



MBF Bioscience: FAIR Segmentation and Annotation V.2

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¹MBF Bioscience

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Works for me

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SUBMIT TO PLOS ONE

ABSTRACT

Segmenting and annotating microscopy image data with MBF Bioscience software using built-in SciCrunch API for up-to-date anatomical terms curated by the SPARC Anatomy Working Group.

PROTOCOL CITATION

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Adding Metadata and Accessing SPARC Vocabularies from SciCrunch

- 1 Launch one of the following MBF Bioscience software with SPARC-mode enabled.

Neurolucida 360

by MicroBrightField Bioscience

Vesselucida 360 v2020.1.1

by MBF Bioscience

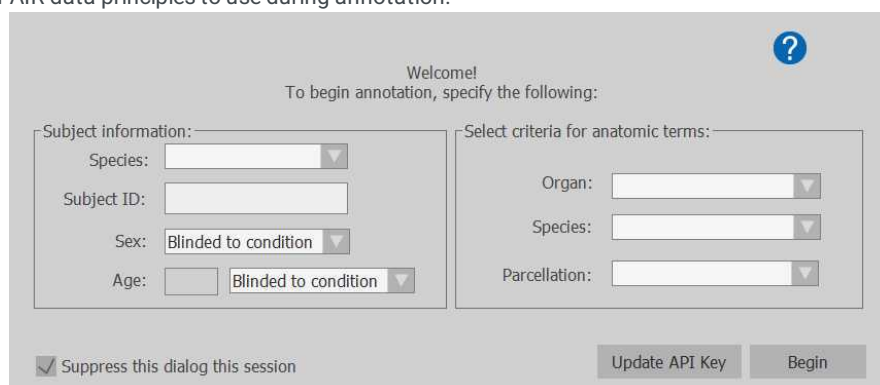
TissueMapper

by MBF Bioscience

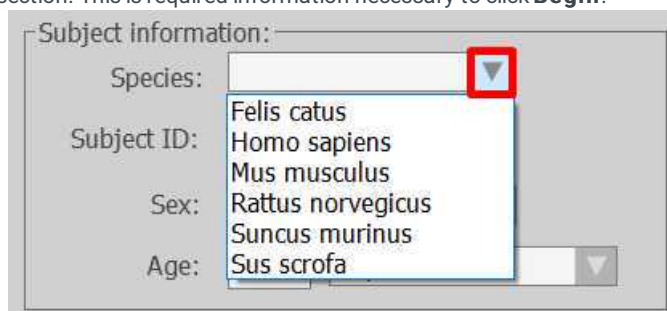
- 2 Open a microscopy image via the **Open** icon, **File>Open**, or dragging and dropping into the program window.

NOTE: MBF Bioscience software supports a variety of image file formats from common microscopy vendors such as JPX/JP2, TIFF, LIF, ND2, IMS, OIF/OIB, almost all CZI, and more.

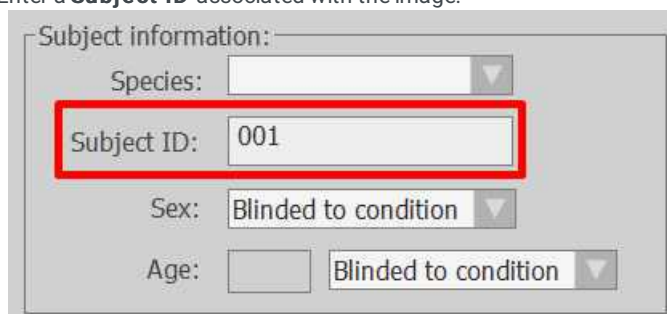
- 3 The **SPARC Vocabulary Services** window will appear. The dialog is displayed so that you can specify subject-specific metadata and gain access to the [SciCrunch](https://scicrunch.org/) database to retrieve anatomical terminology lists compliant with FAIR data principles to use during annotation.



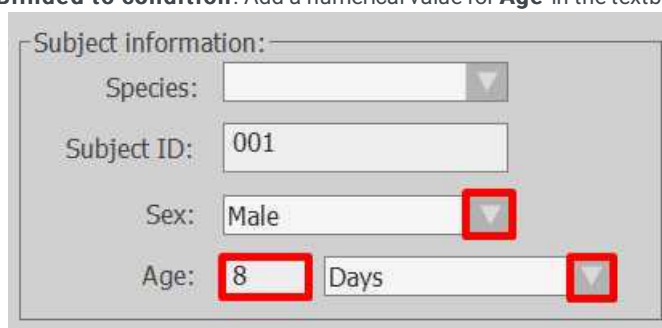
- 3.1 Use the dropdown menu to select the **Species** associated with the image in the Subject Information section. This is required information necessary to click **Begin**.



- 3.2 Enter a **Subject ID** associated with the image.



- 3.3 Use the dropdown menus to select **Sex** and **Age** of the subject associated with the image, or leave as **Blinded to condition**. Add a numerical value for **Age** in the textbox.



Subject information:

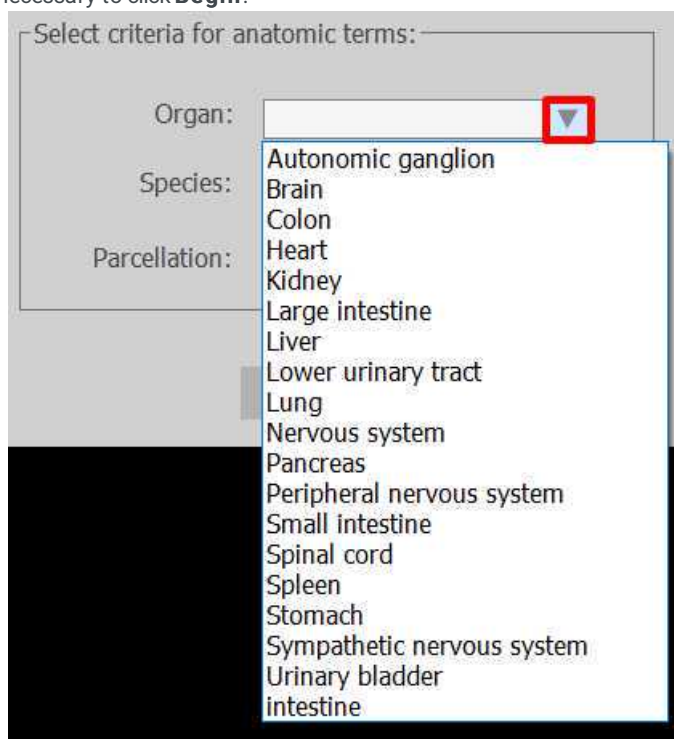
Species:

Subject ID:

Sex:

Age: Days

- 3.4 Use the dropdown menu to select the **Organ** associated with the image. This is required information necessary to click **Begin**.



