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Neurolucida 360: Tree Segmentation

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ABSTRACT

3D neuron reconstruction using Neurolucida 360 software from MBF Bioscience.

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Set-up

- 1 Download and install Neurolucida 360 application (RRID:SCR_016788).

Neurolucida 360 v.2020.1.1 [↗](#)

by MBF Bioscience

Launch the application and set-up SciCrunch connection.

- 2.1 Go to <https://scicrunch.org/> and click REGISTER in the top menu. Fill out the registration form to create an account. From "My account", click API KEYS. Click GENERATE AN API KEY. Copy the key and return to Neurolucida 360.

- 3 Select the "SPARC Vocabulary Services" icon from the *Trace* ribbon.

- 3.1 Right-click to paste the API key in the dialog, then click **SET API KEY**.

Get Started

- 4 Open microscopy image data (2D or 3D) in the application.
- 5 When the SPARC Vocabulary Services dialog appears, select the appropriate items from the drop-down lists and enter the appropriate information regarding the subject to be segmented. Then select **BEGIN**.

5.1 Subject information.

Indicate the species, subject ID, sex, and age of the subject. All entered metadata will be stored o the XML data file. Independent from the cirteria for anatmoic terms selected. Note the "blinded to condition" options for sex and age.

5.2 Criteria for anatomic terms.

ORGAN: Select the organ term list you are interested in annotating with.

SPECIES: The species you are interested in annotating with.

PARCELLATION: The atlas parcellation scheme you are interested in annotating with. If no parcellation exists for the organ and species criteria, select *Species Independent* from the drop-down.

Neuronal Reconstruction: 3D Semi-Automatic Tracing Methods

- 6 In the 3D Environment window, click the **TREE** icon to display the Trace Trees panel.

- 7 Under TRACING MODE, select **USER-GUIDED**.

- 7.1 Select a TRACING METHOD: **Directional Kernels**, **Rayburst Crawl**, or **Voxel Scooping**.

Note: The three tracing methods are based on algorithms that work by tracing along the dendrites/axons one point at a time, and by producing three measurments at each point: (X,Y,Z) coordinate, thickness, estimated position of the next point.

- 8 Hover over the area of the process where you want to place the first point. Click to place the first point. A sphrere represents the first point. Continue to hover over the process. A series of circles is displayed, representing the path detected by the software. When you reach the end of the process, click to place the last point. Continue. Right-click once to end the branch. The traced branch is displayed in 3D.

- 9 To create a bifurcating tree, hover over an existing traced branch (branch A) at the location where the process bifurcates in the image data. A partial sphere appears on branch A (indicating a potential connecting point). Click to place the bifurcation point. Hover over the area of the bifurcating process and trace as you did in step 8.

Note: all tree branches can be traced in either direction, in any order.

Neuronal Reconstruction: 3D Automatic Tracing Methods

- 10 In the 3D Environment window, click the **TREE** icon to display the Trace Trees panel.

- 11 Under TRACING MODE, select **AUTOMATIC**.

- 12 Select a TRACING METHOD: **Directional Kernels**, **Rayburst Crawl**, or **Voxel Scooping**.

Note: The three tracing methods are based on algorithms that work by tracing along the dendrites/axons one point at a time, and by producing three measurements at each point: (X,Y,Z) coordinate, thickness, estimated position of the next point.

- 13 Click the **SHOW SETTINGS** button.

- 13.1 In the DISPLAY SEEDS tab, click the **DISPLAY SEEDS** button.

Optional: To change the seed color, use the color picker next to the HIDE SEEDS button.
To adjust the number of seeds, use the slider and the density buttons.

- 13.2 In the REFINE SEEDS tab, click the **VALIDATE SEEDS** button.

Optional: to decrease the number of unwanted seeds, increase the REFINE FILTER value.
Add or remove seeds manually.

- 13.3 In the TRACE tab, adjust the *SENSITIVITY*, *GAP TOLERANCE*, *REMOVE TRACES SHORTER THAN*, AND *CONNECT BRANCH SEGMENTS* settings. Then click the **TRACE** button to start the automatic trace.

Editing Trees

14 Selecting Trees.

To select a single tree, click the tree.
To select all the trees, click the SELECT ALL button.
To select several adjacent trees, press the CTRL key and draw a marquee around the trees of interest.
To deselect, click away from the selected object.

15 Selecting Points.

To select a single point on a tree, click the POINTS button then click the point of interest.
To select multiple, adjacent points, draw a marquee around the points of interest.
To deselect, click away from the selected options.

16 Connecting and Detaching Trees.

Connecting

1. Zoom in and rotate as needed.
2. Click the CONNECT button. Trees are now represented by a series of points along colored lines.
3. Click and drag an endpoint toward the area you want to connect to. The endpoint briefly turns green and a green

line appears.

4. Release the mouse button to connect the branches.

Detaching

1. Click a tree to select it.
2. Click the DETACH button.
3. Click on the tree to detach.

- If you click a single branch, the tree is split in two.
- If you click a bifurcation, the tree is split in three.

17 Deleting trees

1. Click a tree to select it or click the SELECT ALL button.

Press the DELETE key or click the REMOVE button. You can't undo this action!

18 To save the data file, select **File > Save As** and select **Data File**. Save your data file as an XML Document File (*.xml).

