PELATIHAN
SCANNING ELECTRON MICROSCOPY (SEM)

LPPT-UGM

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Introduction to Electron Microscopy; Scanning Electron Microscopy (SEM)

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OUTLINE

1. THEORY OF SCANNING ELECTRON MICROSCOPE (SEM)

2. SEM SPECIMEN PREPARATIONS

3. CHARACTERIZATION AND INTERPRETATION OF SEM AND SEM-EDS RESULTS

BRIEF INTRODUCTION TO ELECTRON MICROSCOPY

ELECTRON MICROSCOPY:

- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)
- High voltage electron microscopy (HVEM)
- Scanning transmission electron microscopy (STEM)

ANALYTICAL SEM:

- SEM-EDS (energy dispersive x-ray spectroscopy)

Why should we use electron microscope?

Wave length of light and electron

LIGHT

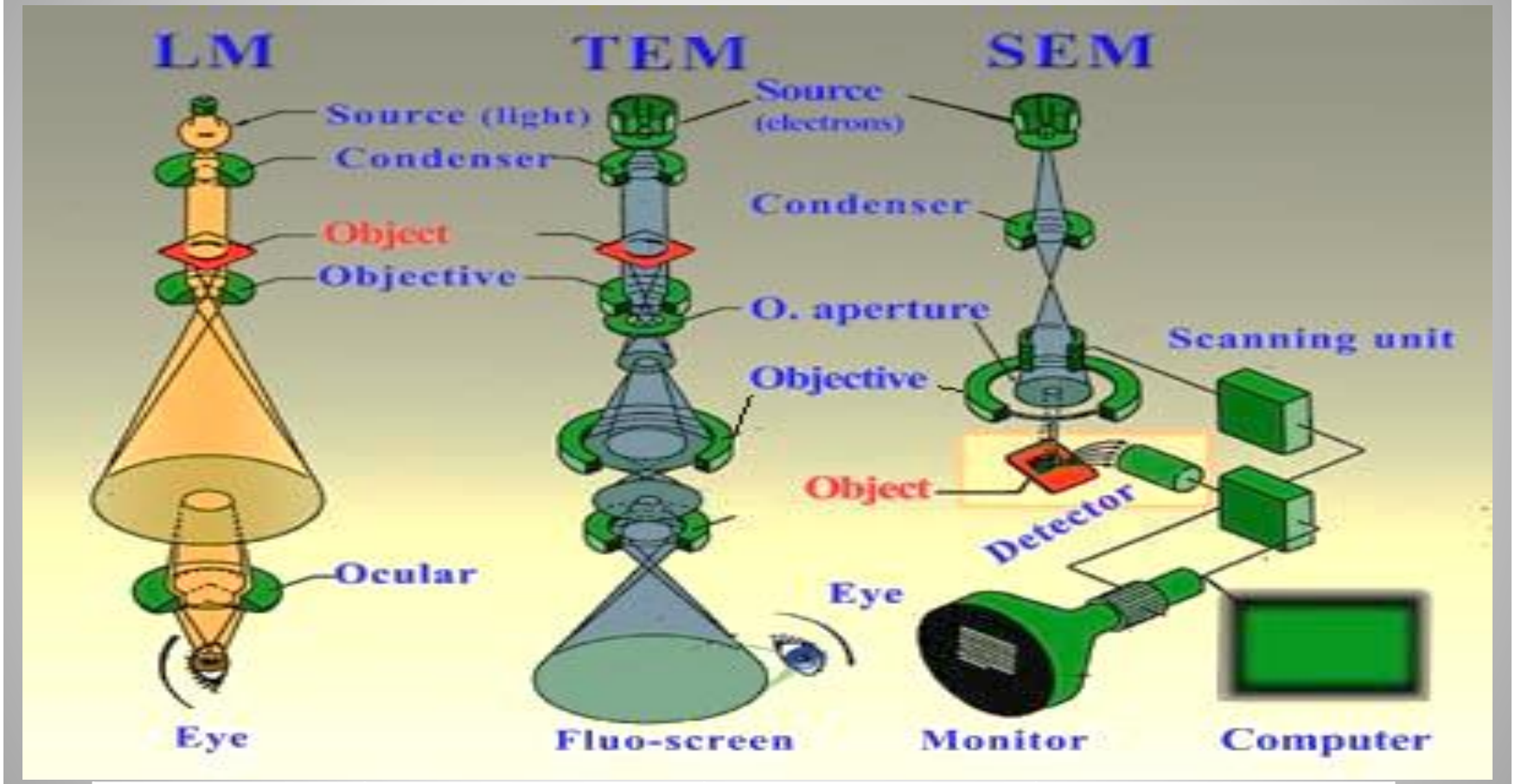
ELECTROMAGNETIC WAVE

Species of light	Wave length (λ, nm)
Electric wave	10^6~10^{12}
Ultra red light	10^3~10^5
Visible light	390~760
Ultra violet light	13~390
X-ray	0.05~10
γ-ray	0.005~0.1

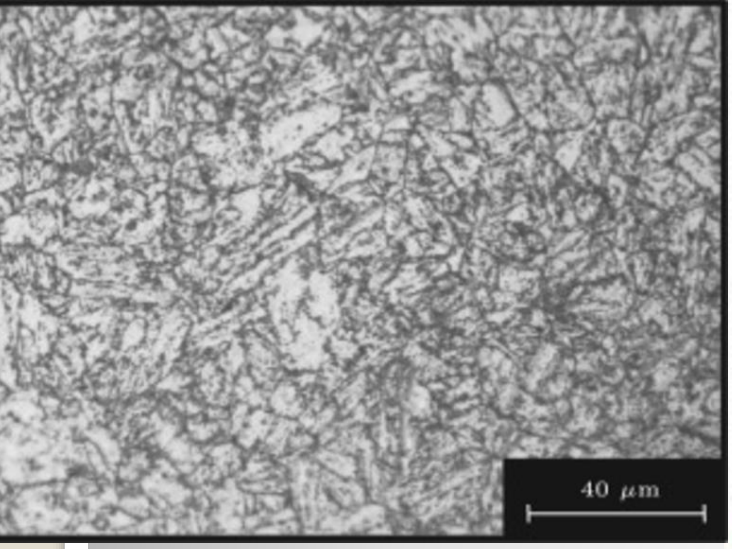
The wave length of **ELECTRON** is dependent on the accelerating voltage

Accelerating voltage	Wave length of electron (λ, nm)
20 kV	0.00859
120 kV	0.00335
200 kV	0.00251
1000 kV	0.00087

The difference between optical and electron microscope



Resolution	0.2 mm (OM)	0.2 nm (TEM)	1.5 nm (SEM)



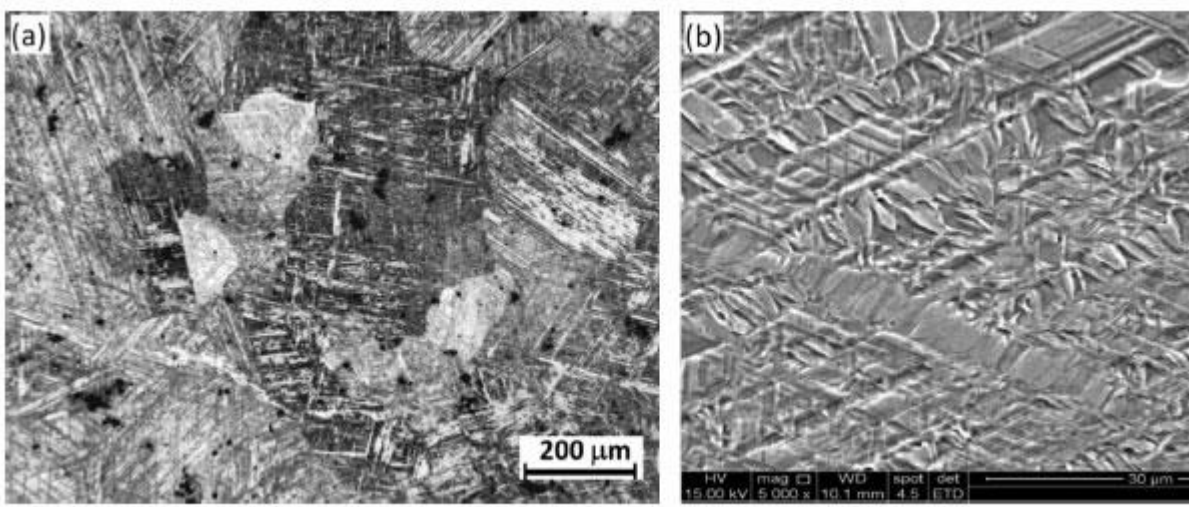
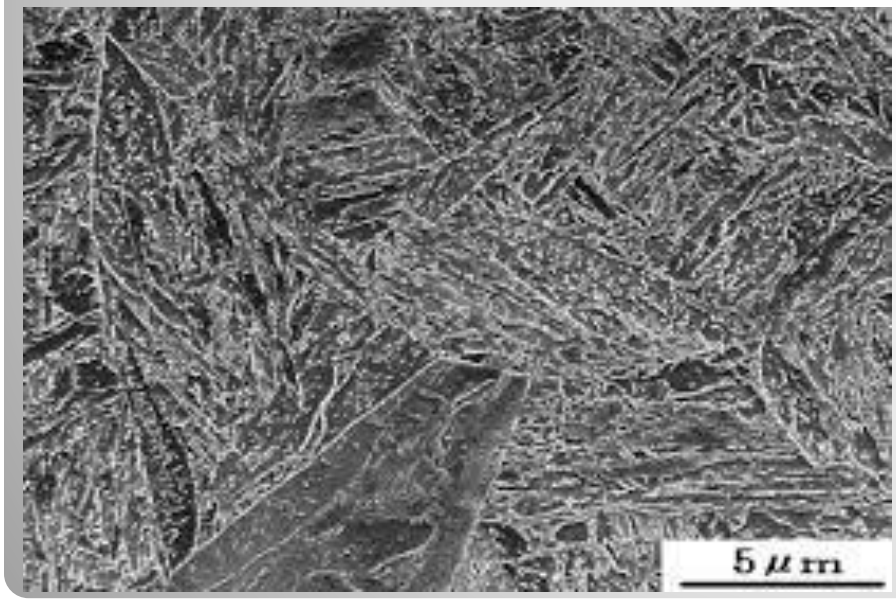
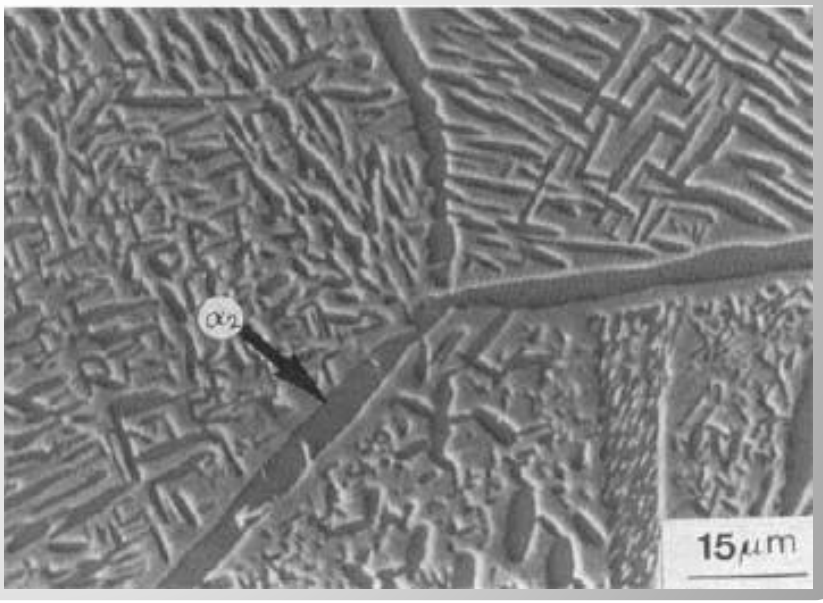
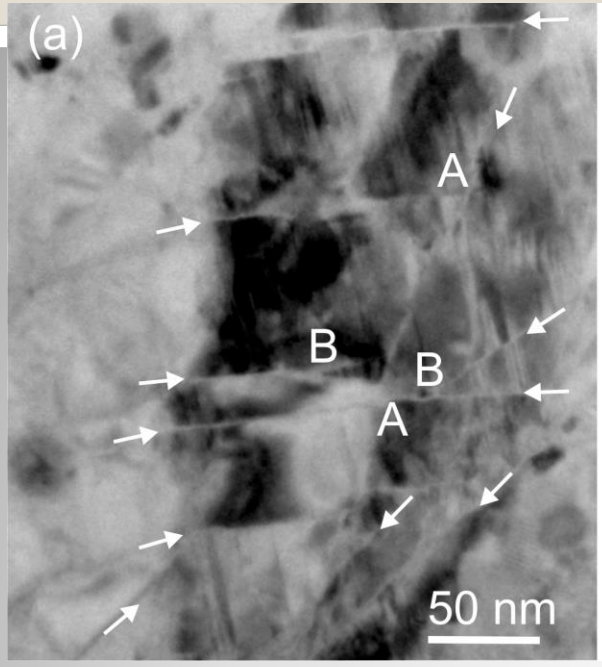


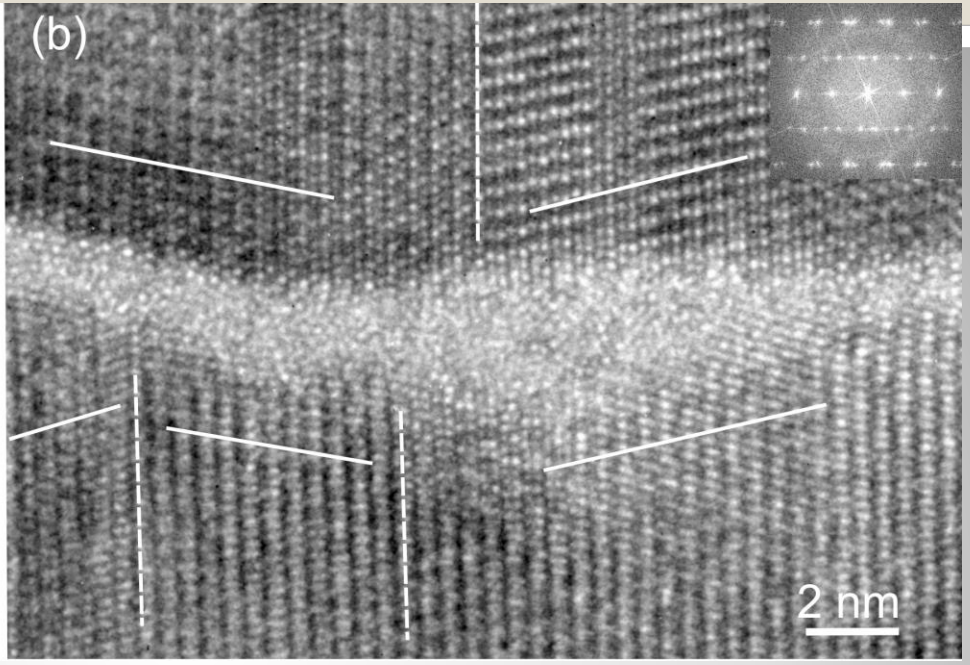
Figure 6. (a) Optical and (b) scanning electron micrographs of hot-rolled sample. Sample was etched with 4% Nital.



