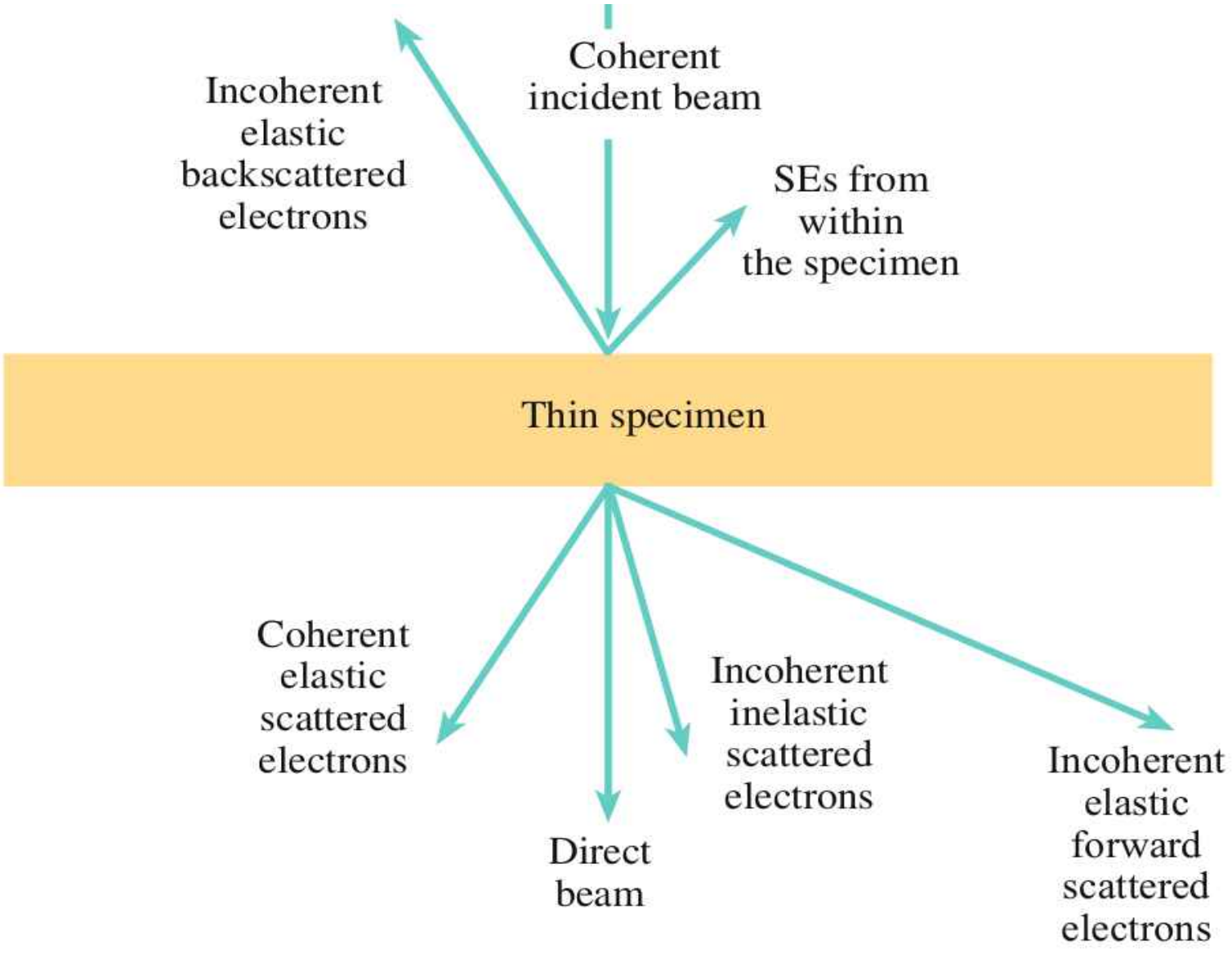
Sample preparation techniques

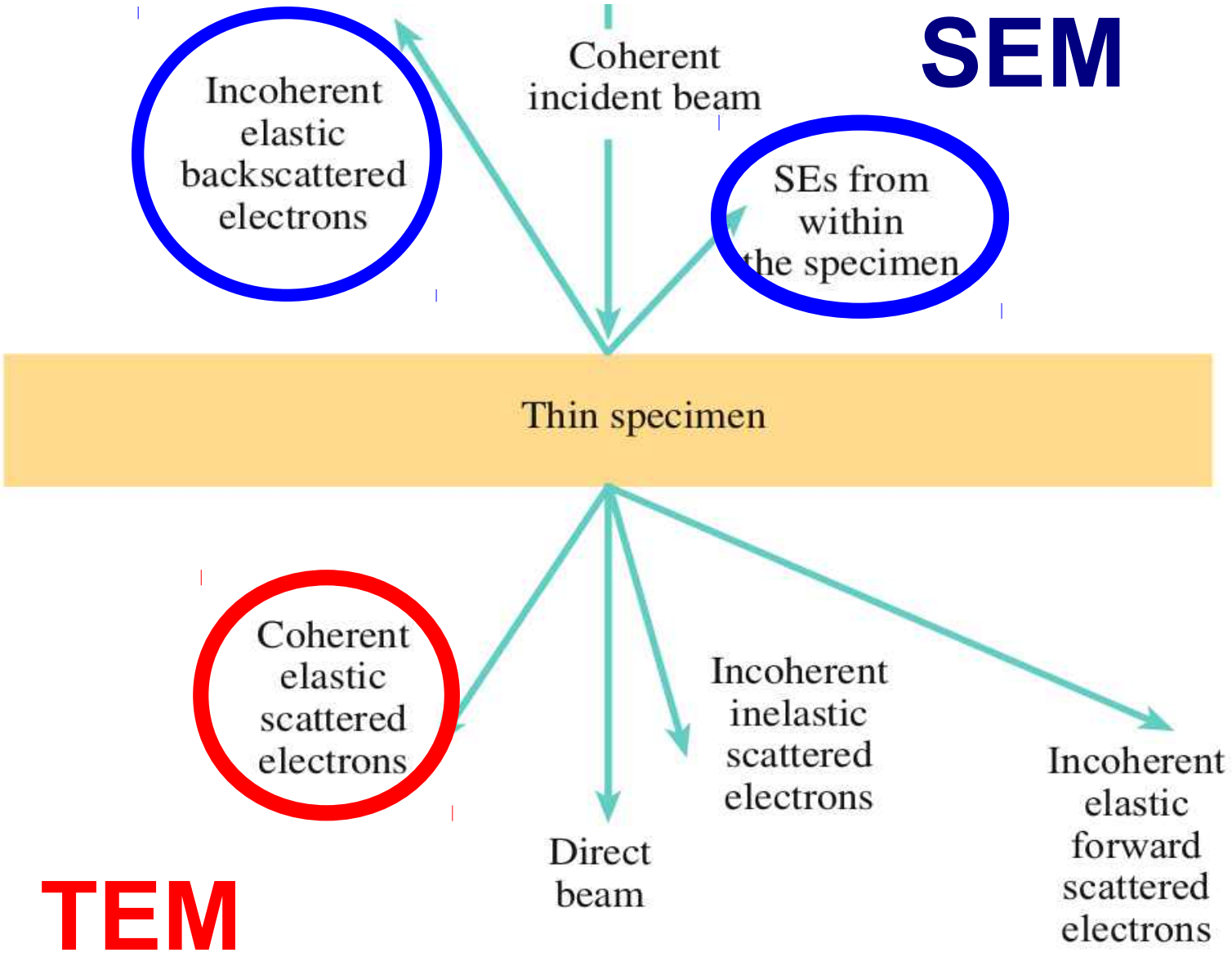
- Thin section methods
- **Heavy metal staining and shadowing**
- **Plunge freezing**
- High pressure freezing
- **Focus ion beam milling**

