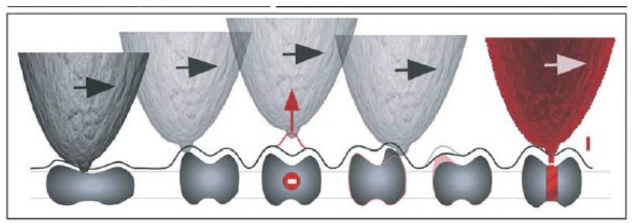
Elettra Sincrotrone Trieste

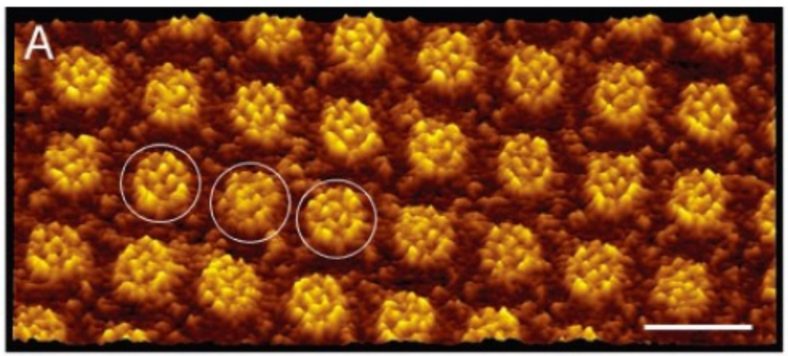
ATOMIC FORCE MICROSCOPY
Imaging in Biology

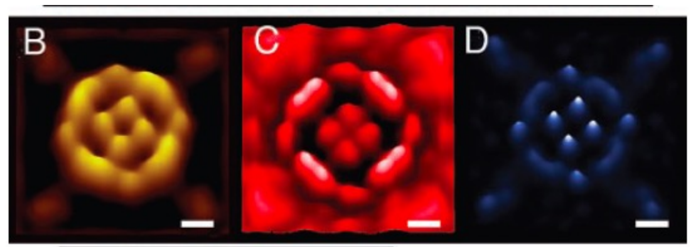
Sources:
Fundamentals of Scanning Probe Microscopy, V. L. Mironov
NanoHub.org

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.



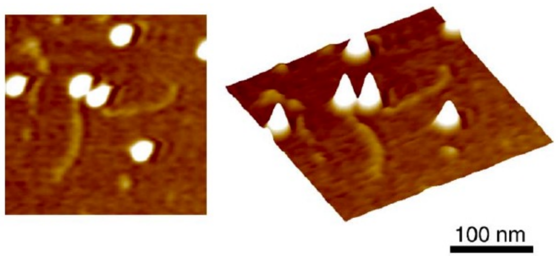


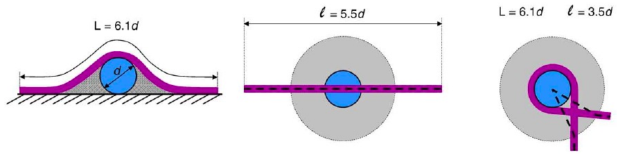


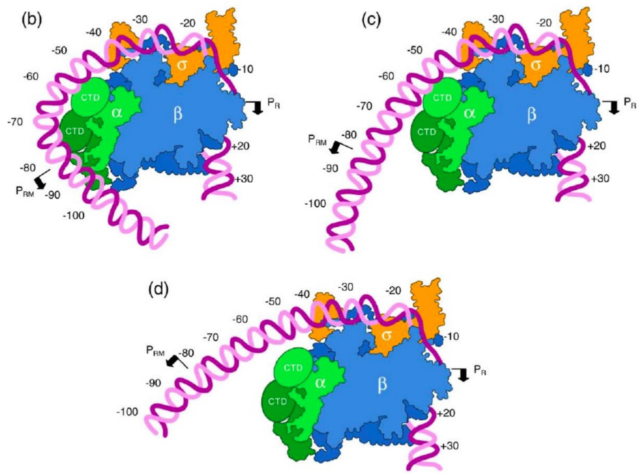
S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

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.



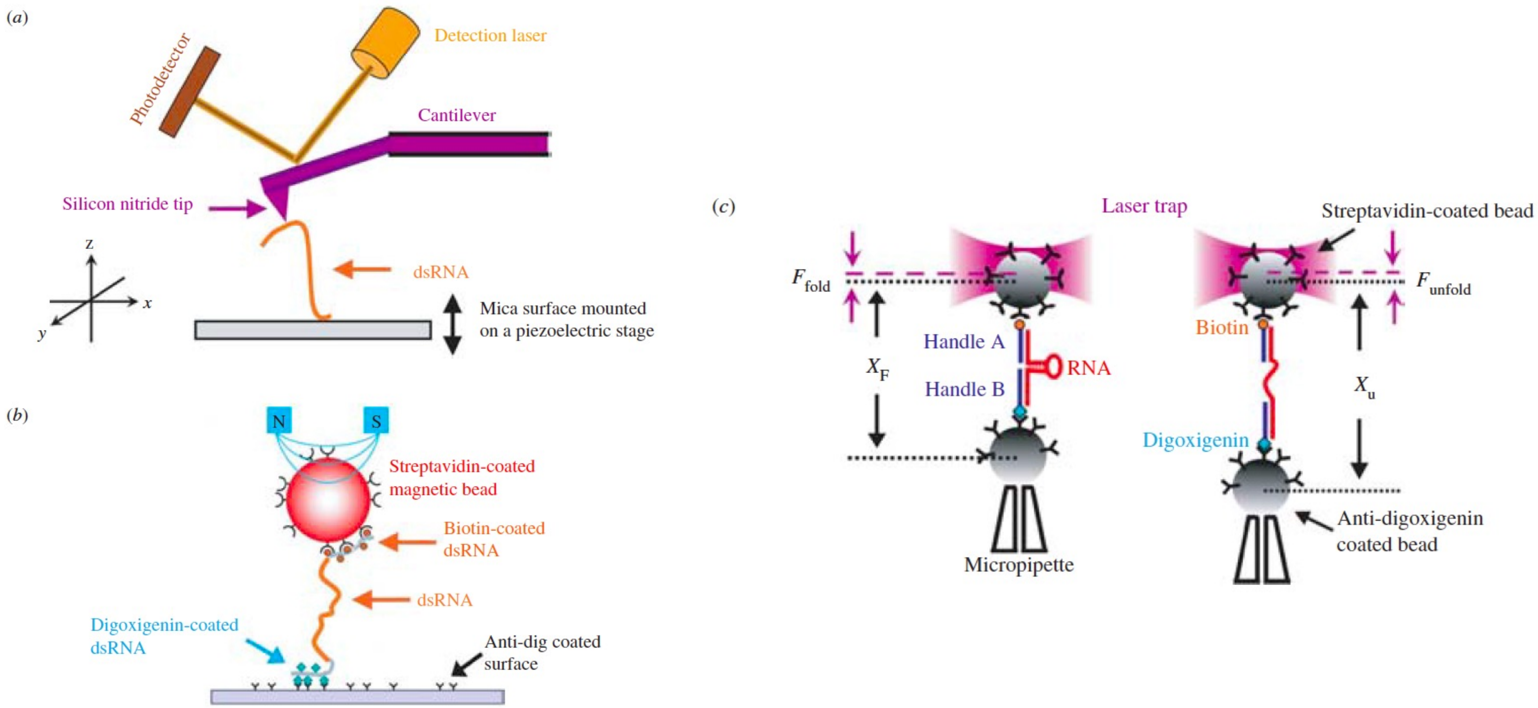




I. Mangiarotti, S. Cellai, W. Ross, C. Bustamante, C. Rivetti, *L. Mol. Biol.* **385**, 748 (2009)

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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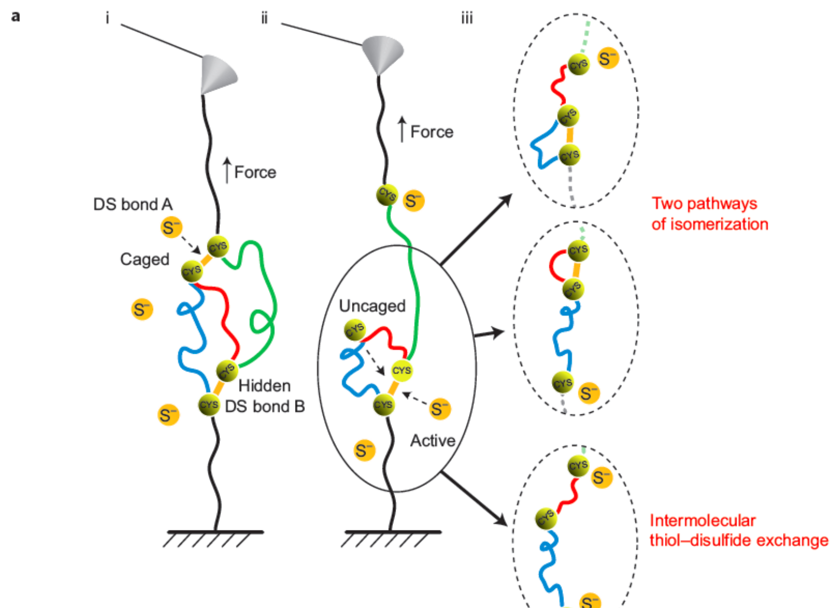
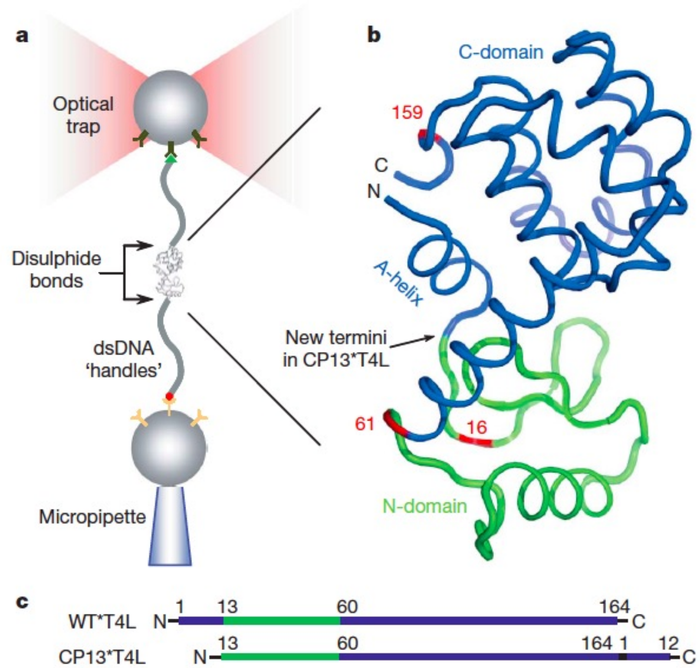
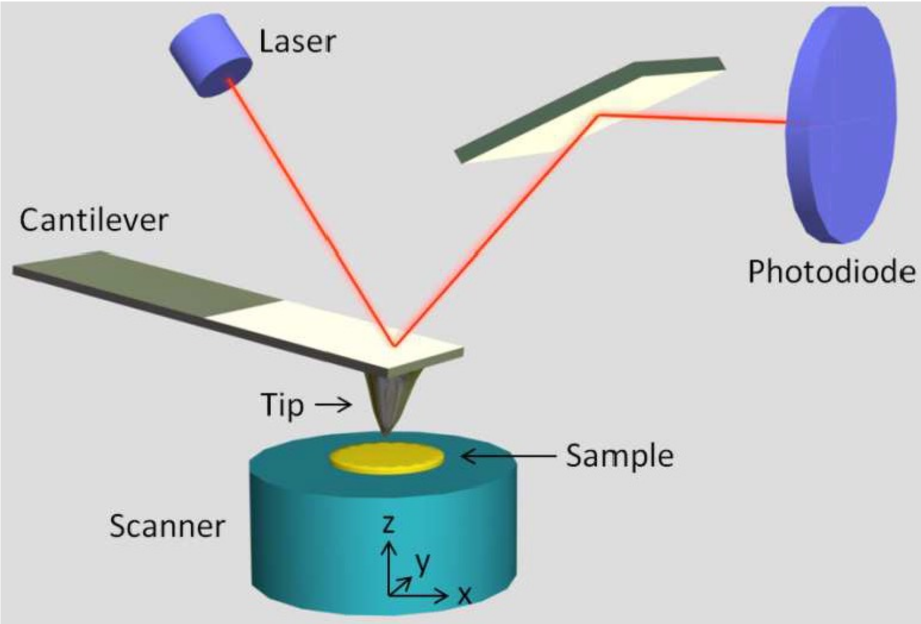


