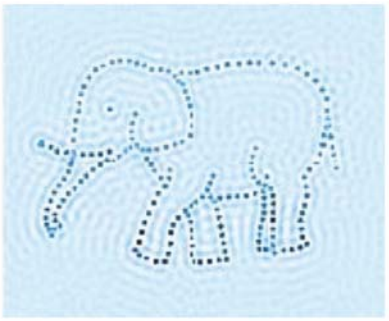


EMBO Practical Course on Image Processing for CryoEM

5th September 2019

Practical 3, Part 2: Tomography Segmentation with IMOD (version 4.9.{x}) – Segmenting microtubules and vesicles from a {human neuronal cell} tomogram.

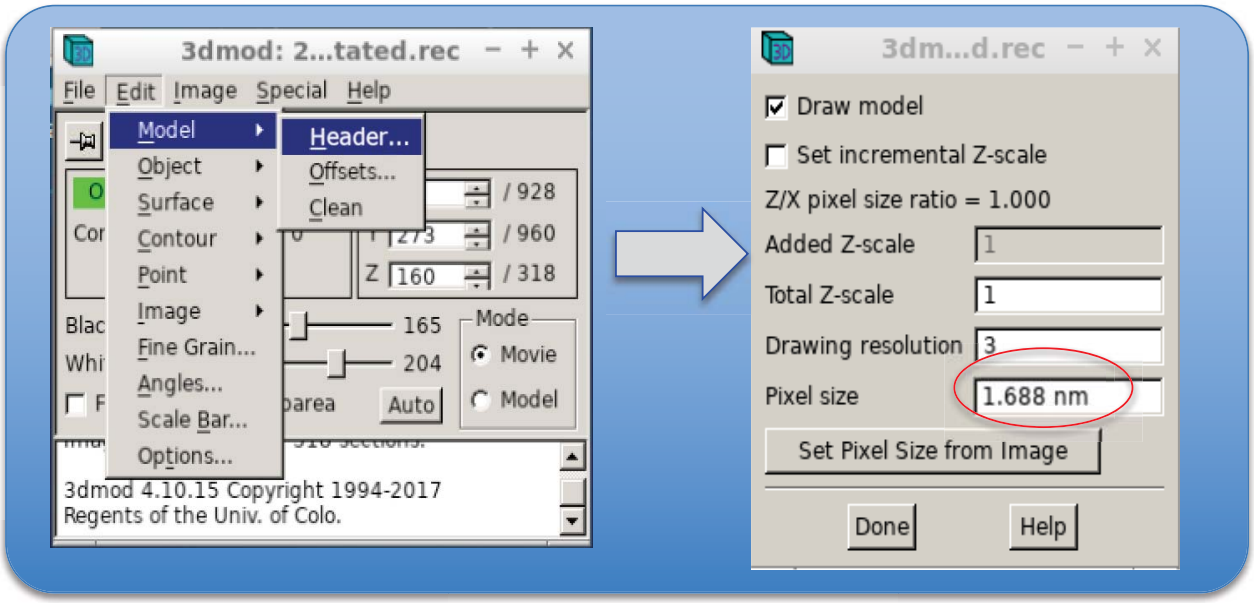
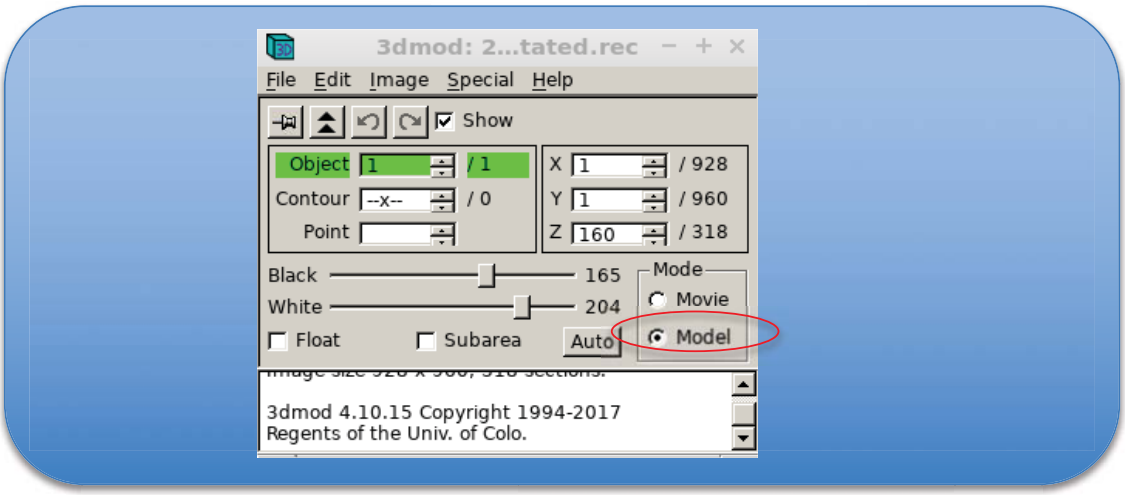
AIMS

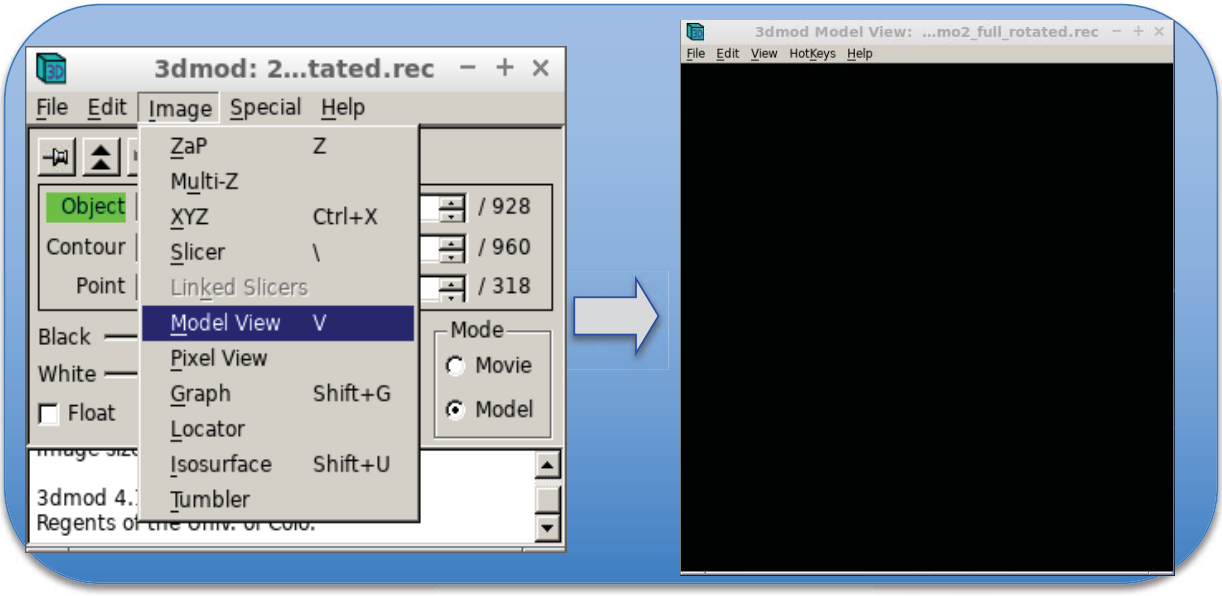
In Part 2 of this Practical you will learn how to use several IMOD tools to highlight biological structures visible in the tomogram reconstructed in Part 1.

