Optical and

Electron Microscopy

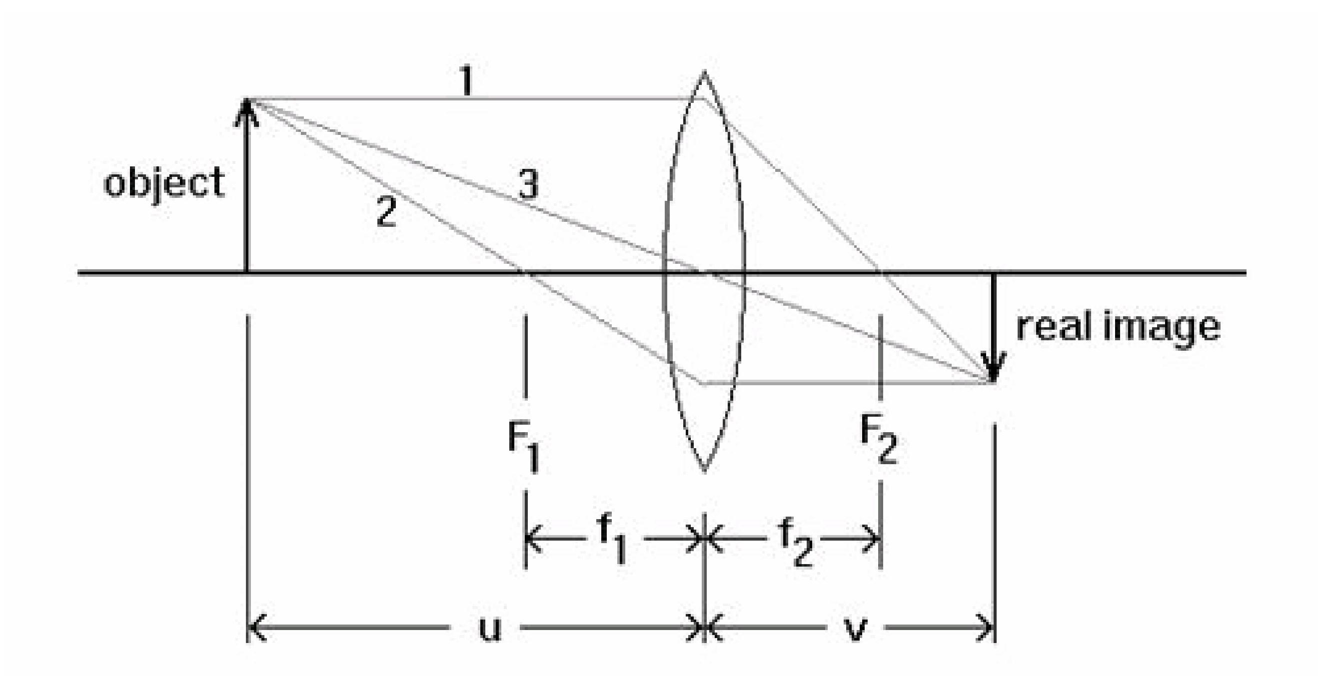
MSN 506

Overview

- Image formation by a Lens
- Anatomy of a microscope
- Electron and Ion optics
- Electron Scattering
- Scanning Electron Microscopy
- Transmission Electron Microscopy
- Ion beam techniques

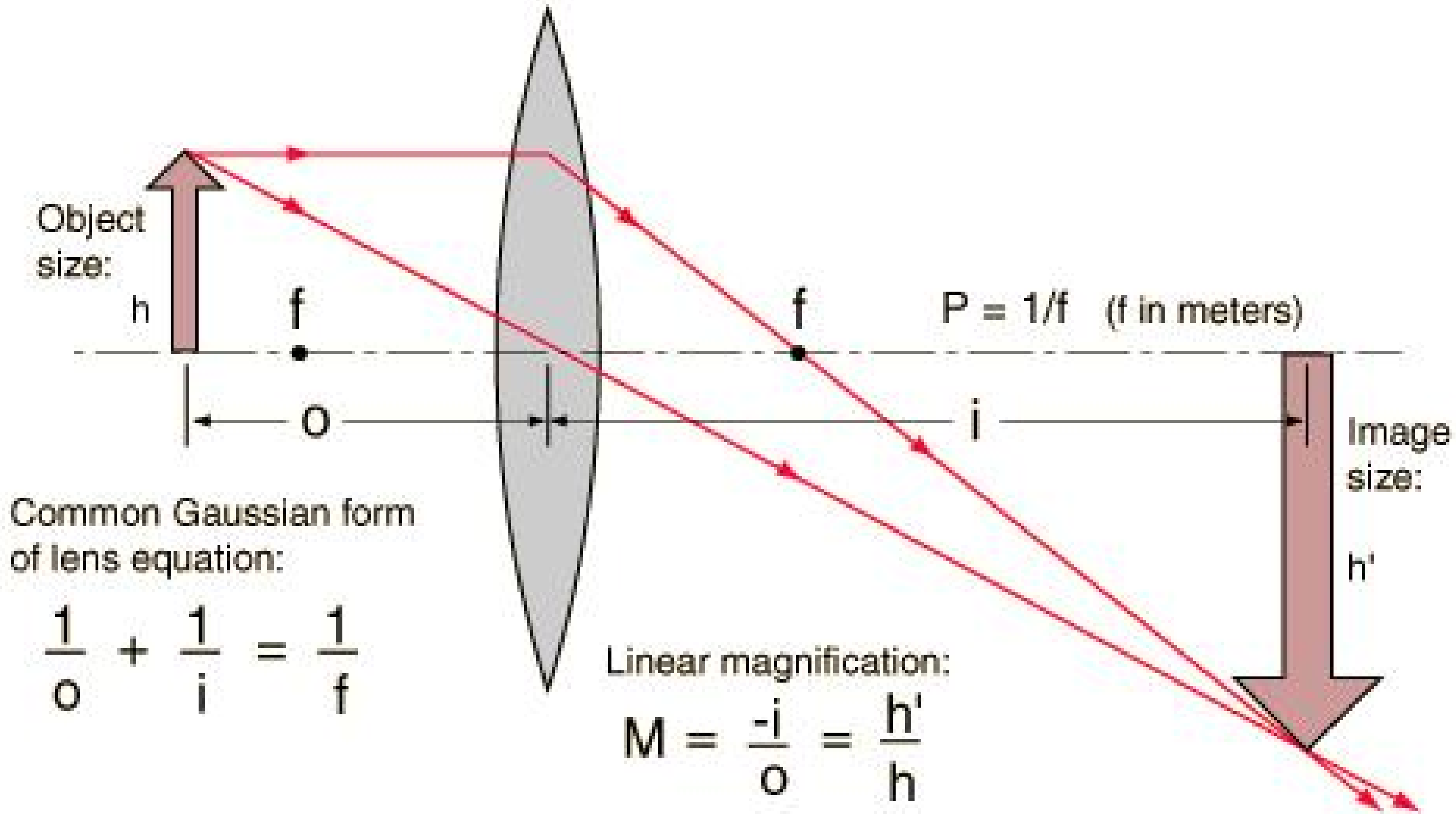
Image formation

- Light rays coming out of an illuminated object diverge from each point on the object
- A lens can be used to refract the rays and converge them at a different location
- This is the basic mechanism of image formation



A lens changes the angle of a beam depending on its incidence angle and location of entrance on the lens

De/Magnification



Ideal Focusing and Point resolution

Generally lens aberrations degrade this limit

- Diffraction limits smallest possible spot size

$$\sin\theta = 1.22\frac{\lambda}{D}$$

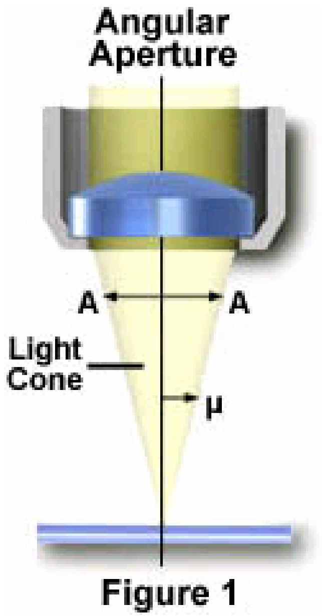
Rayleigh Criterion for angular resolution

$$\Delta l = 1.22\frac{f\lambda}{D}$$

Spatial resolution by a lens

$$R = \frac{1.22\lambda}{2n\sin\alpha}$$

Resolution for a microscope objective



$$\mathrm{NA} = n\sin\theta$$

One often uses the "Numerical Aperture" to characterize The lens resolving power

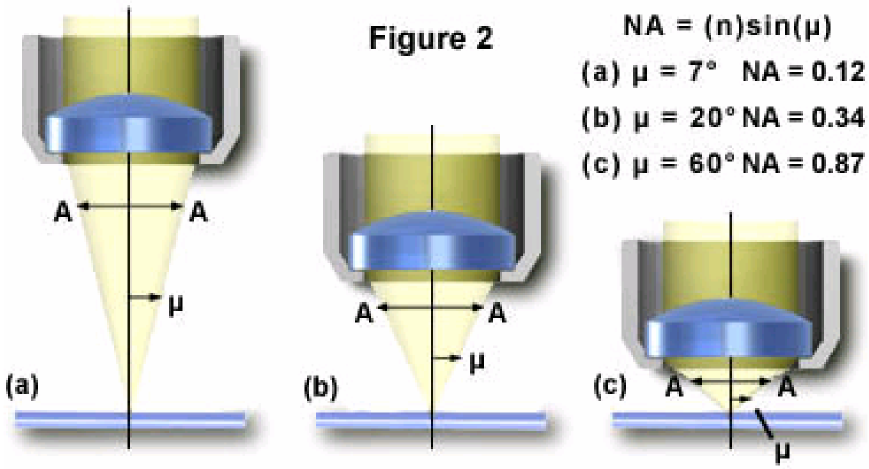
Spot size is related to wavelength

NA is related to the F# of the lens

$$f/\# \approx \frac{1}{2\,NA}$$

$$f/\# = N = \frac{f}{D}$$

Numerical Apertures



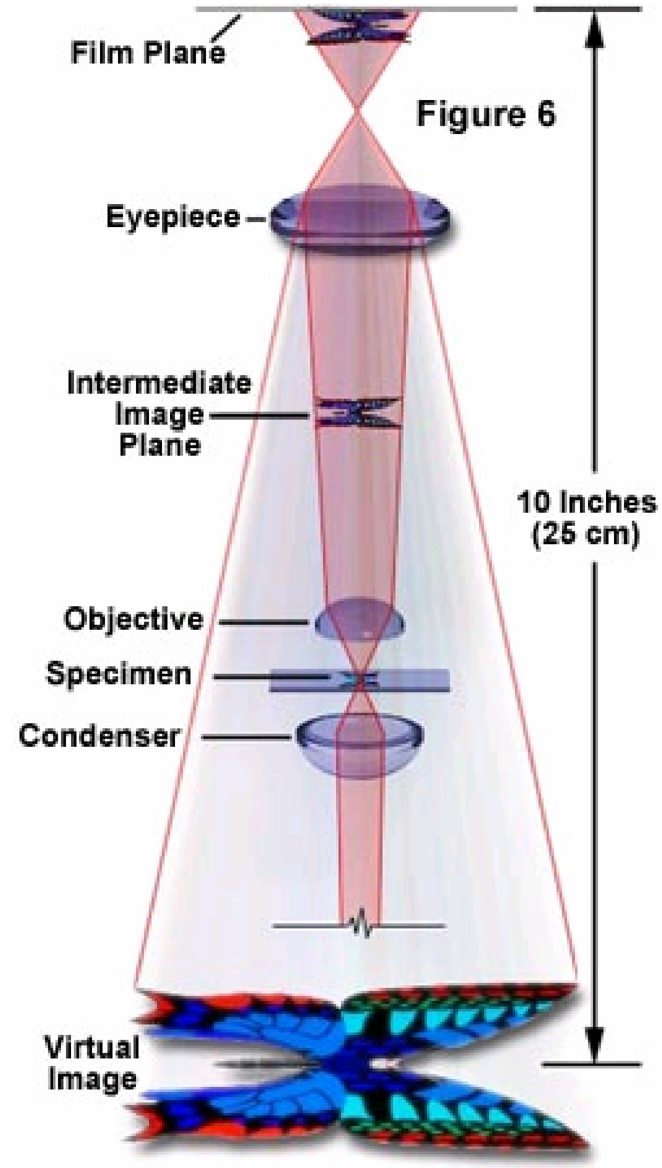
Resolution and Numerical Aperture by Objective Type

	Objective Type					
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A	Resolution (&microm)	N.A	Resolution (&microm)	N.A	Resolution (&microm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20

N.A. = Numerical Aperture

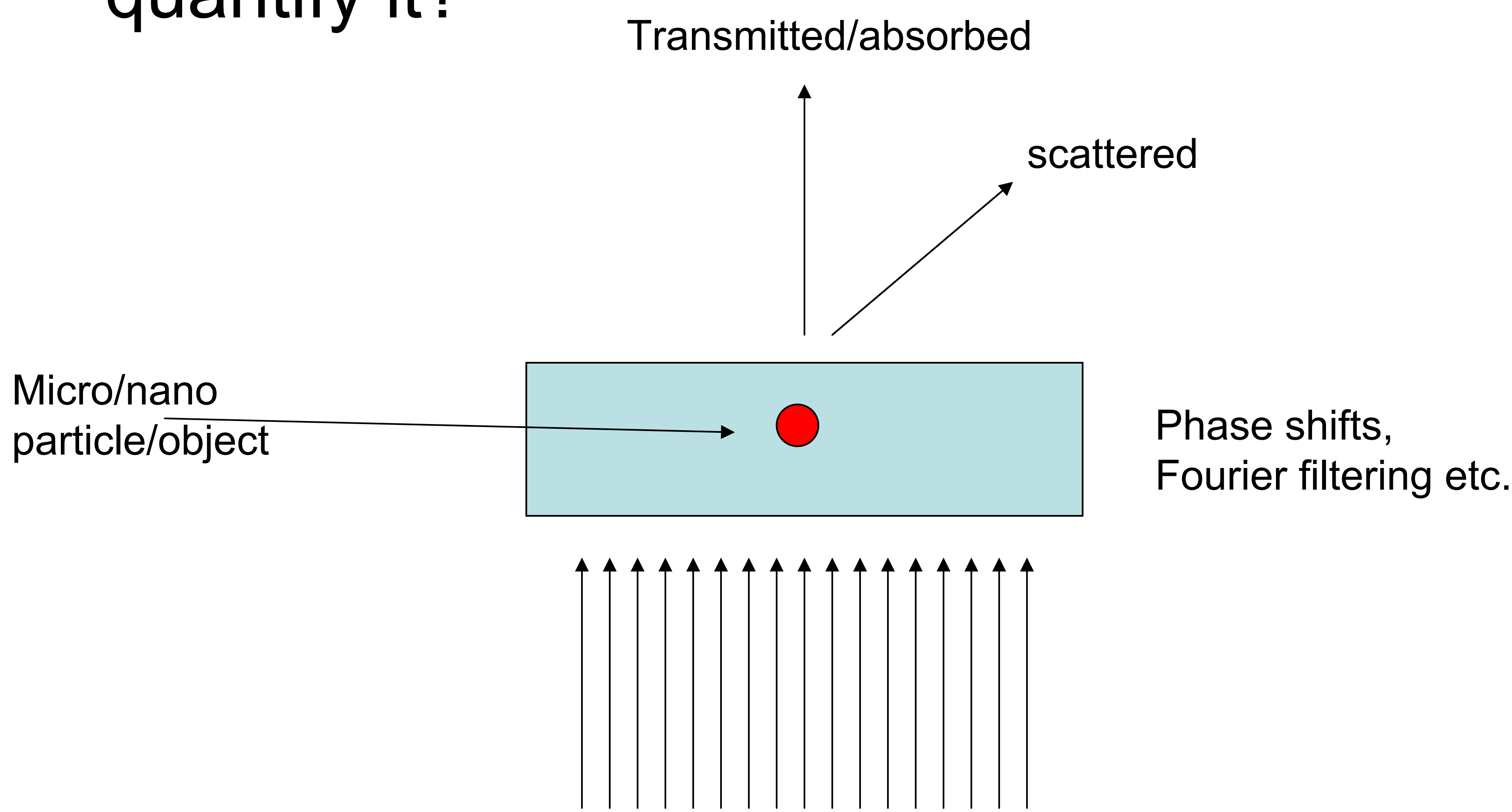
Anatomy of a Light Microscope

- Illumination
  - An even illumination is important for imaging
- Objective Lens
  - Collects light from the sample and nearly collimates it
- Eyepiece
  - Refocuses the light from the objective to form the image



Contrast

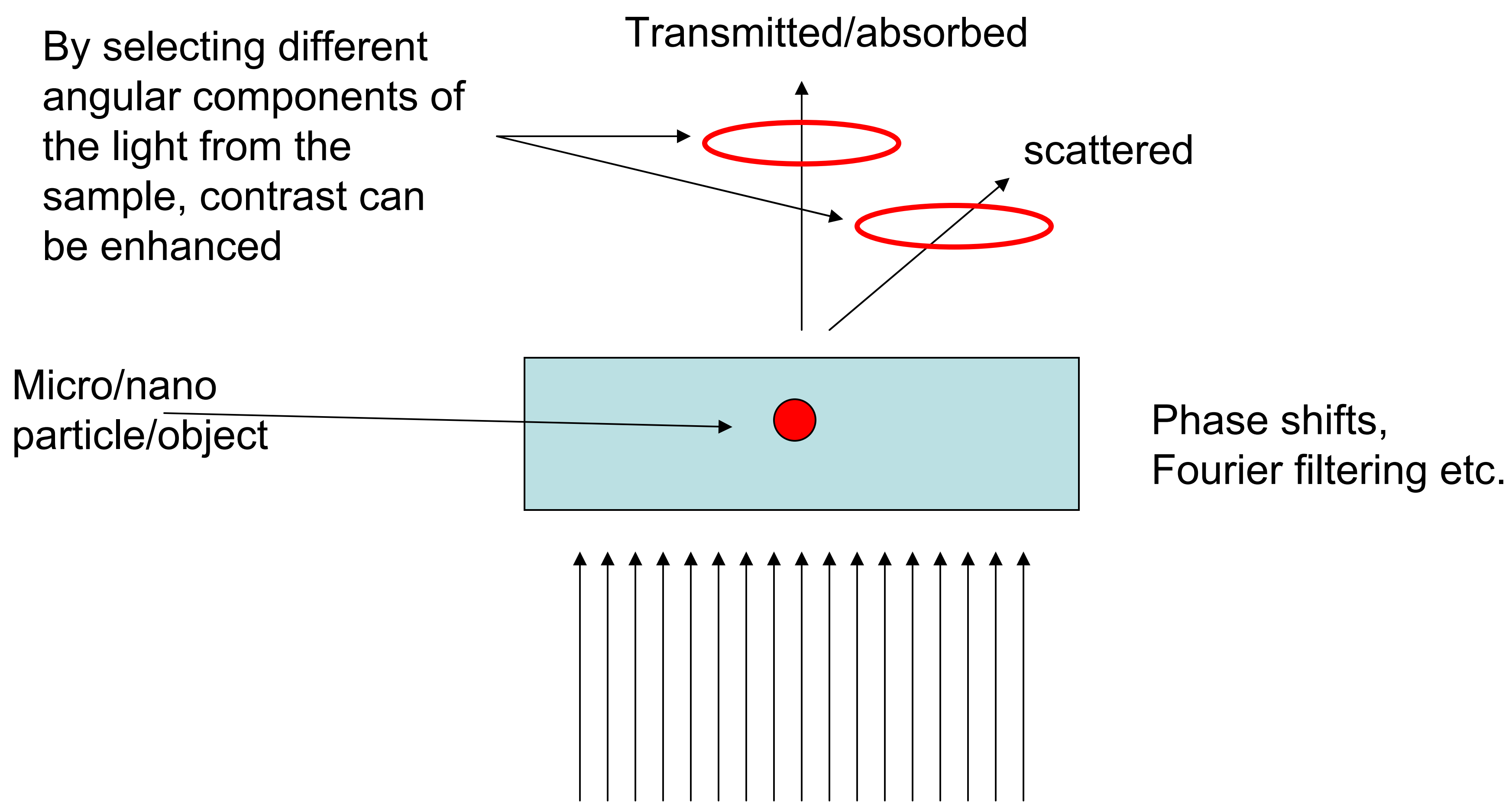
- What causes contrast and how can we quantify it?



Light transmitted or scattered through a path / from a point depends on material properties

Contrast Enhancement

By Placing optical components in the beam path, selective imaging is possible



Modulation Transfer Function

- Is a measure of how much of the constrast is imaged

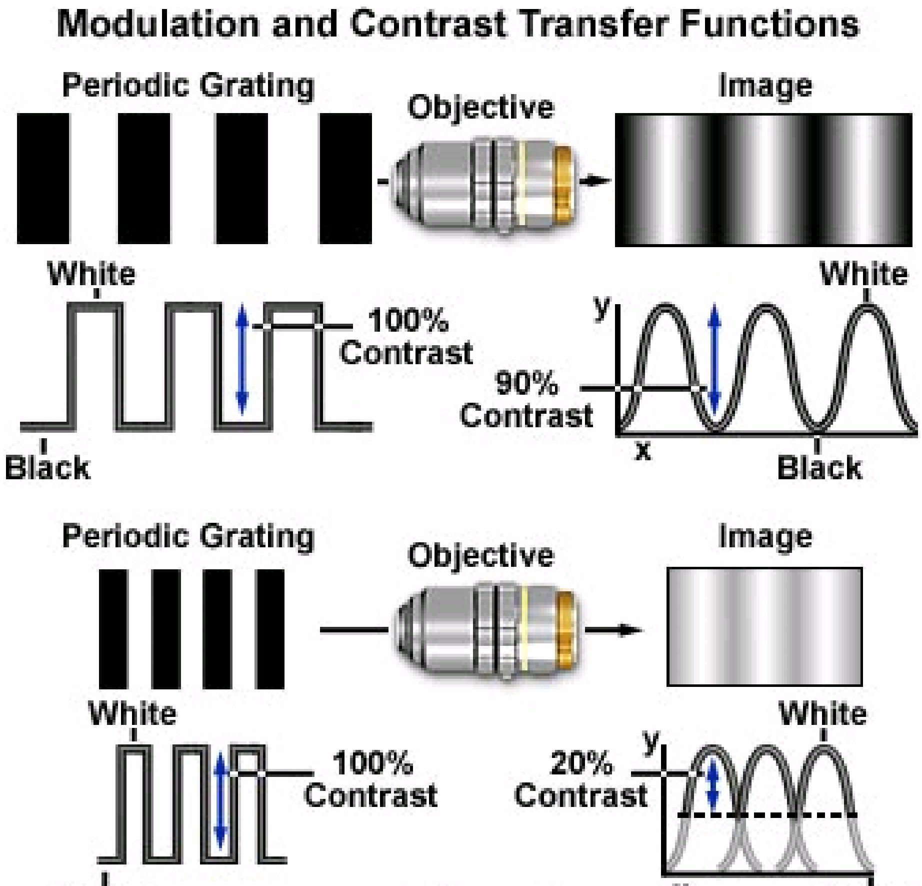


Figure 1

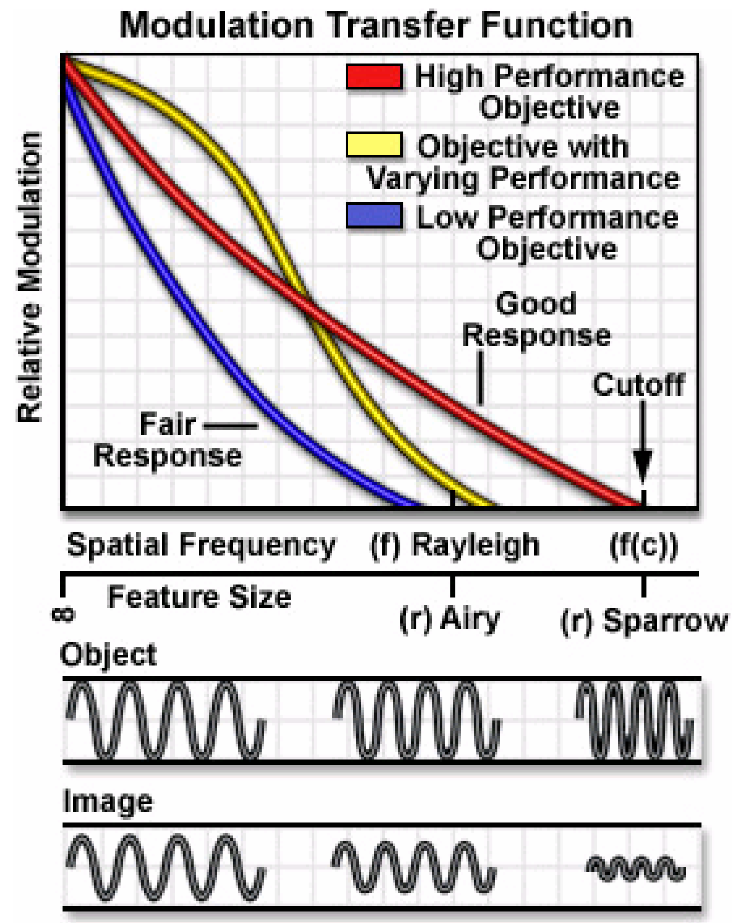
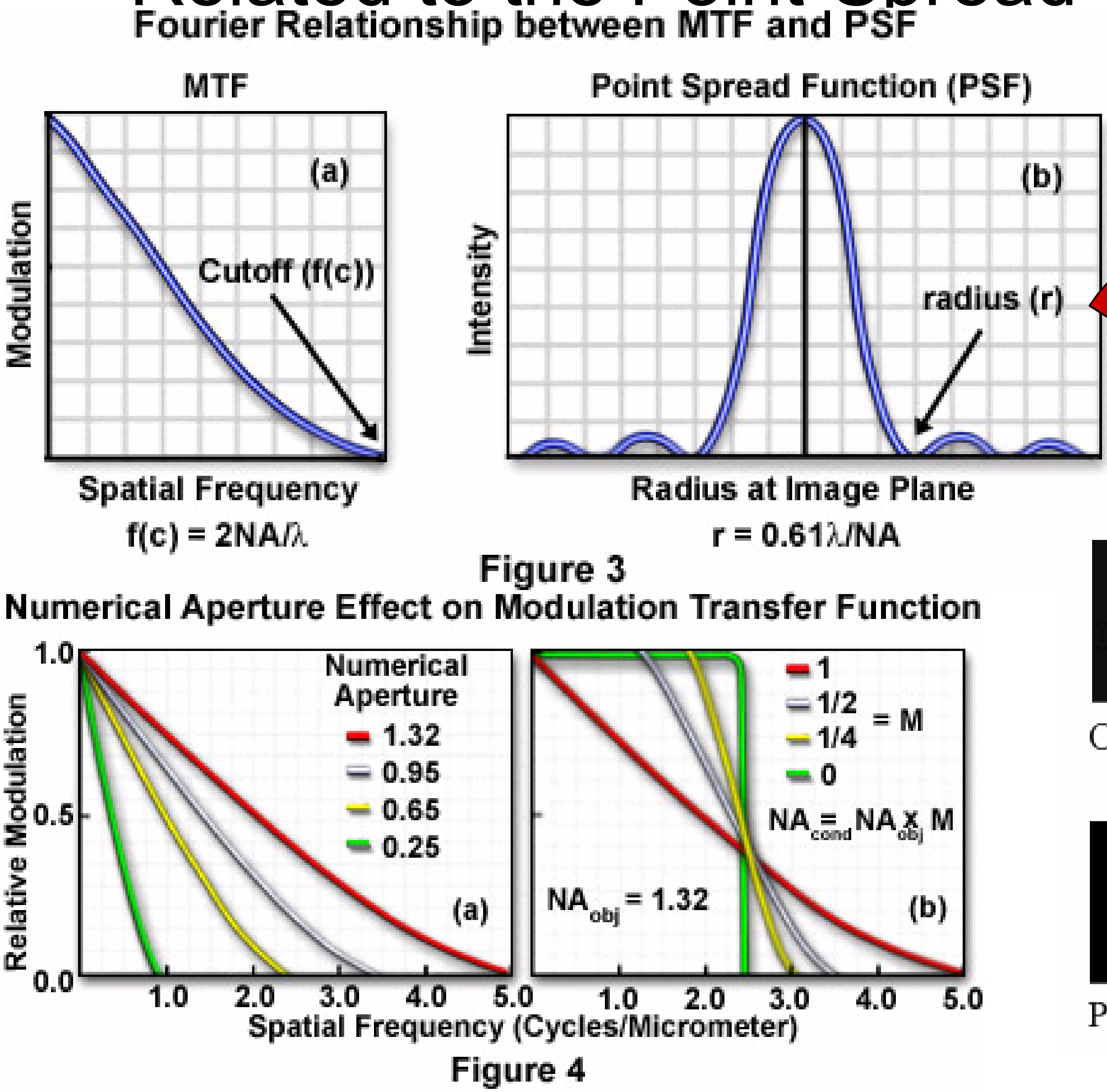
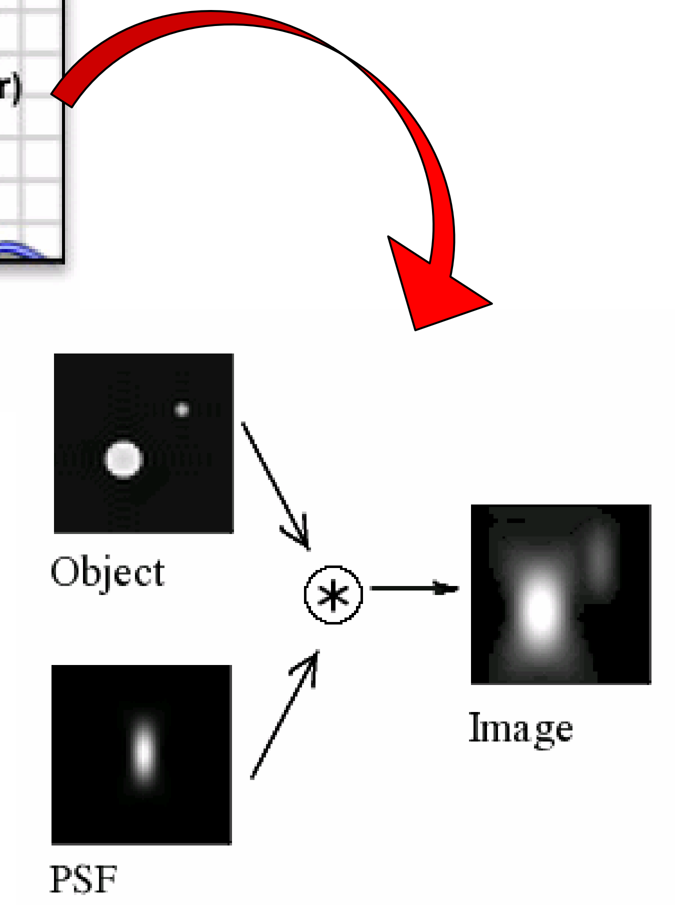


