Electron Microscopy

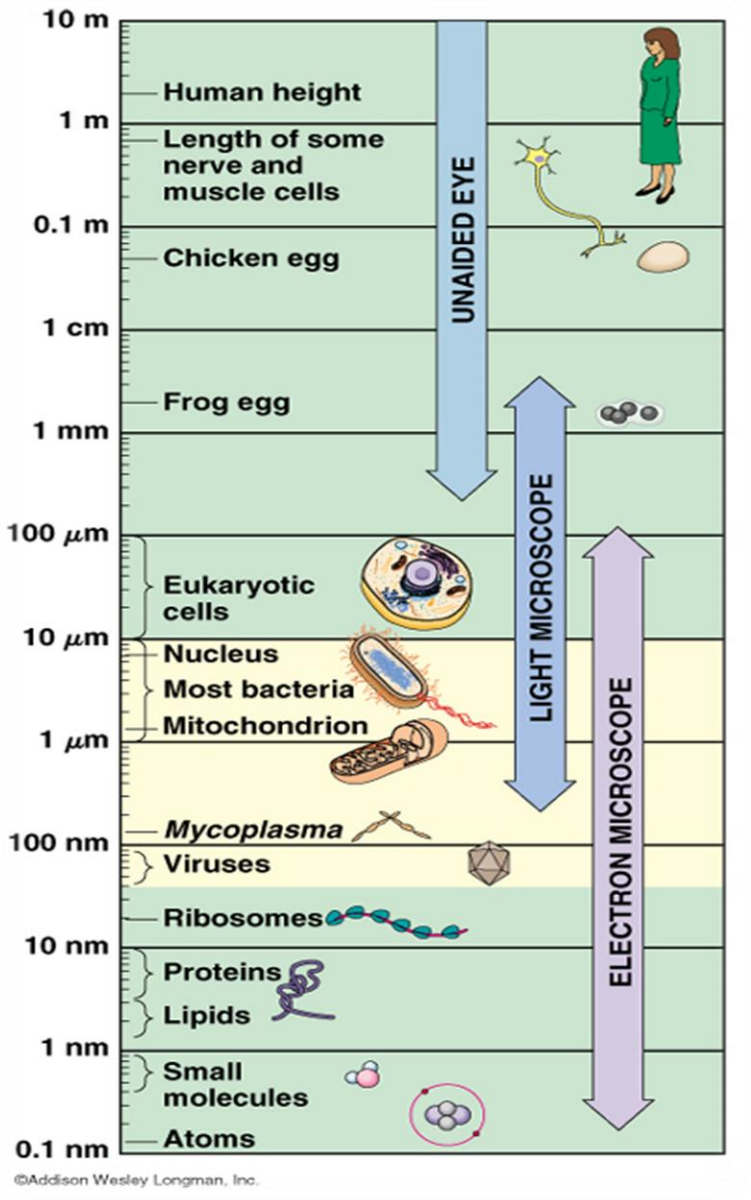
What is Electron Microscopy?

- Electron microscopy offers unique possibilities to gain insights into
  - **structure,**
  - **topology,**
  - **morphology, and**
  - **composition of a material.**

What is an Electron Microscope ?

- A special type of microscope having a high resolution of images, able to objects in nanometres

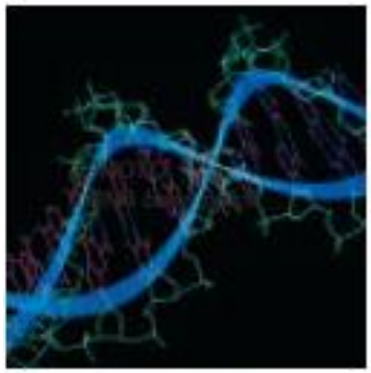
Why were the EMs advented?



