Lecture 2: Electron Microscope

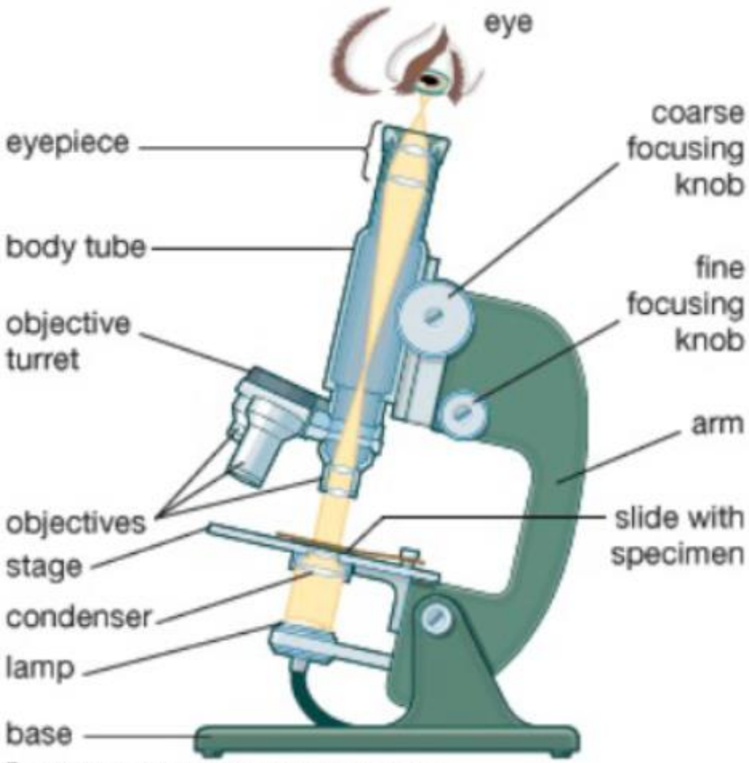
1. Construction of the Transmission Electron Microscope
2. Interaction of Electron and Matter & Image Formation
3. Aberrations and Correctors
4. Construction of the Scanning Electron Microscope

On-line cryoEM courses:

Grant Jensen, CalTec:	http://cryo-em-course.caltech.edu/
Sjors Scheres, MRC:	http://www.ccpem.ac.uk/courses.php
Eva Nogales, UCSF:	https://www.youtube.com/watch?v=nkGRhYv01ag

Optical vs. TEM Microscope

Light Microscope schematic



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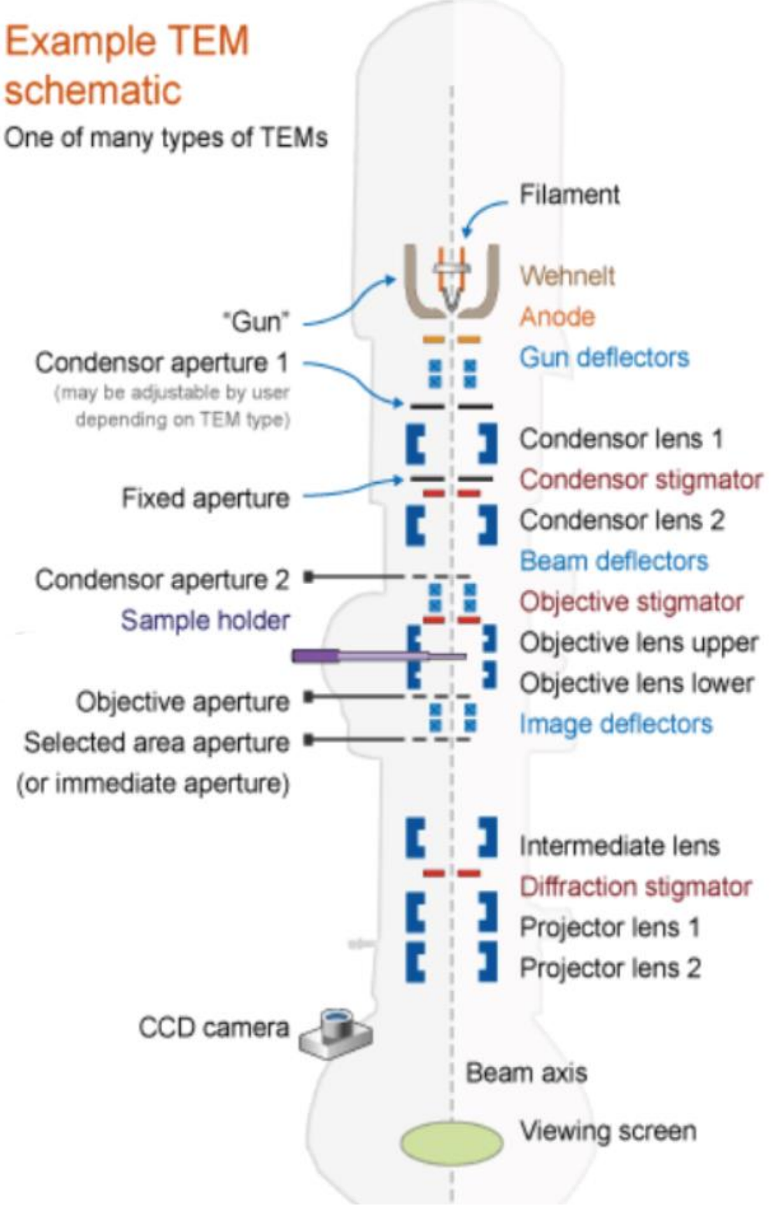
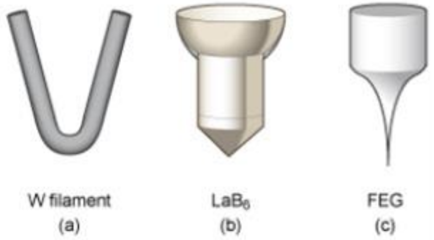
Example TEM schematic

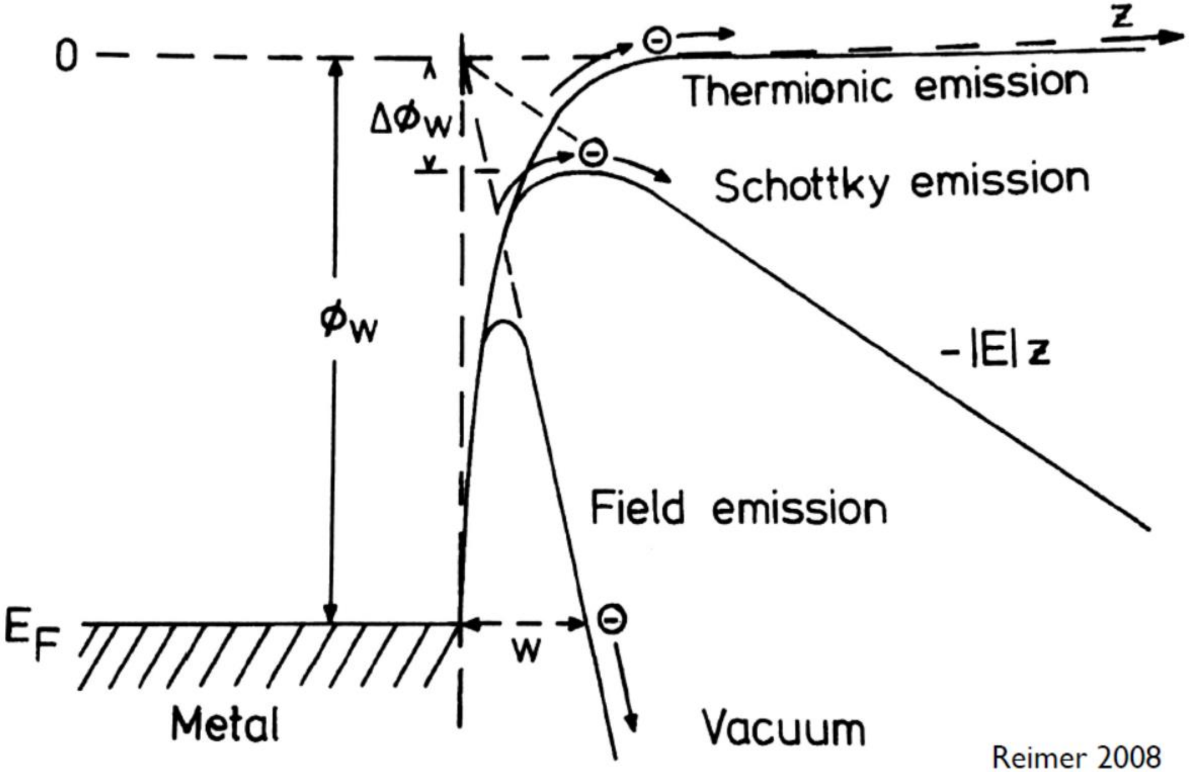
One of many types of TEMs



Source of Electrons – Electron Gun

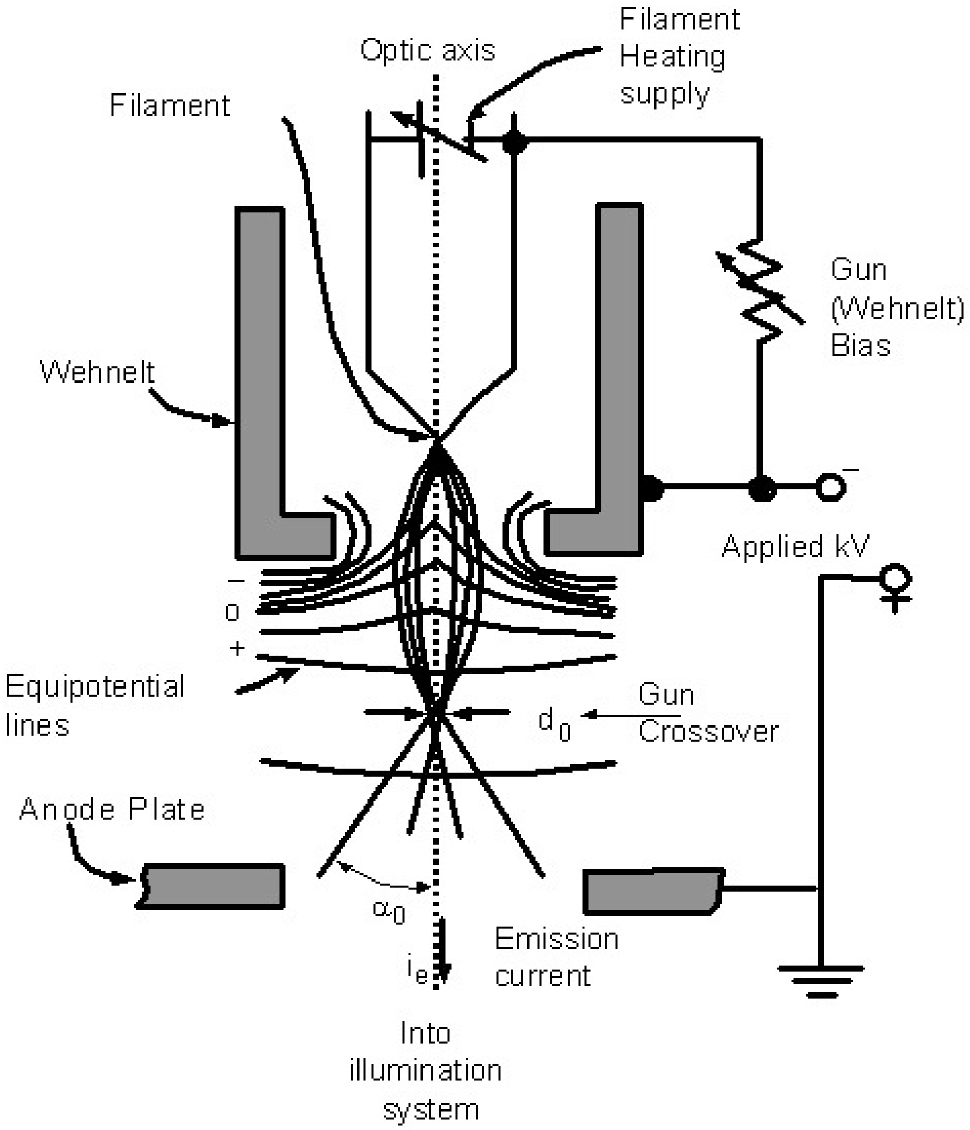
1. **Thermionic emission (Tungsten filament)**
2. **Shottky emission (LaB6 filament)**
3. **Field emission (Field Emission Gun)**

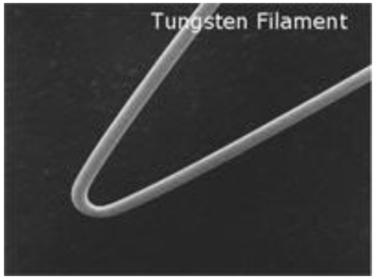


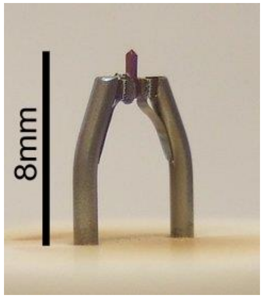


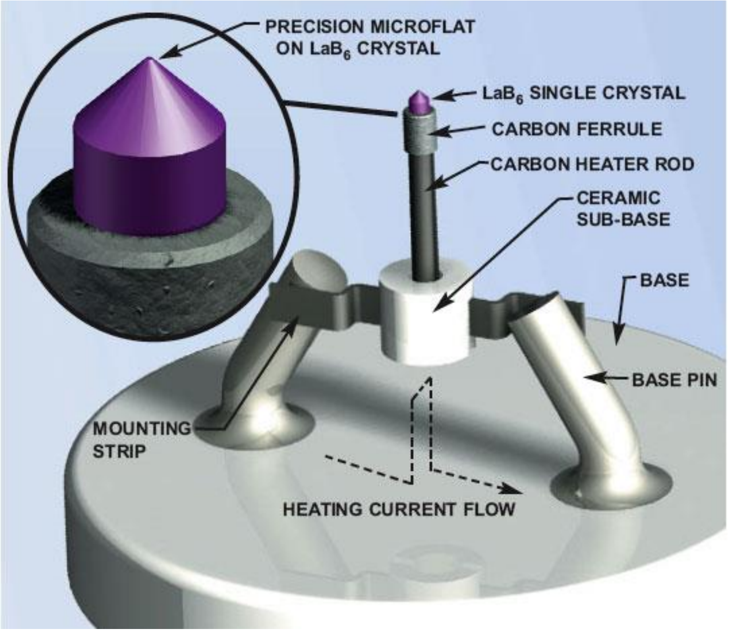
Reimer 2008

Thermal Emission Guns





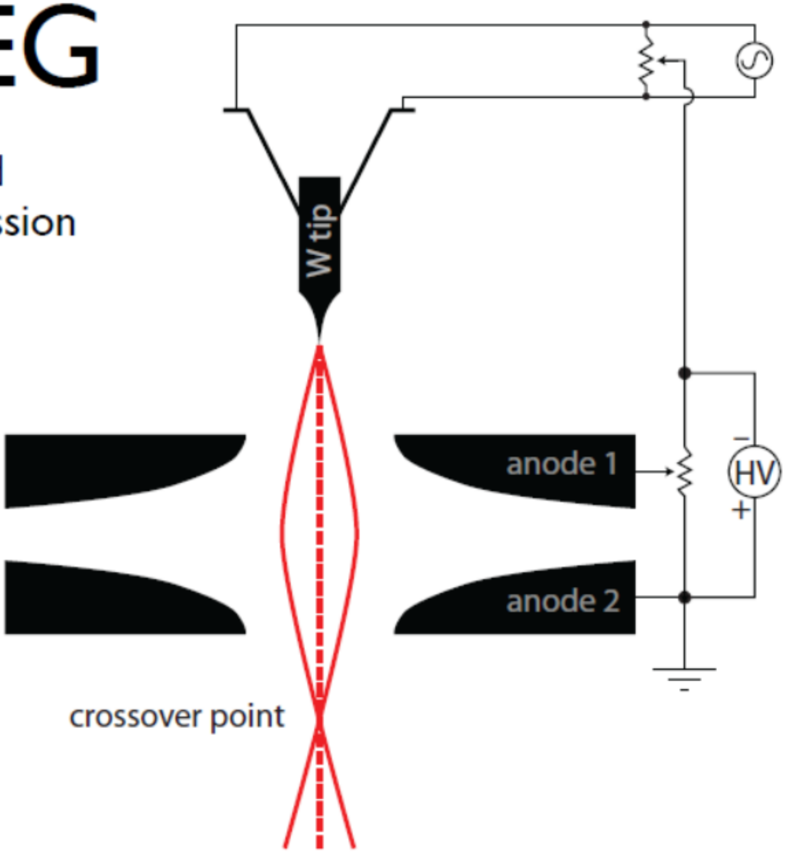


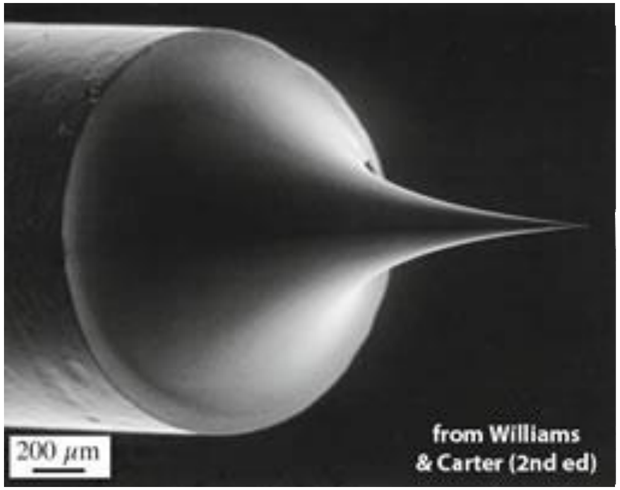


Field Emission Guns

FEG

Field
Emission
Gun

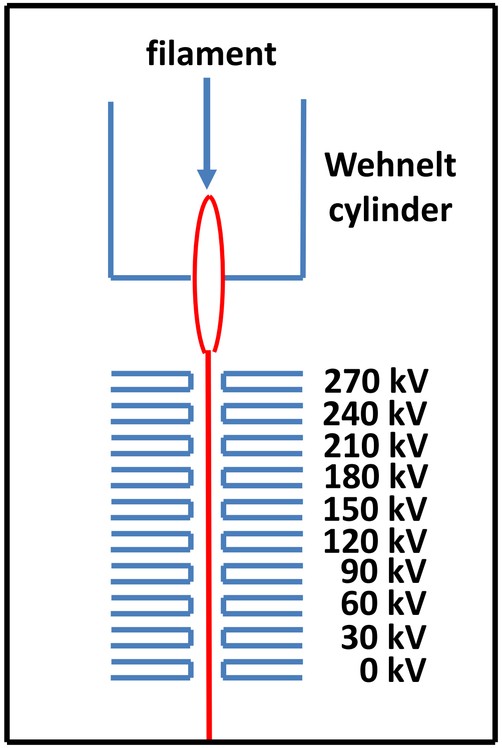


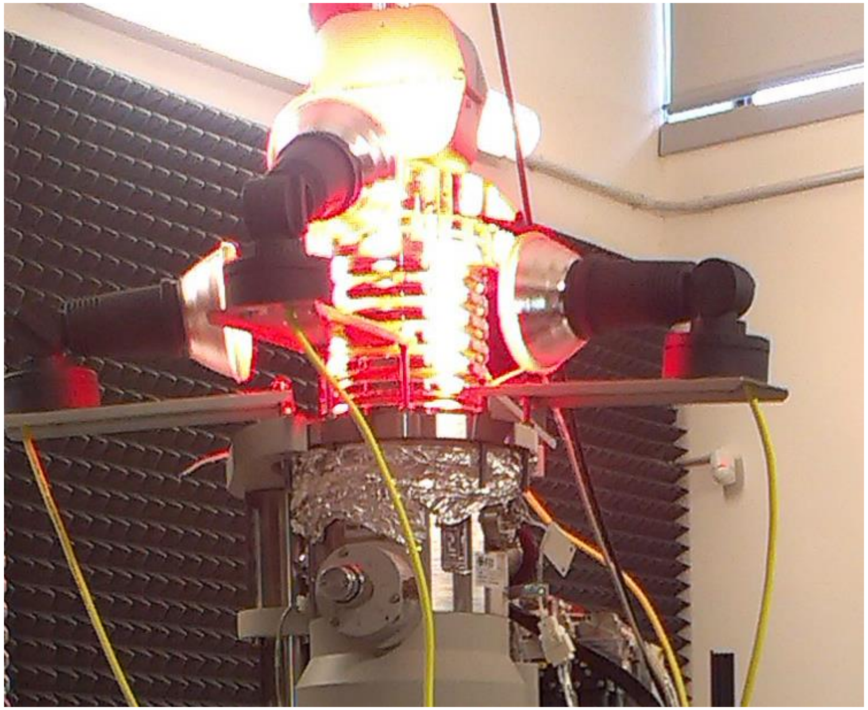


from Williams & Carter (2nd ed)

	Units	Tungsten	LaB_6	FEG
Operating Temperature	K	2700	1700	300
Current Density	A/m^2	5×10^4	10^6	10^{10}
Crossover size	μm	50	10	<0.01
Energy spread	eV	3	1.5	0.3
Stability	% / hr	<1	<1	5
Vacuum	Pa	10^{-2}	10^{-4}	10^{-8}
Lifetime	hr	100	500	>1000

