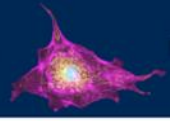
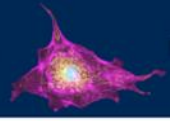


Introduction to image processing and analysis with ImageJ / Fiji. Part 5

Automating Image Analysis

Course by Dale Moulding



Session 5

3 hours
1 hour lecture
2 h for demos & exercises

Learning objectives:

- Correct background for better analysis
- Develop multiple strategies for image analysis
- **Use filters to pre-process images**
- **Cell counting.** Manually and **automatic**
- **Thresholding to generate binary images and masks**
- **Identifying double / triple stained cells**
- Explain the difference between colocalization and co-expression
- Track moving objects

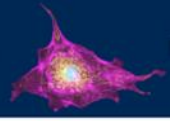


Image segmentation, automatic counting and measurements

Automatic image analysis has benefits and pitfalls..

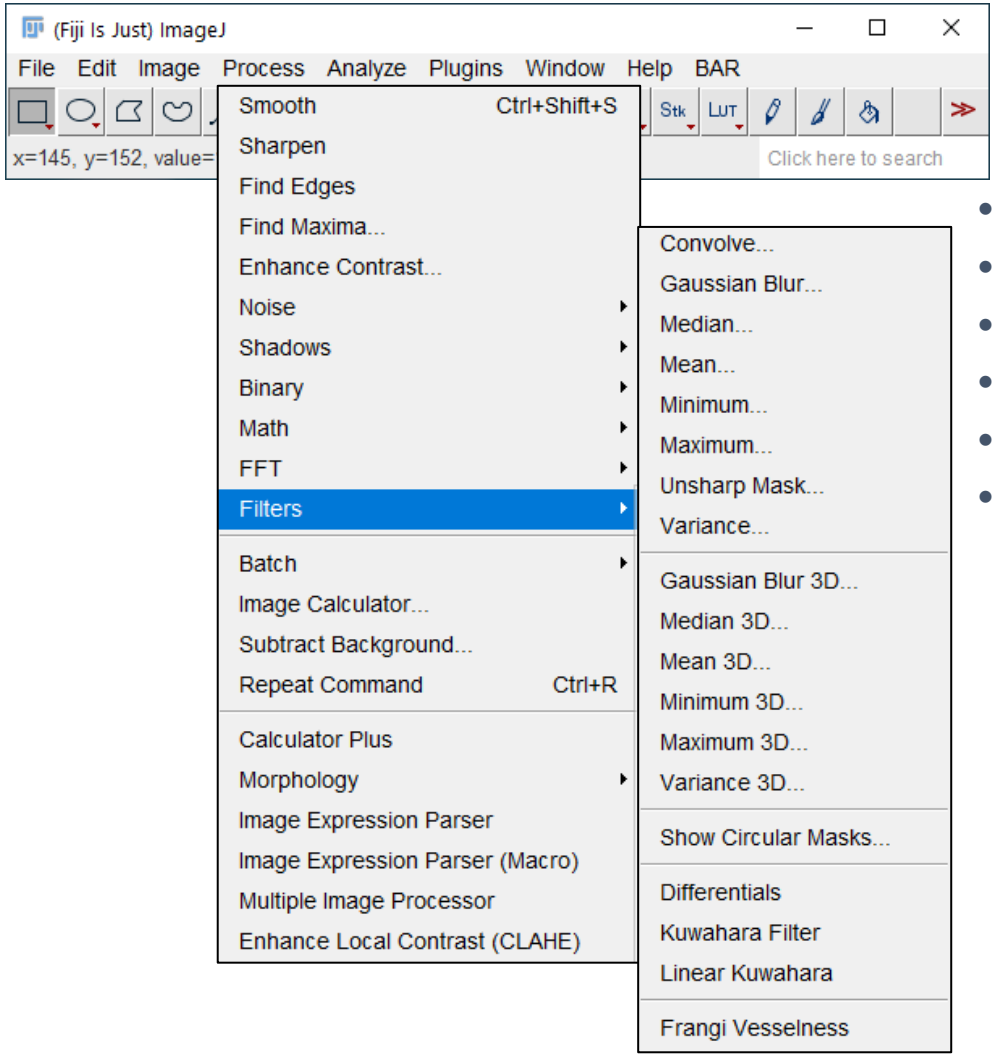
Pros:

- Quick (once methodology is set up)
- Unbiased (computers don't care which sample is which)
- n = lots !

Cons:

- Never 100% accurate (but n = lots !)
- Set up can be time consuming (but then n = lots !)
- Mistakes can be missed (make sure to validate results)

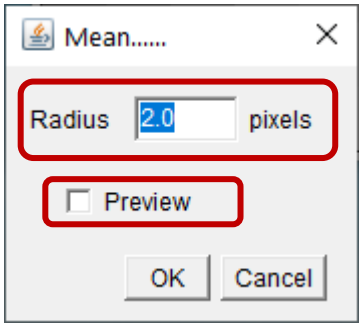
Spatial filters



Process / Filters >

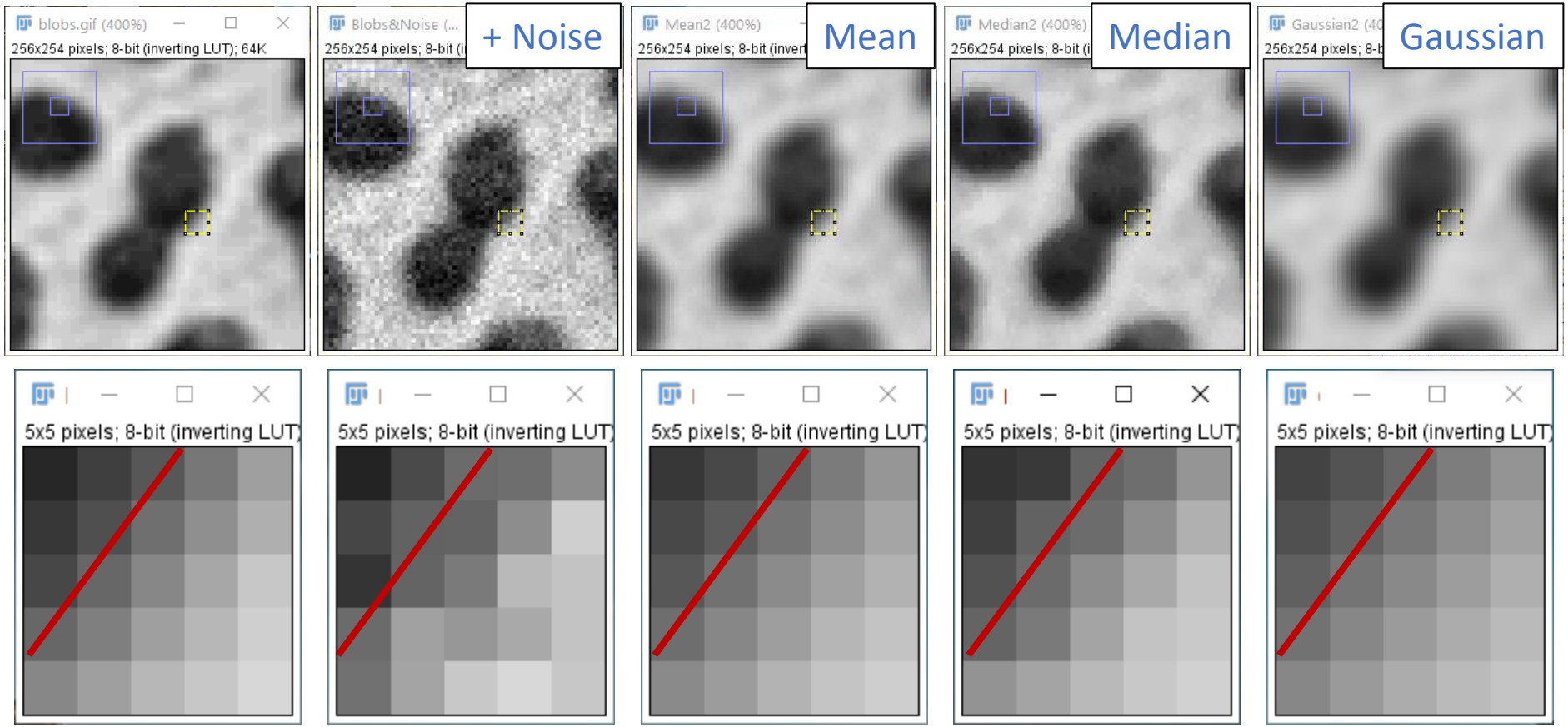
Common filters (2D & 3D):

- Gaussian (fast smoothing)
- Median (slow, keeps edges)
- Mean (fast smoothing)
- Min / Max (~ dims / brightens)
- Unsharp mask (~ enhances edges & noise)
- Variance (~ shows image gradients)

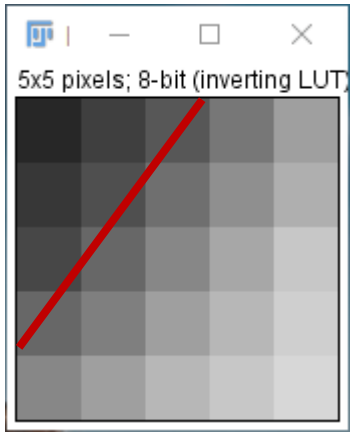


Typically work over a defined distance from each pixel & can be previewed

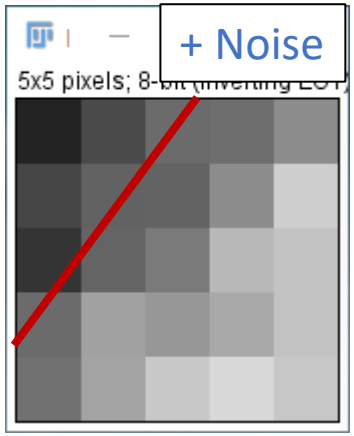
Spatial filters – reduce image noise



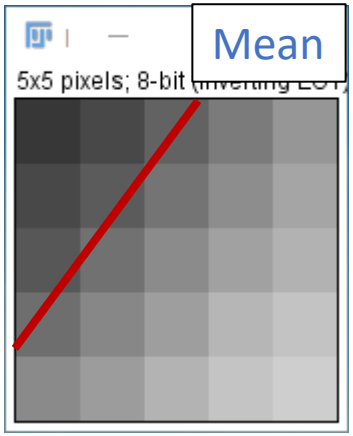
Spatial filters - Mean



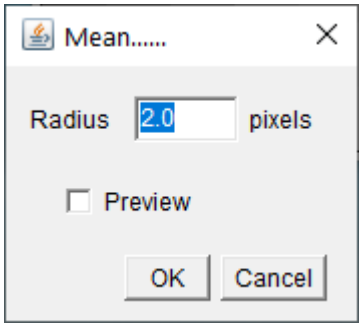
216	192	168	136	96
200	176	144	112	80
184	152	120	88	56
152	128	96	72	48
120	96	72	56	40



220	181	148	145	115
185	157	156	114	48
203	155	133	70	60
148	94	104	86	60
142	91	55	39	56



200	183	157	132	105
183	164	139	114	90
168	142	116	94	77
145	120	97	73	60
117	99	76	59	49



Mean… Radius 2

Changes each pixel's value to the mean value of the surrounding pixels in a 3x3 grid.

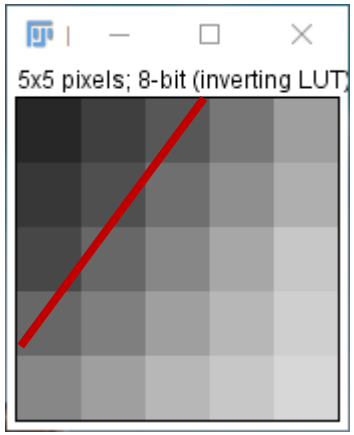
Result:

Less noise

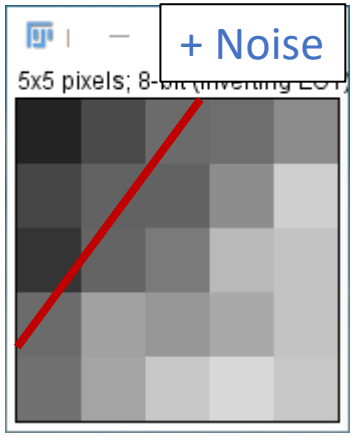
Edges slightly blurred

(depending on radius used)

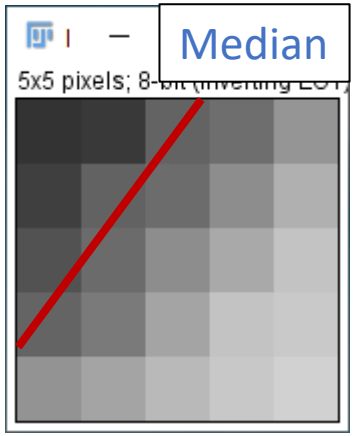
Spatial filters - Median



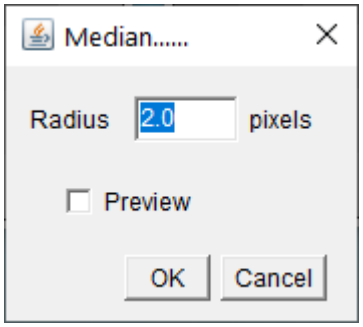
216	192	168	136	96
200	176	144	112	80
184	152	120	88	56
152	128	96	72	48
120	96	72	56	40



220	181	148	145	115
185	157	156	114	48
203	155	133	70	60
148	94	104	86	60
142	91	55	39	56



204	198	156	145	106
192	157	147	114	79
173	148	114	86	60
155	133	91	60	54
108	91	70	55	46



Median… Radius 2

Changes each pixel's value to the median (middle) value of the surrounding pixels in a 3x3 grid.

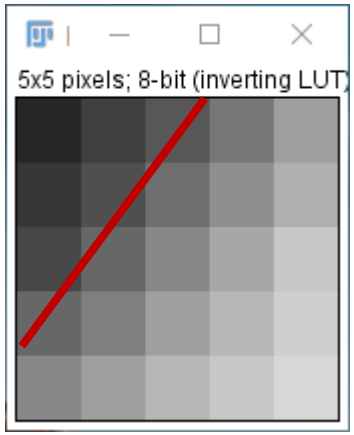
Result:

Less noise.

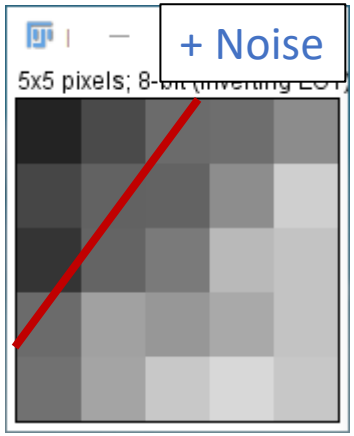
Edges remain defined.

Corners may be lost.

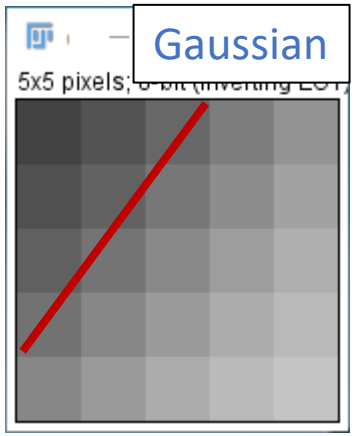
Spatial filters - Gaussian



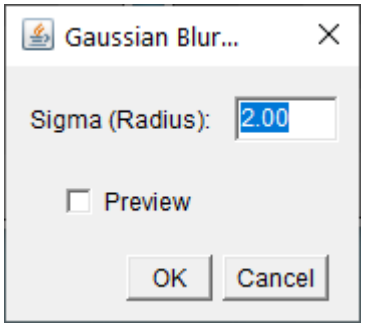
216	192	168	136	96
200	176	144	112	80
184	152	120	88	56
152	128	96	72	48
120	96	72	56	40

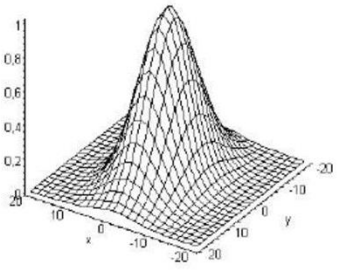


220	181	148	145	115
185	157	156	114	48
203	155	133	70	60
148	94	104	86	60
142	91	55	39	56



188	172	152	130	107
175	157	136	114	93
159	139	118	98	80
141	120	100	83	69
120	101	83	69	59





Gaussian… Radius 2

Changes each pixel's value to the median (middle) value of the surrounding pixels in a 3x3 grid.

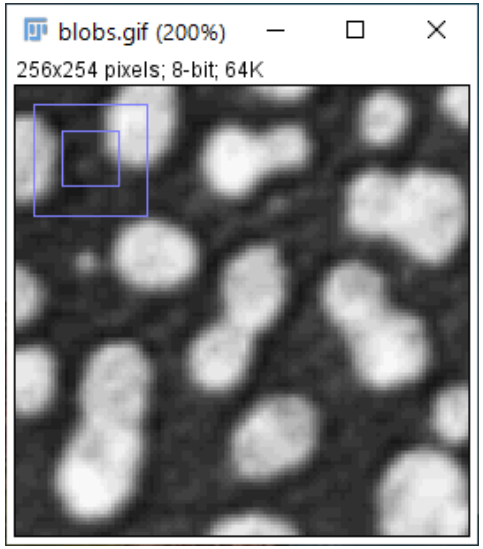
Result:

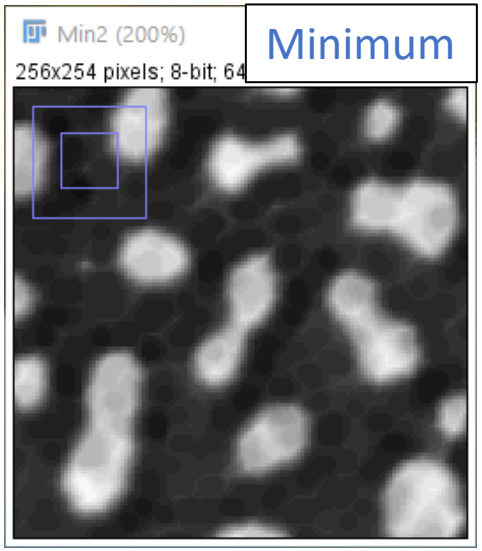
Less noise.

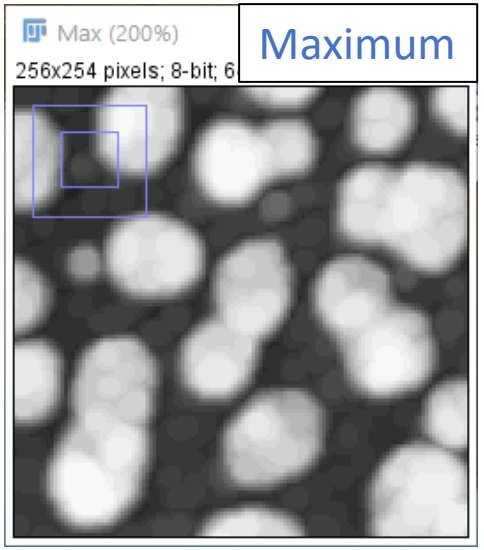
Edges blurred.

