Image Processing

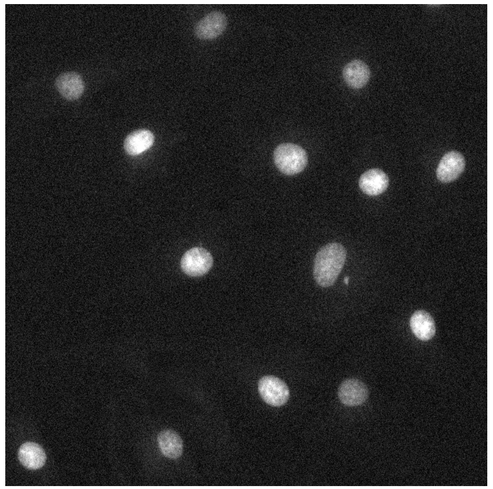
—

Segmentation

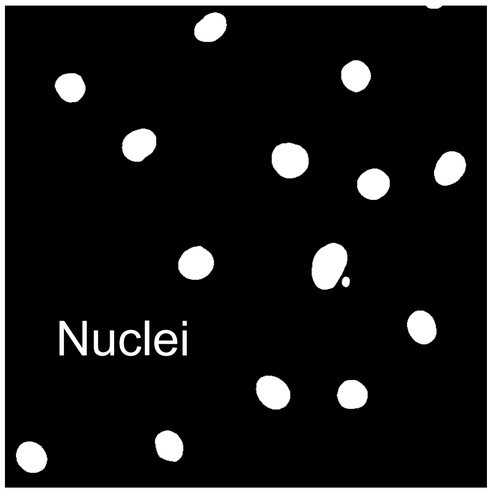
- Segmentation is the **division** of an image into **discrete regions**.

Segmentation

Input

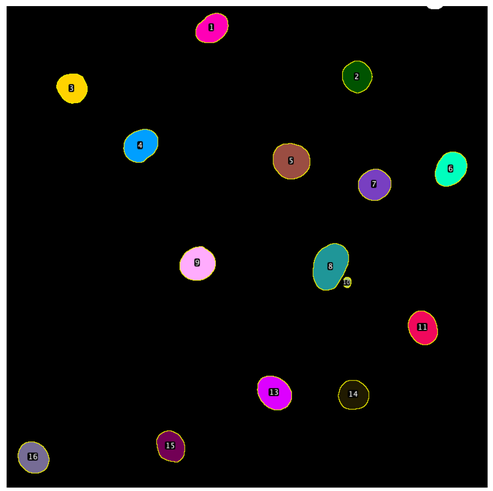


Semantic



Background

Instance



Background
Nucleus 1
Nucleus 2
Nucleus 3
…

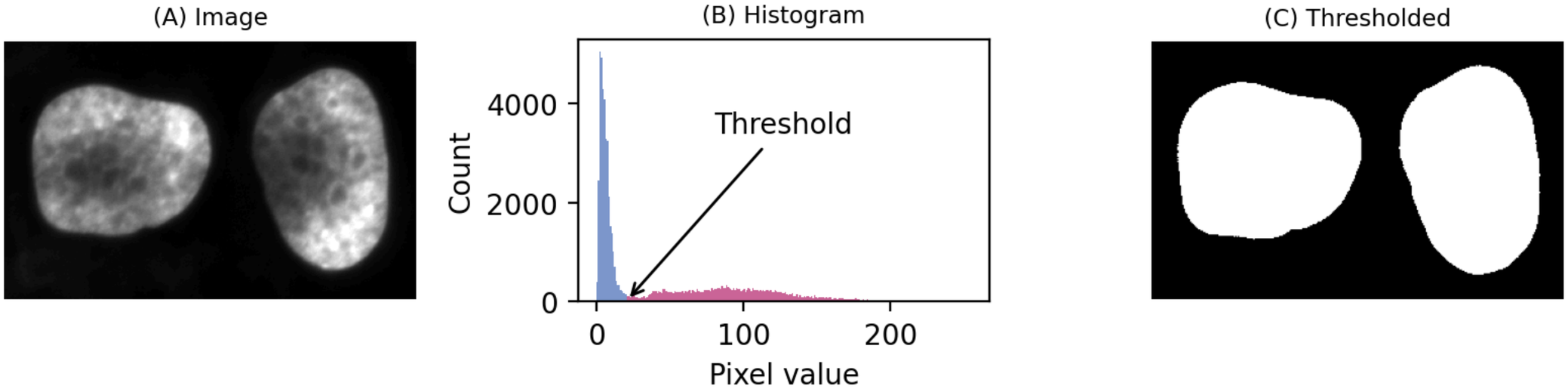
How do we get segments?

