# Introduction to image analysis using ImageJ/Fiji

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*Image Analysis Collaboratory, HMS*

# Get the course materials

## https://hms-iac.github.io/fiji-workshop

One-stop resource for everything we'll cover today

- **Download all slides (PDF)**
- **Download all exercises (PDF)**
- **Download all images (ZIP)**

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# I.A.C. (Image Analysis Collaboratory)
## @ HMS since Sep '22

- **Support** and **collaboration:** for image and data analysis projects
- **Teaching:** at HMS and internationally
- **IT:** access to relevant solutions, open- and closed-source
- **R&D:** centered on methods and tools
- **Locations**: LHRRB 105 & Armenise 531D
- **Consults**: Contact Simon
  - simon@hms.harvard.edu

https://iac.hms.harvard.edu/

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# Who *we* are, currently

Antoine A. Ruzette

Associate

Ranit Karmakar

Specialist Postdoc

Maria Theiss

Specialist Postdoc

Federico Gasparoli

Research Associate

Simon F. Nørrelykke

Director, Lecturer

**Backgrounds in**

Physics
Biology
Microscopy
Bioengineering
Computer Engineering

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# Learning objectives

1. **Motivate** the use of algorithms in image analysis
2. **Introduce** some image-analysis nomenclature
3. **Learn** to use Fiji effectively and reproducibly

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# Reasons for Learning About Image Processing

- Make pretty pictures (processing)
  - publications, talks, websites, ...
- Get numbers out of pictures (analysis)
  - cell sizes, vessel lengths, GPF expression level, ...
- Make experiment possible (automation)
  - whole-genome screen: millions of images
- Objectivity and Reproducibility
  - in science, just do it!

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# Reasons for <u>Not</u> Learning About Image Processing

- …

none

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# Acknowledgements


- Szymon Stoma, ETH Zurich, Switzerland
  - slides and workflows
- Peter Bankhead, Edinburgh University, Scotland
  - slides and graphics


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# Self-introductions

1. My **name** is *Donald Duck*
2. My **position** is as a *10th year postdoc*
3. My **lab** is *Mad City Labs*
4. My model **system** is *blue whales*
5. I have **data** from *confocal microscopy*

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# Optical Illusions

—

## Why should you analyze images with computers

# Color perception and pattern recognition is individual – science less so

https://www.moillusions.com/perfect-circles-optical-illusion/

# Concentric circles !?

https://www.moillusions.com/perfect-circles-optical-illusion/

# Identical central discs?

Our size estimate is strongly influenced by the local neighbourhood

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http://www.brainbashers.com

# Identical central discs?

Yes, the discs are identical

http://www.brainbashers.com

# Is the dot half-way up?

Our sense of distance depends on neighbourhood

http://www.brainbashers.com

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# Is the dot half-way up?

Yes it is!

http://www.brainbashers.com

# Which car is bigger?

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http://www.brainbashers.com

# Which car is bigger?

http://www.brainbashers.com

# Which car is bigger?

All cars are same size

(Erroneous, application of subjective/perceptual constancy algorithm by your brain)

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# Are A and B equally grey?

Intensity perception depends strongly on neighbourhood

http://www.brainbashers.com

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# Are A and B equally grey?

Yes they are!

http://www.brainbashers.com

# Spot the animal

—

Why you should still use your brain
(a pre-trained neural network)

# Pattern Recognition

Yes, that is a cow!

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# Pattern Recognition

Yes, that is a cow!

http://www.brainbashers.com

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# Using Prior Knowledge

Keyword: Dalmatian dog!

http://www.brainbashers.com

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# Using Prior Knowledge

Keyword: Dalmatian dog!

http://www.brainbashers.com

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# A brief history of image analysis

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ImageJ centric

# 37 years ago…

NIH IMAGE

## What is ImageJ?

ImageJ is an open source image processing and image analysis software for multi-dimensional image data with a focus on scientific imaging.

