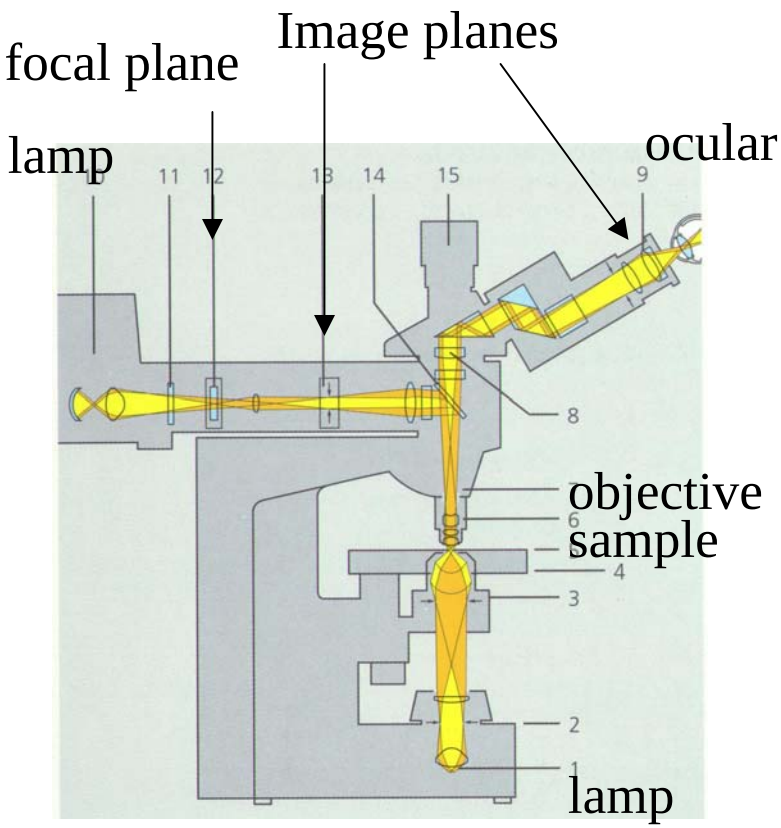
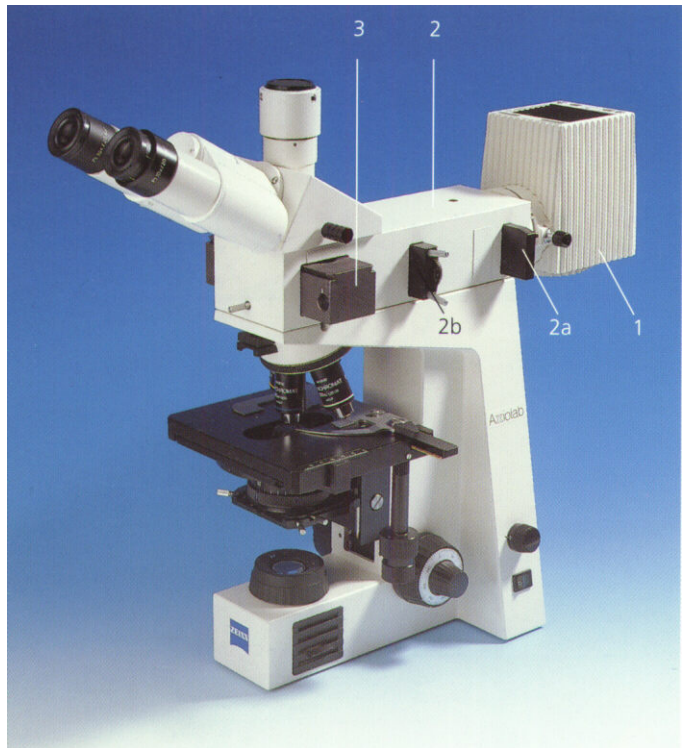
How is working a microscope





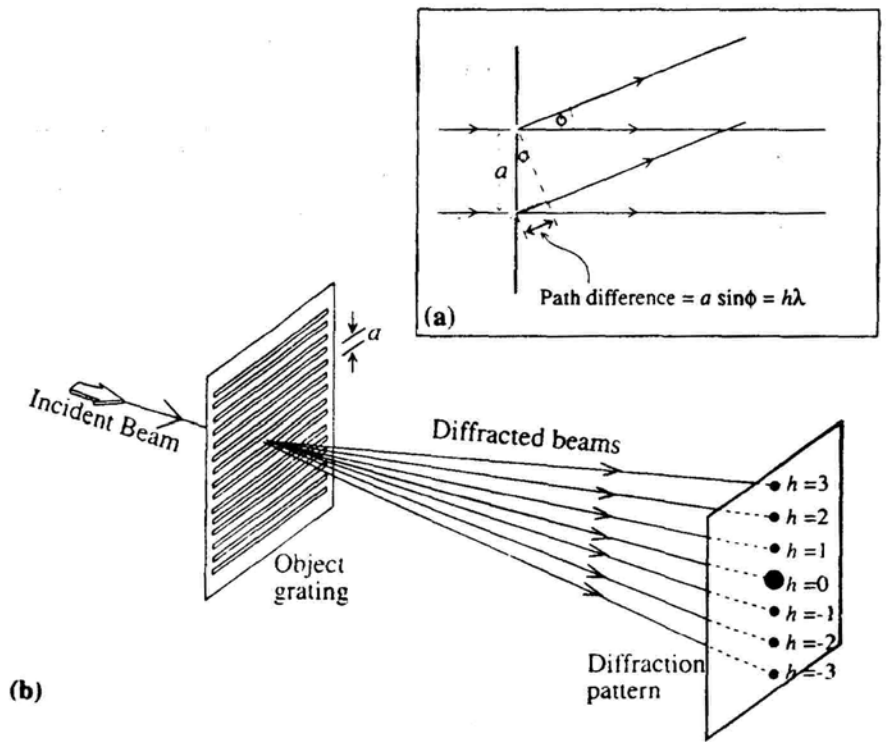
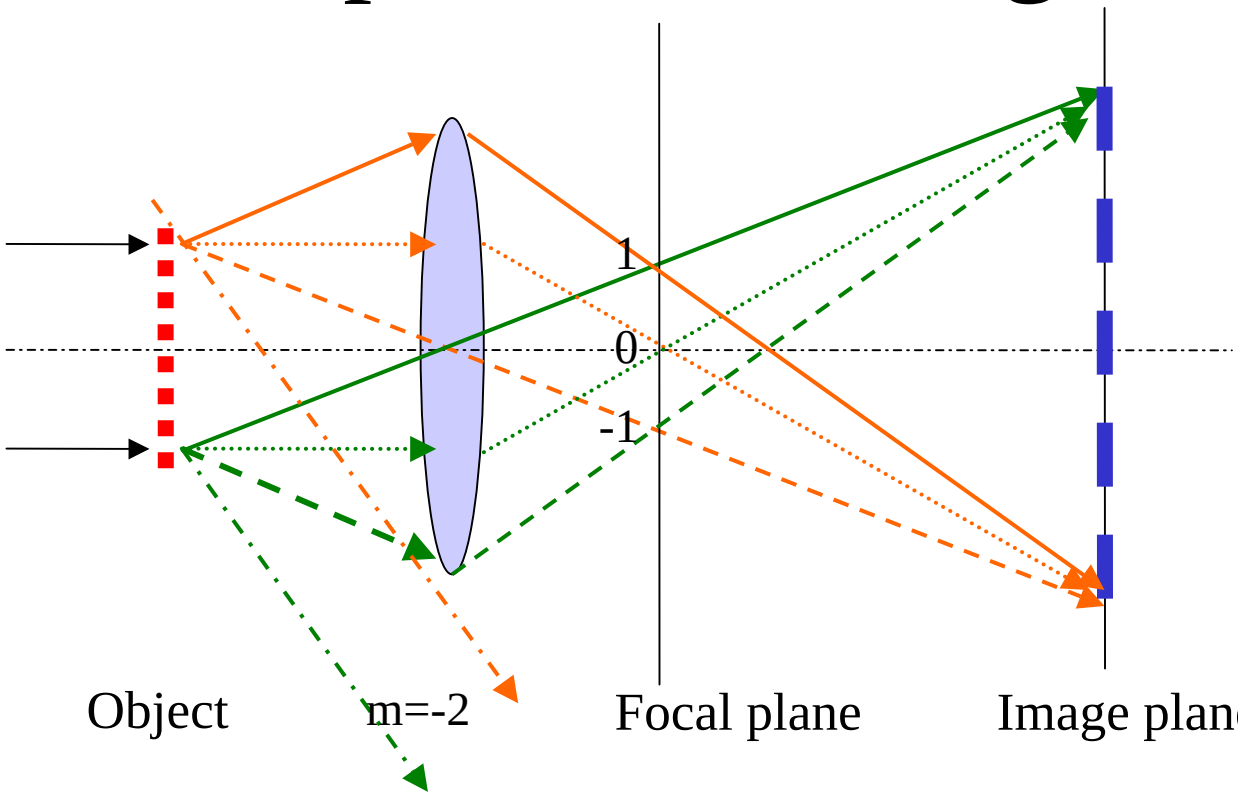
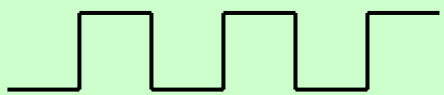


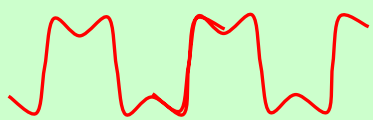
Figure 3.2. The formation of an optical diffraction pattern from a one-dimensional grating illuminated by a laser beam. (a) The condition for constructive interference producing a diffraction maximum, (b) Schematic of the diffraction experiment and the formation of a row of diffraction spots.

Focal plane and image

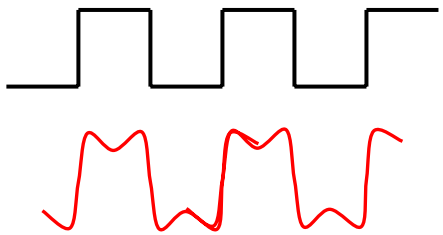




The lack of some components produces an image distorsion

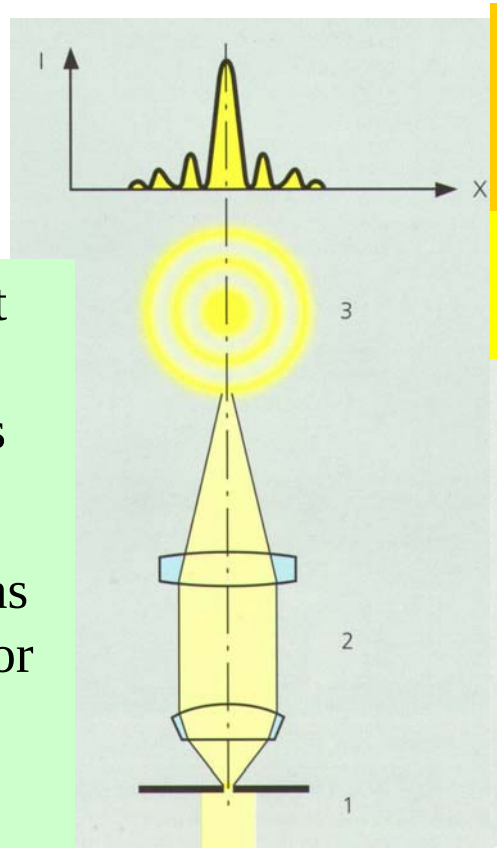


Spatial resolution and diffraction



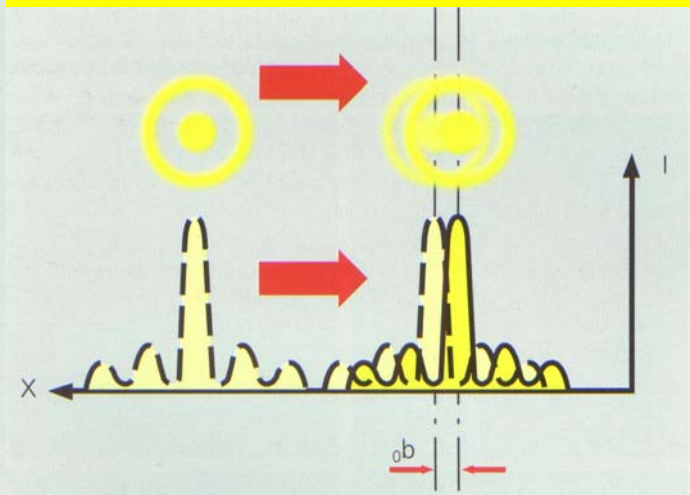
The diffraction of light limits the capability of reproducing the details of an image.

Resolution is defined as the smallest distance for which two different features can be distinguished.



Nunerical Aperture = $n\sin\alpha$ where α is half the opening angle of the objective

Diffraction limit due to the resolution $d_o = 1.22*\lambda / 2*NA$

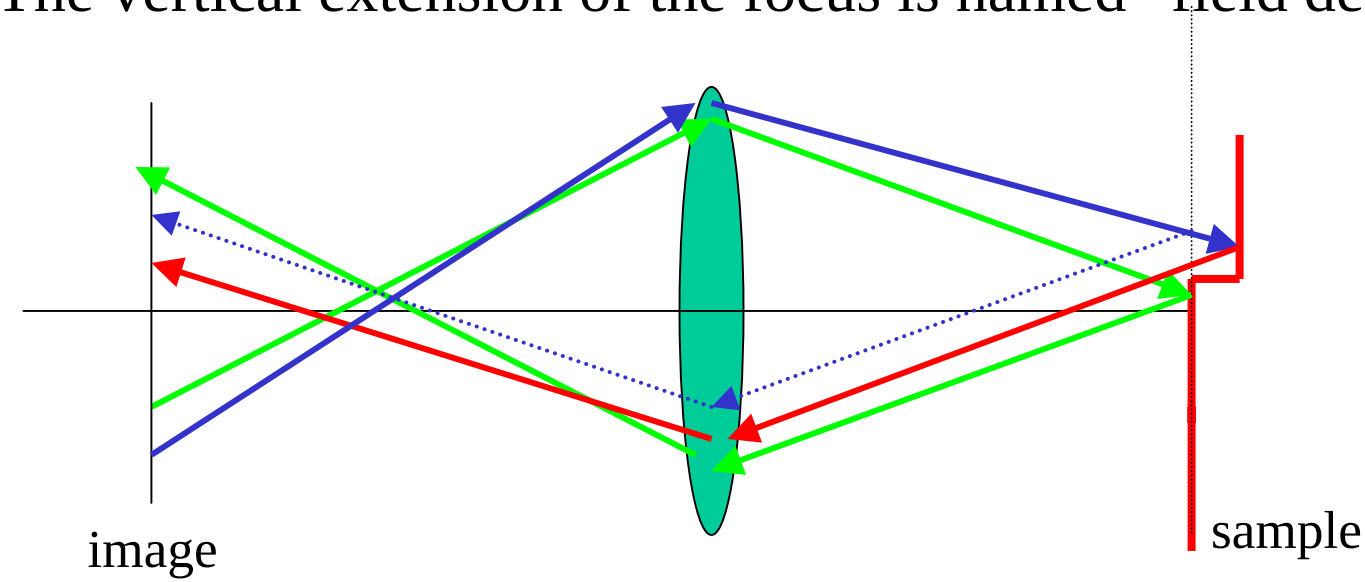


Field view and field deep

The area of sample viewed by the ocular tube is called fiel of view.

