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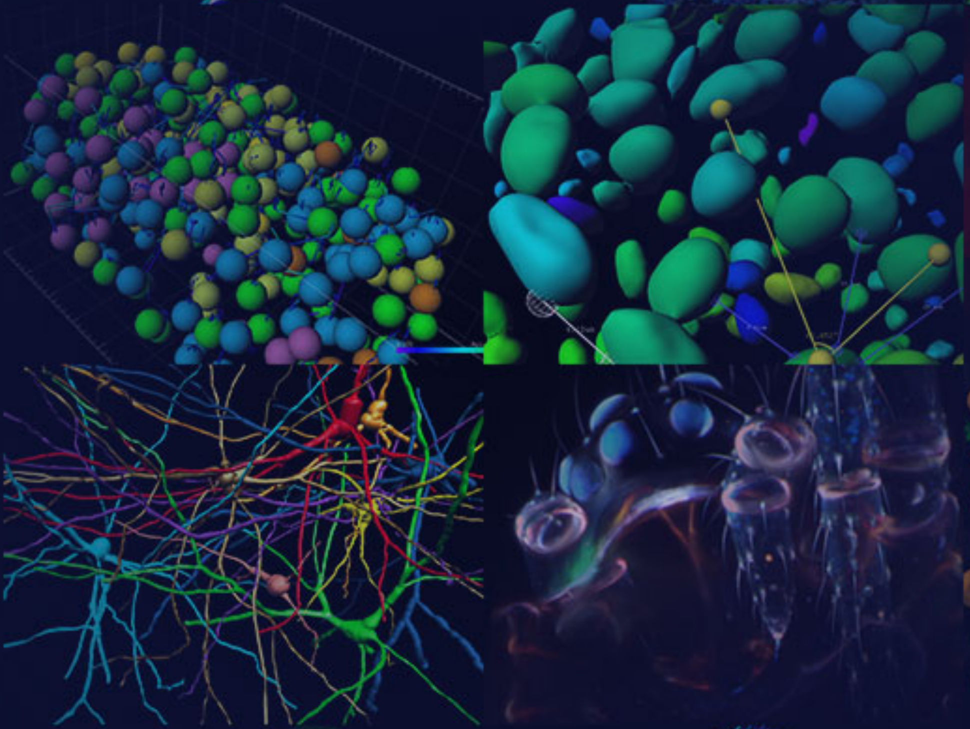

Filament Tracer (+XT)

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Purpose

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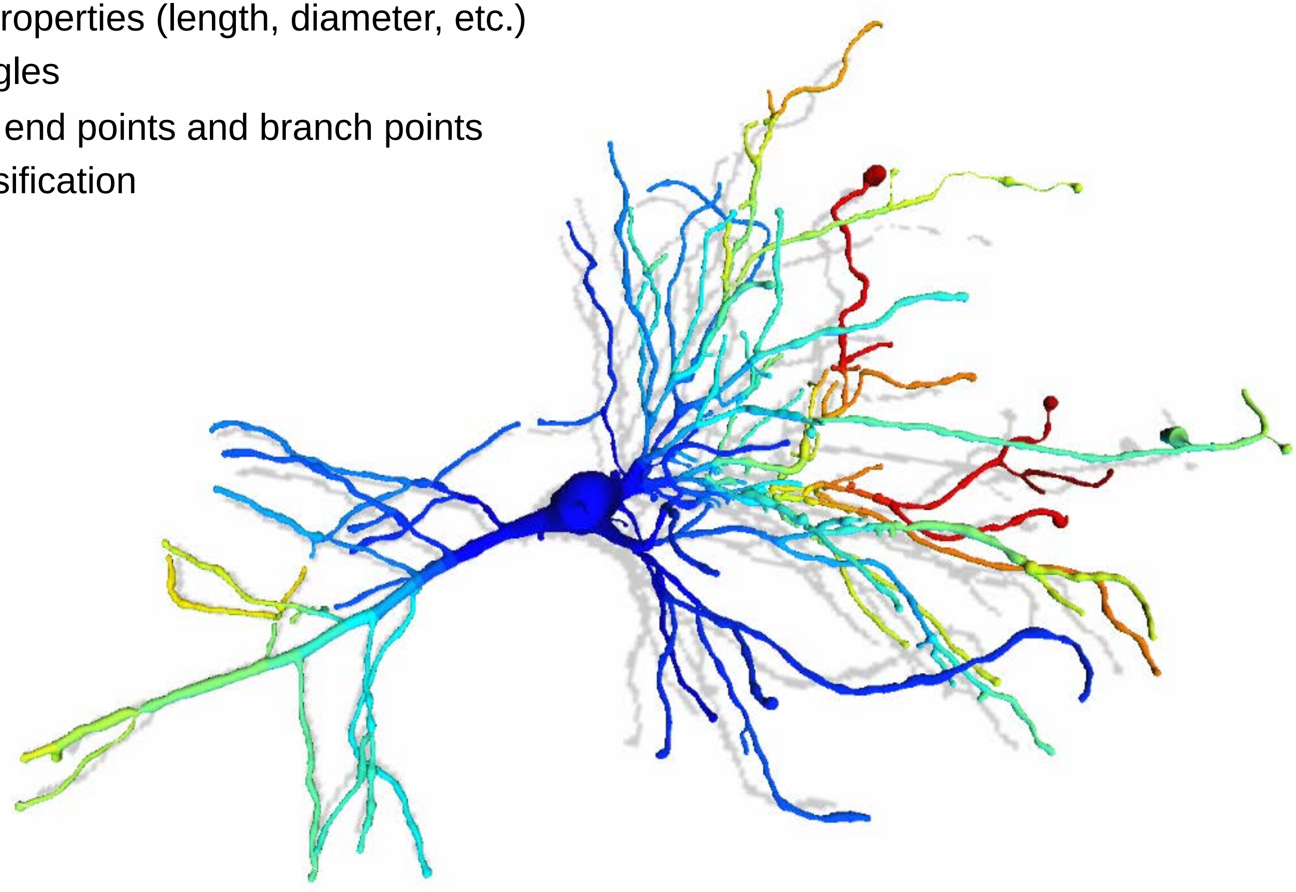
Allows for measurements of neuronal or other branching structures (eg. Mycorrhiza, blood vessels, mitochondria network etc.)

- Segment properties (length, diameter, etc.)
- Branch Angles
- Number of end points and branch points
- Spine classification
- Etc.



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Measurement of Dendritic Tree

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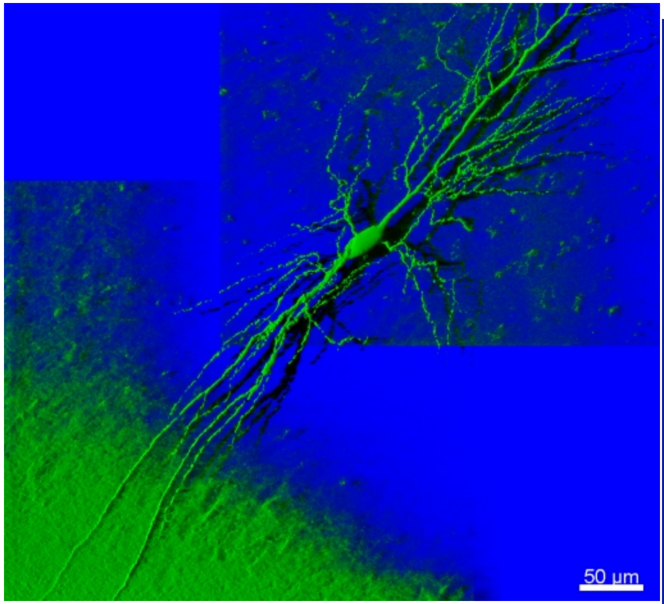
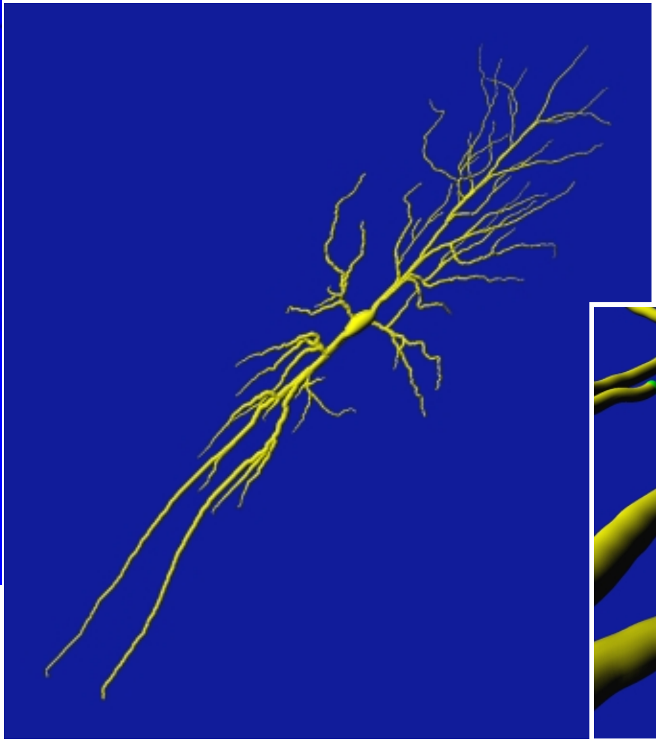
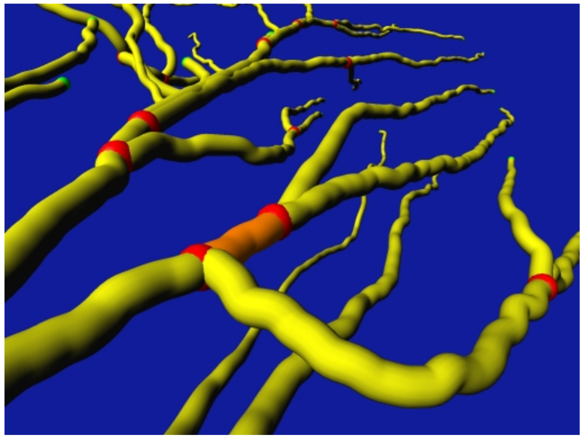


Assembly of two confocal 3D image stacks (shown as volume rendering)



Segmentation using FilamentTracer



Interaction with the modelized structure to measure individual segments or number of branch points

Image: Courtesy of Dr. Anne McKinney, McGill University, Montreal, Canada

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Surface Object Versus Filament

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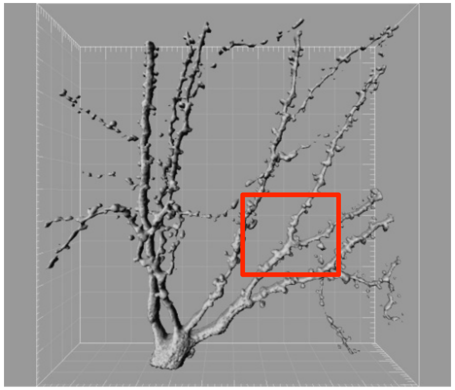
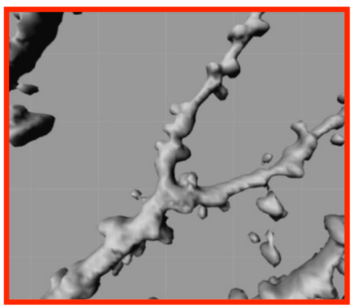
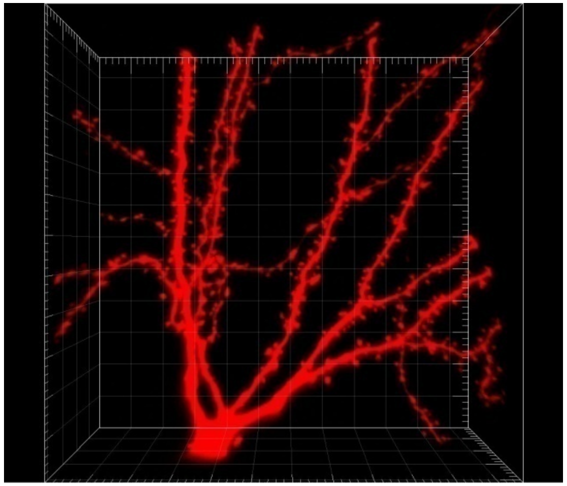
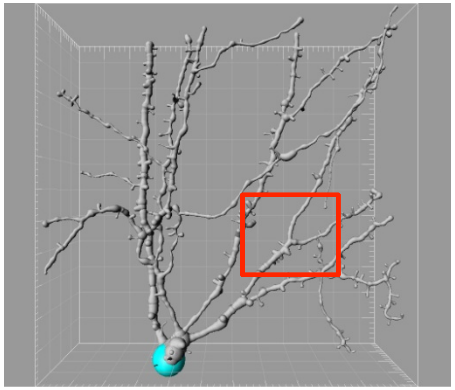
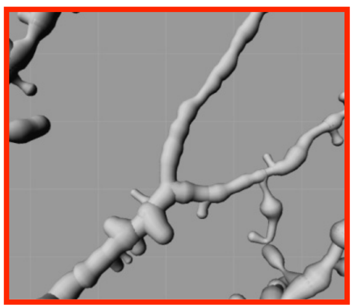


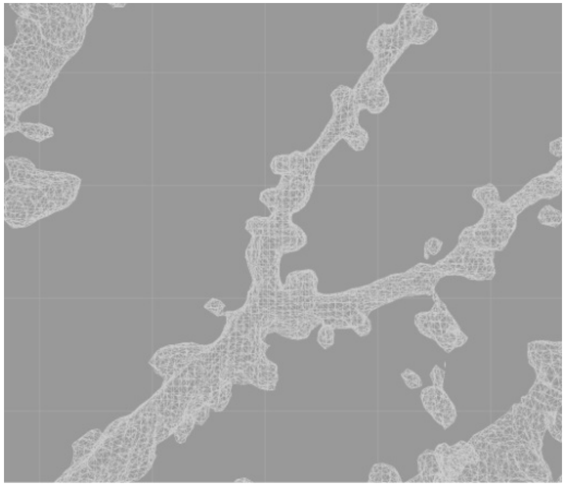




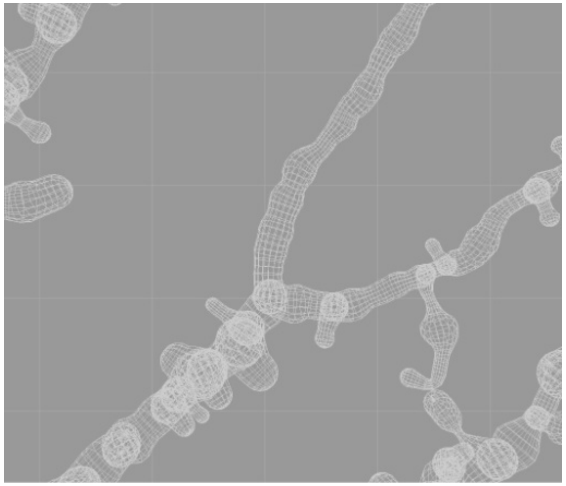




Non-directional



Directional



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FilamentTracer: Tracing Methods

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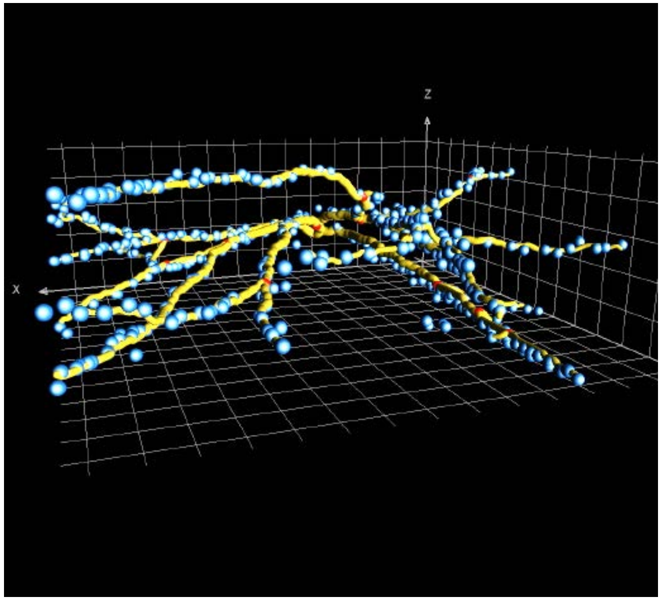

Multiple tracing methods to ensure accurate results:

1. Automatic detection
   a) Fully automatic detection based on intensity threshold (may have loops)
   b) Full automatic (creates tree without loops)
   c) Iterative Tracing
   d) Region of Interst (ROI) Tracing

2. Semi-Automatic – AutoPath

3. Semi-Automatic - AutoNetwork

4. Semi-manual tracing - Autodepth

5. Manual with automatic Z placement

Unique ability to create and edit filaments using any combination of tracing modes + simultaneous visualization of tracing and raw data

➔Accurate and consistent tracing results

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1a. Fully Automatic: Threshold

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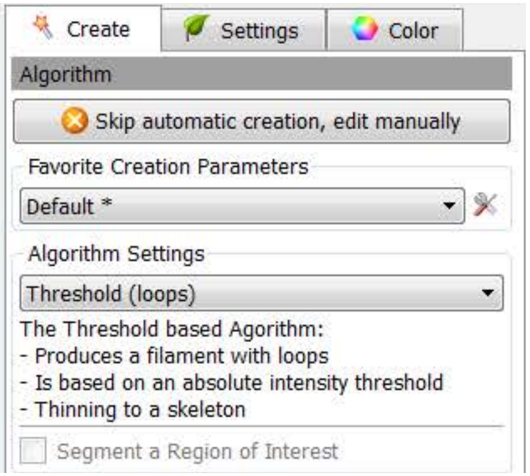
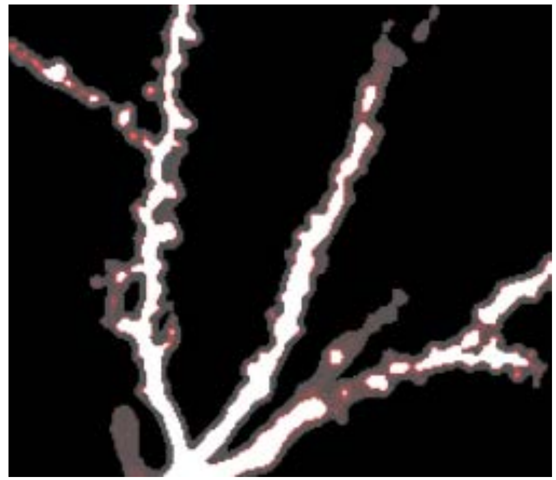
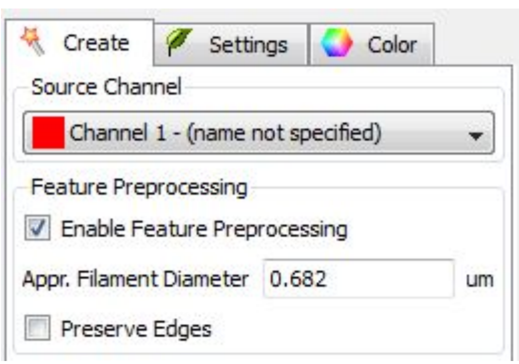
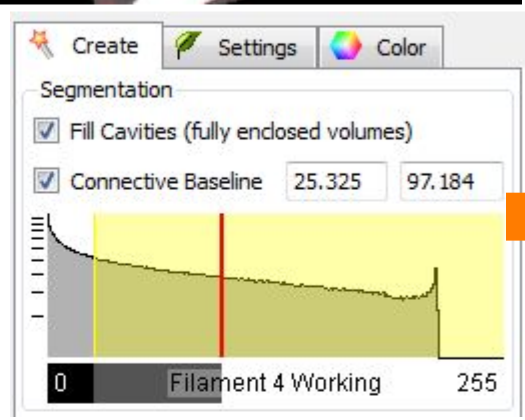


