Microscopic Techniques

Outline

1. Optical microscopy

   Conventional light microscopy, Fluorescence microscopy, confocal/multiphoton microscopy and Stimulated emission depletion microscopy

2. Scanning probe microscopy

   Scanning tunneling microscopy (STM), Atomic force microscopy (AFM), Near-field scanning optical microscopy and others

3. Electron microscopy

   Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Scanning transmission electron microscopy (STEM), Focus ion beam microscopy (FIB)

1. Optical Microscopy

Conventional Optical Microscopy

This is an optical instrument containing one or more lenses that produce an *enlarged image* of an object placed in the focal plane of the lens

Resolution limit: submicron particles approaches the wavelength of visible light (400 to 700nm)

1. Transmission: beam of light *passes* through the sample

e.g. Polarizing or petrographic microscope

Samples are usually fine powder or thin slices (transparent)

2. Reflection: beam of light *reflected* off the sample surface

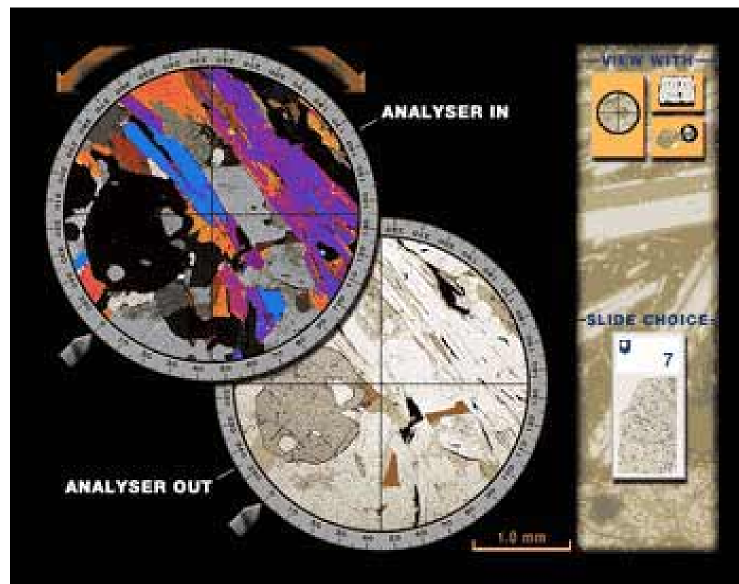
e.g. Metallurgical or reflected light microscope

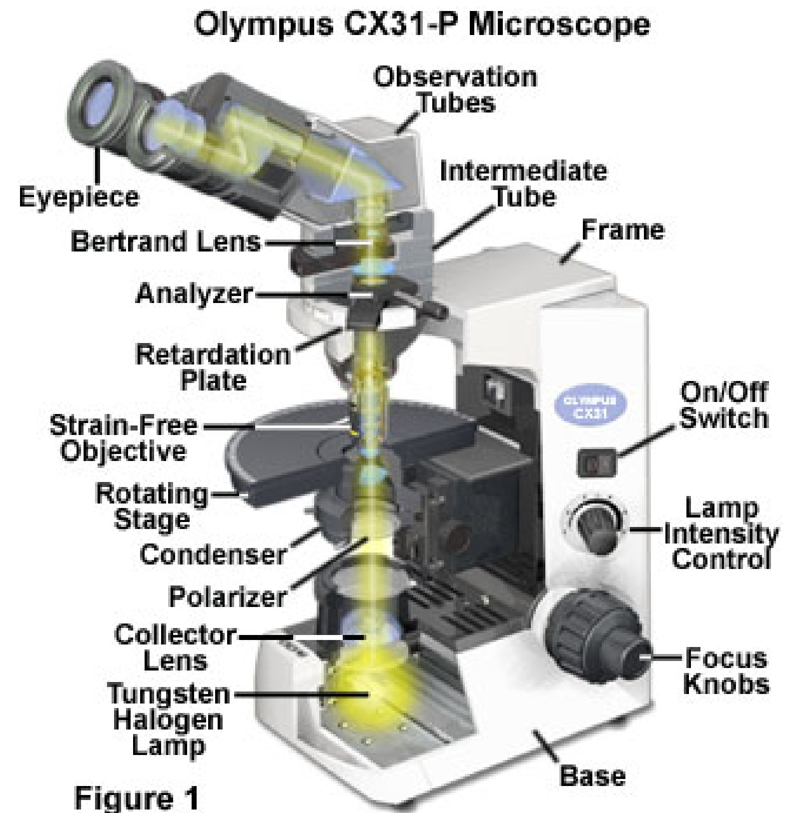
Surface of materials, especially opaque ones

Polarizing Microscope

<u>Polarizer & Analyzer</u>

Only the light component whose vibration direction is parallel to the polarizer is permitted to pass through

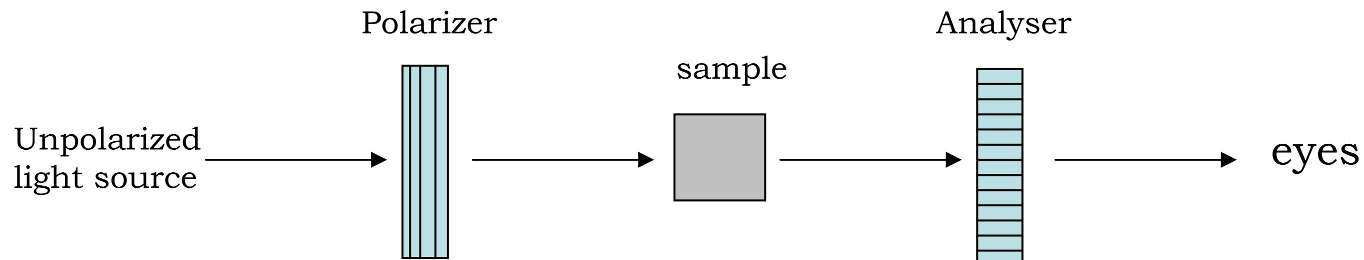




www.olympusmicro.com

Polarized light microscopy is utilized to distinguish between singly refracting (optically isotropic) and doubly refracting (optically anisotropic) media

Principle of Polarizing Microscope

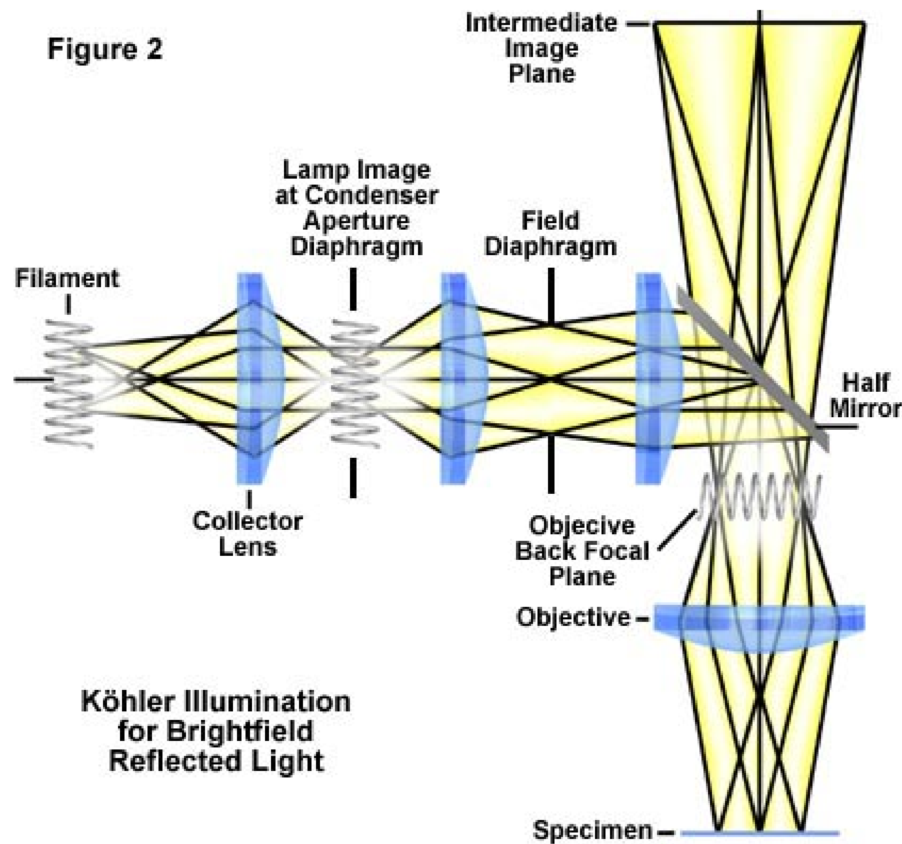


Crossed polars:
1. No sample → black
2. Isotropic sample → black
3. Anisotropic sample → color

The interaction of plane-polarized light with a doubly refracting (*birefringent*) specimen to produce two individual wave components (ordinary ray and extraordinary ray) that are polarized in mutually perpendicular planes.

- Different velocities
- Different propagation direction

Reflected Light Microscope



Half Mirror

Partially reflecting plane glass mirror that deflects light traveling from the horizontal illuminator by 90 degrees into the vertical optical train of imaging components in the microscope.

Objective Lens

- A matching well-corrected condenser properly aligned
- An image-forming objective projecting the image-carrying rays toward the eyepiece

www.olympusmicro.com

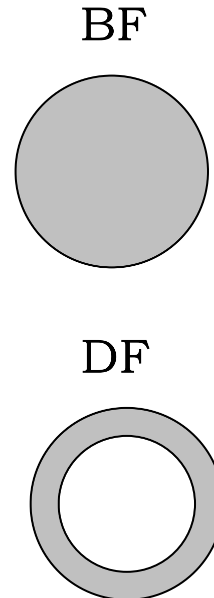
Dark Field *vs.* Bright Field

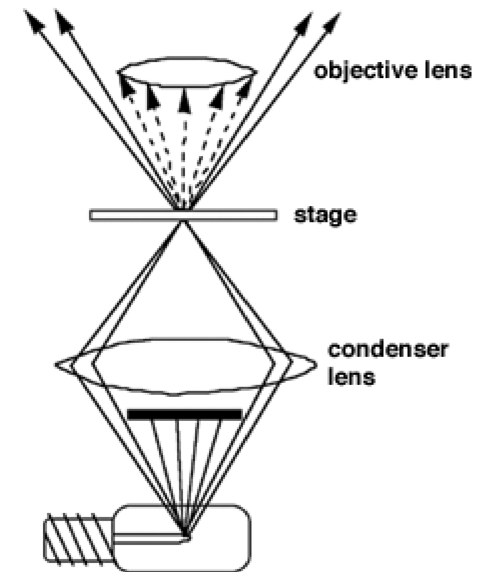
Bright field:

- “normal” wide-field illumination method
- bright background
- low contrast

Dark field:

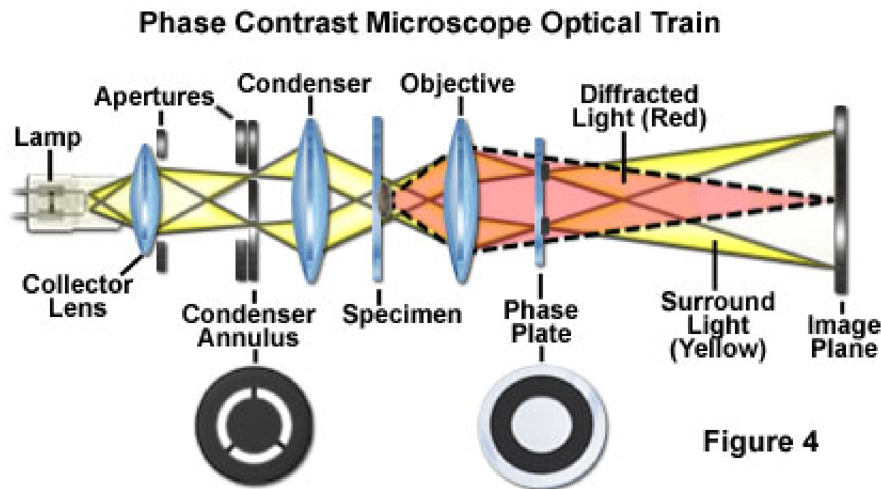
- an opaque disc is placed underneath the condenser lens
- scattered light
- dark background
- high contrast (structural details)





http://www.geog.ucl.ac.uk/~jhope/lab/micro23.stm

Phase Contrast Microscope



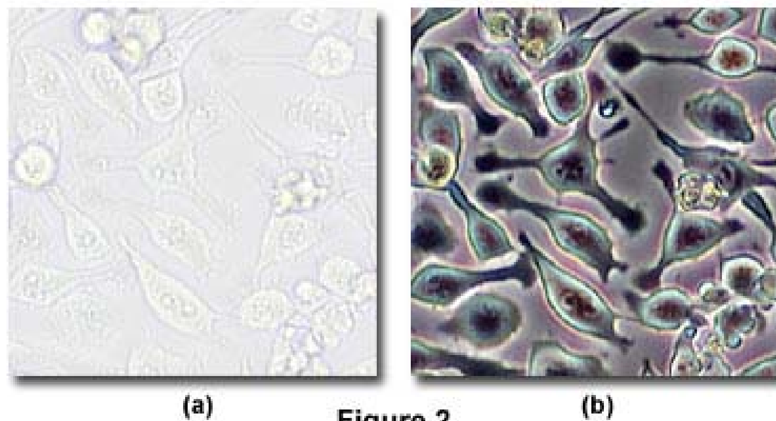


Figure 2

- bright-field
- destructive interference patterns in the viewed image (amplitude and phase difference)
- details in the image appear darker/brighter against a background
- colorless and transparent specimen, such as living cells and microorganisms

$$\mathbf{P = S + D}$$

Optical Path Length (D) = n • t

$$D = (n_2 - n_1) \cdot t$$

$$\delta = \mathbf{2\pi D/\lambda}$$

www.microscopyu.com

Applications of Optical Microscopy

1. Crystal morphology and symmetry

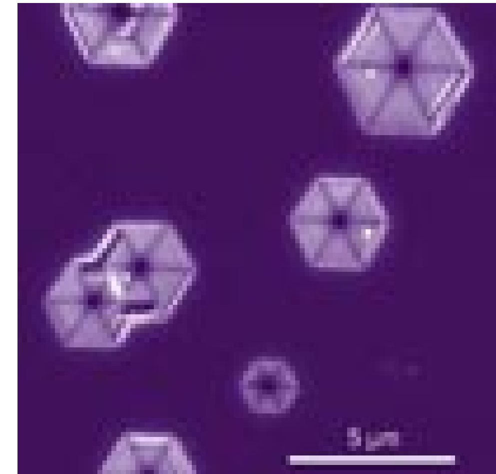
- Crystal fragments (characteristic shape)
- Classify isotropic and anisotropic substances
- Check possible symmetry (parallel extinction)

2. Phase identification, purity and homogeneity

- Standard optical data (refractive indices and optical axes) for comparison
- Phase analysis (impurities with separated crystalline/amorphous phase)
- Single *vs.* twinned crystal

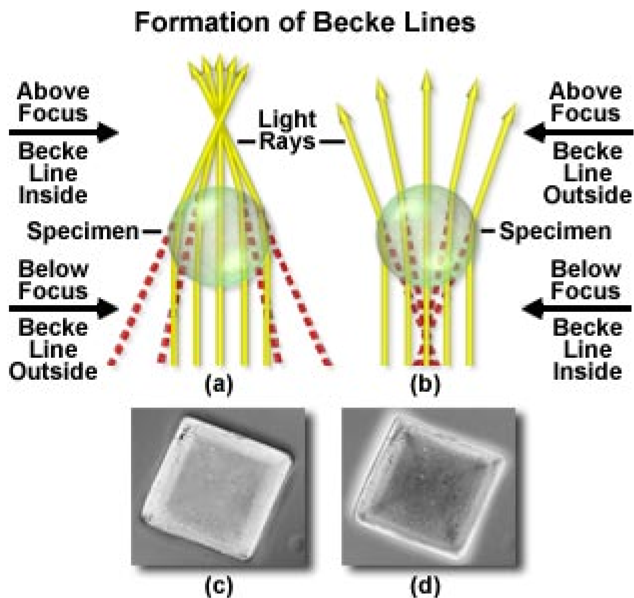
Applications of Optical Microscopy

3. Crystal defects – grain boundaries and dislocations



- Defects always present, even in single crystal
- Chemical etching may preferentially occur at stress sites

4. Refractive index determination



Becke line method:

- Sample (n_1) is immersed in a liquid (n_2)
- Out of focus, light is seen to emerge from region of higher n

Fluorescence Microscope

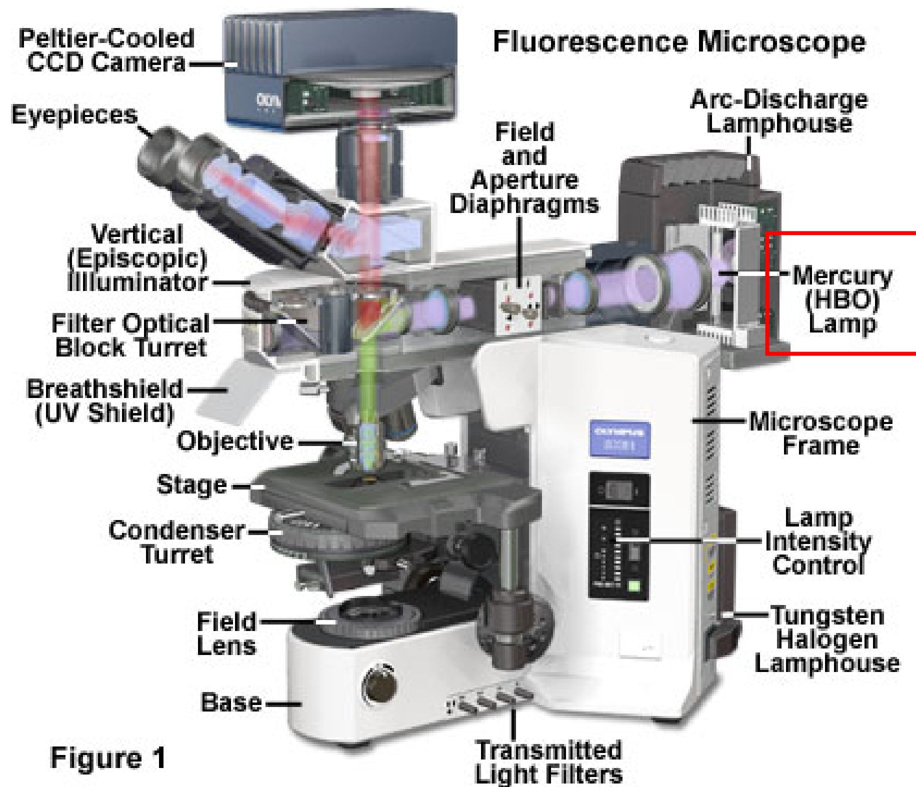
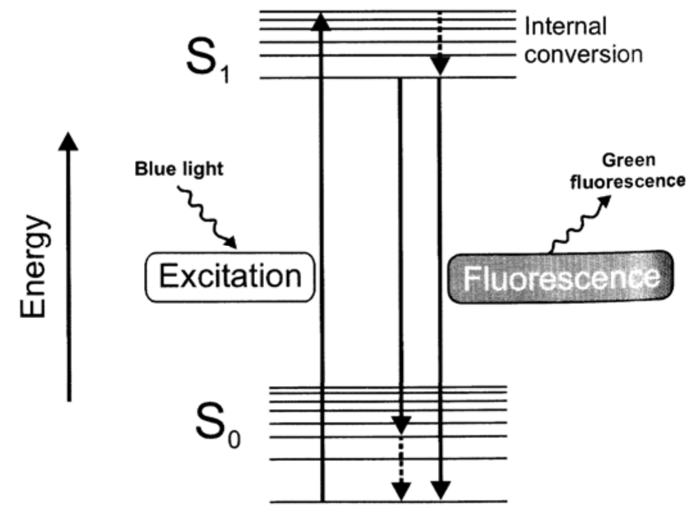


