# Automated Analysis Routines for Voltage Imaging

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## Introduction

The human brain is an amazingly complex organ, governing basic motor movements to higher order thought processes. There exists ~$10^{12}$ synapses in our brain, makes us truly unique individuals. While we have seen dramatic advances in our understanding of the human brain, we are yet limited by existing tools to investigate the brain circuitry. In order to address the challenge of providing a direct measure of electrical dynamics across multiple neurons, the Miller Lab is developing voltage sensitive fluorescent indicators, which output neuronal activity (changes in membrane potential) via changes in fluorescence intensity.

On initial production of a new voltage dye, it is essential to understand the exact relationship between fluorescence intensity and voltage. We perform this characterization in HEK293T cells, where changes in membrane potential are induced via a patch pipette and record a timeseries of the fluorescence changes. We seek to automate the image processing procedure by identifying the cell of interest, reliably calculating the ΔF/F, and plotting against the applied voltage, thus streamlining the char-acterization of a new voltage-sensitive dye.

## Methods

First, the program recognizes the cell of interest and filters out extraneous cells, or objects, or other artifacts from a voltage imaging experiment. A binary mask is finally generated of the region of interest.

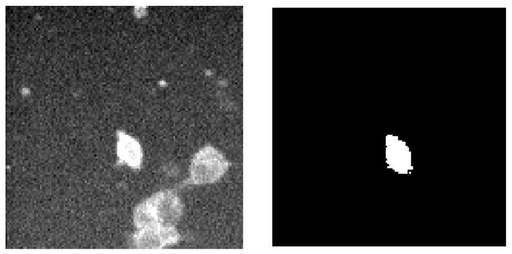
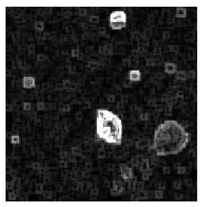


Figure 1. A mask is generated, on right, of the cell of interest in the original recording, on left.

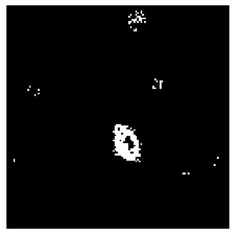
We utilize a multi-step image filtering algorithm.



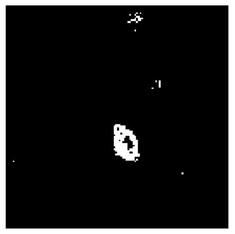
1. Using local range of image to recognize cell membranes: For each pixel, intensity value is replaced with the range value (difference between max and min pixel value in local neighborhood) of it's 3-by-3 pixels.

## Methods (cont'd)

2. Image thresholding using Otsu's method to eliminate noise, based on assumption that background noise pixels have higher correlation with each other than with illuminated cells. Pixels clustered into two classes with minimum intra-class variance.



3. Subtract average intensity of non-responding frame from that of responding frame. Since the intensities of non-responding pixels are relative constant, we are left with only the pixels corresponding to the cell of interest.

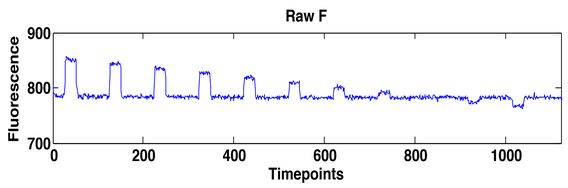


4. Perform "clean" and "spur" morphological operations to further remove isolated pixels and yield a cleaner result.

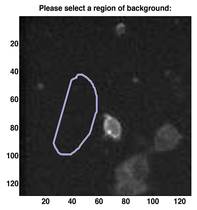
5. Perform an iterative object removal operation: while there is more than one object left in the image, remove the object with the least number of connected pixels. We assume that the responding cell is now the largest and densest object left in the frame.

6. Prior to this point the mask captures the cell membrane of the responding cell. To achieve a better signal to noise ratio, the mask is filled in to represent the entire cell.

After having generated a mask of the cell of interest, we can then conduct an analysis of the fluorescence changes of the cell relative to the amount of voltage applied. The fluorescence profile of the masked cell is generated below.

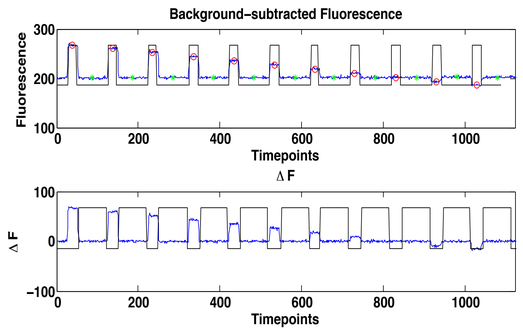



## Analysis

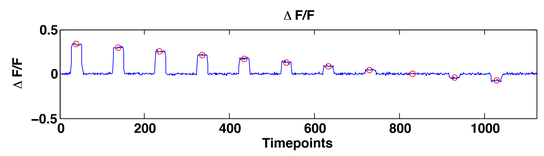



The user is prompted to select a background region. This is used to generate background intensities, which is then subtracted from the raw fluorescence and plotted below.

A protocol is loaded, with slight adjustments to account for drift, to generate the regions that are used to calculate the average intensities of each voltage step and each baseline fluorescence step. The black on-off steps frame the interval which was used to generate the averages, and each red/green data point represents its corresponding calculated average.




A ΔF/F trace is generated by subtracting the baseline fluorescence from the BG-subtracted fluorescence and normalizing. The averages of each interval is also plotted.




## Results

We plot ΔF/F against the voltage applied at each time point and obtain a relative gauge of the sensitivity of the dyes and a measure of how well they predict voltage changes. The signal-to-noise ratio (SNR) is calculated from the +40mV fluorescence change (ΔV=100mV). By fitting a linear regression, we see that the ΔF/F scales linearly with voltage applied.

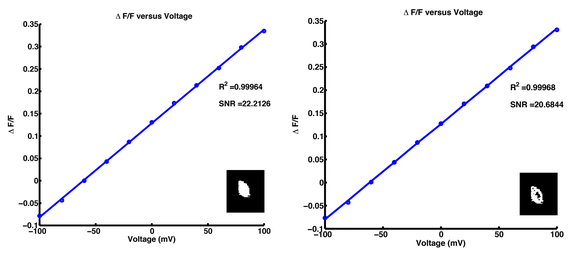



Figure 2. ΔF/F is plotted against voltage applied. The results for both masks are compared above.

## Conclusions and Discussions

Over the duration of this project we sought to automate the process of an aspect of voltage imaging. By correlating ΔF/F with applied voltage we can characterize the sensitivity of a dye. By automating this process using the routines we have developed, we can not only standardize future characterizations, but also greatly reduce the amount of time required in the procedure.

We have run our code against preliminary voltage imaging recordings and it has been consistent in reliable ΔF/F profiles. We have also implemented various displays at each checkpoint of the program, allowing the user full transparency into the mechanisms: generating the mask, intervals selected for obtaining averages, shape of curve, etc.

For the future, we would like to work on batch processing, alignment and image stabilization capabilities, which would be great for perfusion recordings/unstable camera as well as generating masks for multiple cells of interest within one field of view.

## References

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## Acknowledgements

Much love to our postdoc, Ali, who at every step of the way inspired us to do the best we can by her clarity of thought.
Thanks to Rishi and Yi Lin for their data.
Thanks to the rest of the lab for the conversation and occasional celebrations.
Thanks to Vadim for bringing us on and for his wonderful suggestions.
Thanks to Evan for taking us in: we've enjoyed our time as part-time chemists very much and hope to strengthen the bond!
Thanks to URAP as well as the College of Chemistry for hosting us!