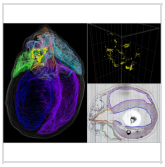

Jul 20, 2020

# Single-Cell ICN Neuron Mapping and 3D Heart Reconstruction with Tissue Mapper

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1 Works for me dx.doi.org/10.17504/protocols.io.bdz5i786

SPARC
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ABSTRACT

This protocol describes the process of using the Tissue Mapper software to map single neurons of the intrinsic cardiac nervous system, annotate or "trace" key cardiac anatomy on select histological sections of an image volume, and to visualize the mapped neurons and traced anatomy in a 3D reconstructed heart.

DOI

dx.doi.org/10.17504/protocols.io.bdz5i786

PROTOCOL CITATION

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KEYWORDS

3D heart reconstruction, Intrinsic cardiac neurons, Intrinsic Cardiac Nervous System, Mapping, Image volume, Image stack, Tissue Mapper, Heart, Rat, KESM



CREATED

Mar 20, 2020

LAST MODIFIED

Jul 20, 2020

PROTOCOL INTEGER ID

34589

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Tissue Mapper | | MBF Bioscience |
| Image stack (.jpx) | | |

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety



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## Sample preparation

1 Rats were anesthetized with 5% isoflurane until the animal was non-responsive to a contralateral toe pinch; then the abdominal cavity was opened. For subjects 54-5 and 54-6, hearts were dissected and fixed overnight in 4% paraformaldehyde prior to whole-mount diffusion staining for seven days in a 0.1% solution of Cresyl Echt Violet (0.05g in 50 mL distilled $H_2O$ and 150 µL of glacial acetic acid) to visualize neurons.

1.1 For the remaining subjects in the study (54-8, 54-9, 54-10, 54-11, 54-13, 54-14), the fixation protocol was updated. Animals were perfused through the abdominal aorta with phosphate-buffered saline at a pressure of 280 mmHg until exsanguinated. Following exsanguination, animals were perfusion-fixed with 200 mL 10% neutral buffered formalin at the same pressure. Hearts were then dissected and further fixed overnight in 10% neutral buffered formalin. Subsequently, the hearts were whole-mount diffusion stained in the same concentration of Cresyl Echt Violet for fourteen days. The hearts were processed in a Sakura Tissue Tek VIP 3000 tissue processor. A syringe was used to inject molten paraffin through the great vessels to fill the chambers and remove air bubbles, and then hearts were embedded in paraffin.

1.2 After the rat hearts were formalin fixed and paraffin embedded (FFPE) into blocks, samples were digitized with a Knife Edge Scanning Microscope (KESM).

## Image stack assembly

2 **Image tile stitching.** The image stack was assembled from individual image tiles (jpg) produced from a whole rat heart by a KESM. Image tiles were stitched together to assemble one section of image data, which corresponds to one plane. Then, each plane of image data was assembled into an image stack. Image stack was compressed 40:1 (lossy).

2.1 Individual jpg tiles from each Z position were stitched together in custom software by MBF Bioscience to create individual planes (uncompressed TIFF format). Images produced from KESM do not require alignment or blending between tiles or planes.

2.2 The uncompressed TIFFs were converted into JP2000 format using an image converter from MBF Bioscience.

2.3 JP2000 planes were assembled into a stack (JPX) using an image converter from MBF Bioscience. 40:1 lossy compression was applied to the stack during the conversion process.

## Software installation setup

3 Have Tissue Mapper installed and licensed on your workstation of choice through an MBF Bioscience representative.

**Tissue Mapper 2020.1.1**
by MBF Bioscience



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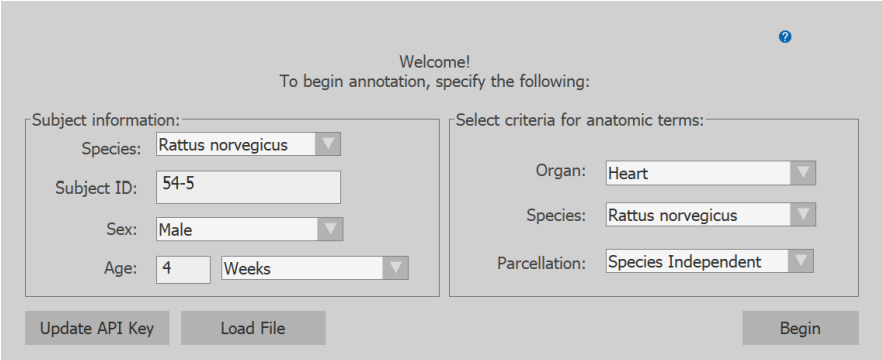
4 After launching Tissue Mapper, set an API key to your profile to pull the ontology list from SciCrunch to annotate your organ of choice with curated SPARC anatomy terms.

