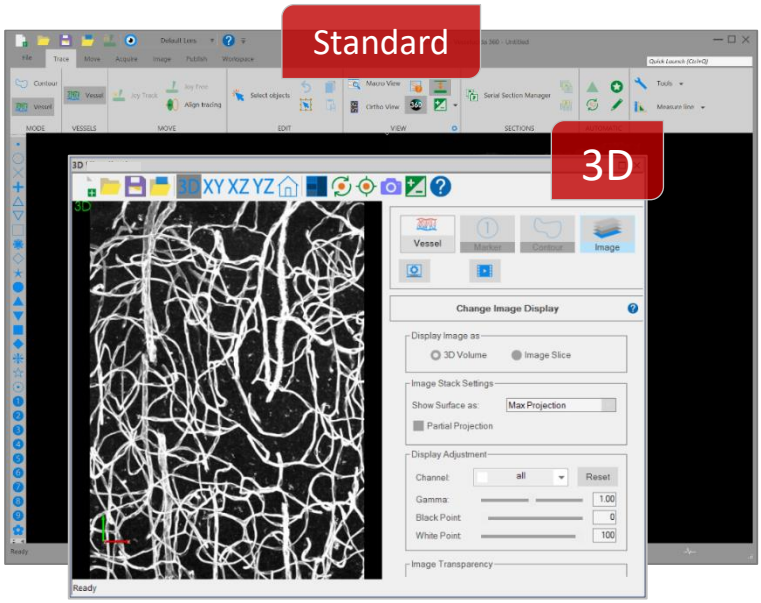
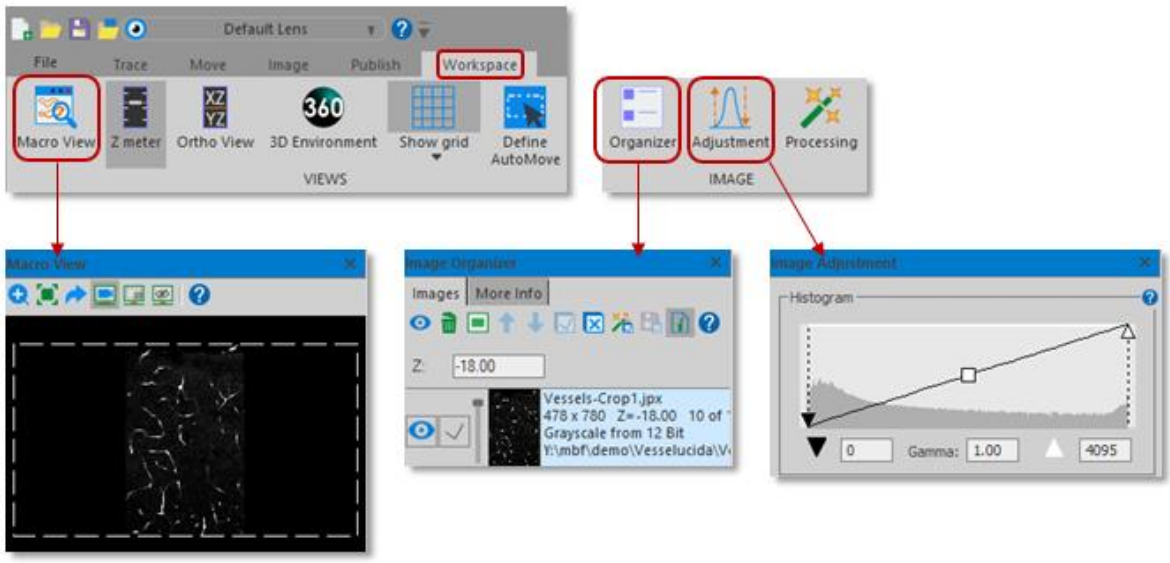
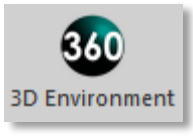
Vesselucida consists of two environments: standard and 3D.



1. Before you start working in 3D, use the “Workspace” ribbon in the standard environment to display useful dockable windows: **Macro View**, **Image Organizer** and **Image Adjustment**

