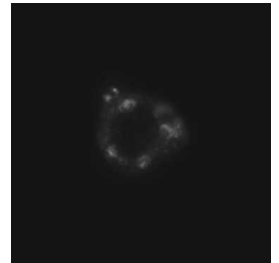May 16, 2023   Version 1

🌐 Using Amira to generate cell body masks in fluorescent light sheet microscopy images of EASI-FISH samples V.1

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We use this protocol and it's working

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Keywords: masks for cell body, cell body masks in fluorescent, cell body mask, fish sample, segment organelles in vem, segment organelle, single molecule spot detection, fish samples this protocol, light sheet microscopy image, cell body, light sheet microscopy images of easi, cell, cellmap project team, assisted iterative fluorescence, classic segmentation workroom, assisted iterative fluorescence in situ hybridization, fluorescence, fish, mask, detection, using amira, fluorescent

Abstract

This protocol describes how **CellMap Project Team** created the masks for cell bodies used in EASI-FISH (Expansion-Assisted Iterative Fluorescence In Situ Hybridization) spot-counting analysis. The **Radial Symmetry-FISH (RS-FISH)** software enables single molecule spot detection in two and three dimensions with high precision. RS-FISH can be installed and run as a Fiji plugin. This protocol features Amira 3D 2021.1 classic segmentation workroom and annotation techniques in the protocol: **Using Amira to manually segment organelles in vEM for machine learning**.

Troubleshooting

Loading the Data

1   This protocol describes how the masks for cell bodies were generated to assist the RS-FISH spot-counting analysis. Please reference the **Using Amira to manually segment organelles in vEM for machine learning** protocol to find details of the annotation techniques and Amira software.

The raw images are in .czi format which can be directly opened in Amira by dragging and dropping the file from the folder to the Amira software (Fig 1.1).
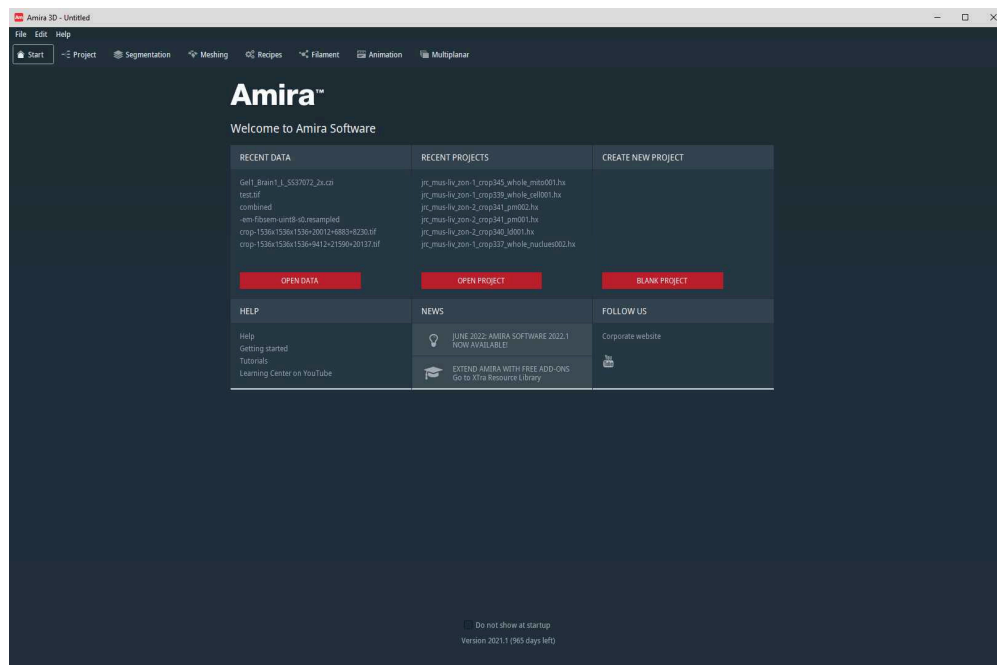

 Fig 1.1

Since the .czi file is large in size, Amira will display a pop-up window with loading policy options to choose from (Fig 1.2). Select the third option, "Read complete volume into memory." and hit "ok" to proceed. If the raw image is split into two datasets (i.e., left and right), load both .czi files onto Amira. The data may take a couple of minutes to load.
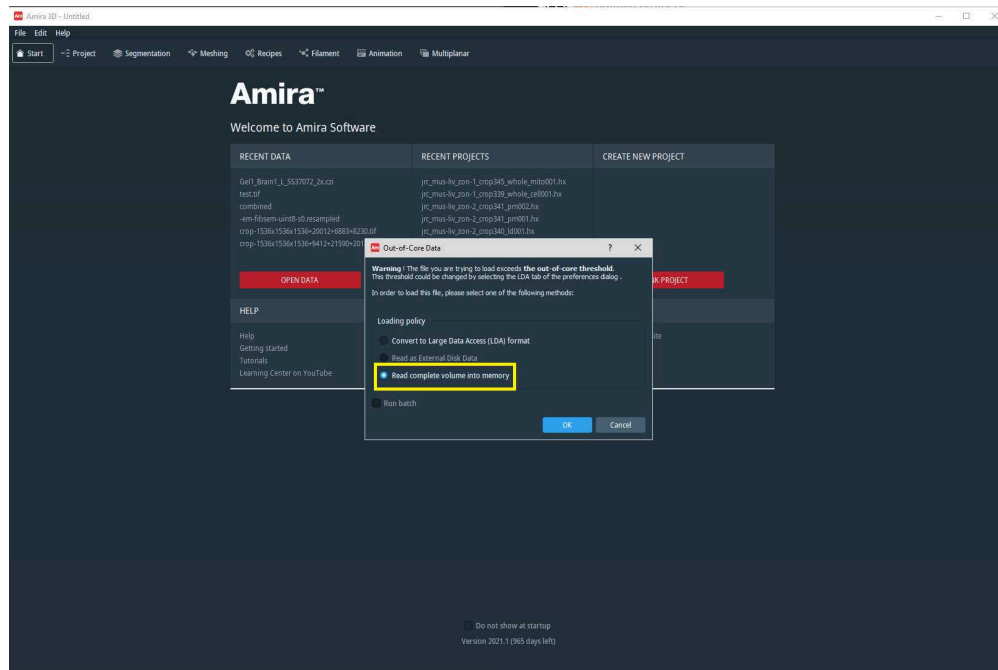
Fig 1.2

