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Electron Microscope

An electron microscope uses an ‘electron beam’ to produce the image of the object and magnification is obtained by ‘electromagnetic fields’; unlike light or optical microscopes, in which ‘light waves’ are used to produce the image and magnification is obtained by a system of ‘optical lenses’.

TEM

- an electron beam from an electron gun is transmitted through an ultra-thin section of the microscopic object and the image is magnified by the electromagnetic fields. It is used to observe finer details of internal structures of microscopic objects like bacteria and other cells.

TEM

- **In a conventional transmission electron microscope, a thin specimen is irradiated with an electron beam of uniform current density. Electrons are emitted from the electron gun and illuminate the specimen through a two or three stage condenser lens system. Objective lens provides the formation of either image or diffraction pattern of the specimen. The electron intensity distribution behind the specimen is magnified with a three or four stage lens system and viewed on a fluorescent screen. The image can be recorded by direct exposure of a photographic emulsion or an image plate or digitally by a CCD camera.**

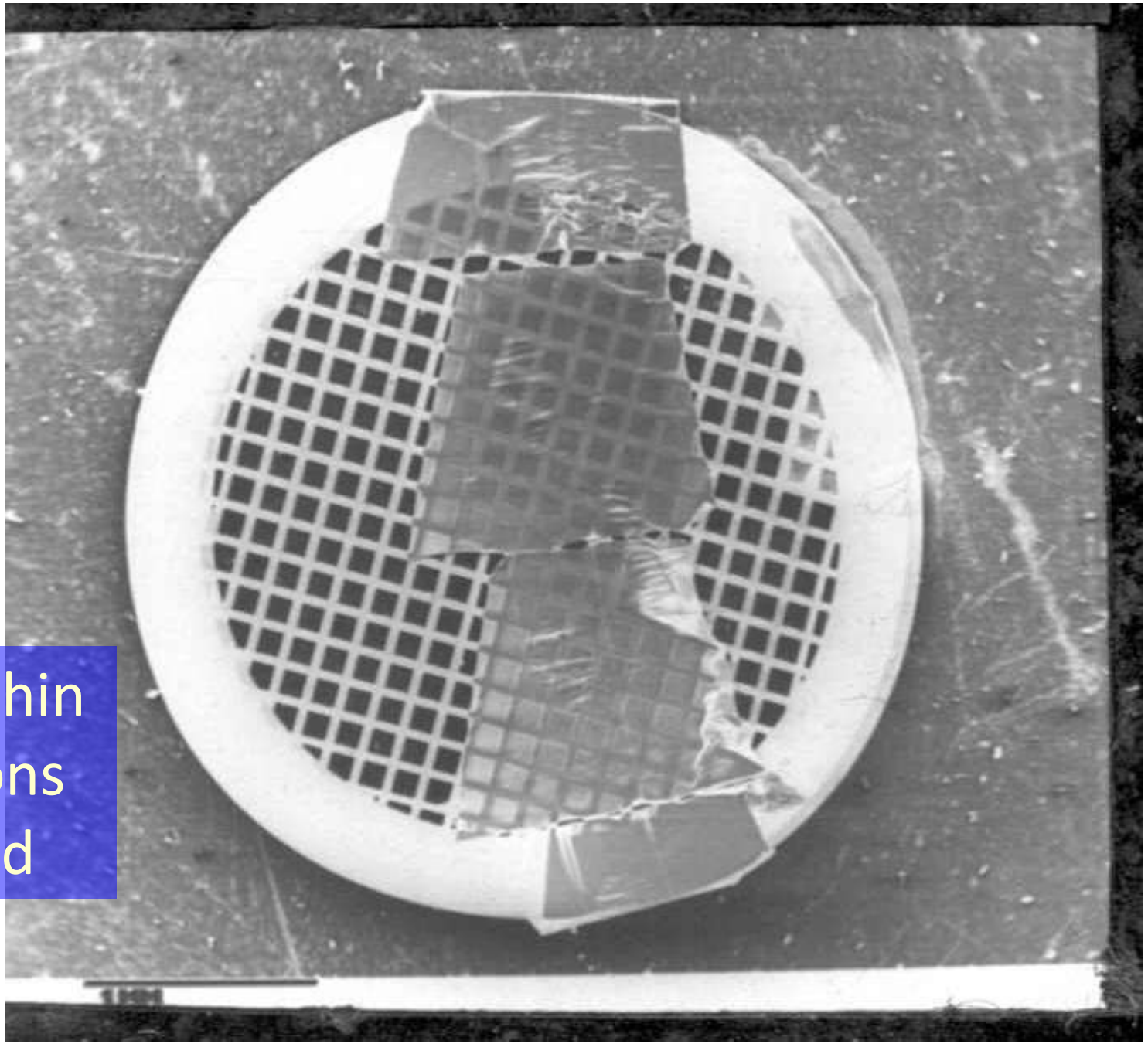
Dates

• The transmission electron microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931.

• The first scanning electron microscope (SEM) debuted in 1938 (Von Ardenne) with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample.

Illumination - Source is a beam of high velocity electrons accelerated under vacuum, focused by condenser lens (electromagnetic bending of electron beam) onto specimen.
Image formation - Loss and scattering of electrons by individual parts of the specimen. Emergent electron beam is focused by objective lens. Final image forms on a fluorescent screen for viewing.

Must be prompt
Cut to 1-2 mm cubes
Use sharp razor blade, avoid crushing
2.5% glutaraldehyde for 4 to 12 hours
Postfixation in 1% osmium tetroxide



Ultrathin sections on grid

The TEM consists of the following major parts:

1. The illumination system

   Electron gun

   Condensers

2. The image forming system

   Objective lens

3. The projective system

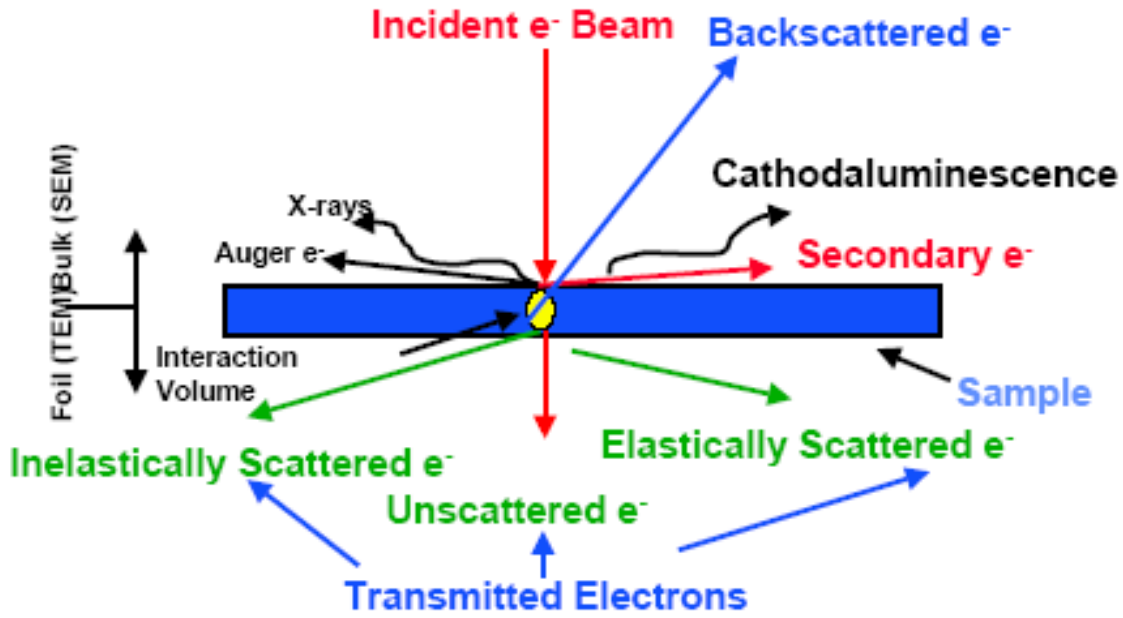
   Several projector lens

4. Apertures