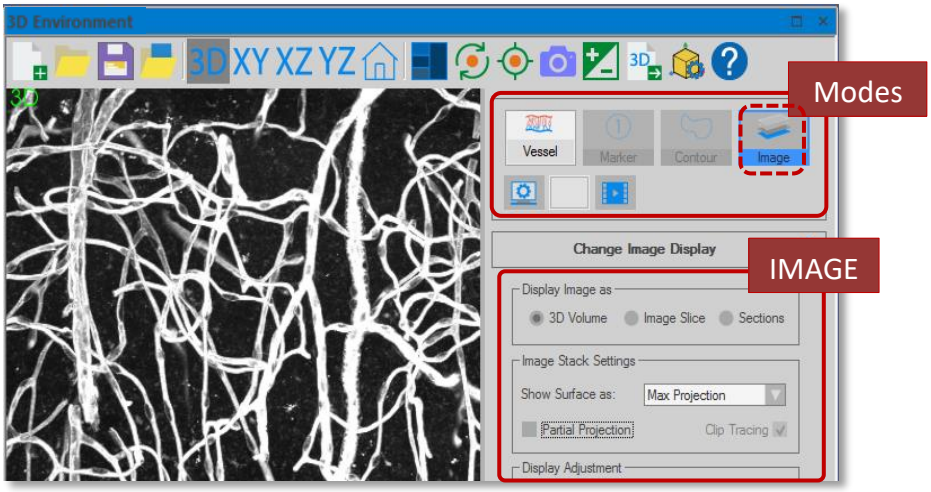


2. Next, start the 3D environment (it might already be open): Click the **360** button in the **Workspace** ribbon.



3. Open your image file: Click the **Open Image File** button in the 3D environment toolbar.

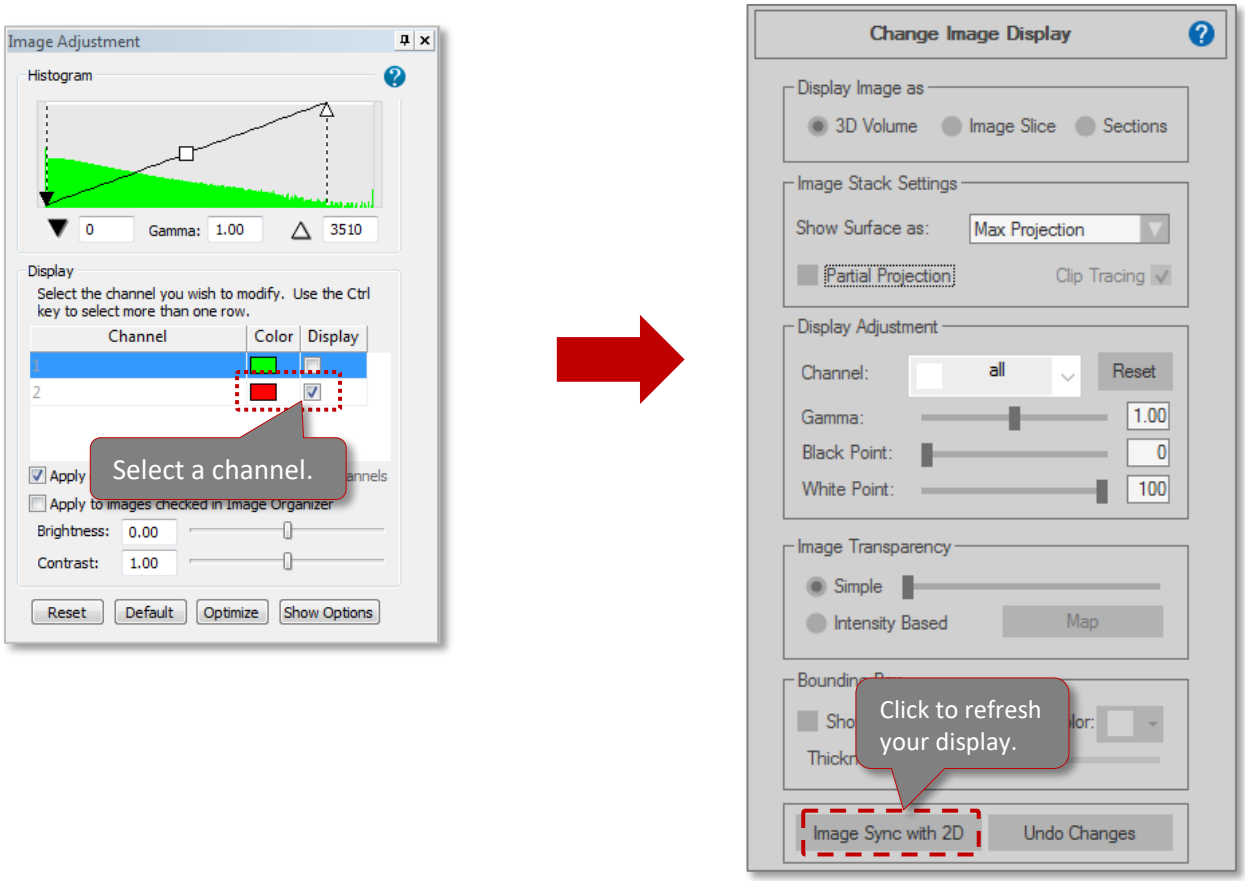
Once your file is loaded, the IMAGE panel is displayed, indicating that the IMAGE mode is active.