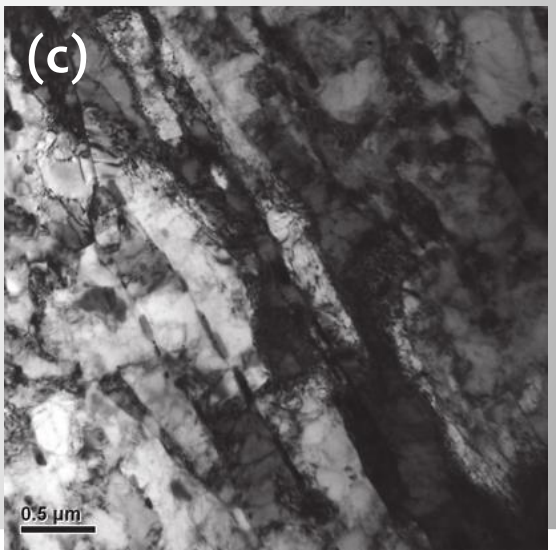


SEM images of martensite structure.





TEM images of martensite structure.
(a) and (c) Bright field (BF TEM images) and
(b) Lattice image.



TEM

Images

BFI (bright field image)

DFI (dark field image)

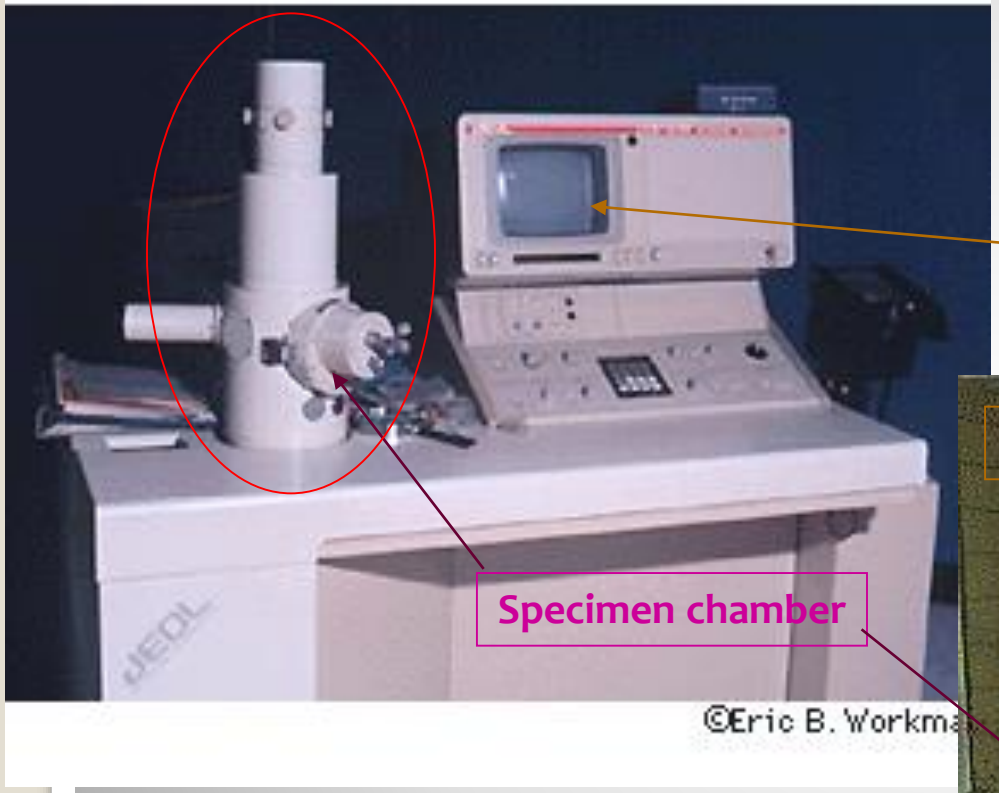
Magnified image

Observation microstructure/ nanostructure (morphology of grain, grain size and distribution, the presence of second phase, dislocations, stacking fault etc.), high resolution image/lattice image.

Electron diffraction patterns

- Crystal structure
- Crystalline or amorphous
- Monocrystalline or polycrystalline
- One phase or more present in the specimen
- Orientation of specimen or individual grain, twin grains, epitaxial relationship

STRUCTURE ⟺ **PROPERTIES**



Specimen chamber

CRT (Cathode ray tube)

X-ray detector

Typical SEM

Conventional SEM


JSM-6510/JSM-6510LV
Scanning Electron Microscope
JSM-6510A/JSM-6510LA
Analytical Scanning Electron Microscop
SEM-EDS
JSM-6510LA
JSM-6510LV

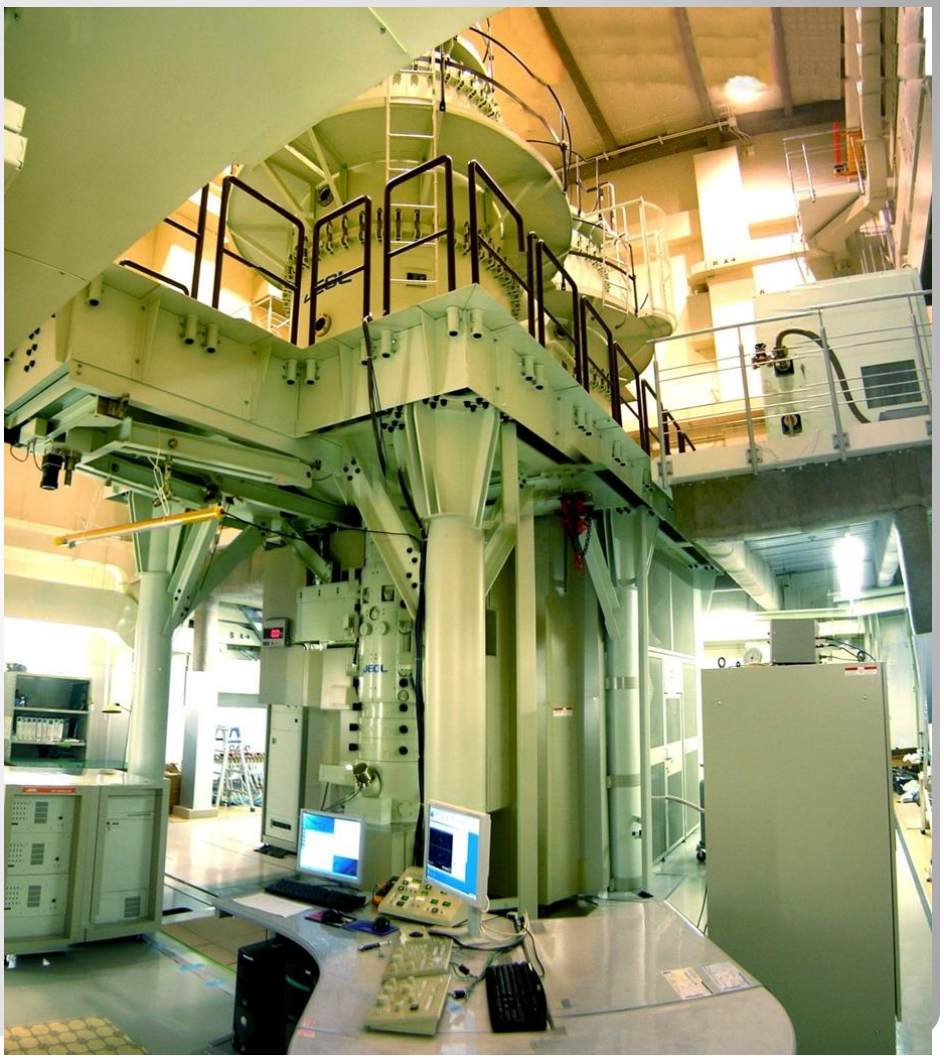
JEM-1400
(MIPA UGM)

Tecnai-20F
Lorentz TEM

Typical TEM

HVEM

JEM-1000 HVEM



HVEM JEM-1300NEF

An electron microscope cannot see the wood for the tree

An electron microscope is just for seeing trees

Electron microscopes have a range of disadvantages :

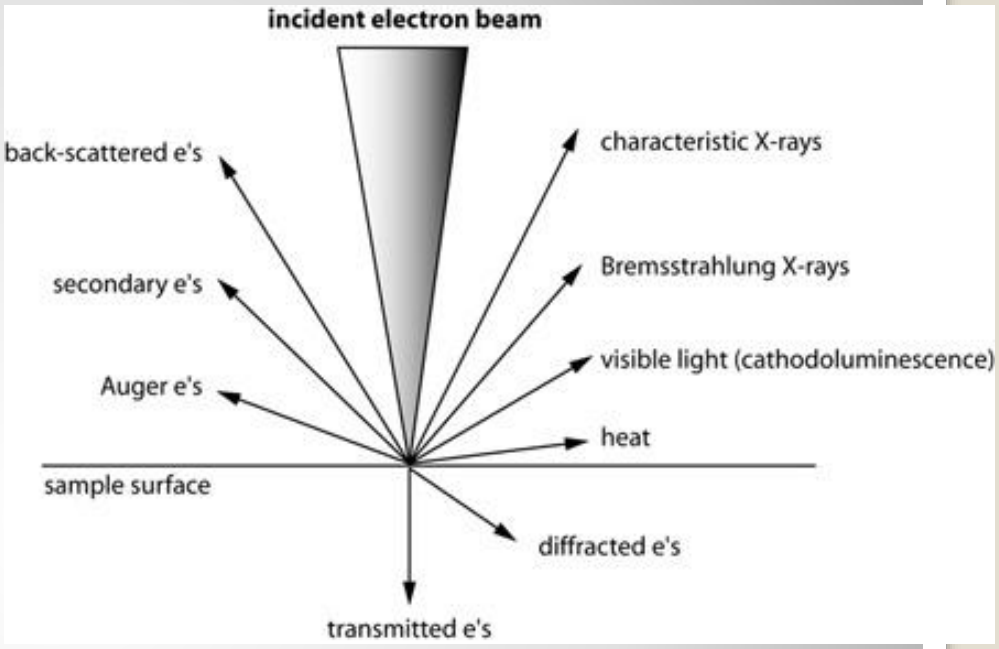
1. They are extremely expensive.
2. Sample preparation is often much more elaborate. It is often necessary to coat the specimen with a very thin layer of metal (such as gold). The metal is able to reflect the electrons.
3. The sample must be completely dry. This makes it impossible to observe living specimens.
4. It is not possible to observe moving specimens (they are dead).
5. It is not possible to observe color. Electrons do not possess a color.
6. The image is only black/white. Sometimes the image is colored artificially to give a better visual impression.
7. They require more training and experience in identifying artifacts that may have been introduced during the sample preparation process.
8. The energy of the electron beam is very high. The sample is therefore exposed to high radiation, and therefore not able to live.
9. The space requirements are high. They may need a whole room.
10. Maintenance costs are high.

SEM
(Theory and Analysis)

What is SEM?

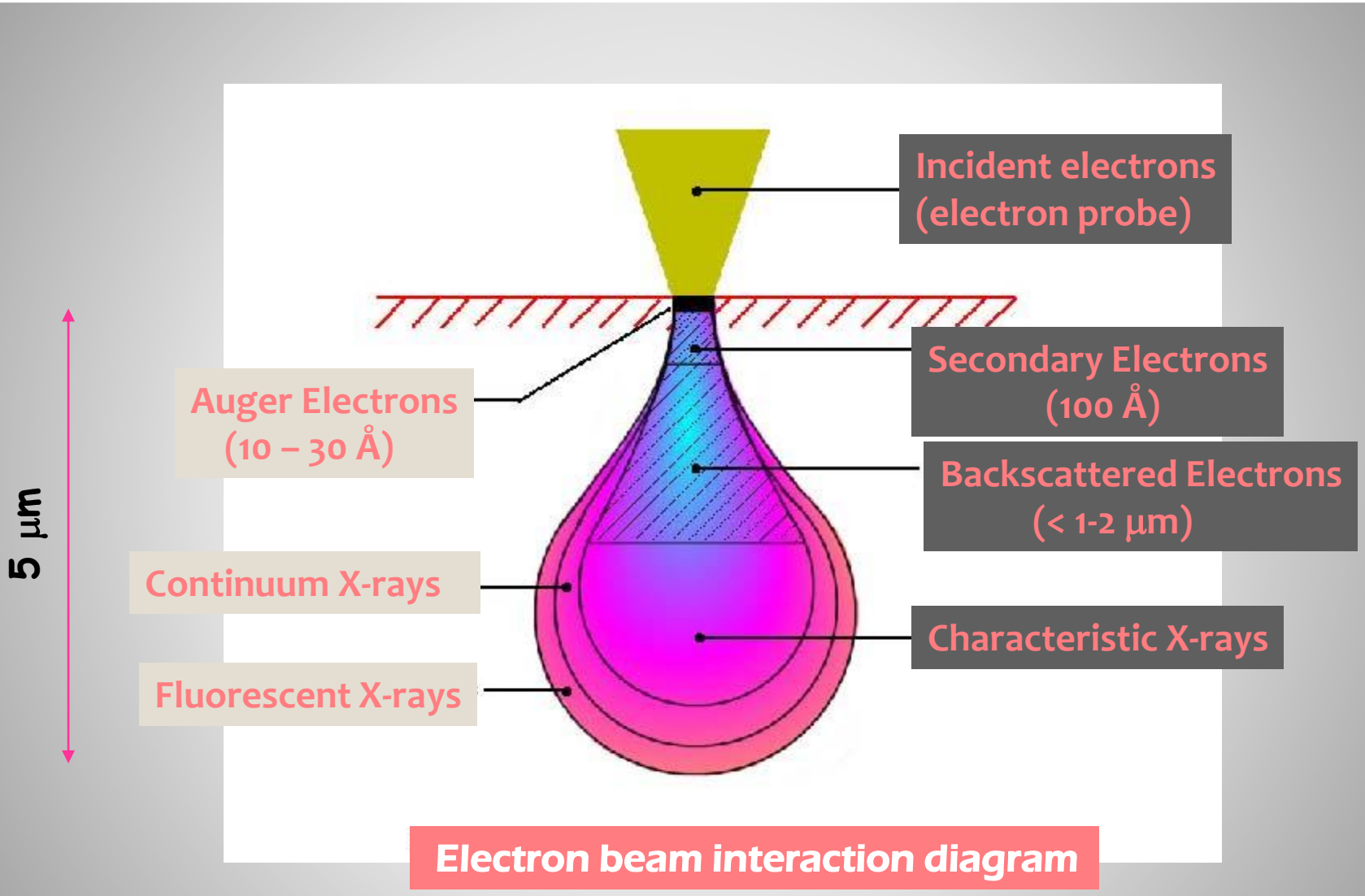
The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.

The signals that derive from **electron and specimen interactions** reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.



