# Image Processing

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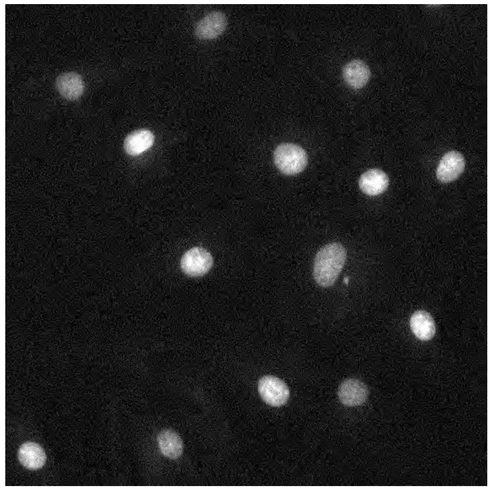
## Segmentation

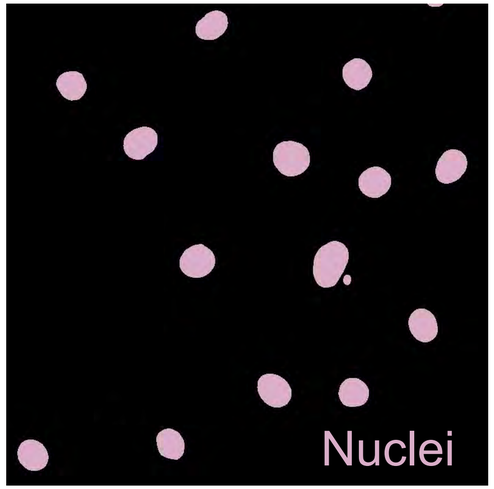
- Segmentation is the **division** of an image into **discrete regions**.

# Segmentation

Input

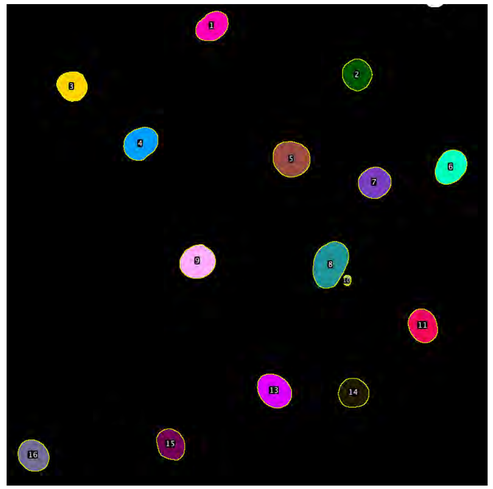


Semantic




Background

Instance



Background
Nucleus 1
Nucleus 2
Nucleus 3
…

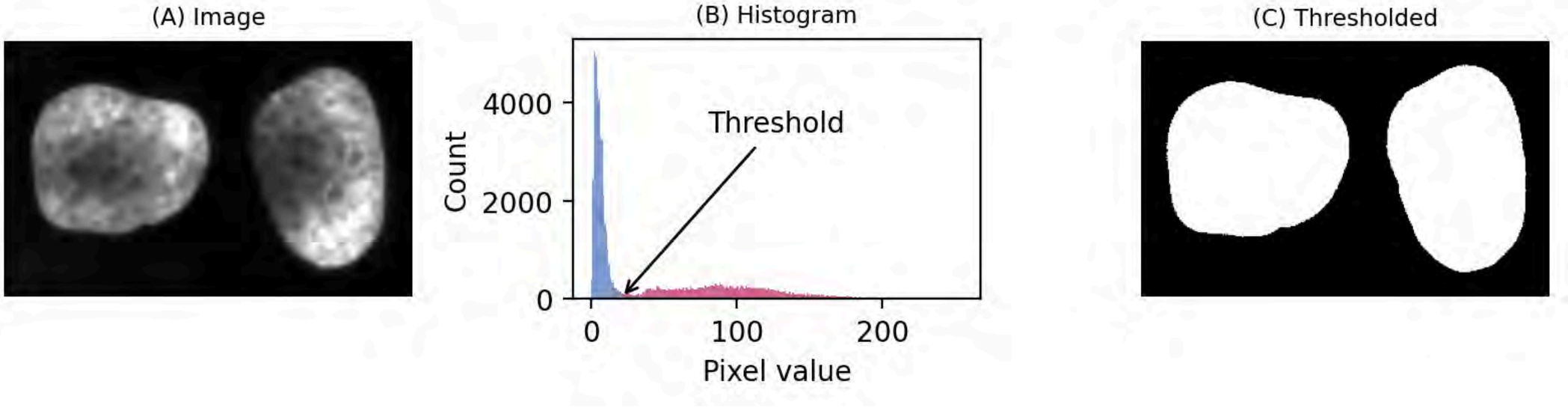
# How do we get segments?

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

# Thresholding

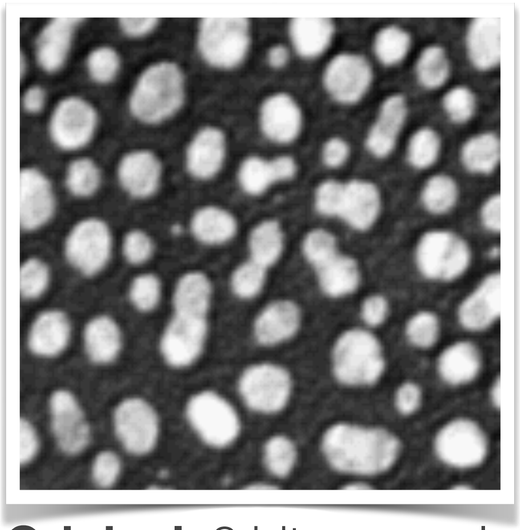
The easiest way to segment an image is often by applying a *global threshold*.

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

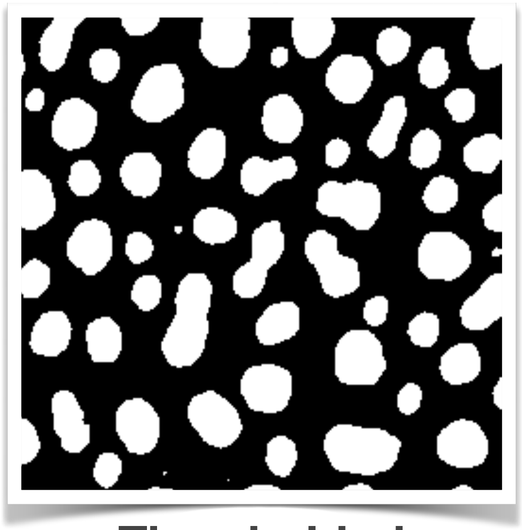



https://bioimagebook.github.io/chapters/2-processing/3-thresholding/thresholding.html

