

# Lecture 16.

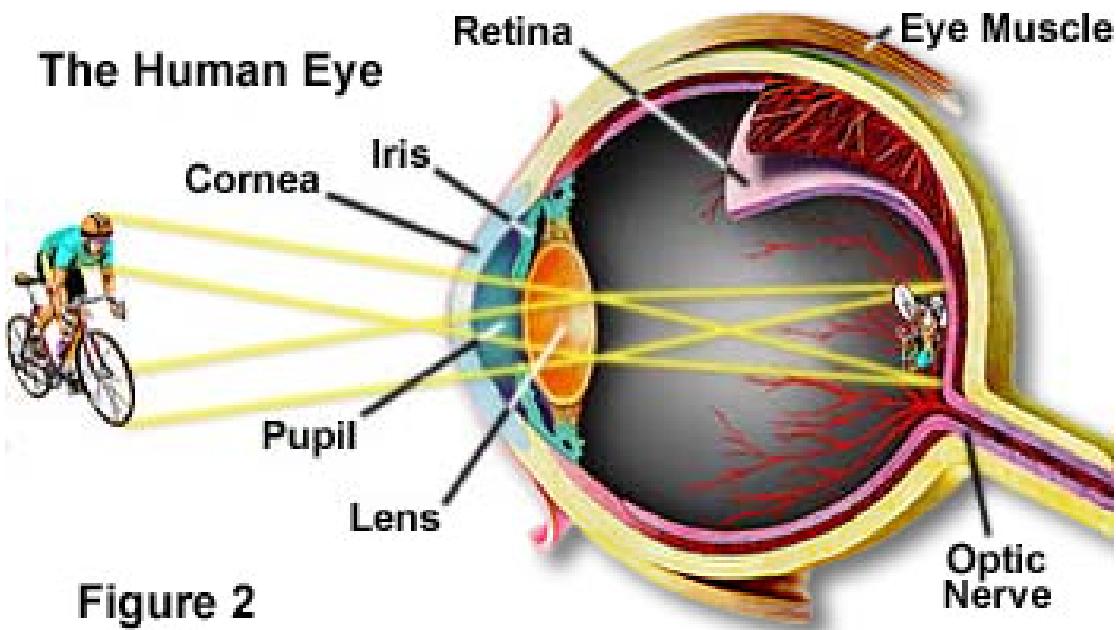
Methods of surface  
characterization:

Scanning probe microscopy

# Method of surface characterization:

- Optical Microscopy
- Fluorescence microscopy
- Scanning Electron Microscopy
- Transmission Electron microscopy
- X-ray diffraction
- Small angle X-ray scattering
- Scanning Probe Microscopy

# Image forms in the eye

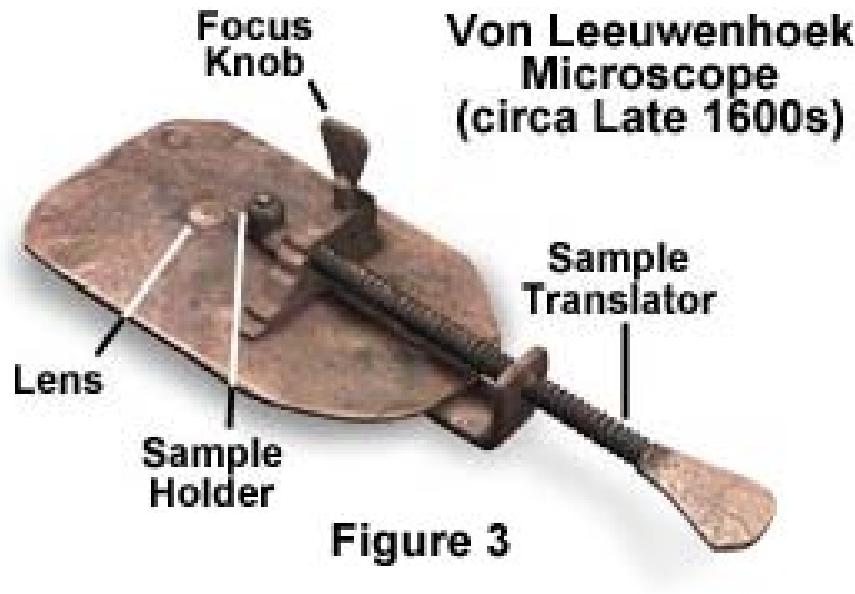


**Figure 2**

For an image to be seen clearly, it must be focused – eye lens.

The front of the eye (see Figure 2), including the iris, the curved cornea, and the lens are respectively the mechanisms for admitting light and focusing it on the retina.

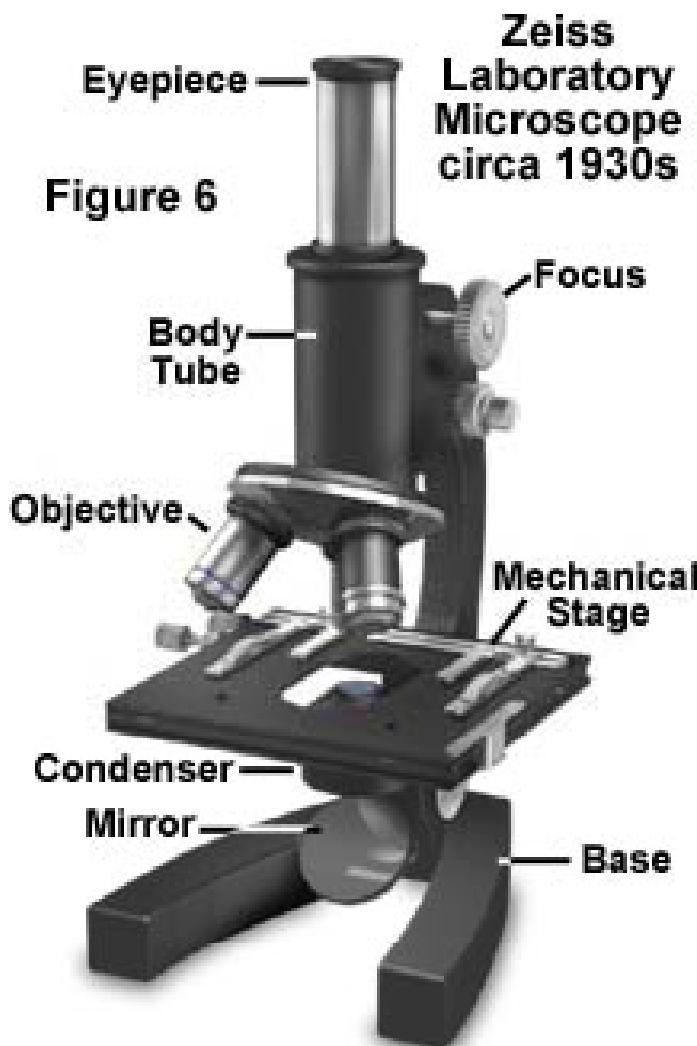
# Microscopes



The "simple microscope" or magnifying glass reached its highest state of perfection, in the 1600's, in the work of Anton von Leeuwenhoek who was able to see single-celled animals (which he called "animalcules") and even some larger bacteria with a simple microscope similar to the one illustrated in Figure 3. The image produced by such a magnifier, held close to the observer's eye, appears as if it were on the same side of the lens as the object itself. Such an image, seen as if it were ten inches from the eye, is known as a virtual image and cannot be captured on film.

- Microscopes are instruments designed to produce magnified visual or photographic images of objects too small to be seen with the naked eye. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera. This group of instruments includes not only multiple-lens (compound microscopes) designs with objectives and condensers, but also very simple single lens instruments that are often hand-held, such as a loupe or magnifying glass.

# Zeiss microscope



A typical microscope of the end of 19<sup>th</sup> century is the Zeiss Laboratory

- Ernst Abbe together with Carl Zeiss published a paper in 1877 defining the physical laws that determined resolving distance of an objective. Known as **Abbe's Law** :

$$d = \frac{\lambda}{2 n \sin \theta}$$

- “minimum resolving distance (*d*) is related to the wavelength of light (*lambda*) divided by the Numeric Aperture, which is proportional to the angle of the light cone (*theta*) formed by a point on the object, to the objective”.

# Modern microscopes

**Olympus Provis AX 70  
(circa 1998)**

**Figure 7**



Olympus Provis AX70 research microscope. This microscope represents the latest state-of-the-art design that incorporates multiple illuminators (episcopic and diascopic), analyzers and polarizers, DIC prisms, fluorescence attachments, and phase contrast capabilities. The photomicrography system is the ultimate in sophistication and performance featuring spot measurement, automatic exposure control, and zoom magnification for flexible, easy framing.

# Magnification

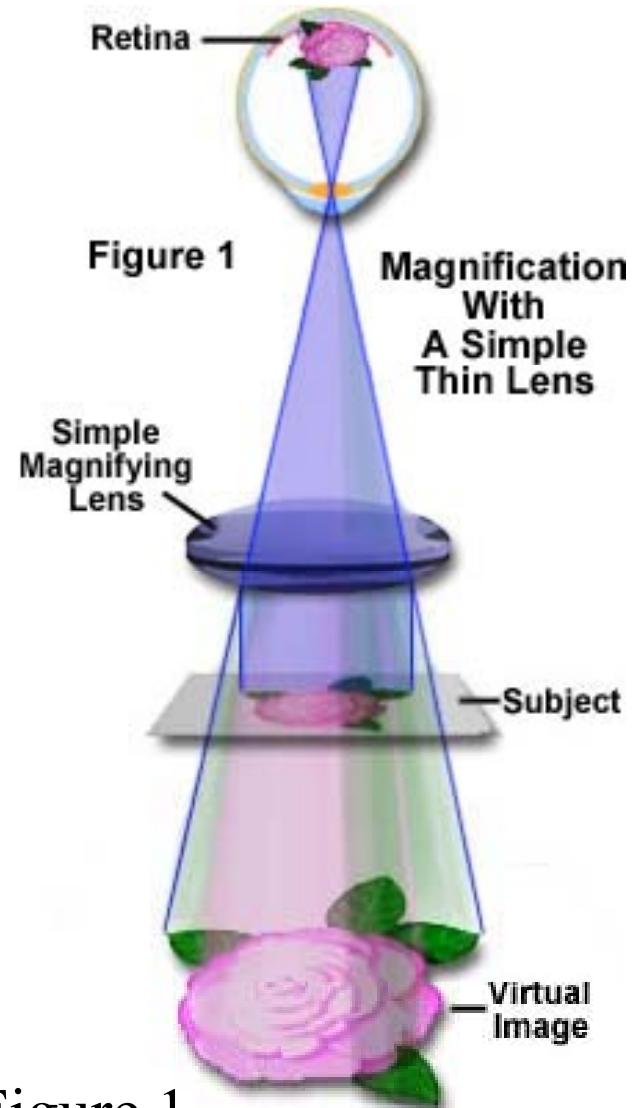


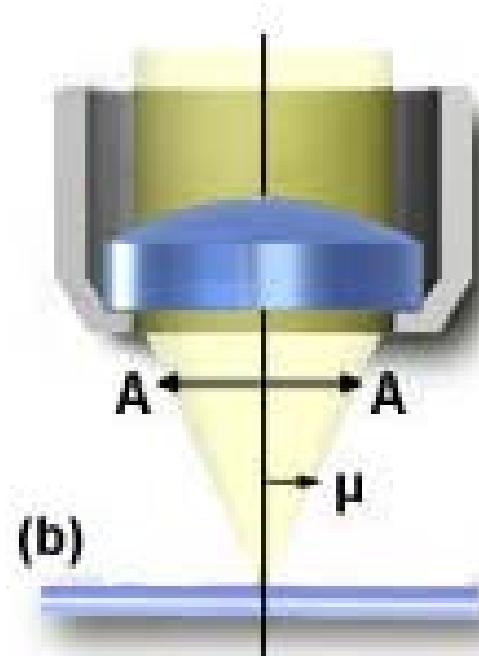
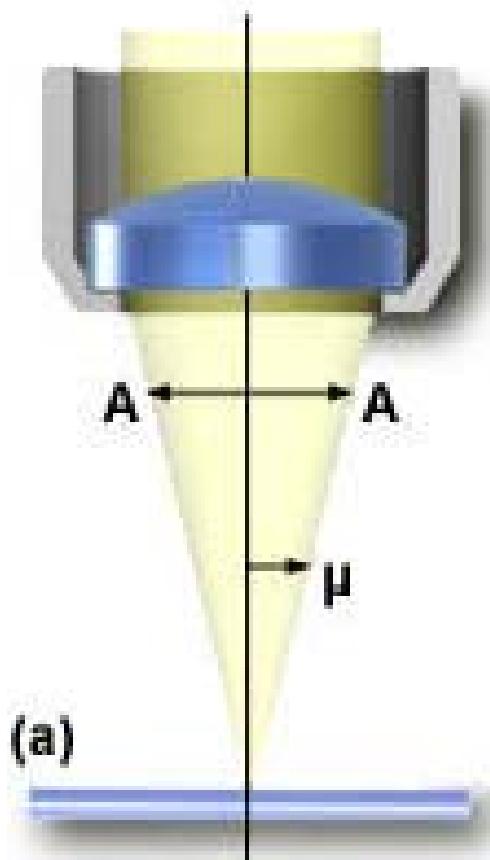
Figure 1

A simple microscope or magnifying glass (lens) produces an image of the object upon which the microscope or magnifying glass is focused. Simple magnifier lenses are bi-convex, meaning they are thicker at the center than at the periphery. The image is perceived by the eye as if it were at a distance of 10 inches or 25 centimeters (the **reference**, or **traditional** or **conventional** viewing distance).

Figure 1 presents an illustration of how a simple magnifying lens operates. The object (a rose) is being viewed with a simple bi-convex lens. Light reflected from the rose enters the lens in straight lines as illustrated in Figure 1. This light is refracted and focused by the lens to produce a virtual image on the retina. The image of the rose is magnified because we perceive the actual size of the object (the rose) to be at infinity because our eyes trace the light rays back in straight lines to the virtual image (Figure 1).

- The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance.

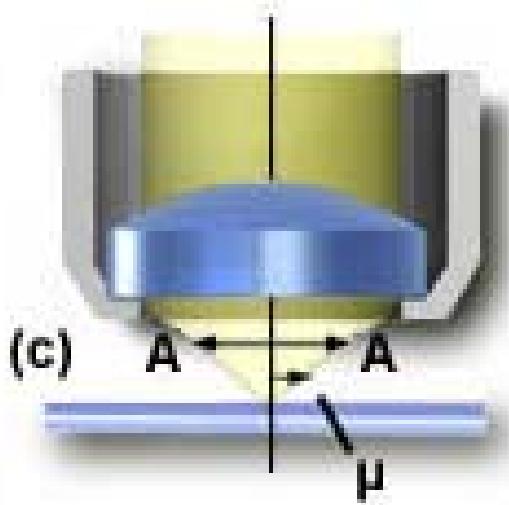
$$(NA) = n \sin \mu$$



**Figure 2**

$$NA = (n)\sin(\mu)$$

(a)  $\mu = 7^\circ$  NA = 0.12  
 (b)  $\mu = 20^\circ$  NA = 0.34  
 (c)  $\mu = 60^\circ$  NA = 0.87

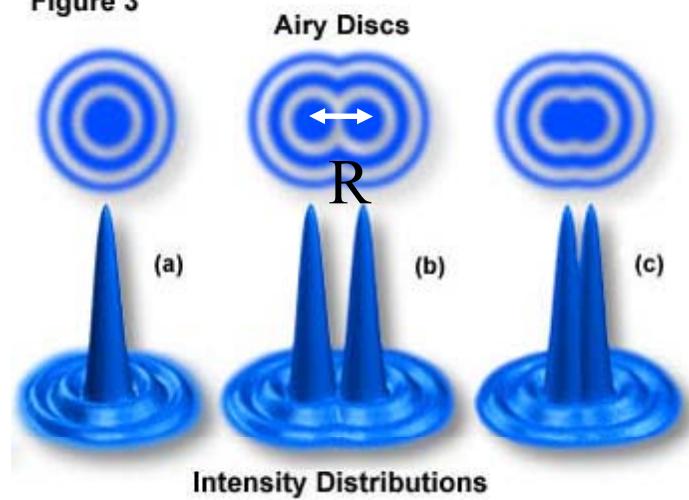


The higher the numerical aperture of the total system, the better the resolution. The wavelength spectrum of light used to image a specimen is also a determining factor in resolution. Shorter wavelengths are capable of resolving details to a greater degree than are the longer wavelengths.

