Neurolucida 360: Quick Surface tool for whole organ annotations

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

How to create contours to map interior and exterior surfaces of intact organs in 3D microscopy images using the Quick Surface in Neurolucida 360.

PROTOCOL CITATION

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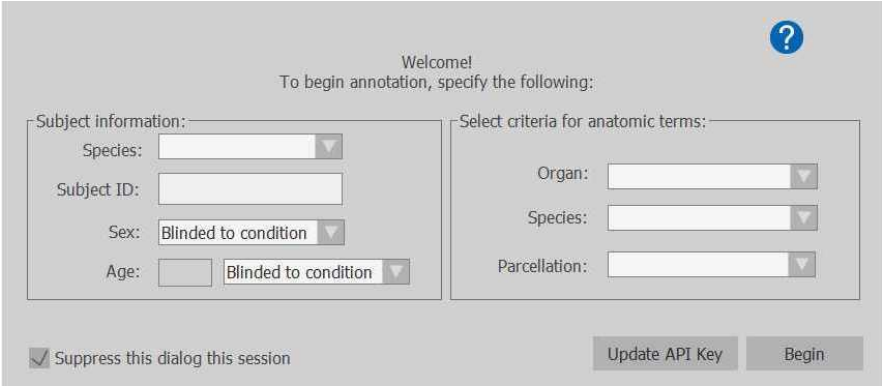
1 Launch Neurolucida 360 with SPARC-mode enabled.

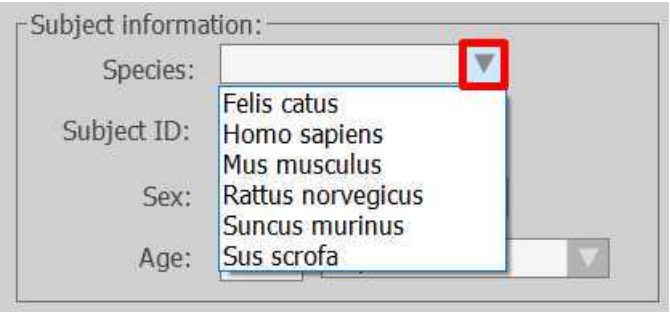
Neurolucida 360
by MicroBrightField Bioscience

Metadata setup.

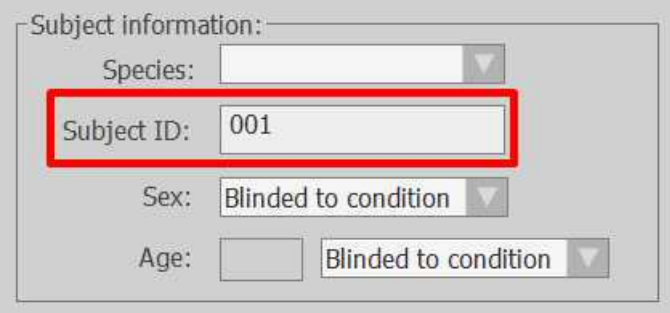
2 The SPARC Welcome Window will appear.



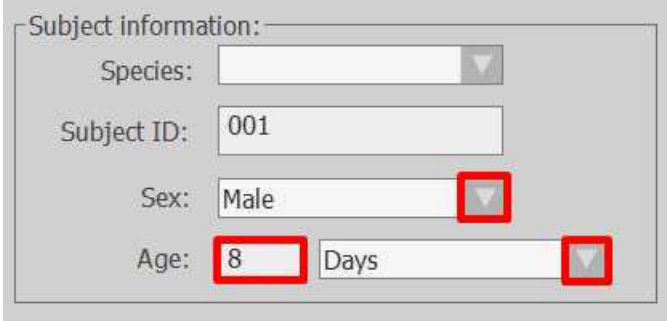
2.1 Use the dropdown menu to select the **Species** associated with the image in the Subject Information section. This is required information necessary to click **Begin**.



