EPFL

Getting started with image analysis in Fiji

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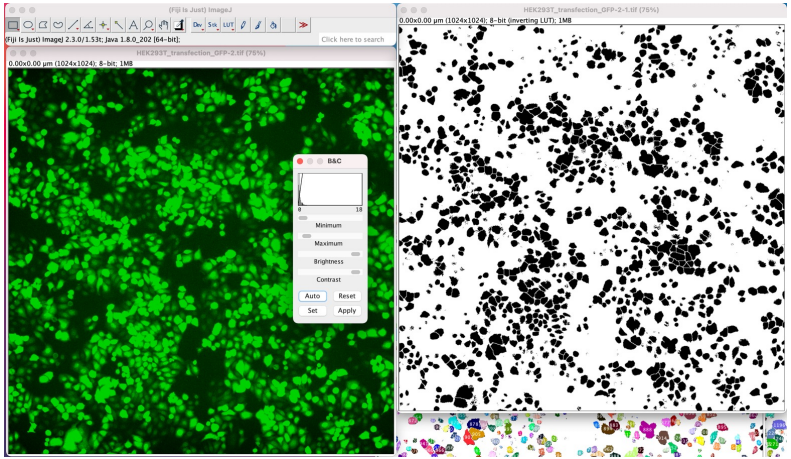
BIO-204

2023

Determine Transfection Efficiency with Fiji

- 24h after transfection (lab 8) cells were fixed and stained with DAPI
- file names
  - transfection = Amy2-CDS-His + GFP
  - mock = no DNA
- identical imaging conditions for transfection and mock
- representative fluorescence images were acquired in two channels
  - GFP: excitation 488 nm
  - DAPI: excitation 405 nm
- download images (Moodle)
- follow step-by-step guidelines

Download image processing software Fiji



open source

Fiji=**Fiji** **i**s **j**ust **I**mageJ (with plugins)

professional software with many functions

https://imagej.net/software/fiji/downloads

Tasks

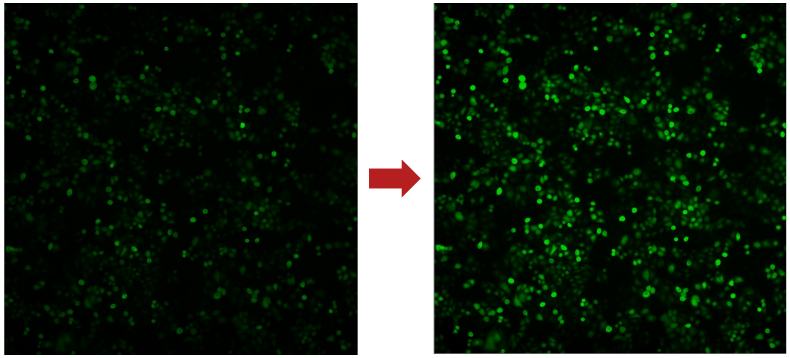
A) Merge DAPI + GFP images

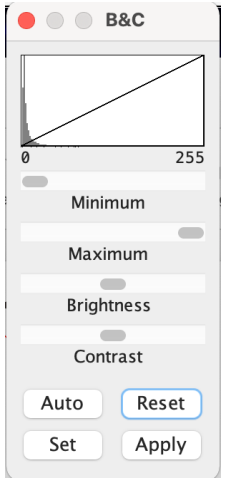
B) Determine Transfection efficiency

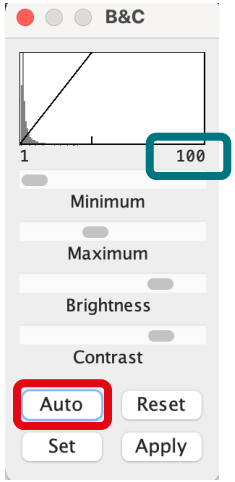
A)
How to merge (overlay) DAPI + GFP images

