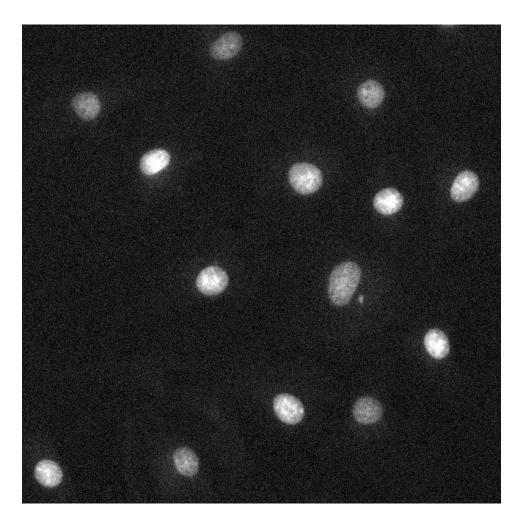


# 

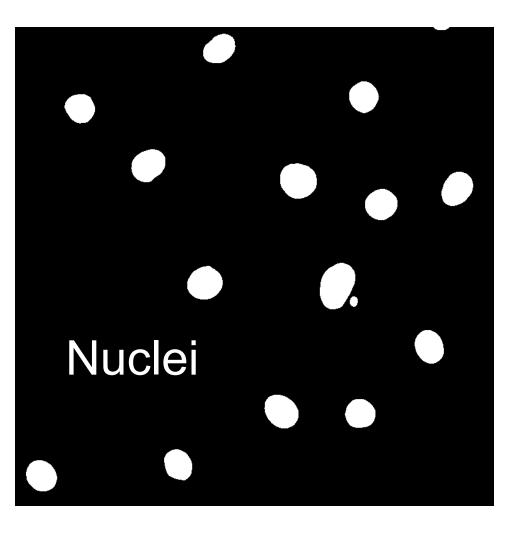
• Segmentation is the division of an image into discrete regions.

### Segmentation

Input

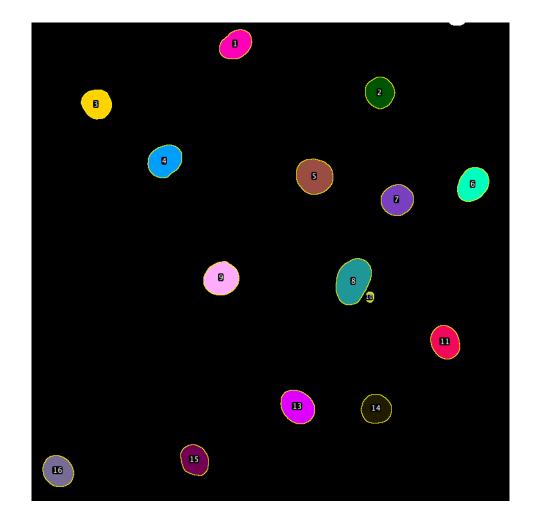


**Semantic** 



Background

### Instance



Background Nucleus 1 Nucleus 2 Nucleus 3

. . .

### How do we get segments?

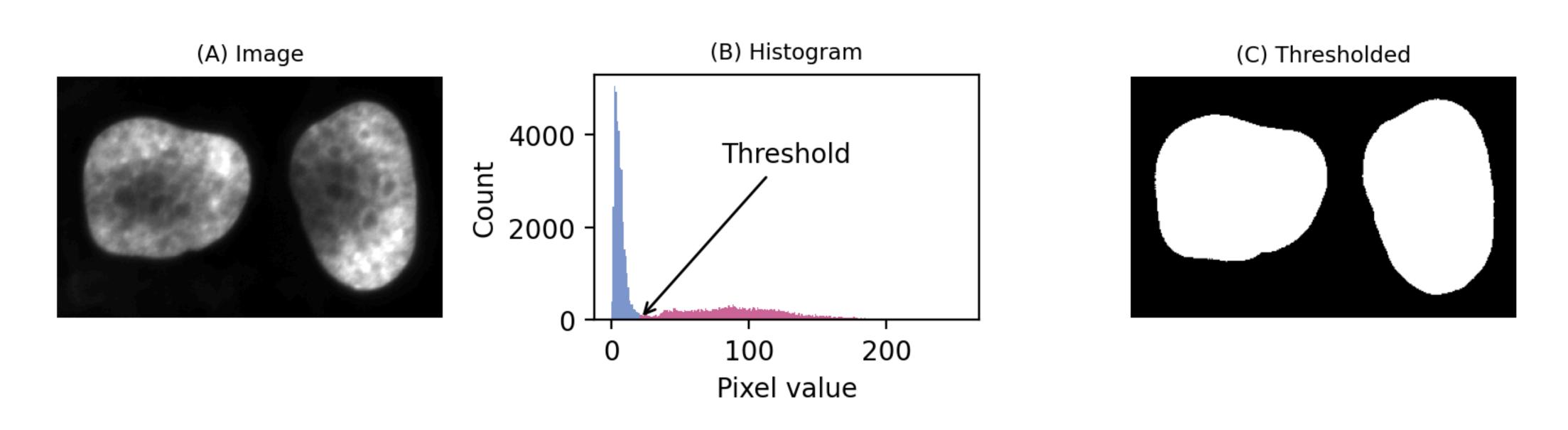
- Thresholding-based
- Interactive tools based on classic machine learning
- Deep-learning based (Stardist, Cellpose)