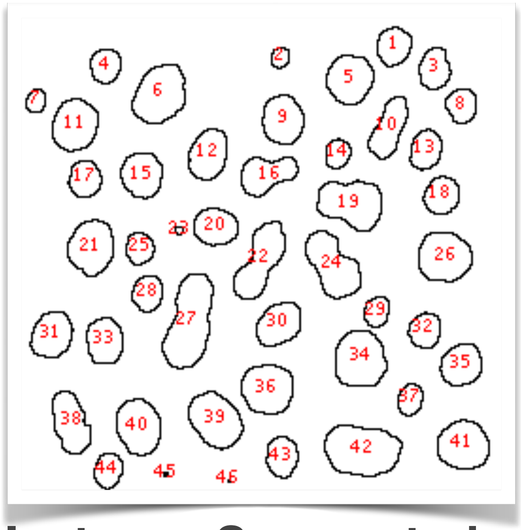
# Thresholding



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



**Thresholded**



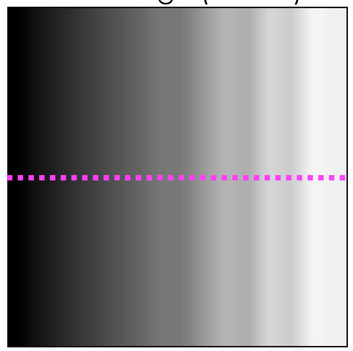

**Instance Segmented**

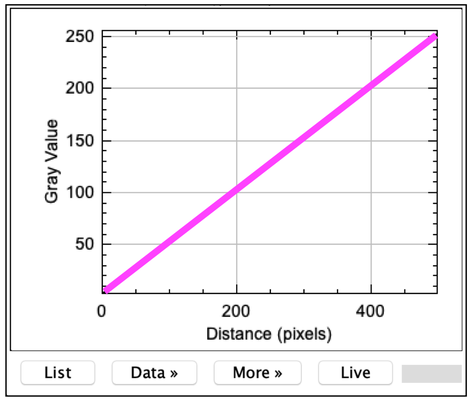
**Instance Segmentation** in FIJI**:** keeping white (*connected-*) objects.

# Thresholding method

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

8 bit image (0 - 255)





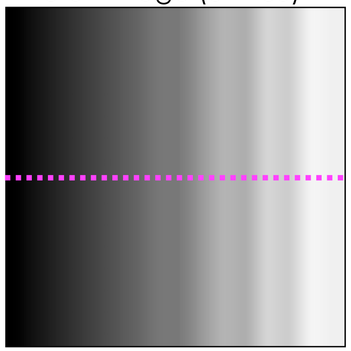
# Thresholding method

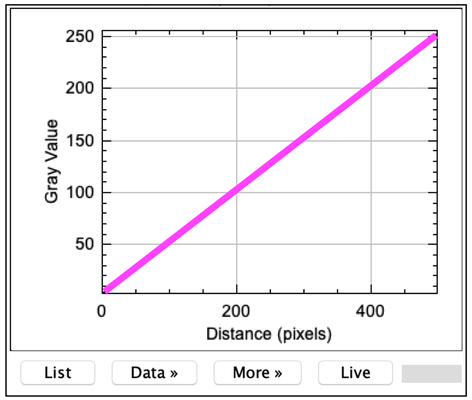
**Select** only a **range** of **digital values** in the image.

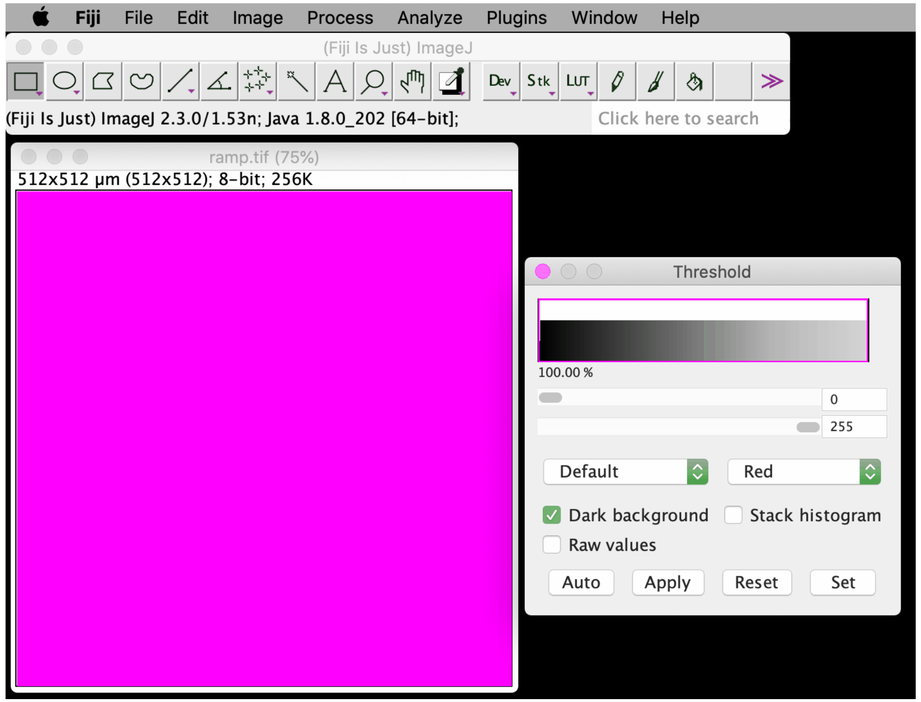
in **Fiji**: **Image** > **Adjust** > **Threshold…**

**(cmd) + shift + t**

8 bit image (0 - 255)





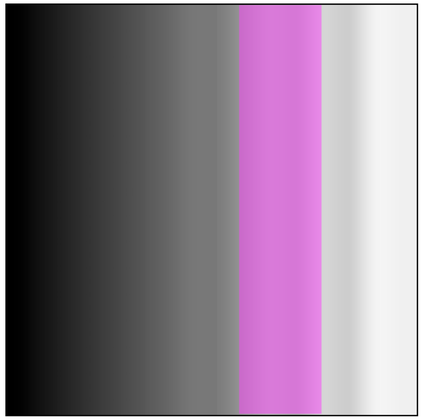




The result of the thresholding process is a **Binary Mask**.

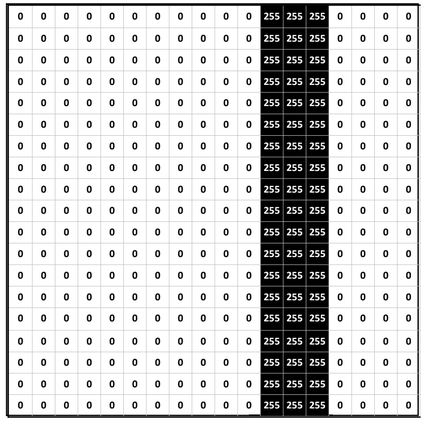
# Thresholding method

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.**

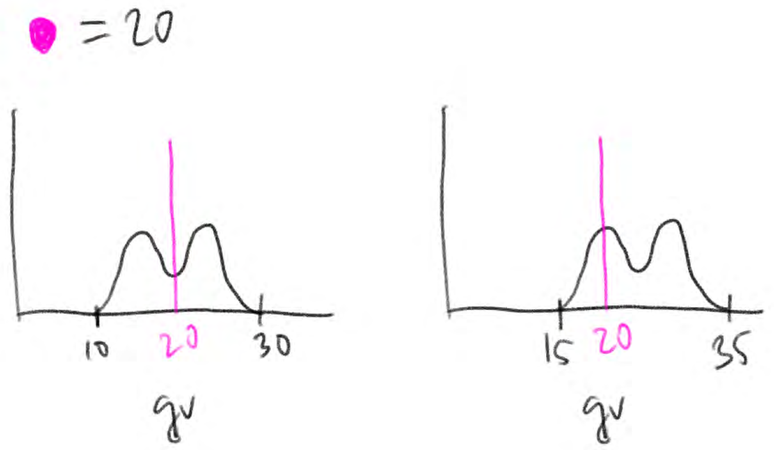
# Segmentation with thresholding—exercises