- adjust contrast of individual images
- merge images

For Good Visibility by Eye: Adjust Contrast





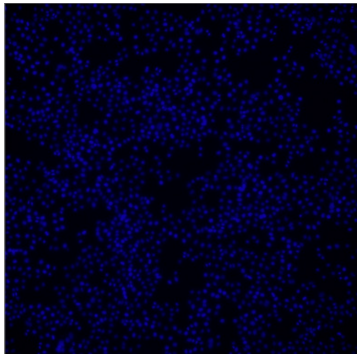


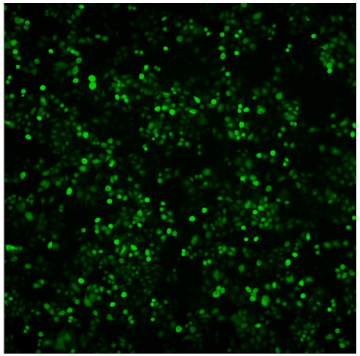
- open image(s)
- image > adjust > brightness/ contrast / auto
  - value can be different for each image
- alternative: move the min/ max for manual adjustment

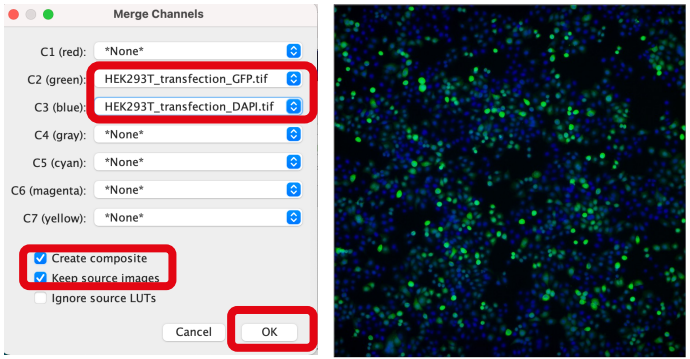
for comparison between different conditions use **same value**

- first set GFP contrast (transfection image)
- then use the **same value** to display the mock image

To Overlay Individual Images: Merge Channels







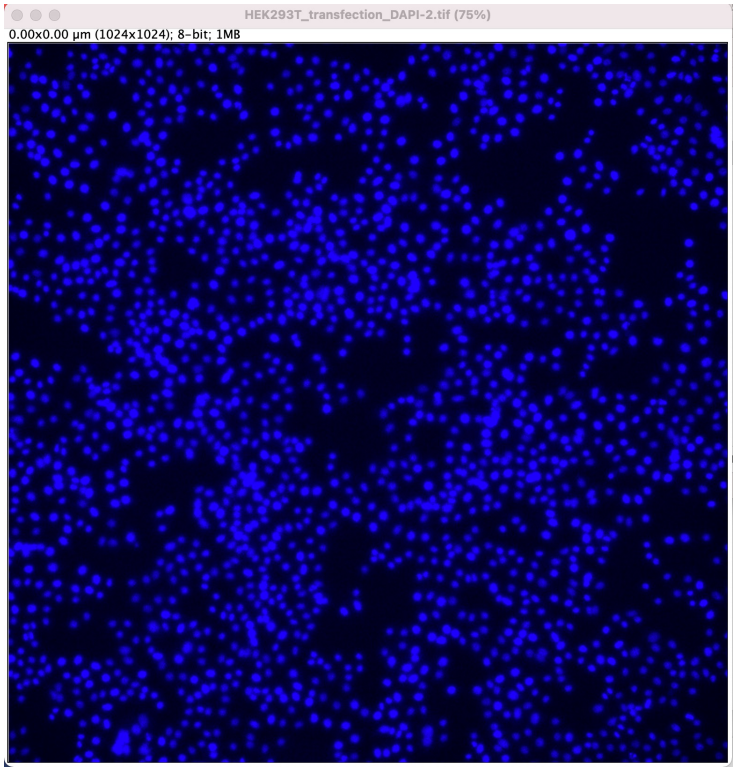
- open images (GFP and DAPI)
- image> color> merge channels
- select files for green and blue channel
- save as> tiff (for scientific processing)
- save as> jpg (compressed format, use for illustration purpose only, ELN OK)
- name: *original filename*_merge)
- now you have 3 image files (DAPI; GFP, merge)

Create a Figure

- see figure guidelines BIO-203
  - panels A, B, C
  - label images (GFP transfected cells, nuclei, merge)
  - write figure legend

B)
How to count objects (pixels, nuclei, cells, ...)