Accelerator Stacks & Coherence



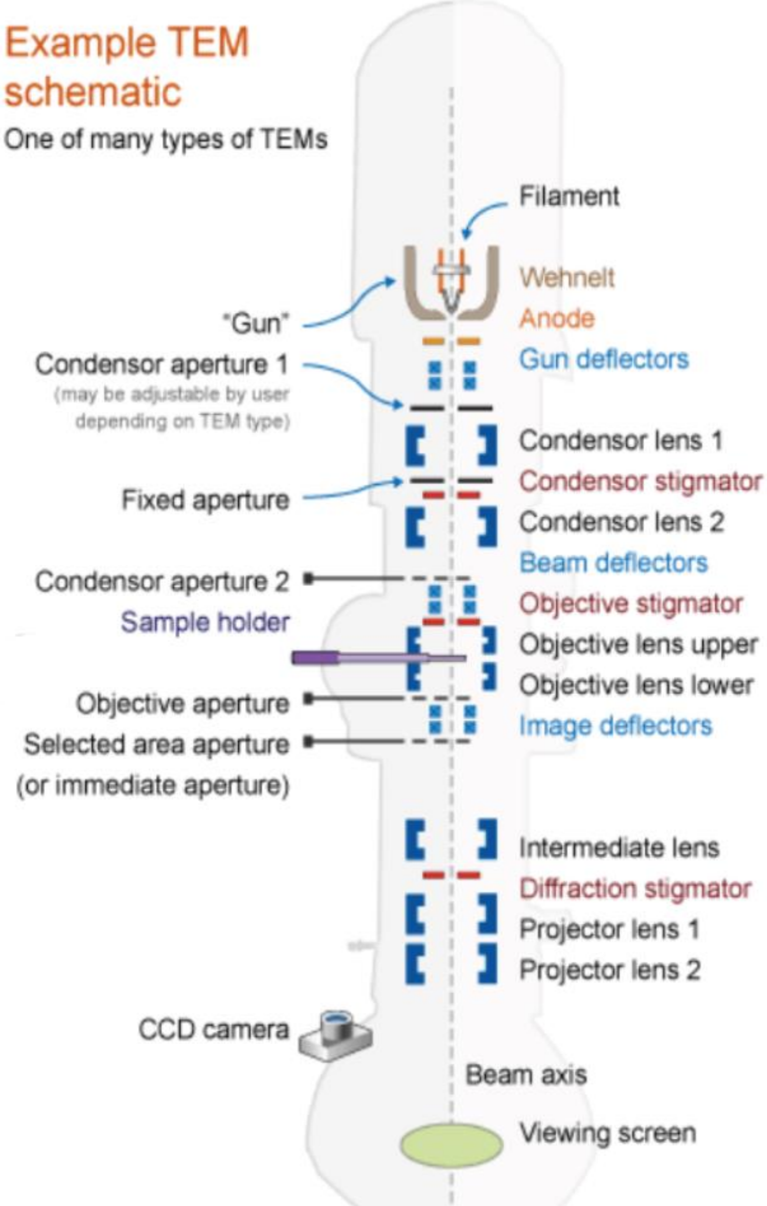


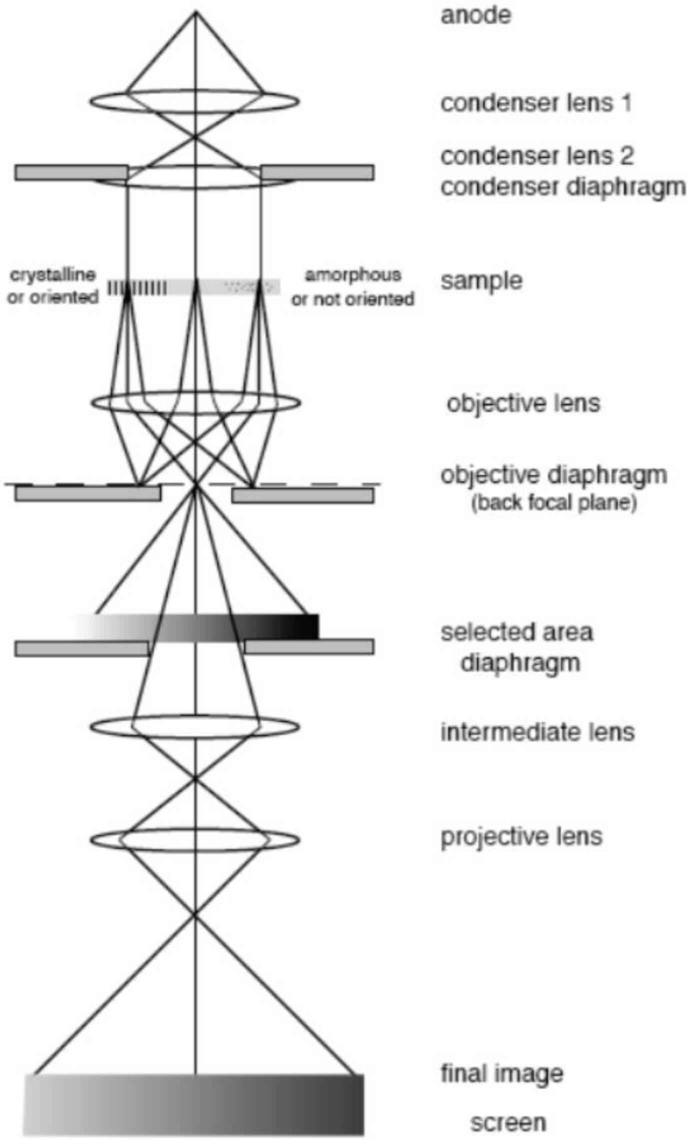
- The accelerator stacks improve both spatial and temporal coherence
- Ultra-high vacuum is required for proper function of the stacks
- Conditioning/baking of the gun chamber may be necessary

Electromagnetic Lenses

Example TEM schematic

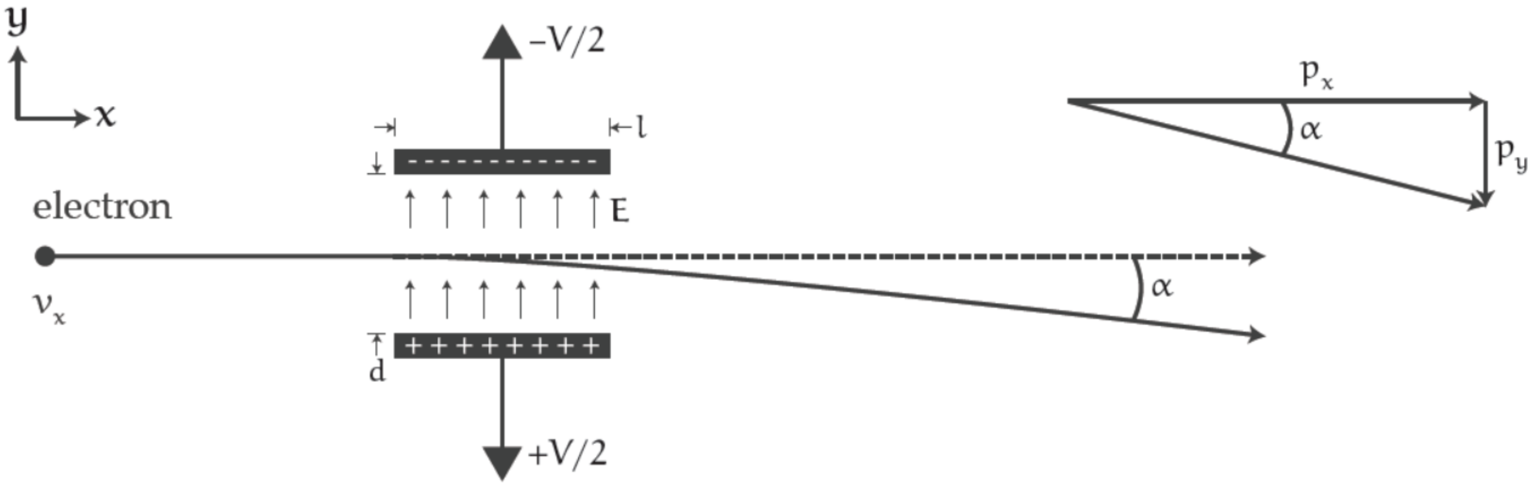
One of many types of TEMs

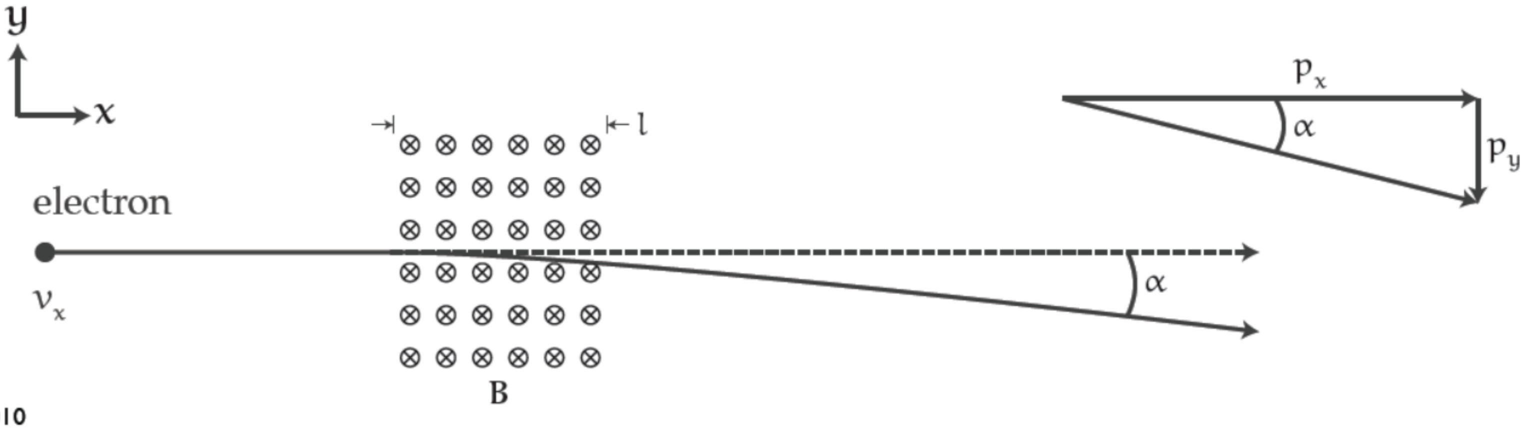




Electromagnetic Lenses

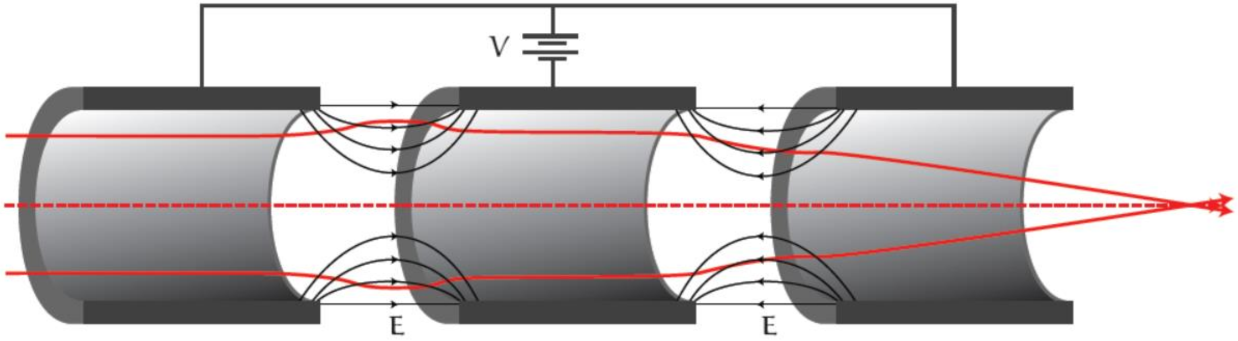
Lorentz Force: $\mathbf{F} = -q_e(\mathbf{E} + \mathbf{v} \times \mathbf{B})$

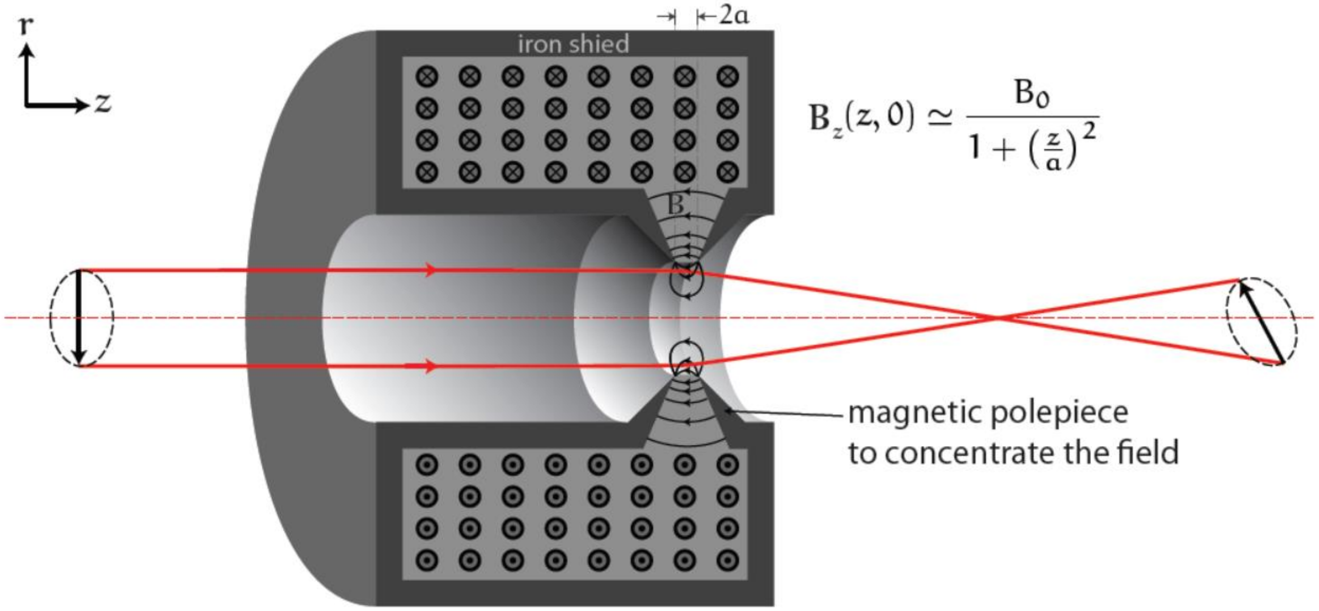




Russo 2010

Electromagnetic Lenses

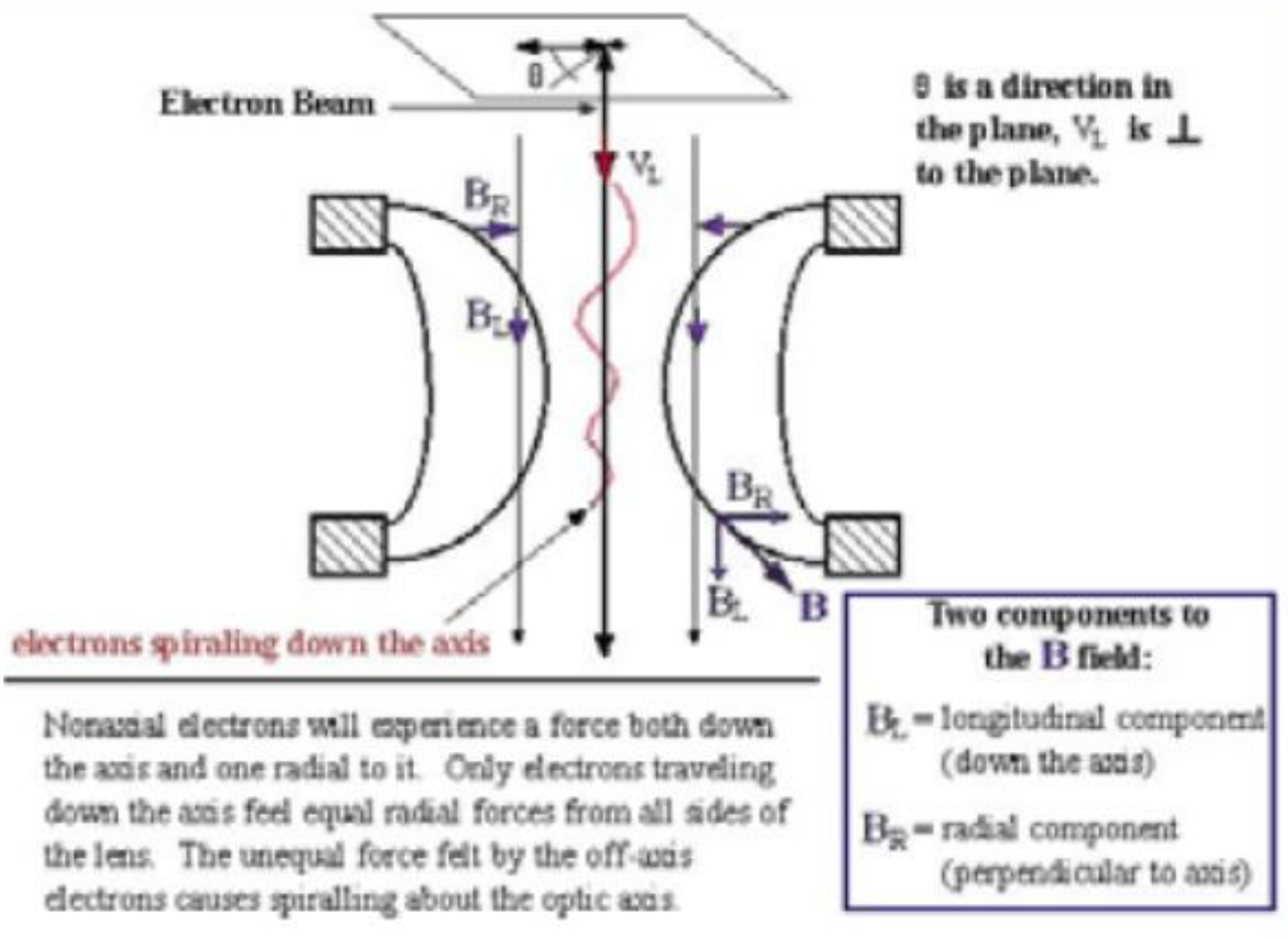




$$B_z(z,0) \simeq \frac{B_0}{1+\left(\frac{z}{a}\right)^2}$$

magnetic polepiece
to concentrate the field

Electromagnetic Lenses



θ is a direction in the plane, $V_\perp$ is ⊥ to the plane.

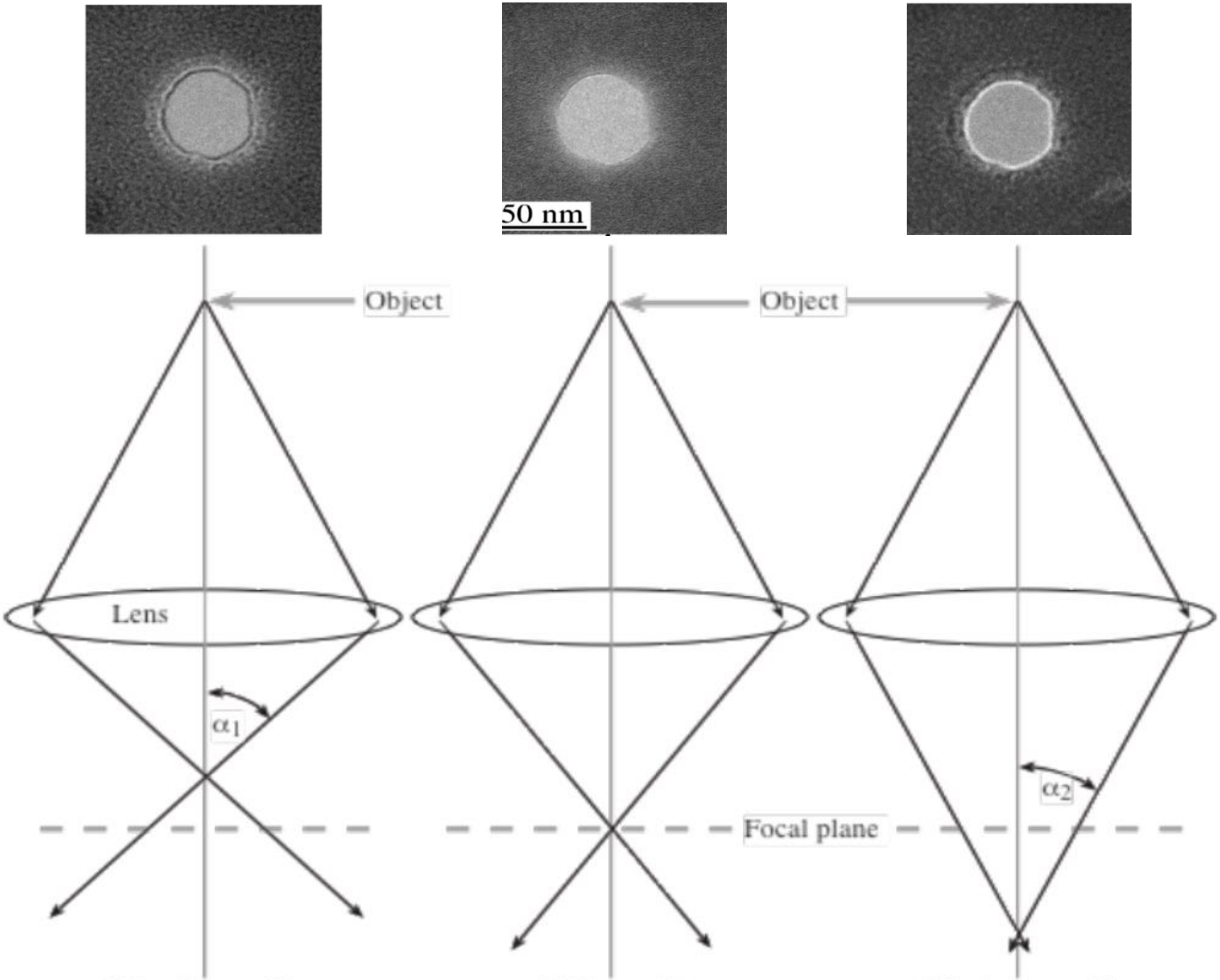
Nonaxial electrons will experience a force both down the axis and one radial to it. Only electrons traveling down the axis feel equal radial forces from all sides of the lens. The unequal force felt by the off-axis electrons causes spiralling about the optic axis.

