequency noise





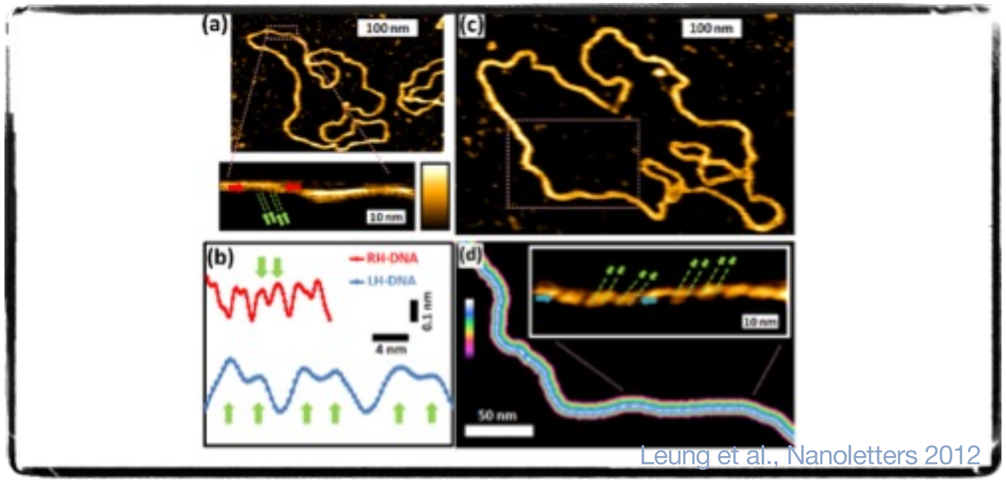




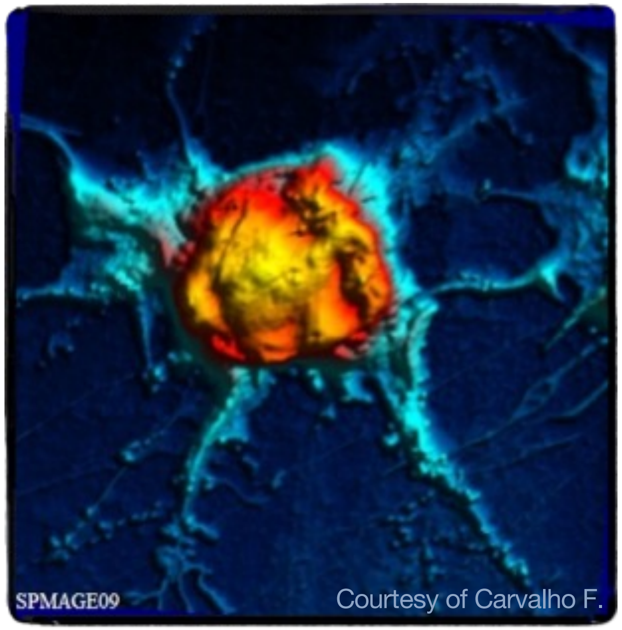


AFM in Biology

DNA



Cells



Proteins