CEITEC

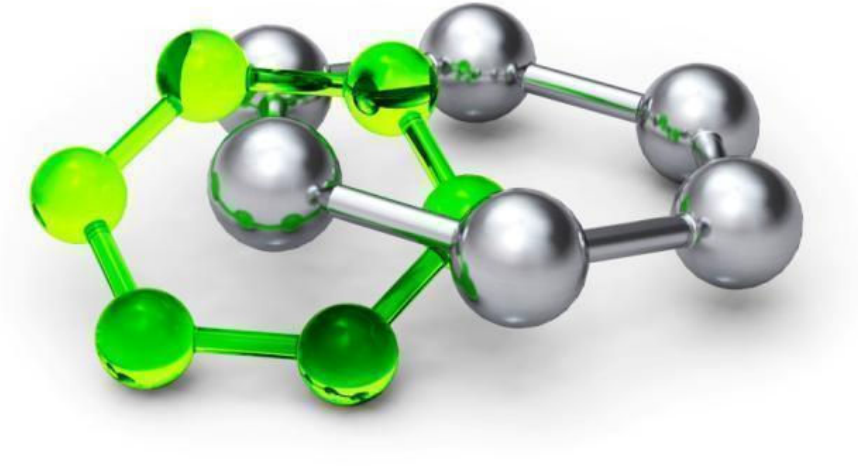
Central European Institute of Technology
BRNO | CZECH REPUBLIC

Nanobiotechnology

Scanning Probe Microscopies

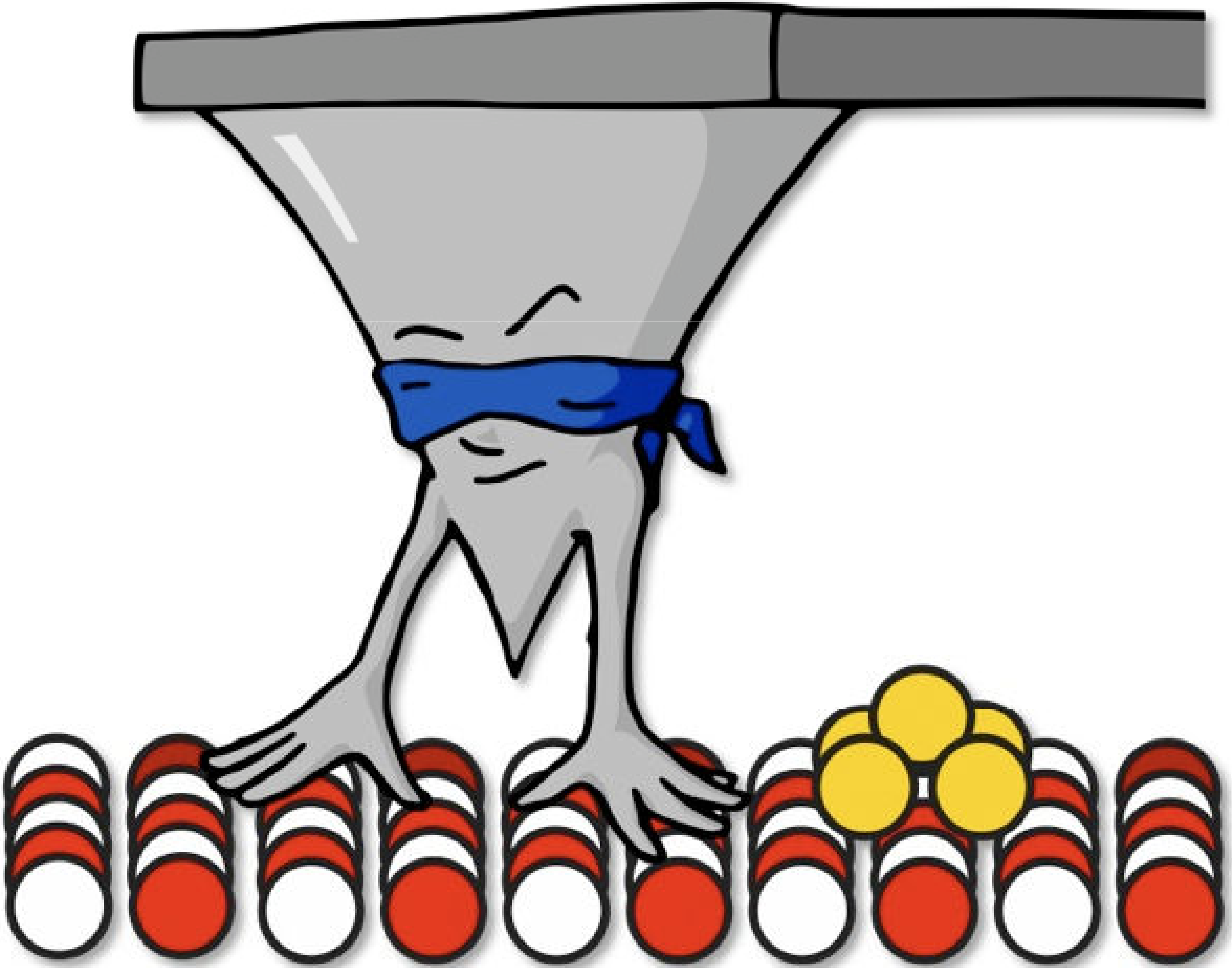
Jan Přibyl

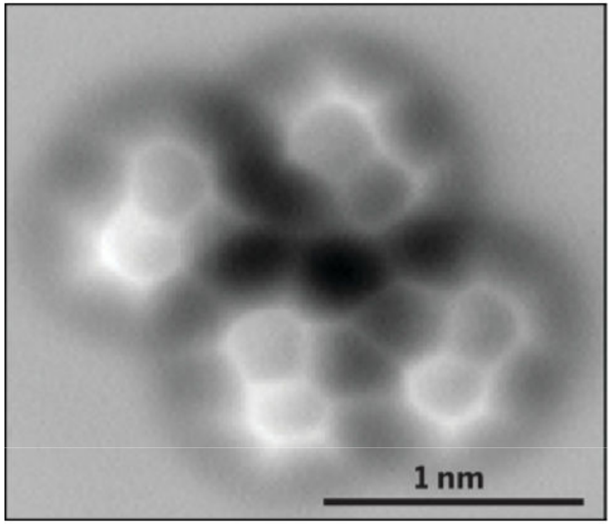
CEITEC MU
Kamenice 5/A35, CZ-62500 Brno
pribyl@nanobio.cz

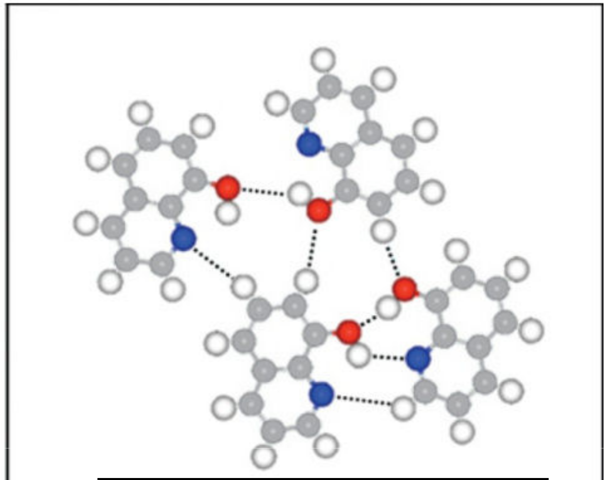


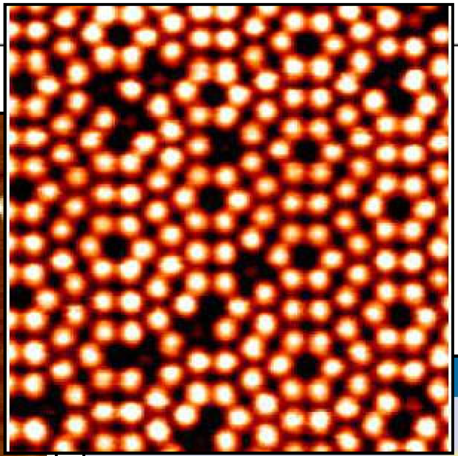
EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

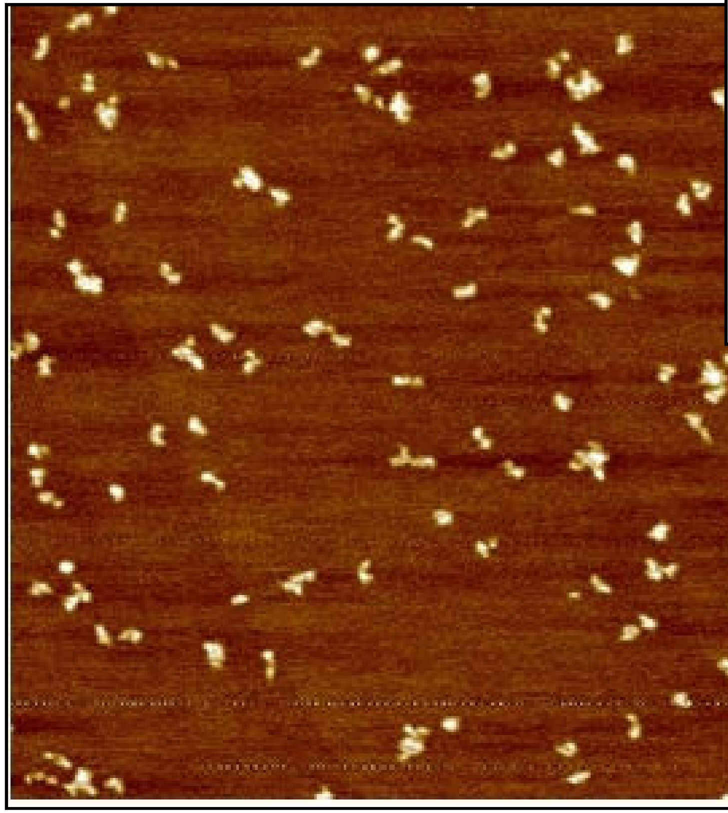
2007–13
OP Research and
Development for Innovation

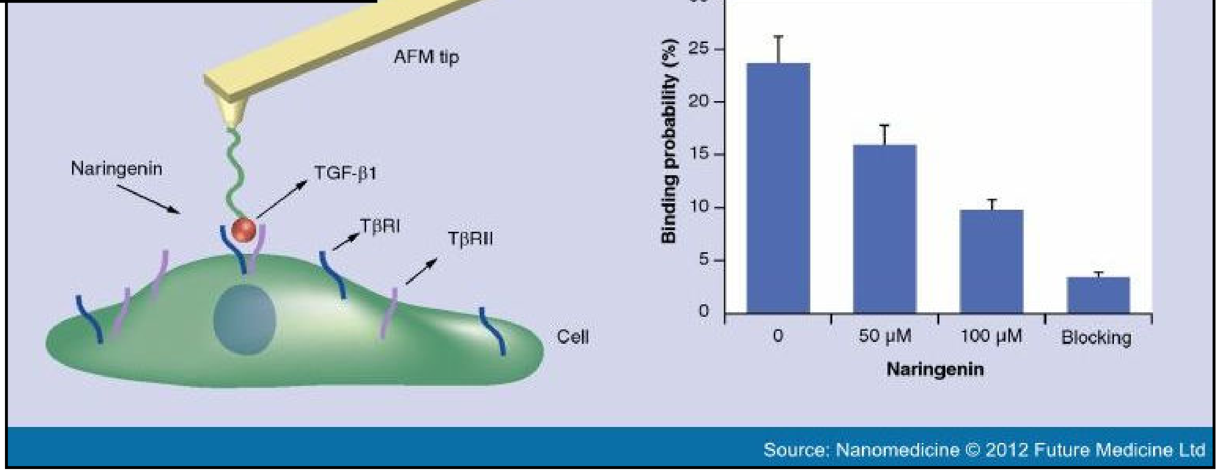






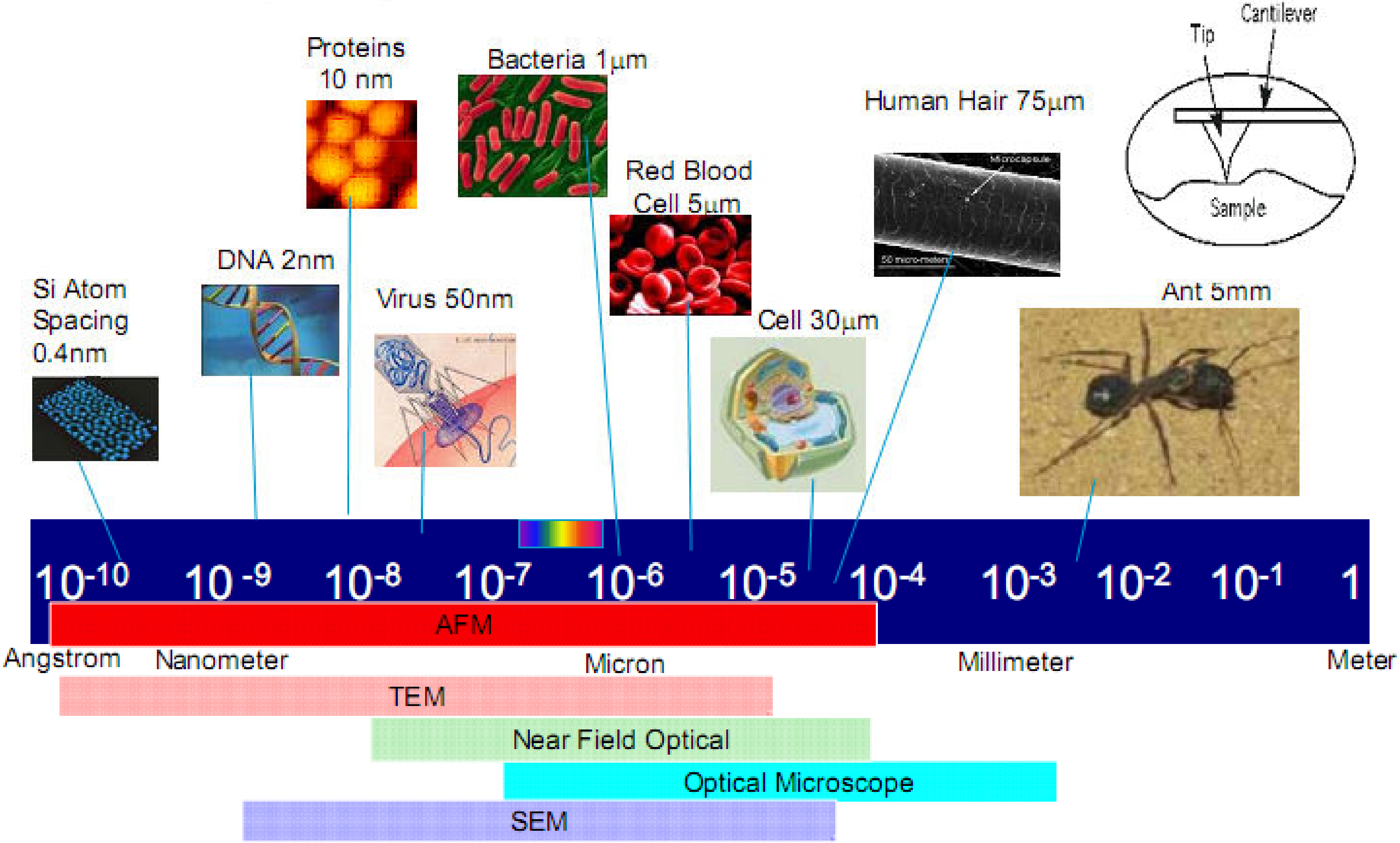






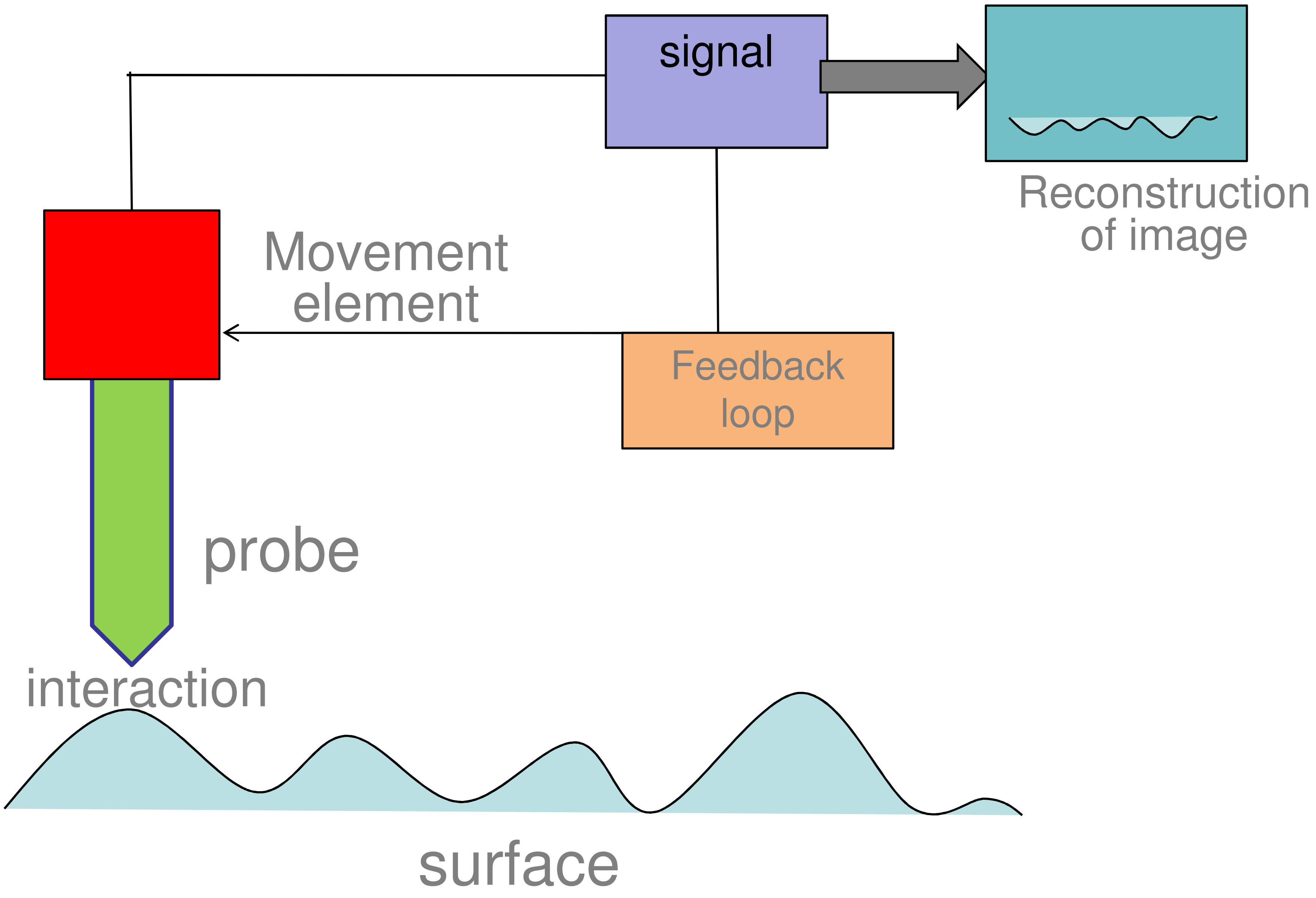
Source: Nanomedicine © 2012 Future Medicine Ltd

Microscopy techniques - resolution

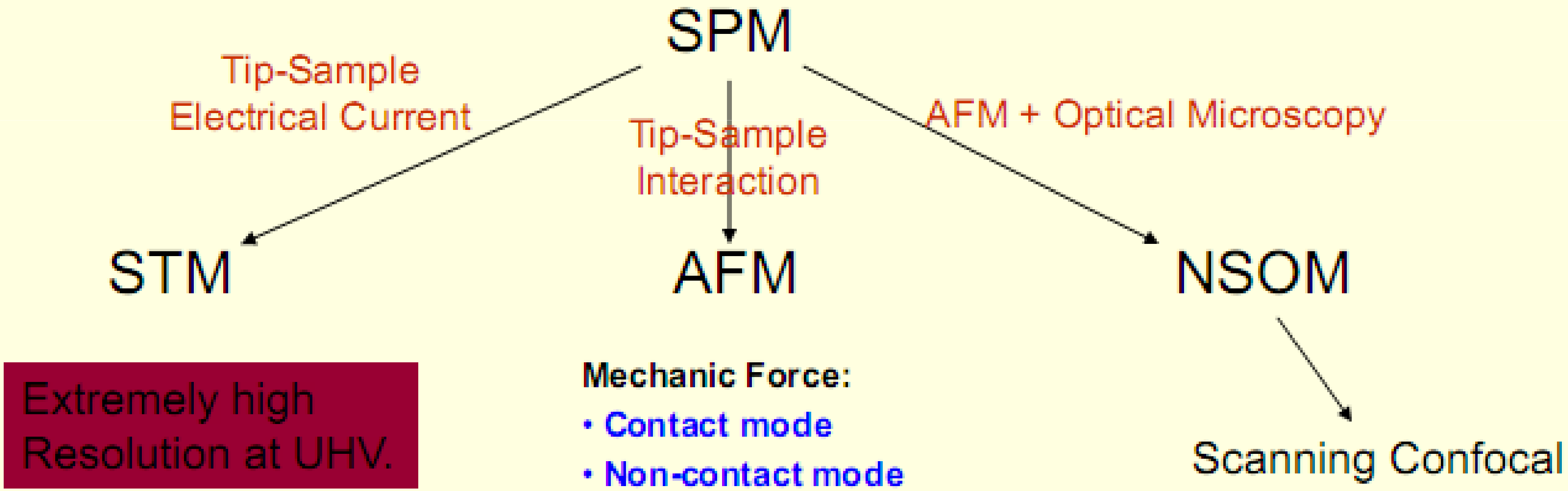


Scanning Probe Microscope

basic scheme



SPM Family



SPM

Tip-Sample Electrical Current

Tip-Sample Interaction

AFM + Optical Microscopy

STM

AFM

NSOM

Extremely high Resolution at UHV.

