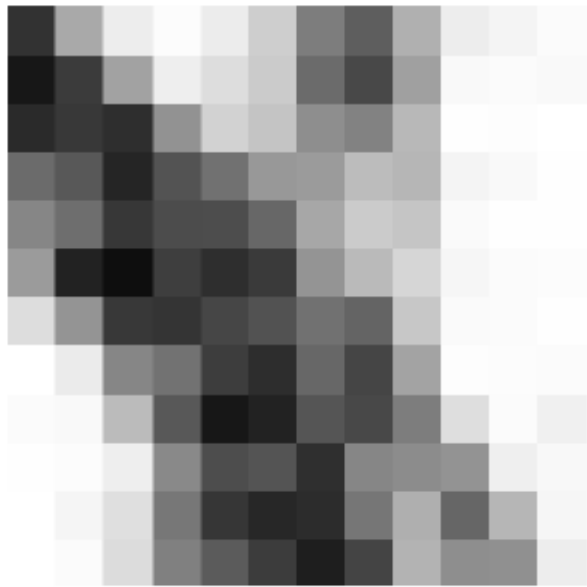


Light microscopy in Cellular Biology

Gabriele Baj
gbaj@units.it



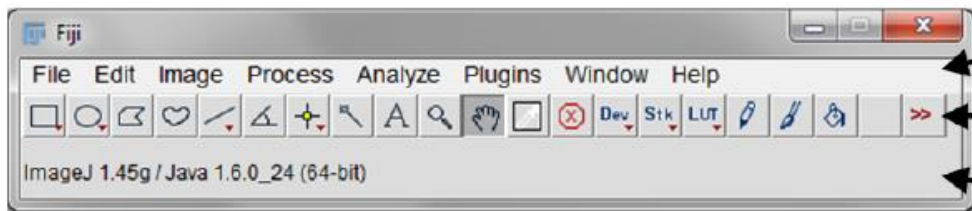
(a) Original image



(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

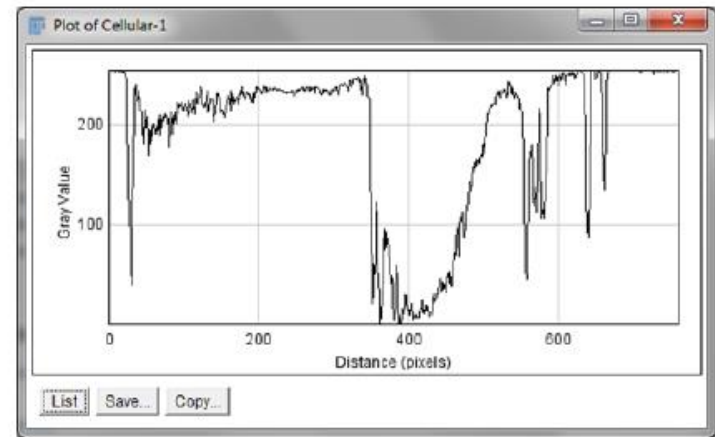
(c) Pixel values of (b)



Menu bar

Tool bar

Status bar



Plot window

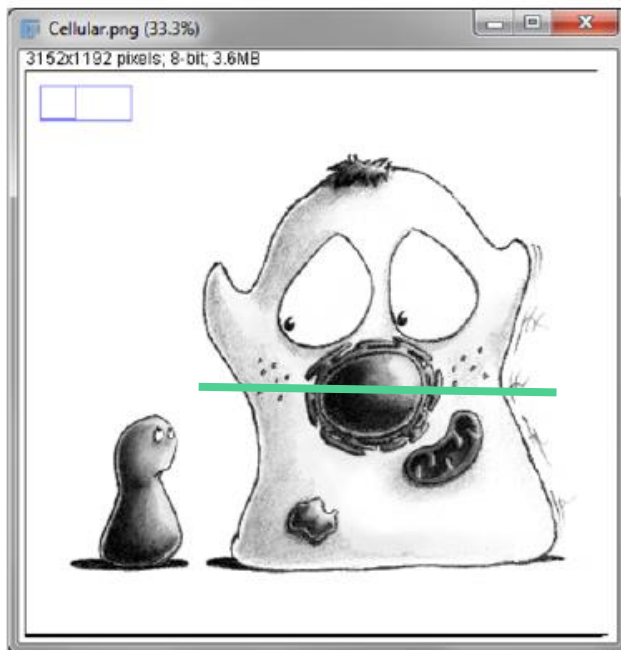
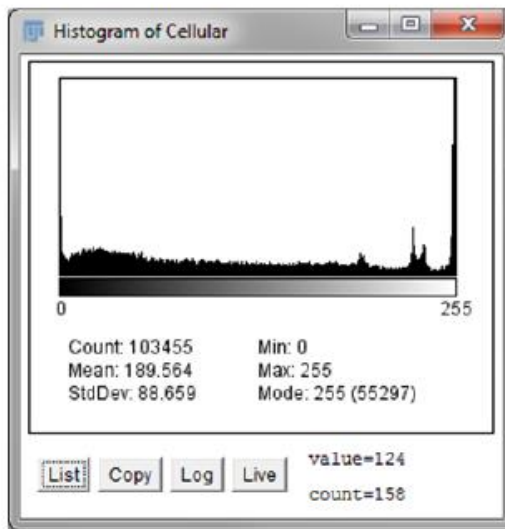


Image window



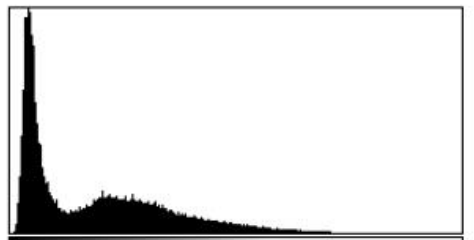
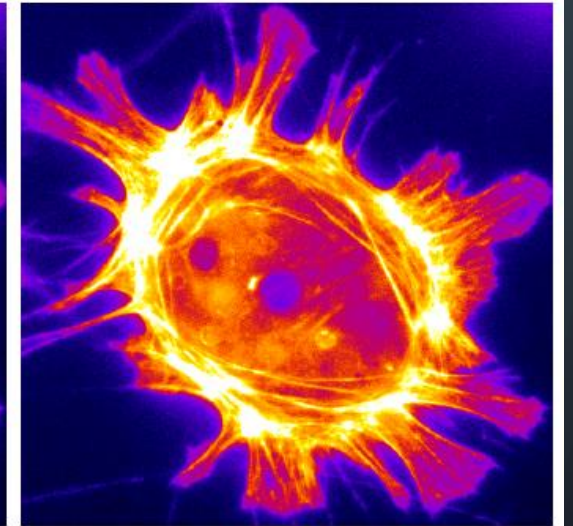
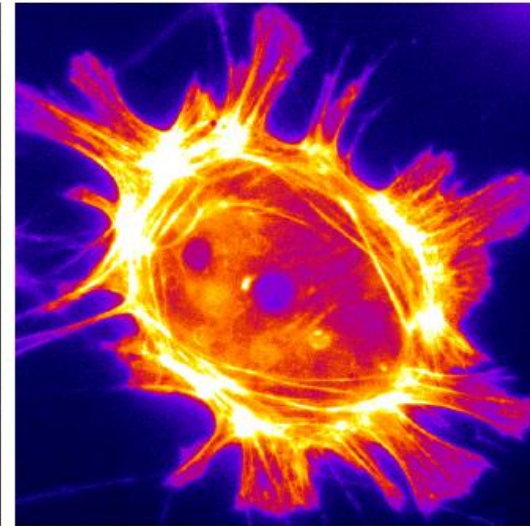
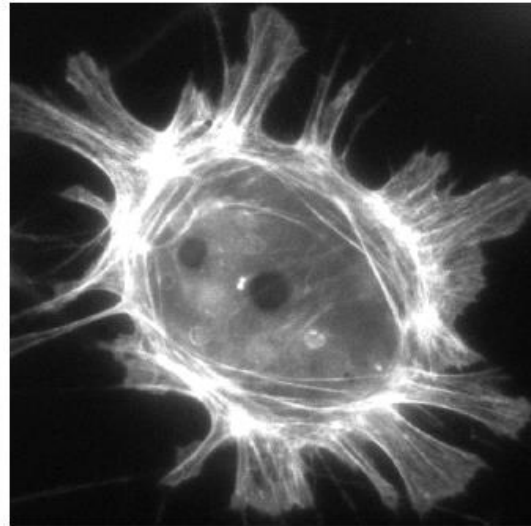
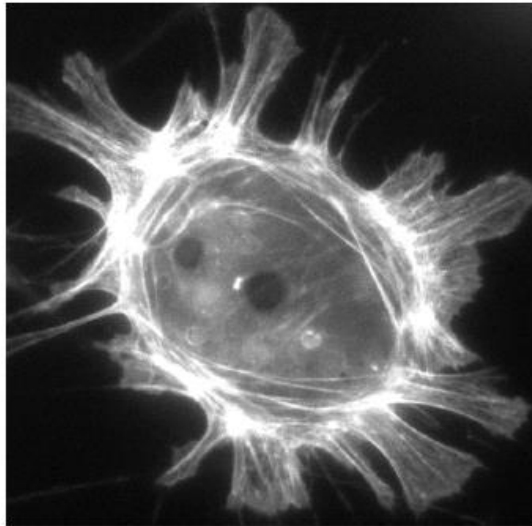
Histogram window

The results table window displays a table with the following data:

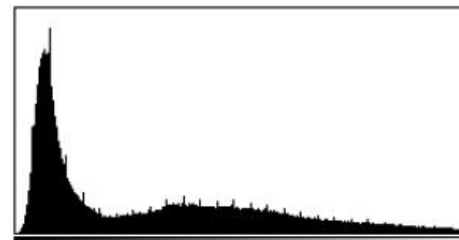
	Area	Mean	StdDev	Min	Max	Major	Minor	Angle
1	18972	249.590	27.457	14	255	209.879	115.095	0
2	42758	140.995	93.854	0	254	329.914	165.016	0
3	4007	128.757	75.740	0	251	80.927	63.043	90.000
4	4705	75.605	43.936	0	255	104.933	57.090	90.000
5	4705	92.641	50.162	0	221	104.933	57.090	90.000

Results table

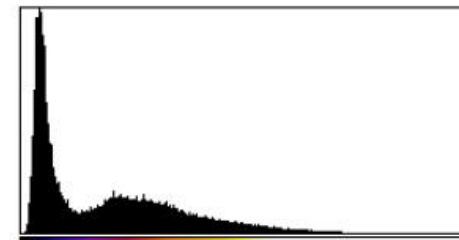
Do not trust your eyes for image comparisons



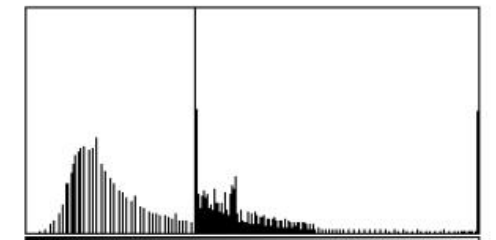
Count: 339864
Mean: 591.429
StdDev: 306.524
Bins: 256
Min: 239
Max: 2090
Mode: 313 (14617)
Bin Width: 7.230



Count: 339864
Mean: 82.006
StdDev: 71.418
Min: 0
Max: 255
Mode: 255 (10308)



