Part 4 - Fundamental Challenges in Biological TEM

Fundamental challenges in biological EM

- Preserving native structure within microscope
- Obtaining 3D information from projections
- Dose limitations

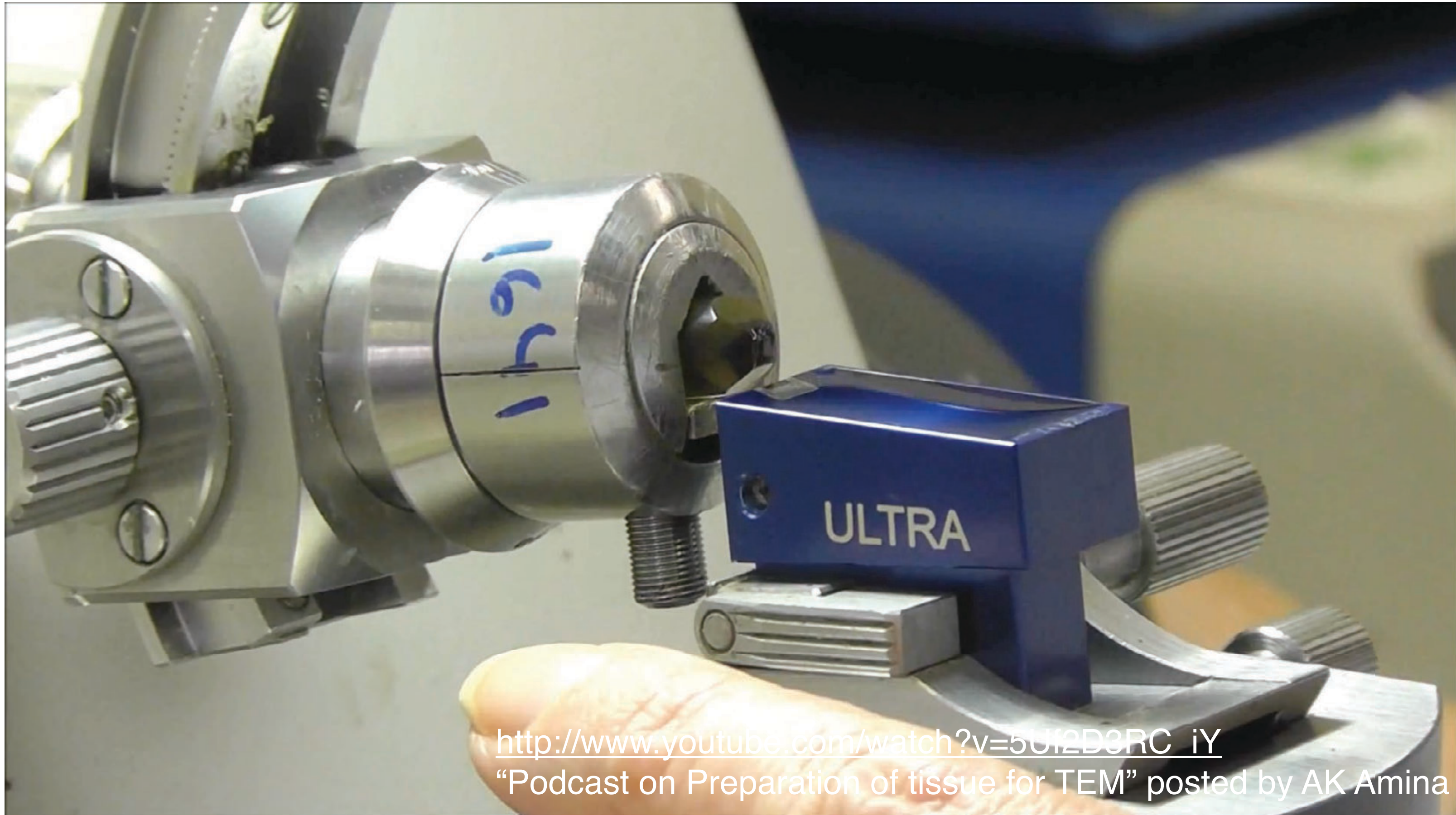
Sample preparation

1. Traditional “thin-section” EM
2. Staining/shadowing
3. High pressure freezing
4. Plunge freezing

“Traditional” EM

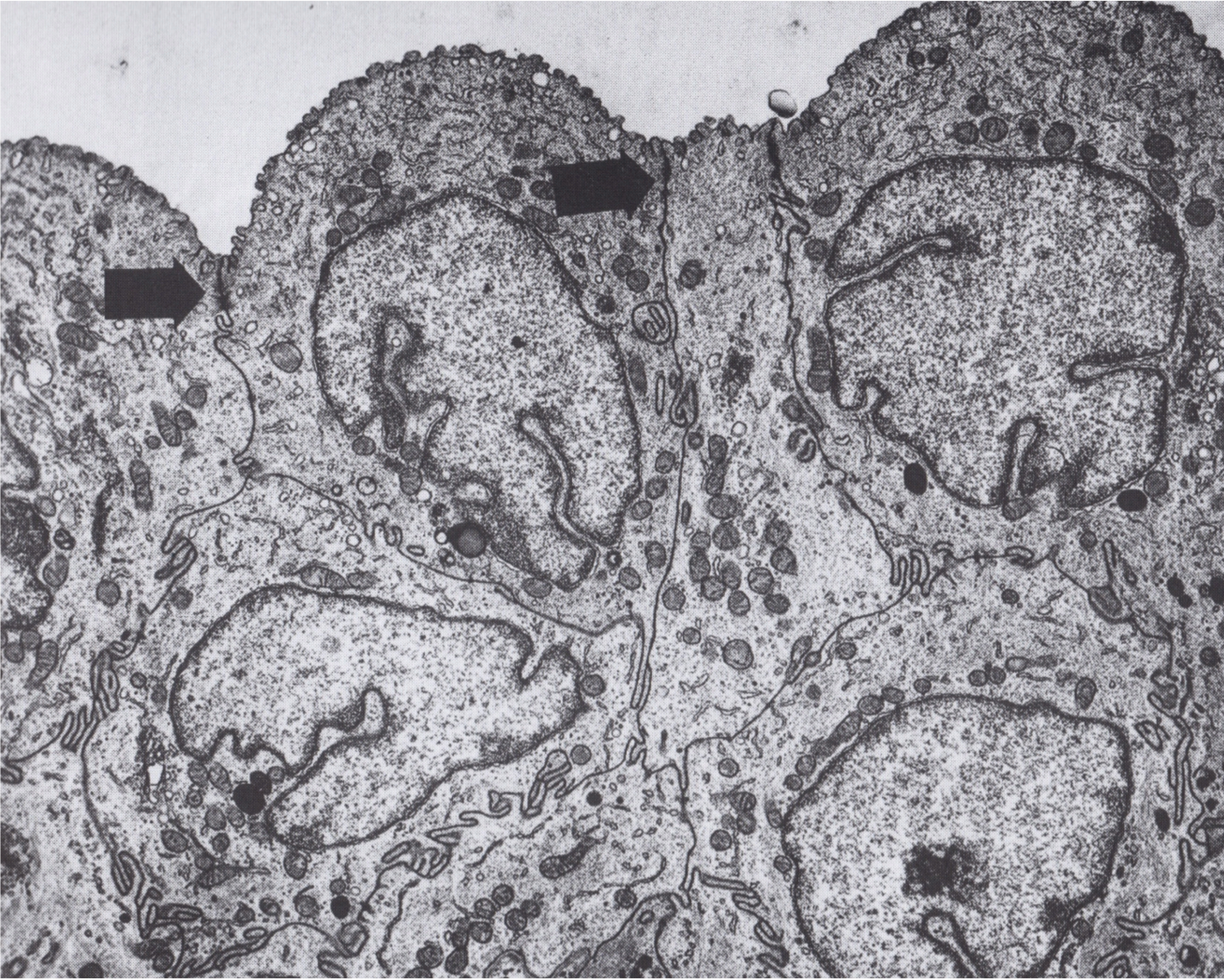
- chemical fixation (aldehydes and osmium)
- dehydration
- plastic embedment (Epon, others)
- sectioning (with a “microtome”)
- staining (uranyl acetate, lead citrate)

Microtomy



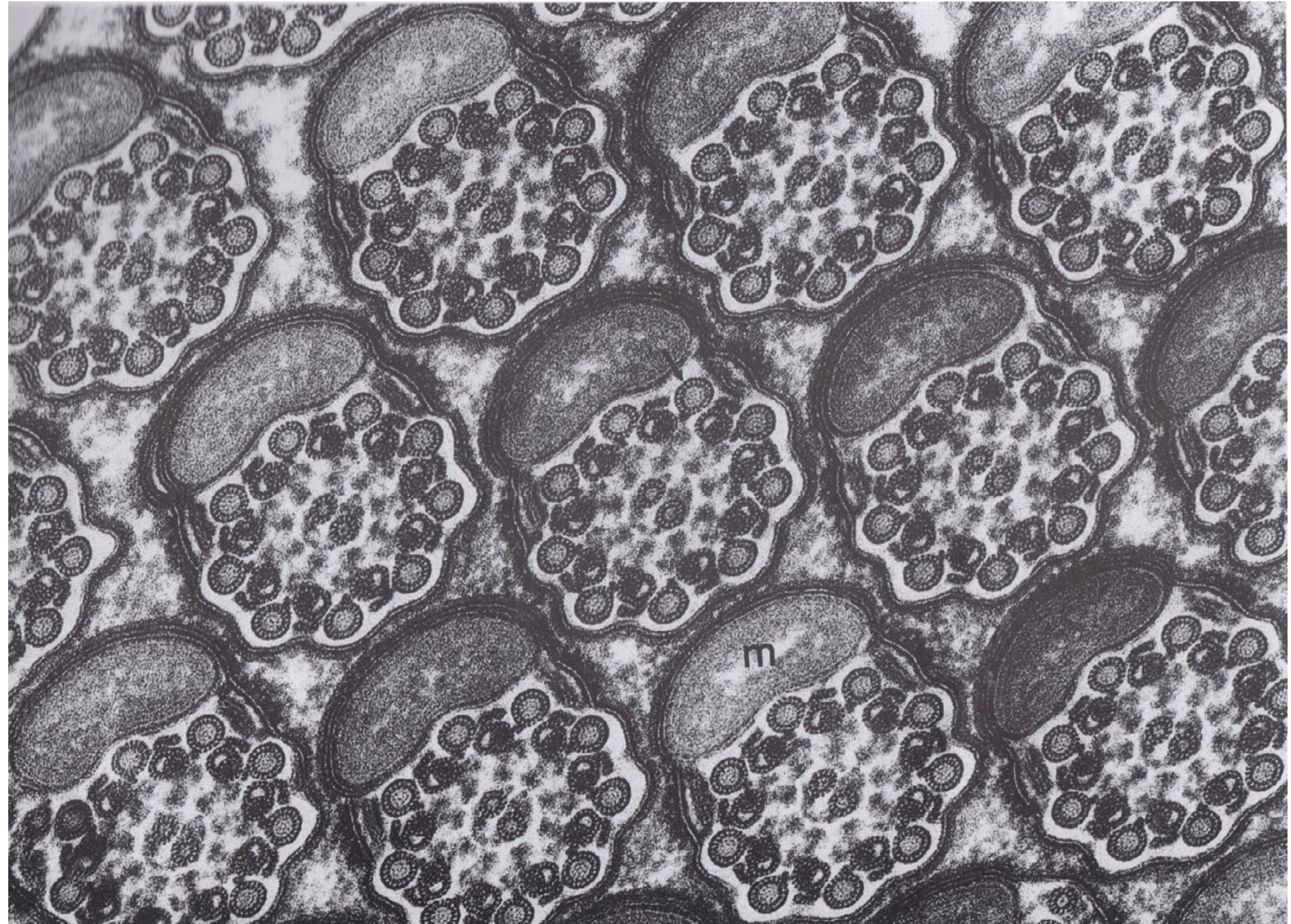
http://www.youtube.com/watch?v=5Uf2D3RC_iY
“Podcast on Preparation of tissue for TEM” posted by AK Amina