1987 - NIH Image: written in Pascal for the Macintosh by Wayne Rasband

1997 - ImageJ 1.x: written in Java and also compatible with Windows PC

Since then, ImageJ has been maintained and upgraded (e.g. ImageJ2, Fiji, …)

| ImageJ GitHub | Fiji GitHub |
|---|---|

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Schneider CA, Rasband WS, Eliceiri KW (2012). "NIH Image to ImageJ: 25 years of image analysis". *Nat Methods*. **9** (7): 671–675. doi:10.1038/nmeth.2089. PMC 5554542. PMID 22930834.

# What is Fiji?

Fiji stands for Fiji Is Just ImageJ

It is a “batteries included” distribution of ImageJ2

Easy to install (“portable” application)

Include a collection of Plugins

Easy to update (https://imagej.net/list-of-update-sites/)

Documentation

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# 12 years ago…

- ***AlexNet*** is published (2012)
  - Wins the *ImageNet Large Scale Visual Recognition Challenge*
  - Error of 15.3%, more than 10.8 percentage points better than #2
  - Possible due to GPUs
- ***CellProfiler*** has been around for 6 years (2006)
- ***Fiji*** (Fiji is just ImageJ) has been around for 5 years (2007)
- ***scikit-image*** released 3 years ago (2009)
- Bitcoin grows from $5 to $13 (remember Silk Road?) (2012)
- U-Net, GANs, and ***Jupyter*** will appear in 2-3 years (2014/15)
- ***AlphaGo*** will beat Lee Sodol in 4 years (2016)
- ***QuPath*** is still 4 years in the future (2016)

CellProfiler™
cell image analysis software

scikit-image
image processing in python

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# 4 years ago…

- CellPose is out (2020)
  - “Cellpose: a generalist algorithm for cellular segmentation”
  - Trained on highly varied images of cells,over 70,000 segmented objects
  - Cells don’t have to be star-shaped
  - Web-platform and Jupyter notebooks
  - https://www.nature.com/articles/s41592-020-01018-x
- ZeroCostDL4Mic available
  - Paper still a year in the future
  - Implementation of common DL technologies to microscopy imaging
  - Relies on GPUs and other infrastructure provided by Google Colab.
  - https://github.com/HenriquesLab/ZeroCostDL4Mic/wiki

- *Vision Transformers* (2020)
  - *“An Image is Worth 16x16 Words: Transformers for Image Recognition at Scale”*, Dosovitskiy et al.
  - https://arxiv.org/abs/2010.11929

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# Today…

## Image Analysis Software

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**forum.image.sc**

# History of Segmentation

https://x.com/BoWang87/status/1773132444717252718

# English as the next programming language

—

LLMs, chatbots and foundation models for bio image analysis

GPT-3.5 GPT-4

# ChatGPT

**Create a workout plan**
for resistance training

**Design a database schema**
for an online merch store

**Explain why popcorn pops**
to a kid who loves watching it in the microwave

**Write an email**
to request a quote from local plumbers

Please write an ImageJ macro that uses StarDist to segment an image from fluorescence microscopy

Free Research Preview. ChatGPT may produce inaccurate information about people, places, or facts. ChatGPT September 25 Version

https://chat.openai.com/

# Using ChatGPT for Image Analysis

SI **You**

please segment the nuclei and show me the relevant code you used

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# Using ChatGPT for Image Analysis

## GPT-4, May 2024

SI You

please segment the nuclei and show me the relevant code you used

## GPT-4o, Oct. 2024

please segment the nuclei and show me the relevant code you used

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# Using ChatGPT for Image Analysis

## GPT-4, May 2024

**ChatGPT**

The nuclei in the image have been segmented using the following steps:

- The image was converted to grayscale to simplify the analysis.
- Gaussian Blur was applied to reduce noise and detail in the image, which aids in thresholding and contour detection.
- Otsu's thresholding was performed to convert the grayscale image into a binary image, which helps in distinguishing the nuclei from the background.
- Contours were found in the binary image, which correspond to the boundaries of the nuclei.
- These contours were then drawn onto the original image to visually segment the nuclei.