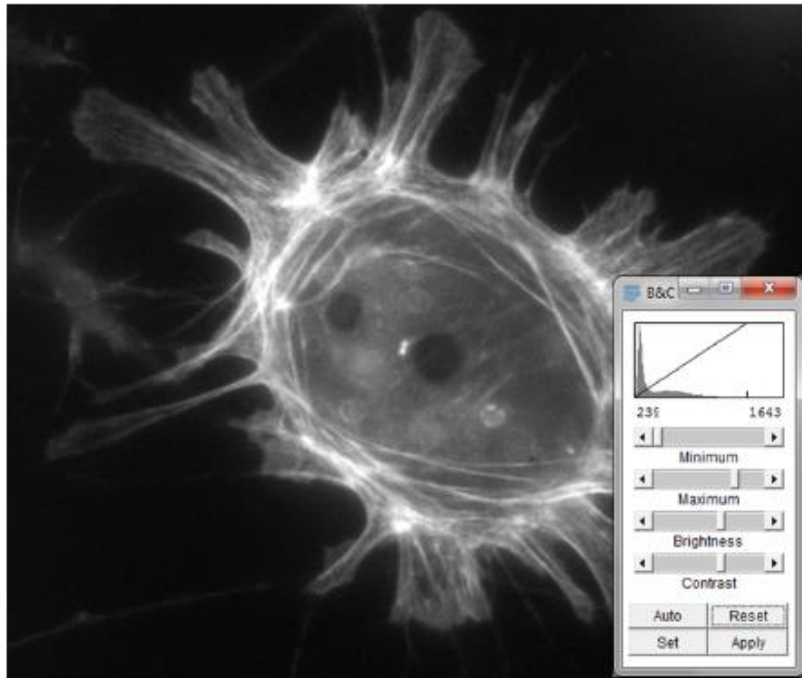
Count: 339864
Mean: 591.429
StdDev: 306.524
Bins: 256
Min: 239
Max: 2090
Mode: 313 (14617)
Bin Width: 7.230



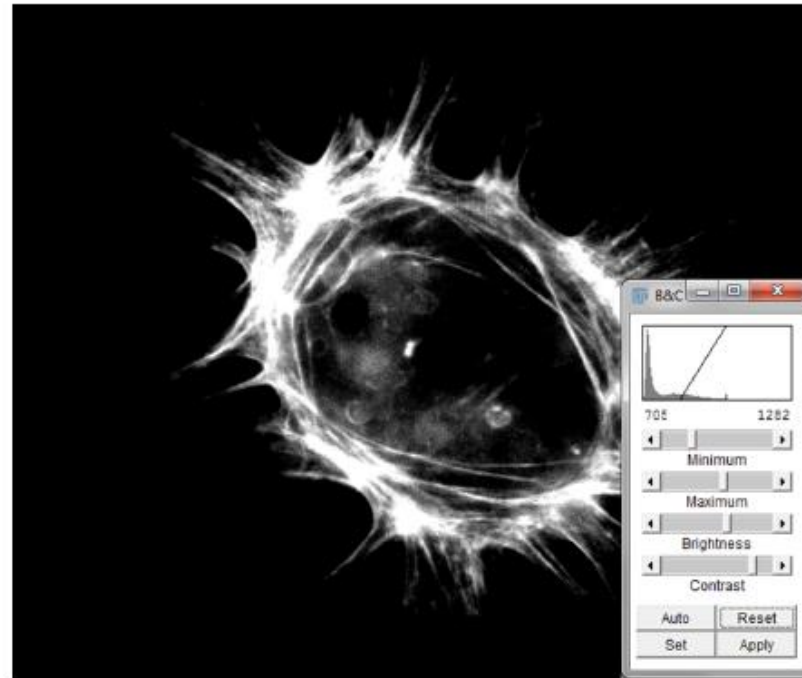
Count: 339864
Mean: 90.544
StdDev: 56.392
Min: 0
Max: 255
Mode: 95 (21862)

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

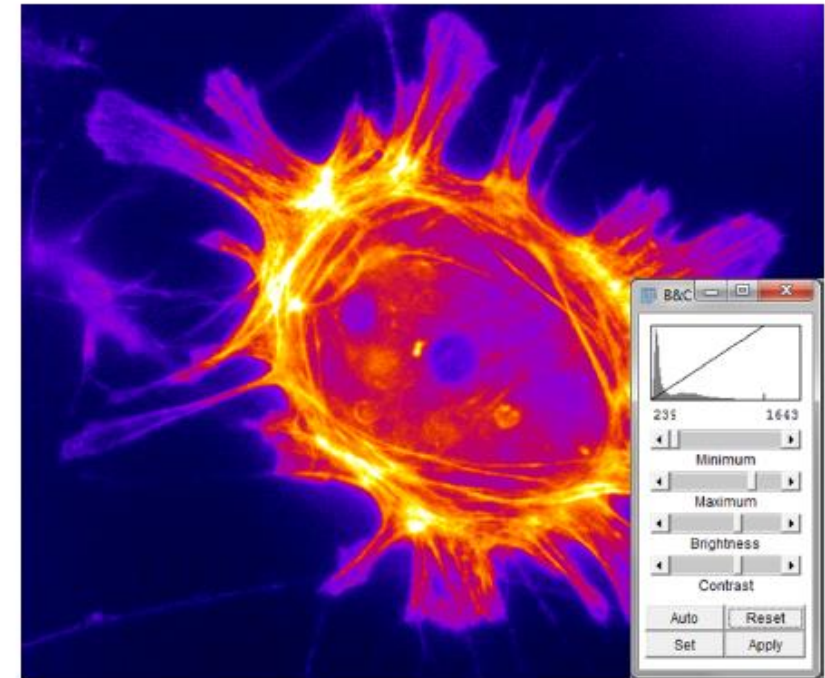
The same image can be displayed in different ways by adjusting the contrast settings or the LUT. Nevertheless, despite the different appearance, the values of the pixels are the same in all three images.



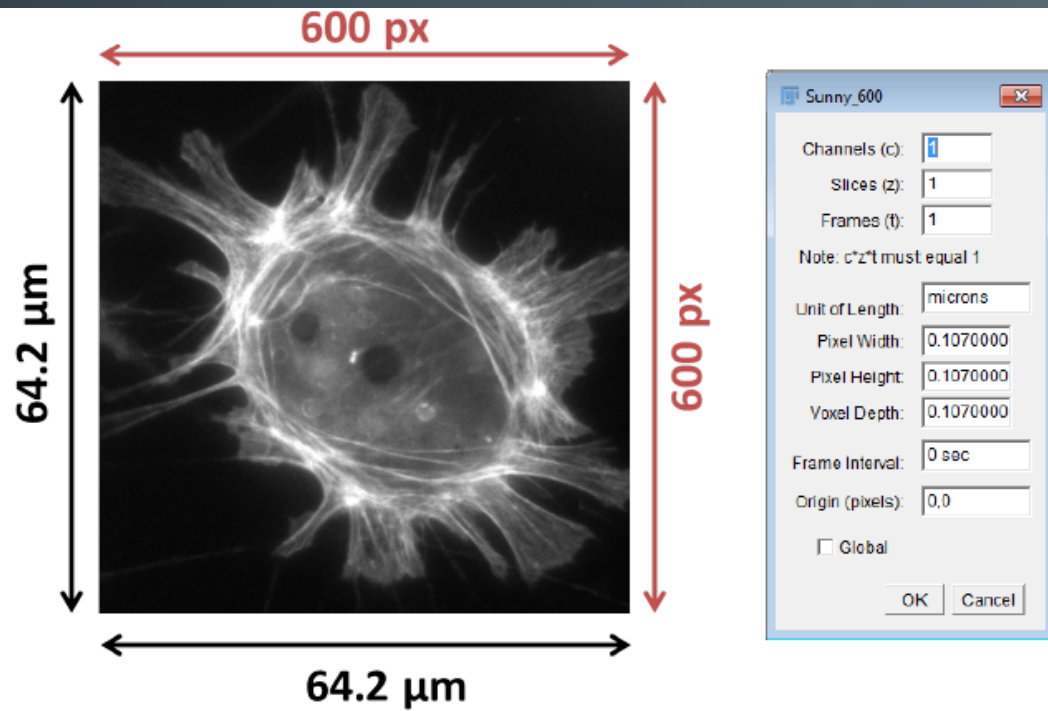
(a) Grayscale



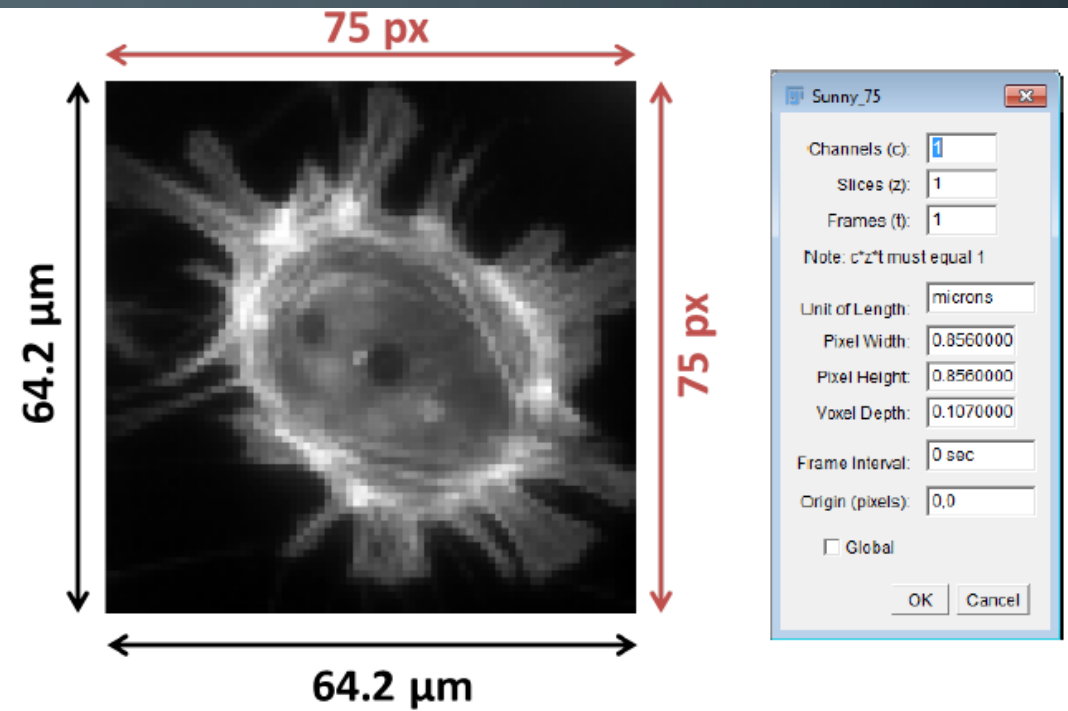
(b) Grayscale (high contrast)



(c) Fire LUT

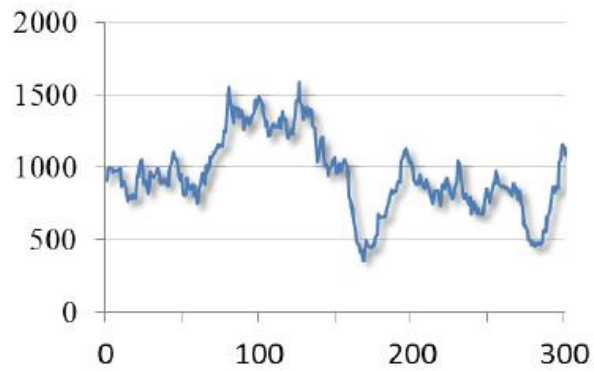


(a) 600×600 pixel image and its properties



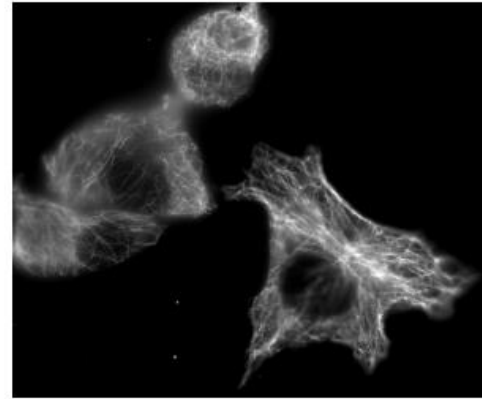
(b) 75×75 pixel image and its properties

