Light microscopy in Cellular Biology

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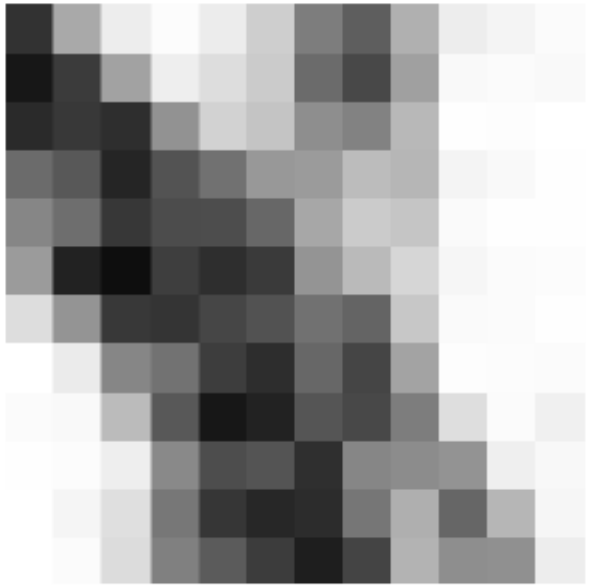
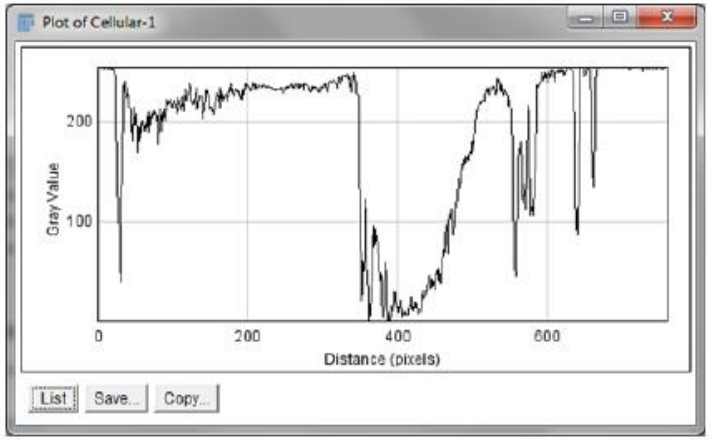
(a) Original image



(b) Enlarged view from (a)

50	169	237	252	236	206	125	94	176	237	244	251
23	59	162	238	221	203	107	72	160	249	251	249
42	56	46	146	210	196	142	130	184	254	253	255
107	88	37	83	113	152	155	188	182	244	249	254
134	110	55	76	77	103	167	203	197	250	254	254
155	34	14	62	46	58	148	186	214	246	251	252
221	148	56	52	70	82	113	100	199	250	251	254
255	235	134	114	61	45	103	69	163	253	252	251
251	249	187	88	23	34	85	72	125	222	251	240
254	252	238	137	77	84	47	134	140	147	239	248
255	245	223	119	54	39	44	118	175	102	182	246
255	251	220	128	91	60	30	68	179	142	144	237

(c) Pixel values of (b)



Plot window

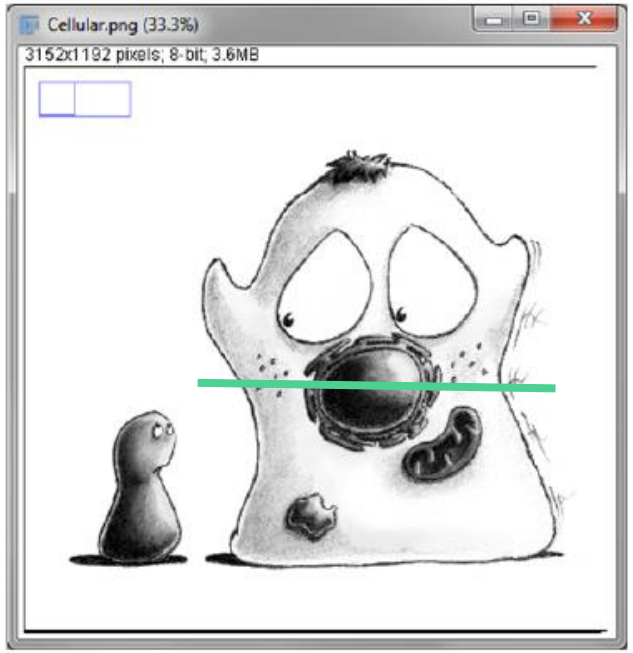
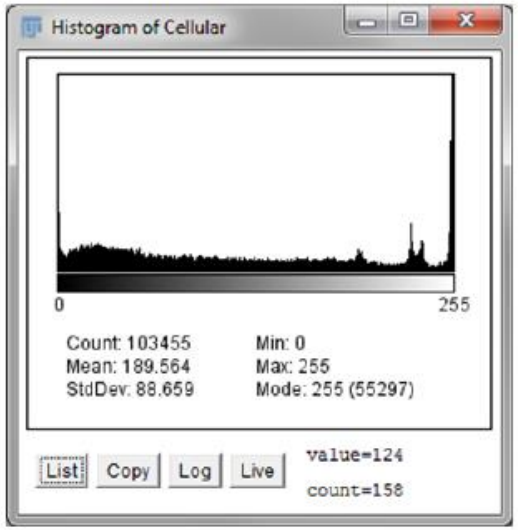
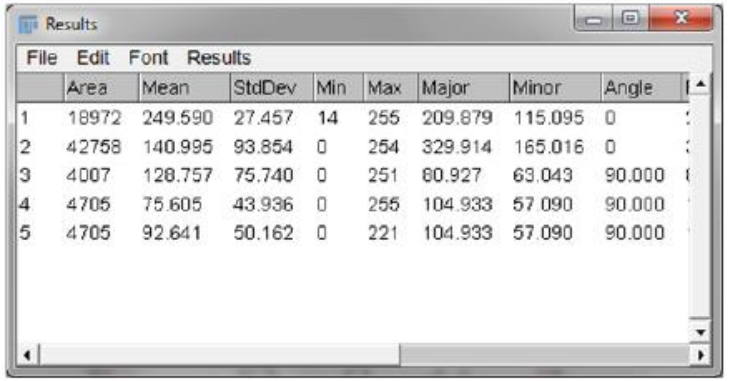


Image window

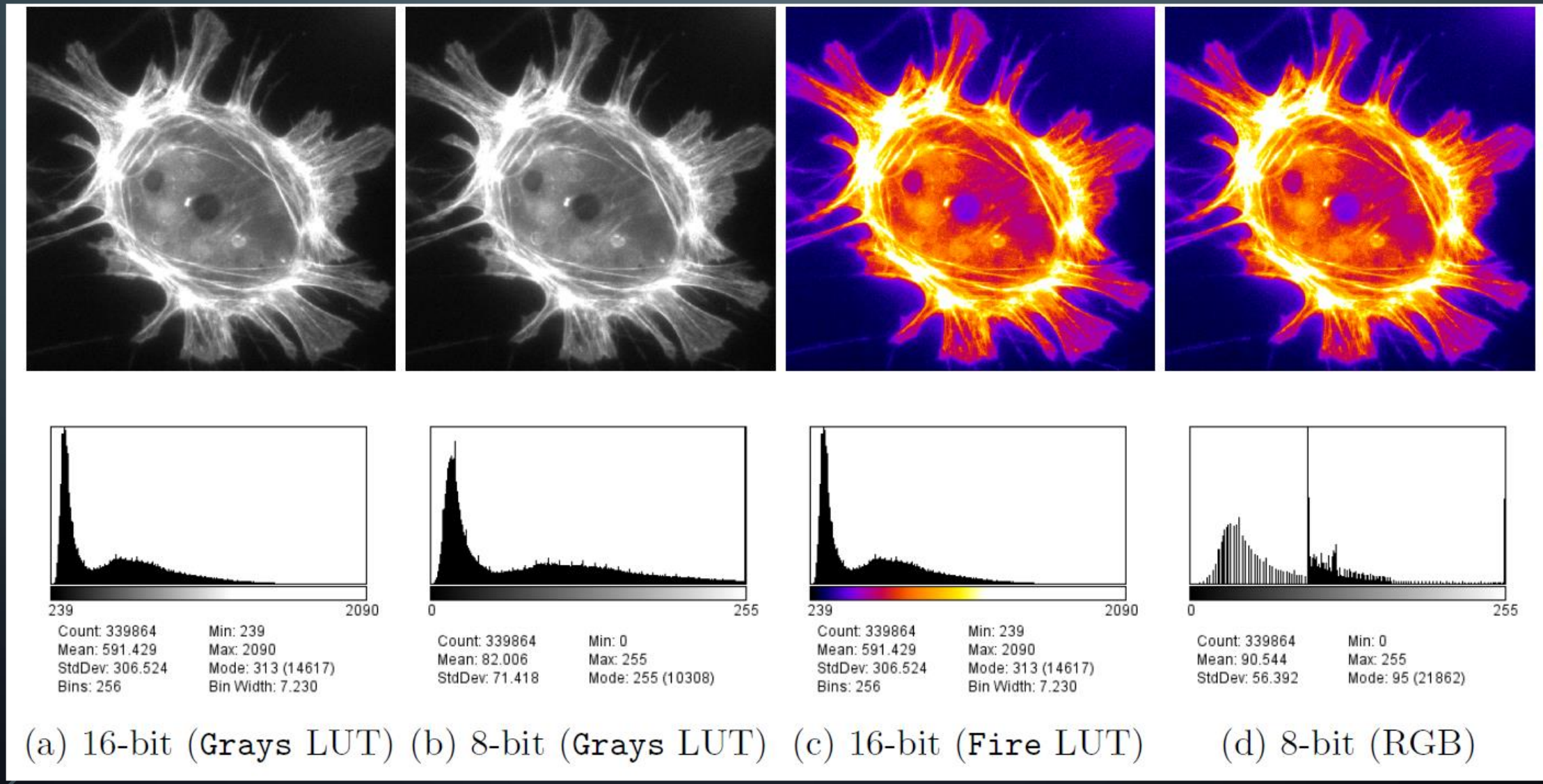


Histogram window



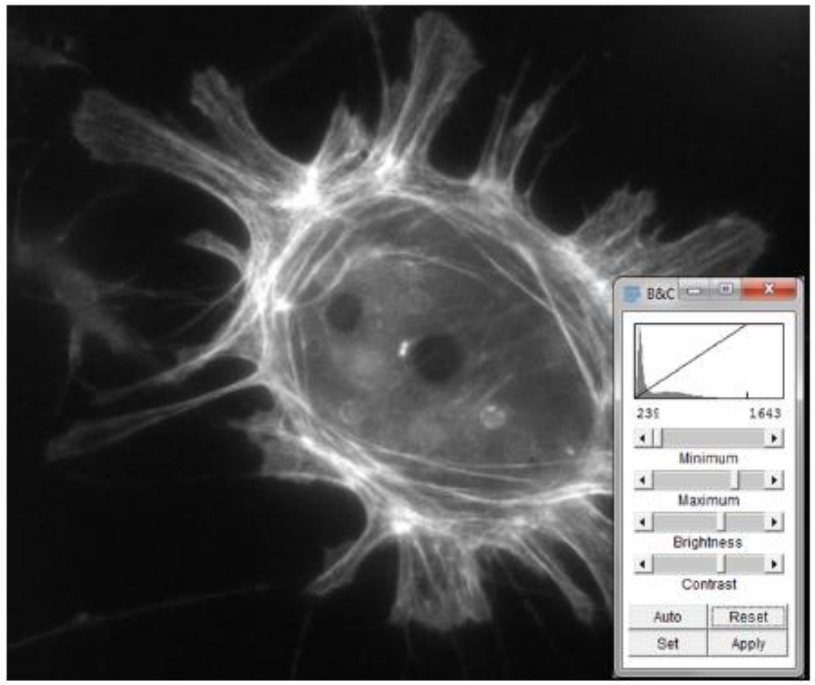
Results table

Do not trust your eyes for image comparisons

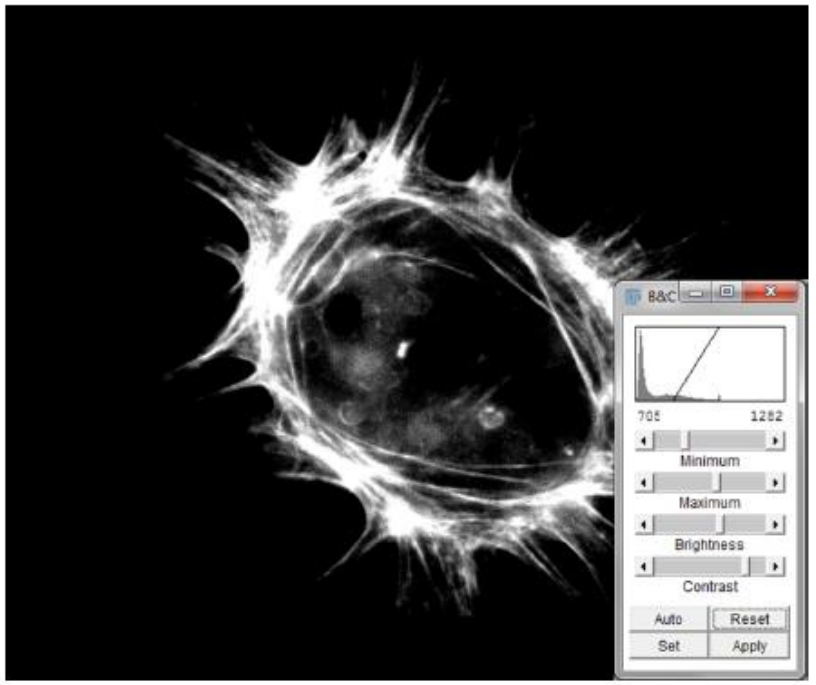


(a) 16-bit (`Grays` LUT) (b) 8-bit (`Grays` LUT) (c) 16-bit (`Fire` LUT) (d) 8-bit (RGB)

