Biomedical Imaging
生物醫學影像學

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牙體技術學系

2013/04/29

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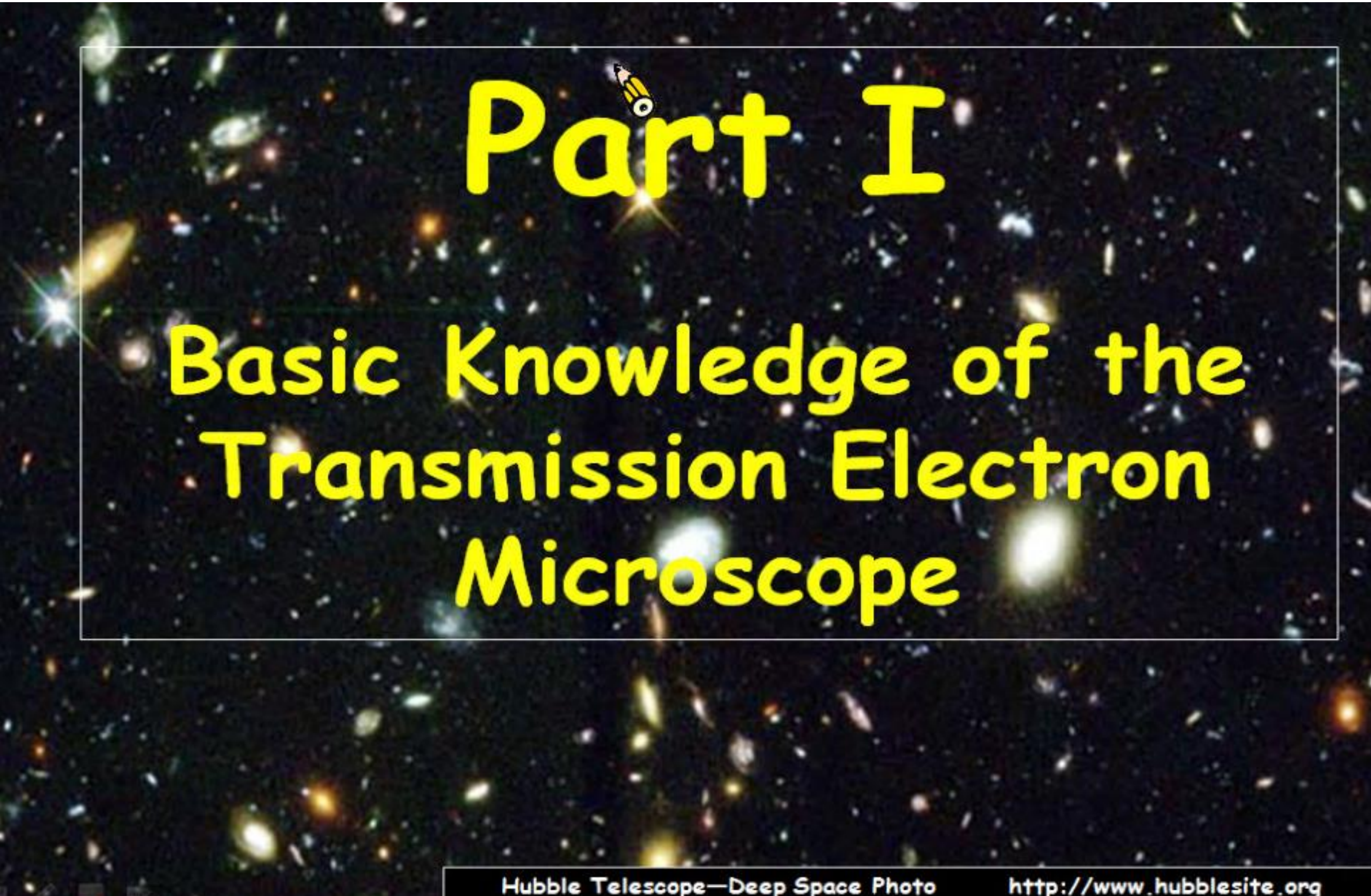
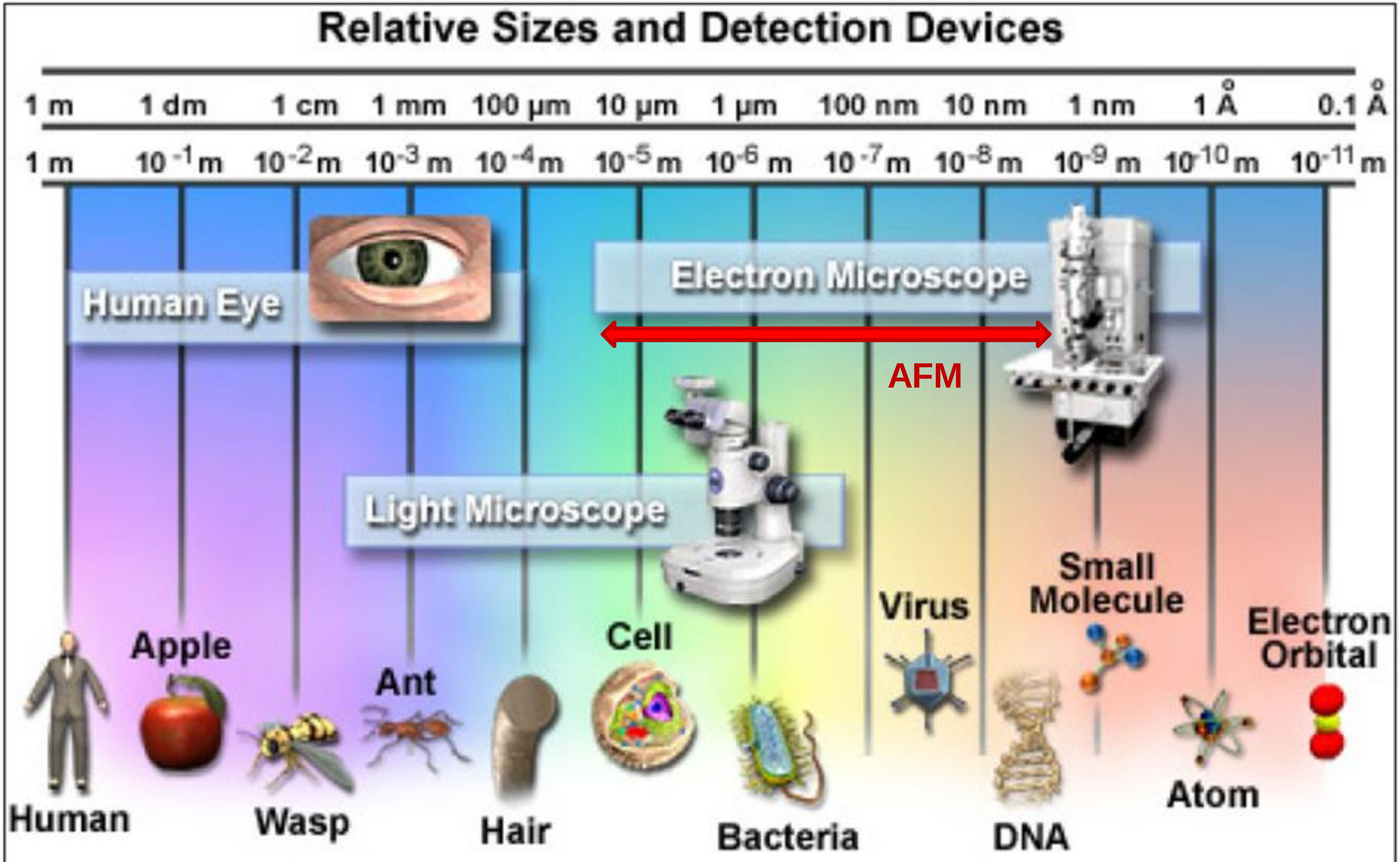
Course Outline

1. Course Introduction
2. Basic Optics and Light Microscopes
3. Fluorescence/Confocal/TIRF Microscopes
4. FRET Techniques and Photo-Spectroscopic Imaging
5. Single Molecule Detection
6. Cell Imaging
7. Atomic Force Microscopy (AFM)
8. Scanning Electron Microscope (SEM)
9. **Transmission Electron Microscopy (TEM)**
10. Digital Image Processing Using MATLAB

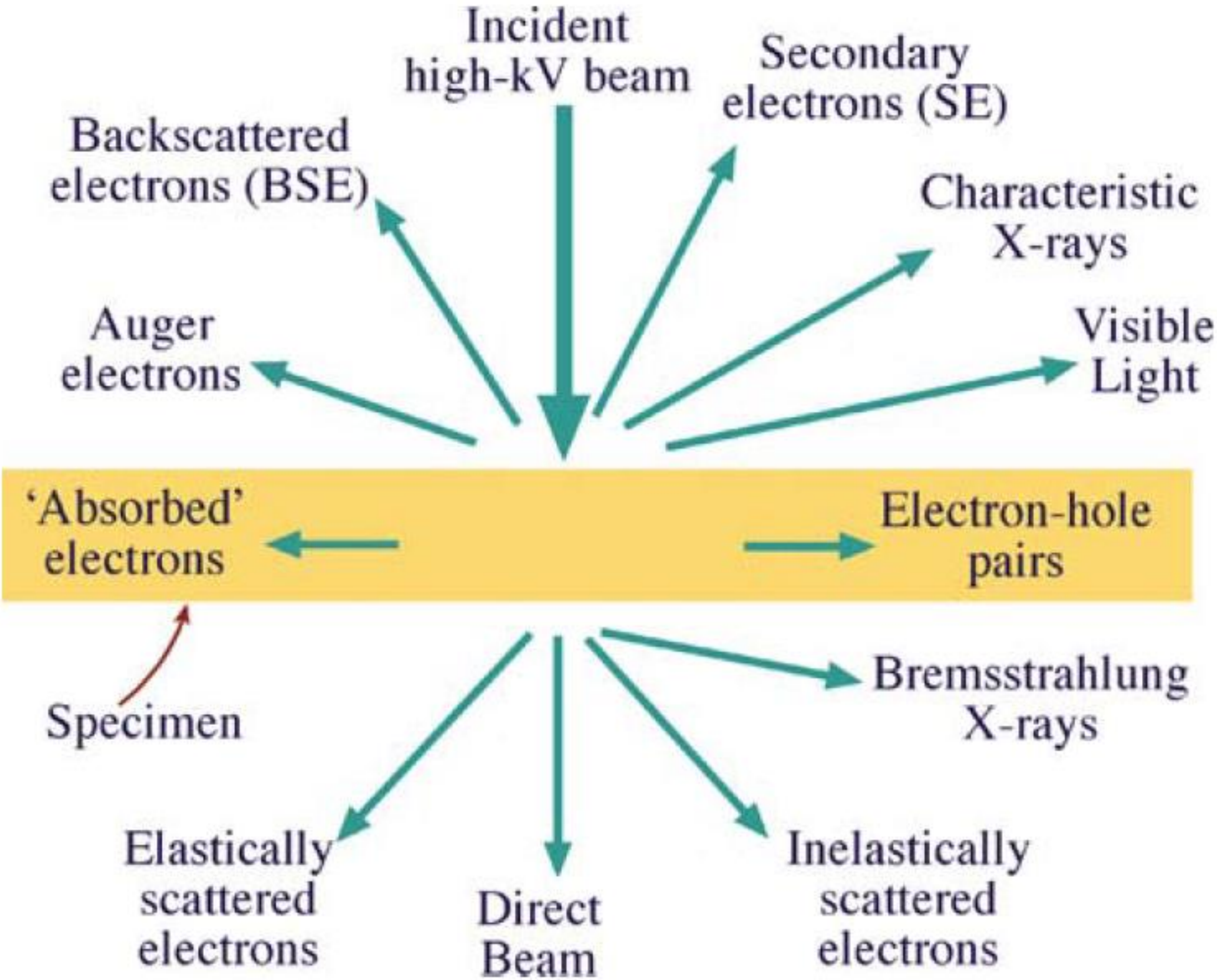

Part I
Basic Knowledge of the Transmission Electron Microscope
Hubble Telescope—Deep Space Photo
http://www.hubblesite.org

Diversity of Optical Bio-Imaging:
principles, technologies, information, applications



Electron Microscopy Signals

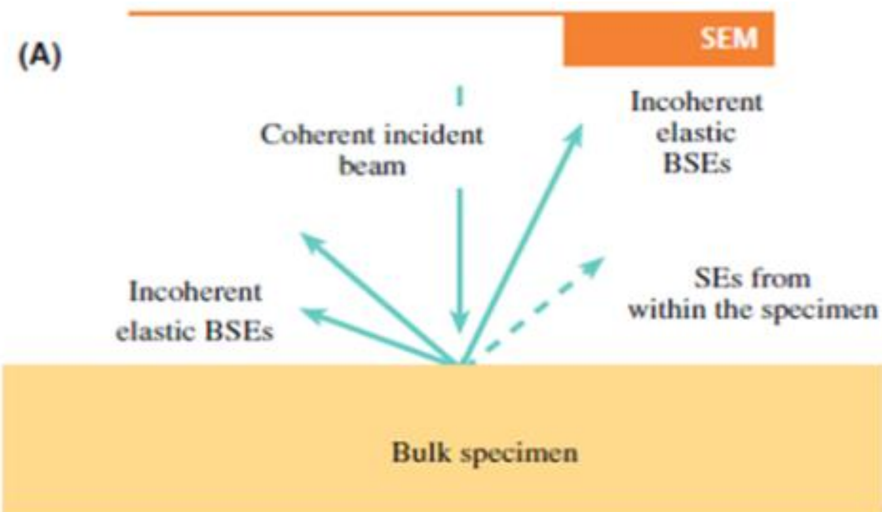
Interaction of electrons with matter

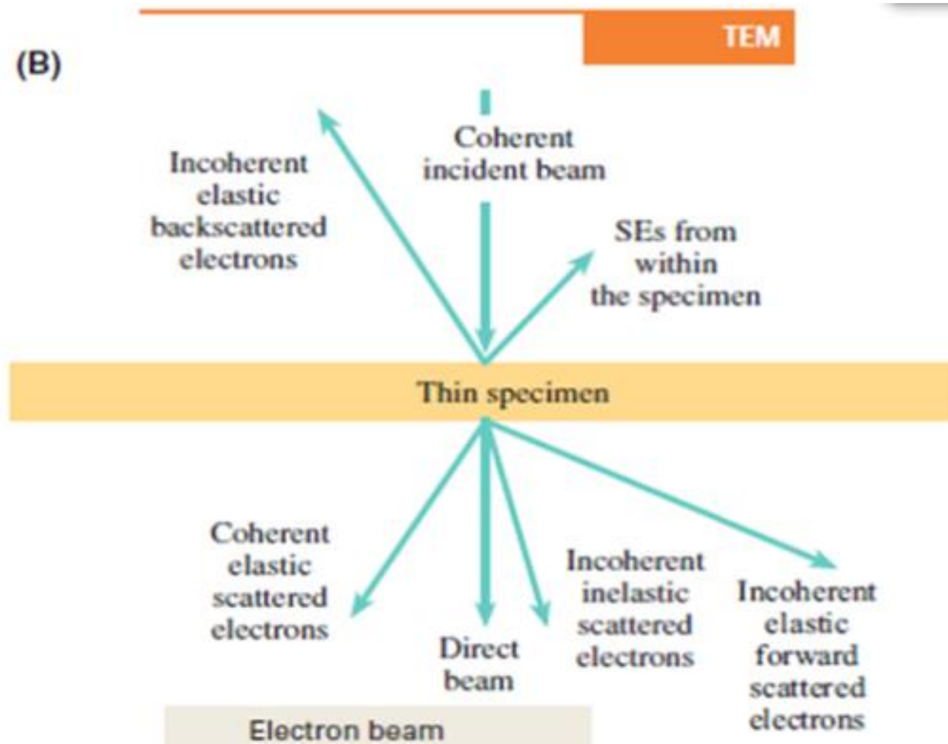


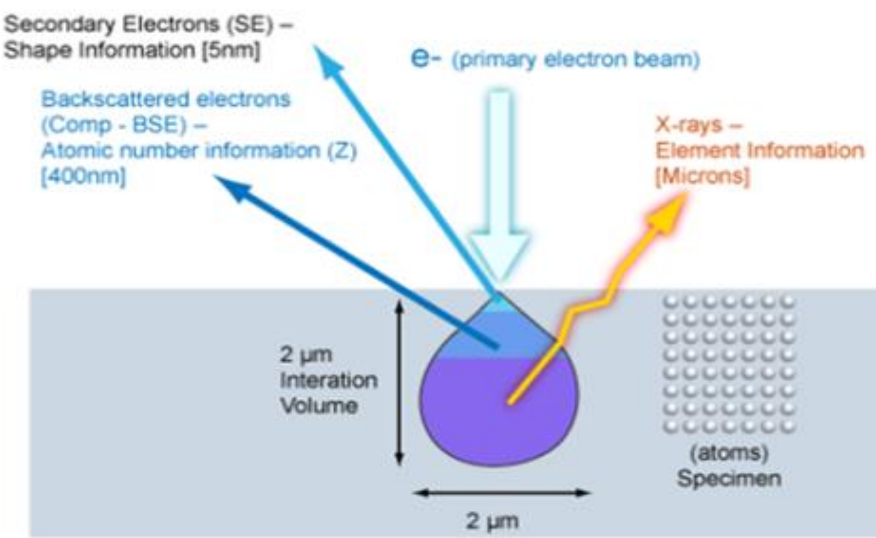
SEM signals

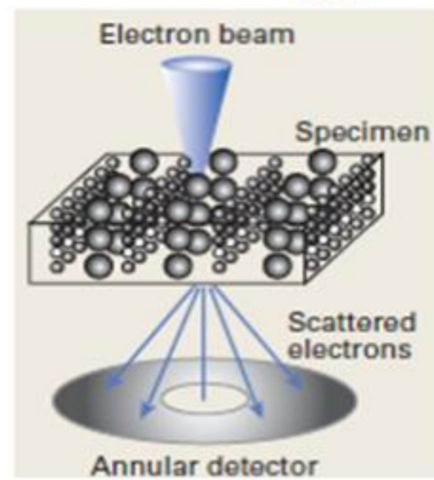
Most important in TEM

Comparison of the SEM with TEM



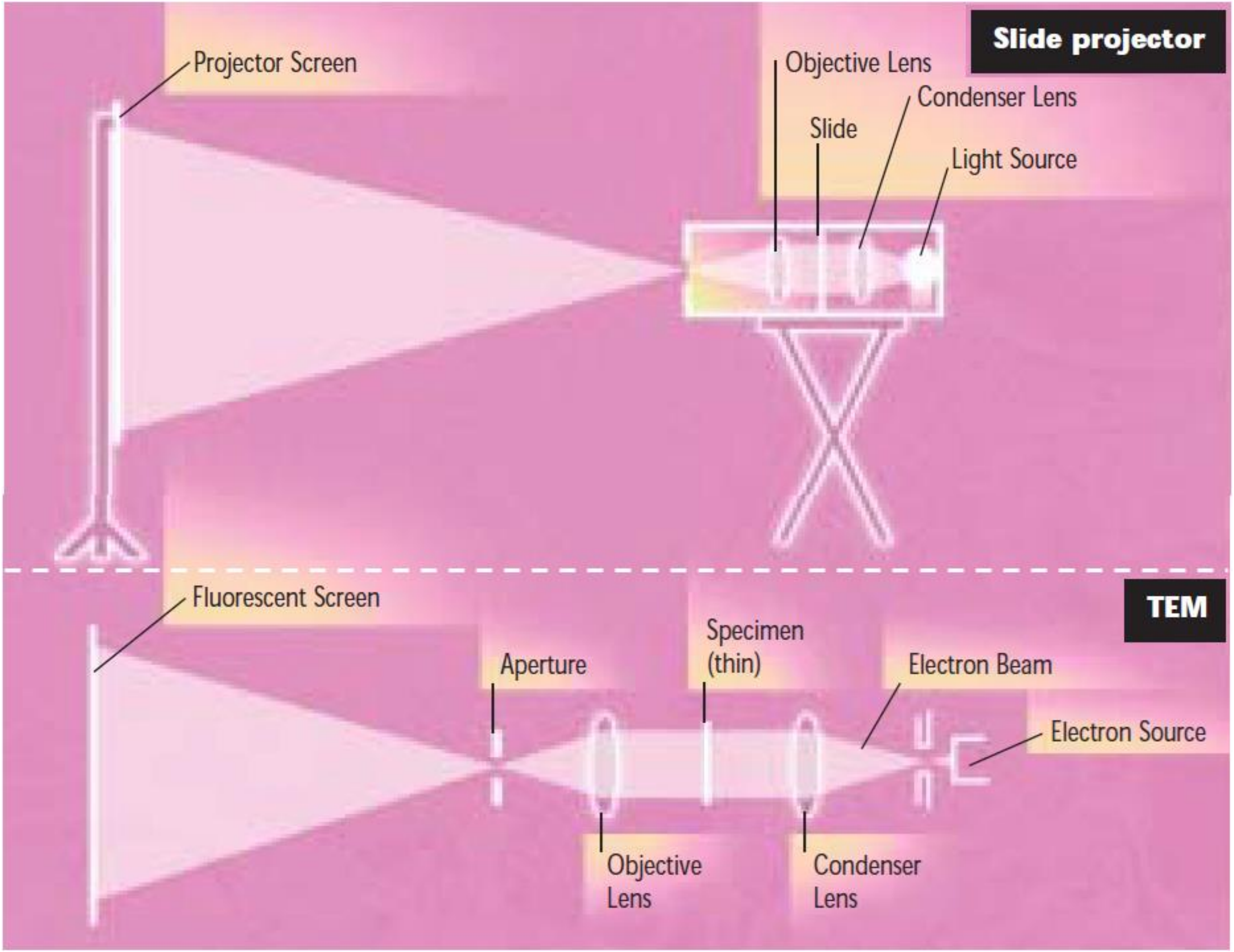






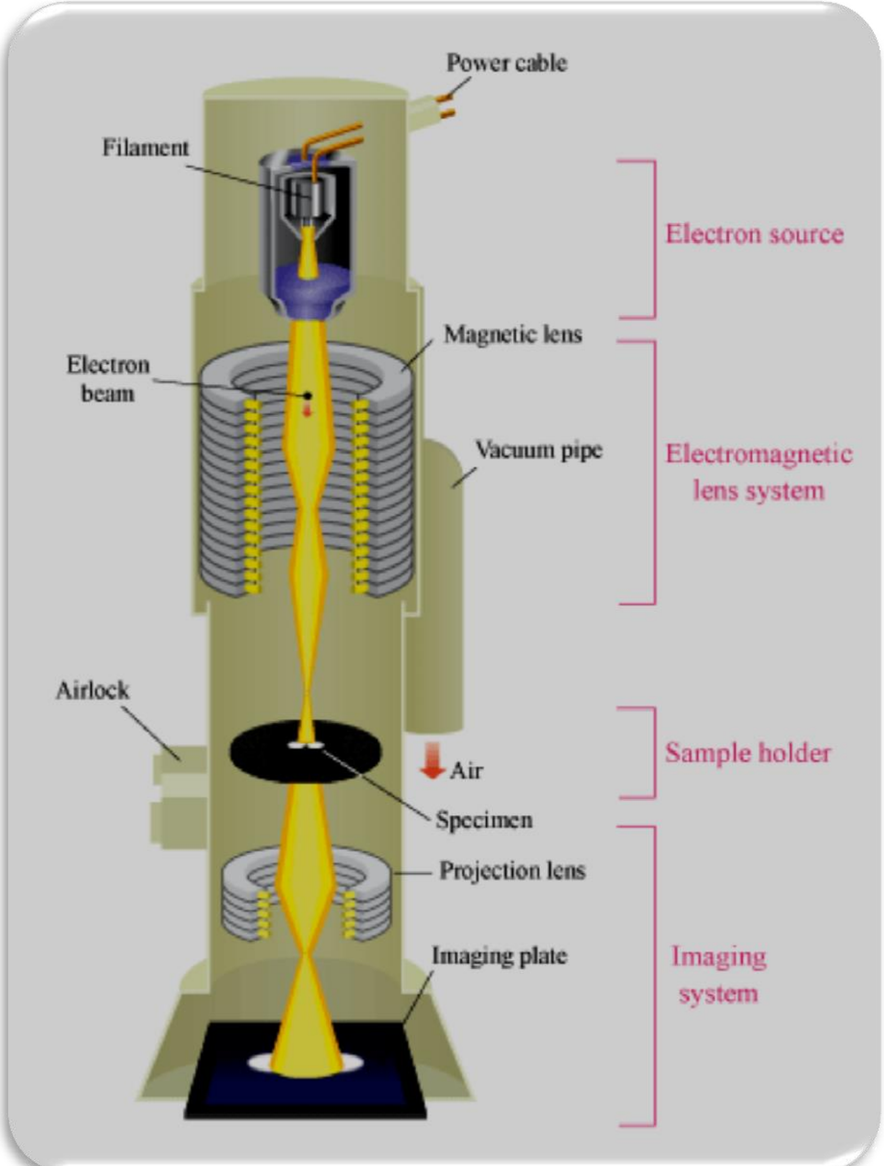
http://www.fei.com/uploadedfiles/documents/content/introduction_to_em_booklet_july_10.pdf

The TEM compared with a slide projector



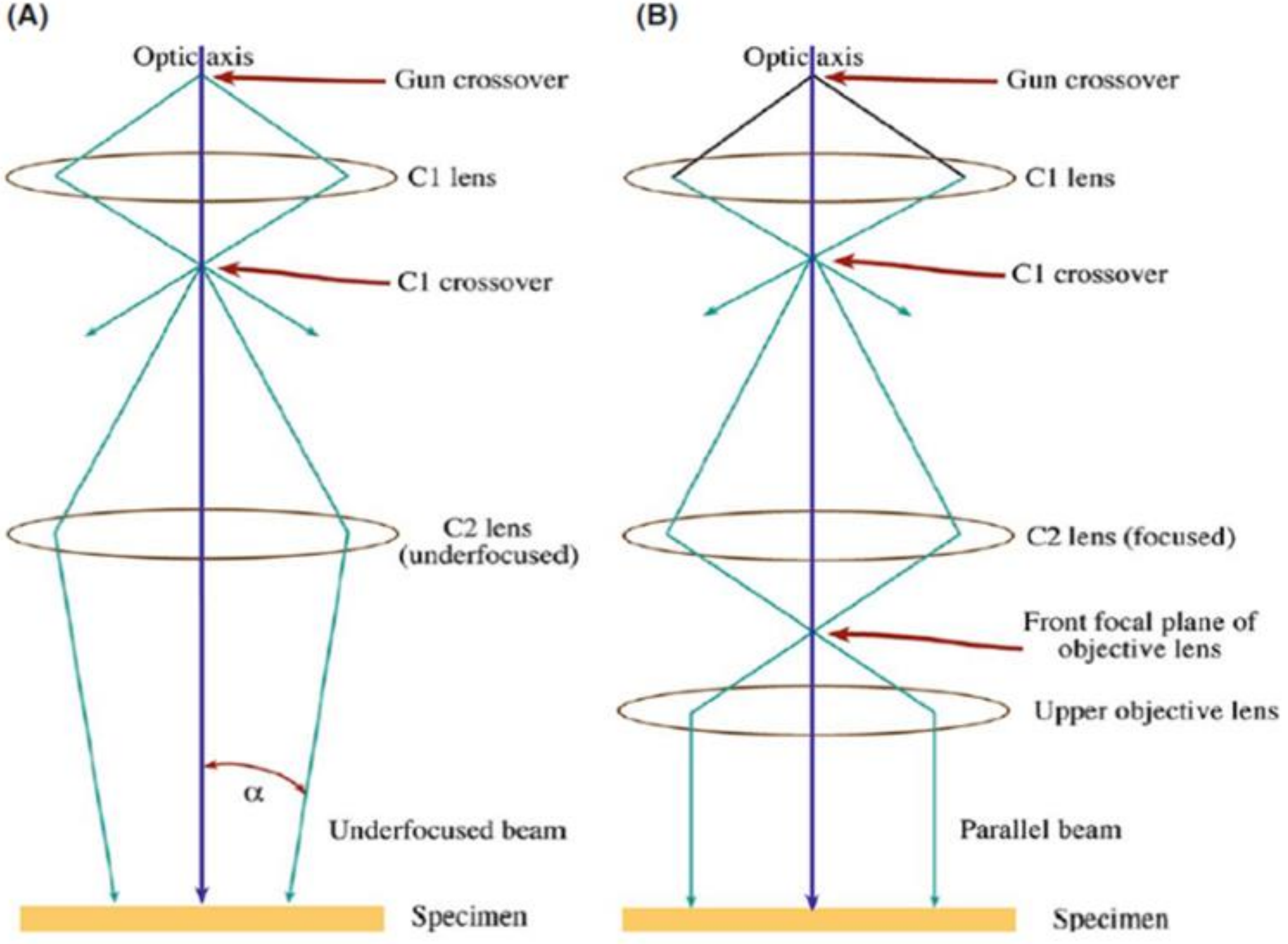
The TEM Instrument

The illumination system, the objective lens/stage, and the imaging system.