Intramolecular thiol-disulfide switching and its role in protein folding (J. Fernandez)



Folding cooperativity and chain topology (C. Bustamante)

Atomic Force Microscopy



Unique characteristics:

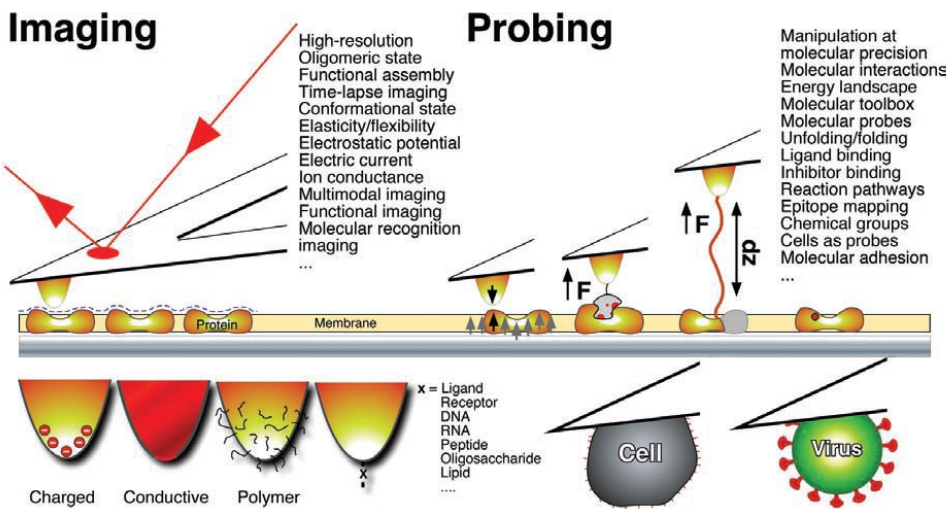
1. built-in atomic scale sensitivity
2. precise motion control technology
3. fabrication technology (nanolithography)

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!

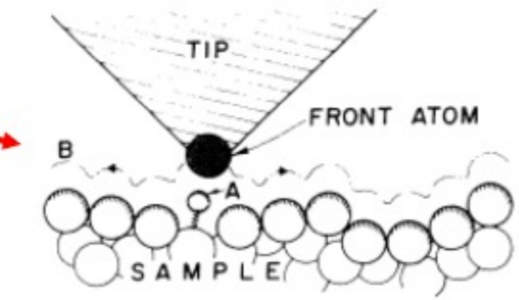
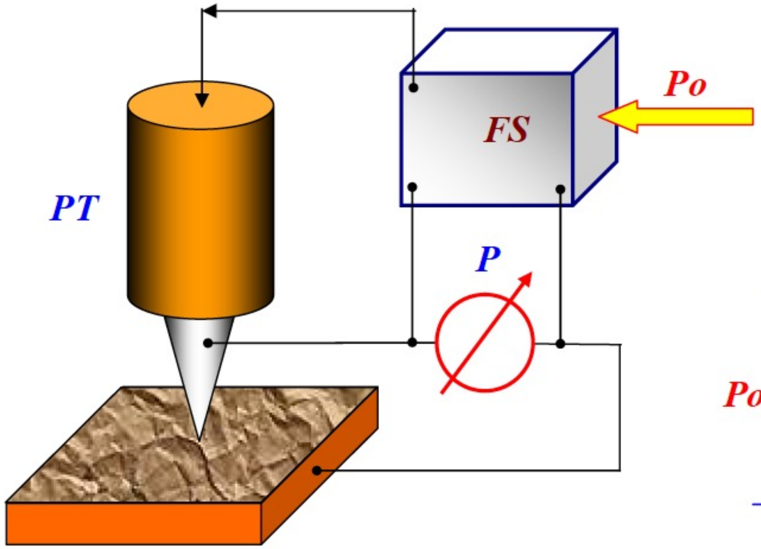


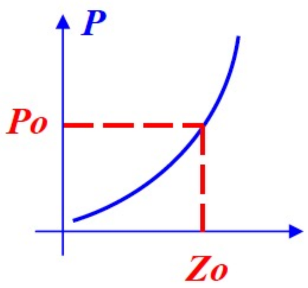
FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM 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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2. precise motion control technology
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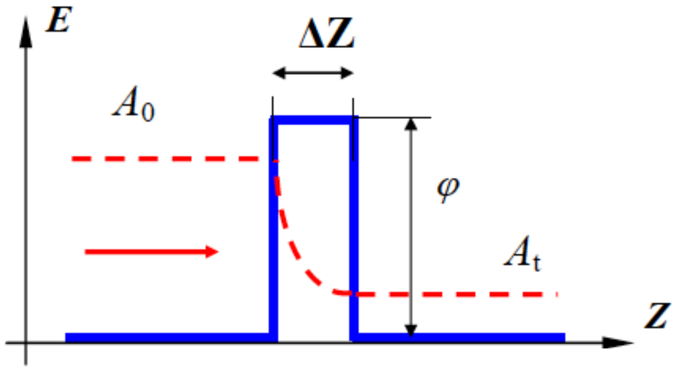
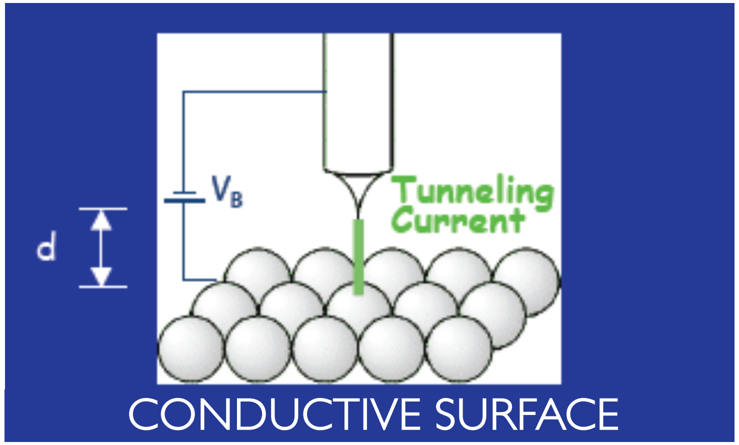
Scanning Tunneling Microscopy





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

average work function ϕ *

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

probability of electron tunneling
(transmission coefficient)

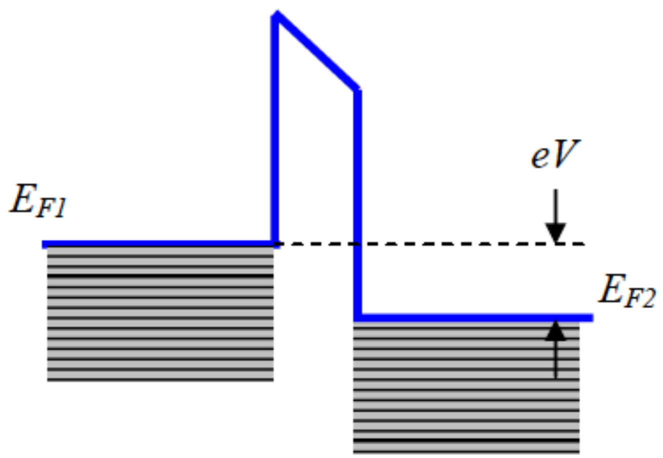
, A = amplitude of electron wave function

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

attenuation coefficient in metals

Scanning Tunneling Microscopy

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



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

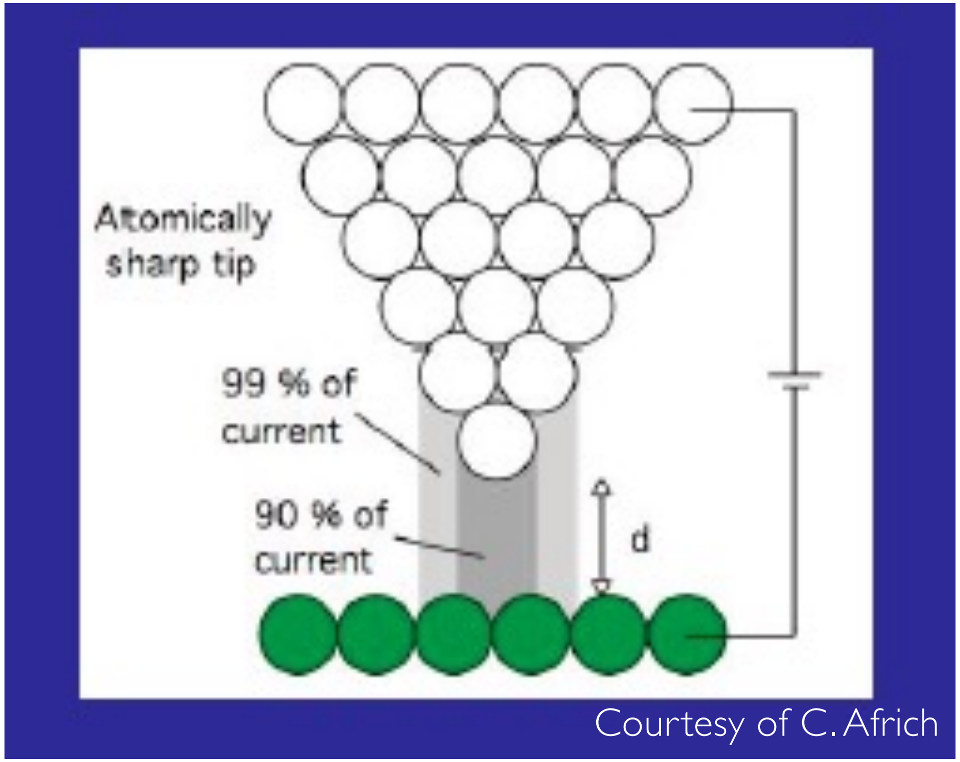
For typical values of the work function

ϕ ~ 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



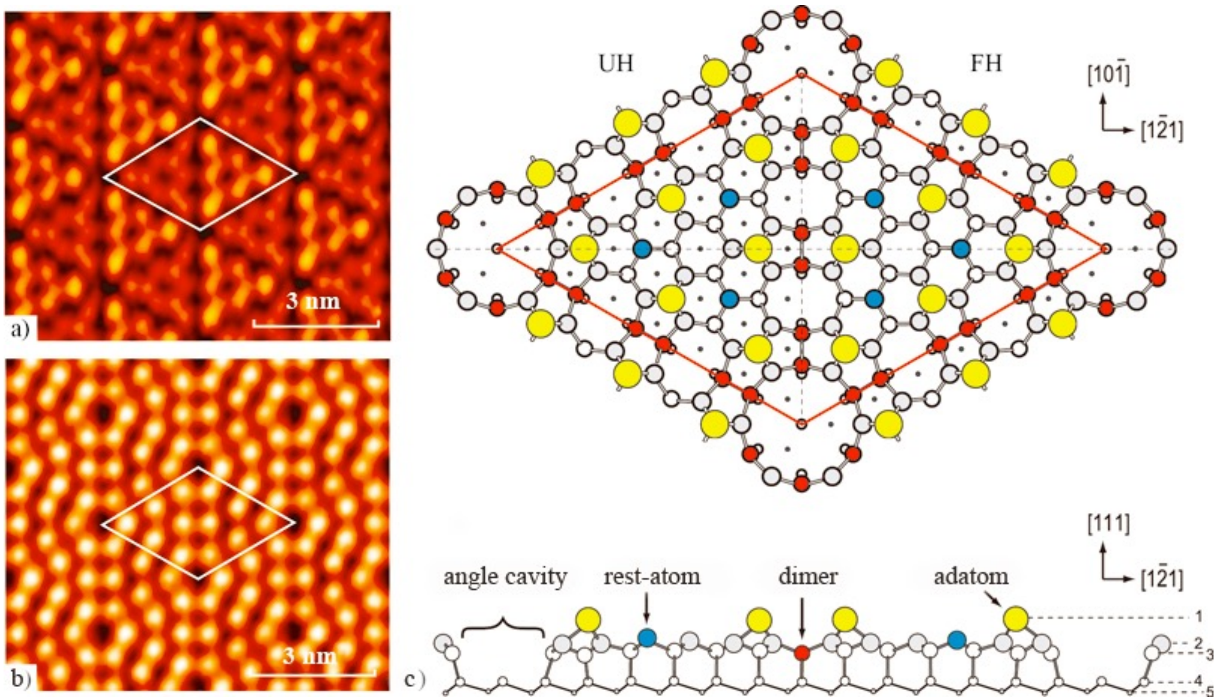
when ΔZ changes of about 1 Å, the current value varies of one order of magnitude!