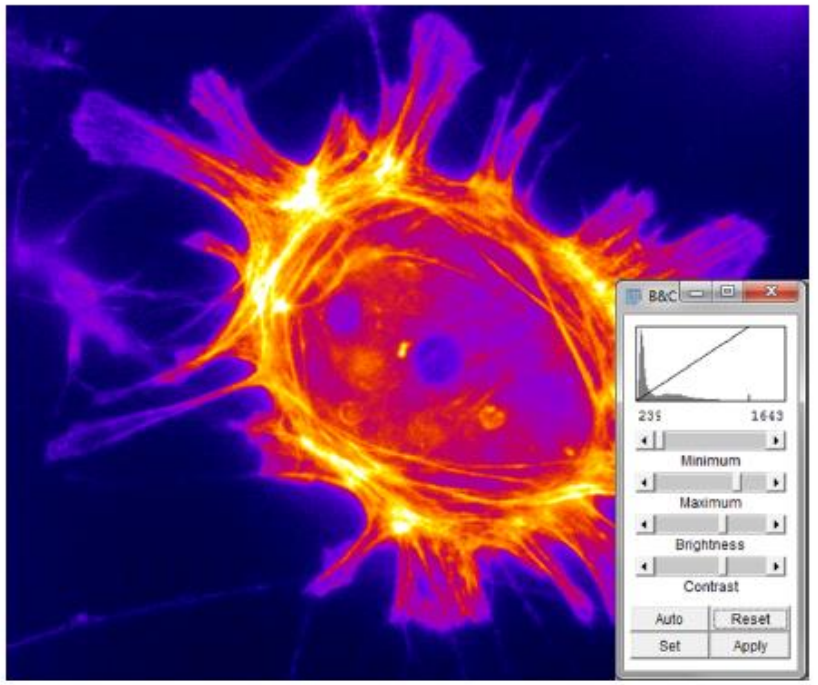
The same image can be displayed in different ways by adjusting the contrast settings or the LUT.
Nevertheless, despite the different appearance, the values of the pixels are the same in all three images.



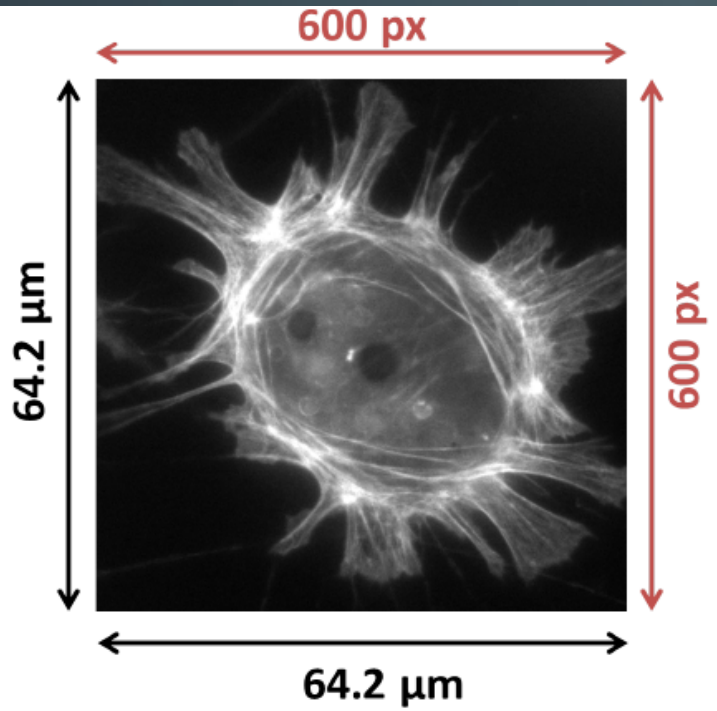
(a) Grayscale

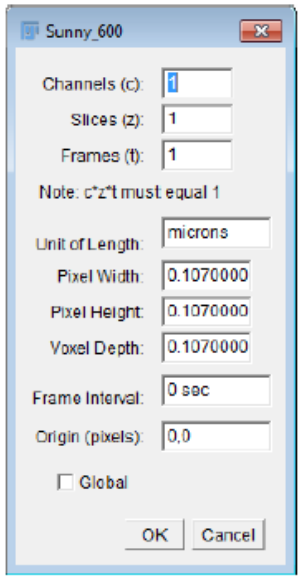


(b) Grayscale (high contrast)

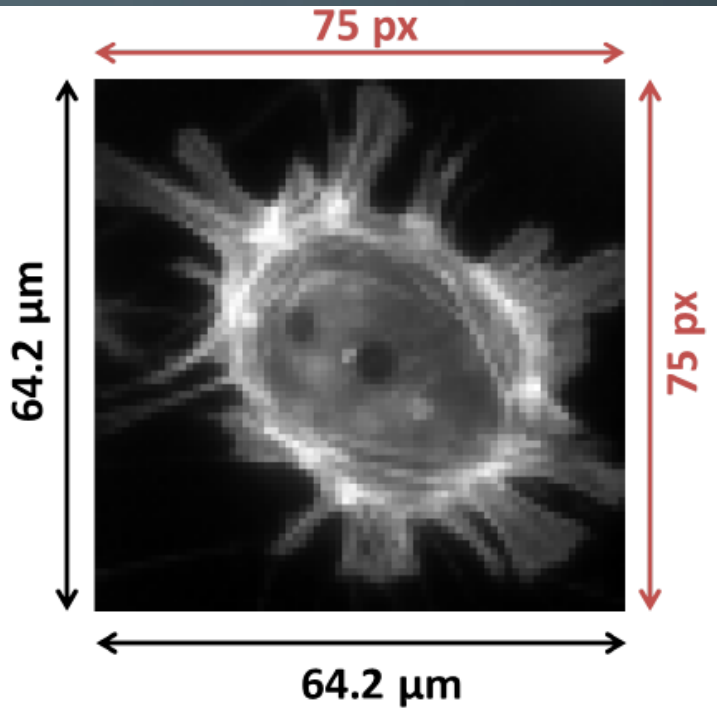


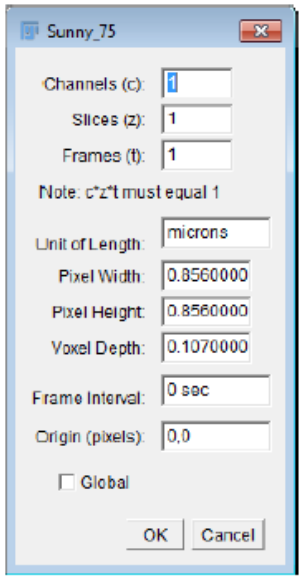
(c) Fire LUT





(a) 600×600 pixel image and its properties

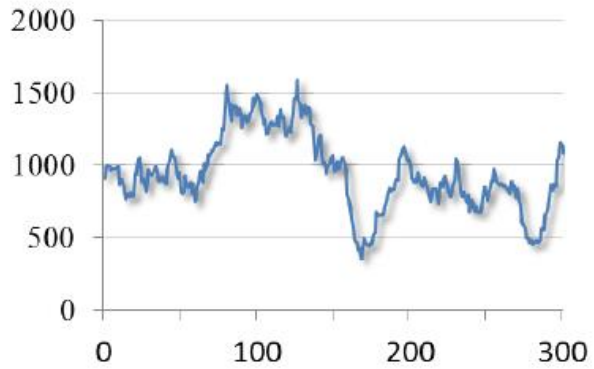




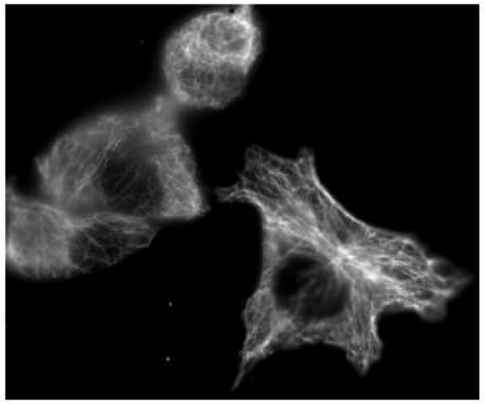
(b) 75×75 pixel image and its properties

