

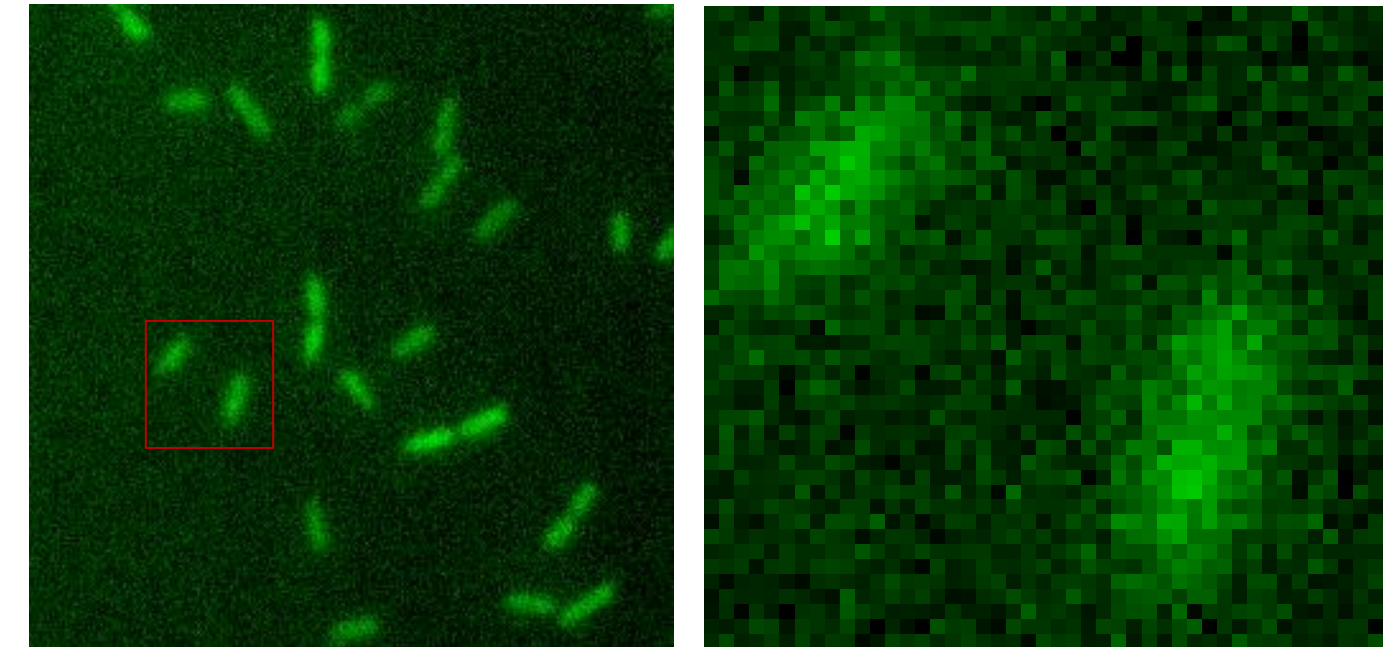
Segmentation Techniques

Image Processing & Analysis for Life Scientists

Olivier Burri, Romain Guiet & Arne Seitz

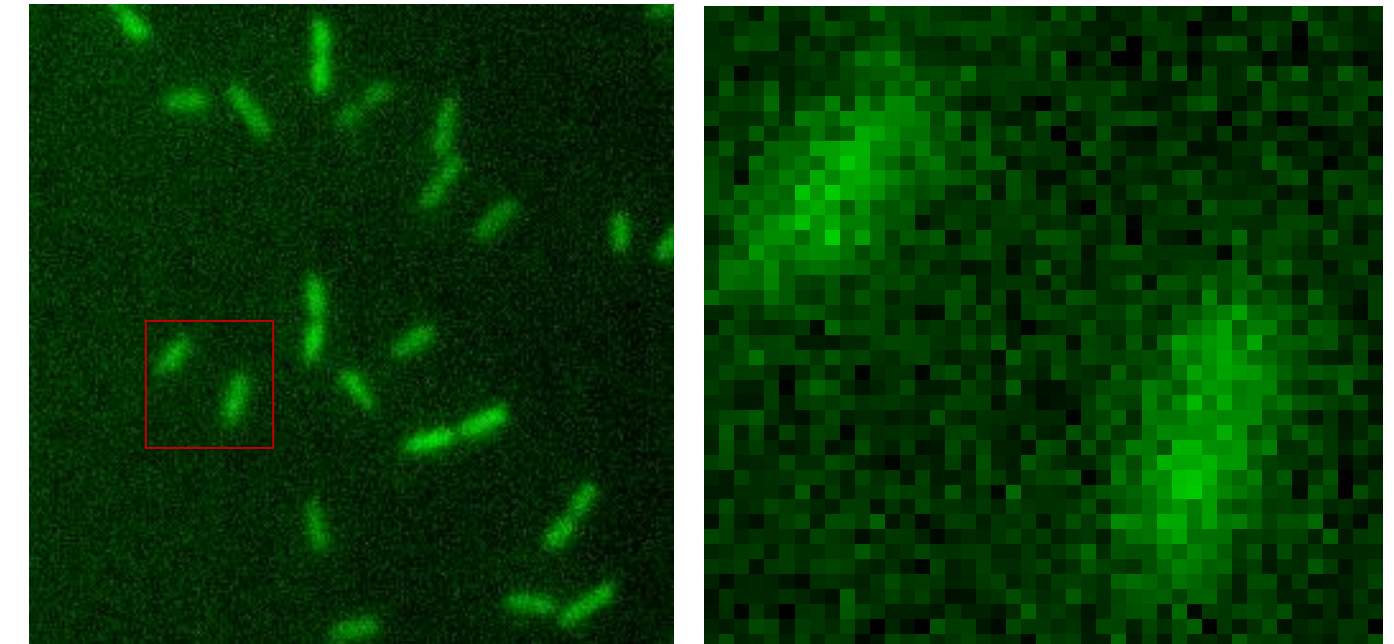
Segmentation Techniques

Image
(*Intensities*)

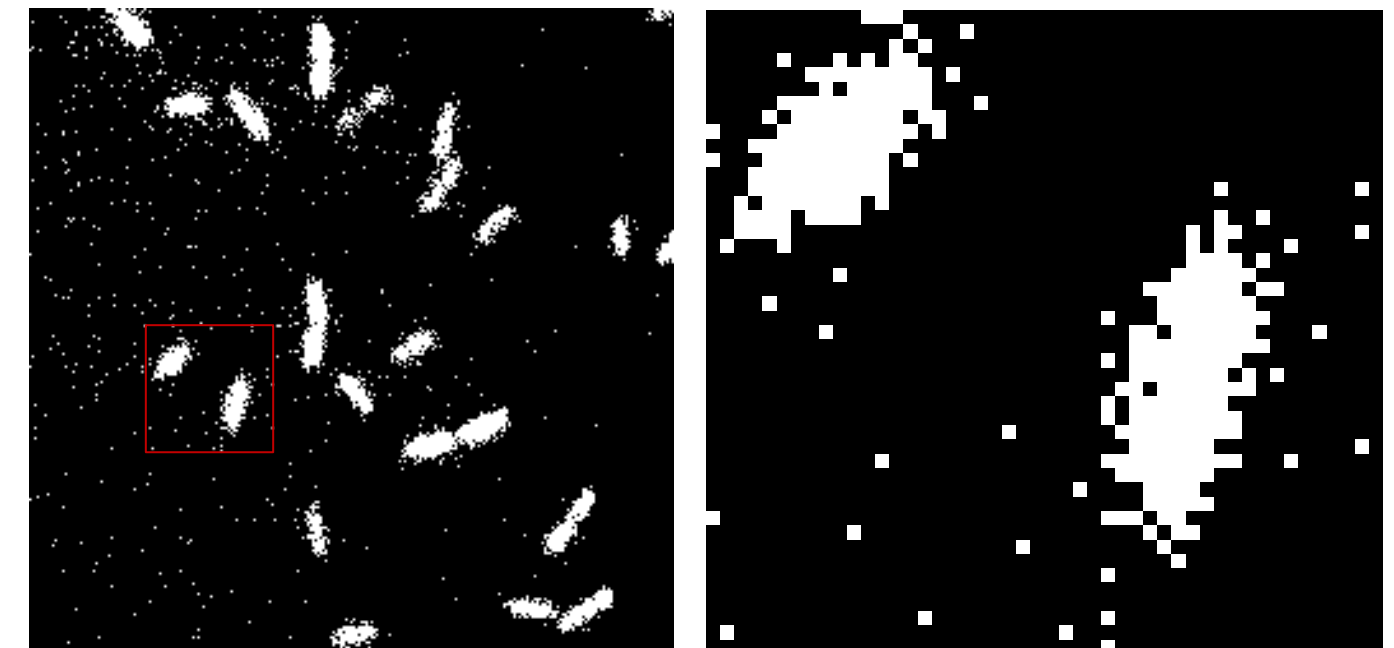


Segmentation Techniques

Image
(Intensities)

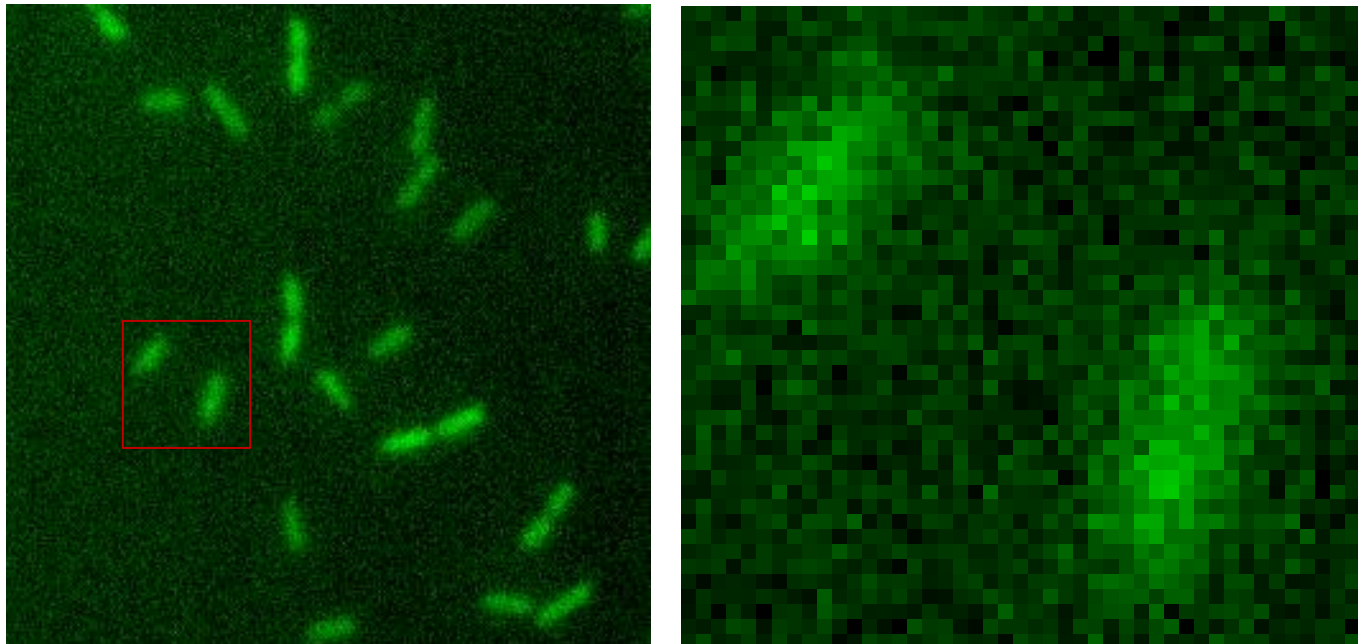


Mask
(Categories)

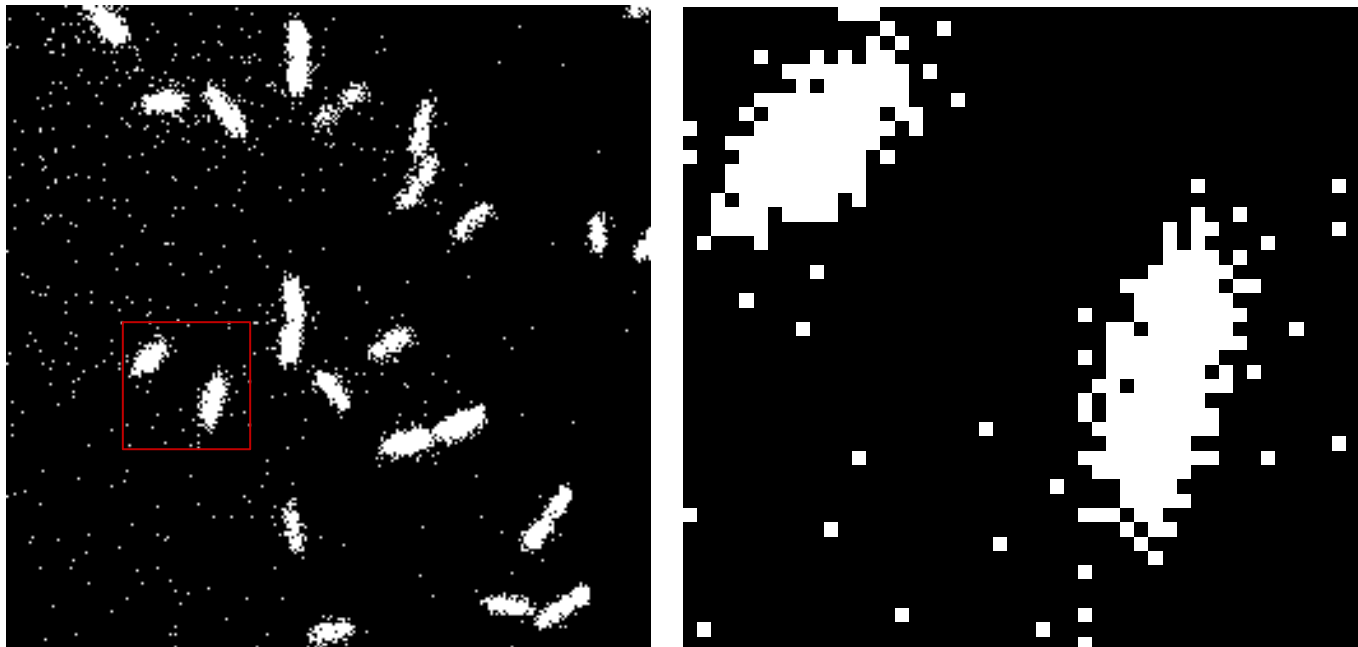


Segmentation Techniques

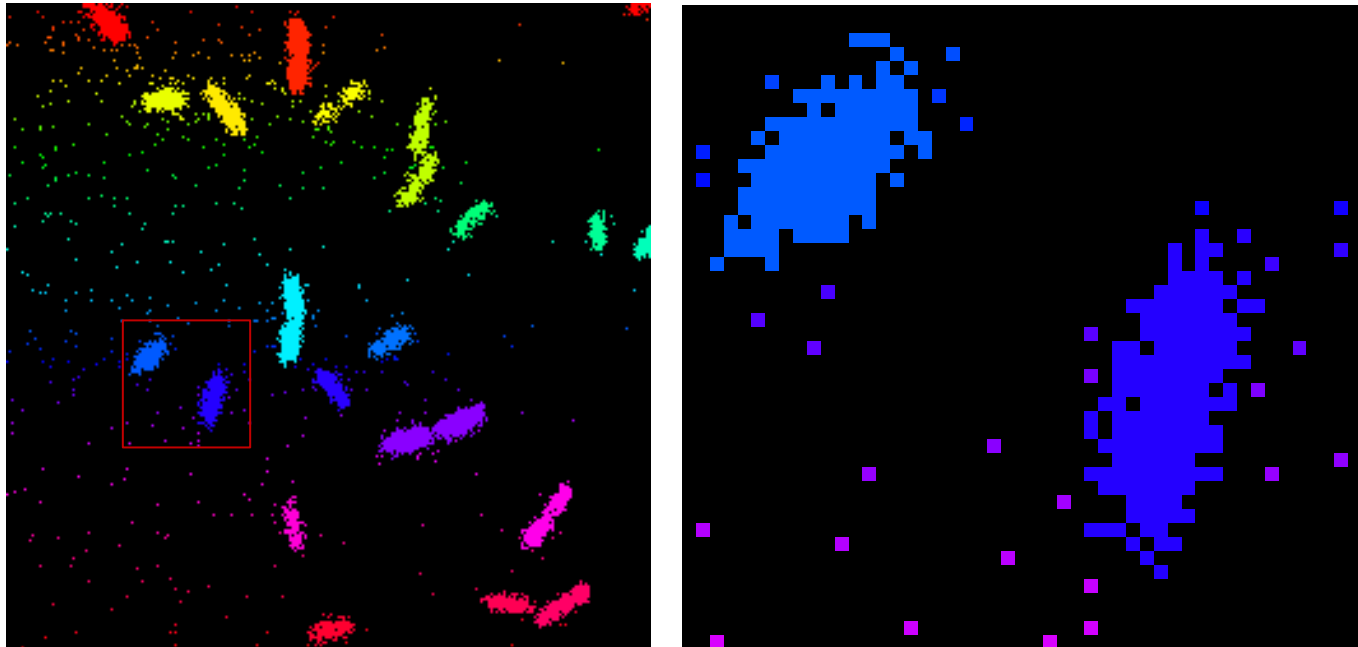
Image
(Intensities)



Mask
(Categories)



Object
(Indexes)



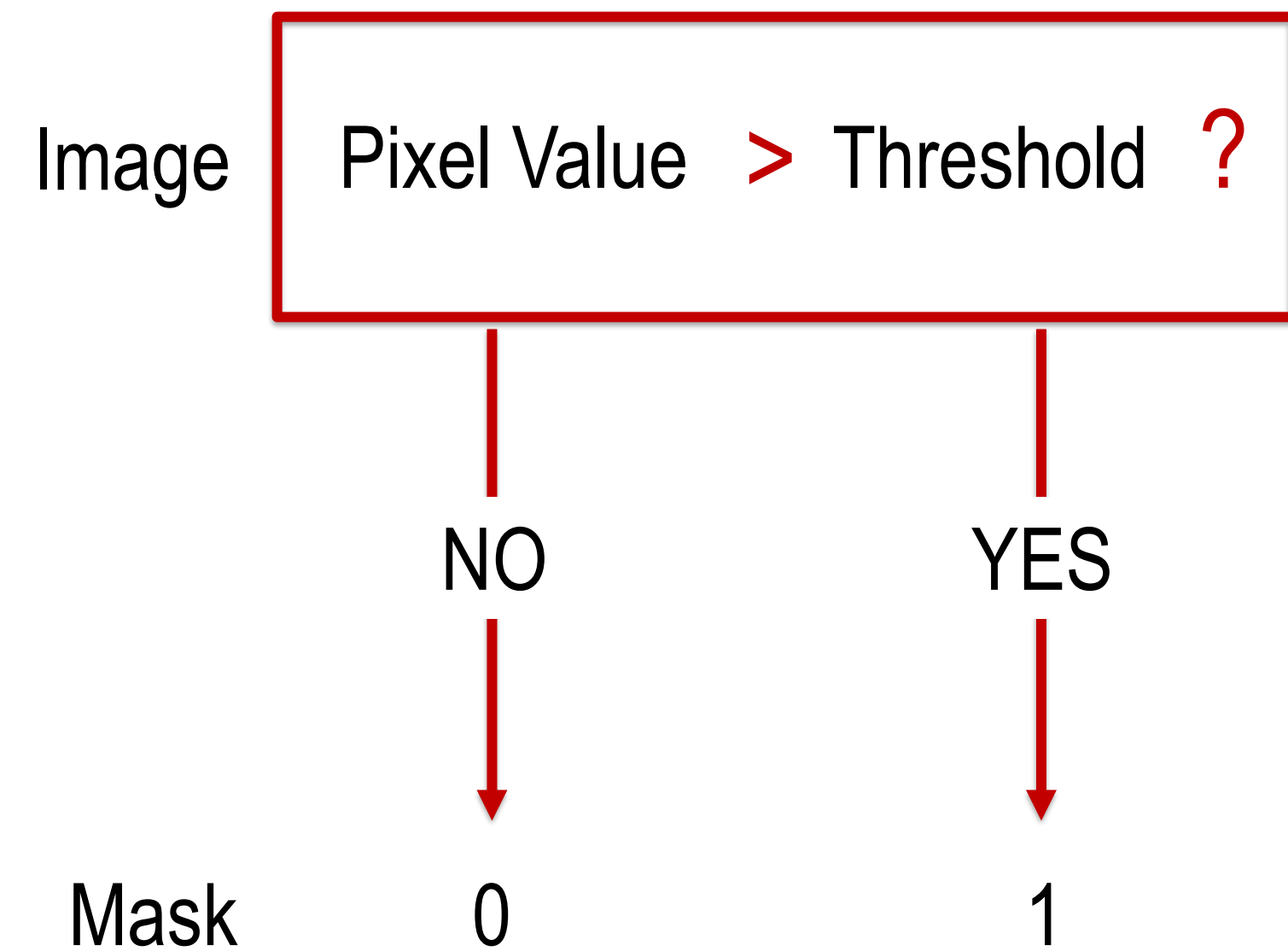
- Thresholding
- Clustering
- Region Growing
- Machine Learning

- Simple Method

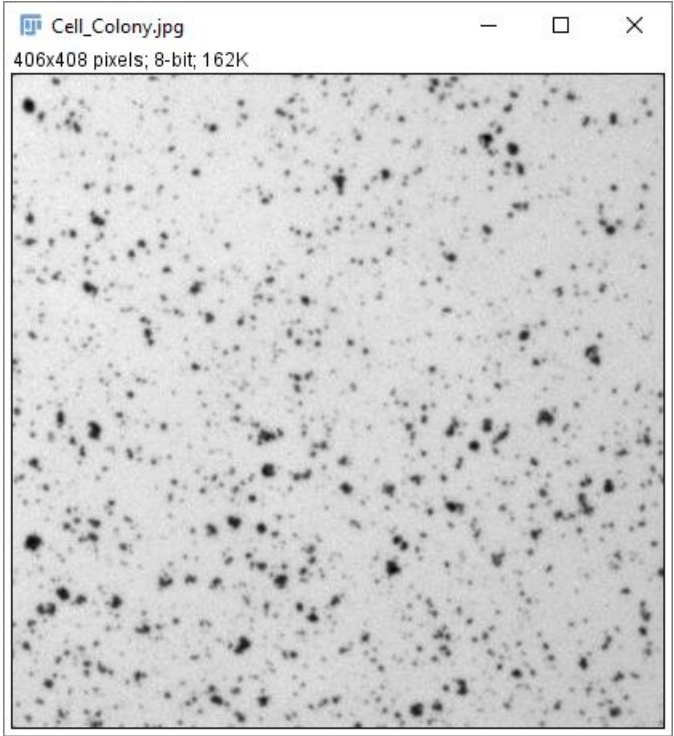
Image

Pixel Value $>$ Threshold ?

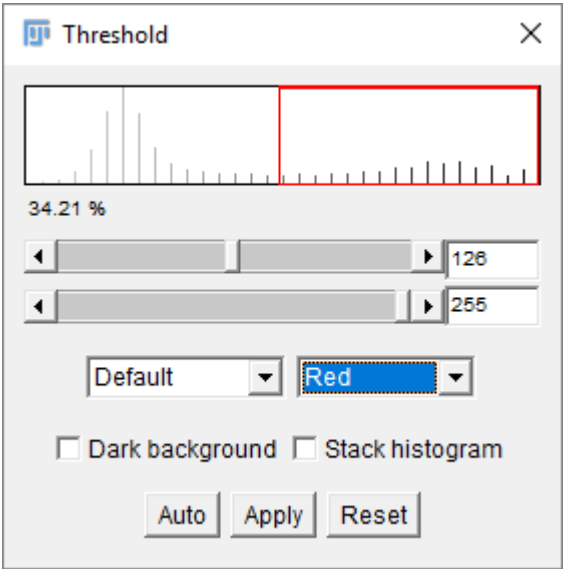
- Simple Method



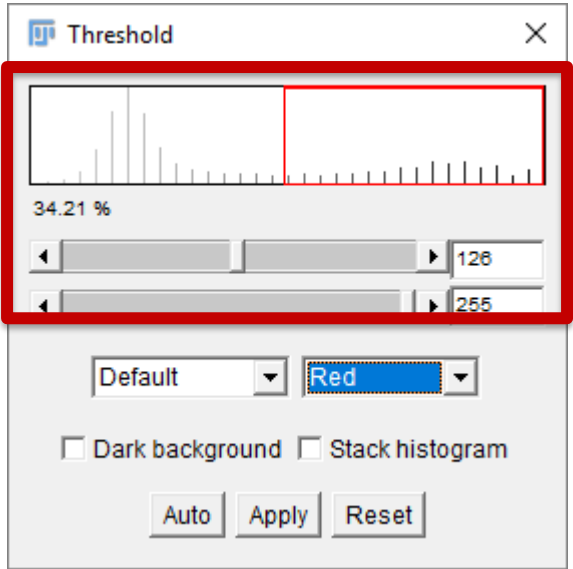
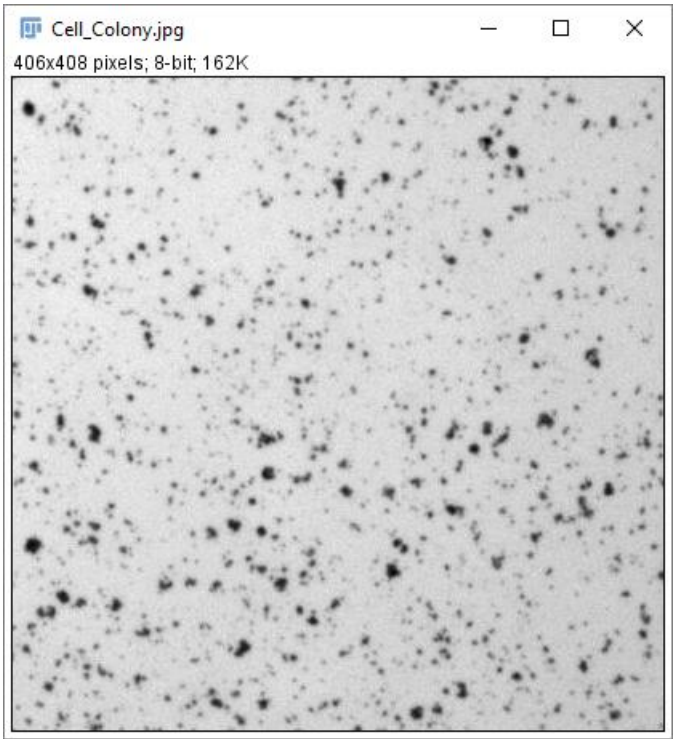
Thresholding



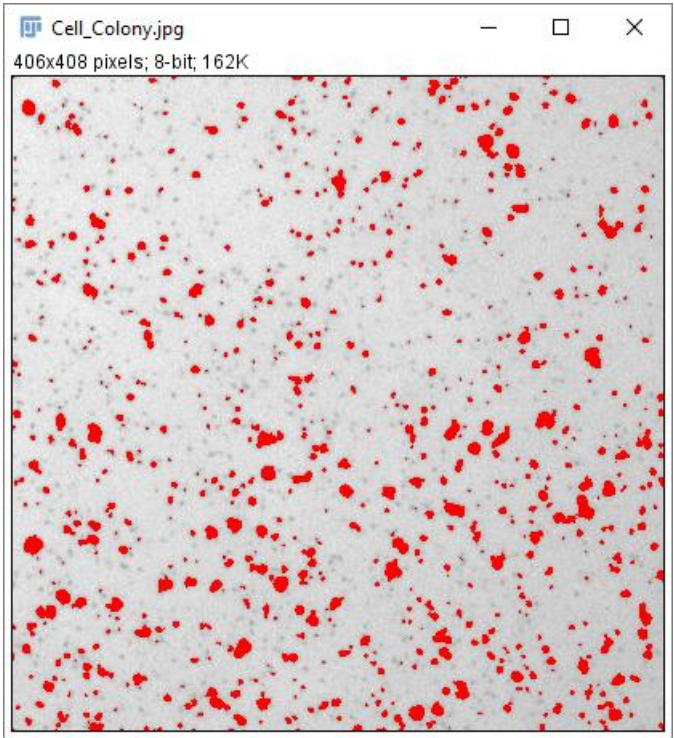
Images



Thresholding

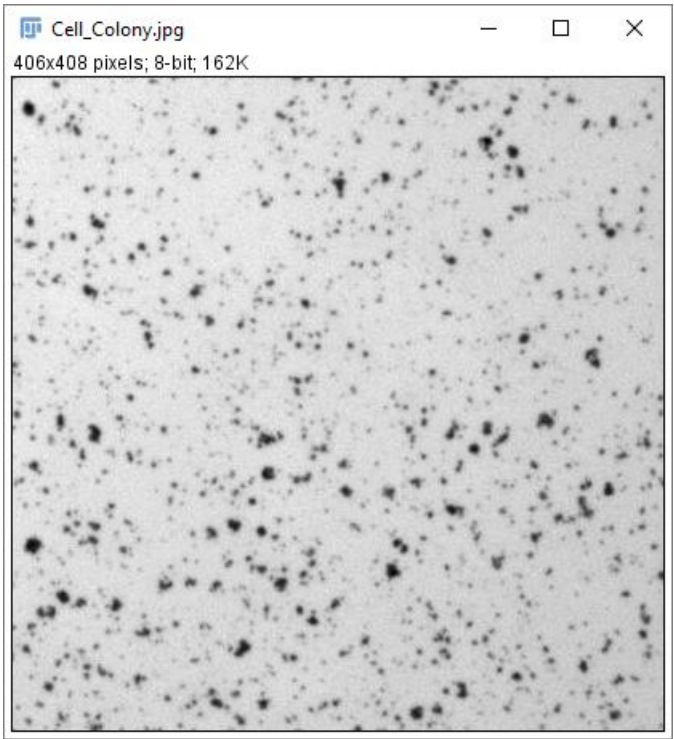


Images

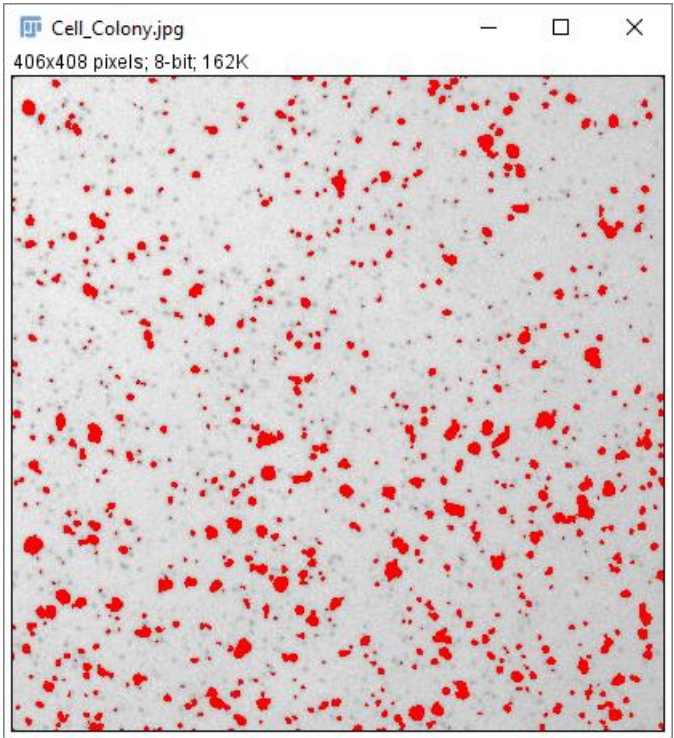
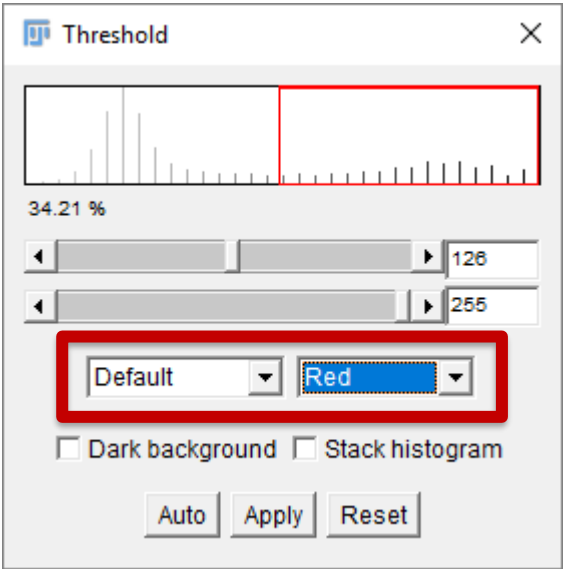