Two components to the B field:

B_L = longitudinal component (down the axis)

B_R = radial component (perpendicular to axis)

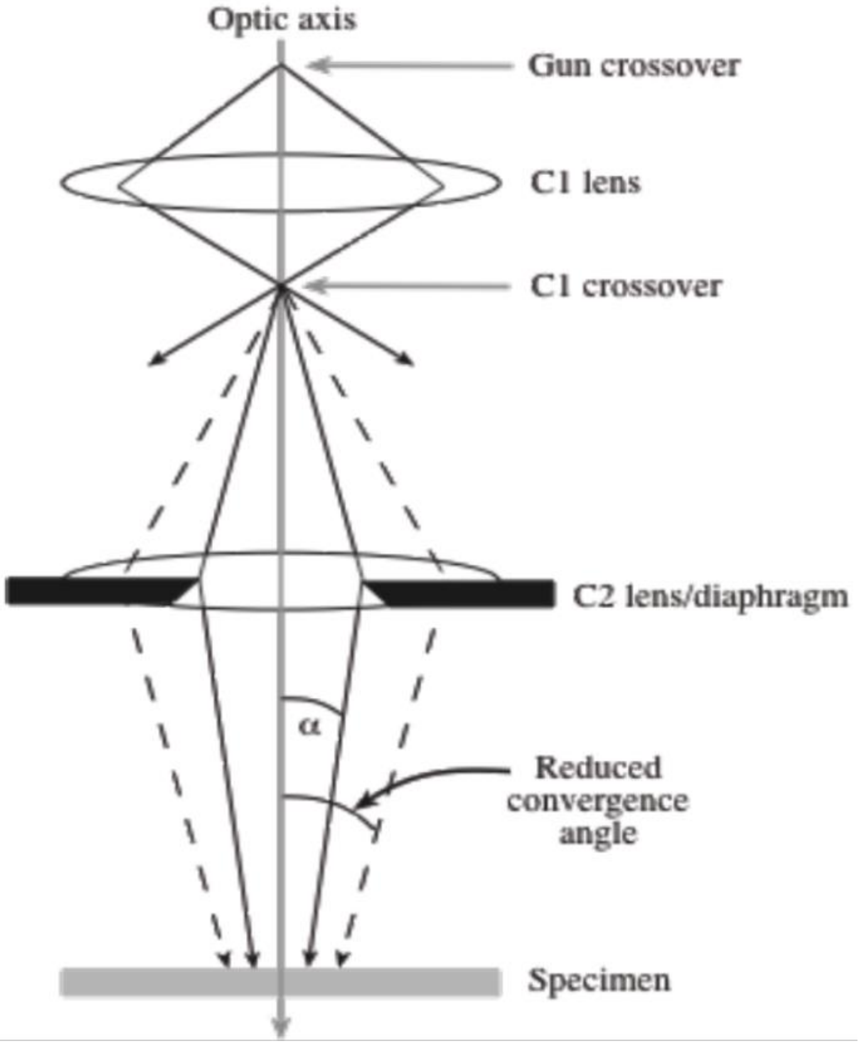
Electromagnetic Lenses



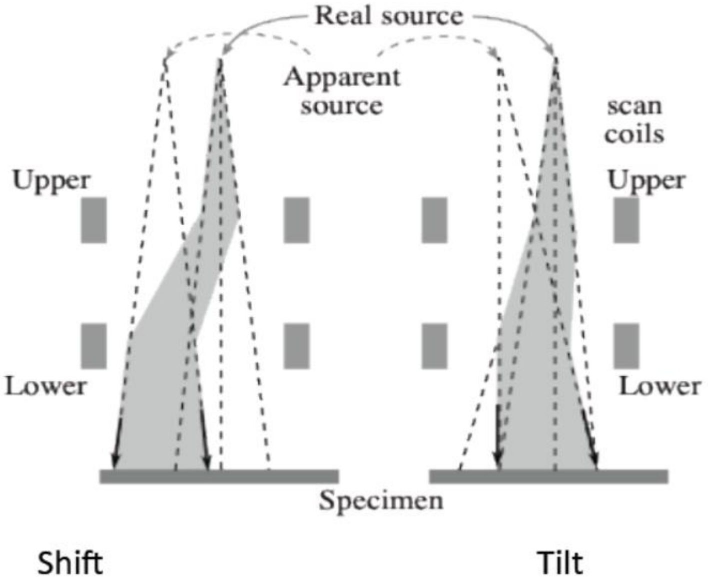
a) Overfocused lens b) Focused lens c) Underfocused lens

Electromagnetic Lenses

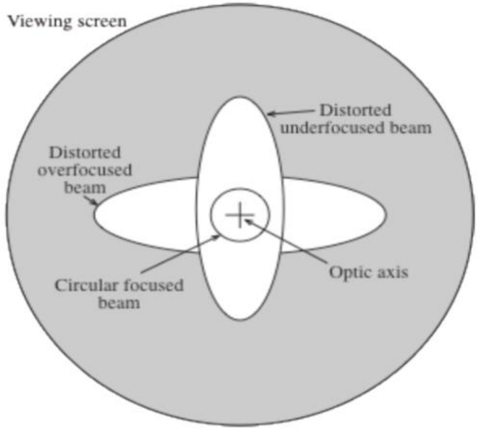
Basic TEM condenser system



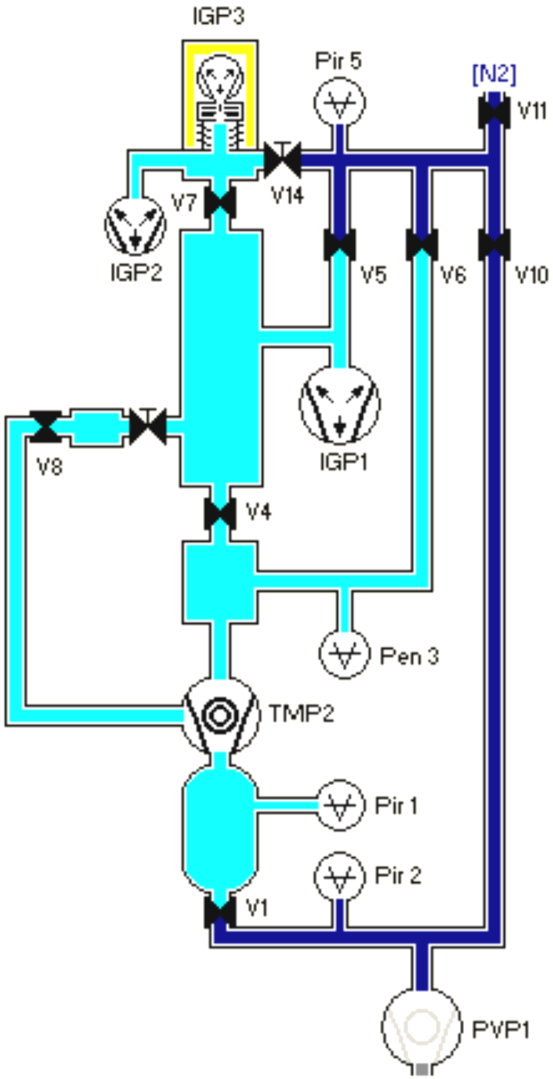
Beam deflectors (tilt/shift)



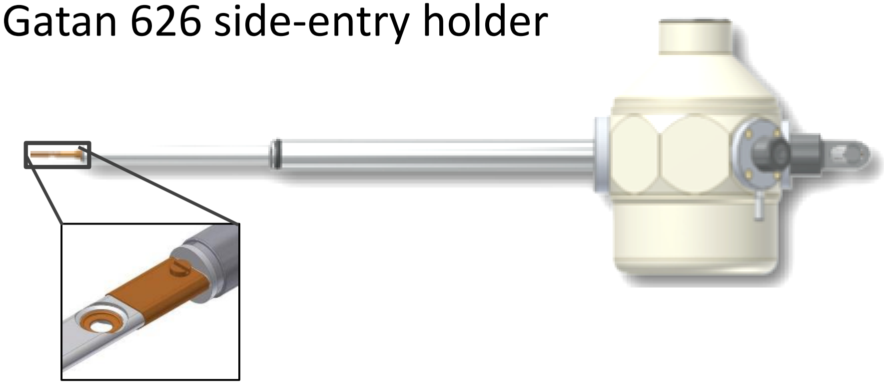
Beam stigmators (astigmatism)

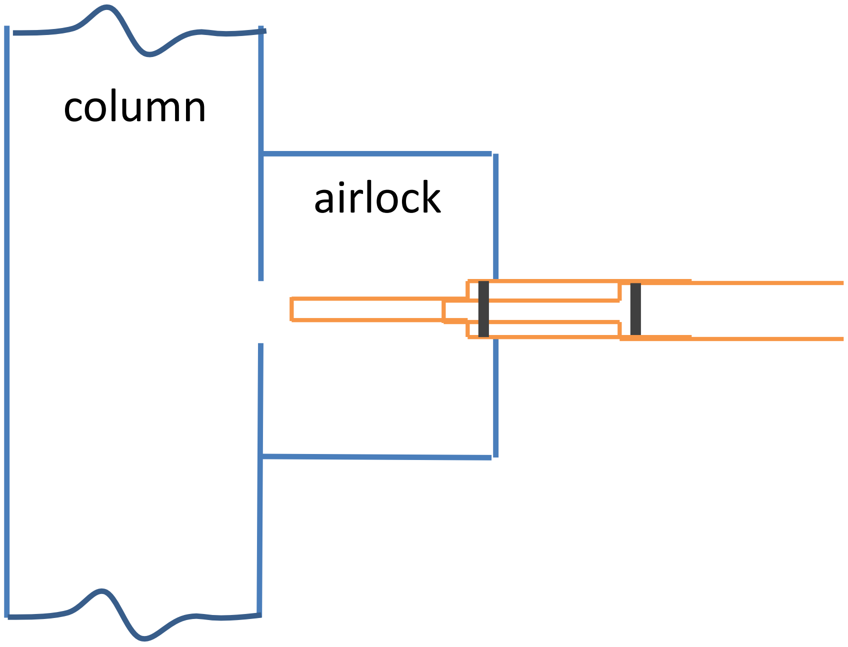


Sample Chamber / Vacuum System



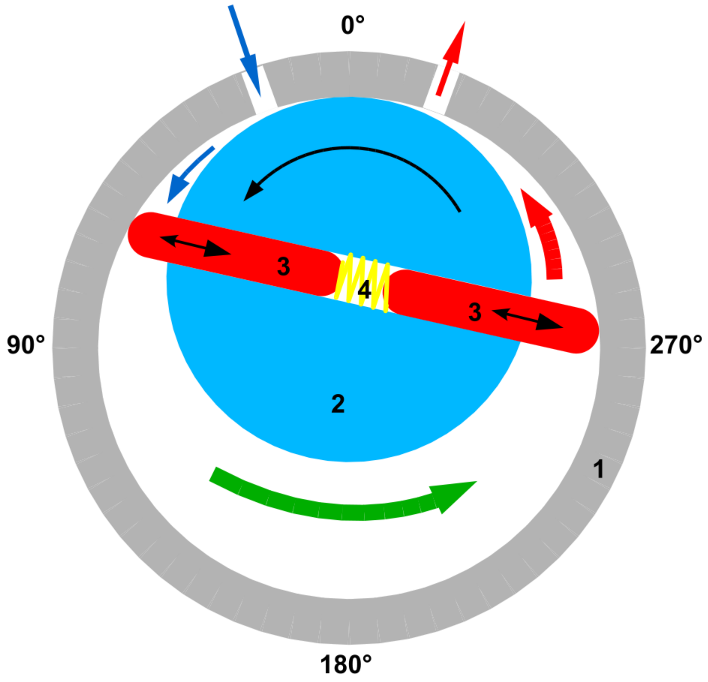
Gatan 626 side-entry holder





Vacuum System

1. Rotary pump (low vacuum up to 10^{5}–10^{-4} Pa)
2. Diffusion pump (high vacuum 10^{0}–10^{-8} Pa)
3. Turbo molecular pump (high vacuum 10^{-2}–10^{-8} Pa)
4. Ion getter pump (ultrahigh vacuum up to 10^{-9} Pa)



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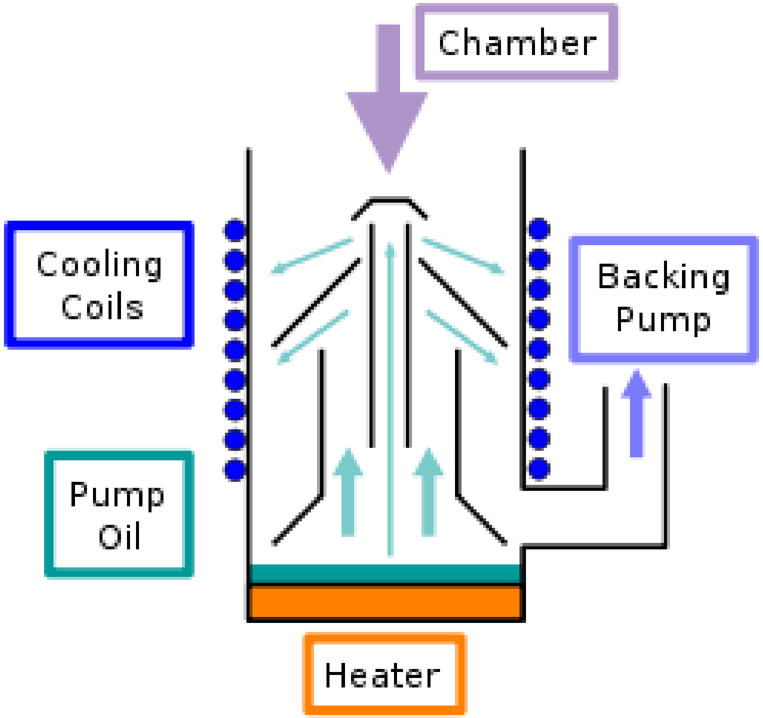

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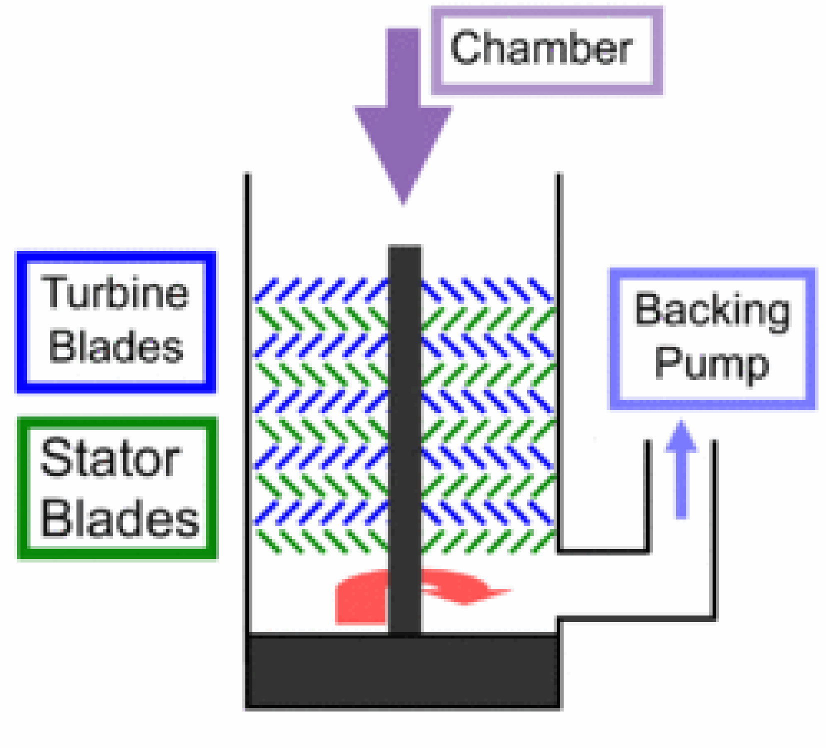

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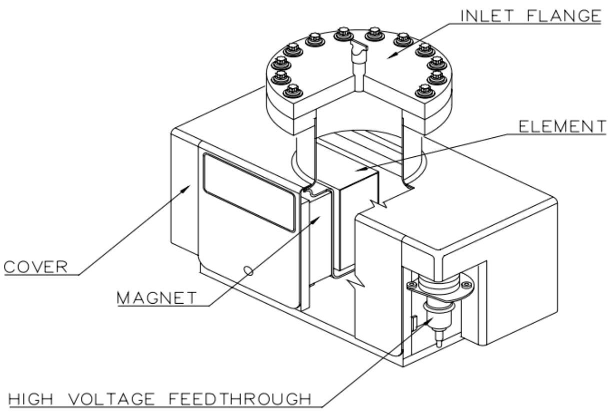
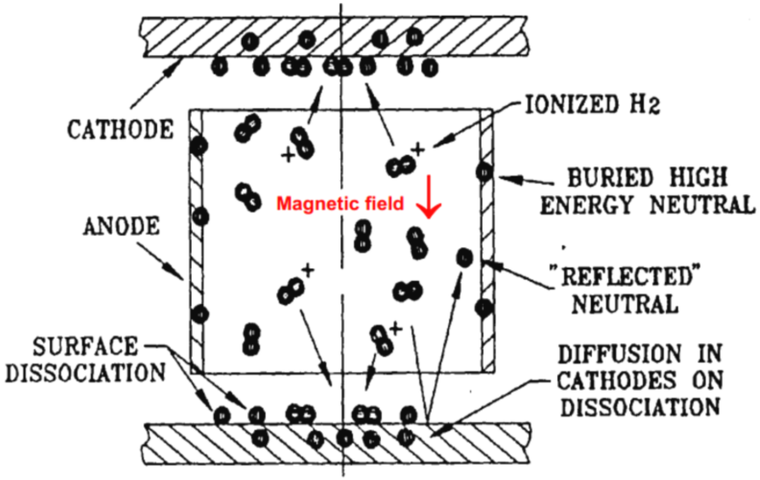
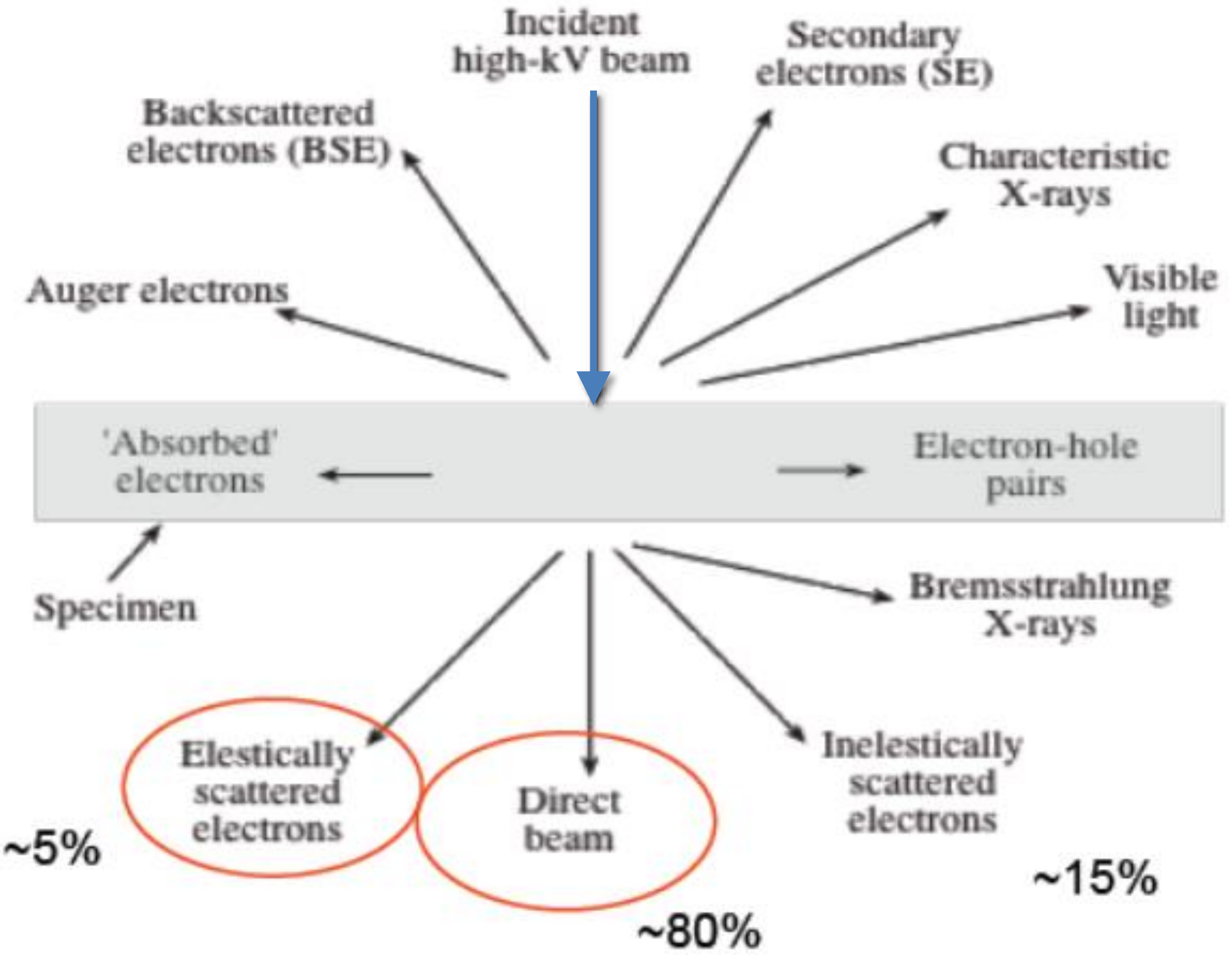