example "traditional thin-section EM" image

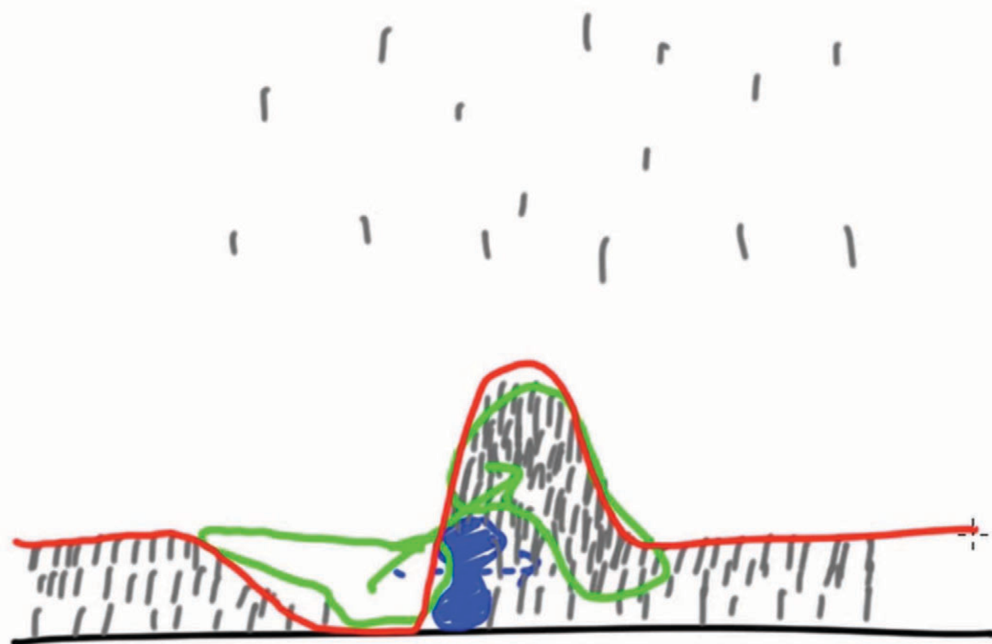


Mouse urethra epithelium
Electron microscopy,
by Bozzola and Russells pg. 493

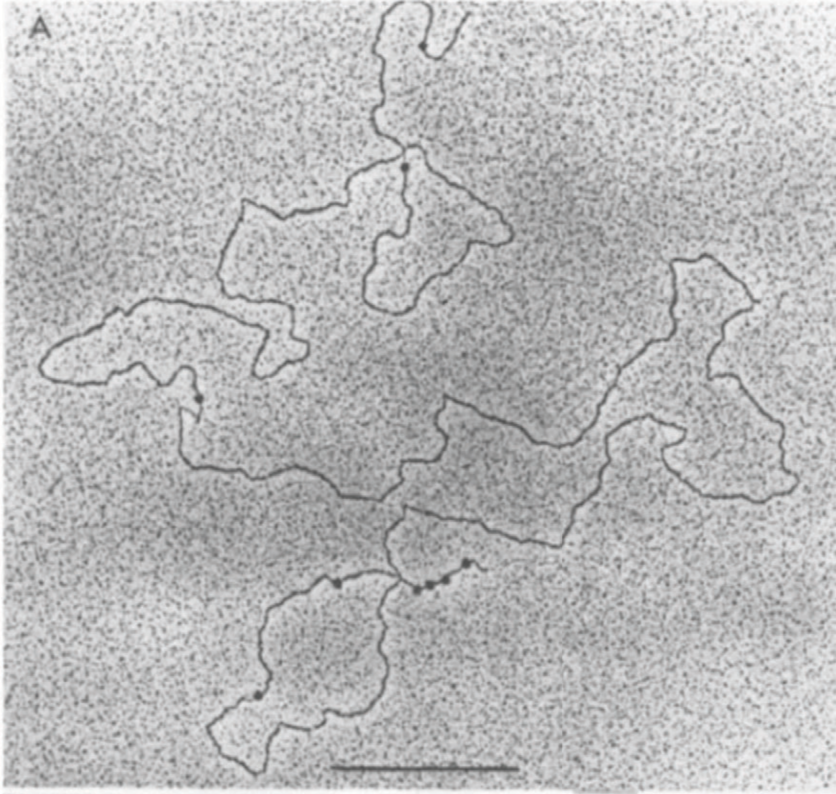
Insect sperm flagella
Electron microscopy by
Bozzola and Russells pg. 553



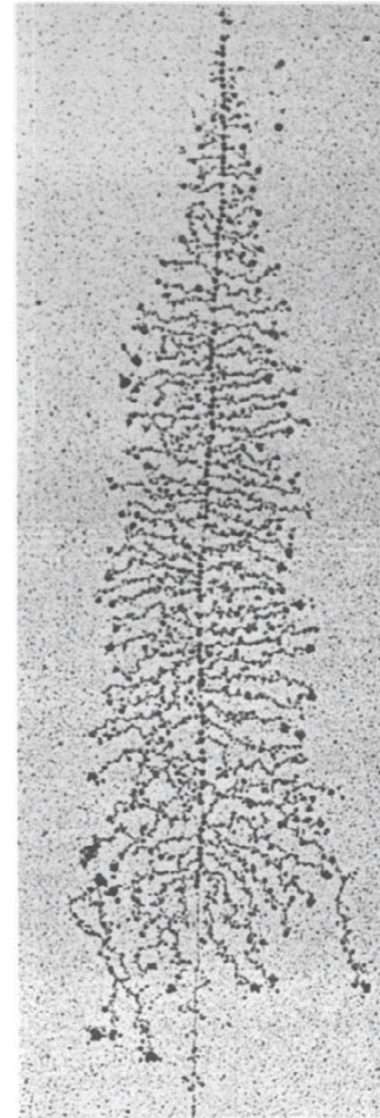
Metal shadowing



Metal shadowing examples



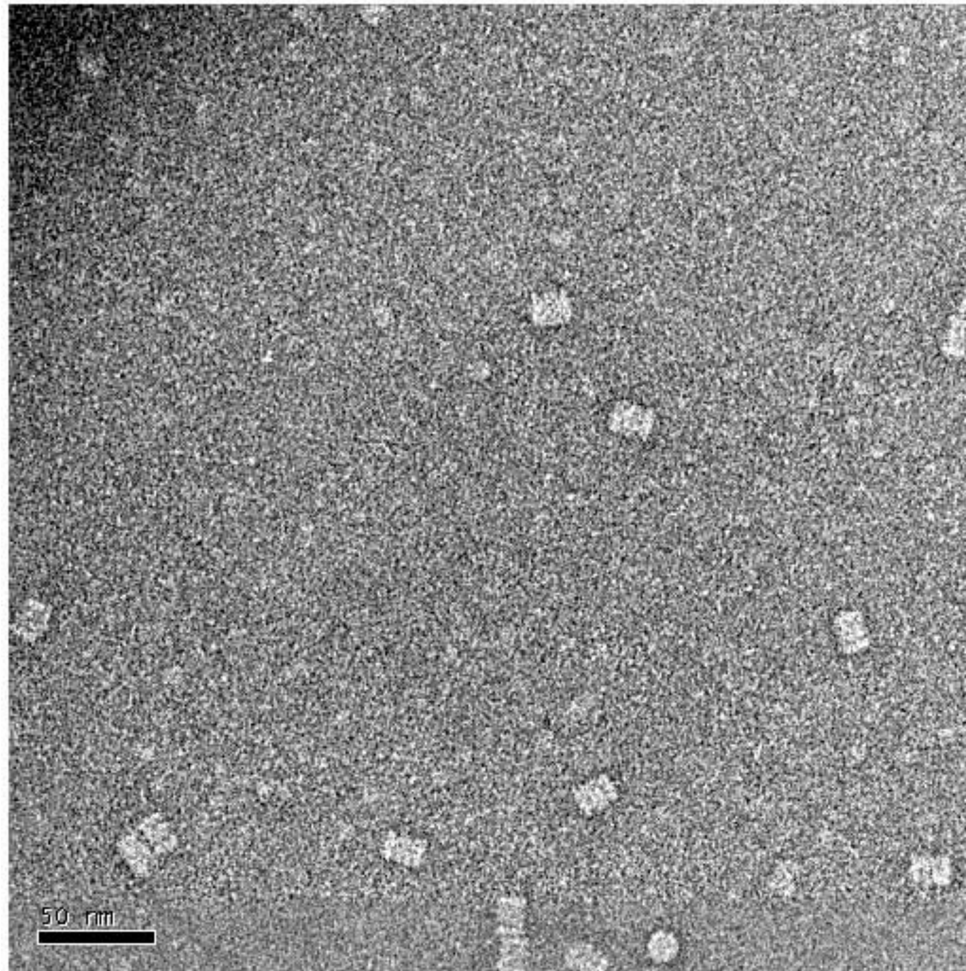
Annu. Rev. Biophys. Bioeng. (1978) 7:19

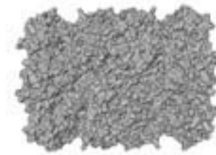


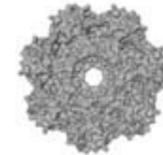
Histochem. Cell Biol. (1996) 106:167

Negative staining

“negative staining” with uranyl acetate







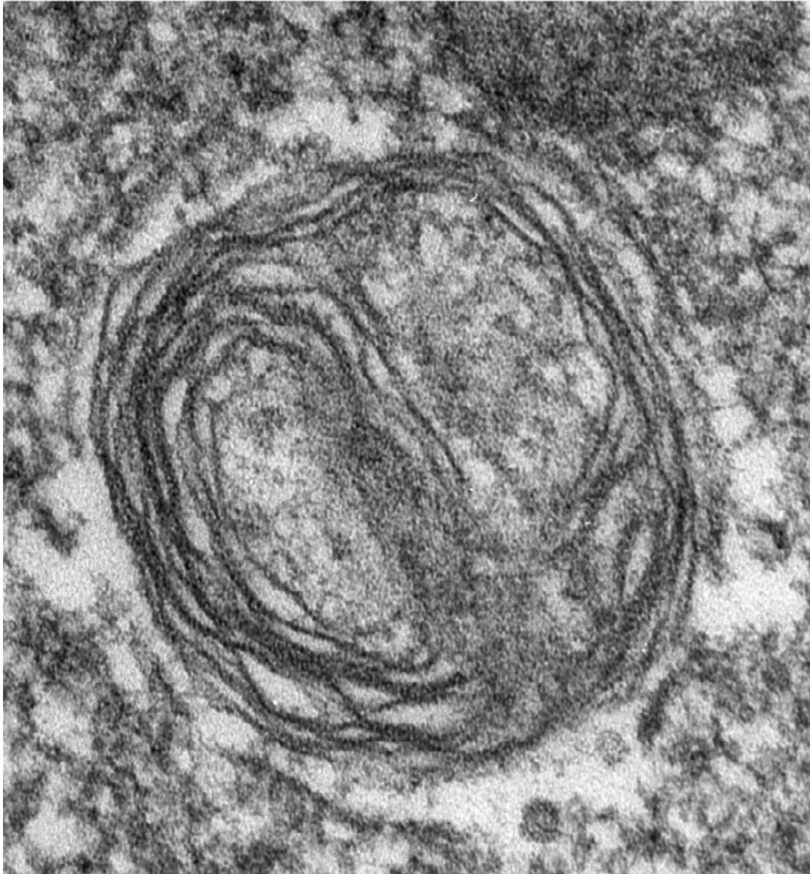
Sample prep - Room temperature methods
Concept check questions:

- Why can't cells just be inserted into the microscope and imaged (without any special preparation)?
- What is "chemical fixation", what agents are used to do it, and what are its advantages and disadvantages?
- Once a cell or tissue is chemically fixed, what else is typically done in preparation for traditional "thin section" EM?
- What metal stains are typically used for thin-section EM, and how do they effect the visibility of sample structures?
- How does "metal shadowing" work?
- How is metal shadowing different from "negative staining"?