1. Select algorithm settings

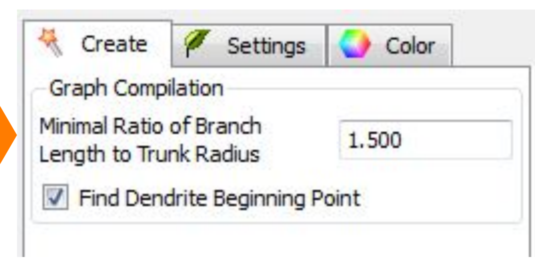




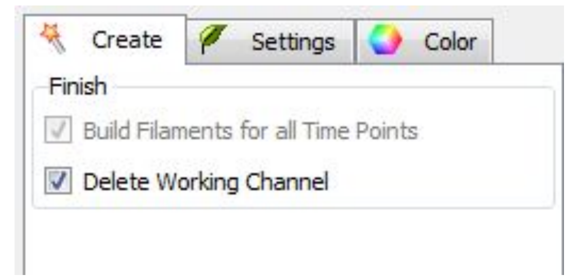
2. Enter filament diameter



3. Adjust thresholds



4. Enter ratio value



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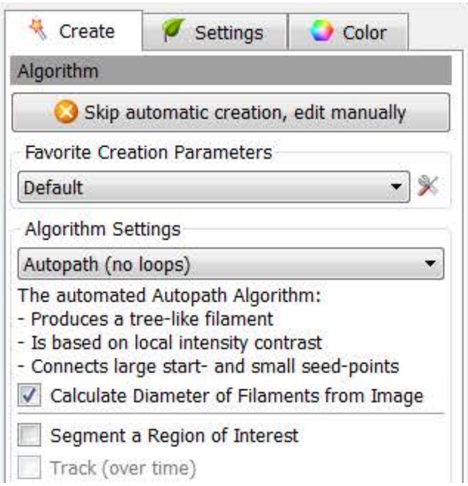
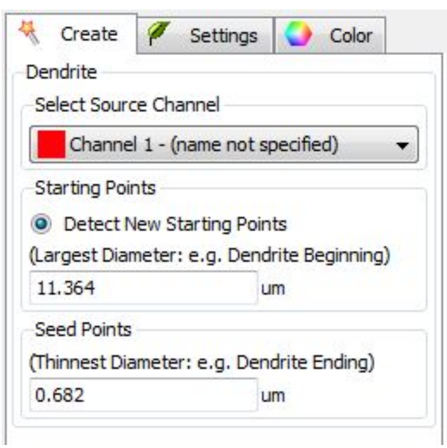
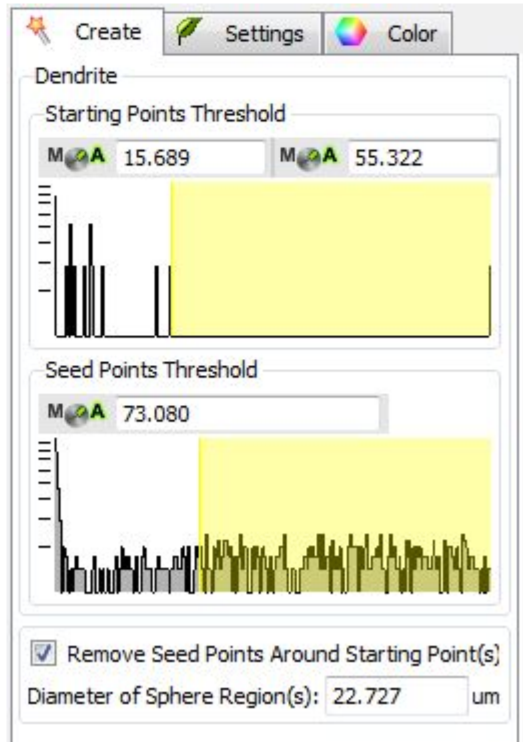
1b. Fully Automatic (no loops)



1. Select algorithm settings

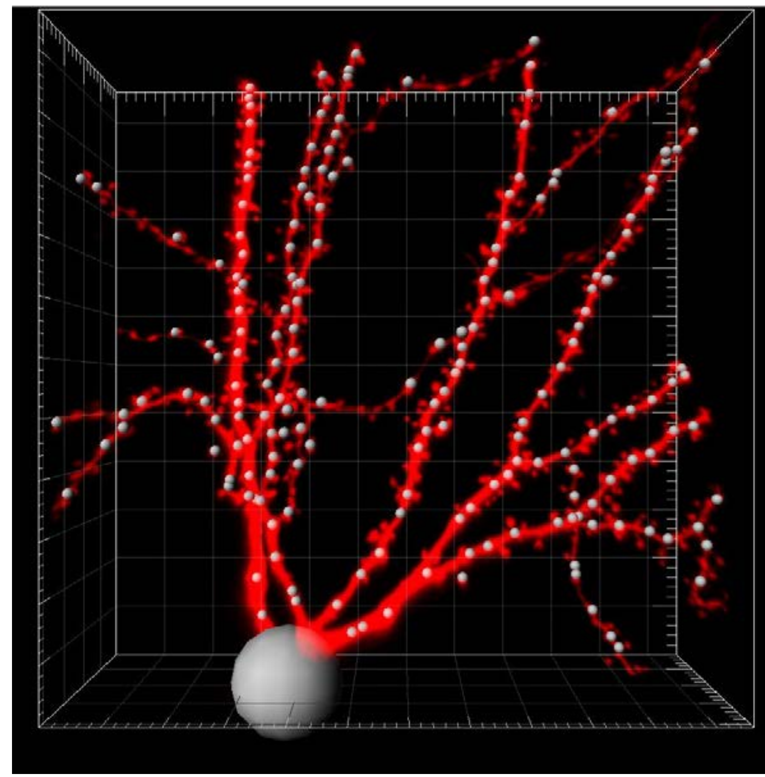


2. Enter Dendrite beginning/End diameter



3. Adjust QUALITY thresholds
-From Histogram
-Manual Editing
-Remove Seed Points

- AutoPath tracing algorithm connects start and end points via shortest path.
- For fully automatic creation we calculate start and end points via spot-detection.



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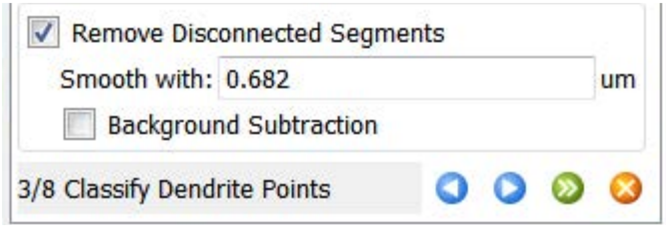
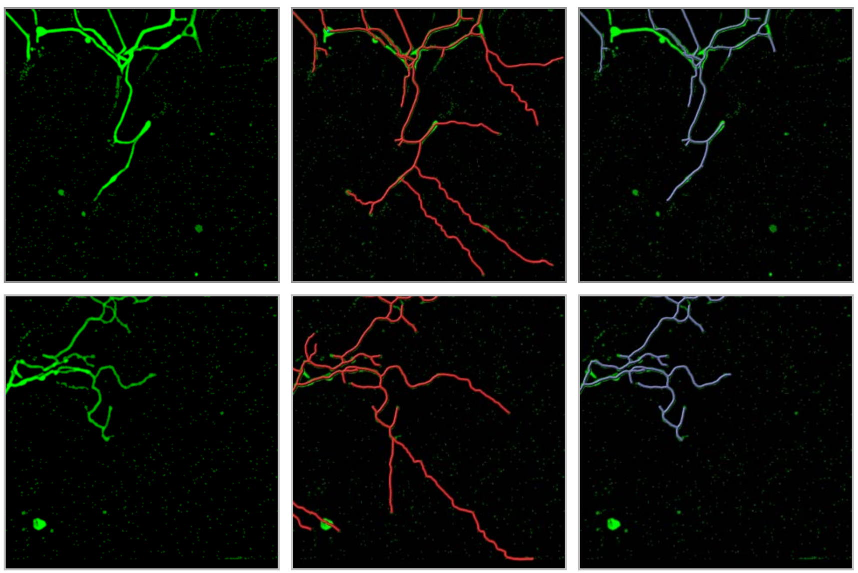
Removal of disconnected segments

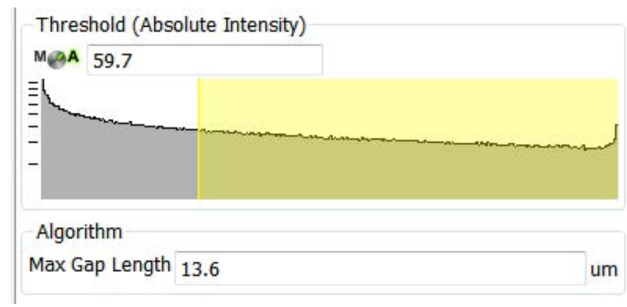




- Original
- without removal
- with removal

- Allows the user to control the behavior of FilamentTracer when it identifies a gap in the image signal

- First mask the original data set and then set a max gap between adjacent masked areas



- If the max gap entered is smaller than the gap between adjacent masked areas FilamentTracer will stop the trace

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Removal of disconnected segments

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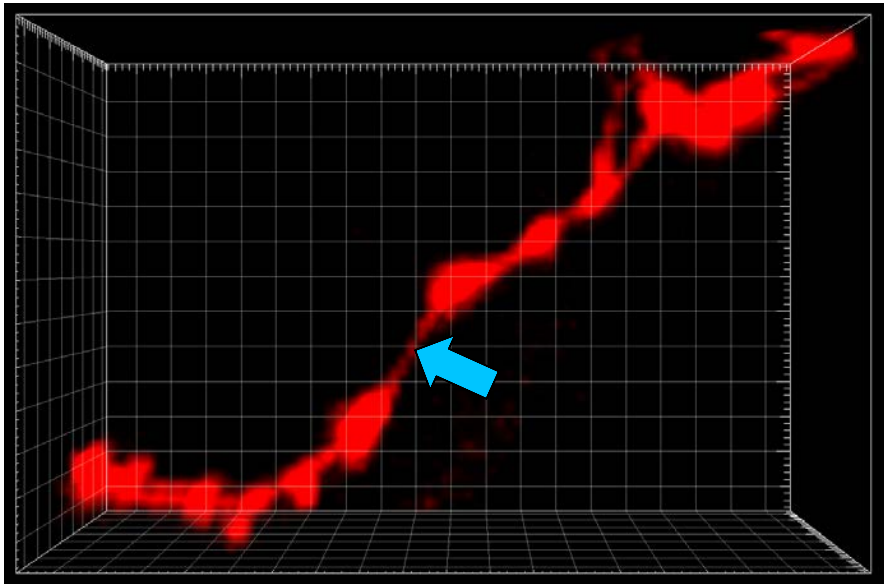
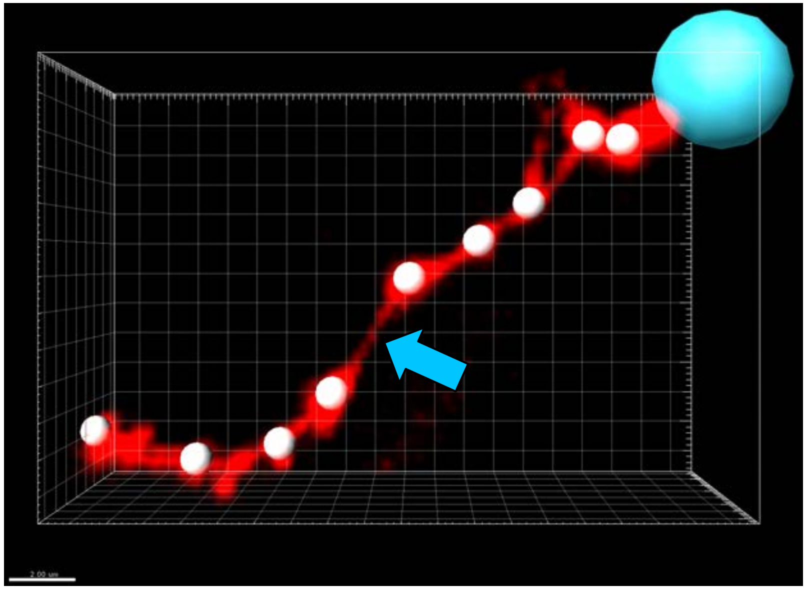
Example:





Filament with some apparent “gaps” (in this case they are not real gaps, just areas with low intensity)

Steps 1,2,3 will get us to this point (seed points and start point visible).

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Removal of disconnected segments (cont)

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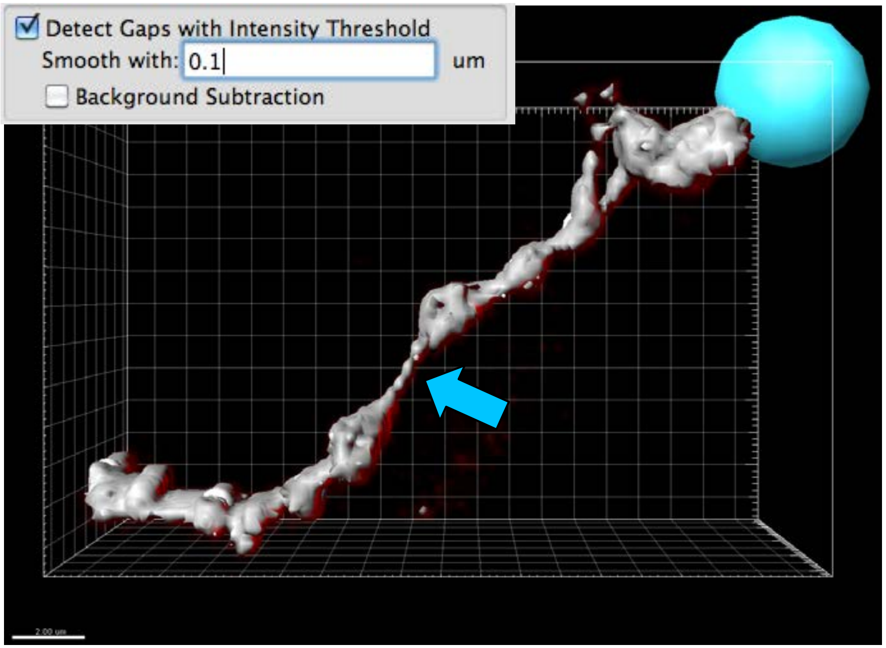
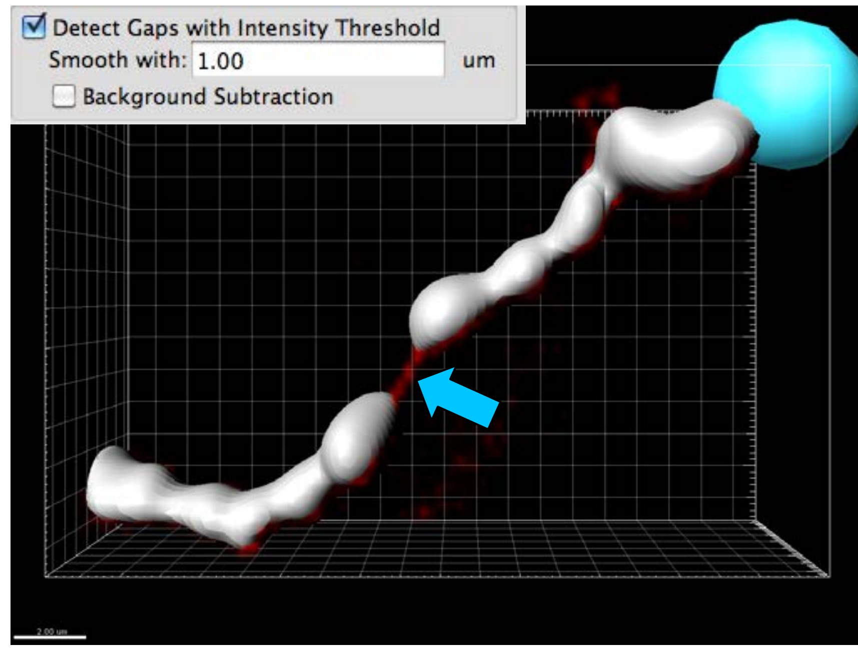
Mask using a small gaussian filter



- Using a smooth factor (gaus. Filter) of 0.1 micron results in a mask that very closely matches the original signal.
- It will include even the "smallest" parts of the filament
- Not ideal if user wants to stop tracing across areas that are connected with a very narrow (but positively stained) filament (see arrow above)
- Ideal if user wants to be very stringent in the use of the "max gap" tool

Mask using a bigger gaussian filter



- Using a smooth factor (gaus. Filter) of 1 micron results in a mask that loosely resembles the original data (small details are no longer included)
- Ideal when user wants to stop traces that would otherwise jump over the visible gap (see arrow above)

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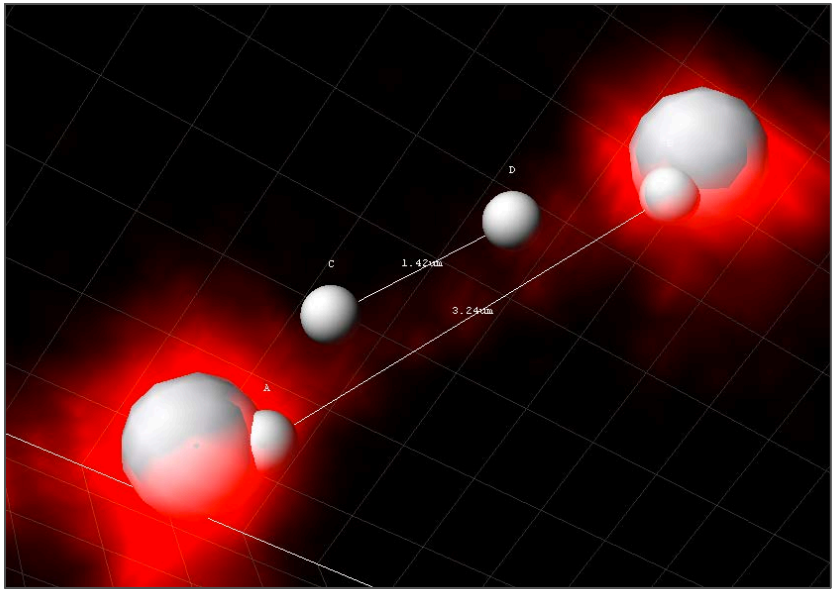
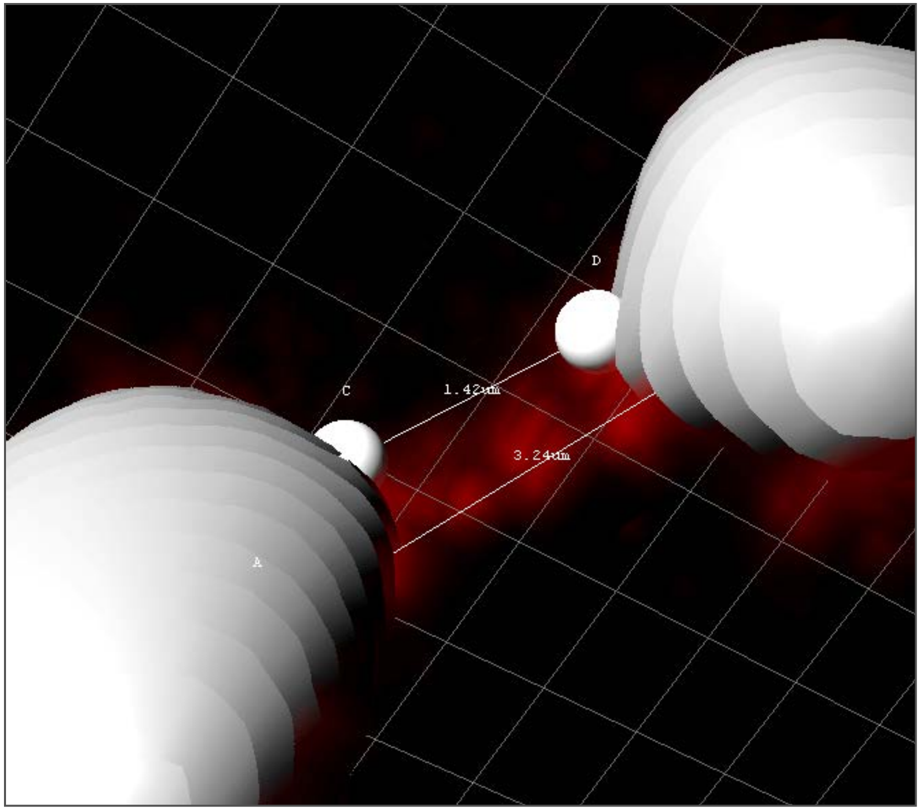
Removal of disconnected segments (cont)

