



A pipeline for neural segmentation and multi-day tracking from two-photon calcium imaging datasets

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Background

Endoscopic two-photon calcium imaging is a method to simultaneously track the activity of spatially restricted neural populations over extended periods of time¹. This technique relies on the large influx of calcium that accompanies neural signaling events to activate a calcium-sensitive fluorescent indicator (GCaMP¹). When paired with a small gradient-index lens lowered into the brain, the microscope allows for high resolution visualization of a very narrow plane within the brain at the single-cell level¹. Returning to this same plane over the course of multiple sessions allows an understanding of how neuronal ensembles evolve their activity over time.

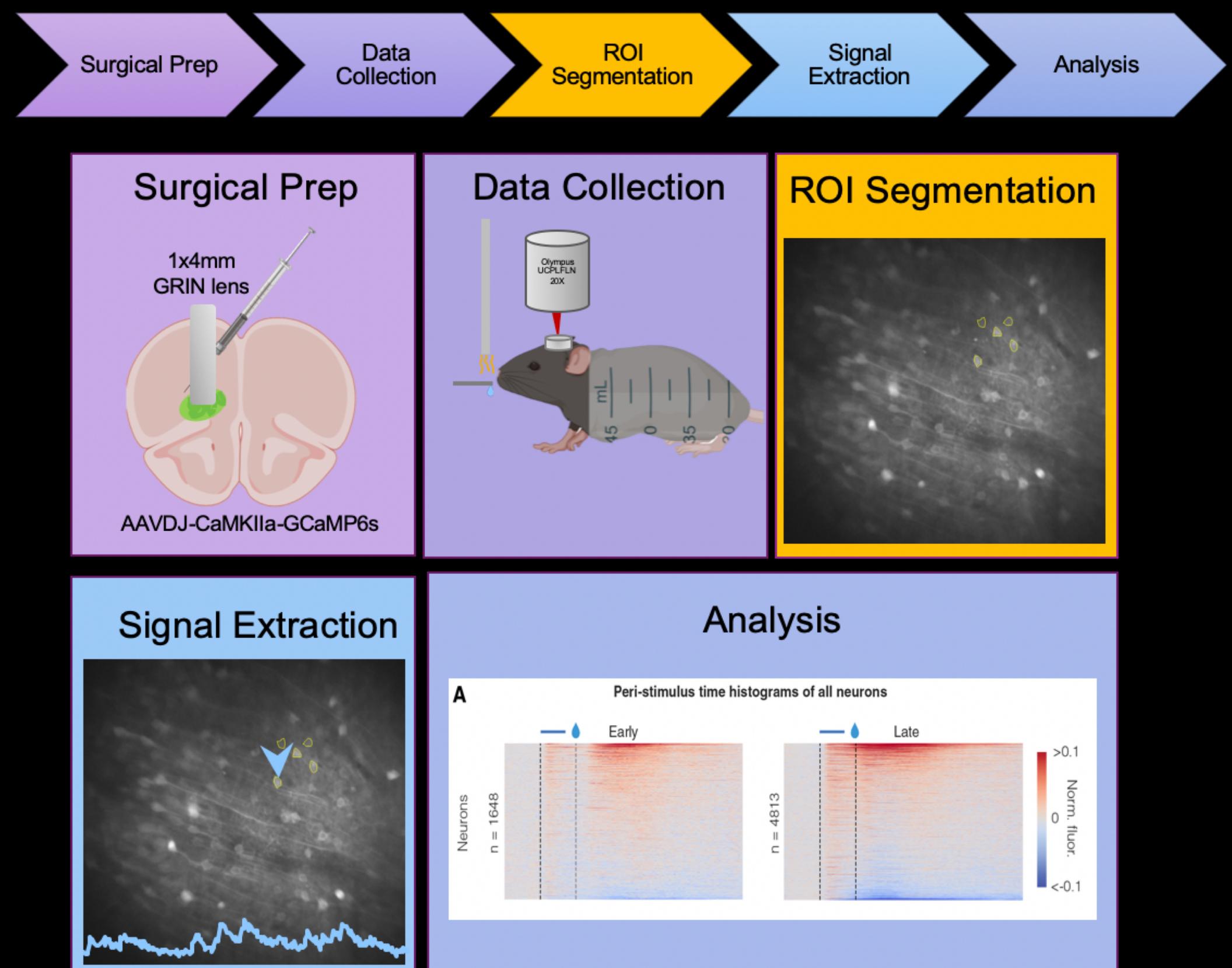


Figure 1. Workflow for two-photon endoscopic calcium imaging experiments

There does not currently exist a reliable algorithm to segment and track neuronal regions of interest (ROIs) that does not make untrue assumptions about neurons or their activity (i.e. that they are always round and tonically active)²