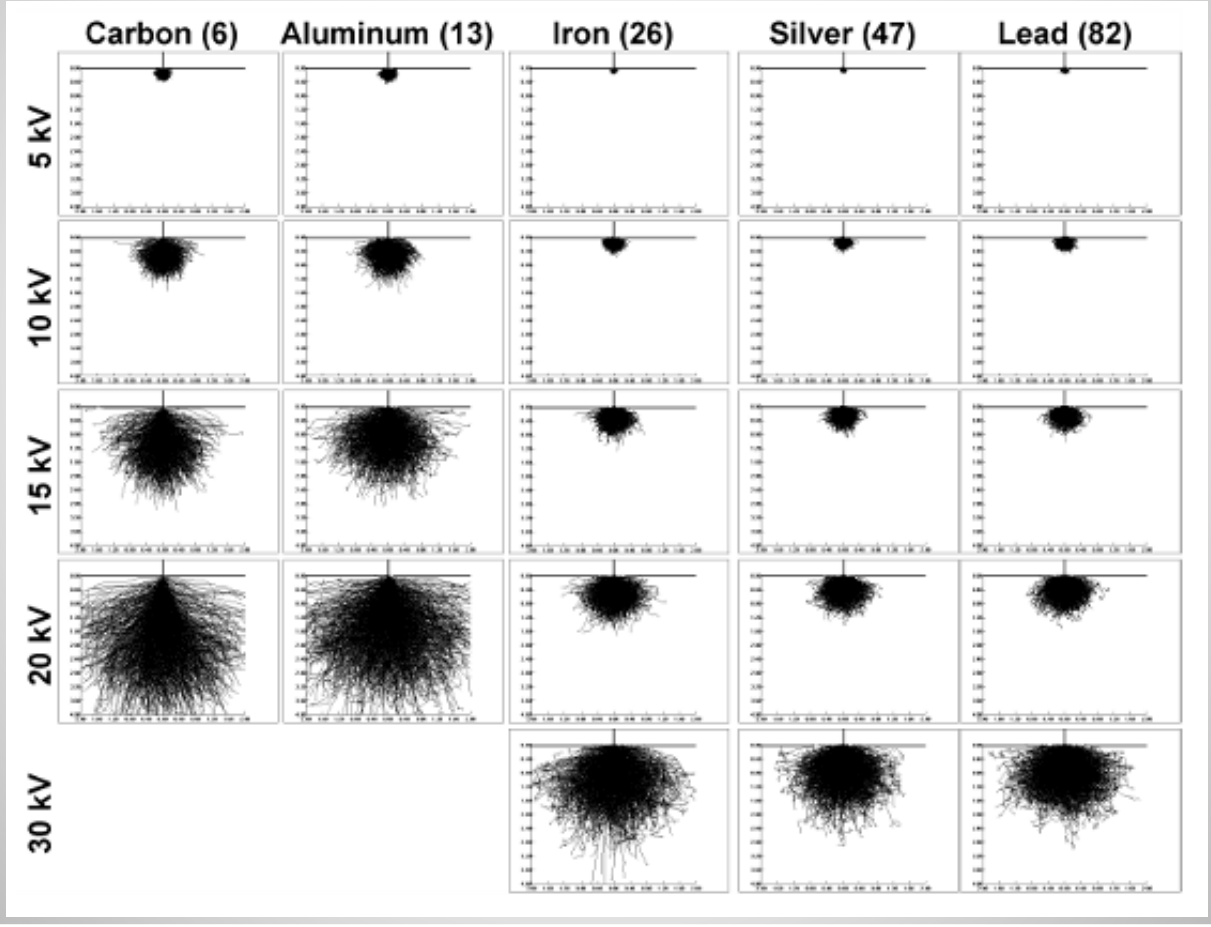
Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm).

The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using **EDS**), crystalline structure, and crystal orientations (using **EBSD, electron backscatter diffraction**). The design and function of the SEM is very similar to the **EPMA (electron probe microanalysis)** and considerable overlap in capabilities exists between the two instruments.



Electron beam interaction diagram

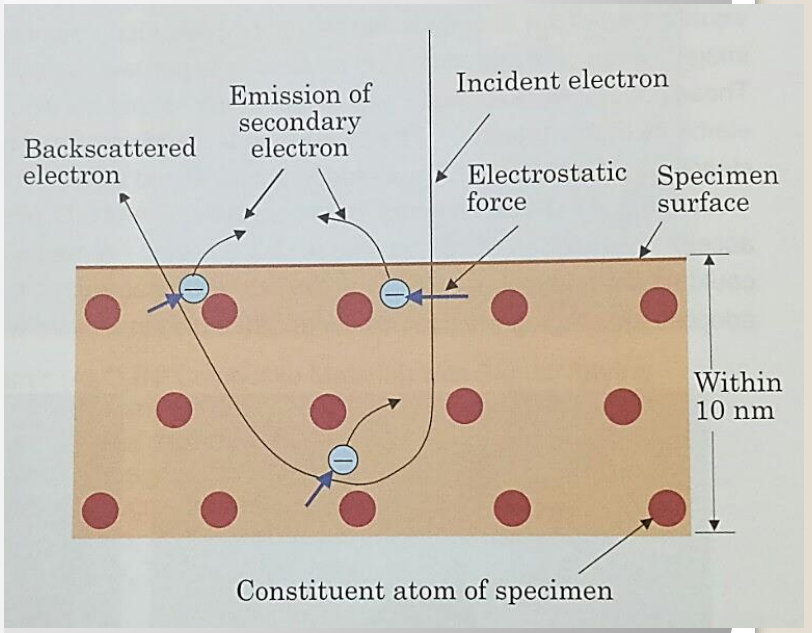
The penetration of the beam into the mass of the specimen is determined by basically 4 parameters: beam current, spot size, accelerating voltage and atomic number of the specimen.



Fundamental Principles of Scanning Electron Microscopy (SEM)

Secondary electrons and backscattered electrons are commonly used for imaging samples: **secondary electrons** are most valuable for showing morphology and topography on the specimen and **backscattered electrons** are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination).

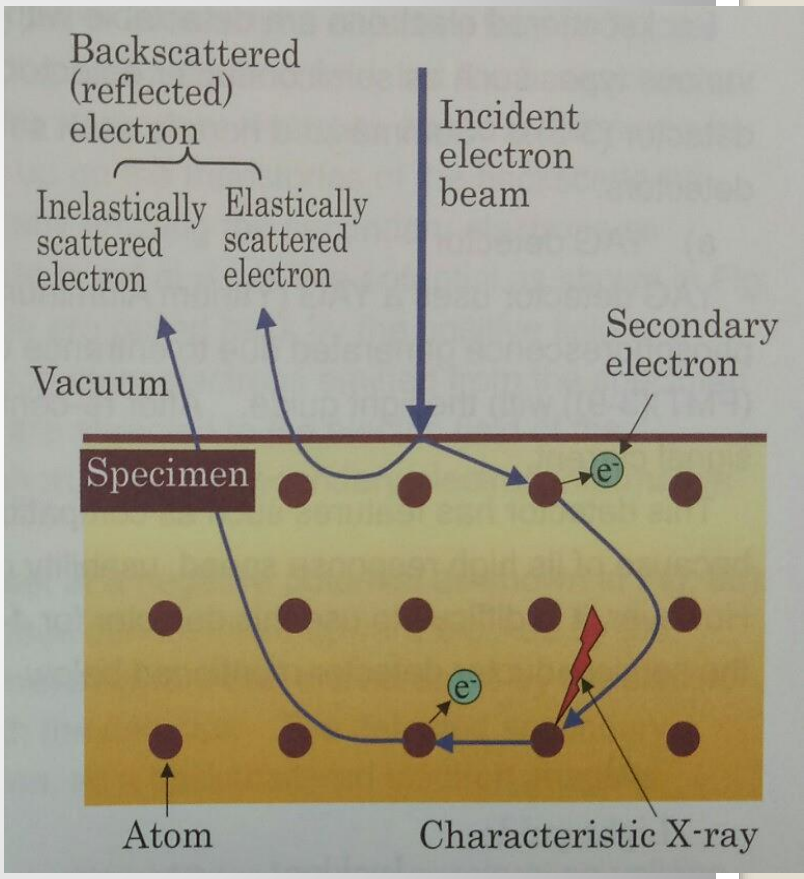
Emission of secondary electrons occurs in the process where incident electrons penetrate a specimen and then inelastically scattered.
A free electron in the specimen may receive an electrostatic force (Coulomb force) from the incident electron or scattered electron and thereby part of the latter's energy and then jump out into the vacuum. This electron Is called **a secondary electron**.
However, the energy received by free electron is as small as a few ten eV max.
Therefore, it has been though that only the secondary electron produced within a depth range about 10 nm below the specimen surface can escape into the vacuum.



Electron scattering in a specimen are classified into 2 types;

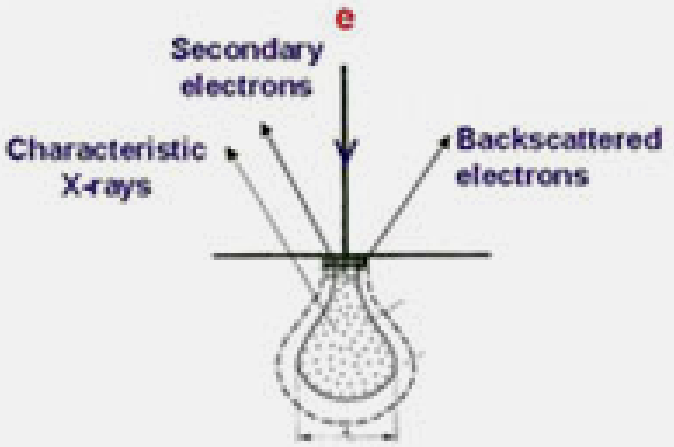
1. elastic scattering in which incident electron are scattered at a large angle with almost no loss in energy.
2. Inelastic scattering in which incident electron Loose energy but are scattered at a small angle.

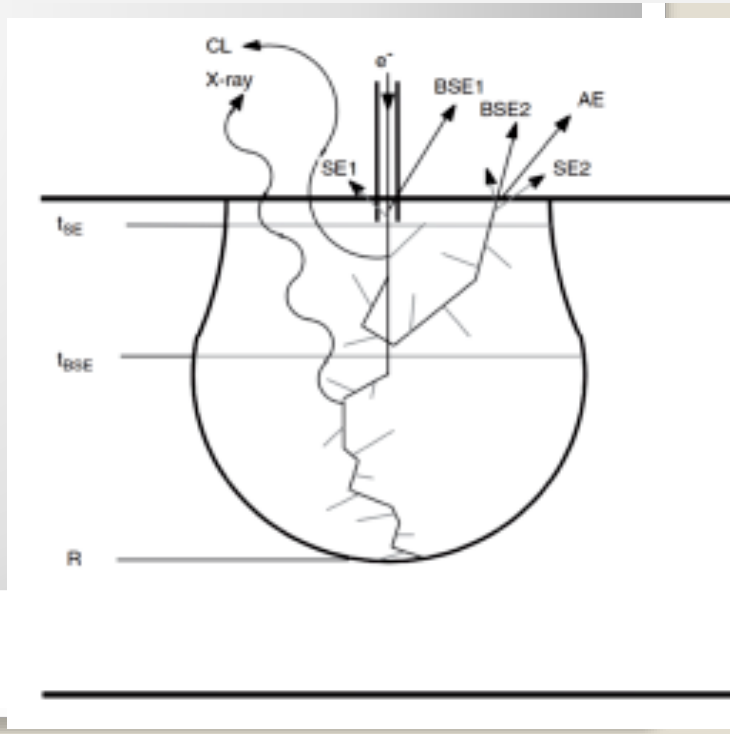
The elastically scattered electrons having approximately the same energy as the incident electrons are scattered from the vicinity of specimen surface into the vacuum, while inelastically scattered electrons which have lost energy substantially are scattered from a Comparatively deep location in the specimen Into the vacuum.

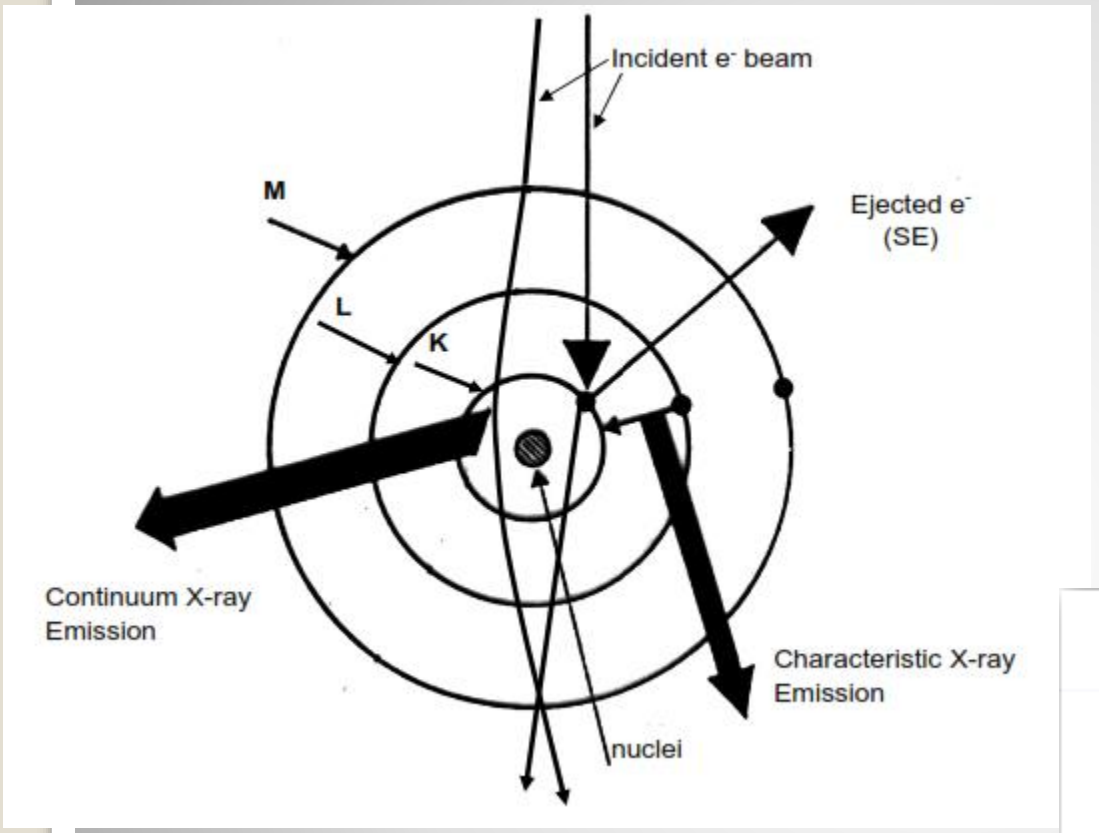


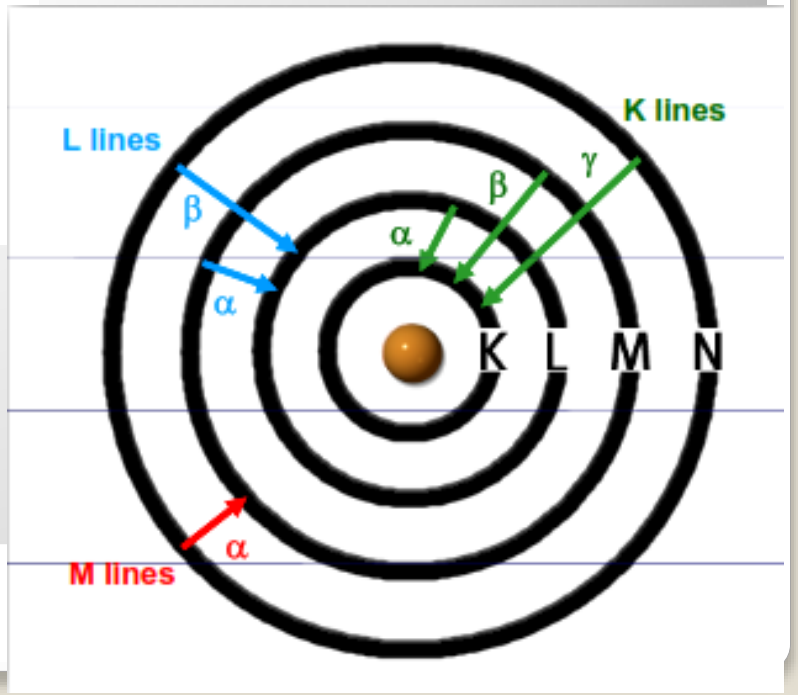
X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete orbitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element).

