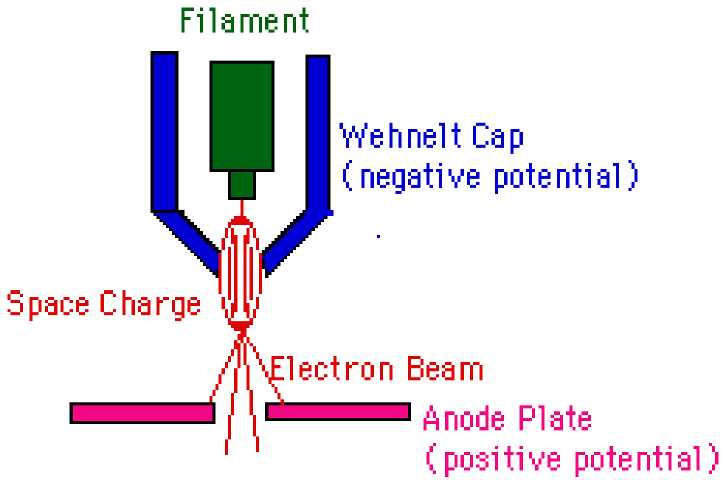
Electron Microscopy

- Electron wavelength:
  - $e\Delta V = 1/2\ mv^2 = p^2/2m$; but $\lambda = h/p$ (de Broglie wavelength) and so

$$\lambda = \frac{h}{\sqrt{2me(\Delta V)}} = \frac{12.3A}{\sqrt{\Delta V(in\ volts)}}$$

  With $\Delta V = 10^4$ V $\lambda = 0.012$ nm; technical problems limit this to 0.2 nm
  - History:
    - 1932 – 1st EM
    - 1934 resolution exceeds light microscope
    - 1938 first practical EM (Siemans)
    - 1942 SEM developed by Zworykin (TV inventor)
    - 1969 STEM developed by Crewe