Select criteria for anatomic terms:

Organ:

Species:

Parcellation:

- Autonomic ganglion
- Brain
- Colon
- Heart
- Kidney
- Large intestine
- Liver
- Lower urinary tract
- Lung
- Nervous system
- Pancreas
- Peripheral nervous system
- Small intestine
- Spinal cord
- Spleen
- Stomach
- Sympathetic nervous system
- Urinary bladder
- intestine

- 3.5 Use the dropdown to select the **Species** associated with the image, matching it to what was selected in step 3.1. The window will reload so as to provide accurate information for step 3.6. This is required information necessary to click **Begin**.

Select criteria for anatomic terms:

Organ:

Species:

Parcellation:

Update API Key Begin

- 3.6 Finally, use the dropdown menu to choose an organ-specific **Parcellation** associated with the image. Note that some may be **Species Independent**.

Select criteria for anatomic terms:

Organ:

Species:

Parcellation:

- 3.7 Click **Begin** to add the selected metadata to the data file when saved and to open the image loaded.

Begin

Adding this metadata to a saved data file will allow for FAIR segmentation to occur!

- 4 You may enable or disable suppression of the SPARC welcome window for the duration of the session you are using the software for by using the **Suppress this dialog this session** checkbox, which is enabled by default.

☒ Suppress this dialog this session

- 5 If your API Key needs to be updated, select the **Update API Key** button.

Update API Key

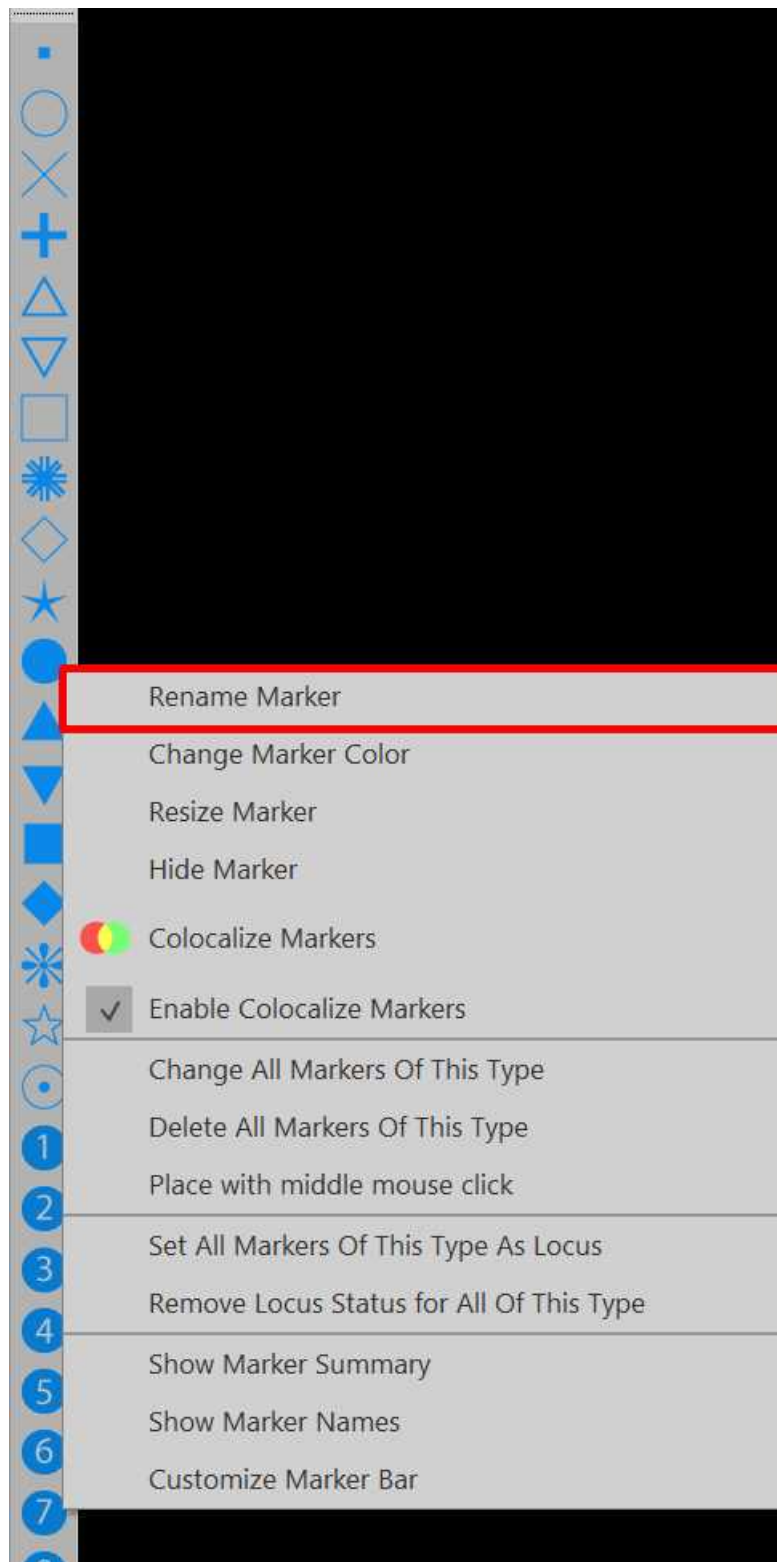
- 6 You may access and update the SPARC Welcome Window information at any time by selecting the **Vocabulary**

Services icon in the Trace ribbon of SPARC mode-enabled MBF software.