Figure 1



Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength

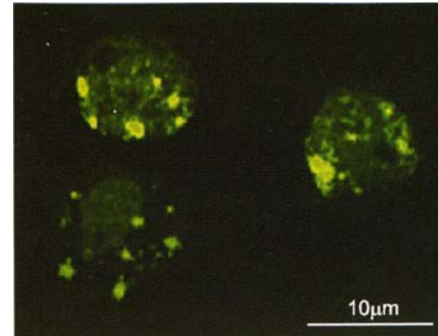
Fluorescence Microscope

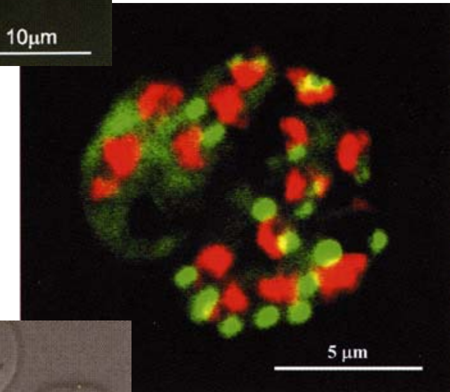
Especially useful in the examination of biological samples:

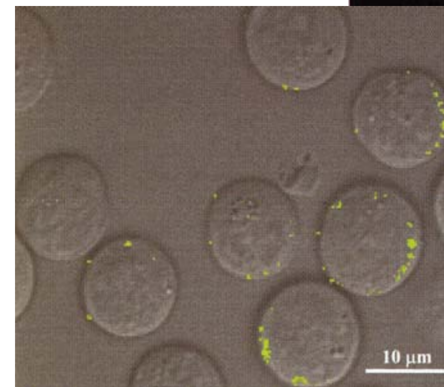
- *Identify* the particular molecules in complex structure (e.g. cells)
- *Locate* the spatial distribution of particular molecules in the structure
- Biochemical dynamics
- High signal to noise ratio
- Both reflected and fluorescence light

Drawback:

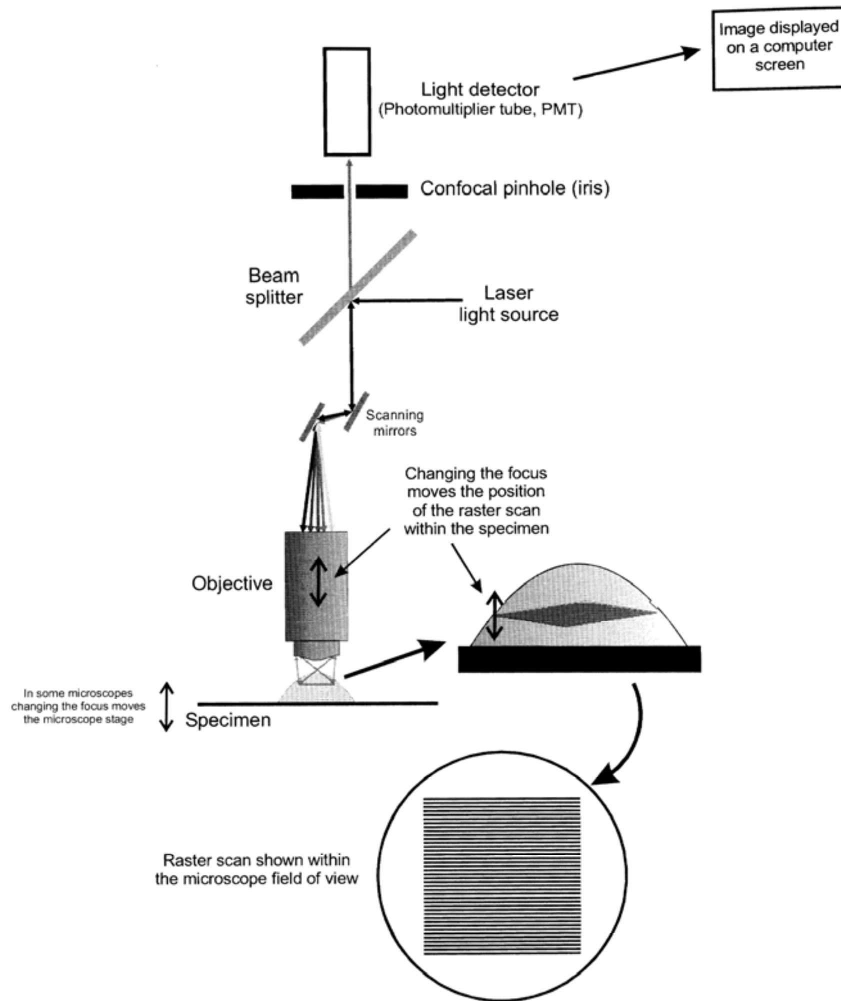
- Chemical labeling



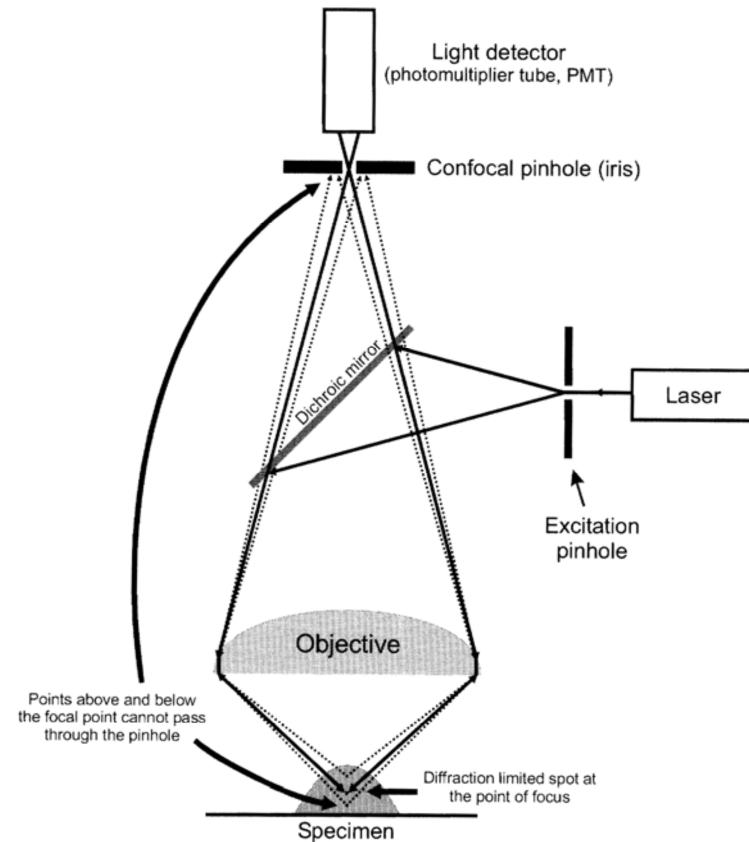




Laser Scanning Confocal Microscope



Scanning a diffraction-limited point of excitation light across the sample



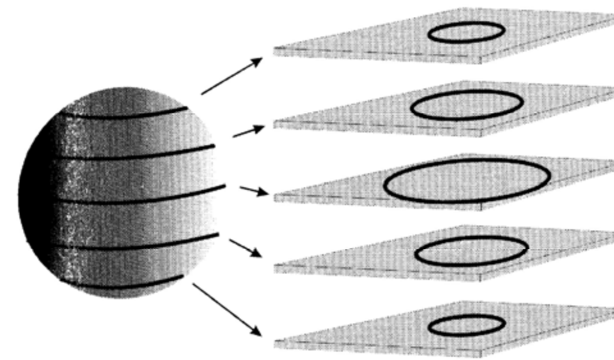
The out of focus light rays are eliminated from the image by the use of a *confocal "pinhole"*

Laser Scanning Confocal Microscope

Important technique for live cell and tissue imaging, the studies of biochemical dynamics!

Advantages:

- Optical sectioning ability
- 3D reconstruction
- Excellent resolution (0.1-0.2 μm)
- Specific wavelengths of light used
- Very high sensitivity



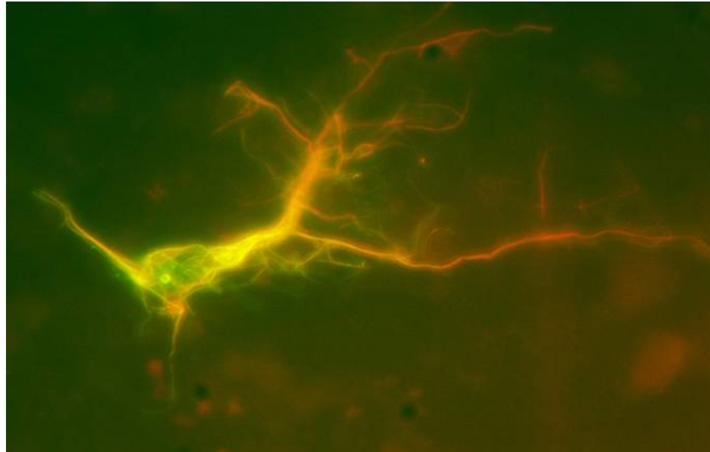
Optical sectioning

Drawbacks:

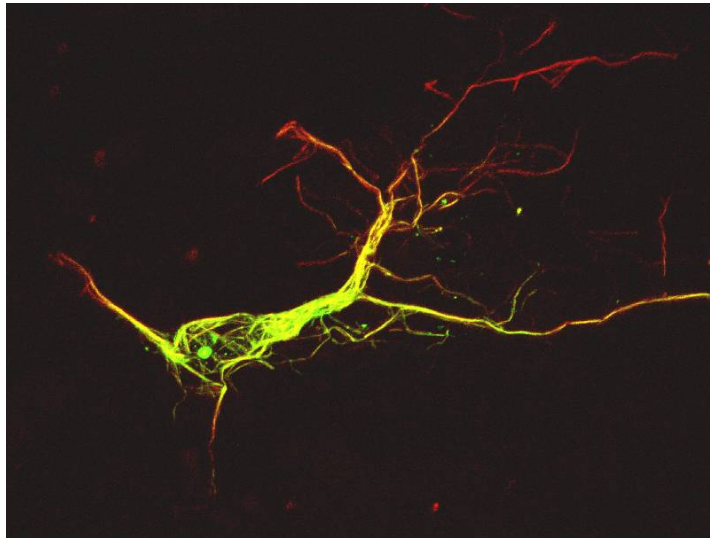
- Expensive
- Complex to operate
- Chemical labeling
- High intensity laser light

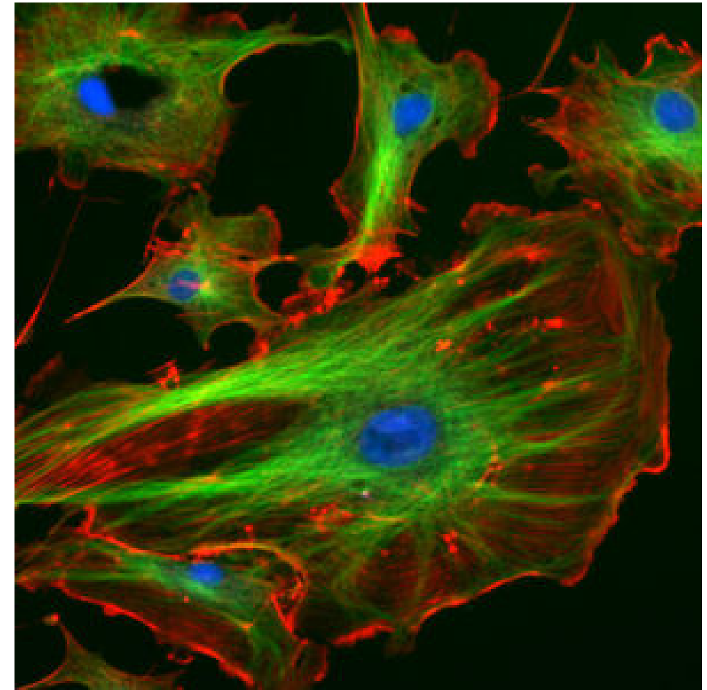
Advantages of Confocal Microscope

Conventional microscope



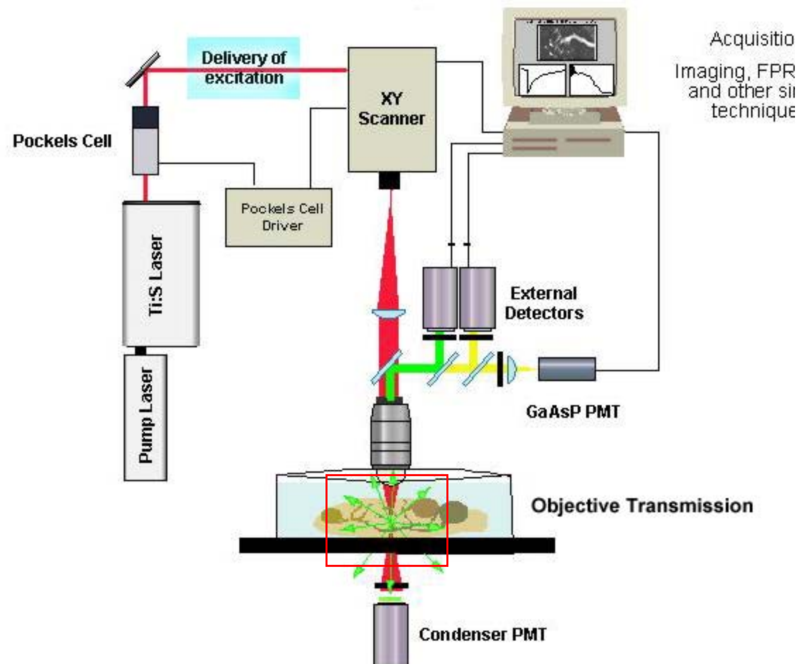
Confocal microscope

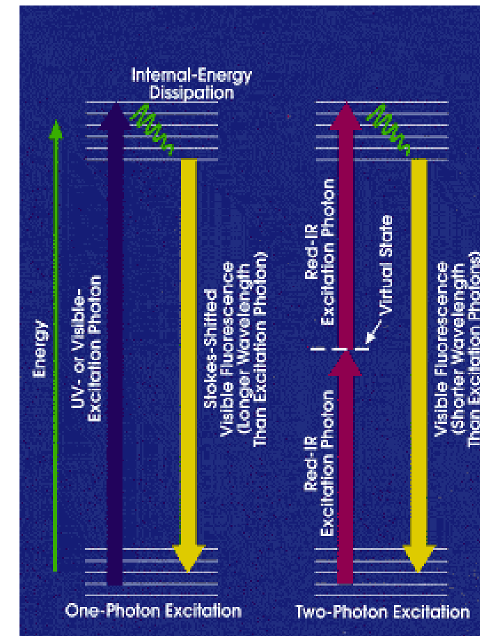


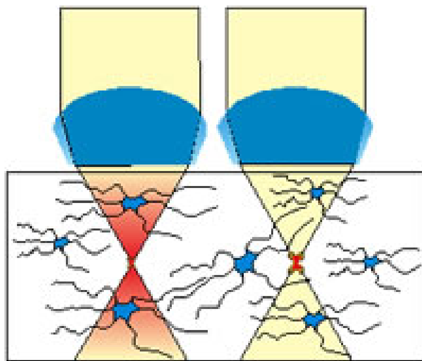


Confocal microscope image

Multiphoton Microscope







Advantages:

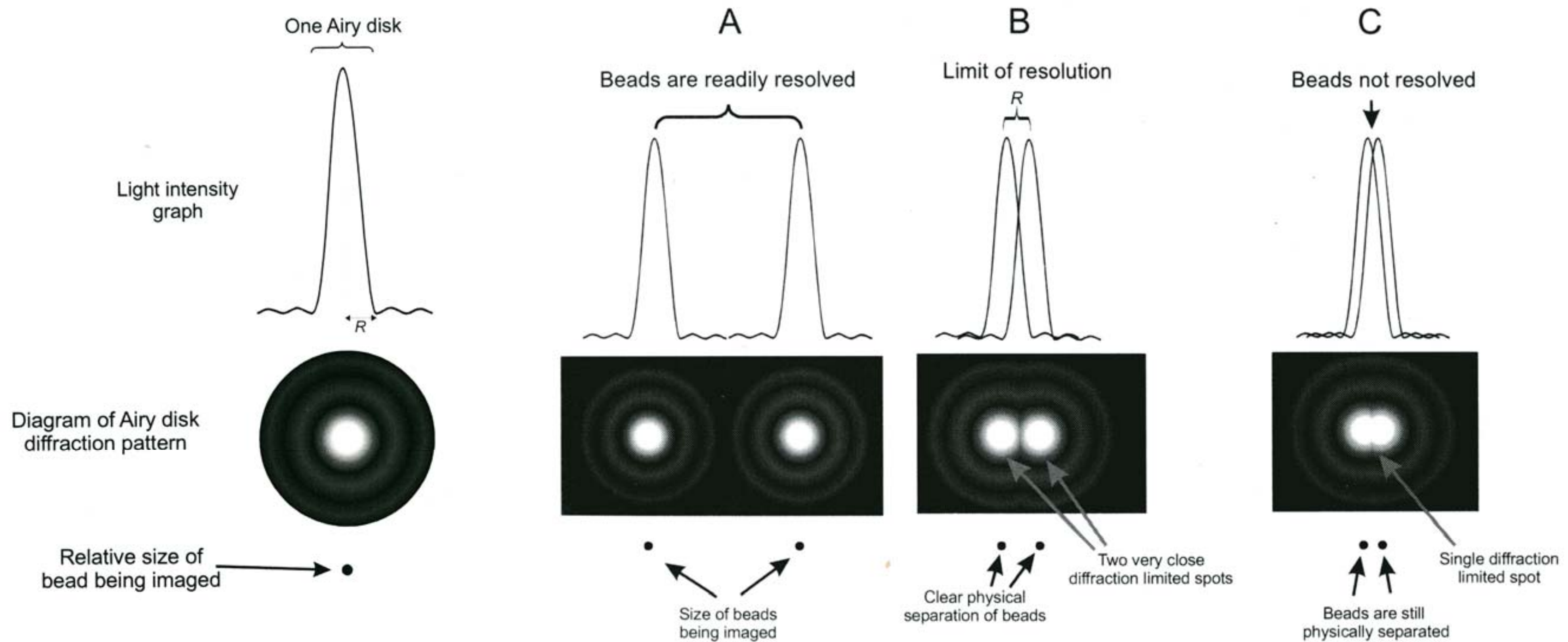
- Fluorescence only occurs at the *focal point*
- Able to image deeper into tissue sample

Drawbacks:

- Even more expensive (pulsed laser)
- Localized heating (photobleaching)

Limitation in Optical Microscopy

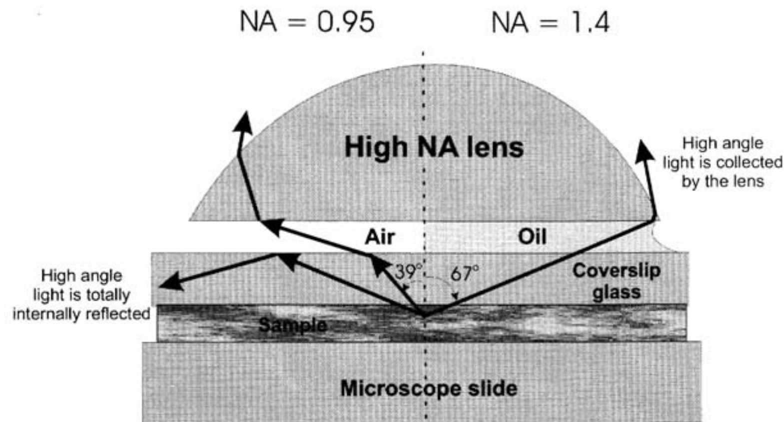
Resolution limited by wavelength of light (diffraction)



$$R = \frac{1.22\lambda}{NA_{objective} + NA_{condenser}} = \frac{1.22\lambda}{2NA_{objective}}$$

NA: numerical aperture

Numerical Aperture



NA = nsinθ   n: refractive index

Lens in air:

n of air: 1
sinθ ⩽ 1
} NA ⩽ 1

Lens in oil:

n of oil >1, similar to coverslip glass (~1.5)

sinθ increase (total internal reflection occur at high θ)