# c machine learning Cellpose)

### Thresholding

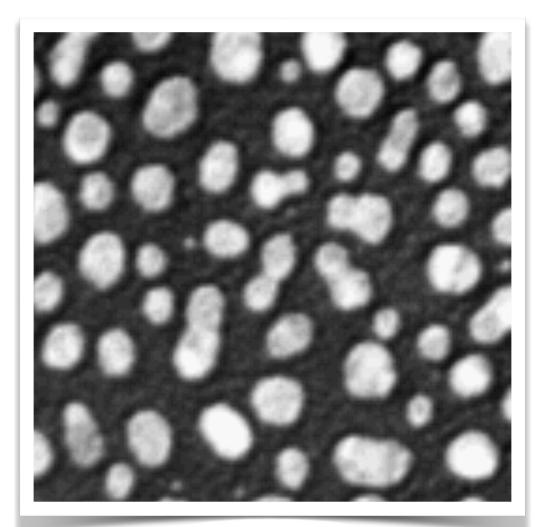
The easiest way to segment an image is often by applying a global threshold.

This identifies pixels that are above or below a fixed threshold value, giving a binary image as the output.

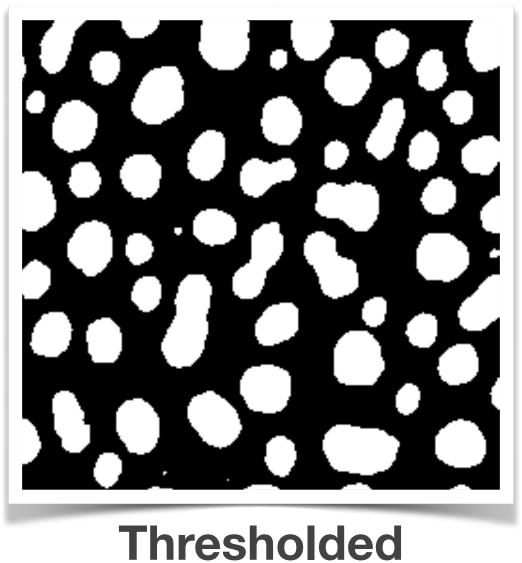


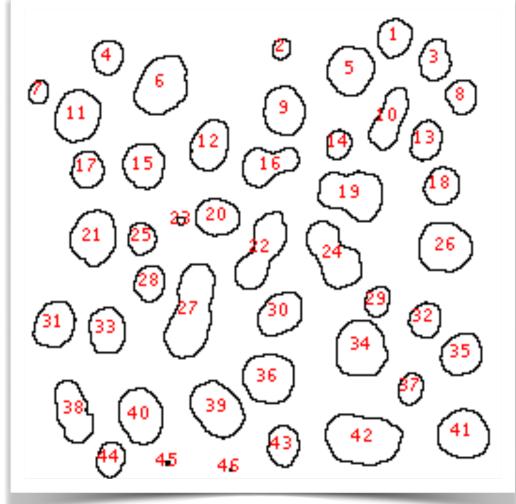
https://bioimagebook.github.io/chapters/2-processing/3-thresholding/thresholding.html

