Fiji/R nuclear fluorescence intensity analysis pipeline (v02.5, 5/5/23)

Pipeline usage overview (steps):

1) Basic image read, processing and ROI measurements
2a) (optional): Extract images for presentation
2) Read ROI data into R
3a) Analysis of individual channels
3b) Analysis of intensities of 2 channels (Ch1 vs Ch2)

Required:

- Fiji
- R (including libraries tidyverse, colorRamps, lmerTest)

Input:

- Leica confocal lif file (Z-stack) 16-bit
    - Other input needs customised input scripts (replacing '01_MaxProj greyscale batch_v02_17-12-20_from lif.ijm', see subfolder 'alternative input and ROI definitition scripts')
    - 8-bit images may require alternative R scripts for proper visualisation (see subfolder 'R scripts optimised for 8-bit images')

Description of pipeline usage steps

1) Basic image read, processing and ROI measurements

- Copy script directory in your main analysis directory
    - For alternative data formats, replace scripts in main script directory with relevant versions from subdirectories (see "Input" above)
- Open images with Fiji

- Run Fiji script "**01_MaxProj greyscale batch_v02_17-12-20_from lif.ijm**"
    - select main analysis directory when asked
    - Will create maximum projection of all opened images in "01_MaxProj greyscale" subfolder
- Run Fiji script "**02_Nuclei ROI measure batch_v021_18-01-15.ijm**"
    - change DAPI channel if required (script line 2)
    - select main analysis directory when asked
    - set DAPI threshold for each image to outline nuclei (background has to be red)
    - will create for each image (folder "02_ImageJ analysis")
        - ROI sets
        - csv files with intensity for each nucleus for each channel

2a) (optional): Extract images for presentation

script "**02a_Greyscale to RGB sel chan adj brightness selected area batch_v022_20-12-15.ijm**")

- Can create RGB images of selected channels in selected colors
- Define output folder in script
- Change parameters in script (channels, colors, intensity range)
- Select input folder when running script

2) Read ROI data into R

- Run R script "**03_R batch input_v02_18-01-15.R**"
  - Define channel names in script line 24 (in order of channels)
  - Reads data from "02_ImageJ analysis" for downstream R analyses in "03_R input" folder
  - Creates "image_groups.csv" where you have to define the image groups for the downstream analysis
    - "group" can be e.g. your experimental conditions
    - "repl" can be e.g. a number or letter to identify your coverslip, if you have multiple technical replicates, to know which image is from which coverslip

3a) Analysis of individual channels

- Run R script **"04_intens distrib 1 Ch 16bit_v023_230301_all channels new plots.R"**
  - Define image groups in "03_R input" folder (see above)
  - Creates QC plots in "04_intens distrib_1Ch" folder, showing intensity distributions for selected channel
- Run R script "**05_threshold testing 1 Ch_v025_230302 with t-test stats.R**"
  - Set the channel you want to analyse in line 27
  - From the QC plots and/or the original max proj images get some thresholds that could make sense
  - Set the thresholds for which you want to get summary statistics in line 29 (you can set as many thresholds as you like to test, separated with commas: "thCh1.test.thresh = c(20, 30, 40)" means it will analyse with 20, 30 and 40 as thresholds)
  - Output:
    - plots and summary csv files for the different thresholds
    - Basic statistic analysis of first group vs other groups (t-test or GLMM stats with lmertest package)

3b) Analysis of intensities of 2 channels (Ch1 vs Ch2)

- Run R script "**04b_intens distrib 2 Ch 16bit_v026_230301_new plots.R**"
  - Define the 2 channels you want to analyse in line 30

    - Gives you folder “04b_intens distrib_2Ch”, with FACS-style scatterplots intensity Ch1 vs Ch2
- Run R script “**05b_threshold analysis 2 Ch_v025_230302 lmer stats included.R**”
    - Define dataset that you want to analyse in line 21
    - Set thresholds for both channels in line 28 (only each one threshold possible: c(thresh_Ch1, thresh_Ch2))
    - Output: folder “05b_treshold analysis_2Ch”, with plots of fraction of Ch1/Ch2 -/-, -/+, … of all cells; and basic stats
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