

# ELECTRON MICROSCOPY

---

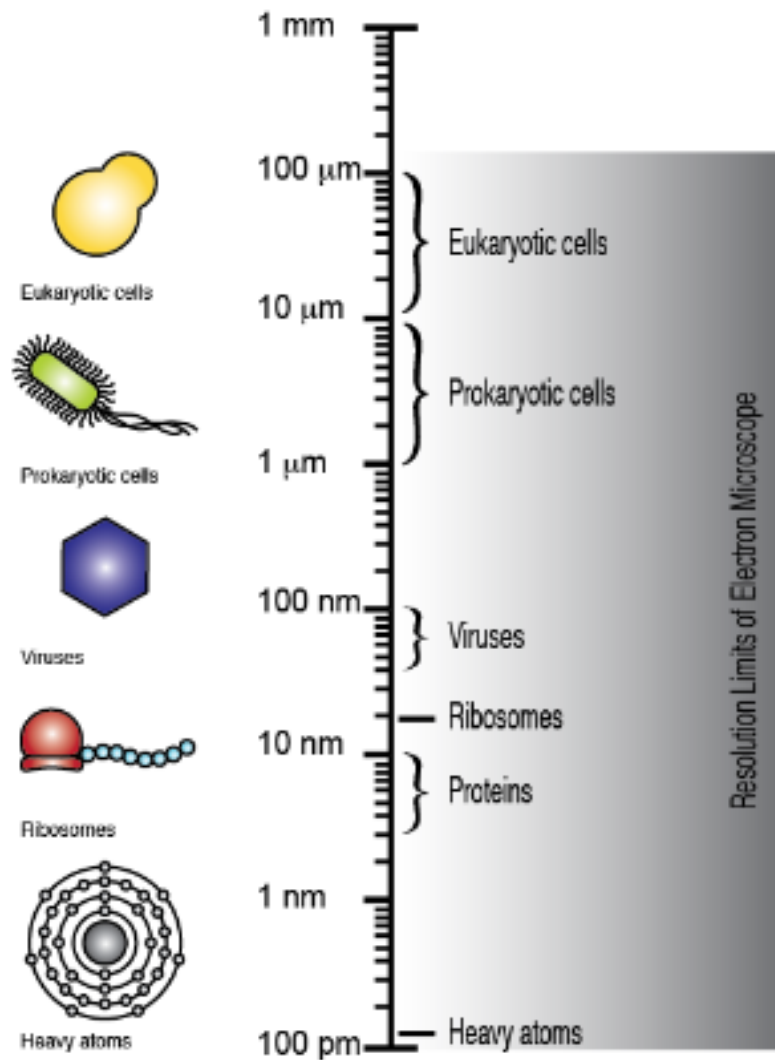
Microbiology – I

BVOC- HCT - 1 semester

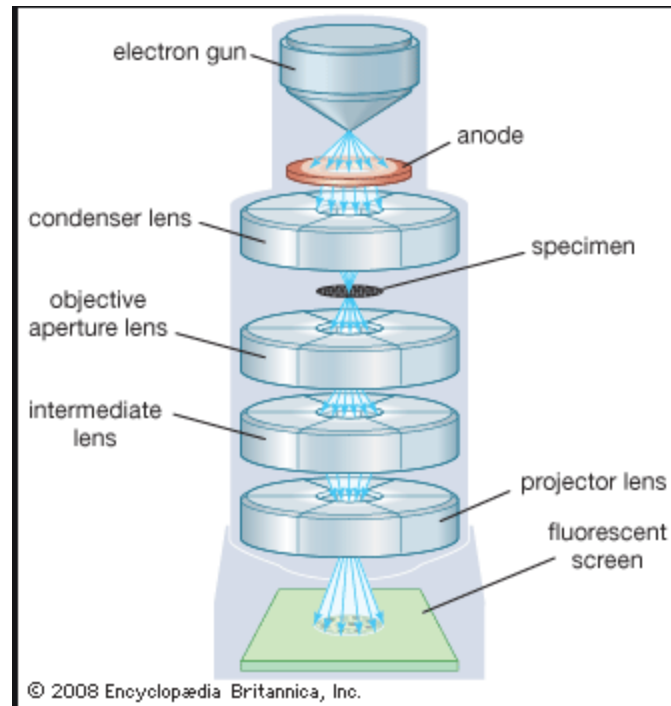
# Electron Microscope

- It uses a beam of accelerated electrons – illumination source .
- Higher resolving power of 0.5 nm – detailed structure of smaller objects - 1  $\mu\text{m}$  bacteria.
- Magnification: 100,000X larger.
- Wavelength of 0.005 nm - 100,000X shorter – visible light
  
- It uses shaped magnetic fields to form electron optical lens systems – as in glass lenses of an optical light microscope.

