![Neurolucida 360 logo] **Neurolucida 360**: Trace neurons in 3D with user-guided and smart manual tracing

In this tutorial, you will follow these steps:

A. Load an image stack in 3D.

B. Detect the soma.

C. Trace trees with the user-guided mode.

D. Trace trees manually (no bifurcation).

E. Trace a bifurcating tree.

F. Edit trees.

G. Save your work.
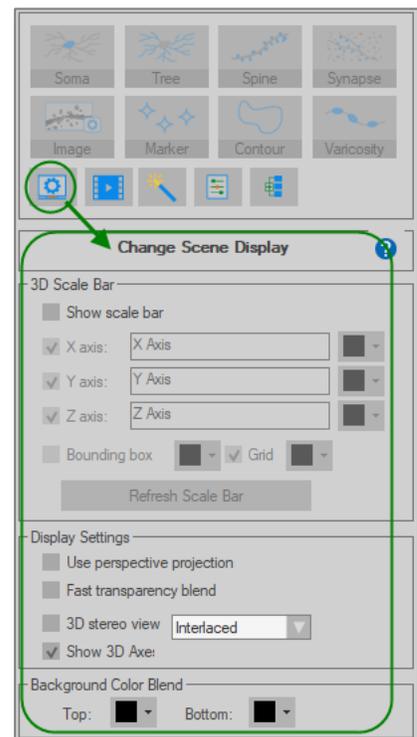
H. Analyze in Neurolucida Explorer

A. Load a demo image stack in 3D

Before loading, the **Scene** panel is active.

1. Click the **Open Image File** button in the toolbar.

2. The **Image Stack Open** window appears with the Neurolucida 360 folder already selected. Select the demo file "MBF_NeuronTracing.jpx" from the list.
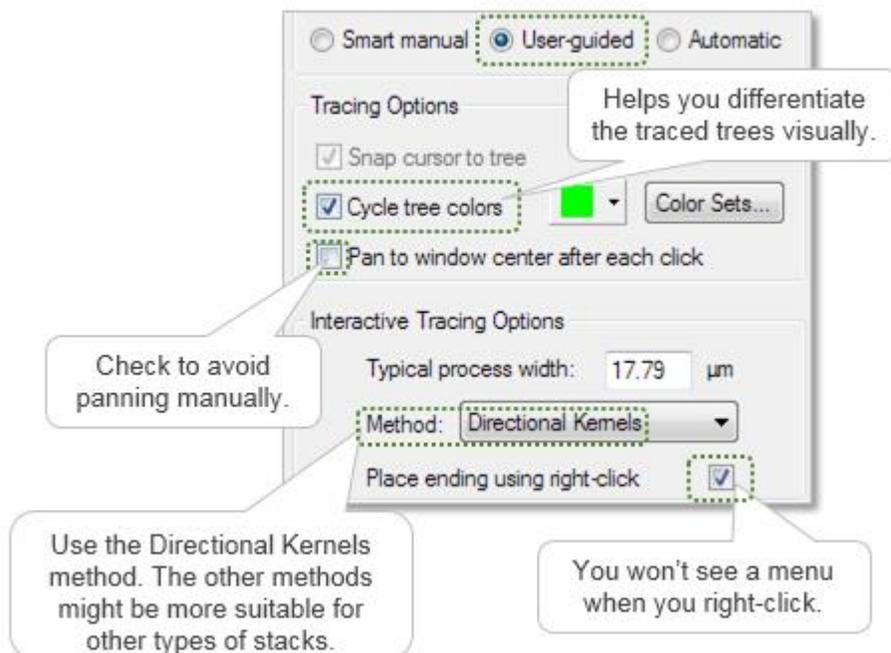
B. Detect the soma

1. Click the Soma button to display the **Detect somas** panel.

2. Resize your cursor to match the size of the soma (hold CTRL and scroll the mouse wheel).

3. Click the soma to model it.

C. Trace trees with the user-guided mode

1. Click the Tree button to display the **Trace Trees** panel.

2. Under **Tracing Mode**, select **User-guided**.

Helps you differentiate the traced trees visually.

Check to avoid panning manually.

Use the Directional Kernels method. The other methods might be more suitable for other types of stacks.

You won't see a menu when you right-click.

3. Zoom in (with the mouse wheel) and pan (holding **SHIFT** down and dragging) until you get a clear view of the branch that we will start with. We'll be tracing from the soma toward an ending.