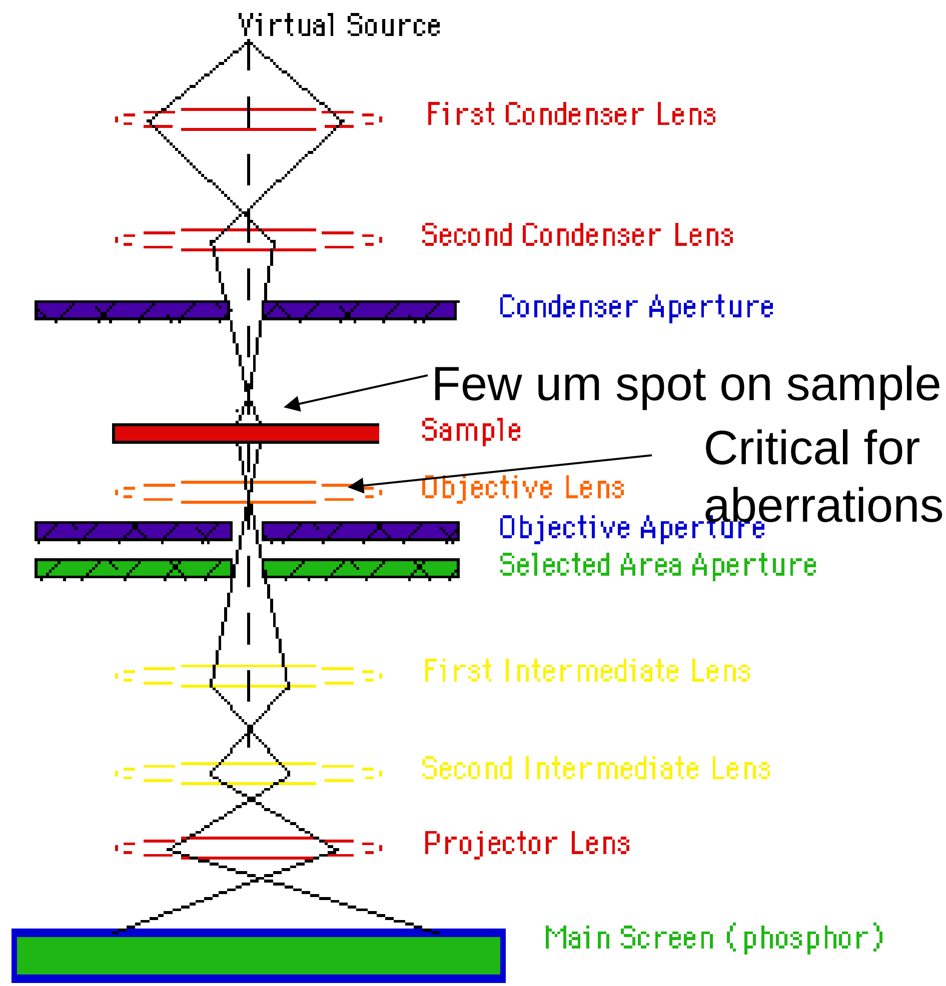
TEM Instrument



Thermionic emission – 2400 K

Accelerate through 40 – 100 kV

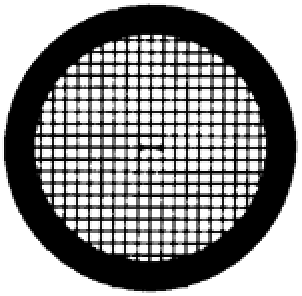
Up to 1 MV in HV-EM



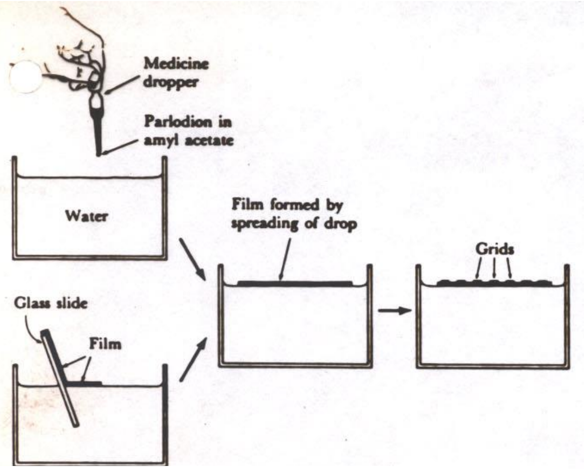
Overall mag = 1000 – 300,000x

Sample Preparation

- Grid with C film by vacuum deposition, or plastic film



- Thin sections or liquid sample
- Need to improve poor intrinsic contrast
- 4 basic methods:
  - Replica – deposit thin layer of metal (Pt) and float off and look at replica
  - Freeze-etching – rapidly freeze and sublime water (to minimize damage)
  - Shadow – evaporate heavy metals (Au)
  - Negative stain with heavy metal salt (U)



Preparation of grids for EM work -

…iderably less concentrated than the solution used with the droplet method. In some cases, the film is a thin layer of carbon that has been evaporated onto the glass or onto a mica sheet. When the slide is lowered into the water, the film comes off the glass and floats on the water. Several grids are then placed on the surface of the film and a piece of absorbent paper is placed on the grids. When the paper is lifted up, the grids adhere to it. An alternative method is to use a vessel with a bottom drain. The grids are placed on a screen platform under the water surface before forming the film. The film is then formed and the vessel is drained. As the water level drops, the film comes in …

Carbon-platinum replica:

Coat with Pt, then C, then float film onto water and pick up on grid

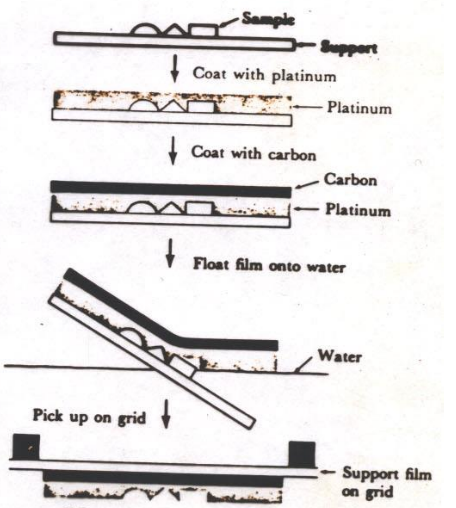


FIGURE 3-5
Formation of a carbon-platinum replica. In some cases, the support film is applied to the carbon layer before floating on water; a bare grid is then used to pick up the film (see legend for Figure …).

