



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.

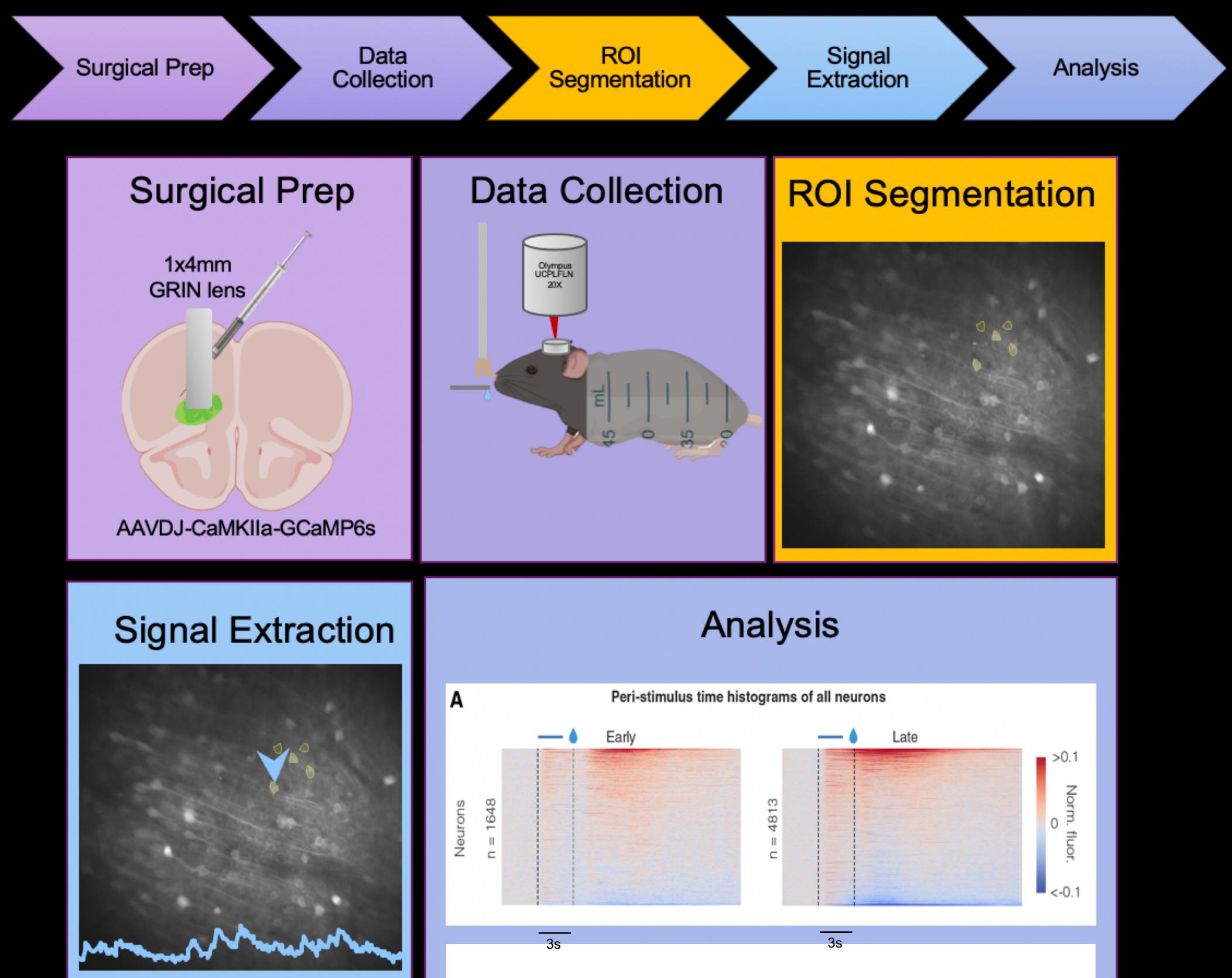


Figure 1. Workflow for two-photon endoscopic calcium imaging experiments. ROI segmentation is time-intensive and performed manually. Sample data is from Reference #2.

