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The steps below describe how to take 2P data and an associated behavioral video, extract the neural data, align the recorded data (based on laser intensity in the video), and interpolate neural data between measurements. All of the codes I used and the output data are in this folder.

**2P data extraction/normalization:**

1. Make a .mp4 from the .pgm images saved by the FlyCap program using the code: **writewholevidmp4.m**
2. Use the code: **getPixelDat\_singleVid.m** (This code will go through all the images and calculate the total pixel intensity of each frame. This intensity data will be used to select the frames when the laser turns on.)
3. At the same time, upload the resulting video into your Google Drive for analysis using your most recent DeepLabCut model and the [**Colab\_AnalyzeNovelVideos**](https://colab.research.google.com/drive/1nE1ilaX1l7VxlmYhH8hphVWph7pemw8g) Google Colab notebook to analyze the video and to convert the resulting .h5 file to a .csv file
4. Download the .csv file
5. Calculate the movement data using the downloaded .csv file and the code: **calcDLCbodypartmvmt\_newModel.m**
6. Make the original mask files using the codes: **write2PhotonProJmask.m** and **write2PhotonProJmask\_slices.m**
7. Look at the tiff files of the automatically generated masks. I have found that the automatically generated masks often do not do a good enough job. If you also feel this way, the next step is to use the code: **uiDrawMasks.m** to manually trace the outline where the intensity of your brain structure of interest is the highest.
   1. At this time, it is also important to pay attention to the top right corner of the frames. This is where the final code will pull data to average and subtract the background. If there is a bright spot in the top left corner of the image, you may need to adjust the area which is used as the background in the final code (i.e., NeuralIntensityExtractionAndBehavioralAlignment.m)
8. At this point, I recommend making a new folder where you can put all of the input files:
   1. The video file
   2. The DLC movement .csv file
   3. The .mat file that results from the getPixelDat\_singleVid.m code
   4. All of the mask files you have generated
9. The final step is to run the code: **NeuralIntensityExtractionAndBehavioralAlignment.m**

**Post-processing/Data Analysis Scripts:**

…will continue to edit/add to these over the next week as I finalize them.

1. **DetectBehaviorsfromDLC.m (version uploaded on 12/16/22)**
   1. This code will take the DLC movement data and detect periods of inactivity that last longer than your specified duration (currently set to 3 mins).
   2. Currently working on adding to this code to also pull out frame numbers when other interesting behaviors occur, e.g., proboscis extension, defecation, etc.