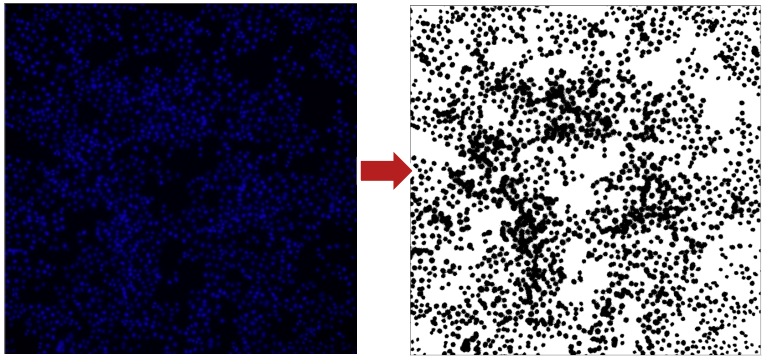
- process image
- analyse

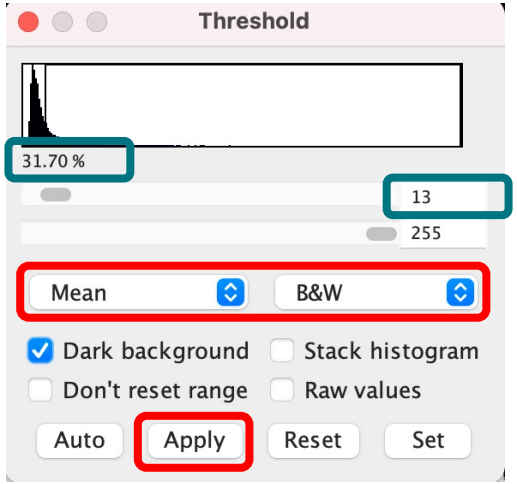


Process DAPI image

- nuclear fluorescence uniform (signal intensity, size, shape...)
- most nuclei are well separated

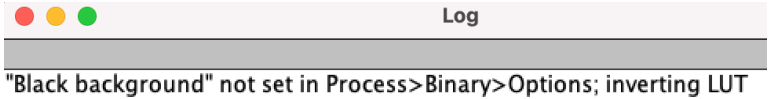
1) Adjust Treshold





- open image (single channel: DAPI)
- convert to grayscale with 254 gray levels
  - image> type> 8-bit
- adjust treshold
  - set to black and white = binary image
  - image> adjust> treshold>mean
  - value can be different for each image

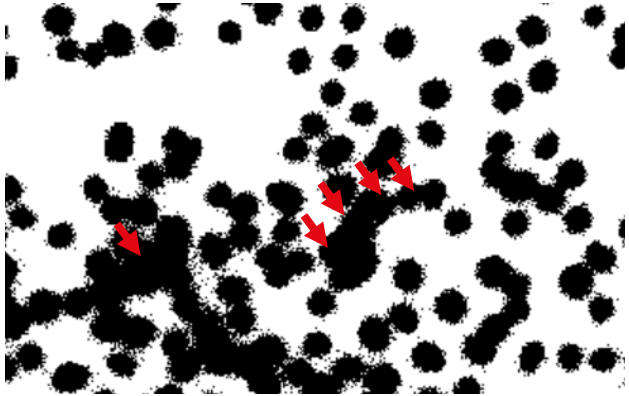
- (possible error message, continue):



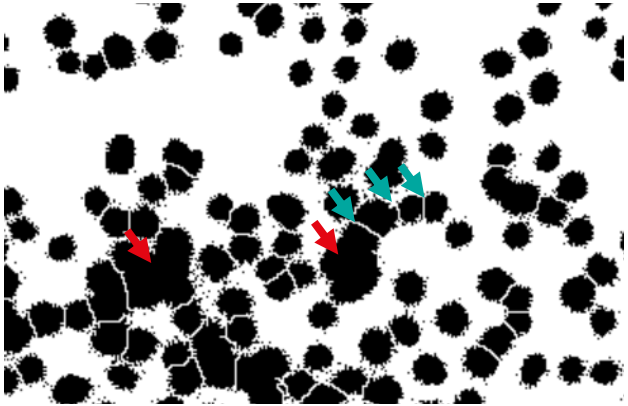
2) Separate Overlapping Objects (nuclei)