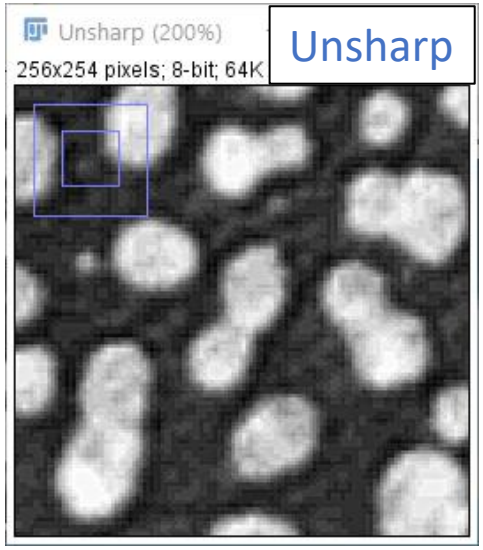
Objects come to a peak.

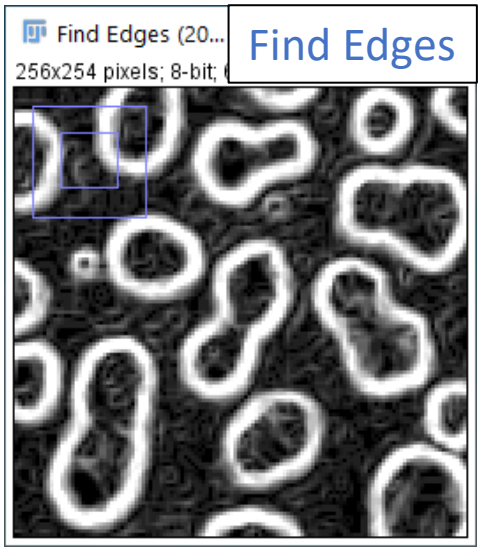
Spatial filters - others

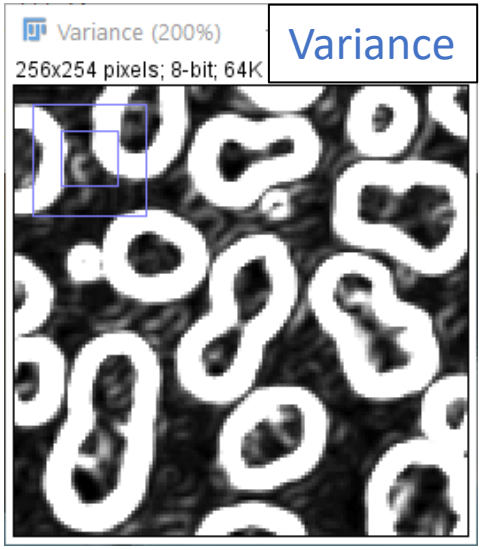






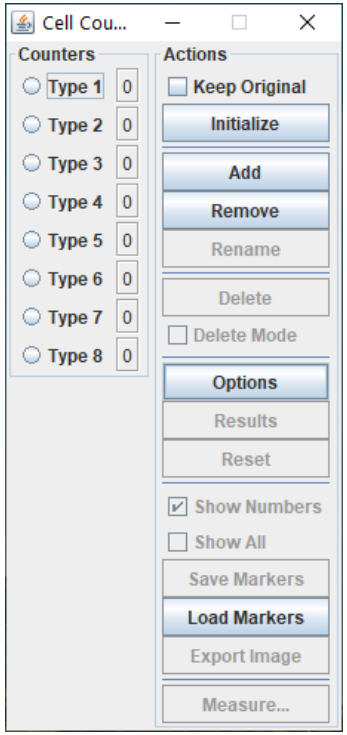


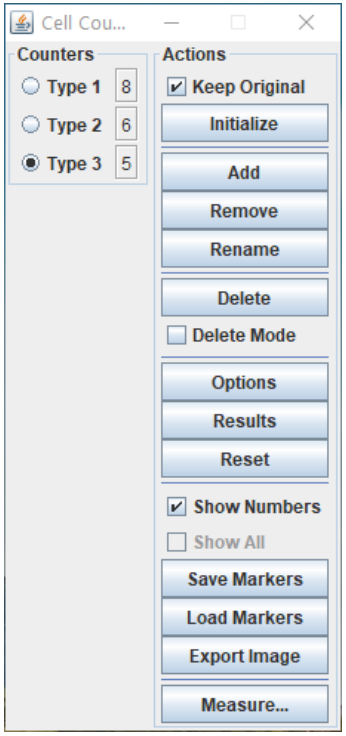




Manual Counting

Plugins / Analyze / Cell Counter





Add / remove Types

Rename types

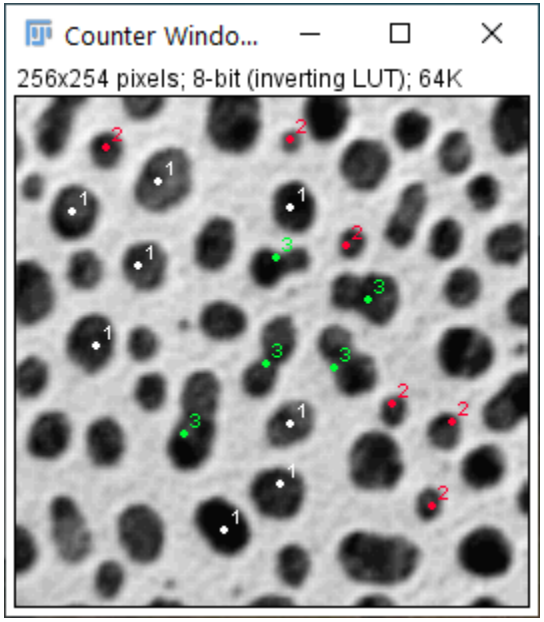
Delate last count /
Delete mode (click to delete)

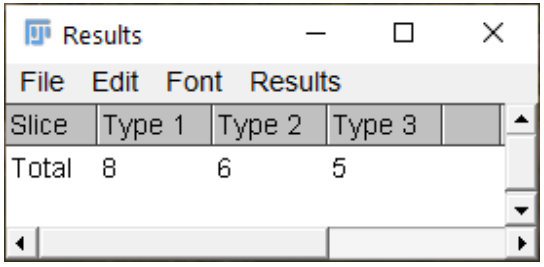
Change counter colours

Results

Save / Load

Measure… more results
(location & intensity)

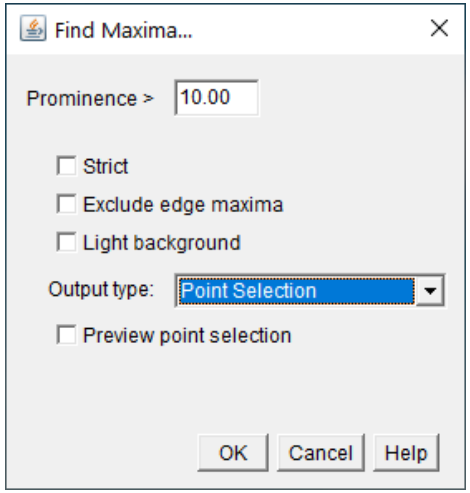


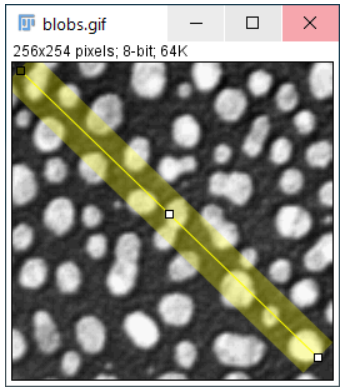


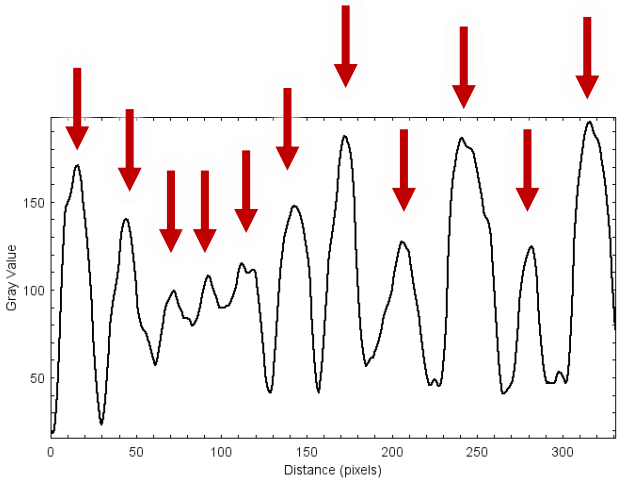
Slow. Accurate? No double counting. Bias?

Automatic counting – Find Maxima

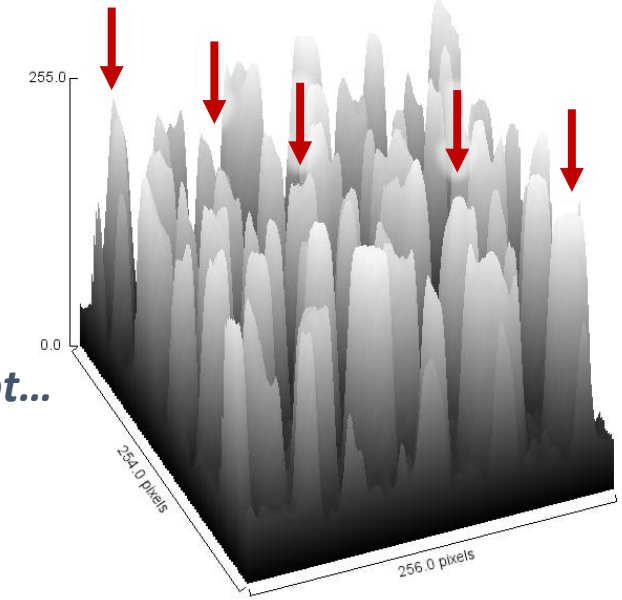
Process / Find Maxima…





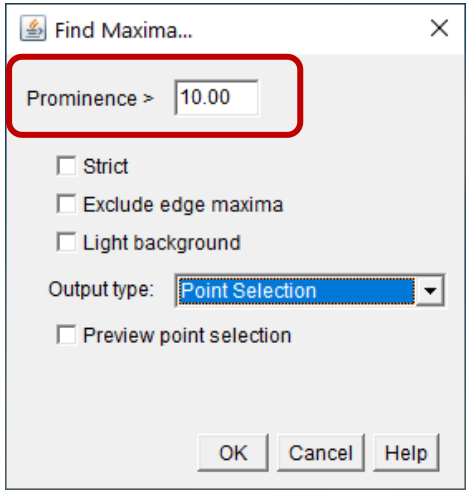


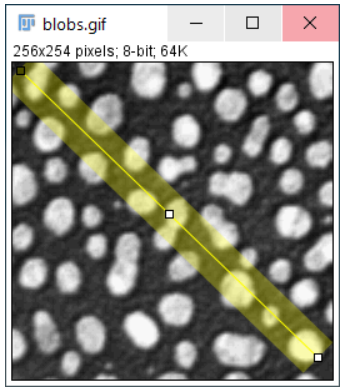
Analyze / Surface Plot…

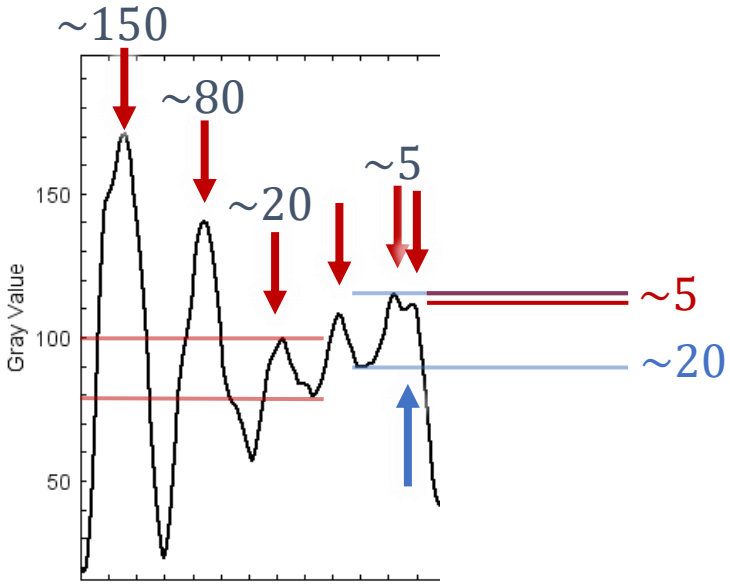


Automatic counting – Find Maxima

Process / Find Maxima…







Prominence: How bright does a maxima need to be?