Scanning Confocal

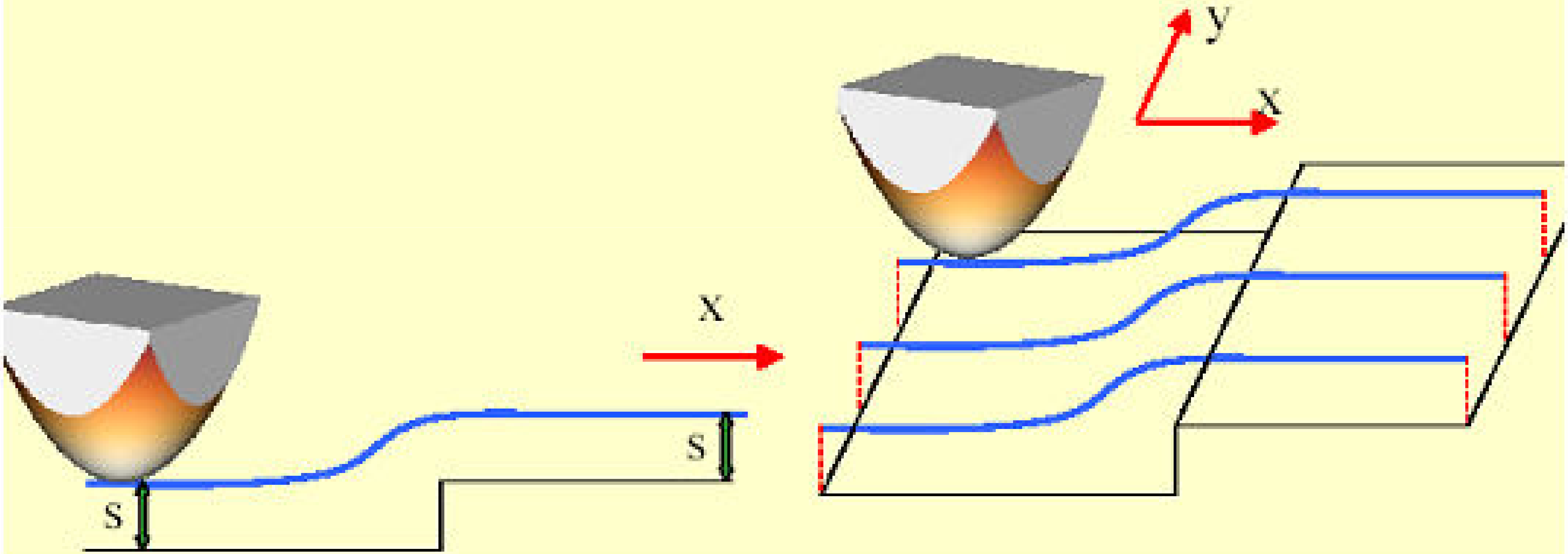
Mechanic Force:

- Contact mode
- Non-contact mode
- Tapping (intermittent) mode

Other Interactions:

- Electrostatic mode (scanning electrostatic potential microscope)
- Magnetic mode
- Chemical Force mode

Obtaining Surface Profiles

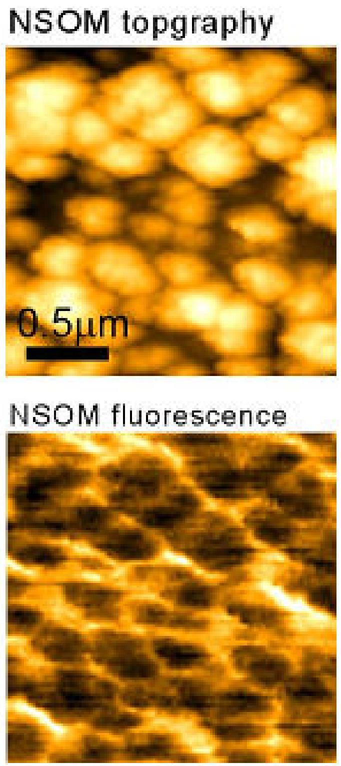


- General components of SPM;
- Tip --- the probe;
- Cantilever --- the indicator of the tip;
- Tip-sample interaction --- the feedback system;
- Scanner --- piezoelectric movement at x,y,z;
- Measurement artifacts: vibration must be isolated.

SNOM (=NSOM)
Scanning NearField Optical Microscopy

SNOM – basic principles

- **STM** measures electric **current**, and **AFM** measures **forces**, neither deals with **light**;
- **Light** - crucial excitation source in both scientific research and mother nature systems.
- Scientific research fields: absorption, fluorescence, photoinduced electron transfer, light-emitting devices, photovoltaic cells.



NSOM topgraphy

TiO_2 particles wrapped in PPV film

NSOM fluorescence

Fluorescence quenching by TiO_2 particles

Why SNOM?

- **Light diffraction limit** - conventional optical microscopy: λ/2 ~ 250 nm (→ Abbe diffraction limit)
- Real cases - **optical resolution** ~ λ, 500 nm
- **NSOM** offers higher resolution around 50 nm (or even < 30 nm), depending on tip aperture size.
- **NSOM** - simultaneous measurements of the:
  - topography
  - \+ optical properties (fluorescence)

  → direct correlation between surface nanofeatures and optical/electronic properties.

- Useful for the **studying**:

inhomogeneous material surfaces (nanoparticles, polymer blends, porous silicon, biological systems)

History of NSOM

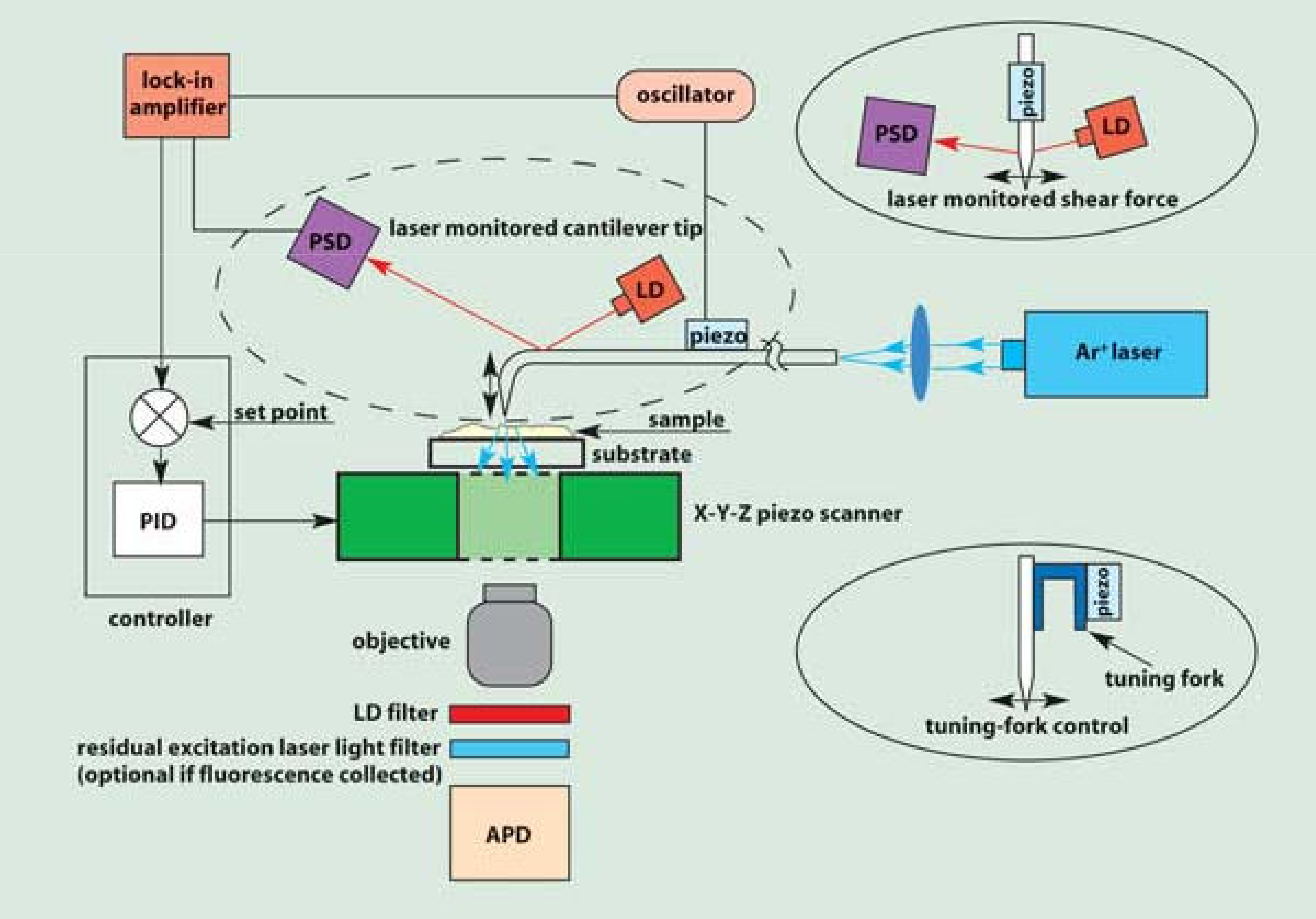
- **1928** roots trace back – letters between Edward Hutchinson Synge and Albert Einstein

- **Ideas started in mid-1980’s:**
  - D.W. Pohl, W. Denk, and M. Lanz, Appl. Phys. Lett. 44, 651-3 (1984).
  - A. Lewis, M. Isaacson, A. Harootunian, and A. Murray, Ultramicroscopy 13, 227 (1984);

- **Technology developed in 1990’s:**
  - Eric Betzig, et al. Science, 262, 1422-1425 (1993).
  - Eric Betzig, et al. Nature, 2369, 40-42 (1994).

- **Prototype commercial available since 2000’s**

Scheme of SNOM apparatus



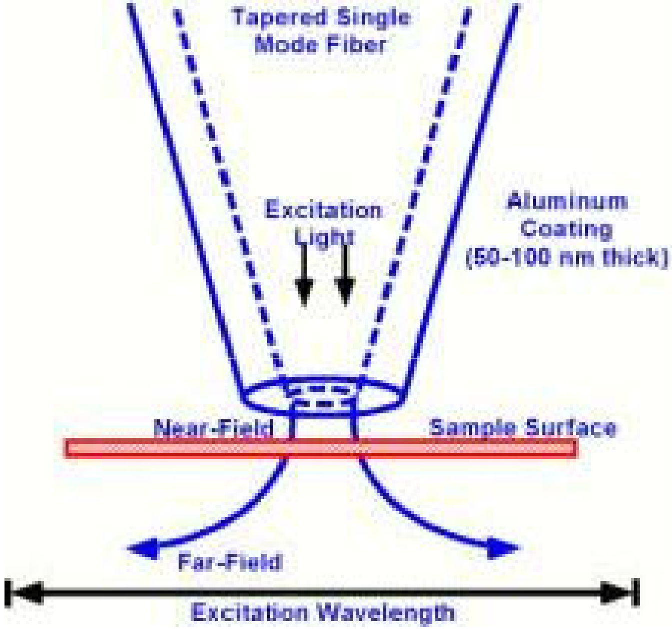
Major components of NSOM

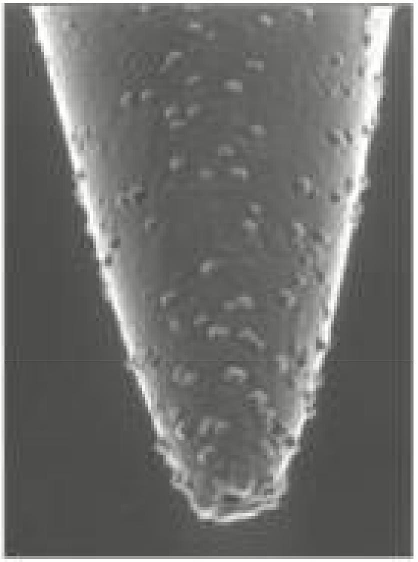
- **Optical**:
  - Light source (lasers: CW and pulsed), Fibers, Mirrors, Lenses, Objectives (oil, large NA)
  - Photon detectors (Photon-Multiplier)
  - Probe (tip)

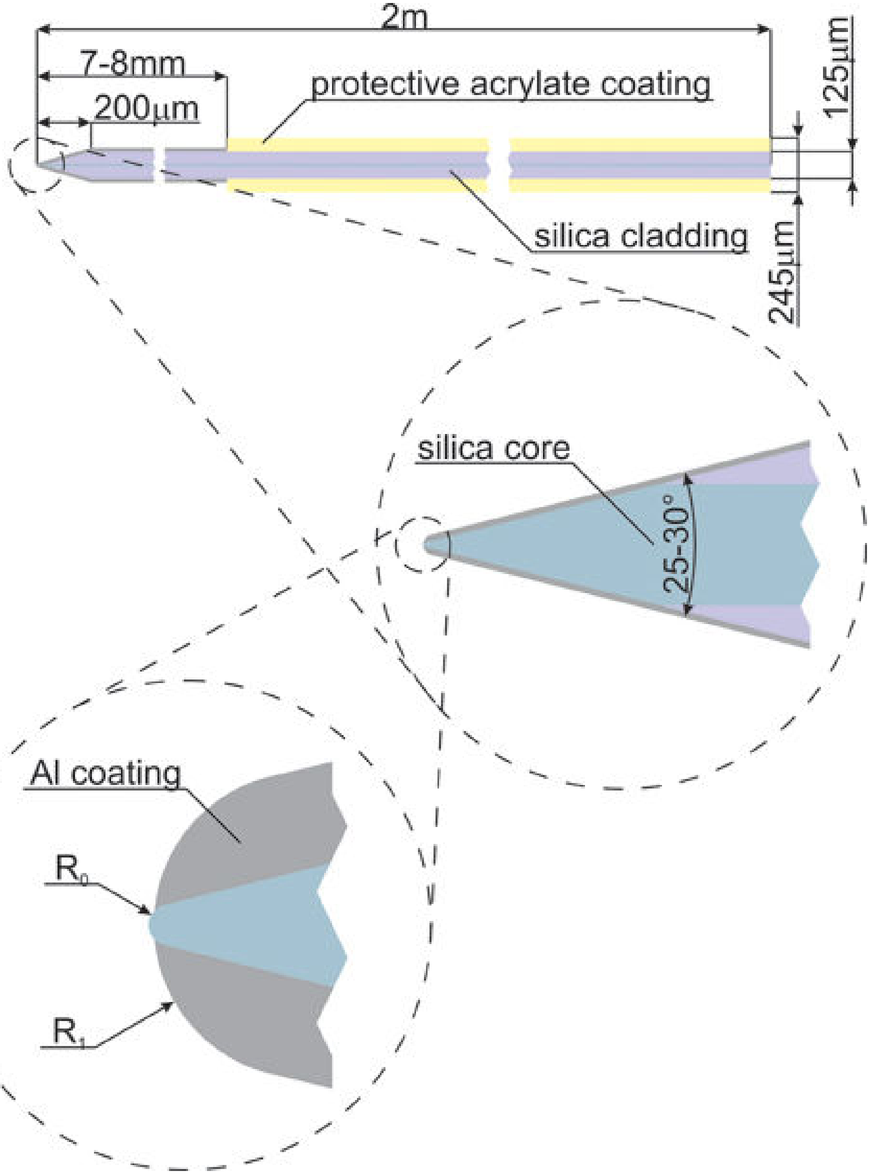
- **Mechanical**:
  - Translation stage, Piezo scanner
  - Anti-vibration optical table

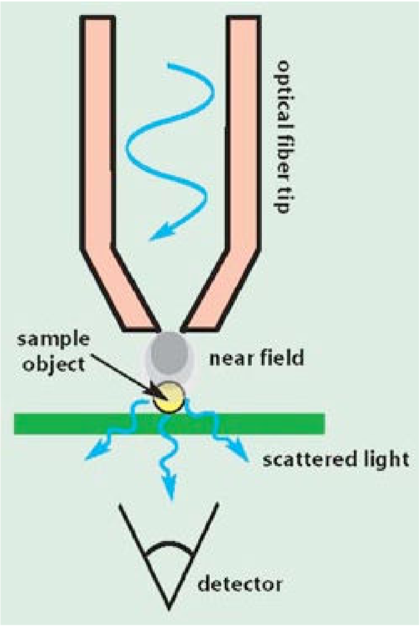
- **Electrical**:
  - Scanning drivers for piezo scanner
  - z distance control (feedback system)
  - Amplifiers, Signal processors
  - Software and Computer

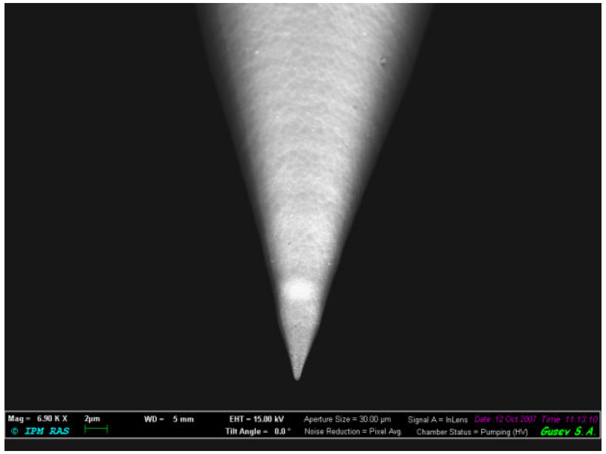
SNOM probe


Tapered Single Mode Fiber
Excitation Light
Aluminum Coating (50-100 nm thick)
Near-Field
Sample Surface
Far-Field
Excitation Wavelength




2m
7-8mm
200μm
protective acrylate coating
125μm
silica cladding
245μm
silica core
25-30°
Al coating
R0
R1