- process> binary> watershed
- separation succesful or failed
- (can be further optimized if needed, here ok)

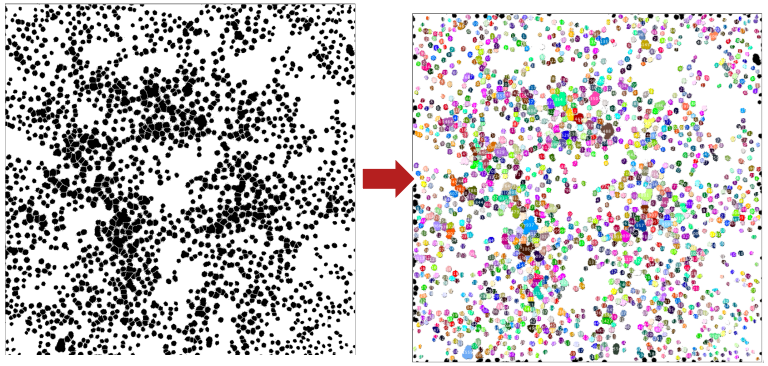
some nuclei are in proximity and difficult to distingush



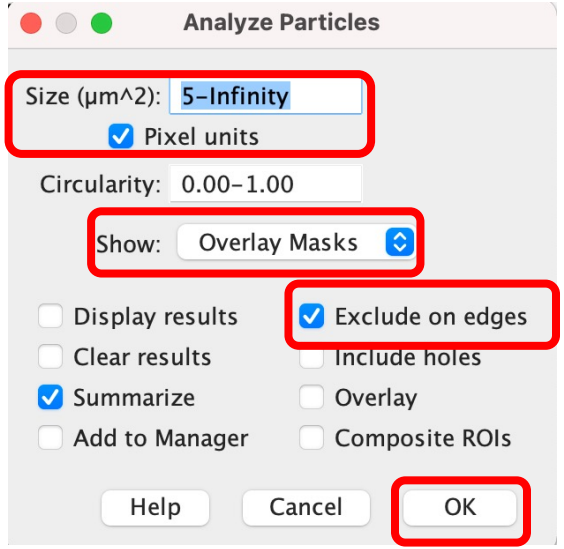
most nuclei separated (see white lines)



3) Count Objects (Nuclei)



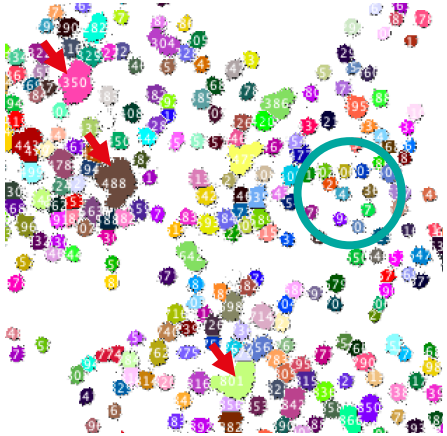
- example: count all black objects with minimum size 5x5 pixels (if only 1-2 pixels are black it is probably not a nucleus)

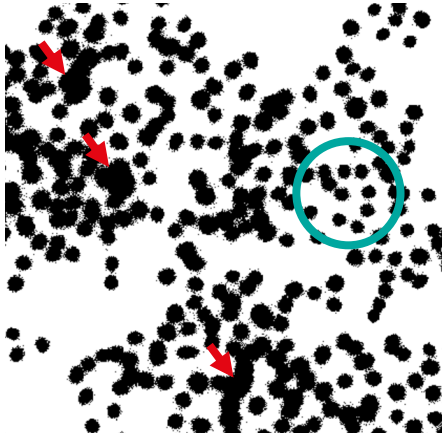


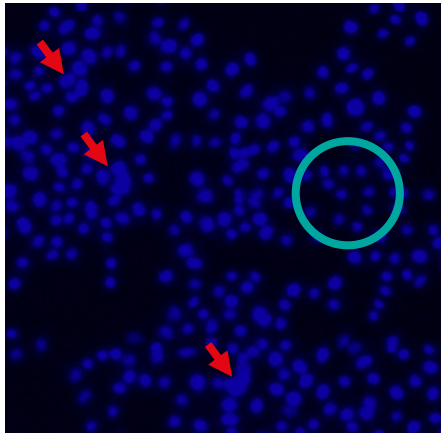
- analyze> analyze particles
  - set size: 5-Infinity
  - exclude on edges (since we lack information for cells that are not entirely inside the image we exclude them from the analysis)

Does the automatic nuclei detection work?