## GPT-4o, Oct. 2024

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# Using ChatGPT for Image Analysis

## GPT-4, May 2024

python Copy code

```python
import cv2
import numpy as np
from matplotlib import pyplot as plt

# Load the image
image_path = '/mnt/data/hela-cells.jpg'
image = cv2.imread(image_path)

# Convert the image to gray scale
gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY)

# Apply GaussianBlur, which is good for edge detection
blurred = cv2.GaussianBlur(gray, (5, 5), 0)

# Perform Otsu's thresholding
_, thresh = cv2.threshold(blurred, 0, 255, cv2.THRESH_BINARY_INV + cv2.

# Find contours which will give us the nuclei boundaries
contours, _ = cv2.findContours(thresh, cv2.RETR_TREE, cv2.CHAIN_APPROX_

# Draw the contours on the original image
contoured_image = image.copy()
cv2.drawContours(contoured_image, contours, -1, (0, 255, 0), 3)

# Save the output image with segmented nuclei
output_path = '/mnt/data/hela-cells-segmented.jpg'
cv2.imwrite(output_path, contoured_image)
```

## GPT-4o, Oct. 2024

python Copy code

```python
from skimage import io, filters, measure, morphology, color
import matplotlib.pyplot as plt
import numpy as np

# Load the image
image_path = '/mnt/data/hela-cells.png'
image = io.imread(image_path)

# Convert to grayscale
gray_image = color.rgb2gray(image)

# Apply Gaussian filter to smooth the image
smoothed = filters.gaussian(gray_image, sigma=1)

# Use Otsu's threshold to create a binary mask of the nuclei
threshold_value = filters.threshold_otsu(smoothed)
binary_mask = smoothed > threshold_value

# Remove small objects and fill holes to improve segmentation
cleaned_mask = morphology.remove_small_objects(binary_mask, min_size=500)
cleaned_mask = morphology.remove_small_holes(cleaned_mask, area_threshold=500)

# Label the segmented regions
labeled_nuclei = measure.label(cleaned_mask)
```

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# Using ChatGPT for Image Analysis

## GPT-4, May 2024

**You**

please use the blue channel for segmenting the nuclei

**ChatGPT**

The nuclei segmentation has been performed using the blue channel of the image with the following steps:

- The blue channel was extracted from the original image since the nuclei appear to be stained blue.
- Gaussian Blur was applied to the blue channel to smooth the image, which helps in reducing noise for thresholding.
- Otsu's thresholding was then used on the blurred blue channel to create a binary image where the nuclei are distinguished from the background.

## GPT-4o, Oct. 2024

# Using ChatGPT-4o for Image Analysis

## GPT-4o, Oct. 2024 - prompted to close larger holes

make sure to close larger holes, and increase smoothing sigma to 3

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# Using ChatGPT-4o for Image Analysis

## GPT-4o, Oct. 2024 - it helps to know what to ask

compute the convex hull for shapes with a low circularity, to approximate the characteristic shape of a nuclei

Original Image

Convex Hull for Low Circularity Nuclei

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# BioImage Chatbot

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https://arxiv.org/abs/2310.18351

https://aicell.io/post/bioimageio-chatbot/

# napari-omega

## Omega – Harnessing for Bioimage Analysi

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10.5281/zenodo.10828225

**Supplementary Figure 3.** Omega's System Architecture. Diagram illustrating the Omega system architecture, encompassing both the user interface and backend components. The user interface is displayed at the top, consisting of the napari viewer, chat window, and AI-enhanced code editor. Key backend elements include the Omega ReAct agent, web server, AI tools within the editor, and the Python code repair module. The architecture employs a cascaded design, with a central dialog loop orchestrated by a primary language model. This model coordinates a suite of specialized tools, each powered by a secondary tool-specific language model that can access online resources, interact with the napari viewer, and integrate functionalities from third-party libraries such as Cellpose and StarDist.