102

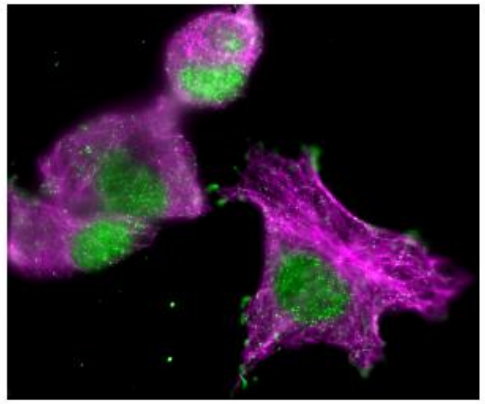
(a) 0 dimensional



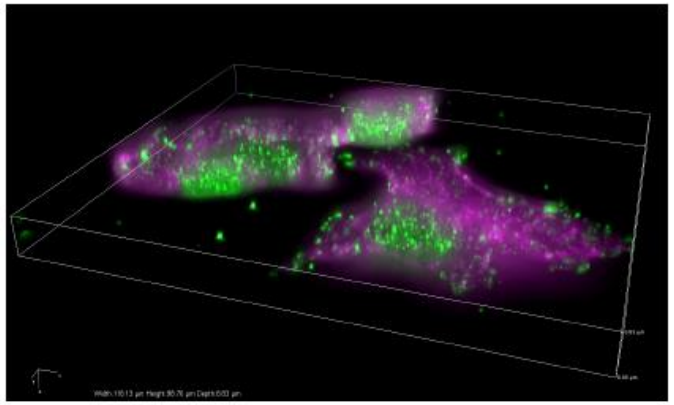
(b) 1 dimensional



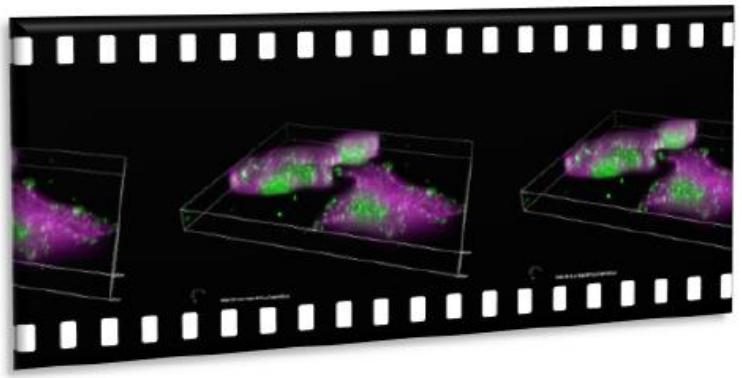
(c) 2 dimensional



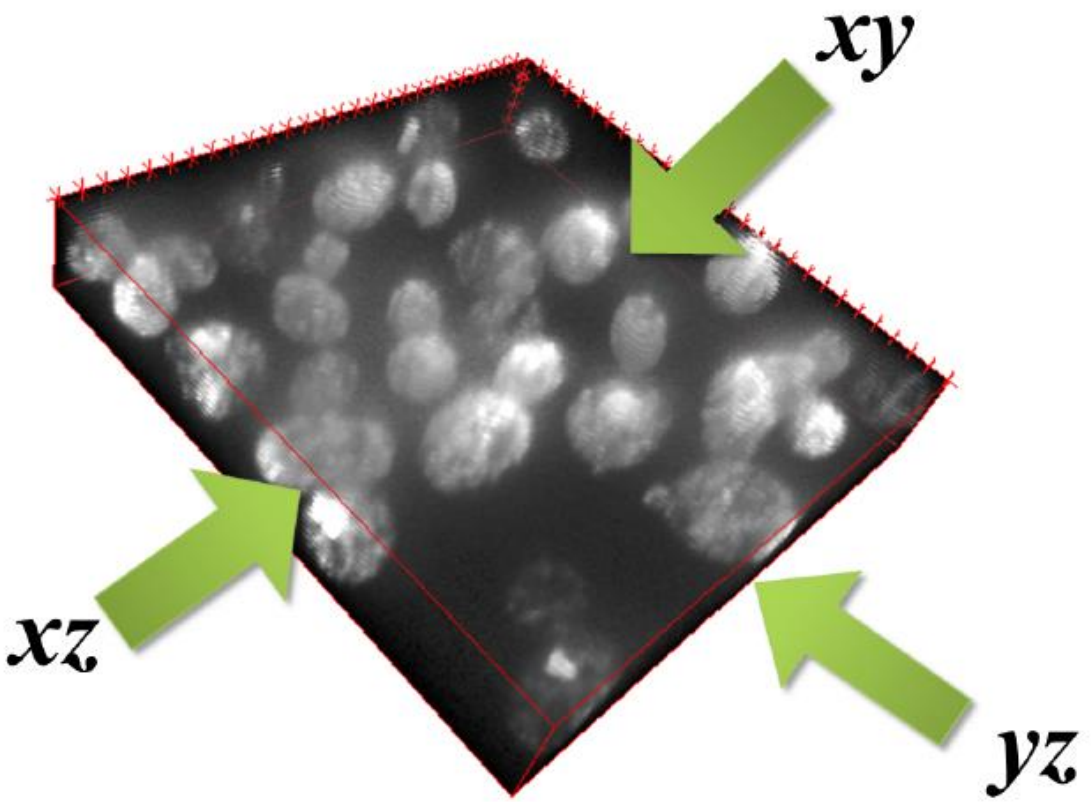
(d) 3 dimensional



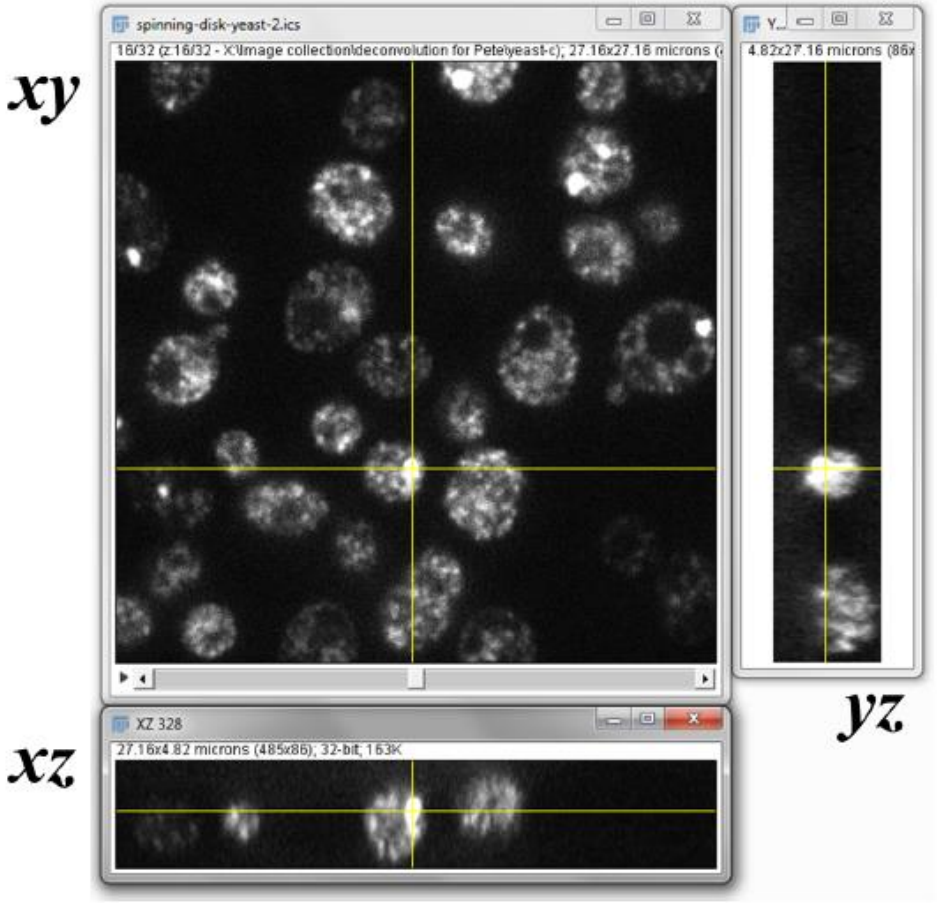
(e) 4 dimensional



(f) 5 dimensional

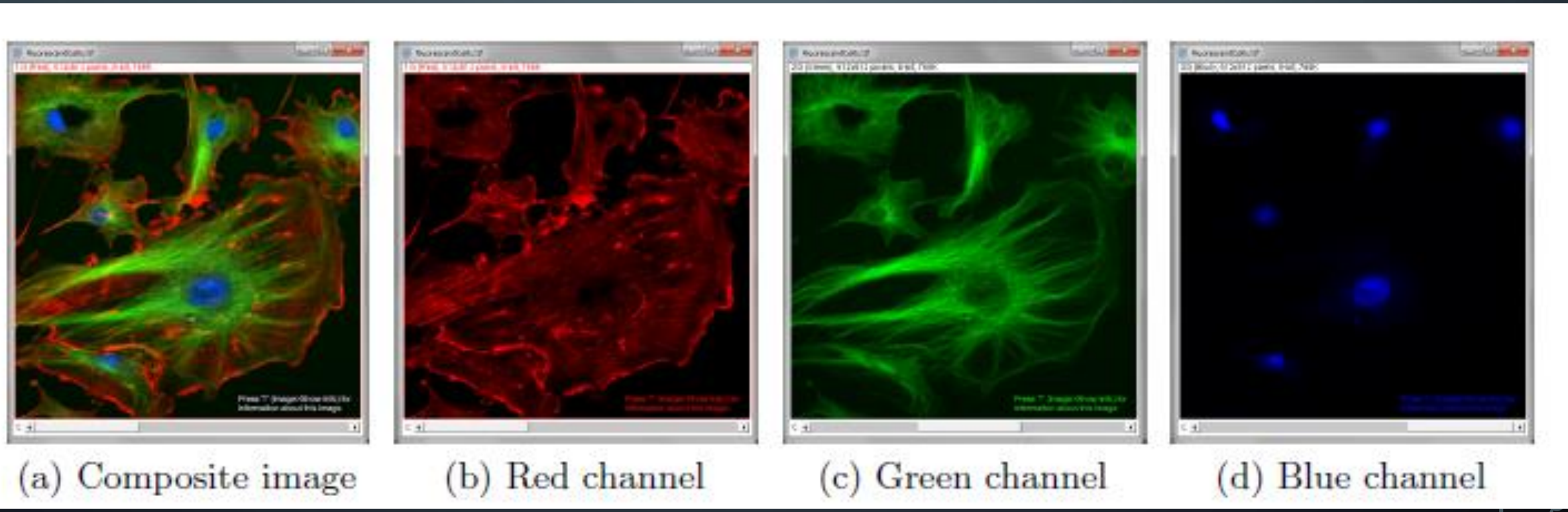


(a) Volume rendering

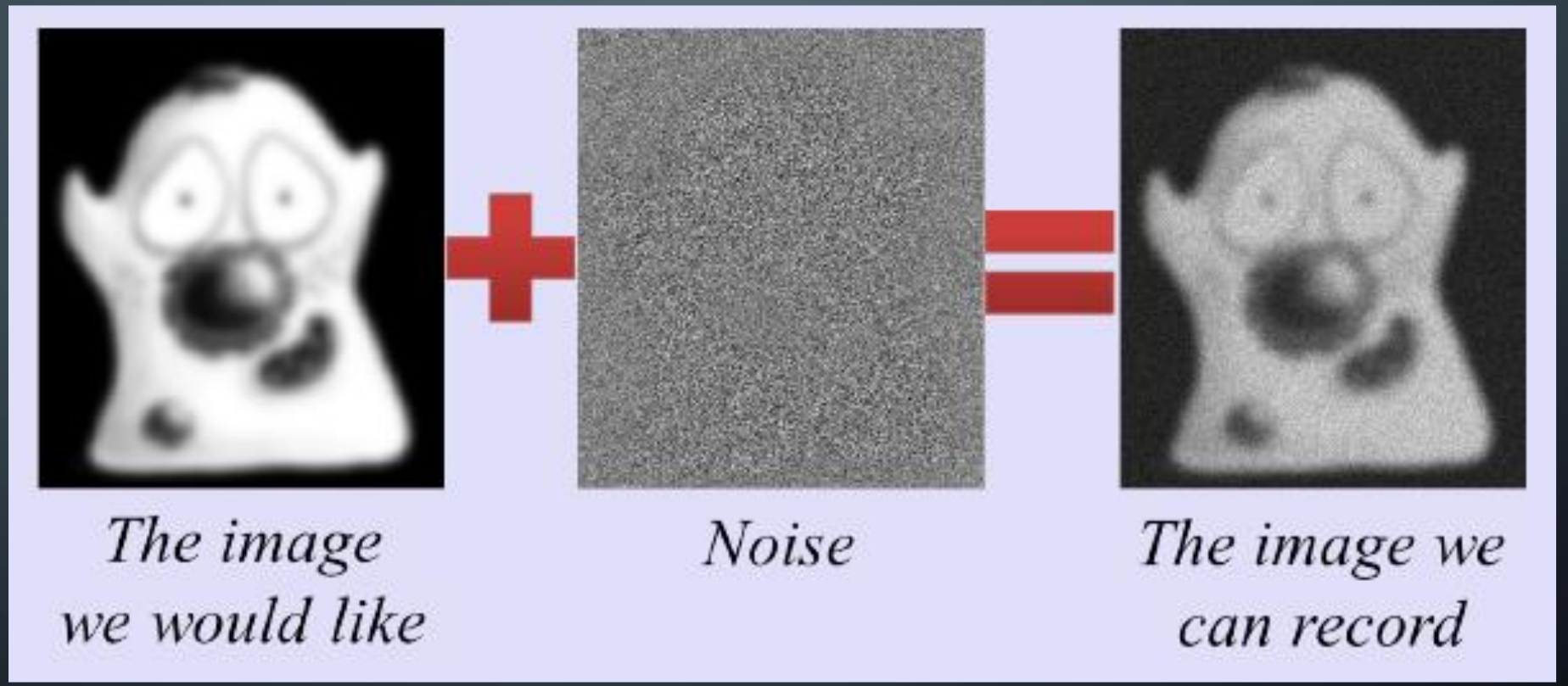


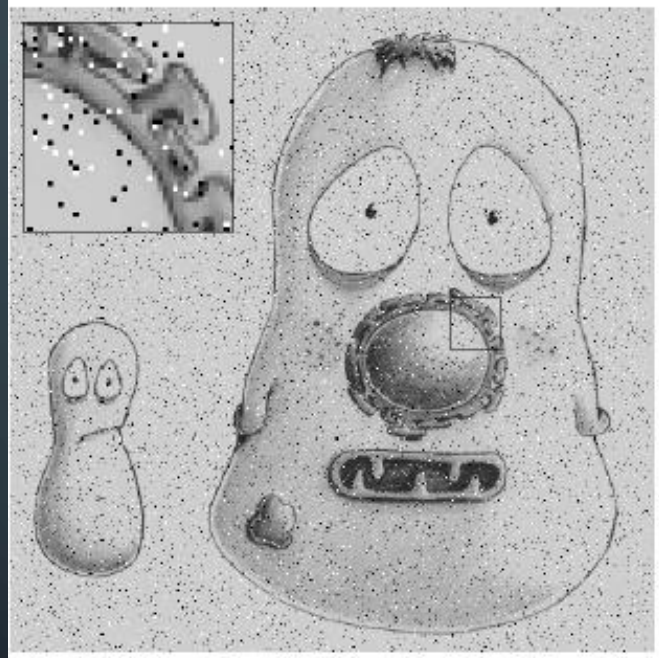
(b) Orthogonal views

ImageJ composite image sample Fluorescent Cells. Using the Channels Tool...
and the slider at the bottom of the window, you can view the channels individually or simultaneously.

