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Overview

Psycho6 (<https://github.com/gsager56/psycho6>) was created to simplify the imaging analysis done by psycho5 and give users more control over each step in the analysis pipeline. It is much more concise, while also being more accurate than psycho5. The biggest improvements are (1) alignment of the recording, (2) Background subtraction, (3) ROI selection, (4) and getting the exponential fit for calculation. The basic analysis pipeline of psycho6 is as follows…

1. LOAD MOVIE: Load in the raw movie and details of the experiment
2. ALIGNMENT: Correct for the movement of the fly in the xy direction by registering each frame of the recording with the neural tissue mask.
3. BACKGROUND SUBTRACTION: Subtract out the intensity of the background from each frame
4. ROI EXTRACTION: Group all pixels into ROIs
5. ROI SELECTION: Throw out extracted ROIs that aren’t responding to the probes
6. FIT : Fit for each selected ROI and only keep “good” fits. What is “good” is up to you. Note, the formula for is , which is the fractional change in the raw intensity relative to the intensity during the interleaves.

Table of Important Variables

|  |  |  |
| --- | --- | --- |
| **Name of Variable** | **Explanation of Variable** | **Dimension of Variable** |
| raw\_movie | Raw intensity values of the unaligned movie | (number of rows) x (number of columns) x (number of time frames) |
| exp\_info | All the details of the experiment saved by the microscope (i.e. time each frame was recorded, which epoch was presented, etc.) | This is a structure where each field is a different kind of information about the experiment |
| aligned\_movie | Raw intensity values of the aligned movie | (number of rows) x (number of columns) x (number of time frames) |
| tissue\_mask | Binary mask where true means that pixel belongs to the neuron you imaged | (number of rows) x (number of columns) |
| filtered\_movie | Aligned and background subtracted raw intensity values of the movie | (number of rows) x (number of columns) |
| mean\_movie | Average intensity value for each pixel over the entire recording | (number of rows) x (number of columns) |
| bkg\_mask | Binary mask where true means this pixel is designated as the background, which is used for background subtraction | (number of rows) x (number of columns) |
| corr\_img | Matrix of how correlated each pixel’s raw intensity over time is with each of it’s neighboring pixels. Strongly positive correlations mean that pixel is likely responding to your stimulus. | (number of rows) x (number of columns) |
| roi\_extract | Matrix of which extracted ROI each pixel belongs to. | (number of rows) x (number of columns) |
| roi\_select | Matrix of which selected ROI (selection is based on how correlated the ROI’s activity is for the probes) each pixel belongs to. 0 means that pixel belongs to no ROI. | (number of rows) x (number of columns) |
| roi\_final | Matrix of which final ROI each pixel belongs to. Final ROIs are slected ROIs that had a good enough fit for the calculation | (number of rows) x (number of columns) |
| roi\_dff | values for each final ROI | (number of frames) x (number of final ROIs) |
| resp | Cell array that stores the final outputs for each analyzed fly. Default final outputs are (1) for each final ROI, (2) which epoch was presented for each frame of the recording, (3) mask of which final ROI each pixel belongs to, and (4) the time (in seconds) each frame was recorded | (number of analyzed flies) length cell array. Fields of the cell array are (1) dff, (2) epoch\_trace, (3) roi\_final, and (4) time |

Setup Directions

1. If you haven’t already, clone the psycho6 repository and create your own analysis branch
   1. Clone the psycho6 repository: <https://github.com/gsager56/psycho6>
   2. Create your own branch in psycho6 to create you own analysis scripts. **DO NOT PUSH ANYTHING TO THE MASTER BRANCH UNLESS YOU KNOW WHAT YOU ARE DOING!!**
   3. Make sure to add the paths of psycho6 and all subfolders
   4. Copy over BaseTemplateAnalysis.m and change what you want to do your analysis. Since you’re working on your own branch, you can also change literally anything you want and never mess up other people’s stuff. However, in case you missed it the first time, **DO NOT PUSH ANYTHING TO THE MASTER BRANCH UNLESS YOU KNOW WHAT YOU ARE DOING!!**
2. Specify the information about what flies/experiments you want to analyze

Detailed Explanation of What Each Analysis Step Does

LOAD MOVIE: Load in the raw movie and details of the experiment

ALIGNMENT: Correct for the movement of the fly in the xy direction by registering each frame of the recording with the neural tissue mask.

BACKGROUND SUBTRACTION: Subtract out the intensity of the background from each frame

ROI EXTRACTION: Group all pixels into ROIs

ROI SELECTION: Throw out extracted ROIs that aren’t responding to the probes

FIT : Fit for each selected ROI and only keep “good” fits