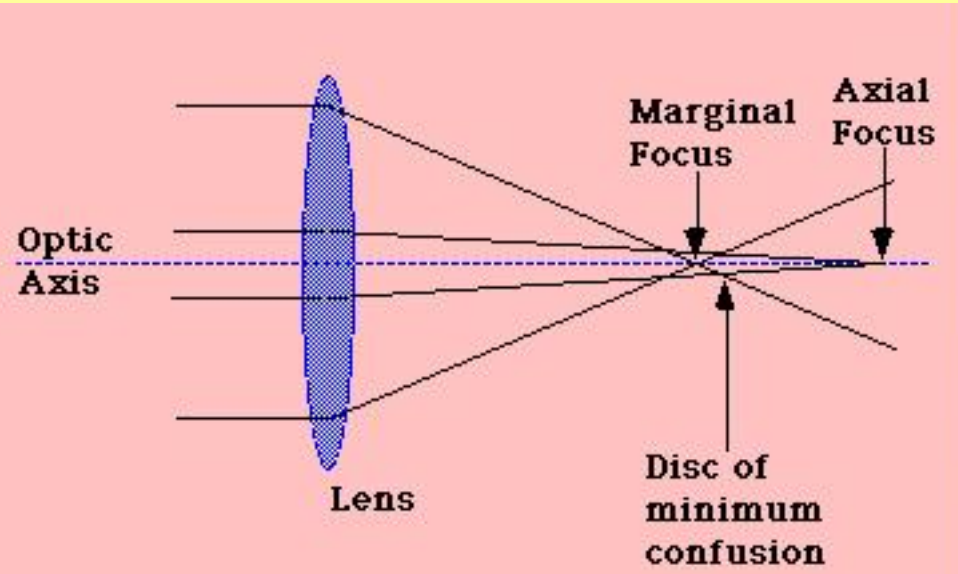
Anyway, not only the lateral resolution is important, but also the vertical features affect the image.

The vertical extension of the focus is named “field deep”

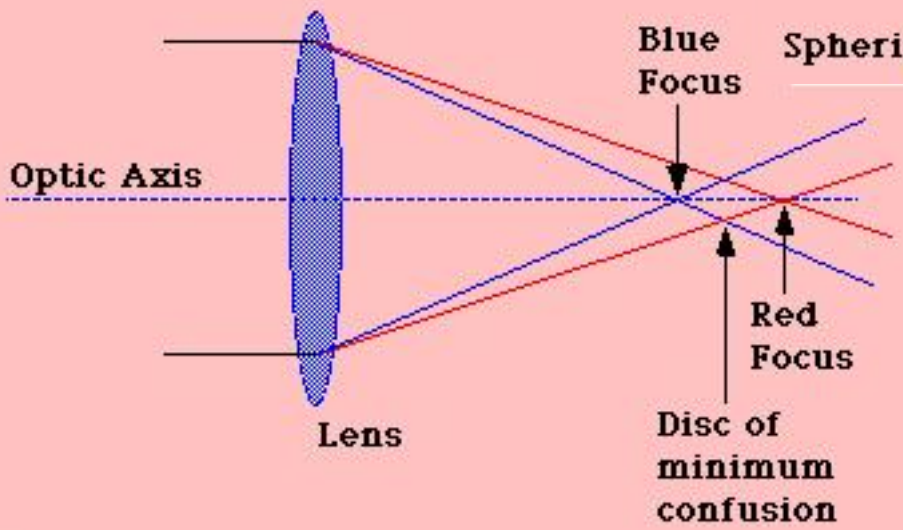


Resolution Limited by Lens Aberrations

Chromatic aberration is caused by the variation of the photon / electron energy and thus photon/electrons are not monochromatic.



Spherical Aberration → **point is imaged as a disk.**



Chromatic Aberration → **point is imaged as a disk.**

Spherical aberration is caused by the lens field acting inhomogeneously on the off-axis rays.

$$r_{min} \approx 0.91 (C_s \lambda^3)^{1/4}$$

Practical resolution of microscope.

C_s–coefficient of spherical aberration of lens (~mm)

Contrast: principles and methods

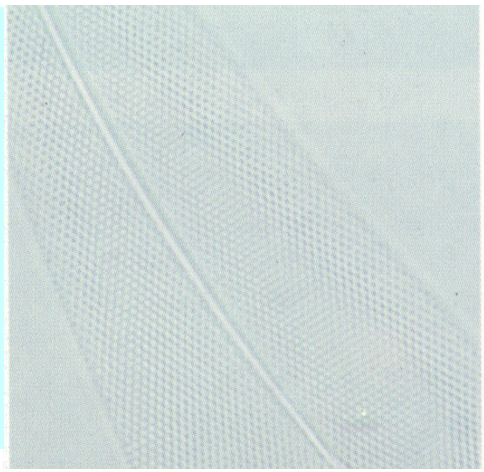
The quality of an image depends on how the interesting details can be evidenced from the backgroung signal. The way to do this is called “contrast” and depends on what kind of features we are looking for.

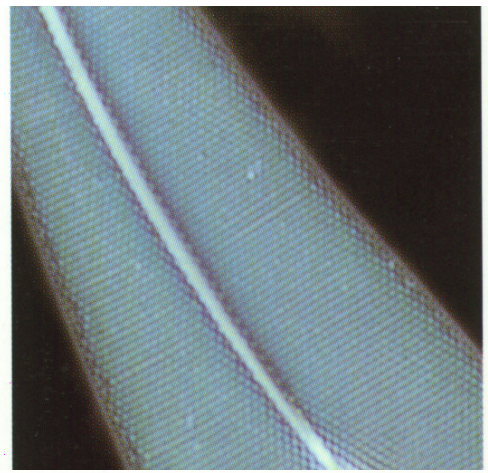
Several methods of contrast exist; the most used are:

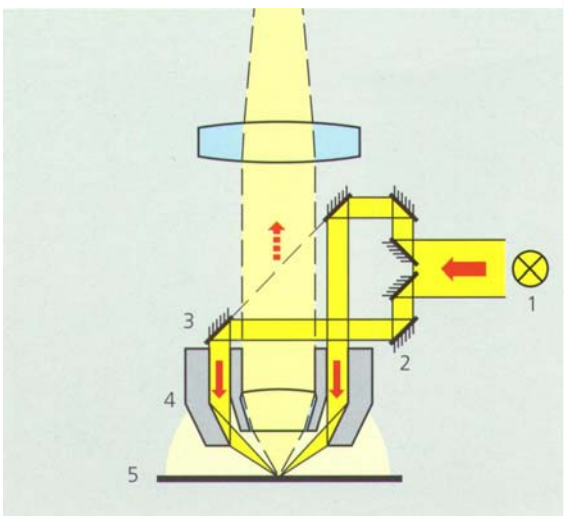
- Bright field
- Dark field
- Polarization contrast
- Phase contrast or also Differential Interference Contrast
- Fluorescence or Luminescence

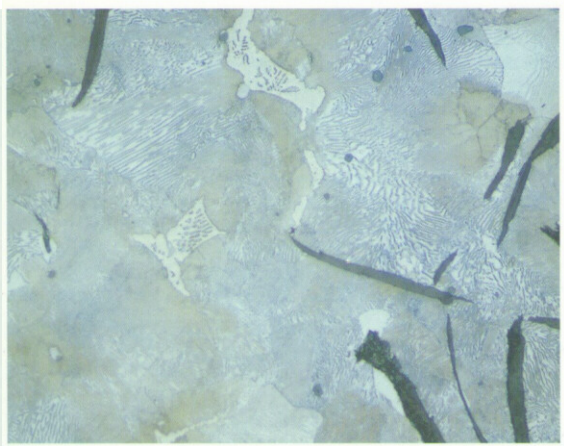
Bright- and dark-field

In some cases it is better to take only the light diffused by the sample (dark field) instead that the light directly illuminating the sample (bright field)

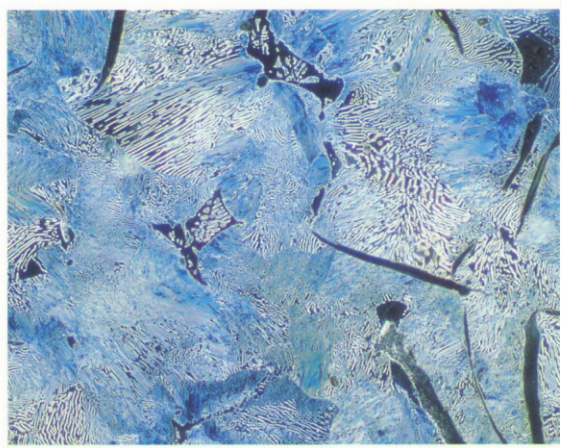






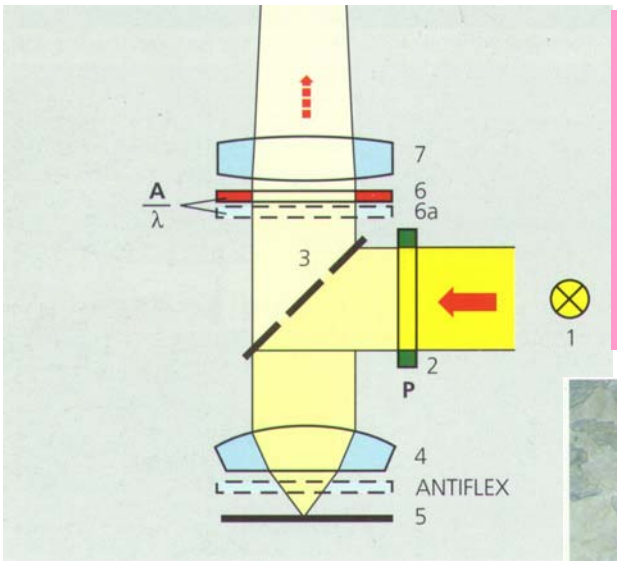


29.1
In reflected-light brightfield, the fine structures in the iron casting are difficult to see.



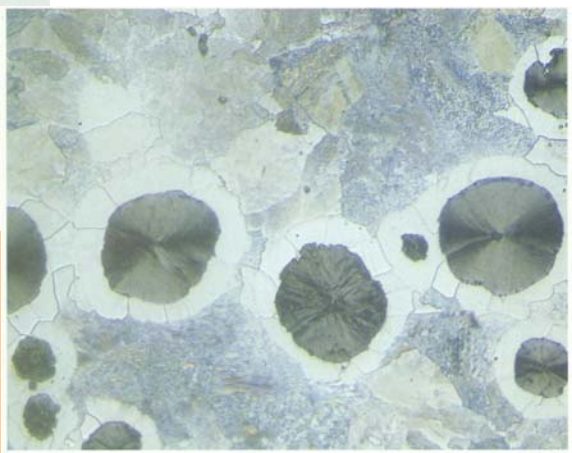
29.2
Only in reflected-light darkfield they are clearly visible in the surface.

Polarization

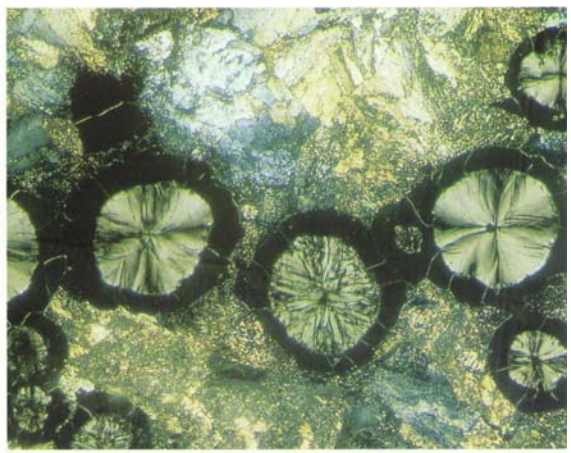


The use of two polarizer, one on the incoming beam (P), the other at the exit towards ocular (A), allows the identification of polarizing (either geometrically or magnetically) regions of the sample

Sample formed by iron spherilites ⇒
Bright field (left)
Polarized light (right)



29.3
The inner structure of these spherulites is barely visible in reflected-light brightfield

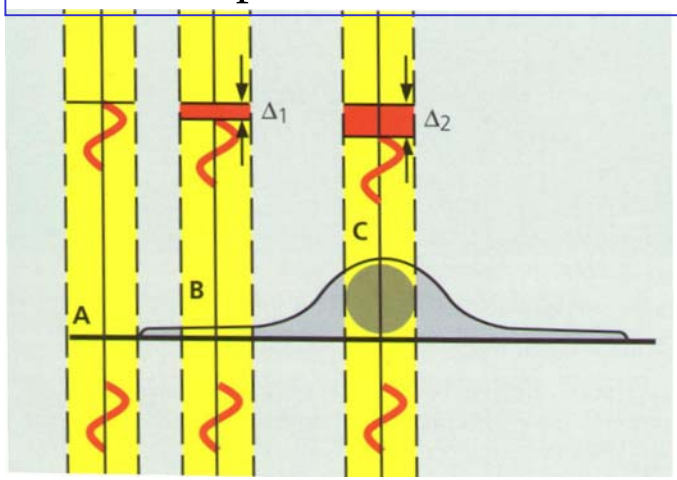


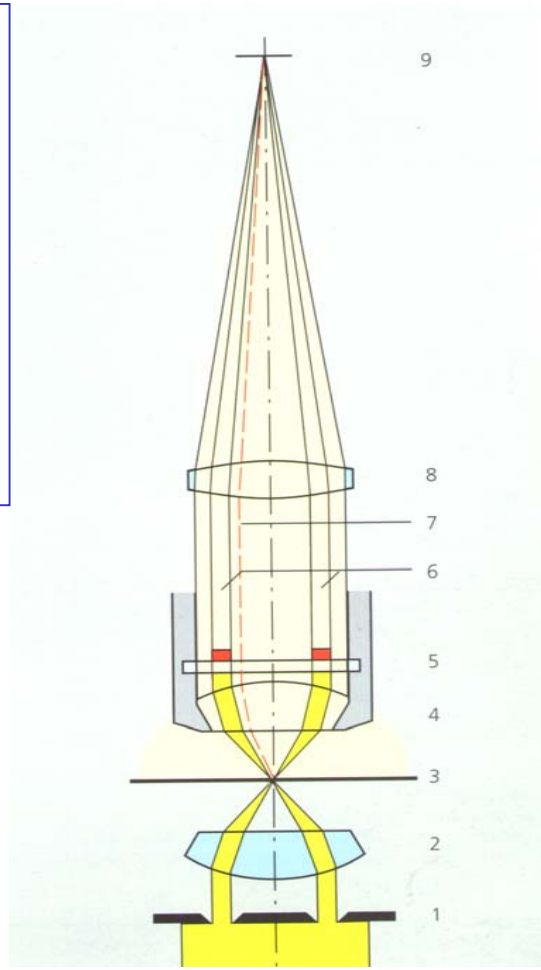
29.4
The structure in the polished iron sample becomes clearly visible in polarization contrast.

Phase contrast

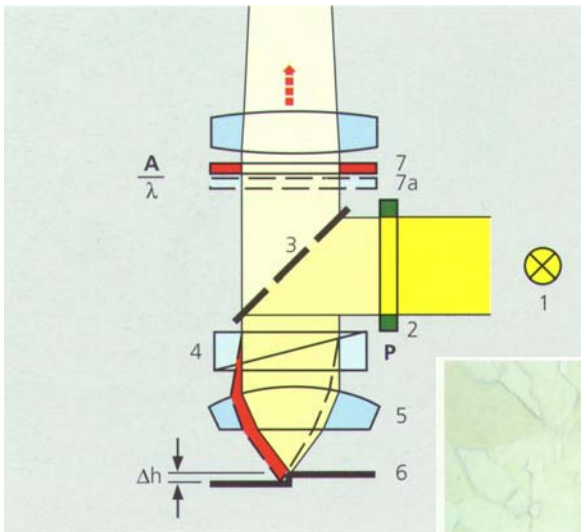
In phase contrast one providing a measure of the phase shift introduced by the sample. This imply to become extremely sensitive to thicknesses and/or changes in the refractive index (in transmission measurements).