   afftect the formation of images and diffraction patterns

Electron-Solid Interactions

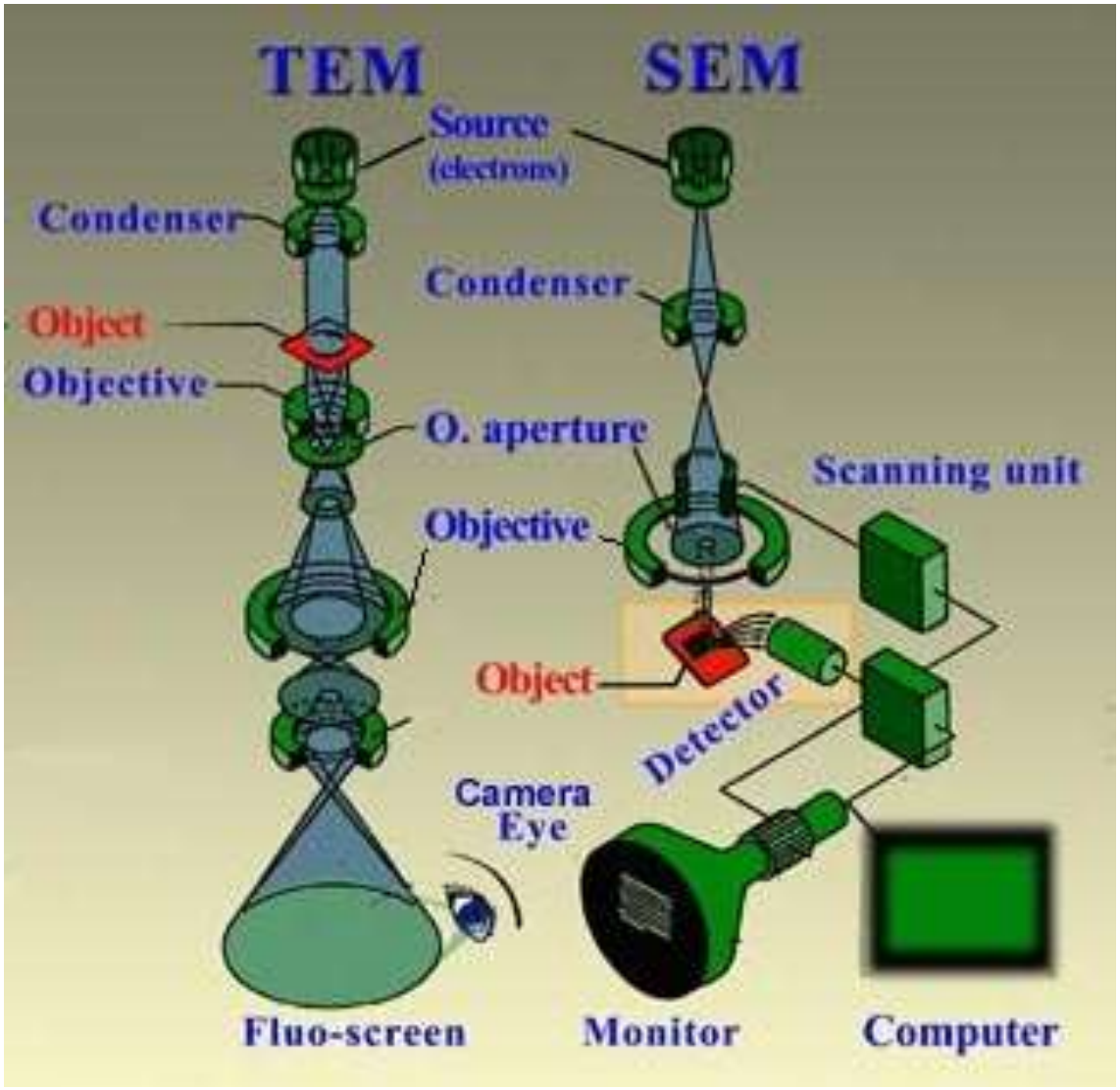
When an electron beam strikes a sample, a large number of signals are generated.