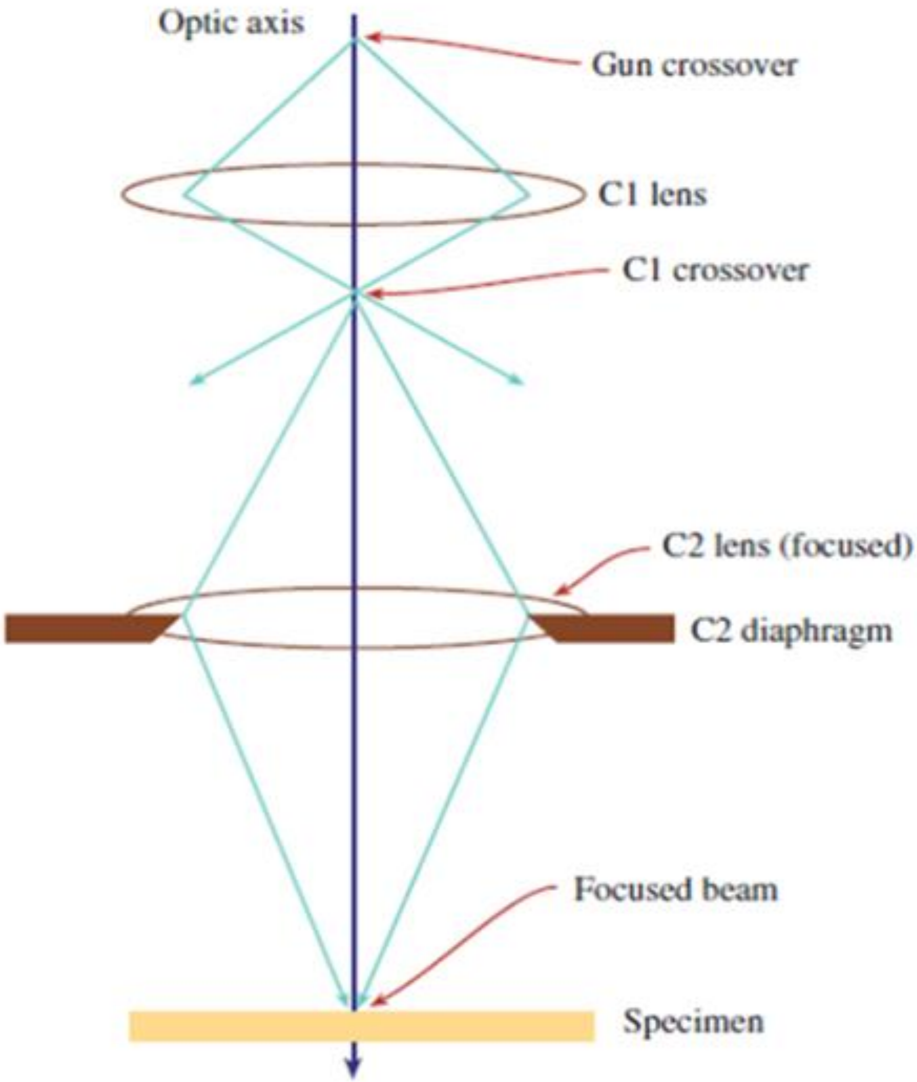
The TEM Instrument (1)

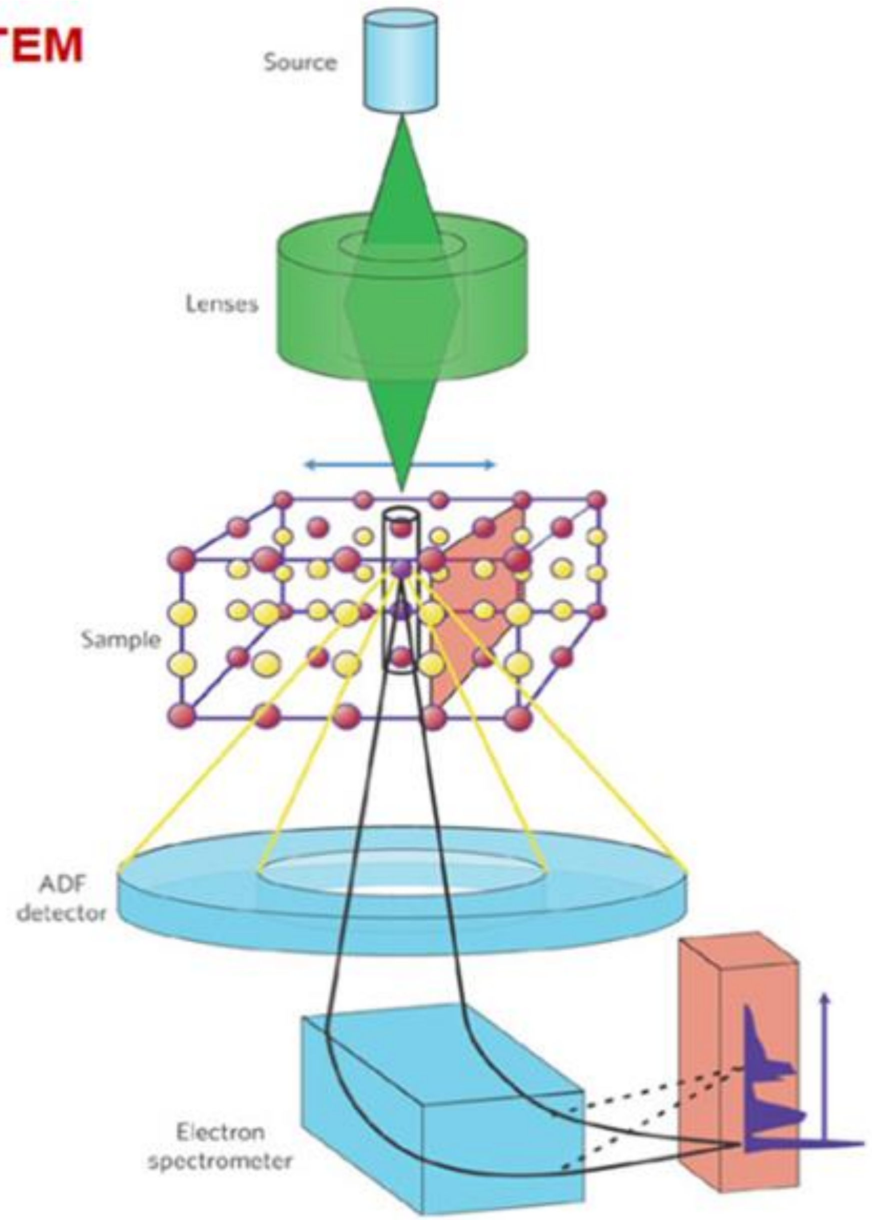
Parallel-beam operation in the TEM



Scanning Transmission Electron Microscope (STEM)

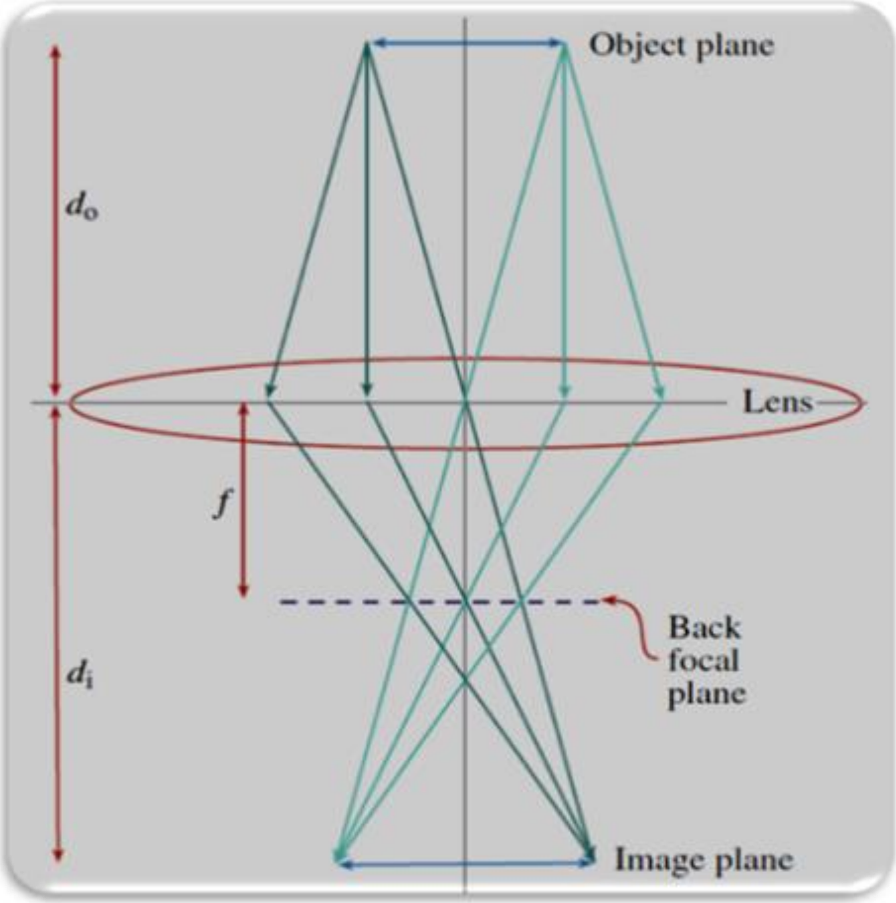
Convergent-beam/probe mode in the STEM

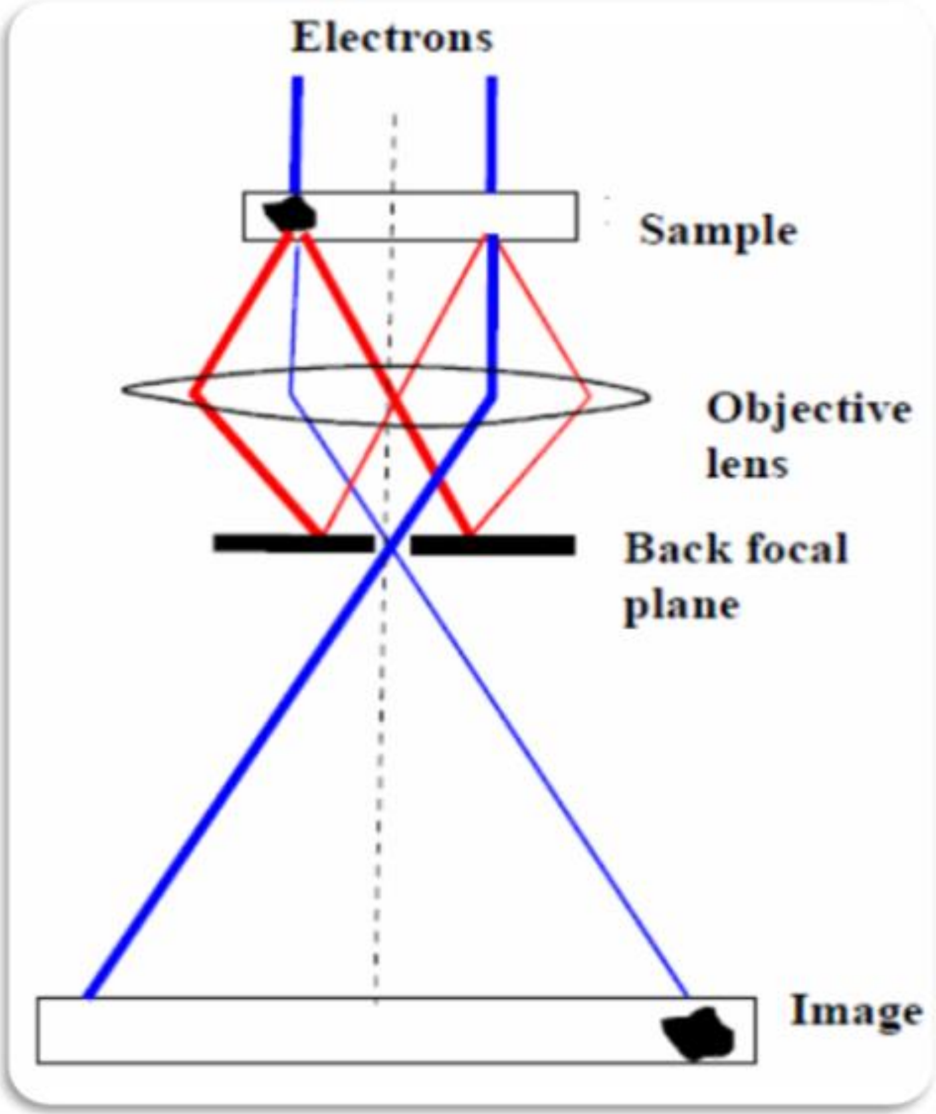




The TEM Instrument (2)

The objective lens/stage





The TEM Instrument (2)

The imaging system of a TEM

Detectors
- Photo film
- Imaging plate
- TV camera
- SS CCD camera

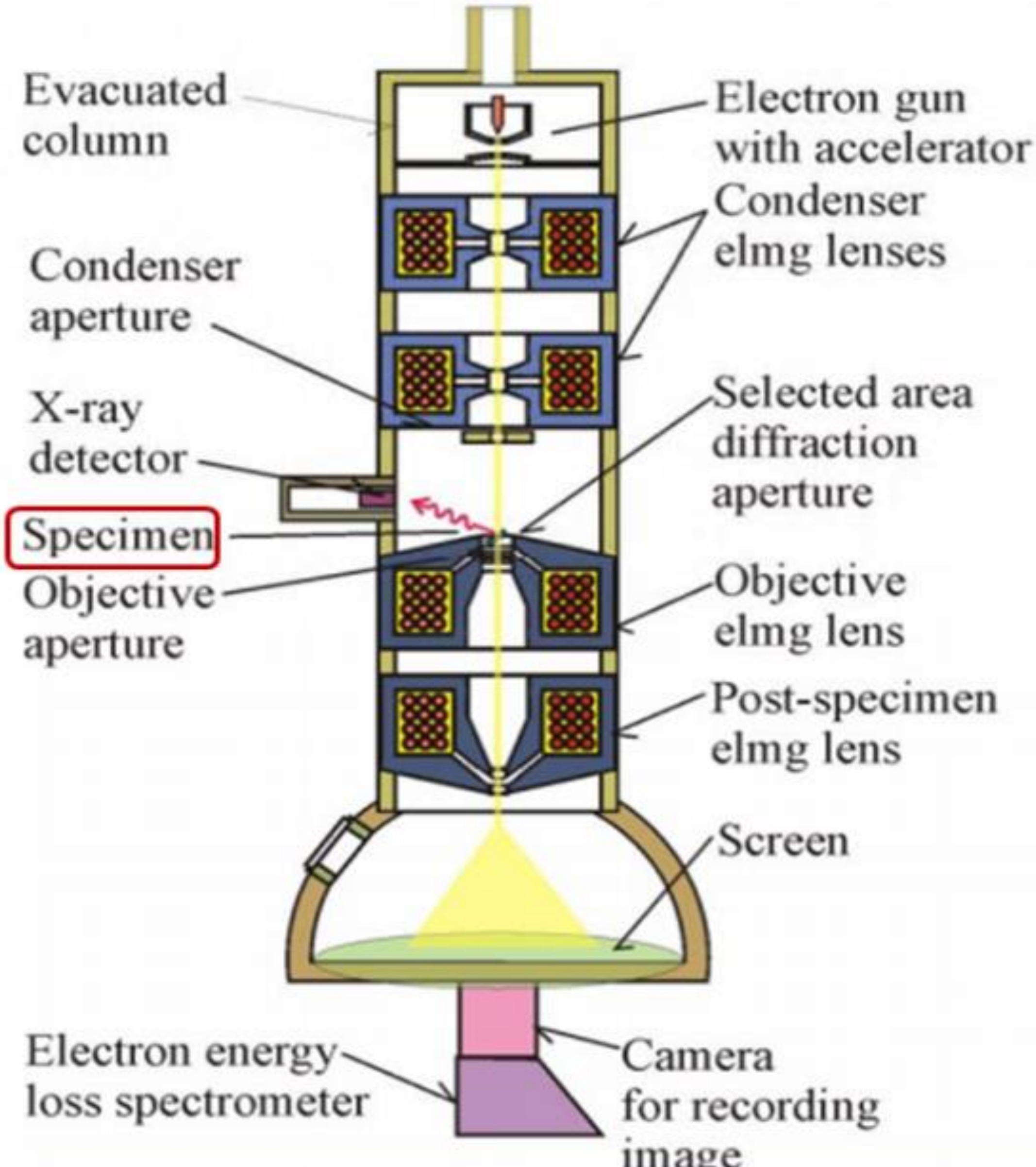
http://www.hk-phy.org/atomic_world/tem/tem02_e.html

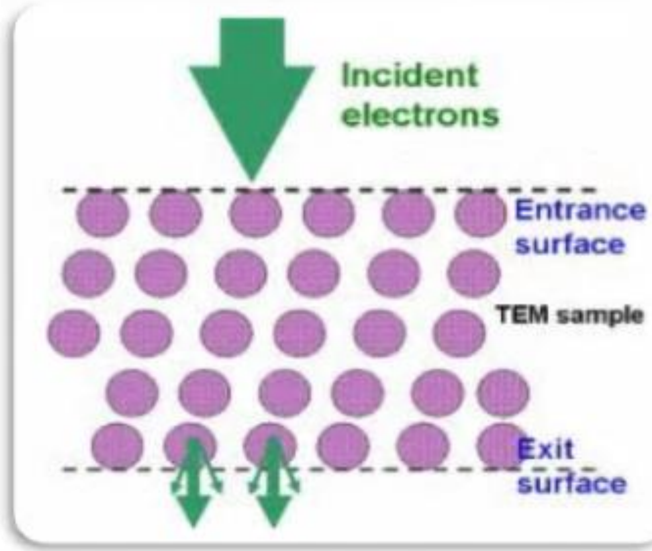
Part II

Sources of TEM Image Information

Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Transmission Electron Microscopy Basics

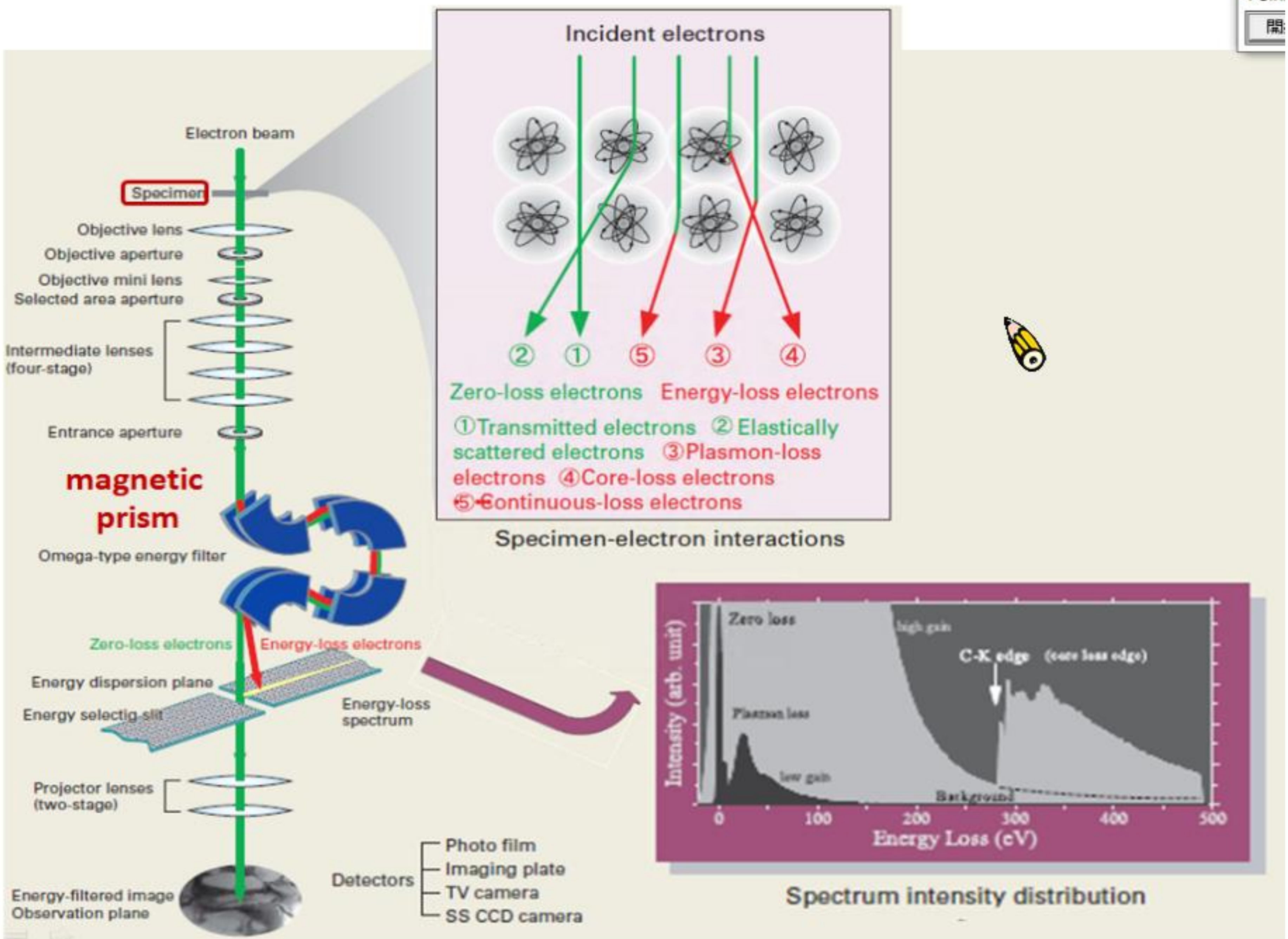




Energy Dispersive X-ray Spectroscopy in TEM

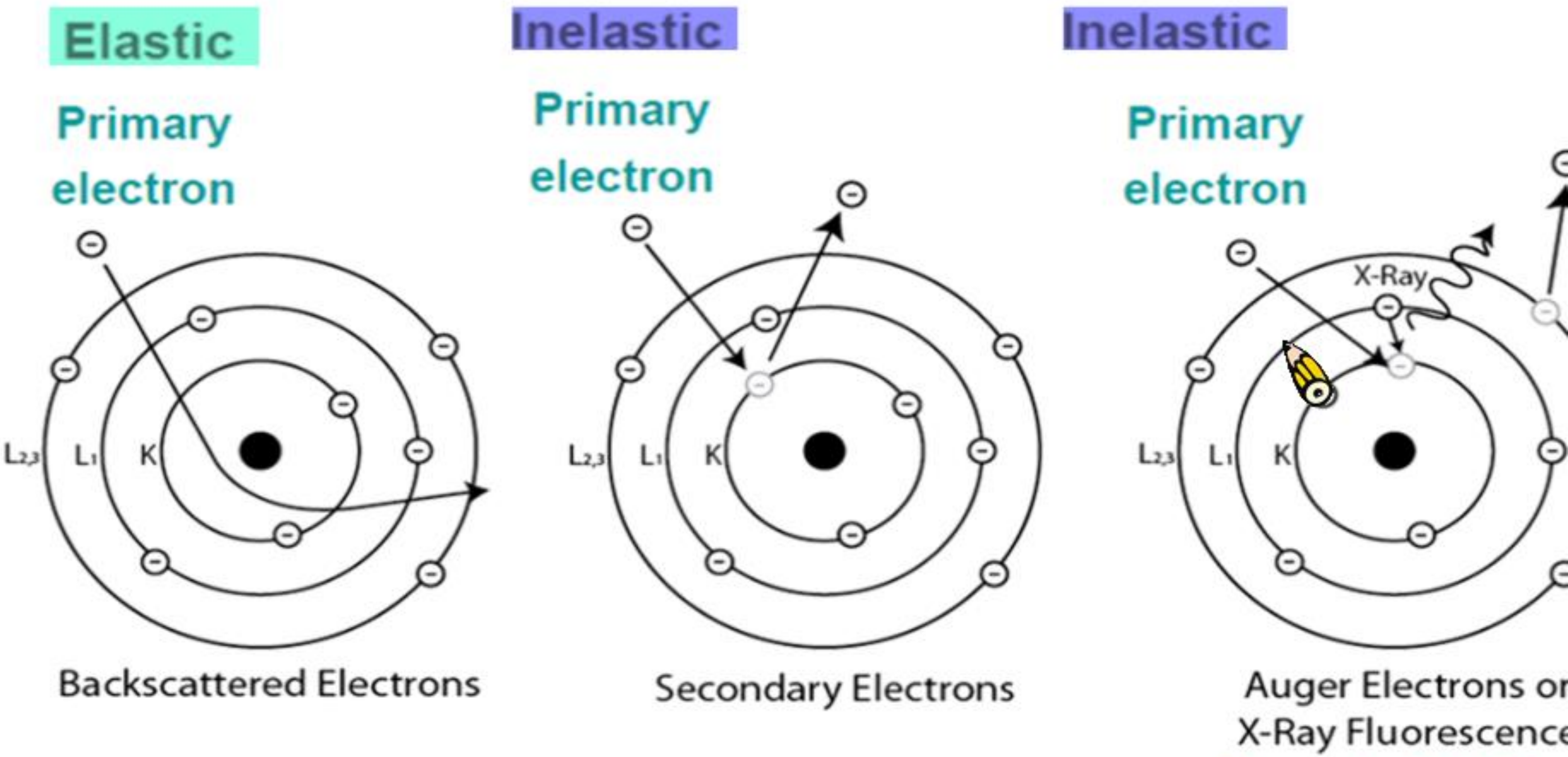
Electron Energy Loss Spectroscopy in TEM

Diffraction in High-resolution TEM


Electron beam
Specimen
Objective lens
Objective aperture
Objective mini lens
Selected area aperture
Intermediate lenses
(four-stage)
Entrance aperture
magnetic
prism
Omega-type energy filter
Zero-loss electrons
Energy-loss electrons
Energy dispersion plane
Energy selectig slit
Energy-loss
spectrum
Projector lenses
(two-stage)
Energy-filtered image
Observation plane
Detectors
Photo film
Imaging plate
TV camera
SS CCD camera
Incident electrons
② ① ⑤ ③ ④
Zero-loss electrons
Energy-loss electrons
①Transmitted electrons ②Elastically scattered electrons ③Plasmon-loss electrons ④Core-loss electrons ⑤Continuous-loss electrons
Specimen-electron interactions
Zero loss
high gain
C-K edge (core loss edge)
Plasmon loss
low gain
Background
Intensity (arb. unit)
0
100
200
300
400
500
Energy Loss (eV)
Spectrum intensity distribution

Emission of Various Electrons and Electromagnetic Waves from the Specime

Electron beam and specimen interaction

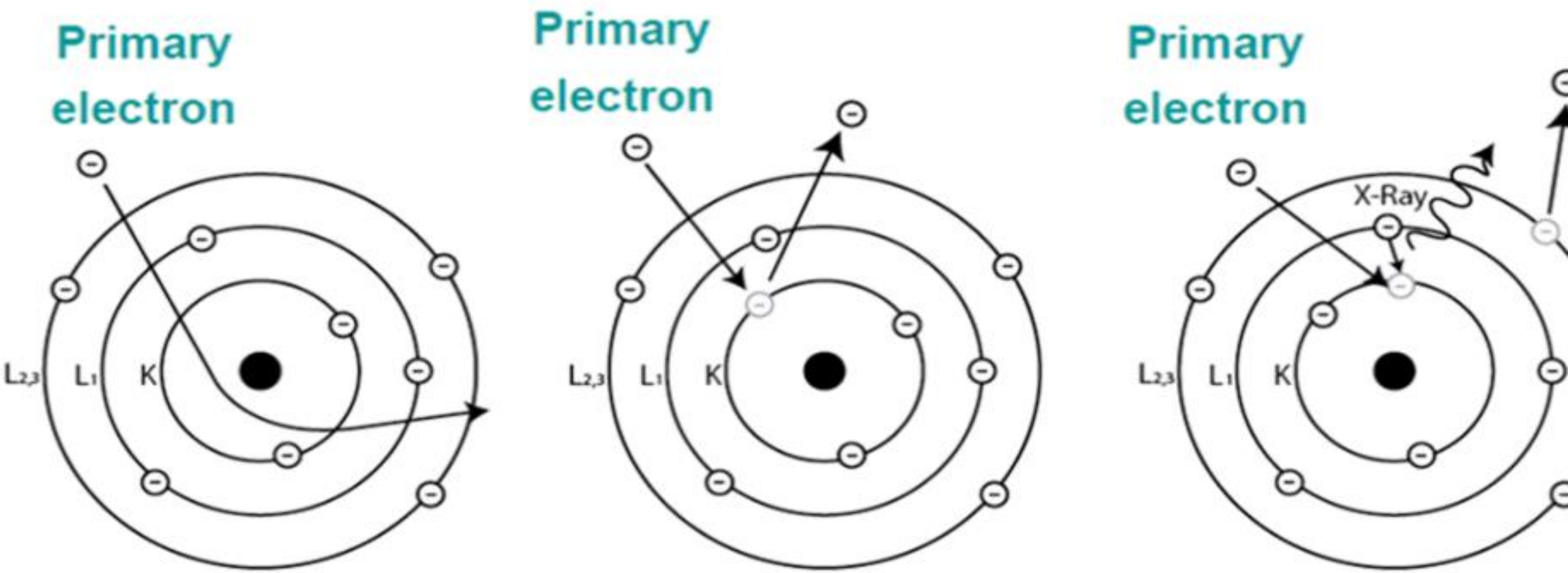


Characteristic X-r

Emission of Various Electrons and Electromagnetic Waves from the Specimen

Electron beam and specimen interactions

Elastic | Inelastic | Inelastic



Backscattered Electrons | Secondary Electrons | Auger Electrons or X-Ray Fluorescence

Characteristic X-r

fsplayer

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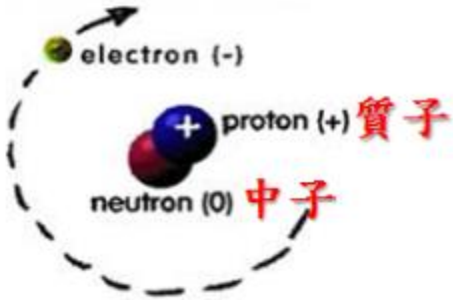
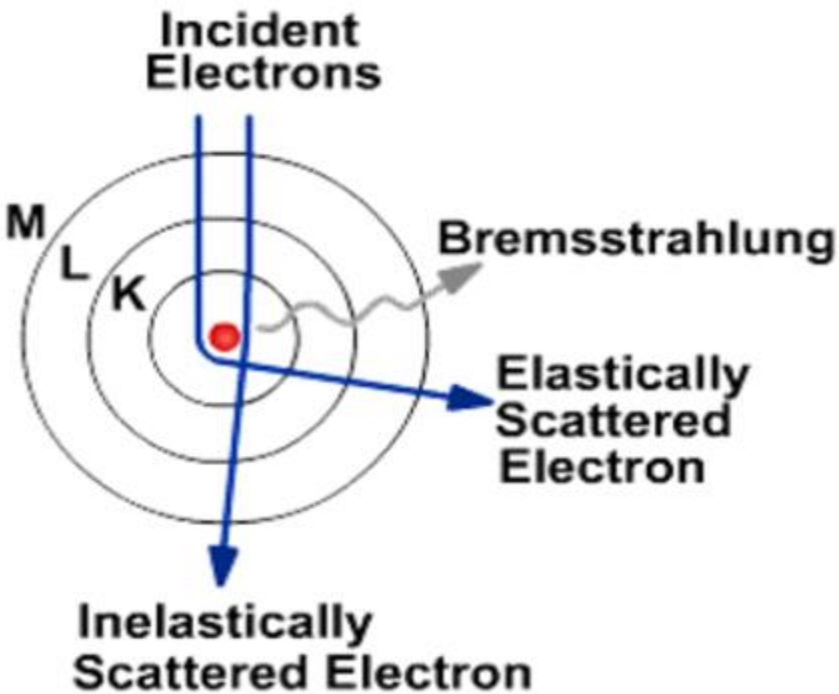
Bremsstrahlung Radiation (制動輻射)

Bremsstrahlung is a German term meaning "braking radiation "