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

Thresholding

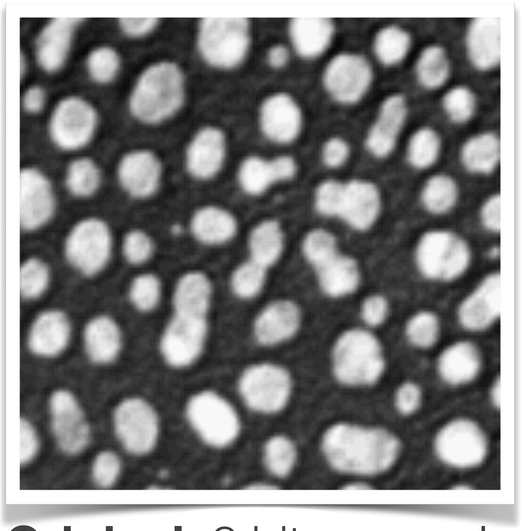
The easiest way to segment an image is often by applying a *global threshold*.

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

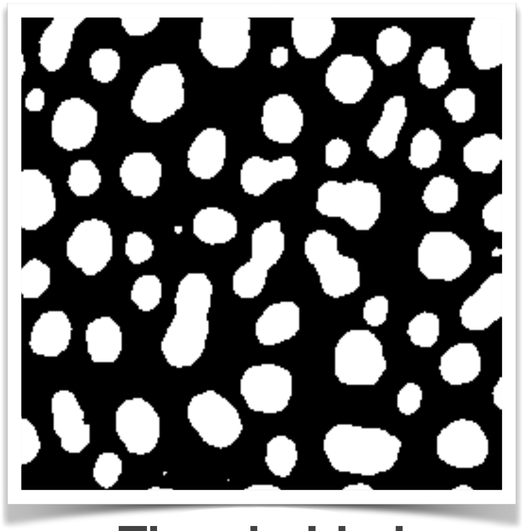


https://bioimagebook.github.io/chapters/2-processing/3-thresholding/thresholding.html