### Thresholding



**Original**, 8 bit grayscale Blobs: Fiji example

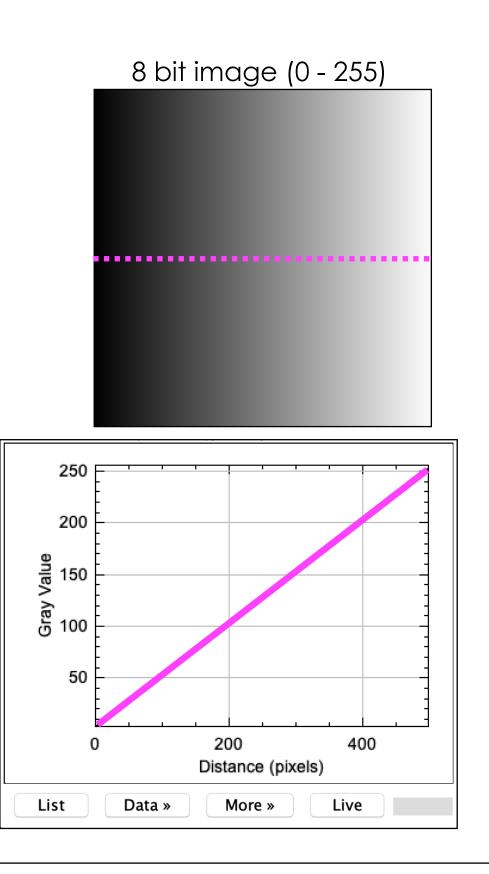




**Instance Segmented** 

**Instance Segmentation** in FIJI: keeping white (connected-) objects.

**Select** only a **range** of **digital values** in the image.







# Analysis Collaboratory





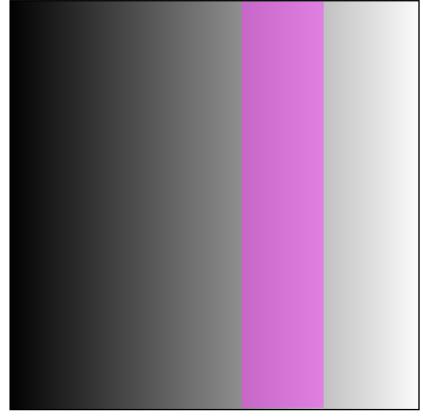
**Select** only a **range** of **digital values** in the image.

The result of the thresholding process is a **Binary Mask**.



### Analysis Collaboratory

8 bit image (0 - 255)

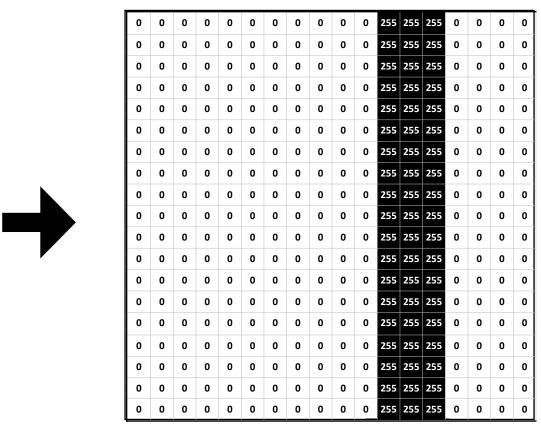


**Binary** because the image has only **two** pixel values, one for the selected pixels and one for the "discarded" pixels.

In Fiji the two pixel values are **0** and **255**.