1) X-ray tubes produce x-ray photons by accelerating a stream of electrons to energies of several hundred kilovolts w velocities of several hundred kilometer per hour and colliding them into a **hea target material**.
2) The abrupt acceleration of the charged particles (electrons) produces **Bremsstrahlung photons**. As a free electron interacts with a **proton (質子)** the electron is slowed (but not capture releasing a photon.
3) The highest-energy x ray produced is for which all of **the electron's energy was converted to photon energy**.

http://www.ndt-

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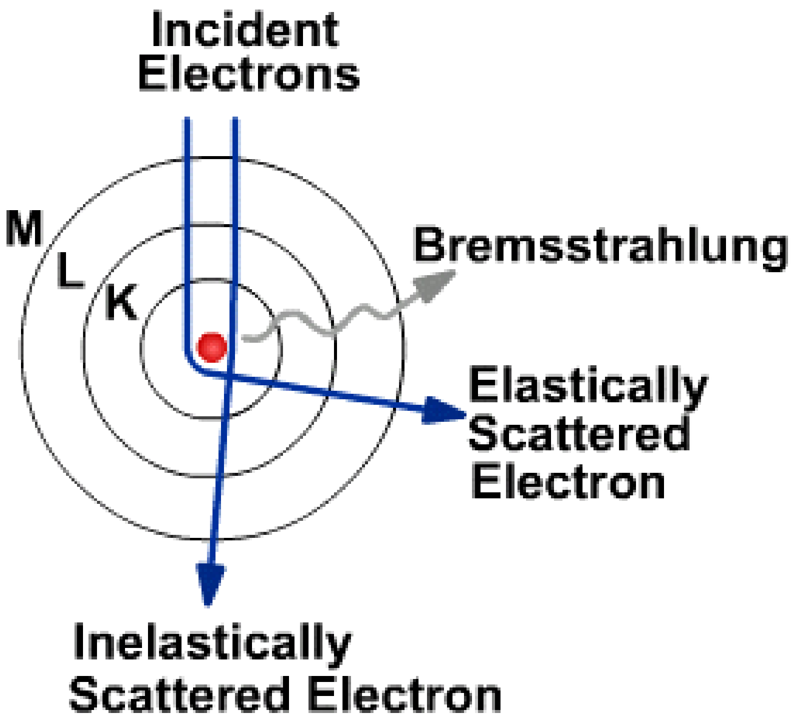
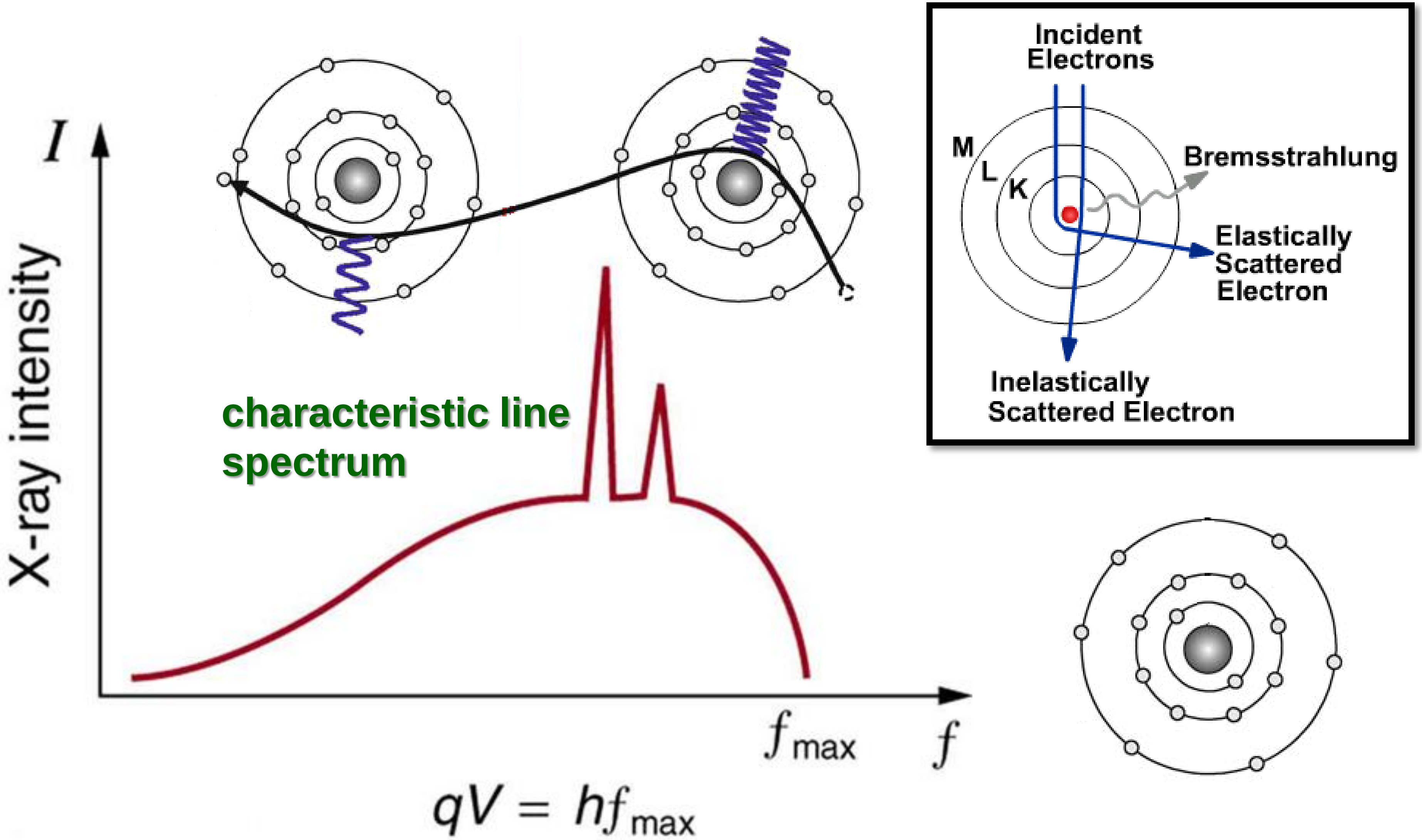


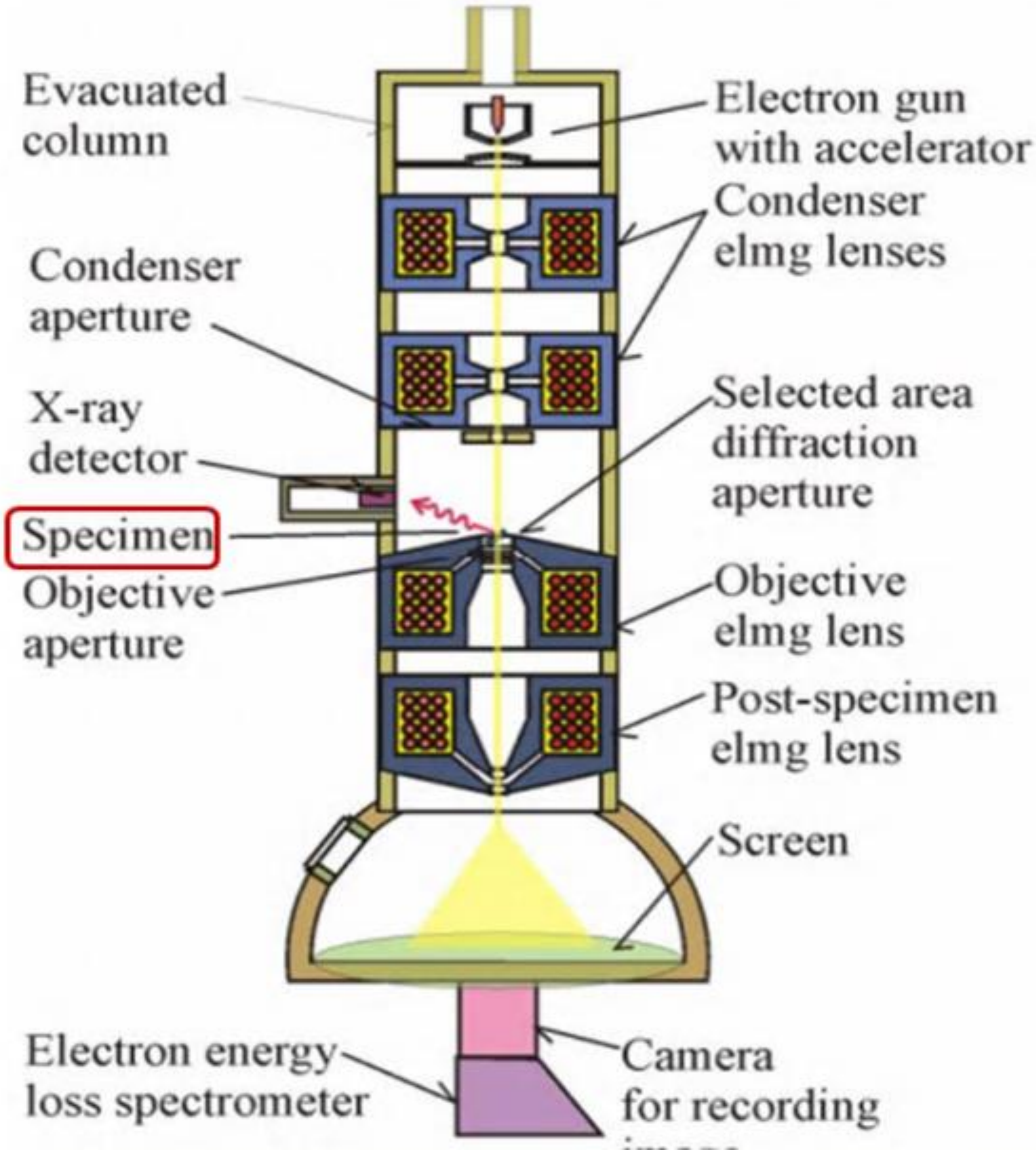
1) X-ray tubes produce x-ray photons by accelerating a stream of electrons to energies of several hundred kilovolts with velocities of several hundred kilometers per hour and colliding them into a **heavy target material**.
2) The abrupt acceleration of the charged particles (electrons) produces **Bremsstrahlung photons**. As a free electron interacts with a **proton (中子)**, the electron is slowed (but not captured) releasing a photon.
3) The highest-energy x ray produced is one for which all of **the electron's energy was converted to photon energy**.
4) Target materials for industrial tubes are typically **tungsten (鎢)**, which means that the wave functions of the bound tungsten electrons are required.

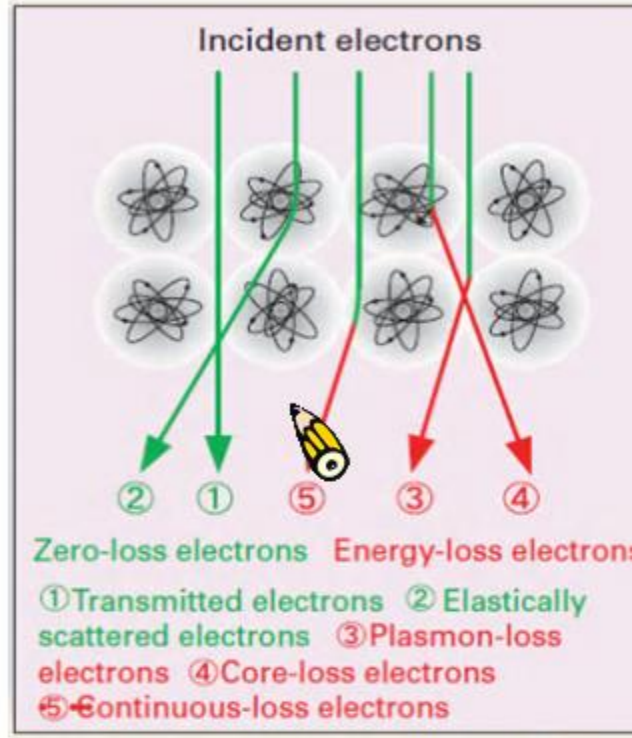
http://www.ndt-ed.org/EducationResources/CommunityCollege/Radiography/Physics/xrays.htm



X-ray spectrum obtained when energetic electrons strike a material, such as in the anode of a CRT. The smooth part of the spectrum is bremsstrahlung radiation, **while the peaks are characteristic of the anode material**. A different anode material would have characteristic x-ray peaks at different frequencies.

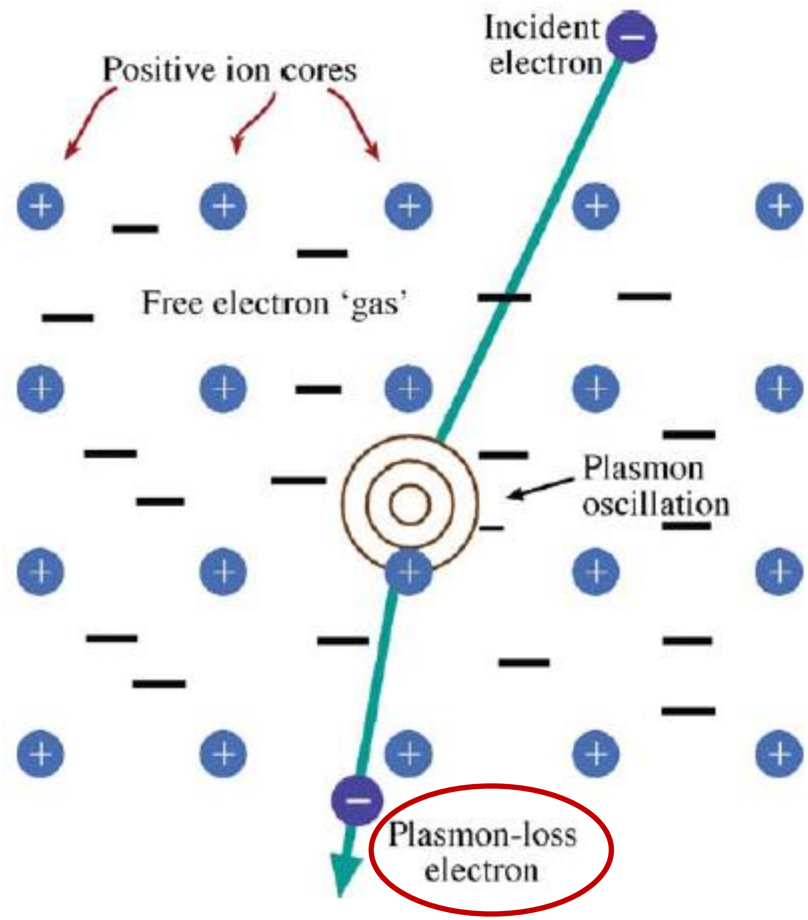
Transmission Electron Microscopy Basics

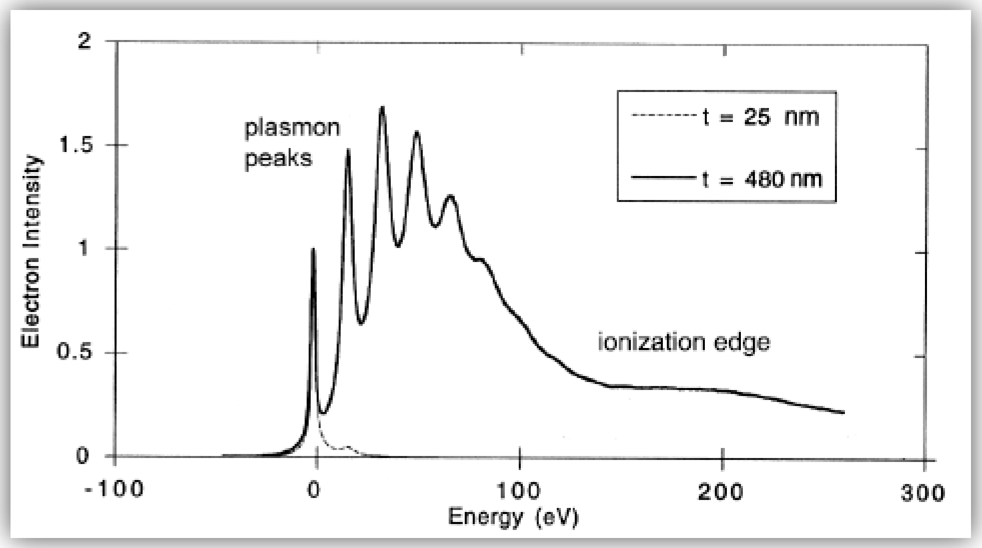




Specimen-electron interactions

(a) Plasmon-loss Electrons



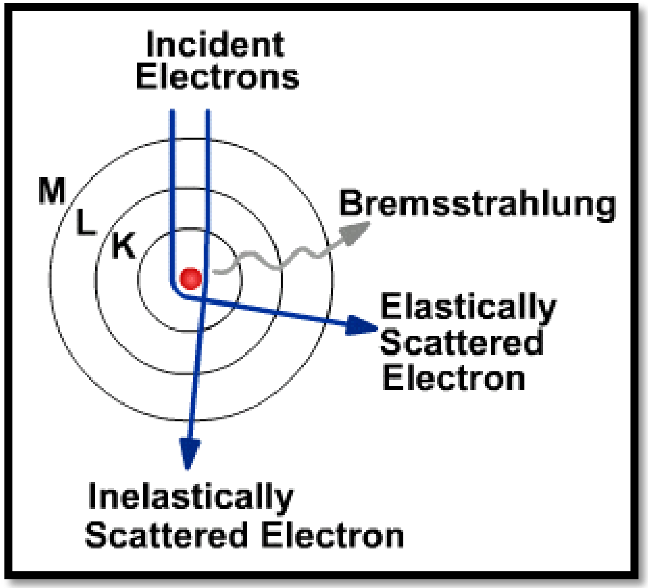


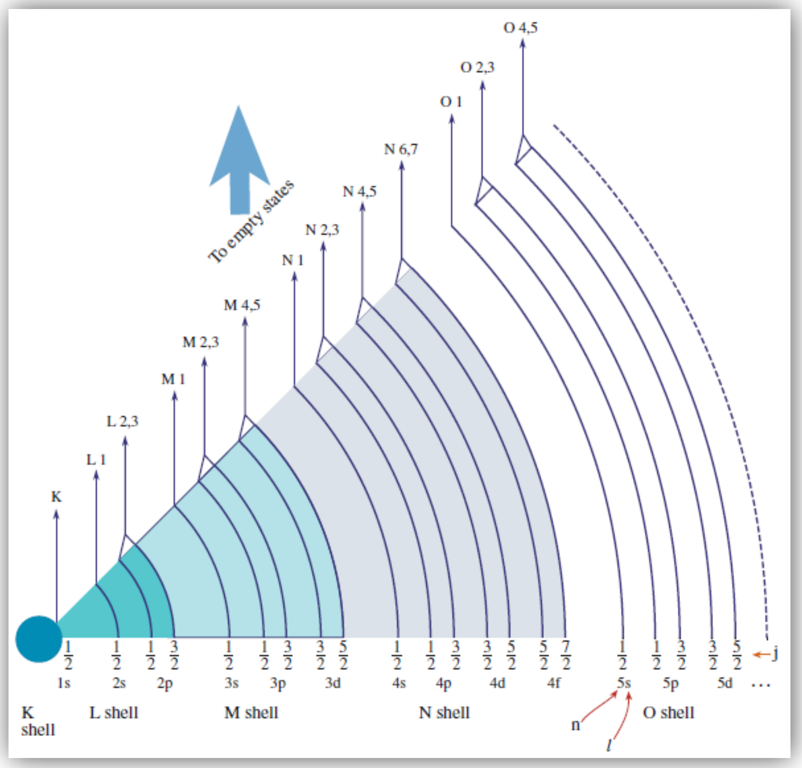
Energy-loss spectrum of a thin region of silicon (thin line) and of a thicker area (thick line), with their zero-loss peaks matched in height. Plasmon peaks occur at multiples of the plasmon energy (Ep = 16.7 eV).

Plasmons can occur in any material with weakly bound or free electrons, but they occur predominantly in metals, particularly ones like aluminum and, therefore, a high free-electron density.

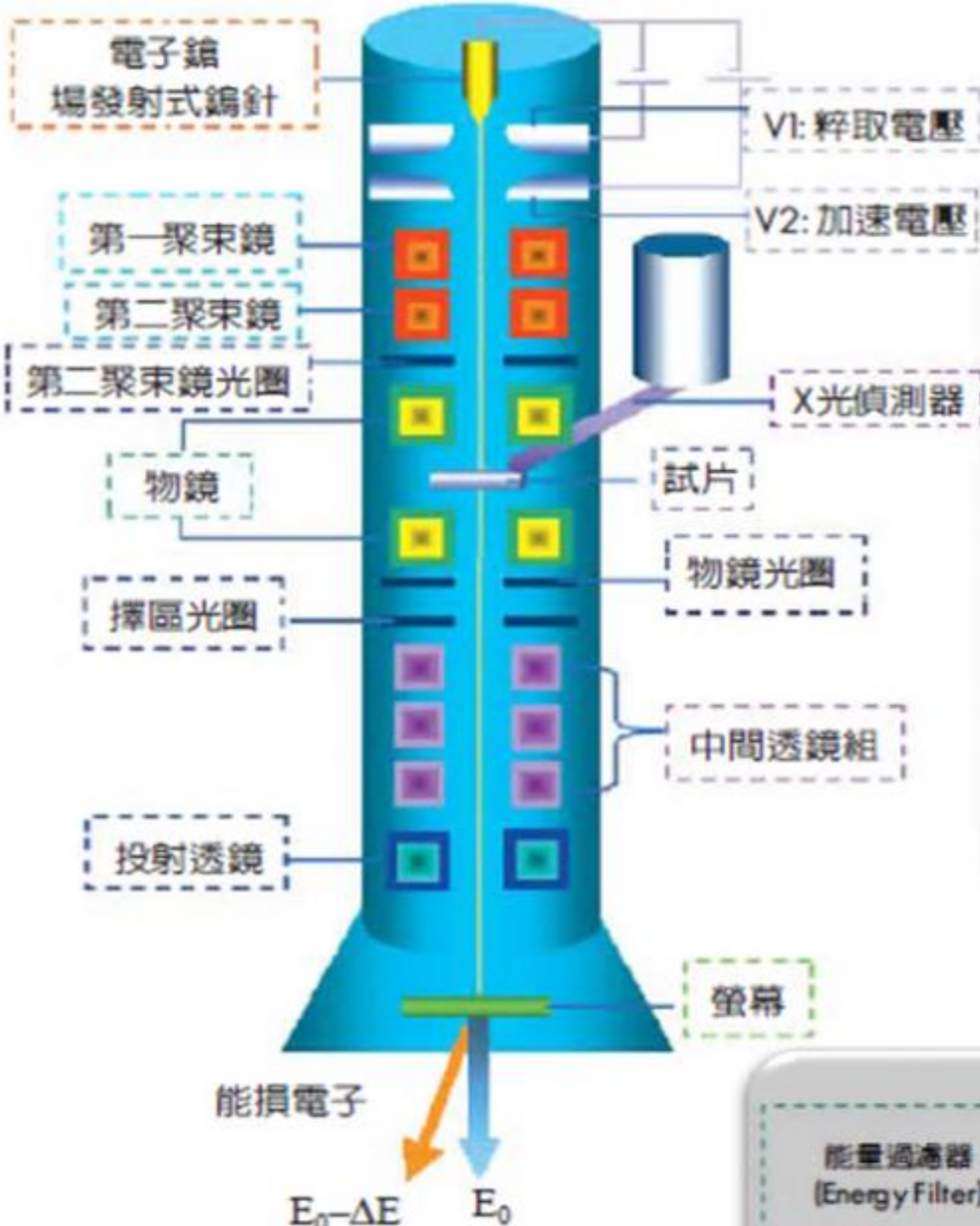
http://tem-eels.ca/egerton-laser/preprint/Egerton%20RPP'09%20EELS%20in%20TEM.pdf

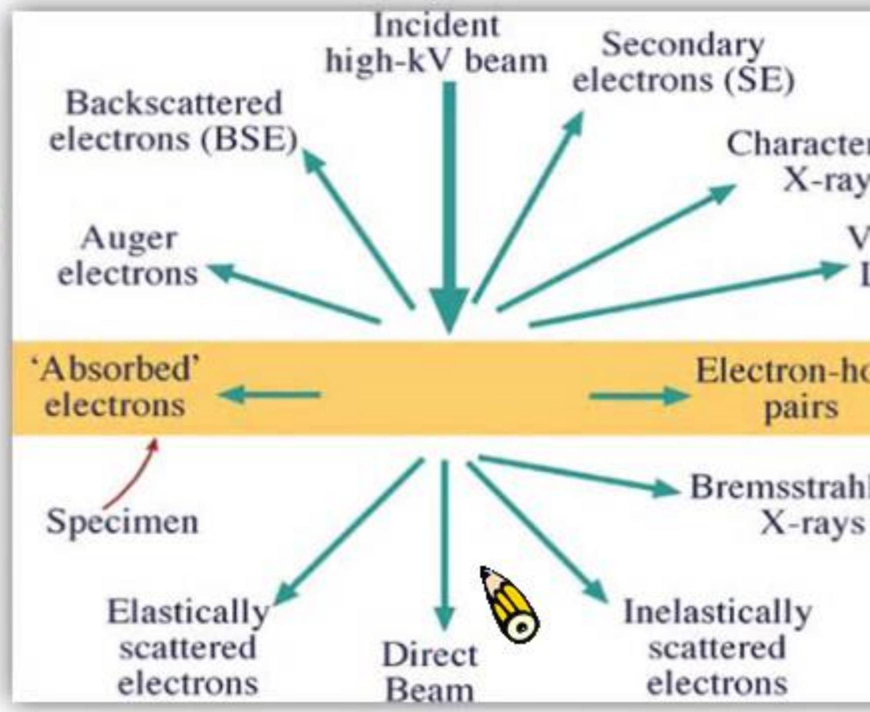
(b) Core-loss Electrons



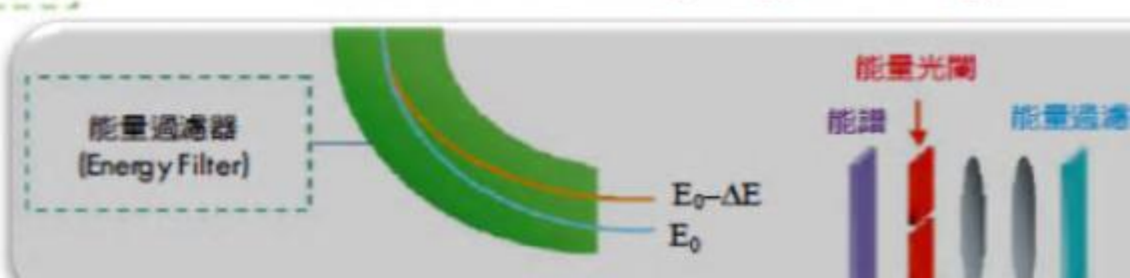


1) When a beam electron transfers sufficient energy to a core-shell electron (i.e., one in the inner, more tightly bound K, L, M, etc., shells) to move it outside the attractive field of the nucleus, the atom is said to be ionized.
2) The atomic electrons that are located in inner have binding energies that are mostly hundreds or thousands of electron volts.
3) We are interested in ionization losses precisely because the process is characteristic of the atom involved and so the signal is a direct source of chemical information.