Figure 2

Modulation Transfer Function

- Related to the Point-Spread-Function





Contrast enhancement and MTF

- Contrast enhancement can significantly alter MTF

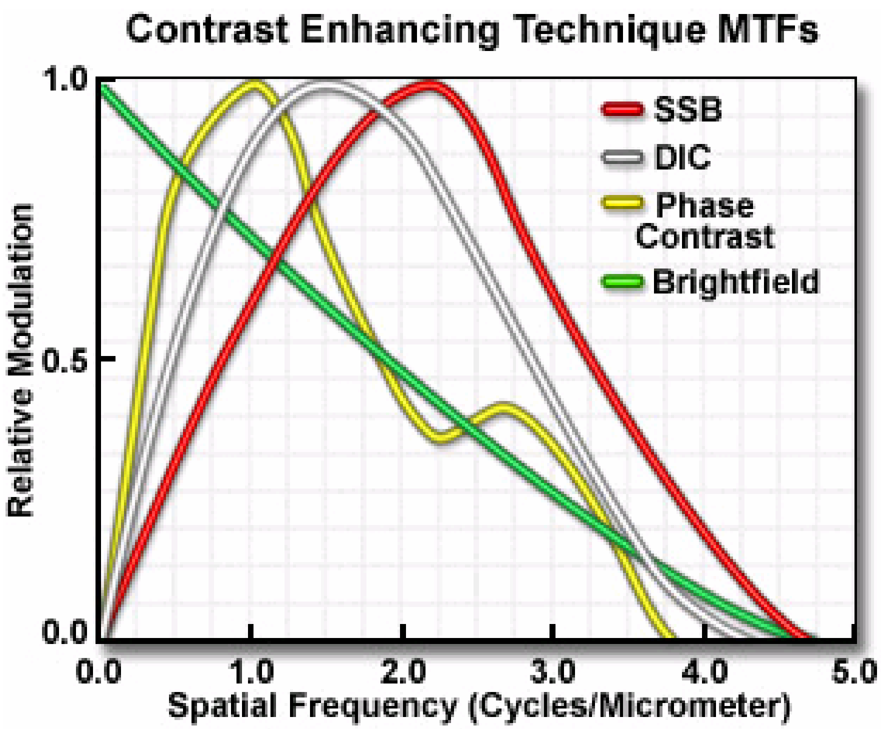


Figure 5

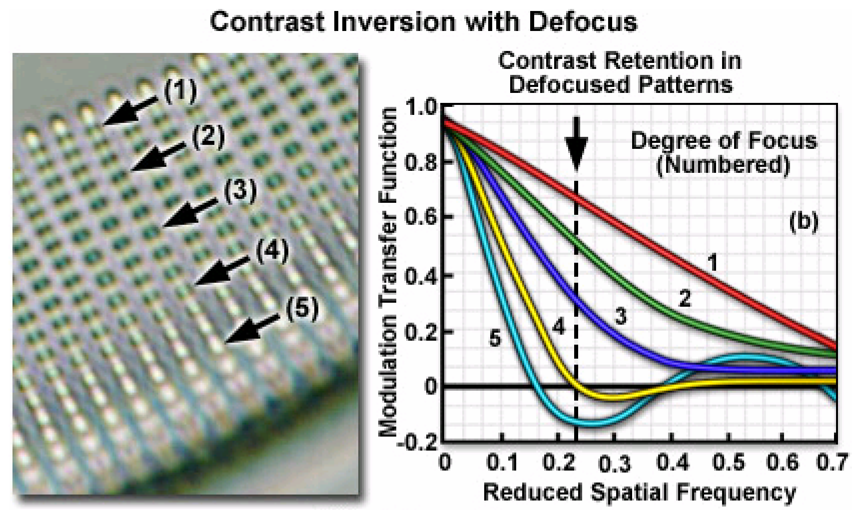
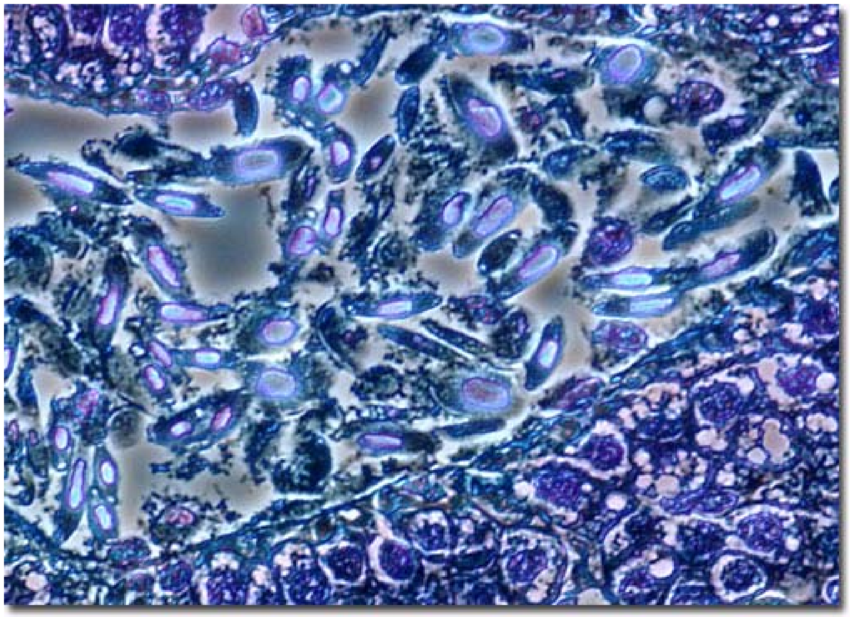


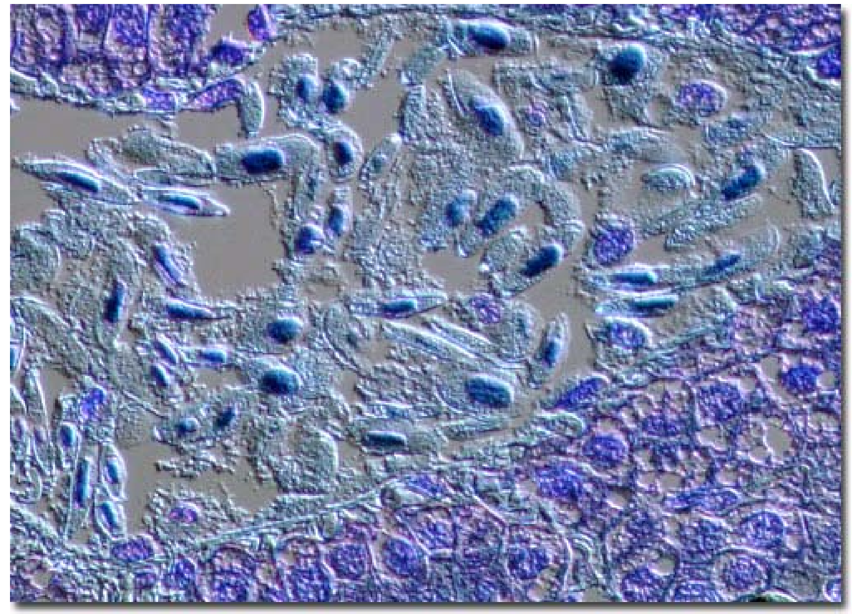
Figure 8

Focus series can be used to get more information

Examples of Contrast enhancement



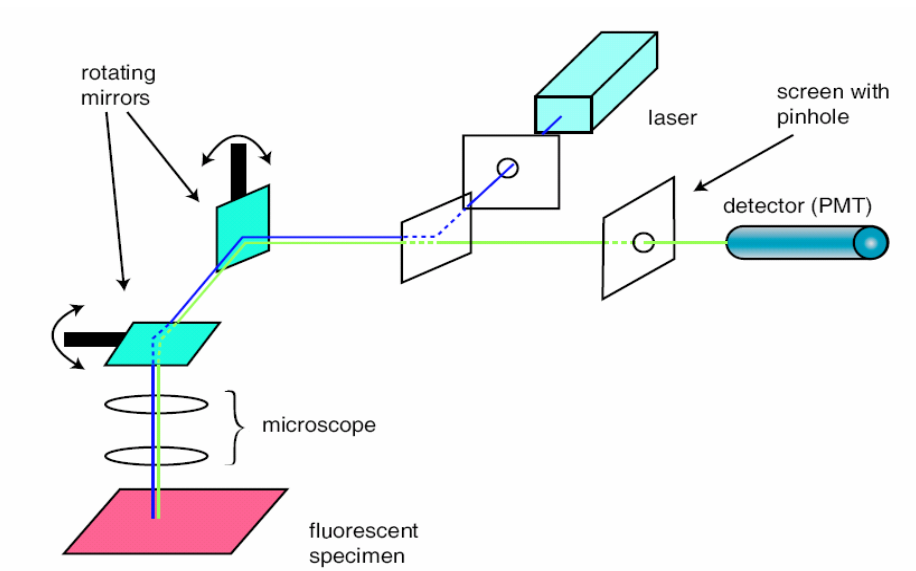
Phase contrast

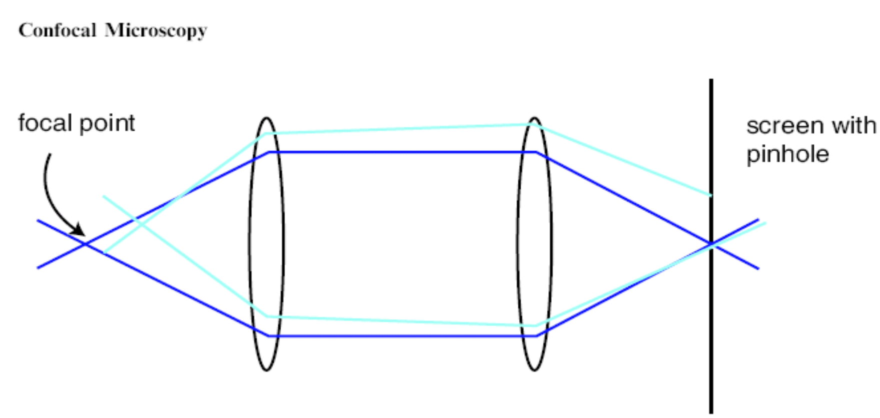


Differential Interference Contrast

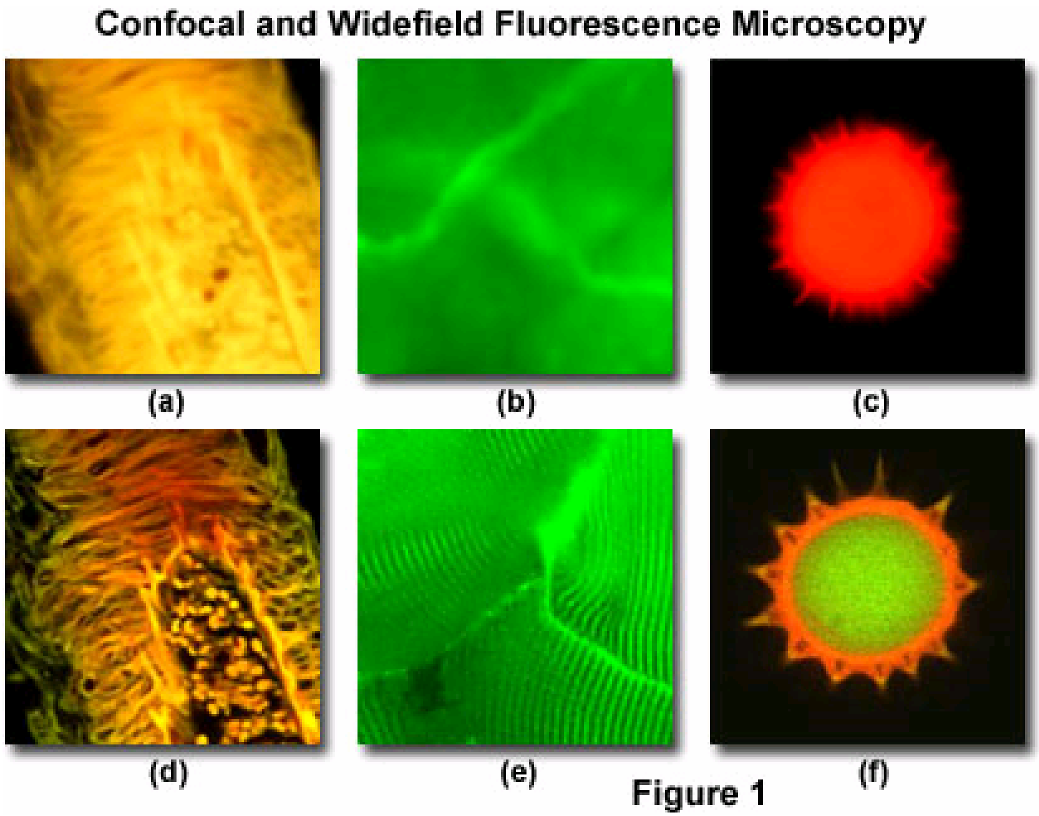
Confocal Microscopy

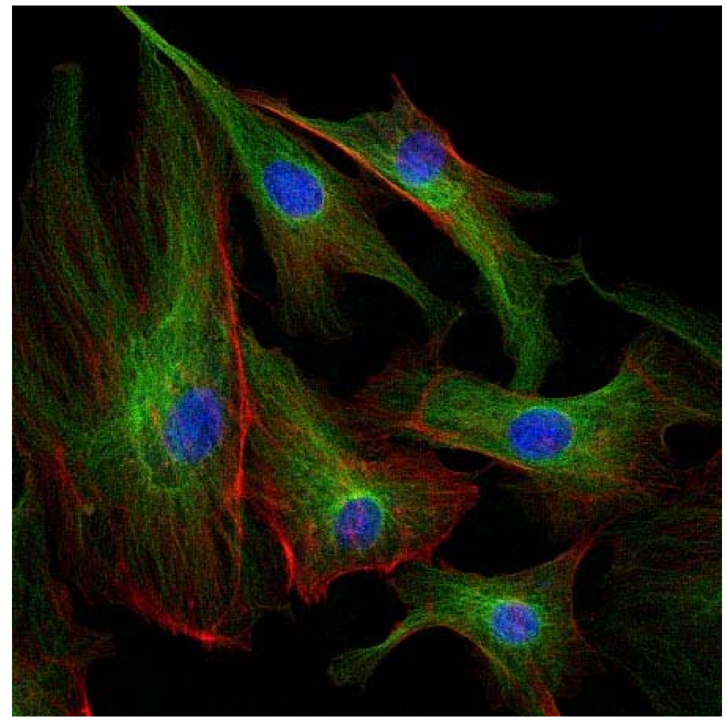
- A laser beam (or sample) is scanned and fluorescence is recorded
- Light is collected from the focused laser spot only
- diffraction limited spot of submicron size





Confocal Microscopy





Magnificent images,
Dyes or quantum dots can be used for fluorescence labeling and functional imaging

Multiphoton Microscopy

Principle of fluorescence induced by one-photon absorption (left) and two-photon absorption (right). While the resolution in two-photon fluorescence mciroscopy (2PFM) is less good, photodamage is lower and penetration depth is higher compared to single-photon (confocal) fluorescence microscopy (1PFM)

Due to nonlinear nature of two-photon absoprtion, signal comes not from the focal cone but from a smaller focal sphere

Why electron microscopy

- Primary reason: Spot size

$$\lambda_B = \frac{h}{p}$$

$$\lambda = \frac{h}{p} = \frac{h}{mv}\sqrt{1 - \frac{v^2}{c^2}}$$

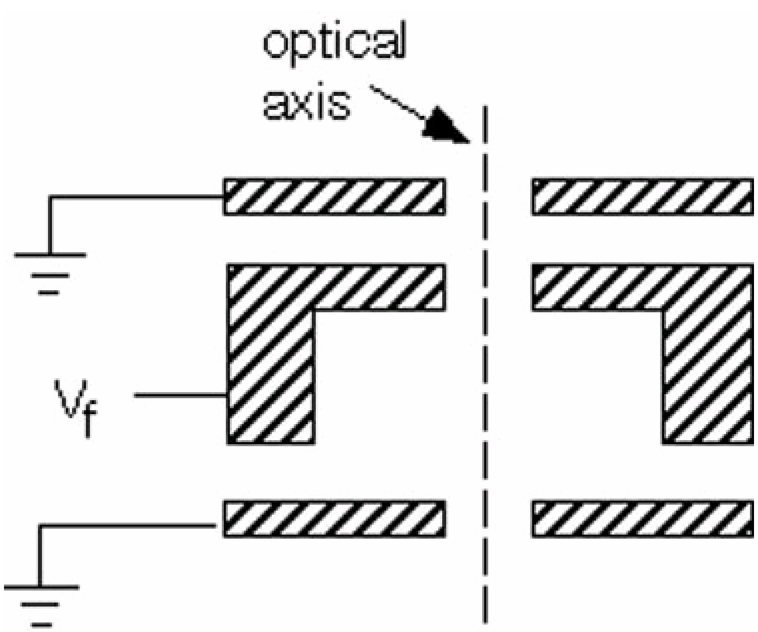
DeBroglie wavelength of a particle

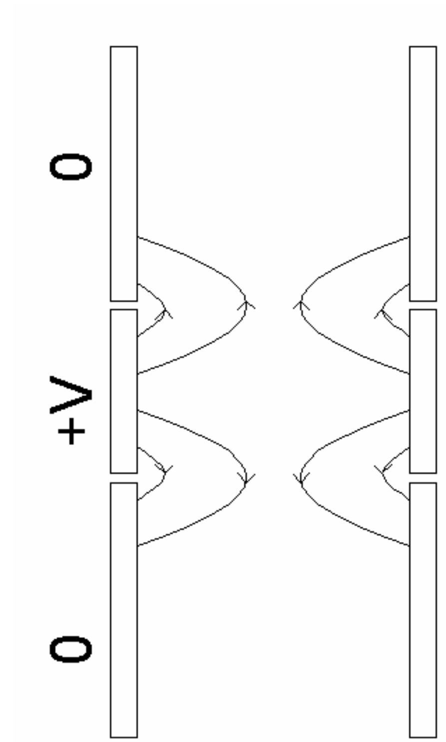
If speeds are large or total acceleration voltage is close to rest mass of particle
You should better use relativistic formulas for energy, momenta etc.

For an electron with KE = 1 eV and rest mass energy 0.511 MeV, the associated DeBroglie wavelength is 1.23 nm, about a thousand times smaller than a 1 eV photon.

Ion and Electron Optics

- We need something that changes the direction of electrons or ions in a beam, depending on initial direction and radial location within the beam





An electrostatic *lens*

Ion and Electron Optics

- Magnetic Lens

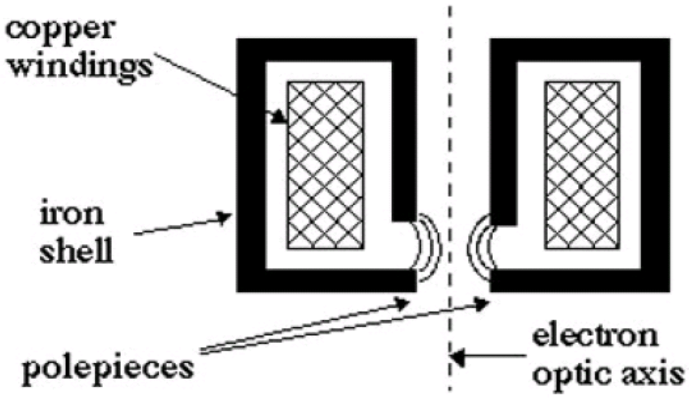
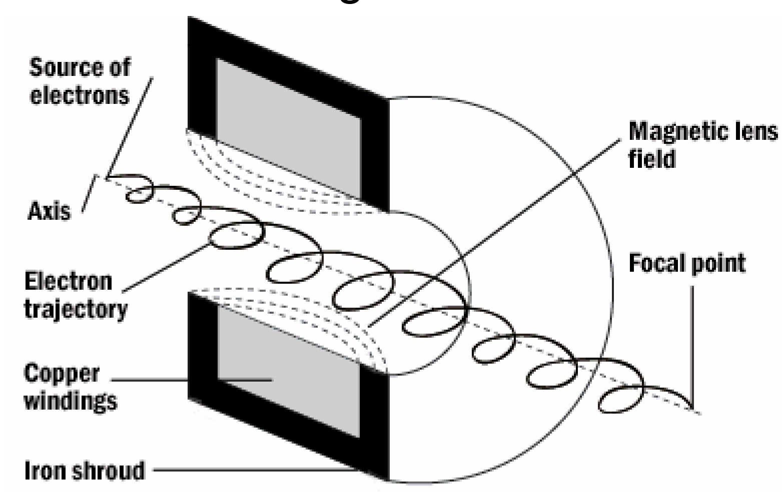
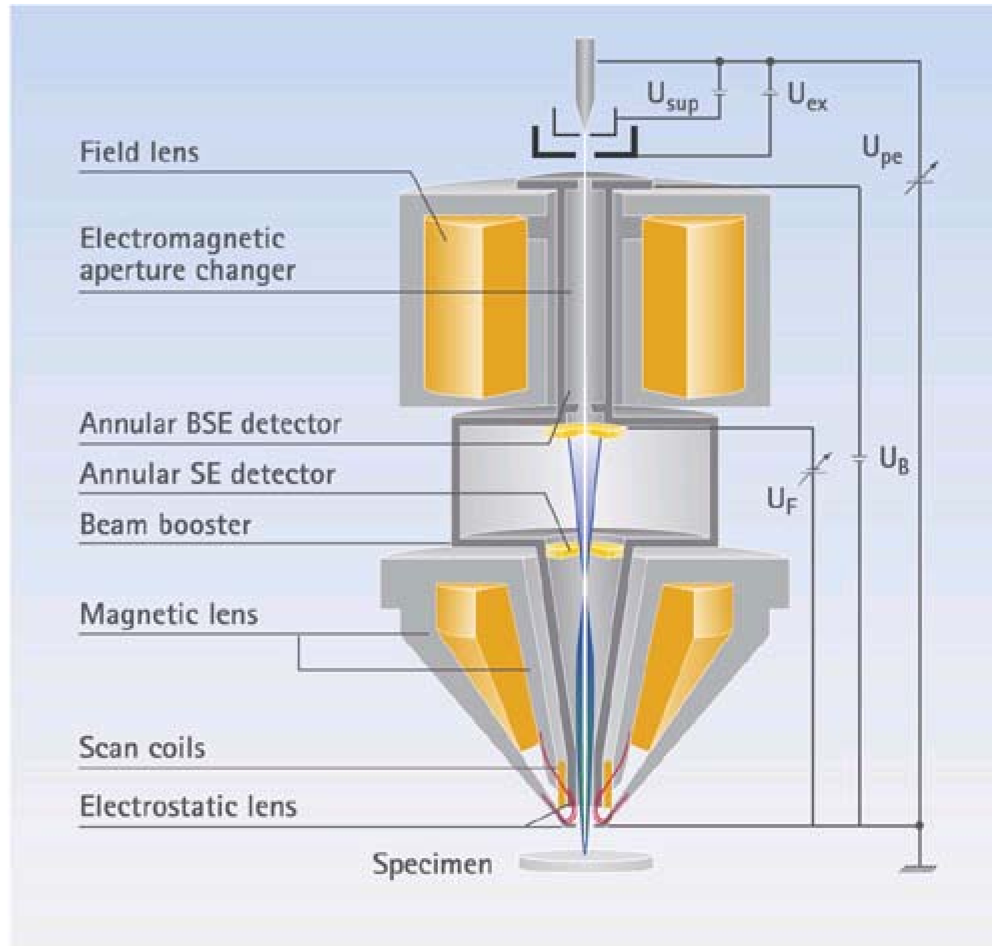


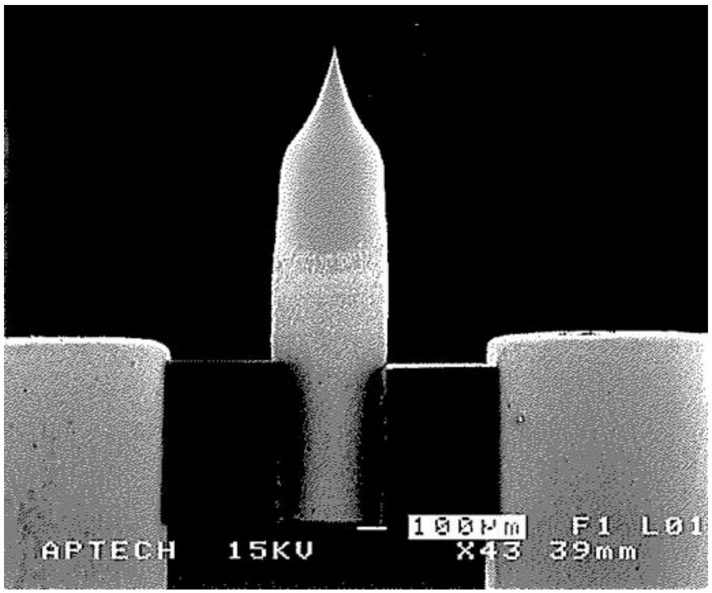
FIGURE 2.6. Cross-section through a magnetic lens with lines showing the magnetic field distribution.

