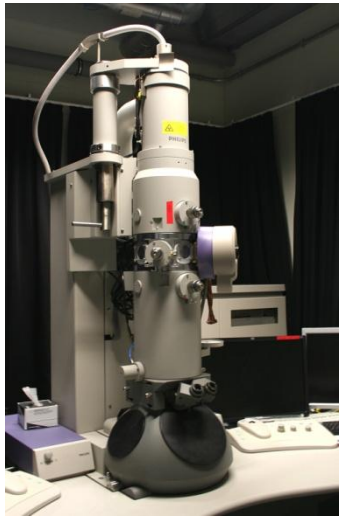
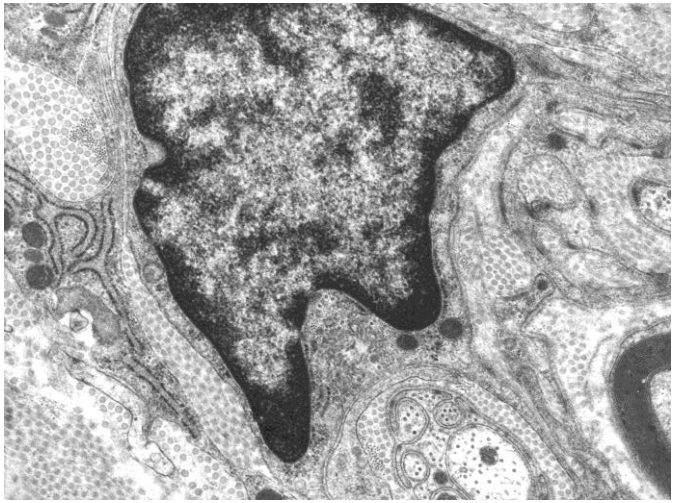
Electron Microscopy

