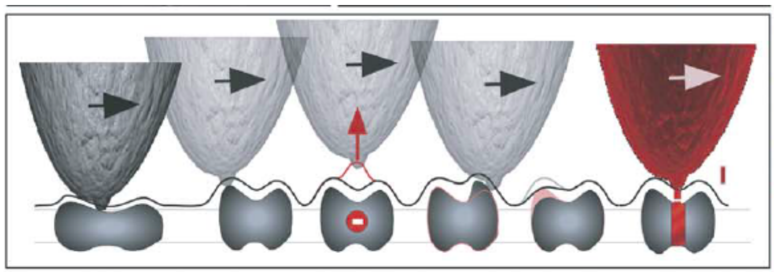
Elettra Sincrotrone Trieste

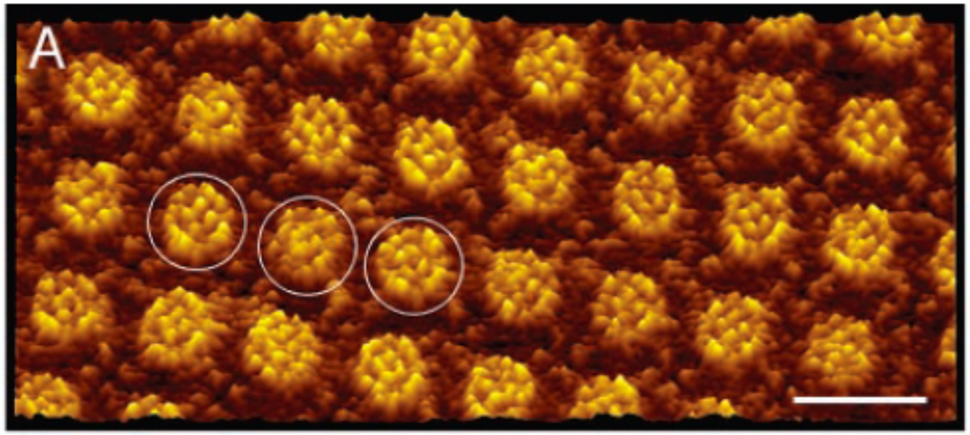
ATOMIC FORCE MICROSCOPY
Imaging in Biology

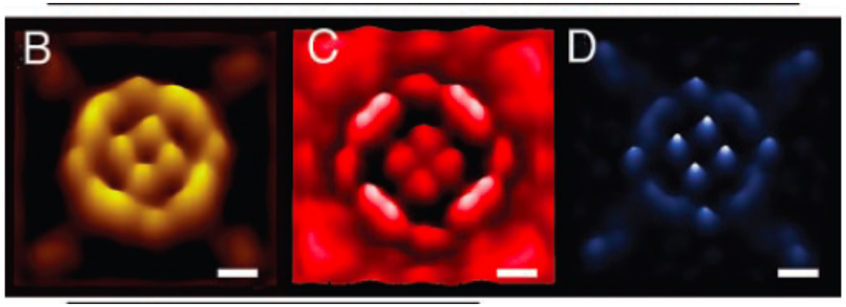
Sources:
Fundamentals of Scanning Probe Microscopy, V. L. Mironov
NanoHub.org

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.



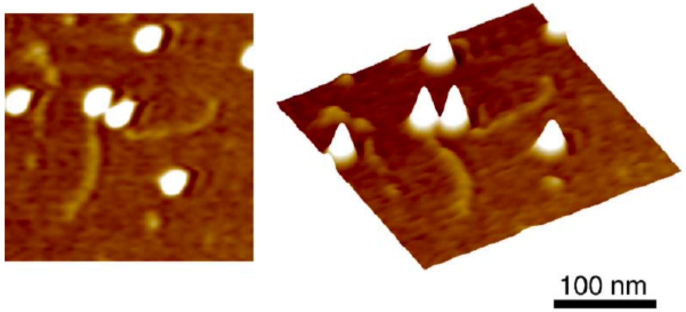


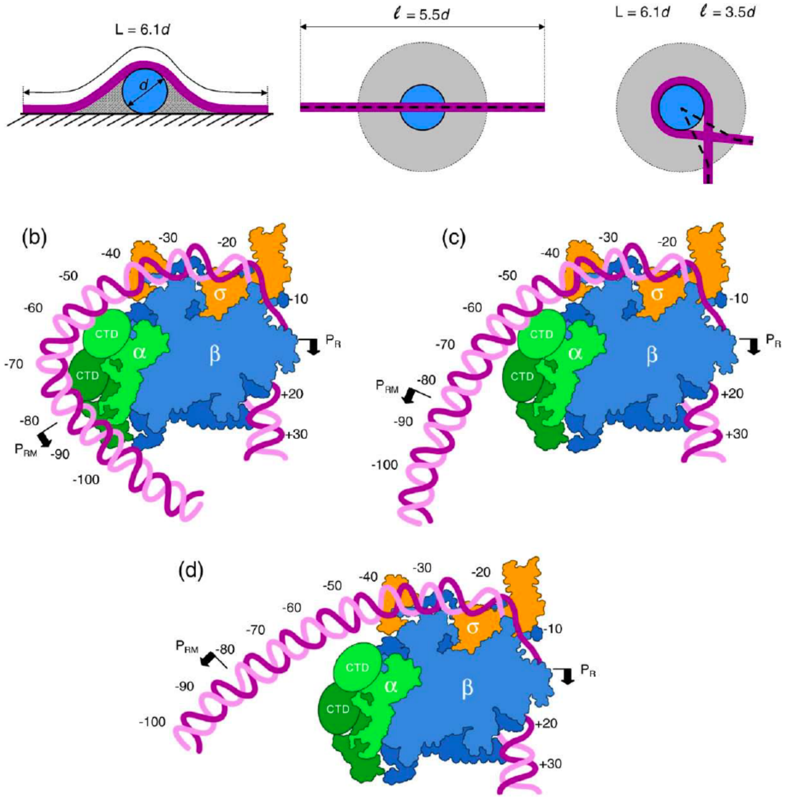


S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

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.

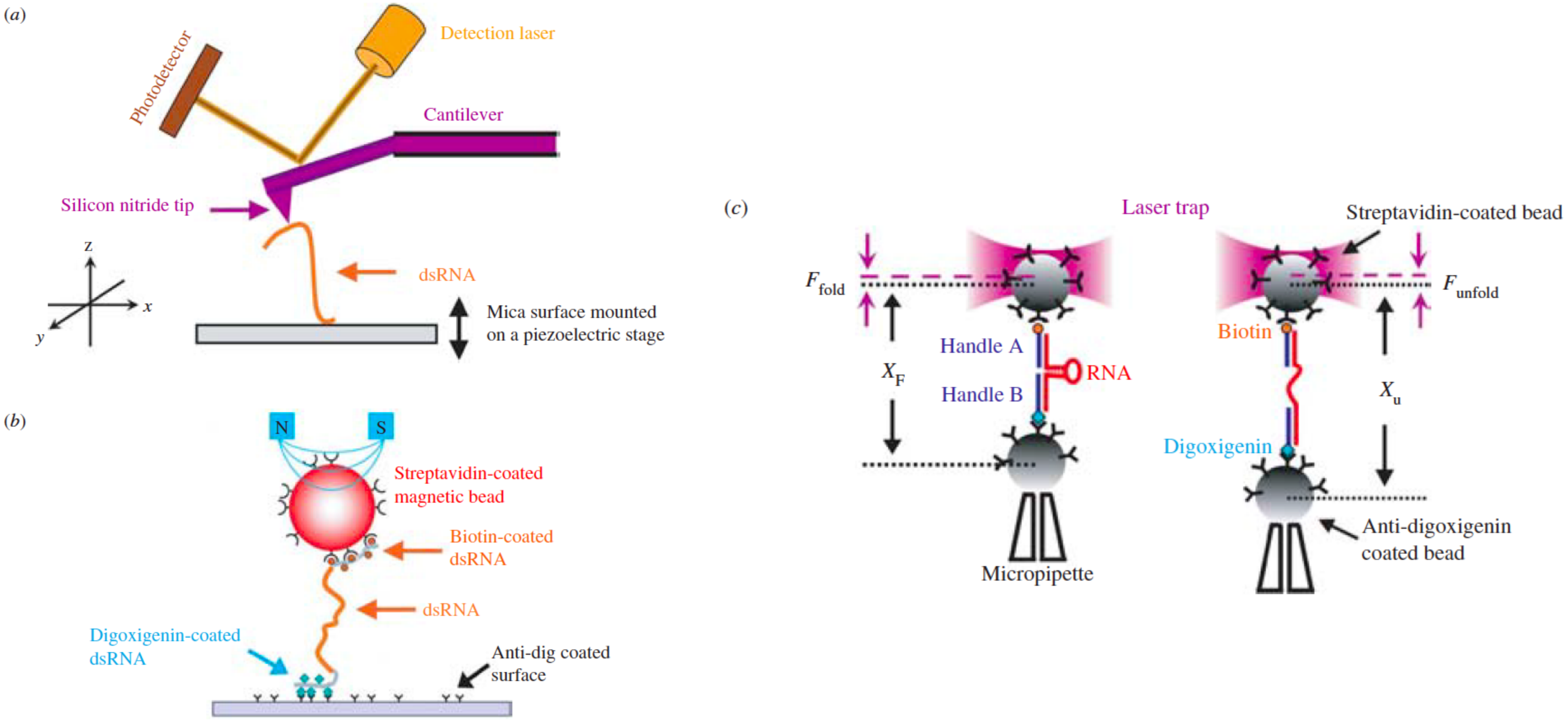




I. Mangiarotti, S. Cellai, W. Ross, C. Bustamante, C. Rivetti, *L. Mol. Biol.* **385**, 748 (2009)

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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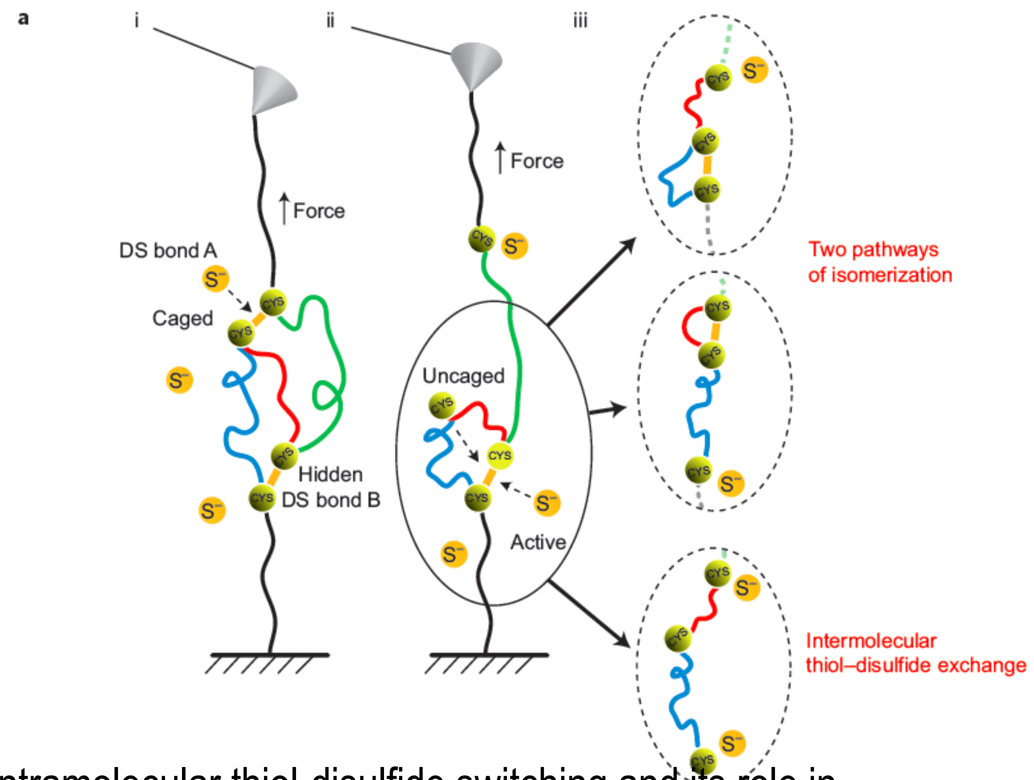
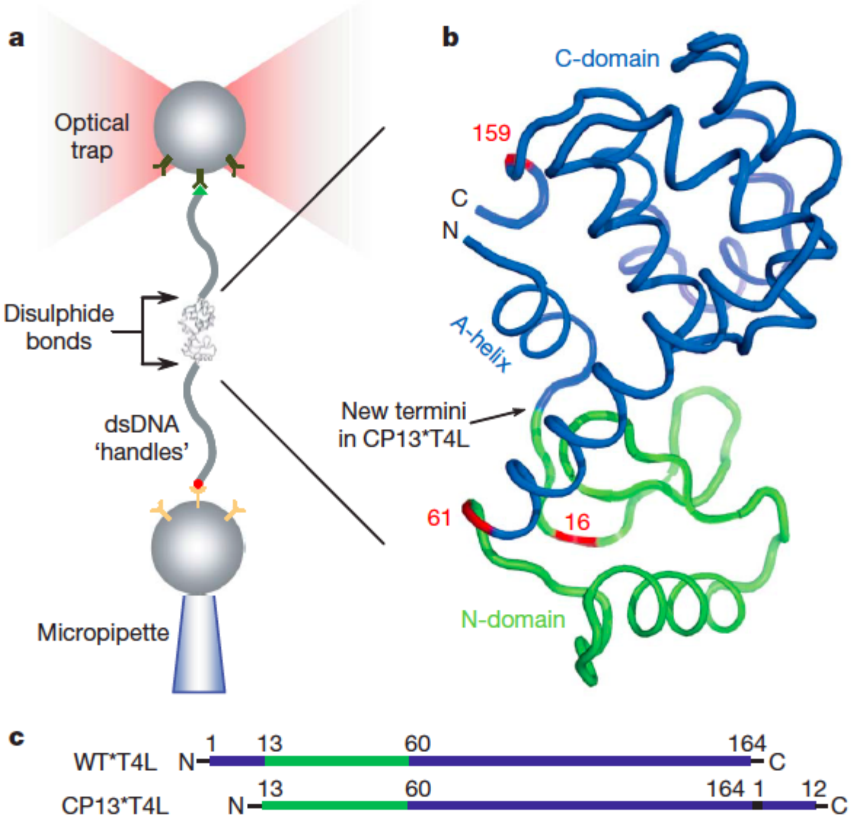
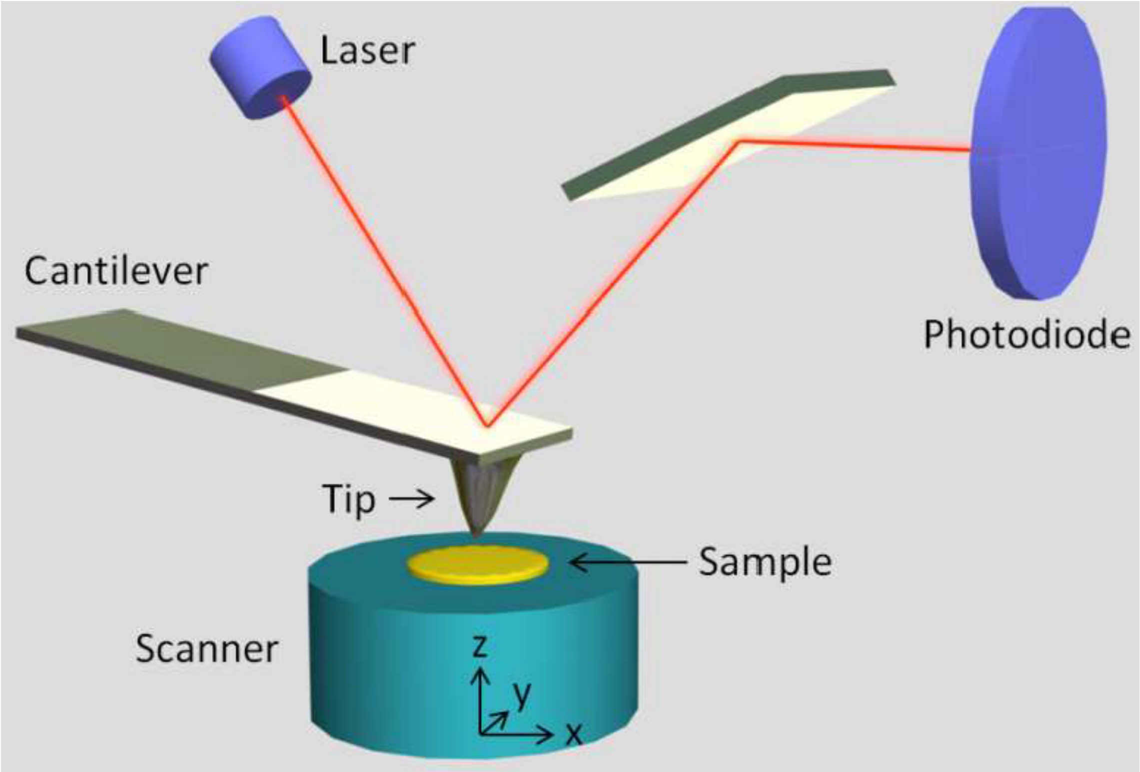


Intramolecular thiol-disulfide switching and its role in protein folding (J. Fernandez)



Folding cooperativity and chain topology (C. Bustamante)

Atomic Force Microscopy



Unique characteristics:

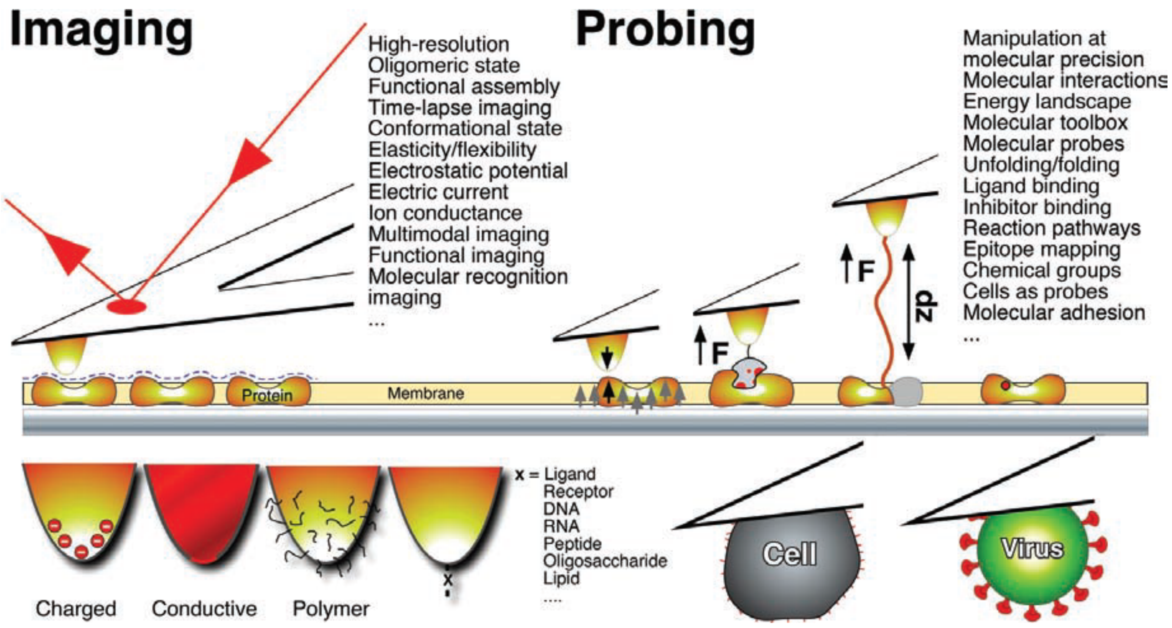
1. built-in atomic scale sensitivity
2. precise motion control technology
3. fabrication technology (nanolithography)

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

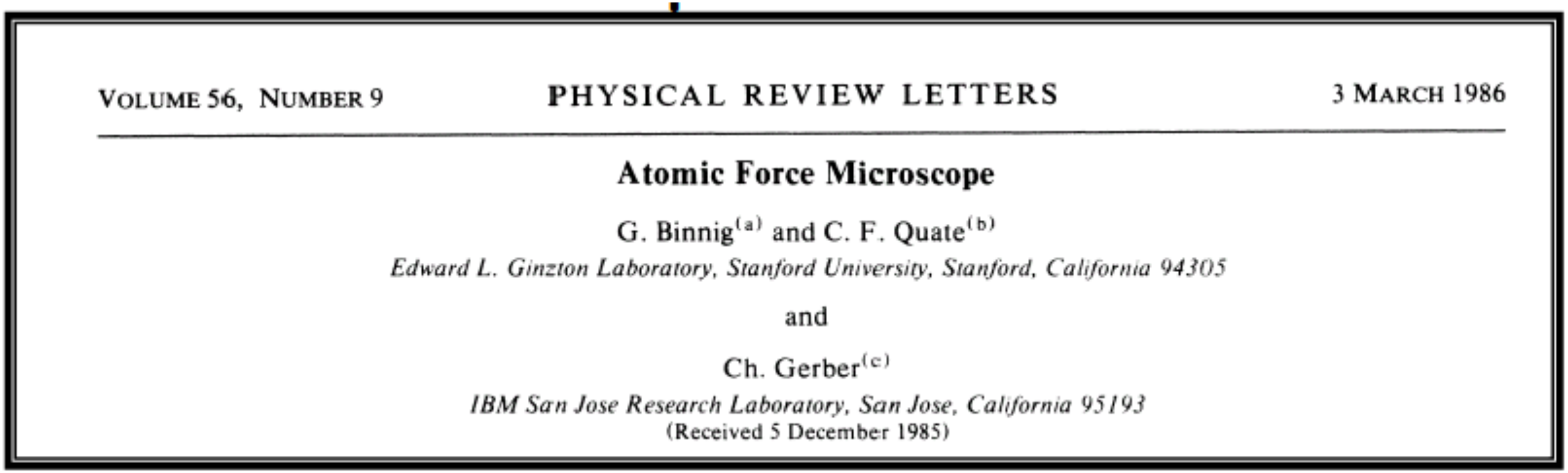
High-resolution AFM has been applied to the imaging of bacterial membrane proteins, deriving the free energy landscape for domains within single protein molecules.

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!

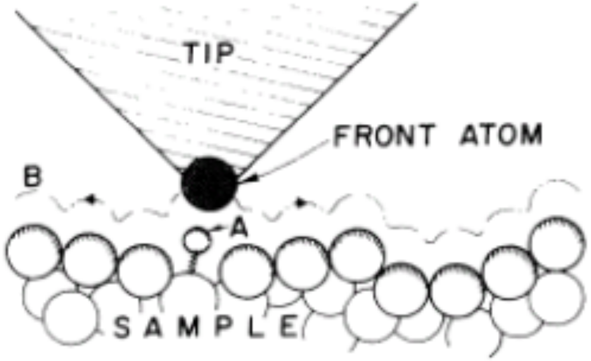
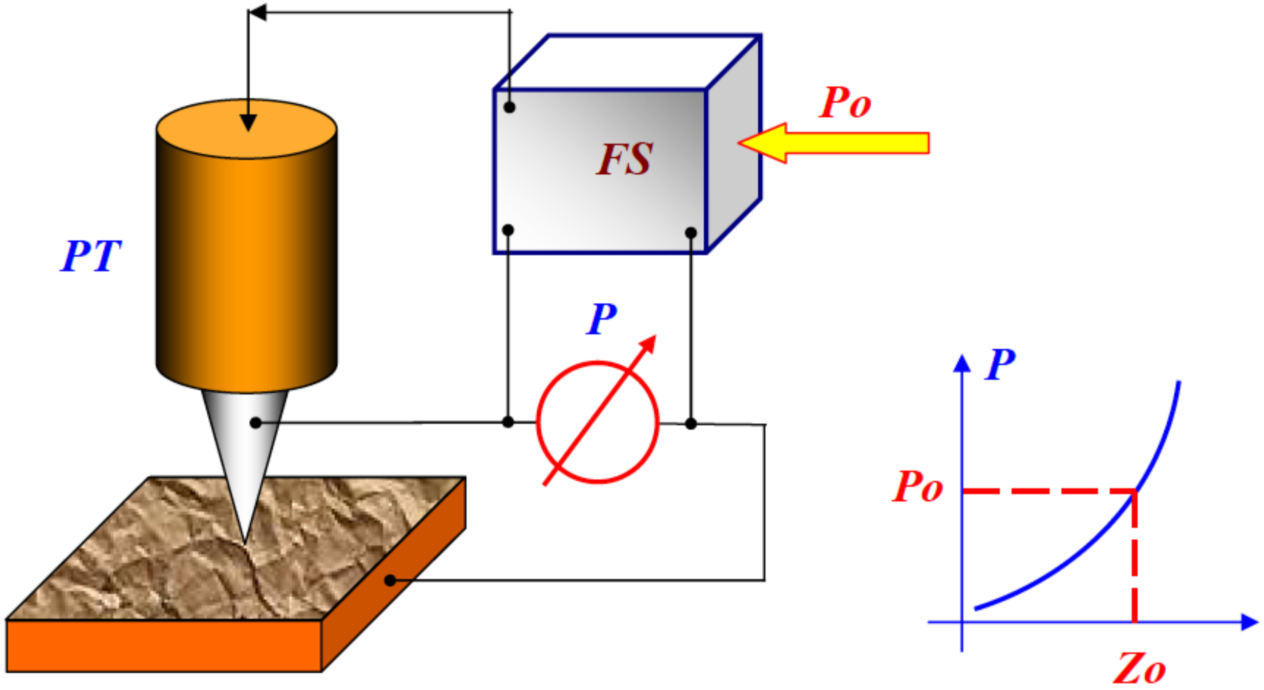


FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM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



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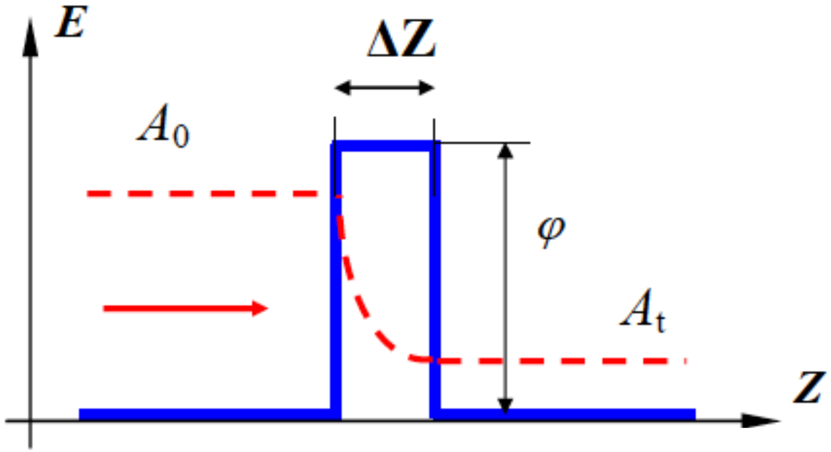
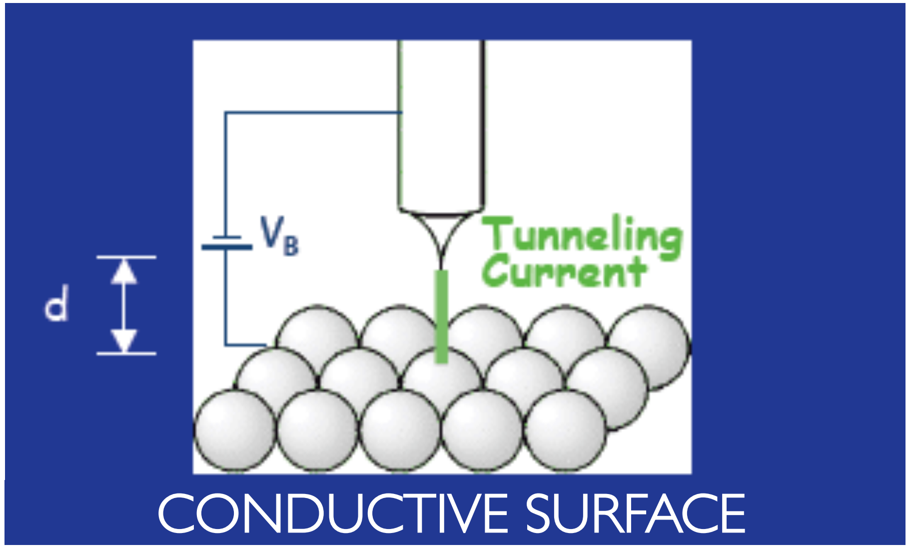
Scanning Tunneling Microscopy





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

average work function ϕ *

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

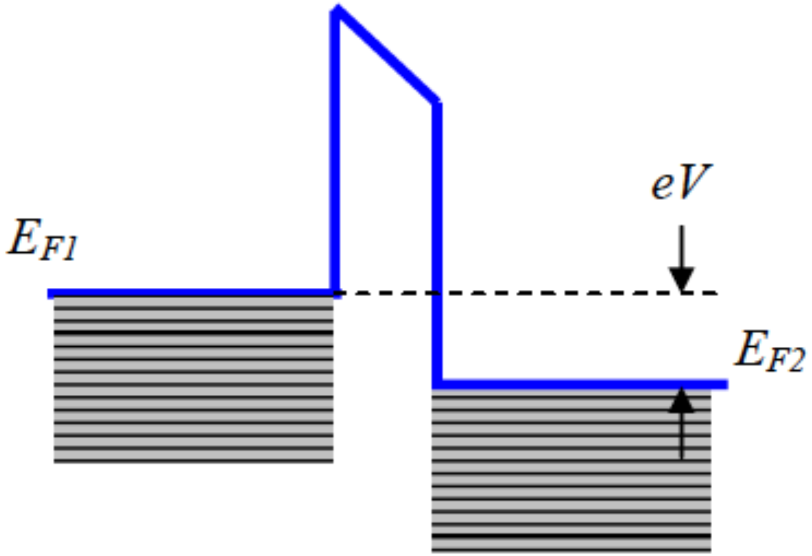
probability of electron tunneling
(transmission coefficient)
A = amplitude of electron wave function