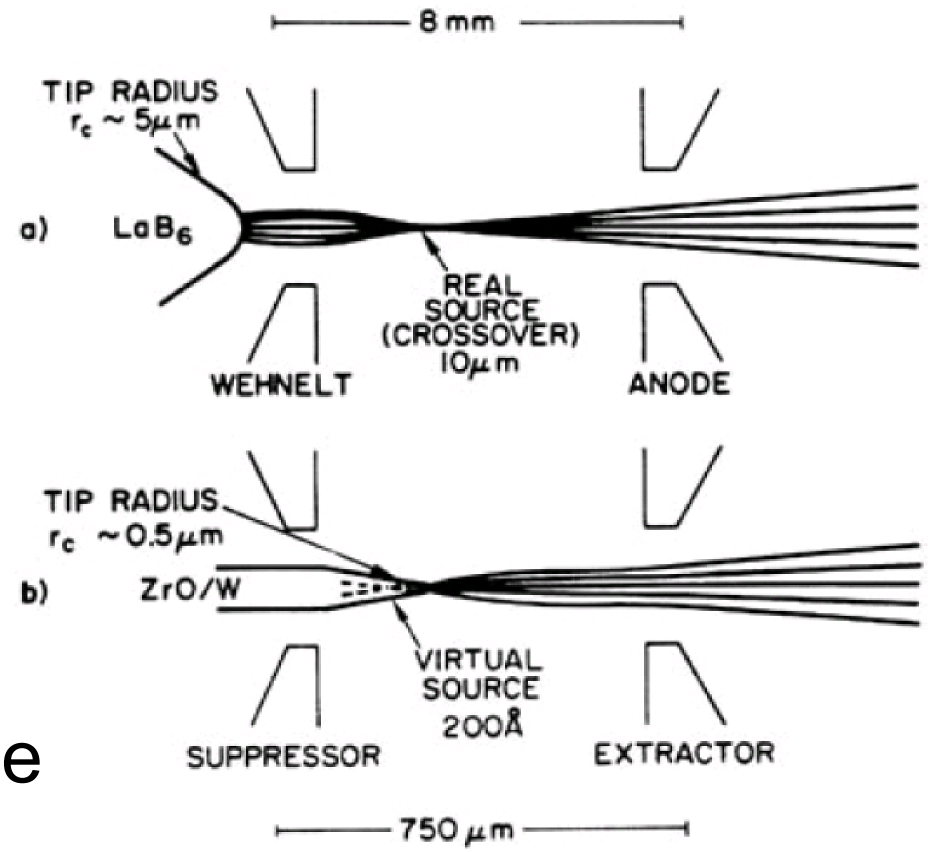
Cylindrically symmetric magnetic
Field with radial gradients





Sources

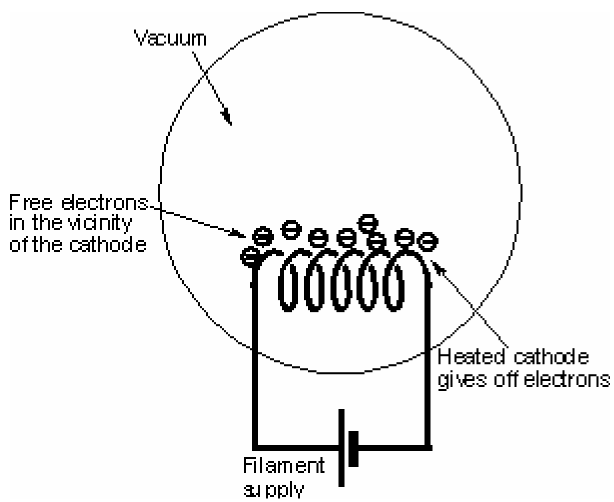


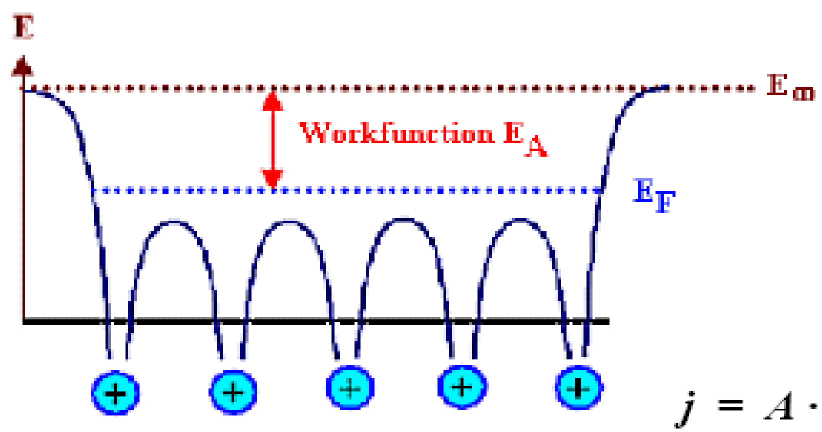


Electron emission can be achieved by different physical mechanisms

Emission

- Thermal emission



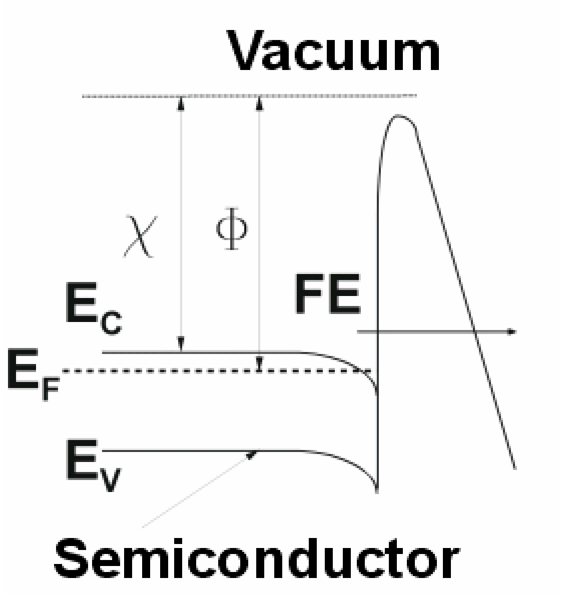


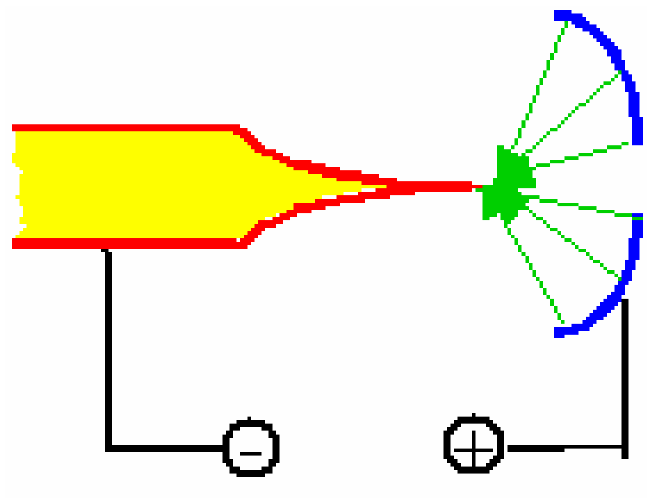
$$j = A \cdot T^2 \cdot \exp - \frac{E_A}{kT}$$

Material	Fe	Ni	Pt	Ta	W	Cs	LaB_6
A [$Acm^{-2}K^{-2}$]	26	30	32	55	60	162	25
E_A [eV]	4,5 - 4,8	5,15 - 5,35	5,65	4,15 - 4,8	4,2	1,8 - 2,14	2,6
T_m [°C]	1 535	1 452	1 755	2 850	3 410	28,4	2 210

Emission

- Field emission





Field emission starts for $E > 10^7$ V/cm
High current density: $J(E) = A \cdot E^2 \varphi \exp(-B \varphi^{1.5} / E)$

Strong nonlinear current-voltage characteristic
Very short switching time (t <ns)

Small spot size due to field enhancement at the tip apex

Ion and Electron Optics

- Electron beam sources

TABLE 2.1 Properties of the electron sources commonly used in electron beam lithography tools.

source type	brightness ($A/cm^2/sr$)	source size	energy spread (eV)	vacuum requirement (Torr)
tungsten thermionic	$\sim 10^5$	25 um	2-3	10^{-6}
LaB_6	$\sim 10^6$	10 um	2-3	10^{-8}
thermal (Schottky) field emitter	$\sim 10^8$	20 nm	0.9	10^{-9}
cold field emitter	$\sim 10^9$	5 nm	0.22	10^{-10}

Source Size and Spot diameter

- The source size can be large (micrometers) and, if so, must be *DEMAGNIFIED* to achieve small (nanometer) spot at the sample plane

Source Stability

- E-beam current must be stable and low noise for clear imaging and stable electron beam manipulation processes

Monochromatic beam is also important

Scanning Electron Microscope

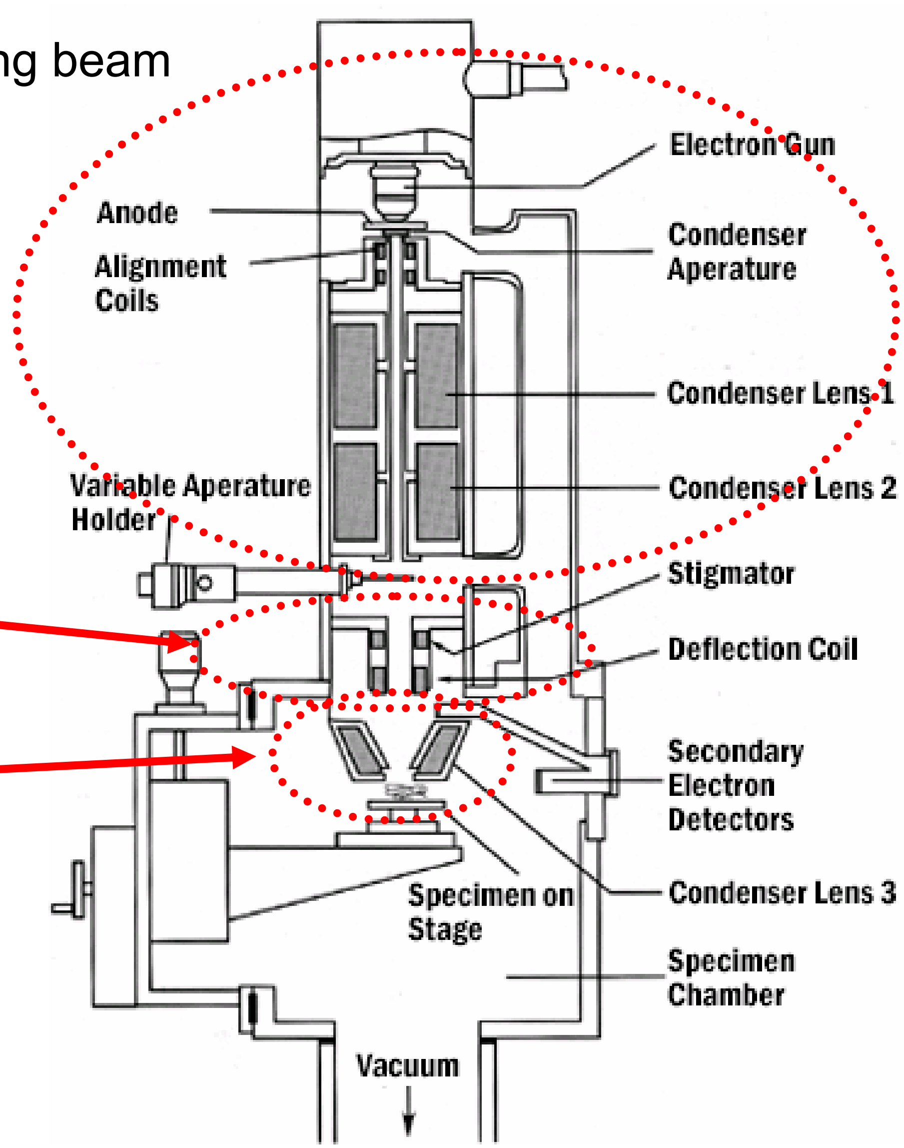
- Sequential imaging similar to the optical scanning confocal microscope
- Can be used in reflection or transmission modes (STEM)

SEM Anatomy

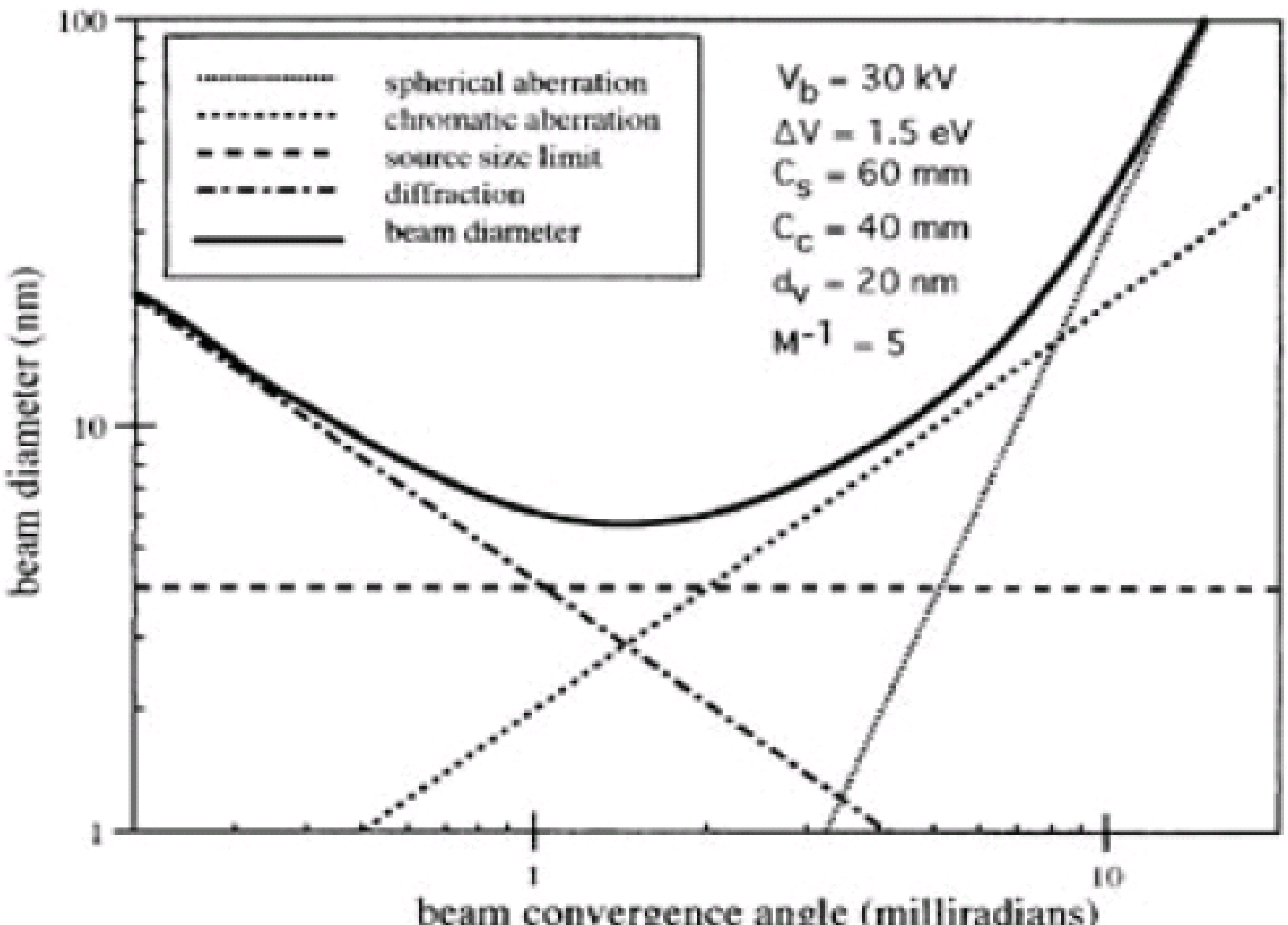
Preparation of proper illuminating beam

XY scanning

Focusing objective

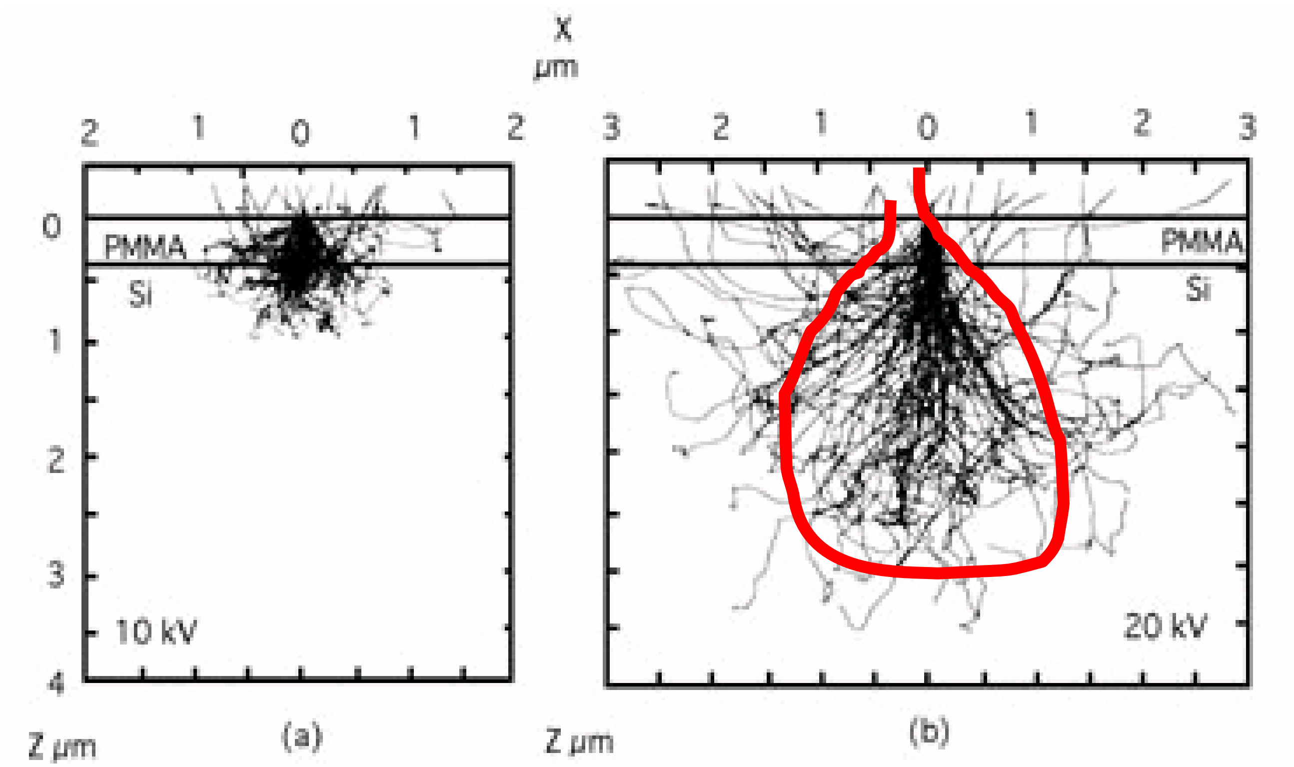


Various factors affecting spot diameter



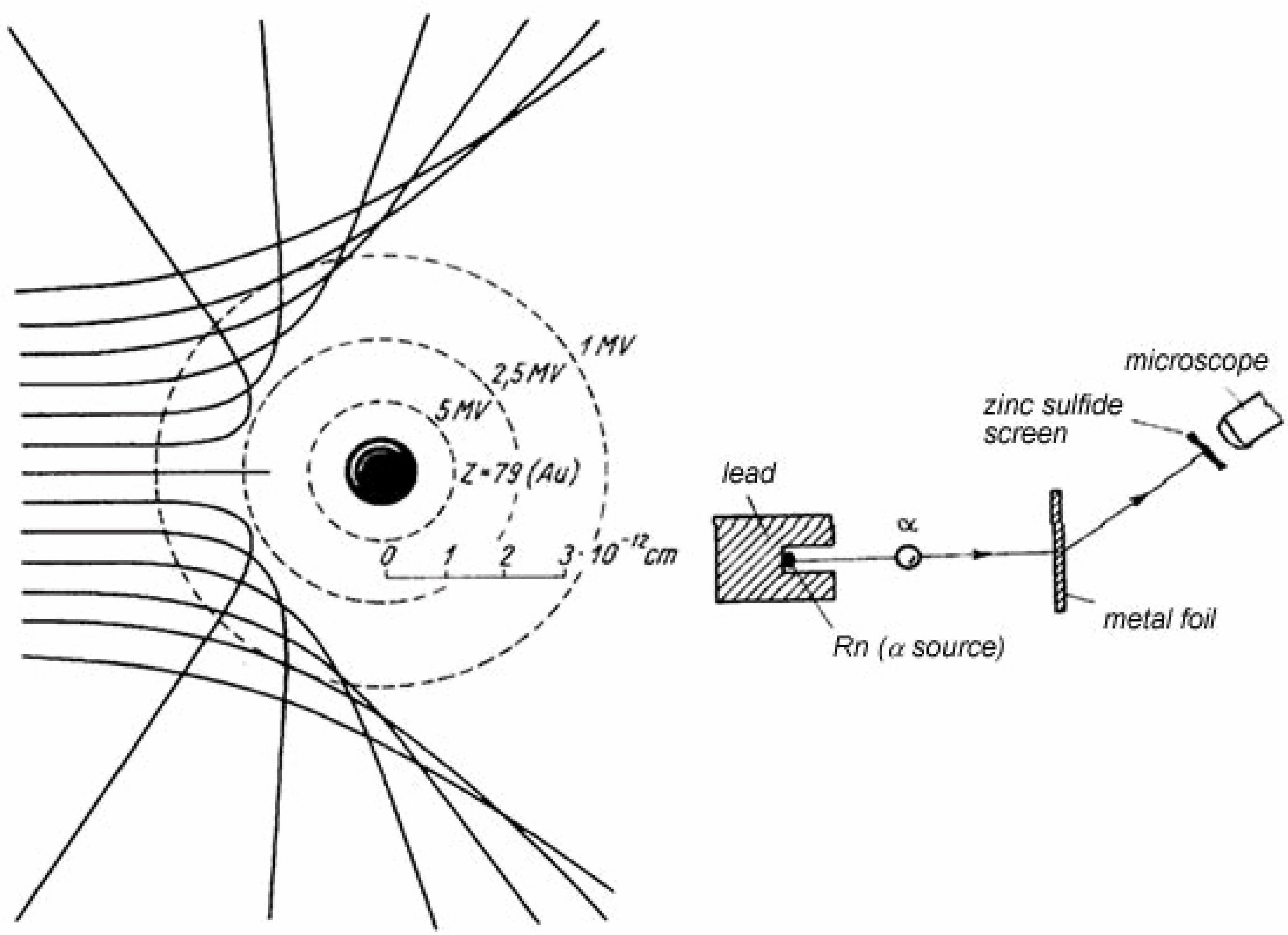
Electron Beam and Sample Interaction

- Depends on energy of beam, material of the sample. The beam penetrates the sample
- Beam Spot size isn't everything