Interaction of electrons with specimen



Williams et al., TEM, Springer

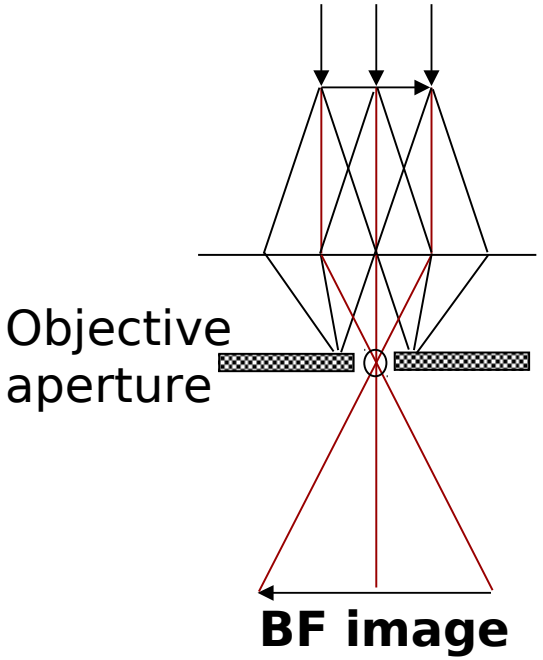
Interaction of electrons with specimen



Williams et al., TEM, Springer

Contrast in EM images

Amplitude contrast

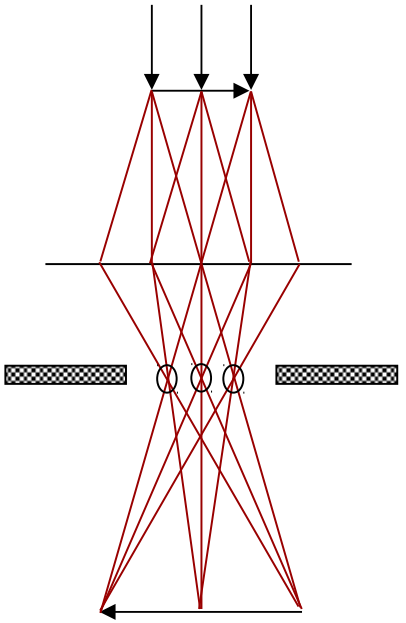


- difference in intensity in two adjacent area

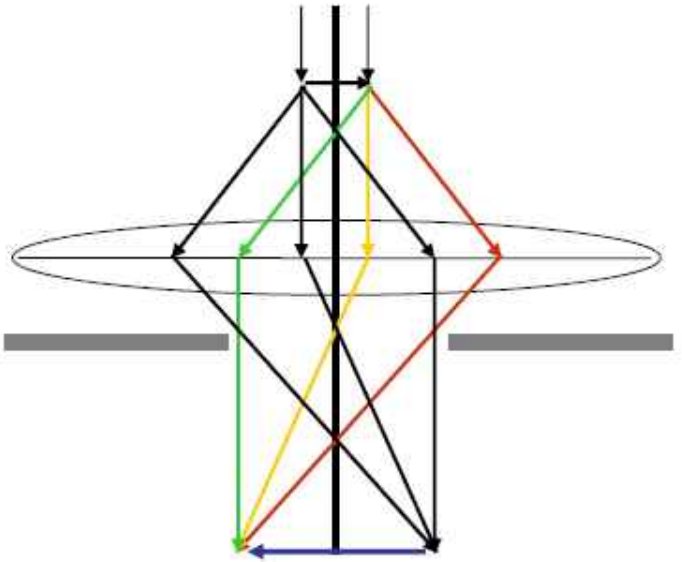
$$C = \frac{(I_2 - I_1)}{I_1} = \frac{\Delta I}{I_1}$$

Contrast in EM images

Phase contrast



- Transmitted and diffracted waves travel through different distances

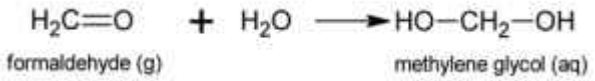


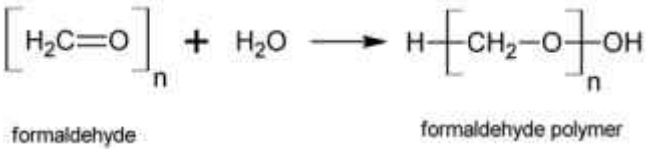
Thin sectioning methods

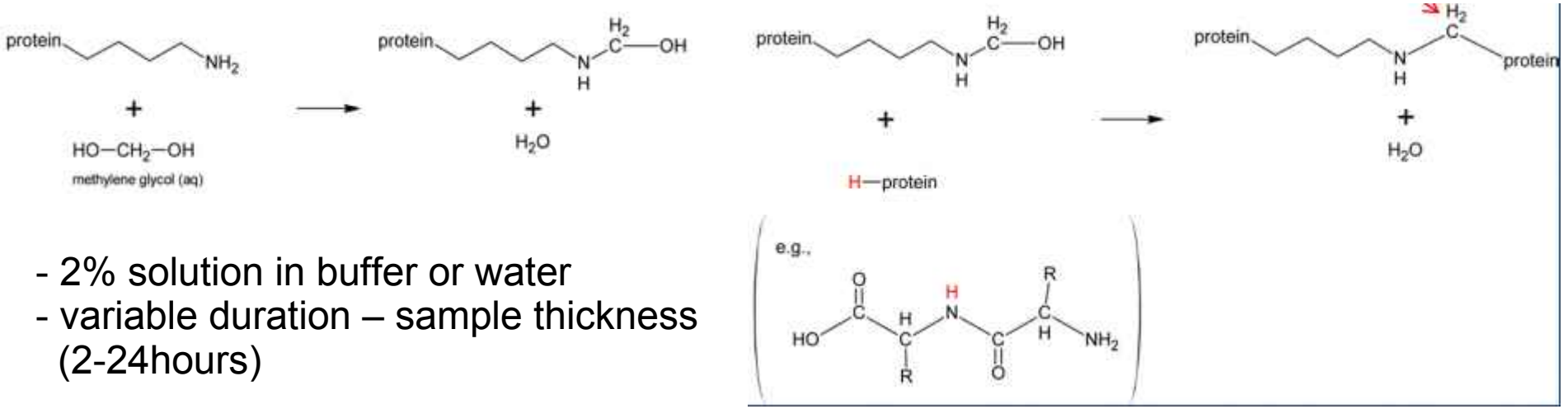