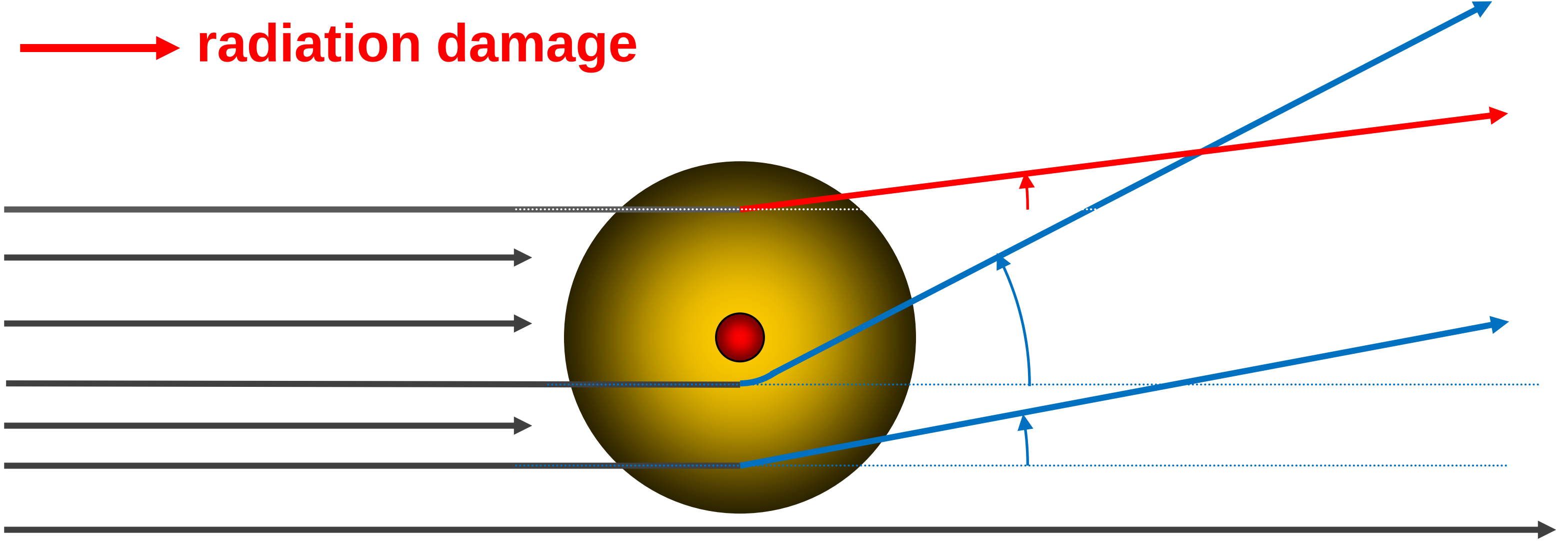
Interaction of Electrons with Matter



Interaction of Electrons with Matter

Inelastic scattering (0-0.001 rad)

→ **radiation damage**



Elastic scattering (0-0.1 rad)

→ **small angles:** **phase contrast**

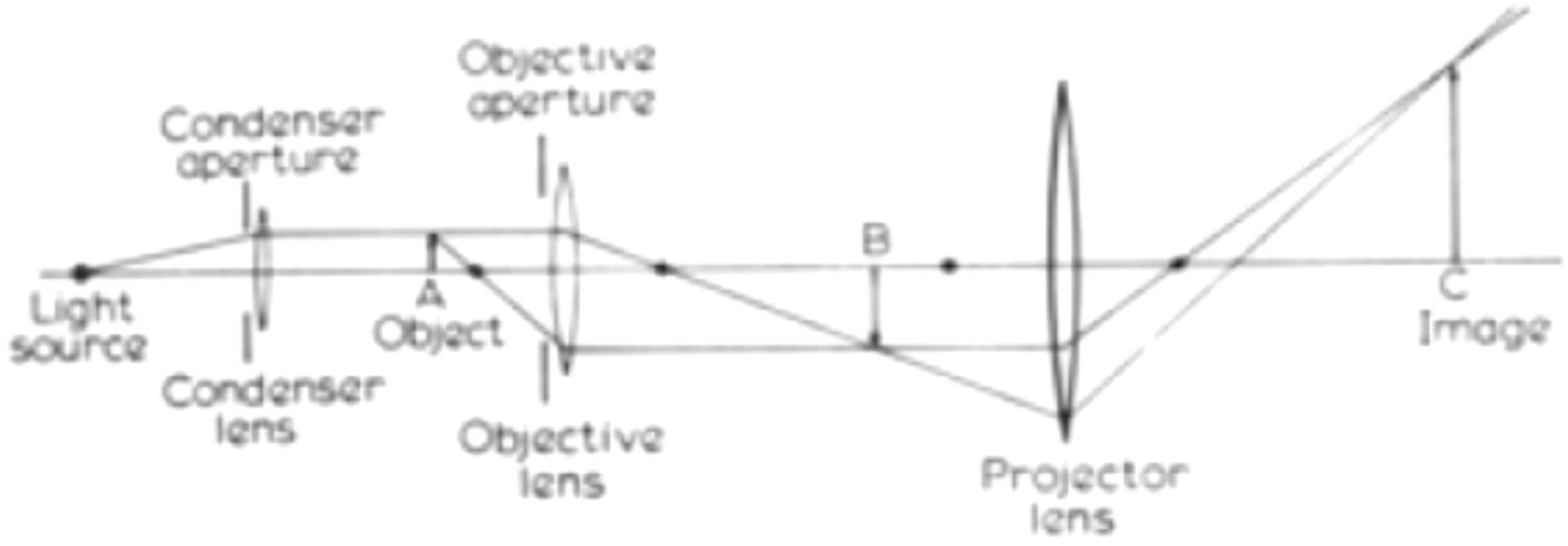
→ **large angles:** **scattering contrast**

- Transmission EM image is formed by the elastically scattered electrons
- Electrons are scattered by local EM field of both electrons and nuclei in the sample
- Electrons are scattered much more than X-rays => can image individual molecules

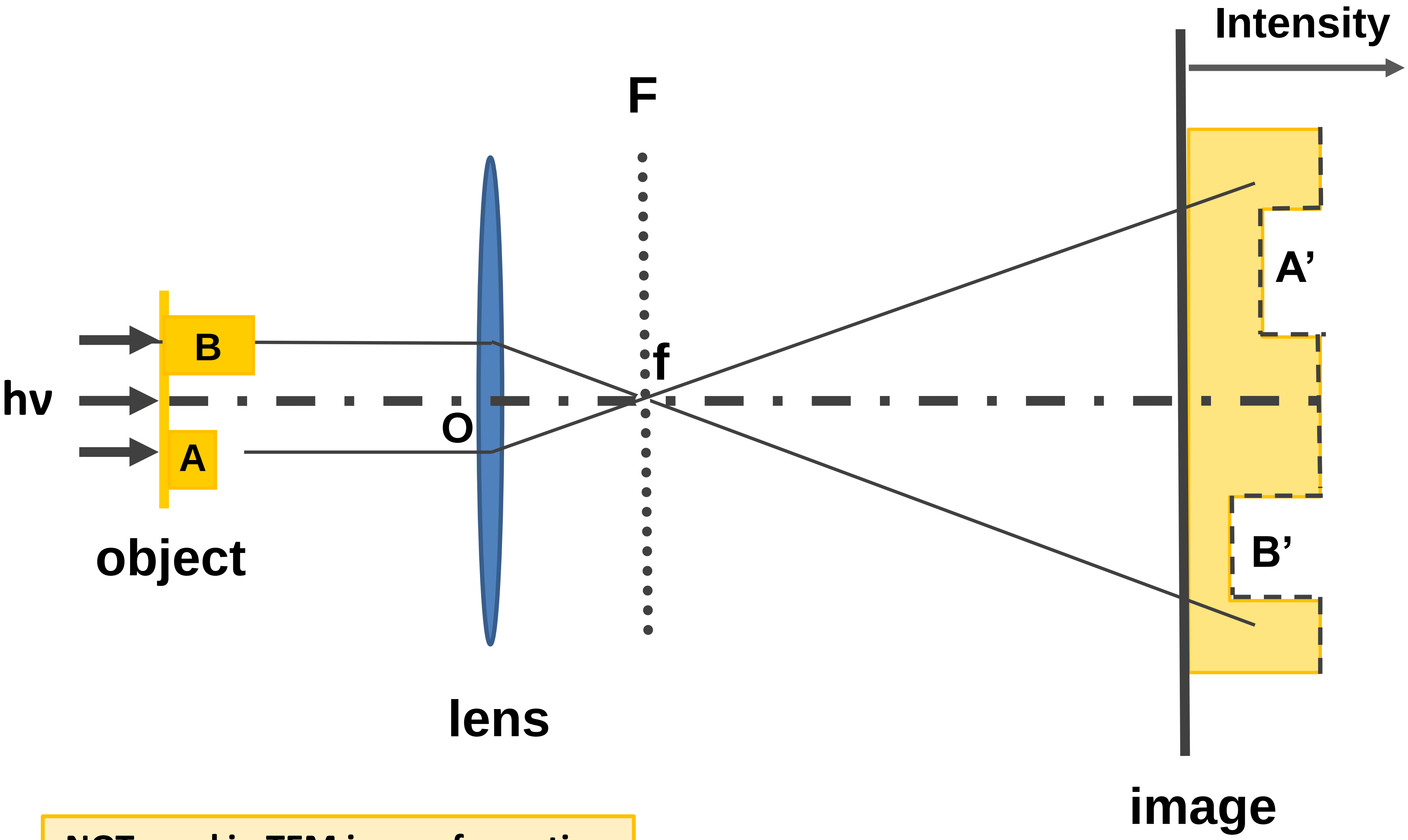
Wave–Particle Duality

- Electrons, as photons, exhibit the wave-particle duality
- Electrons collide with other particles (atoms) and are scattered as particles
- Electrons also behave as (almost) a plane wave and are diffracted by atoms

- TEM image is formed by interference of scattered and non-scattered electrons
- Speed of 300 keV electrons is 0.76 c => electrons exhibit relativistic effects
- At usual currents (0.1 nA), there is only one electron in the column at the time