$$R = \lambda / 2NA - \text{resolution is limited by } \lambda / 2$$

Where **R** - resolution (the smallest resolvable distance between two objects), **NA** - numerical aperture,  $\lambda$ - wavelength, **NA**- the objective numerical aperture,

Figure 3



Numerical Aperture and Airy Disc Size

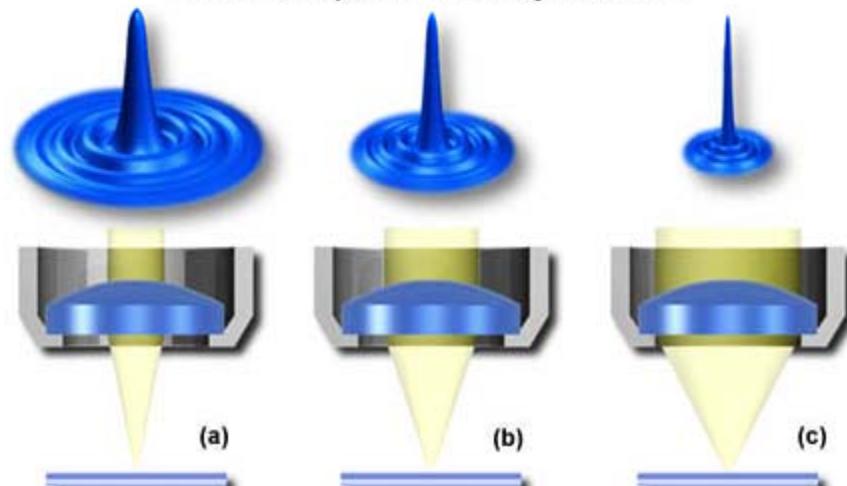
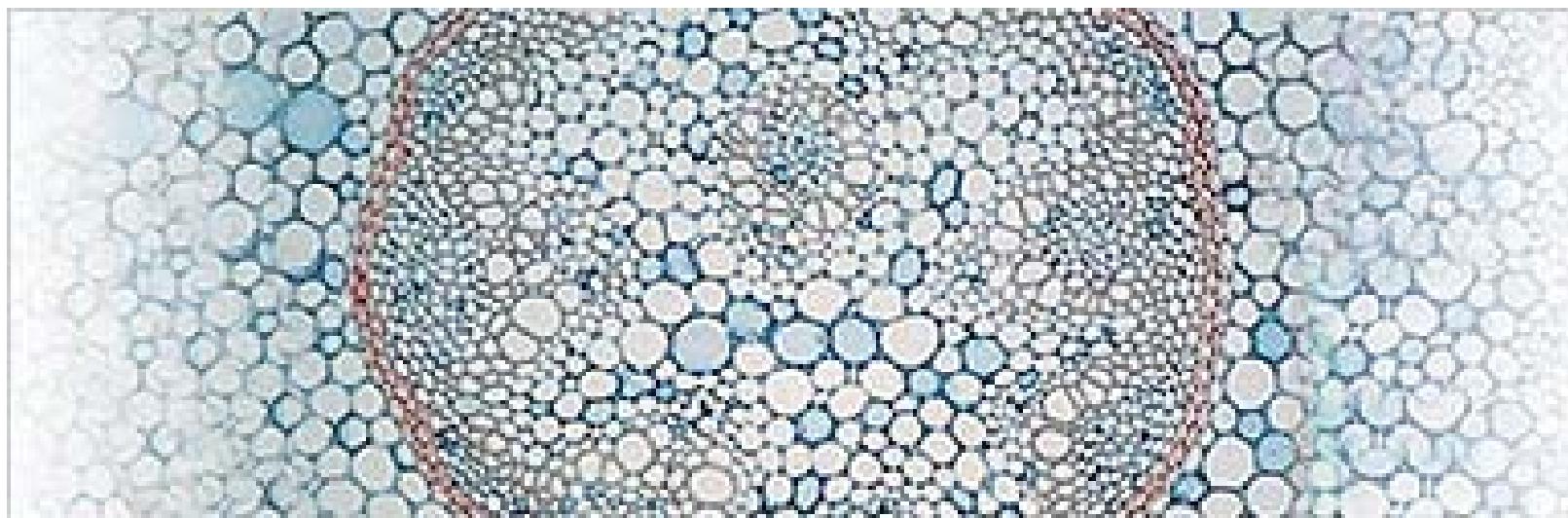


Figure 4

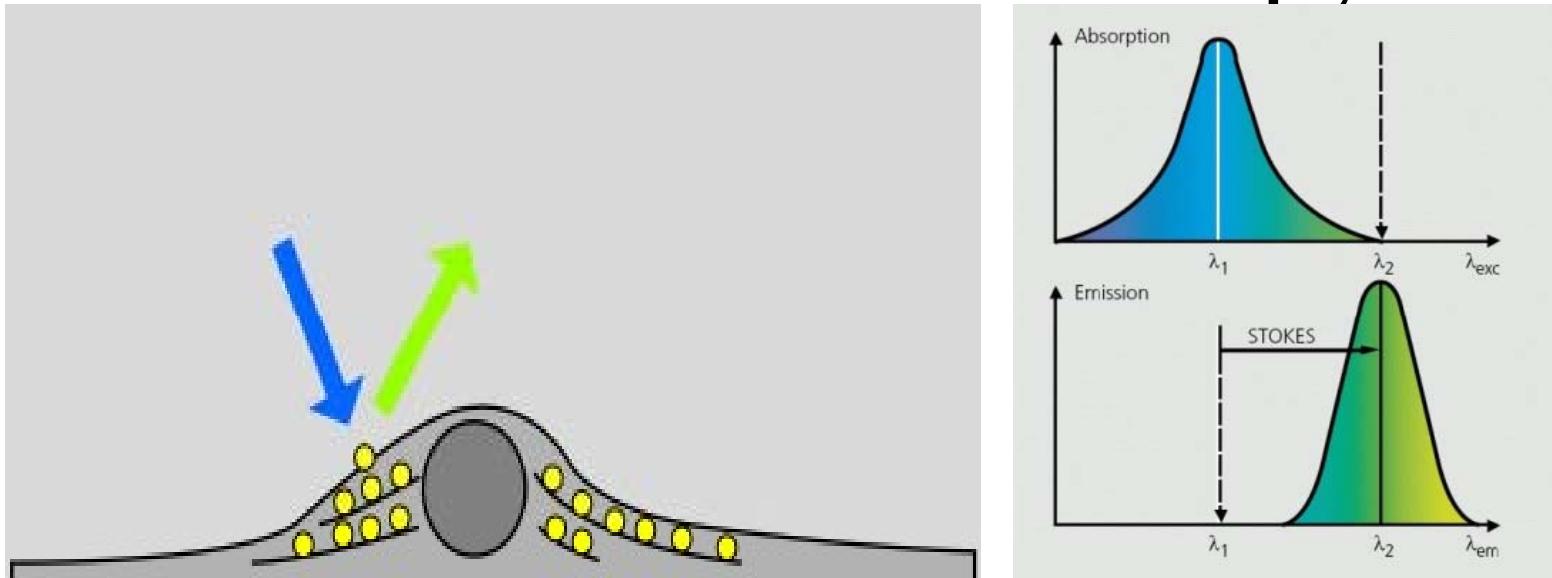
Total magnification = Objective magnification · Eyepiece magnification



The section of the plant stalk

the overall magnification of the microscope is 100x.  
(10x objective with 10x eyepiece).

# Fluorescence Microscopy



- fluorescence microscopy is capable of imaging the distribution of a single molecular species based solely on the properties of fluorescence emission. Thus, using fluorescence microscopy, the precise location of intracellular components labeled with specific fluorophores can be monitored, as well as their associated diffusion coefficients, transport characteristics, and interactions with other biomolecules.

# Fluorescence Microscopy

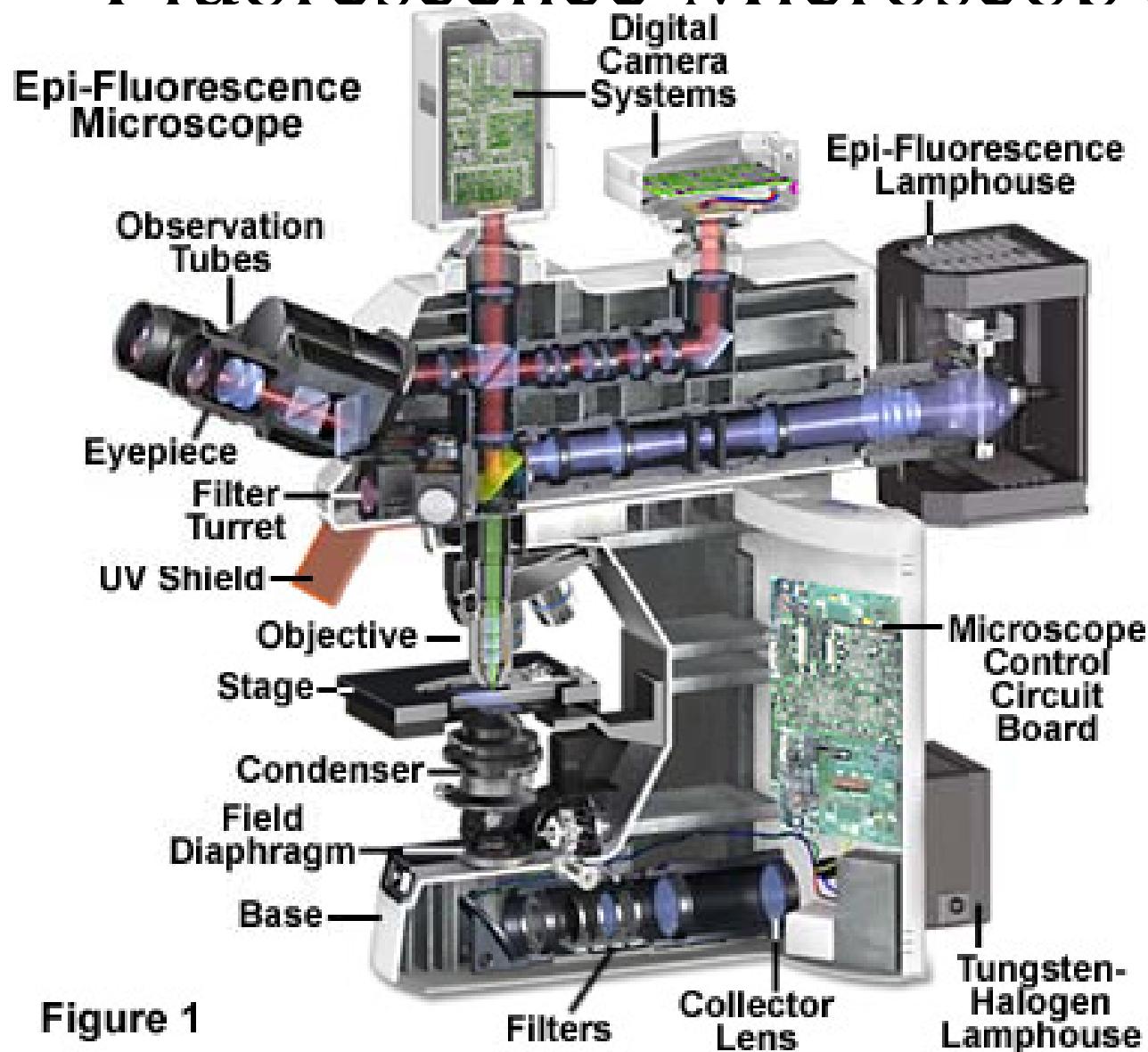
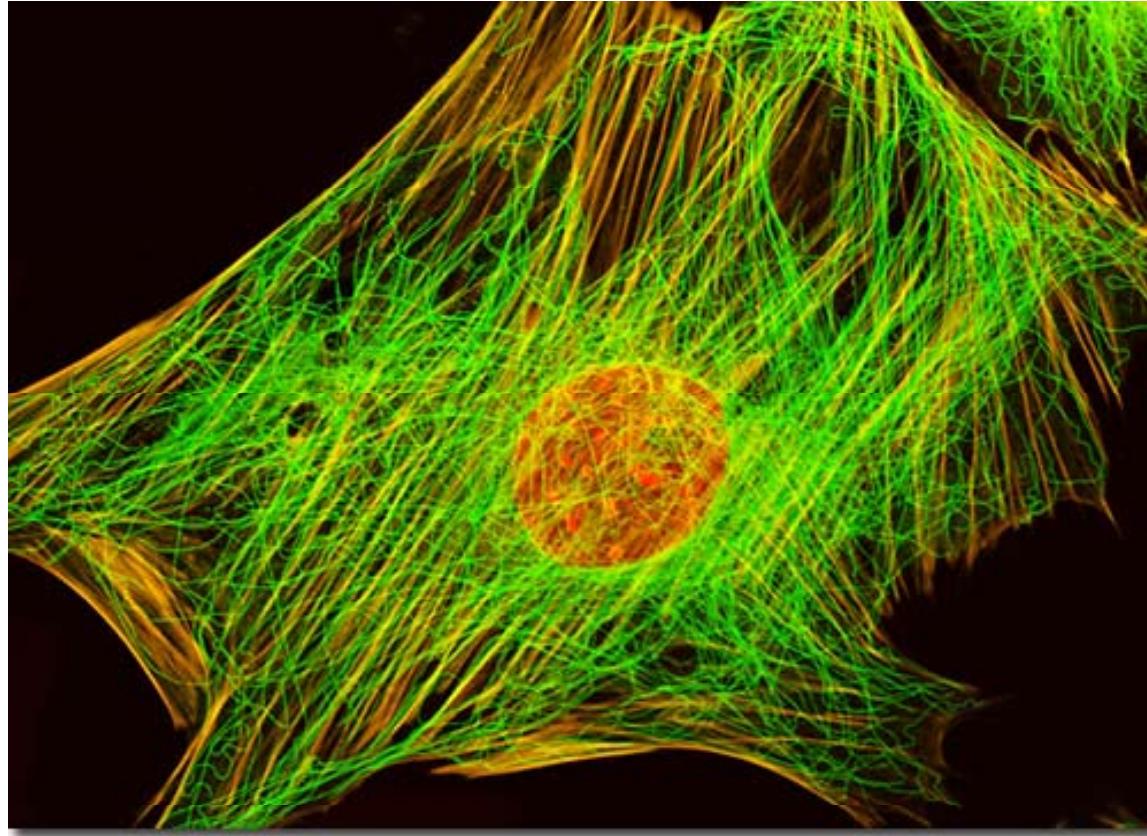


Figure 1

## Example: Embryonic Swiss Mouse Fibroblast Cells



<http://www.olympusconfocal.com/gallery/cells/3t3/3t3large.html>

The isolated 3T3 cell presented in the digital image above was resident in an adherent culture stained for F-actin with Alexa Fluor 546 conjugated to phalloidin, and for DNA with the red-absorbing dye TO-PRO-3. In addition, the culture was immunofluorescently labeled with Alexa Fluor 488 conjugated to antibodies that target *alpha*-tubulin. Images were recorded with a 60x oil immersion objective using a zoom factor of 2.5 and sequential scanning with the 488-nanometer spectral line of an argon-ion laser, the 543-nanometer line from a green helium-neon laser, and the 633-nanometer line of a red helium-neon laser. During the processing stage, individual image channels were pseudocolored with RGB values corresponding to each of the fluorophore emission spectral profiles.

