DCV-pHluorin Toolset

Contents

Introduction

DCV-pHluorin toolset implement different functionality for the analysis of neuropeptide labelled with pHluorin. In particular it was developed and tested using NPY-pHluorin.

The idea of this toolset is to provide a semi-automatic analysis of dense-core vesicles fusion events in primary neuronal culture. It is designed to simplify the analysis and to provide a controlled cross platform workflow for the detection and analysis of fusion of dense-core vesicles. For a complete analysis the user will then use the MATLAB programs SynD and FusionAnalysis2.

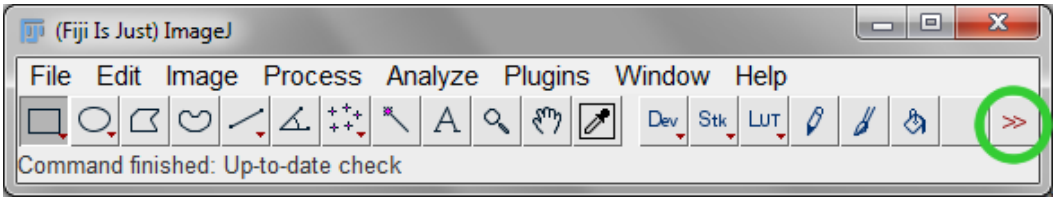
The toolset uses different custom made macros and already existing pluging. In particular StackReg from EPFL MorphoJ Lib from David Legland, Ignacio Arganda-Carreras and ComDet from Eugene Katrukha to which we are extremely grateful

The toolset consists of: fusion detection; exporting as maximum projection for SynD analysis; manual ROI modification; saving, measuring, and navigating ROI; ROI frame reader. There are two sets of option that the end user can adjust for their need. A general option set to handle the graphical setting of the pHluorin analysis, like LUT, zoom, ROIs shape and size and saving functionality, which is accessible from the Tools themselves. A parameter option set, accessible only from the Fusion Detection, where the user can specify the parameter and preference for the detection.

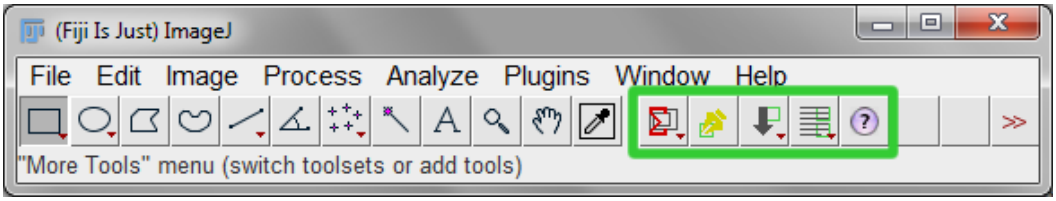
For better performances we recommend to use FIJI instead of ImageJ.

Installation

To install the toolset first add it to the directory Fiji.app\macros\toolsets and run FIJI. To run the toolset click on the right button on FIJI and select the DCV_pHluorin



This will automatically install the 5 macro tools: Start Analysis Menu; Place ROIs Tool; ROIs Interaction Menu; ROIs Frame Reader Menu; Help… Tool.



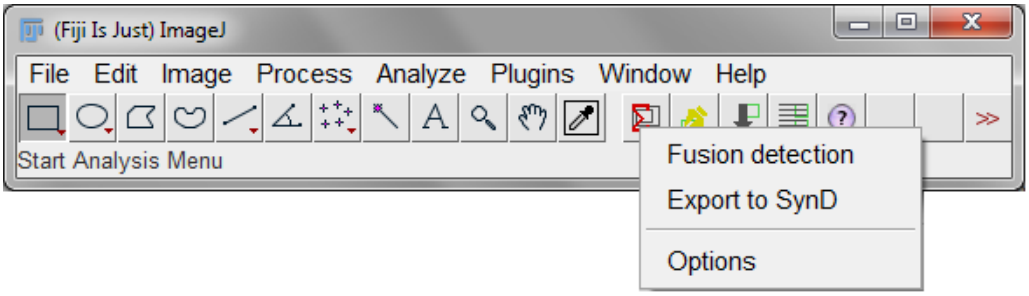
It is also recommended to check for ImageJ and FIJI updates and to make sure ComDet, StackReg and MorphoJ Lib are installed.