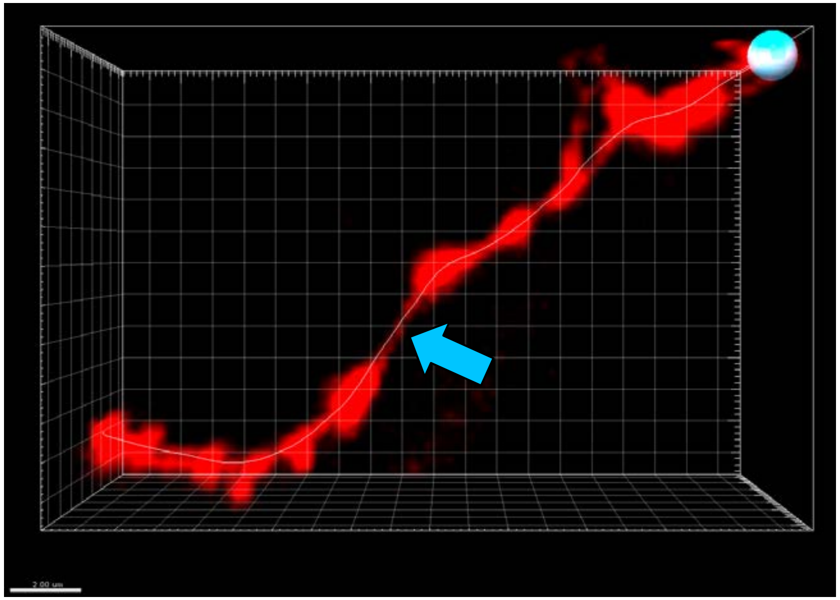


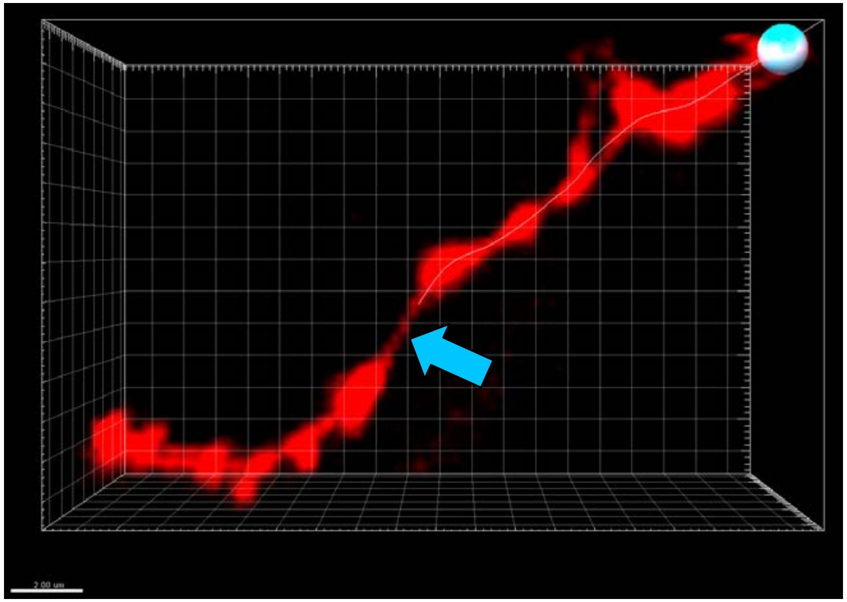
Detail of seed points
The tool does not directly take into consideration the gap between seed points (A and B). Instead it uses the gap between the mask (between C and D)



Detail of Mask
Here the mask is shown (grey). In this case if a “Max Gap Length” of 1.5 was entered, Filament Tracer would jump the gap creating a continuous filament. If the Max Gap Length was set to 1, Filament Tracer would stop before crossing the gap.

Removal of disconnected segments (cont)





- Because the mask included the area with very weak signal (the gap), the filament crossed the gap (see arrow) thus creating a long filament running from the top right to the bottom left

- In this case all the seed point are used for the filament

- Because the mask excluded the area with very weak signal (the gap), the filament did not cross the gap (see arrow) thus creating a short filament running from the top right to center of the image

- In this case only part of the seed points were used (the ones on the top right and center)

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Volume Calculation Threshold Adjustment

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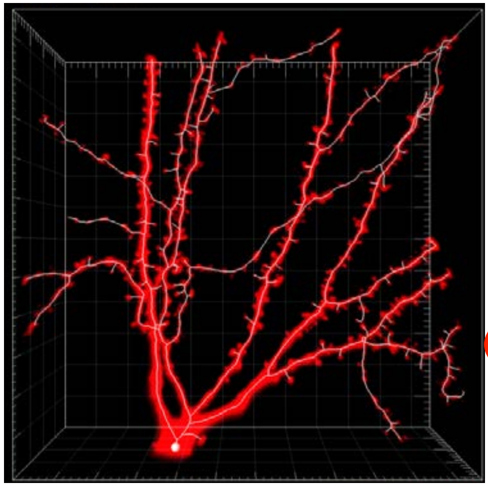
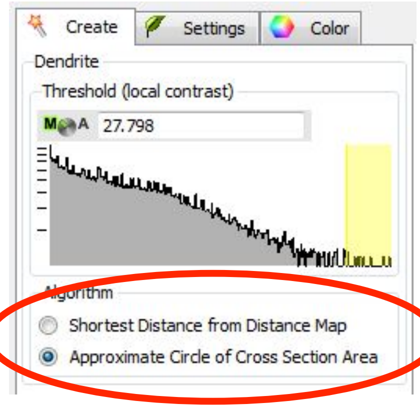
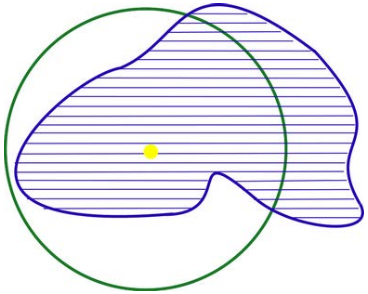
The method of diameter calculation has no effect on the position of the filament center axis, located at the local intensity peak (yellow disk).

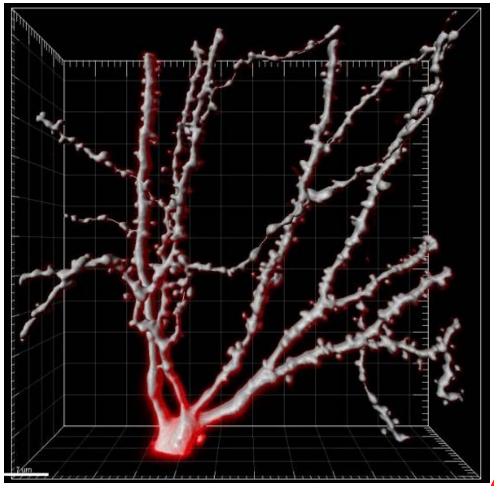


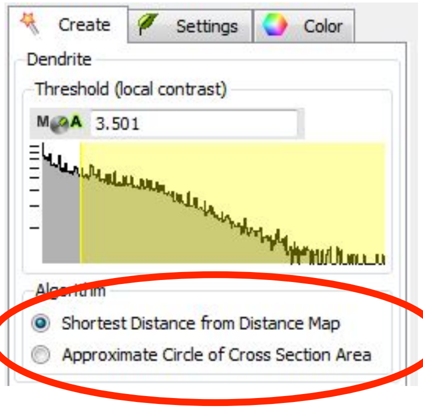


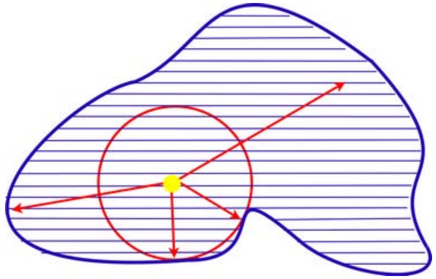


Recommended when the center axis position is not well centered in a large diameter structure e.g large irregular spine heads. The area of the green circle is equal to the cross-section area determined in the threshold step

Choose diameter approximation method







This option considers the radius as the shortest distance from the seed point (yellow disk) to the edge of the filament mask (blue line), in any (x,y,z) direction

This method is typically less sensitive to diameter overestimation due to axial (z) blur. It is recommended when the center axis is overall well centered on the structure and the real structure has a roughly circular cross-section.

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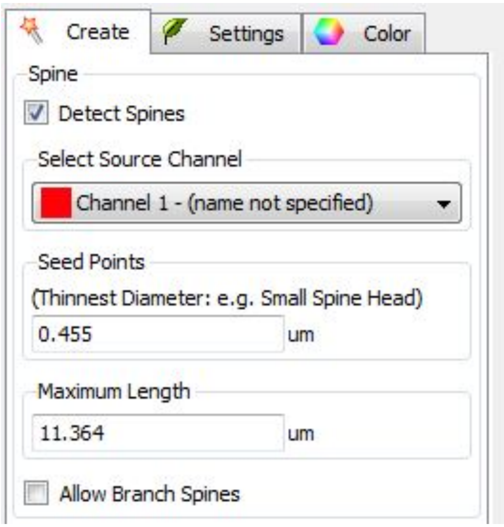
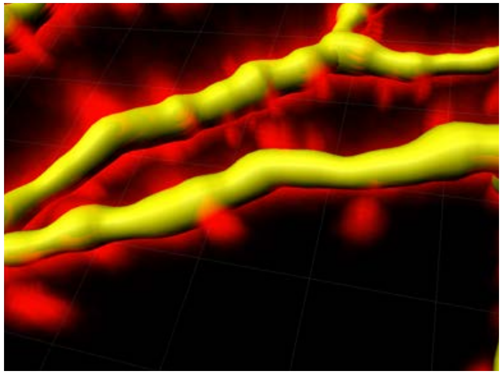
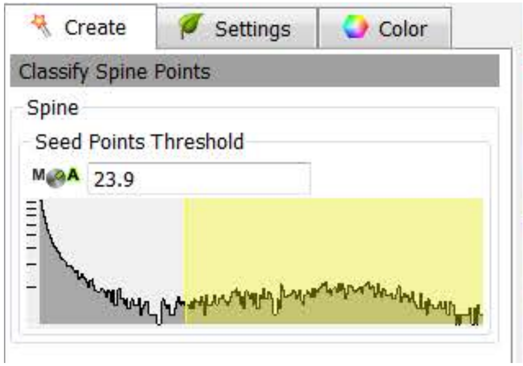
Spine Detection

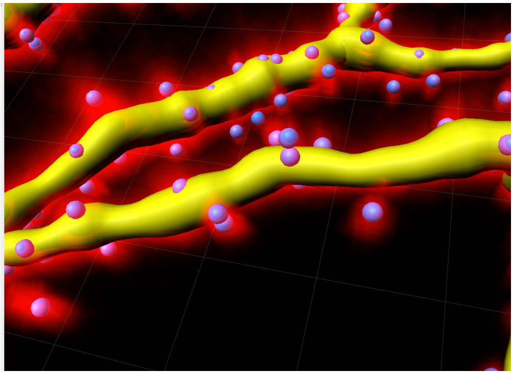


1. Enter seedpoint size and maximum length





2. Adjust number of seedpoints with threshold



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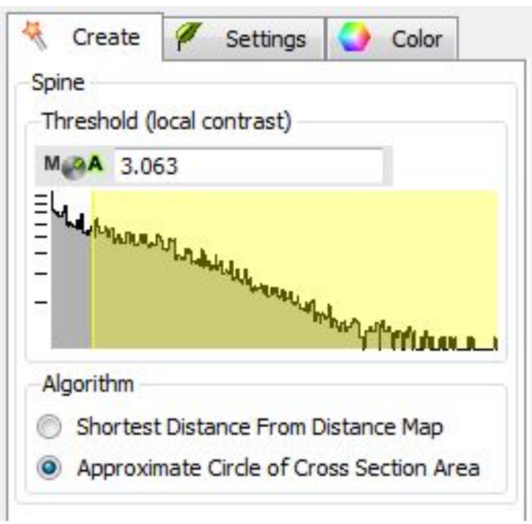
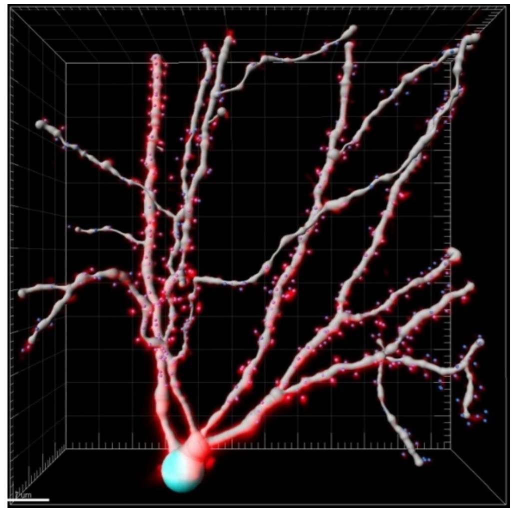
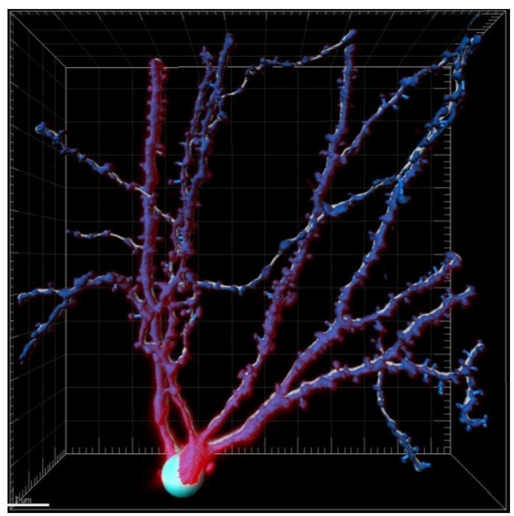
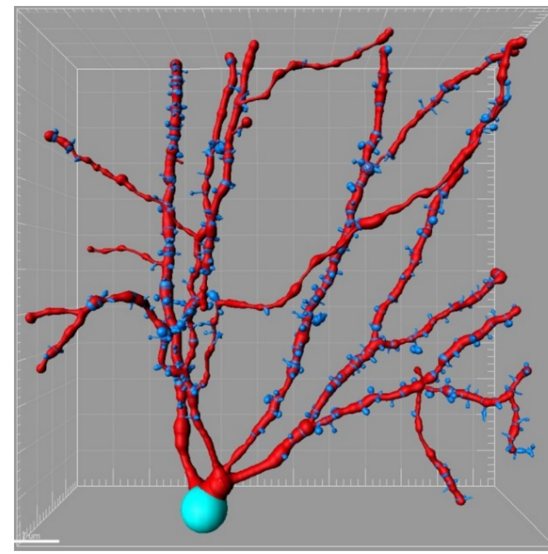
Spine Detection (cont)



3. Define diameter with threshold







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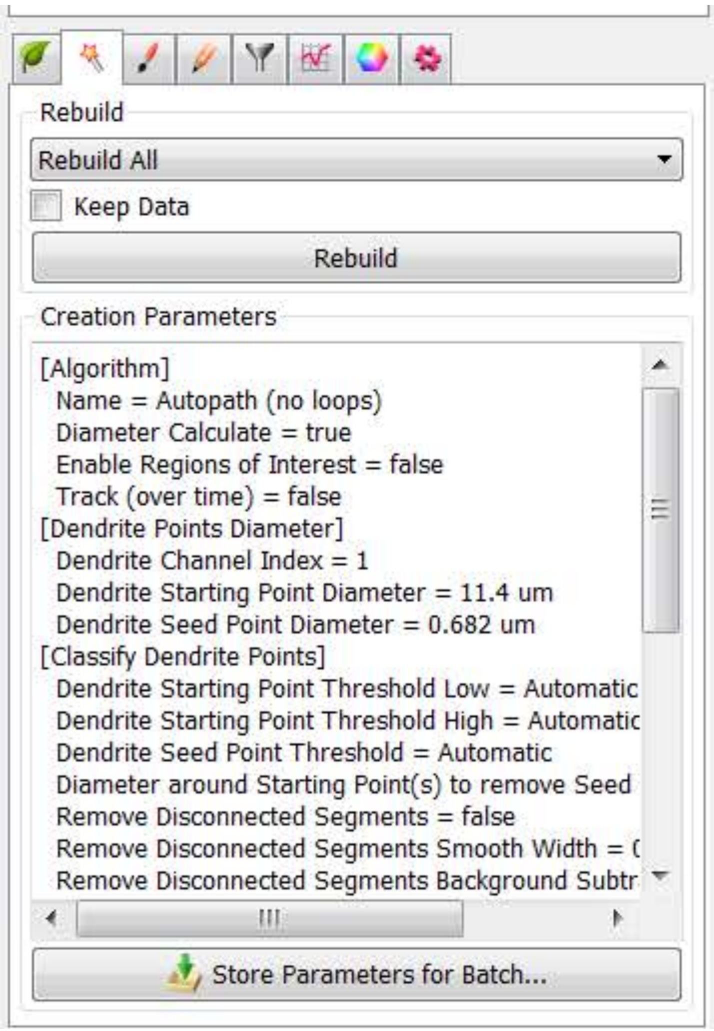
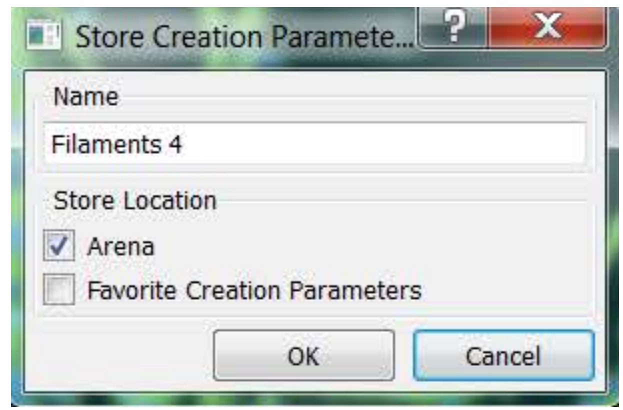
Automatic filament tracing is batchable





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1c. Iterative Automatic Tracing

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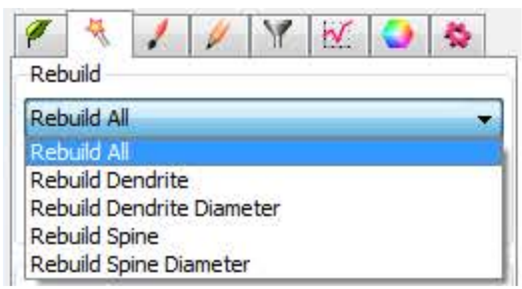
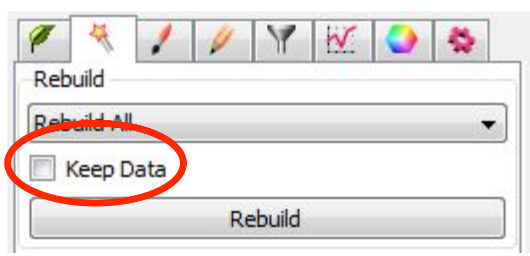
Excellent for tracing complex structures with both fine (small dendrite/spines) and large dendrites or axons

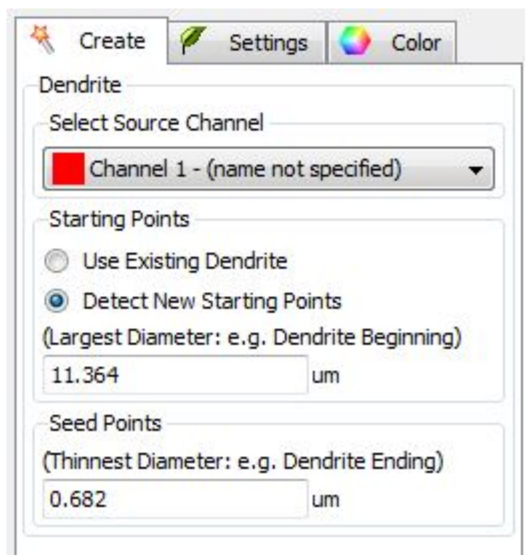
1. *Build a filament using any method.*
2. *Click on the creation tab and select rebuild tab option*