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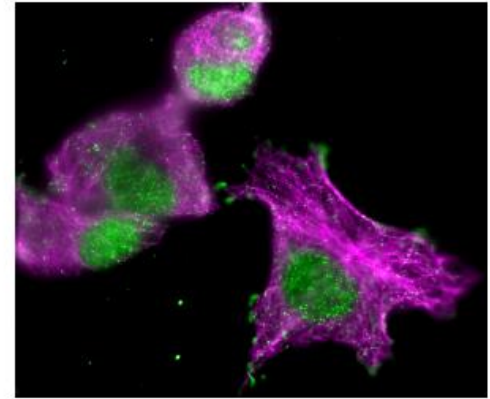


(a) 0 dimensional

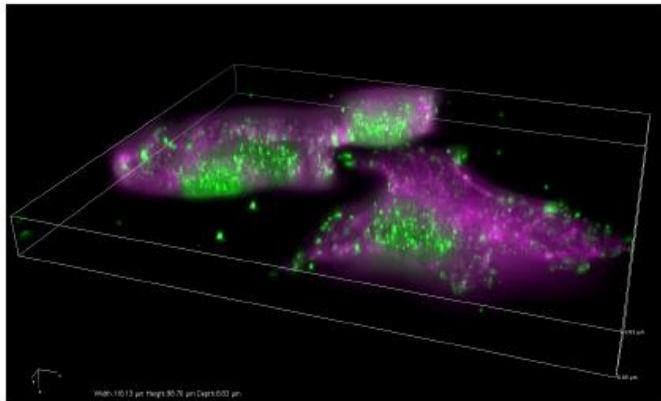
(b) 1 dimensional



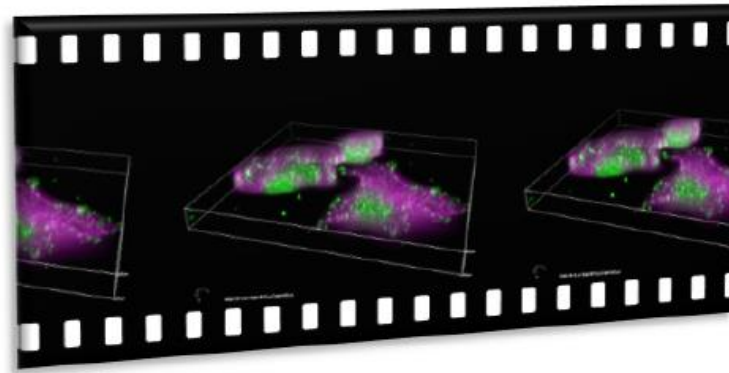
(c) 2 dimensional



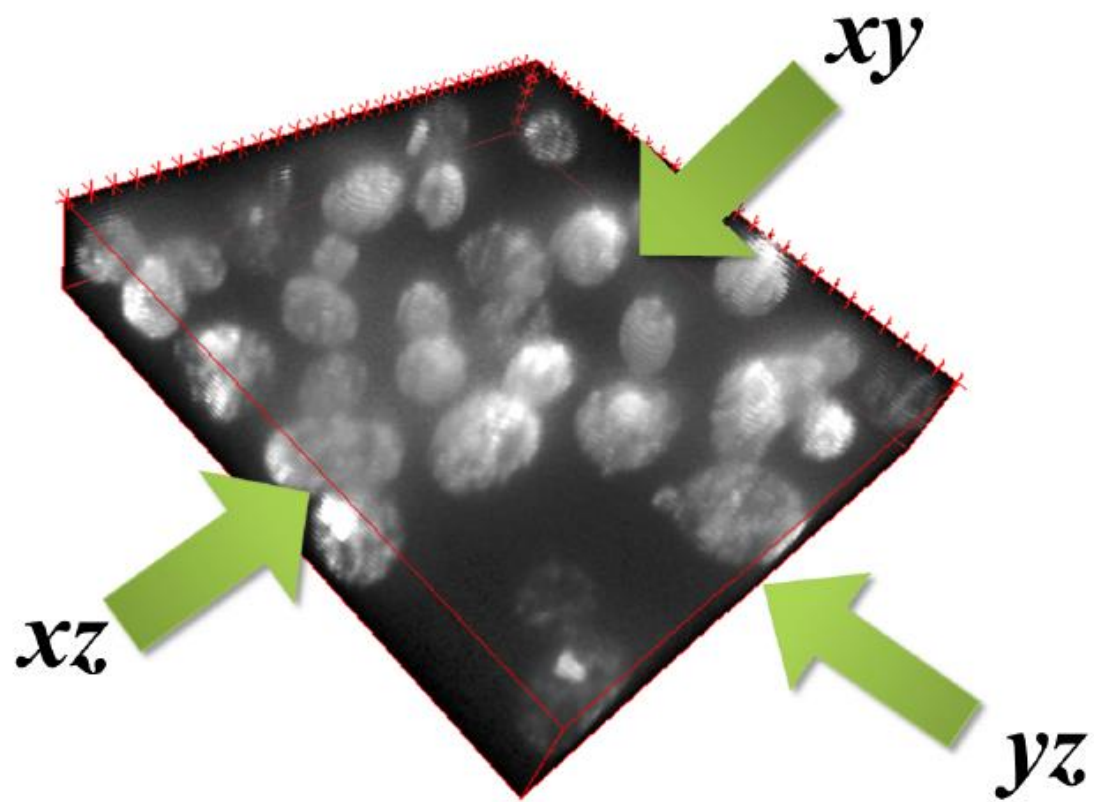
(d) 3 dimensional



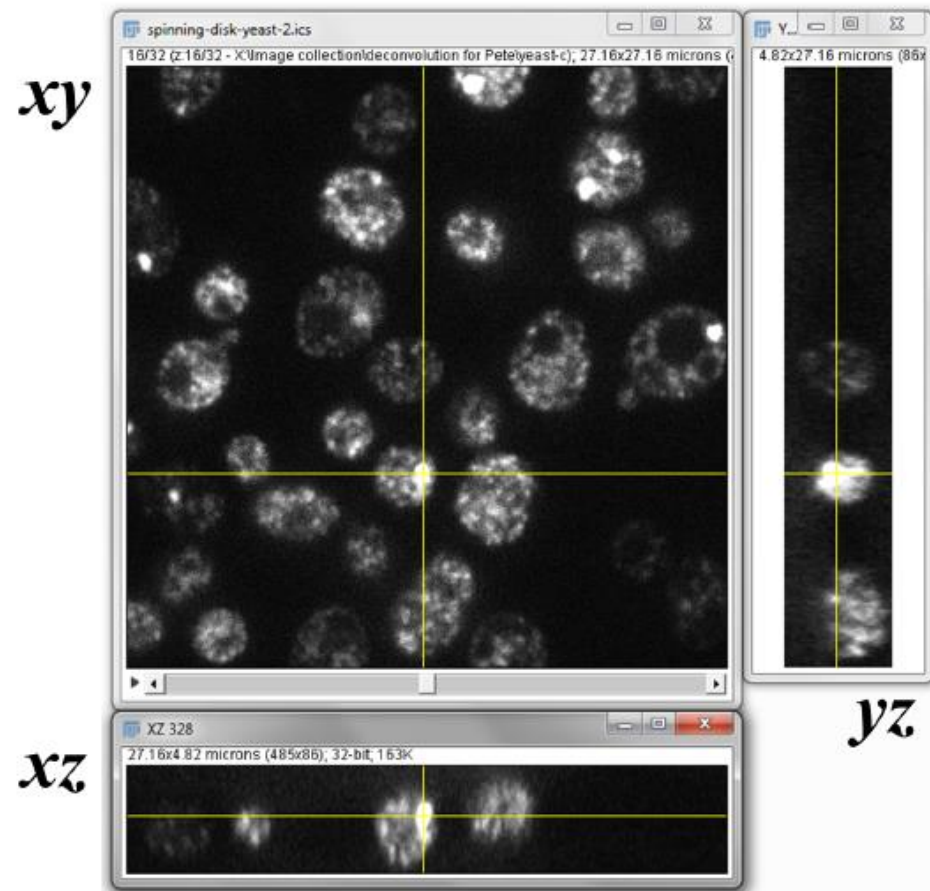
(e) 4 dimensional



(f) 5 dimensional



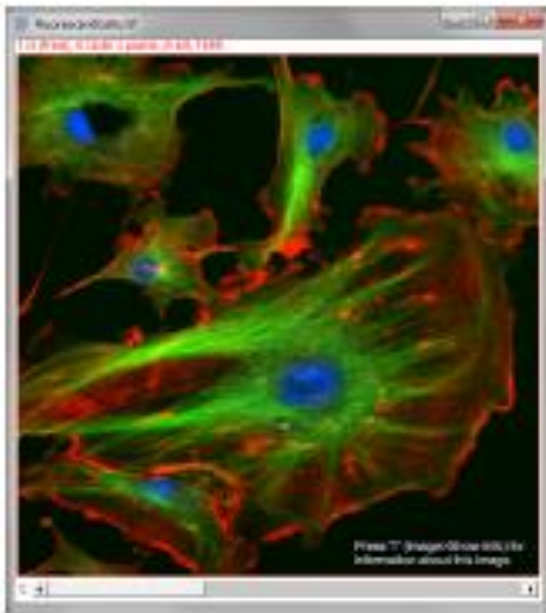
(a) Volume rendering



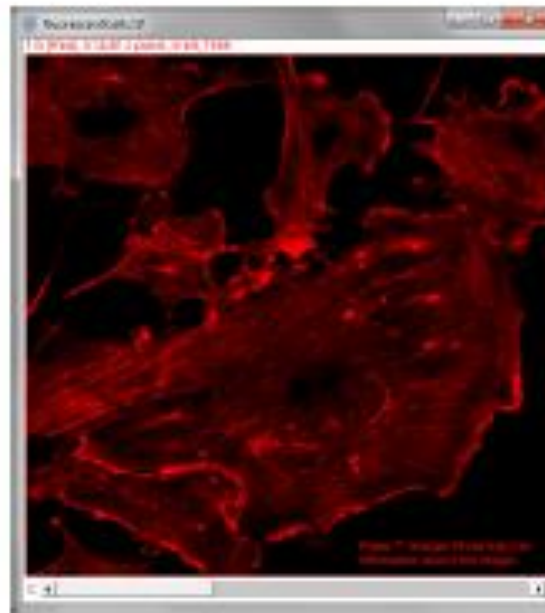
(b) Orthogonal views

ImageJ composite image sample Fluorescent Cells. Using the Channels Tool...

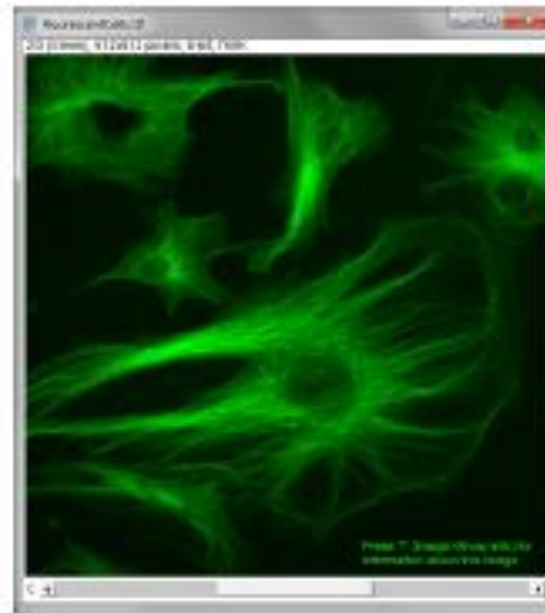
and the slider at the bottom of the window, you can view the channels individually or simultaneously.



