Lesson 16: Visualization

We've used createAlignedModel fairly extensively… for sanity checking, changing voxel size, and symmetrization. It's also used in the same manner when combining results from multiple alignments. However, we have not yet made use of the "Summary" csv file(s) it generates. Here we'll use these file(s) for visualization in conjunction with IMOD programs clonevolume and clonemodel. This can be very helpful in analyzing and understanding your average as well as in preparing figures for publication.

USING CLONEVOLUME

1) `cd $WORKSHOP_HOME/PEET_Labs/MT/PEET/full15Fold`

2) `createAlignedModel *.prm`
Notice from the output that a "Summary" file (*series4-8um_PtsAdded_Tom*_Iter3_Summary.csv*) is generated for each volume.

3)
```
clonevolume -into ../../series4-8um-cor.rec \
-at series4-8um_PtsAdded_Tom1_Iter3_Summary.csv \
full15Fold_AvgVol_3P2528.mrc clonedVolume.mrc
```
where "\" should be followed immediately by **Enter**. The summary file is passed to clonevolume following the "-at" option. It gives the positions and orientations at which each aligned subvolume should be placed. The "-into" option is followed by the name of the volume into which we will be cloning. The final 2 arguments are the name of an mrc file containing the averaged subvolume to be cloned and the desired name for the output file. Several other options are available. Consult the clonevolume man page for more details.

4) `3dmod ../../series4-8um-cor.rec clonedVolume.mrc`
Compare the original and cloned volume at various slice heights and, if you wish, from various perspectives. Exit 3dmod when finished.

USING CLONEMODEL

Cloning can also be used to generate models containing correctly oriented, aligned isosurfaces representing a subvolume average. This is particularly useful for complex structures containing multiple types of components. We will illustrate a simple, single component case, but the concepts are the same. For complex structures, you would first generate separate models for each component and then join them with IMOD's joinmodel.

5) `3dmod full15Fold_AvgVol_3P2528.mrc`

6) Press **Shift+U** or **Image / Isosurface** to display an isosurface in the Model View.

7) In the resulting Isosurface View dialog
   a. uncheck **View Bounding Box**
   b. If desired, adjust the Isosurface **Threshold** slider to get the desired appearance.
   c. Press **Save Object**

8) In the main 3dmod window, press **File / Save Model As**, save the model as *isosurface.mod* and exit 3dmod.

9)
```
clonemodel -x 550,650 -y 1000,1100 -z 0,100 \
-at series4-8um_PtsAdded_Tom1_Iter3_Summary.csv \
isosurface.mod clonedModel.mod
```
(where each \ is immediately followed by **Enter**). Unlike clonevolume, clonemodel always clones into a new model, so there is no "-into" option. The resulting models can be easily combined

using IMOD's joinmodel program. We've specified a limited region of the volume to be cloned with the x, y, and z options. Cloning the entire volume is quite possible, but the resulting isosurface models can have so many facets that they're extremely slow to display and rotate, even with reasonably high-performance graphics cards.

10) Use `3dmodv clonedModel.mod` to examine the results. Exit 3dmodv when finished.

COLORING SURFACES AND PREPARING STILL IMAGES

In this portion, we will be painting an isosurface to distinguish a single pentamer and its neighbors from others on the BPV particle. We will use clonemodel to generate a model with a few pentamers highlighted. We will then load the model on the tomogram for visualization.

11) `cd ../../../BPV_-3/PEET/firstSearch`
`3dmod bpv_AvgVol_4P132.mrc pentamers.mod`
Open an isosurface (**Shift+U**) and turn off **View bounding box**. Open Slicer (\), and turn on **centering** (box within a box icon). From the Model View window, select **Edit / Controls** and check **Link to top Slicer angles**. From the 3dmod info window go to **Model** mode and select **Edit / Object / Type**. Change **Sphere radius for points** to **4**.

12) Rotate your virus particle so that a pentamer is centered in the Model View. From the Isosurface window, select **Paint Obj: 1**. This will now color the portion of the isosurface surrounded by the model spheres to green, the current color of Object 1. To change the color of Object 1, go to the Model View **Edit / Objects** and use the slider at the top to go to **Object 1**. Click on **Line Color** in the menu below and use the **Red**, **Green**, and **Blue** sliders to change the center of the pentamer to any color you want. From the 3dmod info

window choose **File / Save Model As** and save it with a model name of *pentamers-color.mod*.