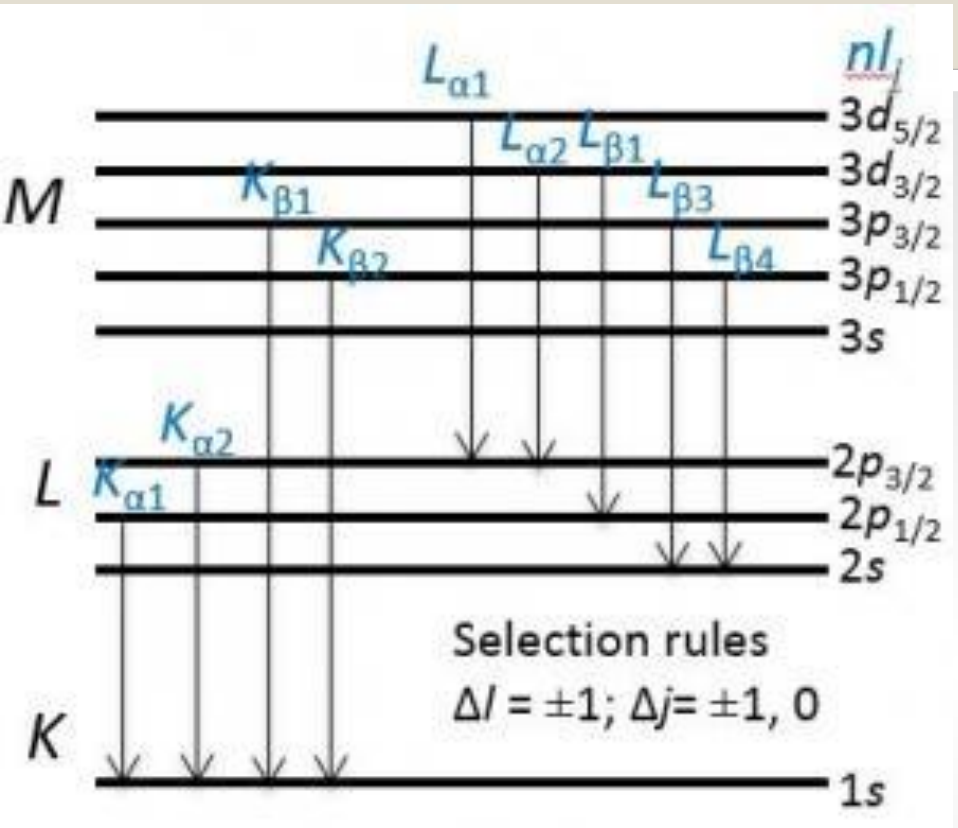
Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.






Incident e⁻ beam
M
L
K
Ejected e⁻
(SE)
Continuum X-ray
Emission
Characteristic X-ray
Emission
nuclei


L lines
K lines
β
β
γ
α
α
K
L
M
N
α
M lines



EDS Principle

Energy Dispersive X-ray Spectroscopy (EDS or EDX) is a qualitative and quantitative X-ray microanalytical technique that provides information on the chemical composition of a sample for elements with atomic number (Z) >3.

Characteristic X-ray Generation

The atoms are ionized by the primary electron beam leading to holes generated on the core shells; following ionization the electrons from outer shells fill the holes and cause the emission of X-ray fluorescence lines.

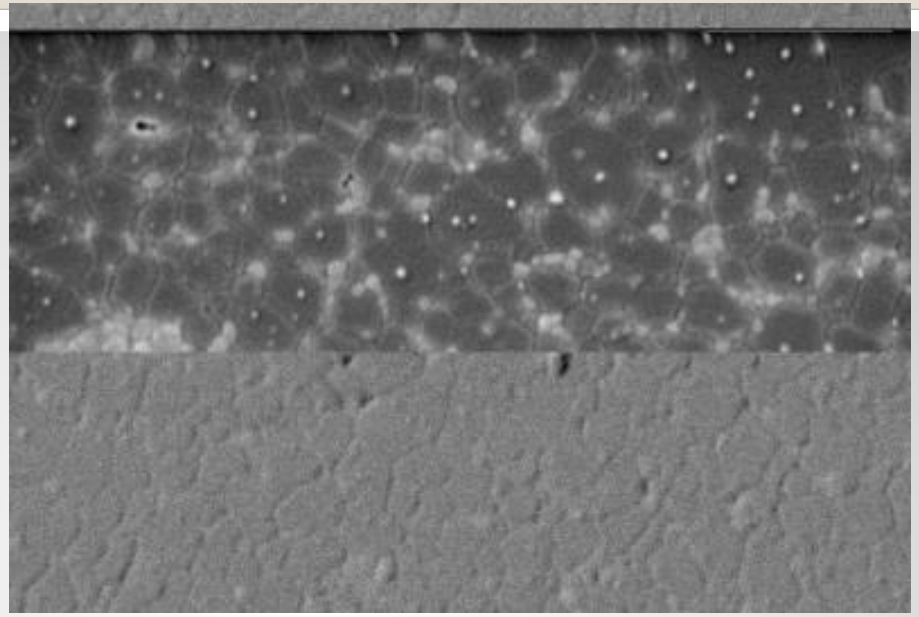
The characteristic X-ray lines are named according to the shell in which the initial vacancy occurs and the shell from which an electron drops to fill that vacancy.

For instance, if the initial vacancy occurs in the K shell and the vacancy filling electron drops from the adjacent (*L*) shell, a K_α x-ray is emitted. If the electron drops from the *M* shell (two shells away), the emitted x-ray is a K_β x-ray. Similarly, if an *L*-shell electron is ejected and an electron from the *M*-shell fills the vacancy, L_α radiation will be emitted.

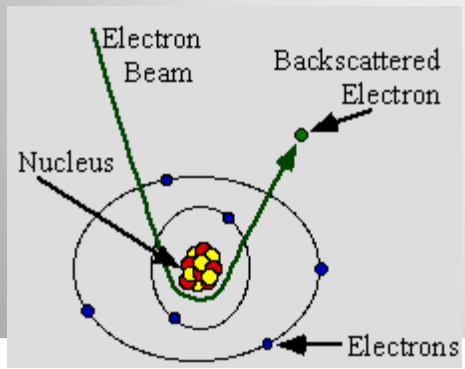
Examples

BSE analysis:
COMPOSITION

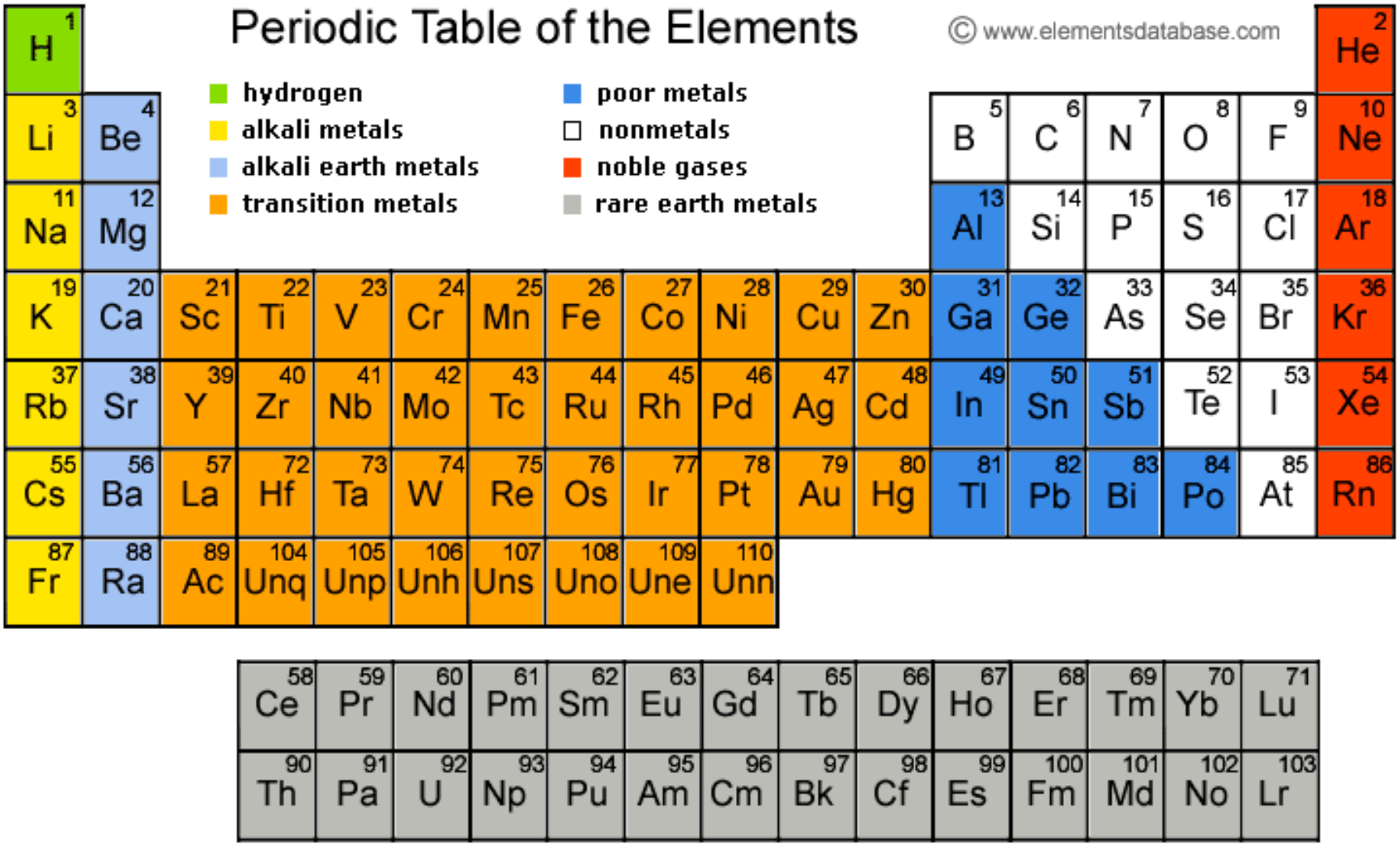
SE analysis:
TOPOGRAPHY



Heavy elements (high atomic number) → backscatter electrons more strongly than light elements (low atomic number) →appear brighter in the image



All the elements have different sized nuclei.
As the size of the atom nucleus increases,
the number of BSE increases.
Thus, BSE can be used to get an image that
showed the different elements present in a sample.


Periodic Table of the Elements
© www.elementsdatabase.com
hydrogen
alkali metals
alkali earth metals
transition metals
poor metals
nonmetals
noble gases
rare earth metals
1 H
2 He
3 Li
4 Be
5 B
6 C
7 N
8 O
9 F
10 Ne
11 Na
12 Mg
13 Al
14 Si
15 P
16 S
17 Cl
18 Ar
19 K
20 Ca
21 Sc
22 Ti
23 V
24 Cr
25 Mn
26 Fe
27 Co
28 Ni
29 Cu
30 Zn
31 Ga
32 Ge
33 As
34 Se
35 Br
36 Kr
37 Rb
38 Sr
39 Y
40 Zr
41 Nb
42 Mo
43 Tc
44 Ru
45 Rh
46 Pd
47 Ag
48 Cd
49 In
50 Sn
51 Sb
52 Te
53 I
54 Xe
55 Cs
56 Ba
57 La
72 Hf
73 Ta
74 W
75 Re
76 Os
77 Ir
78 Pt
79 Au
80 Hg
81 Tl
82 Pb
83 Bi
84 Po
85 At
86 Rn
87 Fr
88 Ra
89 Ac
104 Unq
105 Unp
106 Unh
107 Uns
108 Uno
109 Une
110 Unn
58 Ce
59 Pr
60 Nd
61 Pm
62 Sm
63 Eu
64 Gd
65 Tb
66 Dy
67 Ho
68 Er
69 Tm
70 Yb
71 Lu
90 Th
91 Pa
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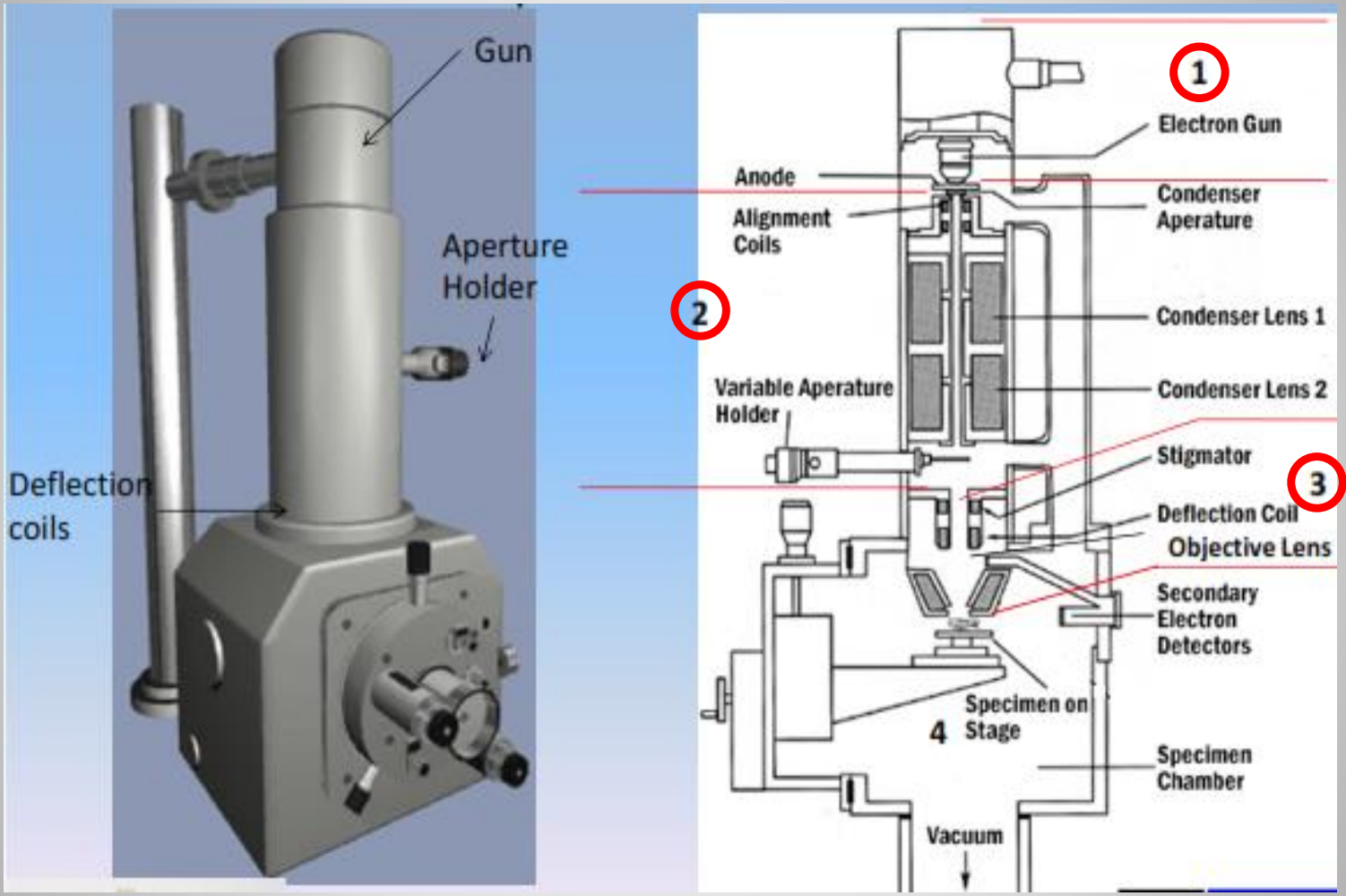
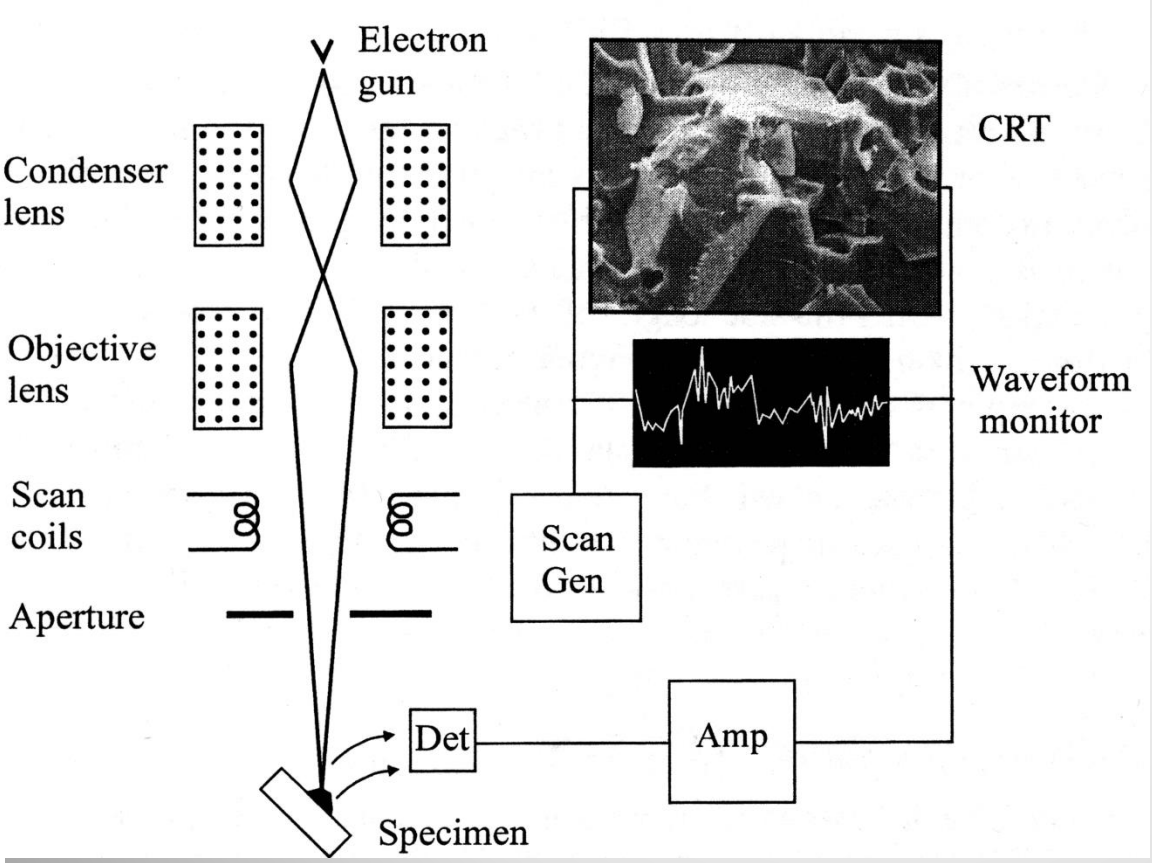
97 Bk
98 Cf
99 Es
100 Fm
101 Md
102 No
103 Lr