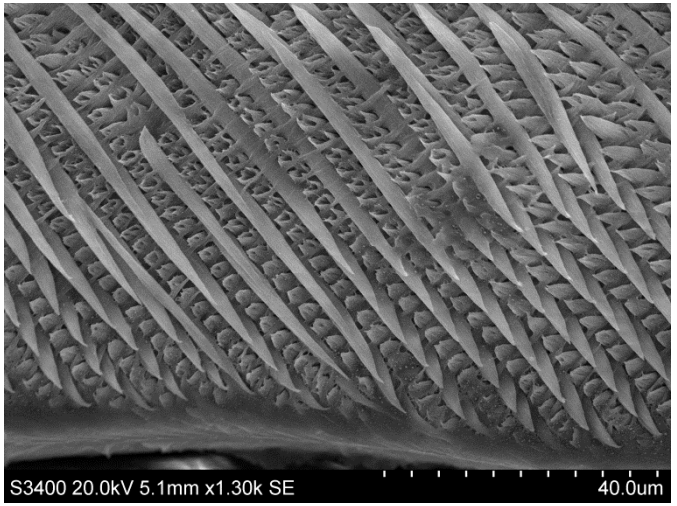
Roger Shore



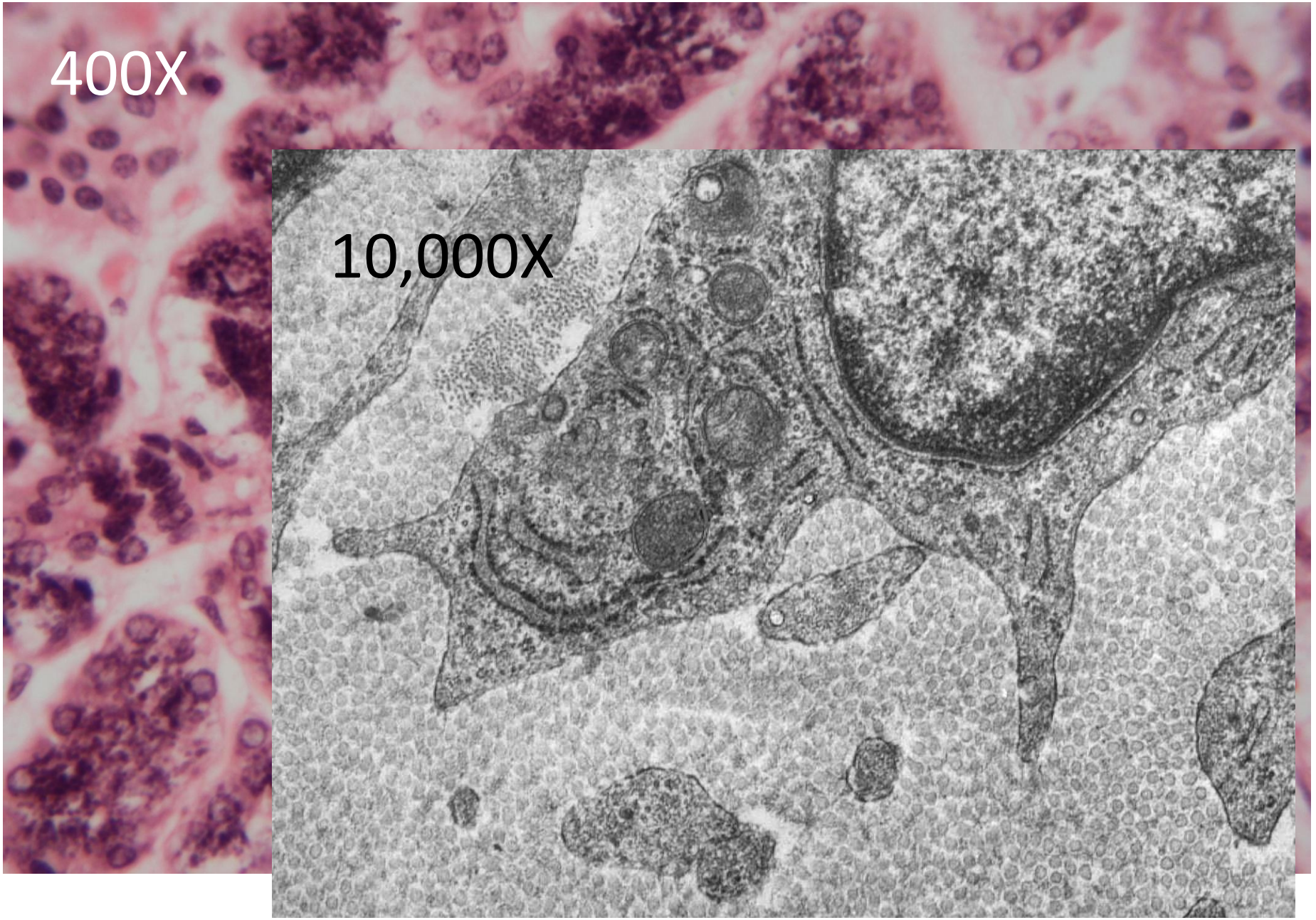
Transmission electron microscopy

Scanning electron microscopy





Transmission electron microscopy


400X
10,000X

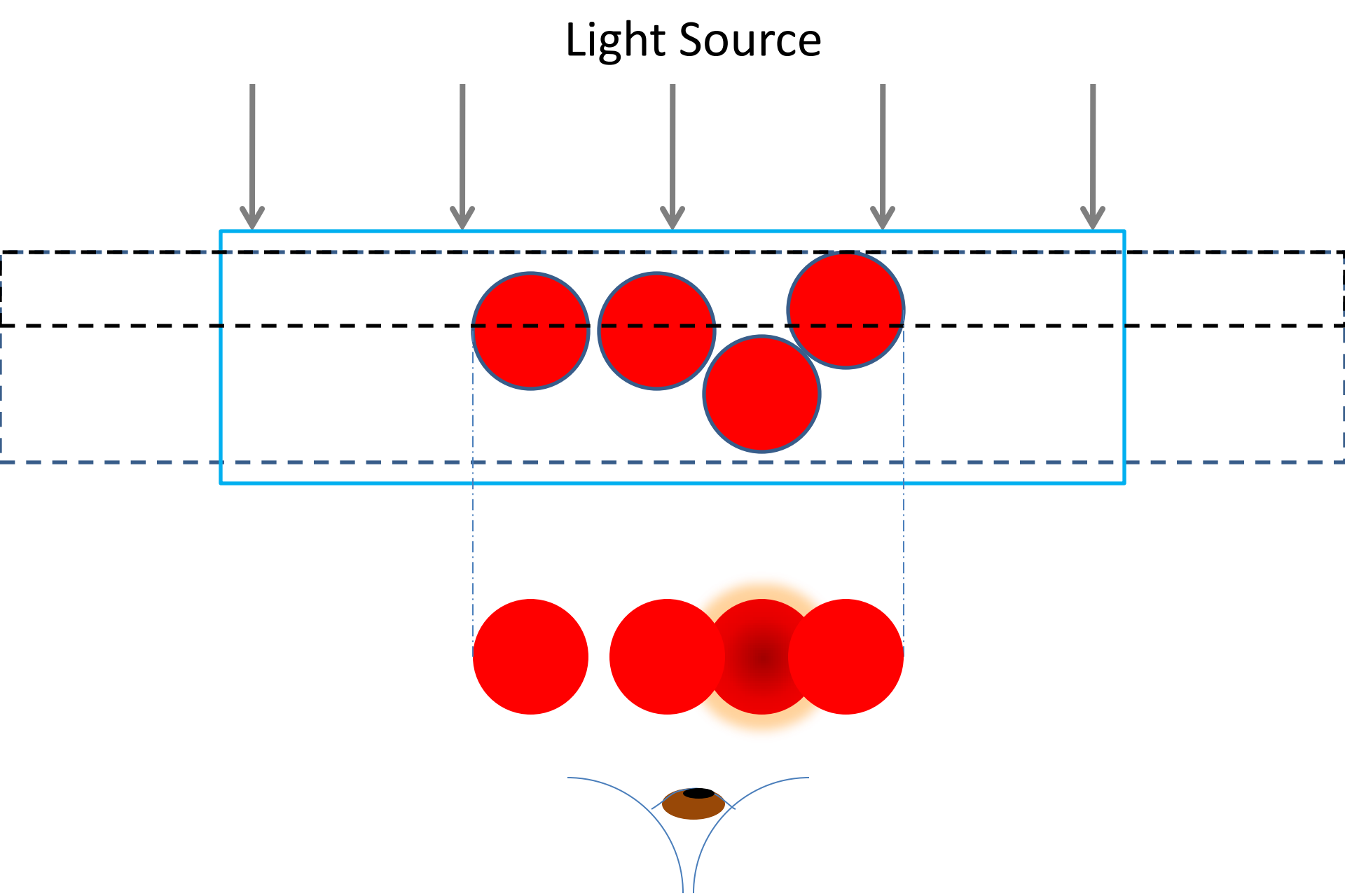
Resolution (r) = λ / 2NA (λ = **wavelength)**

Resolution versus Wavelength

Wavelength (Nanometers)	Resolution (Micrometers)
360	.19
400	.21
450	.24
500	.26
550	.29
600	.32
650	.34
700	.37

To increase the effective resolution:

1. Decrease wavelength of incident beam
    1. monochromatic blue/UV
    2. electrons
2. Increase lens quality
3. Decrease section thickness
    1. Resin embed + ultramicrotomy
    2. confocal microscopy (optical sectioning)


Light Source

For the TEM:

$$\lambda_e \approx \frac{h}{\sqrt{2m_0E\left(1+\frac{E}{2m_0c^2}\right)}}$$

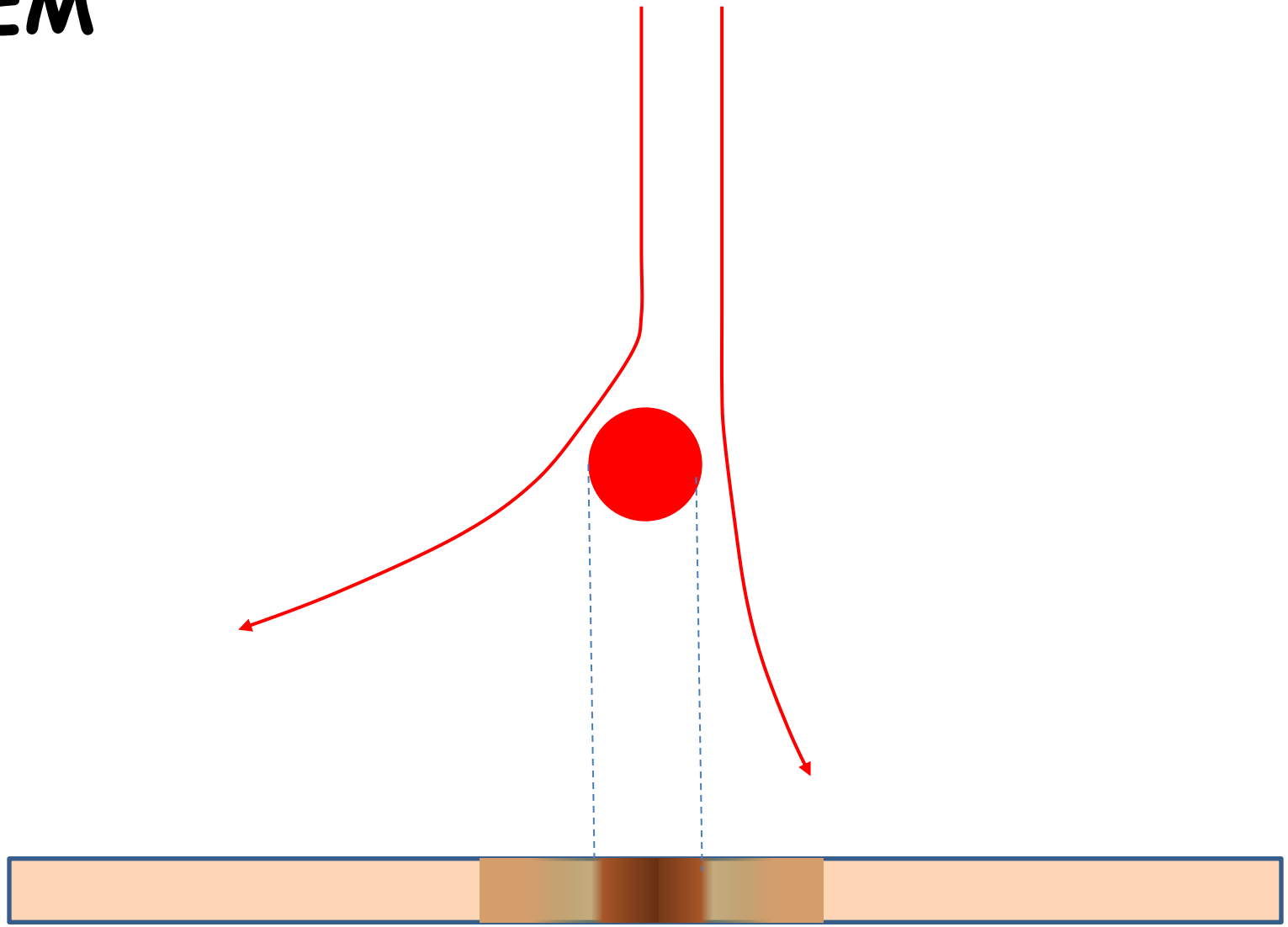
$$d = \frac{0.753}{a\ V^{1/2}}$$

d = resolution in nm
a = half aperture angle
V = accelerating velocity
Solving for 100,000 volts, the result is 0.24 nm or 2.4 Å.

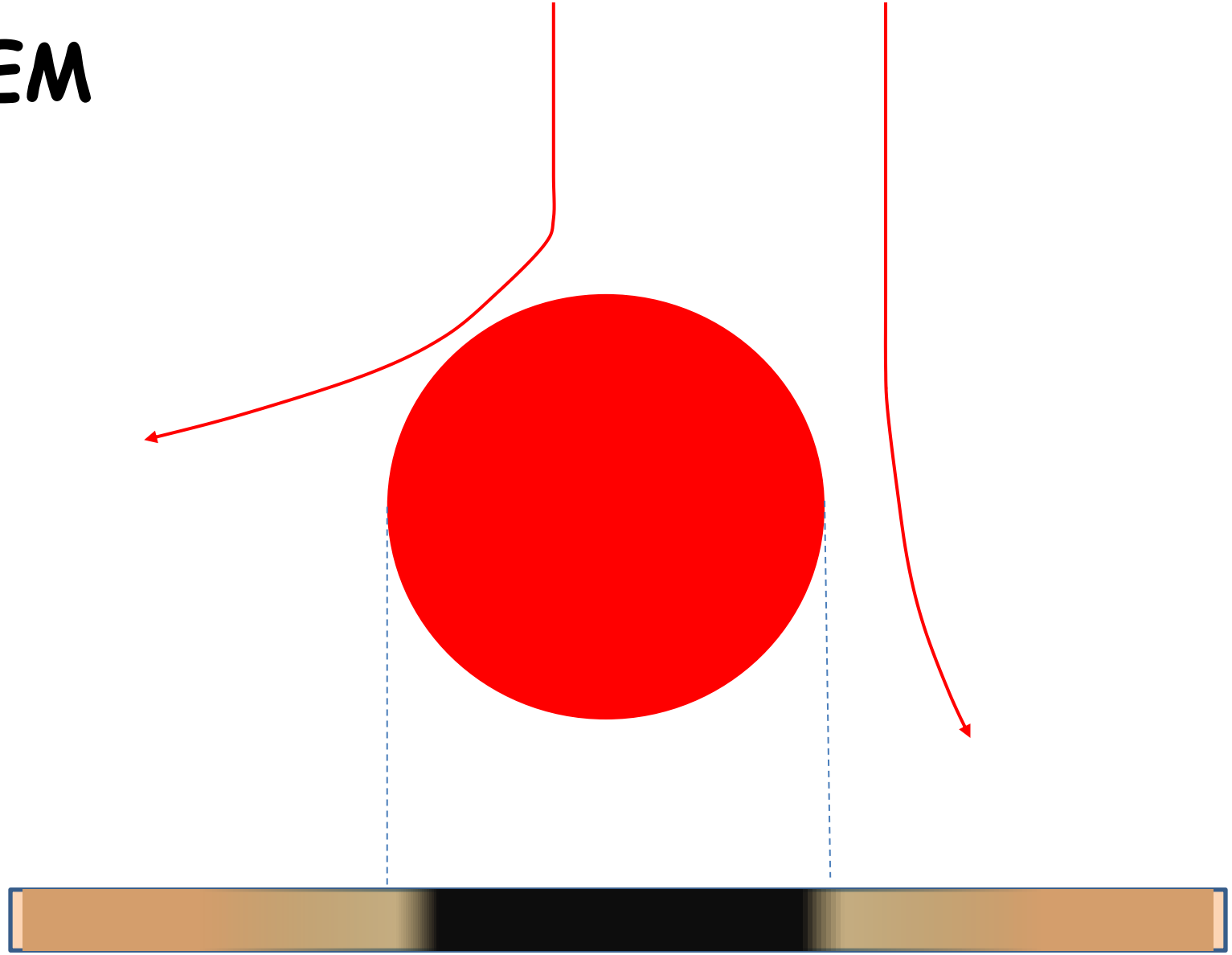
The first TEM was built by Max Knoll and Ernst Ruska in 1931, with this group developing the first TEM with resolution greater than that of light in 1933 and the first commercial TEM in 1939.