Overall NA will increase, >1

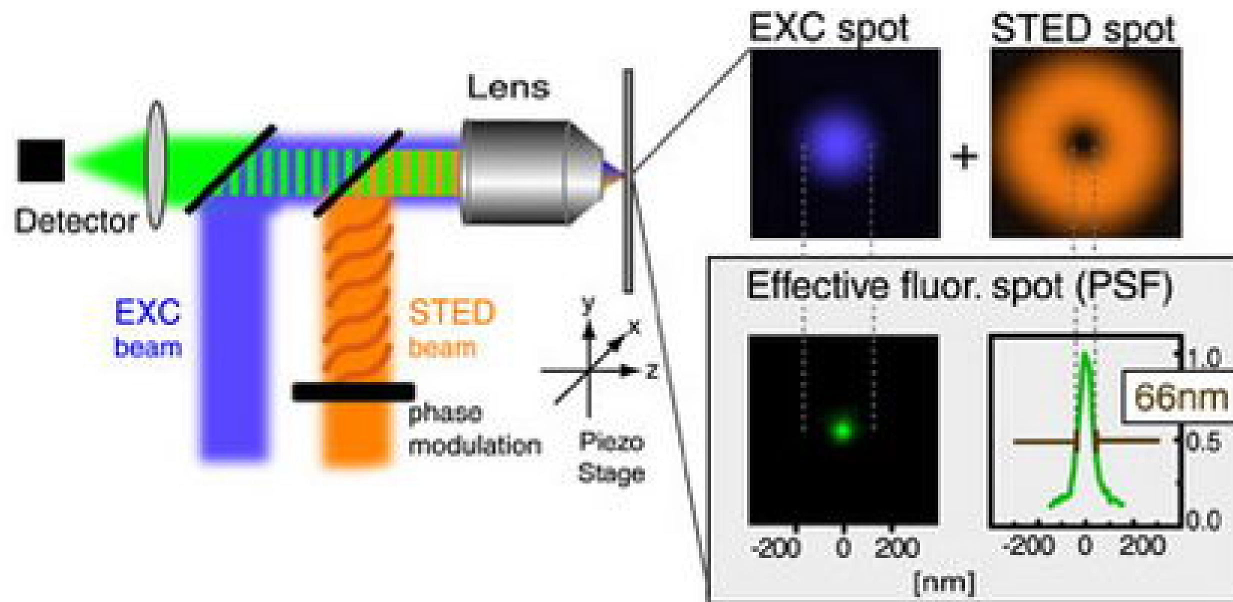
$$R = \frac{1.22l}{2NA_{objective}}$$

$$= \frac{1.22\ (400nm)}{2(1.4)}$$

$$= \sim 175nm$$

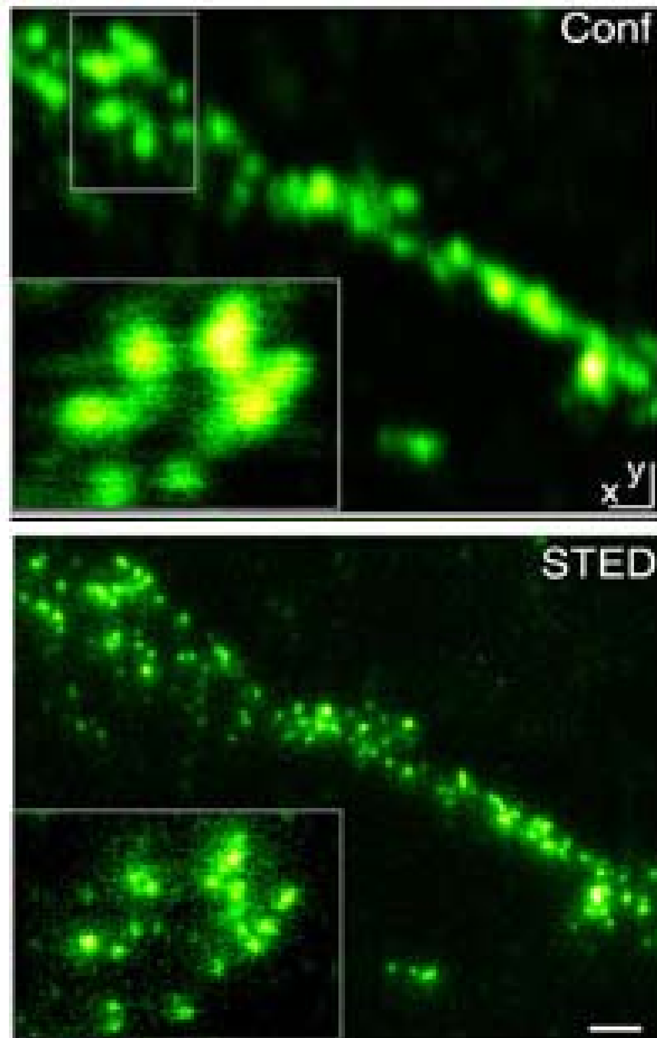
Stimulated Emission Depletion (STED) Microscopy

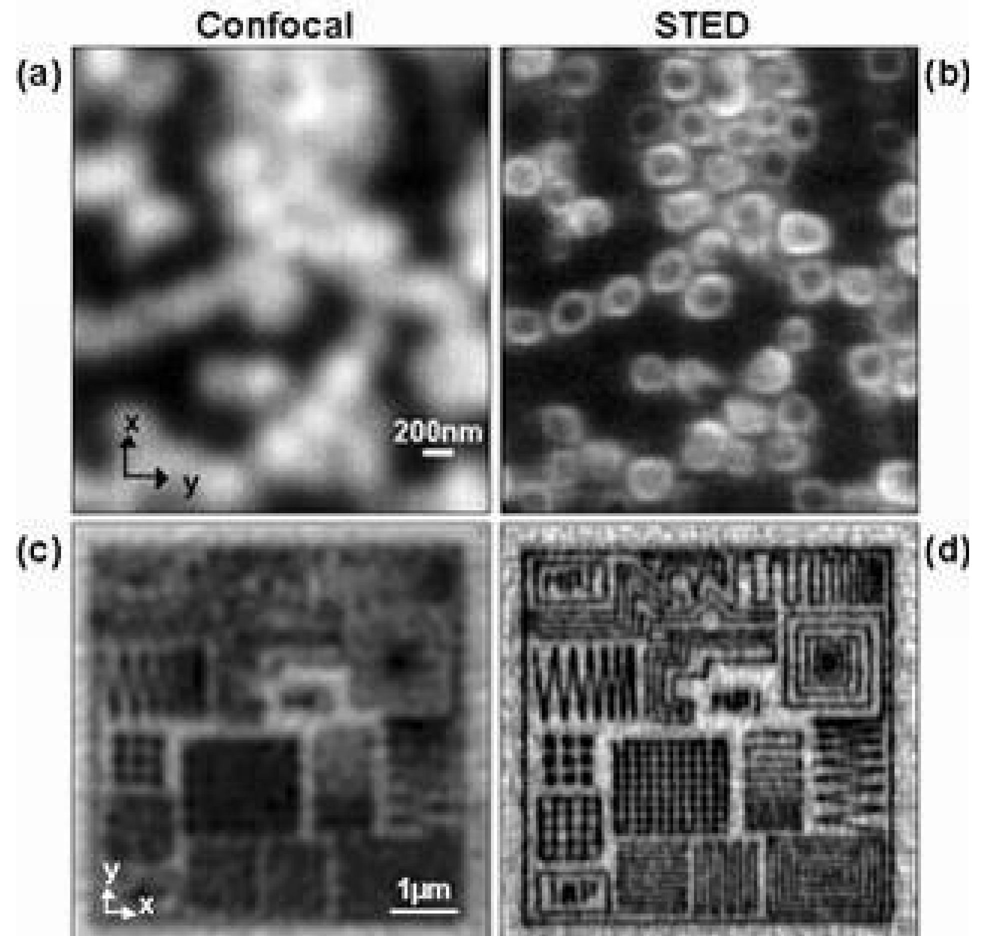
Prof. Stefan W. Hell (Max Planck Institute for Biophysical Chemistry)



- The excitation spot is ~200 nm by focusing with a lens
- A STED beam (doughnut-shaped and centered over the excitation spot) is used to quench the fluorescent markers before they fluoresce
- Very smaller effective fluorescence spot (~60 nm)

Resolution Enhancement using STED





www.physorg.com

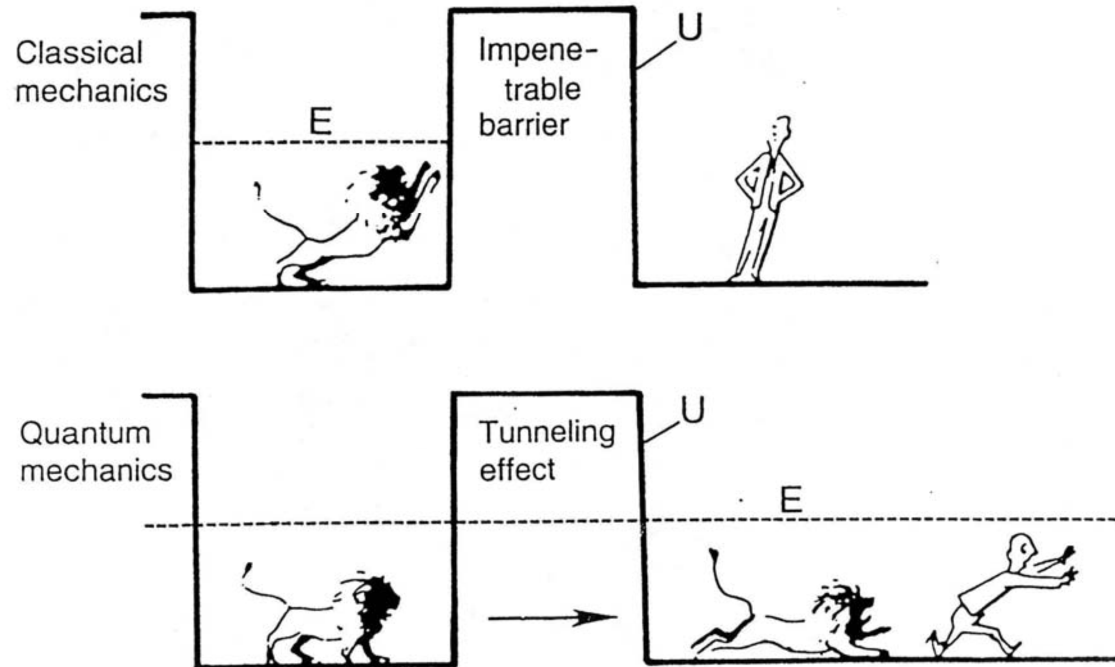
2. Scanning Probe Microscopy

Scanning Tunneling Microscopy (STM)

1986 Nobel Prize in Physics: Drs. Gerd Binning and Heinrich Rohrer (IBM Zurich)

Invention of the STM

Quantum tunneling:

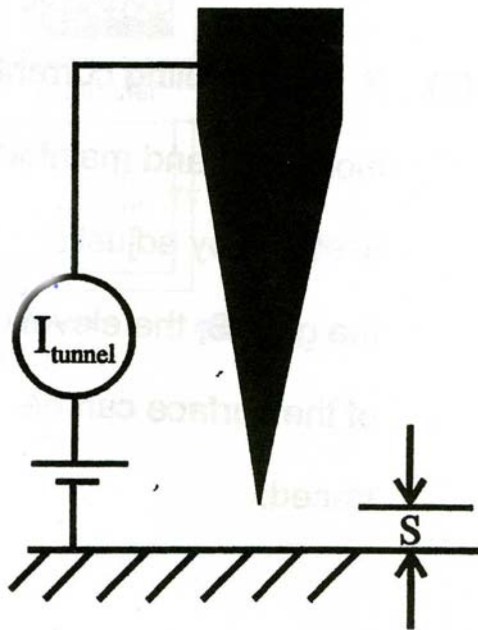


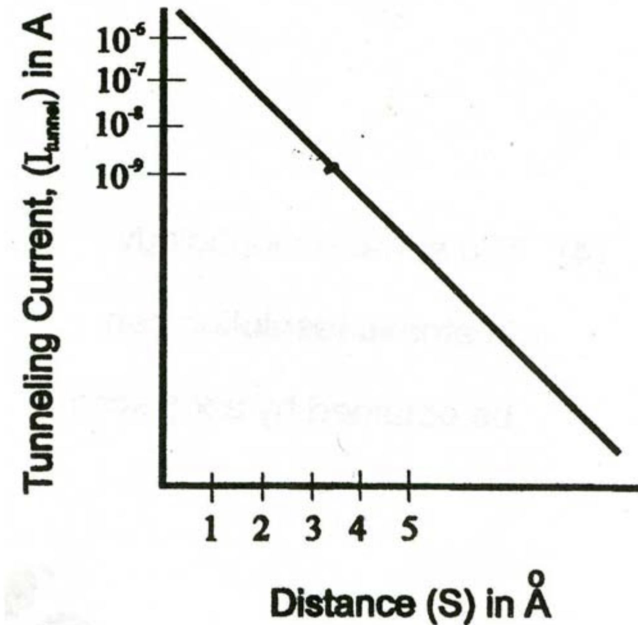
In quantum mechanics, an electron has a non-zero probability of tunneling through a potential barrier

Principle of STM

1. When a conducting tip is very close to a conducting/semiconducting surface and a bias voltage is applied, there will be a tunneling current flowing between the tip and the surface

2. The tunneling current (~pA-nA) is a strong function of the gap between the tip and the surface

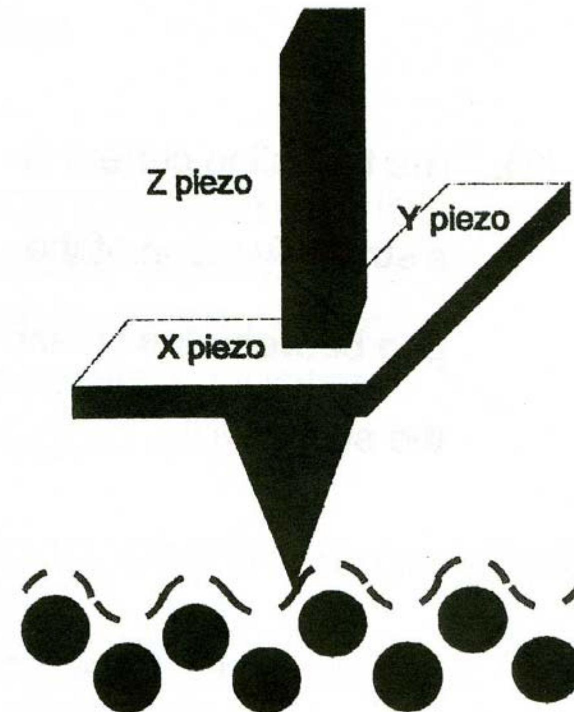




Principle of STM

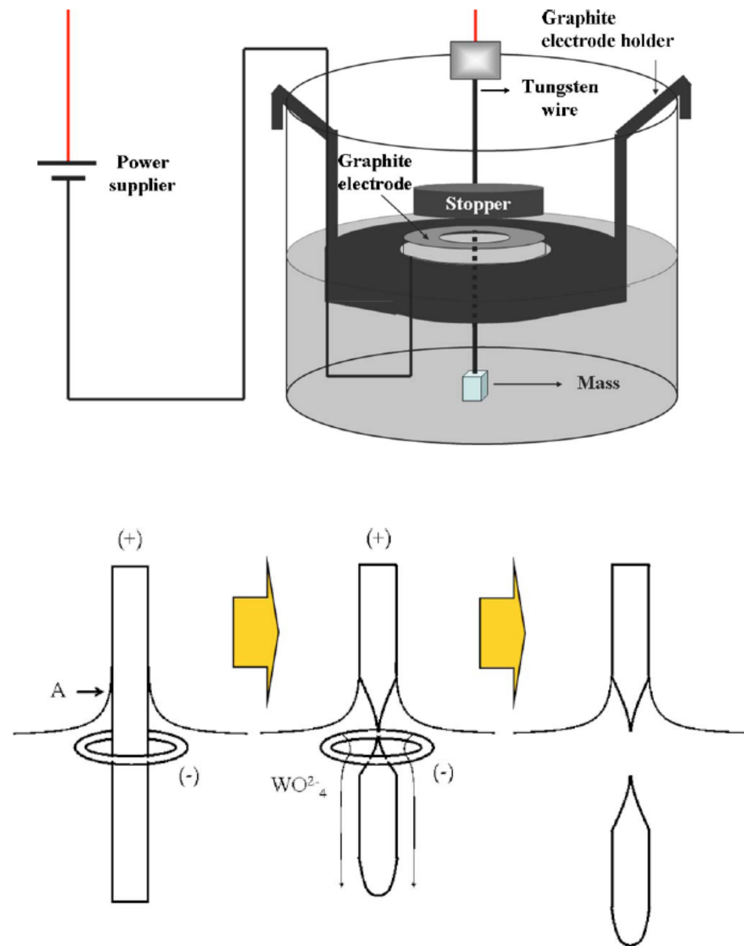
3. If the tunneling current is monitored and maintained constant by adjusting the gap, the elevation of the surface can be traced

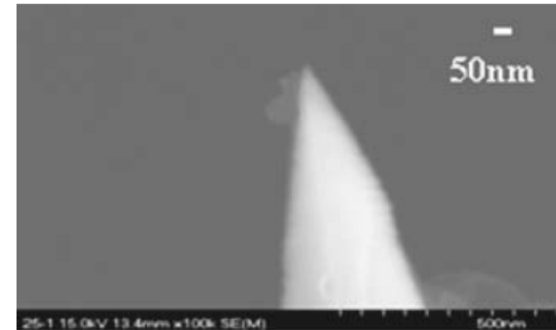
4. The surface morphology in atomic resolution can be obtained by x-y scan

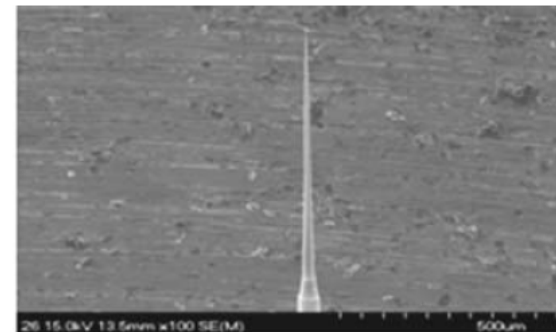


Very Sharp Tungsten Tip

Drop-off Method





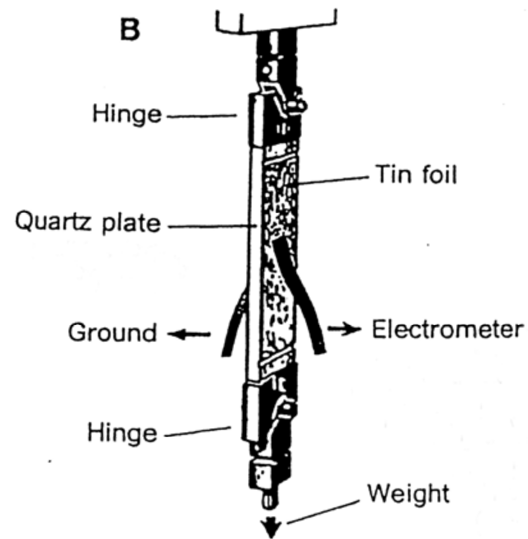


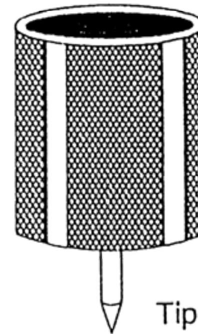
- Electrochemical etching method
- Average radius curvature < 50nm

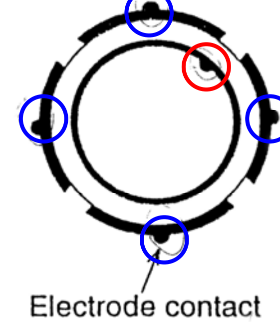
Jeong et al., *review of scientific instruments* **77**, 103706 (2006)