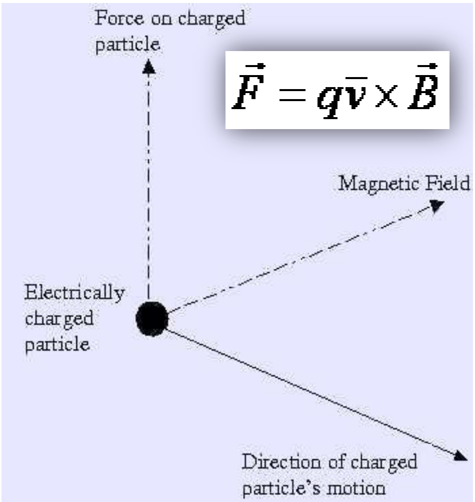




In column omega type Energy filter

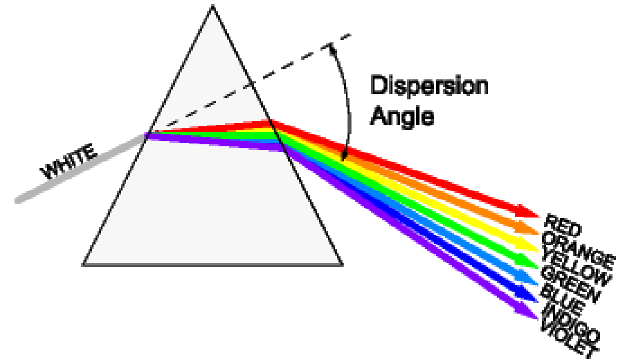


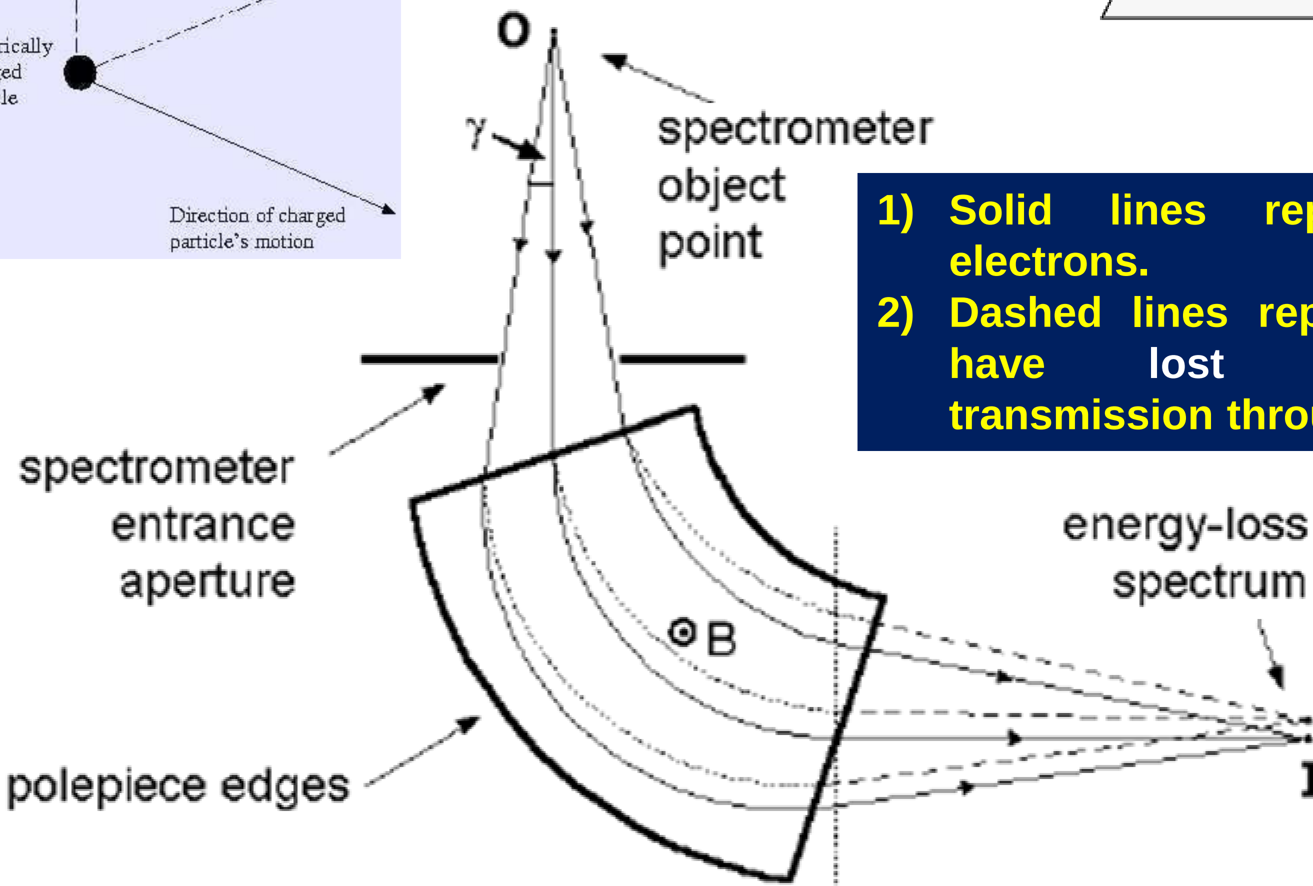
Dispersive and Focusing Properties of a Magnetic Prism



$$F = mv^2/R$$

$$R = (m/e)(1/B)v$$

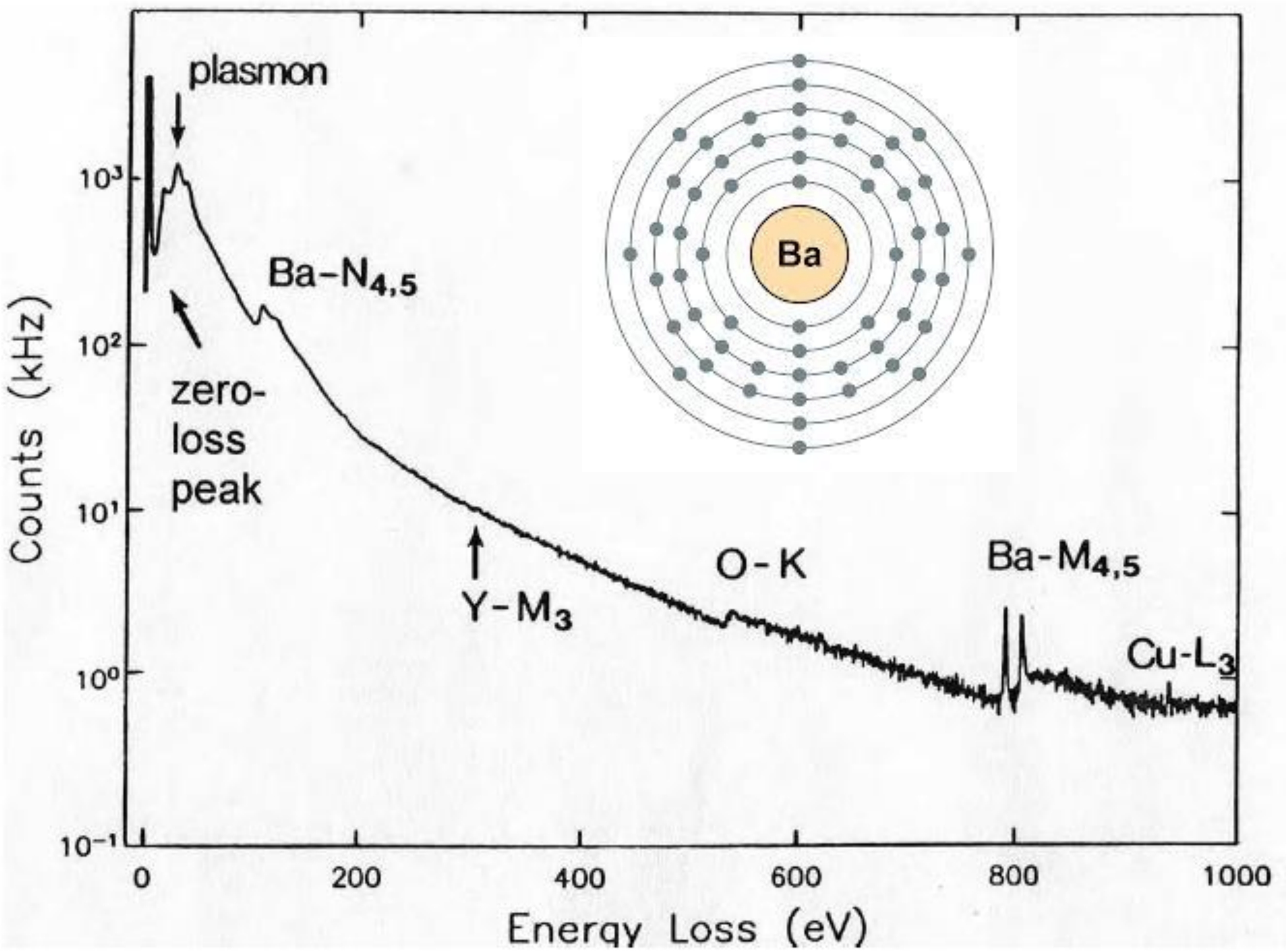




1) Solid lines represent zero-loss electrons.
2) Dashed lines represent those that have lost energy during transmission through the specimen.

http://tem-eels.ca/egerton-laser/preprint/Egerton%20RPP'09%20EELS%20in%20TEM.pdf

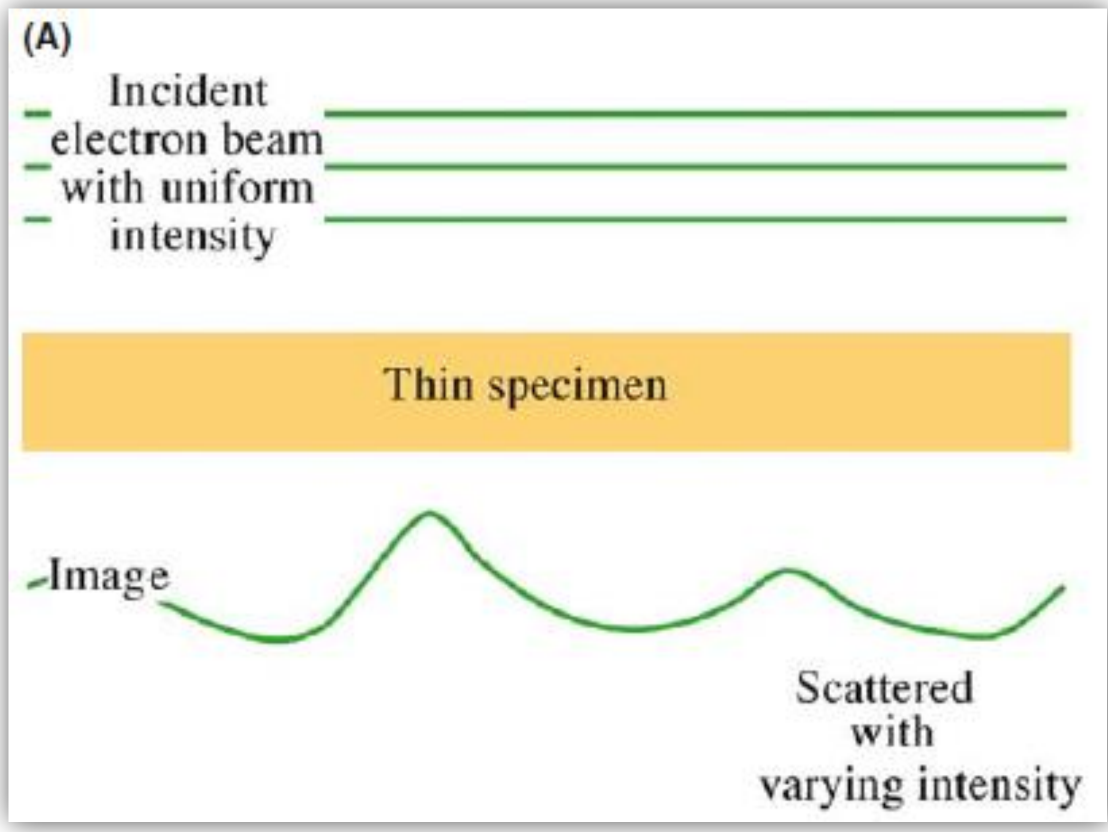
Core-loss Electrons



Electron energy-loss spectrum of a high-temperature superconductor, showing N- and M-shell ionization edges of barium (Ba 鋇), the K-ionization edge of oxygen, and weak ionization edges from copper and yttrium (釔).

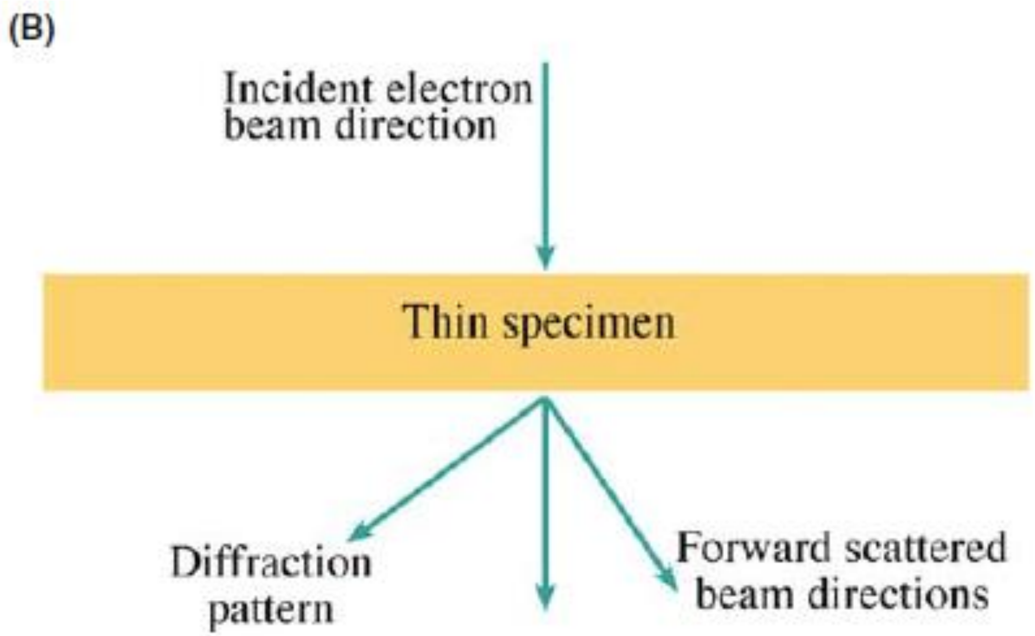
Scattering and Diffraction

- The electron is treated in two different ways: in (A) electron scattering it is a succession of particles, while in (B) electron diffraction it is treated by wave theory.



It is this non-uniform distribution that contains all the structural, chemical, and other information about our specimen.

Scattering and Diffraction



1) The change in angular distribution is shown by an incident beam of electrons being transformed into several **forward-scattered beams**.
2) The angular distribution of scattering can be viewed in the form of scattering patterns, usually called **diffraction patterns (DPs)**.

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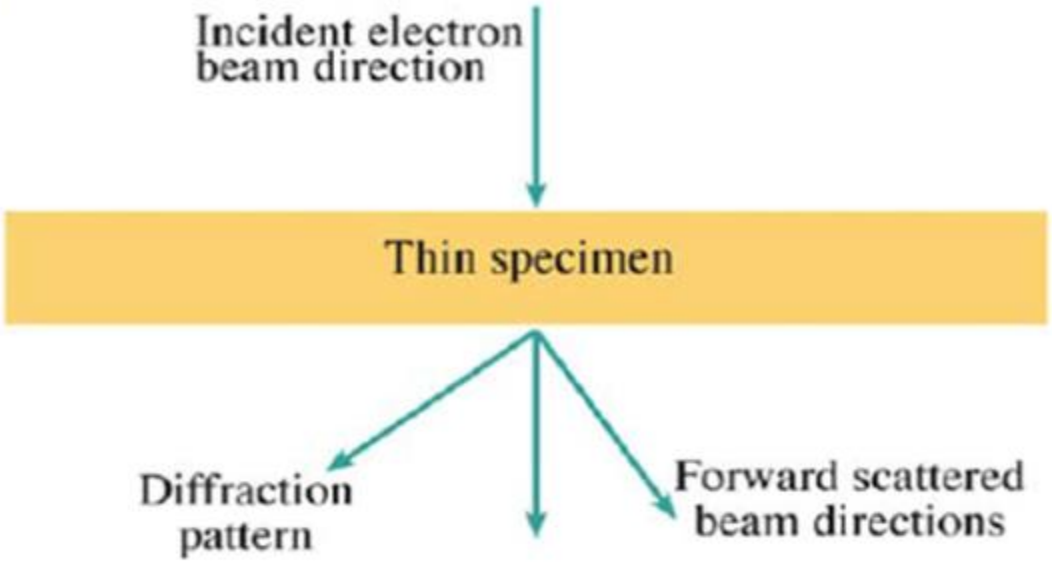
Scattering and Diffraction

(B)



1) **The change in angular distribution is shown by an incident beam o electrons being transformed into several forward-scattered beams.**

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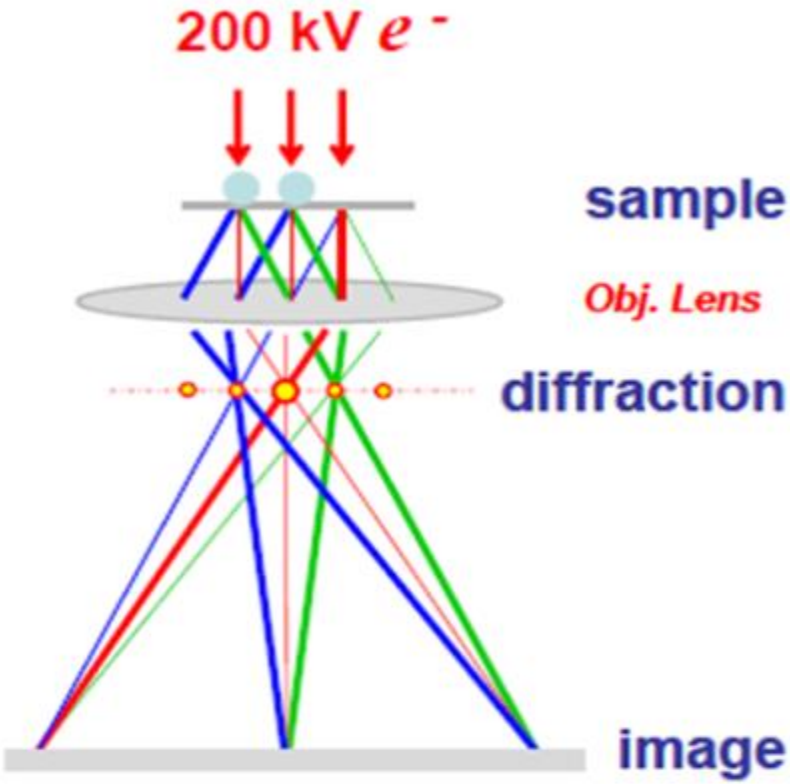
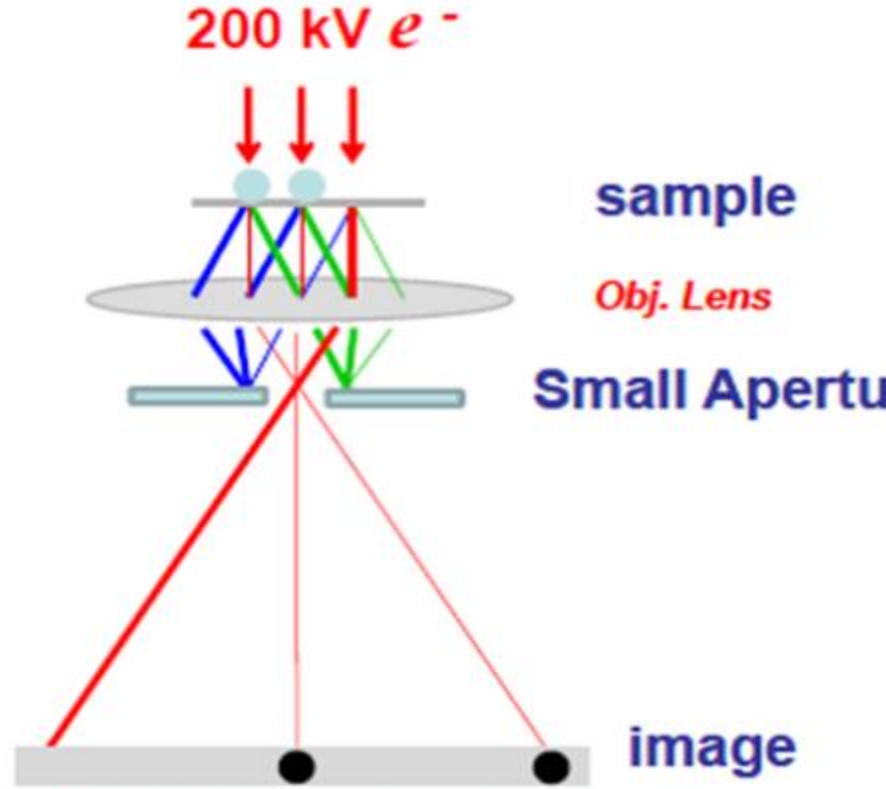
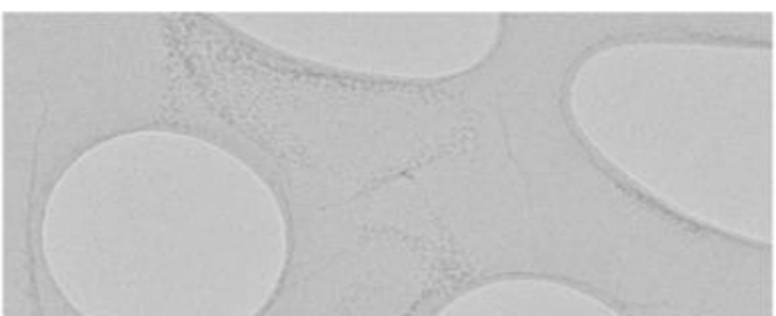
Image Formation and Contrast



No objective aperture



Objective Aperture inserted to select the central direct unscattered beam



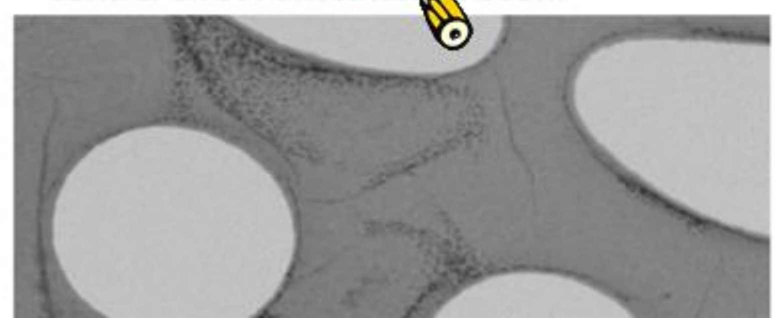
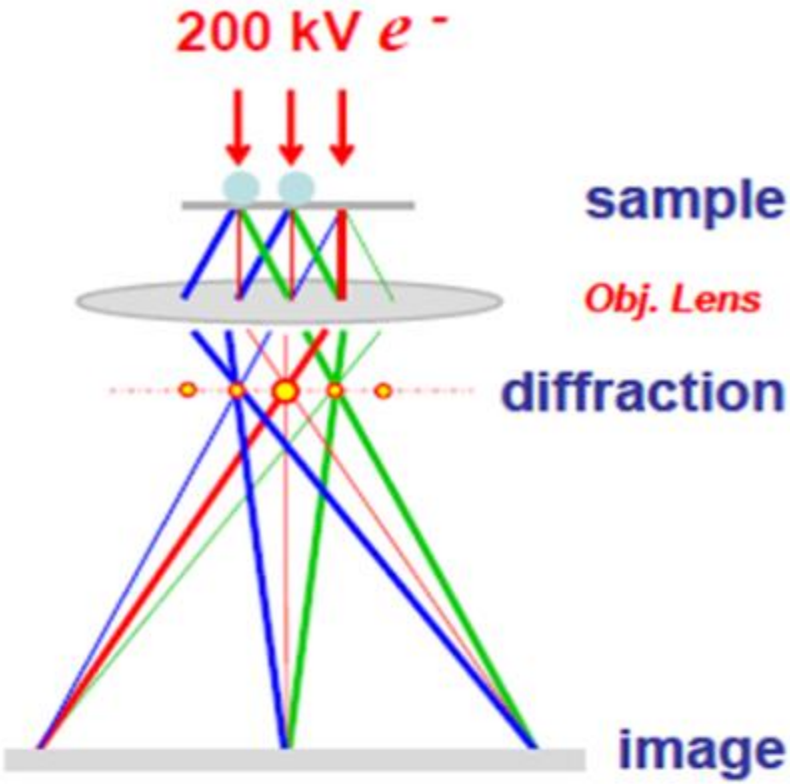


Image Formation and Contrast



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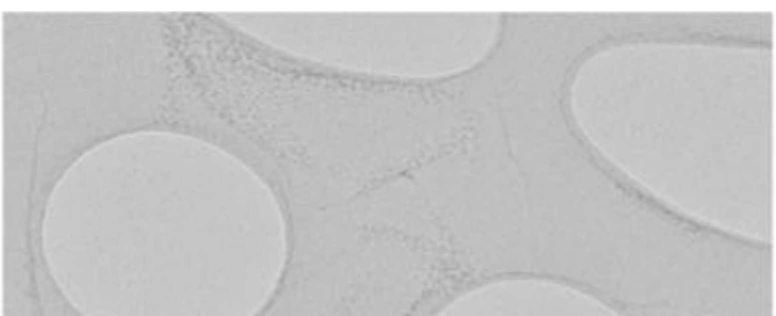

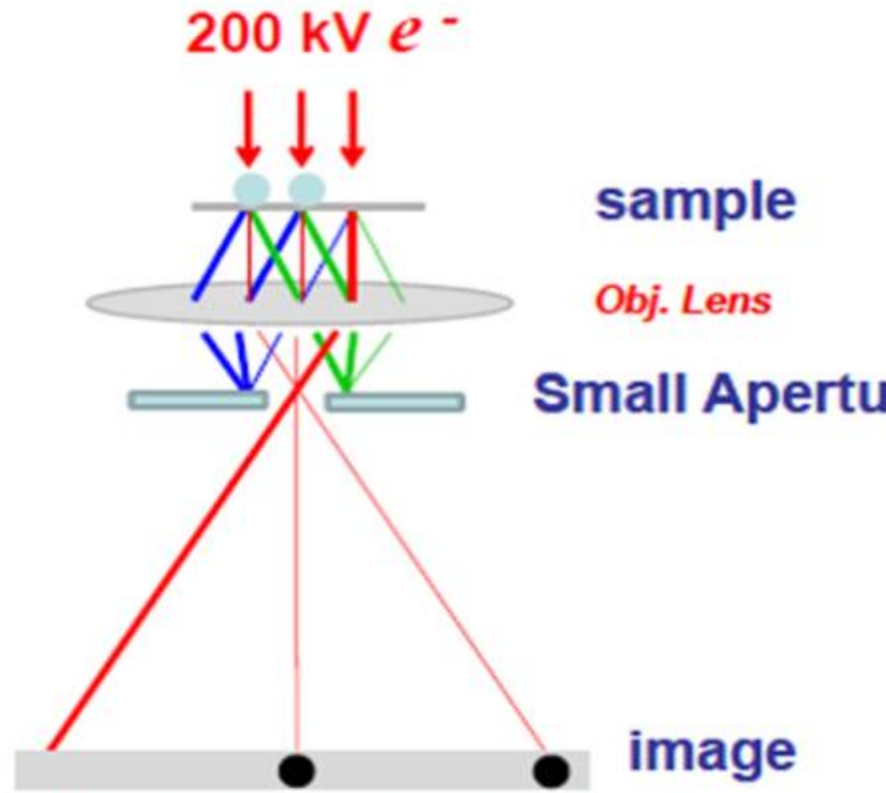

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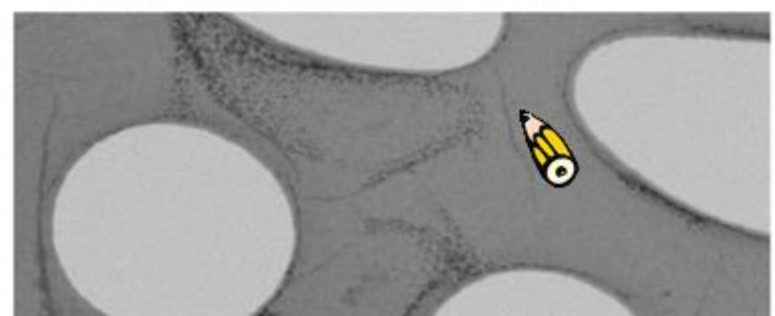
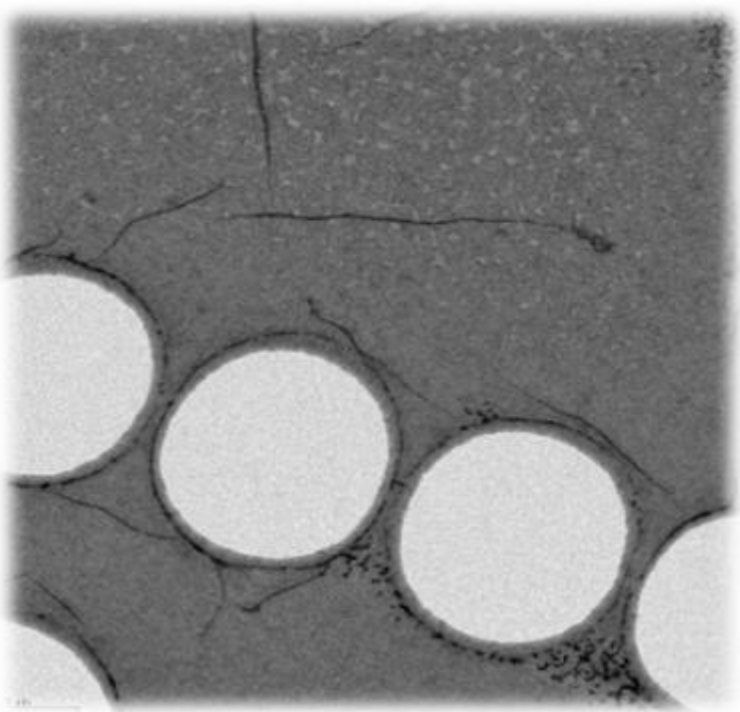
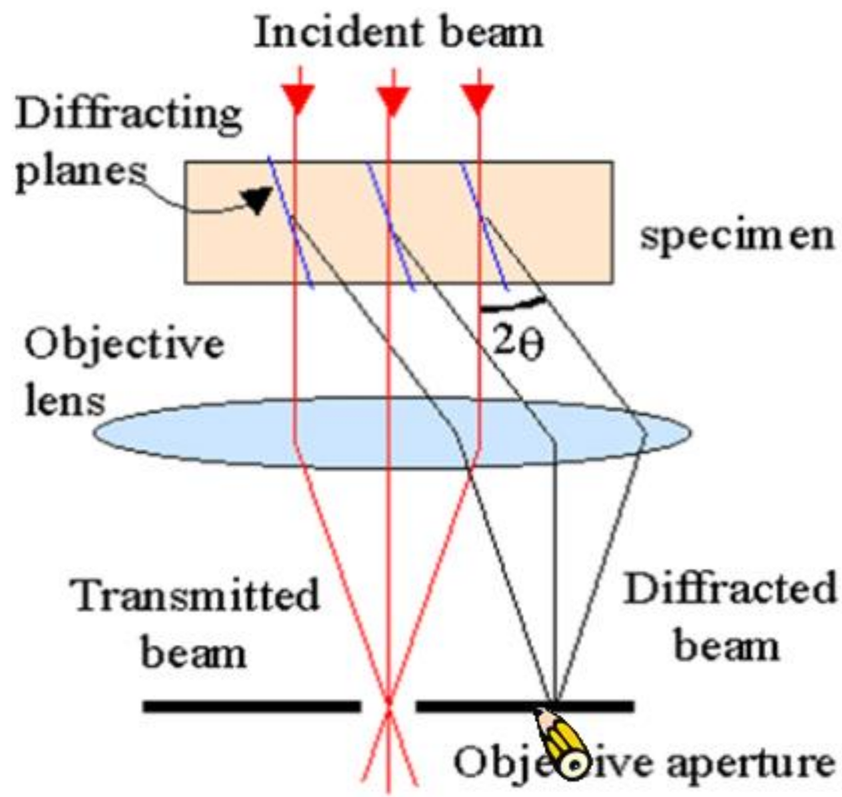


Bright Field Imaging

- If the main portion of the near-forward scattered beam is used to form the image
  - transmitted beam
  - zero-order beam





