

You will follow these steps:

- A. Open an image stack.
- B. Trace the dendrite with the user-guided mode.
- C. Detect the spines automatically.
- D. Classify the spines automatically.
- E. Merge, split, re-color, and delete detected spines.
- F. Re-classify individual spines.
- G. Detect individual spines manually.
- H. Confirm a complex spine model.
- I. Save your work.
- J. Analyze in Neurolucida Explorer

A. Open a demo stack in the 3D environment.

Before opening files, the **Scene** panel is active.

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



2. The Image Stack Open window appears with the NL 360 folder already selected. Select the demo file 10_M415-1-1_S1C4_Seg3_Decon.jpx from the list.





B. Trace the dendrite with the user-guided mode

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

Smart manual is the default.

- 2. Select **User-guided** to change the tracing method and check **Pan to window center** if you don't want to pan manually.
- **3.** The **Typical process width** is determined by the software.
- 4. Use the **Directional Kernels** method.
- 5. Keep Place ending using right-click checked.

Trace Trees (0 trees)			
Tracing Mode			
Smart manua User-guided Automatic			
Tracing Options			
✓ Snap cursor to tree			
✓ Cycle tree colors			
an to window center after each click			
User-guided Tracing Options			
Typical process width: 0.10 µm			
Method: Directional Kernels			
Place ending using right-click			

6. Zoom in (scrolling the mouse wheel forward) and pan (holding down **Shift** and dragging) until the view is suitable for tracing. Hover over a suitable starting point on the left of the dendrite.



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 dendrite)—the color of the circle is the color of the tree being traced.
- **7.** Click to place the starting point. The starting point is marked by a solid sphere and displayed in the window center.

As you hover over the dendrite, the software displays a series of circles indicating the detected path and thickness for the tree.

8. Click to place the next point on the tree. Notice that thickness is displayed (the software automatically determines the thickness) and that the last point clicked in displayed in the window center.



- 9. Rotate (by dragging) to examine the dendrite as you continue to place points.
- 10. When you reach the end of the tree, right-click once to end the tree. There is no menu when you right-click unless you unchecked **Place ending using right-click**.



To verify that you ended the tree, hover the mouse; you should see a sphere at the ending, and partial spheres along the branch:



11. Click the icon



to view the traced tree in the original orientation of the stack.



C. Detect the spines automatically

1. Click the **Spine** button to switch from **Tree** mode to **Spine** mode.



2. The **Detect Spines** panel is now active. Click the **Detect All** button to detect spines with the default settings.



You should see colored spines along the traced dendrite.

- **3.** Adjust the detection settings to refine spine placement—Our assumption is that the tracing of the dendrite is accurate.
 - a. If a spine appears to spread too much over the branch, increase the Minimum height value. Click
 Detect All again to evaluate the adjustment.
 - b. If spurious spines not connected to the traced dendrite are detected, decrease the **Outer range** value. Click **Detect All** again.

Detection Outer range:	2.5
Minimum height:	0.3 Jun
Detector sensitivity:	100 🗘 %
Minimum count:	10 voxels





Show axial smear correction displays the corrected thickness of the tree. Axial smear is automatically calculated after each detection. "Showing axial smear correction" only applies to the display; it doesn't affec t the data.

D. Classify spines automatically

In the **Detect Spines** panel, click the **Classify All** button.

Spines are classified according to four types (filopodium, mushroom, stubby, thin) based on the default settings. The spines are color-coded and each color corresponds to one type.

- To modify the default settings, click the **Settings** button.
- To change the classification colors, click the colors drop-downs.
- To classify manually, use the **Edit Spines** panel.

E. Re-color, merge, split and delete spines

- 1. Click the **Edit** button to display the **Edit Spines** panel.
- 2. Change the color of a single spine.
 - a. Click a spine to select it. Additional options are now visible and the selected spine is overlaid with a wireframe.
 - b. Choose a color from the **Color** drop-down menu.
 - c. Click away from the traced spines to see the tracing without the wireframe.

Classification Keep existing classifications Classify All Settings... Automatic Classification Colors: Mushroom: Stubby: Filopodia:





3. Merge two spines close to each other into a single spine.

You might do this when you notice that the spine detector incorrectly detected two spines instead of a single spine.

- a. Zoom in (scrolling the mouse wheel) and select two spines to merge: Hold CTRL down and click each spine to be merged OR press CTRL and drag the mouse to draw a marquee around the two spines (you can only merge two spines at a time).
- b. Click the **Merge** button.





4. Split a spine.

You would do this when you notice that the spine detector incorrectly detected one spine instead of two spines that are in close proximity.

- a. Click a spine to select it.
- b. Click the **Split** button.
- **5.** Delete a spine.
 - a. Click a spine. The spine is overlaid with wireframe.
 - b. Press the **Delete** key or click the **Remove** button.

F. Re-classify a spine manually

1. The **Edit Spines** panel is displayed. Click a spine to select it.

The type assigned via automatic classification is displayed next to **Type**.

2. Select another type from the **Type** drop-down menu.

G. Detect individual spines manually

- 1. Click the **Edit** button to display the **Detect Spines** panel again.
- 2. Click a spine that you previously deleted. The spine is overlaid with a solid color, indicating that it has been detected.







By clicking to detect a spine, you are overriding the **Minimum height** and **Minimum count** settings.

- **Outer range**: Adjust the maximum distance between the surface of the tree and the potential top of the spine head.
- **Detector sensitivity**: Adjust to identify spines that might be too light or too dark based on • the original image intensity.

Туре:	stubby	•
Show as:	none thin	
Transparency:	mushroom	_
Smooth:	filopodia branched	
Texture:	detached other	





H. Confirm a complex spine model

- 1. Click the **Edit** button to display the **Edit Spines** panel again.
- 2. Click a complex spine to select it.
- 3. In the panel, click the **Points** button. The spine is now represented by five points.
- **4.** To change the spine head position, click and drag the point representing the head (second point from top of the spine). You may also move the two points representing the spine neck.



I. Save your work.

Save the data file.

- a. Click the **Save** button in the toolbar.
- b. In the **Save as** dialog box, enter a filename and select a file type.
- c. Click Save.

J. Analyze in Neurolucida Explorer.

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