In particular, you will learn how to:

- segment elongated/tubular structures, like microtubules (section 2)
- segment globular/spherical structures, like vesicles (section 3)
- make a movie of the final segmentation (section 4)

Section 1: Preparation

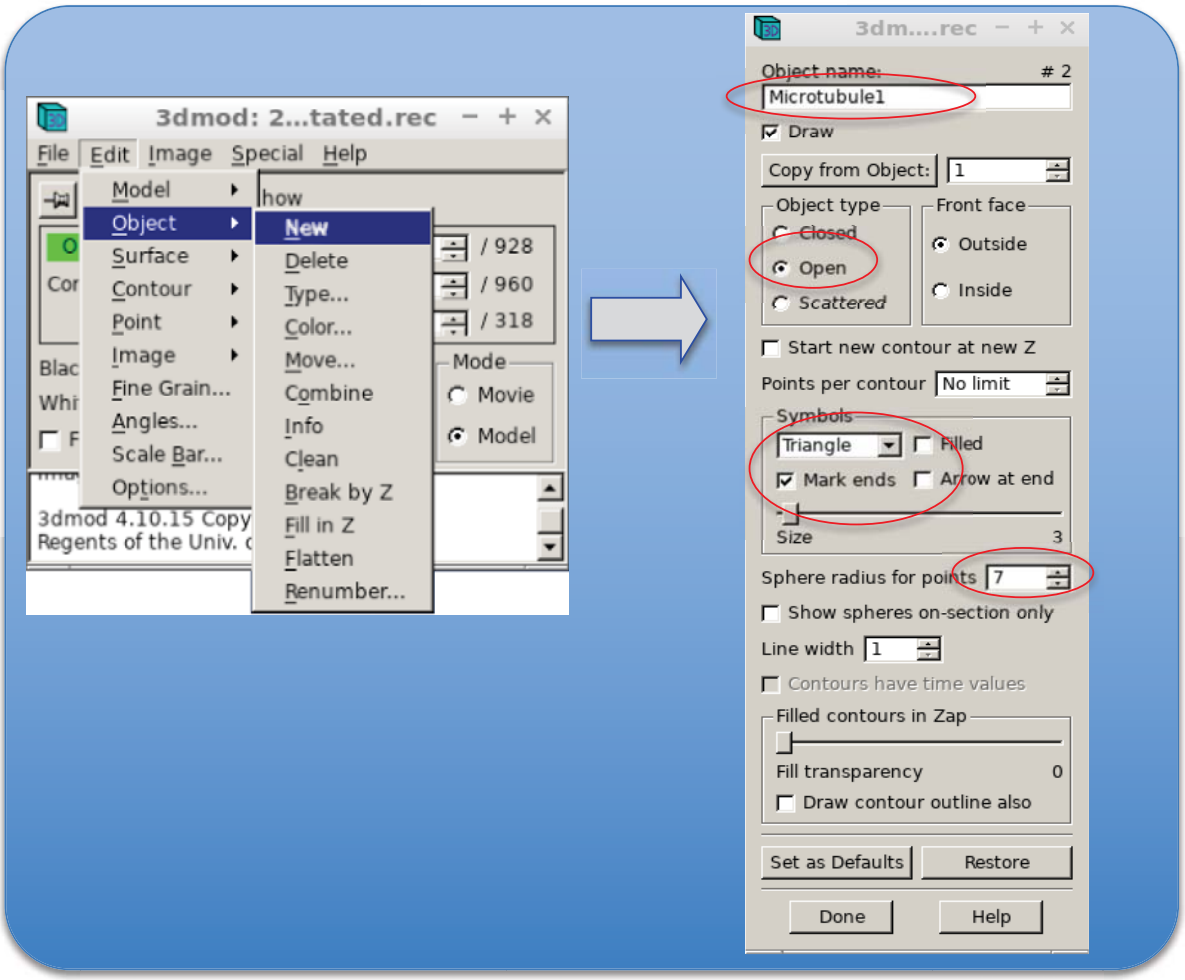
1A	Open your reconstructed tomogram, as explained in the last section of Practical 3, Part 1. Before starting the segmentation, make sure you have setup the correct pixel size for your model. Go to "**Menubar > Edit > Model > Header**". Make sure this contains the correct pixel size of the tomogram, in our case it should be 1.688 nm. 
1B	Be sure you are in "Model" mode, or switch to *Model* in the main "3dmod window". 

1C	Open the “3Dmod Model View” window by pressing the “v” key or by clicking on “**Menubar > Image > Model View**”. 

Section 2: Microtubule Segmentation

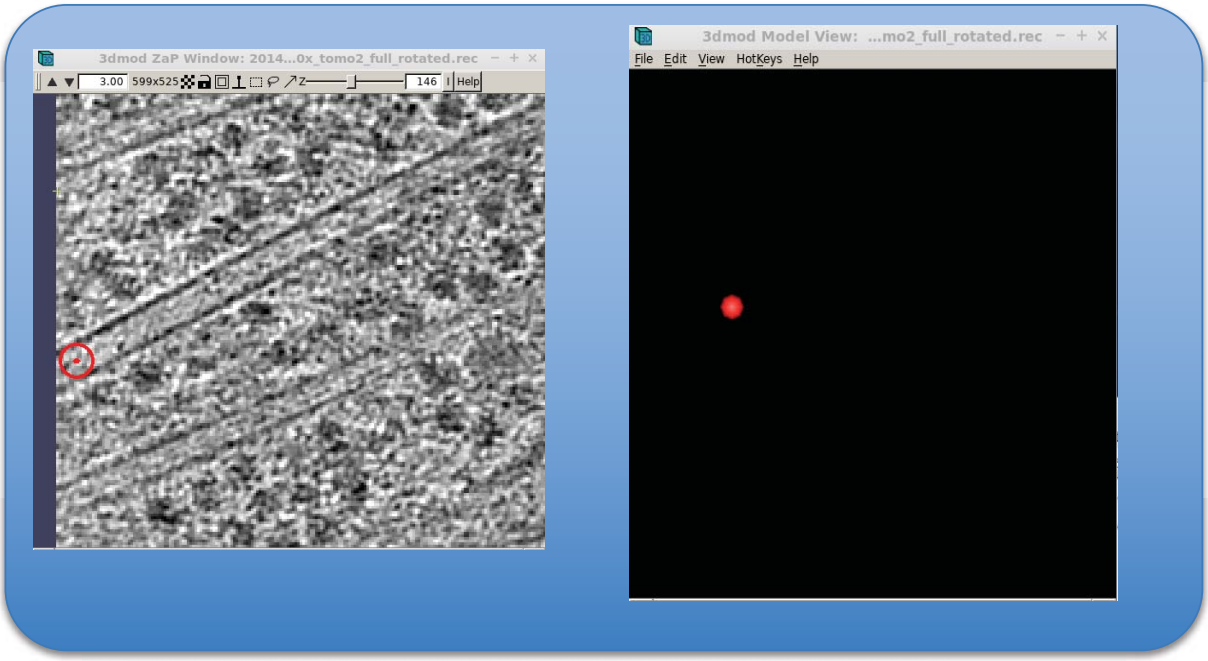
2A	**Create a New Object**: Go to **“Menubar > Edit > Object > New”** and give the object an appropriate name (e.g.: "*Microtubule1*"). Select the “Object type” as *Open*. This option will allow to create an open contour object (e.g. a line) in which the first point is not connected to the last point. Determine the inner radius (half the diameter) of your microtubules in pixels. In our case, it should be approximately 7 pixels. Type the radius in the field “Sphere radius for points”. This sphere size is set just to help you see where you've put points, so don't worry if the spheres are too large.

Another helpful segmentation tool is the choice of a symbol to mark each point. For example, in the "Symbol" field you can choose *triangle* with size 3 and tick (✓) *mark ends*: this will project a triangle over each point and also marks the first and last point in the contour with a red and green cross respectively.