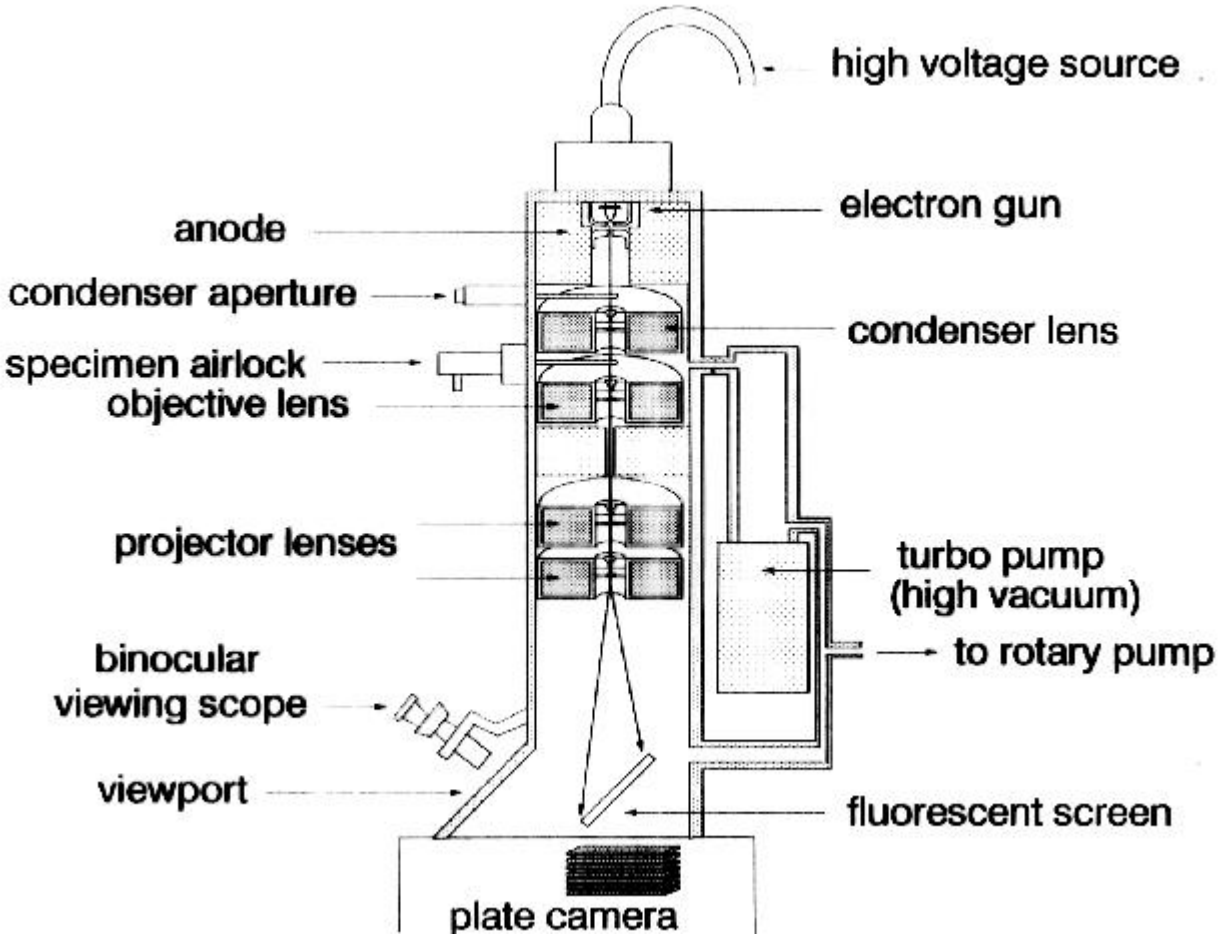
Over time, electron microscopes have become more powerful (in terms of KV), so their resolutions have correspondingly improved from:
10 nanometers (STM, 1989) to
0.05 nm (million KV field emission TEM, 2000 June) >1,000,000X magnification

TEM



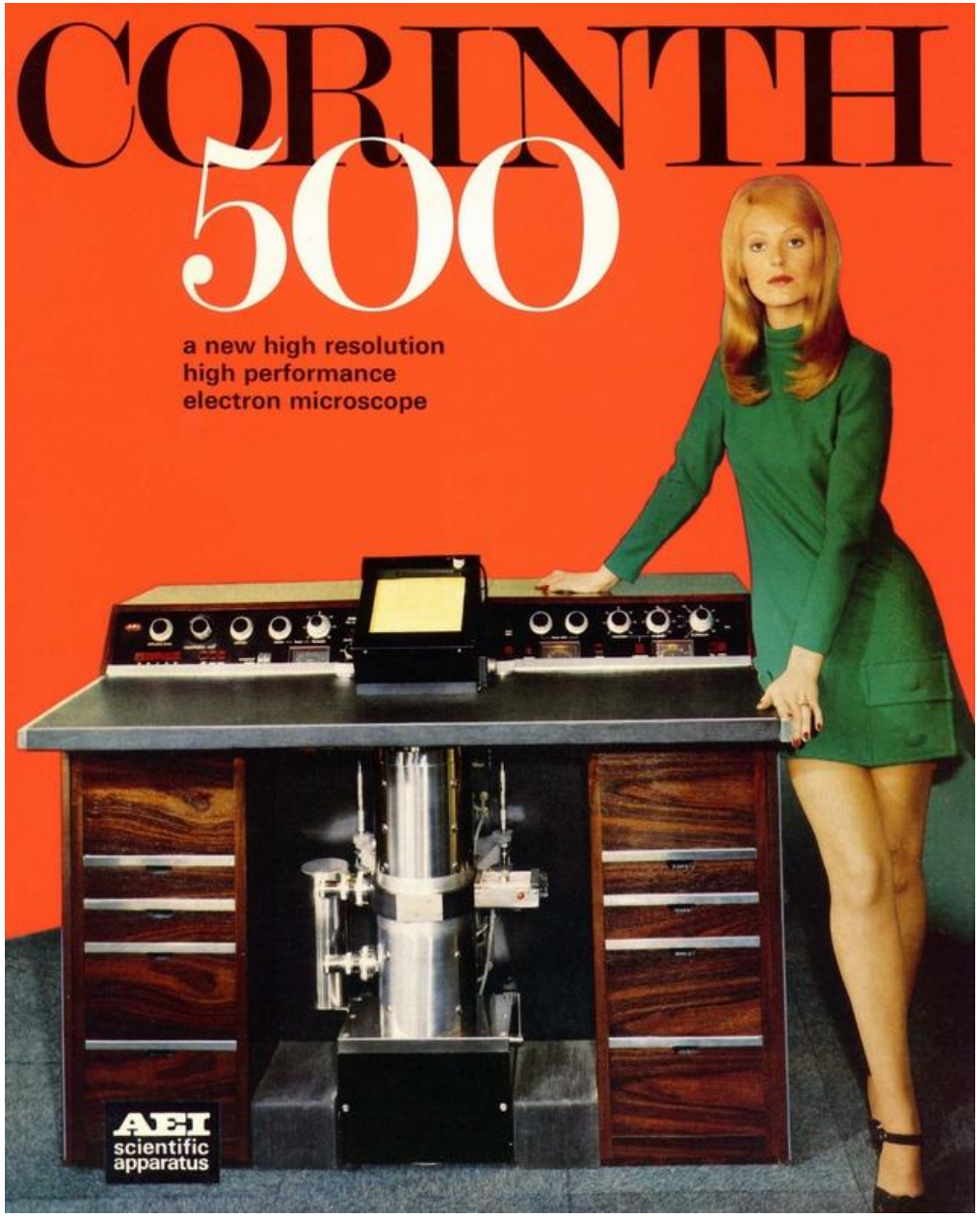
TEM




high voltage source
electron gun
anode
condenser aperture
condenser lens
specimen airlock
objective lens
projector lenses
turbo pump
(high vacuum)
to rotary pump
binocular
viewing scope
viewport
fluorescent screen
plate camera