Start here.

Trace from the soma.
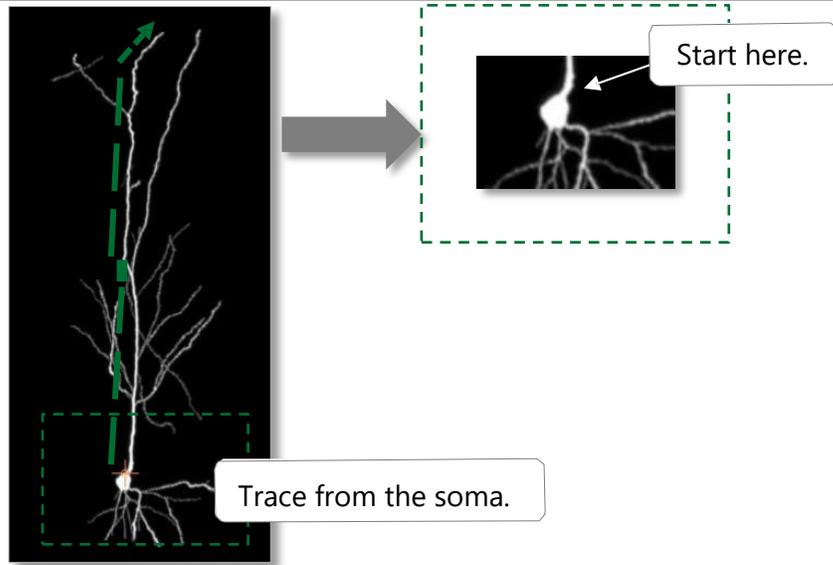
4. Hover over the area where you want to place the originating point.

You should see a red cursor and a circle:

- The red cursor indicates where your mouse cursor is.
- The circle indicates software targeting (i.e., where the software detects the process)—the color of the circle is the color of the tree being traced.

5. Click to place the originating point; the originating point is placed where the targeting cursor (the circle) was displayed. It is represented by the orange sphere.
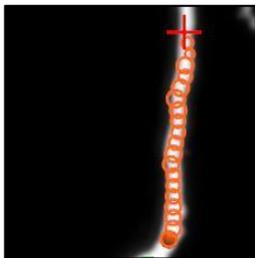
As you hover over the process, the software displays a series of circles indicating the detected path and thickness for the branch.

6. Click to place the next point.

- The software models process thickness automatically while you click to place points.

- If you are satisfied with the path determined by the software, place only one point, the end point.

- As you trace in 3D, your tracing appears in 2D in real time.
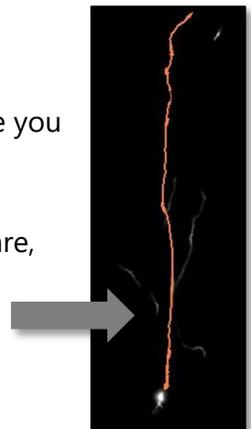
TIP: There is no need to mark the bifurcations; continue to trace the current branch as shown and ignore the bifurcation. You'll learn how to connect branches in **Step 8**.

7. When you reach the end of the branch, end it:

   a. Click to place the last point.

   b. Right-click once to indicate the end of the branch.

   If **Place ending using right-click** is checked in the panel, you won't see a menu.
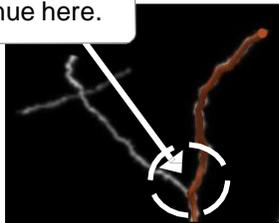
TIP: How can I be sure that I ended the branch?

   •   When hovering over the branch, you see wireframed partial spheres along the branch.

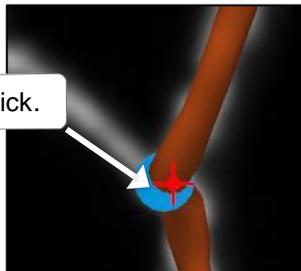   •   The tree count in the right panel has been updated to 1, instead of 0.
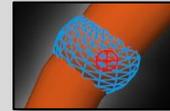
   Trace Trees (1 tree)

8. Trace the branch that connects to the branch you just traced:

   Continue here.

   a. Click this button to return to the original orientation.

   b. Zoom and pan until you see the bifurcation.

   c. Hover the mouse over the bifurcation point and notice the blue partial sphere.

   d. Click at the bifurcation to place the first point.

   e. The cursor color changes to the color of the initial branch, indicating that the branches are connected and part of the same tree.