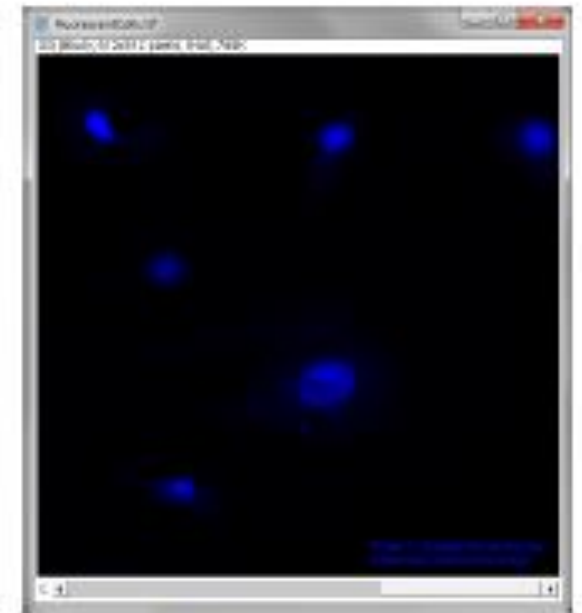
(a) Composite image



(b) Red channel



(c) Green channel



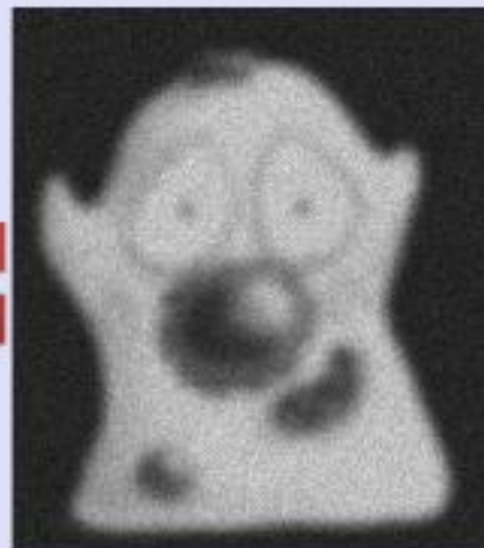
(d) Blue channel



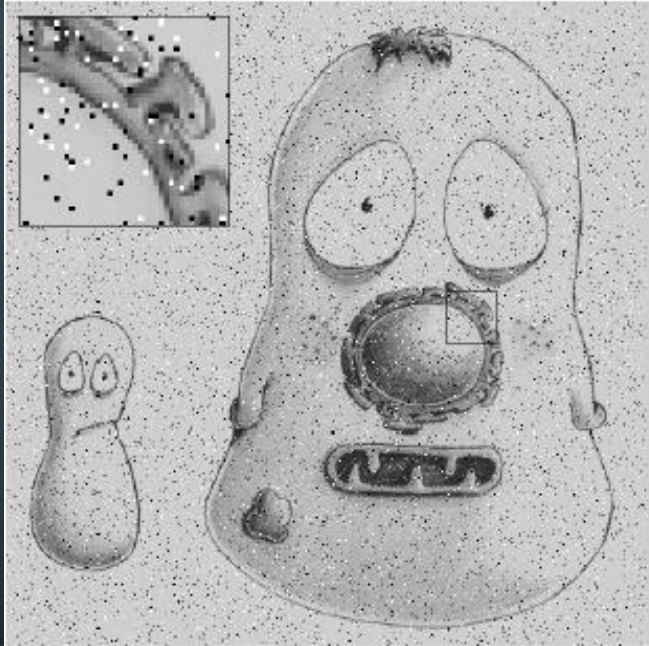
*The image
we would like*



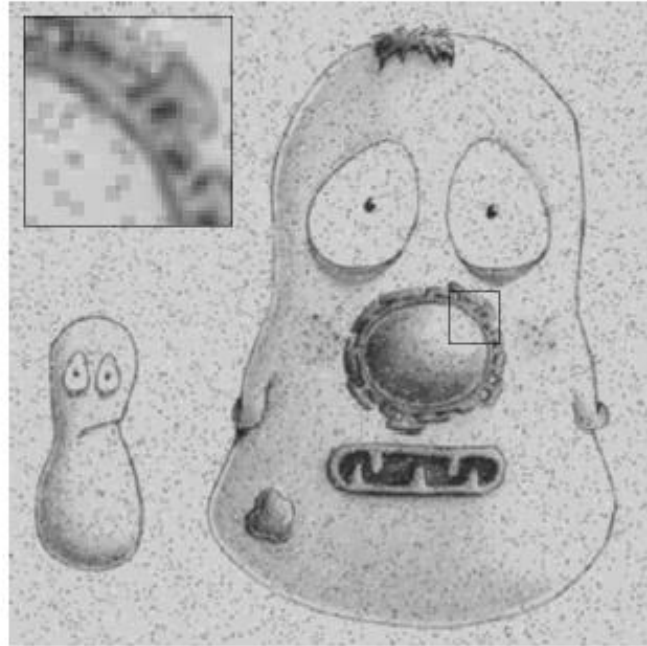
Noise



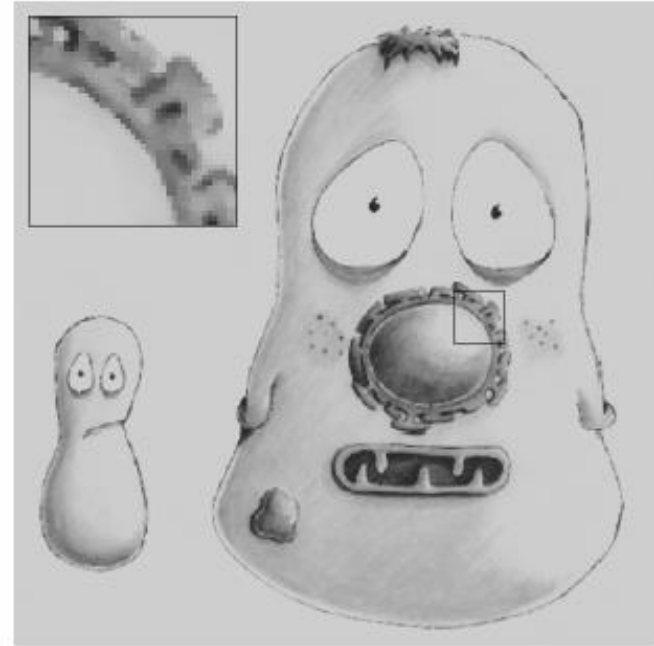
*The image we
can record*



(a) Speckled image



(b) Mean filter



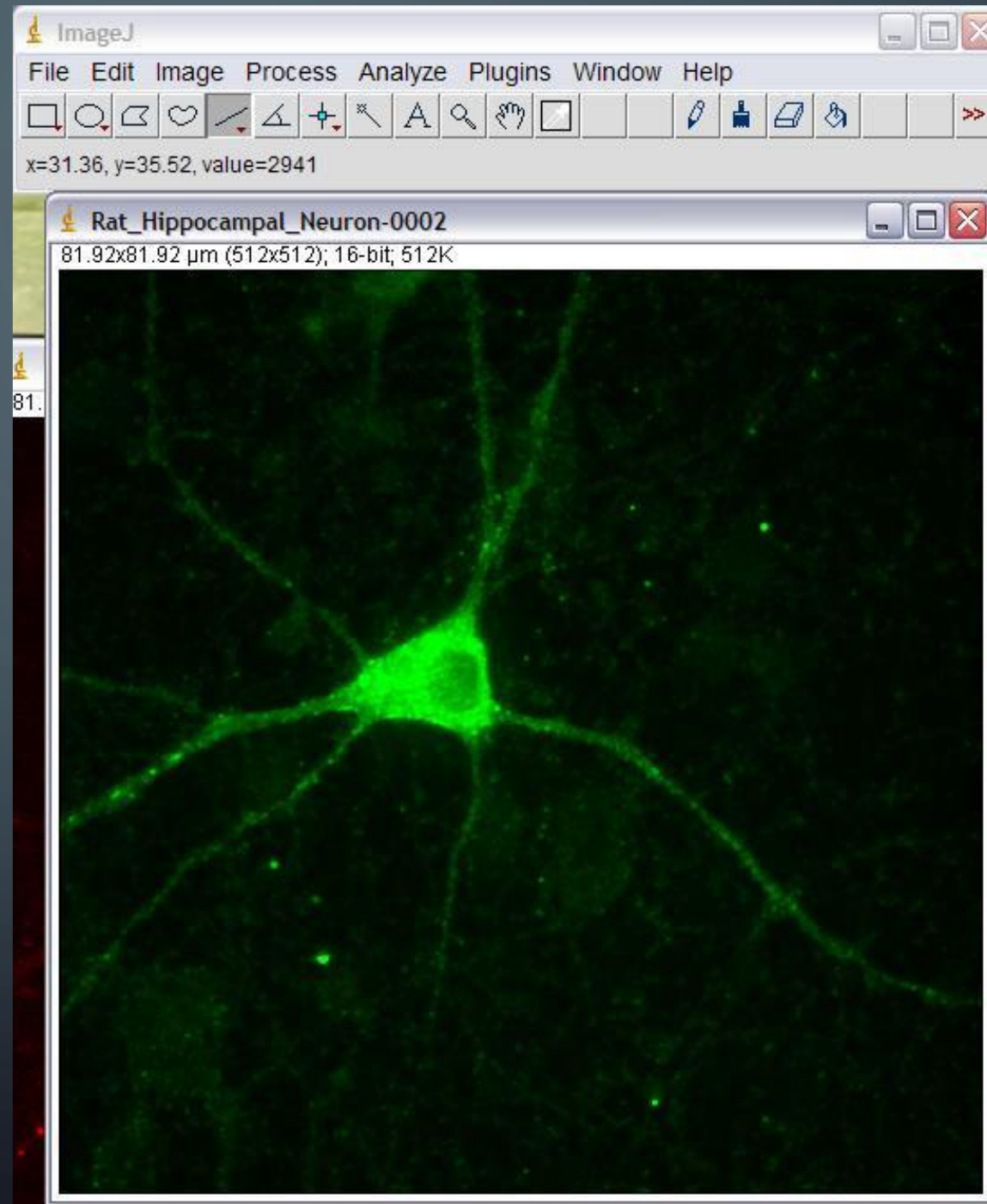
(c) Median filter

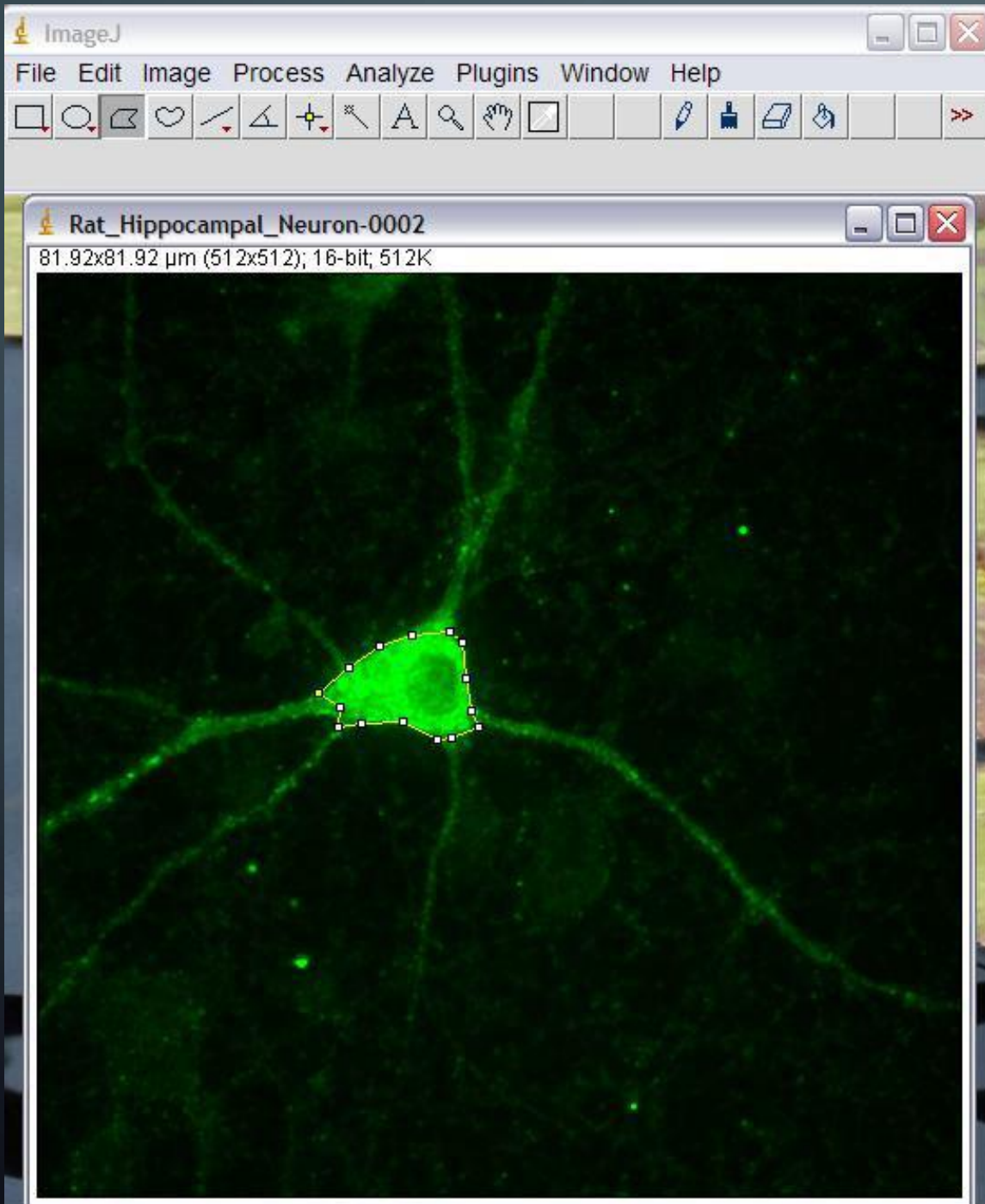
Basic Intensity Quantification with ImageJ

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

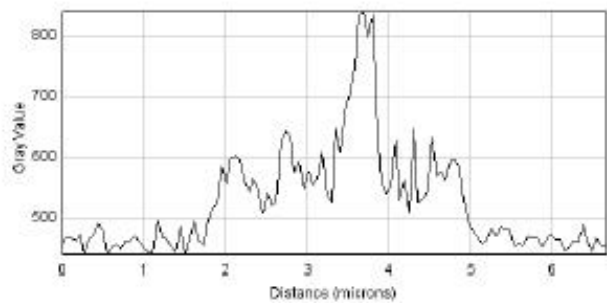
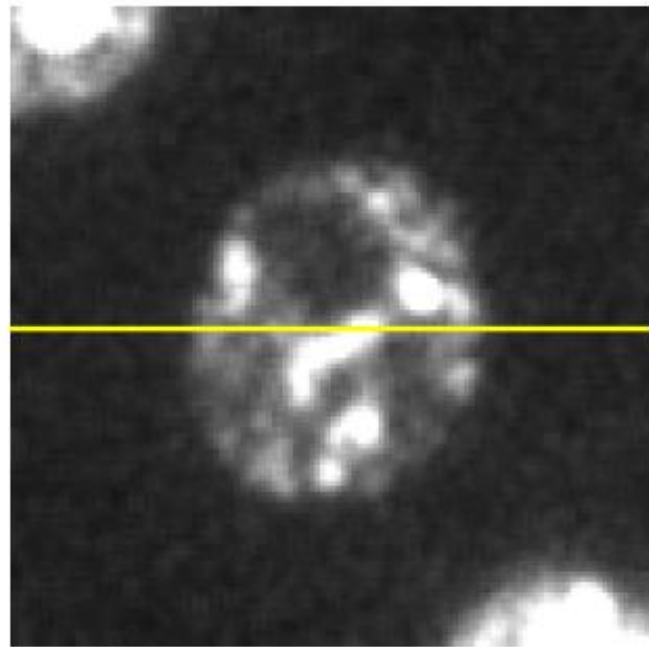
