



Feb 26, 2021

# Neurolucida 360: Importing a 3D Organ Scaffold Model for Fiducial Marking

Maci Heal<sup>1</sup><sup>1</sup>MBF Bioscience**1** *Works for me* This protocol is published without a DOI. Amanda Raymond

SUBMIT TO PLOS ONE

## ABSTRACT

How to import a generic 3D organ scaffold model into Neurolucida 360.

## PROTOCOL CITATION

Maci Heal 2021. Neurolucida 360: Importing a 3D Organ Scaffold Model for Fiducial Marking. **protocols.io**  
<https://protocols.io/view/neurolucida-360-importing-a-3d-organ-scaffold-mode-br9qm95w>

## LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

Feb 09, 2021

## LAST MODIFIED

Feb 26, 2021

## PROTOCOL INTEGER ID

47120

## 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legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](https://protocols.io) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](https://protocols.io), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

## Loading an Image, Adding Metadata, and Accessing Vocabularies

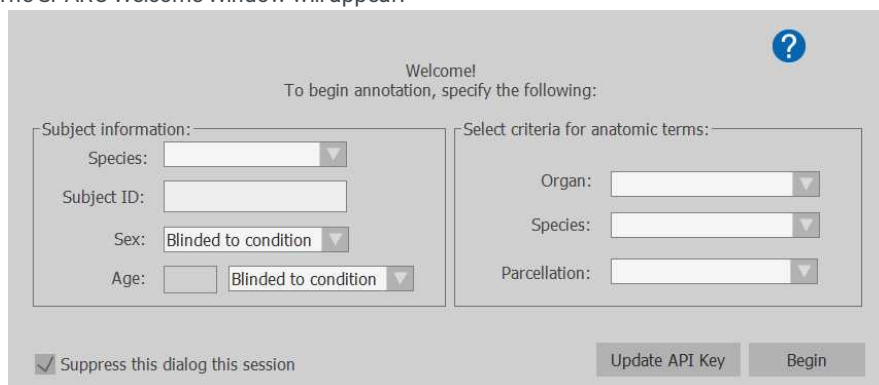
- 1 Launch Neurolucida 360 with SPARC-mode enabled.

**Neurolucida 360** 

by MicroBrightField Bioscience

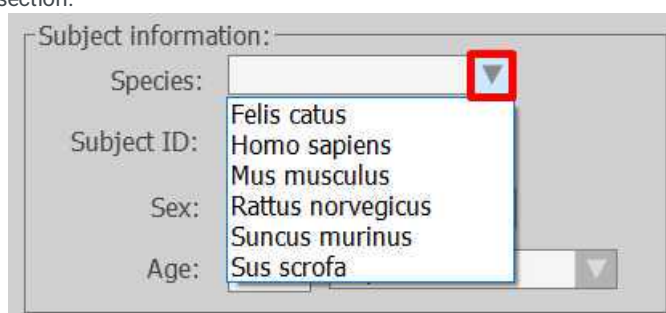
2 Open a microscopy image via the **Open** icon, **File>Open**, or dragging and dropping into the program window.

3 The SPARC Welcome Window will appear.



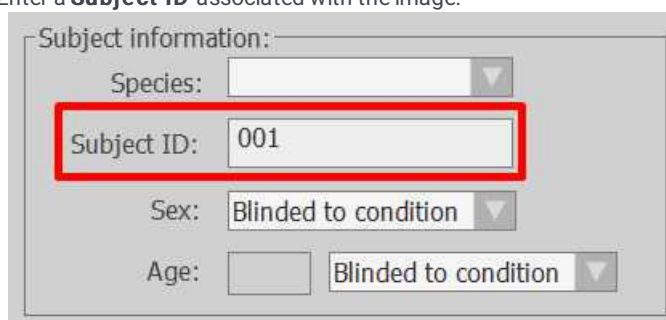
The SPARC Welcome Window is a dialog box with a title bar containing a question mark icon. The main text reads "Welcome! To begin annotation, specify the following:". It is divided into two main sections. The left section, titled "Subject information:", contains four fields: "Species:" with a dropdown menu, "Subject ID:" with a text input field, "Sex:" with a dropdown menu set to "Blinded to condition", and "Age:" with a text input field and a dropdown menu set to "Blinded to condition". The right section, titled "Select criteria for anatomic terms:", contains three fields: "Organ:" with a dropdown menu, "Species:" with a dropdown menu, and "Parcellation:" with a dropdown menu. At the bottom left is a checkbox labeled "Suppress this dialog this session". At the bottom right are two buttons: "Update API Key" and "Begin".

3.1 Use the dropdown menu to select the **Species** associated with the image in the Subject Information section.



This image shows a close-up of the "Species:" dropdown menu in the "Subject information:" section. The dropdown is open, displaying a list of species: "Felis catus", "Homo sapiens", "Mus musculus", "Rattus norvegicus", "Suncus murinus", and "Sus scrofa". A red square highlights the dropdown arrow icon.

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



This image shows a close-up of the "Subject ID:" text input field in the "Subject information:" section. The field contains the text "001". A red rectangle highlights the entire "Subject ID:" label and its corresponding text input field.

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.

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:

- Felis catus
- Homo sapiens
- Mus musculus
- Rattus norvegicus
- Suncus murinus
- Sus scrofa

Update API Key

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

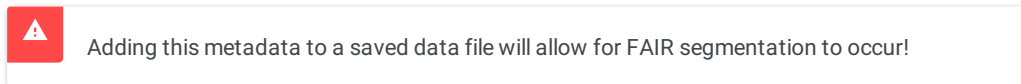
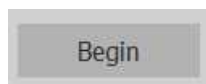
Select criteria for anatomic terms:

Organ:

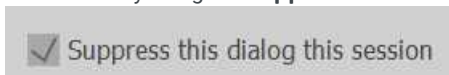
Species:

Parcellation:

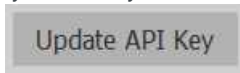
3.7 Click **Begin** to add the selected metadata to the data file when saved and to open the image loaded.



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.



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

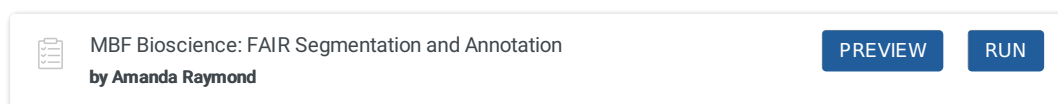


6 You may access and update the SPARC Welcome Window information at any time by selecting the **Vocabulary Services** icon in the Trace ribbon of SPARC mode-enabled MBF software.

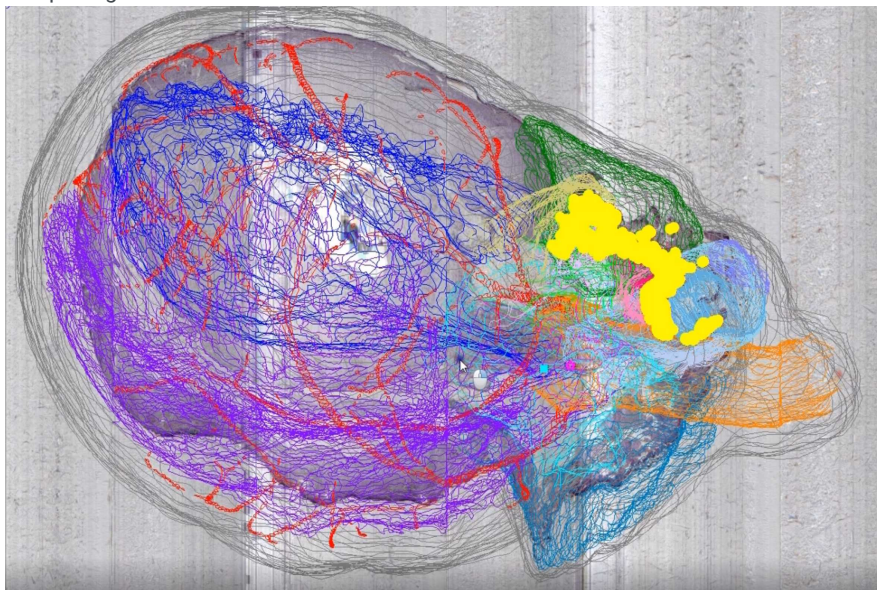


## Segment

7 Add contours, makers, and/or trees to your image with SPARC vocabulary terms. Save the data as an **XML Document File (\*.xml)**. See below protocol for more about this process.



Example segmentation of a rat heart.



Data courtesy of Drs. James Schwaber and Zixi Cheng labs.