A further phase sensitive method is DIC



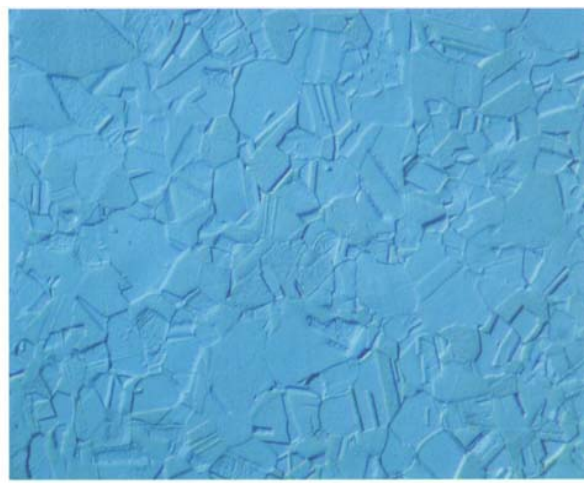


Differential Interpherence Contrast (DIC)



A beam of polarized light is splitted by a birifrangent prism in to two slightly displaced beams. Any path difference (typically produced by e.g. a step on the surface) causes a polarization change in the beam at the exit, before a second polarizer.

29.5
The structure of this brass sample can be seen only very faintly in reflected-light brightfield.



29.6
The same sample surface looks like a three-dimensional relief image in Differential Interference Contrast (DIC).

Enhancing the resolution

Several problems can affect the resolution even before to reach the diffraction limit:

- Chromatic or spherical aberration of the optical components (usually affecting the uniformity of the field view)
- Difficulty in finding the focus because of roughness and scarcely defined deep of field
- Superposition to the signal of unwanted reflections (e.G. from the objective lenses) or unexpected phase effects.

Two techniques allows to overcome a large part of such problems and to reach the diffraction limit:

Confocal microscope and Interference microscope

Scanning the image

Both the confocal and the interference techniques can enhance the resolution by taking the signal from a proportionally small portion of the sample surface.

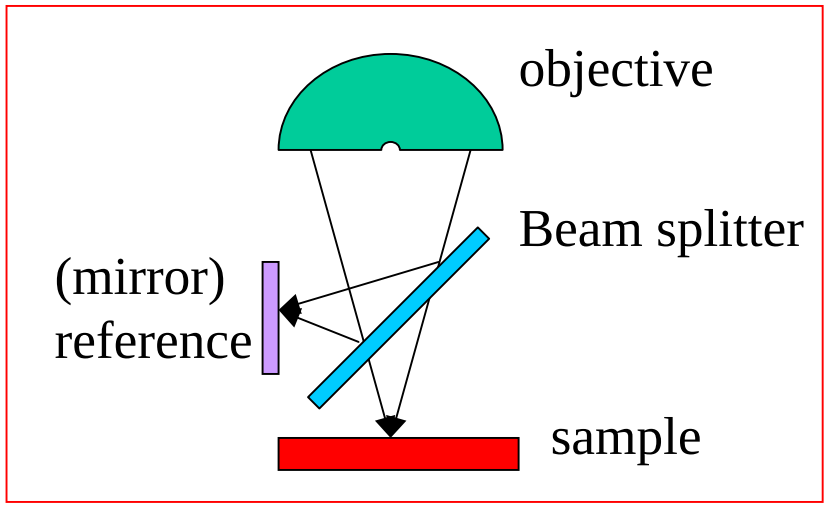
There is then the need, in order to reconstruct the image of the sample, of proceed with a scanning of the surface.

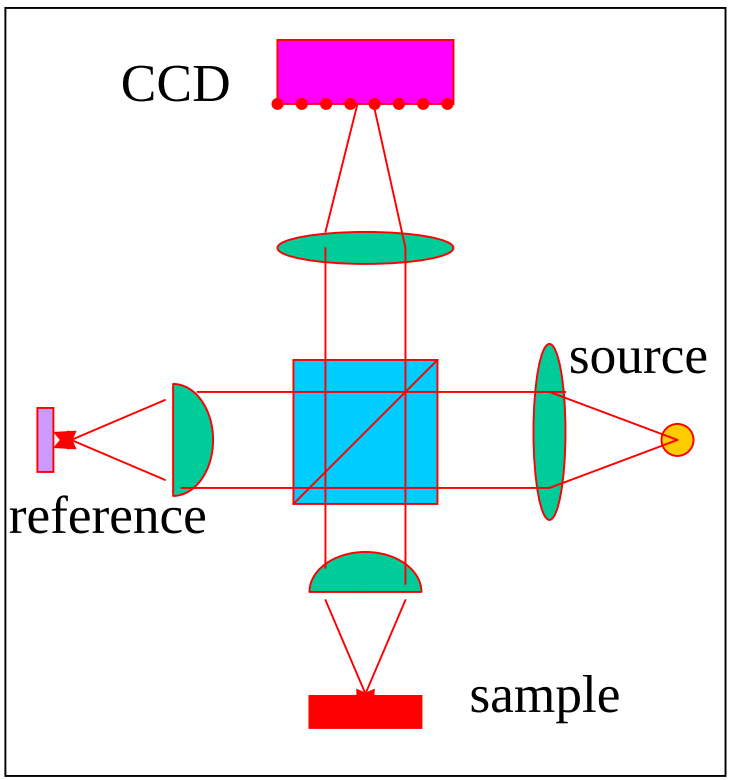
This can be achieved either by

- Moving the sample or by
- Moving the light spot (with a Nipkow disk, a galvanometer mirror, an acusto-optic system, …)

Interference microscope

A simplified scheme of an interpherence microscope ⇒



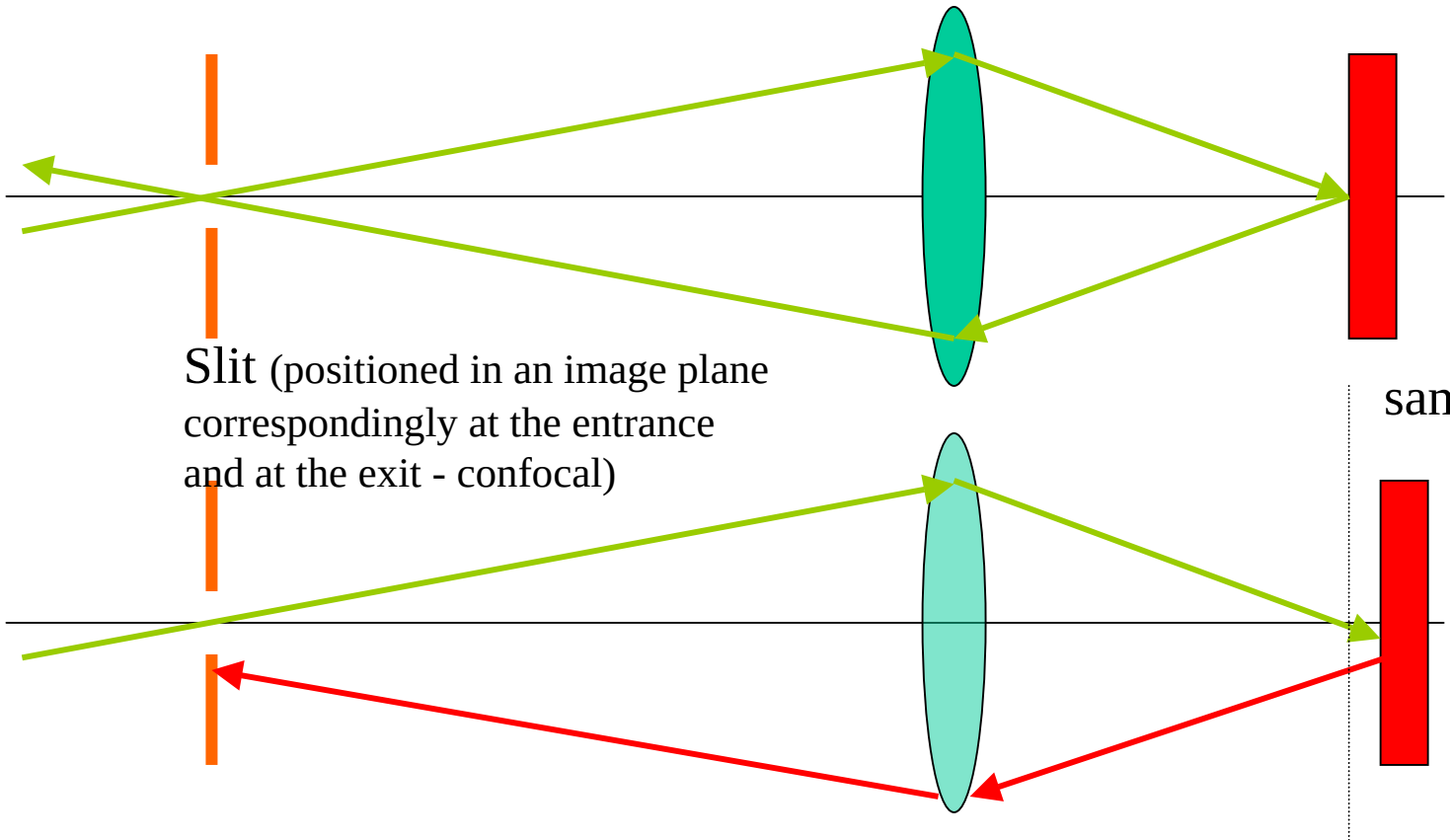


Coherence probe microscope

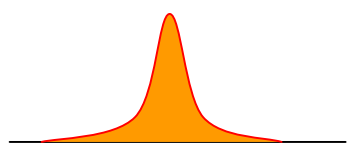
⇐

The interpherence image formed on the detector is extremely sensitive to the focussing of the beam

Confocal microscope



The idea is in enhancing the vertical sensitivity by introducing two pinholes which restrict the field of view (and the field deep)



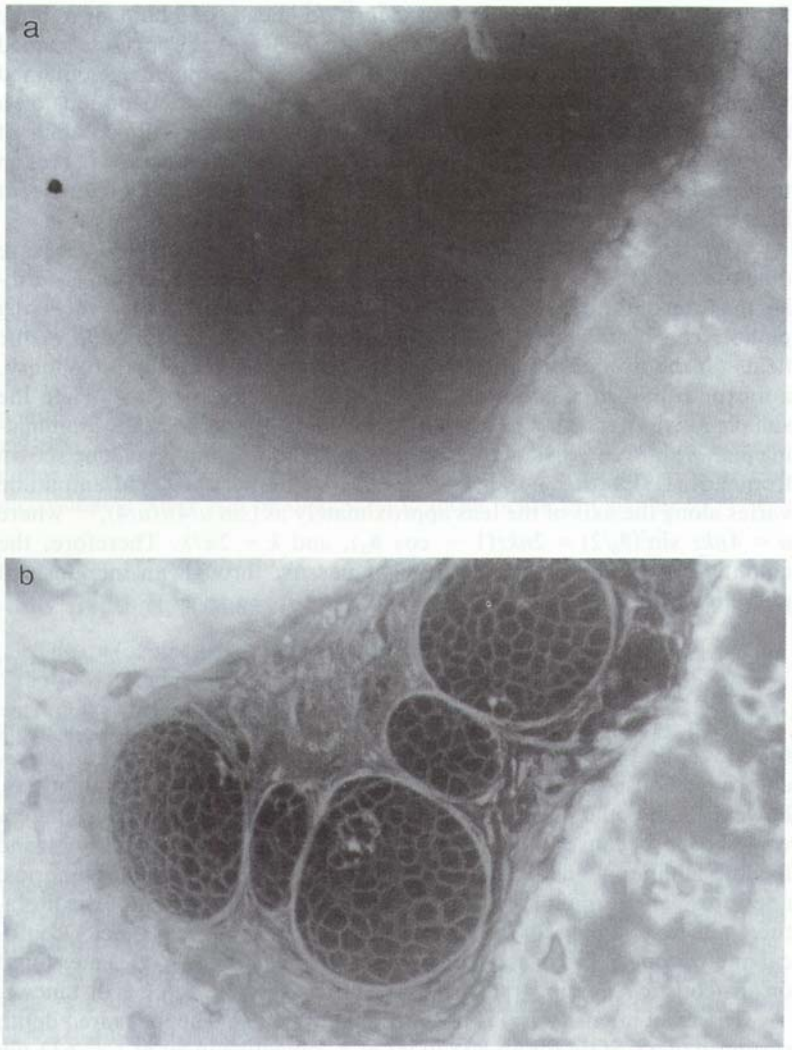
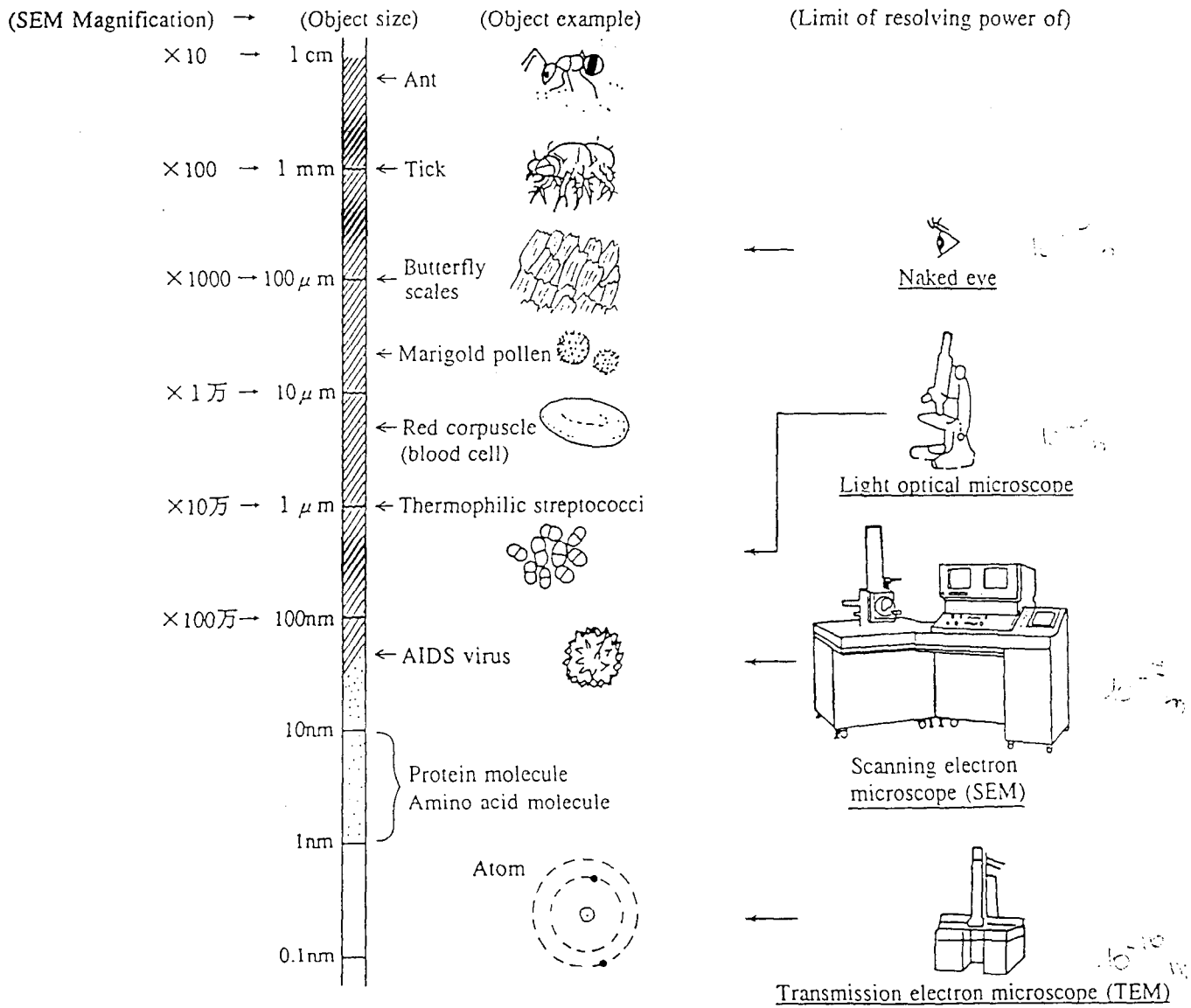
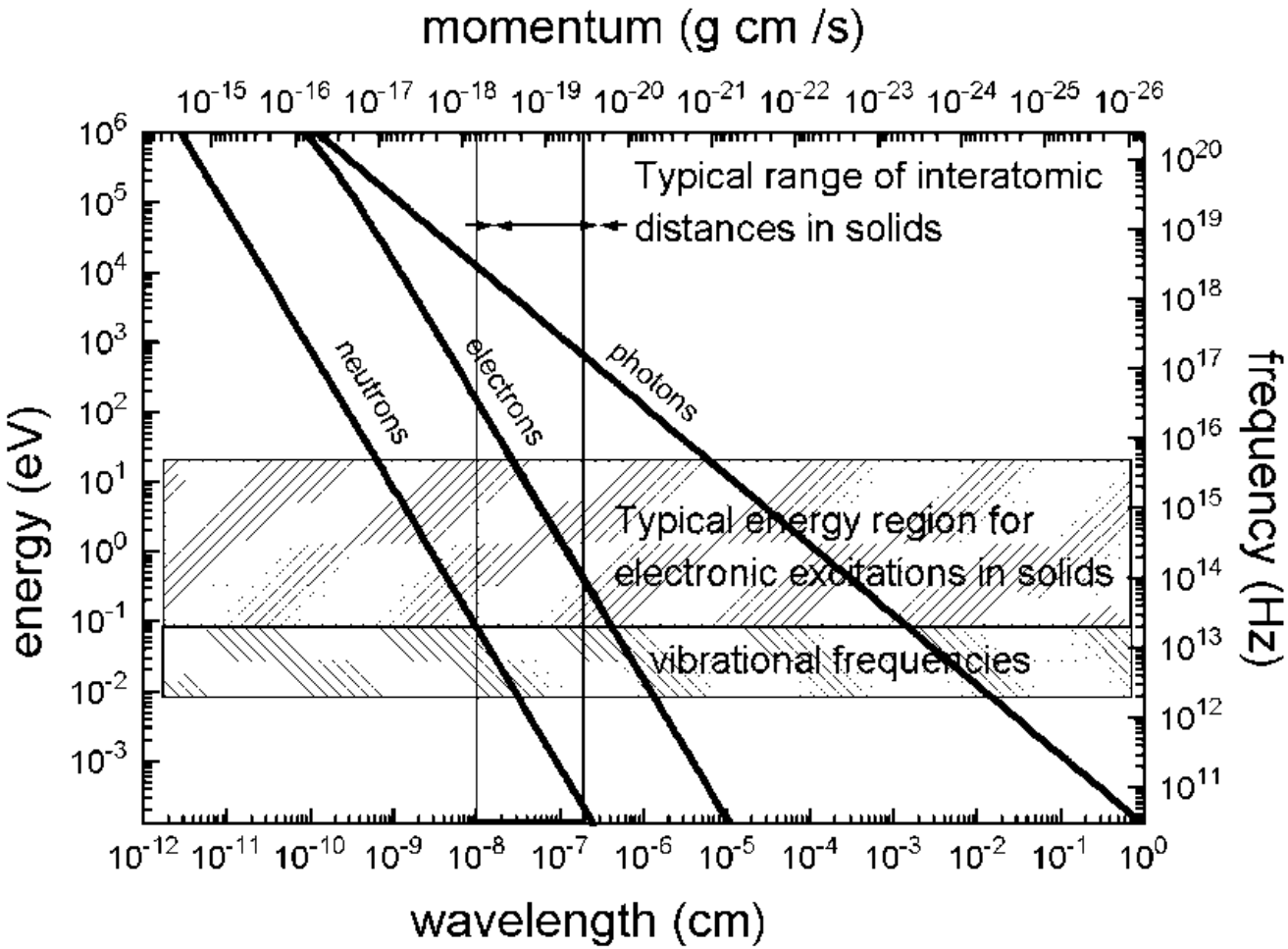