We can divide the signals into two *broad* categories:

a) electron signals, b) photon signals

(SEM) and TEM


TEM
SEM
Source
(electrons)
Condenser
Condenser
Object
Objective
O. aperture
Scanning unit
Objective
Object
Detector
Camera
Eye
Fluo-screen
Monitor
Computer

Scanning Electron Microscopy (SEM)

Scanning electron microscopy is used for inspecting topographies of specimens at very high magnifications using a piece of equipment called the scanning electron microscope. SEM magnifications can go to more than 300,000 X but most semiconductor manufacturing applications require magnifications of less than 3,000 X only.
During SEM inspection, a beam of electrons is focused on a spot volume of the specimen, resulting in the transfer of energy to the spot. These bombarding electrons, also referred to as primary electrons, dislodge electrons from the specimen itself. The dislodged electrons, also known as secondary electrons, are attracted and collected by a positively biased grid or detector, and then translated into a signal.

To produce the SEM image, the electron beam is swept across the area being inspected, producing many such signals. These signals are then amplified, analyzed, and translated into images of the topography being inspected.

JEOL 6700F Ultra High Resolution Scanning Electron Microscope

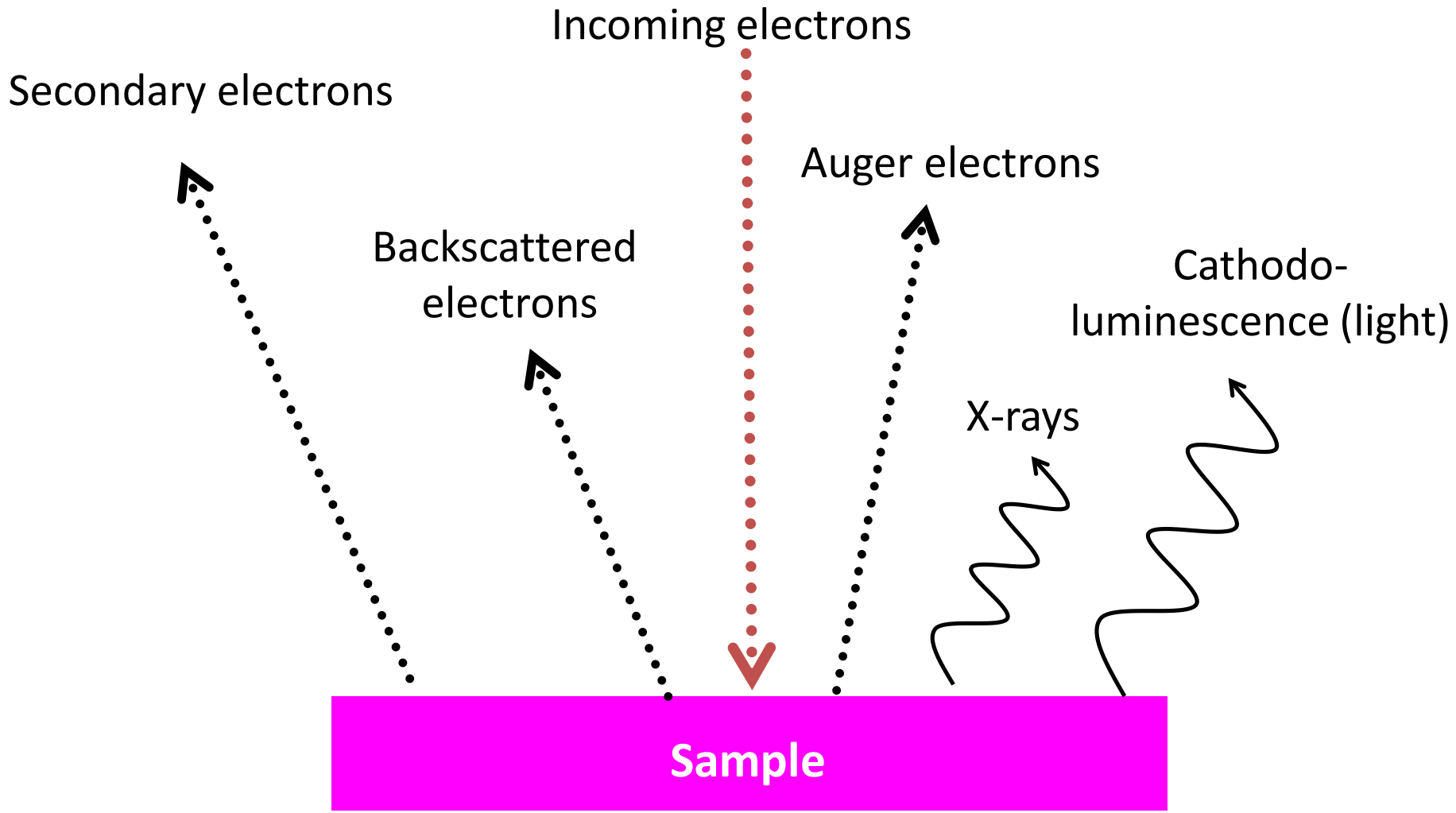
- It is a microscope that produces an image by using an electron beam that scans the surface of a specimen inside a vacuum chamber.