## 7.1 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

## 7.2 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.*

## 7.3 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.

Welcome!  
To begin annotation, specify the following:

Subject information:

Species:

Subject ID:

Sex:

Age:

Select criteria for anatomic terms:

Organ:

Species:

Parcellation:

☒ Suppress this dialog this session

7.3.1 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**.

Subject information:

Species:

Subject ID:

Sex:

Age:

Felis catus  
Homo sapiens  
Mus musculus  
Rattus norvegicus  
Suncus murinus  
Sus scrofa

7.3.2 Enter a **Subject ID** associated with the image.

Subject information:

Species:

Subject ID:

Sex:

Age:

7.3.3 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:

Use the dropdown menu to select the **Organ** associated with the image. This is required information

7.3.4 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

7.3.5 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:

- Felis catus
- Homo sapiens
- Mus musculus
- Rattus norvegicus
- Suncus murinus
- Sus scrofa

Update API Key

7.3.6 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:

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

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

7.4 The SPARC Welcome Window will appear.

Your connection has timed out. Please reinput to API key to verify its correctness. 

Subject information:

Species:

Subject ID:

Sex:

Age:

Select criteria for anatomic terms:

Organ:

Species:

Parcellation:

☒ Suppress this dialog this session

7.4.1 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**.

Subject information:

Species:

Subject ID:

Sex:

Age:

7.4.2 Enter a **Subject ID** associated with the image.



Subject information:

Species:

Subject ID:

Sex:

Age:

- 7.4.3 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

- 7.4.4 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

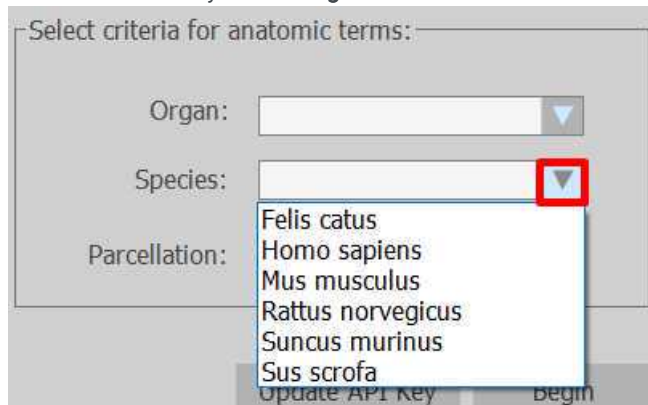
Sympathetic nervous system

Urinary bladder

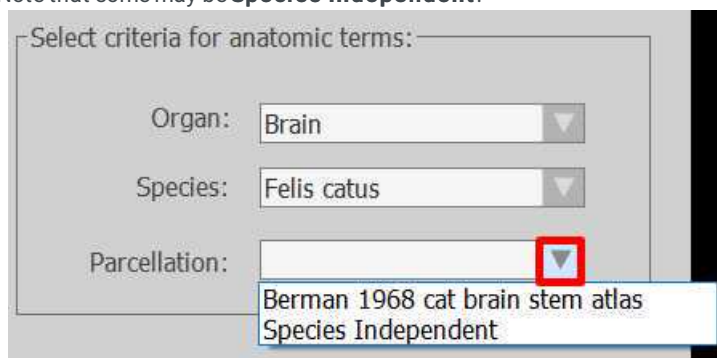
intestine

- 7.4.5 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

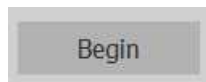
information necessary to click **Begin**.




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



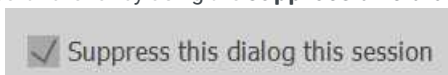
- 7.4.7 Click **Begin** to add the selected metadata to the data file when saved and to open the image loaded.



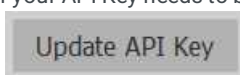


The subject information entered through this dialog will be written to the data file after saving.

- 7.5 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.



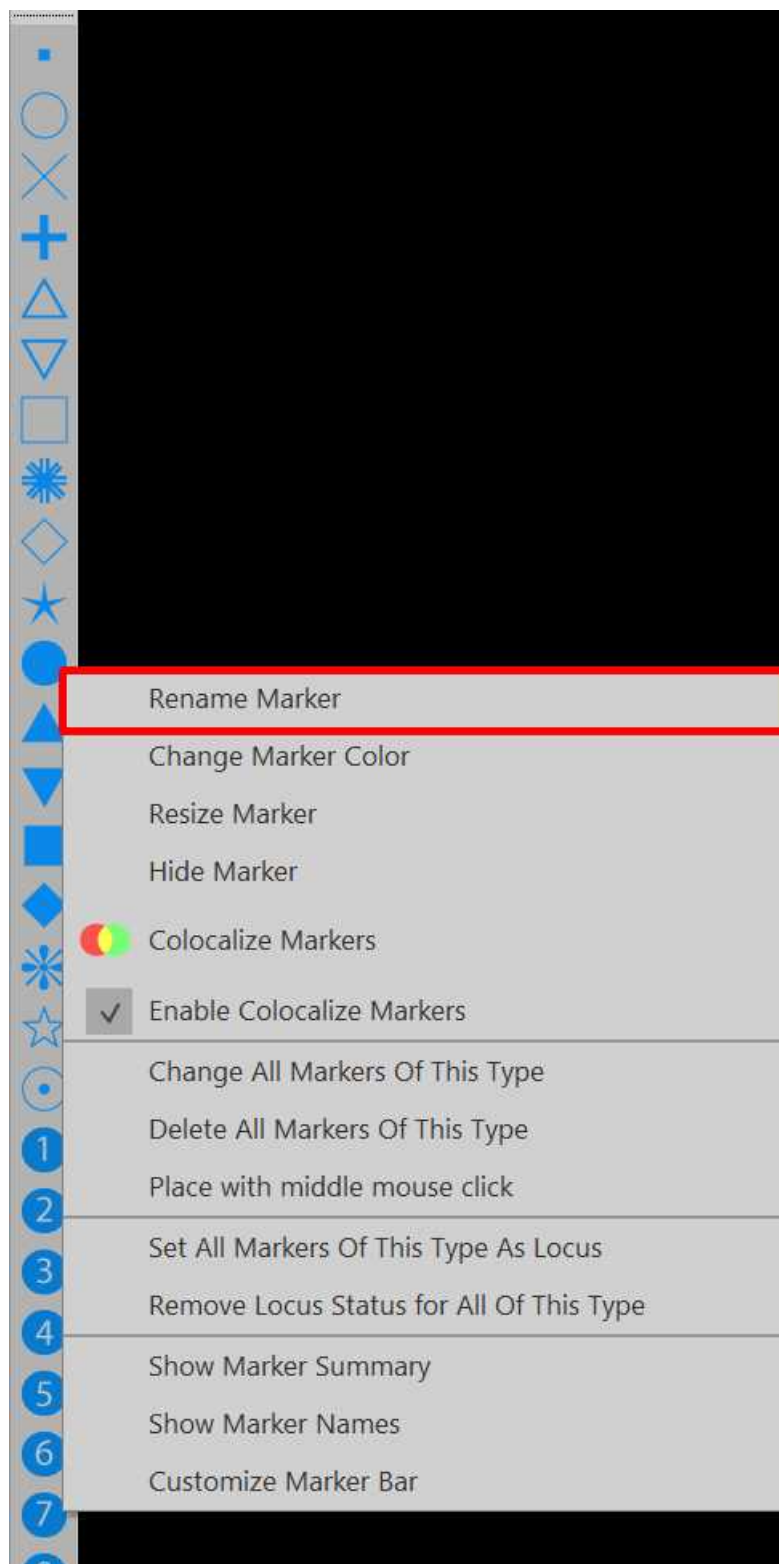
- 7.6 If your API Key needs to be updated, select the **Update API Key** button.



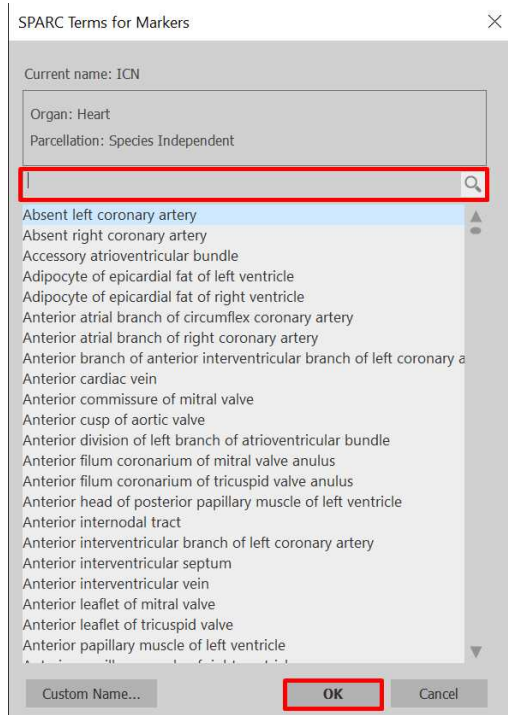
- 7.7 You may access and update the SPARC Welcome Window information at any time by selecting the **Vocabulary Services** icon in the Trace ribbon of SPARC mode-enabled MBF software.