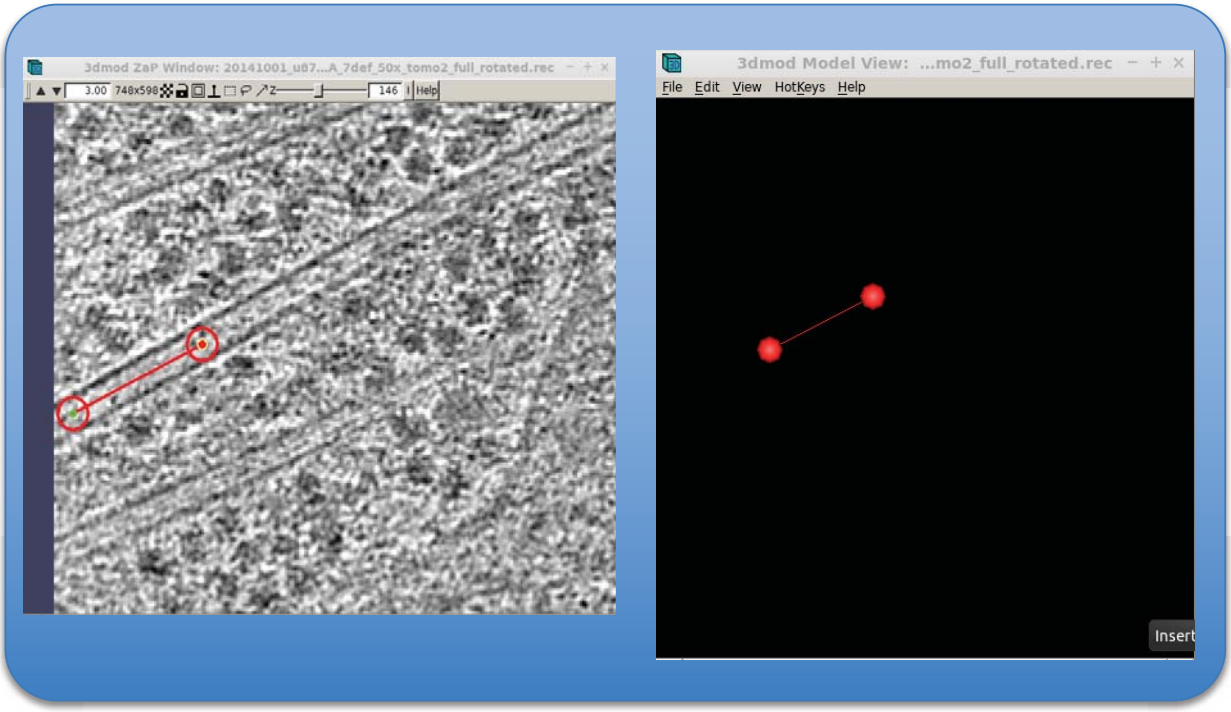
2B **Draw points as contours.**

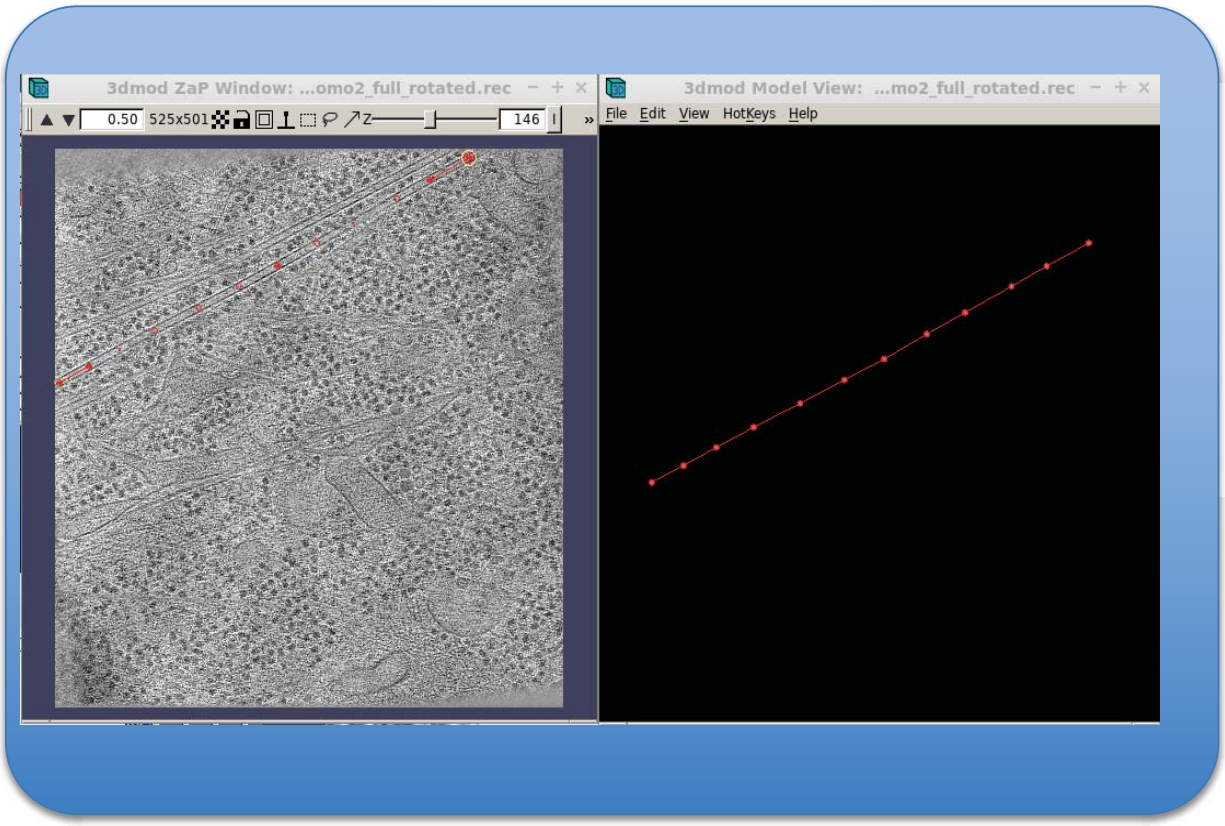
In the ZAP window of the tomogram, use the keys **[PgUp]** and **[PgDown]** to locate one end of a microtubule and the key **[+]** or **[-]** to zoom/unzoom in the area. Once you locate it, left-click at the

	centre of the microtubule. Place the first point by clicking the middle mouse button. You will be able to see a point in the "3dmod Model View" window and you can use the scroll wheel of the mouse to zoom in and out of the model. 
2C	**Draw a line by placing multiple points in the same contours.** Place the second point at a distance of approximately 4-6 times the diameter of the microtubule by middle-click. In the "3dmod Model View" you will see two dots joined by a line. This means that the two points are part of the same contour (group of points). If you don't see the line connecting the two dots in the "3dmod Model View" you accidentally created a new contour. To avoid creation of new contours, be sure the last inserted point is selected: usually a yellow cross or circle surrounds the centre of the last placed point, making it selected.

Once a new point is created by middle-click, it should be also automatically selected. However, sometimes a left-click in the ZAP window may cause the deselection of the last point, resulting in creating a new contour, breaking the line.

In this case the easiest thing to do is to delete the last point inserted, select the second-last inserted point by left-click, and place the new point using the middle-click, as usual. Alternatively, two contours can be joined by selecting the separate segments using **[CTRL+left click]** and by joining them using **[SHIFT+j]**.