- Chemical fixation (formaldehyd, glutaraldehyde, osmium tetraoxide)
- Dehydration (EtOH, aceton)
- Plastic embedding
- Sectioning

Thin section methods

Chemical fixation - formaldehyd







- 2% solution in buffer or water
- variable duration – sample thickness (2-24hours)

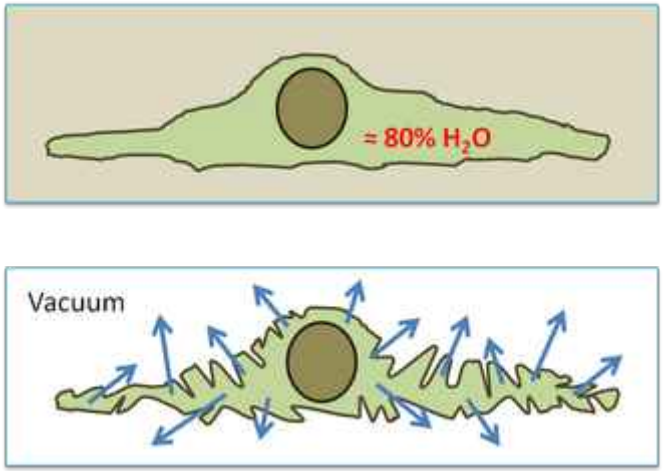
Thin section methods

Dehydration

- high vacuum in the miscorscope
- EtOH, aceton
- succesive increase of dehyd. agent concentration

30% aceton - 15 mins
50% aceton - 15 mins
70% aceton - 15 mins
90% aceton - 15 mins
100% aceton - 3 changes

Drawbacks:
- contraction of protein lipids
- sample shrinking up to 40%
- fromation of various artefacts