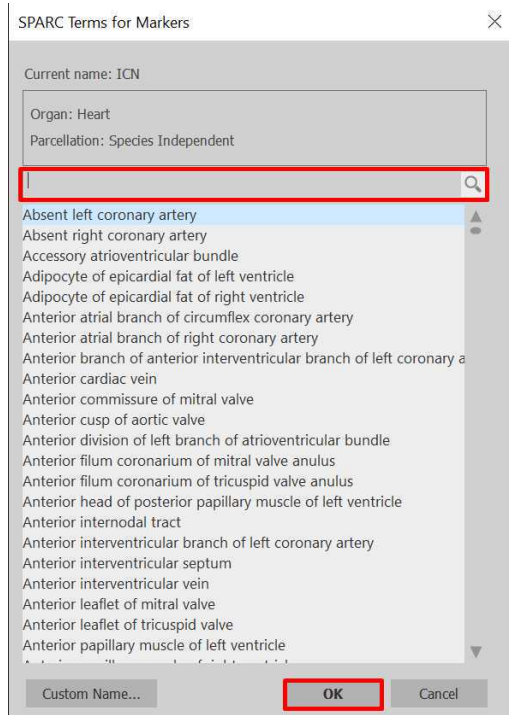


Marking with FAIR terminology

- 7 In the marker toolbar on the left side of the program, right-click on the marker type you would like to use. Select the option to **Rename Marker**.

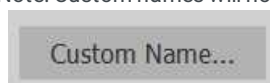


- 8 The **SPARC Terms for Markers** window will appear. Search for and/or select the term you would like to name the marker and click **OK**. The example below shows the SciCrunch terms associated with the rat heart.



You may also add a marker name by using the **Custom Name...** button. Simply type in the custom marker name and select **OK** to apply the custom marker name. Select **OK** once again in the **SPARC Terms for Markers** window.

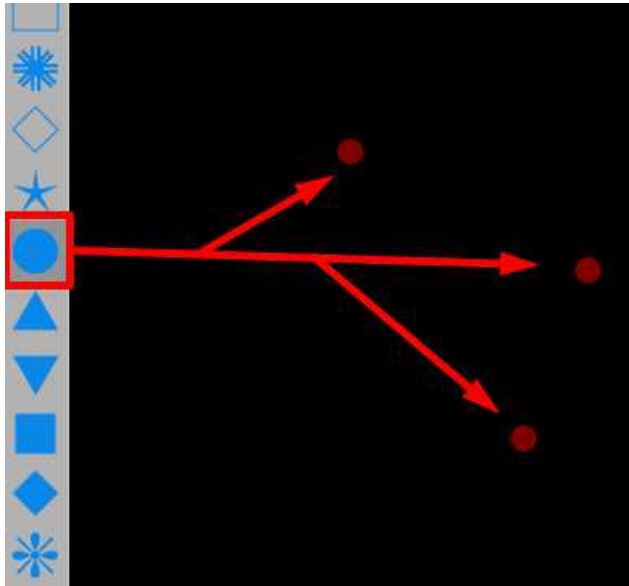
Note: Custom names will not have persistent, unique identifiers from SciCrunch.



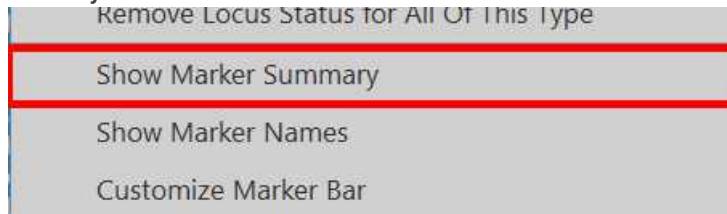
You can see what the current name of the marker is here:



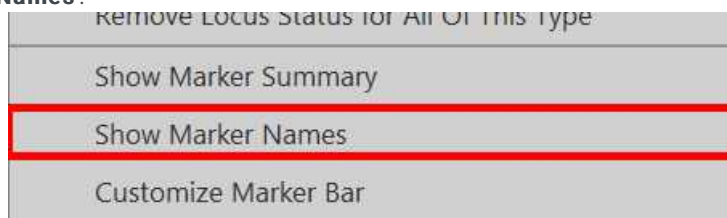
- 9 You may now select the marker from the marker toolbar and click in the 2D window to place the marker on the image.



To show the number of each marker placed, right-click anywhere on the marker toolbar and enable '**Show Marker Summary**'.



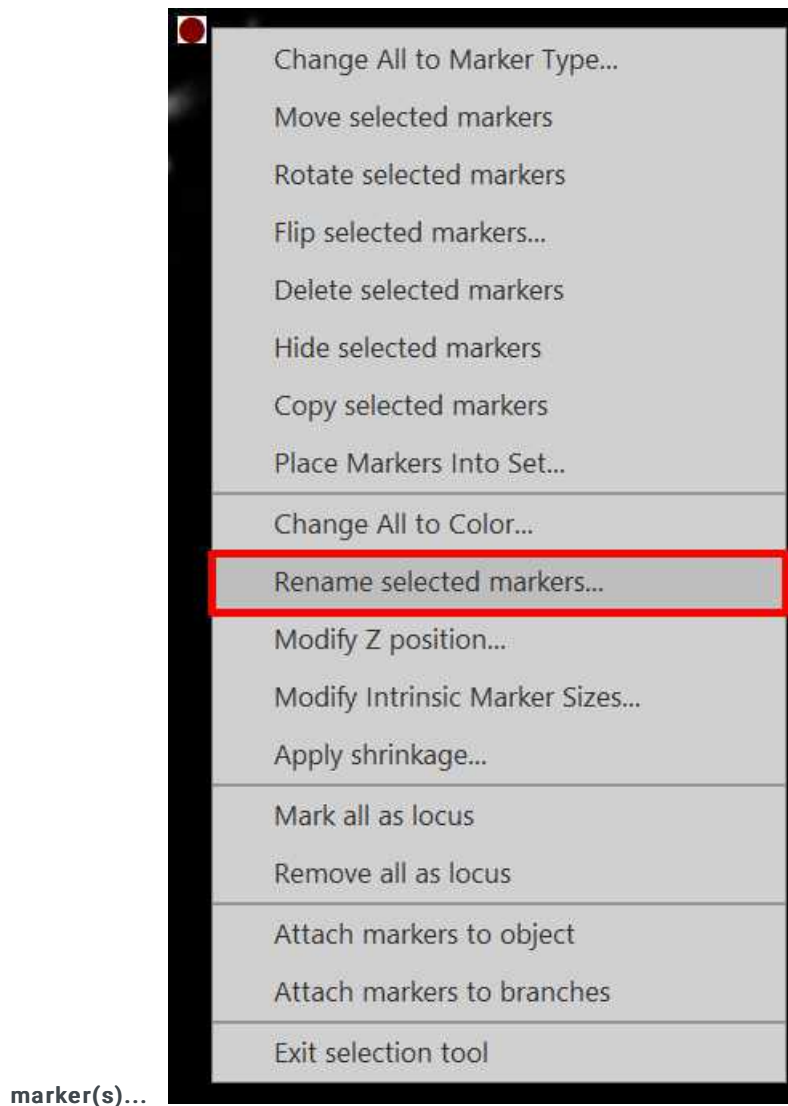
To show the names of each marker, right-click anywhere on the marker toolbar and enable '**Show Marker Names**'.