Bright Field Imaging

fsplayer

File Control Help

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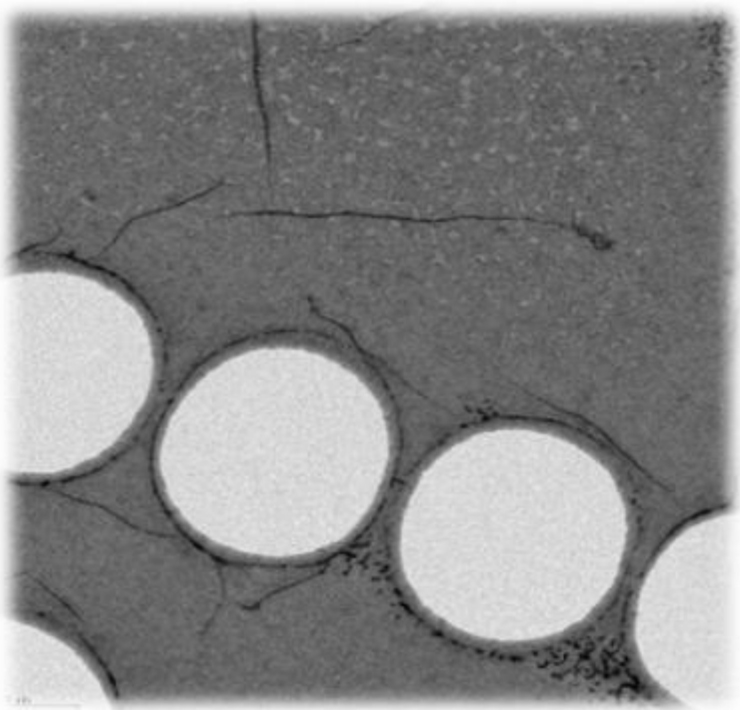
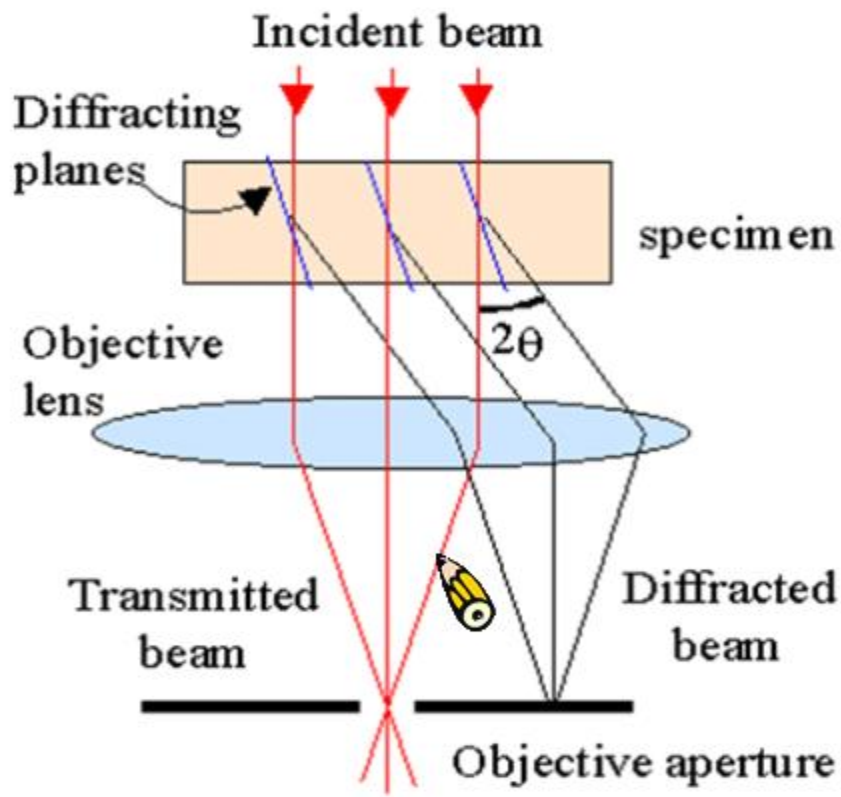
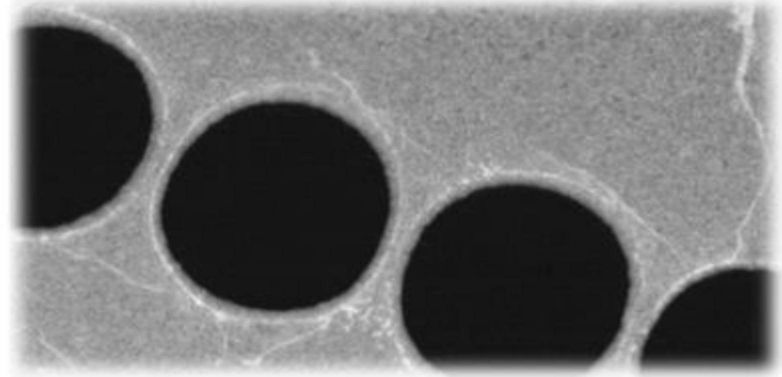


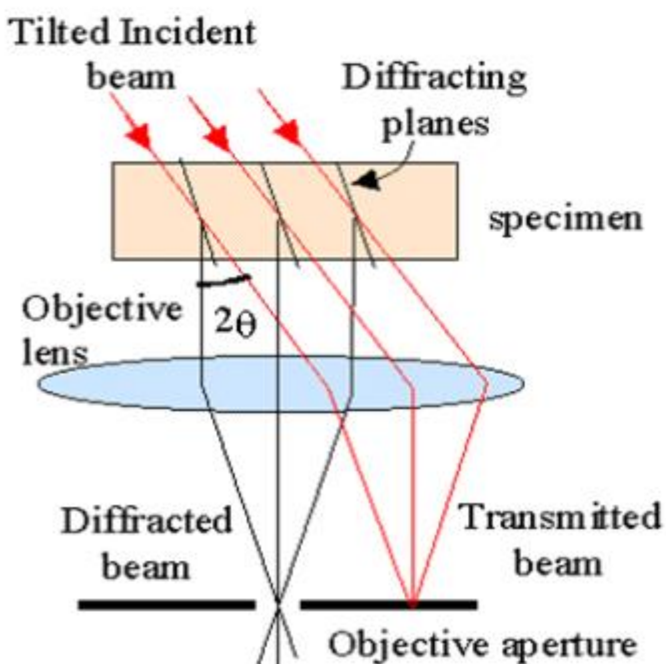


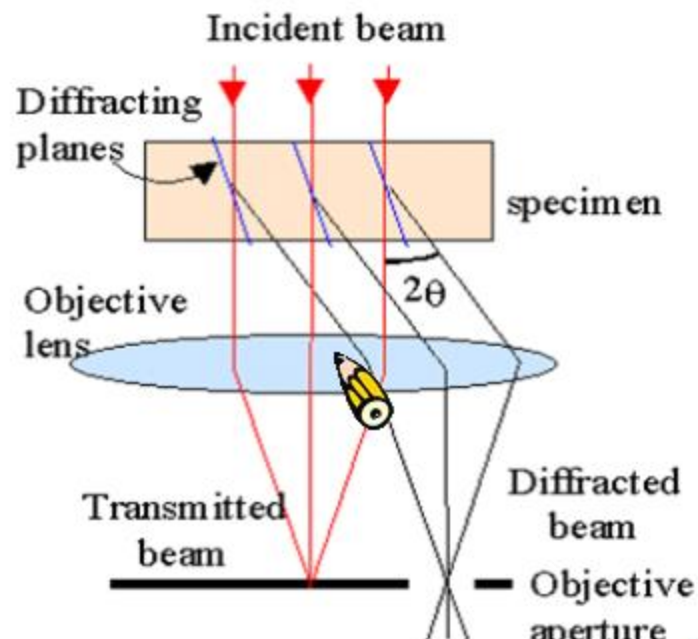
Bright Field Imaging

Dark Field Imaging

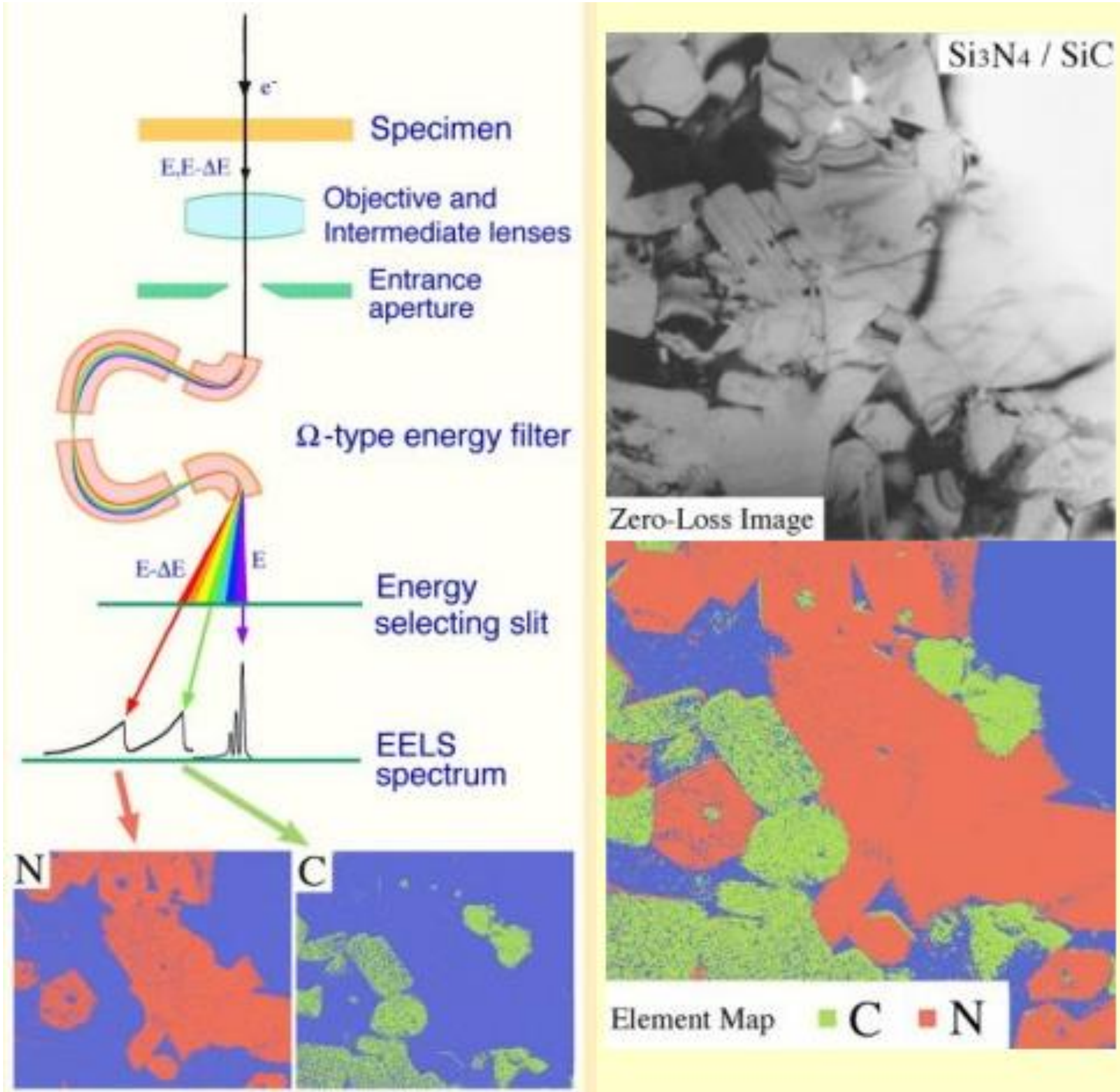
- If the transmitted beam is excluded from the imag formation process
  - off-axis imaging
  - tilted beam imaging







Element Mapping by Ω-Type Energy Filter TEM



http://www.tagen.tohoku.ac.jp/labo/terauchi/research/instruments/jem2010fef.html

Interpreting Transmission Images

projection-limitation

1) TEM information is averaged through the thickness of the specimen. In other words, a single TEM image has no depth sensitivity.

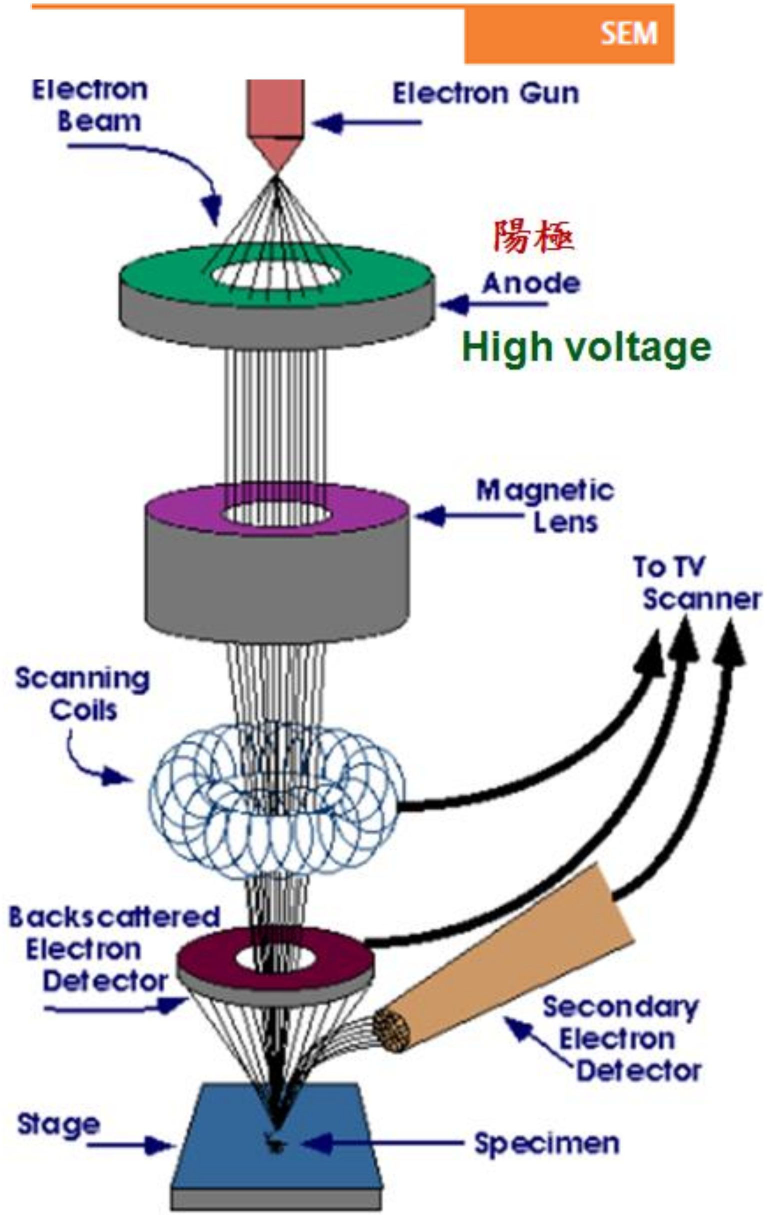
2) it is an axiom in TEM that, almost invariably, thinner is better and specimens <100 nm should be used wherever possible.

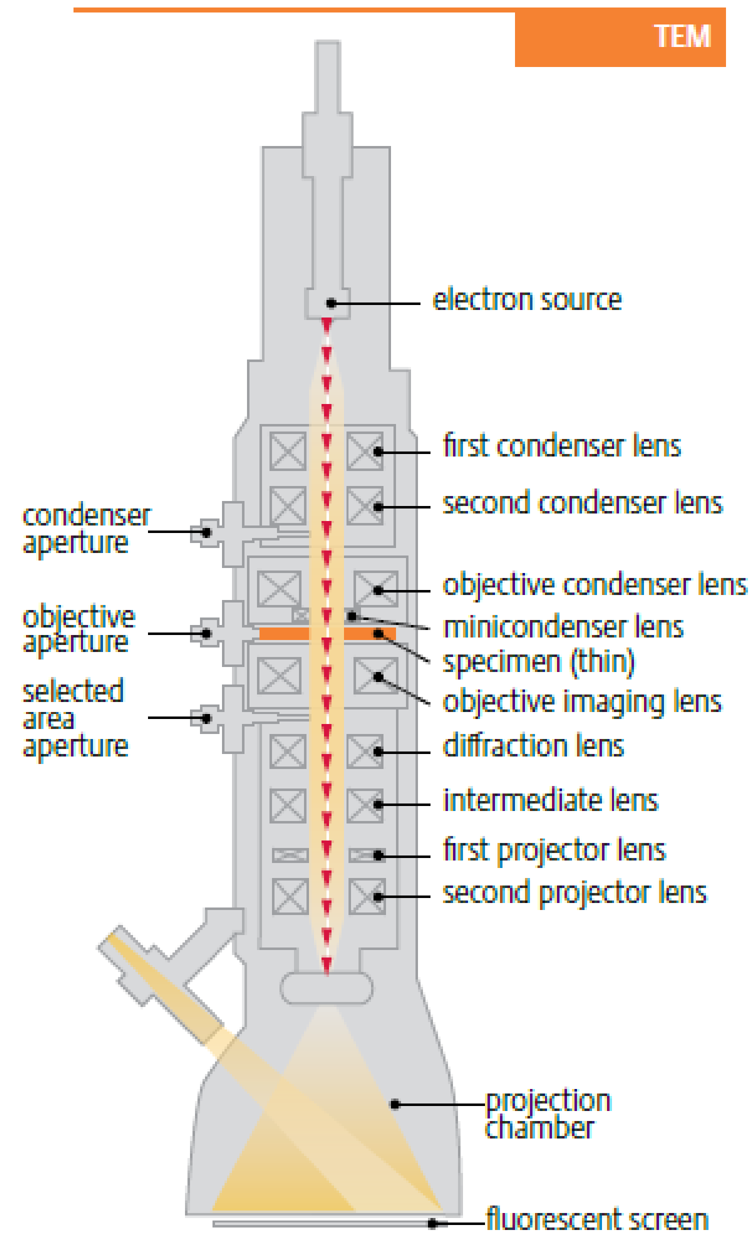
3) In extreme cases such as doing electron spectrometry, specimen thicknesses < 50 nm (even < 10 nm) are essential. These demands become less strict as the beam voltage increases, but this is offset by the production of beam damage.

Thanks For Your Attention

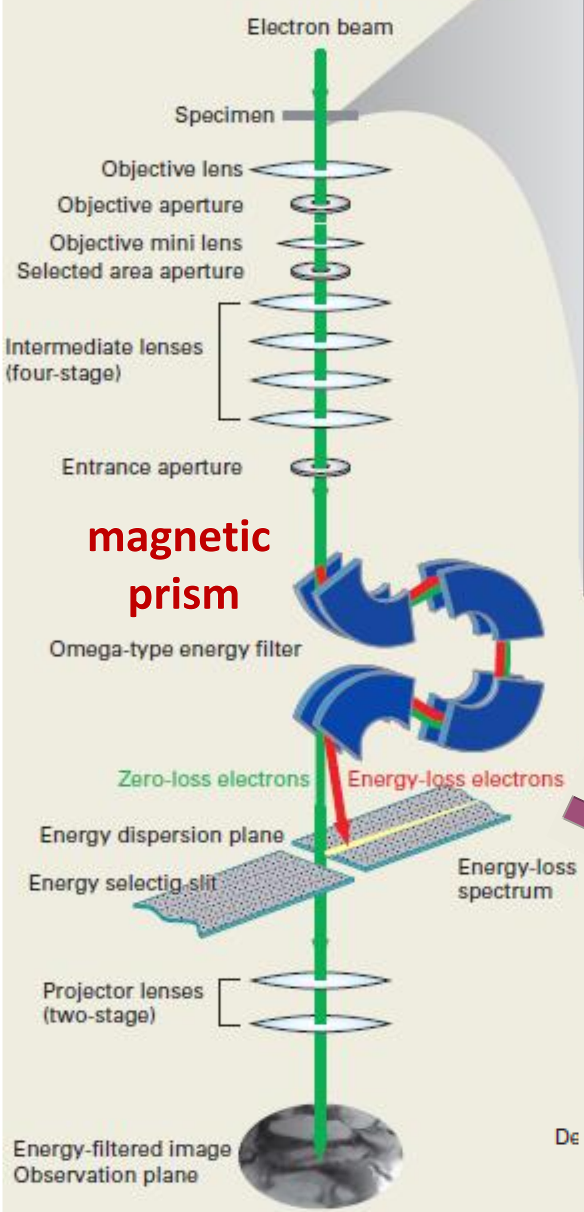
37

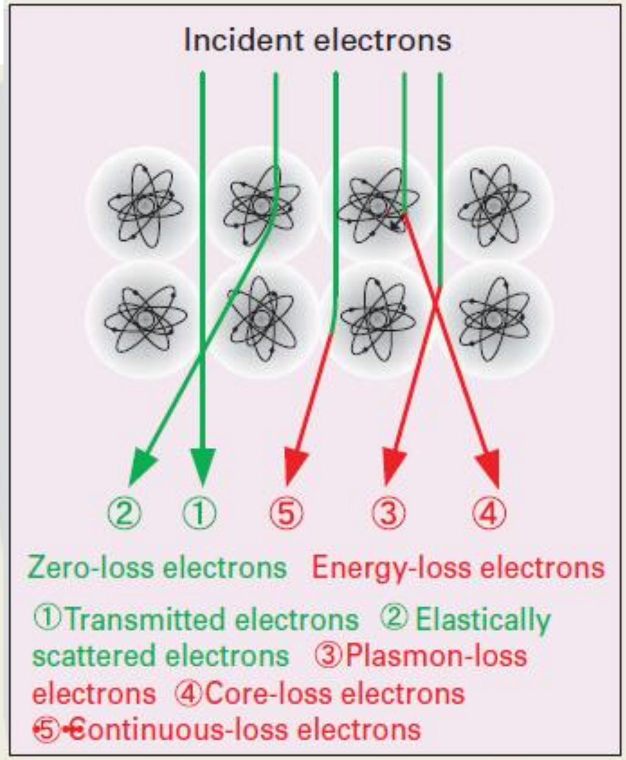
Comparison of the SEM with TEM



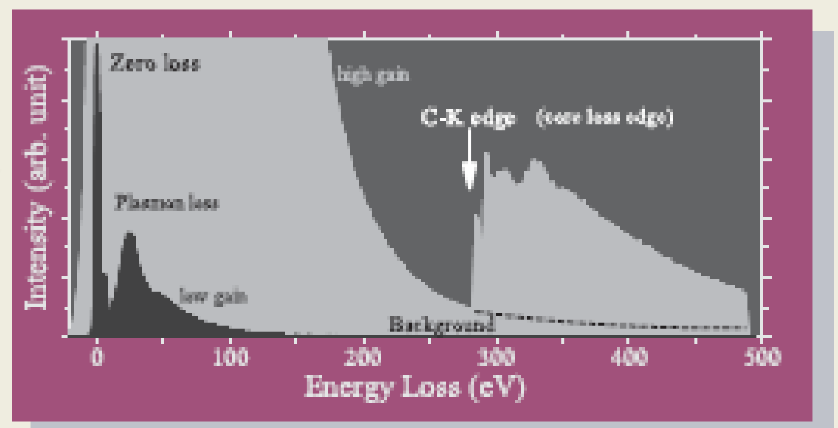


http://www.fei.com/uploadedfiles/documents/content/introduction_to_em_booklet_july_10.pdf



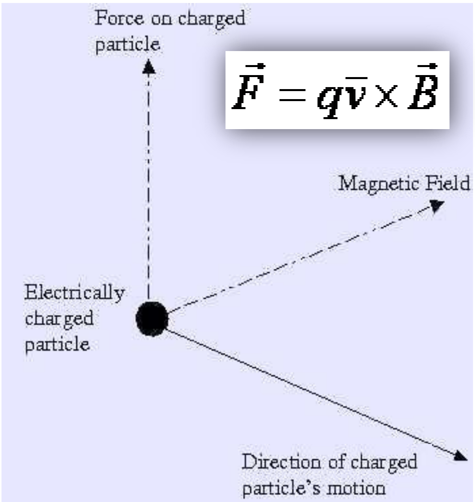


Specimen-electron interactions



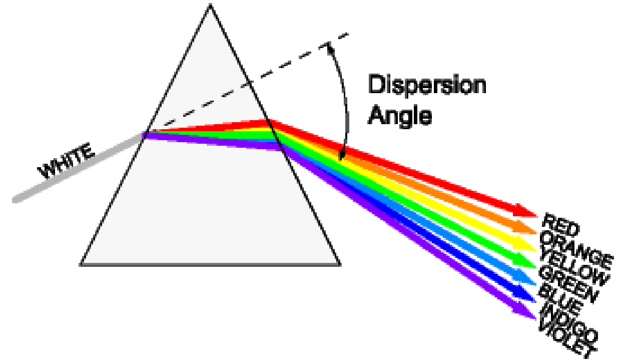
Spectrum intensity distribution

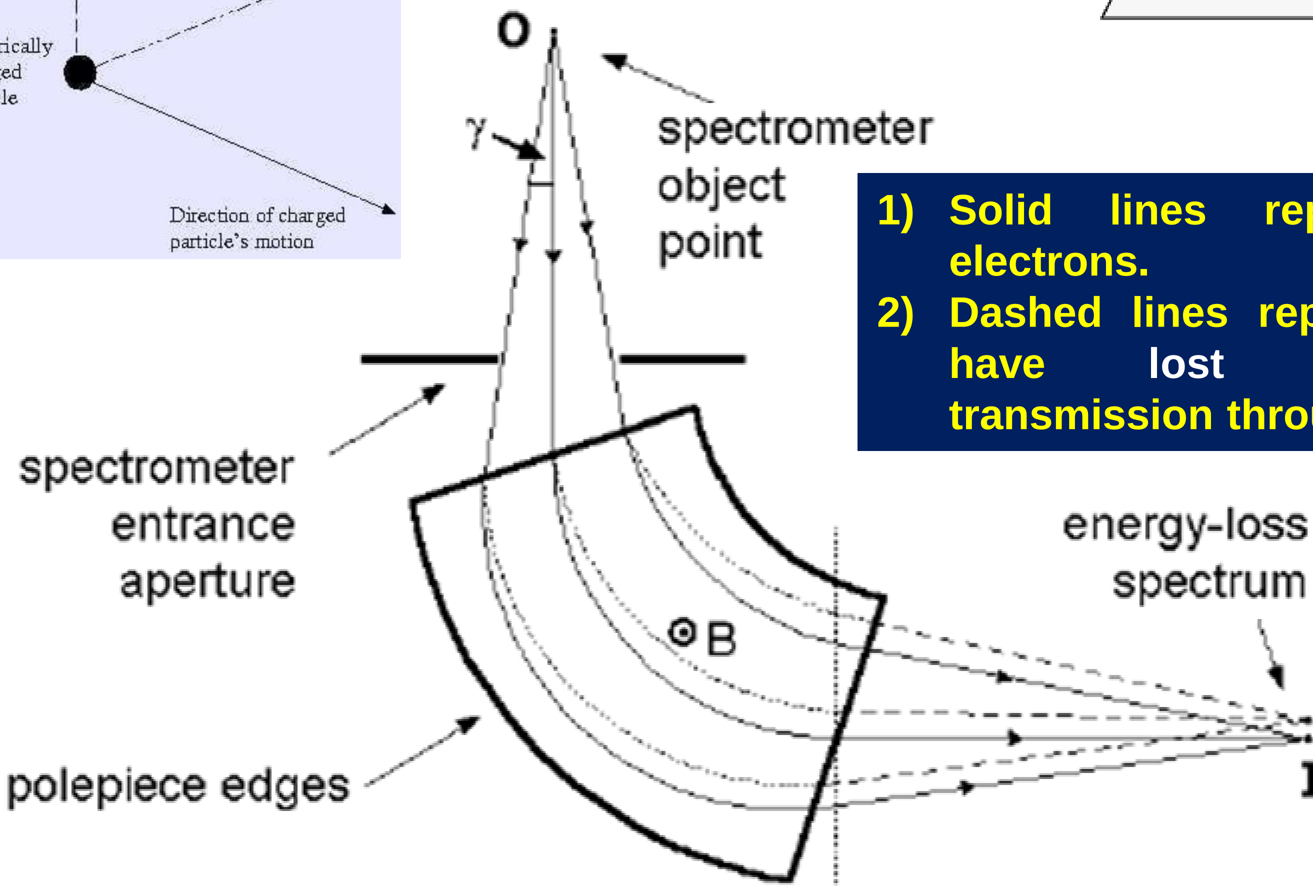
Dispersive and Focusing Properties of a Magnetic Prism



$$F = mv^2/R$$

$$R = (m/e)(1/B)v$$



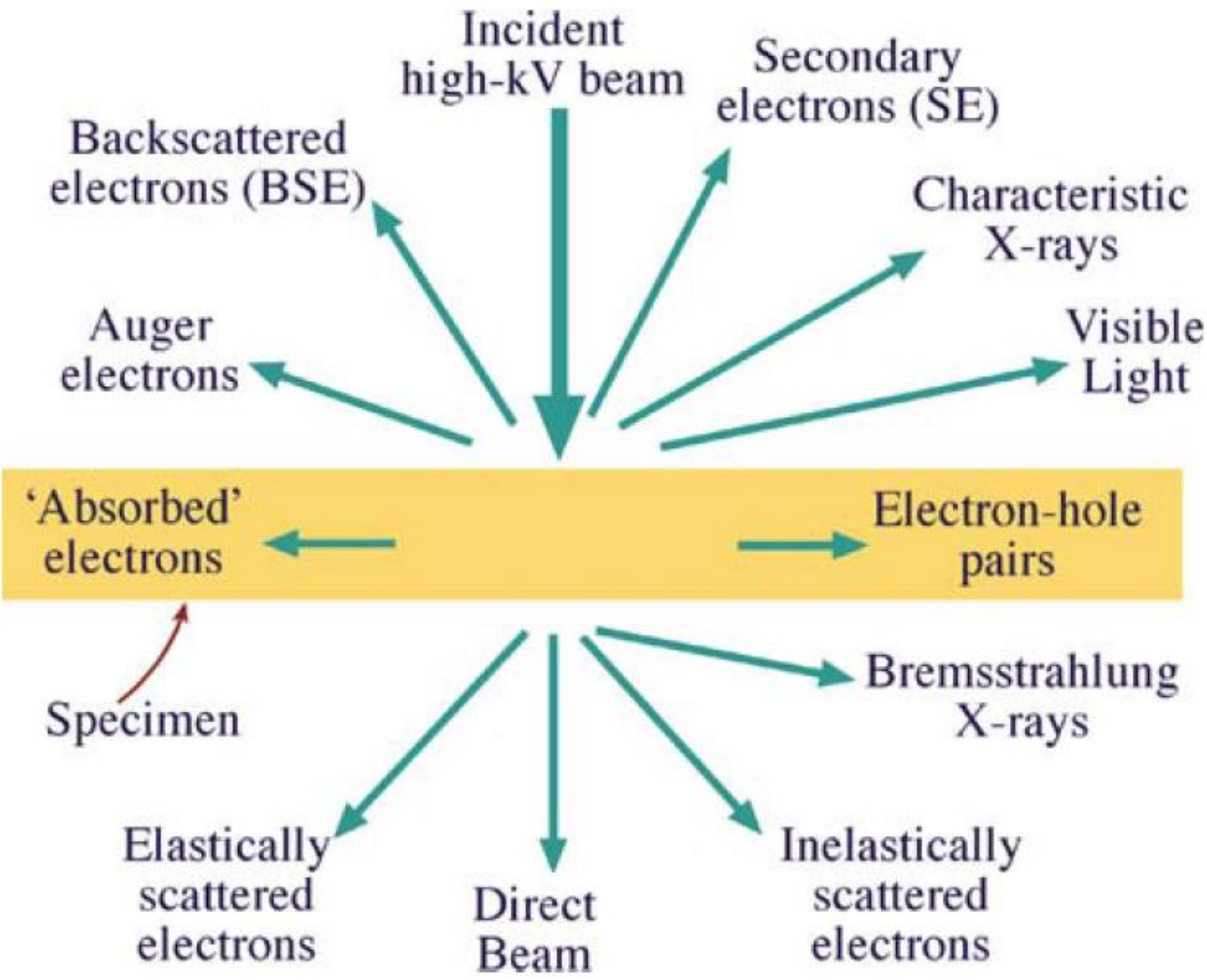


1) Solid lines represent zero-loss electrons.
2) Dashed lines represent those that have lost energy during transmission through the specimen.