The purpose of this project was to generate such a pipeline with the three overarching goals:

Aim 1: To diminish the contribution of out-of-plane fluorescence from the overall signal

Aim 2: To segment individual neurons

Aim 3: To track segmented neurons over time

Methods

Aims 1 and 2 were addressed using the default version of python, scikit image and matplotlib in google colab. Aim 3 was performed in the desktop version of python with a custom environment. This environment, all analysis notebooks, and this poster can be accessed at: https://github.com/mmgrayCHEME599/CHEME599_Brain. Before analysis began, I calculated average intensity time projection images of the neuronal data. All figures were generated using the linked code except Figure 3C which was processed with a 5 pixel disk rank median filter for better visualization on this poster.

Results

Aim 1: Background Reduction

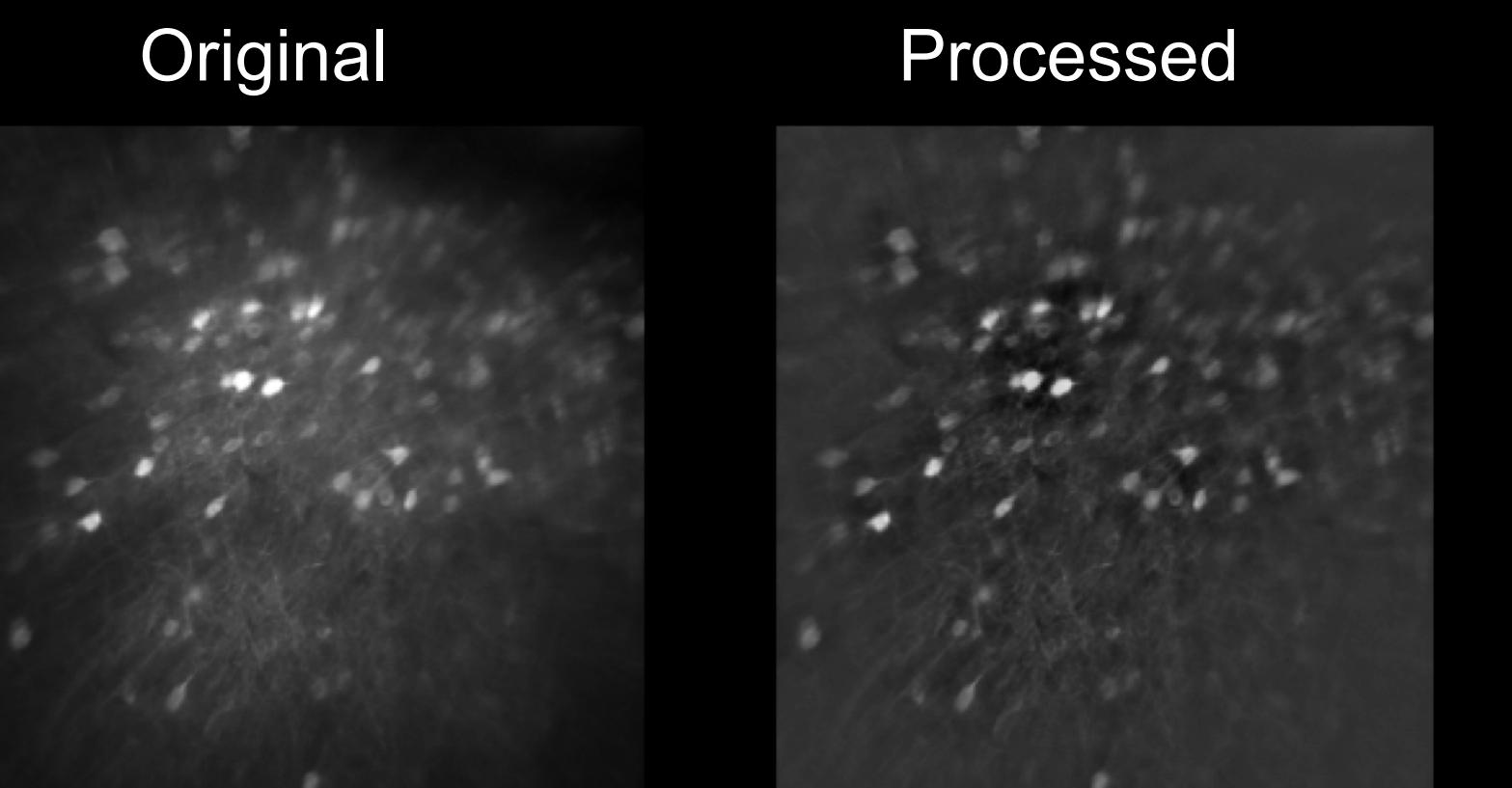


Figure 2: Results of background reduction algorithm. Note that the processed image has a much more uniform background and similar intensity across neuronal ROIs.