Scanning Tunneling Microscopy

VOLUME 50, NUMBER 2 PHYSICAL REVIEW LETTERS 10 JANUARY 1983

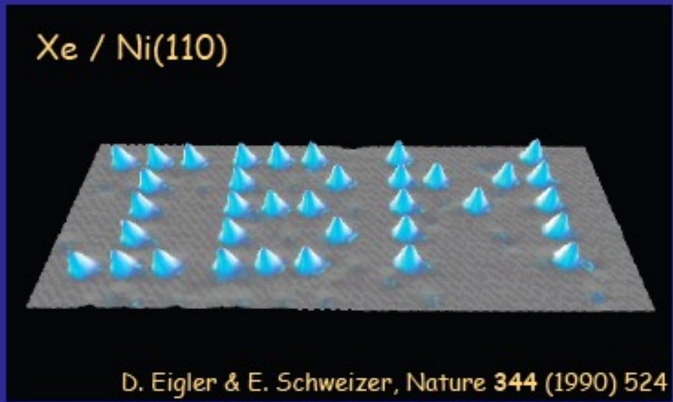
7 × 7 Reconstruction on Si(111) Resolved in Real Space

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

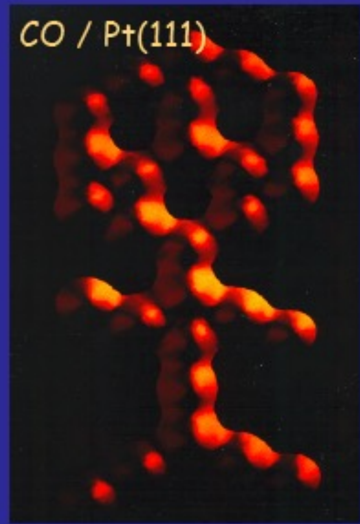


Surf. Sci. 1985. V. 164. P. 367. 7x7 rec. reduces dangling bonds from 49 to 19

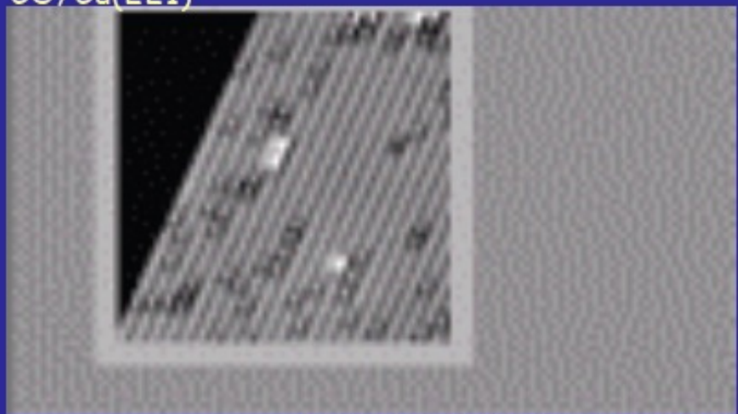
Manipulation by STM



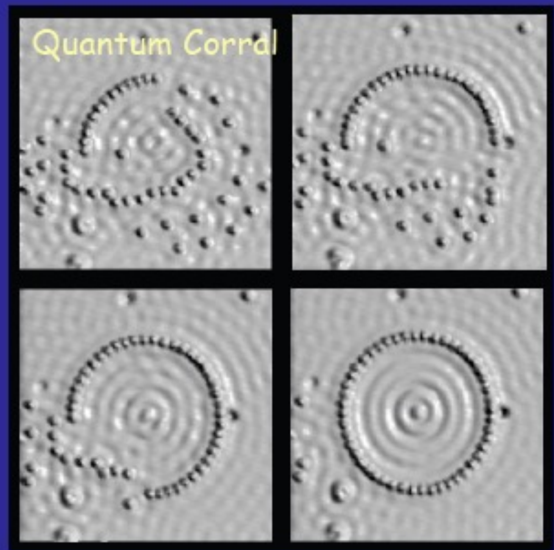
D. Eigler & E. Schweizer, Nature **344** (1990) 524



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

VOLUME 56, NUMBER 9 PHYSICAL REVIEW LETTERS 3 MARCH 1986

Atomic Force Microscope

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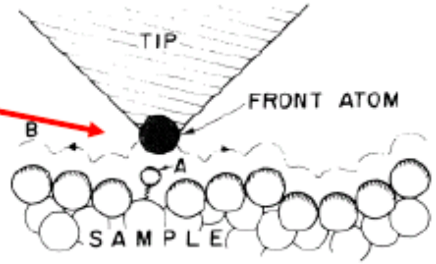
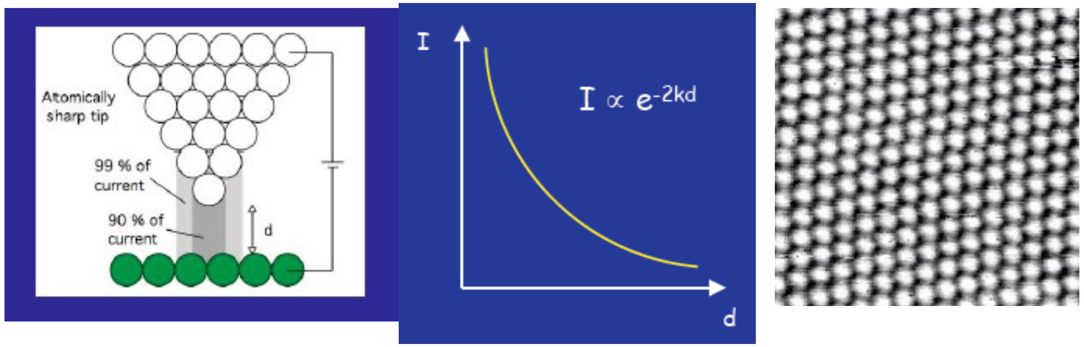
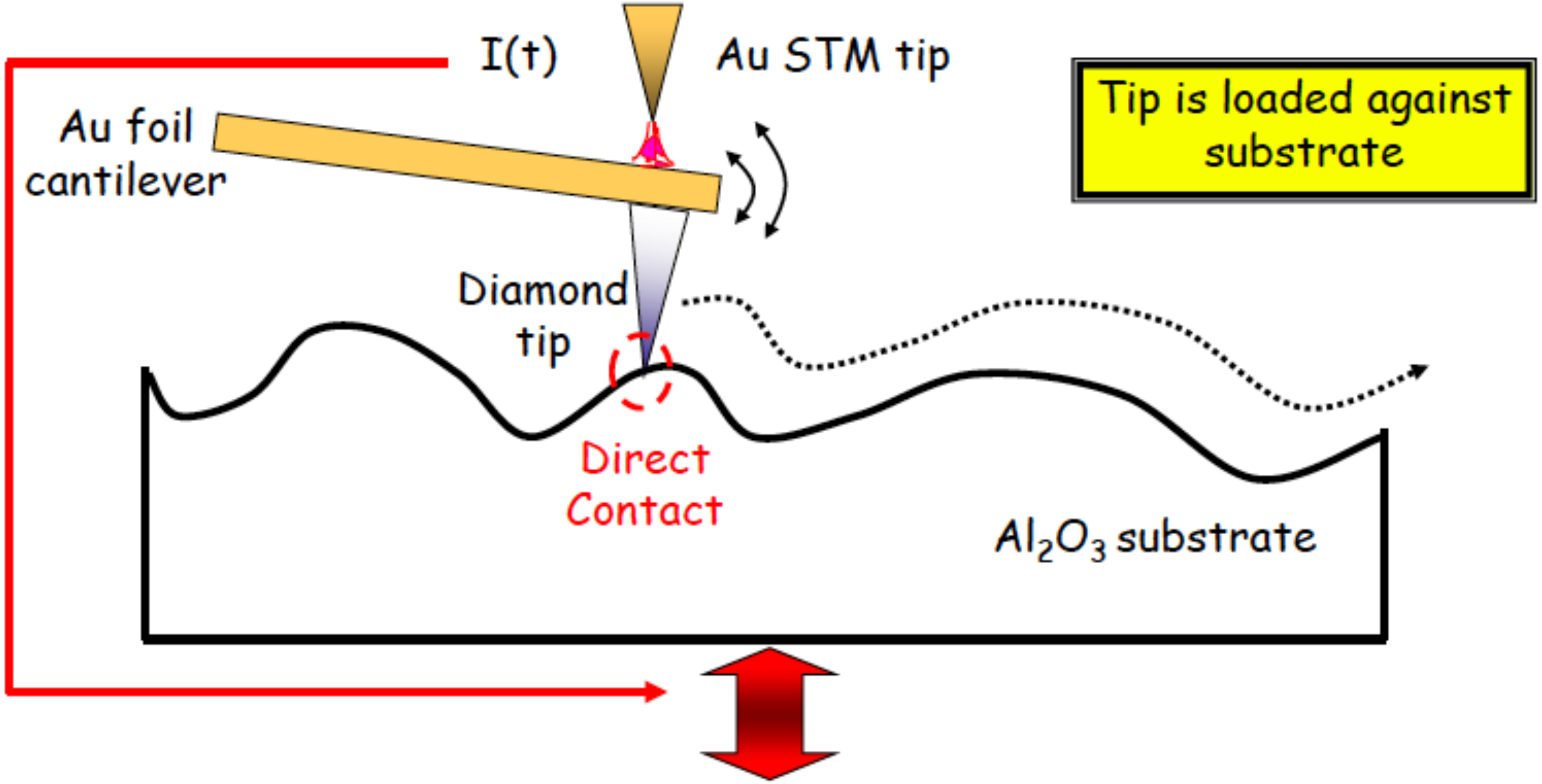


FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM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

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.