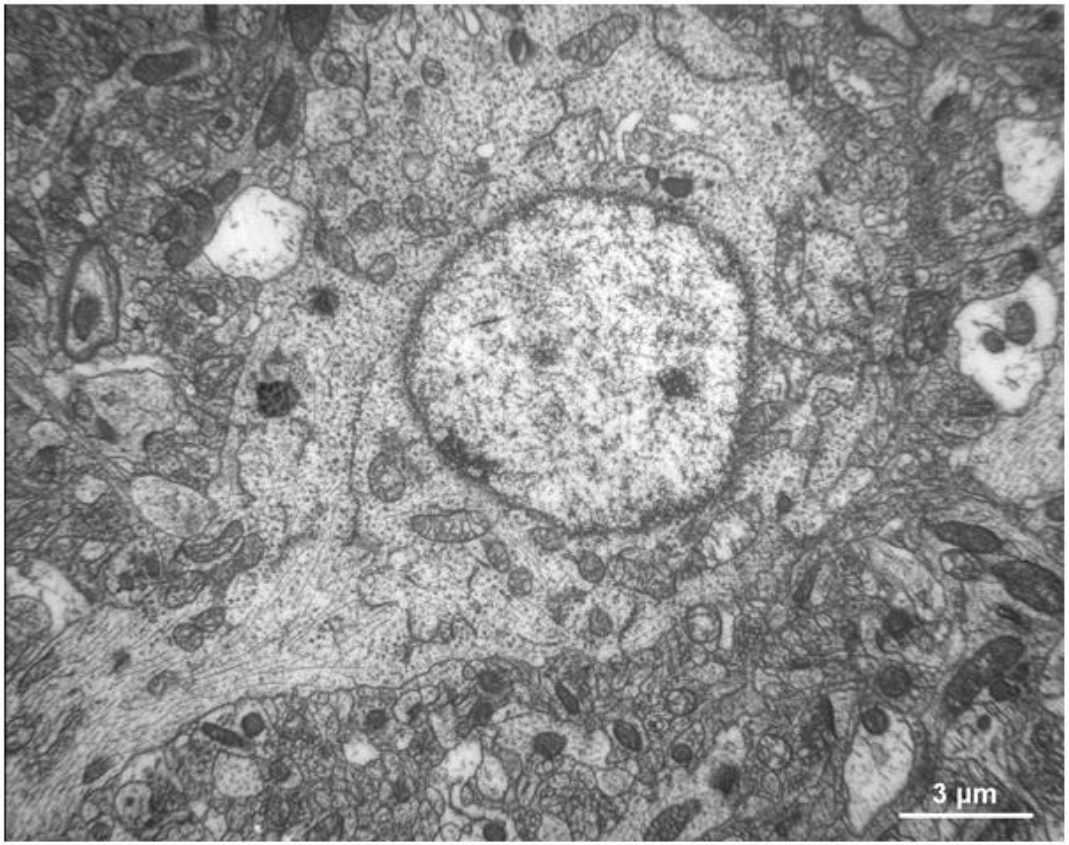
CORINTH 500

a new high resolution
high performance
electron microscope




DANGER

LVEM5



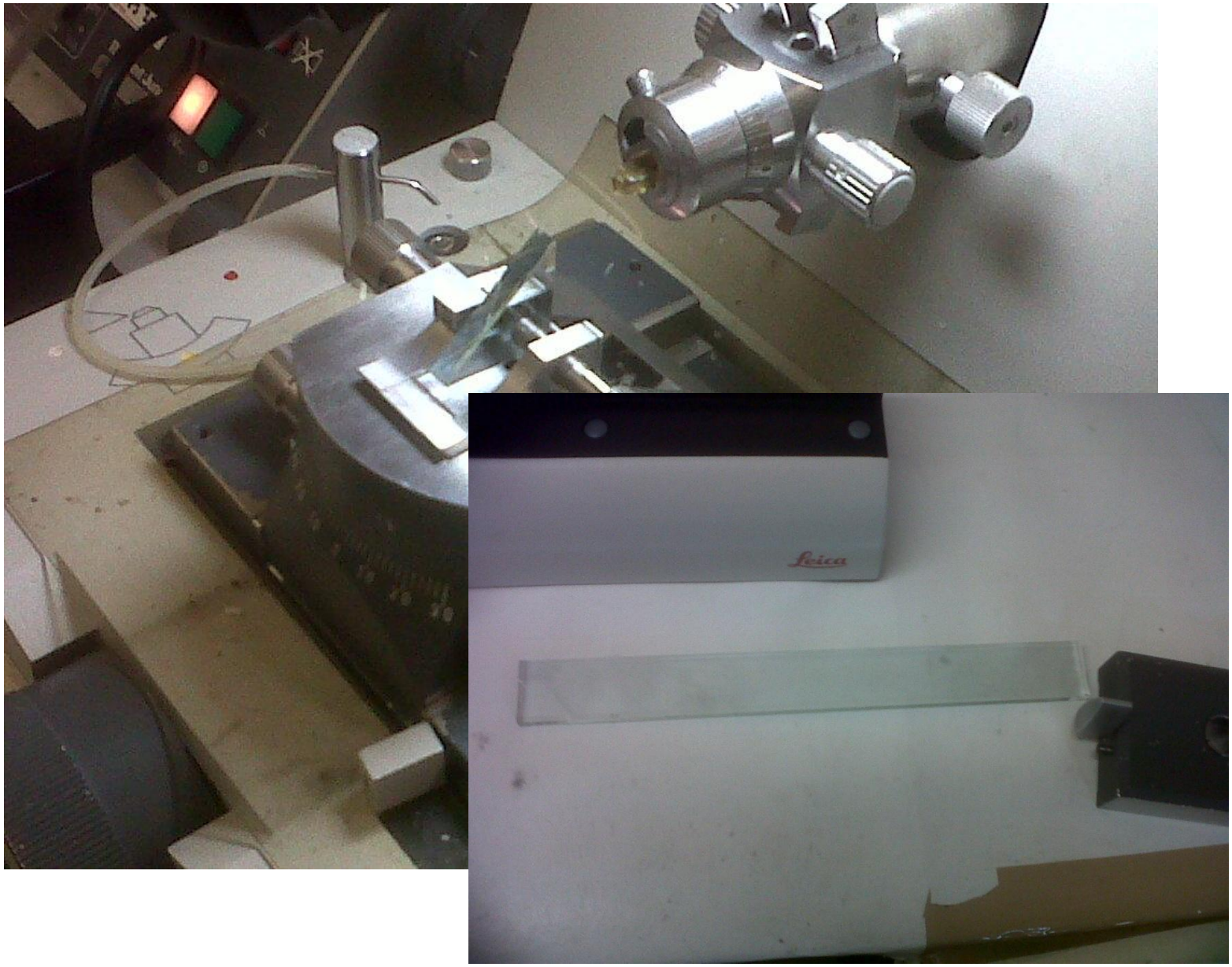
Bird brain tissue
226/394

What are problems/differences between TEM compared to LM?