Prominence = 20 would give 5 peaks on the graph

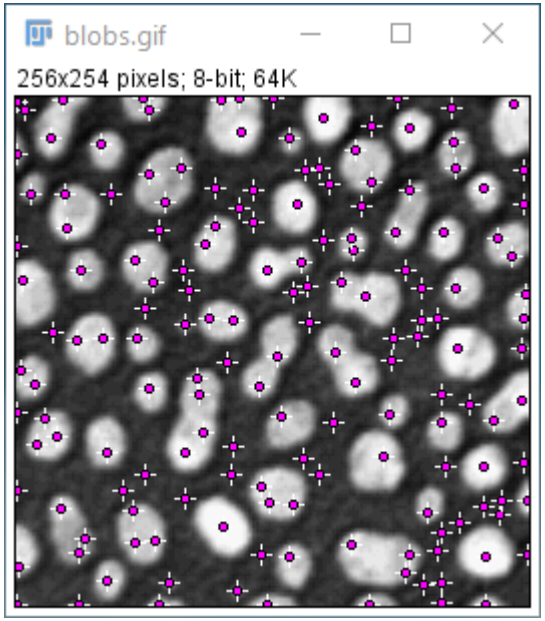
Prominence = 5 would give 6 peaks

Prominence = 80 would give 2 peaks

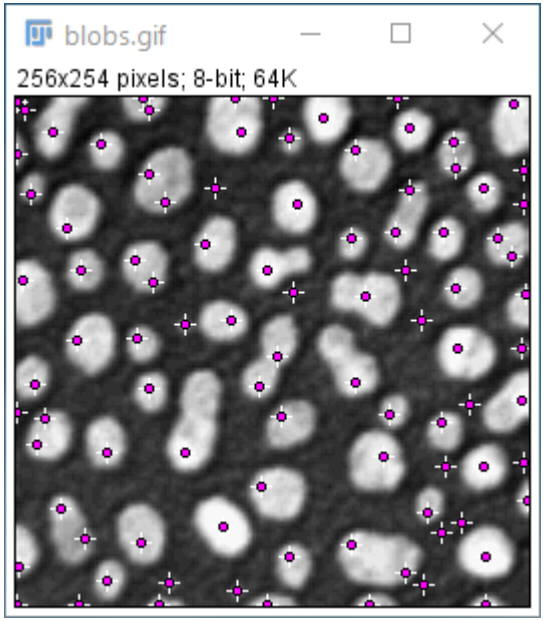
Automatic counting – Find Maxima

Process / Find Maxima…

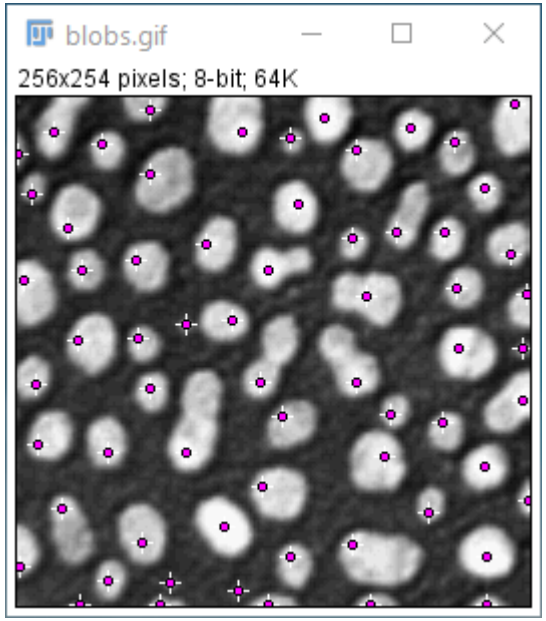
Prominence = 10



Prominence = 20



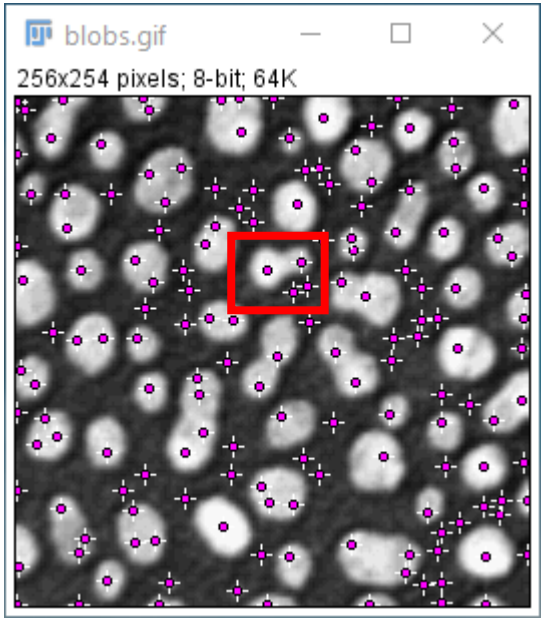
Prominence = 50

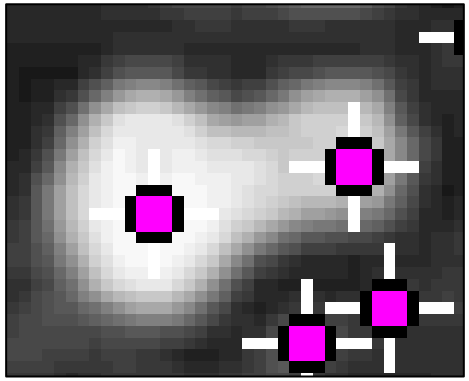


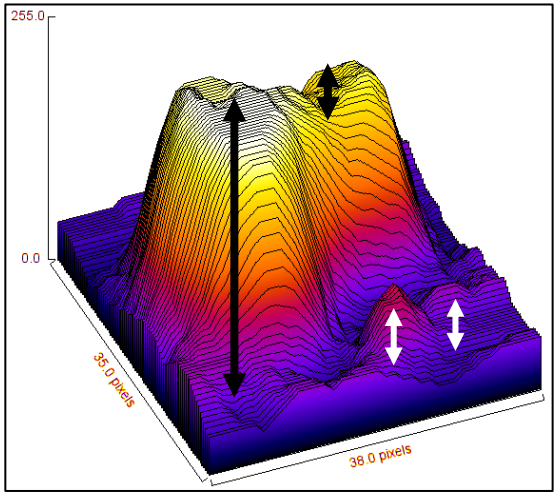
Automatic counting – Find Maxima

Process / Find Maxima…

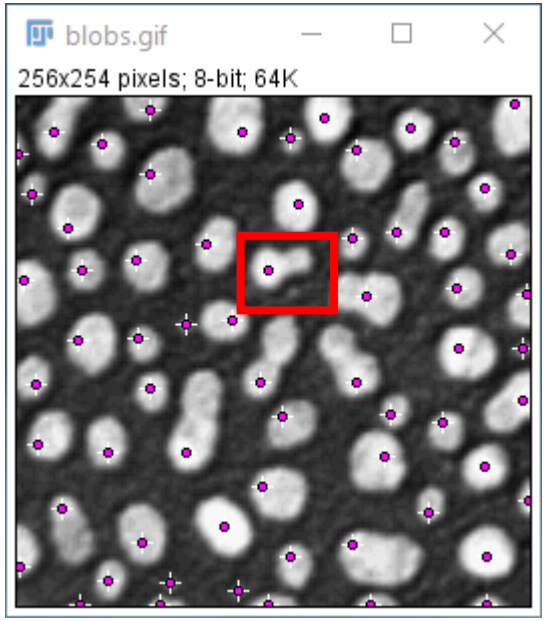
Prominence = 10







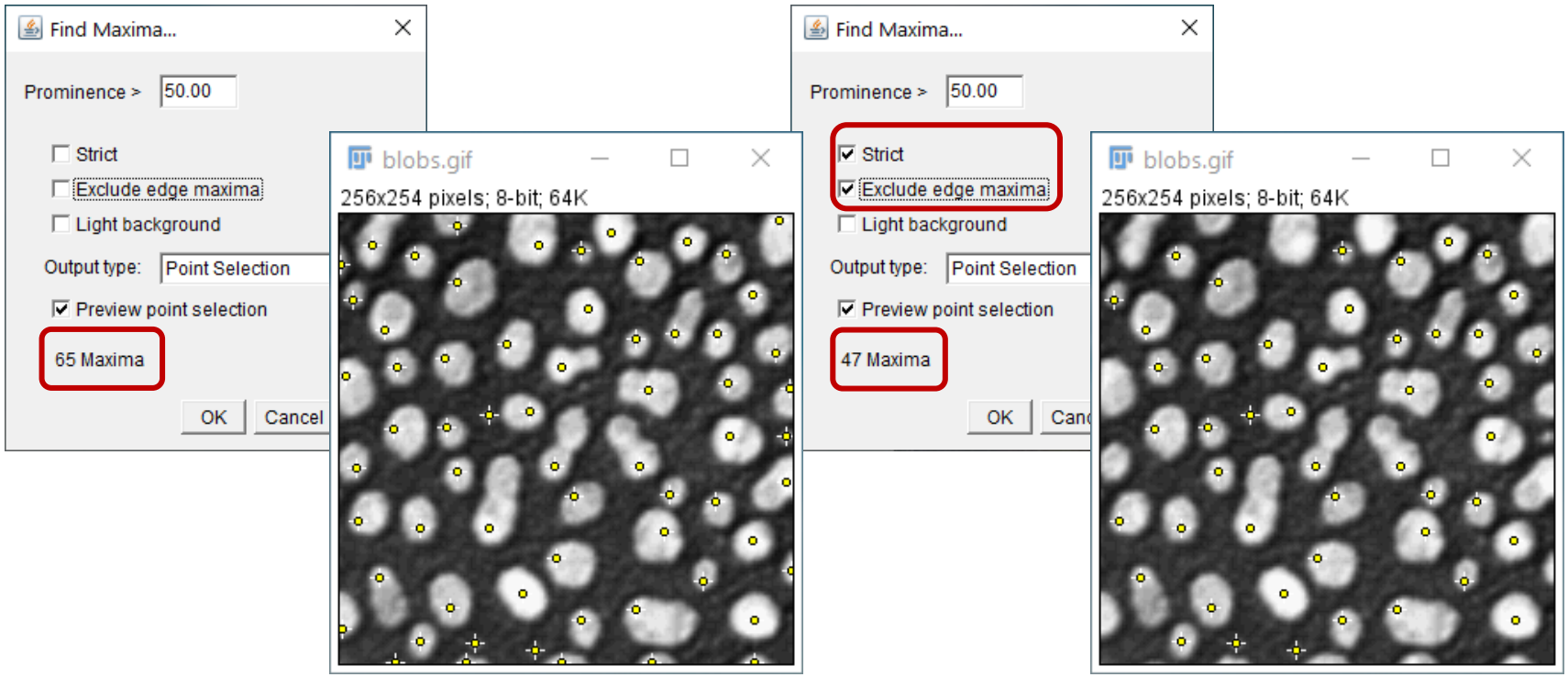
Prominence = 50



Analyze / Surface Plot…

Automatic counting – Find Maxima

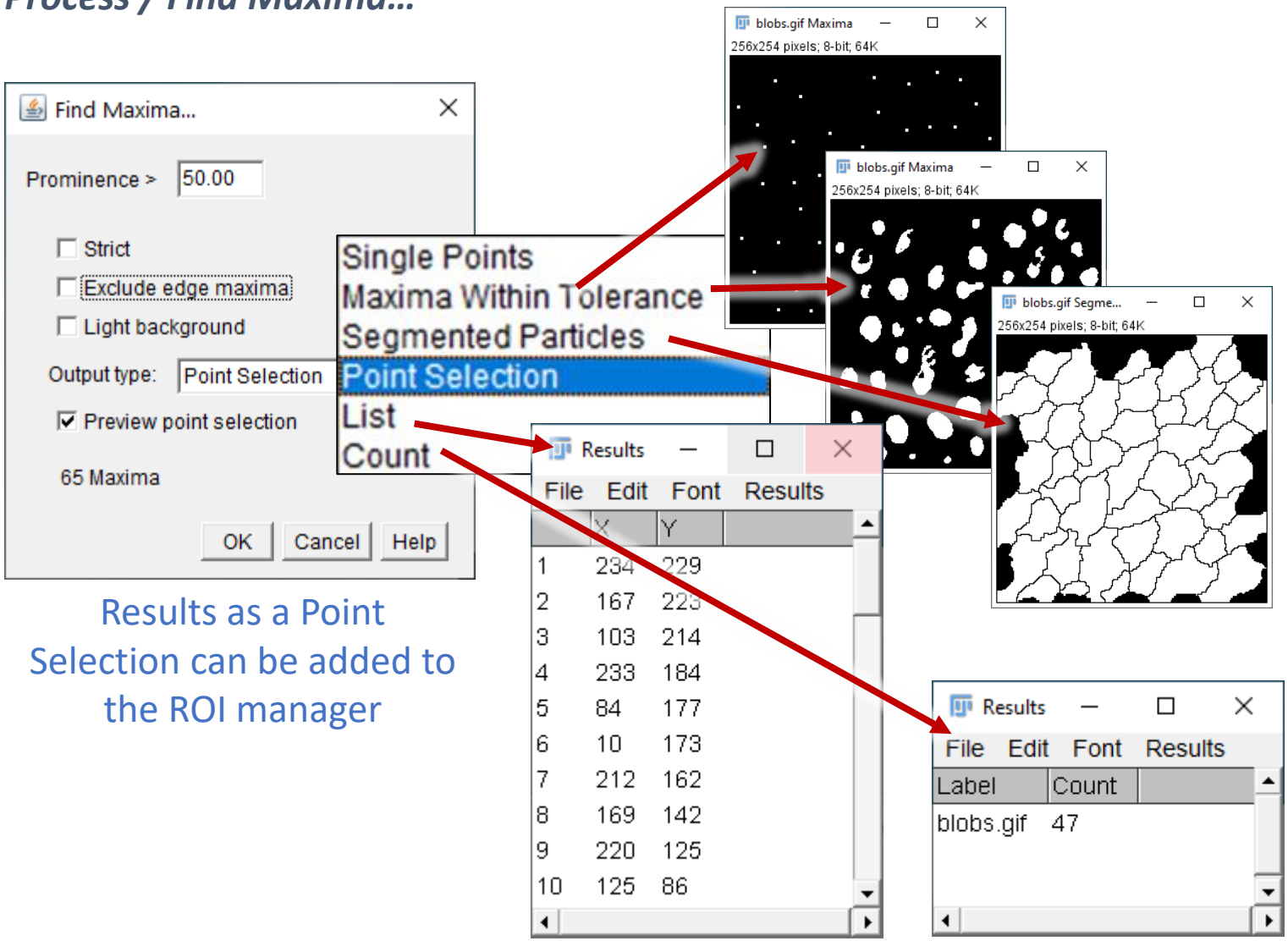
Process / Find Maxima…



Results as a Point Selection can be added to the ROI manager

Automatic counting – Find Maxima

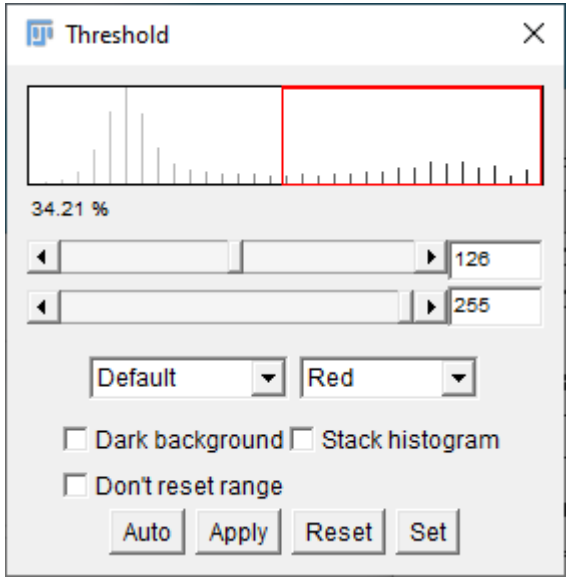
Process / Find Maxima…



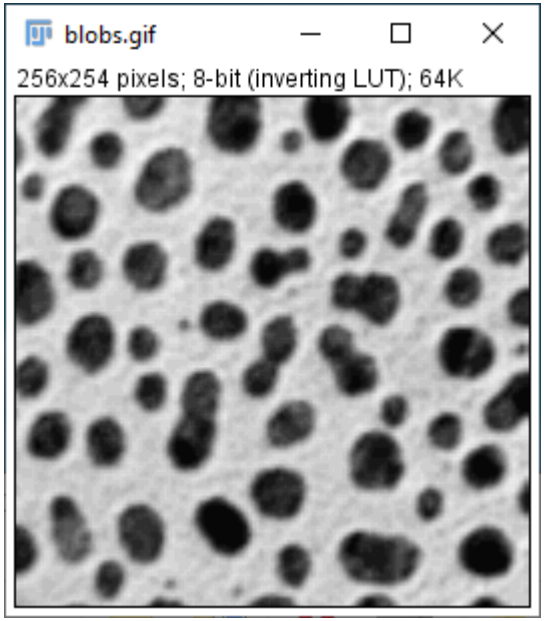
Results as a Point Selection can be added to the ROI manager

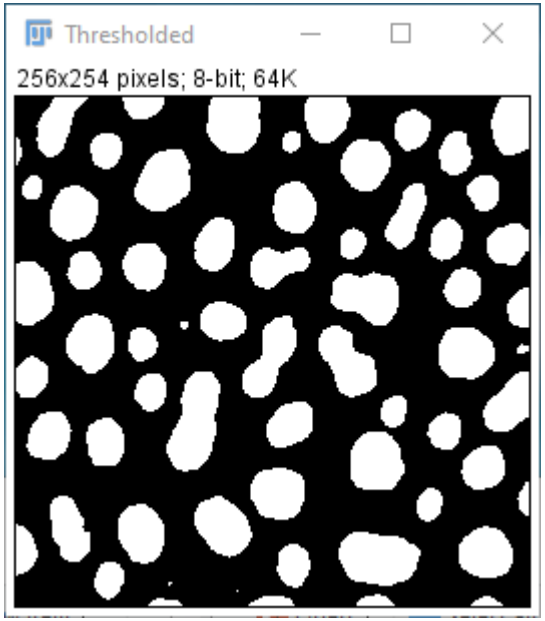
Automatic counting – Thresholding

Image / Adjust > Threshold…



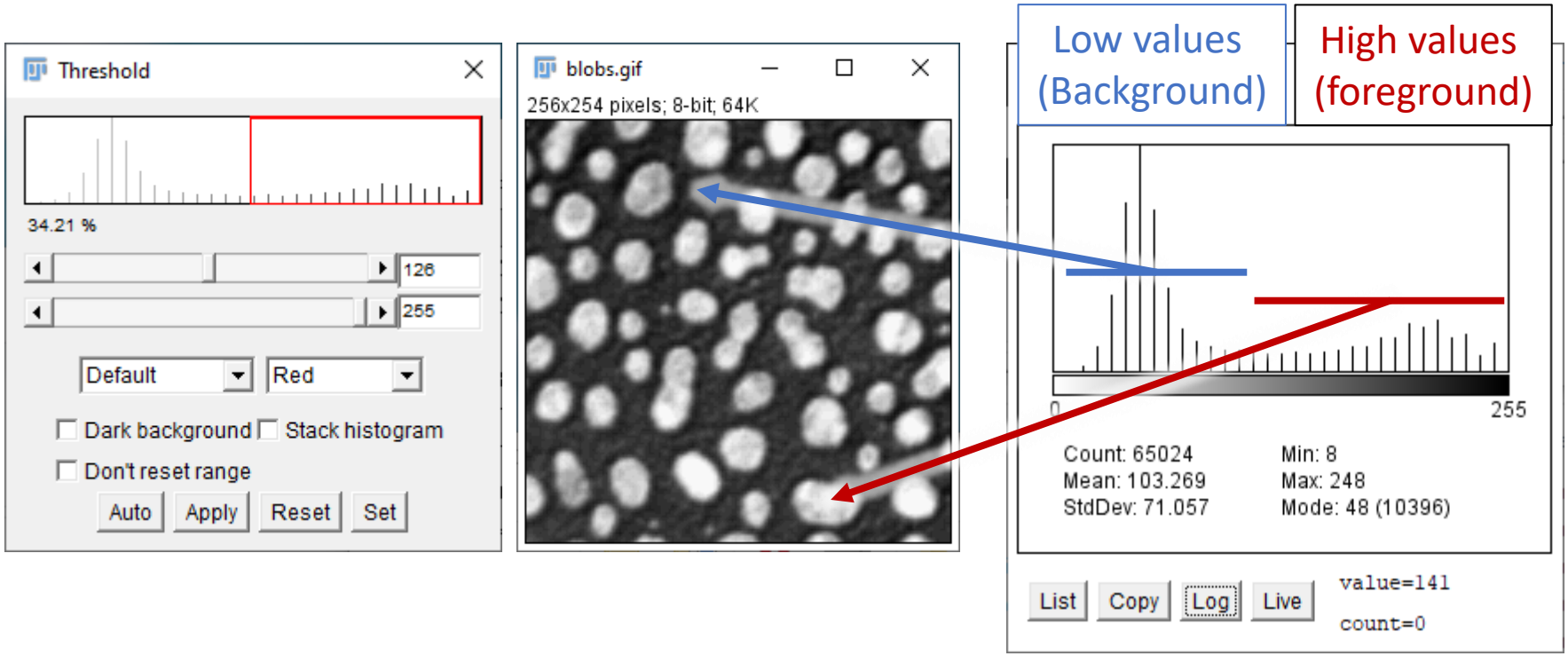
- Thresholding is a key step in many image analysis workflows.
- Make a binary (2 colour) mask of your image