I was able to best remove neuropil fluorescence using an unsharp masking algorithm followed by intensity rescaling. This removes the non-uniform background fluorescence and allows for clearer visualization of neuronal ROIs, especially those at the periphery

Aim 3: Neuronal Tracking

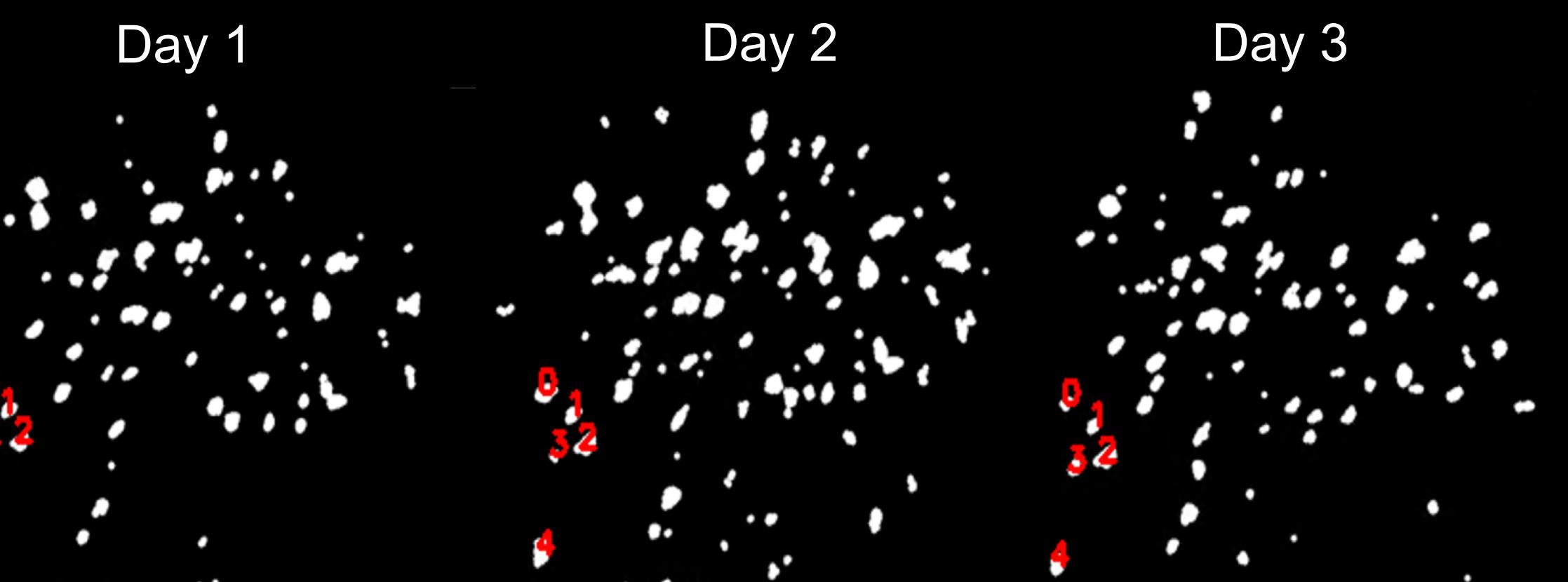


Figure 4: Sample tracking window with numbered cells

I developed a pipeline that combines neuronal tracking and manual selection/exclusion of ROIs from multi-day datasets based of cv2 mouse click registration. Training a classifier for so few images would be overly time-intensive and prone to overtraining. Only annotated ROIs are kept for the final analysis.



Figure 3: Visualization of segmentation steps on a representative image

Repeating this technique over different sessions in the multi-day datasets generated the data for use in Aim 3

References and Acknowledgements

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[1] Grienberger and Konnarth, *Neuron* Volume 73, Issue 5, P862-885, March 08, 2012
[2] Pachitariu et al, *bioRxiv* 061507; doi: <https://doi.org/10.1101/061507>