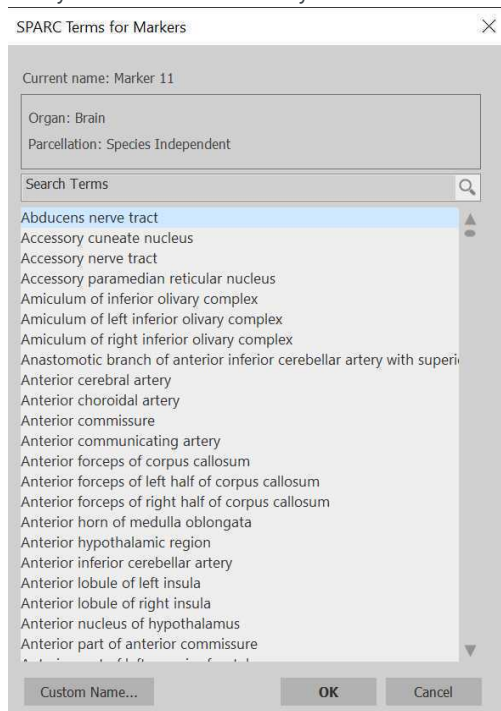
- 10 To rename markers that are already placed, click on the **Select objects** icon in the Trace ribbon of the software and select one or more markers in the 2D window you would like to classify.



10.1 Right-click with your mouse to open the marker options menu. Select **Rename selected**



- 10.2 Search for and/or select the marker name you would like the selected marker(s) to be renamed as. When you've selected the term you would like to be associated with the marker(s), click **OK**.



You may also add a marker name by using the **Custom Name...** button. Simply type in the custom marker name and select **OK** to apply the custom marker name. Select **OK** once again in the **SPARC Terms for Markers** window.

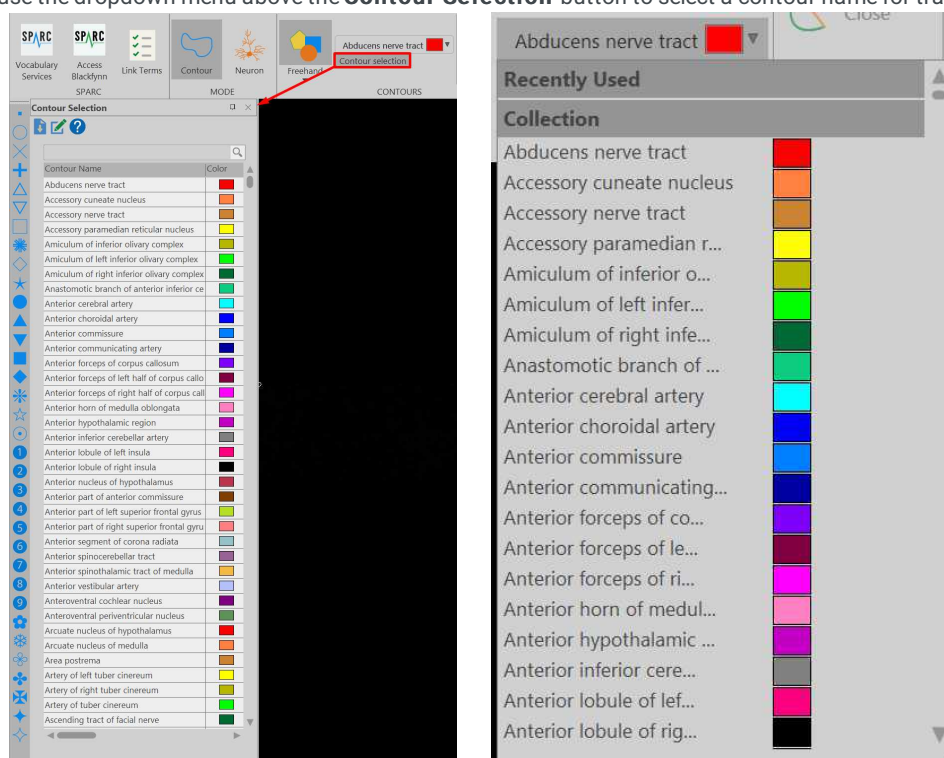
Custom Name...

Contouring with FAIR terminology

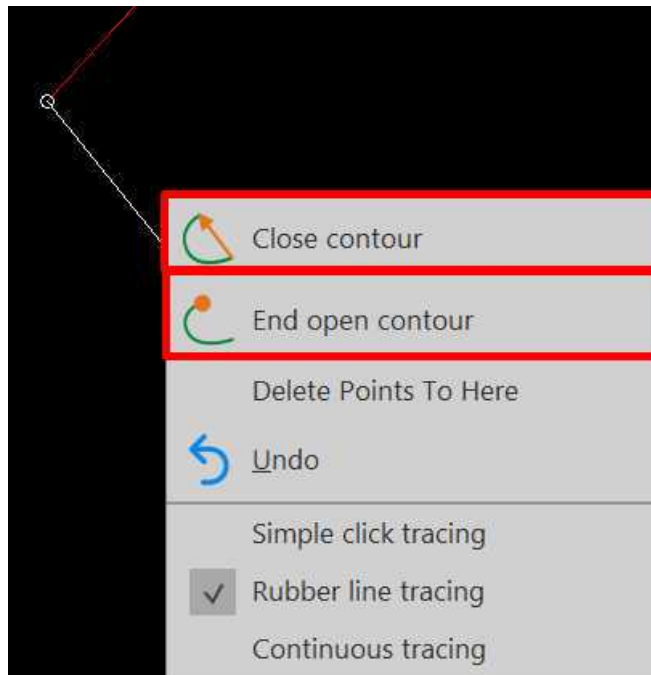
- 11 Select the **Contour** icon in the Trace ribbon of the software.



