Tomography notes

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Are structures in the cell variable and how do we deal with this?

Yes. Celebrate diversity!

Is 40A data useful?

Yes, but not for everything.

What are the prospects for getting to 20A resolution?

Unclear. Need direct detectors, phase plates, aberration correctors, ultrafast EM, and CTF-correction.

How do we glean general principles of cellular and subcellular structure from small data sets (n < 10, say)?

Become a biologist.

Can larger datasets (larger numbers of tomograms) be obtained, and what new problems does this create?

Yes, given lots of microscope time and sophisticated automation software like TOM, SerialEM, or Leginon. Need automatic reconstruction software like RAPTOR and a data management system like the "Caltech tomography" or "Cell-Centered" Databases.

Given the limitations of the method (missing wedge) how can one reliably detect heterogeneity?

Must understand point-spread-function. Do not "cherry-pick" your favorite sub-volume like a constellation in the night sky - base conclusions on statistically significant numbers of examples. If in doubt, test methods and hypotheses by simulation.

How do we sort populations and average?

Cross-correlation with references or *ab initio* classification. Beware reference bias!

What are the considerations during averaging of sub-volumes?

Don't let missing wedge bias alignments. Be sure to average (not sum) in Fourier space.

How were the various problems that were encountered solved?

Reconstruct or include in the sample a control. Use all available external clues.

What is in the pipeline in terms of new approaches?

Correlated light and electron microscopy, metal tags, cryosectioning, FIB milling, ultrafast EM.

What does NOT work?

Averaging things that shouldn't be averaged and aligning subvolumes to wrong references.

Is helium temperature useful for tomography?

No.

How does one communicate the data in an objective manner?

Show large numbers of examples. Make movies to show 3-D data.