- in the output image each object is shown with a color (count number in white can be ignored here)
- Compare by eye if the color mask corresponds to what you see in raw image
  - succesful counting in areas with low nuclei density (majority of nuclei)
  - less accurate when nuclei are close (signal not separated)







Results are in Table

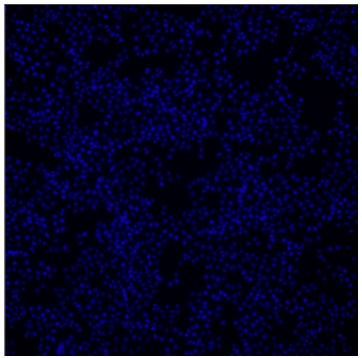
Summary

Slice	Count	Total Area	Average Size	%Area	Mean	IntDen
HEK293T_transfection_DAPI-5.tif	1624	1.680E-6	1.034E-9	29.547	255	2.637E-7

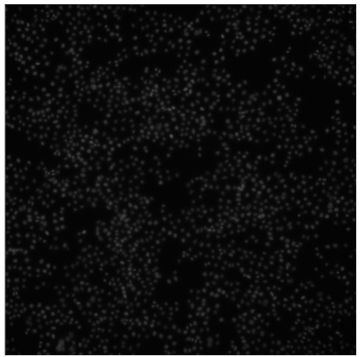
- Count: 1624 objects (here: nuclei)

Summary:
Image Processing for Nuclei Count (DAPI)

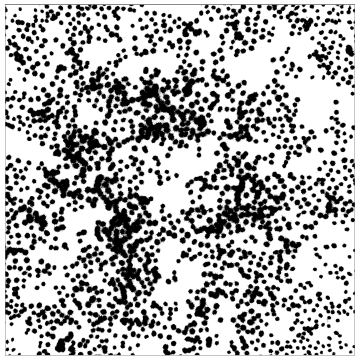
raw image



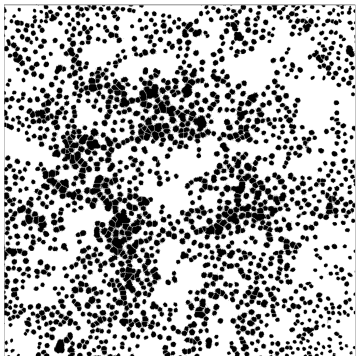
greyscale (8-bit)



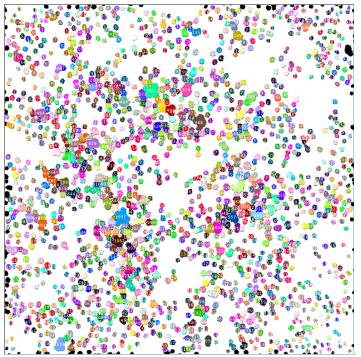
treshold (black + white)