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

attenuation coefficient in metals

Scanning Tunneling Microscopy

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



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

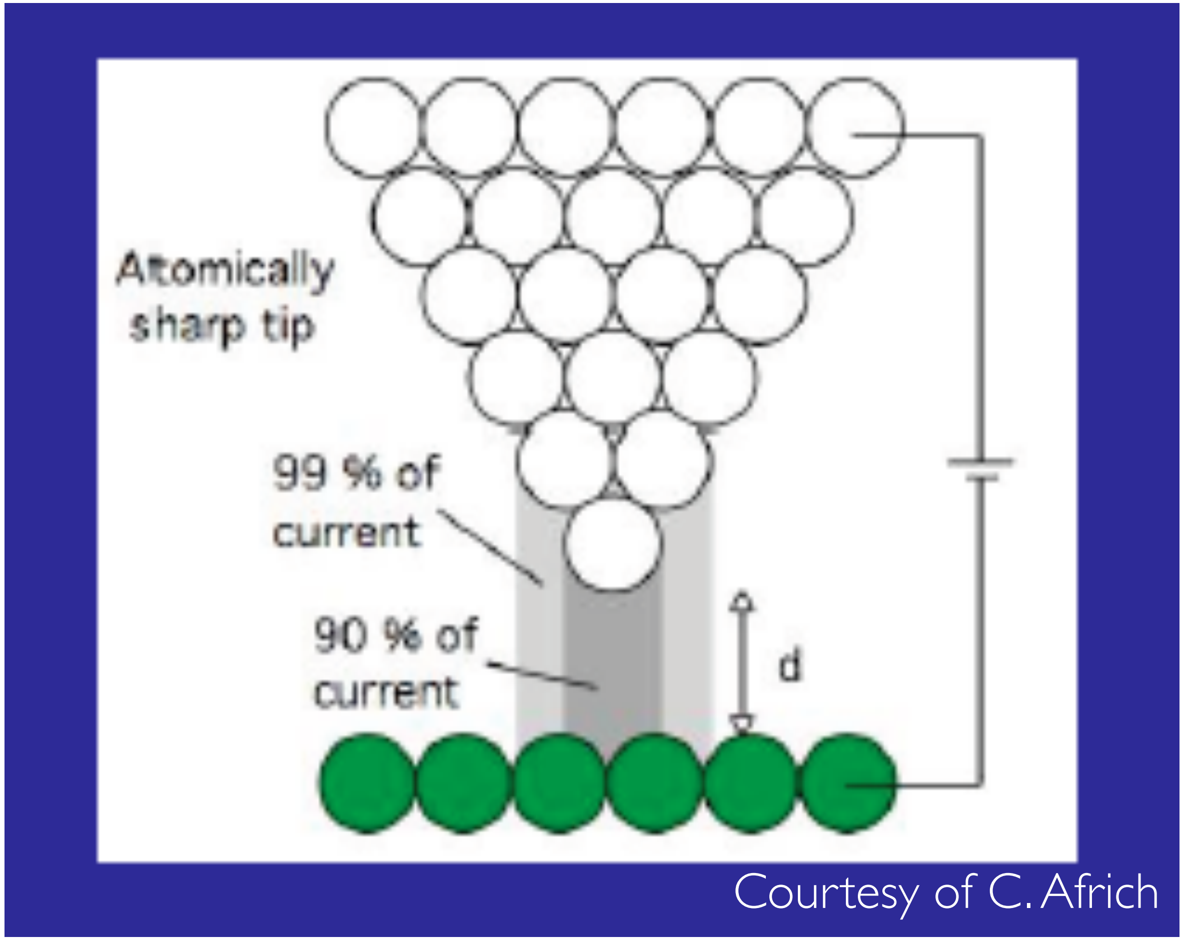
For typical values of the work function

$\phi \sim 4$ 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



Courtesy of C. Africh

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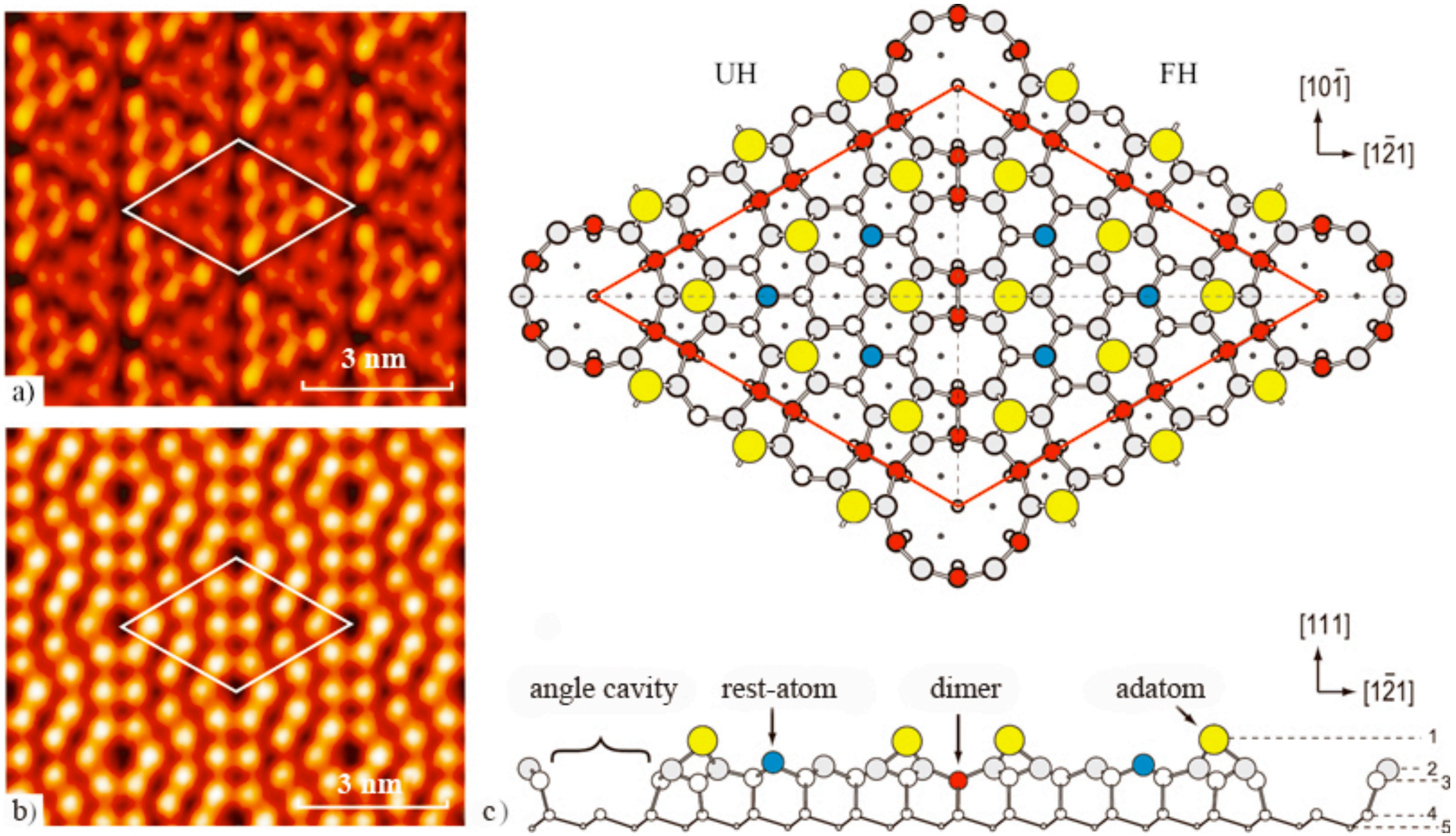
Scanning Tunneling Microscopy

VOLUME 50, NUMBER 2 PHYSICAL REVIEW LETTERS 10 JANUARY 1983

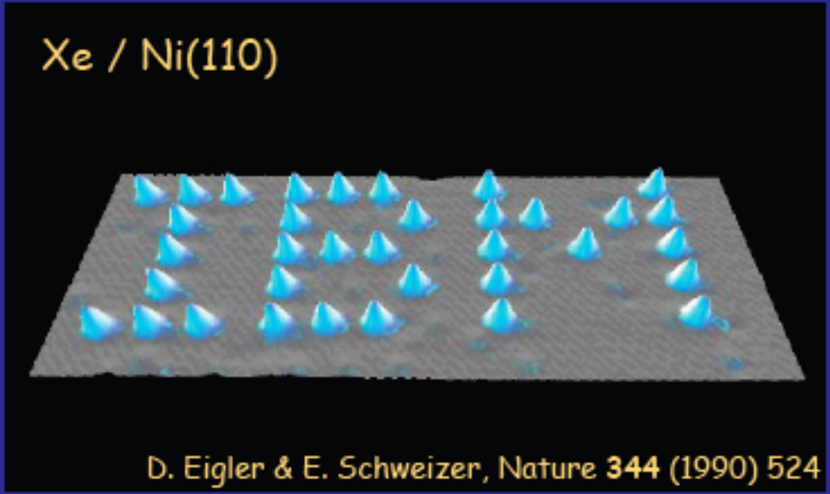
7 × 7 Reconstruction on Si(111) Resolved in Real Space

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

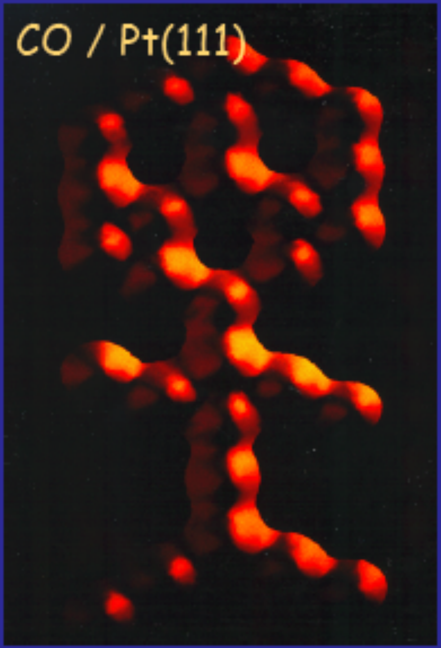


Surf. Sci. 1985. V. 164. P. 367. 7x7 rec. reduces dangling bonds from 49 to 19

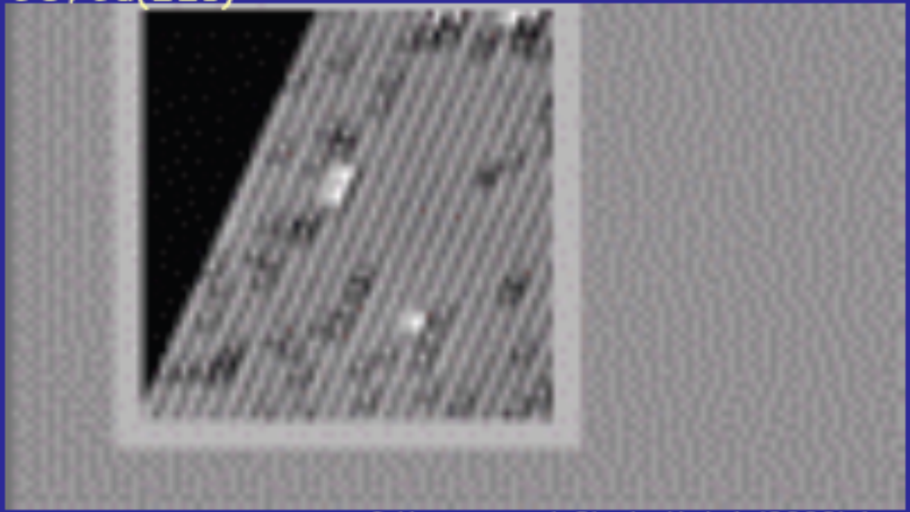
Manipulation by STM

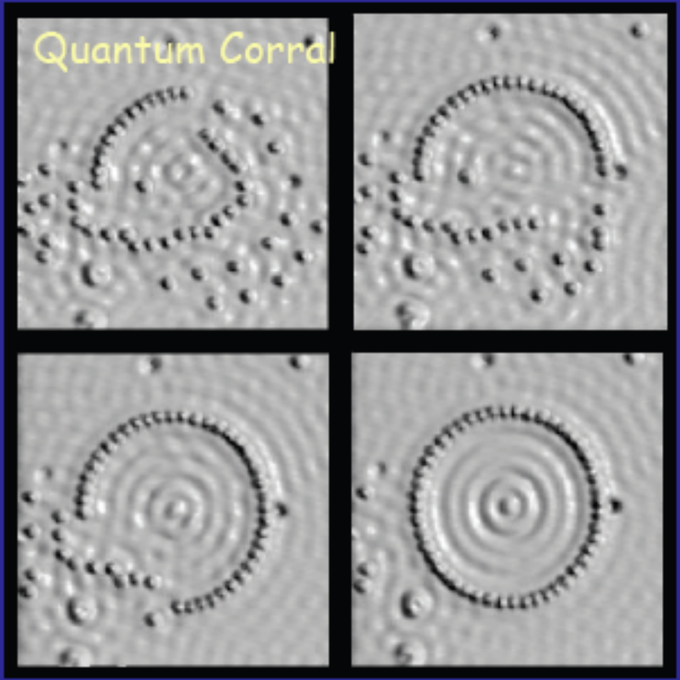

Xe / Ni(110)

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


CO / Pt(111)

CO/Cu(221)

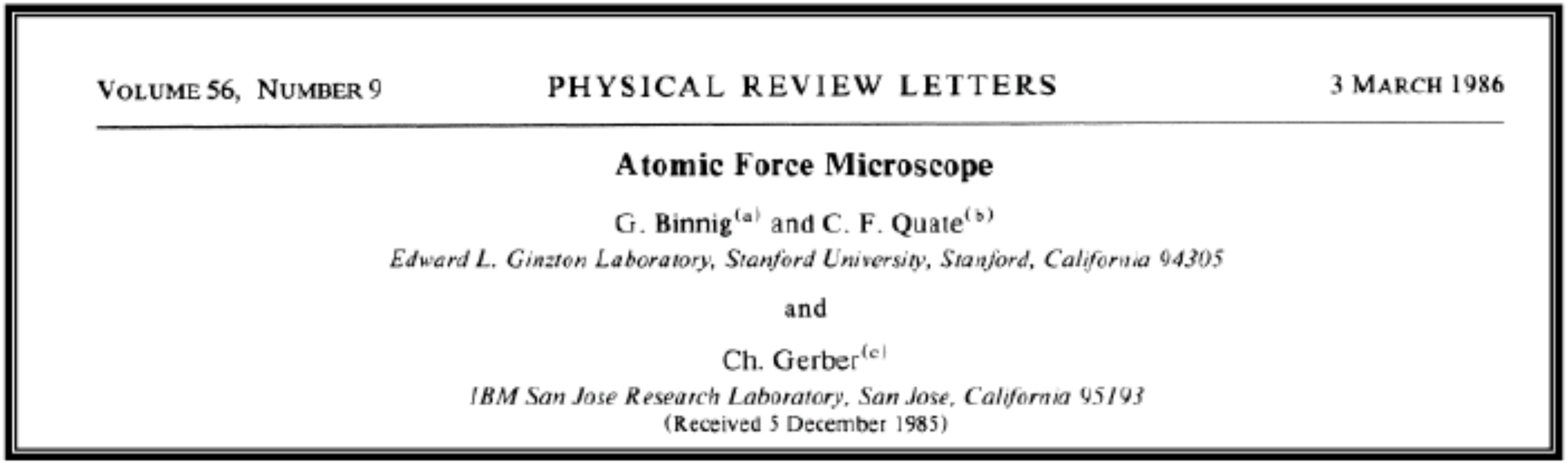

G.Meyer *et al*, Single Mol. **1** (2000) 1
http://www.physik.fu-berlin.de/~ag-rieder/LT-STM2/


Quantum Corral

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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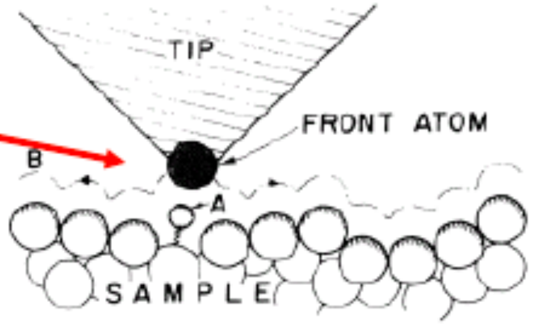

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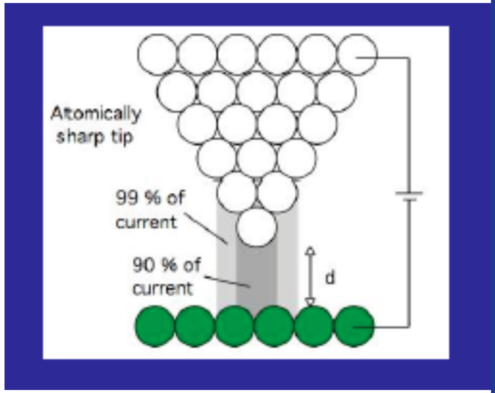
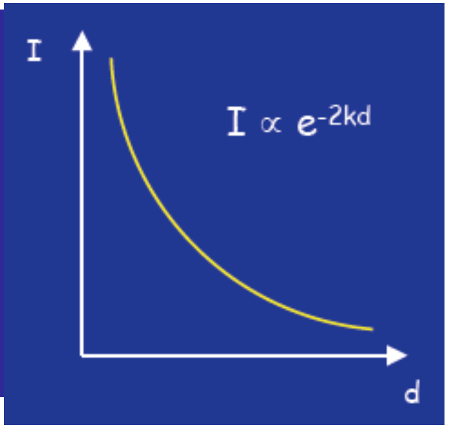
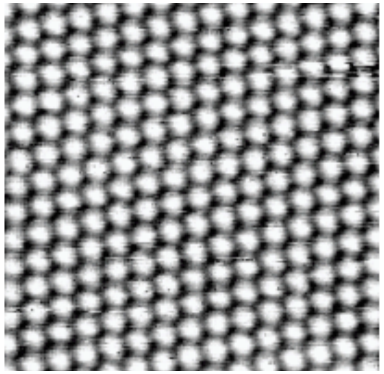
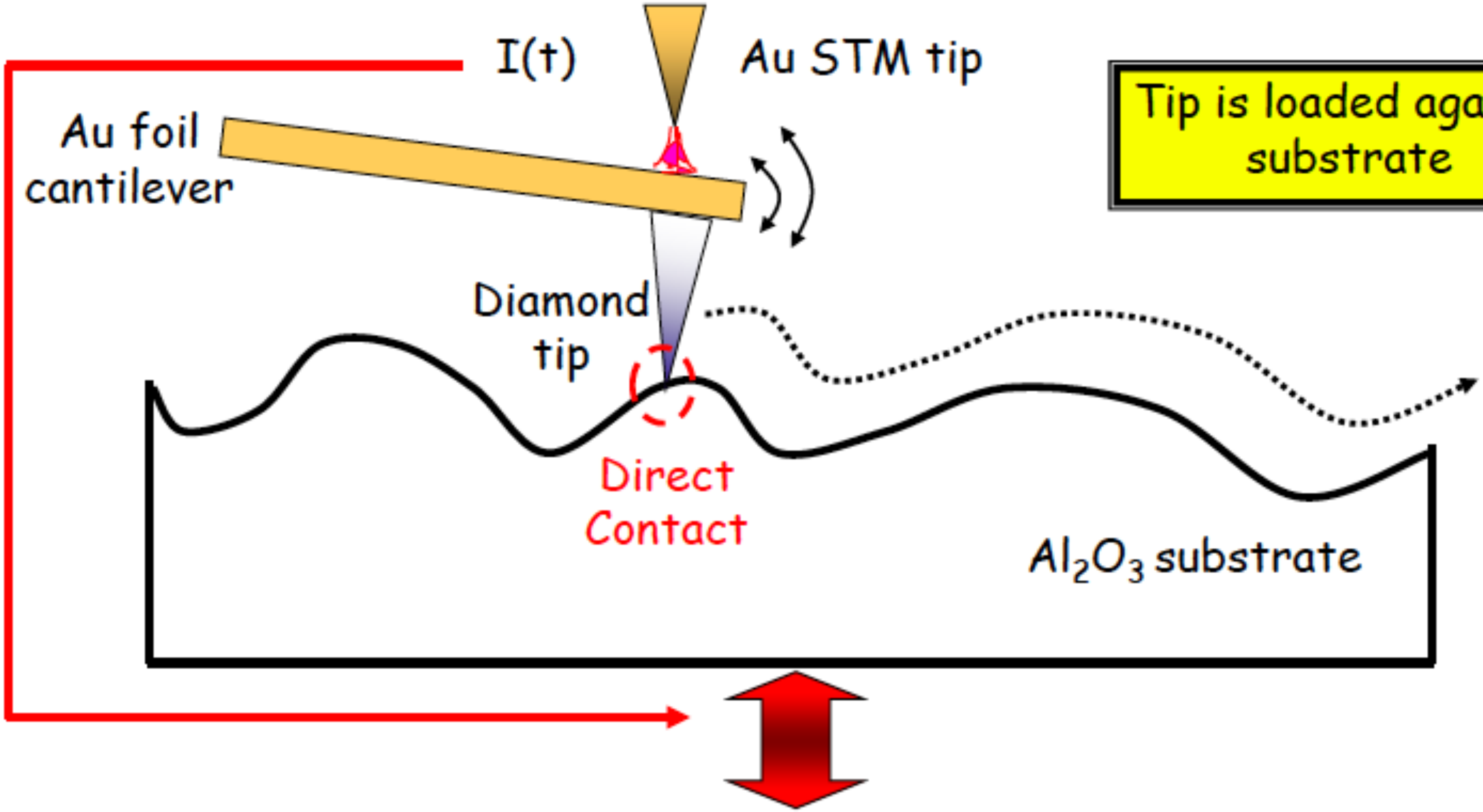
Key Idea: use sensitivity of STM to measure the rise and fall of a tip mounted on a cantilever when rastered across an insulating substrate.









I(t)

Au STM tip

Au foil cantilever

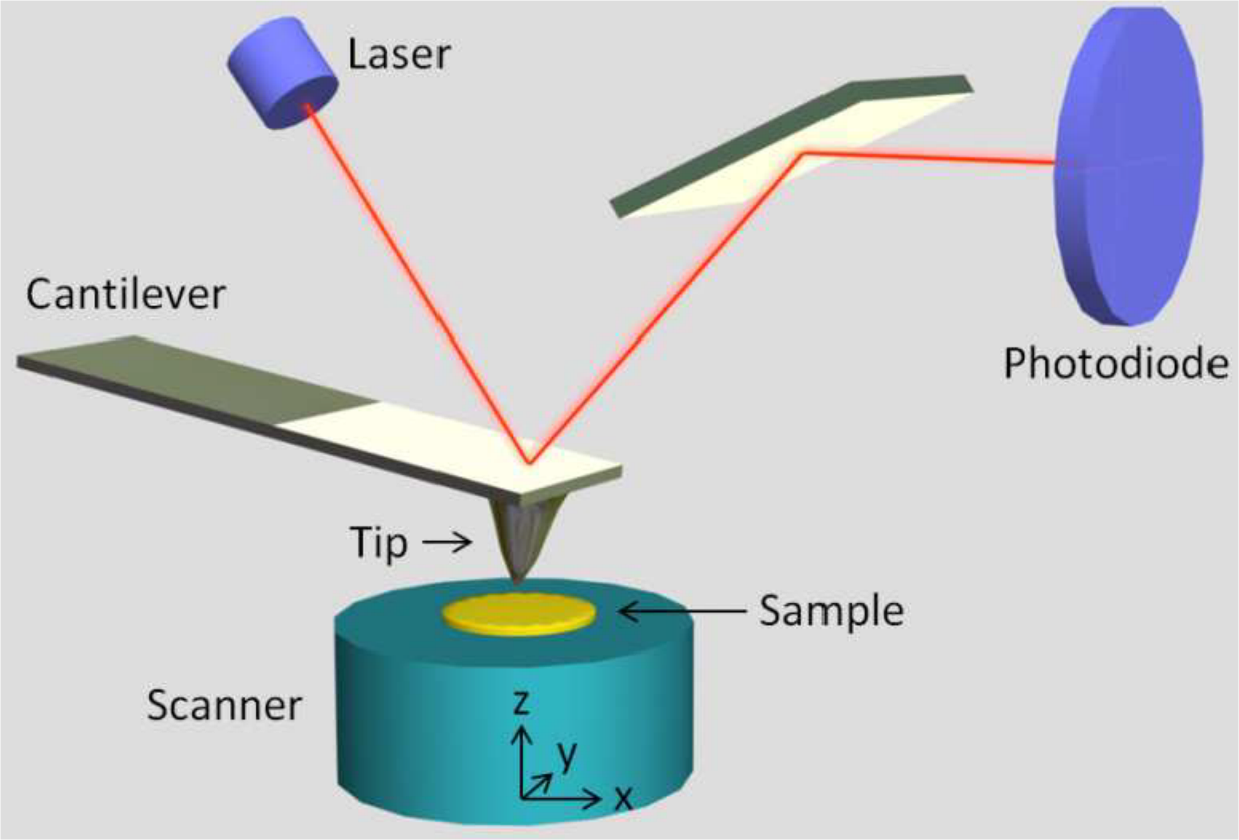
Diamond tip

Direct Contact

Al_2O_3 substrate

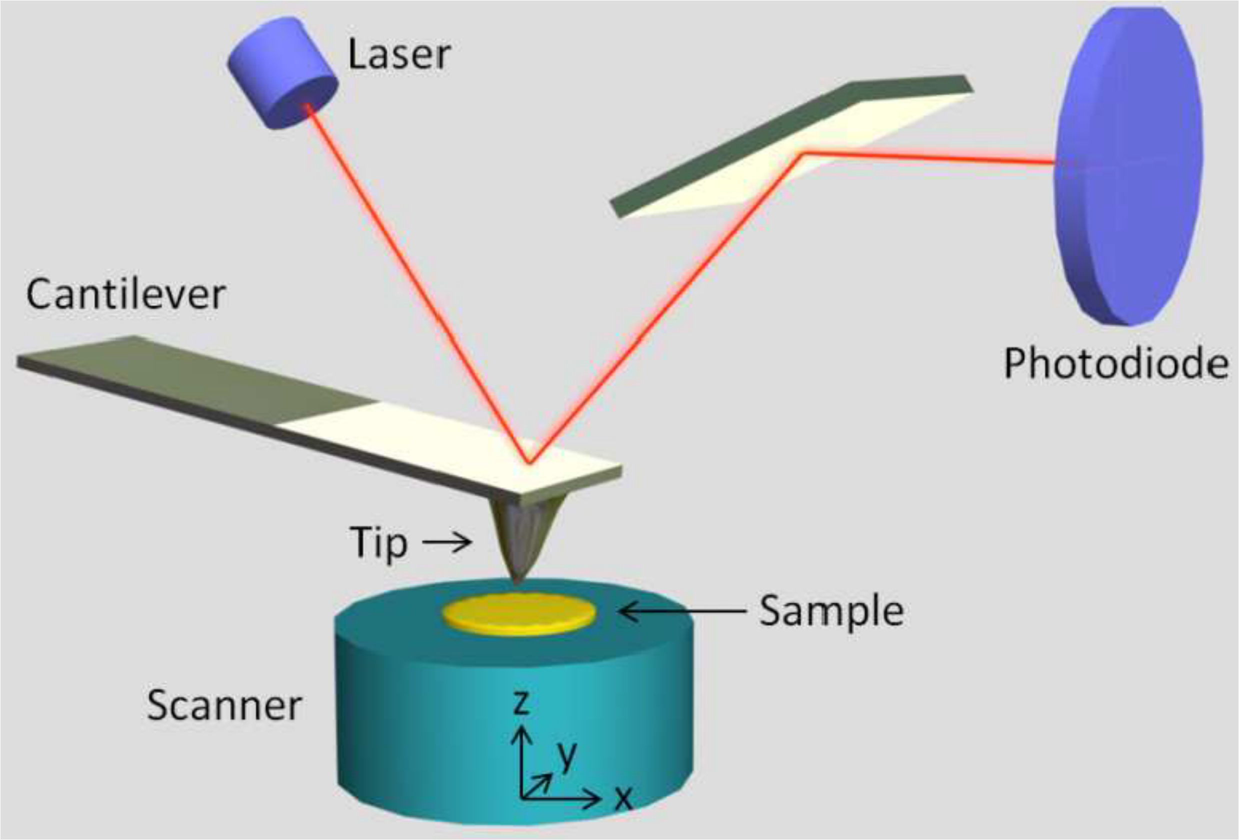
Tip is loaded against substrate

Atomic Force Microscopy



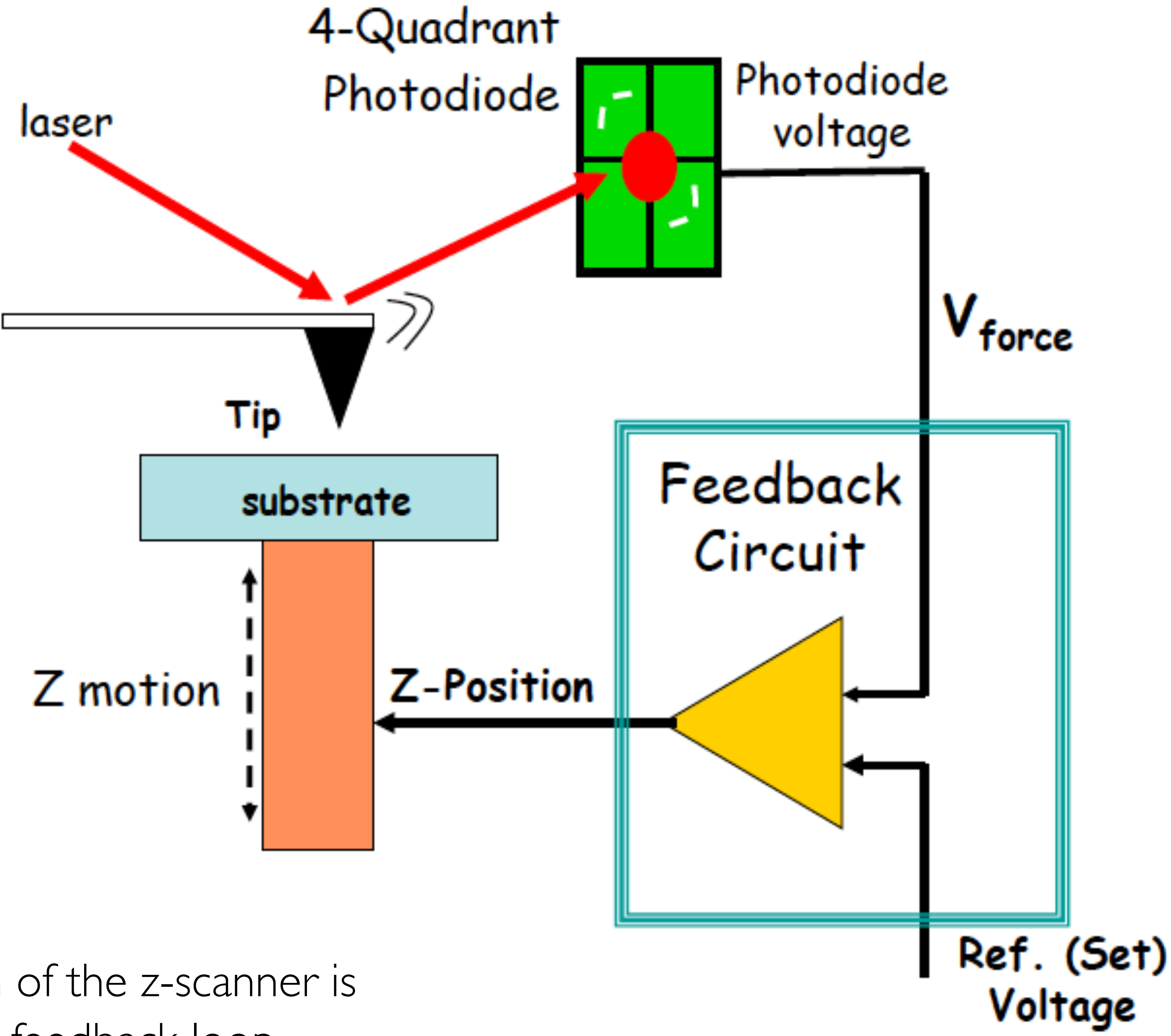
AFM is a mechanical imaging instrument that derives the **3-D profile (topography)** and the physical properties of a surface by measuring the **INTERACTION FORCES** with a scanning, nanometer sized probe.

