spikeMapper: Methods and User Guide

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1 Introduction

spikeMapper is a spike detection tool written in MATLAB. It performs spike detection on a voltage imaging experiment video and allows the user to analyze multiple ROIs and choose the best based on desired results.

2 Methods

The spikeMapper source code has 4 main modules:

2.1 Pre-processing

The spike detection algorithm is applied to each pixel of the input video individually. when the resolution of the input video is too high, to keep the running time of the program reasonable, the user is asked to allow for block processing. A square block size is proposed based on equation (1). The block size is asked on each run of the program and can be set arbitrary. If chosen 1, no block processing is applied.

$$\text{block size} = 0.04 \times \sqrt{width \times height} \tag{1}$$

2.2 Bleach Correction

Since many voltage imaging experiments include some level of photo bleaching through the length of the experiment, a bleach correction step is required before applying any spike detection algorithm to the trace.

Spike detection is performed according to the following steps:

1. A least squared distance polynomial regression was used to find a polynomial that best fits the original trace. This method almost always fits to the traces baseline. The only case with a potential for problematic regression is when a burst, instead of narrow spikes, is present in the trace. In this case, the polynomial order can be tweaked to achieve a better fit (the default is a polynomial of order 10).
2. For each block (or pixel, if block size is chosen to be 1):
   (a) A polynomial regression is applied on function $I(t)$: time step $\rightarrow$ *intensity*. This polynomial is assumed to represent the baseline of the raw trace.
   (b) The minimum value of the fitted polynomial ($R(t)$: time step $\rightarrow$ evaluated intensity) is found.
   $$min(R(t))$$
   (c) The minimum from part b is subtracted from each value of the fitted line.
   $$R_z(t) = R(t) - min(R(t))$$
   The result is the fitted polynomial of part a, shifted down to have its minimum at zero ($R_z(t)$).

(d) Each value of the part c result is subtracted from each value of the main trace. The result is the bleach corrected trace ($B(t)$).

$$B(t) = I(t) - R_z(t), \forall t$$

2.3 Spike Detection and Counting

For each block (or pixel, if block size is chosen to be 1):

1. A thresholding is applied to the bleach corrected trace ($B(t)$) to detect the spikes:

   $$\textit{detected spikes} = B(t) - \textit{median}\{B(t)\} + 3 \times \textit{standard deviation}\{\textit{B(t)}\}$$

   A zeroing of negative results is performed after the above thresholding to eliminate the detected negative spikes, then, the time steps at which the the spikes are detected (all non-zero values) is found and counted.

2. An optional re-arming step can be performed after the previous step, with an arbitrary re-arming factor parameter which can be set by the user. If the parameter is set to 1, no re-arming is performed. If set non-zero n, then it will consider all spikes that are at most n time steps apart a single spike:
   A function $SpikeTimes(t)$ is defined as

   $$SpikeTimes(t) : SpikeNumber \rightarrow \textit{detected spikes}$$

   $$\textit{(e.g. SpikeTimes(1) = first detected spike)}$$

   Re-arming is done by thresholding dSpikeTimes(t) (differential) with the re-arming factor parameter, thus

   $$\textit{re-armed spike times = spike numbers at which dSpikeTimes(t)} > \textit{re-arm parameter}$$

2.4 Mapping the detected spikes

After bleach correction and spike detection steps are performed on each block, the results are shown to user as a heat map where the blocks are distinguished by different colors based on their counted spikes. The user, then, can select arbitrary regions of interest and see the original trace of those regions along with a bleach corrected trace using the same method as part 2.1. User can save each trace if desired and can add each ROI trace to a final raster plot.

3 User guide

3.1 Requirements

This tool has been tested on R2015b on Window 8.1 and 10 operating systems. Contact kkarbasi@berkeley.edu if there is any problem with other platforms/versions.
To run, all the following files should be in the same folder. Run *spikeMapper_v1.m* to start the program:

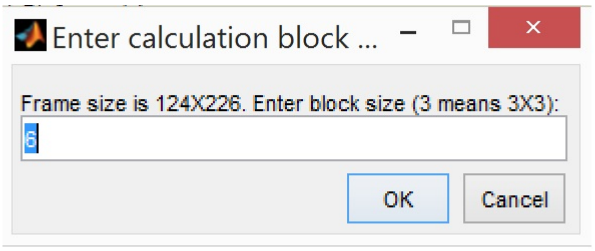
- traceFlattener.m
- spikeMapper_v1.m

- spike_counter.m
- save_traces.m
- burstAggregator.m
- applyMask2TiffStack.m
- tiffStackReaderFast.m

If spikeMapper is run on MATLAB R2014b and older, file repelem.m should also be included in the running folder.
A select file window is popped up prompting for an input .tif or .tiff video file.

3.2 Choose processing block size

A default block size is proposed by the program based on section 2.1. It can be changed by simply typing the desired block size, or 1 if no block processing is demanded:


Enter calculation block ...

Frame size is 124X226. Enter block size (3 means 3X3):

6

3.3 Selecting ROI(s)

A heat map of the input video is shown in which counted spikes of each processing block are color mapped to distinguish areas of different spiking activity. You can freely select a region of interest on the map:

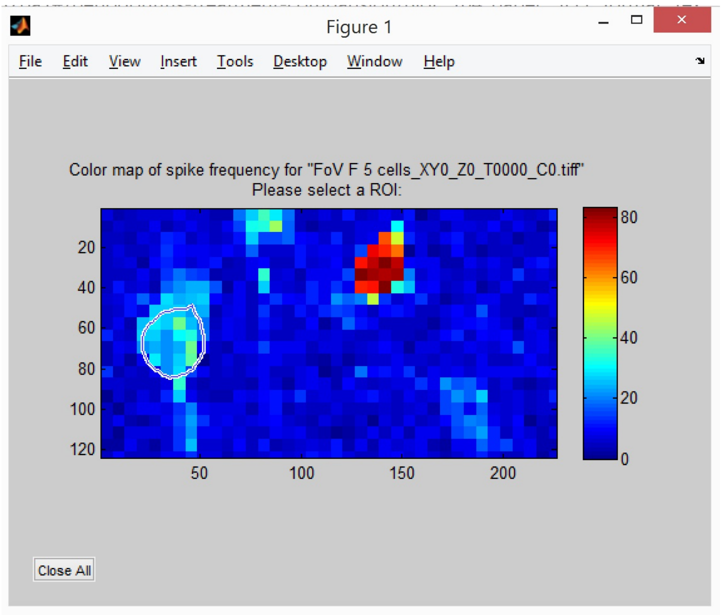
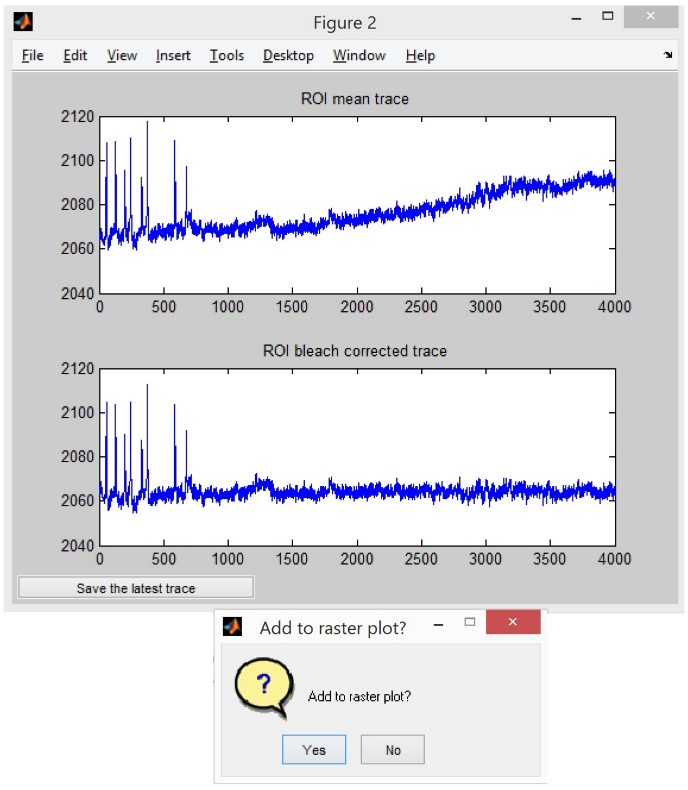


Figure 1

Color map of spike frequency for "FoV F 5 cells_XY0_Z0_T0000_C0.tiff"
Please select a ROI:

After selecting a ROI, a trace of the mean intensity of the selected region along with its bleach corrected trace is shown and user is prompted if this ROI should be added to the final raster plot.



After this step, user is asked if the program should continue, save the current trace, or stop and plot the final raster plot. If *Continue* is pressed, another ROI can be selected on the heat map. if *Save and Continue* is pressed, the current raw trace, its bleach corrected trace and the detected spikes (using same method as 2.3) are save in external files. If *Stop and Plot Raster* is pressed, the program plots the final raster plot and exits. The ROIs that user has added to the raster plot are numbered and their corresponding activity can be found on the same row number (on y axis) on the raster plot:

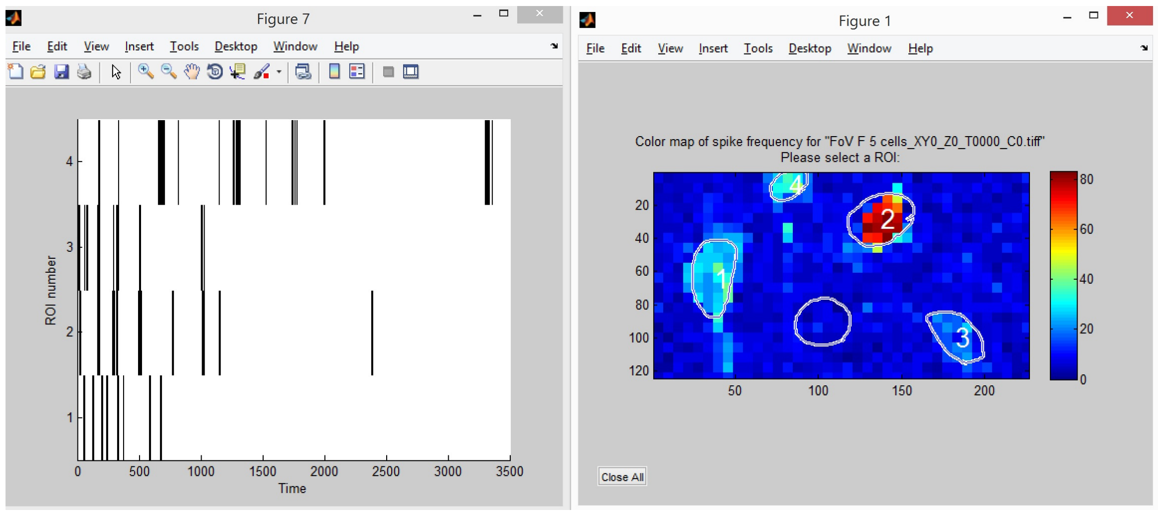


Figure 7
File Edit View Insert Tools Desktop Window Help
ROI number
Time
Figure 1
Color map of spike frequency for "FoV F 5 cells_XY0_Z0_T0000_C0.tiff"
Please select a ROI:
Close All

3.4 Setting Parameters

At the top of spikeMapper_v1.m, a section is marked as *Configuration Variables*:

- **polynomial_order** is the order of the polynomial that is fitted to the baseline trace in the regression step (default = 10).
- **sd_treshold** is the standard deviation coefficient in thresholding step (default = 3).
- **re_arm_factor** is the re-arming factor parameter, explained in 2.3 (default = 1 (no re-arming))