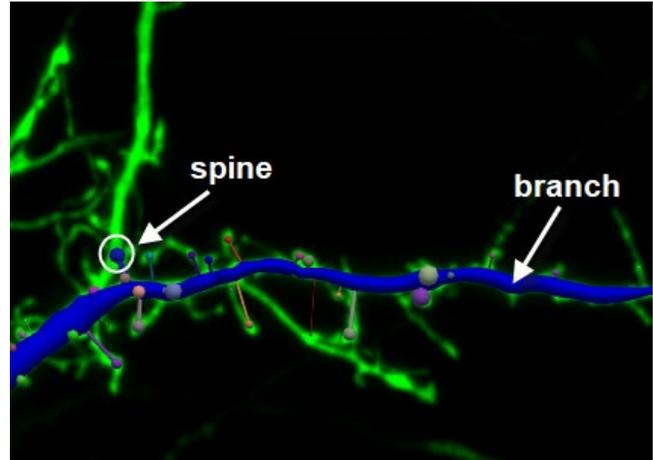


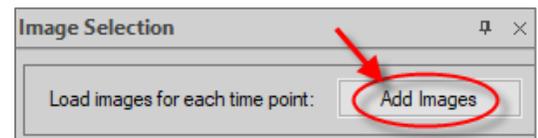
This guide walks you through using MicroDynamix software to compare morphometric changes in dendritic spines over three time points (A, B, C).

In this example, we examine spines on a single branch.

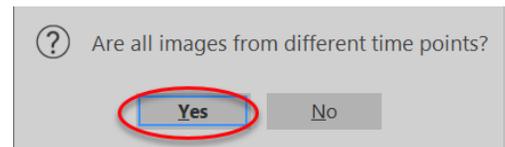


1. Load images corresponding to three time points:

- a. Click the **Add images** button in the **Image selection** panel on the right.



- b. Click **yes** to the prompt, *Are all images from different time points?*



- c. Navigate to the location of the files. Hold down **Ctrl** and click to select the three files. Then click **Open**.

- d. Loading the images will take a moment because MicroDynamix software aligns or “registers” the images (B and C in this example) relative to the first image (A) while loading.

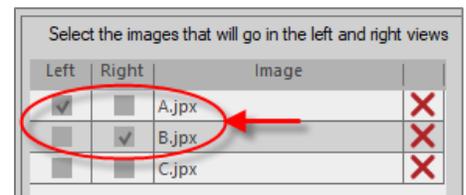
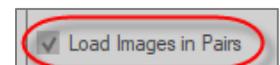


Image A is displayed in the left viewport while image B is displayed in the right viewport, as indicated in the **Image selection** panel.

- e. Check **Load images in pairs** for easier navigation between images later.



2. Positioning and navigating images:

- Zoom: Scroll the mouse wheel to zoom.
- Pan: Hold down SHIFT and drag with your mouse to pan.
- Rotate in 3D: Drag with your mouse to rotate images in 3D.

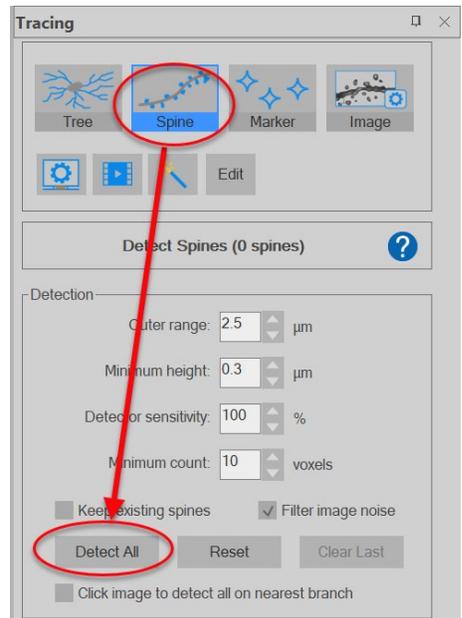
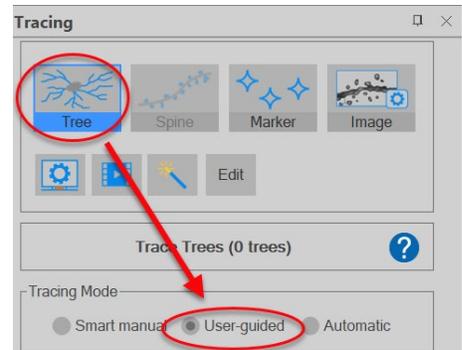
- Change the 3D rotation axis:
 - Click the **pivot point** tool in the general section of the ribbon to select it. 
 - Click in the image to set the new pivot point.
 - Drag to rotate relative to the new point.
- Rotate in 2D:
 - Click the **2D rotation** tool in the ribbon to select it. 
 - Drag to spin (rotate) images.
- Return to the original orientation: Click the **reset** button. 

3. Trace one branch and detect spines in the first image:

- a. Click the **Tracing** button in the in Tracing section of the ribbon bar to open the **3D Tracing** panel.