The "Multi-Channel-Field" consists of four different channels (Fig 1.3). The mask annotation will use channel 0 with the cell bodies as the image source. Other channels may be deleted (Fig 1.4).
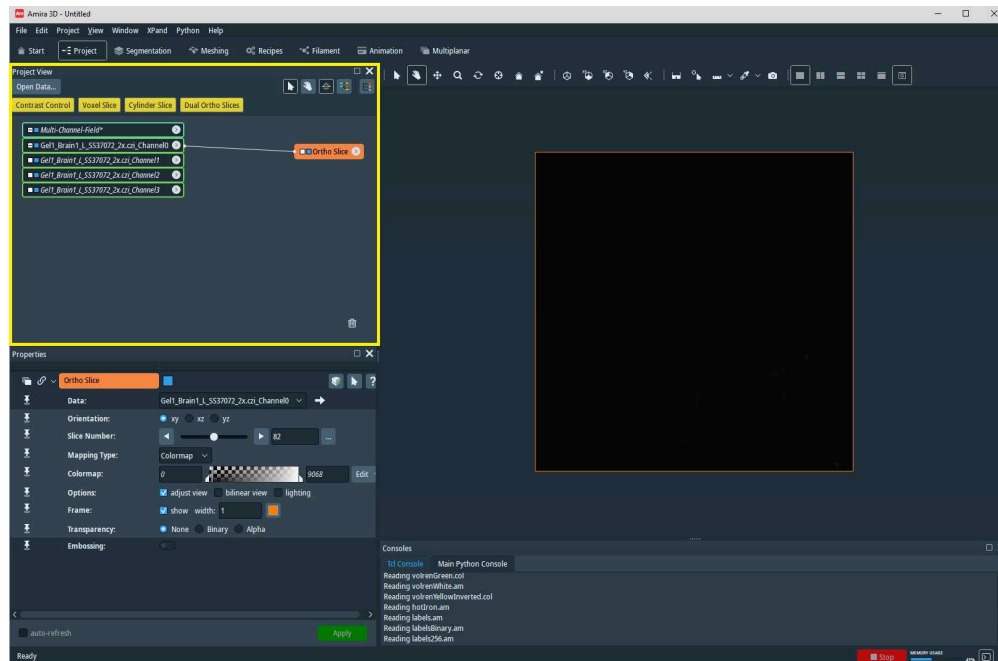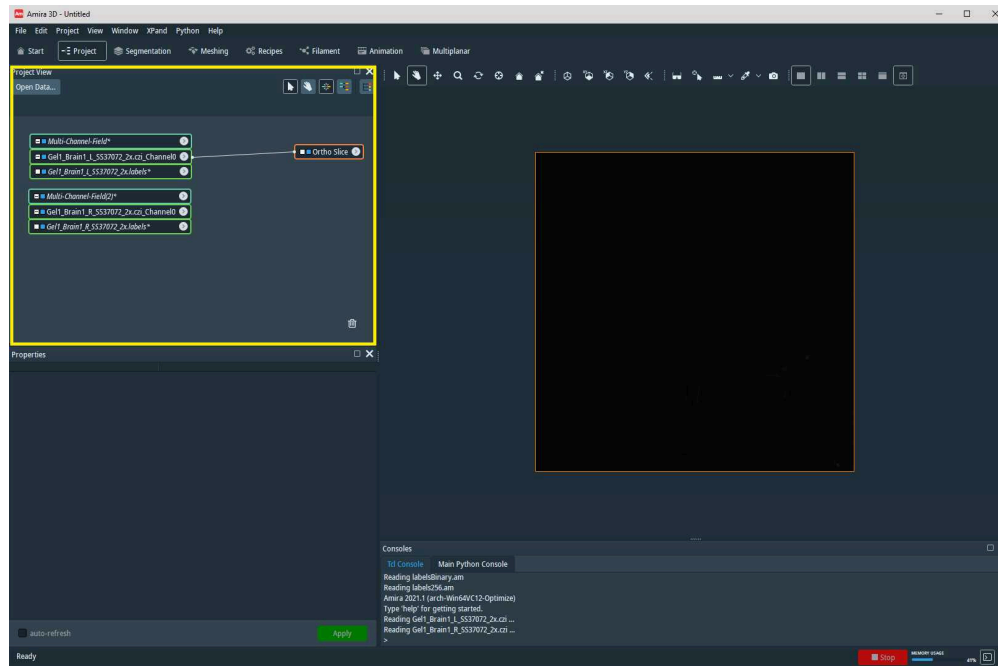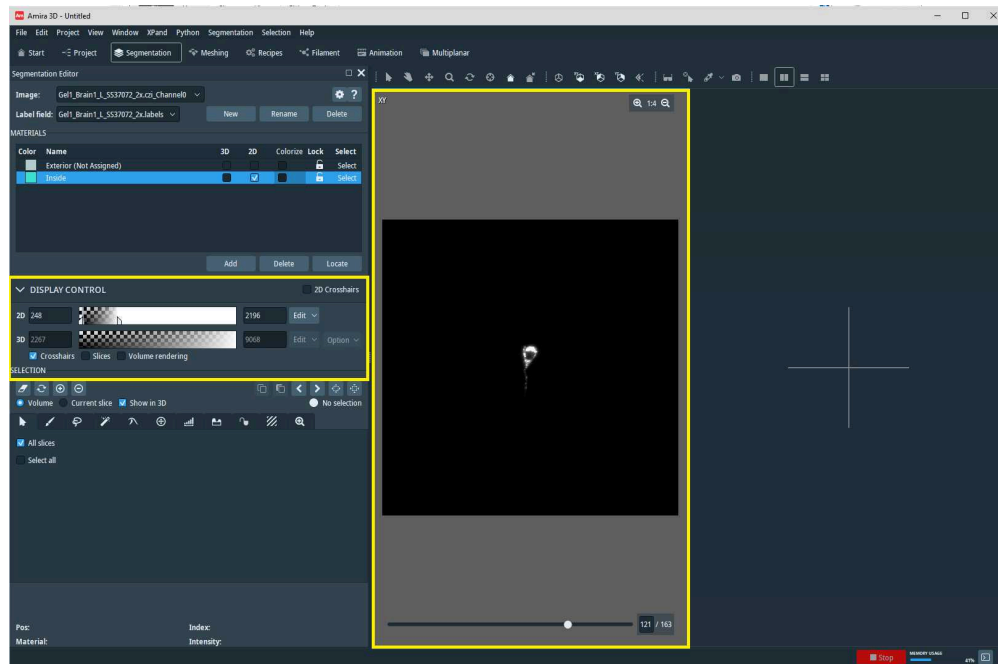