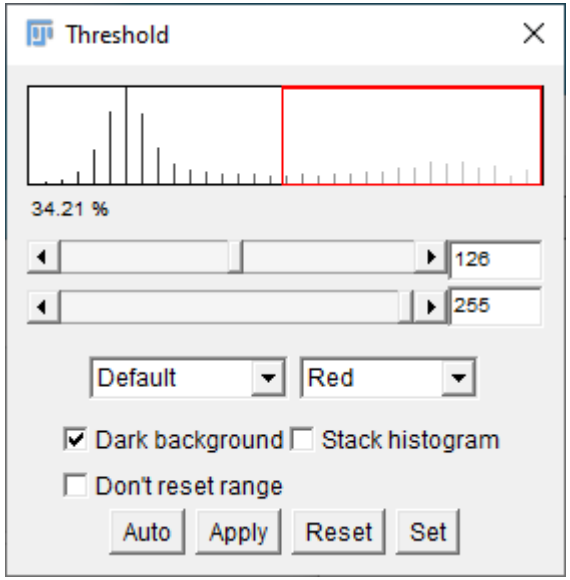
Automatic counting – Thresholding

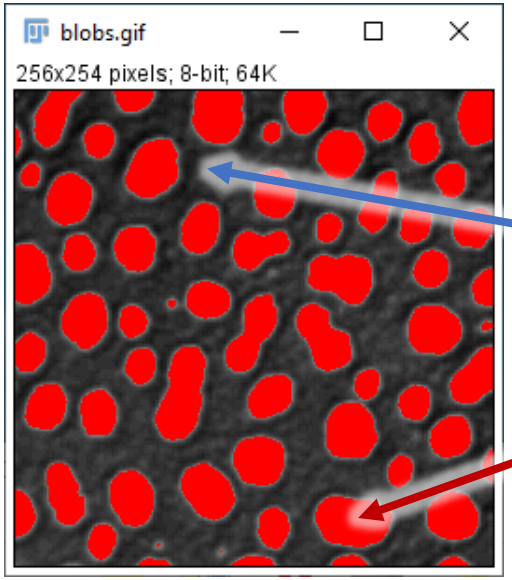
Image / Adjust > Threshold…

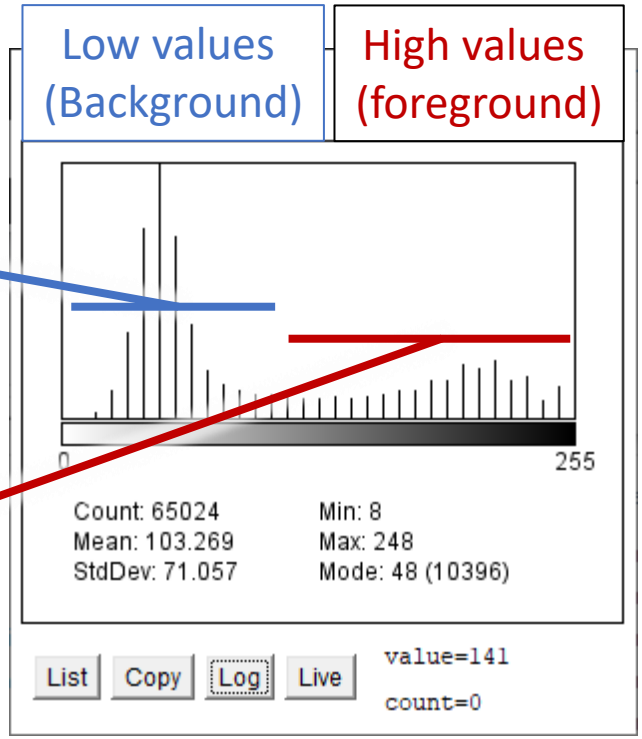


Automatic counting – Thresholding

Image / Adjust > Threshold…

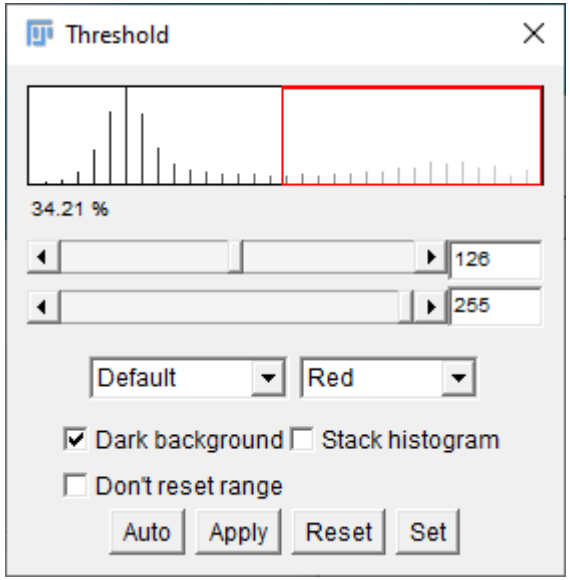


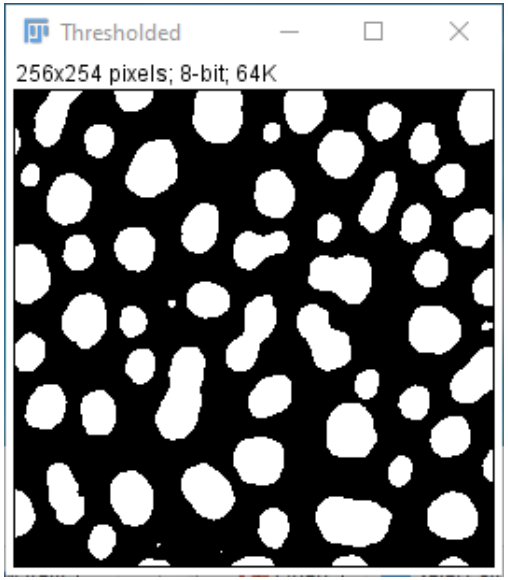




Automatic counting – Thresholding

Image / Adjust > Threshold…

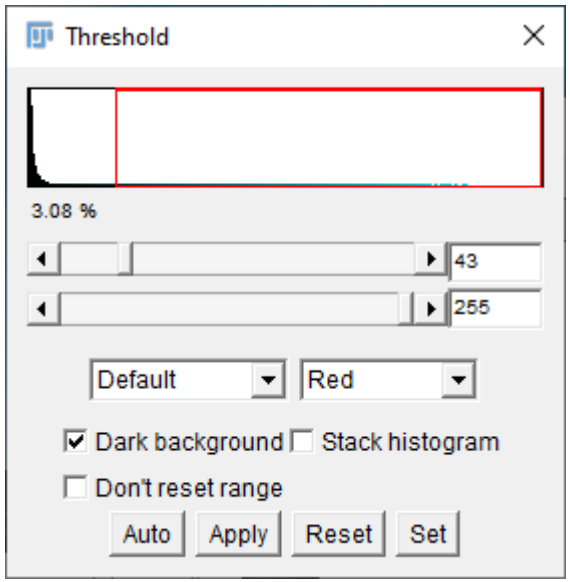


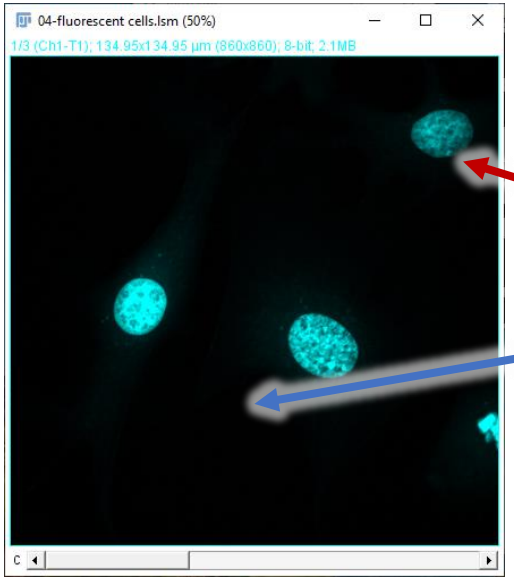


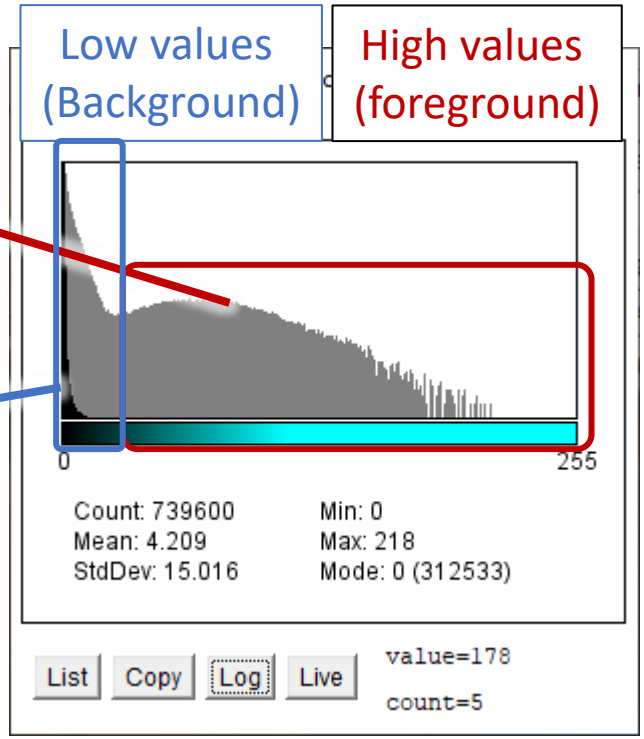
Binary (2 colour) mask

Automatic counting – Thresholding

Image / Adjust > Threshold…

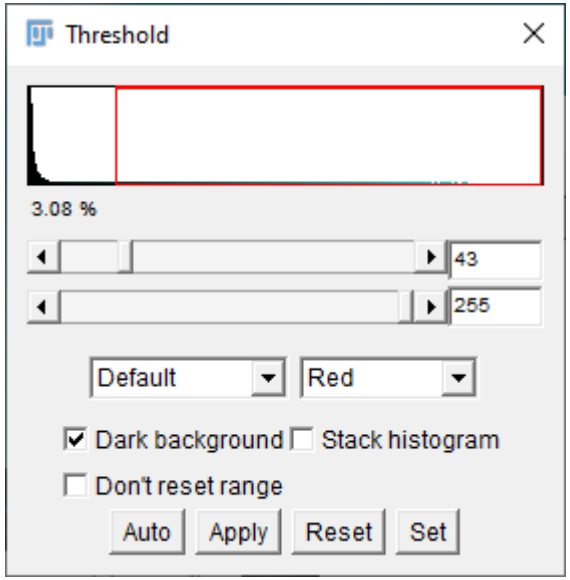




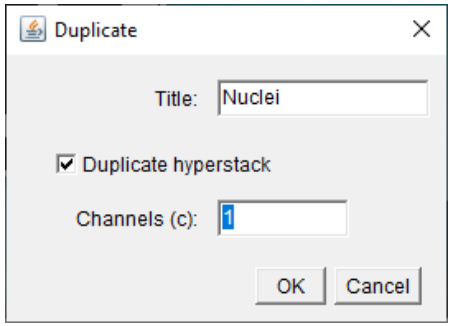


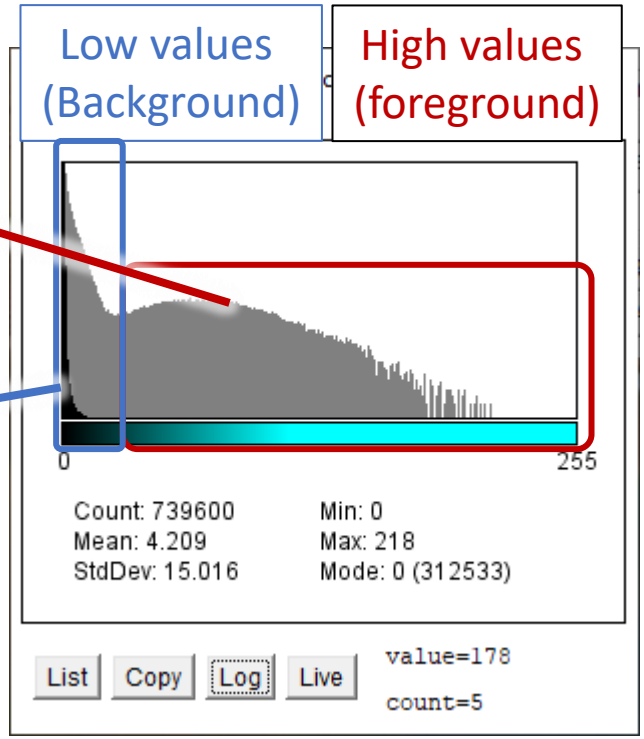
Automatic counting – Thresholding

Image / Adjust > Threshold…



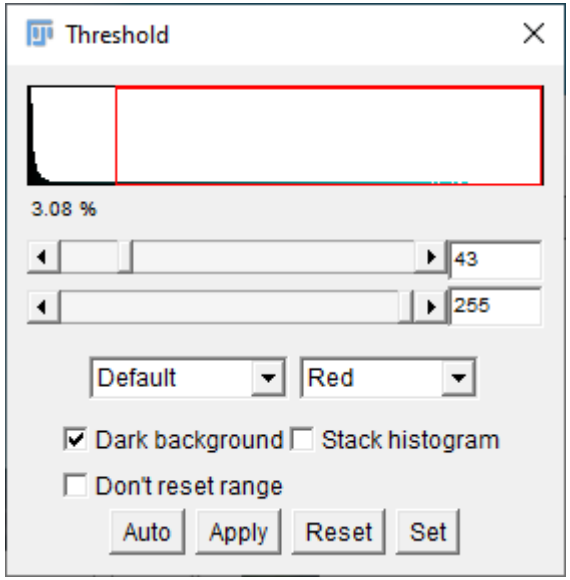
Must be a single channel image

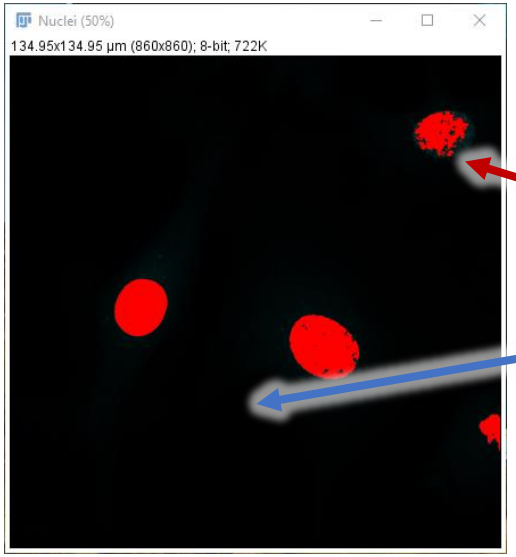


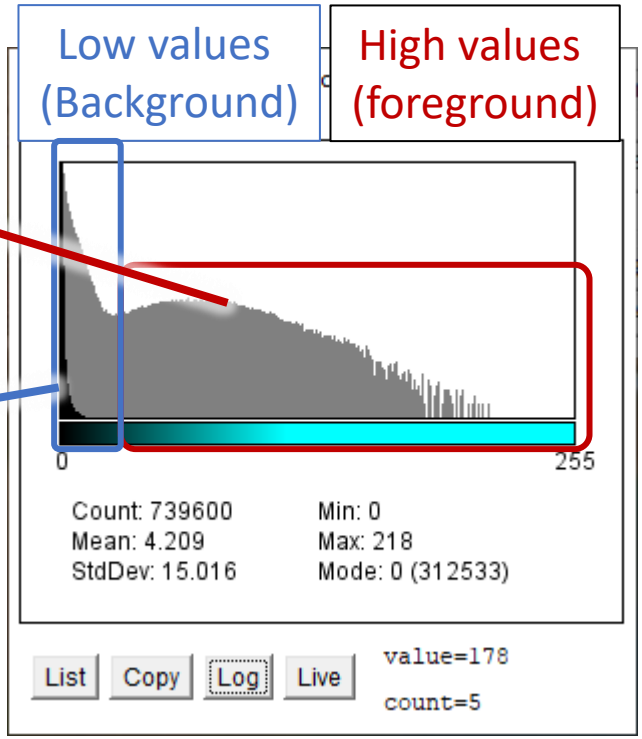


Automatic counting – Thresholding

Image / Adjust > Threshold…

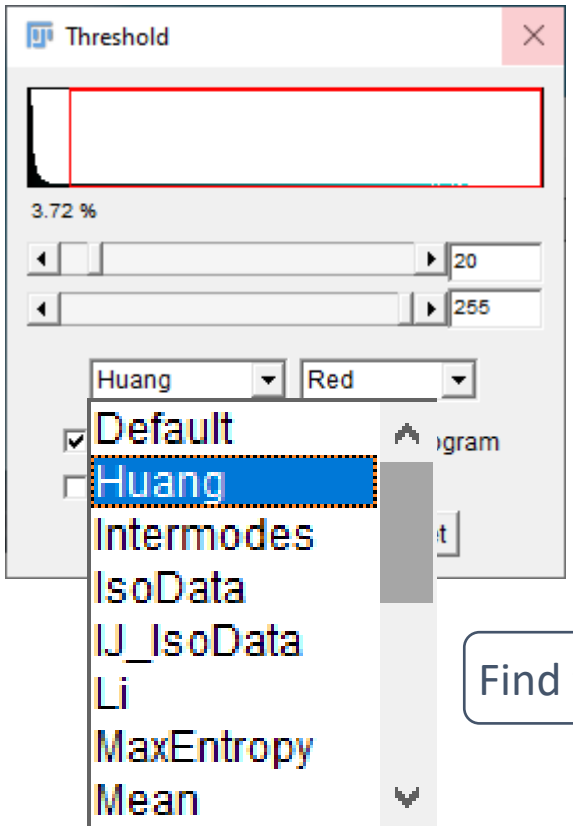


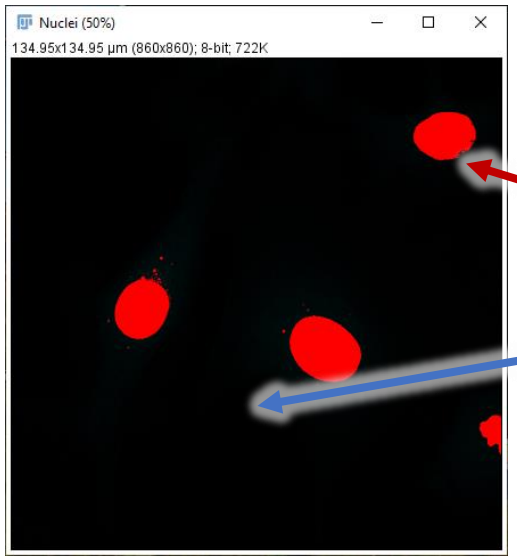


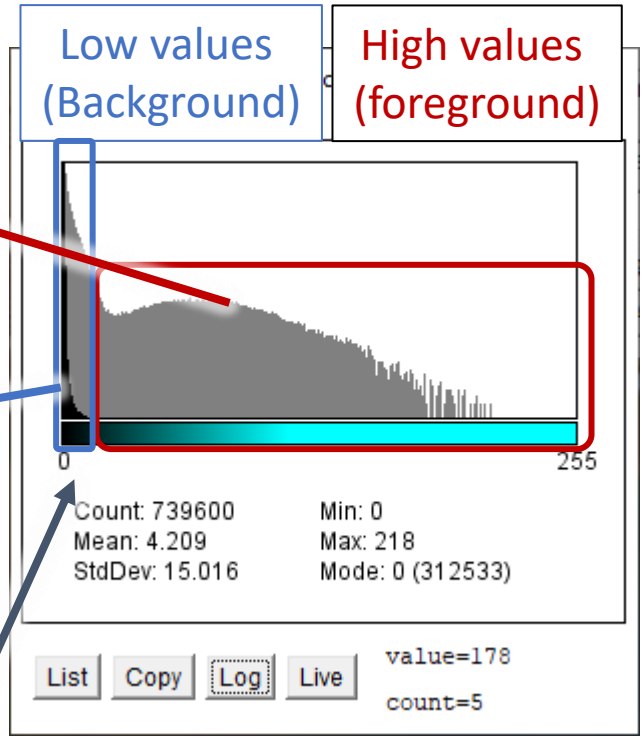


Automatic counting – Thresholding

Image / Adjust > Threshold…



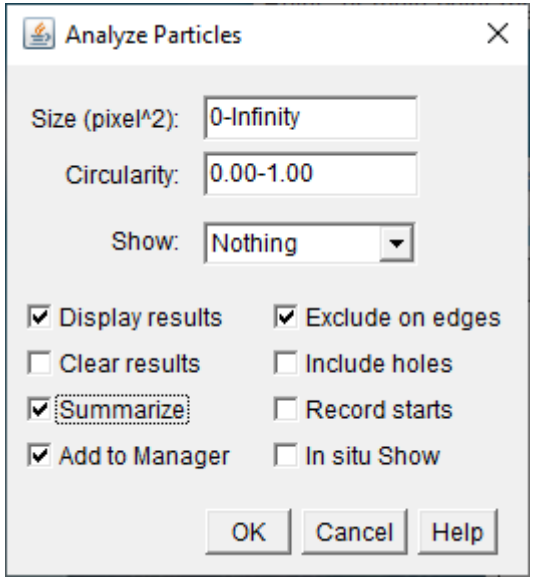


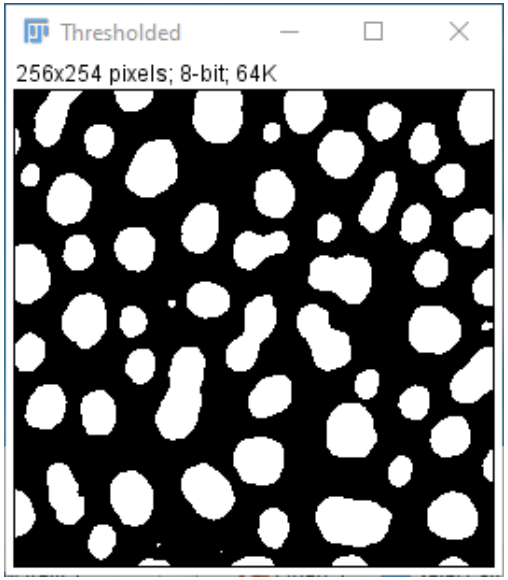


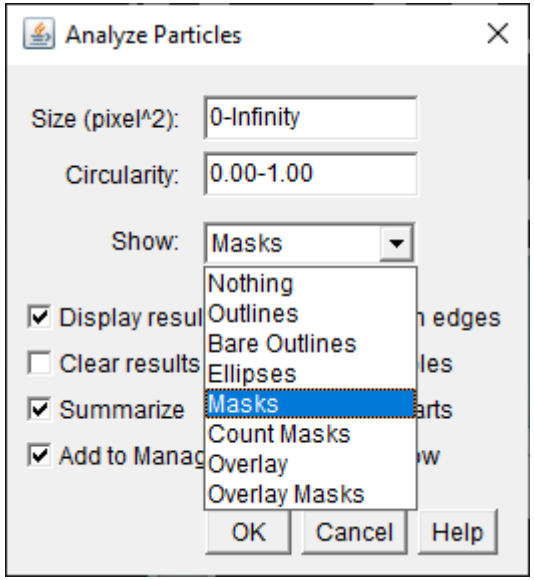
Find the appropriate algorithm

Automatic counting – Analyze particles…

Analyze / Analyze particles…

