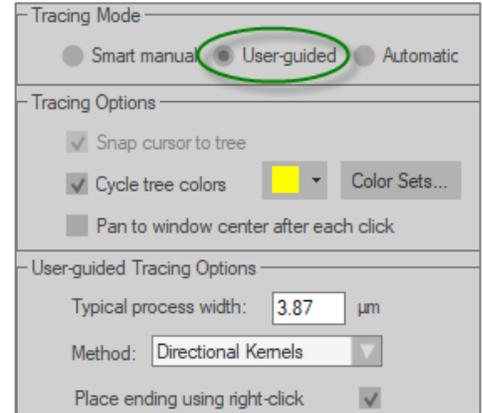## Before you start

- You already loaded the correct image stack.
- Click the **Tree** button to display the **Trace Trees** panel. Under **Tracing Mode**, `select` **User-guided**.

    > **Typical process width**: As detected by the software.

    > **Method**: If **Directional Kernels** doesn't seem to detect as accurately as you would expect, try the other methods.

You will learn to…

- Trace a tree (no bifurcation)
- Trace a bifurcating tree
- Abort new trees
- Resume the tracing of a branch
- Switch to smart manual tracing
- Check the accuracy of the tracing

Trace a tree (no bifurcation)

1. Optional: Zoom in by scrolling the mouse wheel and select **Pan to window center after each click** to avoid panning manually (to pan manually, hold down **SHIFT** and drag).

2. Hover over the area of the process where you want to place the first point. When zoomed in, you see:

    - A red cursor indicating the location of your mouse pointer.
    - A circle indicating targeting (i.e., where the software detected the process); the diameter of the circle corresponds to the detected diameter of the process at that point.

3. Click to place the first point. A sphere represents the first point.

4. Hover over the process. A series of circles is displayed, representing the path detected by the software.

5. Keep hovering until you reach the end of the process then click to place the last point. The traced branch is displayed in 3D.
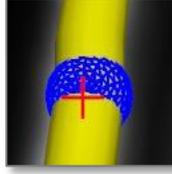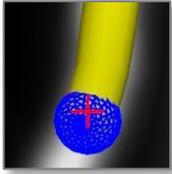
    You may need to place several points on the process to improve the accuracy of the tracing.

6. Right-click once to end the branch.
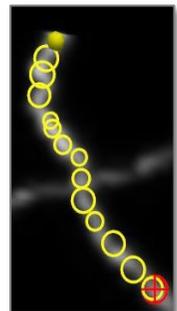
If you checked **Place ending using right-click**, there is no right-click menu.

When the branch is ended:

▸ You can see a sphere at the end of the branch or partial spheres along the branch as you hover.

▸ The tree count is updated. Trace Trees (1 tree)

TIP: To undo your most recent tracing action before you ended the tree, press **CTRL-Z**.

To see the right-click menu, uncheck **Place ending using right-click**.

Trace a bifurcating tree

In this example, trace process 1 (red), then trace process 2 (orange), and finally connect 1 and 2. Trace from end of process toward soma.

Under **Tracing Options**, keep **Cycle tree colors** selected to easily visualize potential connections between branches.

Cycle tree colors automatically selects a new color each time you start a new tree.
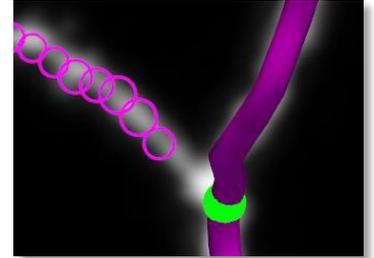
1. Trace branch 1:

    a. Hover over the area at the end of the process where you want to place the first point.

    b. Click to place the first point.

    c. Hover over the process to see the detected path.

    d. As you reach the bifurcation, ignore it— You will connect the branches in step **3.d** —and keep hovering until the process meets the soma.

    e. Click to place the last point.