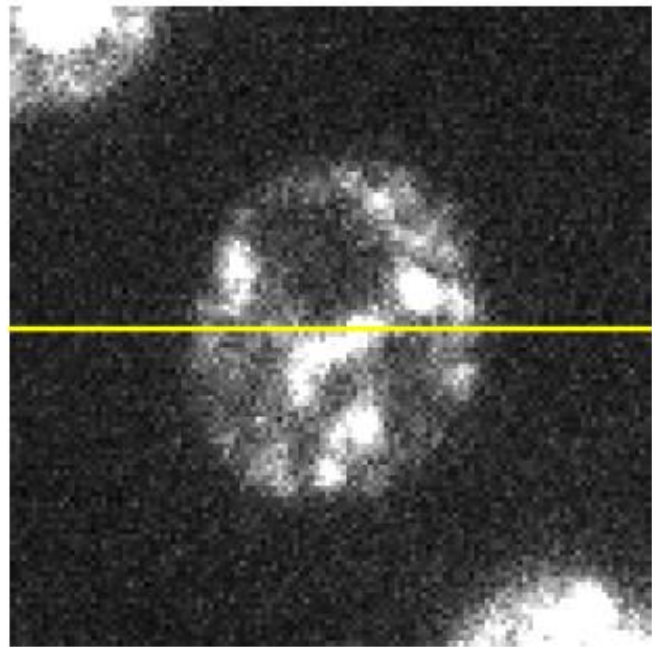




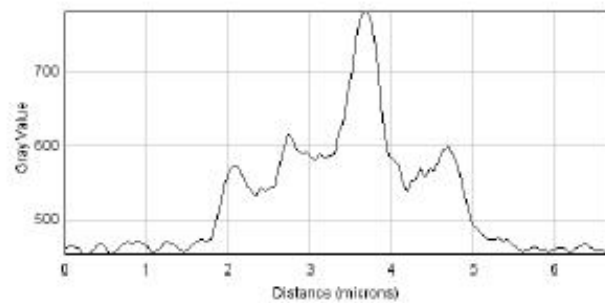
If you want to limit your measured area to just your object you draw a region of interest (ROI) around your object with one of the drawing tools (in the toolbar) and then

Analyze

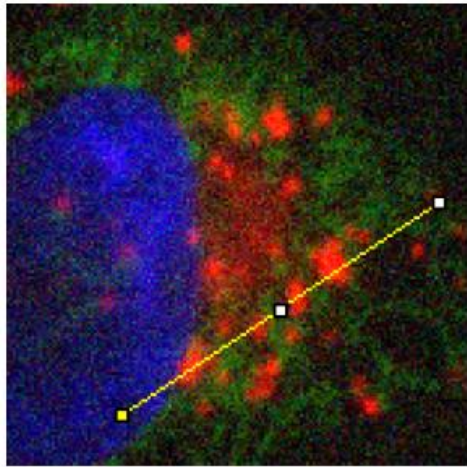
Measure



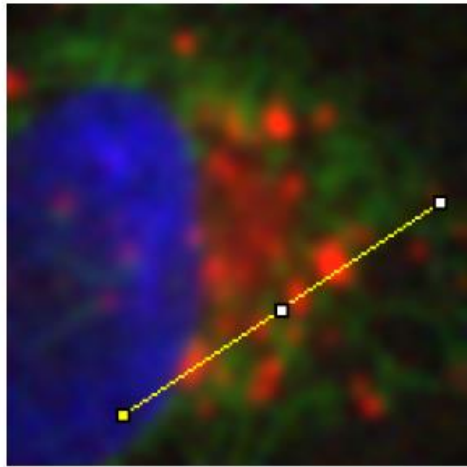
(a) Original image



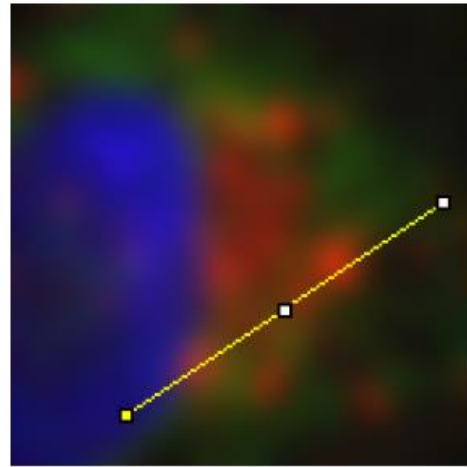
(b) Mean filtered



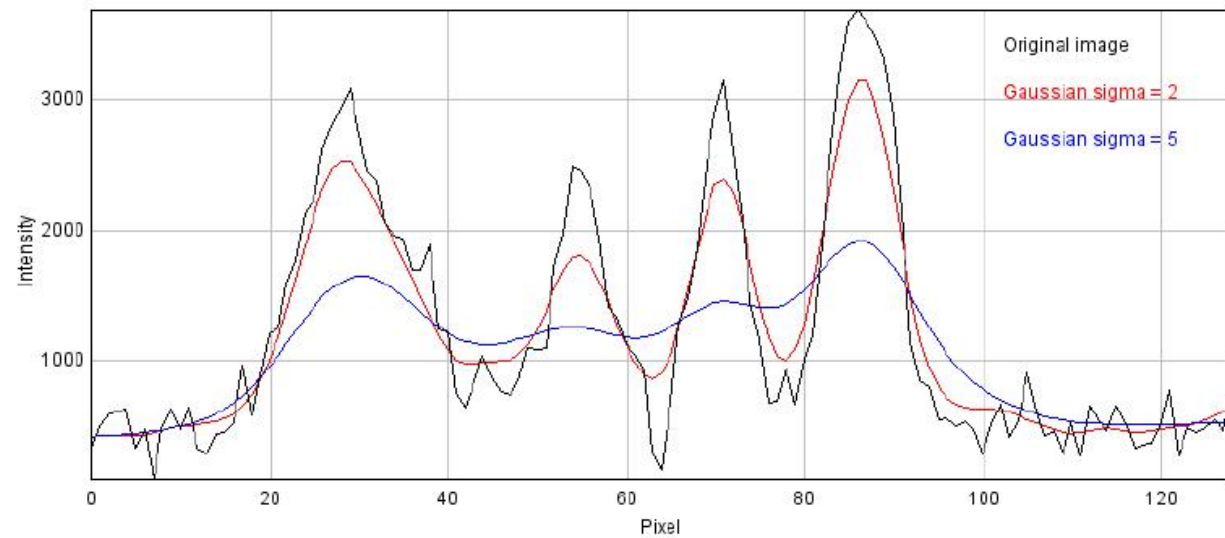
(a) Original image



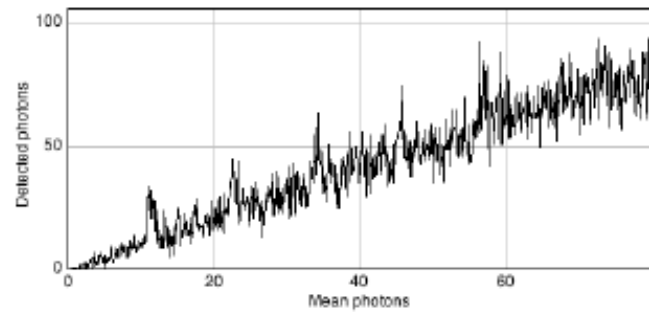
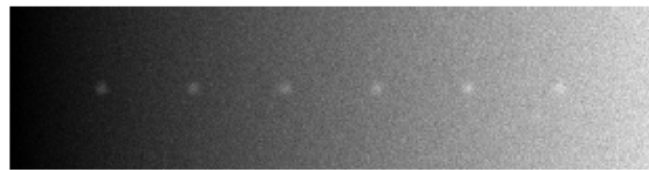
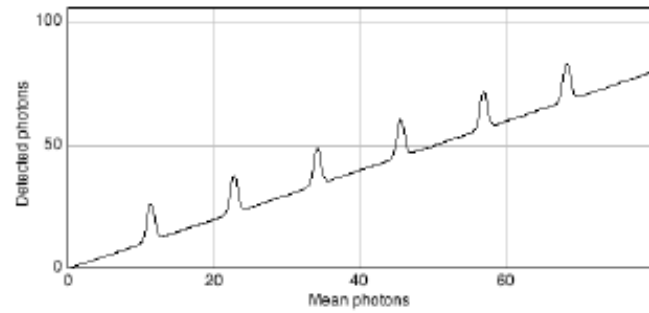
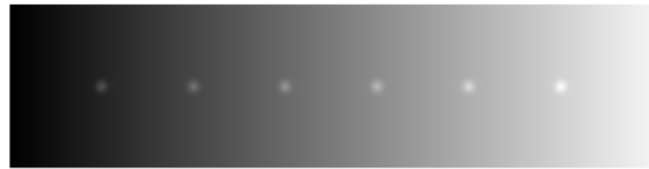
(b) Gaussian $\sigma = 2$



