

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



Input





Semantic

Background

Instance



Background Nucleus 1 Nucleus 2 Nucleus 3

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Image Analysis 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.







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







Select only a range of digital values in the image.

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





Generate a **binary mask**.

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**.



0 **or** 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.











interested in.



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

- 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



Annotate



Predict





Standalone: Ilastik



https://www.ilastik.org/

For histopathology



https://qupath.github.io/

As a Fiji plugin: Labikit



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





in **Fiji**:

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Image Analysis

Collaboratory

Plugins

- > Labkit
- > Open Current Image With Labkit



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in **Fiji**:

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Image Analysis

Collaboratory

Plugins

- > Labkit
- > Open Current Image With Labkit



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Labeling
backgrou
foreground
+ Add label
Segmentation
#4 - Labkit Pixe
+ Add classifi





Image Analysis Labkit Segmentation

in **Fiji**:

Collaboratory

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Plugins

- > Labkit
- > Open Current Image With Labkit





	Pixel Classification Settings
U accelera	tion: (experimental, requires CLU2 and NVIDIA GPU)
ions:	2D
igmas:	1.0; 2.0; 4.0; 8.0
Basic Filt	ters Il image
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differen	nce of gaussians (for each sigma)
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Customia	zable Filters
Deprecat	ed Filters

Cancel

OK



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Image Analysis









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Image Analysis











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Image Analysis



Segmentation $\overline{\mathbf{a}}$ #3 - Labkit Pixel Clas... n





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Image Analysis







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4.3 DAPI segmentation with Labkit

Breakpoint at 12: Create results