Atomic Force Microscopy



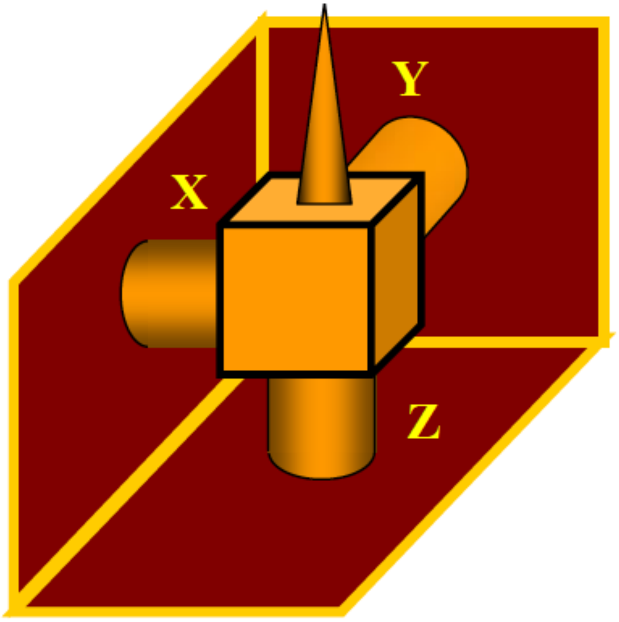
Topographic image of the sample obtained by:
•plotting the deflection of the cantilever versus scanner x,y position (seldom);
•plotting the height position of the translation stage versus versus scanner x,y position.
Image contrast arises because the force between the tip and sample is a function of both tip–sample separation and the material properties of tip and sample.

Maintaining a constant force



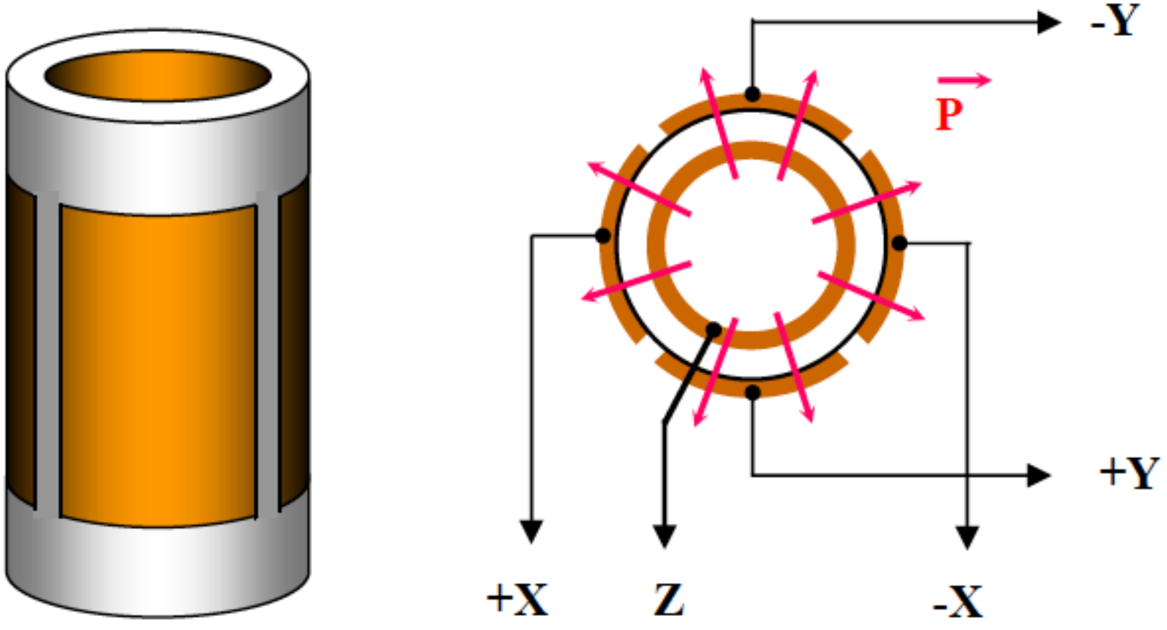
Height position of the z-scanner is controlled by a feedback loop

Key element of the feedback system: piezoscanner



Tripods : strongly asymmetic

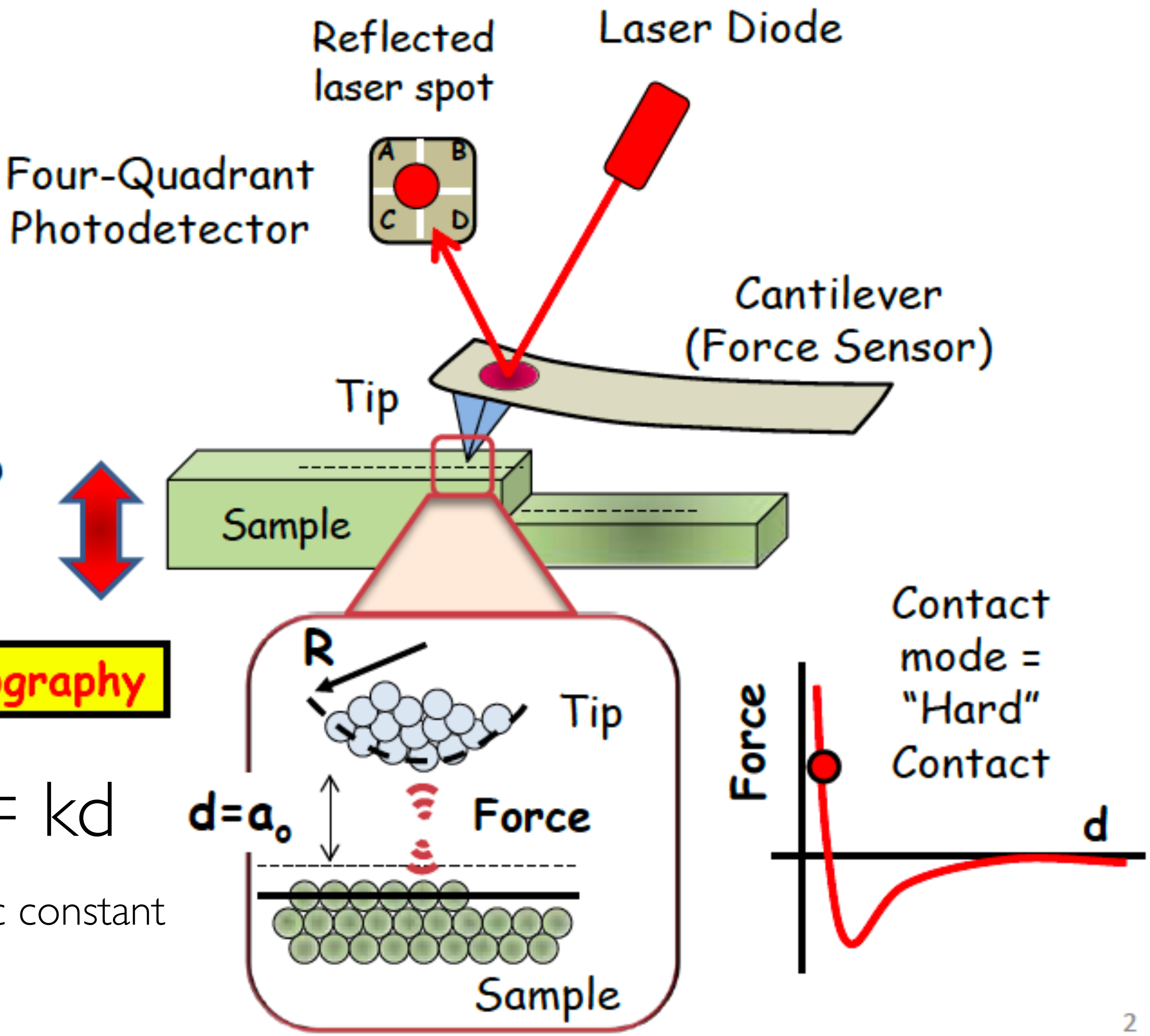
Single tube scanner



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



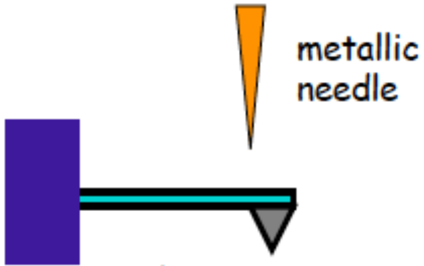
Move sample to keep deflection of cantilever constant

Sample Motion = Topography

$$F = kd$$

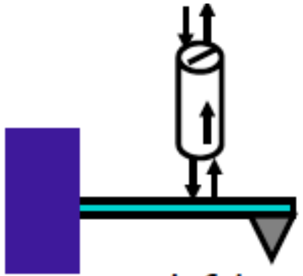
K= cantilever elastic constant

AFM: the deflection detection system



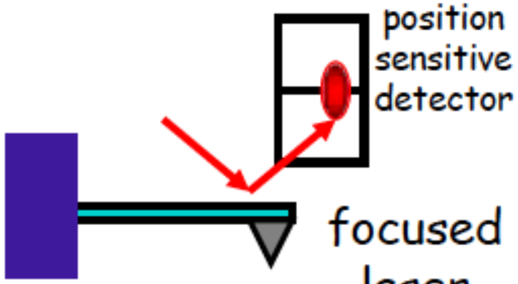
STM

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



optical fiber

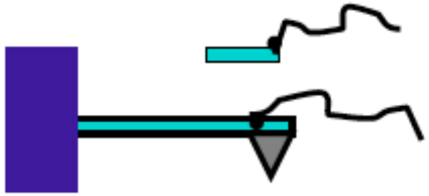
Martin *et al.*, J. App. Phys. **95** (1987)



focused
laser

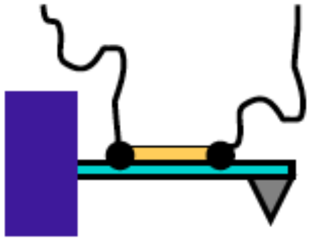
Marti *et al.*, J. Microscopy, **152** (1986)

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



capacitance

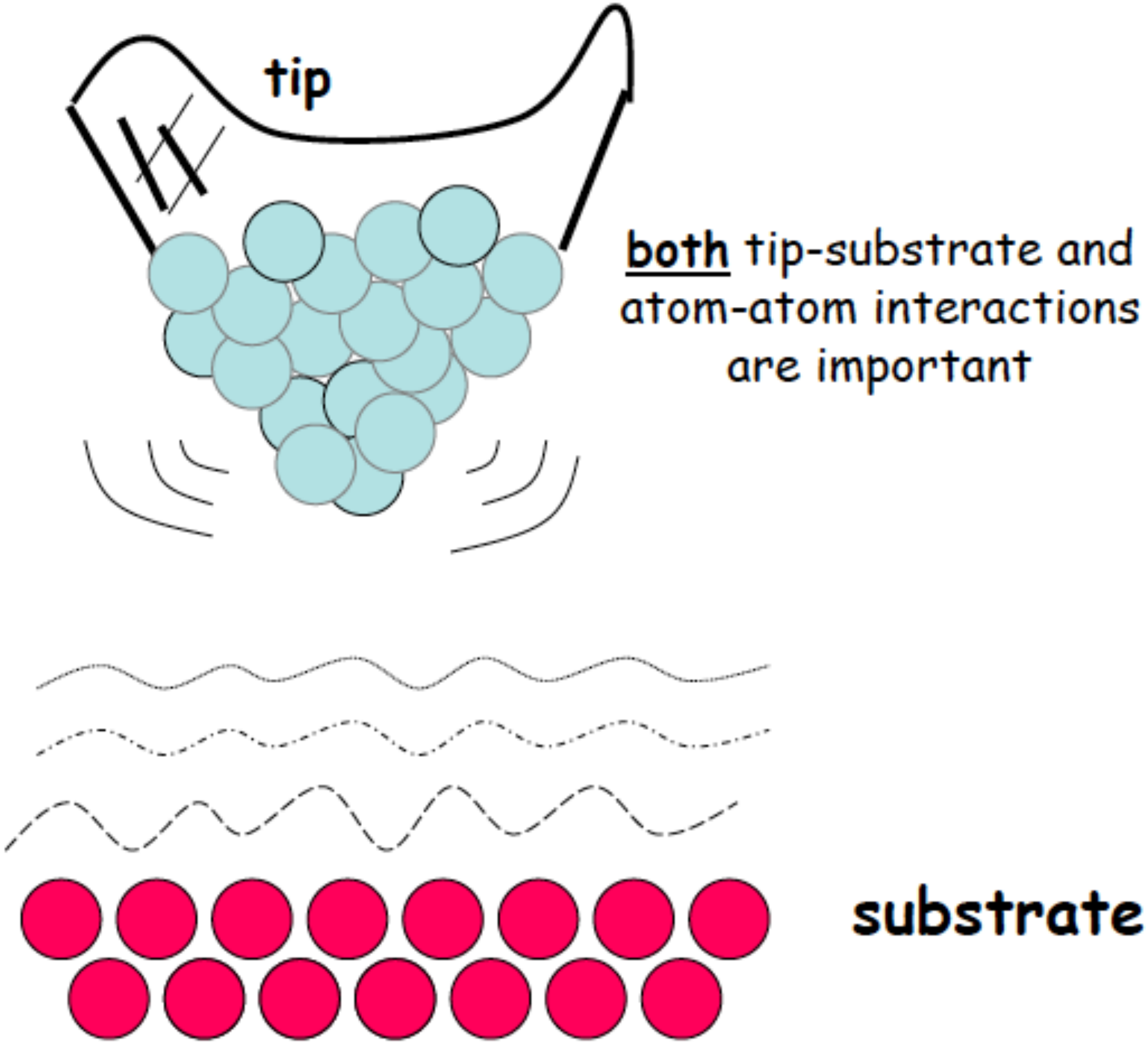
Göddenhenrich *et al.*, J. Vac. Sci. Technol. **A8** (1990)



piezoresistance

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

Tip-Substrate Interactions



both tip-substrate and atom-atom interactions are important

Electrostatic,magnetic

van der Waals (1873)

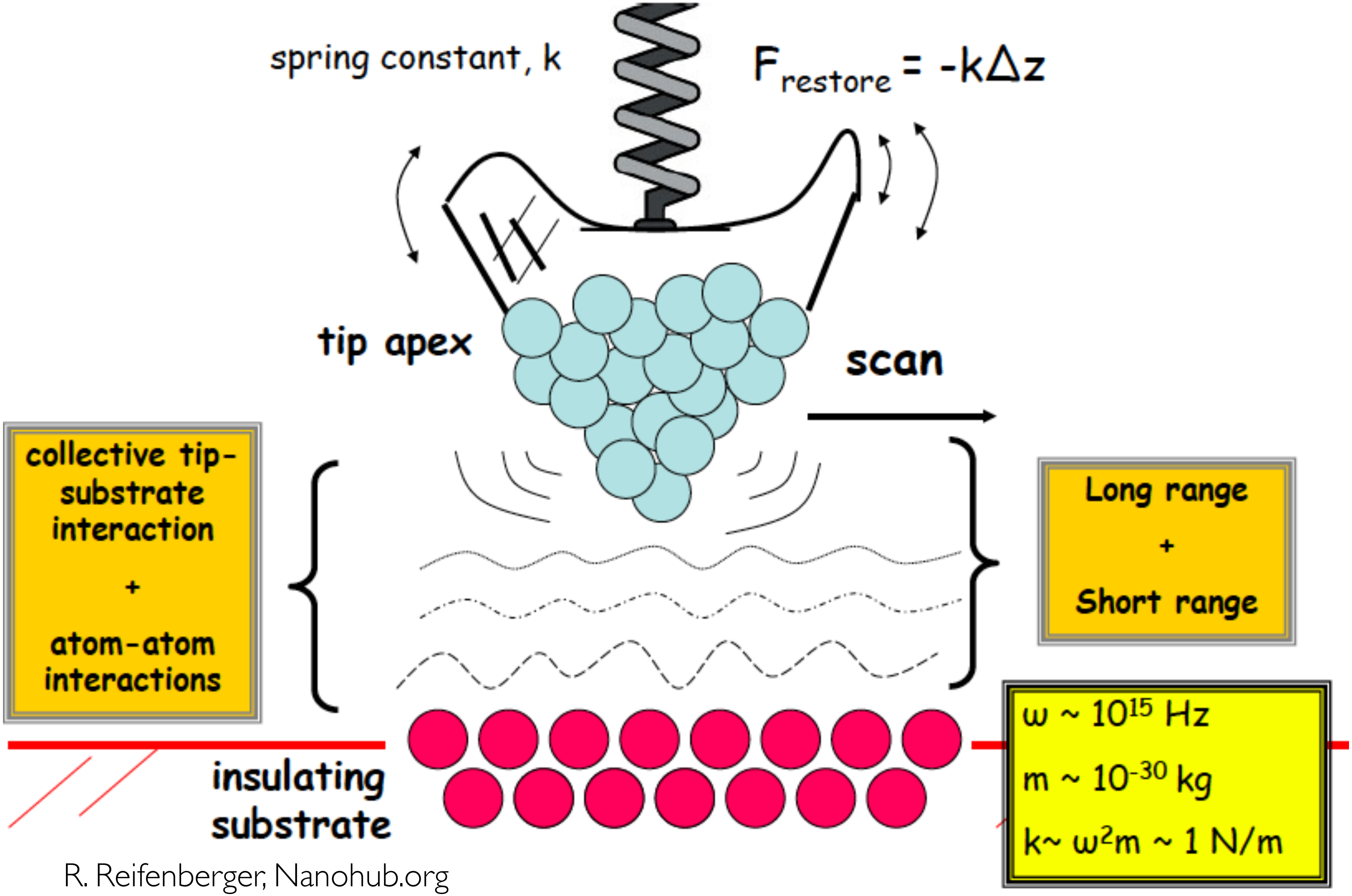
Capillary

Chemical:
(chemisorbed,physisorbed)

Friction

Deformation (indentation)

What controls the atomic force?



collective tip-substrate interaction

\+

atom-atom interactions

Long range

\+

Short range

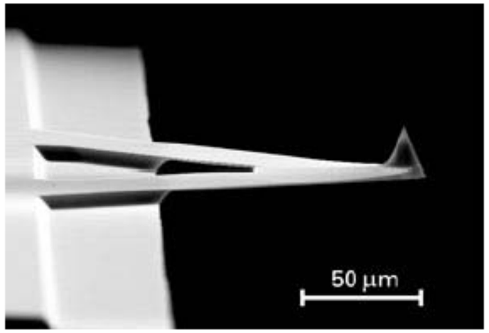
$\omega \sim 10^{15}$ Hz

$m \sim 10^{-30}$ kg

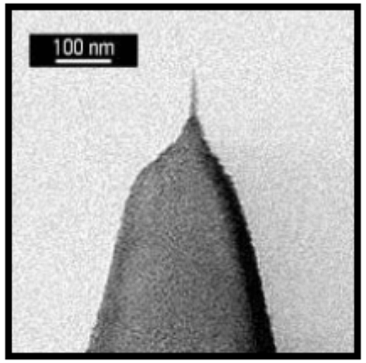
$k \sim \omega^2 m \sim 1$ N/m

R. Reifenberger, Nanohub.org

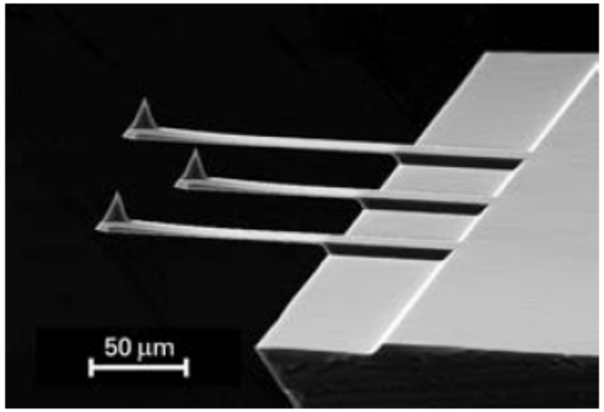
AFM probes



www.spmtips.com



µmasch



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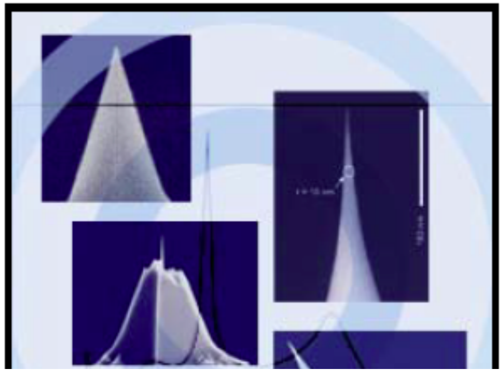
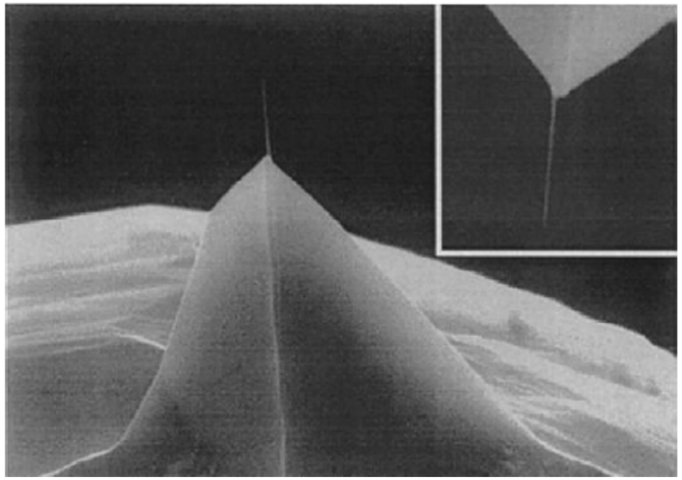
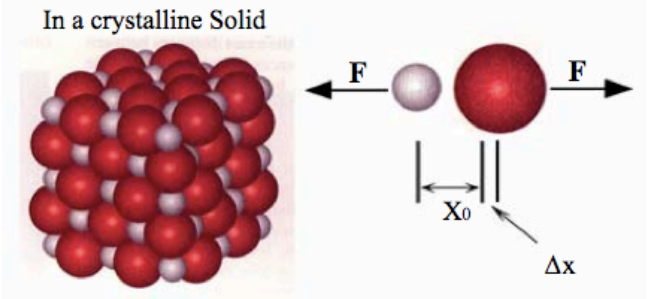




CNT tip

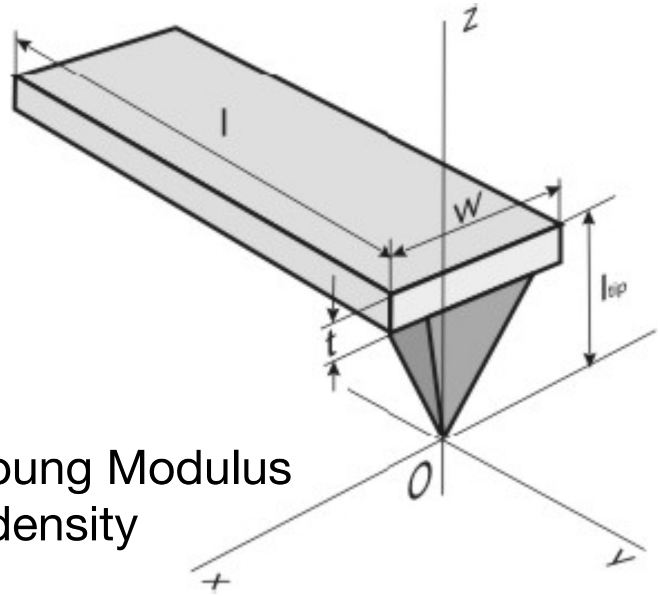
Typical use	k (N/m)	f_o (kHz)
Non-contact	10-100	100-300
Intermittent contact	1-10	20-100
Contact	0.1-1	1-50



$F = k\Delta x$

$k \sim 10$ N/m

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.