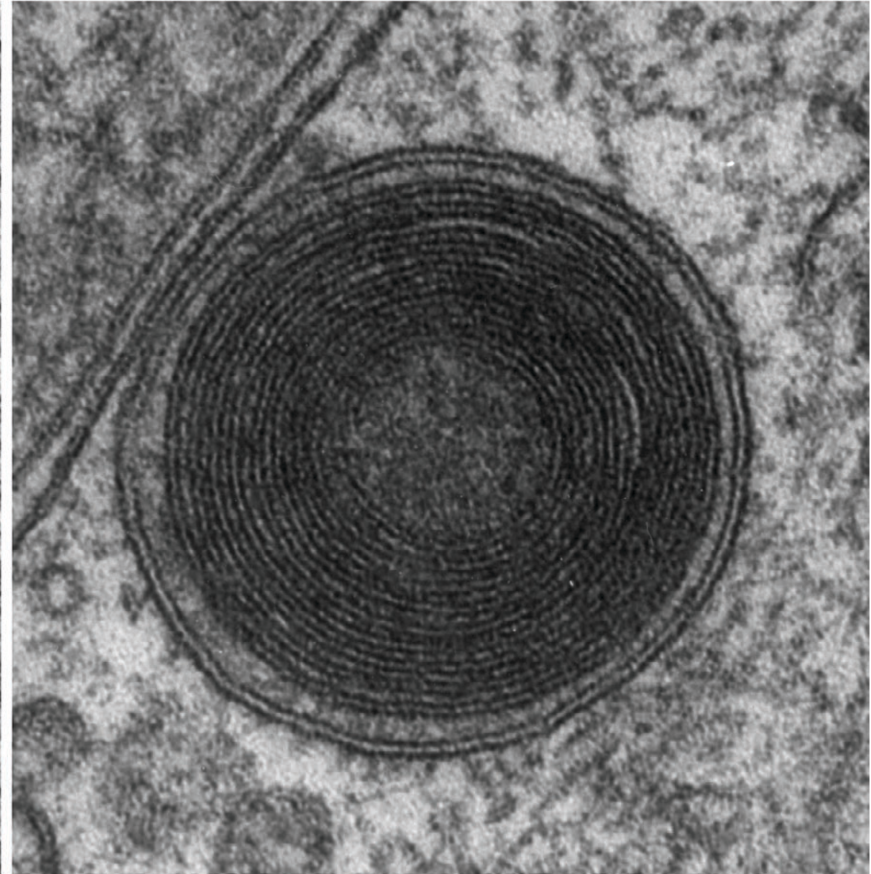
High pressure freeze/freeze-substitute/ low-temperature embed

- pressure prevents ice crystal formation
- dehydrate, fix, embed slowly at -20° C
- use low-temp polymerizing plastic

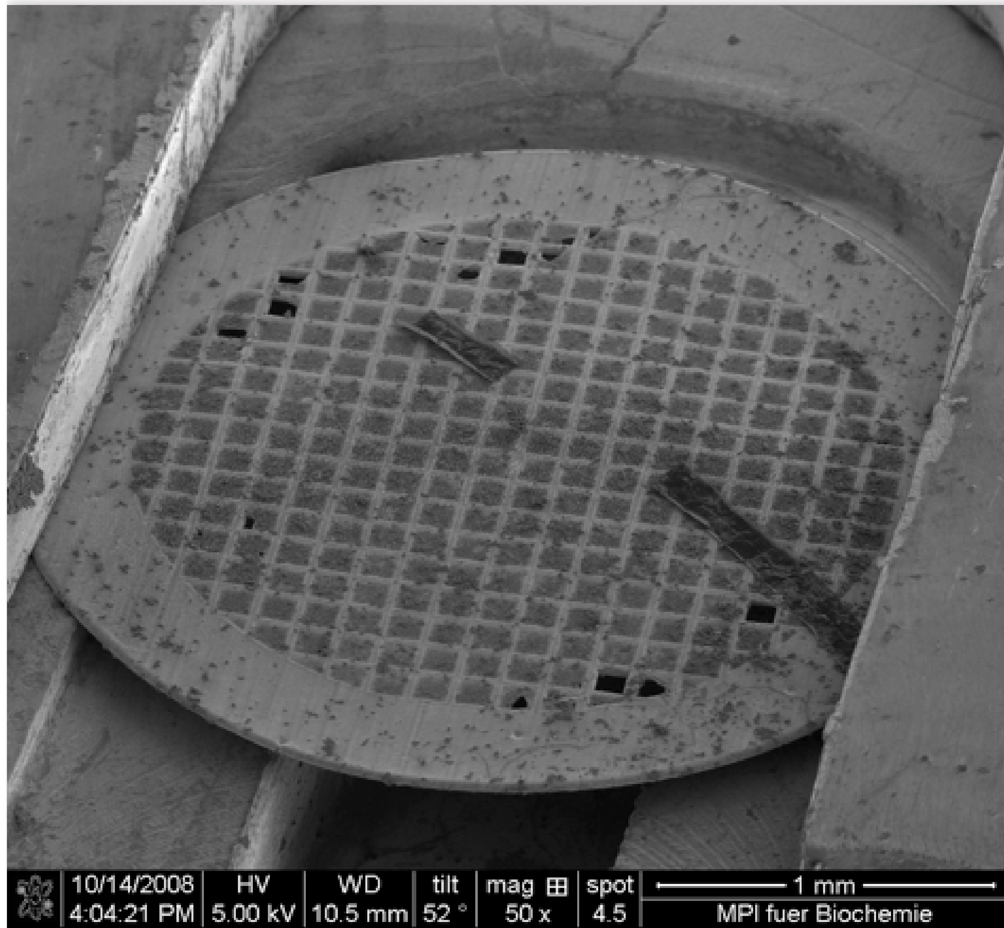
Multivesicular body images courtesy Mark Ladinsky



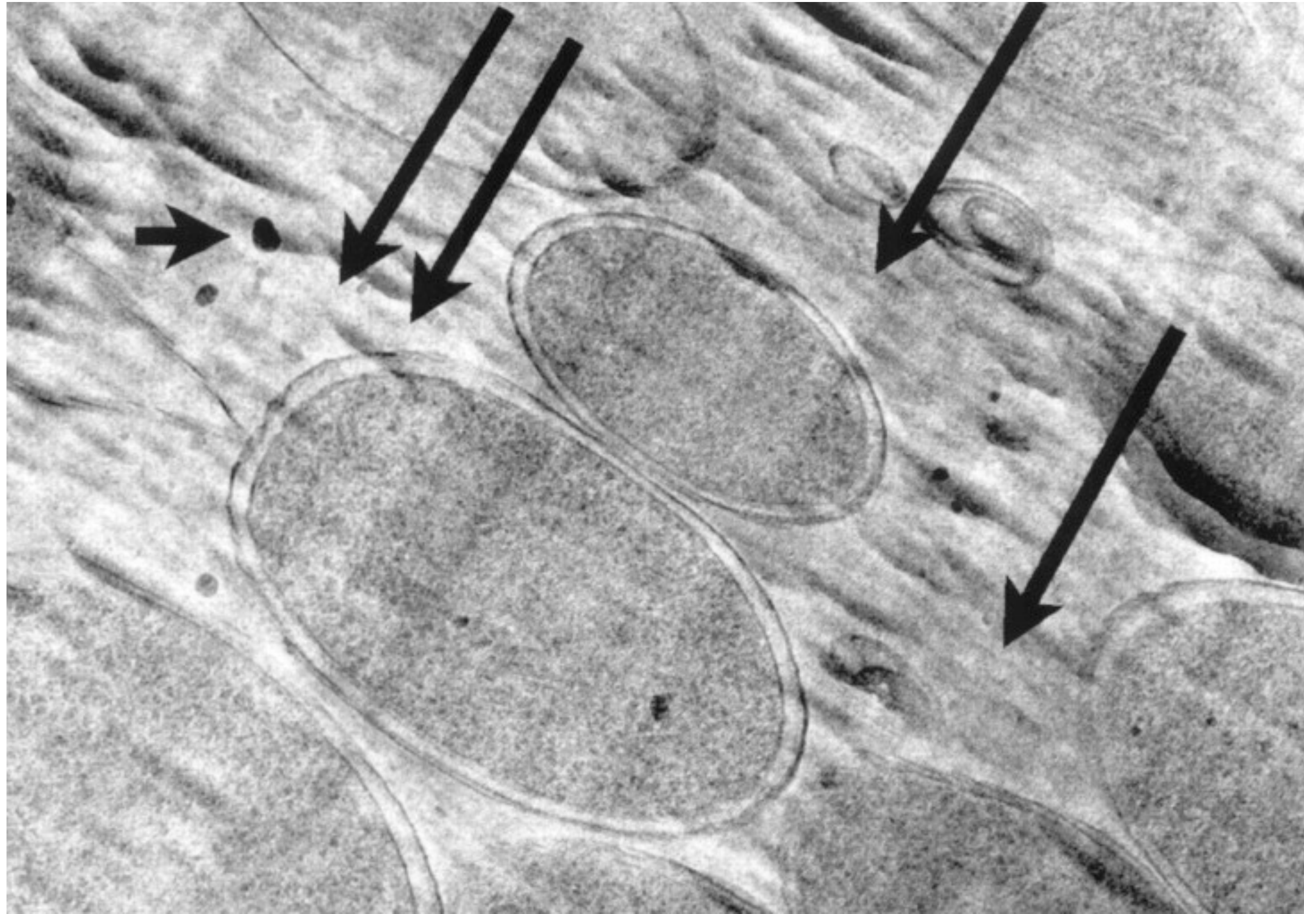
Traditional Chemical Fixation



Ultra-Rapid Freezing and Freeze-Substitution



Juergen Plitzko, MPI Biochemistry



Matias et al., J. Bacteriology 185:6112 (2003)