### Generate a **binary mask**.



0 **or** 255



### Image Analysis Collaboratory

### Segmentation with thresholding—exercises



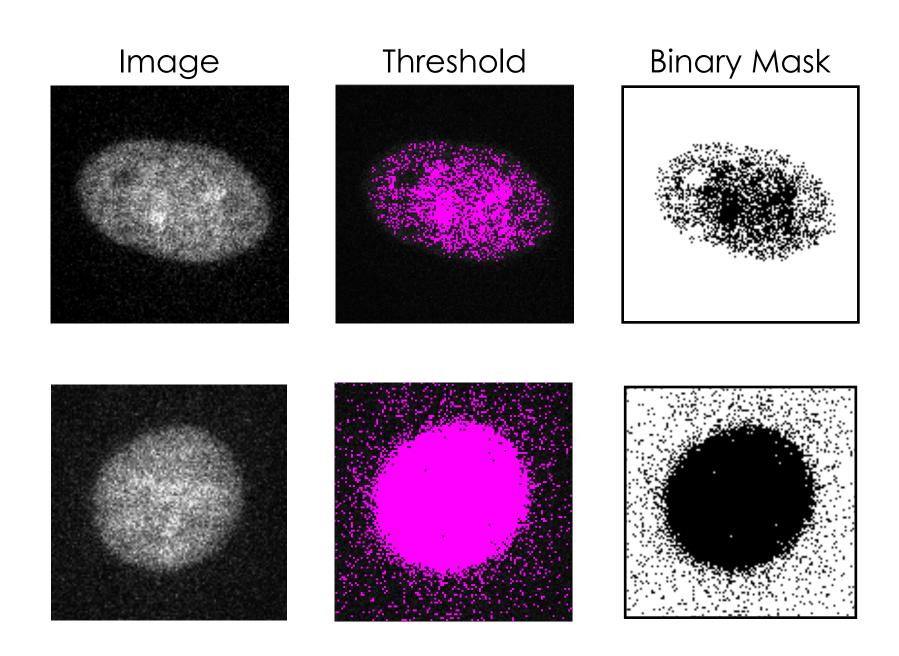


### 7.1 DAPI segmentation with thresholding

### skip "Analyze Particles"



Usually, if you apply **thresholding** to the "ORIGINAL" image (the one you get out of the microscope), you won't be able to precisely select all/only the pixels you are interested in.



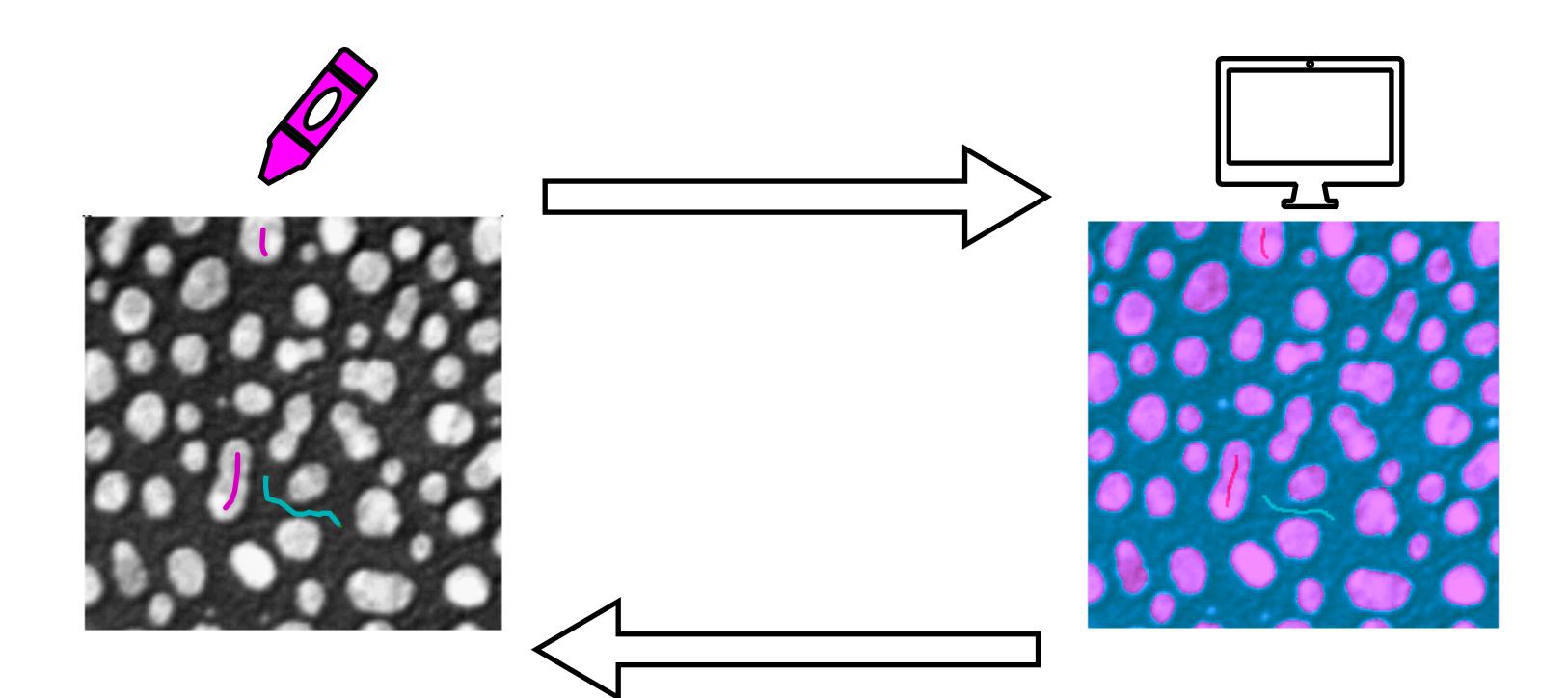


What can go wrong?