**Supplementary Video 1. Omega can segment nuclei with StarDist and perform follow-up analysis.** The video showcases Omega's ability to segment cell nuclei in a 2D image using Stardist. Omega successfully segments the nuclei and adds a label layer to the napari viewer. With further instructions, Omega can count the segmented nuclei and create a CSV file on the desktop folder of the machine. This file contains coordinates and areas of all segments, sorted by decreasing area, with one segment per row. Omega also opens the file using the system's default CSV viewer. The video has been sped up by a factor of 2.

**Supplementary Video 2. Omega can segment nuclei in a 3D image.** This video shows how Omega segments the nuclei in a 3D image displayed in the napari viewer. Omega uses a specialized tool for cell and nuclei segmentation and employs a 'classic' approach that combines single thresholding, specifically Otsu, with watershed splitting to prevent under-segmentation. After segmentation, Omega adds a labels layer to the viewer, and we inquire about the number of segments detected. The response is 27. The video has been sped up by a factor of 2.

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Human red blood cells
DIC microscopy

"BBBC009v1 from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]."

https://segment-anything.com/

https://segment-anything.com/

# Segment Anything in ImageJ/Fiji

Released around March 14th, 2024
Graphical User Interphase
No coding required

Example of point annotations

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https://github.com/segment-anything-models-java/SAMJ-IJ

# Image Analysis Workflow

- There are typically *five* steps in an image analysis
- Often a good idea to structure work along these lines before starting

Think of this even ***before*** you acquire the images!

otherwise image analysis may become only a *post-mortem* on your experiment

Pre-processing → Processing → Post-processing → Measuring → Analysing & Visualising

- **Data-wrangling in general**
  - Image format conversion, incl. lossless compression
  - Renaming
- **Restoration and Reconstruction**
  - Tomographic reconstruction
  - Registration (3D stacks)
  - Stitching (tiled images)
  - Illumination correction
  - Intensity normalisation
  - Deconvolution
  - Filtering (smoothing, sharpening, etc)
  - Grayscale morphological operations
- **Color deconvolution**
- **Channel alignment (chromatic aberrations)**
- …

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Pre-processing → **Processing** → Post-processing → Measuring → Analysing & Visualising

- **Image Segmentation**
  - Thresholding
  - Pixel Clustering
  - Edge detection
  - Region growing
  - Level set methods
  - Watershed
  - Model based
  - Multi-scale
  - Trainable pixel-classification
- **Spot detection**
- **Tracking**
- …

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Pre-processing → Processing → **Post-processing** → Measuring → Analysing & Visualising

- **Filtering** detected objects based on
  - Size, shape, intensity
  - Texture, location, neighbours
- **Binary morphological operations**
  - Open/close
  - Skeletonise
  - Hit-or-miss transform
- …

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Pre-processing → Processing → Post-processing → **Measuring** → Analysing & Visualising

- **Object measures**
  - Size, shape, intensity
  - Texture, positions
  - Local neighbourhood
- **Image measures**
  - Object count
  - Area covered
  - Total intensity
- **Co-localisation**
- …

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Pre-processing → Processing → Post-processing → Measuring → **Analysing & Visualising**

- Exploratory data analysis
- Filtering of numerical data based on measured values
- Summary statistics
- Plotting numerical values
- Making movies and montages of images
- Record entire work-flow for later reference
  - Write down what you did and why (each step)
  - Use a version control system
  - Document your code
- …

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# Processing vs. Analysis

**Image Formation**
object in → image out

**Image Processing**
image in → image out

**Image Analysis**
image in → features out

| Obj | Area | Perim |
|---|---|---|
| 1 | 324.2 | 98.5 |
| 2 | 406.7 | 140.3 |
| 3 | 487.1 | 159.2 |
| 4 | 226.3 | 67.8 |
| 5 | 531.8 | 187.6 |
| 6 | 649.5 | 203.1 |
| 7 | 582.6 | 196.4 |
| 8 | 498.0 | 162.9 |
| 9 | 543.2 | 195.1 |