13) From the 3dmod info window, select **Edit / Fine Grain**. Now, make a new contour (hotkey **N**) and make sure you are in **Contour 2**. In the Model View window, right-click on a subunit adjacent to the central, colored one and the Slicer window should now move to that position. Place a new model point (middle-click) in the Slicer window and then right-click in Slicer until you have the point centered over this subunit in the isosurface. The selected subunit should now be the same color as the center of the pentamer. To change the color which will be used for this and the other subunits surrounding the central one, go to the **Fine Grain** dialogue and select **Contour** in the **Edit** box. Then, under **Line Color**, press **Set** and change the **Red**, **Green**, and **Blue** sliders to a different color. Press **Done** on the color changer when finished.

    Repeat this process of right-clicking in the Model View on other subunits that surround the center of the pentamer and adding a well-centered point in Slicer. You should end up with the subunit at the center of the pentamer one color and the surrounding neighbors a different color. The 5 surrounding subunits should be in Contour 2, while the central one should be in Contour 1.

14) In the Isosurface window, uncheck **View user model**. Now you can see the painted model without interference from the spheres. Adjust the **Threshold** of the isosurface to your liking and press **Save Object**. Close the Isosurface window. In Model View, you should now see a 'blue' BPV particle with your painted pentamers and the model points as spheres. To turn off the spheres, at the top of the **Objects** window, make sure only **2** is checked. Then change the color of Object 2 by selecting **Object 2** with the slider and go to **Line Color**. Adjust the **Red**, **Green**, and **Blue** sliders to a color you

like.. Save your model from the 3dmod info window by selecting **File / Save Model**. Close all 3dmod windows.

15) `createAlignedModel *.prm`

16) `clonemodel -x 473,549 -y 339,420 -z 33,83 -at \`
`bpv_bin2_Tom1_Iter4_Summary.csv pentamers-color.mod \`
`clonebpv.mod`

17) `3dmod ../../bpv_bin2.rec.nad clonebpv.mod`
Isosurfaces—even those saved as an object—are not displayed in the ZaP or Slicer windows, so you need to visualize them in the Model View window with hotkey **v**. You should see 2 BPV particles with the painted pentamer in various orientations. As noted before, too many isosurfaces will cause your computer to run very slowly and possibly even hang or crash. From Model View, open **Edit / Controls**. Reset **X**, **Y**, and **Z** angles to **zero**.

PREPARING IMAGES FOR PUBLICATION

Images captured from IMOD windows are limited by screen resolution. We will demonstrate how to create higher resolution images suitable for publication as well as smaller images suitable for movie-making.

18) Minimize the Model View window. When preparing still images for publication, it is best to save your images in their own directory. From the 3dmod info window, select **File / Set Snap Dir**. In the resulting file chooser, make a new folder called *Stills* and press **Choose**.

19) Open **Edit / Scale Bar** and uncheck **Color ramp**. Notice at the bottom of the scale bar dialogue that the scale bar is in pixels. This model lacks pixel size information. The tomogram has the appropriate pixel size information (1.52 nm). Go to **Edit / Model /**

Header and press **Set Pixel Size from Image**. Then press **Done**. Save your model with the hotkey **s**. The Scale bar dialogue now shows the length of the bar in nm for both the ZaP and Model View windows.

20) In the ZaP window, zoom to **2**, resize the window as desired, and center a group of particles so that you have a nice image such as you might want to use for publication. Notice the scale bar value changes dynamically as you zoom in and out. Left-click anywhere in the image. You should see a yellow cross where you clicked. To turn off the cross, press **Shift+T**. To save a low-resolution image suitable for presentations, select the ZaP window and press **Ctrl+S**. This saves a tif image (*zap000.tif*) in the *Stills* directory. Confirmation of this will be displayed in the 3dmod info window. Similarly, Shift+S saves a jpeg image. I often rename the file immediately to something more meaningful and often include the scale bar size in the file name.
`mv Stills/zap000.tif Stills/BPV-1_50nm.tif`
For this step and subsequent steps, substitute the correct value for your own scale bar if it's something other than 50 nm.

21) Next, we will create larger images suitable for publications using montaging. In the 3dmod info window, select **File / Movie/Montage**. This is the 3dmod Movie window. Check **Montage** by **2** in **Zap montage snapshots** near the bottom of the dialog box. Select the ZaP window and press **Ctrl+S**. You will see 4 images flash by as a seamless montaged image is created. Rename the file with:
`mv Stills/zap000.tif Stills/BPV-2_50nm.tif`.

22) Restore the Model View window and press **Ctrl+S**. A message will print in the terminal window that your image (*modv0000.tif*) was saved in the *Stills* directory. Rename using
`mv Stills/modv0000.tif Stills/model-1_50nm.tif`.