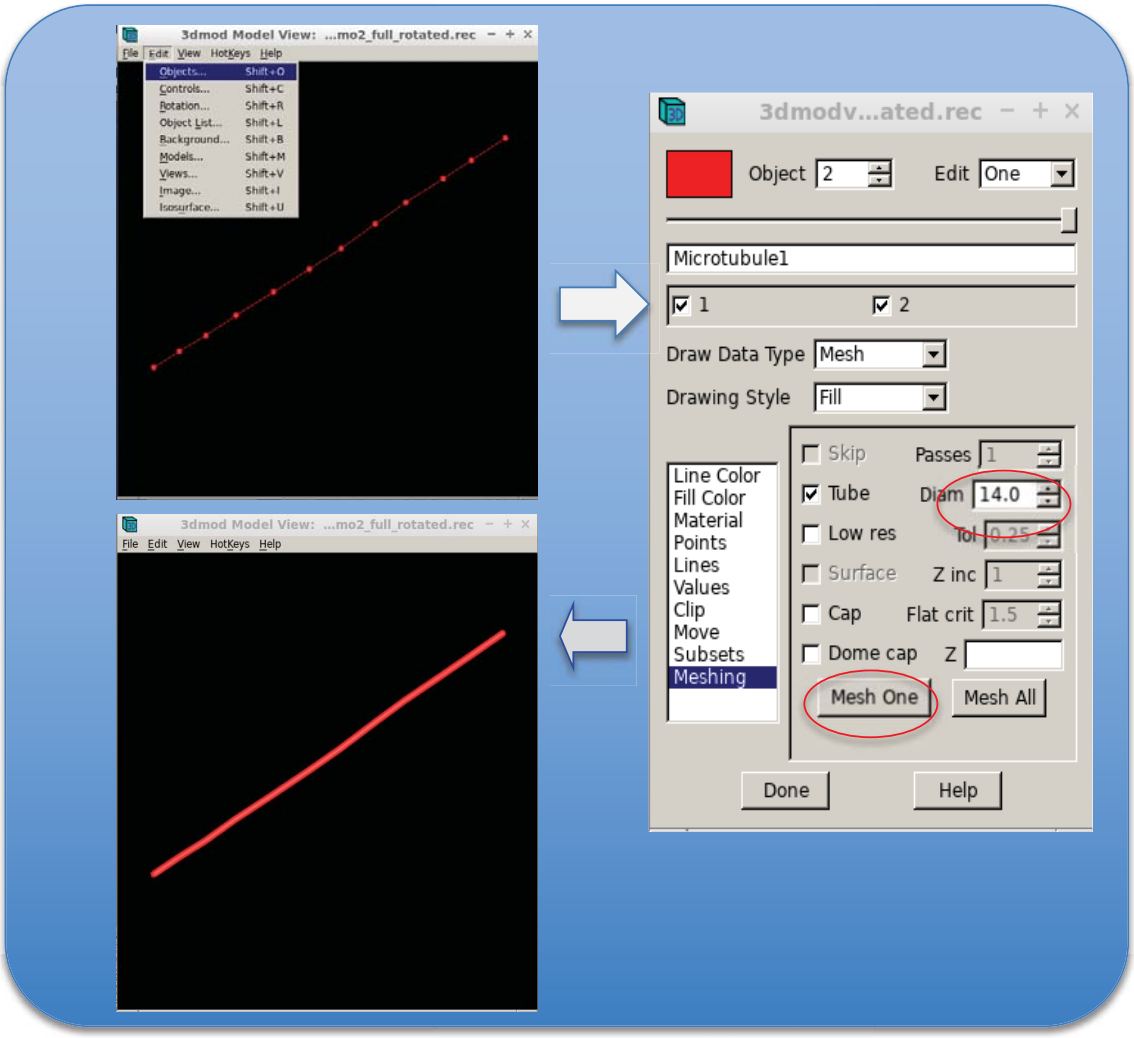
2D	Keep placing points till you reach the other end of the microtubule, trying to avoid the creation of new contours. If the microtubule is not perfectly centred in the plane of the section, use the **[PgUp]** and **[PgDown]** to slice the tomogram in the Z direction, and place the point at its correct centre. If any of the point is slightly misplaced from the microtubule, you can select it with left-click and move it with right-click at the amended position. 

2E

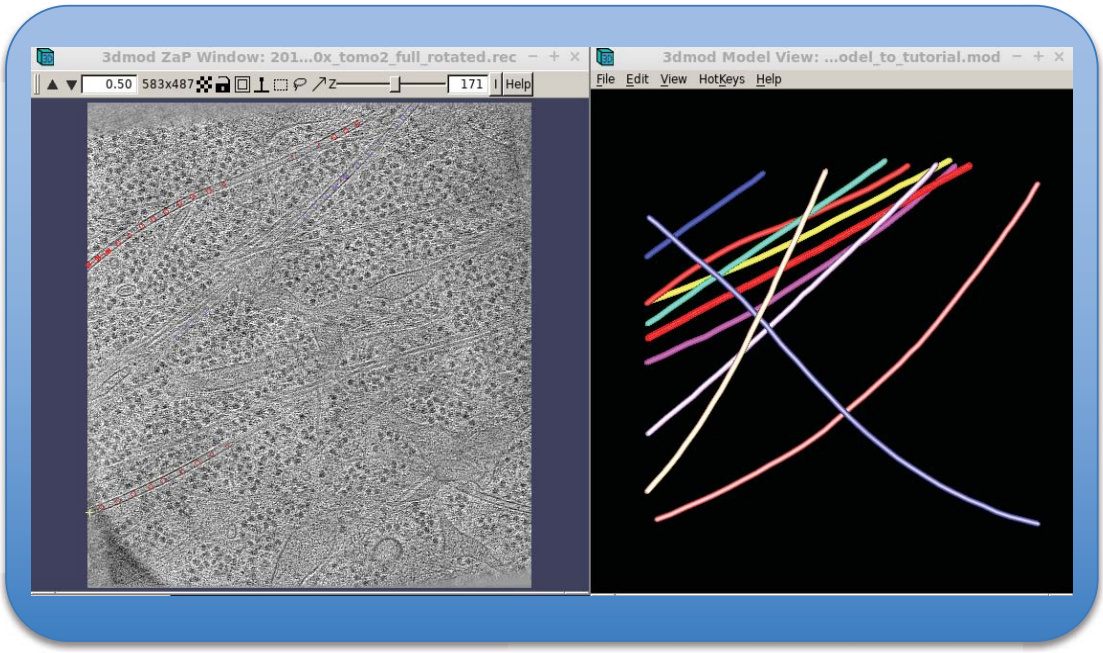
Meshing the model

To create a tube mesh, select the Model View Window then click "**Menubar > Edit > Objects ...**". In this objects window, tick (✔)*Tubes* in the "Meshing" field and enter the value of diameter in pixels in the *Diam* box, to set the diameter of the tube.

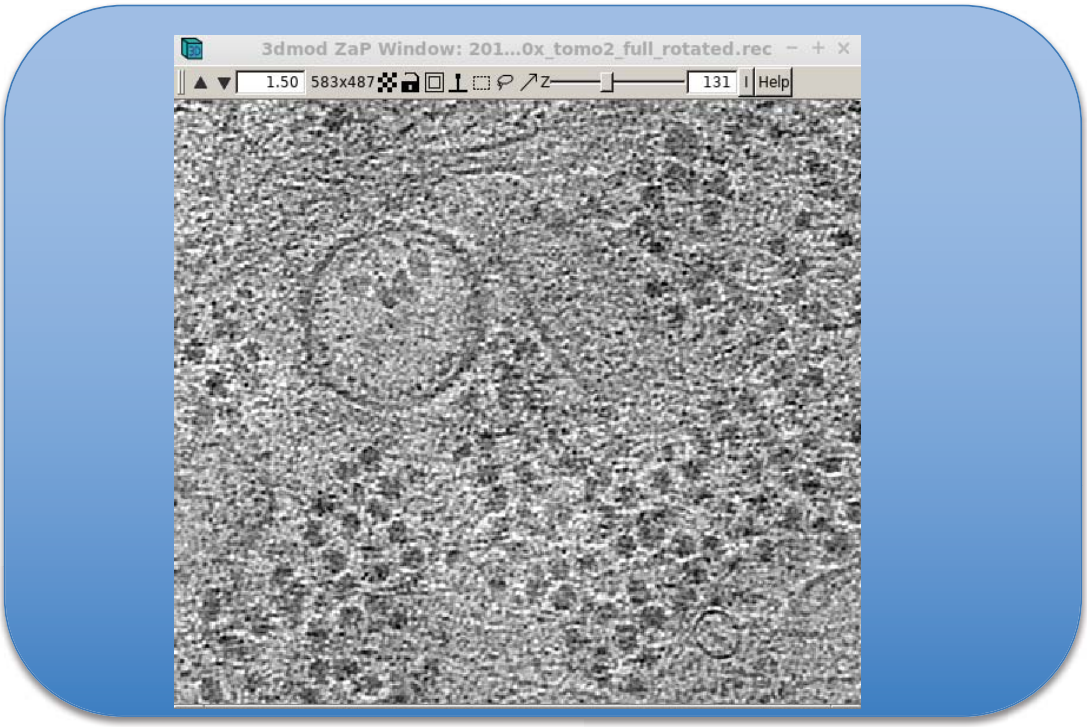
We calculated the diameter of the microtubule earlier, approximately 14 pixels. Once the correct diameter is set, click "**Mesh One**" and you will see your tubes generate as mesh.