# Fluorescence and photobleaching

## Photobleaching Rates in Multiply Stained Specimens

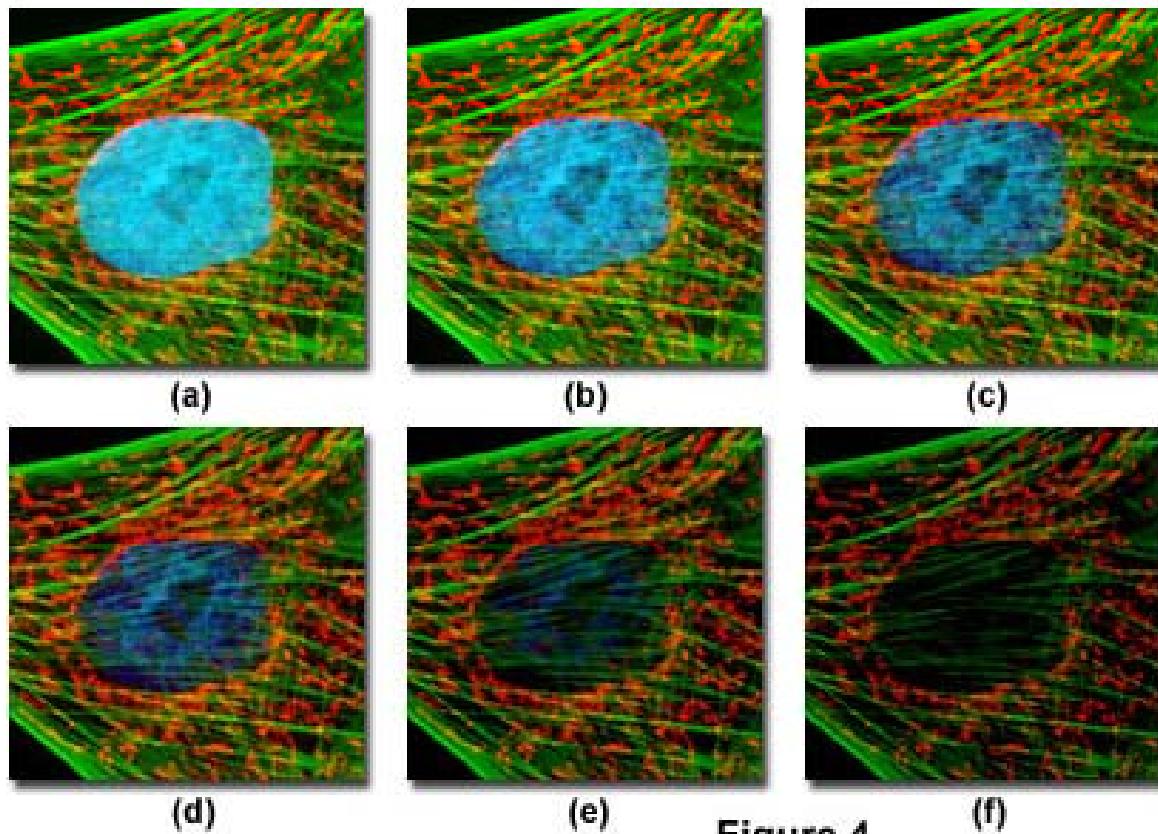


Figure 4

multiply-stained culture of Indian Muntjac deer epidermis fibroblast cells. The nuclei were stained with a bis-benzimidazole derivative (Hoechst 33258; blue fluorescence), while the mitochondria and actin cytoskeleton were stained with MitoTracker Red CMXRos (red fluorescence) and a phalloidin derivative conjugated to Alexa Fluor 488 (green fluorescence), respectively.

Presented in Figure 4 is a typical example of photobleaching (fading). Time points were taken in two-minute intervals using a fluorescence filter combination with bandwidths tuned to excite the three fluorophores simultaneously while also recording the combined emission signals. Note that all three fluorophores have a relatively high intensity in Figure 4(a), but the Hoechst fluorophore (blue) intensity starts to drop rapidly at two minutes and is almost completely gone at 6-8 minutes. The mitochondrial and actin stains are more resistant to photobleaching, but the intensity of both drops significantly over the course of the timed sequence (10 minutes).

# Scanning Electron Microscopy

- Focused e-beam scans the surface
- e interact with the sample surface
- This results to emission of photons and e from the sample surface, which are analyzed
- Imaging at nm scale, samples need to be dried and coated with metal

# Transmission Electron microscopy

- e beam penetrates thin sample
- Becomes deflected or undeflected, scatter
- High resolution
- Chemical information
- Samples need to be dried

# X-ray diffraction

- X-rays are diffracted by crystalline ordered structure
- information about crystalline structure
- Small angle X-ray scattering

# Scanning Probe Microscopy methods

- Allow 3D imaging at subnanometer and atomic resolution
- no diffraction limit as for optical microscopy
- Can image single molecules, not restricted to crystalline specimens
- Simple sample preparation (adsorption to flat support), no coating required
- Biologically friendly environment
- Nanomanipulation
- Nanosensing

# Scanning Probe Microscopy methods

resolution is limited by the sharpness of the probe:

- Atomic Force Microscopy
- Scanning Tunneling Microscopy
- Near Field Optical Scanning Microscopy
- Electrostatic Scanning Probe Microscopy
- Kelvin probe Force Microscopy
- Magnetic Scanning Probe Microscopy
- Ion Conductance Scanning Probe Microscopy
- Electrochemical Scanning Probe Microscopy and Nanolithography

## • STM

- tunneling of electrons between 2 electrodes under electric field
- requires conductive surface
- works in air or vacuum

## • AFM

- van der Waals interaction between tip and sample
- can image non conductive surfaces
- works in air and in liquid media

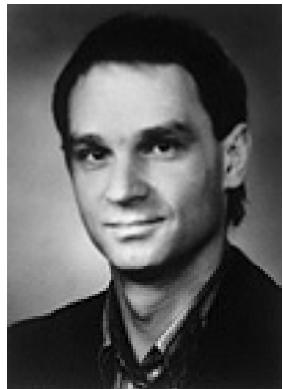
## • NSOM

- interaction of light with the sample at the near-field
- requires fluorescence label
- works in air and in liquid media

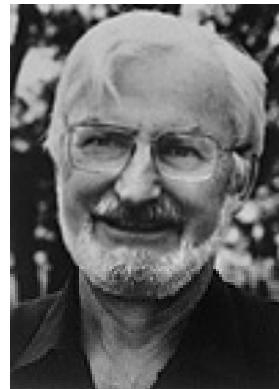
# Scanning Tunneling Microscopy (STM)

Allows for the imaging of the surfaces of metals and semiconductors at the atomic level.

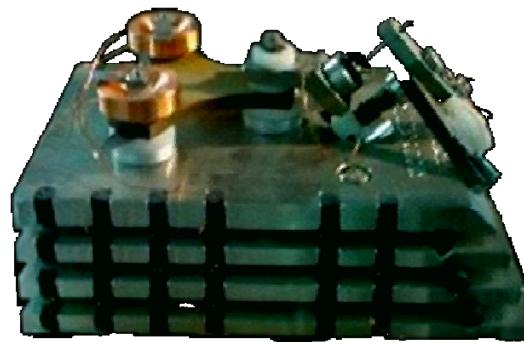
Developed by Gerd Binnig and Heinrich Rohrer at the IBM Zurich Research Laboratory in 1982.



Binnig



Rohrer

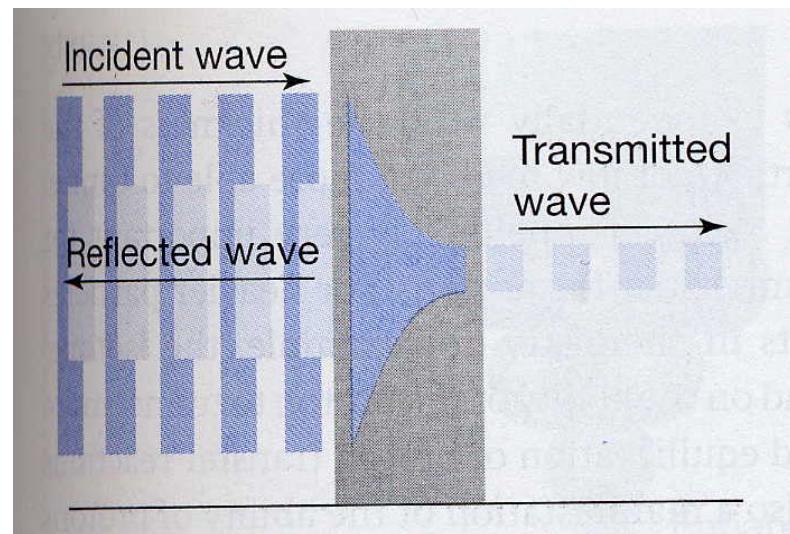
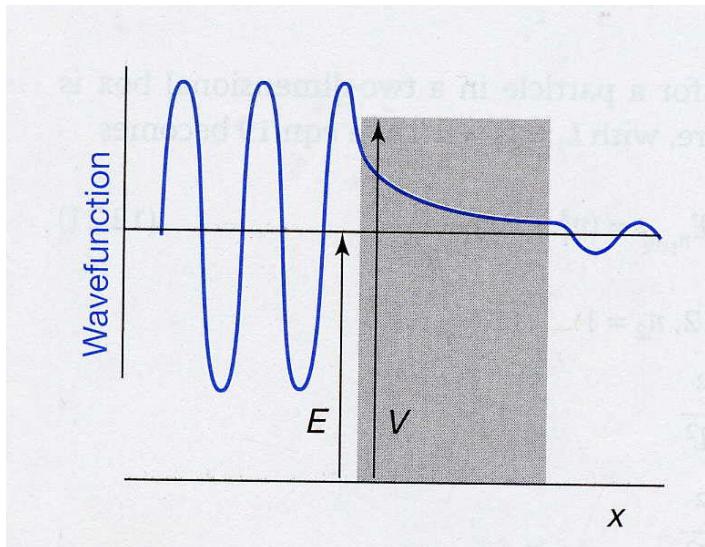


Pocket-size STM  
Ch.Gerber, G.  
Binnig and H.  
Rohrer, IBM  
Rueschlikon

1986 Nobel Prize in physics for developing STM.

STM has fathered a host of new atomic probe techniques: Atomic Force Microscopy, Magnetic Force Microscopy, Scanning Acoustic Microscopy, etc.

# An Introduction to Quantum Mechanical Tunneling

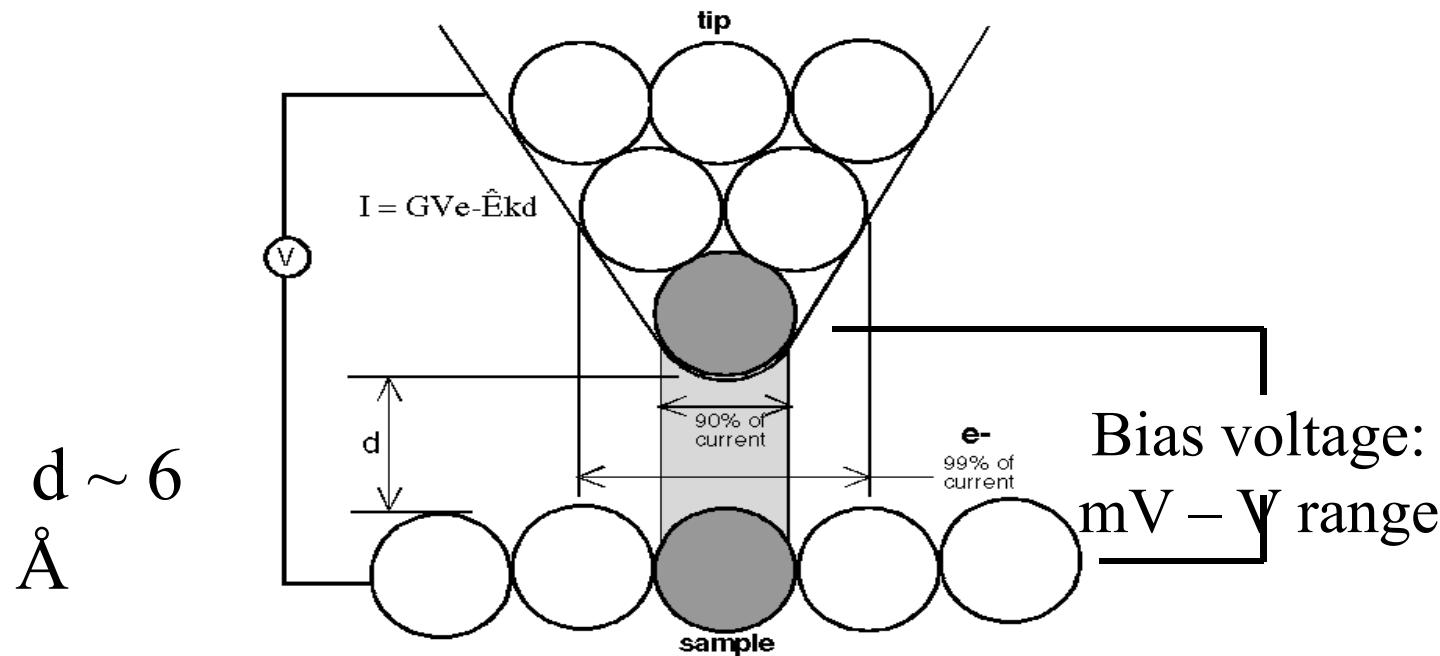


Quantum mechanics allows a small particle, such as an electron, to overcome a potential barrier larger than its kinetic energy.

Tunneling is possible because of the wave-like properties of matter.

Transmission Probability:  $\sim e^{-2\kappa L}$

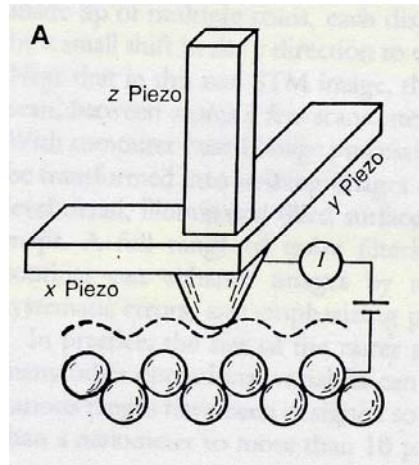
# Basic Principles of STM



Electrons tunnel between the tip and sample, a small current  $I$  is generated (10 pA to 1 nA).

$I$  proportional to  $e^{-2kd}$ ,  $I$  decreases by a factor of 10 when  $d$  is increased by 1 Å.

# Instrumental Design: Controlling the Tip

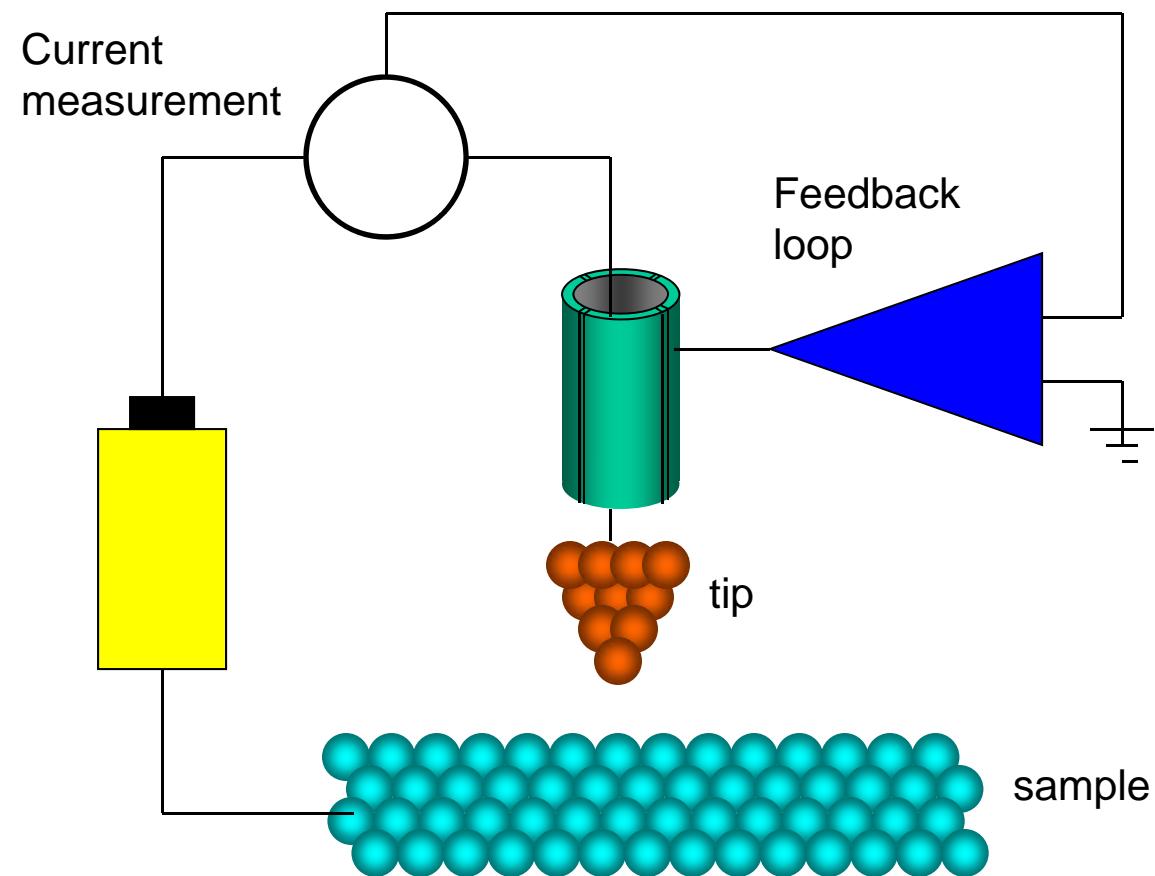


Precise tip control is achieved with  
Piezoelectrics

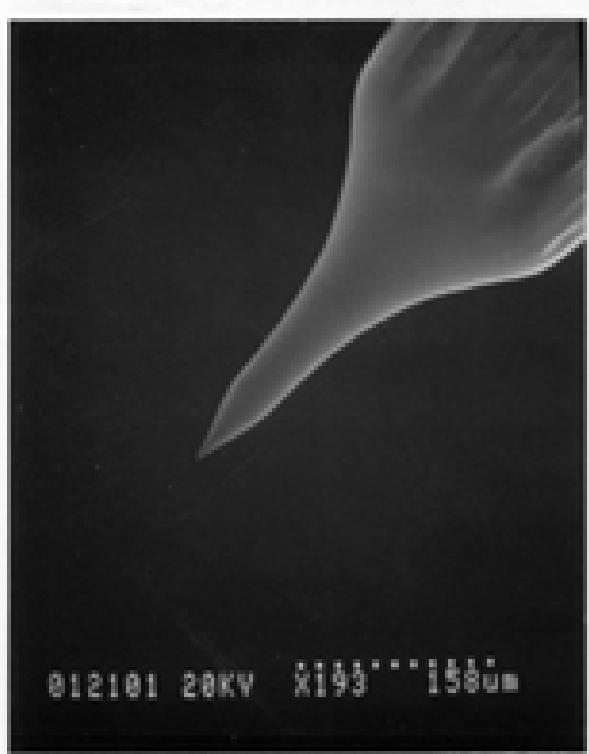
Displacement accurate to  $\pm .05 \text{ \AA}$

The principle is to measure the displacement of the tip while holding the tunneling current constant. The measured displacement allows a computer to generate a map of surface topography.