Fig 1.3

Fig 1.4

Fig 1.5

Once the appropriate .czi files and channels are successfully loaded onto the "Project" workroom, the image can be viewed from the "Segmentation" workroom (Fig 1.5). Adjust

the toolbar from the "Display Control" to visualize the cell bodies. The optimal display setting may vary between each cell body or dataset.
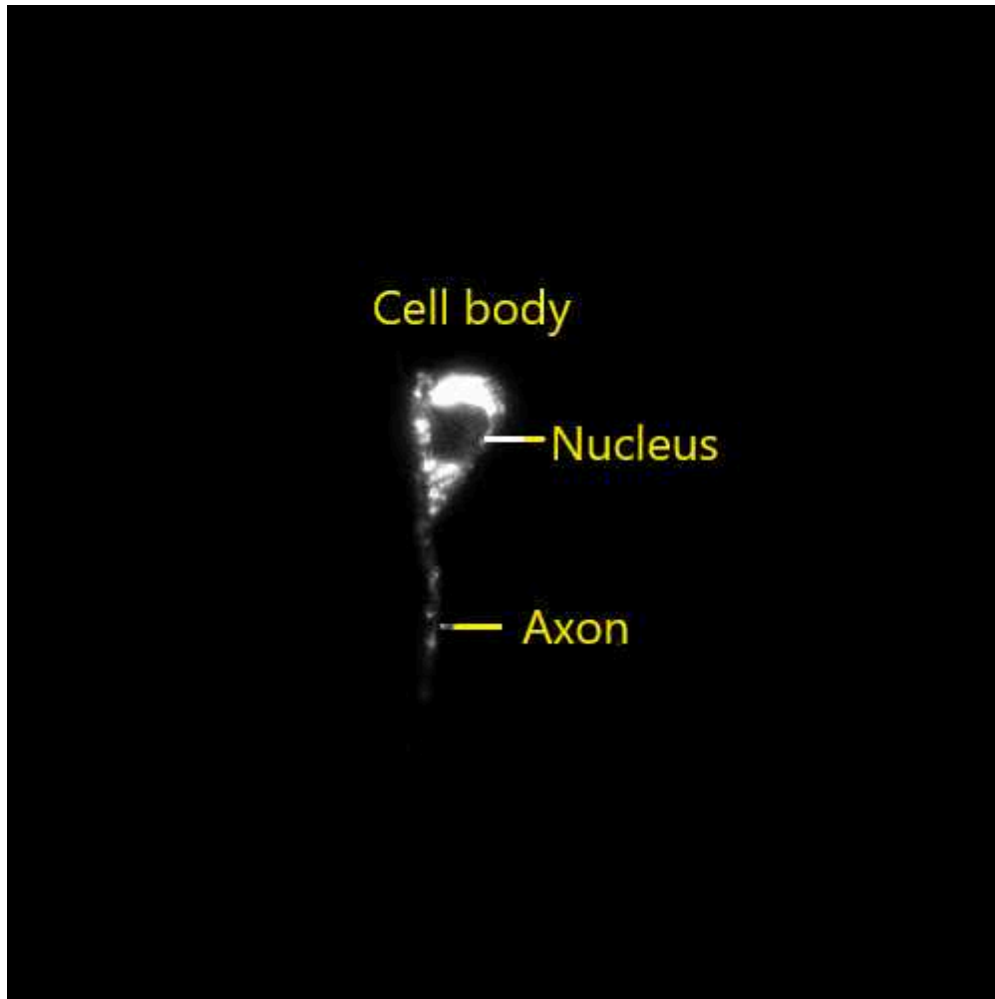
Creating Mask