**Computer Graphics**
numbers in → image out

| X | Y | I |
|---|---|---|
| -3.54 | -2.32 | 0.50 |
| -2.78 | -1.90 | 0.12 |
| -1.15 | 0.42 | 3.09 |
| 0.45 | 1.65 | 5.89 |
| 1.83 | 2.18 | 7.72 |
| 2.98 | 3.33 | 2.07 |
| 4.21 | 3.96 | -4.58 |
| 5.62 | 4.54 | -11.45 |
| 7.16 | 5.02 | -3.63 |

**Computer Vision**
image in → interpretation out

The series shows microtubule growth in a live neuron. The average speed of the distal ends is comparable in the cell body, dendrites, axons, and growth cones.

**Visualization**
image in → representation out

Meijering and van Cappellen. Biological Image Analysis Primer. (2006)

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# Debugging Fiji and getting help — a workflow

1. **Restart** Fiji
2. **Ask** you right neighbor
3. **Ask** you left neighbor
4. **Ask** an instructor
5. **Search** https://forum.image.sc/

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# Install and Update Fiji

# Download Fiji

https://fiji.sc/

https://imagej.net/Fiji

**Fiji is distributed as a portable application,**

**which means that you do not have to run an installer.**

**Just download (zip file), unpack and start it.**

# Update Fiji

(and install/uninstall plugins)

**Help > Update…**

**To update Fiji and/or the plugins.**

***Apply Changes*:** Install/Update the listed plugins

***Manage Update Sites*:** opens a list of plugins, you can select which one(s) to install in Fiji

**You can have more than one Fiji app!**

# Graphic User Interface (GUI)

# Graphic User Interface (GUI)

**Search Bar**
(L key shortcut)

**Plugins > Shortcuts > List Shortcuts - list of the default shortcuts*

***Plugins > Utilities > Find Commands - search for Fiji Commands*

# Open a file in Fiji

***Drag and Drop the file you want to open onto the Status Bar.***

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https://imagej.net/Bio-Formats

# Bio-Format Plugin

## import a file - options

***Import data from many life sciences file formats (e.g. @NIC .nd2 format)***

# Bio-Format Plugin

## import a file - options

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https://imagej.net/Bio-Formats

# Bio-Format Plugin

## import a file

**Drag and Drop**

***If you have a tiff file, "Drag and Drop" does not open Bio-Format Importer.***

**File > Import > Bio-Formats**

**or**

**Plugins > Bio-Formats > Bio-Formats Importer**

# what is an image?

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# A digital image is a matrix of numbers!

