




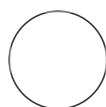
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 Analysis of immofluorescence images in ImageJ 

 In 1 collection

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## ABSTRACT

Analysis at a single cell level of images taken on confocal

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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 acquired 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  $\text{integrated density measurement} - (\text{cell area} * \text{mean average background fluorescence})$ .