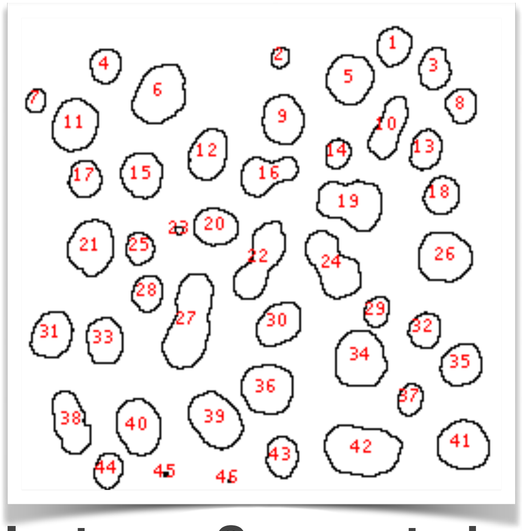
Thresholding



Original, 8 bit grayscale
Blobs: Fiji example



Thresholded



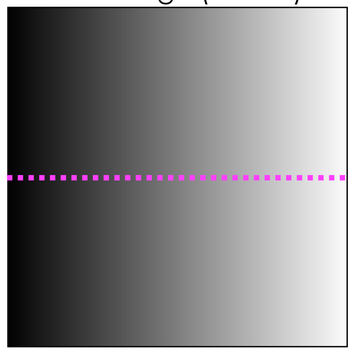
Instance Segmented

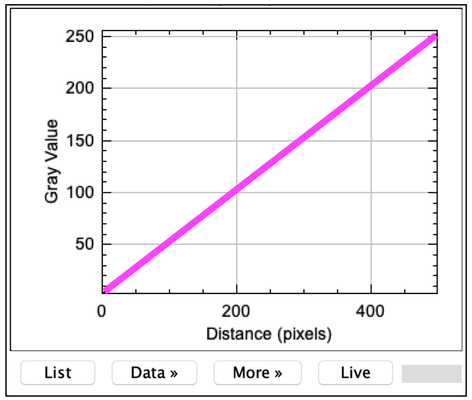
Instance Segmentation in FIJI**:** keeping white (*connected-*) objects.

Thresholding method

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

8 bit image (0 - 255)





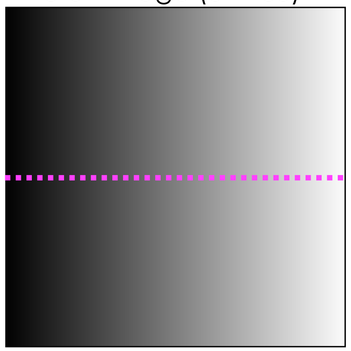
Thresholding method

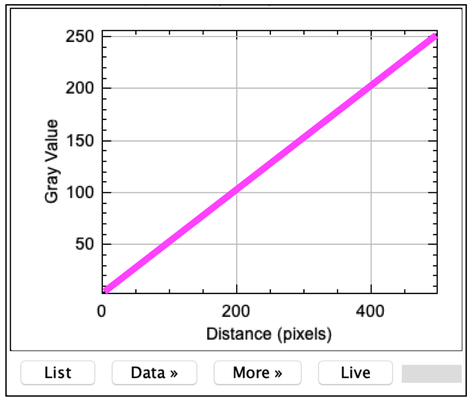
Select only a **range** of **digital values** in the image.

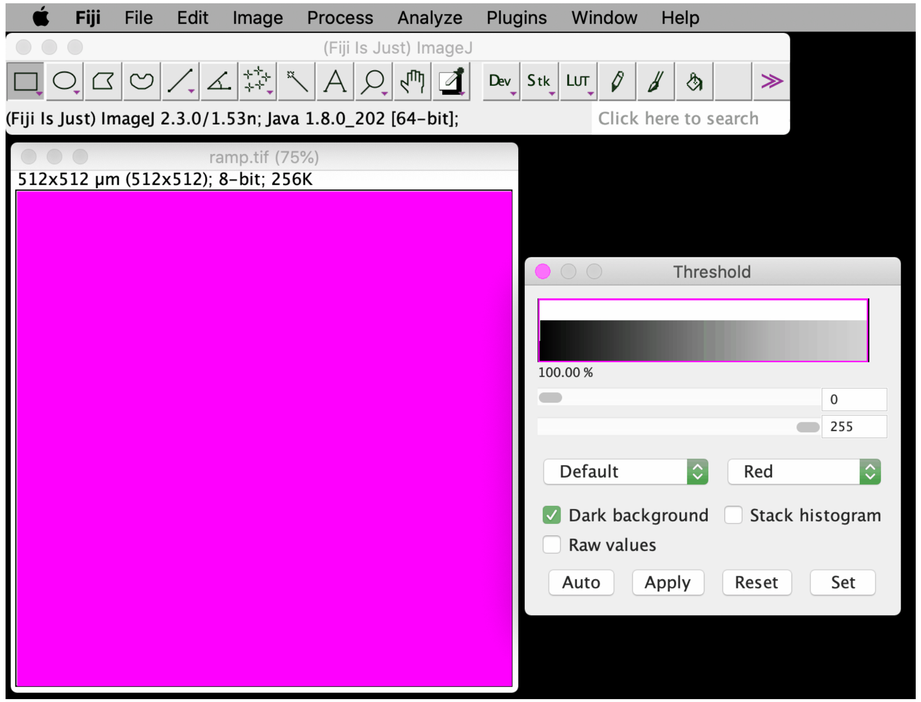
in **Fiji**: **Image** > **Adjust** > **Threshold…**

