ELECTRON MICROSCOPY

Electrons and the Electromagnetic/Electrostatic lenses

Electron Microscope (EM)

- The overall design of an electron microscope is similar to that of a light microscope
- In the electron microscope, the light is substituted with electrons and the glass lenses are substituted with electromagnetic/electrostatic lenses
- So, Electron microscope uses a beam of accelerated electrons as a source of illumination
- It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen
- Ernst Ruska (1906-1988), a German engineer and academic professor, built the first Electron Microscope in 1931, and the same principles behind his prototype still govern modern EMs

Principle

- Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition
- The electron gun generates electrons
- Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam
- To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between tungsten filament and anode
- The specimen to be examined is made extremely thin, at least 200 times thinner (20-100 nm) than those used in the optical microscope
- The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen
- The denser regions in the specimen scatter more electrons and therefore appear darker in the image in contrast, transparent regions are brighter
- The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image
- The ocular lenses then produce the final further magnified image

Parts of EM

EM is in the form of a tall vacuum column which is vertically mounted

It has the following components:

1. **Electron gun**

- The electron gun is a heated tungsten filament, which generates electrons

2. **Electromagnetic lenses**

- Condenser lens focuses the electron beam on the specimen
- A second condenser lens forms the electrons into a thin tight beam
- The electron beam coming out of the specimen passes down the second of magnetic coils called the objective lens, which has high power and forms the intermediate magnified image
- The third set of magnetic lenses called projector (ocular) lenses produce the final further magnified image
- Each of these lenses acts as an image magnifier all the while maintaining an incredible level of detail and resolution

3. Specimen Holder

- The specimen holder is an extremely thin film of carbon held by a metal grid

4. Image viewing and Recording System

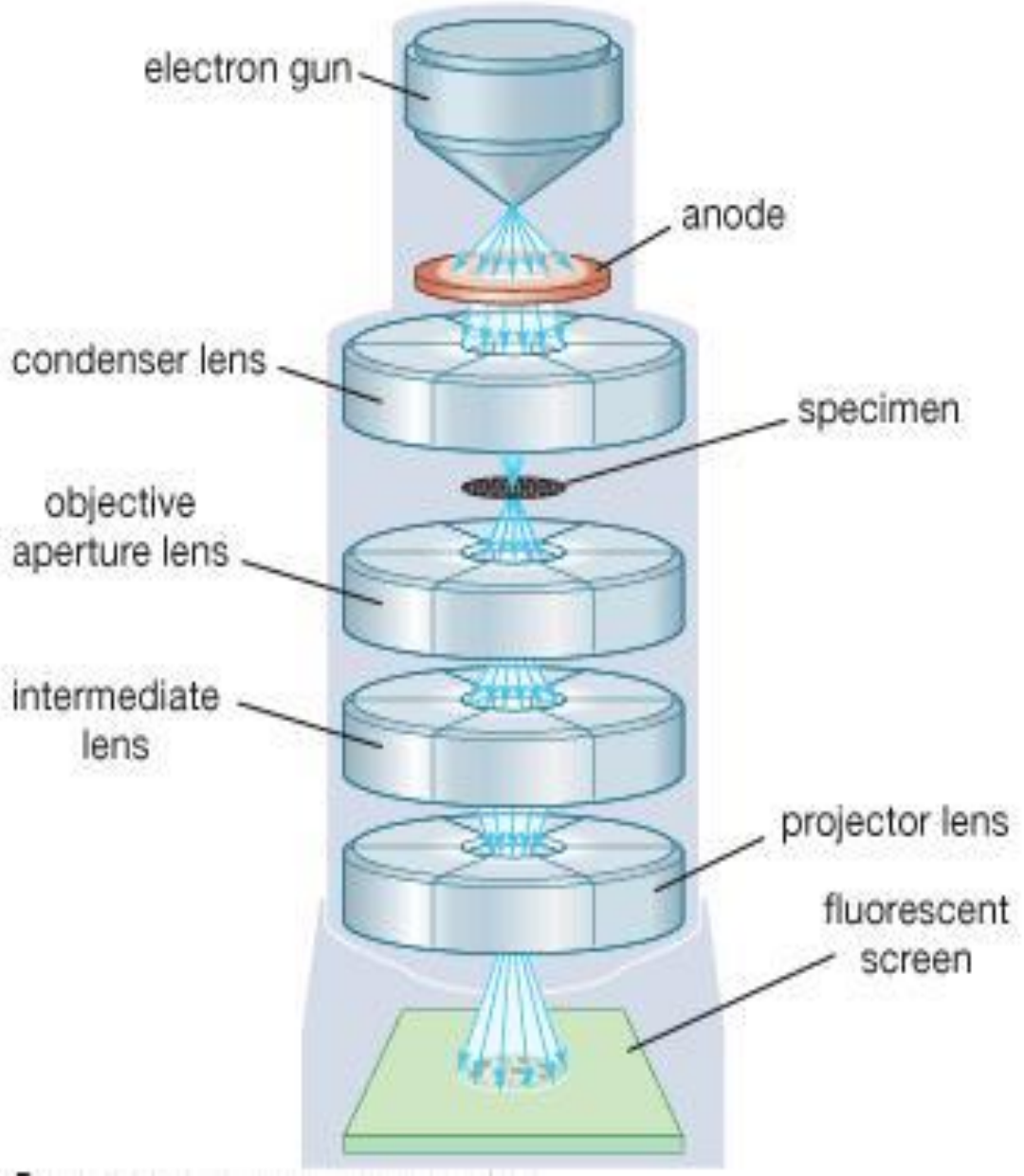
- The final image is projected on a fluorescent screen
- Below the fluorescent screen is a camera for recording the image