Shadowing – vacuum deposition

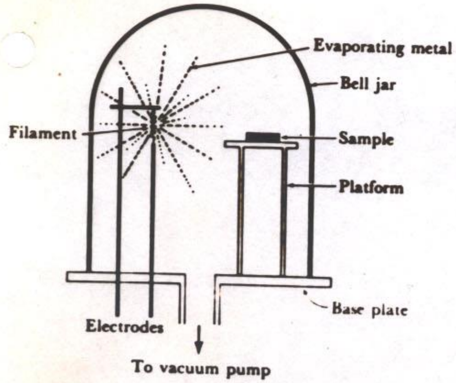


FIGURE 3-8
Apparatus for vacuum evaporation or shadow-casting. The bell jar is evacuated by vacuum and diffusion pumps. The tungsten filament, around which the metal wire to be evaporated is wrapped, is heated to the boiling point of the metal. In 10 or 15 seconds, the metal is boiled away and forms a film on the sample. The thickness of the film is proportional to the amount of metal put on the filament and inversely proportional to the square of the distance from filament to sample.

Kleinschmidt technique for DNA preparation for EM

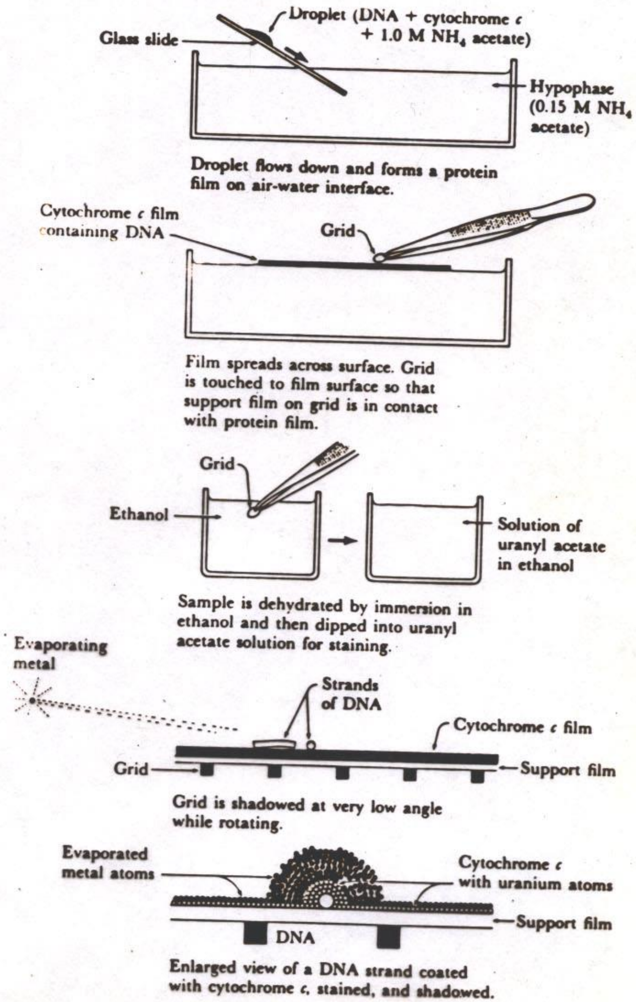
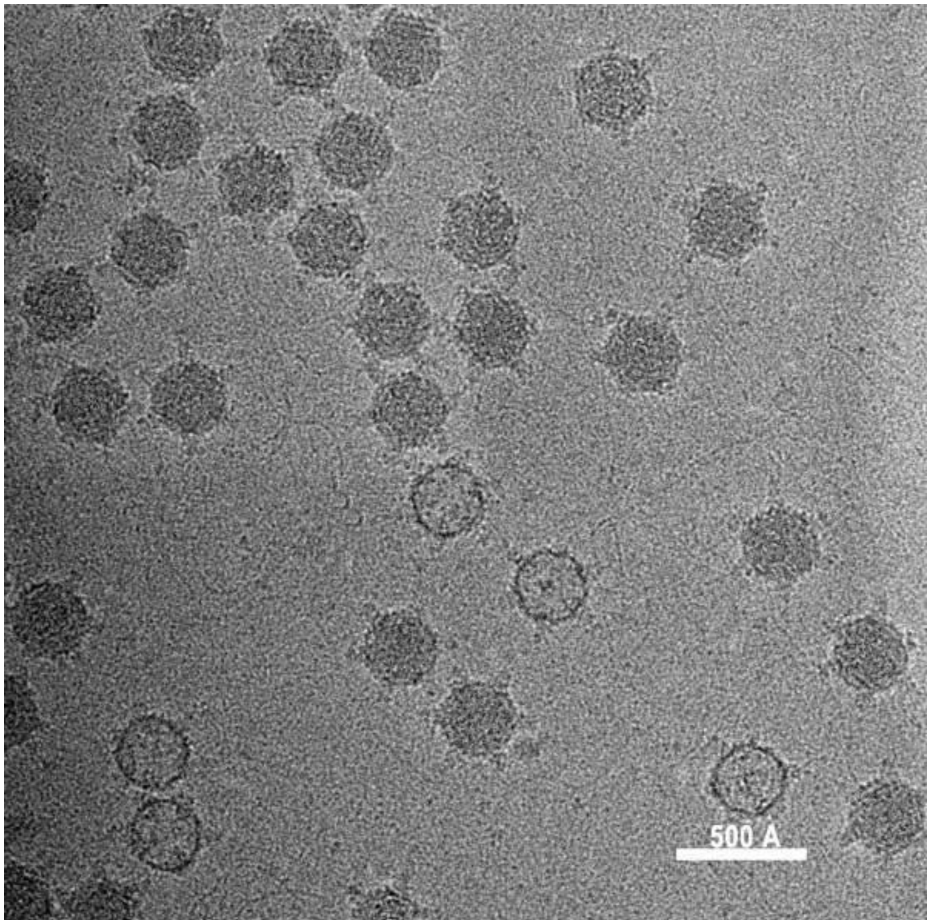
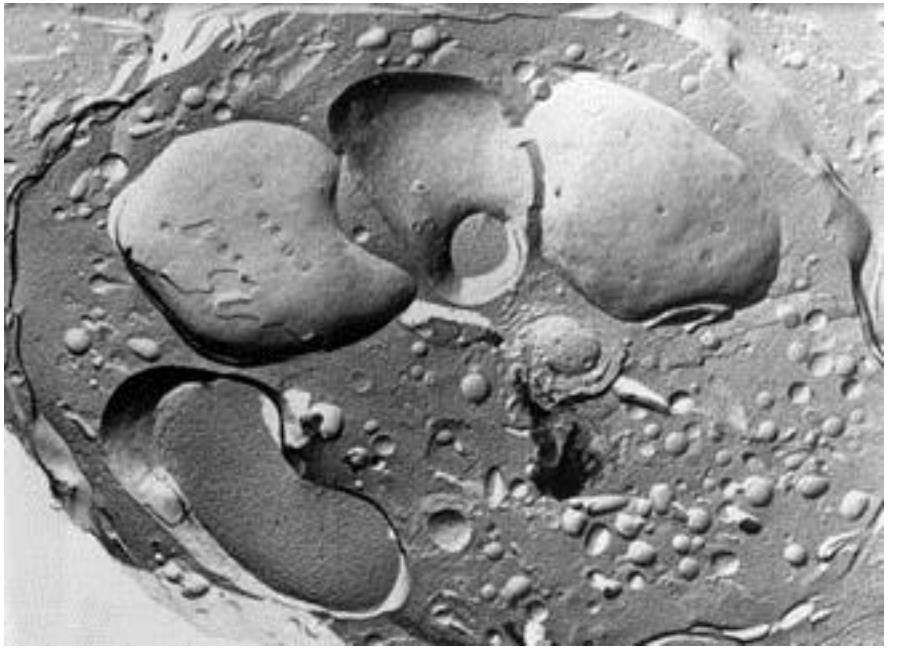


FIGURE 3-14
Preparation of DNA for electron microscopy, using the Kleinschmidt method.