http://tem-eels.ca/egerton-laser/preprint/Egerton%20RPP'09%20EELS%20in%20TEM.pdf

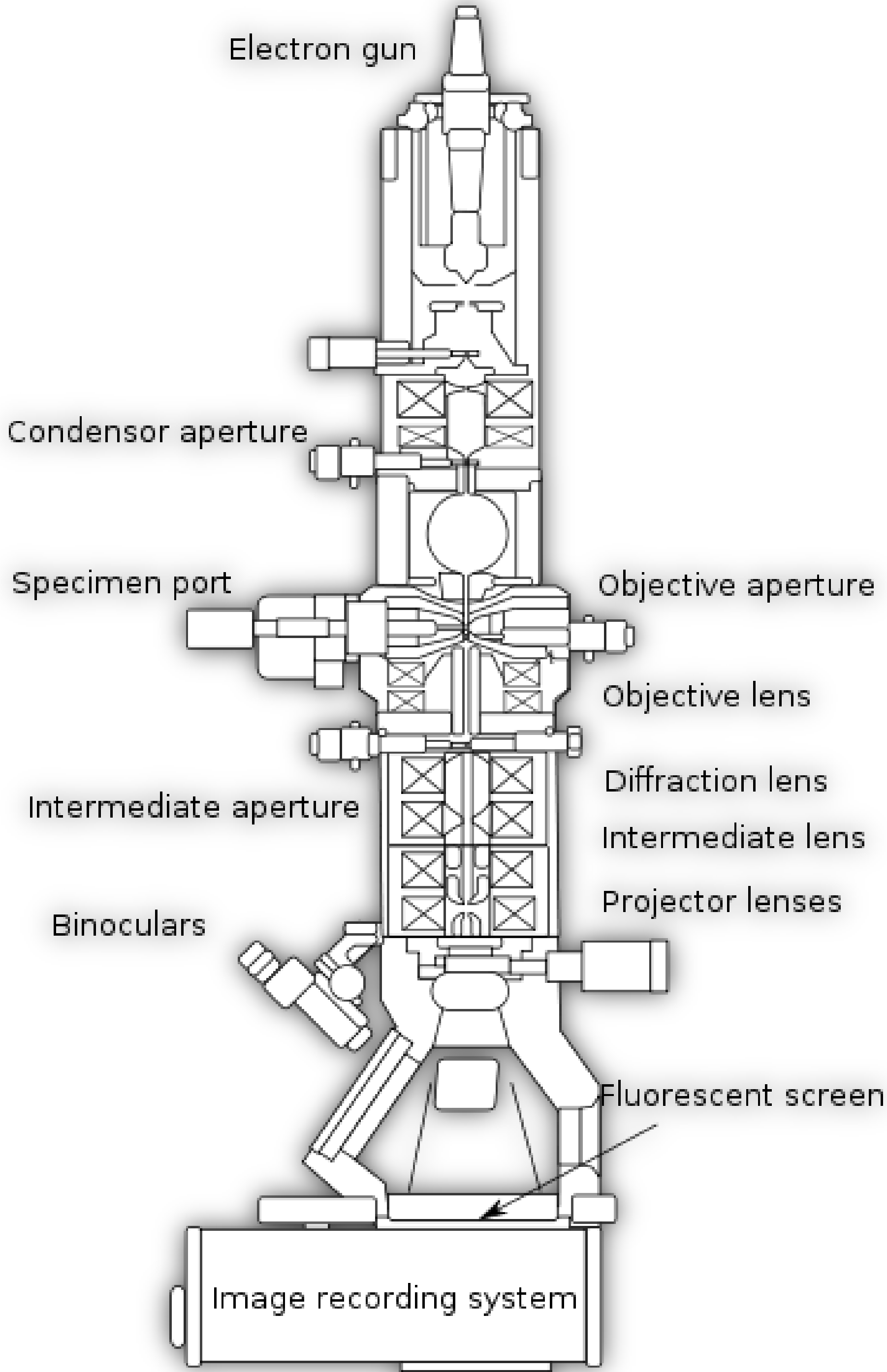
Electron Microscopy Signals

Interaction of electrons with matter

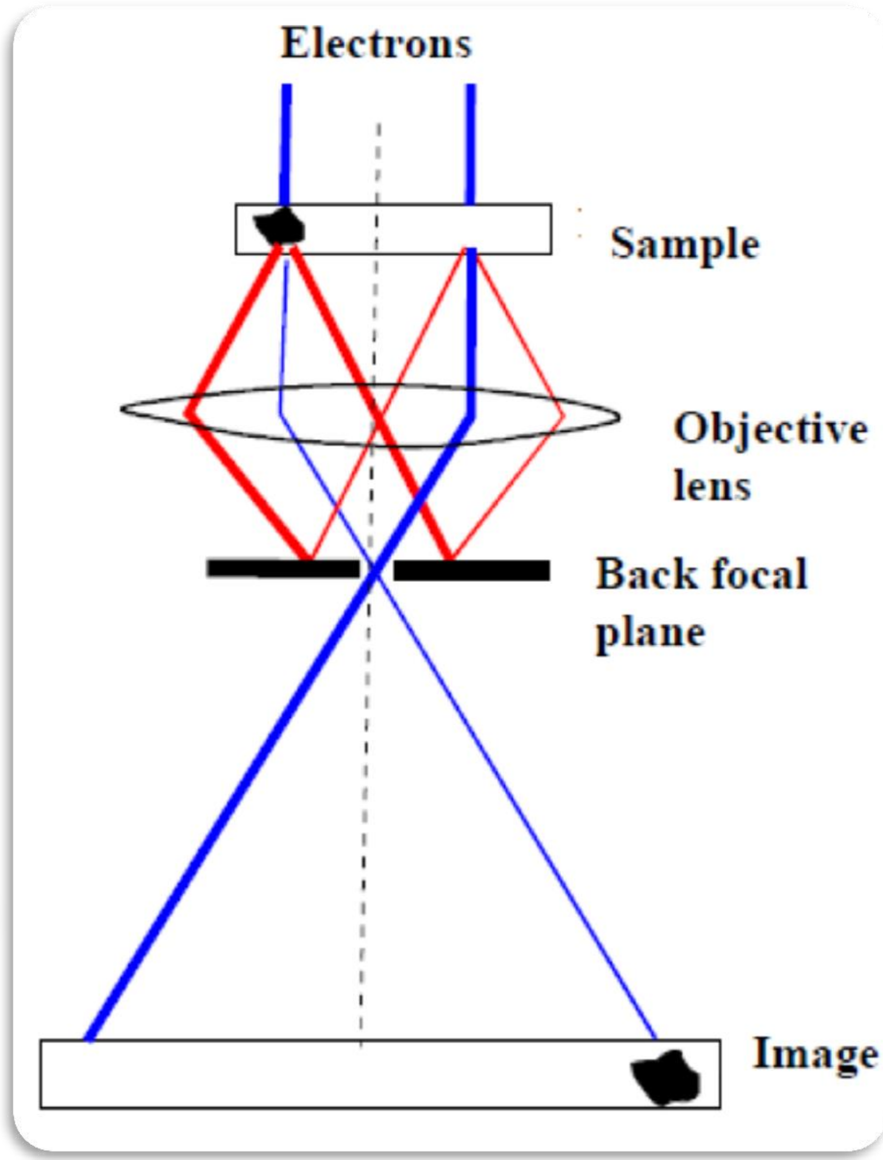


SEM signals

Most important in TEM

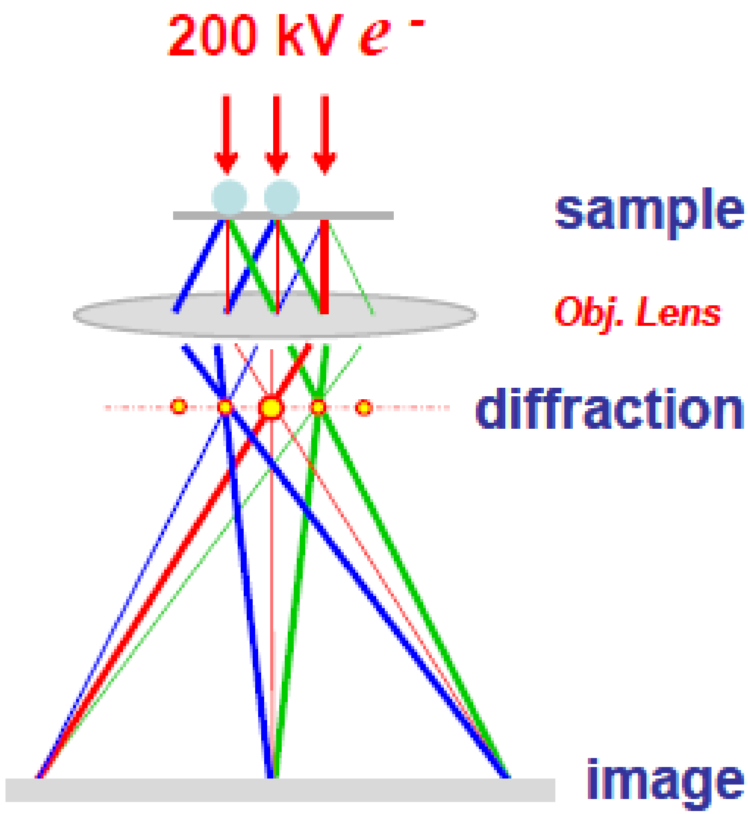


Electron gun
Condensor aperture
Specimen port
Objective aperture
Objective lens
Diffraction lens
Intermediate aperture
Intermediate lens
Projector lenses
Binoculars
Fluorescent screen
Image recording system

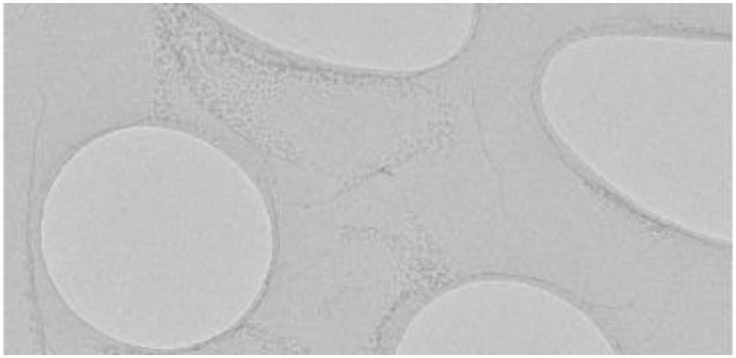


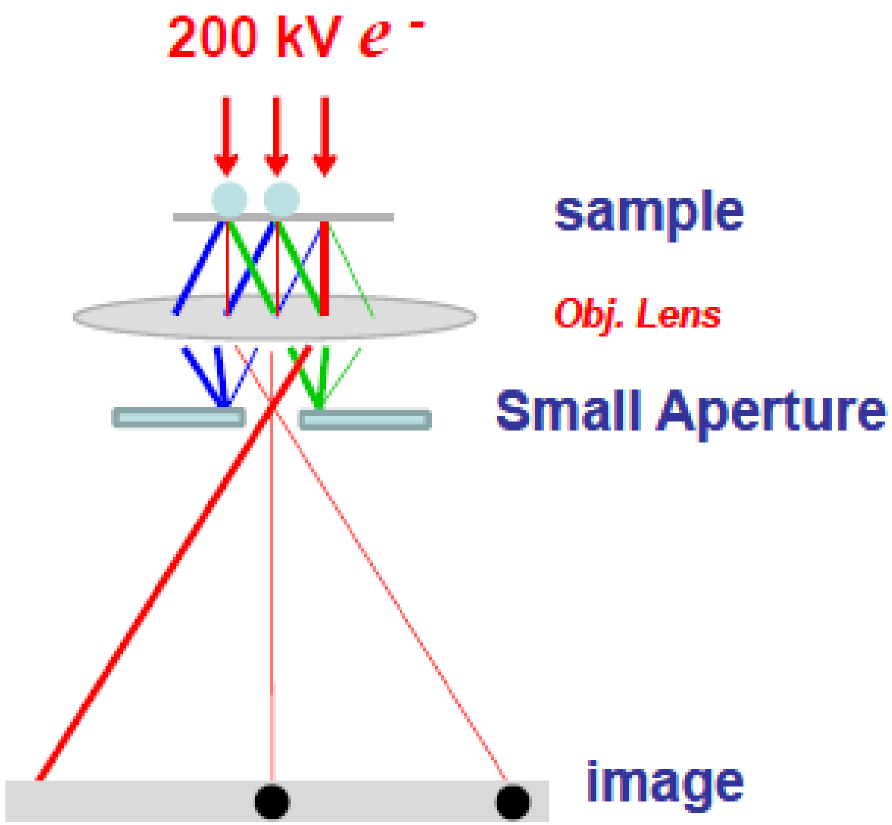
Electrons
Sample
Objective lens
Back focal plane
Image

Image Formation and Contrast

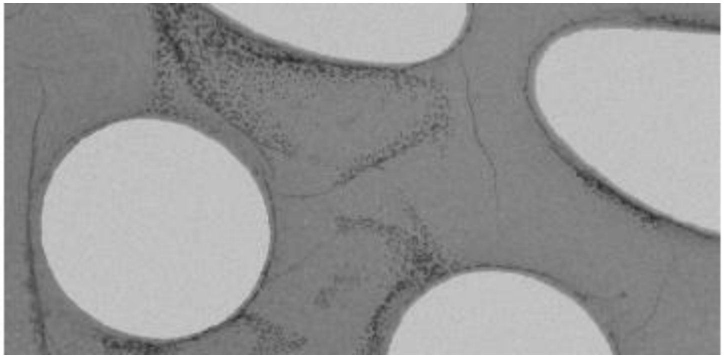


No objective aperture

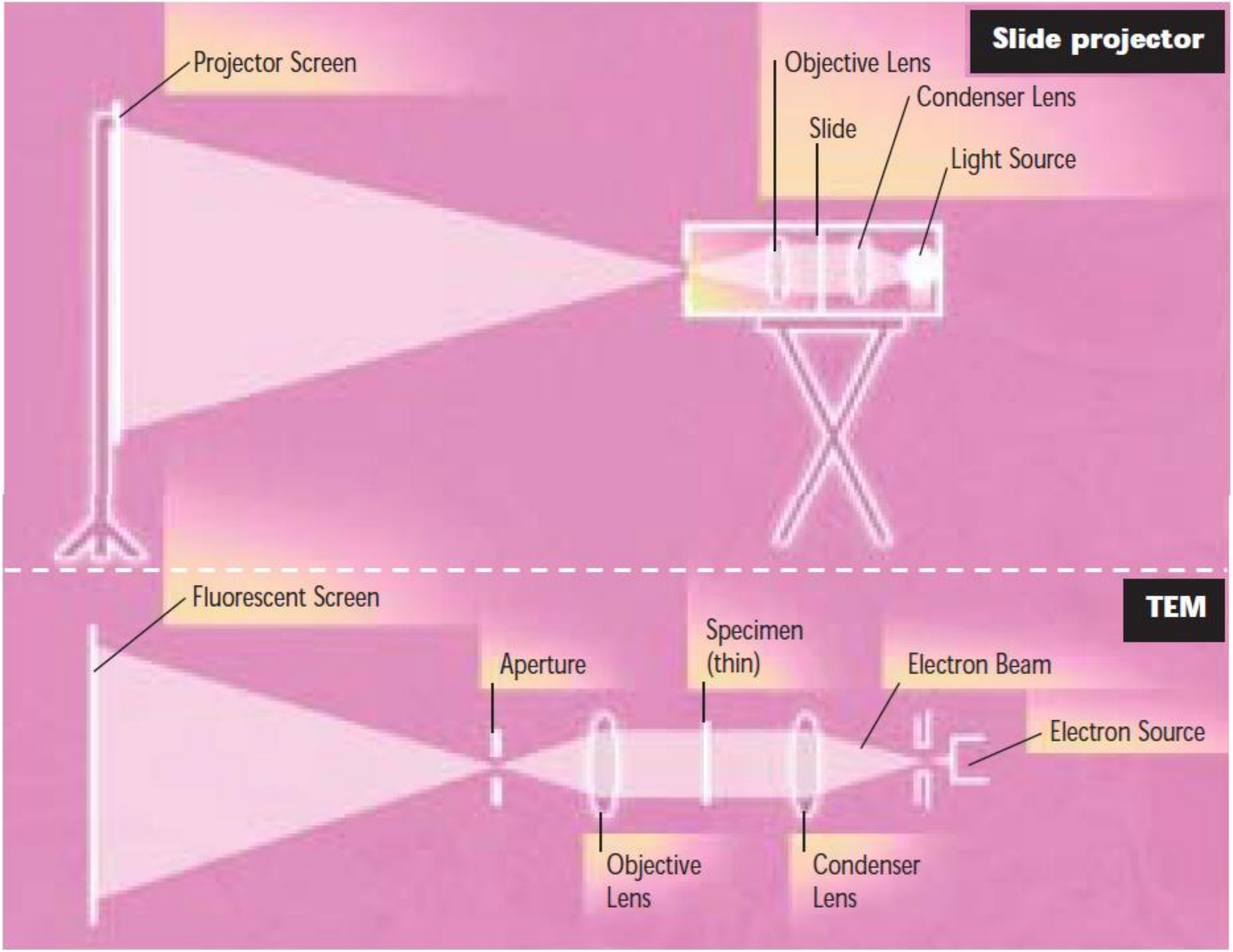


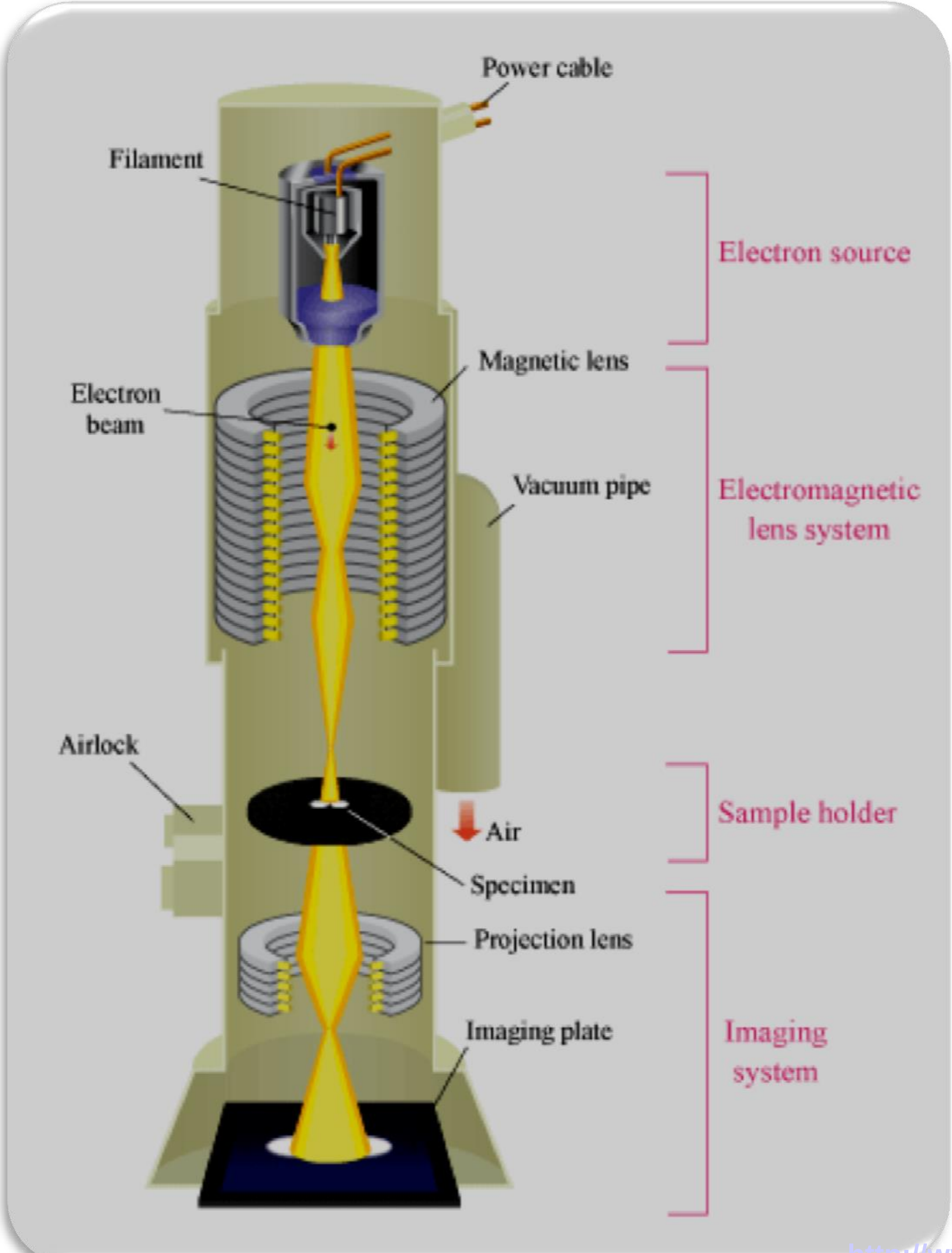


Objective Aperture inserted to select the central direct unscattered beam



The TEM compared with a slide projector





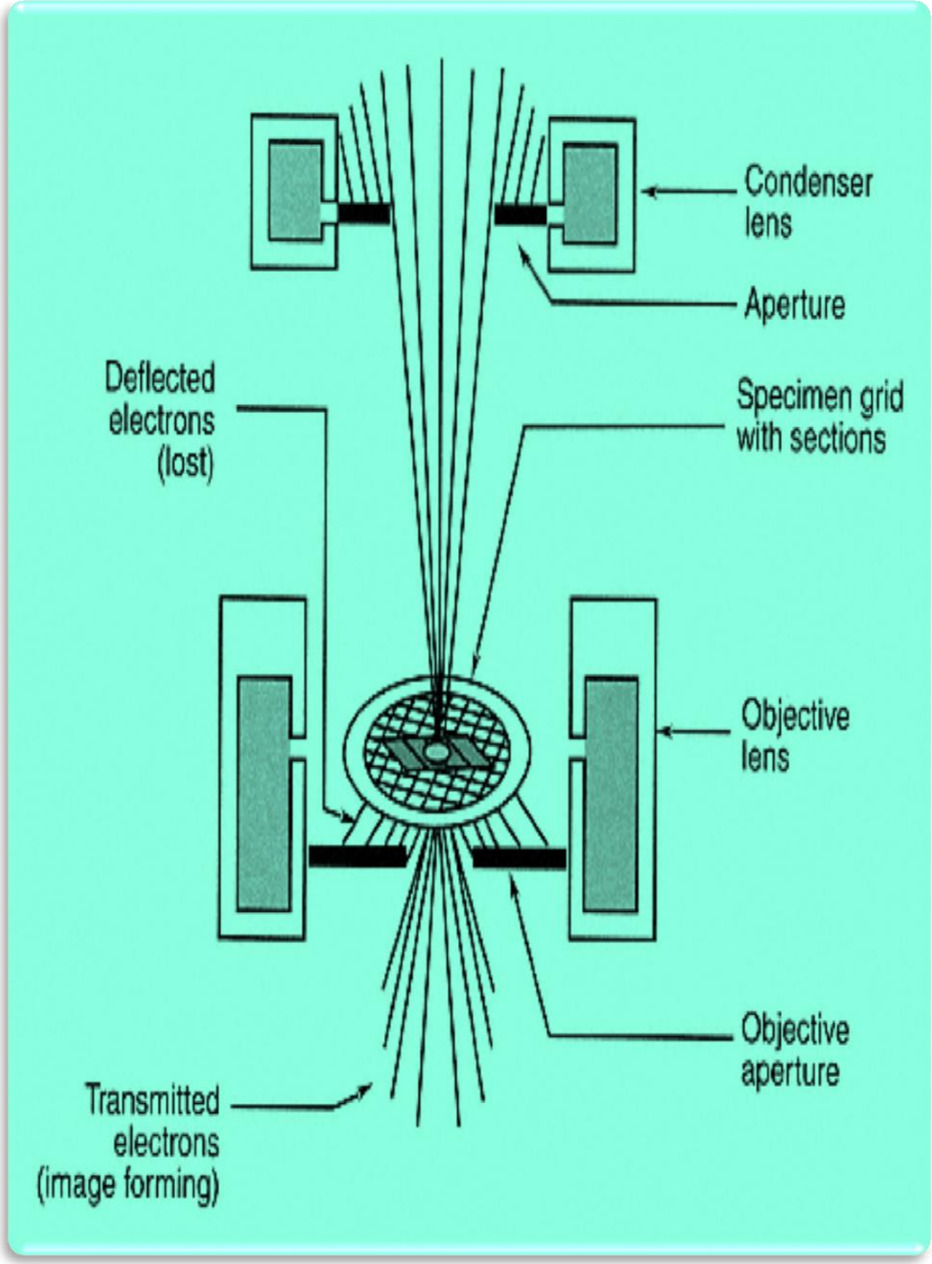
The imaging system of a TEM.

http://www.hk-phy.org/atomic_world/tem/tem02_e.html

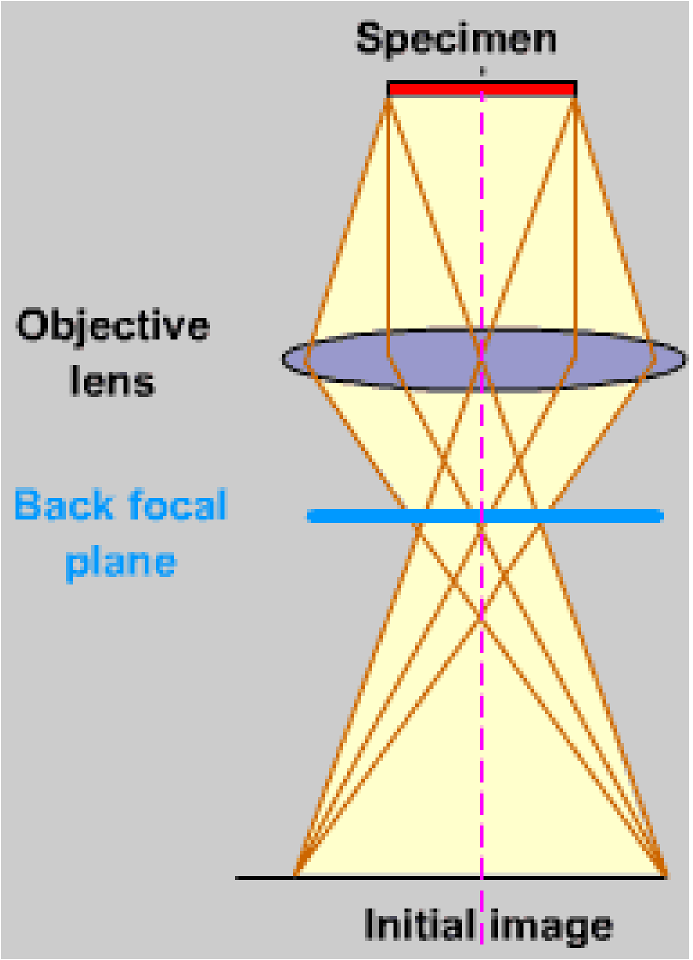
TEM Imaging System

1) This part of the microscope includes the objective, intermediate, and projector lenses.

2) It is involved in the generation of the image and the magnification and projection of the final image onto a viewing screen or camera system of the microscope.



Objective Lens of the TEM

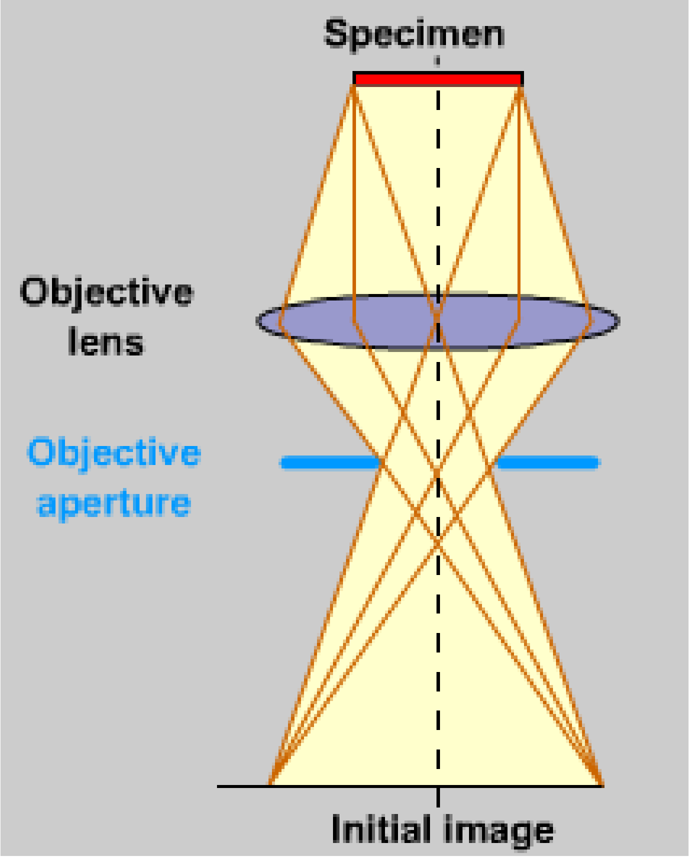


The objective lens forms an inverted initial image, which is subsequently magnified.