Tools

Start Analysis Menu

This menu will prompt to a submenu with the Fusion detection; Export to SynD and Option.

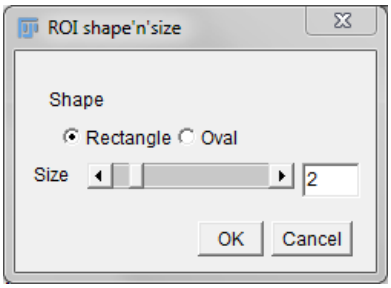
Fusion Detection will bring the user to the automatic detection of fusion events. Export to SynD will create an image to be anylized in SynD to quantify the total pool of dense-core vesicles.



Place ROIs Tool

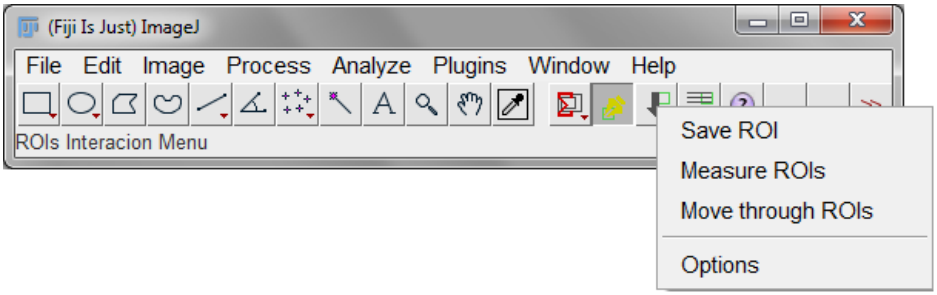
This is a selection tool, it allows creating fixed sizes region of interest and automatically adding them to the Roi Manager. With "*Alt* + *click*" inside a ROIs the tool will look for the first ROI which contains the selected pixel and delete it. Left click on the tool button opens the ROI shape and size option dialog.

The supported shapes are rectangular and circular ROI with size from 1x1 to 10x10 pixels.



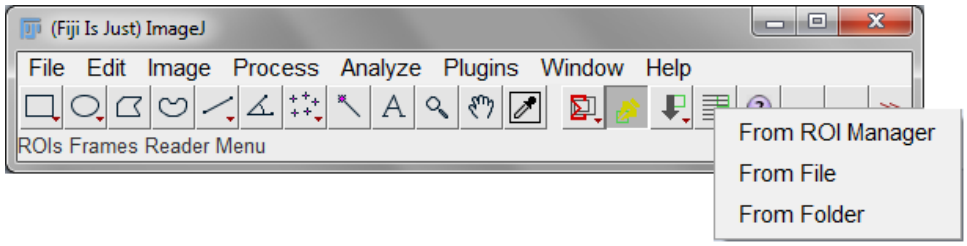
ROIs Interaction Menu

This menu allows the user to save the ROIs, measure them and navigate them; it also has an access to the options.



ROIs Frame Reader Menu

This menu allows the user to read the frame of the ROIs from the Roi Manager, from a file or a folder.