***4.1 DAPI segmentation with thresholding***

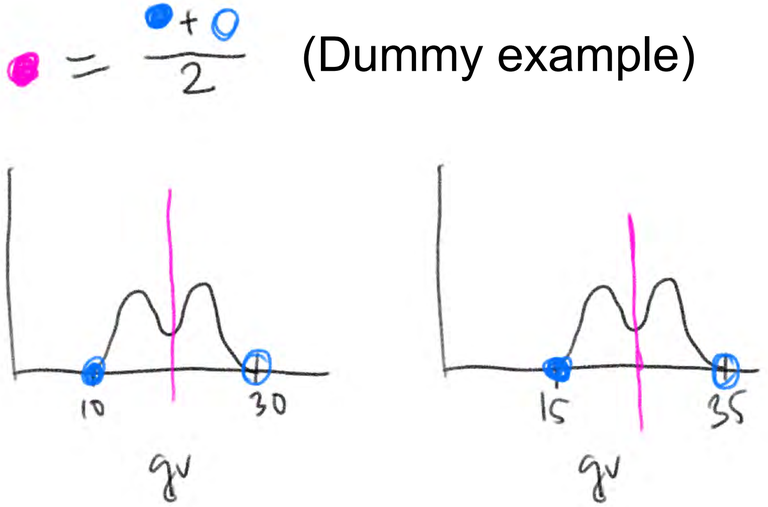
***Stop at (including) point 10,***
***Do not continue further***

# Manual vs. automated thresholding

Manual thresholding is nonreproducible.
Typically, each image requires a different threshold




Automatic thresholding uses histogram information to set a threshold.

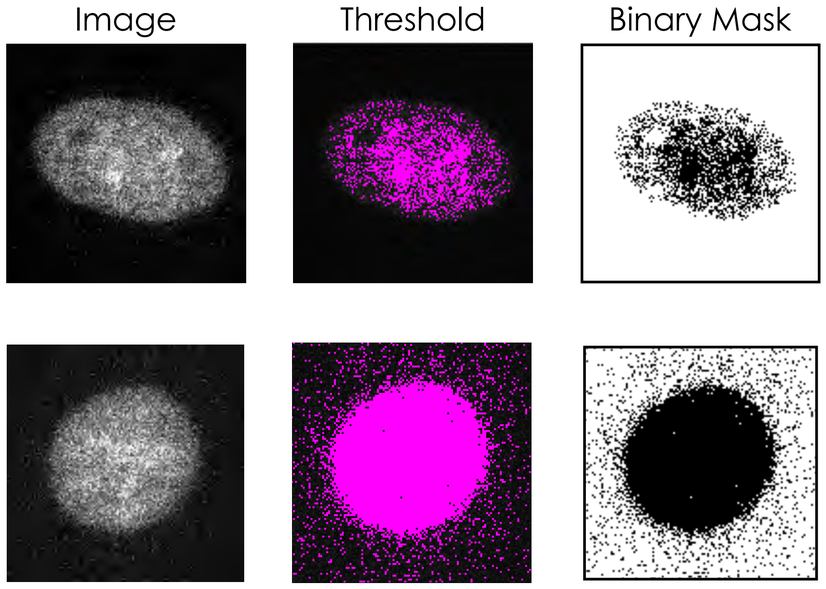



(Dummy example)

# Thresholding method

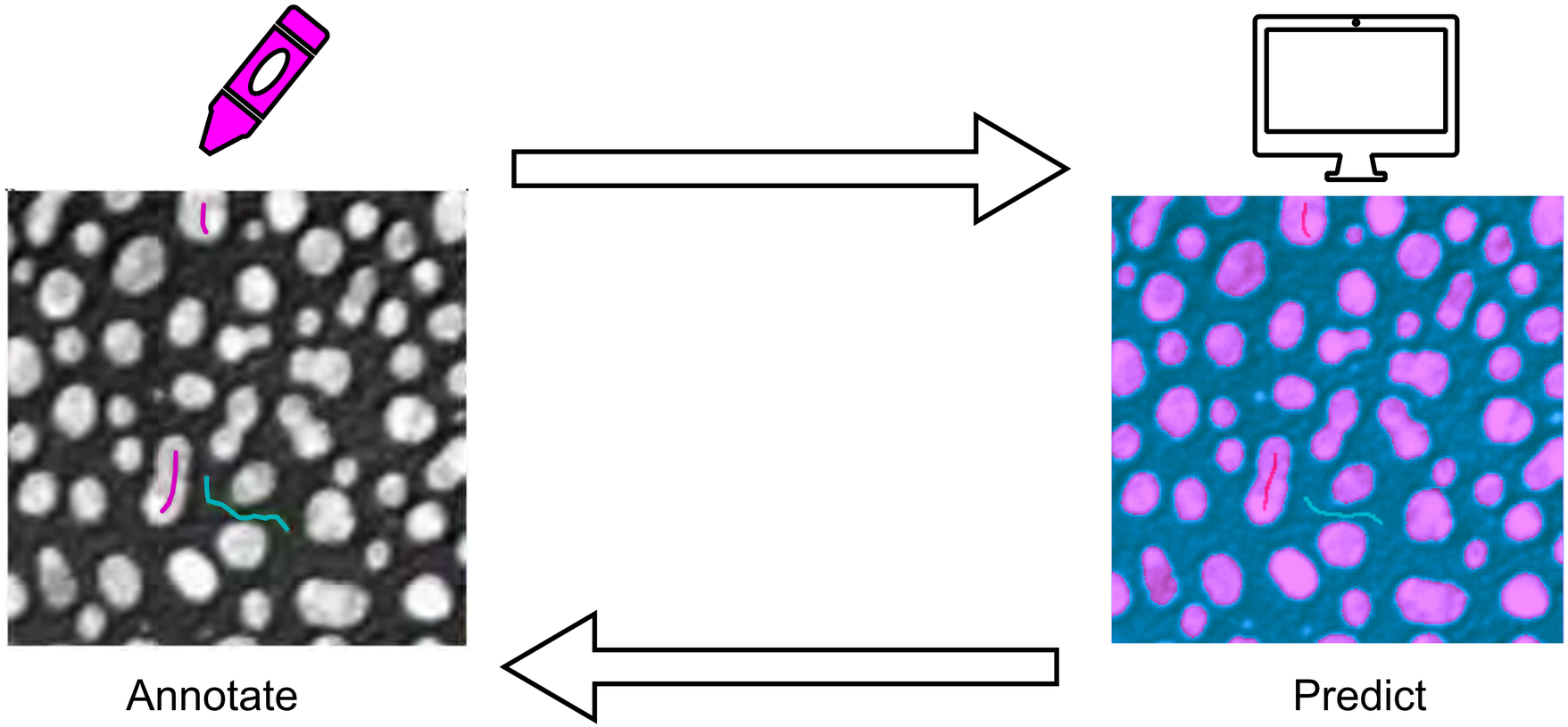
What can go wrong?