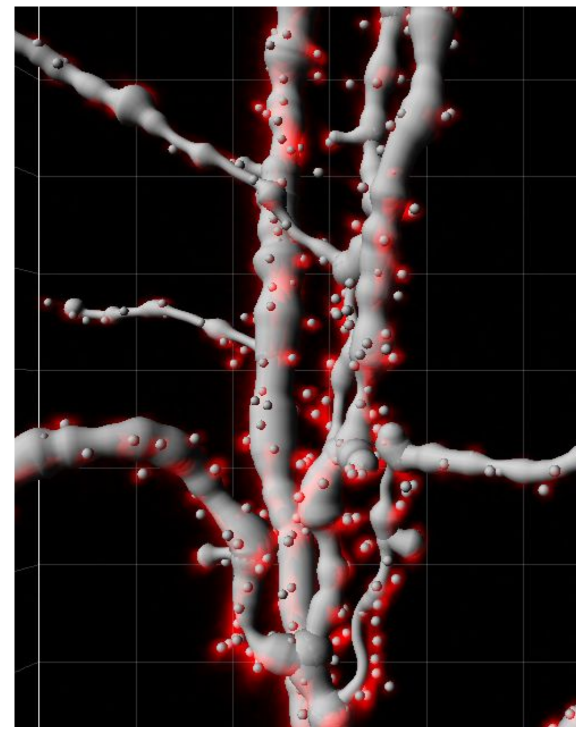
3. *Choose whether or not to keep data*



4. *Choose "use existing dendrite" if desired and set your new end point diameter*



5. *Adjust threshold to choose additional end point to be added to existing filament.*



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1d. Region of Interest (ROI) Tracing

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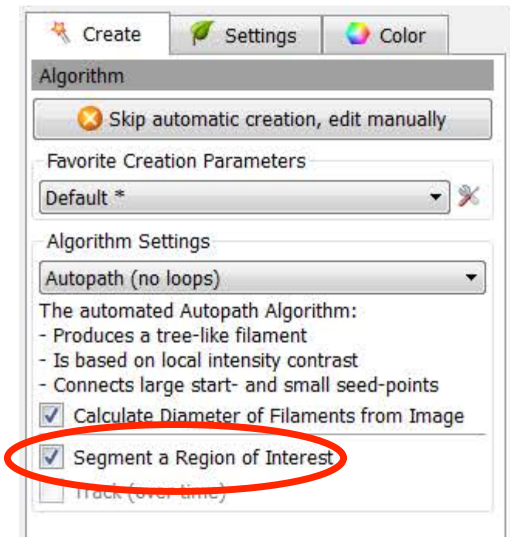
Good for tracing portions of large complicated data or data that has regions with different parameters

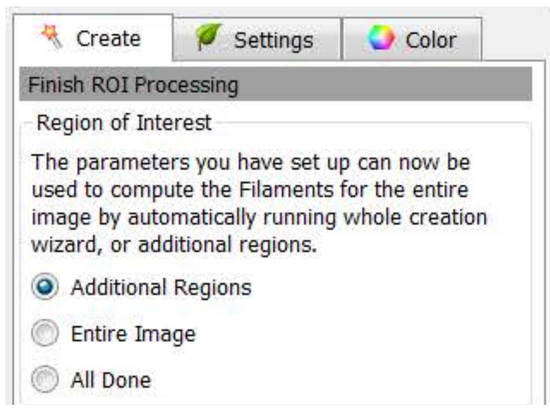
1. Create a new filament and check "segment a region of interest"



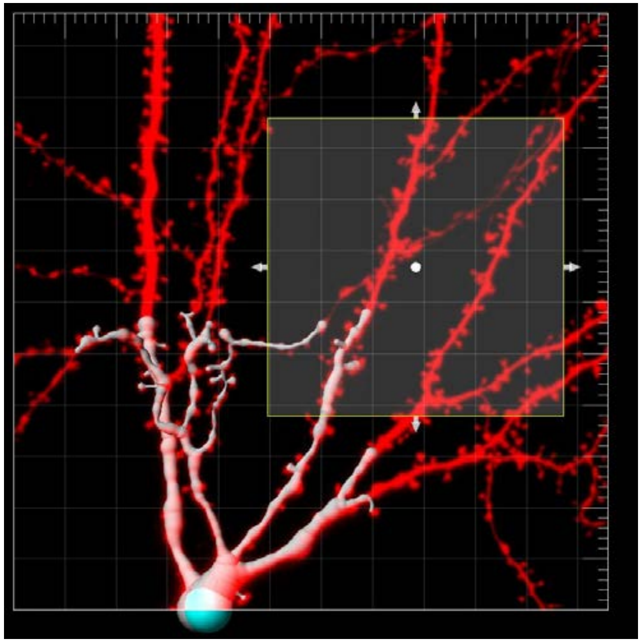
2. Select a ROI. (Switch to orthogonal view for easy ROI navigation)

3. Proceed with processing as normal

4. On "last" step, select additional regions.



5. Select ROI to overlap with existing filament to continue to build on existing structure.



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1d. Region of Interest (ROI) Tracing (Cont)

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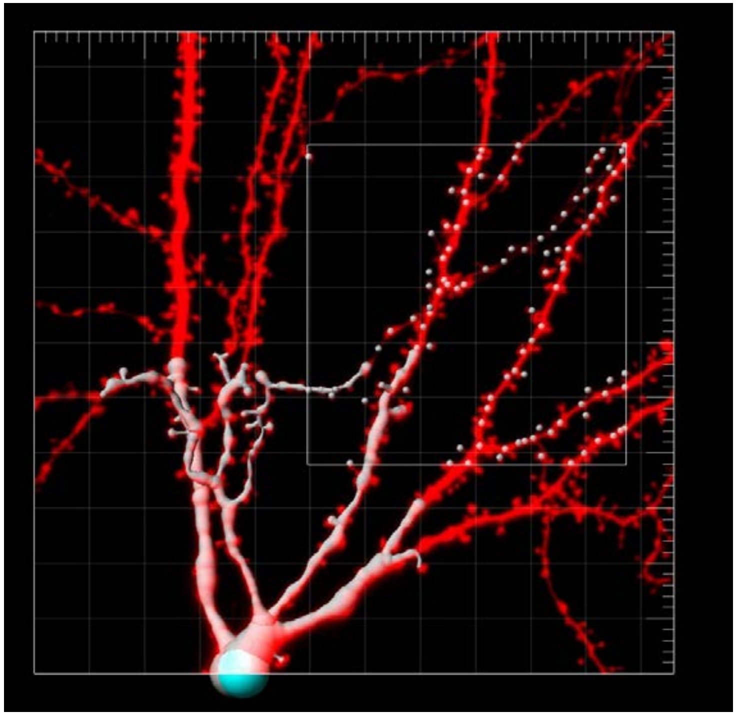
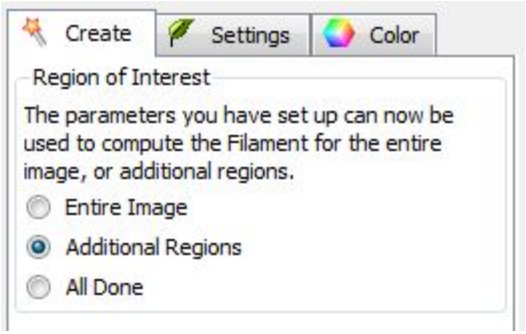
6. *Choose "use existing dendrite" and set your new end point diameter. Adjust threshold.*



7. *Calculate diameter. Repeat process from step 4. When finished, select "All done" to complete process.*



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2. Semi-Automatic AutoPath

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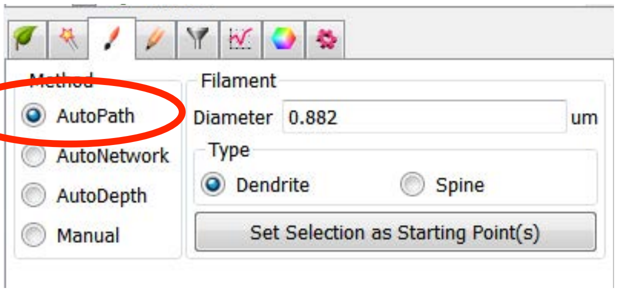
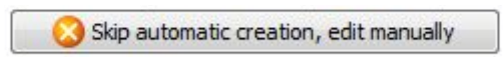
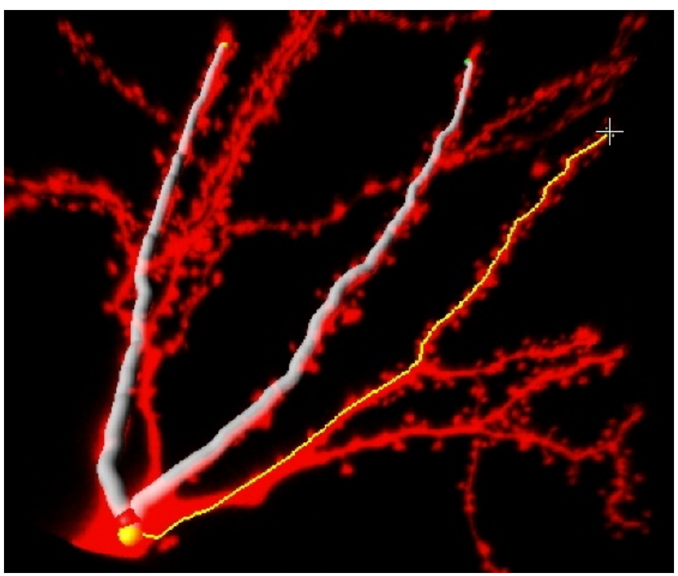


- To start with Semi-Automatic Tracing, cancel fully automatic creation.



OR

- Select “Draw” tab and method “AutoPath”



- Switch Pointer to selection mode (Esc-key)
- Shift+RightClick onto cell body in 3D volume
- Wait for calculation
- Move Mouse around and see traced paths in real-time.
- Shift+LeftClick to add path to filament.

Algorithms described in:
E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling, and M. Unser, 2004
Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images, *Cytometry*, vol. 58A, no. 2, April 2004, pp. 167-176

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Semi-Automatic AutoPath: How It Works

- When you press Shift+RightClick to set a starting point, the FastMarching Algorithm propagates a front outward from this starting point. The speed of propagation depends on image intensity, faster propagation occuring at brighter voxels.

- Finally the paths are traced backward from the current mouse position by steepest descent.

- Upon Shift-Left Click paths are traced backward until they reach the filament. Here they are merged with the existing filament to create a tree without loops.

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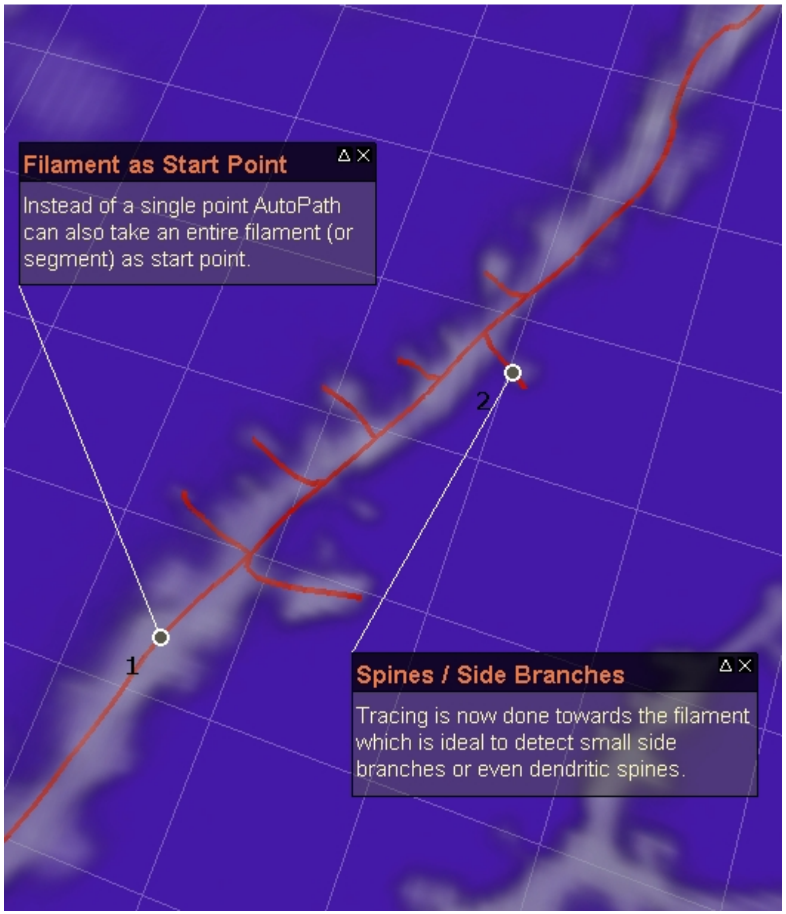
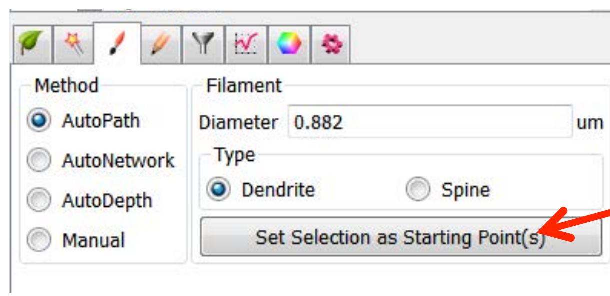
Using Filament as Start Point in AutoPath



- The center segment in the left picture was taken as start point from which AutoPath calculated the highest intensity paths.

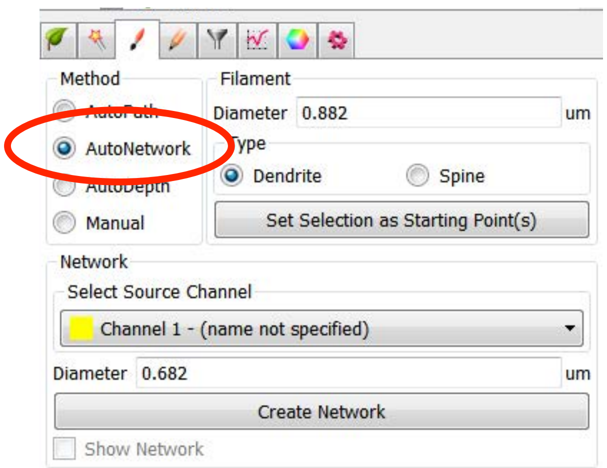
- Tracing is then carried out towards the center filament and allows to capture side branches with a single click.

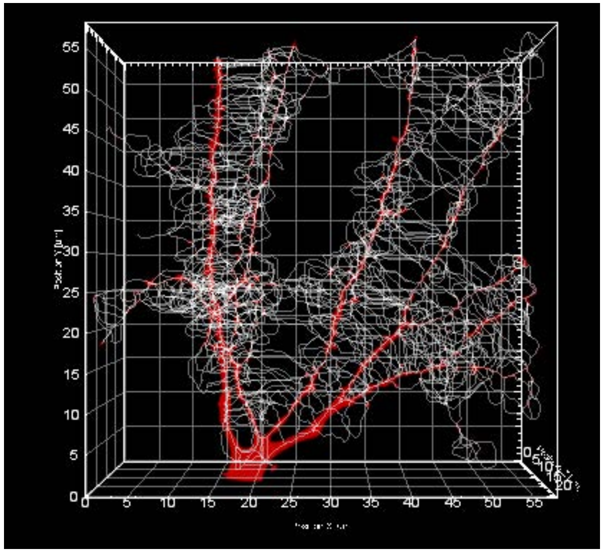


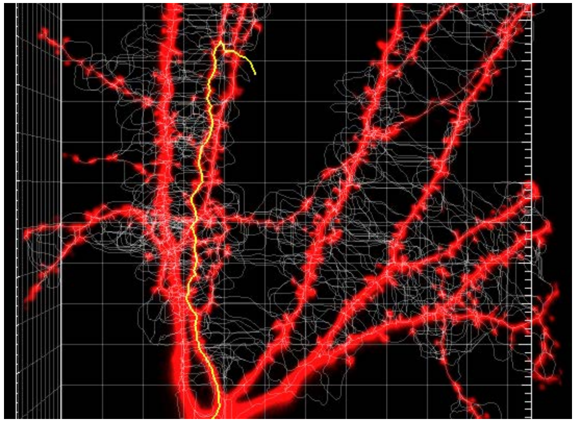
1. Highlight segment, pt, or branch to use
2. Click

3. Semi-Automatic AutoNetwork

- Similar to AutoPath with the exception that intermediate «way-points» can be set after having first created a Network







- Select the source channel and the minimum (smallest) diameter to be detected
- To set the starting point press **Shift + right-click** on the preferred location of the structure.
- Place the mouse at the ends of the structure and the path is displayed.
- To draw the displayed path press Shift + left click.
- Please note: To create „way-points“ press **Shift+left-click** at the location of the way-point, and **simultaneously press Shift-right-click** (at the same location) in order to move forward toward the real end-point.

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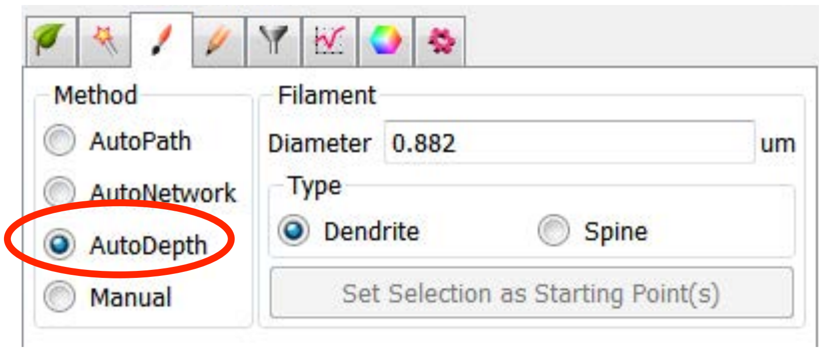
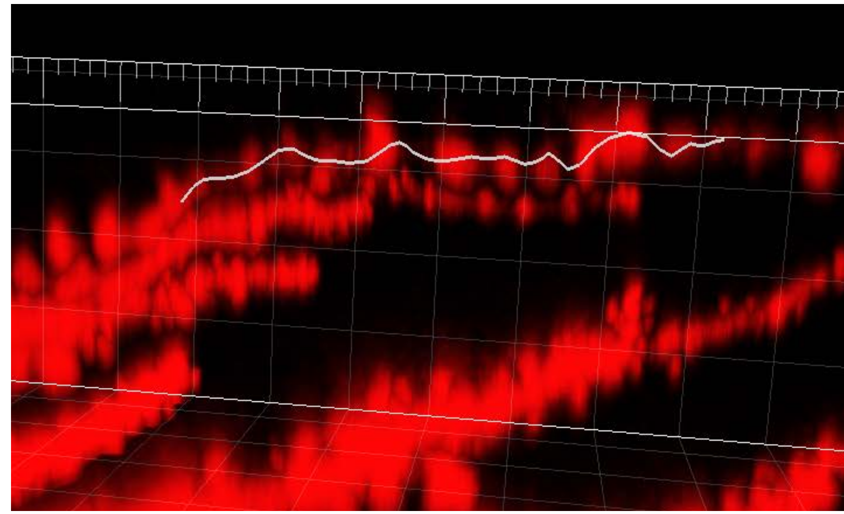
4. AutoDepth Tracing





1. *On the“Draw“ tab switch method to “AutoDetph“.*
2. *Switch the Surpass view to “Selection“ mode (ESC key)*
3. *Turning the mouse wheel or entering a value will alter the cursor box size (resulting filament diameter only visible in certain modes).*
4. *Left-click+shift at the desired starting point and draw along a filament. Imaris automatically determines the 3rd dimension using maximum intensity along the viewing ray.*

- The resulting filament is of constant diameter (unless the wheel is turned during drawing) and follows the structure in 3D
- Good for fast, accurate and reproducible manual tracing

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5. Manual Drawing

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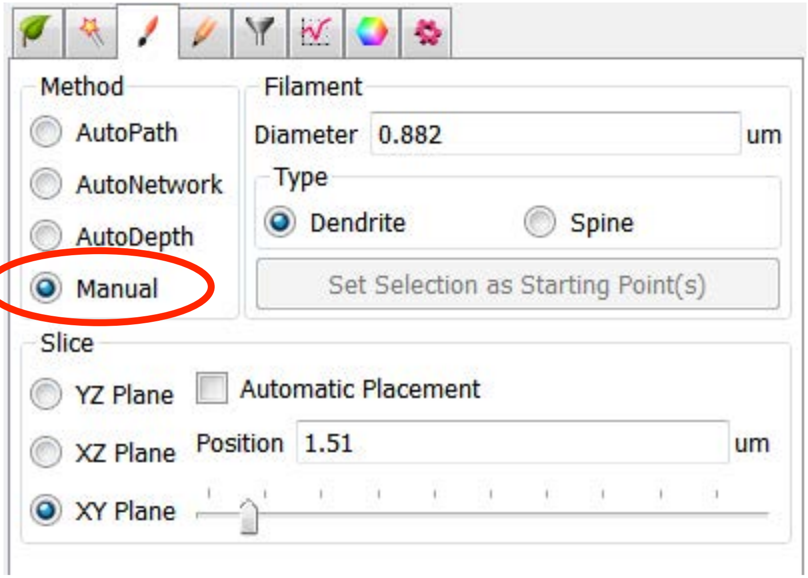