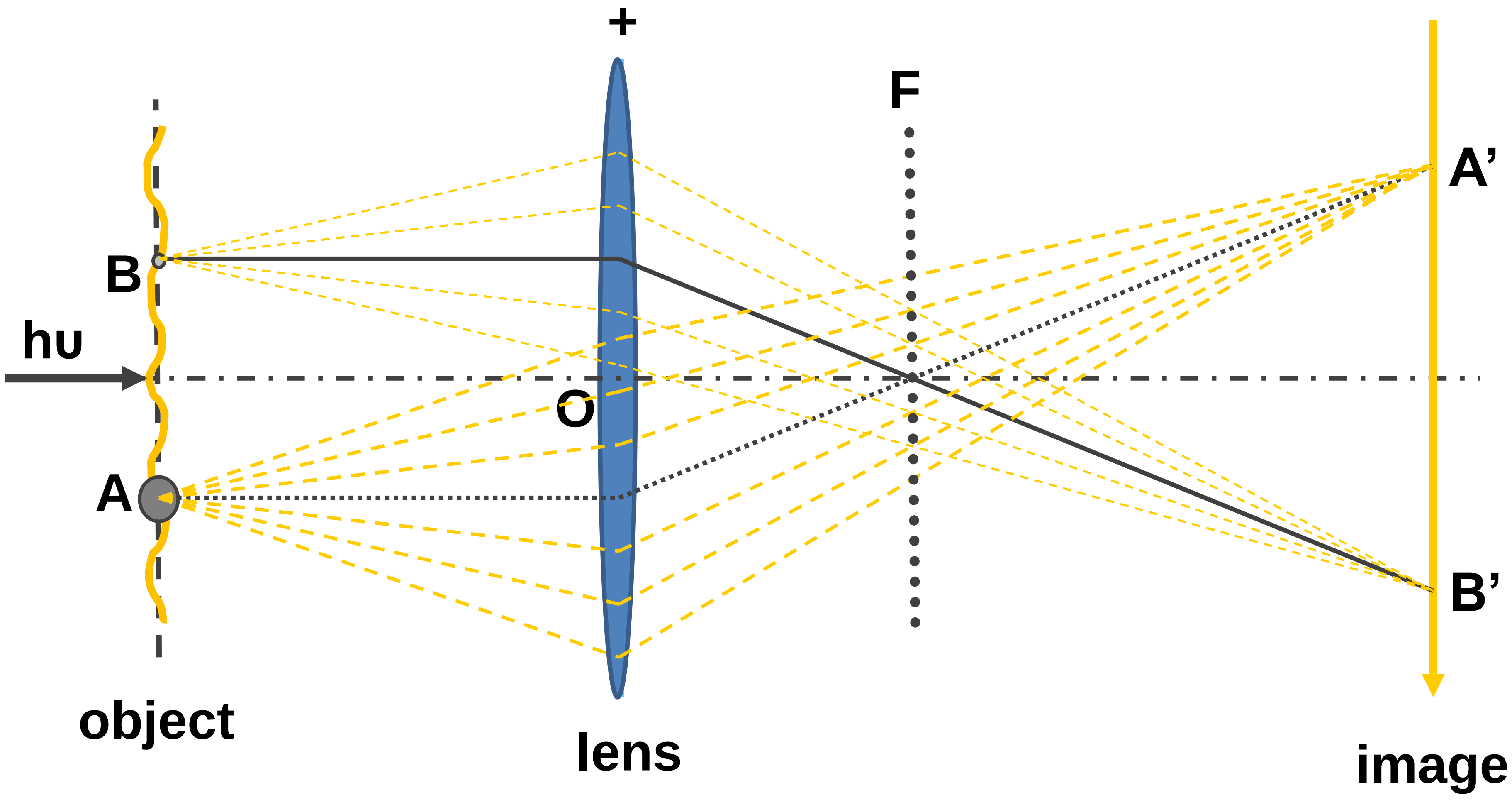


Absorption Contrast

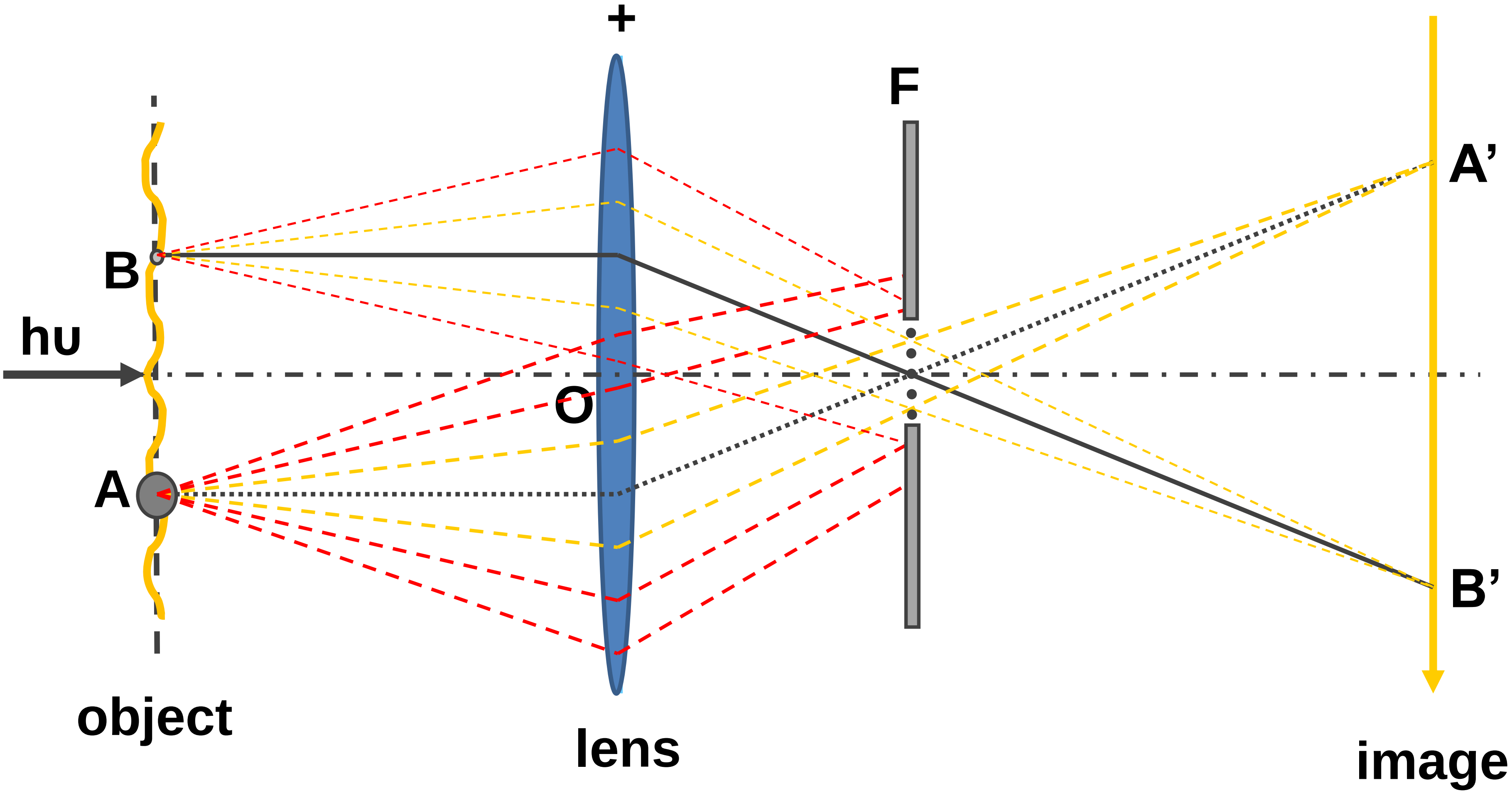


NOT used in TEM image formation

Scattering Contrast

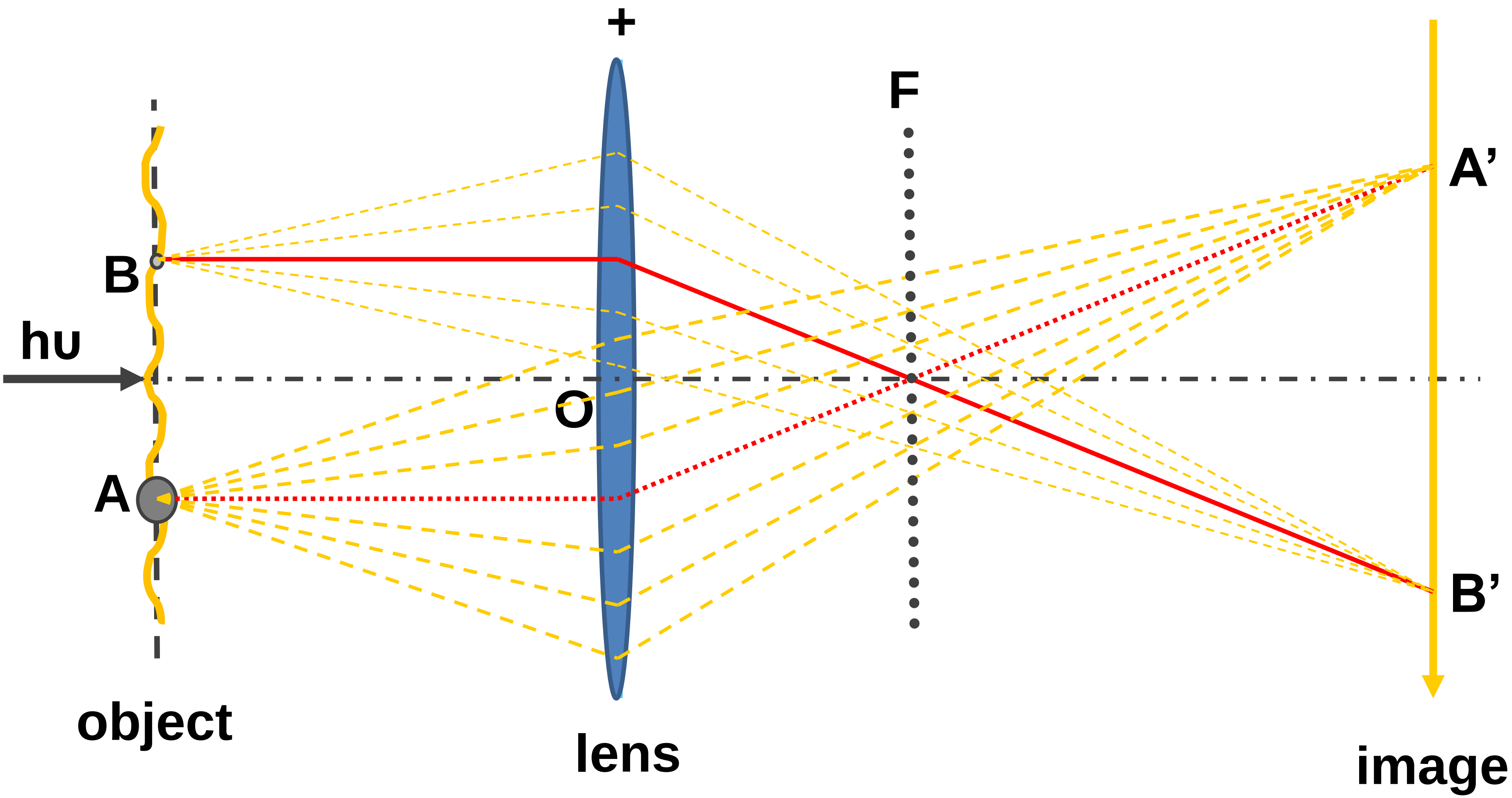


Scattering Contrast



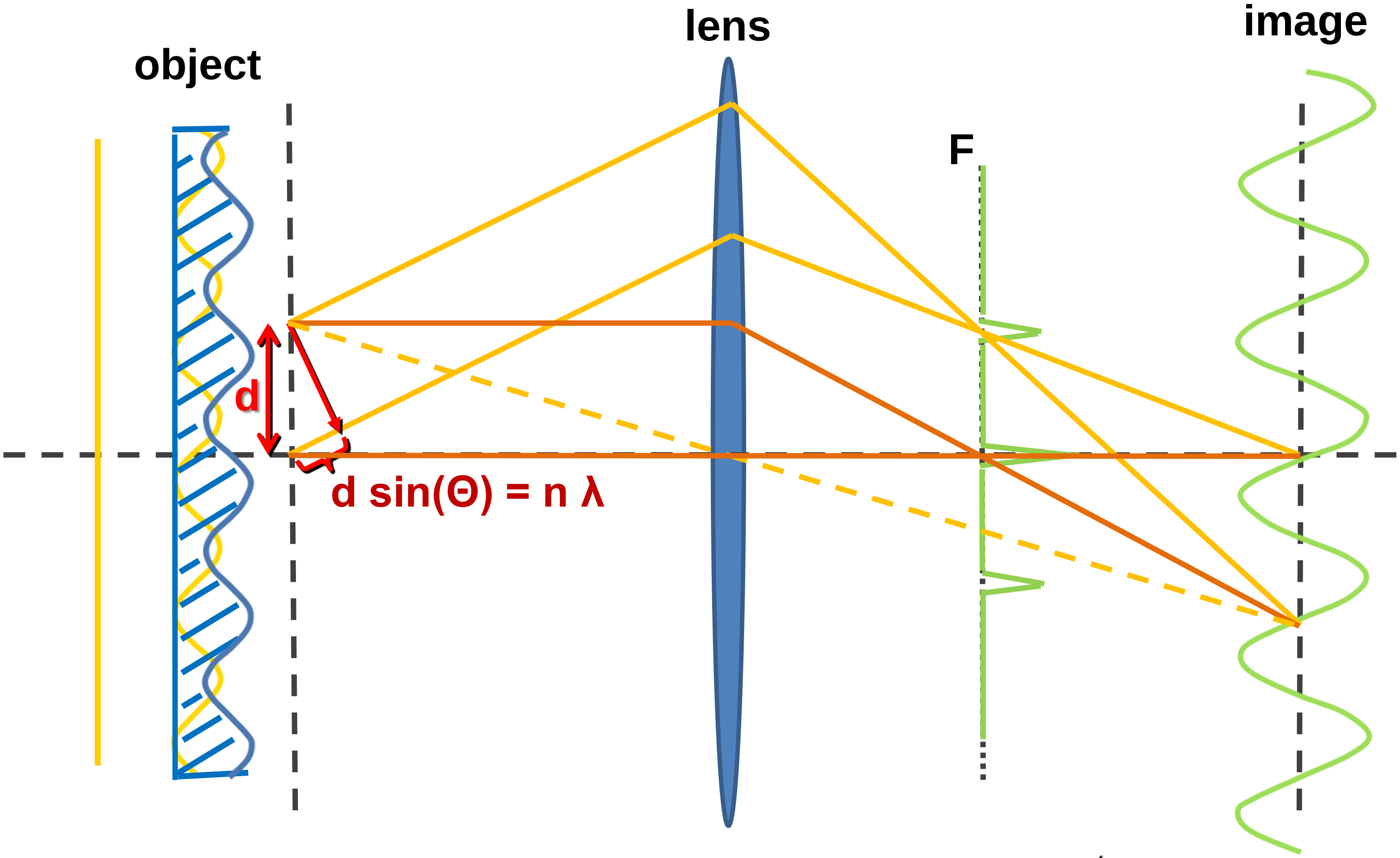
The scattering area appears dark in the image.

Phase Contrast



The image contrast is described by Phase Contrast Transfer Function.

EM Image and Diffraction Formation

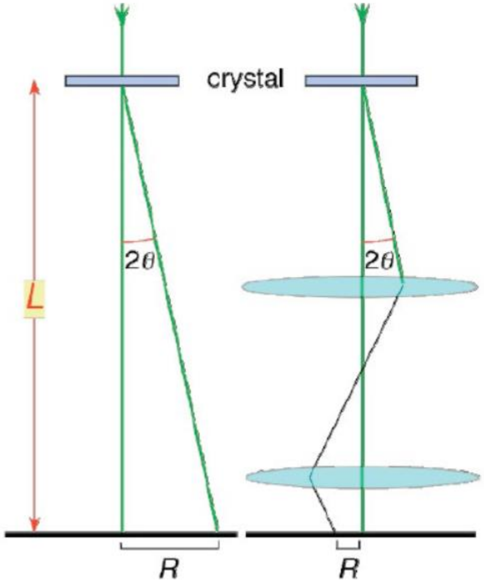


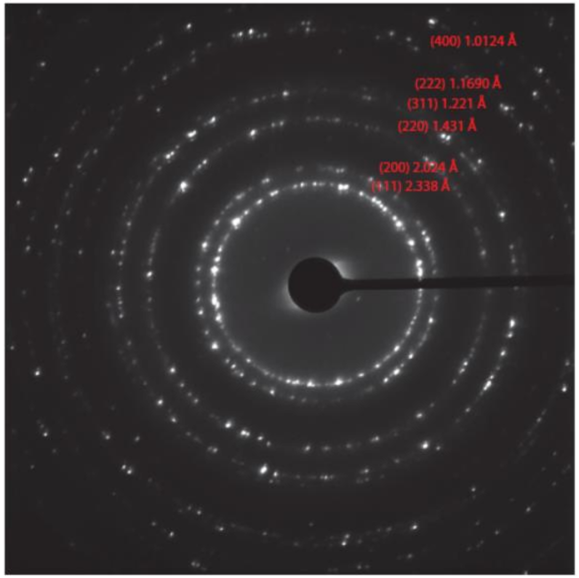
Thin phase object approximation: $I(x,y) = \int_{z=0}^{t} V(x,y,z)\partial z$

EM Diffraction

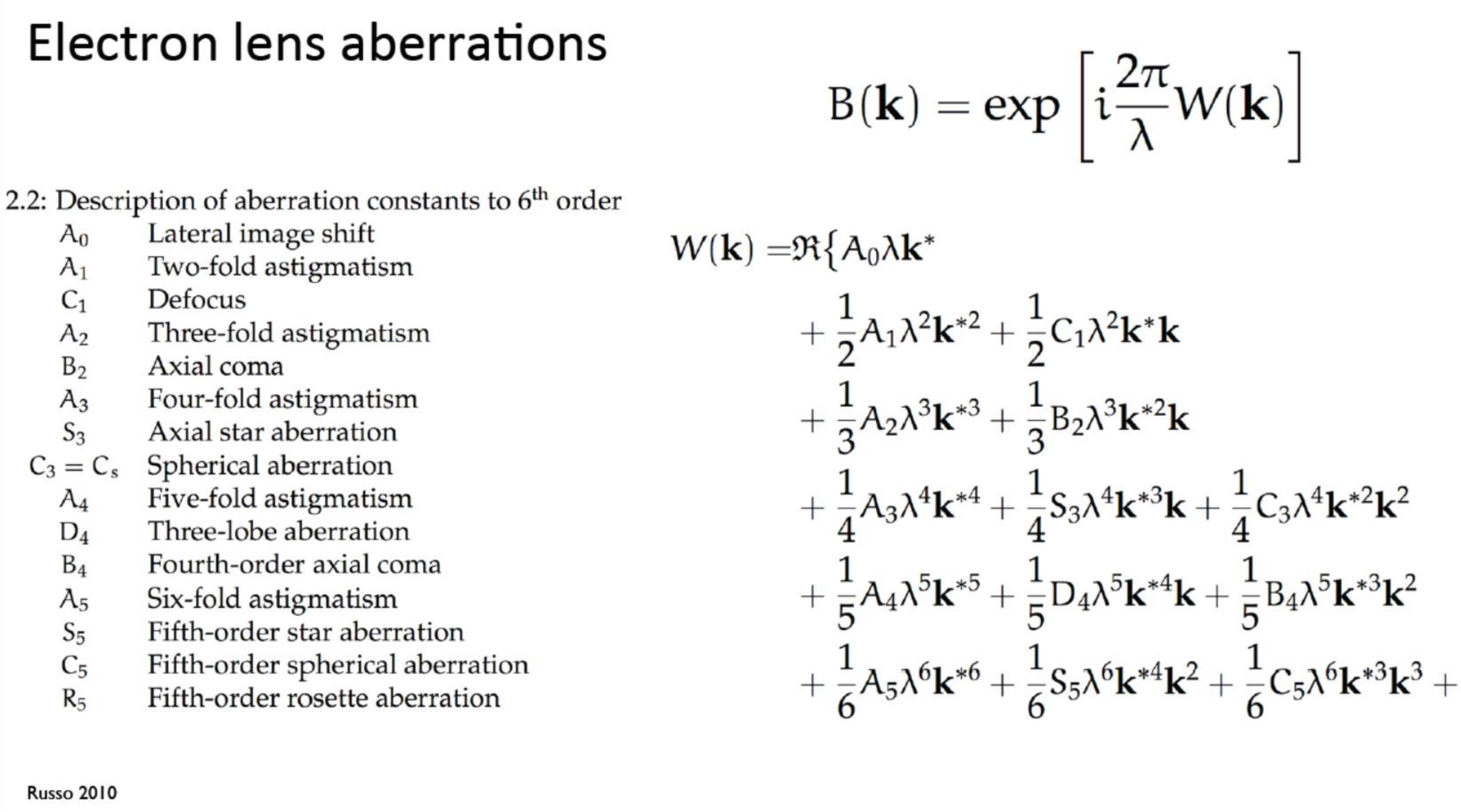
- Magnification of diffraction pattern
- Distance between object and screen
- Effective length with imaging lenses included
- L= R/2q

- Good: very high resolution
  - 2 Å on a T12!
- Bad: phase problem; need a crystal





CTF and Aberrations



Electron lens aberrations

$$B(\mathbf{k}) = \exp\left[i\frac{2\pi}{\lambda}W(\mathbf{k})\right]$$

2.2: Description of aberration constants to 6th order

A_0	Lateral image shift
A_1	Two-fold astigmatism
C_1	Defocus
A_2	Three-fold astigmatism
B_2	Axial coma
A_3	Four-fold astigmatism
S_3	Axial star aberration
$C_3 = C_s$	Spherical aberration
A_4	Five-fold astigmatism
D_4	Three-lobe aberration
B_4	Fourth-order axial coma
A_5	Six-fold astigmatism
S_5	Fifth-order star aberration
C_5	Fifth-order spherical aberration
R_5	Fifth-order rosette aberration

$$\begin{aligned} W(\mathbf{k}) = \Re\{ & A_0\lambda\mathbf{k}^* \\ & + \frac{1}{2}A_1\lambda^2\mathbf{k}^{*2} + \frac{1}{2}C_1\lambda^2\mathbf{k}^*\mathbf{k} \\ & + \frac{1}{3}A_2\lambda^3\mathbf{k}^{*3} + \frac{1}{3}B_2\lambda^3\mathbf{k}^{*2}\mathbf{k} \\ & + \frac{1}{4}A_3\lambda^4\mathbf{k}^{*4} + \frac{1}{4}S_3\lambda^4\mathbf{k}^{*3}\mathbf{k} + \frac{1}{4}C_3\lambda^4\mathbf{k}^{*2}\mathbf{k}^2 \\ & + \frac{1}{5}A_4\lambda^5\mathbf{k}^{*5} + \frac{1}{5}D_4\lambda^5\mathbf{k}^{*4}\mathbf{k} + \frac{1}{5}B_4\lambda^5\mathbf{k}^{*3}\mathbf{k}^2 \\ & + \frac{1}{6}A_5\lambda^6\mathbf{k}^{*6} + \frac{1}{6}S_5\lambda^6\mathbf{k}^{*4}\mathbf{k}^2 + \frac{1}{6}C_5\lambda^6\mathbf{k}^{*3}\mathbf{k}^3 + \end{aligned}$$

Russo 2010

Microscope Aberrations and CTF

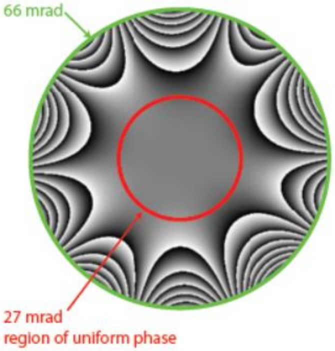
Lens aberrations can also be visualized using Zernike polynomials

Aberrations are corrected with additional lenses in the microscope or in software after the image is collected (“CTF correction”)

Complete set of orthogonal functions

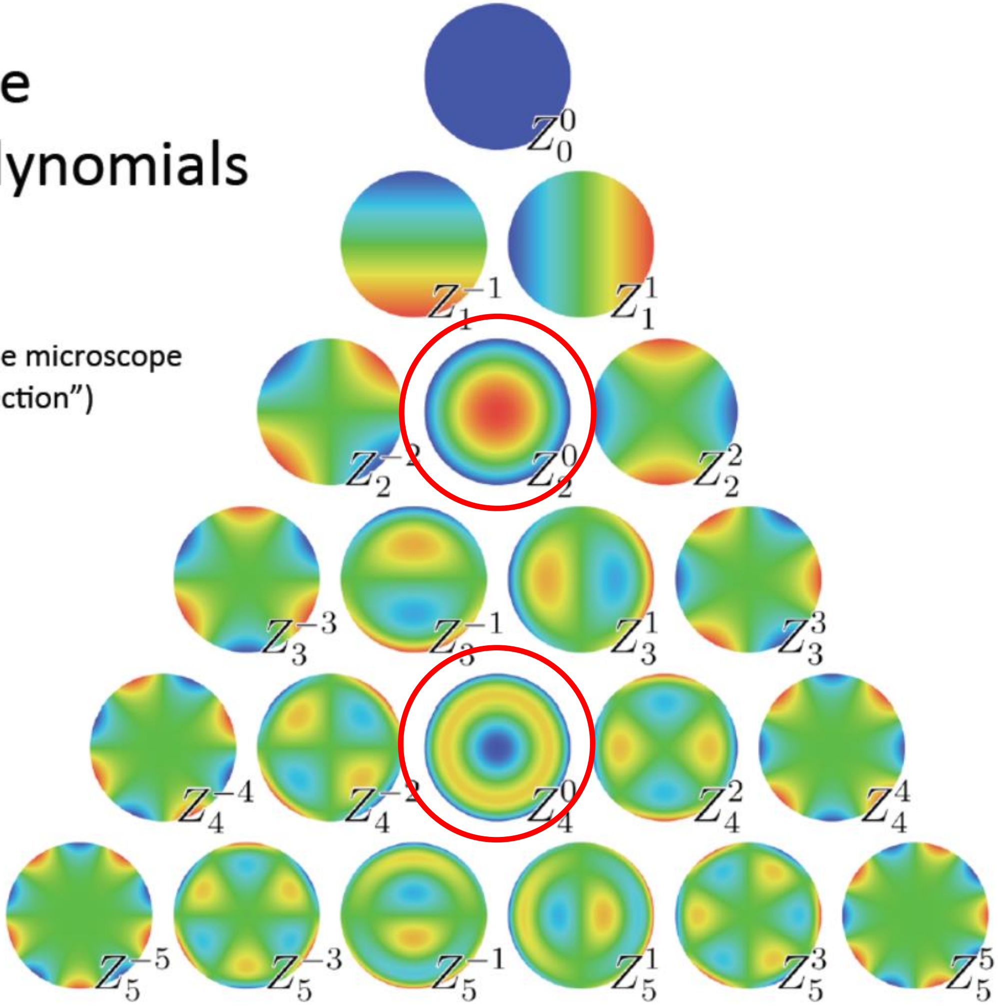
Zernike transform analogous to Fourier transform

Wikipedia 2014



66 mrad

27 mrad region of uniform phase



Microscope Aberrations and CTF

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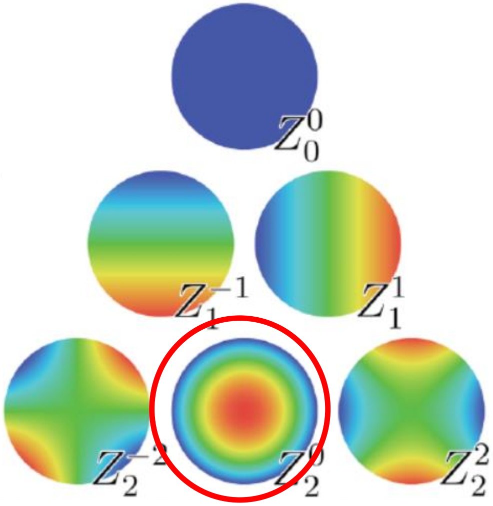