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, …)

# Interactive tools based on classic machine learning



# Examples

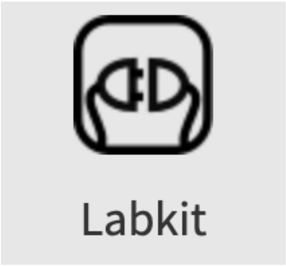
## Standalone: Ilastik

https://www.ilastik.org/

## For histopathology

https://qupath.github.io/

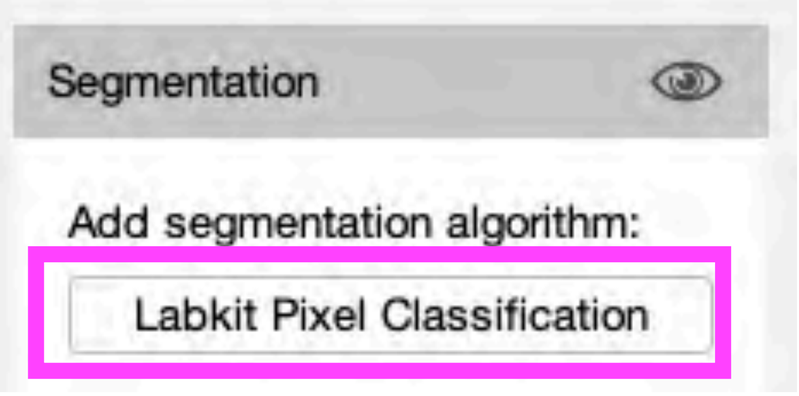
## As a Fiji plugin: Labikit

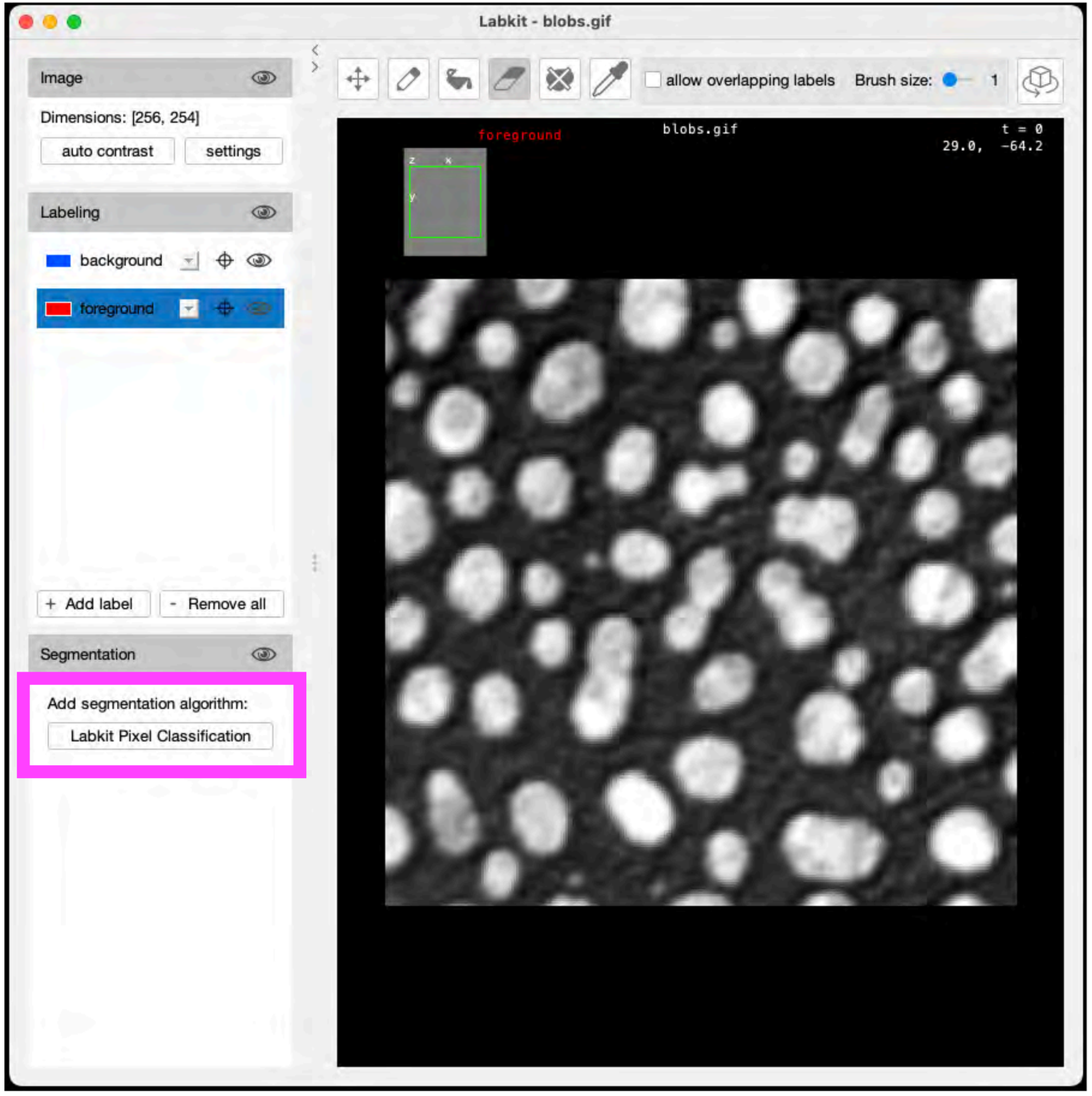



https://imagej.net/plugins/labkit/

# Labkit Segmentation

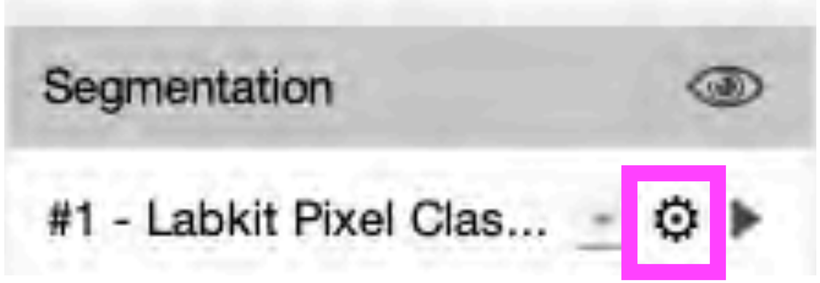
in Fiji:
**Plugins**
> **Labkit**
> Open Current Image With Labkit