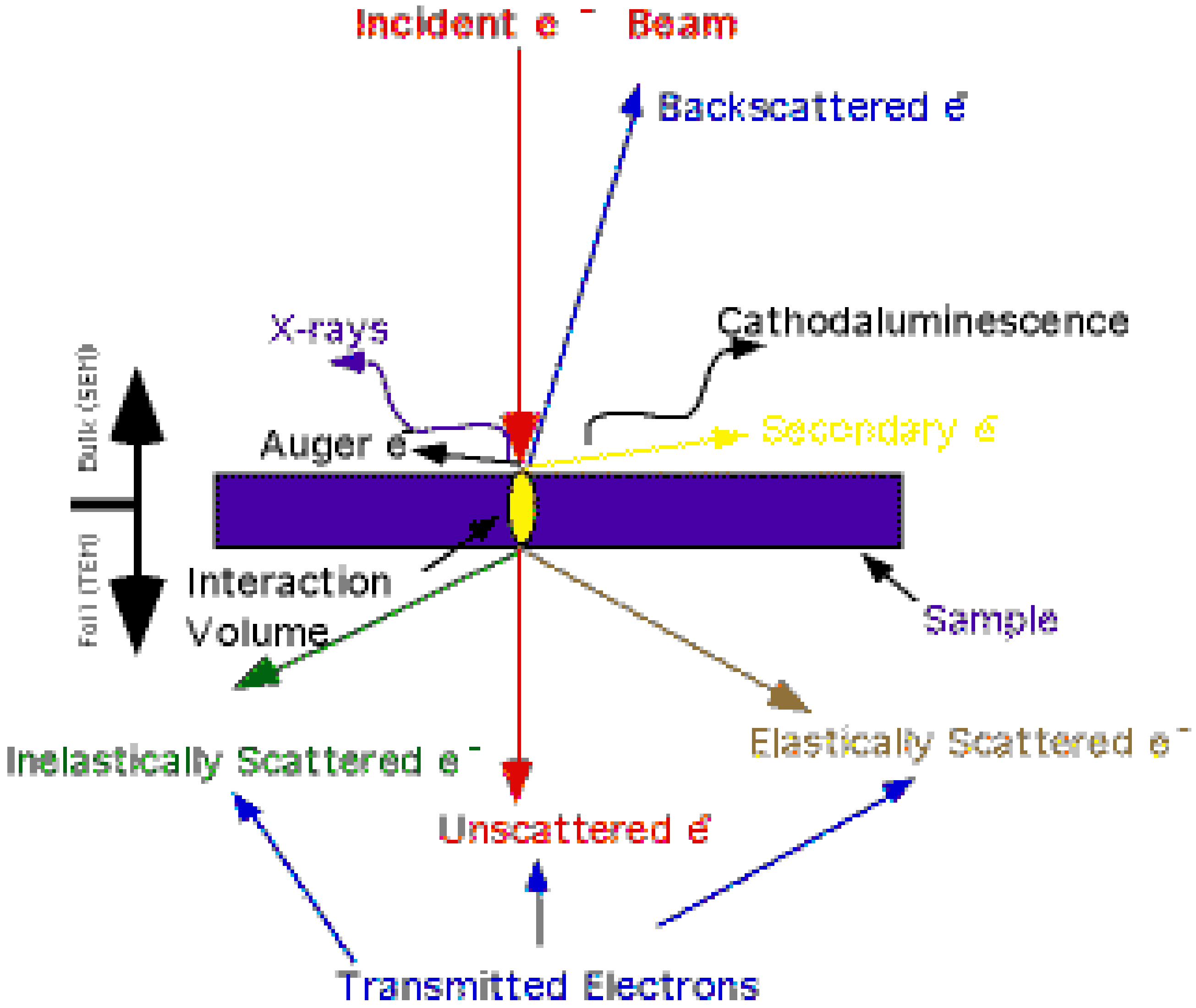
CPV (cytoplasmic polyhedrosis virus) Courtesy of Baylor College of Medicine, NCMI