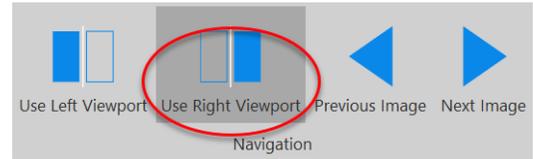


- b. Click the **Tree** button and select **User-guided** under *Tracing Method*.
- c. In image A, trace one branch: Click over the dendrite to place the first point, click along the dendrite to place more points, then right-click to end the tree.
- d. In image A, detect spines on the tree you just traced: Click the **Spine** button and click **Detect all**.



4. Trace the corresponding branch and detect spines in images from the later two time points.

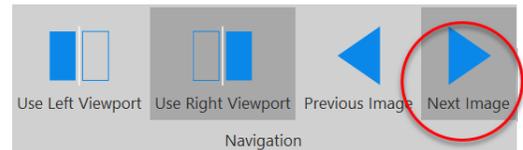
- a. Click **Use Right Viewport** image in the Navigation section of the ribbon to switch to image B. Alternatively, you can press the space bar on your keyboard.



- b. Trace the same branch identified in image A at the later time point represented in image B:
Click the **Tree** button and select **User-guided** under *Tracing Method*. Click over the dendrite to place the first point, click along the dendrite to place more points, then right-click to end the tree. Notice that the tracing of the tree becomes the same color as the tree in image A while you trace. This means that MicroDynamix has “recognized” the branch.

- c. Detect spines on the tree you just traced: Click the **Spine** button and click **Detect all**.

- d. To show image C, click **Next image** in the ribbon bar. C is now shown on the right, and B on the left.



- e. Trace the same branch in image C and detect the spines (repeat steps 3.b and 3c).

5. Review the associations between images to get a sense of how trace association works.

- a. Click the **Association** button in the ribbon bar.

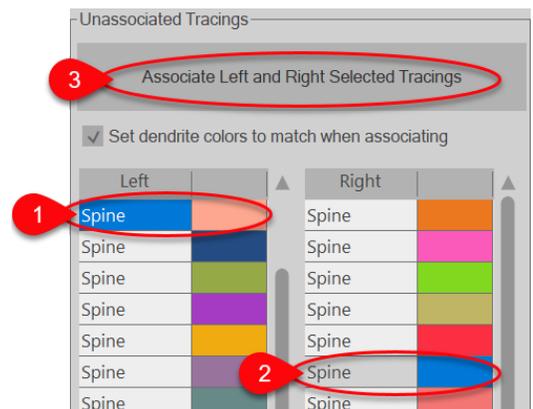


- b. In the **Associated tracings** panel, you can review the dendrites and spines that are recognized by MicroDynamix as being associated. If the association is incorrect, you can *break* it by clicking the **X**.

Associated Tracings			
Left		Right	Break
Dendrite		Dendrite	X
Spine		Spine	X
Spine		Spine	X

Note that you can click on the table row that represents a traced structure to highlight it in the images.

- c. In the **Unassociated tracings** panel, you can see the structures that the program has identified in one image but not in the other. To associate two structures, click a structure on the left, then the corresponding structure on the right, then click the **Associate Left and Right Selected Tracings** button.



6. Review the spine morphometric changes.

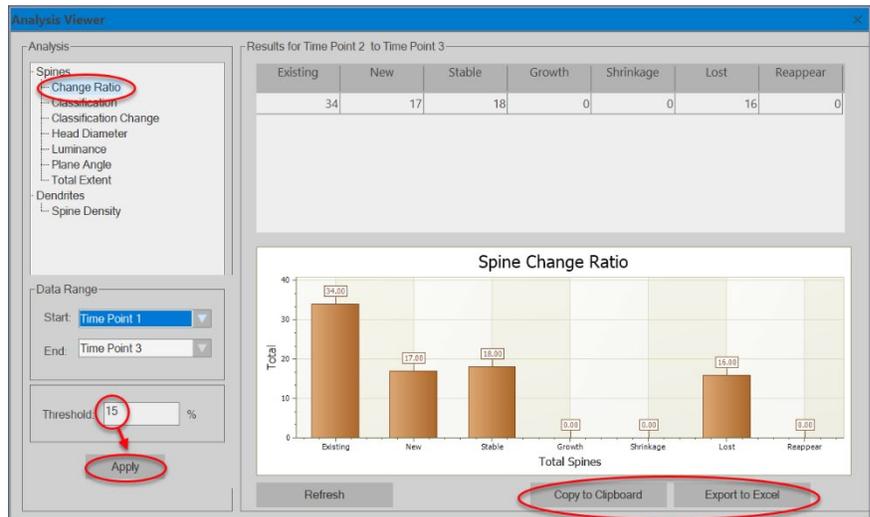
- a. Click the **Analysis** button in the ribbon bar.



- b. In the **Analysis viewer**, select the **Spines—Change ratio** analysis.

This analysis creates a summary report of dendritic spines detected across time points.

- c. You can change the default **threshold** (percentage a spine can change to estimate growth, shrinkage, and appearance/disappearance) and click **Apply**.



- d. Click **Export to Excel** to open a spreadsheet with the results for the analysis selected or click **Copy to Clipboard** copy the currently displayed graph to your clipboard so that you can paste it into another software program.

7. Save your reconstructed trees and spines: Go to **File > Save as > Data File**.

Or click the save icon in the Quick Access Toolbar.



When you're ready to resume work, open the data file—it stores both your images and traced structures so that you can pick up right where you left off.