Quantitating nuclear fluorescence

Note: Check that images are not saturated (over-exposed) ie outside the linear range of the CCD camera. Images should not be converted to another format (should be the original TIFF images). Images should all be collected on the same day as the lamp intensity changes with age. Ensure that the field intensity is as even as possible (can do this by comparing background levels or fluorescent beads).

Obviously all images need to have been collected with the same filter and exposure.

Make sure you use the latest version of ImageJ otherwise the “add to overlay” option is not available.

1. Open images to be quantitated.

set ROI for image analysis

2. For a circular ROI (for nuclear analysis): Use Analyze>Tools>ROI manager>Specify

set width and height (eg 25) so that larger than all images to be measured. (check ‘circular ROI’)

Add ROI to ROI manager in case you need to restore it (can also use Edit>Selection>Restore selection)

3. Analyze>Set measurments - check parameters to be measured eg mean grey value and check ‘add to overlay’.

4. Drag ROI to say 20 background regions and Command-M to measure. Check that levels are not too dissimilar.

Copy bg levels to excel spreadsheet and calculate mean bg level

5. Repeat for eg nuclei or regions to be quantitated.

Copy results to excel and subtract mean bg level.

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