Charged particle scattering

- Example: Rutherford Scattering



Charged particle scattering

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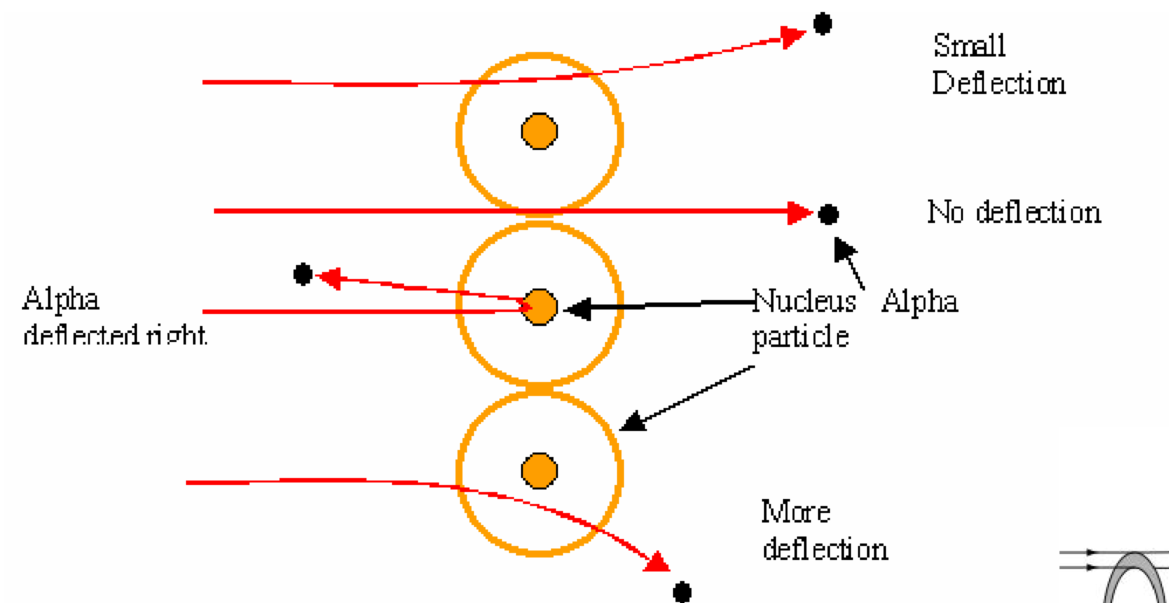
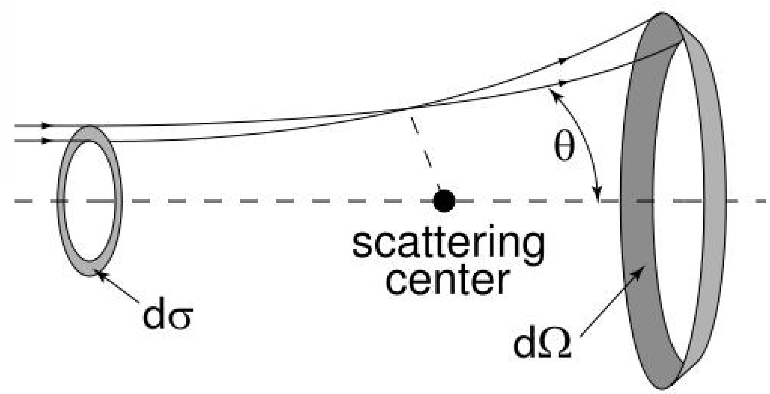


$$\frac{d\sigma}{d\Omega} = \frac{q^2}{16} \sin^{-4}(\theta / 2)$$

$$q = 2Ze^2 / E$$



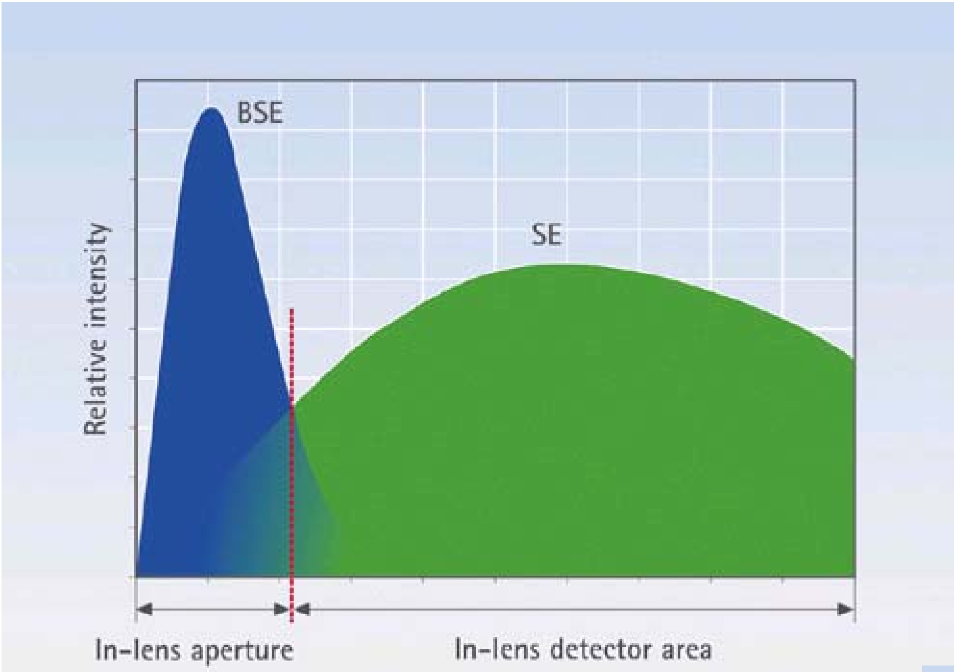
Pure classical calculation of scattering

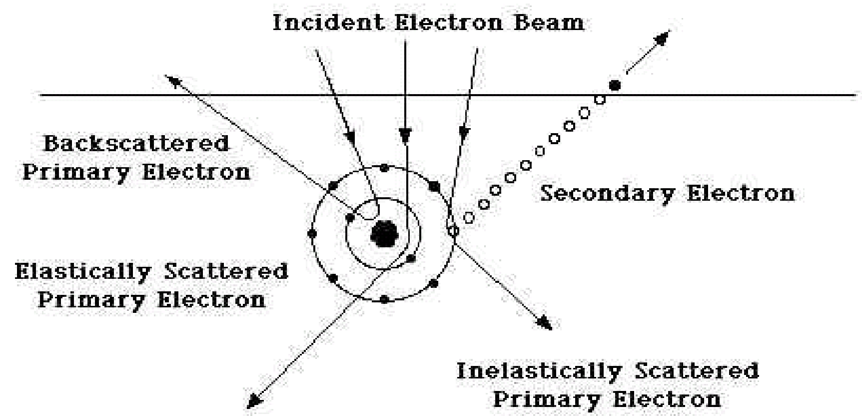
Elastic scattering

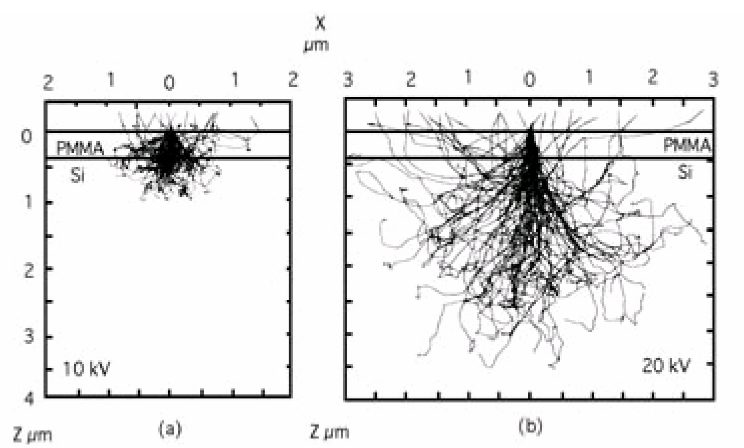
Angular scattering Dependent on particle energy, Mass of nucleus

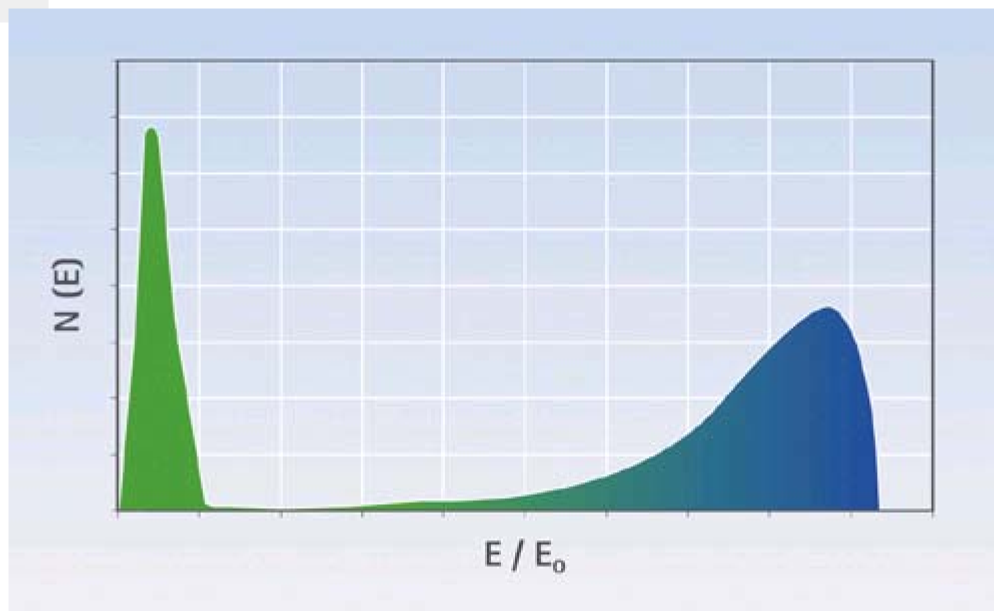
BSE (Back-scattered Electrons) give better Z contrast

Charged particle scattering

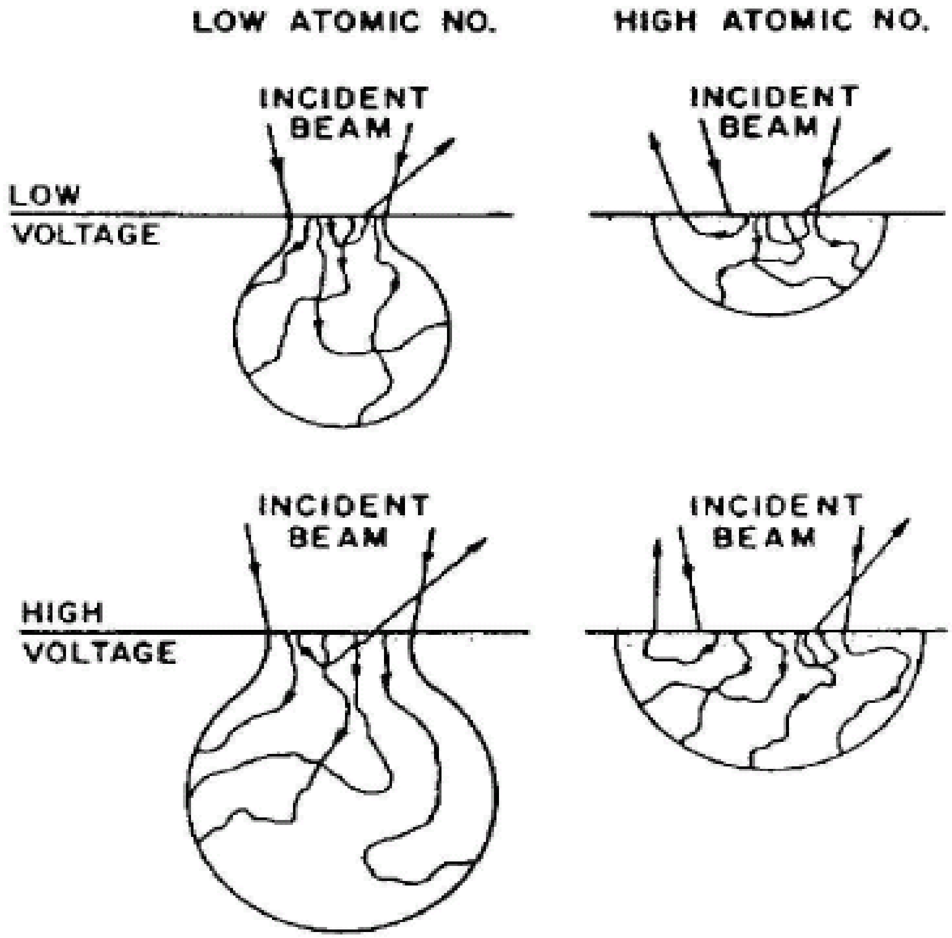








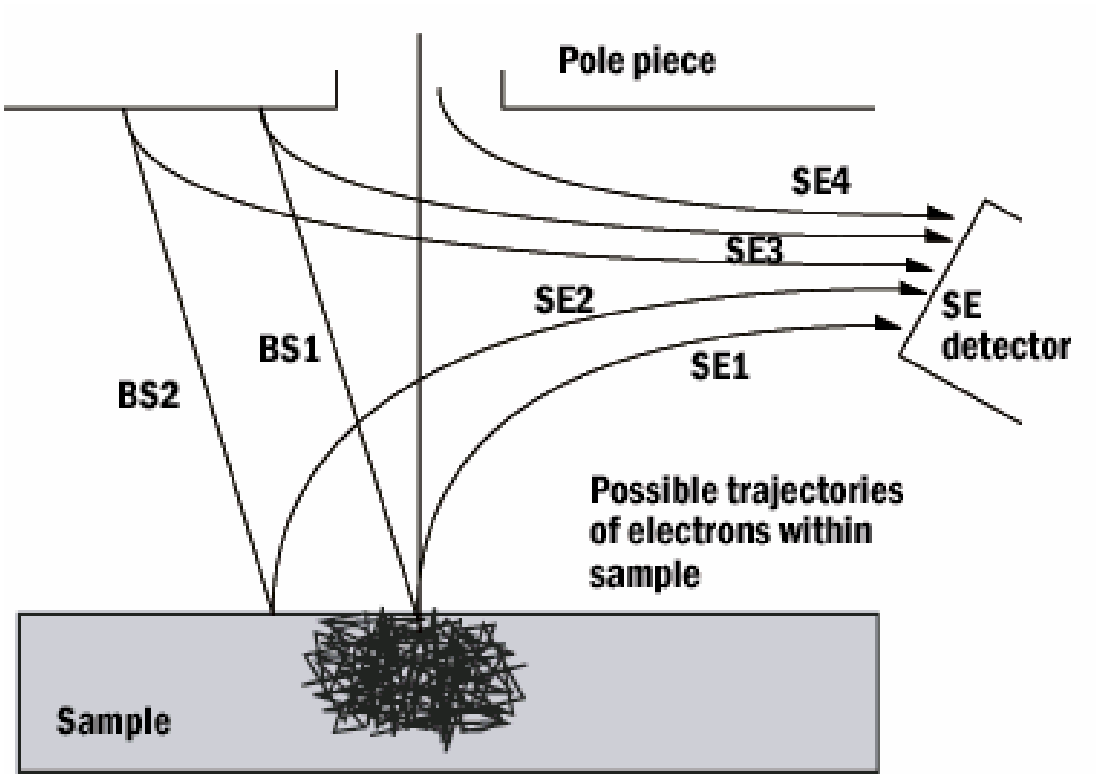
Electron and sample interaction



High voltage: Large penetration, more sample damage

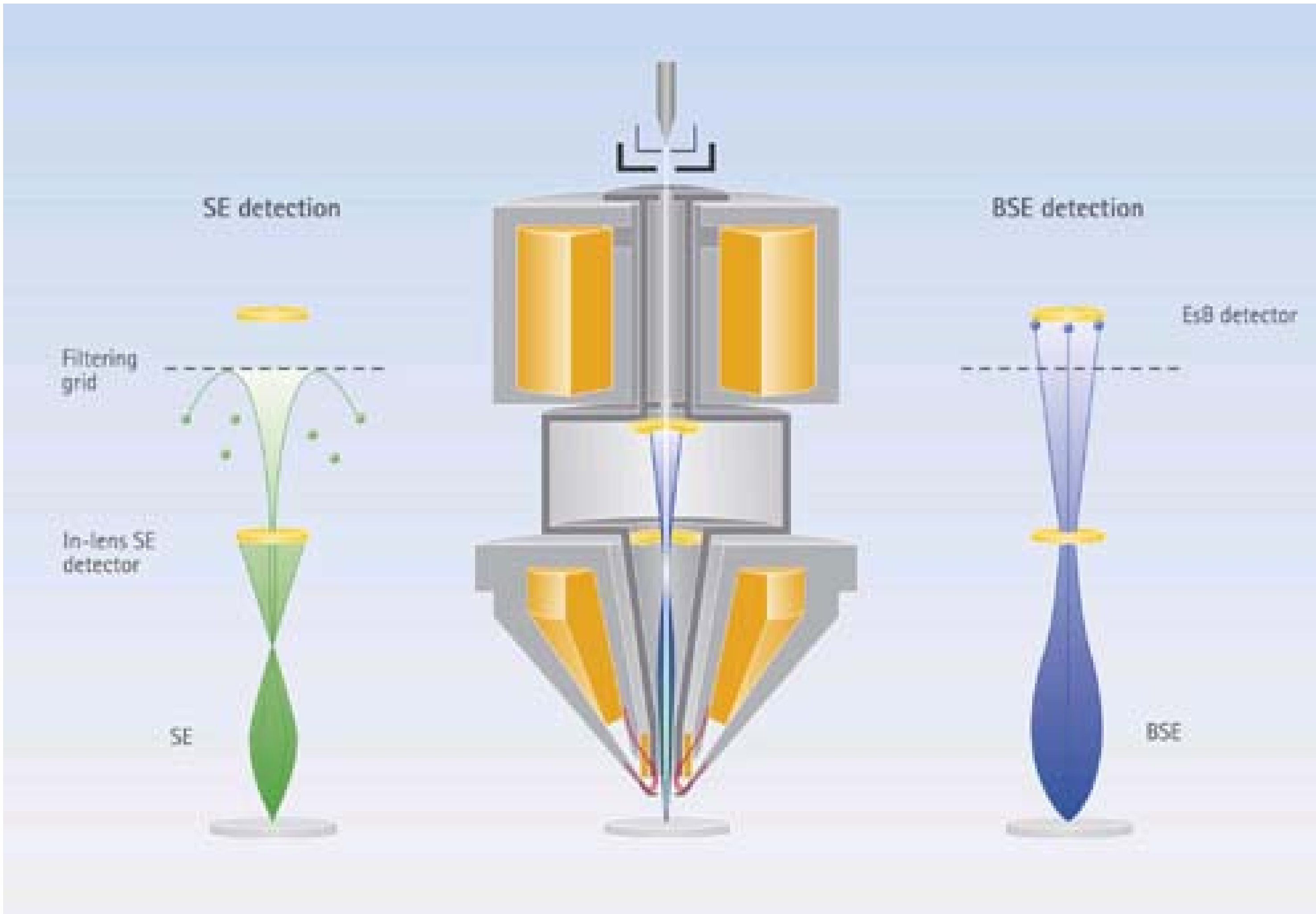
Large current: More damage, more carbon deposition, better X-ray signal

Detectors: Secondary Elec. Det.

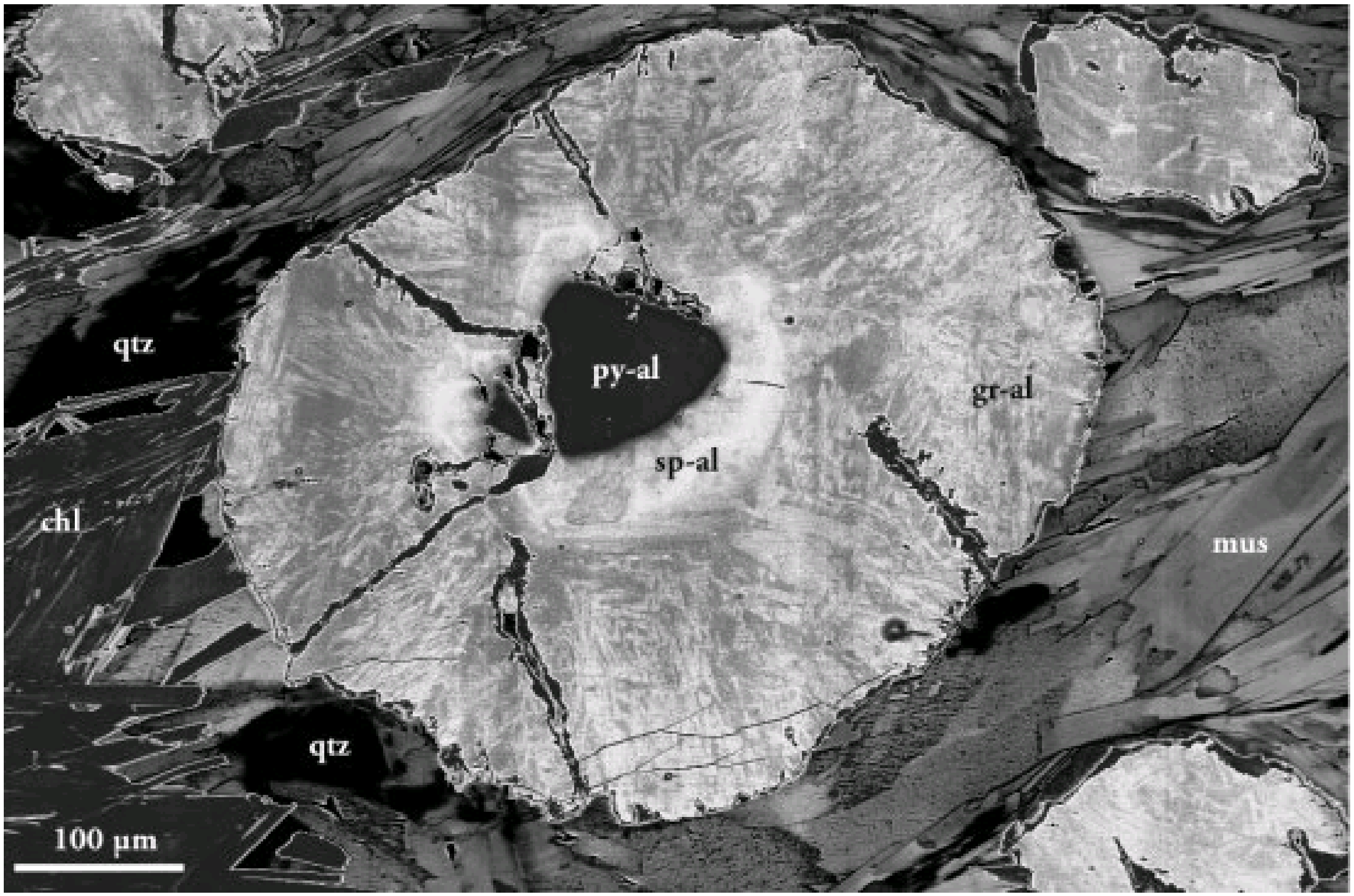


- Secondary electrons generally low energy (50-100 eV)
- Trajectories can be bent easily by biasing the detector
- Low Z contrast