Thin section methods

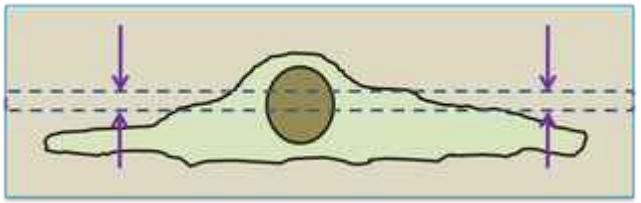
Resin embedding

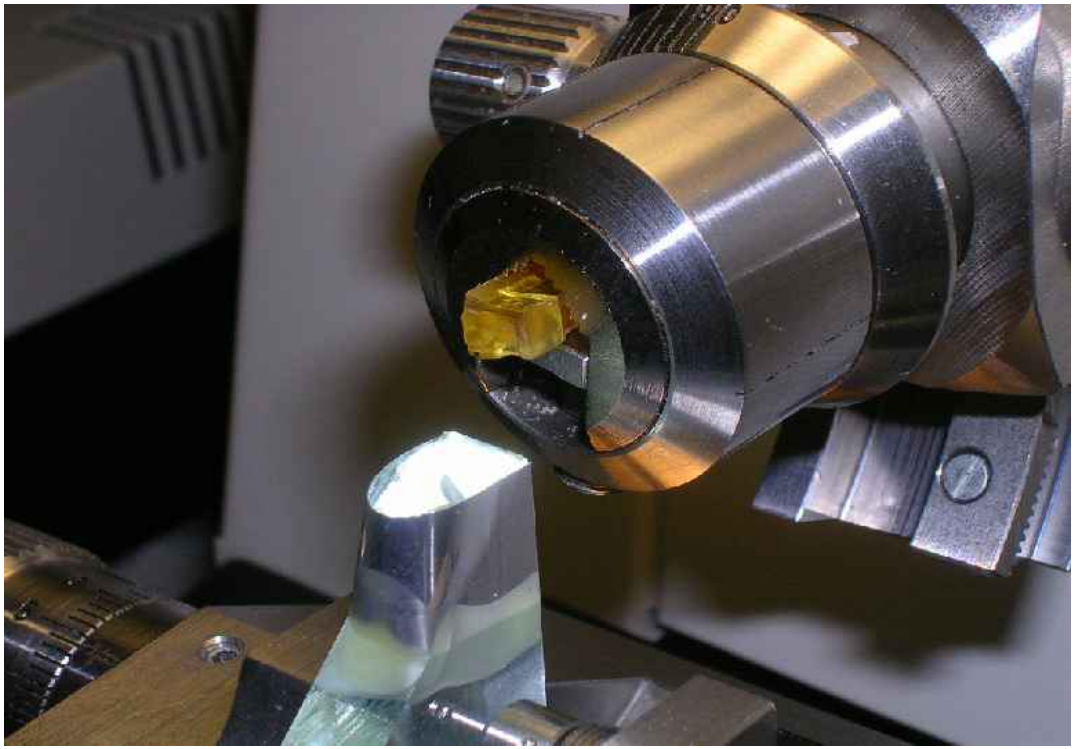
Resin infiltration: 2:1 mix of propylene oxide:resin (1h)
1:1 mix of propylene oxide:resin (1h)
1:2 mix of propylene oxide:resin (1h)
100% resin overnight