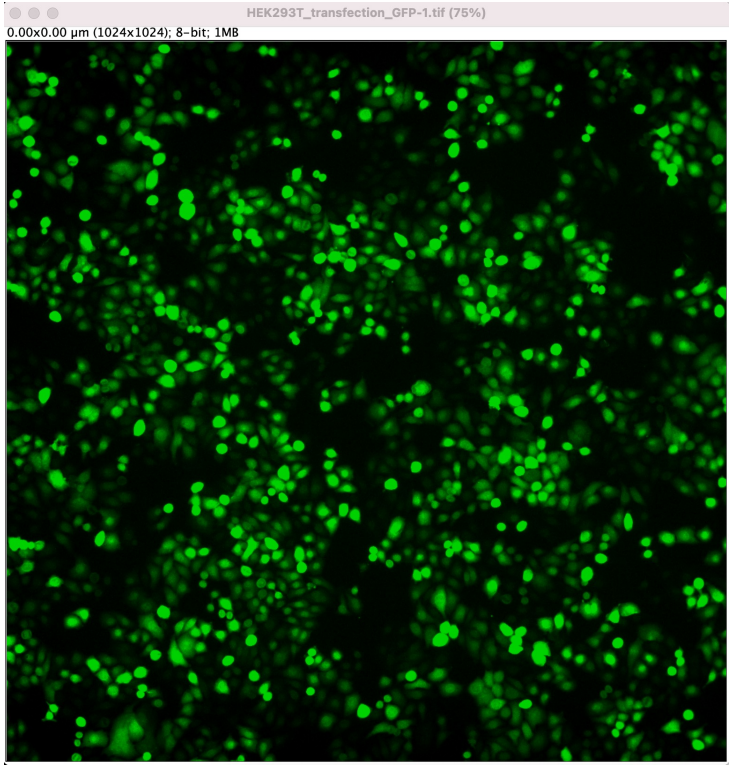
separate (watershed)



count objects



Slice	Count
HEK293T_transfection_DAPI-5.tif	1624



Process GFP image

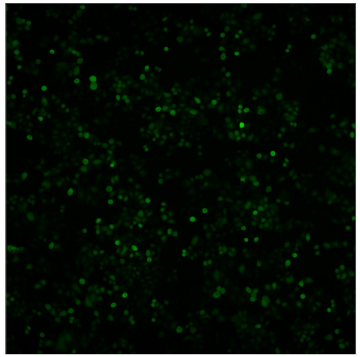
(same steps as with DAPI image)

- intensity of GFP fluorescence is variable: strong vs weak expression

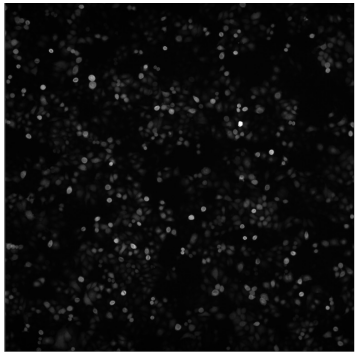
- difficult to count
  - cells in close contact
  - weak expressing cells
  - strong expressing cells

Summary:
Image Processing for GFP Expressing Cells

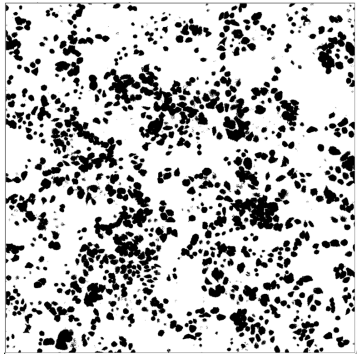
raw image



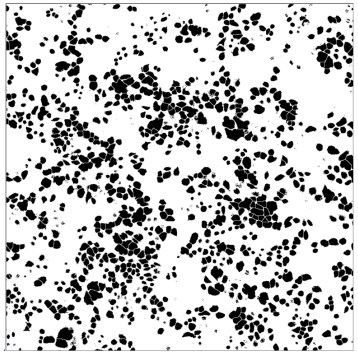
greyscale
(8-bit)



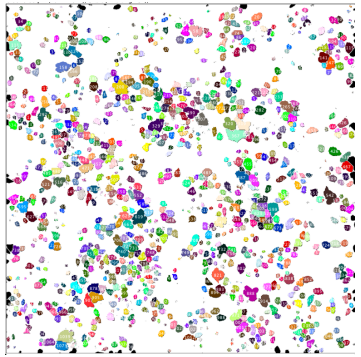
treshold
(black + white)



separate
(watershed)