In the back focal plane of the objective lens a diffraction pattern is formed. The objective aperture can be inserted here.

http://www.matter.org.uk/tem/lenses/objective_aperture.htm

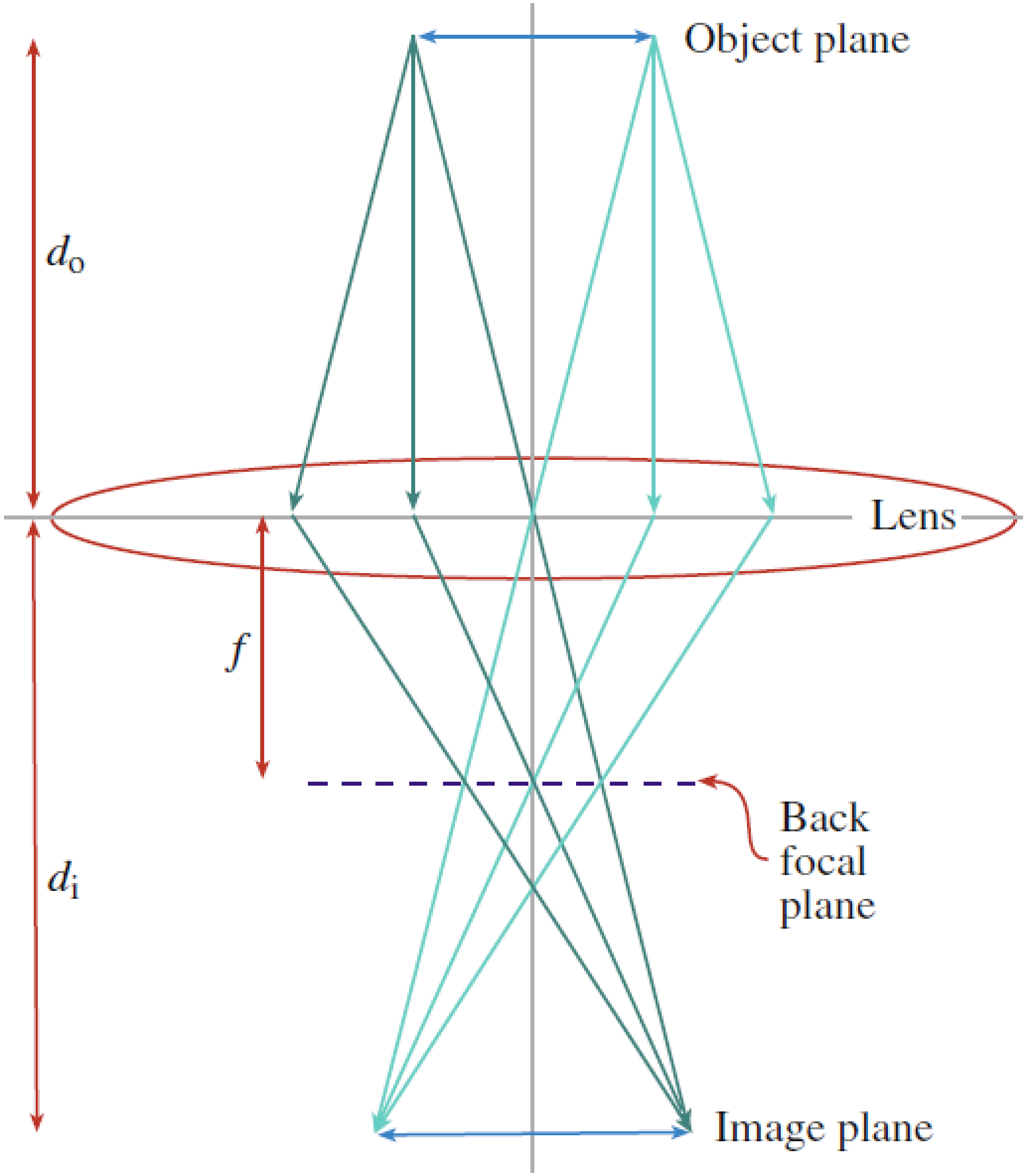
Objective Lens of the TEM



The objective aperture is placed in the back focal plane of the image.

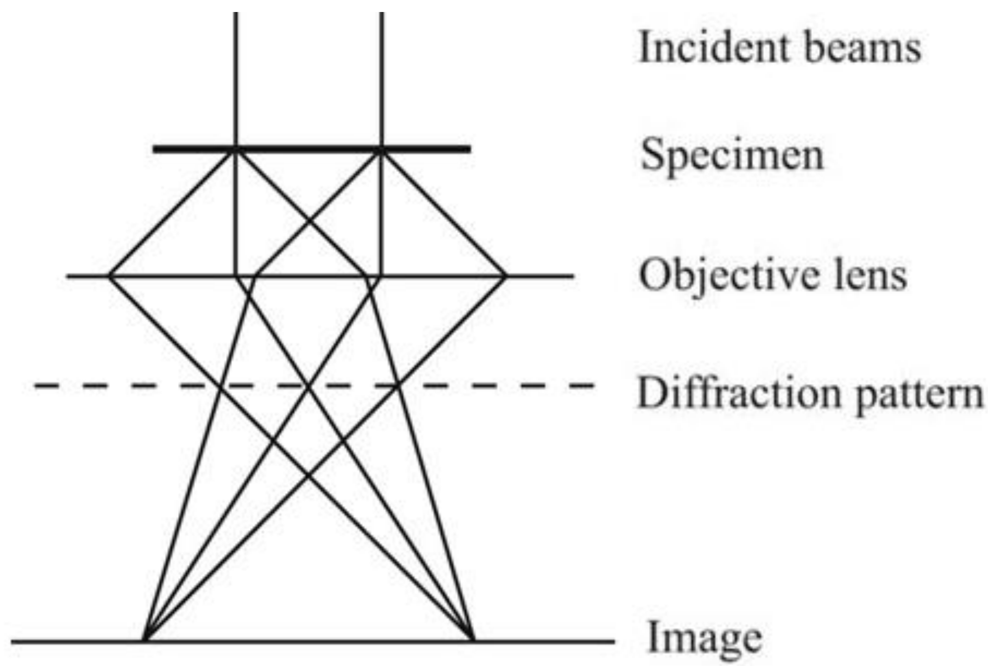
Its function is to:

- **Select those electrons which will contribute to the image, and thereby affect the appearance of the image.**
- **Improve the contrast of the final image.**


Object plane
d_o
Lens
f
Back focal plane
d_i
Image plane

After the electrons have left the sample they pass through the electromagnetic objective lens. This lens acts to collect all electrons scattered from one point of the sample in one point on the fluorescent screen, causing an image of the sample to be formed.

We note that at the dashed line in the figure, electrons scattered in the same direction by the sample are collected into a single point. This is the back focal plane of the microscope, and is where the diffraction pattern is formed. By manipulating the magnetic lenses of the microscope, the diffraction pattern may be observed by projecting it onto the screen instead of the image.



http://en.wikipedia.org/wiki/Electron_diffraction

Imaging Modes in the TEM

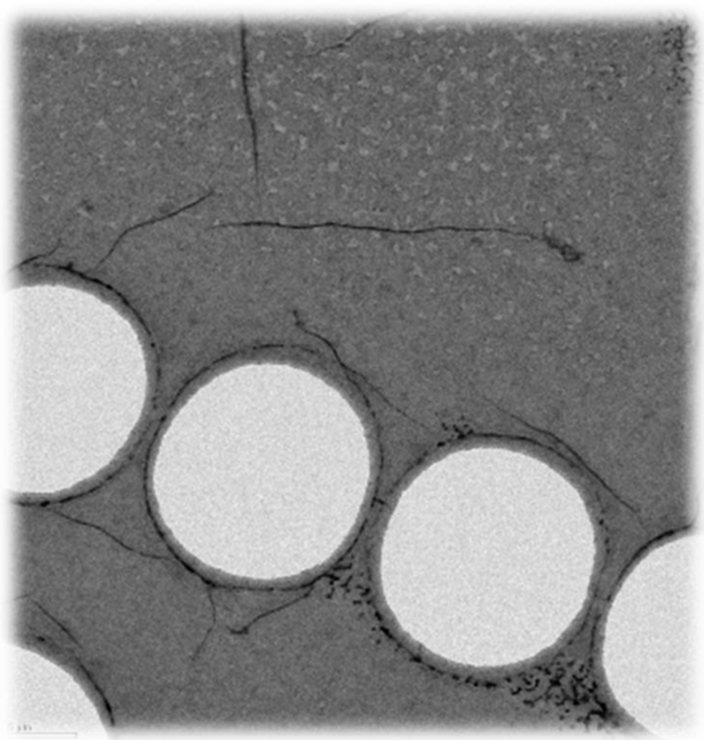
Bright Field Mode

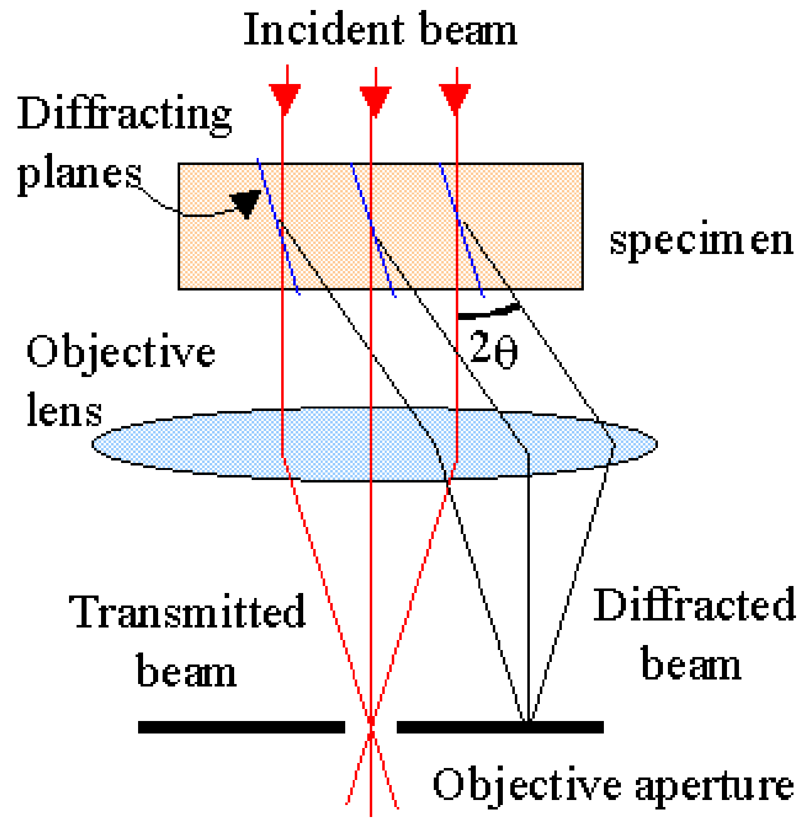
Dark Field Mode

Diffraction Mode

Bright Field Imaging

- If the main portion of the near-forward scattered beam is used to form the image
  - transmitted beam
  - zero-order beam

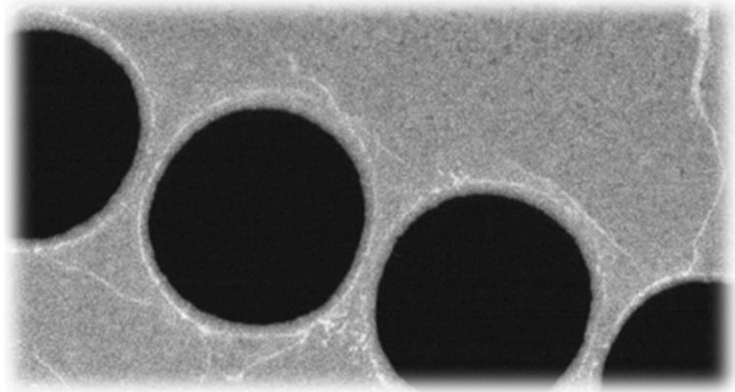


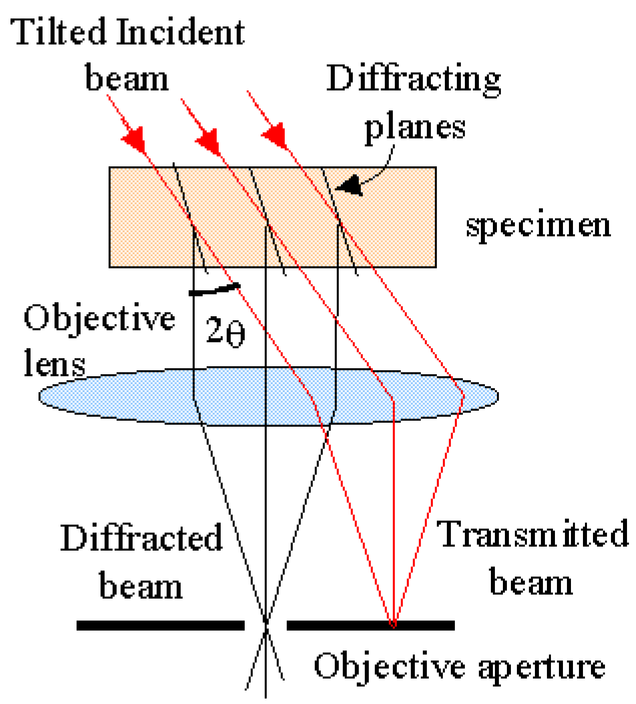


Bright Field Imaging

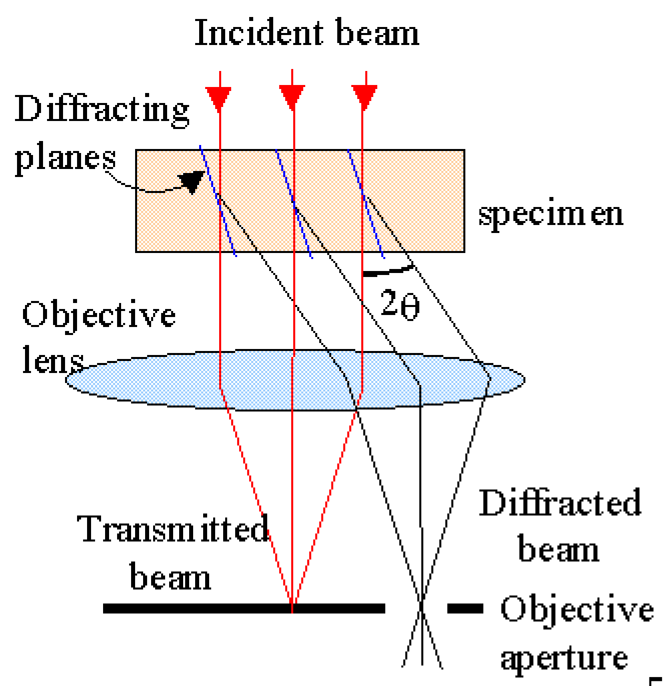
Dark Field Imaging

- If the transmitted beam is excluded from the image formation process
  - off-axis imaging
  - tilted beam imaging





On-axis Dark Field



Off-axis Dark Field

Image formation and contrast: Phase Contrast

High resolution lattice imaging

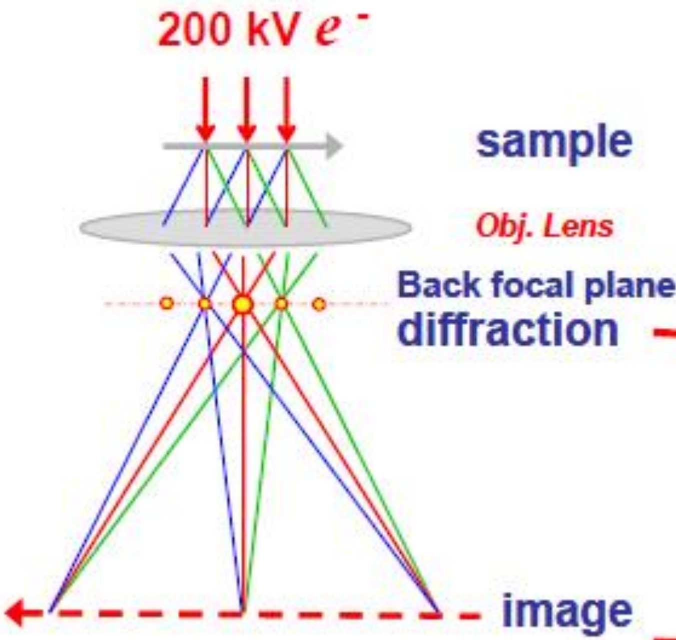
Phase Plate technology in TEM

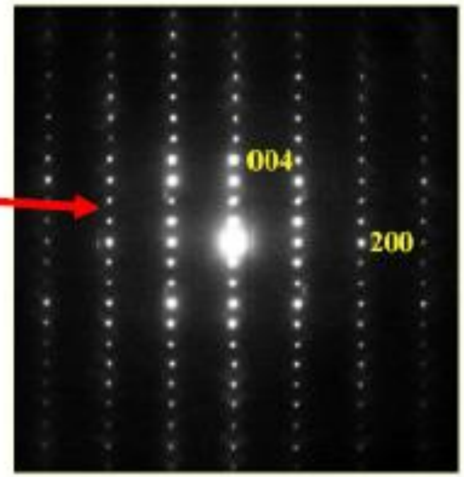
Electron Diffraction

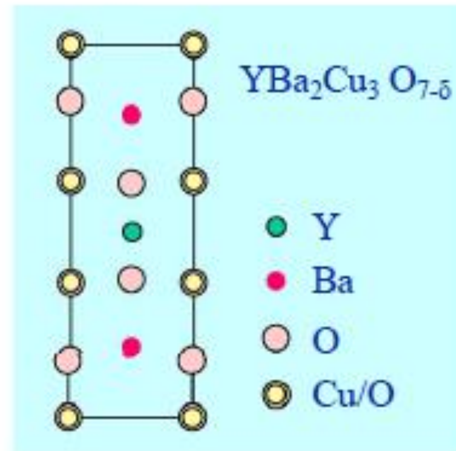
- Four conditions in Back Focal Plane (BFP) of the objective lens:
  - No sample — No reflections (only transmitted beam)
  - Amorphous — Transmitted beam + random scattering
  - Polycrystal — Transmitted beam + rings
  - Single crystal — Transmitted beam + spots

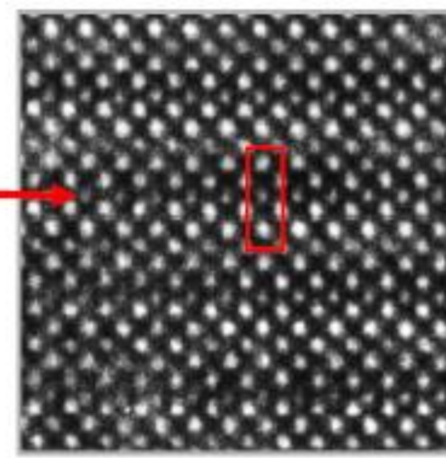
Amorphous: 沒有特定結晶方向面的結構，稱之為非晶體
Polycrystal: 多晶體

Diffraction & Imaging (Direct beam and elastic scattering..)



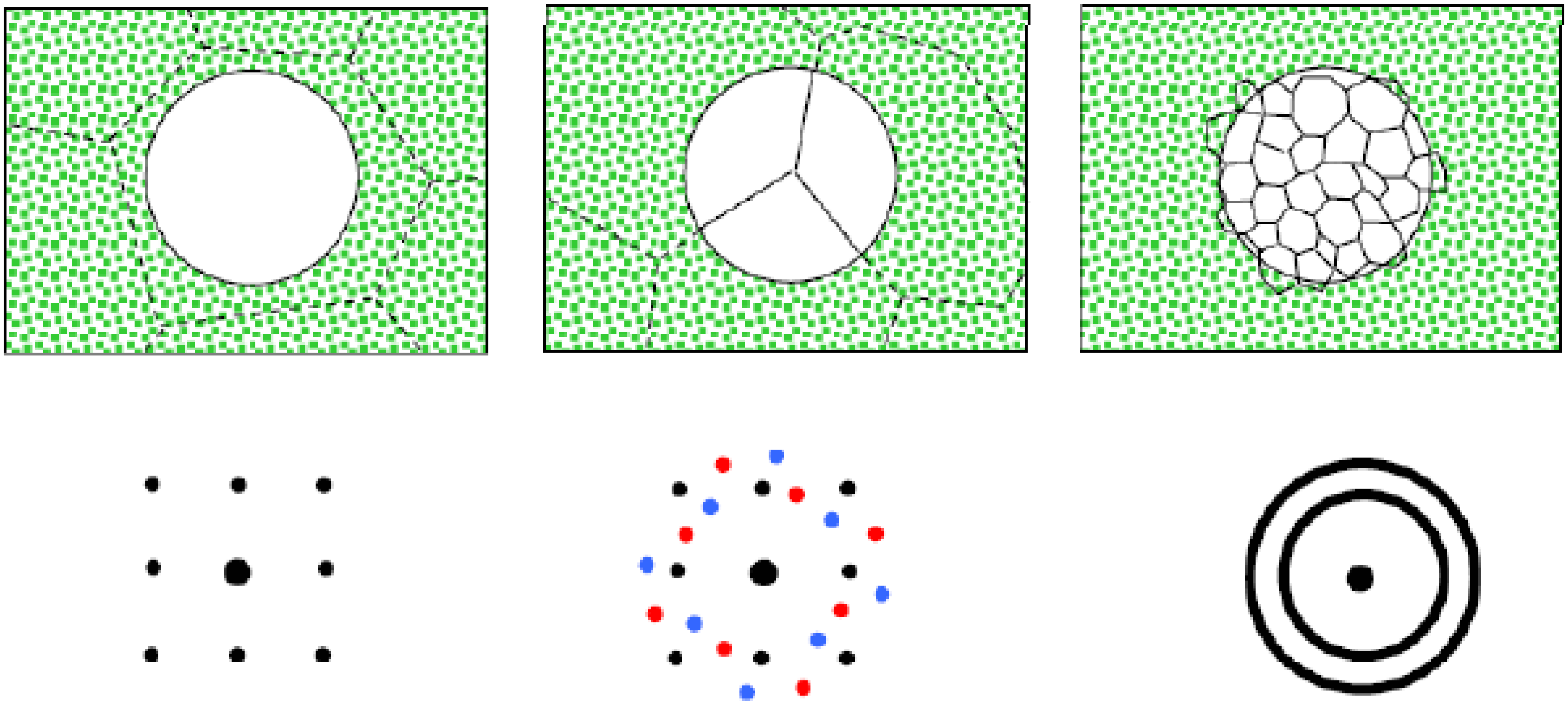




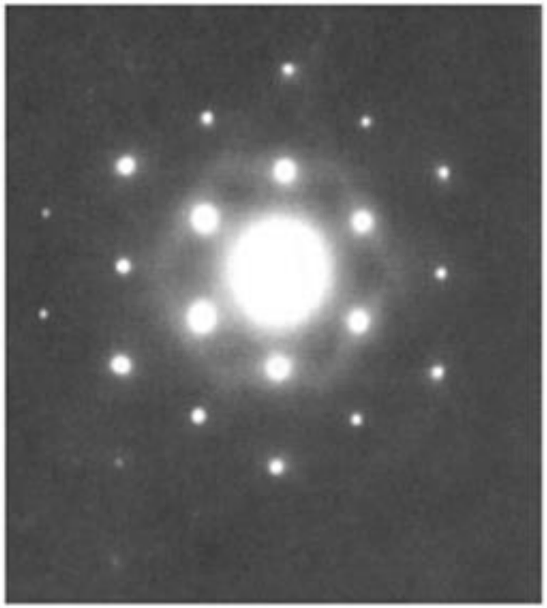


- An image represents the structure in real space at a certain resolution;
- The diffraction is an reproduction of the structure in reciprocal space.

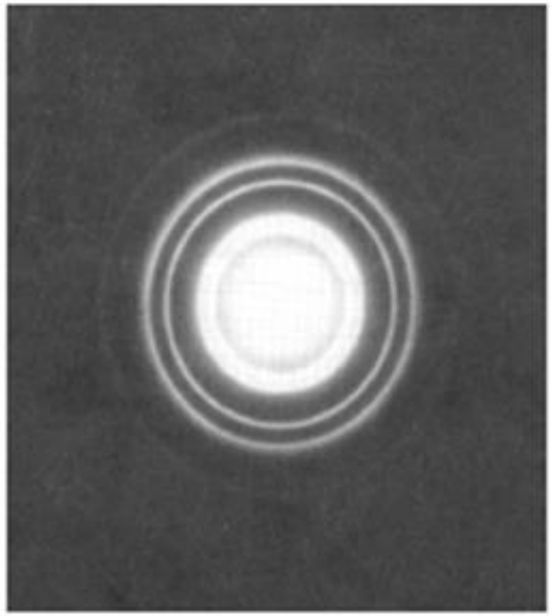
Electron Diffraction Pattern--Spot to Ring



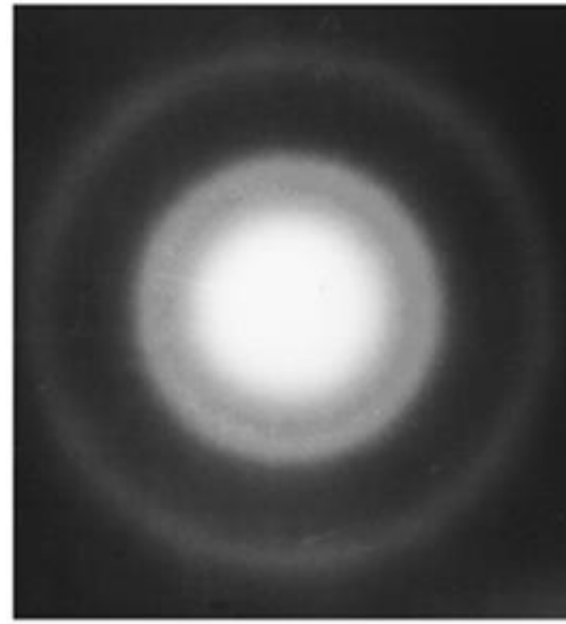
(a) (b) (c)