Figure 1.23 Fluorescent images of a human rib bone stained with brilliant sulphaflavine: (a) standard optical microscope image; (b) RSOM image. [Courtesy: G. Q. Xiao, "Confocal Optical Imaging Systems and their Applications in Microscopy and Range Sensing," Ph.D. Dissertation, Department of Physics, Stanford University, Stanford, California, USA (December 1989).]

Standar-vs.confocal-microscope

Microscopi e microscopie




momentum (g cm /s)
10^{-15} 10^{-16} 10^{-17} 10^{-18} 10^{-19} 10^{-20} 10^{-21} 10^{-22} 10^{-23} 10^{-24} 10^{-25} 10^{-26}
10^{6} 10^{5} 10^{4} 10^{3} 10^{2} 10^{1} 10^{0} 10^{-1} 10^{-2} 10^{-3}
energy (eV)
10^{20} 10^{19} 10^{18} 10^{17} 10^{16} 10^{15} 10^{14} 10^{13} 10^{12} 10^{11}
frequency (Hz)
Typical range of interatomic distances in solids
neutrons
electrons
photons
Typical energy region for electronic exoitations in solids
vibrational frequencies
10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0}
wavelength (cm)

Tecniche di scattering

fascio emesso / fascio incidente	Raggi X	Fotoni	Elettroni	Ioni
Raggi X	XRD Fluorescence		ESCA	
Fotoni		Optical Microscopy	Photoemission pectroscopy	
Elettroni	Energy dispersive analysis	Cathodo-luminescence	TEM, SEM, LEED, HEED, STEM, EELS	
Ioni	Ion-induced XRD	Ion-induced photon analysis	Ion-induced secondary electron em.	RBS, SIMS nuclear reaction analysis

ANALISI DEGLI EFFETTI PRODOTTI DALL'INTERAZIONE CON IL CAMPIONE DI UN FASCIO DI PARTICELLE

Why Electrons?

Resolution!

In the expression for the resolution (Rayleigh's Criterion)

$$r = 0.61\lambda / n\sin\alpha$$

λ-wavelength, $\lambda = [1.5/(V + 10^{-6}V^2)]^{1/2}$ nm

V-accelerating voltage, n-refractive index

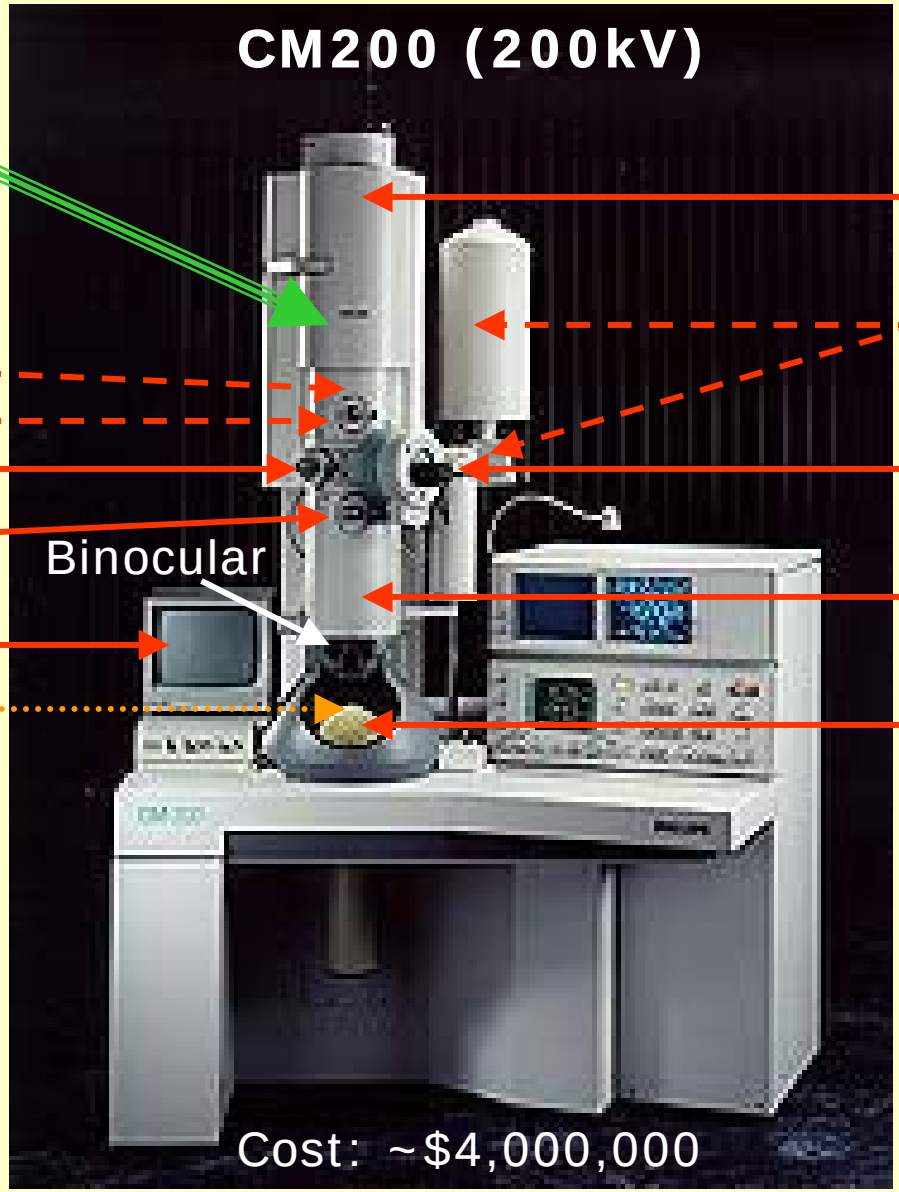
α-aperture of objective lens, very small in TEM

$\rightarrow \sin\alpha \rightarrow \alpha$ and so $r = 0.61\lambda/\alpha$ $\alpha \sim 0.1$ radians

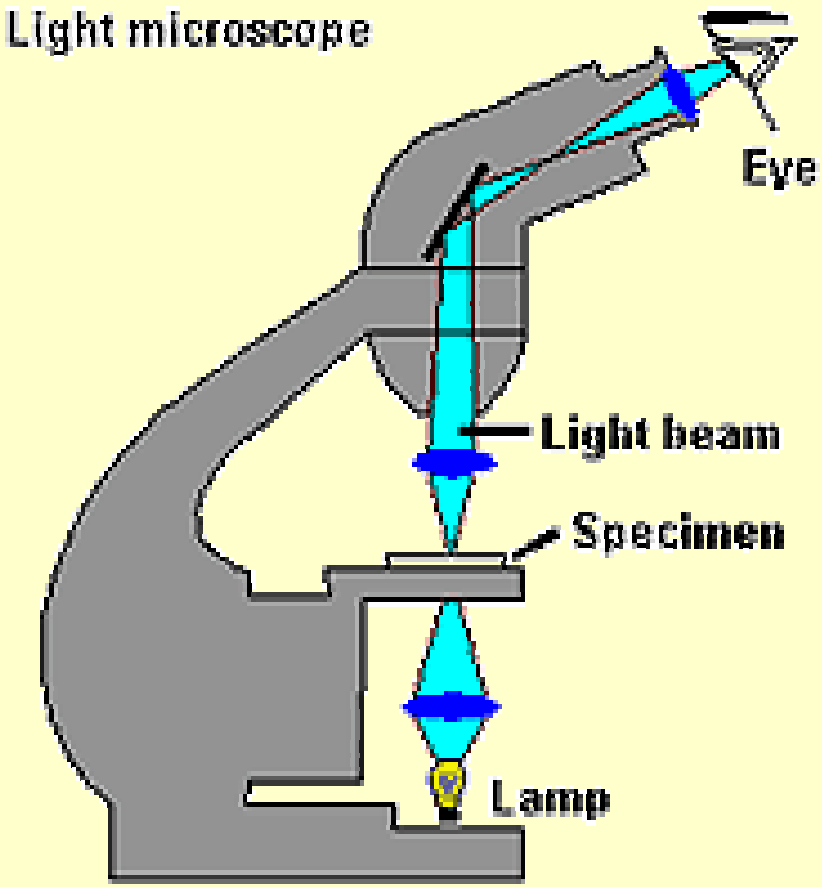
Green Light
$\lambda \sim 400$nm
n~1.7 oil immersion
r~150nm (0.15μm)

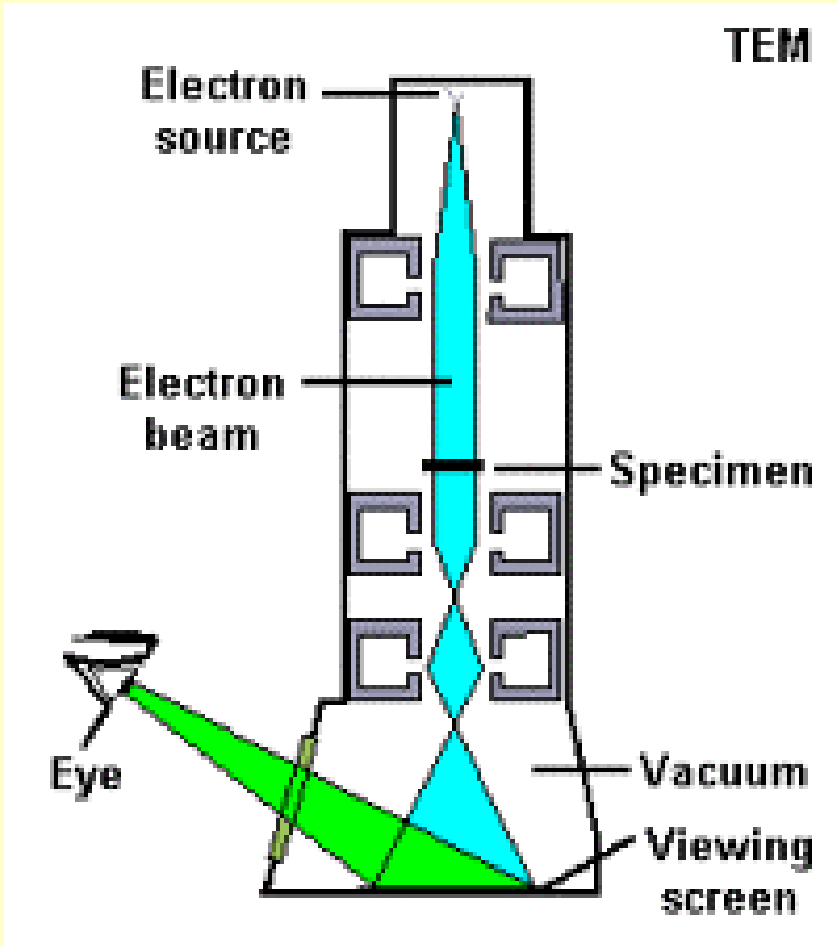
200kV Electrons
$\lambda \sim 0.0025$nm
n~1 (vacuum)
r~0.02nm (0.2Å)
1/10th size of an atom!
UNREALISTIC! WHY?