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)³

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, I calculated average intensity time projection images of the neuronal data. All figures represent the data from successive steps in the workflow code Figure 3C which was processed with a 5 pixel disk rank median filter for better visualization. The neural datasets used for this project derive from layer V of the prelimbic cortex in the mouse brain

Results

Aim 1: Background Reduction

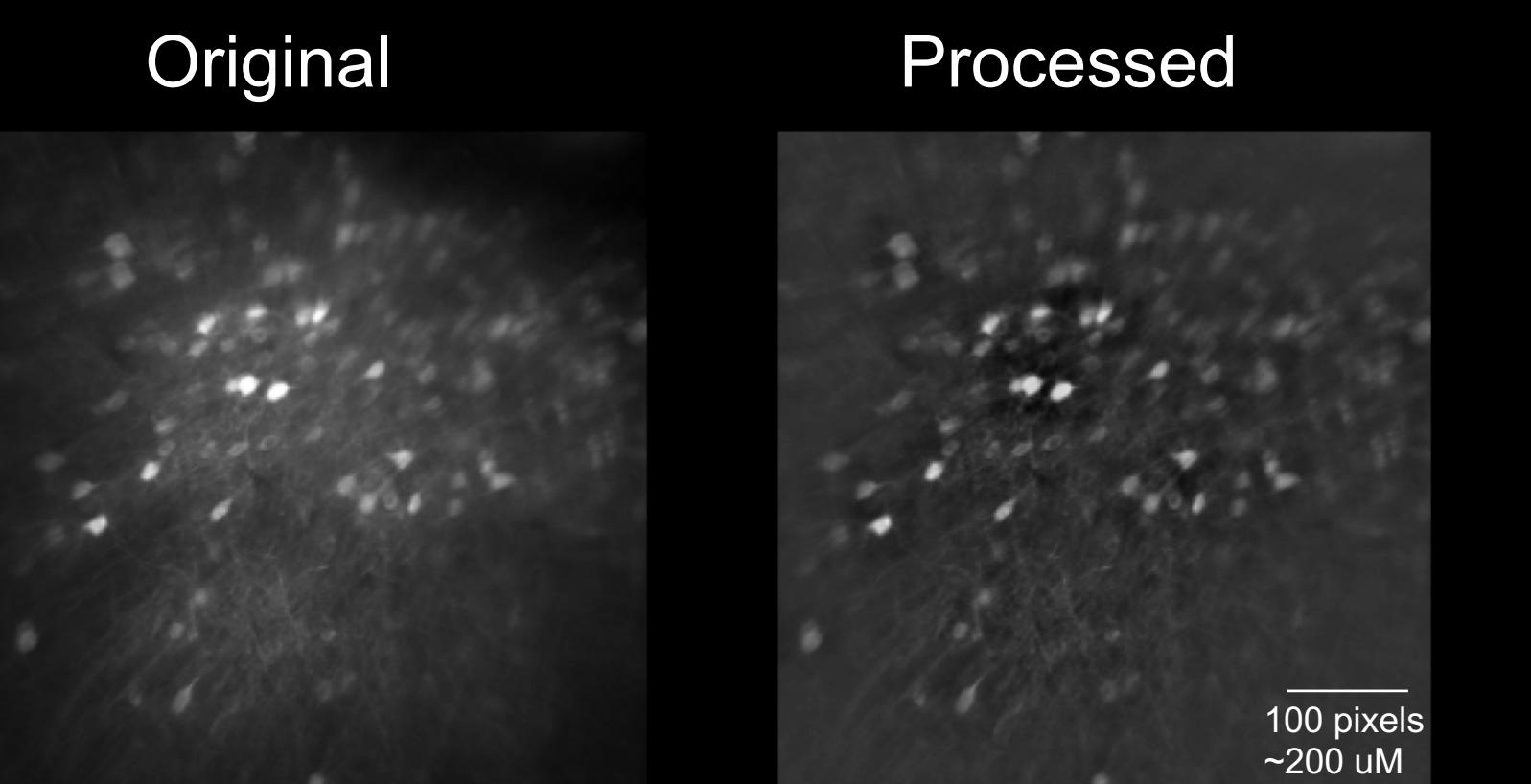


Figure 2: Results of background reduction algorithm. Note that the processed image has a much more uniform background and similar intensity across neuronal ROIs.

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

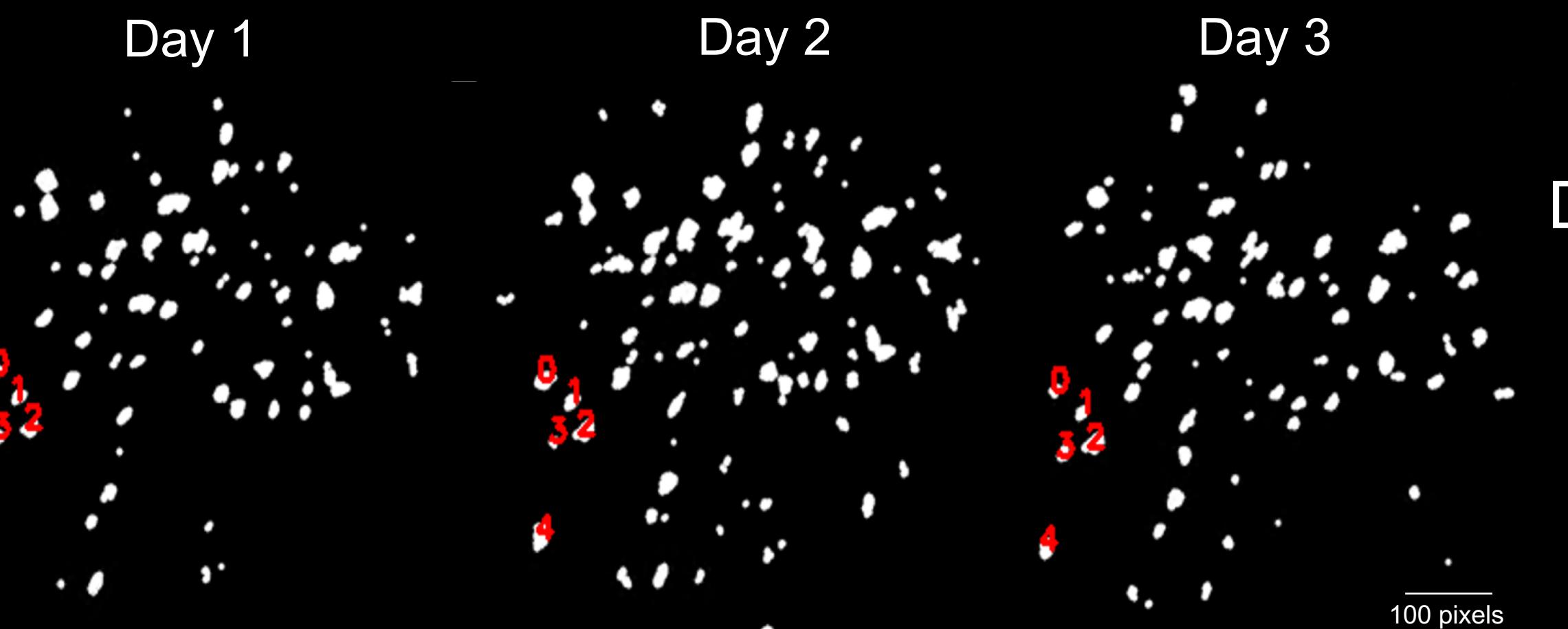
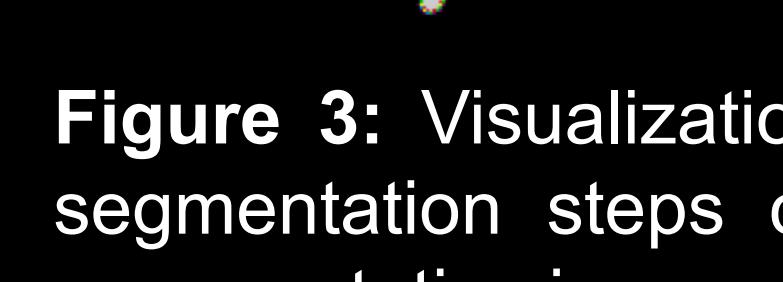
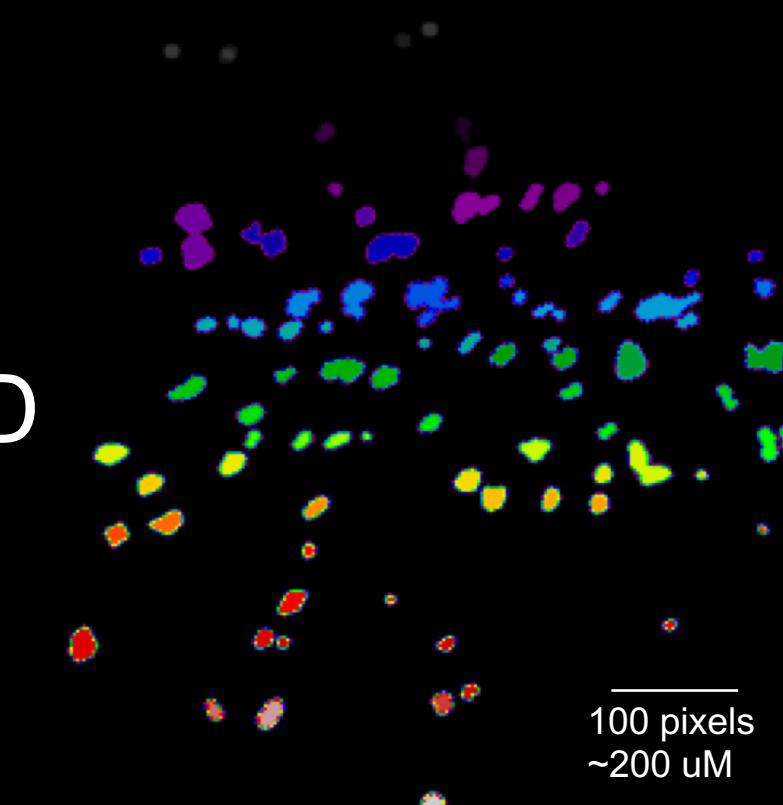
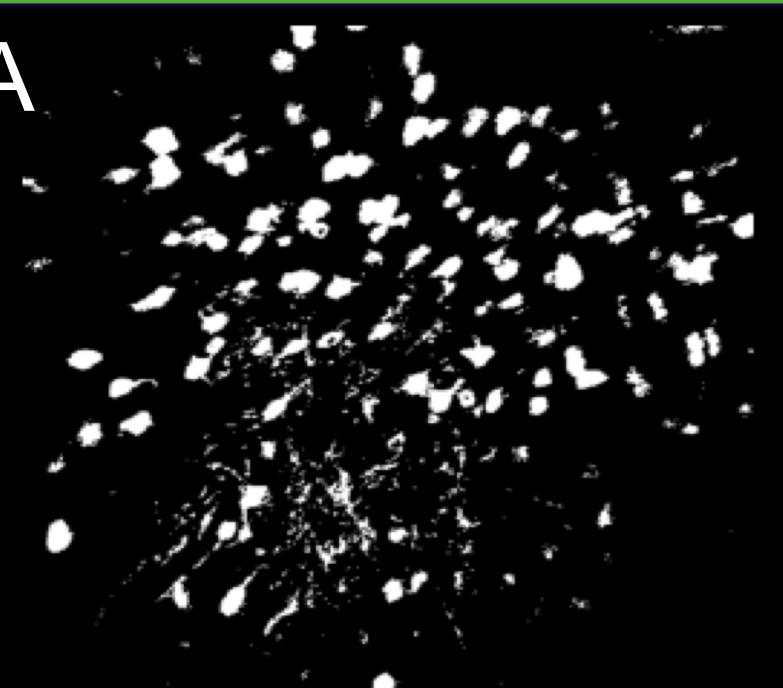


Figure 4: Sample tracking window with numbered cells

I developed a pipeline that combines neuronal tracking and manual selection/exclusion of ROIs from multi-day datasets based off the mouse click registration in OpenCV. Masks from Aim 2 that overlap with Aim 3 will be used for signal extraction and analysis in future experiments. Together, these innovations dramatically expedite my workflow.



100 pixels
~200 μM

Figure 3: Visualization of segmentation steps on a representative image

Aim 2: Neuronal Segmentation

Segmenting neurons took multiple steps. First, I had to set a minimum intensity level to generate a binary image that separate foreground (neurons) from background (neuropil)

Logical operator to threshold the neural image by removing any intensity below third quartile

Next, I had to remove parts of the image that were the same intensity as neurons but not part of the neural cell bodies I wanted to segment.

Binary opening of image A with a disk radius equal to neuron radius (8 pixels)

To seed my watershed algorithm, I performed a distance transform and identified the center of objects that were at least one neural radius apart.

Distance transform with local maxima detection using image B

Finally, I performed my watershed algorithm and segmented the neuronal ROIs. The algorithm performed well and was able to parse cells even if they were partially overlapping

Watershed algorithm of image in B with mask from C

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

I successfully segmented individual neurons with this technique. Future experiments with data from other brain areas can determine how translatable segmentation parameters are across the brain

References and Acknowledgements

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