Model View images will save in the same directory (*Stills*) you already chose for the ZaP images; you can move or save them to a different directory if you so choose.

23) From the Model View window, select **File / Movie/Montage**. This is called the 3dmodv Movie window. Notice that this window is different than the 3dmod Movie window accessed from the 3dmod info window. Near the bottom of the window, change to **Montage** and press **Make**. Again, you will see a series of 4 images flash on the screen as the montage is created. Rename the file with `mv Stills/modv0000.tif Stills/model-2_50nm.tif`

24) To see the size of the files, type `ls -l -h Stills/*.tif`. The –h option says to list file sizes in human readable format. Notice how the first images are considerably smaller than the second images. The first files are appropriate for presentations and the larger files are appropriate for scaling to generate high quality images for publication. You can also make smaller files by using a different format such as png, especially for model images.

MAKING A SERIES OF IMAGES FOR A MOVIE

25) Now you are going to save a set of files for a simple movie showing different slices through the tomogram. Select **File / Set Snap Dir** and create a folder named *ZaPs*. Go to the ZaP window and determine where the main layer of the BPV particle starts and stops (~ slices **36-88**). Resize the ZaP window so that it is about 5x5 inches on the screen. From the **3dmod Movie** window, change the **Start** and **End** sliders to the numbers you just determined. Make sure that **None** is selected under **Snapshot** and uncheck **Montage**. You can middle- or right-click in the ZaP window to preview what your movie will look like. Once you are satisfied, change the **Snapshot** from None to **JPEG**. Middle-click in the ZaP window. You will see the file names *zap000-zapnnn.jpg* displayed in the 3dmod info

window. When all the images have been saved, be sure to **change Snapshot** back to **None** or you may accidentally write more files.

26) Switch to the Model View window and change back from **Montage** to **Movie** in the **3dmodv Movie** window. Switch the **Save as** selection from TIFFs to **PNGs**. Go to **File / Set Snap Dir** and make a new folder called *model*.

27) For more complicated movies, the Movie Sequence window lets you save information about a series of movie segments, and record them all in sequence. This is particularly useful if there is any chance that you might need to remake the movie later. A movie segment is just the continuous set of operations that happen when you press Make in the 3dmodv Movie window. Press the **Sequence** button to open this window. We will now make a movie in the Model View window where we slice down through the tomogram image without out the isosurfaces present, back up, then down again, this time with the isosurfaces visible. We will then remove the image and rotate the isosurfaces a full 360° around the Y axis.

Go to **Edit / Image** and check **View Z image** and press **Use 3dmod Black / White**. In the **Controls** window, be sure that all of the angles are **0**. Go to **Edit / Objects** and turn off all objects by unchecking them. In the **Image** window change the **Z** slider to **81**. On the **3dmodv Movie** window, press **Set Start**. Now change the **Z** slider to **31** and press **Set End**. Change the **# of movie frames** to **25**. Press **Make**. In the **Movie Sequence** window, press **Add After** to save this as the first segment. In the **Movie Sequence** table, **Label** this as **Movie Down (81-31)**.

Now, we need to reverse this process by pressing **Set Start**. Then change the **Z** slider to **81** and press **Set End**. Press **Make** followed by **Add After**. **Label** as **Movie Up**.

Let's turn on the isosurfaces. In the **Objects** window, check **Objects 2 and 5**, then press **Set Start**. Move the **Z** slider to **31** and press **Set End**. Press **Make** and **Add After**. **Label** as **Movie Down with isosurface**.

Uncheck **View Z image** and press **Full 360 Y**. Change # **of movie frames** to **60**. Press **Make** and **Add After**. **Label** as **Full 360 Y** (this should happen automatically).

28) Notice that in most cases you need to define the end of the previous segment as your new start by pressing Set Start; this was not necessary in the case of Full 360 Y, which takes care of this automatically. Additionally, meaningful labels will help you remember what you did.

29) From the **Movie Sequence** window, press **Run All**. This is a preview of your movie sequence in order. You can change # **Frames** for any of the segments now, and then press **Run All** to see how the appearance changes. Naturally, the more frames you have, the larger the movie. Once you are pleased with your movie sequence, from the **3dmodv Movie** window, check the **Write files** box. This time, when you press **Run All**, the files will be written to the *model* folder. This image series can now be loaded into your favorite movie making software.

30) To save the sequence, press the **Save** button in the **Movie Sequence** window with the file name *ModelSequence.txt*. You can reload this file later, modify if desired, and remake the movie. Close all 3dmod windows and save your model when asked.