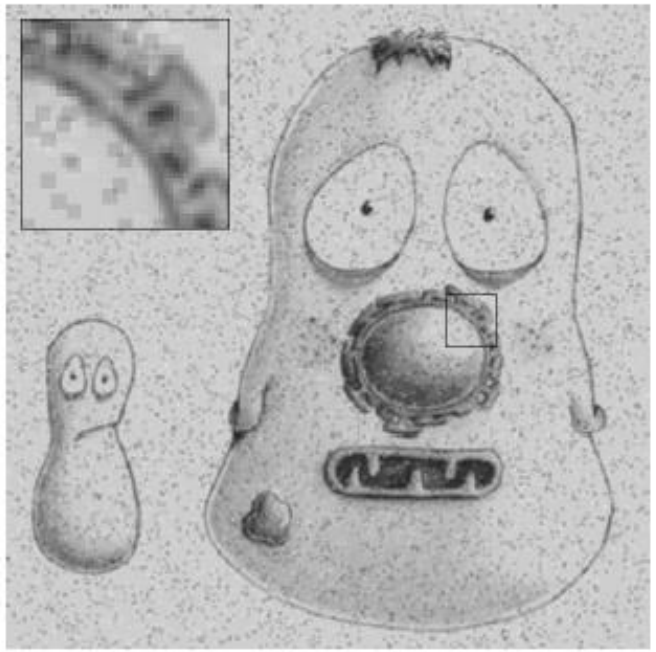


(a) Composite image (b) Red channel (c) Green channel (d) Blue channel

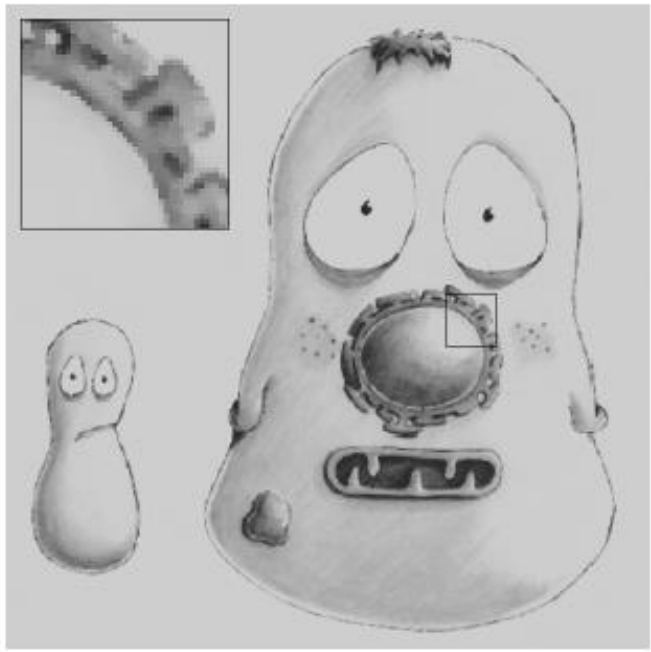

The image we would like
Noise
The image we can record



(a) Speckled image



(b) Mean filter



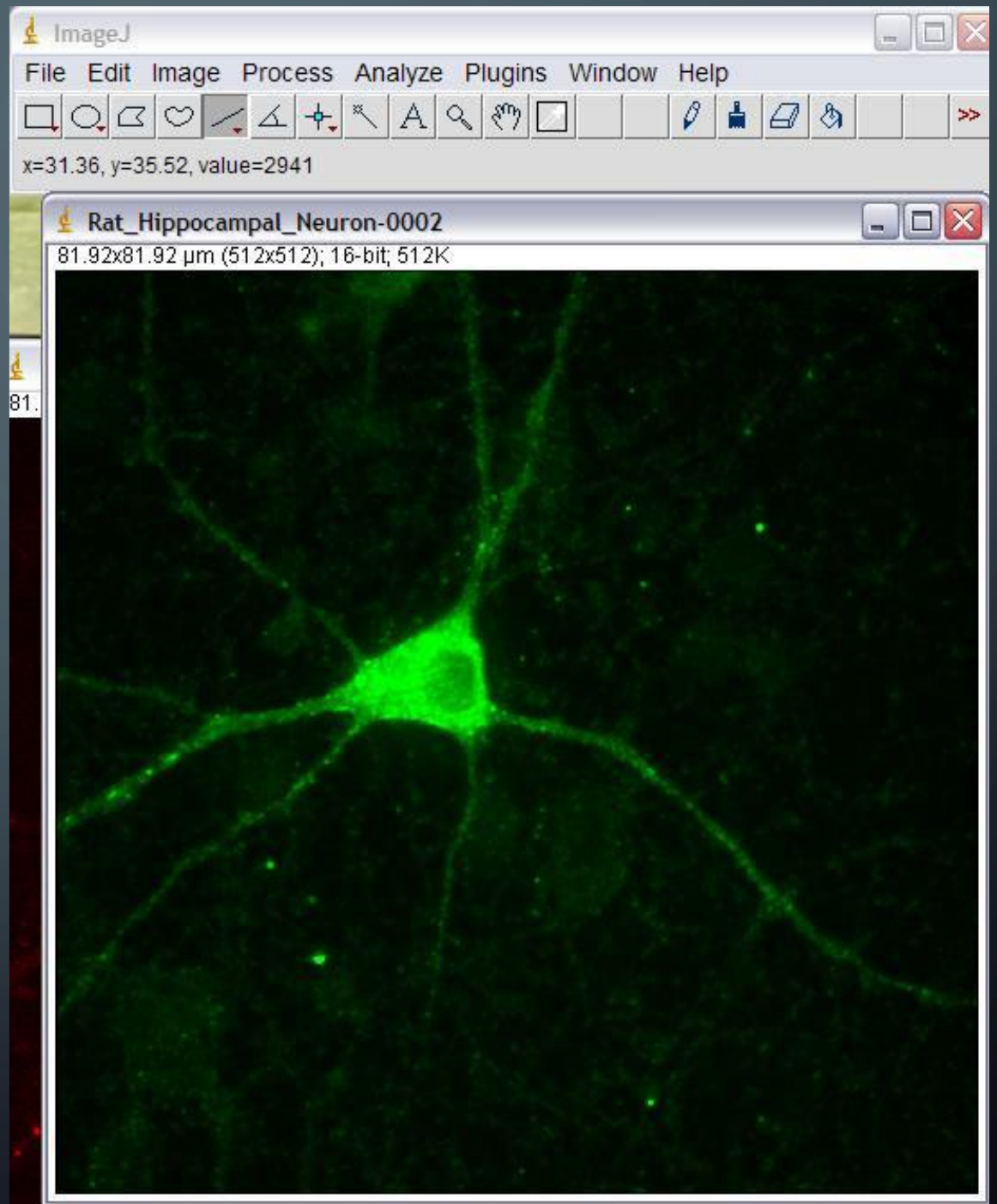
(c) Median filter

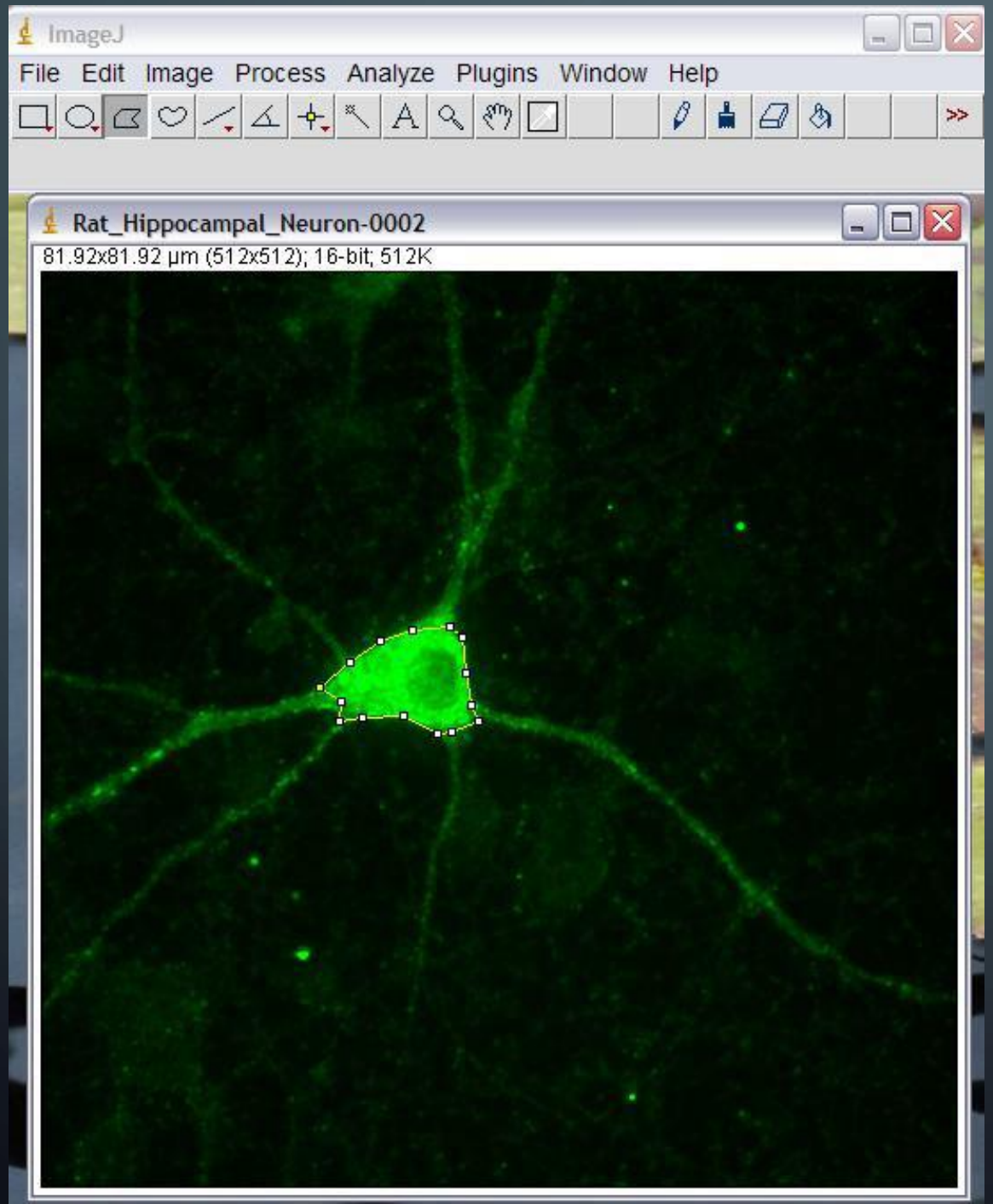
Basic Intensity Quantification with ImageJ

Pretty pictures are nice, but many times we need to turn our images into quantifiable data. ImageJ is useful for getting information from images, including pixel intensity.

There are a number of different ways to get intensity information from images using the base package of ImageJ (no plugins required).