## Cell marking and image annotation

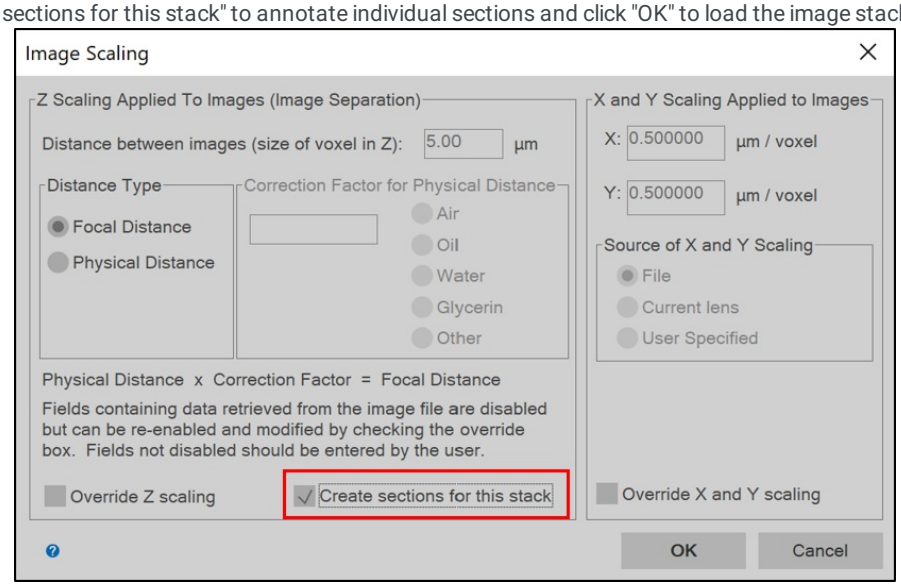
5 **Metadata setup.** To begin, create an empty document by filling in the subject information of your sample with the species, sample ID, sex, and age on the left side of the dialog. Then specify the species and organ in which you are annotating on the right side of the dialog to access the curated SPARC anatomy terms and press "Begin" when you are done. For the purposes of our annotation, the organ is the "Heart" and the species is "Rattus Norvegicus."




More information on SPARC vocabulary services can be found here:
https://www.mbfbioscience.com/help/tissuemapper/Default.htm#cshid=8044

5.1 Manually inputting information about your sample only occurs once when you first create an empty document to annotate your sample. After you save your work as an .xml file and load it into the program, the metadata dialog will autopopulate and you can resume your work.

6 **Configuring and opening the image file.** Open the image stack (.jpx) through the "File" ribbon and under the "Open" menu, select "Image stack," and select the desired .jpx file. A dialog will appear to verify or adjust the XYZ scaling properties of the image stack. If the scaling properties are correct and do not need adjustment, check the "Create sections for this stack" to annotate individual sections and click "OK" to load the image stack.




Instructions for adjusting the image scaling can be found here:
https://www.mbfbioscience.com/help/tissuemapper/Default.htm#cshid=1185

7 **Workspace setup.** For ease of annotation, it is recommended to set up your workspace with dockable windows. Under the "Workspace" ribbon, enable the "AutoMove" function and dock the "Macro view," "Image organizer," and the