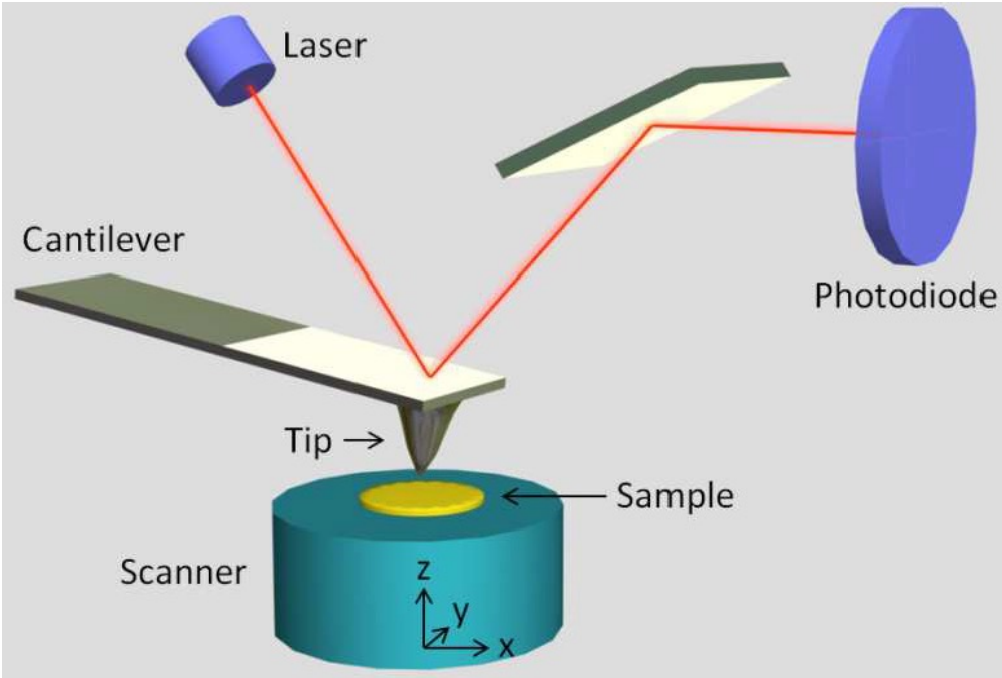


Atomically sharp tip
99 % of current
90 % of current
d
I
$I \propto e^{-2kd}$
d



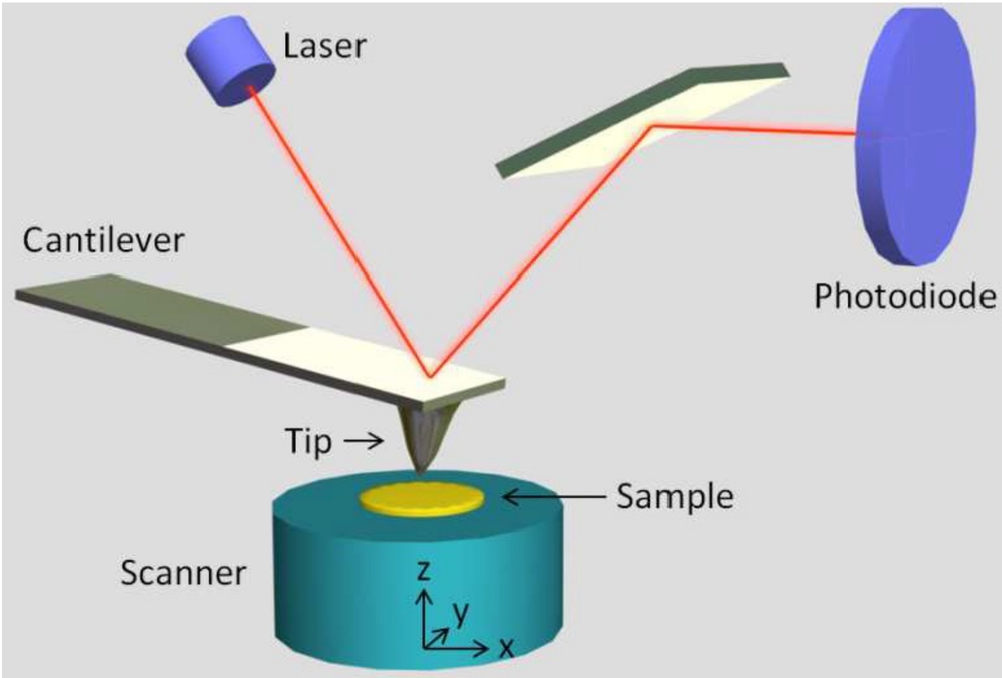
I(t)
Au STM tip
Tip is loaded against substrate
Au foil cantilever
Diamond tip
Direct Contact
Al_2O_3 substrate

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



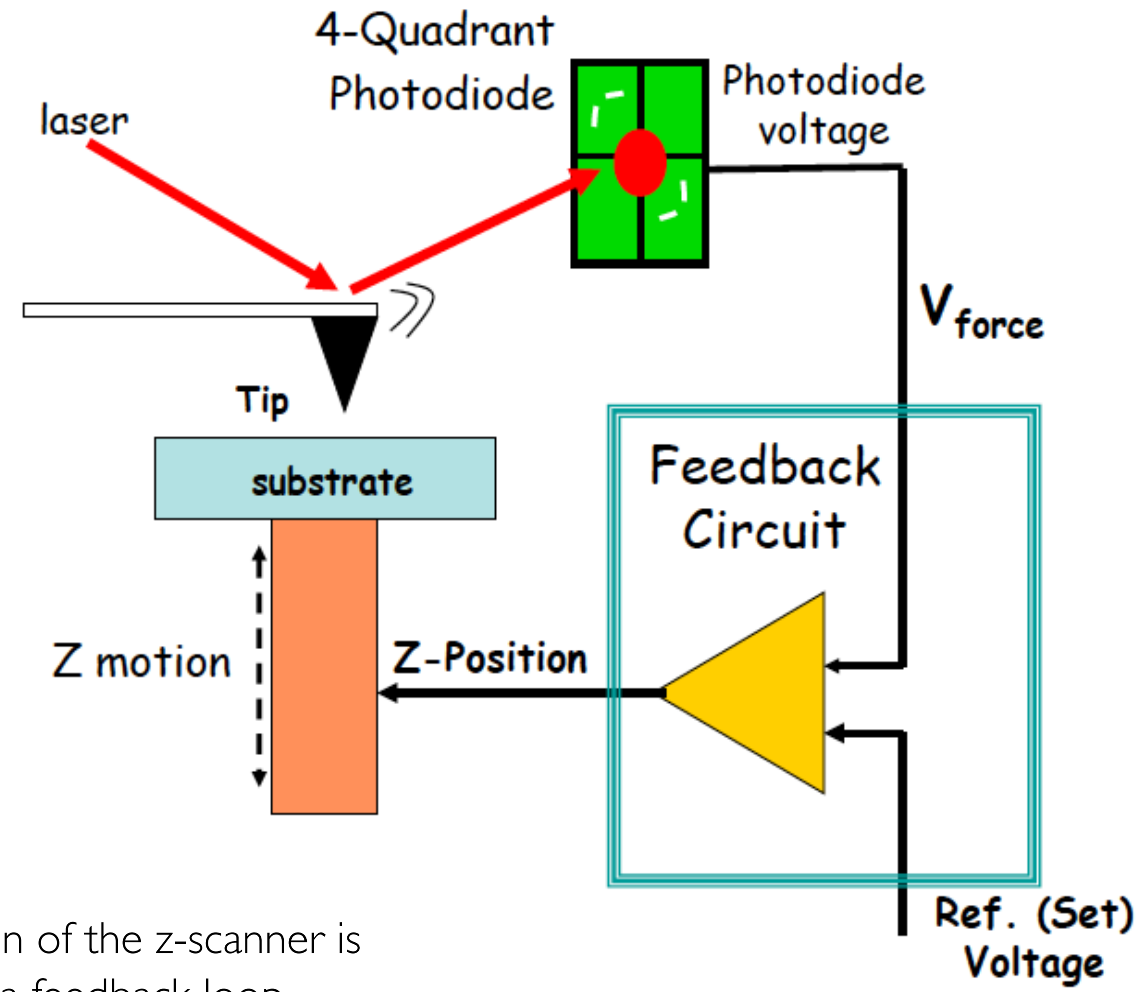
Topographic image of the sample obtained by:

•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.

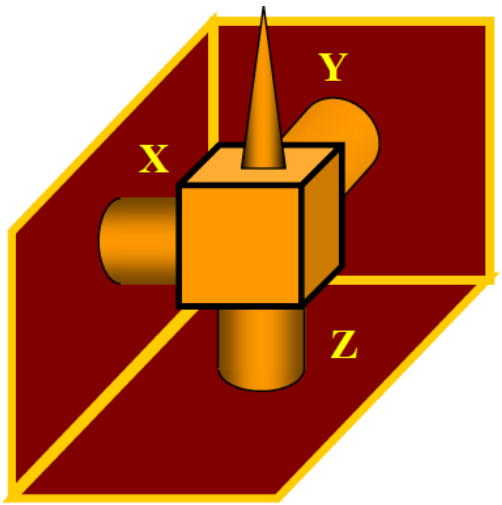
Image contrast arises because the force between the tip and sample is a function of both tip–sample separation and the material properties of tip and sample.

Maintaining a constant force



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

Key element of the feedback system: piezoscanner

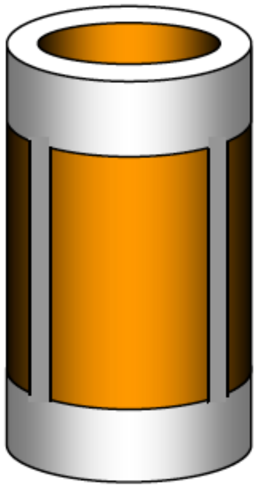


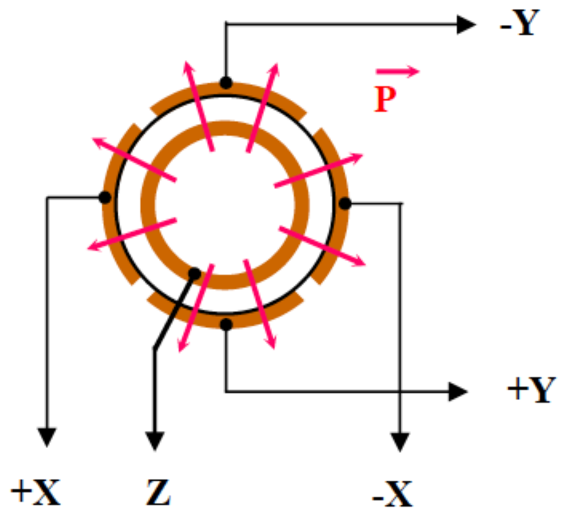
Tripods : strongly asymmetic

Single tube scanner

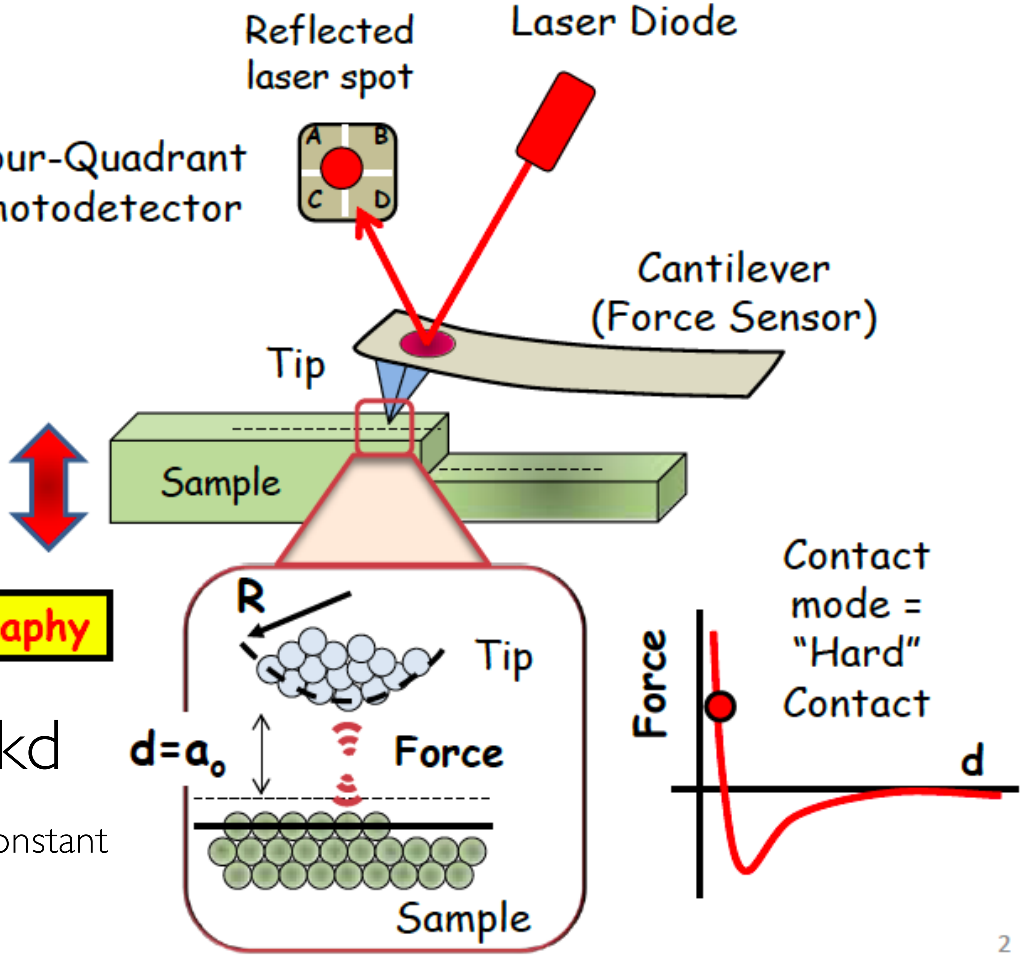
The polarization vector (ceramic) is radially directed