# STM – Experimental schematic



# Tips



Cut platinum – iridium wires

Tungsten wire electrochemically etched

Tungsten sharpened with ion milling

Best tips have a point a few hundred nm wide

# Applications of STM

Surface Structure: Compare to bulk structure

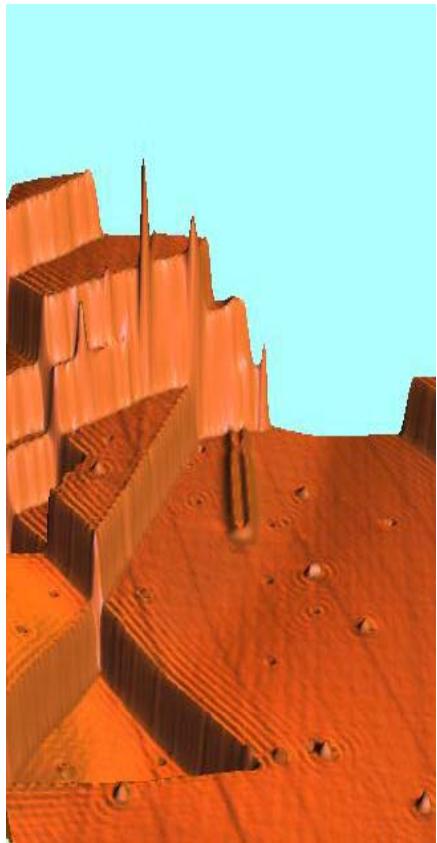
Semiconductor surface structure, Nanotechnology,  
Superconductors, etc.

Metal-catalyzed reactions

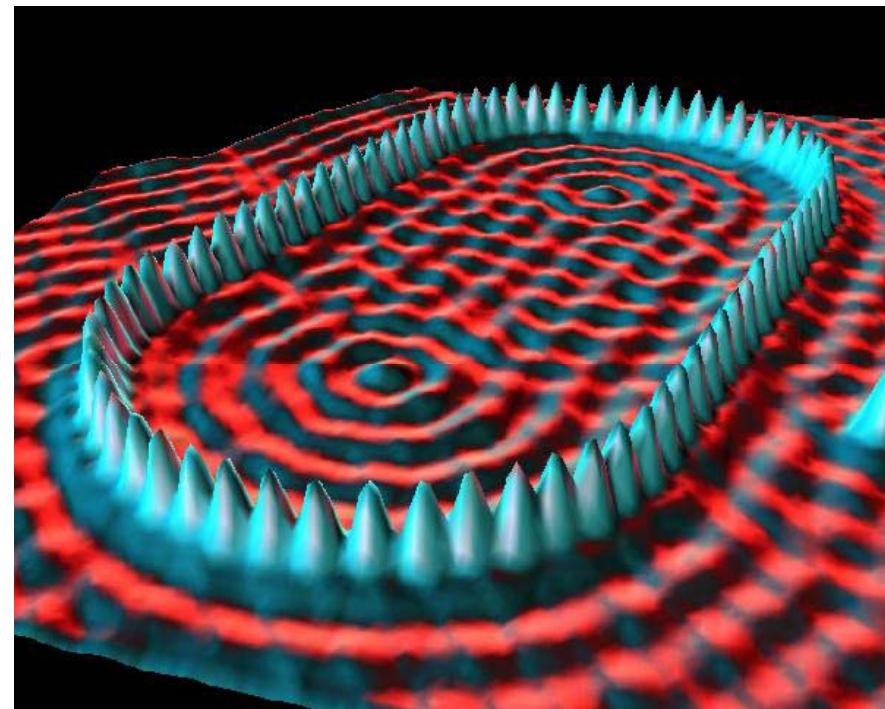
Limited biological applications: Atomic Force Microscopy

Nanomanipulation – moving atoms, molecules, creating  
holes, etc

# Examples of STM images



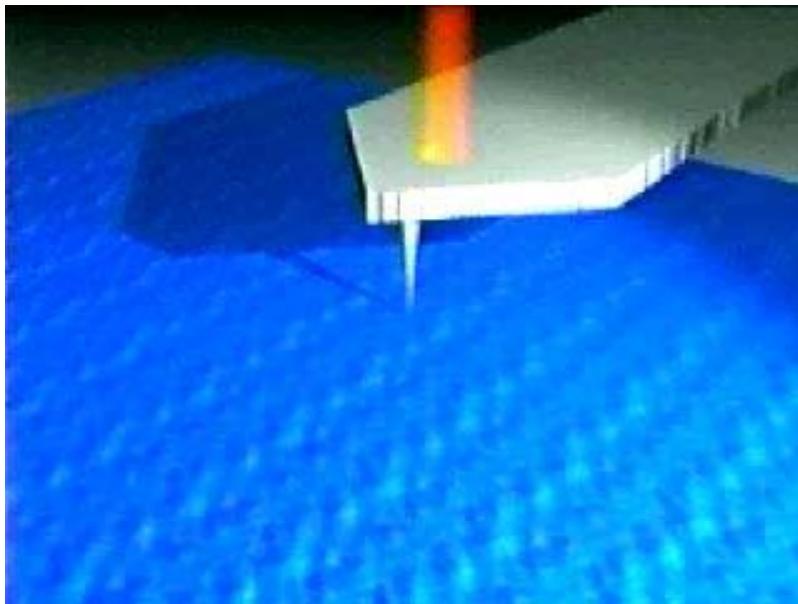
Copper Surface



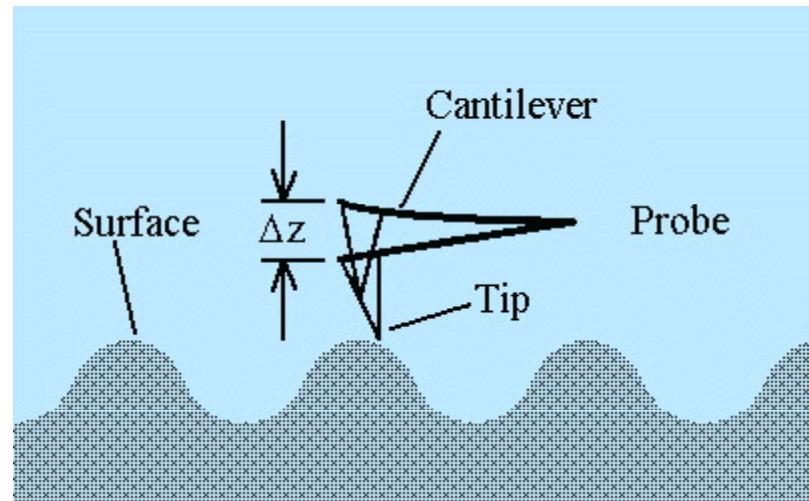
Iron on Copper

# Atomic Force Microscopy

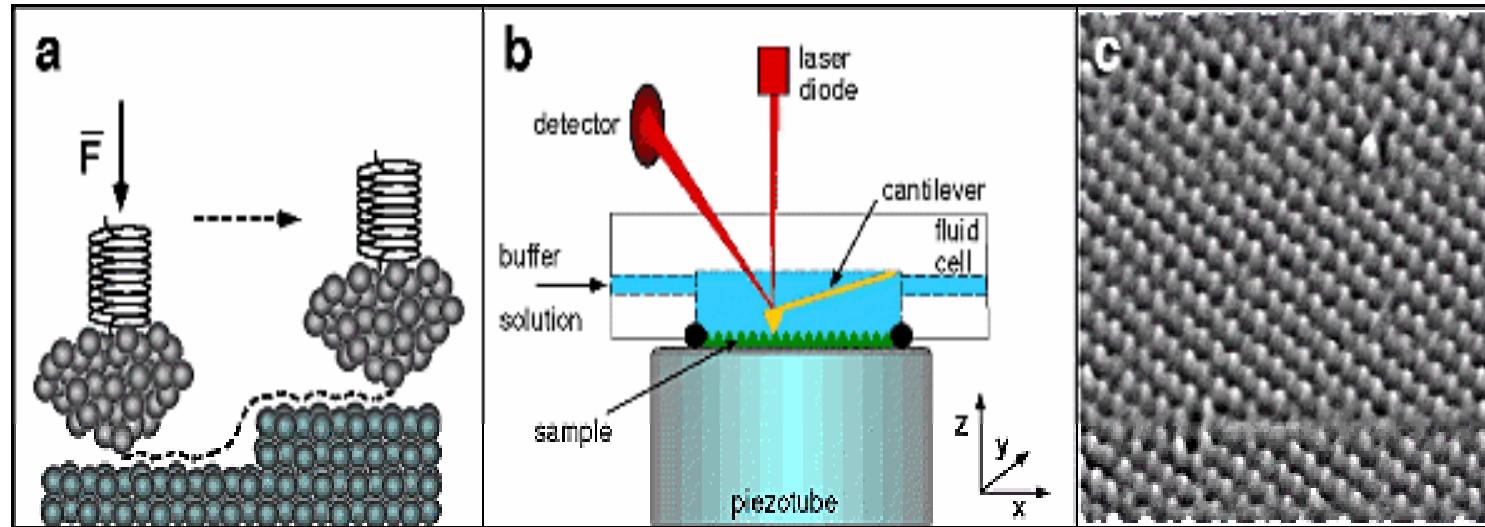
Imaging – scanning probe



- In AFM scanning probe is a sharp stylus (radius 10-20 nm)

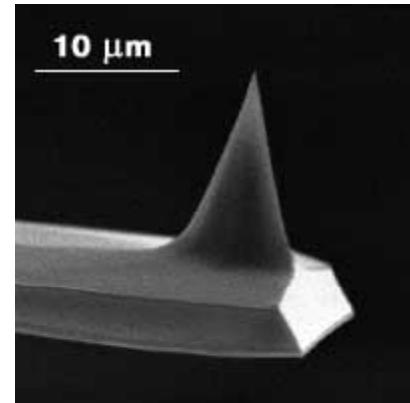


The principle of AFM is to measure the deflection of cantilever while holding the force of interaction as a constant. The measured cantilever deflections allow a computer to generate a map of surface topography.

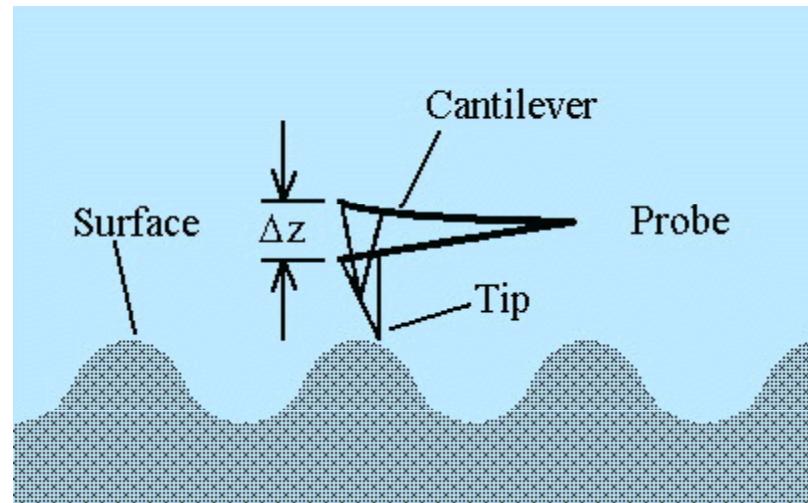


interatomic forces, instead of tunnelling current , no requirement for conductivity

# The Tip and Cantilever



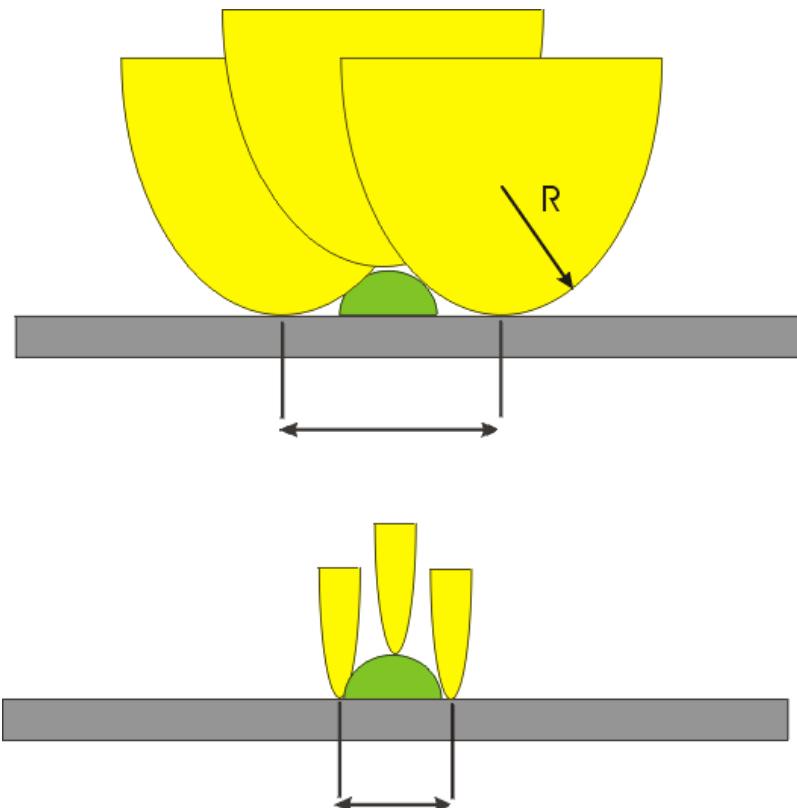
- The tip is made of Silicon Nitride,
- Tip is usually pyramidal
- Requirements - must be sharp, thin and durable
- Cantilever is a thin flexible beam which holds the tip.