Basic features of A Modern TEM

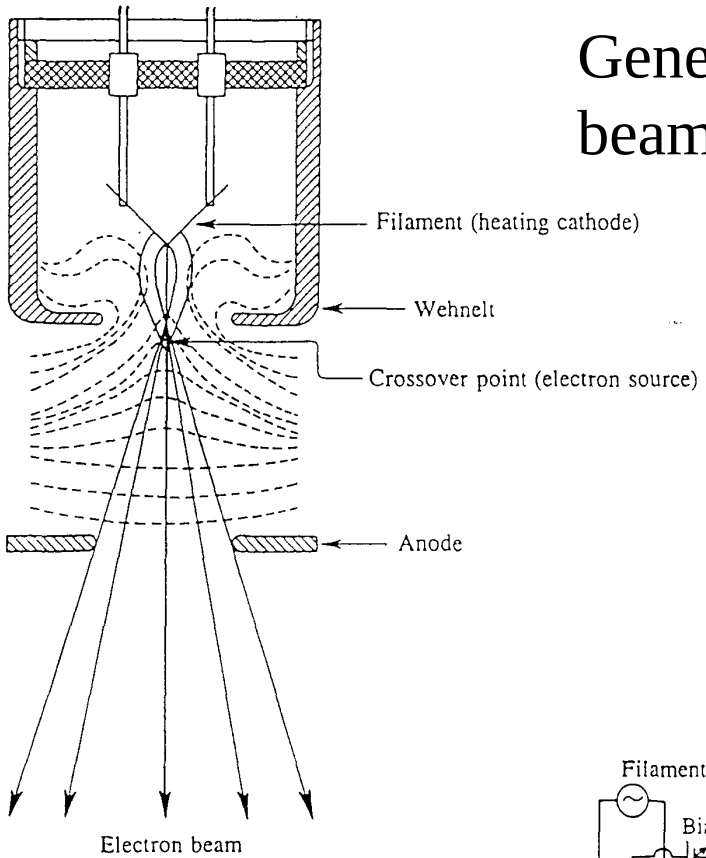


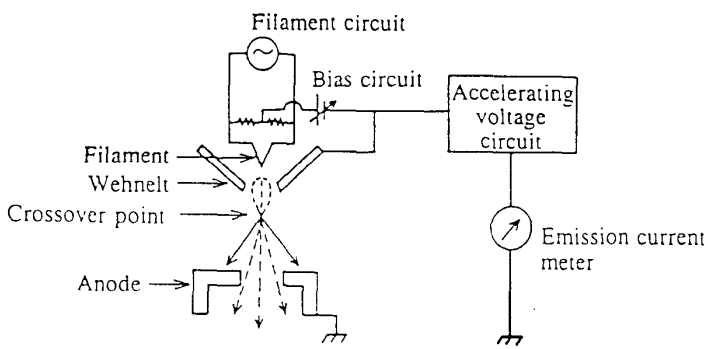
A better comparison





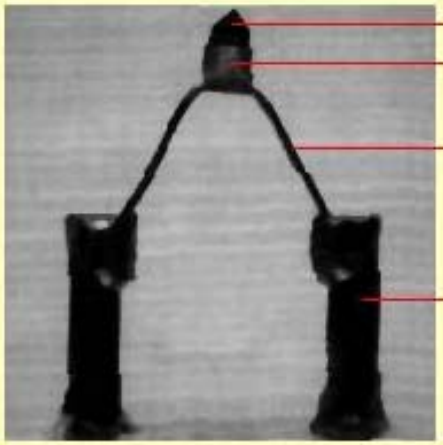
Generation of electron beam by electron Gun



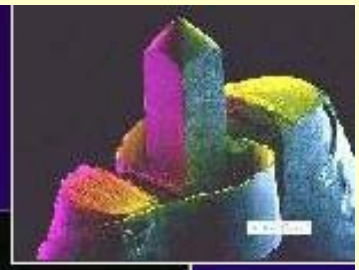


A SEM fitted with a field emission electron gun can provide higher brightness and higher resolution.

Electron Beam Source



Lanthanum Hexaboride Single Crystal Filament.



Electron Beam Source

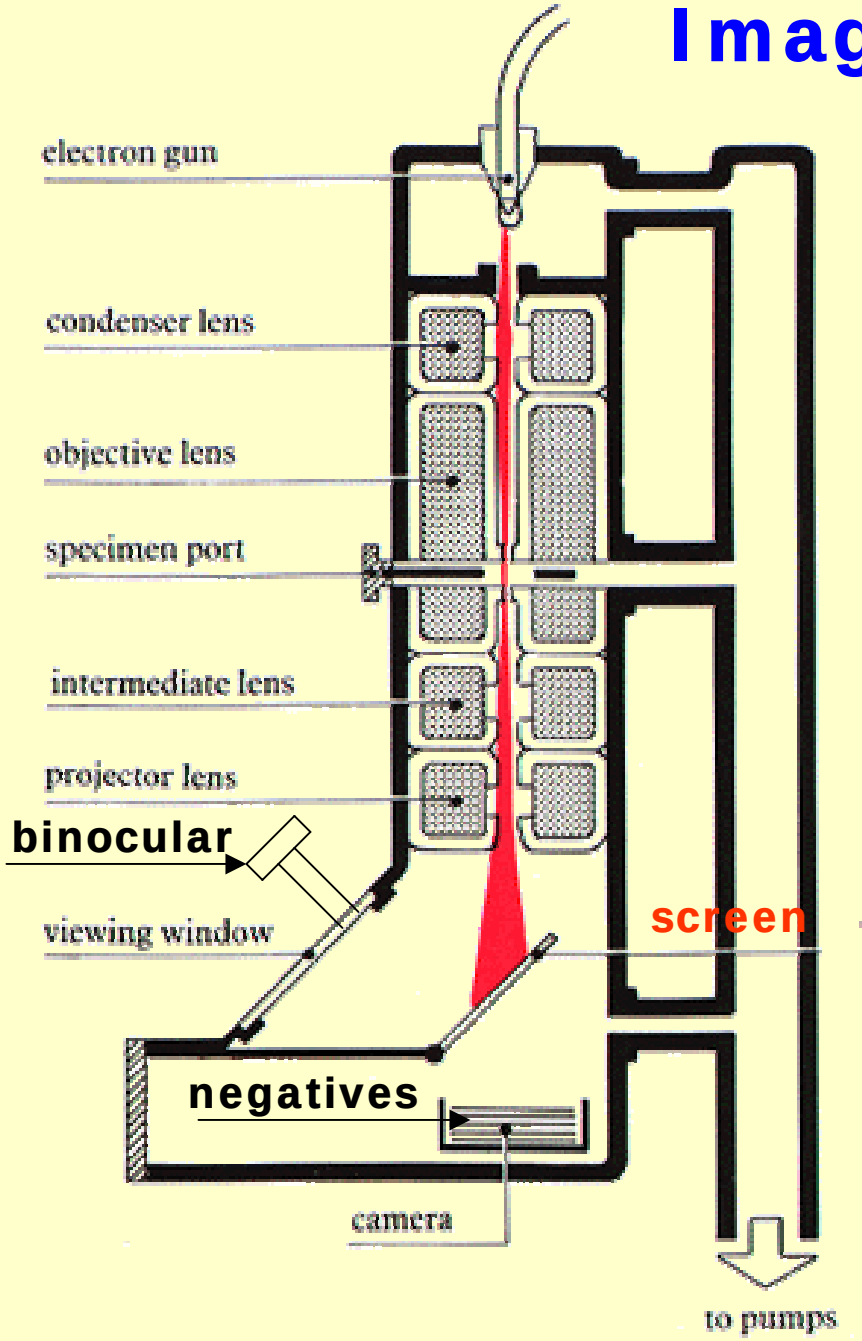
W Filament - Current density ~10A/cm^2, probe size ~5.0nm

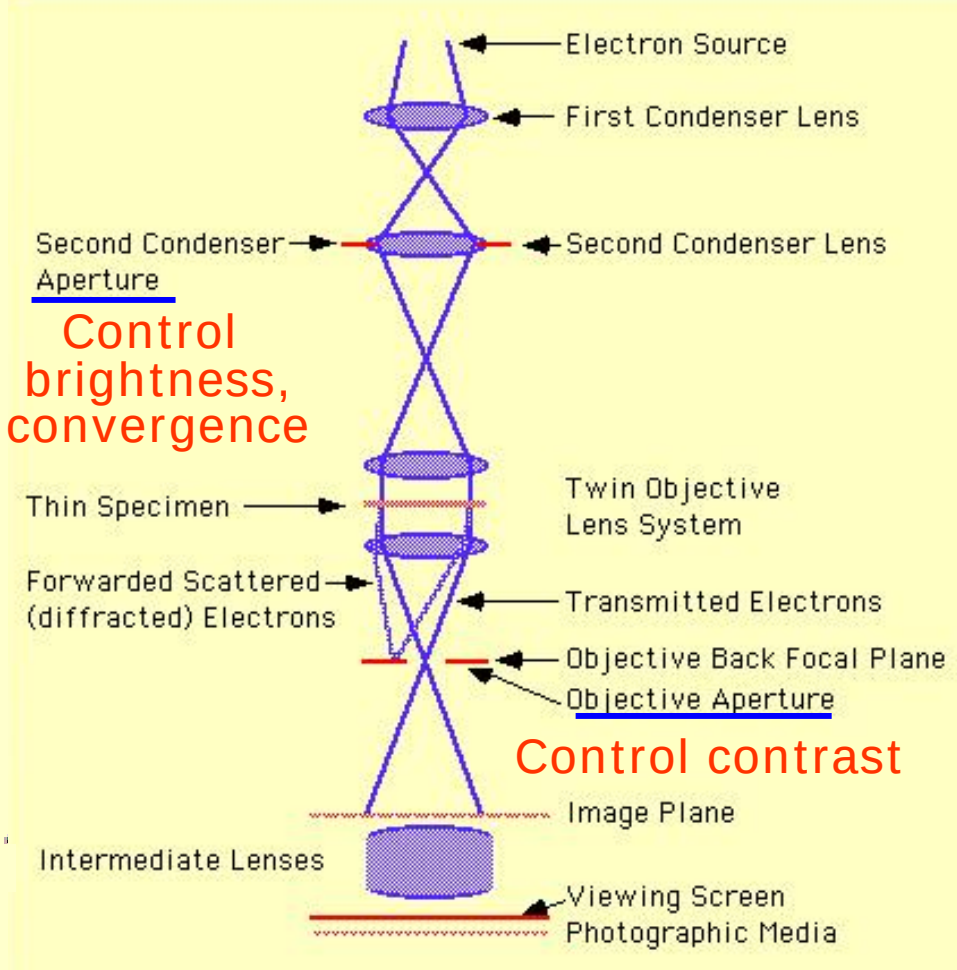
LaB_6 - Current density ~10^3A/cm^2, probe size ~1.5nm

FEG - Current density ~10^5A/cm^2 , probe size ~<1.0nm

EMAL

Image Formation in TEM





Ray Diagram for a TEM

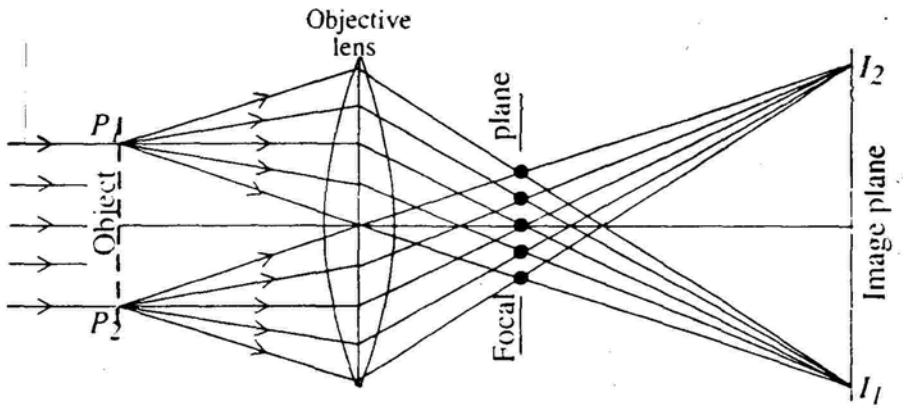
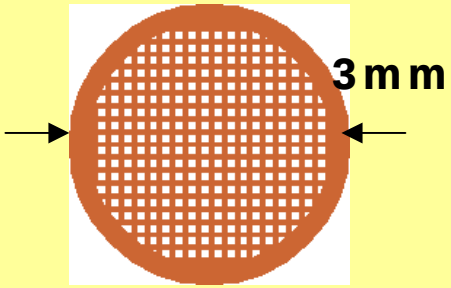


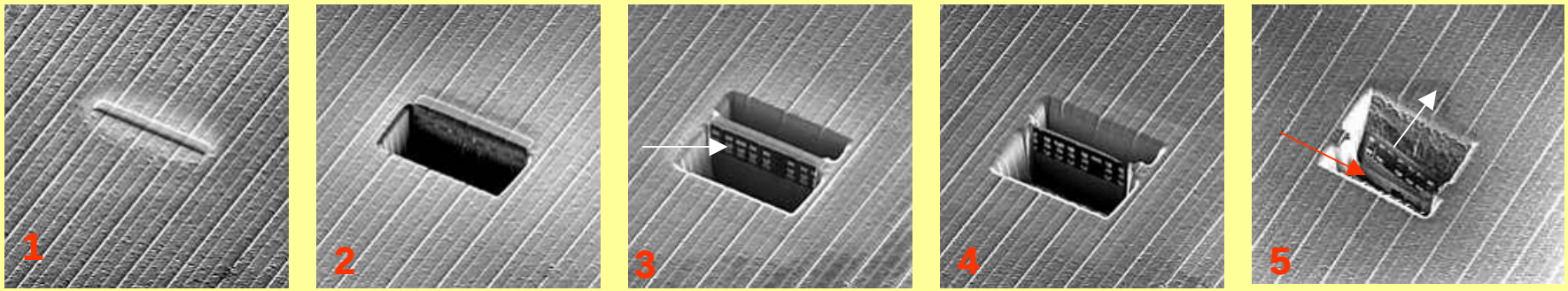
Figure 3.1. Simple imaging system illustrating the two stage process in image formation: first, the formation of the diffraction pattern in the focal plane of the objective lens, and second, the recombination of the diffracted beams to form the image.

Specimen Preparation-Destructive

Dispersing crystals or powders on a carbon film on a grid



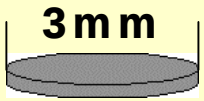
Making a semiconductor specimen with a Focused Ion Beam (FIB)



1. a failure is located and a strip of Pt is placed as a protective cover.
2. On one side of the strip a trench is milled out with the FIM.
3. The same is done on the other side of the strip (visible structure).
4. The strip is milled on both sides and then the sides connecting the strip to the wafer are cut through.
5. The strip is tilted, cut at the bottom and deposited on a TEM grid.