Schematic cross-section of SEM

Components of the SEM

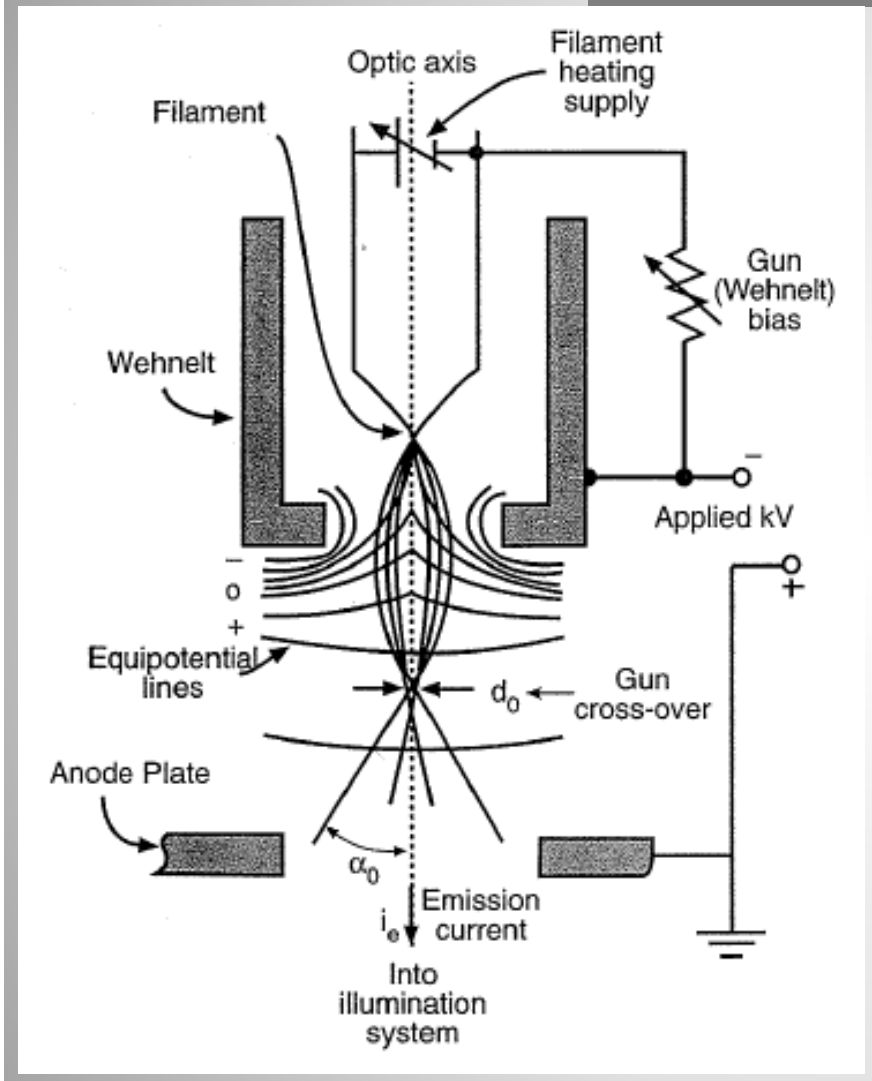


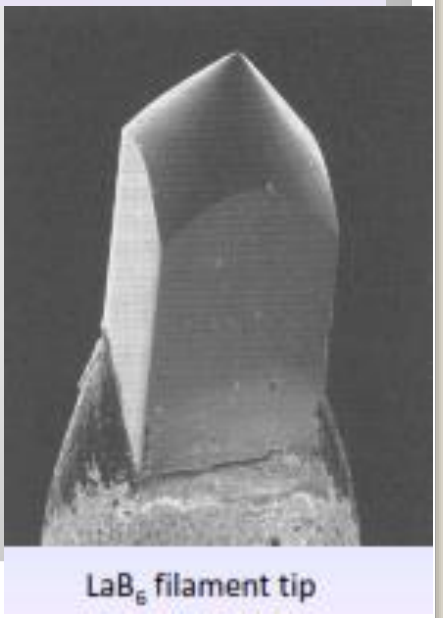
Electrons emitted by the gun are accelerated typically by 20 kV.

They pass through condenser and objective lenses, and then through a set of scan coils and an aperture. A scan is simultaneously generated on a computer monitor.

Electrons emitted by the specimen are detected, amplified and the signal is then used to produce an image.

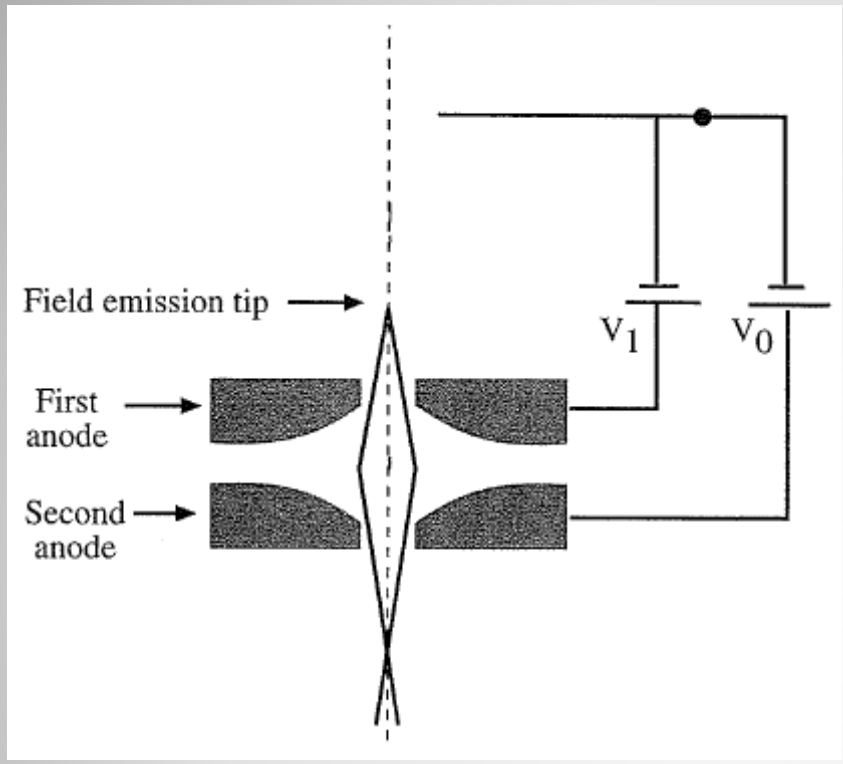
ELECTRON GUN

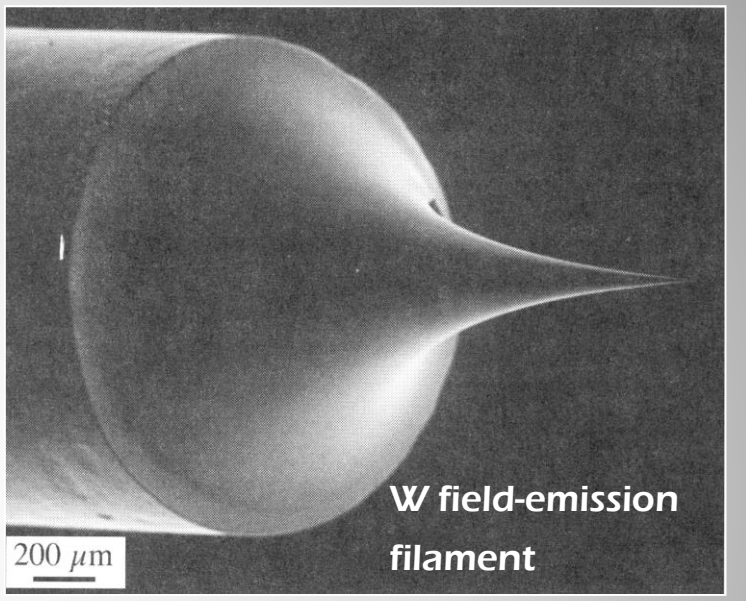




Schematic illustration of electron paths from a thermionic electron gun

Thermionic source (from heating)





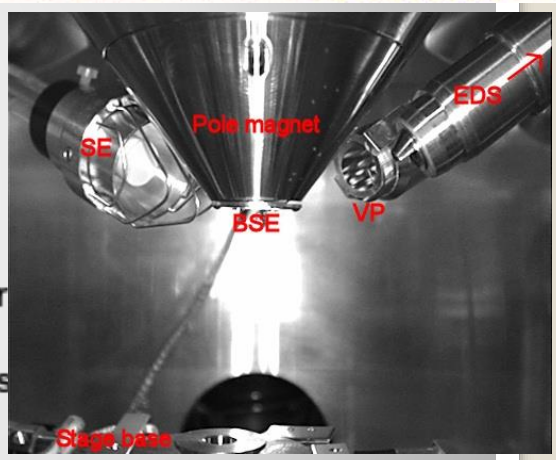
Schematic illustration of electron paths from a field emission gun

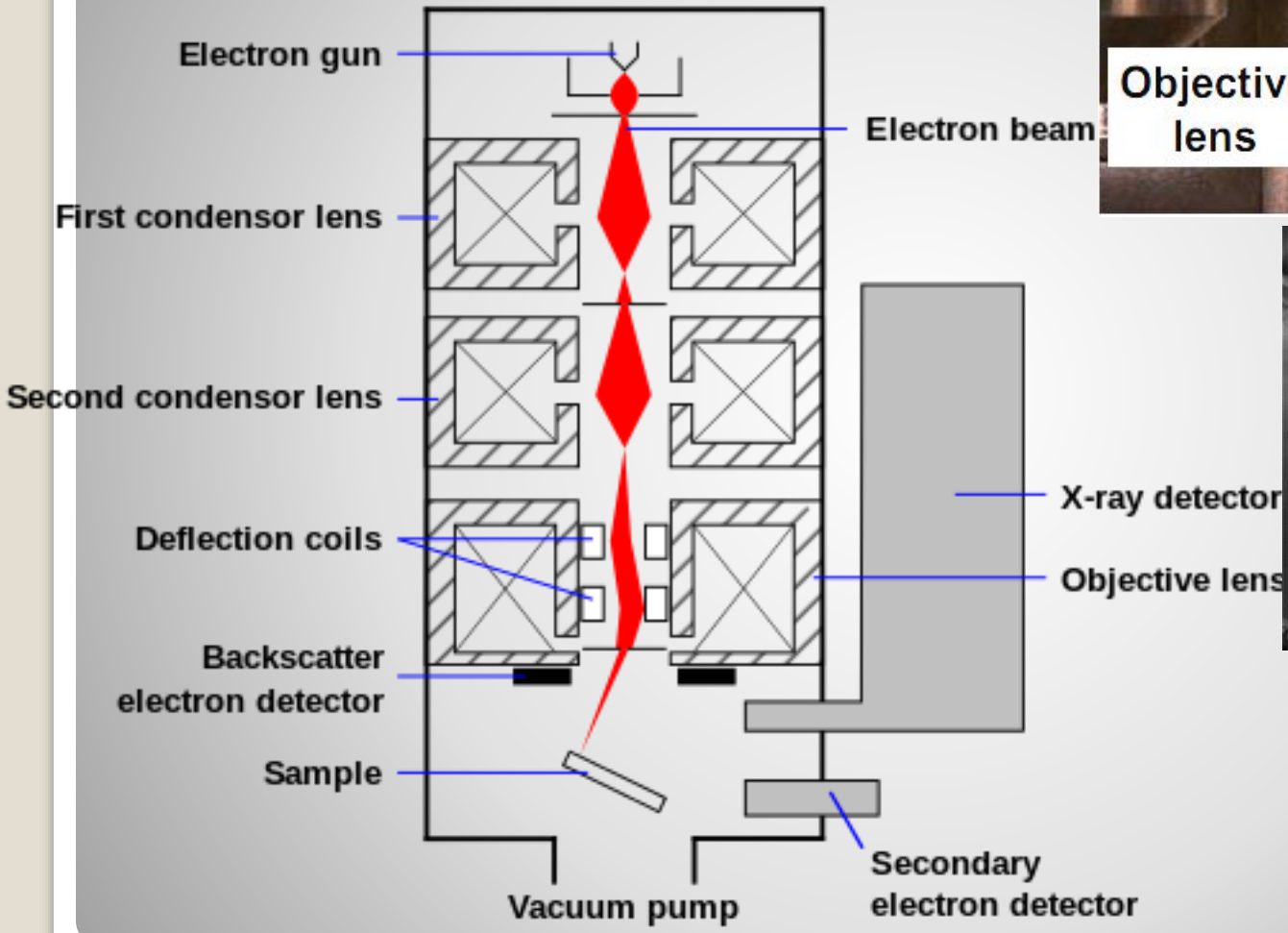
Field-emission source
(from electric field)

Comparison of three types of source operating at 100 kV

	Units	Tungsten	LaB_6	Field Emission
Work function, Φ	eV	4.5	2.4	4.5
Richardson's constant	A/m^2K^2	6×10^5	4×10^5	
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
Brightness	A/m^2sr	10^9	5×10^{10}	10^{13}
Energy spread	eV	3	1.5	0.3
Emission current stability	%/hr	<1	<1	5
Vacuum	Pa	10^{-2}	10^{-4}	10^{-8}
Lifetime	hr	100	500	>1000


EDS Detector
Secondary Detector
Objective lens
Backscatter Detector


SE
Pole magnet
EDS
BSE
VP
Stage base


Electron gun
Electron beam
First condensor lens
Second condensor lens
Deflection coils
X-ray detector
Objective lens
Backscatter electron detector
Sample
Secondary electron detector
Vacuum pump

Vacuum system

Category of vacuum (1 Torr≈130 Pa, 1 Pa= 7.5x10-3 Torr)

- Rough vacuum: 100 to 0.1 Pa (~1 to 10^{-3} Torr)
- Low vacuum: 0.1 - 10^{-4} Pa (~10^{-3} - 10^{-6} Torr)
- High vacuum: 10^{-4} - 10^{-7} Pa (~10^{-6} - 10^{-9} Torr)
- Ultra High vacuum: if the pressure is lower than 10^{-7} Pa (~ 10^{-9} Torr)

All electron microscopes are operated under vacuum:

-To keep specimen clean (less contamination)
-To not reduce lifetime of filament
-To avoid contaminant in the column
-To improve image resolution

Resolution in the SEM

- The resolution of the SEM is determined by the size of the incident beam. This can be reduced by introducing an aperture unto the beam path and by reducing the probe size using the condenser lens. Note that reducing the probe size using the condenser lens also reduces the beam current (for an explanation, see Goodhew et al., p. 131). Therefore, as you reduce the probe size you eventually reach a point where imaging is impossible. For a typical SEM operating at 20 kV, the minimum usable probe size is of the order of 1 – 3 nm.