- Fluorescence label (e.g. DAPI)
- **Background** (uneven illumination, out-of-focus light, aberration, ...)
- Noise (detector read noise, Poisson noise, ...)



### Interactive tools based on classic machine learning







# Analysis Collaboratory

### Standalone: Ilastik







https://www.ilastik.org/

https://qupath.github.io/

WEKA: Waikato Environment for Knowledge Analysis: collection of free machine learning and data analysis software developed by the University of Waikato, NZ



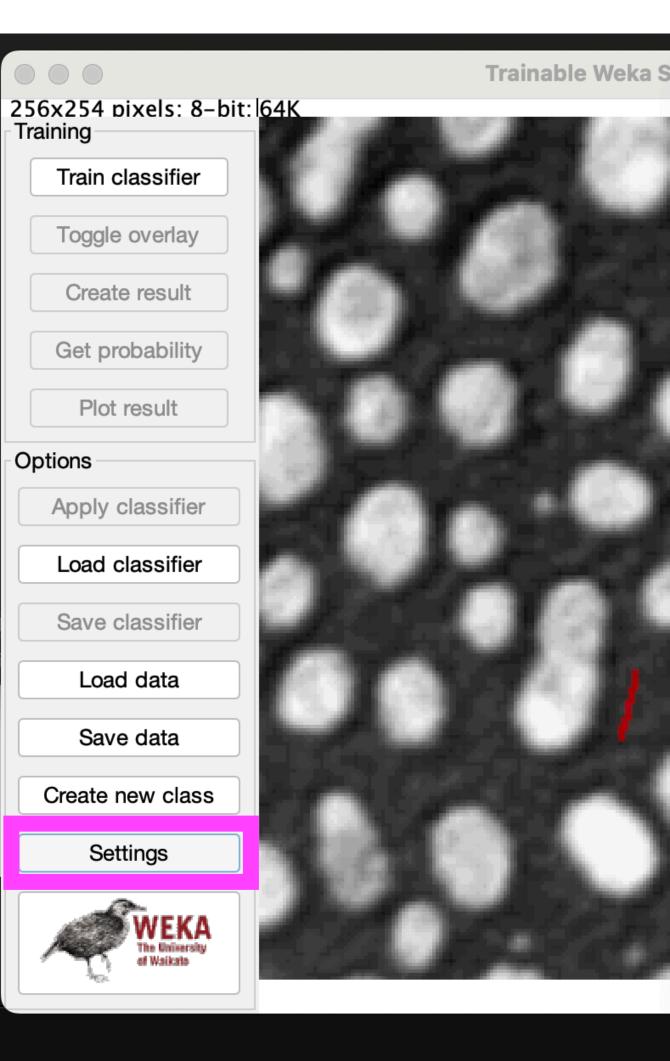
### As a Fiji plugin



https://imagej.net/plugins/tws/



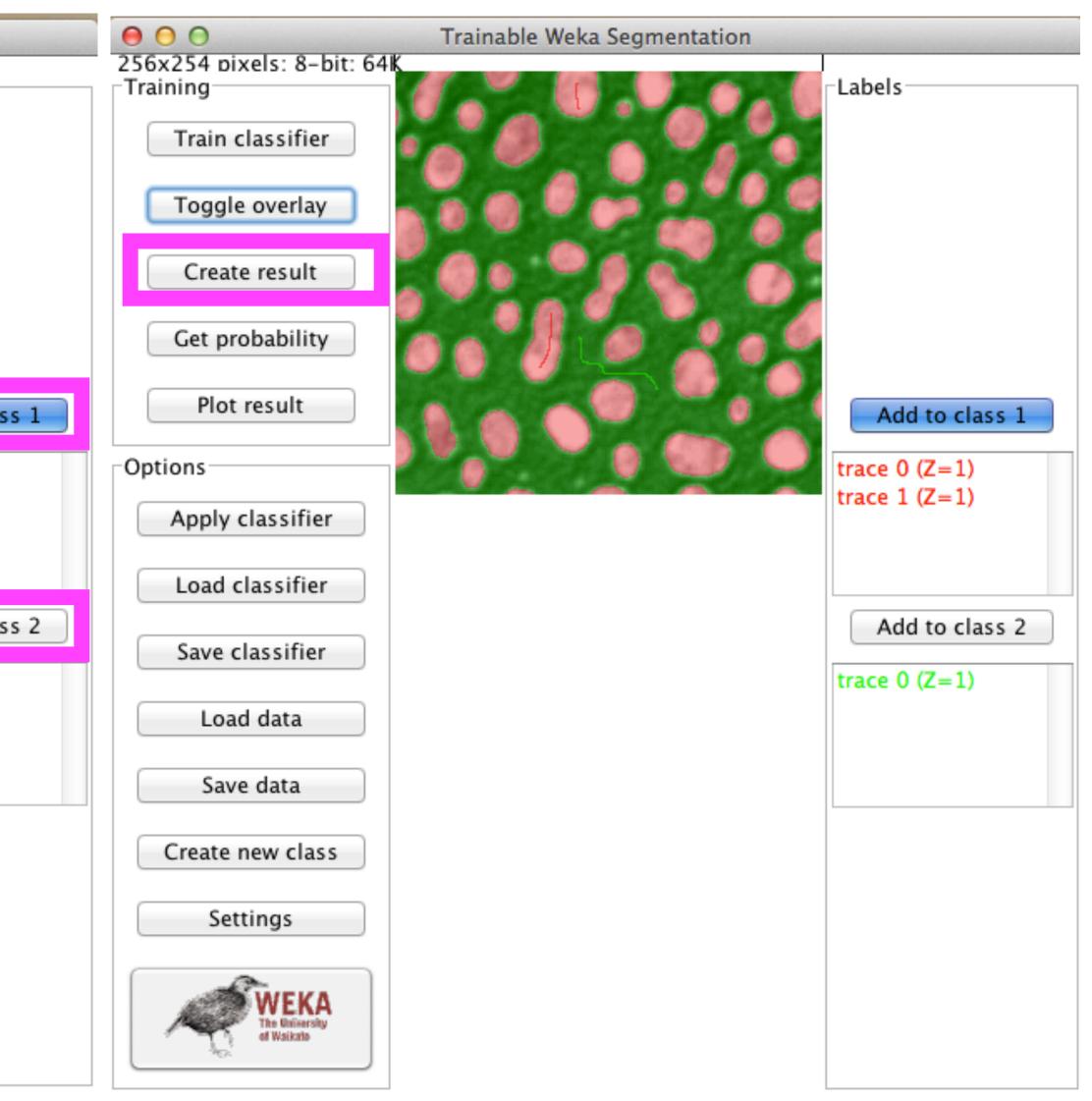