Preview

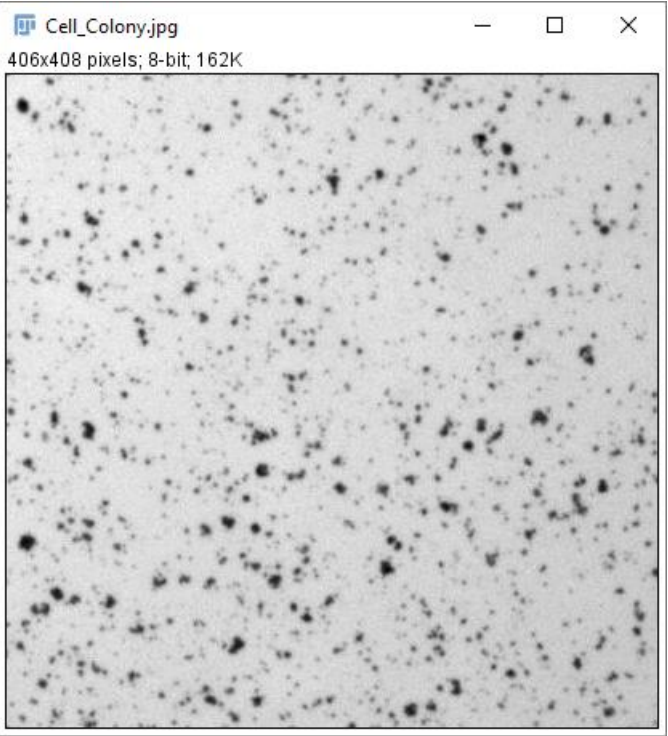
Thresholding



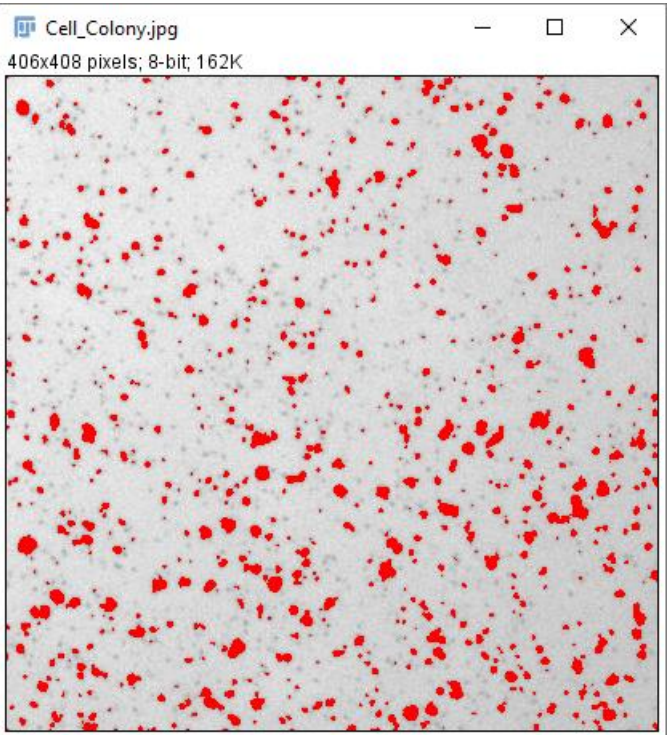
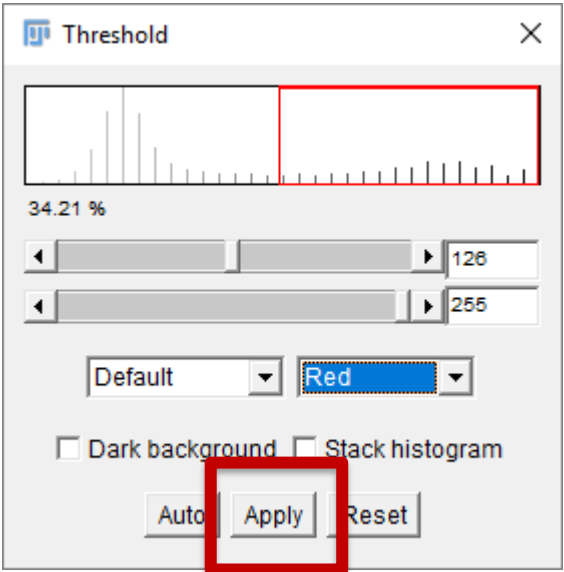
Images



Preview

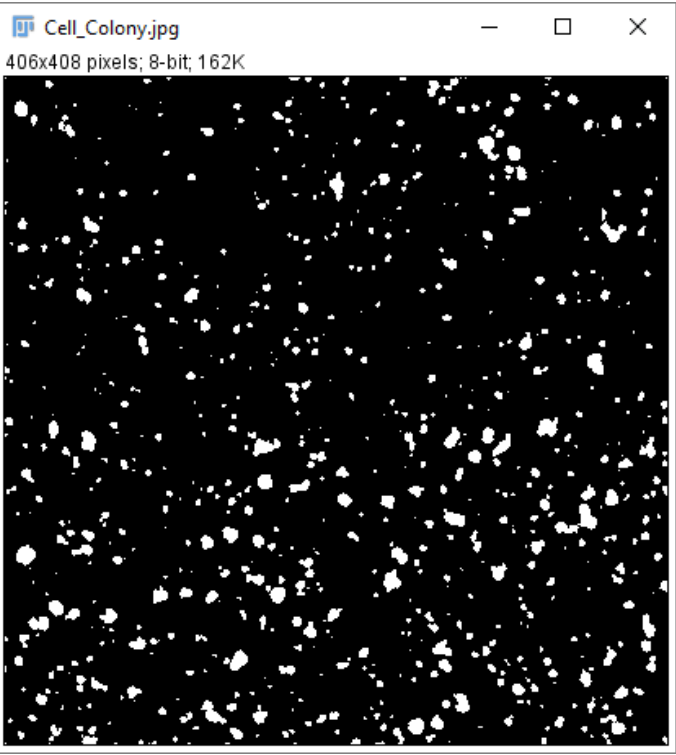


Images



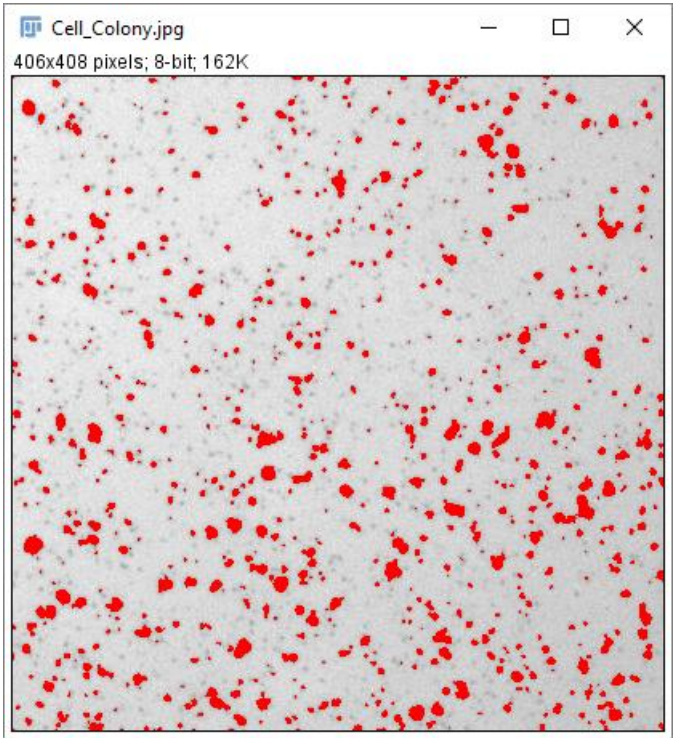
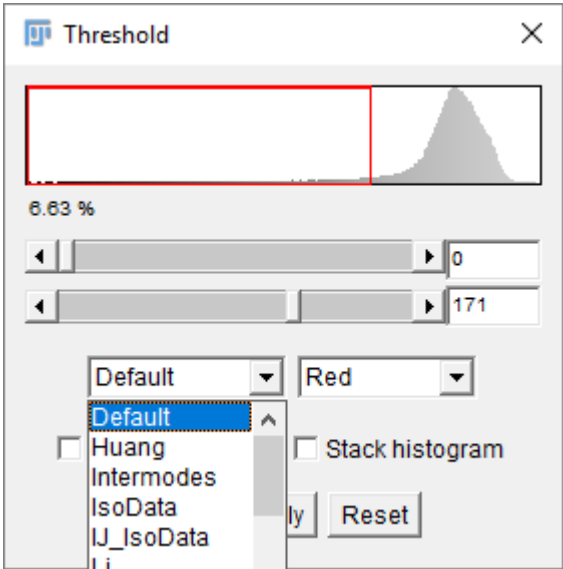
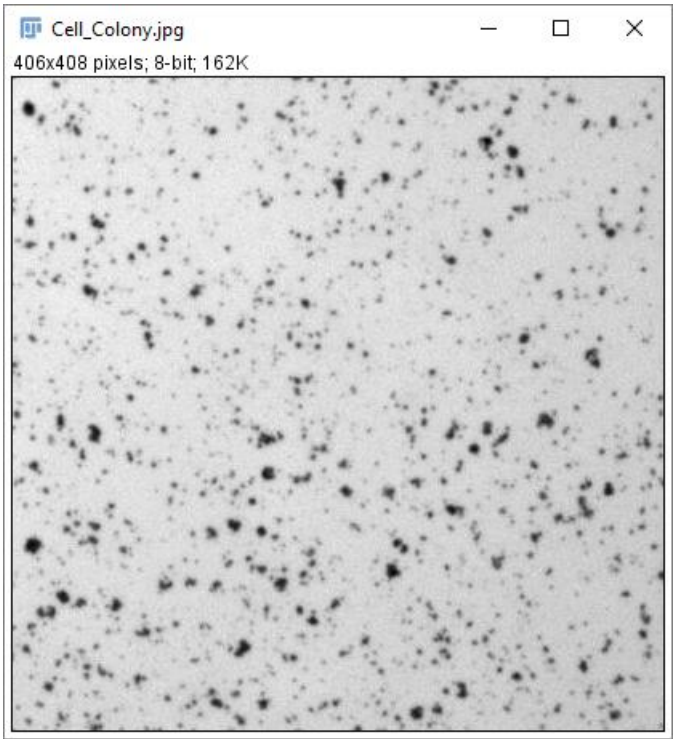
Preview

Apply
→

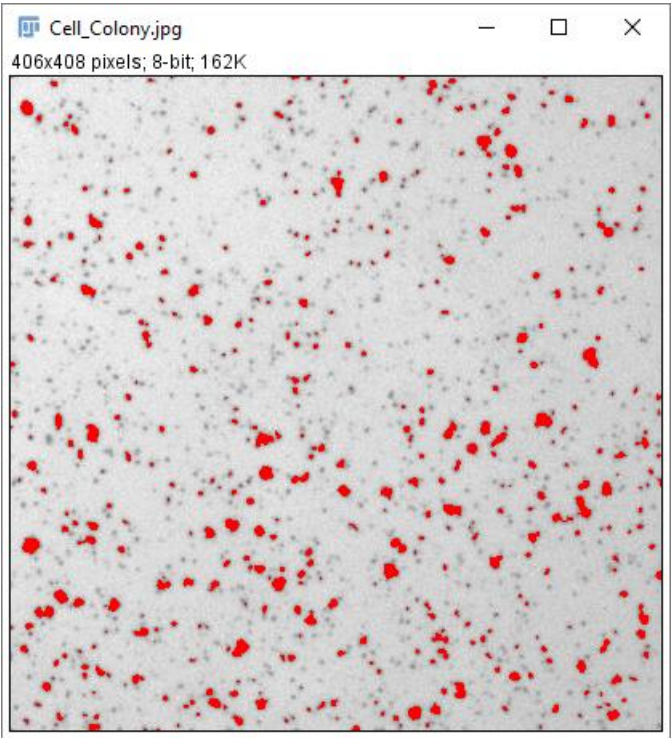


Mask

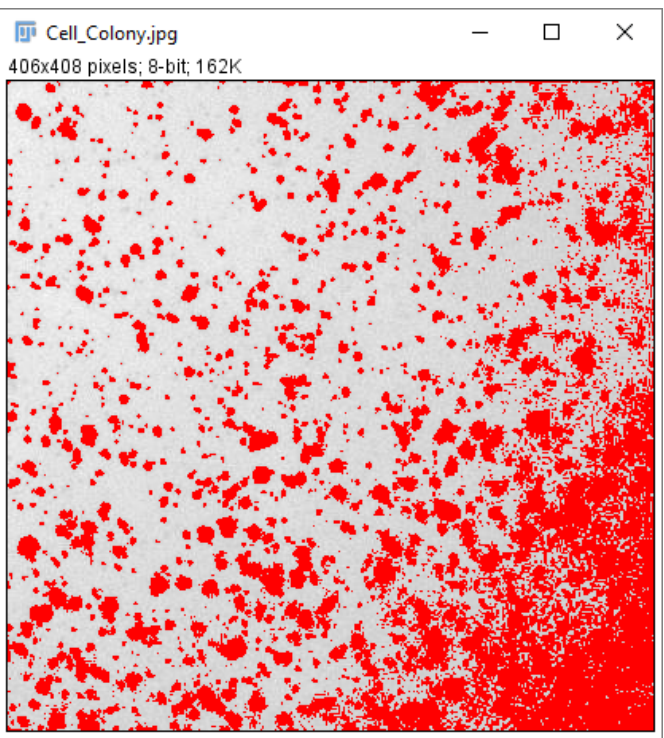
Different Methods



Default

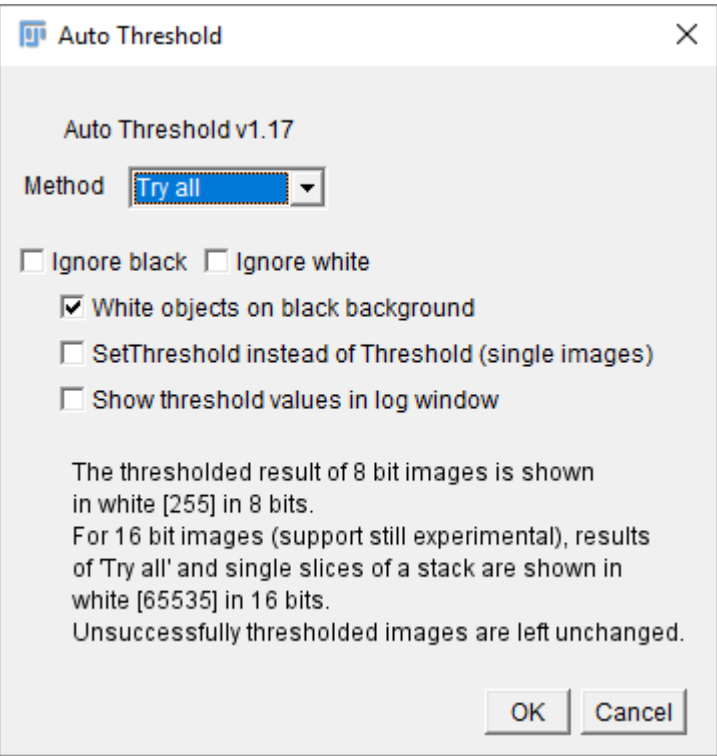
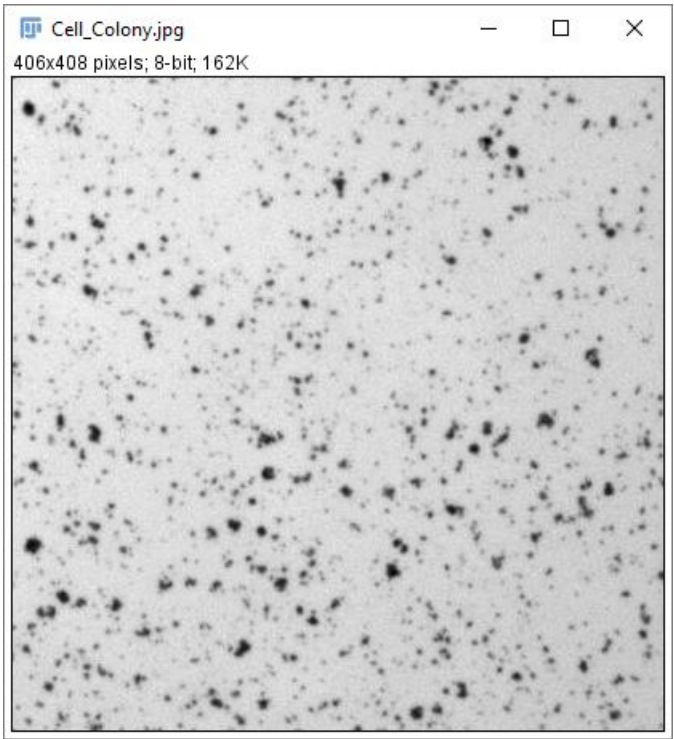


Intermodes

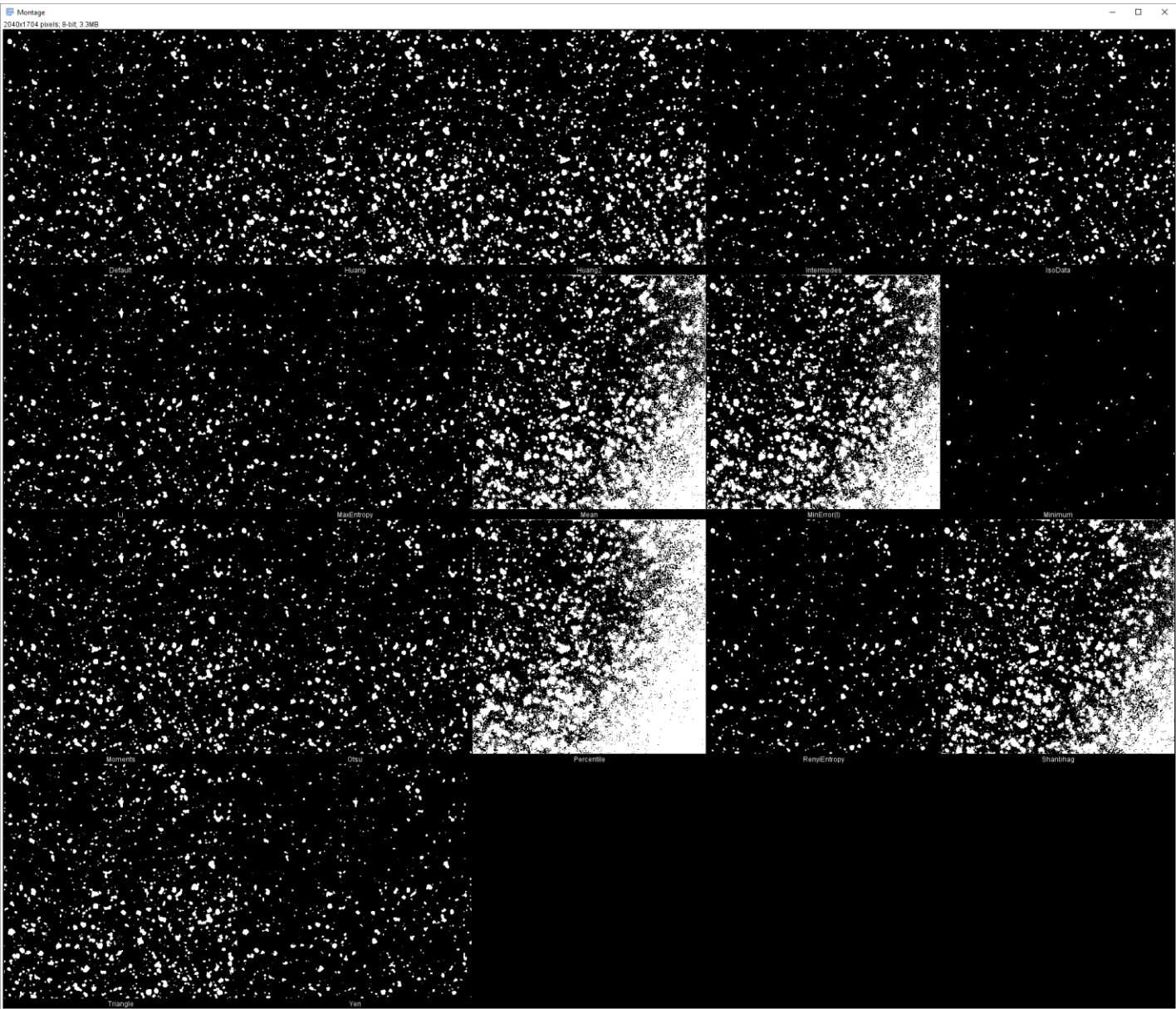
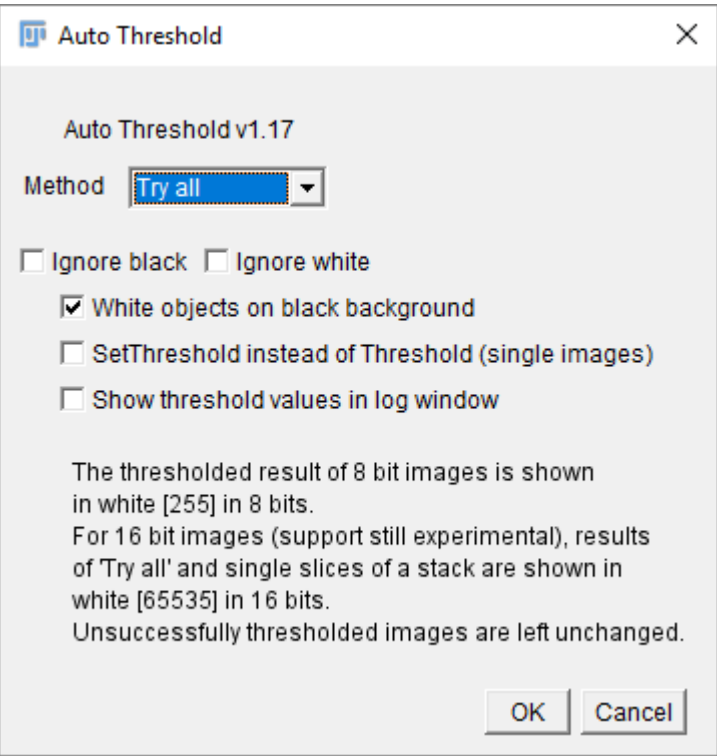
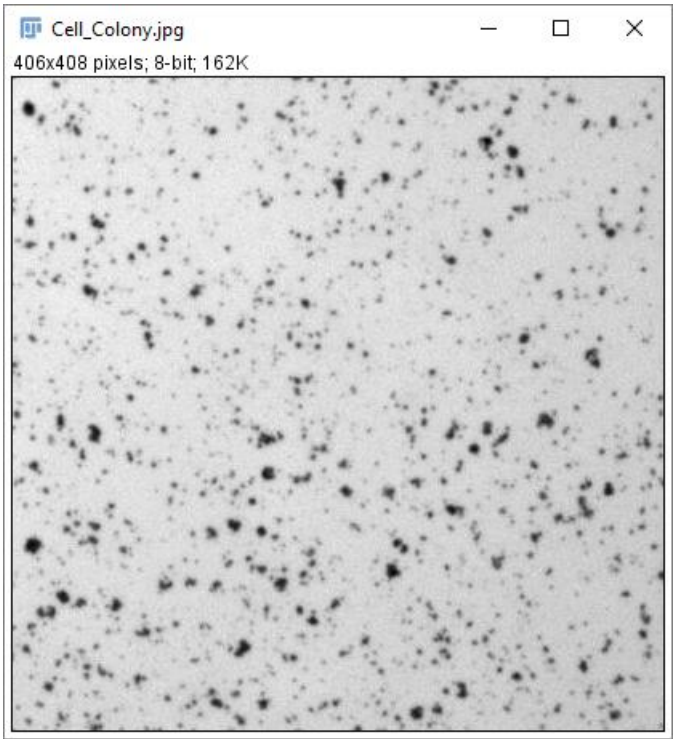


Shanbhag

How to choose ?

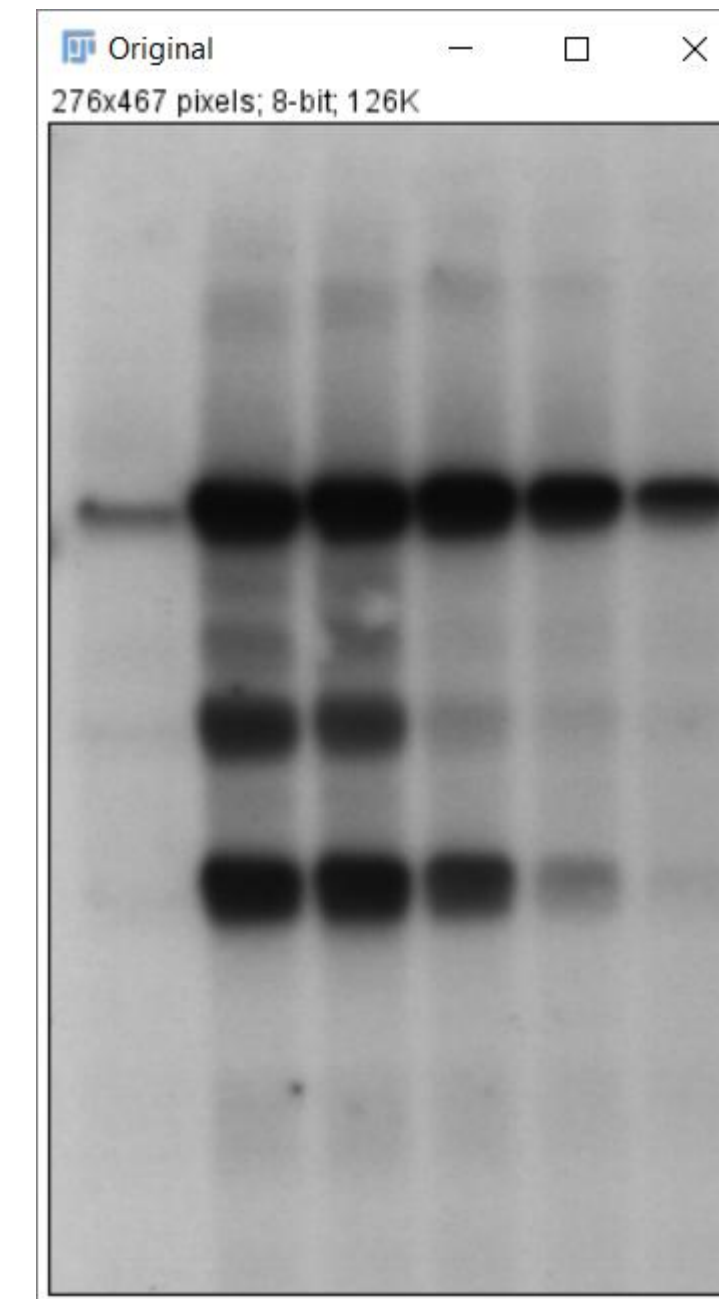


How to choose ?

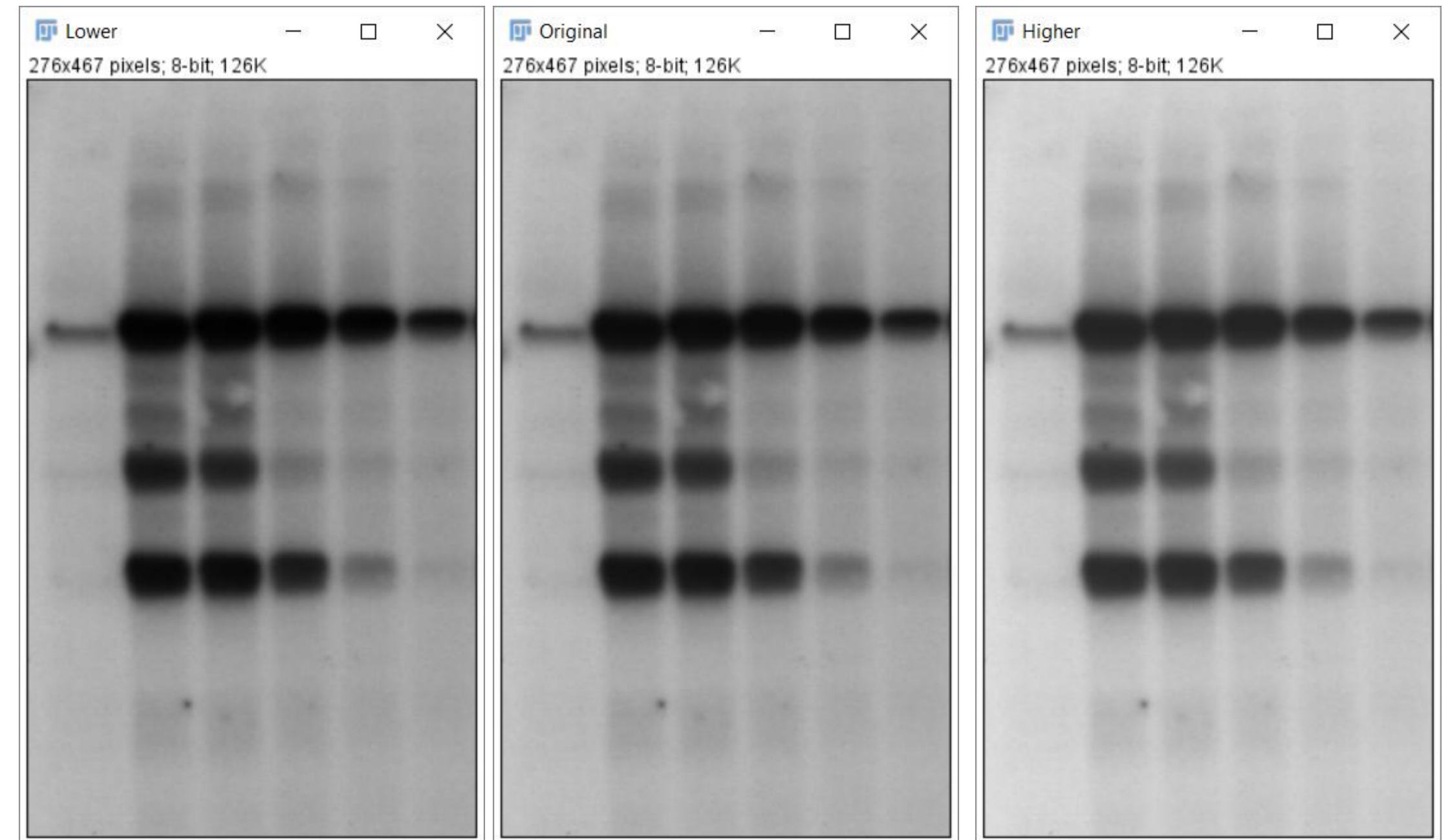


Automatic Threshold : Why using them ?

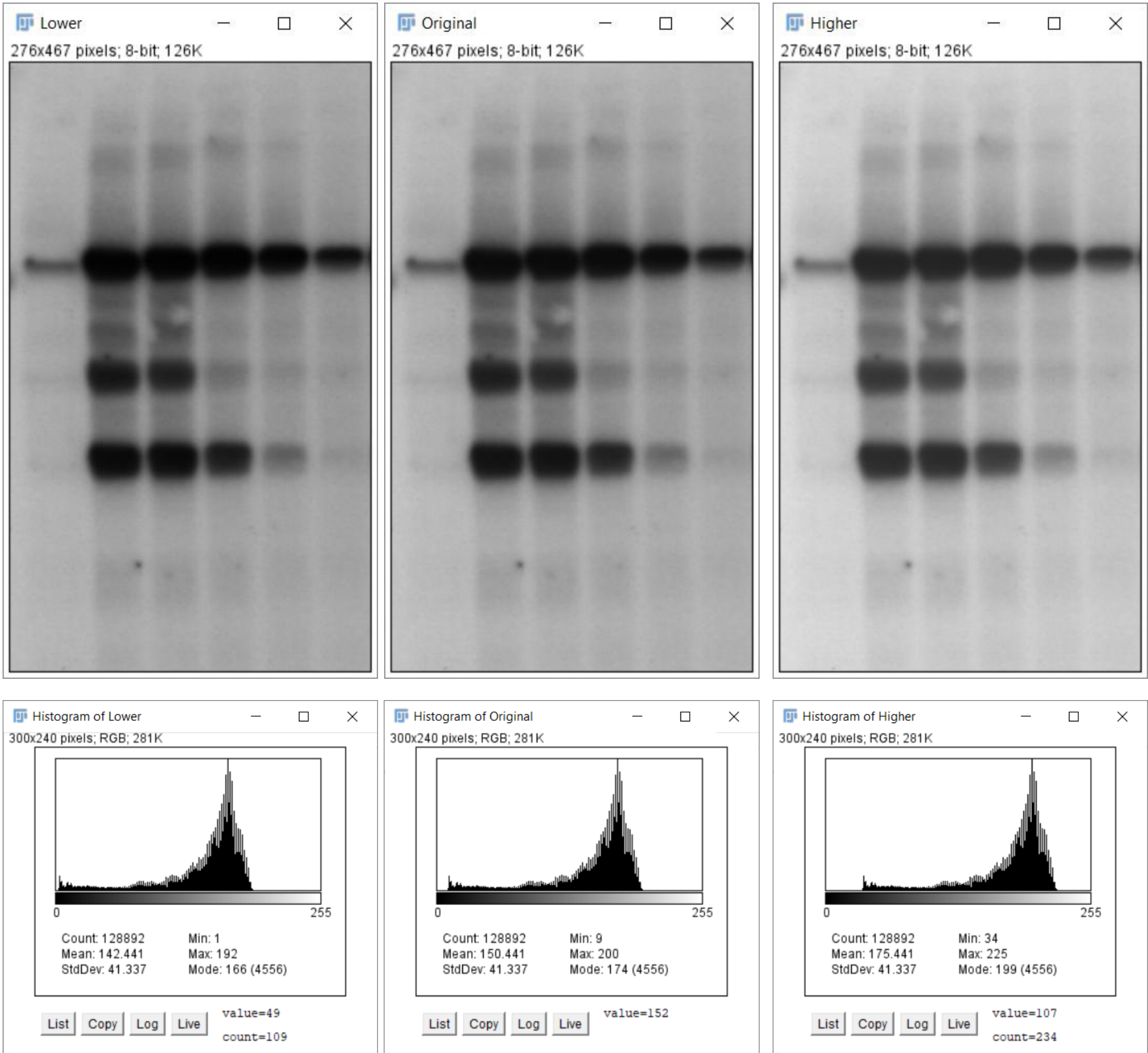
Automatic Threshold : Why using them ?