(cmd) + shift + t

8 bit image (0 - 255)







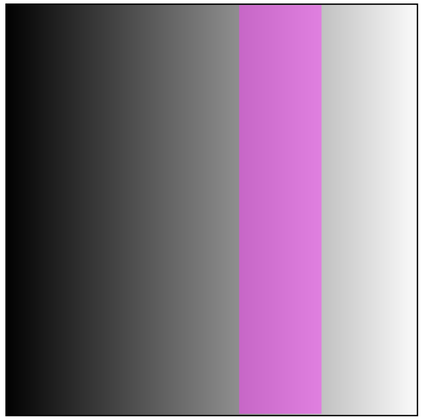
The result of the thresholding process is a **Binary Mask**.

Image Analysis Collaboratory

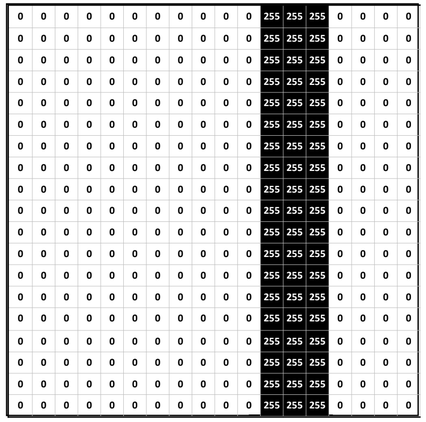
Thresholding method

Generate a **binary mask**.

8 bit image (0 - 255)



0 **or** 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.**

Image
Analysis
Collaboratory

Segmentation with thresholding—exercises

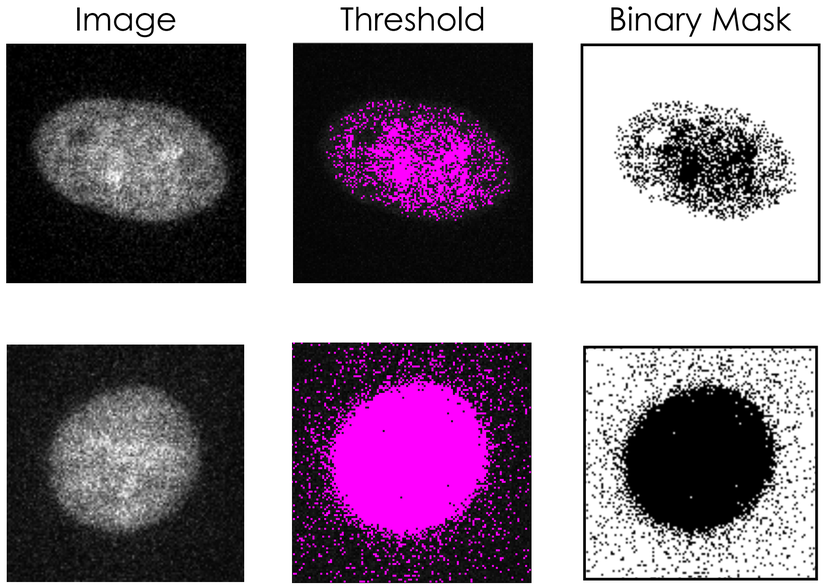
7.1 DAPI segmentation with thresholding

skip “Analyze Particles”

Thresholding method

What can go wrong?

