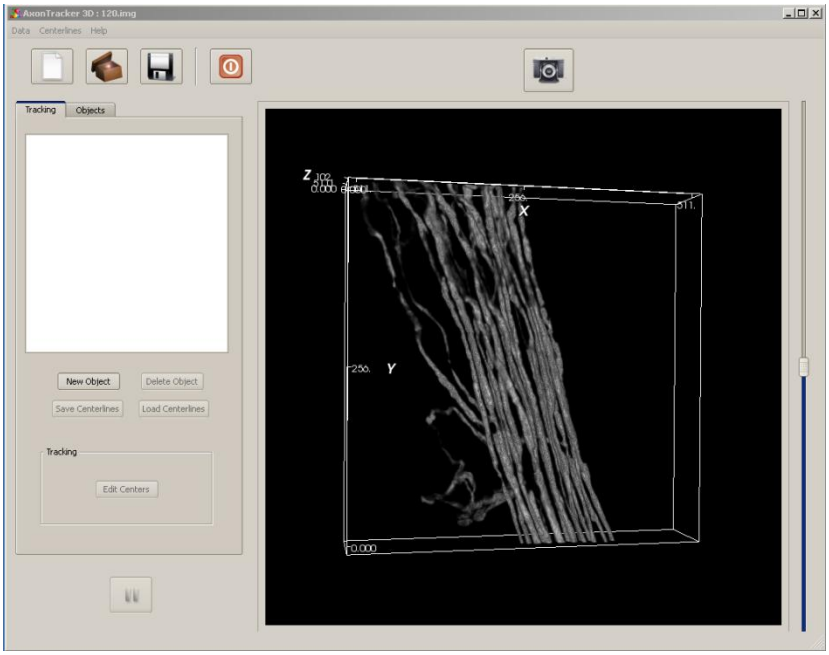
CENTER FOR BIOENGINEERING AND INFORMATICS

# AxonTracker3D Manual

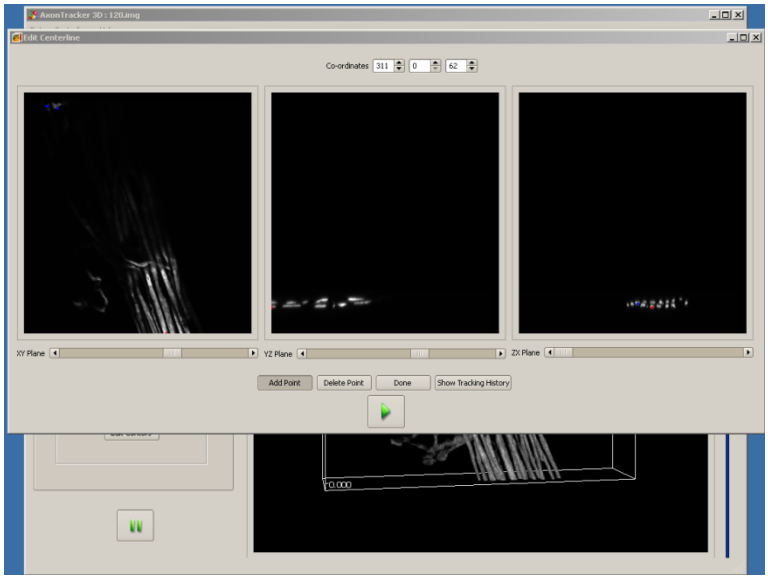
## I. Loading the data

In order to load the data, click on the “Load Data” option from the “Data” menu. Alternatively, data can also be loaded using the “New” shortcut in the main window. Once the data is loaded, it is rendered in the main window along with a binding box, coordinates and labeled axes for reference. The intensity of the rendered data can be changed with a vertical slider bar provided next to the rendering area to the far right. Moving the slider up or down will change the brightness of the rendered data.




## II. Tracking the centerlines

To begin tracking the axon centerlines, an object must first be added to the list of objects (Alternatively, an object can be deleted by clicking “Delete Object” button). For each object created, an initial point must be entered to begin tracking. This can be done by clicking on the “Edit centers” button. This will open a 2D view of the dataset at three orthogonal directions. The locations of these 2D images can be adjusted by the respective sliders under each of these images. By enabling the “Add Point” button in this window, a center point in one of the three images can be chosen. Once a point



is chosen, the remaining two windows automatically adjust their position to show this center. The chosen seed is displayed as a red dot. “Delete Point” button can be used to remove the point that has been added.