Specimen Preparation-2

Ion-milling a ceramic

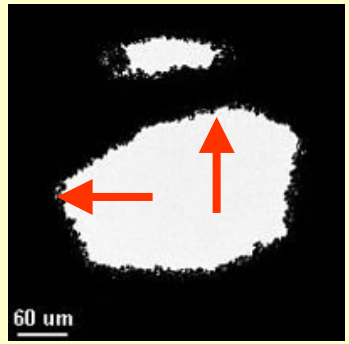


Ultrasonic cut grind

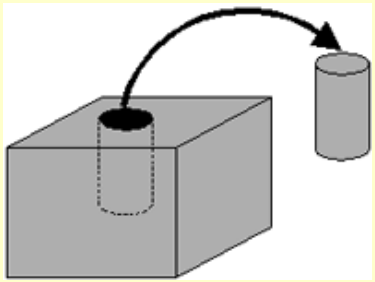
Dimple center part of disk to ~5-10μm

Ar (4-6keV, 1mm A)

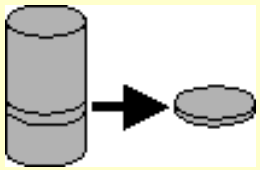
ion-mill until a hole appears in disk



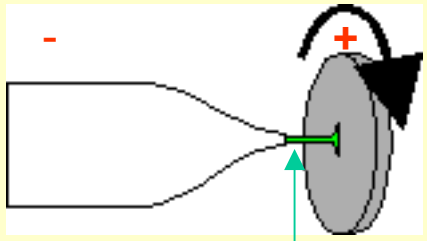
Jet-polishing metal



Drill a 3mm cylinder

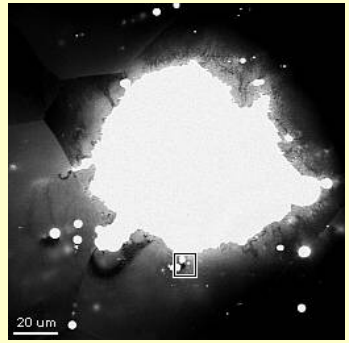


Cut into disks and grind



a thin stream of acid

A disk is mounted in a jet-polishing machine and is electropolished until a small hole is made.



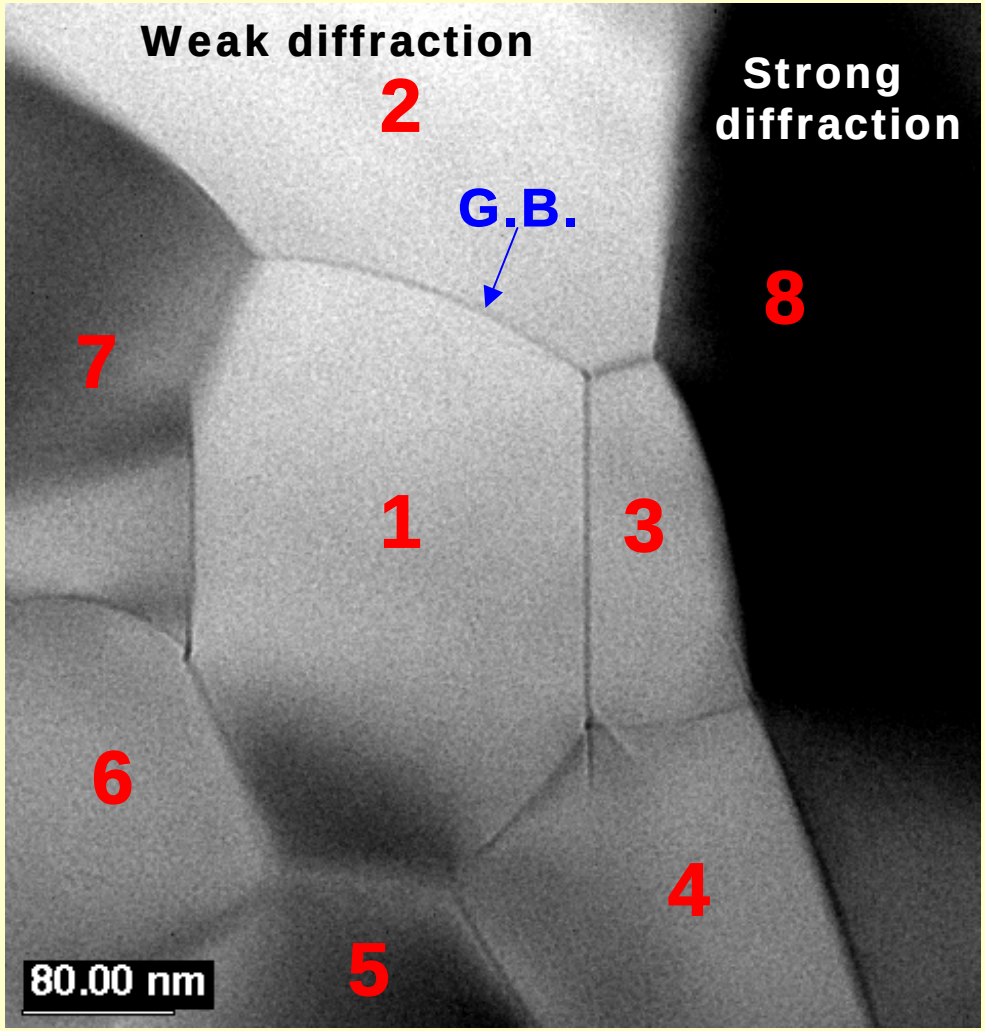
Ultramicrotomy-using a (diamond) knife blade

Mainly for sectioning biological materials.

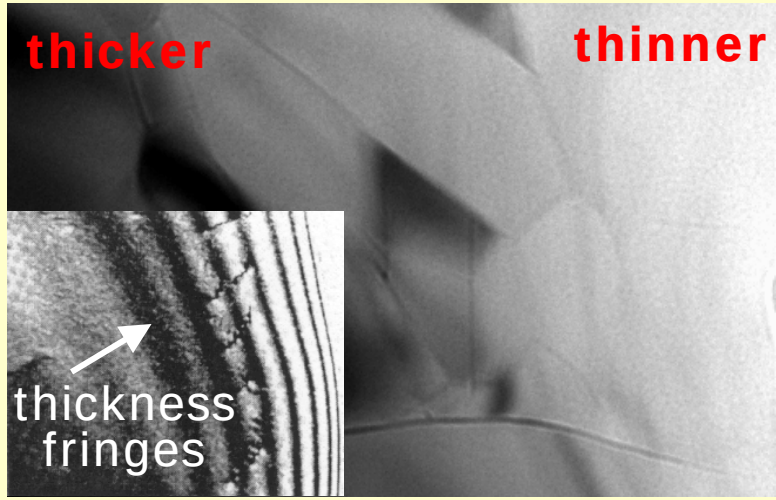
To avoid ion-milling damage ultramicrotome can also be used to prepare ceramic TEM specimens.

Diffraction, Thickness and Mass Contrast

BF images

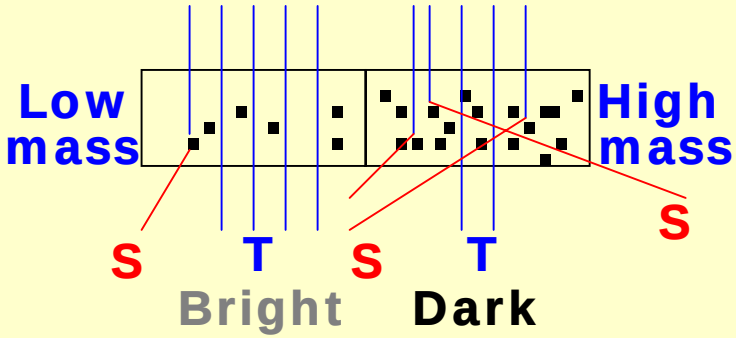


8 grains are in different orientations or different diffraction conditions

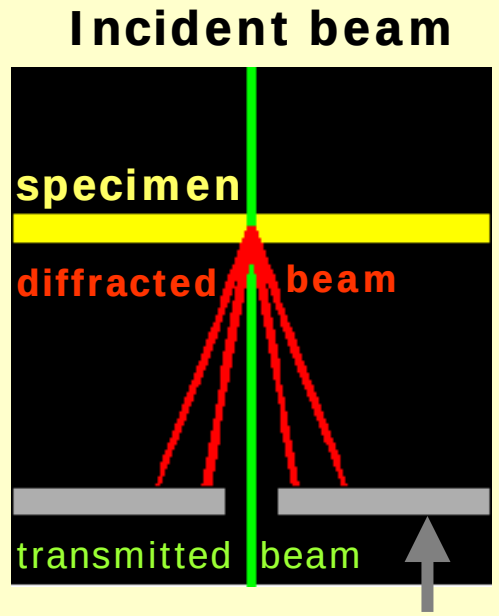


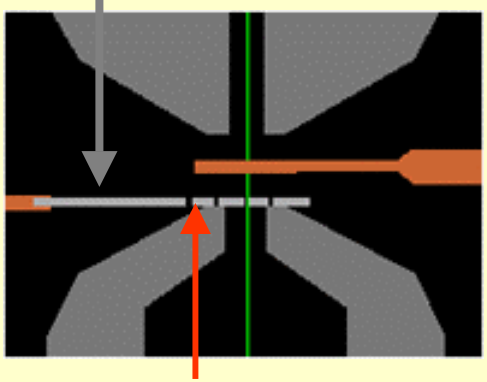
thickness

Disk specimen



Bright Field (BF) and Dark Filed (DF) Imaging



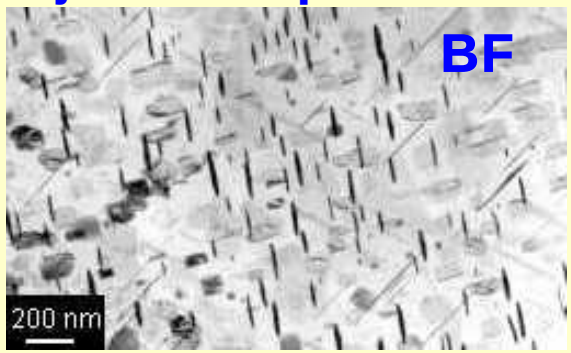


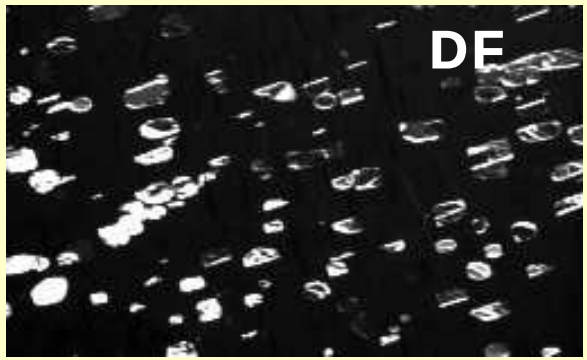
hole in objective aperture(10-100μm)

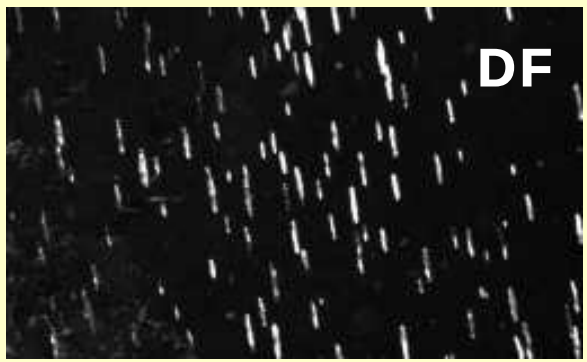
BF imaging-only transmitted beam is allowed to pass objective aperture to form images.

mass-thickness contrast

DF imaging only diffracted beams are allowed to pass the aperture to form images.



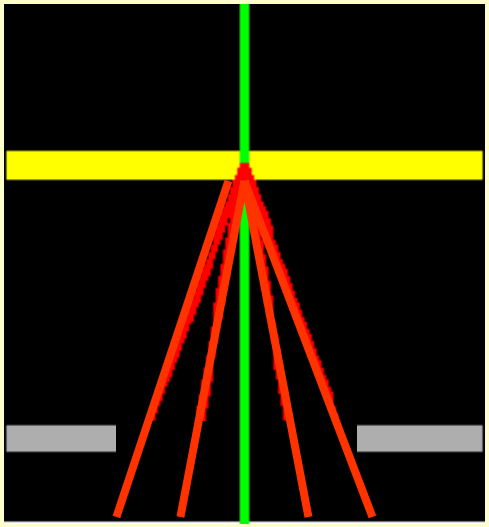




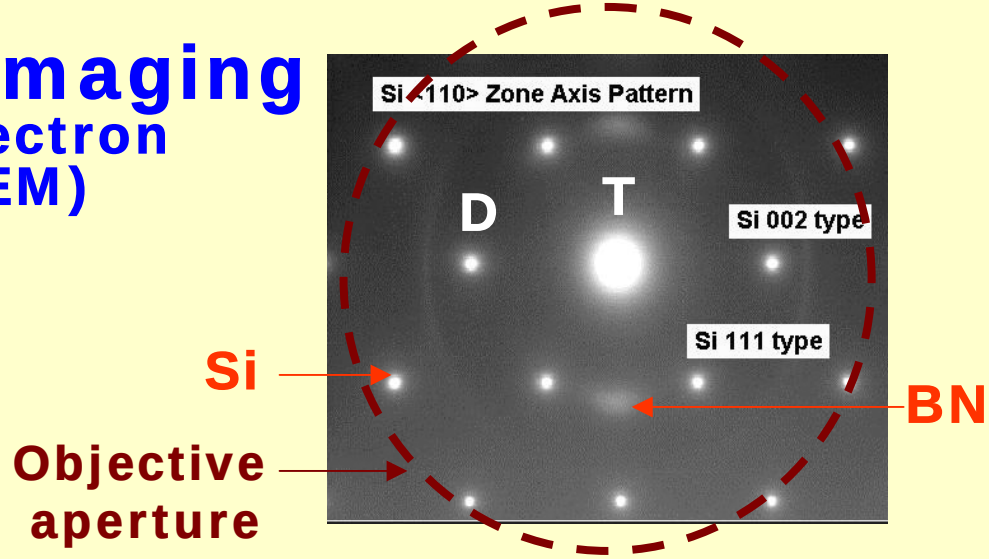
Particles in Al-Cu Alloy.
thin platelets ll e
Vertical, dark Particles ⊥e.