# Resolution limits of electron microscope

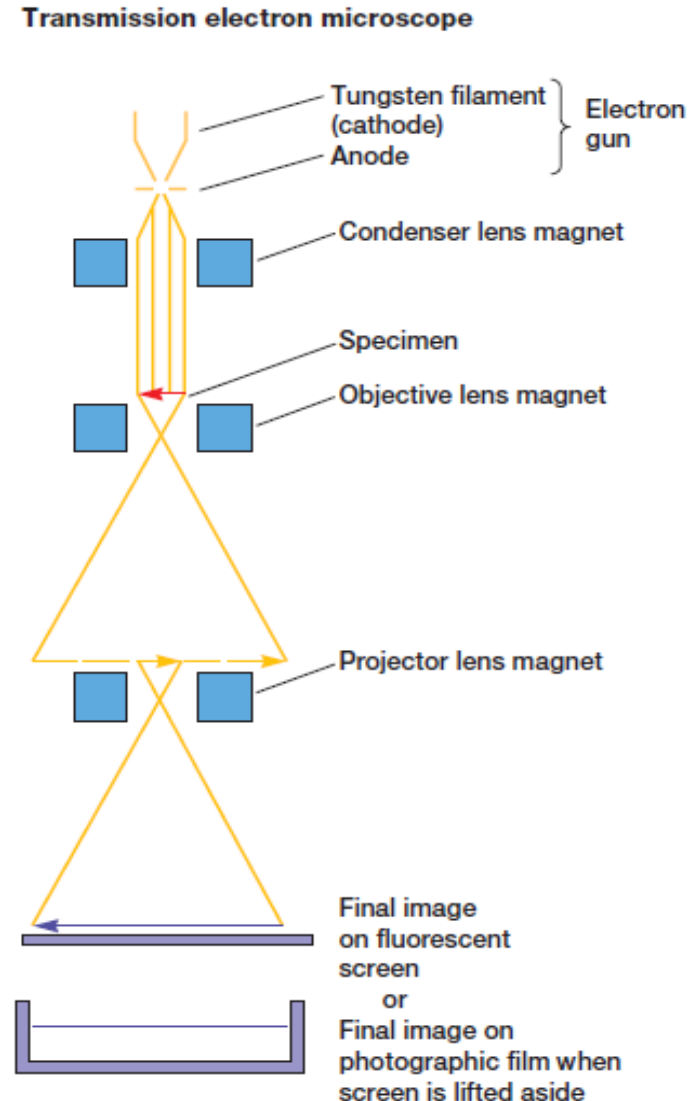


# Transmission electron microscope (TEM)



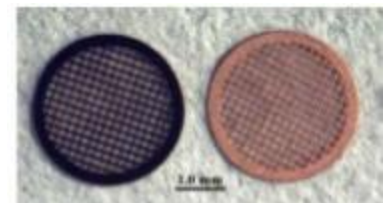
# Transmission electron microscope (TEM)

- Heated tungsten filament produces beam of electrons
- Electrons are focused on the specimen by condenser
- Beam is focused by magnetic lenses
- Vacuum in the chamber obtain a clear image
- Electron falls on the specimen scatters electrons and focused by magnetic lenses.
- The image formed is enlarged, visible image on the fluorescent screen
- Dense regions of the specimen appear dark and electron-transparent regions are brighter
- Image is captured on the photographic film as a permanent record.