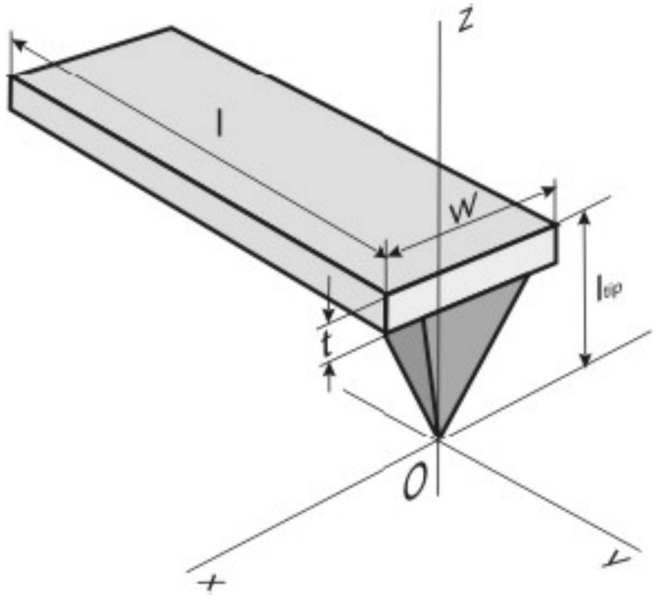
Typical E value: 1.5 x 10^{11} N m^{-2} in silicon nitride

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

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



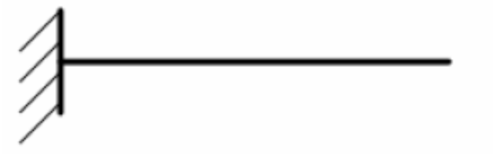
$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} = \frac{1}{4\pi}\frac{t}{l^2}\sqrt{\frac{E}{\rho}}$$

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

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

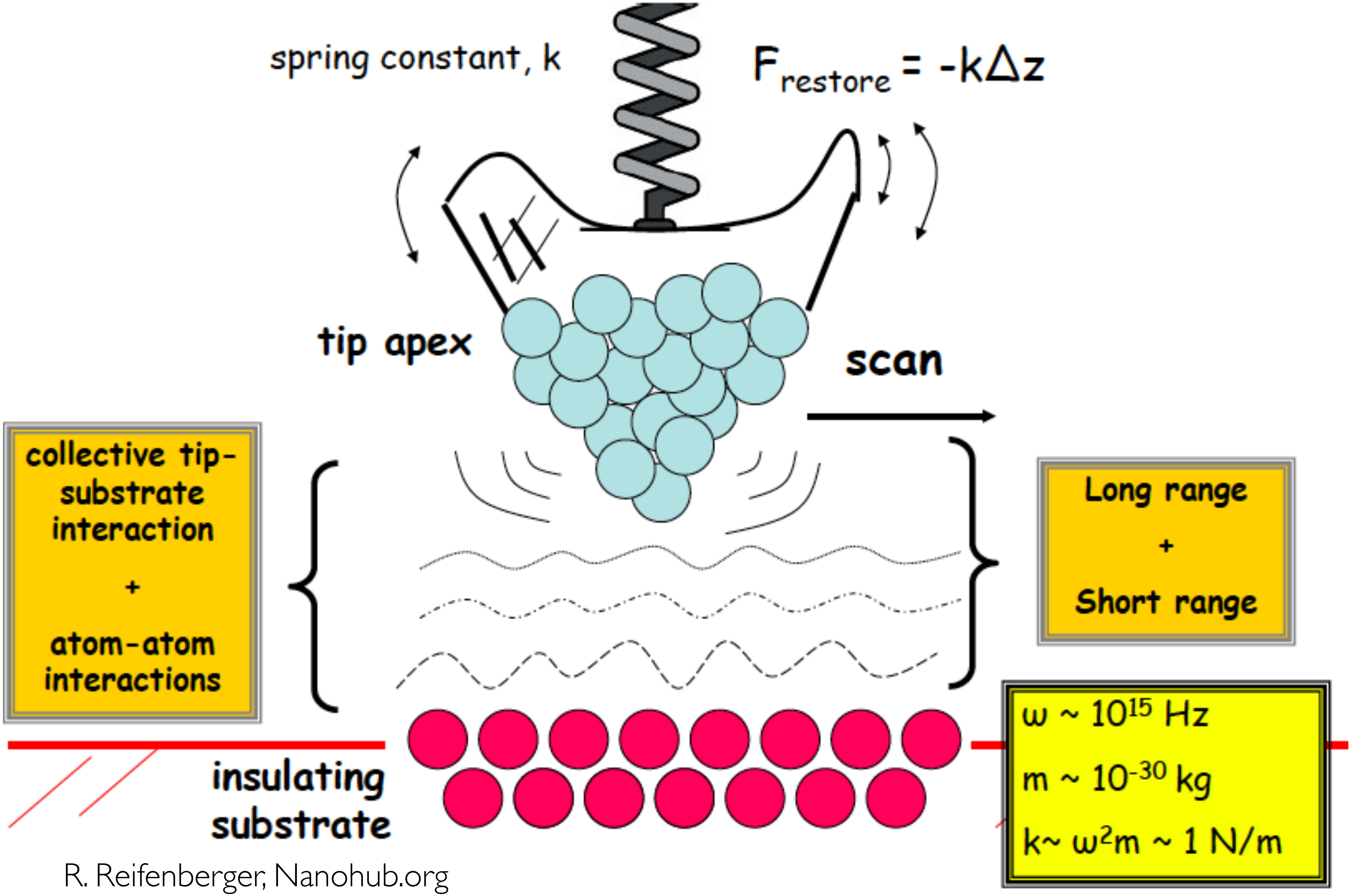


$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4 \rho}}$$

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

make cantilevers short to increase ω_0 and thinner to restore k

What controls the atomic force?



collective tip-substrate interaction

+

atom-atom interactions

Long range

+

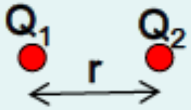
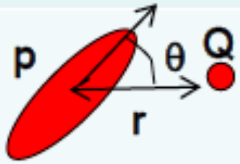
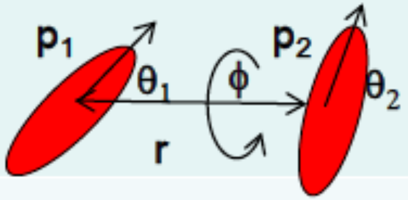
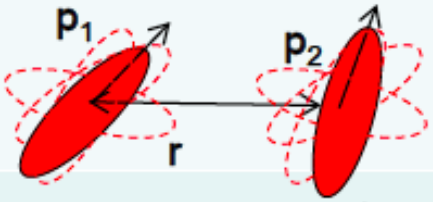
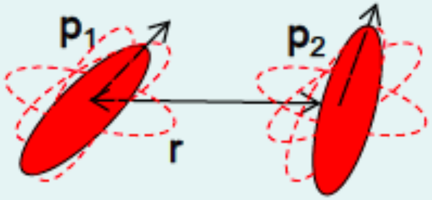
Short range

$\omega \sim 10^{15}$ Hz

$m \sim 10^{-30}$ kg

$k \sim \omega^2 m \sim 1$ N/m

R. Reifenberger, Nanohub.org

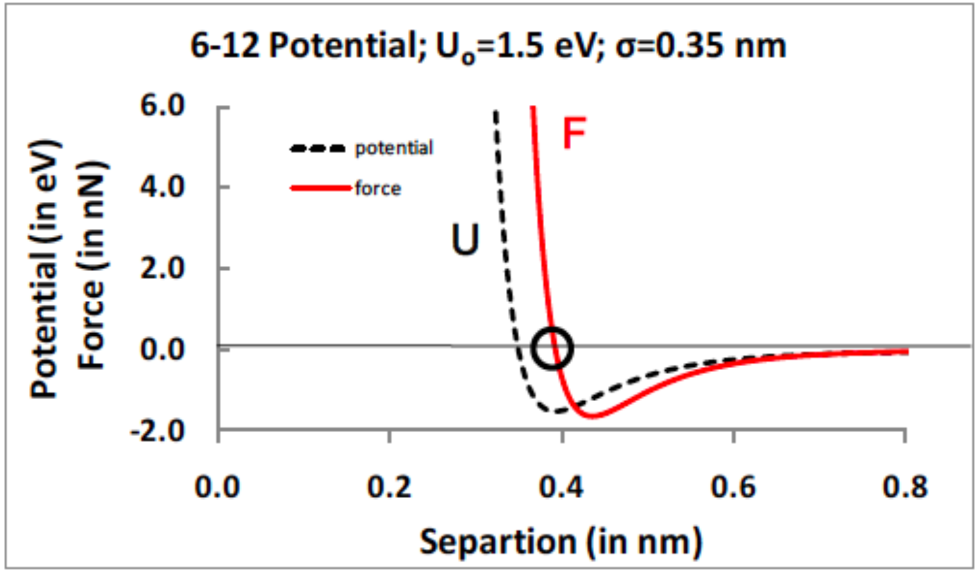
	Type of interaction
	Ion-ion electrostatic $U(r)=\dfrac{Q_1Q_2}{4\pi\varepsilon\varepsilon_0 r}$
	Dipole-charge electrostatic $U(r)=-\dfrac{Qp\cos(\theta)}{4\pi\varepsilon\varepsilon_0 r^2}$
	Dipole-dipole electrostatic $U(r)=-\dfrac{p_1p_2\left[2\cos(\theta_1)\cos(\theta_2)-\sin(\theta_1)\sin(\theta_2)\cos(\phi)\right]}{4\pi\varepsilon\varepsilon_0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

Intermolecular interactions probed by AFM

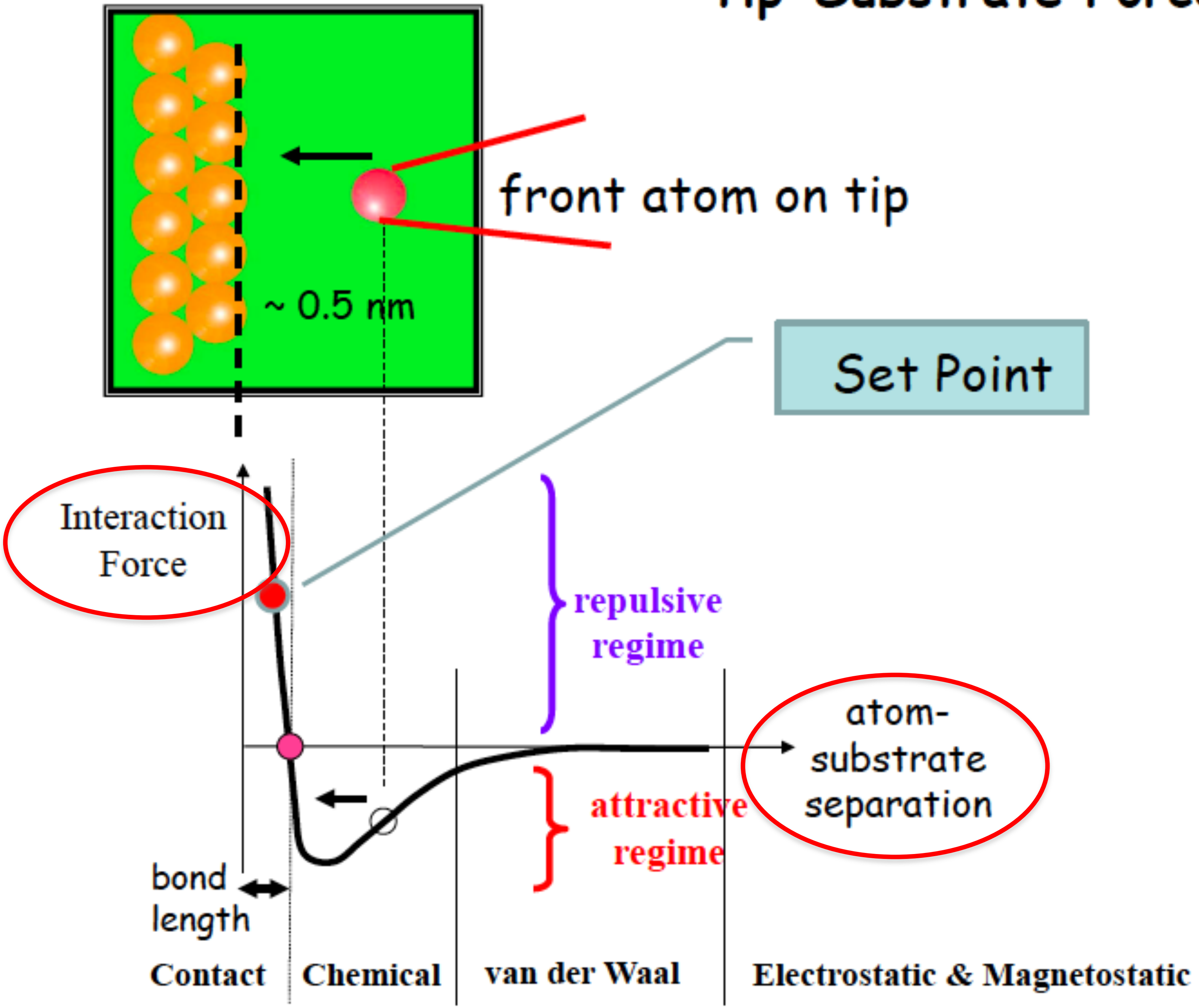
- Simple *ad hoc* model that tries to couple dispersion forces and Pauli repulsion.

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

- 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 Forces



front atom on tip

~ 0.5 nm

Set Point

Interaction Force

repulsive regime

attractive regime

atom-substrate separation

bond length

Contact

Chemical

van der Waal

Electrostatic & Magnetostatic

AFM imaging modes

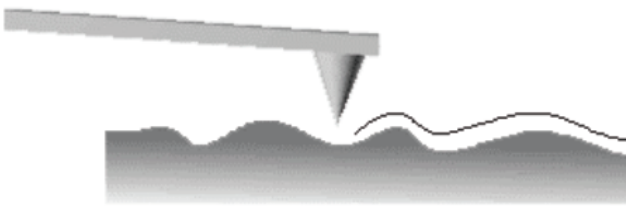
Contact Mode:

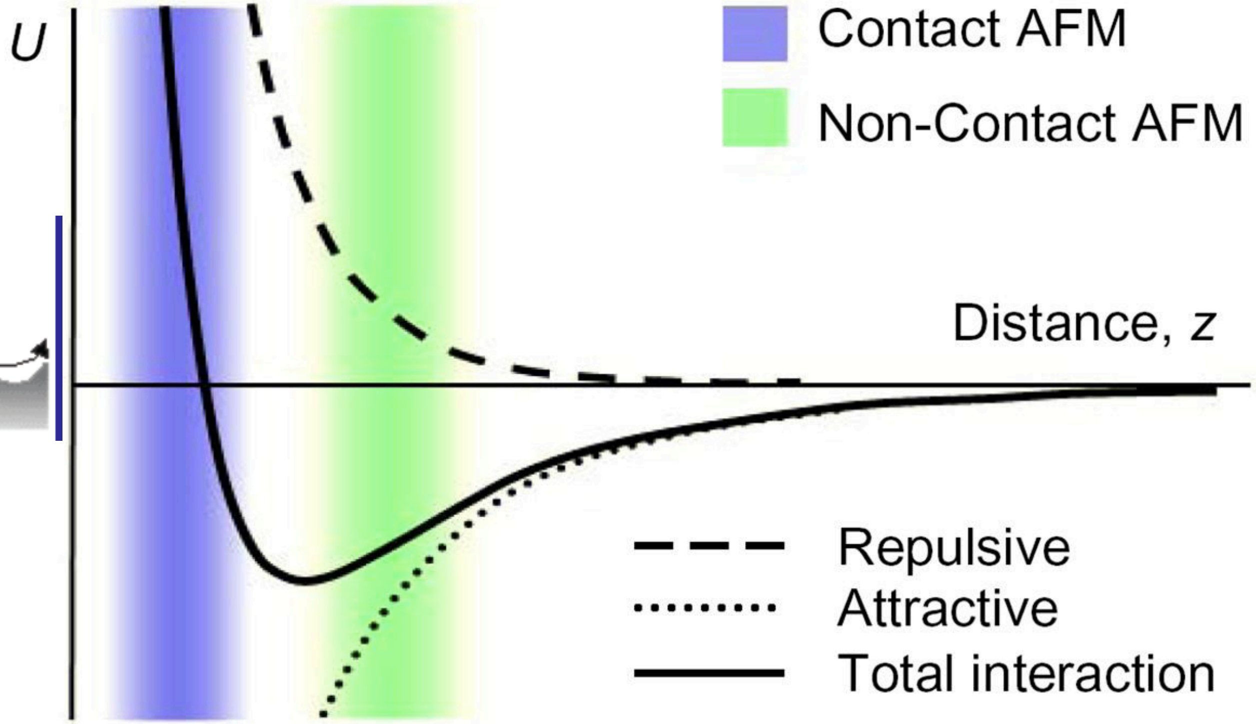
d < 5 Å

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

Atomic resolution

Problems: frictional forces, capillary fc



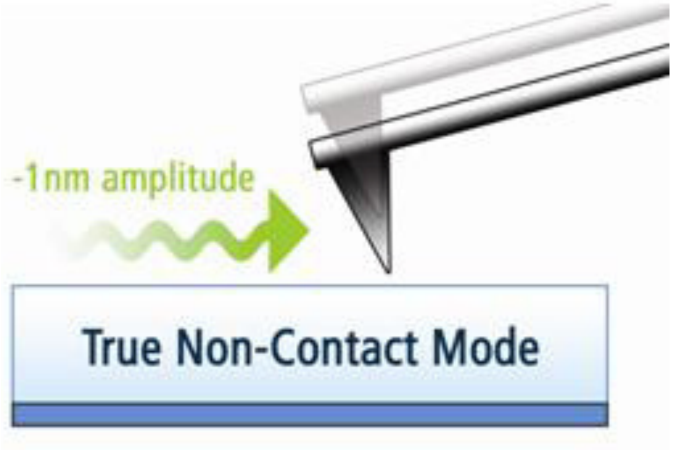


Non-Contact Mode:

d = 10÷100 Å

Actractive forces - ~10^{-12} N

Soft, elestic materials



Tapping Mode:

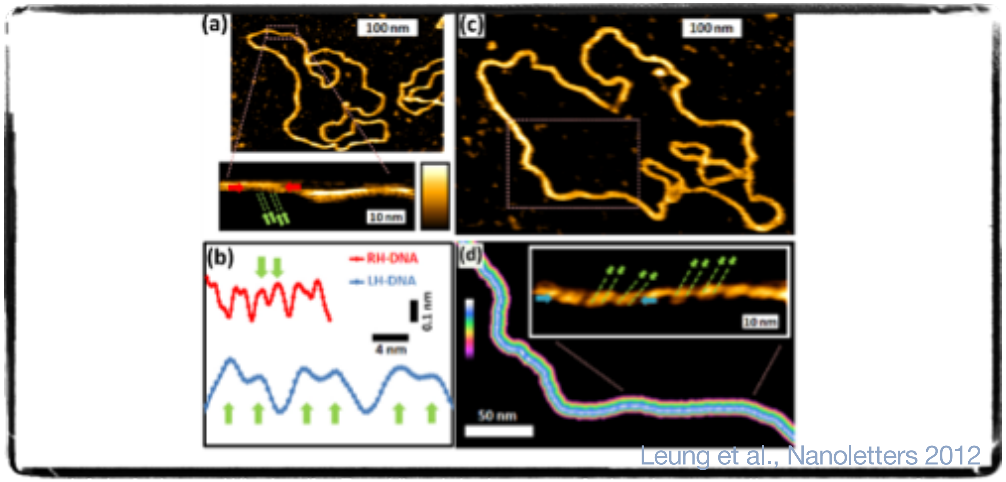
d = 5÷20 Å

Intermitting contact

Big scanning areas, no friction

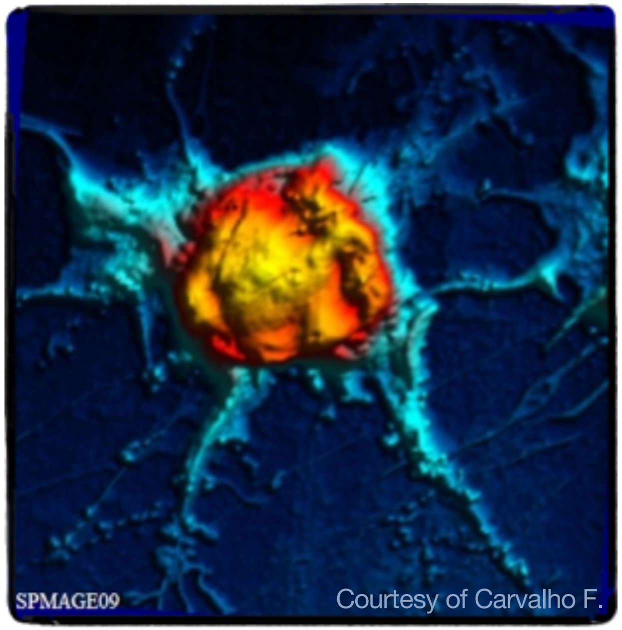
AFM in Biology

DNA



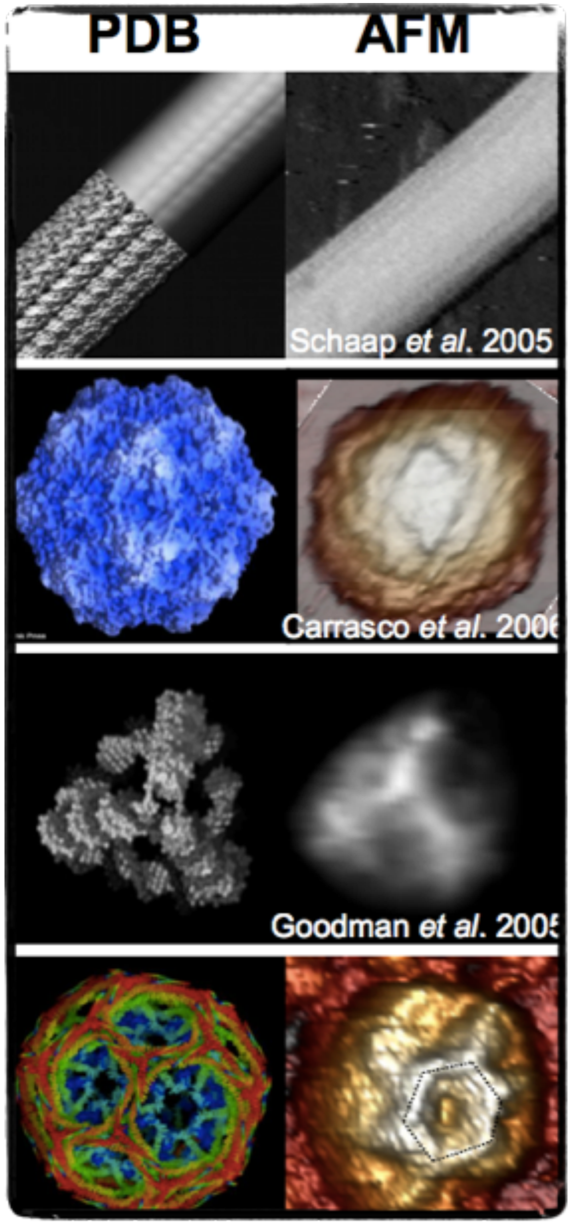
Leung et al., Nanoletters 2012

Cells



Courtesy of Carvalho F.

Proteins



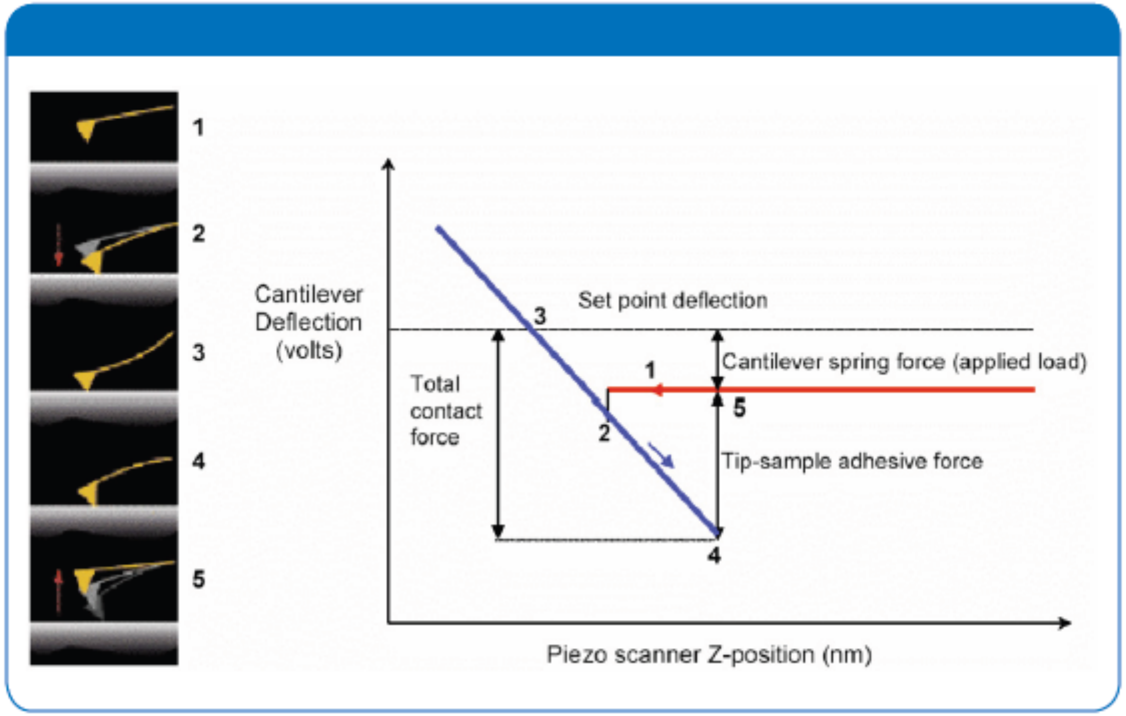
Schaap *et al.* 2005

Carrasco *et al.* 200

Goodman *et al.* 200

Calibration: force-distance curves

A basic AFM operation is the force-distance curve. No feedback in z!!!!
The cantilever is brought from a point within the range of the z-piezo toward the surface until the tip contacts the surface and back. 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



Force-distance curves

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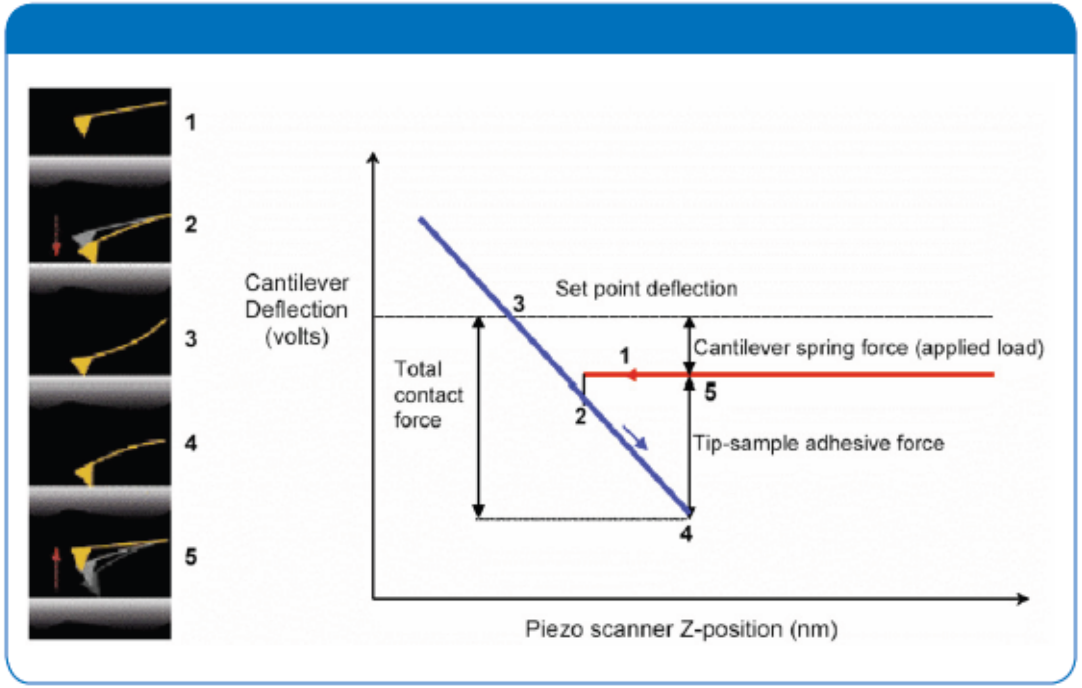
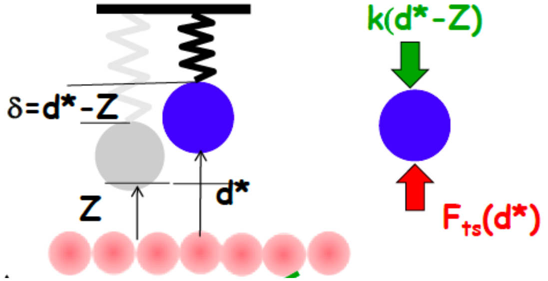
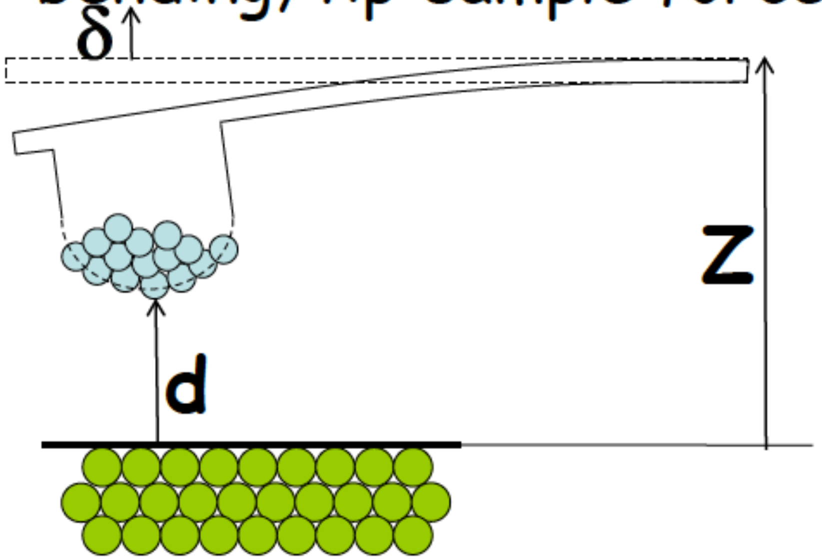


Points 2 and 4 describe two important occurrences in a loading curve. These are the points where the tip-sample interaction force is not balanced by the restoring force of the cantilever, i.e., dF/dx > k at point 2.



Force-distance curves

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

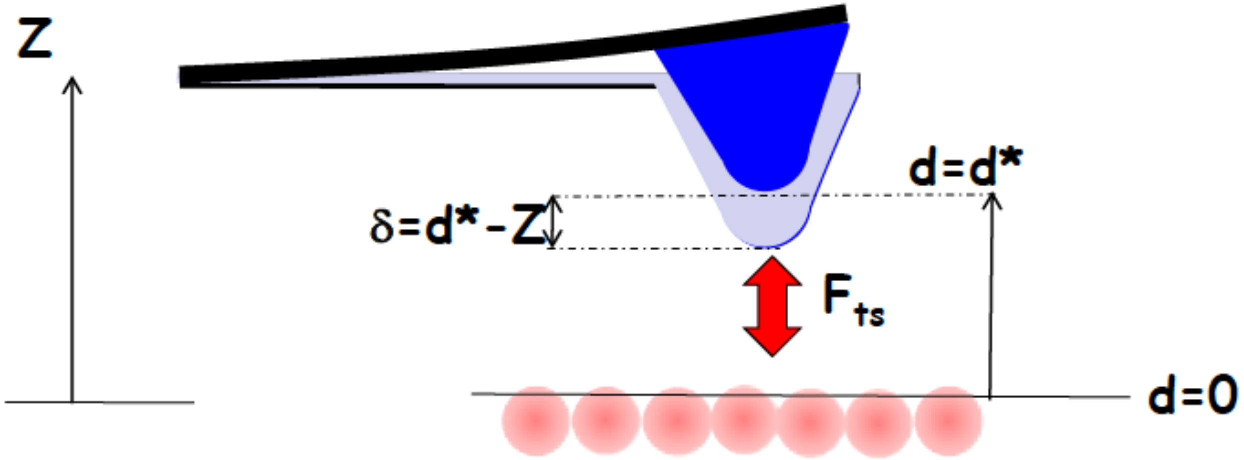


AFM's measure F_{ts} vs. Z !!

