Semi-Automatic Particle Picking

Manual Particle Picking Is Not Fun

- Can it be avoided? Yes, sometimes!
  - Ideal cases: just need to pick 1 particle!
  - More typical:
    - Manually pick a sample
    - Align and average
    - Use to find similar particles… *e.g.* by template matching

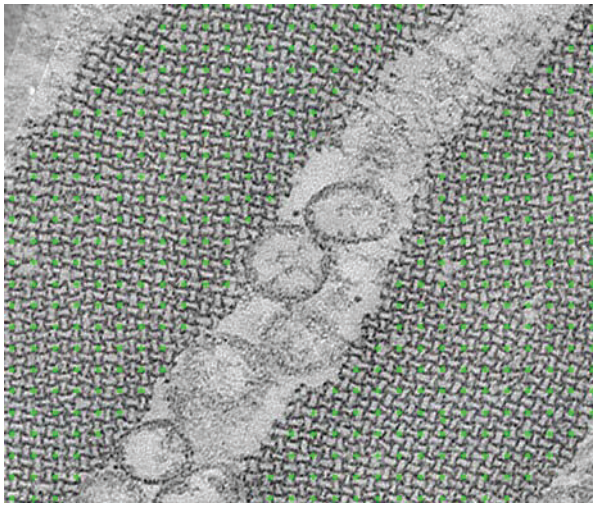
Can I Pick Particles Using Program <X>?

- Yes! If you can translate the results to the format PEET needs:
  - Pixel coordinates of subvolume centers
    - Point2model can convert these to a 3dmod model
  - Initial motive list Euler angles
    - Angles can be all 0's (*e.g.* if using spherical search)
  - Rotation axes
    - Easily manipulated csv format
    - Can just use tomogram Y if doing spherical search

Semi-Automated Picking in PEET

- PEET searches position and orientation
  - Finds single best alignment in a limited region
- Not well suited for
  - Finding many candidates
  - In a very large volume
- A Workaround…
  - Split large volume up into many small volumes
  - Seed each with a candidate particle
  - After alignment, choose the best candidates

A Case Where 1 Point Suffices!

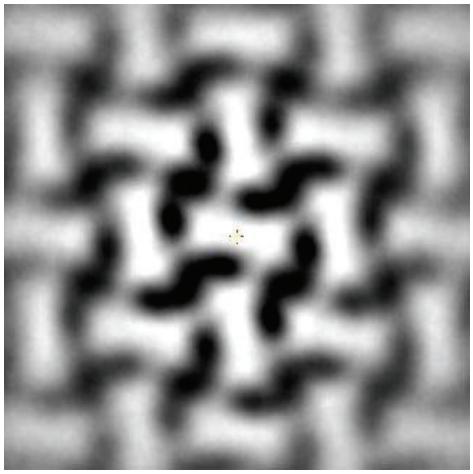


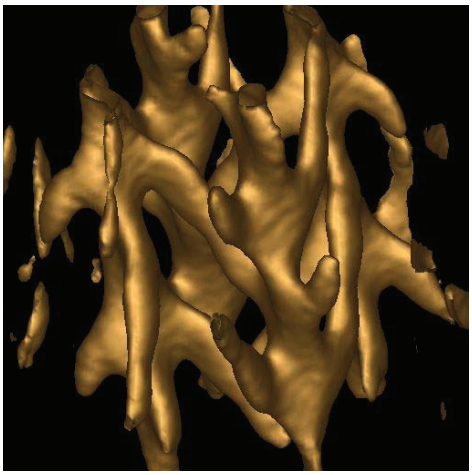
~30 nm Slice of Sonic Muscle Z-line (with Pradeep Luther)

Sonic Muscle Picking Strategy

- Seed points with gridInit
- Drawing tools eraser to clean up initial seeds
- 1 manually picked point as a starting reference
- Align and average
- Threshold by cross-correlation
  - *E.g.* use selectClassID and createAlignedModel
- Manually remove bad points – not needed here
- Symmetrize

Sonic Muscle Results





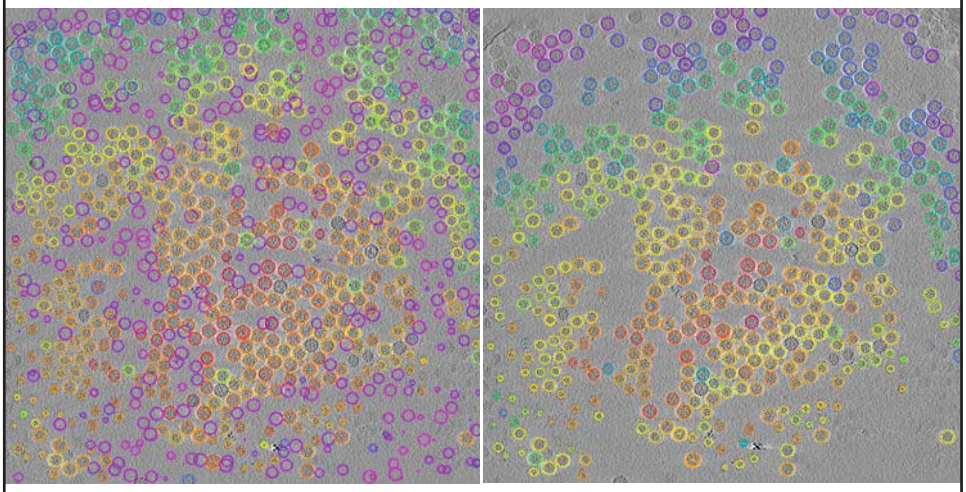
More Typically…

- Align and average several hundred particles
- Use the resulting average as a template to find additional particles (often in other volumes)
- Template matching in PEET
  - Select “No reference refinement” in Etomo
  - “flgNoReferenceRefinement = 1” in prm file
- Beware of Reference Bias
  - Bin or low-pass filter the template
  - Final locations should look as if manually picked

Semi-automated Particle Picking

- Widely applicable
  - 2D crystals
  - Isolated particles (will explore BPV in exercise)
  - Spikes / fusion proteins
  - Membrane associated proteins
- PEET tools / programs are fairly flexible
- Strategy / details will vary with application

Isolated Particles: Binned BPV



Before Thresholding After Thresholding

Pseudo-colors (inserted by createAlignedModel) reflect cross-correlation coefficient

Questions?