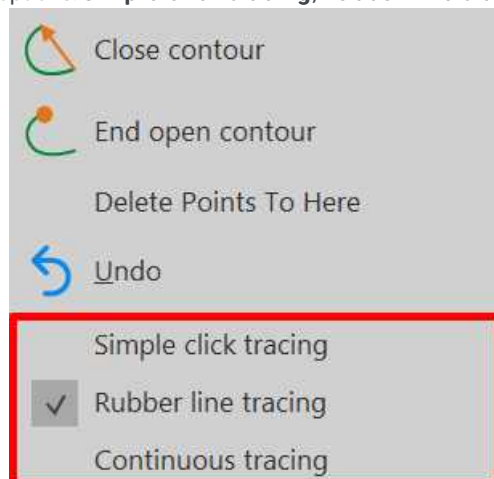
- 12 Select the **Contour Selection** button to open the Contour Selection window to choose a contour name for tracing **OR** use the dropdown menu above the **Contour Selection** button to select a contour name for tracing.



- 12.1 To place a contour on the image in the 2D window, simply click in the image to place contour points. When you have completed your contour, right-click and select either **Close Contour** to finish your closed contour or **End Open Contour** to finish your open-ended contour.



Change the method of tracing a contour by right-clicking and selecting one of the following options: **Simple click tracing**, **Rubber line tracing**, or **Continuous tracing**.



Simple click tracing will only show the last point traced and you will click to place the next point.

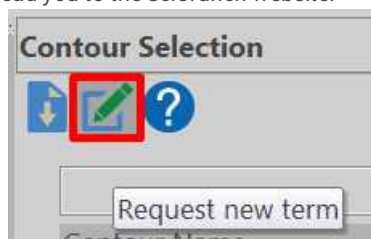
Rubber line tracing will show the last point traced as well as a rubber line connected to the cursor to indicate where the next point will be placed after clicking.

Continuous tracing allows for the cursor to be used as a continuous pen stroke as long as the mouse button is held down.

12.2 Use the **Load in Terms** icon to load in a .csv or .txt file of terms you would like to add to the contour



- 12.3 Request new terms be added to the term list by clicking on the **Request new terms** icon. This will lead you to the SciCrunch website.

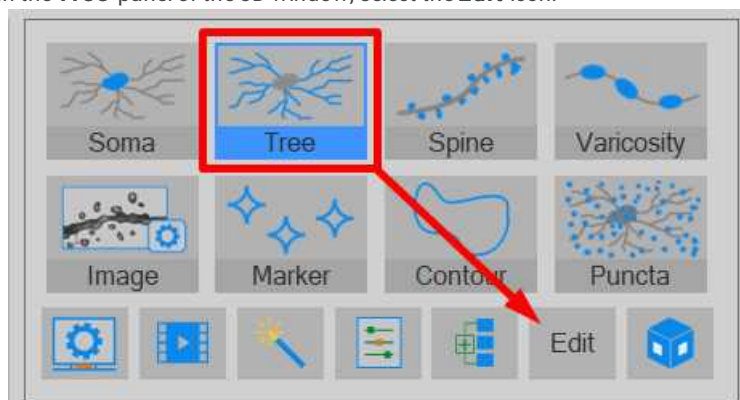


- 13 Repeat contouring process for all anatomical annotations in your sample.

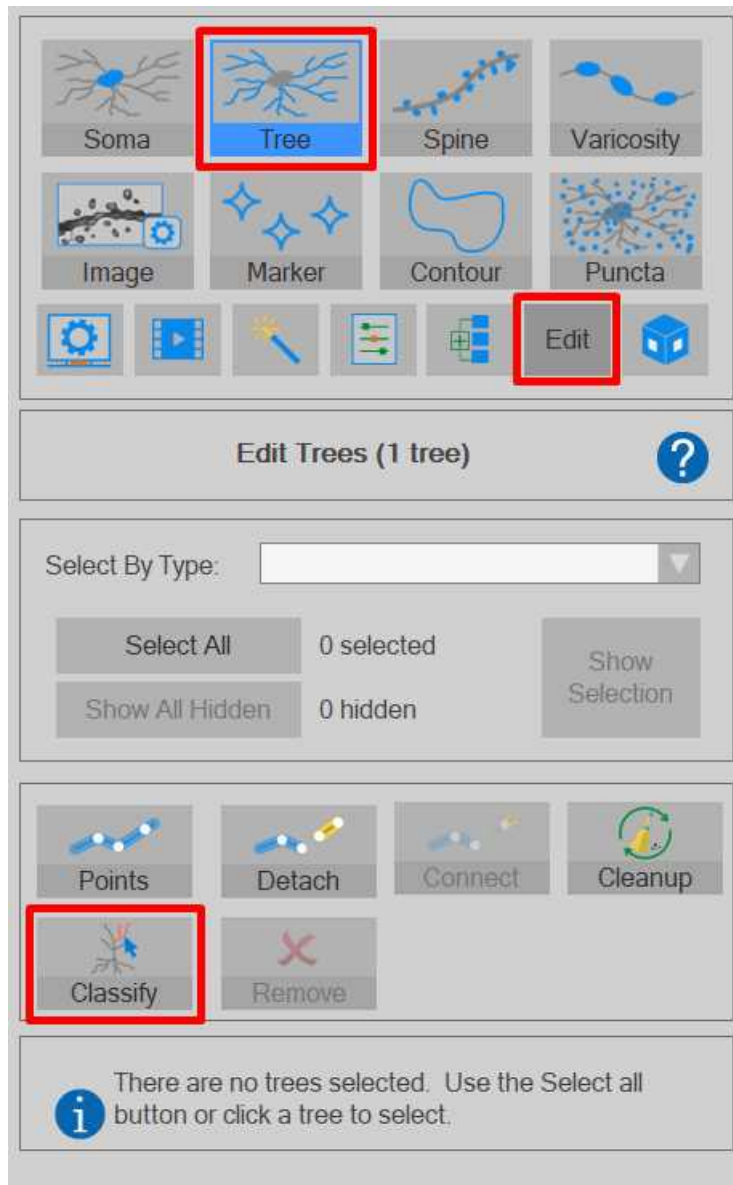
Classifying trees with FAIR terminology

- 14 After tracing the trees or loading in a data file containing trees, you will be able to classify the trees by using the **Classify** function in the **Edit Trees** panel of the 3D window.