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

$$F = kd$$

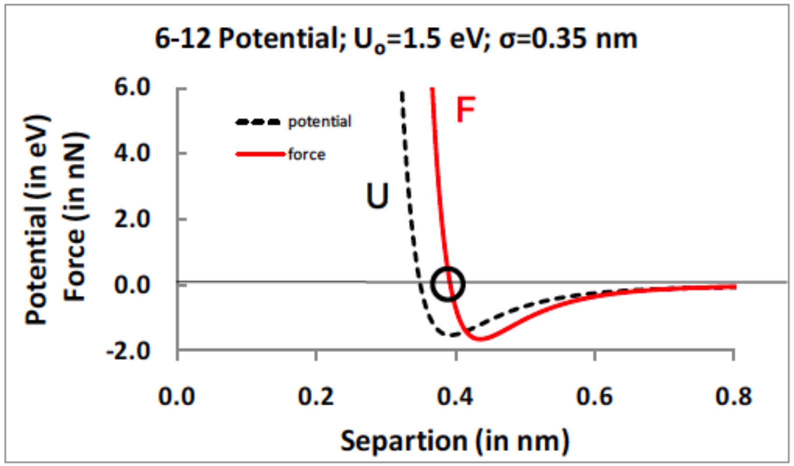
K= cantilever elastic constant

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

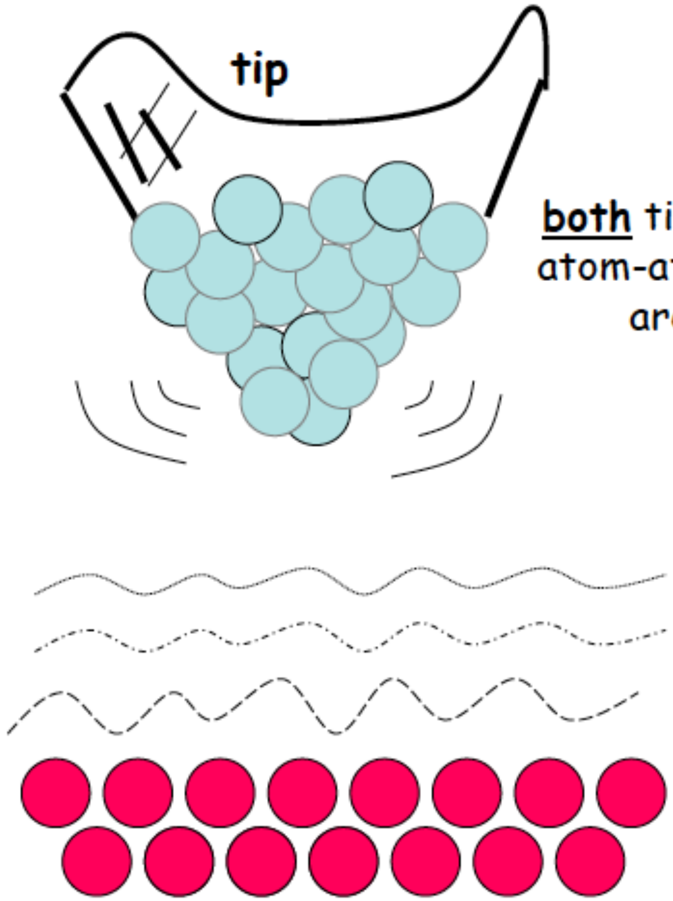
van der Waals (1873)

Capillary

Chemical:
(chemisorbed,physisorbed)

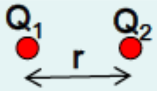
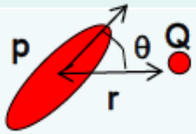
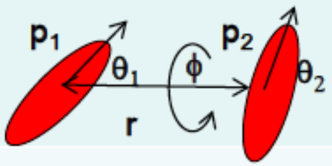
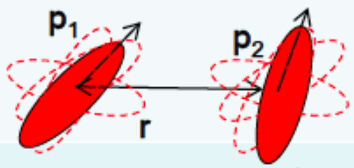
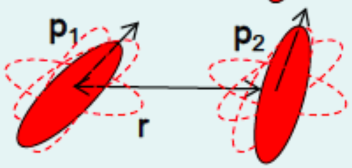
Friction

Deformation (indentation)



both tip-substrate and atom-atom interactions are important

substrate

	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) $U_{Keesom}(r) = -\frac{p_1^{\,2}p_2^{\,2}}{3(4\pi\varepsilon\varepsilon_0)^2 k_BT}\frac{1}{r^6}$
	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) $U_{London}(r) = -\frac{3}{2}\frac{\alpha_{01}\alpha_{02}}{(4\pi\varepsilon_0\varepsilon)^2}\frac{(I_1)(I_2)}{I_1+I_2}\frac{1}{r^6}$

Intermolecular interactions probed by AFM

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

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

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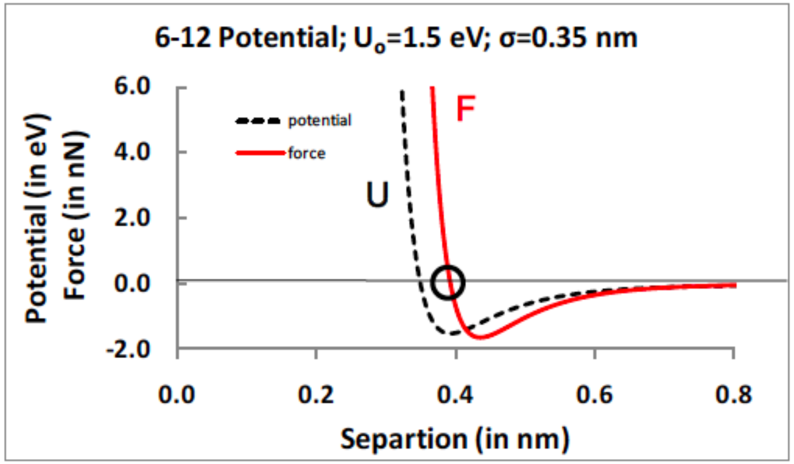

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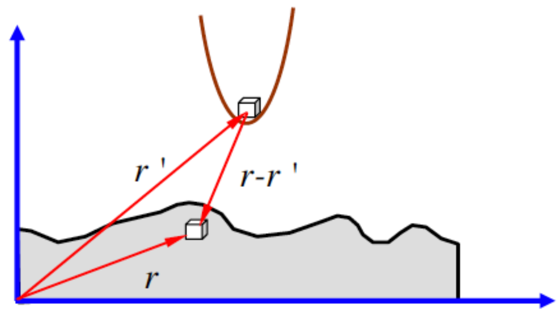
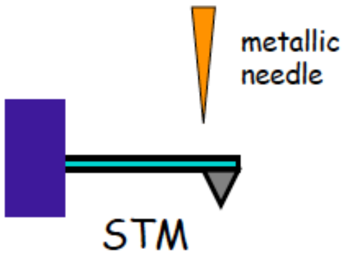


Fig. 61. How to calculate the energy of interaction between tip and sample atoms

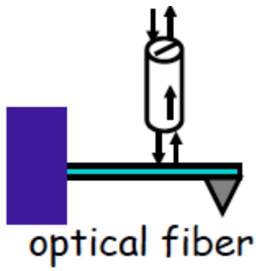
$$W_{PS} = \iint_{V_P V_S} U_{LD}(r - r')\, n_P(r')\, n_S(r)\, dV\, dV'$$

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

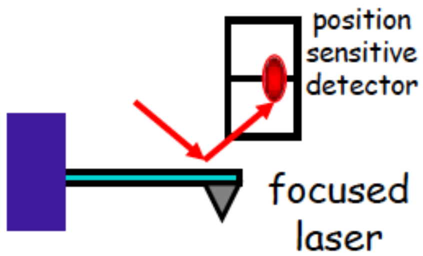
AFM: the deflection detection system



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

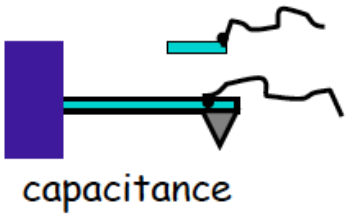


Martin *et al.*, J. App. Phys. **95** (1987)

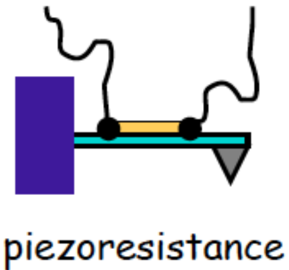


Marti *et al.*, J. Microscopy, **152** (1986)

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)

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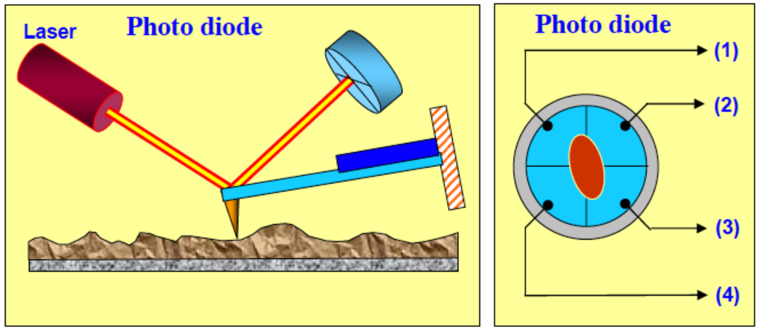


Fig. 62. Schematic description of the optical system to detect the cantilever bending

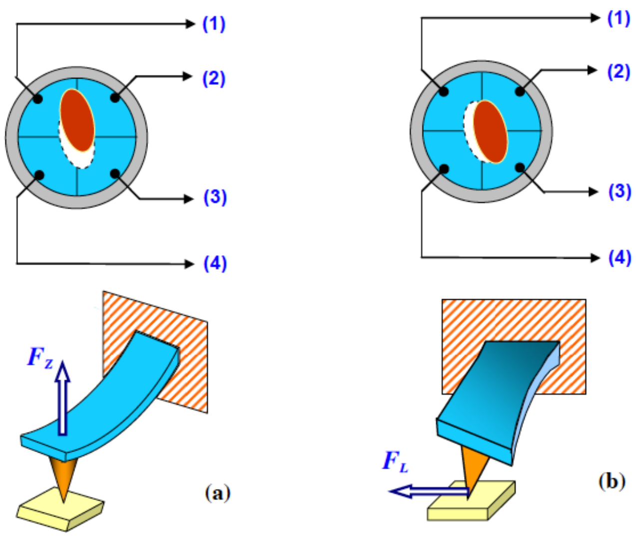


Fig. 63. Relation between the types of the cantilever bending deformations (bottom) and the change of the spot position on the split photodiode (top)

Normal forces:

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

Lateral forces:

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

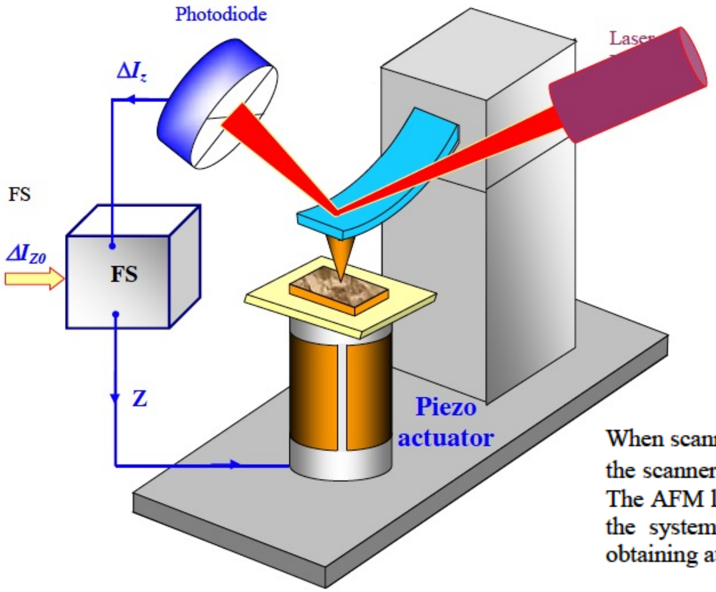
With ΔI_Z used as input to the feedback loop

The feedback system (FS) keeps ΔI_Z = const with 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.

