1P/2P Cell Matching - Fall 2024

Creator: @Enrico Piperno

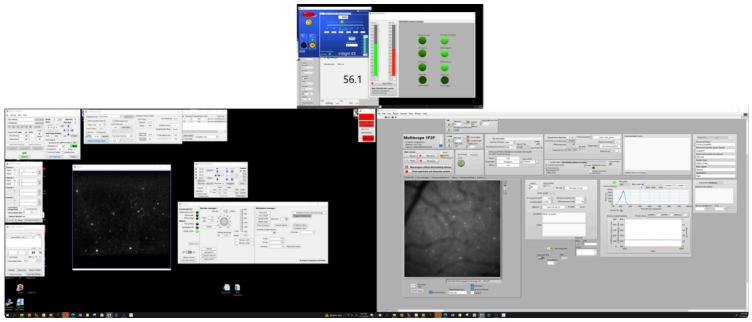
Lab Book Pages: Q3 2024 1p in vivo - ex vivo alignment

Summary: This was a rotation project in which I attempted to match cells taken with the Multiscope in the concurrent 1P/2P Spinning Mirror Modality. That is, can we identify cells imaged in 1P with cells taken in 2P. The purpose of the matching is to retrieve z-information from the 2P cells, since in 1P the optical sectioning is poor due to the physical properties of 1P excitation. The overarching goal was to image a large field of view with the BFM, and then transfer the same mice to the Multiscope to retrieve the z-information of the cells found in the BFM. This would require tiling the FOV to fit into the smaller FOV of the multiscope and scanning the 2P z-axis over the 1P axis. That is, while the 1P "z-depth" is held fixed, we need to scan the deformable mirror under and over the set "z-depth" to retreive the z-coordinate of each cell. Once we know all the x,y,z coordinates of the cells taken in the BFM, we can then perform ex-vivo slicing to return the transcriptomic data of those cells. This would allow for us to match the cell's activities with their cell-types in a large FOV.

In reality, only the 1P-2P cell matching algorithm was attempted.

1P-2P Cell Matching Algorithm Description:

Example Data Collection:



In this example, we set-up the 1P-2P imaging modality.

The imaging details are located here. To summarize,

- Mouse: GCamP? (need to find details)
- Objective: Nikon 16X0.8NA
- 1P Imaging Depth: -200um
- 2P Stack:
  - Zmin: -110um
  - Zmax: 185um
  - NPlanes: 10

Raw Image Files:

- 1P Movie (Movies too large to share here)
- 2P Movie flashing to the different z-planes

Motion Correction and Cell Extraction:

- NormCorre Motion Correction
- Cell Extract with cells numbered
  - 1 X 1P Cell Extraction (since they are on the same plane)

    - 10 X 2P Cell Extraction (I divided the movie into 10 different movies of each plane, and performed the cell extraction on the individual planes)
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Pre-Processing:

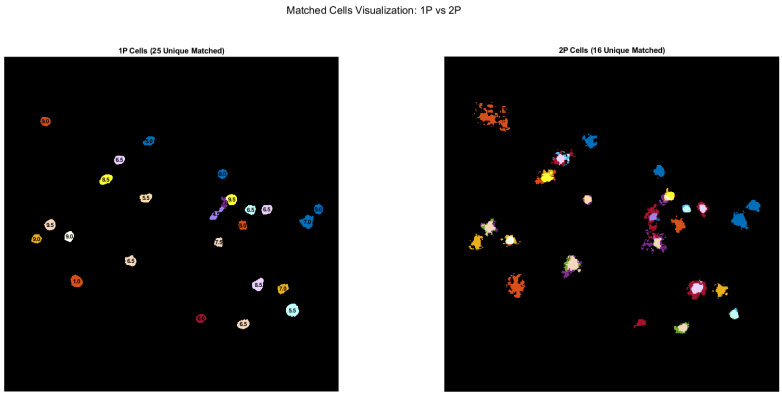
- Images in 1P and 2P have distortion between them, as a result first we need to align the two images
- I did this by first manually matching cells based on cell shape and “constellation” from 1P and 2P, and noted down their coordinates. Then, I computed a Affine Matrix (might be overkill) to align the two images.
- Pre and Post Affine Transform: 
- Then, I interpolated 2P data to have same spatial and temporal resolution as 1P 

Cell Matching:

- Input: S_p1, T_p1, S_p2, T_p2 (S refers to spatial information of the extracted cells, while T refers to traces)
- Output: 1P Matched Cell ID, 2P Matched Cell Z-plane
- For every 1P Cell,
    - Temporal cross-correlation with a possible lag
    - Spatial cross-correlation using dot-product of flattened spatial data
- Parameters: Thresholds for Spatial and Temporal Correlations
- Accept cells with correlation exceeding thresholds

Results:

- Movie of Matched Cells (Too large to share)
- XYZ Projection of Matched Cells



Example 1P-2P cell matching. On the 1P cells I calculated the z-plane from which these cells were matched from. Once we calculate the z-coordinate of the planes after doing some DM calibration, this refers to the z-coordinate of those cells.