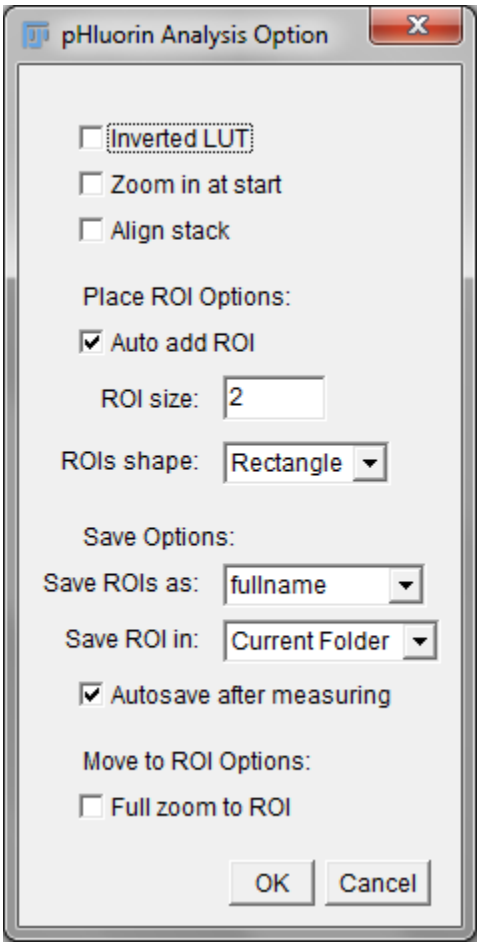
Help…

Prompt to the help dialog with information on the version and several links.

Options

pHluorin Analysis Option

This options allows to personalize the appearance of the analysis. They can be access from the Start Analysis Menu and ROIs Interaction Menu.



Inverted LUT: when the program finishes the automatic analysis inverted the LUT. If it is check the Measure ROIs will inverted back the LUT before measuring the ROIs.

Zoom in at start: if checked when the program finishes the automatic analysis increase to zoom of the image.

Align stack: if checked before running the Fusion Detection run Stackreg to correct from drifting.

Auto add ROI: if checked will automatically add to the Roi Manager the ROI placed with the Place ROI Tool.

ROI size: the size of the Place ROI Tool. The size is always even (as 2x2 3x3 pixel).

ROI shape: the size of the Place ROI Tool, the user can choose between Rectangle and Oval.

Save ROIs as: save the Roi Manager as a *.zip file. The options are: *fullname* which save as RoiSet_*imageName*.zip; if the image name is *YYMMDD_condition_cs*ID_*cell*ID the file could be saved as RoiSet_*cs*ID_*cell*ID, with the option *cs and cell ID*.

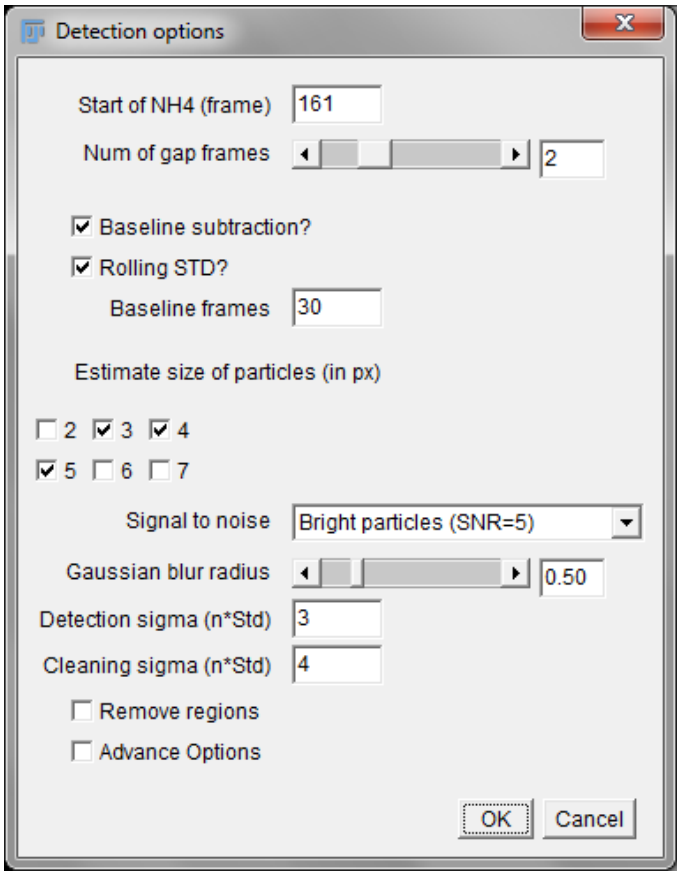
Save ROI in: select the folder where to save the Roi Manager between *current folder*, the same folder as the image, *specific or new folder* which will prompt to a new specify folder.