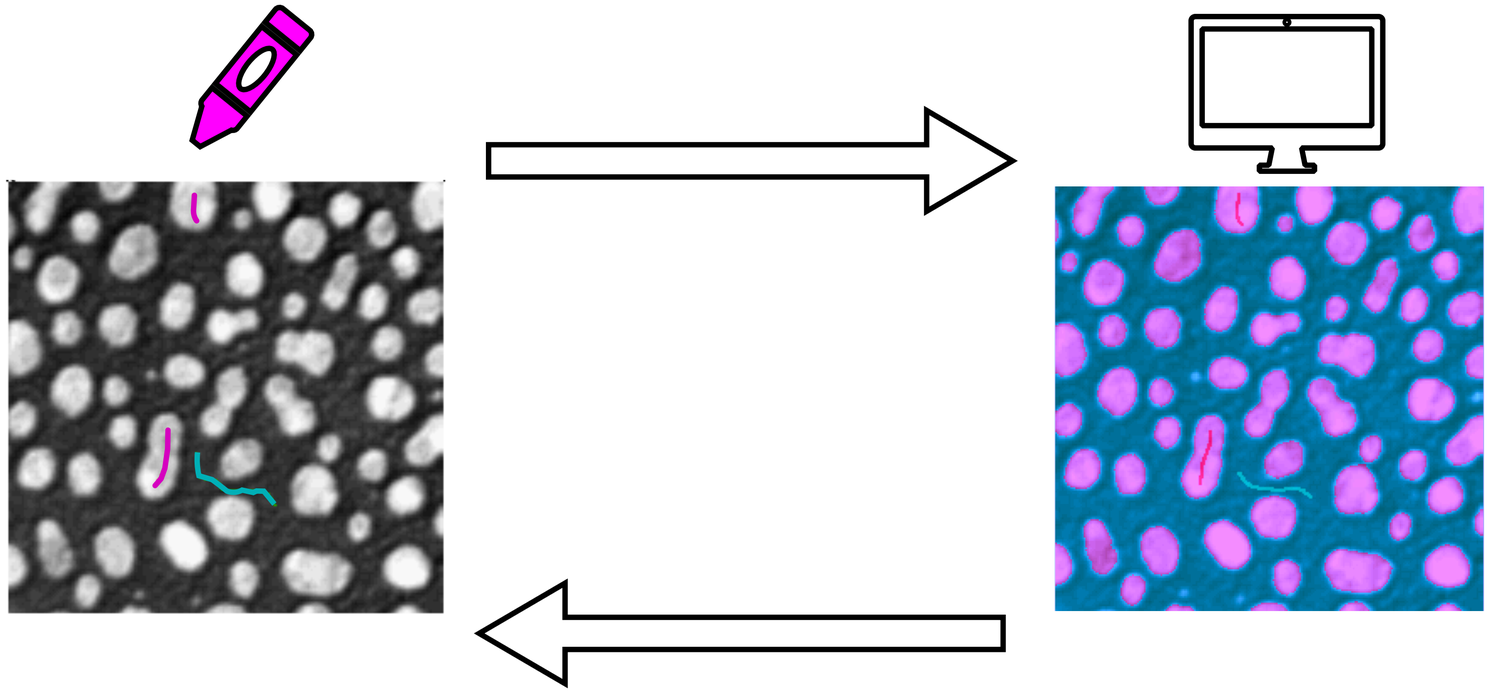
Usually, if you apply **thresholding** to the **"ORIGINAL" image** (the one you get out of the microscope), you won't be able to precisely **select all/only the pixels** you are interested in.