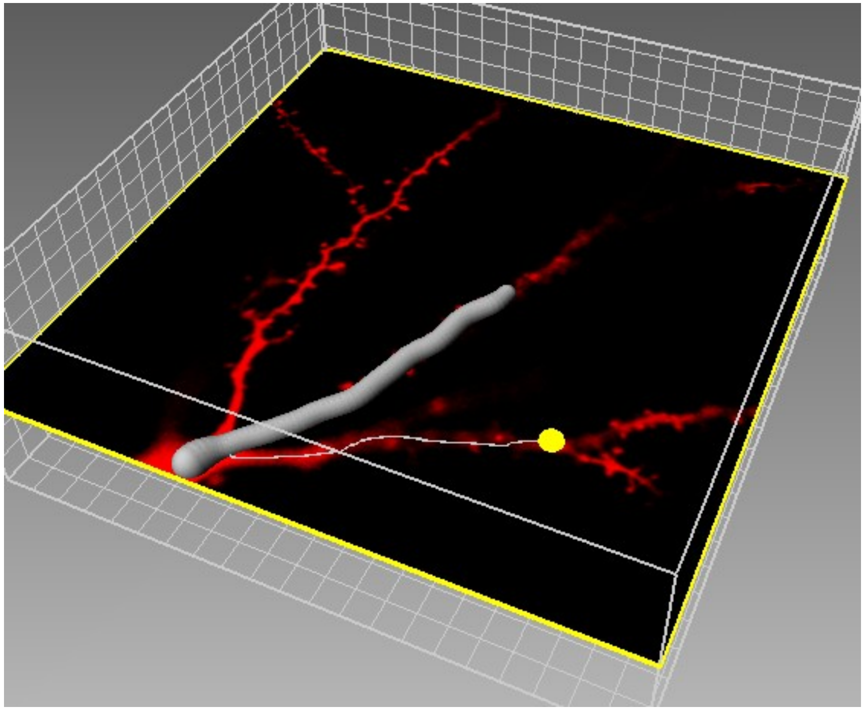

- In this mode users can manually trace filaments in xy, in a single z-plane.
- the z-position can be determined:
  - manually (when Automatic Placement is switched off) using the +/- keys or up/down keys.
  - Automatically via Automatic Placement in which a single z-plane moves based on cursor position
- Good for tracing in complex trees where one wants to focus on one specific portion of the structure

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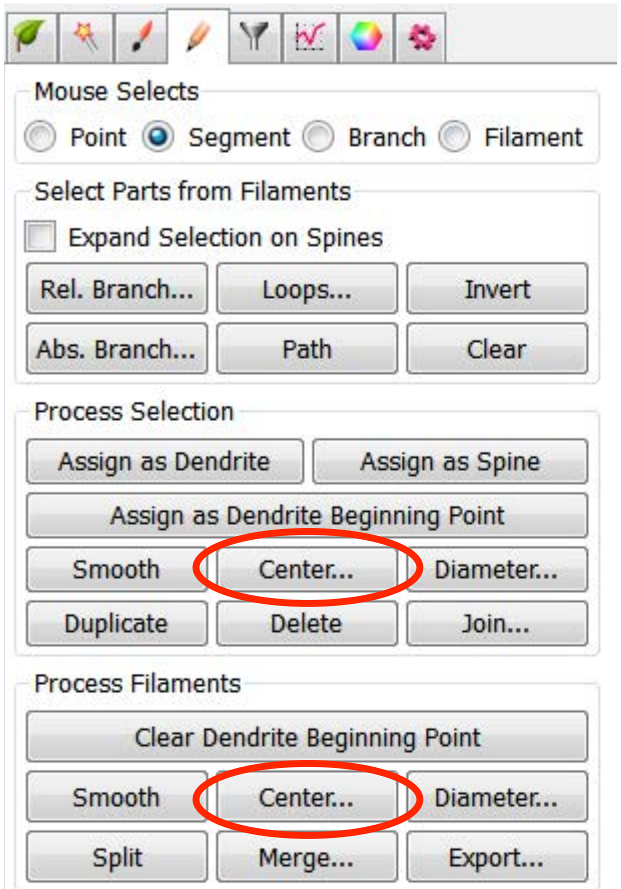
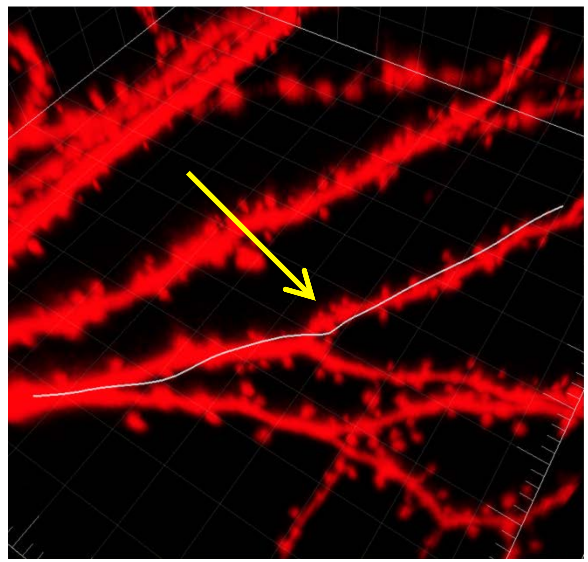
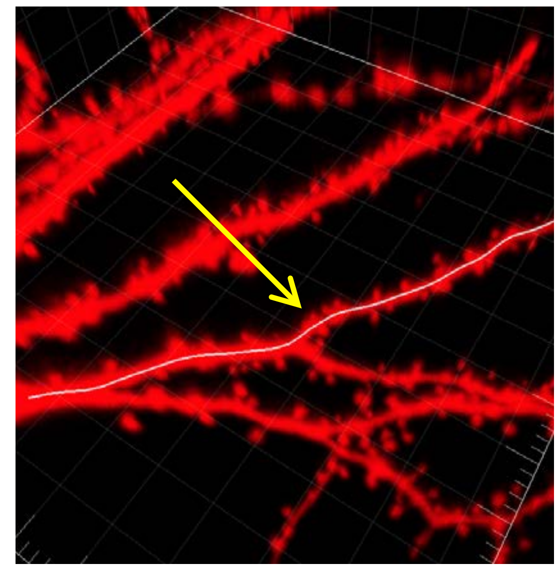
Path Centering



Before Centering



After Centering



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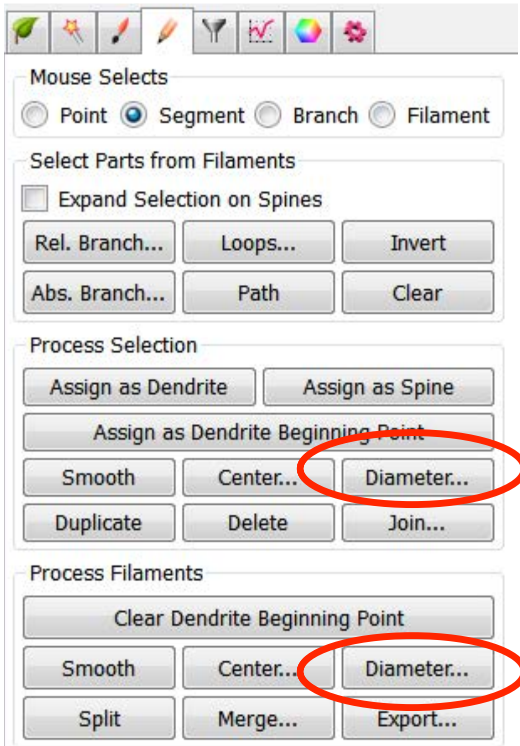
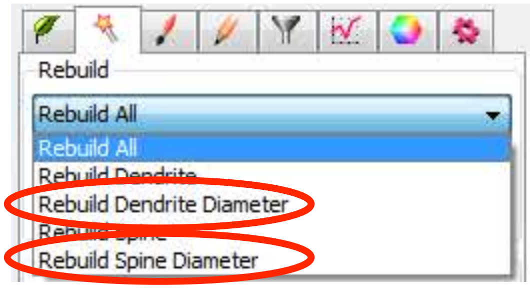
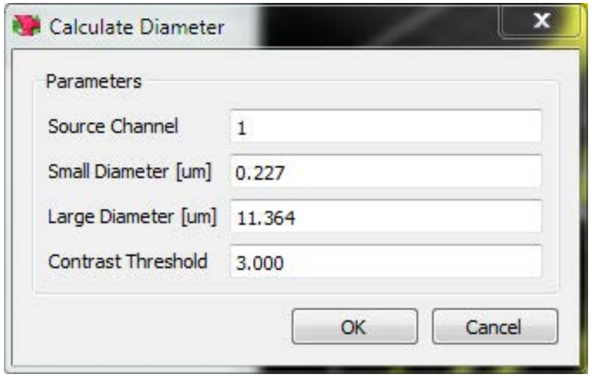
Diameter Calculation

- Can rebuild diameter from Creation tab - *BEST METHOD*
- Diameter Calculation is also available on the Edit tab - *Only to be used to change individual dendrite segments or spines*







1. Select the source channel
2. Type-in smallest Filament diameter in selected region
3. Type-in largest Filament diameter in selected region
4. Type-in the contrast threshold in the selected region

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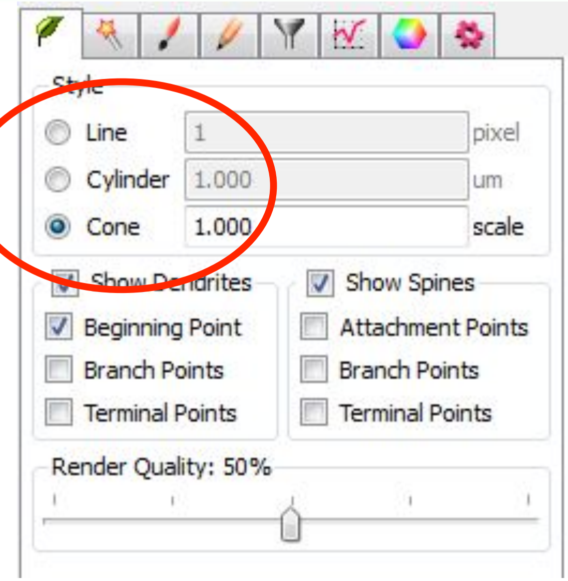
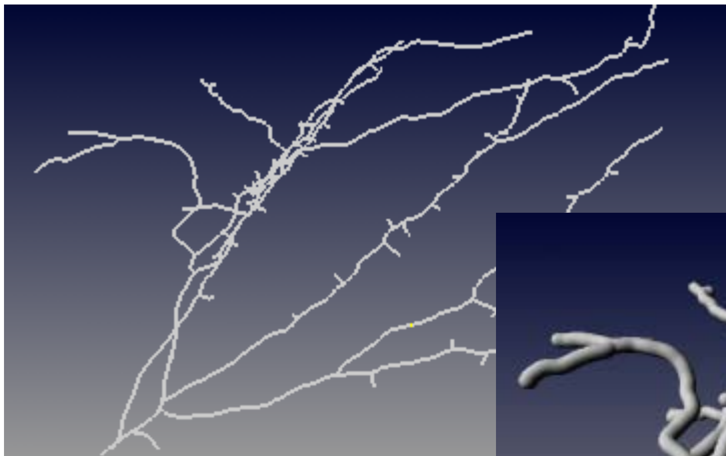
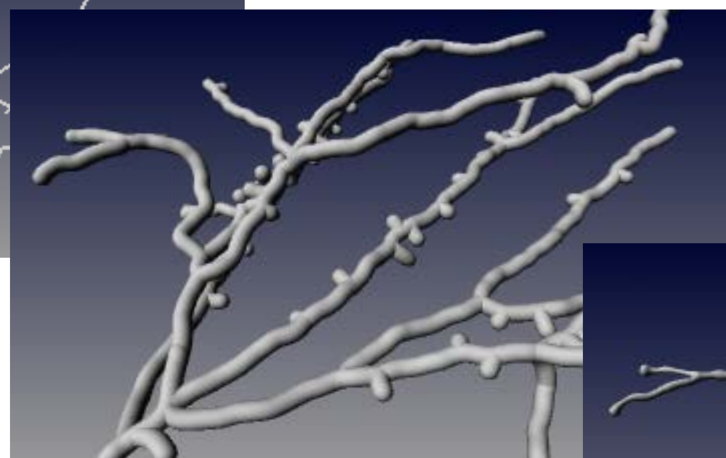
Filament Visualization Styles

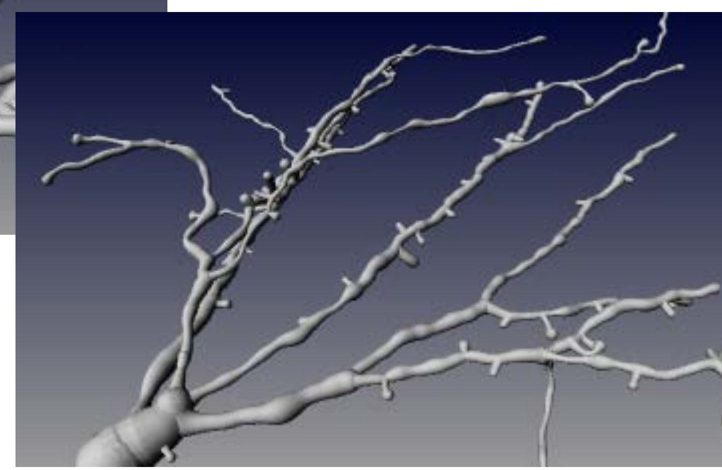




Line-skeletonized filament



Cylinder-”tube-like” filament



Cone-filament with volume measurement

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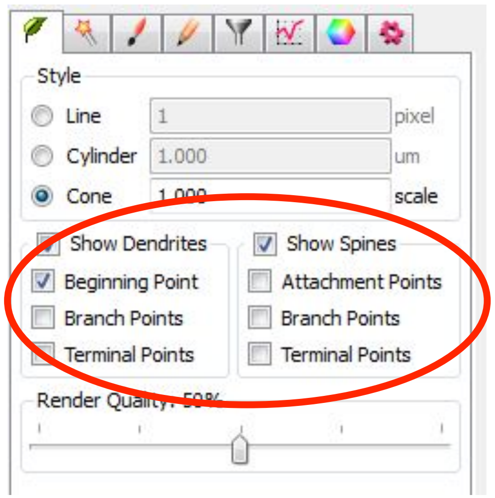
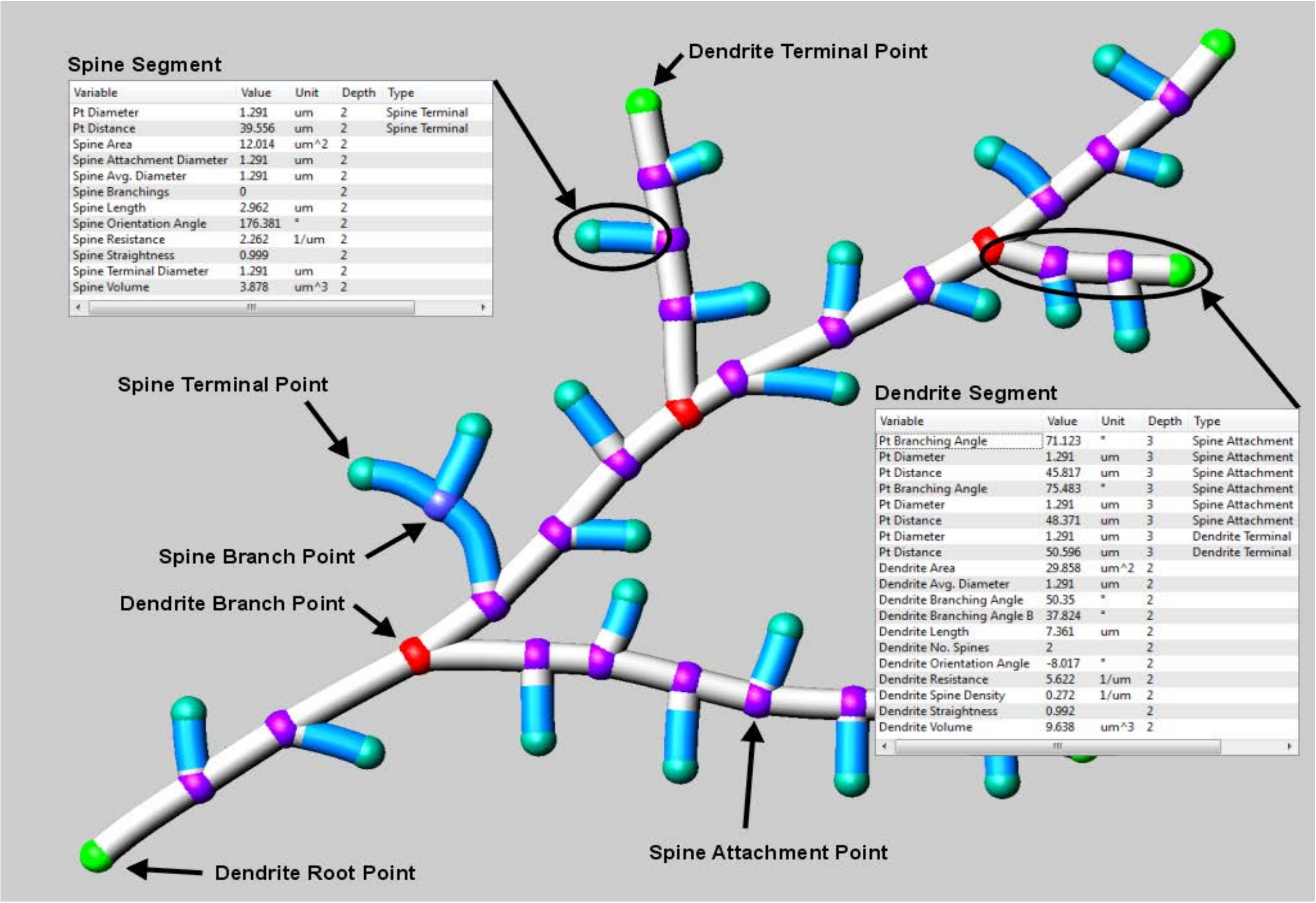
Filament Element Visualization





Spine Segment

Variable	Value	Unit	Depth	Type
Pt Diameter	1.291	um	2	Spine Terminal
Pt Distance	39.556	um	2	Spine Terminal
Spine Area	12.014	um^2	2	
Spine Attachment Diameter	1.291	um	2	
Spine Avg. Diameter	1.291	um	2	
Spine Branchings	0		2	
Spine Length	2.962	um	2	
Spine Orientation Angle	176.381	°	2	
Spine Resistance	2.262	1/um	2	
Spine Straightness	0.999		2	
Spine Terminal Diameter	1.291	um	2	
Spine Volume	3.878	um^3	2	

Dendrite Segment

Variable	Value	Unit	Depth	Type
Pt Branching Angle	71.123	°	3	Spine Attachment
Pt Diameter	1.291	um	3	Spine Attachment
Pt Distance	45.817	um	3	Spine Attachment
Pt Branching Angle	75.483	°	3	Spine Attachment
Pt Diameter	1.291	um	3	Spine Attachment
Pt Distance	48.371	um	3	Spine Attachment
Pt Diameter	1.291	um	3	Dendrite Terminal
Pt Distance	50.596	um	3	Dendrite Terminal
Dendrite Area	29.858	um^2	2	
Dendrite Avg. Diameter	1.291	um	2	
Dendrite Branching Angle	50.35	°	2	
Dendrite Branching Angle B	37.824	°	2	
Dendrite Length	7.361	um	2	
Dendrite No. Spines	2		2	
Dendrite Orientation Angle	-8.017	°	2	
Dendrite Resistance	5.622	1/um	2	
Dendrite Spine Density	0.272	1/um	2	
Dendrite Straightness	0.992		2	
Dendrite Volume	9.638	um^3	2	