2

Fig 2.1

The critical classifiers when identifying cell bodies are the dark, round nucleus in the center and the "tail" that proceeds to form the axon (Fig 2.1).

Instead of outlining the cell bodies, the mask will remain inside the plasma membrane and follow the cell body from the first frame of the image to the last, including all abnormal projections.

Some cell bodies may be very dim. If the morphology of the cell body is unclear, conservatively follow the cell boundaries.
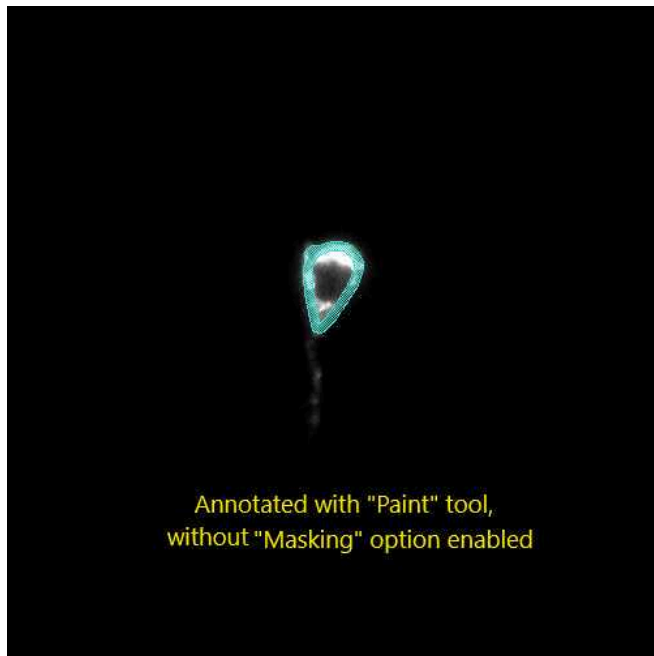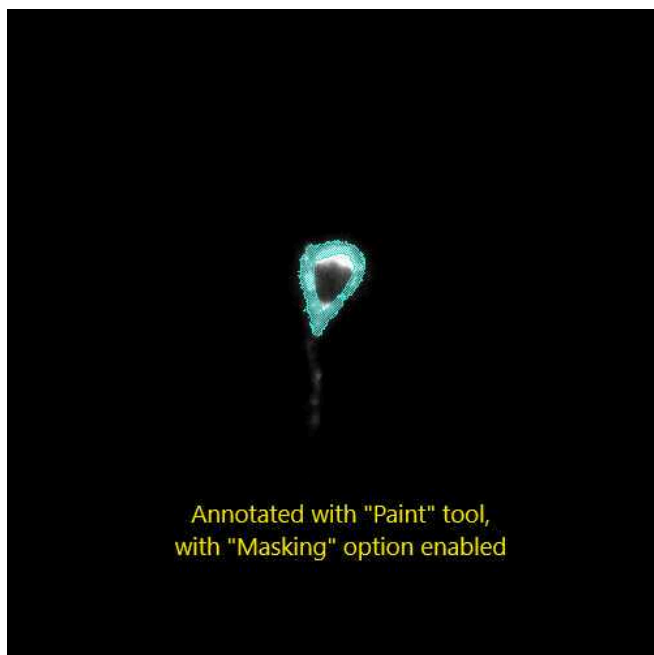
Fig 2.2

