Analysis of *C. elegans* neuronal activation using a calcium sensor
Matlab tutorial

These codes have been created for tracking timelapse recordings of neuronal pairs (left and right) expressing a calcium sensor such as GcAMP, as in Quintin S., Aspert T. and Charvin G., 2021 BioRxiv. Below are described the sequential functions to run under Matlab.

Upon acquisition, movies should be cropped as small as possible to minimize processing time and should be save as .tif files.
Note: we recommend to first make a 4D-projection of the original movie, draw the smallest ROI around the neurons and transfer it to the original movie, to ensure that all frames are properly included in the cropped selection.

1. Movie registration

—> readworm_PHA ('movie_name.tif')

After ROI selection with the 2 neurons of interest, the function readworm_PHA processes each movie time point and performs the alignment of neurons, generating two files:

movie-registered.mat
movie.mp4

The mp4 allows the user to verify quickly that the movie has been correctly aligned.

2. Neuron segmentation, selection and tracking

—> viewworm_2('movie_name')

The function opens a figure project with several images and buttons.
The top left image allows movie traveling in the registered movie for each frame, using the right and left keyboard arrows, or typing directly the frame number in the box at the bottom of the window. To better visualize neurons, the intensity threshold of the image viewer can be adjusted with the top and bottom keyboard arrows.

Using the '**Pixel Train**' button, the user teaches the program which pixels should be selected for neuronal segmentation by painting them in green (left mouse click). The pixels which should not be included for segmentation are painted in red (right mouse click). This needs to be done for several time points to obtain the best segmentation results, especially during the neuronal response. When this teaching has been done, the user clicks '**Classify pixels**', leading to the **neuron segmentation** as defined by the user. Then the user defines each neuron in the bottom segmented image, by clicking the '**Select neurons**' button:

right click—> red selection (left neuron);
left click —> blue neuron (right neuron);

Upon pressing the '**Track'** button, red and blue neurons will be tracked for all movie time points. Upon pressing '**Plot'**, the **mean fluorescence intensity** is indicated as a function of time, generating the red and blue curves in the bottom right panel.

Important note : this step allows the **correction of tracking errors** at every frame. The quality control is performed by the user by selecting the appropriate neuron and clicking '**Track'** again until all tracking errors are corrected (green pixels are excluded from the quantification).

When the movie is properly tracked to the end, click '**Save'** button to obtain the .fig files which will be used for subsequent analyses. The user needs to indicate the T0 in the command window, which corresponds to the time point at which the stress/ stimulus was applied.

Finally click on '**Movie'** to generate an mp4 movie showing the registered, segmented and tracked movies altogether, allowing an assessment of the global quality of the analysis.

Once all movies have been successfully processed, create a folder containing all the individual.fig, which will be used for the average curve.

3. Average curve processing

—> averageGCamp2('folder_name')

In the command window, assign [low, high, offset]=averageGCamp2('folder_name')

—> processAverage('low,high,offset')

This function will create average curves for red and blue neurons, for raw and normalized data.

Finally to retrieve individual intensity mean values at the response peak, run the **ExportSingleWormData** function which will produce an Excel sheet with all individual peak values.