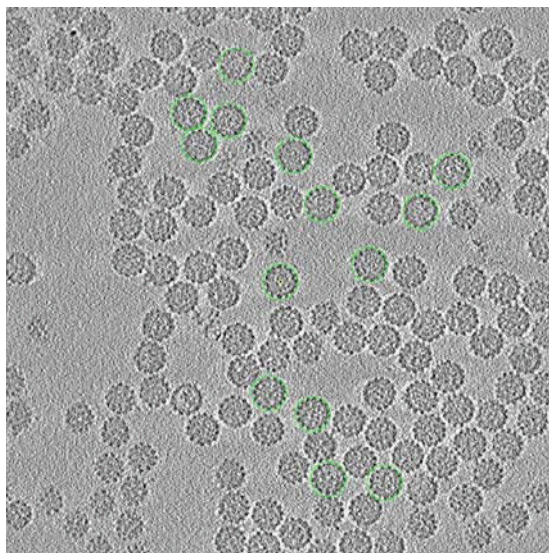# Aligning Isolated Particles: BPV
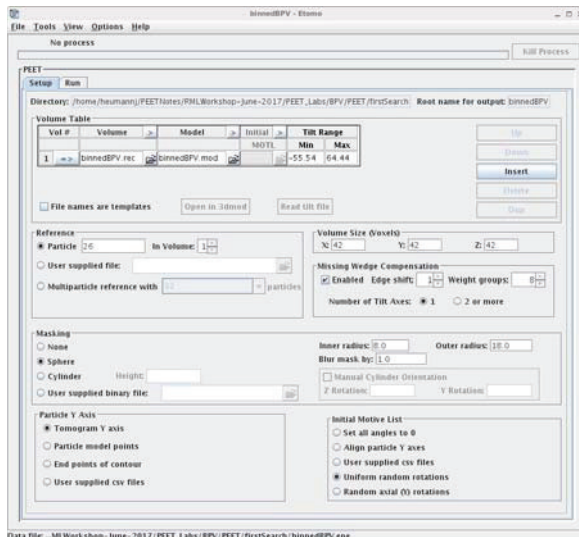

2X Binned BPV Sample -8 μm Defocus

Modeling BPV

- Always try to model accurately
  - improves the odds of a good alignment / average
- Center points in XY and Z as well as you can
- Setting "Sphere Radius for Points" and paging up / down helps with centering points in Z
- May wish to start with binned or binned and NAD-filtered data

Alignment Settings – Setup Tab

- Volume(s)
- Model(s)
- Tilt range
- Reference
- Size to average
- Mask Definition
- Particle Y Axis
- Initial Motive List

Alignment Settings – Run Tab
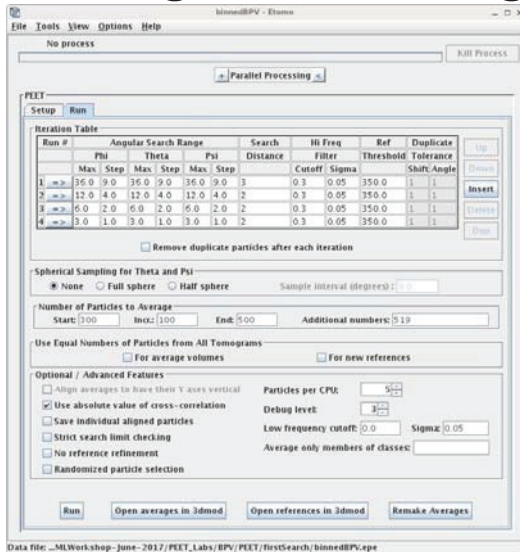
- Parallel Processing
- Iteration Table
- Spherical Sampling
- Number to Average
- Equal Numbers?
- Optional / Advanced Features

Iteration Table

- Search settings based on model uncertainty
- Iteration n Max ~ 1.5X iteration n-1 Step
- 3:1 ratio Max:Step good for speed / accuracy
- Angular settings decrease 2X row-to-row

Finest Angular Resolution?

- R sin(dΘ) ≈ R dΘ = 1 [voxels]
- dΘ ≈ 1 / R [radians] = 180 / (π R) [degrees]
- In this case, mask radius = 18
  so dΘ ≈ 3°
- Actually searched down to 1°
- Last 2 iterations shouldn't change much
- This is the case, as we'll verify in the lab

Optional / Advanced Features

Optional / Advanced Features

- ☐ Align averages to have their Y axes vertical
- ☑ Use absolute value of cross-correlation
- ☐ Save individual aligned particles
- ☐ Strict search limit checking
- ☐ No reference refinement
- ☐ Randomized particle selection

Particles per CPU: 5
Debug level: 3
Low frequency cutoff: 0.0   Sigma: 0.05
Average only members of classes:

- Align vertical: not available with Tomogram Y
- Absolute value:|CCC| instead of CCC
  - avoid Einstein-from-the-noise
  - problematical with some pattern… *e.g.* Zebra stripes
- Strict search limits: never exceed max step
- No reference refinement: template matching

Optional / Advanced Features

Optional / Advanced Features

☐ Align averages to have their Y axes vertical   Particles per CPU: 5
☑ Use absolute value of cross-correlation   Debug level: 3
☐ Save individual aligned particles   Low frequency cutoff: 0.0   Sigma: 0.05
☐ Strict search limit checking   Average only members of classes:
☐ No reference refinement
☐ Randomized particle selection

- Randomized selection: not based on CCC
- Particles per cpu: determines number of chunks
- Debug level: amount of detail, seldom used
- Low frequency cutoff: attenuate shading gradients, seldom used
- Average only: for use with heterogeneous data

Questions?