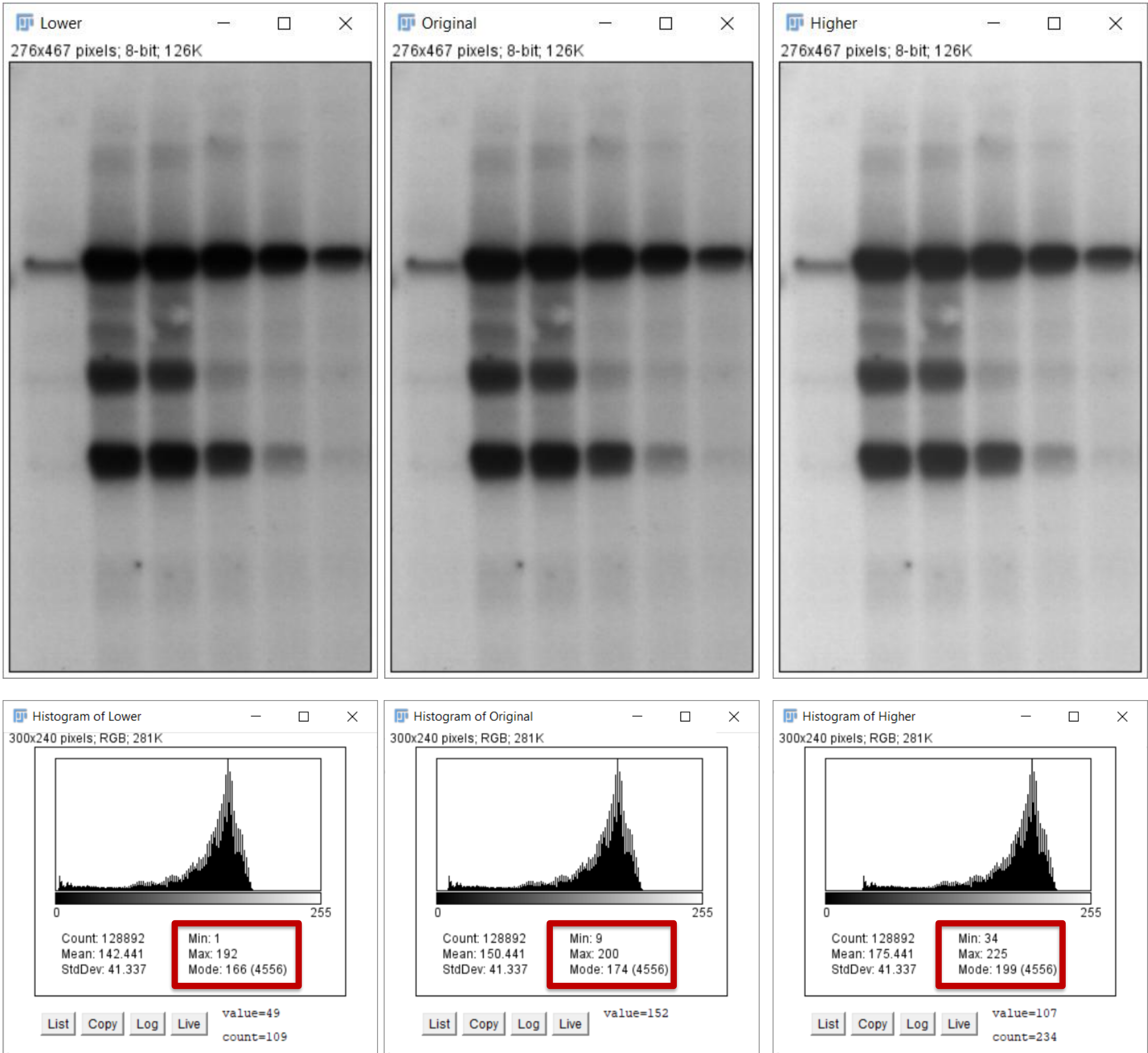
Automatic Threshold : Why using them ?



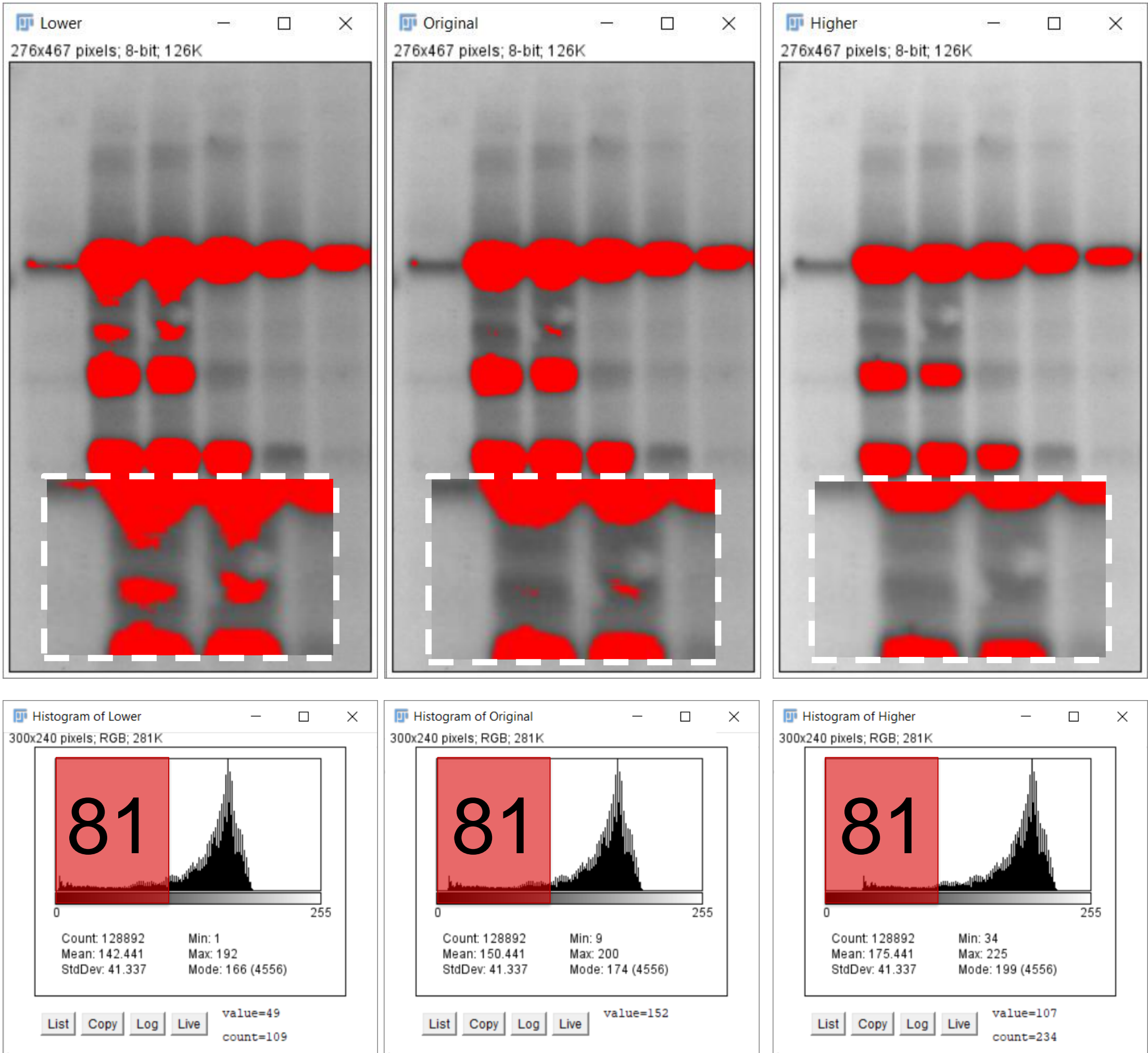
Automatic Threshold : Why using them ?



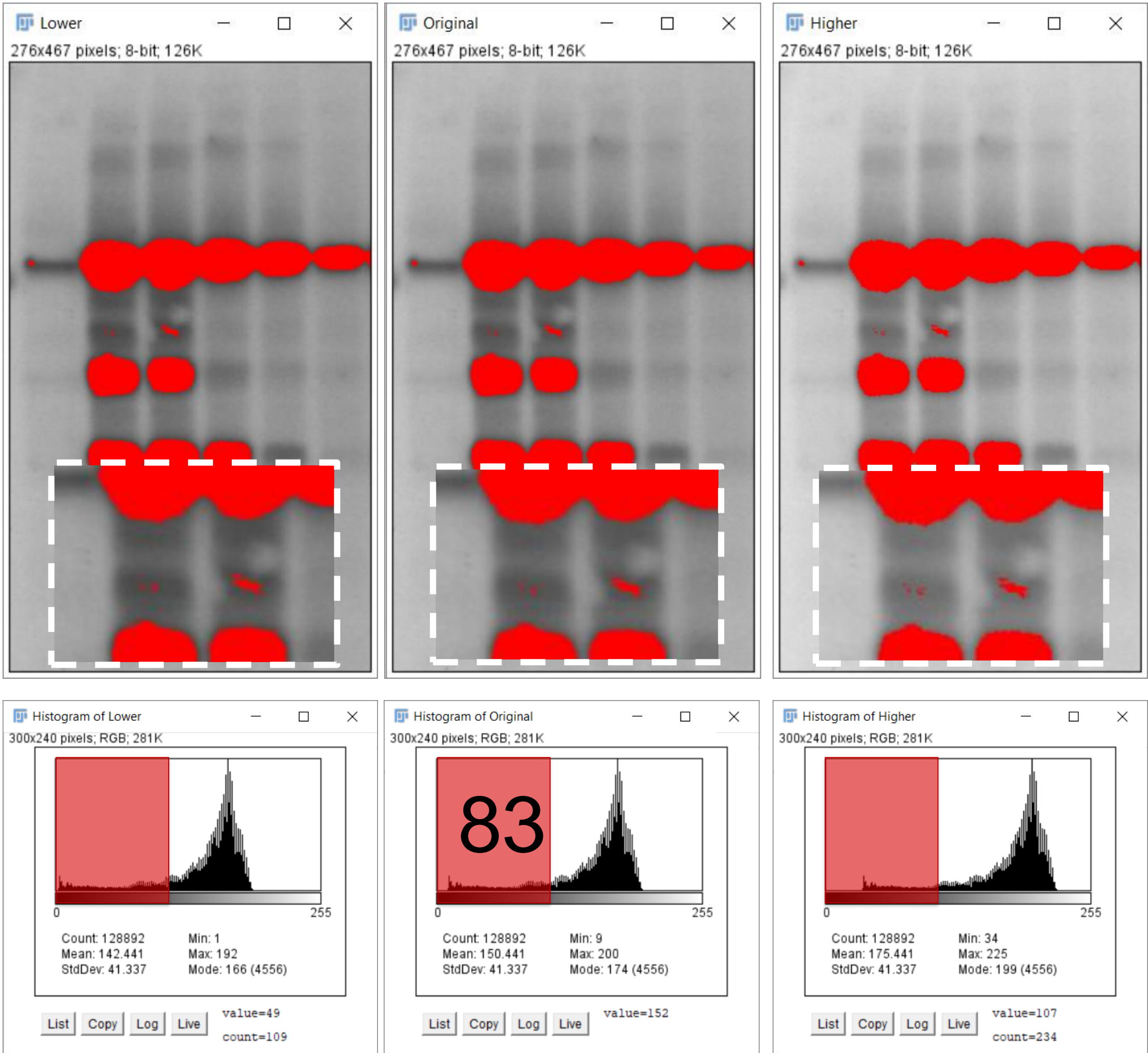
Automatic Threshold : Why using them ?



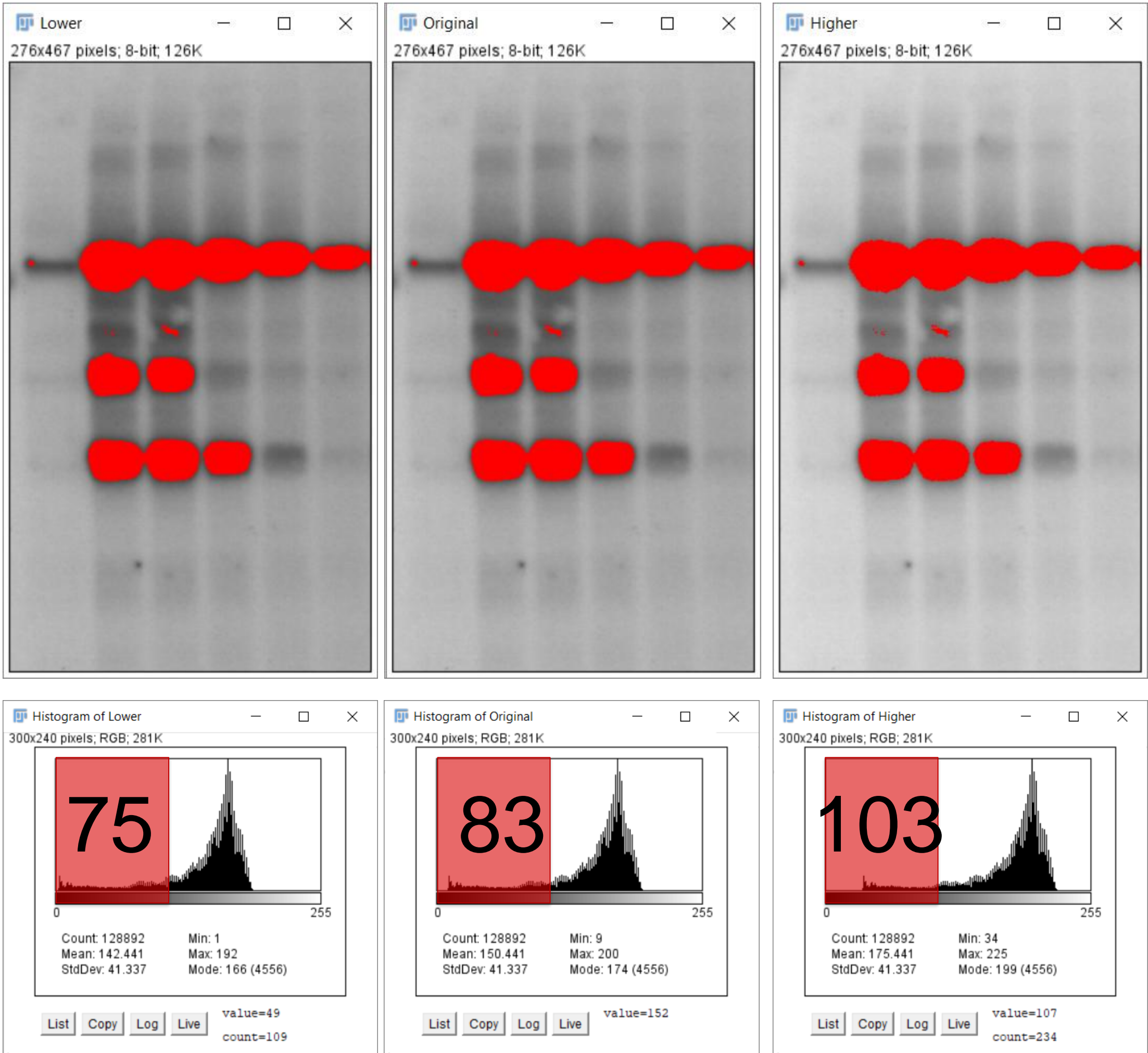
Automatic Threshold : Why using them ?



Automatic Threshold : Why using them ?

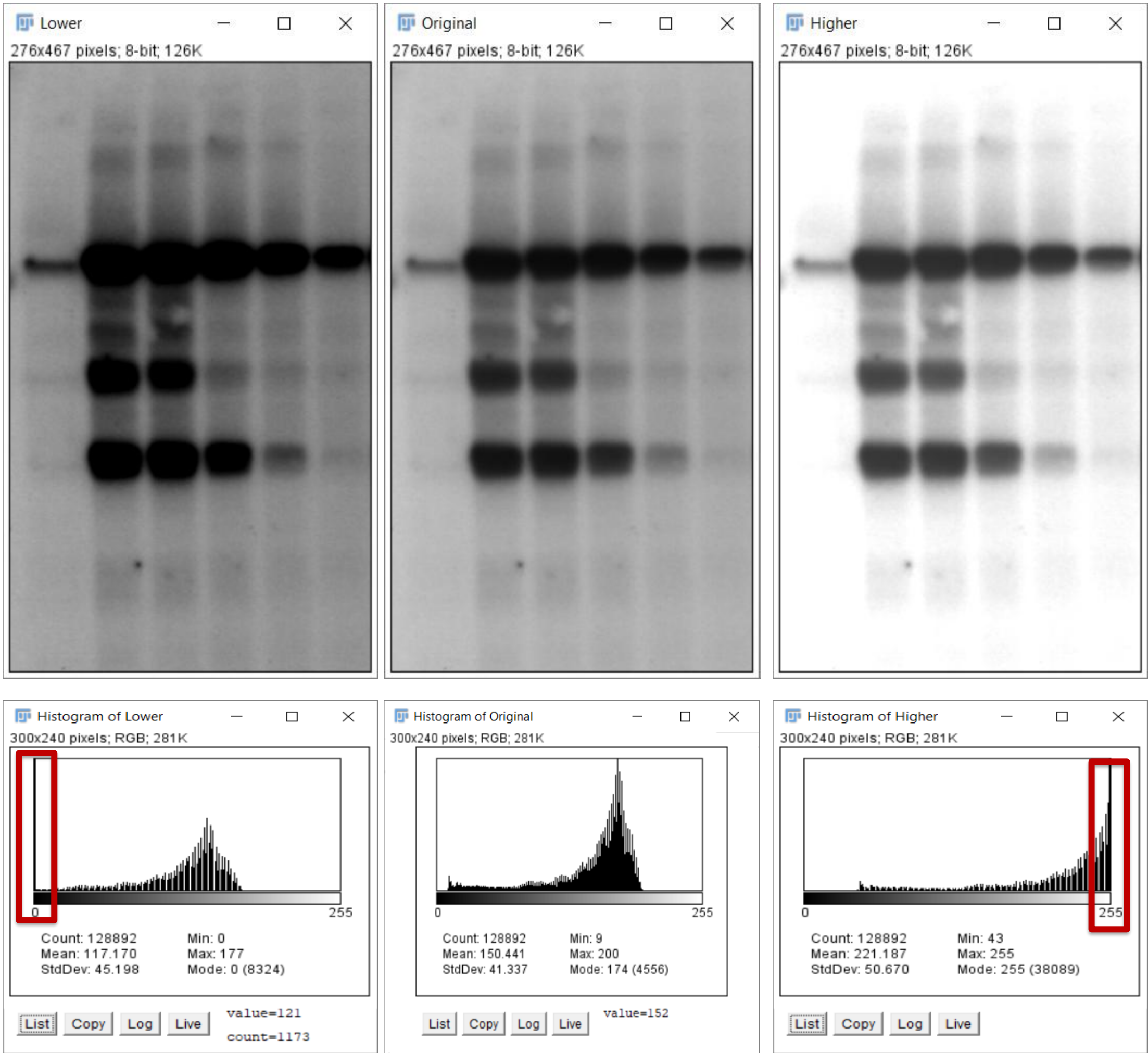


Automatic Threshold : Why using them ?

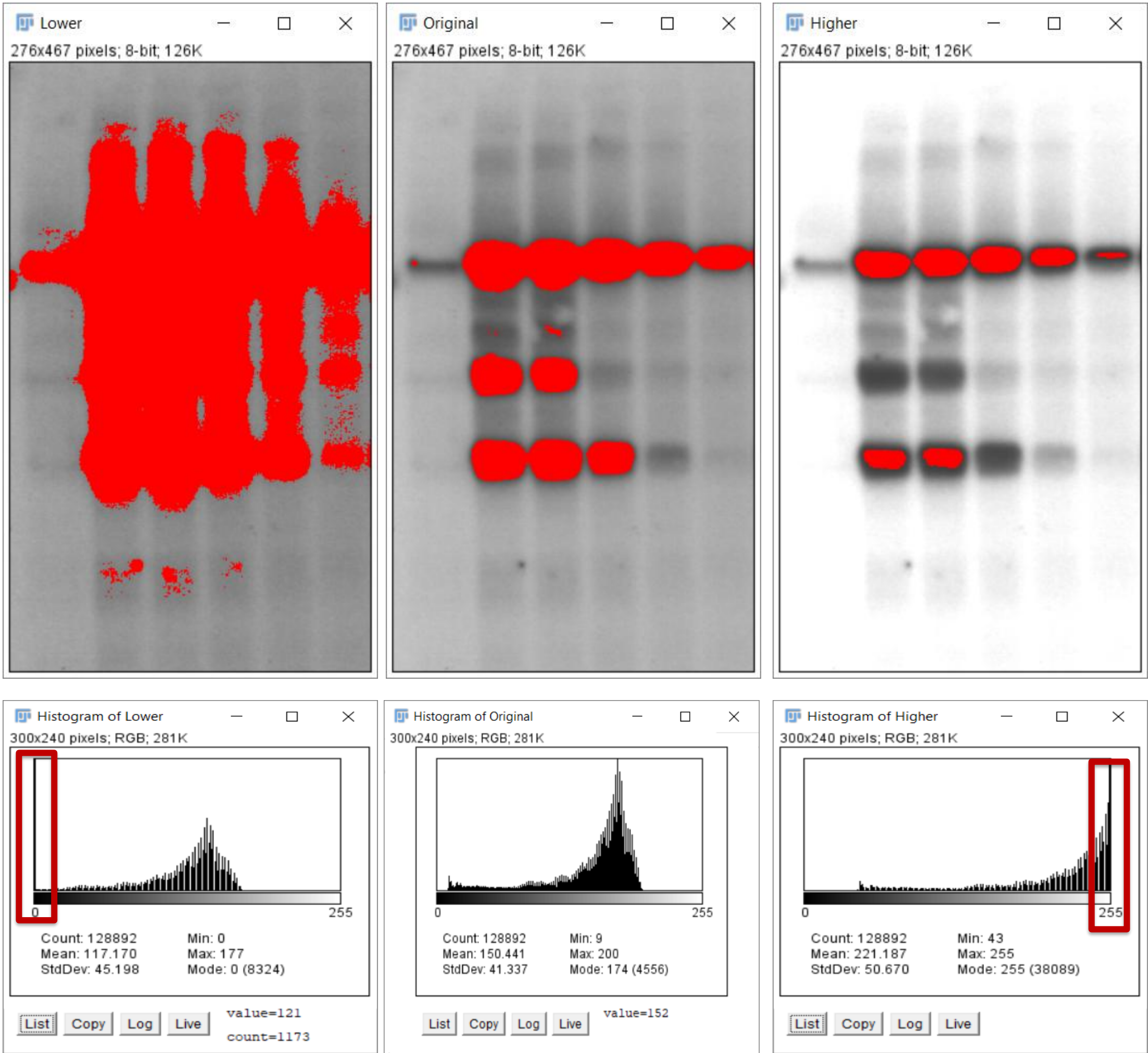


Automatic Threshold : Limitations

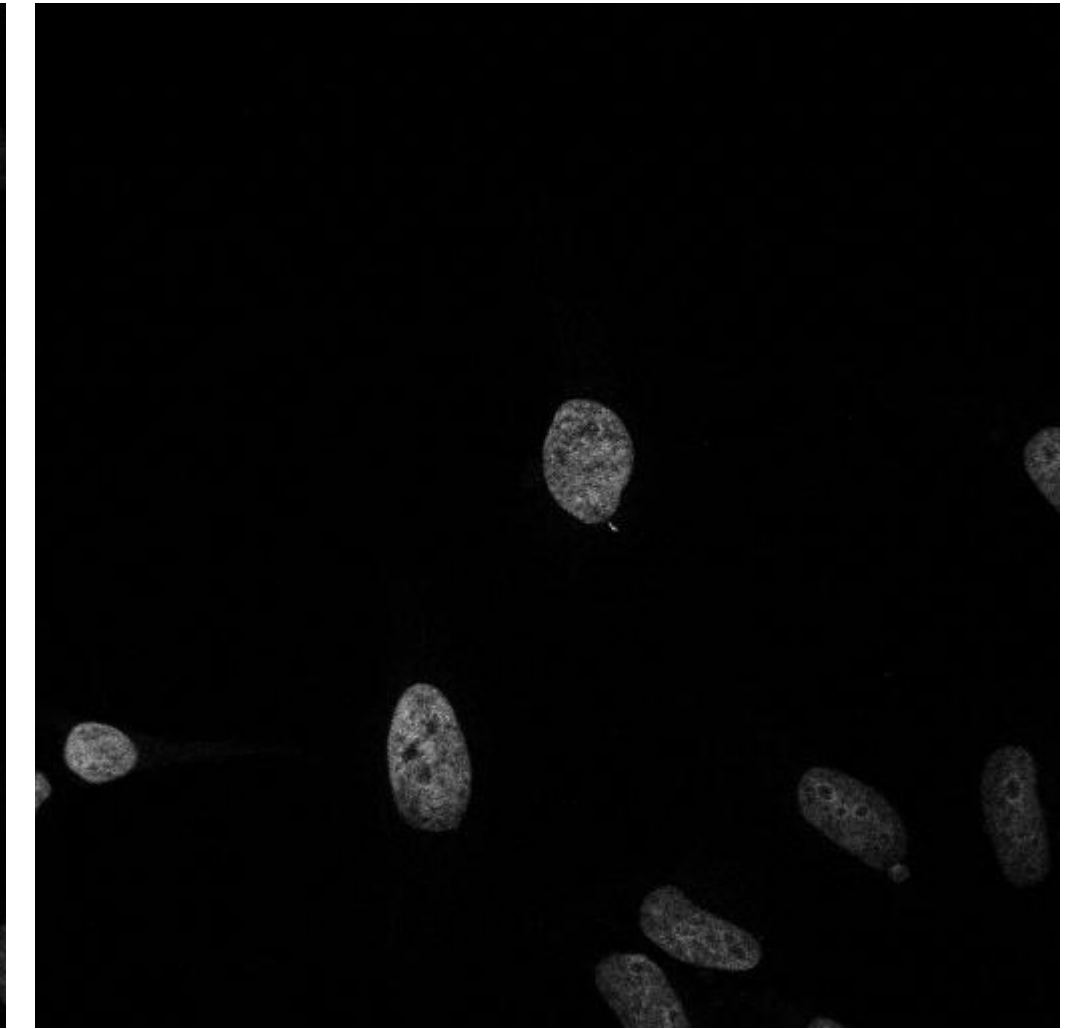
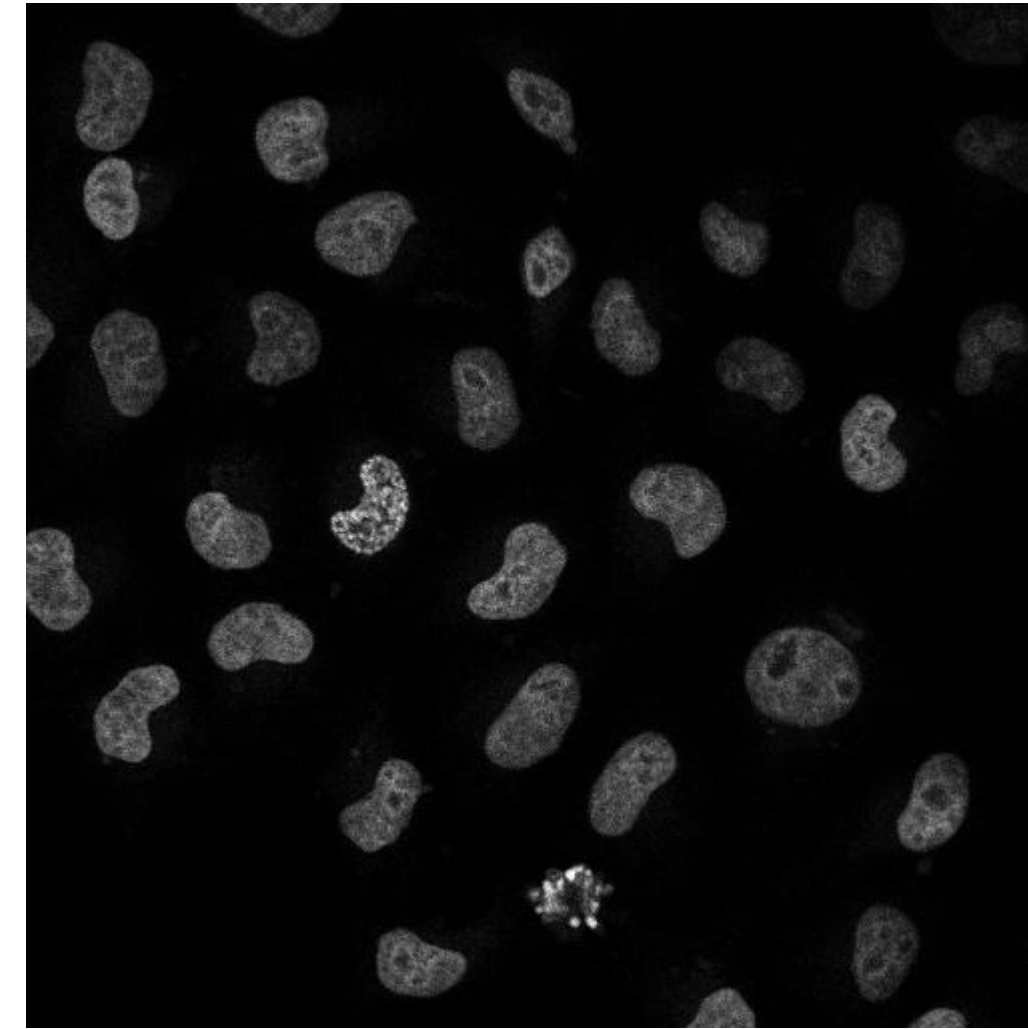
Automatic Thresholding : Limitations