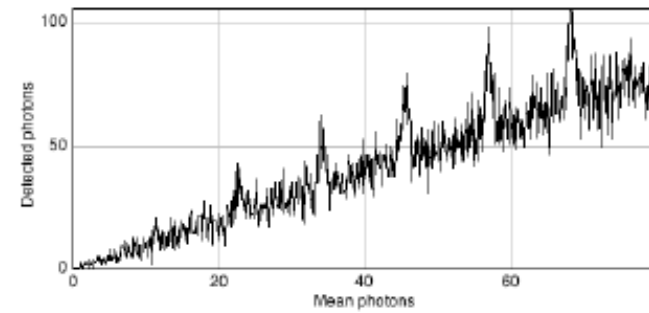
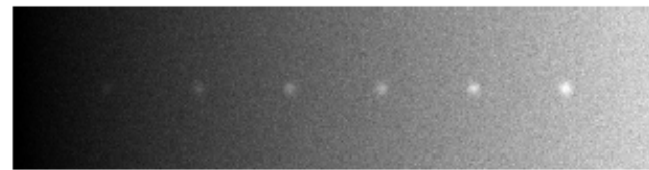
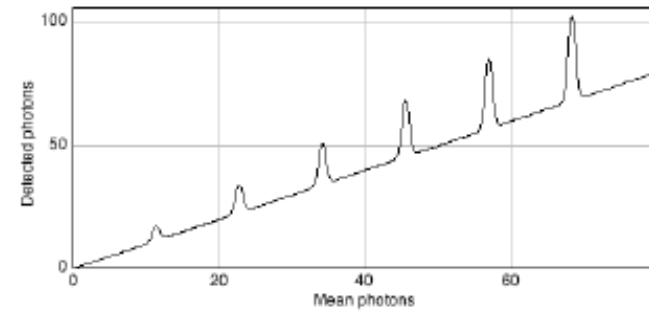
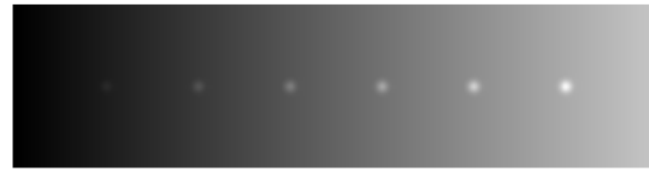
(c) Gaussian $\sigma = 5$



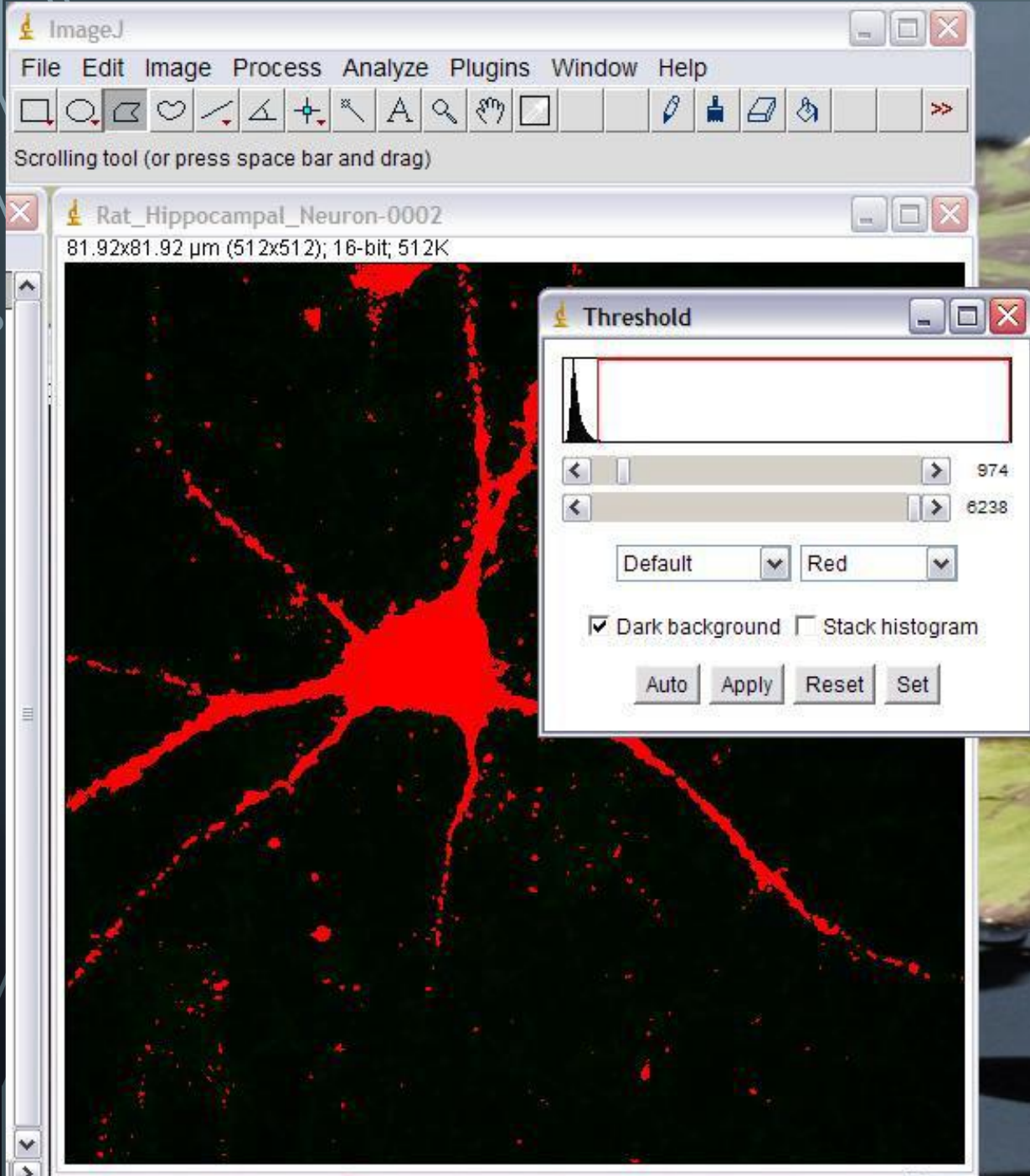
(d) Profile plots of the intensity in the red channel of the image



(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.”