	LM	TEM
Atmosphere	Air	High vacuum
Specimen size	>1cm^3	1mm^3
Fixation	Formaldehyde (immersion)	Glutaraldehyde (perfuse)
Section thickness	<>10µm	60-80nm
Embedding	Wax	Resin
Sectioning	Steel knife	Glass or diamond knife
Mounting	Glass slide	Copper grid (3mm)
Stain	Organic basis	Heavy metals (Pb, Ur)


Reichert Jung

Sectioning - ultramicrotome


Leica


ultra 35° Jumbo

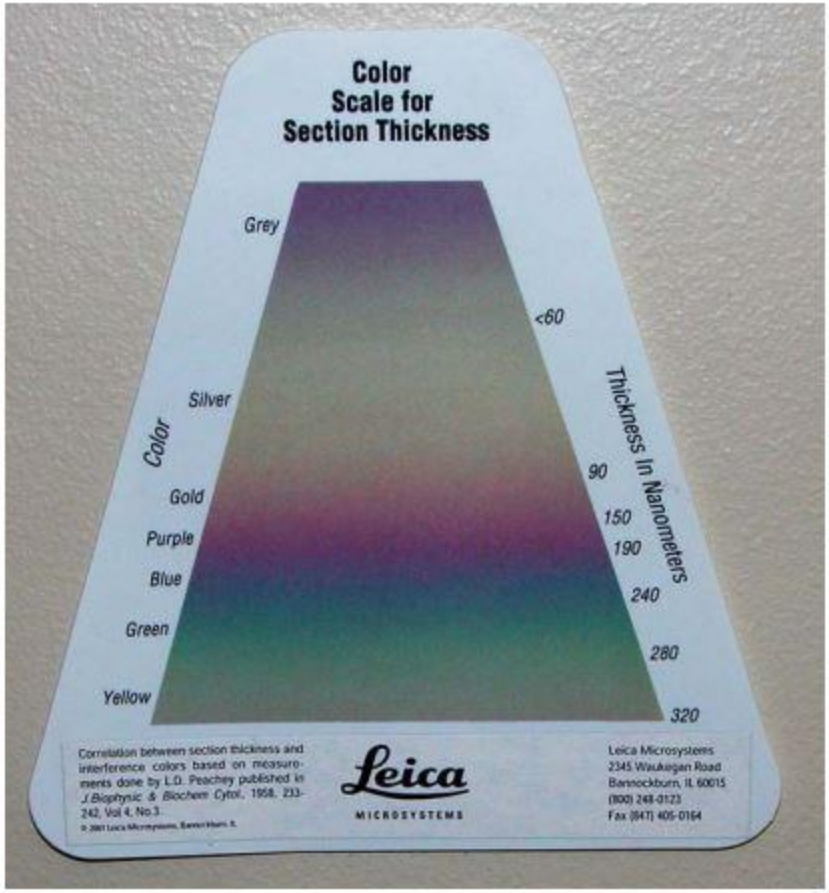


Figure 17. Interference card for determination of section thickness.

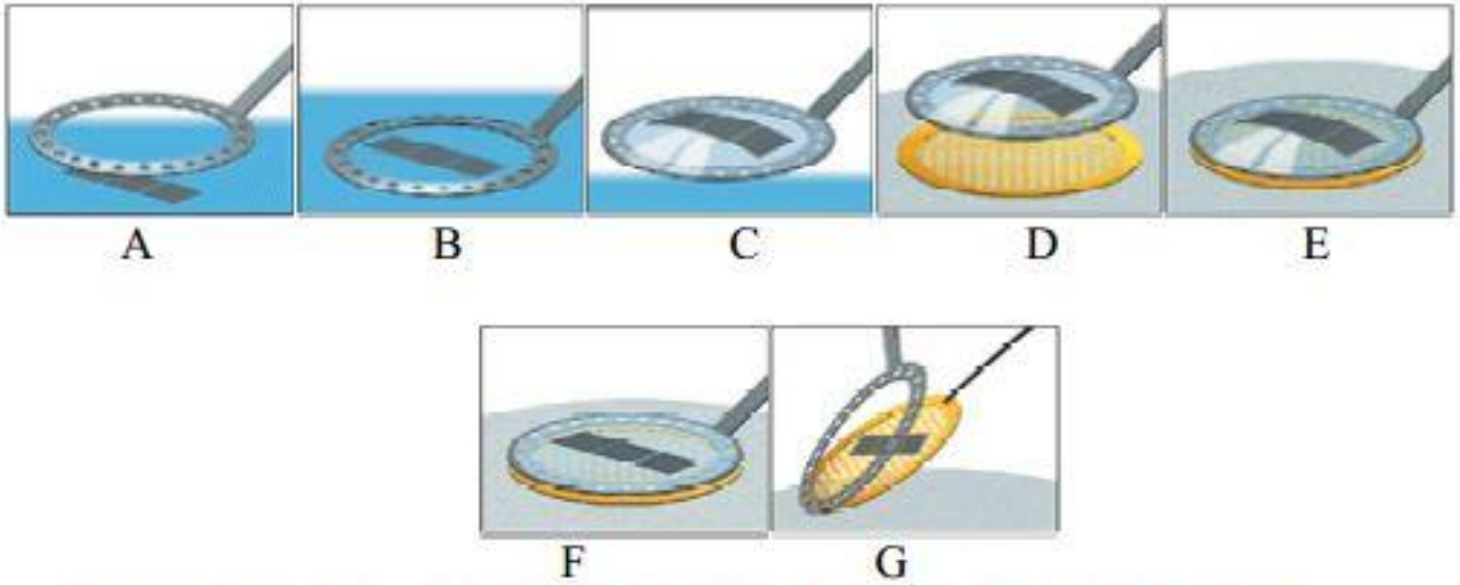
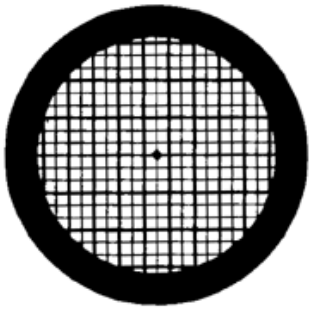


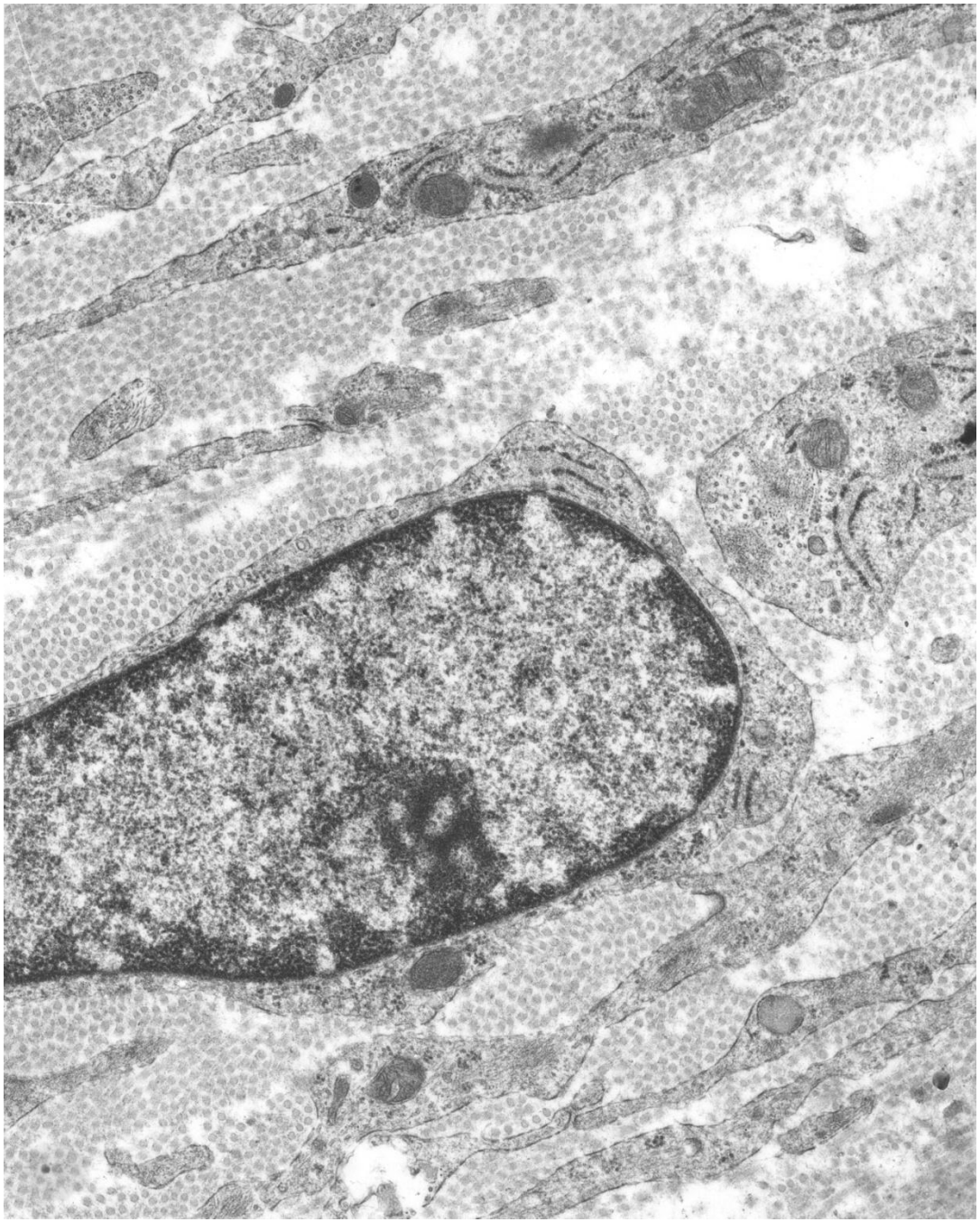
Figure 22. A-G. Diagrams illustrating the use of a Perfect Loop