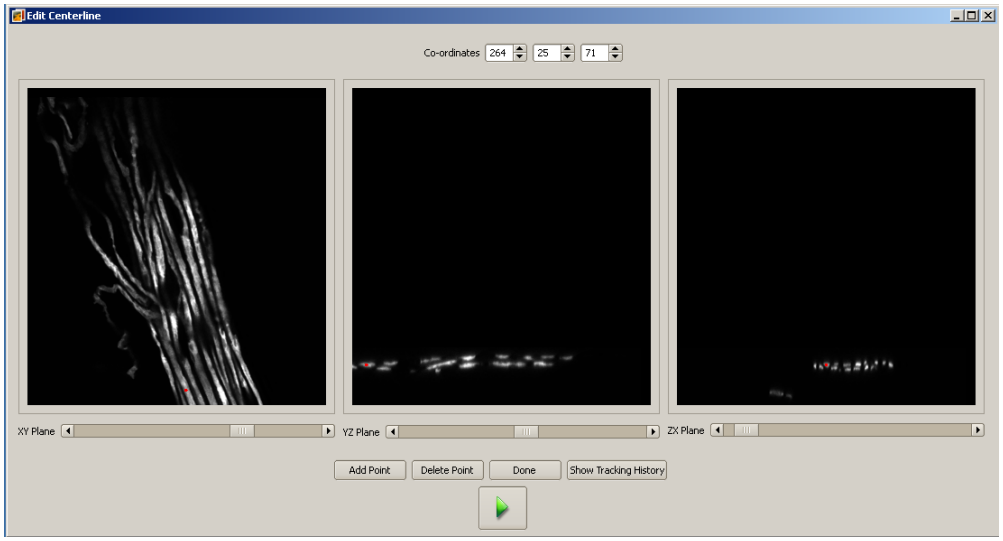
A “Play/Pause” button has been provided in both the main window and the orthogonal view window for convenience. This button starts the tracking of the object selected in the main window (the centerline being edited in the orthogonal view window). Please note that *there is a small delay before the actual tracking begins (usually about 3-4 minutes)*, as the software estimates the most probable locations of the centers. This is done only once for each dataset and there is no delay for subsequent objects being tracked. The data can be preprocessed to remove any noise present, before starting the tracking. This can be done by “Data->Pre Processing” menu.

As the objects are tracked, the (3D) centerlines are shown in the render window in real time. If the orthogonal views are open, the new center is shown as a red dot in window. If any previously tracked centers are present in the current view (2D), they are shown by green dots.

## III. Editing the centerlines

In case of a failure to find the next center point due to low contrast and/or intensity, a notification will pop up asking to add more points to the current centerline. In such a case, add at least 2-3 points on the axon to guide the centerline. When done adding the centers, click on the play/pause button to resume tracking.

If during tracking, the centerline “jumps” from one axon to another due to poor contrast at the boundaries, the tracking can be paused and the wrong centers can be deleted and corrected using “Delete Point” button and “Add Point” button. This can be observed by having the orthogonal views (2D) open during tracking, or by clicking “Show Tracking History” button at any time during the tracking (Disabled while “play/pause” button is active).



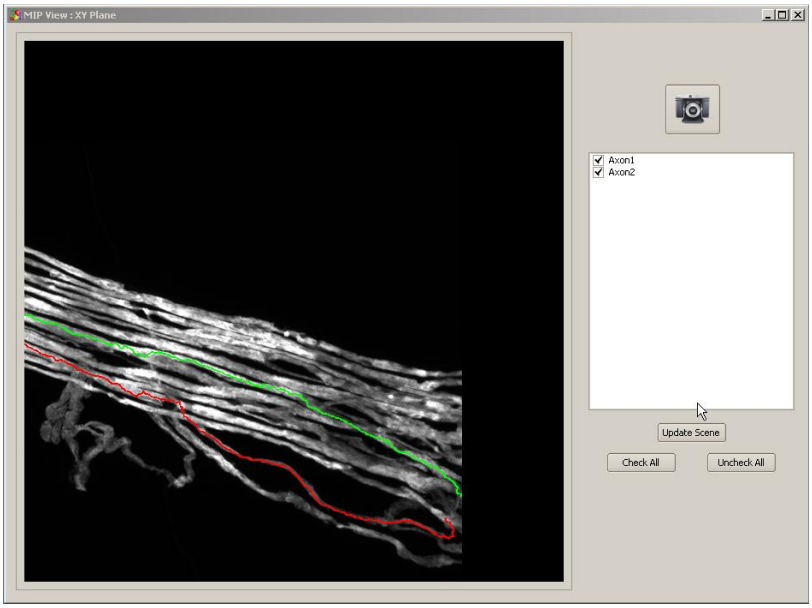
***Note: While adding new points, add them close to the last center tracked by the algorithm. Spacing the points too much causes errors in tracking.***

***Note: Adding new points in the opposite direction of tracking will cause erroneous results.***

## IV. Visualizing the tracking results

The tracking results can be visualized by clicking on the "Objects" tab. The objects that have been tracked so far will be listed along with a check-box on their left hand side. To visualize an object from the list, check the object and click the "Update Scene". "Check all" and "Uncheck All" buttons have been provided for convenience. The 3D rendering window will be updated according to the selection. A "Snapshot" button has been provided just above the render window to save the 3D rendering, along with any centerlines added to the scene, in a ".png" format.

Alternatively, the tracked objects can be visualized in 2D as well. To do this, click on the radio button next to one of the MIP (Maximum Intensity Projection) views. This opens up a 2D MIP image along with similar controls for 3D case. To visualize an object, check the object from the list, and click "Update Scene". The "Snapshot" button serves the same function as in the 3D case.




## V. Saving your work

To save the tracked centerlines to disk, click on the "Save Centerlines" button, which is present in the "Tracking" tab. Alternatively, this can be done by clicking on the shortcut "Save Tracking" or by "Centerlines->Save Centerlines". The tracking can be paused at any time and saved to disk

in this manner. Loading the saved centerlines can be done in a similar fashion with "Load centerline" button, "Open saved centerlines" shortcut, or "Centerlines->Load Centerlines". Please note that after loading centerlines, a*ll the current centerlines in the list will be erased.*

The centerlines can be saved in two formats, Binary and Comma Separated Value (CSV) format (can be read by Microsoft Excel). The former can be used for saving and loading the centerlines, whereas the latter can just be used for saving the centers.