AFM: the deflection detection system

ΔI_Z is used as input to the feedback loop

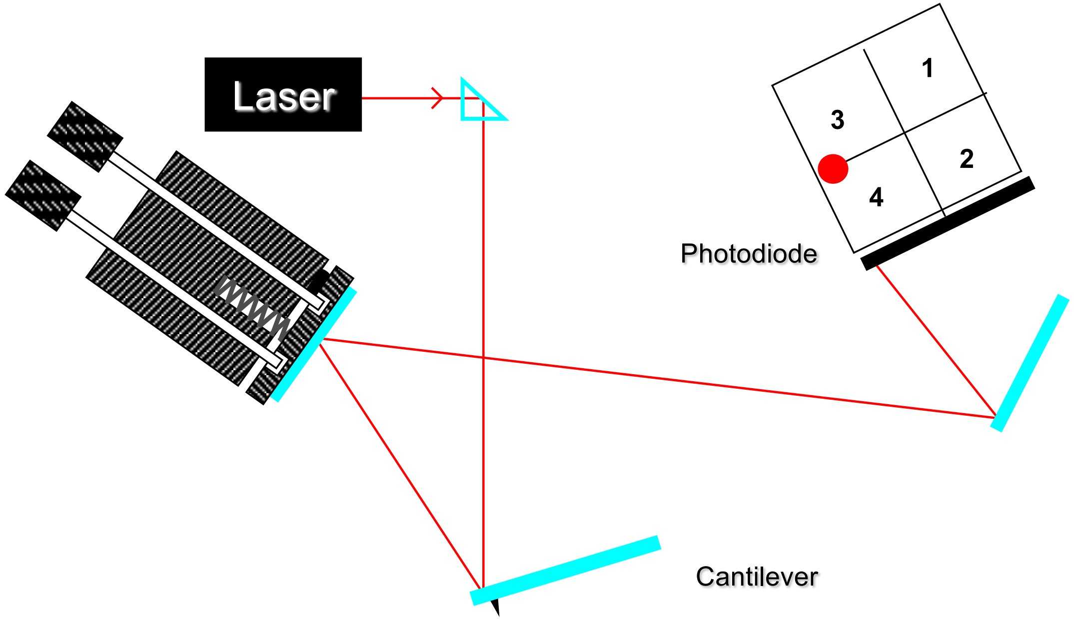
The feedback system (FS) keeps ΔI_Z = const with the help of a piezoelectric transducer (scanner), which controls the tip-sample distance in order to make the bending ΔZ equal to the value ΔZ_0 preset by the operator.

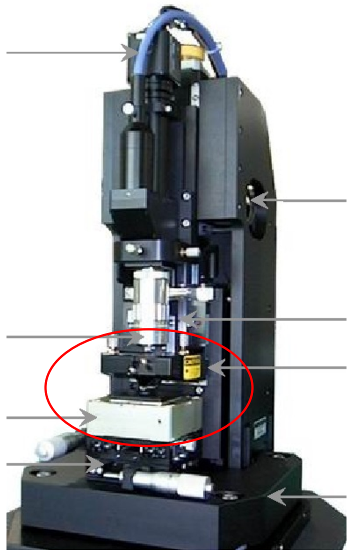


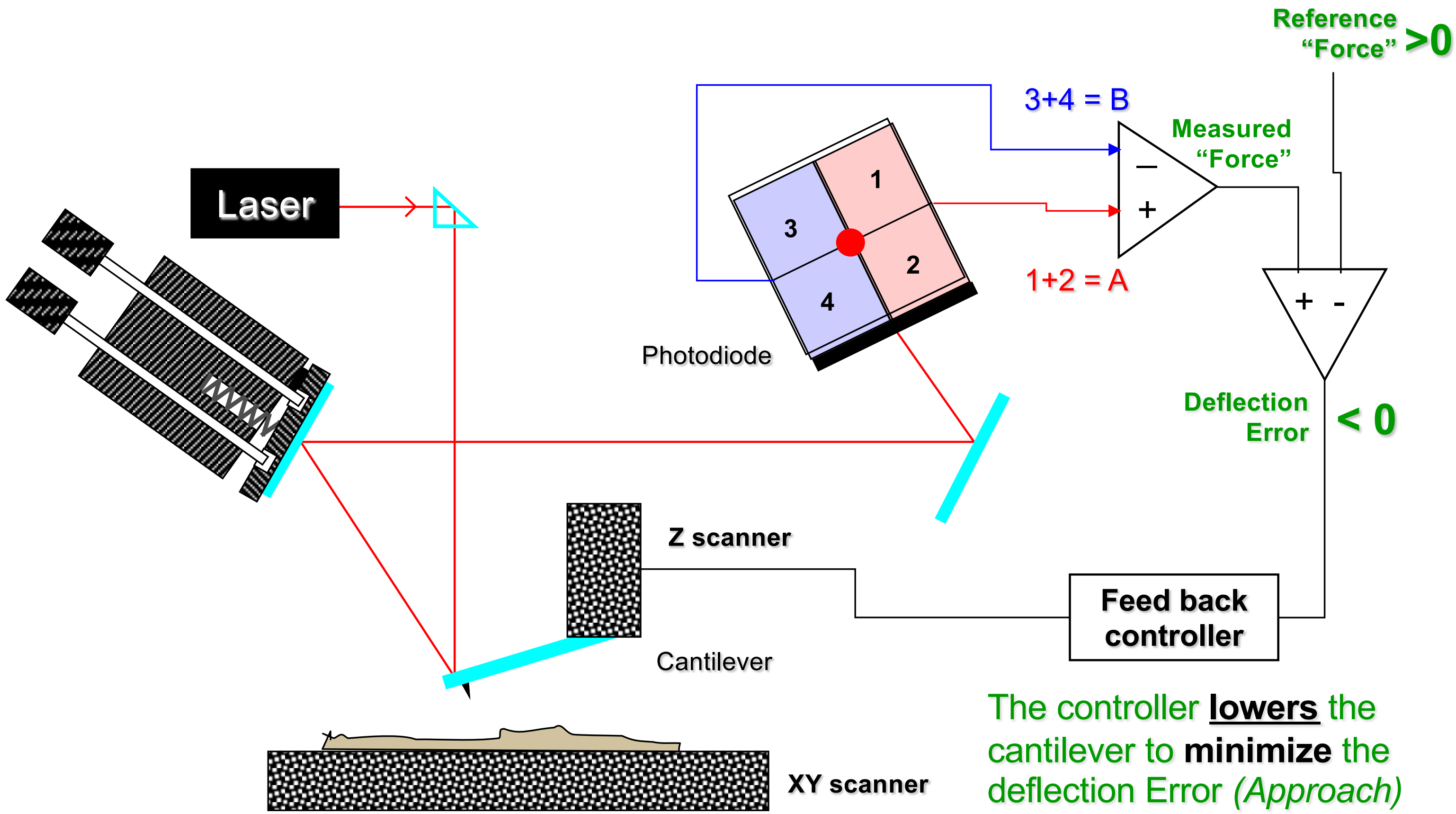
When scanning a sample in a $\Delta Z = const$ mode the tip moves along the surface, thus the voltage on the scanner Z-electrode is recorded in the computer memory as a surface topography $Z = f(x, y)$. The AFM lateral resolution is defined by the radius of curvature of the tip and by the sensitivity 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*