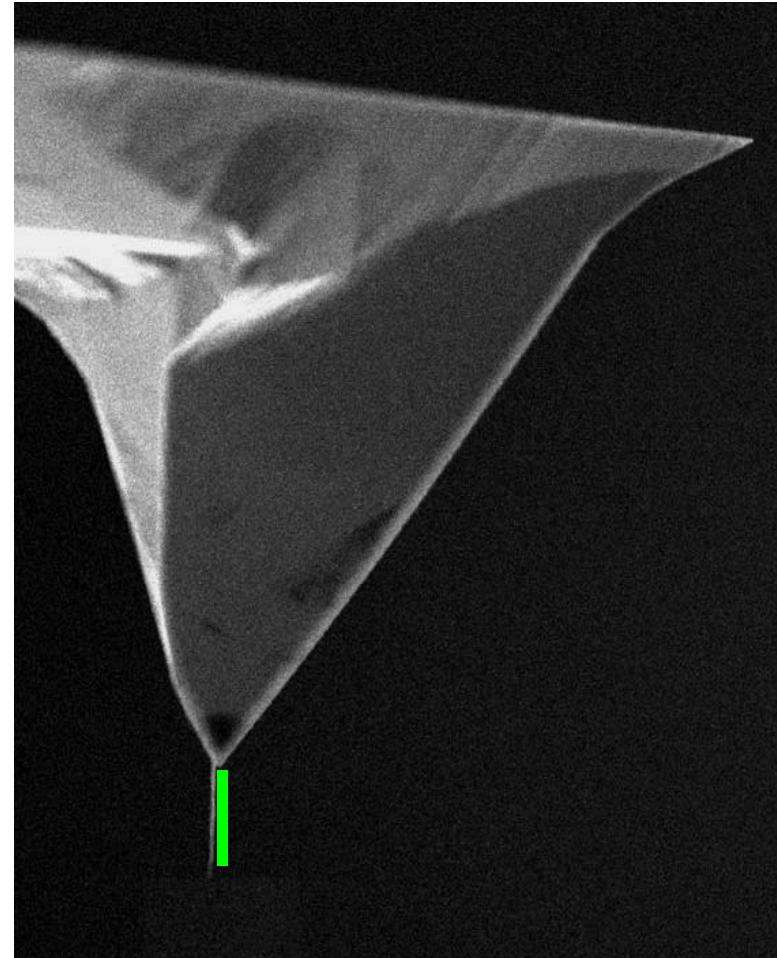
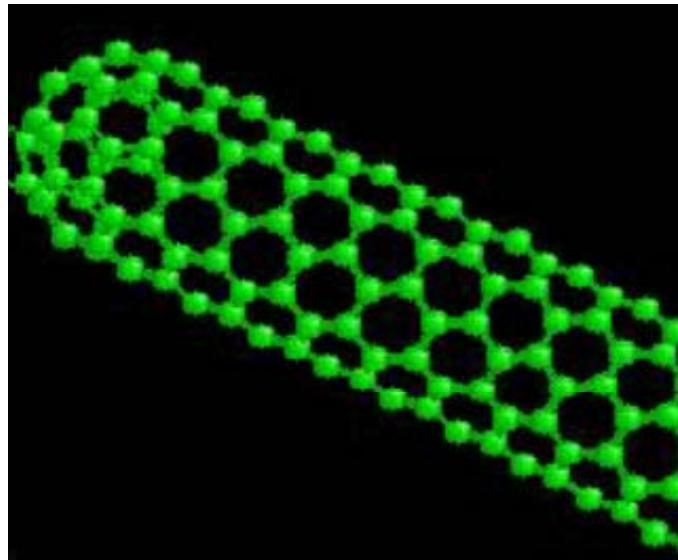
Interaction force  $F = kx$

## Resolution is limited by sharpness of the tip

- When radius of curvature of the tip is higher than surface features the lateral dimensions of the feature can not be detected correctly - tip broadening
- trends - improve tip sharpness



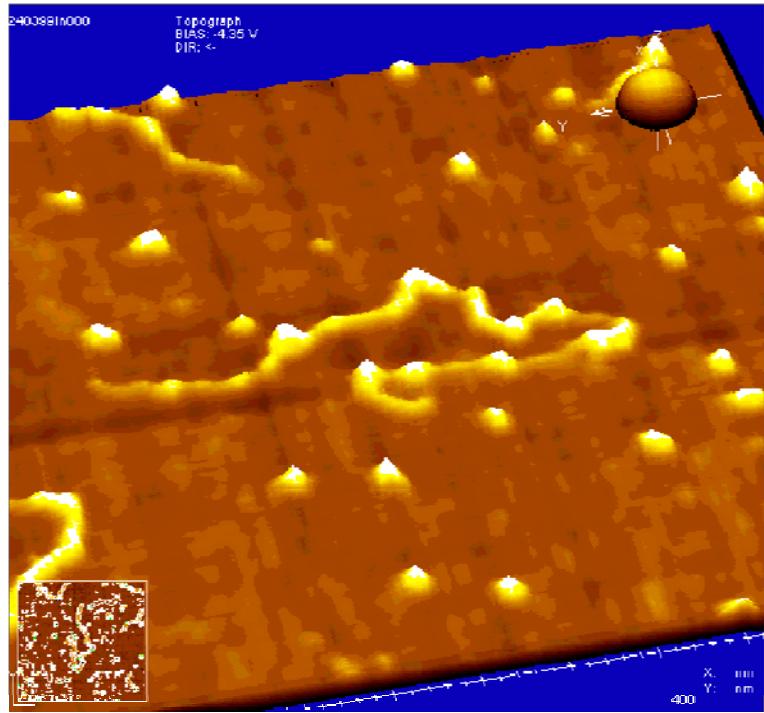
- Use carbon nanotube (CNT) as a tip



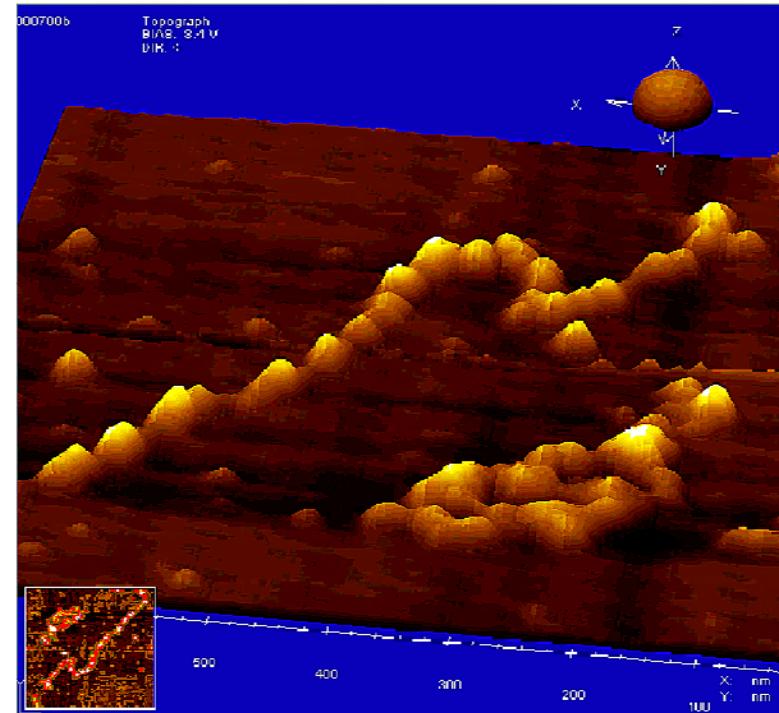
# Applications of AFM in biosciences

- DNA and RNA analysis;
- Protein-nucleic acid complexes;
- Chromosomes;
- Cellular membranes;
- Proteins and peptides;
- Molecular crystals;
- Polymers and biomaterials;
- Ligand-receptor binding.

# AFM imaging



10 min



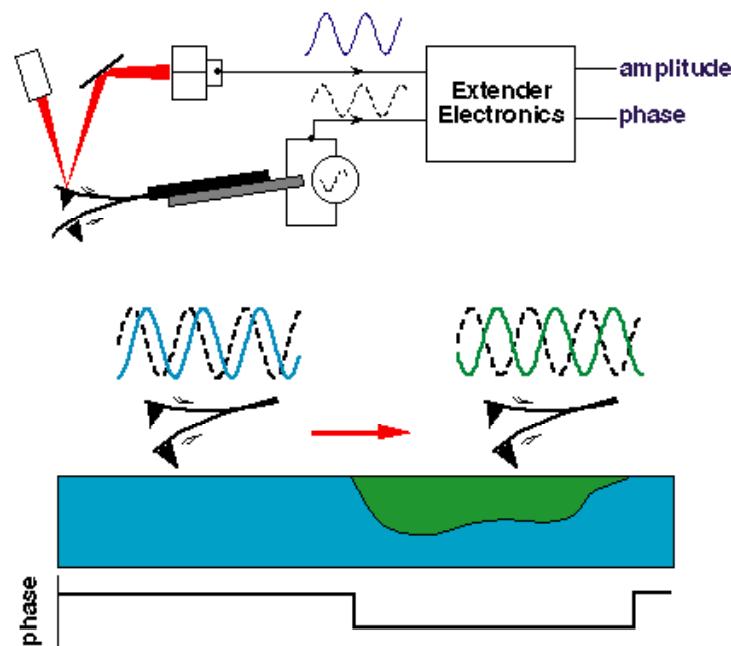
30 min

DNA binding by the repair protein Ku

Leonenko D. Merkle, L. Shamrakov, S.P. Lees-Miller, D. Cramb ,  
Biosensors and Bioelectronics, 20, 918-924, 2004.

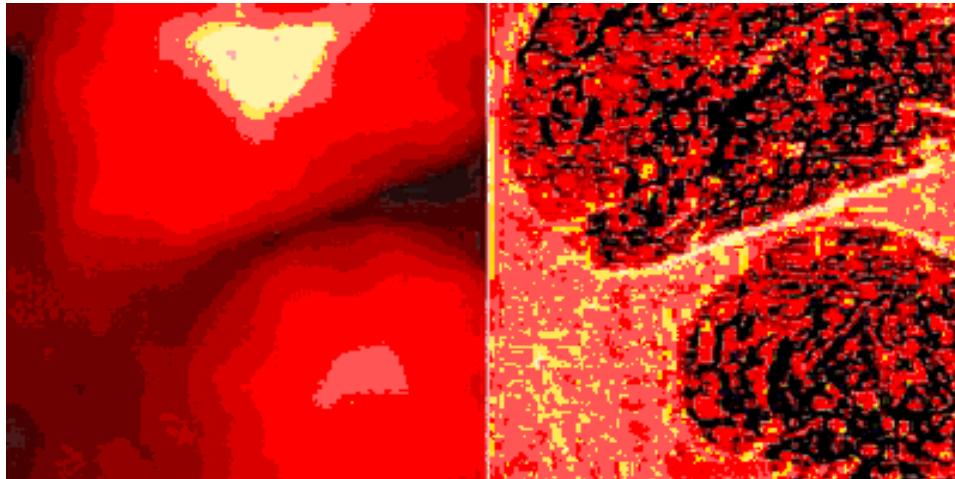
# Phase Imaging

- Accessible via TappingMode
- Oscillate the cantilever at its resonant frequency. The amplitude is used as a feedback signal. The phase lag is dependent on several things, including composition, adhesion, friction and viscoelastic properties.
- Identify two-phase structure of polymer blends
- Identify surface contaminants that are not seen in height images
- Less damaging to soft samples than lateral force microscopy



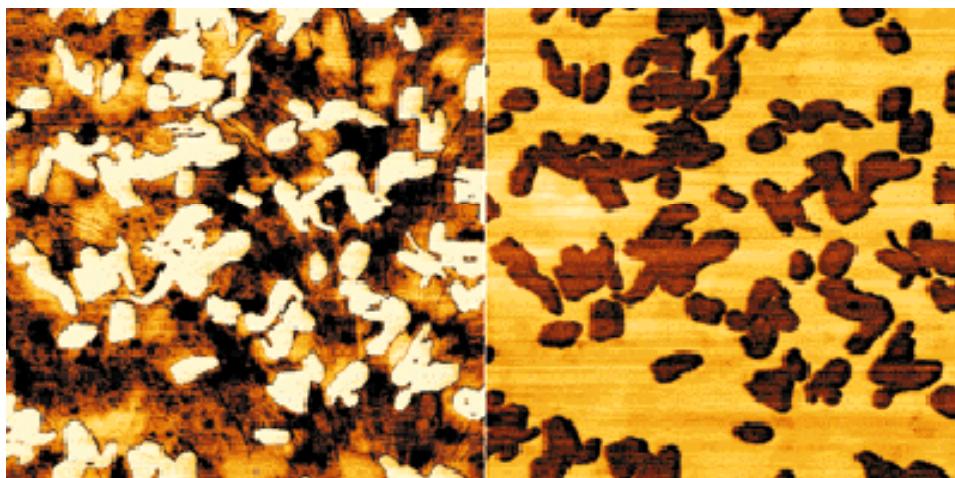
Image/photo Digital Instruments, Santa Barbara  
,CA

# Phase Imaging



Composite polymer  
imbedded in a matrix

1 micron scan



$\text{MoO}_3$  crystallites  
on a  $\text{MoS}_2$  substrate

6 micron scan

# Atomic Force Spectroscopy

- Measures forces of interaction between end of the probe (tip) and sample
- when AFM tip is moved close to the sample surface and away from the sample surface

# Van der Waals Force

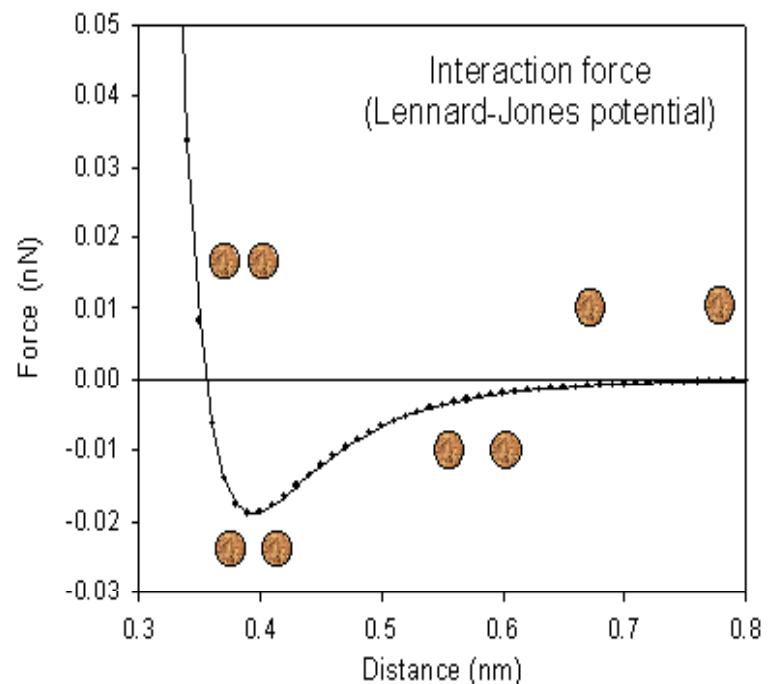
Lennard-Jones potential: (interaction between two atoms)

$$w(r) = -A/r^6 + B/r^{12},$$

r is the separation of the two atoms, A and B are interaction constants.  
Interaction force

$$F = -dw(r)/dr = -6A/r^7 + 12B/r^{13}$$

A and B are known to be  $10^{-77} \text{ Jm}^6$   
and  $10^{-134} \text{ Jm}^{12}$



# INTERATOMIC/ INTERMOLECULAR FORCES

- **CHEMICAL FORCES**

- a) covalent: electronic cloud overlap
- b) coulombic: ionic attraction

quantum origin, very strong and short range

## ELECTROSTATIC FORCES

Between charged molecules. **strong in the long range**

- **MAGNETIC FORCES**

Between molecules possessing magnetic moments. **long range forces, weak**

- **SURFACE TENSION (CAPILLARY CONDENSATION)**

from moisture and adsorbates. **strong in the long range**

- **HYDROGEN BONDING**

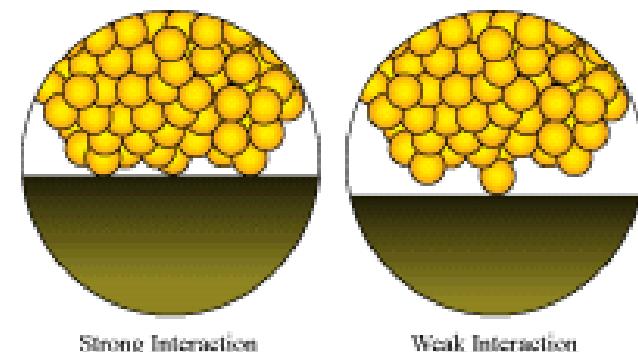
H covalently bonded to O, F, Cl or N is **polarized** (+)  
attracts nearby O, F, Cl or N