- 14.1 In the **Tree** panel of the 3D window, select the **Edit** icon.



- 14.2 In **Tree Edit** mode, select the **Classify** icon.



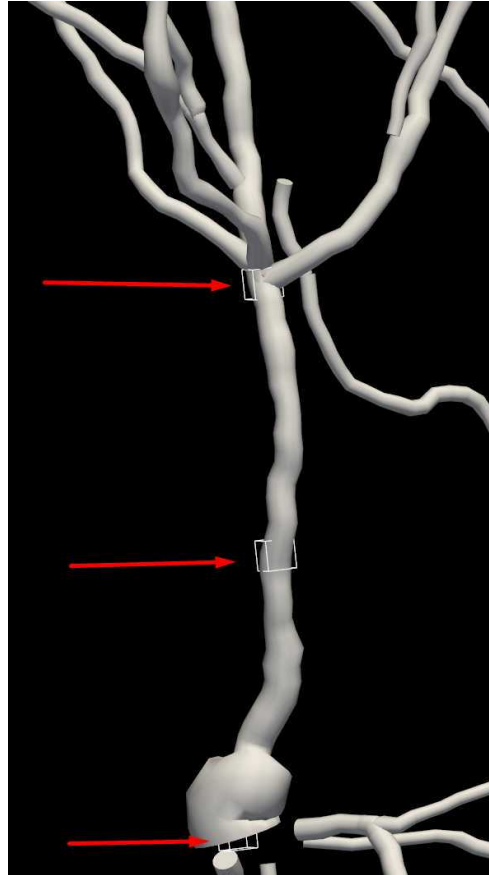
14.3 In **Classify** mode, select the tree segment(s) in the 3D window you would like to classify.

Once entering **Classify** mode, all the trees will change to the color gray. Unclassified trees will remain gray.

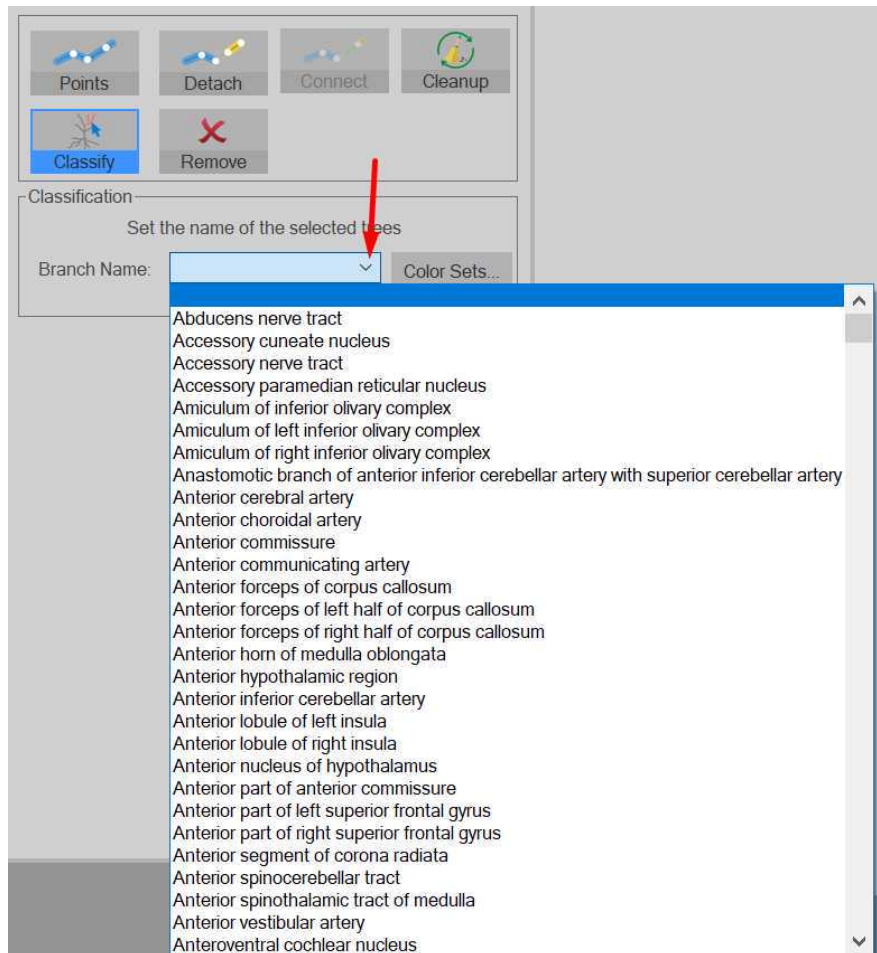


Select a tree segment by simply clicking on a tree in the 3D window. When selected, a tree segment will show three squares: one on each end of the segment and one in the middle. A tree segment is not always the whole tree, as seen below.

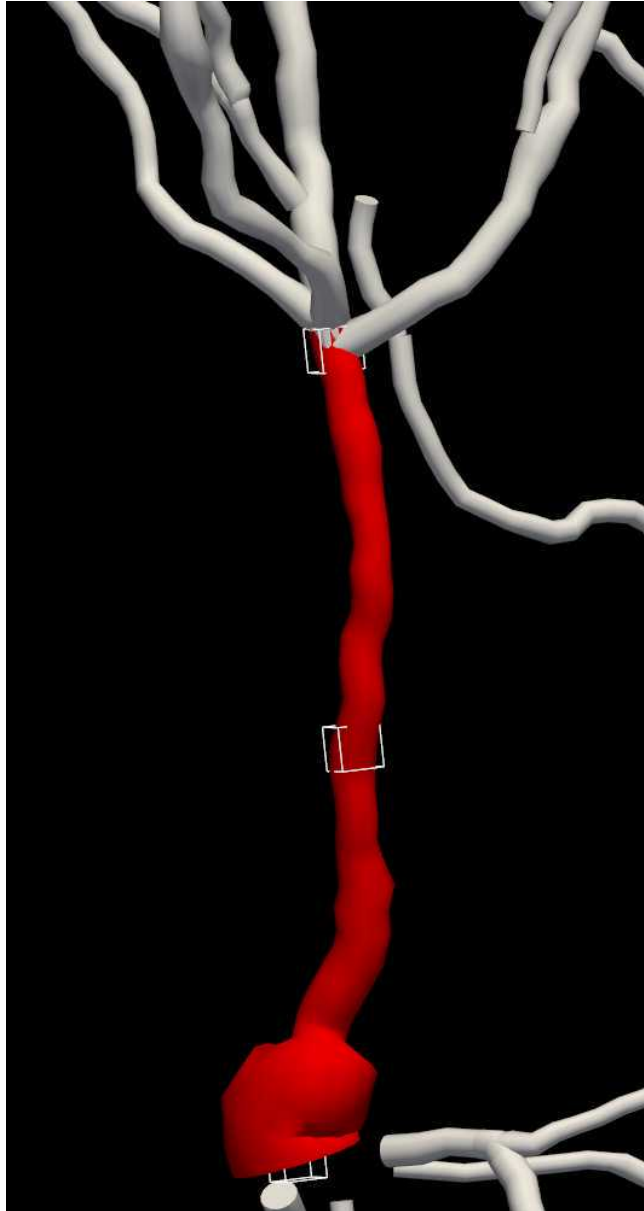
To select multiple segments, hold down the Ctrl button and click to select the segments.