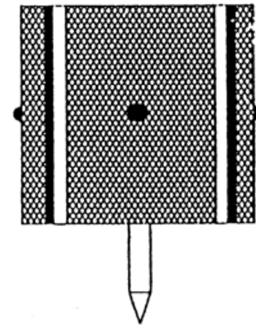
Piezoelectric Scanner

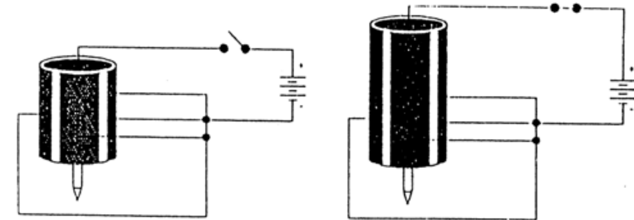
Piezoelectric effect



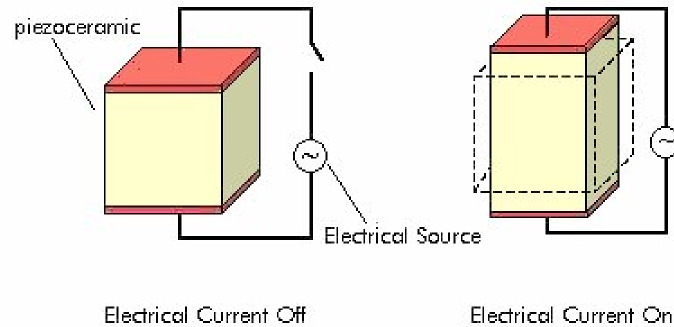


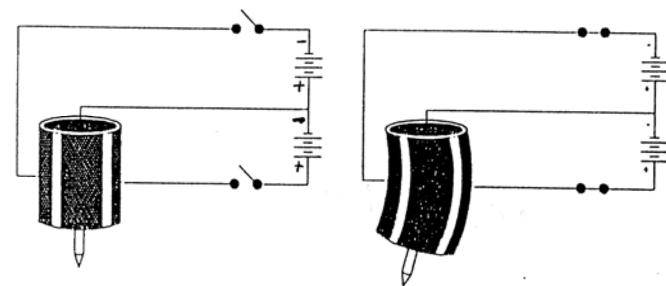




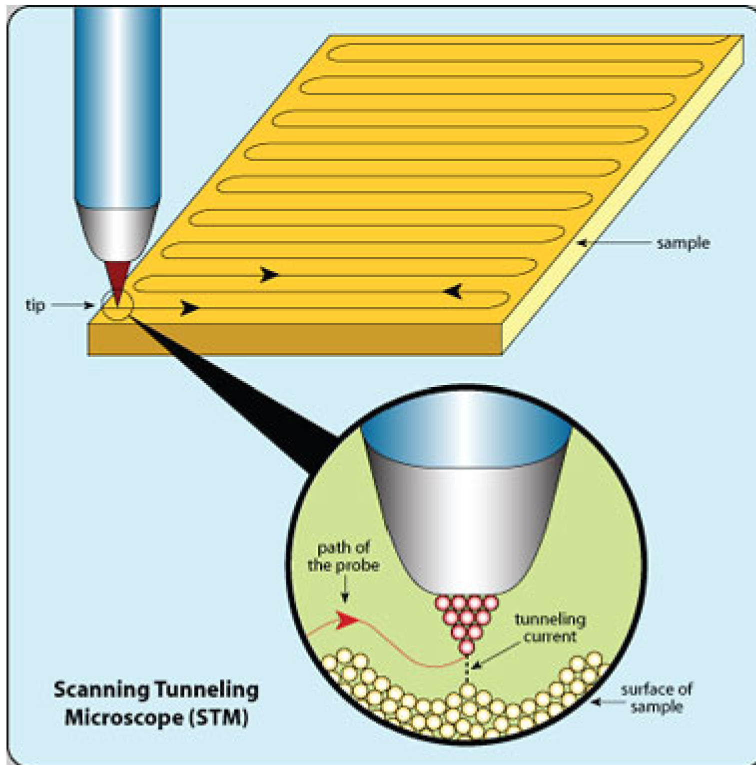


Inverse piezoelectric effect

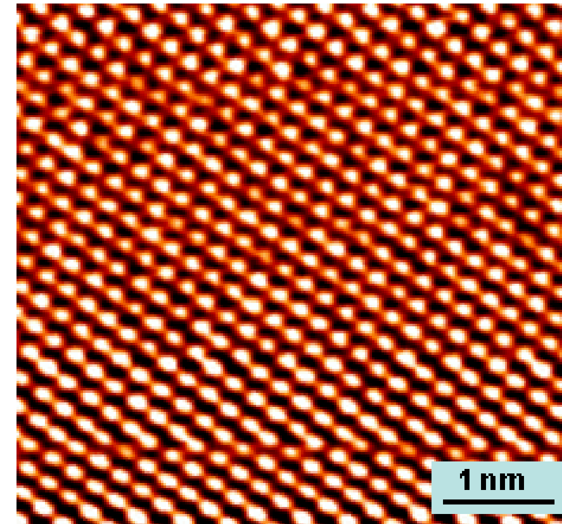


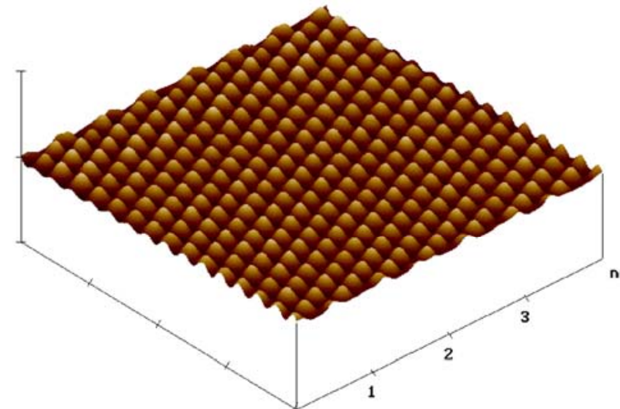


STM Imaging



HOPG surface (atomically flat)

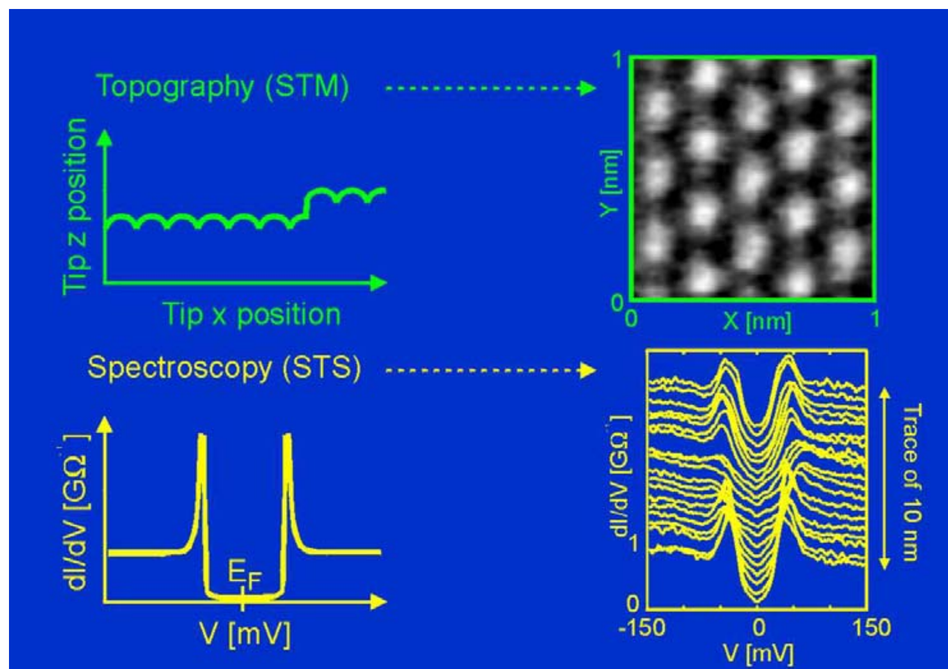




Atomic resolution (0.1nm)

Scanning Tunneling Spectroscopy (STS)

By ramping the bias voltage, or distance of the tip from surface, the current signal can reveal the local electronic character of the substrate.



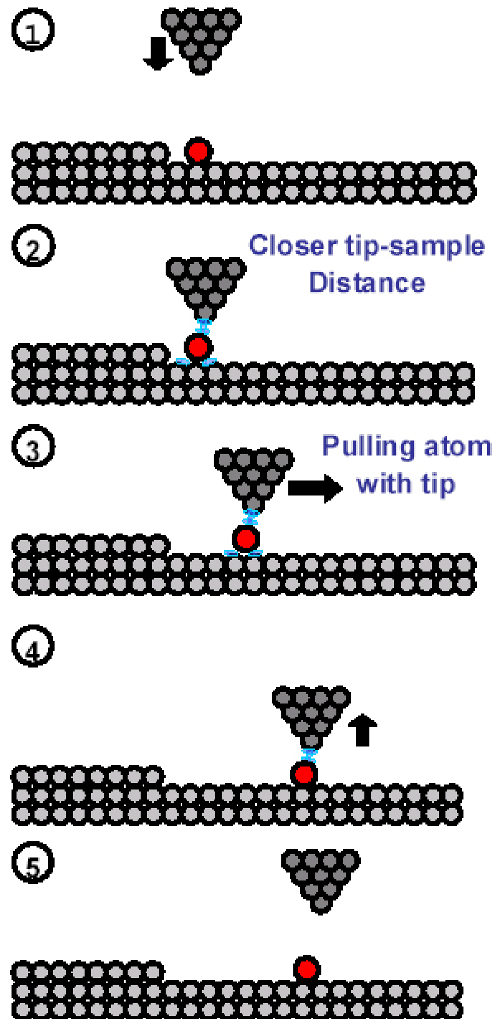
Can determine:

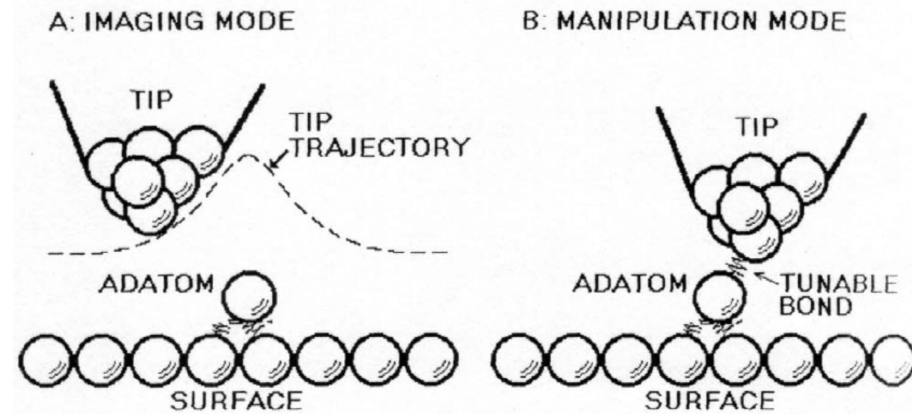
- Conductivity
- Bandgap
- Work function
- Density of State

Prof. Øystein Fischer's research group

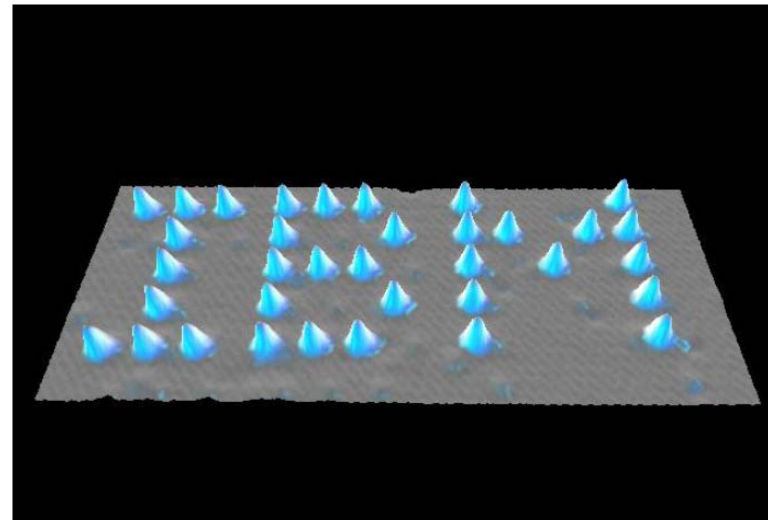
http://dpmc.unige.ch/gr_fischer/

Manipulation of Atoms





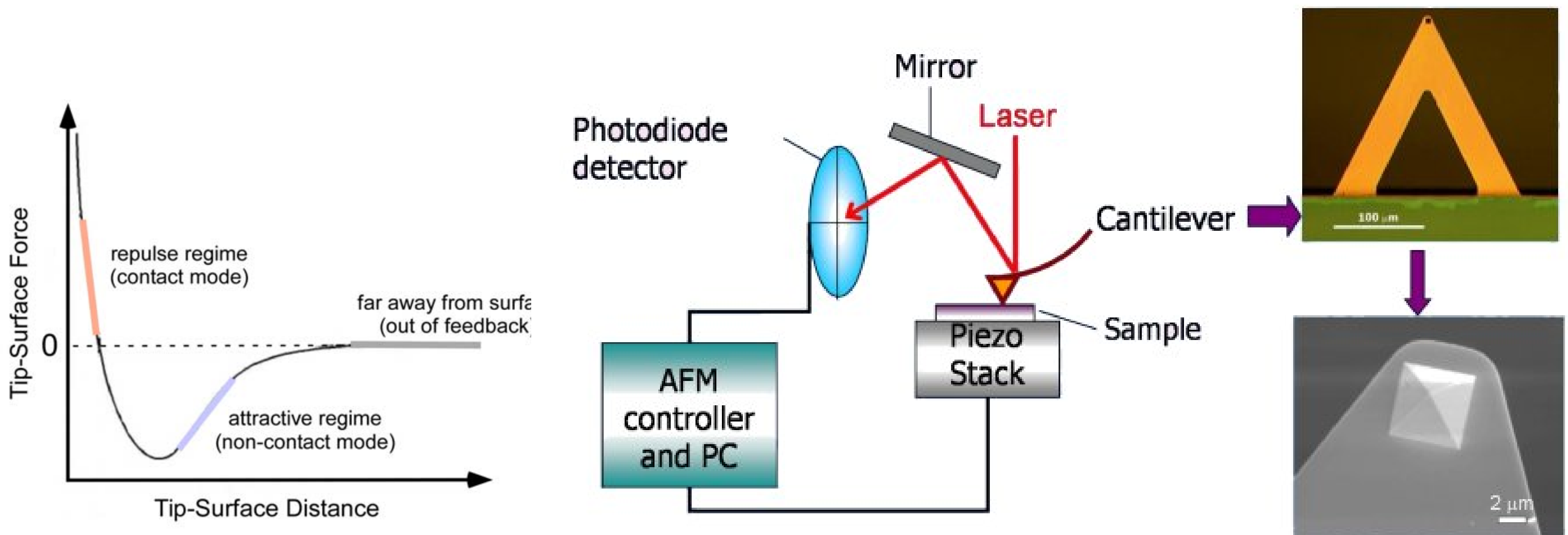
Xenon atom on Ni (110)



http://www.almaden.ibm.com

Atomic Force Microscopy (AFM)

Principle:



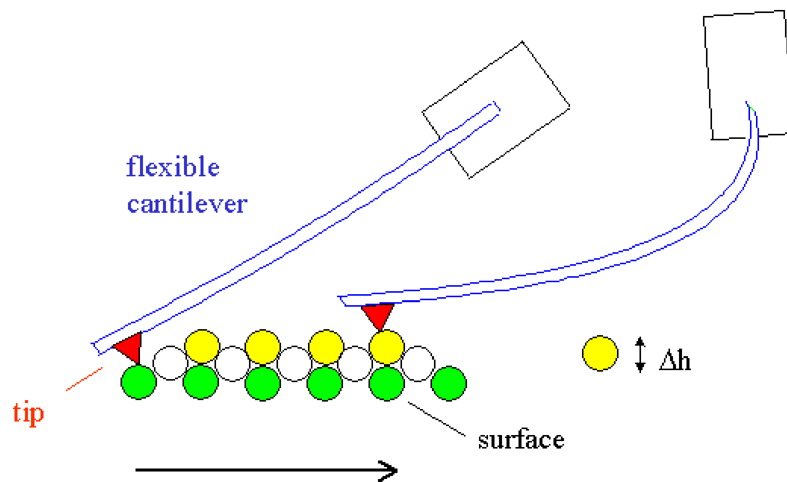
1. The molecular force is a strong function of the separation between two object

2. The force can be monitored by the deflection of a cantilever (100-200mm long) which is in turn amplified by the deflection of a laser beam

3. Constant force is maintained by adjusting the z-position of the surface. A x-y scan will produce the morphology

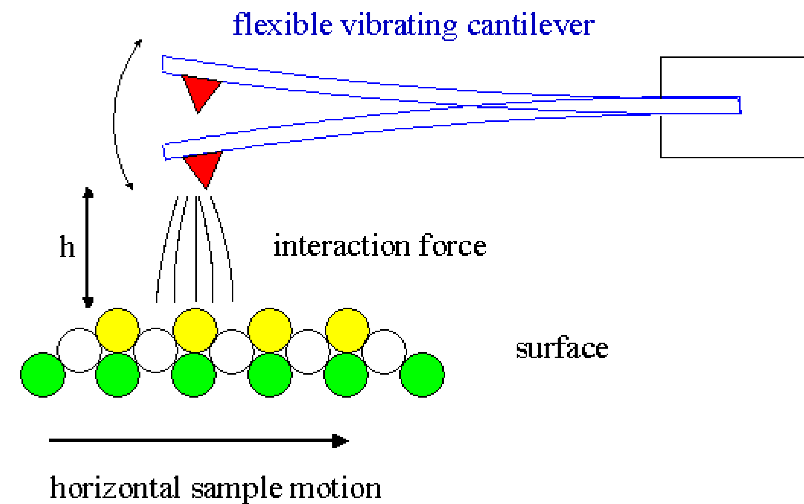
Operation Modes of AFM

I. Contact mode



- Tip touching surface
- Interaction force is repulsive (10^{-8} -10^{-6}N)

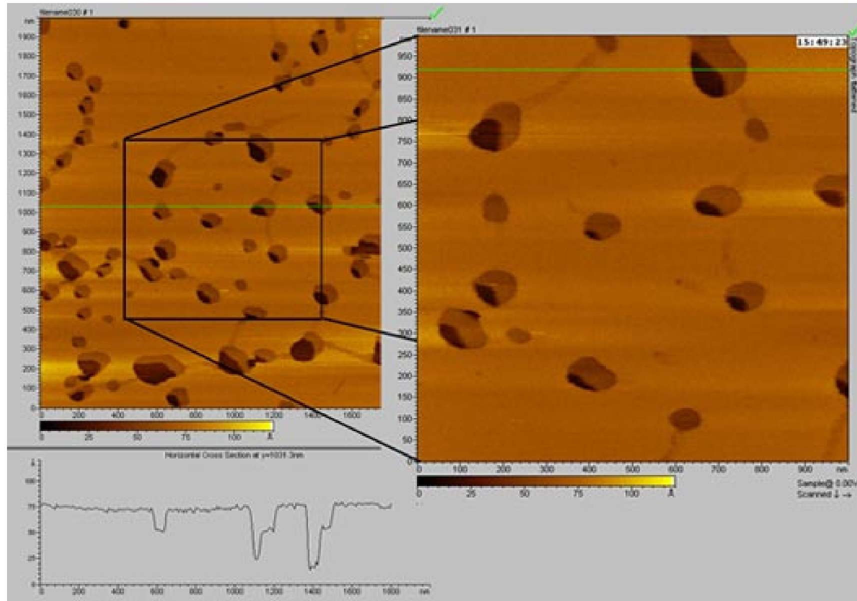
II. Tapping mode



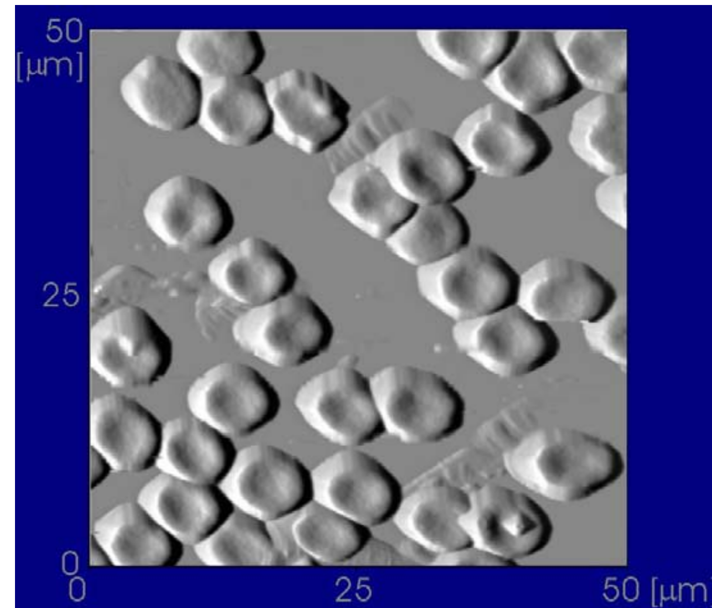
- >10nm above surface, no contact
- Cantilever set into vibration
- Detect changes in the resonant frequency of cantilever
- Feedback control of height