Artifacts arising from cryo-sectioning

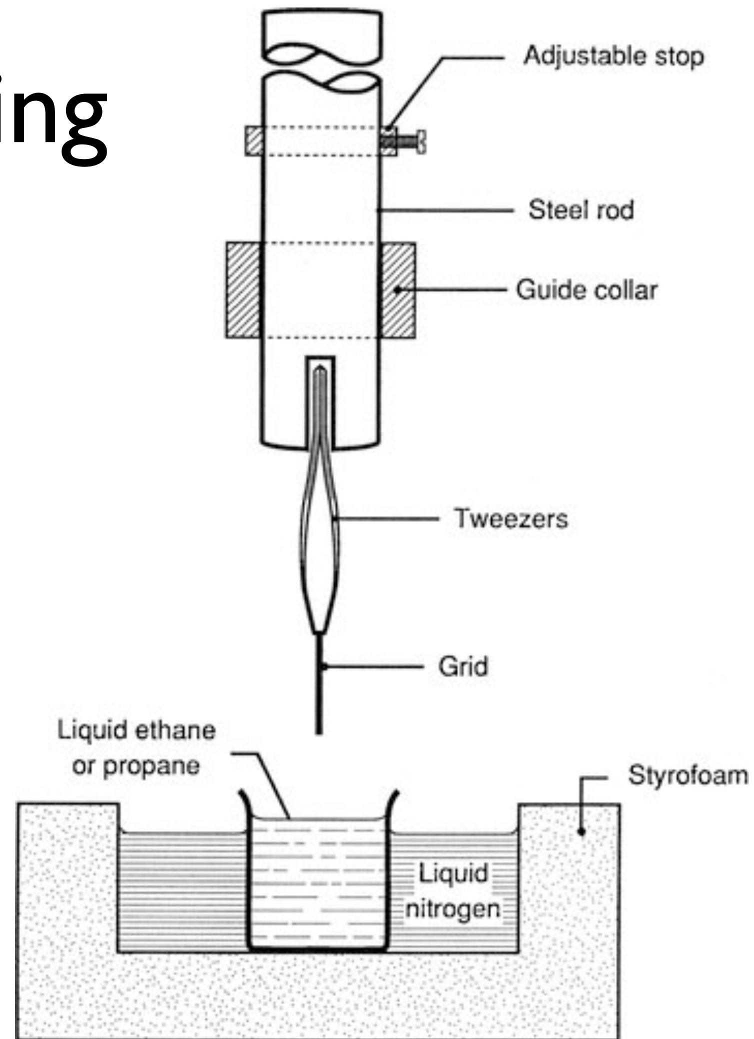
Crevassing

compression

knife marks

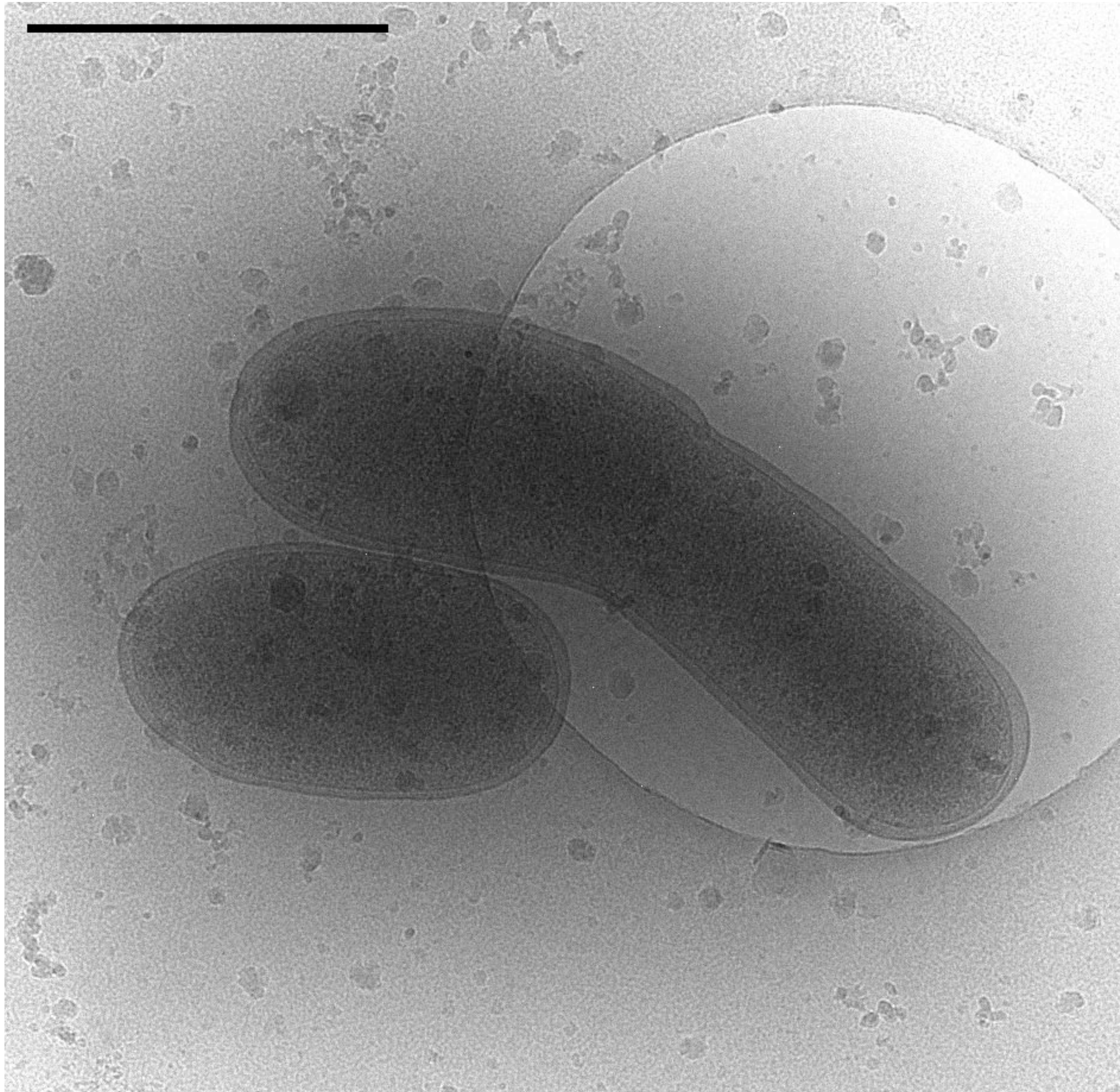
11:03 / 27:46

Plunge-freezing



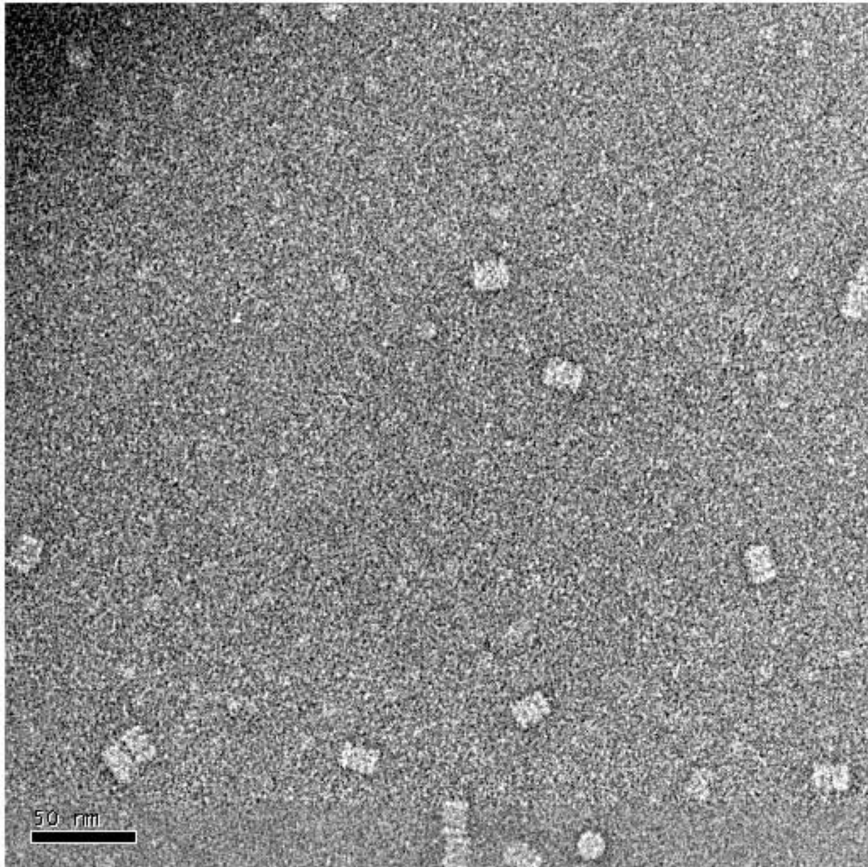
Stewart, 1991

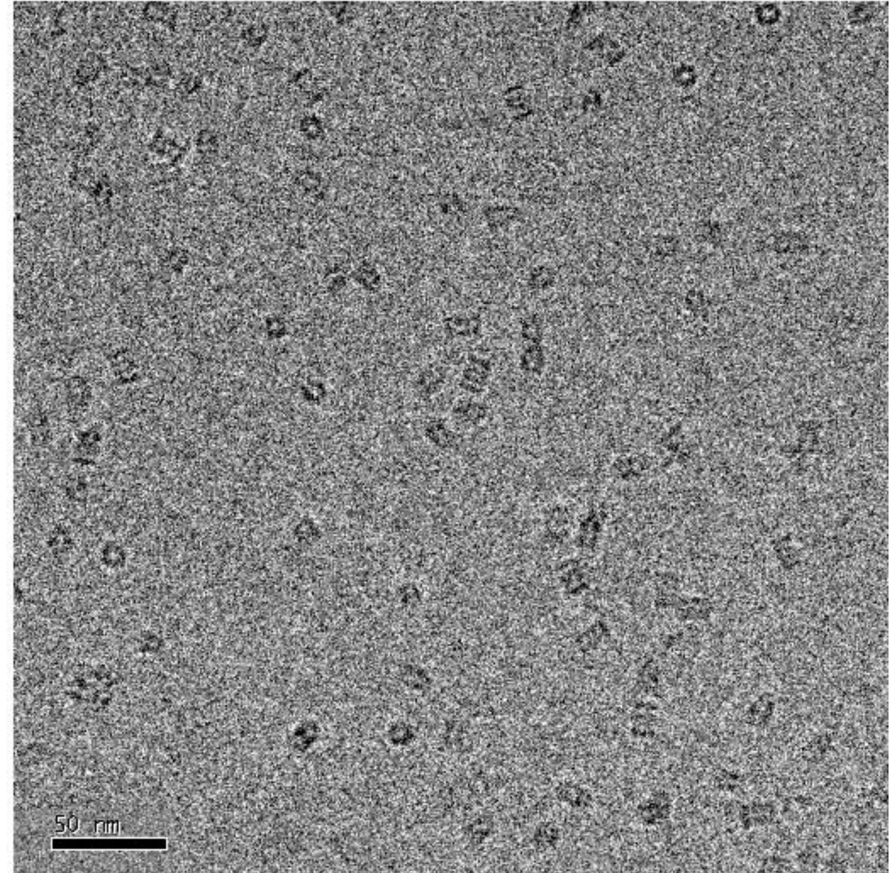
Example plunge-frozen cell

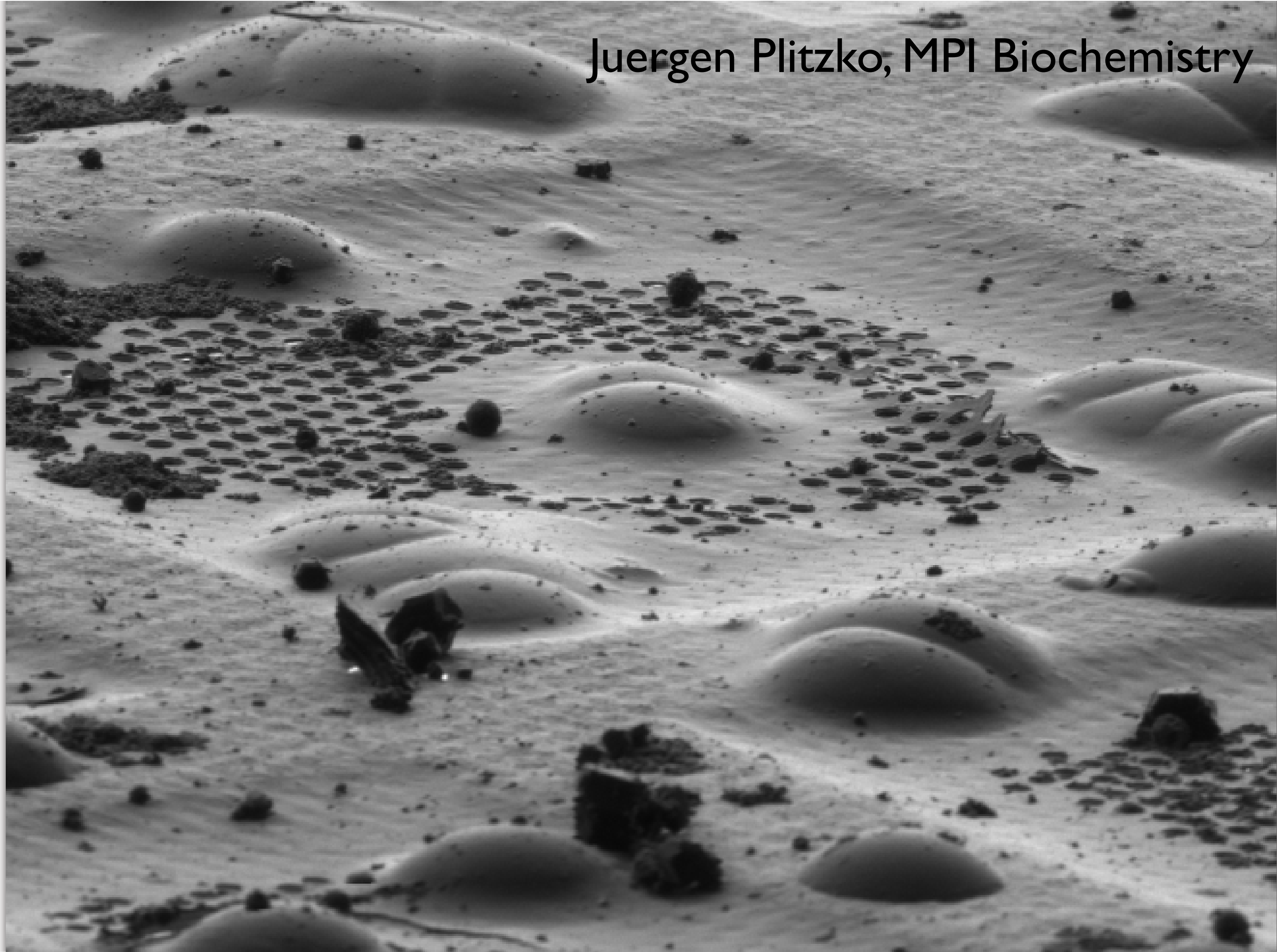


negative staining

plunge-freezing

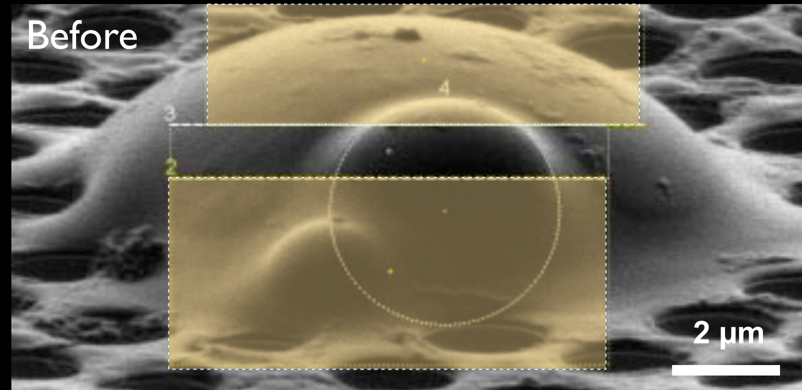


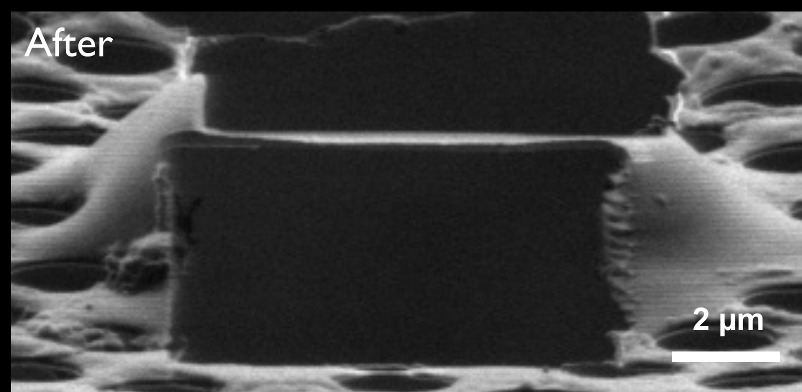



Juergen Plitzko, MPI Biochemistry

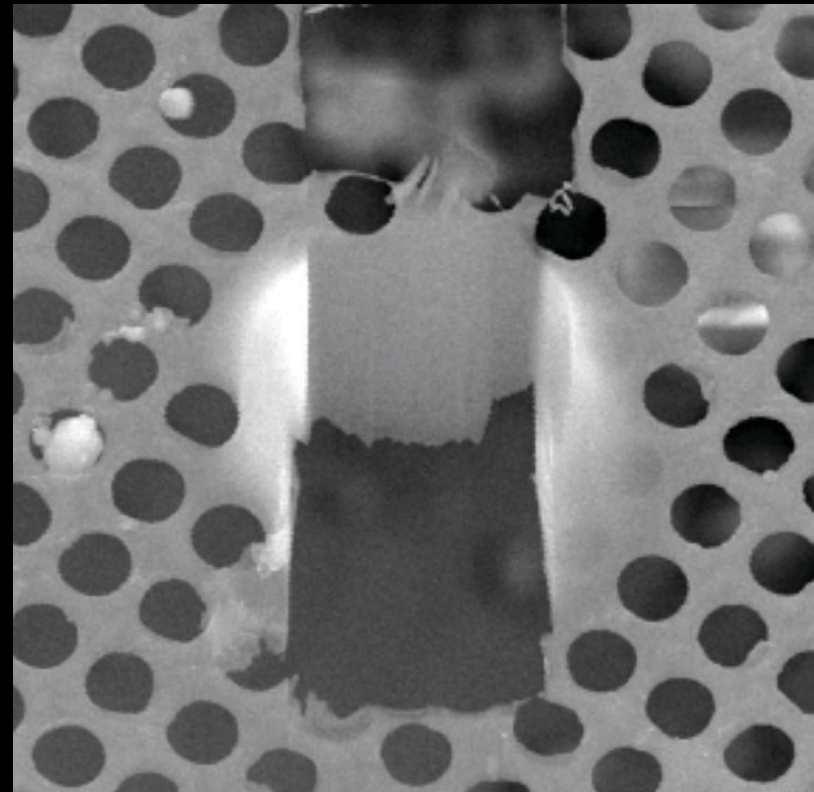
Focused Ion Beam Milling

Milling area