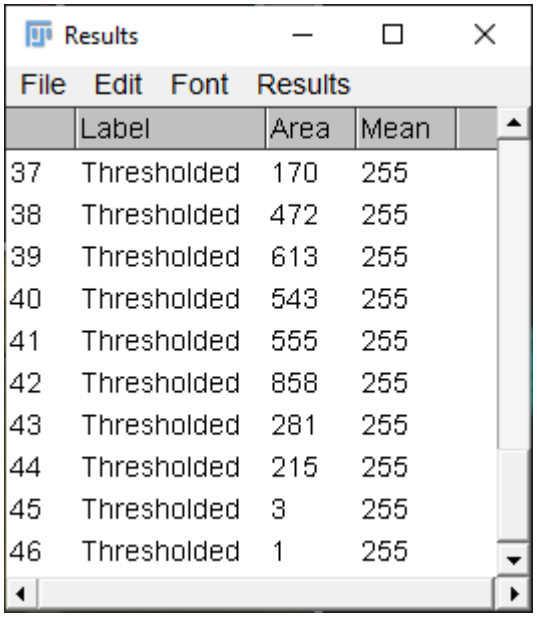




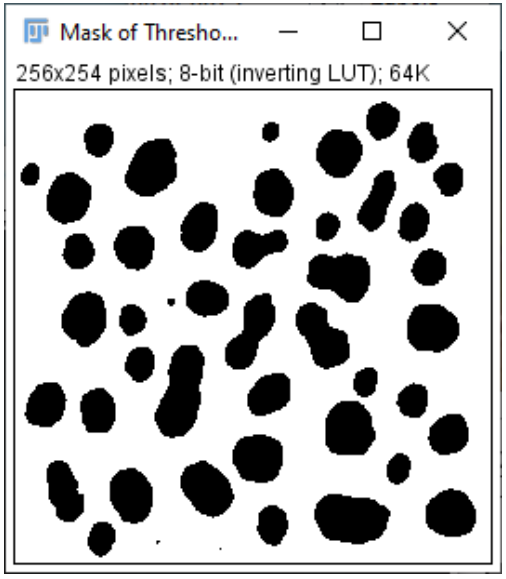


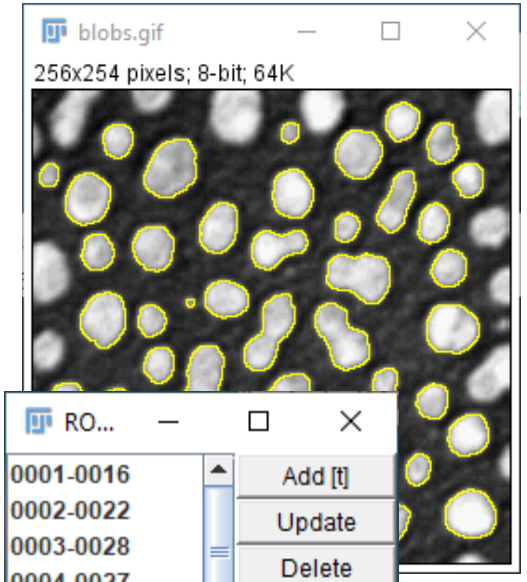
Automatic counting – Analyze particles…

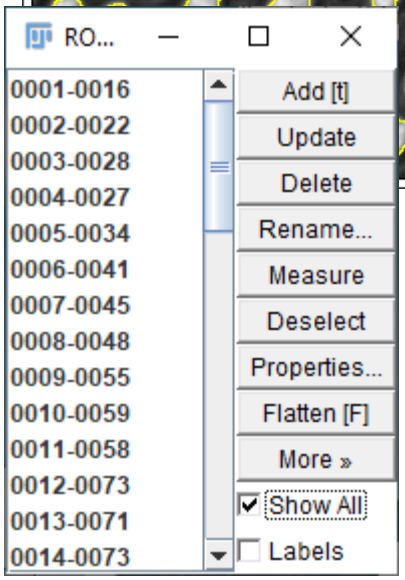
Analyze / Analyze particles…

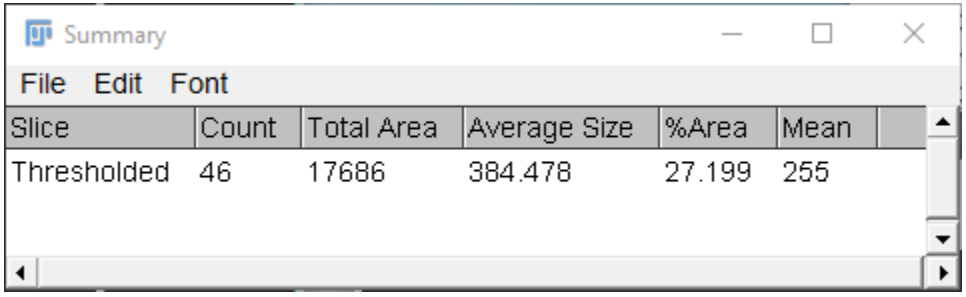


	Label	Area	Mean
37	Thresholded	170	255
38	Thresholded	472	255
39	Thresholded	613	255
40	Thresholded	543	255
41	Thresholded	555	255
42	Thresholded	858	255
43	Thresholded	281	255
44	Thresholded	215	255
45	Thresholded	3	255
46	Thresholded	1	255





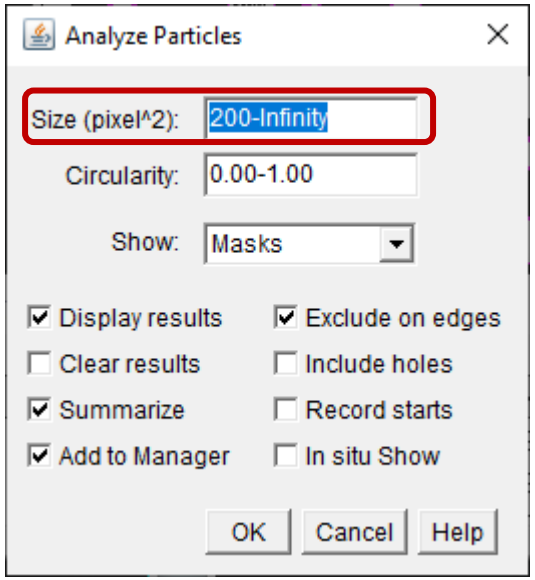


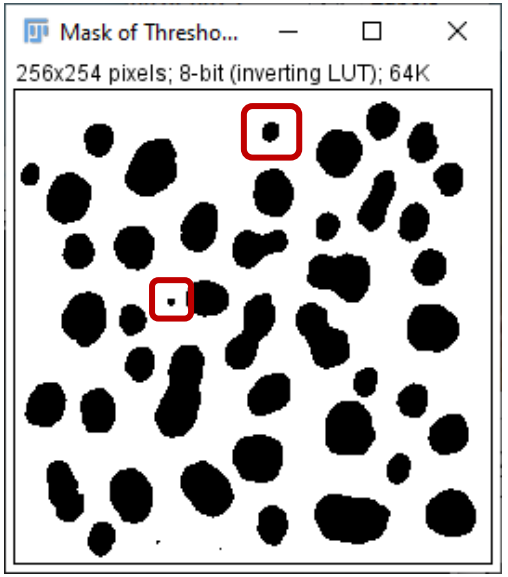


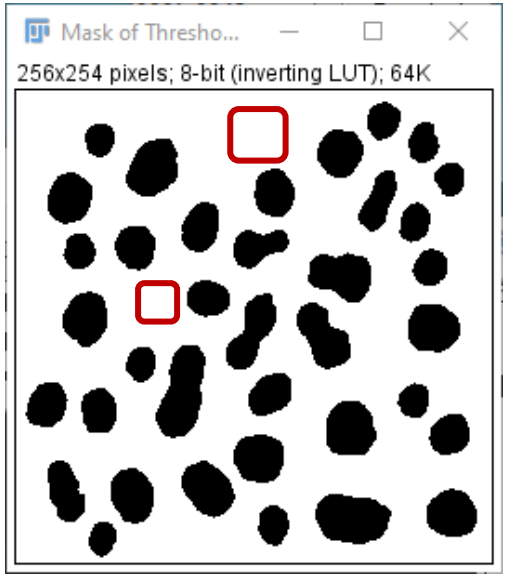
Slice	Count	Total Area	Average Size	%Area	Mean
Thresholded	46	17686	384.478	27.199	255

Automatic counting – Analyze particles…

Analyze / Analyze particles…



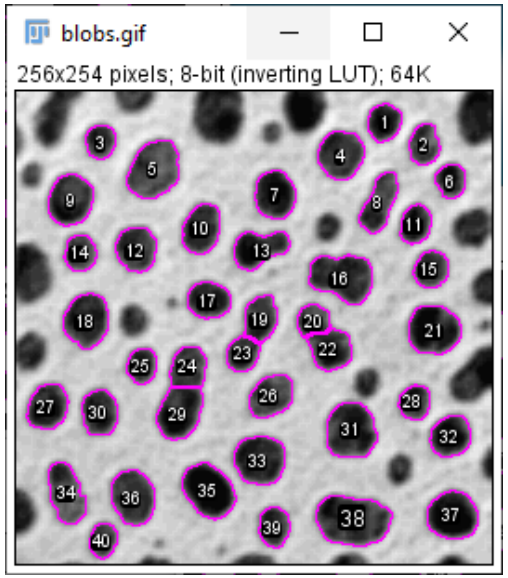


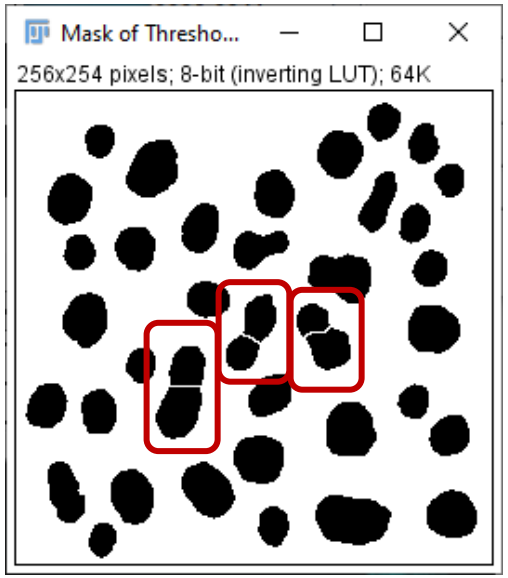


Specify a size range

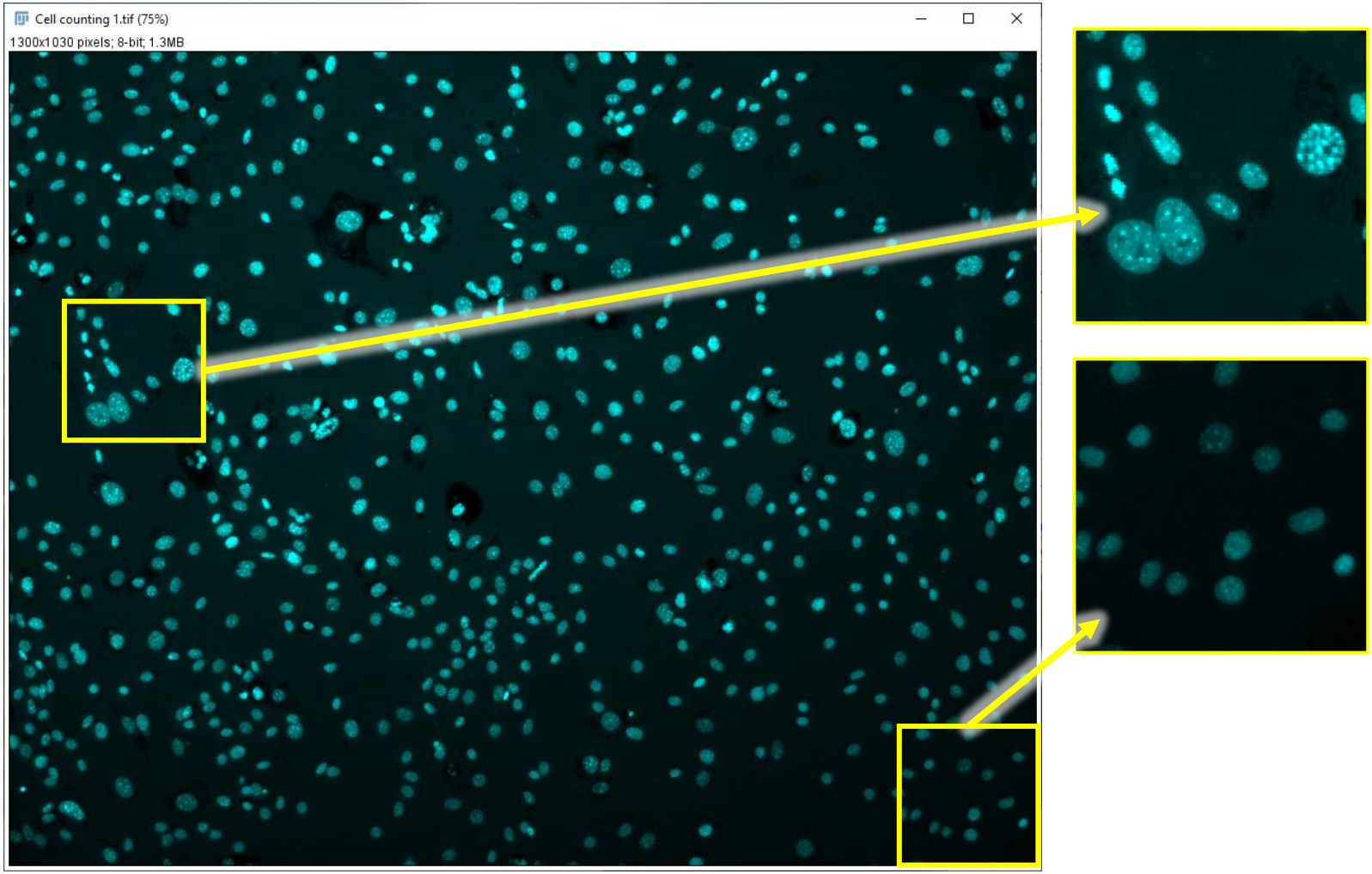
Automatic counting – Split overlapping objects

Process / Binary > Watershed

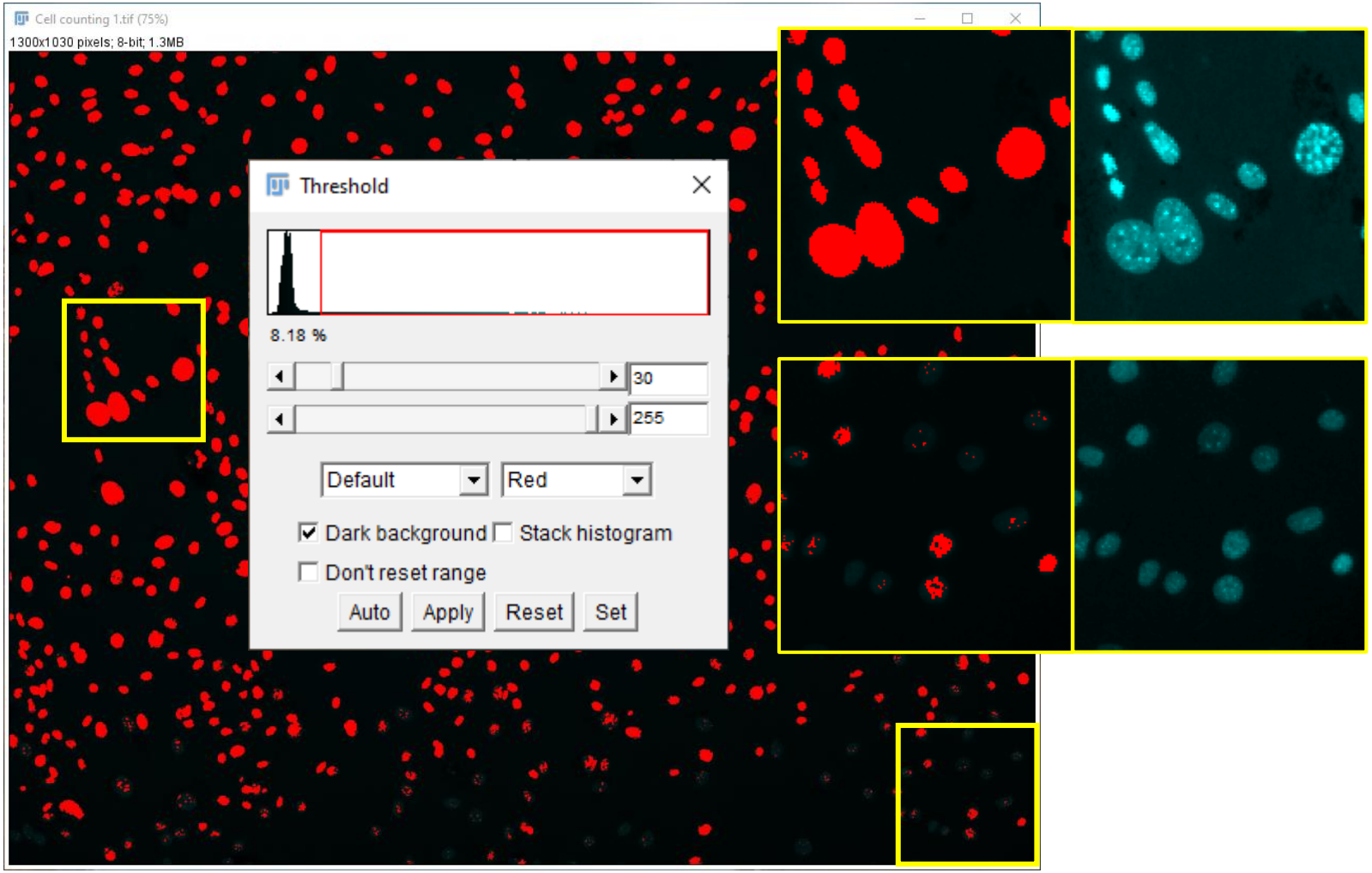




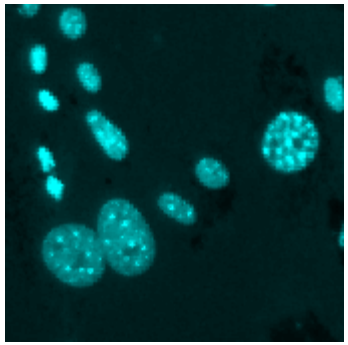
Automatic counting – Real samples

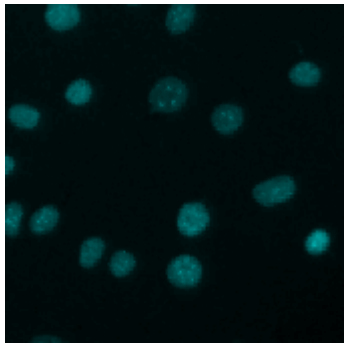


Automatic counting – Real samples



Automatic counting – Real samples





Pre-process the image before thresholding / find maxima.

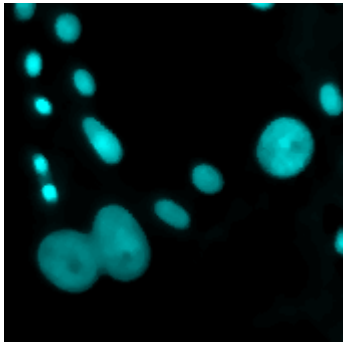
Subtract background

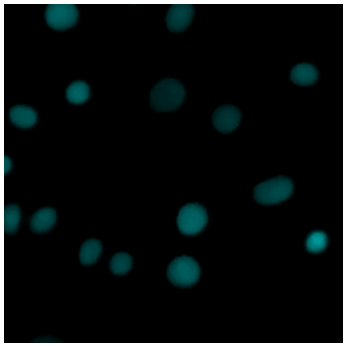
Filter:
Median / Gaussian / Mean?