- 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 $d=Z+\delta$, to within an arbitrary constant

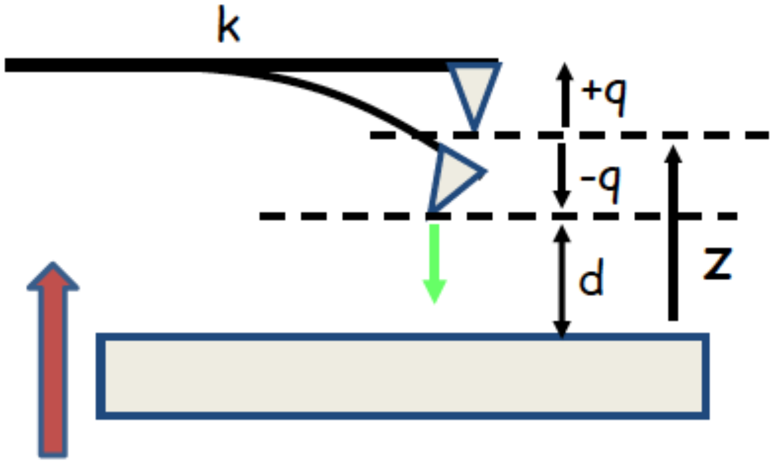
Force-distance curves

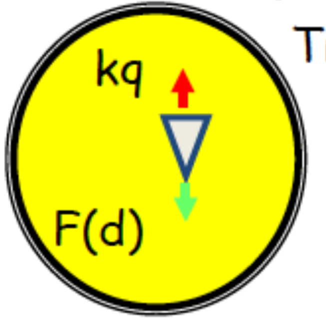
equilibrium positions



- 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

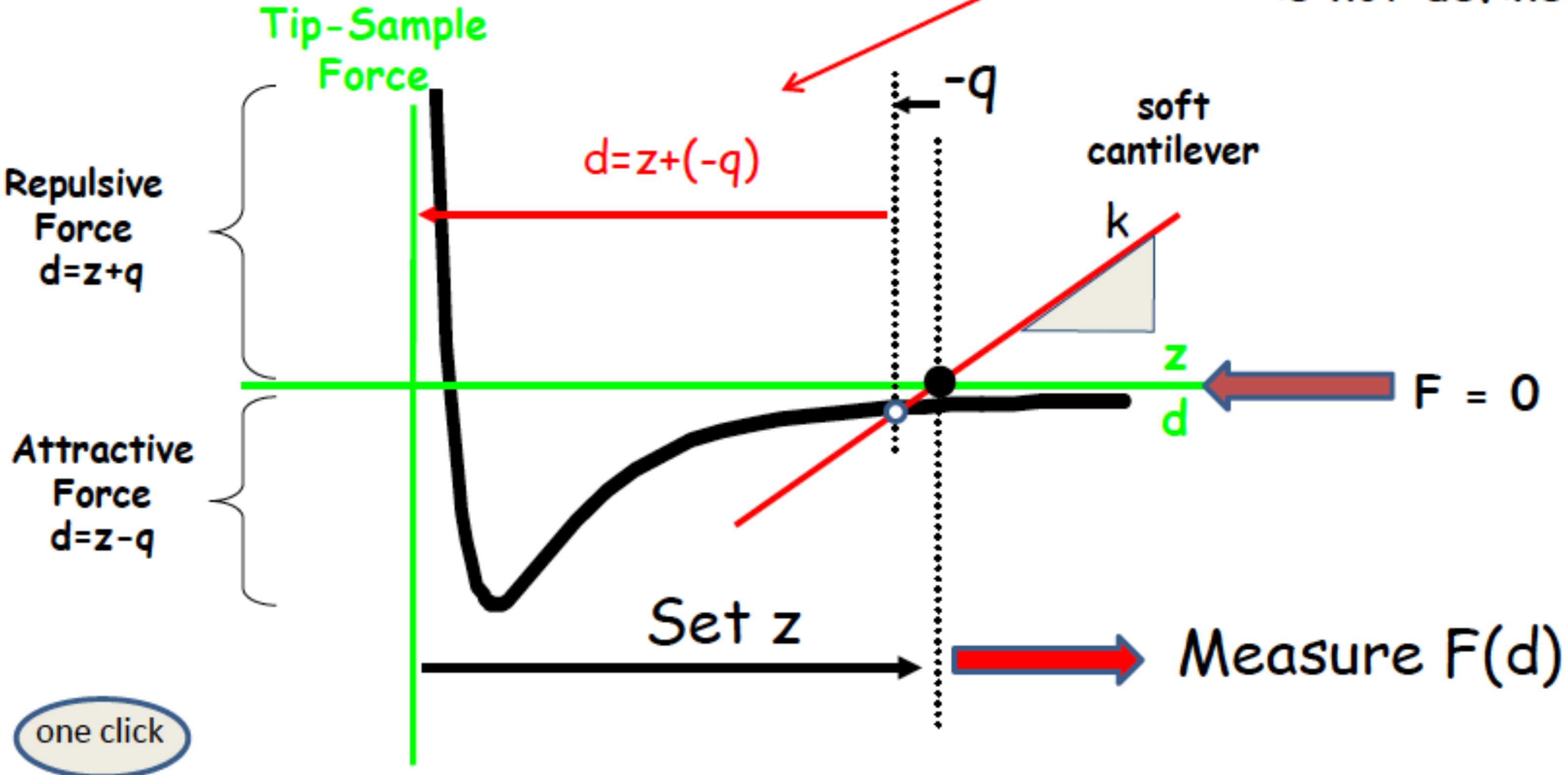
Measuring Force vs. z-displacement





Tip Free-body Diagram

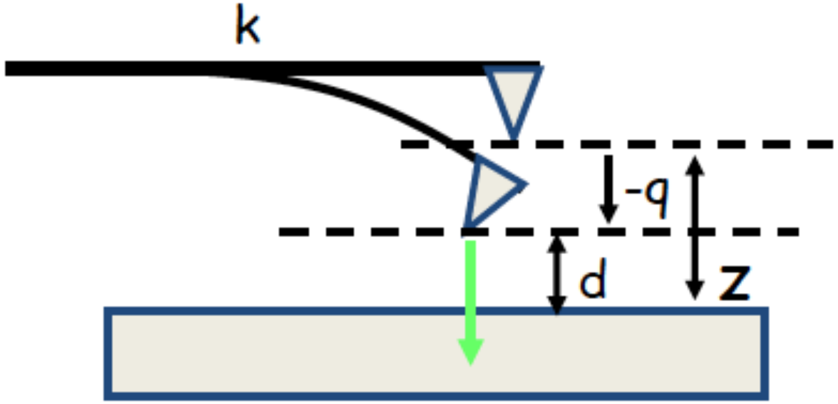
Important: Since z=0 is arbitrary (i.e. unknown), d=0 is not defined!

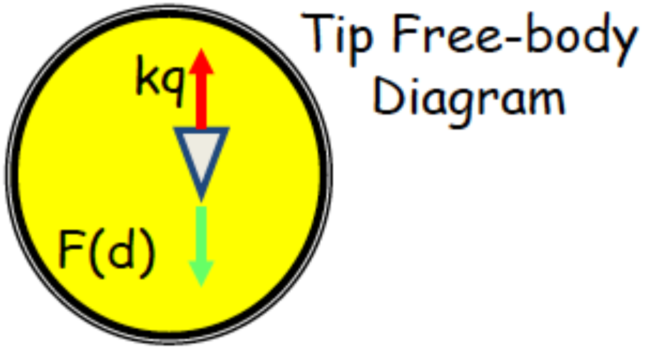


one click

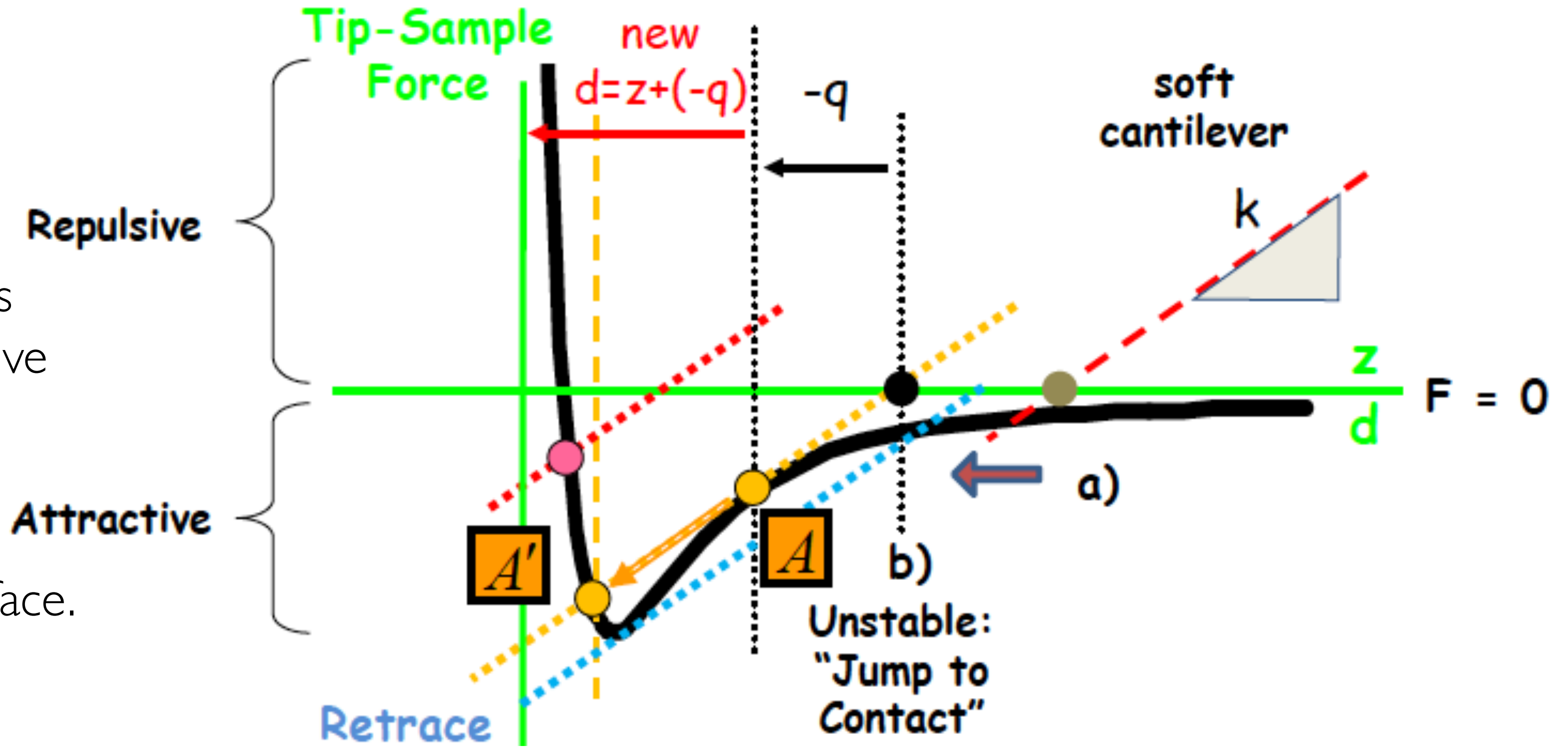
P1_Wk4_L2

Jump to Contact



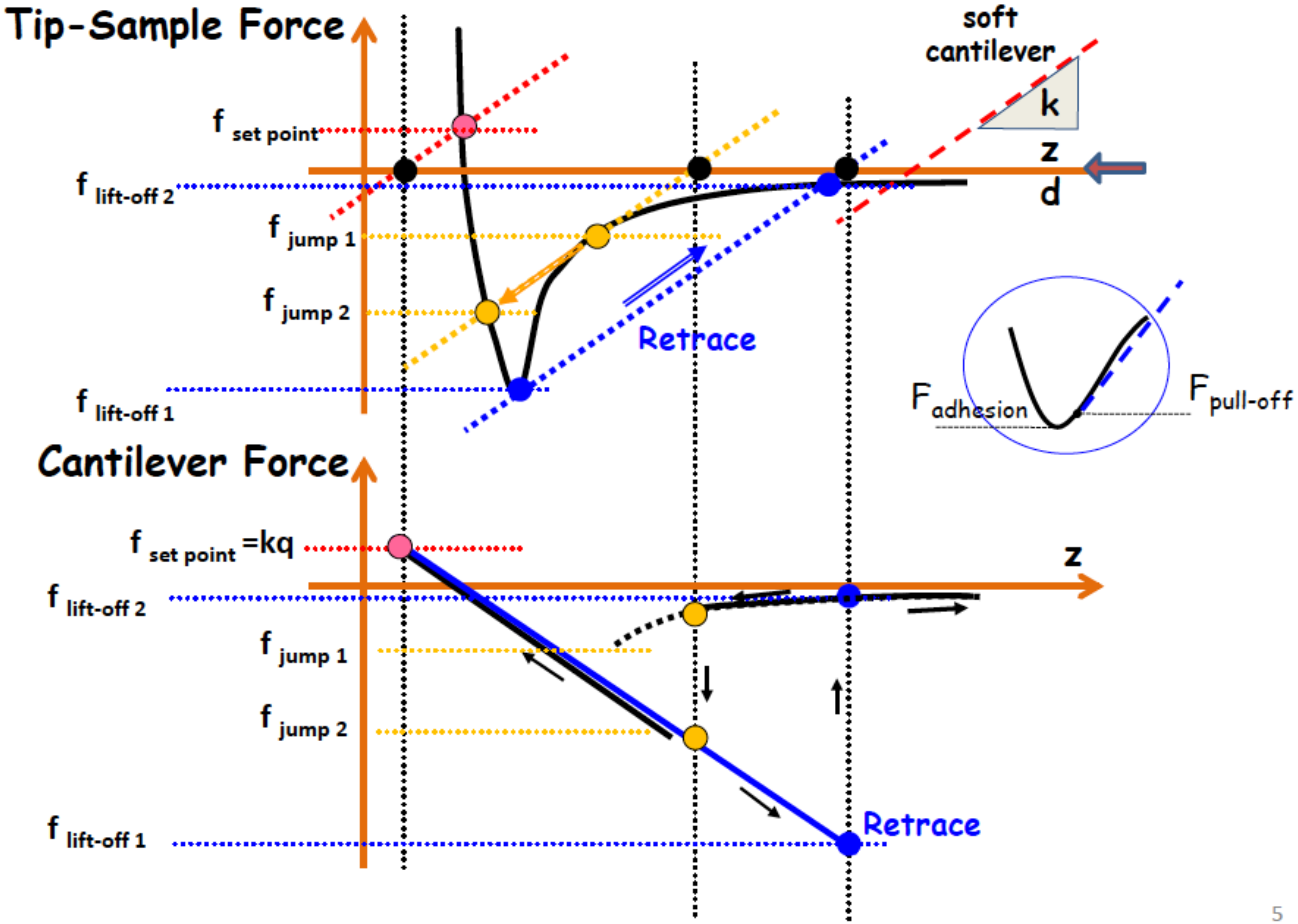


If the force gradient is larger than the effective elastic constant, the cantilever becomes unstable and "jumps" onto the surface.

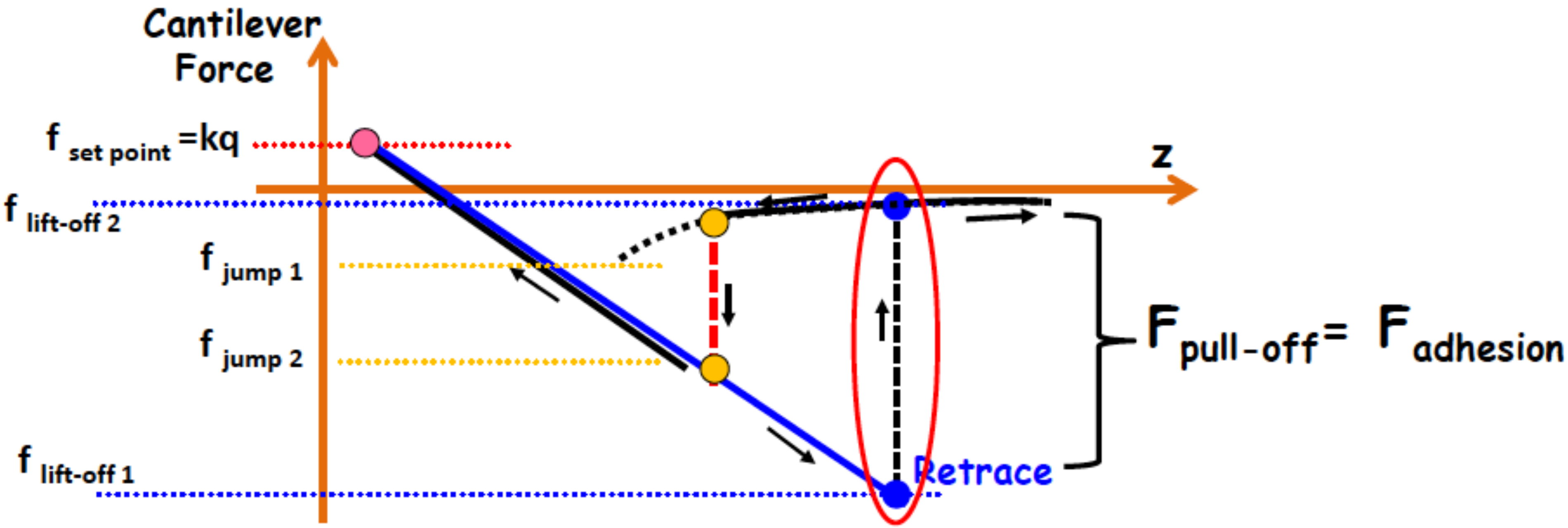


- With soft cantilevers (small k) it is not possible to measure entire 'd' range

Force vs. Separation Curve



The pull off force feature



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

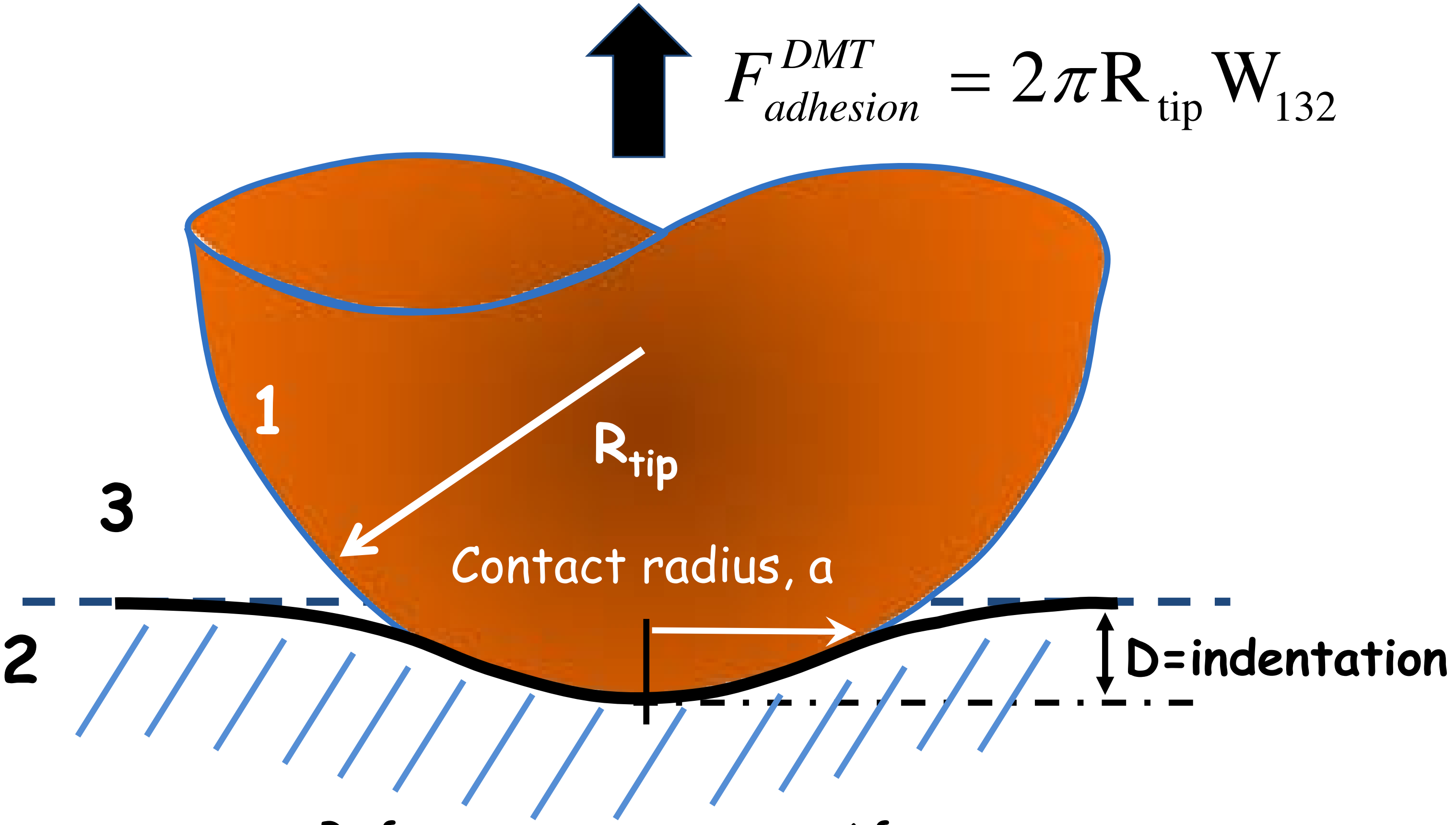
$$F_{adhesion} = F_{electrostatic} + F_{vdW} + F_{capillary} + F_{chemcial} + \ldots$$

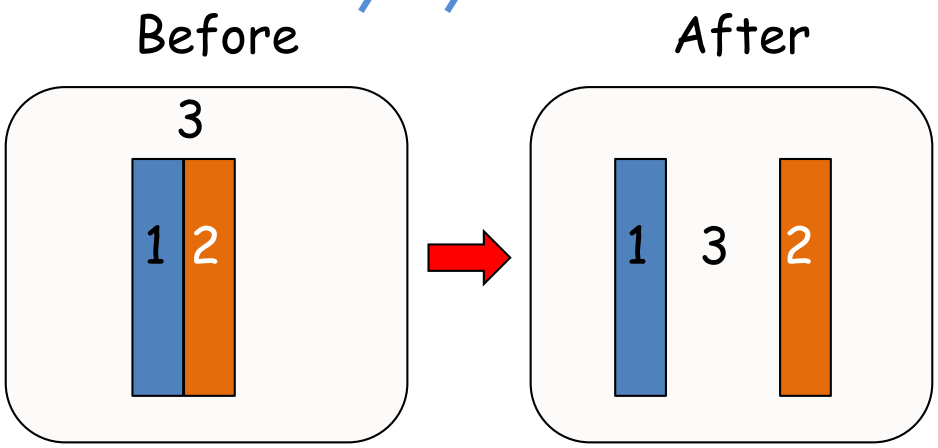
1. To understand $F_{pull\text{-}off}$, the tip-sample deformation, adhesion and contact area are required
2. **If** vdW forces dominate, then , e.g, the DMT model predicts

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

Review: Adhesion and the DMT Model

$$F_{adhesion}^{DMT} = 2\pi R_{tip} W_{132}$$

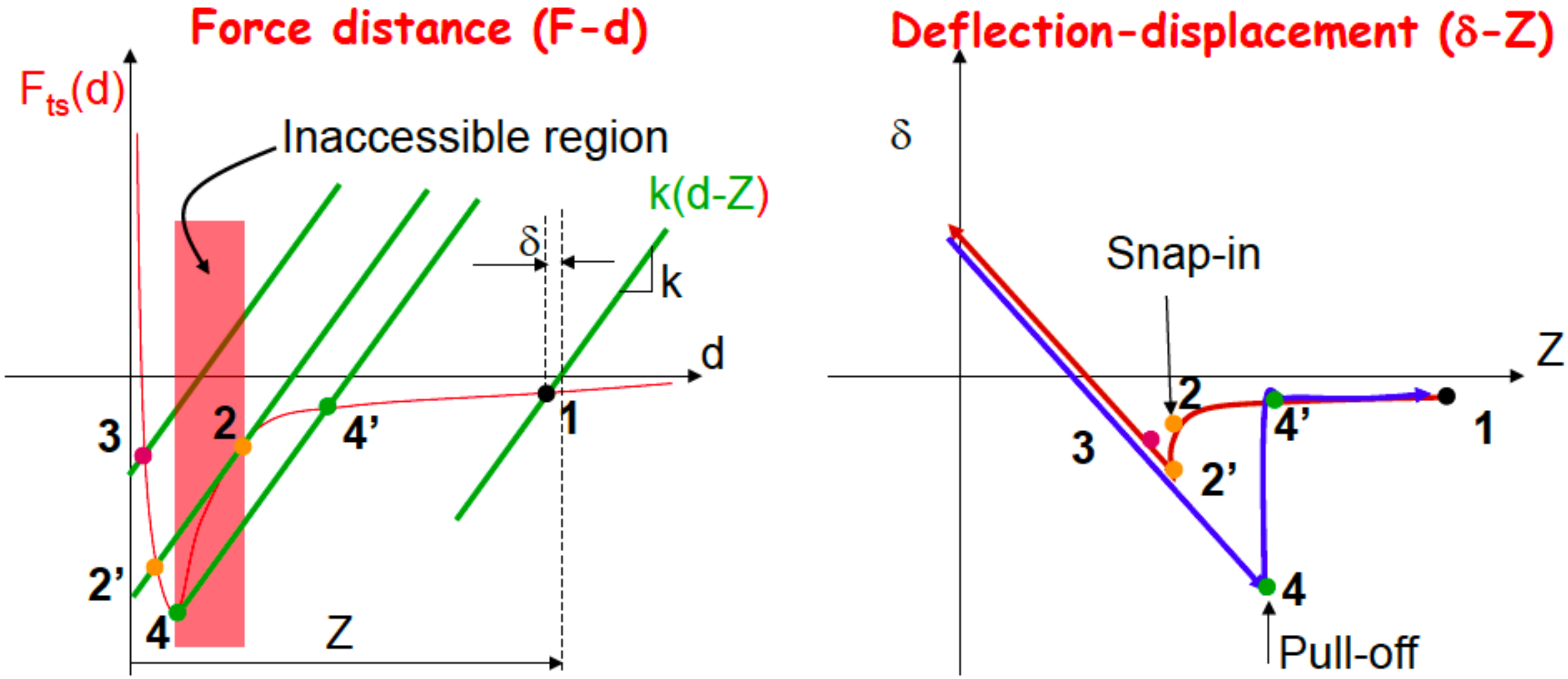




$$W_{132} = \gamma_{13} + \gamma_{23} - \gamma_{12}$$

Force-distance curves

F-d F-z conversion

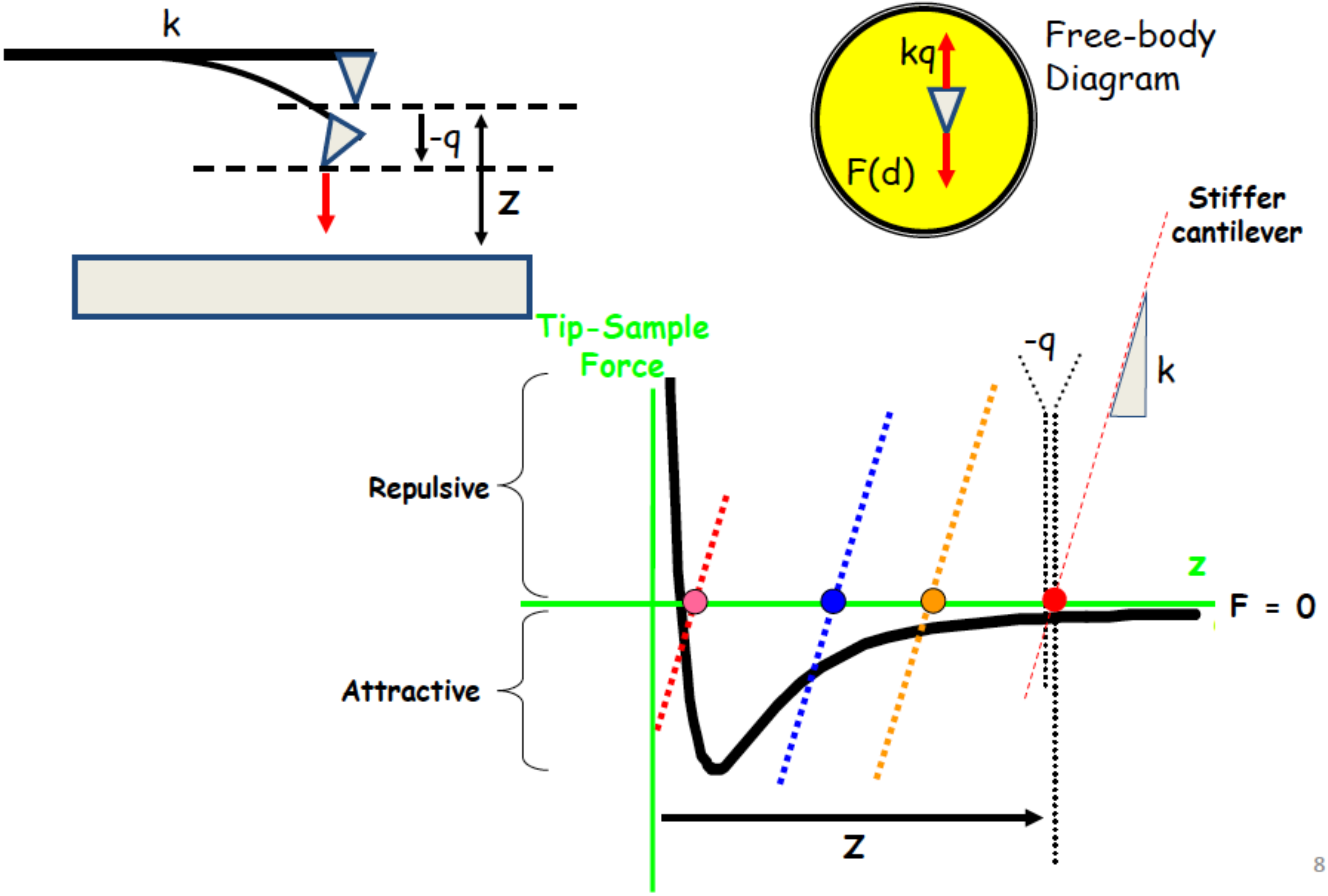


Force distance (F-d)