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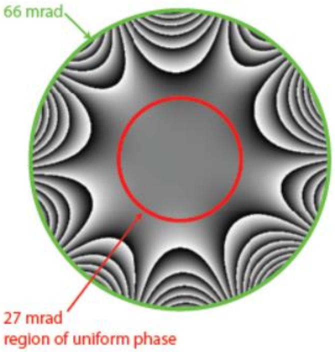
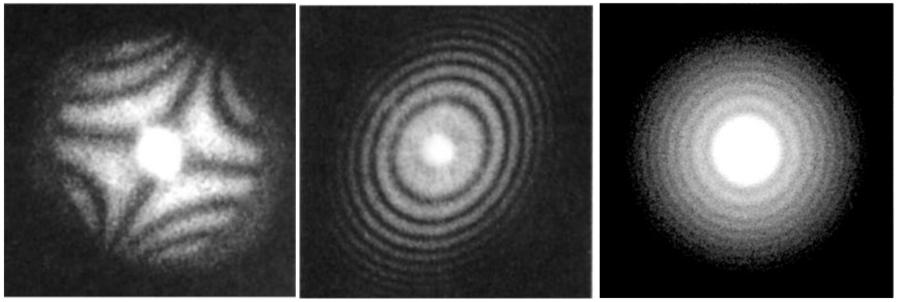
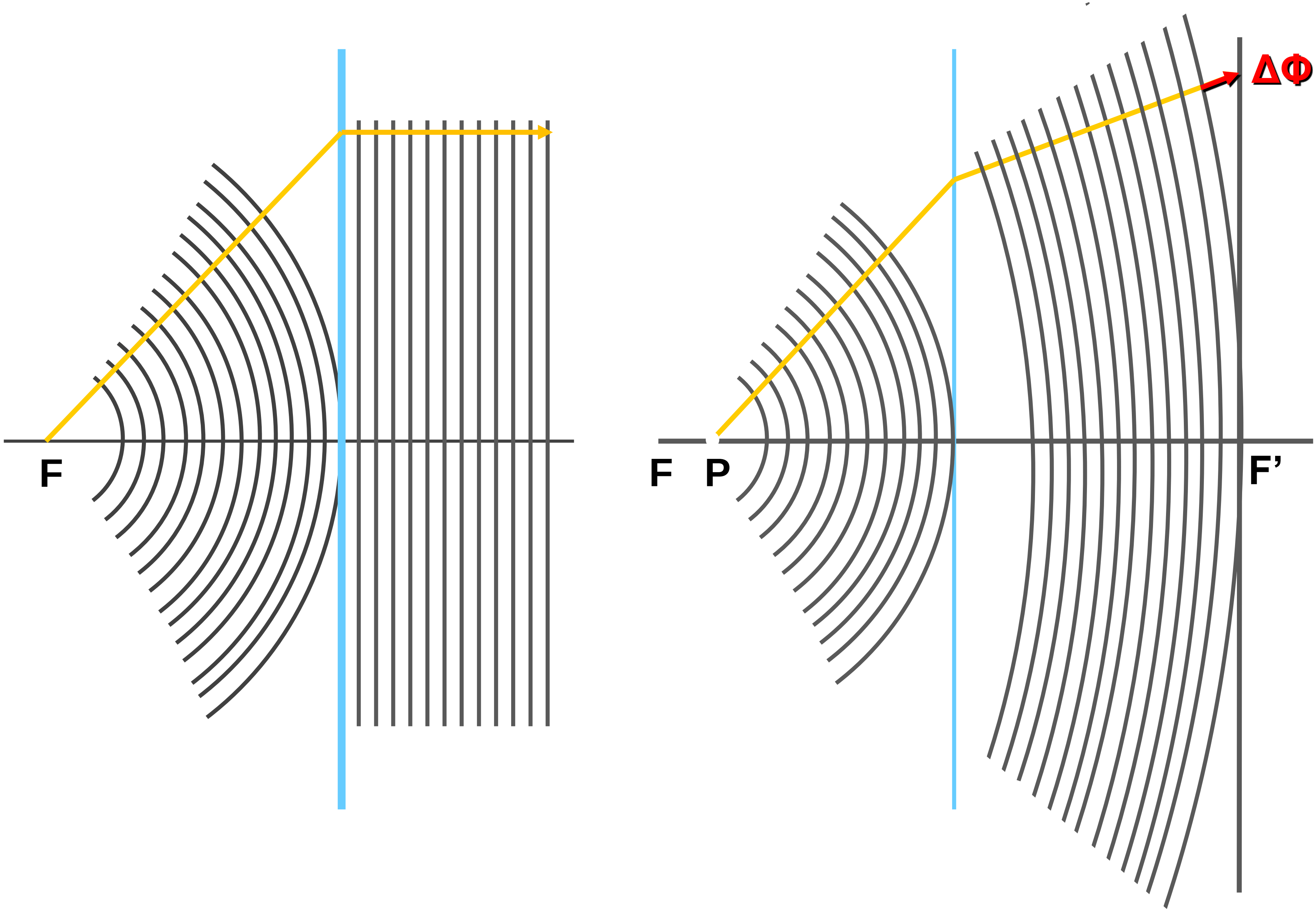


Objective astigmatism:



CTF Induced by Defocus



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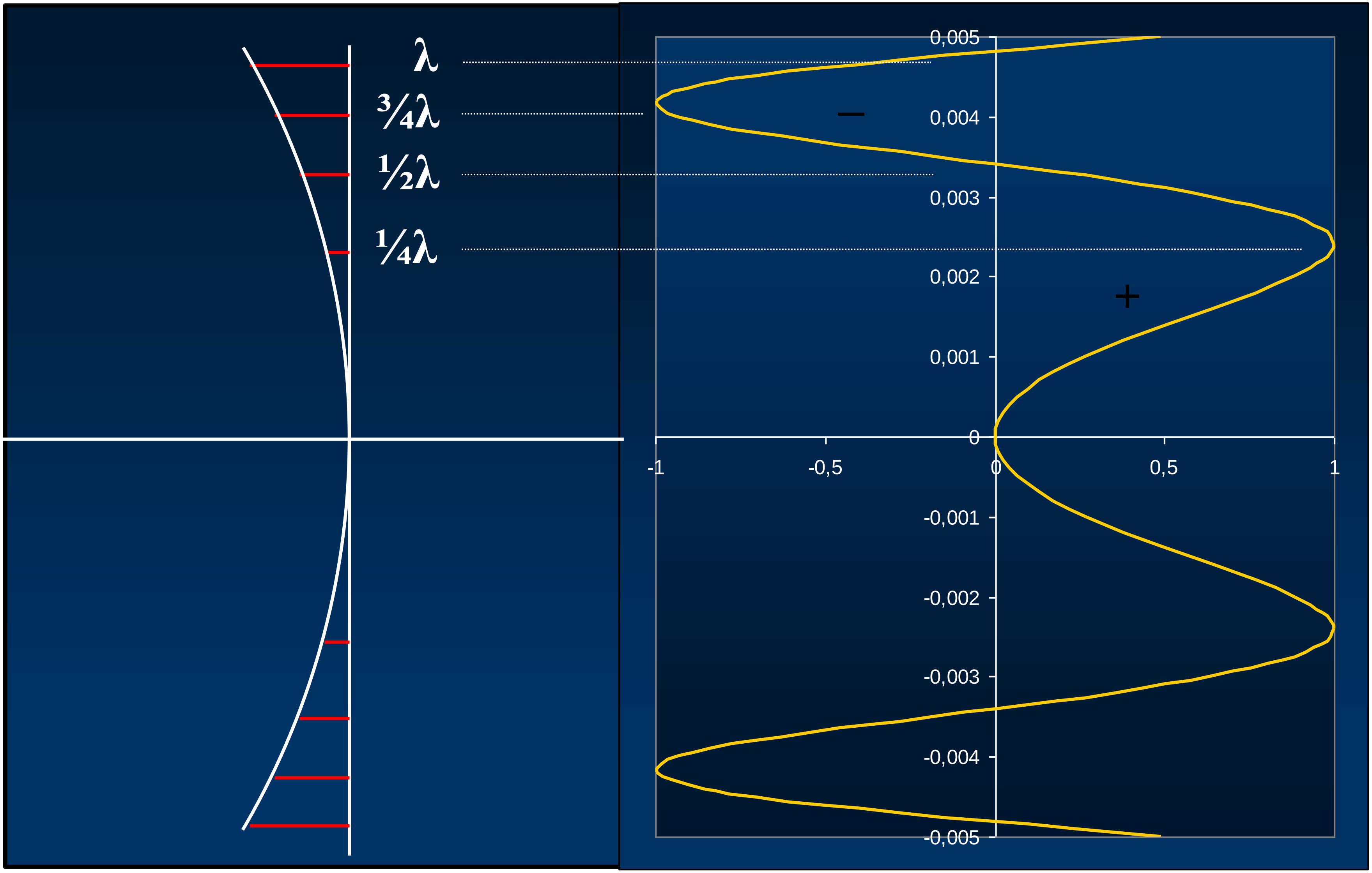
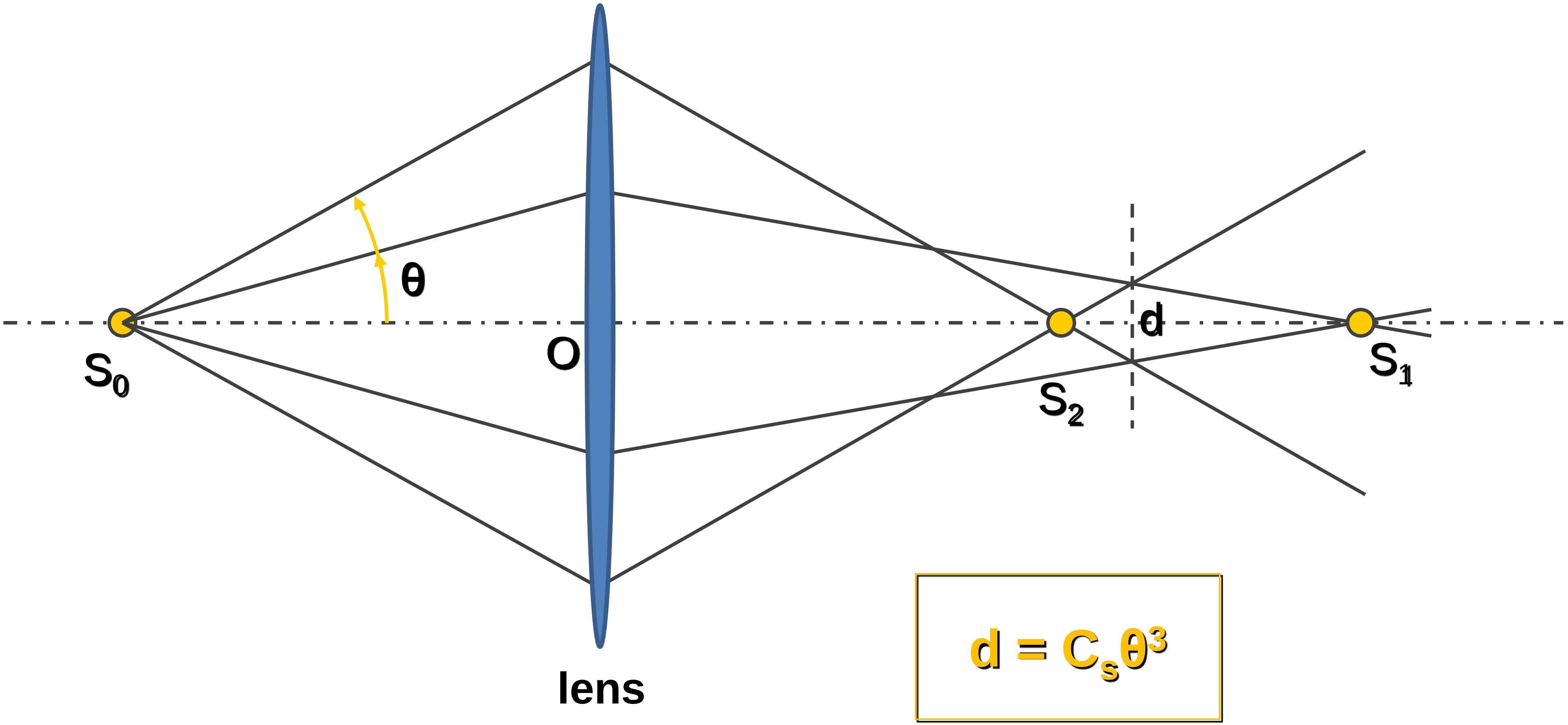
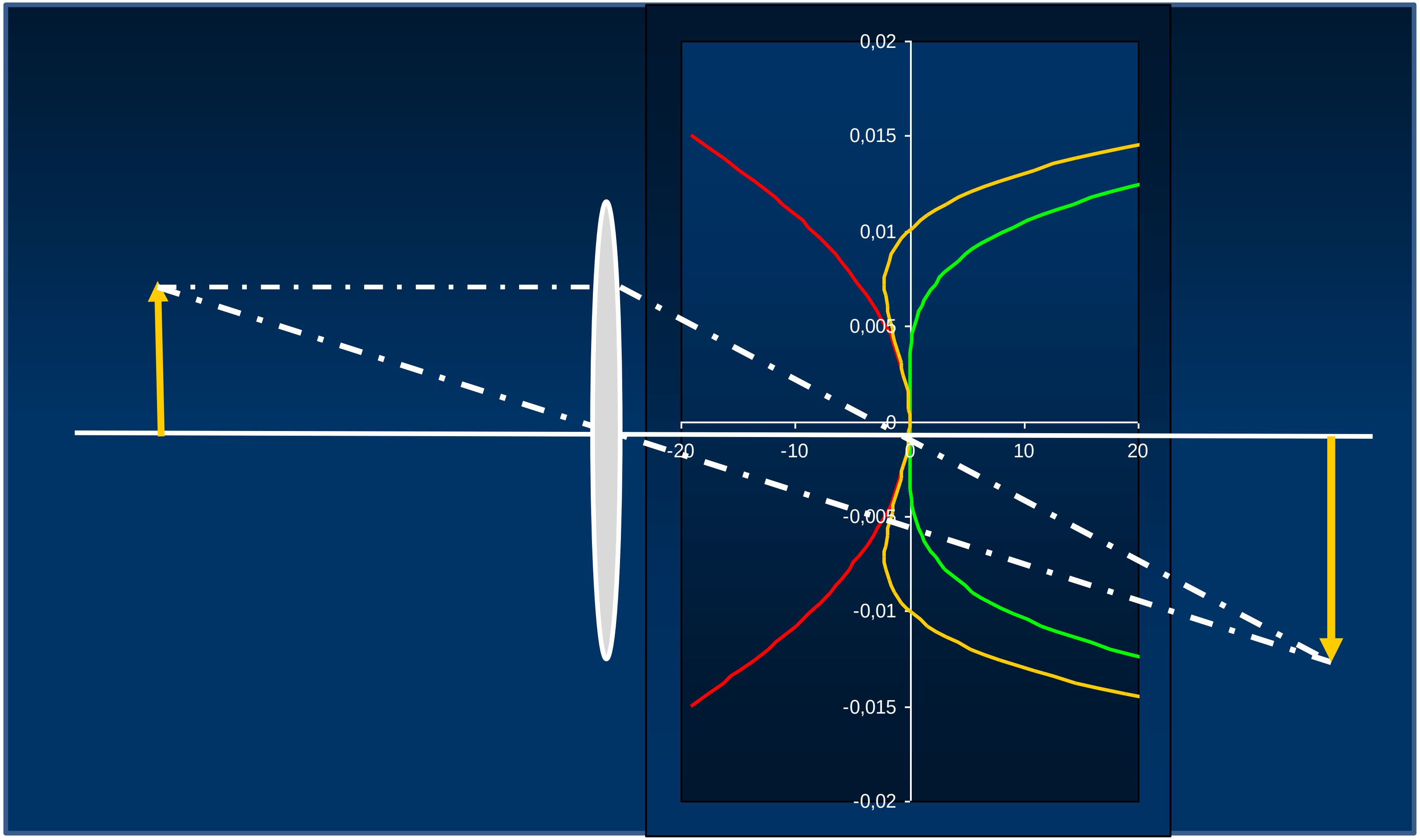


CTF Induced by Spherical Aberration



$$d = C_s\theta^3$$

Phase Contrast Transfer Function



$$\mathrm{Sin}[X(\nu,\theta)] = \mathrm{Sin}[2\pi/\lambda\ (C_s \lambda^4 \nu^4/4 - \Delta z(\theta)\ \lambda^2 \nu^2/2)]$$

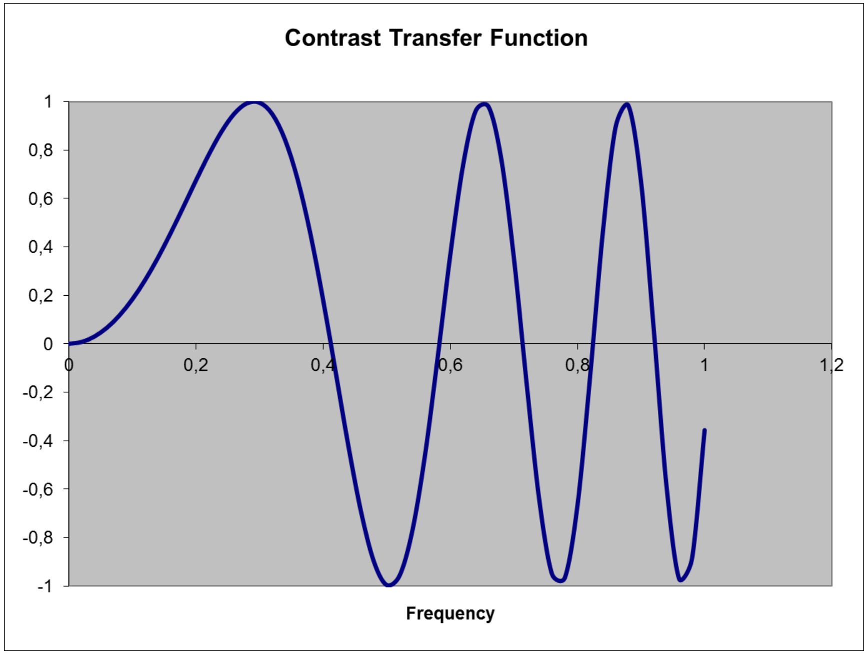
Scherzer focus: $\mathrm{Sin}[X(\nu,\theta)] = 0$

Amplitude and Phase CTF

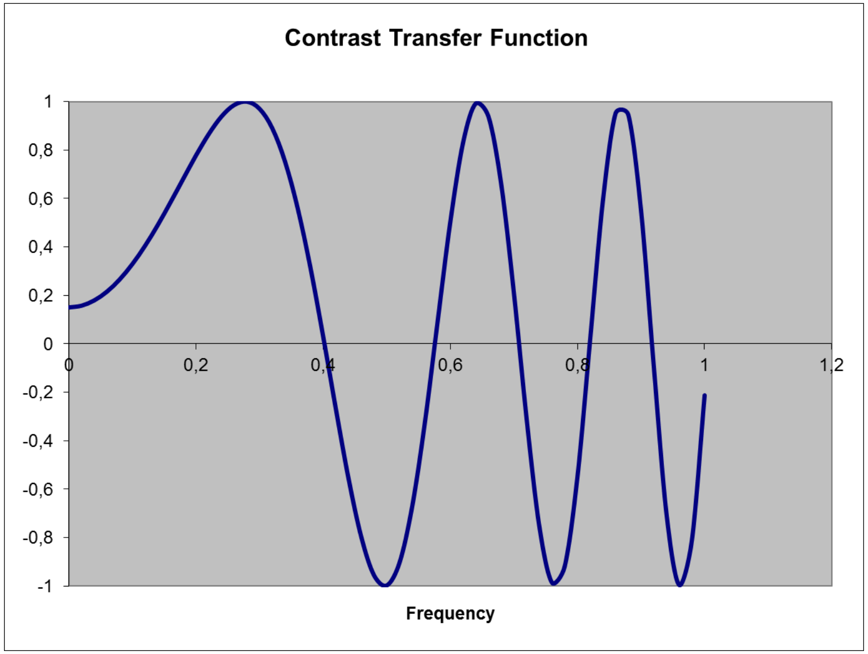
$$\mathrm{CTF}(\nu) = B \sin(X(\nu)) + A \cos(X(\nu))$$

$$\mathrm{CTF}(\nu) = \sqrt{(1-A^2)} \sin(X(\nu)) + A \cos(X(\nu))$$

$$X(\nu,\theta) = 2\pi/\lambda \left(C_s \lambda^4 \nu^4/4 - \Delta z(\theta)\, \lambda^2 \nu^2/2\right)$$



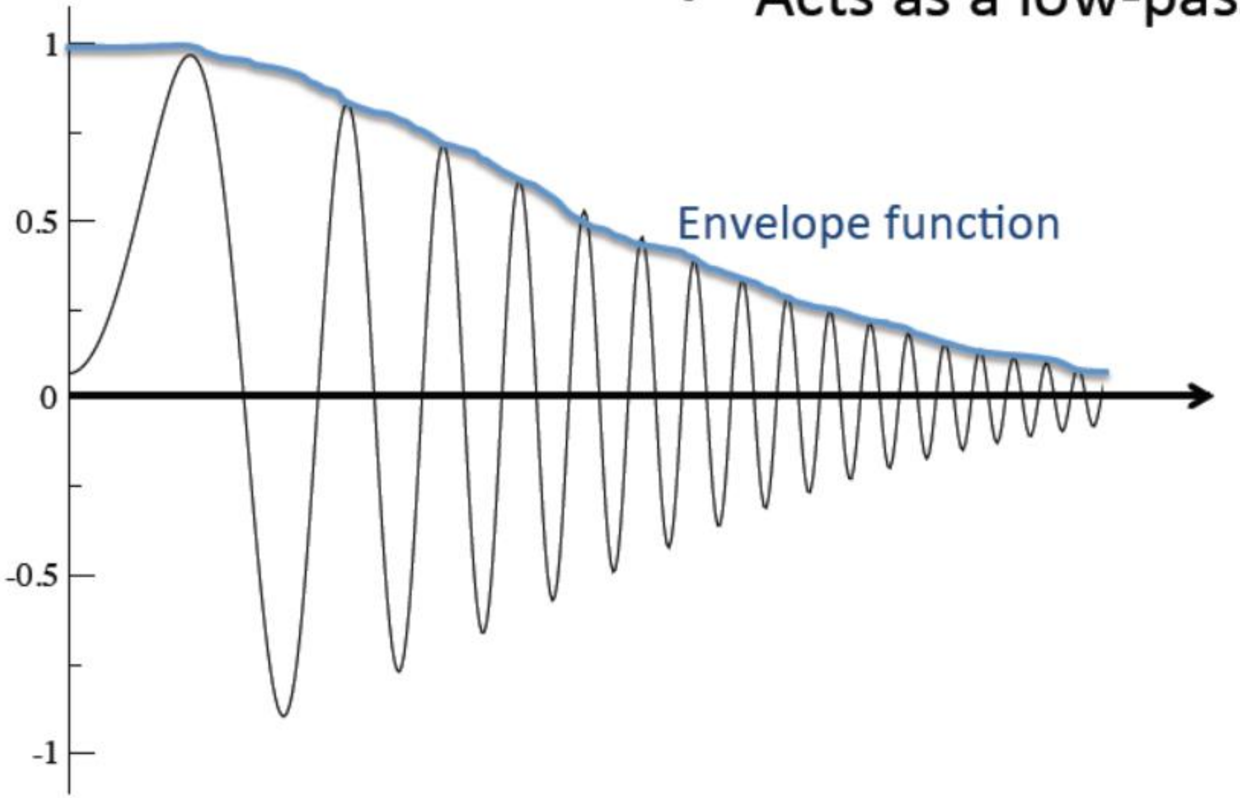
CTF with zero amplitude contrast



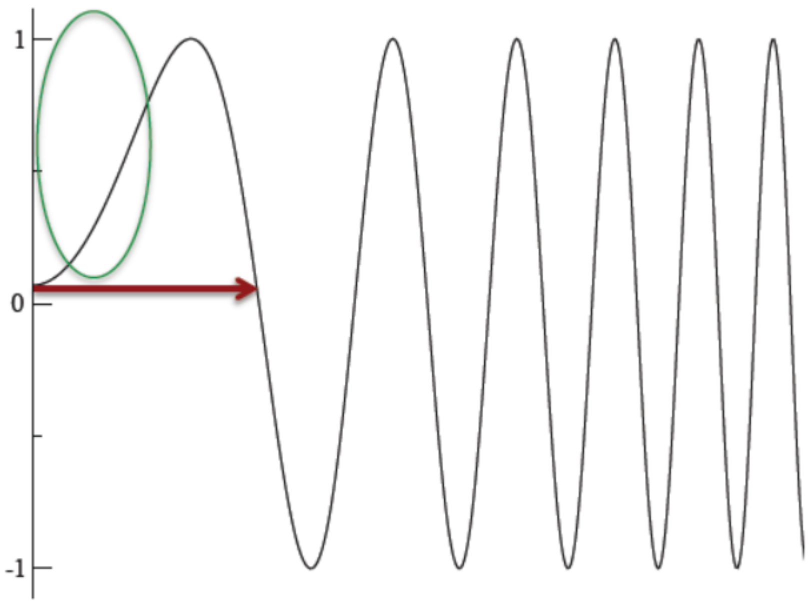
CTF with 15% amplitude contrast

Envelope Function

- Caused by imperfect imaging
- Acts as a low-pass filter!