Applications of AFM

1. Imaging



Red blood cell

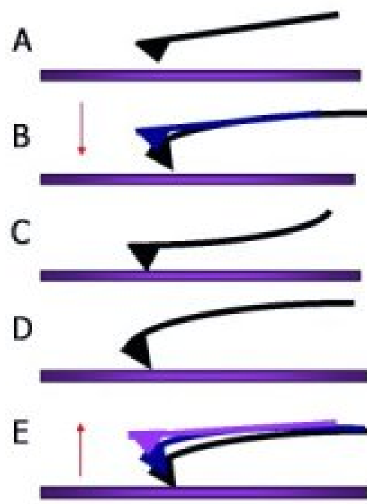


www3.imperial.ac.uk/

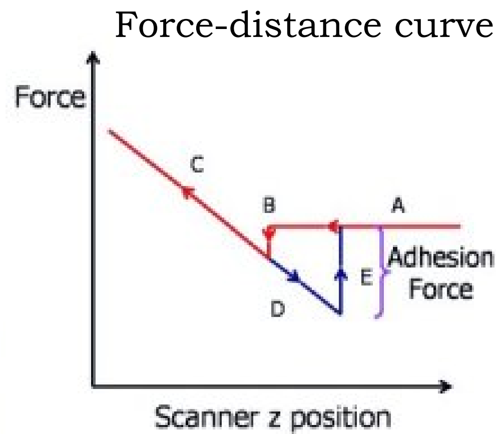
- Resolution ~nm
- Topology
- Able to image non-conducting materials e.g. polymer and biological samples

Applications of AFM

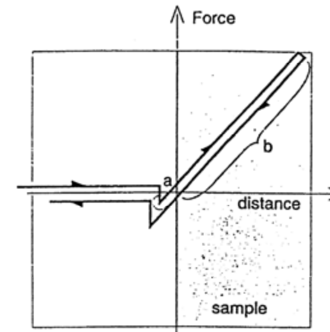
2. Force mapping



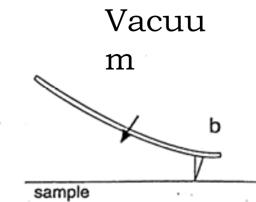
Force-distance curve

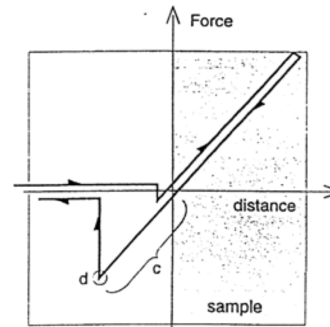


- To detect the variation of softness, elasticity and stickiness on sample surface
- Useful for composite materials

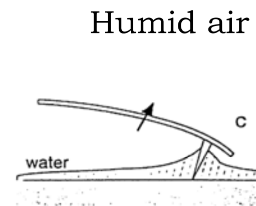


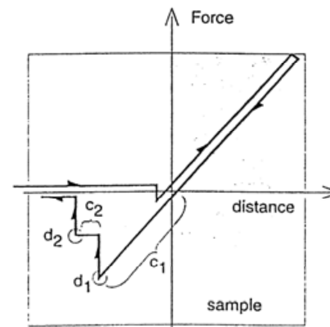
Vacuum



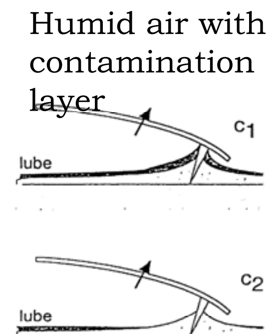


Humid air



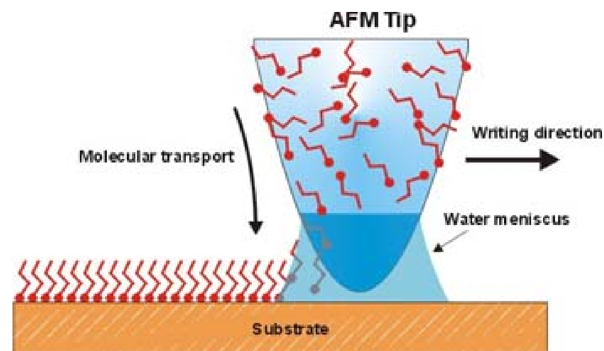


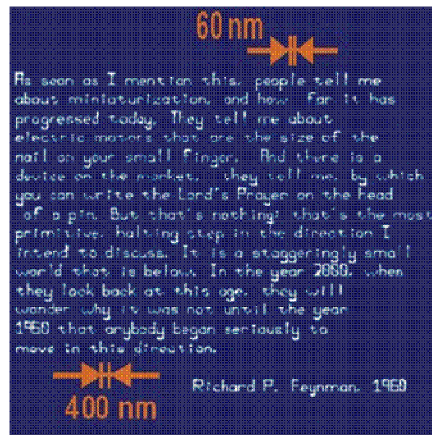
Humid air with contamination layer



Applications of AFM

3. Dip-Pen Nanolithography

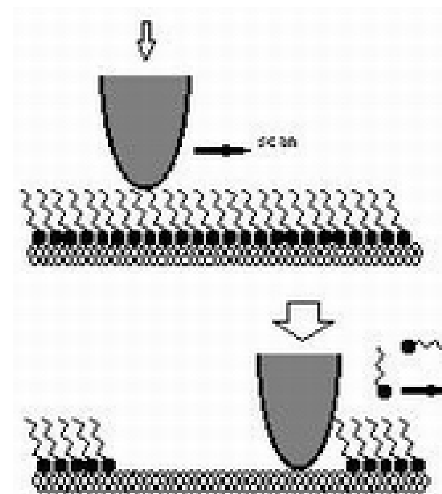




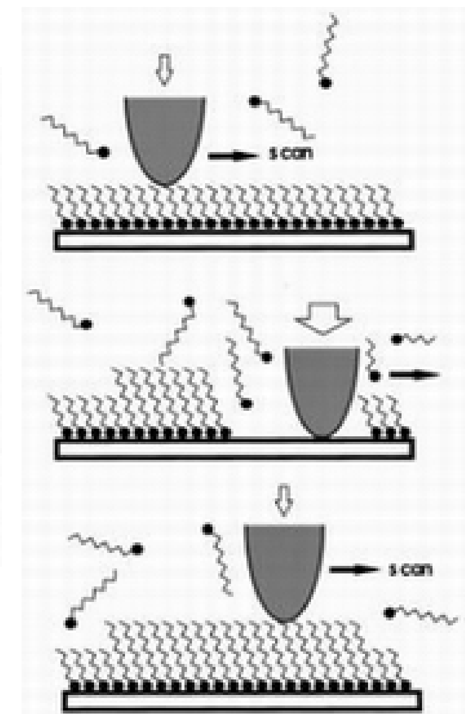
Prof. Chad A. Mirkin research group

4. Nanofabrication

Nanoshaving



Nanografting



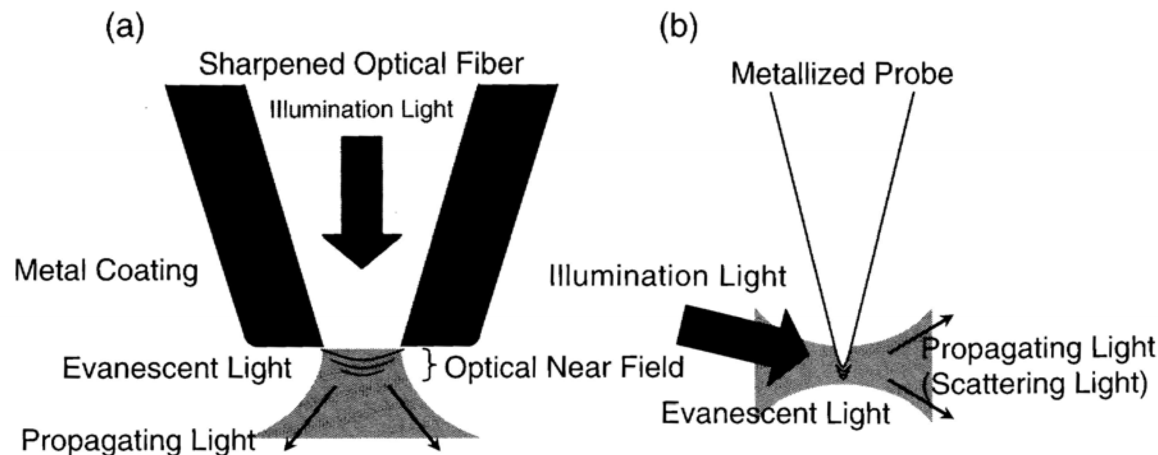
- Pattern molecules in high resolution
- Functionalize surfaces with patterns of two or more components

Summary of STM and AFM Functions

	STM	**AFM**
Instrumentation	Tip, scanner, controller	Cantilever, scanner, optics, controller
Conducting samples	Yes	Yes
Non-conducting samples	No	Yes
Resolution in vacuum	<0.1 Å	~ Å
In dry air	< 1 Å	~ nm
In liquid	~ nm	~ 10 nm
Operation in liquid	Tip coating	No coating needed
Modes of operation	Constant height	Constant height
	Constant current	Constant force
		Contact mode
		Tapping mode
Applications	Imaging	Imaging
	Tunneling spectroscopy	Force mapping
	Manipulation of atoms/molecules	nanolithography

Near-field Scanning Optical Microscope (NSOM)

Principle of NSOM: Can be simply modeled by the electromagnetic interaction of two very closely positioned nano-objects, which represent a probe and sample



<u>Aperture-type</u>

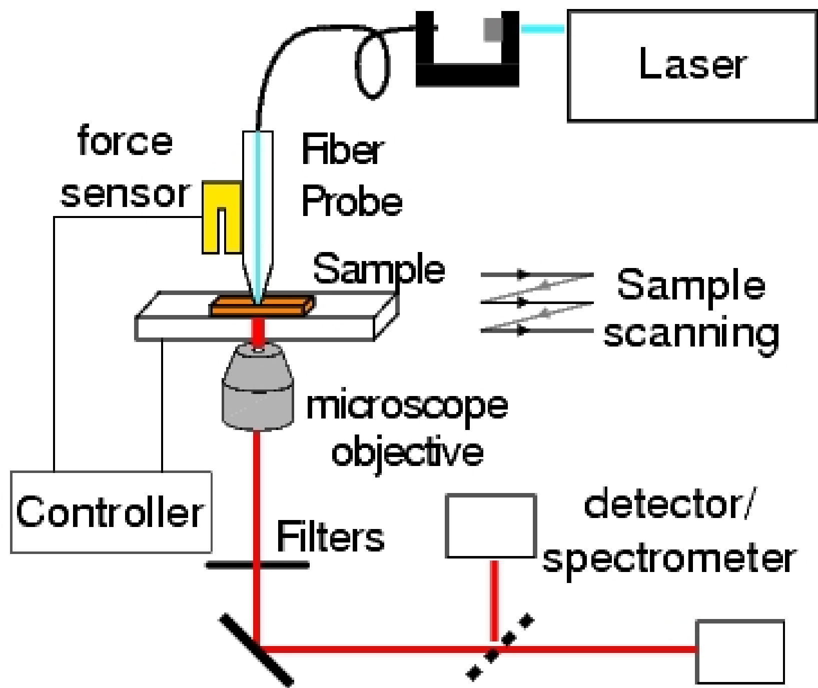
- Nanoscale light spot same as aperture size
- Aperture-sample distance is regulated at < 10 nm

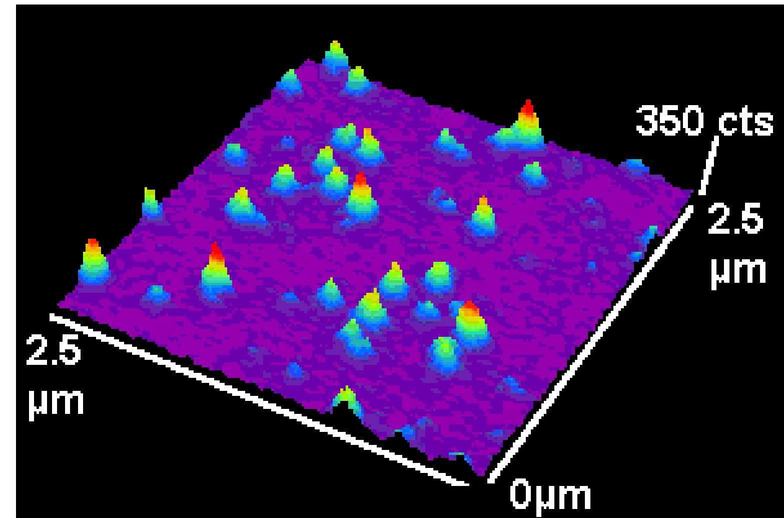
<u>Scattering-type</u>

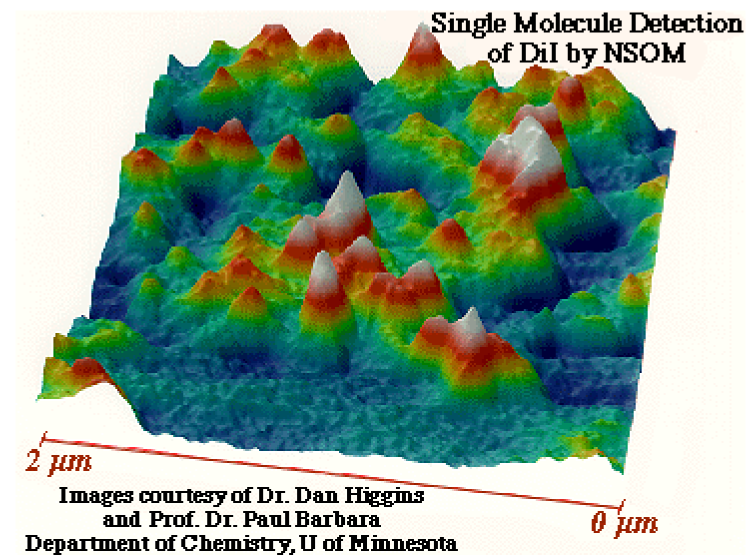
- Sharpened homogeneous metal tip, with enhanced electric field
- Spatial resolution defined by apex diameter

Review paper: Lewis A., *Nature Biotech.* **21**, 1378 (2003)

Single Molecule Fluorescence Imaging



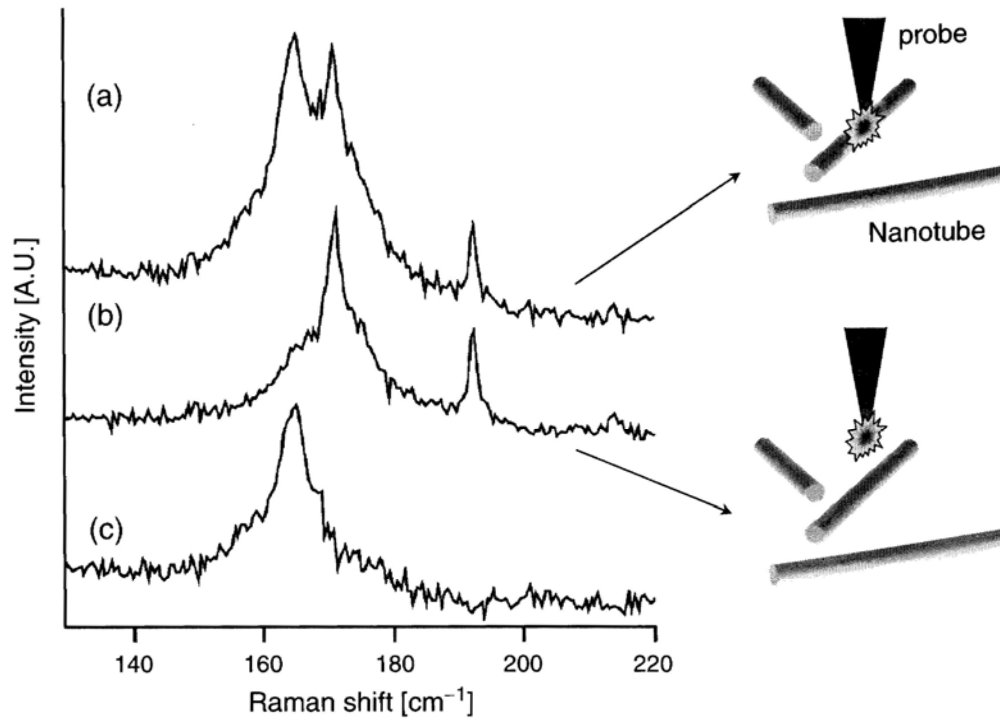




- Spatial resolution ~10-30nm
- Single molecule, quantum dot

Near-field Optical Spectroscopy

NanoRaman Spectroscopy



- Enhanced electric field at the tip
- Resolution as high as 15 nm

3. Electron Microscopy

Transmission Electron Microscopy, by David B. Williams and C. Barry Carter (Plenum Press, New York, 1996)

ISBN: 0-306-45247-2

Resolution and Abbe's Equation

Abbe's equation:

Resolution

Numerical aperture

$$R = 0.612\,\lambda\ /\ n \sin \alpha$$

Wavelength of imaging radiation

Wavelength of Electron:

$$\lambda = h\,(2meV)^{-1/2}$$

Planck's constant mass charge accelerating voltage

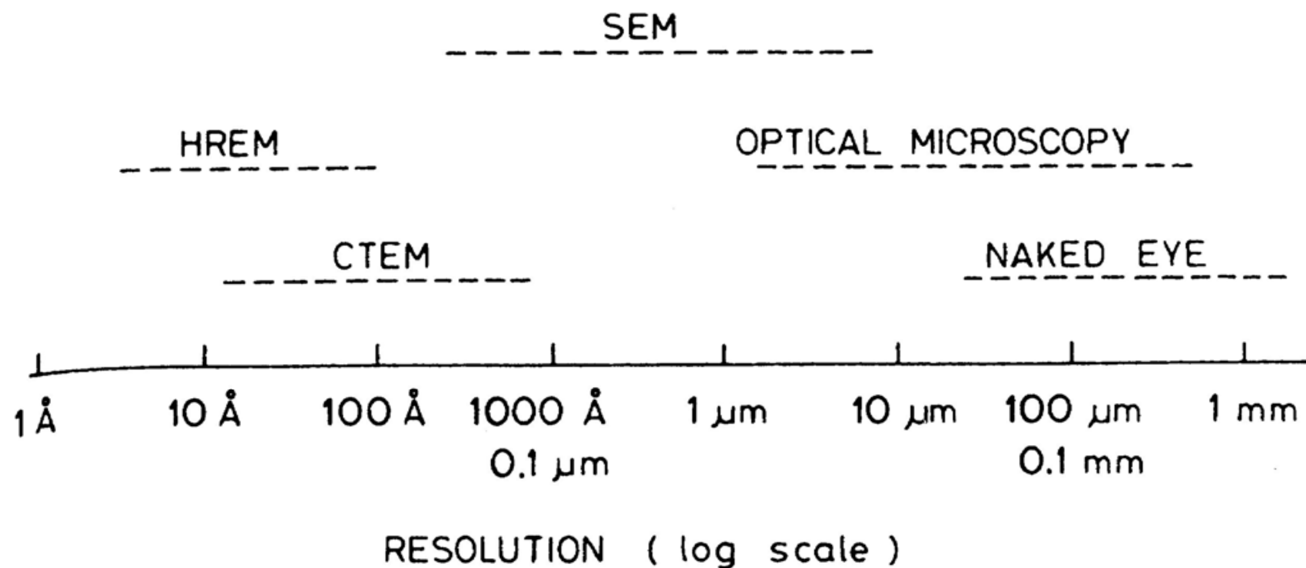
Electron microscopy:

- Very short wavelength (depends on accelerating voltage, ~0.04 Å at 100 kV)
- Can be deflected by magnetic field (focusing)