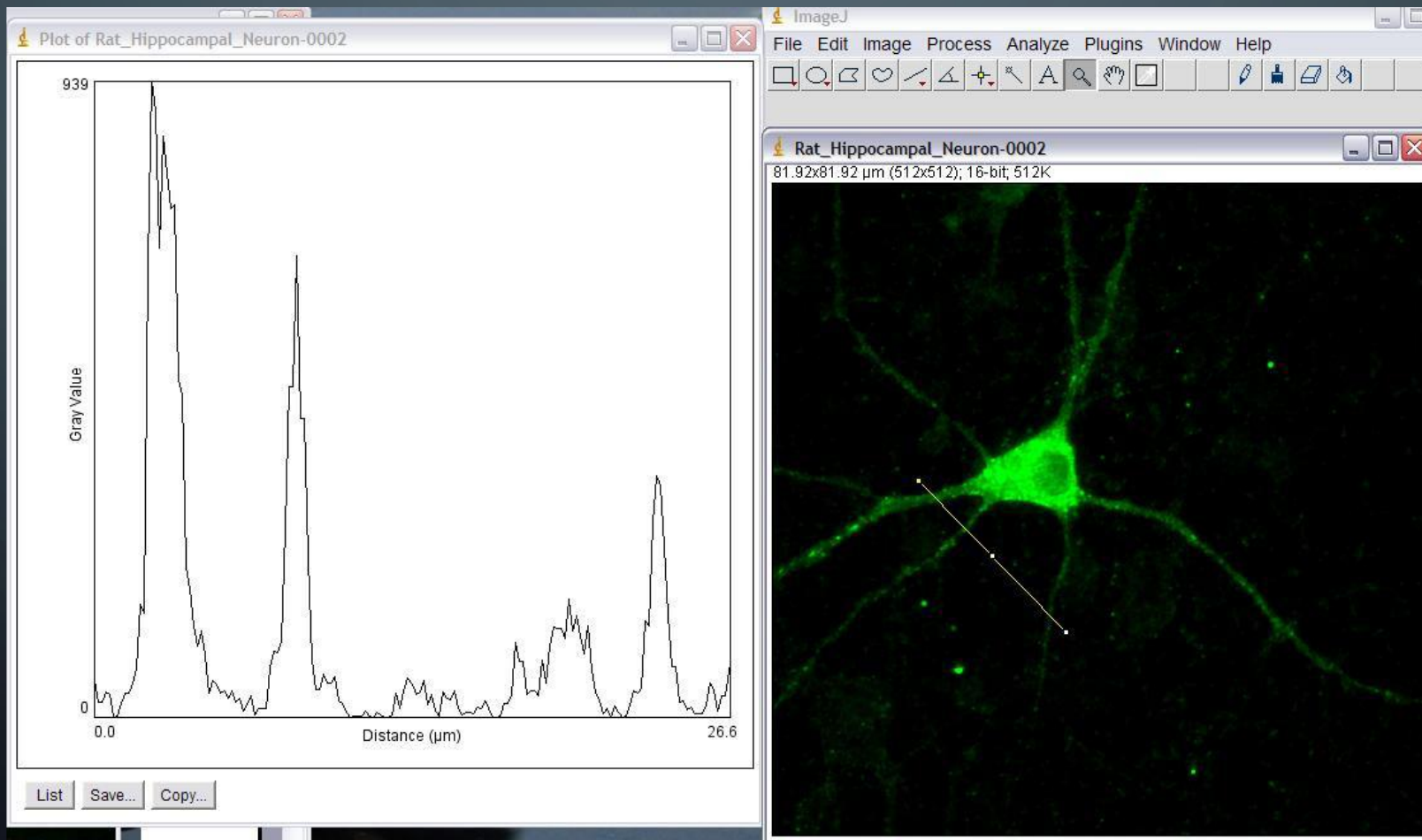
Then use
Image > Adjust > Threshold

to highlight the area you want to
analyze, and then

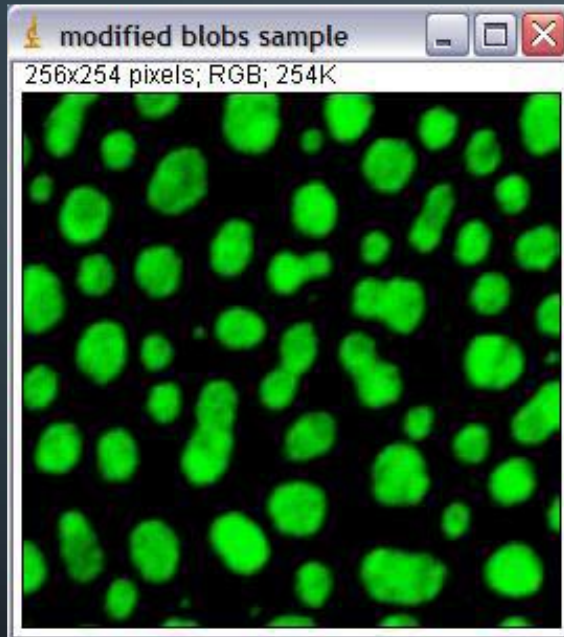
>
Analyze

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

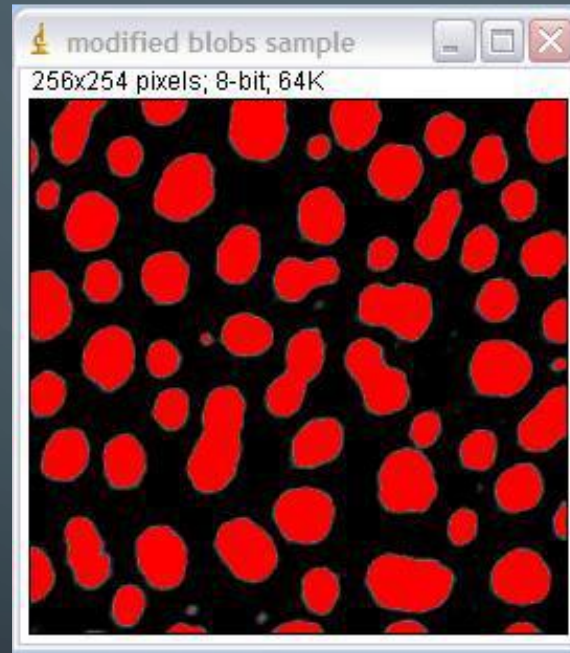
In the example below, the plot gives the intensity values along the line drawn across three cell processes.



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



convert to grayscale

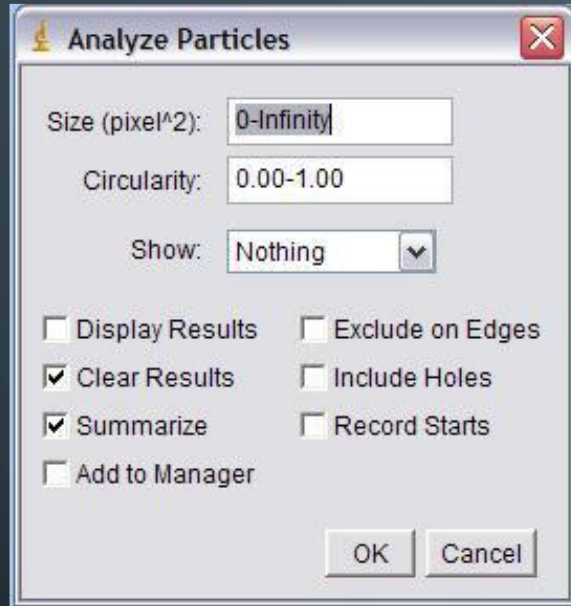


create a binary image,



Process > Binary > Watershed

Analyze > Analyze Particles



modified blobs original i...
256x254 pixels; 8-bit; 64K

modified blobs sample
256x254 pixels; 8-bit (inverting LUT); 64K

Summary

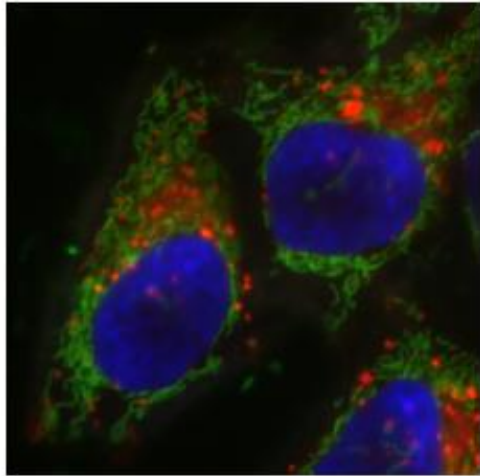
Slice	Count	Total Area	Average Size	Area
modified blobs sample	72	21286.00	295.64	32.7

Drawing of modified blob...
256x254 pixels; 8-bit; 64K

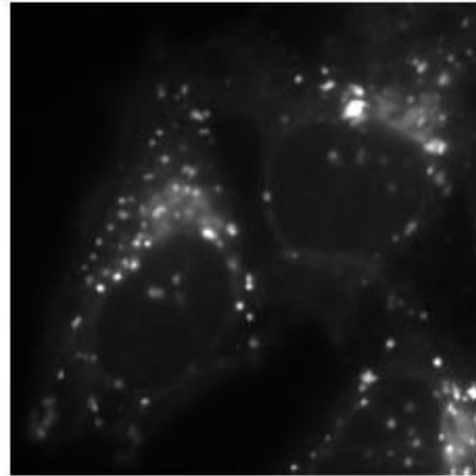
Results

	Area	Mean
1	71	216.76
2	181	196.73
3	646	227.85
4	426	233.20
5	465	235.85
6	330	212.84
7	273	225.50
8	74	175.35
9	264	192.43
10	221	210.10
11	25	156.68
12	485	210.86
13	639	186.82
14	92	179.97
15	216	215.79
16	432	229.84
17	139	187.53
18	503	215.34
19	234	204.85
20	407	219.49
21	257	223.14
22	345	211.14
23	149	205.60
24	399	207.22
25	294	228.37
26	100	206.66
27	245	198.83
28	494	220.19
29	272	210.67
30	187	216.55
31	454	225.26

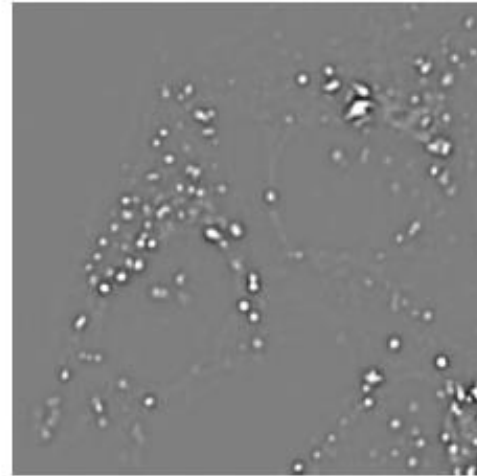
An simple image analysis workflow for detecting and measuring small spots, applied to the red channel of the sample image HeLa Cells.



(a) Original image



(b) Extract channel



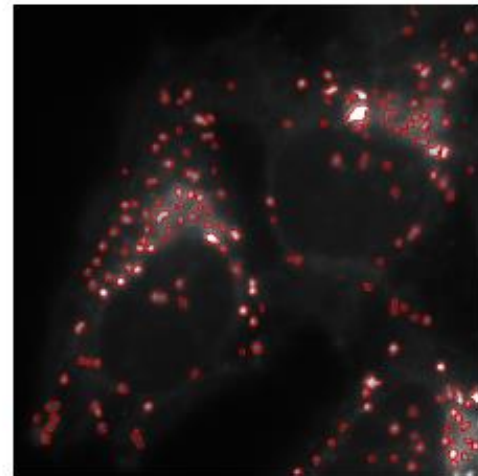
(c) Apply filters



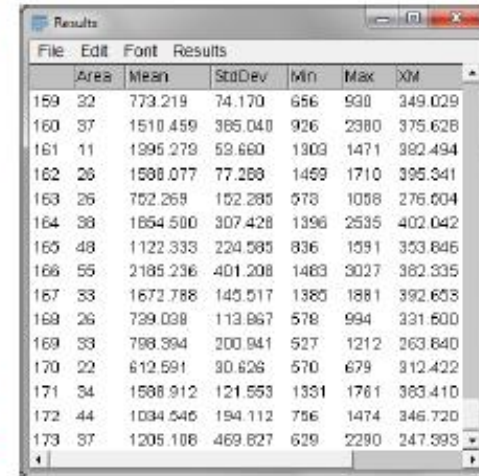
(d) Apply threshold



(e) Refine detection

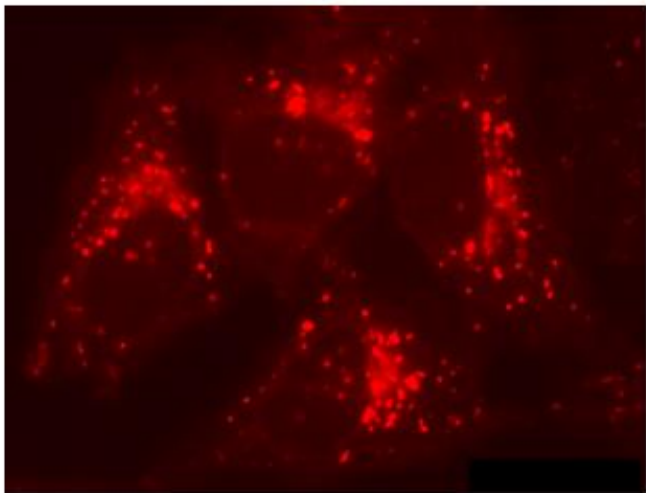


(f) Relate (e) to (b)



File	Edit	Font	Results			
Area	Mean	StdDev	Min	Max	XM	
159	32	773.219	74.170	656	930	349.029
160	37	1510.459	365.040	926	2380	375.628
161	11	1395.279	59.660	1303	1471	382.494
162	26	1588.077	77.268	1459	1710	395.341
163	26	762.269	152.285	578	1058	276.604
164	38	1654.500	307.428	1396	2535	402.042
165	48	1122.333	224.585	836	1591	353.846
166	55	2185.236	401.208	1483	3027	362.335
167	33	1672.788	145.517	1380	1881	392.653
168	26	729.038	113.867	578	994	331.600
169	33	798.394	200.941	527	1212	263.840
170	22	612.691	90.626	570	679	312.422
171	34	1588.912	121.553	1331	1761	383.410
172	44	1034.546	194.112	796	1474	346.720
173	37	1205.108	469.627	629	2290	247.993