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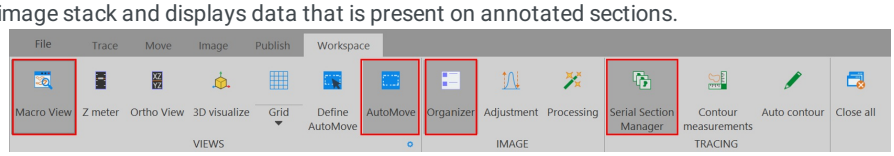
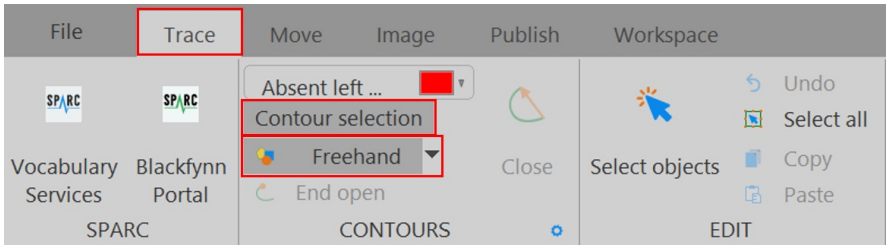
"Serial Section Manager." The AutoMove feature will define a working area and automatically reposition your image to the next adjacent region when your contour moves beyond the bounds of the working area. The "Macro view" acts as a mini-map in a separate window to show your location on the image section and to also take you to any region of interest. The "Image organizer" allows you to show, hide, save, or discard the image data and is especially helpful for visualizing the contours in 3D. Lastly, the "Serial Section Manager" enables movement through individual sections of the image stack and displays data that is present on annotated sections.

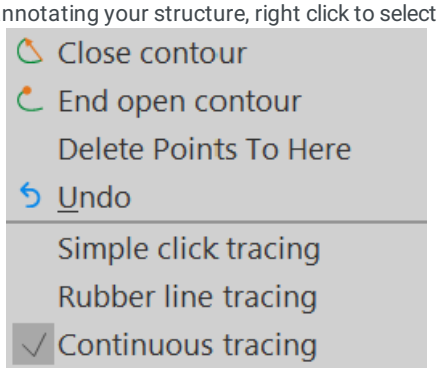



More information on setting up dockable windows can be found here: https://www.mbfbioscience.com/help/tissuemapper/Default.htm#About/WindowsSetup.htm%3FTocPath%3DWorkspace%2520overview%7C____4

8 **Contour preparation.** To begin contouring or tracing anatomical features, go to the "Trace" ribbon and in the "CONTOURS" submenu, click "Contour selection" to display the list of curated SPARC anatomy terms. Under the "Contour selection" button, you will want to set tracing options to "Freehand" to manually contour the anatomical structures of interest.




9 **Section annotation**. Select the desired anatomical structure that you wish you annotate under the "Contour Selection" list. Navigate to a section of image data with the "Serial Section Manager," zoom in on the image as needed, and contour your desired structure by clicking along the structure to place points and draw lines. Alternatively, you can also continously draw a contour by right clicking and selecting the "Continuous tracing" option. This method of tracing works best when paired with a Wacom tablet or other digital tablet drawing device. When you are finished with annotating your structure, right click to select "Close contour" or "End open contour."




Right-click menu for contouring and tracing options

10 **Additional section annotation.** Move to a different section in the image stack with the Page up (PgUp) or Page down (PgDn) keys and repeat step 9. As the image sections become more populated with contours and tracing data, it will be best to only keep the contours on each section visible by clicking the "Show current section" button in the "Serial Section Manager" docked window. The "Show current section" button will look grey when it is selected.



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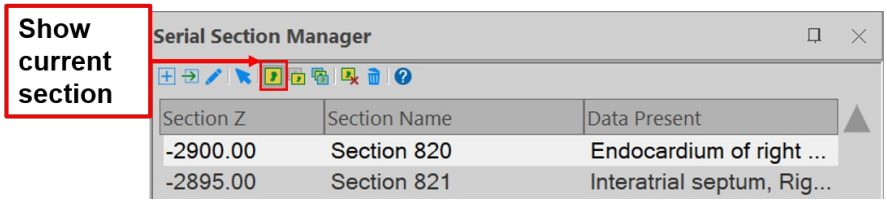
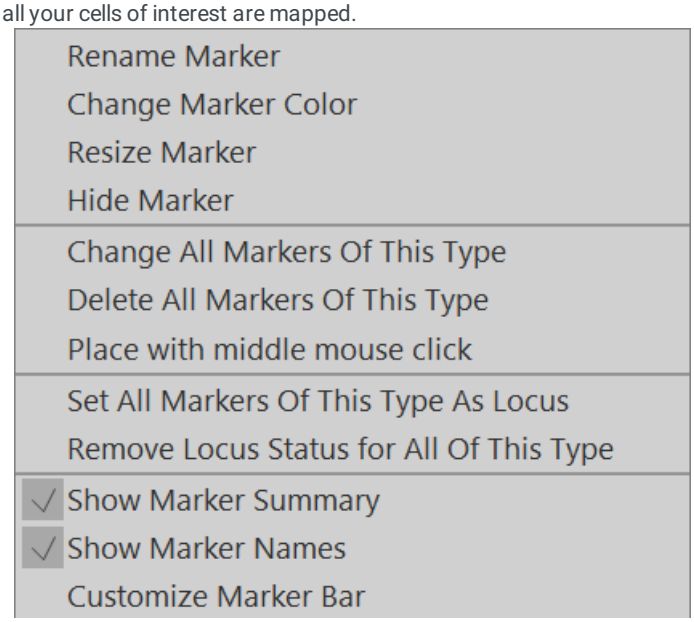