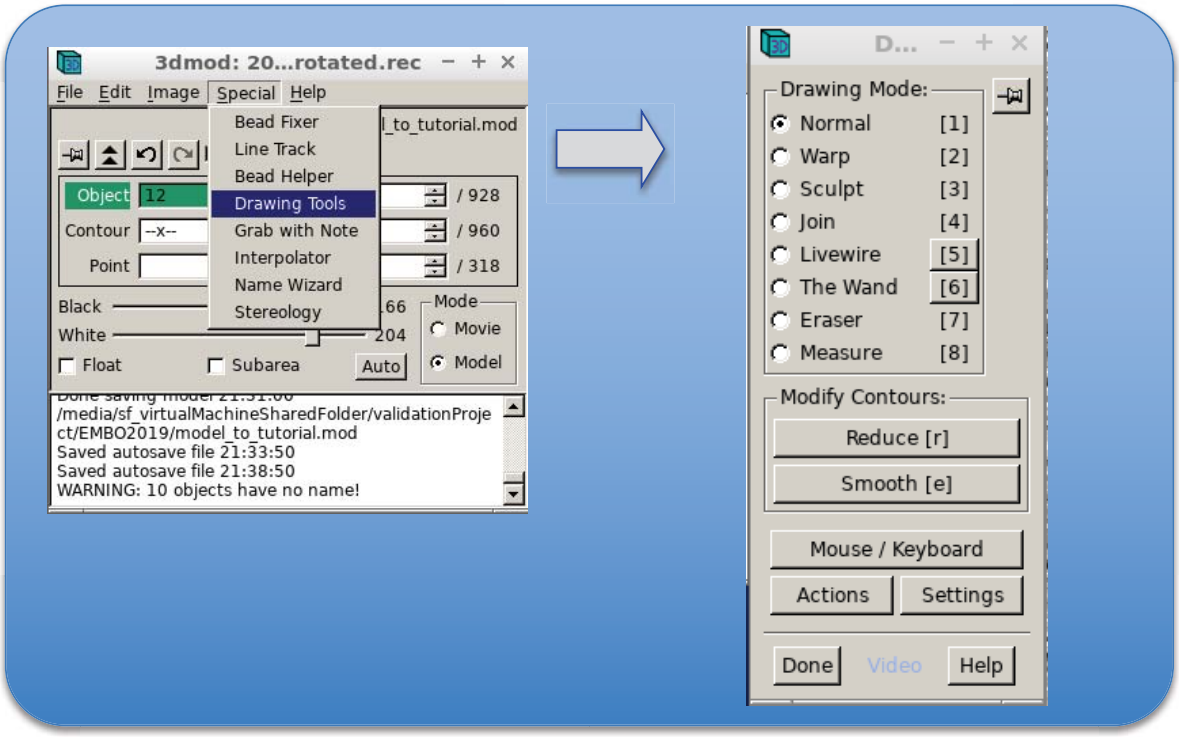


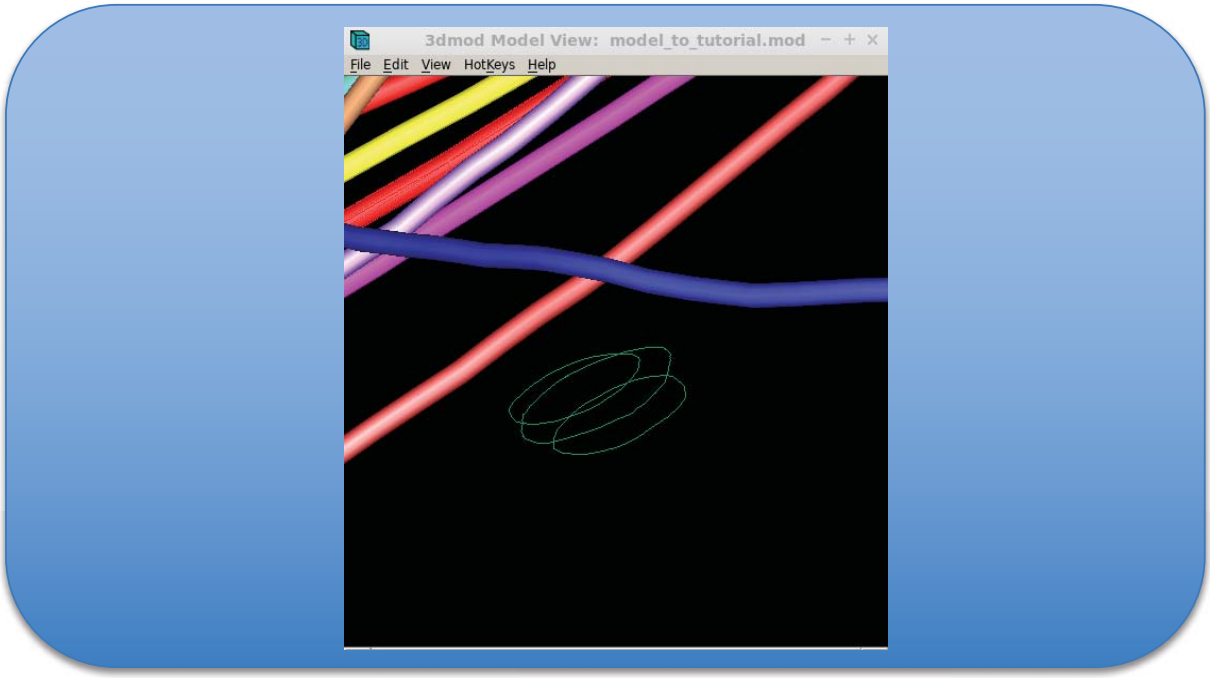
	Note that you will have to click "**Mesh One**" to regenerate this mesh each time, and you can switch back to the normal view by changing the "*Draw Data Type*" between *Mesh* and *Contour*. You might also want to hide spheres by clicking *Points* and then tick the option (✔)*Skip if drawing mesh*. **OPTIONAL:** To change the mesh color, click on "**Fill Color**" and tick the (✔)*Use fill color* option, then slide Red, Green and Blue bars until the preferred color is displayed. To make the 3D effect pop, click on "**Material**" and play with the bars to adjust lights and shadows of the model until the preferred effect is reached.
2F	**Segment more microtubules** You can now segment more microtubules, this can be done in two alternative ways • by creating a new object (go to point 2A) • by creating a new contour (go to point 2B) Creating a new contour instead of a new object is quicker, as you don't have to select every time the "Open", "Object type" and "Sphere radius for points". However, the advantage of segmenting new microtubules as new objects is that you can deal with each microtubule separately, with different colours, or excluding a subset of objects from visualization. To get the result shown below, we segmented the different microtubules as different objects as from point 2A.


3dmod ZaP Window: 201...0x_tomo2_full_rotated.rec
0.50
583x487
171
Help
3dmod Model View: ...odel_to_tutorial.mod
File Edit View HotKeys Help

Section 3: Vesicle Segmentation

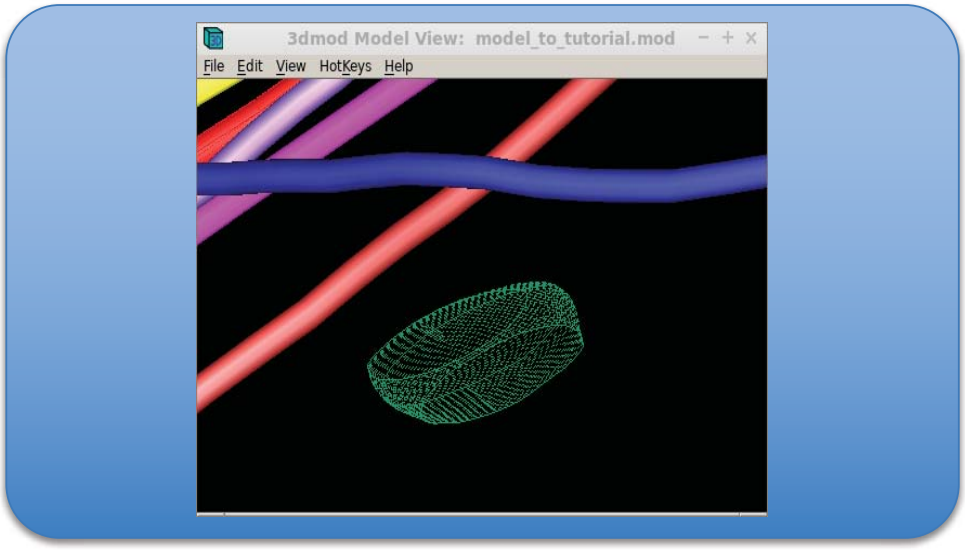
3A	**Getting started** Keep the "3dmod Model View" window open and create a new object **(Menubar > Edit > Object > New),** You may name it as "vesicle 1" and in this case, make sure that the Object type is marked as "Closed".
3B	**Localize a vesicle** Localize a vesicle in the tomogram using **[PgUp]** and **[PgDown]** to slice it, as usual. Going through the sections you may notice that for some sections the edges of some vesicles are barely visible, if not visible at all. This is due to the missing wedge effect, an intrinsic ET technique artefact caused by the restricted range of tilting angles, which limits the amount of information obtainable from the object. Here we first segment the visible part of the vesicle, and then we show how to interpolate not-visible regions. Although it is good practice to avoid segmenting what you cannot see, it is up to the investigator to decide whether or not segmenting those regions. 