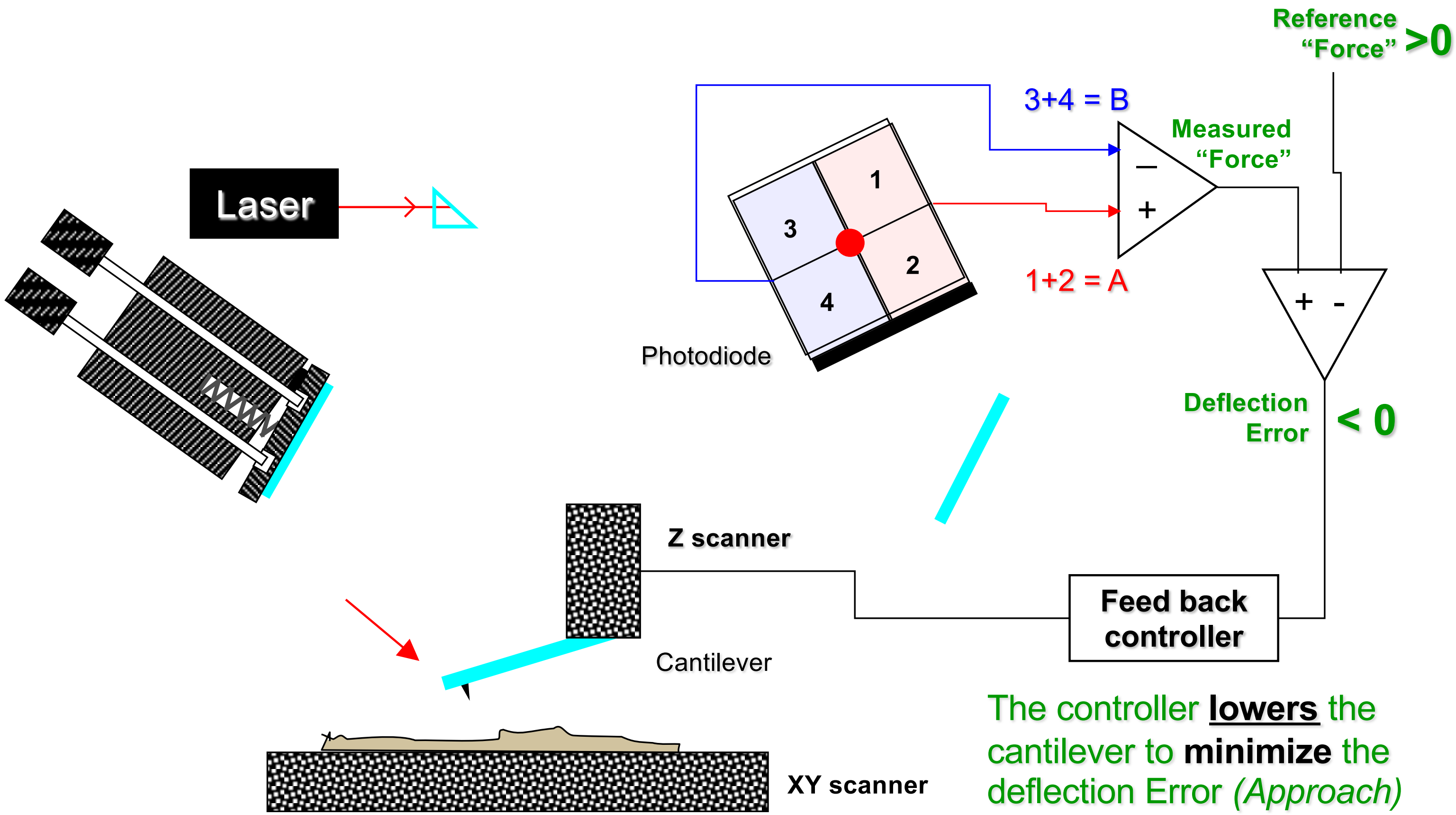






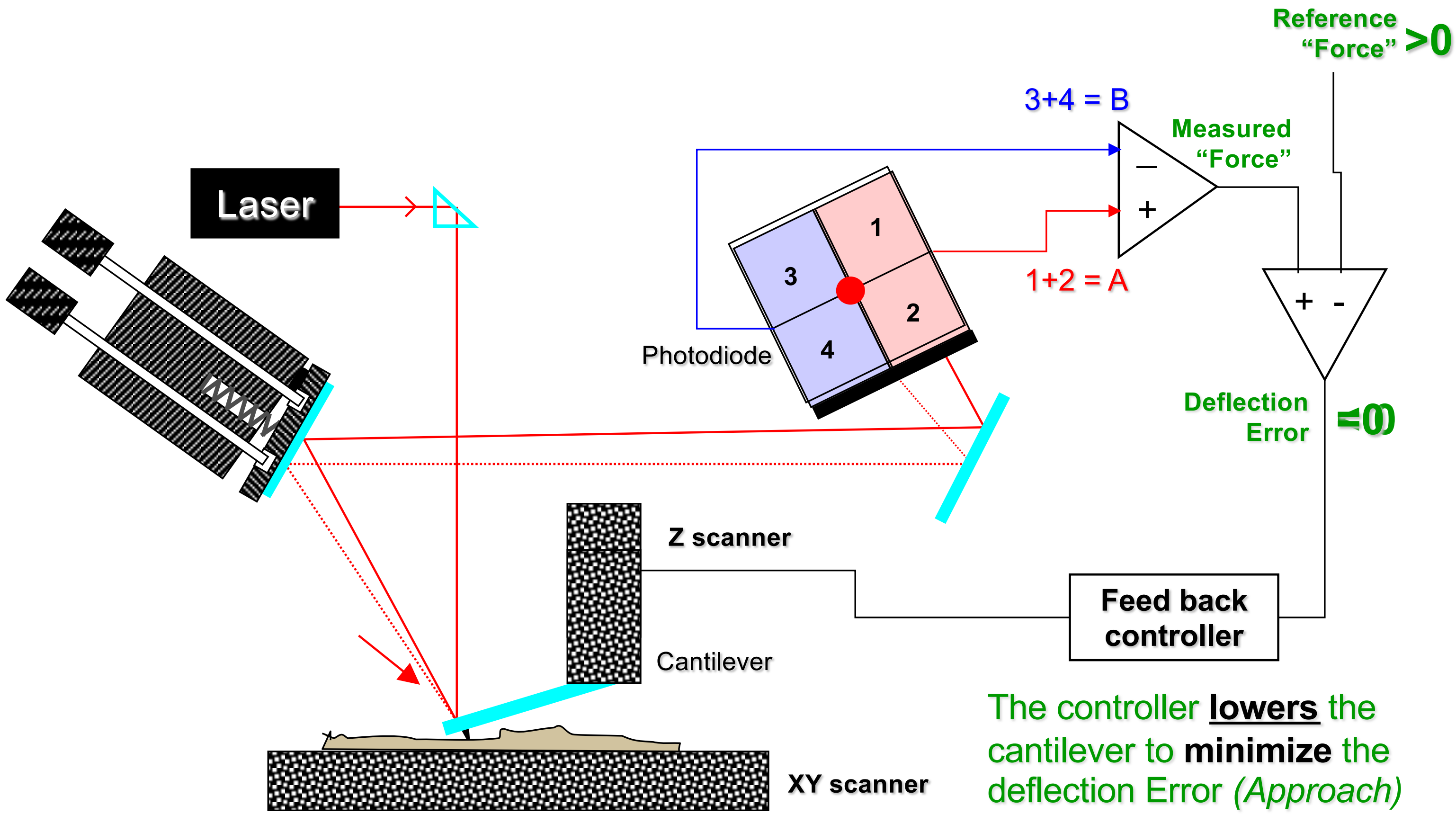
The controller **<u>lowers</u>** the cantilever to **minimize** the deflection Error *(Approach)*

Force Measurement



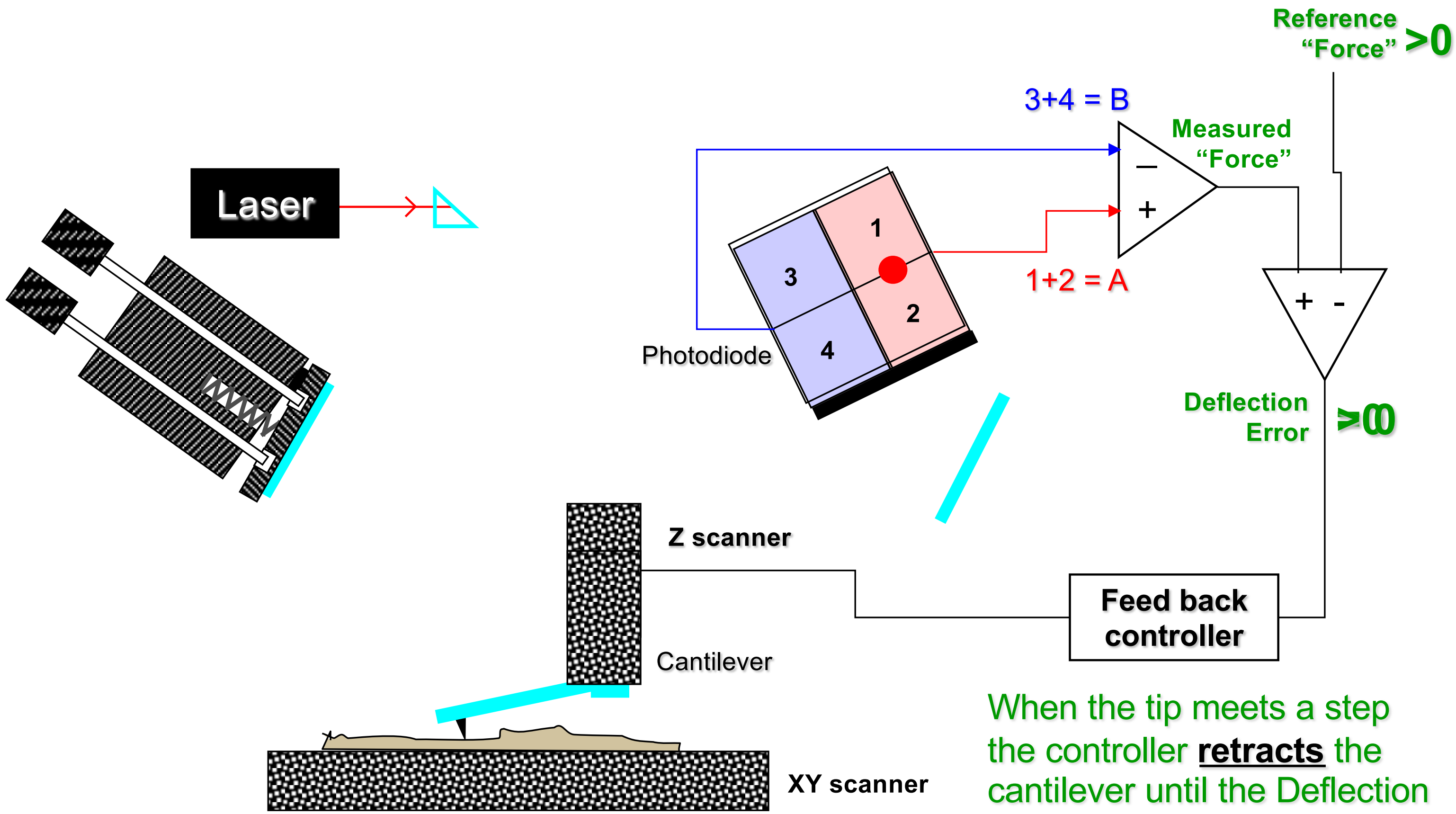
The controller **lowers** the cantilever to **minimize** the deflection Error *(Approach)*

Force Measurement



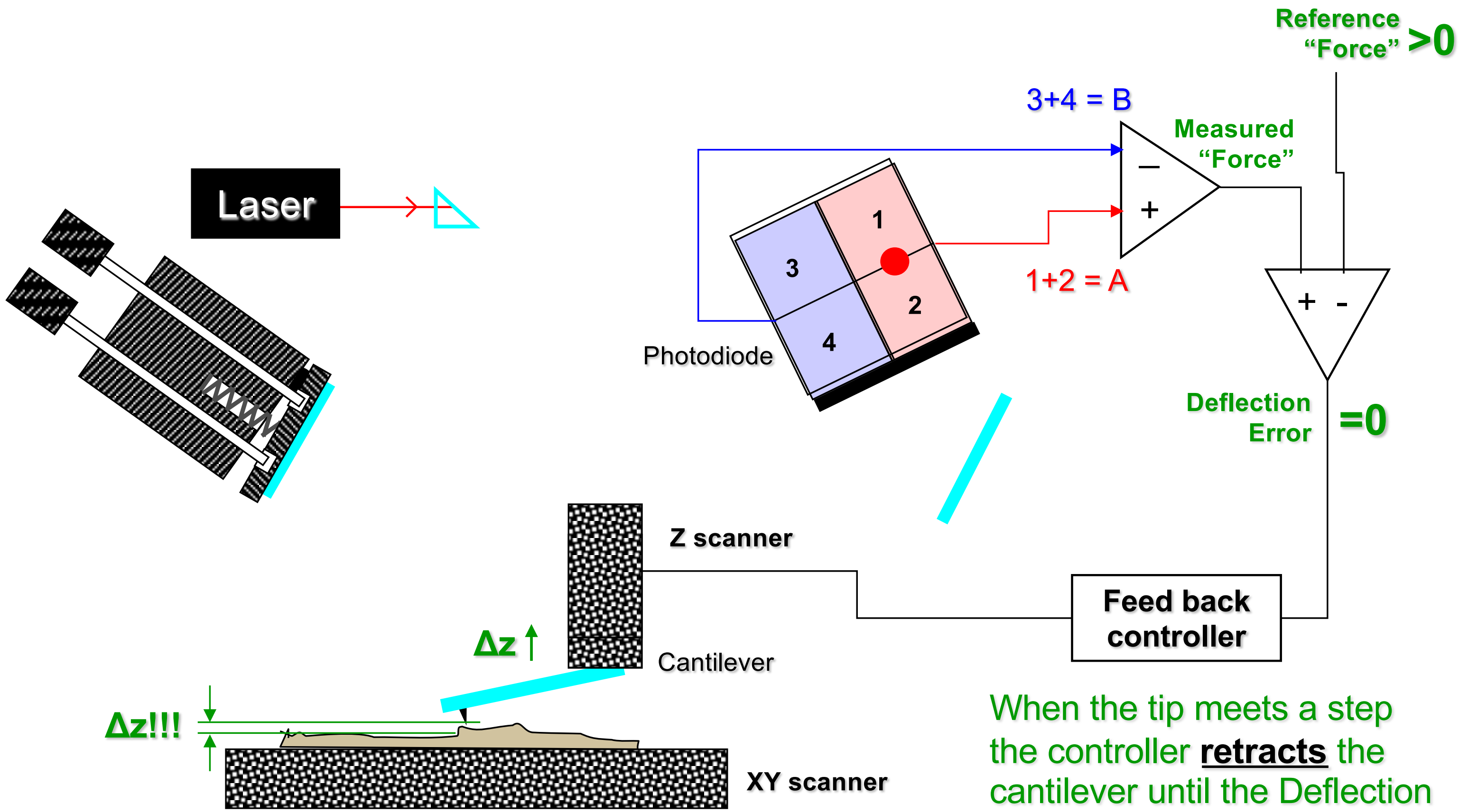
The controller **lowers** the cantilever to **minimize** the deflection Error *(Approach)*

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

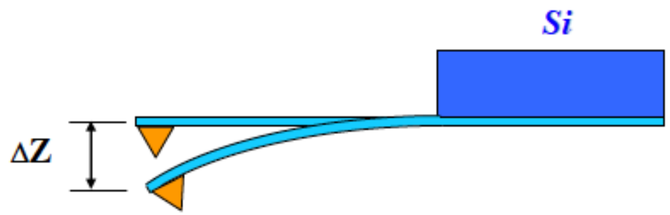


Fig. 65. Schematic picture of the AFM probe

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.

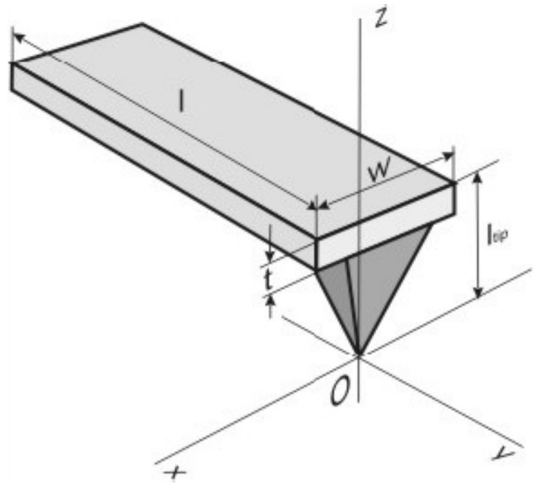
The angle near the tip apex is 10 ÷ 20º.

