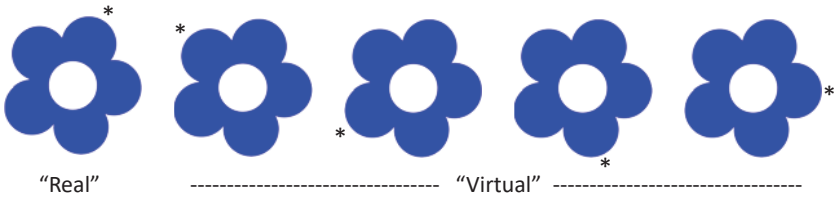
Using Symmetry

Symmetrization with Virtual Particles

"Real"

- Initial average of real particles

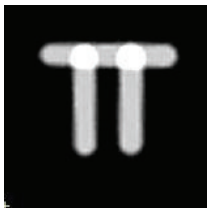
Symmetrization with Virtual Particles



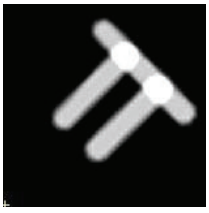
- Apply symmetry operations (rotations / translations) to generate virtual particles
- Re-align including virtual particles
- Improves SNR, missing wedge

Rotating / Translating Particles

- modifyMotiveList
  - Generates a new, modified motive list
  - Specify desired rotations / translations for average



Z-Y-X Rotation
(in order X,Y,Z)
0,0,-45

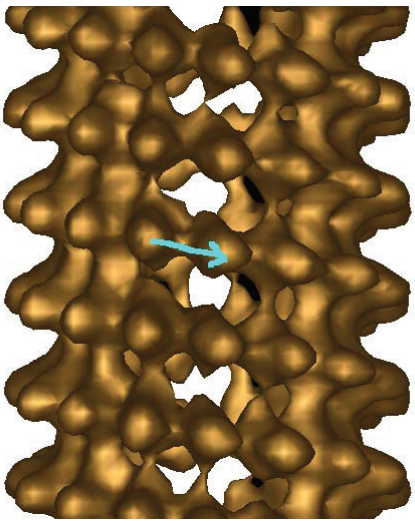


  - Re-make averages or re-align to see effects

Before Symmetrization…

- Center and align the starting average!
  - Use modifyMotiveList for this too
  - Okay to use repeatedly before aligning / averaging
- Align averages vertically (used in some labs)
  - Affects final averages but not in final motive list
  - Get vertical alignment angles from *finish.log and apply with modifyMotiveList before symmetrizing

15 PF Microtubule Symmetrization



360/15=24° Y rotation

-16 /15=-1.07 nm shift

(1.07 nm ≈ 1.18 voxels)

15X effective particles

15 PF Microtubule Script

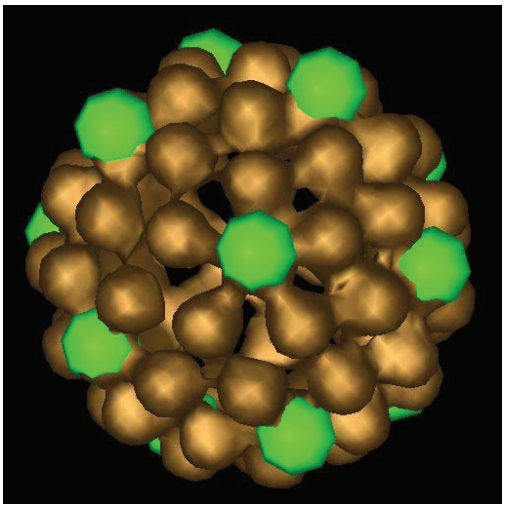
reorient.ksh - Notepad

File Edit Format View Help

```
# First, modify the final motive list from firstSearch to vertical
# orientation, using the Slicer angles found in *finish.log. Also
# center in in XY, by shifting -1 voxel in X.
modifyMotiveList ../firstSearch/series4_8um_MOTL_Tom1_Iter3.csv \
 initMOTL1.csv "0.01,-0.0,-28.13" "0,0,-1"

# Next generate the rotations and translations for 15-fold symmetrization
# Y shift calculated as 16 nm / (15 * 0.906 nm / voxel) = 1.17734 voxels
modifyMotiveList initMOTL1.csv initMOTL2.csv "0,24,0" "0,-1.17734,0"
modifyMotiveList initMOTL1.csv initMOTL3.csv "0,48,0" "0,-2.35467,0"
modifyMotiveList initMOTL1.csv initMOTL4.csv "0,72,0" "0,-3.53201,0"
modifyMotiveList initMOTL1.csv initMOTL5.csv "0,96,0" "0,-4.70935,0"
modifyMotiveList initMOTL1.csv initMOTL6.csv "0,120,0" "0,-5.88668,0"
modifyMotiveList initMOTL1.csv initMOTL7.csv "0,144,0" "0,-7.06402,0"
modifyMotiveList initMOTL1.csv initMOTL8.csv "0,168,0" "0,-8.24135,0"
modifyMotiveList initMOTL1.csv initMOTL9.csv "0,192,0" "0,-9.41869,0"
modifyMotiveList initMOTL1.csv initMOTL10.csv "0,216,0" "0,-10.596,0"
modifyMotiveList initMOTL1.csv initMOTL11.csv "0,240,0" "0,-11.7734,0"
modifyMotiveList initMOTL1.csv initMOTL12.csv "0,264,0" "0,-12.9507,0"
modifyMotiveList initMOTL1.csv initMOTL13.csv "0,288,0" "0,-14.128,0"
modifyMotiveList initMOTL1.csv initMOTL14.csv "0,312,0" "0,-15.3054,0"
modifyMotiveList initMOTL1.csv initMOTL15.csv "0,336,0" "0,-16.4827,0"
```


BPV Symmetrization



12 5-fold (c5) sites

Each to top center

5 orientations each

60X effective particles

Two Paths To Symmetrization

- modifyMotiveList on output motive lists
  - Simpler, fewer steps, usually effective
- createAlignedModel, then modifyMotiveList
  - Separates position (model) and orientation (motive lists)
  - Also revises particle Y axes estimates if present
    - Effective when initial alignment is good, not always
  - Easiest path when changing voxel size

Axial Symmetrization – *e.g.* Spikes

- Goal: preserve axial symmetry even when axis is not yet accurately located
- Kuybeda *et al* (2013) JSB 181:116-127
- In some cases, symmetrized axial-only search can find ~correct rotation, even when axis is well- centered or oriented

Axial Symmetrization in PEET

- Specify symmetry and iteration to apply by manually editing prm file
  - yAxisSymmetry = [1, 3, 1, 1]
- Corresponding iteration much be in ϕ only, *e.g.*
  - Phi Max = 60, Step = 6
- When to do this? Judgement / Trial and Error!
- Not a replacement for use of virtual particles.

Questions?