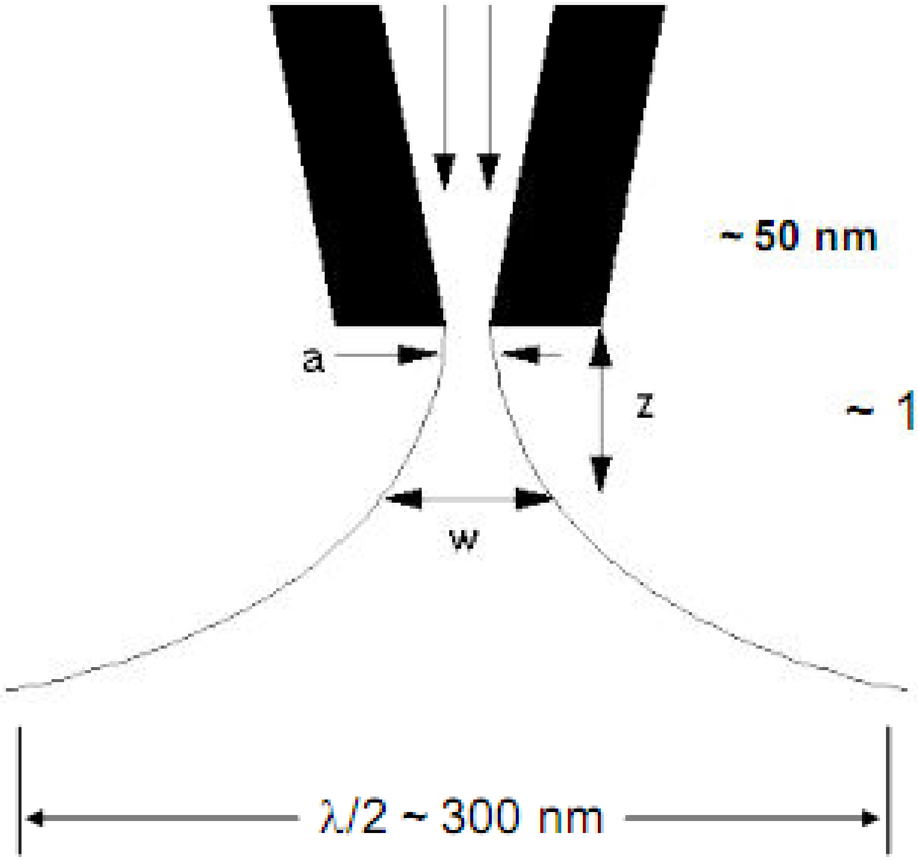

optical fiber tip
sample object
near field
scattered light
detector

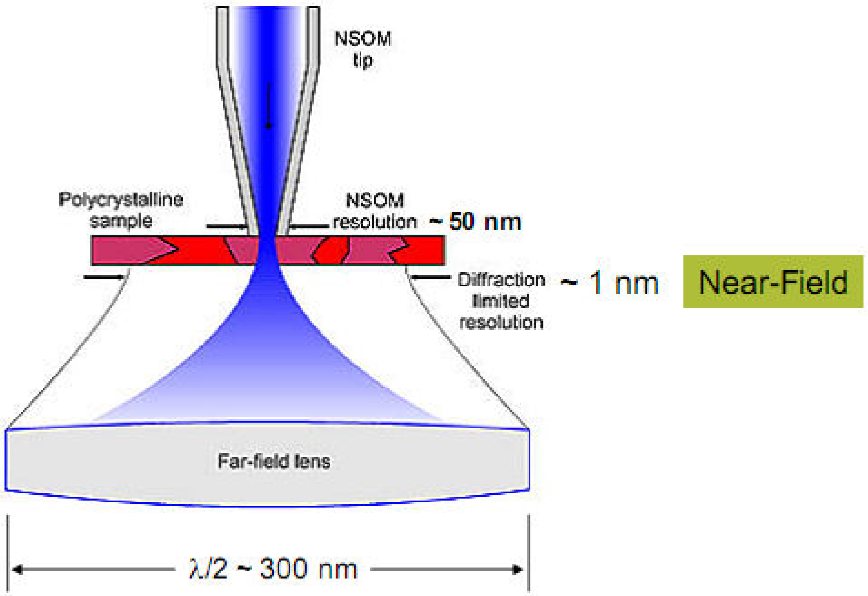

Mag = 6.90 K X
2μm
WD = 5 mm
EHT = 15.00 kV
Tilt Angle = 0.0 °
Aperture Size = 30.00 μm
Noise Reduction = Pixel Avg.
Signal A = InLens
Chamber Status = Pumping (HV)
Date 12 Oct 2007
Time 11:13:10
© IPM RAS
Gusev S. A.

What is Near-Field?

- requires a nanometer sized aperture (much smaller than the light wavelength).
- A specimen is scanned very close to the aperture.
- As long as the specimen remains within a distance less than the aperture diameter, an image with sub-wavelength resolution (aperture size) can be generated.
- There is a tradeoff between resolution and sensitivity (light intensity)
- --- aperture size cannot be too small.

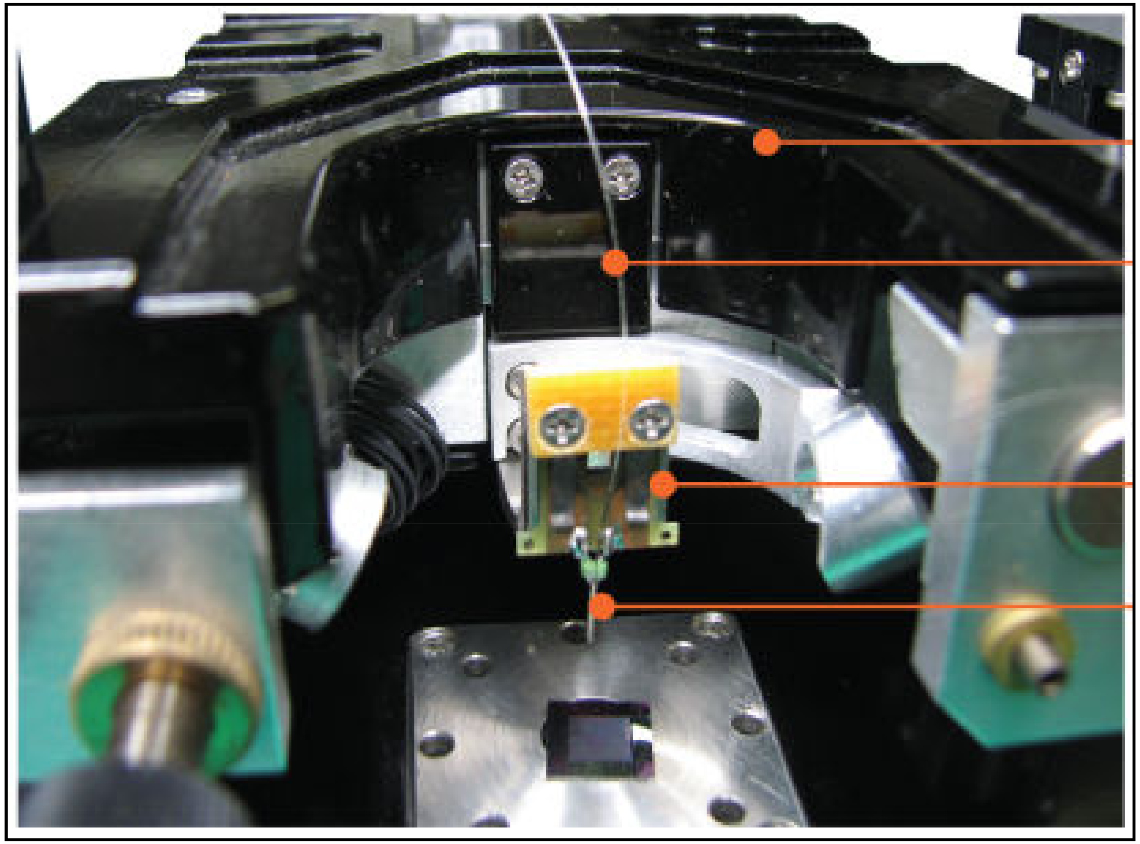
What is Near-Field?

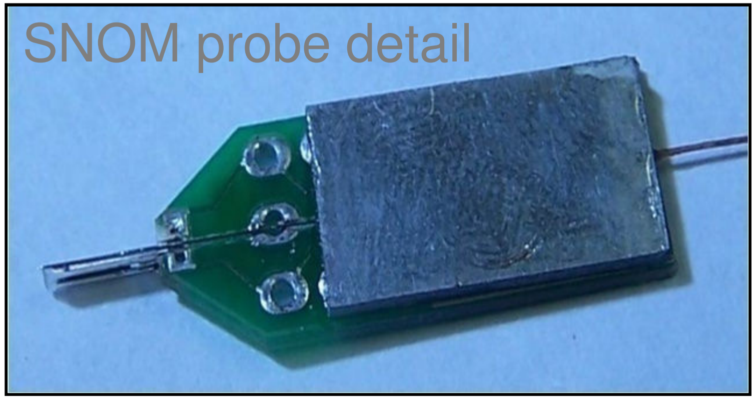


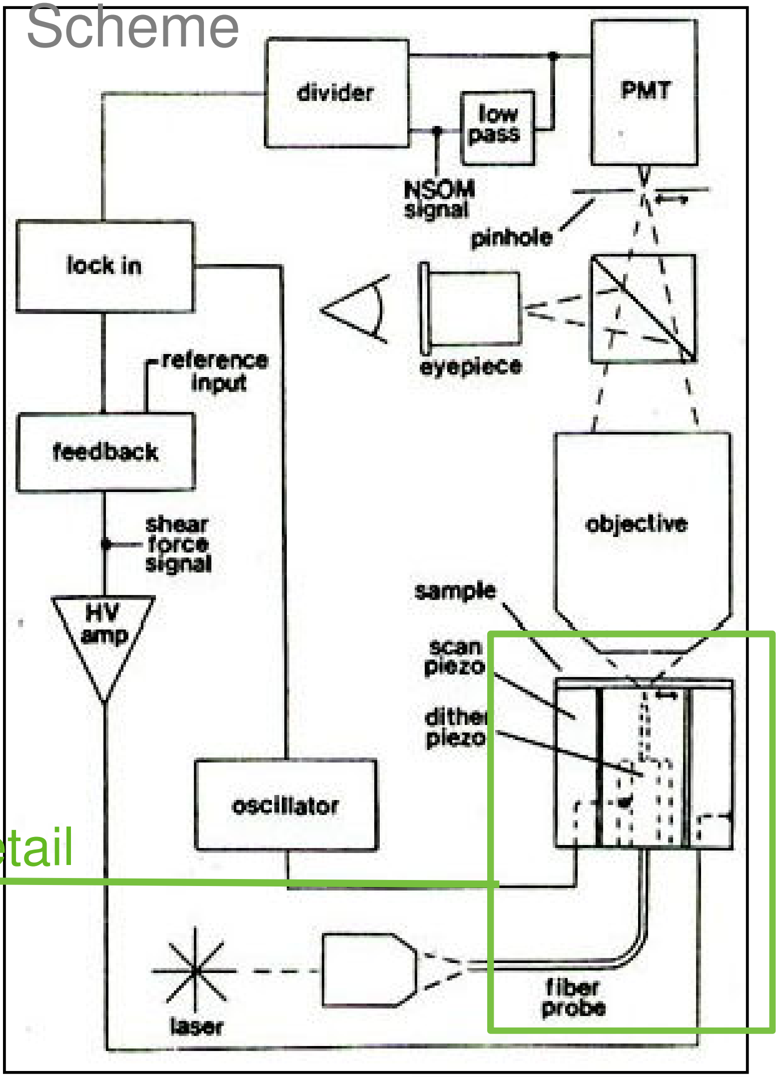


Near-field: For high spatial resolution, the probe must be close to the sample

NSOM
in operation




SNOM probe detail


Scheme

Detail

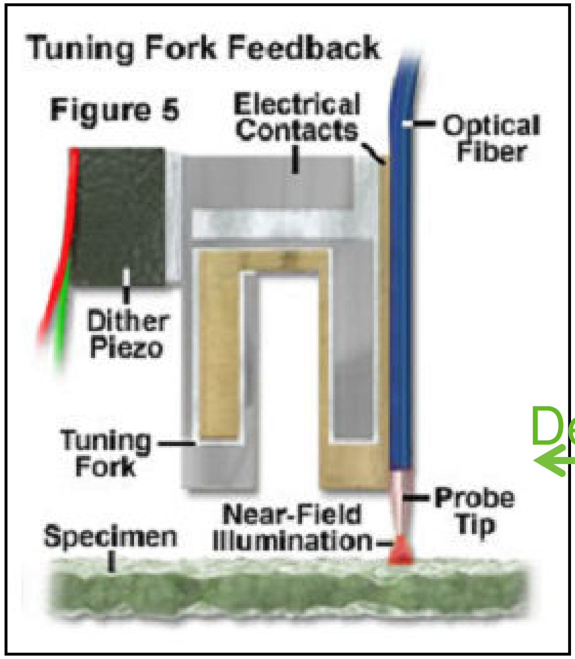
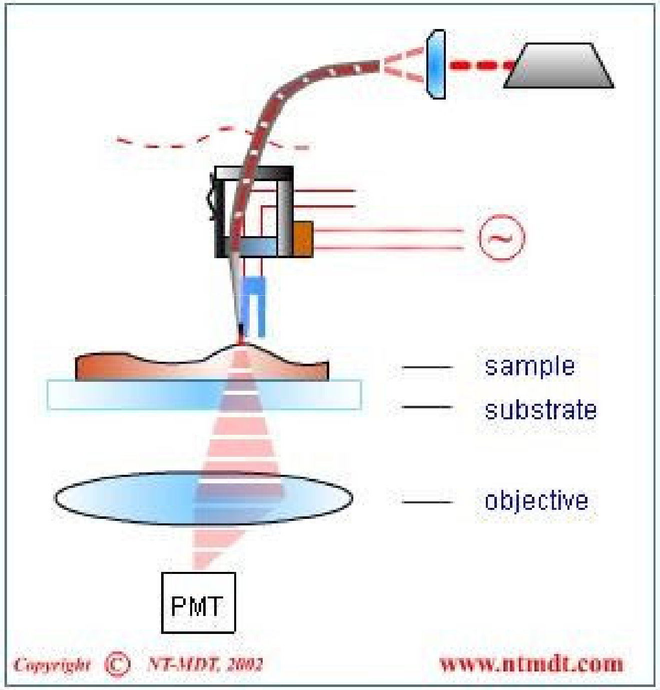
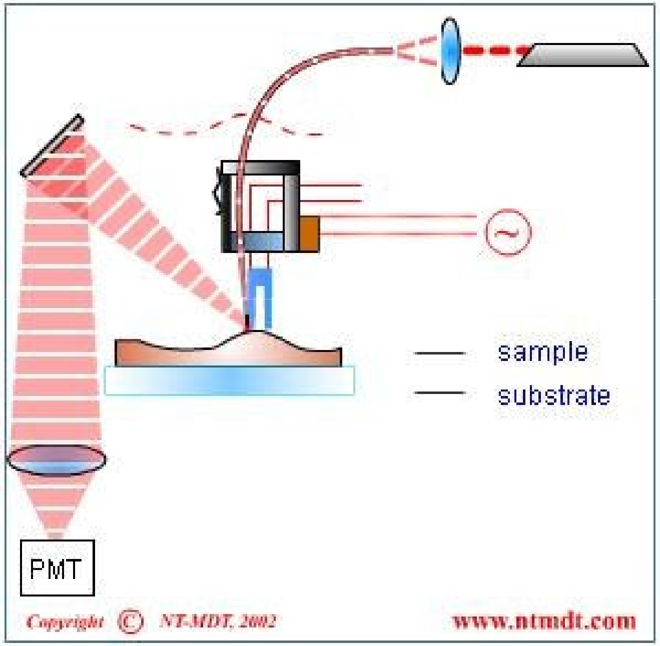

Tuning Fork Feedback

Figure 5

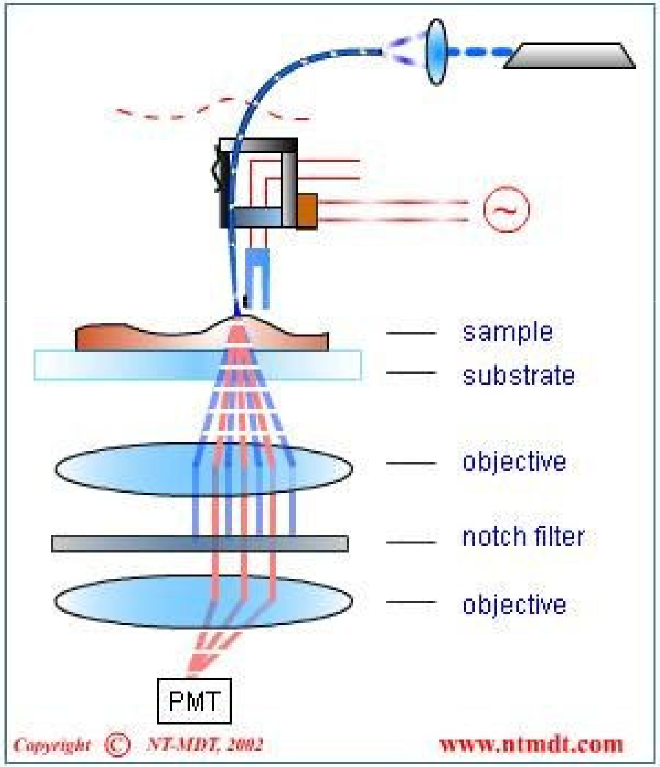
Operation Modes



Transmission mode



Reflection mode



Luminescence mode

Simoultaneously with **Shear Force Microscopy** (SFM)

→ Piezodriver via quartz tuning fork (change of oscillation amplitude is monitored → AFM-like imaging)

Key point is to use "AFM technology" to bring the light very close to the surface (1-10 nm, distance<probe diameter)

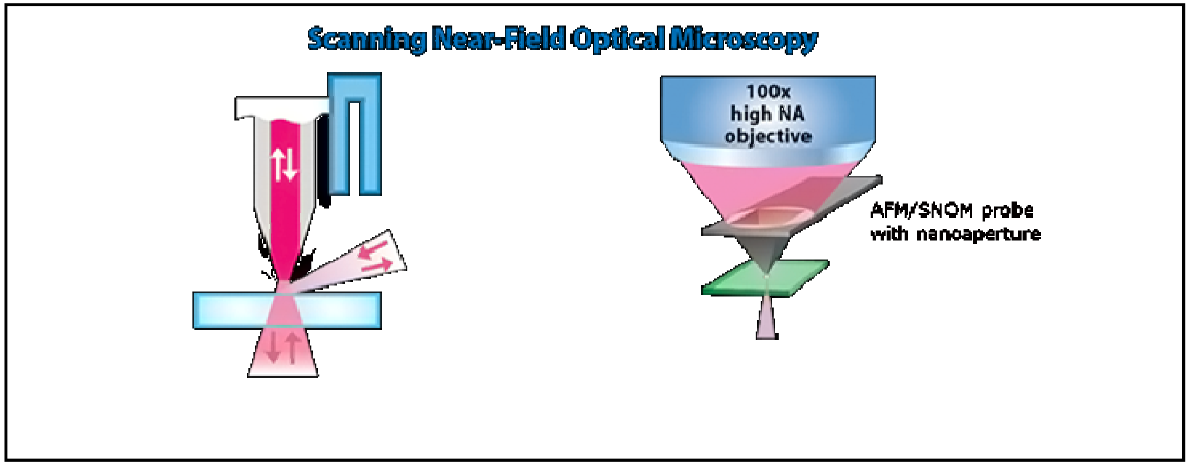
Real instrument example

Ntgra Vita AURA, Ntgra Vita SPECTRA (NTMDT, Zelenograd, Russia)