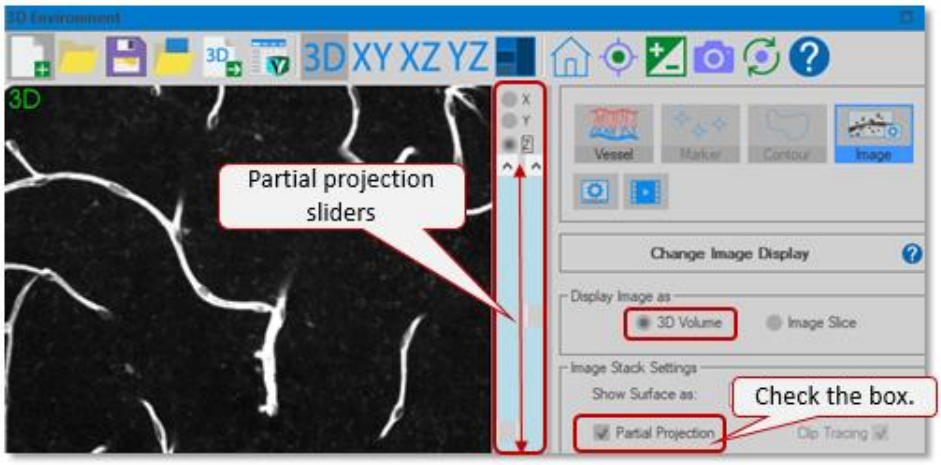
4. Practice navigating your image with the mouse.

a. Drag the mouse to rotate.
b. Scroll the mouse wheel to zoom.
c. Hold down **Shift** and drag to pan.

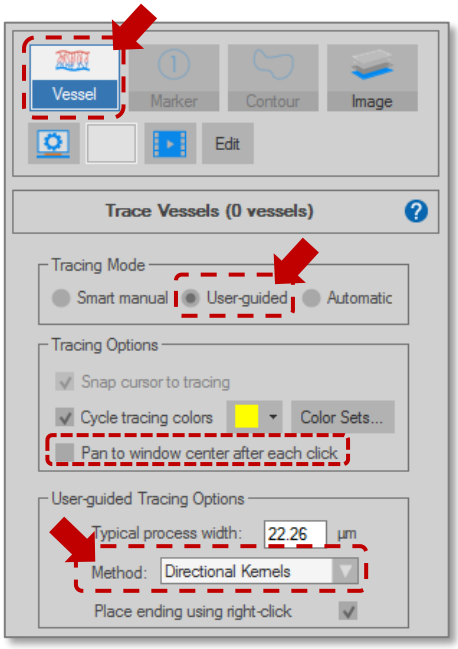
5. OPTIONAL: If you're working with a multichannel image and are only interested in a single channel in the image, use **Image Adjustment** to select a channel. Then return to the 3D environment and click **Image Sync with 2D** in the IMAGE panel to apply the changes.



6. In the IMAGE panel, keep **3D Volume** selected and check **Partial projection**. Partial projection displays a subset of the data in X,Y or Z. You will use the sliders to adjust the subset and reveal more of the structures while tracing.



7. Click the **Vessel** button to switch to VESSEL TRACING mode. In the TRACE VESSELS panel, select the **User-guided** tracing mode.



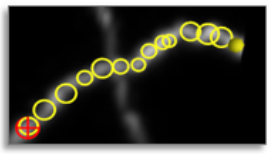
8. Start tracing.

a. OPTIONAL: Select **Pan to window center after each click** to avoid panning manually.

b. Place the cursor over the area of the vessel where you want to place the first point. When zoomed in, you see the red cross-hair cursor and a circle. The circle diameter represents the segment width detected.

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

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



e. Click along the vessel to place more points.

f. When the vessel is no longer visible, drag one of the **partial projection** sliders to reveal more of the vessel and continue tracing.

g. Click to place the last point. This may be the point where a loop closes.

h. Right-click once to end the vessel. When the branch is effectively ended, you can see a sphere at the end of the vessel or partial spheres along the vessel as you hover and the vessel count is updated.

Note that you have three tracing methods; if you're not satisfied with the results produced by **Directional Kernels**, try another method.

9. Once you're done tracing the vessels, save your work.

a. Click the **Save tracing** icon in the toolbar.

b. Save your file as an XML document.

10. Optional: Analyze your date with Vesselucida Explorer: Click in the toolbar to open your data file and access the analyses.