Quantify Gray Levels Across an Entire Image or Single Object/Region

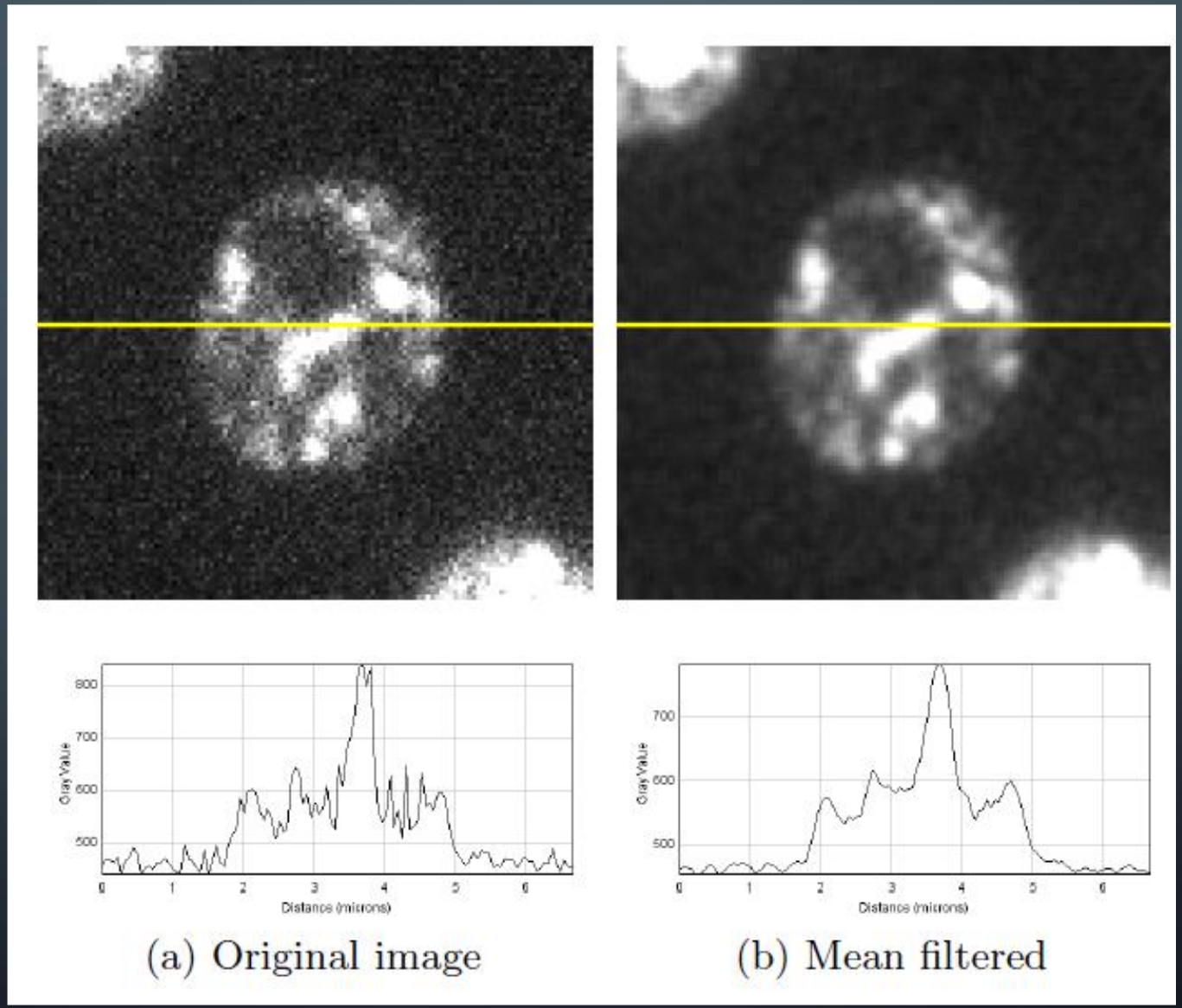




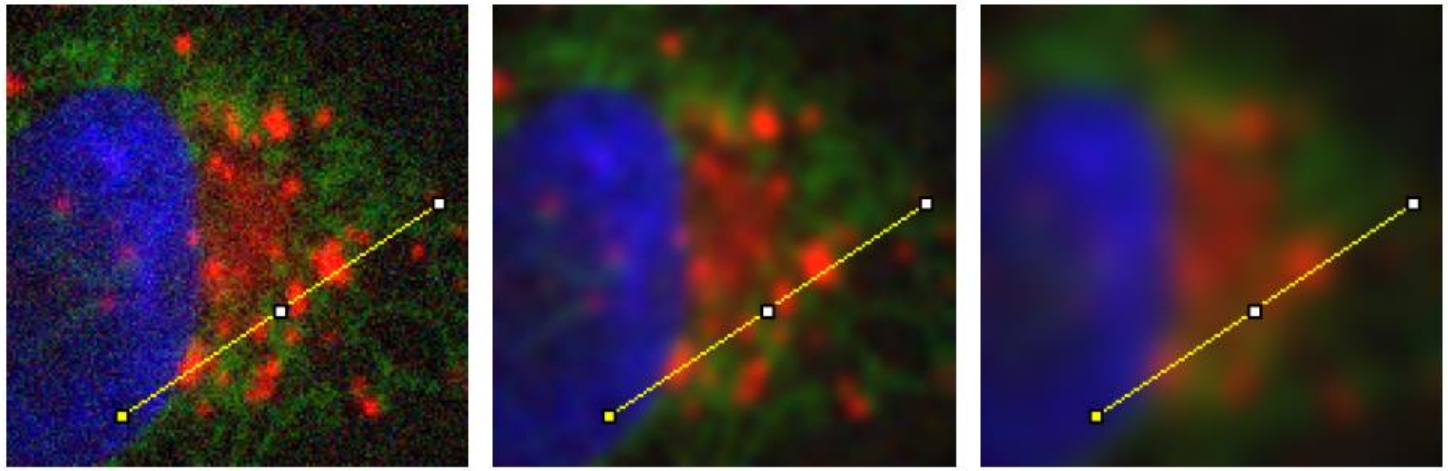
If you want to limit your measured area to just your object you draw a region of interest (ROI) around your object with one of the drawing tools (in the toolbar) and then

Analyze

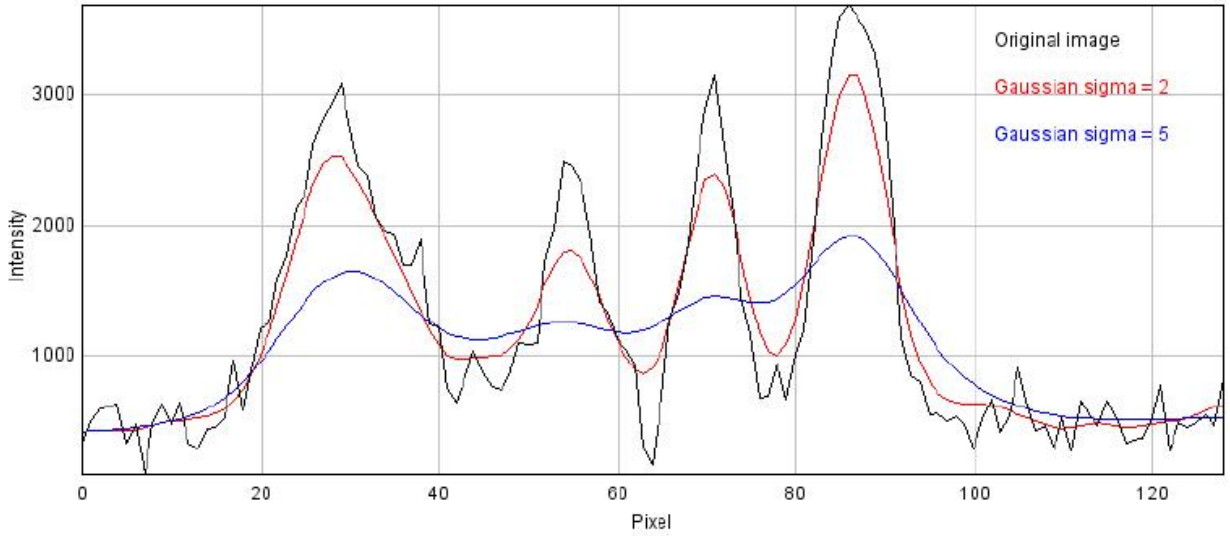
Measure



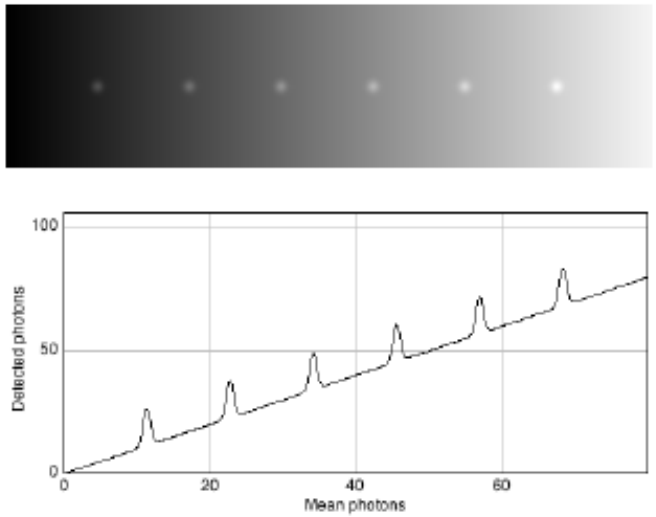
(a) Original image (b) Mean filtered

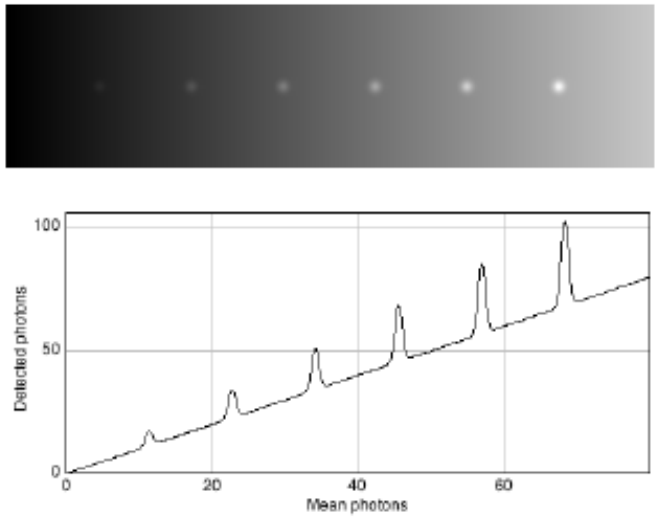


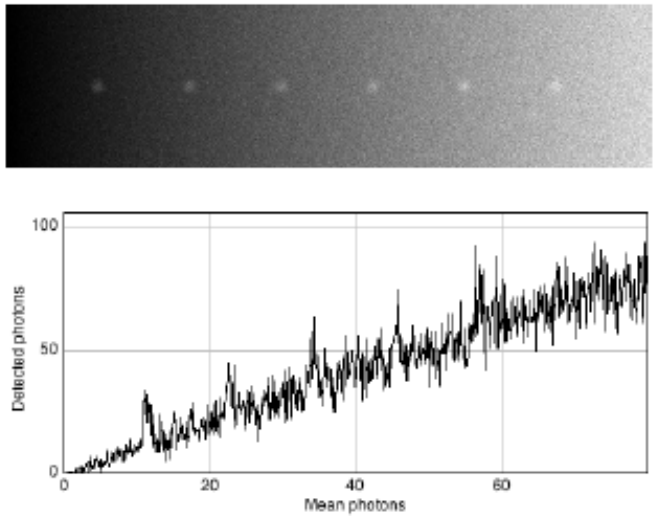
(a) Original image (b) Gaussian $\sigma = 2$ (c) Gaussian $\sigma = 5$



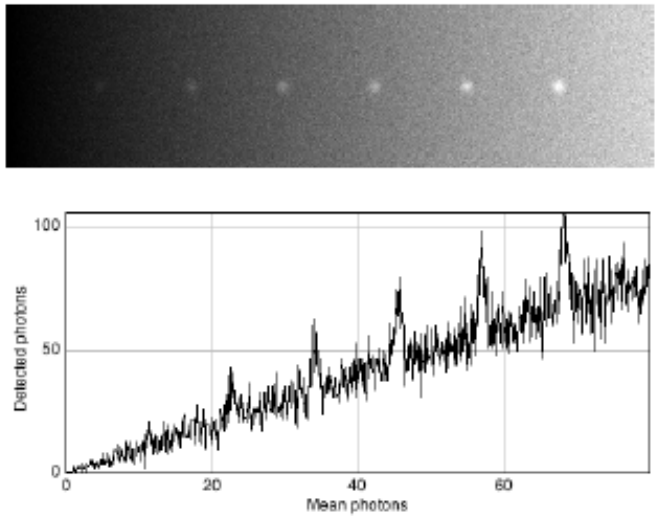
(d) Profile plots of the intensity in the red channel of the image