Fig 2.3

The "Paint" tool is used to annotate the cell bodies (Fig 2.2). Depending on the image's contrast, the "Masking" feature can be turned on (Fig 2.3). This will only select the voxels within the set threshold. The "halo" around the cell body is not part of the membrane and should not be annotated. To find more details on how to use the "Paint" tool, refer to the **Amira Annotation Protocol**, Section 3.
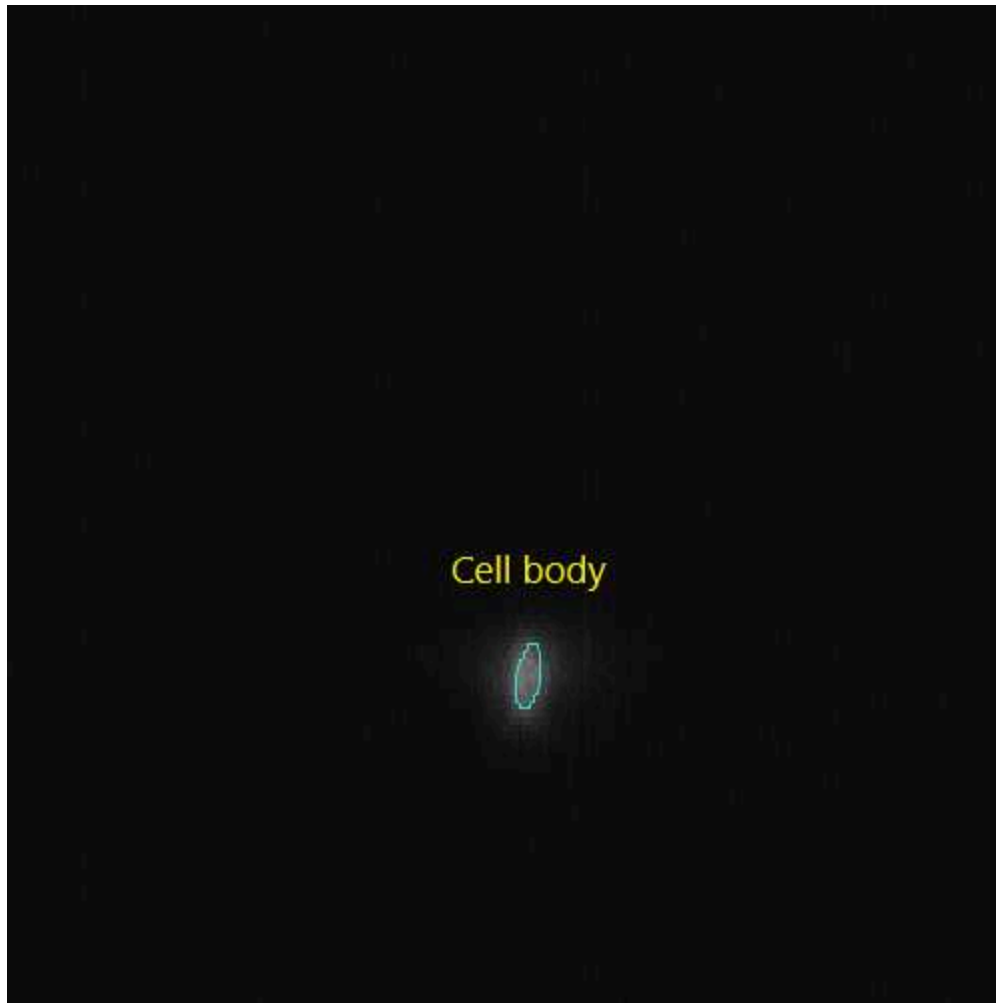
Fig 2.4

Follow the cell body all the way, and cut off when the diameter reaches a consistent size. The uniform, elongated structure attached to the cell body is the axon which is not part of the mask.

Use the up and down arrow keys to navigate through the image frames. Make an annotation on one frame, then skip five slices to make another annotation. "Interpolation" automatically interpolates all frames between each selection. Review the result after selection, and make any needed changes before adding the selection to the material.

Change your orientation frequently to capture the three-dimensional cell body in all planes. Since the annotation is done on one plane at a time, when the orientation is changed, the annotation may appear jagged and irregular on other planes.

Use the "Smoothing" function to even out the membrane edges. The kernel size determines the intensity of smoothing. The bigger the size is, the more intense the

smoothing effect becomes. Start with a smaller kernel size, 2, then examine the result in all three planes before increasing the kernel size. In Cellmap, the typical smoothing kernel size ranges from 2-5.
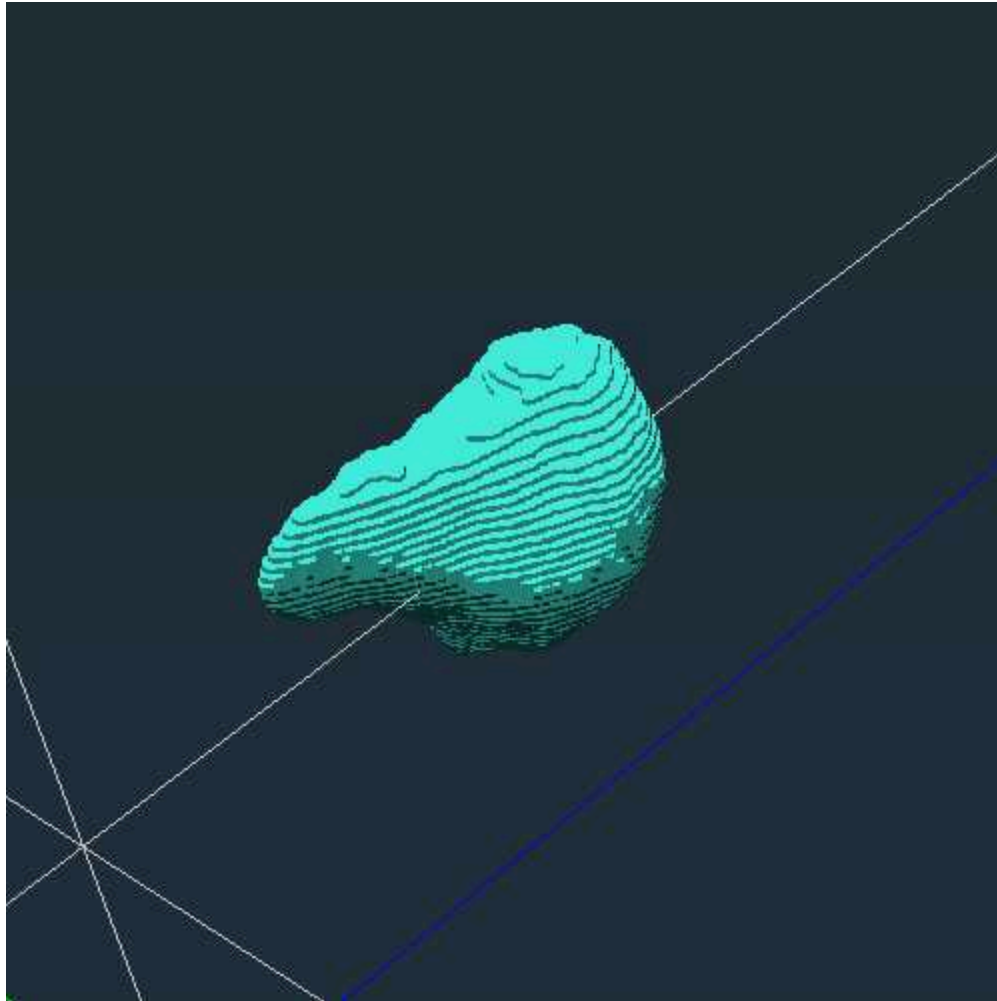