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

Image
Analysis
Collaboratory

Interactive tools based on classic machine learning



Standalone: Ilastik

https://www.ilastik.org/

For histopathology

https://qupath.github.io/

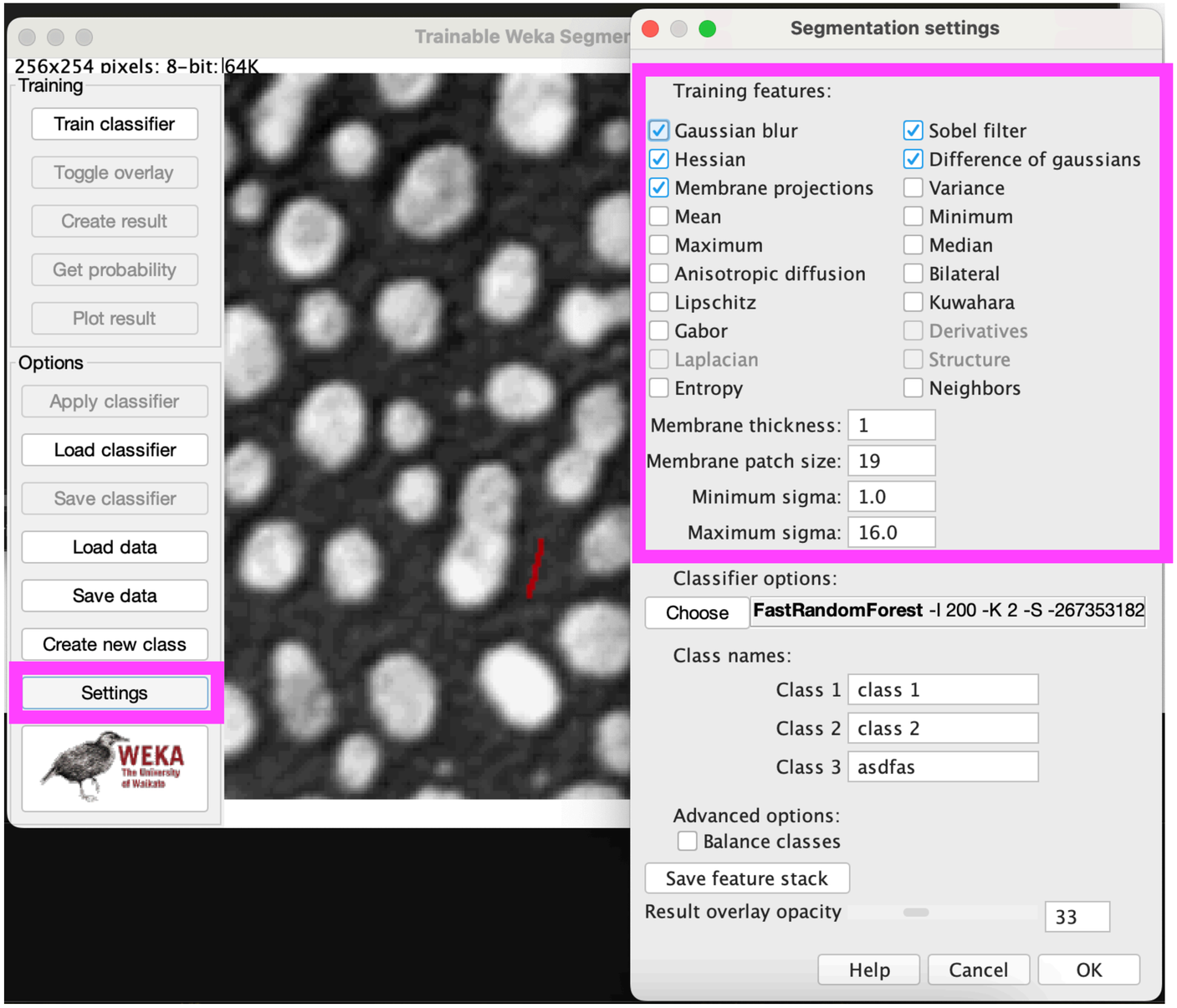
As a Fiji plugin

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