- 7.8 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**.

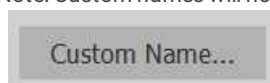


7.9 The **SPARC Terms for Markers** window will appear. Search for and/or select the term you would like to name the marker and click **OK**. The example below shows the SciCrunch terms associated with the rat heart.



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.

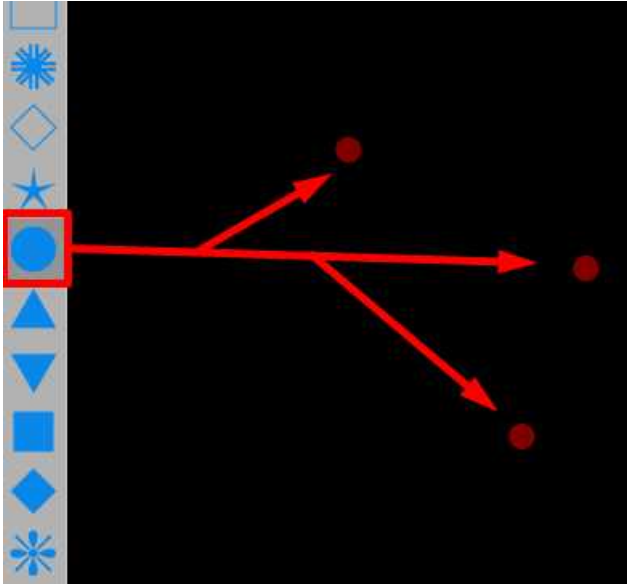
Note: Custom names will not have persistent, unique identifiers from SciCrunch.



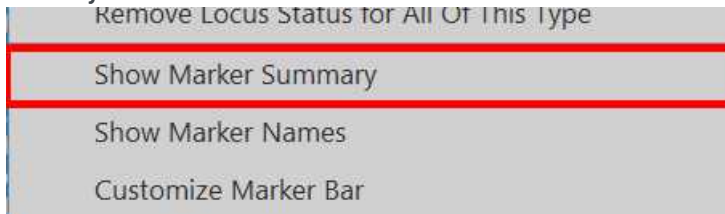
You can see what the current name of the marker is here:



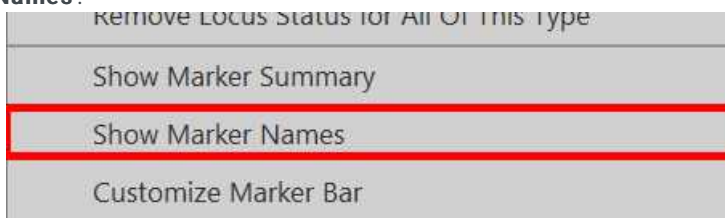
**7.10** You may now select the marker from the marker toolbar and click in the 2D window to place the marker on the image.



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



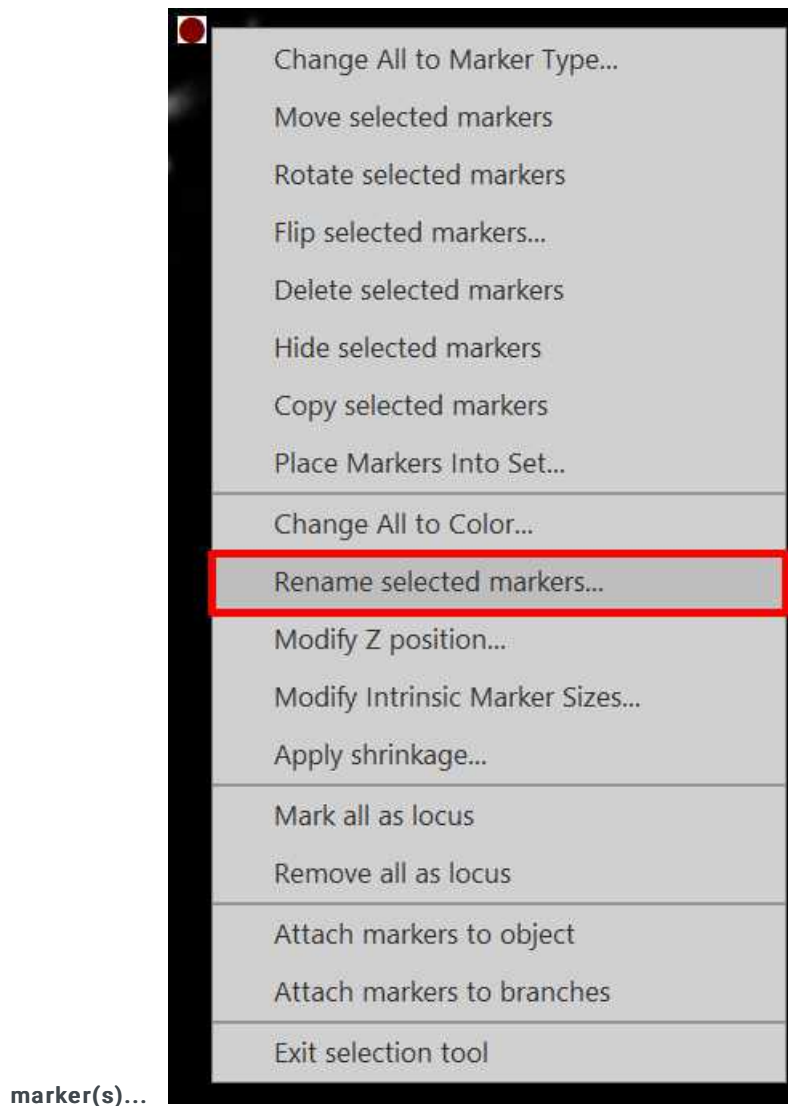
To show the names of each marker, right-click anywhere on the marker toolbar and enable '**Show Marker Names**'.



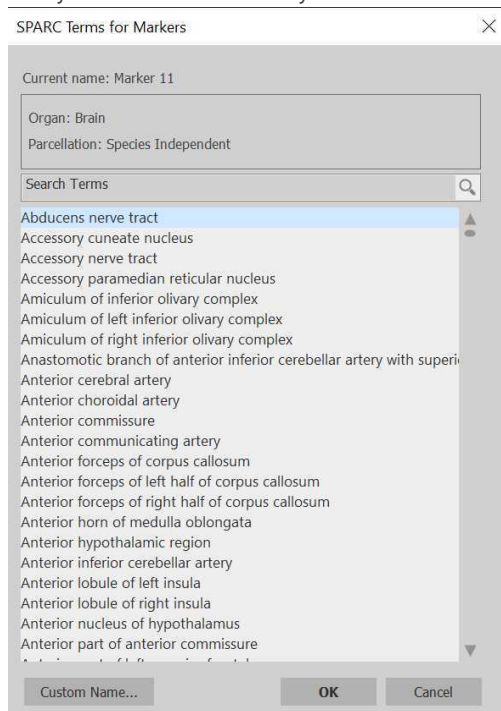
- 7.11 To rename markers that are already placed, click on the **Select objects** icon in the Trace ribbon of the software and select one or more markers in the 2D window you would like to classify.



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



- 7.11.2 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**.





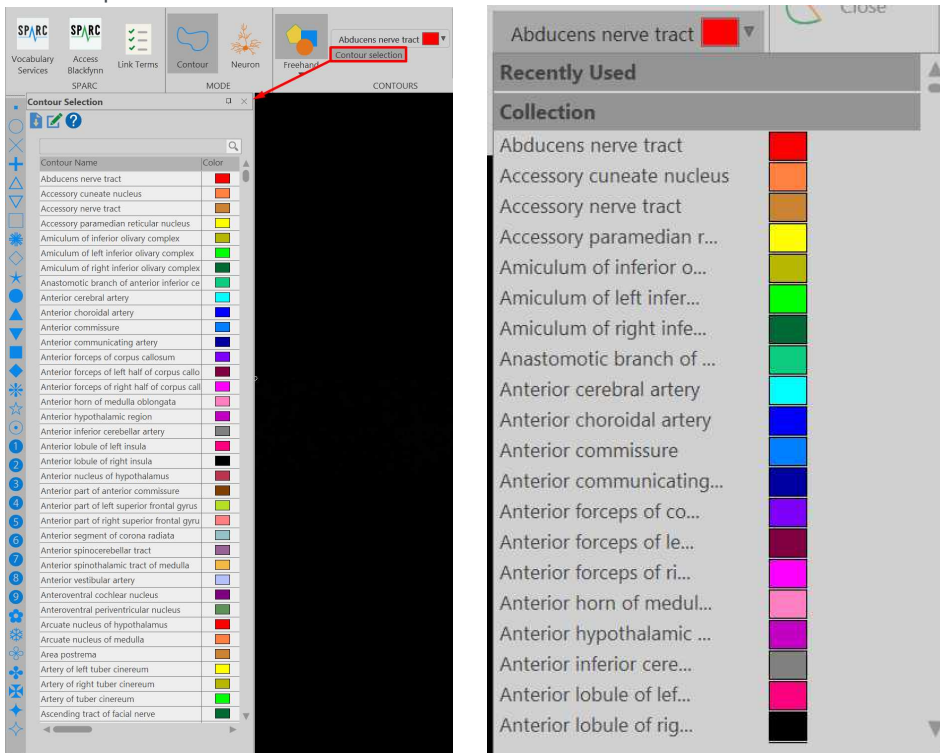
You may also add a marker name by using the **Custom Name...** button. Simply type in the custom marker name and select **OK** to apply the custom marker name. Select **OK** once again in the **SPARC Terms for Markers** window.