Median – keeps edges – good for thresholding

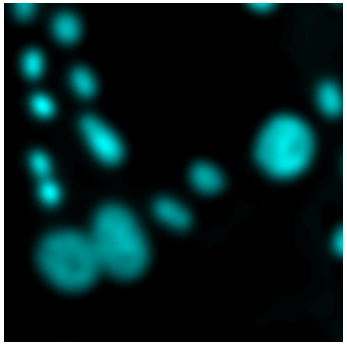
Gaussian – blurs & softens edges – good for find maxima

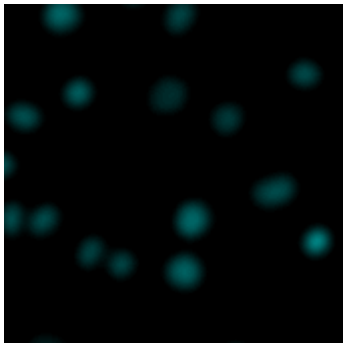
Median





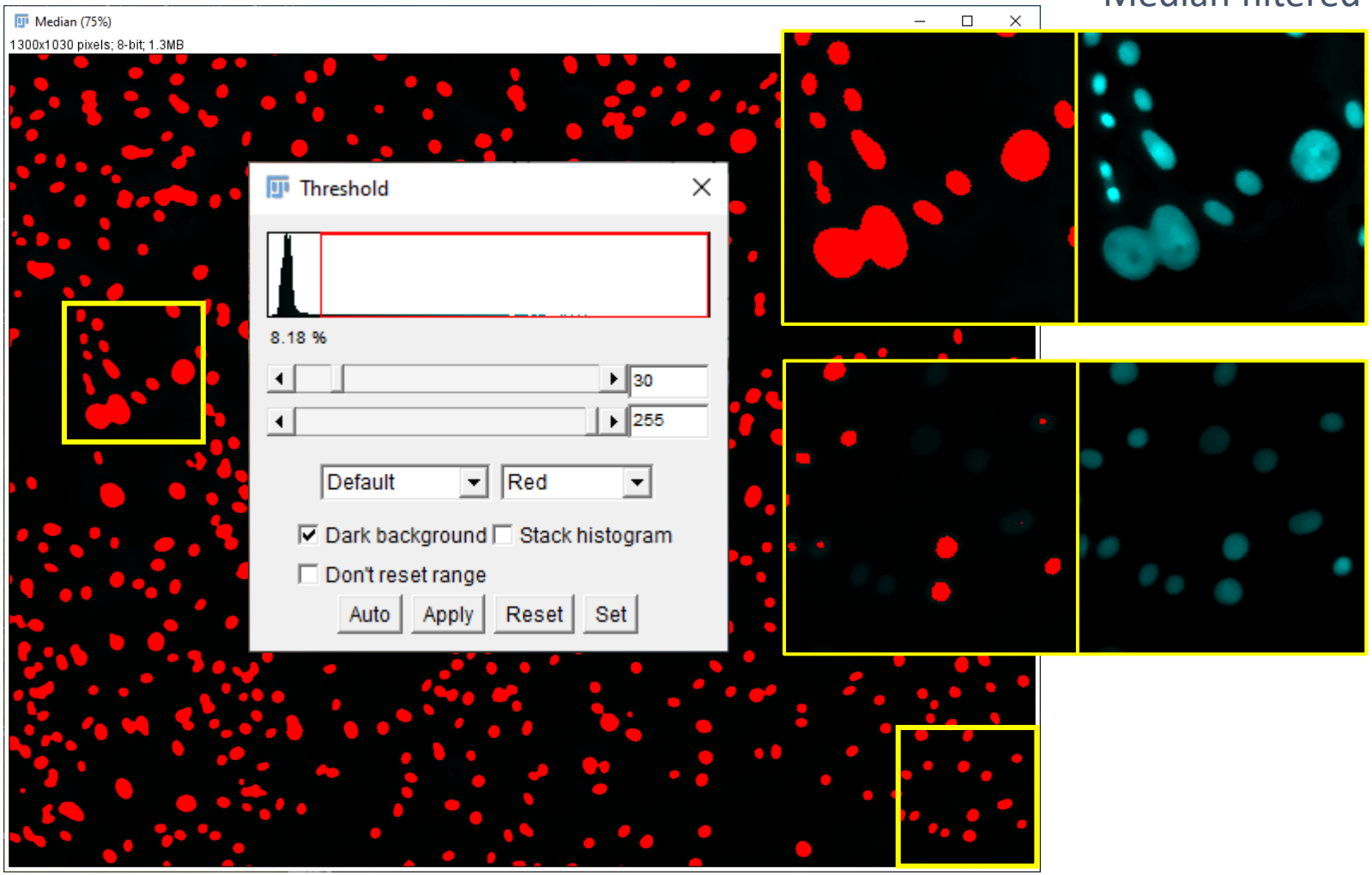
Gaussian





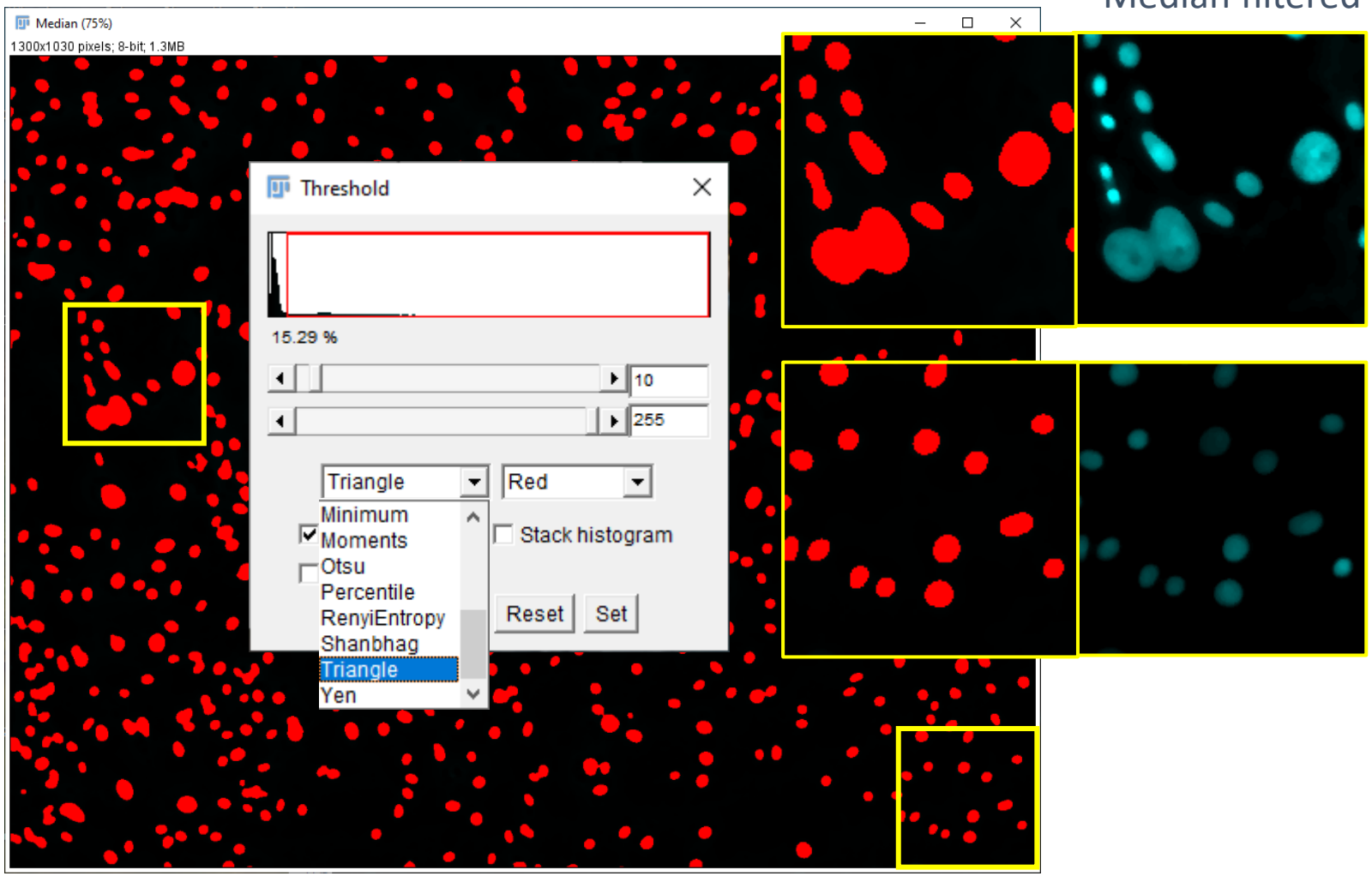
Automatic counting – Real samples

Median filtered

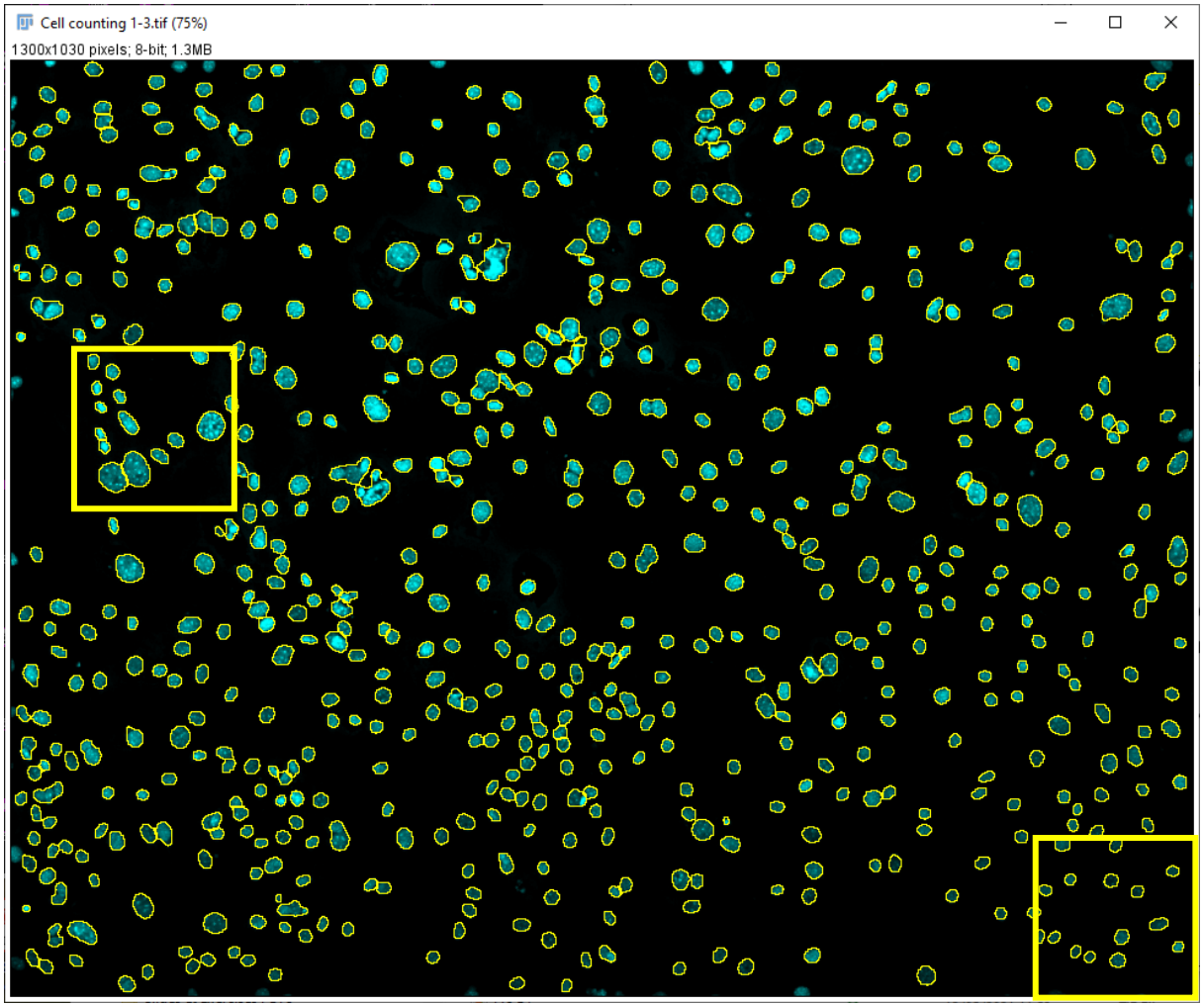


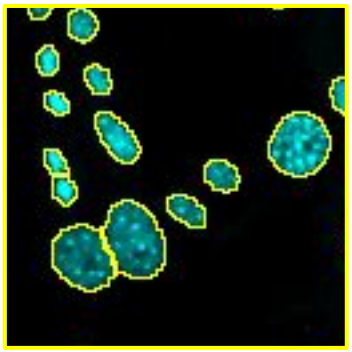
Automatic counting – Real samples

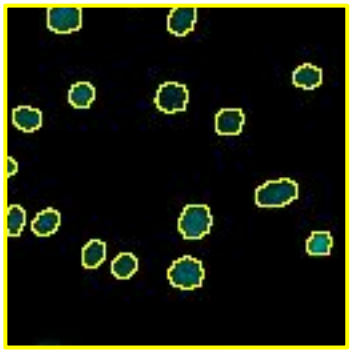
Median filtered



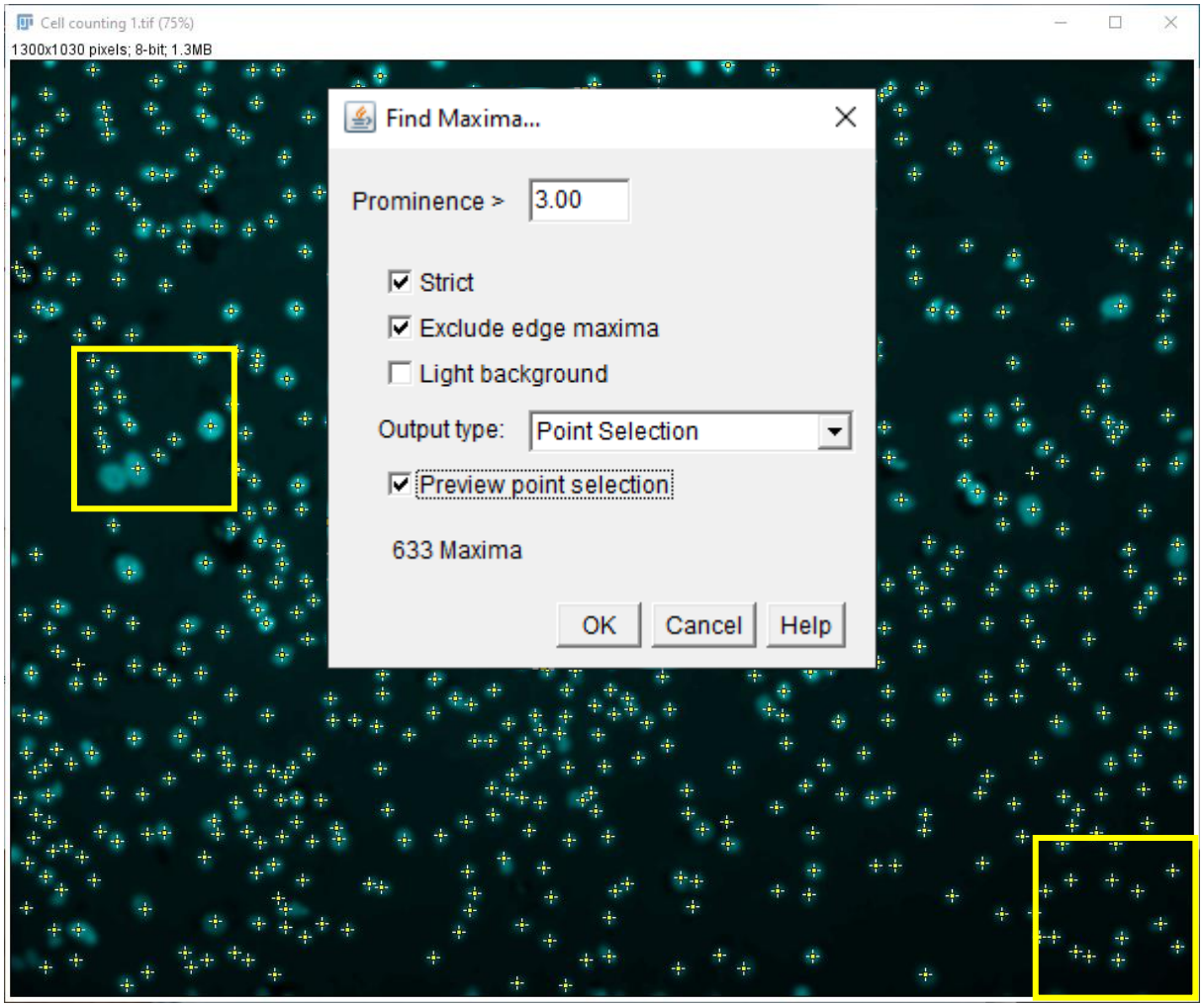
Automatic counting – Real samples

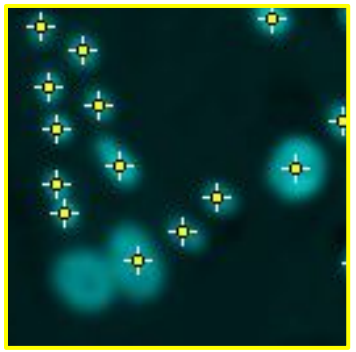


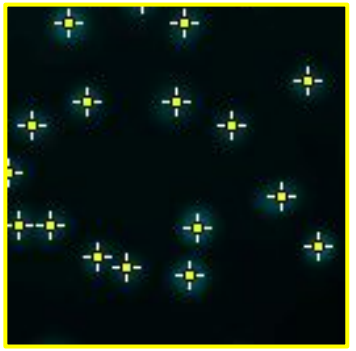




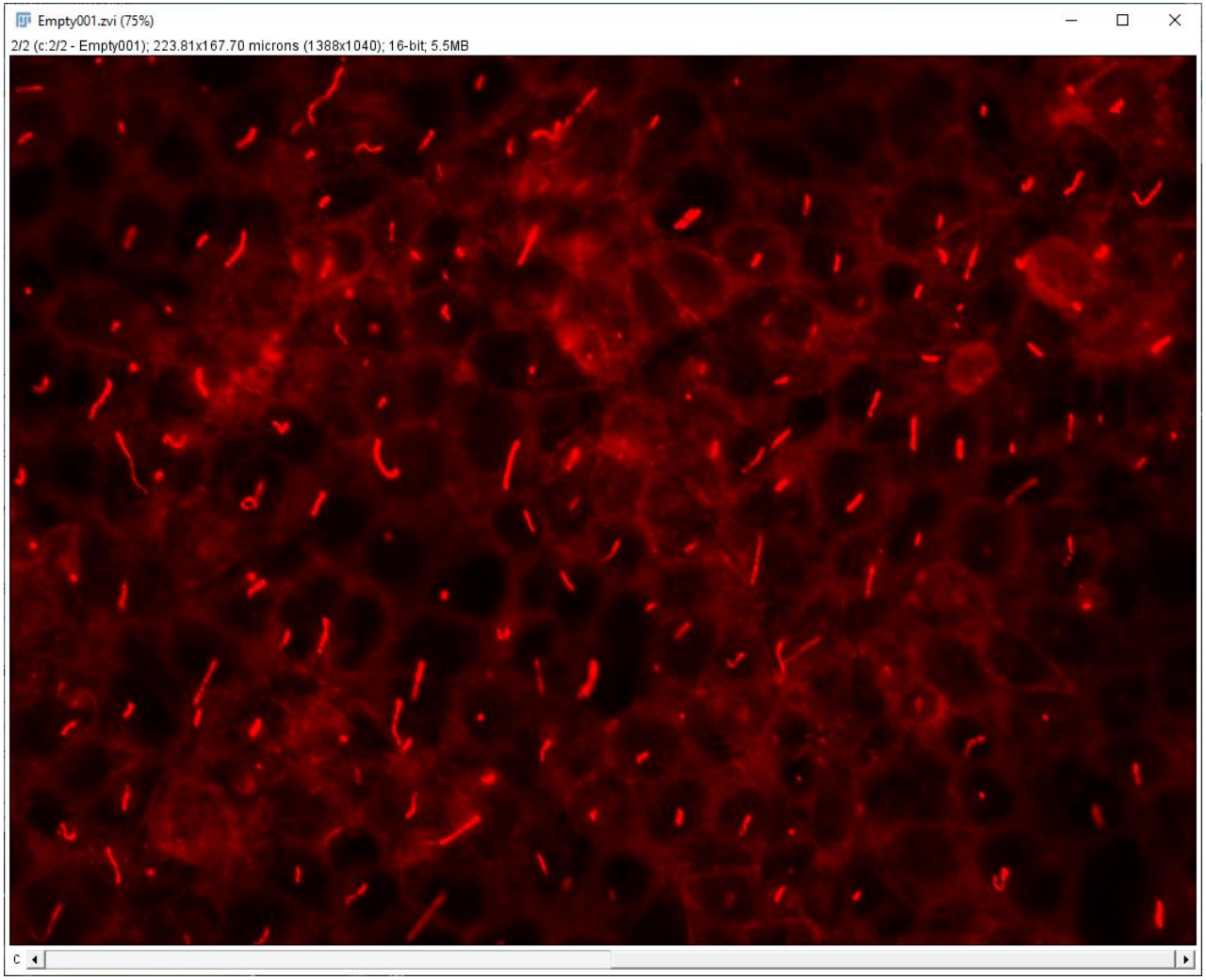