Fig 2.5

Once the cell body is annotated in all frames and smoothed, the annotation will appear as a round sphere with a peak on one end in the 3D viewer (Fig 2.5).

Then with the "Pick & Move" tool, select any region inside the cell body that is enclosed by the plasma membrane. Add the selection to the material to complete the mask. If there is a break in the annotation, the selection will overfill and select every unassigned voxel. Scroll through the frames to locate the problematic frame with the break in the membrane and enclose it.

Before completing the mask, there are a few common mistakes to double-check for. The halo around the cell body should not be included in the annotation (Fig 2.6).
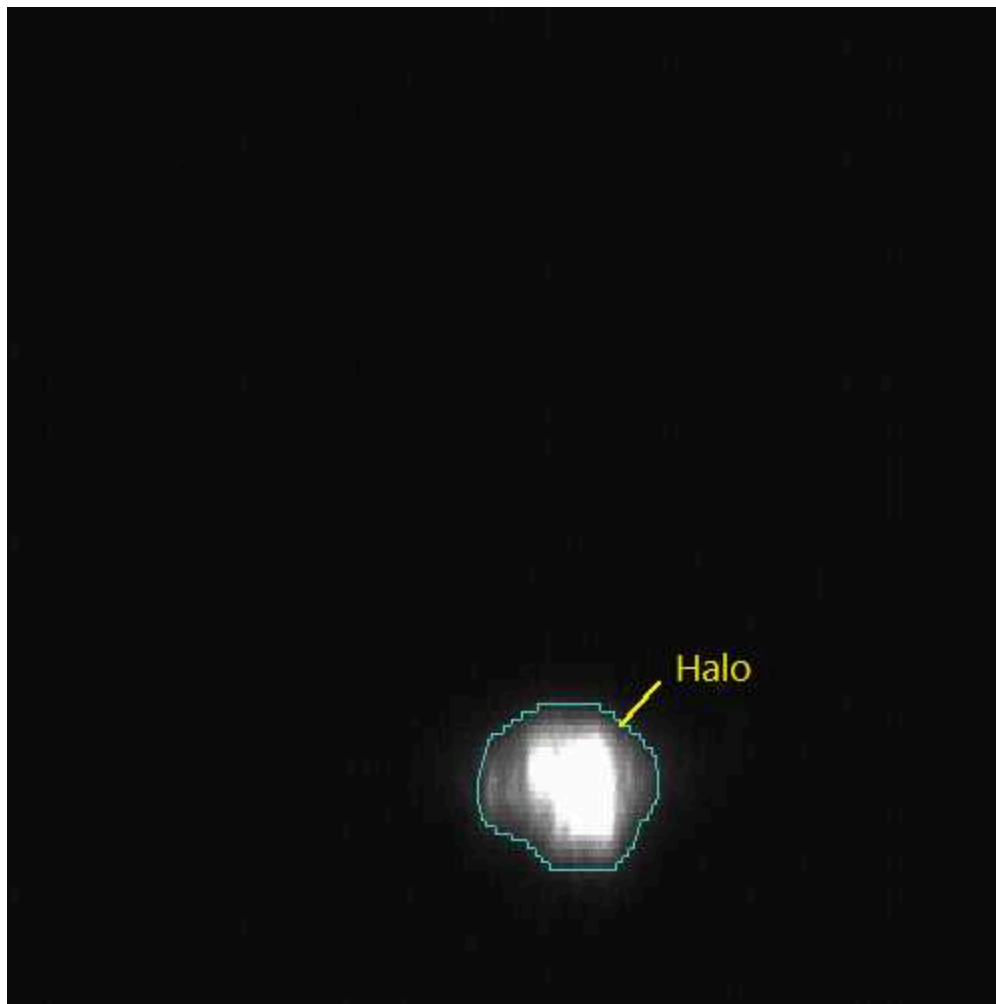
Fig 2.6
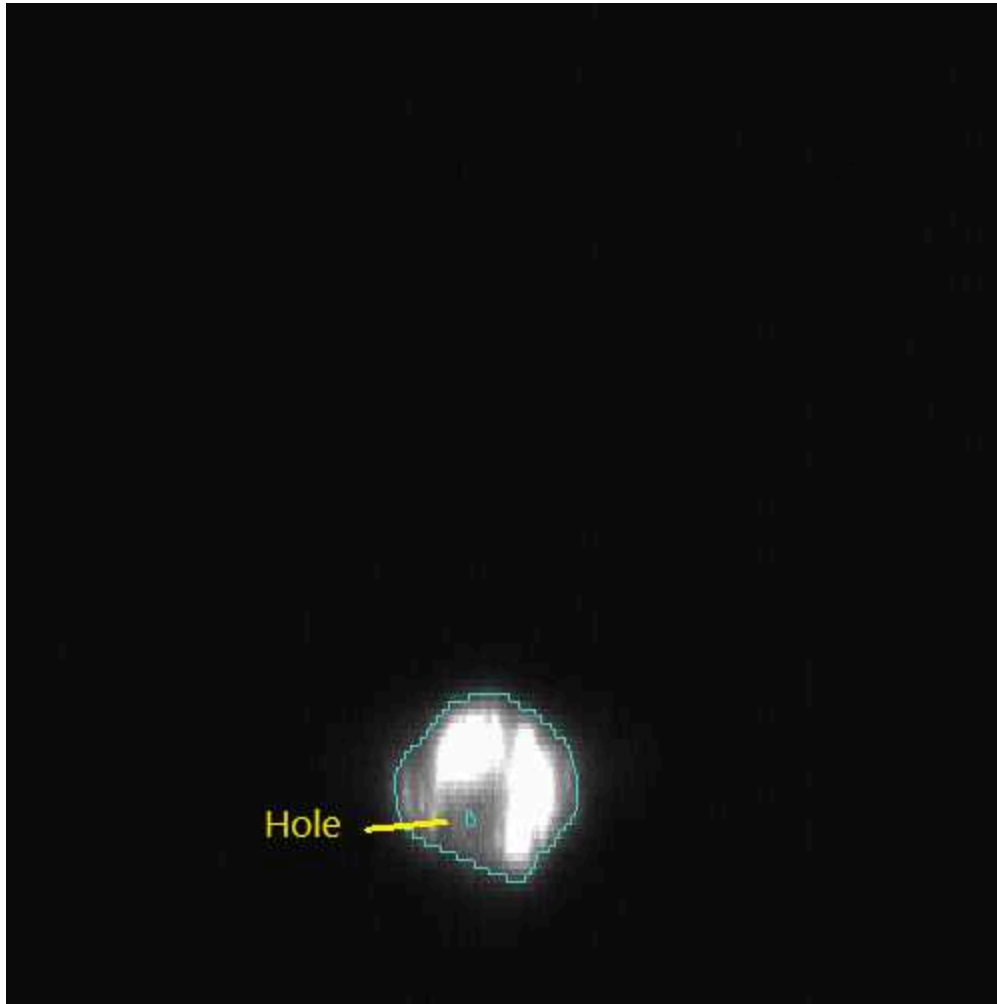
There should be no holes in the mask (Fig 2.7)