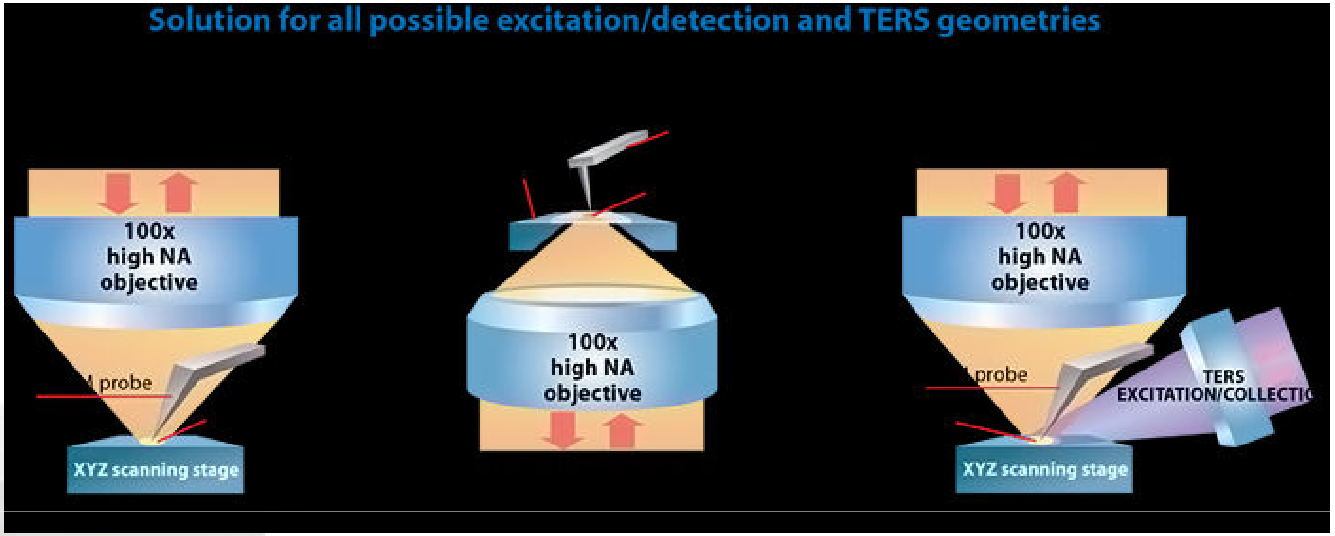
Bringing light close to the surface

Optical fiber
+ tuning fork

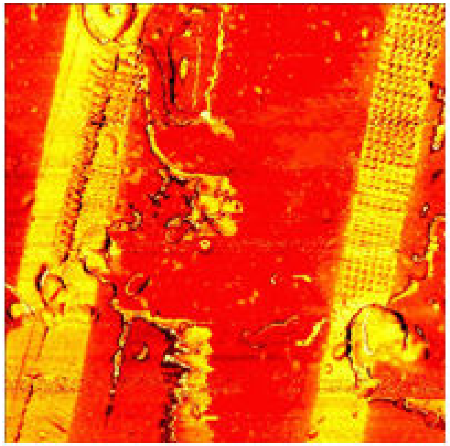
AFM transparent
probe
+ light via
objective



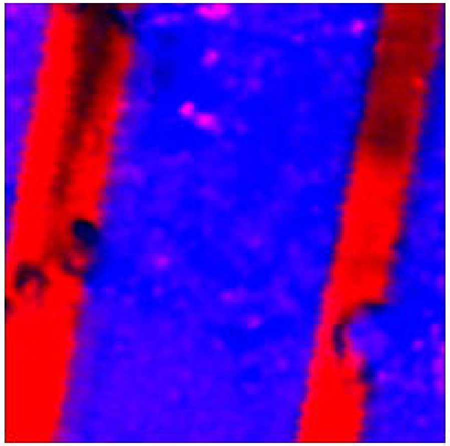
TERS



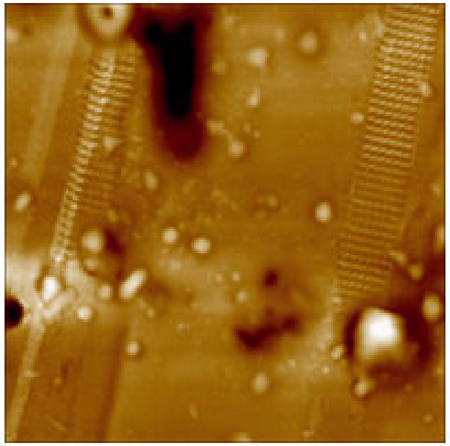
Tip **E**nhanced **R**aman **S**pectroscopy



Stiffness of HDPE/LDPE polymer sandwich cut by microtome

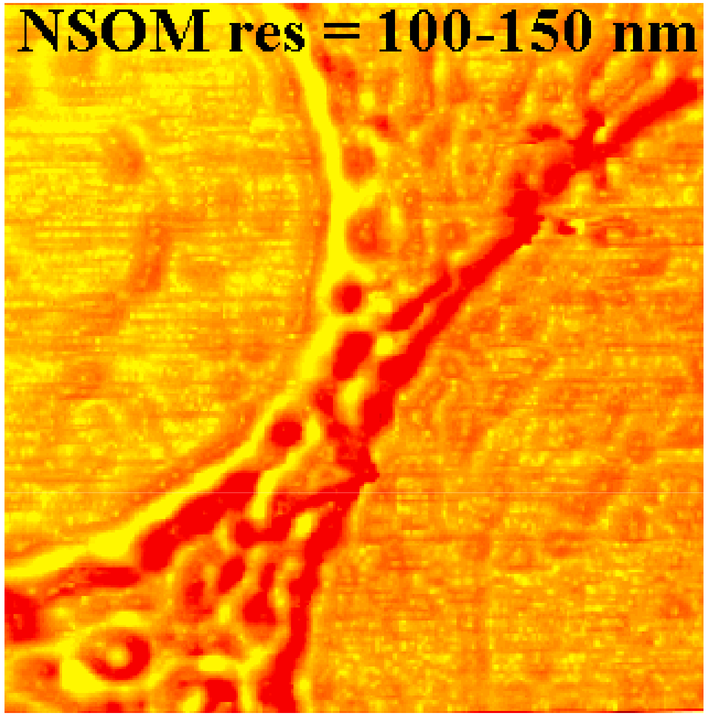


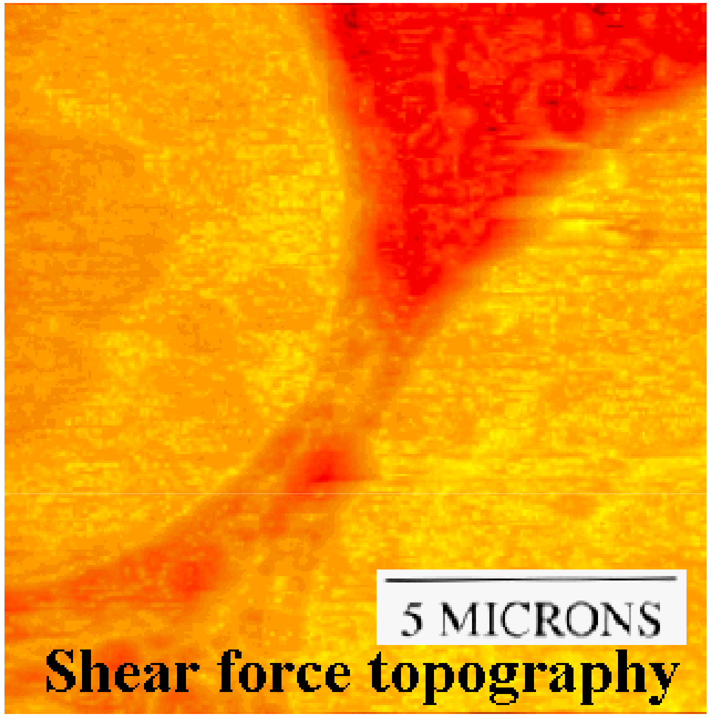
Overlap of Raman maps: HDPE (red), LDPE (blue)



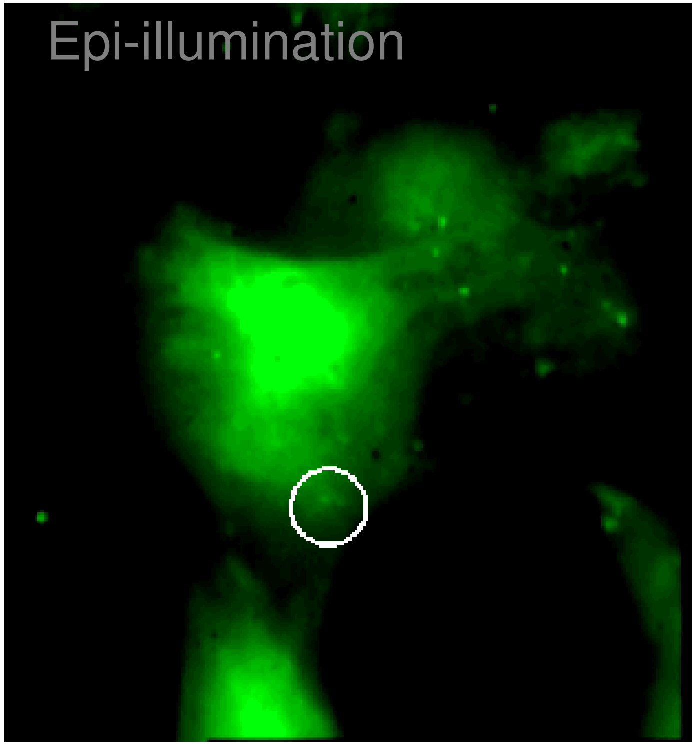
AFM topography

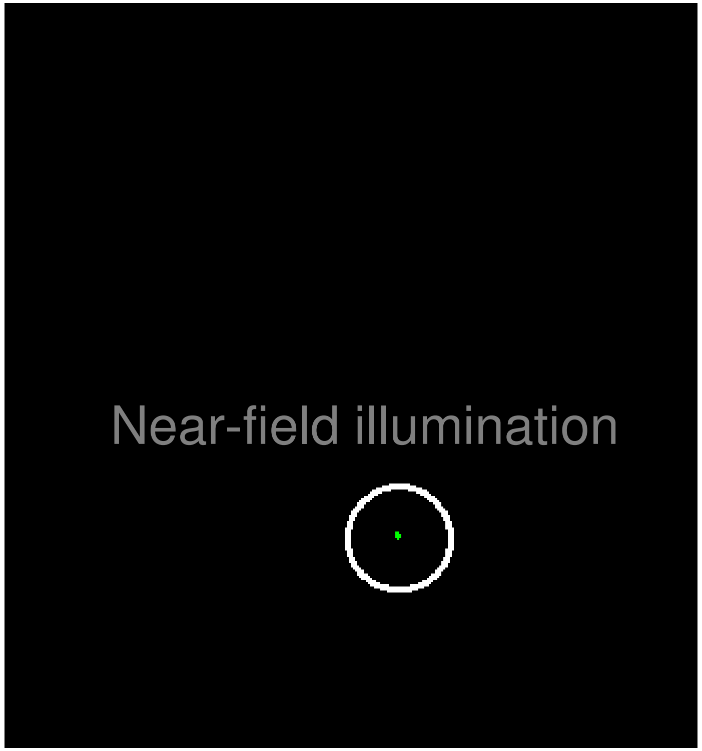
NSOM images





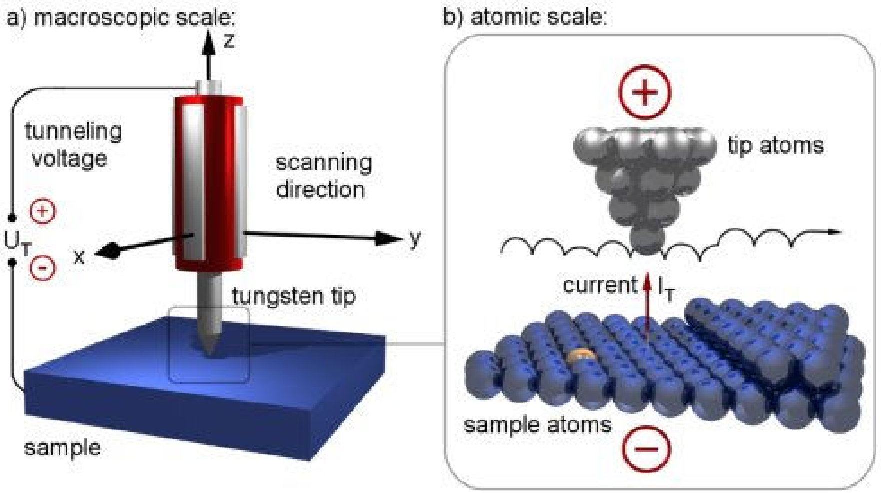
Tissues images





Living cells

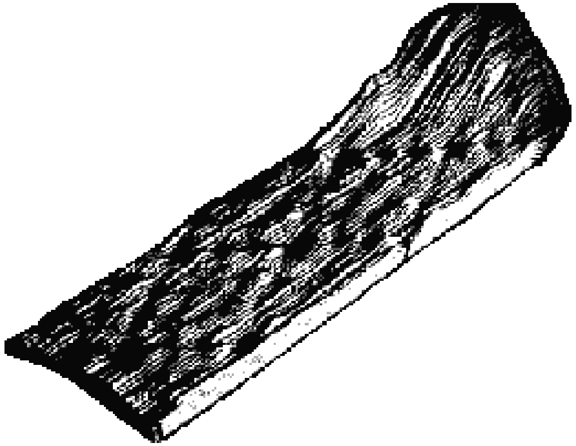
Scanning Tunnelling Microscopy
STM



The Nobel Prize in Physics 1986

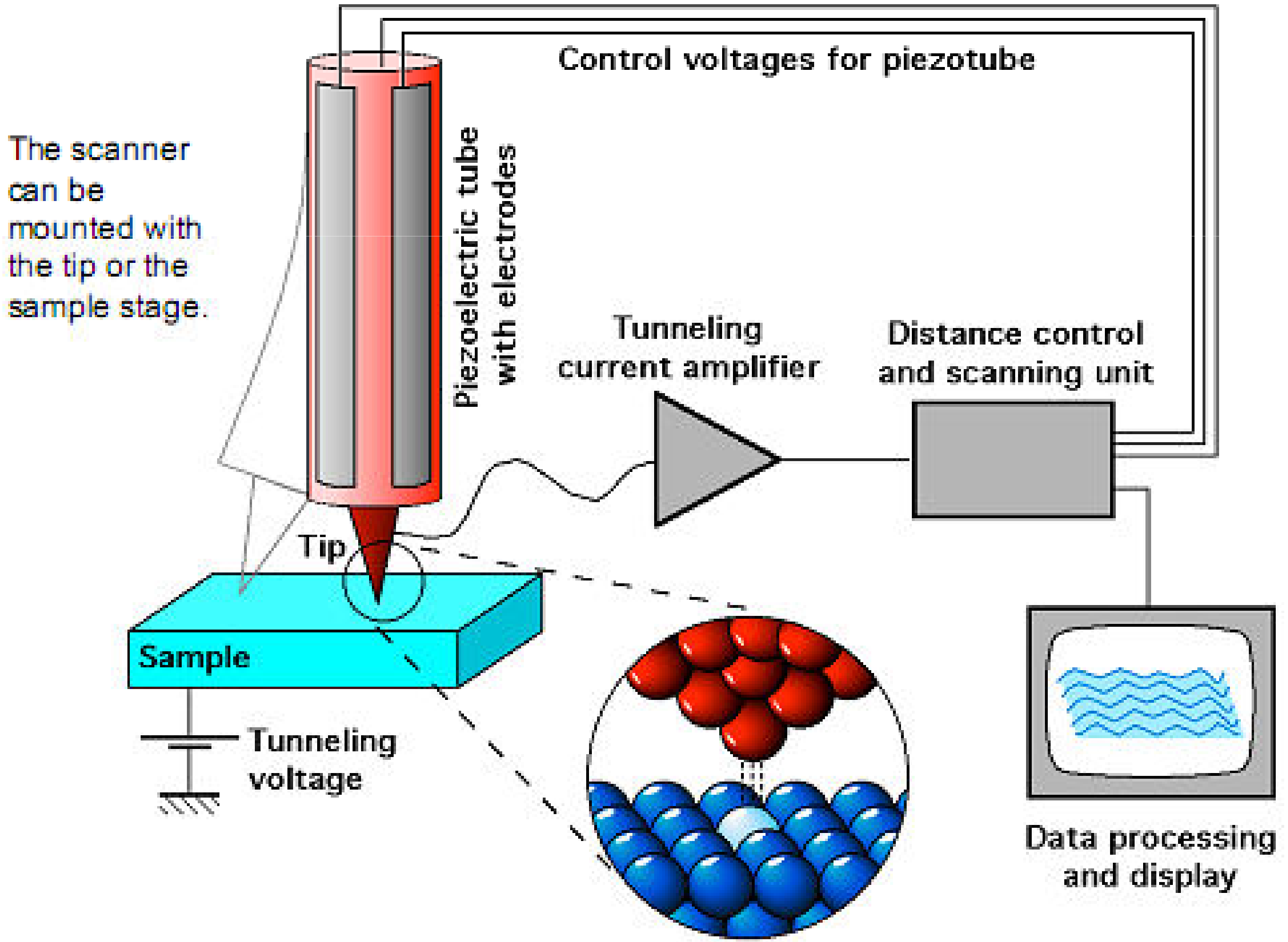
Nobel Laureates Heinrich Rohrer and Gerd Binnig

- **STM** - the **first** member of **SPM** family
- Developed in **1982** by Gerd Binnig and Heinrich Rohrer members of IBM in Zurich (Phys. Rev. Lett., 1982, vol 49, p57)
- **1986 -** Nobel prize in physics for their brilliant invention



1982 *- Triumph of Scanning Probe Microscopy - image of silicon surface 7x7 reconstruction.*

Basic components of STM



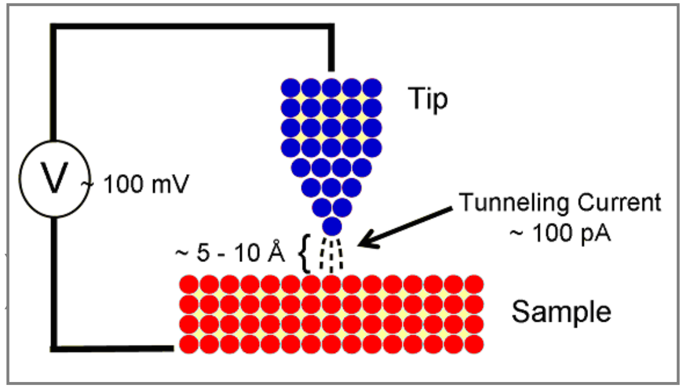
Five basic components:

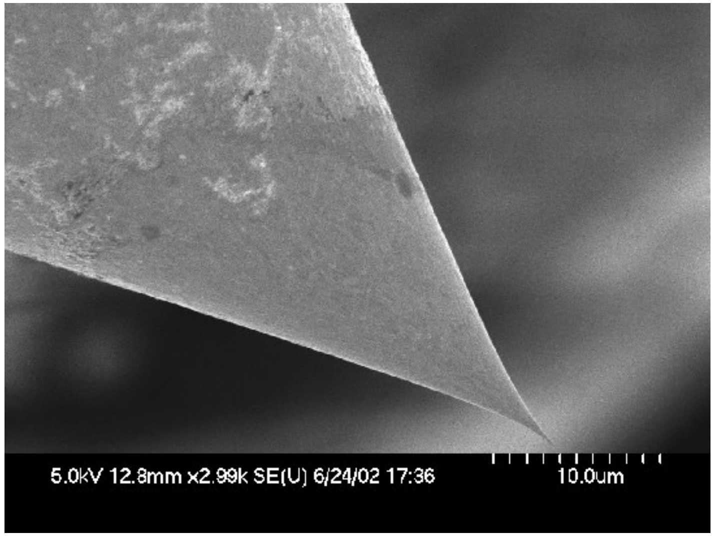
1. Metal tip,
2. Piezoelectric scanner,
3. Current amplifier (nA),
4. Bipotentiostat (bias),
5. Feedback loop (current).