- The SEM uses electrons instead of light to form an image.
- A beam of electrons is produced at the top of the microscope by heating of a metallic filament.
- The electron beam follows a vertical path through the column of the microscope. It makes its way through electromagnetic lenses which focus and direct the beam down towards the sample.

The electrons interact with atoms in the sample, producing various signals that can be detected

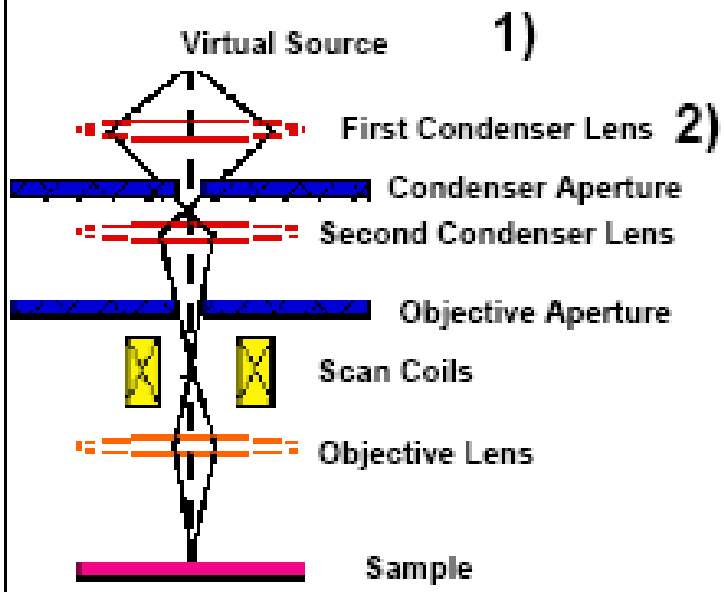
- Once it hits the sample, other electrons (backscattered or secondary) are ejected from the sample. Detectors collect the secondary or backscattered electrons, and convert them to a signal that is sent to a viewing screen similar to the one in an ordinary television, producing an image.

Signals from the sample



- the specimen is exposed to a narrow electron beam from an electron gun, which rapidly moves over or scans the surface of the specimen (Figure 4.13). This causes the release of a shower of secondary electrons and other types of radiations from the specimen surface.
- The intensity of these secondary electrons depends upon the shape and the chemical composition of the irradiated object. These electrons are collected by a detector, which generates electronic signals. These signals are scanned in the manner of a television system to produce an image on a cathode ray tube (CRT).