# Trainable WEKA Segmentation (in ImageJ/Fiji)



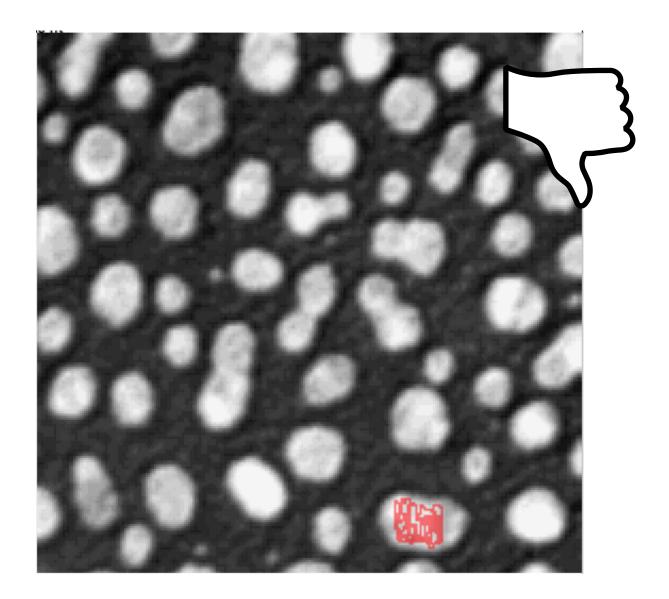
egmer	Segmentation settings
	Training features:   ✓ Gaussian blur ✓ Sobel filter   ✓ Hessian ✓ Difference of gaussians   ✓ Membrane projections Variance   Mean Minimum   Maximum Median   Anisotropic diffusion Bilateral   Lipschitz Kuwahara   Gabor Derivatives   Laplacian Structure   Entropy Neighbors   Membrane thickness: 1   Membrane patch size: 19   Minimum sigma: 1.0
	Maximum sigma: 16.0
	Classifier options: Choose <b>FastRandomForest</b> -I 200 -K 2 -S -267353182
	Class names: Class 1 class 1 Class 2 class 2 Class 3 asdfas Advanced options: Balance classes Save feature stack Result overlay opacity 33
	Help Cancel OK