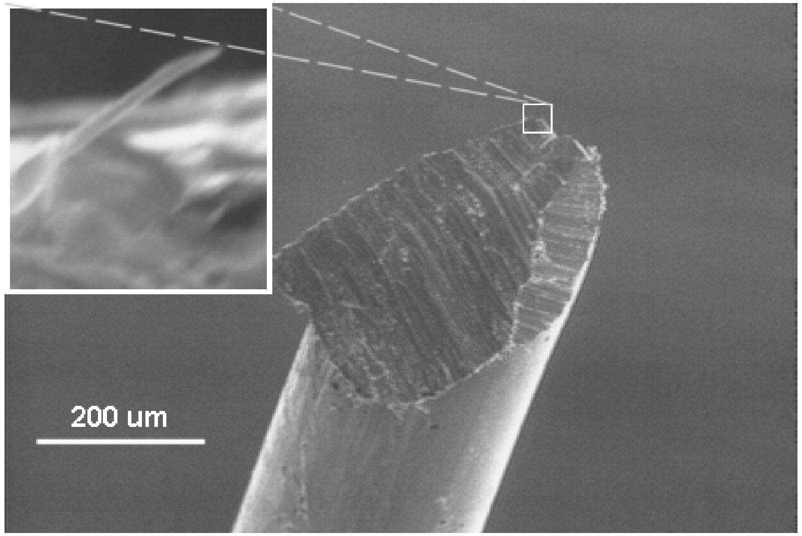
STM tip

- **STM tip** - conductive (metals - Pt, W, Pt/Ir)
- **STM** microscopy uses the very top (outermost) atom at the tip and the nearest atom on sample

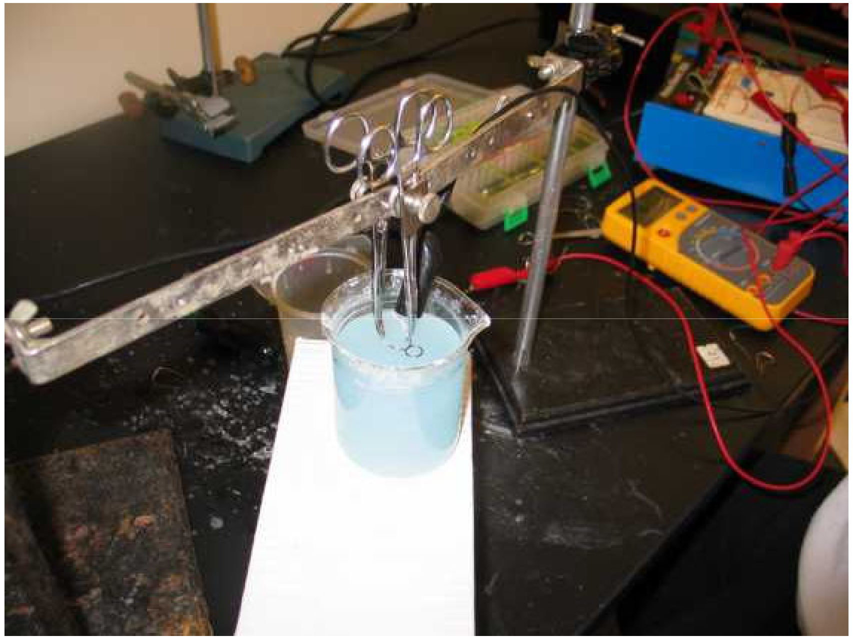
  Tip is not necessarily very sharp in shape (different from AFM)
- **Tip preparation**:
  - Cutting with scissors
  - Electrochemical etching
  - Other techniques such as FIB (and combination)

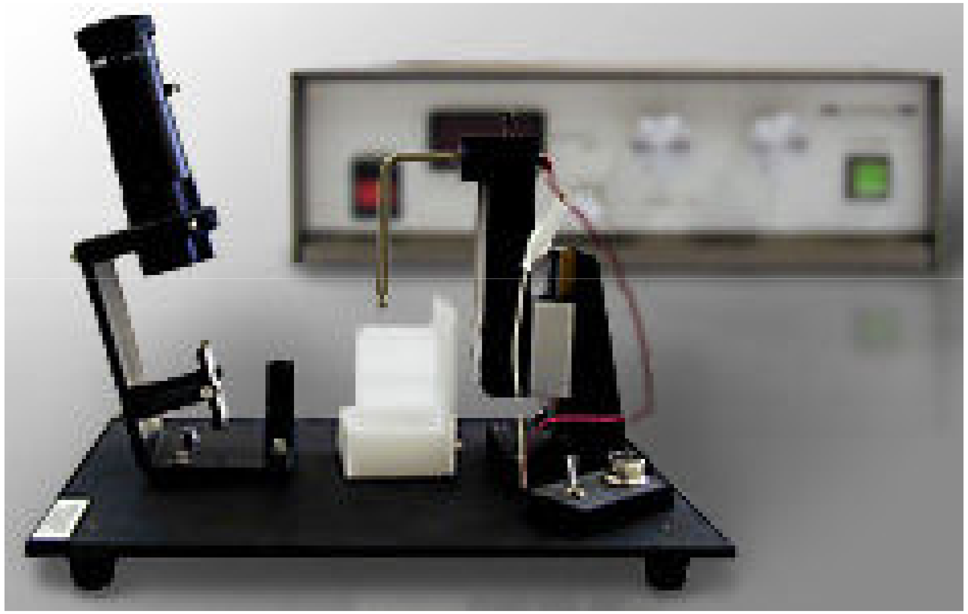


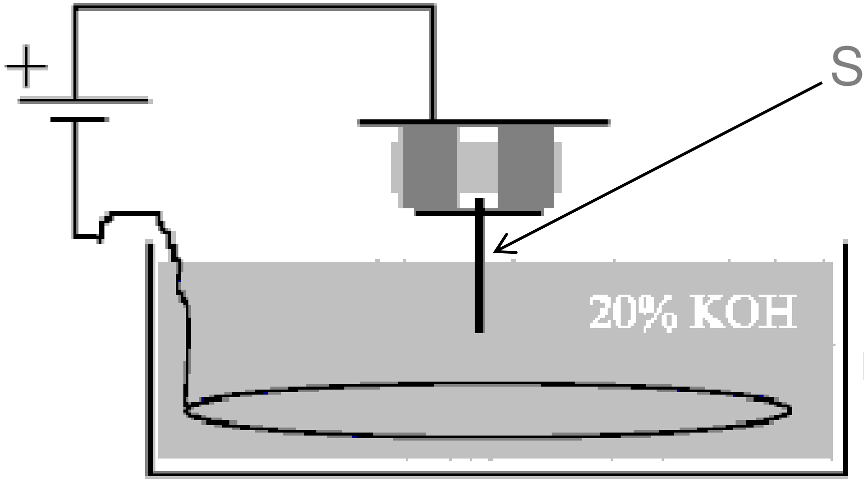




STM tip electrochemical etching







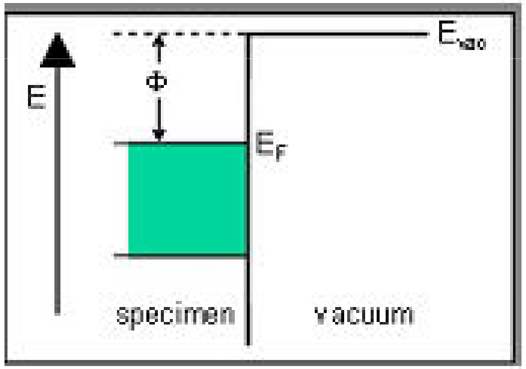
Surface tension helps to create tip shape

After etching the tip-including part of wire remains in holder, remaining part falls to bottom

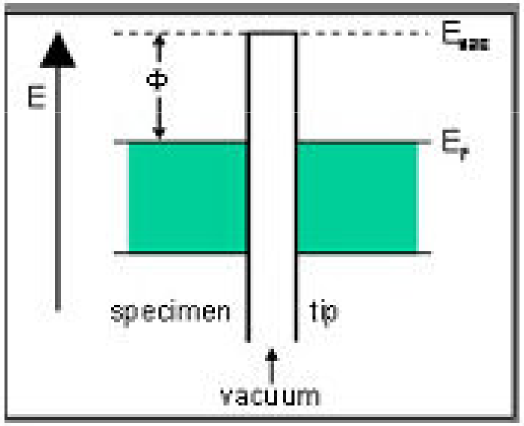
Or…

Tunneling current

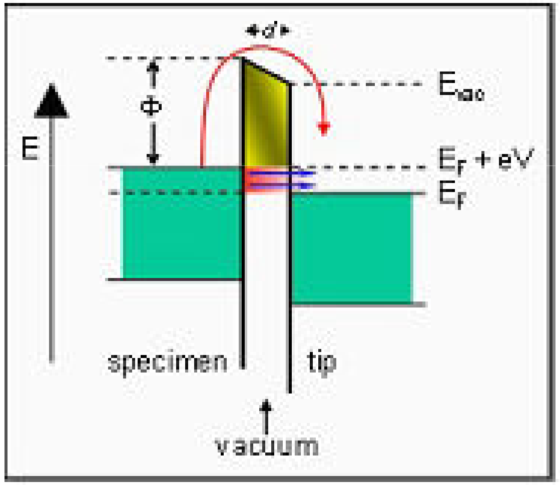
Transfer of electrons **without a contact** of conductive is not possible → according to classical mechanics → **tunneling**



In a metal, the energy levels of the electrons are filled up to a particular energy, known as the 'Fermi energy' E_F*. In order for an electron to leave the metal, it needs an additional amount of energy* Φ*, the so-called 'work function'.*

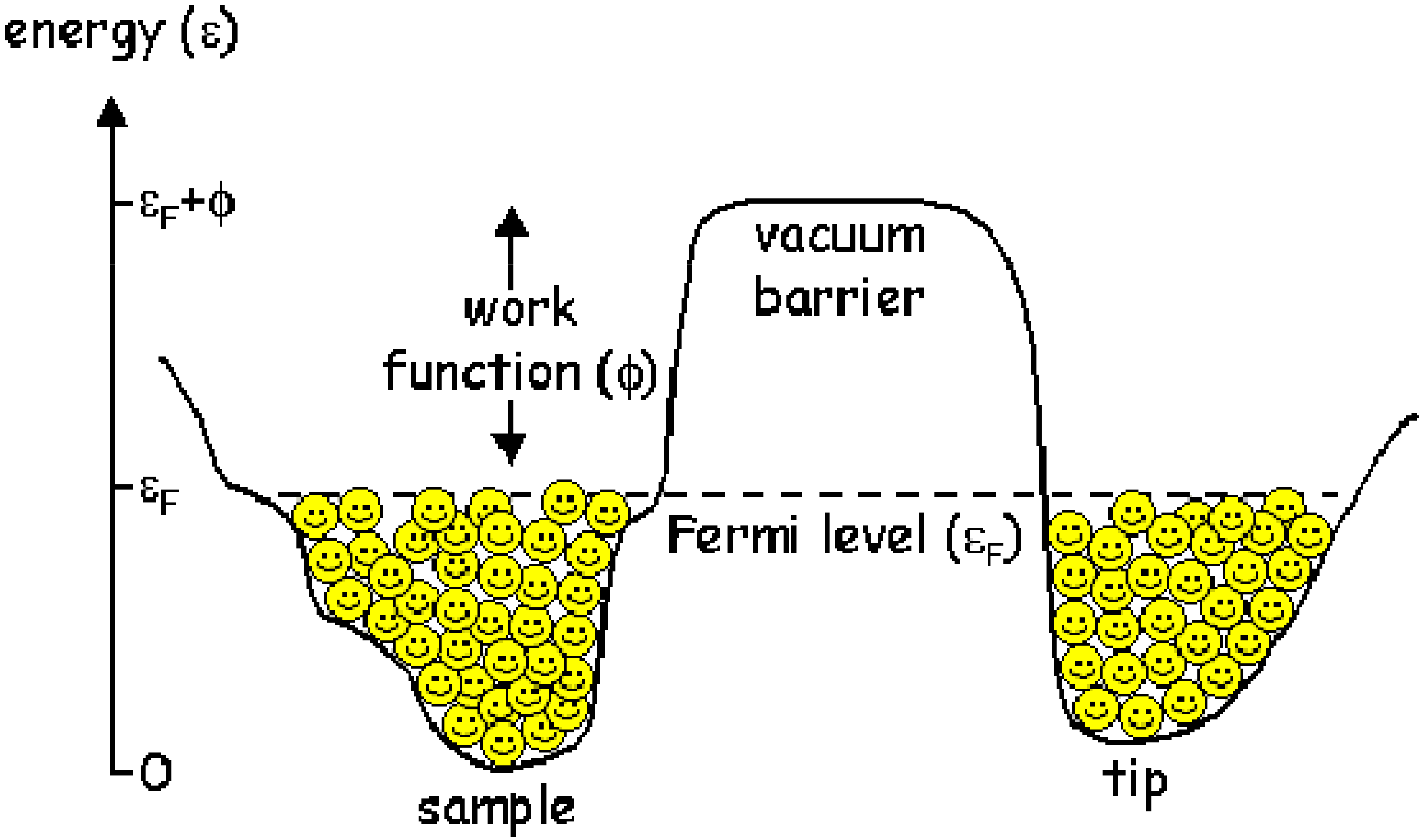


When the specimen and the tip are brought close to each other, there is only a narrow region of empty space left between them. On either side, the electrons are present up to the Fermi energy. They need to overcome a barrier Φ *to travel from tip to specimen or vice versa*



If the distance d between specimen and tip is small enough, electrons can 'tunnel' through the vacuum barrier. When a voltage V is applied between specimen and tip, the tunneling effect results in a net electron current. In this example from specimen to tip. This is the tunneling current.

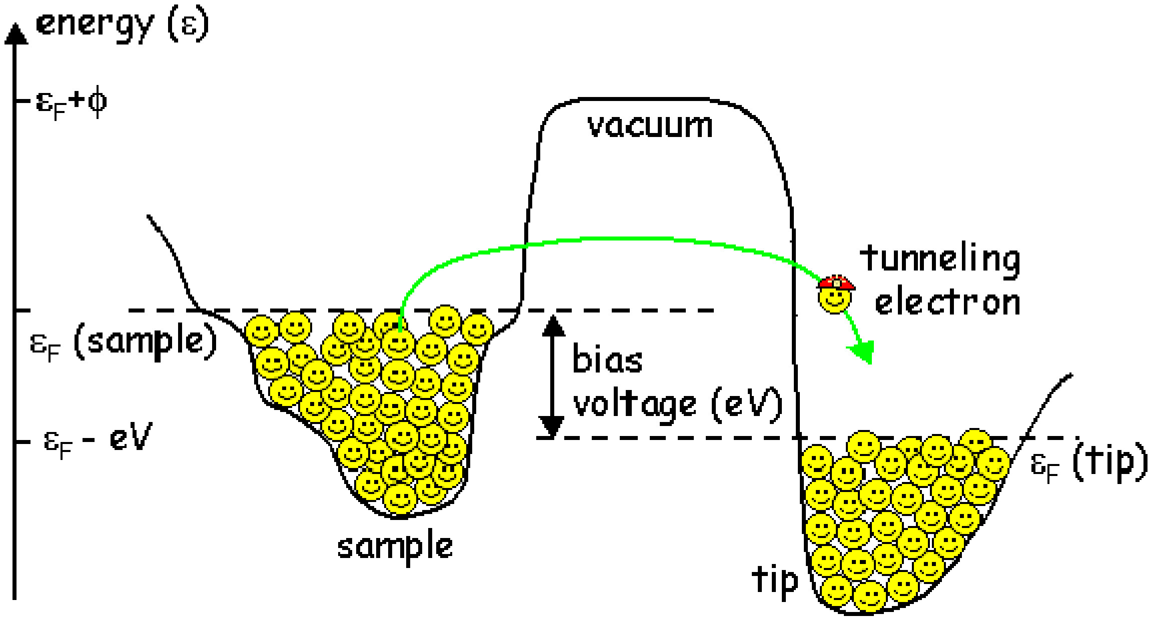
Tunneling of electrons… Fermi level



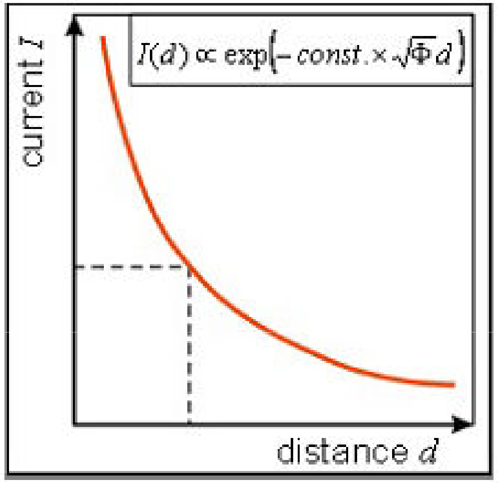
The electrons in the tip and the sample are sitting in two separate valleys, separated by a hill which is the vacuum barrier.

Electron density of states - Fermi level

- Electrons are happy sitting in either the tip or the sample
- It takes energy to remove an electron into free space - vacuum around the tip as an energy hill that the electron would need to climb in order to escape. The height of this energy hill is called the work function, φ.
- In order to bring an electron up and over the vacuum energy barrier from the tip into the sample (or vice versa), we would need to supply a very large amount of energy.
- Quantum mechanics tells us that the electron can tunnel right through the barrier. Note: this only works for particles!
- As long as both the tip and the sample are held at the same electrical potential, their Fermi levels line up exactly. There are no empty states on either side available for tunneling into! This is why we apply a bias voltage.



- A thin metal tip is brought in close proximity of the sample surface. At a distance of only a few Å, the overlap of tip and sample electron wavefunctions is large enough for an electron tunneling to occur.
- When an electrical voltage *V* is applied between sample and tip, this tunneling phenomenon results in a net electrical current, the ‘tunneling current’. This current depends on the tip-surface distance *d*, on the voltage V, and on the height of the barrier *Φ*:
- This (approximate) equation shows that the tunneling current obeys Ohm’s law, i.e. the current I is proportional to the voltage *V*.
- The current depends exponentially on the distance *d*.
- For a typical value of the work function *Φ* of 4 eV for a metal, the tunneling current reduces by a factor 10 for every 0.1 nm increase in *d*. This means that over a typical atomic diameter of e.g. 0.3 nm, the tunneling current changes by a factor 1000! This is what makes the STM so sensitive.
- The tunneling current depends so strongly on the distance that it is dominated by the contribution flowing between the last atom of the tip and the nearest atom in the specimen
- **Single-atom imaging!**

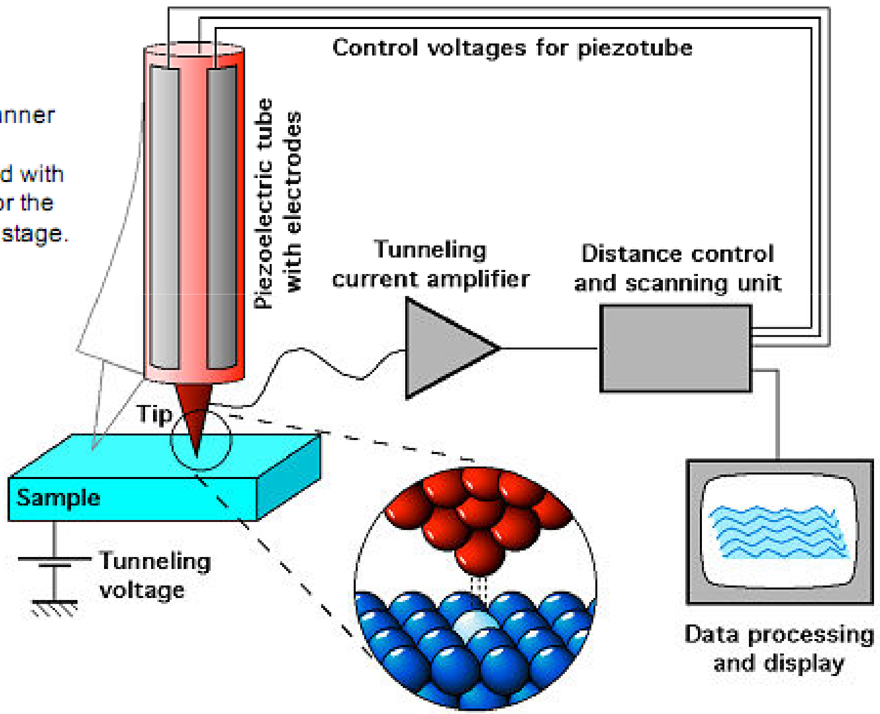


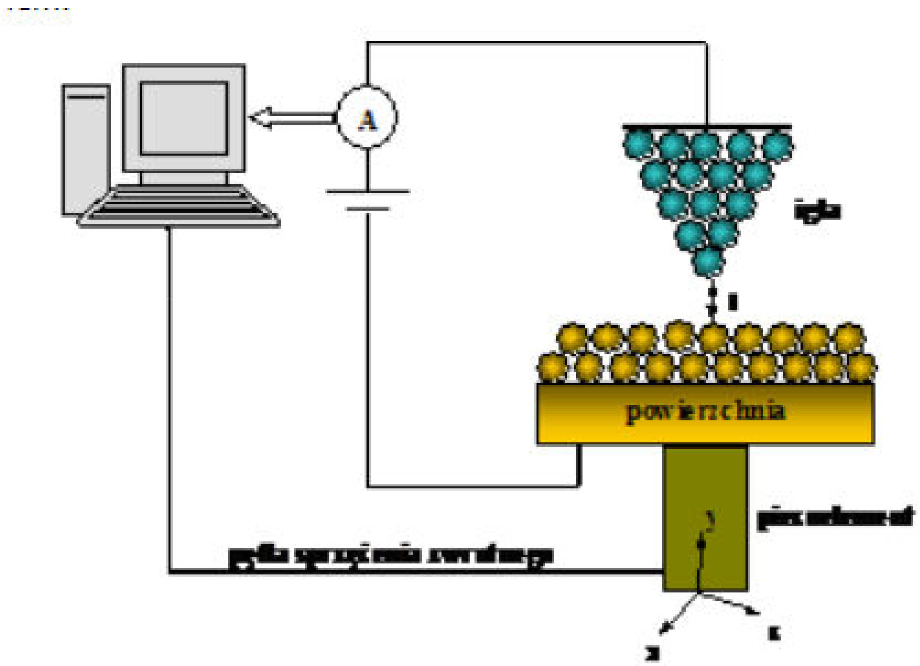
$$I(d) = constant \times eV \exp\left(-2\frac{\sqrt{2m\Phi}}{\hbar}d\right)$$

Φ - the work function (energy barrier)

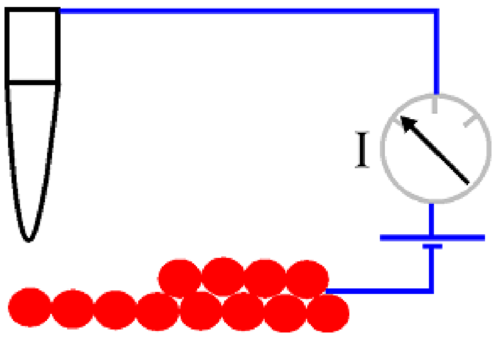
D - tip-sample distance

STM modes

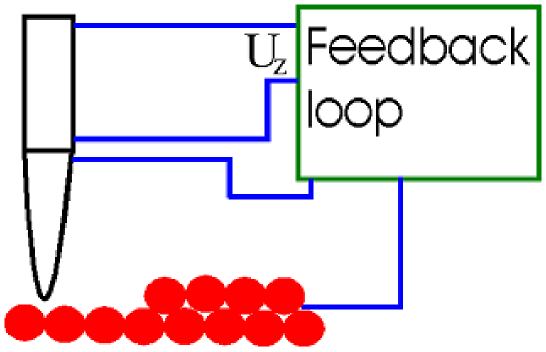

The scanner can be mounted with the tip or the sample stage.