- To study objects of < 0.2 micrometer
- For analysis of sub cellular structures
- Intra cellular pathogens - viruses
- Cell metabolism
- Study of minute structures in the nature

Greater resolving power of the EMs than light microscope

- An EM can magnify structures from **100 – 250000 times than light microscopy**



Nanometers

(b)

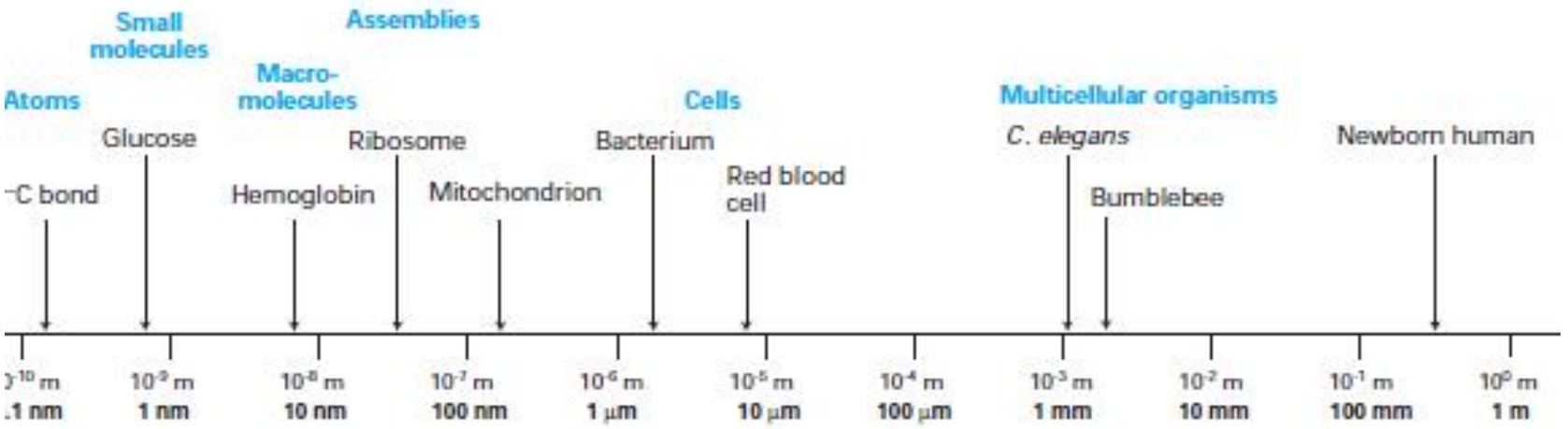
Micrometers

(c)

Millimeters

(d)

Meters



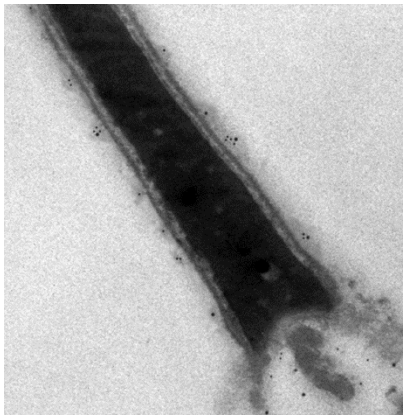
The novelty of EMs from others

- **Beam of Electrons** …… instead of a beam of light

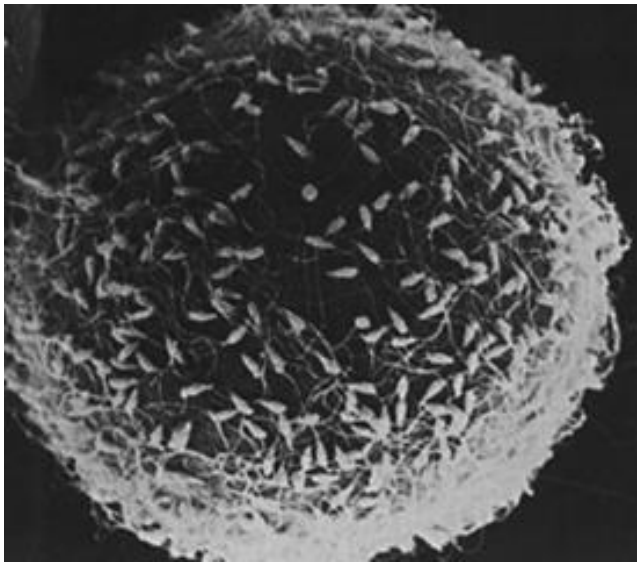
- **Cylindrical Vacuum column** - Electrons should travel in vacuum to avoid collisions with air molecules that cause scattering of electrons distorting the image