Polymerization: 12-24 hours at 60-70C

Thin section methods

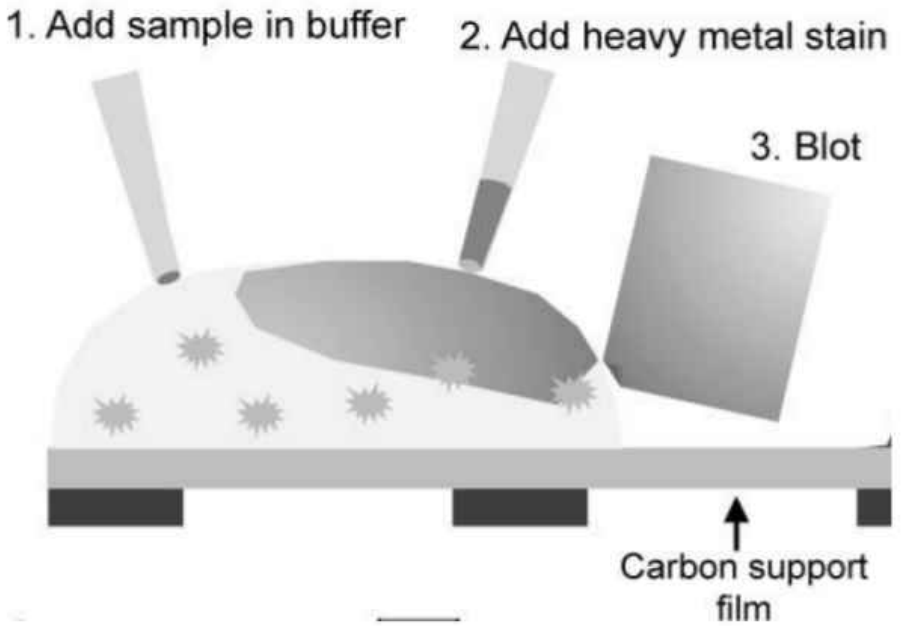
Sectioning

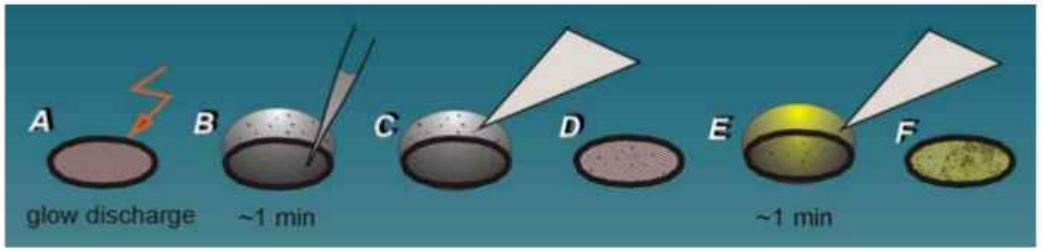




Heavy metal staining and shadowing

Negative staining

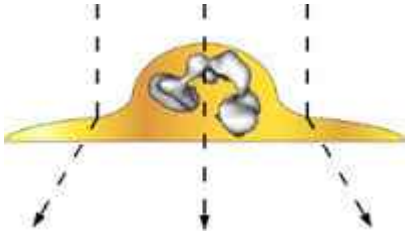


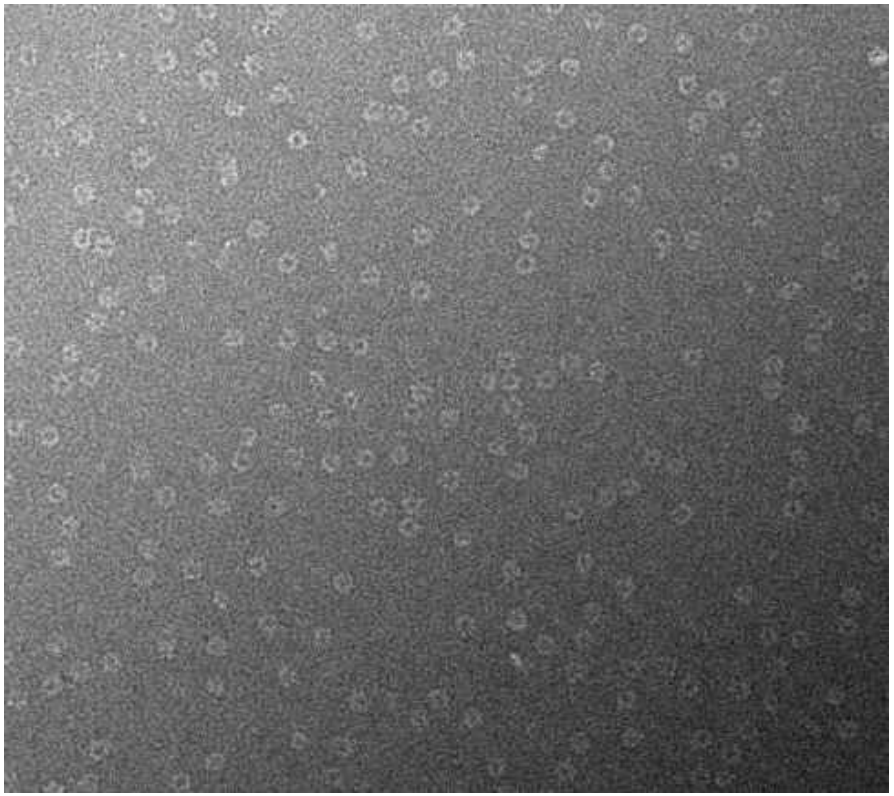


Stains: uranyl acetate (pH=4)
uranyl formate (pH=4)
ammonium molybdenate (pH=7)
phosphorus thungstanate (pH=7)