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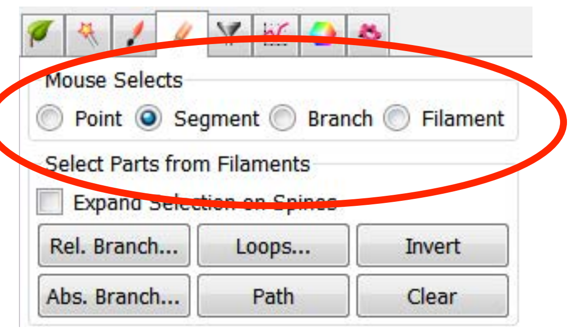
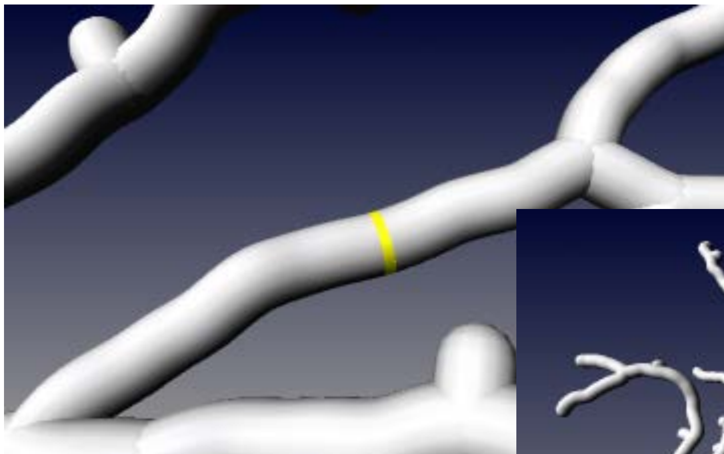
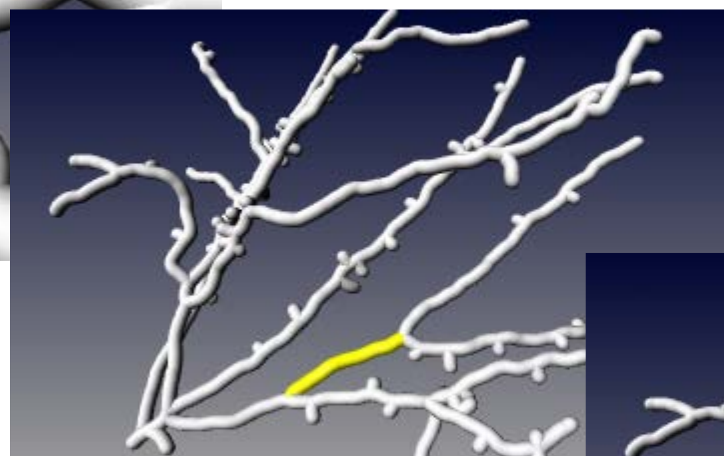
Filament Editing Selection

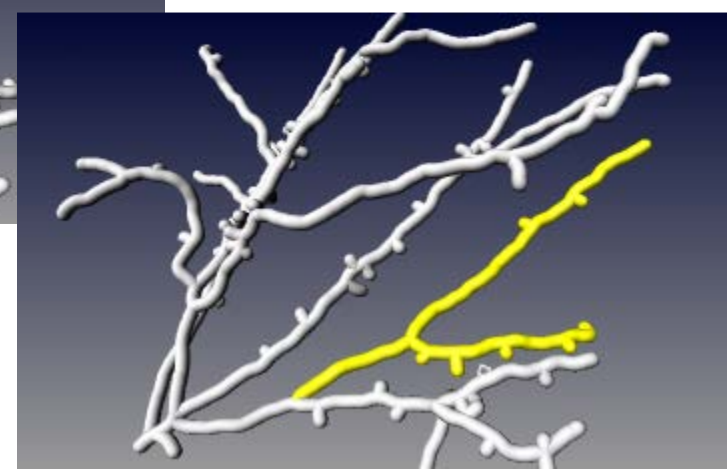




Point



Segment



Branch

Note : *Holding "Ctrl" while clicking allows you to multiple select items*

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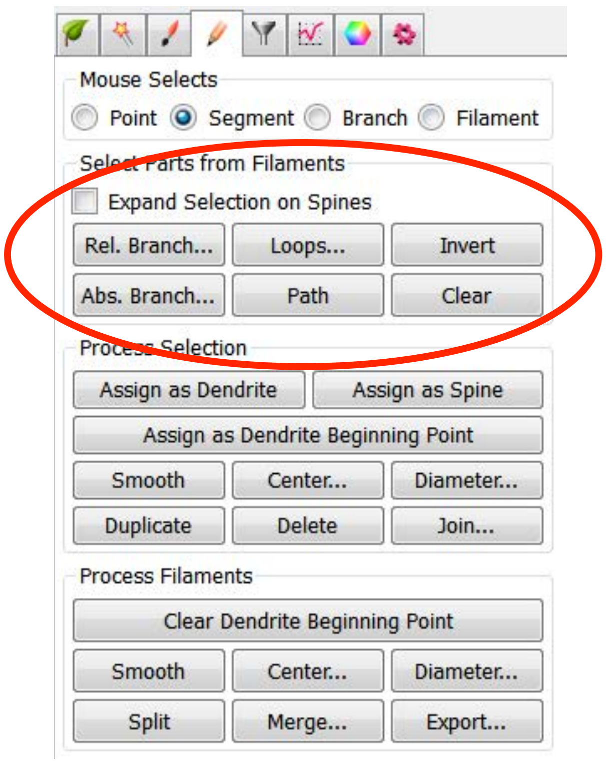
Automatic Filament Selection



Rel. Branch- selects short parts defined by the ratio of the branch length to trunk radius

Loops- finds circular closed filaments limited by a maximum length.

Invert- selects unselected parts and deselects selected parts.

Abs Branch- selects branches shorter than a defined length.

Path- selects shortest path between two selected points

Clear- clears current selection.

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Filament Processing

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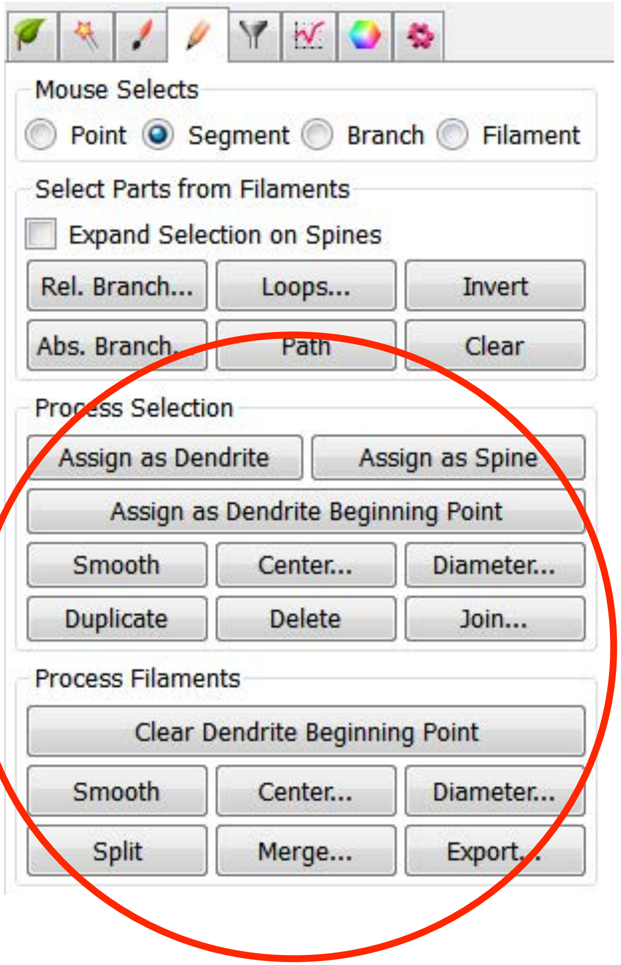

1. "Assign as ___"-marks the selection as a dendrite, spine, or dendrite beginning point
2. Smooth- removes roughness of the filament/selection
3. Center-re-centers the filament/selection
4. Diameter-recalculates the filament diameter
5. Duplicate-Copies the selected portions to a new filament object
6. Delete-deletes the selected portion
7. Join-Connects two parts of a filament
8. Split-Splits unconnected parts of the filament into separate filament objects. Allows individual coloring and individual statistics.
9. Merge-connects separate filament objects
10. Export-Stores filament graph in the Neuron file format (*.hoc).

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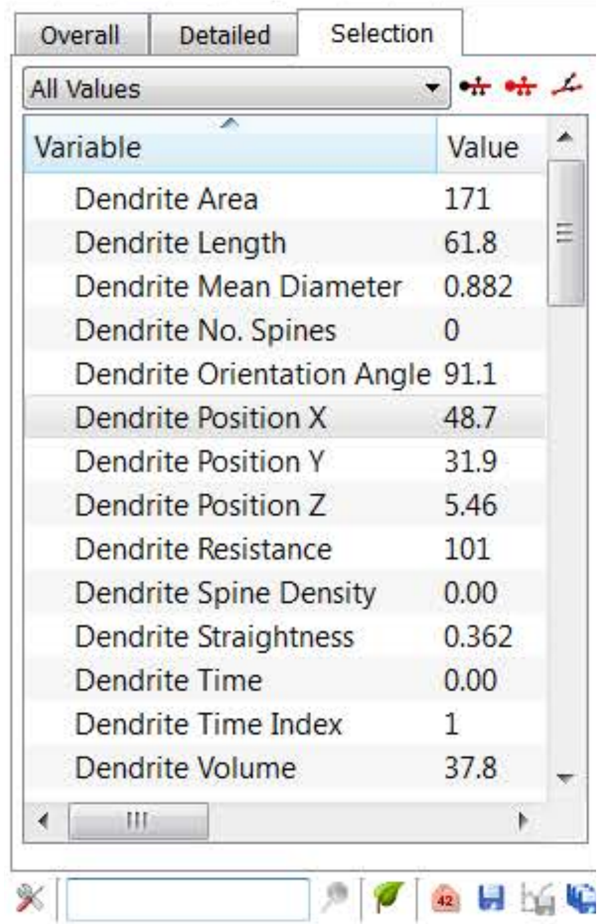
Filament Statistics

Statistics displayed for:

- -entire filament (overall)
- -particular statistic (detailed)
- -the selected portion

Specific categories can be selected to be shown or to be removed from the list:

- dendrite
- filament
- spine



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Available Preset Statistics

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Configure list of available
visible statistic values

Show Statistic Values

- Filaments
  - Dendrite Area
  - Dendrite Branch Depth
  - Dendrite Branch Level
  - Dendrite Branching Angle
  - Dendrite Branching Angle B
  - Dendrite Length
  - Dendrite Mean Diameter
  - Dendrite No. Spines
  - Dendrite Orientation Angle
  - Dendrite Position X
  - Dendrite Position Y
  - Dendrite Position Z
  - Dendrite Resistance
  - Dendrite Spine Density
  - Dendrite Straightness
  - Dendrite Time
  - Dendrite Time Index
  - Dendrite Volume
  - Edge Diameter
  - Edge Length
  - Filament Acceleration
  - Filament Acceleration X
  - Filament Acceleration Y
  - Filament Acceleration Z
  - Filament Area (sum)
  - Filament BoundingBoxAA Length X
  - Filament BoundingBoxAA Length Y
  - Filament BoundingBoxAA Length Z
  - Filament BoundingBoxOO Length A
  - Filament BoundingBoxOO Length B
  - Filament BoundingBoxOO Length C
  - Filament Dendrite Area (sum)
  - Filament Dendrite Length (sum)
  - Filament Dendrite Volume (sum)
  - Filament Displacement X
  - Filament Displacement Y
  - Filament Displacement Z
  - Filament Displacement^2
  - Filament Full Branch Depth
  - Filament Full Branch Level
  - Filament Length (sum)
  - Filament No. Dendrite Branch Pts
  - Filament No. Dendrite Branches
  - Filament No. Dendrite Segments
  - Filament No. Dendrite Terminal Pts
  - Filament No. Edges
  - Filament No. Sholl Intersections
  - Filament No. Spine Branch Pts
  - Filament No. Spine Segments
  - Filament No. Spine Terminal Pts
  - Filament No. Unconnected Parts
  - Filament No. Vertices
  - Filament Number of Filaments per Time Poin
  - Filament Number of Tracks
  - Filament Position X
  - Filament Position Y
  - Filament Position Z
  - Filament Speed
  - Filament Spine Area (sum)
  - Filament Spine Length (sum)
  - Filament Spine Volume (sum)
  - Filament Time
  - Filament Time Index
  - Filament Total Number of Filaments
  - Filament Track Ar1 Mean
  - Filament Track Ar1 X
  - Filament Track Ar1 Y
  - Filament Track Ar1 Z
  - Filament Track Displacement Length
  - Filament Track Displacement X
  - Filament Track Displacement Y
  - Filament Track Displacement Z
  - Filament Track Duration
  - Filament Track Length
  - Filament Track Number of Branches
  - Filament Track Number of Filaments
  - Filament Track Number of Fusions
  - Filament Track Position X Mean
  - Filament Track Position X Start
  - Filament Track Position Y Mean
  - Filament Track Position Y Start
  - Filament Track Position Z Mean
  - Filament Track Position Z Start
  - Filament Track Speed Max
  - Filament Track Speed Mean
  - Filament Track Speed Min
  - Filament Track Speed StdDev
  - Filament Track Speed Variation
  - Filament Track Straightness
  - Filament Velocity X
  - Filament Velocity Y
  - Filament Velocity Y
  - Filament Velocity Z
  - Filament Volume (sum)
  - Pt Acceleration
  - Pt Acceleration X
  - Pt Acceleration Y
  - Pt Acceleration Z
  - Pt Branching Angle
  - Pt Diameter
  - Pt Displacement X
  - Pt Displacement Y
  - Pt Displacement Z
  - Pt Displacement^2
  - Pt Distance
  - Pt Number of Points per Time Point
  - Pt Number of Tracks
  - Pt Position X
  - Pt Position Y
  - Pt Position Z
  - Pt Speed
  - Pt Time
  - Pt Time Index
  - Pt Total Number of Points
  - Pt Track Ar1 Mean
  - Pt Track Ar1 X
  - Pt Track Ar1 Y
  - Pt Track Ar1 Z
  - Pt Track Displacement Length
  - Pt Track Displacement X
  - Pt Track Displacement Y
  - Pt Track Displacement Z
  - Pt Track Duration
  - Pt Track Length
  - Pt Track Number of Branches
  - Pt Track Number of Fusions
  - Pt Track Number of Points
  - Pt Track Position X Mean
  - Pt Track Position X Start
  - Pt Track Position Y Mean
  - Pt Track Position Y Start
  - Pt Track Position Z Mean
  - Pt Track Position Z Start
  - Pt Track Speed Max
  - Pt Track Speed Mean
  - Pt Track Speed Min
  - Pt Track Speed StdDev
  - Pt Track Speed StdDev
  - Pt Track Speed Variation
  - Pt Track Spine Area Max
  - Pt Track Spine Area Mean
  - Pt Track Spine Area Min
  - Pt Track Spine Length Max
  - Pt Track Spine Length Mean
  - Pt Track Spine Length Min
  - Pt Track Spine Volume Max
  - Pt Track Spine Volume Mean
  - Pt Track Spine Volume Min
  - Pt Track Straightness
  - Pt Velocity X
  - Pt Velocity Y
  - Pt Velocity Z
  - Spine Area
  - Spine Attachment Pt Acceleration
  - Spine Attachment Pt Acceleration X
  - Spine Attachment Pt Acceleration Y
  - Spine Attachment Pt Acceleration Z
  - Spine Attachment Pt Branching Angle
  - Spine Attachment Pt Diameter
  - Spine Attachment Pt Displacement X
  - Spine Attachment Pt Displacement Y
  - Spine Attachment Pt Displacement Z
  - Spine Attachment Pt Displacement^2
  - Spine Attachment Pt Distance
  - Spine Attachment Pt Position X
  - Spine Attachment Pt Position Y
  - Spine Attachment Pt Position Z
  - Spine Attachment Pt Speed
  - Spine Attachment Pt Time
  - Spine Attachment Pt Time Index
  - Spine Attachment Pt Velocity X
  - Spine Attachment Pt Velocity Y
  - Spine Attachment Pt Velocity Z
  - Spine Branch Depth
  - Spine Branch Level
  - Spine Branchings
  - Spine Length
  - Spine Max Diameter
  - Spine Mean Diameter
  - Spine Min Diameter
  - Spine Neck Length
  - Spine Neck Max Diameter
  - Spine Neck Mean Diameter
  - Spine Branch Depth
  - Spine Branch Level
  - Spine Branchings
  - Spine Length
  - Spine Max Diameter
  - Spine Mean Diameter
  - Spine Min Diameter
  - Spine Neck Length
  - Spine Neck Max Diameter
  - Spine Neck Mean Diameter
  - Spine Neck Min Diameter
  - Spine Neck Volume
  - Spine Orientation Angle
  - Spine Position X
  - Spine Position Y
  - Spine Position Z
  - Spine Resistance
  - Spine Straightness
  - Spine Terminal Pt Acceleration
  - Spine Terminal Pt Acceleration X
  - Spine Terminal Pt Acceleration Y
  - Spine Terminal Pt Acceleration Z
  - Spine Terminal Pt Diameter
  - Spine Terminal Pt Displacement X
  - Spine Terminal Pt Displacement Y
  - Spine Terminal Pt Displacement Z
  - Spine Terminal Pt Displacement^2
  - Spine Terminal Pt Distance
  - Spine Terminal Pt Intensity Center
  - Spine Terminal Pt Position X
  - Spine Terminal Pt Position Y
  - Spine Terminal Pt Position Z
  - Spine Terminal Pt Speed
  - Spine Terminal Pt Time
  - Spine Terminal Pt Time Index
  - Spine Terminal Pt Velocity X
  - Spine Terminal Pt Velocity Y
  - Spine Terminal Pt Velocity Z
  - Spine Terminal Pt Volume
  - Spine Time
  - Spine Time Index
  - Spine Volume

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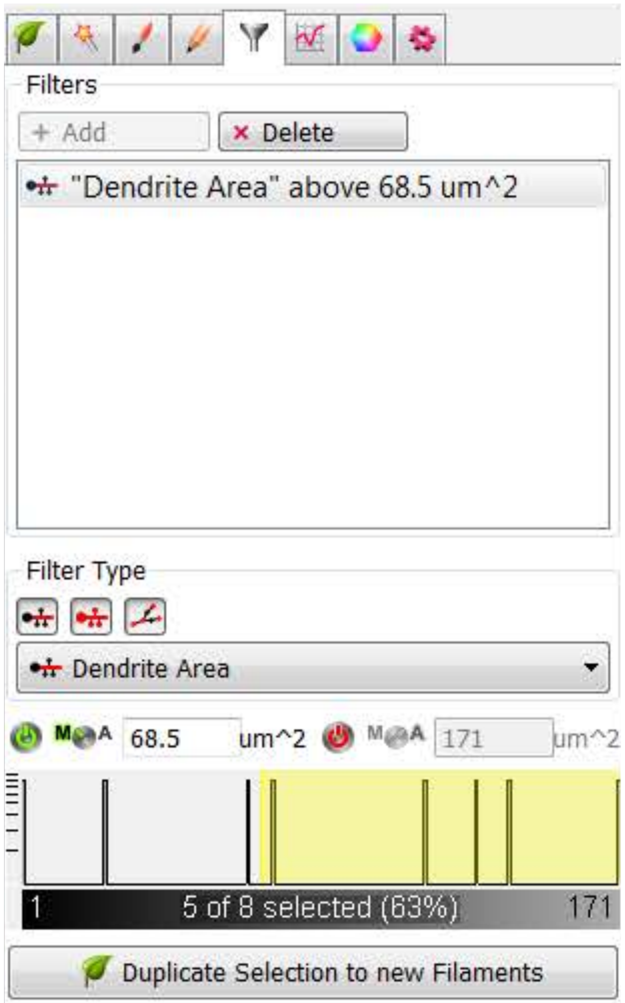
Filament Filters



- Allows for filtering the data based on a specific statistic using any of the available statistics (ex. Dendrite length, spine volume, etc.)
- Multiple filters can be made
- Can copy the filter selection to a new filament object
- Filters list can be reduced by selecting:
  - Dendrite filters
  - Spine filters
  - Filament Filters