count objects

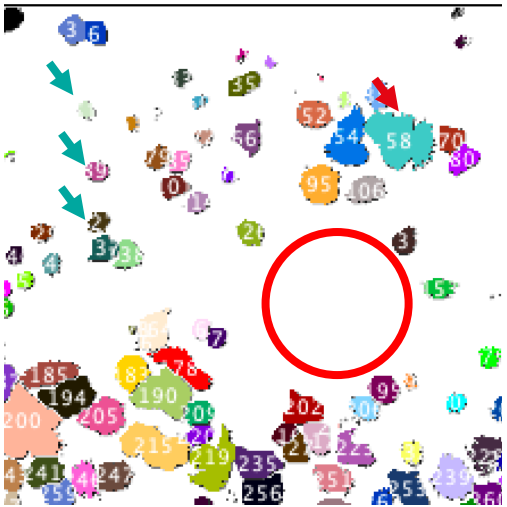


Slice	Count
HEK293T_transfection_DAPI-5.tif	1624
HEK293T_transfection_GFP.tif	1100

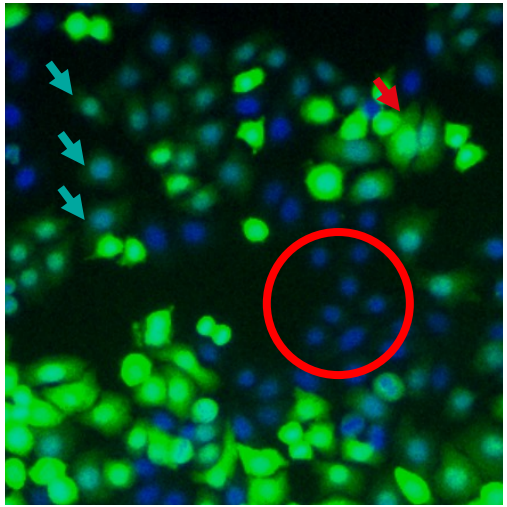
Note Some GFP Detection Issues

- Compare by eye color mask and raw data
- succesful counting for cells with ‘mean’ expression level
- unterestimation of cell counts due to:
  - very weak expression - below treshold- not counted
  - very strong expression, high cell density: cells not well separated

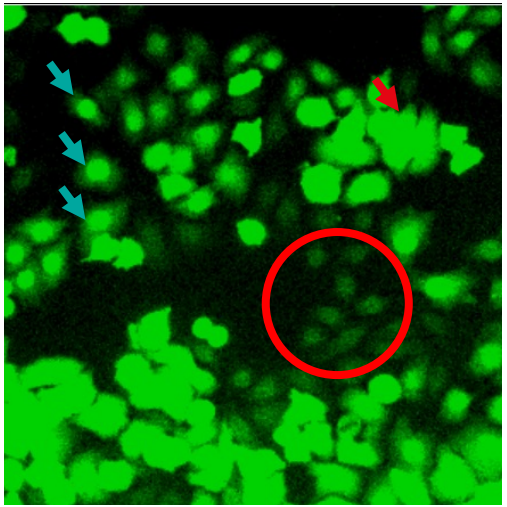
count

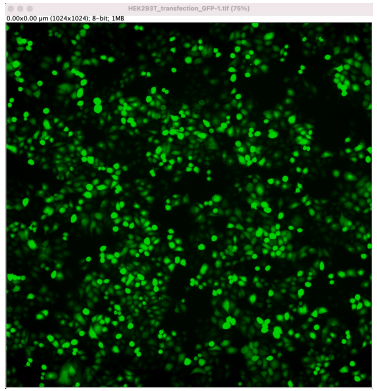


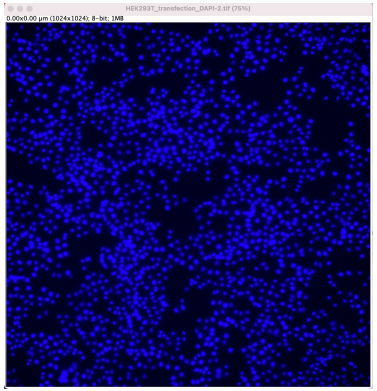
merge GFP + DAPI



GFP enhanced contrast







Calculate % Transfection Efficiency

Transfected Cells (GFP): 1100

All cells (DAPI nuclei): 1624

Transfection efficiency

1100/1624 = 68%

Every image is different

- optimize !
  - experimental setup (cell density, expression duration...)
  - image acquisition
  - processing
- if you have acquired many images use batch processing