Tell if same atoms

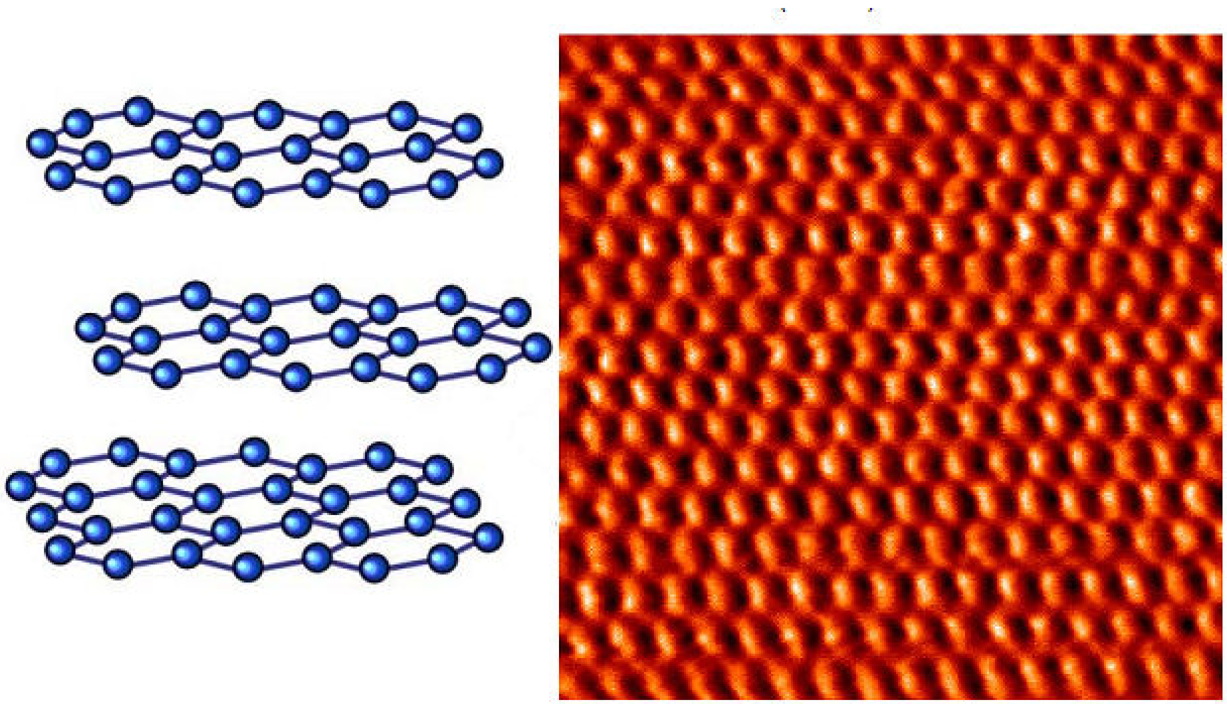


Tell heights for the same atoms



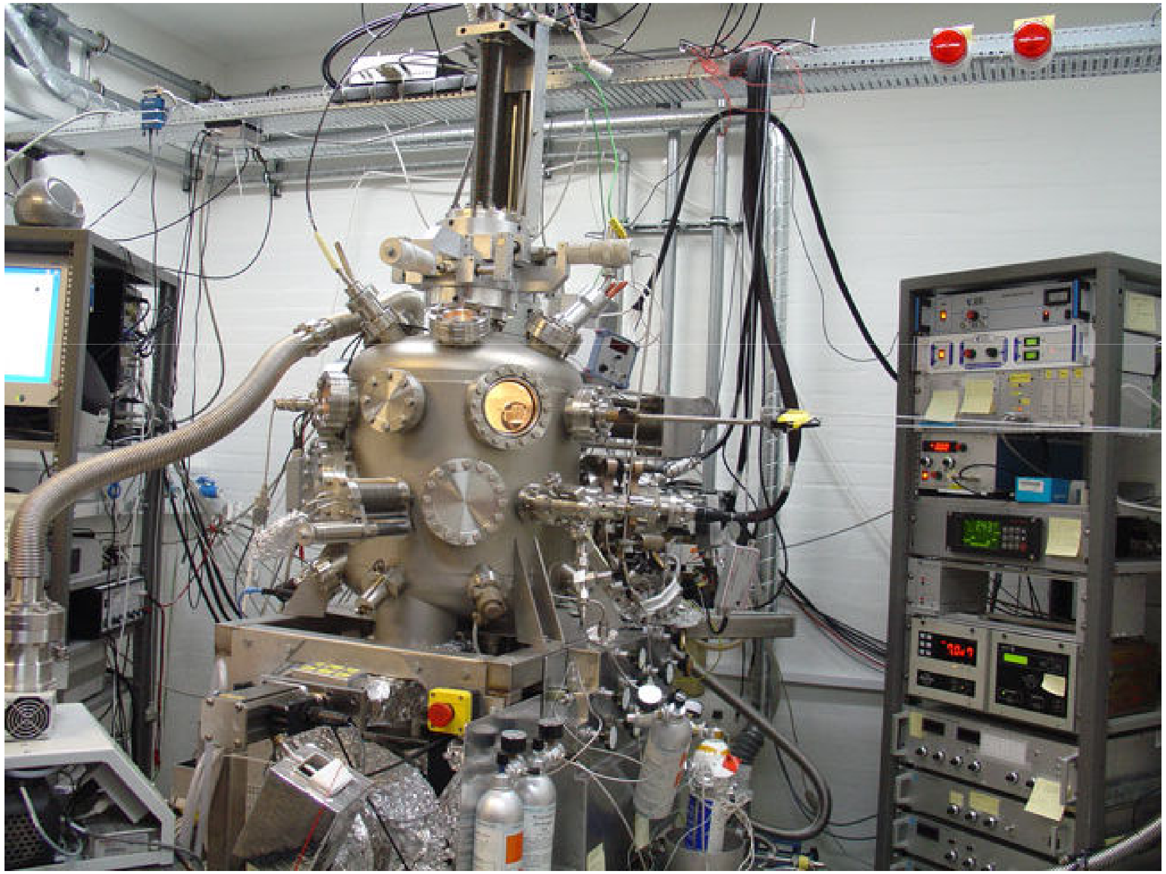
Factors affecting STM imaging

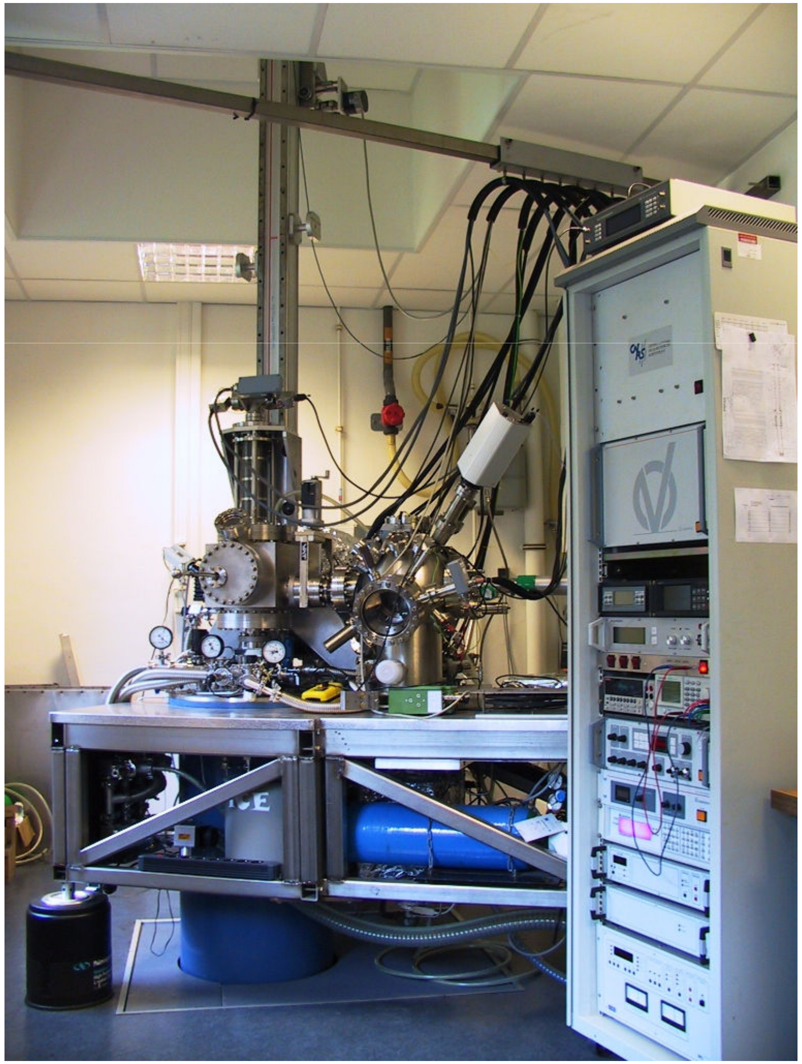
- **Corrugation** - how much the electron density of surface atoms varies in height above the surface.
- **Thermal drift** – change of temperature cause extension of material



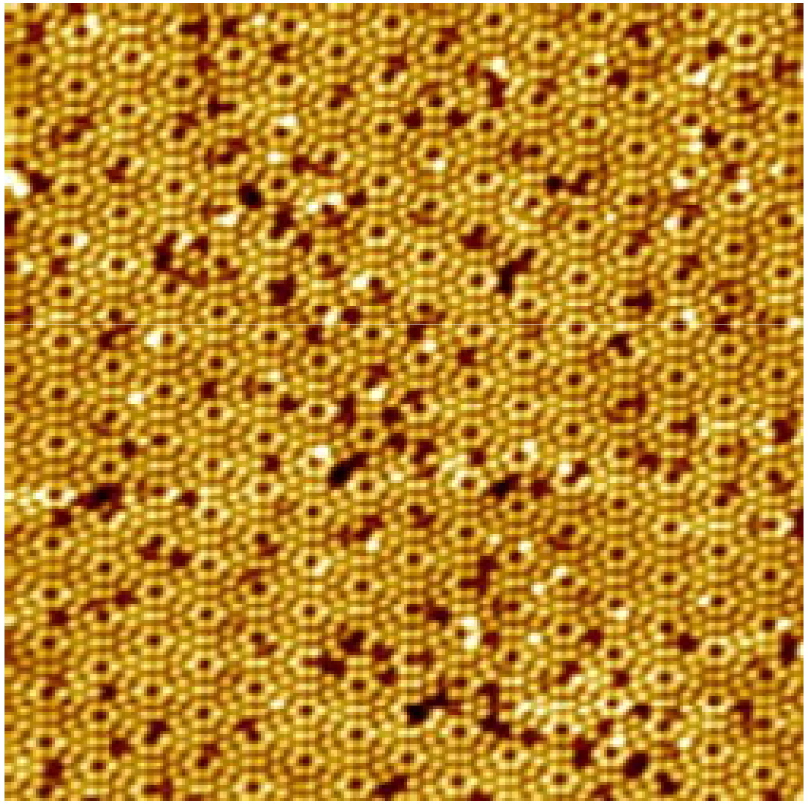
Graphite has a large corrugation, and is very planar, and thus is one of the easiest materials to image with atomic resolution. (see next slide for example)

UHV-STM (UltraHigh Vacuum STM)

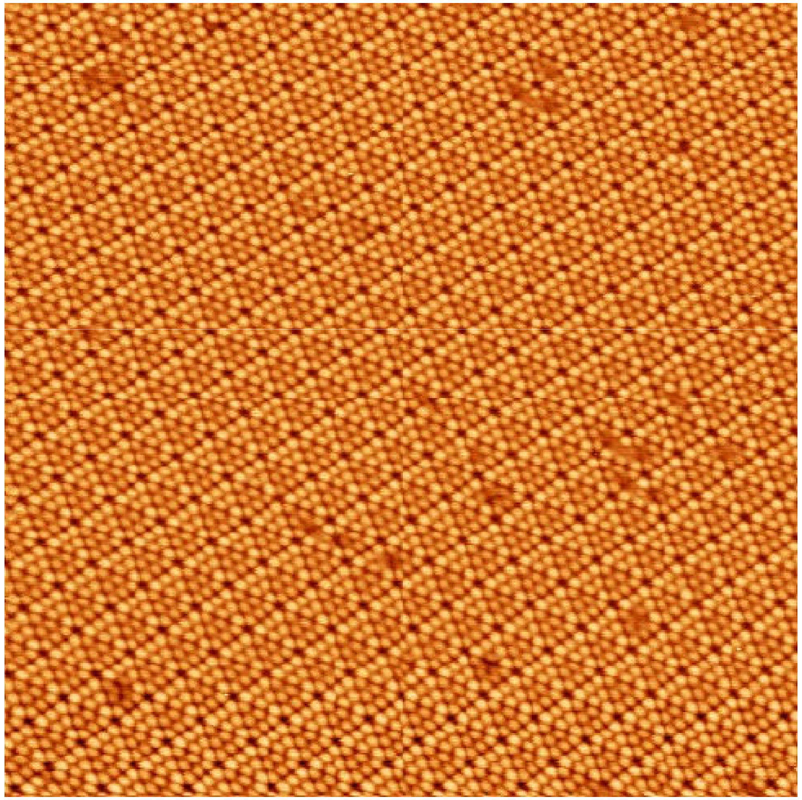




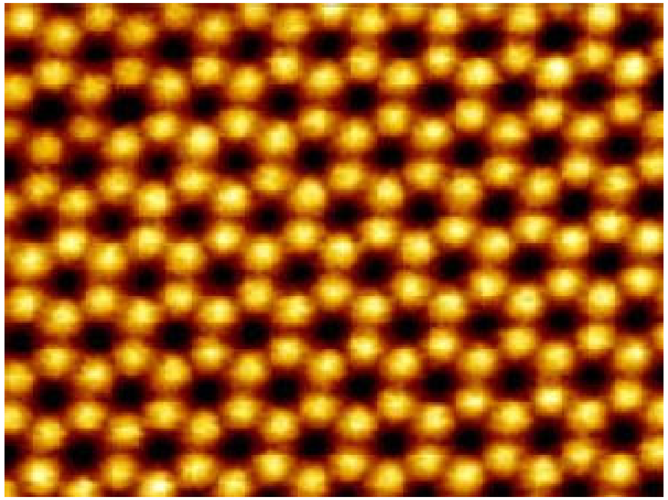
UHV-STM examples



Si (111) 7x7, 40nm
empty states image,
room temperature, dark
spots represent missing
atoms or adsorbates



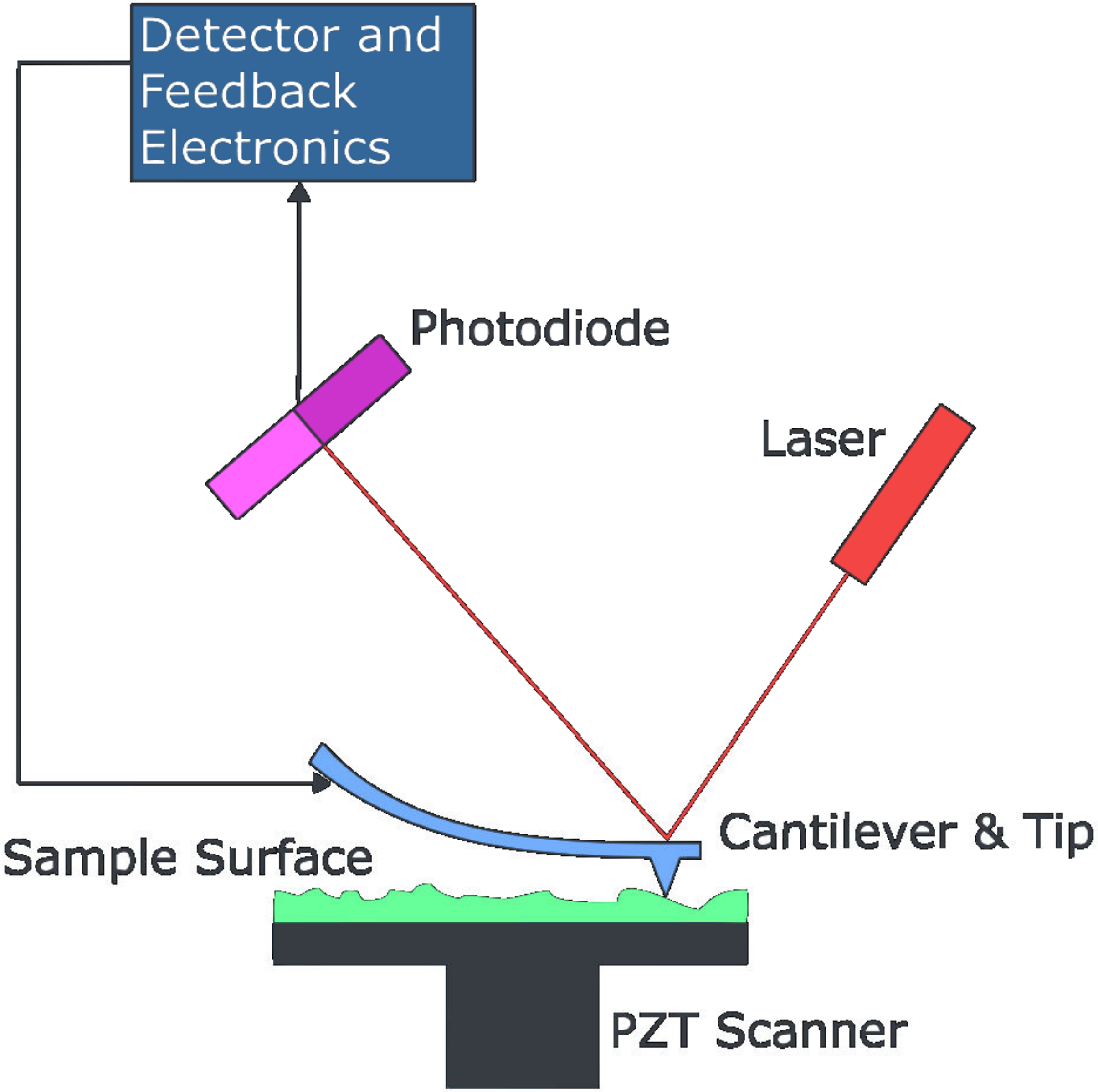
Clean Si(111)
reconstructed to
(7x7); STM image 50
x 50 nm2