1. Structure – *qualitative* –, classic TEM

2. Structure – *quantitative 'size'* image analysis TEM

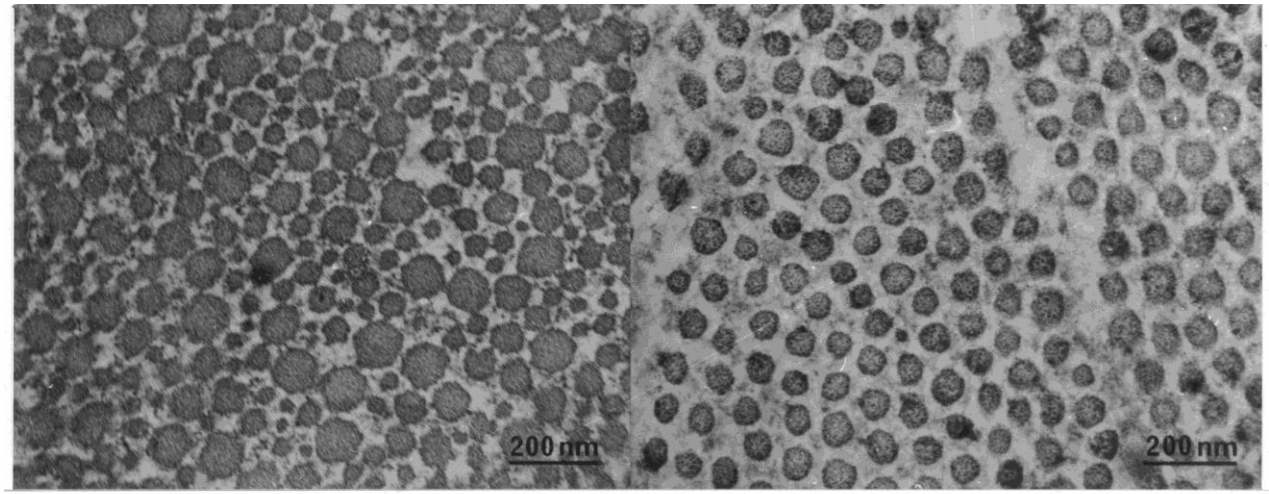
3. Structure – *quantitative 'composition'*
   a) elemental – Energy Dispersive Xray Spectroscopy (EDS or EDX)
   b) protein – immunocytochemistry

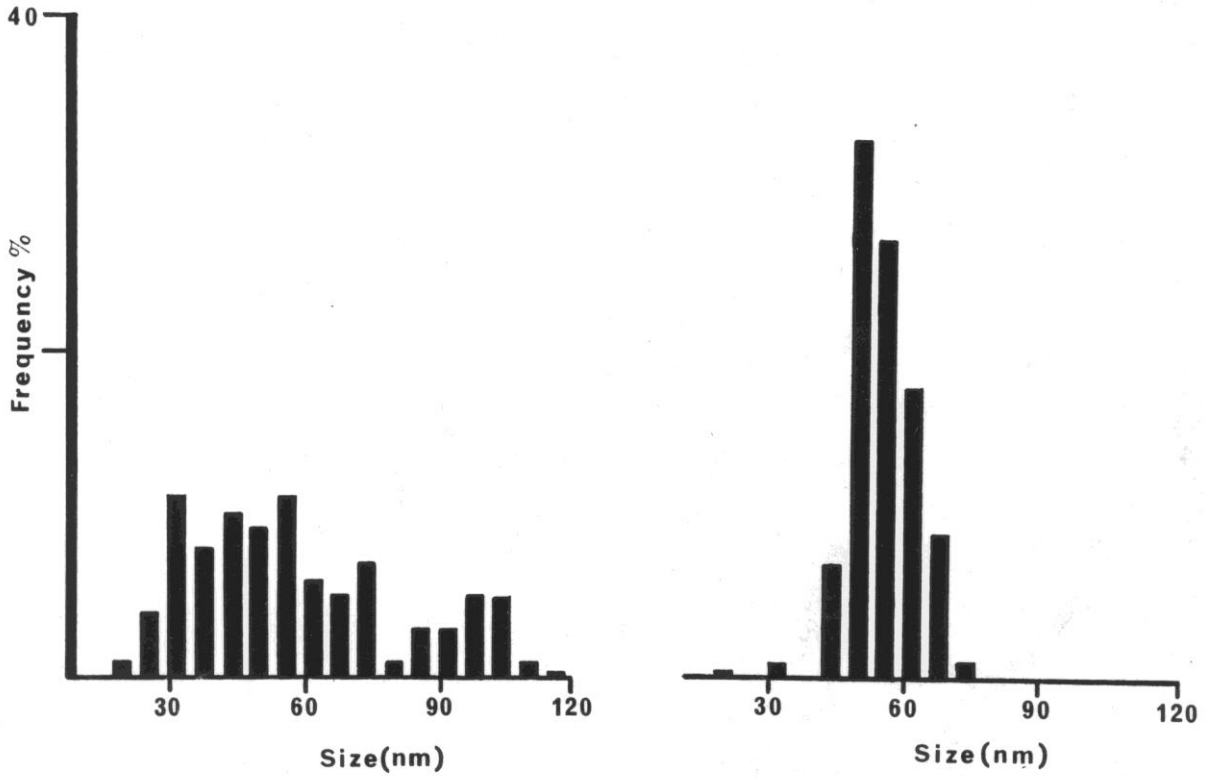


1. Structure – *qualitative* –, classic SEM, TEM

2. Structure – *quantitative 'size'* image analysis TEM

3. Structure – *quantitative 'composition'*
   a) mineral – Energy Dispersive Xray Spectroscopy (EDS or EDX)
   b) protein – immunocytochemistry