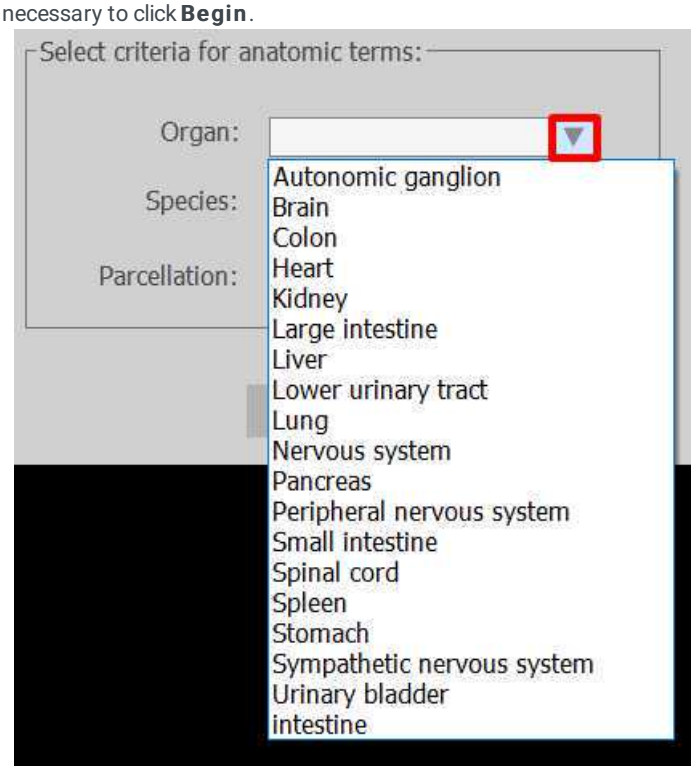
2.2 Enter a **Subject ID** associated with the image.



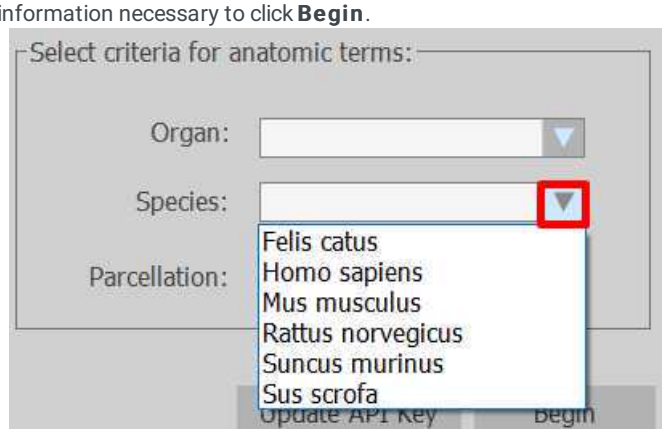
2.3 Use the dropdown menus to select **Sex** and **Age** of the subject associated with the image, or leave as **Blinded to condition**. Add a numerical value for **Age** in the textbox.



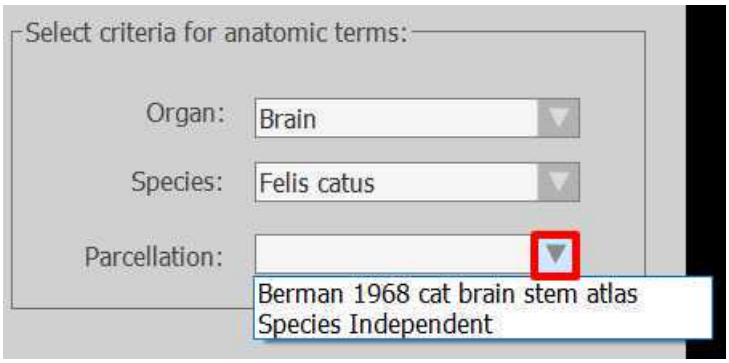
2.4 Use the dropdown menu to select the **Organ** associated with the image. This is required information necessary to click **Begin**.



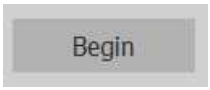
2.5 Use the dropdown to select the **Species** associated with the image, matching it to what was selected in step 3.1. The window will reload so as to provide accurate information for step 3.6. This is required information necessary to click **Begin**.



