

This program has been designed for the detection of pH sensitive GFP netrin (Netrin1-SEP) exocytosis events. The program is divided into two parts, first 'Exocytose_PeakDetector.m' detects the clusters or spots appearing on the movie stack then the script 'Exocytose_Analysis.m' loads the detected spots and display them to the user so false positives are removed.

The program was build for two channels analysis; it expects a .tif movie of exocytose events and another tiff or z-stack from a synaptic or morphological marker. Exocytose_PeakDetector loads the exocytose movie then remove background, the background is defined as the average of the first 25 frames of the movie. Typically, the exocytosis event are bright spots that appear between two frames in an all or none fashion. For Netrin-1-SEP, they are bright spots that can last for minutes on the neuronal membrane. To detect the events the software use a backward subtraction of the frames. i.e. frame $t=2$ subtract to frame $t=1$ and so on. This creates a movie of appearing spots and the peak exocytosis is detected by 'f_ExoSpots.m'. For each detected spots we then compute the $\Delta F/F$ of the event we define a baseline of 3 frame and a decay time of 10 frames. We then compute the area under the curve for each event and this value will help discriminate noise to real exocytosis events.

'Exocytose_Analysis.m' is the second part of the program, it will be automatically launched after the peakdetection has finish running. The first action the user need to take is to click on 'Get Data' button, the user will need to select the exocytosis movie .tif and the synaptic marker z-stack or single plane tif file. Then a movie player will appear and a figure named 'display spots'. In order to continue with the analysis the user will need to click on the slider of the figure 'display spots'. The value that is displayed correspond to the area under the curve of the previously computed $\Delta F/F$. This is the threshold value used for discerning real exocytosis events to noise. After looking at the movie in the player, the user should lower the Max area under the curve threshold so to they are a little bit more events detected in display spot than visualized in the movies. Then the user can click on 'Get events' button. Then many figures will appear, all of them have created so the user can make a choice on keeping and classifying the exocytose events. Based on the red channel abd max event overlay the user can select if this is a synaptic, dendritic or an unclassified (NaN) event, it is also possible to delete the events. We typically select event that show a sharp increase in fluorescence and a slow stable decay for Netrin-1. The events are sorted in a decreasing area under the curve order so the brightest events will be displayed at the beginning. If many false positive are displayed at the end of the analysis, it means that the user has used a sufficiently low enough threshold, all events should have been detected. The results are saved in a .txt file, we save the frequency, the keepers events : [x y frame eventnumber localisation Decaytype Amplitude Amp1 tau1 Amp2 tau2] and all the $\Delta F/F$ events : [x y frame eventnumber $\Delta F/F$ localisation].

The program has been developed in Matlab 2012a, with Image Processing Toolbox Version 8.0, Statistics Toolbox Version 8.0 and Optimization Toolbox Version 6.2.

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