

# Manual for Bundle Trac

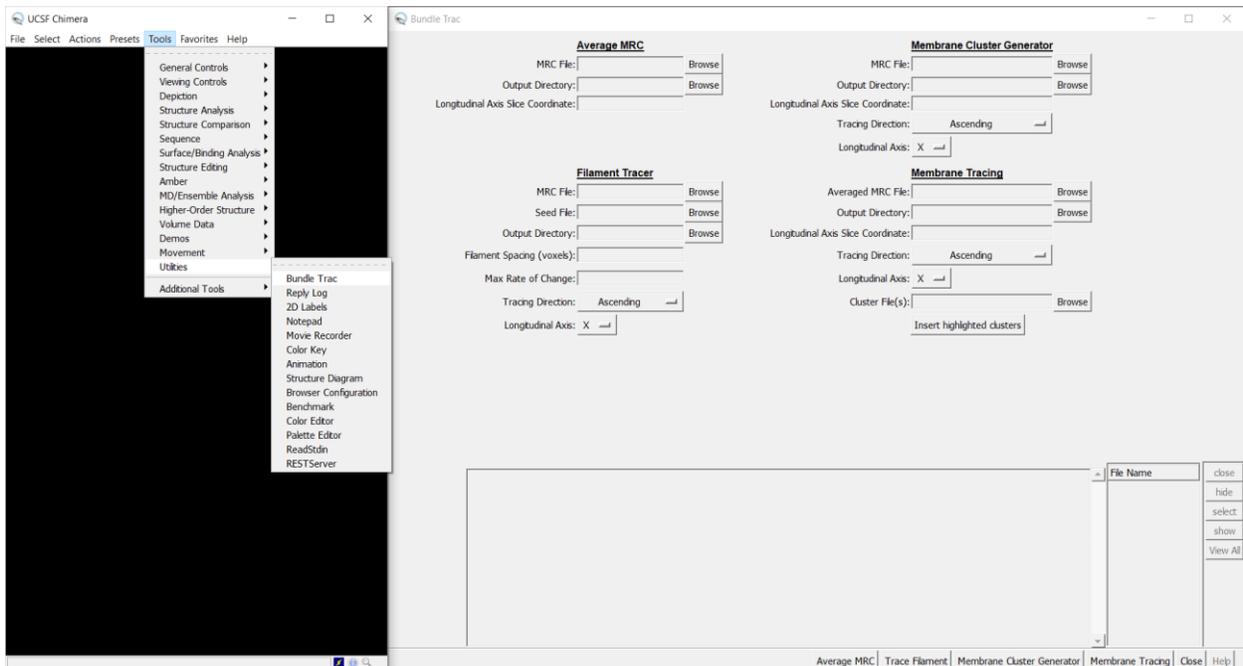
## Usage within UCSF Chimera

### Setup:

To use BundleTrac in Chimera, open **Preferences** under Favorites → “Add to Favorites/Toolbar...”. Under Third-party plugin locations, press the “**Add...**” button and select the location of the “Chimera\_Plugin” folder. At the bottom of the Preferences window. Press **Save** and then **Close**.

BundleTrac will be available for use under Tools → Utilities → Bundle Trac

Files can be selected for each program by hitting each field’s corresponding “Browse” button.



## **Average MRC:**

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**Description:** Averages three-dimensional electron tomography density maps along Axis.

### **Required Input:**

MRC File

Output Directory

Longitudinal Slice Coordinate (Currently only Y-axis supported.)

### **To Run:**

After providing the necessary input, hit the button "Average MRC"

### **Output:**

Output will be located in the specified output folder under <name of input mrc file>\_AveragedMRC\_Output named averaged\_map.mrc

## Filament Tracer:

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**Description:** Filament Tracer is a tool for tracing actin filament bundles in three-dimensional electron tomography density maps.

### Required Input:

**MRC File** - MRC map needs to be averaged before filament tracing

**Seed File** - Seed file contains first coordinates of all the filaments, needs to be in .CMM format.

### Output Directory

**Tracing Direction** – Direction in which to trace the filaments. (Ascending: low y-index to high Y-index) or (Descending: high y-index to low Y-index)

**Longitudinal Axis** - Currently only Y-axis supported.

### Optional Input:

**Filament Spacing (vowels)** - Not used currently, will be added in later version.

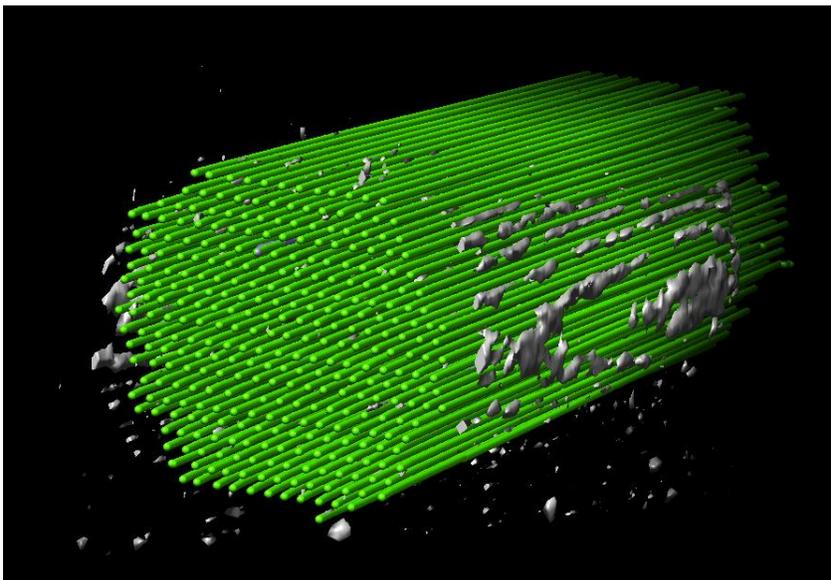
**Max Rate of change** - Not used currently, will be added in later version.