Energy filtered detection of electrons

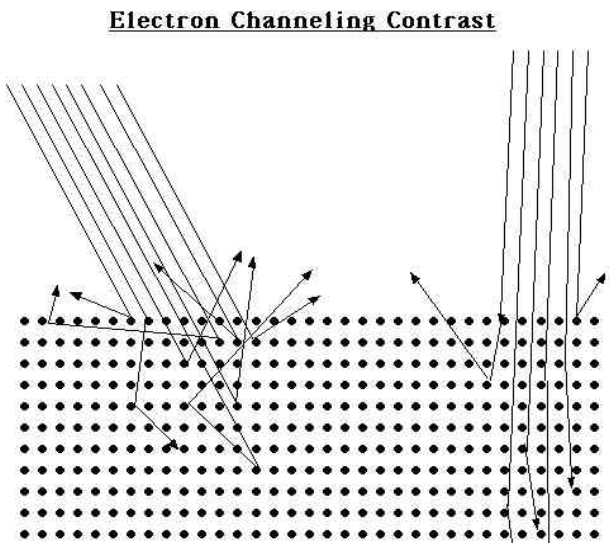


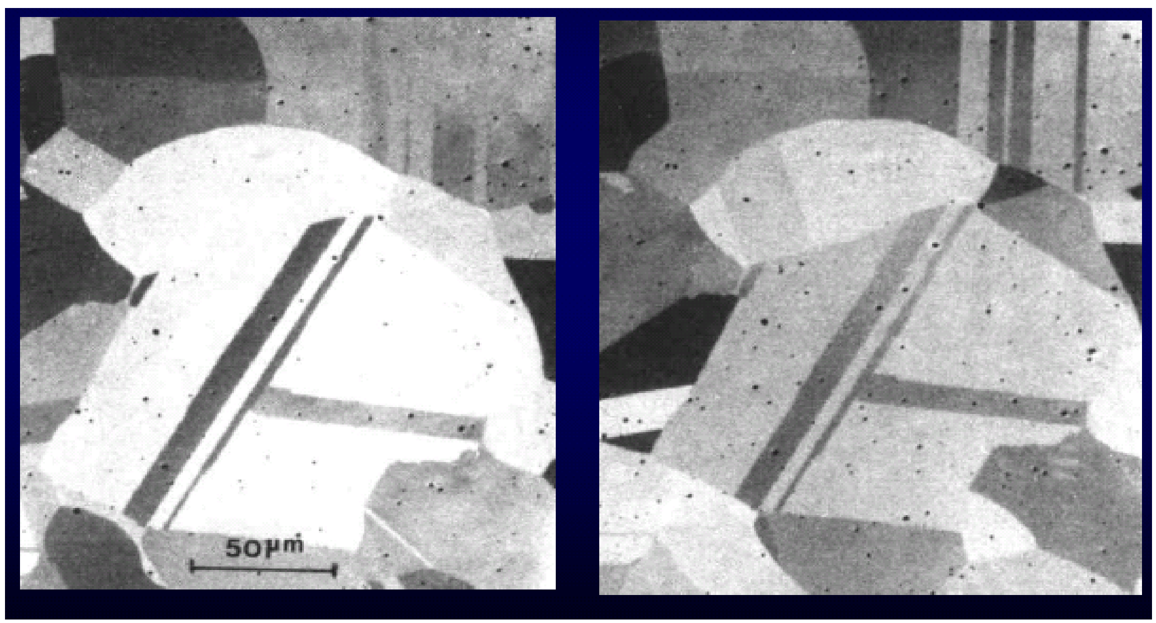
Z-Contrast in BSE signal



Metallurgical sample

Channeling Contrast

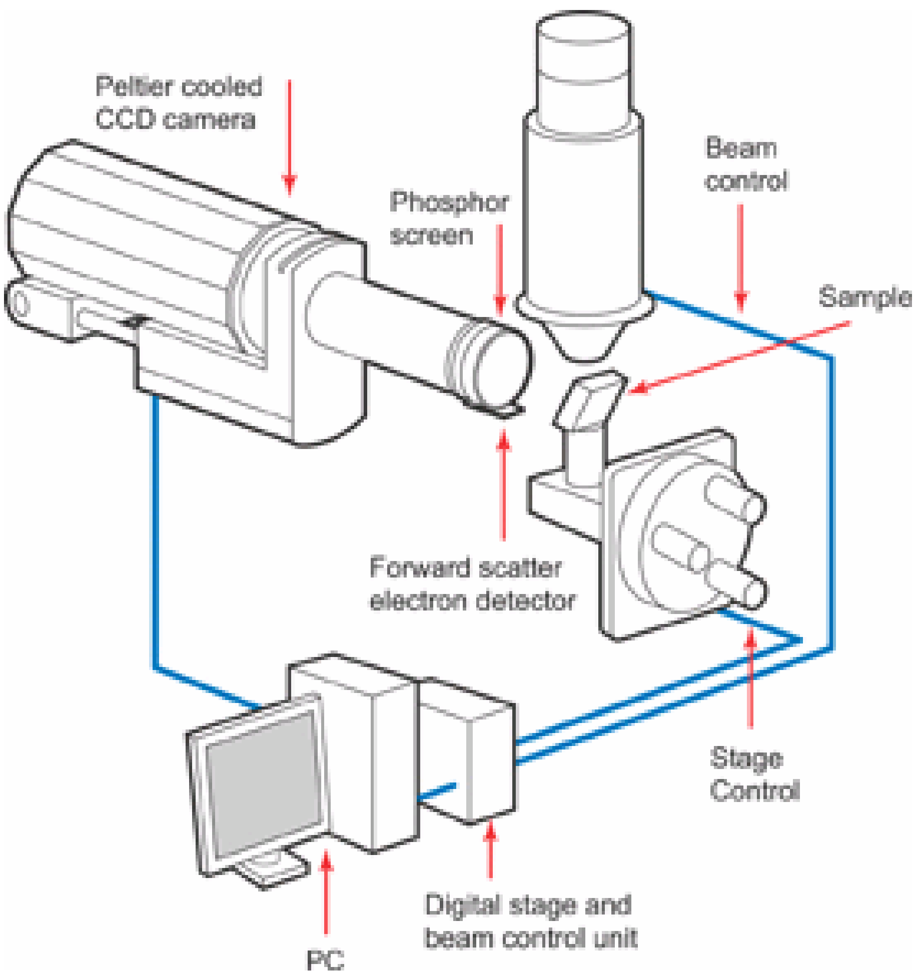


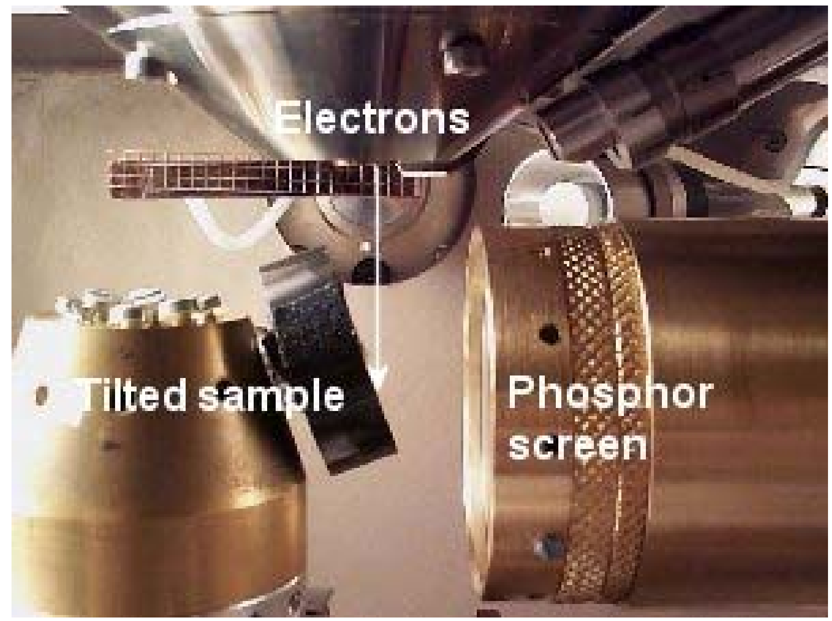


Analytical tools in SEM

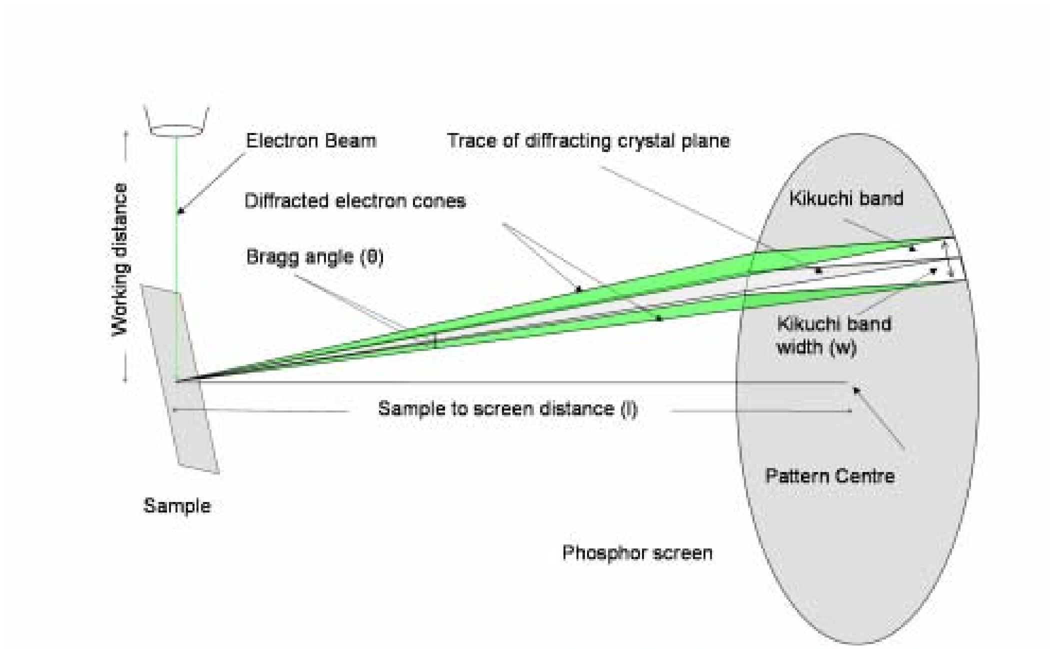
- Obtain information about
  - Composition
  - Crystal structure/orientation
- Methods include
  - EBSD (Electron back scatter diffraction)
  - EDX (Energy-dispersive X-ray analysis)
  - AES (Auger Emission Spectroscopy)

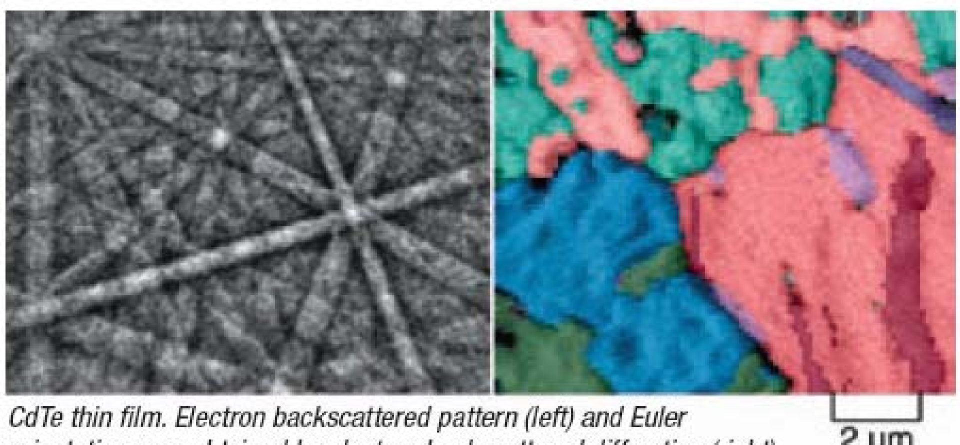
EBSD


Peltier cooled
CCD camera
Phosphor
screen
Beam
control
Sample
Forward scatter
electron detector
Stage
Control
Digital stage and
beam control unit
PC

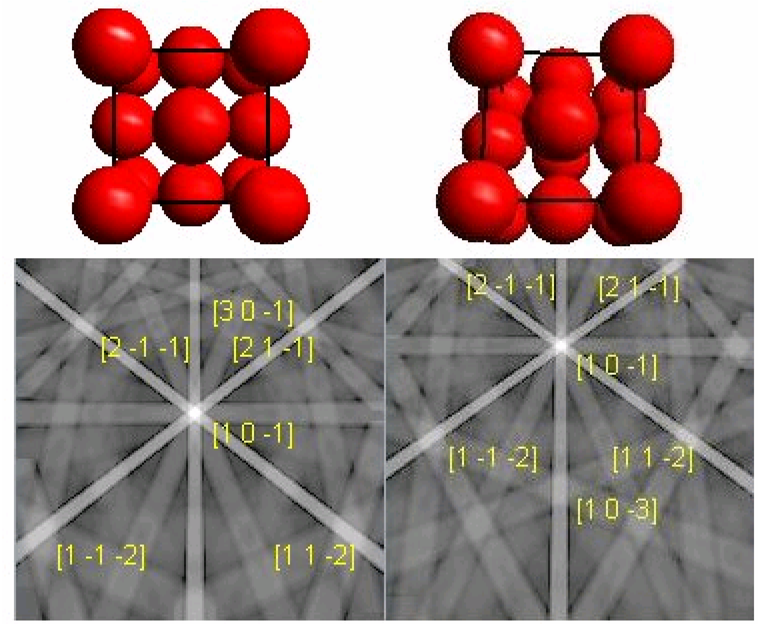

Electrons
Tilted sample
Phosphor
screen

EBSD





CdTe thin film. Electron backscattered pattern (left) and Euler orientation map obtained by electron backscattered diffraction (right).



Changes in the crystal orientation result in movement of the diffraction pattern. The simulated diffraction pattern is from a sample tilted 70° to the horizontal and the crystal orientation is viewed along the direction perpendicular to the sample.

EDX

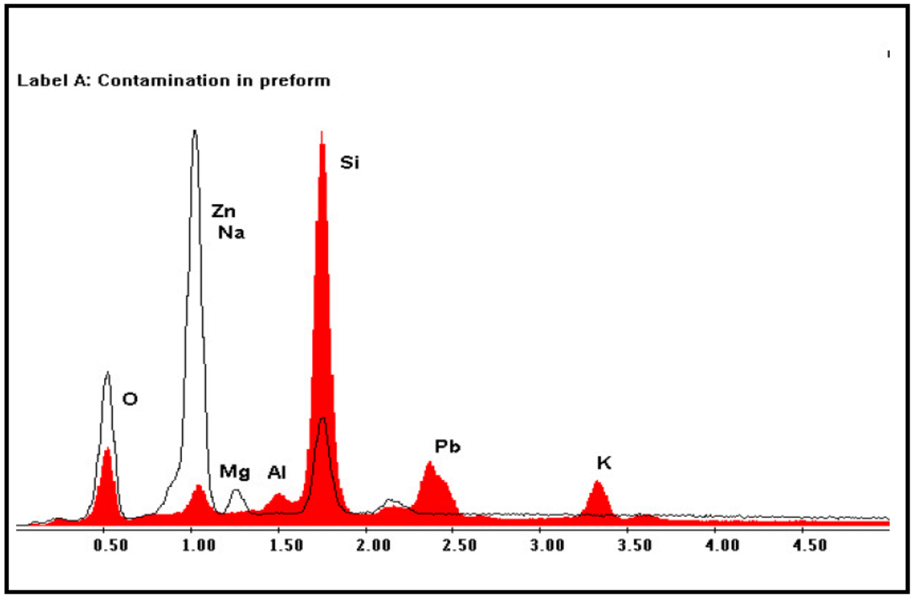


Fig. 4. EDX spectra of particle (red) and the normal ceramic (black outline)

Can be used to identify composition
On a micron scale

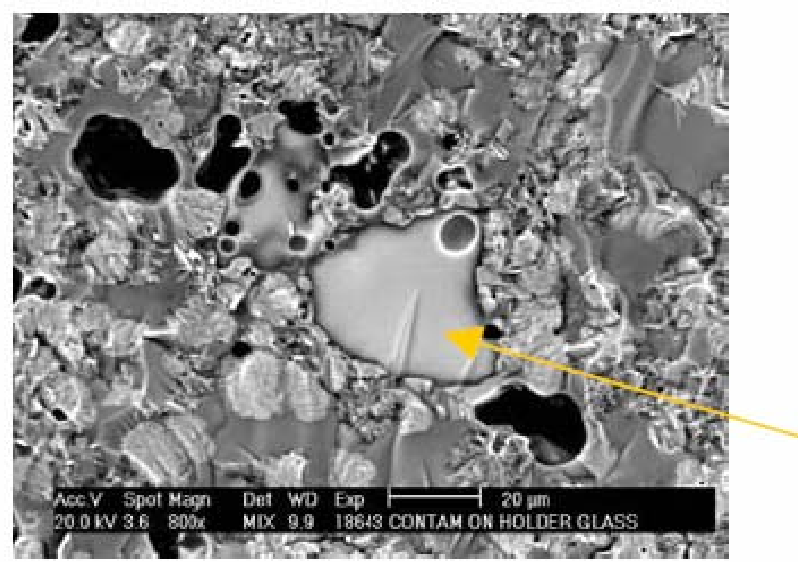
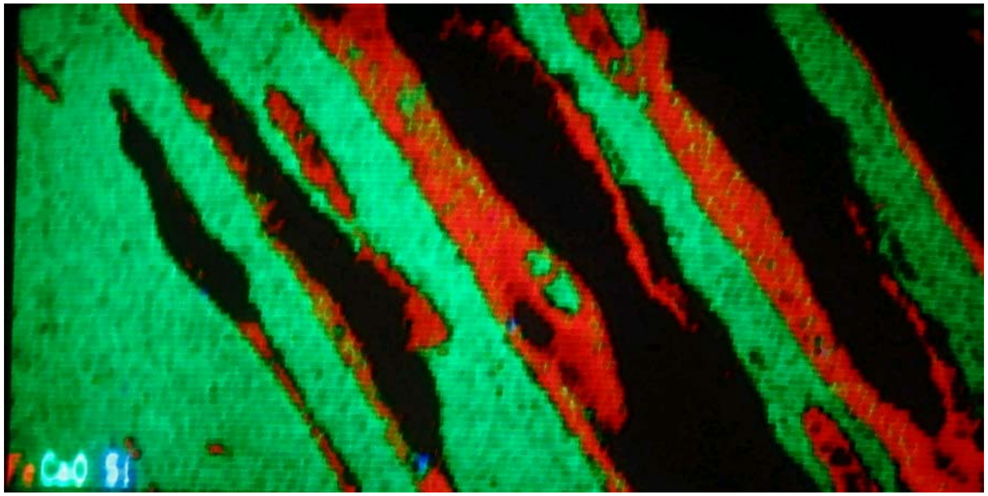
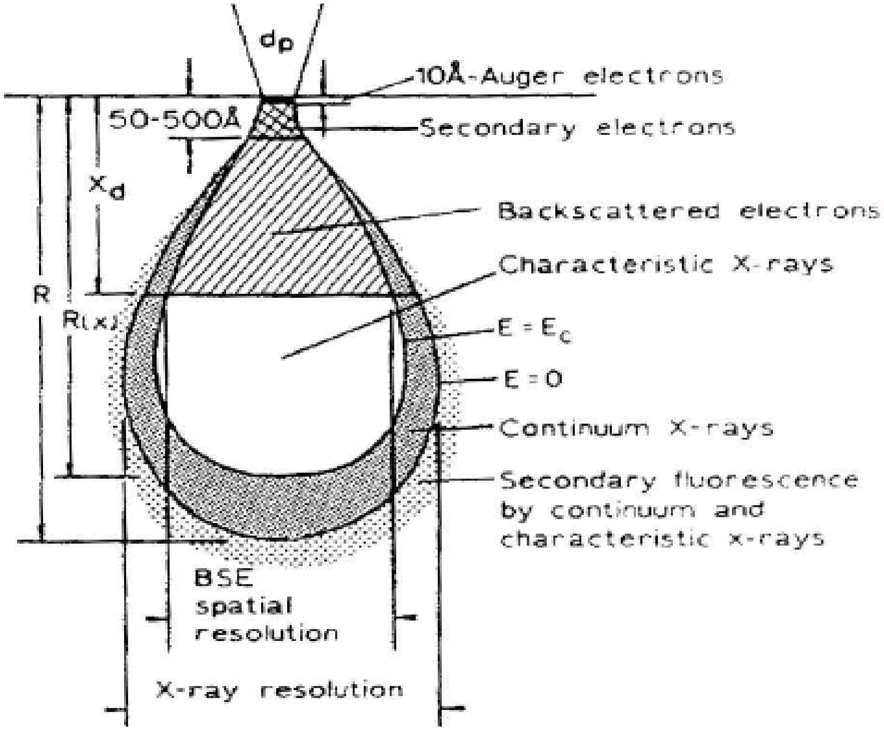


Fig. 3. Contamination inside the holder ceramic

EDX elemental mapping



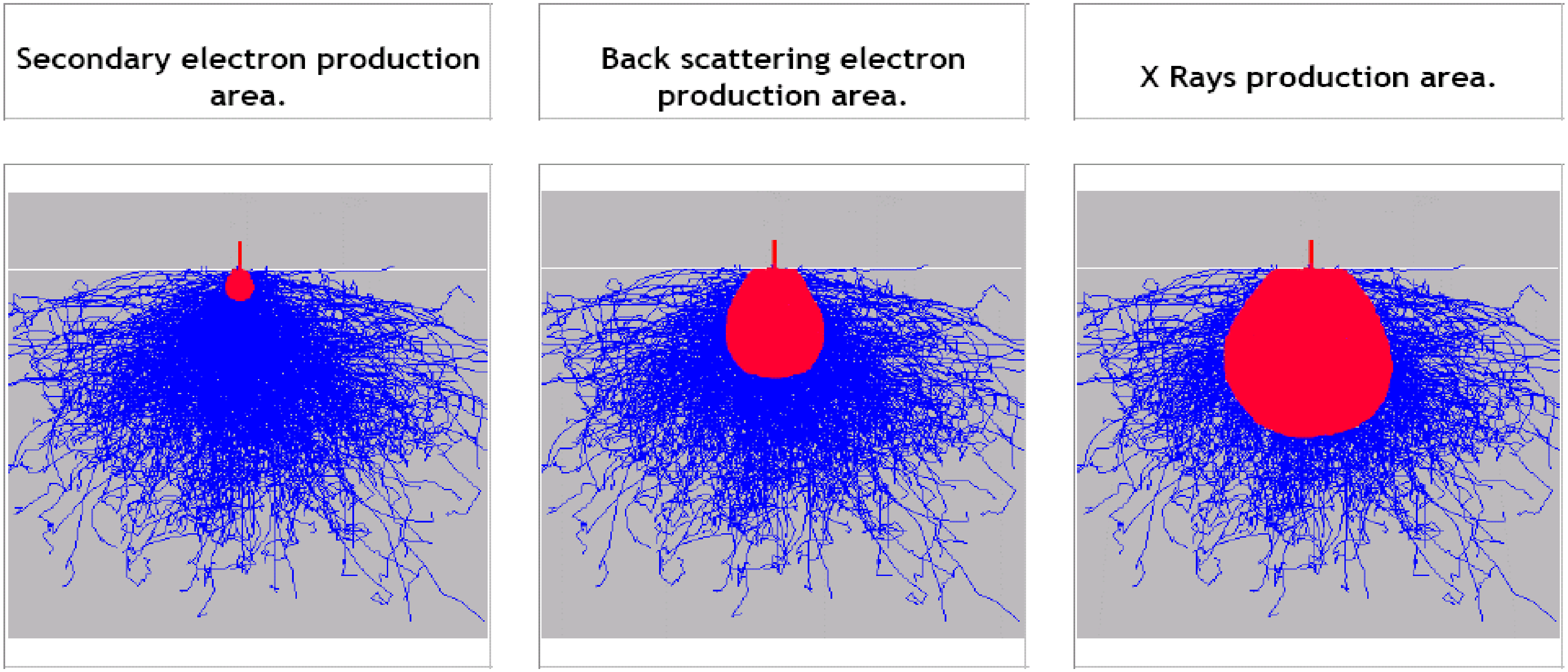
Shown here is a piece of fossil bone, red is iron and green is calcium.



Resolution is somewhat Limited due to large interaction Volume

Can be improved by thinning The sample

Electron Interactions

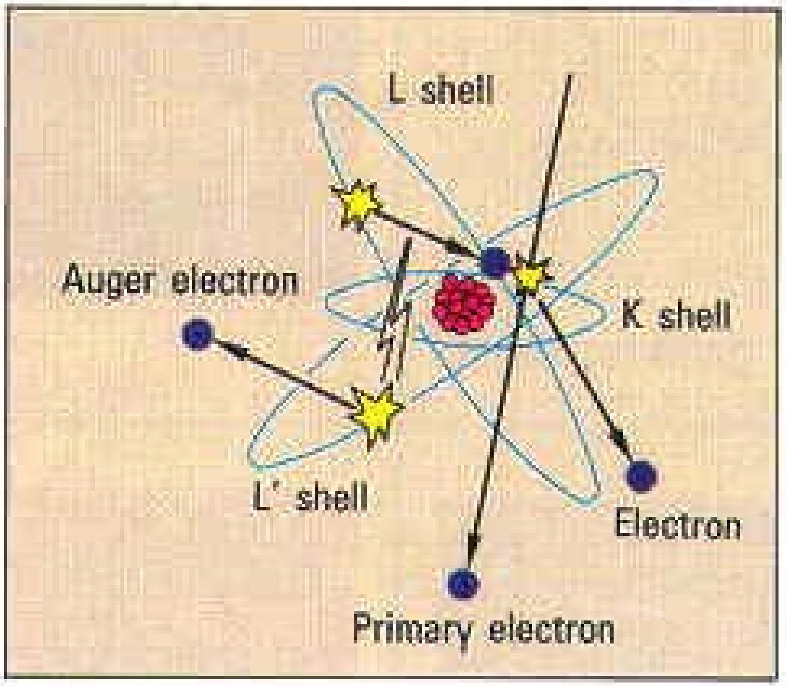


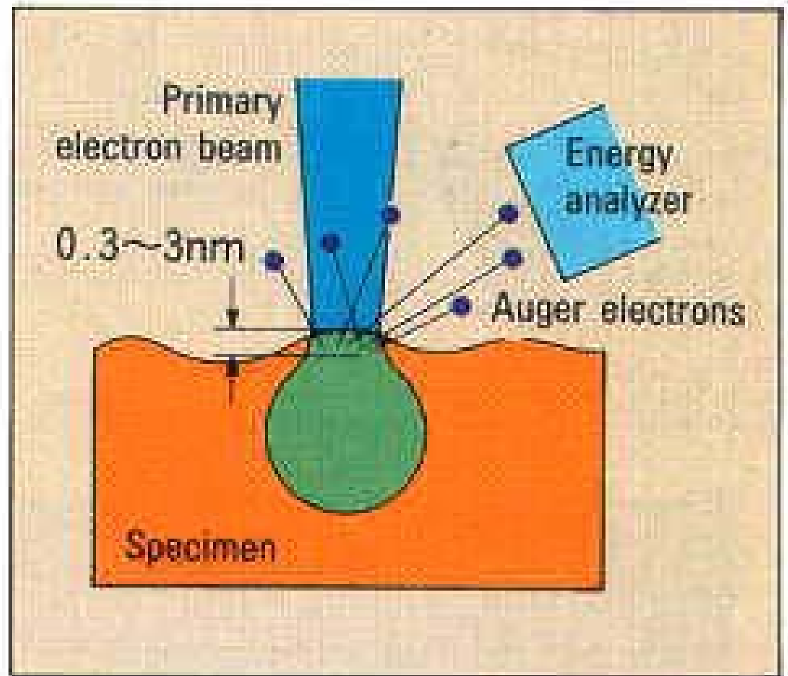
Best spatial resolution

Better Z contrast

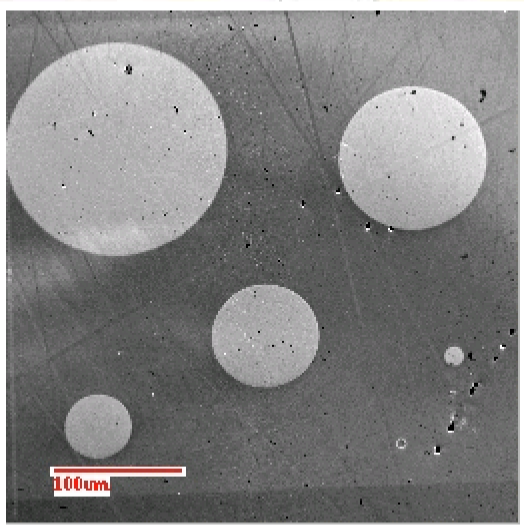
Best analytical

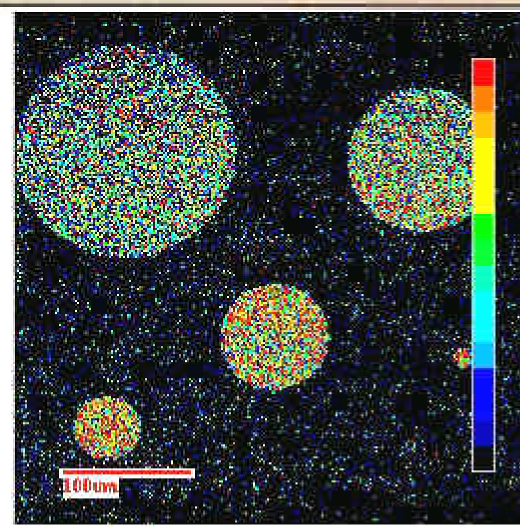
Auger Electron Spectroscopy



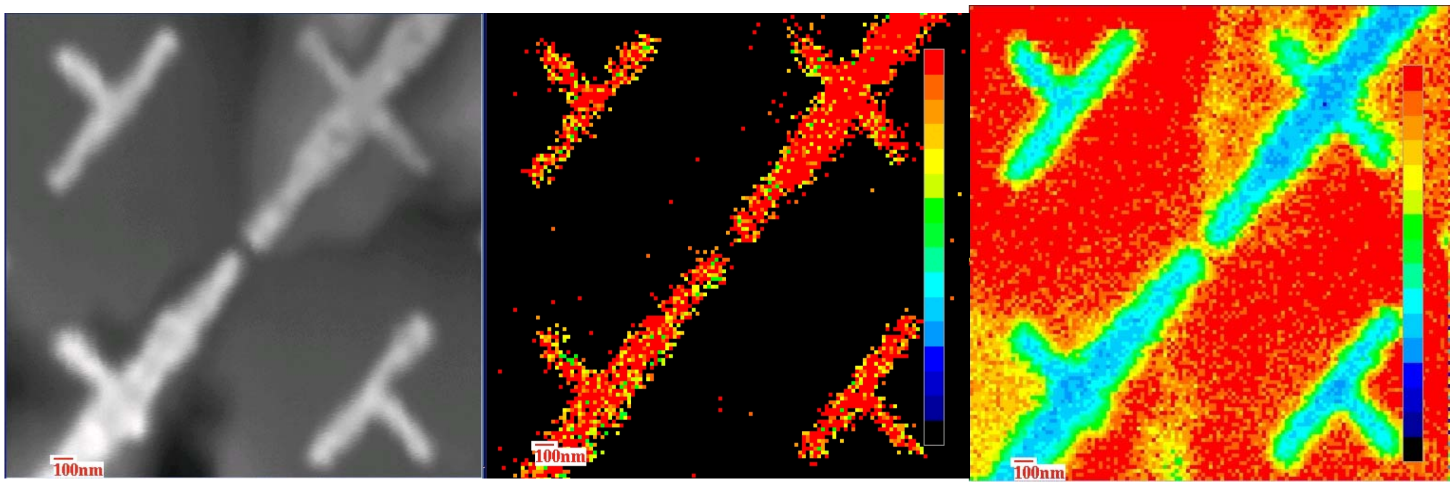


Oxidation of Ni3Al with variable beam size (SAM map of O)