Fig 2.7

The elongated section of the cell body that proceeds to form the axon (Fig 2.8) should not be part of the mask.
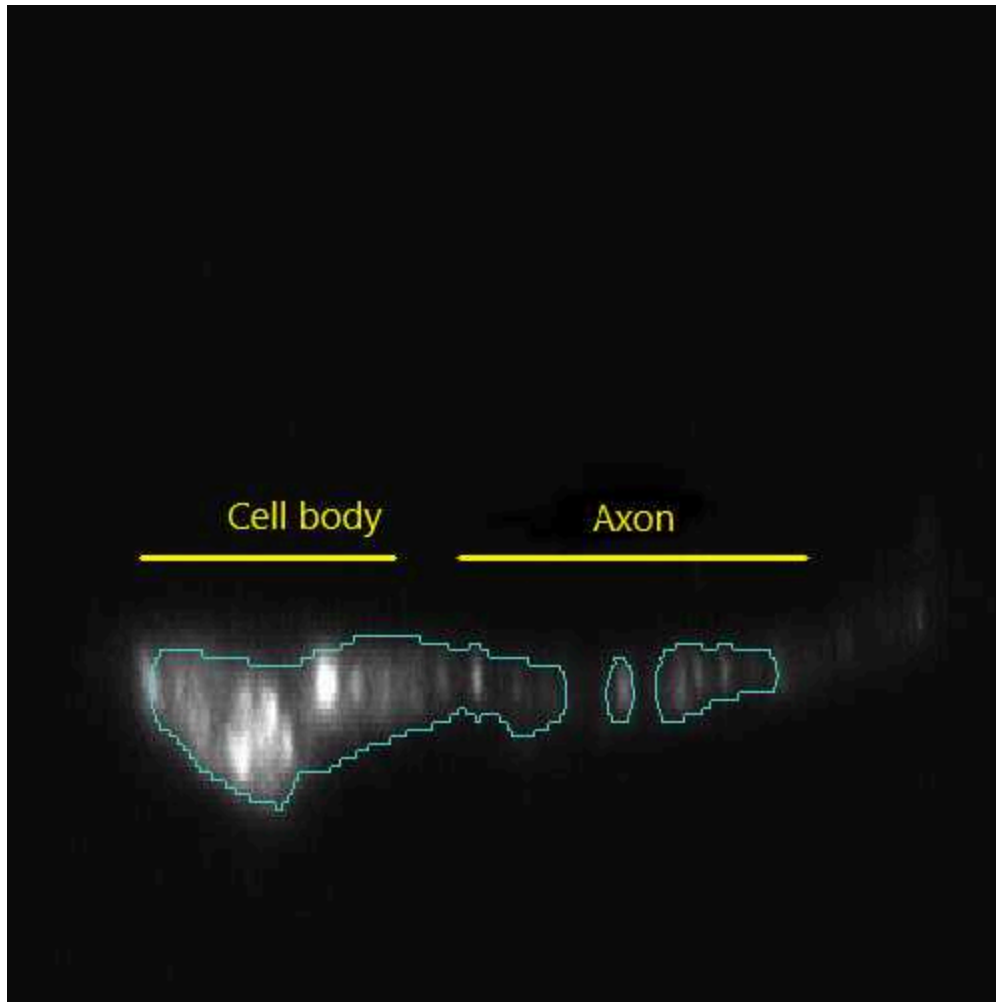
Fig 2.8