Automatic counting – Real samples



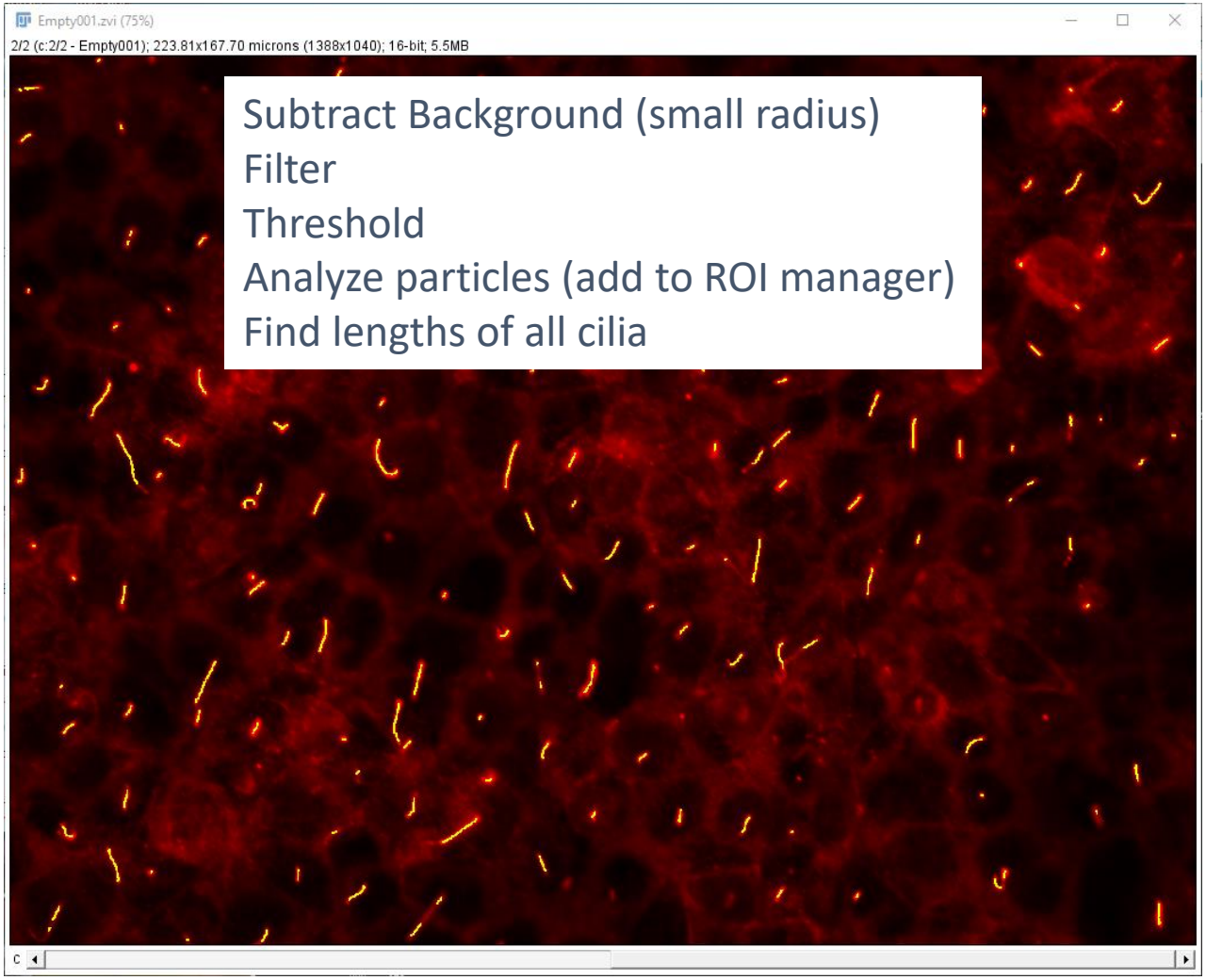




Automatic counting – Real samples - Cilia

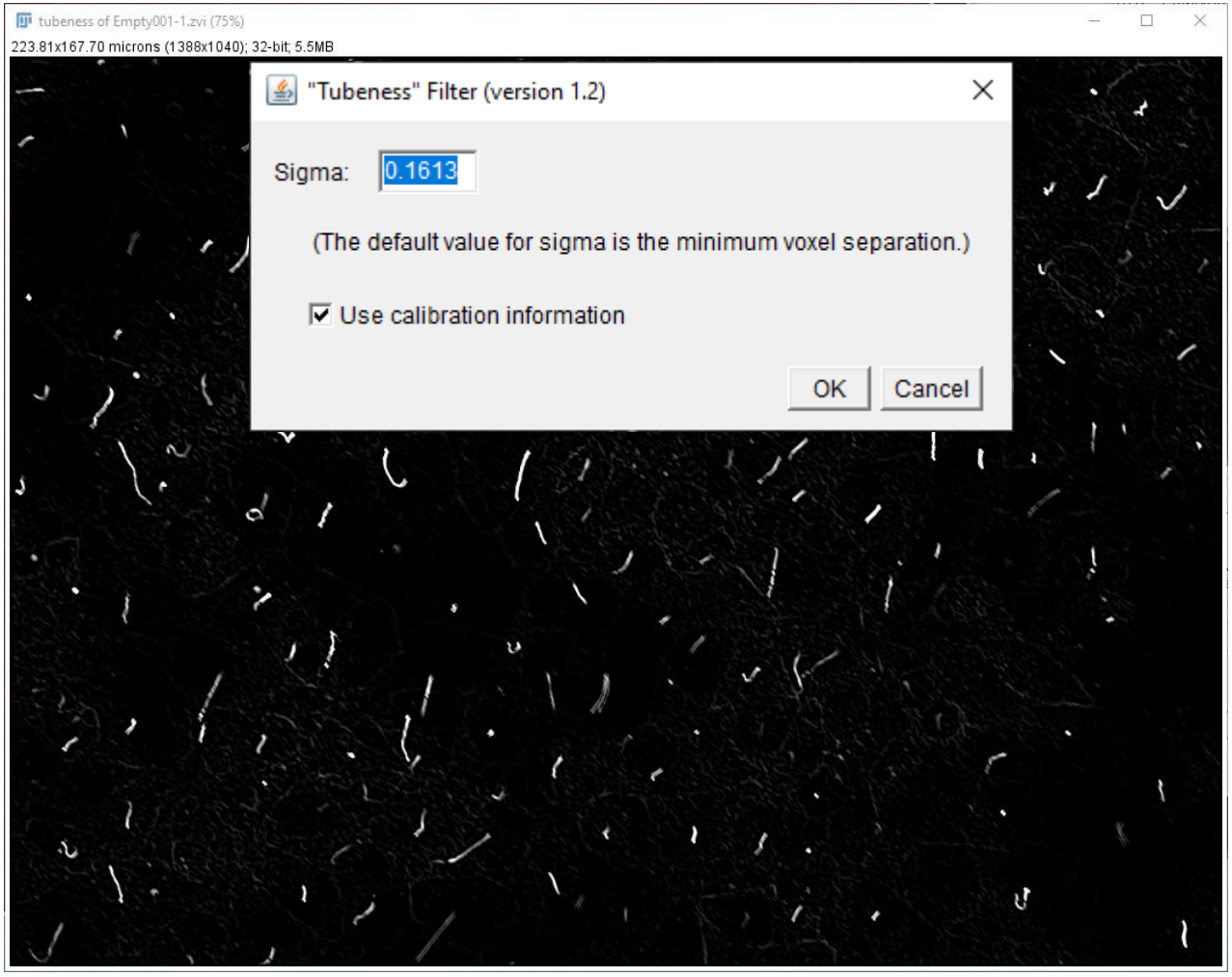


Automatic counting – Real samples - Cilia



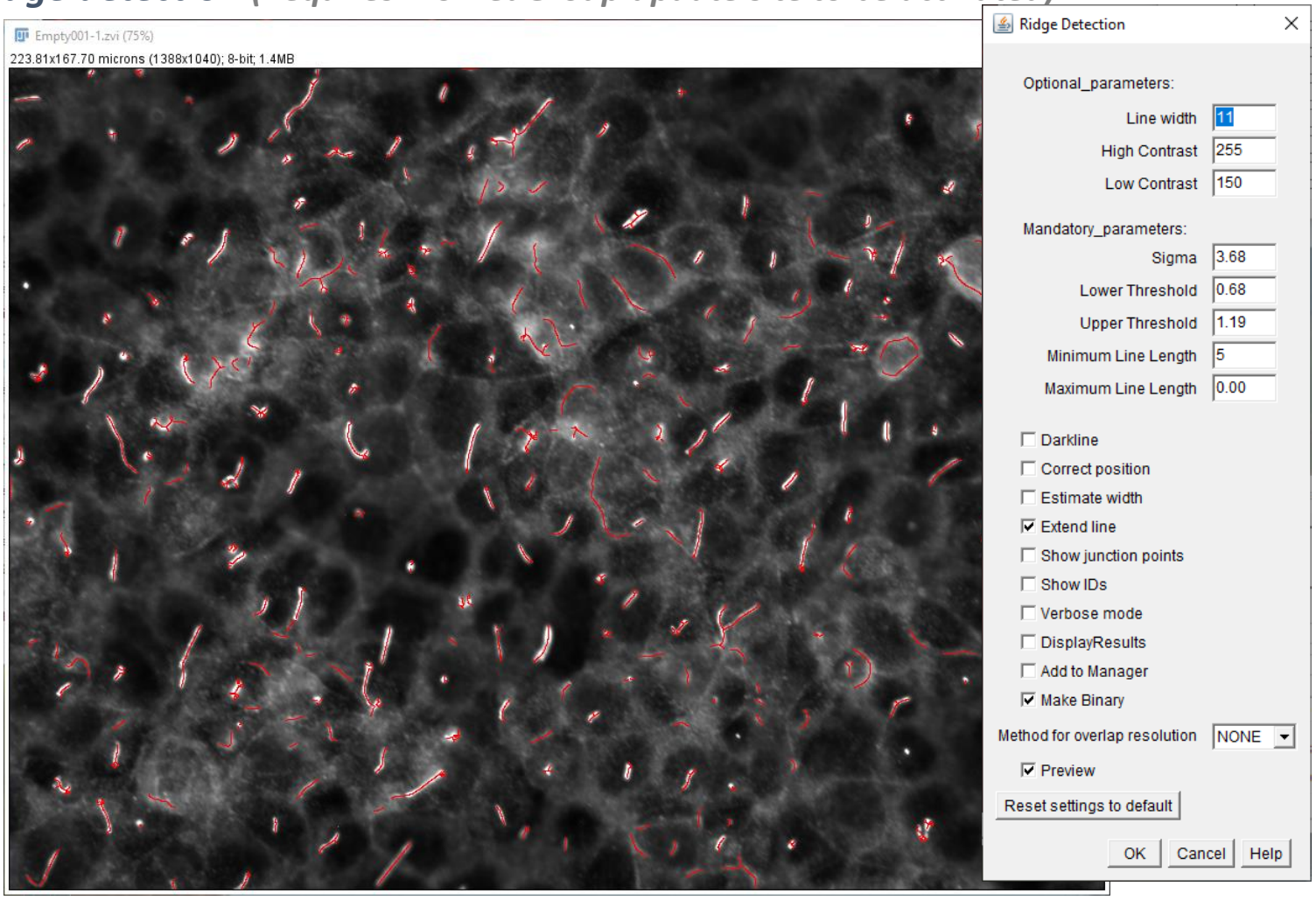
Alternative strategies – look for tube like structures…

Plugins / Analyze > Tubeness

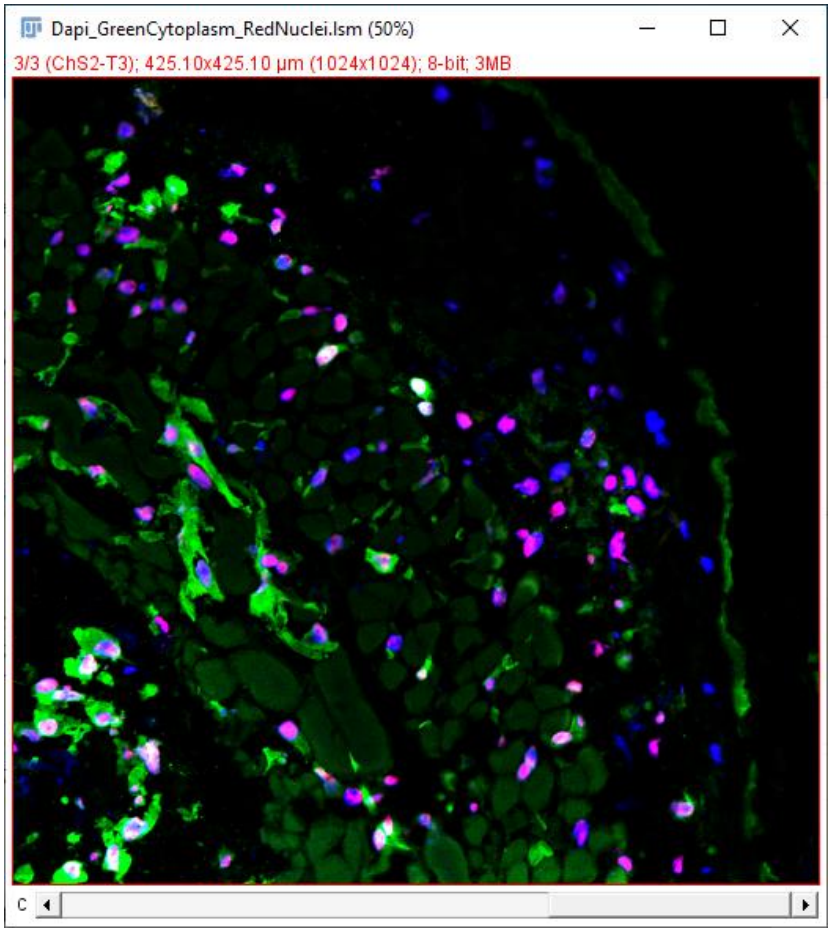


Alternative strategies – look for ridges…

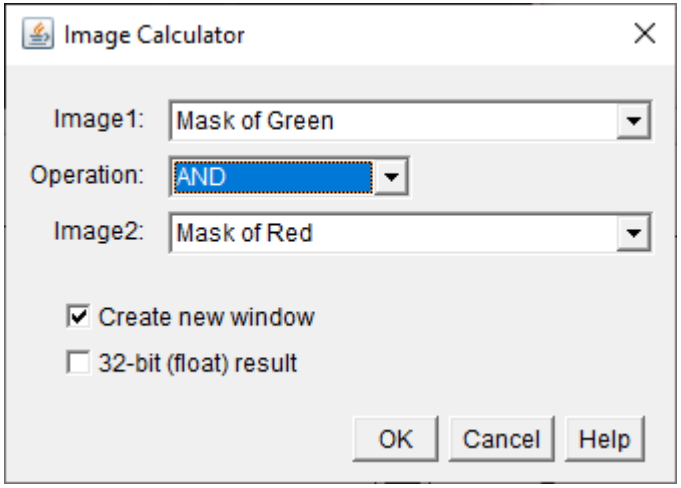
Plugins / Ridge detection *(Requires BioMedGroup update site to be activated)*



Double & Triple stained cells

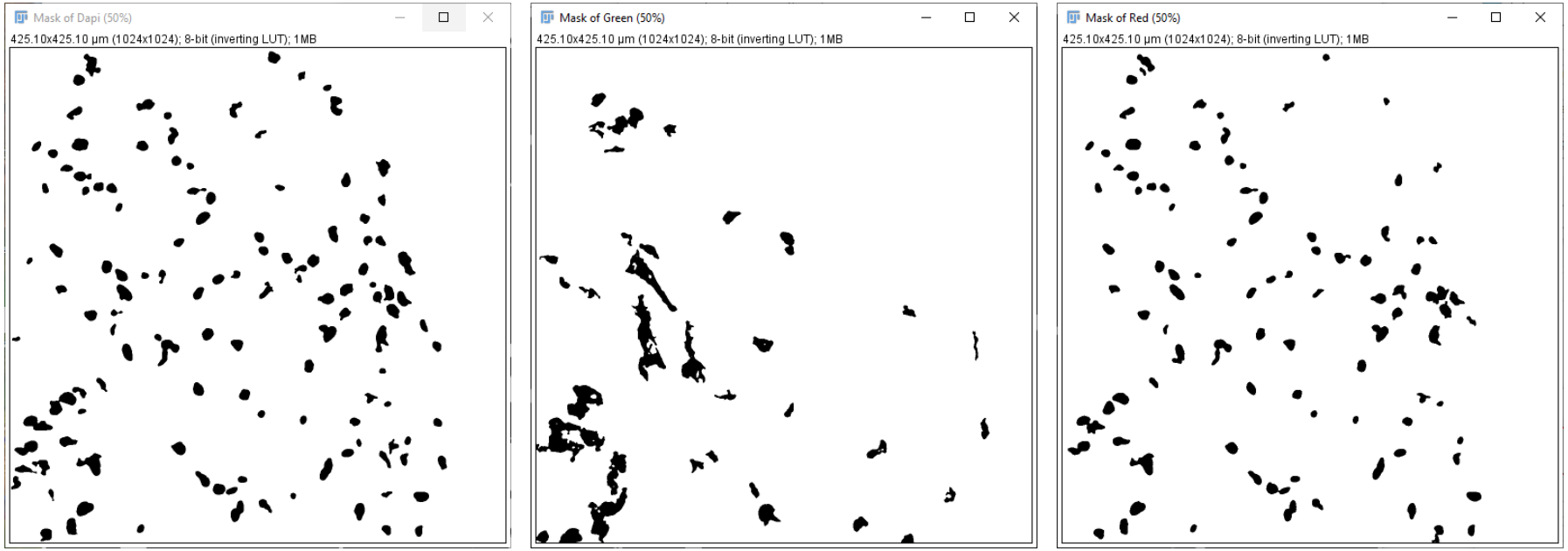


Identifying and counting double (or triple) stained cells is as simple as thresholding, with a single additional step…