Auger Electron Spectroscopy



SEM for nanocontacts ***SAM-W*** ***SAM-Si***

Uses an energy dispersive electron detector to map elements with about 100 nm

High pressure / Environmental SEM

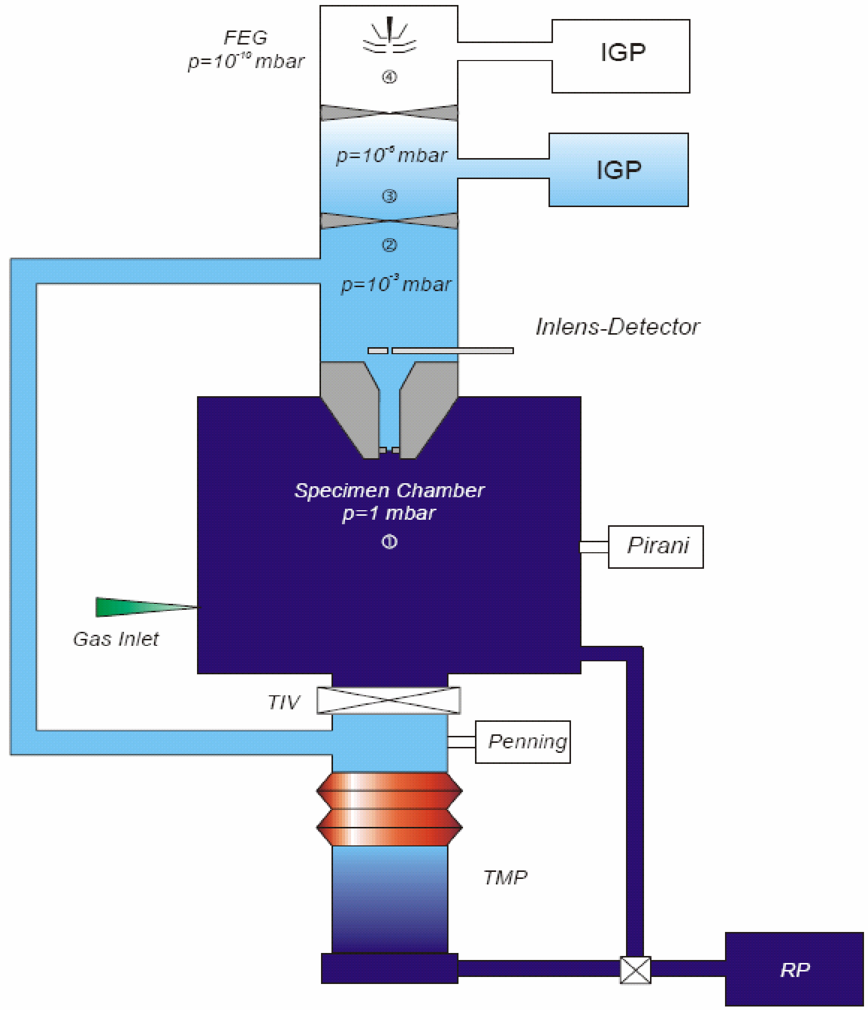
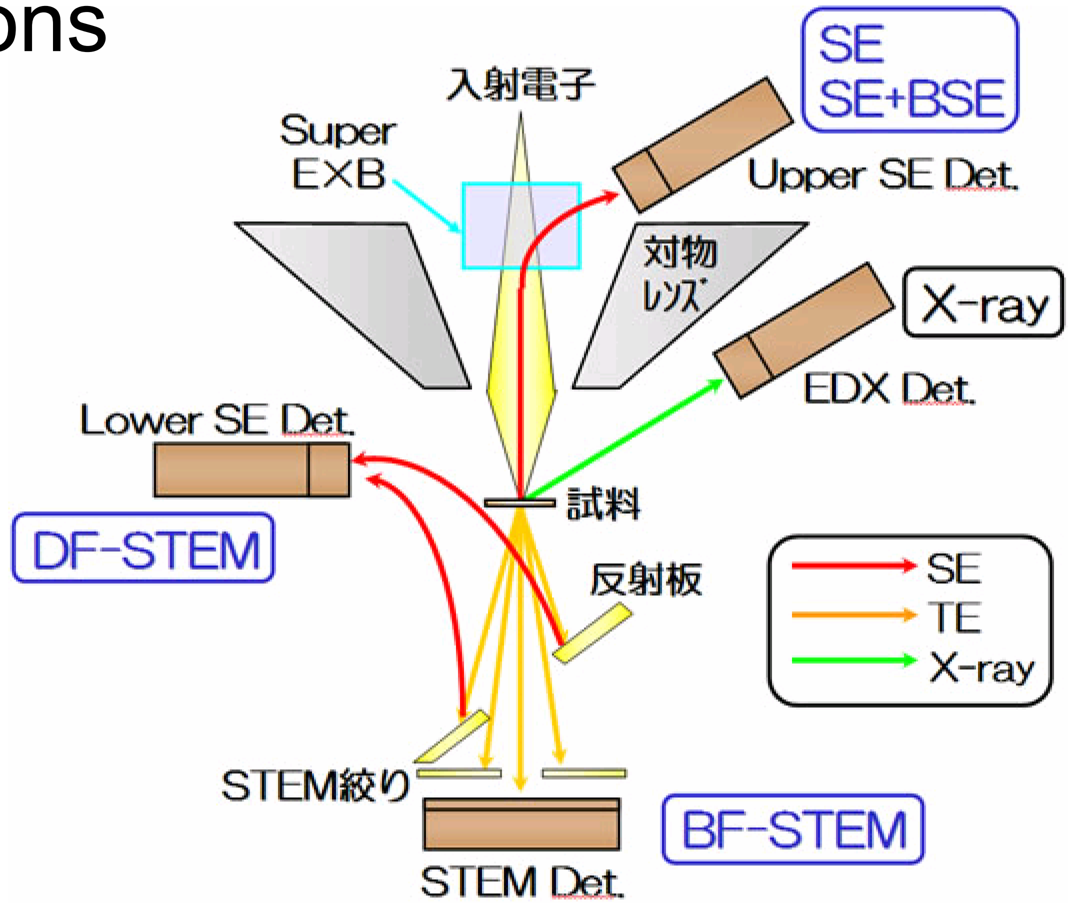


Figure 2: Vacuum System of the 1500 VP FESEM in VP-Mode: The four pressure regions (1)-(4) are separated by three pressure limiting apertures. The vacuum in the FEG area and the upper part of the column is maintained by two ion-getter-pumps, the lower part of the column is pumped by a turbomolecular pump (TMP). The specimen chamber is separated from the TMP-vacuum by a turbo-isolation-valve (TIV). The pressure in the specimen chamber is adjusted by using a dry rotary pump in combination with a needle valve.

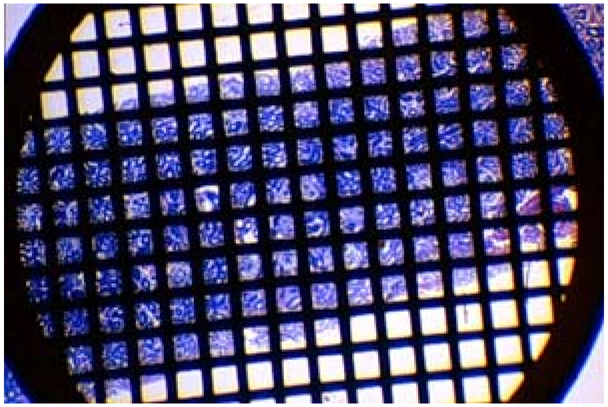
Scanning Transmission SEM

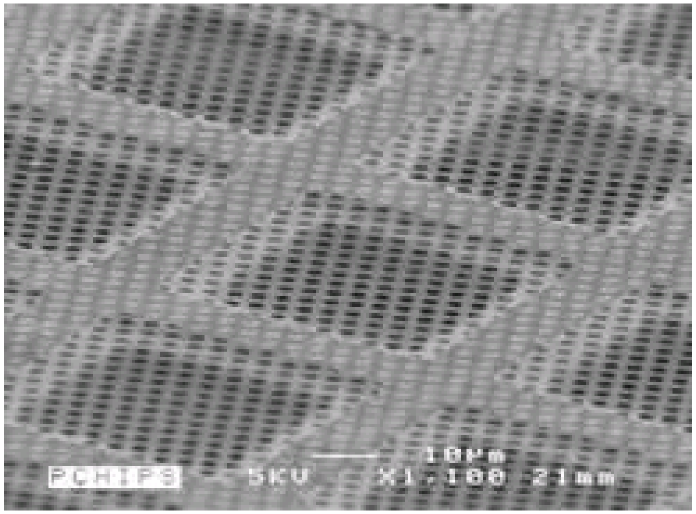
- A thinned sample (less than 100 nm)
- A detector to collect the transmitted electrons

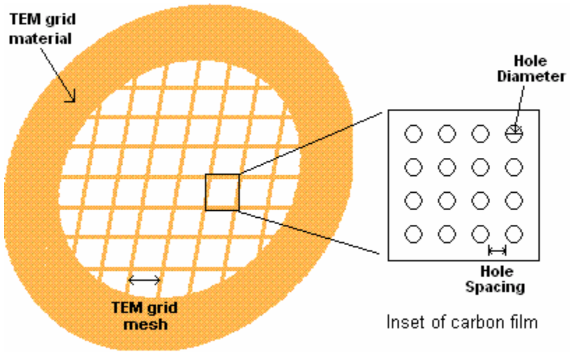


Scanning Transmission SEM

- For small particulate samples (biomolecules etc.), use TEM grids

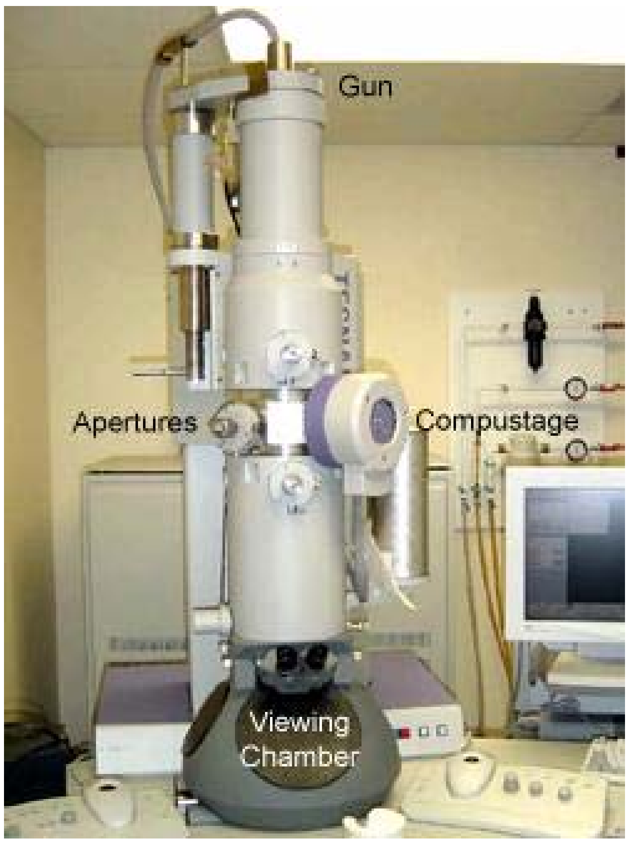






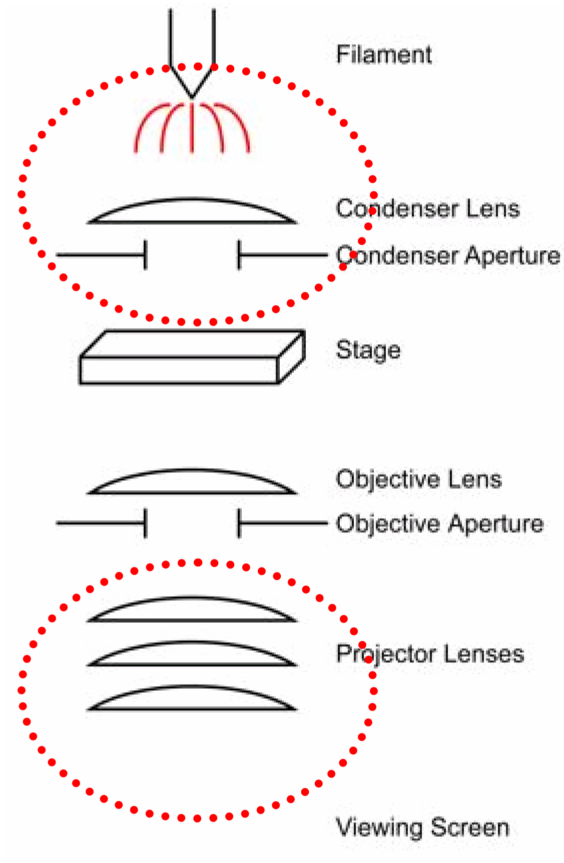
Transmission Electron Microscope

- More analogous to an optical microscope

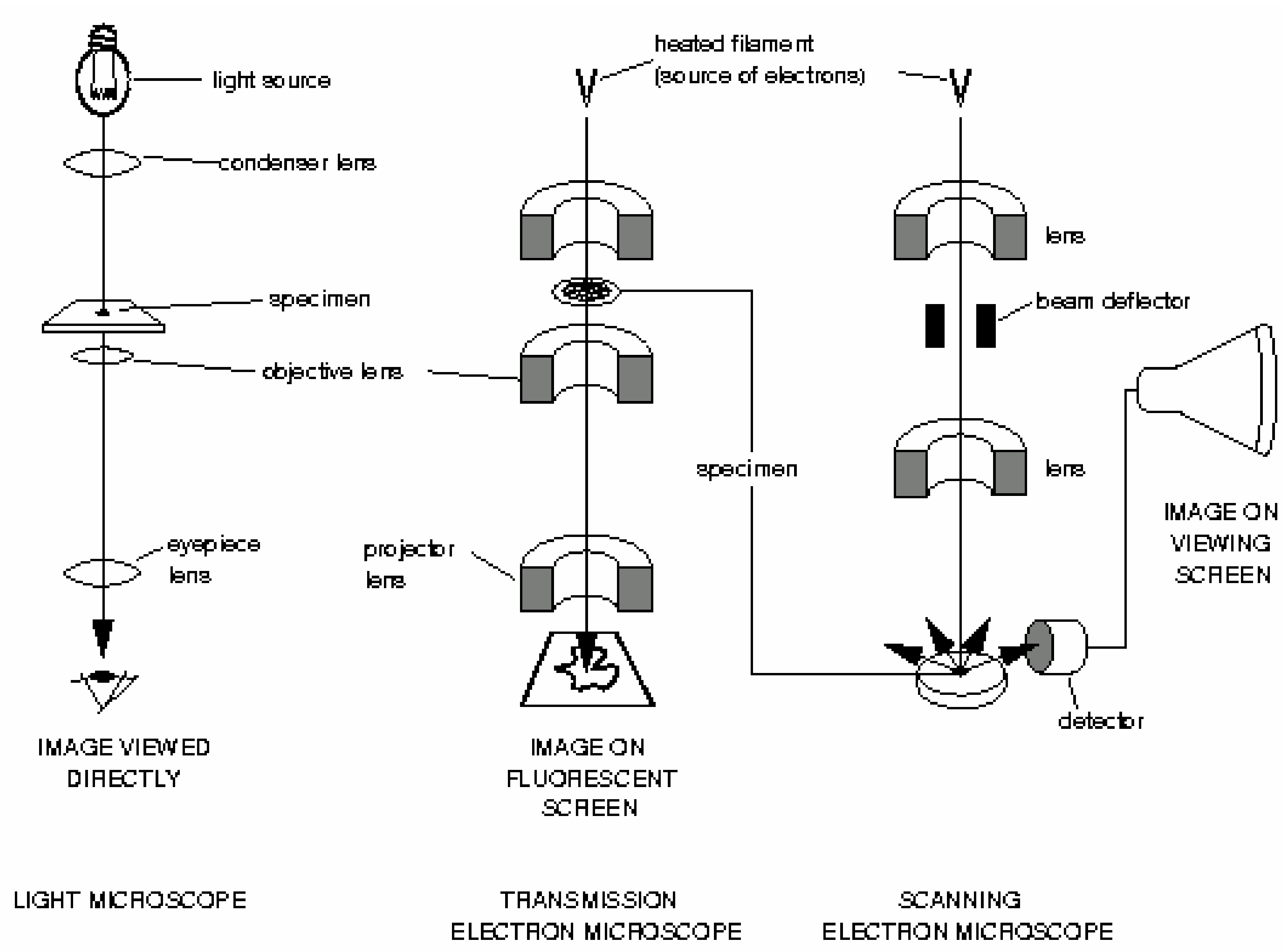


illumination

"eyepiece"

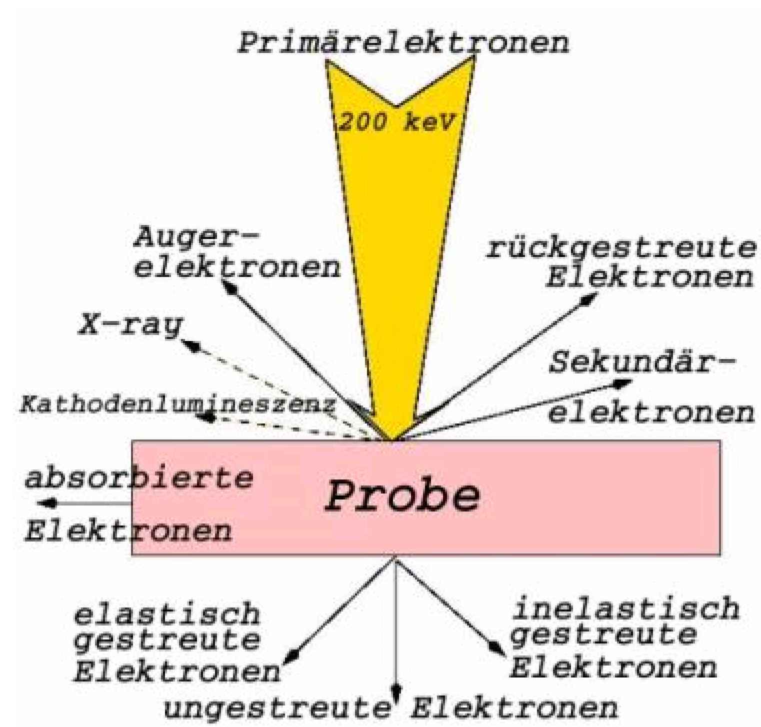


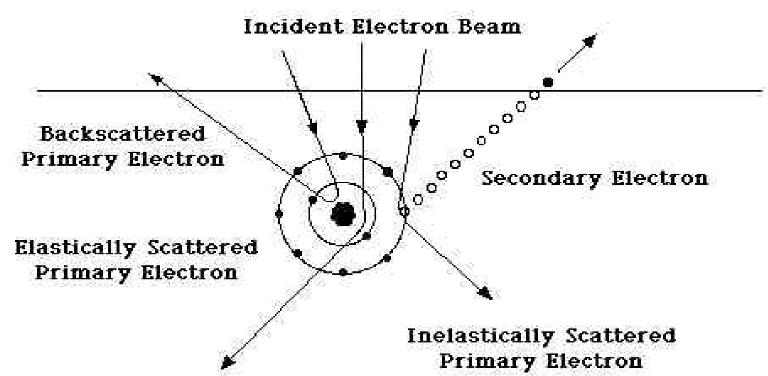
Transmission Electron Microscope



TEM

- An electron beam illuminates the sample, and transmitted beam is imaged in a very similar way to optical microscopy but with electrons of 300 KeV energy
- Gives sub nanometer resolution
- Requires extensive sample preparation for high resolution imaging (TEM *"Lamella"*)
- Analytical capabilities (Z contrast / mapping)
- Contrast depends on electron wave scattering from nuclei and electronic states of the sample





TEM MTF (CTF) / PSF

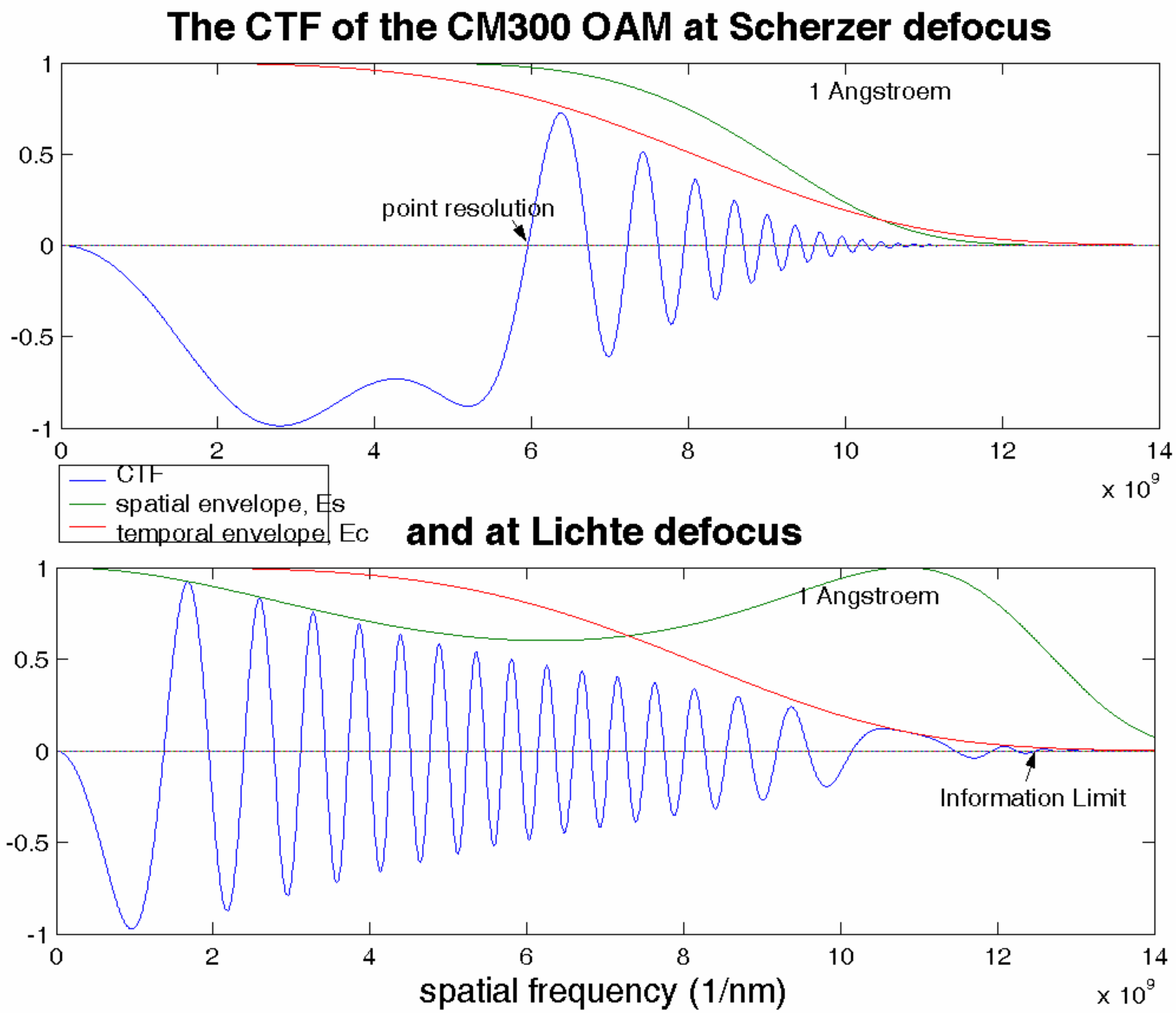


Image interpretation

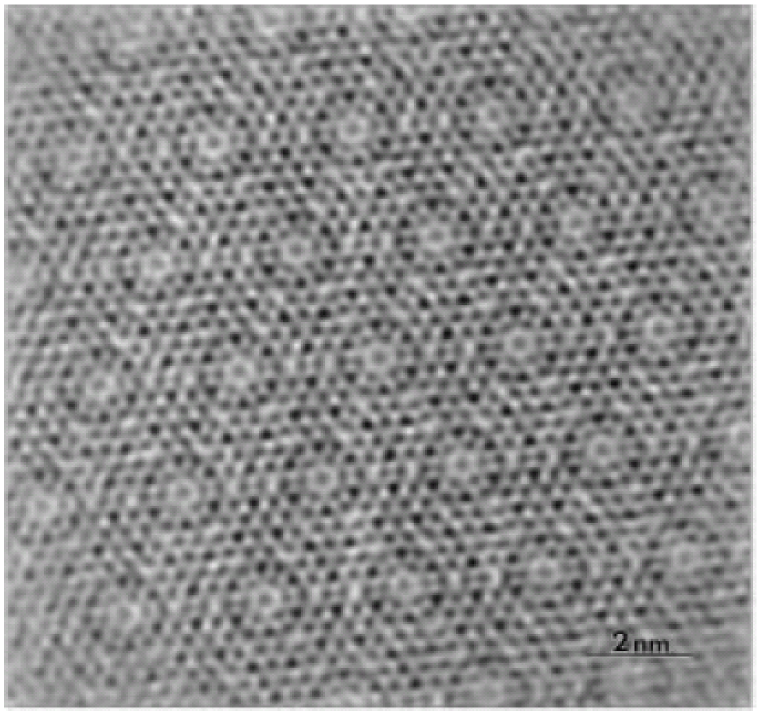


FIG. 1. A separated part of the image 1 from a 1024 × 1024 pixel region after rotational (threefold) averaging. Atoms are black, with slightly darker features at the adatom locations where two atoms are superimposed.

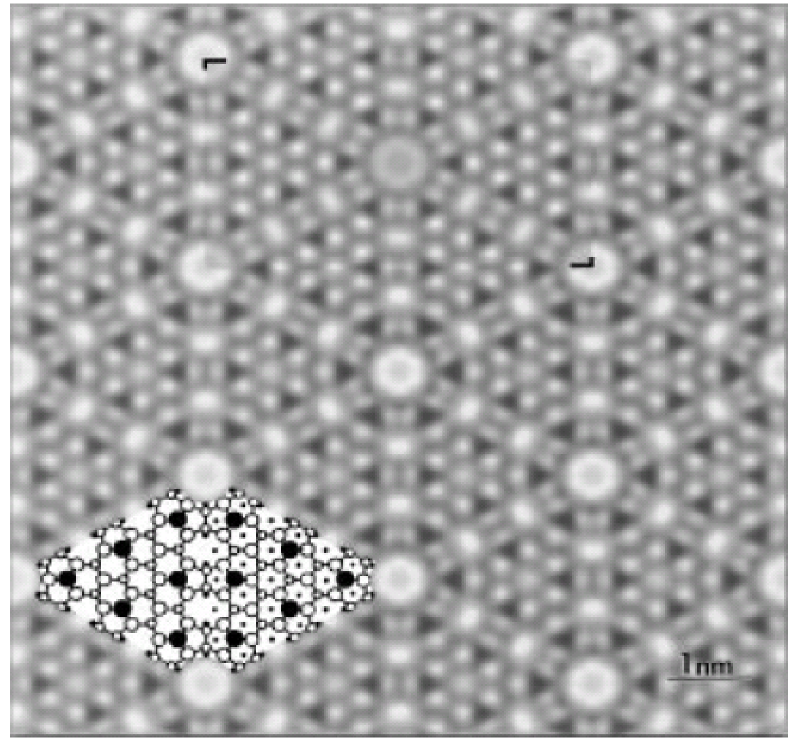


FIG. 2. A separated, rotationally (sixfold) and translationally averaged image from image 1. The inset is an image simulation for a defocus of −36 nm. Atoms are black, with slightly darker features at the adatom locations where two atoms are superimposed.