(g) Measure



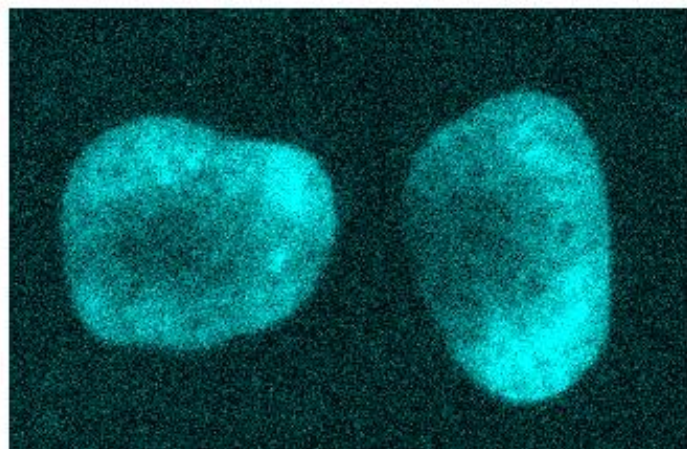
(a) Image



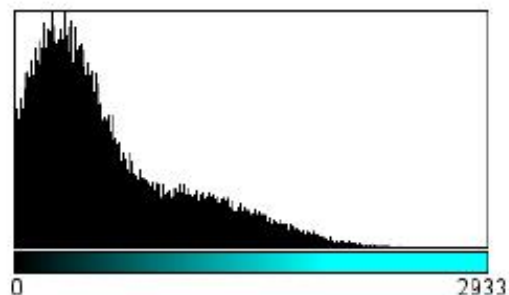
(b) Low threshold



(c) High threshold

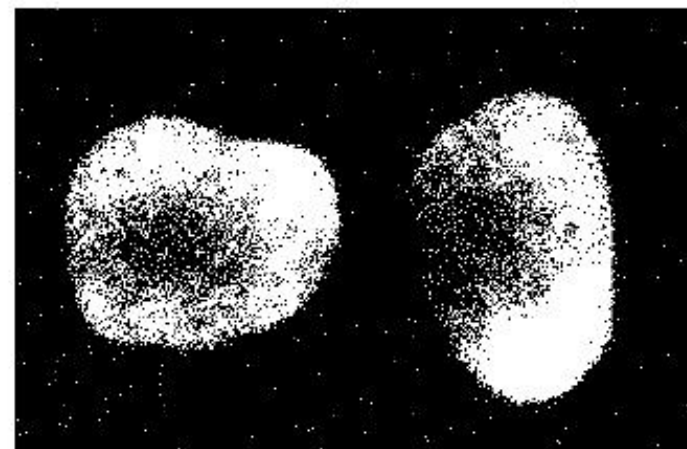


(a) Noisy image

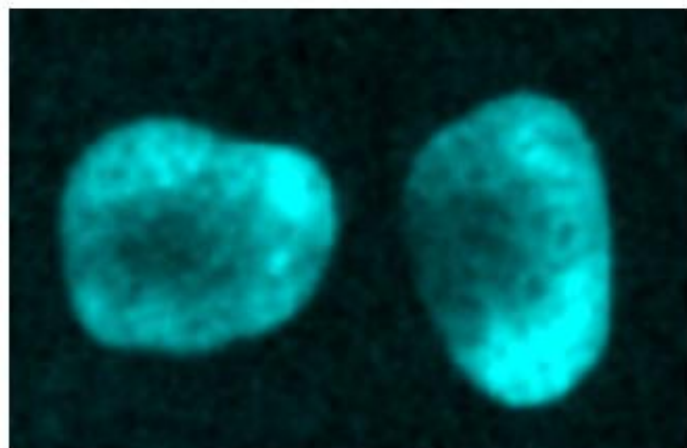


Count: 75702
Mean: 601.936
StdDev: 483.337
Bins: 256
Min: 0
Max: 2933
Mode: 236 (1164)
Bin Width: 11.457

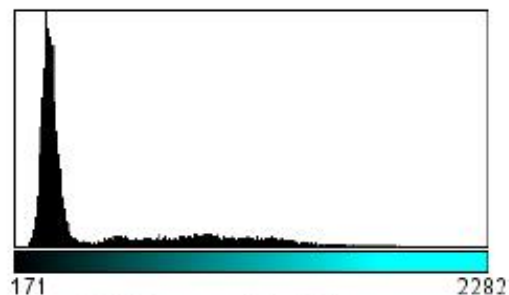
(b) Histogram of (a)



(c) Threshold applied to (a)



(d) Gaussian filtered image

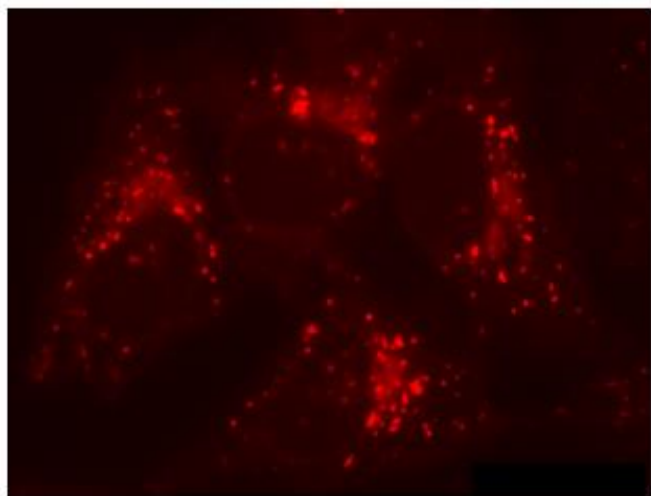


Count: 75702
Mean: 601.913
StdDev: 425.297
Bins: 256
Min: 171
Max: 2282
Mode: 318 (4747)
Bin Width: 8.246

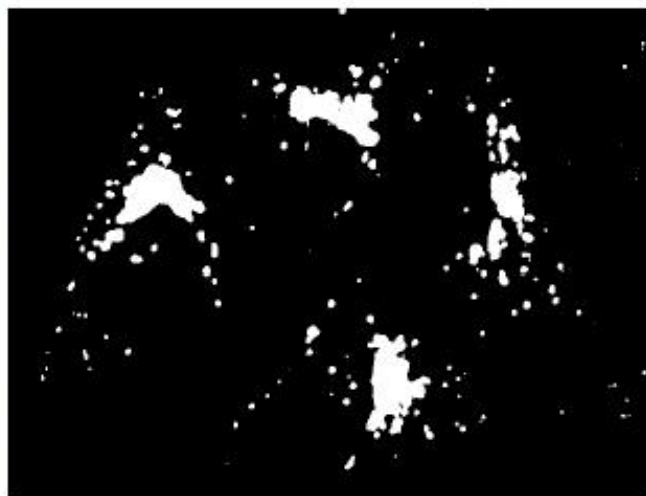
(e) Histogram of (d)



(f) Threshold applied to (d)



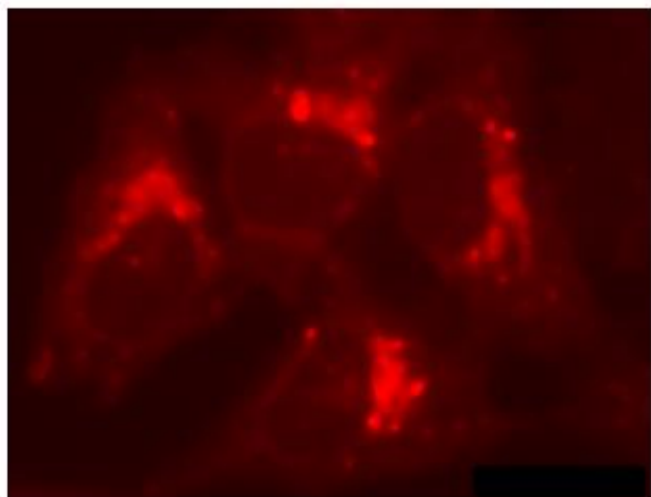
(a) Original image



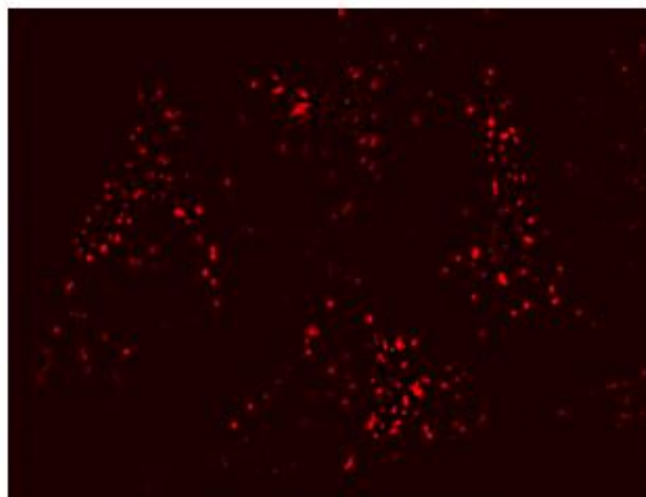
(b) Otsu's threshold



(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>

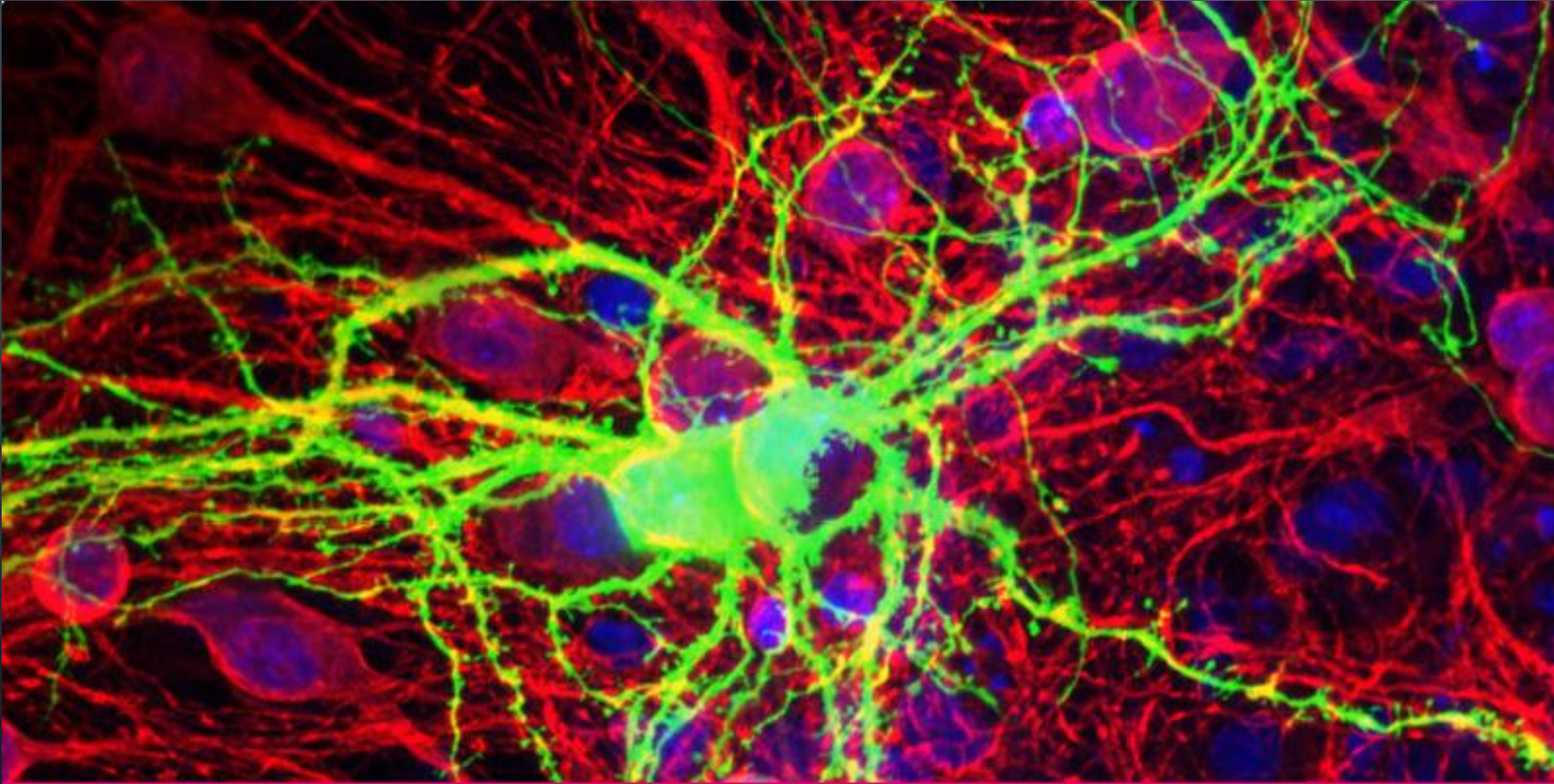


Short introduction to histogram processing

https://youtu.be/nIRhHb04u_k



Question?



Thanks for your attention!

Gabriele Baj
gbaj@units.it