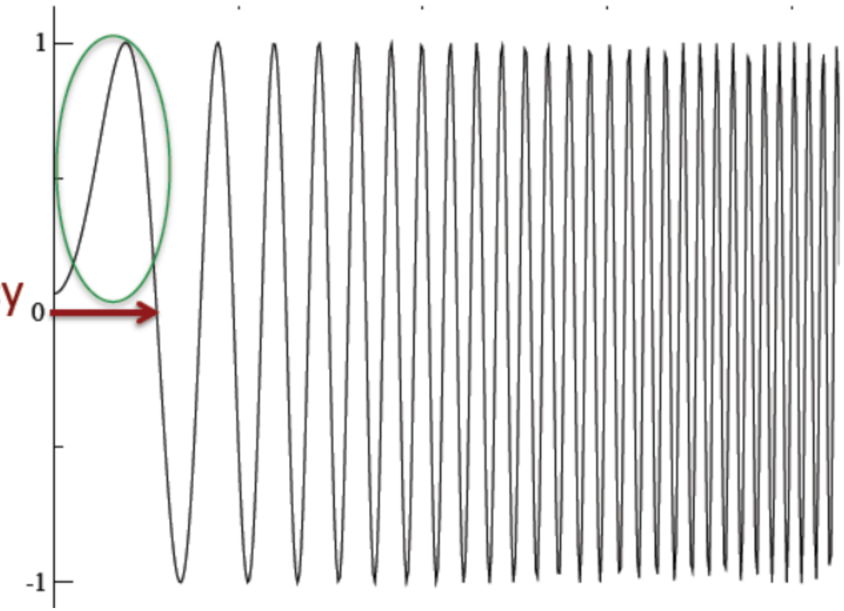


Real CTF and Defocus Settings



Low defocus

High defocus

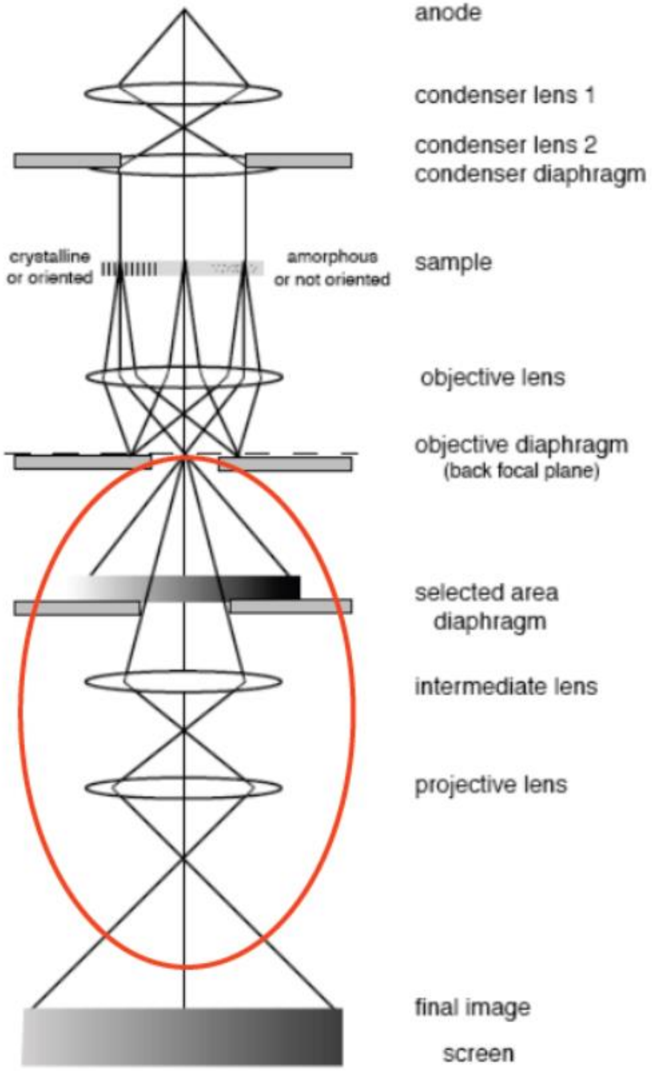


– First zero-crossing at lower frequency
Much faster modulations
Often stronger envelope

+ Stronger low-frequency signal!
Different zero-crossings

Projector Lenses

- Magnify the image formed by the objective lens with minimal distortions.
- Very small illumination angles assure almost perfect imaging.
- The magnified imaged is projected to the detector.

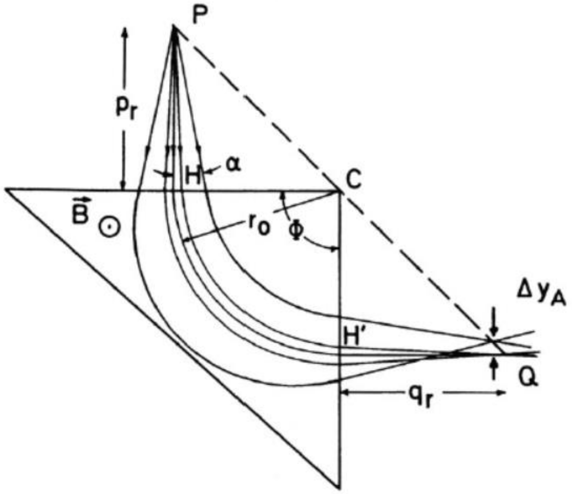


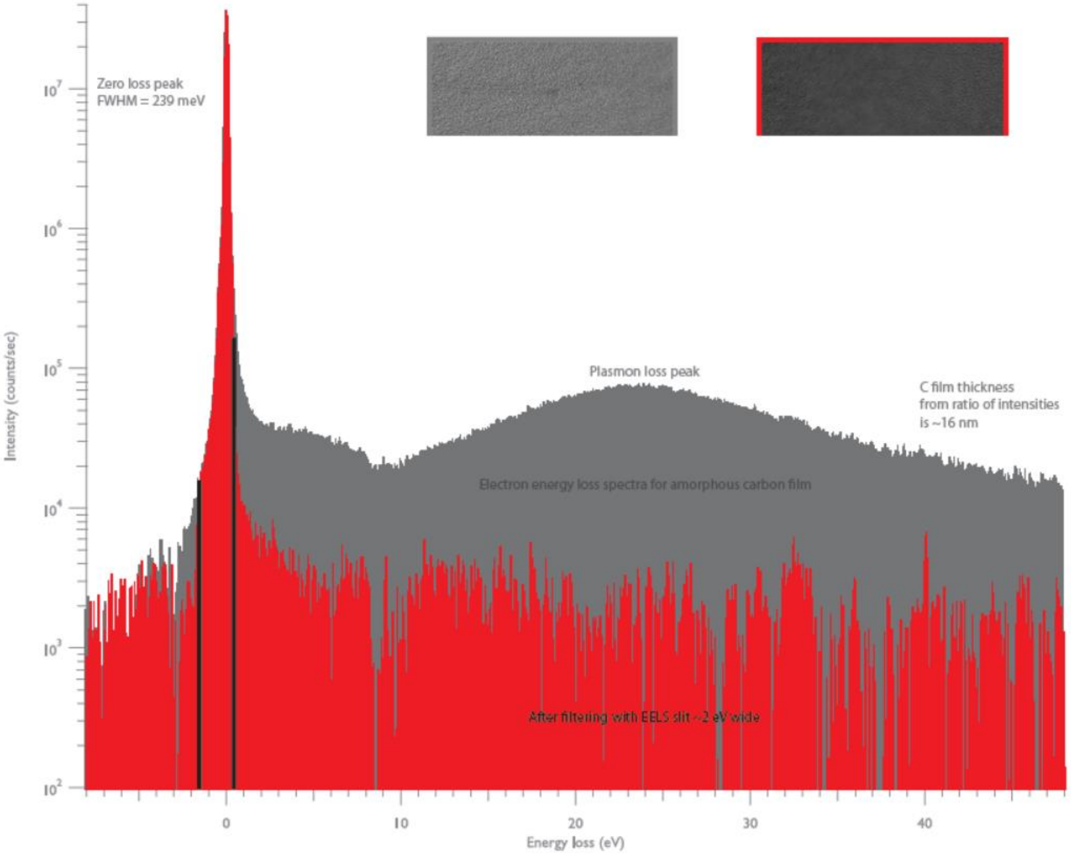
Energy Filter

Energy filter

Think: "prism"

$$\mathbf{F} = -q_e(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$





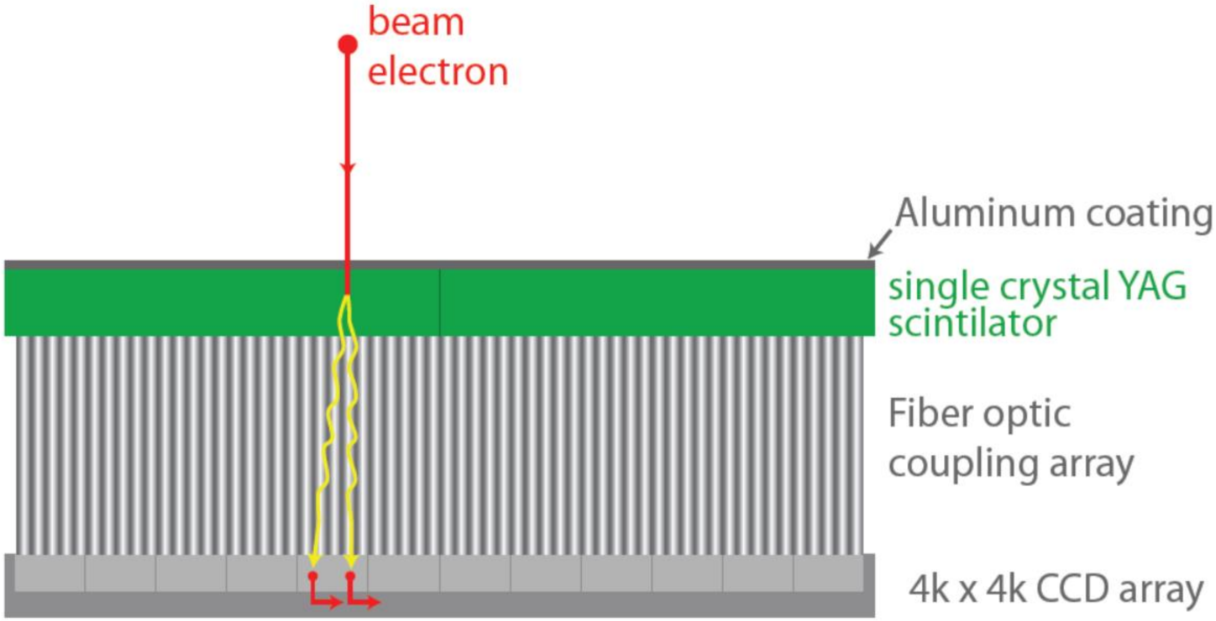
Detectors

Silver

The original direct electron detector

reduce silver halides exposed to electrons to silver

"Conventional" electron microscope camera

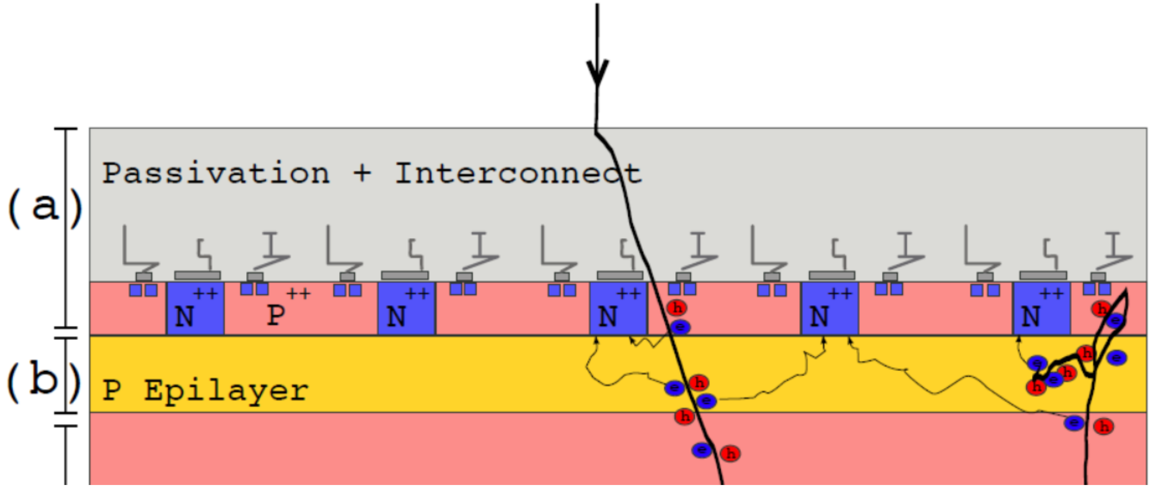


Charge
Coupled
Device

convert electrons to photons and back to electrons

Detectors

Direct electron detector



"CMOS detector"

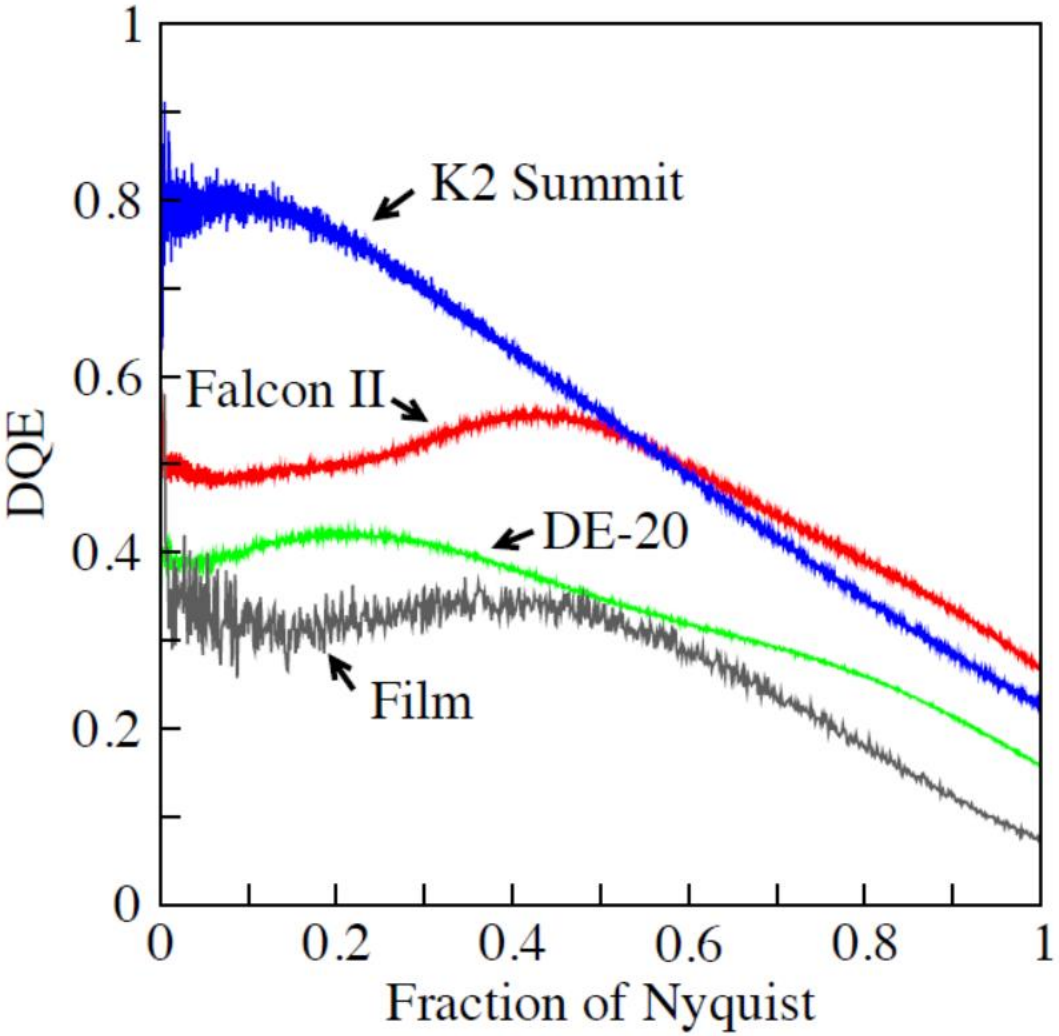
Complementary
Metal
Oxide
Semiconductor

Backed-thinned direct electron detector

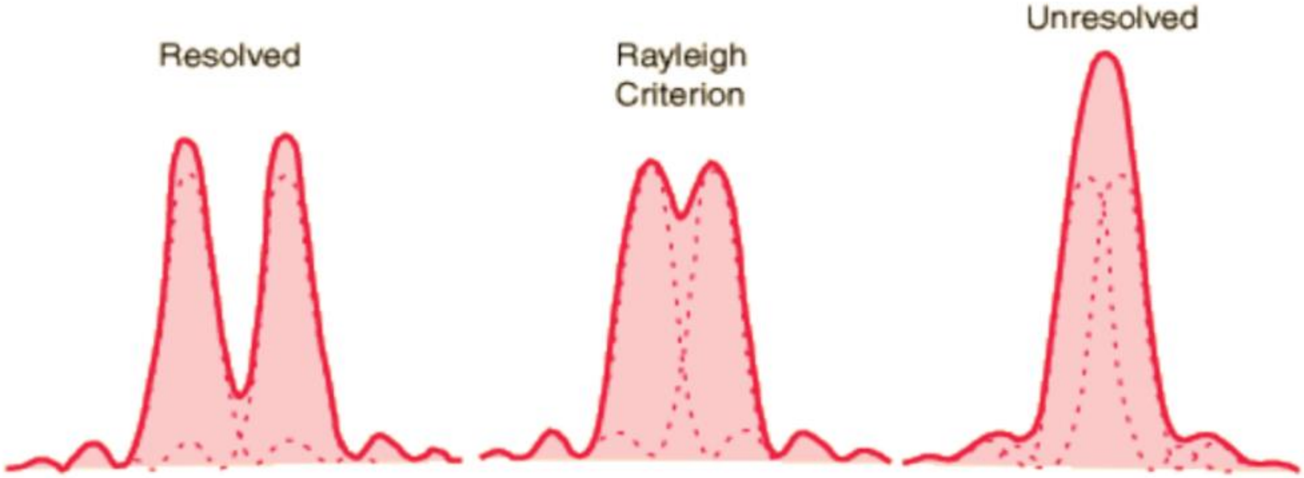
courtesy of G. McMullan

Detectors

Detector quantum efficiency



Resolution



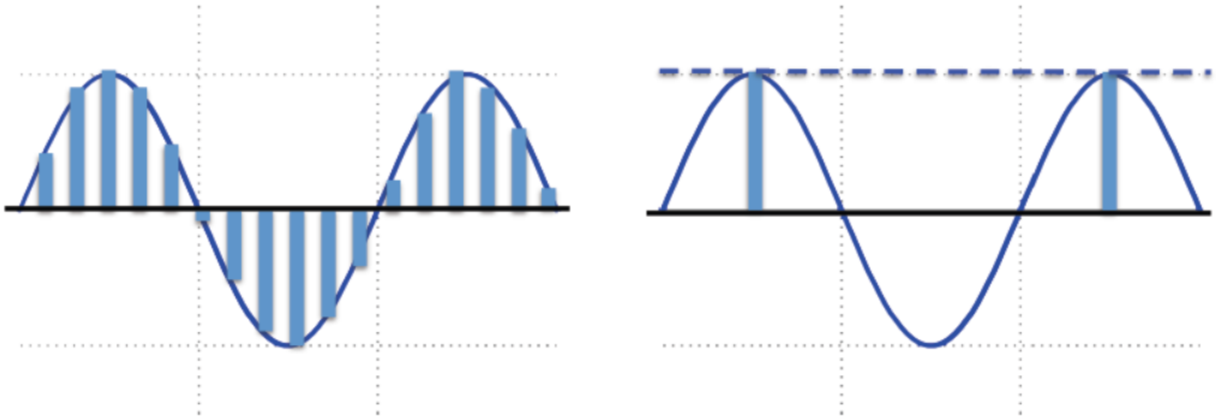
$$R = d/2 = 0.61\,\lambda / n \sin(\alpha) = 0.61\,\lambda / \text{N.A.}$$