- **VAN DER WAALS FORCES**

**Dipole forces, generally attractive. and long range**

- **HYDROPHILIC AND HYDROPHOBIC FORCES**

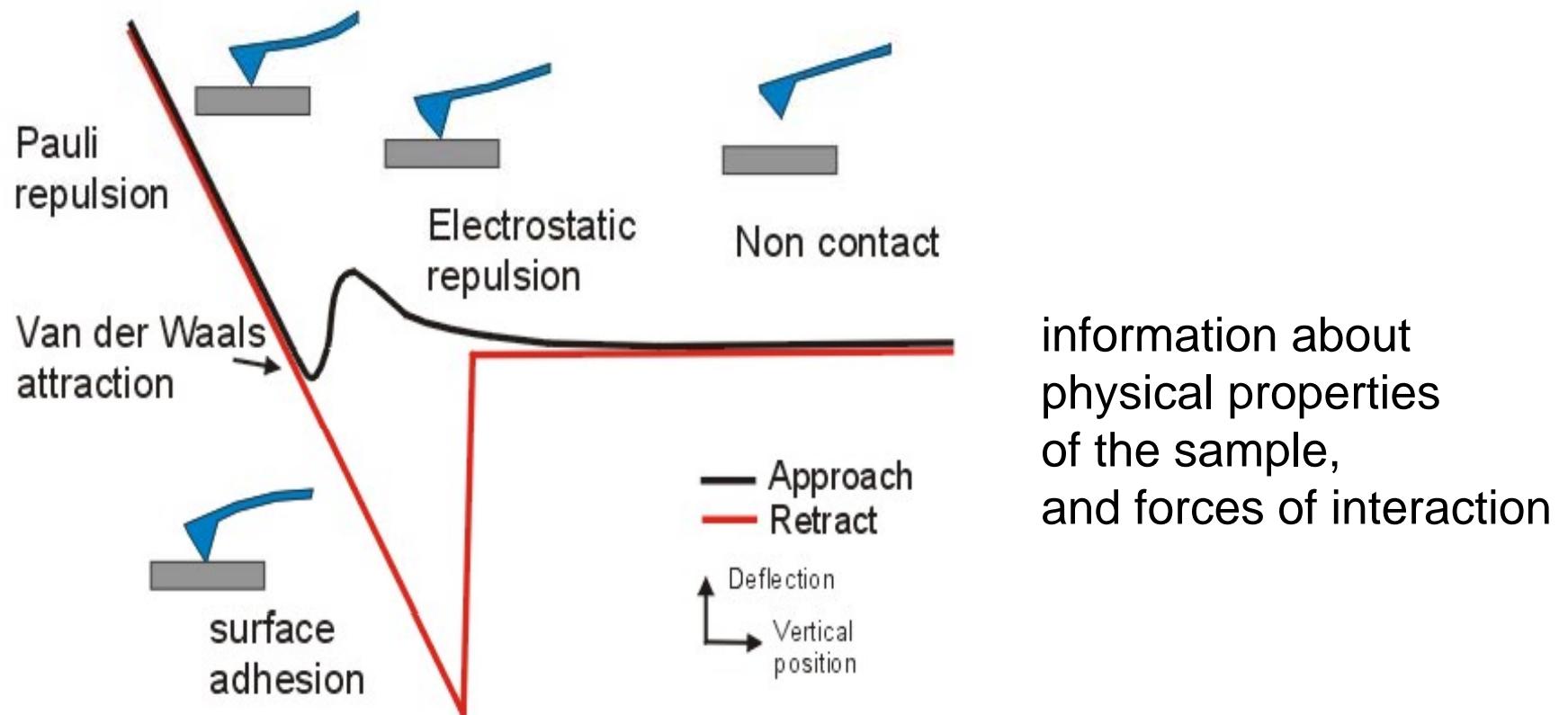
In AFM few atoms – 100 atoms interact with the sample



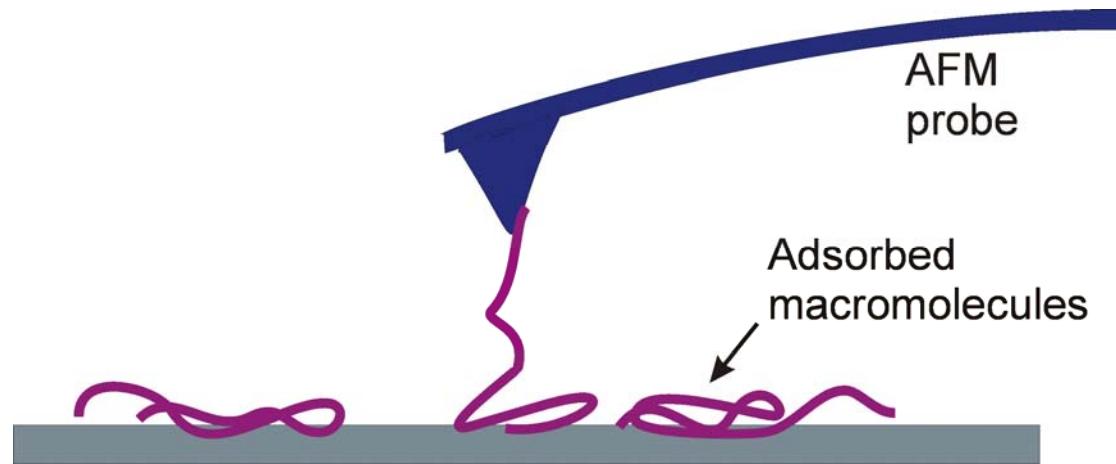
# AFM Force measurements

$$F = -kx$$

measure these forces of interaction between the end of the probe and the sample through the deflection of the cantilever



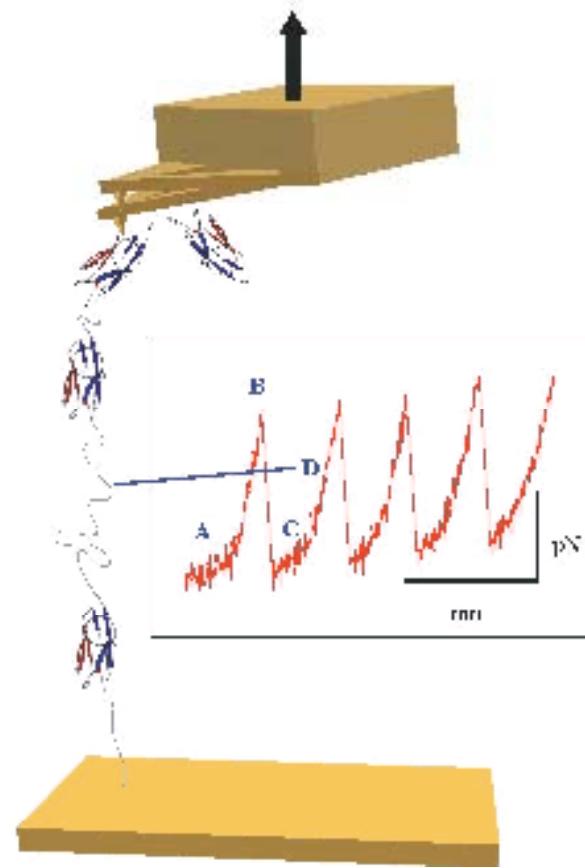
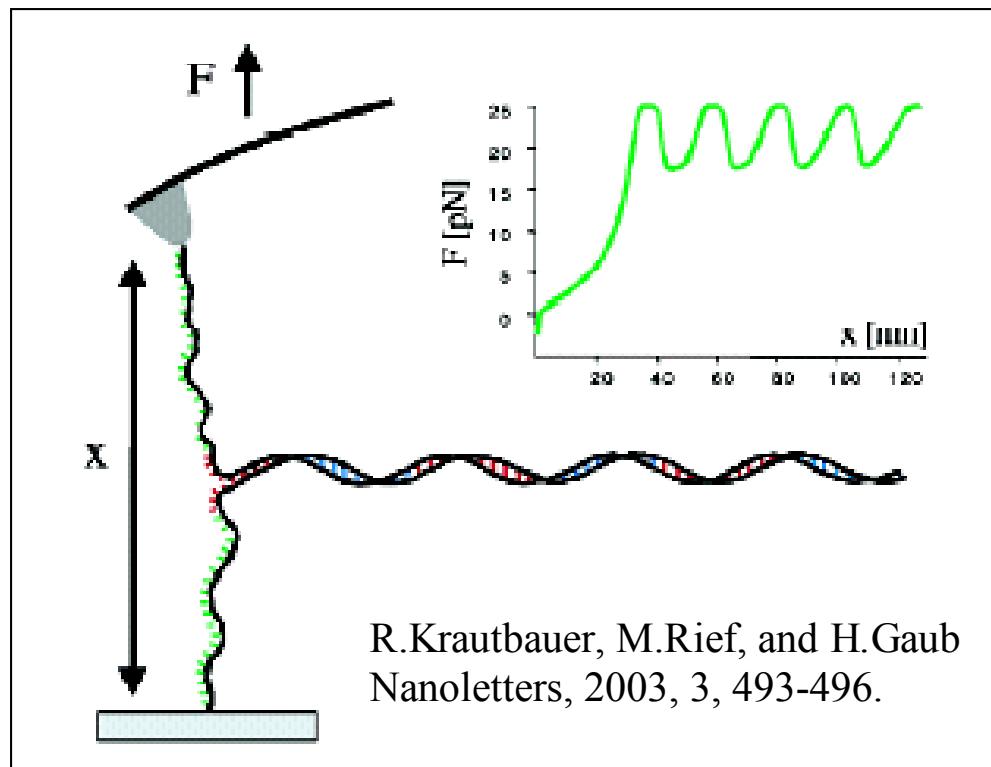
# AFM Molecule manipulation



- Unfolding of macromolecules - proteins, DNA, polymers, etc.
- Sensitive force measurements detects  
**structural transitions and elastic properties**

# Protein unfolding

## DNA unzipping

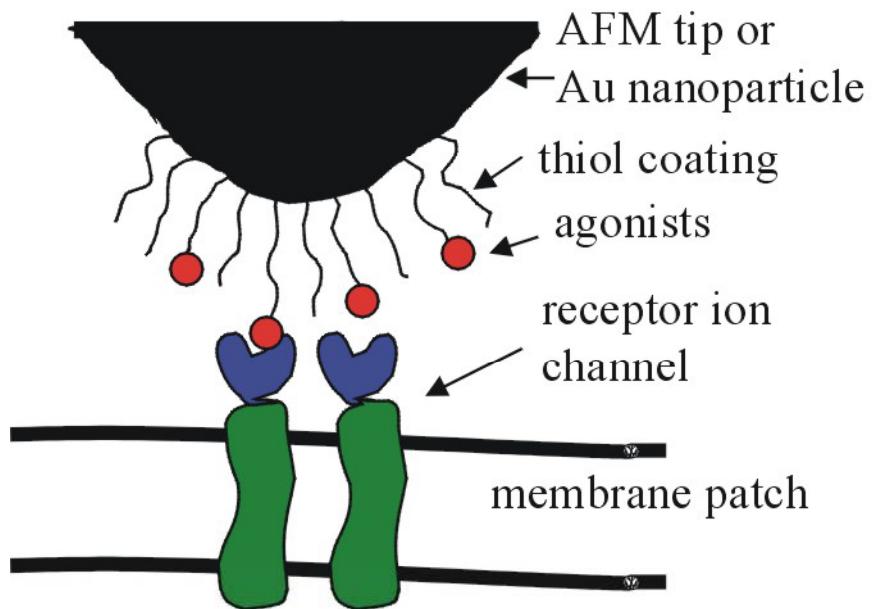


# Chemical recognition

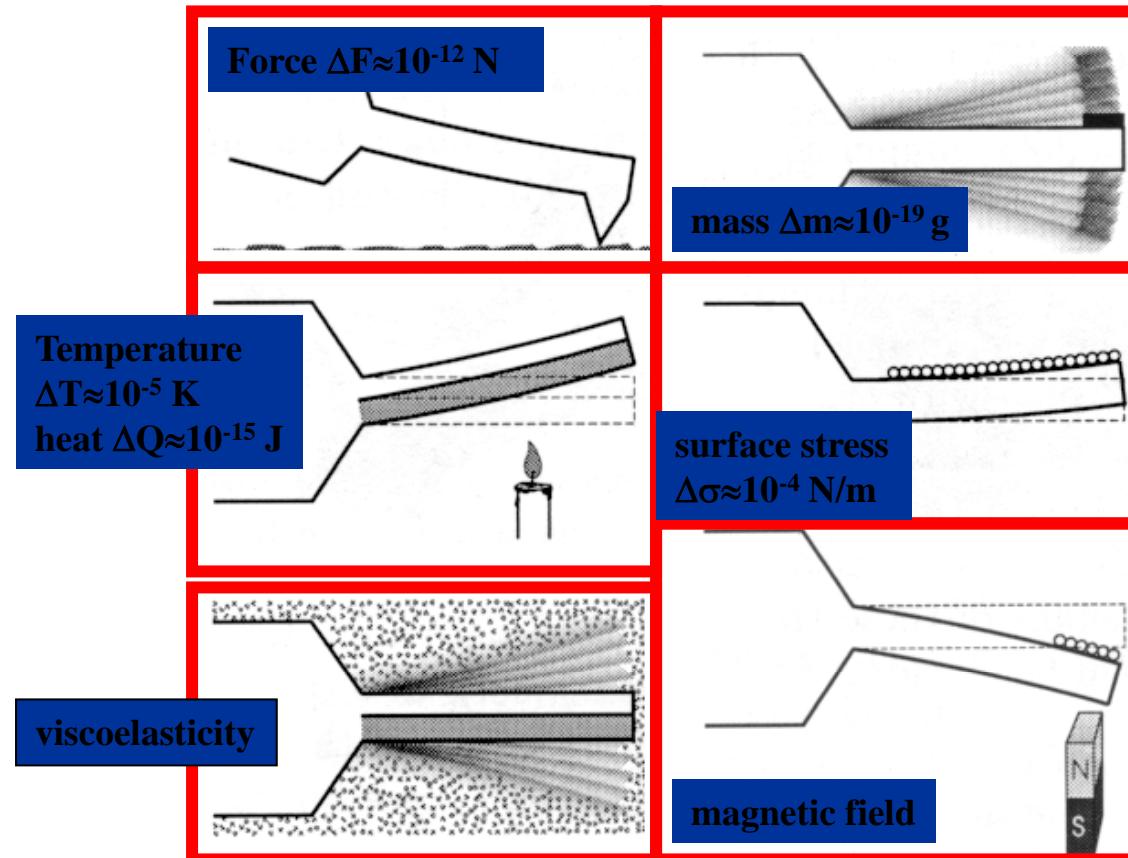
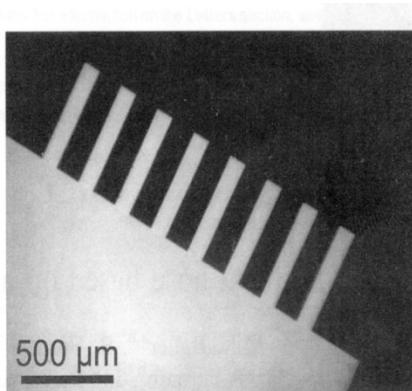
silicon nitride probe - non-specific interaction

chemically modified probe - specific interaction:

hydrophilic,  
hydrophobic,  
charged,  
specific interactions (ligand-receptor, antigen-antibody)

