

Red Dot in Green Cells macro (version 14b)

Overview

This macro analyzes fluorescence microscopy images produced by the proximity ligation assay (PLA), also known as DuoLink. The PLA signal consists of fluorescent dots in images of cells or tissue. More specifically, the macro counts red dots only in green cells (expressing GFP, for example). A report including red dot counts and green cell areas is saved after analyzing a whole folder of images. The latest version can be downloaded for free from <http://biologic.xyz>.

Technical details

The macro can be edited within Fiji or using a text editor. In the current version, images are expected to be acquired with the 63X objective of a Zeiss confocal microscope and saved as Blue Green Red Z stack lsm files. Images are analyzed as follows:

1. maximal intensity projection.
2. channel splitting (1 blue, 2 green and 3 red).
3. segmentation using the "Statistical Region Merging" plugin provided by Fiji, followed by thresholding, which detects all but the faintest green cells.
4. masking the red image with the green one to remove dots that don't overlap green areas.
5. counting and outlining remaining red dots with the "Analyze Particles" feature of ImageJ.
6. generating an image by merging 3 color channels and white outlines of detected red dots.
7. preparing a table to summarize red dot counts and green cell areas.

Note that it is best to avoid taking pictures where green and non-green cells overlap because red dots produced by negative cells would be counted in overlapping regions.

Works with Fiji / ImageJ (Version: 2.0.0-rc-15/1.49m from 2014-10-06), which runs under Intel Mac OS X 10.5 or later, Windows XP or later and Linux. Fiji is available for free from <http://imagej.net/> or <http://fiji.sc/Fiji>.

Installation

1. Start Fiji and choose:
Plugins > Install Plugin...
2. When prompted, open the provided "Red_Dots_in_Green_Cells.ijm" file.
3. Quit/Exit Fiji.

Using the software

Copy the provided "Example_Image.lsm" file to a new folder/directory.

Start Fiji and choose:

Plugins > Red Dots in Green Cells (at the bottom of the menu)

When prompted, choose the folder containing the "Example_Image.lsm" file. It will be analyzed and results will be saved into a subfolder as images and a "results.xls" summary table. One "marked" image shows counted red dots outlined in white over green cells as in the included "RedDotsGreenCells.jpg" file. Additional images include individual red, green and blue channels and a binary image showing cells detected in the green channel. Please inspect these images to verify the accuracy of green and red detection. The summary, which can be opened with Excel or a text editor, includes red dot count, green cell area and their ratio for each original image file. Please save the summary as a native Excel format to preserve your formulas.