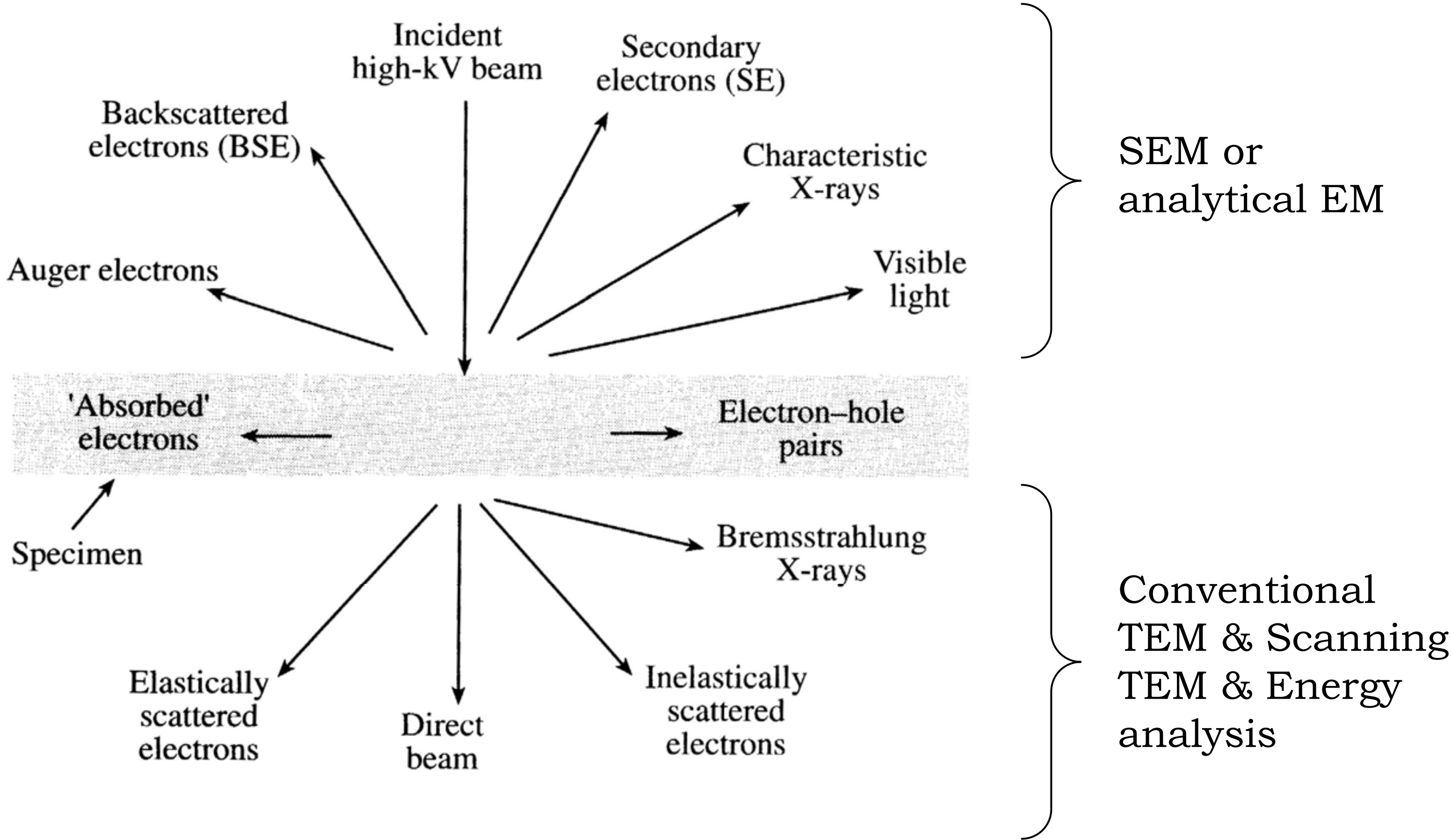
Fundamentals of Electron Microscopy

Scanning electron microscopy (SEM): For studying the texture, topography and surface feature, resolution ~ 10 nm

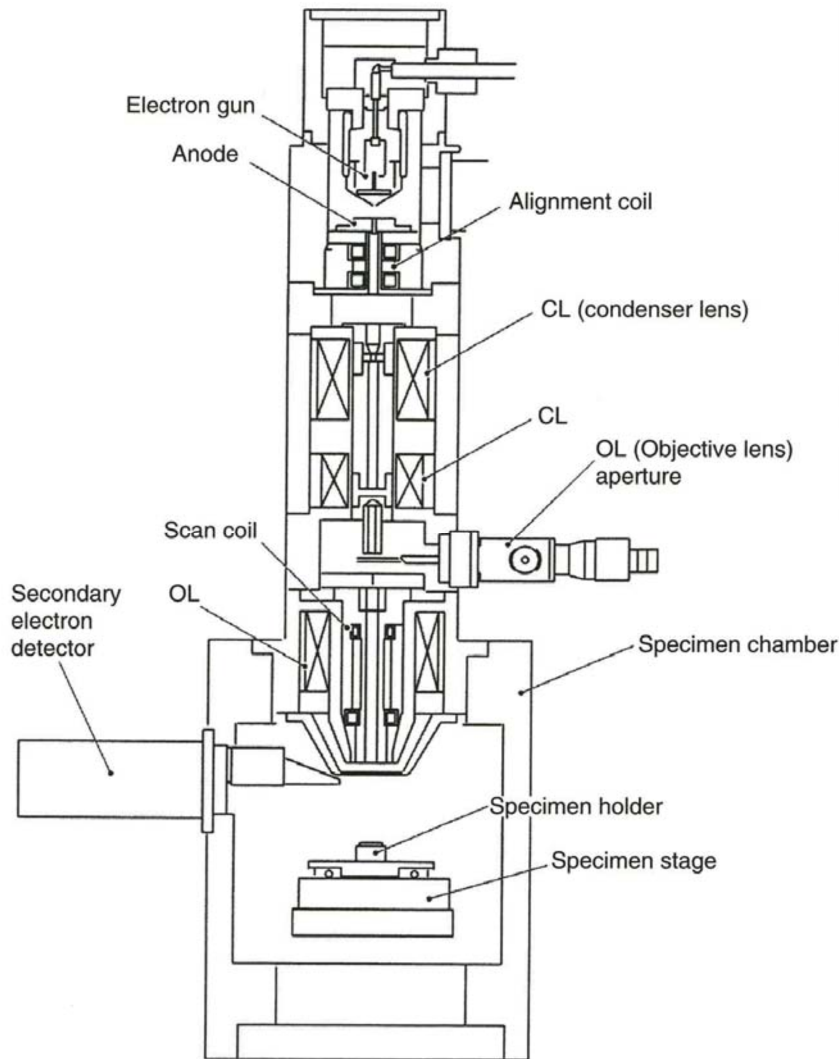
Transmission electron microscopy (TEM): Lattice imaging, resolution < 0.2 nm

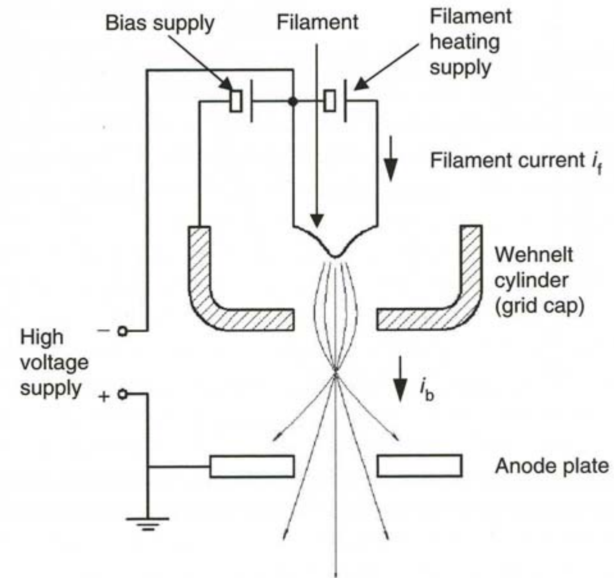


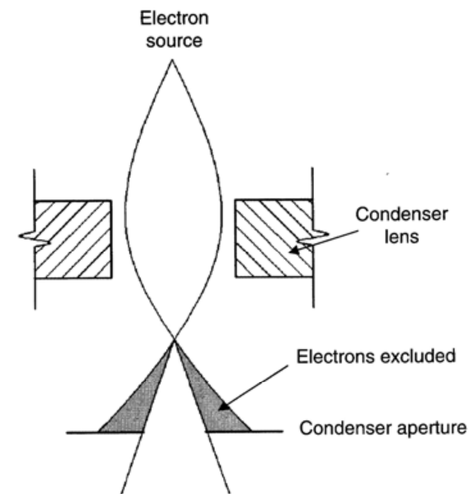
Interaction of Electron with Samples



Configuration of SEM

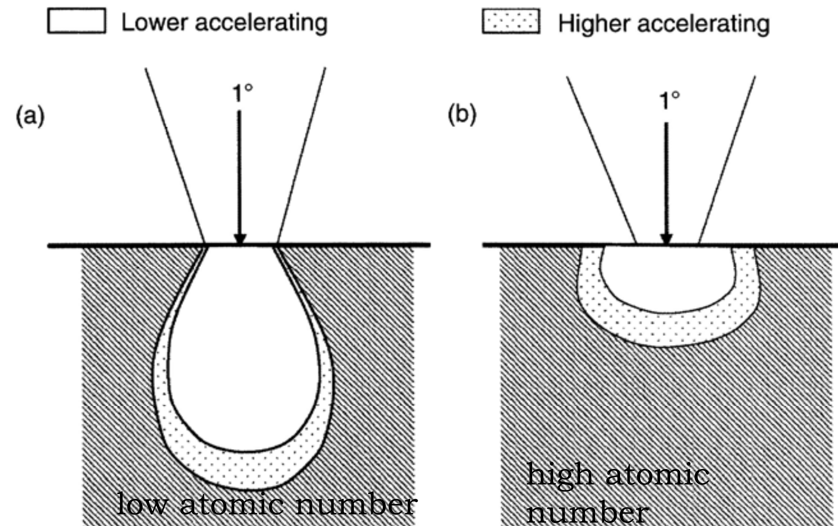


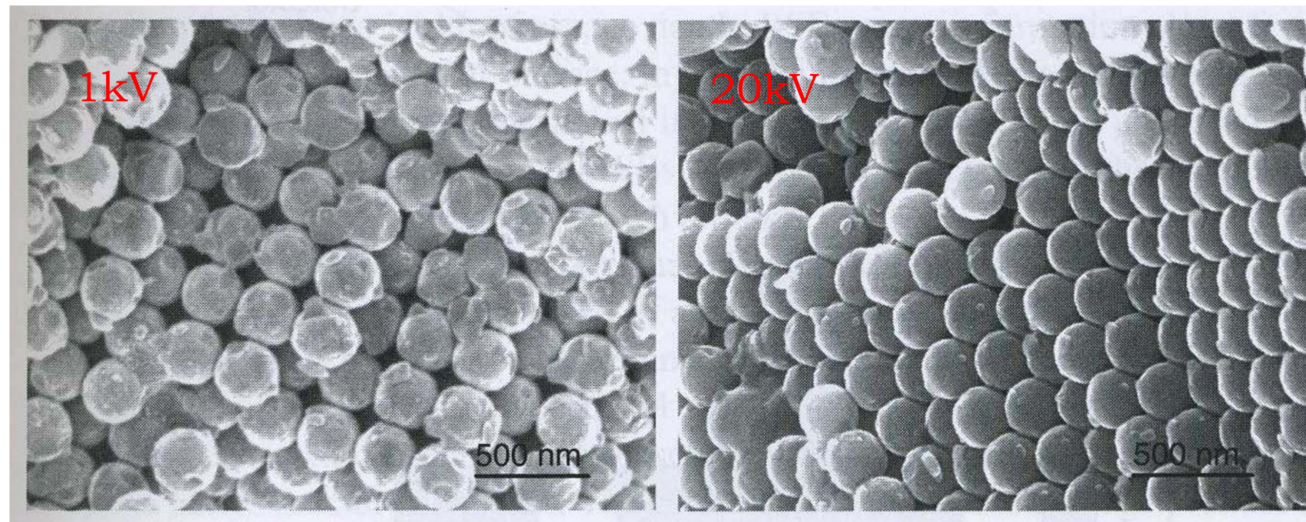




Secondary electrons

- Low energy
- Topographic contrast (surface texture and roughness)
- Resolve surface structure down to 10nm
- Excitation region depends on the accelerating voltage

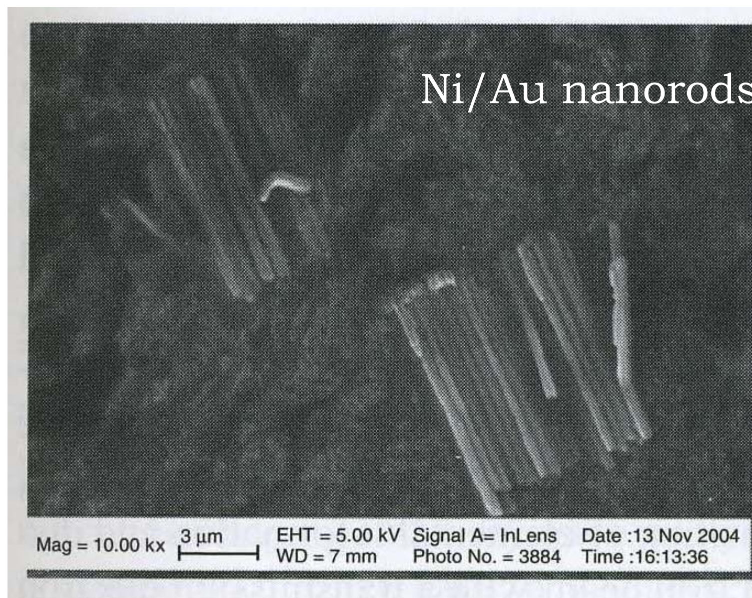




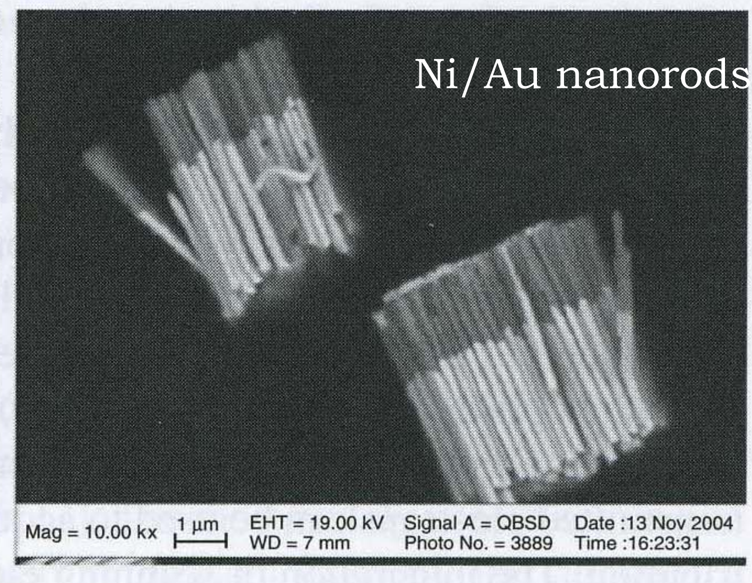
Backscattered electrons

- High energy
- Both Compositional and Topographic information
- Atomic number contrast
- Lateral resolution is worse than secondary electron image

Secondary electron image

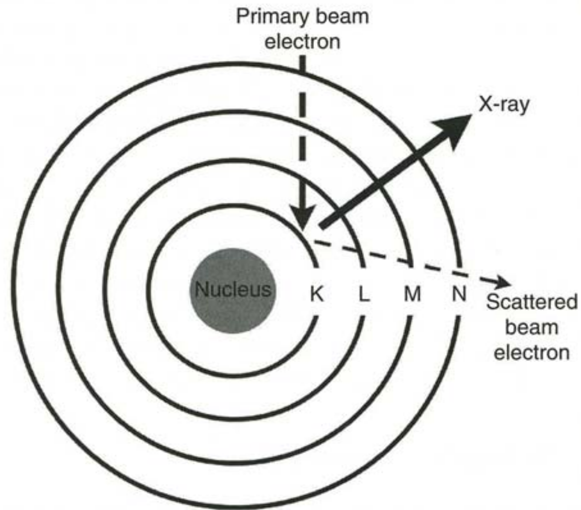


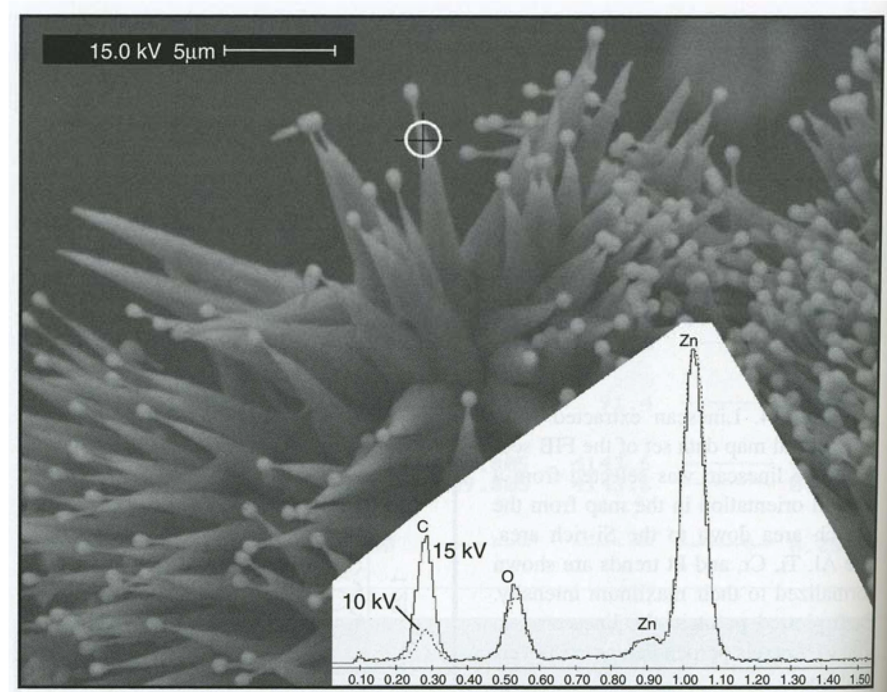
Backscattered electron image



Characteristic X-ray

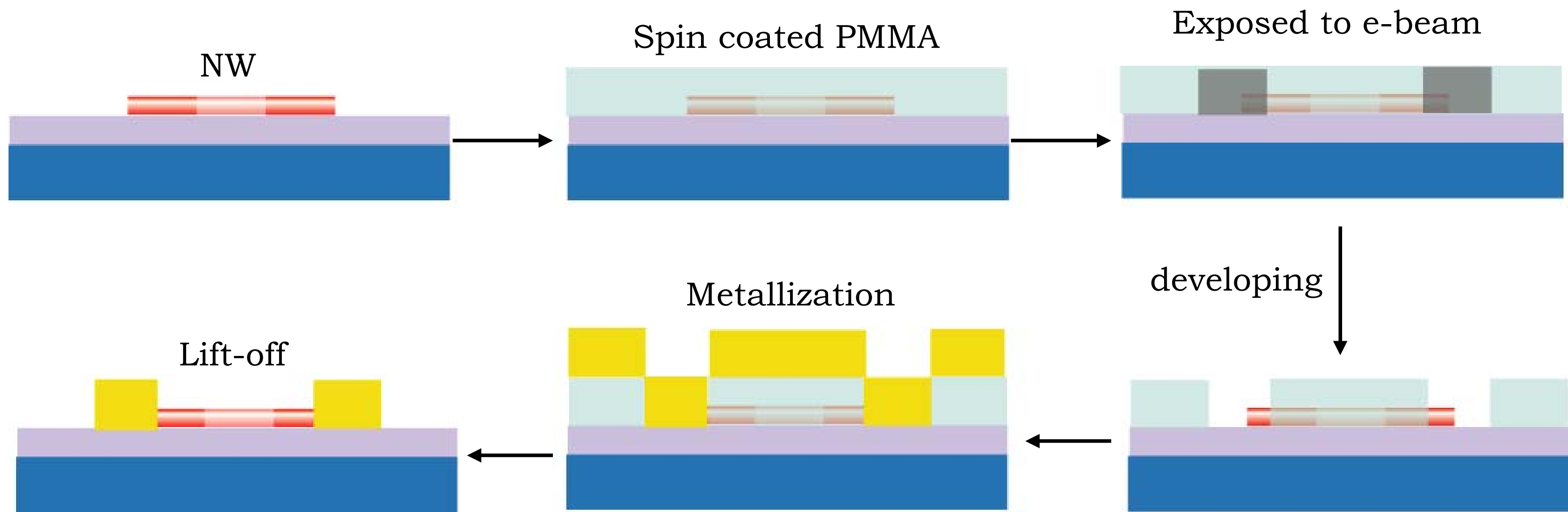
- Chemical information of sample
- Energy Disperse X-ray Spectroscopy (EDS)