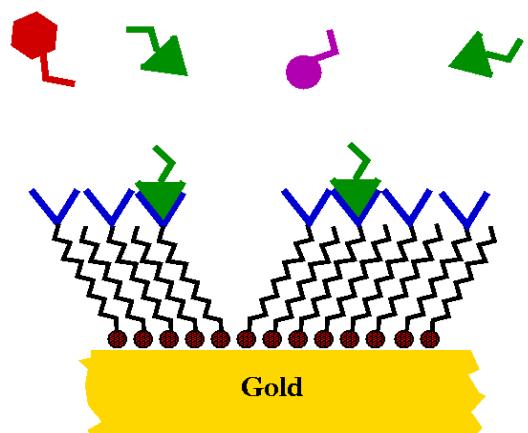


# AFM Biosensing at nanoscale

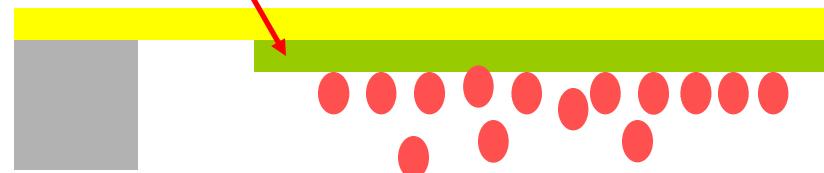


# Cantilever based sensing

Making a Surface  
Selective and  
Responsive



Molecule recognition  
layer



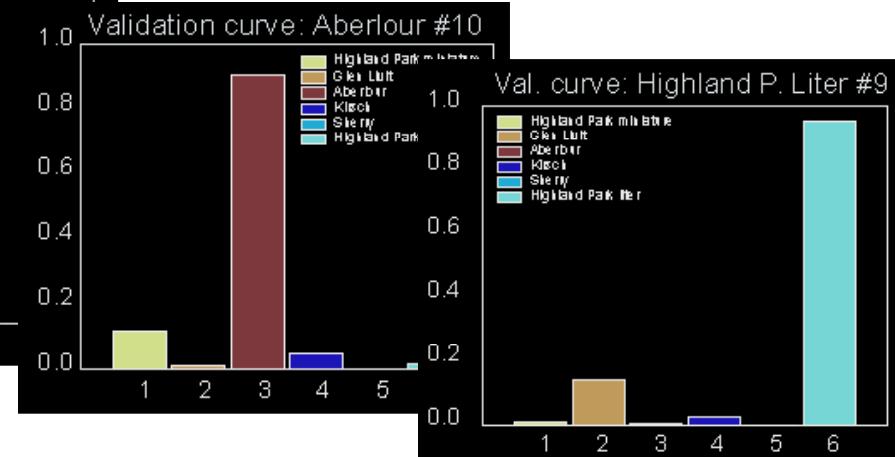
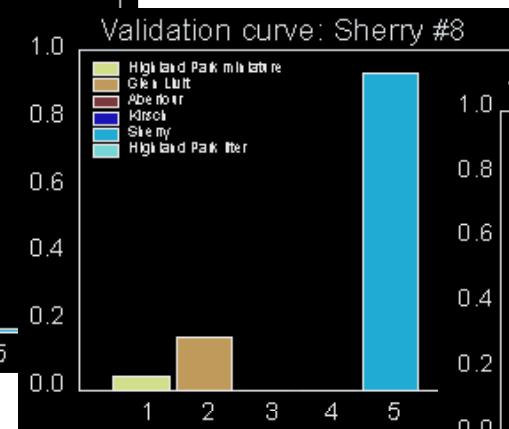
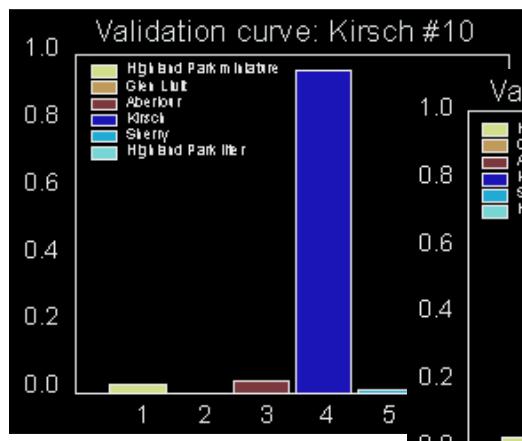
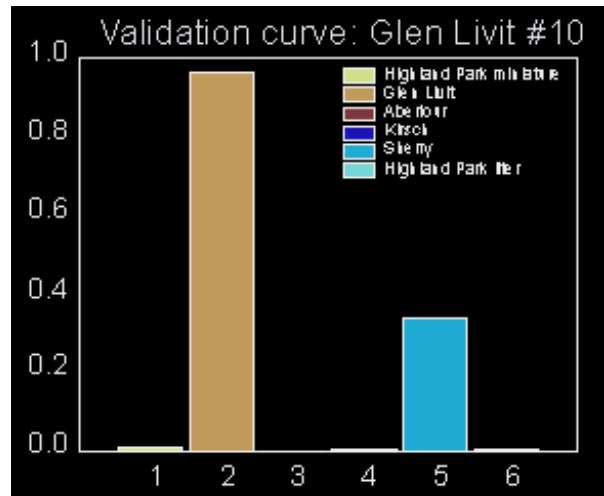
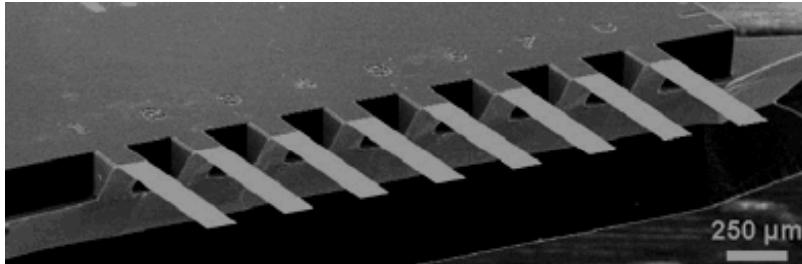
Analyte  
molecules

analytes

immobilized  
Receptors

thiol  
SAMs,

## Nano Lab: Artificial Nose



H.P. Lang, M.K. Baller, R. Berger, Ch. Gerber, J.K. Gimzewski, F.M. Battiston, P. Fornaro, J.P. Ramseyer, E. Meyer, and H.-J. Güntherodt, "An Artificial Nose Based on a Micromechanical Cantilever Array," *Analytica Chimica Acta*, 393, 59 (1999).

# Other AFM related techniques

Electrostatic force microscopy

Kelvin probe force microscopy

Magnetic force microscopy

Electrochemical force microscopy

AFM based force measurements

Nanolithography:

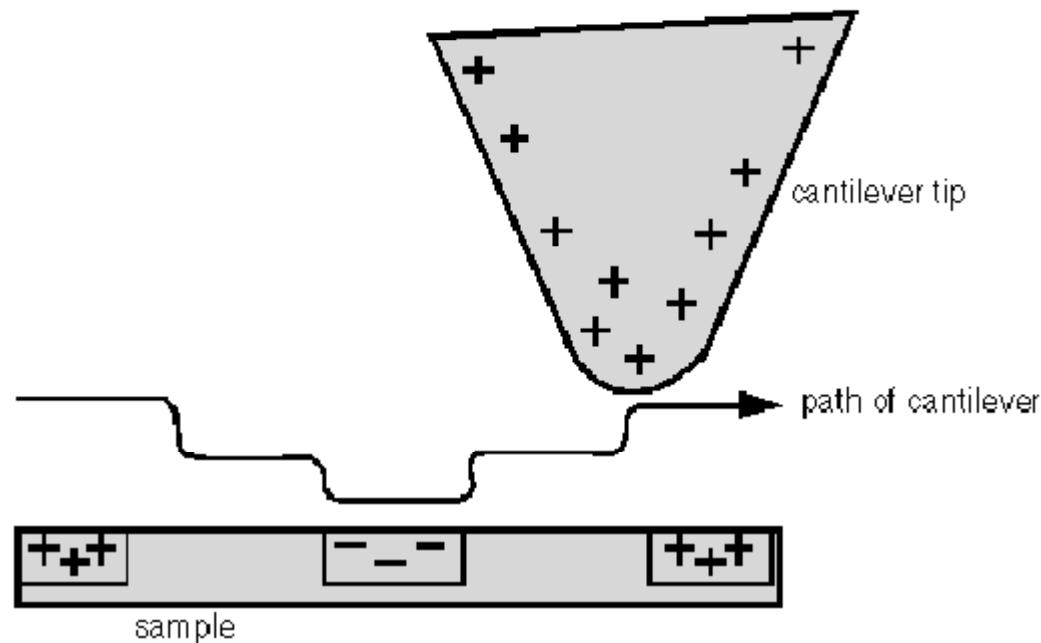
T- lithography

Electrochemistry,

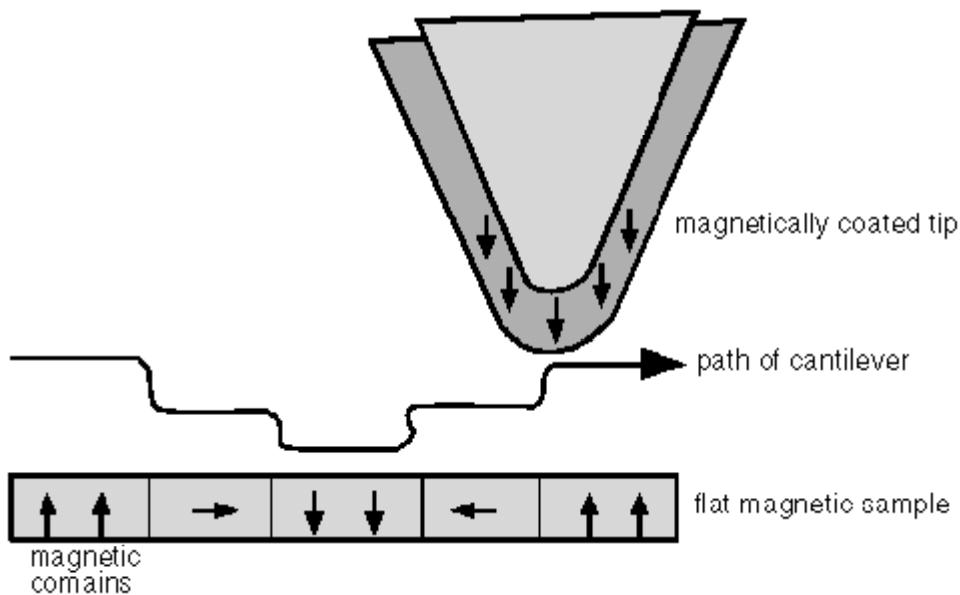
Piezoresponce SPM

# Electrostatic Force Microscopy

Measures electric field gradient and distribution  
above the sample surface  
-detection of charges as small as single electron

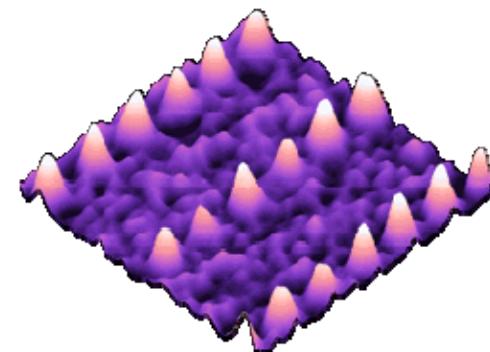


# Magnetic Force Microscopy



Gradients in the magnetic forces on the tip induce changes in the resonant frequency of the cantilever  
-images the stray magnetic fields on a sample surface

Magnetic memory



Magnetic bits written with an MFM probe on perpendicular Co-Cr media with a NiFe sublayer. The bits are about 180nm in size spaced 370nm, giving an equivalent area density of ~5Gbits/in<sup>2</sup>.

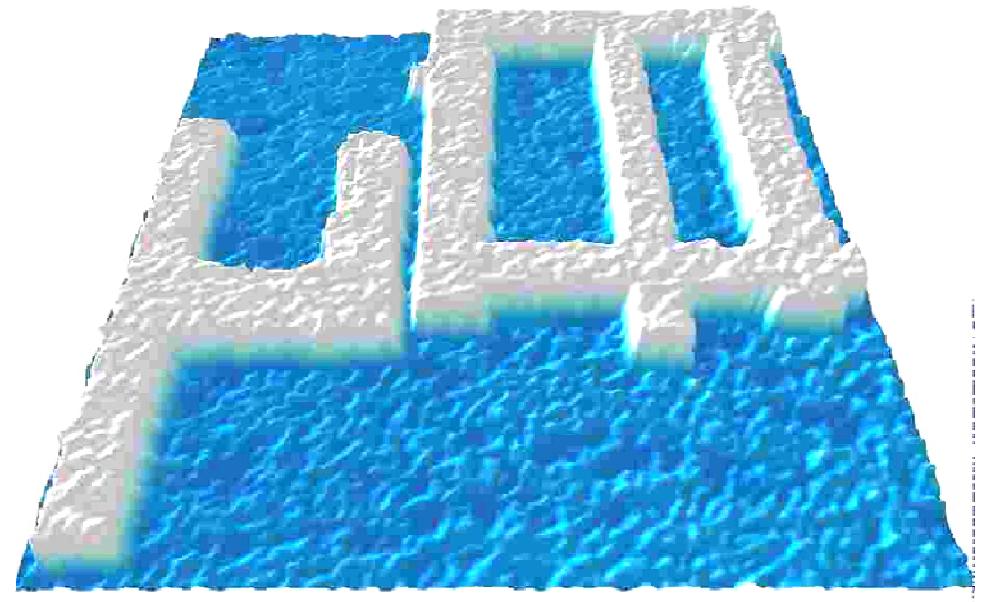
2.3μm scan ,Michael Azarian,  
Censtor Corporation.

# Piezoresponce Force Microscopy, PFM

Polarization sensitive mode SFM,  
ferroelectric domain writing and switching

PFM image of written  
domains

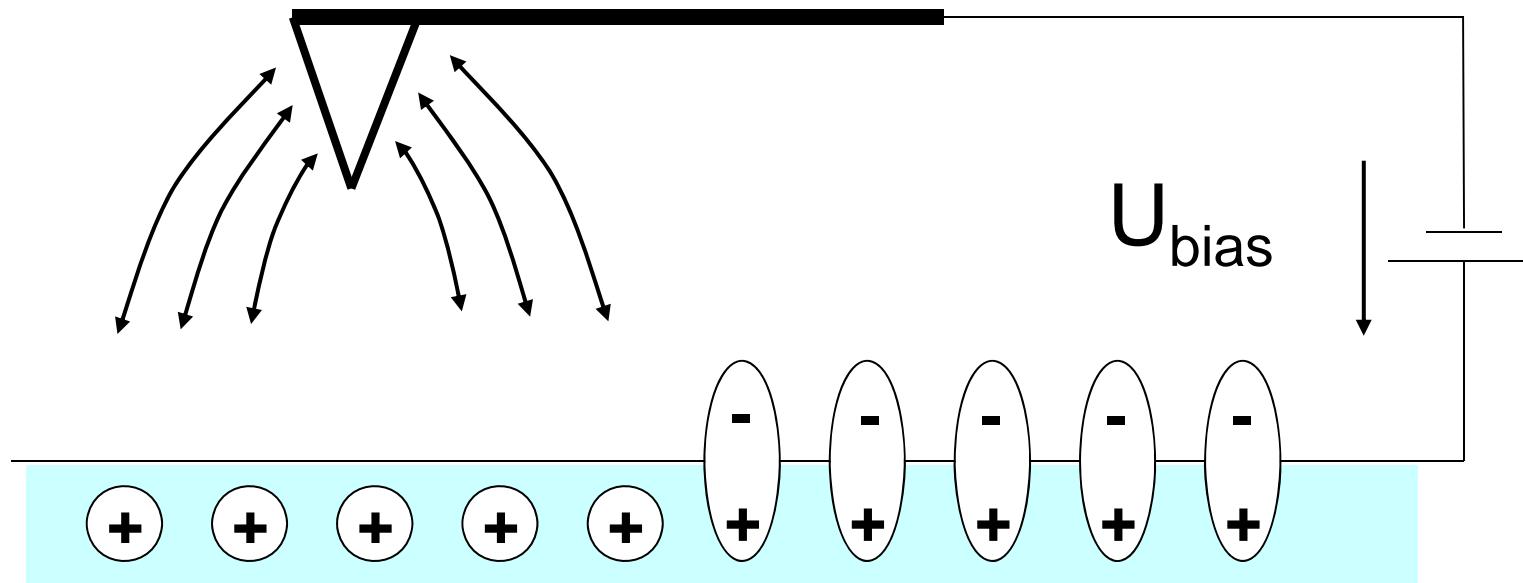
C. H. Ahn et al., Science 276, 1100 (1997)



# Kelvin Probe Force Microscopy: KPFM

combines kelvine method and probe microscopy-  
lateral resolution at nm and single molecule level

- workfunction change
- surface potential



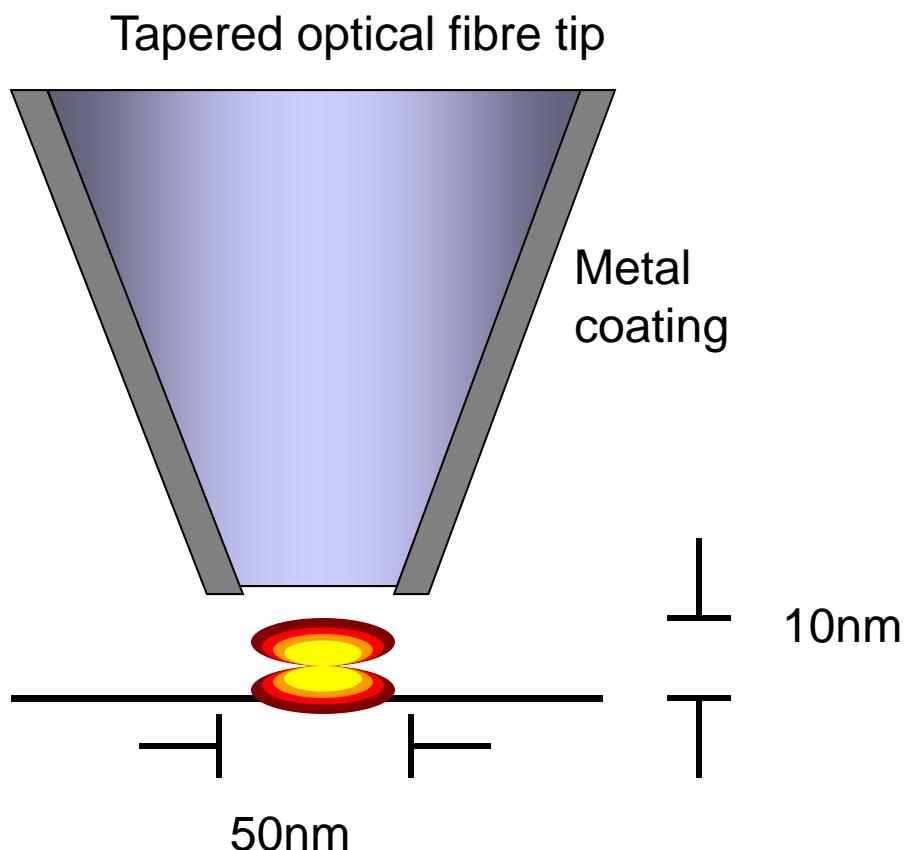
# Electrochemical force microscopy. Nanolithography

is the art and science of etching, writing, or printing at the microscopic level, where the dimensions of characters are on the order of nanometers. This includes various methods of modifying semiconductor chips at the atomic level for the purpose of fabricating integrated circuits.