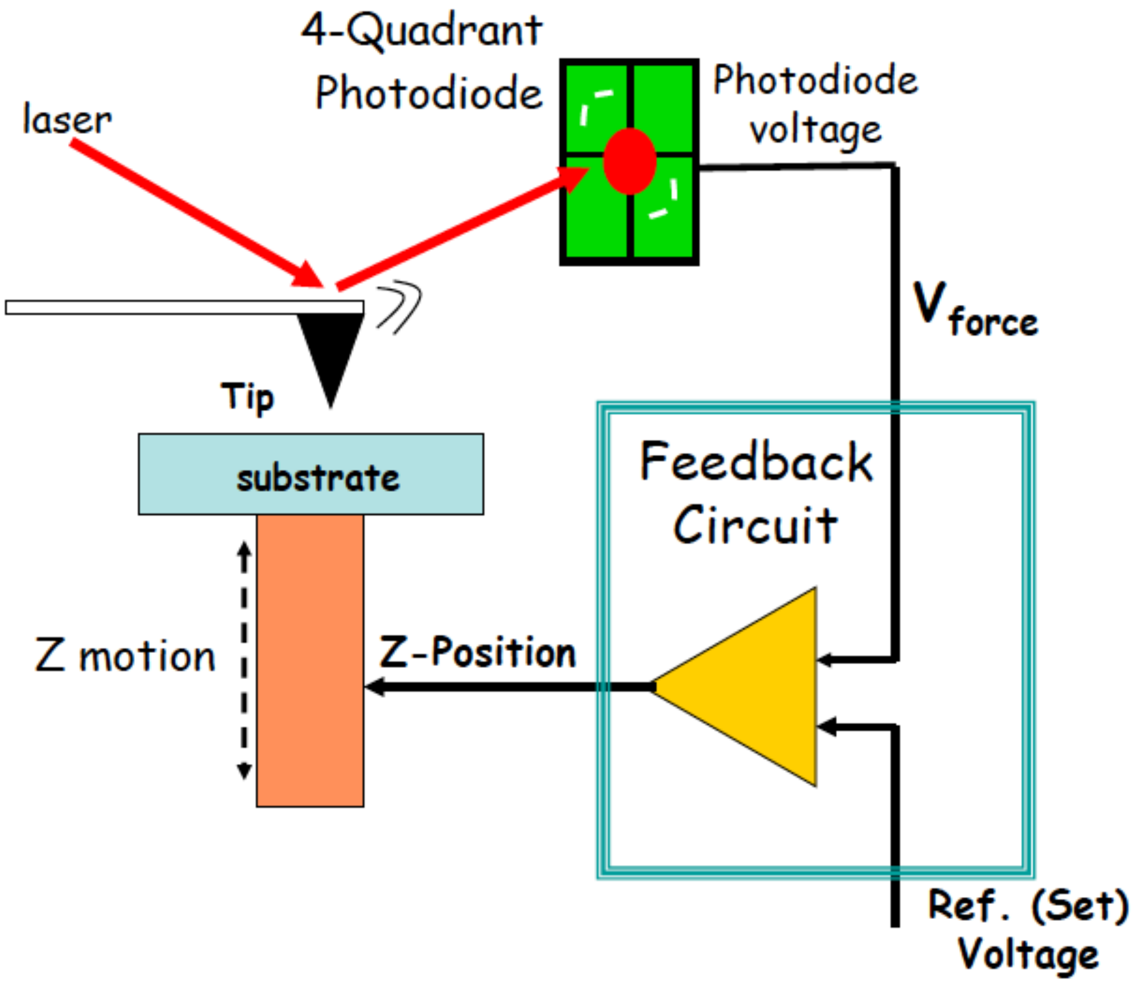
Deflection-displacement (δ-Z)

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

Stiffer Cantilever

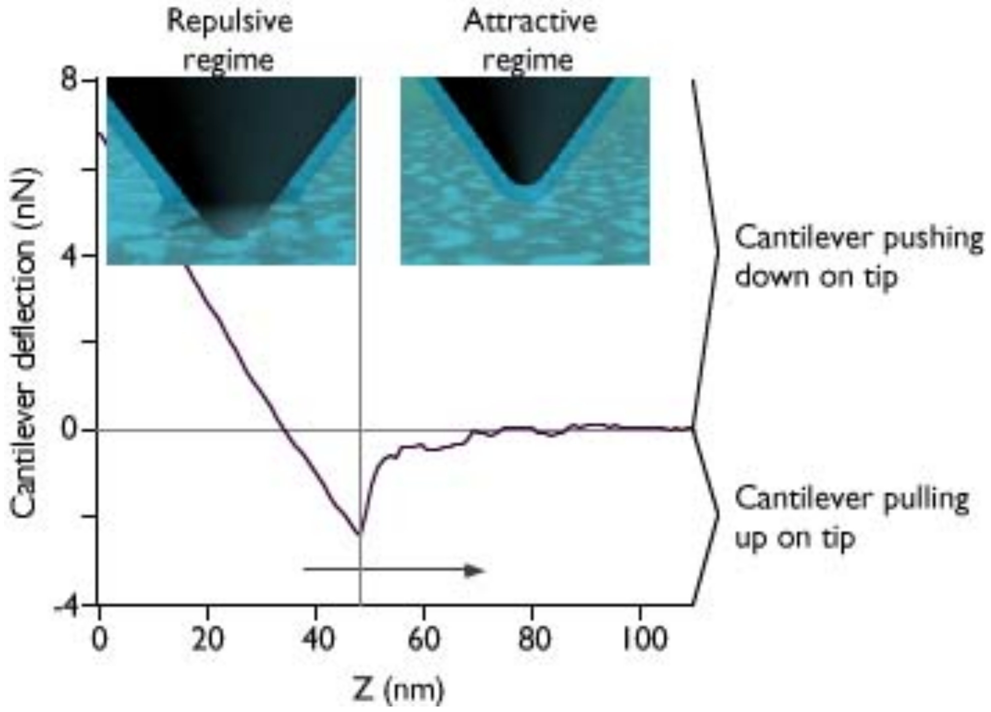


Feedback system requests



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 !

Contact mode AFM



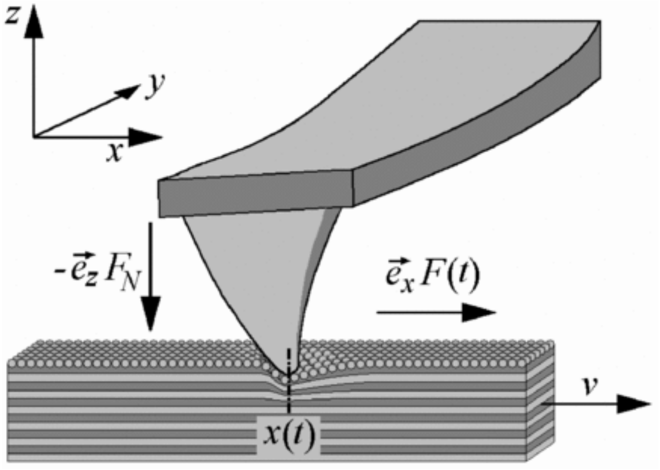
- Useful for scanning non deforming materials
- "Soft" (low force constant) cantilevers are more sensitive
- Applicable for operation in liquids without complication

Problems:

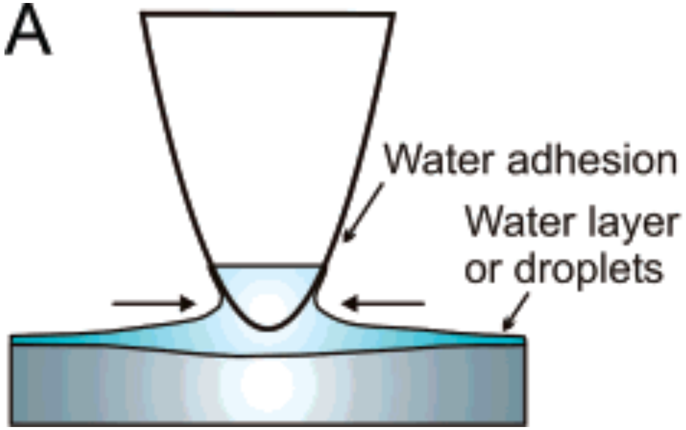
1) Frictional forces
2) Water mesiscus in air: adhesion forces
3) Dragging forces, important for biological samples which are usually loosely bound and easily damageable

AFM Contact mode-associated imaging modes

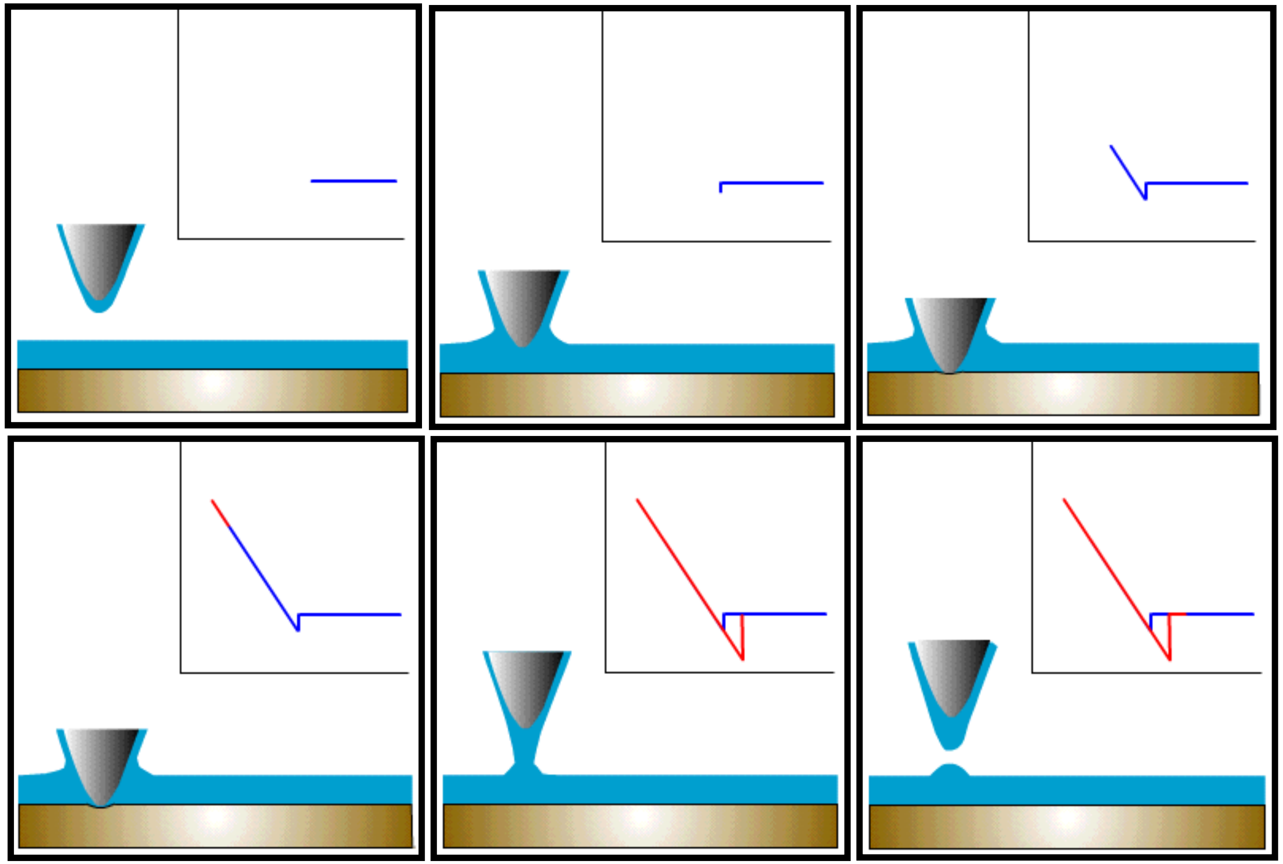
Friction:
The cantilever bends laterally due to a friction force between the tip and the sample surfaces.



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.

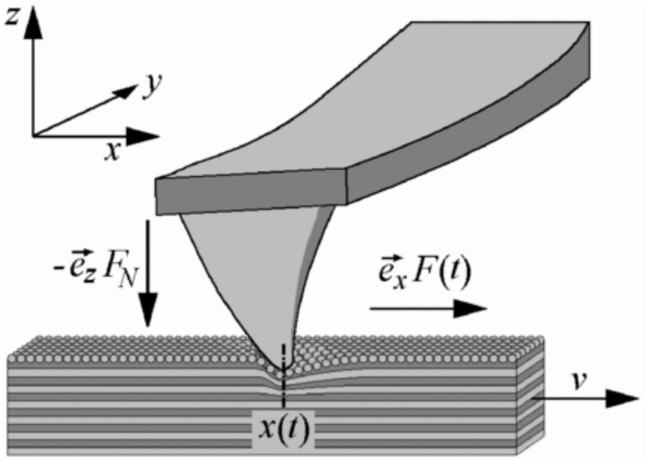


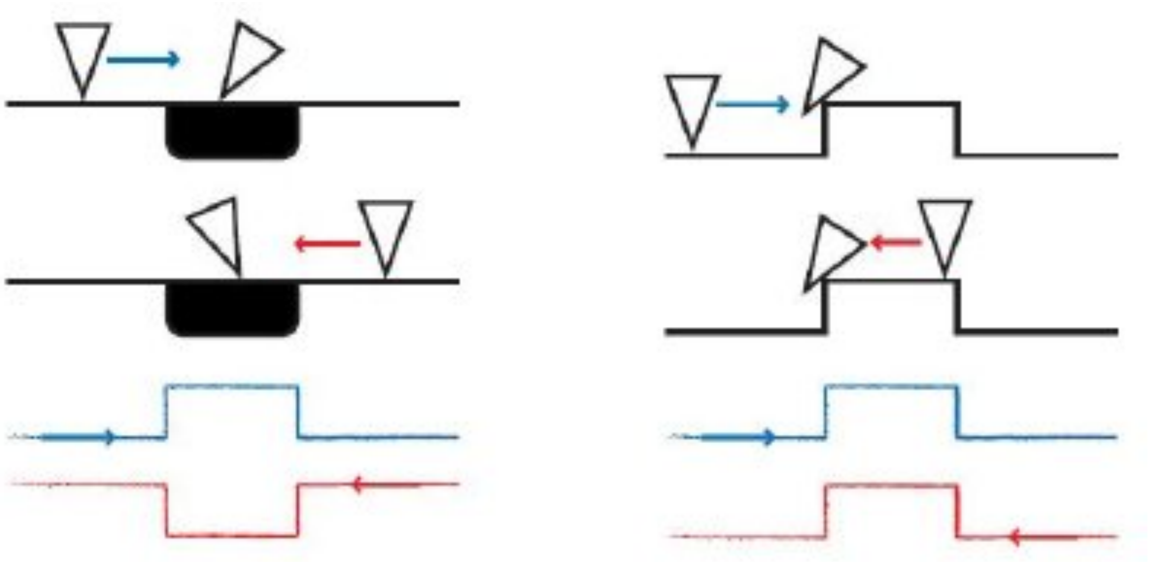
Water meniscus



Lateral Force Microscopy

sample friction

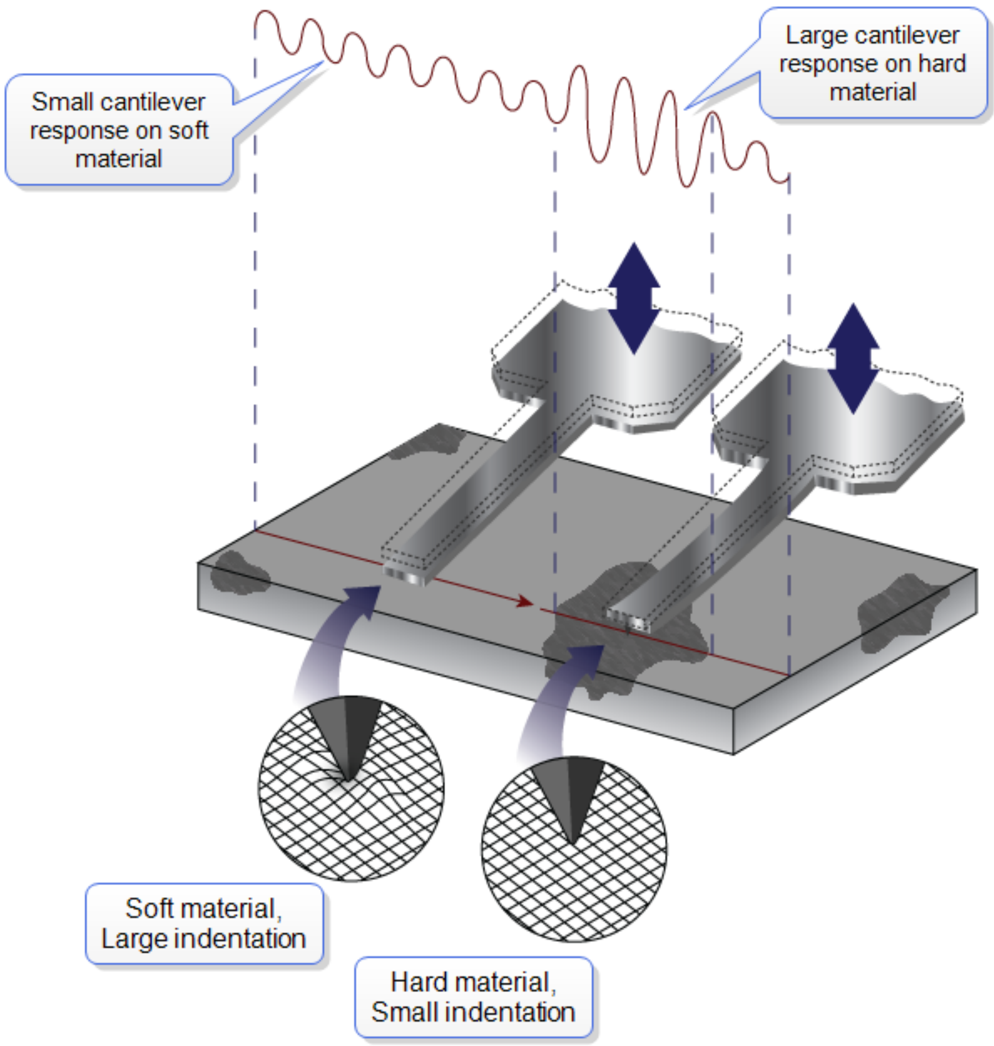




Differences between lateral forces caused by friction and the ones caused by topographic features of the scanned surface. (Left) Mirroring of lateral deflection due to frictional forces. (Right) No mirroring with topographically induced lateral deflection. All forward scan traces are in blue, backward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

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.

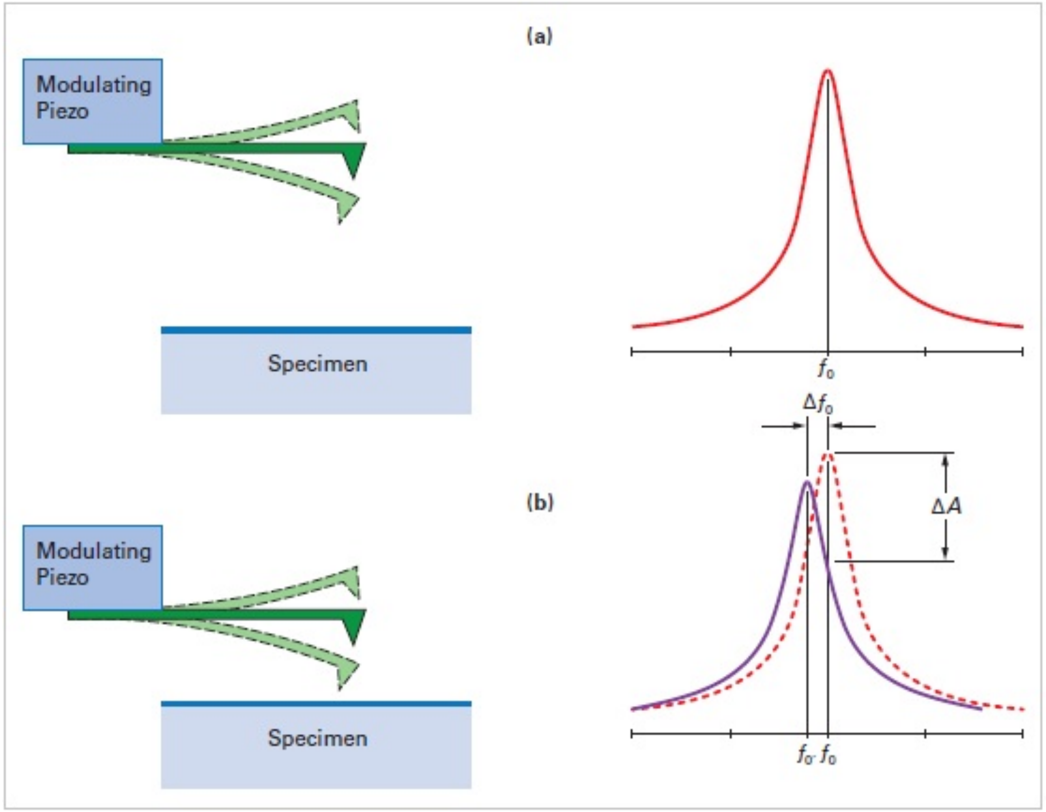
- From the changes in the amplitude of cantilever modulation, the system generates a force modulation image → a map of the sample's elastic properties.

Contact mode AFM

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

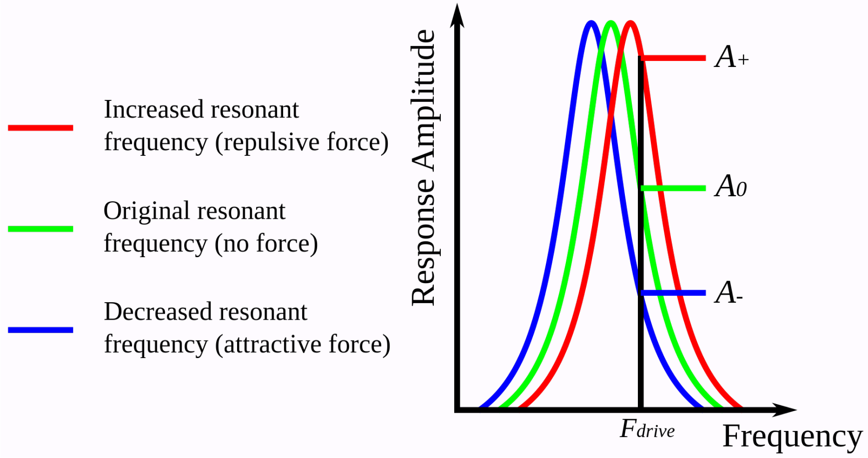
- damage induced
- molecular dragging (single molecules)

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.

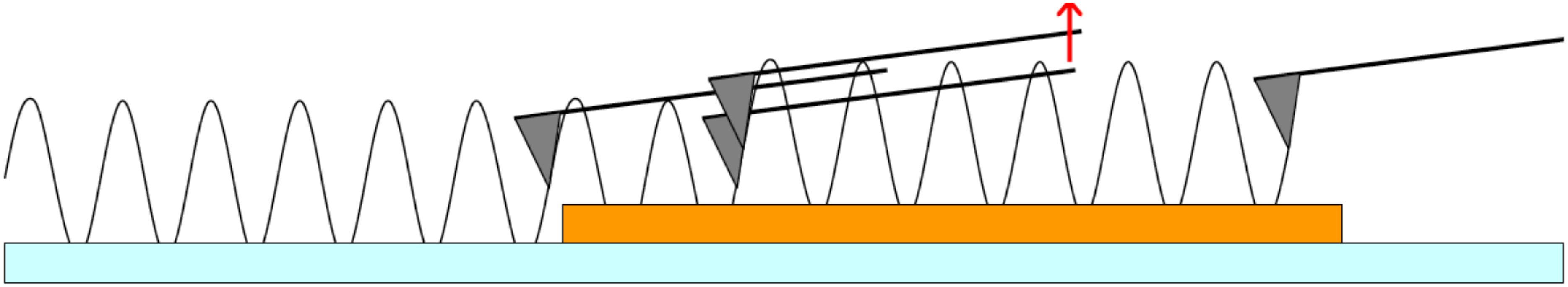
Frequency or amplitude are used as feedback parameter

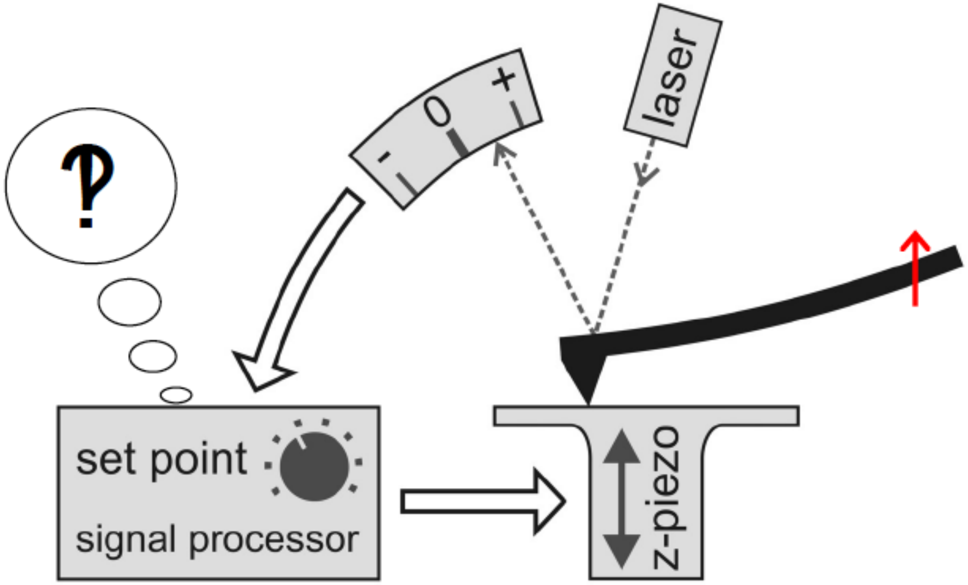


$f_0=(k_{eff}/m)^{1/2}$

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

amplitude modulation imaging





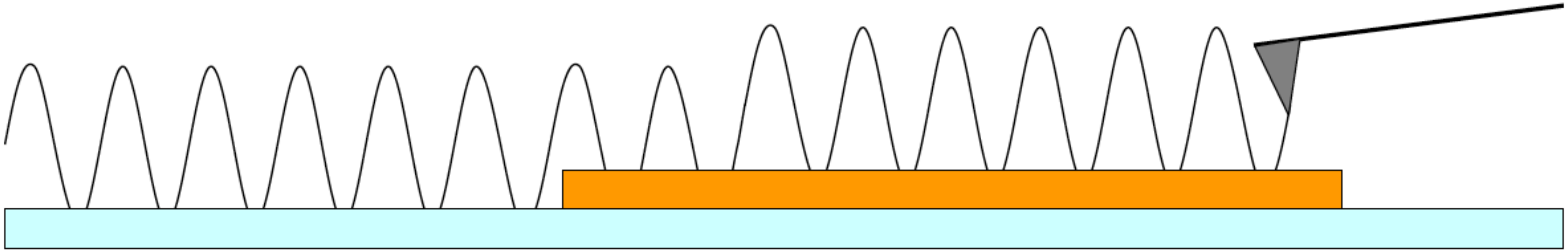
adjust piezo height (z) to keep amplitude constant

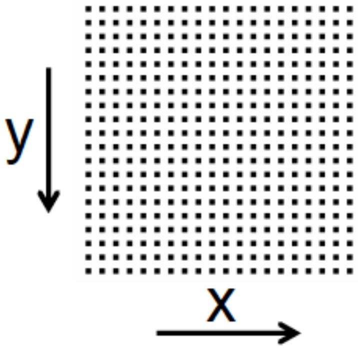
piezo motion gives height info

peak force at height changes

lower lateral/drag forces

Courtesy of I. Schaap



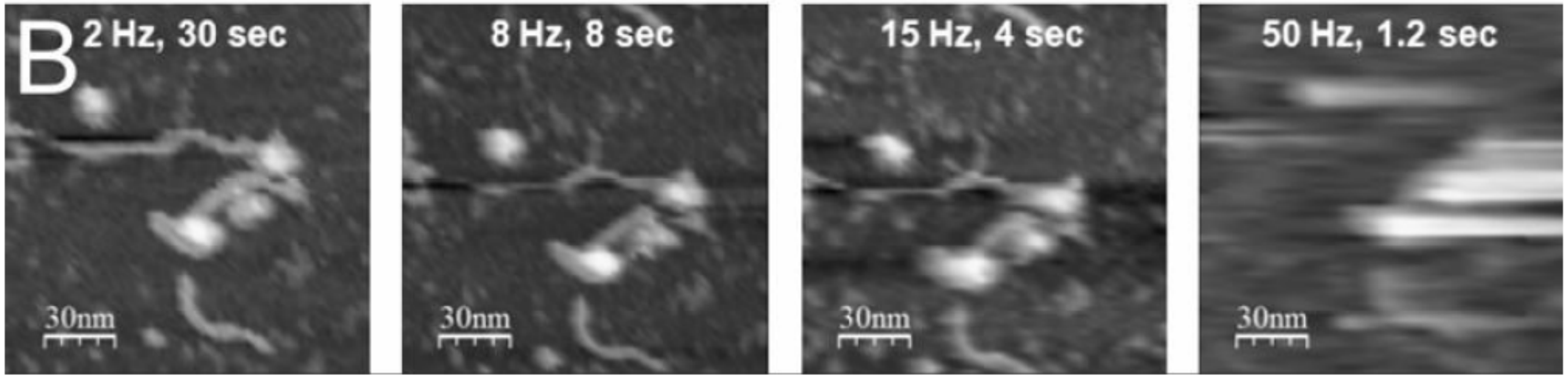


64 x 64 = 4096 px

need a few oscillations per pixel (~5)

fast bio-cantilever ~ 25 kHz

→ 0.04 ms * 5 * 4096 = 1 s

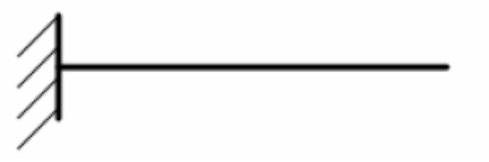


(there are other limiting factors (z-piezo, feed-back loop)