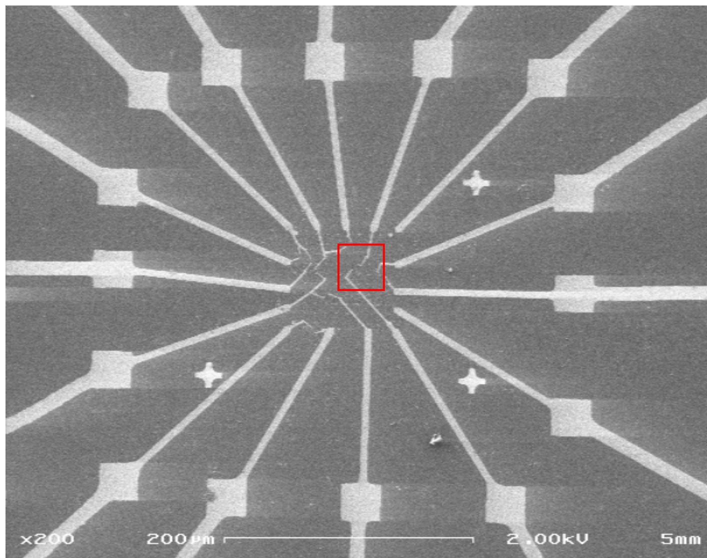


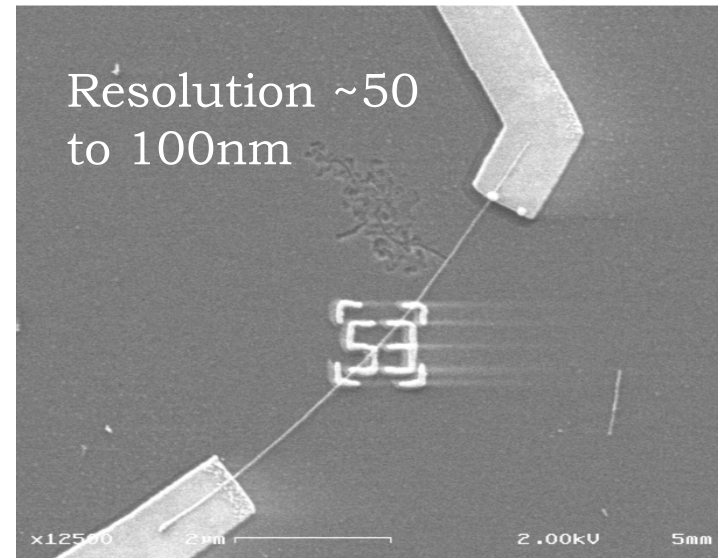


Detection area is limited by the resolution of SEM (accelerating voltage of electron)

E-beam Lithography



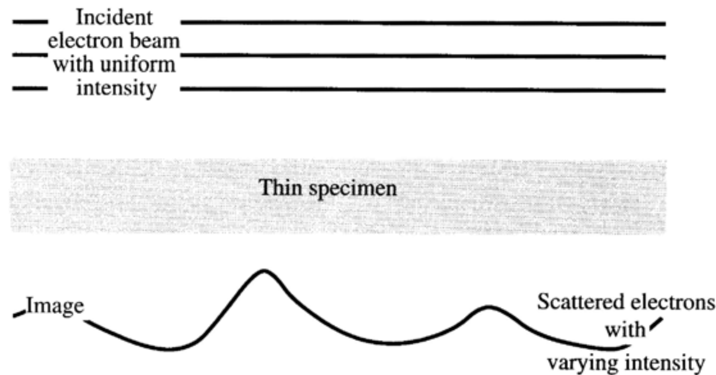




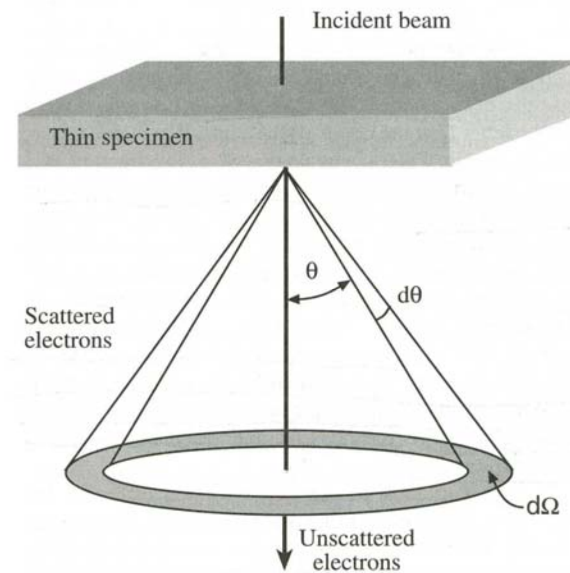
Transmitted electrons

In the TEM, we utilize the electrons that go through a very thin specimen (<200nm)

- Scattering electrons (strong interaction between electrons and matter)
- Image, diffraction pattern, x-ray spectrum and electron energy loss spectrum



Non-uniform distribution of electrons contains all the structural & compositional information

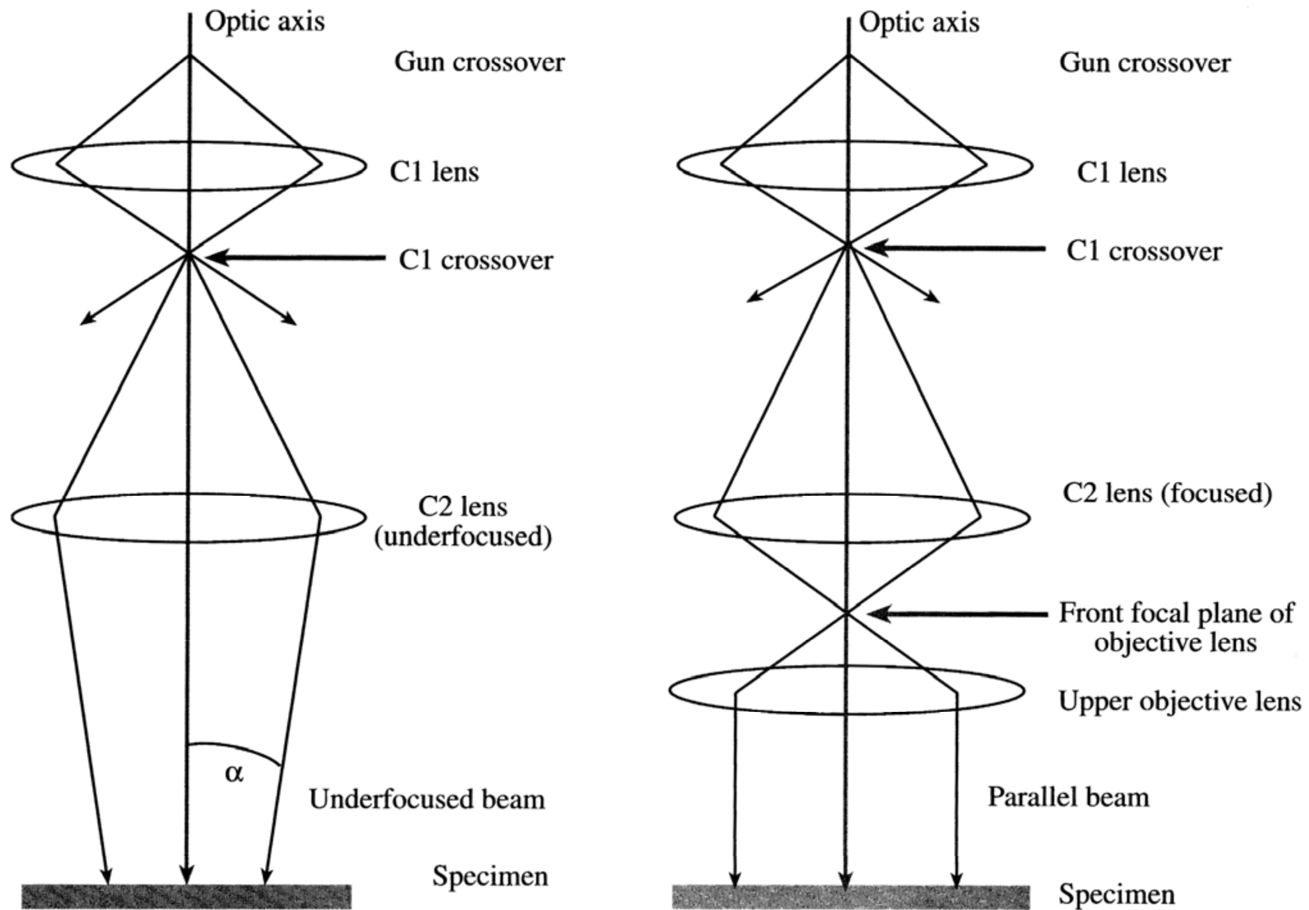


$$2d\sin\theta = n\lambda$$

When d >> λ, sinθ become very small!