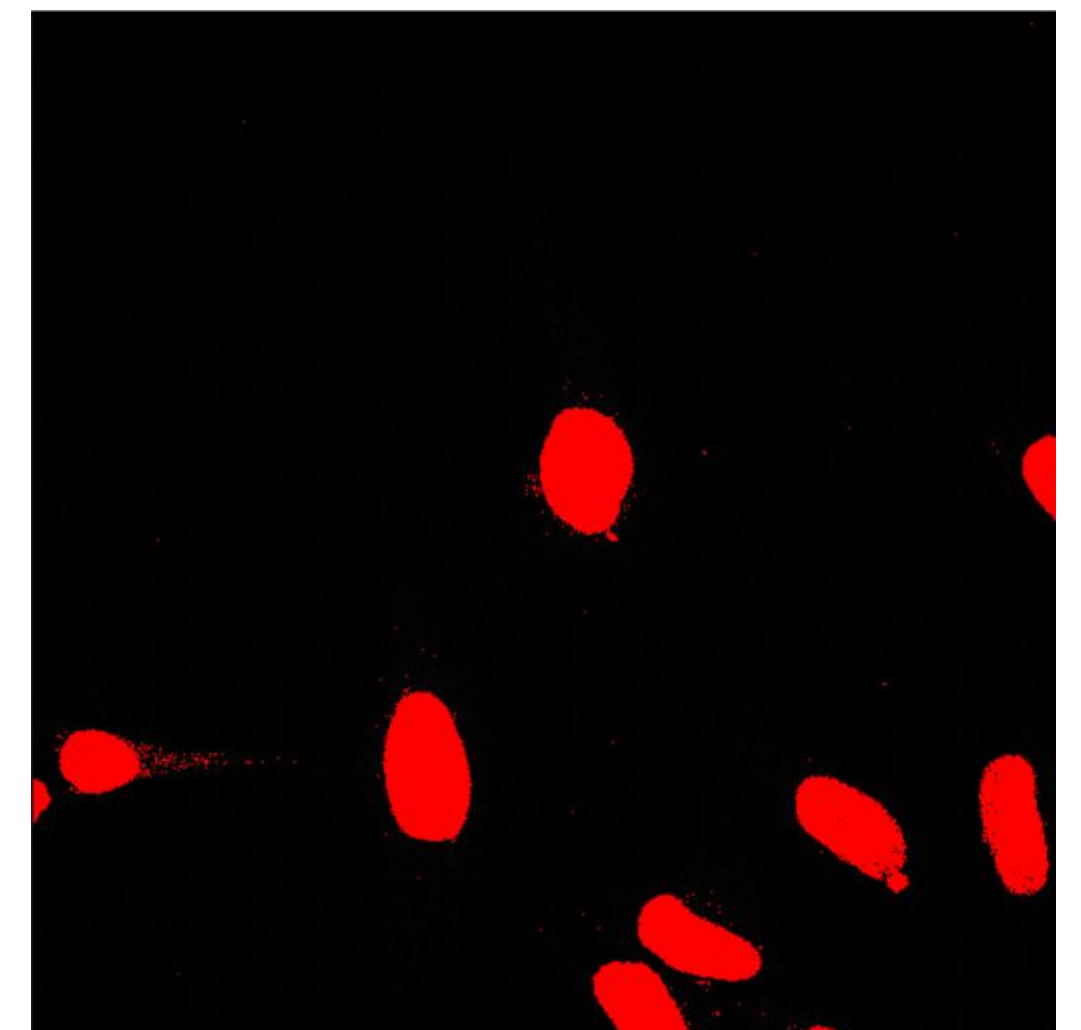
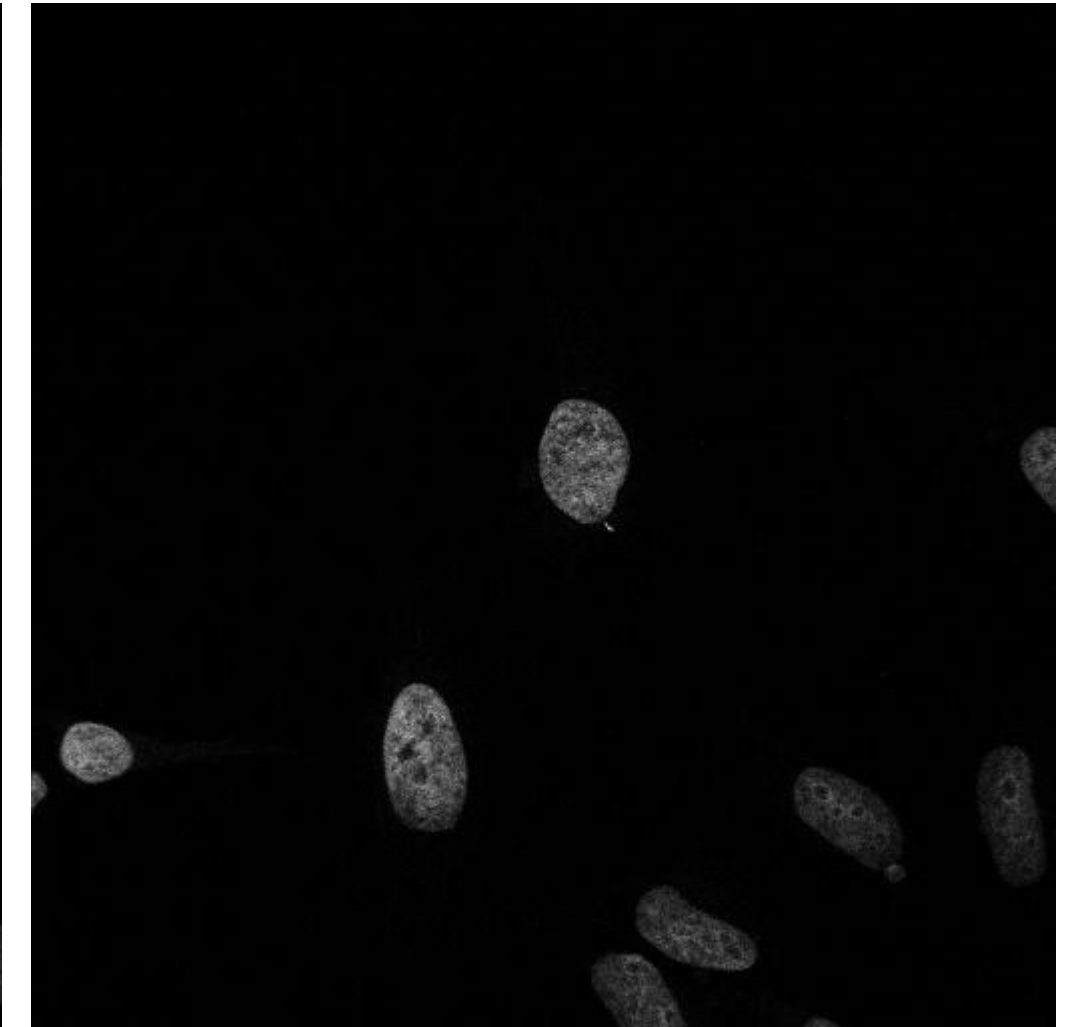
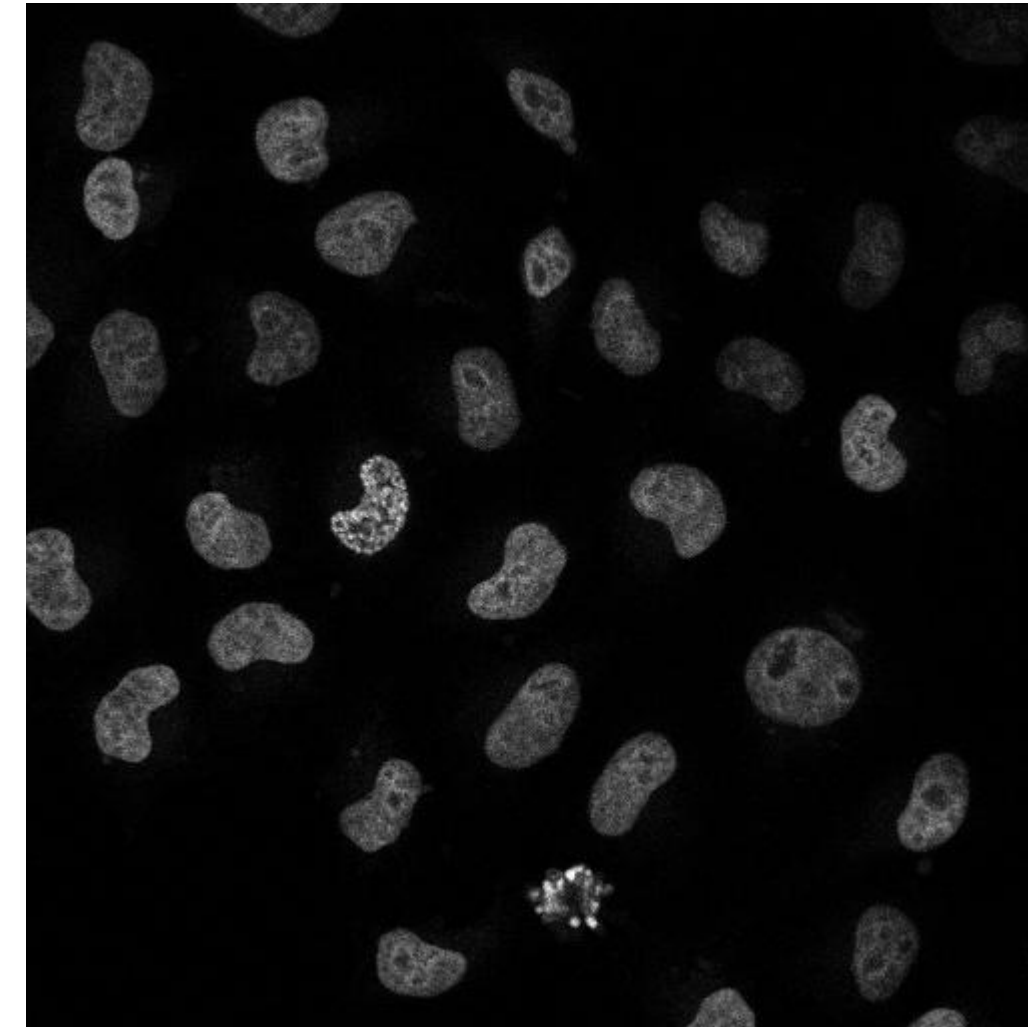
Automatic Thresholding : Limitations



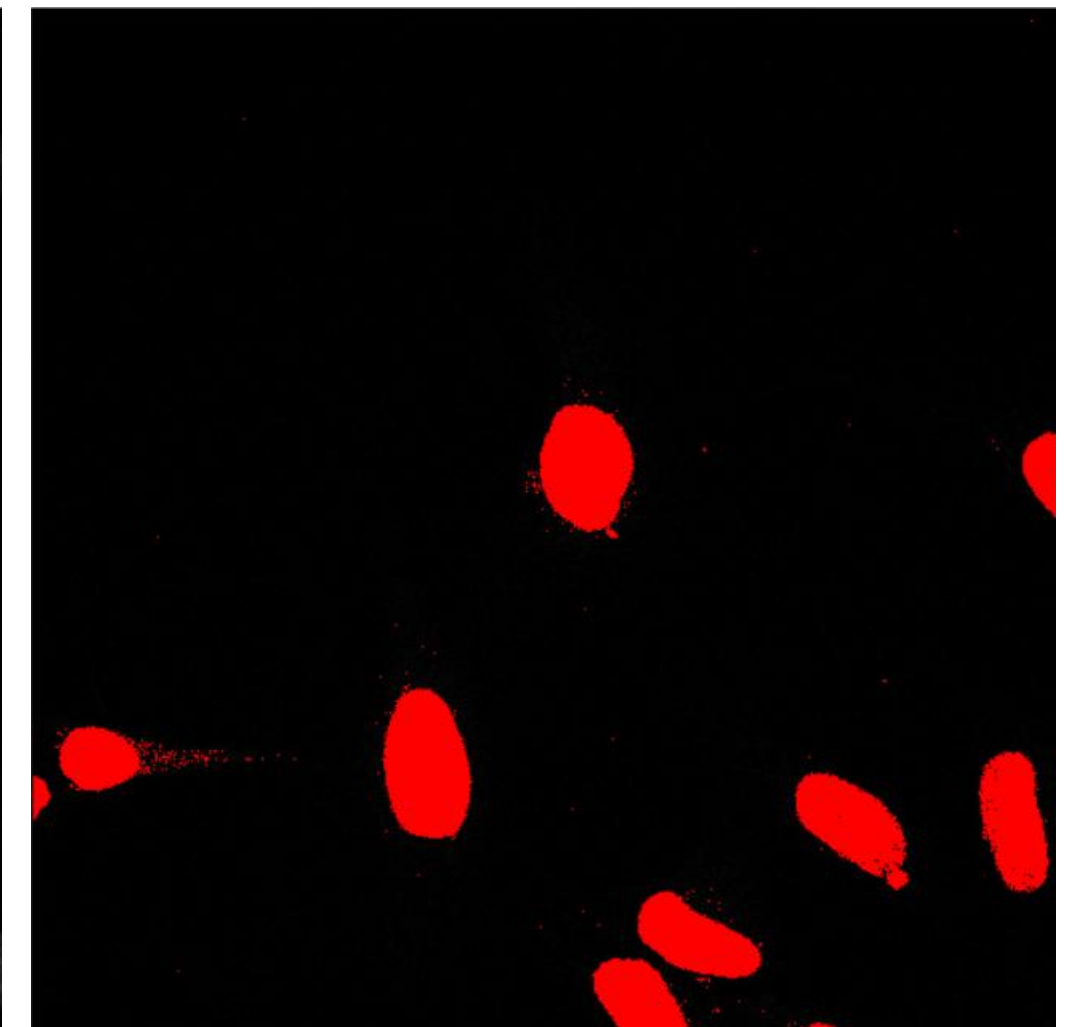
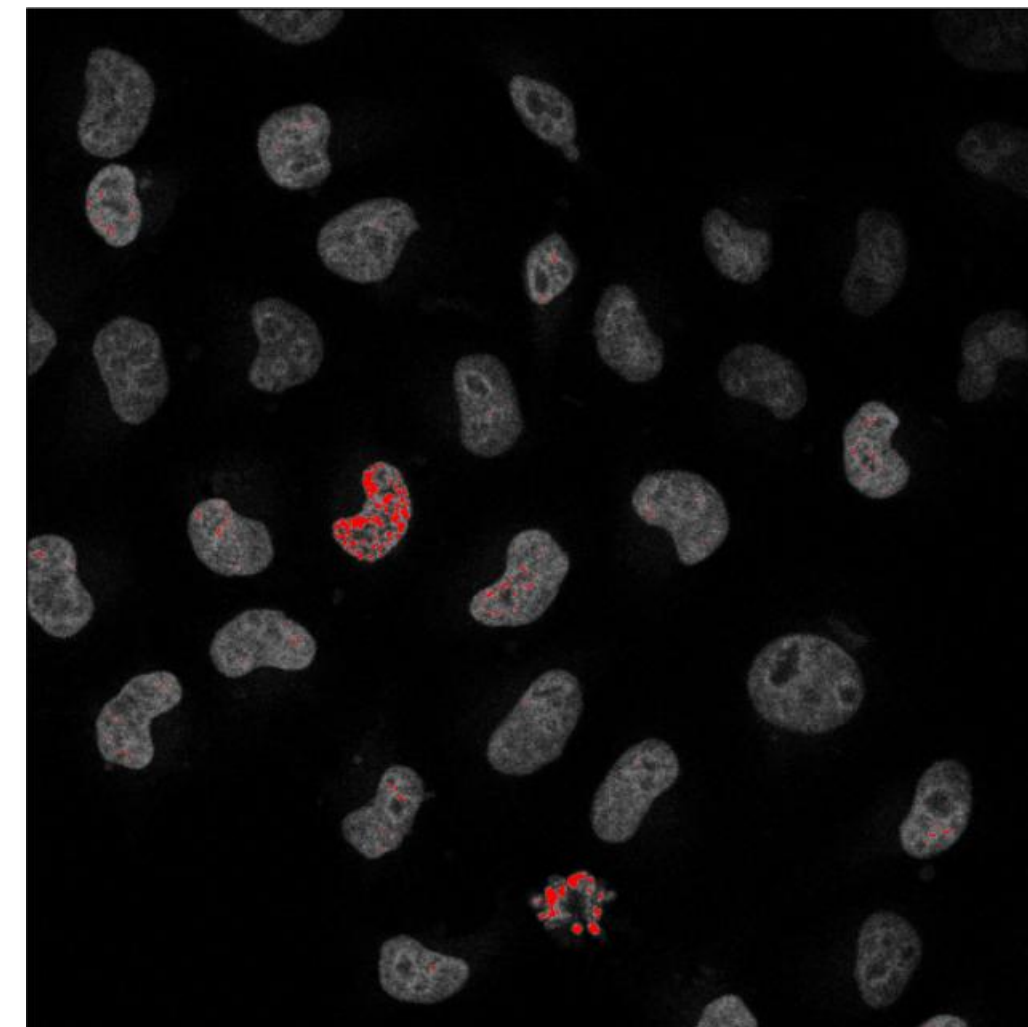
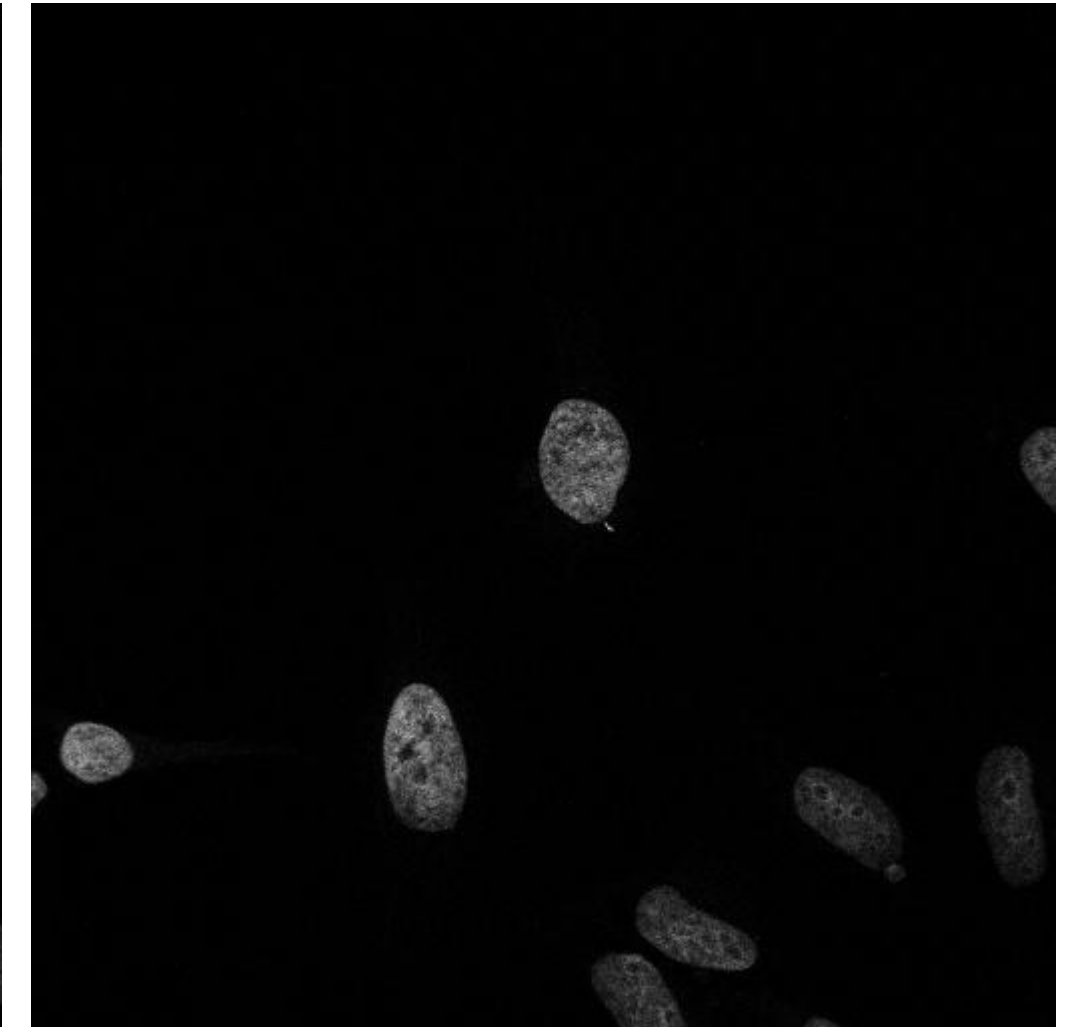
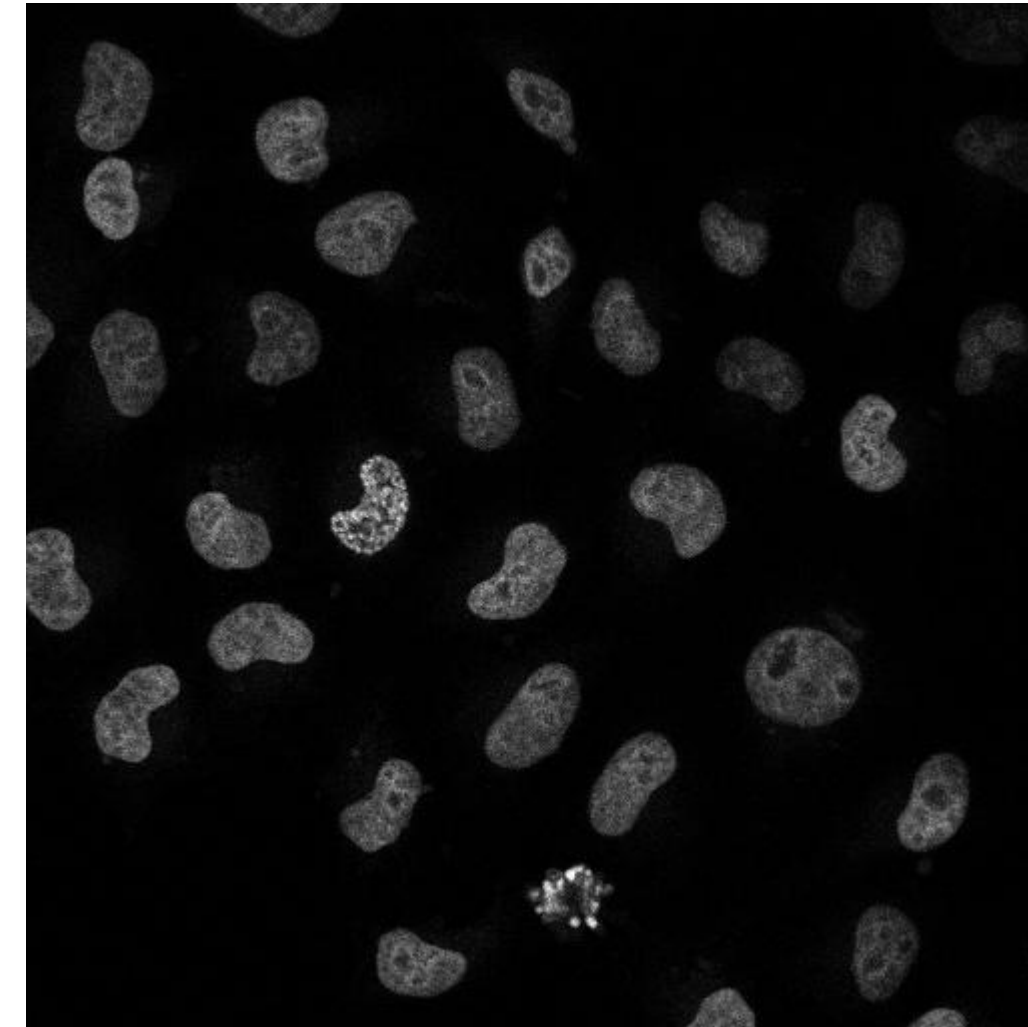
Automatic Thresholding : Limitations



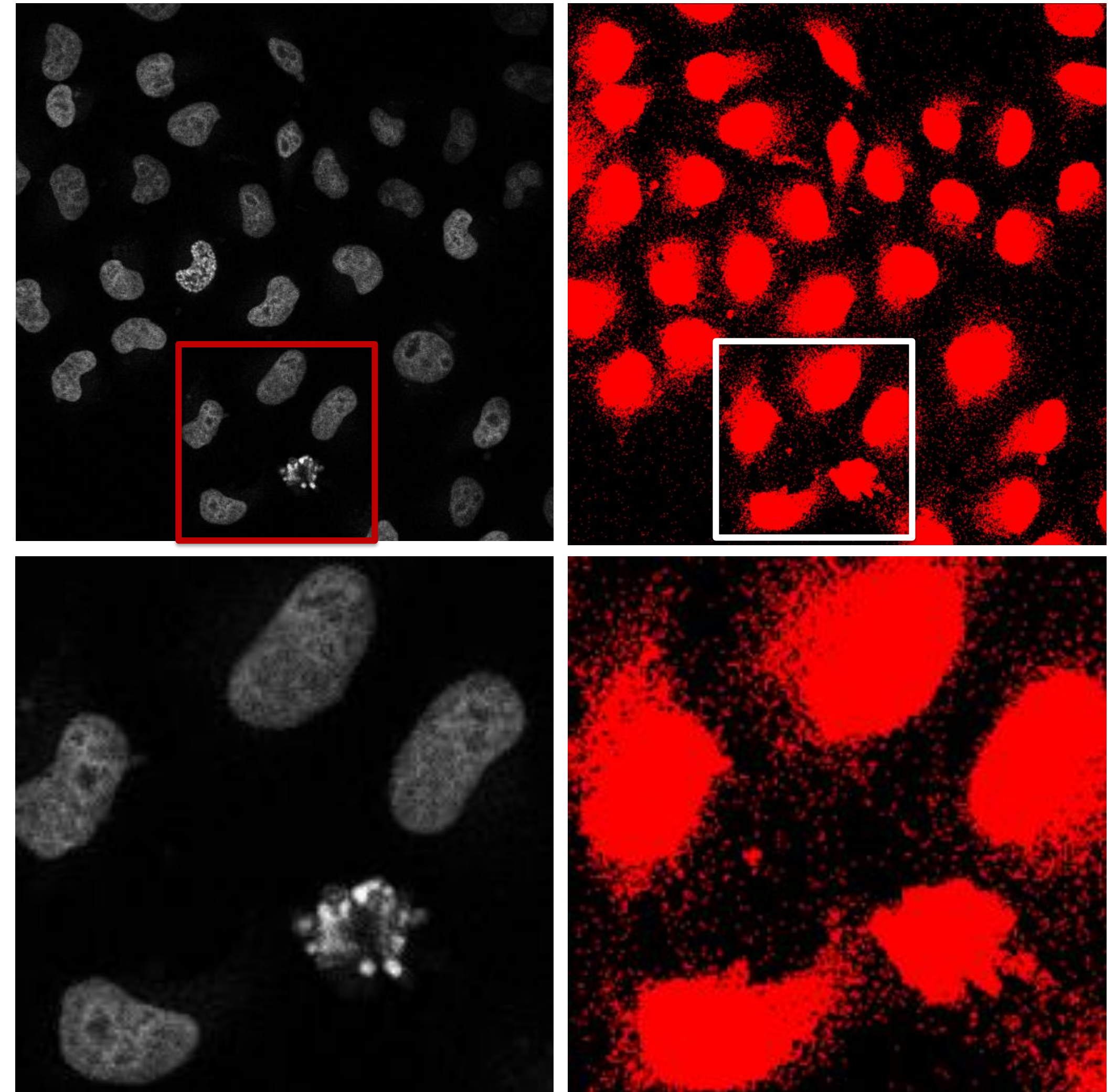
Automatic Thresholding : Limitations



Automatic Thresholding : Limitations



Automatic Thresholding : Limitations

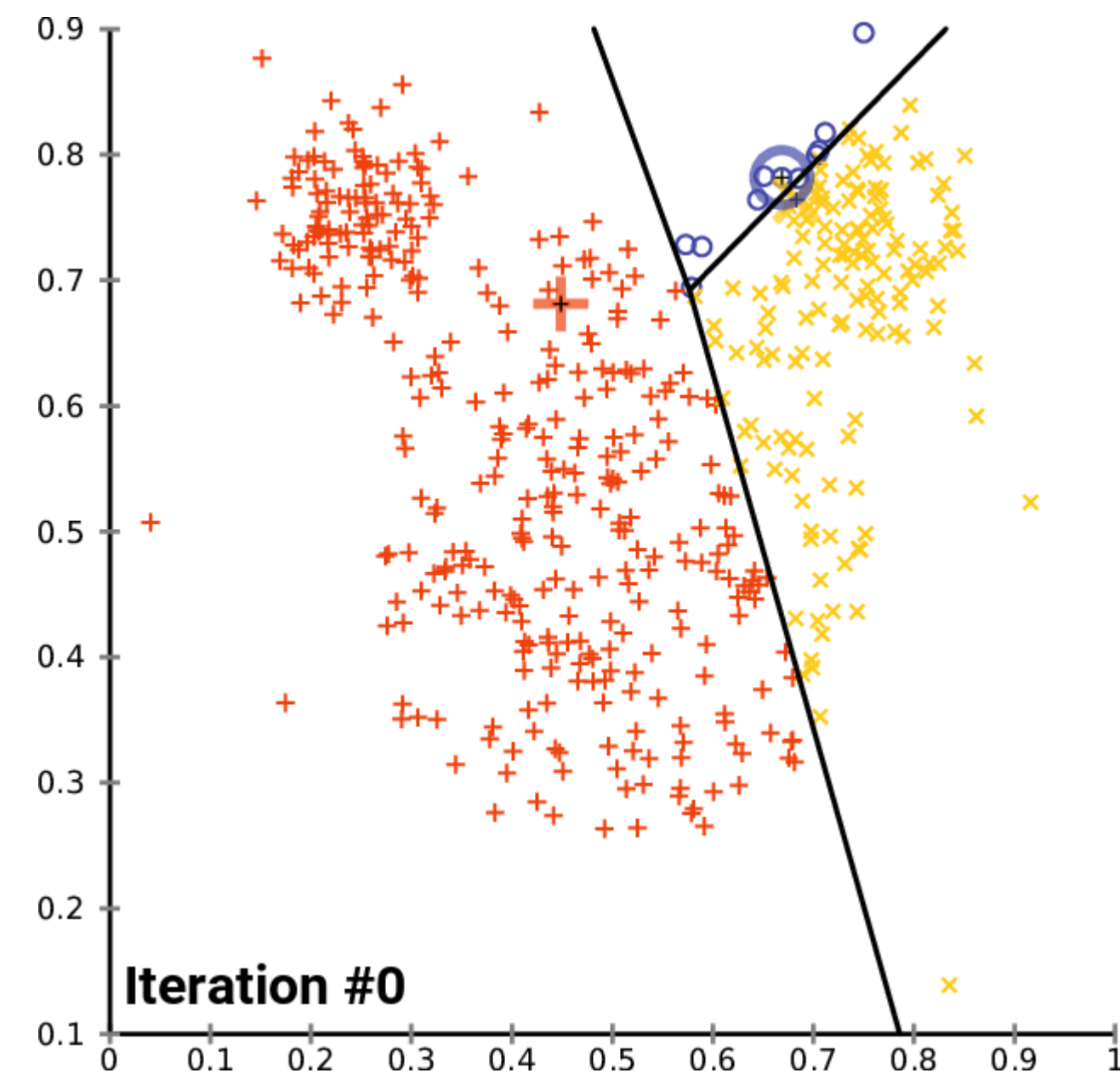


- Pros :
 - Simple
 - Automatic Threshold using Algorithm
- Cons :
 - Noise
 - Variability
 - Saturation

- Thresholding
- Clustering
- Region Growing
- Machine Learning

- Mathematical Algorithm
that will make Cluster of Pixels
 - k-means

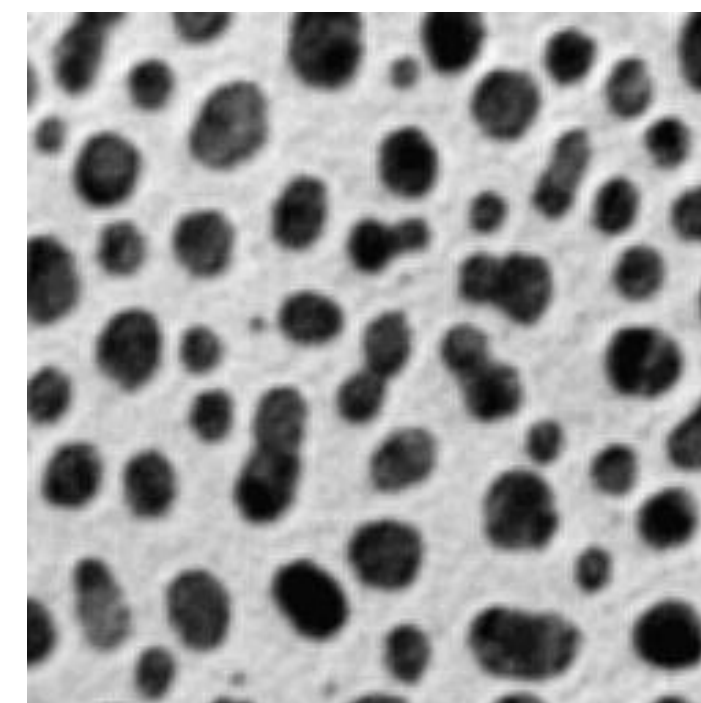
- Mathematical Algorithm that will make Cluster of Pixels
- k-means



- Mathematical Algorithm that will make Cluster of Pixels
 - k-means (centroid)
 - Expectation-maximization (Distribution)
 - ...