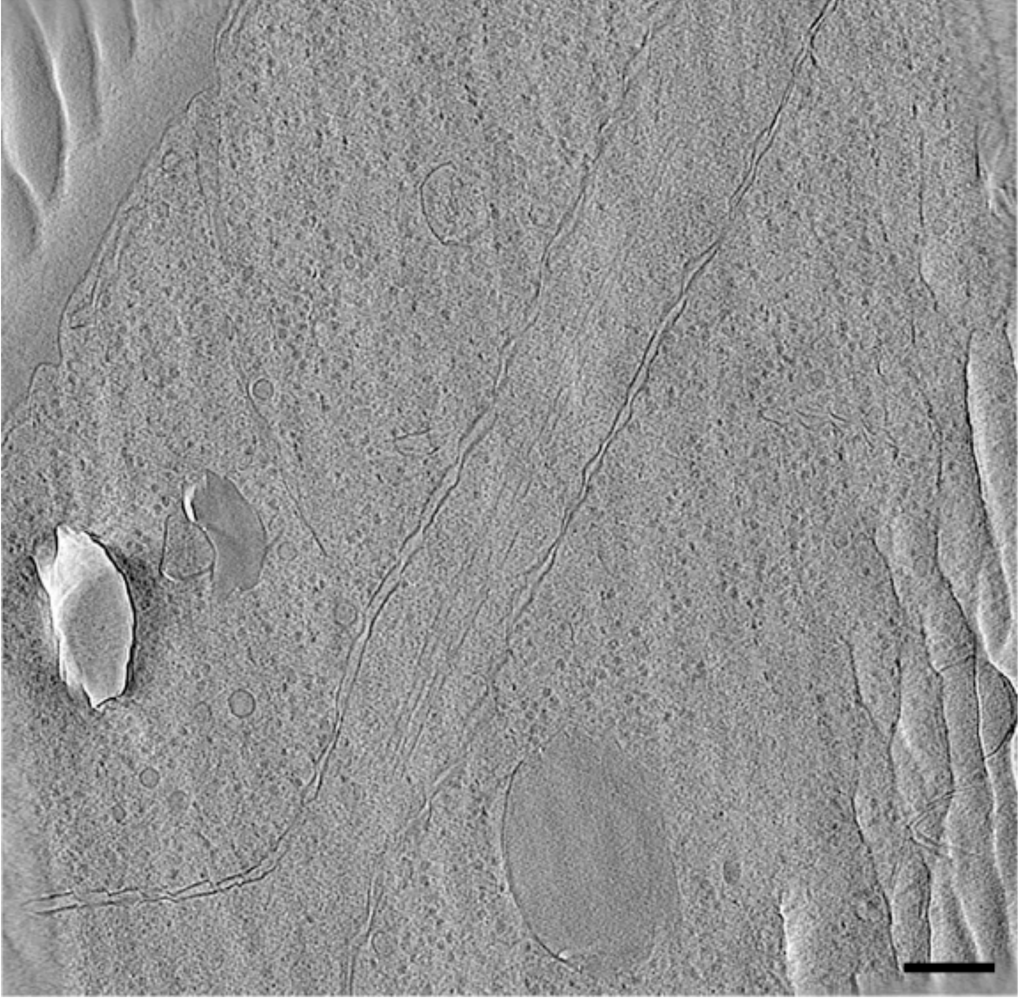


Thinned region: Cryo-SEM (top view)

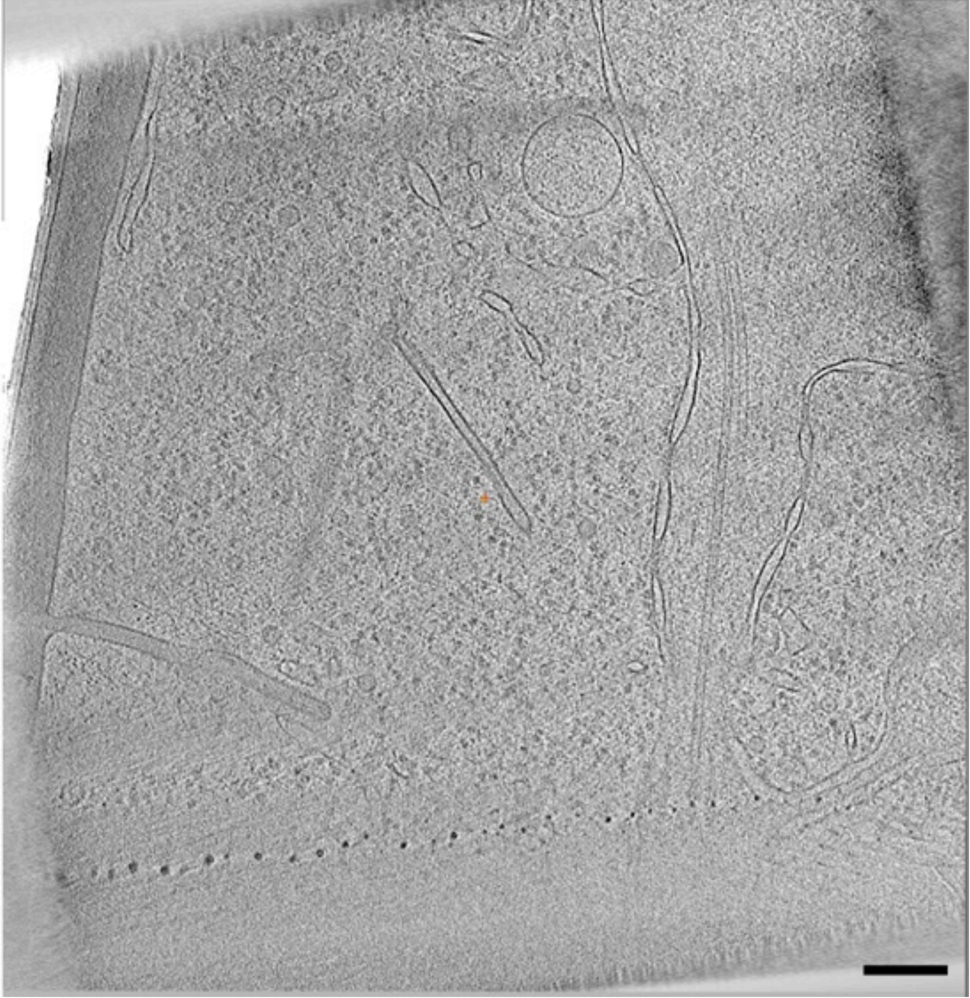


A. Rigort, F. J.B. Bäuerlein, E. Villa, M. Eibauer, T. Laugks, W. Baumeister and J. M. Plitzko
Focused Ion Beam micromachining of eukaryotic cells for cryoelectron tomography
Proceeding of the National Academy of Sciences USA, March 5, 2012 Doi:10.1073/pnas.1201333109.

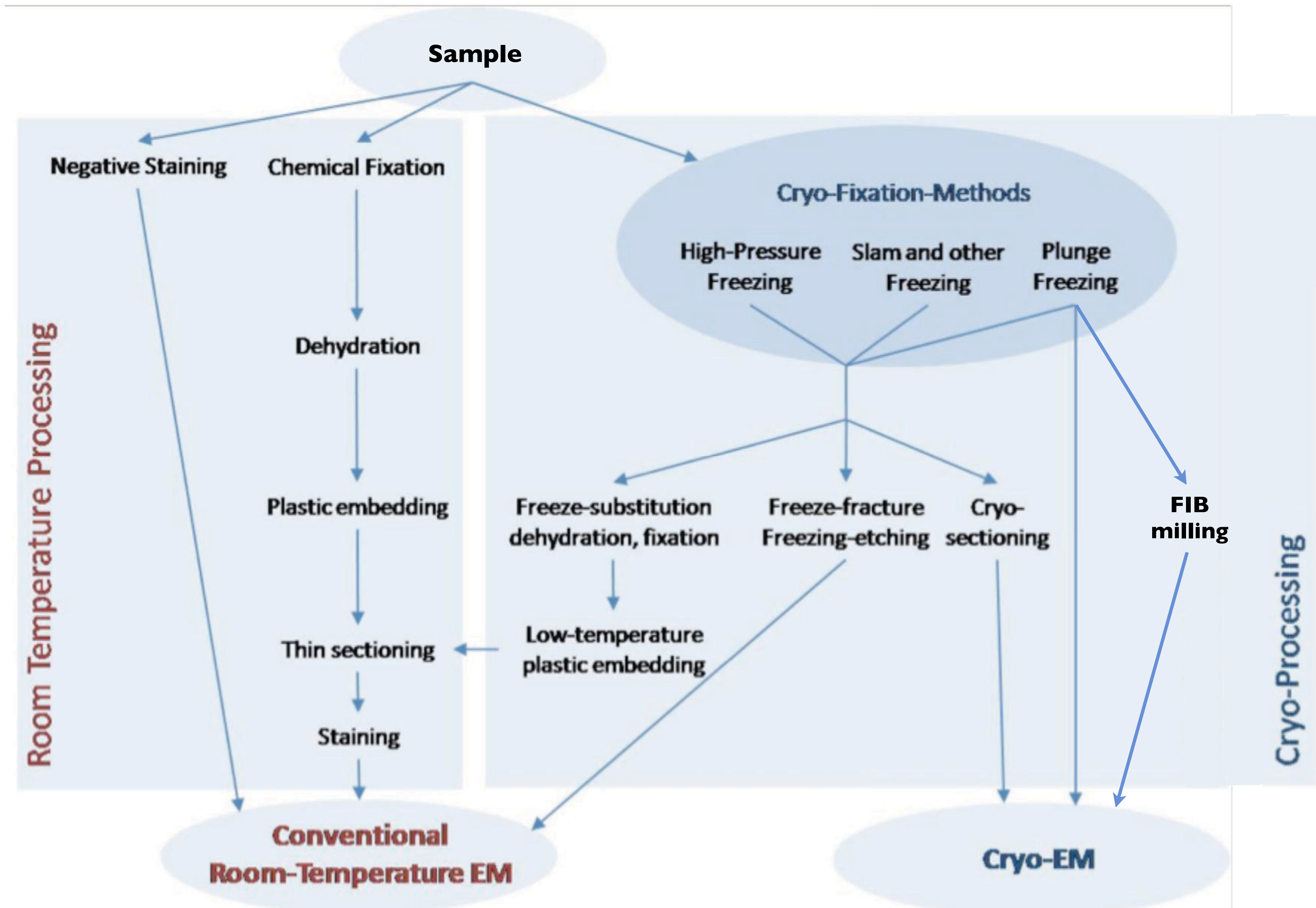
Cryosection



FIB Milled

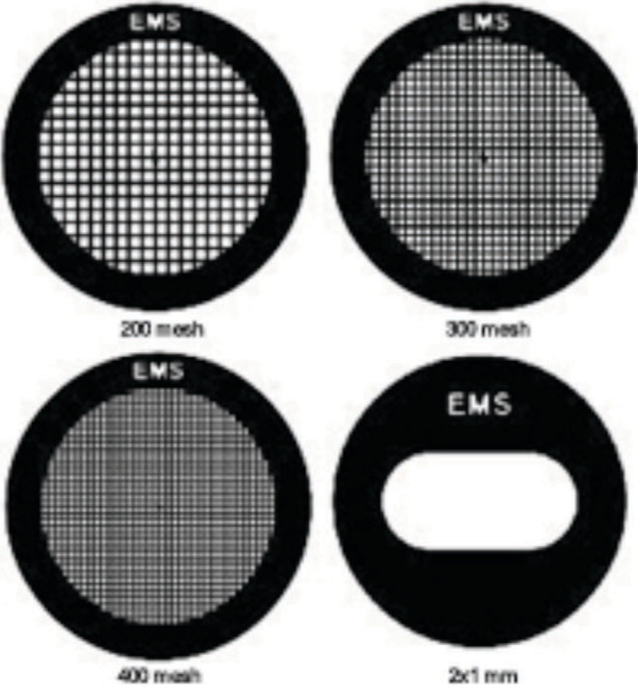


adapted from Pilhofer et al., MCB 2010



Sample prep - Methods involving freezing
Concept check questions:

- What problem does high pressure freezing solve?
- What is "low-temperature" embedding?
- What is "cryo-sectioning," and what artifacts (3) and challenges (several) are associated with it?
- What kinds of samples can be "plunge-frozen"?
- How can focussed ion beams be used to prepare cryo-EM samples?



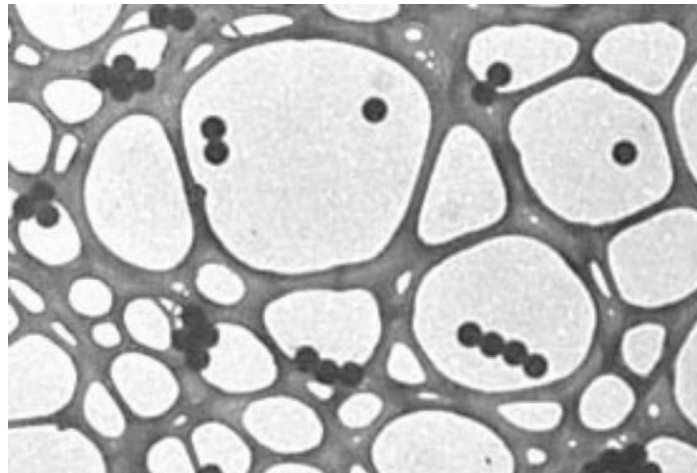
www.emsdiasum.com

“Finder” grids

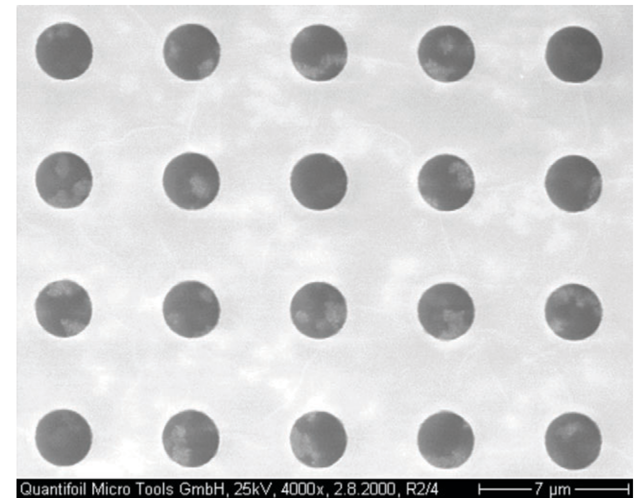
www.fullam.com

Coatings

- Formvar
- Carbon
- Holey carbon
- “Quantifoil”
- Holes covered with thin carbon



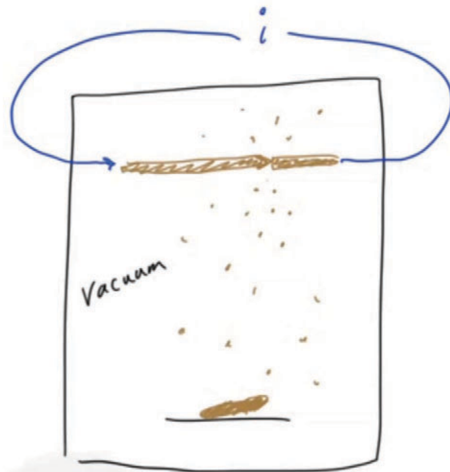
www.tedpella.com

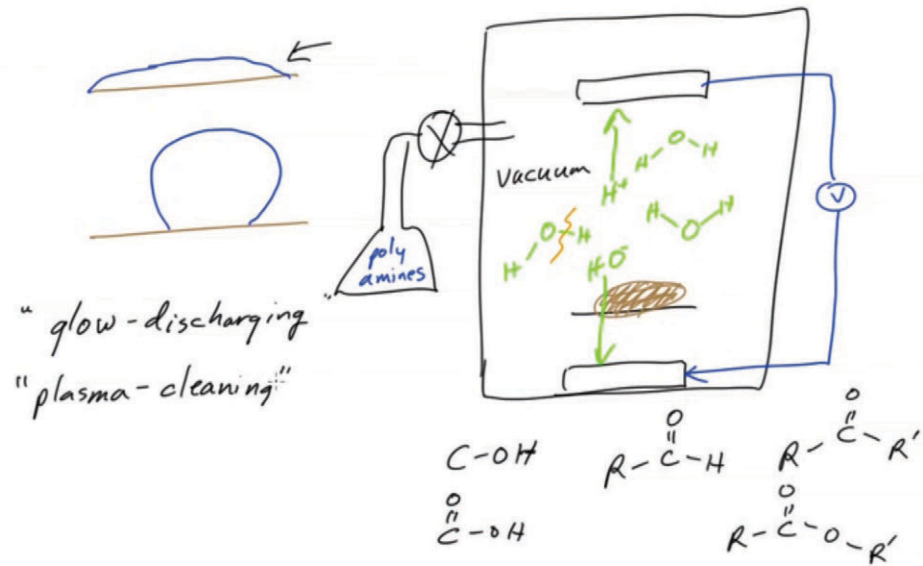


www.2spi.com

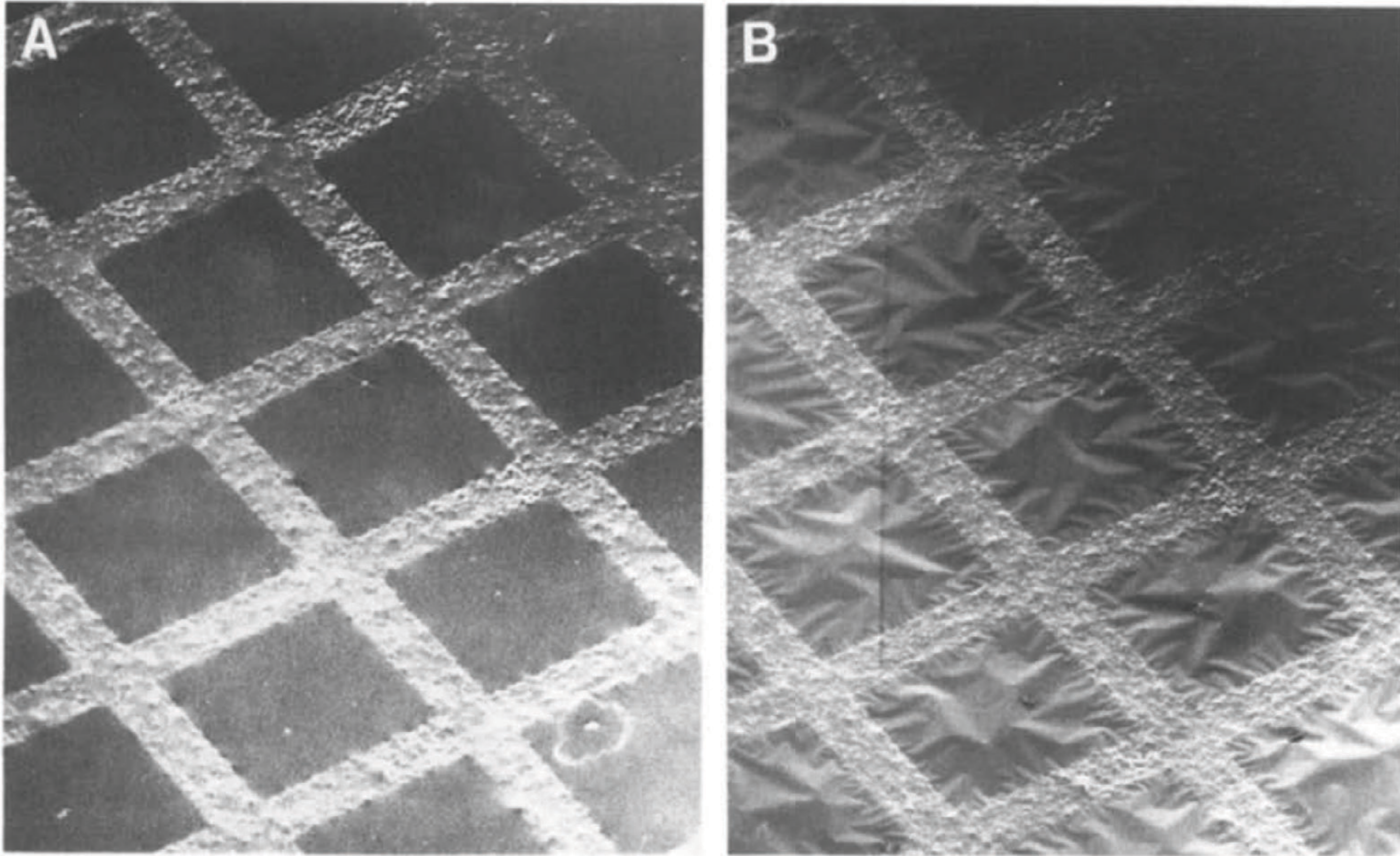
carbon evaporation

glow discharging





“Cryo-crinkling”

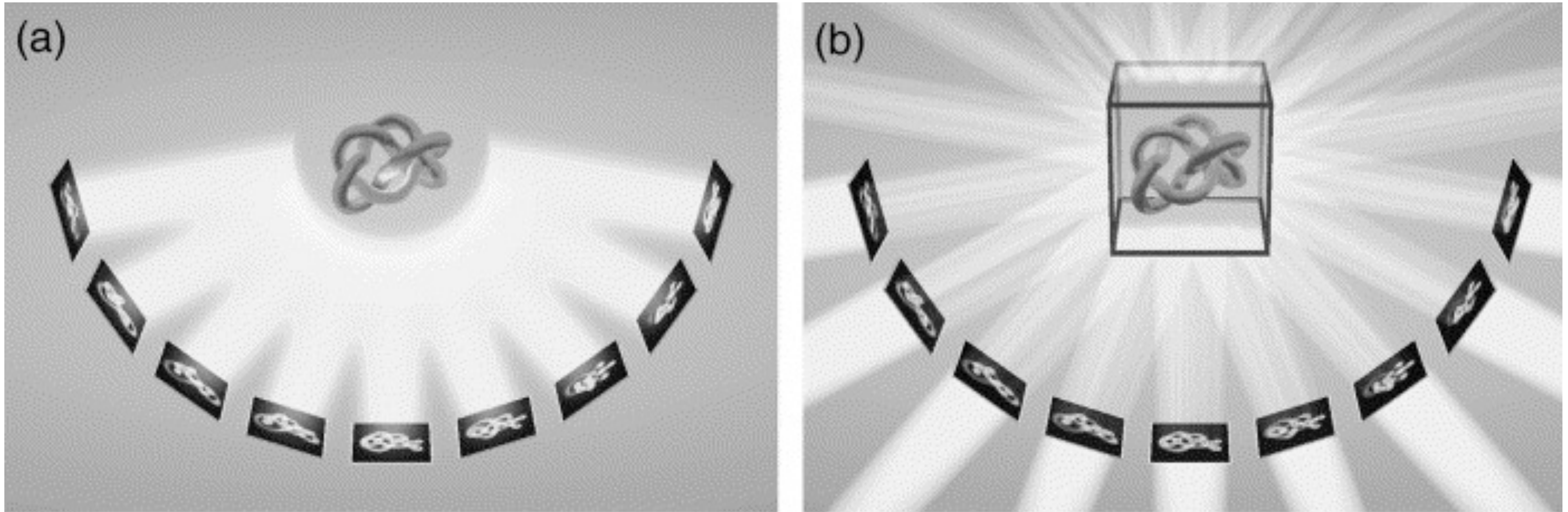


Booy and Pawley, Ultramicroscopy 1993

Sample prep - Grids
Concept check questions:

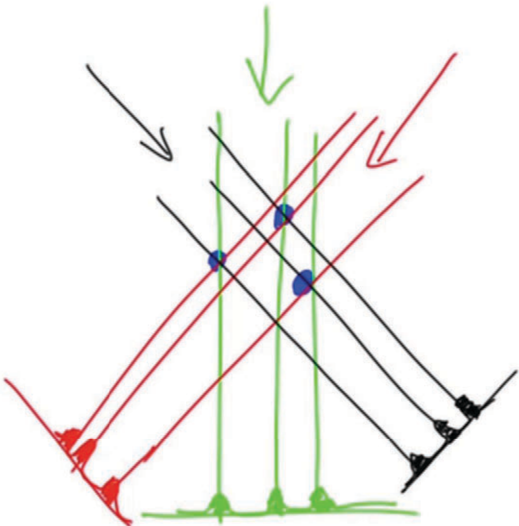
- What are the most common materials used to make grids?
- If you wanted to culture cells on grids, which grids would be better - copper or gold?
- What does "250 mesh" mean?
- What is a "slot" grid? A "finder" grid?
- What is formvar?
- What is the difference between "holey" carbon and "Quantifoil" coatings?
- What is a carbon evaporator, and how does it work?
- What is "glow discharging," and why is it done?
- What is "cryo-crinkling", and what are some ways to reduce it?