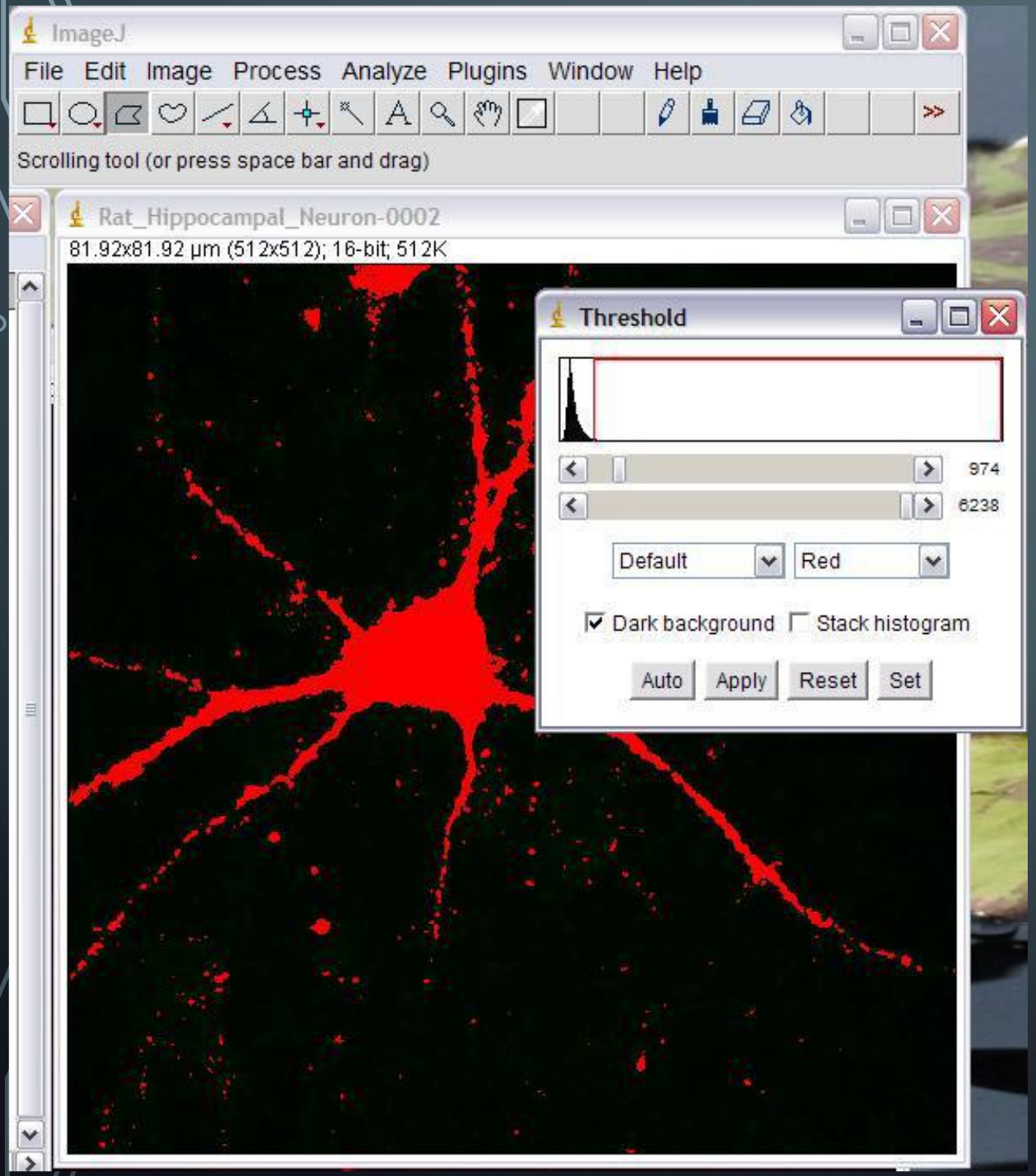




(a) Seeing spots with the same absolute brightness



(b) Seeing spots with the same relative brightness



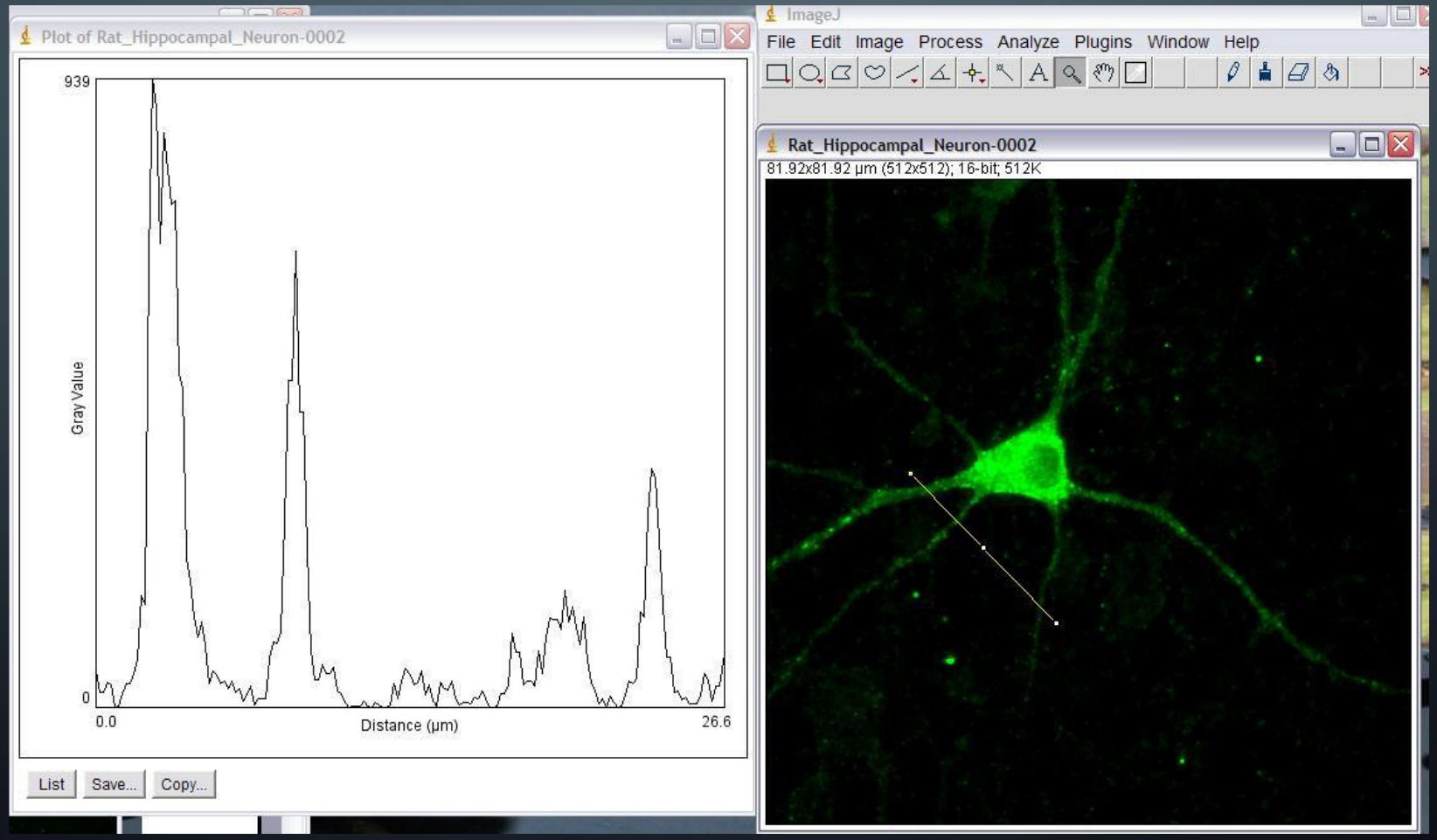
Alternatively, you can go to Analyze > Set Measurements

and check off the box next to “Limit to Threshold.”
Then use
Image > Adjust >Threshold

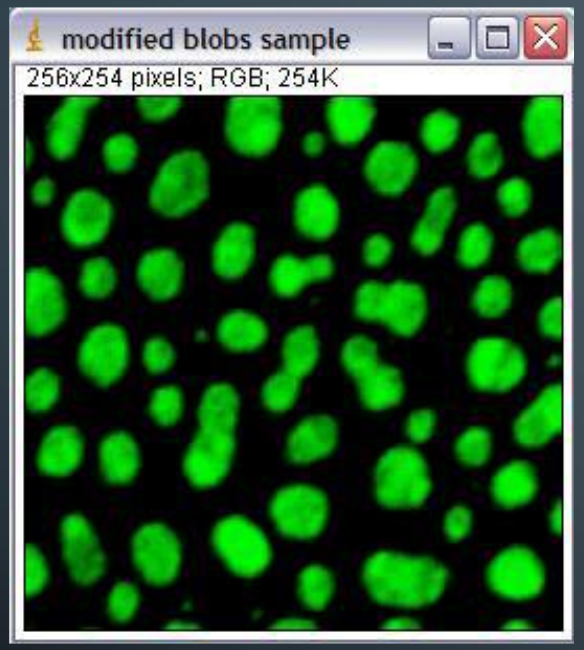
to highlight the area you want to analyze, and then
>
Analyze

You can use Analyze > Plot Profile to create a plot of intensity values across features in your image.

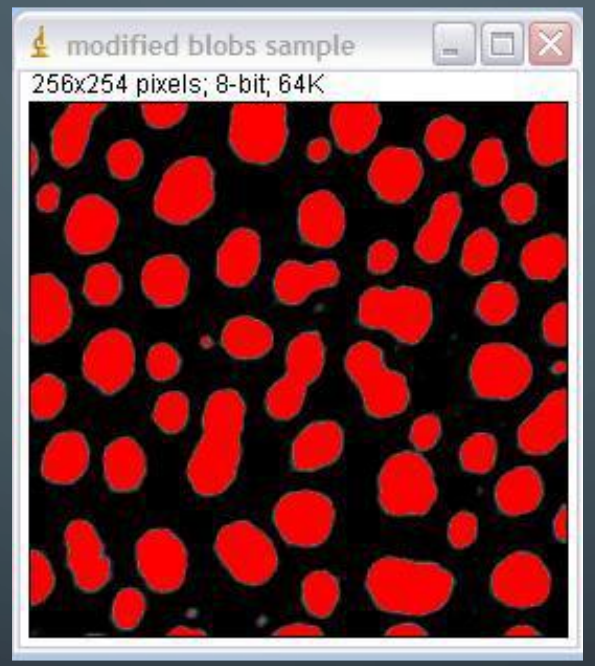
In the example below, the plot gives the intensity values along the line drawn across three cell processes.