Ag-Si (111) 10nm

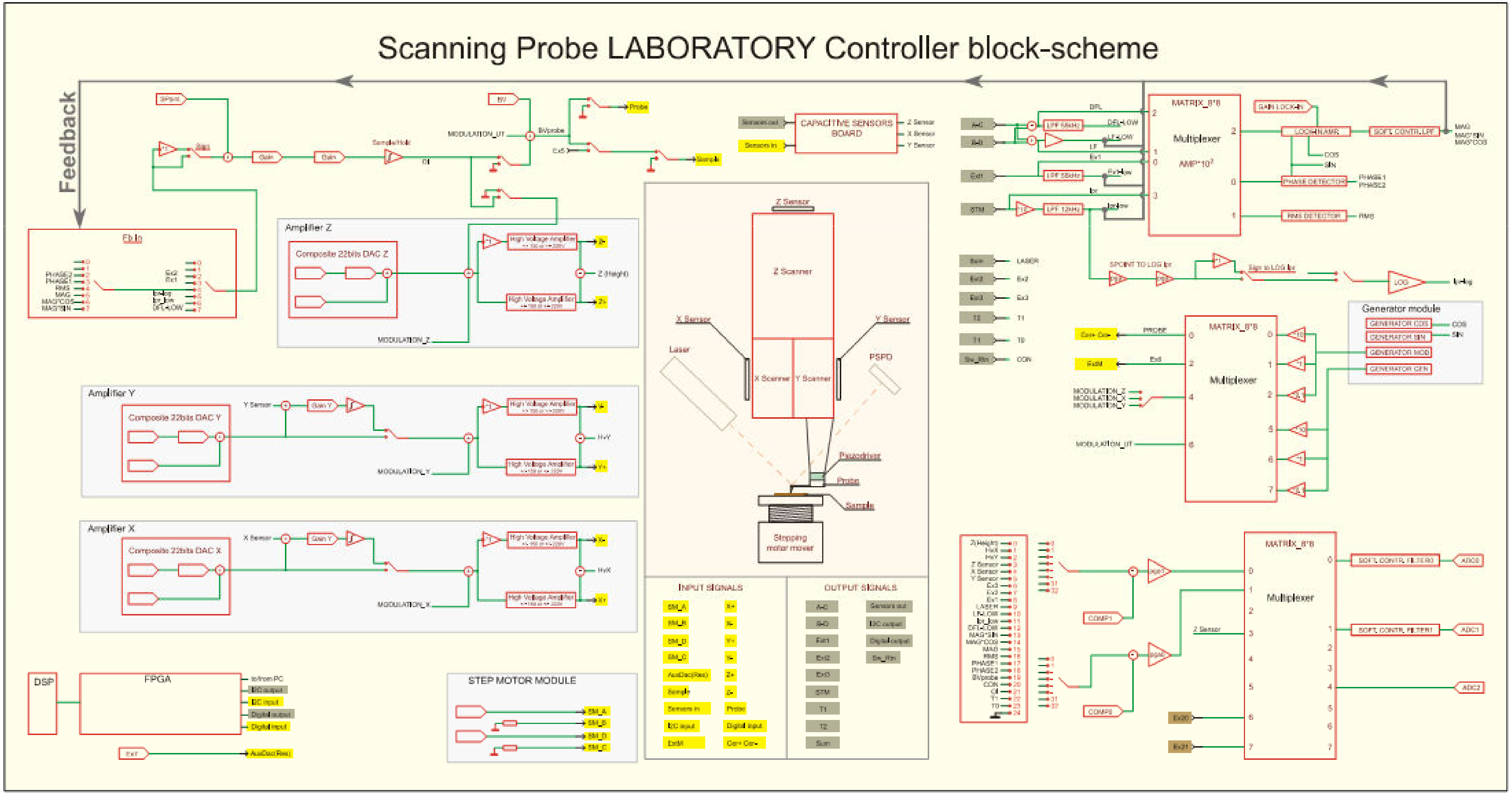
Atomic Force Microscopy
AFM

AFM microscope basic scheme



Detector and Feedback Electronics

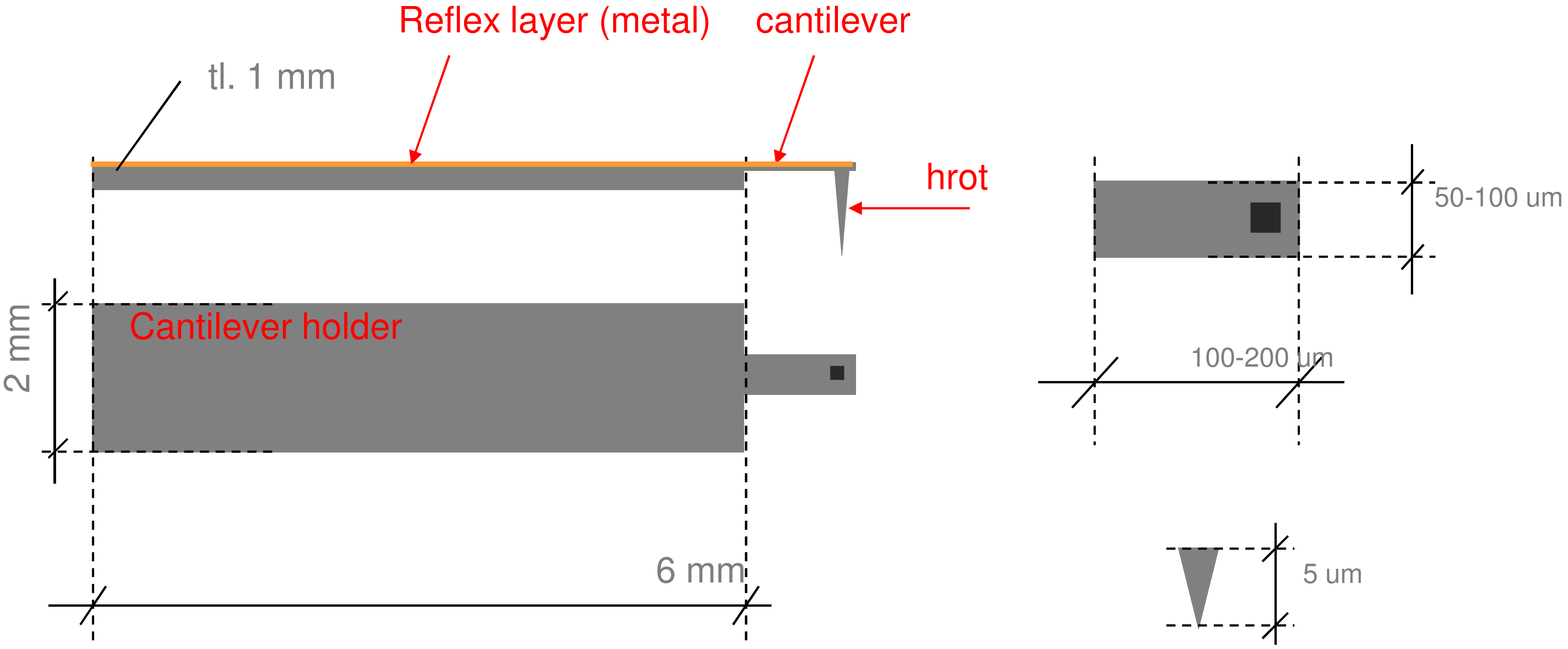
AFM microscope block scheme



CEITEC

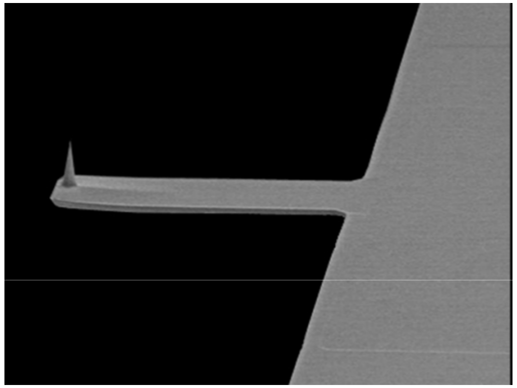
Tip and cantilever

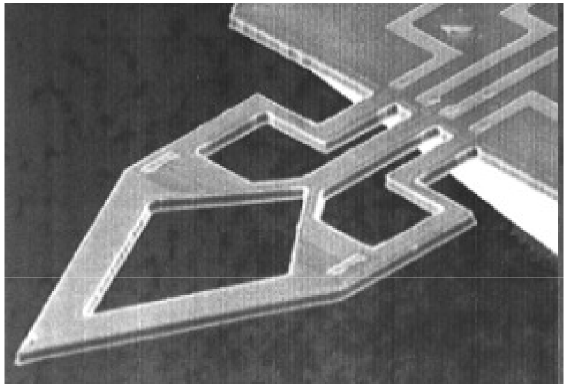
Cantilever and tip

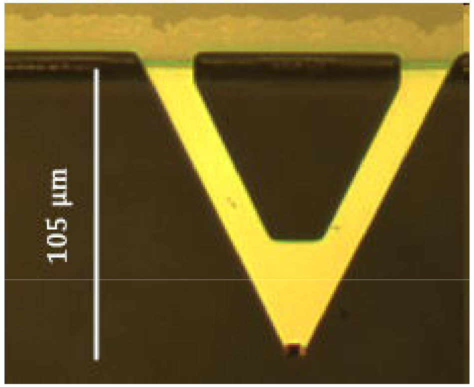


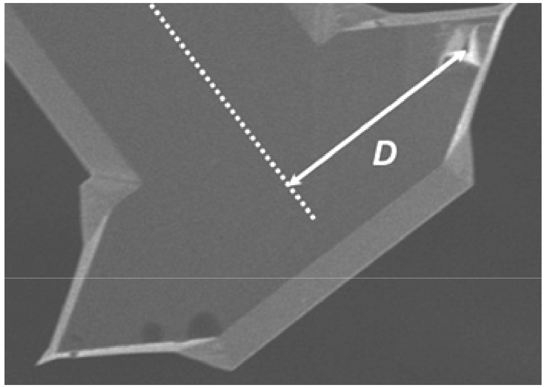
- Cantilever holder is quite universal
- Cantilever and tip – a variety of various types

Cantilevers







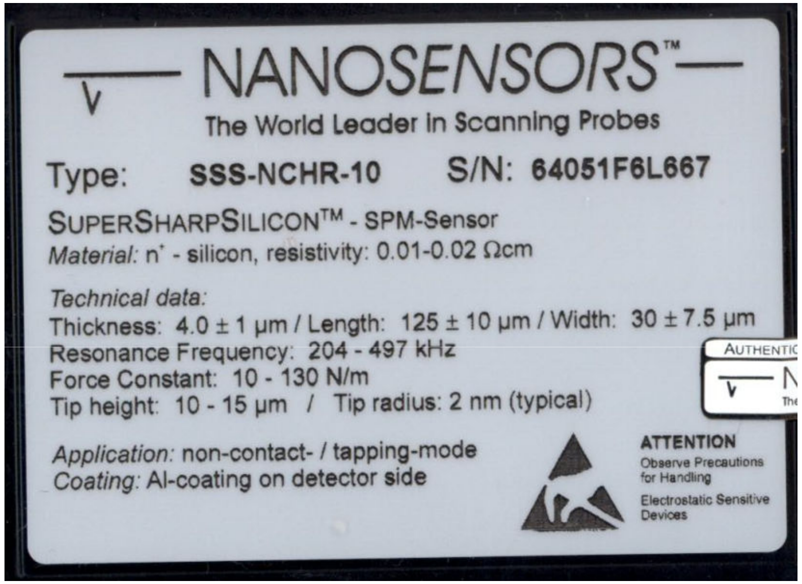


Material properties – Stiffness **Force Constant [N/m]**

Force const.[N/m]	10-130	1-10	0.1-1.0	0.005-0.1
Material	cryst. silicon	pol. silicon	glass	Si_3N_4
Res. f. [kHz]	200-500	100-200	15-100	1-20

Special applications – conductive, colloid, magnetic, tip less, …

Cantilever
characterization you may find on box



NANOSENSORS™
The World Leader in Scanning Probes

Type: **SSS-NCHR-10** S/N: **64051F6L667**

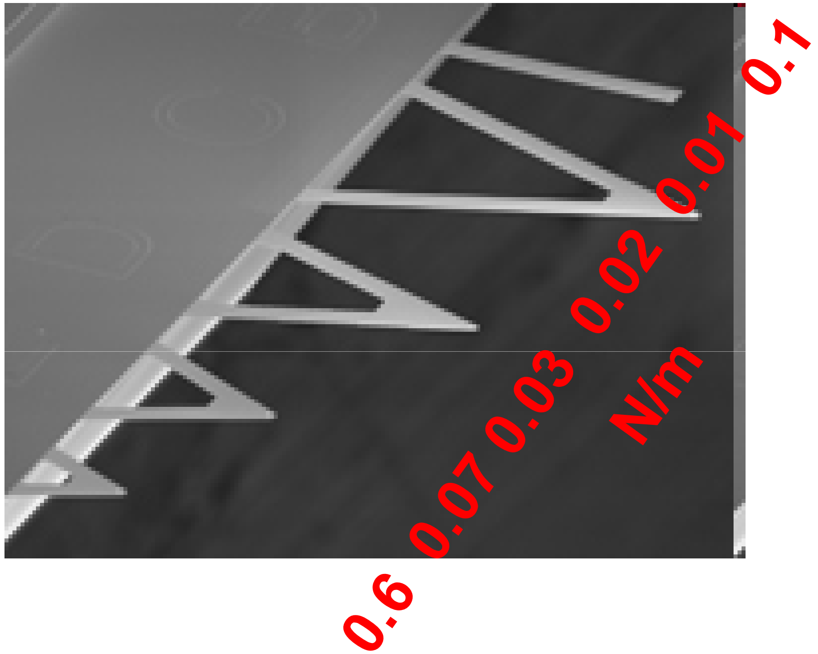
SuperSharpSilicon™ - SPM-Sensor
Material: n^+ - silicon, resistivity: 0.01-0.02 Ωcm

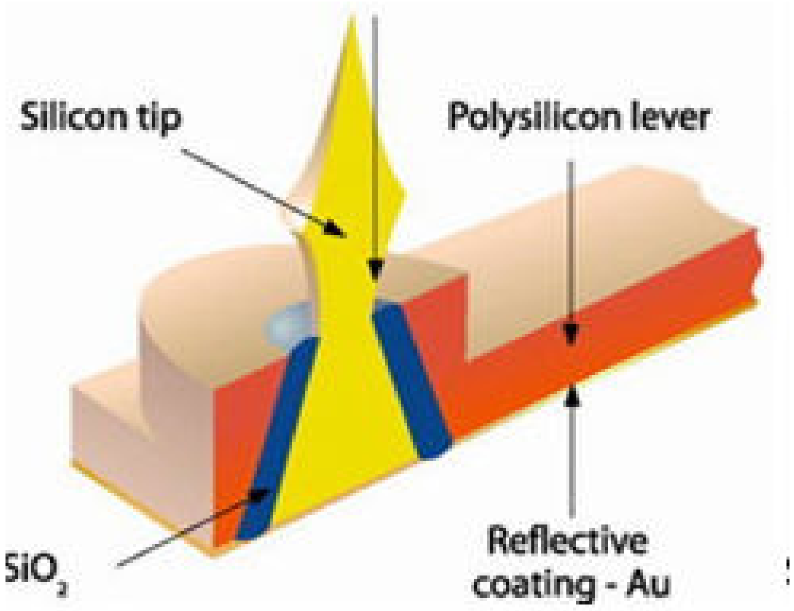
Technical data:
Thickness: 4.0 ± 1 µm / Length: 125 ± 10 µm / Width: 30 ± 7.5 µm
Resonance Frequency: 204 - 497 kHz
Force Constant: 10 - 130 N/m
Tip height: 10 - 15 µm / Tip radius: 2 nm (typical)

Application: non-contact- / tapping-mode
Coating: Al-coating on detector side

ATTENTION
Observe Precautions for Handling
Electrostatic Sensitive Devices

Cantilever field
choose the one you like/need





AFM probes (**micro)fabrication** is quite complex

Tip properties

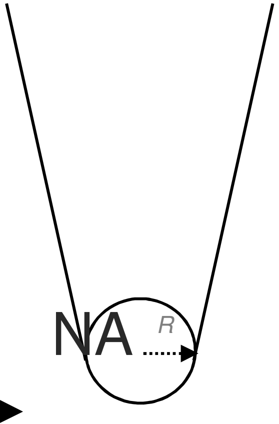
Shape – Curvature Radius *R [nm]*

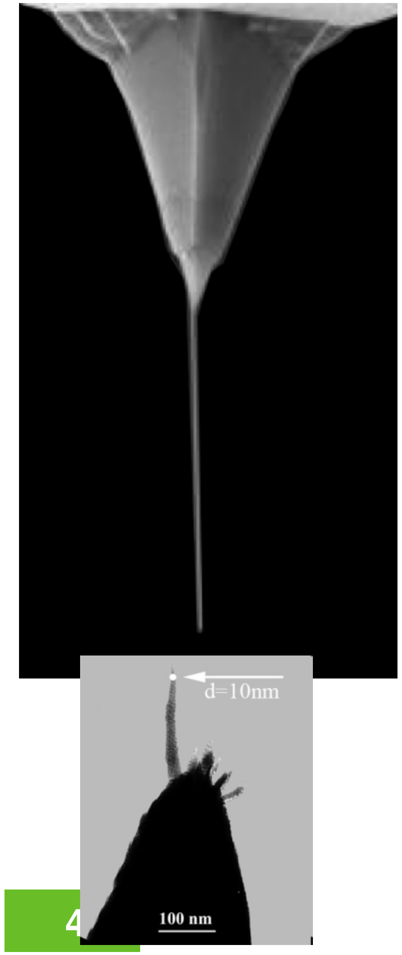
R 1 nm — Supersharp

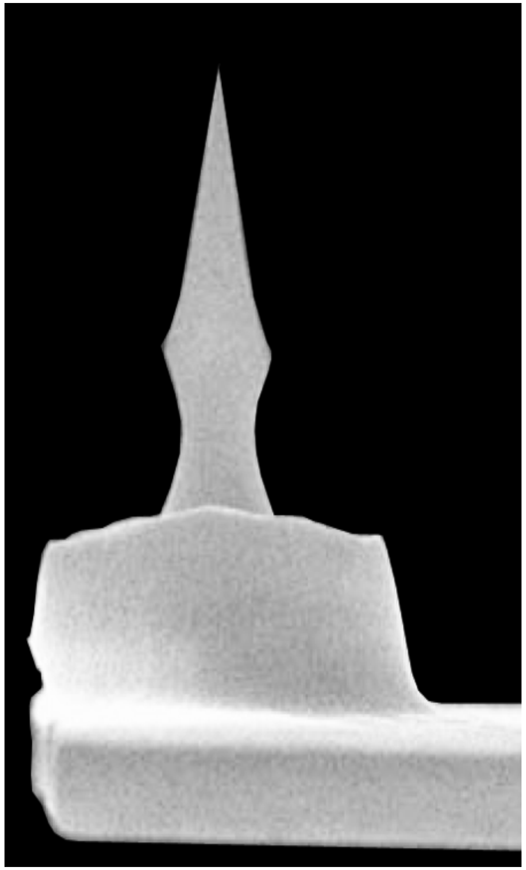
10 nm — Standard

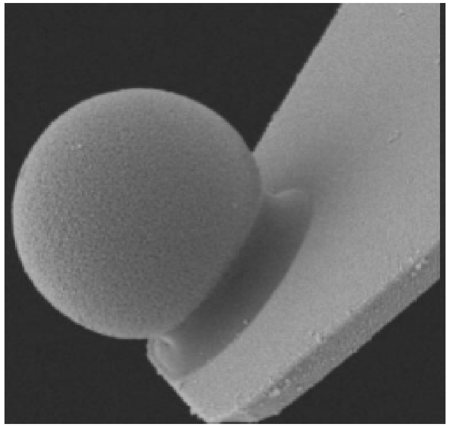
100 nm — Special app.