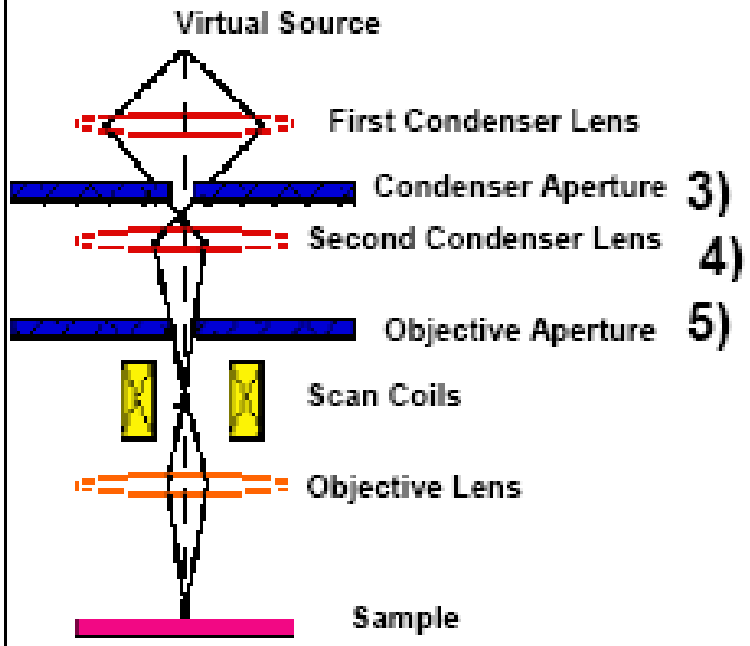
Scanning Electron Microscope



1) The "Virtual Source" at the top represents the electron gun, producing a stream of monochromatic electrons.

2) The stream is condensed by the first condenser lens (usually controlled by the "coarse probe current knob"). This lens is used to both form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture to eliminate the high-angle electrons from the beam.

Scanning Electron Microscope

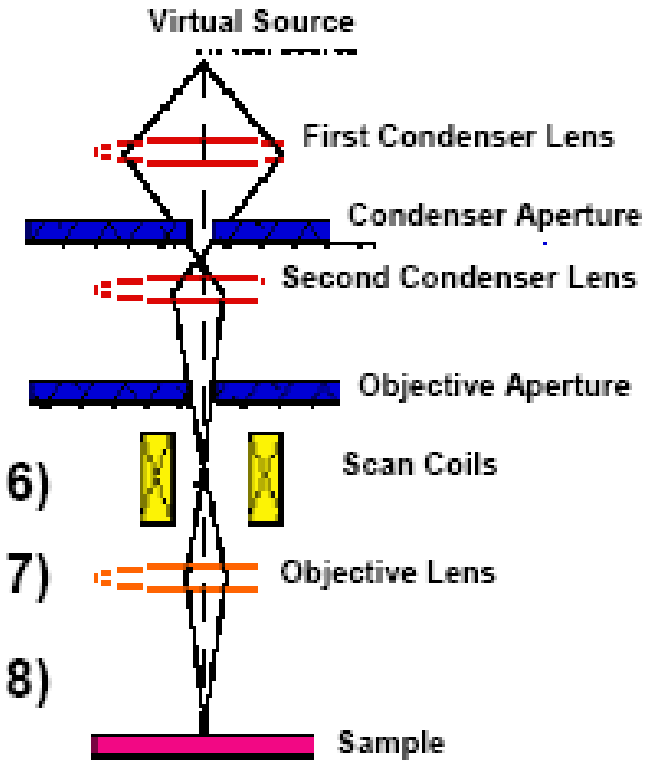


3) The beam is then constricted by the condenser aperture (usually not user selectable), eliminating some high-angle electrons.

4) The second condenser lens forms the electrons into a thin, tight, coherent beam and is usually controlled by the "fine probe current knob".

5) A user selectable objective aperture further eliminates high-angle electrons from the beam.