Instruments used in nanolithography include the electrochemical scanning probe microscopy (ESPM) and the atomic force microscopy (AFM).

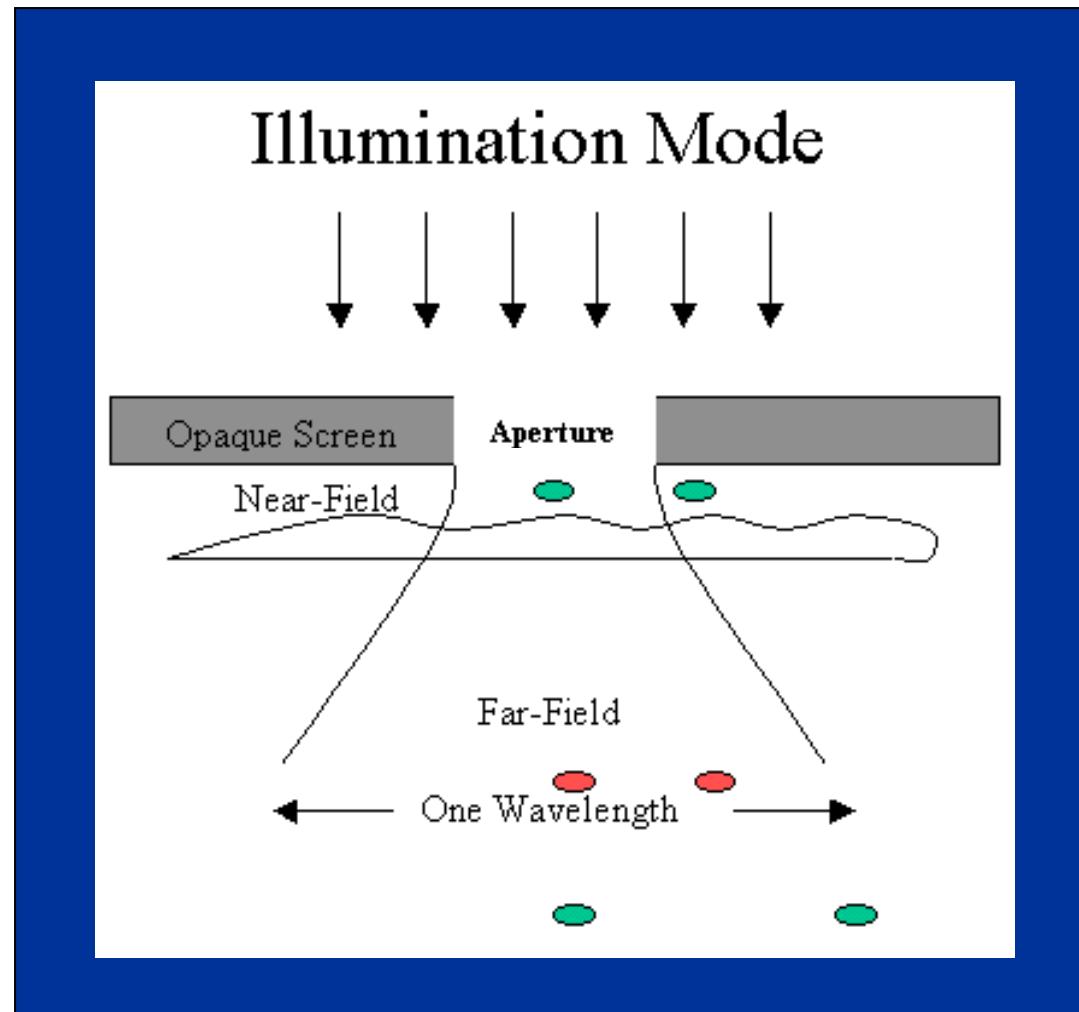
# Scanning Near-Field Optical Microscopy – SNOM or NSOM

- Tip held ~10nm from surface using AFM techniques
- Light comes through the tip and excites the dye molecule –fluorescence label
- no diffraction limit
- requires fluorescence label



In  
optical microscopy  
resolution is limited  
to  
 $1/2$  of wave length,

in NSOM -  
diameter of aperture





**Rhodamin Nanokristalle**  
**Scansize: 10 μm**

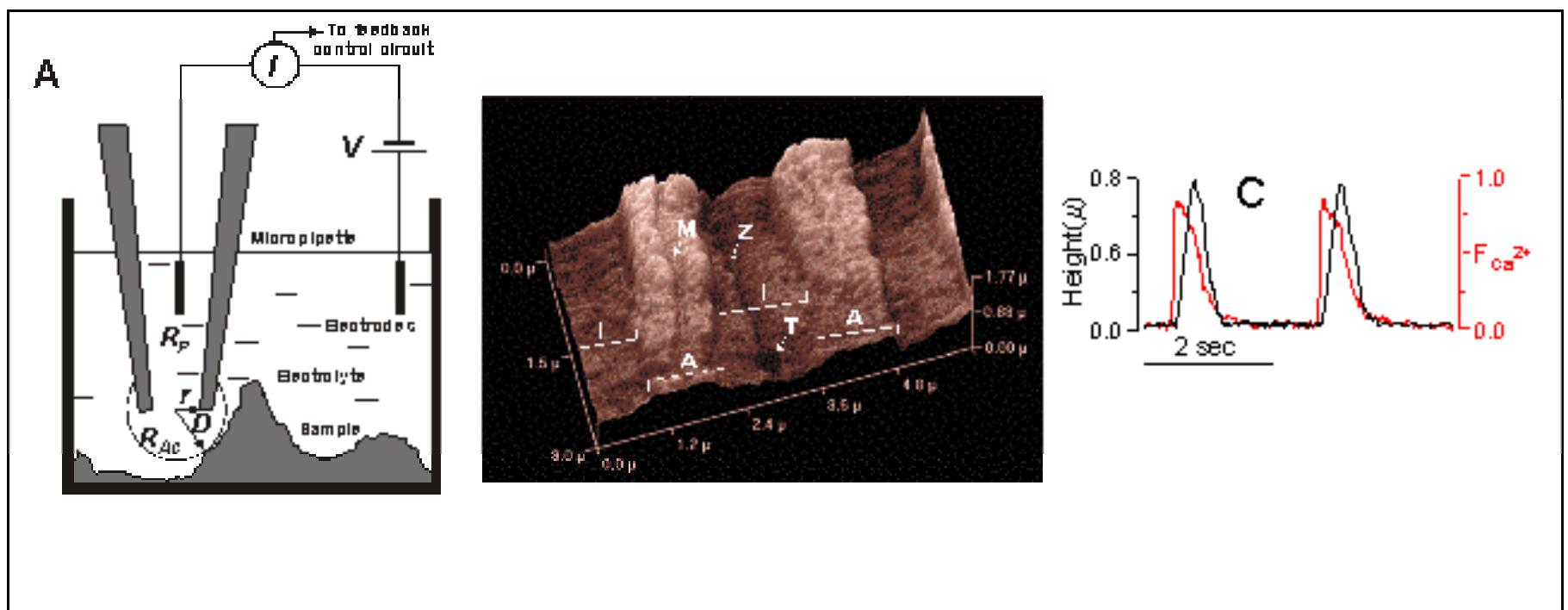
SPM<sup>2</sup> group  
courtesy of  
Dr. L.Eng, IAPP,  
TU Dresden

# Scanning Ion Conductance Microscopy

## CISM

scanning probe - nanopipette.

resolution is 50 nm



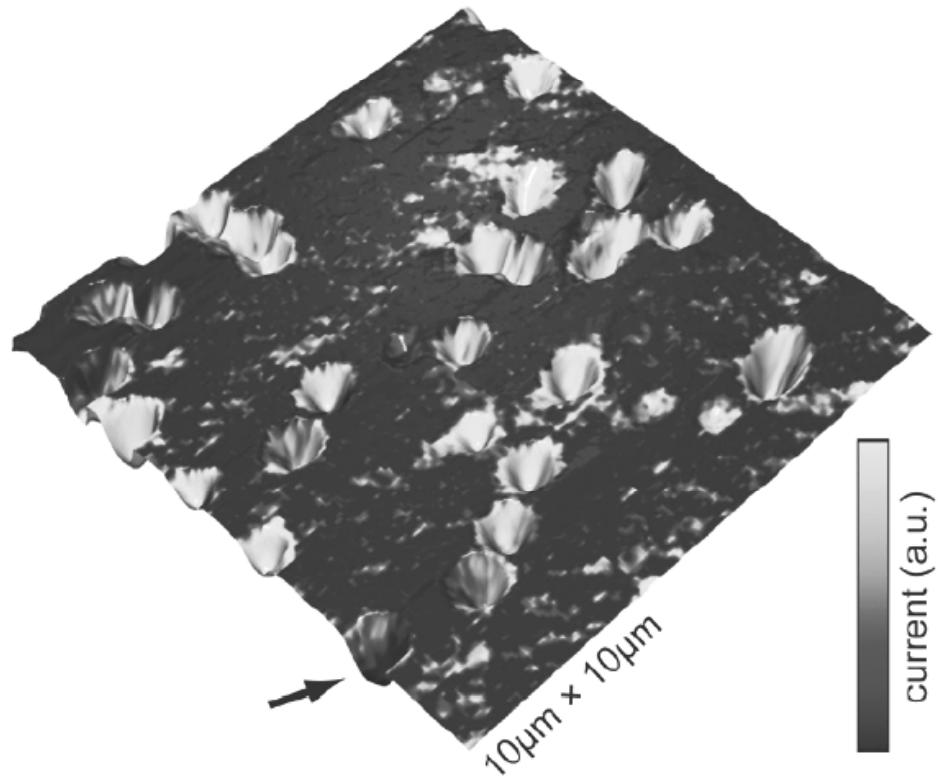
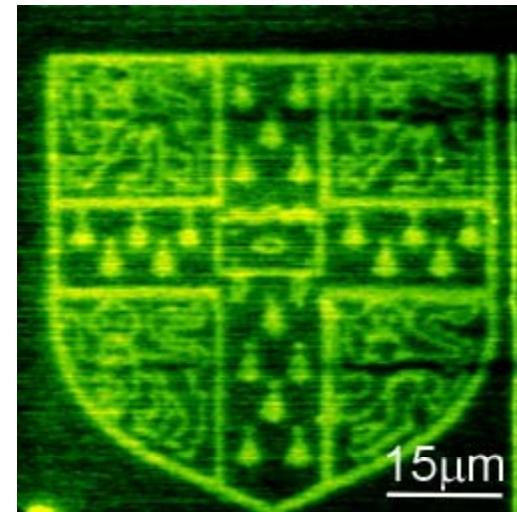
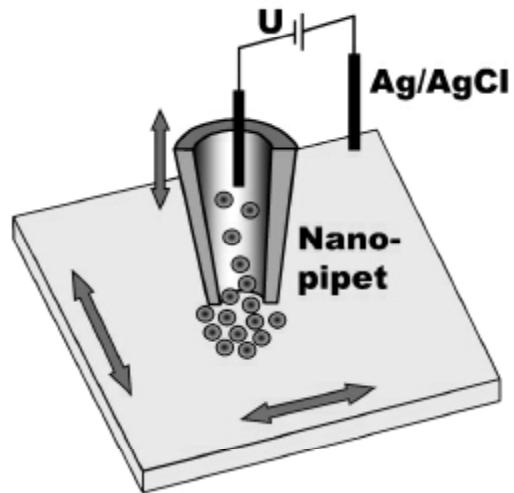


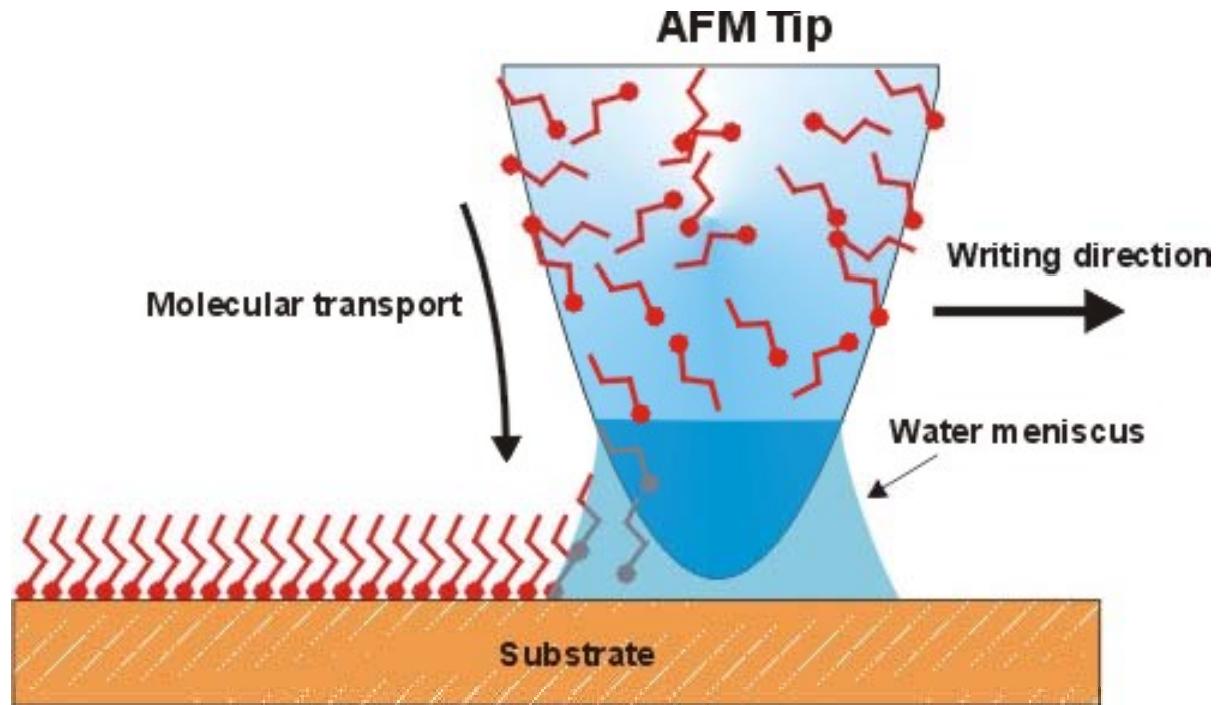
Figure 2. SICM image of topography (relief) and ion current (color) recorded simultaneously on a porous polycarbonate membrane. Brighter colors correspond to larger currents. The pores can clearly be identified in both topography and ion current.

using a nanopipette like a nanopen - controlled delivery of biomolecules to create novel patterns, arrays and structures on the nanometer scale



<http://www.ch.cam.ac.uk/CUCL/staff/dk.html>

# Dip Pen Nanolithography



Mirkin group <http://www.chem.nwu.edu/~mkngrp/dippen.html>

# SXM - scanning anything microscopy

- Any property that can be locally measured when special kind of sensitive probe is used to image
- Scanning ion conductance microscopy
- Scanning magnetic force microscopy
- Scanning electrochemical microscopy
- Scanning anything microscopy
- You name it...