To Quantify Gray Levels for Each Object in Images with Multiple Objects



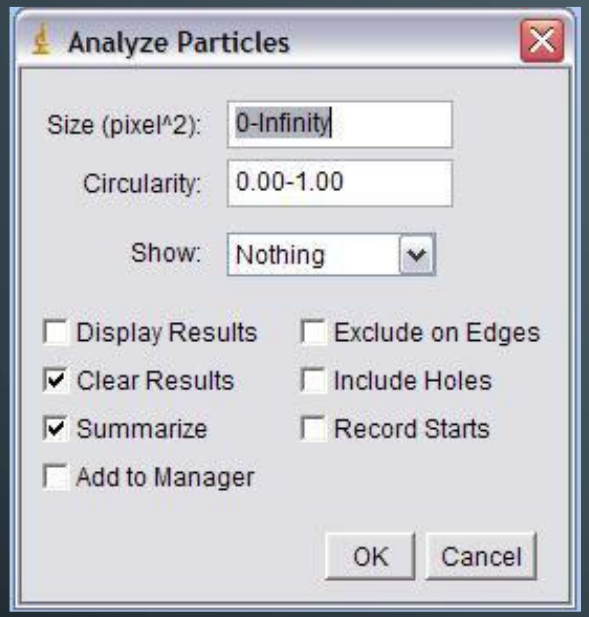
convert to grayscale



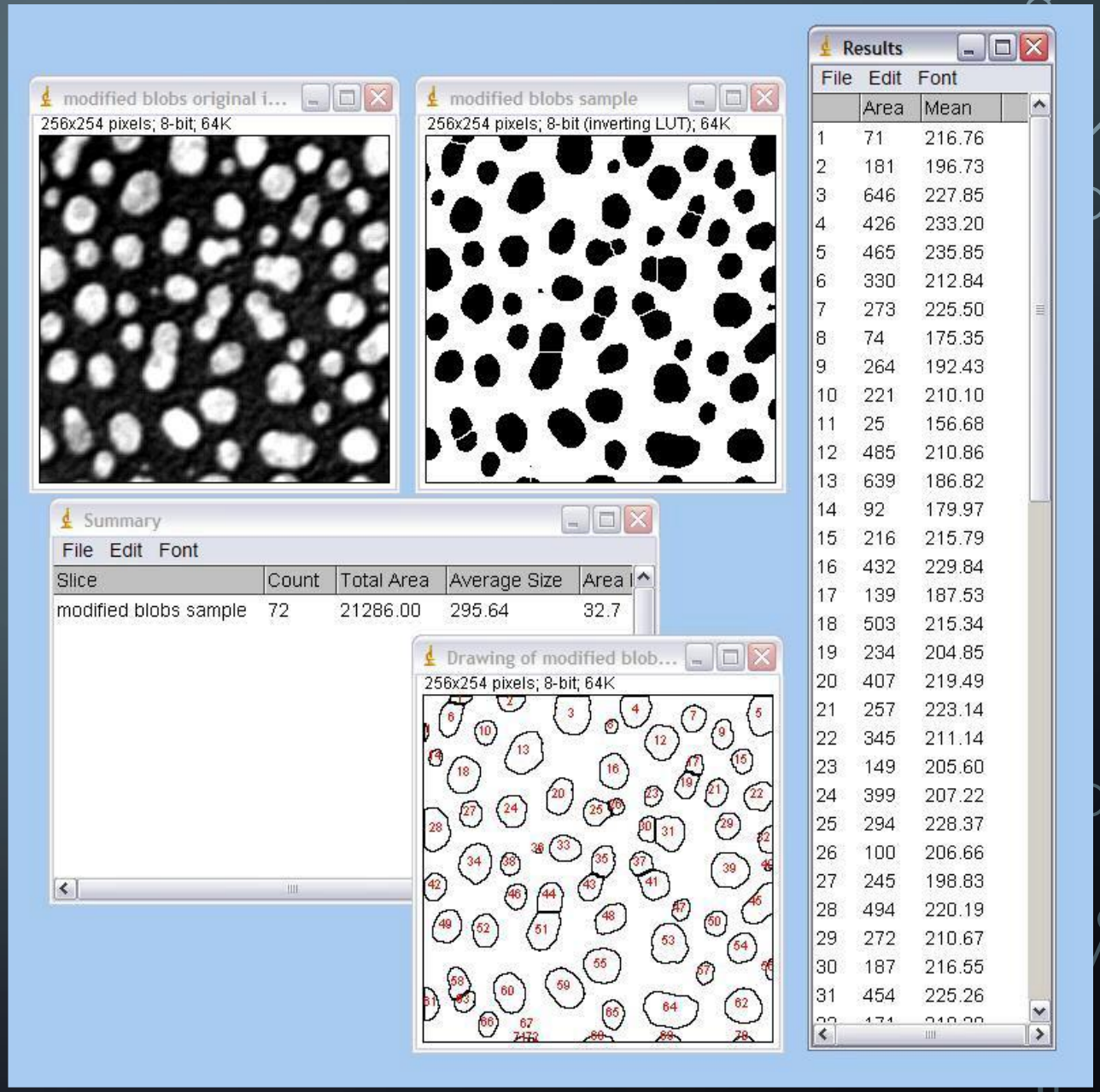
create a binary image,

Process > Binary > Watershed

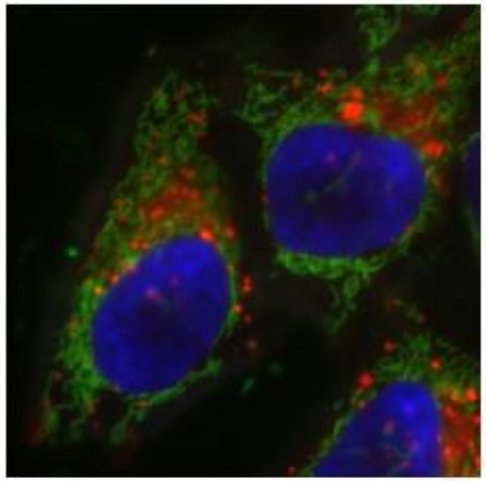
Analyze > Analyze Particles



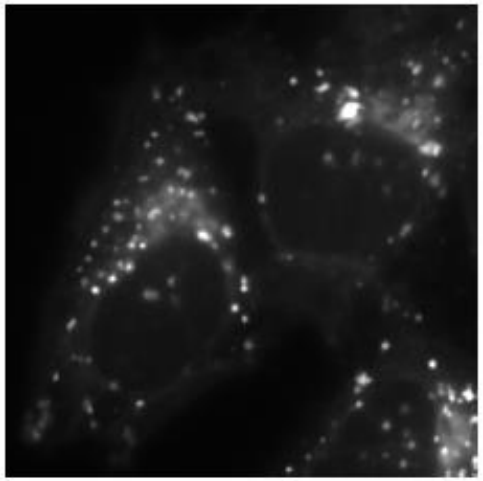
>



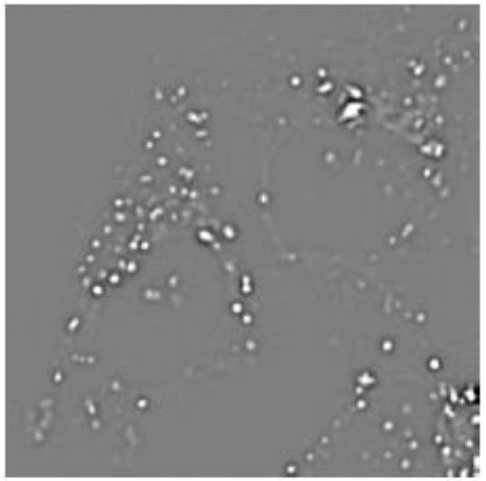
An simple image analysis workflow for detecting and measuring small spots, applied to the red channel of the sample image HeLa Cells.



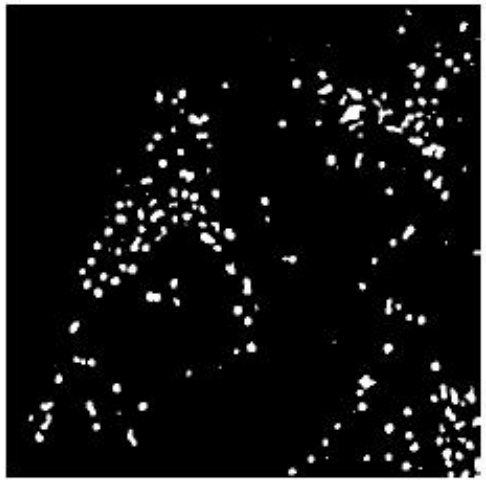
(a) Original image



(b) Extract channel

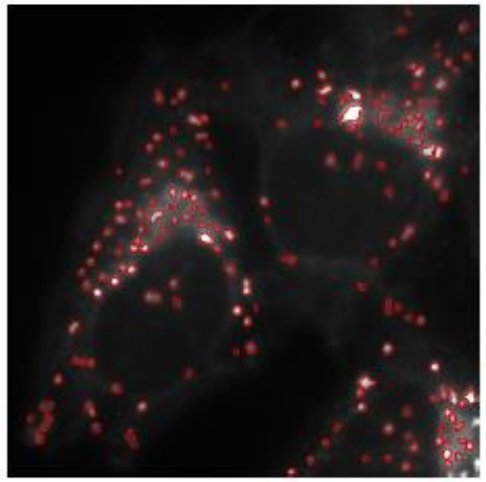


(c) Apply filters

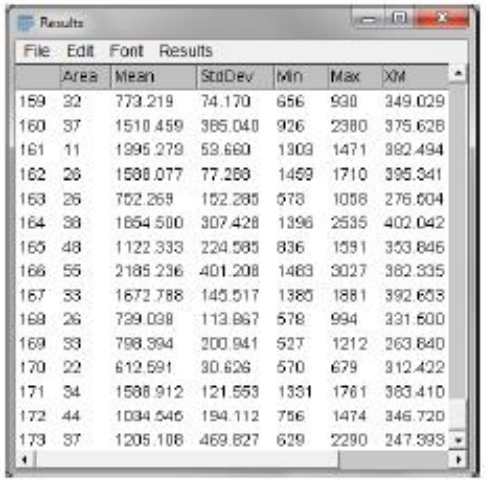


(d) Apply threshold

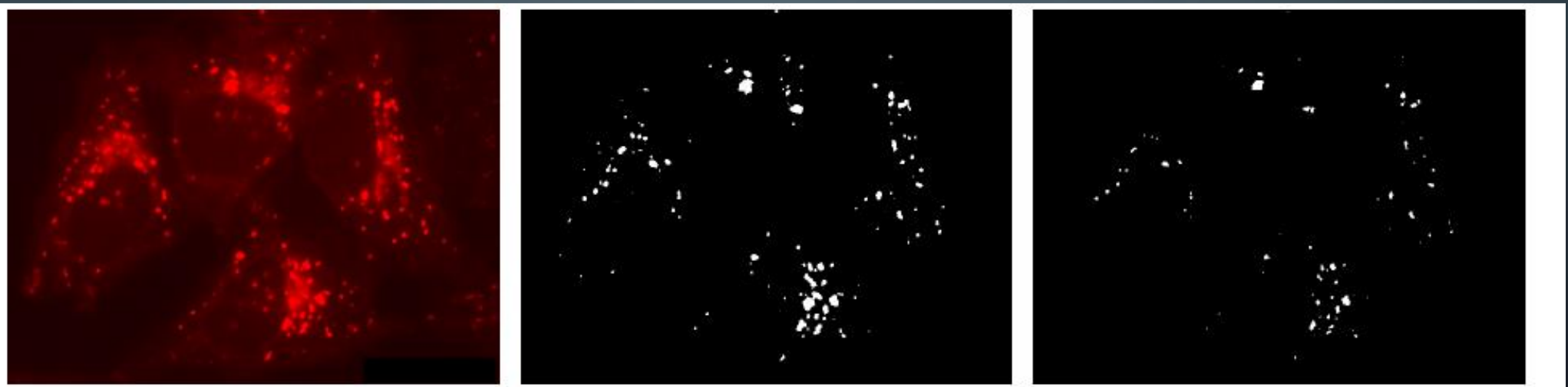
(e) Refine detection



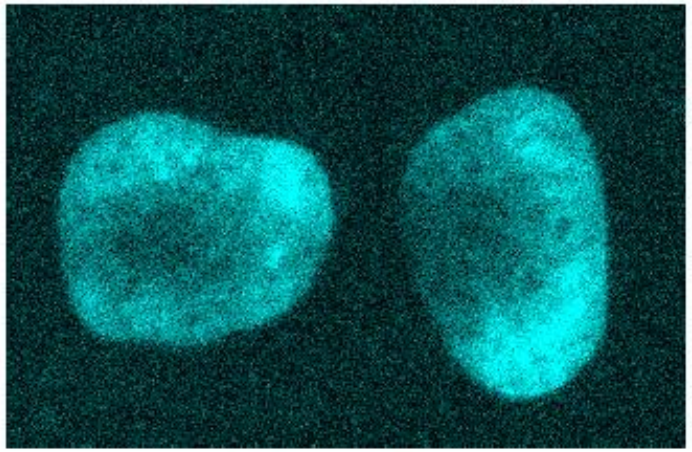
(f) Relate (e) to (b)



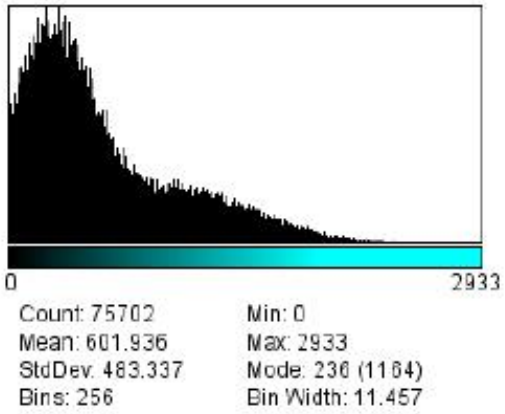
(g) Measure



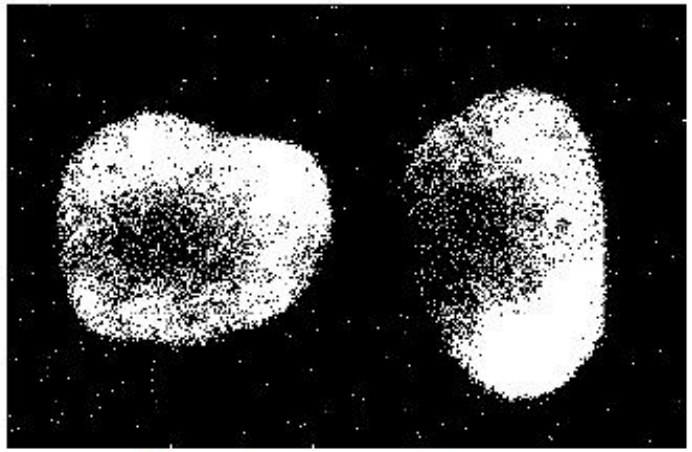
(a) Image (b) Low threshold (c) High threshold



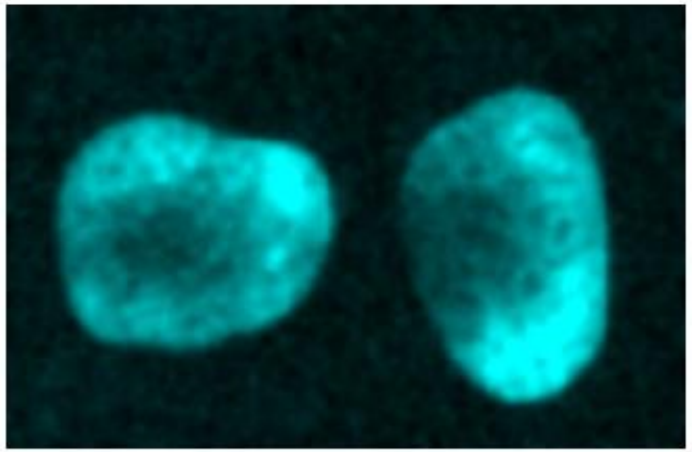
(a) Noisy image



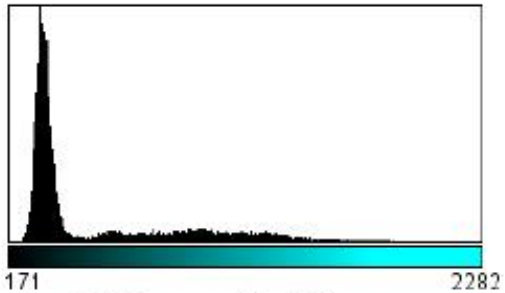
(b) Histogram of (a)