# Specimen Preparation for TEM

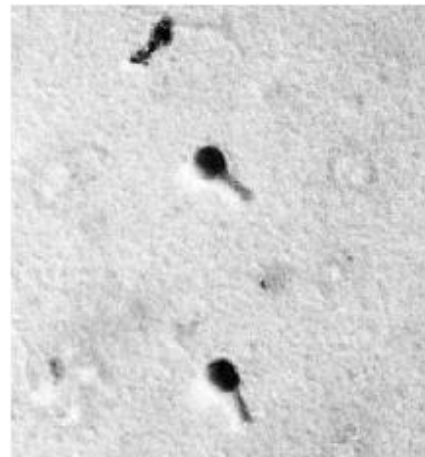
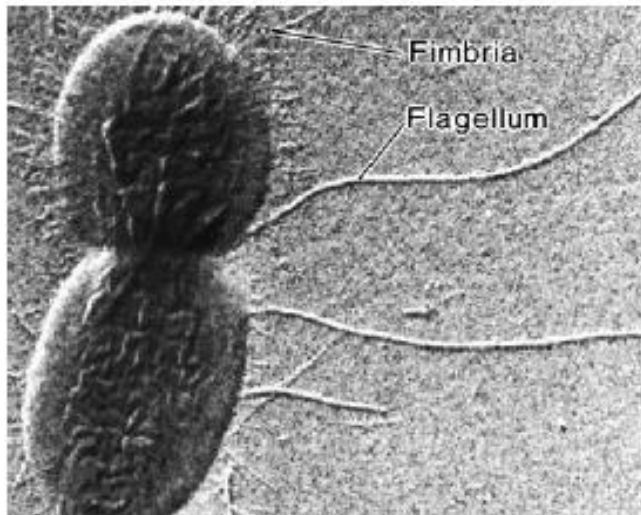
- **Samples thickness:** microbial specimen extremely thin slices - 20 -100 nm thick. It is embedded on a plastic support.
- **Staining:** Specimens are soaked in solutions of heavy metal salts like lead citrate and uranyl acetate.
- **Fixation:** glutaraldehyde or osmium tetroxide to stabilize cell structure.
- **Dehydration:** organic solvents e.g., acetone or ethanol.
- **Embedding in resin:** Specimen is soaked in unpolymerized, liquid epoxy plastic until it is completely permeated, the plastic is hardened to form a solid block.
- **Trimming /ultrathin sectioning:** Thin sections are cut from this block with a glass or diamond knife using an ultramicrotome.
- **Mounting:** specimens mounted on tiny copper grids and viewed.



Copper grids

## Staining methods for sample observation:

- **1. Negative staining:** using heavy metals gives background dark, the specimen appears bright in photographs. Ex. viruses, bacterial gas vacuoles.
- **2. Shadowing:** coated with a thin film of platinum or other heavy metal on one side.
- The area coated with metal scatters electrons and appears light in photographs, the shadow region created by the object is dark.