More information on the workspace setup and annotation instructions can be found here: https://www.mbfbioscience.com/help/tissuemapper/Default.htm#About/TMgetStarted.htm%3FTocPath%3DCreate%2520annotations%2520(contours)%7C____1

10.1 **Heart anatomy contouring.** In an image stack with more than 1000 sections, heart anatomy was contoured from start to finish in intervals ranging between 10-20 sections until the entirety of one anatomical structure was complete. Steps 9+10 were repeated until the atria, ventricles, and major blood vessels were entirely contoured. Each anatomical structure is represented by a different color that is defined either automatically with preset values or with user-defined values.

11 **Marker placement and cell mapping.** In the Markers toolbar, select a marker that you like to use to best represent the cells (neurons) or region of interest to be pinpointed. To rename it, right click and select "Rename" to specify what the selected marker represents. To change the color of a marker, right click and select "Change Marker Color" and you can choose from preset colors or a user-defined color, click "OK" when you are finished. Next, select your desired marker and go to a section that has the cell or area of interest and click on the image to place a marker. Repeat this step until all your cells of interest are mapped.




Right-click menu for Markers toolbar options

11.1 **Neuron mapping.** Mapping neurons of the intrinsic cardiac nervous system (ICN) was performed by using the Markers toolbar in Tissue Mapper. A circle marker was renamed as "ICN" and changed to a yellow color to represent the neurons. Neurons were marked every 4th section where the nucleus was most prominent to avoid double-counting. To keep track of the total number of neurons mapped, the "Show Marker Summary" and "Show Marker Names" were enabled in the Markers Toolbar.

12 **Saving your work.** To preserve your progress in mapping and annotation, go to the "File" ribbon and under the tab "Save as," select "Data File" and save your work as an XML document file (.xml). The associated metadata from the initial dialog box will autopopulate when you load the file to resume your work.



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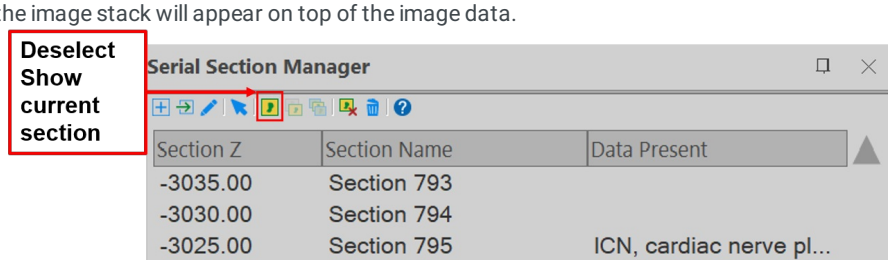
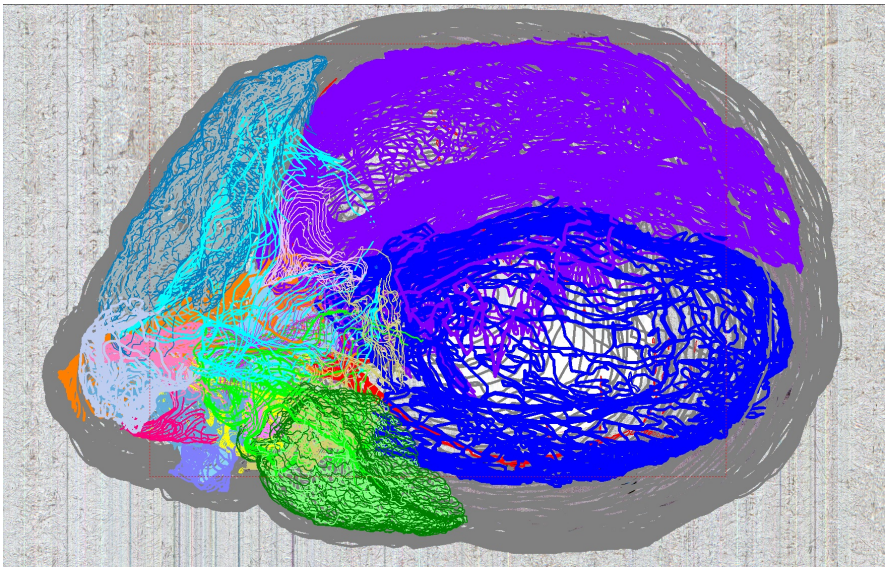
13 **3D visualization of mapped neurons and contours.** Visualization of the contours and mapped neurons of the entire annotated image stack in the 3D viewer are achieved through three steps and will be described in sub-points below.