- Mathematical Algorithm that will make Cluster of Pixels
 - k-means (centroid)
 - Expectation-maximization (Distribution)
 - ...
- Implementation for ImageJ
 - k-means clustering in **IJ Plugins Toolkit**
 - Expectation-maximization in **xLib**

k-means clustering in IJ Plugins Toolkit



Define the number of clusters

K-means Configuration

Number of clusters

Cluster center tolerance

☐ Interpret stack as 3D

☒ Enable randomization seed

Randomization seed

☒ Show clusters as centroid value

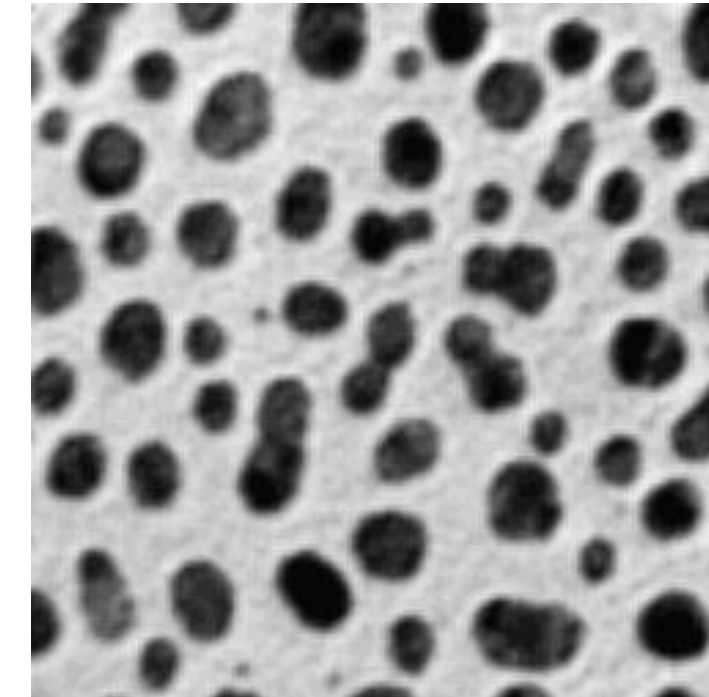
☒ Enable clustering animation

☒ Print optimization trace

☒ Send to results table

OK Cancel Help

k-means clustering in IJ Plugins Toolkit



Define the number of clusters

K-means Configuration

Number of clusters

Cluster center tolerance

☐ Interpret stack as 3D

☒ Enable randomization seed

Randomization seed

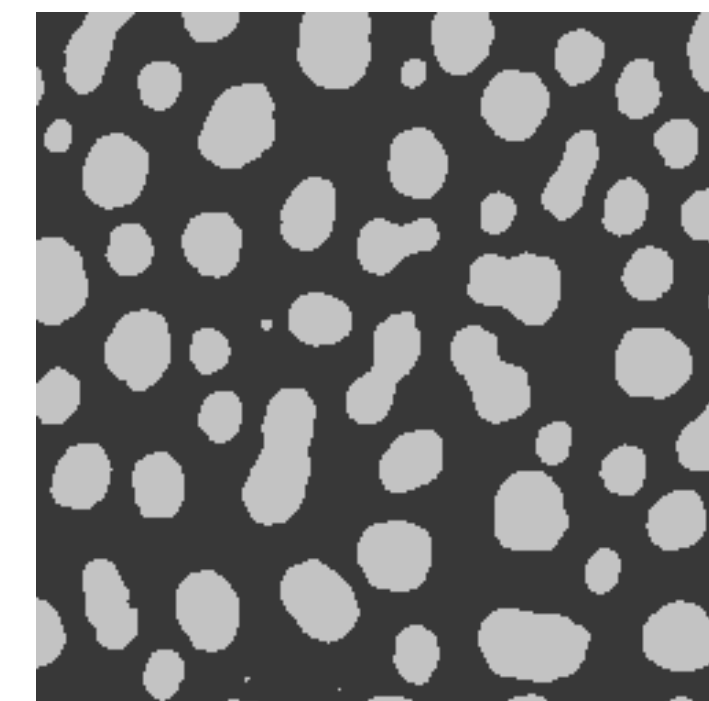
☒ Show clusters as centroid value

☒ Enable clustering animation

☒ Print optimization trace

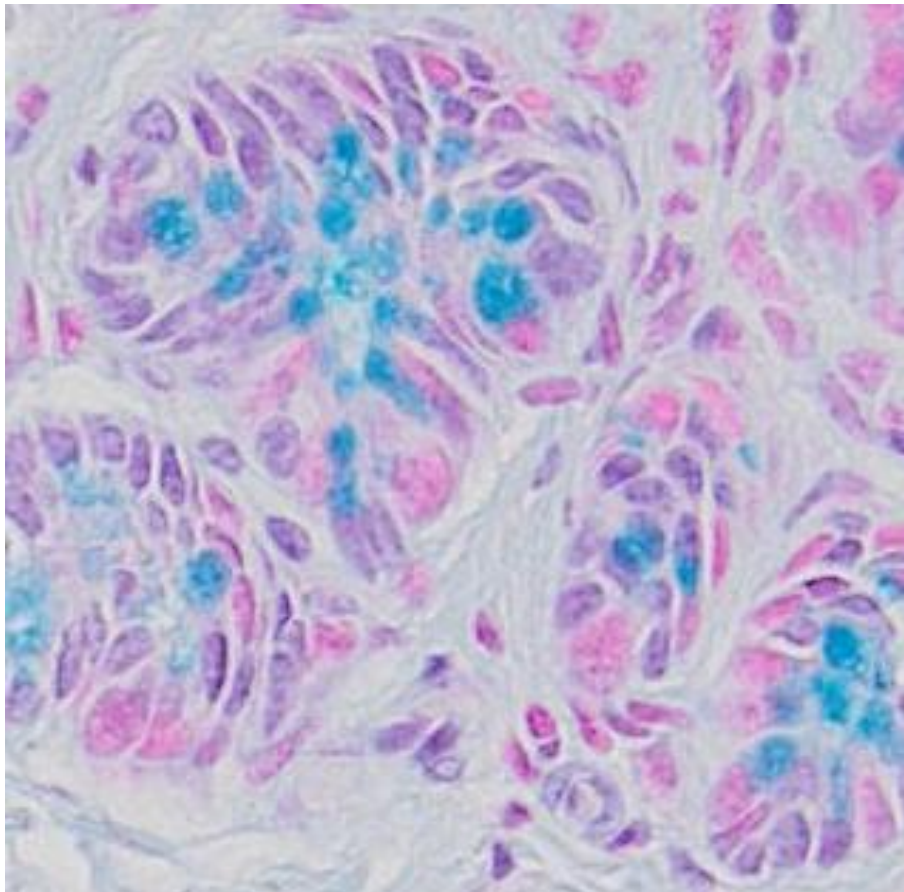
☒ Send to results table

OK Cancel Help

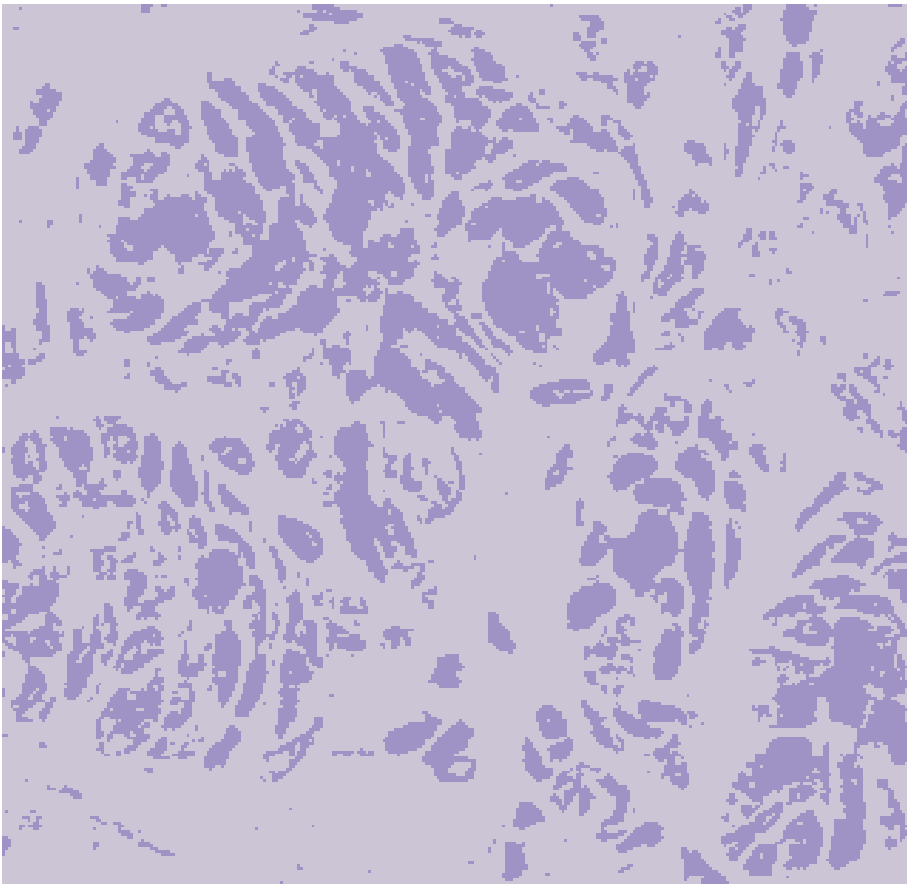


k-means clustering in IJ Plugins Toolkit

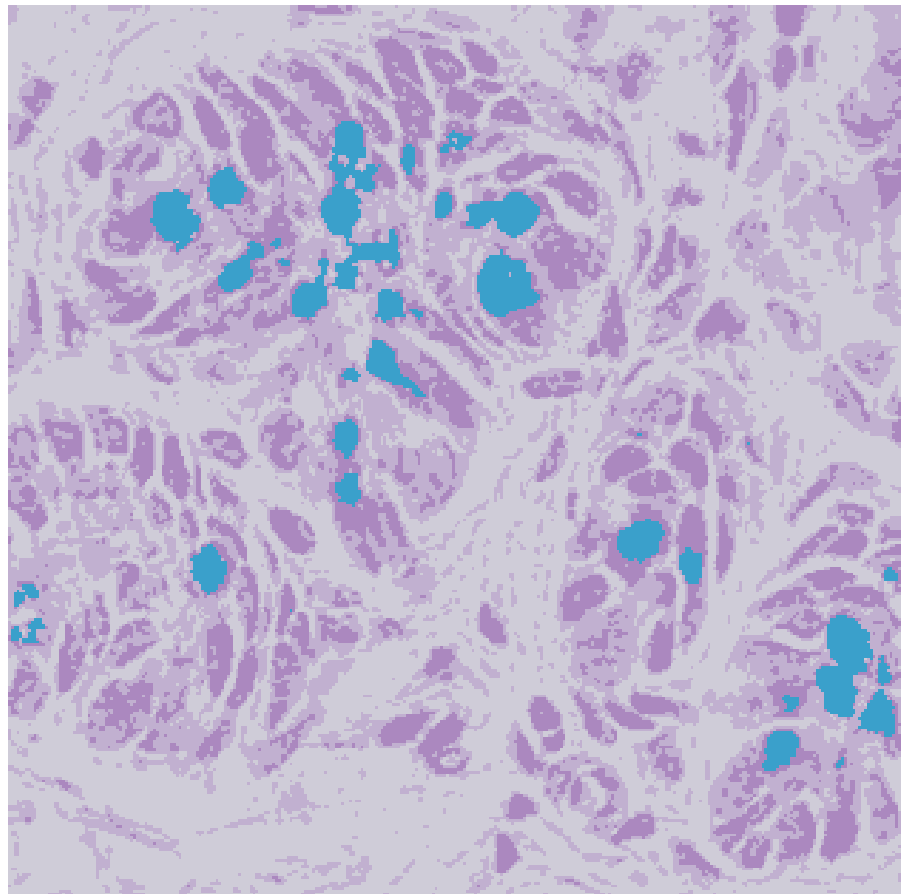
Original



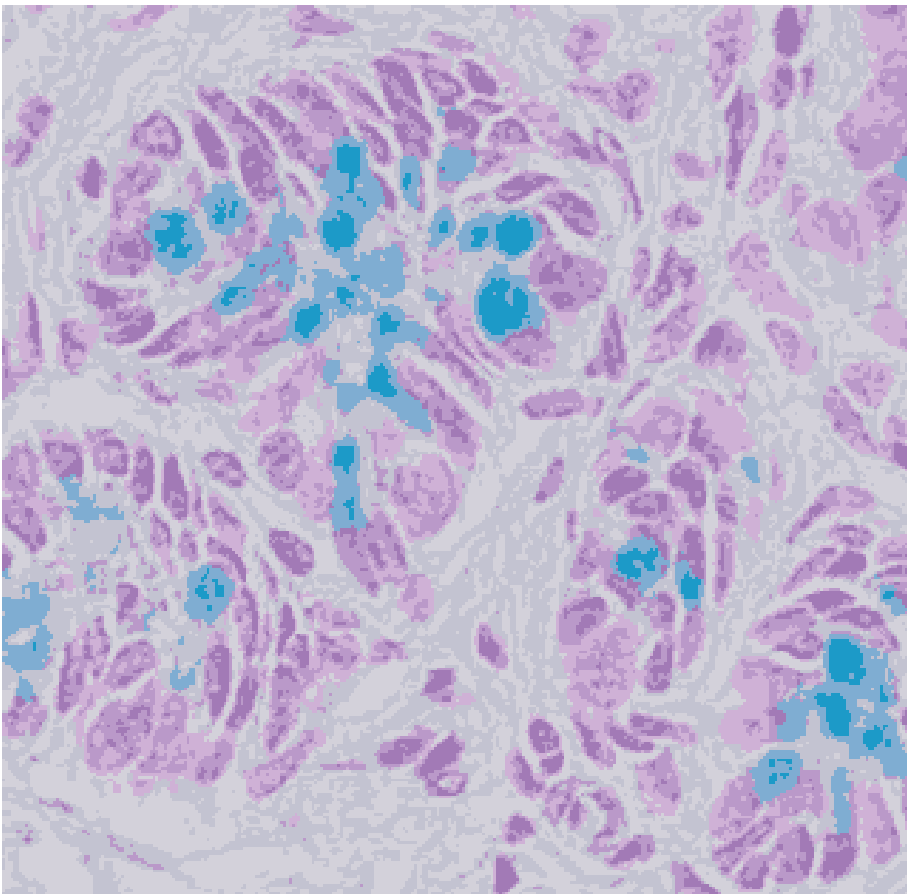
K=2



K=4



K=7



- Pros

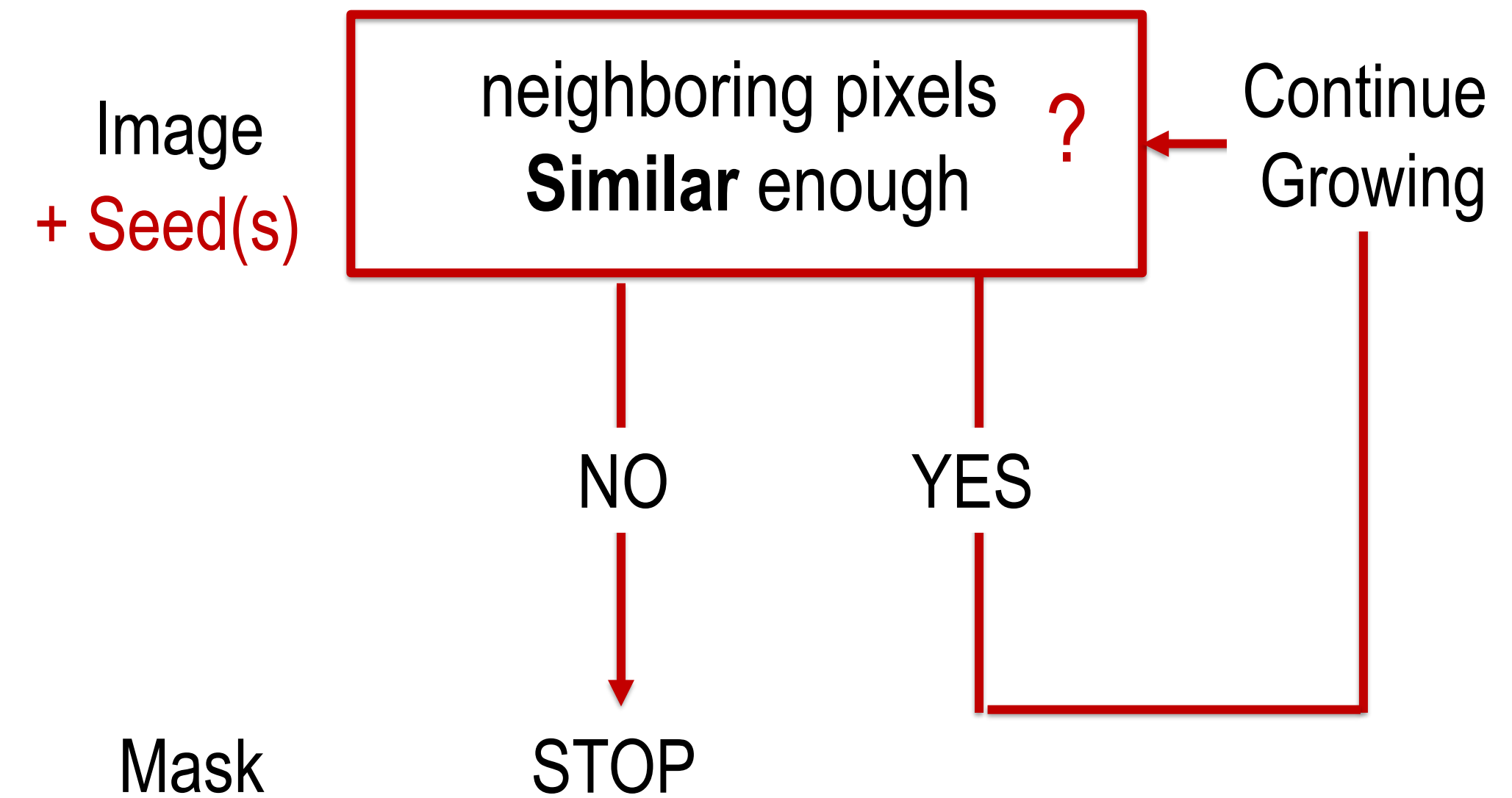
- More than 2 categories
- Works on RGB image

- Cons

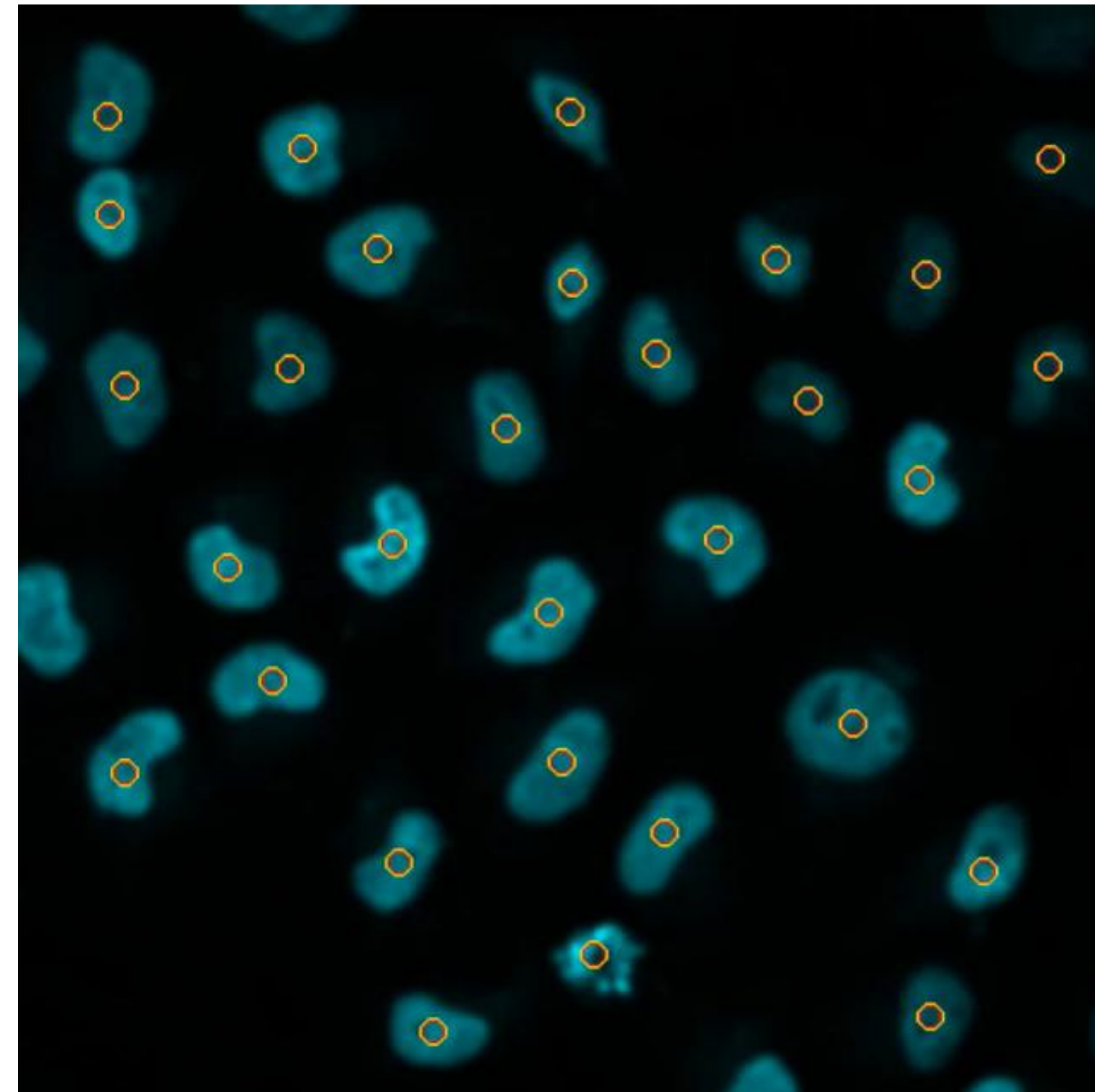
- Needs apriori knowledge (categories number)
- Initial Centroids
- Local Minima

Region Growing

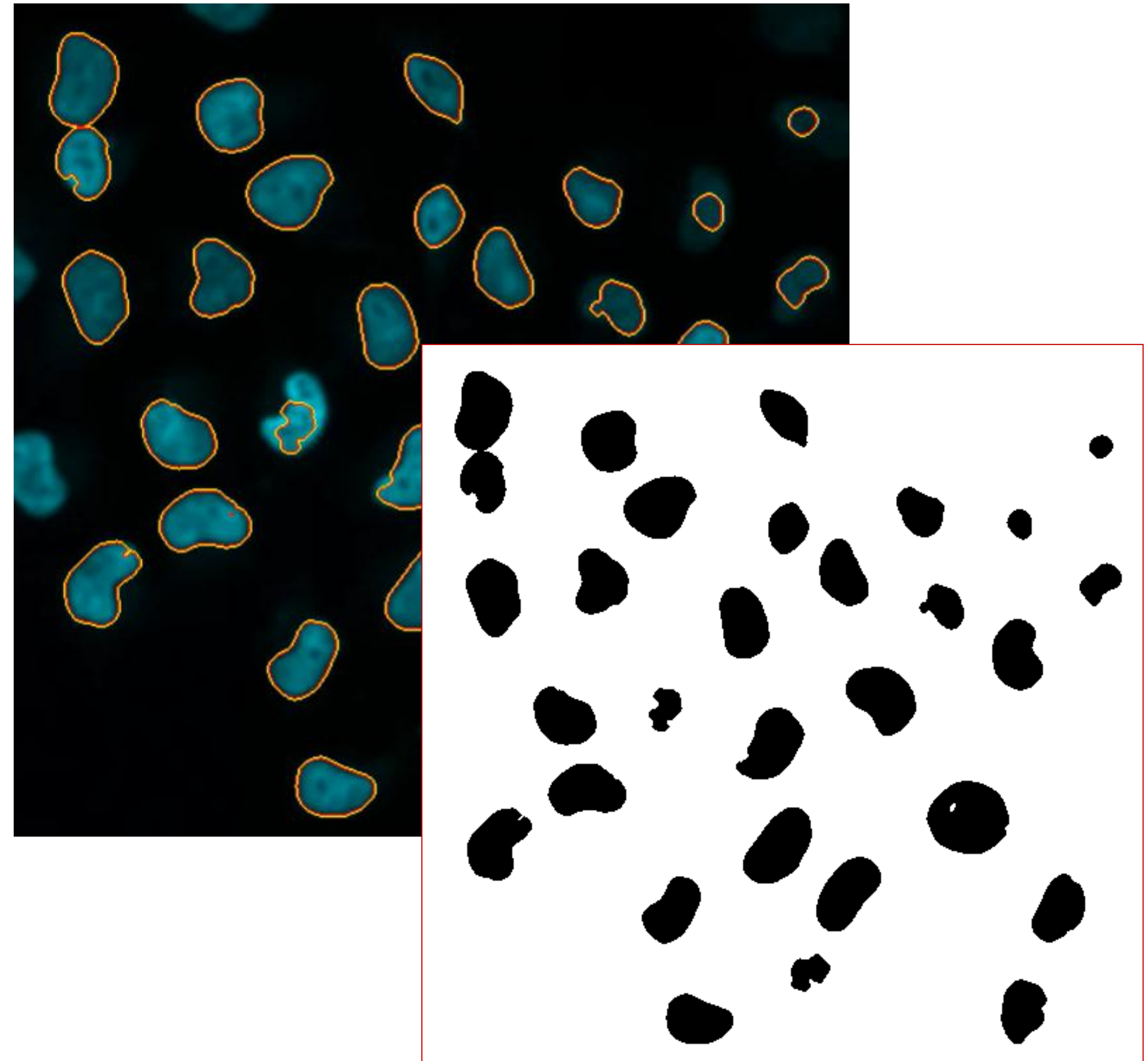
Region Growing : Principle



Region Growing

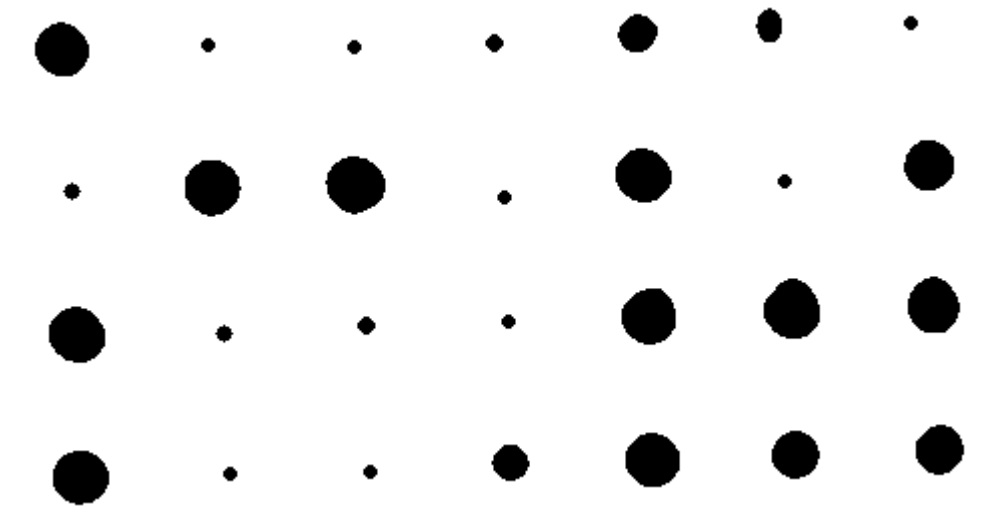
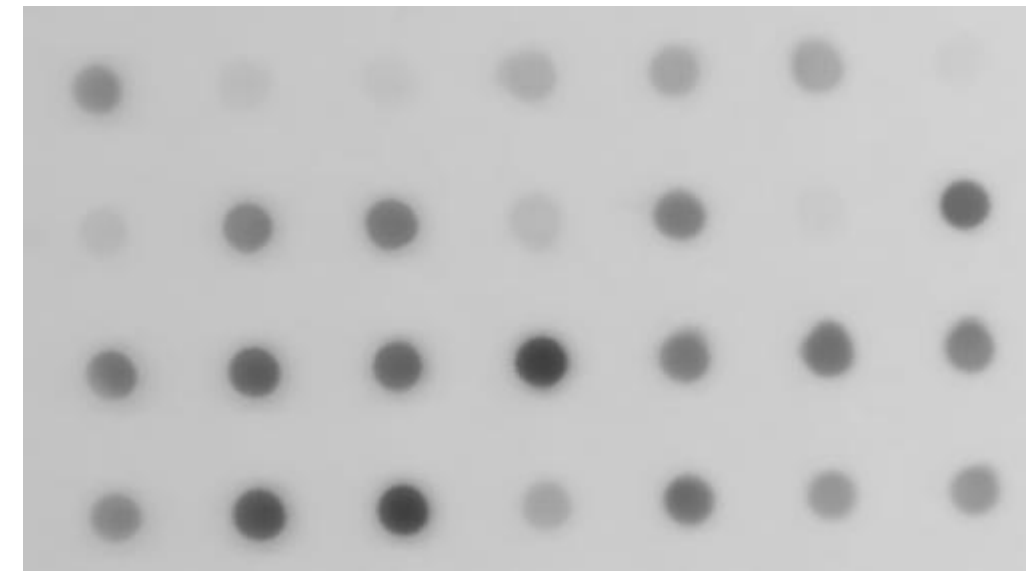


Region Growing



Region Growing : Limitations

- Large Variety of Foreground Pixels



- "Slow"

- Pros
 - Multiple Criteria
- Cons
 - Needs seeds
 - Foreground pixels with large range
 - Slow

Machine Learning



Image + Annotations
+ Algorithm



Image + Annotations
+ Algorithm  Classification
Rules

Confront Pixels to the **Rules**



Mask

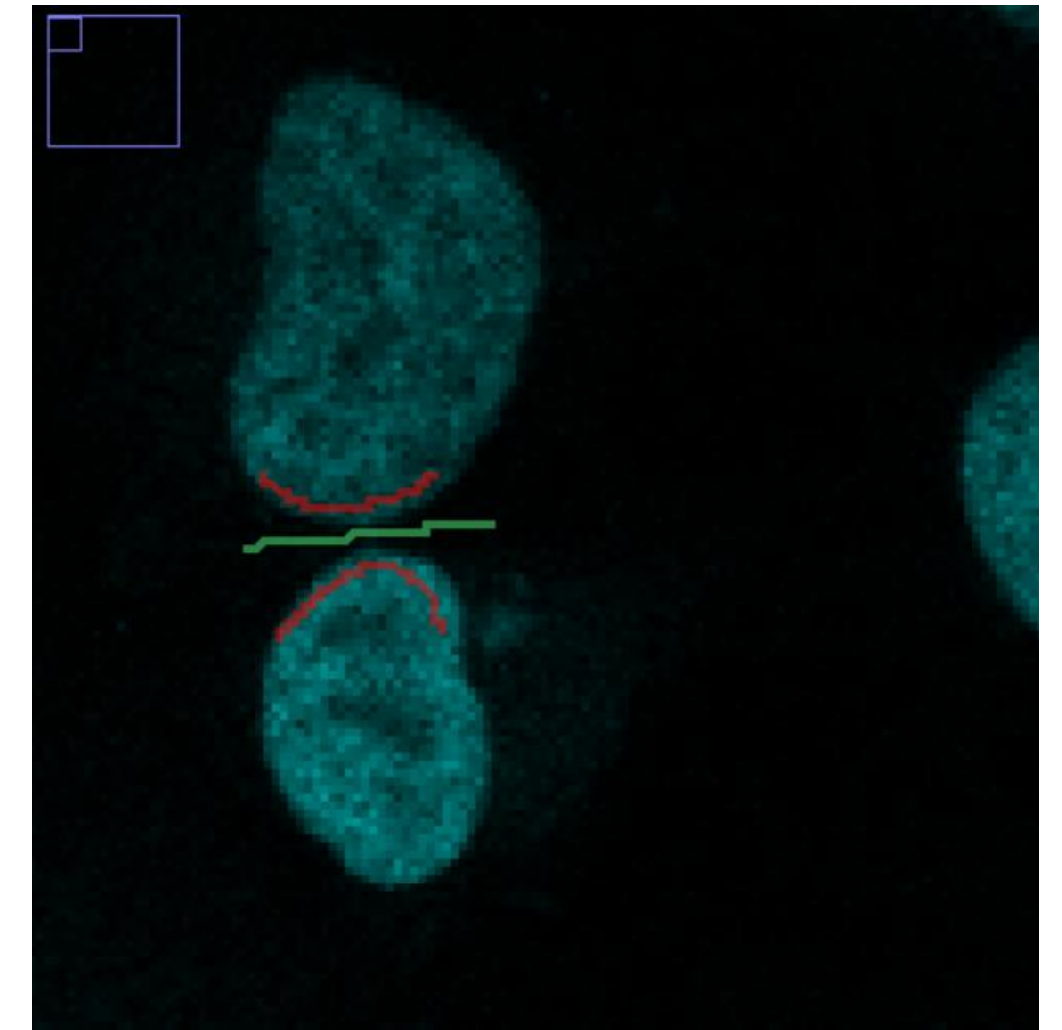
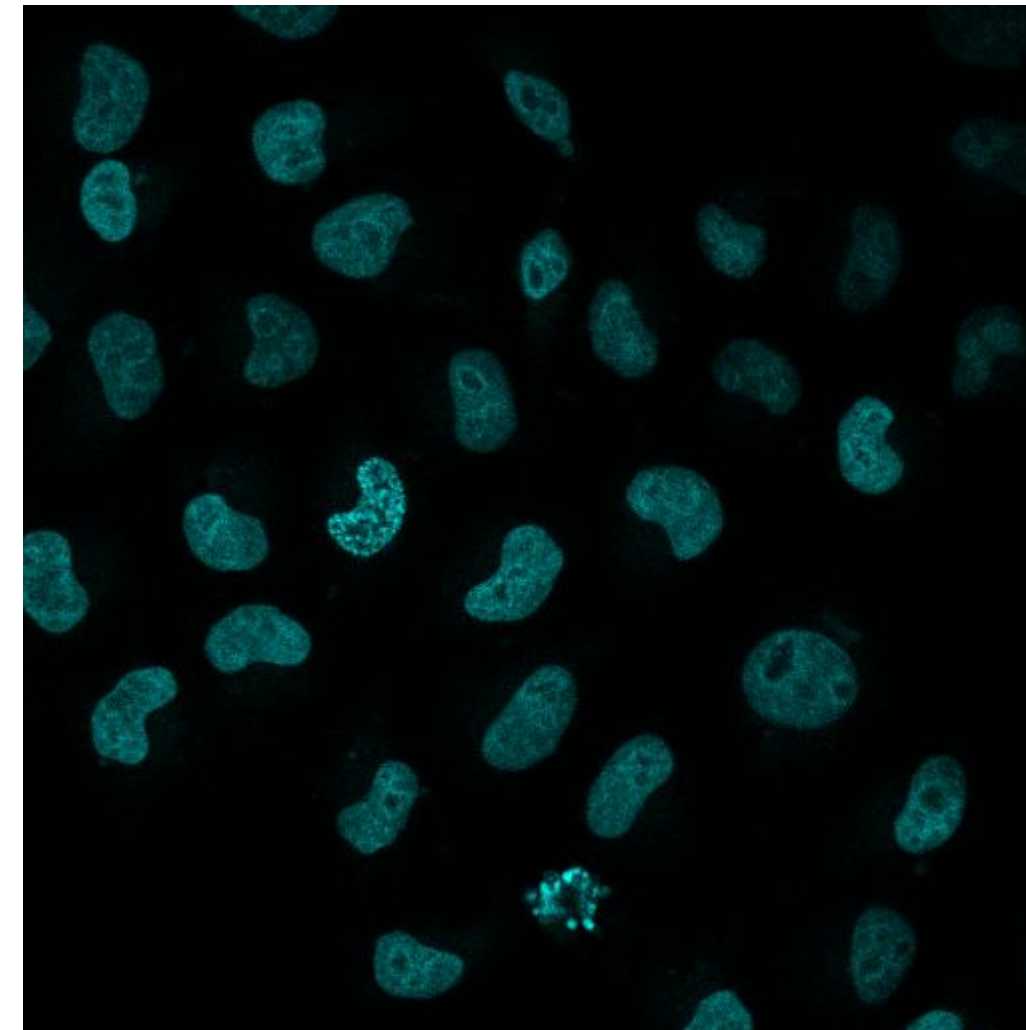
Image + Annotations
+ Algorithm  Classification
Rules