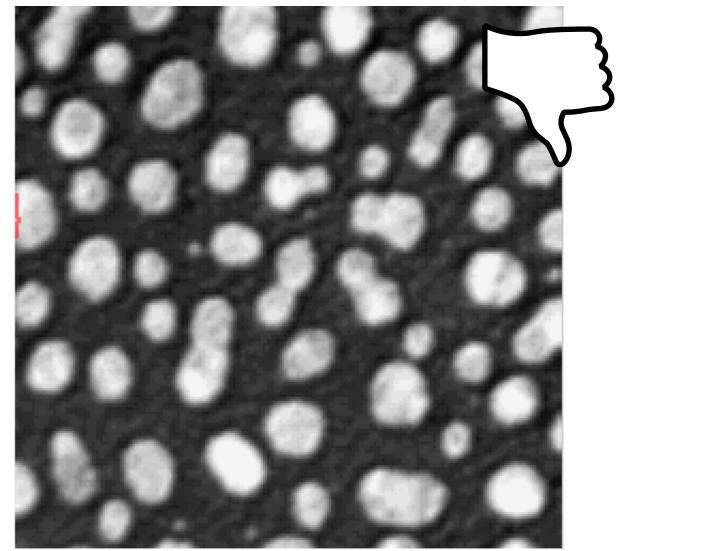
### Trainable WEKA Segmentation (in ImageJ/Fiji)

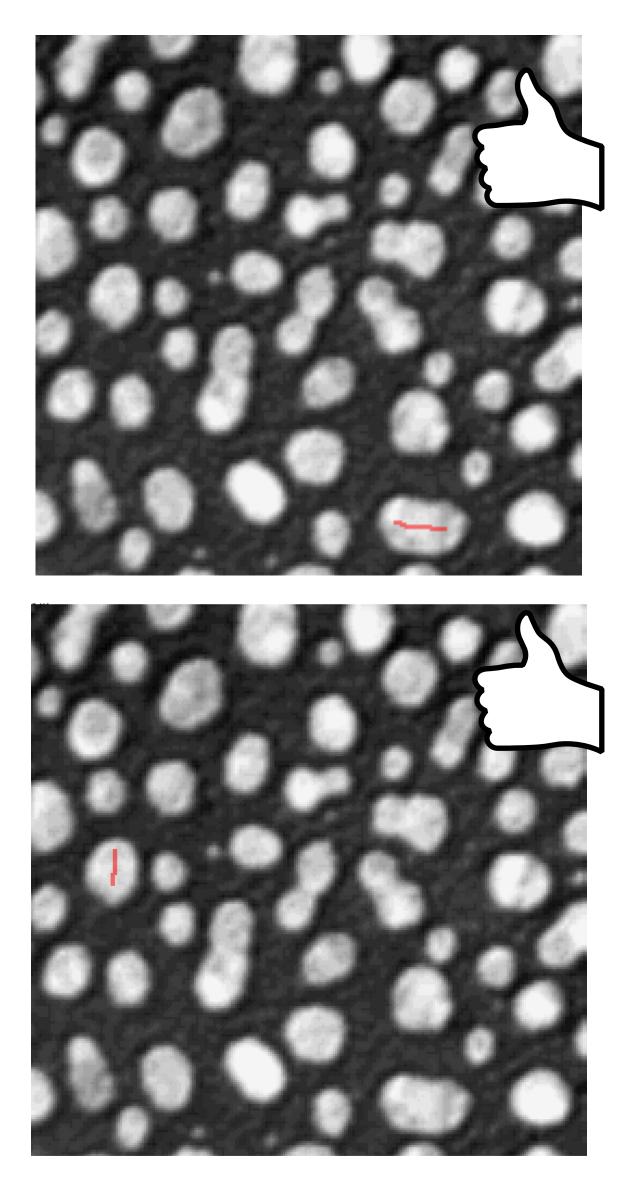
0.0.0		
0 0 0 256x254 pixels: 8-bit: 64	Trainable Weka Segmentation	
Training		Labels
Train classifier		
Toggle overlay		
Create result		
Get probability		
Plot result	and the second second second second	Add to clas
		Add to clas
Options	And the second second second	trace 0 (Z=1)
		trace 1 (Z=1)
Apply classifier		
Load classifier		
		Add to clas
Save classifier		
		trace 0 (Z=1)
Load data		
Save data		
Create new class		
Settings		
The University		
of Waikato		