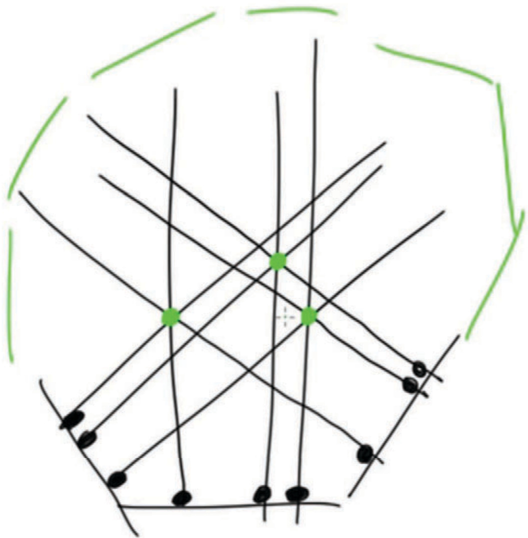
Back-projection



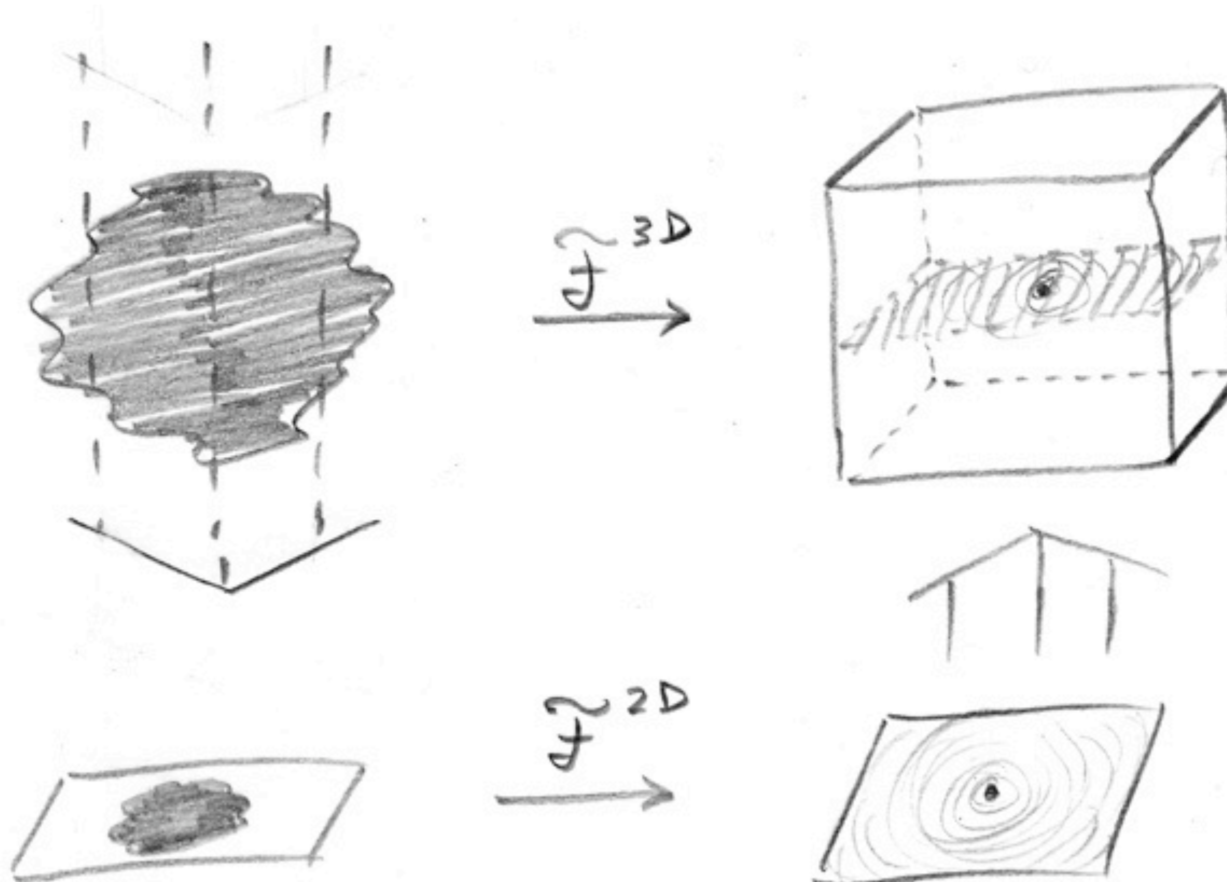
Baumeister et al., Trends in Cell Biology 9:81

back-projection

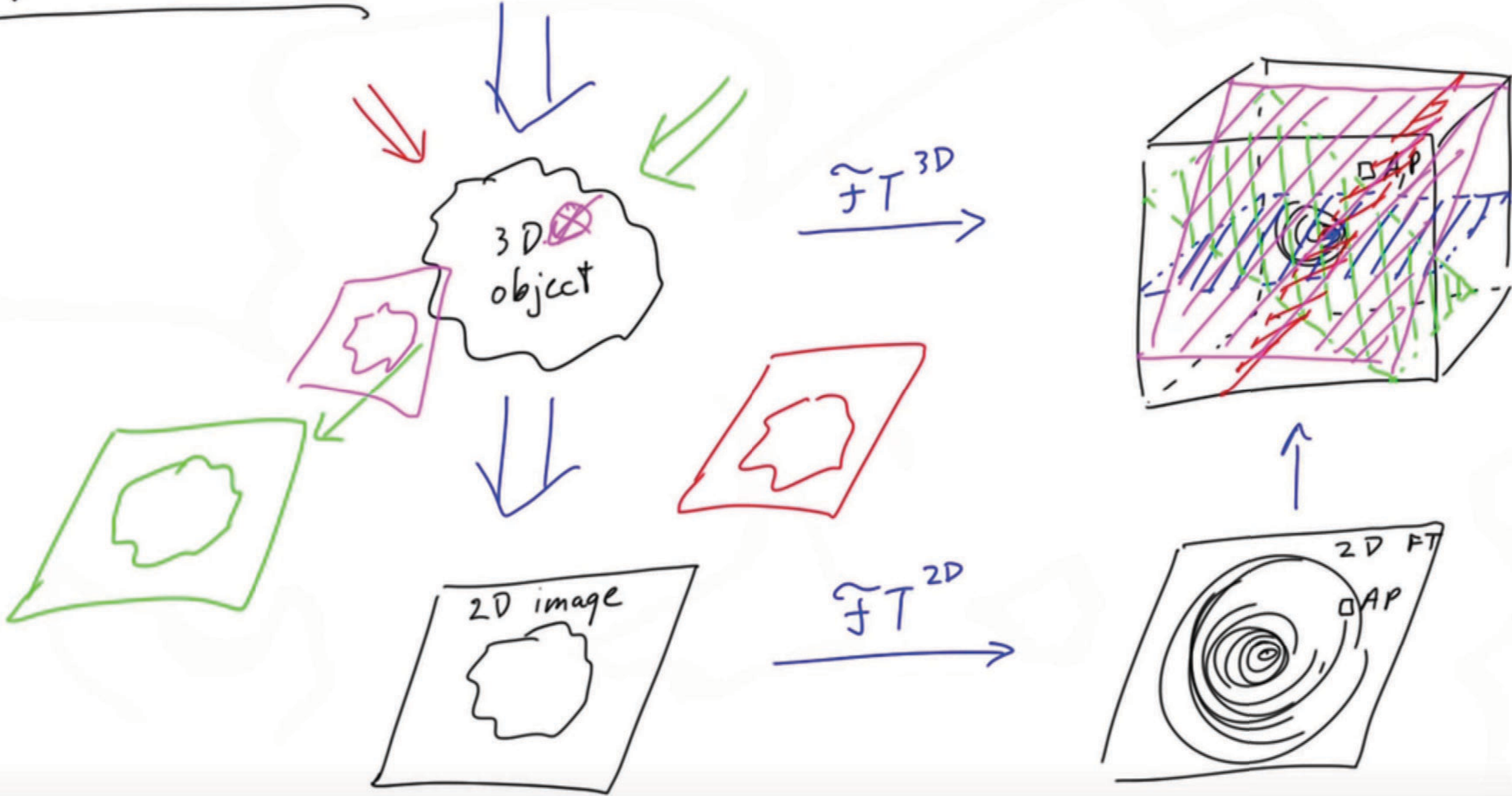




The projection theorem



The projection theorem



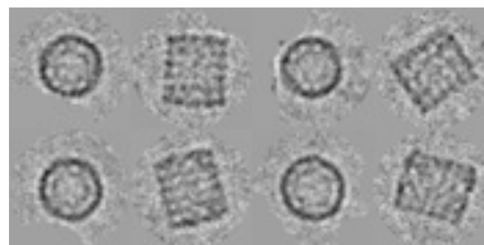
10:00 / 11:17

3D reconstruction
Concept check questions:

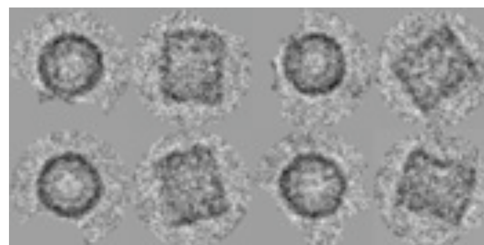
- How can 3-D reconstructions be calculated from 2-D projections in real space?
- What is the "projection theorem"? Draw it.
- How are 3-D reconstructions calculated from 2-D projections in reciprocal space?