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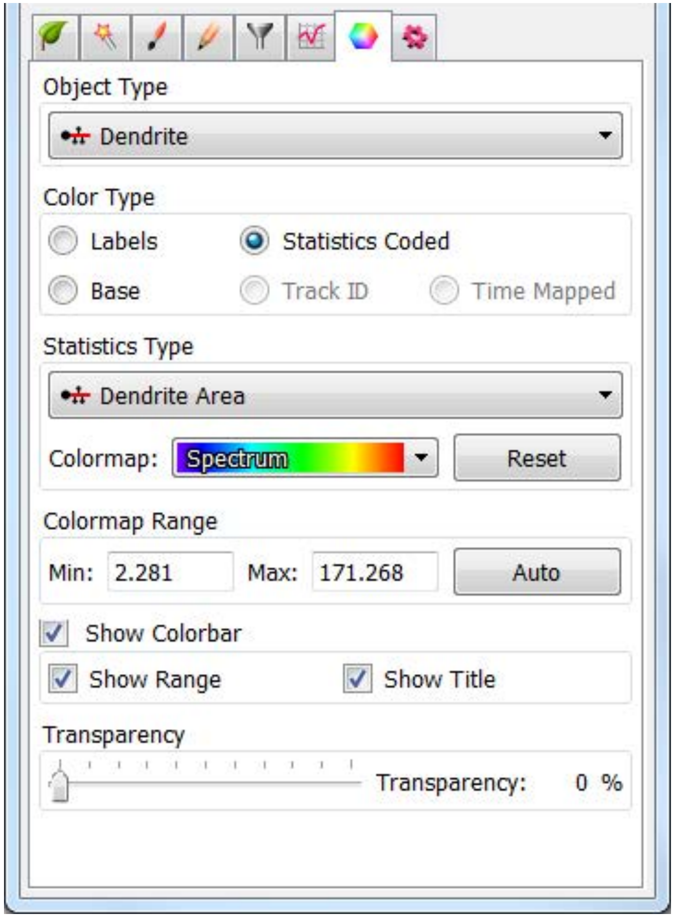
Filament Coloring

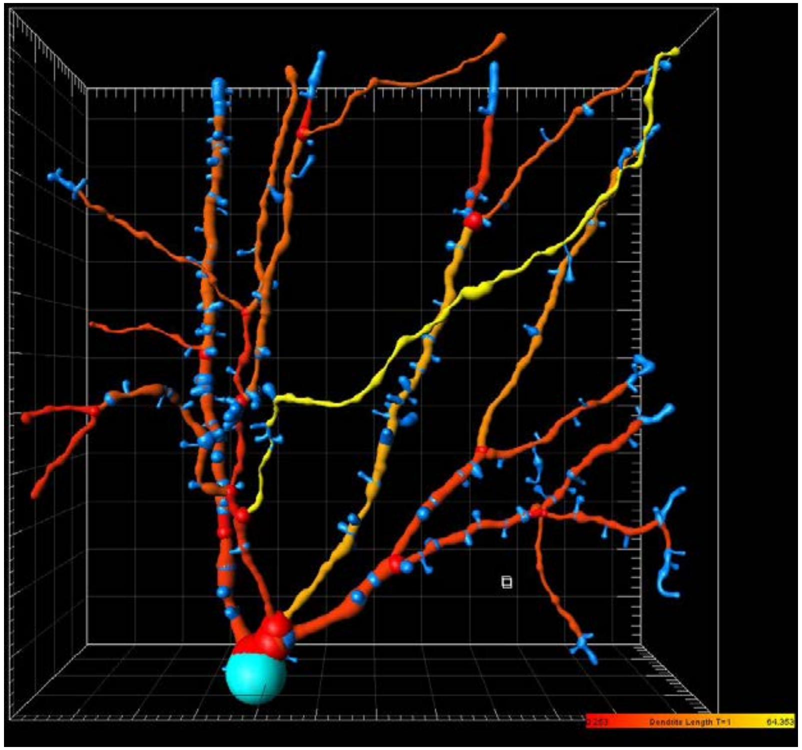


- *Base Color Type:*

  Solid color filament

- *Statistics Coded Color type:*

  Colors each portion according to statistical values



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Filament Time Series

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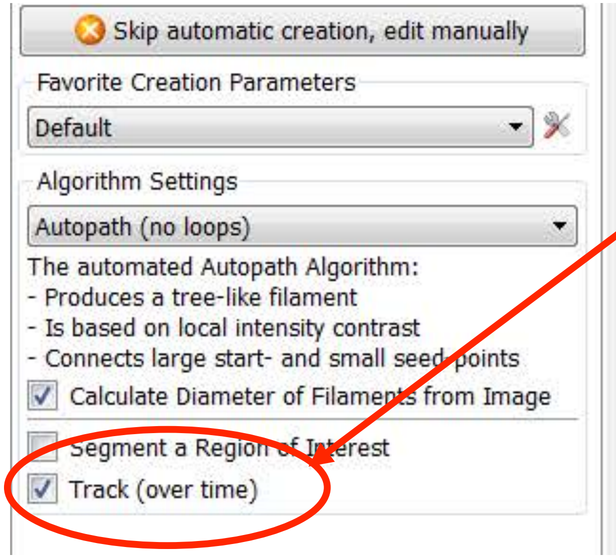
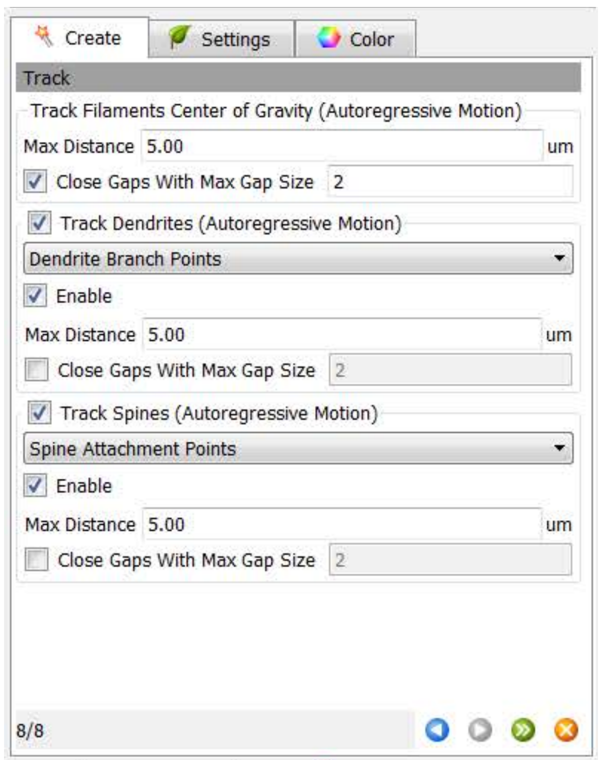
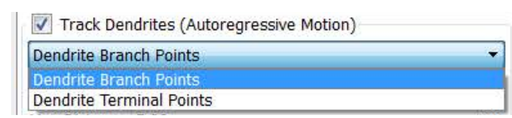
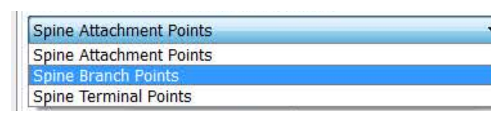


To track filaments (dendrites) and/or spines over time, check *Track (over time)* and run through the creation steps.



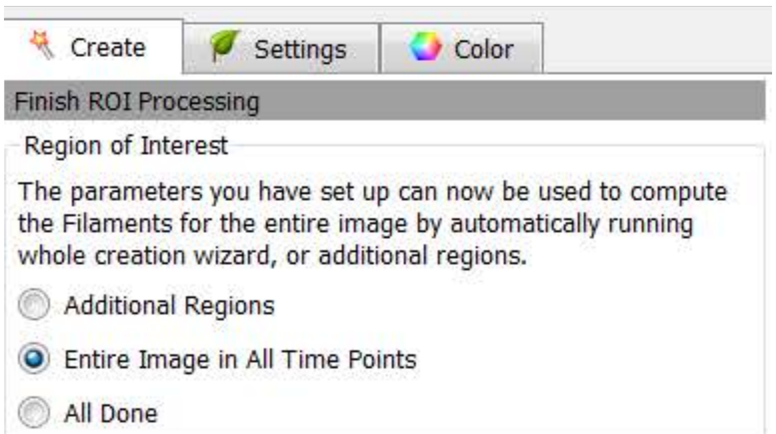
In the last step enable or disable objects to be tracked and set the tracking parameters.



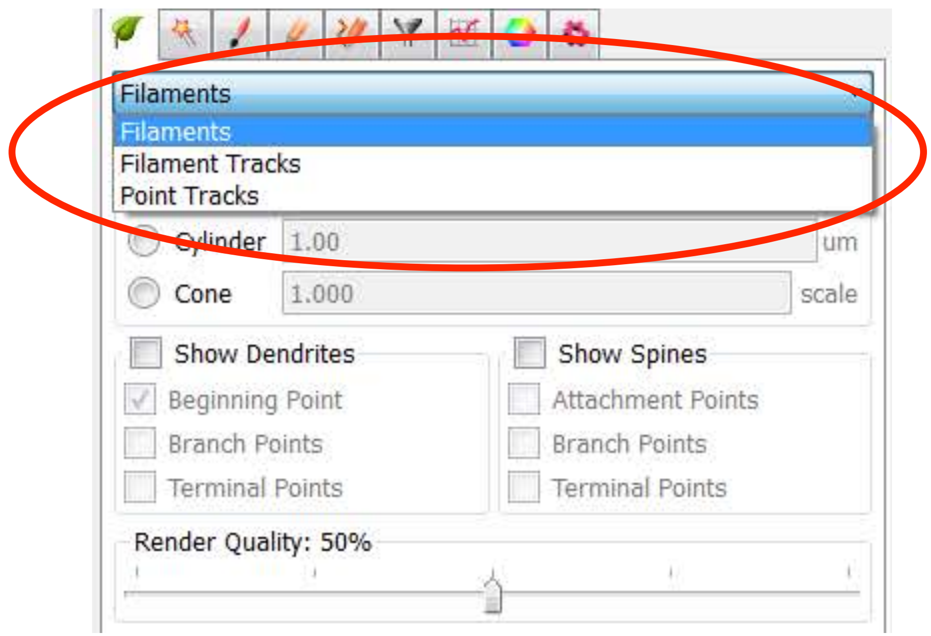


Filament Time Series (cont)

When working with ROIs following options are available:



Once the filaments created, you can switch between the standard Filament visualization options and Filament Tracks



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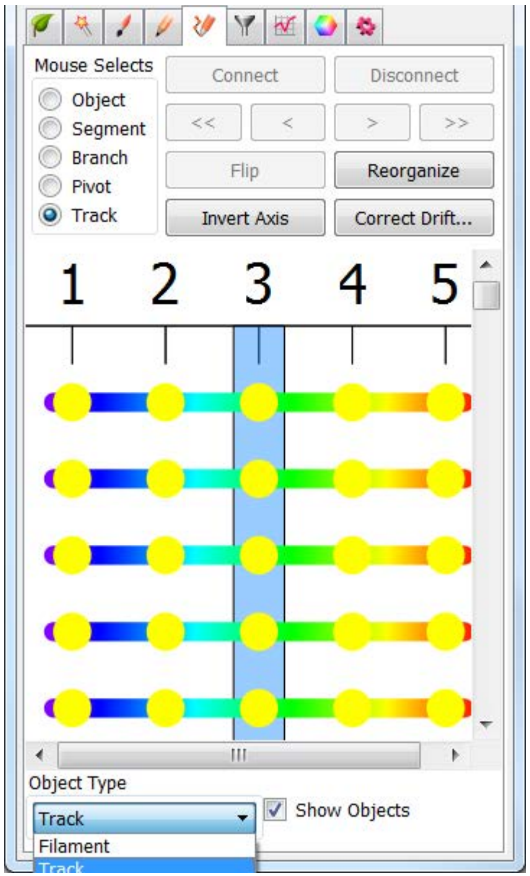
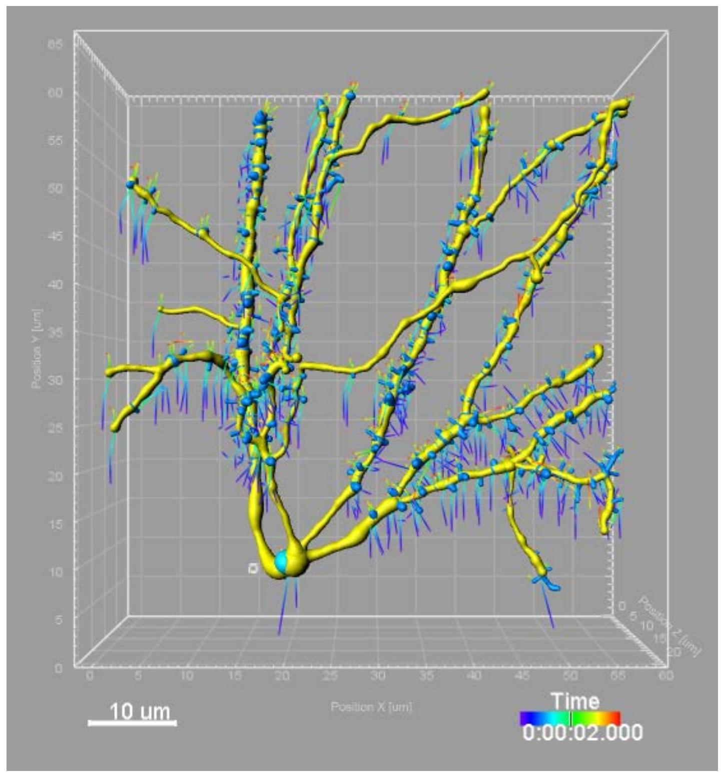
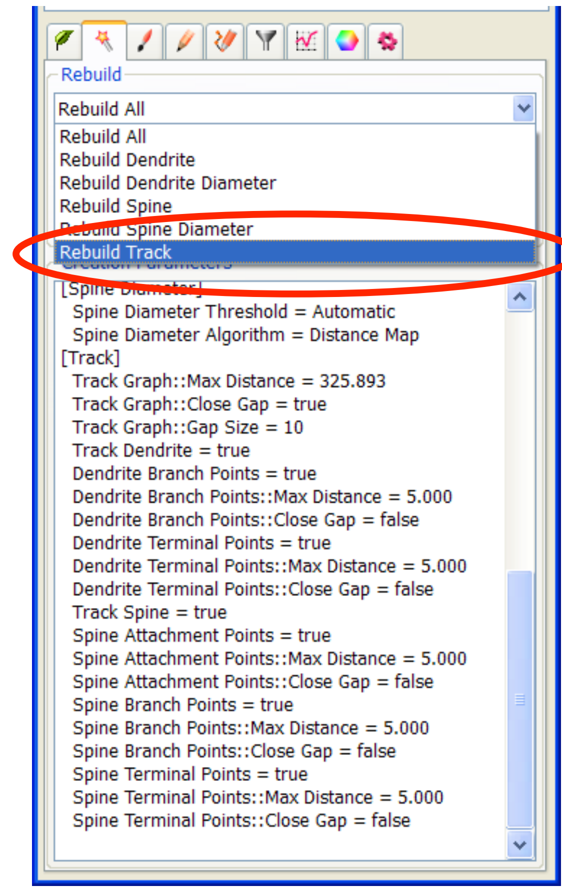
Filament Time Series (cont)

The Track Editor is similar to Track Spots or Surfaces





Tracks can easily be rebuild



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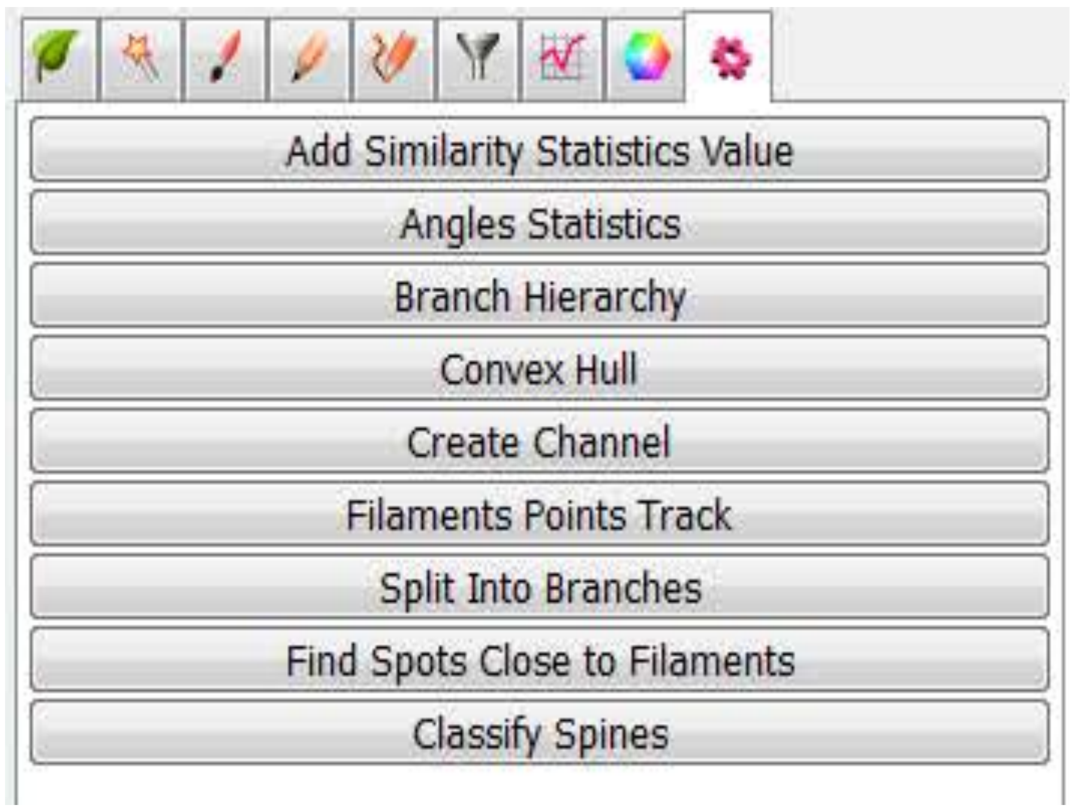
Filament Options with ImarisXT

Customize Imaris for your specific research applications or use pre-existing XTensions