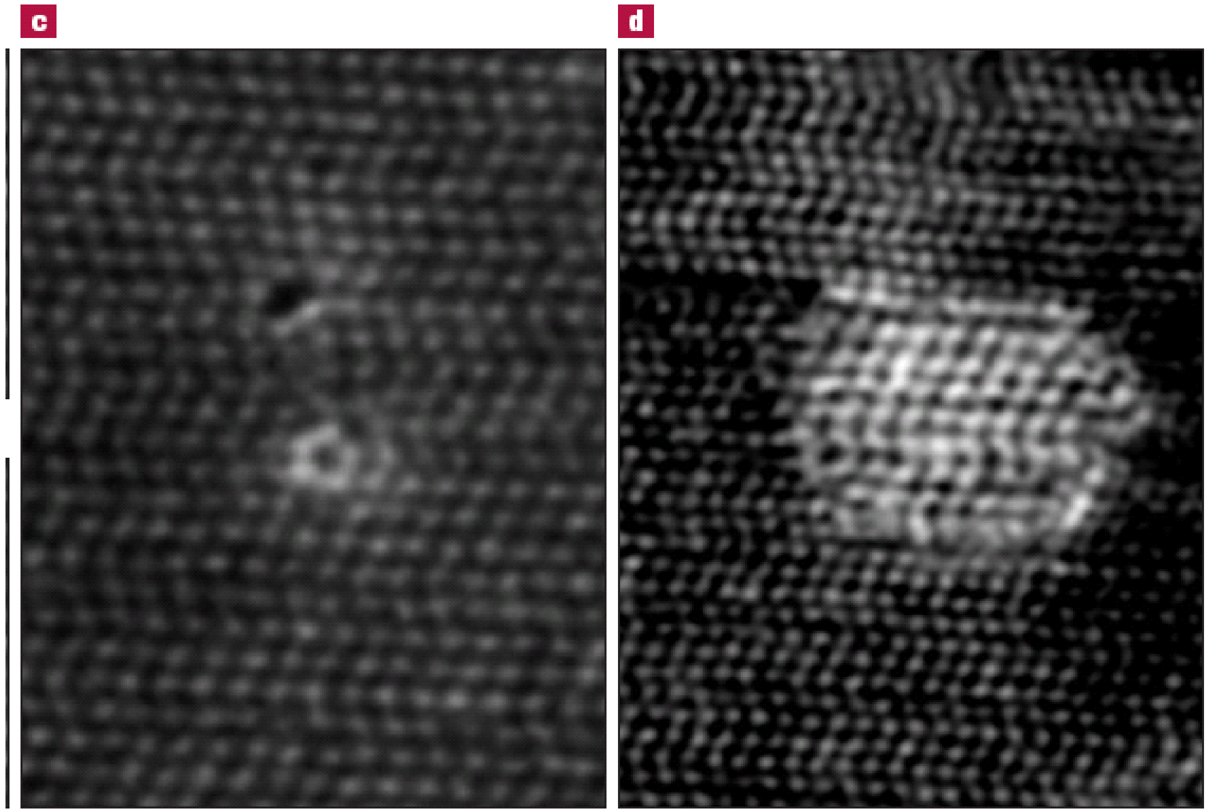
Imaging the Dimers in Si(111)-(7 × 7)

PHYSICAL REVIEW LETTERS

E. Bengu, R. Plass, and L. D. Marks

11 NOVEMBER 1996

Direct imaging of atoms / defects



Not only periodicity but actual individual atoms or defects can be imaged

Direct observation of defect-mediated cluster nucleation

U. KAISER*[1], D. A. MULLER[2], J. L. GRAZUL[2], A. CHUVILIN[3] AND M. KAWASAKI[4]

Tomography

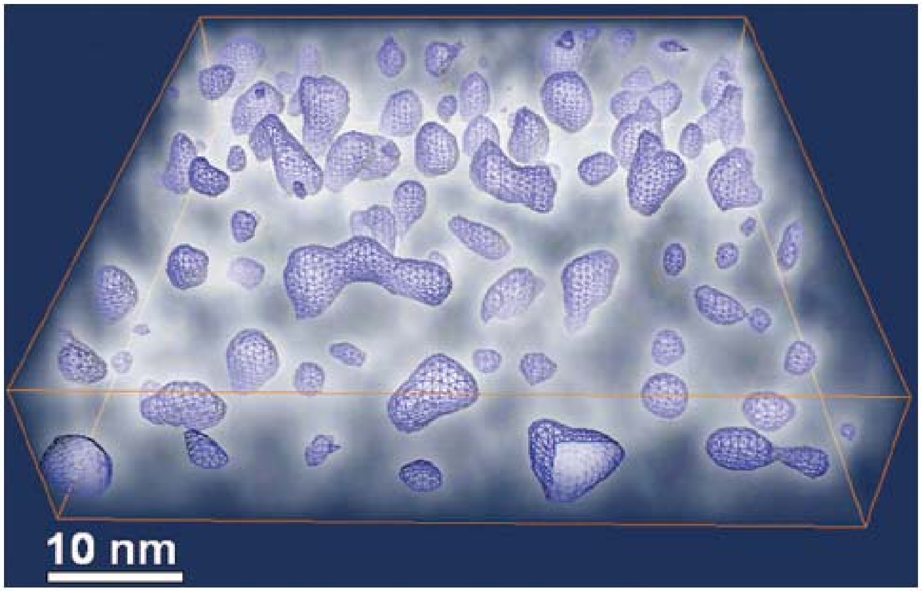


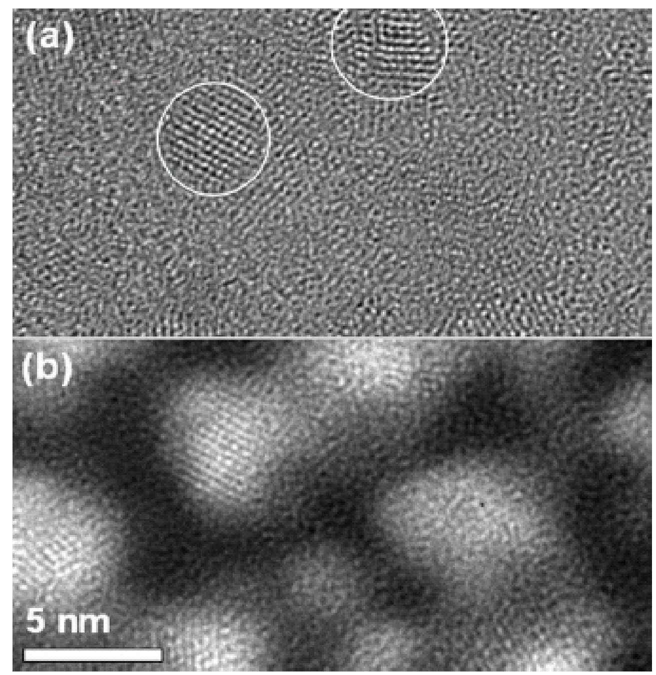
FIG. 3. (Color) Tomographic reconstruction from a series of plasmon loss images recorded at different sample tilts on the Tecnai F20. Nanoparticles are visualized by isosurface rendering at fixed threshold (blue shapes), while the actual reconstructed plasmon loss signal at 17 eV is visualized by volume rendering (white "fog"). Complex, nonspherical morphologies are dominant.

APPLIED PHYSICS LETTERS **89**, 151920 (2006)

Three-dimensional imaging of nonspherical silicon nanoparticles embedded in silicon oxide by plasmon tomography

Aycan Yurtsever,[a] Matthew Weyland, and David A. Muller
School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14850

- Through rotations of the sample, and focus series, multiple images can be used to reconstruct a 3D image



Energy Contrast

- Energy loss due to characteristic electronic properties of material under the beam
  - Zero-loss
  - Plasmonic
  - Core excitations

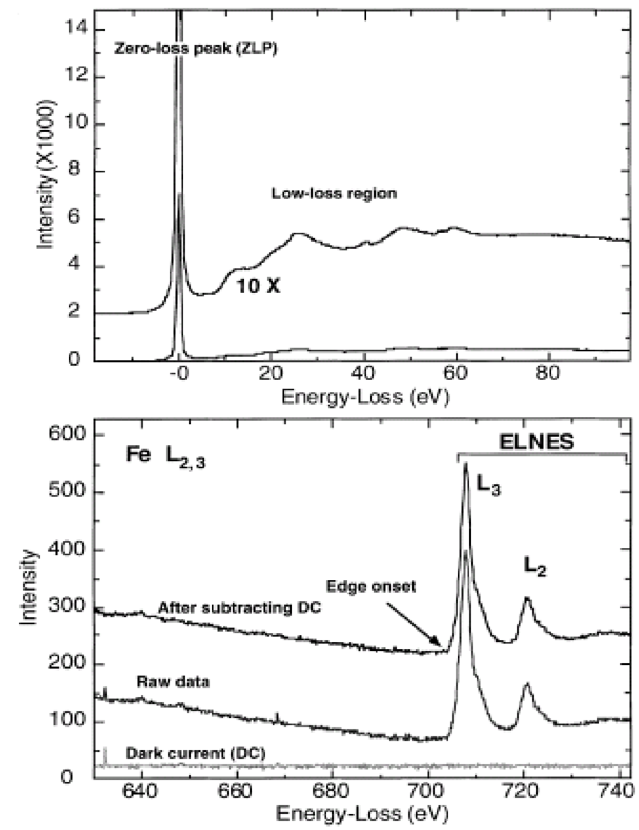
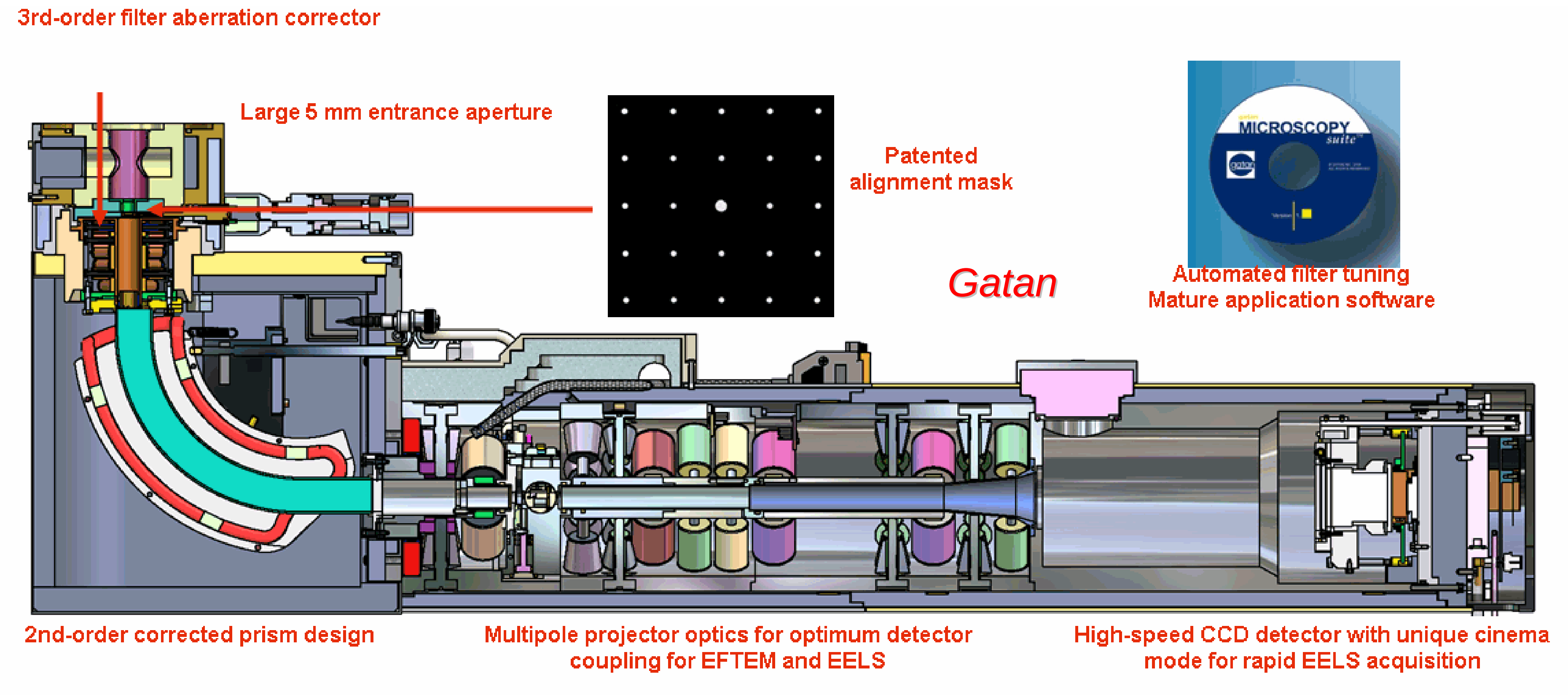


Fig. 1. EELS spectra showing zero-loss peak (ZLP) and low-loss region (upper) and a core-loss edge (lower) from a mineral ilmenite ($FeTiO_3$).

Energy filters

- Gatan Image Filter (GIF)
  - Post filtering below the fluorescent screen
- Zeiss Omega filter
  - Filtered before fluorescent screen



Energy filters

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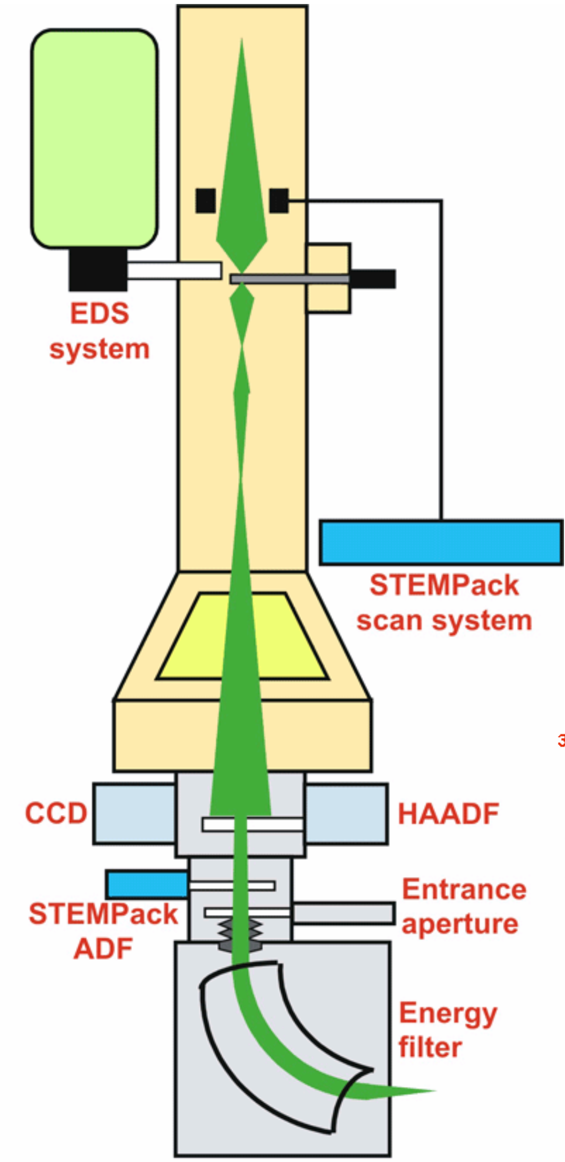
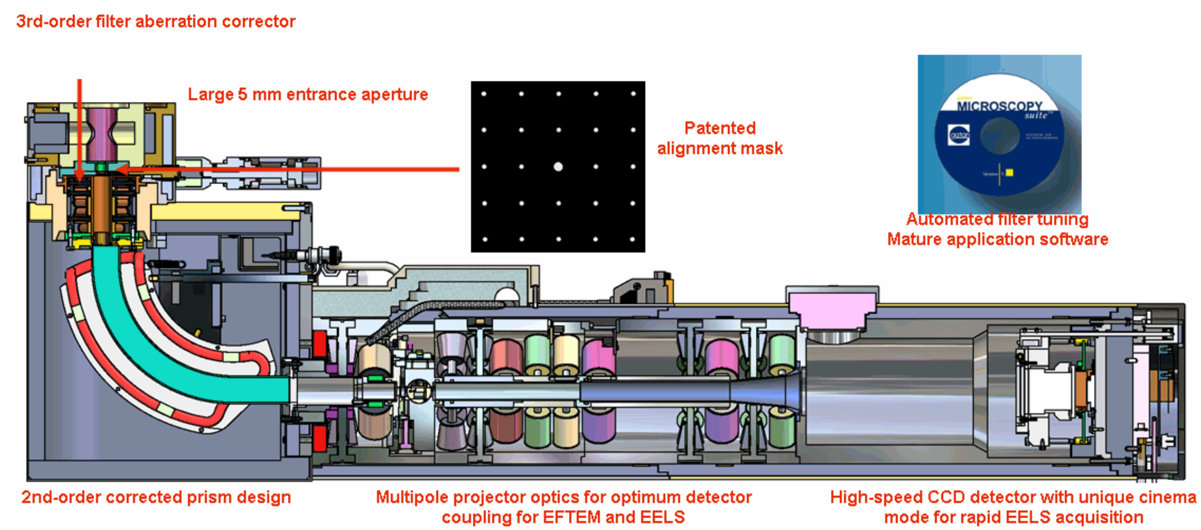
  - Post filtering below the fluorescent screen
- Zeiss Omega filter
  - Filtered before fluorescent screen





Energy filters

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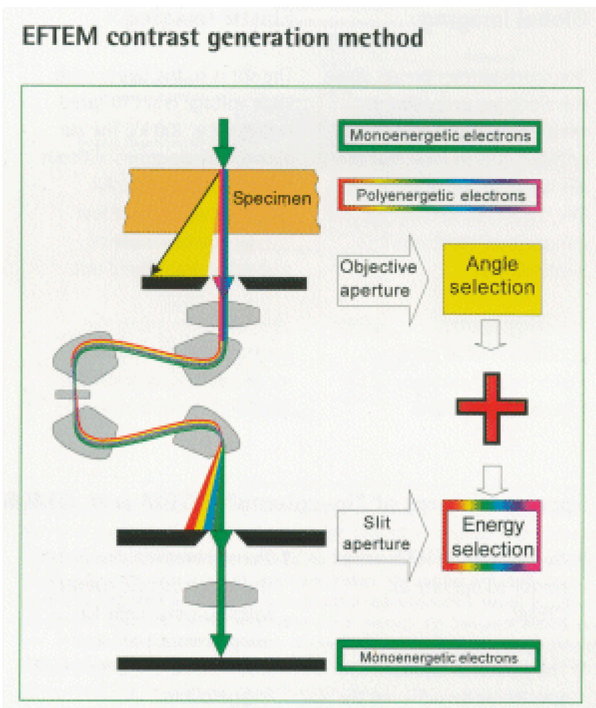
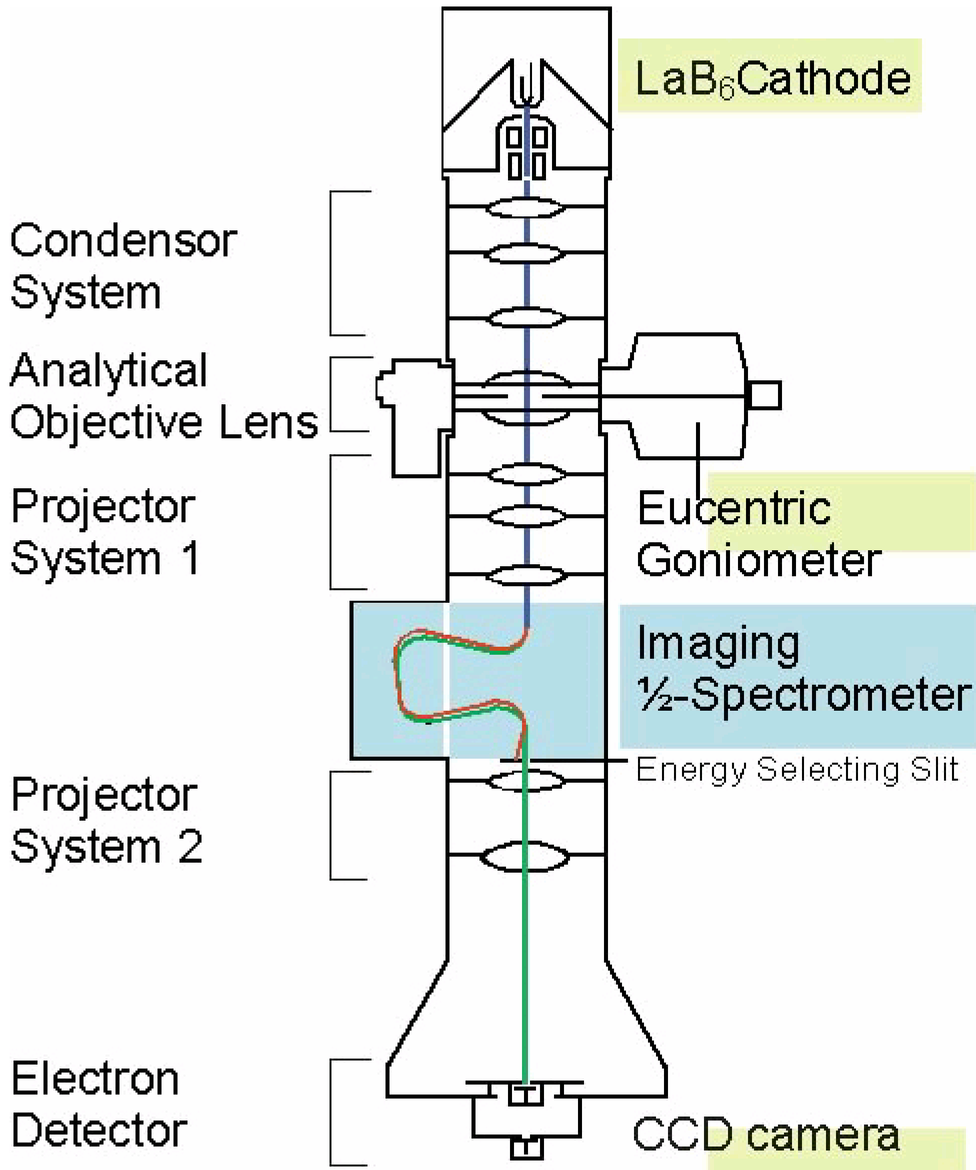
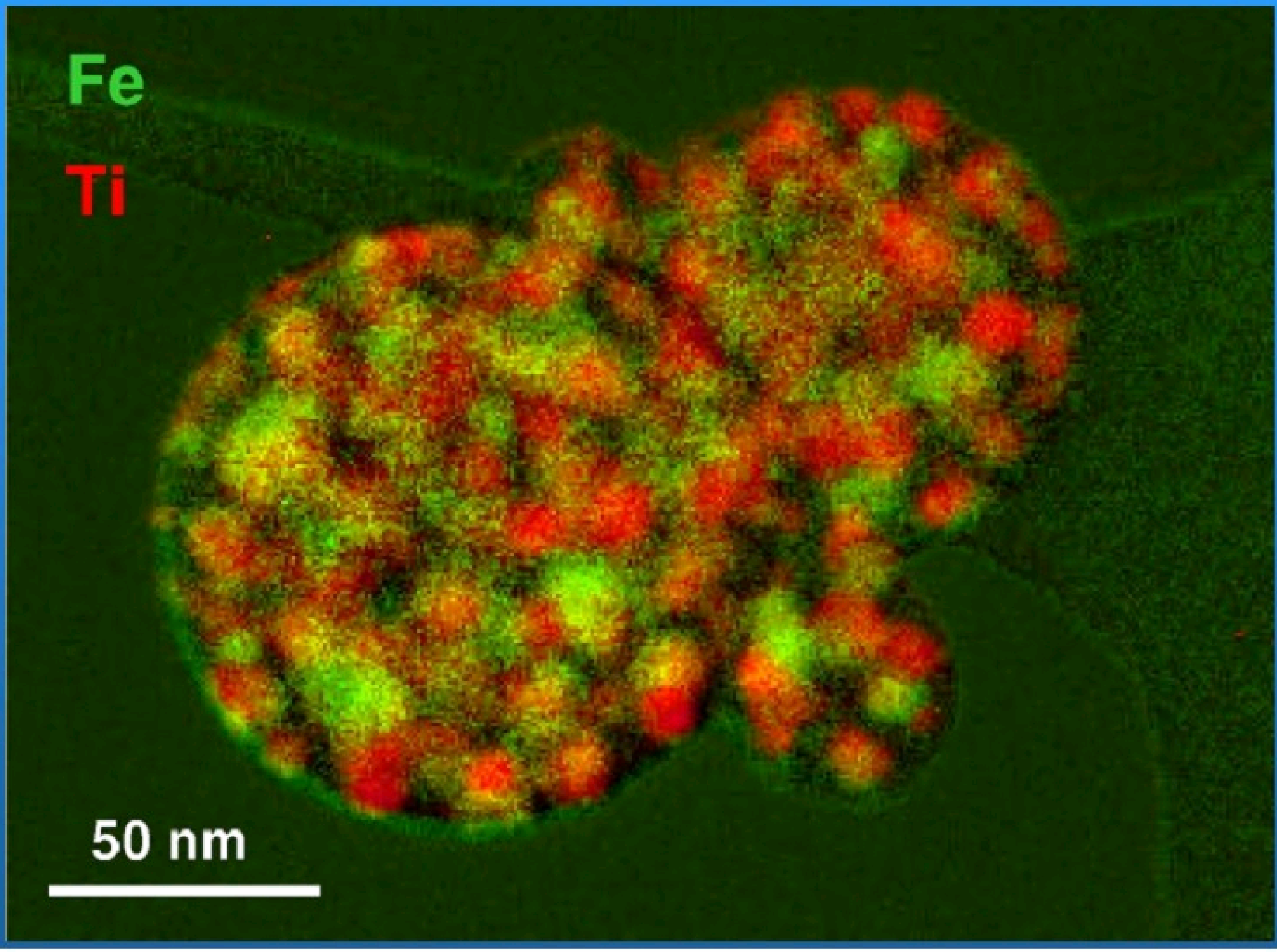
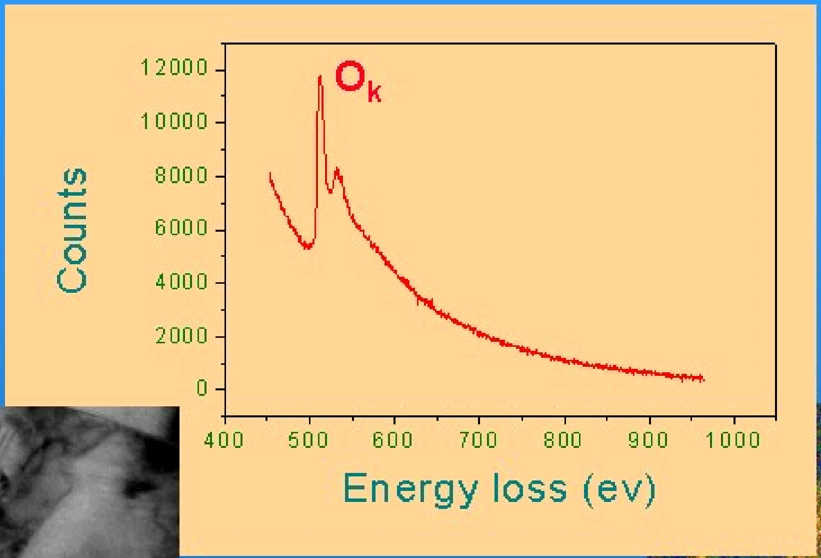