200 nm
200 nm


40
Frequency %
30
60
90
120
Size(nm)
30
60
90
120
Size(nm)

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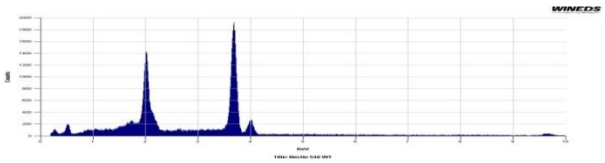
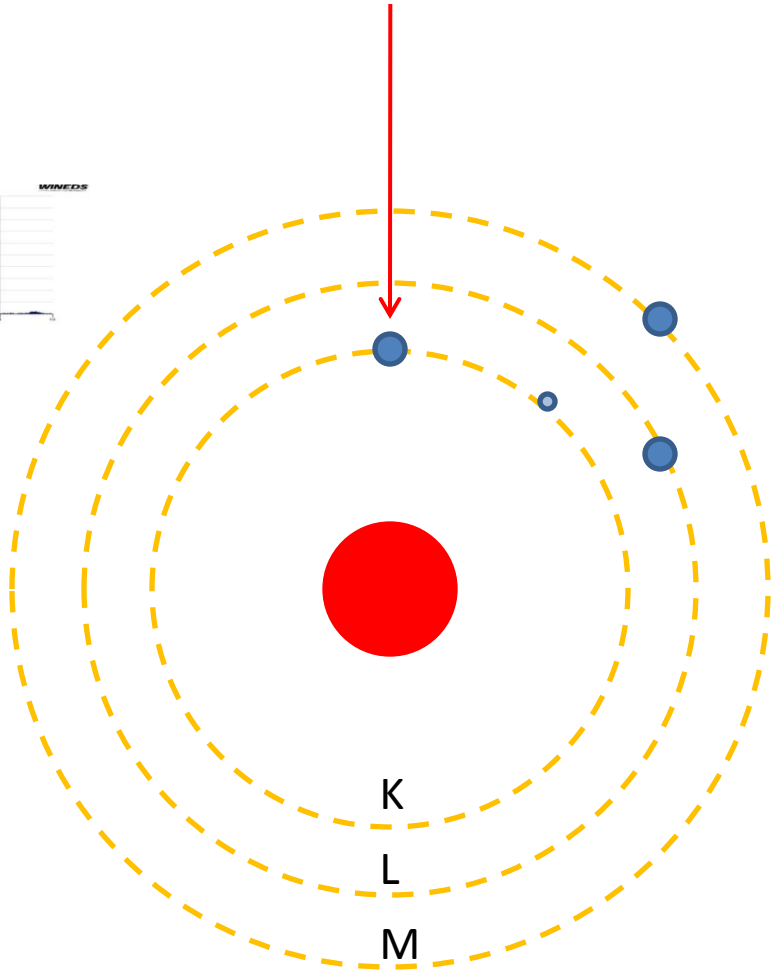
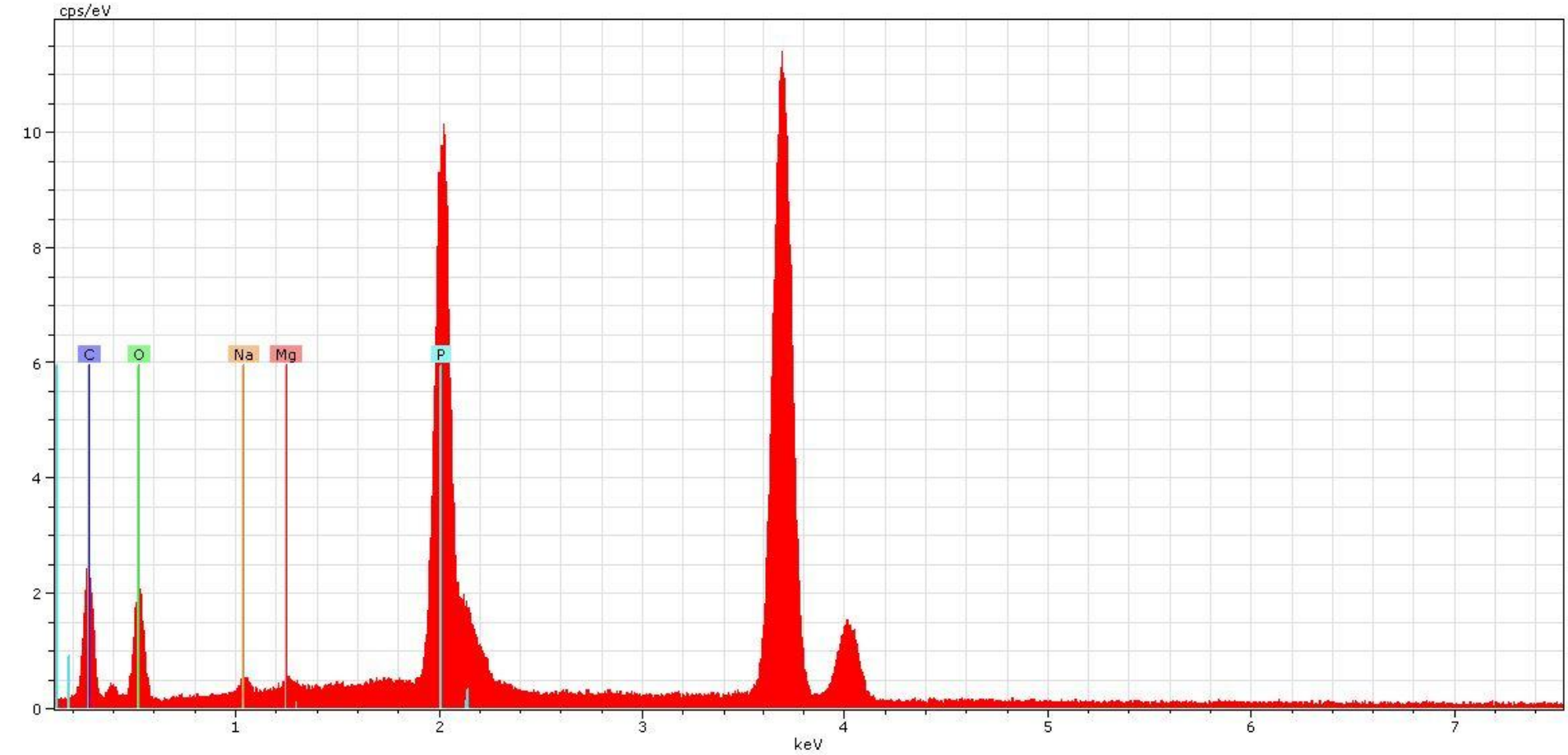
EDS





X-ray Detector

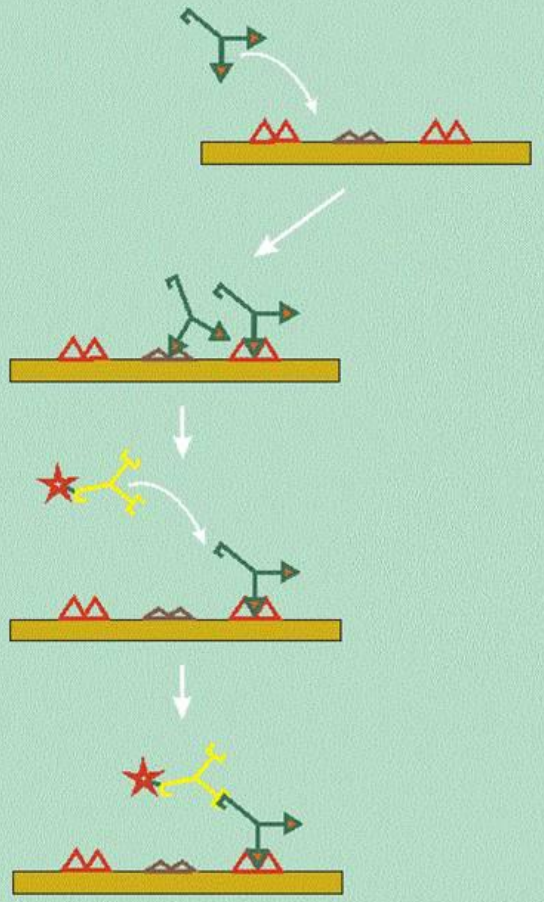

cps/eV
10
8
6
4
2
0
C
O
Na
Mg
P
1
2
3
4
5
6
7
keV

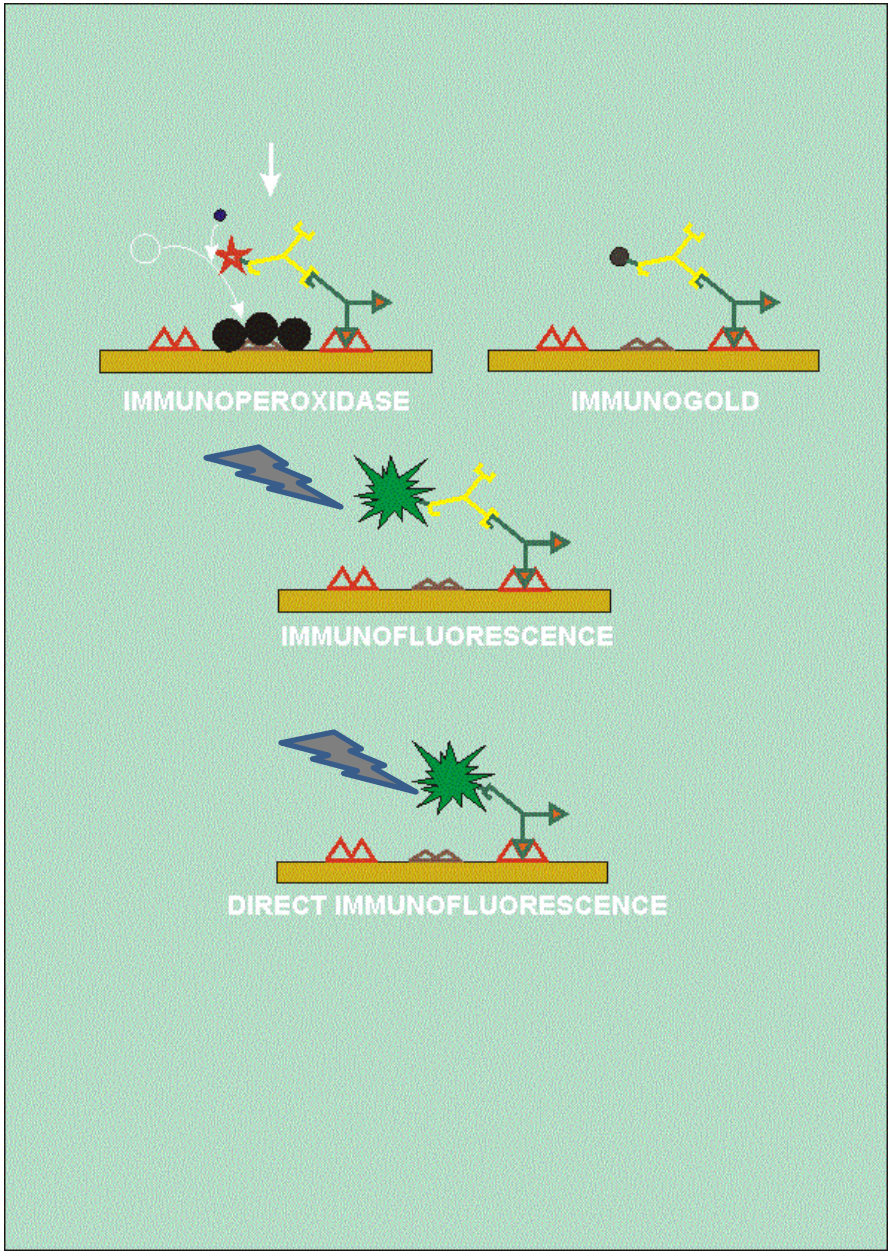
1. Structure – *qualitative* –, classic SEM, TEM