Heavy metal staining and shadowing

Negative staining

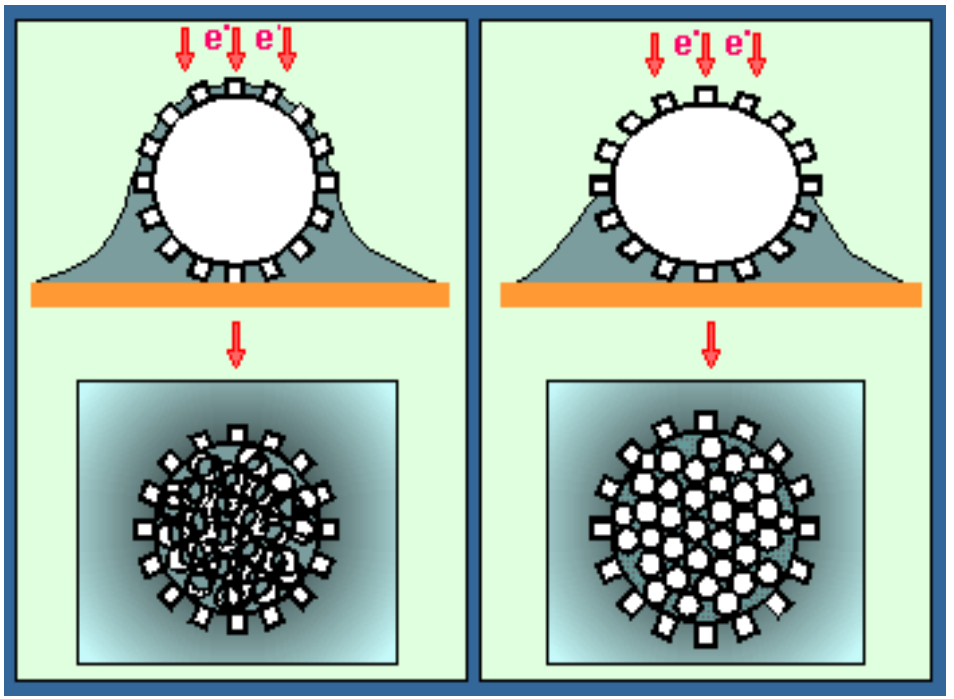




Pros: quick sample screening
high amplitude contrast
less prone to beam damage

Heavy metal staining and shadowing

Negative staining



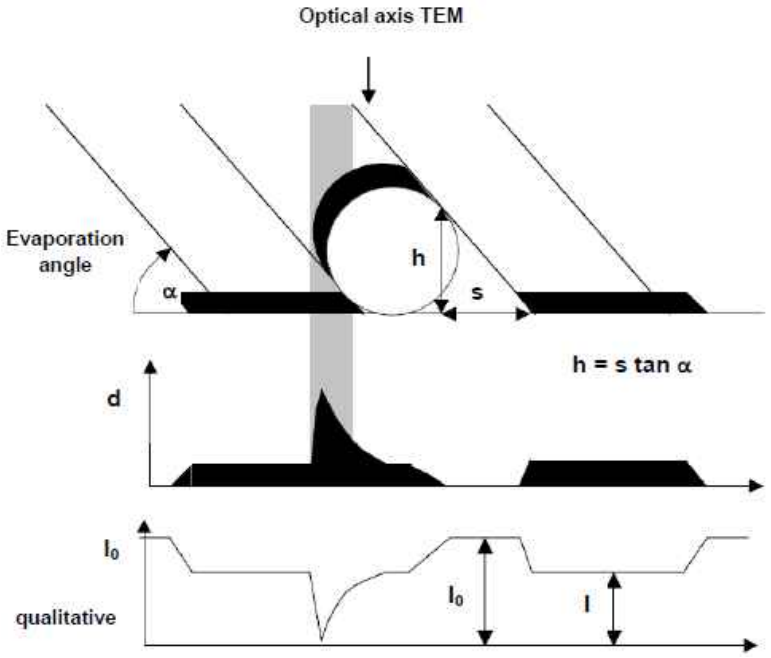
Pros: quick sample screening
high amplitude contrast
less prone to beam damage

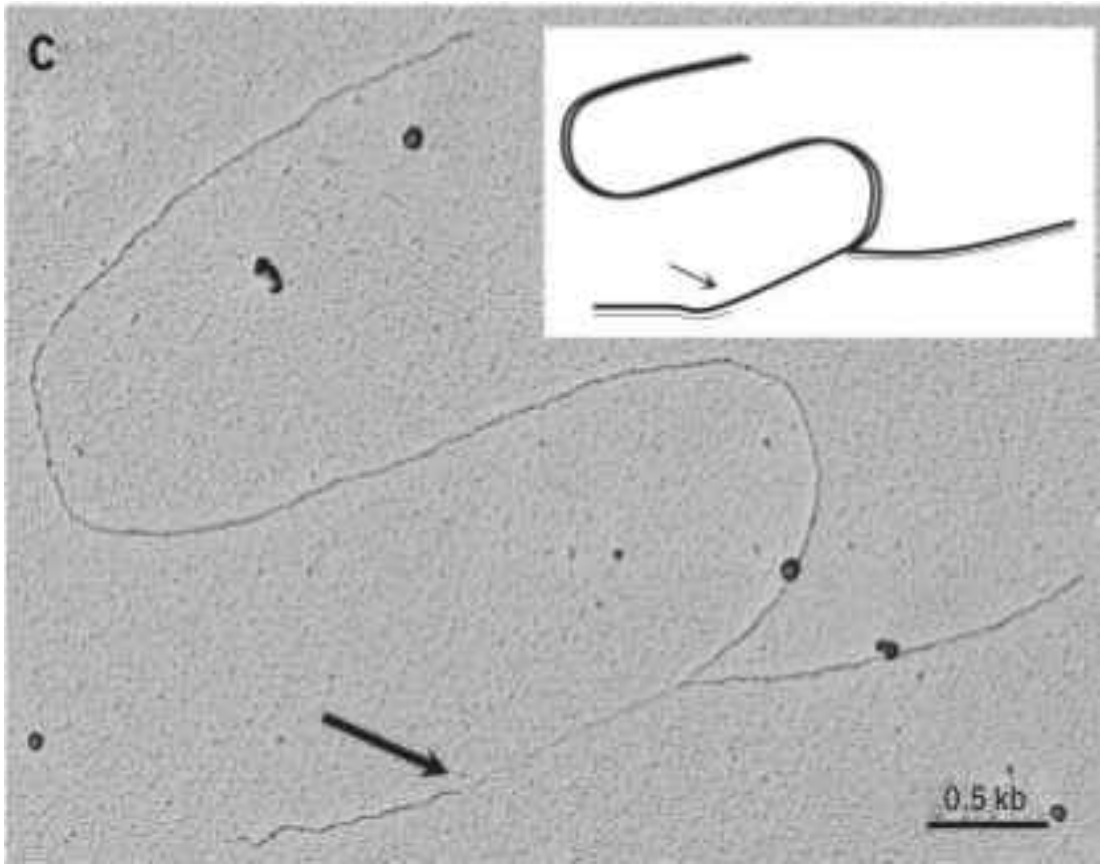
Cons: limited resolution (20A)
flattening artefacts
denaturation of proteins