Scanning Electron Microscope

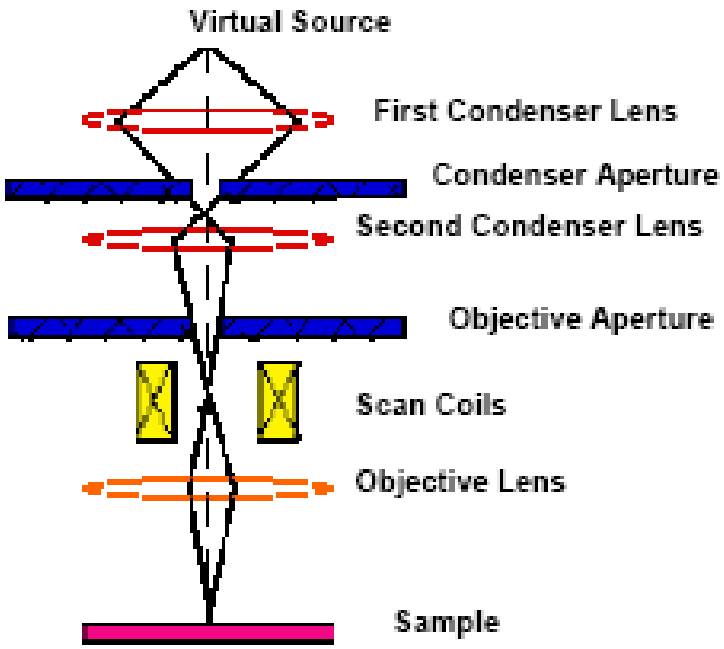


6) A set of coils then "scan" or "sweep" the beam in a grid fashion (like a television), dwelling on points for a period of time determined by the scan speed (usually in the microsecond range).

7) The final lens, the objective, focuses the scanning beam onto the part of the specimen desired.

8) When the beam strikes the sample (and dwells for a few microseconds) interactions occur inside the sample and are detected with various instruments.

Scanning Electron Microscope



9) Before the beam moves to its next dwell point these instruments count the number of e^- interactions and display a pixel on a CRT whose intensity is determined by this number (the more reactions the brighter the pixel).

10) This process is repeated until the grid scan is finished and then repeated, the entire pattern can be scanned 30 times/sec.

Scanning Electron Microscopy (SEM)

- The energy of the primary electrons determines the quantity of secondary electrons collected during inspection. The emission of secondary electrons from the specimen increases as the energy of the primary electron beam increases, until a certain limit is reached. Beyond this limit, the collected secondary electrons diminish as the energy of the primary beam is increased, because the primary beam is already activating electrons deep below the surface of the specimen. Electrons coming from such depths usually recombine before reaching the surface for emission.

- The primary electron beam results in the emission of backscattered (or reflected) electrons from the specimen. Backscattered electrons possess more energy than secondary electrons, and have a definite direction. As such, they can not be collected by a secondary electron detector, unless the detector is directly in their path of travel. All emissions above 50 eV are considered to be backscattered electrons.

Scanning Electron Microscopy (SEM)

- Backscattered electron imaging is useful in distinguishing one material from another, since the yield of the collected backscattered electrons increases monotonically with the specimen's atomic number. Backscatter imaging can distinguish elements with atomic number differences of at least 3, i.e., materials with atomic number differences of at least 3 would appear with good contrast on the image.

- A SEM may be equipped with an EDX analysis system to enable it to perform compositional analysis on specimens. EDX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen.

1.1 Characteristic Information: SEM

Topography

The surface features of an object or "how it looks", its texture; direct relation between these features and materials properties

Morphology

The shape and size of the particles making up the object; direct relation between these structures and materials properties

Composition

The elements and compounds that the object is composed of and the relative amounts of them; direct relationship between composition and materials properties

Crystallographic Information

How the atoms are arranged in the object; direct relation between these arrangements and material properties

- **high magnification at high resolution**
- technique largely standardized
- some ultrastructural features are highly specific for certain cell types or diseases

- equipment expensive
- procedures time consuming (staff costly)
- small samples lead to possible sampling error and misinterpretation
- optimum tissue preservation requires special fixative and processing
- much experience is needed for interpreting the results