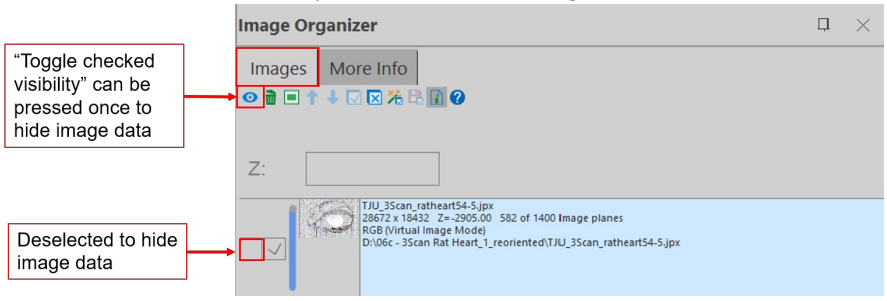
13.1 **First,** deselect the "Show current section" button in the docked "Serial Section Manager window." The "Show current section" button will not look grey and a maximum projection of the contours throughout the image stack will appear on top of the image data.



Maximum projection of all the contours overlaying the image data in the tracing workspace after deselecting the "Show current section" button.

13.2 **Second,** in the docked "Image Organizer" window under the "Images" ribbon, deselect the eye or press the "Toggle Checked Visibility" button to hide the image data. In the main tracing workspace, only the contours and markers should be present with a black background.




Two options for hiding the image data.



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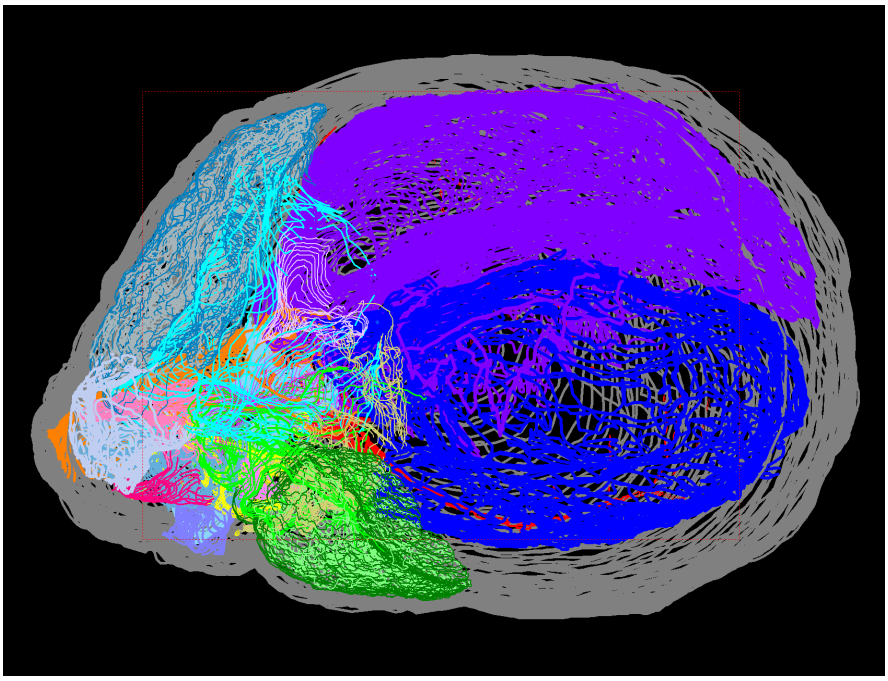
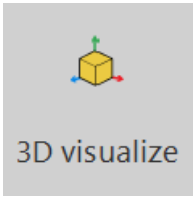


How the main workspace appears after hiding the image data to show only the contours and markers.