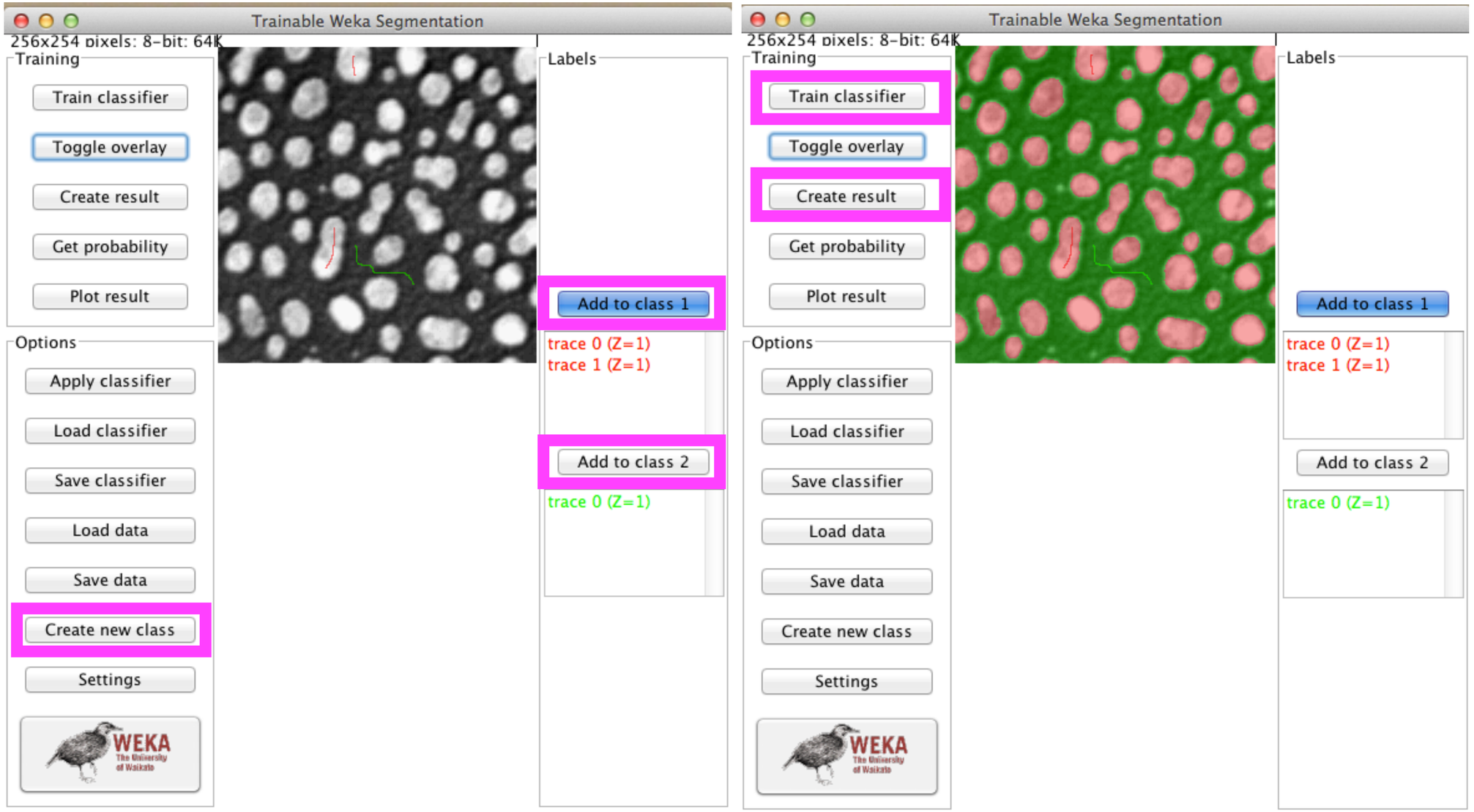
WEKA: Waikato Environment for Knowledge Analysis: collection of free machine learning and data analysis software developed by the University of Waikato, NZ

Image Analysis Collaboratory

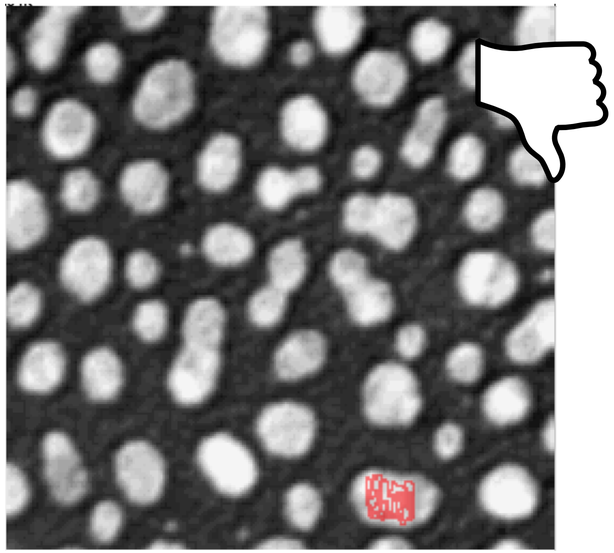
Trainable WEKA Segmentation (in ImageJ/Fiji)