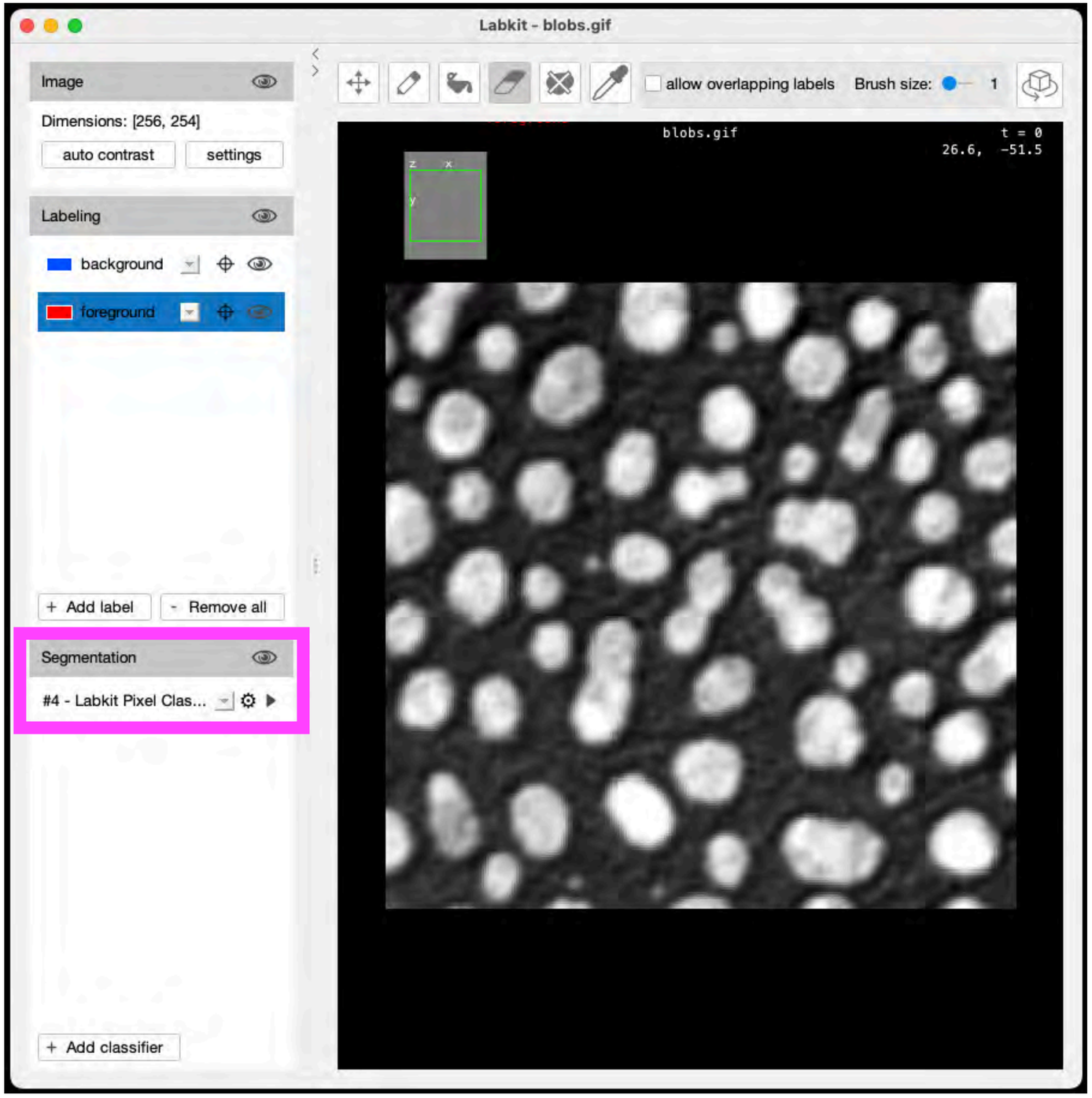


# Labkit Segmentation

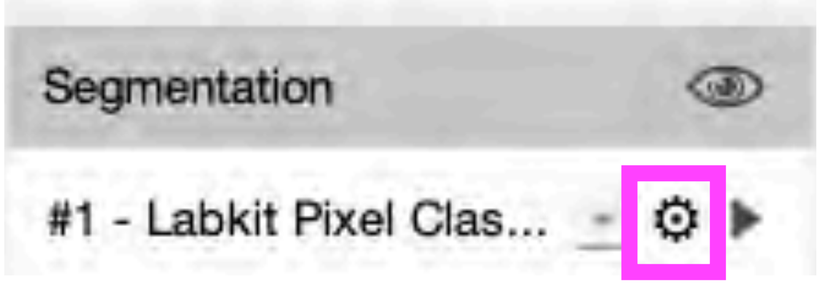
in Fiji:
**Plugins**
> **Labkit**
> Open Current Image With Labkit

