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Analysis of immofluorescence images in ImageJ

In 1 collection

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ABSTRACT

Analysis at a single cell level of images taken on confocal

Keywords: ASAPCRN

Funders Acknowledgement:

ASAP

1 Import image into ImageJ as a TIFF file.

2 Set scale for Image by going to Analyze --> set scale. Set distance in pixels and unit of length to appropriate values for objective images were collected with.

3 Convert image to 8 bit going to Image --> type --> 8 bit.

4 Open up ROI manager using Analyser --> tools --> ROI manager.

5 Use freehand selection tool to carefully draw around the outline of each cell. For each cell outline add to ROI manager by selecting ADD.

6 Set up measurement parameters in Analyse --> set measurements --> select AREA, MIN & MAC GREY AREA, INTEGRATED DENSITY, MEAN GREY VALUE AND LIMIT TO THRESHOLD.

7 If a multichannel image convert to composite.

8 Go to IMAGE --> ADJUST --> THRESHOLD. From drop down menu select intermodes.

9 Use a consistent threshold for all images,set to threshold and in ROI manager select MEASURE.

10 Calculate a background fluorescence of each image aquired by drawing 3 ROI not including a cell. Take a measurement of background using the same threshold settings.

11 Calculate corrected total cell fluorescence using = integrated density measurement - (cell area * mean average background fluorescence).