Autosave after measuring: if checked after using the Roi Measure will save the Roi Manager with the Save ROIs as and Save ROI in options.

Full zoom to ROI: if checked during the ROIs navigation will automatically zoom to the selected ROI.

Detection Options

Those are the basic option for the Fusion Detection. The dialog will appear every time the Fusion Detection is lunched.



Start of NH4 (frame): Indicate the last frame to be analyzed.

Num of gap frames: Indicate how many frames to skip for the stack subtraction. If greater than 1 allow for a better identification of events that rise in more than 1 frame.

Baseline subtraction: If checked will calculate the mean and standard deviation value of the baseline as use it for background noise cleaning in the subtracted stack.

Rolling STD: If checked will use a walking mean + STD to detect the start of the event.

Baseline frames: number of frames to be considered as baseline, it will be use for the baseline subtraction, rolling STD and frame detection.

Estimate size of particles (in px): indication or the area of the potential particles to be detected, considering that the stack subtraction will enlarge or shrink different population of events.

Signal to noise: an indicative lower value for the signal to noise, the lower the more particle will be detected with the possibility of increasing the false positive rate and computation time.

Gaussian blur radius: radius of the Gaussian curve in pixel to filter the camera shot noise, bigger radius favors bright events.

Detection Sigma (n*Std): number of standard deviation the signal needs to be above the mean to be out of the noise range. If the baseline subtraction is checked uses this parameter to calculate which pixels are above the baseline noise.

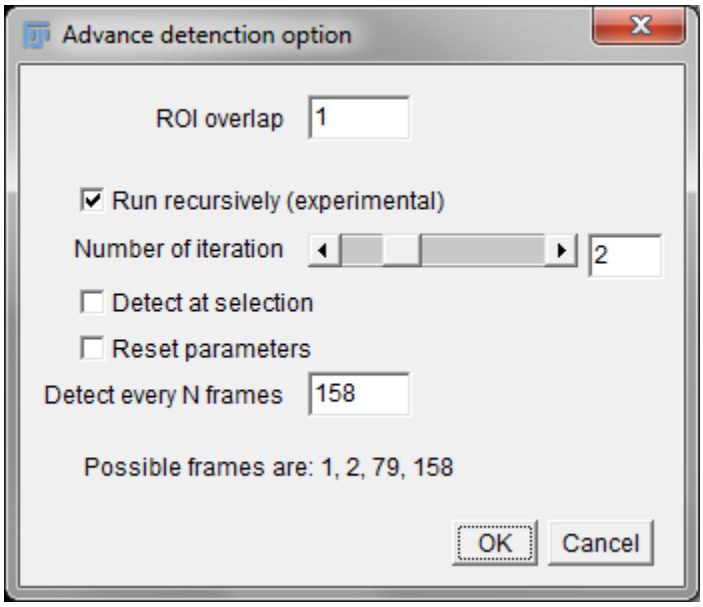
Cleaning Sigma (n*Std): number of standard deviation the first derivative of the signal needs to be above the mean to be considered as fusion. If the Rolling STD is unchecked the program will calculate the fusion event start using the gradient in the first derivative of the signal and uses this parameter to clean for false positive.

Remove regions: if checked the user can manually select region to be excluded by the Fusion Detection, for example the soma.

Advance Options: prompt to the Advance Detection Options.

Advance Detection Options

This dialog will appear if the corresponding check box is checked in the Detection Options allowing the user to modify settings that might influence the overall performance of the program.



ROI overlap: maximum number of pixel two ROIs could have in common.

Run recursively: if checked will perform the fusion detection a number of time indicated by the Number of Iteration, every time it will mask the already detected pixel.

Number of iteration: more iteration longer processing and more false positive, 2 seems the sweet spot.

Detect at selection: opposite of the Remove regions option, if there is a neurite mask it can be load and use it to remove everything else.

Reset parameters: set all the Options to their default values.

Detect every N frames: this parameter uses a clustered Z projection to increase the number of frames it uses for the detection, lower values means more frame to detect thus longer processing. If the value does not match the possible frames the file is processed using the maximum number of frames.

Remarks

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