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

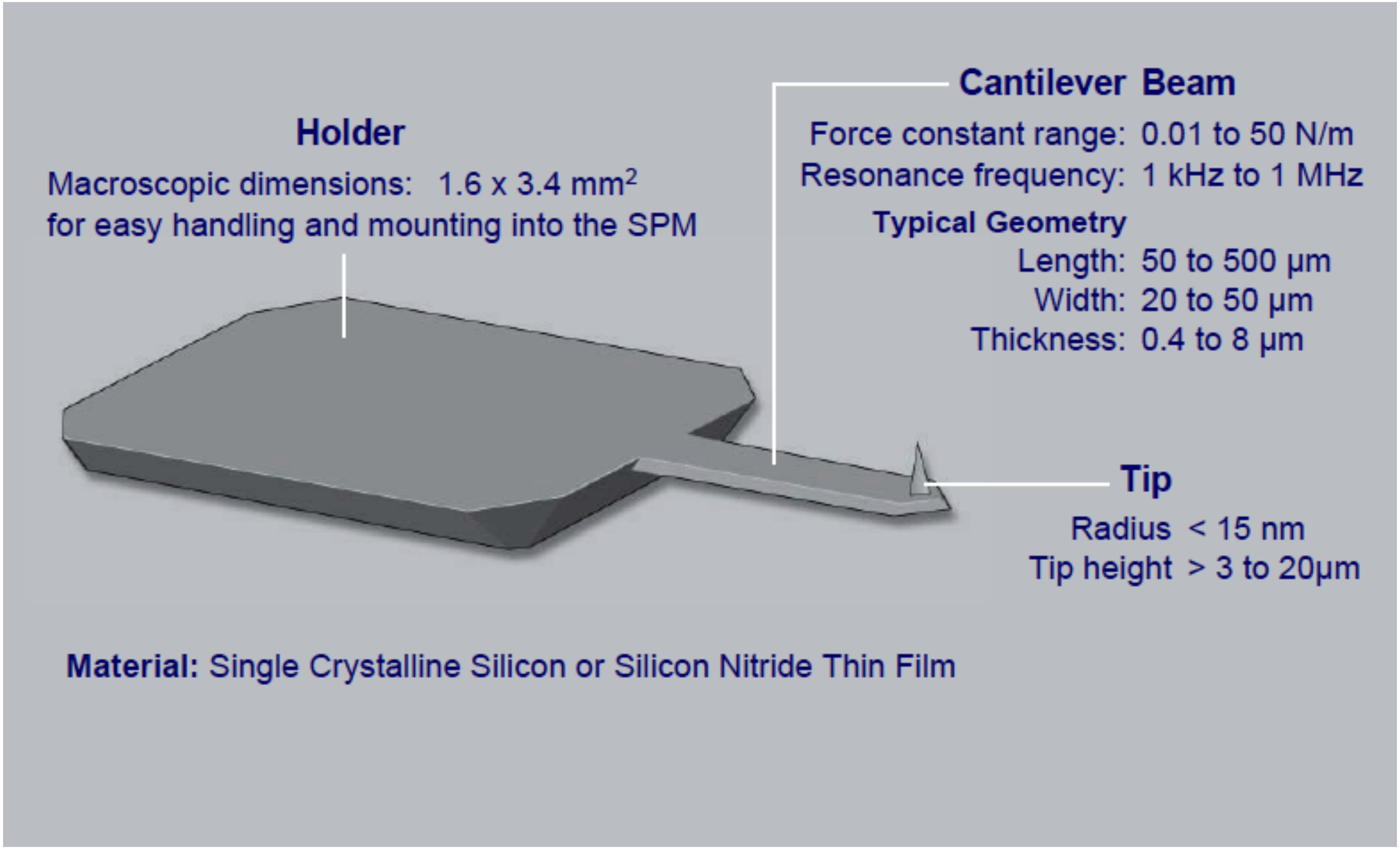


$$\omega_0 = \sqrt{k/m} = \sqrt{\frac{Et^2}{l^4 \rho}}$$

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

make cantilevers short to increase ω_0 and thinner to restore k

AFM probes



Holder

Macroscopic dimensions: 1.6 x 3.4 mm^2
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

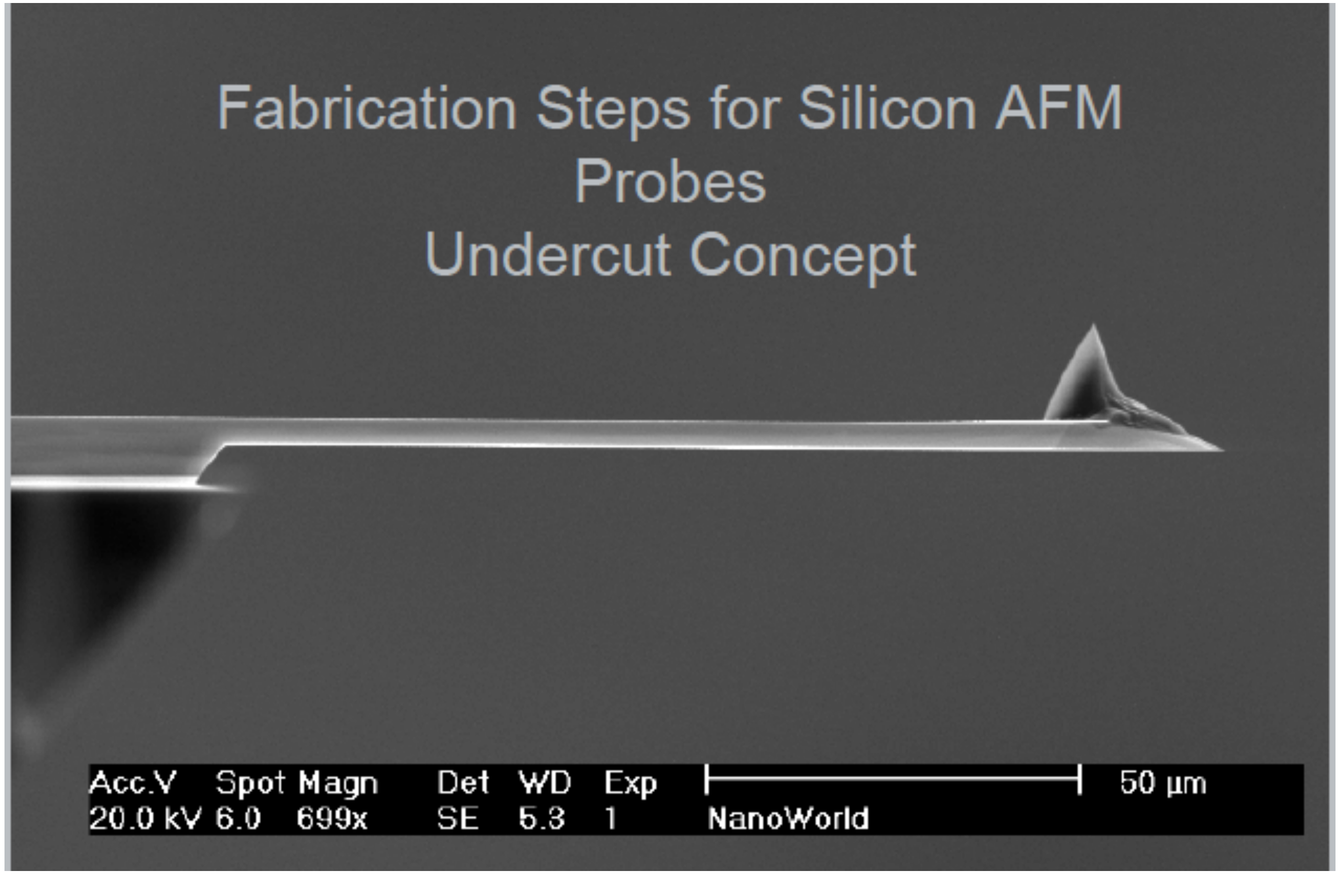
Length: 50 to 500 μm
Width: 20 to 50 μm
Thickness: 0.4 to 8 μm

Tip

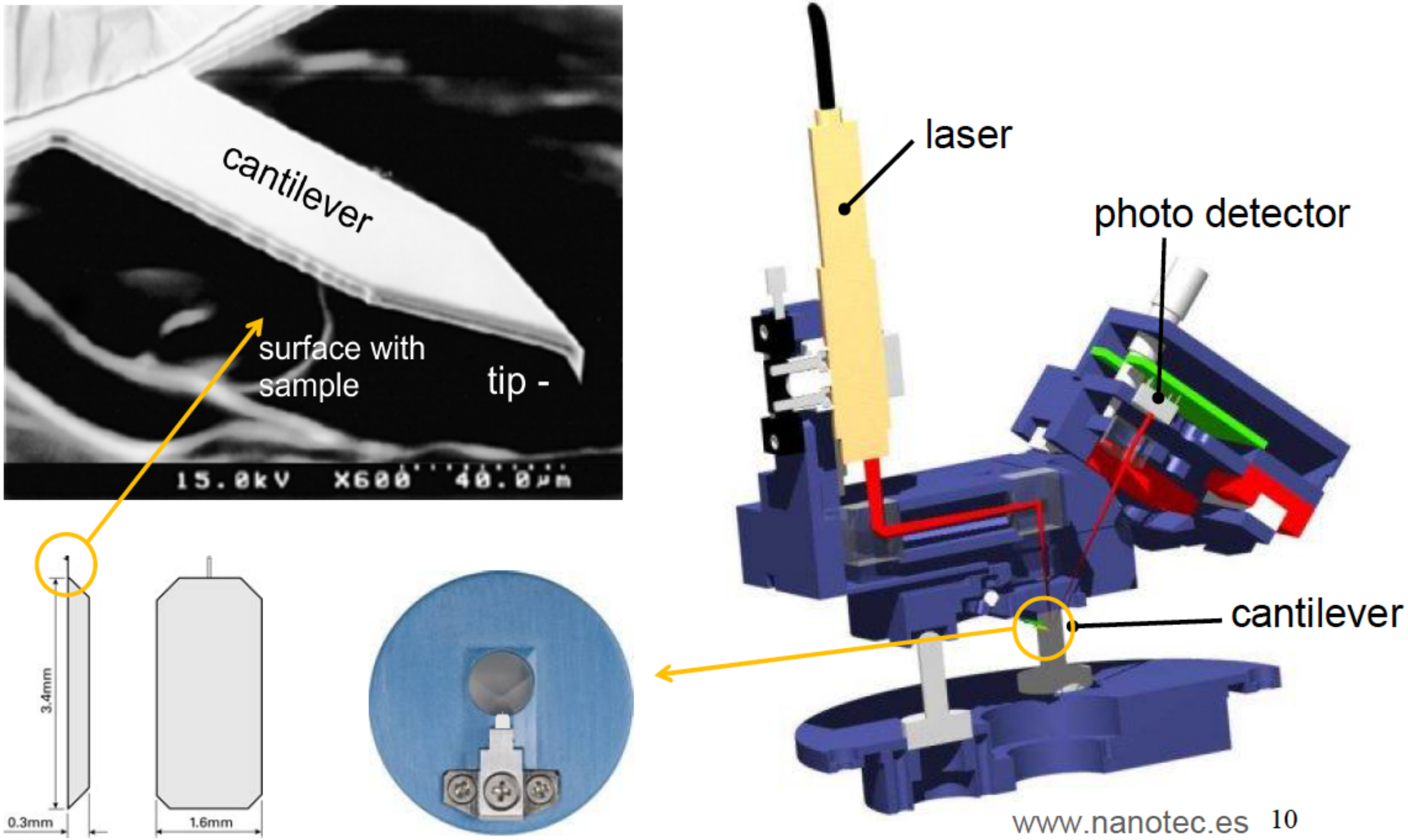
Radius < 15 nm
Tip height > 3 to 20μm

Material: Single Crystalline Silicon or Silicon Nitride Thin Film

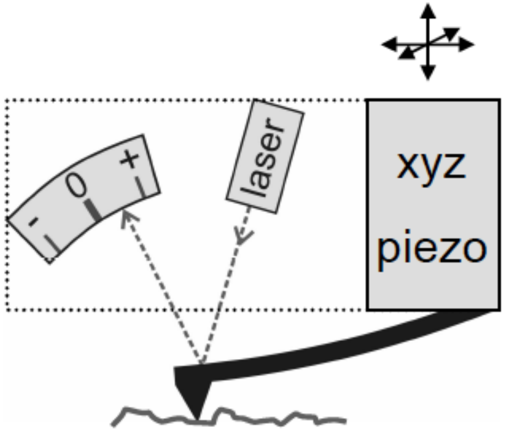
AFM probes

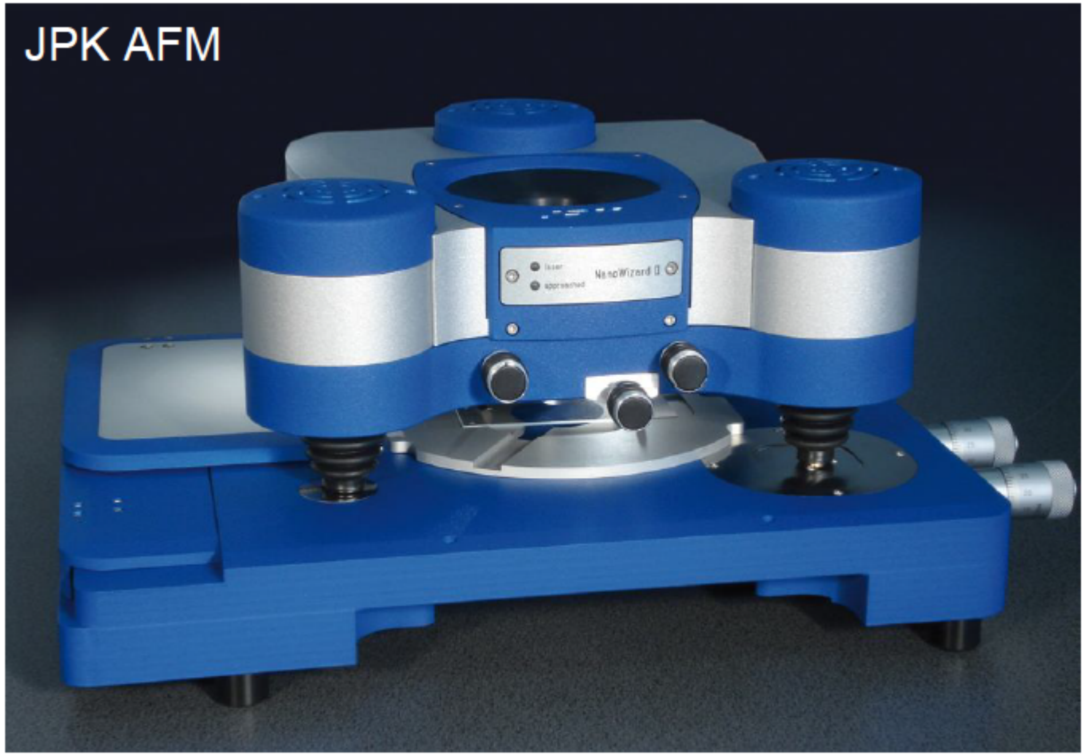


SEE NANO WORLD SLIDES


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

top xyz scanner



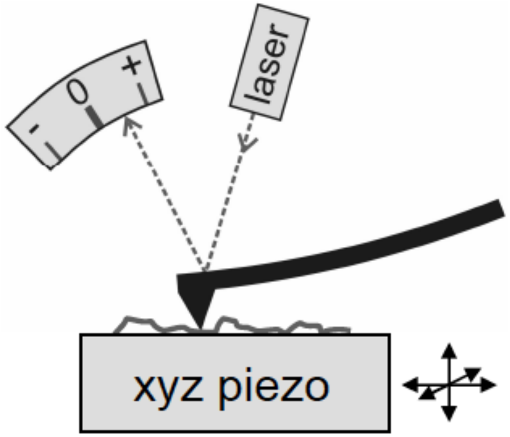


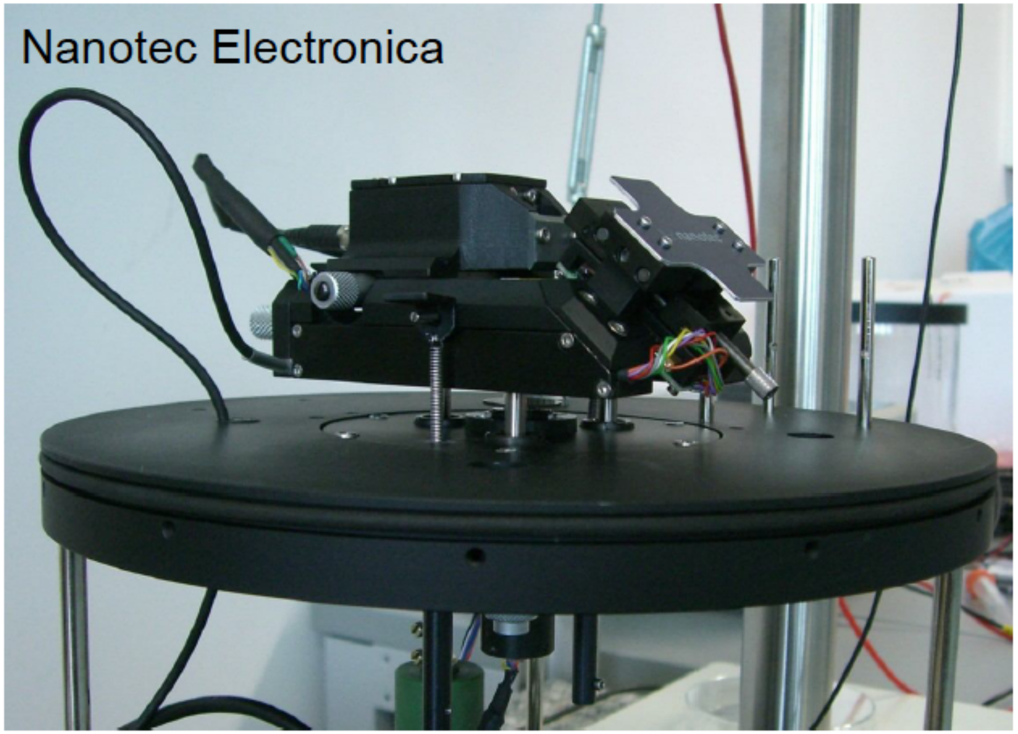
complicated design, not so fast

optical access from below

Courtesy of Iwan Schaap

base xyz scanner





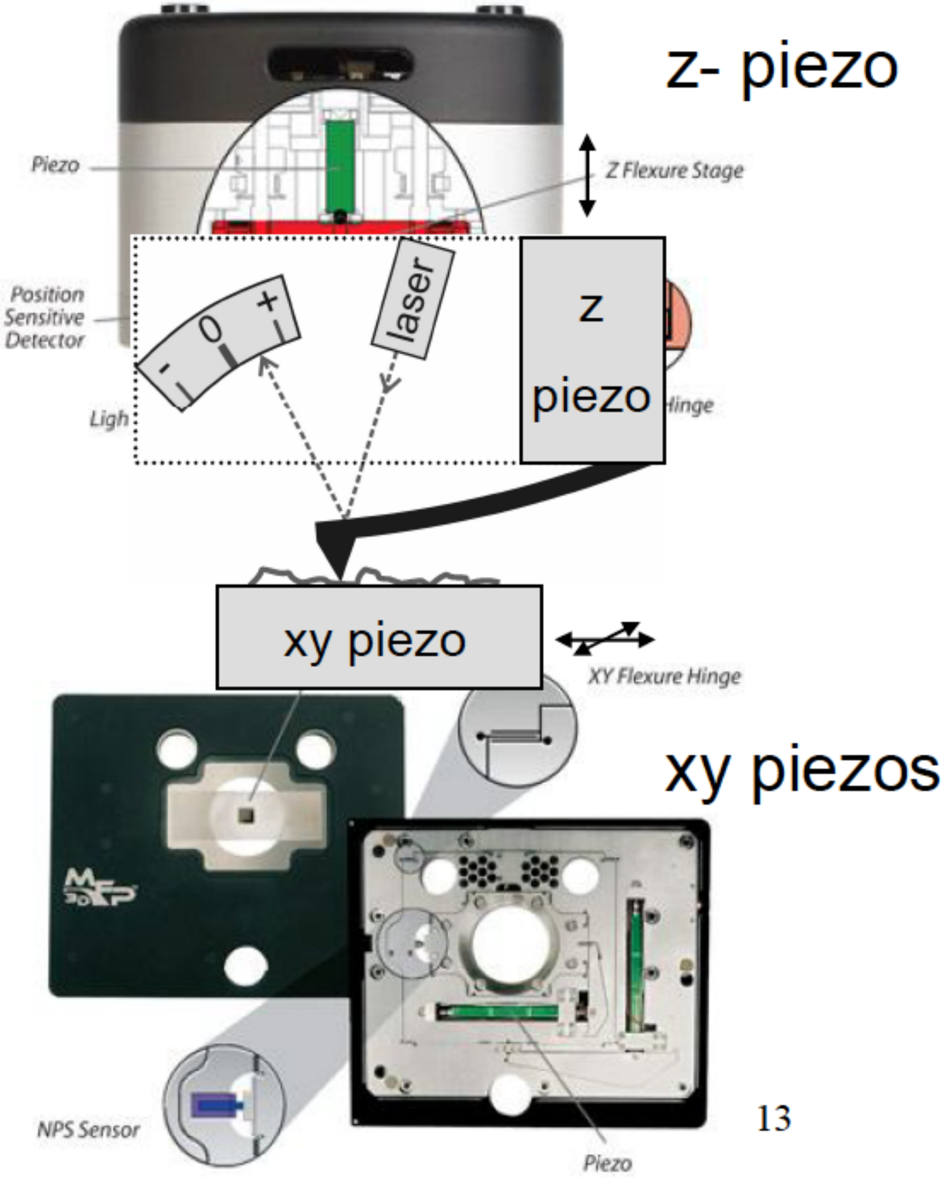
less complex and faster, atomic resolution

