

Nov 17, 2023

Analysis of immofluorescence images in ImageJ

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9py4zg3e/v1>

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Protocol Citation: rachel.bates 2023. Analysis of immofluorescence images in ImageJ. **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: July 30, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 85694

Keywords: ASAPCRN, analysis of immofluorescence image, immofluorescence image, imagej analysis, image, single cell level, cell

Funders Acknowledgements:

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