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

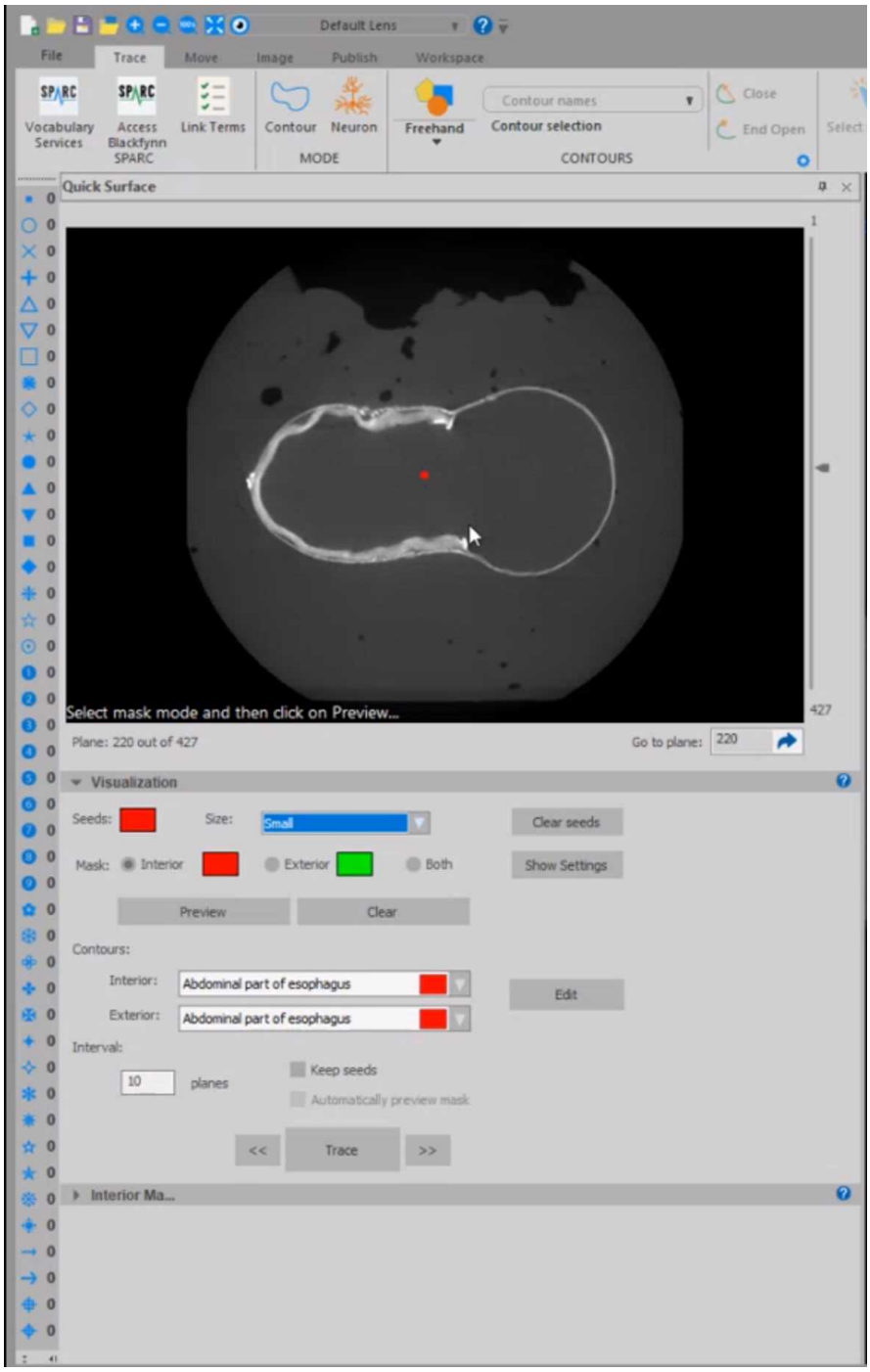


2.7 Click **Begin** to add the selected metadata to the data file when saved and to open the image loaded.



Quick Surface

3 In the **Trace** ribbon, select **Quick Surface**. The **Quick Surface** window will appear.



- The top portion of the window displays the image stack plane-by-plane. On the right side, there is a Z-plane slider and below that, a Z-position jump box.
- Tools and settings are in the bottom portion of the window.
- As the image stack is opened, Neurolucida 360 software will auto populate the serial section manager with a section for each plane in the image stack.

4 Navigate to an image plane with an easily visible interior and exterior surface using the Z-plane slider or by typing the plane in the Z-position jump box.

4.1 Adjust settings for the interior contour:

Click inside the region or regions of interest with your mouse to place a **seed point.**

To create your interior mask, make sure the 'Interior' option is selected and a seed is placed. Then select "Preview." 

A mask that represents a preview of the contour is displayed.

4.2

Click the SHOW SETTINGS button to view and customize the **Interior Mask** Settings. Adjust the mask settings so that the outer edge of the mask corresponds to the inner contour of the structure; the preview will update in real time as you move the Mask Settings sliders.


Adjustments to the mask settings will be represented on the preview screen.