Heavy metal staining and shadowing

Metal shadowing

- DNA visualization





Hashimoto et al., NMSB 2010

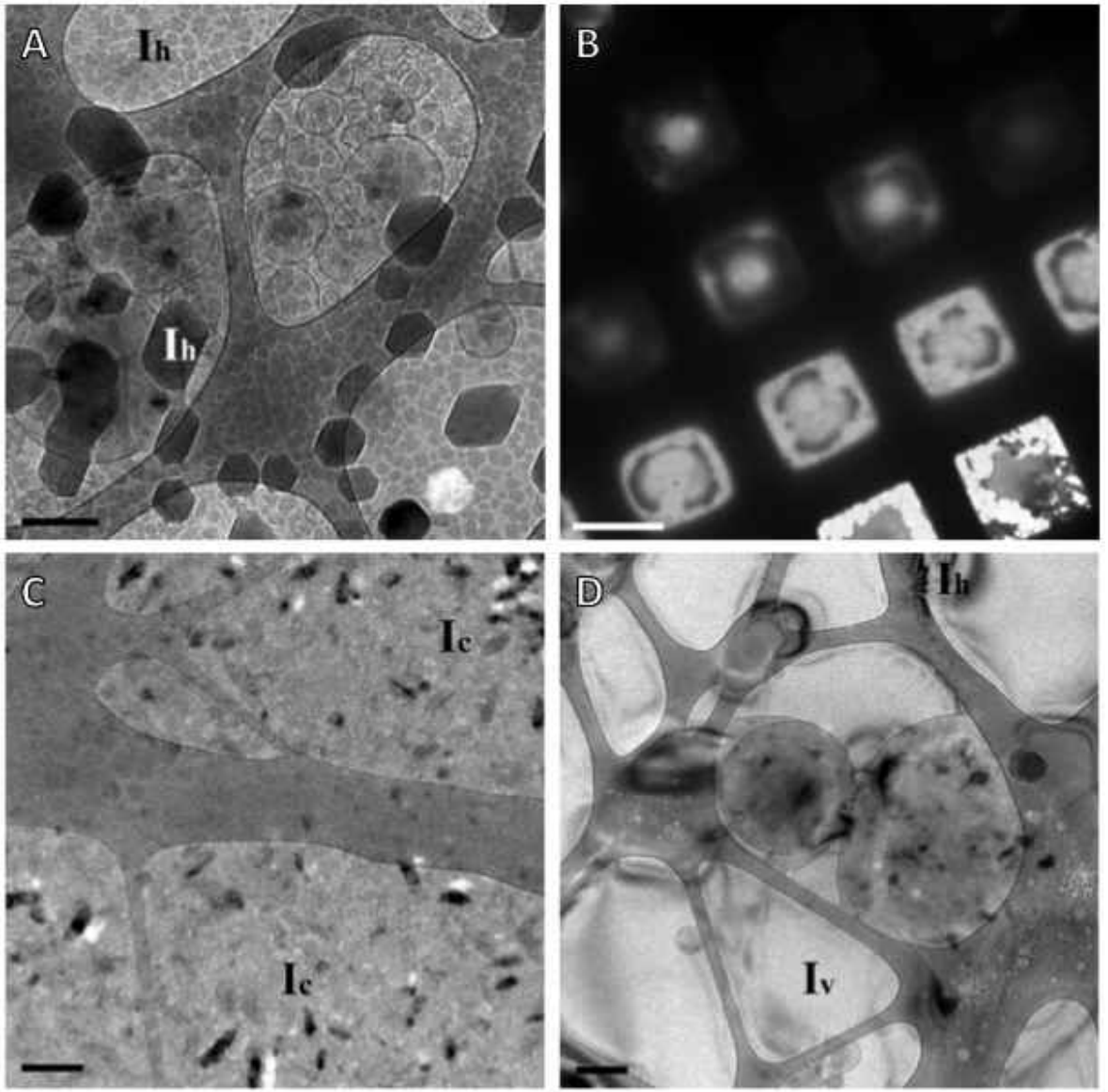
Plunge freezing

- rapid immersion of buffered sample into cryogen
- cryogens: liquid ethane,
  ethane:propane mixture

-vitrification has to be fast ~10000 K/s

=> amorphous ice
=> thin layer (200-600nm)