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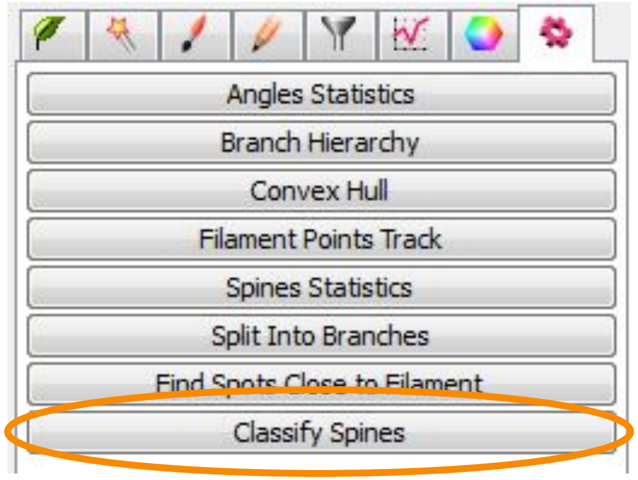
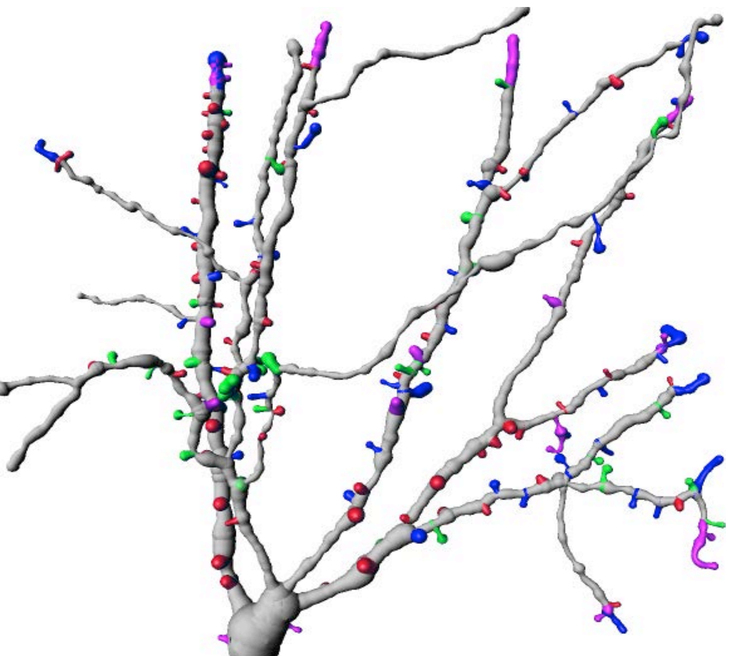
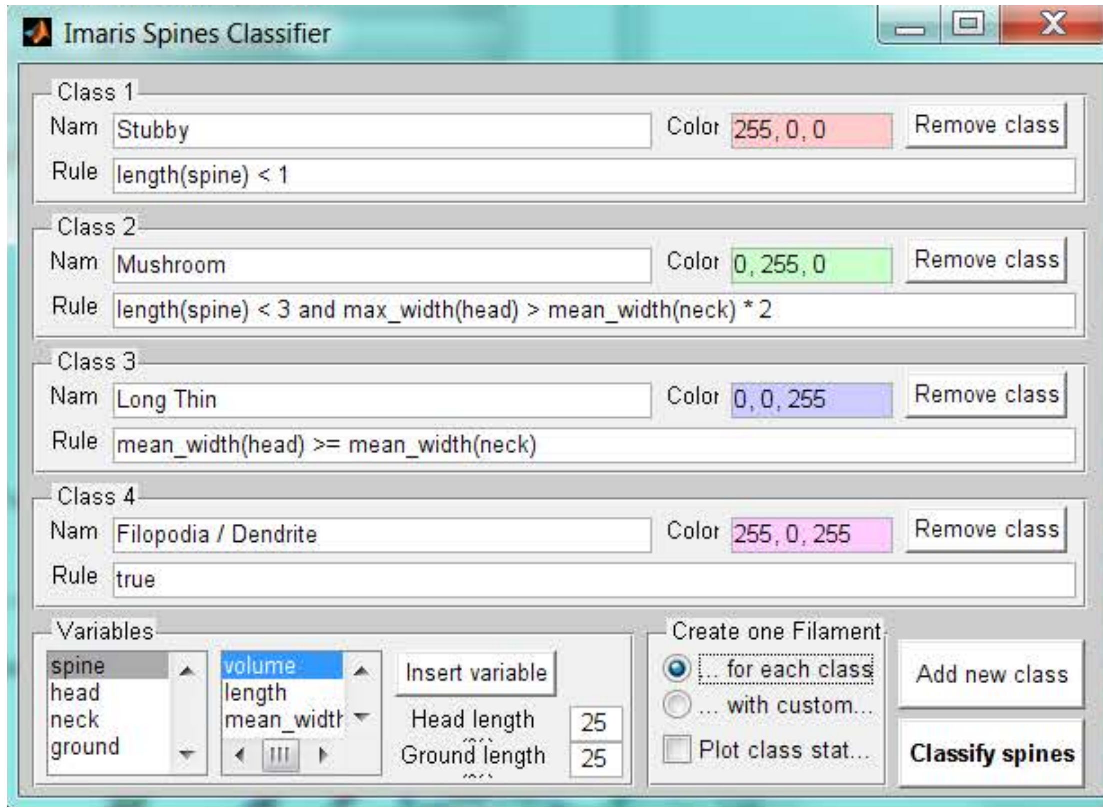
Filament Spines Classifier







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Filament Branch Hierarchy

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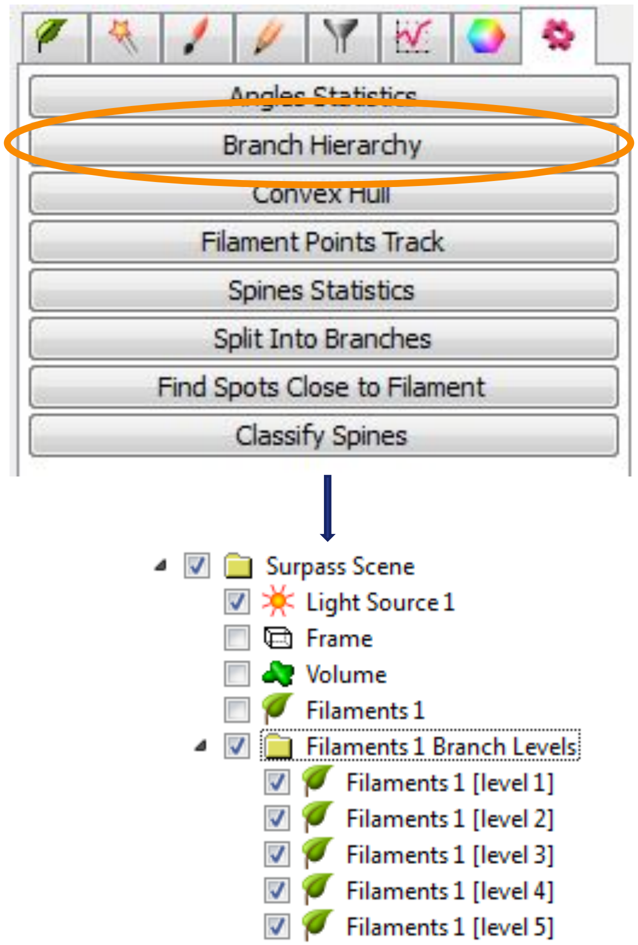

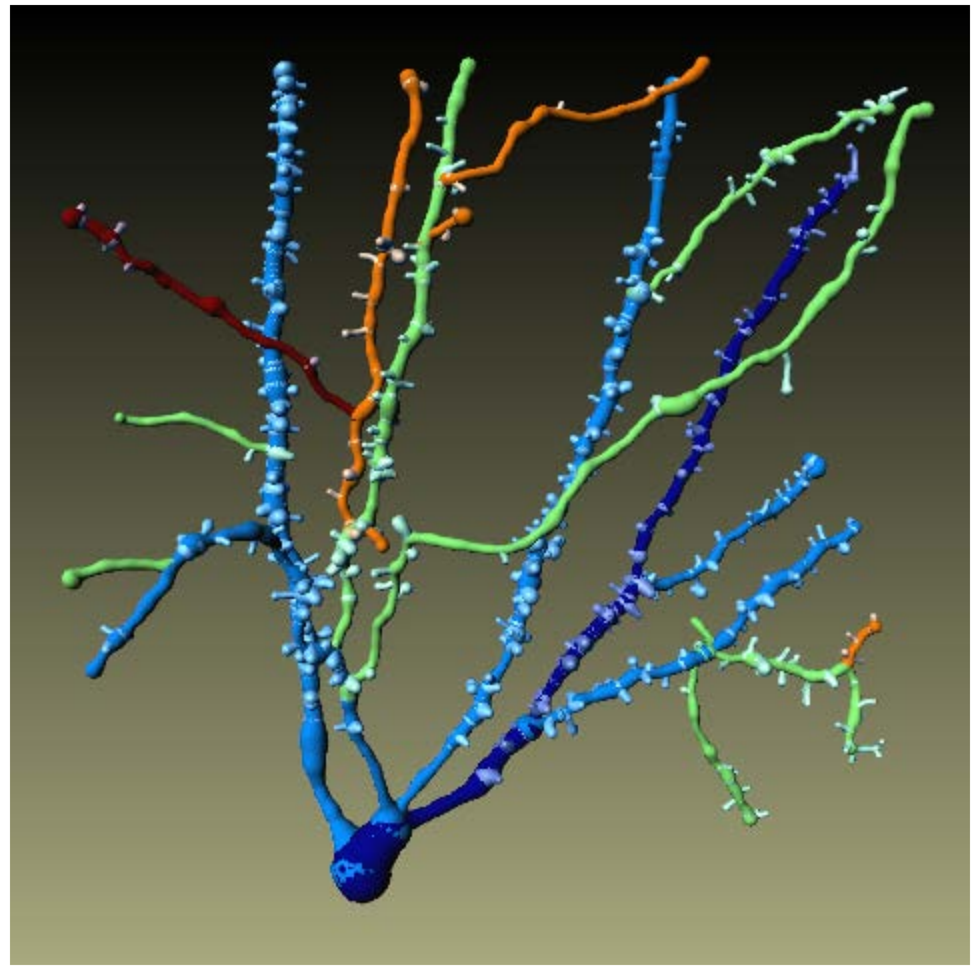

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Find Spots Close to Filament

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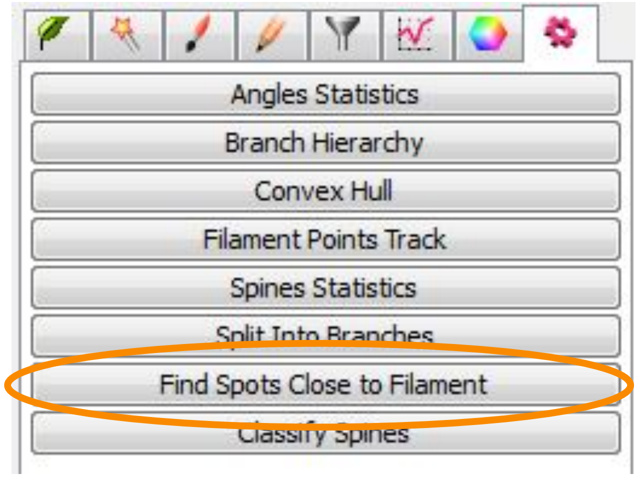

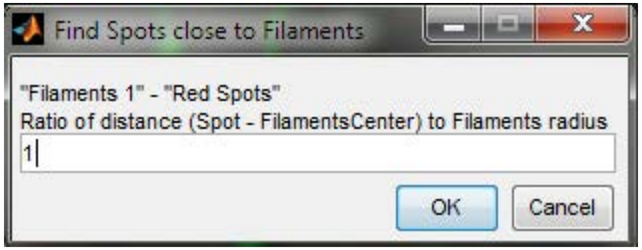

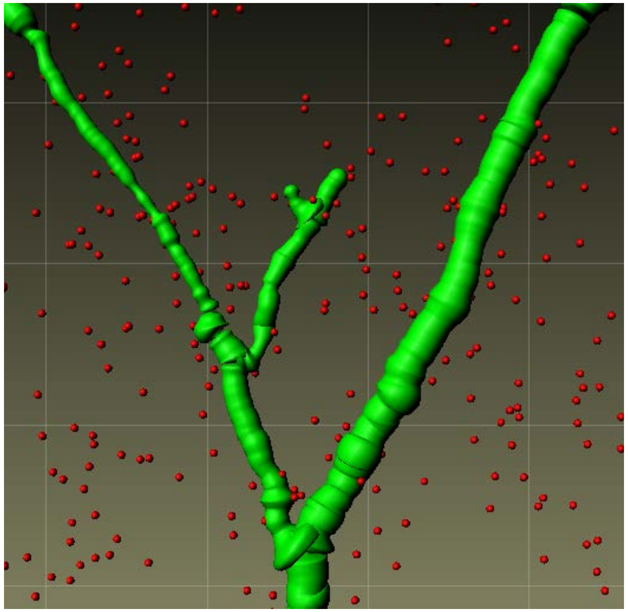

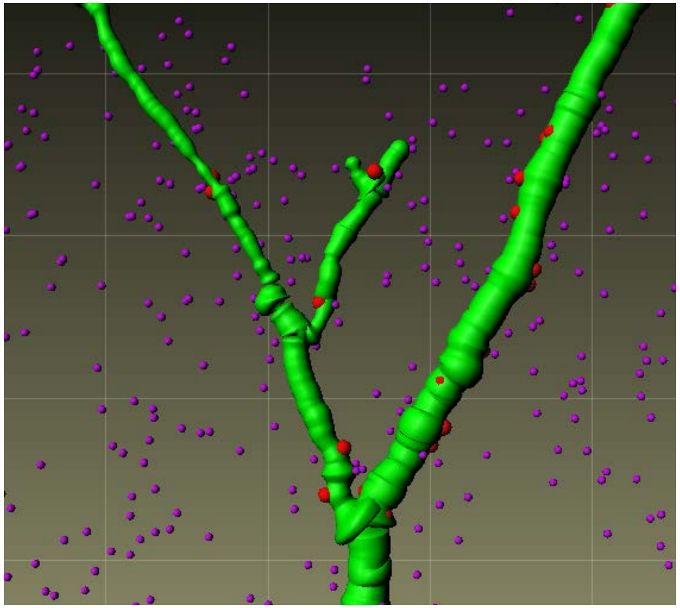

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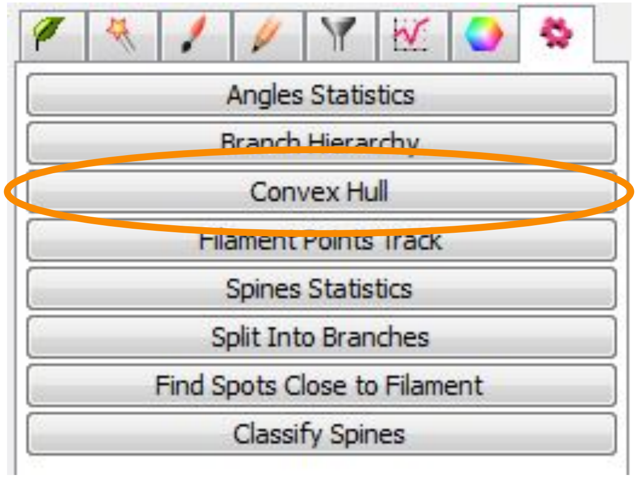
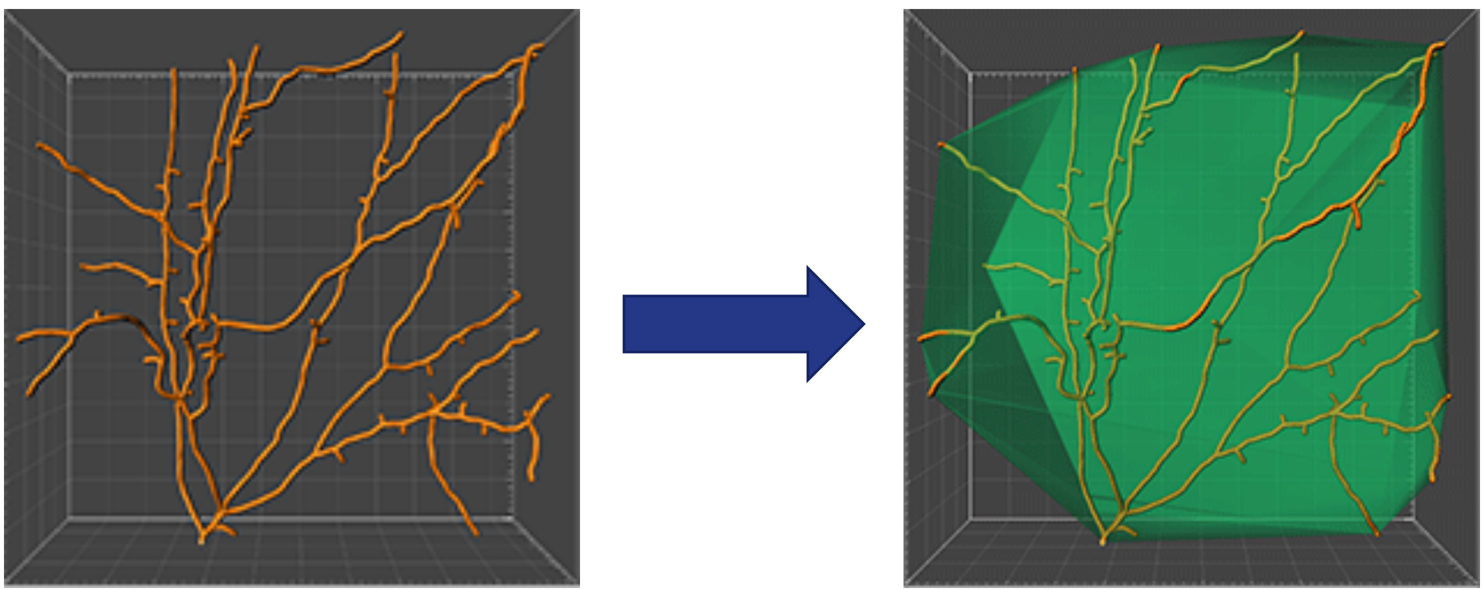
Filament Convex Hull



Find a minimal convex surface which includes all points of a filament. This is a reliable and robust measurement of dendrite spread.



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Excercise 1 (Classify Spines)

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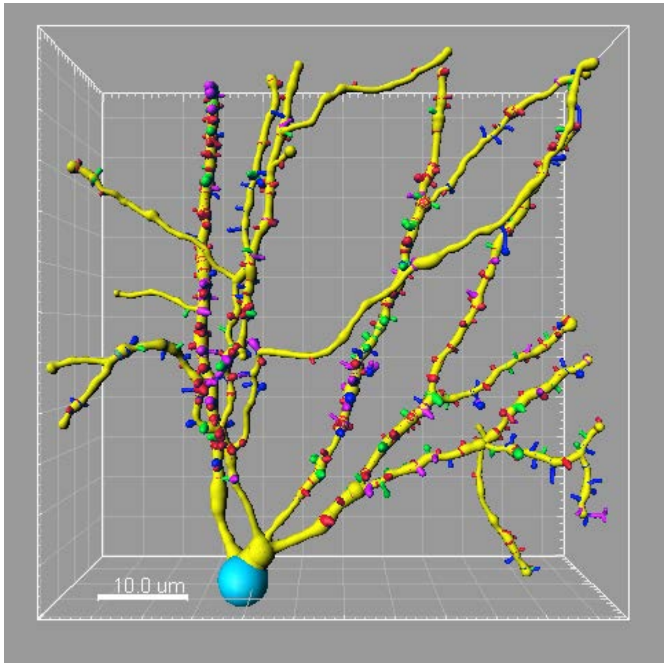
PyramidalCellRed.ims

1. Generate a Filaments object including Spines.

2. Use the Classify Spines XTension to get a plot of the number of spines in the different classes (option inside the XTension).

3. Display the shapes of the spines in Imaris

4. Do a second run to find stubby spines which are shorter than 0.8 µm



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Exercise 2

SR-BRC-nosoma.ims

1. Skip Automatic Creation

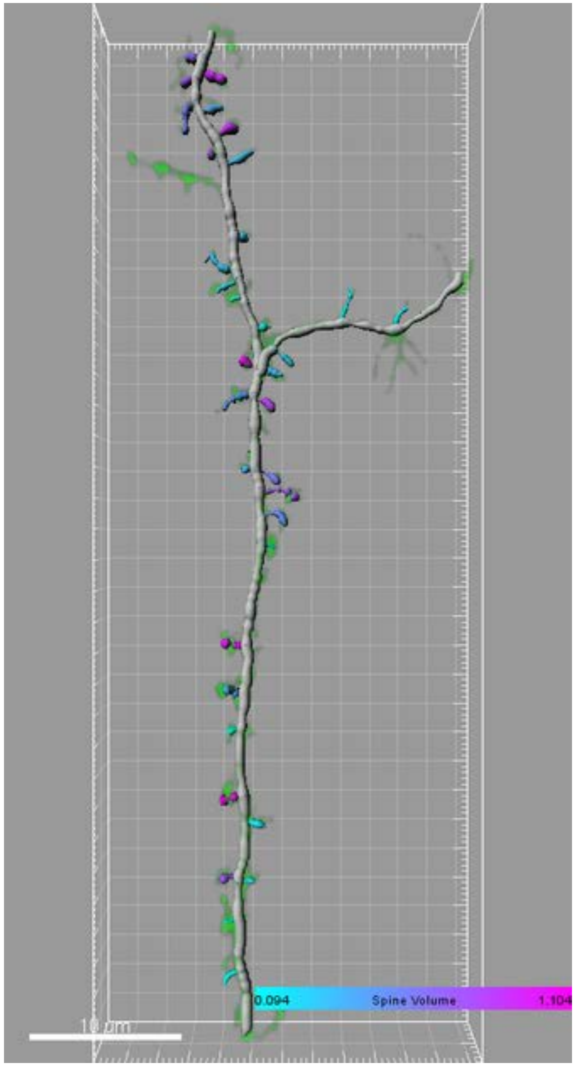
2. Use the AutoPath drawing mode to draw the dendrites

3. Use Rebuild *Dendrite* Diameter to detect the dendrite diameter (but not spines)

4. Go back to Draw tab, set dendrite you have drawn to the AutoPath starting point

5. Use Rebuild *Spine* Diameter to detect the diameter of the spines you have drawn

6. Color-code the Spines according to some statistic (e.g. Spine Volume)



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