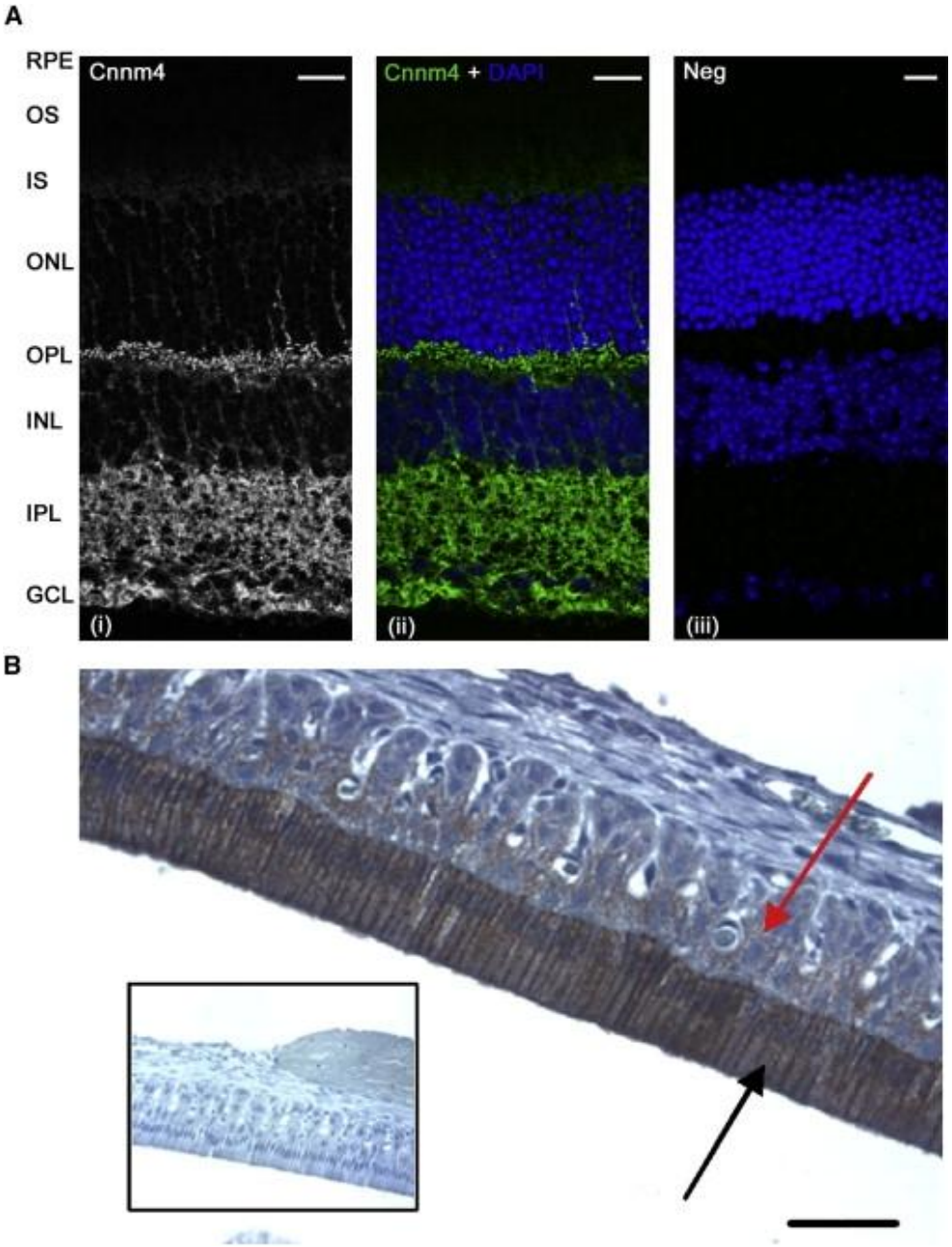
2. Structure – *quantitative 'size'* image analysis TEM

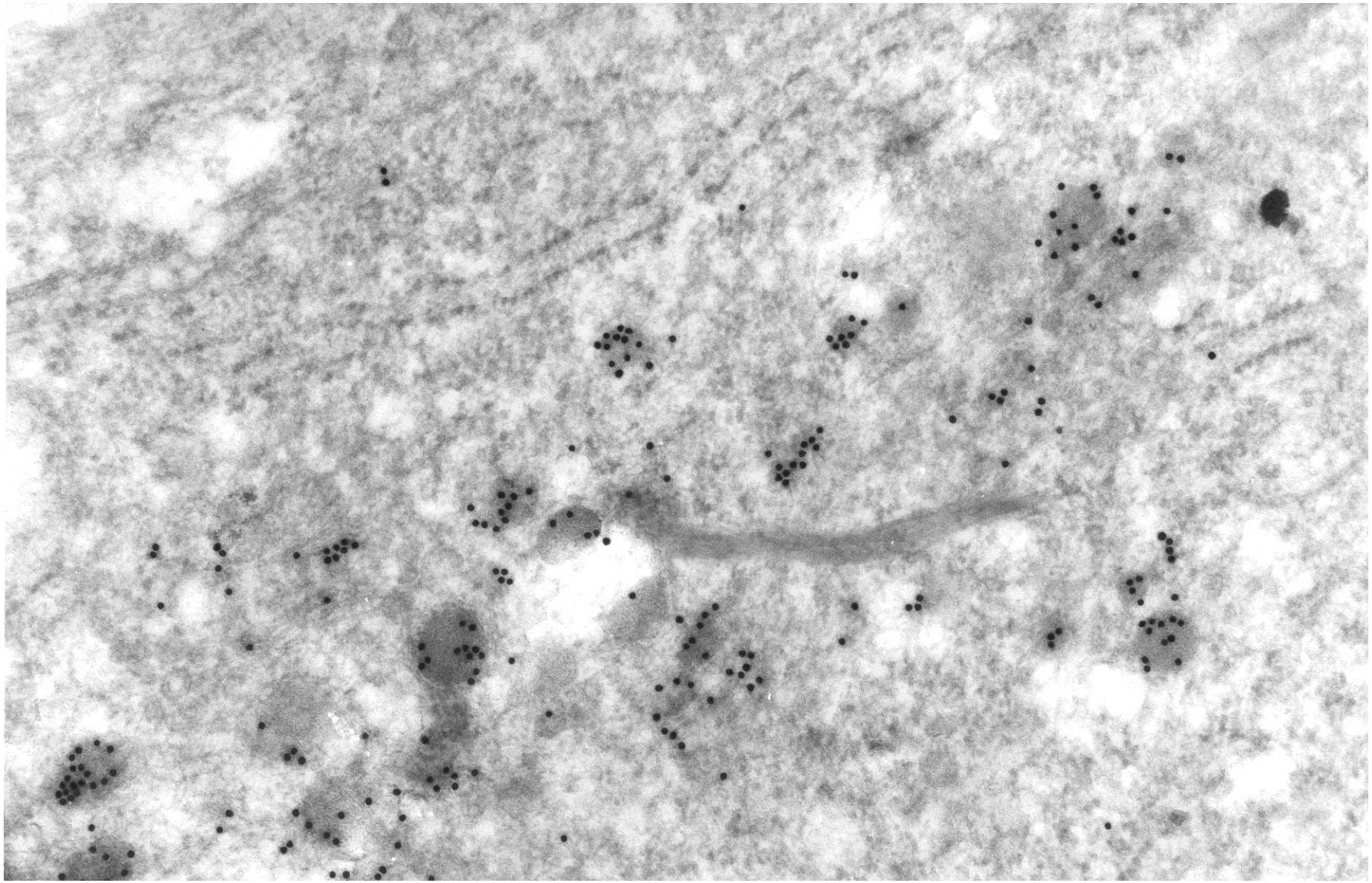
3. Structure – *quantitative 'composition'*
   a) elemental – Energy Dispersive Xray Spectroscopy (EDS or EDX)
   b) protein – immunocytochemistry

IMMUNOHISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY




IMMUNOPEROXIDASE
IMMUNOGOLD
IMMUNOFLUORESCENCE
DIRECT IMMUNOFLUORESCENCE


A
RPE
OS
IS
ONL
OPL
INL
IPL
GCL
Cnnm4
Cnnm4 + DAPI
Neg
(i)
(ii)
(iii)
B



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