=

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 13 | 19 | 6 | 19 | 13 | 9 | 19 | 9 | 6 | 9 | 6 | 16 | 16 | 6 | 16 | 13 | 132 | 229 | 103 | 19 | 16 | 13 | 23 | 9 | 9 |
| 19 | 19 | 6 | 13 | 13 | 13 | 13 | 16 | 16 | 19 | 9 | 13 | 9 | 6 | 16 | 16 | 49 | 192 | 216 | 106 | 23 | 13 | 16 | 16 | 23 | 13 |
| 13 | 9 | 4 | 13 | 13 | 16 | 19 | 36 | 66 | 93 | 79 | 26 | 13 | 13 | 6 | 16 | 113 | 209 | 196 | 113 | 29 | 19 | 36 | 49 | 36 | 33 |
| 19 | 13 | 19 | 13 | 16 | 13 | 26 | 89 | 123 | 136 | 152 | 116 | 76 | 33 | 13 | 46 | 159 | 162 | 159 | 126 | 79 | 96 | 189 | 229 | 226 | 212 |
| 16 | 16 | 9 | 6 | 13 | 19 | 26 | 93 | 156 | 179 | 106 | 66 | 79 | 136 | 106 | 152 | 179 | 93 | 29 | 13 | 16 | 23 | 79 | 156 | 123 | 49 |
| 16 | 6 | 13 | 13 | 16 | 13 | 23 | 69 | 103 | 69 | 19 | 16 | 6 | 109 | 209 | 236 | 179 | 43 | 9 | 16 | 9 | 13 | 13 | 19 | 13 | 13 |
| 9 | 9 | 16 | 19 | 13 | 13 | 19 | 13 | 26 | 16 | 16 | 13 | 6 | 103 | 179 | 189 | 132 | 33 | 19 | 16 | 16 | 9 | 9 | 6 | 6 | 6 |
| 13 | 9 | 4 | 13 | 13 | 13 | 16 | 19 | 13 | 23 | 6 | 16 | 23 | 123 | 186 | 192 | 169 | 126 | 26 | 16 | 19 | 13 | 6 | 13 | 16 | 13 |
| 13 | 13 | 9 | 16 | 9 | 6 | 13 | 19 | 16 | 19 | 6 | 19 | 63 | 199 | 192 | 106 | 29 | 149 | 162 | 113 | 119 | 53 | 9 | 13 | 6 | 13 |
| 13 | 9 | 16 | 6 | 6 | 19 | 13 | 9 | 23 | 13 | 9 | 6 | 119 | 182 | 149 | 36 | 6 | 39 | 196 | 196 | 176 | 73 | 16 | 9 | 9 | 9 |
| 6 | 19 | 13 | 9 | 19 | 16 | 13 | 13 | 19 | 9 | 9 | 23 | 142 | 179 | 109 | 13 | 16 | 9 | 39 | 59 | 23 | 19 | 13 | 4 | 9 | 9 |
| 19 | 13 | 9 | 9 | 16 | 16 | 16 | 9 | 9 | 13 | 6 | 66 | 169 | 172 | 43 | 16 | 9 | 9 | 9 | 13 | 13 | 19 | 16 | 16 | 16 | 9 |
| 9 | 9 | 6 | 9 | 13 | 9 | 6 | 13 | 4 | 9 | 19 | 116 | 196 | 89 | 9 | 9 | 16 | 16 | 19 | 19 | 9 | 16 | 6 | 16 | 9 | 9 |
| 13 | 13 | 9 | 23 | 19 | 13 | 9 | 9 | 9 | 6 | 26 | 159 | 219 | 59 | 23 | 9 | 13 | 9 | 6 | 13 | 6 | 19 | 16 | 13 | 16 | 13 |
| 9 | 23 | 13 | 6 | 6 | 23 | 9 | 19 | 13 | 16 | 66 | 206 | 179 | 13 | 6 | 16 | 13 | 13 | 13 | 16 | 9 | 13 | 9 | 9 | 16 | 13 |
| 13 | 13 | 23 | 16 | 19 | 19 | 6 | 9 | 19 | 13 | 142 | 255 | 103 | 19 | 13 | 6 | 19 | 9 | 16 | 9 | 16 | 9 | 16 | 13 | 23 | 9 |
| 6 | 13 | 23 | 9 | 13 | 16 | 13 | 6 | 9 | 53 | 229 | 246 | 39 | 9 | 13 | 13 | 13 | 13 | 9 | 9 | 19 | 13 | 16 | 13 | 13 | 13 |
| 13 | 19 | 59 | 76 | 26 | 9 | 16 | 16 | 13 | 99 | 249 | 142 | 6 | 19 | 13 | 13 | 13 | 13 | 19 | 4 | 13 | 13 | 6 | 26 | 9 | 13 |
| 16 | 113 | 229 | 219 | 93 | 9 | 26 | 83 | 23 | 159 | 219 | 59 | 9 | 9 | 6 | 13 | 16 | 13 | 16 | 13 | 6 | 9 | 9 | 16 | 23 | 9 |