Each cell should have its own material. So, for example, if there are 11 cell bodies in the dataset, there should be 11 materials (Fig 2.9)
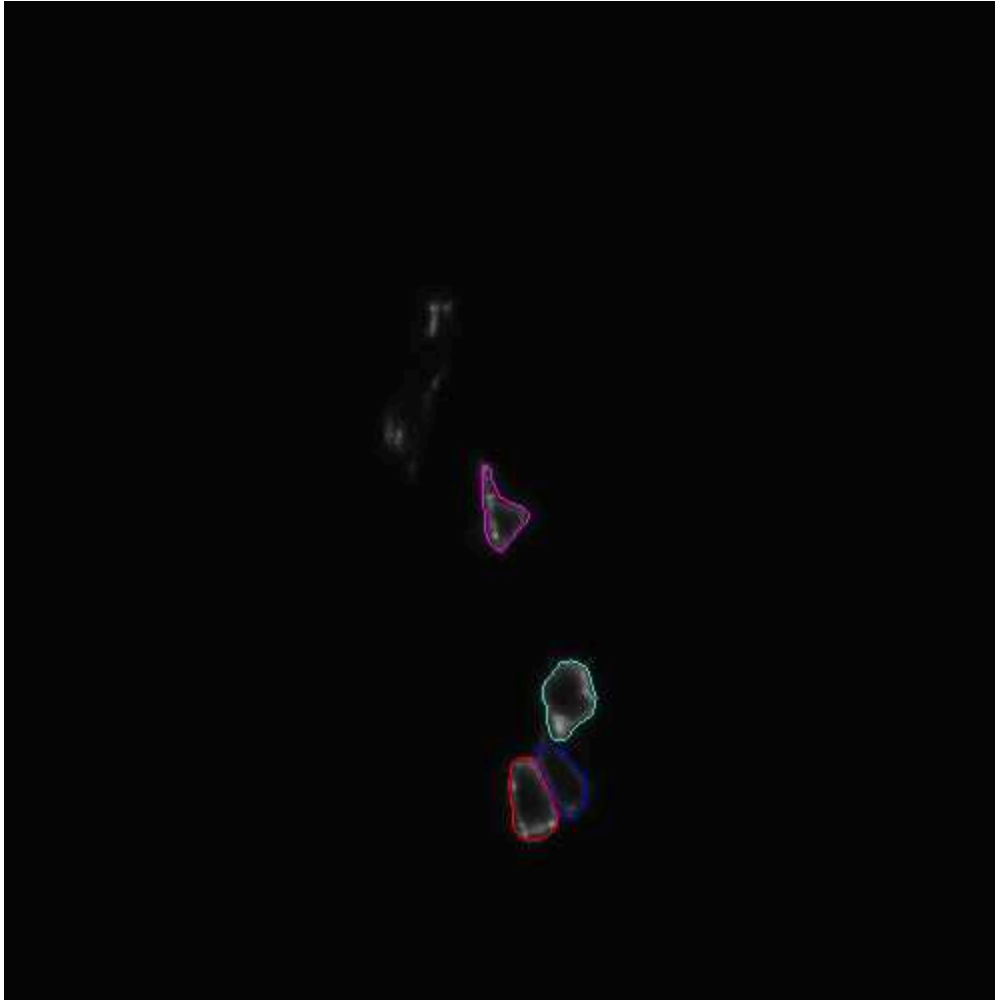
Fig 2.9

Exporting Mask

3   The completed mask annotation needs to be exported as a 3D tiff file. In the "Project" workroom, right-click on the label source with the mask annotation. Then from the pop-up window, hit "Export Data As" then choose 3D tiff from the "Save as type" dropdown menu.

Protocol references

RS-FISH: precise, interactive, fast, and scalable FISH spot detection

Using Amira to manually segment organelles in vEM for machine learning V.2/

FIJI

Amira