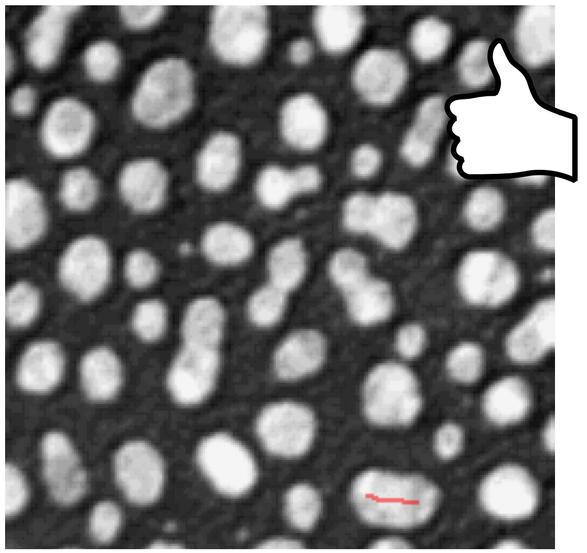


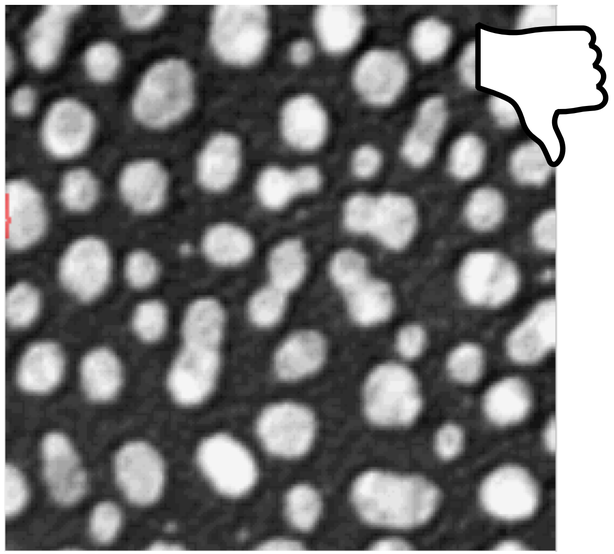
Trainable WEKA Segmentation (in ImageJ/Fiji)

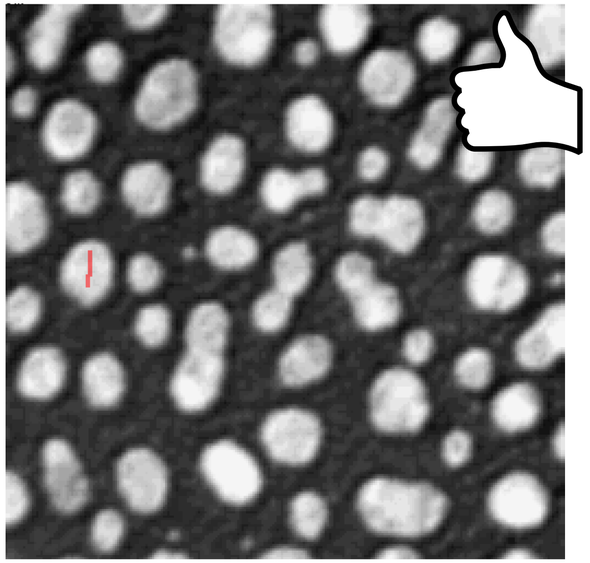


Tips









Segmentation with pixel based classifier—exercises

7.2 DAPI segmentation with Weka

Breakpoint at 12: Create results