Types of Electron Microscope

There are two types of electron microscopes, with different operating styles:

1. The Transmission Electron Microscope (TEM)

- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image
- The TEM is analogous in many ways to the conventional (compound) light microscope
- TEM is used, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture)

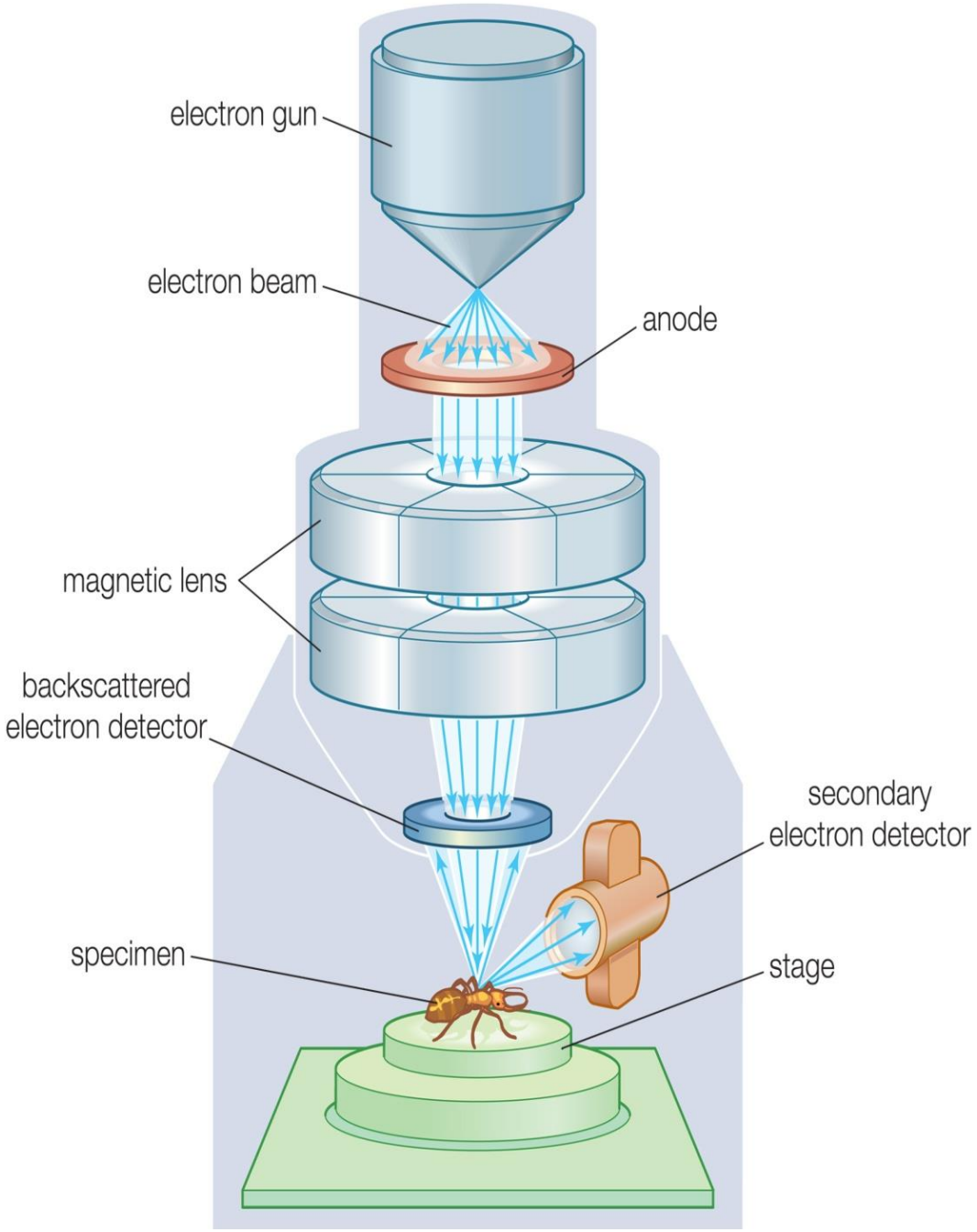


Transmission Electron Microscope (TEM)

- Gives excellent view of internal structures.
  - Magnification: 100,000 X or more
  - Resolving power: 2.5 nm or better
  - Two dimensional image
- Drawbacks of TEM:
  - Due to limited penetrating power of electrons, can only view very thin slices (70-90 nm) of specimen
  - Must slice, fix, dehydrate, and view specimen under a vacuum
  - Staining may be used to enhance image contrast
  - Treatments kill specimen and may cause shrinkage and distortion of cells

