

## Before you start

- You already loaded your image stack/series of images.
- If applicable, detect somas.
- Click the **Tree** button to display the **Trace Trees** panel. Under **Tracing Mode**, select **Automatic**.

## Implementing automatic tracing

There are two ways to implement automatic tracing:

- For a tracing using the defaults, click the **Trace** button.
- For a more accurate or faster tracing, click the Show Settings button, adjust the settings, then click the Trace button. The settings give you the opportunity to review seed placement, which is used to initiate the automatic tracing.

## Tracing after adjusting the settings



Click the Refine Seeds tab.

available.

- 2. In the Display Seeds tab, click the Display Seeds button.
  - **Optional**: To change the seed color, use the color picker next to the **Hide Seeds** button.
  - To adjust the number of seeds, use the slider and the density buttons.

a. Click the Refine Seeds button. Neurolucida applies an

algorithm to evaluate seed placement. The manual

b. Optional: To decrease the number of unwanted seeds.

options below the Refine Seeds button are now



Display Seeds Refine Seeds Trace	
Automatic tracing uses seeds to initiate	
Display Seeds	
Sensitivity	
·	70
Density	
Coarse	
Medium	
O Dense	





d. Optional: Remove seeds.

color picker.

increase the Refine filter value.

i. Select the Remove radio button.

c. Optional: Add seeds by clicking in the image.

ii. Hover the mouse over a branch to see the circular cursor.

To change the color of manually added seeds, use the

- iii. Hold down CTRL and scroll the mouse wheel to resize the cursor.
- iv. Click in the image to remove seeds within the cursor radius OR hold down CTRL and drag to remove contiguous seeds.



- 4. Click the **Trace** tab to modify the tracing settings:
  - a. Select a tracing method from the drop-down menu.
  - b. Modify the settings:
    - Sensitivity: Use the slider or type a value to adjust sensitivity to dim and low-contrast structures.
    - **Gap tolerance**: Use the slider to change the maximum acceptable distance between two segments to make a connection.
    - **Remove traces shorter than**: Discard segments shorter than the specified value.
    - Connect branch segments: Check to have Neurolucida connect branches according to the Branch Connections Criteria set in the Advanced Settings:

Display Seed	Is Refine Seeds Trace	
Method:	Directional Kernels	-
	Directional Kernels	
Sensitivity	Voxel Scooping	
-	0	70
Gap Tolera	ance :	
Small 🛛		Large
Remove	traces shorter than 1.93	μm
Connect	t branch segments	
Advanced	Settings	

- Largest gap: Determines the largest distance that the software will "jump" to make a connection.
- **Max deviation angle**: This is the maximum angle considered by when connecting branches. The maximum value is 180°.
- Min ratio of diameters: When two branches are considered for connection based on their respective location in XYZ space, the software compares the branch diameters of each branch's end point. If the ratio of the diameters is greater than the specified value, the software connects the two branches and recolors according to the branch that is closest to the soma (if the soma is traced).

*Example*: The software compares Branch A and Branch B.

- Branch A's diameter at end point (D<sub>A</sub>) > Branch B's diameter at end point (D<sub>B</sub>).
- The default ratio is 50%.

The software connects A and B if  $D_B >= (D_A/2)$ 

5. Click the **Trace** button to start the automatic tracing.

If the automatic traces are incomplete, use the **userguided tracing** mode to continue tracing.

Tracing Mode Smart manual O User-guided Automatic		
<ol> <li>Detect somas before tracing.</li> </ol>		
Method: Directional Kernels		
,,		
Trace		