

Before you start

- Trace the trees.
- To display the panel, click the **Spines** button in the 3D environment.

Detecting spines automatically

- 1. Optional: In the Detect Spines panel...
 - If your image is "noisy," check the Filter image noise box.
 - To detect spines in addition to spines previously detected manually, check the Keep existing spines box.
- 2. Click Detect All for an initial detection with the default settings. Automatic detection may take a few seconds.
- 3. Adjust the detection settings to refine spine detection.
 - **Outer range**: Adjust the maximum distance between the surface of the tree and the potential top of the spine head.
 - **Minimum height**: Adjust the minimum distance between the surface of the tree and the potential top of the spine head.
 - **Minimum count**: Adjust to ignore objects that are too small to be considered spines. Works in conjunction with **Minimum height**.
- 4. Optional: To detect spines in addition to spines already detected, check **the Keep existing spines** box.
- 5. Click **Detect All** to detect again.

NOTE: To detect spines on a single branch, check the "Click to detect all spines on nearest branch" under **Options** then click near the desired branch.

Classifying 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 modify a default color, click a color swatch under Automatic classification colors.
- To classify manually, use the Edit Spines panel.

Detecting individual spines

- 1. Zoom, rotate, and pan as needed to view the spine of interest clearly.
- 2. Click the spine of interest. The detected spine is displayed with a color overlay.

Optional: Adjust the Detector sensitivity.



Tree



Now, let's explore the spine editing tools to: merge or split spines, change their color, classify them manually, delete them, confirm complex spine models, and re-assign spines to a different branch.

Click the **Edit** button to display the **Edit Spines** panel.

Merging spines

To override one spine that is modeled as two spines, use the **Merge** button.

- 1. Select two spines:
 - Hold CTRL down and click each spine.

OR

- Press **CTRL** and drag the mouse to draw a marquee around the spines.
- 2. Click the **Merge** button.

Splitting spines

To override two spines that are modeled as one spine due to physical proximity, use the **Split** button.

- 1. Click the spine you want to split to select it.
- 2. Click the Split button.

In most cases, the split location is unambiguous and only one split operation is re quired to obtain the two desired spines.

For more complex cases, you may need to perform several Split and Merge operations to obtain a satisfactory model.

Changing spine color and other display settings

Click the **Select All** button or click a spine in the tracing window to select it.

Selected spines are displayed with a wireframe. Appearance settings are displayed.

- 1. Select a color from the color drop-down menu.
- 2. Use the sliders to adjust transparency or smoothness.

Classifying spines manually

- Click a spine to select it.
 If you used automatic classification, the type is displayed next to Type.
- 2. Select another type from the **Type** drop- down menu.

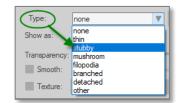
If you used automatic, the type you select overrides the type assigne d by Neurolucida 360.













Deleting spines

- 1. Select the spines to delete:
 - To select all the spines, Click the Select All button.
 - To select a single spine, click a spine in the tracing window.
 - To select neighboring spines, press **CTRL** and draw a marquee (rectangle) around the spines.

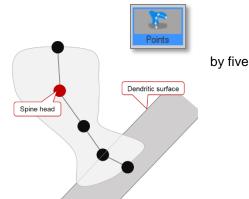
Selected spines are displayed with a wireframe.

2. Press the **Delete** key on the keyboard OR click the **Remove** button.

Confirming complex spine models

- 1. Click a spine to select it.
- 2. In the panel, click the **Points** button. The spine is represented points.
- 3. 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.





Re-assigning a spine to a different branch

- 1. Inspect the spines to verify their assignments. To make the inspection easier:
 - a. Click Select All.
 - b. From the **Show as** drop-down menu, select the **Stick** type. The sphere at the end of the stick represents the spine head.
- 2. Click anywhere away from the spines to de-select the spines.
- 3. Click the spine that is not assigned to the correct branch to select it.
- 4. Click the **Points** button. The spine is now represented by five points.
- 5. Click the insertion point (first point starting from the dendrite) to select it then drag it to the centerline of the correct branch. Drag the other points as necessary to obtain a more accurate spine model.