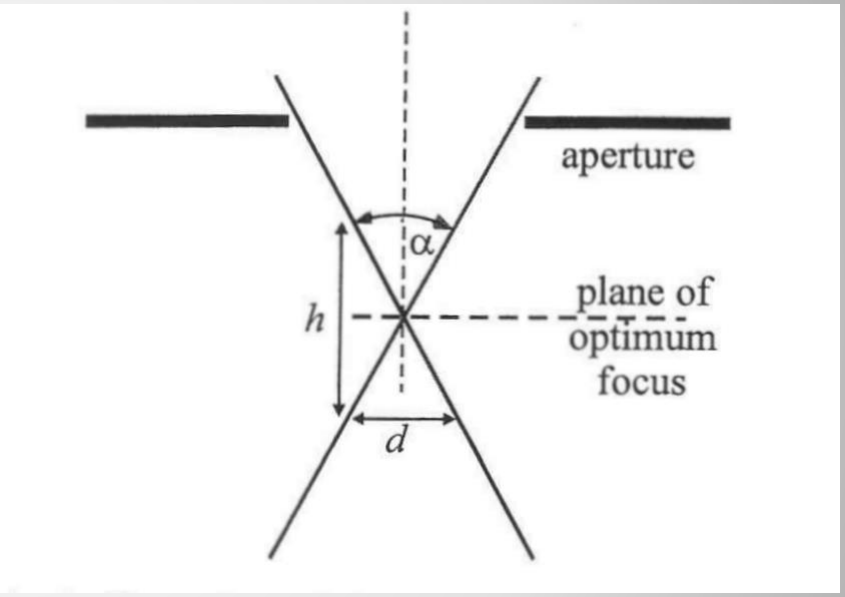
- Resolution also depends on accelerating voltage. This is because higher energy electrons experience less spherical aberration when they pass through the lenses. Resolution is also improved by reducing the working distance, up to a certain point. Beyond that point the lenses may not be able to focus the beam on the sample.

- As already noted, images obtained with backscattered electrons have a lower resolution than these obtained with secondary electrons, because they originate from deeper within the specimen.

Depth of field 1

Depth of field is the distance above and below the plane of optimum focus within which the image is in focus.

In the diagram on the right, *d* represents the diameter of the electron beam at the specimen. The depth of field is *h*, since it makes no difference to the sharpness of the image if the object is anywhere within the range *h*.

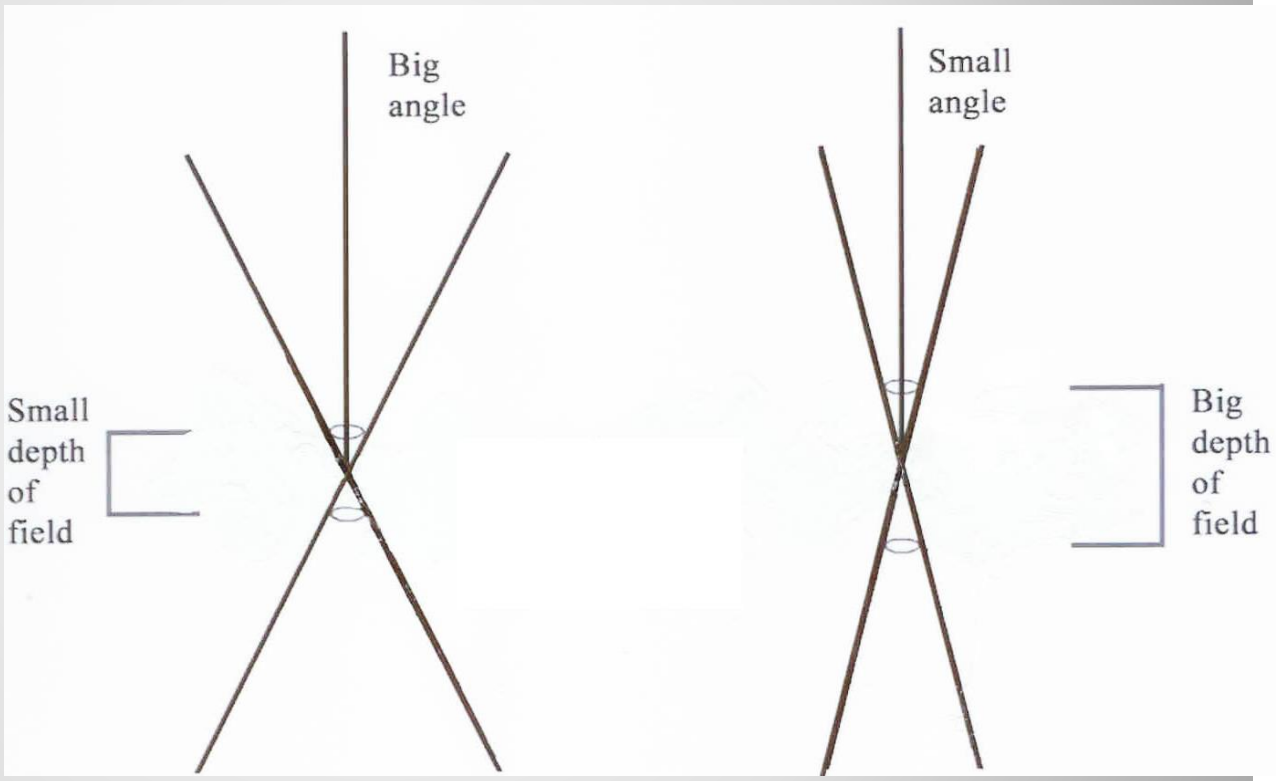


Reducing the angle α increases the depth of field. This can be achieved by using a smaller aperture or by increasing the working distance.

Depth of field 2

These diagrams illustrate the effect of the convergence angle α on the depth of field.

Because of the geometry of the imaging system, scanning electron microscopes have a much greater depth of field than optical microscopes .

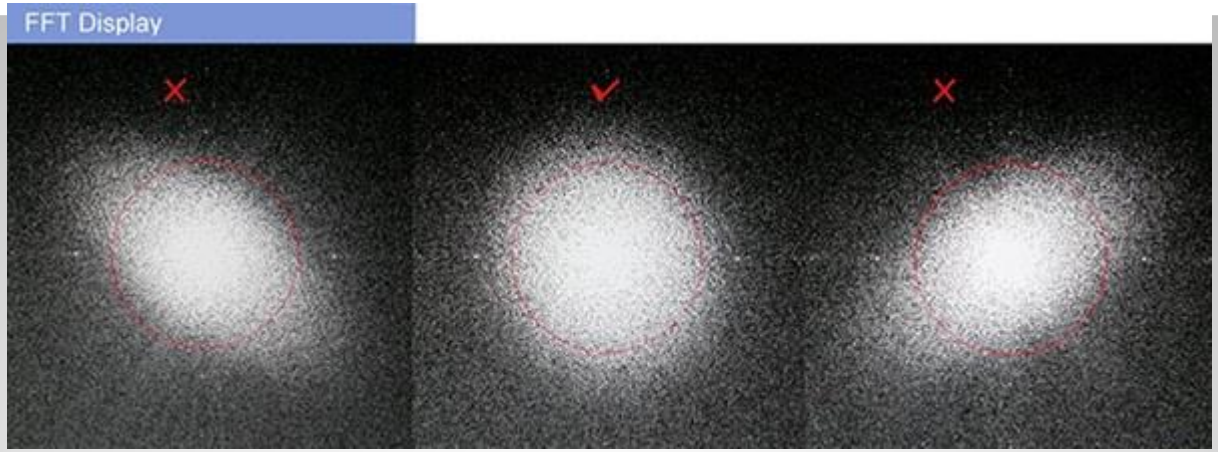


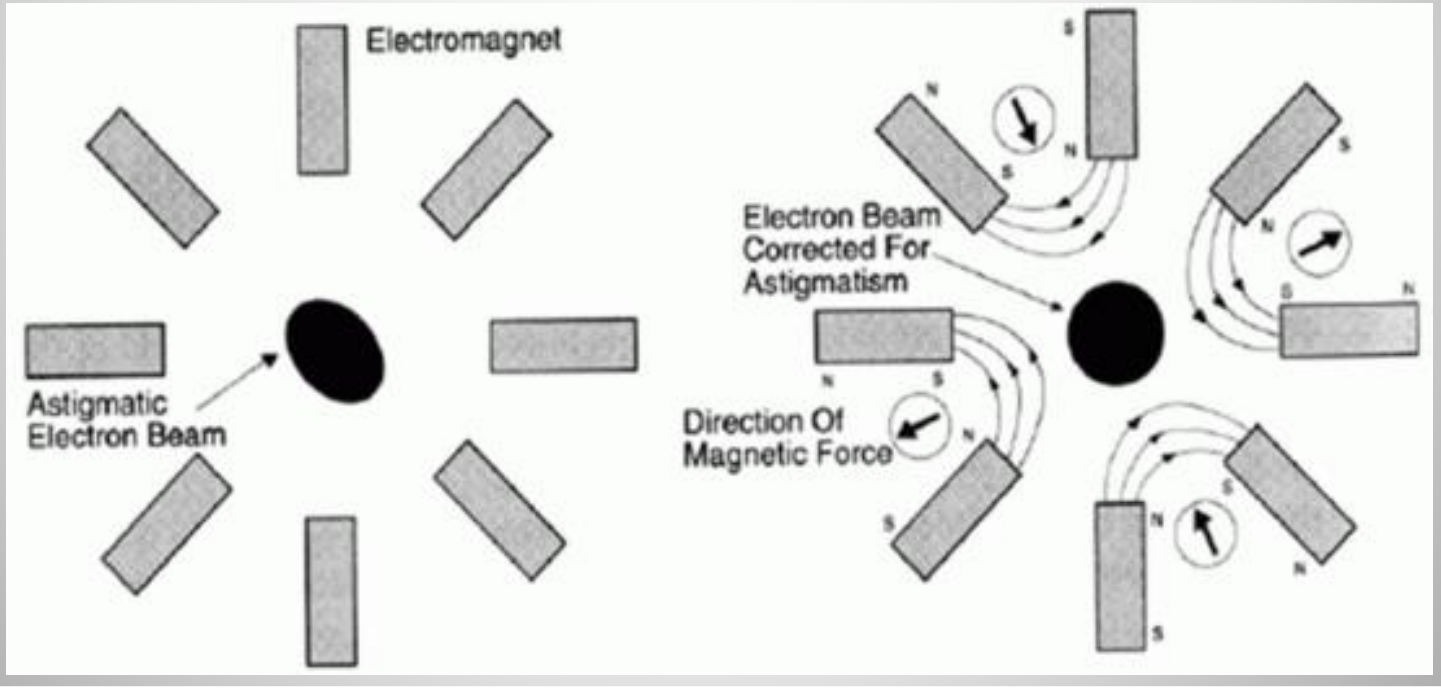
Astigmatism 1

Astigmatism is a problem that is commonly encountered in SEM (and TEM). It is an aberration of lenses that causes rays in a plane parallel to the optical axis to be focused at a different focal point from rays in a plane at 90° to it. The effect of astigmatism is that objects in the image generally appear "stretched" in one direction, and then in the other direction as you go through focus.

All electron microscopes are equipped with stigmators, which allow the user to correct the astigmatism, as shown in the next slide. Properly corrected astigmatism is essential in achieving high resolution images.

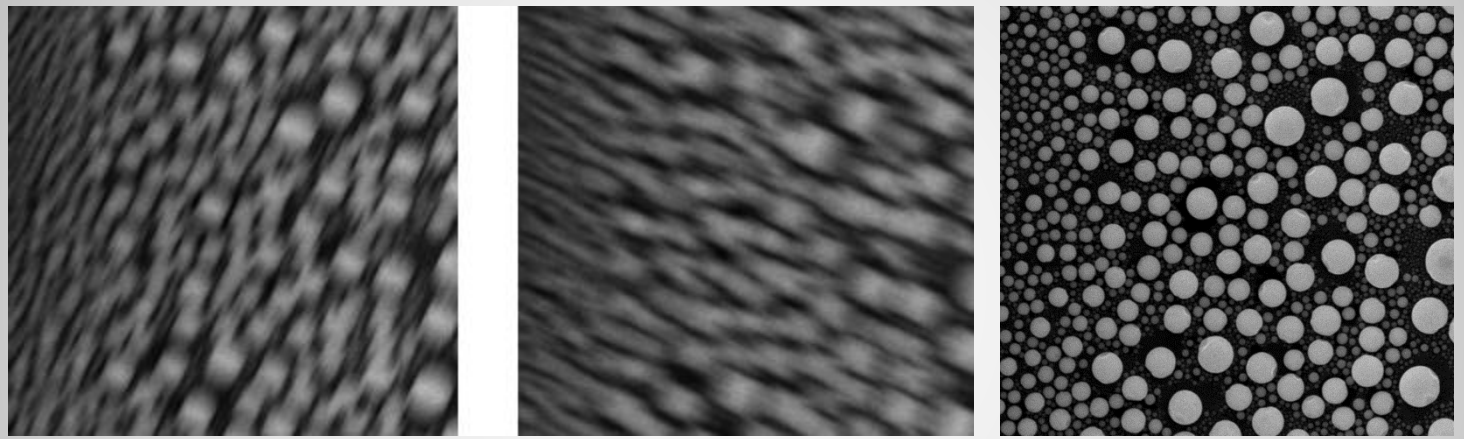
FFT Display



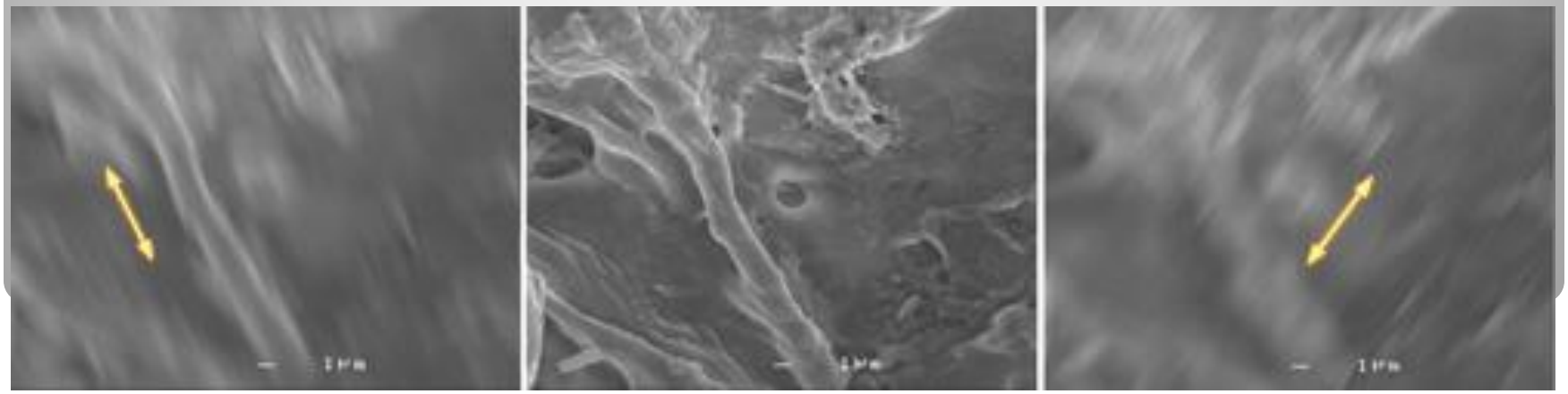

Electromagnet
Astigmatic
Electron Beam
Electron Beam
Corrected For
Astigmatism
Direction Of
Magnetic Force

Astigmatism 2

The SEM images shown below left illustrate how astigmatism affects the image as you go through focus. On the right is shown the image following correction with the stigmators.