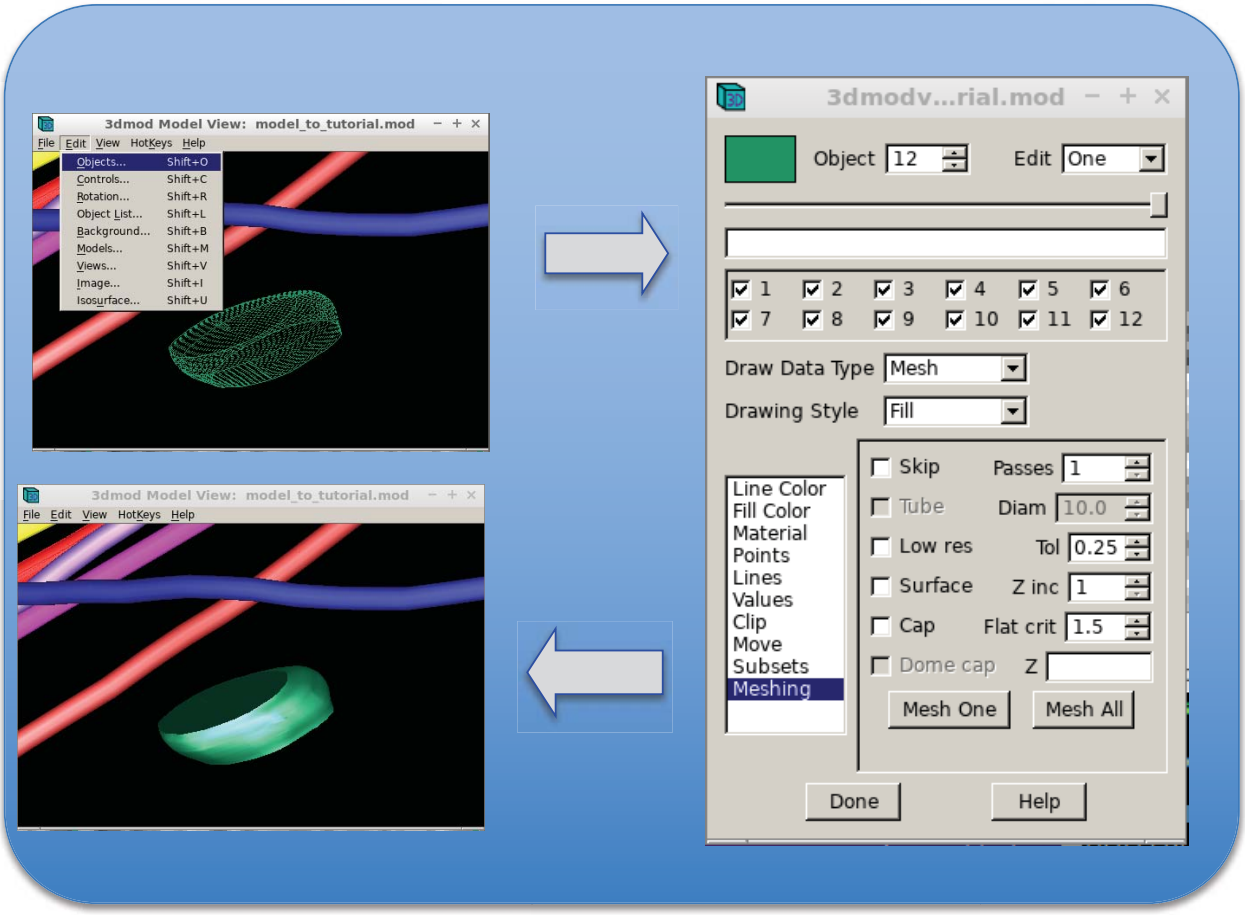
3C	Open “**Menubar > Special > Drawing Tools**” and select the “Normal” option in the *Drawing Mode* field. 
3D	**Draw a line around the vesicle.** To draw a line, you first need to left-click on the vesicle, then with the middle mouse button clicked, draw a complete circular line. You will be able to see the circular line in the “3dmod Model View” window.
3E	**Draw lines around the vesicle at different Z-coordinate planes** Navigate through the sections and draw other complete circles along the vesicle as different contours, as just described in point 3D: first, left-click to de-select the contour at the previous section and then use the middle mouse button to actually draw the circle. Every circle in every section should be a different contour. In the “3d Model View” you can zoom by scrolling the mouse, and you should see several circles, corresponding to the vesicle segmented at different sections:

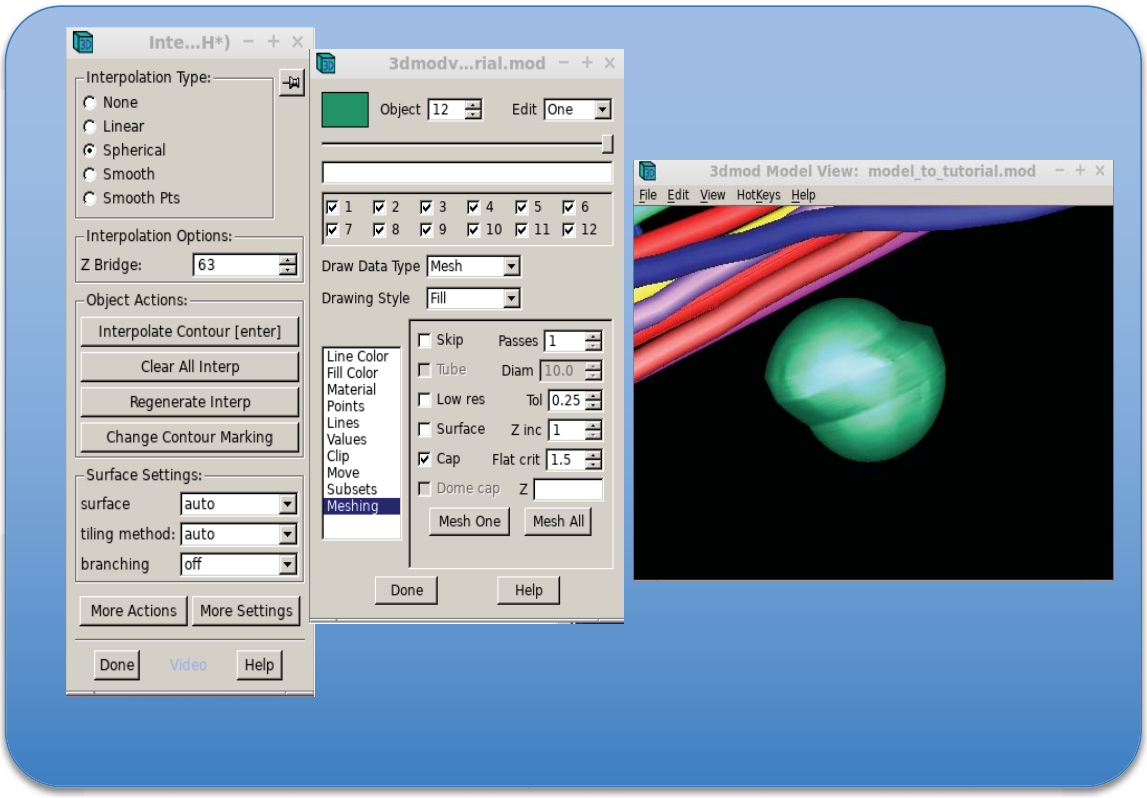


3F **Interpolate intermediate contours**

To interpolate missing segmented sections, the "**Menubar > Special > Interpolator**" is a very useful tool. Select "Smooth" in the *Interpolator Type* field. Then left-click on a vesicle contour to select it. Click on "Interpolate Contour" or press the **[Enter]** key to apply the interpolation. The interpolated contours are now visible on the "3dmod Model View".

