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=5Uj2D3RC_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


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
Matias et al., J. Bacteriology 185:6112 (2003)
Artifacts arising from cryo-sectioning
Plunge-freezing

Stewart, 1991
Example plunge-frozen cell
negative staining

plunge-freezing
Another possibility: FIB milling thick cells

Milling area

Thinned region: Cryo-SEM (top view)

Before

After

2 µm

2 µm

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.
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 focused ion beams be used to prepare cryo-EM samples?
“Finder” grids

www.emsdiasum.com

www.fullam.com
Coatings

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

www.tedpella.com
www.2spi.com
“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
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⁻/Å²

120 e⁻/Å²

200 e⁻/Å²

350 e⁻/Å²
Diffraction mode
Effects of temperature on radiation damage

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

10 or 20 e⁻/Å²

120 e⁻/Å²

200 e⁻/Å²

350 e⁻/Å²

82 K (liquid N₂ cooling)

12 K (liquid He cooling)
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?