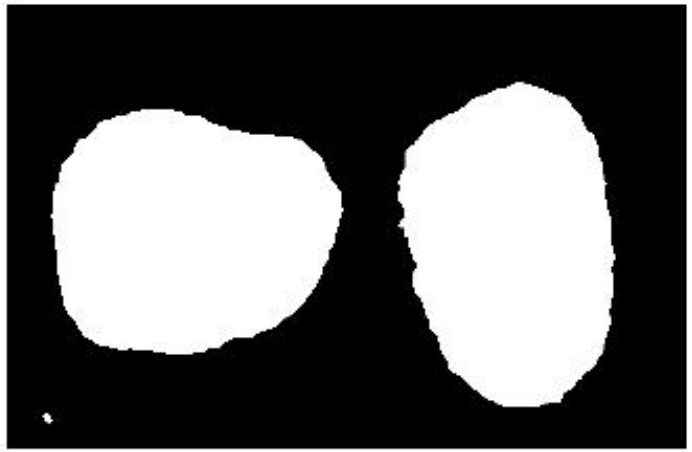
(c) Threshold applied to (a)



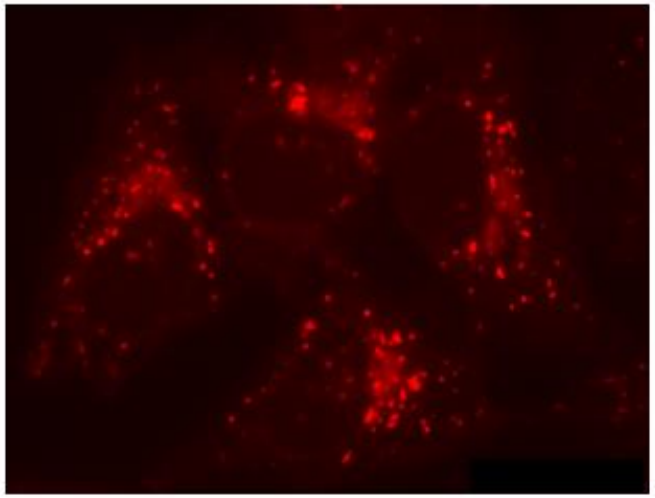
(d) Gaussian filtered image



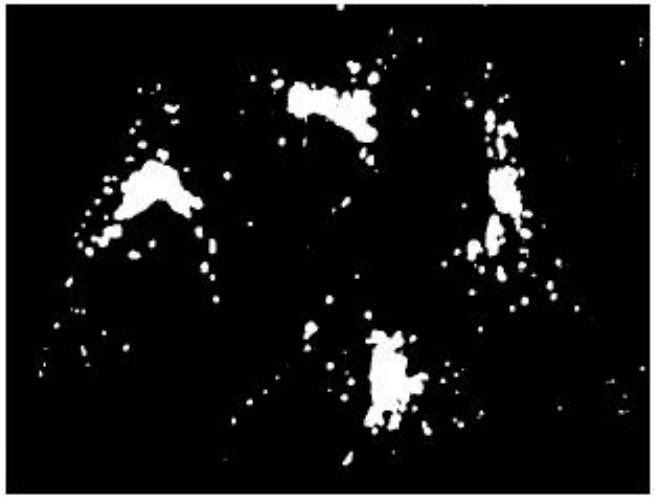
(e) Histogram of (d)



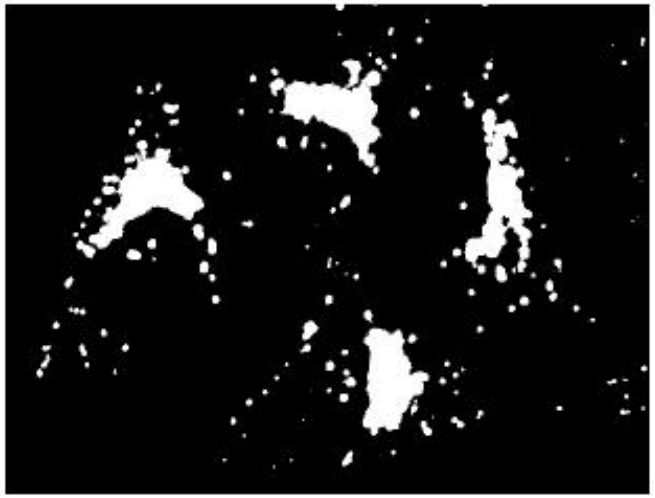
(f) Threshold applied to (d)



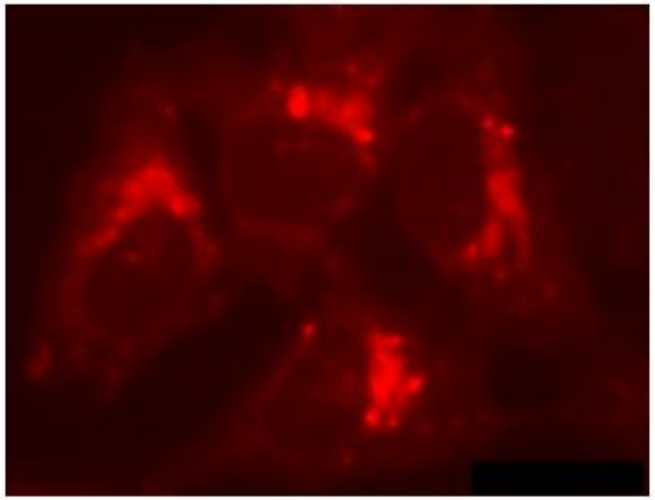
(a) Original image



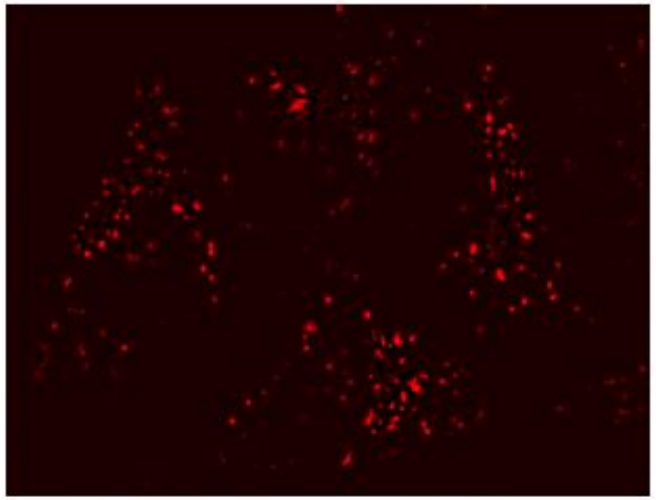
(b) Otsu's threshold



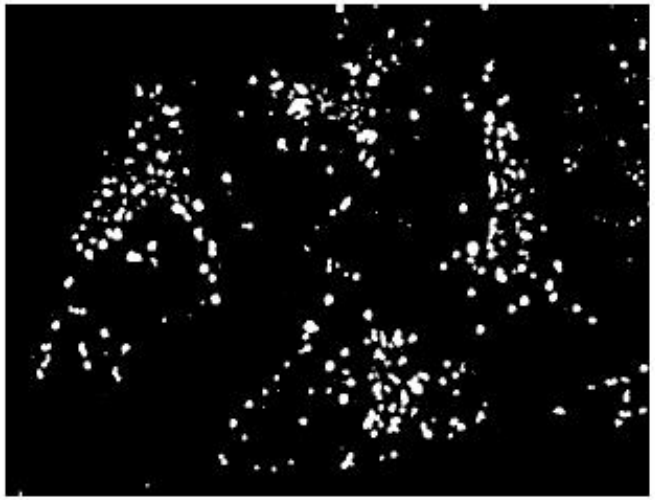
(c) Triangle threshold



(d) Median filtered image



(e) Result of (a)-(d)



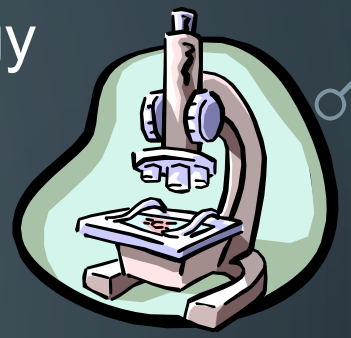
(f) Triangle threshold of (e)

ImageJ How to Measure Mean Fluorescence Intensity Over Timelapse Image Stack

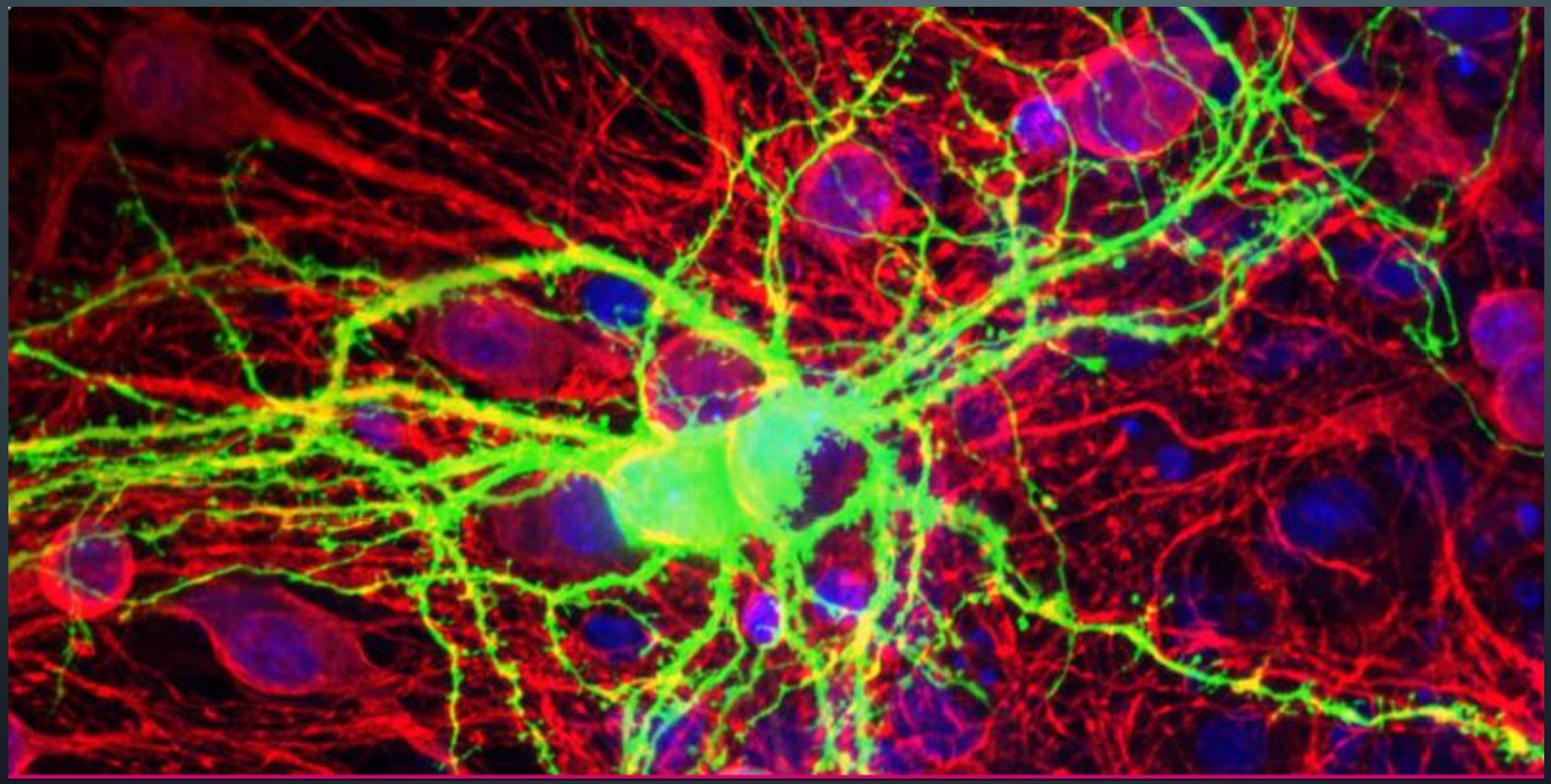
https://youtu.be/GHvndpGQKe4

Short introduction to histogram processing

https://youtu.be/nlRhHb04u_k



Question?



Thanks for your attention!

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