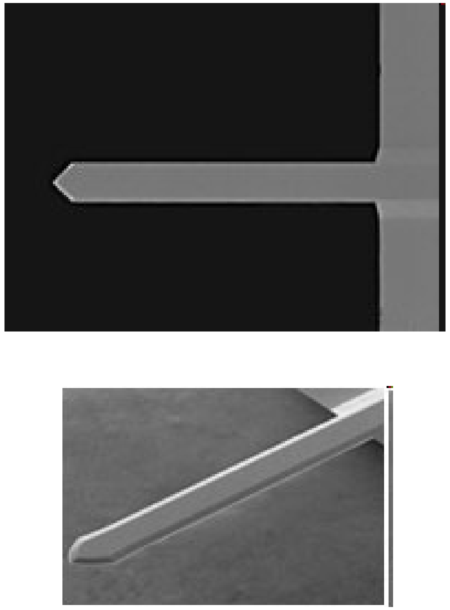
NA — Tip less



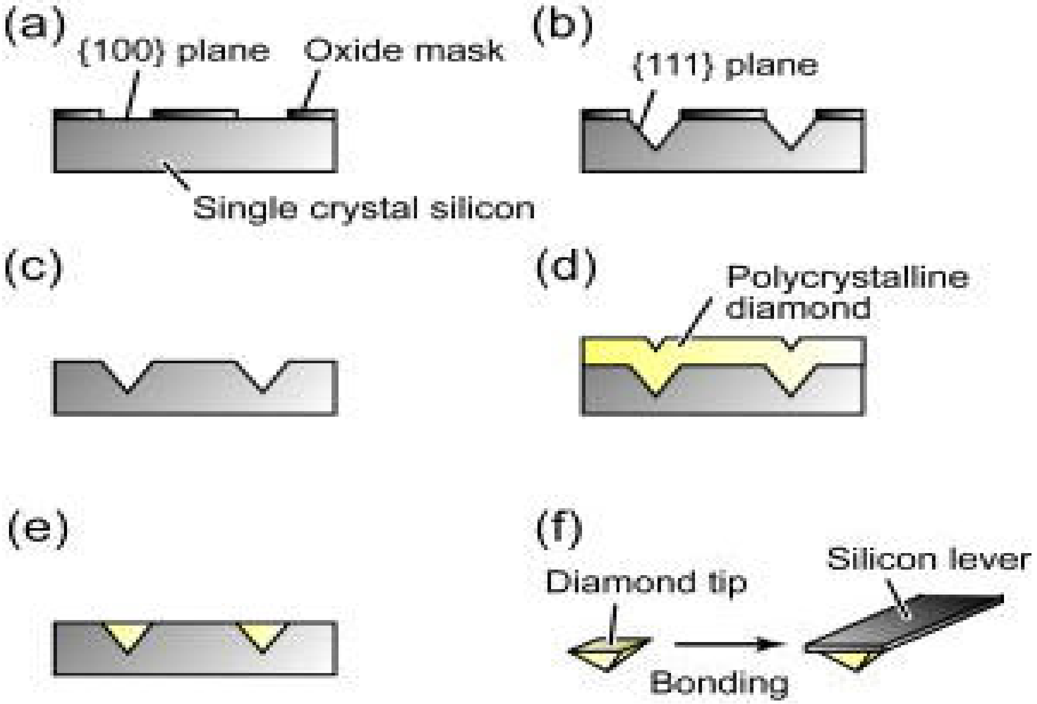




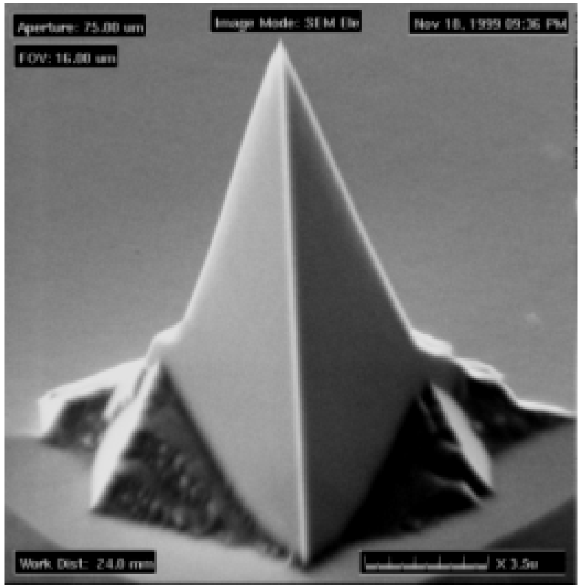


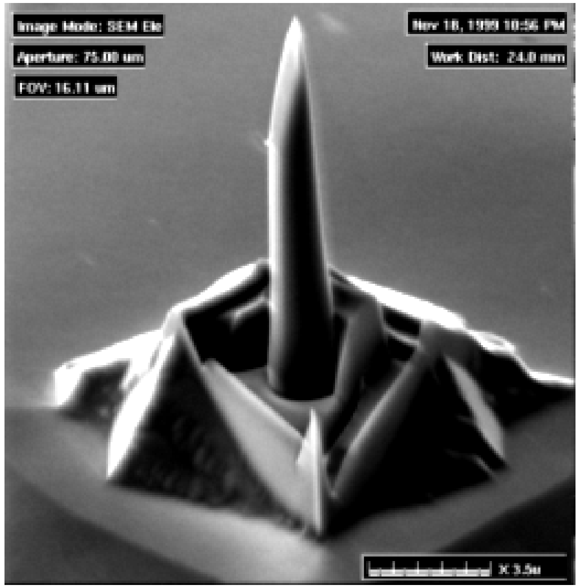


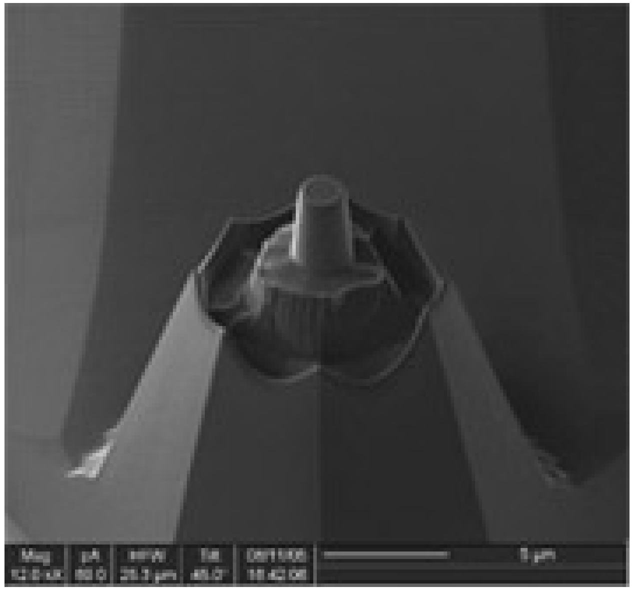
Cantilever **fabrication**



FIB (Focus Ion Beam) post-fabrication of AFM probes (tip)

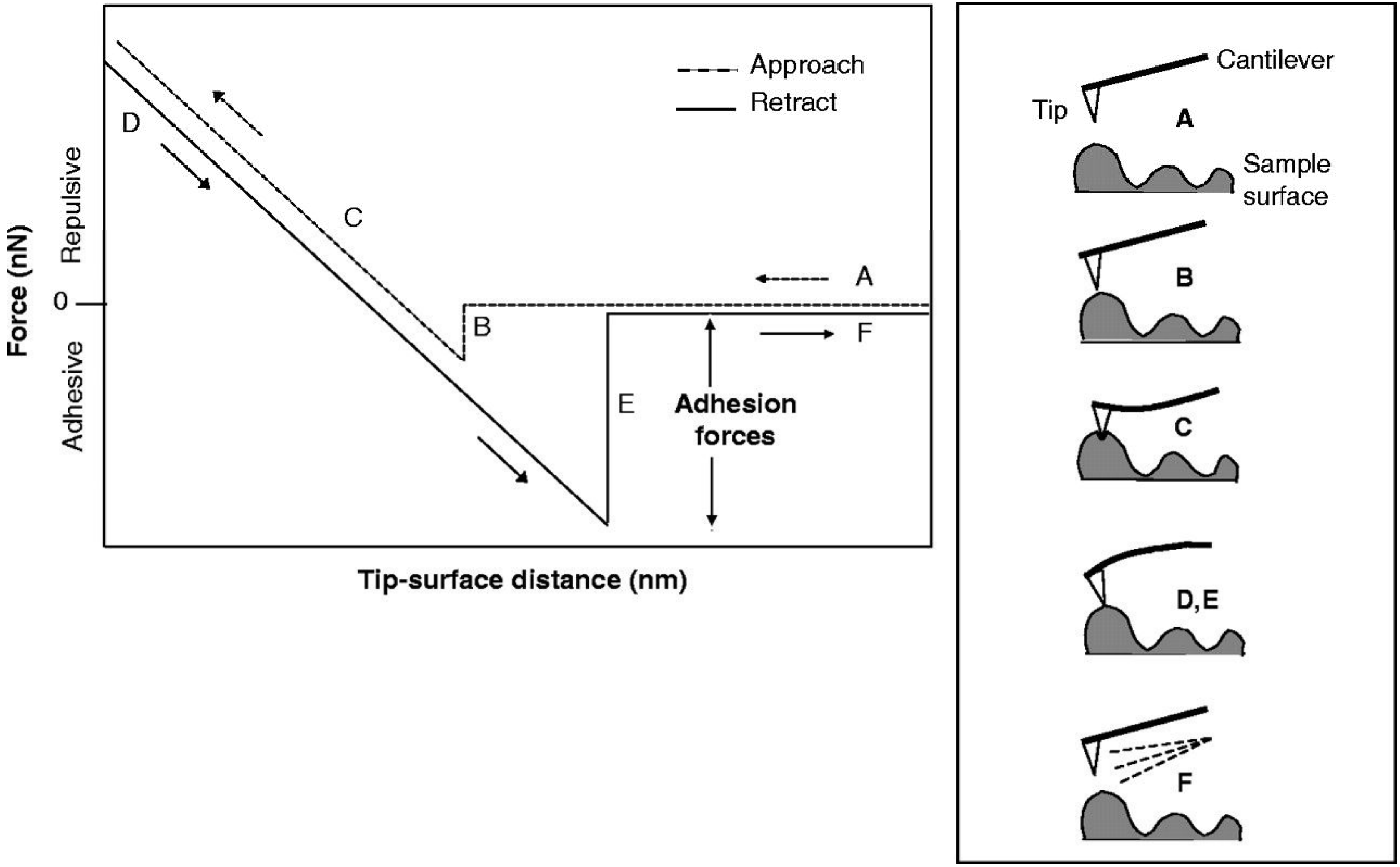






Plateau Tip

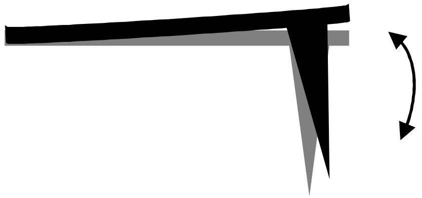
Idealized force-distance curve describing a single approach-retract cycle of the AFM tip, which is continuously repeated during surface scanning.



Victor Shahin et al. J Cell Sci 2005;118:2881-2889

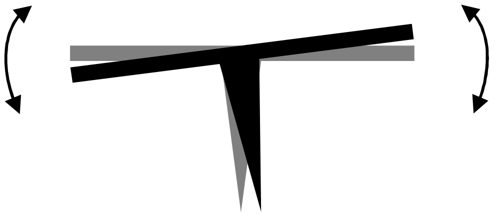
Cantilever bending – how to detect

Contact with surface

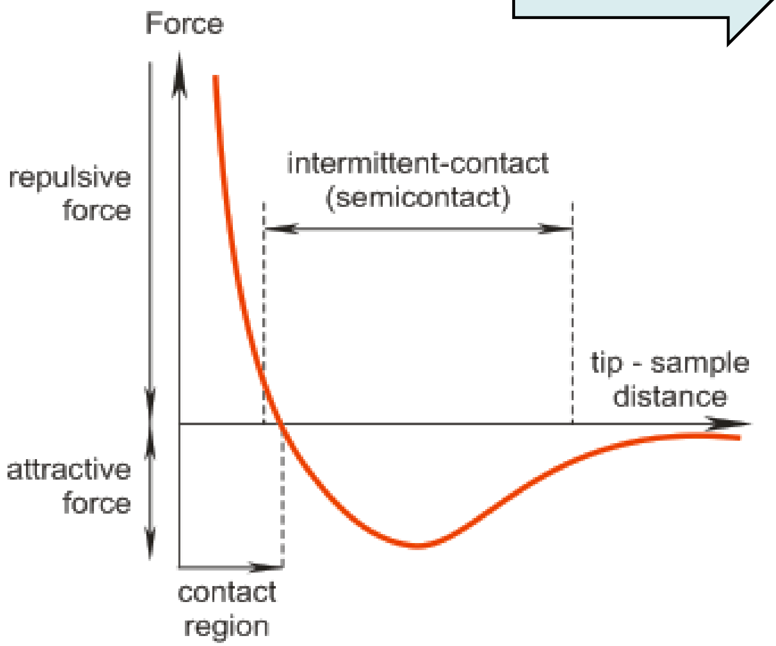


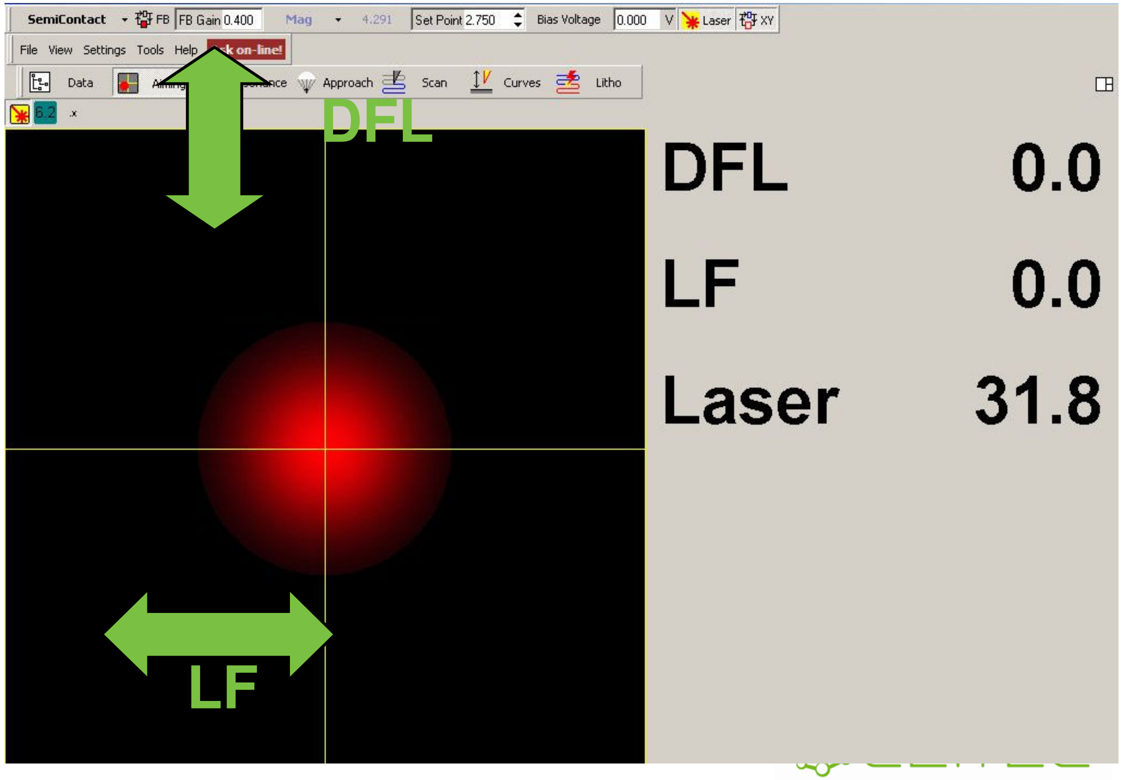
(**DFL** deflection)

Torsion forces (**LF** latheral forces)



Change of cantilever properties (DFL/LF) is detected by laser beam





FB Gain 0.400

Set Point 2.750

Bias Voltage 0.000 V

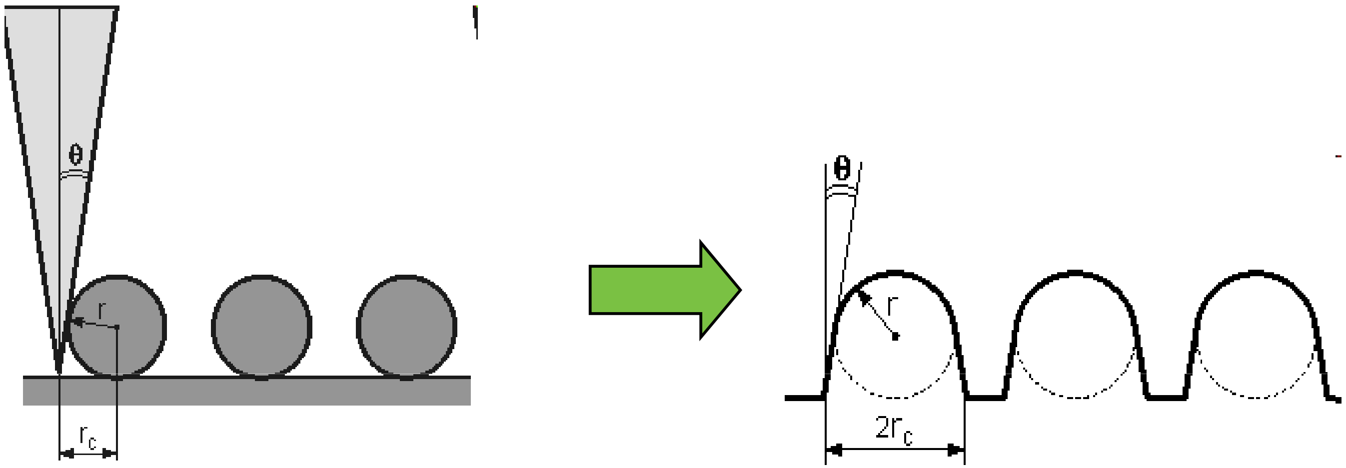
DFL 0.0

LF 0.0

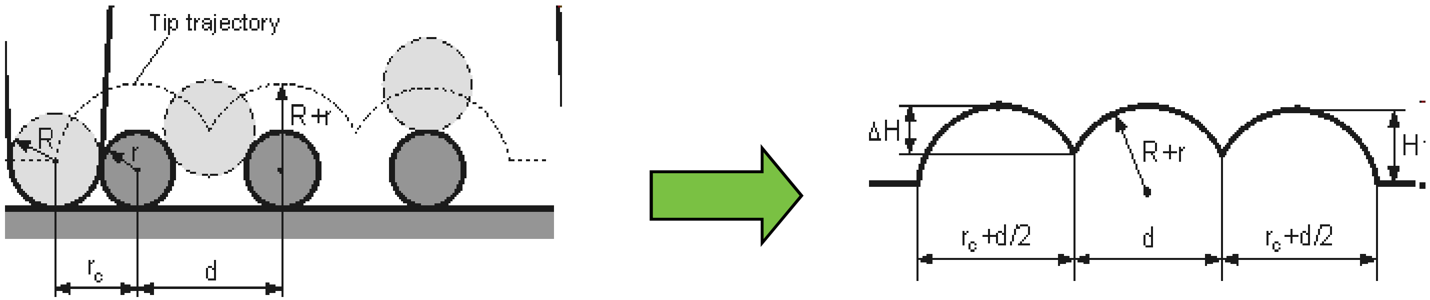
Laser 31.8

Curvature radius (R) effect

SuperSharp tip
= real image



Standard tip
= R ~ 5-10nm



Blunt tip
= affecting real shape
and size