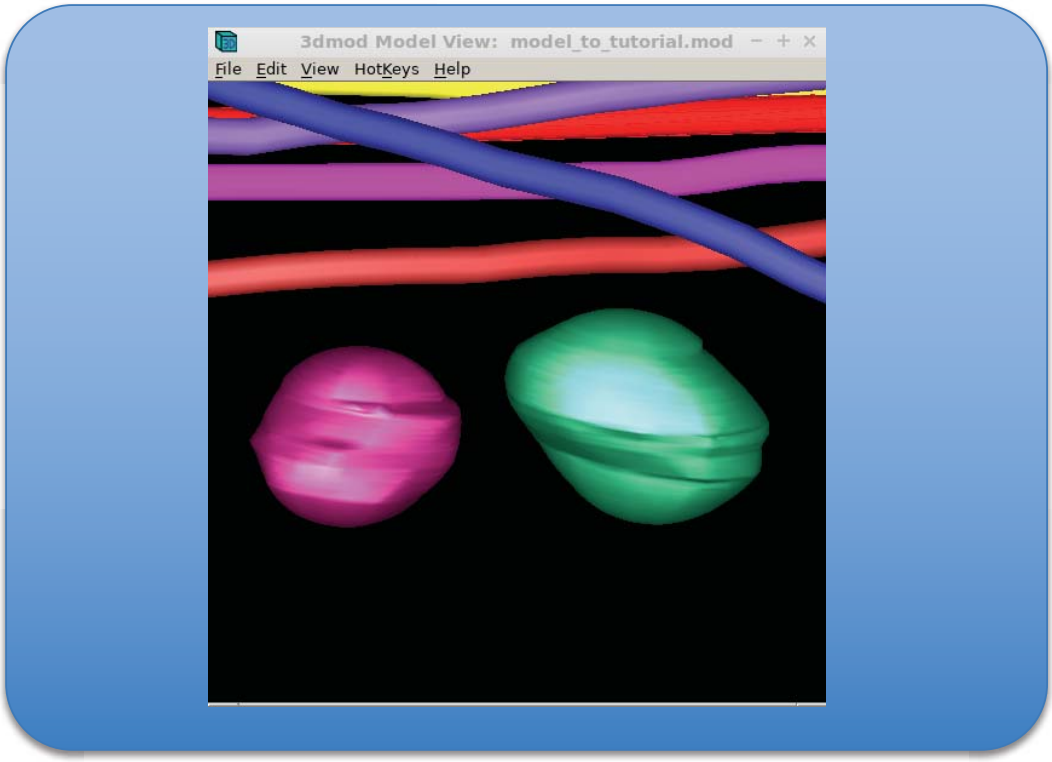


3G	**Mesh the contours** You can now mesh the contours using the Object Window in the "**3dmod Model View > Edit > Objects**": select the vesicle object, go to the *Meshing* tab and click "Mesh One". 
3H	**Interpolate missing regions (i.e. guess)** If you want to interpolate the vesicle regions invisible because of missing wedge, you can use the "Spherical" option as type of interpolation in the "Interpolator" window. This assumes the object is spherical and approximates the missing regions to a sphere. You can click "Interpolate Contour" or hit the **[Enter]** key to create the interpolation, and then apply again the mesh effect to see the result in the "3dmod Model View" window:

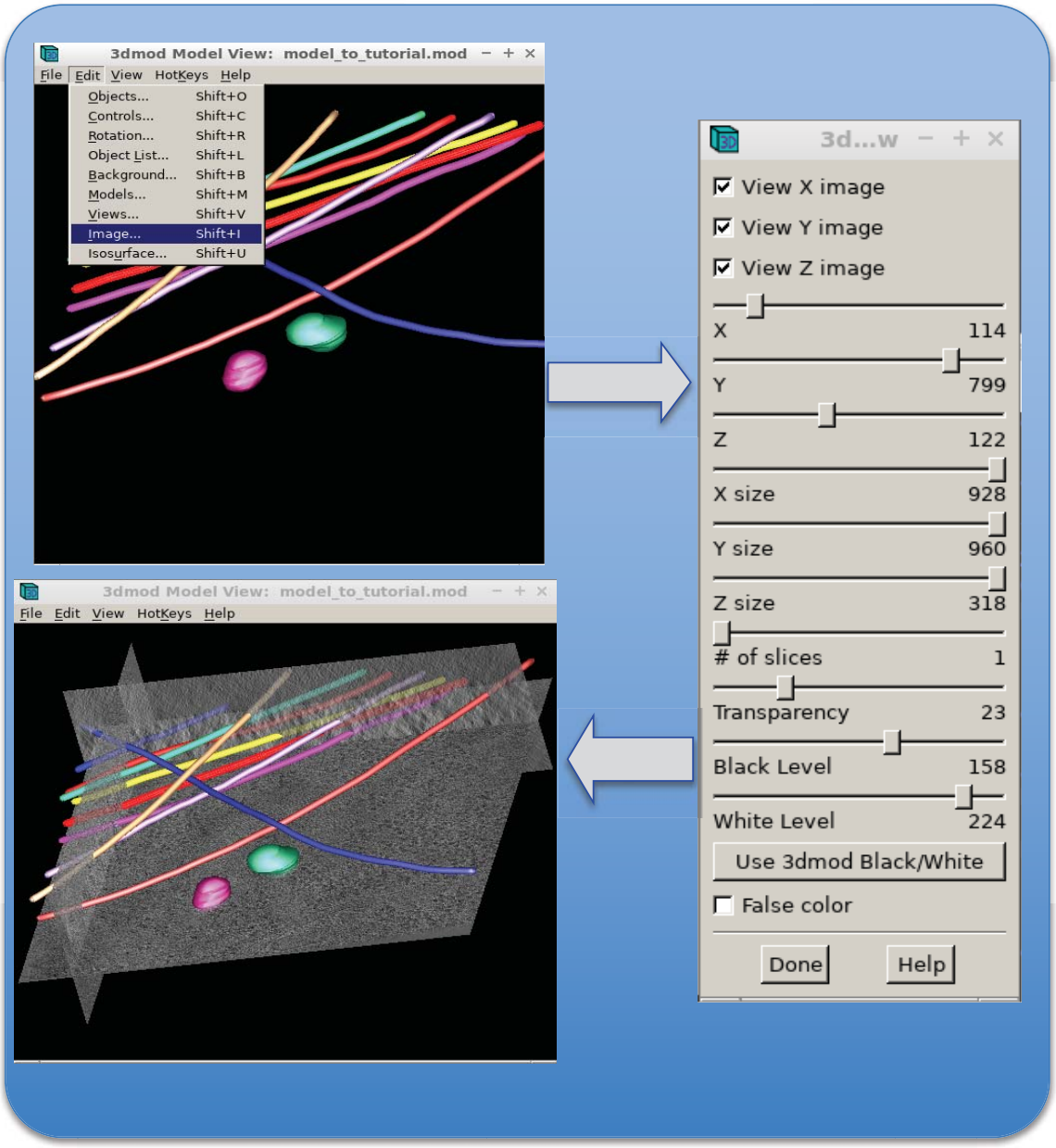


To smoothen the interpolation lines, tick *Surface* in the *Meshing* tab and increase the "Z inc" value to 3 or 4.

You can scroll through the sections using **[PgUp]** and **[PgDown]** and use the drawing tools "**Special > Drawing Tools**" to refine the interpolated contours. At this point, check whether you are happy with the interpolated sections, especially with the "guessed" ones. You may remove undesired contours by using **[delete]**, or accept correct contours you are happy about by pressing **[enter]**. Moreover, you can try new interpolation types and create new interpolations until you are satisfied with the tomogram segmentation.