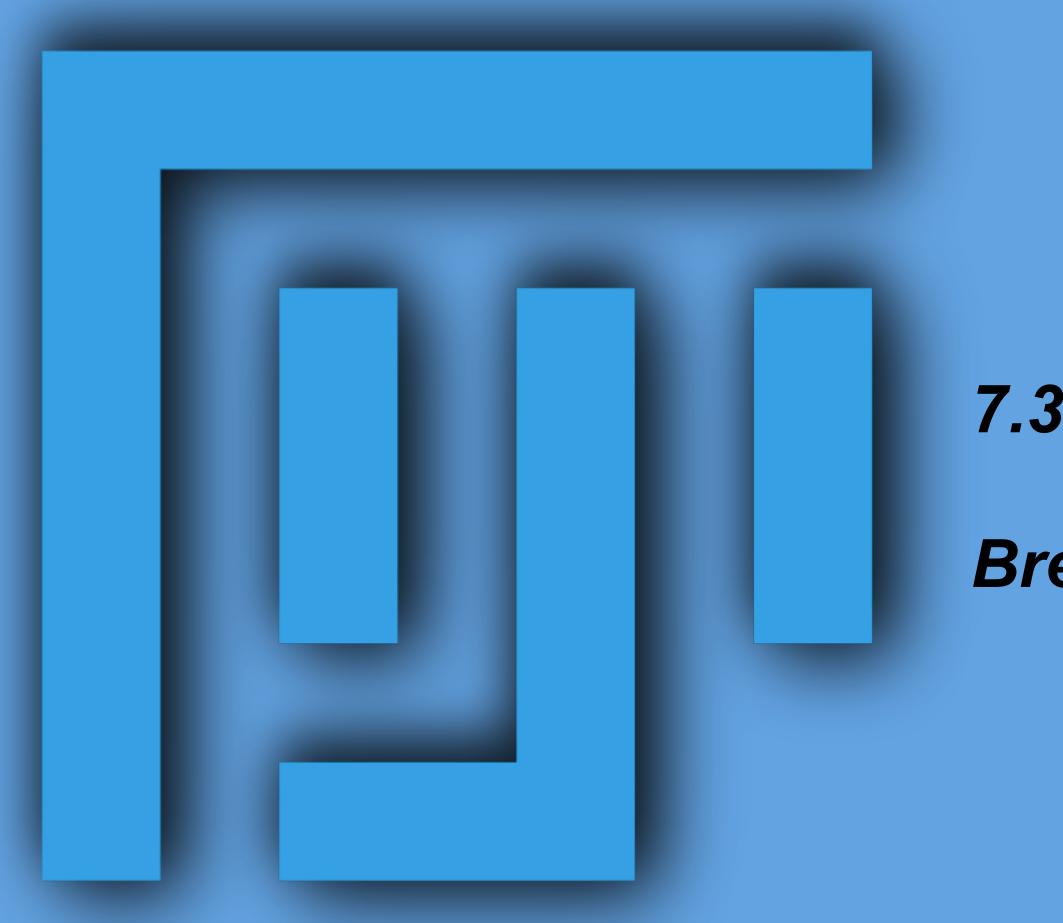
### Tips







### Segmentation with pixel based classifier—exercises



### 7.3 DAPI segmentation with Weka

### Breakpoint at 12: Create results