Phase Contrast Imaging

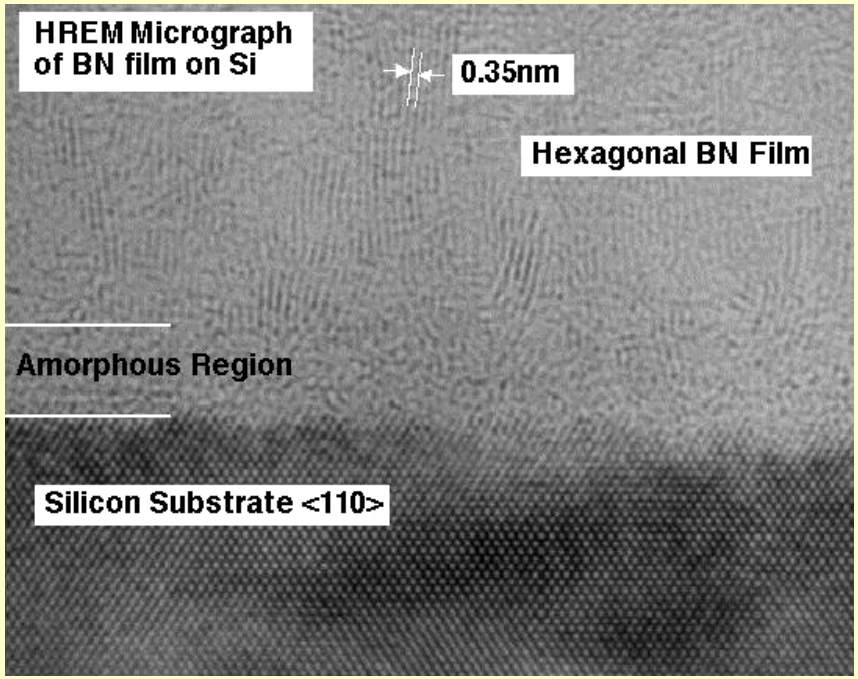
High Resolution Electron Microscopy (HREM)

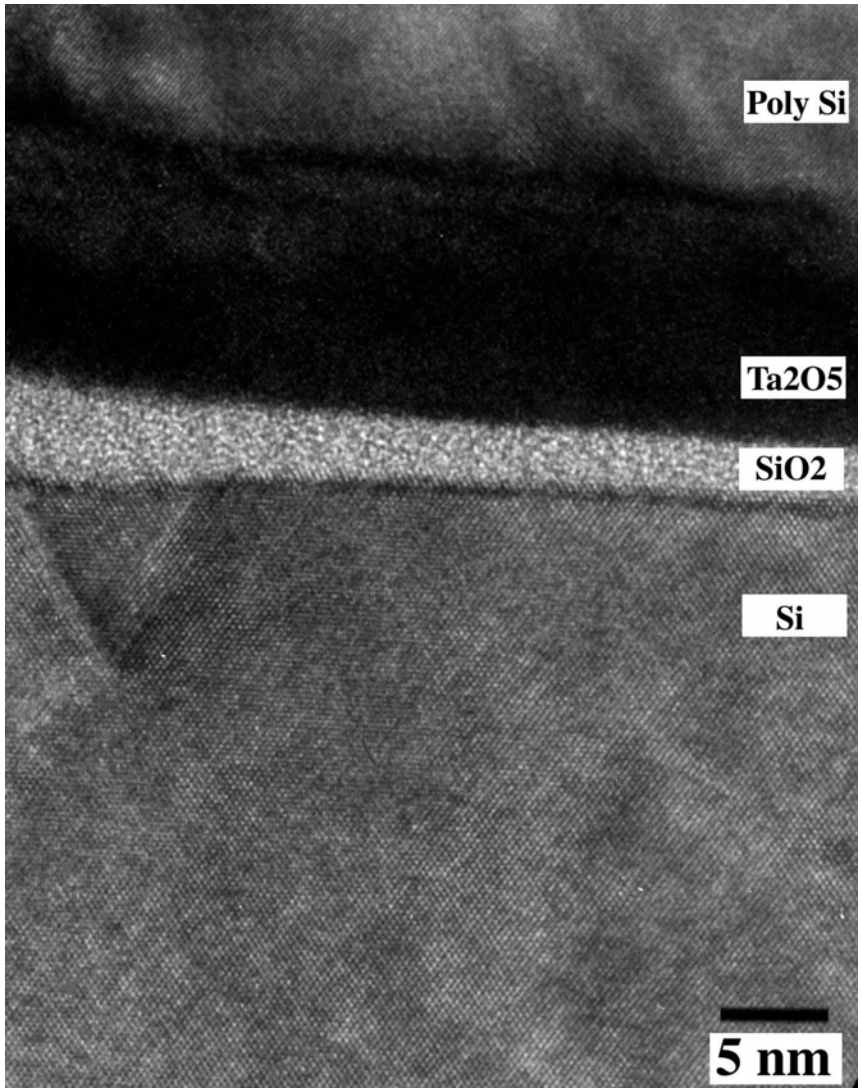


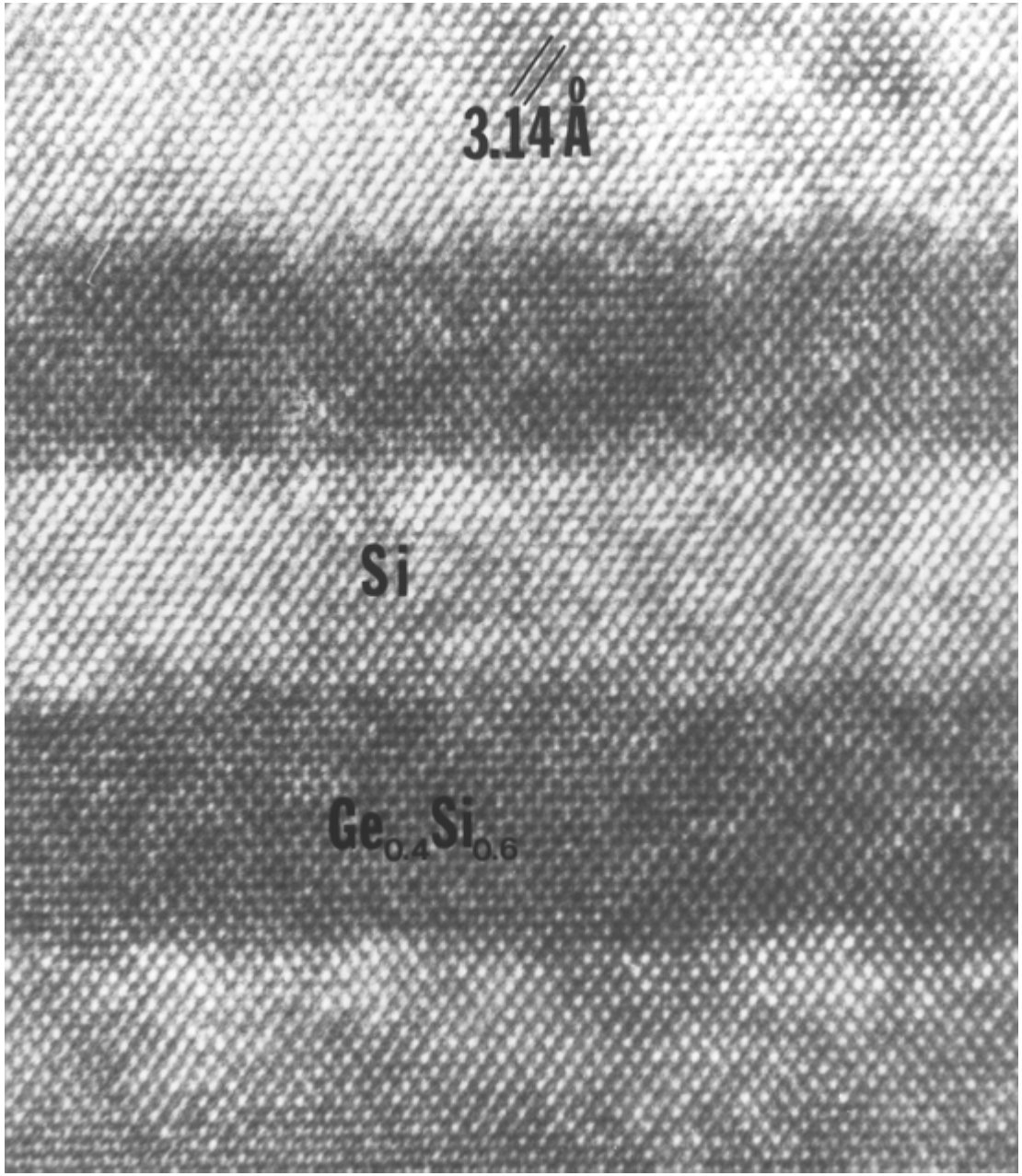
Use a large objective aperture.
Phases and intensities of diffracted and transmitted beams are combined to form a phase contrast image.

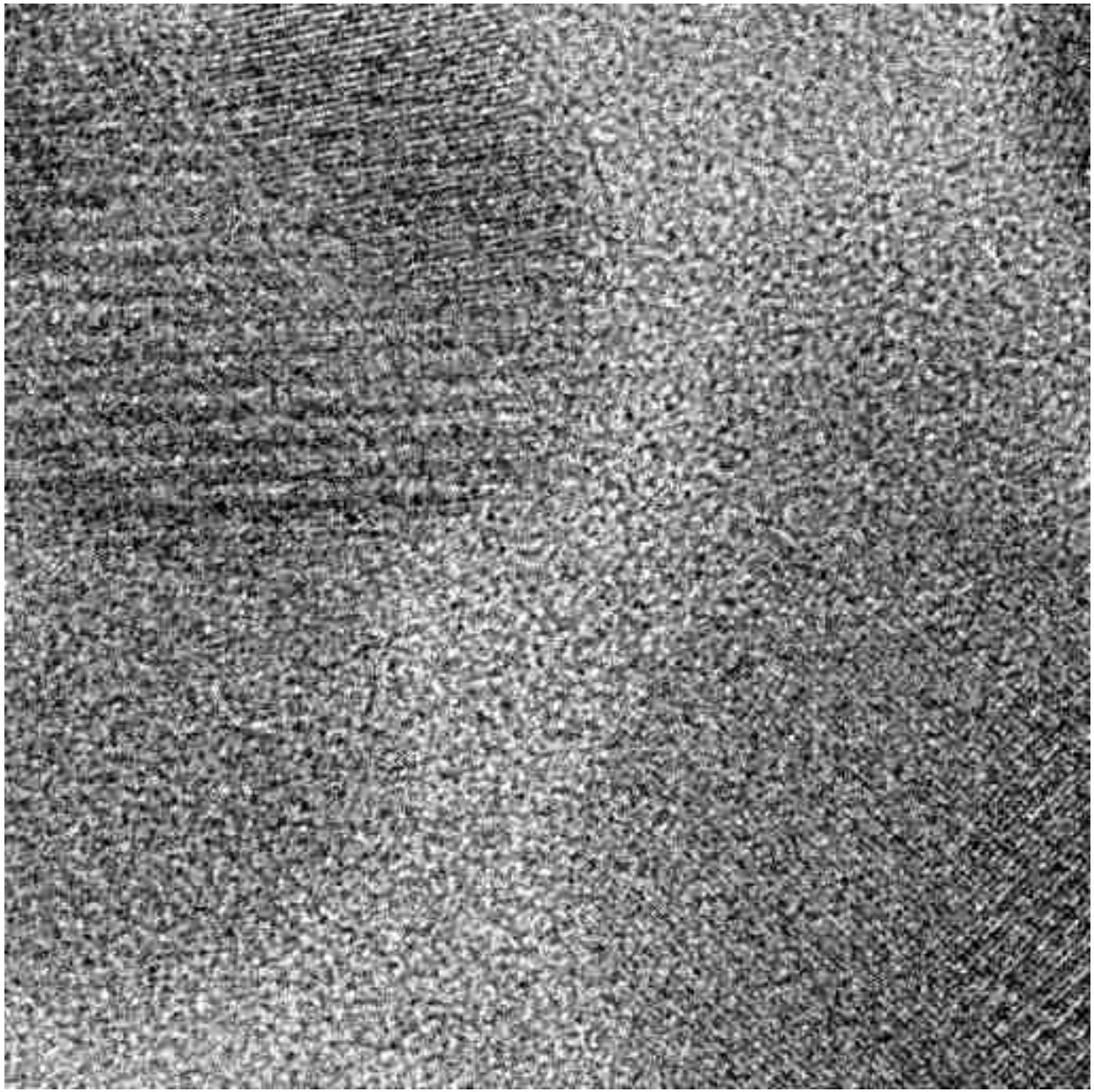


Electron diffraction pattern recorded From both BN film on Si substrate.

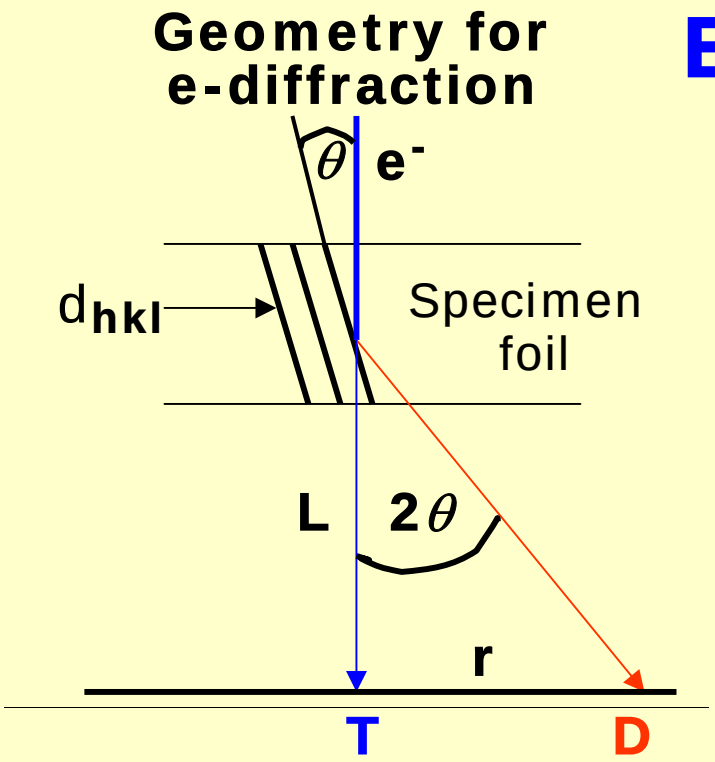



Poly Si
Ta2O5
SiO2
Si
5 nm


3.14 Å
Si
$Ge_{0.4}Si_{0.6}$



Electron Diffraction



Bragg's Law: $\lambda = 2d\sin\theta$

λ = 0.037Å (at 100kV)

θ = 0.26° if d = 4Å

$$\lambda = 2d\theta$$

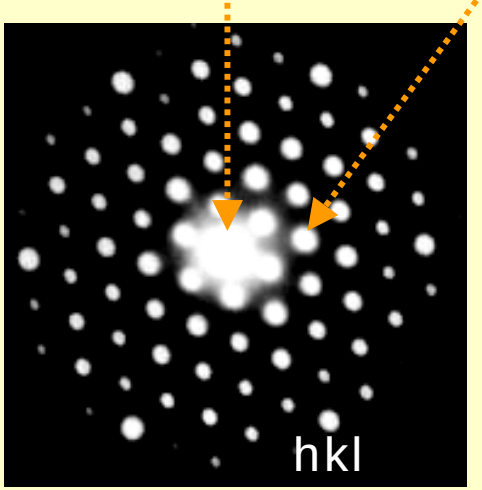
$r/L = \sin 2\theta$

as $\theta \rightarrow 0$

$r/L = 2\theta$

$r/L = \lambda/d$ or

$$r = \lambda L x \frac{1}{d}$$



[hkl] SAED pattern

Reciprocal lattice

L -camera length

r -distance between **T** and **D** spots

1/**d** -reciprocal of interplanar distance(Å^{-1})

