I. Introduction to Subvolume Alignment / Averaging with PEET

Subvolume Alignment & Averaging

- Aligning and averaging large numbers of "identical" 3D subvolumes to
  - Fill in (partially or completely) missing data in Fourier space (*a.k.a.* the "missing wedge")
  - Improve signal-to-noise ratio (SNR)
- Subvolume and particle are synonyms here
  - Need not be an isolated particle.
  - *E.g.* repeating subunits along a higher order structure

"Identical" Subvolumes?

- Biological samples are usually heterogeneous
  - We will discuss checking for and handling this
- Eliminate unnecessary variation beforehand!
  - Mixed samples
  - Imaging conditions… magnification, HV, etc.
  - Contrast
  - Ice thickness
  - Filament polarity
  - And so on…

SVA Compared to SPA

- SVA
  - Uses 3D volumetric data from each article
  - Is good for *in vivo* or *in situ* data and portions of higher order structures, where unobstructed projections of individual particles are difficult or impossible to obtain
  - Typically yields lower resolution results than SPA for a given amount of input data
- Note on terminology: SVA ≈ STA ≈ SPT

Rough Guidelines / Rules of Thumb

- Use voxel size ≤ (target resolution) / 3
- 100 well-oriented particles can greatly suppress missing wedge artifacts
- SNR (in amplitude) scales like $\sqrt{n}$
- Typically, need 500-5000 particles for SNR
- n = 5000-10,000 often yields ~2.5 nm resolution
- Sub-nm requires n>10,000 and careful handling
- Subvolumes from 32^3 to 128^3 voxels are typical

PEET: Subvolume Alignment / Averaging

- Separate package, for use with IMOD
- Open source, freely available from http://bio3d.colorado.edu/PEET
- Online guides, man pages, tutorial videos, and discussion group
- Supports 64-bit Linux, OS X, and Windows
- IMOD's Etomo Graphical UI for common operations
- Command line for flexibility / advanced operations

PEET: Subvolume Alignment / Averaging

- Computationally demanding
  - At least 12-64 cores are useful for typical applications
- Parallelism via IMOD mechanisms:
  - Multiple cores on local machine
  - Passwordless ssh to nodes on local network
  - Cluster computing (Pbs, Pbs-Maui, Sge, and Slurm supported)
    - Configured via cpu.adoc. (See IMOD documentation for details… especially cpuadoc and processchunks man pages).
  - Currently no GPU support

PEET: Key Alignment Features

- Initial subvolume centers in an IMOD model
  - All Points in Object 1 = centers
  - No need to “box out” subvolumes
- Rotations / translations: Motive Lists
  - Typically in file(s) named *MOTL*.csv
- Reference volume: something to align to
- Per-particle rotation (Y) axis
- May be missing, defaulted, or automatically generated but effectively always present!

Multiple Coordinate Systems

- Global / Tomogram coordinates
  - Fixed
  - Used for subvolume centers, motive list rotations / translations, and search distances
- Per-particle coordinates
  - Attached to and move with each particle
  - Particle Y = particle rotation axis
  - Used to specify angular searches

3D Rotations In PEET

- Etomo angular search ranges: Y(ϕ)-Z(Θ)- X(Ψ) in particle coordinates (fixed at start of search)
- Motive list entries: Z-X-Z Euler angles in global coordinates
- IMOD Slicer angles: Z-Y-X angles in global coordinates
- Confusing variety!
- You will seldom have to deal with this directly

Questions?

A Few Words on Logistics…

- Presentations will cover concepts
- Lab exercises will explore details
  - We will announce time available for each lab
  - Try to pace yourself and complete topics of greatest interest
  - Okay if you can't complete all the exercises
  - Labs designed and available for your later use
  - We will skip most alignments to save time