TYPES OF Electron Microscopy

- **Transmission Electron Microscopy (TEM)**



- **Scanning Electron Microscopy (SEM)**



Transmission Electron Microscopy

The first TEM was built by Max Knoll and Ernst Ruska in 1931, with this group developing the first TEM with resolving power greater than that of light in 1933 and the first commercial TEM in 1939.

TEM - Definition

TEM is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through.

An image is formed from the interaction of the electrons transmitted through the specimen;

the image is magnified and focused onto an imaging device, such as a fluorescent screen detected by a CCD camera.

Applications

- TEMs are capable of imaging at a significantly higher resolution than light microscopes.
- to examine fine detail.
- application in Biological sciences like cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

Limitations of TEM

- Many materials require extensive sample preparation
- Difficult to produce a very thin sample
- relatively time consuming process with a low throughput of samples.
- The structure of the sample may change during the preparation process.
- Small field of view may not give conclusive result of the whole sample.

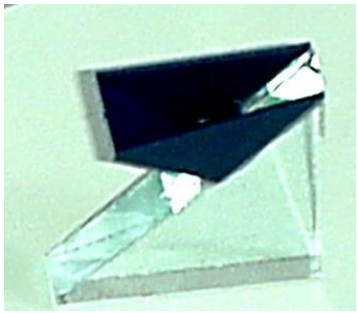
Biological Sample preparation

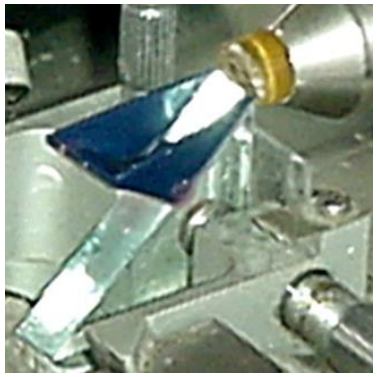
- Sample thickness – less than 1 mm^3
- Rapid fixation with least cell damage – Gluteraldehyde and Osmium tetroxide
  - **Gluteraldehyde** has aldehyde groups which bonds with amino groups of proteins, forming insoluble complexes
  - **OsO_4** binds to cell membranes containing fatty acids
- Dehydration – alcohol series - transition solvent" such as propylene oxide
- Embedding – Epoxy resins (Epon or araldite)
  - Acrylic resins (London Resin White-LRW)
- Section – 0.1 micrometer using ultramicrotome

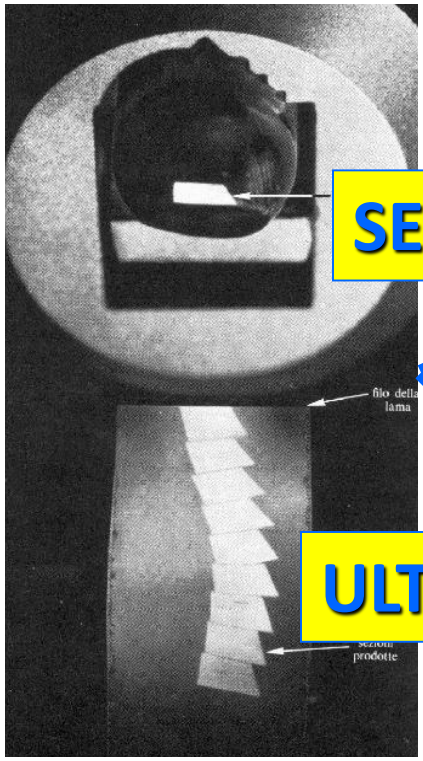
(Ideal – 70-90 nm thickness)