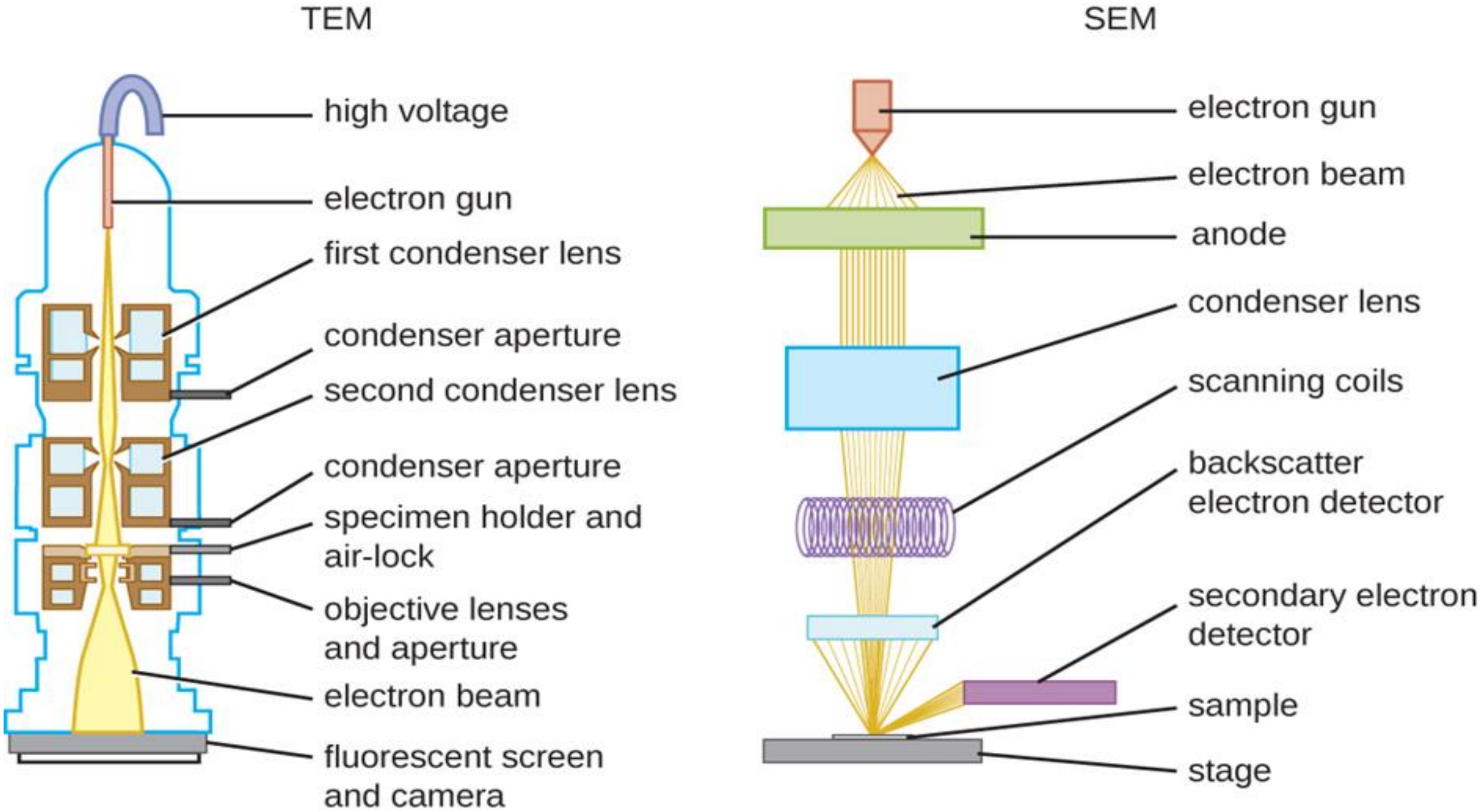
2. Scanning Electron Microscope (SEM)

- Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specime
- It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control
- It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern



Scanning Electron Microscope (SEM)

- Gives excellent view of external surface
- Magnification: 10,000 X or more
- Resolving power: 20 nm or better
- Three dimensional image
- More recent invention than TEM
- Used mainly to observe the surfaces of cells and viruses
- Specimens are covered with a layer of heavy metal (gold or palladium)
- A narrow beam of electrons (primary electron beam) is swept across specimen surface
- Electrons on the specimen surface are knocked out, creating a secondary electron beam which is collected and amplified to produce an image


TEM
high voltage
electron gun
first condenser lens
condenser aperture
second condenser lens
condenser aperture
specimen holder and
air-lock
objective lenses
and aperture
electron beam
fluorescent screen
and camera
SEM
electron gun
electron beam
anode
condenser lens
scanning coils
backscatter
electron detector
secondary electron
detector
sample
stage