### To Run:

After providing the necessary input, hit the button “Trace Filament”.

### Output:

Output will be located in the specified output folder under final\_filaments\_smoothed”.



## Membrane Cluster Generator:

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**Description:** Membrane Cluster Generator is a tool for generating Membrane Clusters in three-dimensional electron tomography density maps. The clusters generated are used as input to the Membrane Tracing program. The purpose of this to allow the user to view which clusters are correctly aligned with the Membrane of the maps provided and choose which clusters to trace from.

### Required Input:

**MRC File** - MRC map needs to be averaged before filament tracing

**Output Directory**

**Longitudinal Slice Coordinate** - Y-axis index from which to begin tracing

**Tracing Direction** – Direction in which to trace the filaments. (Ascending: low y-index to high Y-index) or (Descending: high y-index to low Y-index)

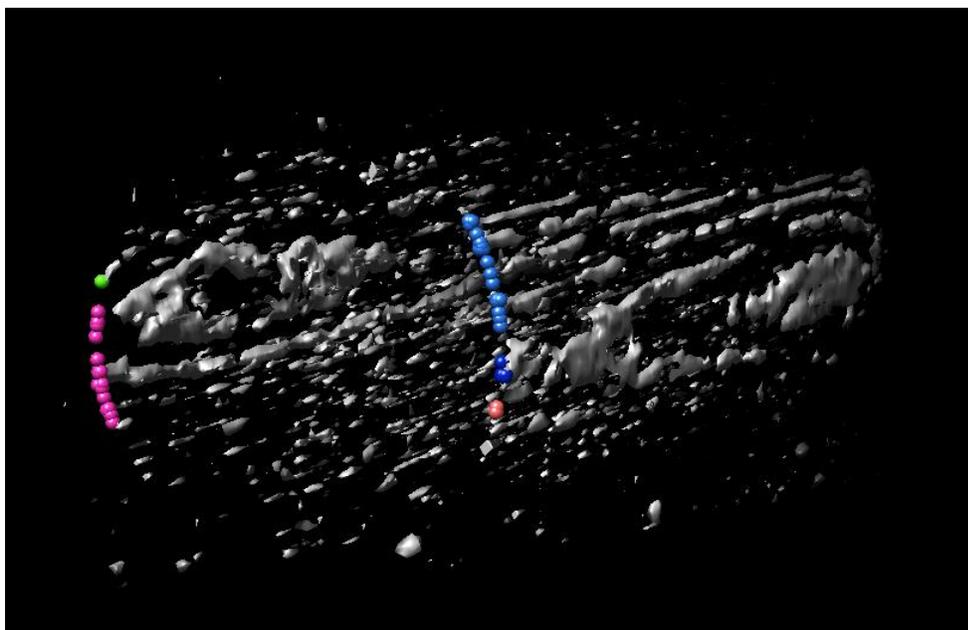
**Longitudinal Axis** - Currently only Y-axis supported.

### To Run:

After providing the necessary input, hit the button “Membrane Cluster Generator”.

### Output:

Output will be located in the specified output folder under “<input MRC filename>\_Cluster\_Output”.



## Membrane Tracing:

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**Description:** Membrane Tracer is a tool for tracing the membranes in three-dimensional electron tomography density maps. The Membrane Tracer uses output from the Membrane Cluster Generator as input. The user selects the clusters to trace from along the Membrane.

### Required Input:

**Averaged MRC File** – The averaged map of the Membrane Cluster Generator is used as input for Membrane Tracing

### Output Directory

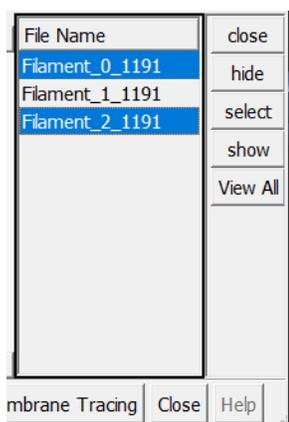
**Longitudinal Slice Coordinate** – Y-axis index from which to begin tracing

**Tracing Direction** – Direction in which to trace the filaments. (Ascending: low y-index to high Y-index) or (Descending: high y-index to low Y-index)

**Longitudinal Axis** - Currently only Y-axis supported.

**Cluster File(s)** – The cluster files (.CMM) produced from Membrane Cluster Generator are used as input for Membrane Tracing.

**To input Cluster File(s)** – You may either select multiple files using the “browse” option or you can insert cluster files already open in Chimera (Ideal for if you have just run the Membrane Cluster Generator). To insert cluster files already open in Chimera, select the desired cluster files in the GUI under “File Name”. Then, select the button “Insert highlighted clusters”.



### To Run:

After providing the necessary input, hit the button “Membrane Tracing”.

**Output:**

Output will be located in the specified output folder under “<input MRC filename>\_Traced\_Output”.