Custom Name...

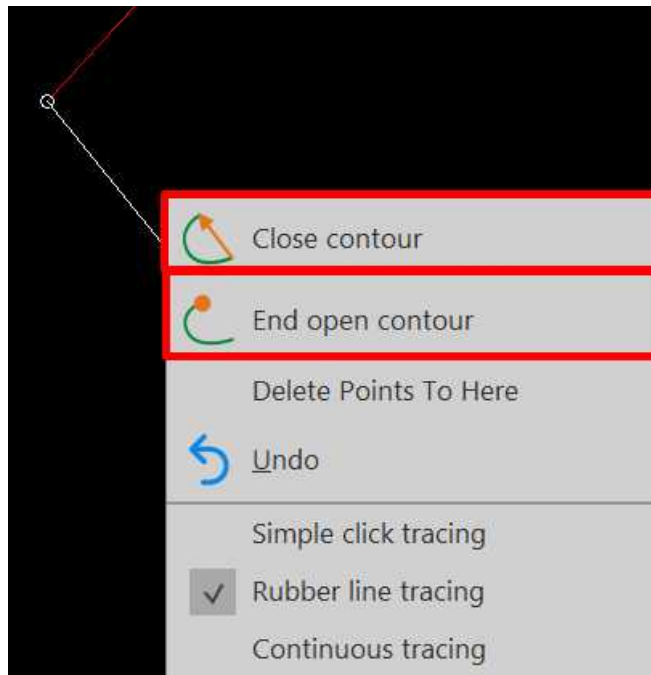
7.12 Select the **Contour** icon in the Trace ribbon of the software.



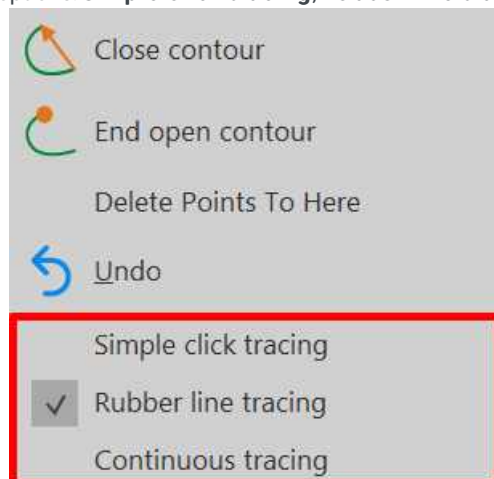
7.13 Select the **Contour Selection** button to open the Contour Selection window to choose a contour name for tracing **OR** use the dropdown menu above the **Contour Selection** button to select a contour name for tracing.



7.13.1 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.

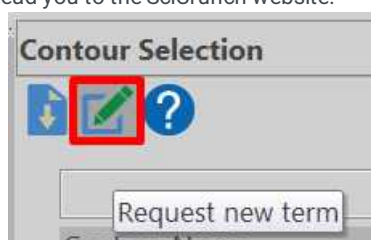
**Rubber line tracing** will show the last point traced as well as a rubber line connected to the cursor to indicate where the next point will be placed after clicking.

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

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



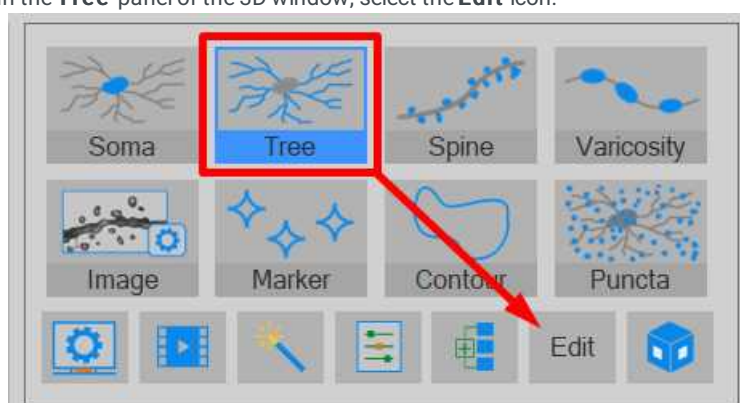
- 7.13.3 Request new terms be added to the term list by clicking on the **Request new terms** icon. This will lead you to the SciCrunch website.



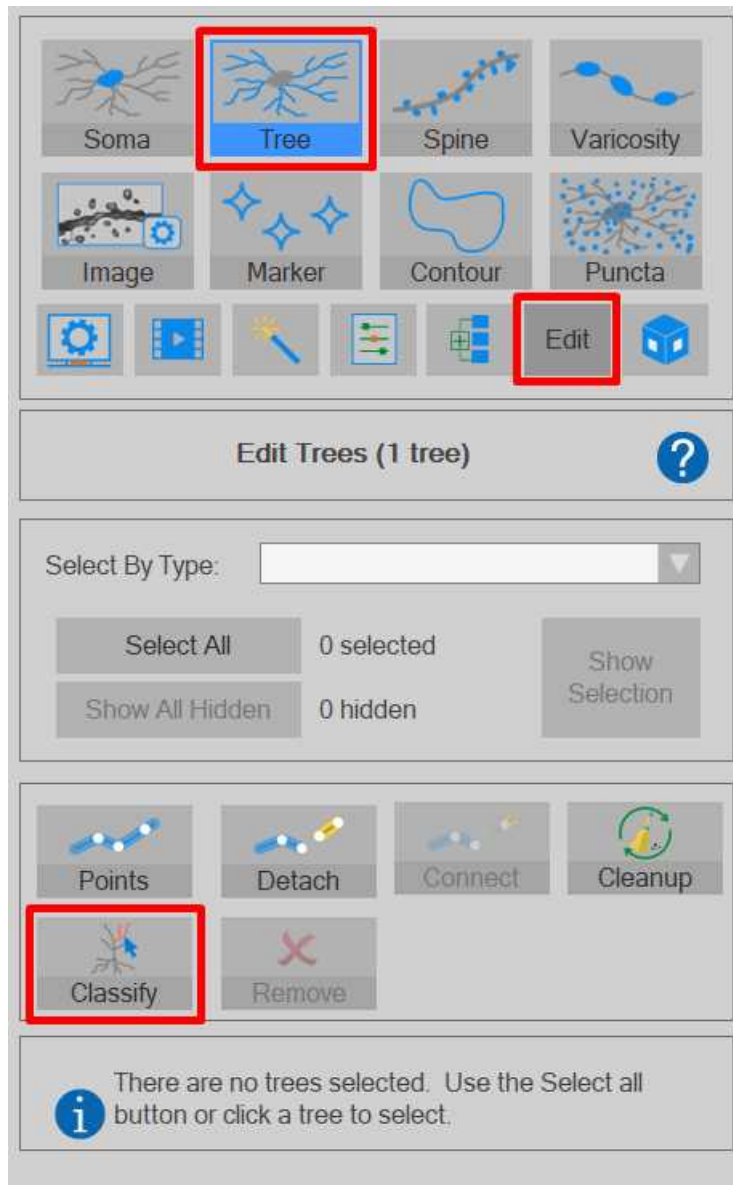
- 7.14 Repeat contouring process for all anatomical annotations in your sample.

- 7.15 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.

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

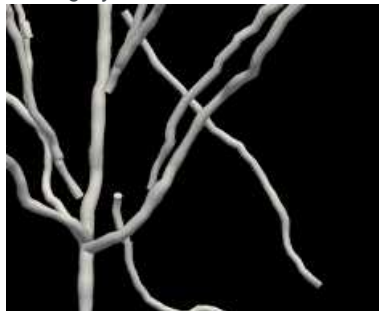


- 7.15.2 In **Tree Edit** mode, select the **Classify** icon.