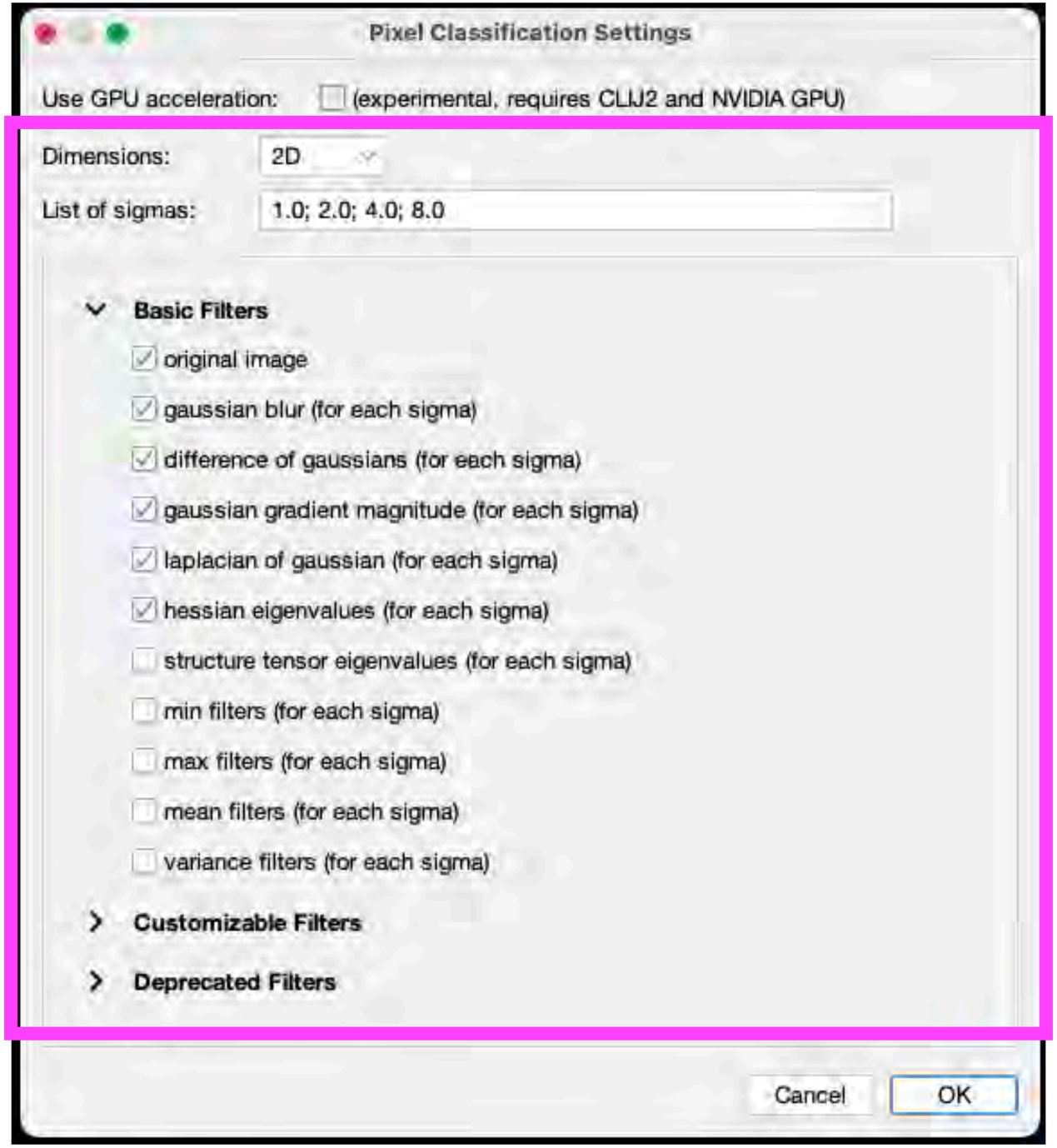


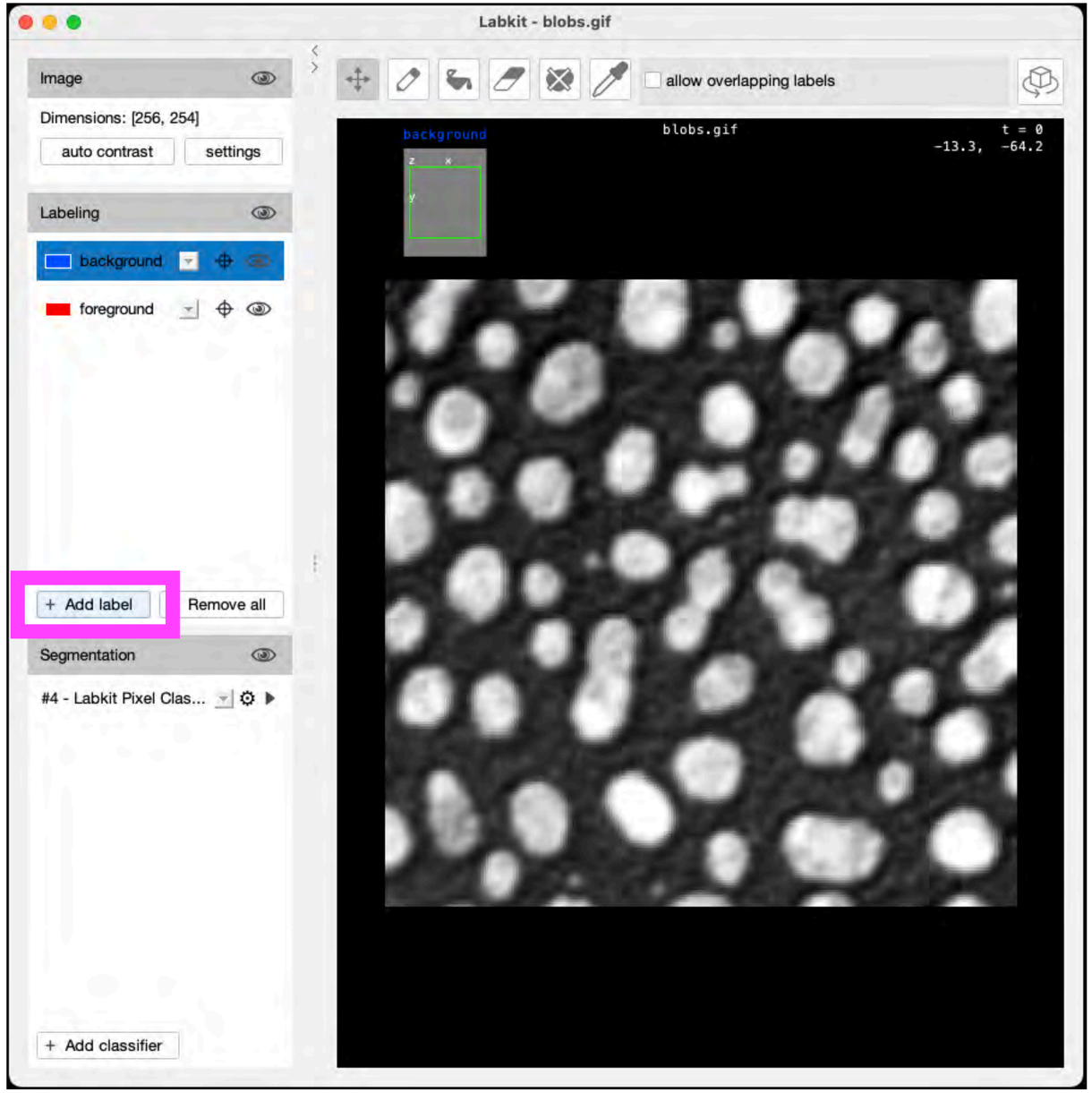
# Labkit Segmentation

in Fiji:
**Plugins**
> **Labkit**
> Open Current Image With Labkit

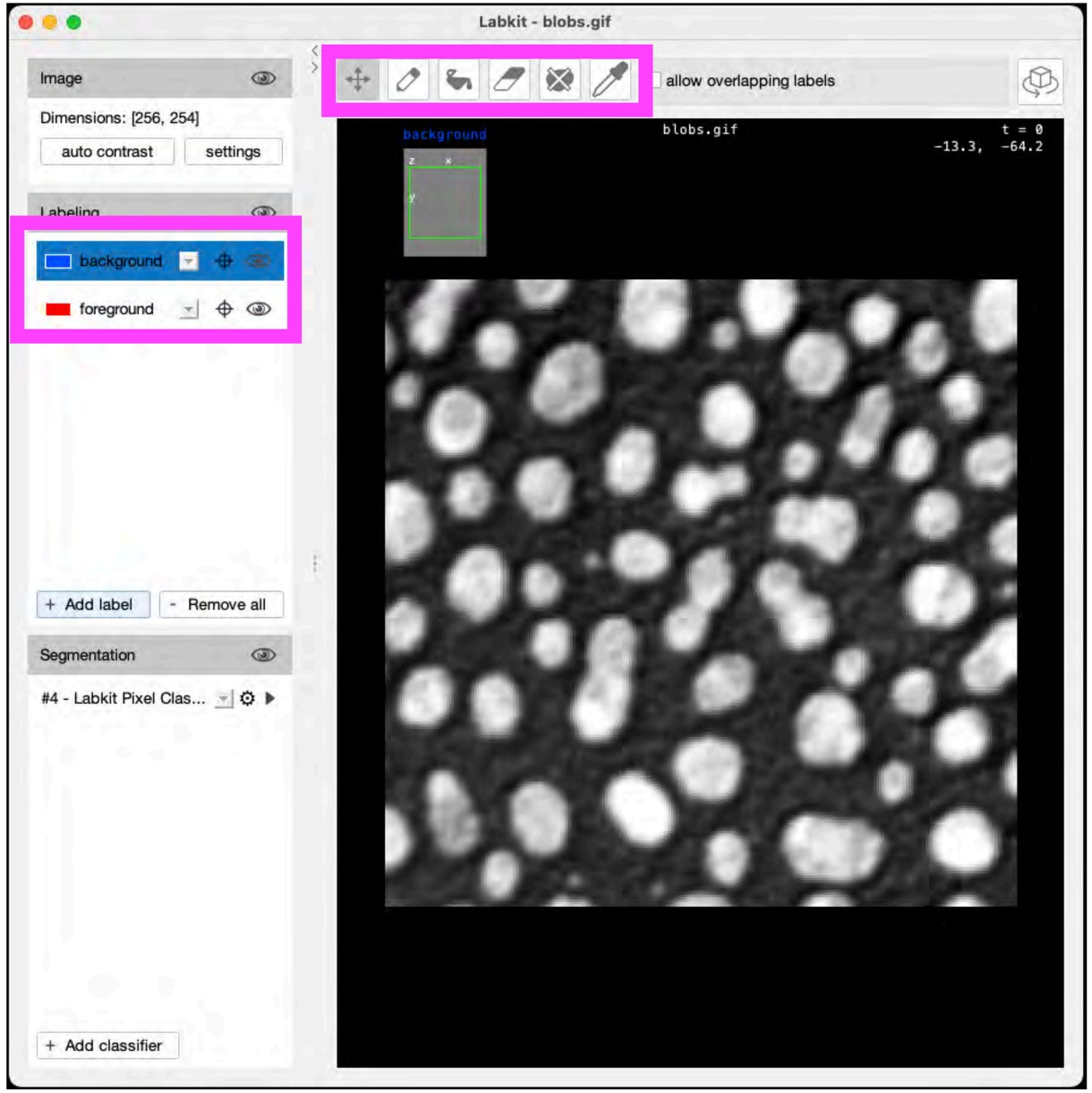


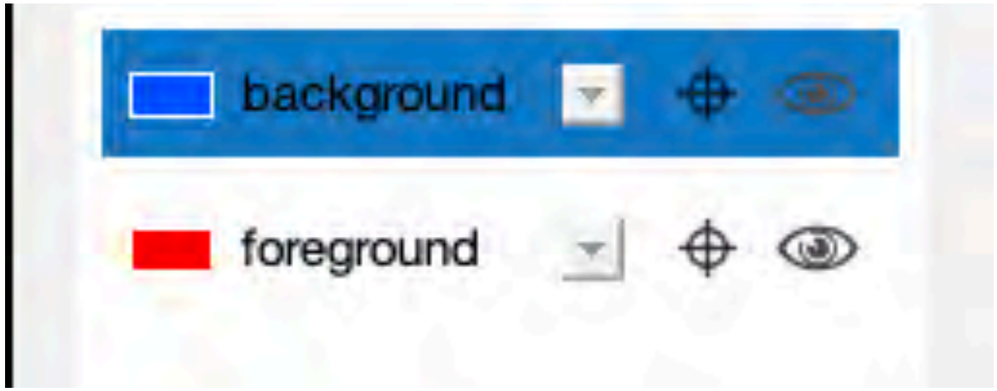