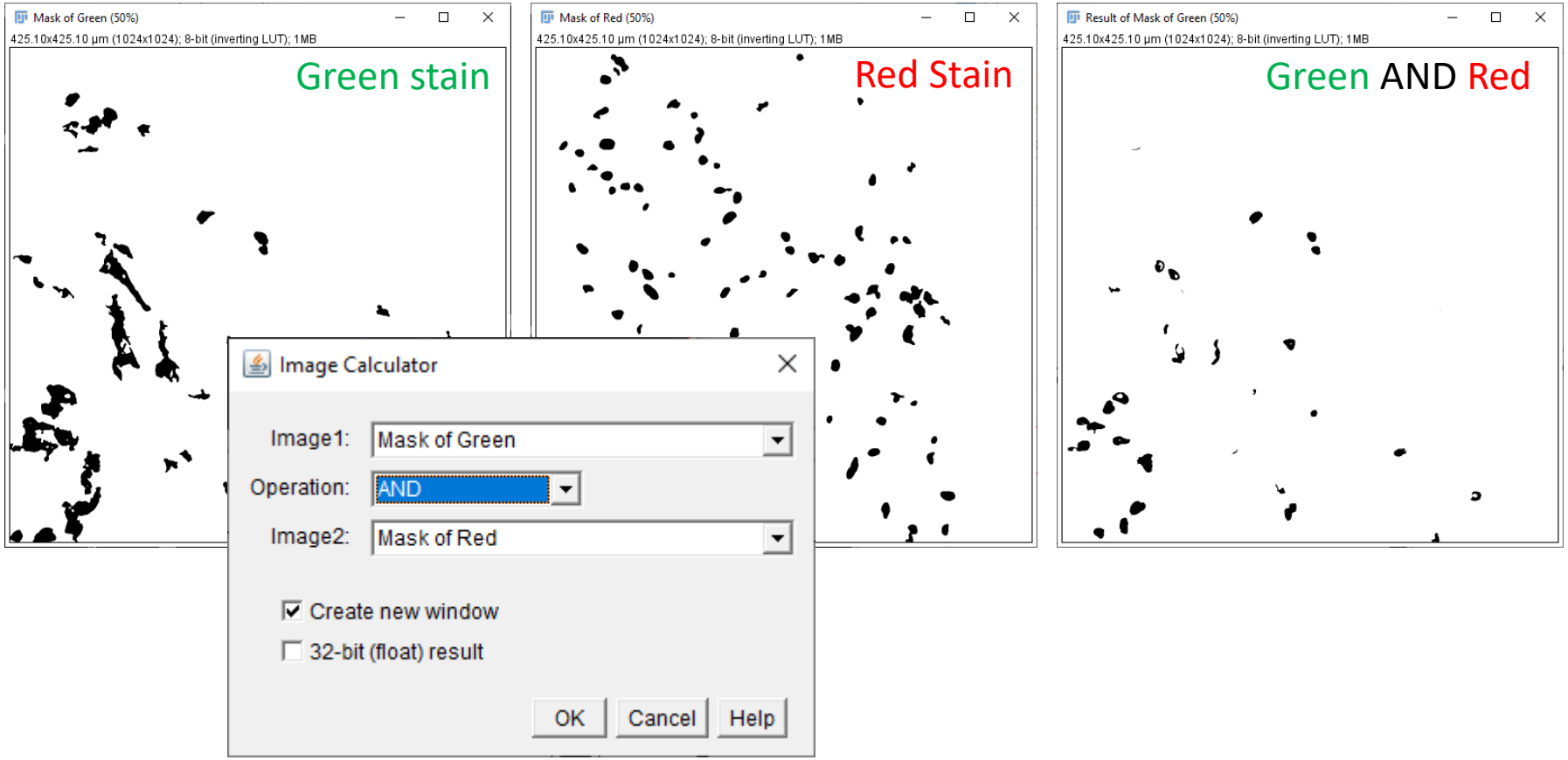
Double & Triple stained cells

Threshold to binary images, then find overlaps between images…

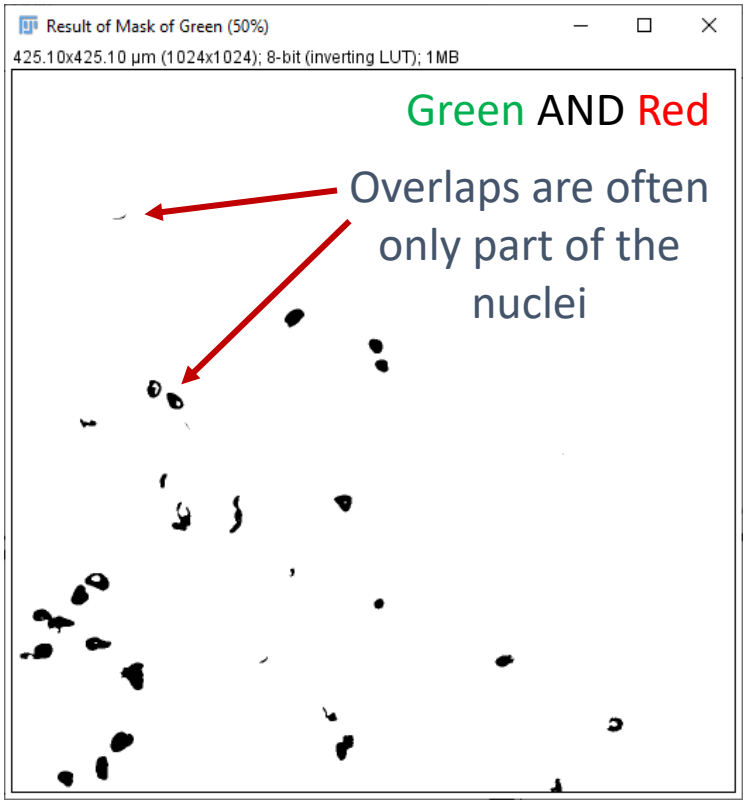


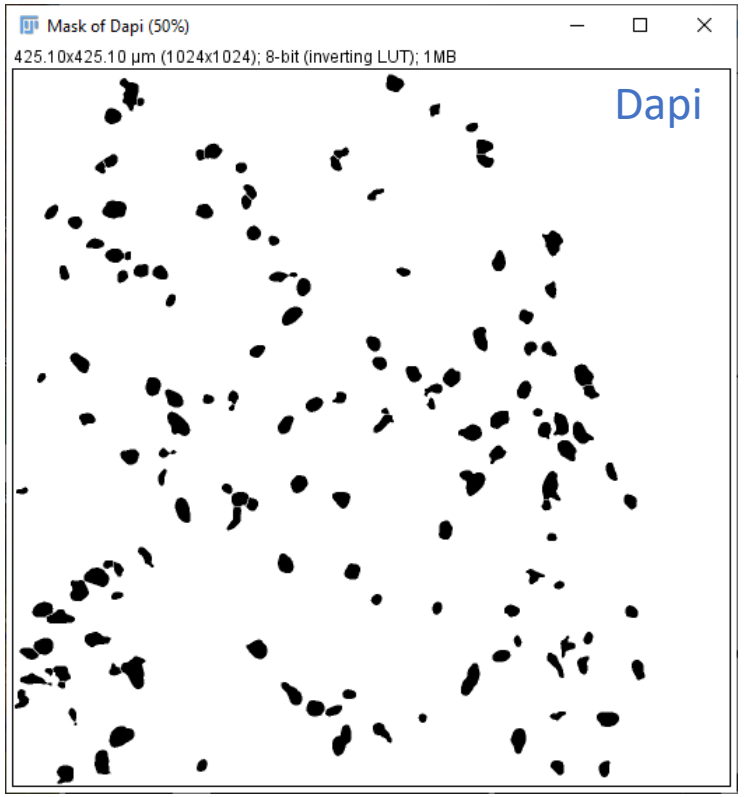
Double & Triple stained cells

Find overlaps between images…



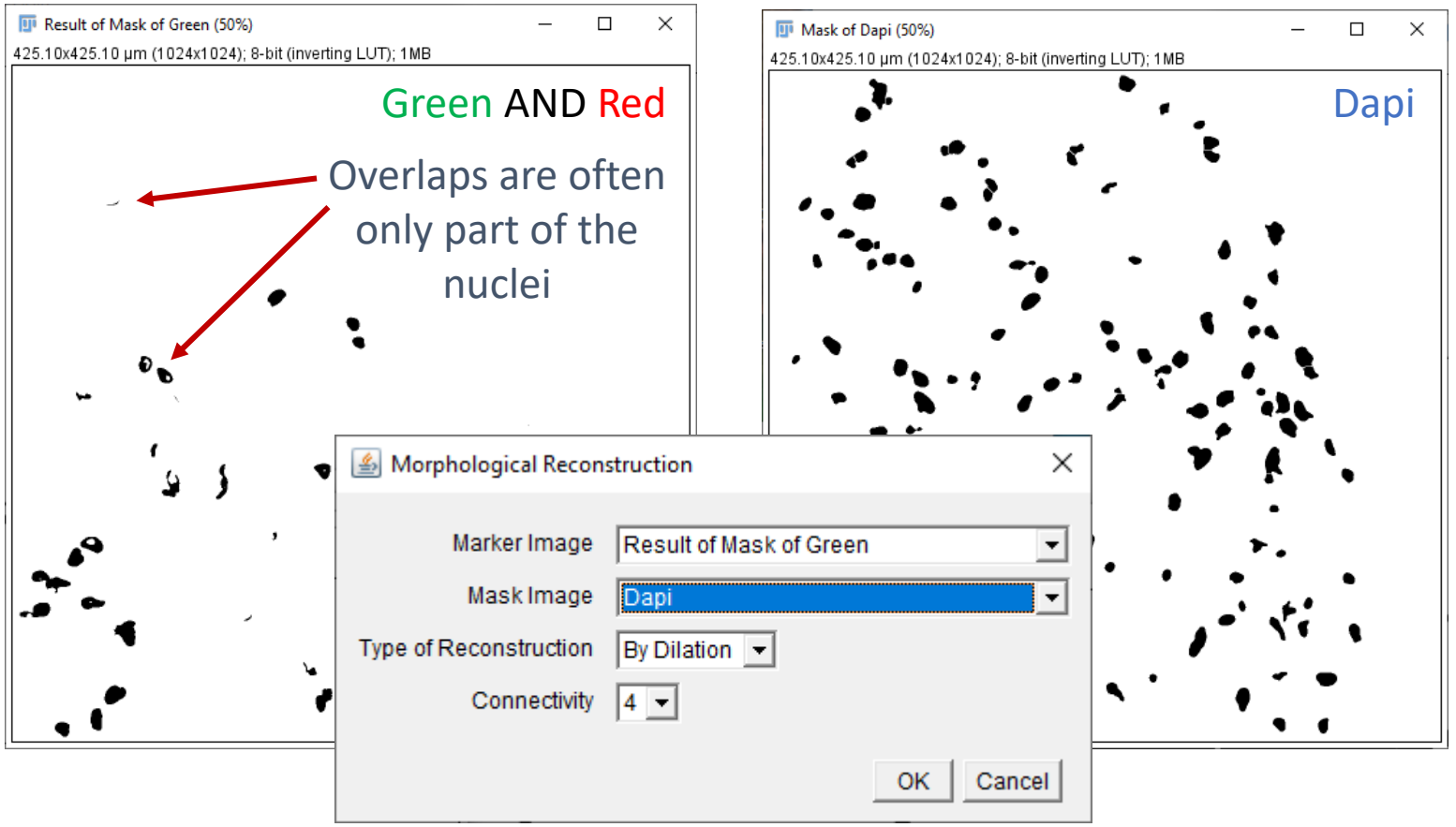
Double & Triple stained cells





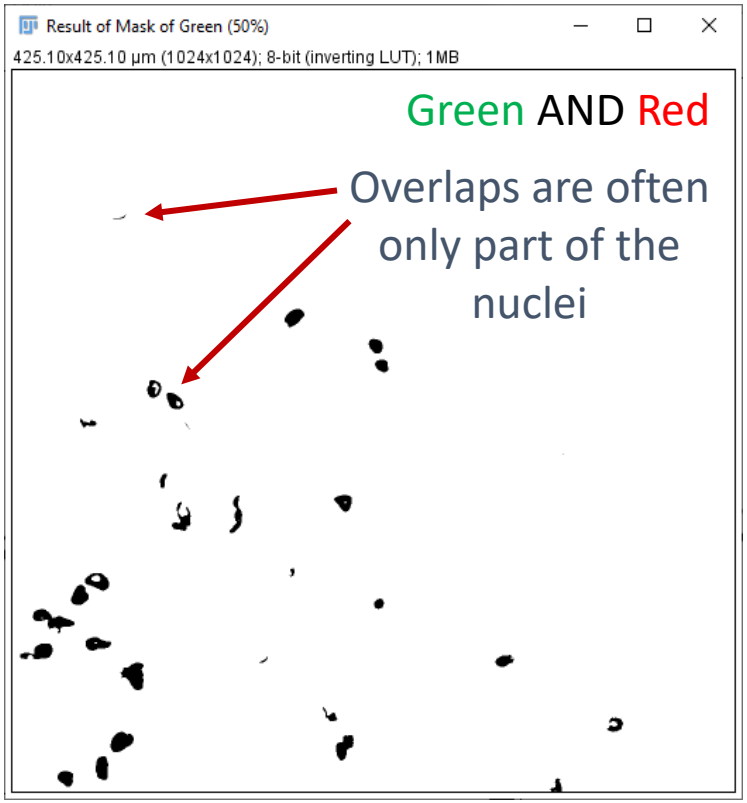
Double & Triple stained cells

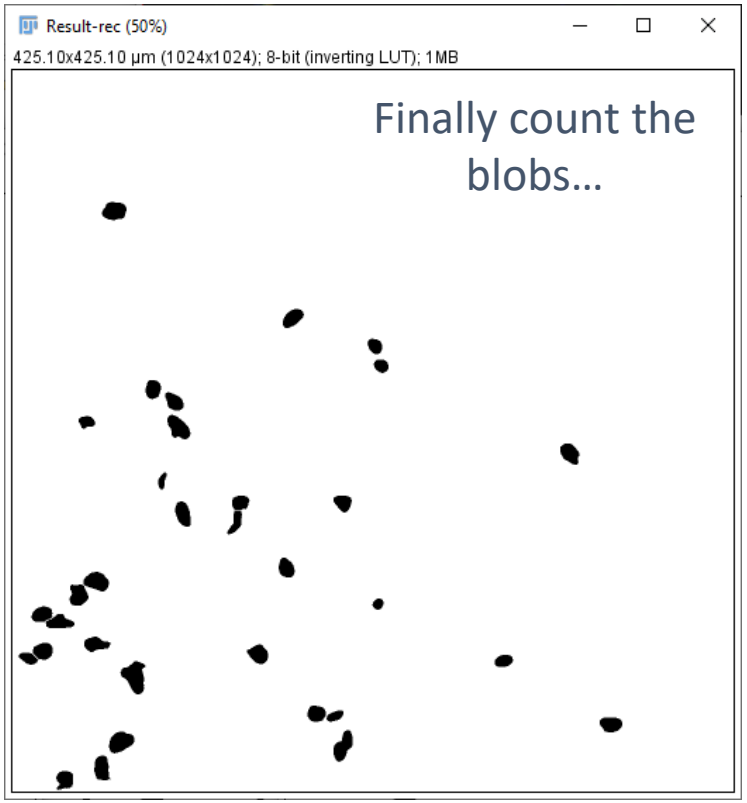
Plugins / MorpholibJ / Morphological Reconstruction



Double & Triple stained cells

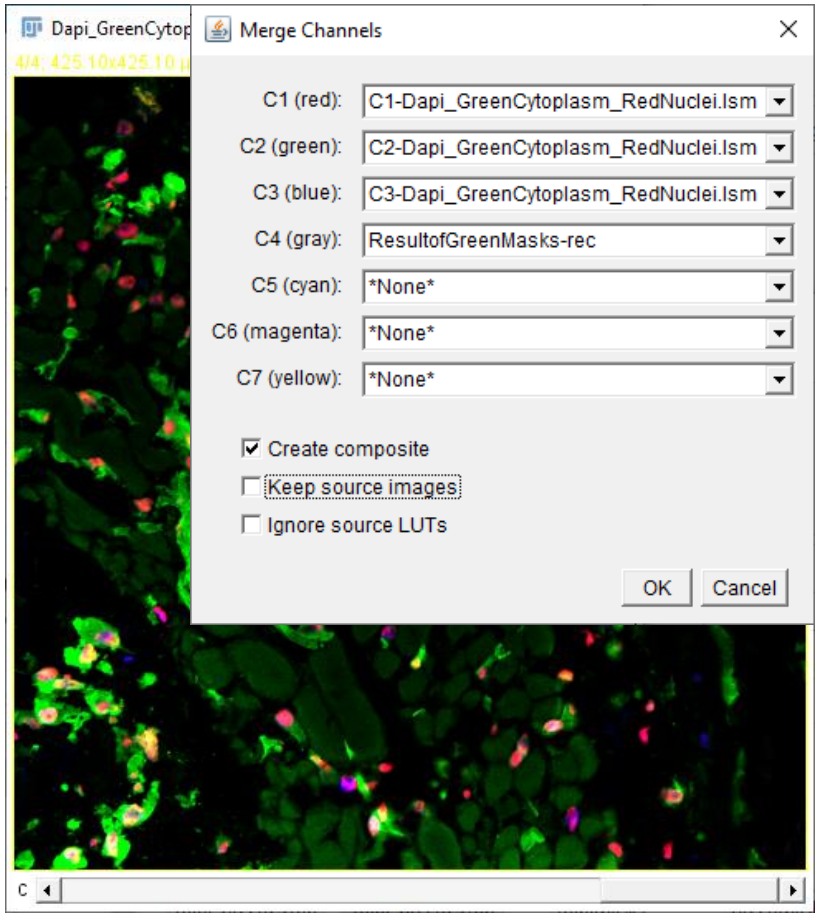
Plugins / MorpholibJ / Morphological Reconstruction

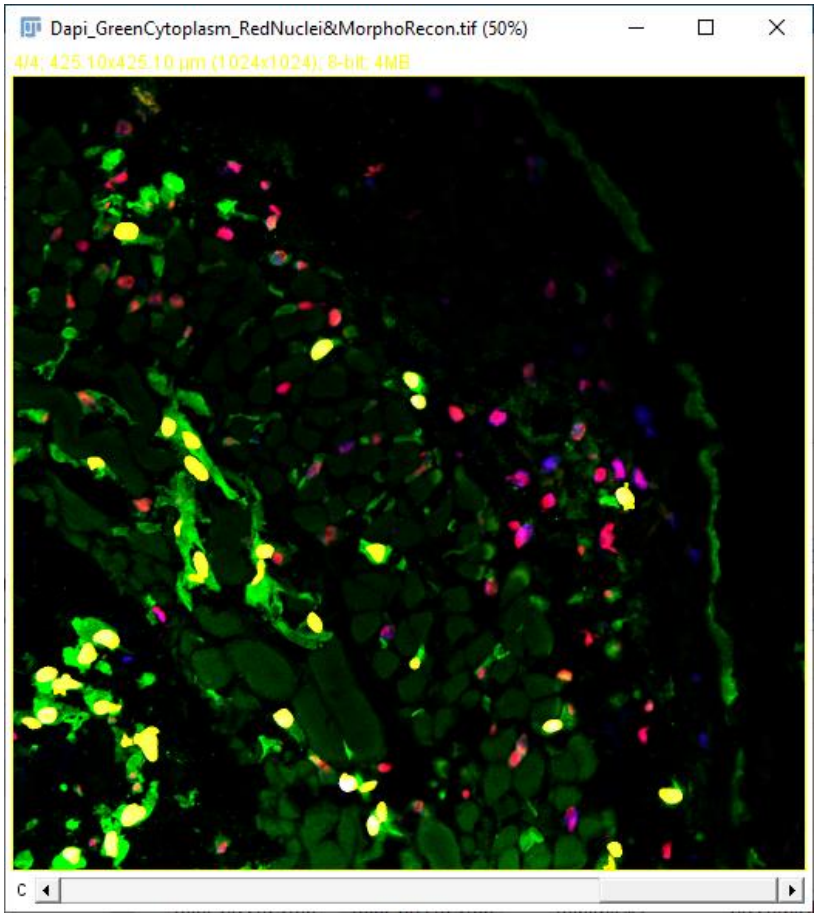


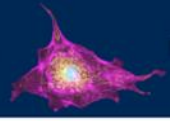


Double & Triple stained cells

Make an image to show the result







Exercises Session 5 – Thresholding, Particle Analyzer and Find Maxima

Counting tools, filters, thresholding etc
2 hour for exercises

13) Run a macro to check out some filters.

14) Count Blobs, thresholding

15) Count real samples – Nuclei

16) Cilia!

17) Double stained cells