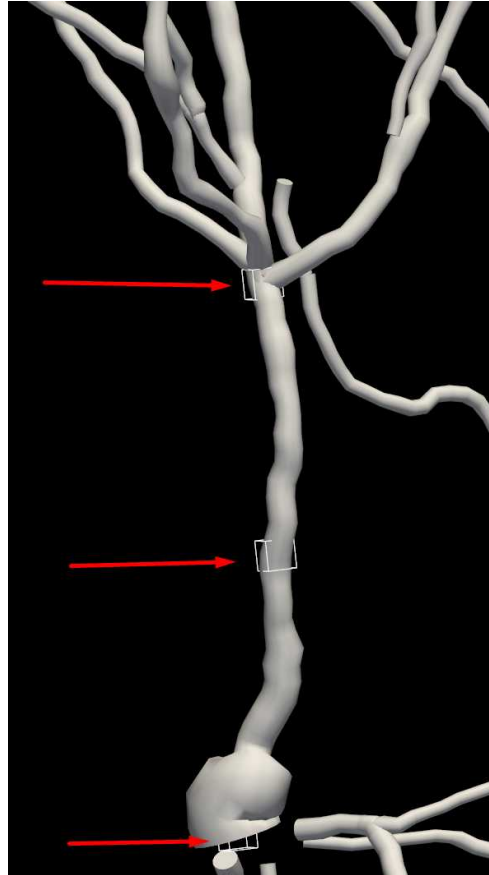
7.15.3 In **Classify** mode, select the tree segment(s) in the 3D window you would like to classify.

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

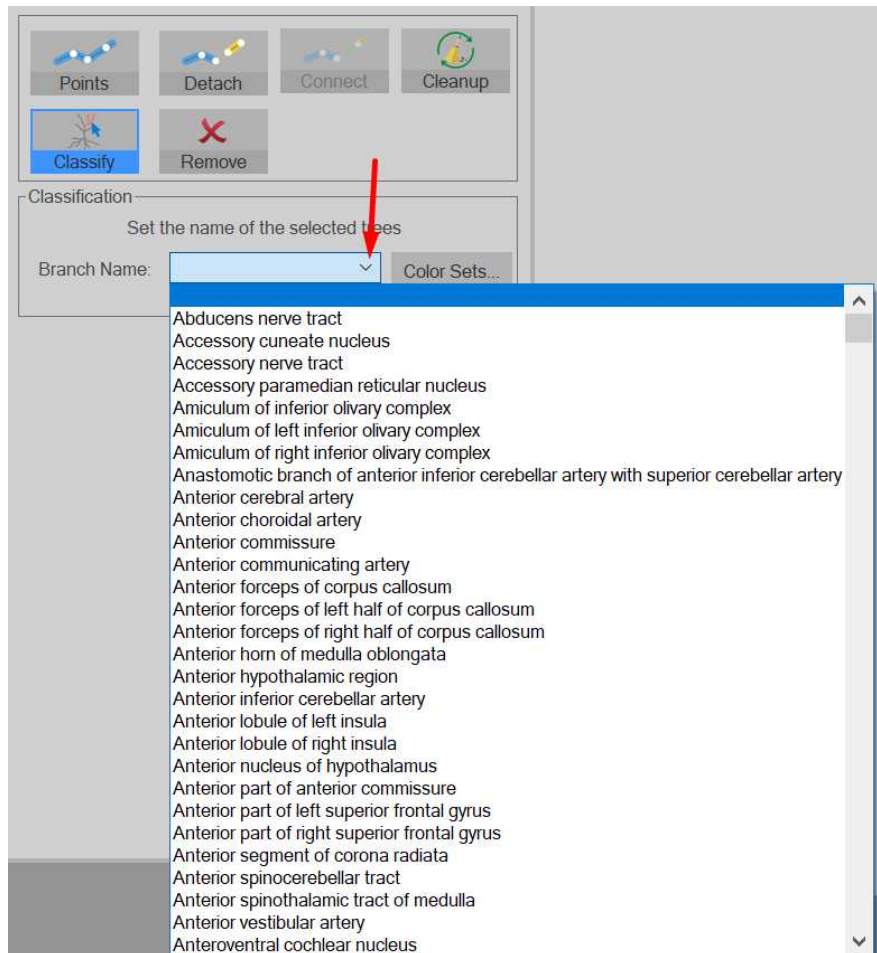


Select a tree segment by simply clicking on a tree in the 3D window. When selected, a tree segment will show three squares: one on each end of the segment and one in the middle. A tree segment is not always the whole tree, as seen below.

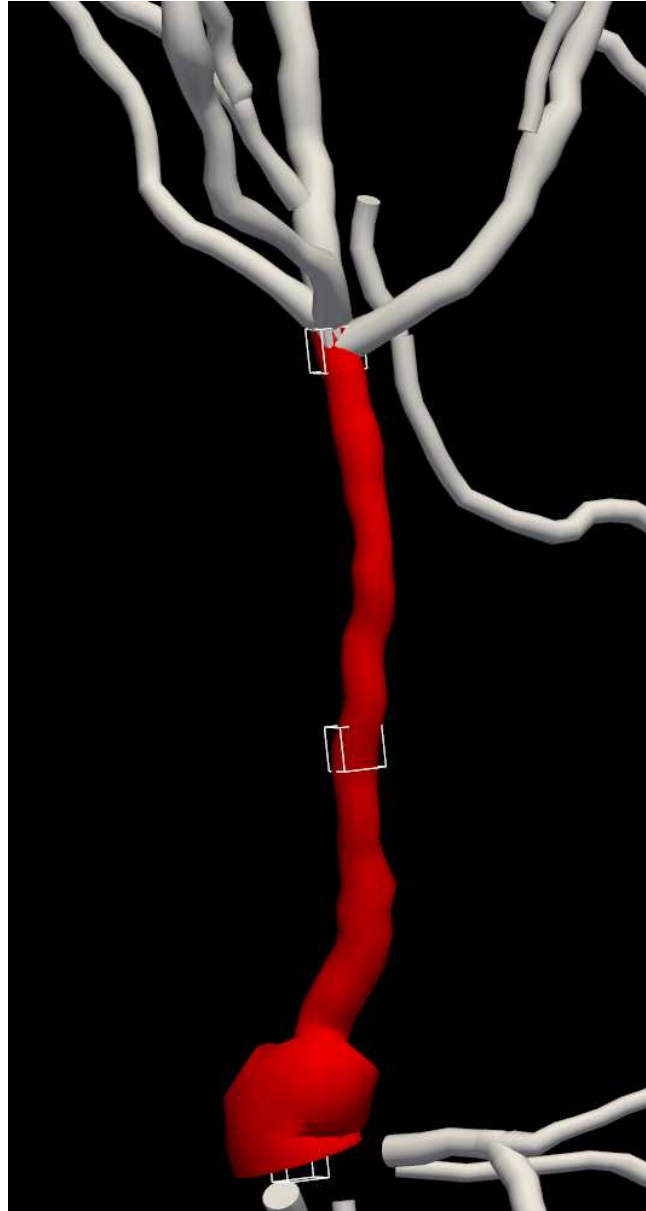
To select multiple segments, hold down the Ctrl button and click to select the segments.