Astigmatic **Corrected**



SEM SPECIMEN PREPARATION

SPECIMENS:

1. **Powder**

2. **Bulk Specimens:**
   - **Metals/alloys**
   - **Ceramics**
   - **Minerals**
   - **Natural fibers**
   - **Nanofiber membranes etc.**

3. **Biological materials**

4. **Specimens containing oil**

SPECIMEN PREPARATION

1. For small particles (powder, <100 nm)

2. For small particles (powder, >100 nm)

MOST IMPORTANT: DISPERSION !!

Example:

Nanocrystalline Cellulose Studied with a Conventional SEM
(2014 International Conference on Physics, ICP 2014)

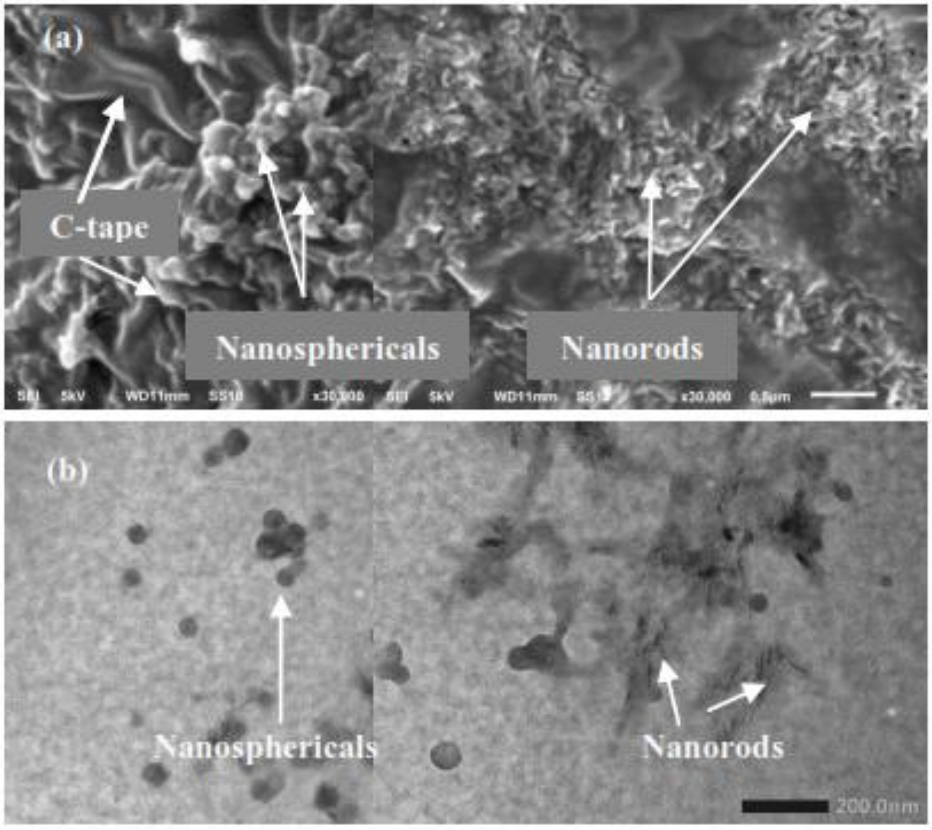


Figure 3. SEM image of nanocrystalline cellulose prepared on a C-tape which dried imperfectly (a) and TEM image of the same specimen (b).

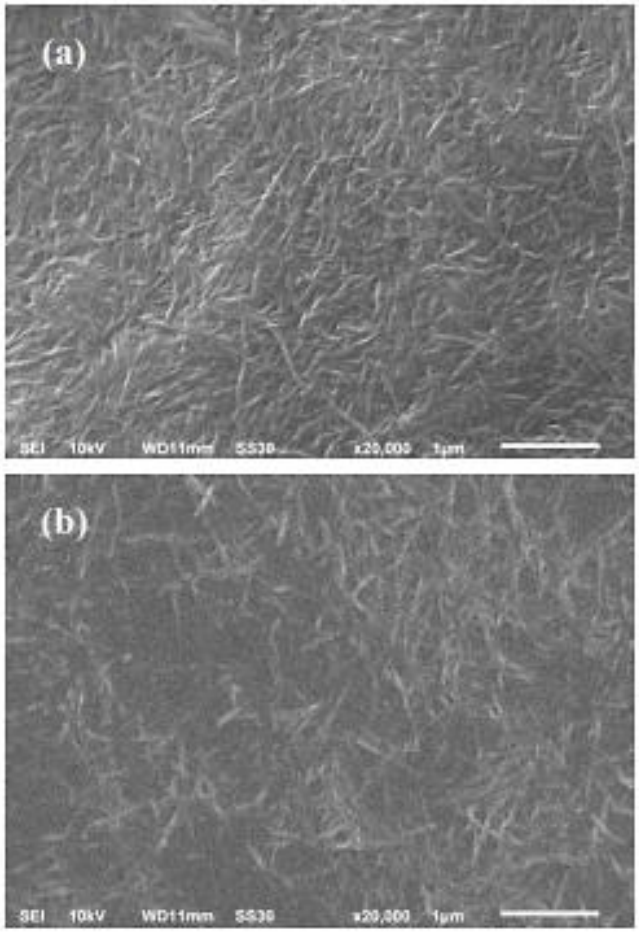


Figure 4. SEM images of nanocrystalline cellulose prepared on perfectly dry of a C-tape (a) and a Si plate (b) substrates.

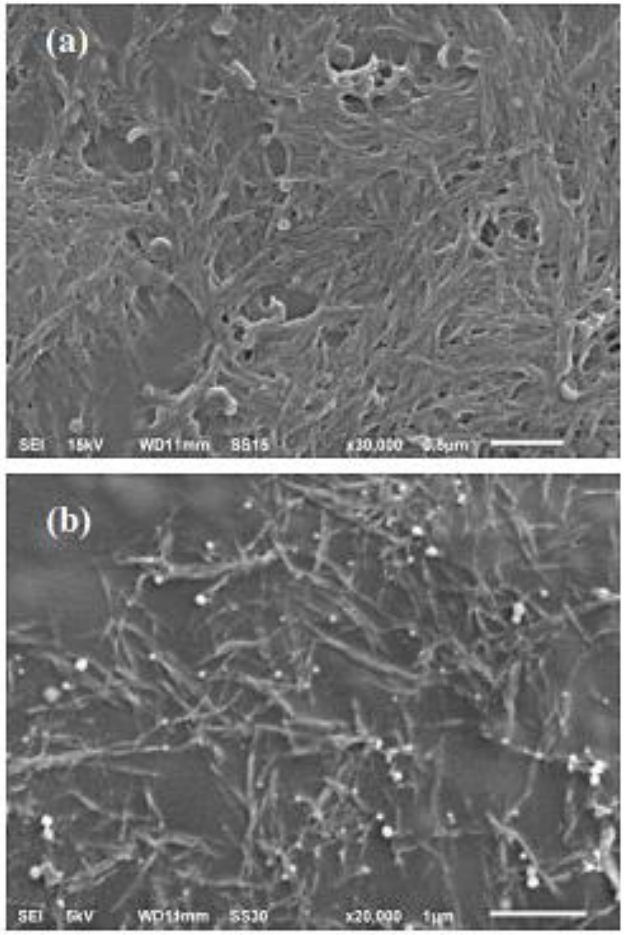
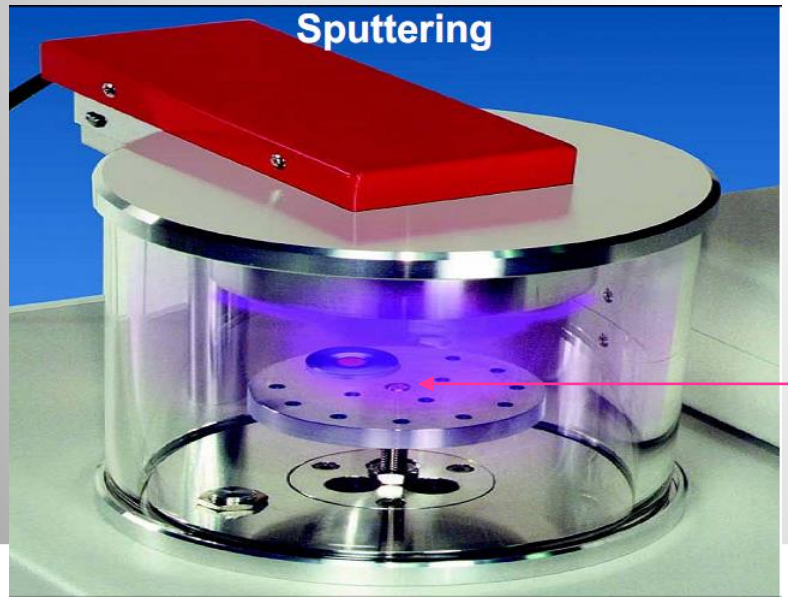


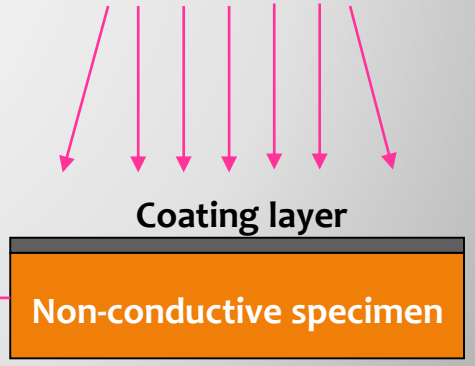
Figure 5. SEM images of nanocrystalline cellulose prepared on a carbon coated Cu-grid with different dispersion time; (a) 10 min and (b) 30 min.

For conventional imaging in the SEM, specimen must be electrically conductive at least at the specimen surface and electrically grounded to prevent the accumulation of electrostatic charge at the surface during electron irradiation.

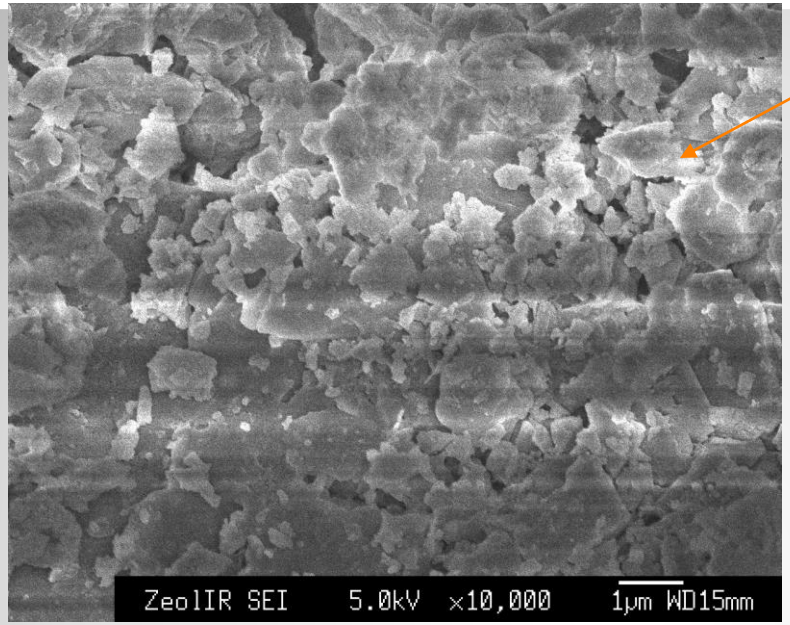
For the specimen that is enough conductivity, two important reasons for coating are to maximize signal and improve the spatial resolution.