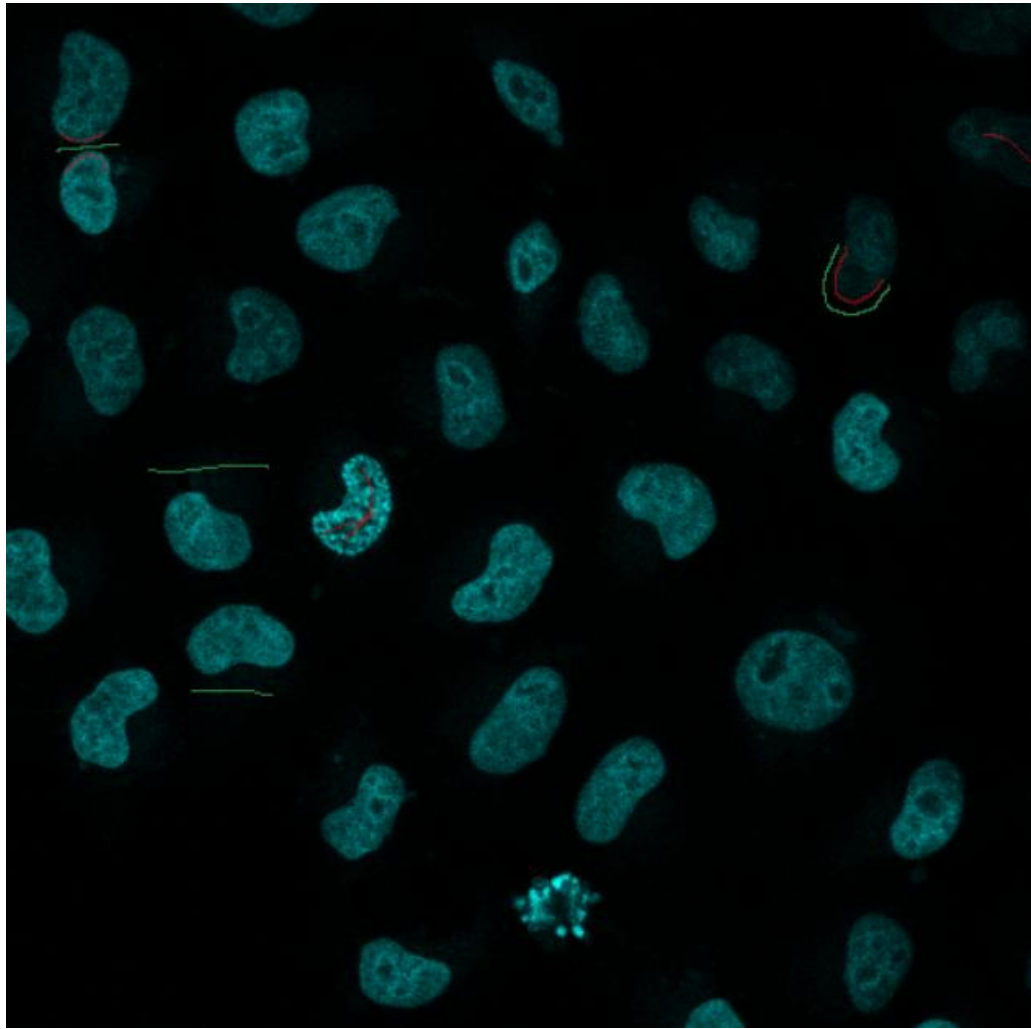
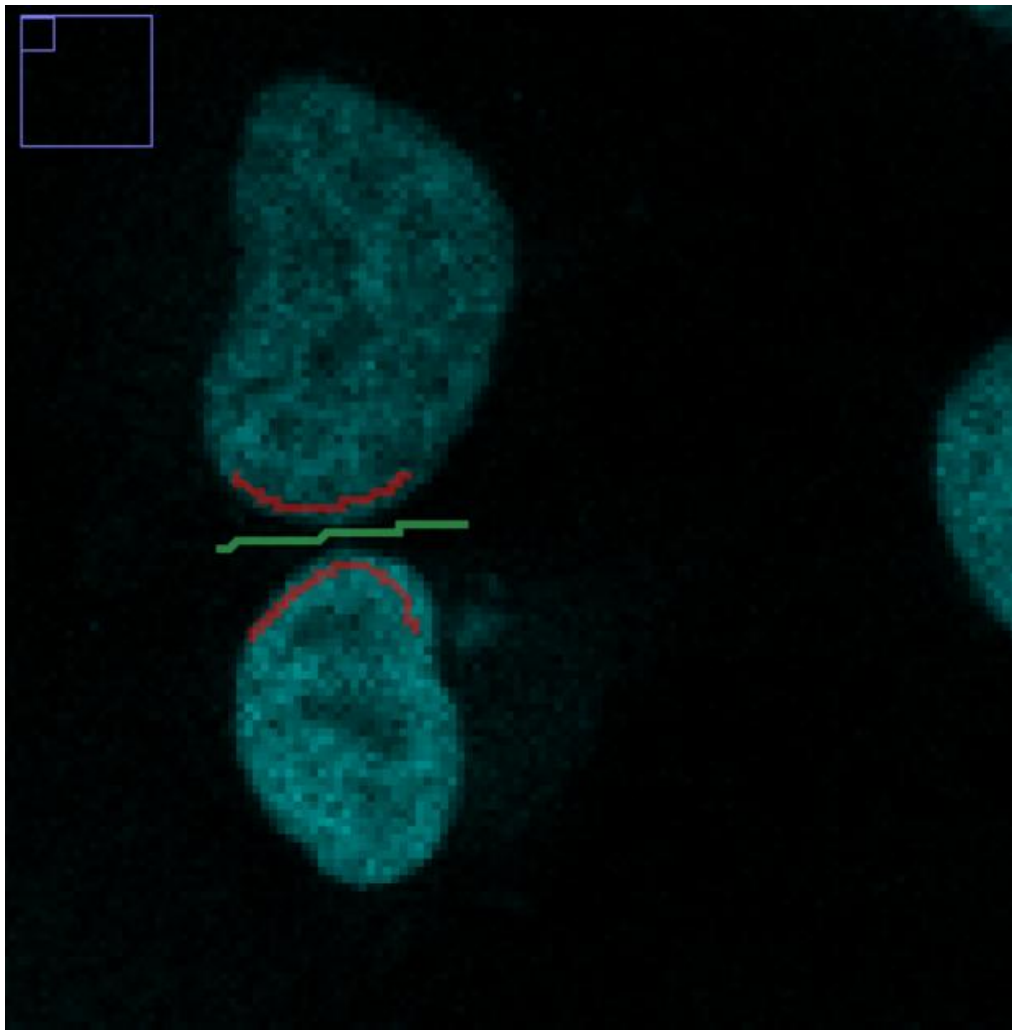
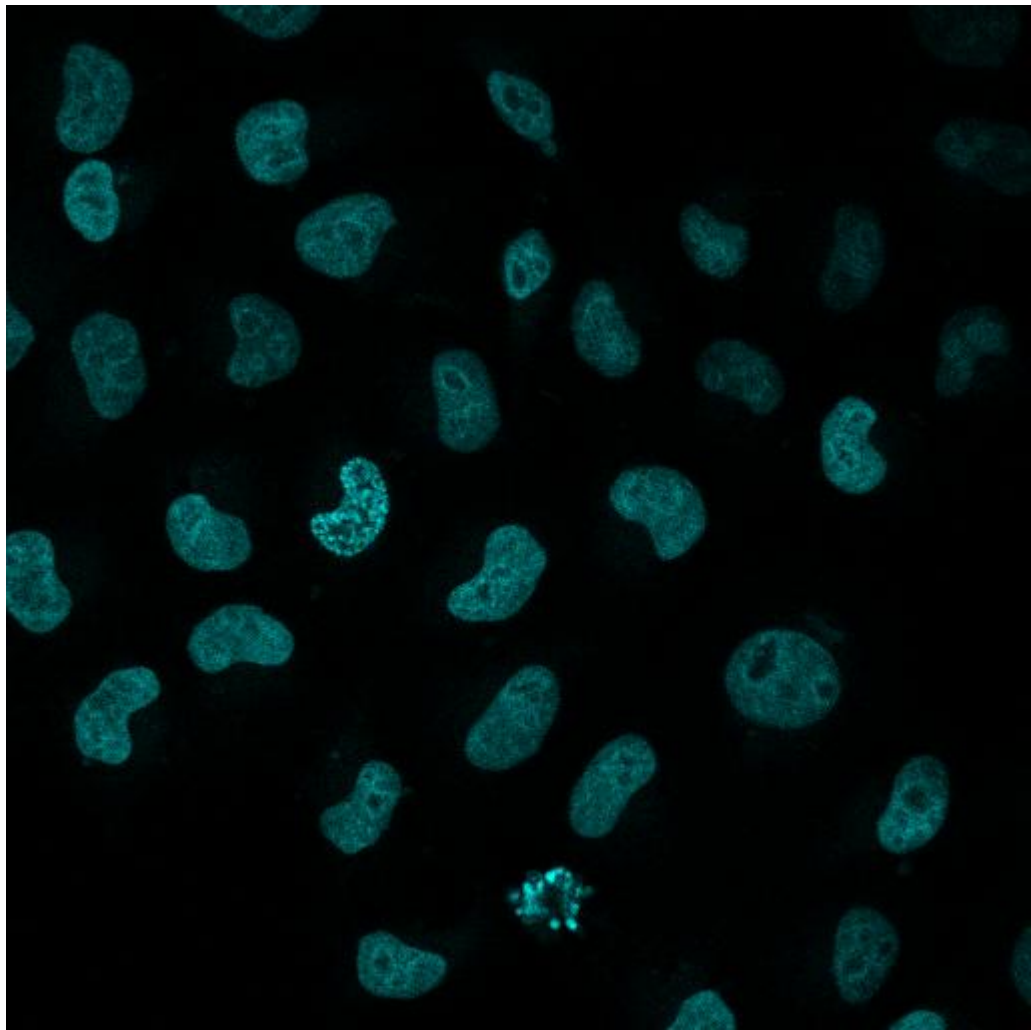
Confront Pixels to the **Rules**

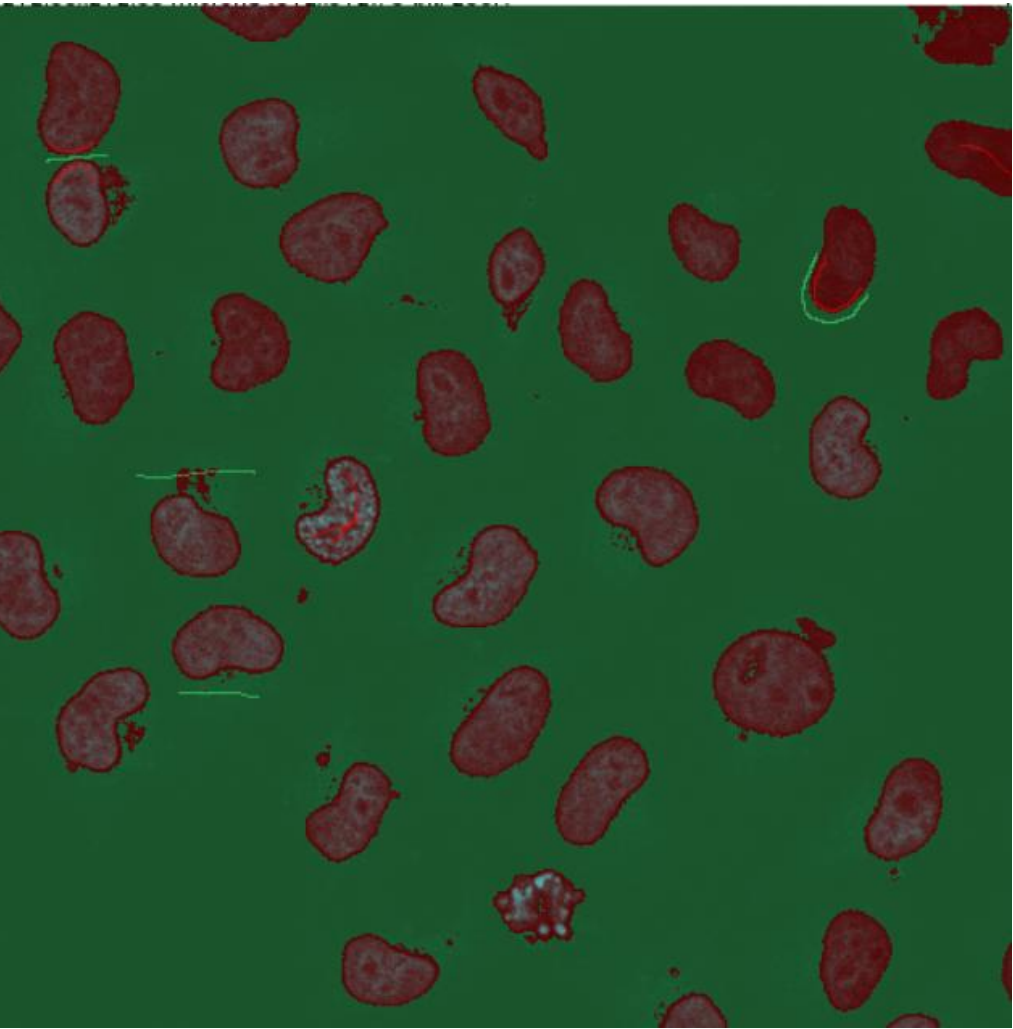
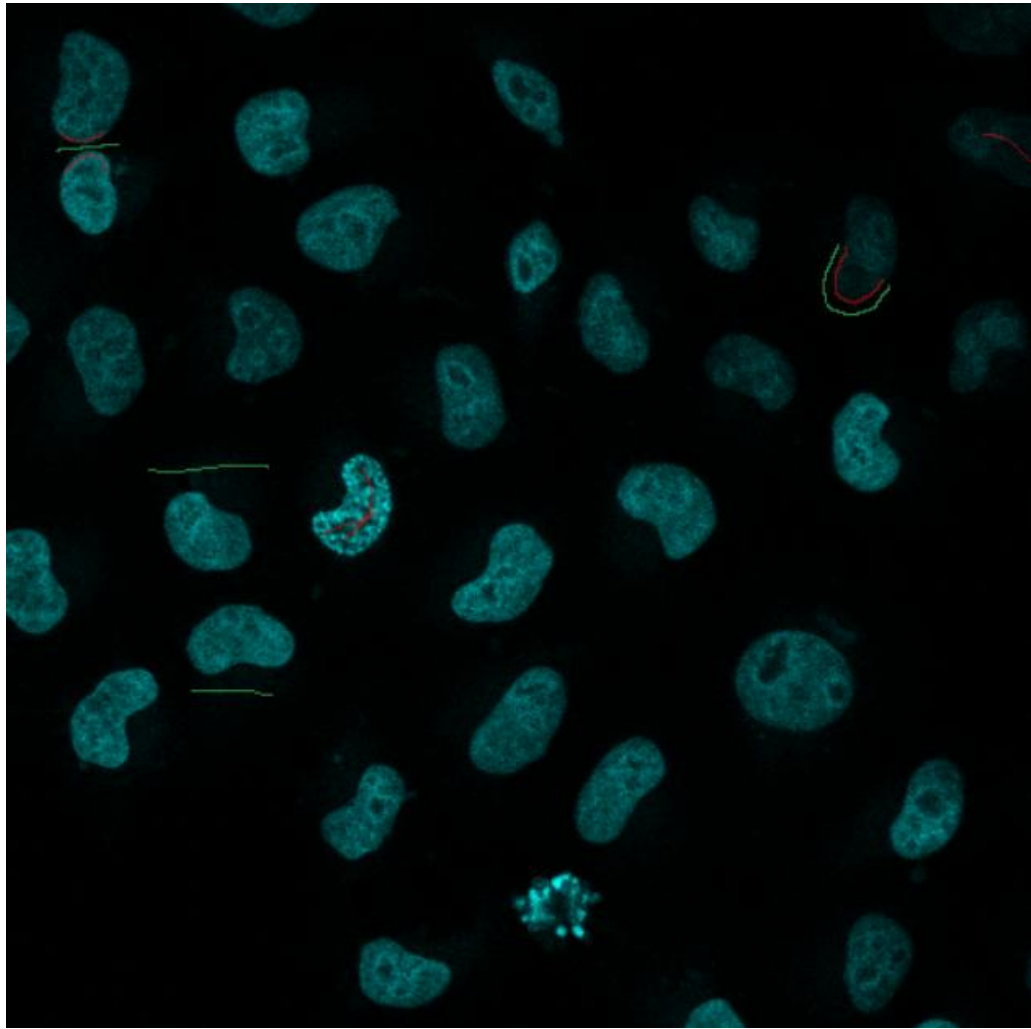
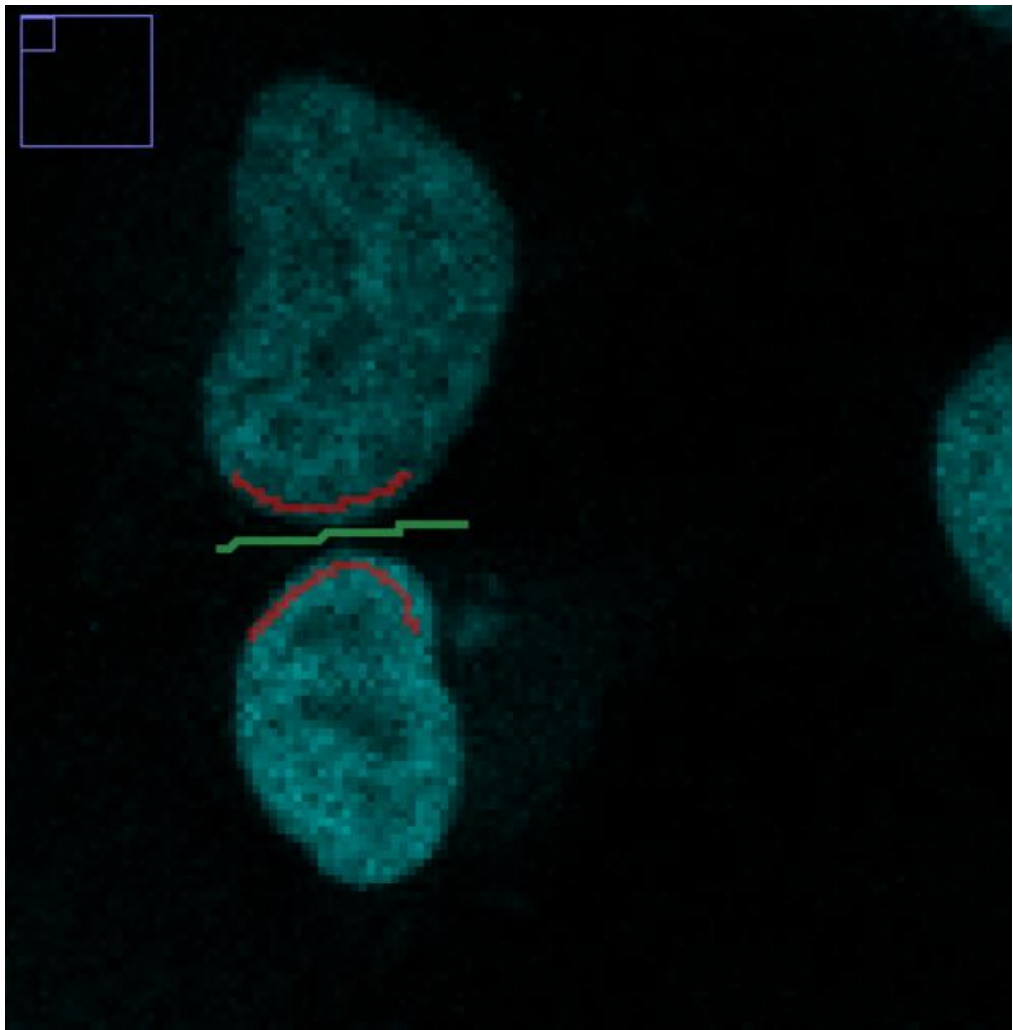
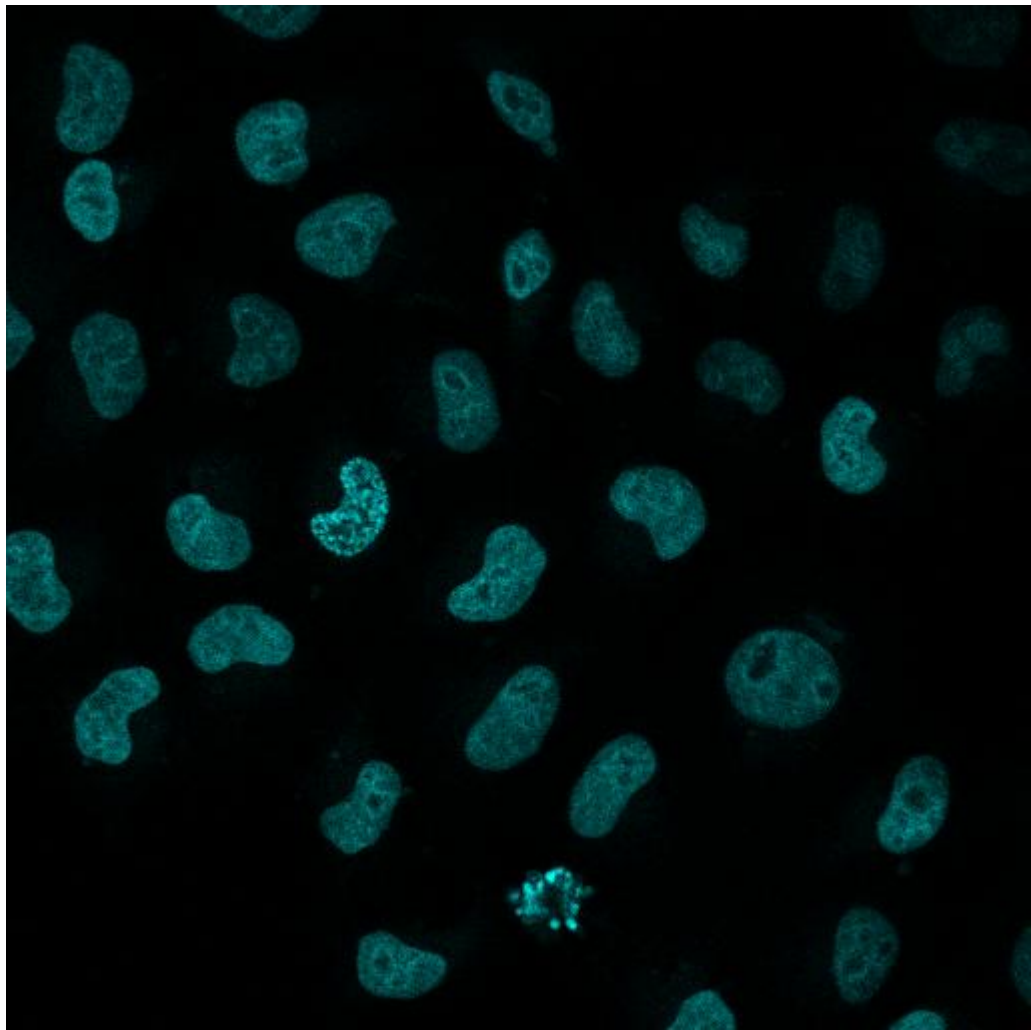
Mask

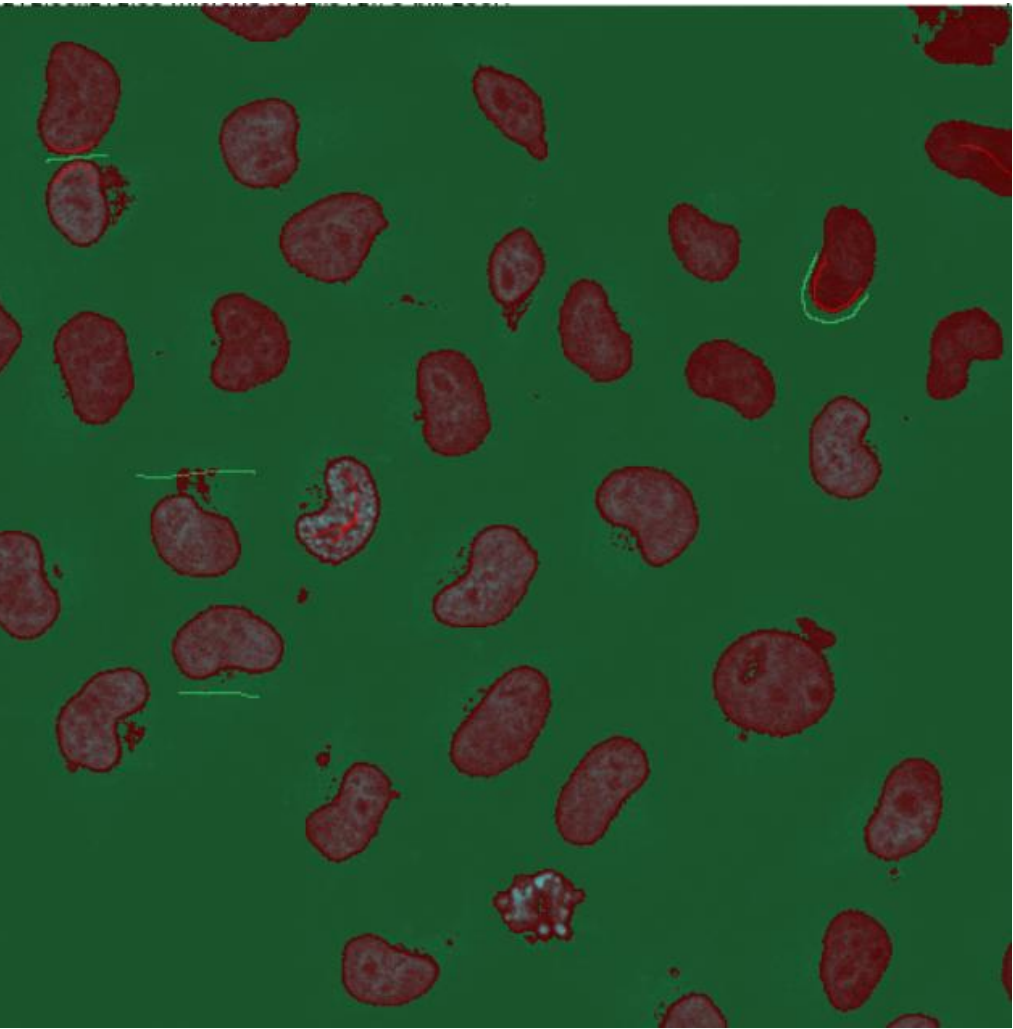
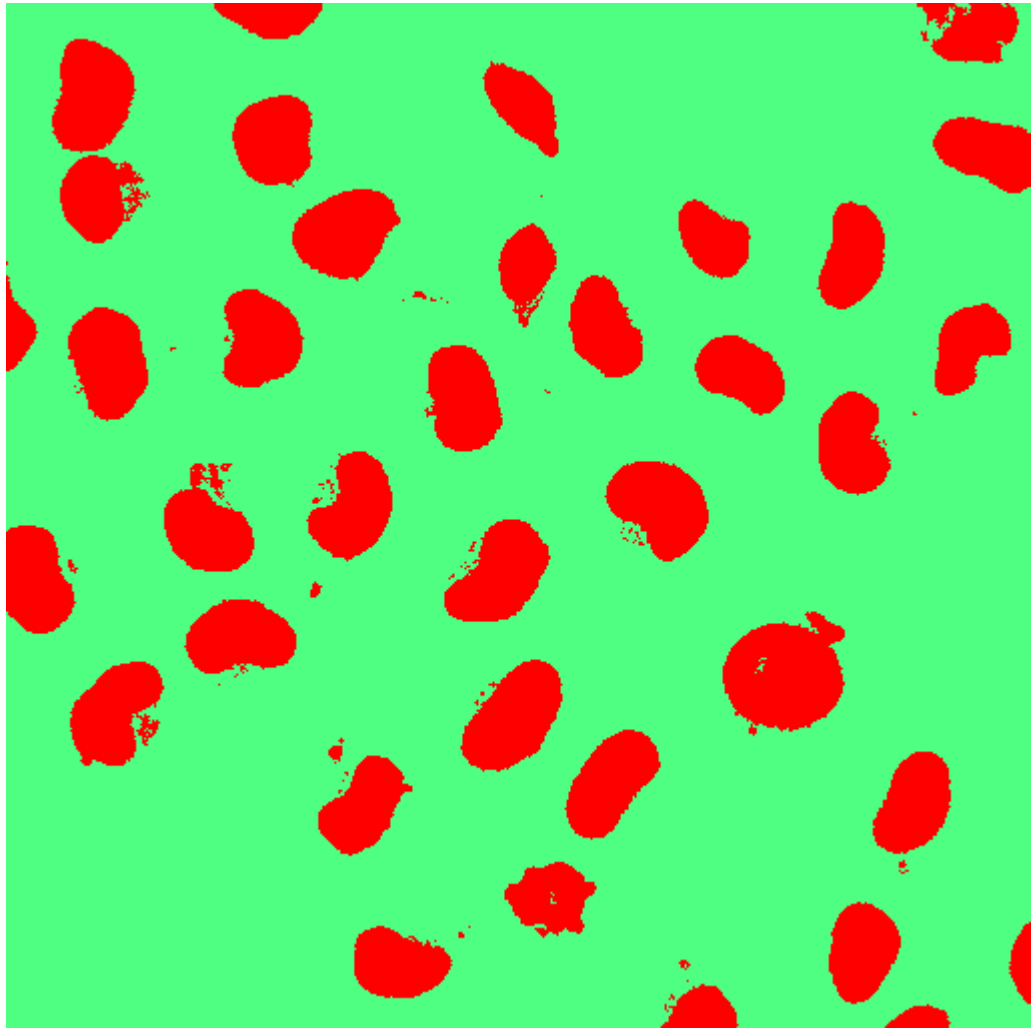
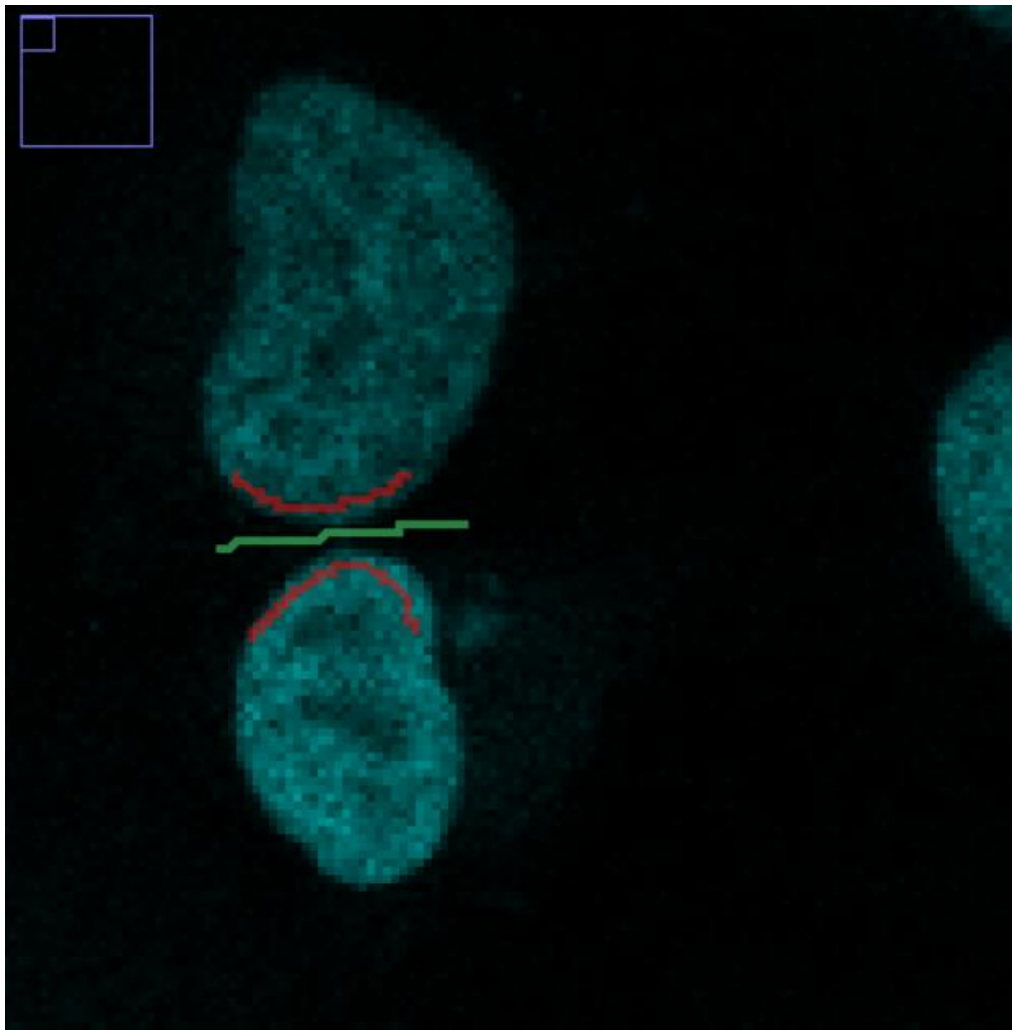
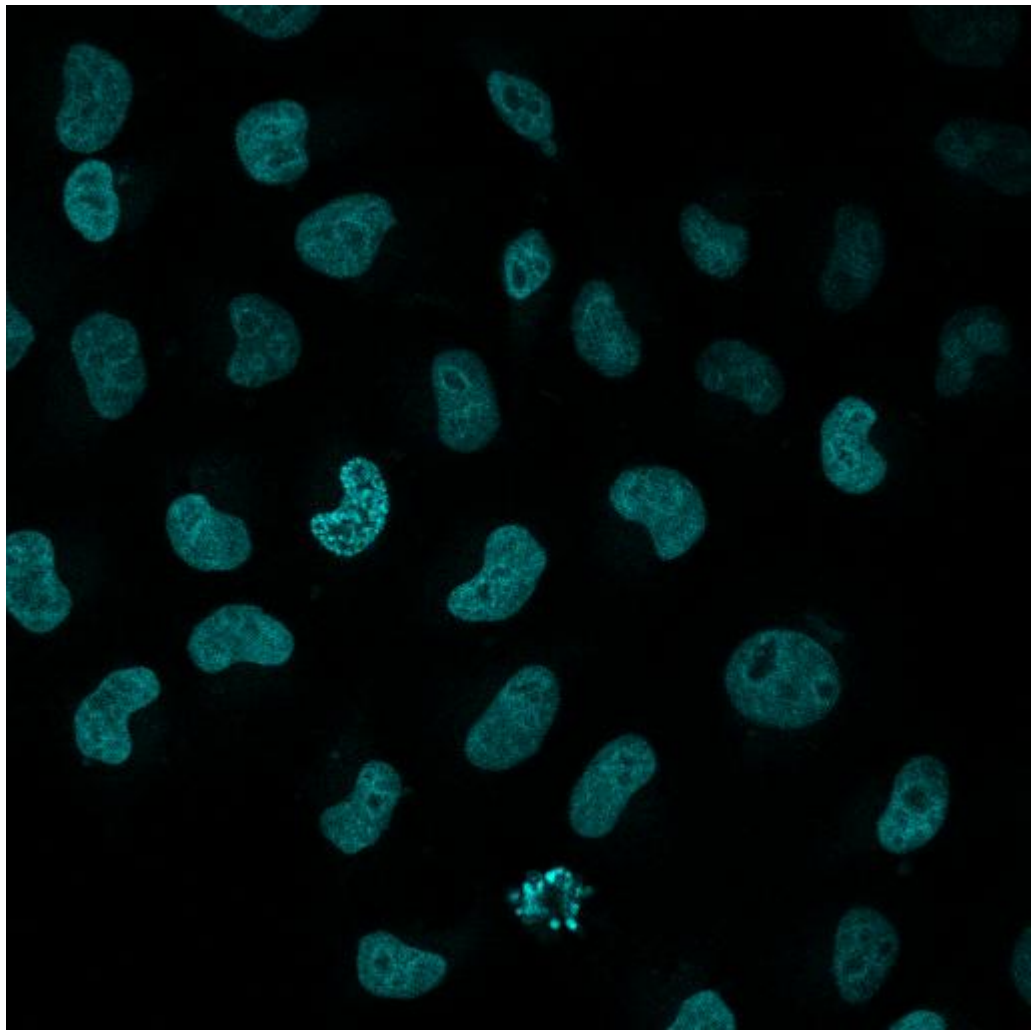
(Probability Map)