#### 7.15.4 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.

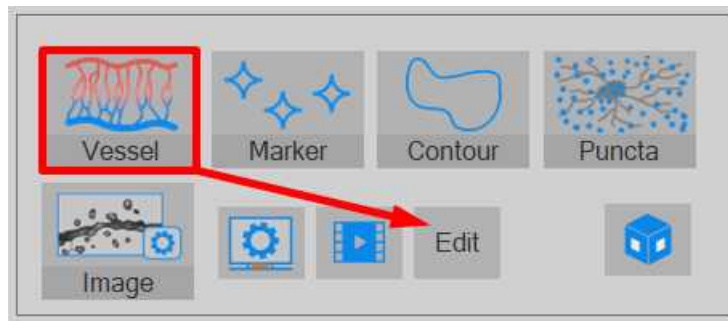


7.16 Repeat step 15 for all trees or tree segments you wish to classify.

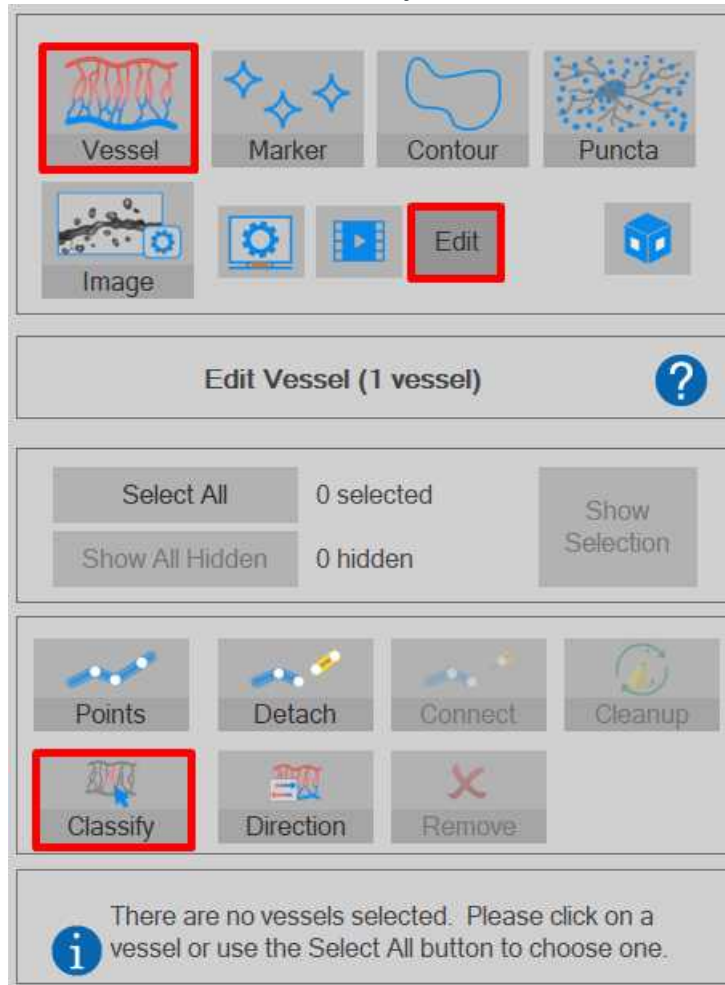
7.17 After tracing the vessels or loading in a data file containing vessels, you will be able to classify the vessels by using the **Classify** function in the **Edit Vessels** panel of the 3D window.

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





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



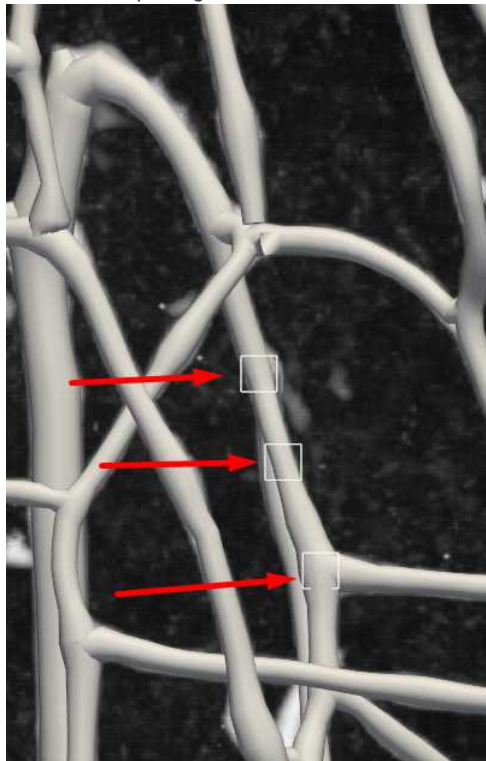
7.17.3 In **Classify** mode, select the vessel(s) in the 3D window you would like to classify.

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

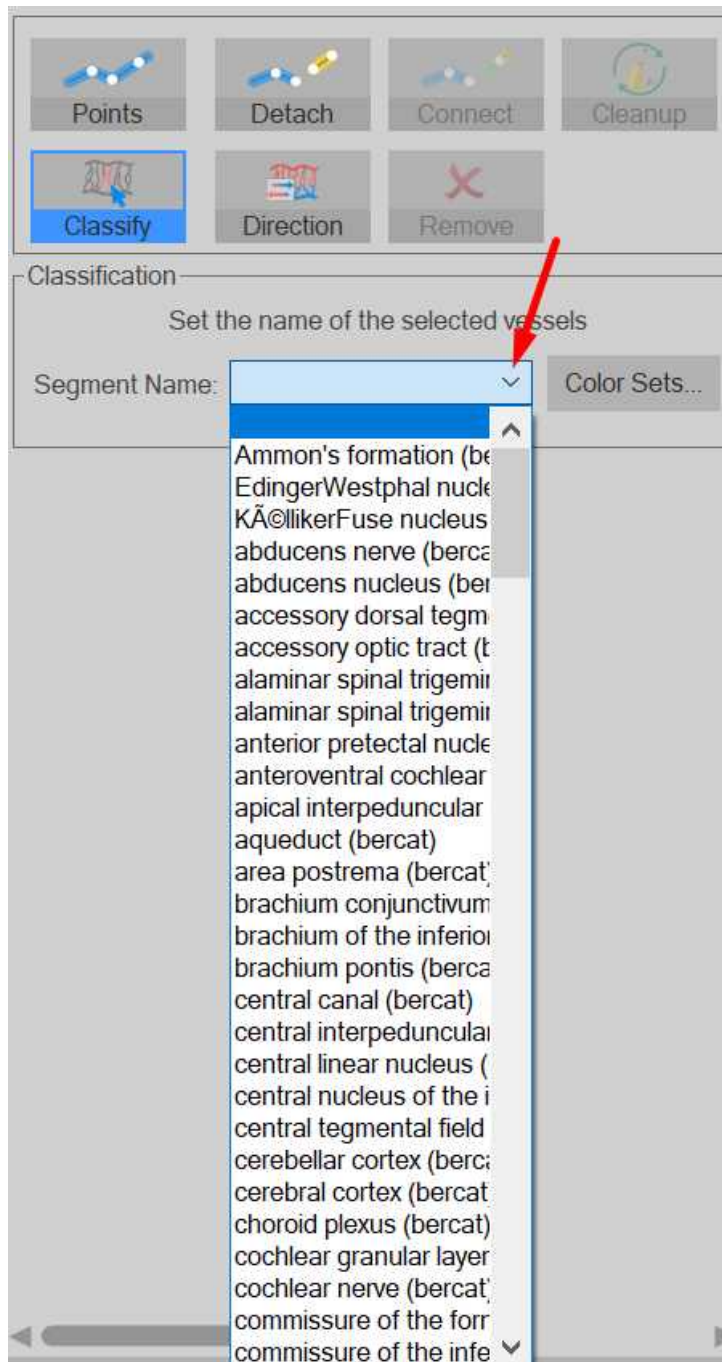


Select a vessel segment by simply clicking on a vessel in the 3D window. When selected, a vessel segment will show three squares: one on each end of the segment and one in the middle. A vessel segment is not always the whole vessel, as seen below.

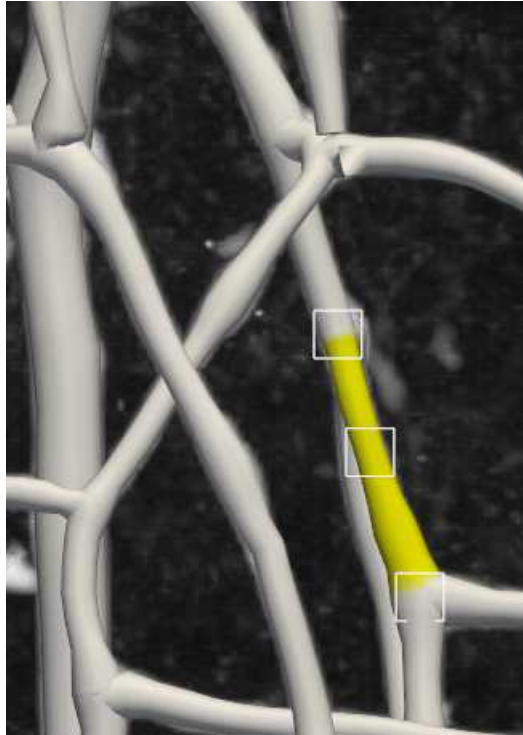
To select multiple segments, hold down the Ctrl button and click to select the segments.