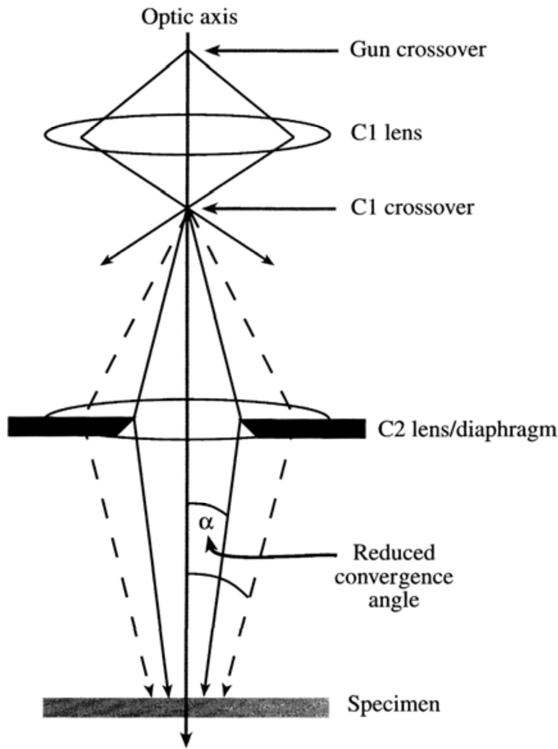
Illumination System

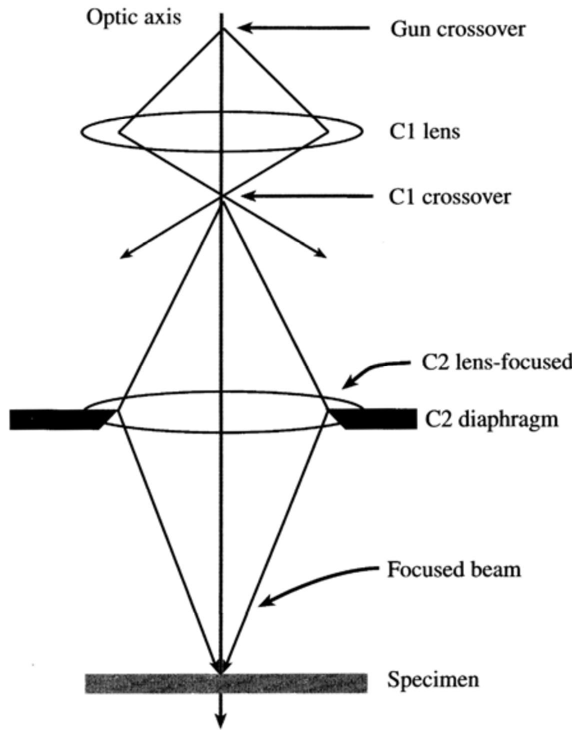
TEM operation using a *parallel* beam



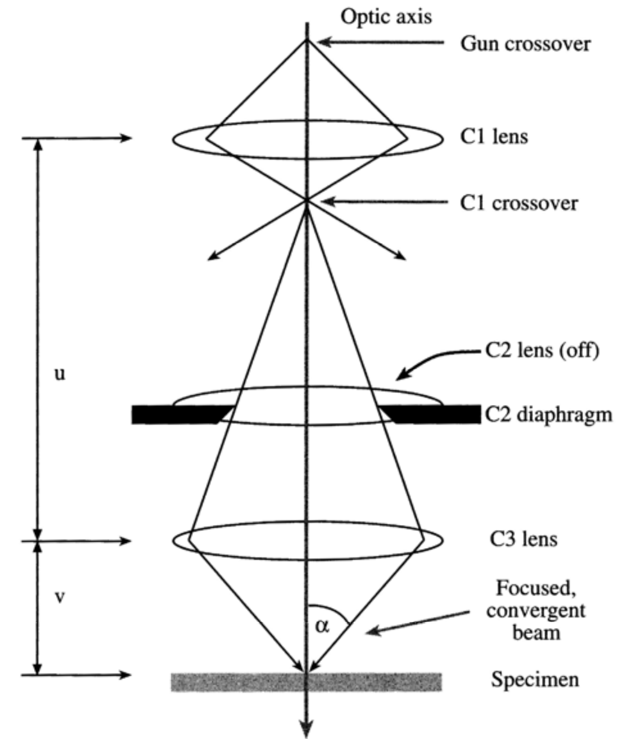
Illumination System

Function of C2 condenser aperture





Convergent beam for (S)TEM

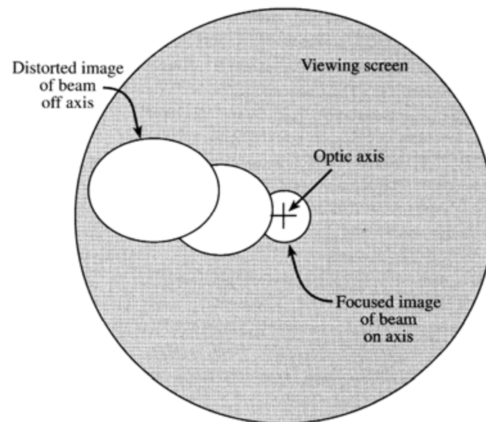


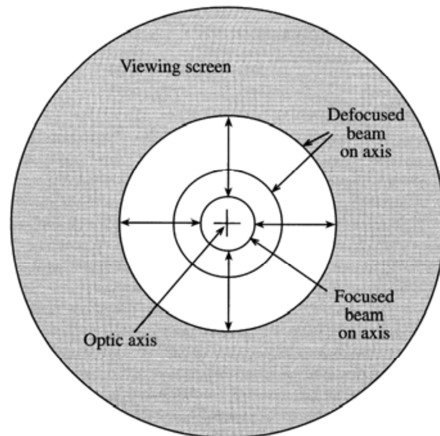
$M = v/u$

Alignment and Adjustment

1. Gun alignment: Electron should follow a straight line through the lens and apertures until it hit the specimen

2. Alignment of C2 aperture

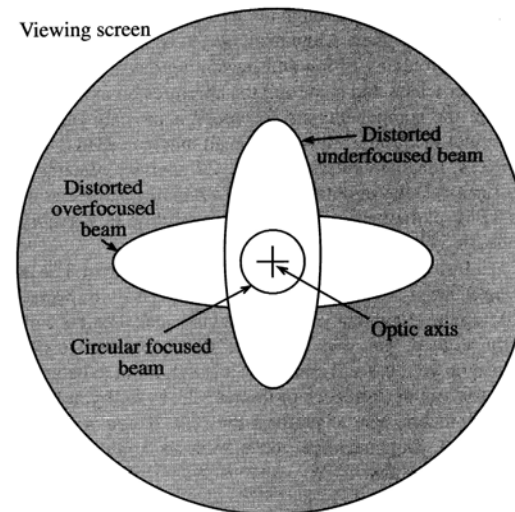




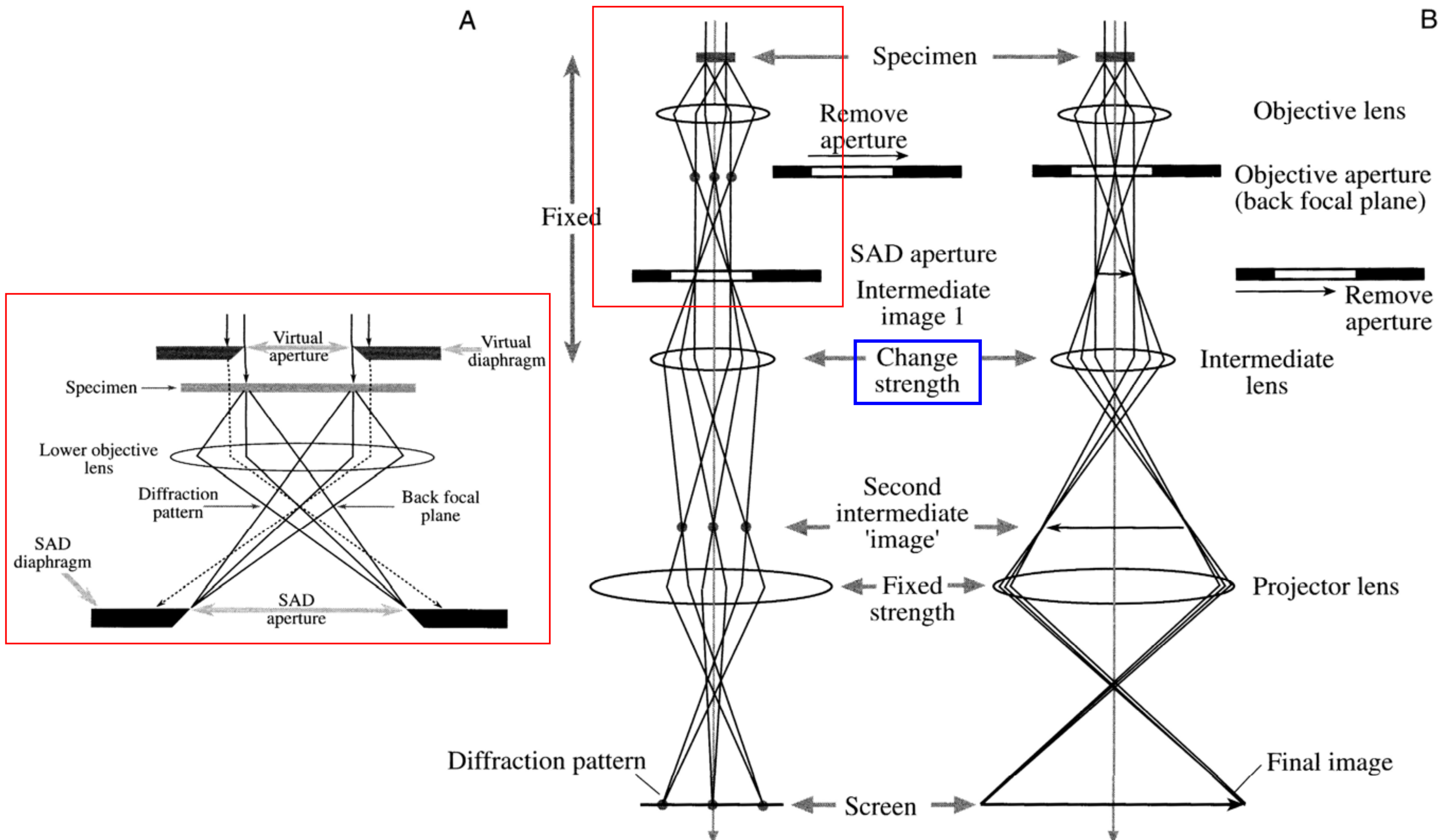
3. Lens aberration

- Control the minimum possible probe size
- Aberration corrected TEM

4. Astigmatism



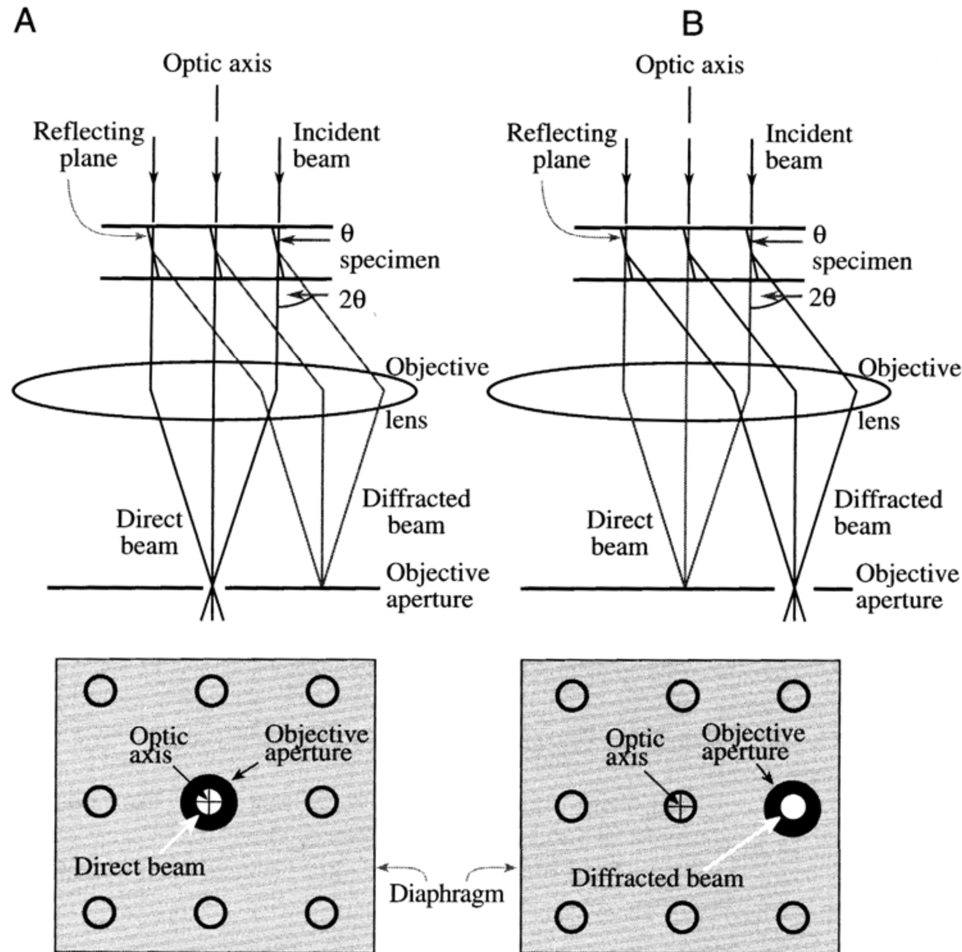
Imaging *vs.* Diffraction Modes



Bright Field *vs.* Dark Field

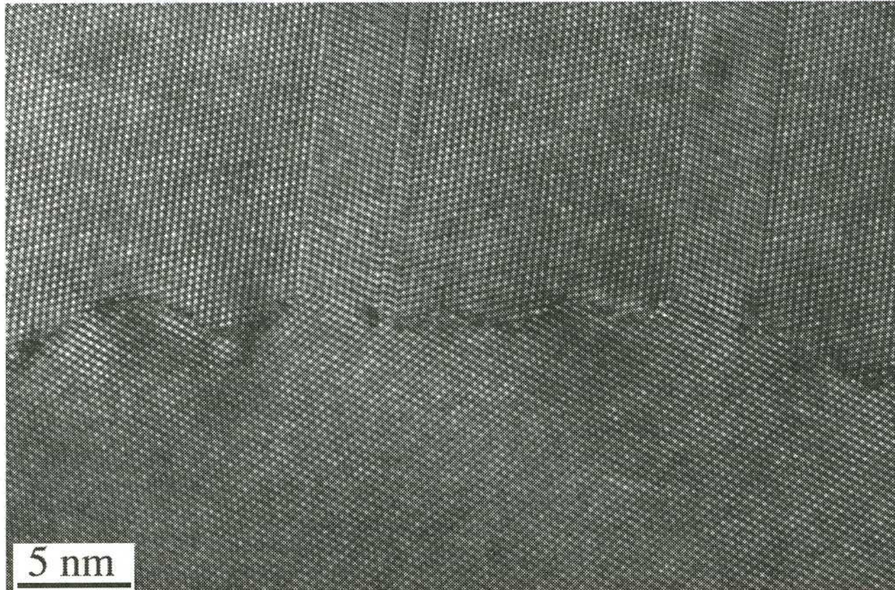
Bright field

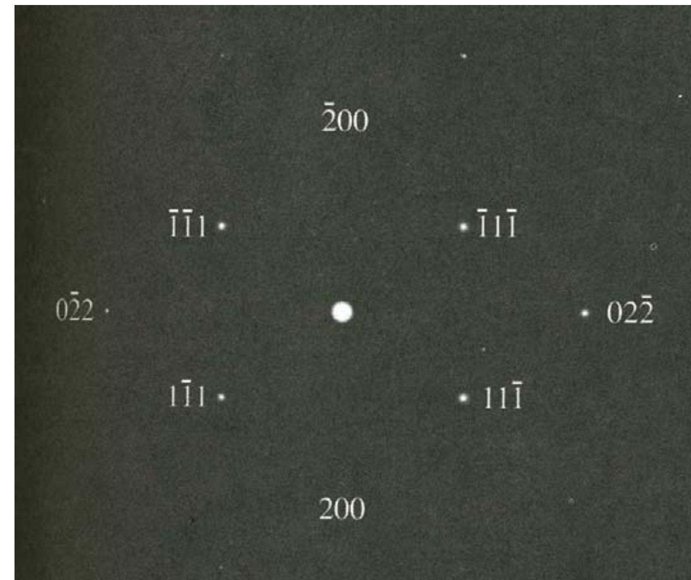
Dark field



To select the electrons to form the image by inserting an objective aperture into the back focal plane of the objective lens

High Resolution Imaging and Diffraction

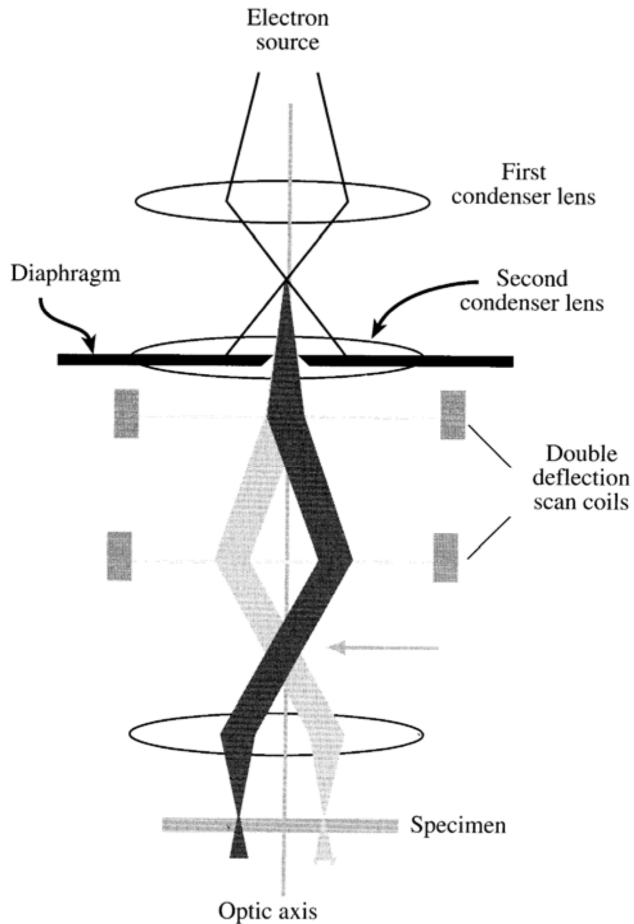


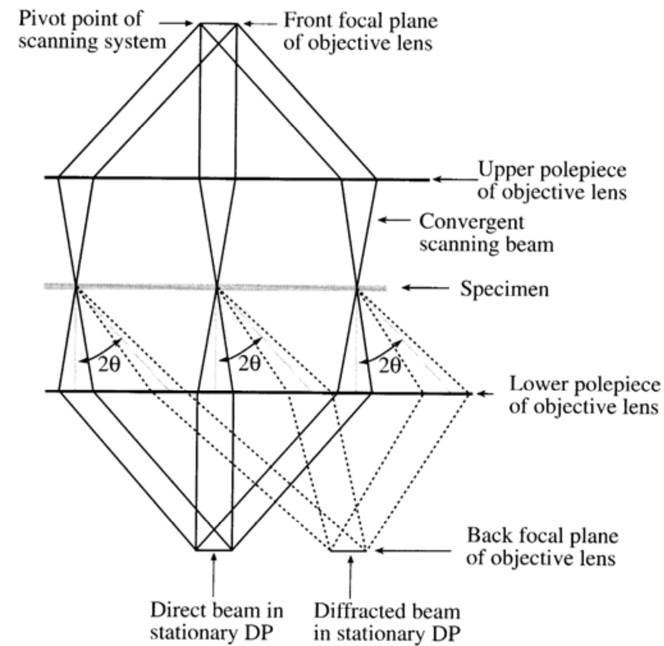


- Atomic resolution < 0.16 nm
- Lattice spacing, atomic structure
- Interface (different phases, crystal structure)
- Combined with computer simulation

- Crystalline vs. amorphous materials
- Single *vs.* polycrystalline materials
- Crystal structure and orientation
- Crystal phases, facet

Scanning TEM

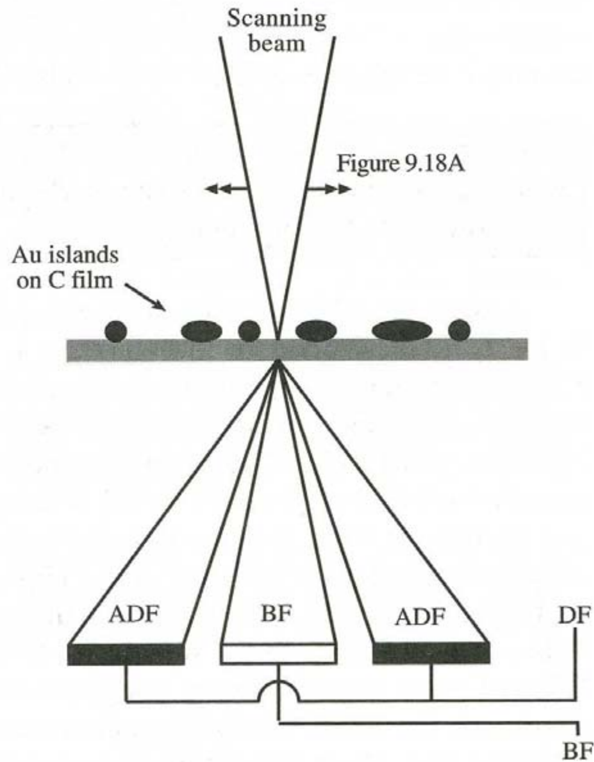


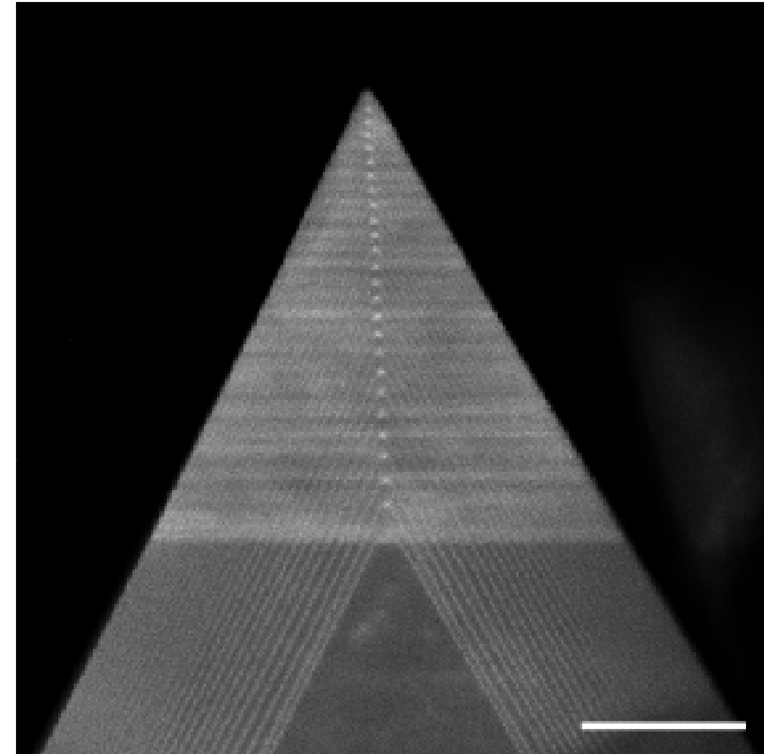


- Beam has to scan parallel to the optic axis at all times

STEM signal generated at any point on the specimen is detected, amplified and a proportional signal is displayed at an equivalent point on CRT

Scanning TEM





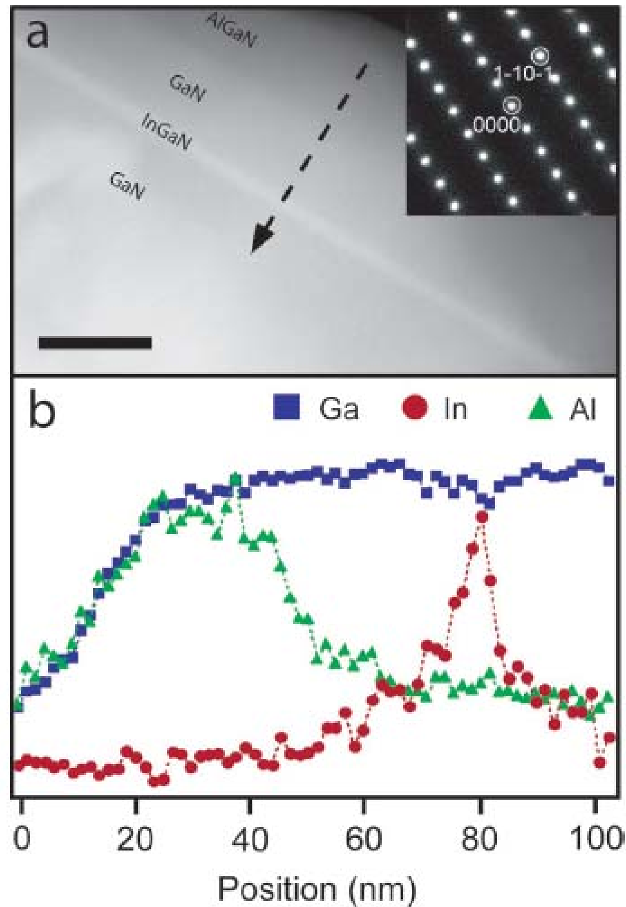
Unpublished result, Qian, Li and Lieber

Dark-field STEM image:

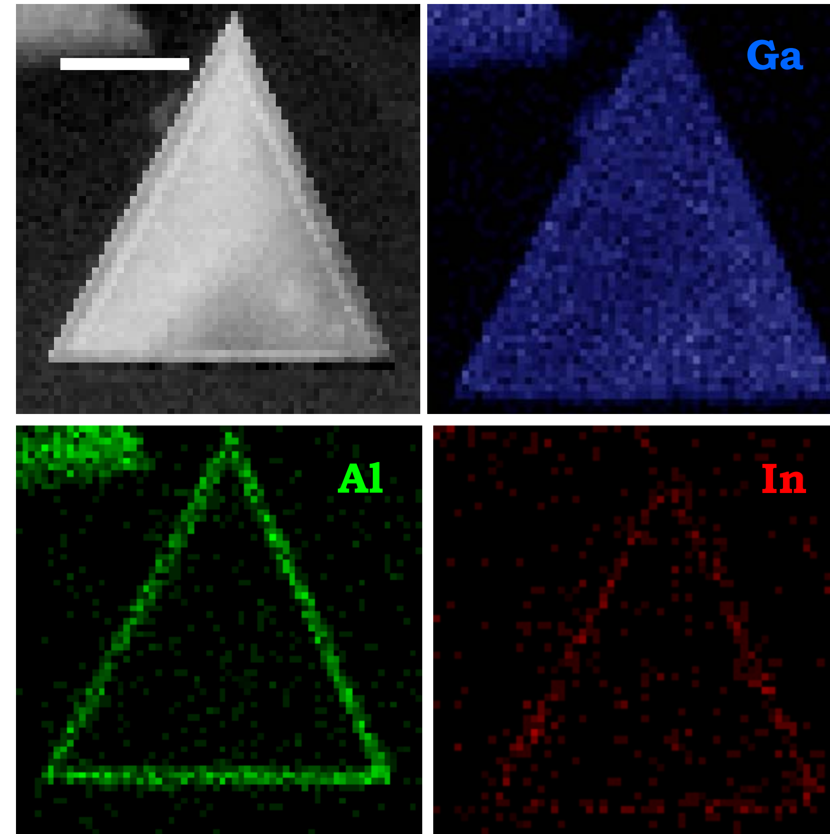
- Annular detector, surrounds the BF detector
- Image contrast is sensitive to the atomic number of imaged materials
- Possible to detect impurities (dopant) using high resolution STEM

Energy Disperse X-ray Spectroscopy (EDS)

Line scan



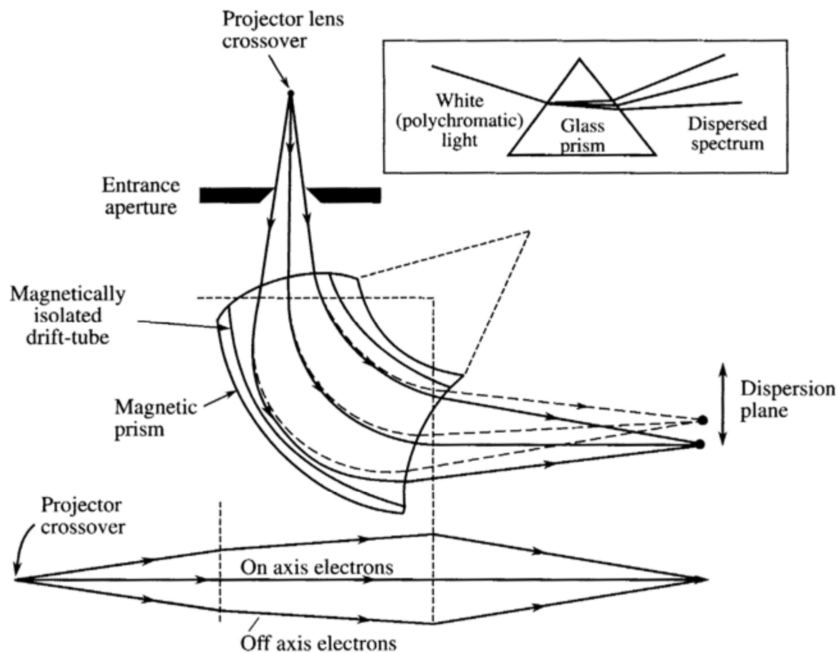
Elemental mapping

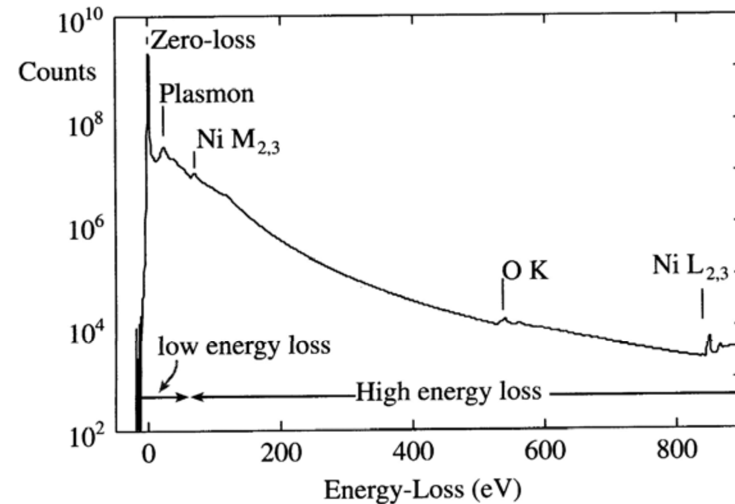


Highly resolved spatial distribution of elements in specimen

Electron Energy Loss Spectroscopy (EELS)

Magnetic prism spectrometer





- Absorption spectroscopy
- Inelastic scattered electrons

- Complementary to EDS
- High energy resolution
- Atomic composition, chemical bonding, valence and conduction band electronic properties and surface properties
- Ability to fingerprint different forms of the same element

Summary

Microscopy: Optical microscopy,
Scanning probe microscopy
Electron microscopy

Functions:

- Imaging (fluorescence, lattice-resolved and topography)
- Chemical analysis
- Structure determination
- Manipulation of atoms and molecules
- Nanolithography, e-beam lithography
- Spectroscopy: surface, electrical and optical properties