Staining – Uranyl acetate , Lead citrate

ULTRAMICROTOME



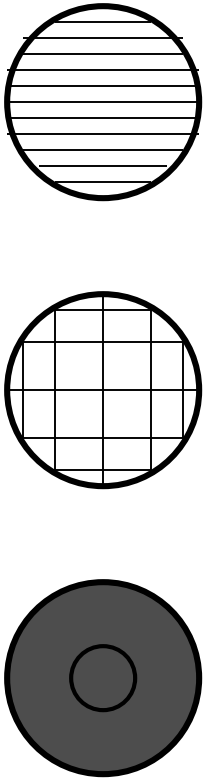


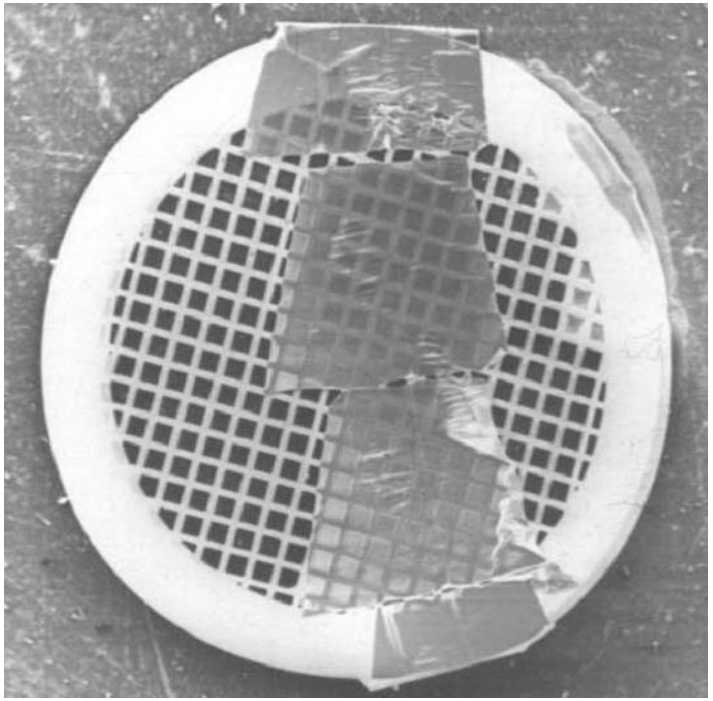


SEMITHIN

ULTRATHIN

METAL GRIDS

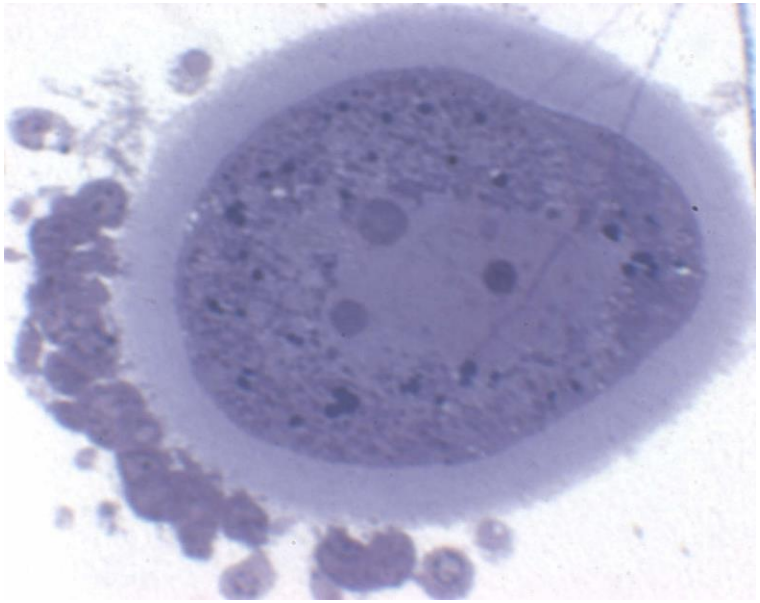




Semithin sections stain

↓

Toluidine Blue



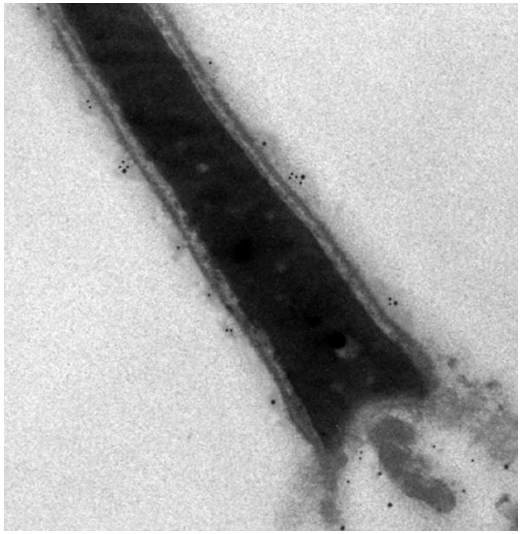
Ultrathin section contrast

↓

uranyl acetate

+

lead citrate



Definition of SEM

- An electron microscope that produces images of a sample by scanning over it with a focused beam of electrons.