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

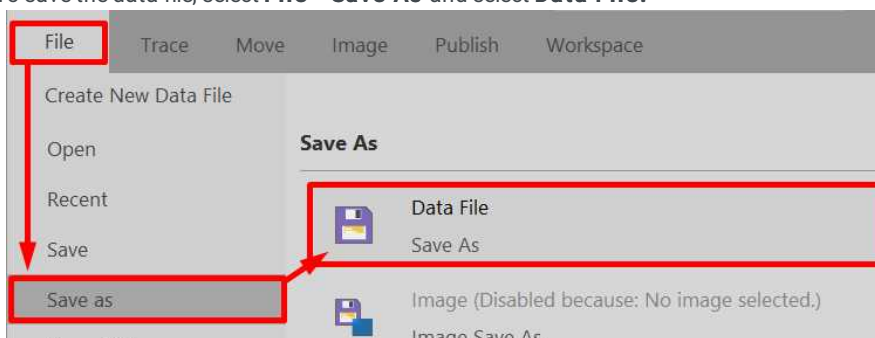


When a vessel segment has been classified, it will change to the color associated with the term selected. Unclassified segments of the vessel will remain gray.

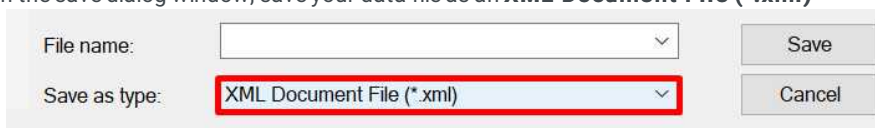


7.18 Repeat step 17 for all vessels or vessel segments you wish to classify.

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

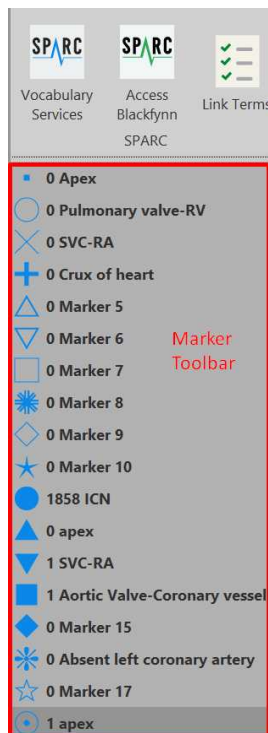


7.20 In the save dialog window, save your data file as an **XML Document File (\*.xml)**

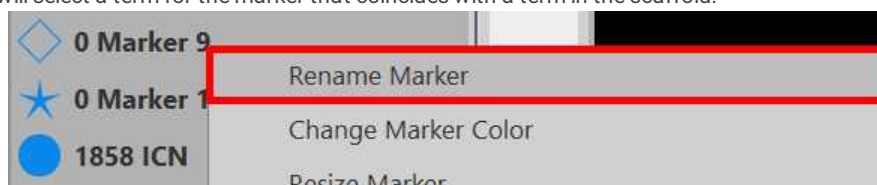


#### Add Fiducial Marker(s)

- 8 On the left side of the program, you will see a marker toolbar. Right-click and select **Show Marker Summary** to show the number of each marker placed on the image and **Show Marker Name** to show the names of each marker.



- 9 Right-click on a marker and select **Rename Marker** to open the SPARC Terms for Markers window. This is where you will select a term for the marker that coincides with a term in the scaffold.



This will become your **fiducial marker**, a unique anatomical point.

- 9.1 In the SPARC Terms for Markers window, select the anatomical term for your fiducial point. You can search for a term in the search bar and select your term by clicking **OK**.

SPARC Terms for Markers ✕

Current name: apex

Organ: Heart

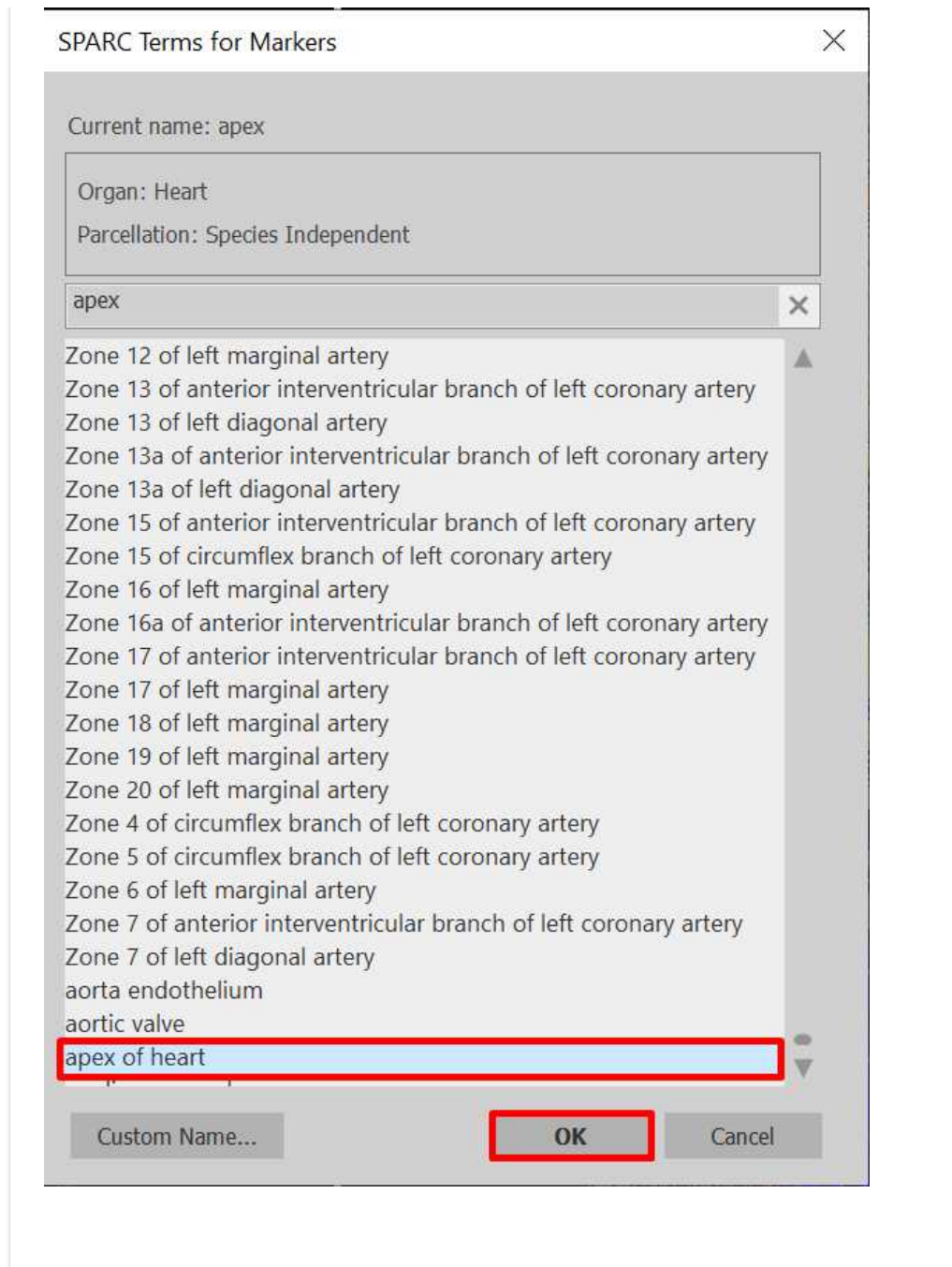
Parcellation: Species Independent

- Absent left coronary artery
- Absent right coronary artery
- Accessory atrioventricular bundle
- Adipocyte of epicardial fat of left ventricle
- Adipocyte of epicardial fat of right ventricle
- Anterior atrial branch of circumflex coronary artery
- Anterior atrial branch of right coronary artery
- Anterior branch of anterior interventricular branch of left coronary artery
- Anterior cardiac vein
- Anterior commissure of mitral valve
- Anterior cusp of aortic valve
- Anterior division of left branch of atrioventricular bundle
- Anterior filum coronarium of mitral valve anulus
- Anterior filum coronarium of tricuspid valve anulus
- Anterior head of posterior papillary muscle of left ventricle
- Anterior internodal tract
- Anterior interventricular branch of left coronary artery
- Anterior interventricular septum
- Anterior interventricular vein
- Anterior leaflet of mitral valve
- Anterior leaflet of tricuspid valve
- Anterior papillary muscle of left ventricle

Custom Name... **OK** Cancel

In this example, the **apex of the heart** has been selected.