# Labkit Segmentation

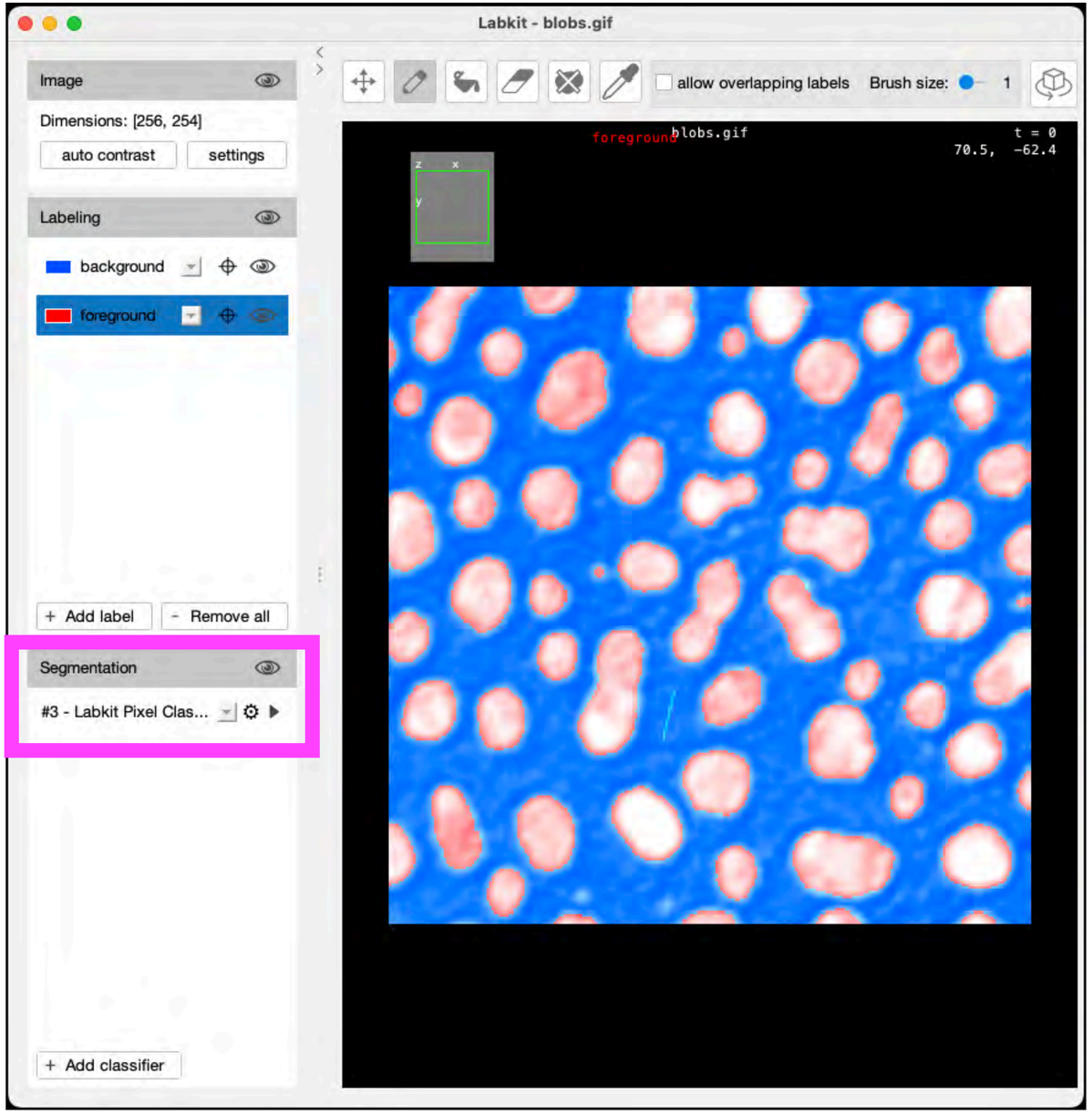


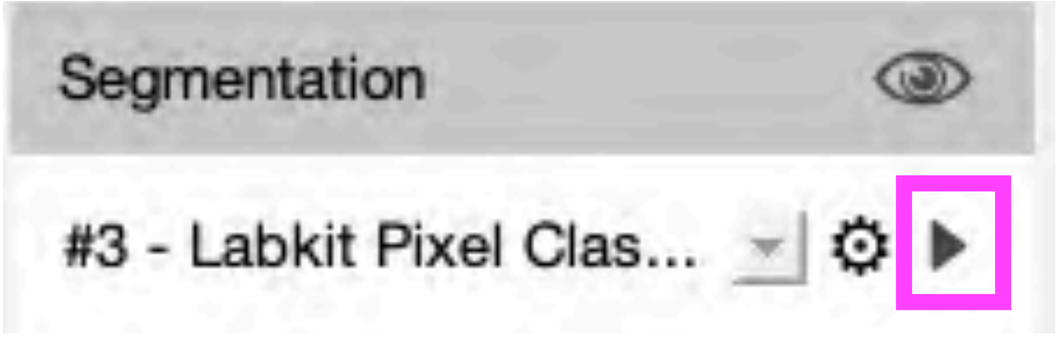
# Labkit Segmentation



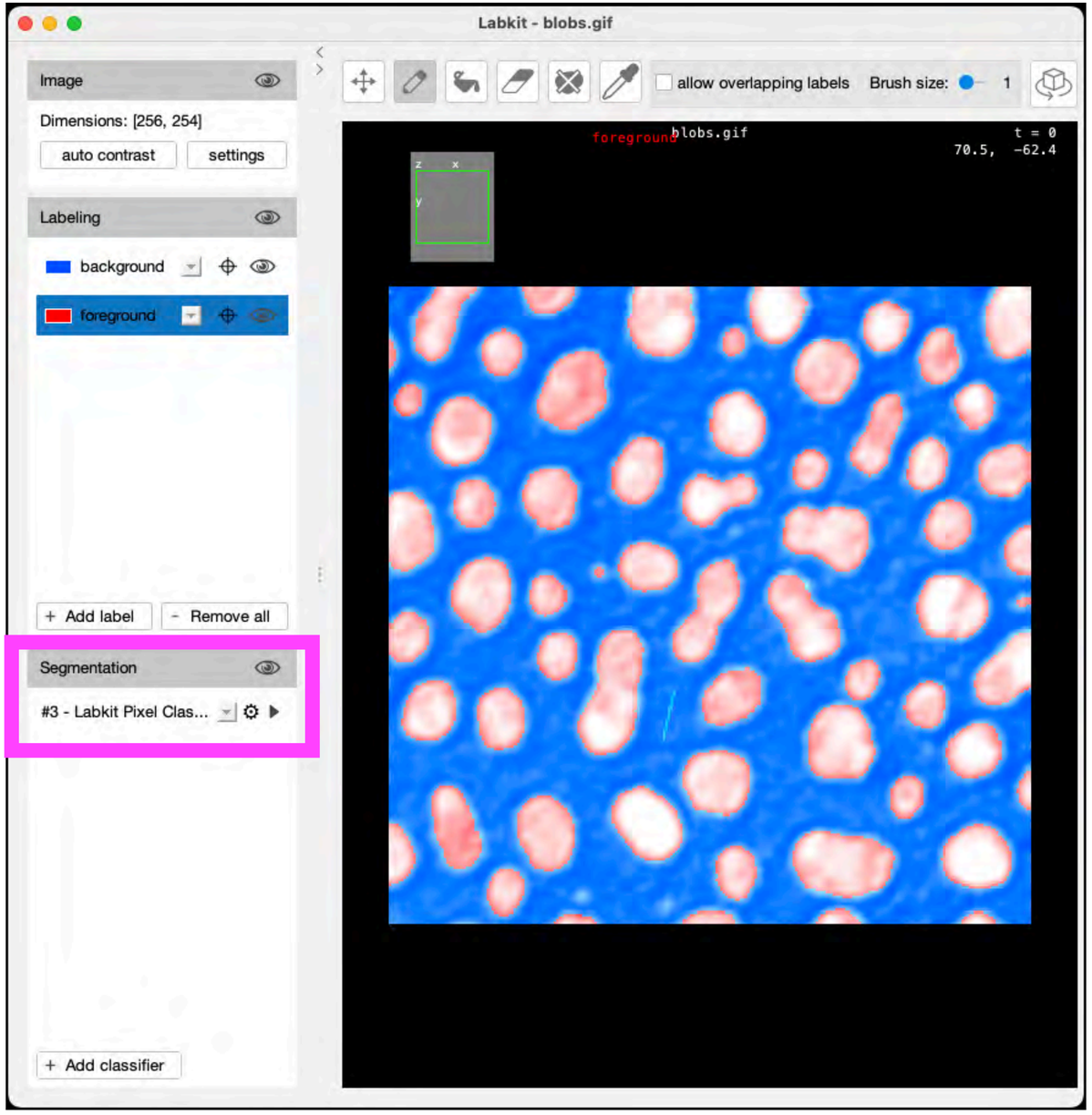


# Labkit Segmentation



# Labkit Segmentation



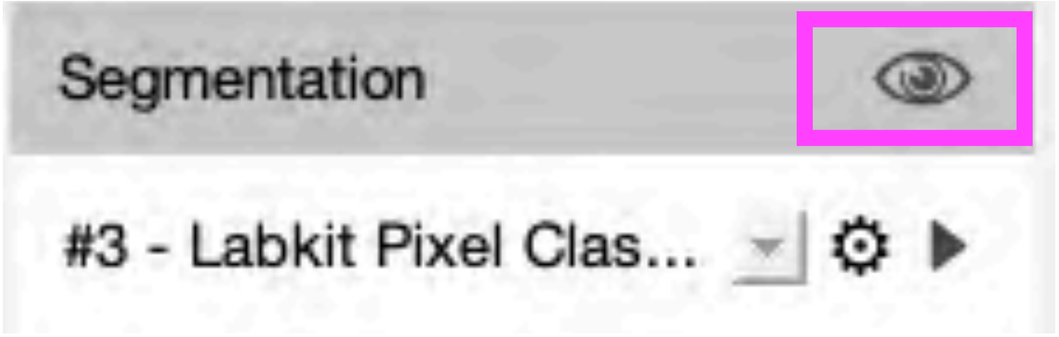