Target: Au, Au-Pd alloy, Pt, W, C



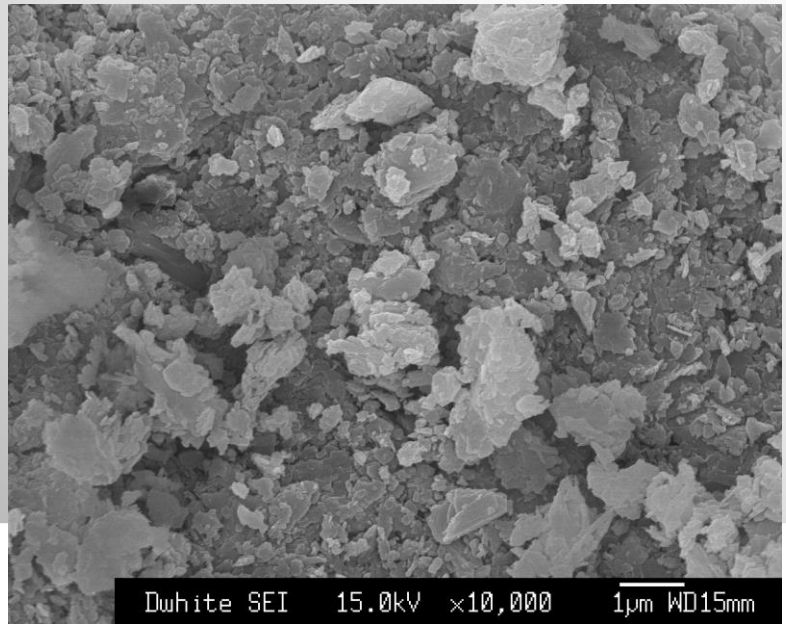


Zeolites

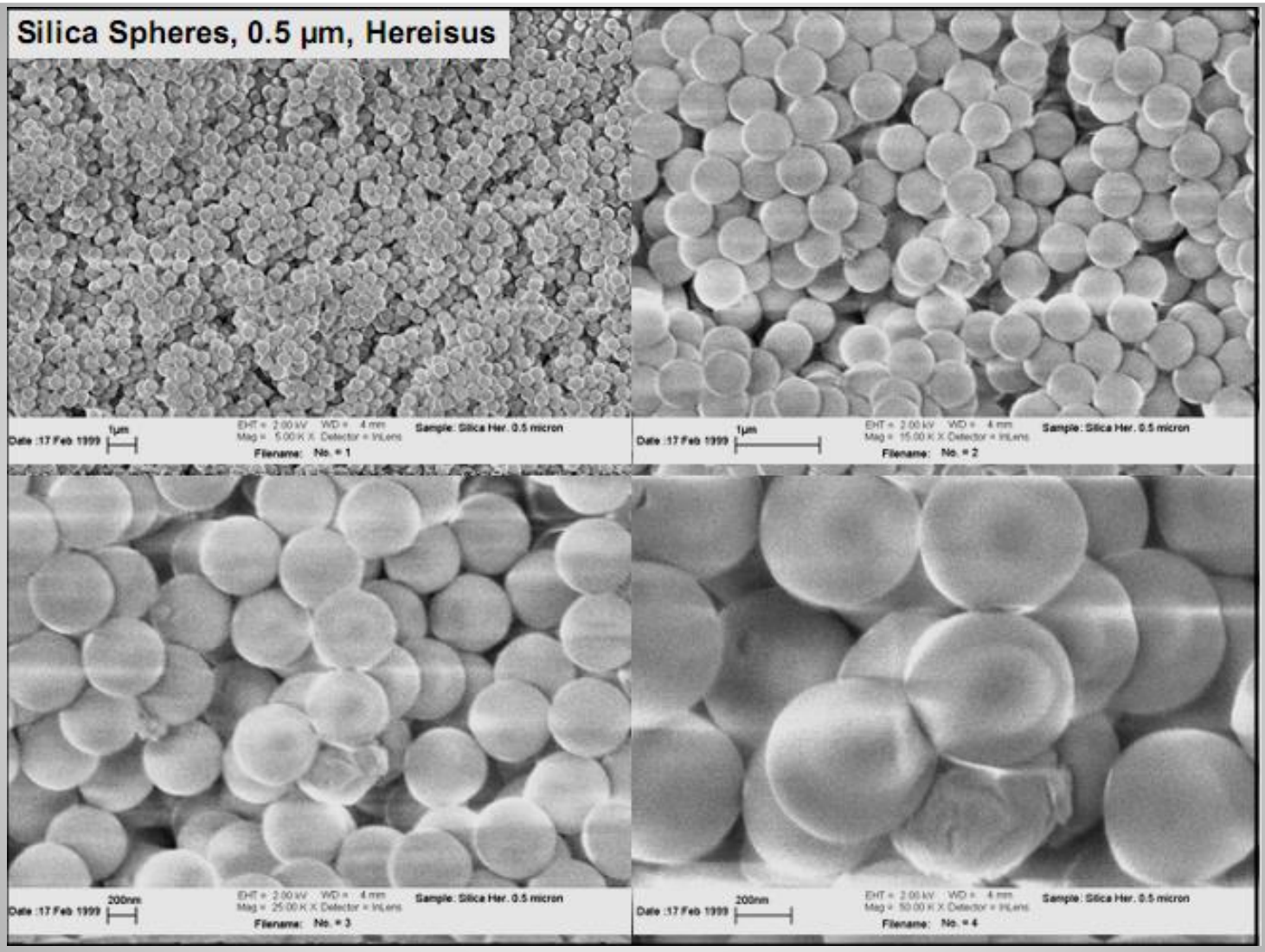


Charge-up

- No dried in an oven (120°C, 1 h)
- C-coating



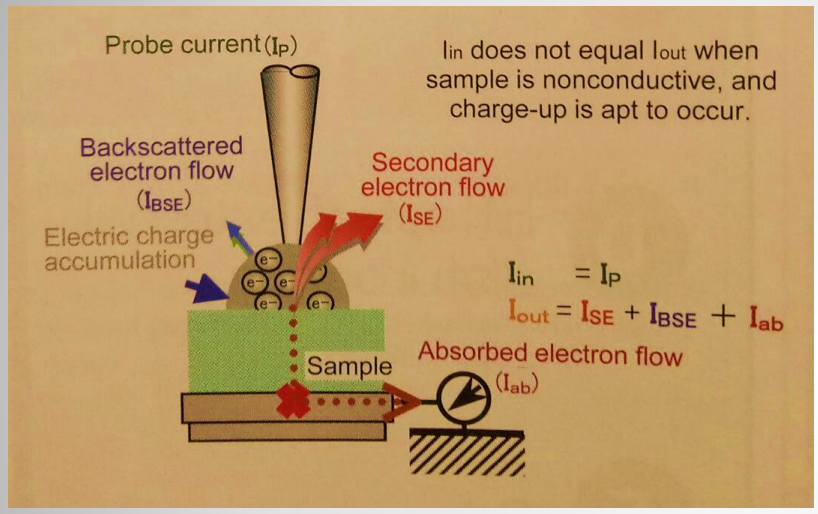
- dried in an oven (120°C, 1 h)
- C-coating

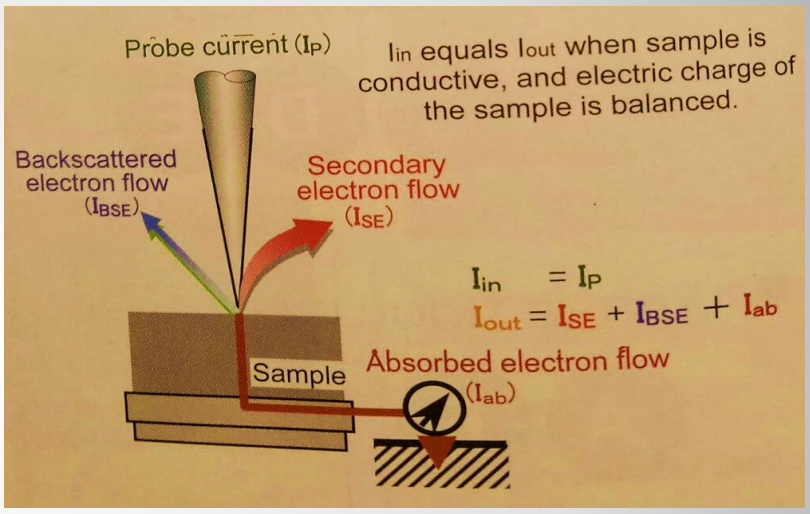


Specimen with non-conducting features

What is the charge-up phenomenon ?

Charge-up occurs during observation of non-conductive specimens





The charge-up phenomenon can be overcome as follows.

1. Reduce the accelerating voltage
2. Reduce the specimen irradiating current
3. Apply the metal coating
4. Observe image in low vacuum mode
5. Utilize a low-acceleration BSE signal.

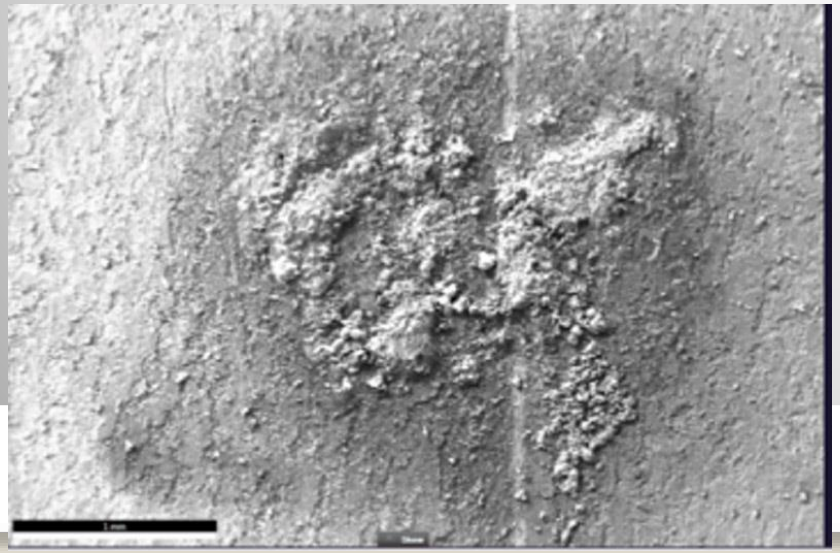
What is contamination?

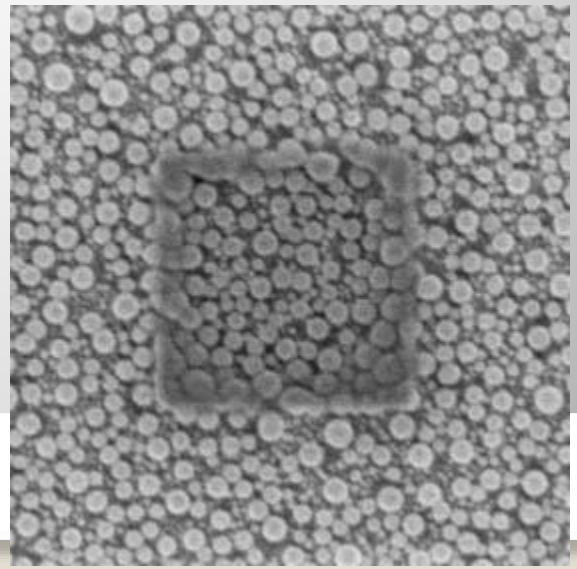
Specimen contamination remains one of the major SEM issues to be overcome.

Contamination results from many places:

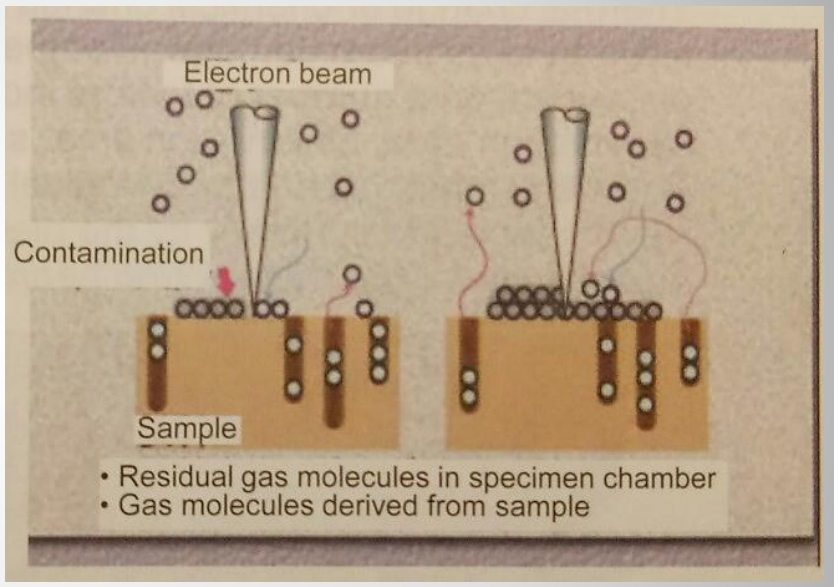
- Vacuum system
- Specimen surface
- Specimen handling
- Processing chemistry
- Specimen itself

Example of contaminated specimens



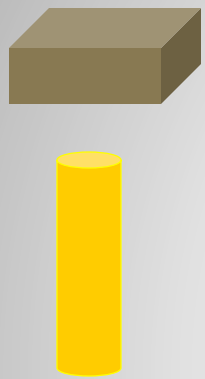


The phenomenon by which gas molecules of hydrocarbons existing around the specimen due to electron beam irradiation, then bond together and adhere to the specimen surface is referred to as contamination.
With the electron beam irradiating the sample, the clarity of the image at that area decreases and become darker due to the matter accumulated on the specimen surface suppresses the discharge of secondary electron from the specimen.

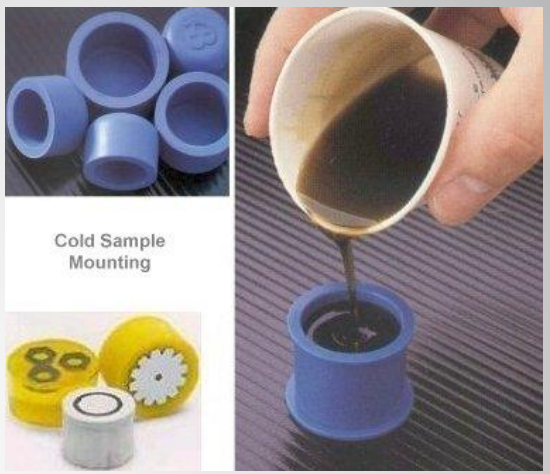


Contamination can be reduced by the following methods:
- Reduction of residual gas molecules in the specimen chamber
- Reduction of gas molecules derived from specimen.

Metals/alloys



Cutting machine



600, 800, 1000, 1500, 2000#

Emery papers

Alumina or diamond paste (30, 15, 5, 1, 0.1 μm)

Polishing grinder

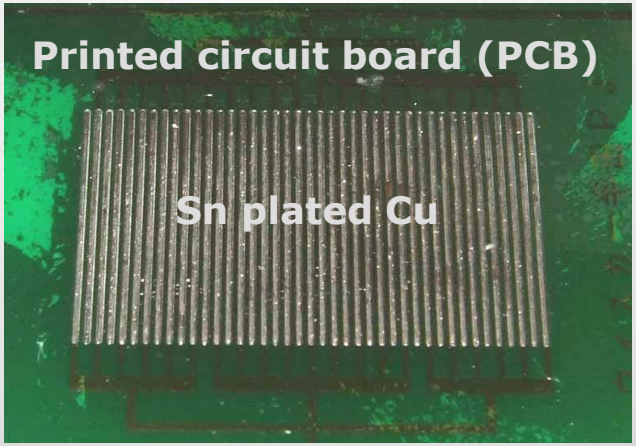
Etching

Flat
Mirror surface
Scratch free

CLEAN !!

Zeolite

Kenaf fibers



Plan-view of oxidized/corroded surface

General preparation for biological specimens

Such Specimens are usually observed in dried condition since the Interior of the electron microscope is evacuated.

Perfusion fixation
(Substitution of fixative solution with blood and glutsraldehyde, formalin, etc.

↓

Fine sectioning of specimen

↓

Immersion fixation (Glutaraldehyde, formalin etc)

↓

Post-fixation (Osmium tetraxide)

↓

Conductive staining (Tannic acid-osmium tetroxide etc.)

↓

Dehydration (Ethanol series, etc.)

↓

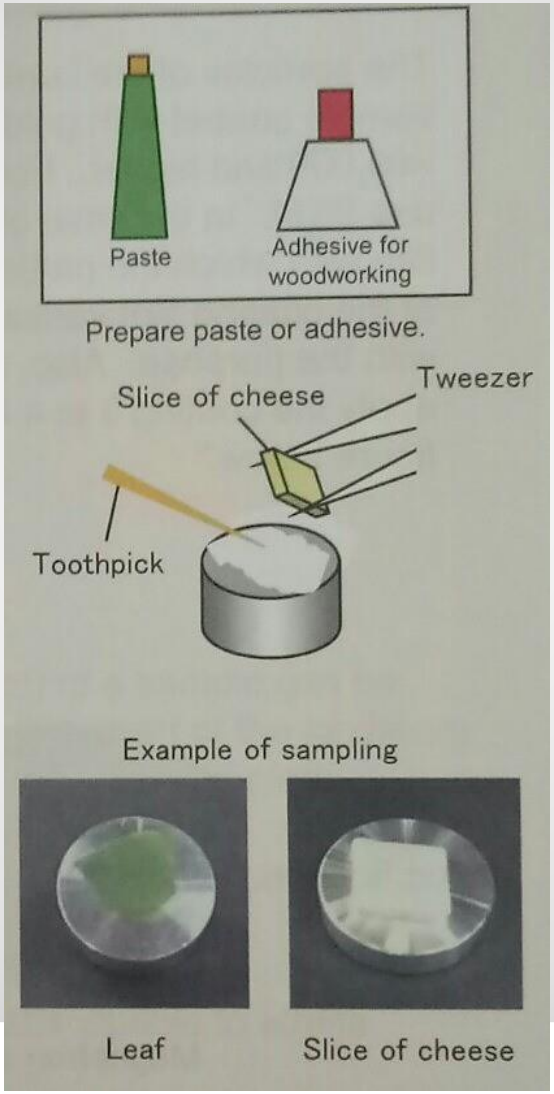
Drying (Critical point drying, freeze drying etc.)

↓

Observation with a SEM

Oily specimens

These are observed with a low vacuum SEM. In some cases, a cool stage may be used for cooling the sample for observation.



Apply a little paste or adhesive to the specimen stub and fix the sample (cut to a small size) onto this.

CHARACTERIZATION AND INTERPRETATION OF SEM AND SEM-EDS RESULTS

Some examples and discussion