SAED –selected area electron diffraction

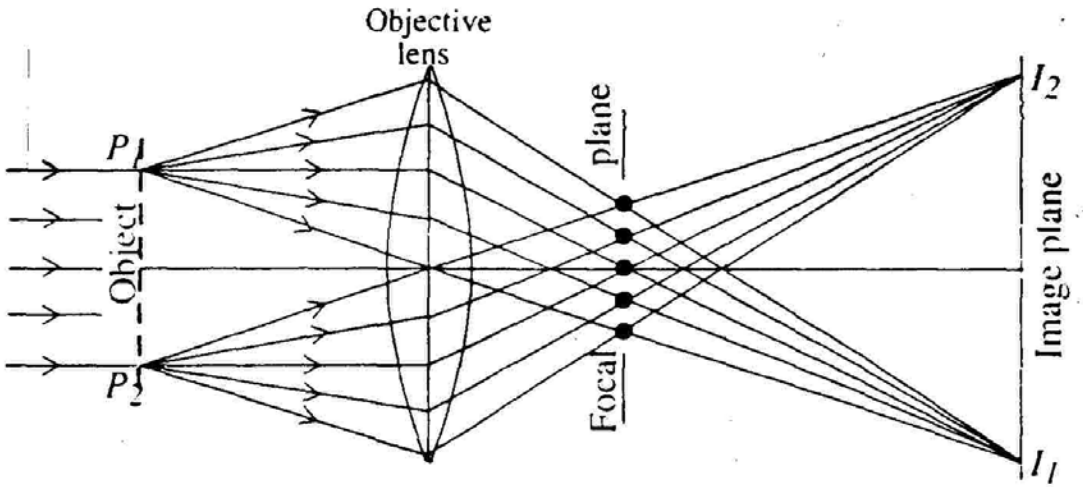
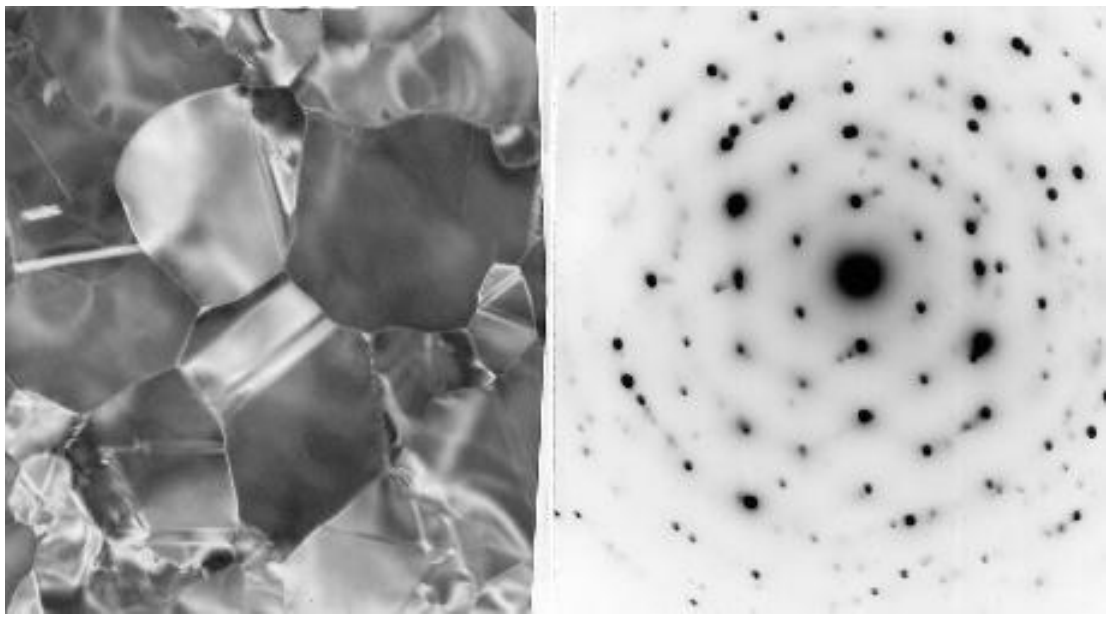
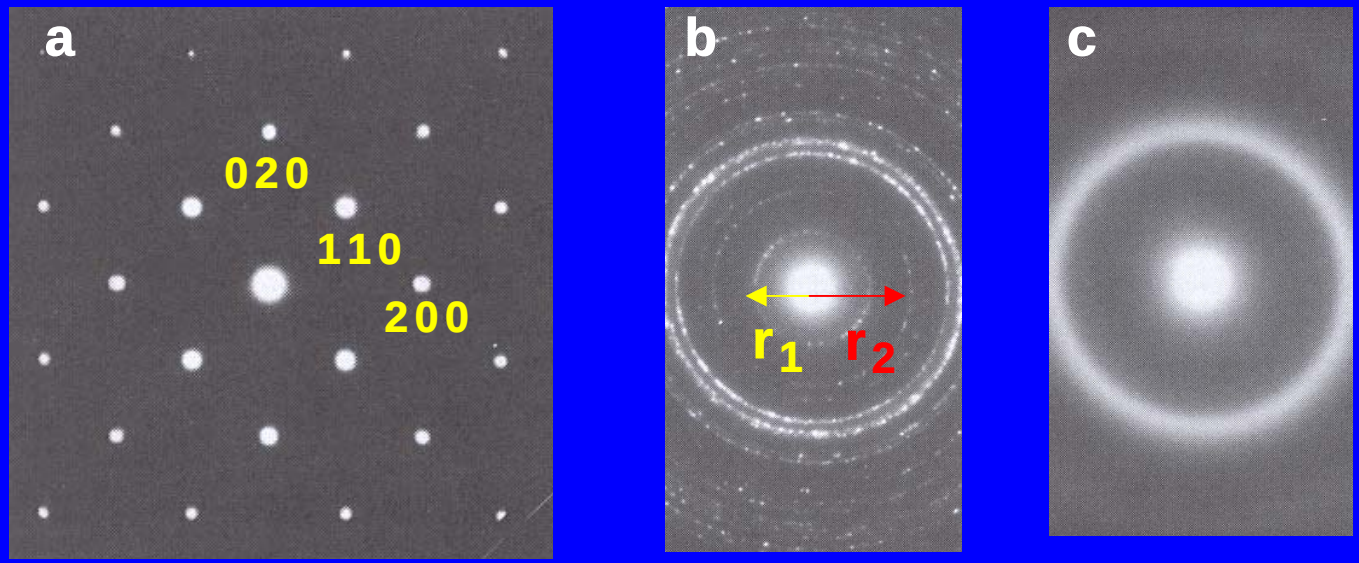


Figure 3.1. Simple imaging system illustrating the two stage process in image formation: first, the formation of the diffraction pattern in the focal plane of the objective lens, and second, the recombination of the diffracted beams to form the image.



SAED Patterns of Single Crystal, Polycrystalline and Amorphous Samples



a. **Single crystal Fe (BCC) thin film-[001]**
b. **Polycrystalline thin film of Pd_2Si**
c. **Amorphous thin film of Pd_2Si. The diffuse halo is indicative of scattering from an amorphous material.**

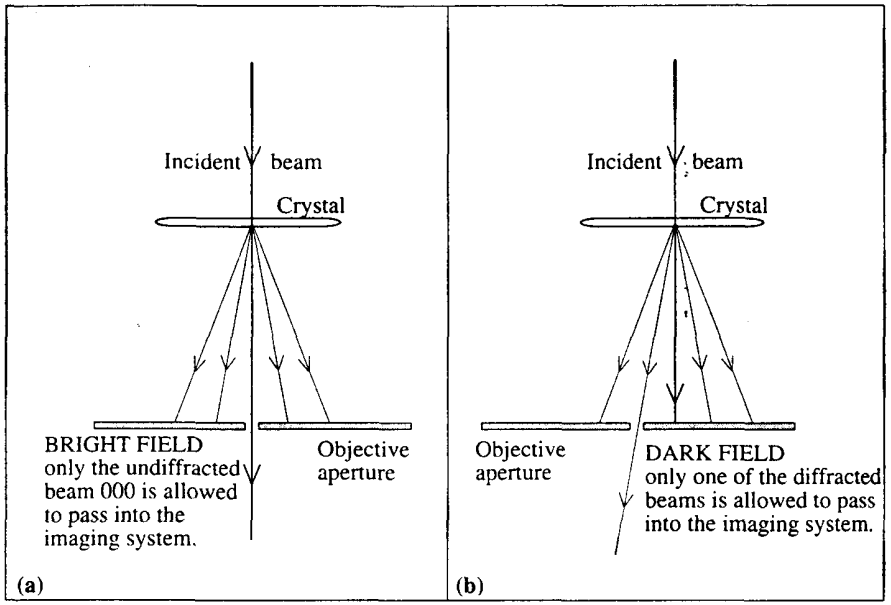


Figure 3.40. The position and function of the objective aperture for (a) a bright field image and (b) a dark field image. In practice the incident beam is tilted to obtain a dark field image, rather than the aperture off-centred, so that the selected diffracted beam is parallel to the microscope column.

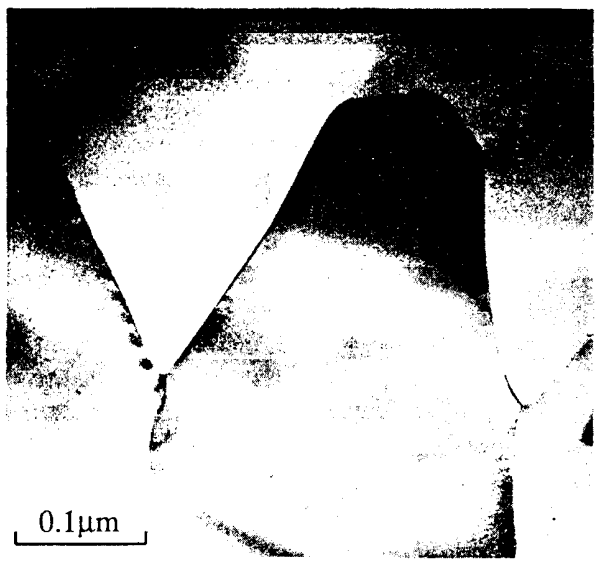


Figure 3.41. Bright field image of a polycrystalline specimen of cordierite, in which the different crystals are in different orientations, and hence diffract differently. This shows up the grain boundaries.

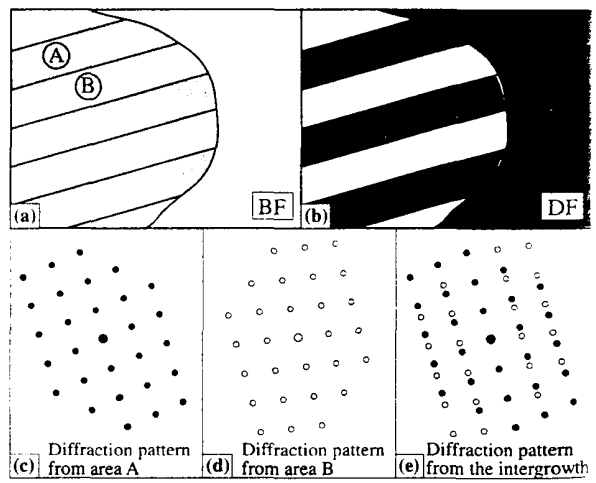


Figure 3.42. A schematic illustration of diffraction contrast imaging. (a) A bright field image showing a lamellar intergrowth, set A diffracting more strongly than B. (c), (d) and (e) are diffraction patterns from individual lamellae and over the whole intergrowth respectively. The dark field image in (b) is formed using one of the diffracted beams from set B, i.e. one of the spots drawn as open circles.

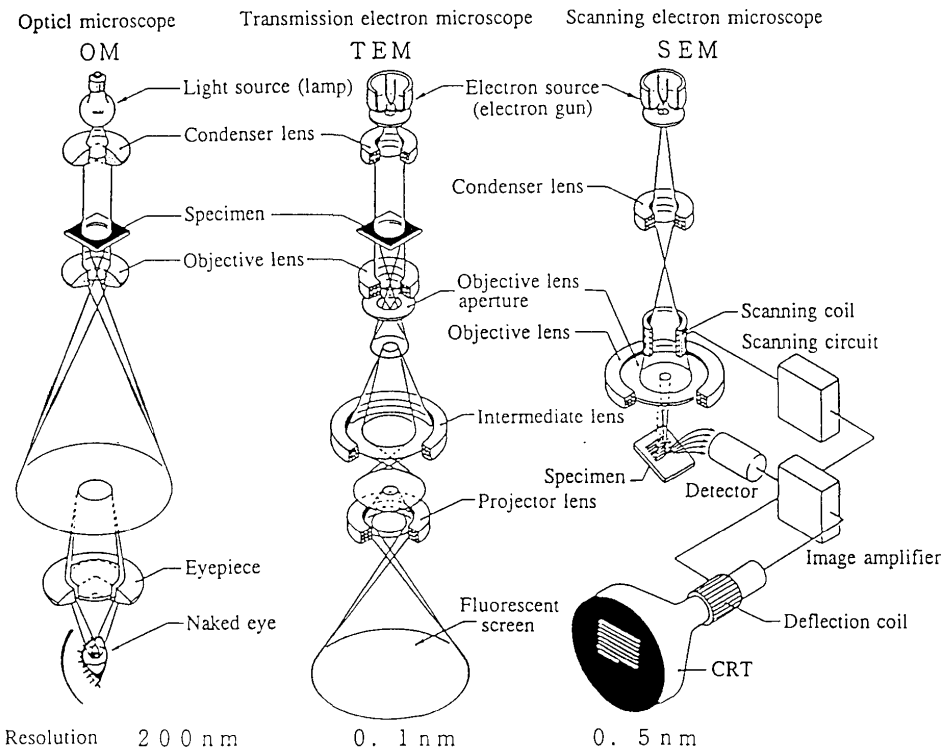
6. Comparison of Scanning Electron Microscope with Optical Microscope and Transmission Electron Microscope

6.1 Comparison by Principle Diagrams

Below are compared three types of microscopes that are used generally.

The optical microscope (OM) taken up here is for observing transmitted images. With this OM, the light passing through a sufficiently thin specimen is magnified through glass lenses. When observing a specimen with a transmission electron microscope (TEM), a specimen that is made thin enough to transmit an electron beam is prepared, and a beam which has passed the specimen and scattered is observed after being magnified by electron lenses.

In scanning electron microscope (SEM) observation, an electron beam that is finely focused by electron lenses is scanned over the specimen and the brightness on the CRT is modulated by the signals obtained.

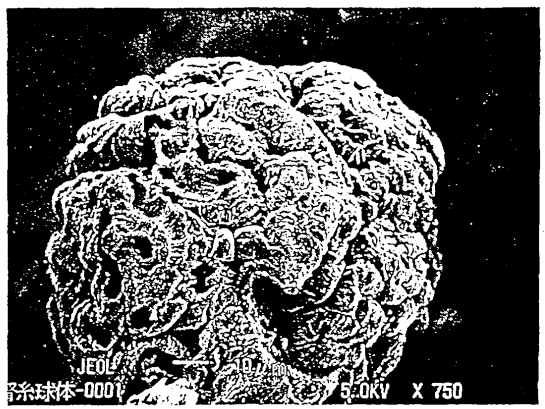


6.2 Comparison of SEM Image with OM and TEM Images

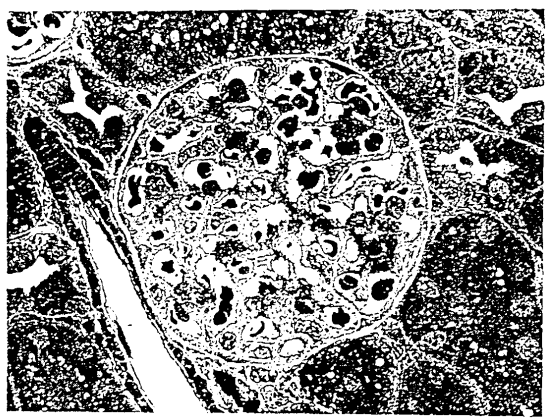
We compared photos of glomerulus obtained with an OM, SEM and TEM. With a bulk-state specimen, a SEM image has a large focal depth as compared with an OM image.

OM image: × 200
Specimen: Rat's glomerulus (free cell)
O_sO_4 stained

OM image: × 200
Specimen: Rat's glomerulus (section)
HE stained



SEM image: × 750 (secondary electron image)
Specimen: Rat's glomerulus
Surface morphology of
the bulk-state specimen is observed.



TEM image: × 750
Specimen: Rat's glomerulus
The internal structure of
a thin section specimen is observed.