# Lesson 8: Microtubules

## SIMPLE FILAMENT MODELING

In this exercise, we'll look at modeling a simple, untwisted microtubule using an accessory program, addModPts.

1) `cd $WORKSHOP_HOME/PEET_Labs/MT`

2) Open the tomogram along with a new model.
`3dmod series4-8um-cor.rec mySimpleModel.mod`

3) Switch to **Model** mode, select **Edit / Object / Type**, and choose **open**.

4) In the ZaP window, set zoom to 1.0 and center the microtubule in Z (Z = ~45). Drag the image with the mouse so the top left corner of the microtubule is in view. Starting at the upper left, and working down and to the right, middle-click to place 5-7 model points sequentially along the length of the tube near the center of the lumen. You will need to change Z height to follow the center of the tube since it is not quite in the XY plane, especially near the bottom right corner. Do this relatively quickly, not spending too much time to accurately center the model points, since we'll be correcting their location in the next step. You should end up with all your points in Contour 1. Save the model with hotkey **s**.

5) Open a Slicer window (**Image / Slicer** or hotkey \). Select **interpolation** (checkerboard) and **centering** (box within a box) modes by clicking the **1st** and **3rd** buttons at the top of the Slicer window. You may need to change the point selection for centering to take effect. Select either the first or last model points. Set the

zoom to **2.5** and adjust the **Z rotation** until the tube is vertical (approximately **-27**°).

6) Set **X rotation** in the Slicer window to **-90**° so you are looking down on an XZ cross-section of the tube. Set **Img** to **30** to display a 30-slice thick view (approximately 27 nm). If the current model point does not appear well centered, right-click in the center of the tube to move the point location. You can right-click repeatedly until you are satisfied. As in a previous lab, you may optionally wish to set **Mod** to **10** and **Sphere radius for points** to ~**12** as an aid to more easily visualize when the point is well centered.

7) Adjust the remaining points by using the **scroll buttons** in the 3dmod info window or move to the previous or next point using the **[** and **]** hotkeys. With tubes more curved than this one, you might need to adjust the Z rotation when changing points. This tube is sufficiently straight so that no such adjustment is necessary. Accurate modeling is worth the effort! In difficult cases, it can make the difference between success and failure. Save your model when finished.

8) Next we want to insert model points approximately every 8 nm along the center of the tube, since this is the pitch of the $\alpha$-$\beta$ tubulin dimers. The voxel size is 9.06 Å, so we'll place a point every round(8 / 0.906) = 9 voxels, where "round" denotes the nearest integer.

```
addModPts mySimpleModel.mod 9
```

Notice that addModPts prints both the name of the output file (*mySimpleModel_PtsAdded.mod*) and the final number of points.

9) Open *mySimpleModel_PtsAdded.mod* on your tomogram by going to **File / Open Model** via the 3dmod info window to verify that you now have points evenly spaced along the length of the microtubule.

Adjusting Sphere radius for points will make visualization easier. Exit 3dmod when finished. You've now completed a simple model suitable for aligning and averaging either a helical microtubule, such as the 15 PF sample we're currently working with, or a 13 PF tube with a seam. We'll look at the settings you might use for such alignments momentarily. First, let's see how to model a more complicated case in which we need to account for twisting of the tube about its axis.

## MODELING A TWISTED FILAMENT

We'll consider how to model a twisted microtubule using programs addModPts and modTwist2EM. We need to create a model with 2 objects in point-to-point correspondence, with Object 1 following to the center of the microtubule and Object 2 tracing paths following contours indicating twist on the tube surface. We'll use protofilaments on the upper surface of the tube for the latter and will trace a given protofilament only until it starts to wrap around either side of the tube and becomes hard to follow in an XY cross section; then we'll start a new contour and switch to another protofilament. This process is not difficult, but is slightly tedious, so we've done this portion of the modeling for you to save time. Let's open the starting model, so you can see this initial model and how you would create such a model yourself.

10) `3dmod series4-8um-cor.rec stage1.mod` and select **Model** mode.

11) Open a Slicer window, set **zoom** to **1.0**, select **interpolation** (checkerboard) and **centering** (box within a box) modes, and set **Img** to **15** and **Mod** to **5**. Rotate the microtubule to be vertical and adjust the size of the Slicer window so that it fills your screen from top to bottom, allowing you to visualize a significant fraction of the microtubule's length. The 15-section thick slice makes the protofilaments readily visible when focused on the upper (or

lower) surface of the microtubule. Additionally, we've already set Line thickness and Sphere radius for points to aid visualization of neighboring points and contours. This is often not necessary when modeling, but it's good to know that you can always make similar adjustments when you find them helpful. Open the Model View window by using the hotkey, **v**. Right-click on any model line in the Model View window to center that same line in the Slicer window.