   Click.

   f. Continue hovering until the end of the branch.

   g. Click to place the last point.

   h. Right-click to end the branch.

4

9. Trace the other branches connected to the apical dendrite following the same method (tracing toward the processes endings).

Next, for an overview of smart manual tracing, we'll trace the basal processes. First we'll trace a simple tree (process 1), then we'll trace a tree with bifurcation (processes 2 and 3).
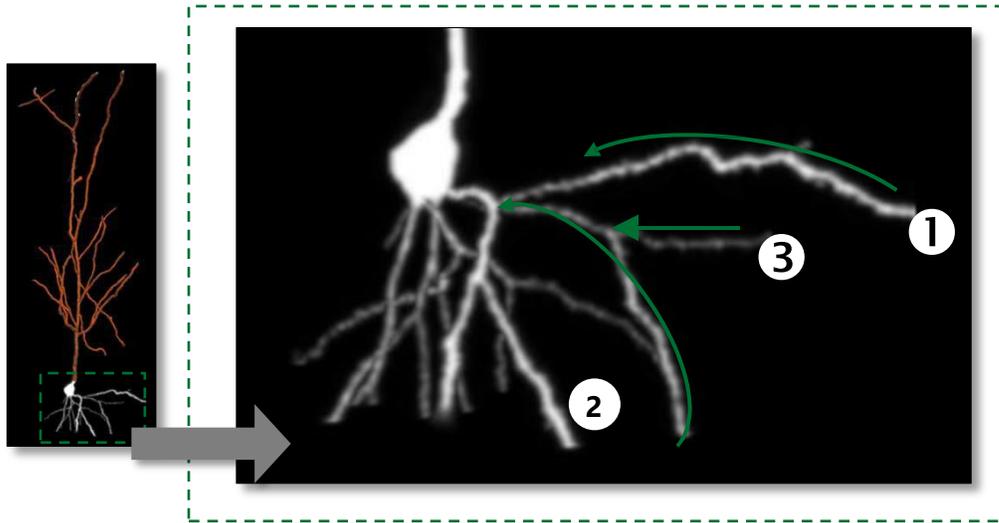

D. Trace a tree manually (no bifurcation)

1. Zoom in (with the mouse wheel) and pan (holding down SHIFT and dragging) until you get a clear view of the soma and the basal processes.

2. Under **Tracing Mode**, select **Smart manual**.

   Under **Tracing Options:**

   - Check **Snap cursor to tree** to trace with 2 cursors:

     The red cursor indicates the location of your mouse pointer.
     The circle indicates targeting (i.e., where the software detects the presence of a process). When you click, the software places a point at the center of the circle.

   - Optional: Check **Pan to window center** to avoid panning.
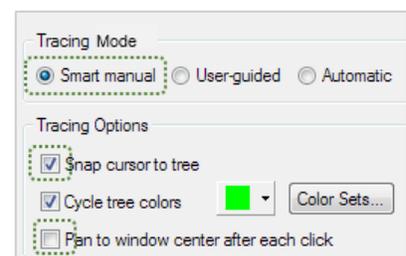
3. Trace process 1 partially. End it before you reach the bifurcation.
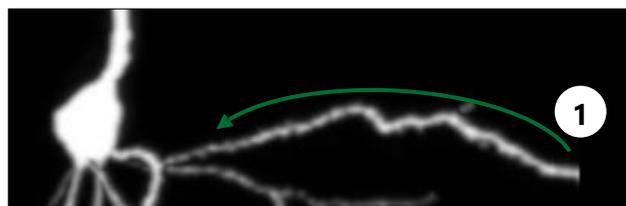
a. Zoom in as needed and, before placing the first point, adjust the thickness.

Unlike 2D tracing, there is no need to focus.

    i.   Hover over the starting point.

    ii.   Hold down CTRL and scroll the mouse wheel until the circle on the cursor matches the apparent process diameter.

b.   Click to place the first point. A solid sphere indicates the first point.

c.   Hover over the next point, adjust the thickness, and click to place the point. The software displays the thickness for the segment. Repeat for each point until you get close to the bifurcation.

d.   End the branch:

    i.   Click to place the last point.

    ii.   Right-click and select Ending from the right-click menu.