Machine Learning

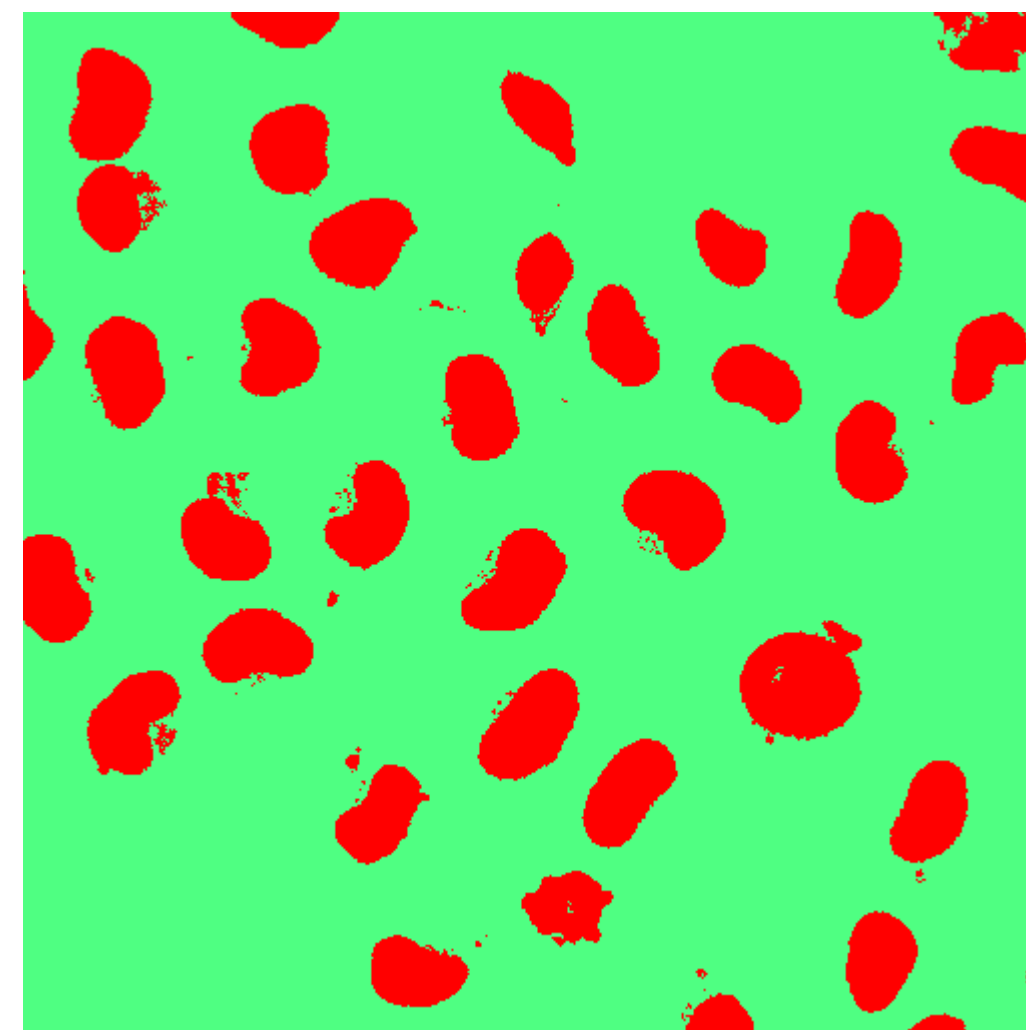
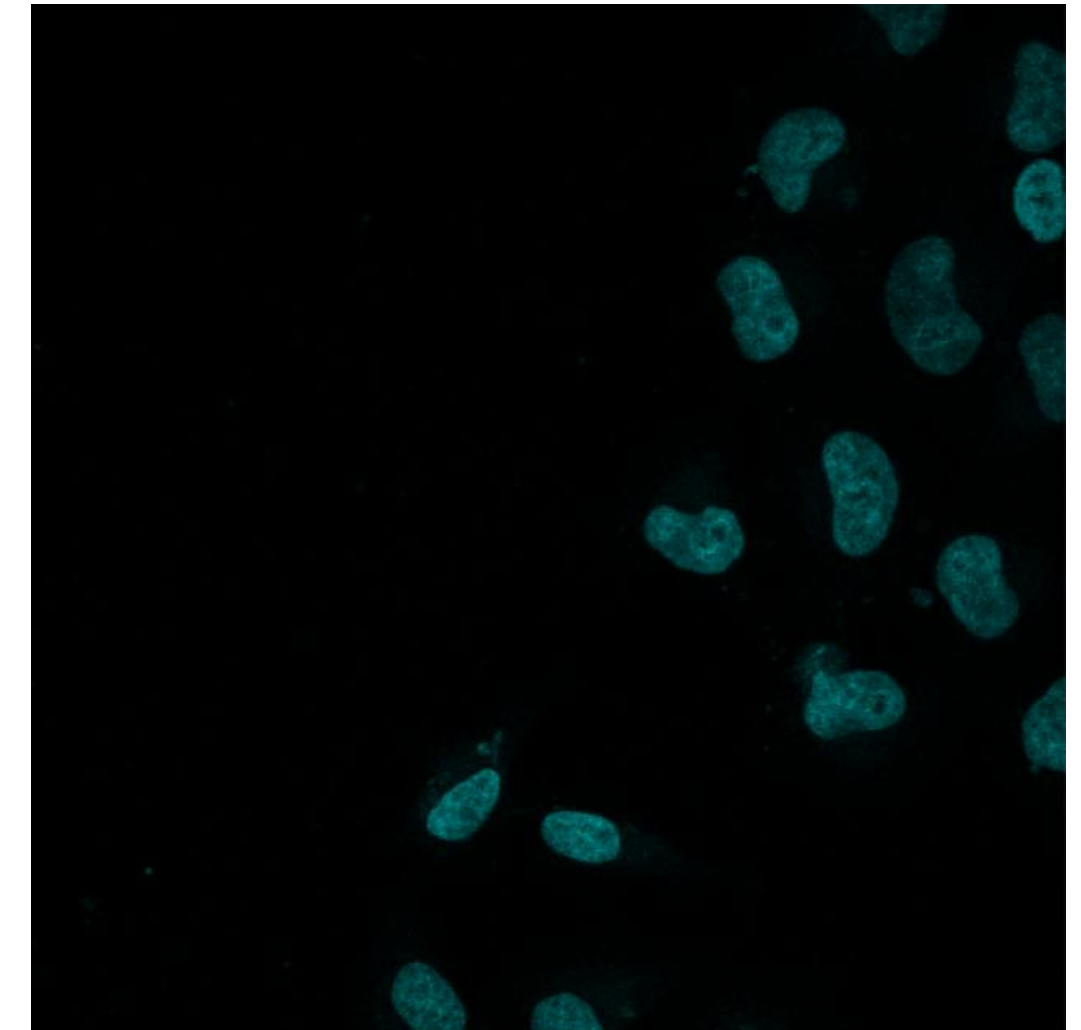
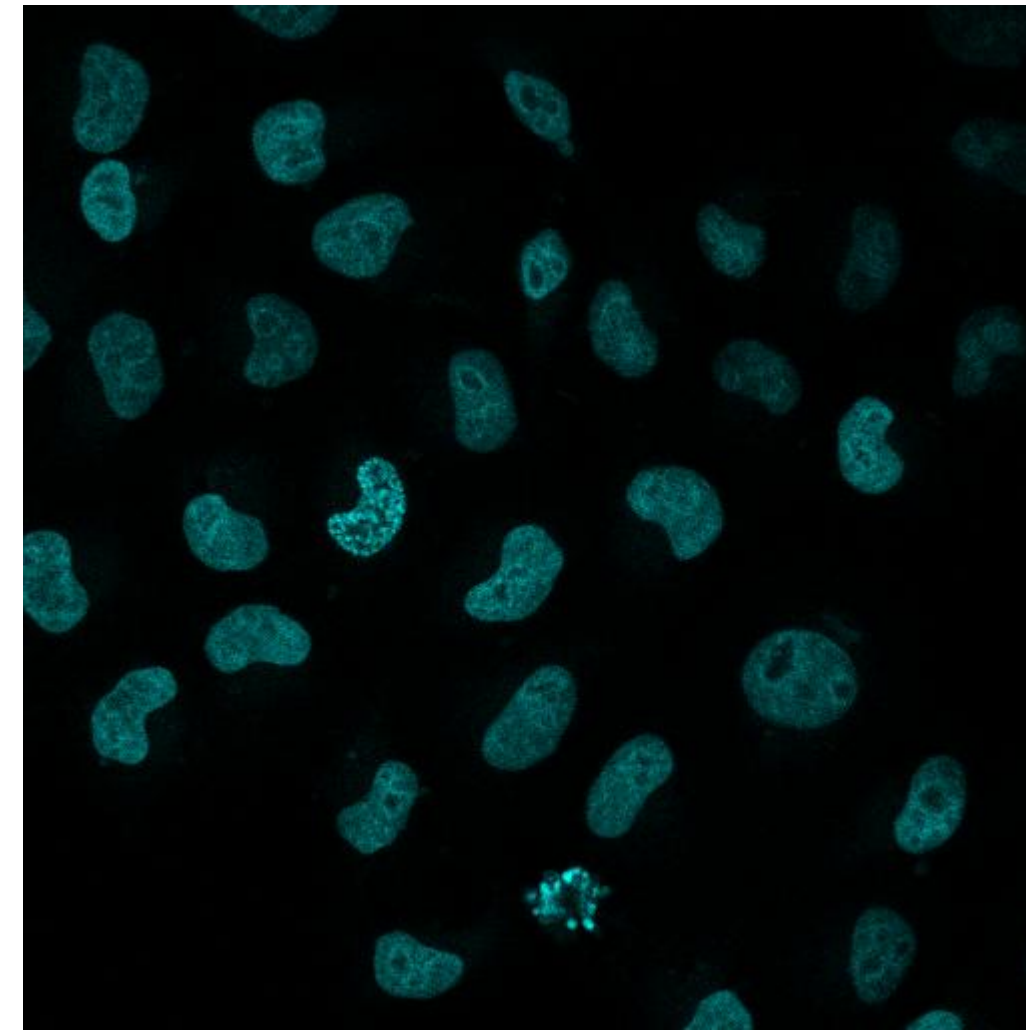




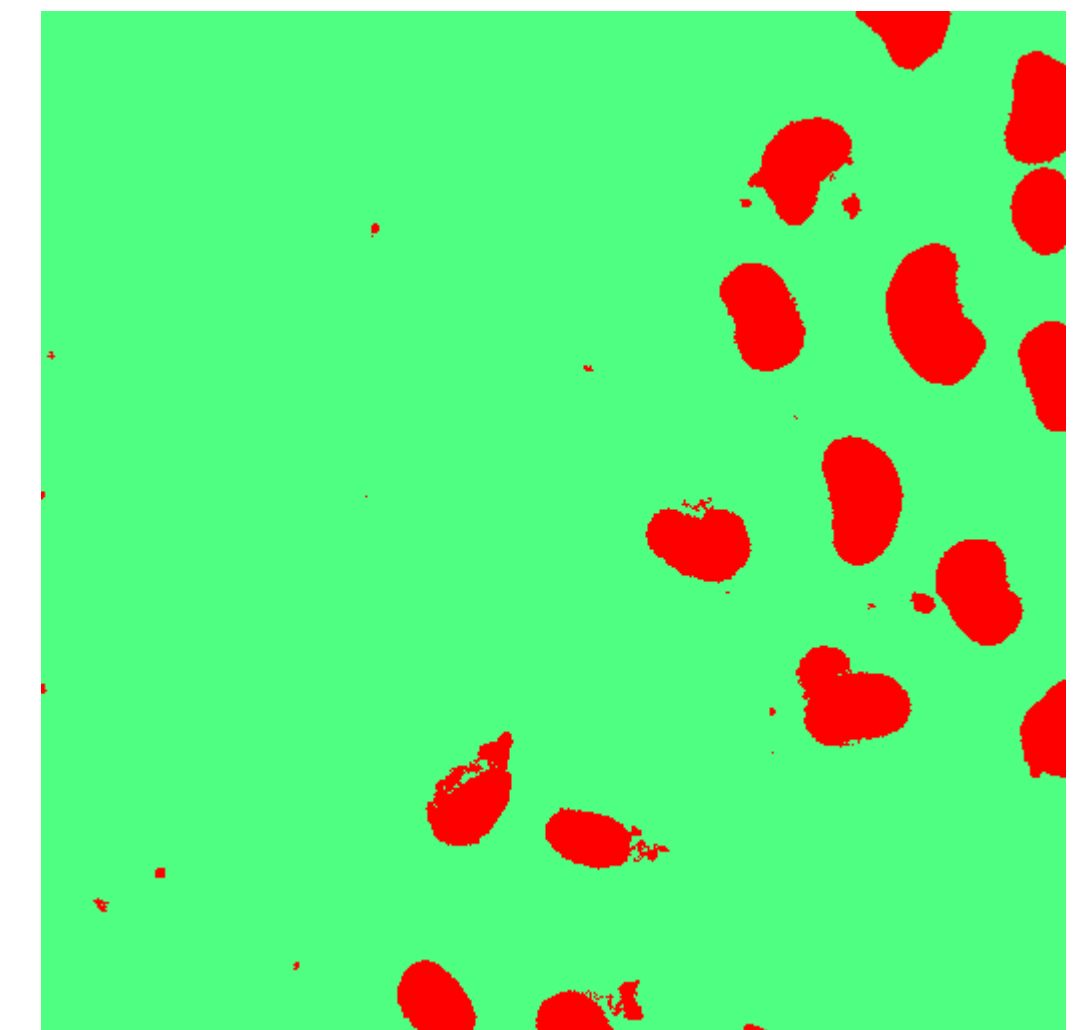
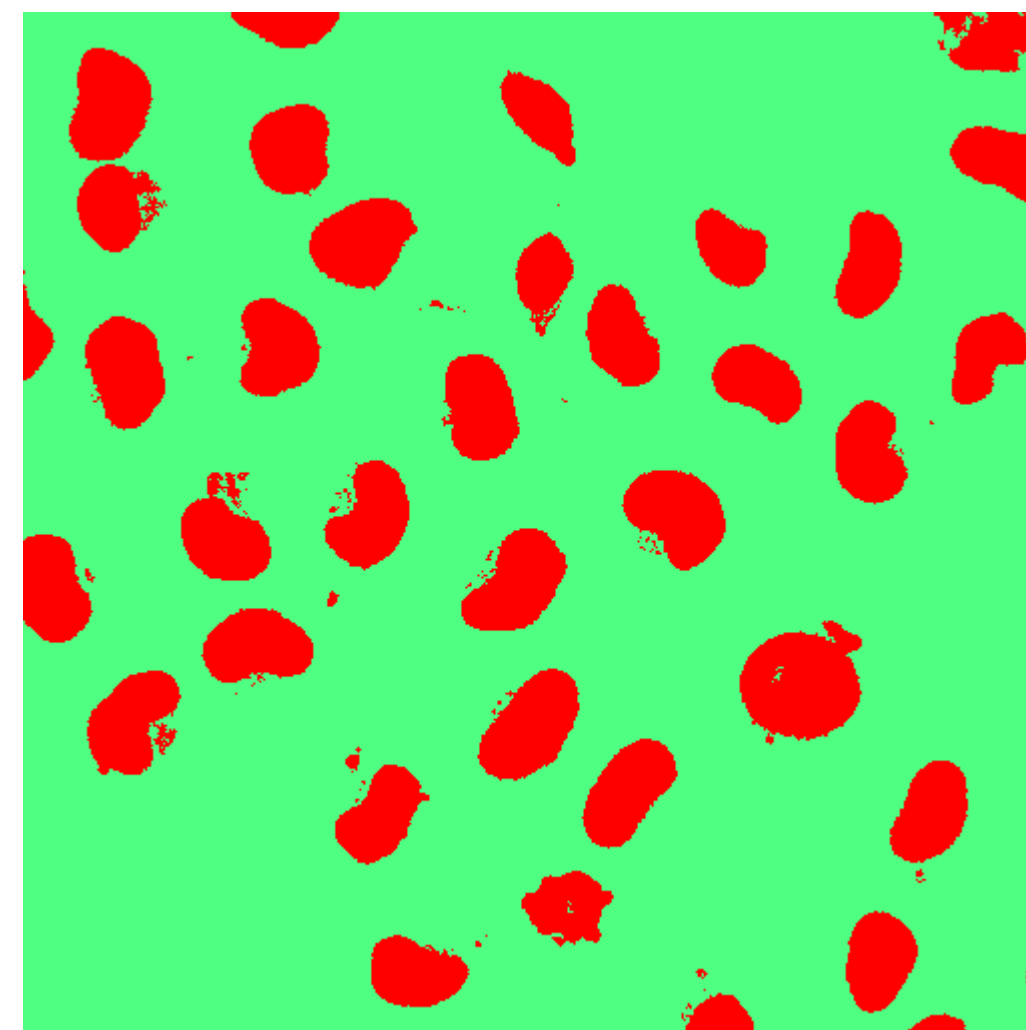
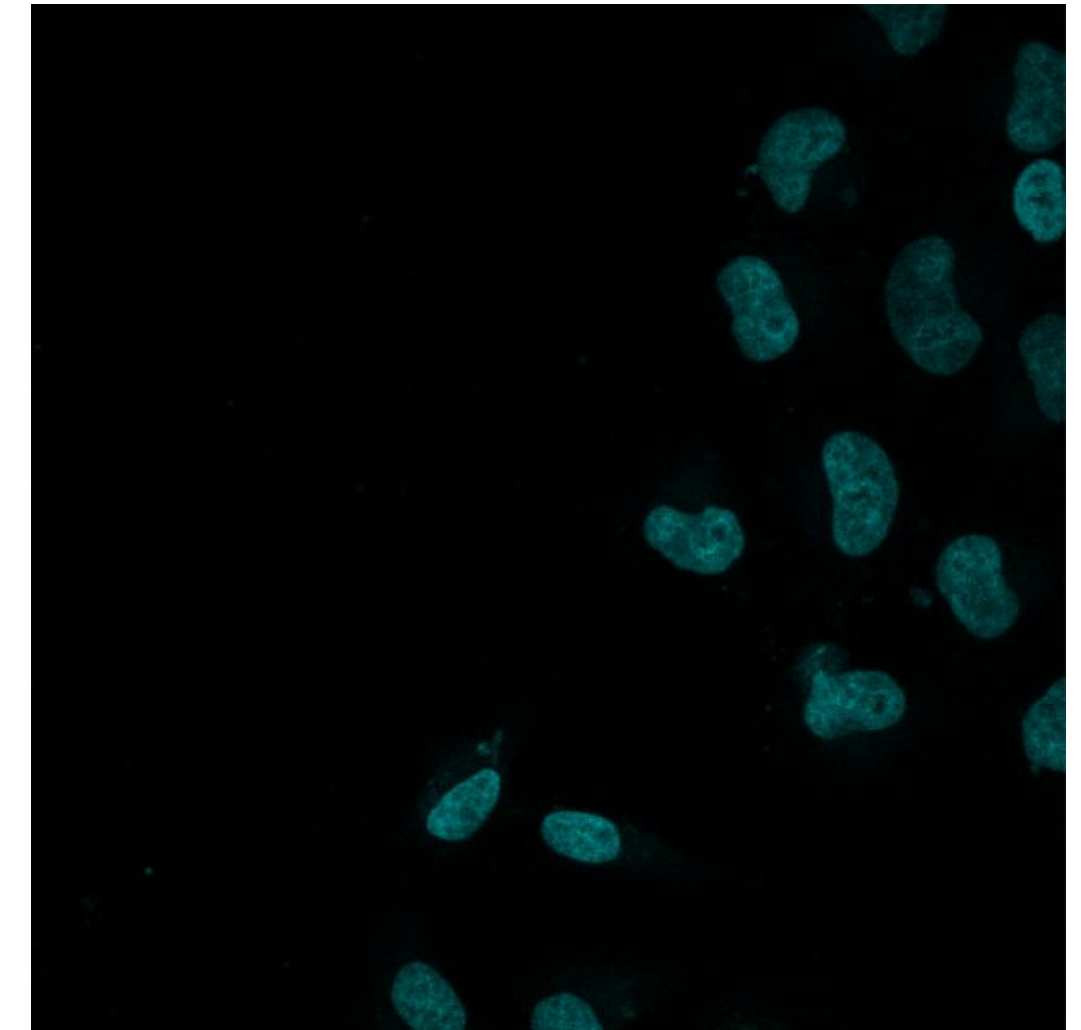
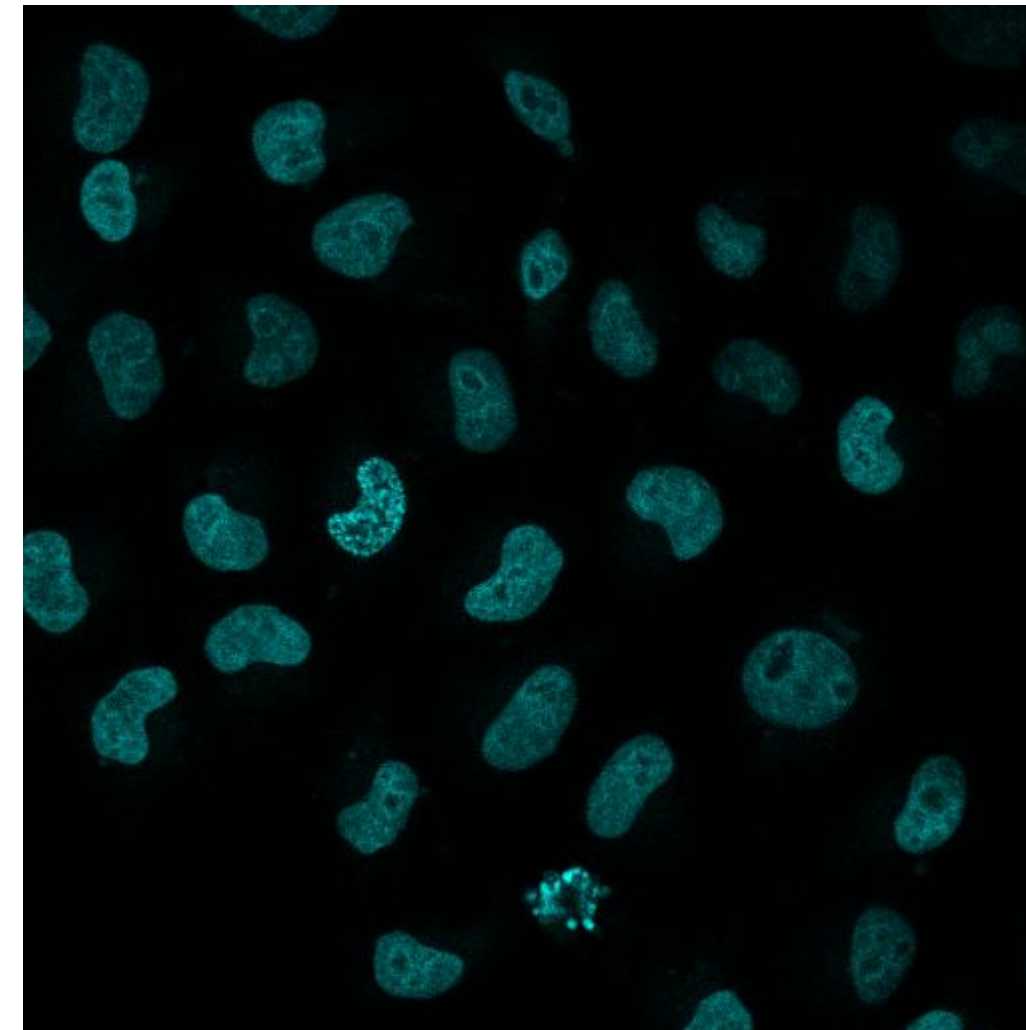




Machine Learning



Machine Learning



- Pros
 - Complexe classification
- Cons
 - Needs «Good» Annotations
 - Robust Imaging Condition

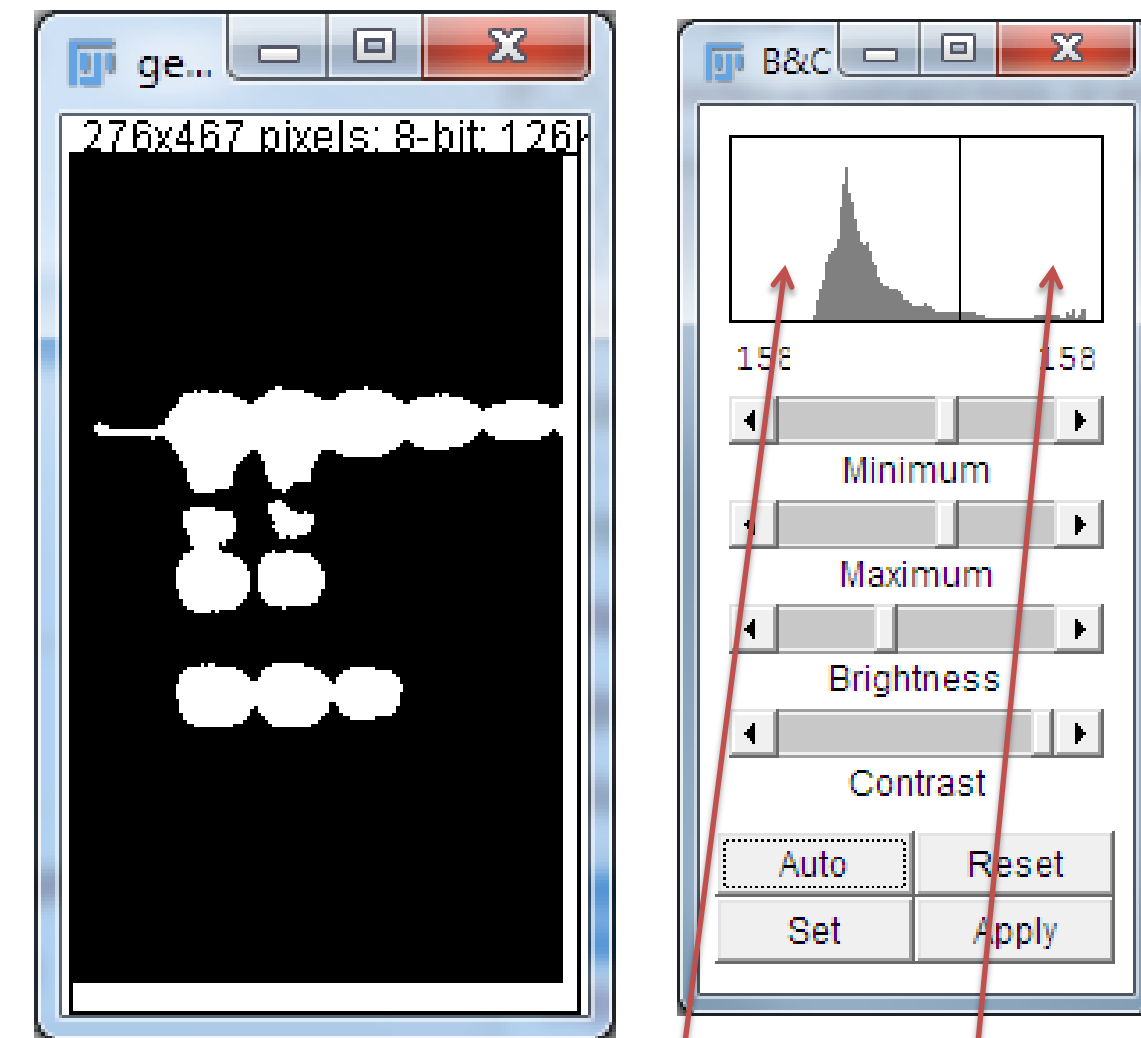
- Thresholding
- Clustering
- Region Growing
- Machine Learning

Segmentation: Thresholding

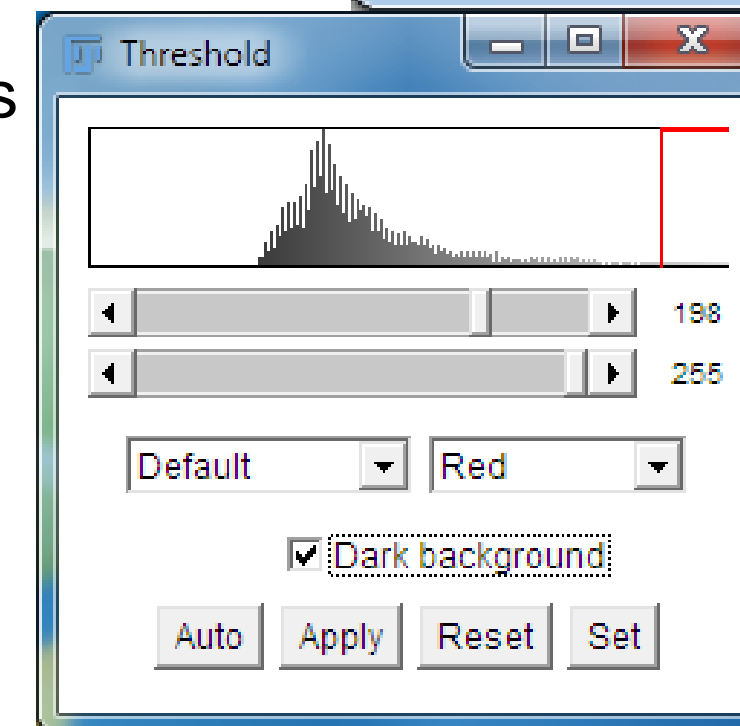
- Goal: Be able to use threshold based image segmentation

