# How to measure fluo staining in nucleari area inside cells using FIJI ImageJ?

Logics:

1. Use "wand" tool to manually select nucleus across the whole image (threshold nuclear channel first).
2. Use "ROI manager" to manage all ROIs (also nucleus in this case): Analyze/Tools/ROI Manager)
3. After the above 2 steps, direct measuring to the channel you like to measure fluo (need to spilt channels at the very beginning: Image/Color/Spilt Channels).
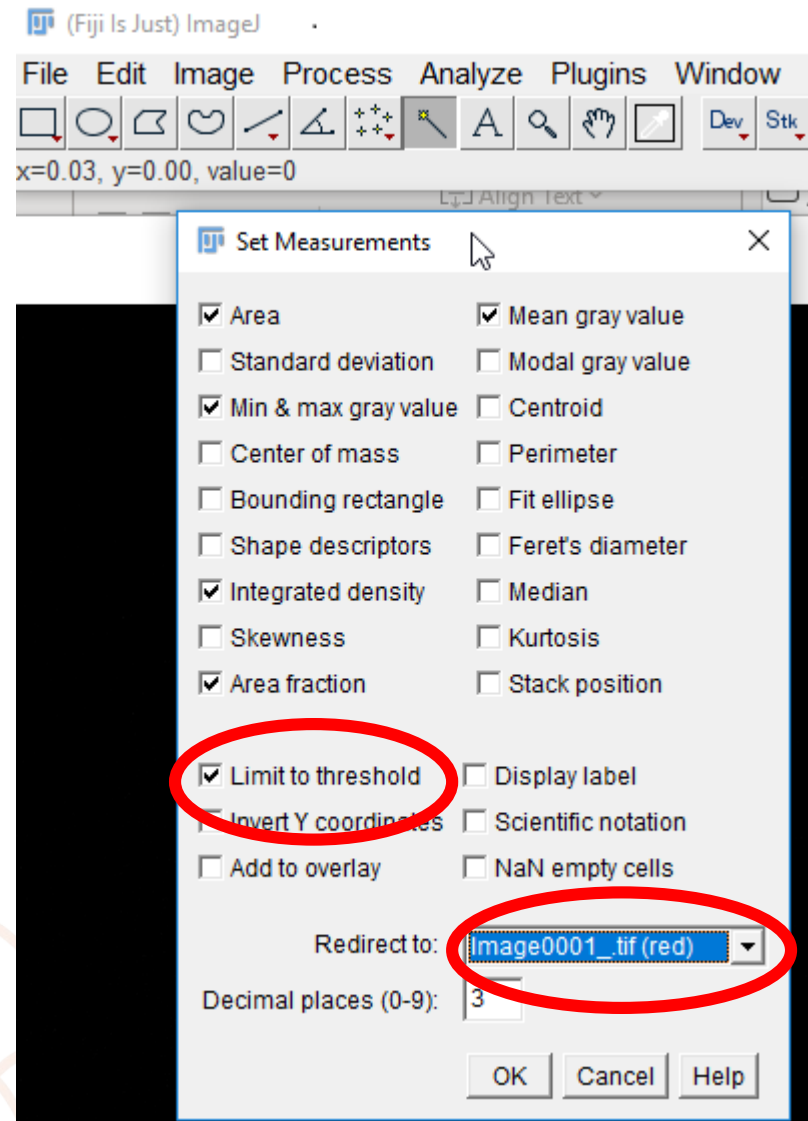
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Steps

1. Open your RGB image-split channels(FIJI/Image/Color/Split channels)
2. Threshold DAPI channel: Image/Adjust/threshold (no need to press "apply" after threshold adjustment, otherwise, you get a binary image which you may not necessarily need in this case).
3. Activate the Wand tool (this tool works easier after threshold adjust in order to select the interested area), click in the middle of the threshold-selected area i.e. nuclear to select nucleus.
4. Then go to ROI manager (FIJI/Analyze/tools/ROI manager), you will have a ROI Manager window popping up- check "select all" to view all ROIs. Then click "add" in the ROI Manager (or shortcut "t").
5. Repeat Steps 3 & 4 to get all ROIs added/registered in ROI Manager
6. Threshold the channel where fluo is to be measured i.e. red channel in this case

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7. Set up measurements: Analyze/Set Measurements

**Notes:**
- direct measuring to the previously split channel you like to measure fluo i.e. red channel
- tick "limit to threshold" (this way, only thresholded areas in red channel will be calculated against ROIs (nucleus in this case).

# 8. Measure from ROI Manager (Can rename the ROIs here)



Red staining is selected by adjusting threshold
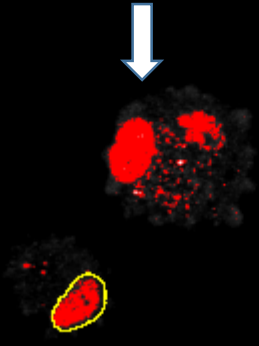
ROIs selected based on DAPI staining