        When you hover over the branch, you see a series of partial spheres around the branch, indicating that the branch was ended (only visible if **Snap cursor to tree** was selected).
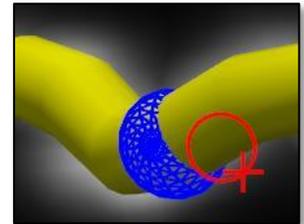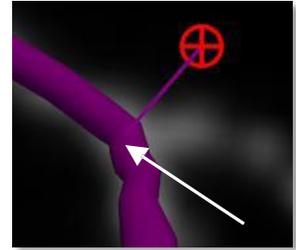
E. Trace a bifurcating tree.

1.   Trace process 2.

    a.   Adjust the thickness and click to place the first point.

    b.   Hover over the next point, adjust the thickness, and click to place the point. Repeat for each point until you reach the bifurcation.

    c.   At the bifurcation, right-click and select **Bifurcating node**.

    d.   Continue placing points until you reach the next bifurcation.

    e. Click to place the last point before the bifurcation then right-click and select **Ending** to end the tree.
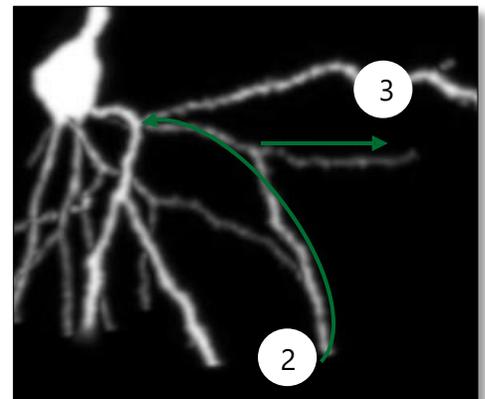
Neurolucida re-directs you to the bifurcating node from which you can start tracing (when you move the mouse, you see a rubber-band line between the cursor and the node).

2. Trace process 3, starting at the node.

   a. From the node, adjust the thickness and click to place the first point.

   b. Hover over the next point, adjust the thickness, and click to place the point. Repeat for each point until you reach the outer edge of process 3.

   c. Click to place the last point then right-click and select **Ending** to end the tree. Branch 2 and 3 are now connected.
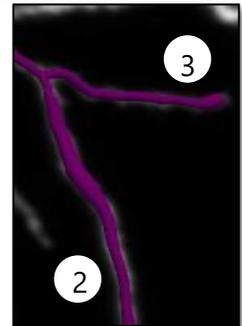
F. Edit trees

First, we'll verify that the origins of the trees are set correctly for branch ordering. Then, we'll connect and detach some branches. Finally, we'll correct a bend.

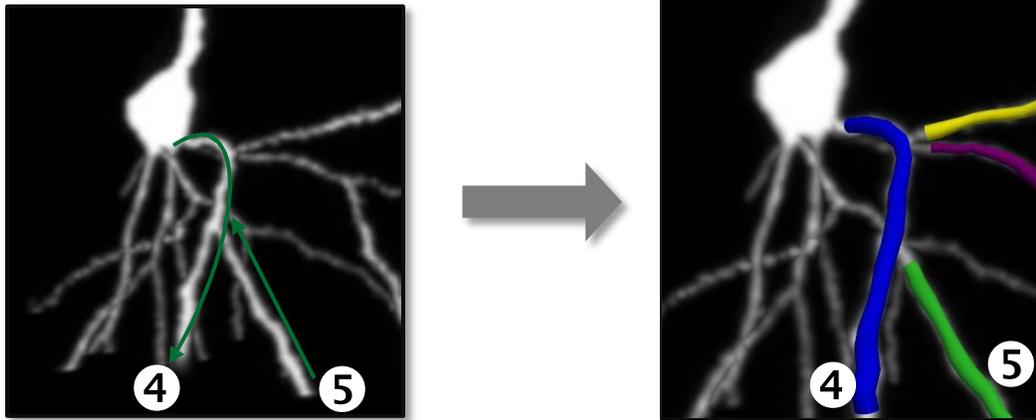1. Click the **Edit** button to display the **Edit Trees** panel.