Exercise:

1. Open **Gel.tif**
2. Go to the **Adjust Brightness/Contrast** menu first
Set 'minimum' and 'maximum' to the same value and move 'brightness' and look at the change. Any pixel above the vertical bar is white, any pixel below is black.
3. Now use **File→Revert** and do **Image→Adjust→Threshold**
Red: White pixels
Play with the settings and the auto threshold methods the differences
All methods explained at:
http://pacific.mpi-cbg.de/wiki/index.php/Auto_Threshold



Black/White



Machine Learning

Trainable Weka Segmentation v3.2.28

c:1/3 - #157+edu24hOnly #2: 212.55x212.55 microns (512x512): 8-bit: 256K

Training

Train classifier

Toggle overlay

Create result

Get probability

Plot result

Options

Apply classifier

Load classifier


Save classifier

Load data

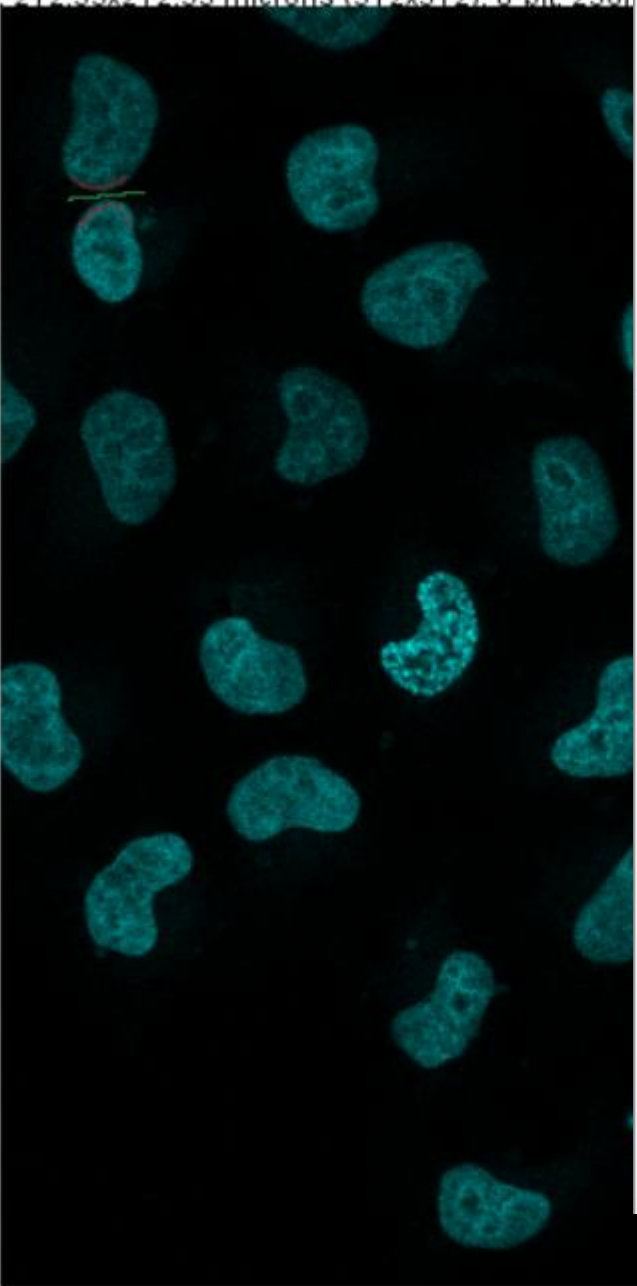
Save data

Create new class

Settings



WEKA
The University of Waikato



Labels

Add to class 1

Trainable Weka Segmentation v3.2.28

c:1/3 - #157+edu24hOnly #2: 212.55x212.55 microns (512x512): 8-bit: 256K

Training

Train classifier

Toggle overlay

Create result

Get probability

Plot result

Options

Apply classifier

Load classifier


Save classifier

Load data

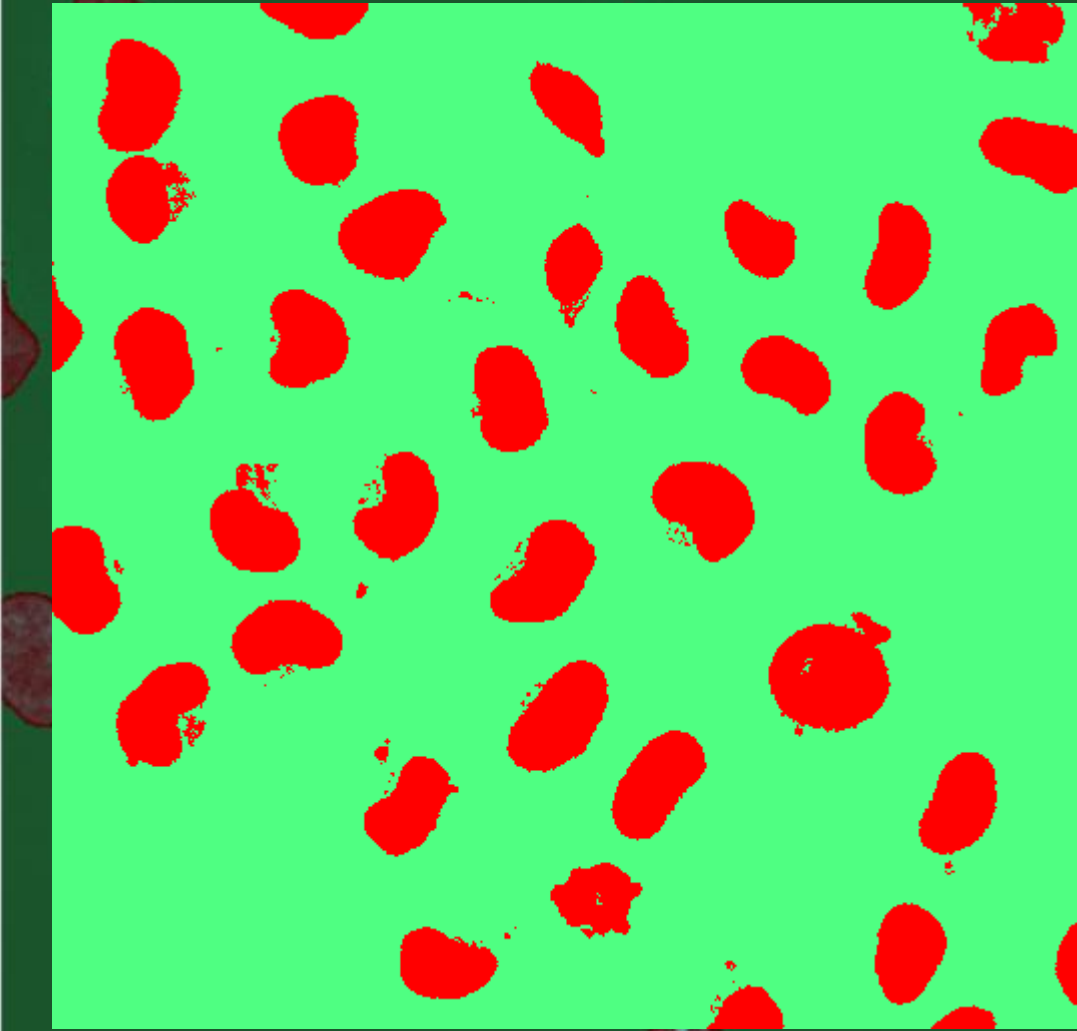
Save data

Create new class

Settings



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Labels

Add to class 1

trace 0 (Z=1)
trace 1 (Z=1)
trace 2 (Z=1)
trace 3 (Z=1)
trace 4 (Z=1)

Add to class 2

trace 0 (Z=1)
trace 1 (Z=1)
trace 2 (Z=1)
trace 3 (Z=1)

Trainable Weka Segmentation v3.2.28 (400%)

c:1/3 - #157+edu24hOnly #2: 212.55x212.55 microns (512x512): 8-bit: 256K

Training

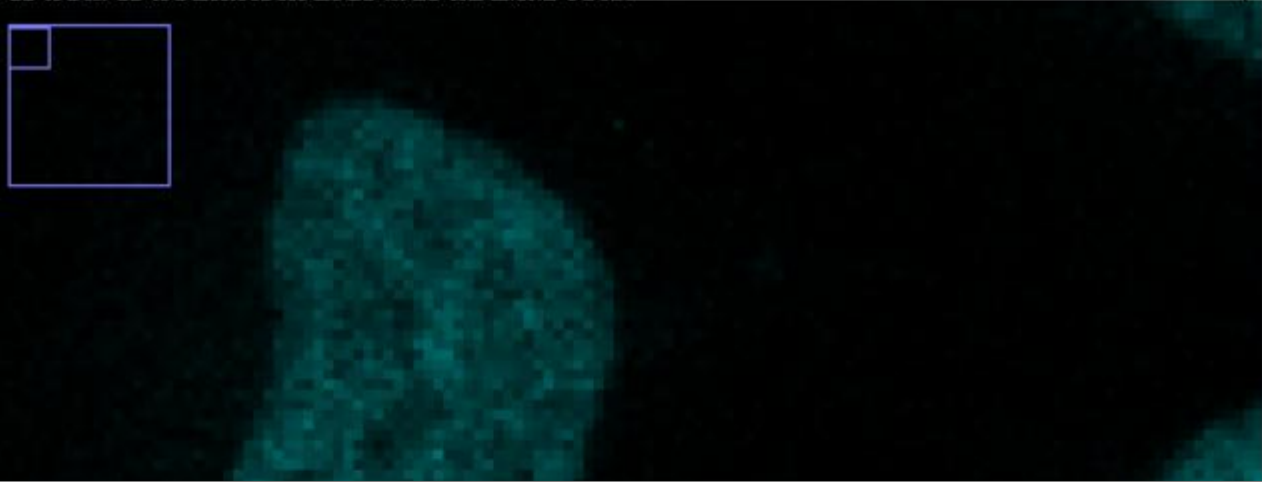
Train classifier

Toggle overlay

Create result

Get probability

Plot result



Labels

Add to class 1

Trainable Weka Segmentation v3.2.28 (300%)

c:1/3 - #157+edu24hOnly #2: 212.55x212.55 microns (512x512): 8-bit: 256K

Training


Train classifier

Toggle overlay

Create result

Get probability

Plot result



Labels

Add to class 1

Trainable Weka Segmentation v3.2.28

c:1/3 - #157+edu24hOnly #2: 212.55x212.55 microns (512x512): 8-bit: 256K

Training

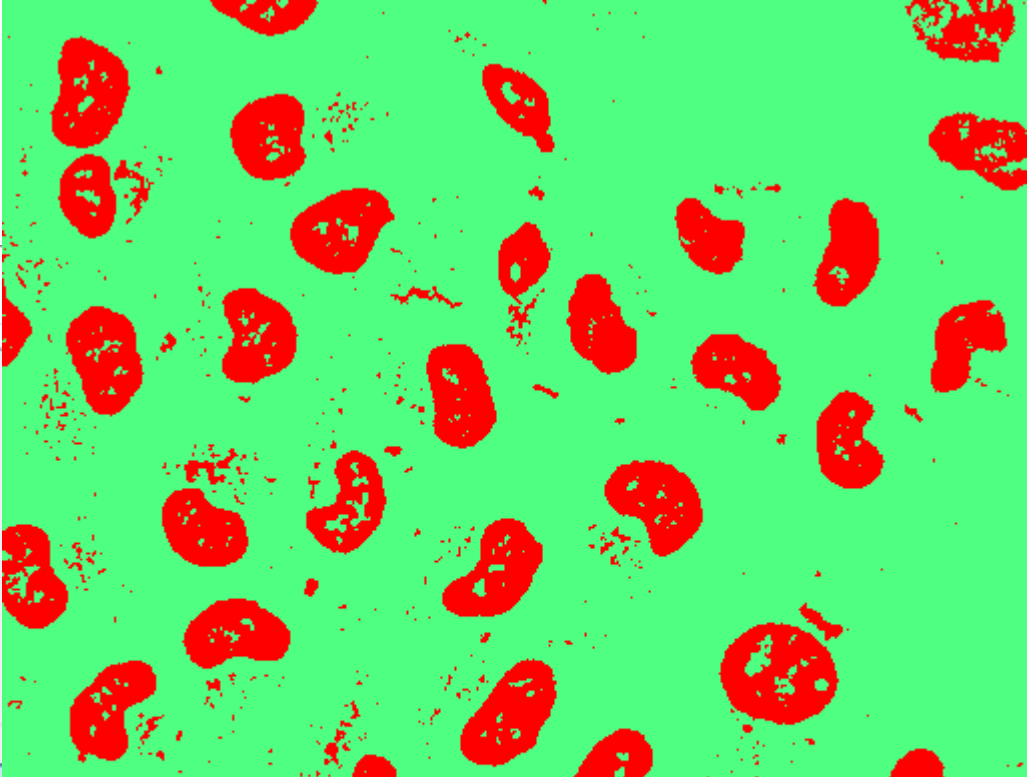
Train classifier

Toggle overlay

Create result

Get probability

Plot result




Labels

Add to class 1

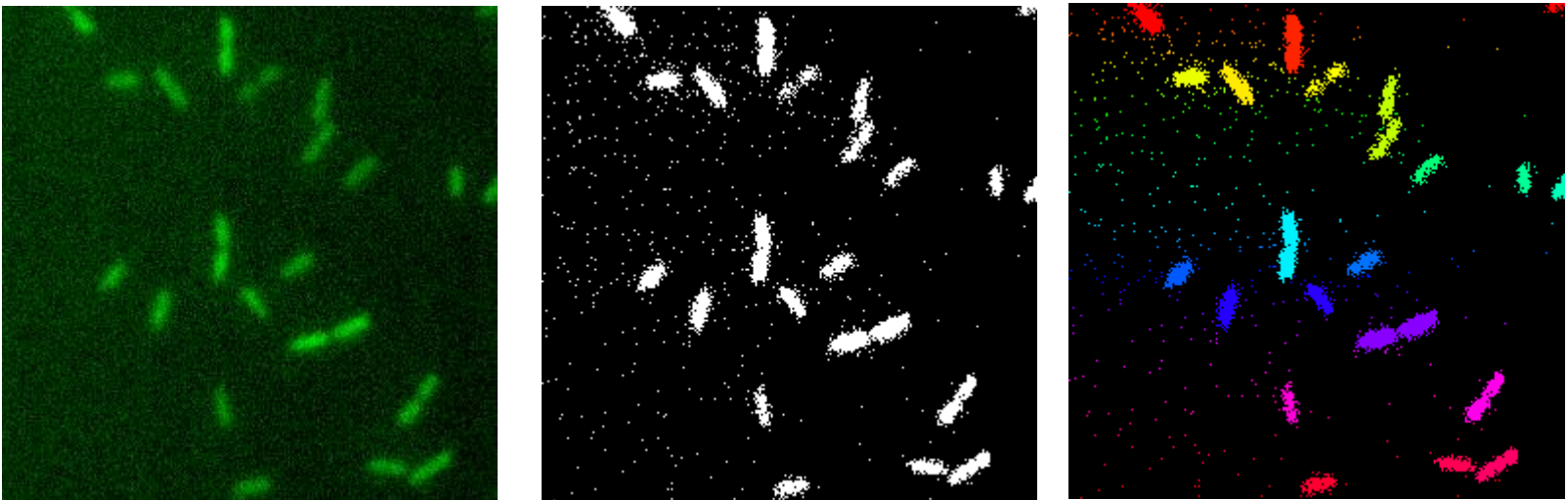
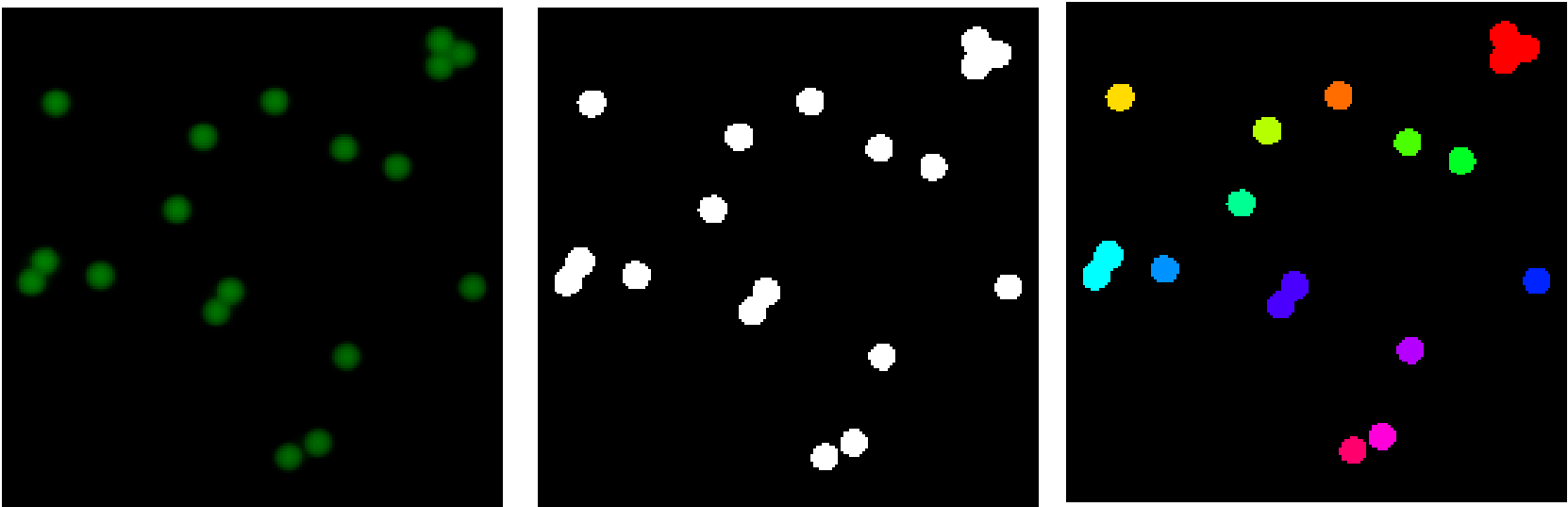
Save data

Create new class

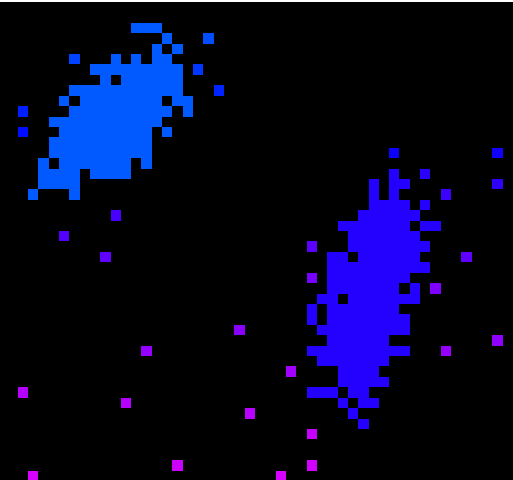
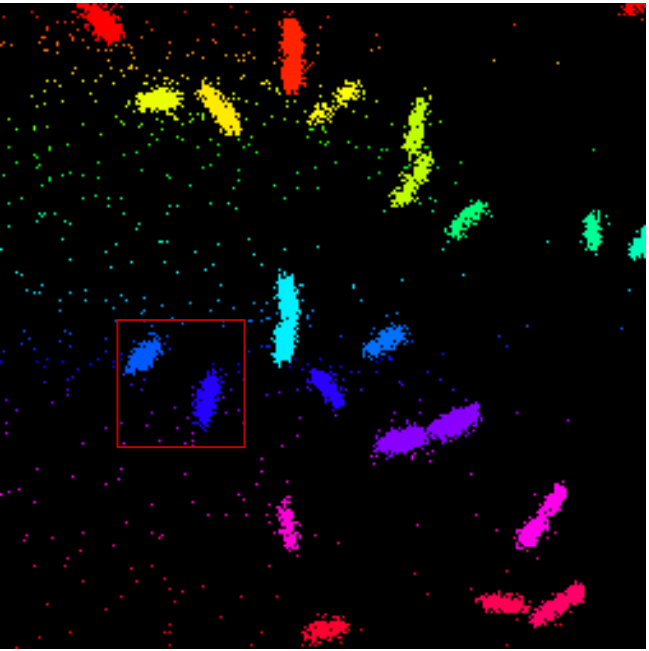
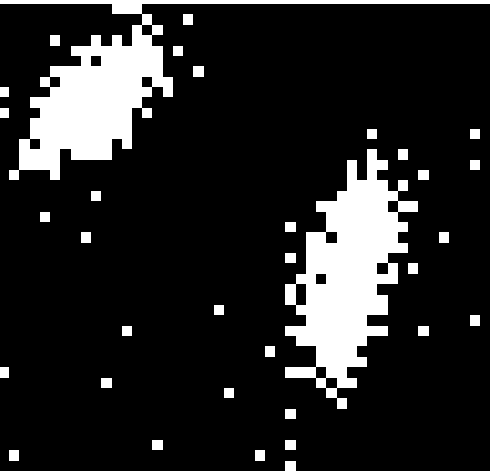
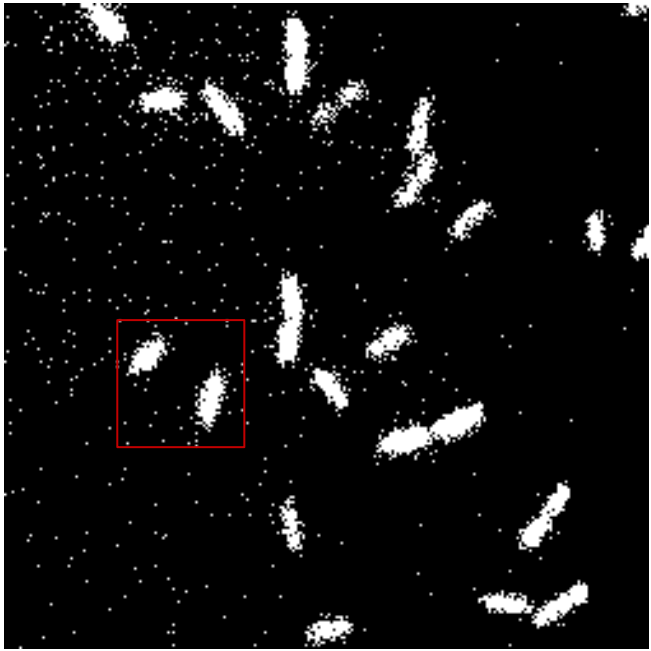
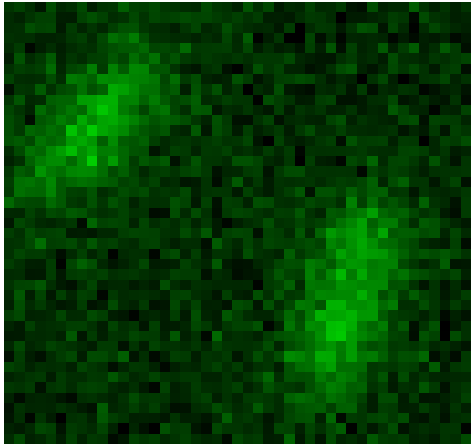
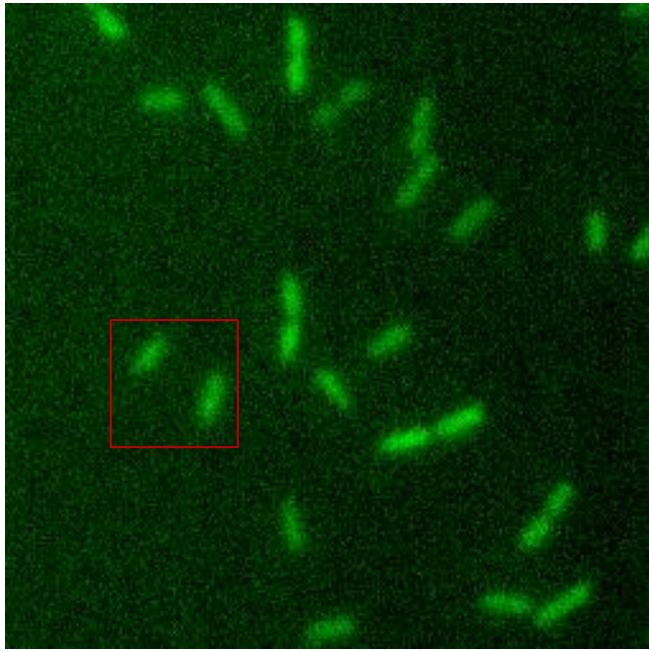
Settings



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DESCRIBE WORKFLOW from image to objects

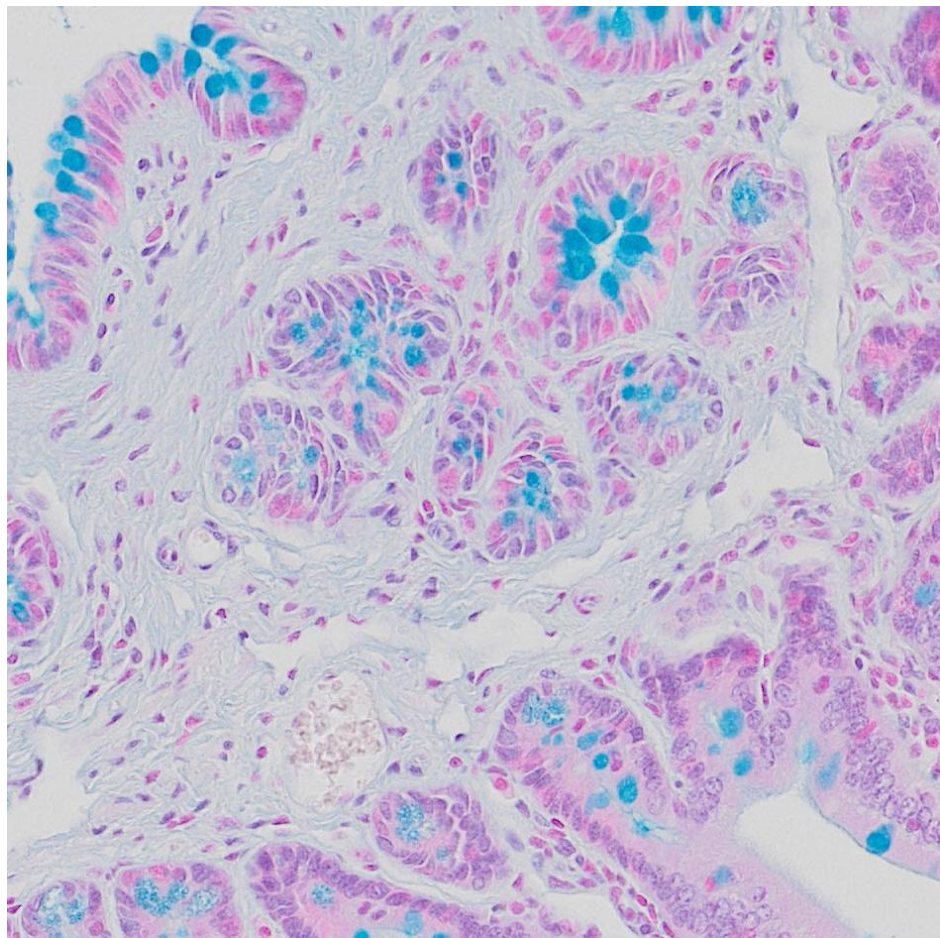


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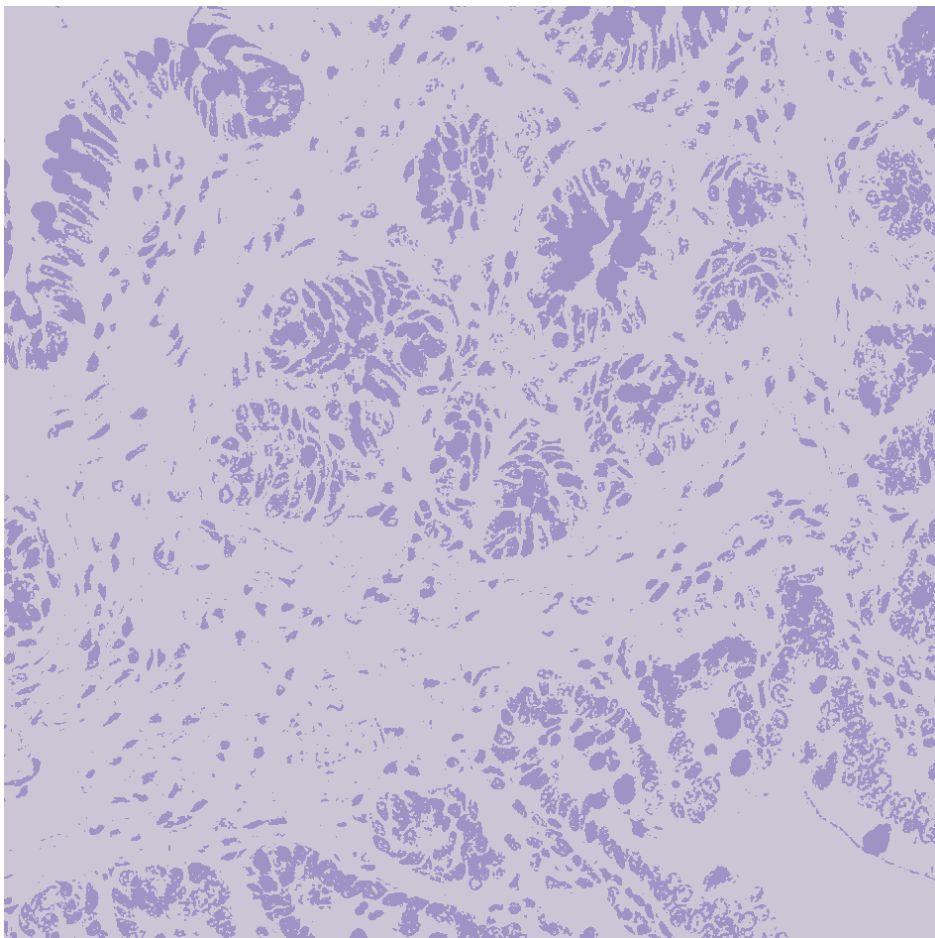
[illegible][illegible]

K-means clustering in IJ Plugins Toolkit

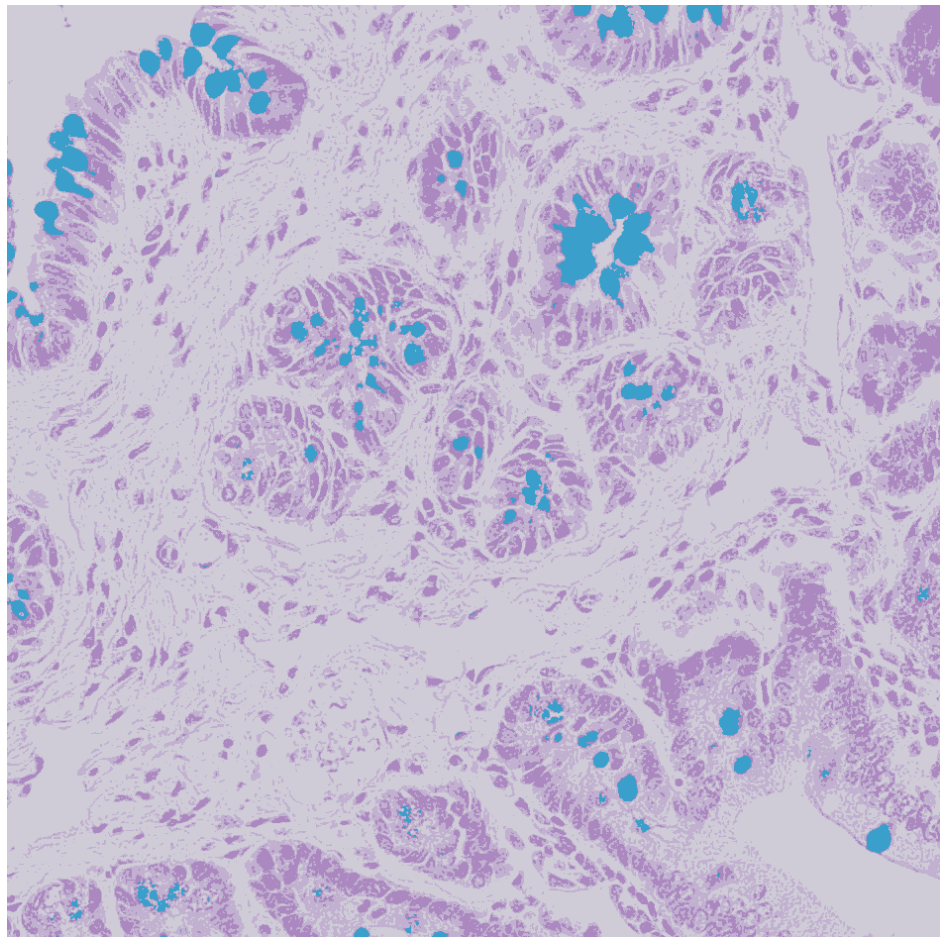
Original



K=2



K=4



K=7