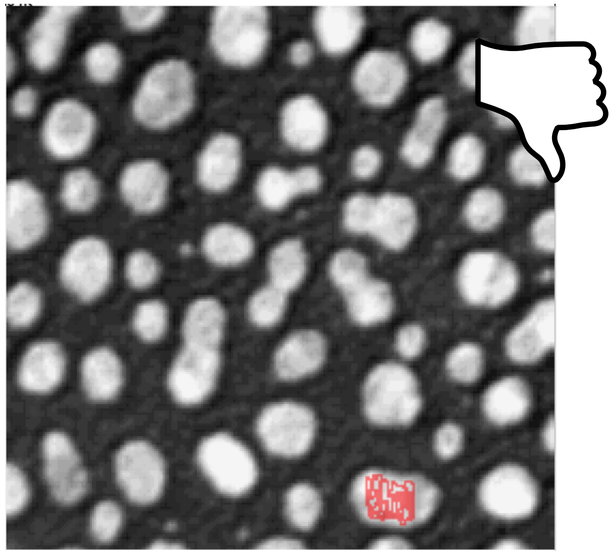
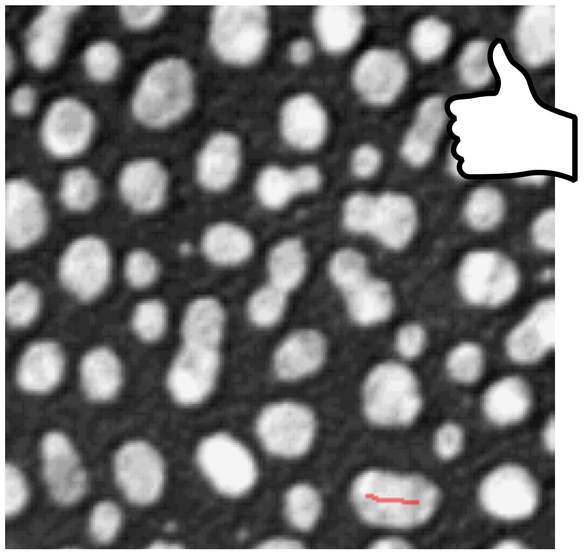
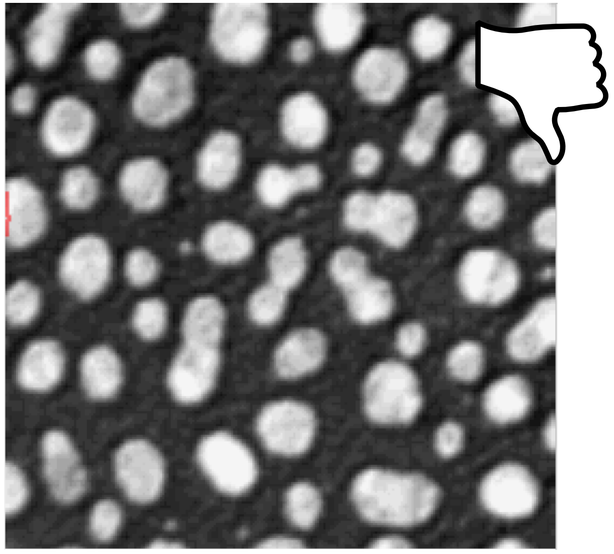



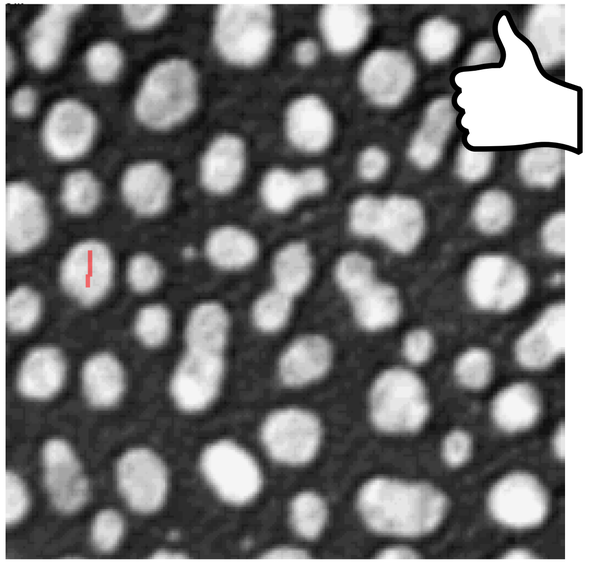
Image Analysis Collaboratory

# Tips









## *4.3 DAPI segmentation with Labkit*

## *Breakpoint at 12: Create results*