Freeze-fracture + rotary shadowing

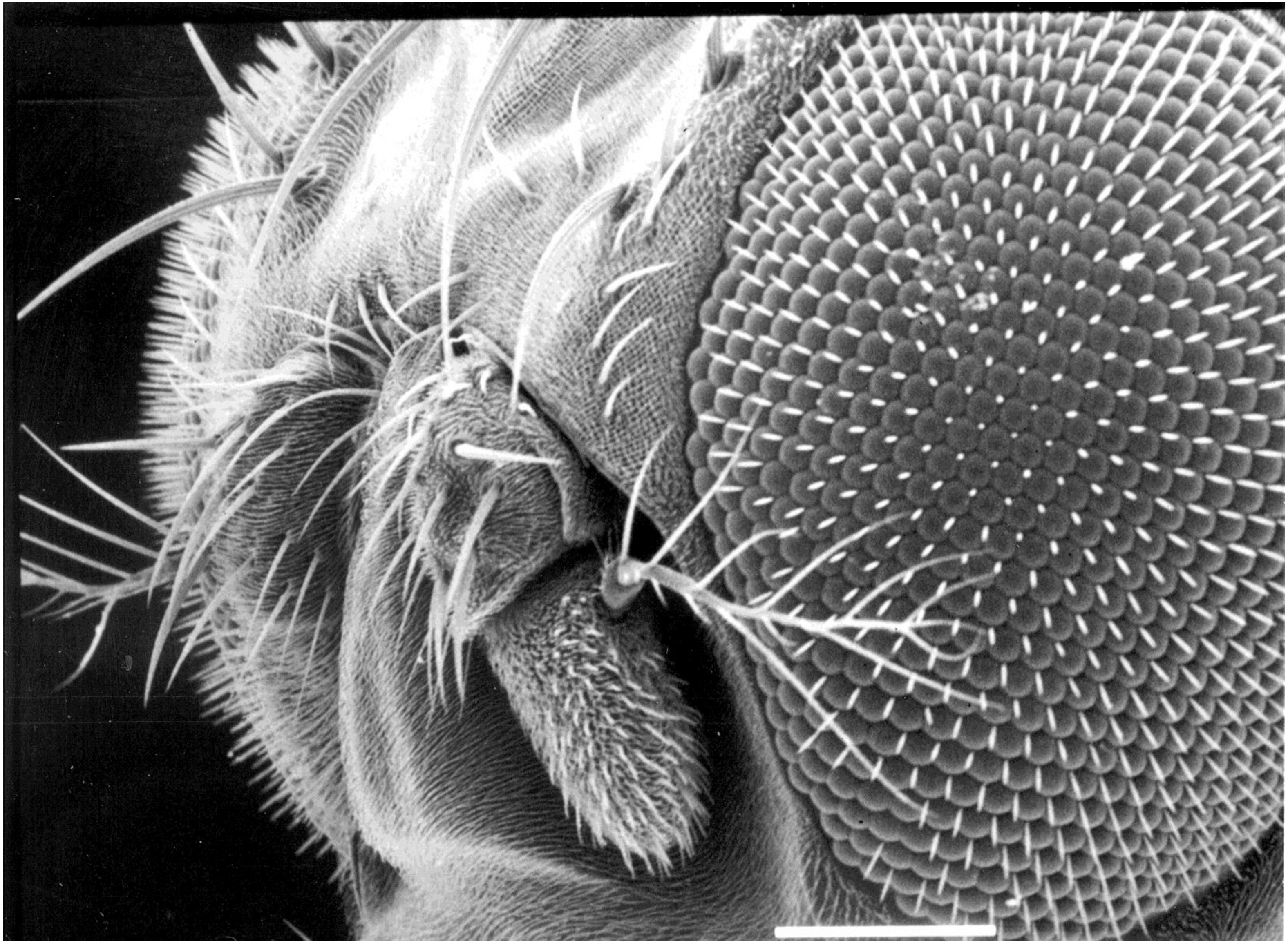
human amnion cell

SEM – Scanning EM



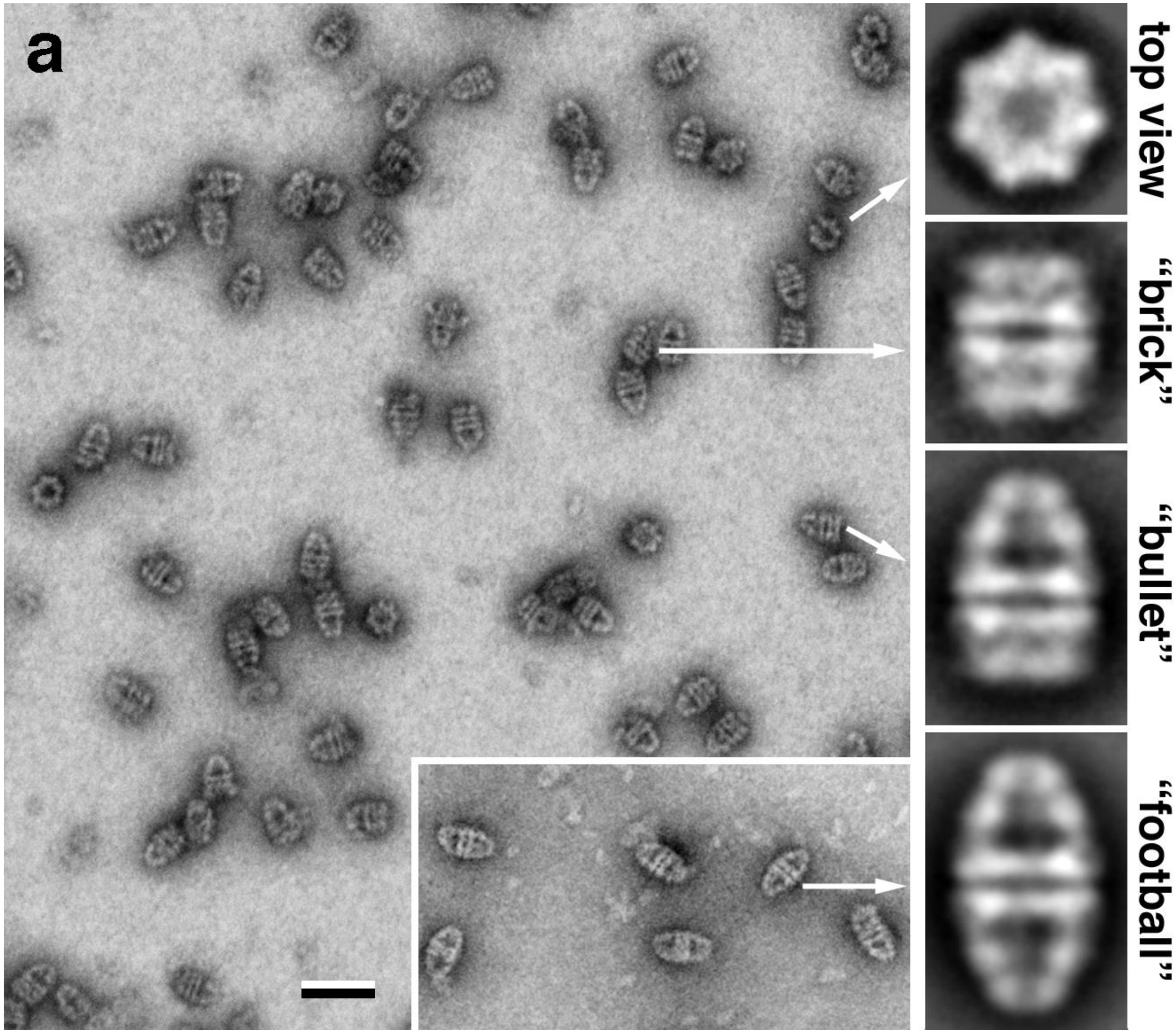
Details on SEM

- Electron beam comes in off-axis at ~ 10 nm spot
- Scanned across surface in raster pattern
- Detectors sense a number of signals – backscattered e-, x-rays, fluorescence, also transmission
- Signals are displayed as 2-D image on CRT
- Samples must be coated and resolution is limited to ~ 10 nm – but depth of focus is extremely high



STEM

- Record both elastic and inelastic scattering – ratio is characteristic of each element and so can do elemental analysis
- Uses tightly focused beam (0.5 nm) and scans as in SEM, but at each point measures ratio of $I_{elastic}/I_{inelastic}$ – very high resolution and elemental analysis



Chaperonin – bar = 20 nm; reconstruction on right