limited optical access

Courtesy of Iwan Schaap

separated xy and z scanner

Asylum Research



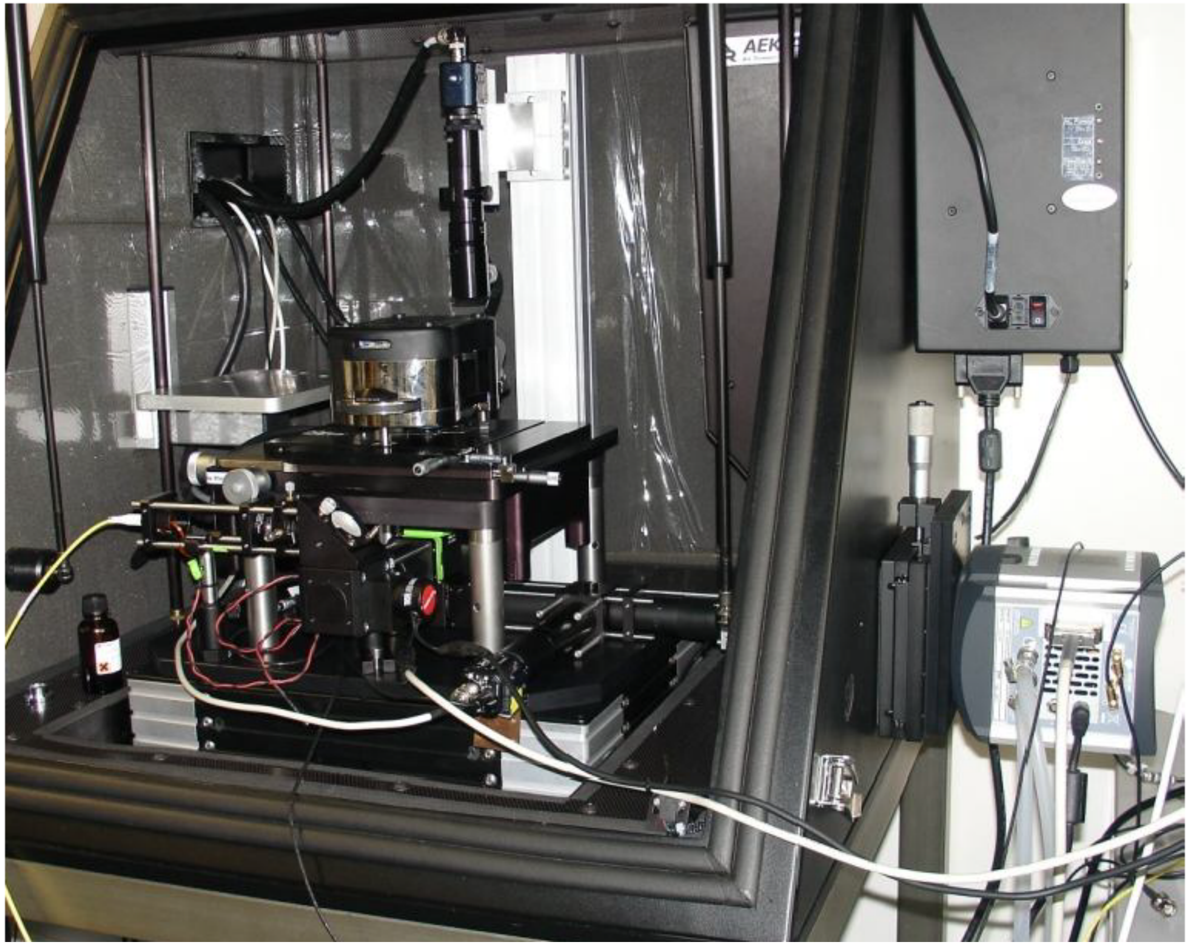
xy and z are mechanically decoupled

optical access from below

not so fast

Courtesy of Iwan Schaap

combining AFM with optical microscopy



Courtesy of Iwan Schaap

camera mechanically and thermally isolated

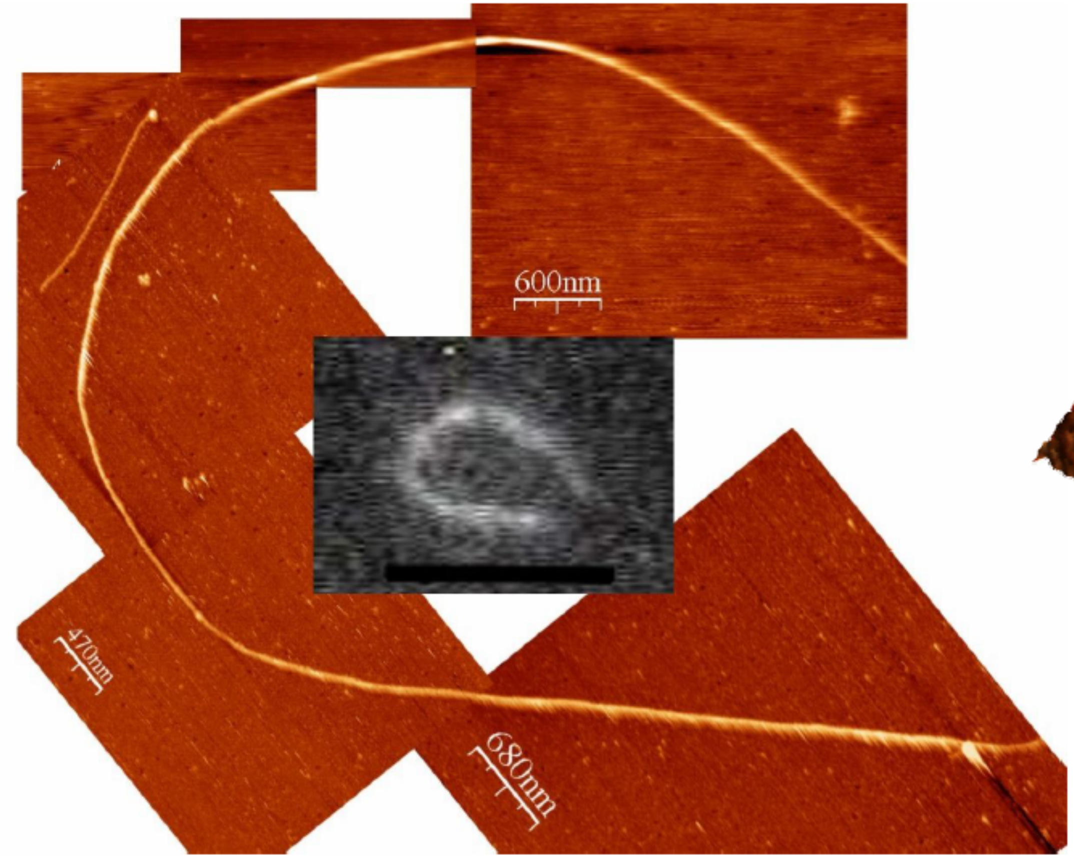
no resonance body

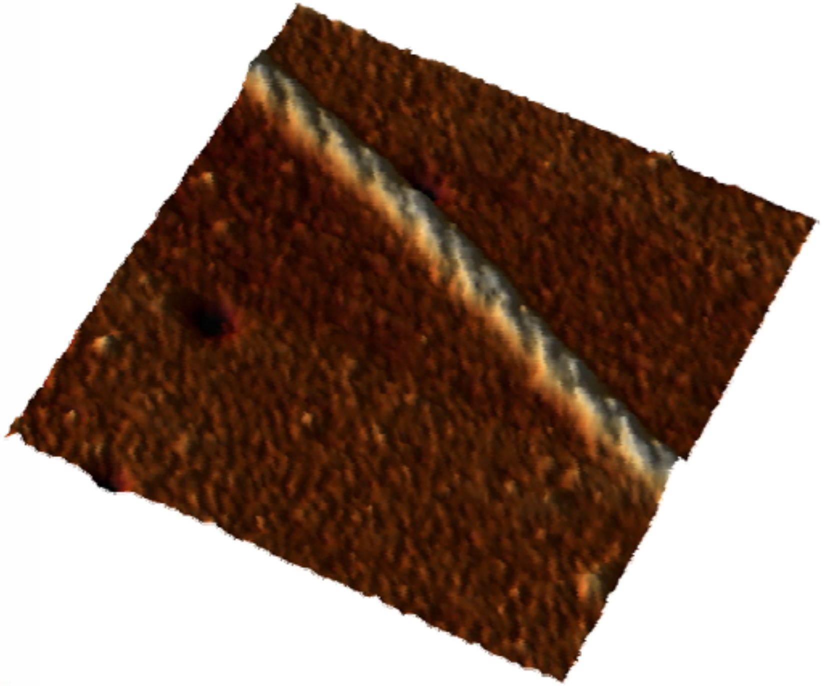
noise z_{RMS}: 0.35 nm

still worse than a simple AFM

noise z_{RMS}: 0.23 nm

combining AFM with optical microscopy





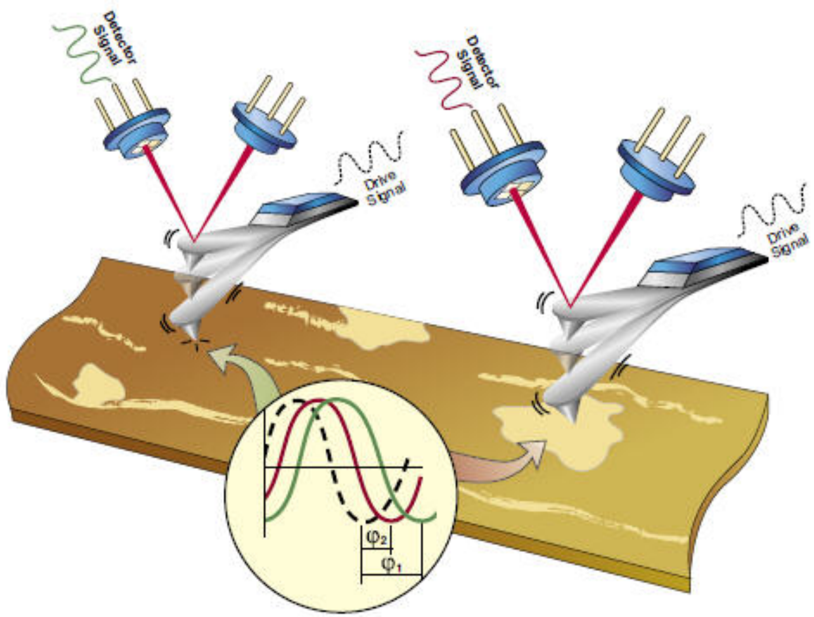
- localization
- identification

Courtesy of Iwan Schaap

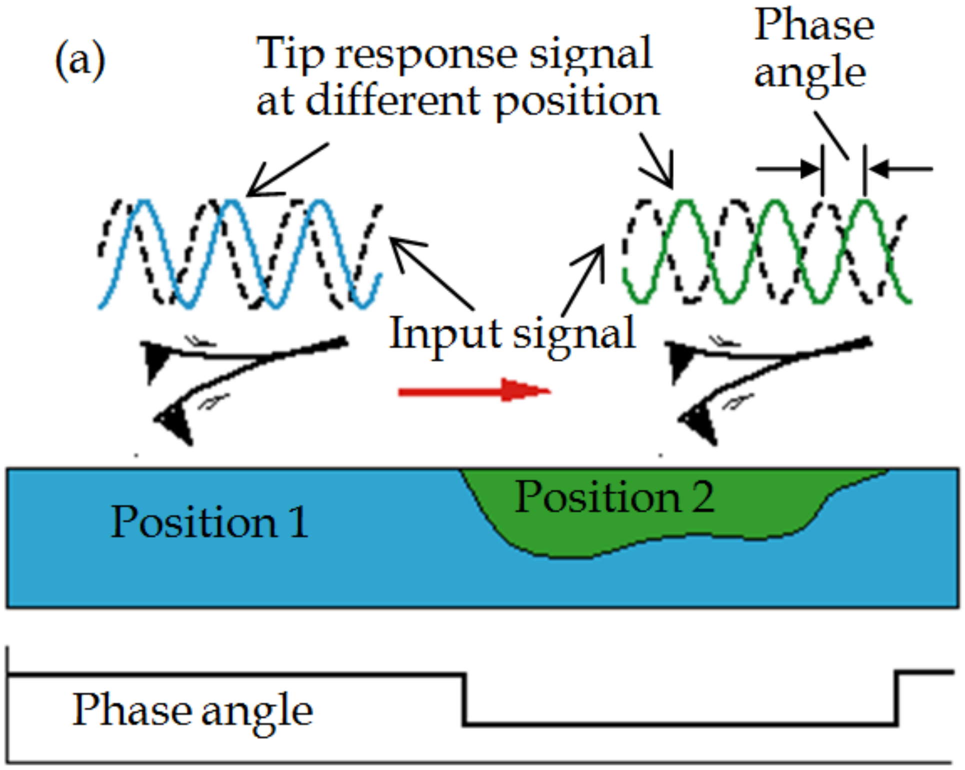
Other AFM Imaging Modes

AC mode: phase imaging

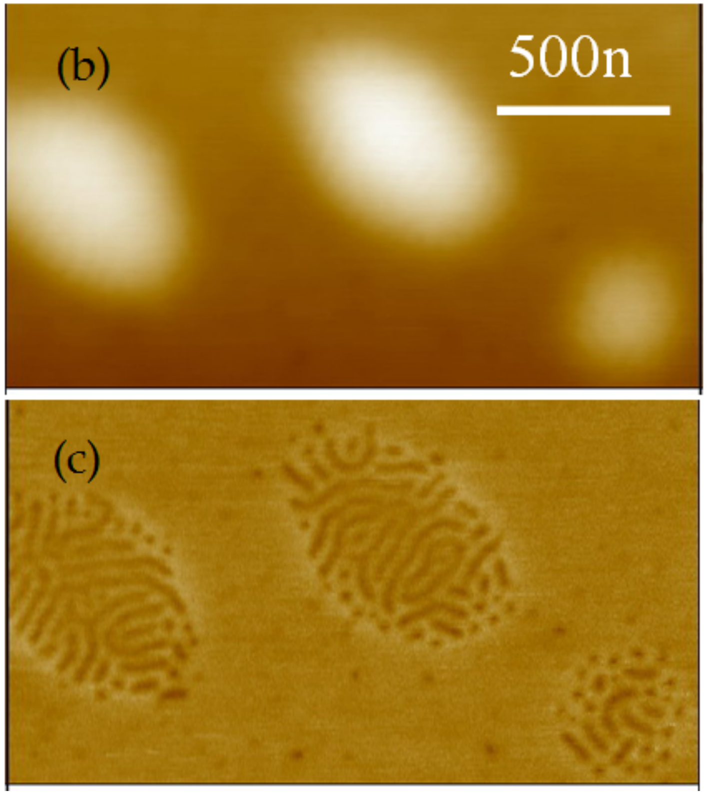
Phase imaging monitors the phase lag between the signal that drives the cantilever to oscillate and the cantilever oscillation output signal. Phase detection images can be produced while an instrument is operating in any vibrating cantilever mode.



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.



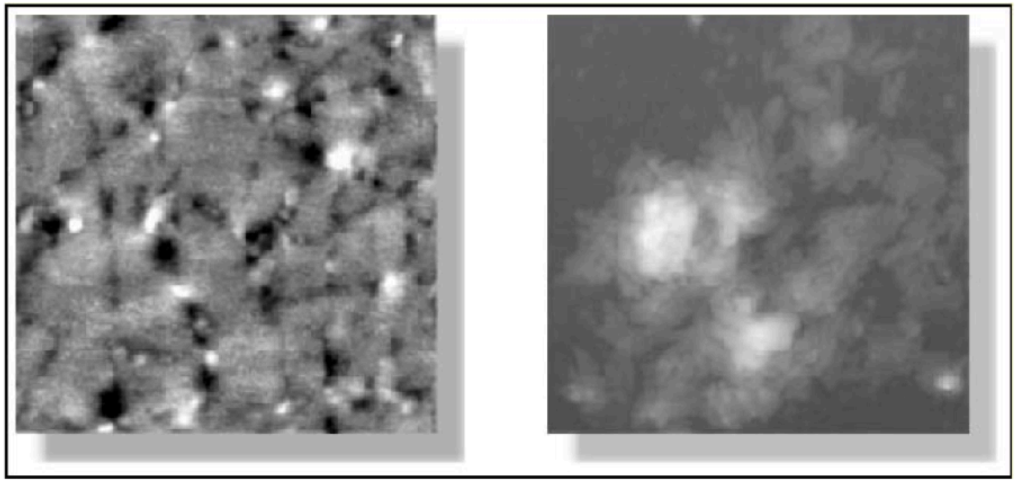
AC mode: phase imaging



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.

PLICATIONS:

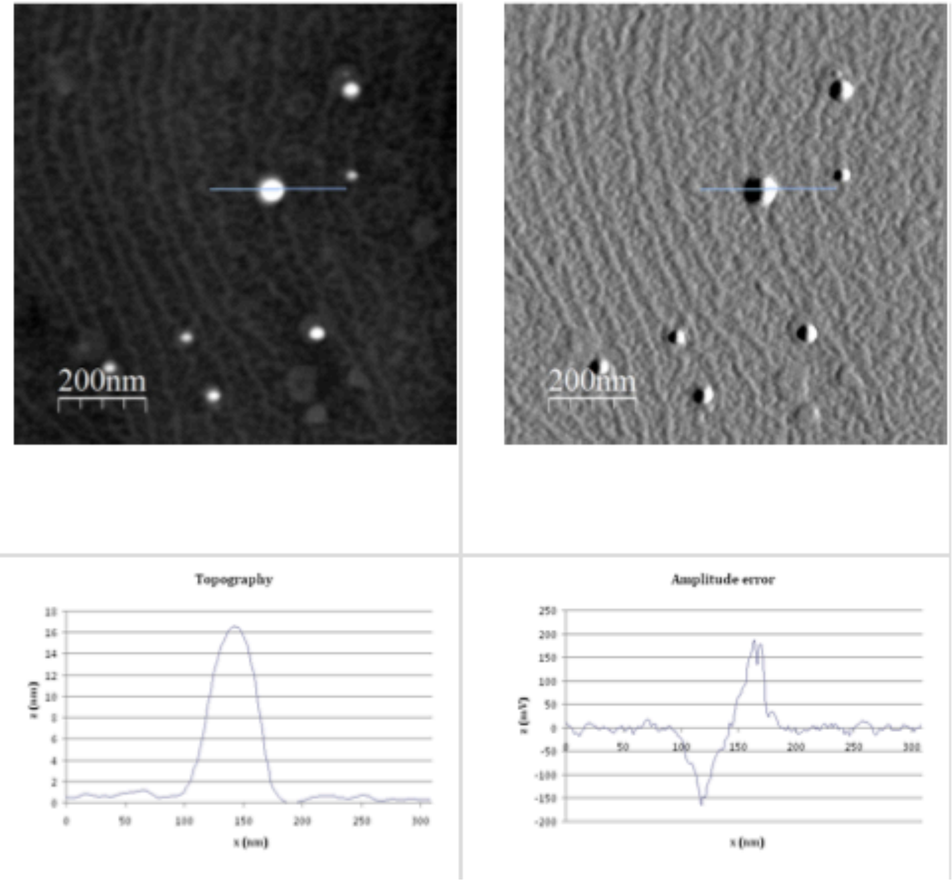
Identification of contaminants;
Mapping of different components in composite materials;
Differentiating regions of high and low surface adhesion or hardness;
Mapping of electrical and magnetic properties with wide-ranging implications in data storage and semiconductor industries.



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.

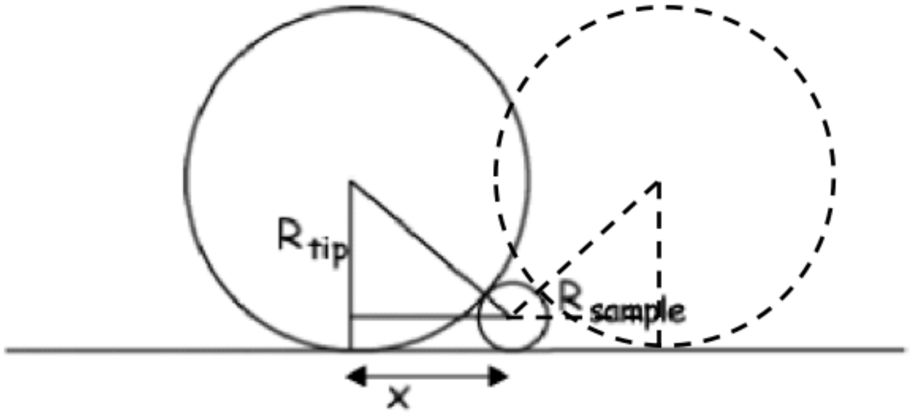


PARAMETERS AFFECTING THE IMAGING:

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

Resolution and artifacts

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

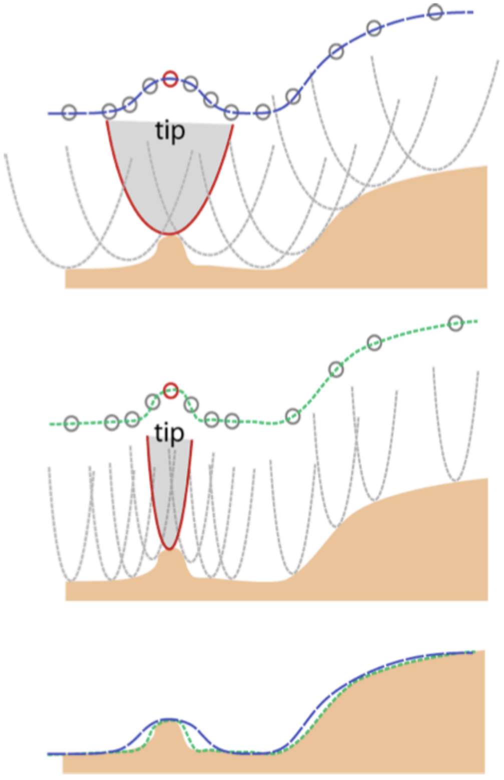


$$x^2 = (R_{tip} + R_{sample})^2 - (R_{tip} - R_{sample})^2$$

$$x^2 = \cancel{R_{tip}^2} + 2R_{tip}R_{sample} + \cancel{R_{sample}^2} - \cancel{R_{tip}^2} + 2R_{tip}R_{sample} - \cancel{R_{sample}^2}$$

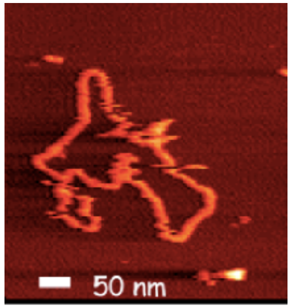
$$x = 2\sqrt{R_{tip}R_{sample}}$$

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



DNA: 2 nm.

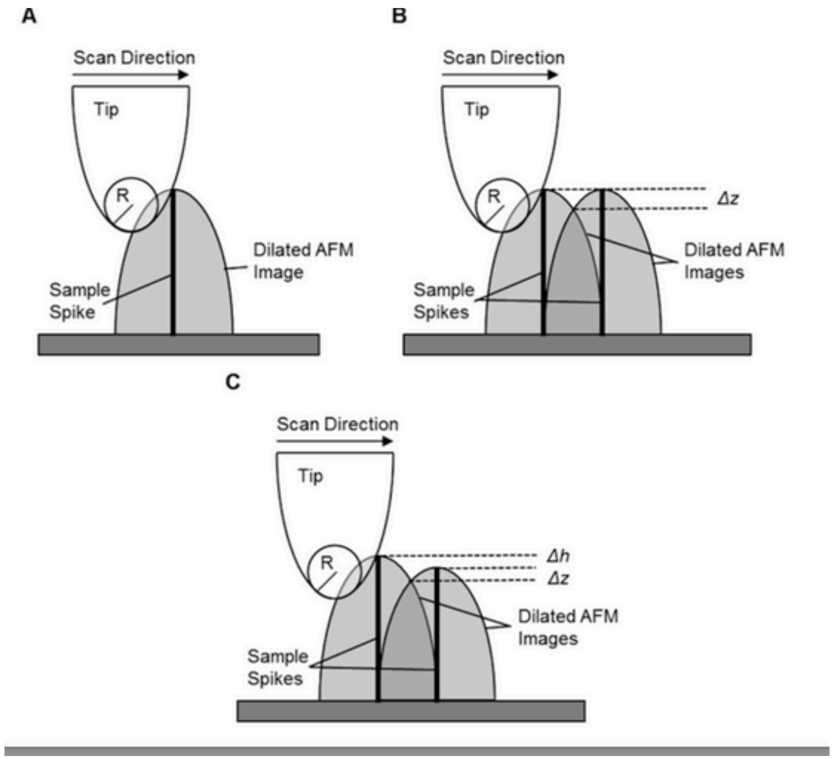
tip ~ 20 nm => w = 25 nm
tip ~ 10 nm => w = 18 nm



Resolution and artifacts

The Lateral resolution is defined as the minimum separation d for which the dimple height Δz is higher than the noise

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



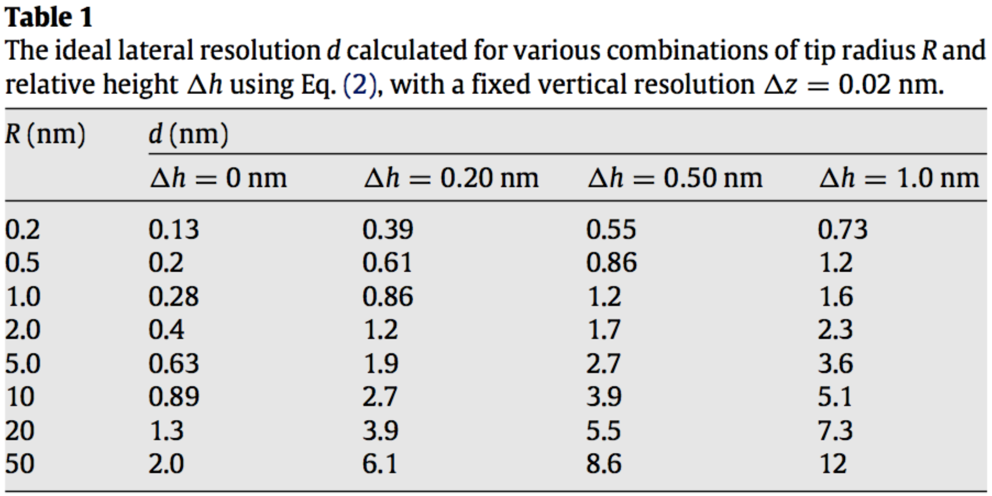


Table 1
The ideal lateral resolution d calculated for various combinations of tip radius R and relative height Δh using Eq. (2), with a fixed vertical resolution $\Delta z = 0.02$ nm.

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

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

Thermal noise in AC mode AFM

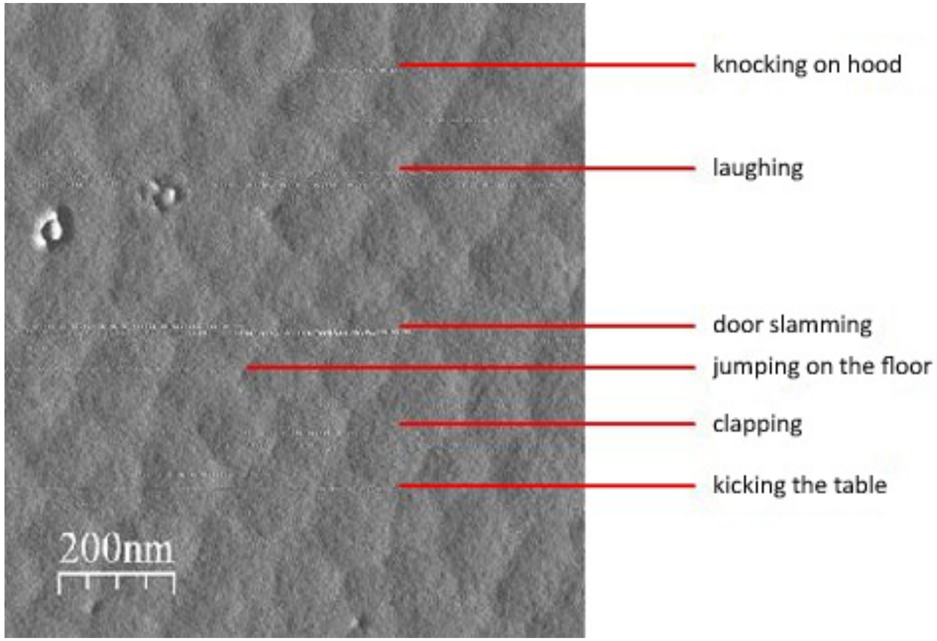
$$\Delta z = \sqrt{\frac{4k_B TQB}{\pi f_0 k}}$$

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

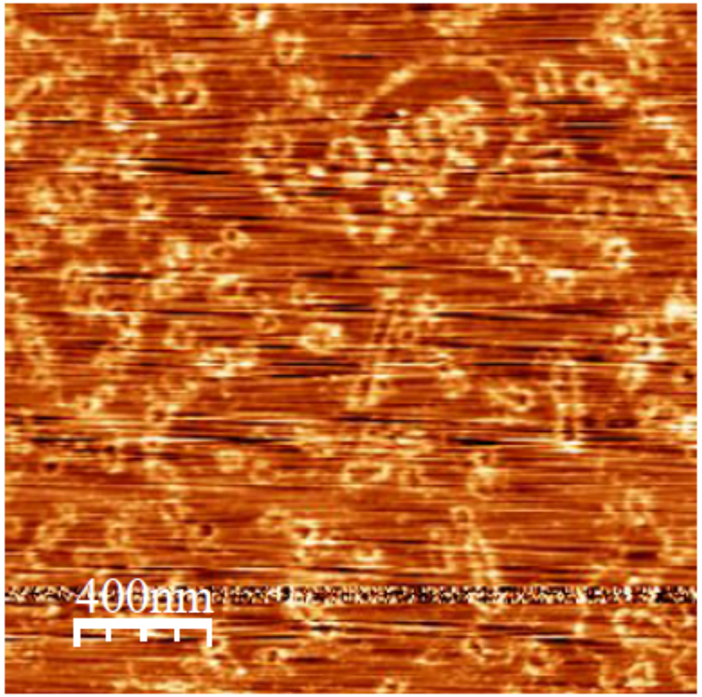
Resolution and artifacts
Vibrations



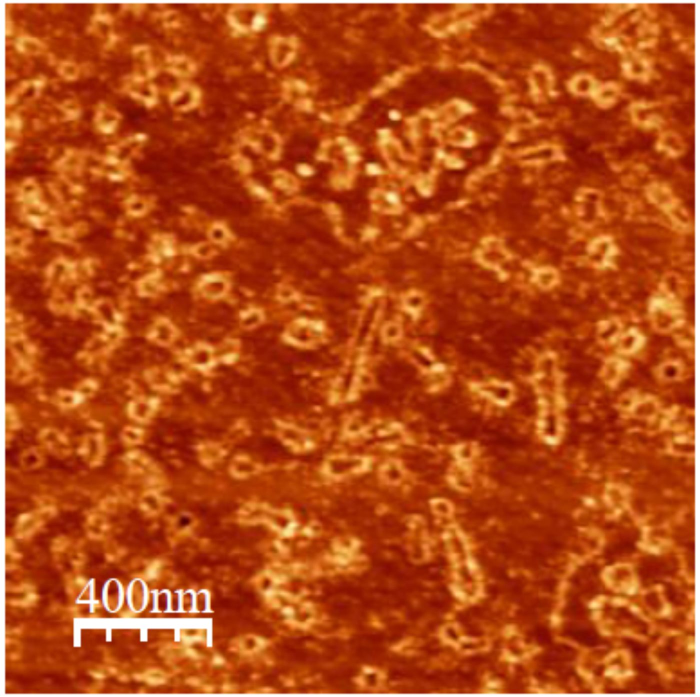
These vibrations may be transmitted through the floor, for example from footsteps or the use of a lift. These can be minimised by the use of a vibrational isolation table, and locating the AFM on a ground floor or below.

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

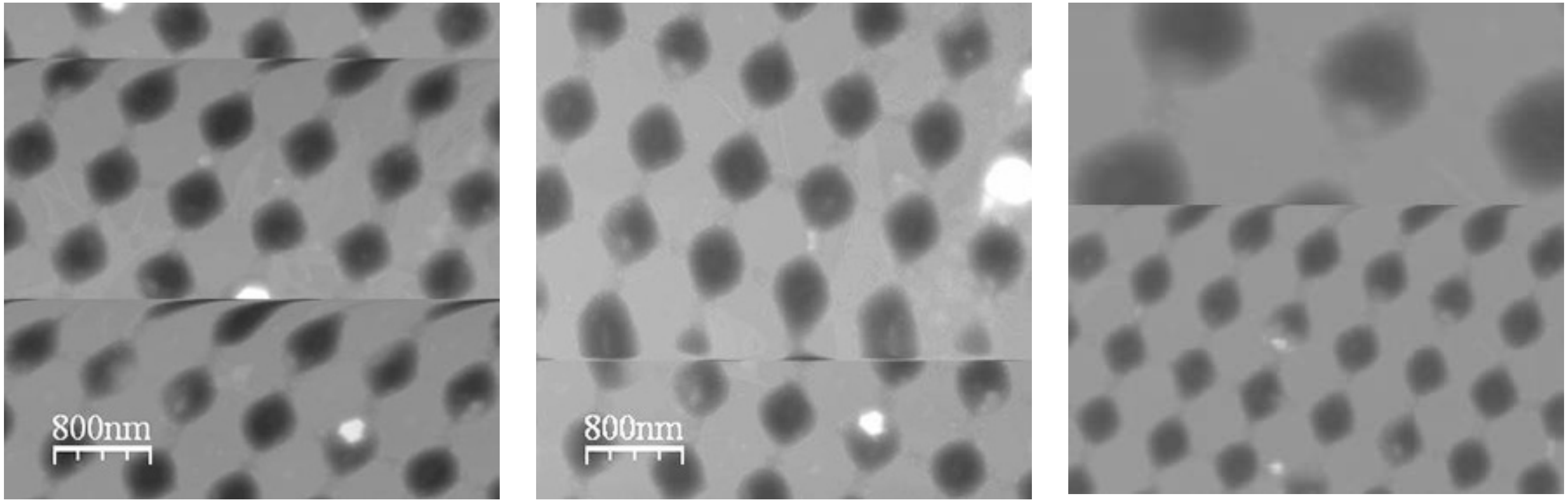


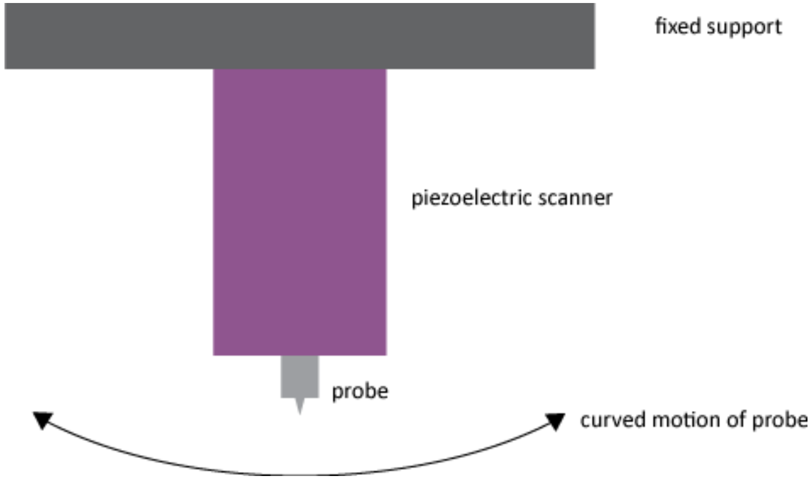
quiet room,
no vibration isolation



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

Resolution and artifacts
Scanner creep

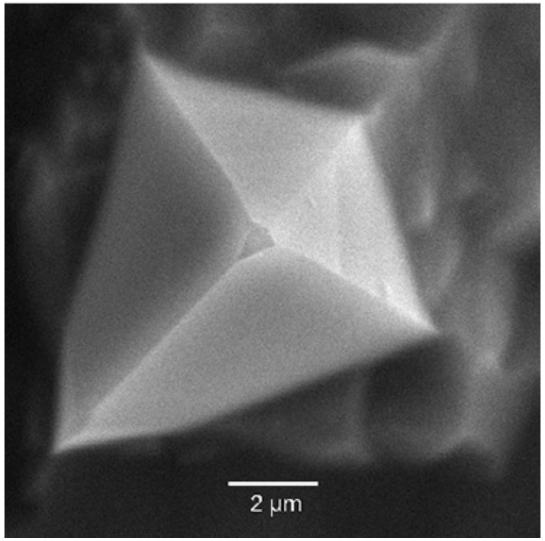


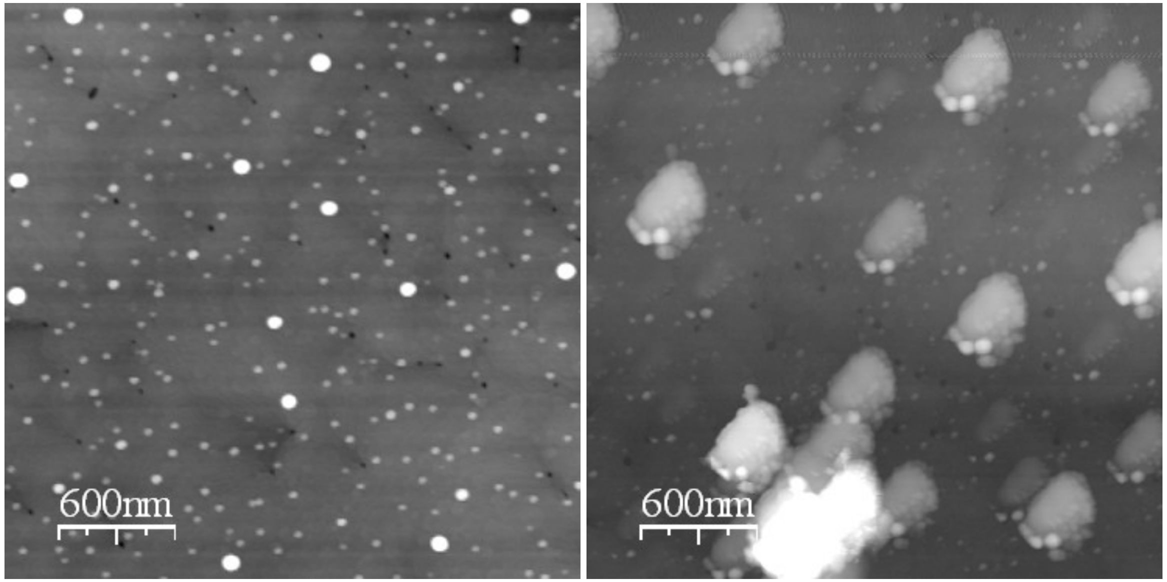


Because of the construction of the piezo-scanner, the tip does not move in a perfectly flat plane. Instead its movement is in a parabolic arc, as shown in the image below. This causes the artefact known as *scanner bow*. Also the scanner and sample planes may not be perfectly parallel, this is known 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