13.3 **Third,** to visualize all the contours and markers in the 3D viewer, press the "3D Visualize" button under either the "Trace" or "Workspace" ribbon. A separate window will open to allow interation with the 3D reconstructed heart model. In the 3D viewer, the user can selectively show, hide, and edit specific contours and markers, create video clips of the data, and export the 3D representations.




3D visualize button can be found in either the Trace or Workspace ribbon.



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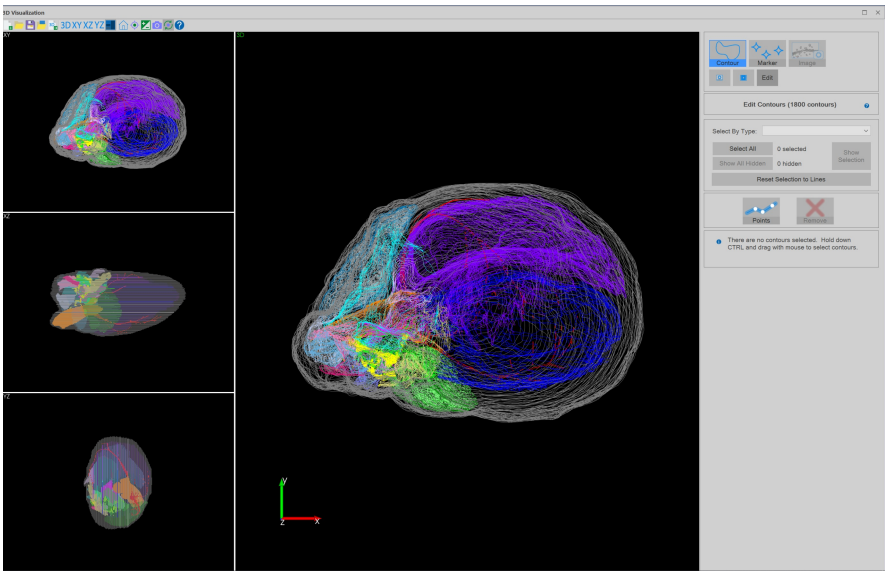
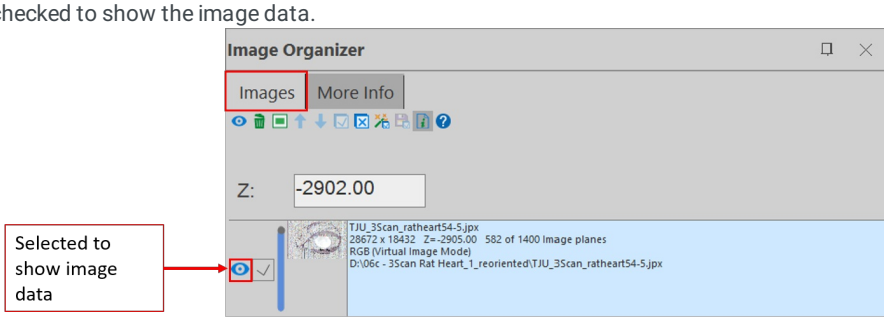


3D Visualization window for manipulation and interaction of contours and markers in the 3D space.

More information on 3D Visualization capabilities are described here: https://www.mbfbioscience.com/help/tissuemapper/Default.htm#3Dvisualization/3D_Tour.htm%3FTocPath%3DWorkspace%2520overview%7C____2

13.4 **3D visualization of mapped neurons, contours, and image data.** It is also possible to load the 3D image data along with the markers and contours. To have the image stack load with the contours, go to the docked "Image Organizer" and under the "Images" ribbon, make sure to have the eye checked to show the image data.




Then, press "3D Visualize" to load the image volume along with the contours and markers. Depending on the size of the image volume and computer processing power, it may take some time for the 3D visualization to load. Once loaded, the image data and associated contours can be resliced to visualize selective slabs of image data in the XY, XZ, and YZ planes for additional 3D analysis.



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