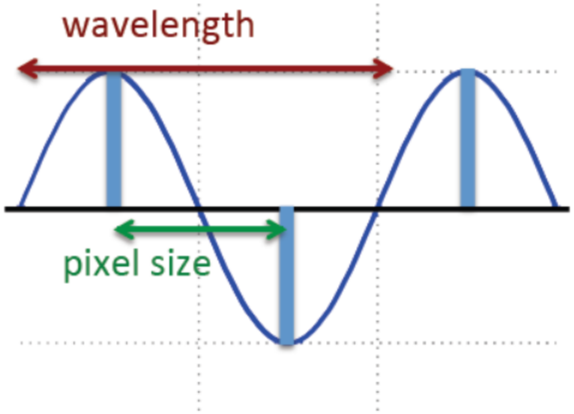
- **The wavelength of used radiation (visible light, 400-700 nm) is the limiting factor in the achievable resolution of images (200 nm).**
- **In TEM, the wavelength of 300 keV electrons is 2 pm.**
- **The limiting factor in achievable resolution of biological specimen is beam damage to the sample and consequent poor SNR of images.**

Resolution

pixelised

Discrete images



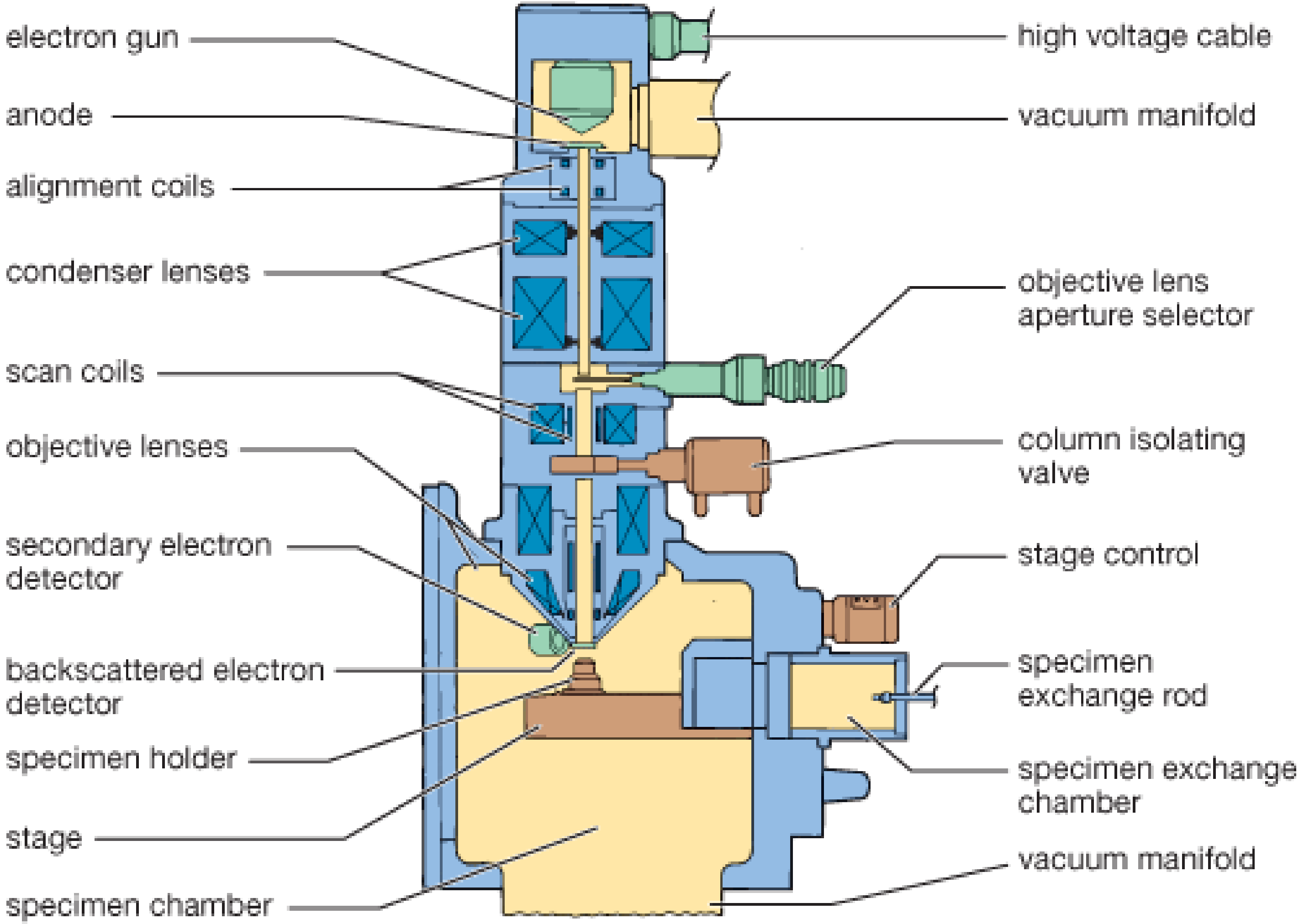


If pixel size = 2 Å then smallest sine wave that can be described has a wavelength TWICE that value, e.g. 4 Å

1/4 Å is ***Nyquist* frequency**

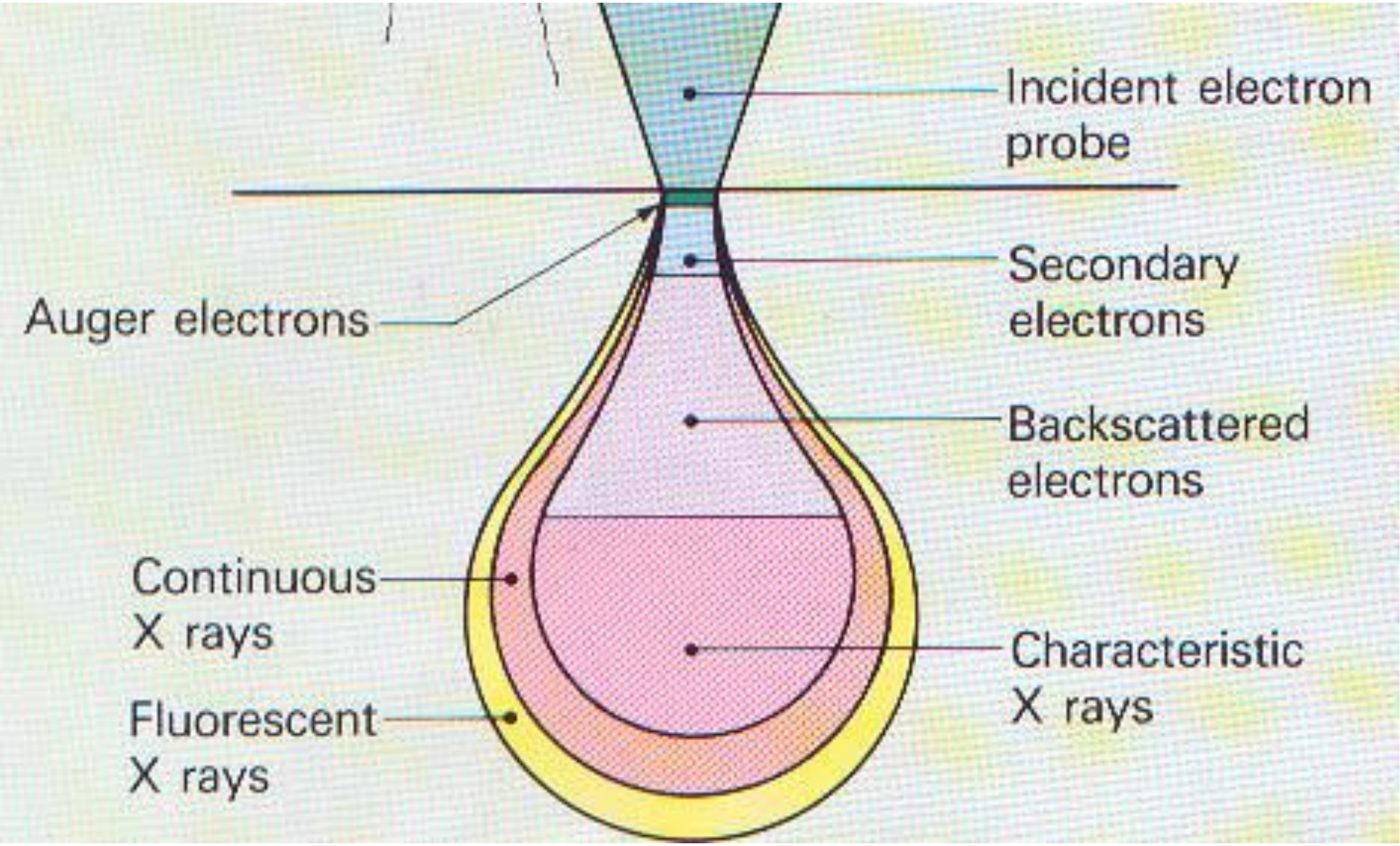
Scanning Electron Microscope

Parts of a scanning electron microscope

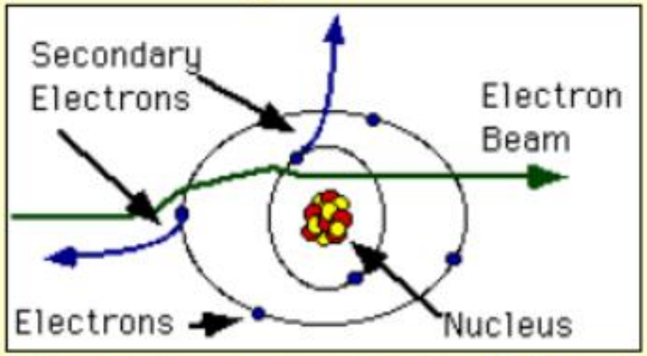


Source: JEOL U.S.A., Inc.

Scanning Electron Microscope



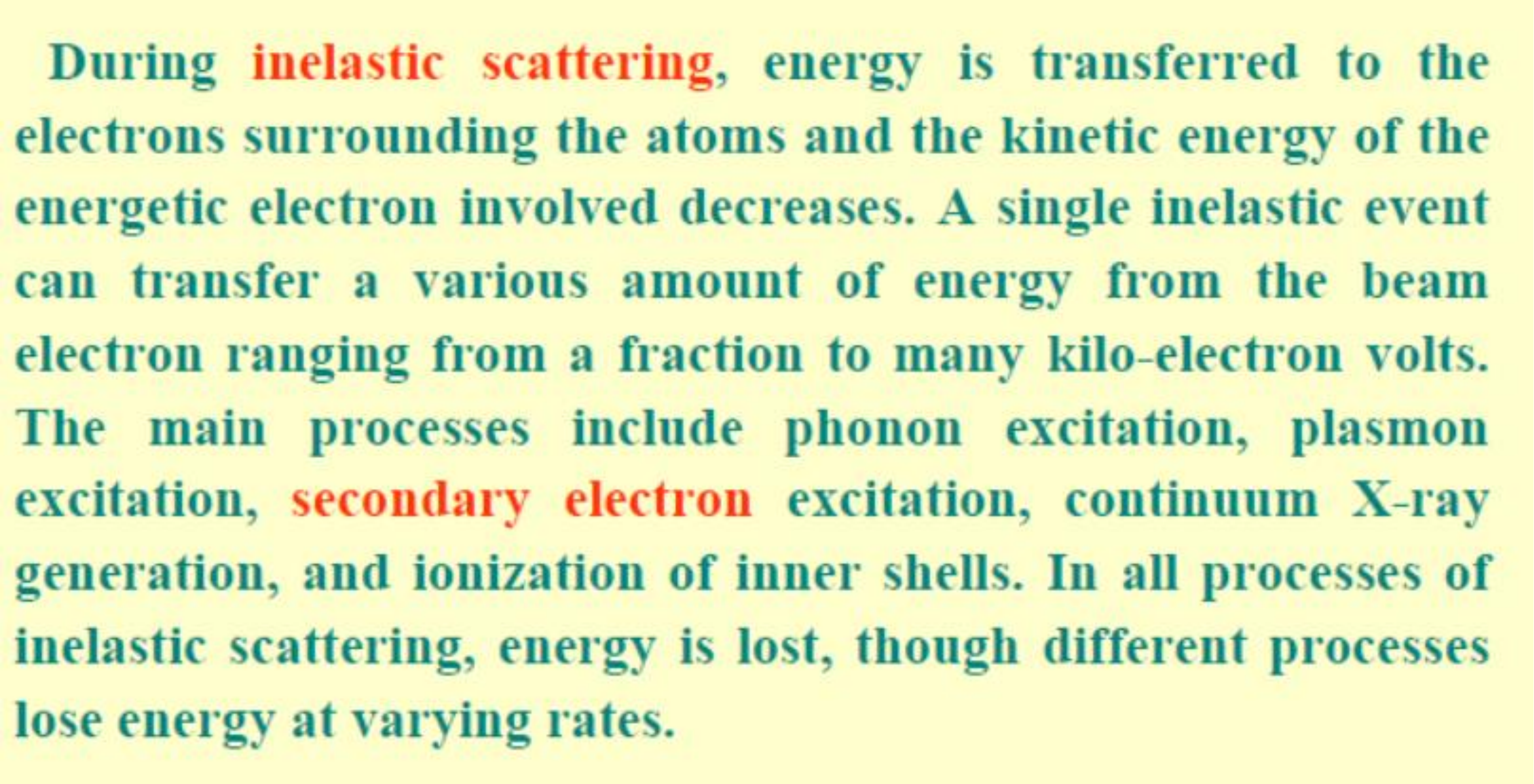
Secondary Electrons



1. **Secondary electrons** are predominantly produced by the interactions between energetic beam electrons and weakly bonded conduction-band electrons in metals or the valence electrons of insulators and semiconductors.

2. There is a great difference between the amount of energy contained by beam electrons compared to the specimen electrons and because of this, only a small amount of kinetic energy can be transferred to the secondary electrons.

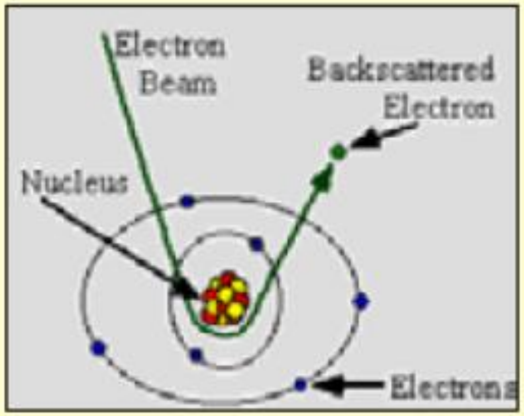
Secondary Electrons



During inelastic scattering, energy is transferred to the electrons surrounding the atoms and the kinetic energy of the energetic electron involved decreases. A single inelastic event can transfer a various amount of energy from the beam electron ranging from a fraction to many kilo-electron volts. The main processes include phonon excitation, plasmon excitation, secondary electron excitation, continuum X-ray generation, and ionization of inner shells. In all processes of inelastic scattering, energy is lost, though different processes lose energy at varying rates.

SE are specimen electrons that obtain energy by inelastic collisions with beam electrons. They are defined as electrons emitted from the specimen with energy less than 50 eV.

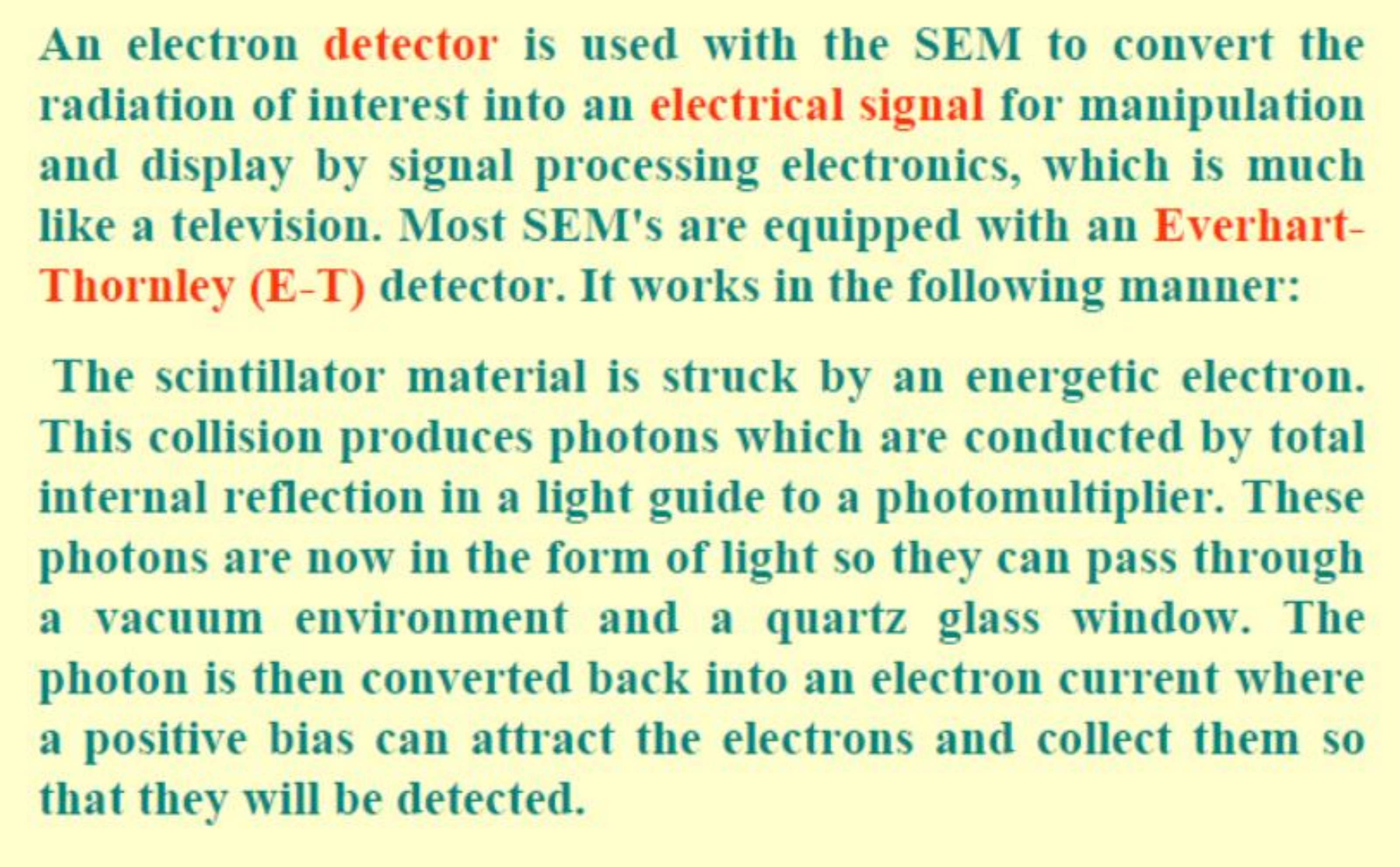
Backscattered Electrons



As the name implies, elastic scattering results in little (<1eV) or no change in energy of the scattered electron, although there is a change in momentum. Since momentum, p=mv, and m doesn't change, the direction of the velocity vector must change. The angle of scattering can range from 0-180 degrees, with a typical value being about 5 degrees.

Elastic scattering occurs between the negative electron and the positive nucleus. This is essentially Rutherford scattering. Sometimes the angle is such that the electron comes back out of the sample. These are backscattered electrons.

Everhart-Thornley Detector



An electron detector is used with the SEM to convert the radiation of interest into an electrical signal for manipulation and display by signal processing electronics, which is much like a television. Most SEM's are equipped with an Everhart-Thornley (E-T) detector. It works in the following manner:

The scintillator material is struck by an energetic electron. This collision produces photons which are conducted by total internal reflection in a light guide to a photomultiplier. These photons are now in the form of light so they can pass through a vacuum environment and a quartz glass window. The photon is then converted back into an electron current where a positive bias can attract the electrons and collect them so that they will be detected.

Everhart-Thornley Detector

An electron **detector** is used with the SEM to convert the radiation of interest into an **electrical signal** for manipulation and display by signal processing electronics, which is much like a television. Most SEM's are equipped with an **Everhart-Thornley (E-T)** detector. It works in the following manner: