**Installation**

Required Software:  
Matlab (the code has been used on version R2021a)  
Femtonics MES software (the code has been used on version 6.4.8309)

Required non-standard hardware: none

1. Install Matlab
2. Install Femtonics MES software, downloadable from [www.femtonics.eu](http://www.femtonics.eu)
3. In Matlab, make sure to add the MES folder and folders containing the mtGCaMP\_analysis script and required Functions to the path (‘Home’, ‘Set Path’, ‘Add with Subfolders…’
4. Dependencies (all included in the “Functions” folder):
   1. For the drift correction, scripts are used that is described in (Pnevmatikakis and Giovannucci, 2017)
   2. The script natsort is needed to sort filenames

**Using the code**

1. Start MES and open your MES file (e.g. ‘mt-GCaMP\_ExampleData.mes’)  
   If MES doesn’t start automatically when you open Matlab, type ‘mes’ and press Enter
2. Select your recording of interest (e.g. ‘ExampleRecording’) and export into the folder of your choice (separate file for each channel, i.e. UG and UR)
3. In Matlab, navigate to the folder where you exported the files in step 5
4. When done, the next step is to extract the action potential traces.  
   Right click your recording of interest in MES and select PropertyCollect  
   Set Format to Curves  
   Set Mestag properties to AUXi1 (you need to select it even if it is already highlighted)

Click ‘Do’

Click ‘To curve analysis’  
On the left hand side, select all traces of interest  
On the right hand side, click ‘Export…’ and select ‘Curves to variable’

Name the variable ‘ephys\_traces’  
In the Matlab Command Window: type save(‘ephys\_traces’) and press Enter  
The Matlab Workspace (which should only contain the objected ephys\_traces) is now saved in you active folder

1. At the top of the code, provide the start and stop time of stimulation (in ms, should be the same for all trials so only provide one number for start and one for stop), the maximum and minimum y-values of the ephys trace (according to MES), the threshold for an AP, the threshold to define a response, and the number of rows for the subplots
2. Press ‘Run’
3. Name your recordings – if you use ‘Soma’, ‘Dendrite’, ‘Axon’ or ‘En passant’ in the name the script will provide an excel file with averaged responses for each subcellular compartment
4. After drift correction has finished, you will be asked:
   1. To draw an ROI on the mitochondrion. Do this in the image on the top. This ROI will automatically be copied to the image on the bottom. Here, move the ROI to a background location, without changing its size. You have 5 seconds for this, but can change this duration in the script in line 138.
   2. To provide the rate of decay (t) for the bleach correction. Play around with this number if bleach is not corrected well. Bleach is corrected well if the black line follows the baseline signal and continues as a nice, curved line. Pay attention to all plots/trials.

To accept the bleach correction or repeat it. If you press ‘No, do it again’, try different values for t (min/max) in order to improve the correction

1. All of the rest of the code should run automatically
2. If you wish to make a heatmap of a specific recording, uncomment the ‘Heatmap z-stack’ section (starting from line 1366). Enter the number of the stimulation you wish to make a heatmap of (open the variable S to see a list). Change the caxis to change to color range (also change the range of the colorbar in c.Ticks).

**Expected output**

The output consists of:

* See “Example\_Analysis\Analyses\220404
* A Data.mat file that contains all the settings you used for your analysis
* ROI.jpg files that indicate your chosen ROIs
* If applicable, a TIF file with a heatmap
* Drift corrected TIF file
* PDF files with the AUC, Peak height, Peak location, trendline, AP frequency, bleach correction, calcium responses and peak detection
* If applicable, these will also be calculated for repetition trials
* If applicable, an Excel file with averaged responses for each subcellular compartment
* Two Excel files (prestim\_data and stim\_data) showing the measure parameters for each recording either before (prestim\_data) or during (stim\_data) the stimulation. Stim\_data.xlxs also contains the traces
* The demo run should be done within 5 minutes

Note:

* Use ImageJ to check if drift correction worked well. This might be important if you for example observe a spontaneous decrease in dF/F signal
* For the detection of peaks in the ephys trace (code lines 803 – 847), MinPeakHeight might not always be optimal. Try MinPeakProminence alternatively. Use figure 12 and 13 to check what is marked as a peak (indicated by a blue arrow)

Potential errors:

* Bleach correction: 'Exiting due to infeasibility' -> try changing upper-c to inf and lower-c to -inf

**References**

Pnevmatikakis EA, Giovannucci A (2017) NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data. J Neurosci Meth 291:83–94.