- 14.4 Using the **Branch Name** dropdown menu, select the branch name you would like to classify the selected branches as.



When a tree segment has been classified, it will change to the color associated with the term selected. Unclassified segments of the tree will remain gray.

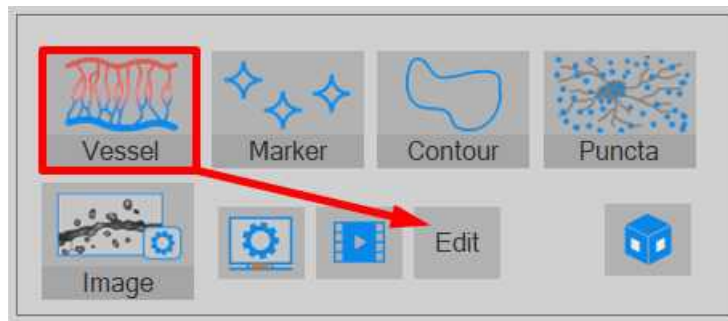


- 15 Repeat step 15 for all trees or tree segments you wish to classify.

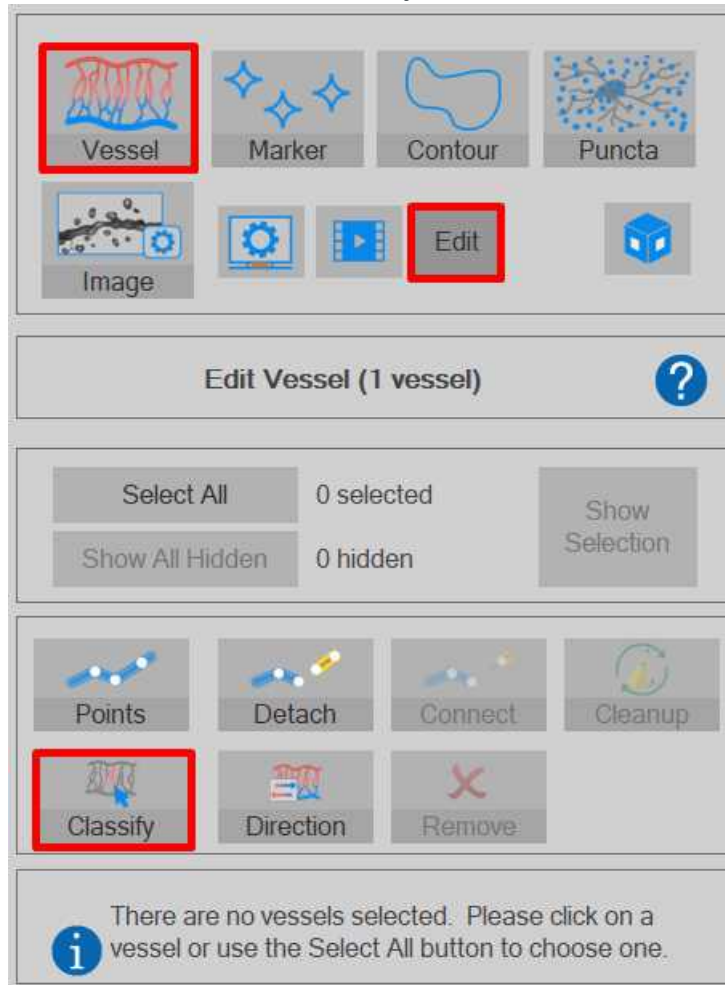
Classifying vessels with FAIR terminology

- 16 After tracing the vessels or loading in a data file containing vessels, you will be able to classify the vessels by using the **Classify** function in the **Edit Vessels** panel of the 3D window.

- 16.1 In the **Vessel** panel of the 3D window, select the **Edit** icon.



16.2 In **Vessel Edit** mode, select the **Classify** icon.



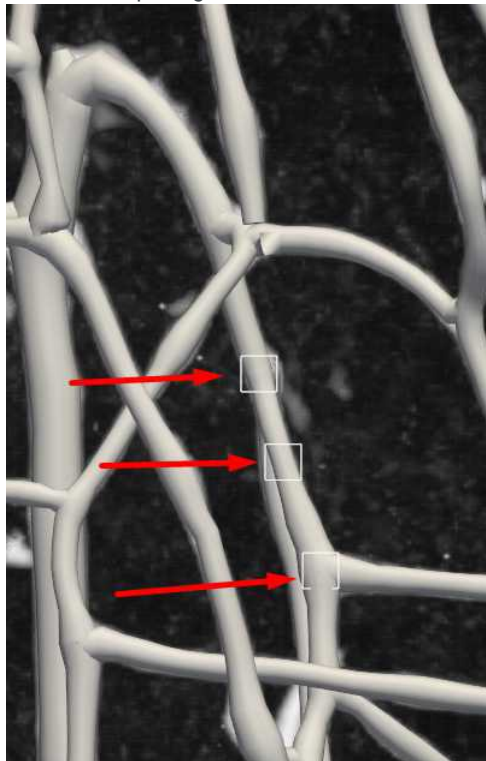
16.3 In **Classify** mode, select the vessel(s) in the 3D window you would like to classify.