    f. Right-click once to end the branch.
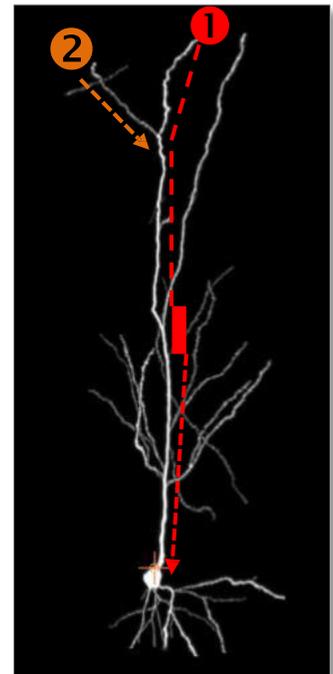
2. Trace branch 2 and connect to branch 1.

    a. Place the first point at the end of the process then hover over the process until you reach the bifurcation.
    b. At the bifurcation, a partial sphere appears on branch 1 (indicating a potential connecting point) and the series of circles changes color to reflect the color of branch 1.
    c. Hover over the bifurcation area until you see a partial sphere where you want to place the bifurcation point.
    d. Click to connect branch 1 and branch 2.

        -   Notice that the branches are the same color.
        -   There is no need to end branch 2. It is automatically done by connecting to branch 1.

Abort new trees
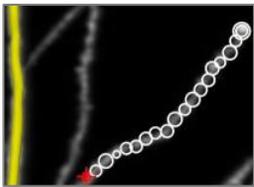
To abort a tree started independently of an already traced tree, right-click OR press **CTRL-Z**.
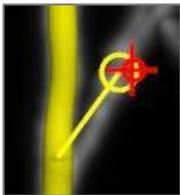
To abort a tree started from another tree, right-click AND press **CTRL-Z**.

Resume the tracing of a tree

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. Hover over the end of the branch until you see a sphere marking the end.

2. Click the sphere to resume tracing.

3. Click to place the last point and right-click once to end the branch.

Switch to smart manual tracing

Right-click and click the check mark to end the user-guided tracing mode.

Check the accuracy of the tracing

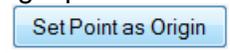There are two aspects you can verify: directionality and node placement.

1. **Directionality**

   For proper branch ordering, specify the tree origin (directionality):

   a. Click the **Tree** or **Edit** button to display the **Edit Trees** panel.
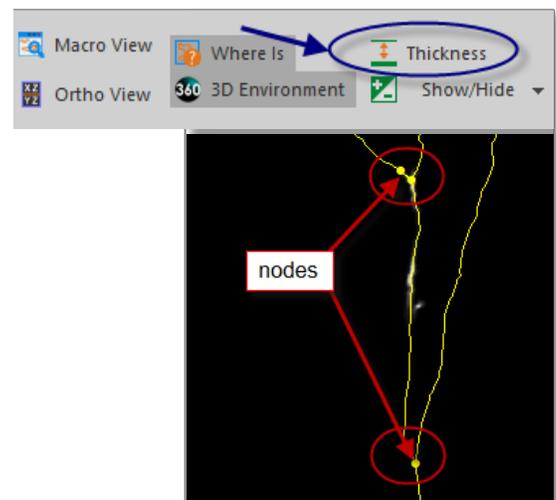
   b. Click the button

   c. Click the desired origin point on the tree (it must be an end point).

   d. Click the button   Set Point as Origin
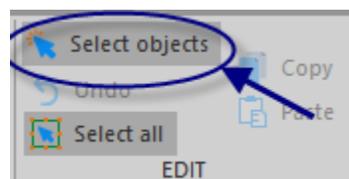
2. **Nodes**

   To view and correct node placement, leave the 3D environment and use the standard editing options available from the **Trace** ribbon:

   a. Click **Thickness** to turn it off. Nodes are displayed as solid circles.

   b. Click **Select Objects** to display the **Edit Tool** panel.

   c. In the **Edit Tool** panel, check **Individual Points**.

   d. Click the node you want to delete to select it.

   e. Right-click and select **Eliminate node**.

   f. Return to the 3D environment to resume tracing.