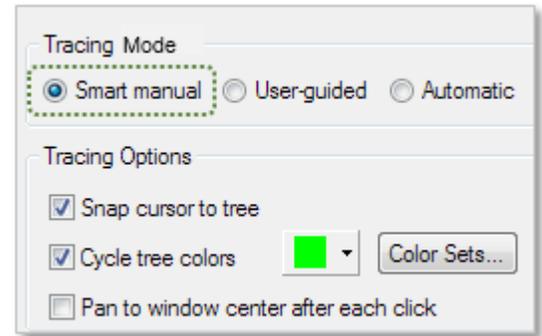


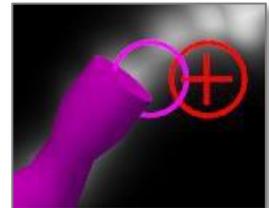
What you will learn

- Tracing a tree (no bifurcation)
- Tracing a bifurcating tree (method 1)
- Tracing a bifurcating tree (method 2)
- Resuming the tracing of a branch
- Switching to user-guided tracing



Tracing a tree (no bifurcation)

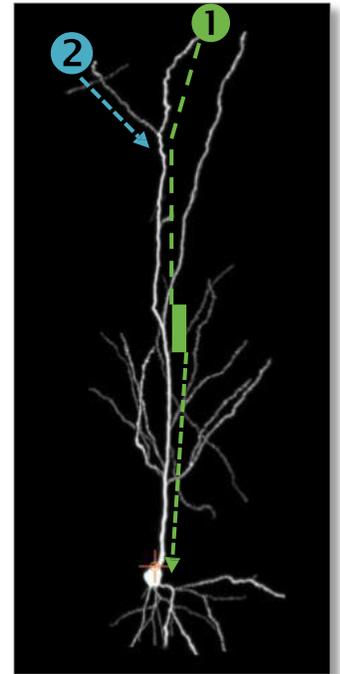
1. With the desired image stack already opened, click the **Tree** button. The **Trace Trees** panel is active; the tracing method selected is **Smart manual**.
2. **Optional:** Under **Tracing Options**, check **Snap cursor to tree**.
 - Useful to easily connect branches while tracing (See **Tracing a bifurcating tree—Method 1**).
 - Two cursors are visible: the red cursor (crosshair in a circle) indicates the location of your mouse pointer; the circle indicates targeting (i.e., the software suggests a location in the image where it detects the presence of a process).
3. **Optional:** Under **Tracing Options**, check **Pan to window center** to avoid manual panning when zoomed in.
 - To pan manually, hold down **SHIFT** and drag.
4. Zoom in by scrolling the mouse wheel until the magnification is suitable for manual tracing.
5. Hover over the starting point of the process and record the thickness.
Unlike 2D tracing, there is no need to focus.
 - To adjust thickness, hold the **CTRL** key and scroll the mouse wheel until the circle of the red cursor matches the apparent process diameter.
6. Click to place the starting point. A sphere represents the starting point.
7. Hover over the next point, adjust the thickness, and click to place the next point.
 - To undo the last click, press **CTRL-Z**.
8. Repeat step **6** for each point.
9. Click to place the last point.
10. Right-click and select **Ending** to end the tree.



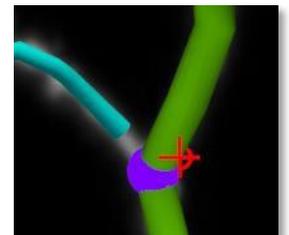
Tracing a bifurcating tree—Method 1

In this example, we trace process 1 (green) then process 2 (blue), and we finally connect 1 and 2.

1. Under **Tracing Options**, select **Snap cursor to tree** and **Cycle tree colors**.
Cycle tree colors automatically selects a new color each time you start a new tree.
2. Trace branch 1:
 - a. Hover over the area at the end of the process where you want to place the first point.
 - b. Adjust the thickness and click to place the first point.
 - c. Hover over the next point, adjust the thickness, and click to place the point. Repeat for each point until you reach the bifurcation.
 - i. To undo the last click, **press Ctrl-Z**.
 - d. As you reach the bifurcation, ignore it— You will connect the branches in step **3-c** —and keep placing points until the process meets the soma.
 - e. Click to place the last point.
 - f. Right-click and select **Ending** from the menu.



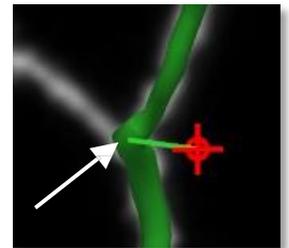
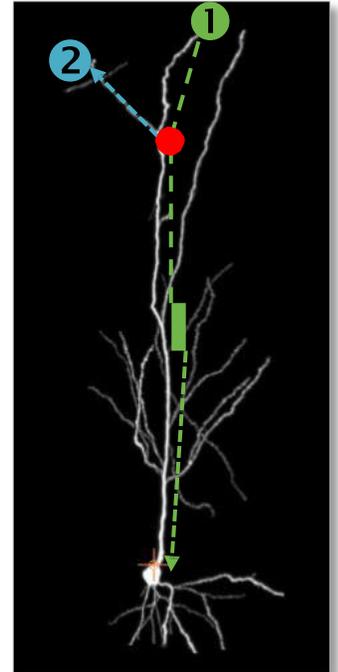
3. Trace branch 2 and connect to branch 1.
 - a. Start from the outer edge of the process and place points until you reach the bifurcation.
 - b. At the bifurcation, hover over branch 1. A partial sphere (purple here) appears on branch 1, indicating a potential connecting point.
 - c. Click to connect branch 1 and branch 2.
 - Notice that the branches are now the same color.
 - There is no need to end branch 2. It is automatically done by connecting to branch 1.



Tracing a bifurcating tree—Method 2

In this example, start tracing process 1 (green), mark the bifurcation, continue tracing process 1, then trace process 2 (blue) starting from the bifurcating node.

1. **Optional: Snap cursor to tree** is checked.
2. Zoom in and pan as needed.
3. Trace branch 1:
 - 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 end of the process.
 - e. Click to place the last point then right-click and select **Ending** to end the tree. NeuroLucida re-directs you to the bifurcating node (red here) from which you can start tracing (when you move the mouse, you see a rubber-band line between the cursor and the node).
4. Trace branch 2:
 - 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 2.
 - c. Click to place the last point then right-click and select **Ending** to end the tree. Branch 1 and 2 are now connected.



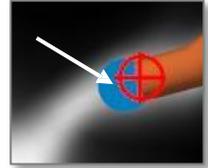


Resuming the tracing of a branch

In this example, the orange branch was traced earlier and you realize that you missed a portion of the process. You resume tracing to include the last portion.



1. In the **Tree** panel, the **Snap cursor to tree** box is checked.
2. Hover over the end of the branch until you see a sphere marking the end.
3. Click the sphere to resume tracing.
4. Click to place the last point.
5. Right-click and select **Ending** to end the branch.



Switching to user-guided tracing

Right-click in the tracing window and select **User-guided Tracing**.