Lesson 7:
BPV

SIMPLE MODELING WITH SPHERICAL PARTICLES

1) `cd $WORKSHOP_HOME/PEET_Labs/BPV_-3`

2) Open the tomogram and create a new model by typing:
   `3dmod bpv_bin2.rec.nad myBPV.mod`

3) Select **Model** mode

4) Press **Edit / Object / Type** and select **Scattered**. Leave the Object Edit window open.

5) Adjust zoom and the ZaP window size to settings you find comfortable for modeling. Notice how particle diameters change as you **PgUp / PgDn**, reaching a maximum when the slice cuts through the center of the particle.

6) Adjust Z slice for maximum diameter of some particle near the center of your view, which is not touching other particles or obscured by gold or contamination. Place a model point near the center of this particle by clicking with the middle mouse button.

7) In the **Object Edit** dialog, increase **Sphere radius for points** until the resulting green circle is just large enough to just include the entire virus particle (~**19**). This circle makes it easier to see if your model point is correctly centered. If you find the green circle difficult to see, try switching to the Slicer window (hotkey \) and increasing **Mod** to ~**10** or increase the **line width** in the **Edit / Object / Type** dialog. In the Slicer window, adjust the position of the point by right clicking until you are satisfied that it is well-centered (in all 3 dimensions!). In both the Slicer and ZaP windows, left, middle,

and right mouse clicks, respectively, select an existing model point, add a new one, or move an existing one. In ZaP movement by right-clicking is limited to the plane containing the point, while in Slicer it is not.

8) Try adding at least 10 more points, leaving the sphere radius as you've set it. You'll find this makes accurate modeling in all 3 dimensions much easier. Choose particles that are not touching other particles, gold, or contamination and are at least half a particle diameter away from any edge of the volume. When finished, you can save your model (**File / Save Model**). Examine the supplied model by going to **File / Open Model** and choosing *bpv_bin2.mod*. Exit 3dmod when finished.

CHECKING MASK SIZE AND LOCATION

9) *PEET/firstSearch* contains an already completed PEET run on the 2X binned data. PEETCleanup has already been run to remove intermediate files, leaving important output files. Recall that when using a mask, one of the first things to do is verify that the mask is correctly sized, located, and (in the case of non-spherical masks) oriented.

```
cd PEET/firstSearch
etomo *.epe
```

On the Setup tab, notice that we've chosen a spherical mask with inner and outer radii of 10 and 22 voxels to be blurred with a Gaussian of standard deviation 1 voxel. Close Etomo.

10) `3dmod bpv*Ref*.mrc`

This opens all of the masked references and we will go through them one-by-one using the **4th D** arrows (hotkeys **1** and **2**) in the ZaP window.

11) Examine *bpv_Ref1.mrc* and notice that the mask is well centered and that the inner and outer radii seem appropriately set to include all the capsid, with minimal interference from the central nucleic acid.

Note also the soft mask edges. When using masking, it is a good idea to check the mask settings shortly after starting the run. Recall that the **Ref1*.mrc* initial reference files are created very quickly. If the mask settings are incorrect, it makes sense to kill the run and fix them rather than waiting for the run to complete.

VERIFY ANGULAR SEARCH STEPS

12) The angular resolution calculation we discussed in the lecture suggested that the last 2 iterations, with search steps of 2 degrees and 1 degree, were probably superfluous and unlikely to improve results. Verify that this is the case by comparing references **Ref3.mrc* through **Ref5.mrc* and observing that they are almost unchanged from **Ref2.mrc*. Exit 3dmod when finished.

SET UP A SPHERICAL SEARCH

For BPV, icosahedral symmetry allowed us to limit our initial angular search range. In this exercise, we'll examine how the settings would be different if that were not true. First we'll copy the existing firstSearch project settings, and then modify them appropriately for randomly oriented particles with no symmetry.

13)
```
mkdir ../mySphericalSearch
cd ../mySphericalSearch
```

14) As in the Introductory lab, run etomo and copy project settings from **../firstSearch/bpv.epe** (step 12 in the Introductory exercise).

15) On the lower right corner of the **Setup** tab, change **Initial motive list** from Uniform random rotations to **Set all angles to 0**. Since we are now assuming a full spherical search will be required, random rotations serve no purpose in suppressing missing wedge artifacts; the full search would allow the artifacts to simply re-align. Randomization is typically only helpful when a limited angular search can be used.

16) Switch to the **Run** tab.

17) Under **Spherical Sampling for Theta and Psi**, select **Full sphere**. Notice how the Theta and Psi fields for Iteration 1 in the Iteration Table are now greyed out and unavailable. These will be handled automatically by the spherical search algorithm.

18) We will use the same Phi step (9°) as previously. Since we're doing a full spherical search, we need to search from -180° to +180°; change the **Phi Max** setting at Iteration 1 from 36.0 to **180.0**.

19) Search parameters for Theta and Psi will be set automatically, but we need to specify the **Sample interval (degrees)** or finest search step to be used. Normally, this is just set equal to the Phi step at Iteration 1. In this case, that's **9.0** which happens to be the default, so we don't need to change anything.

20) We found above that possibly Iteration 3 and certainly Iteration 4 weren't really necessary. In the **Iteration Table**, Click on the **=>** to the right of the Iteration number (**Run** #) 4, and then press the **Delete** button at the right side of window to remove this row. Similarly, delete the row for Iteration 3 if you wish.

21) Exit Etomo and your settings will be automatically saved.

22) `cd ../sphericalSearch` to see the results of a completed spherical search run that we've already done. Examine the results in 3dmod if you like by typing `3dmod *AvgVol*.mrc`. You may also wish to open the Isosurface view (**Shift+U** or **Image / Isosurface**). Command line options are available to specify in advance which 3dmod views to open; consult the 3dmod man page for details. A spherical search run will take longer and may suffer more from missing wedge artifacts than the limited search range approach. Close 3dmod when finished.