2. Verify the origin of a tree:

   a. Click the traced apical dendrite to select it.

   b. Click the **Points** button to display the **directionality** option.

   c. Zoom in until you can see the origin point then click the origin point. It is shown in green.

   d. Click the button **Set point as origin** if the desired point is not already defined as the origin point.

   e. Repeat steps a-d for the basal trees.

   f. Click the **Points** button again to turn off the **Edit Points** mode.
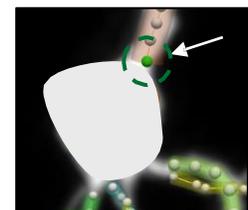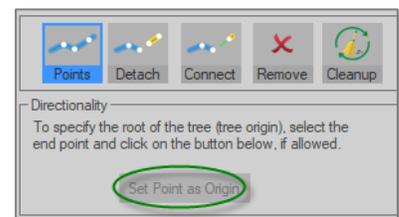
3. Connect branches.

Let's trace processes 4 and 5 with the interactive method and connect them manually.

a. Click the **Tree** button to display the **Trace Trees** panel again and select the **User-guided mode.**

b. Trace process 4 inward toward the soma (ignoring the bifurcation).

c. Trace process 5 inward toward the soma and end the branch shortly before the bifurcation.

d. Click the **Edit** button to display the **Edit Trees** panel.

e. Click the **Connect** button. The **Points** button is automatically selected and branches now display as a series of points along colored lines.

f. Click the end point on the green branch and drag toward the blue branch. The end point on the green branch briefly appears.

g. Drag to the point on the blue branch that you want to connect to.You should see a green line between the points you want to connect.
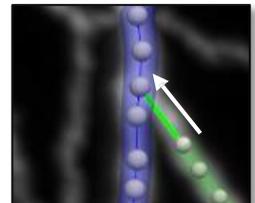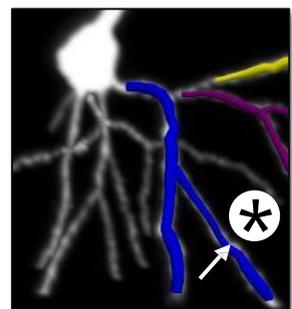

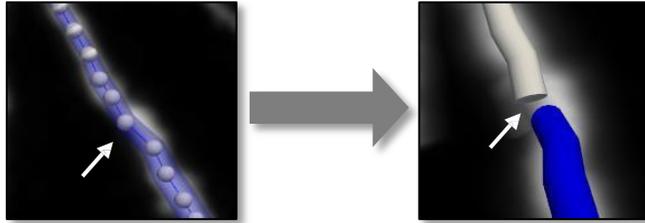h. Release the mouse to connect. Both branches are now blue, indicating that they belong to the same tree.

4. Detach branches: We're going to detach at the "star."

a. With the **Points** mode still selected, click the **Detach** button.

NOTE: You can also detach without selecting the Edit **Points** mode.

b. Click the point where you want to split the branch. Two segments are now displayed.

c. Click the **Points** button to disable the **Edit Points** mode and the **Detach** button to turn off the **Detach** mode.

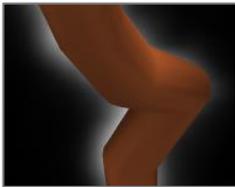5. Correct a bend: We're going to correct a bend that wasn't traced with enough accuracy.

a. In the **Edit Trees** panel, click the **Points** button.
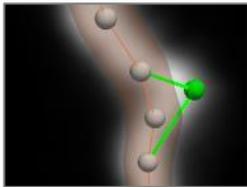
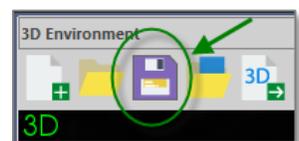b. Click and drag the point you want to move into the bend that wasn't closely traced.
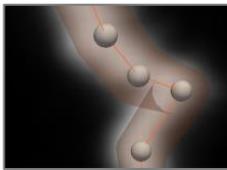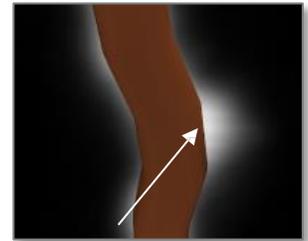
c. Release the mouse button. The point has been moved.

d. Click the **Points** button again to turn the functionality off.

G. Save your work.

Click the **Save** icon in the toolbar to save the data file.

H. Analyze in Neurolucida Explorer.

Click the button cicled in green to open the data file directly into Neurolucida Explorer.