**Guide for processing calcium data**

**Preprocessing:**

Files exported by the Miniscope device are commonly exported as compressed video files ie: ffv1 avi. These approximately halve the size of the video file but hinder accessibility to image processing tools such as FIJI. Prior to analysis with CaImAn, we convert these ffv1 files into cropped tif files. To remove ffv1 compression we must first use FFmpeg to convert ffv1 avi to rawavi format.

In command prompt use “cd” function to find the directory housing the video files of interest. Then use the line below to convert from ffv1 to rawavi with the name #\_x.

ffmpeg -i INPUT.avi -c:v rawvideo OUTPUT.avi (for one video)

for /L %G IN (0,1,#) do ffmpeg -i %G.avi -c:v rawvideo %G\_x.avi

(for several videos)

Note that the \_x is to designate the rawvideo avi files, they can be renamed in command prompt

for /L %G IN (0,1,#) do ren %G\_x.avi msCam%G.avi

These rawavi files are then concatenated using the line below to generate an avi file that contains a file of all of the recorded data for that session. You must add a text file named “list.txt” which ffmpeg will use to call each file to concatenate. Note that this video may be massive, make sure you have ample harddrive space prior to performing this step. This file can be used as a reference to assess the quality of data and locate any timepoints of noise, motion artifact, ambient light, etc. Once analysis is complete it is recommended to delete all rawavi files.

Format for list.txt (terminal/command prompt must be in dir with videos)

file ‘0\_x’

file ‘1\_x’

file ‘2\_x’

Line to concatenate all video files in list.txt:

ffmpeg -f concat -i list.txt -c copy concat.avi

In order to improve caiman analysis this concatenated rawavi file is imported into FIJI and cropped to reduce the amount of dark background around the ganglion (contrast can be a source of noise). The image sequence function is used to export a sequence of 1000 frame tif files which are renamed to msCam# (starting from 0). You may change the msCam naming scheme, just make sure to change the fname section of the caiman code to call the correct files.

You may also use the batch processing function in FIJI to change and export many avi files at the same time. For example, this code calls all files in a folder, spatially downscales them by a factor of 2 into 376 x 240 pixels (edit these numbers based on the resolution of your Miniscope files), and exports tif files with the same name.

dir1 = getDirectory(“Choose Source Directory “);

dir2 = getDirectory(“Choose Destination Directory “);

list = getFileList(dir1);

setBatchMode(true);

for (i=0; i<list.length; i++) {

showProgress(i+1, list.length);

open(dir1+list[i]);

run("Scale...", "x=.5 y=.5 z=1.0 width= 376 height=240 depth=1000 interpolation=Bilinear average process create");

saveAs("TIFF", dir2+list[i]);

}

**CaImAn Considerations**

Installing CaImAn can be finicky, make sure to house the caiman\_data folder in its own environment as recommended in the [CaImAn documentation](https://caiman.readthedocs.io/en/master/Installation.html).

It is recommended to place the two files “Caiman Ganglion Analysis.ipynb” and “Calcium Trace Component Pipeline.ipynb” into your caiman\_data/demos/notebooks folder.

To launch CaImAn, open anaconda prompt and use the following commands:

conda activate caiman

jupyter notebook --NotebookApp.iopub\_data\_rate\_limit=1.0e10

Make sure when inputting the file names in the fnames list to make the last number of the list one larger than your last file name. For example, if you wish to analyze 6 files from msCam0 to msCam5 then your last number in the list should be 6. For our datasets we analyzed 6000 frames per stimulus. The first 1000 frame were baseline after a saline wash, followed by 4000 frames of response, then a final 1000 frames of another saline wash. You have to adjust the number of videos analyzed at a time depending motion artifacts, changes in ambient lighting and experimental design.

Running this CaImAn code will generate several very large (several GB) files including memmap files, motion corrected files, DFF files. Thus, it is highly recommended to have several terabytes of hard drive space available when doing this analysis. If you wish to rerun the CaImAn analysis these files must be deleted prior to rerunning the code. Once the pkl files are exported for this dataset it is highly recommended to delete the memmap, motion\_corr, and DFF files to conserve space.

Once the neuron number graph is plotted, you must pick out the index number of the good ROIs as fits your dataset. For our datasets, good ROIs were relatively quiet prior to the stimulus, had a response with amplitude of > 0.05 within 2000 frames of exposure to the stimulus, and had a well-defined spatial ROI that was not touching the border of the FOV.

**Common Issues**

Occasionally CaImAn may have issues with plotting graphs, reinstalling opencv may resolve this:

conda install -c conda-forge opencv\

If there is sample drift throughout the recording the motion correction step may generate a visual artifact on the border of the FOV. If this is severe, then CaImAn may detect hundreds of bad ROIs on the border. Consider cropping the visual artifacts from the motion corrected tifs in FIJI and rerunning CaImAn on the new tif files.

Occasionally, mice will gasp, which creates a brief breathing artifact where the FOV shifts for a few frames, then returns to its original position. This may drag the spatial ROIs (very noticeable on the spatial correlation plots) and disrupt analysis. Consider removing the frames of the breathing artifact in FIJI and reexporting the tif files prior to rerunning analysis.

Depending on the activity across of the ganglion, two important parameters to keep in mind in CNMF-E are the min\_corr and min\_pnr. A min\_corr of 0.6 and min\_pnr of 4 are robust in most jugular-nodose ganglion recordings, however, if activity is too low then CaImAn will crash when attempting to render the neuron number plot. Try adjusting the min\_corr & min\_pnr to better fit the output of those graphs and rerun CaImAn.

**Calcium Trace Component Pipeline**

Make sure to edit all instances of FILENAME with the name of the pkl file exported by the Caiman Ganglion Analysis file. Likewise, make sure to change any instances of “cap” to accurately reflect the stimulus applied on the nerve.

By default, this pipeline processes 6000 frames of data where the first 1000 frames is the baseline and frames 1000-3000 are analyzed for the response. Adjust these parameters to fit your experimental design.