Once entering **Classify** mode, all the vessels will change to the color gray. Unclassified vessels will remain gray.

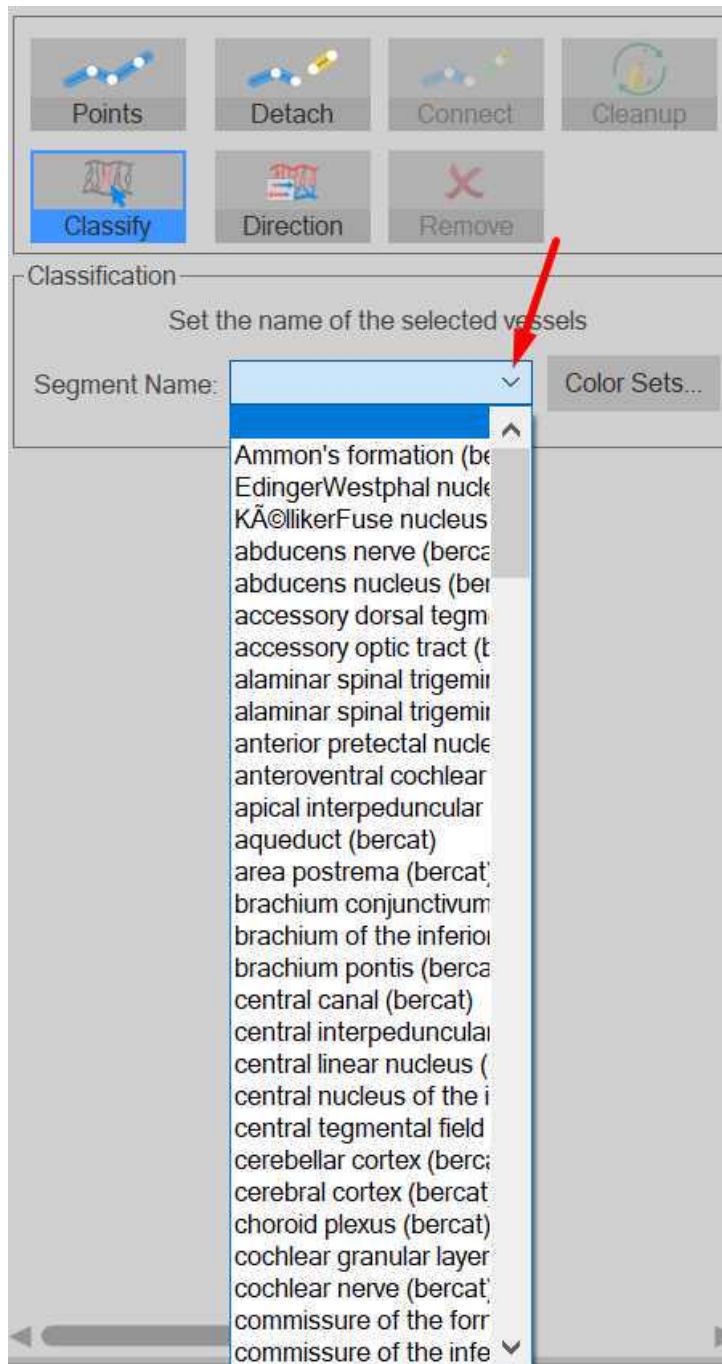


Select a vessel segment by simply clicking on a vessel in the 3D window. When selected, a vessel segment will show three squares: one on each end of the segment and one in the middle. A vessel segment is not always the whole vessel, as seen below.

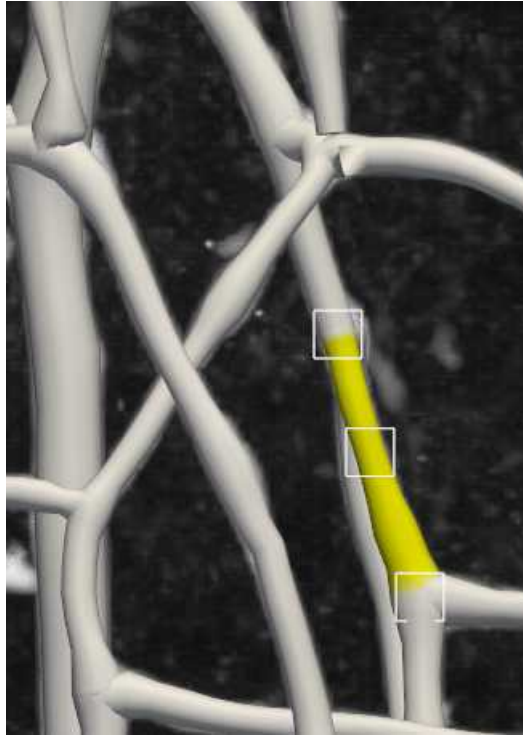
To select multiple segments, hold down the Ctrl button and click to select the segments.



- 16.4 Using the **Segment Name** dropdown menu, select the segment name you would like to classify the selected vessel segments as.



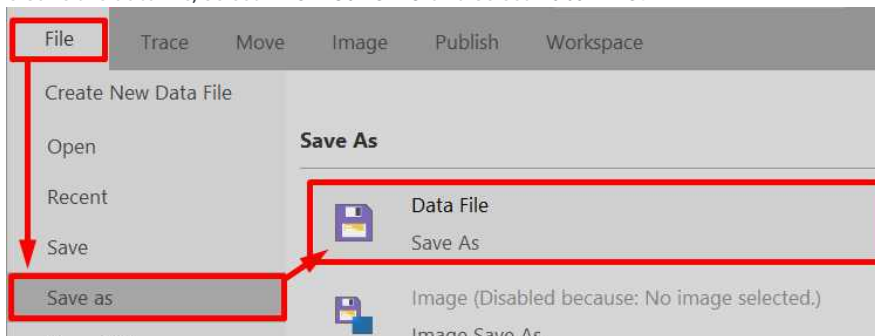
When a vessel segment has been classified, it will change to the color associated with the term selected. Unclassified segments of the vessel will remain gray.



- 17 Repeat step 17 for all vessels or vessel segments you wish to classify.

Saving your Data as an XML

- 18 To save the data file, select **File > Save As** and select **Data File**.



- 19 In the save dialog window, save your data file as an **XML Document File (*.xml)**

