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Analysis of immofluorescence images in ImageJ

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Protocol status: Working
We use this protocol and it's working

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ASAP

Abstract

Analysis at a single cell level of images taken on confocal

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).