- The incident electrons interact with electrons in the sample, producing various signals that can be detected and
- contain information about the sample's surface topography and composition.

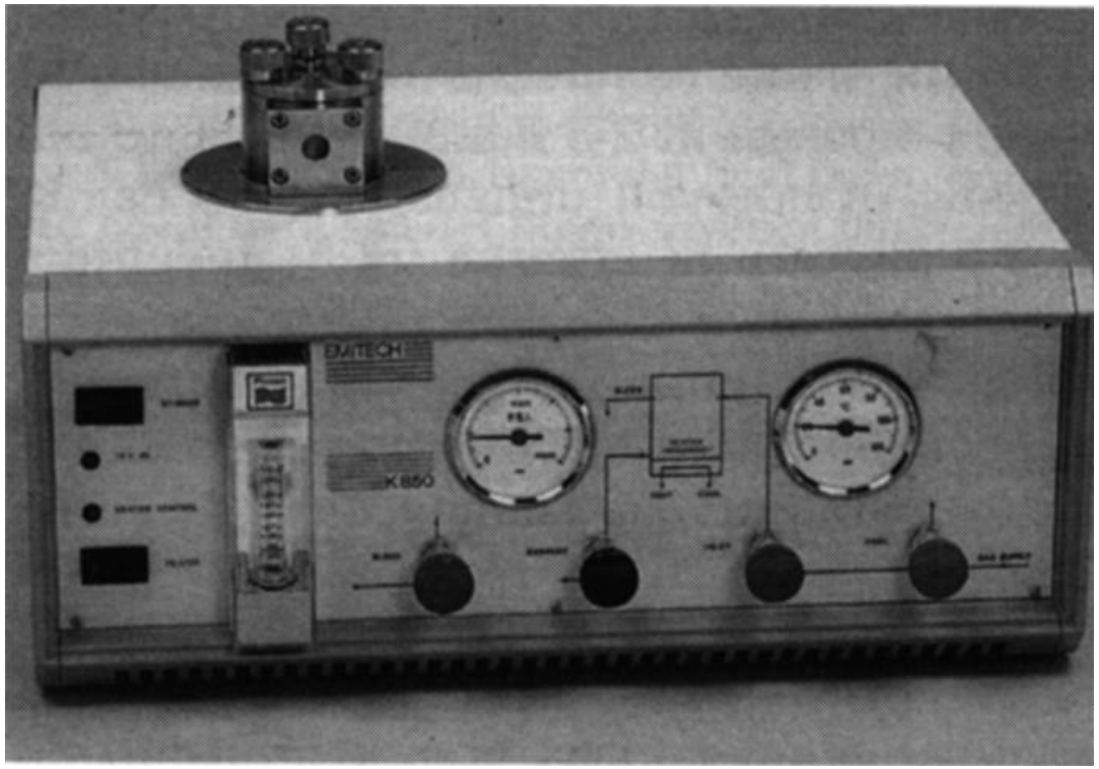
Salient features

- Electrons are used to create images of the surface of specimen - **topology**
- Resolution of objects of nearly **1 nm**
- Magnification upto **500000 x** (250 times > light microcopes)
- Gives **3D views** of the exteriors of the objects like cells, microbes or surfaces