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



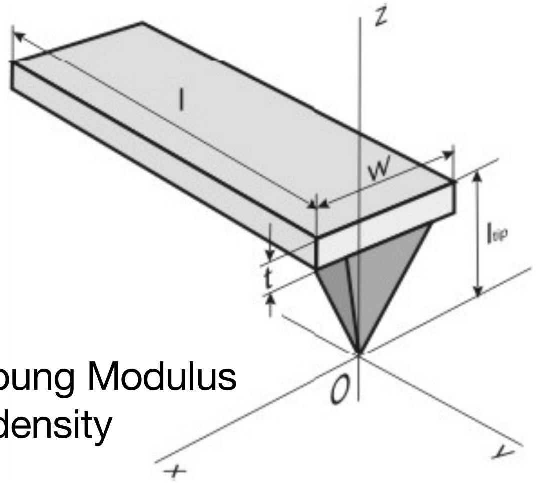
E= Young Modulus
ρ = density

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

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



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

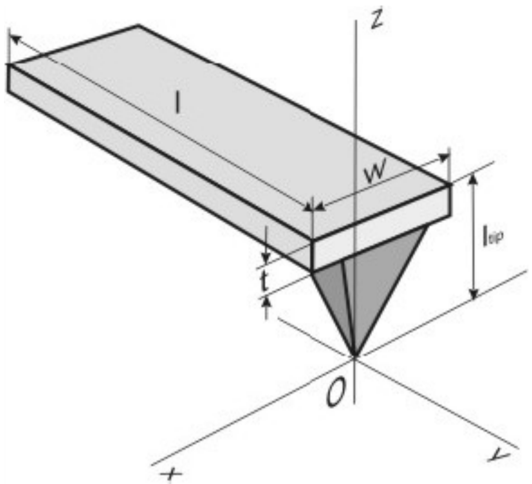
Typical E value: 1.5×10^{11} N m^{-2} in silicon nitride

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

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

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

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

M probes

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

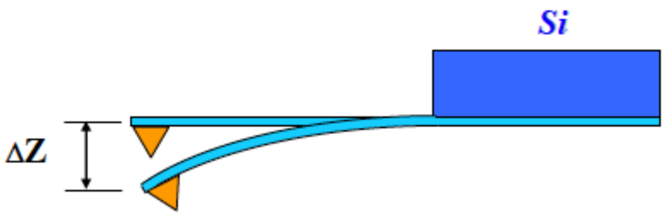


Fig. 65. Schematic picture of the AFM probe

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 ÷ 20º.

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;
λ_i a numerical coefficient (1÷100), depending on oscillations mode.

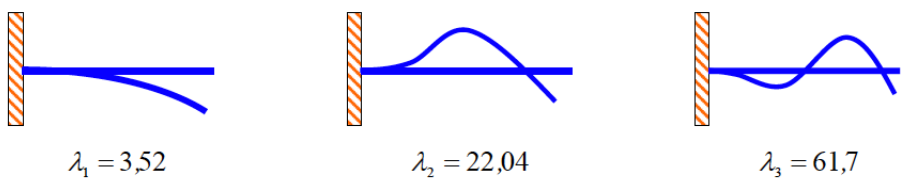
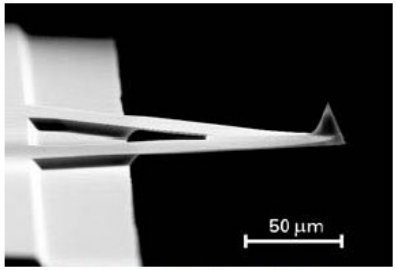


Fig. 66. Main cantilever oscillations modes

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



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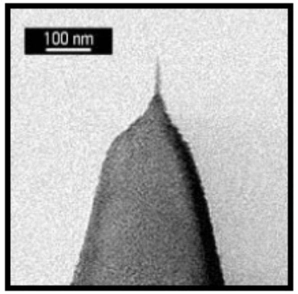
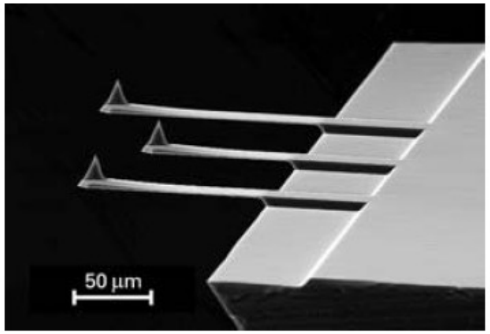


µmasch



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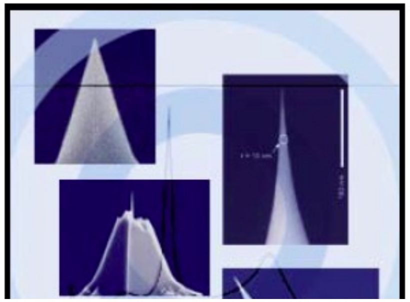
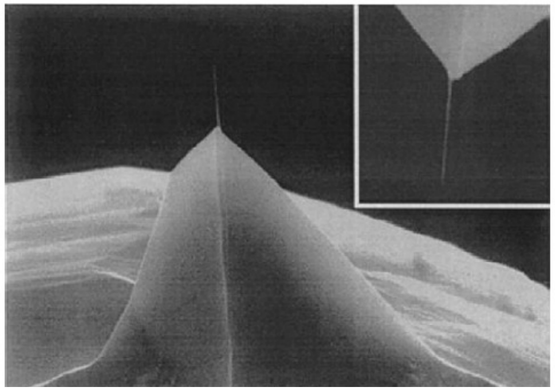
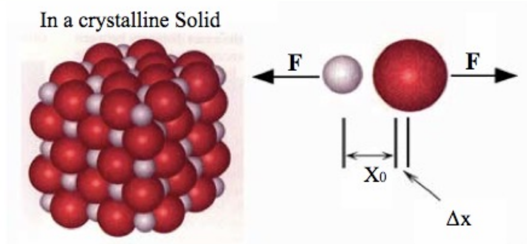


Typical use	k (N/m)	f_o (kHz)
Non-contact	10-100	100-300
Intermittent contact	1-10	20-100
Contact	0.1-1	1-50



CNT tip

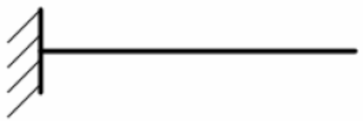


$F = k\Delta x$

$k \sim 10$ N/m

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

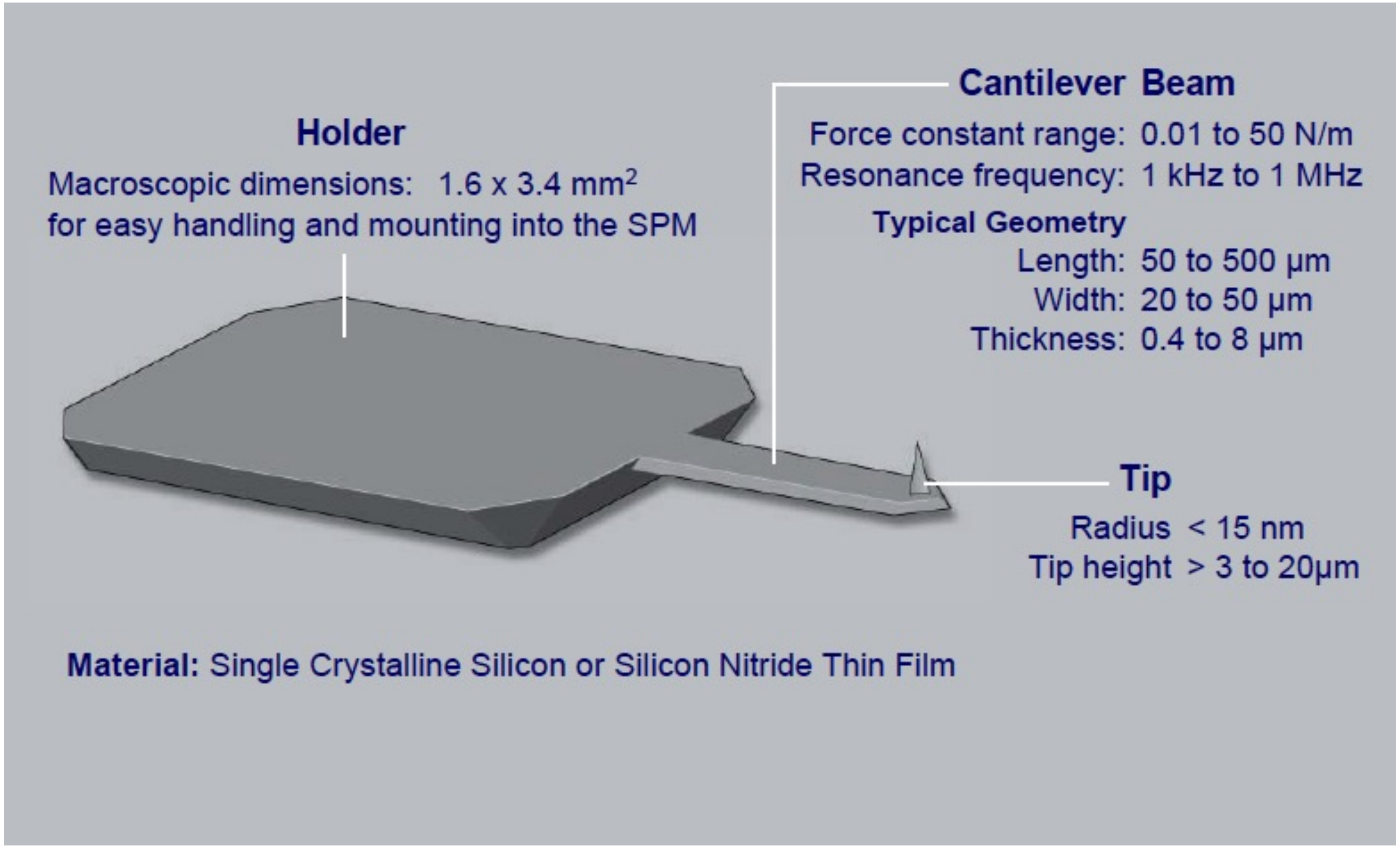


$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4 \rho}}$$

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

make cantilevers short to increase ω_0 and thinner to restore k

AFM probes



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
Holder
Macroscopic dimensions: 1.6 x 3.4 mm2
for easy handling and mounting into the SPM
Tip
Radius < 15 nm
Tip height > 3 to 20µm
Material: Single Crystalline Silicon or Silicon Nitride Thin Film

AFM probes

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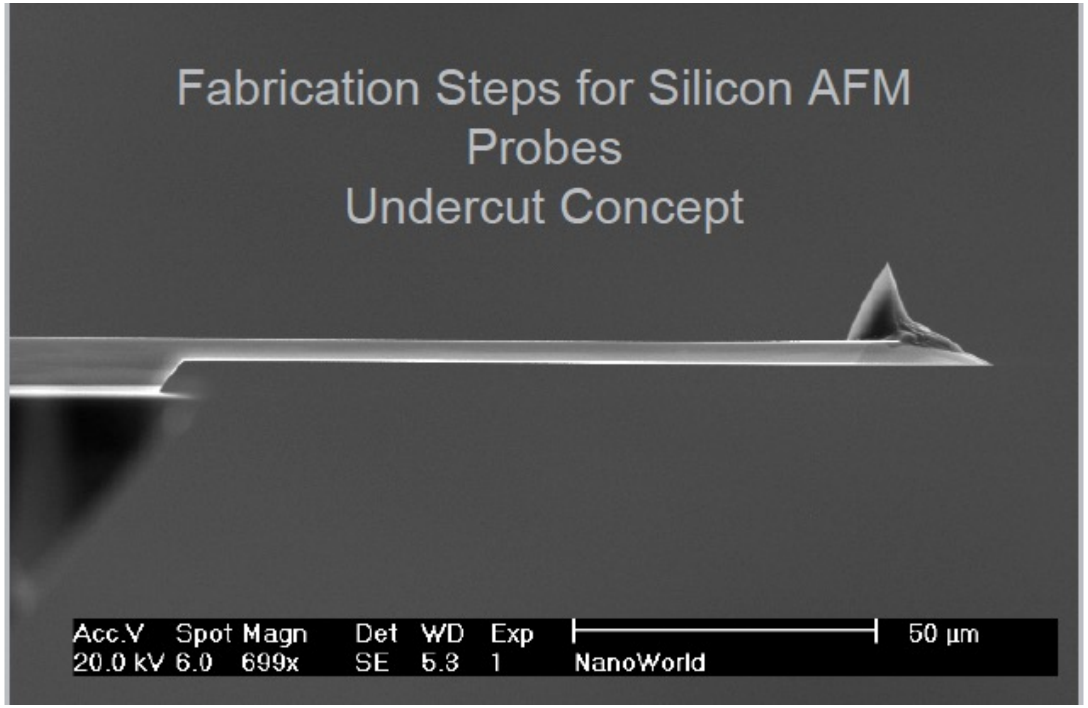

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