# Tissuecyte processing steps

## Copy image processing folder into mouse’s folder on the server.

Create an empty folder in the mouse’s data folder on the server called “histology”. Copy the “image\_processing\_X” folder into the histology folder.

## Prepare to run AIBSOPT software in the command line.

1. Open Cmder.
2. Run: conda activate OPT
3. Run: cd C:\Users\lesliec\code\AIBSOPT\Software\Analysis

## Create colored volume.

1. In the command line, run: python TC\_make\_color\_volume.py.
2. Use the GUI to adjust the color intensities (mainly red and green) until the probes are visible. You can scroll through the volume using the slider below the brain image or the arrow keys.
3. Once you reach the desired image colors, click the “Save” button.
4. This can be run multiple times to over-write the saved colored volume.

## Annotate the probe locations.

In the command line, run “python annotation\_app\_TC.py”. When the GUI launches, click “Load” in the bottom right corner. Navigate to the mouse’s “histology” folder and double-click the “resampled\_color\_volume.npy” file. Track the probes and the stim electrode using the probe letter associated with its location. Keep track of the experiment days and which dye color.

## Create files needed to refine the boundaries.

1. Open “TC\_align\_to\_ephys.py” in an editor.
2. Update the location of the histology folder (line 30) with the current mouse.
3. Update the location of the data (line 35). Enter the experiment’s folder (\computer\mouseX\estimX) or None for annotation 1 and 2.
4. In the command line, run: python TC\_align\_to\_ephys.py.

## Refine the boundaries.

In the command line, run “python refinement\_app\_TC.py”. When the GUI launches, click “Load” button on the right side. Navigate to the mouse’s “histology” folder and double-click the “initial\_ccf\_coordinates\_expX.csv” file. The probes will be plotted in their respective spots (A, B, C, D, E, F). Refine the boundaries by clicking the boundary label (the line will highlight green) then click where you want to place the boundary. ***You should not change the order of the boundaries; currently downstream programs cannot process it.*** When finished, click “Save” on the right side.

What ephys features are plotted:

* Pink dots: power in low frequencies in LFP band
* Turquoise shaded line: number of units localized to each channel
* Purple line: multi-unit spike rate

Some potentially useful documents:

* Check out the [Mouse Brain Atlas](https://mouse.brain-map.org/static/atlas). Or see the [Mouse CCF whitepaper](http://help.brain-map.org/display/mouseconnectivity/Documentation) (click on Mouse CCF, Refence Atlas, Version 3, 2017) – this has a table of the acronyms and associated structures.
* [Allen CCF published paper](https://www.sciencedirect.com/science/article/pii/S0092867420304025) – for information about how the CCF was created.
* [Accurate Localization of Linear Probe Electrode Arrays across Multiple Brains](https://www.eneuro.org/content/8/6/ENEURO.0241-21.2021)
* [Survey of spiking in the mouse visual system reveals functional hierarchy](https://www.nature.com/articles/s41586-020-03171-x) – see Methods section for details about how the NPX team localized the probes using OPT technique.
* [Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals](https://journals.physiology.org/doi/full/10.1152/jn.00785.2013)

## Update the probe and unit files with probe locations.

1. Open “TC\_save\_locations.py” in an editor.
2. Update the location of the histology folder (line 27) with the current mouse.
3. Update the location of the data (line 32). Enter the experiment’s folder (\computer\mouseX\estimX) or None for annotation 1 and 2.
4. Update the location of the stimulating electrode (line 40).
5. In the command line, run: python TC\_save\_locations.py.
6. Enter the CCF coordinates into Excel log files.