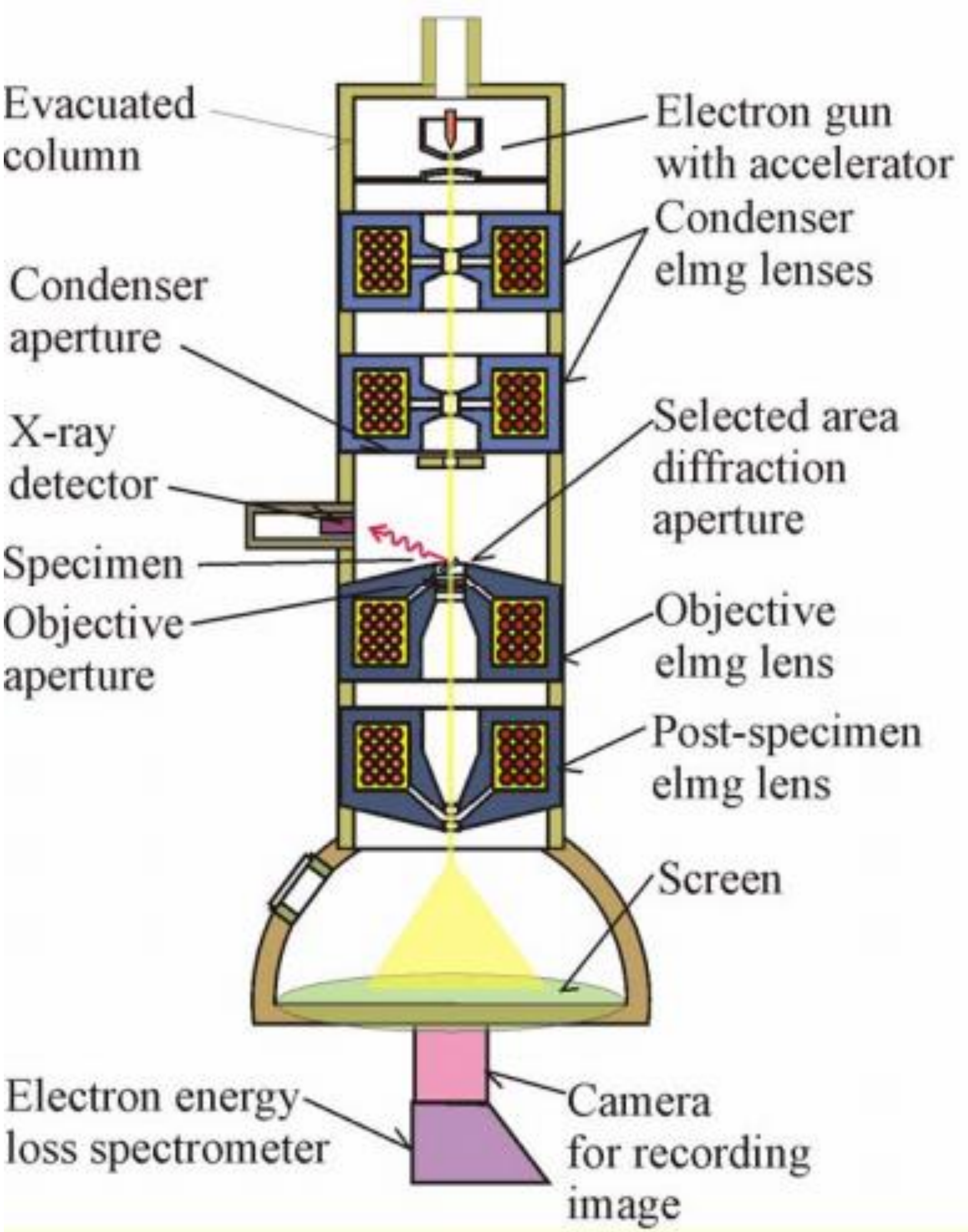
Single crystal



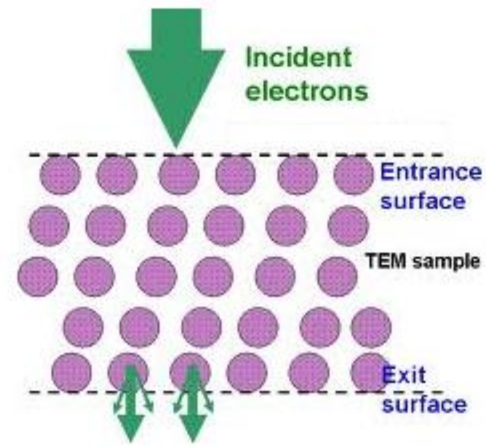
Poly crystalline



Amorphous - disordered

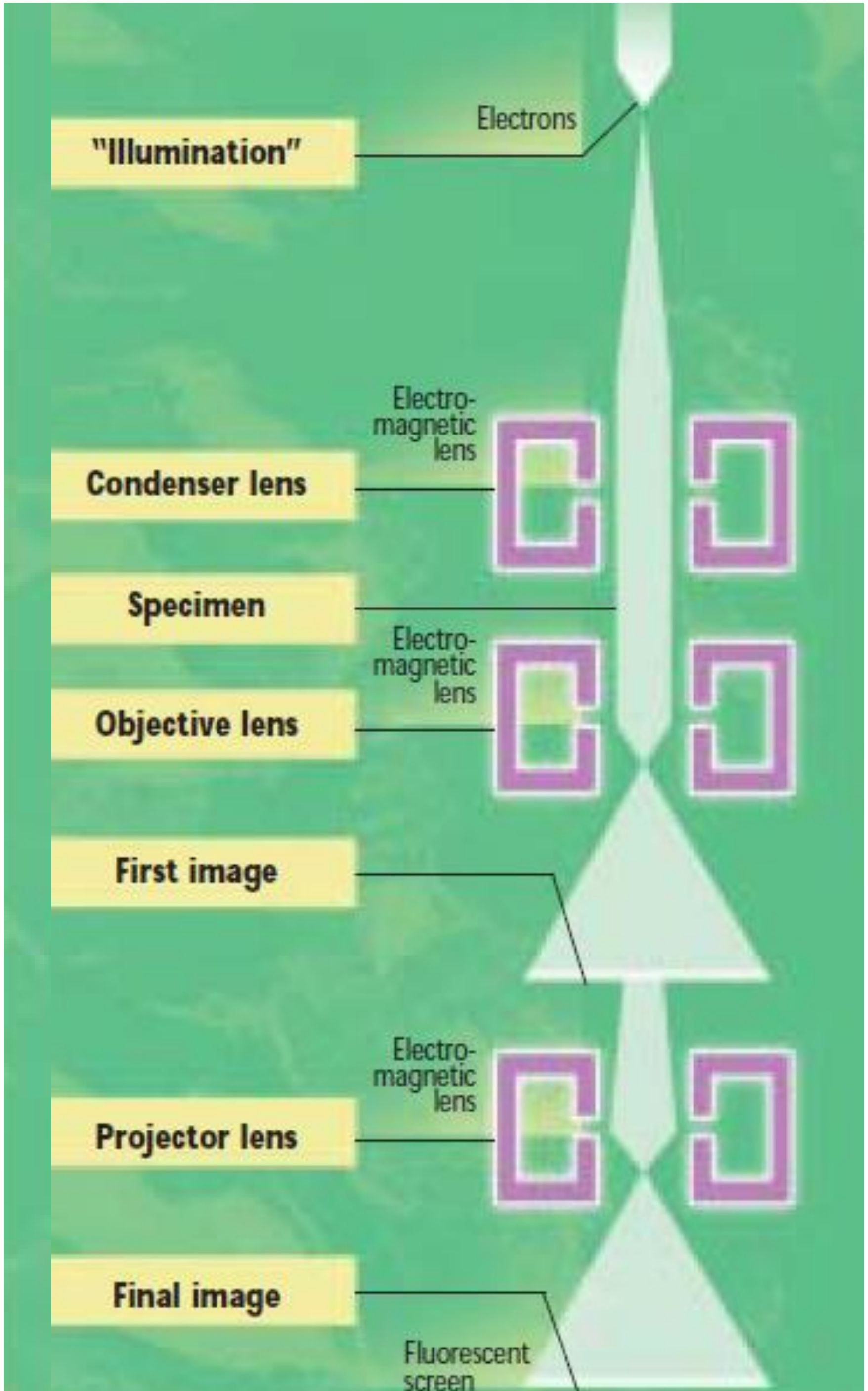


Energy Dispersive X-ray Spectroscopy in TEM



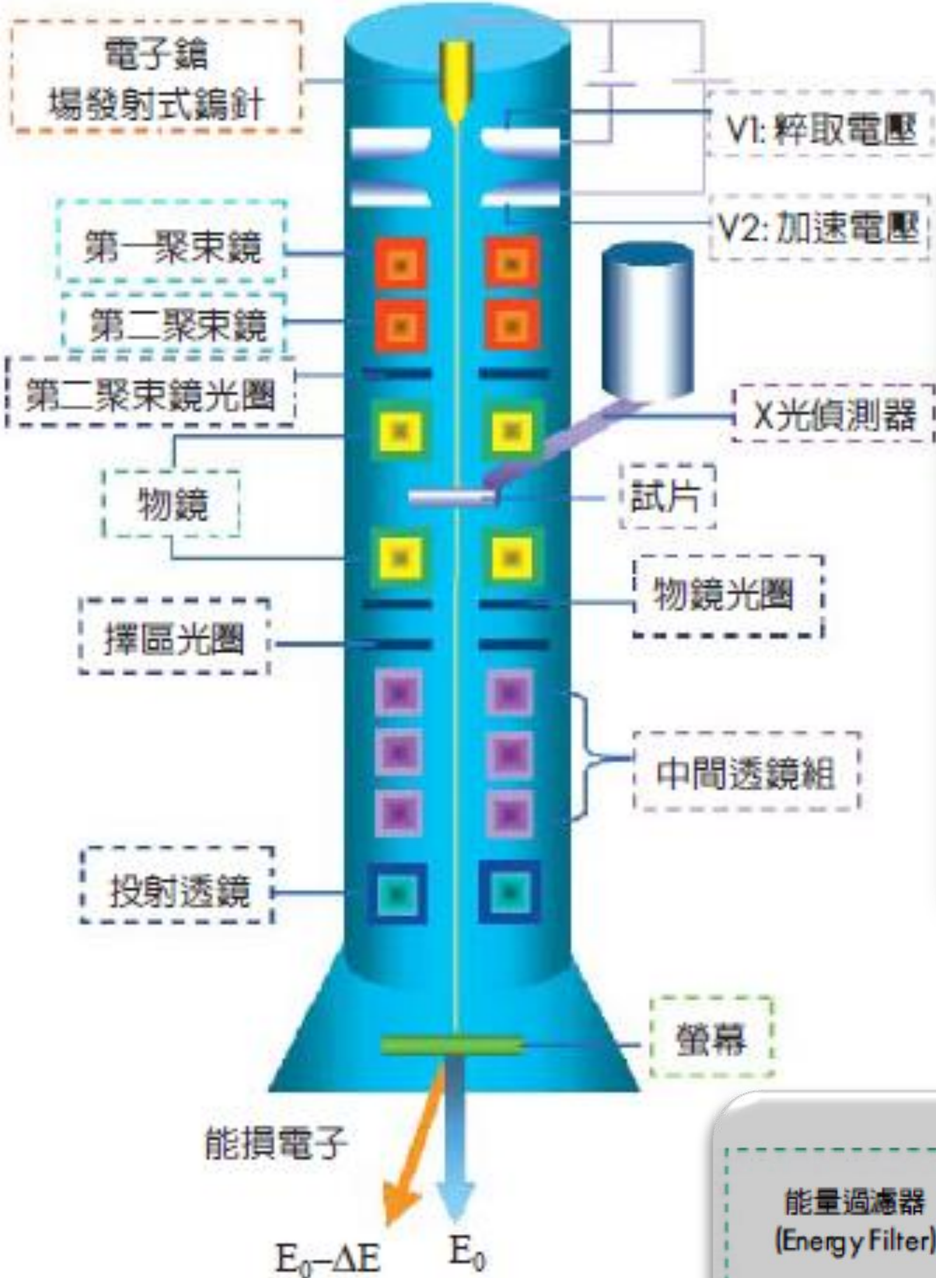
Electron Energy Loss Spectroscopy in TEM

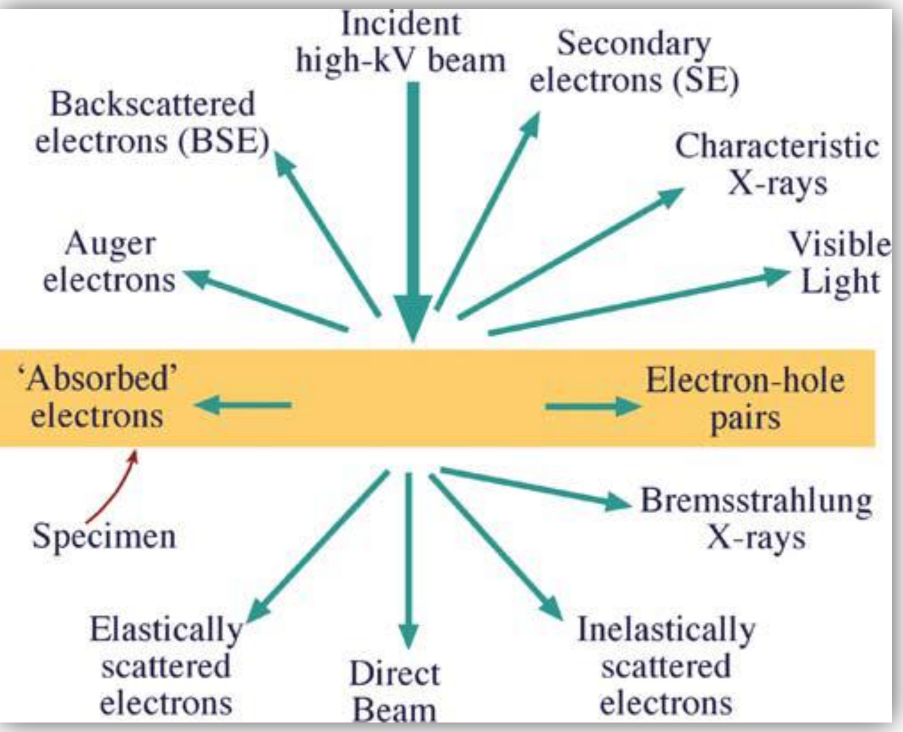
Diffraction in High-resolution TEM



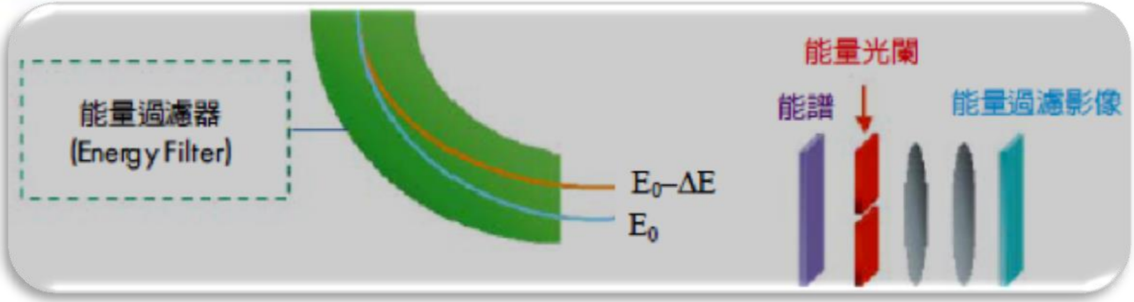
Electron Density

How many electrons impinge on the specimen? A typical electron beam has a current of about 1 picoampere (10^{-12} A). One ampere is 1 coulomb/sec. The electron has a charge of 1.6×10^{-19} coulomb. Therefore approximately 6 million (百萬) electrons per second impinge on the specimen.

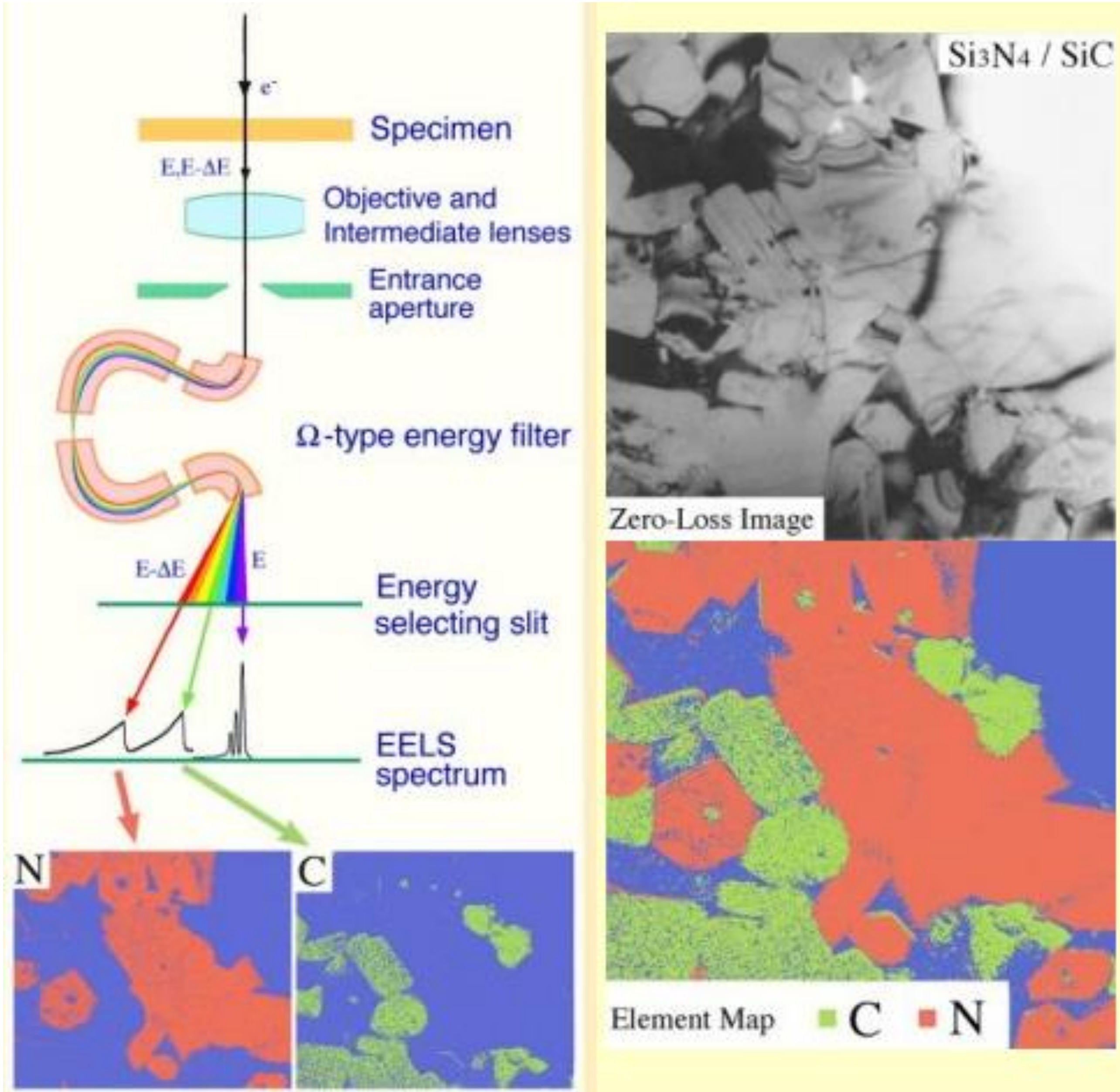




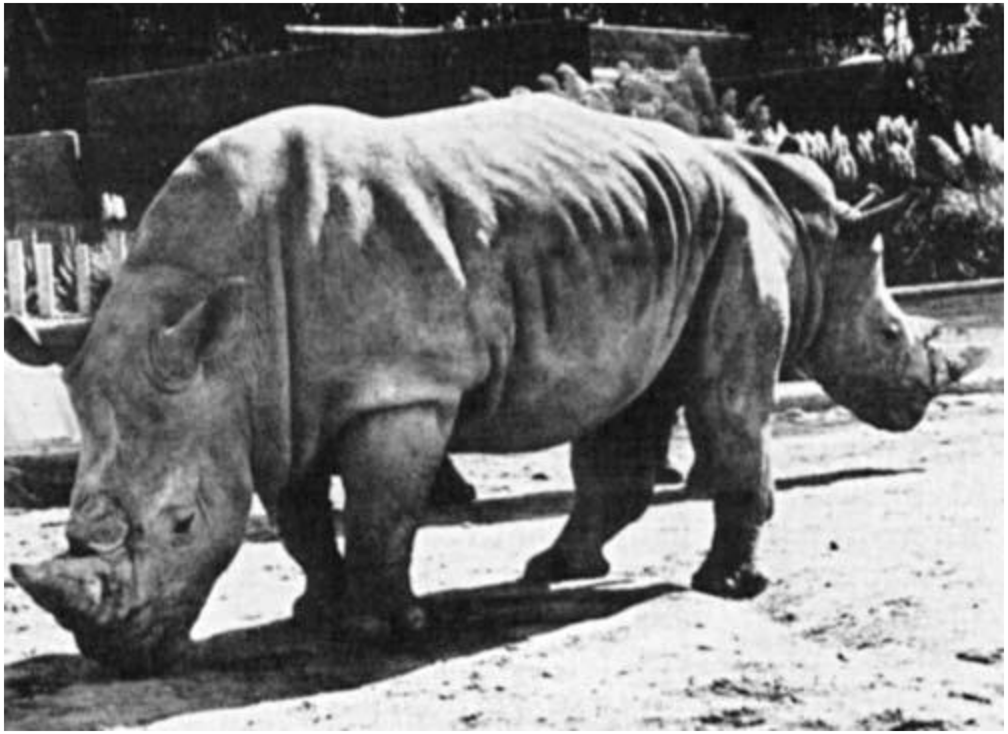
In column omega type Energy filter



Element Mapping by Ω-Type Energy Filter TEM



http://www.tagen.tohoku.ac.jp/labo/terauchi/research/instruments/jem2010fef.html



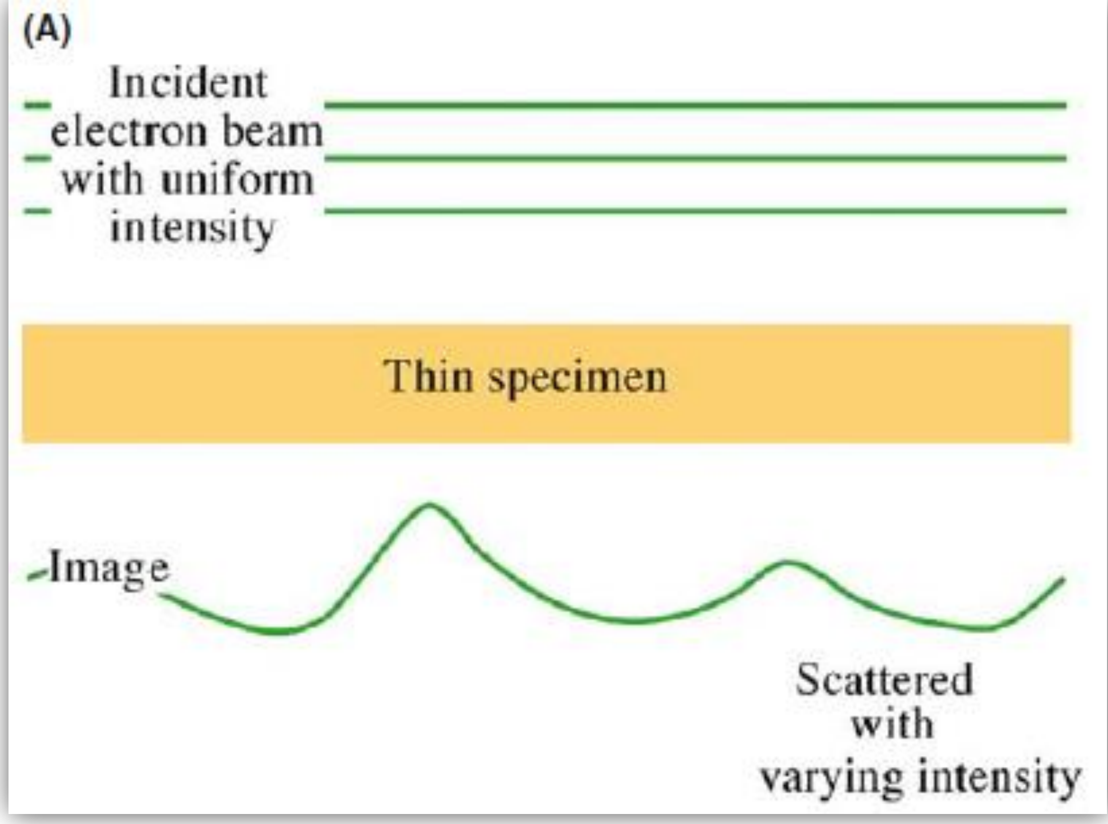
1) Our eyes and brain routinely understand reflected light images but are ill-equipped to interpret TEM images and so we must be cautious.

2) This problem is well illustrated by the picture of the two rhinoceros side by side such that the head of one appears attached to the rear of the other.

Photograph of two rhinos taken so that, in projection, they appear as one two-headed beast. Such projection artifacts in TEM images are easily mistaken for 'real' features.

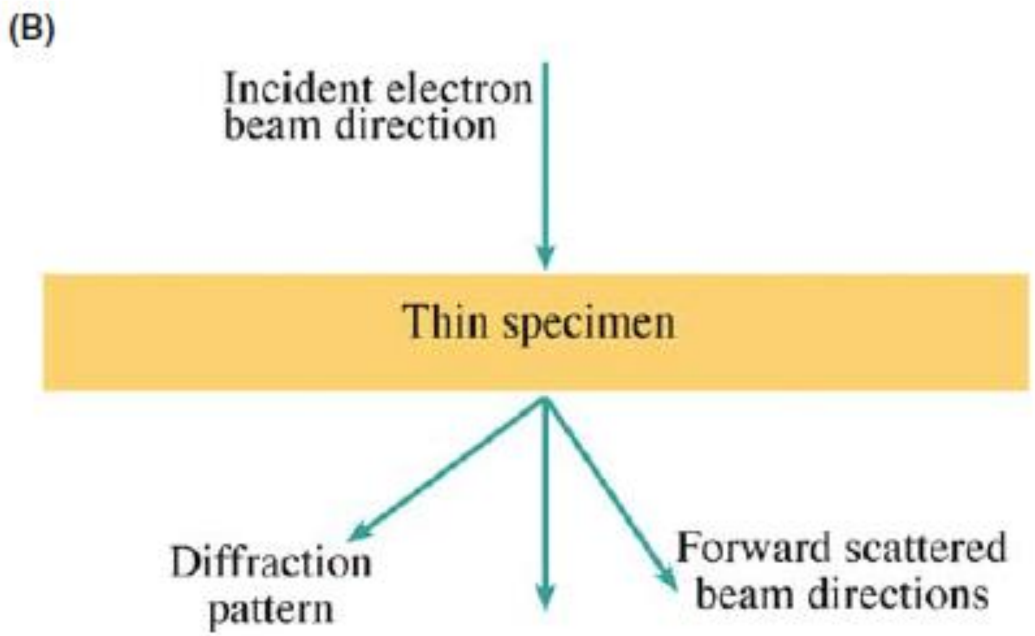
Scattering and Diffraction

- The electron is treated in two different ways: in (A) electron scattering it is a succession of particles, while in (B) electron diffraction it is treated by wave theory.

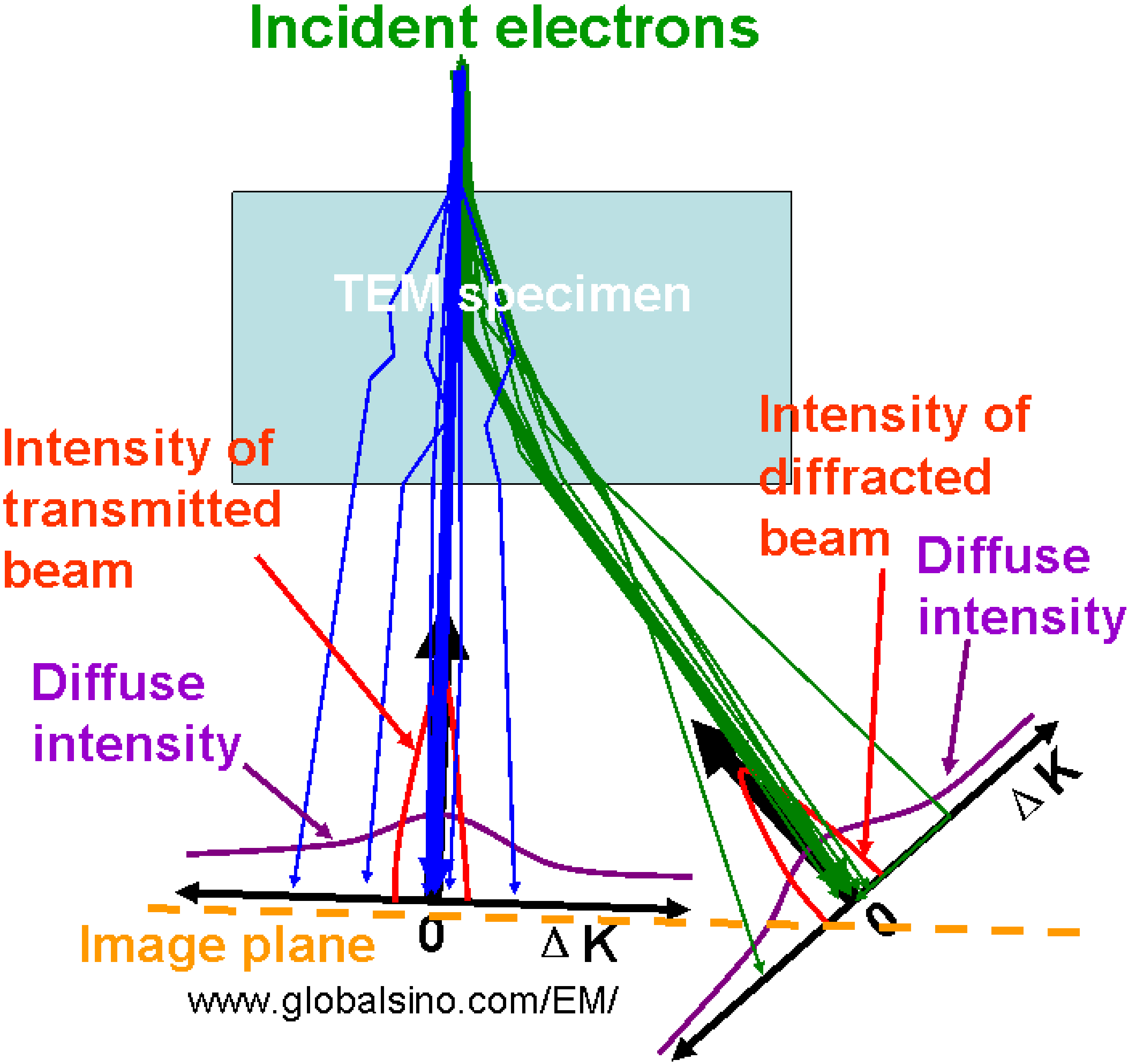


It is this non-uniform distribution that contains all the structural, chemical, and other information about our specimen.

Scattering and Diffraction



1) The change in angular distribution is shown by an incident beam of electrons being transformed into several forward-scattered beams.
2) The angular distribution of scattering can be viewed in the form of scattering patterns, usually called diffraction patterns (DPs).



http://www.globalsino.com/EM/page4127.html

1) Inelastic scattering of electron beam by TEM specimen, and intensities of the transmitted beam and a diffracted beam superimposed on diffuse backgrounds.
2) The tilt of the diffracted beam is exaggerated due to the shorter distance between the specimen and the image plane in the schematic illustration here.

Interpreting Transmission Images

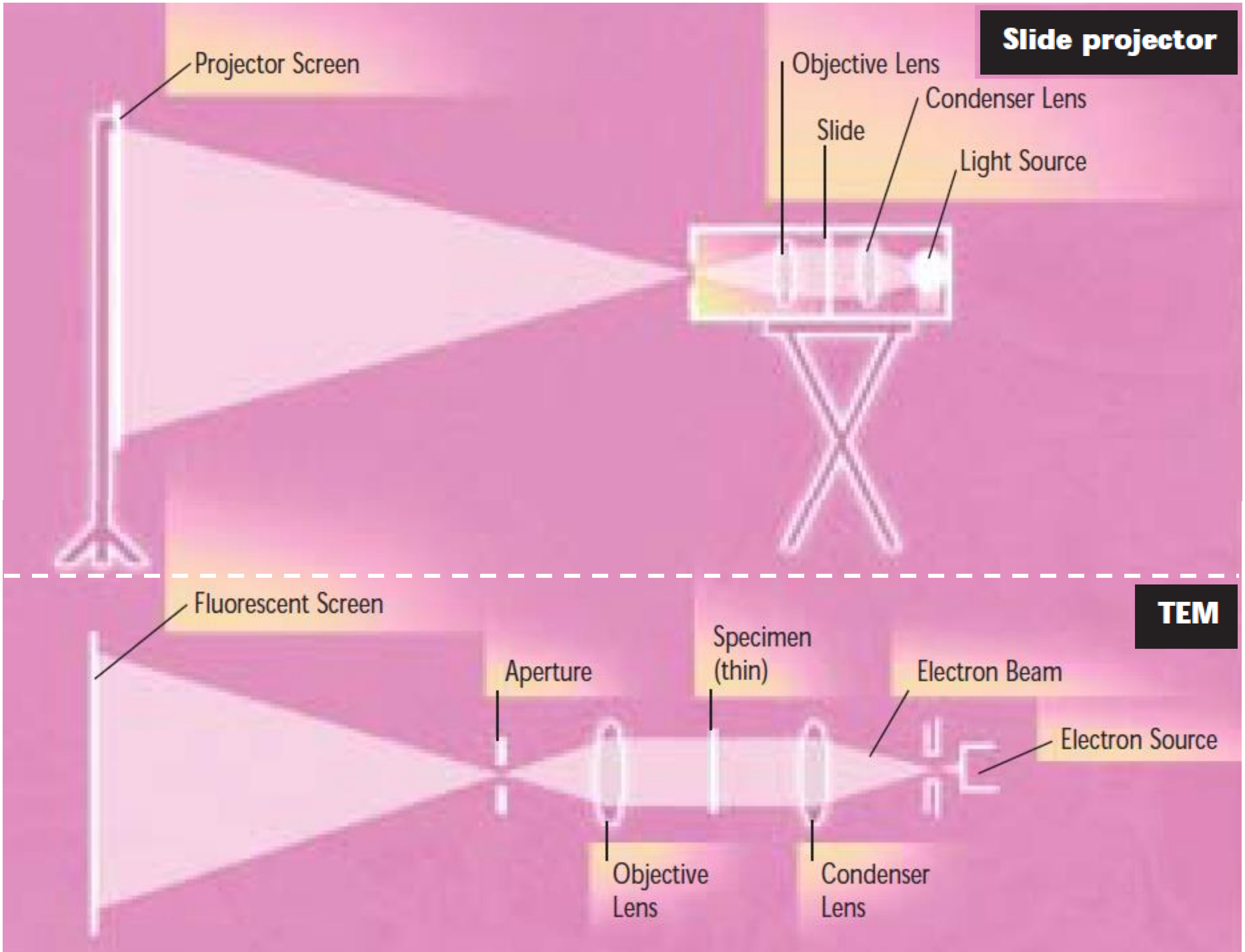
projection-limitation

1) TEM information is averaged through the thickness of the specimen. In other words, a single TEM image has no depth sensitivity.

2) it is an axiom in TEM that, almost invariably, thinner is better and specimens <100 nm should be used wherever possible.

3) In extreme cases such as doing electron spectrometry, specimen thicknesses < 50 nm (even < 10 nm) are essential. These demands become less strict as the beam voltage increases, but this is offset by the production of beam damage.

Scanning Transmission Electron Microscope


Slide projector
Projector Screen
Objective Lens
Condenser Lens
Slide
Light Source
TEM
Fluorescent Screen
Specimen (thin)
Aperture
Electron Beam
Electron Source
Objective Lens
Condenser Lens