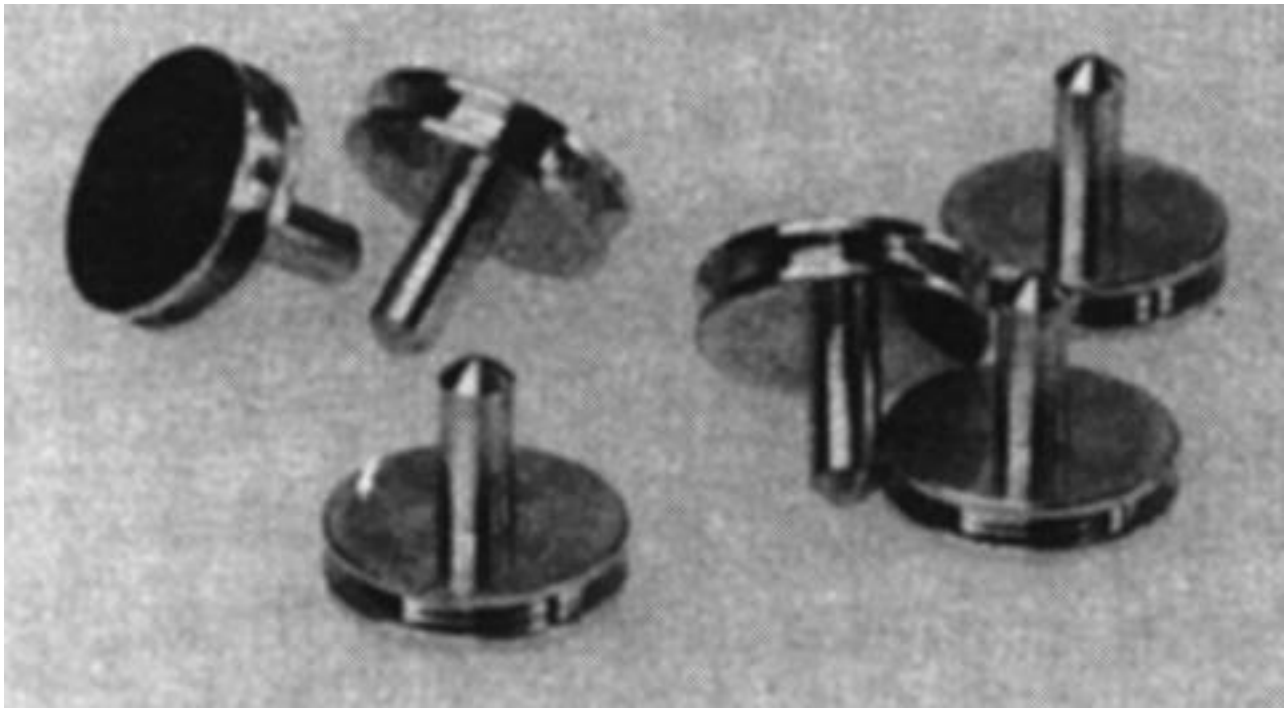
Biological sample preparation

- Chemical fixation with Gluteraldehyde, optionally with OsO4 – for soft tissues
- No fixation needed for dry specimen like bones, feathers etc
- Dehydration by replacement of water in the cells with organic solvents such as ethanol or acetone, and dryed by critical point drying.
- The dry specimen is mounted on a specimen stub
- coating done by low-vacuum sputter coating.
- Conductive materials in current use for specimen coating include **gold**, **platinum**, **tungsten**, and **graphite**.

CRITICAL POINT DRYING



DRY SPECIMEN IS MOUNTED ON A SPECIMEN STUB



SPUTTER COATER