12) Examine several points / contours. Notice:
    a) How the first point of a new contour is always placed at approximately the same axial position as the last point of the previous contour, and
    b) how we can skip one or more protofilaments when starting a new contour.

    PEET uses the single point overlap between neighboring contours to distinguish rotation due to supertwist from that caused by the jump from one protofilament to another.

13) Next, we will make a second object, with the same settings as Object 1. Select **Edit / Object / New**, press **Copy from object 1**, and **Done**. Somewhat counter-intuitively, this creates a new, but still empty Object 2. Select Object 1 by left clicking on any existing model point or by choosing **Object 1** in the 3dmod info window. **Press Edit / Contour / Copy**, select **Copy to Object#**, enter **2**, choose **All contours in object**, press **Apply**, and then press **Done**. You now have two identical objects, 1 and 2, following protofilaments along the surface of the tube. Recall, however, that we want the points in Object 1 to follow the center of the tube. We'll next modify Object 1 to accomplish this.

14) Once again, set the Slicer window **Z rotation** to **-27**° to orient the tube vertically, and **X rotation** to **-90**° to obtain a cross-sectional view. Adjust slice thickness as desired using the **Img** field.

15) Select **Object 1, Contour 1, Point 1** on the 3dmod info window. As before, right-click in the center of the tube to move this point to the center of the tube. When satisfied, proceed to the next point, continuing until all the points in Object 1, Contour 1 have been centered. **Do *not* center the points in Object 2!** Those should remain at the surface of the tube where we initially placed them. Repeat this process for the remaining contours. Use the model view window to verify that you moved all the points.

16) When finished, save your model as *myStage2.mod* by going to **File / Save Model As**. If you wish, compare your results with the provided *stage2.mod* by typing `3dmodv stage2.mod`. They should be very similar. Close all 3dmod windows.

17) Next run `addModPts myStage2.mod 9 T`. The final "T" argument tells addModPts to run in a special mode in which it will process both center points in Object 1 and surface points in Object 2.

18) Run `3dmod series4-8um-cor.rec myStage2_PtsAdded.mod`, and select **Model** mode. Next we need to select a nice-looking, but representative contour / point near the middle of the tube to use as a reference. For this example, we will use point 36 of contour 3. Exit 3dmod.

19) Run `modTwist2EM myStage2_PtsAdded.mod 36 3` and notice the information printed by modTwist2EM. The "EM" in the name comes from an old format previously used instead of csv files for motive lists. The name has persisted, even though the format has changed.

    This yields both a model, *myStage2_PtsAdded_Twisted.mod*, from which overlaps between contours have been eliminated, and a corresponding initial motive list, which compensates for the supertwist named *myStage2_PtsAddedRefP129_initMOTL.csv*. These are suitable as inputs into a PEET alignment for a twisted tube.

## EXPLORING THE ALIGNMENT SETTINGS AND RESULTS

20) `cd PEET/firstSearch`

21) `etomo *.epe`

22) Examine the settings chosen for the alignment search, and see if they make sense to you. Specifically, these settings are for the 15 PF helical case using the first, simpler modeling strategy from step 8 above. Axial randomization is used with a Phi search range limited to 360 / 15 = 24°.

    The model used here actually contains 2 objects and is also suitable as input to modTwist2EM. PEET ignores objects other than the first when doing alignments, however, and the few overlapping points between adjacent contours, while not desirable, do not cause any significant problems in this case.

23) One of the first things you should do during an alignment run is to verify that the reference, and if applicable, masking is appropriate by running `3dmod *_Ref1.mrc` and checking in appropriate views. Depending on your situation, you may need to check multiple slices and in several orientations. In this case, because we're examining output files from an already completed run, you can simply press **Open references in 3dmod** at the bottom of the Etomo **Run** tab. It's also instructive to scroll through the references from the various iterations. Typically, you should see monotonically improving images, ideally with rapid convergence. References that get worse are often an indication of an incorrectly set up alignment, even if things eventually converge.

24) Next, consider how you might change the alignment settings for a 13 PF microtubule, again assuming use of the simple search strategy without tracing protofilaments. You would simply change the Initial Motive List selection from Random axial (Y) rotations to

Align particle Y axes, and, optionally, choose a more restricted initial search range for φ (Phi), reflecting only the uncertainty in tracing protofilaments rather than the 24° spacing between protofilaments for a 15 PF microtubule. Close all Etomo and 3dmod windows.

25) Similarly, to use the files generated by modTwist2EM from step 19 for the more complex strategy described above you would
    a. copy / rename the model and initial motive list generated by modTwist2EM if desired (often directly into the new project directory, although this is not required),
    b. change the Initial Motive List setting to User supplied csv files, and
    c. add the path to the initial motive list csv file in the Initial MOTL column of the volume table.

    As above, a more restricted φ search would suffice.