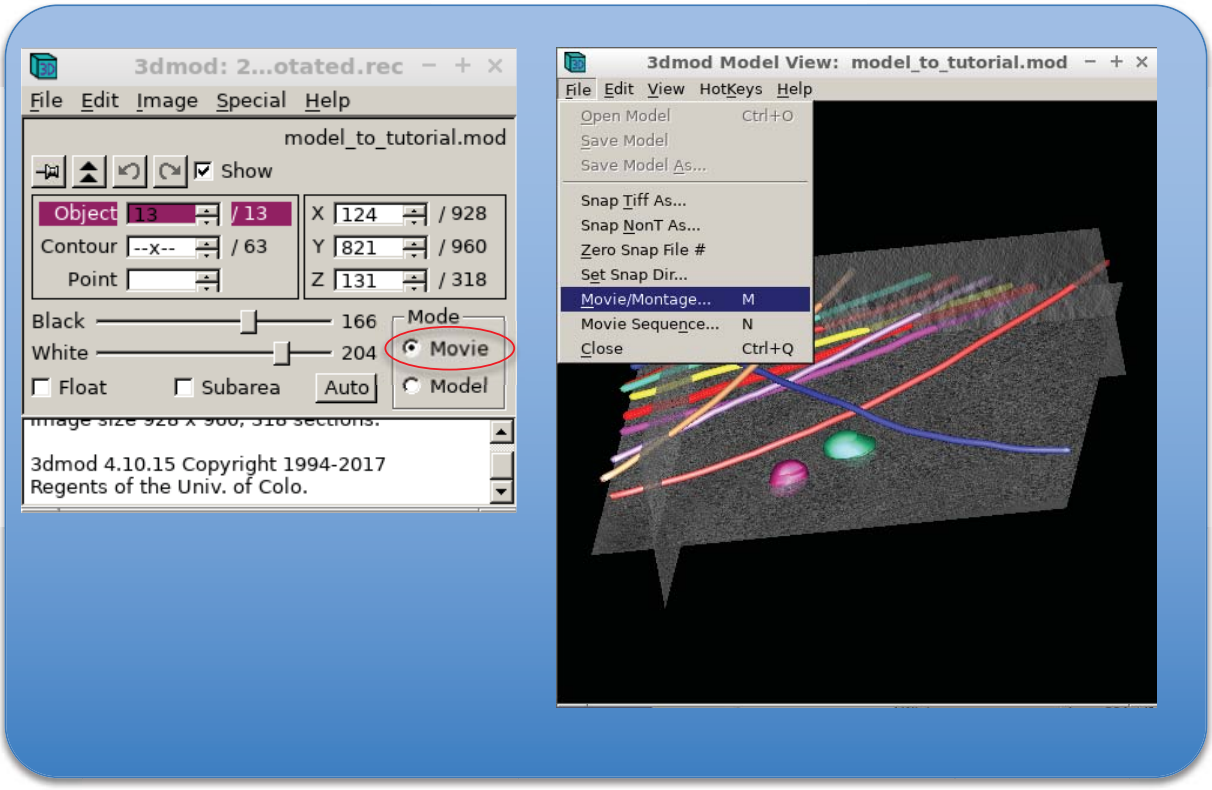
3I	**Segment more vesicles** As an exercise you can segment other vesicles as new objects. Below, the result of the segmentation of two vesicles: 
3J	**Display extra information** To include tomographic views in the model window, go to "**Edit > Image**" in the "3dmod Model View" and check the boxes (✓)"view X image", (✓)"view Y image" and (✓)"view Z image". This will make the planes of the tomogram appear in the black background of the "3dmod Model View", in between the segmented objects. Adjust the X, Y, Z slice positions, contrast and transparency of the tomogram using the *Black Level/White level* and *Transparency* bars. To auto-adjust the contrast simply press "Use 3dmod Black/White".

If you need to reorient the tomogram in the original position, in "3dmod Model View" click "**HotKeys > Model Orientation and Zoom > Show top of the model**".



When you are satisfied with the segmented model and you have chosen the perfect position of the tomogram to visualize your features of interest (microtubules, vesicles, both…), you can start acquiring a movie of your 3D model.

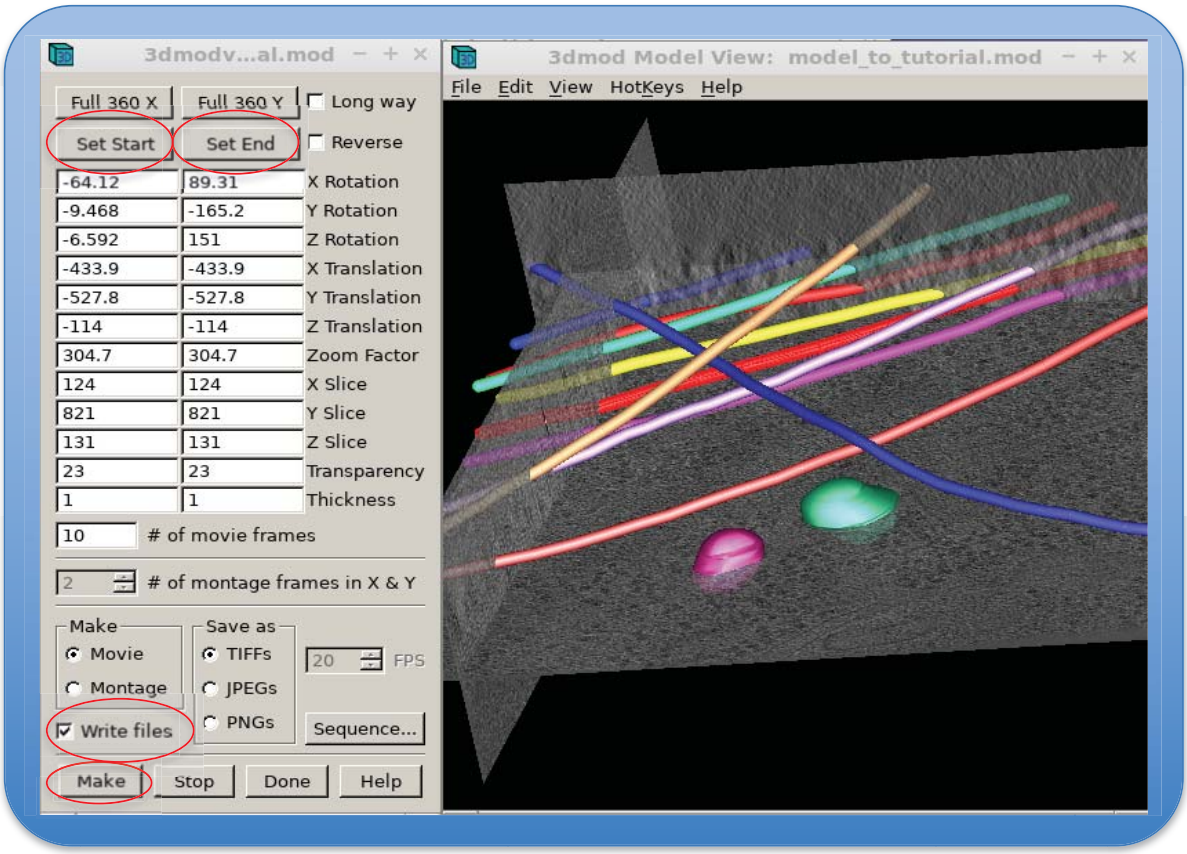
Section 4: Make a Movie (requires ImageJ/Fiji)

4A	**Getting started** In the "3dmod window" set the "Mode" as *Movie* and open "**Menubar > File > Movie/Montage**". 
4B	**Set the scene and generate movie frames** In the "3dmod Model View" window adjust the model in the preferred position to show main features of interest (e.g. microtubules, vesicles…). When satisfied with the position, scroll the tomogram to set the starting frame of the movie and click *Set Start* in the "Movie/Montage" window. Do the same for the ending frame and click *Set End*. In the *"#of movie frames"* box, set the length of the movie by giving the total number of frames to be captured from start to end: more

frames for longer and more detailed movies (maximum number of frames is the total selected frames), less frames for short and more dynamic movies. It is anyways possible to speed up or slow down the movie by adjusting the number of frames-per-second in the *FPS* box: ~28 FPS or more for fast movies, ~20 or less for slower movies.

If you want the movie to loop back and forth, tick the *Reverse* option (close to *Set End*).

Click "Make" to preview the movie. If you are satisfied with the preview, select (✓)"Write files" then click again on "Make": this will write all the frames of the movie in the correct order inside your chosen working directory.



4C	To make more sequences of the movie, click on *Set Start* to start where the previous sequence ended, and repeat the sequence explained in 4B.
4D	Arrange saved movie frame into an AVI movie (using imagej) The frames are written by default in the project directory, so you need to use an external program to make the sequence of frames into a real movie. One of the programs that can easily do that is ImageJ/Fiji. Simply open it by typing Fiji in a terminal and import the images using "**File > Import > Image Sequence**". The root of the filename by default is "modv", so you can input it in the "File name contains" field, along with the starting and ending frame numbers. Once the full sequence of images is imported, just save it using "**File > Save As > AVI...**" and you will create an .AVI movie of your final 3D segmentation.