Radiation damage

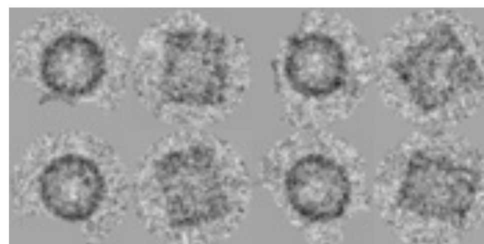
10 or 20 e^-/A^2



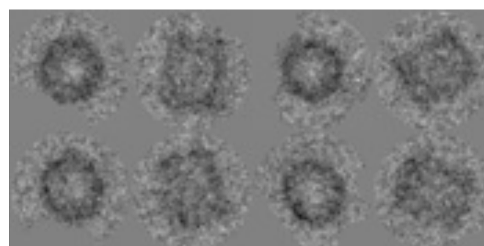
120 e^-/A^2



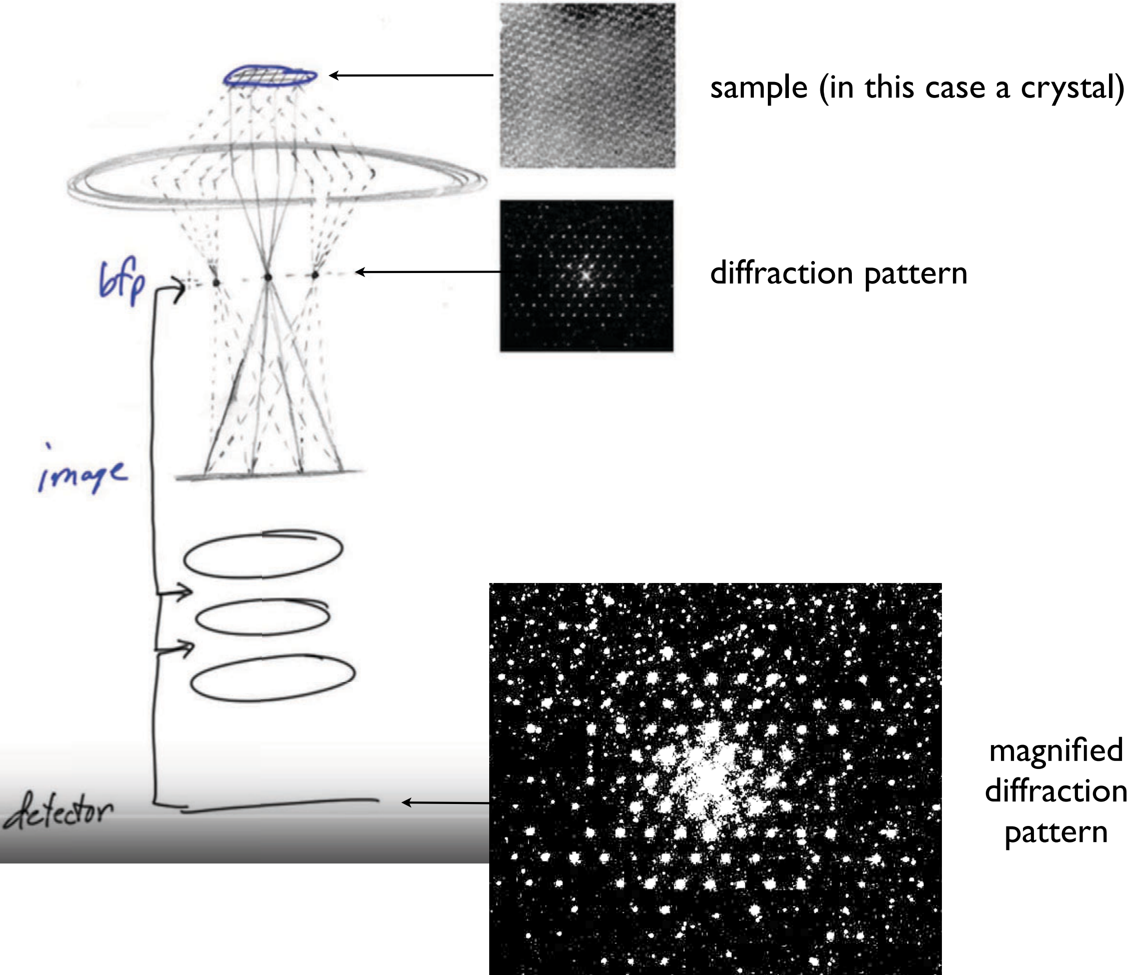
200 e^-/A^2



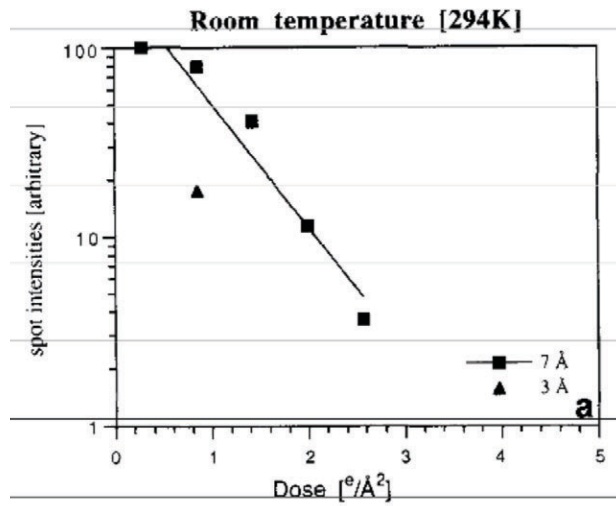
350 e^-/A^2

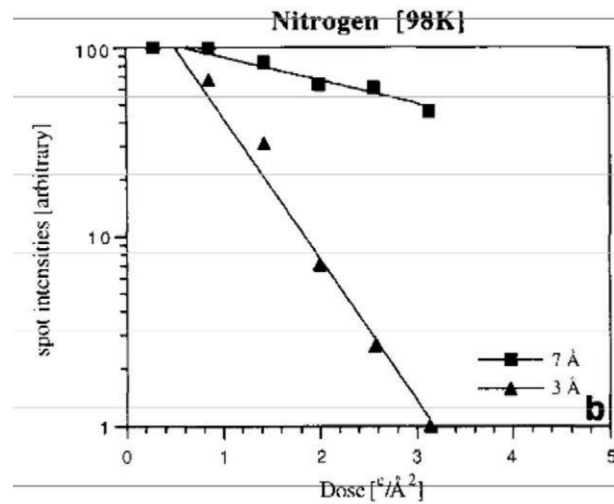


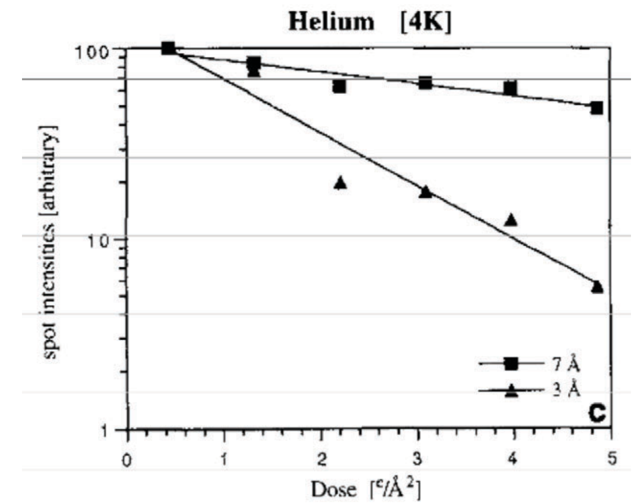
Diffraction mode



Effects of temperature on radiation damage

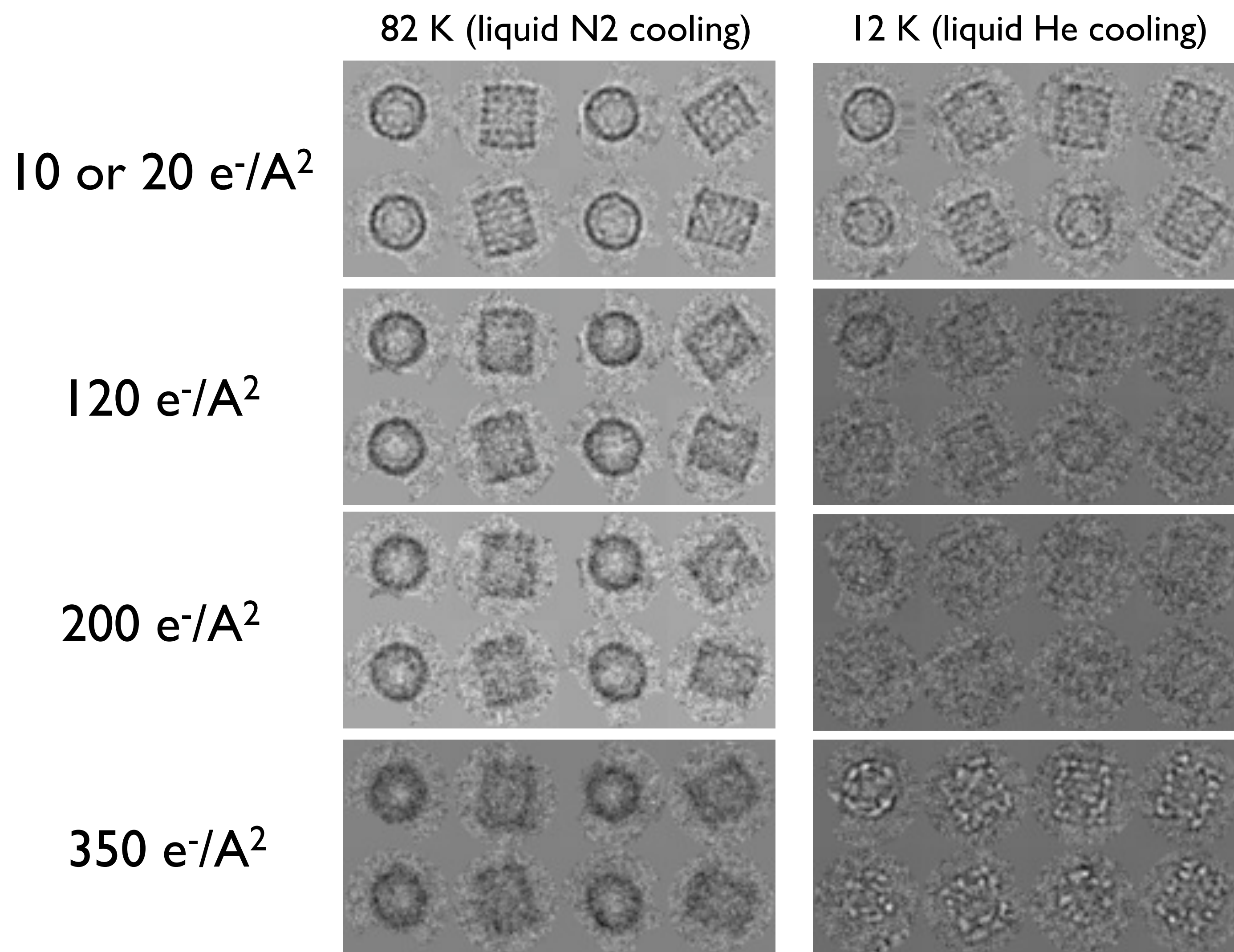




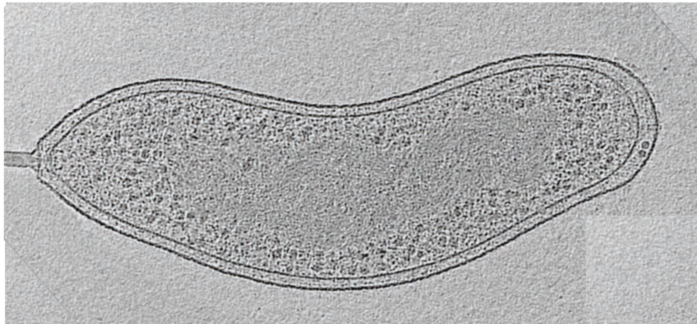


Stark et al., Ultramicroscopy 63:75 (1996)

Radiation damage

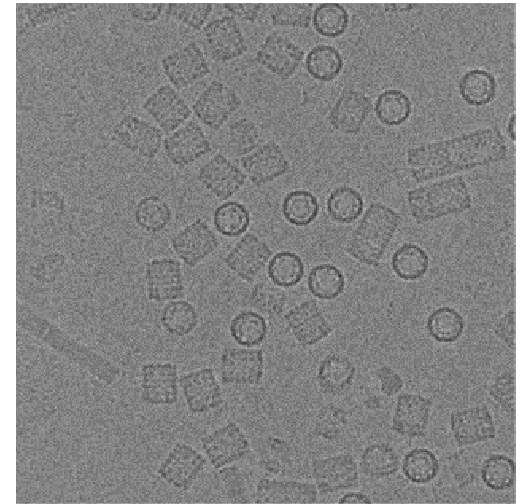


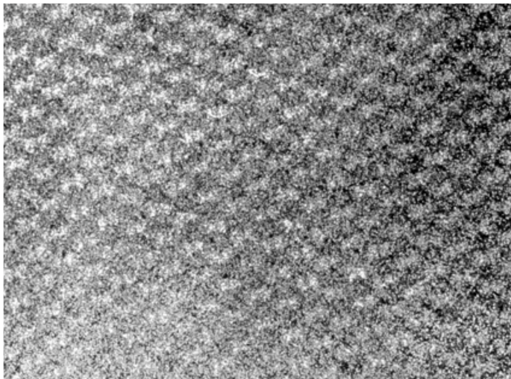
Basic approaches in cryo-EM



Tomography

Single particle analysis





2D crystallography

Dose limitations
Concept check questions:

- How do imaging electrons damage biological samples?
- How can radiation damage be recognized in images?
- How can the rate of this damage be assessed quantitatively?
- What is the effect of temperature on the rate of radiation damage?
- What disadvantage is there to imaging at temperatures less than 40K?
- For what kinds of samples can radiation damage be overcome? How?
- What are the three basic modalities of cryo-EM? How are they different? What kinds of resolutions can be expected from each? Why?