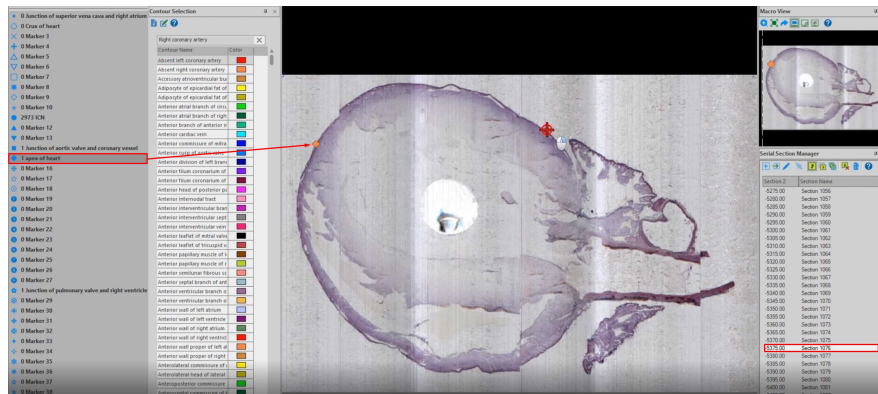




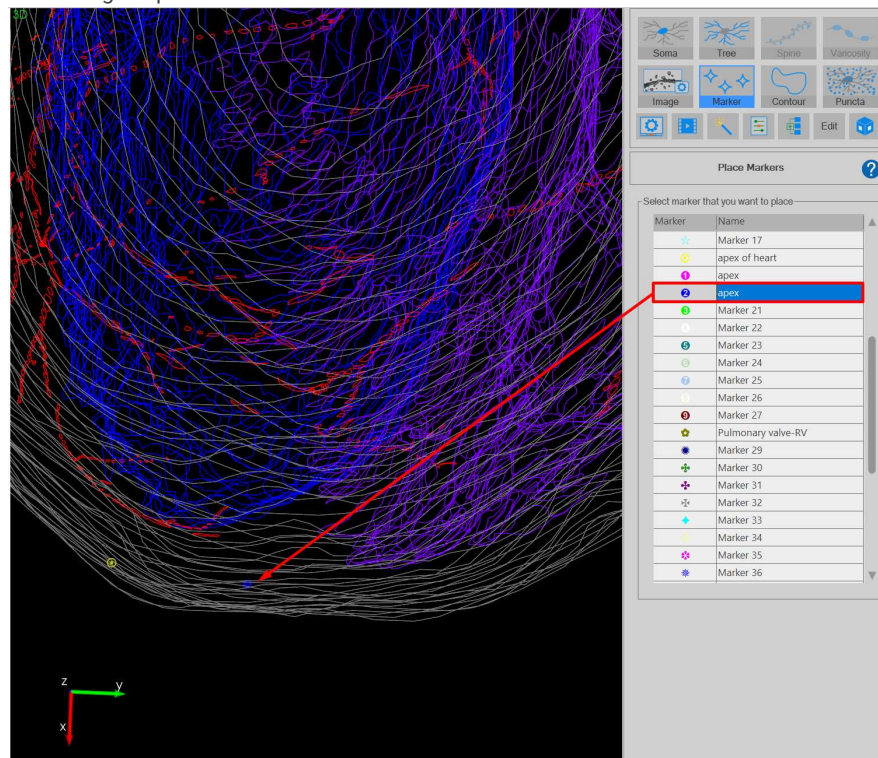
10 Place the fiducial marker in the appropriate location of your image. This can occur either in the 2D window or the 3D window.

10.1 In the 2D window, navigate to the proper plane and click to place the appropriate fiducial marker from step 9. In the example below, the diamond marker has been renamed **apex of the heart**.





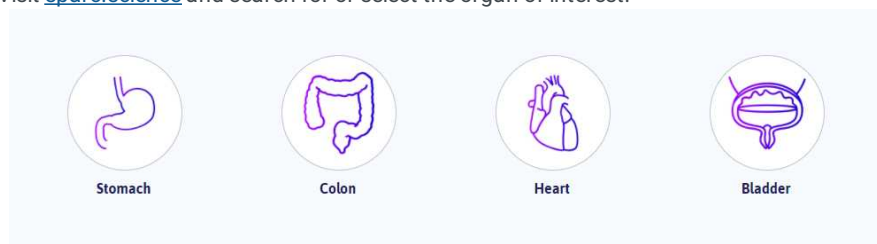
10.2 In the 3D window, select the appropriate fiducial marker from step 9 in the **Marker** panel and click on the 3D image to place the marker.



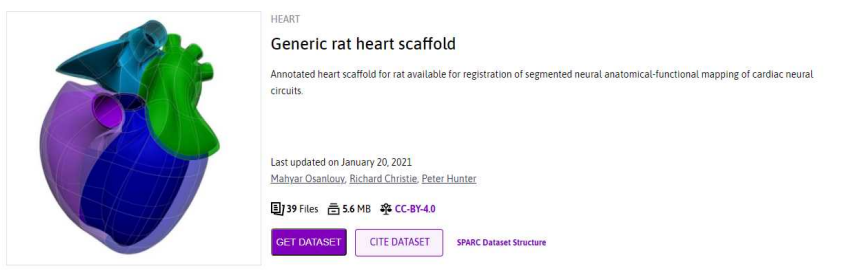
11 Repeat steps 8-10 as necessary for each fiducial marker.

Download a Scaffold from the SPARC Portal

12 Visit [sparc.science](https://sparc.science) and search for or select the organ of interest.



13 Find and select the scaffold dataset of interest. The example below shows the [generic rat heart scaffold](#).

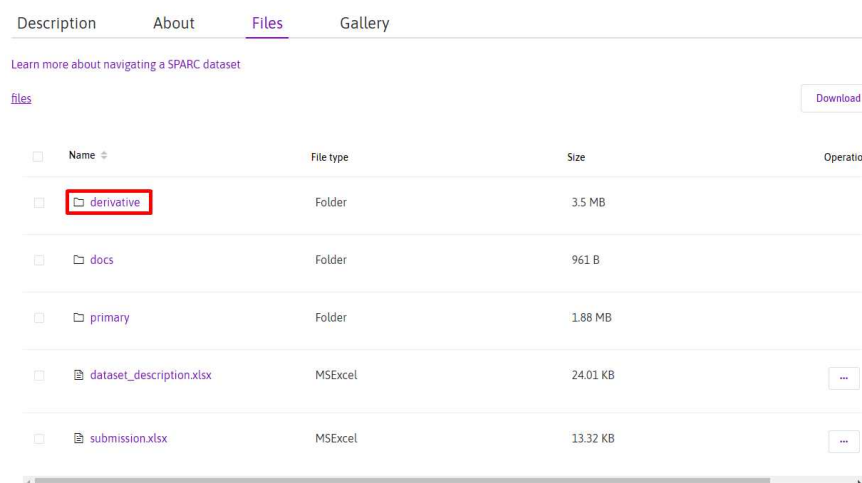


14 Download the scaffold **.vtk** file from the derivative folder.

14.1 Select **Files**.



14.2 Select the **Derivative** folder.



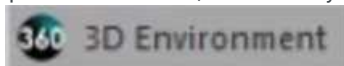
14.3 Select the three dots to the right of the 'mesh'.**.vtk** file and click **Download**. The example below shows the scaffold **.vtk** file for the Rat Heart.

Description   About <u>Files</u> Gallery				
Learn more about navigating a SPARC dataset				
<a href="#">files / derivative</a>				<a href="#">Download</a>
<input type="checkbox"/> Name	File type	Size	Operation	
<input type="checkbox"/> Scaffold	Folder	2.02 MB		
<input type="checkbox"/> manifest.xlsx	MSEXcel	10.73 KB	...	
<input type="checkbox"/> meshMarker.csv	CSV	363 B	...	
<input type="checkbox"/> <b>ratHeartMesh.vtk</b>	GenericData	1.47 MB	...	<a href="#">Download</a>

- 14.4 Download the meshMarker.csv file, if one exists, from the **derivative** folder. Save both .vtk and .csv files in the same directory.

#### Comparing the Segmentation Data to the Scaffold

- 15 Open the 3D window, if not already open.

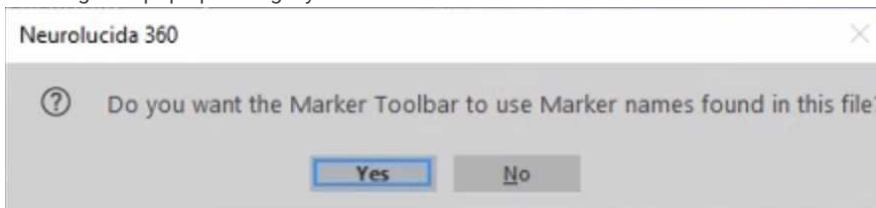


- 16 Select the **Import 3D Model** icon in the 3D window icon toolbar.



- 17 Navigate to and select the **.vtk** mesh scaffold file downloaded in step 14.

- 18 A message will pop up asking if you would like to use the marker names found in the file. Select **Yes**.



- 19 The 3D scaffold will load on the left viewport of the 3D window. You will now be able to move each file separately in 3D to compare the scaffold model to your segmentation data.

The example below shows the scaffold and segmentation file for a rat heart, where the apex of the heart is labeled **Apex** and is orange to match the marker of the same name.

