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Drawing ROIs in ITK-Snap

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Protocol status: In development
We are still developing and optimizing this protocol

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Introduction

- 1 This protocol will show you two different methods for drawing ROIs in ITK-SNAP. The first method will be drawing ROIs manually and the second method will show the automatic ROI with the snake tool.

Note: The example in this protocol uses the Fornix Fimbria in a ferret brain as the ROI.

Manual ROIs

- 2 Open up ITK-SNAP by typing:

Command

```
cd /rsgrps/hutchinsone/Singularity_Containers
module load singularity
singularity run nklab-neurotools-v0.4.sif itksnap
```

- 3 Open NIFTI file on ITK-SNAP.

- 3.1 Identify the ROI you want to segment on your brain.

There are resources on the internet, and in lab to help identify the tracts. For this protocol a Brain Atlas of the Ferret was utilized.

- 4 Zoom into the brain until you are satisfied with your view.
To zoom in select the "Zoom/Pan Mode" button, and then the up arrow boxed in the image below.

- 5 Select the "Polygon Mode" and on your ROI trace an outline.

Once you are happy with your shape select the accept button in the bottom right hand corner.

- 6 Continue the previous step through the slices till your ROI is finished.
- 7 Lastly, save your ROI as a NIFTI file, and open it in ITK-SNAP to ensure there are no mistakes.

7.1 Save the ROI by clicking on Segmentation>Save Segmentation Image.

Title your ROI and make sure to delete the ".gz" at the end. You want a ".nii" file, not a ".nii.gz" file.

Auto ROIs

8 Open up ITK-SNAP by typing:

Command

```
cd /rsgrps/hutchinsone/Singularity_Containers  
module load singularity  
singularity run nklab-neurotools-v0.4.sif itksnap
```

9 Open NIFTI file on ITK-SNAP.

10 Select the snake tool in the top left corner.

Then select the Segment 3D button.

- 11 Change the Presegmentation mode to Classification from Thresholding.

- 12 Zoom into the brain until you are satisfied with your view.
To zoom in select the "Zoom/Pan Mode" button, and then the up arrow boxed in the image below.
- 13 Starting with Label 1, use **either** the polygon or paintbrush mode mark the tract/ROI.

The labels are identified by color on the right hand side of the above image. Create another label, and mark an area that is **not** your ROI. Select "Train Classifier."

- 13.1** The goal is to completely single out your tract/ROI onto a single ROI from the surrounding grey matter (and other white matter tracts). This is achieved by adding labels to the area surrounding the ROI and updating the "Train Classifier" until the desired effect is achieved.

Typically you will end up using 3-5 labels, but that is dependent on the brain and selected ROI.

- 13.2** Note: Should for any reason you wish to clear the marks after training the classifier, select "Clear Examples" to delete marks made with the brush or polygon mode.

- 14** Once you are happy with the trained classifier, select "Next" in the bottom right hand corner.

- 15** Move the cursor to your ROI on the image and select "Add Bubble at Cursor." Ensure that your bubble is correctly sized and placed on the desired region. Select Next.

15.1 Grow the bubble by selecting the play button.

As the bubble grows, you can select update at the bottom of the left hand corner to see a 3D visualization of your ROI.

15.2 Select Finish.

16 Lastly, save the ROI by clicking on Segmentation>Save Segmentation Image.

Title your ROI and make sure to delete the ".gz" at the end. You want a ".nii" file, not a ".nii.gz" file.

Above is the Snake-Tool ROI.