Plunge freezing



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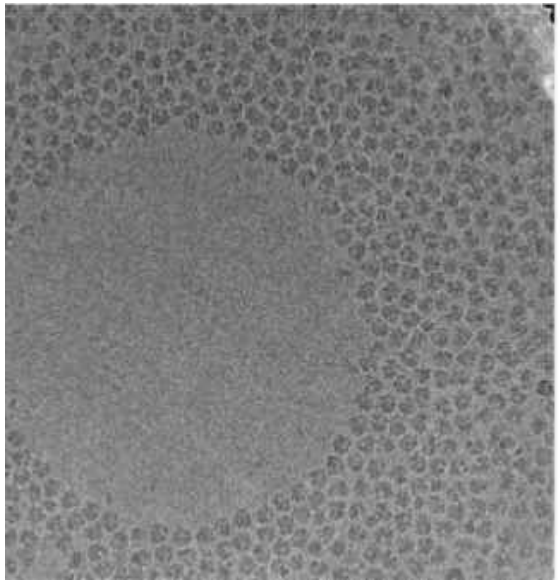
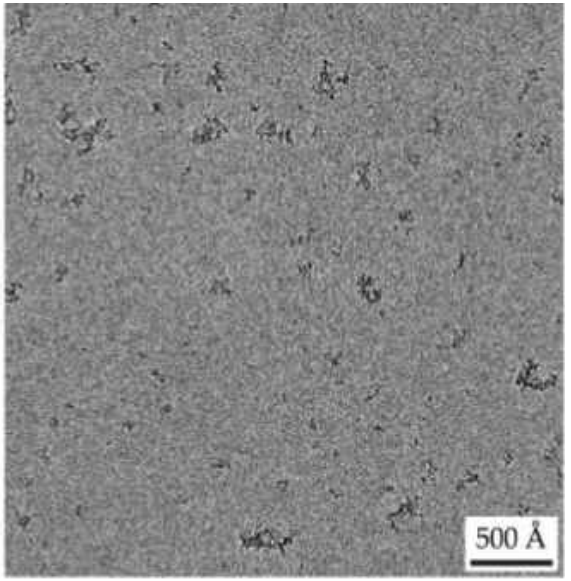
Pros: - sample in frozen hydrated state (native)
- internal structures can be visualized
- high resolution information preserved

Cheng et al. Cur. Microscopy 2010

Plunge freezing



Extrusion of particles from thin ice



Denaturation at air water interface

- rapid immersion of buffered sample into cryogen
- cryogens: liquid ethane, ethane:propane mixture

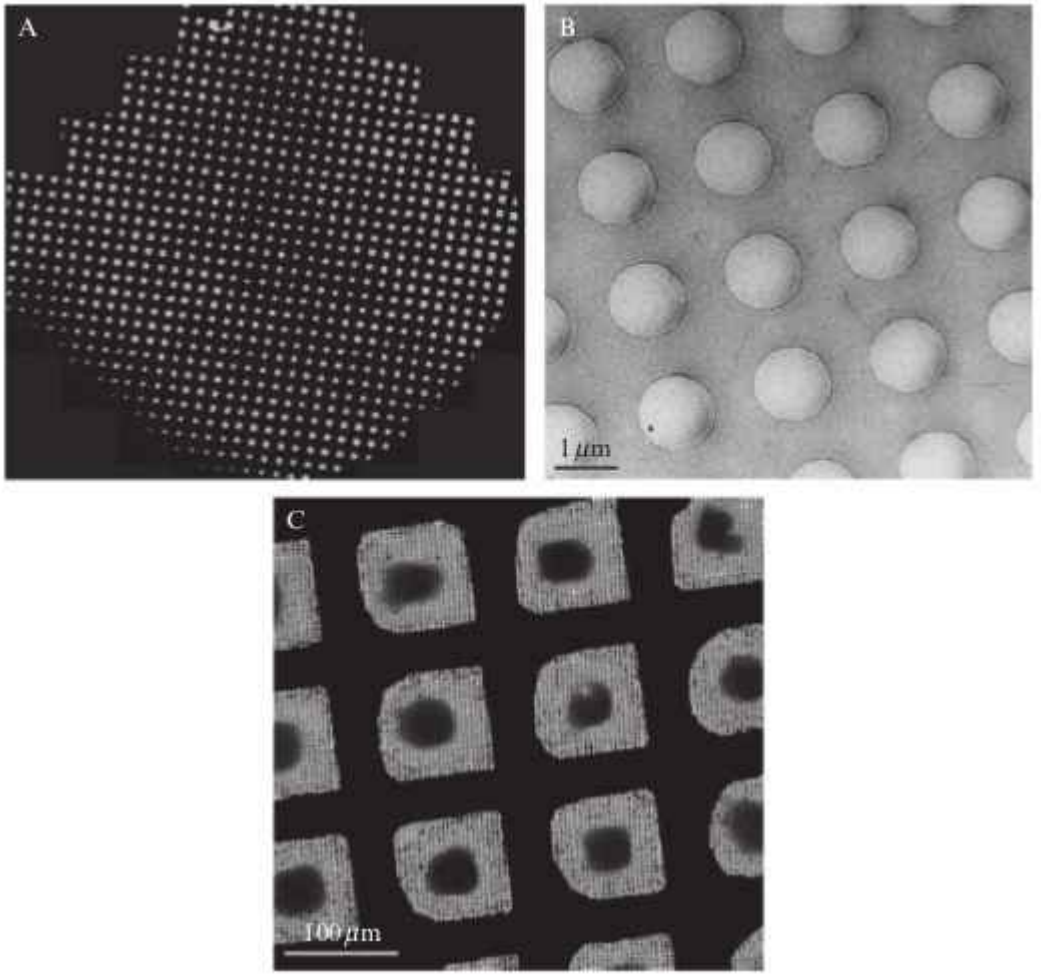
-vitrification has to be fast ~10000 K/s

=> amorphous ice
=> thin layer (200-600nm)

Pros: - sample in frozen hydrated state (native)
- internal structures can be visualized
- high resolution information preserved

Cons: - low signal to noise
- prone to radiation damage
- sample handling more difficult

Plunge freezing



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- high resolution information preserved

ace

High pressure freezing