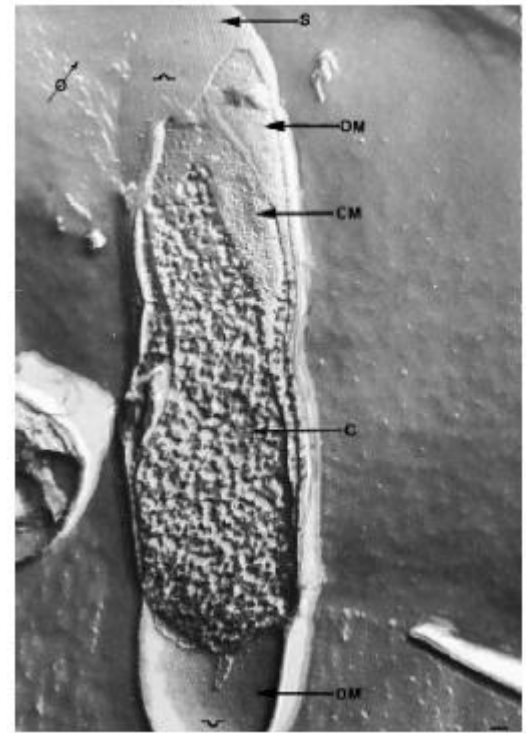
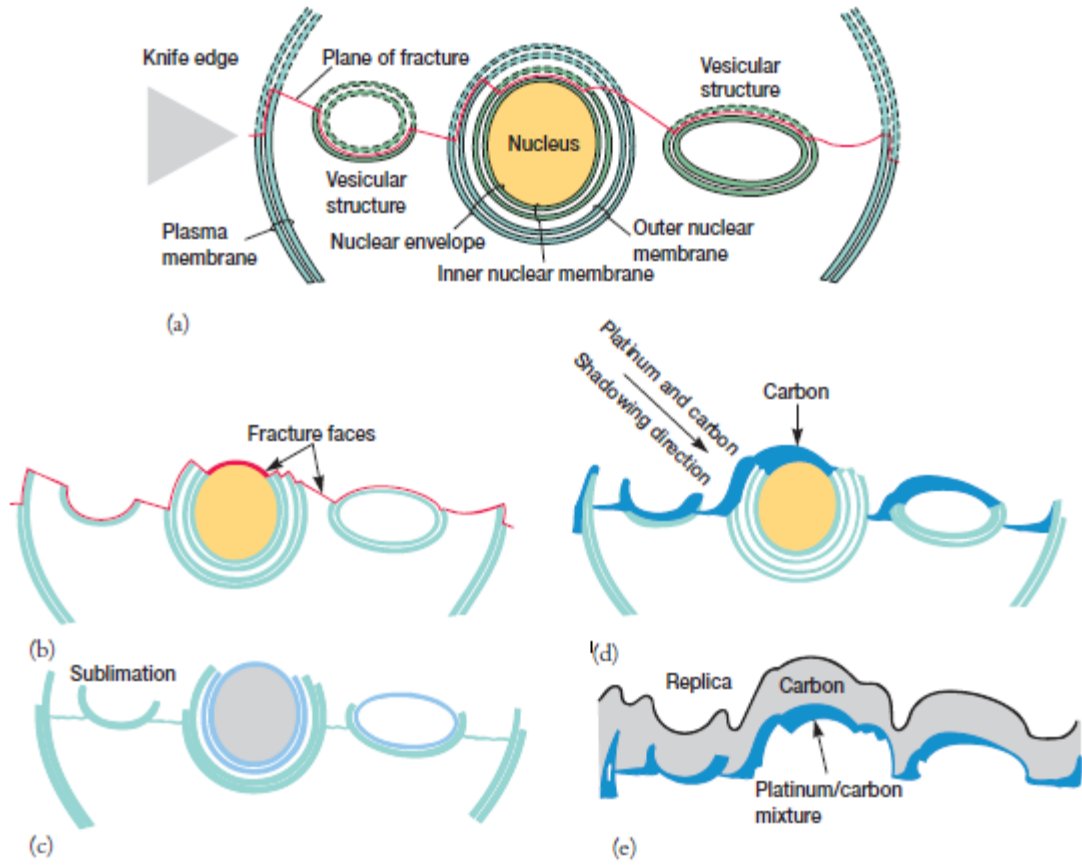


Specimen shadowing

## Freeze-etching:

- To observe shape of organelles within microorganisms.
- Cells are rapidly frozen in **liquid nitrogen** and then warmed to  $-100^{\circ}\text{C}$  in a vacuum chamber.
- Knife – precooled with liquid nitrogen ( $-196^{\circ}\text{C}$ ) fractures the frozen cells, brittle which easily break.
- Specimen are placed under high vacuum for 1 min, ice sublimates.
- Exposed surfaces are shadowed and coated with layers of platinum and carbon to form a replica of the surface.
- Specimen is chemically removed - replica is studied TEM
- Image: detailed, three-dimensional view of intracellular structure
  
- **Advantage:** it minimizes artifacts because the cells are frozen quickly rather than being subjected to chemical fixation, dehydration, and
- plastic embedding.





TEM image

The Freeze-Etching Technique.

## Advantages & Disadvantages of TEM

### **Advantages**

- TEMs offer very powerful magnification and resolution.
- TEMs have a wide-range of applications and can be utilized in a variety of different scientific, educational and industrial fields
- TEMs provide information on element and compound structure .
- Images are high-quality and detailed.

### **Disadvantages**

- TEMs are large and very expensive.
  - Laborious sample preparation.
  - Operation and analysis requires special training.
  - Samples are limited to those that are electron transparent.
  - TEMs require special housing and maintenance.
  - Images are black and white .
-

## Applications of TEM

- In medicine as a **diagnostic tool** – important in renal biopsies.
  - **Cellular tomography**
    - Tomography refers to imaging by sectioning, through the use of any kind of penetrating wave.
    - Information is collected and used to assemble a three dimensional image of the target.
    - Used for obtaining detailed 3D structures of subcellular macromolecular objects.
  - **Cancer research** - studies of tumor cell ultrastructure .
  - **Toxicology** – to study the impacts of environmental pollution on the different levels of biological organization.
-