Applications of EM

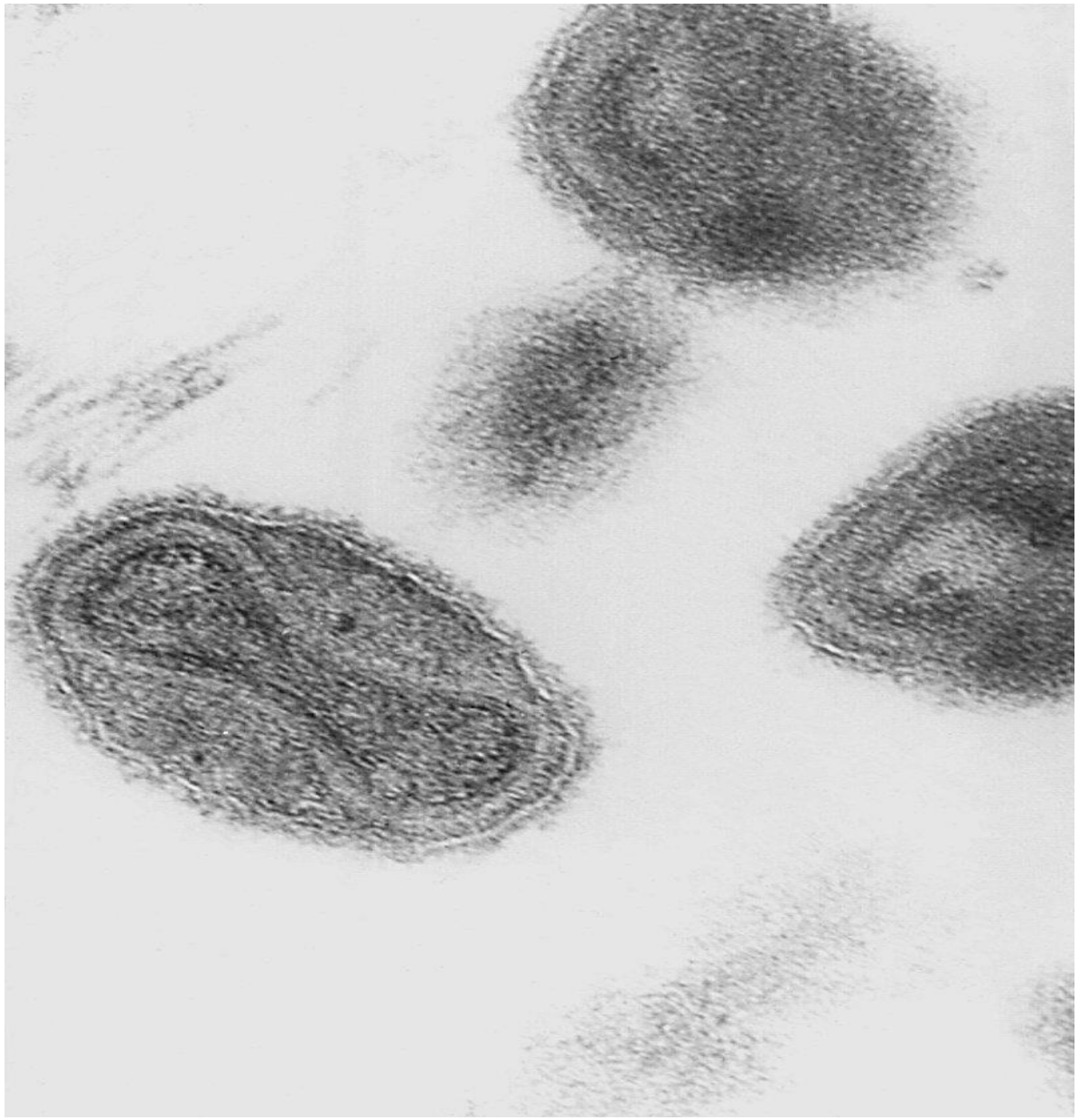
- Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals
- Industrially, electron microscopes are often used for quality control and failure analysis
- Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the images
- Science of microbiology owes its development to the electron microscope, study of microorganisms like bacteria, virus and other pathogens have made the treatment of diseases very effective

Advantages and Limitations

- Very high magnification
- Incredibly high resolution
- Material rarely distorted by preparation
- It is possible to investigate a greater depth of field
- Diverse applications

- The live specimen cannot be observed
- As the penetration power of the electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation
- As the EM works in a vacuum, the specimen should be completely dry
- Expensive to build and maintain
- Requiring training for handling
- This type of microscope is a large, cumbersome extremely sensitive to vibration and external magnetic fields

TEM Image of Small Pox Virus



SEM Image of soil Bacteria