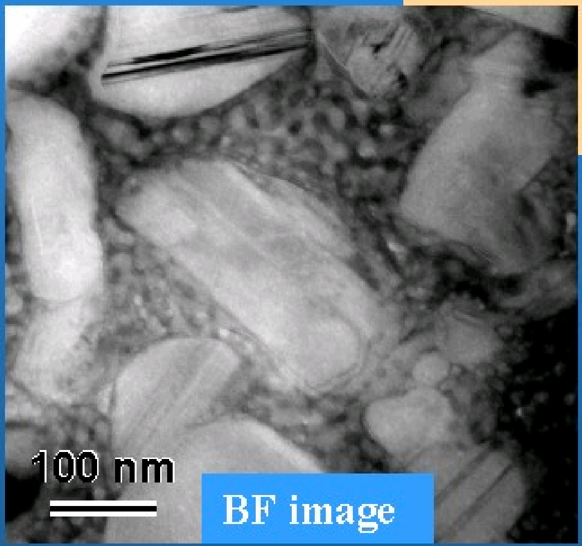


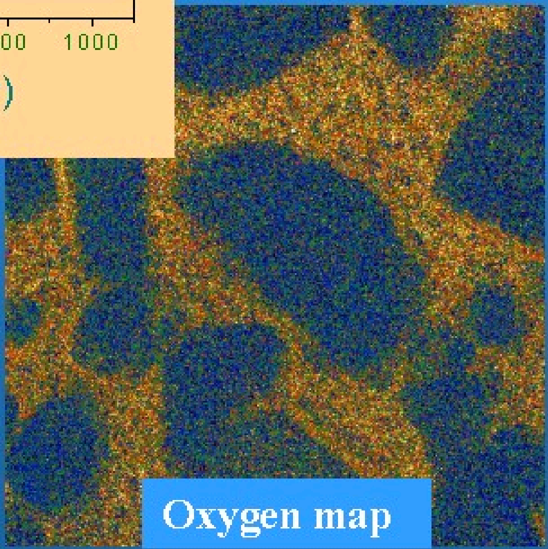
Phase Segregation by EFTEM



Mapping Elements – EFTEM



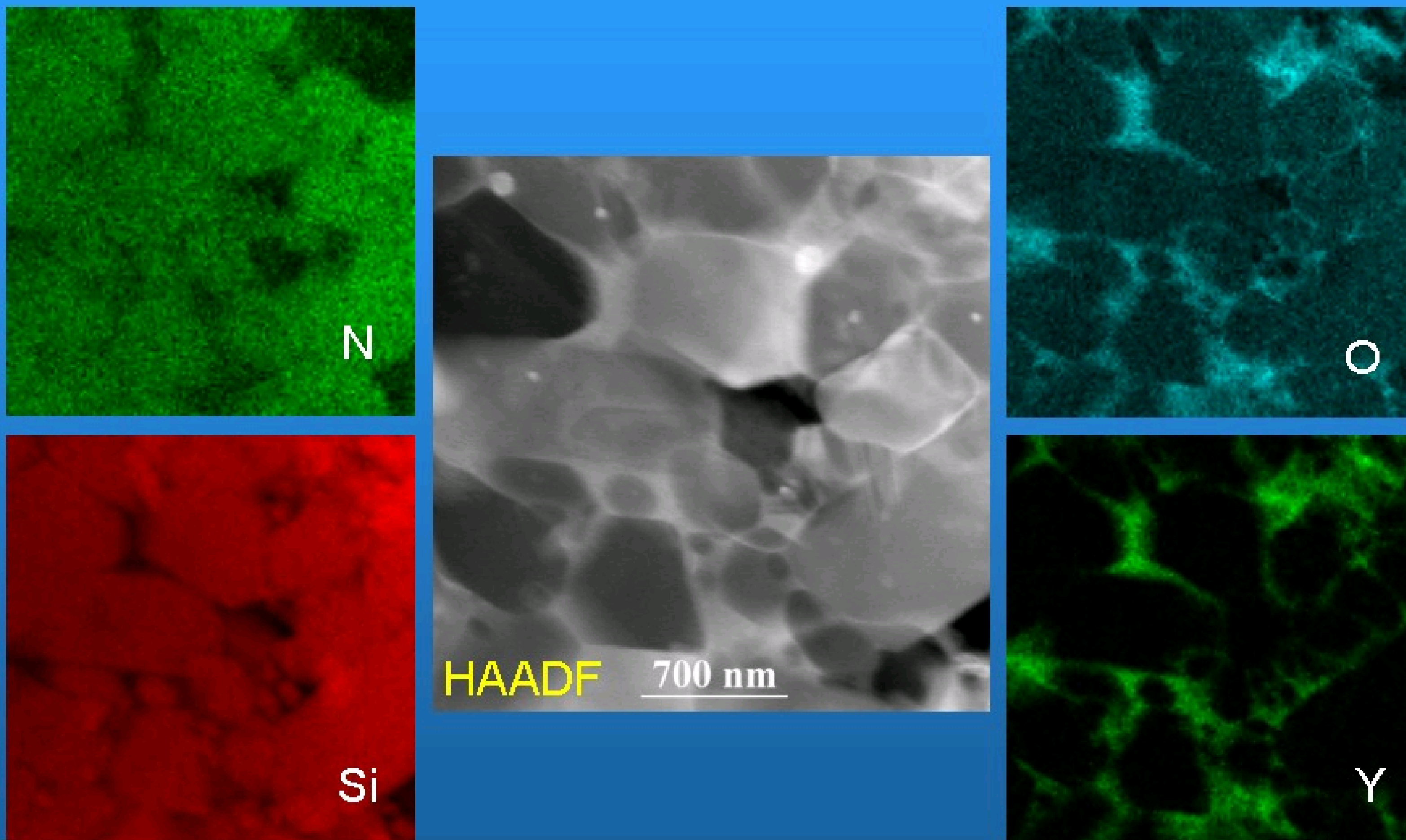




Alternative Analytical Tools to EFTEM

- X-Rays!
- EDX can be fitted on to a TEM
- TEM is operated in a scanning fashion (STEM) and at each point the X-ray spectrum is recorded to construct an image of elements
- STEM provides better resolution because the sample is already very thin
- Damage to the sample is possible at high energy and high current density

Mapping Elements – EDS

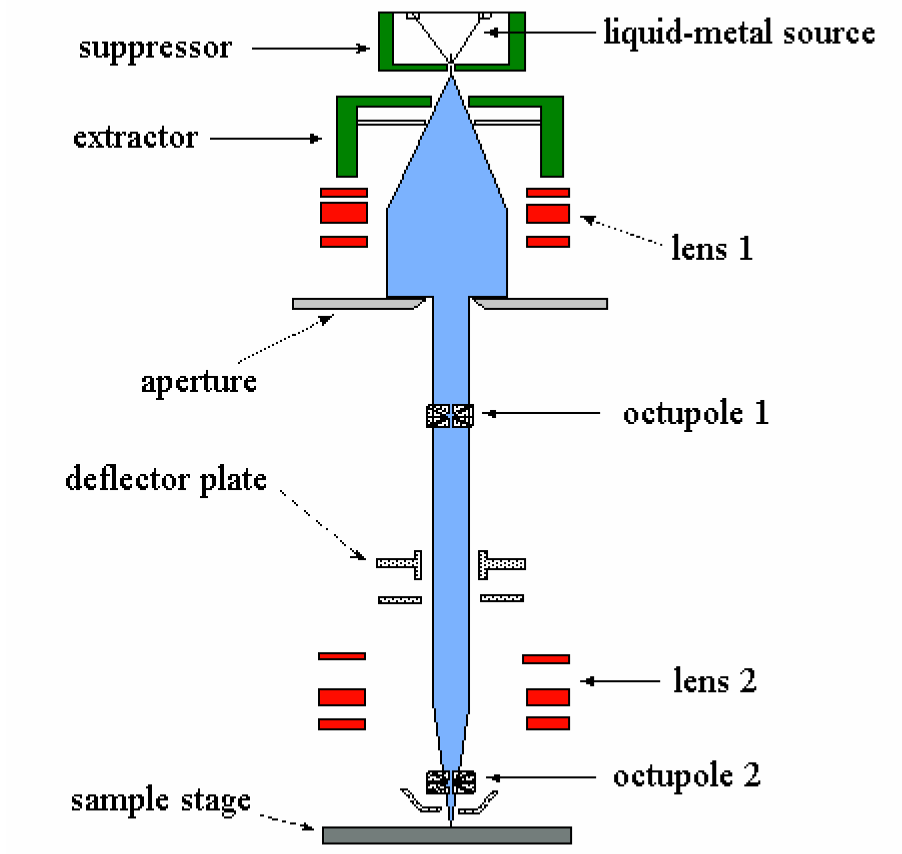


TEM Sample Preparation

- Ultrasonic disk cutters
- Abrasive Dimplers
- Argon ion gun thinners
- Plasma cleaners (to clean thin lamella surface)
- Time consuming process, handwork

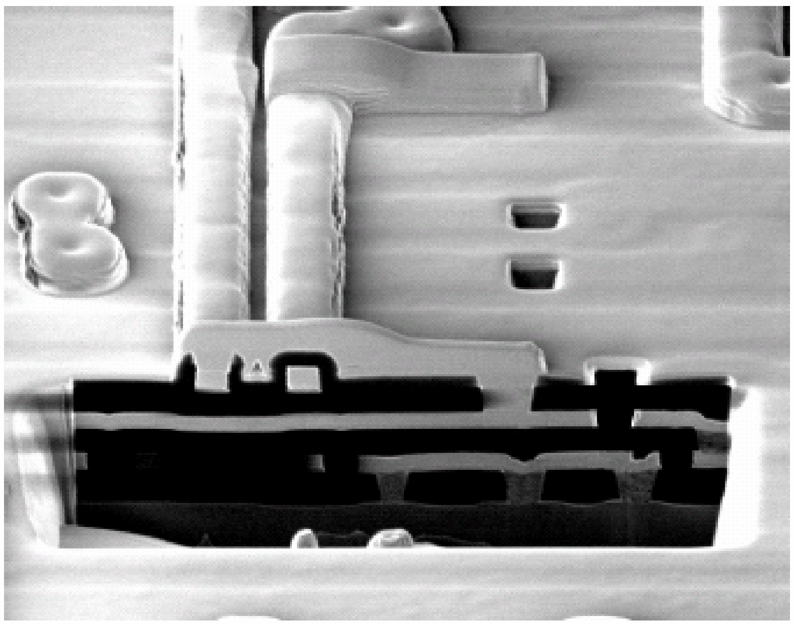
FIB (Focused Ion Beam)

- Ga ions (heavy nuclei) melt at low temperature
- Can be used to mill (etch by impact) with 20 nm resolution
- Works like an SEM



FIB cutting

- **Cross Sectioning & Microsurgery**



Can be used to image cross sections in a Ion/Electron dual beam system

Added analytical tools (EDX) can be used to generate 3D elemental maps

FIB TEM sample prep

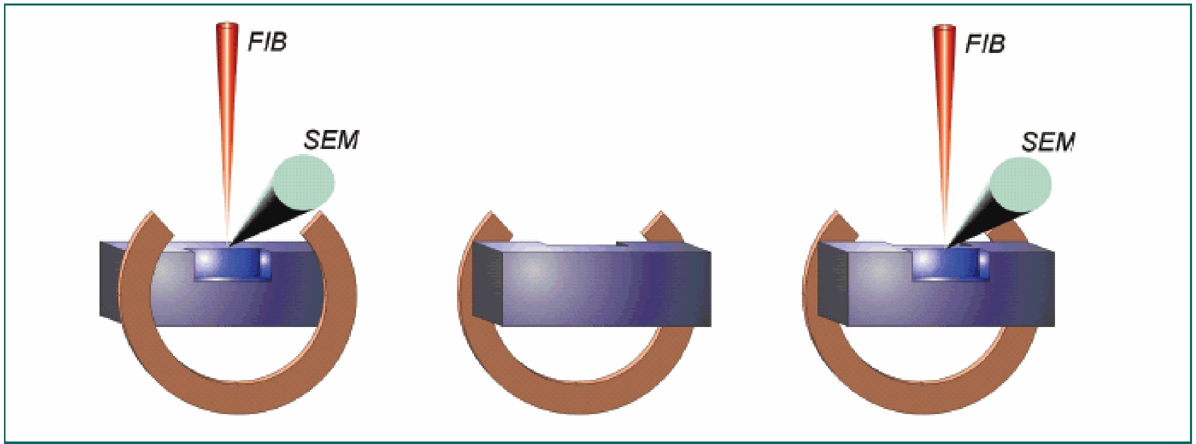


Figure 4. Steps for a pre-thinned TEM sample preparation using the CrossBeam technology.

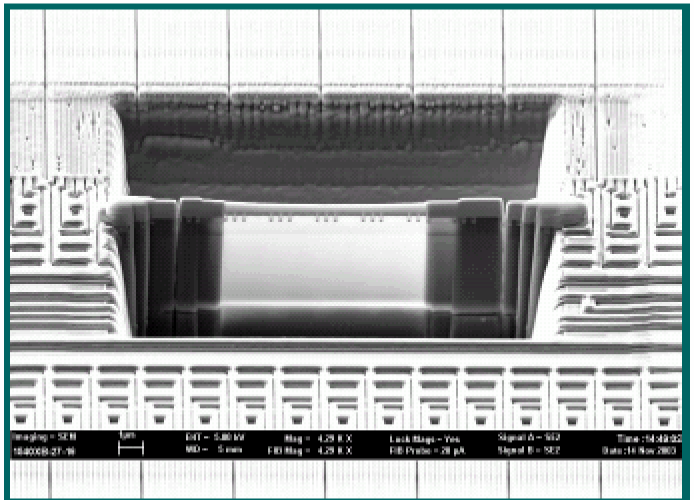


Figure 7. TEM lift-out sample after milling and polishing. The sample is cut out of the substrate and is ready for lift-out. Note the electron transparency of the thin area.

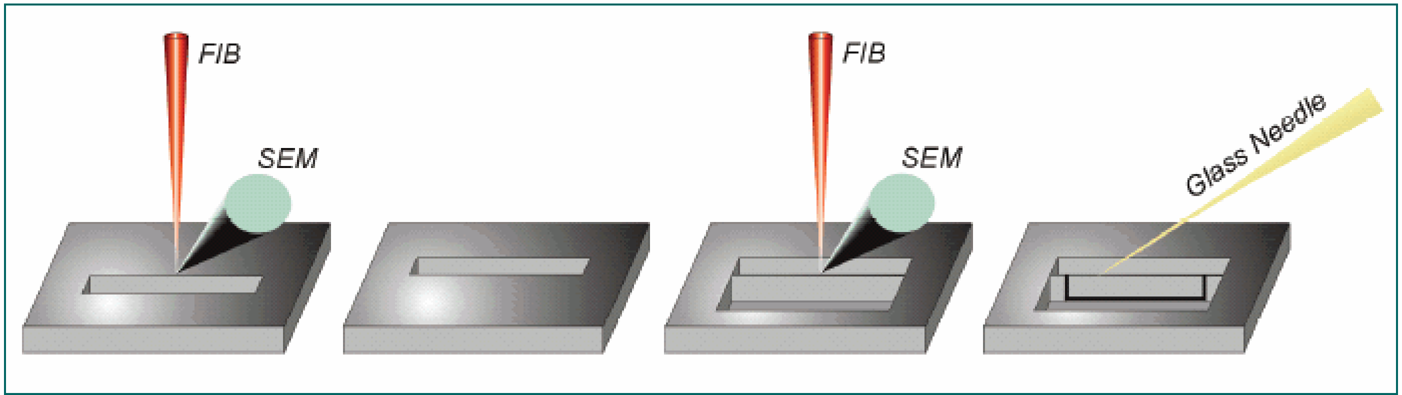


Figure 6. TEM lift-out sample preparation using the CrossBeam technology. After the final polish the lamella is cut out of the substrate by three cuts and is transferred to a TEM grid by use of a micromanipulator and a glass needle.

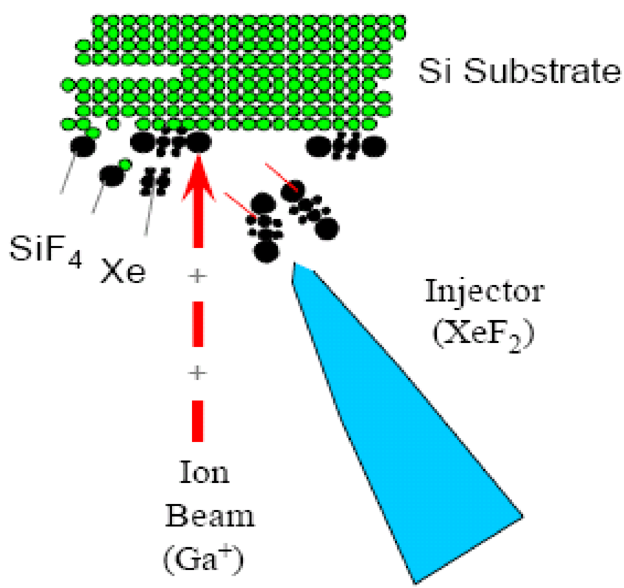
Much Easier and Faster automated process

F IB/EB assisted etching/deposition

- Gas injectors provide reactive gases

Gas-Assisted Etching of Si:

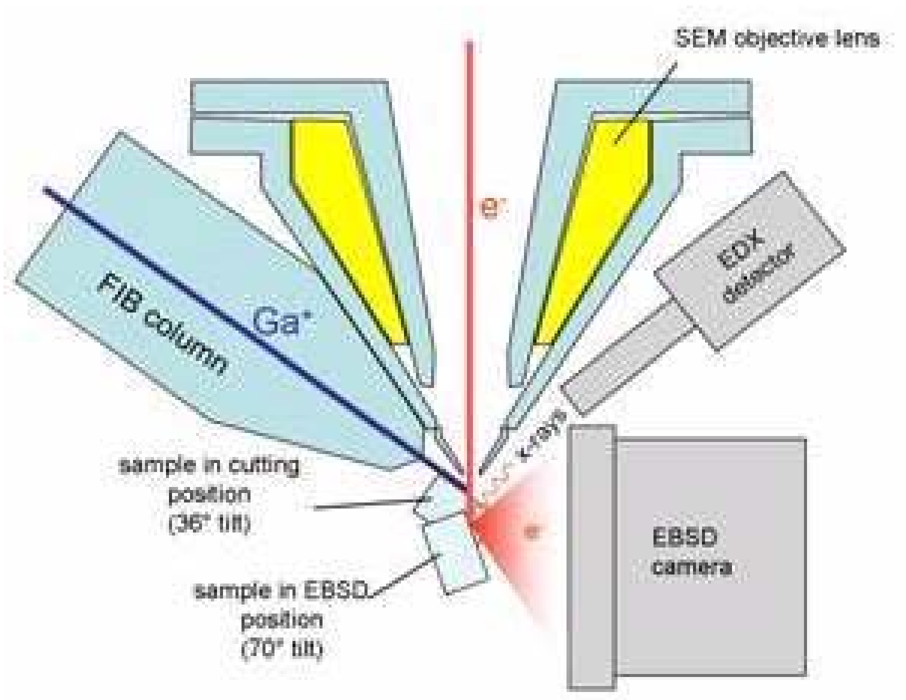
Si + 2 XeF2 -> SiF4 + 2 Xe

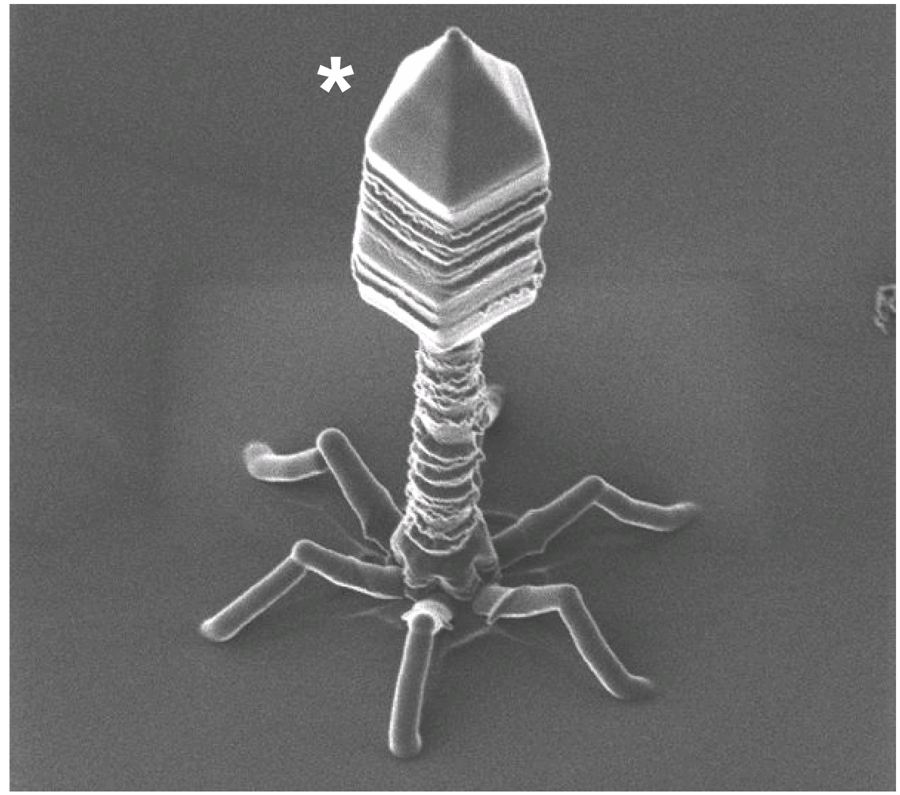


Can be used to deposit:
Carbon, Gold, Platinum etc.

Can be used to etch surfaces

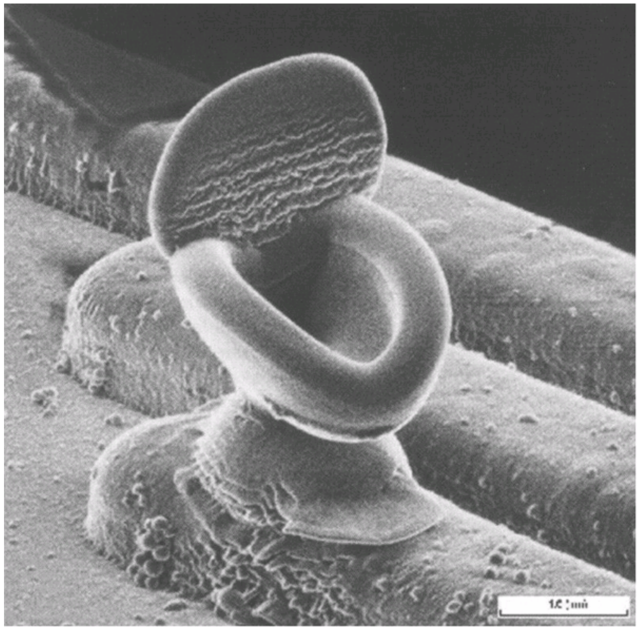
F IB/EB assisted etching/deposition

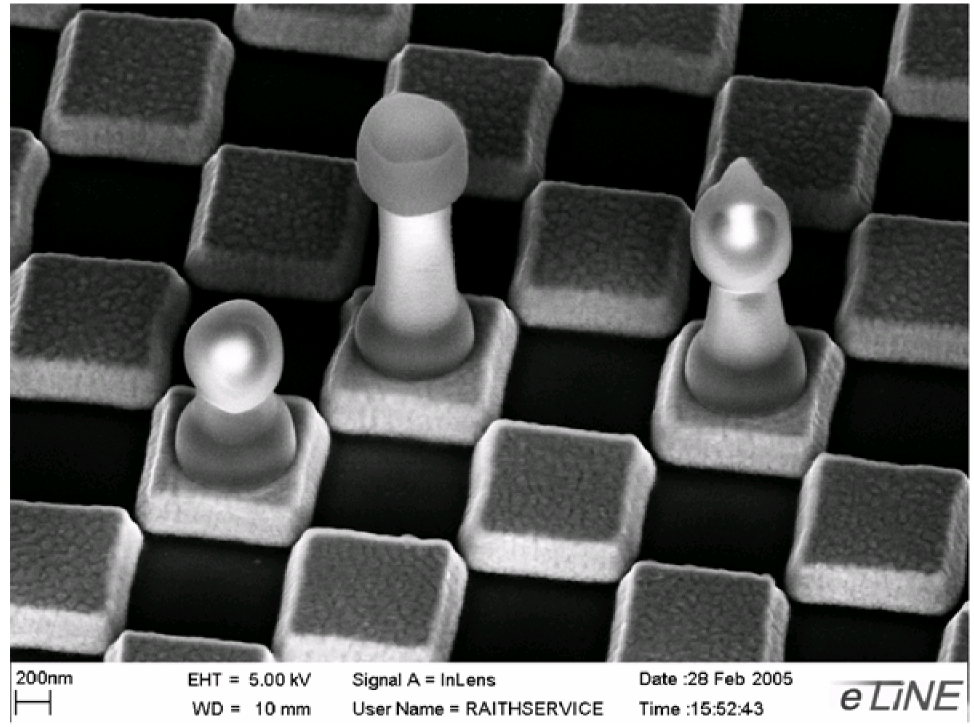


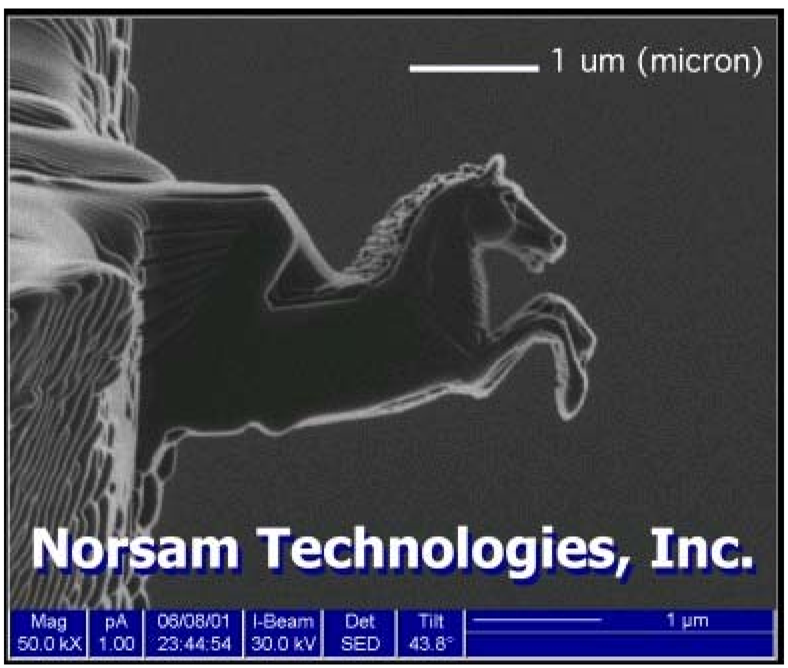


3D structures

* .. not a real virus!







Demonstrating FIB and EBID

(Scientists with a lot of free time)