- Edge Thickness: (affects external masks only) Controls the maximum distance to extend the exterior surface beyond the interior. This is useful when objects appear close together to avoid merging.
- Smoothing: Controls how tightly the generated contour hugs the tissue; lower values will adhere more closely; larger values will smooth over irregularities and gaps. This parameter impacts the contour locally.
- Sensitivity: Controls how much difference between foreground (tissue) and background is needed to define a contour. Lower values require a greater difference in intensity, higher values

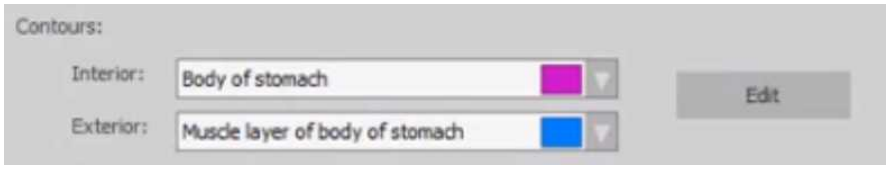
require less difference. This parameter impacts the contour globally.

- Point Density: This controls how many points are included in the contours. It provides a trade-off between the number of points in the contour and how accurate the contour is relative to the tissue edge.
- Transparency: Controls the transparency of the mask displayed on top of the preview image.

4.3

Repeat these steps 4.1 - 4.3 to preview and edit your **Exterior** masks.

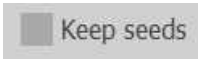
5 Select contour names for **Interior** and **Exterior** contours using the drop-down menus.



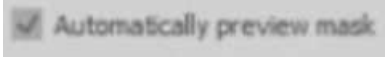
The contour terms are pulled from the organ, species, and parcellation selection from SciCrunch (selected during step 2). To change the terms seen in the Quick Surface dialog to another organ, species, and/or parcellation, click on the **SPARC Vocabulary Services** icon from the **TRACE** ribbon.

6 Lastly, before tracing your contours, there are some options for you to quickly and consistently contour your tissue of interest.

6.1 You can choose to keep your placed seeds for your interior and exterior masks consistent throughout your tissue image by checking "**Keep seeds.**"

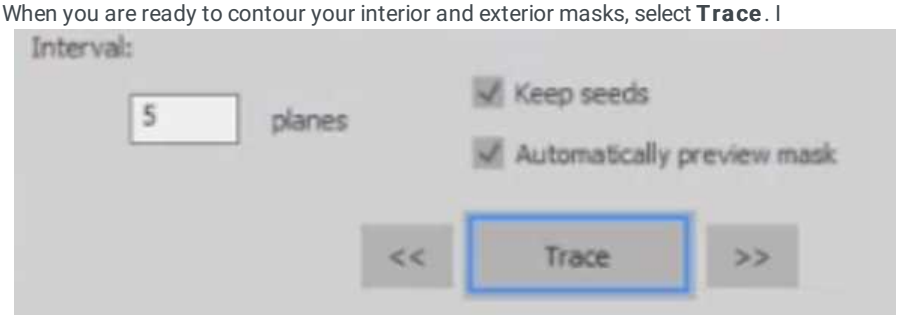


6.2 Checking "**Automatically preview mask**" will keep your previewed mask displayed while you travel through z planes of your image.



6.3 You can also change the interval of those z planes that you contour by inputting a number in the "**Interval**" box.

7 When you are ready to contour your interior and exterior masks, select **Trace**. I

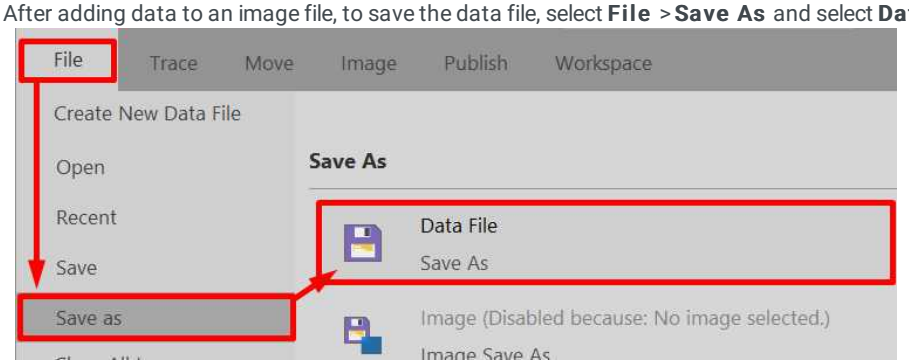


In the main window, the exterior and interior contour(s) will appear on the corresponding plane of the image stack.

8 To continue contouring through the image stack, click on the "<<" or ">>" to move to the next or previous plane at the specified interval. Continue to click trace at each interval until the entire region of interest has been contoured.

Saving your data as an XML

9 After adding data to an image file, to save the data file, select **File** >**Save As** and select **Data File**



10 In the save dialog window, make sure your data file saves as an **XML Document File (*.xml)**, to adhere to SPARC FAIR data standards