=

| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

## Pixel = Picture Element

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Images in publications and presentations
should be used to ***communicate*** a finding...
not ***be*** the finding

*this* is your **data**

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 13 | 19 | 6 | 19 | 13 | 9 | 19 | 9 | 6 | 9 | 6 | 16 | 16 | 6 | 16 | 13 | 132 | 229 | 103 | 19 | 16 | 13 | 23 | 9 | 9 |
| 19 | 19 | 6 | 13 | 13 | 13 | 13 | 16 | 16 | 19 | 9 | 13 | 9 | 6 | 16 | 16 | 49 | 192 | 216 | 106 | 23 | 13 | 16 | 16 | 23 | 13 |
| 13 | 9 | 4 | 13 | 13 | 16 | 19 | 36 | 66 | 93 | 79 | 26 | 13 | 13 | 6 | 16 | 113 | 209 | 196 | 113 | 29 | 19 | 36 | 49 | 36 | 33 |
| 19 | 13 | 19 | 13 | 16 | 13 | 26 | 89 | 123 | 136 | 152 | 116 | 76 | 33 | 13 | 46 | 159 | 162 | 159 | 126 | 79 | 96 | 189 | 229 | 226 | 212 |
| 16 | 16 | 9 | 6 | 13 | 19 | 26 | 93 | 156 | 179 | 106 | 66 | 79 | 136 | 106 | 152 | 179 | 93 | 29 | 13 | 16 | 23 | 79 | 156 | 123 | 49 |
| 16 | 6 | 13 | 13 | 16 | 13 | 23 | 69 | 103 | 69 | 19 | 16 | 6 | 109 | 209 | 236 | 179 | 43 | 9 | 16 | 9 | 13 | 13 | 19 | 13 | 13 |
| 9 | 9 | 16 | 19 | 13 | 13 | 19 | 13 | 26 | 16 | 16 | 13 | 6 | 103 | 179 | 189 | 132 | 33 | 19 | 16 | 16 | 9 | 9 | 6 | 6 | 6 |
| 13 | 9 | 4 | 13 | 13 | 13 | 16 | 19 | 13 | 23 | 6 | 16 | 23 | 123 | 186 | 192 | 169 | 126 | 26 | 16 | 19 | 13 | 6 | 13 | 16 | 13 |
| 13 | 13 | 9 | 16 | 9 | 6 | 13 | 19 | 16 | 19 | 6 | 19 | 63 | 199 | 192 | 106 | 29 | 149 | 162 | 113 | 119 | 53 | 9 | 13 | 6 | 13 |
| 13 | 9 | 16 | 6 | 6 | 19 | 13 | 9 | 23 | 13 | 9 | 6 | 119 | 182 | 149 | 36 | 6 | 39 | 196 | 196 | 176 | 73 | 16 | 9 | 9 | 9 |
| 6 | 19 | 13 | 9 | 19 | 16 | 13 | 13 | 19 | 9 | 9 | 23 | 142 | 179 | 109 | 13 | 16 | 9 | 39 | 59 | 23 | 19 | 13 | 4 | 9 | 9 |
| 19 | 13 | 9 | 9 | 16 | 16 | 16 | 9 | 9 | 13 | 6 | 66 | 169 | 172 | 43 | 16 | 9 | 9 | 9 | 13 | 13 | 19 | 16 | 16 | 16 | 9 |
| 9 | 9 | 6 | 9 | 13 | 9 | 6 | 13 | 4 | 9 | 19 | 116 | 196 | 89 | 9 | 9 | 16 | 16 | 19 | 19 | 9 | 16 | 6 | 16 | 9 | 9 |
| 13 | 13 | 9 | 23 | 19 | 13 | 9 | 9 | 9 | 6 | 26 | 159 | 219 | 59 | 23 | 9 | 13 | 9 | 6 | 13 | 6 | 19 | 16 | 13 | 16 | 13 |
| 9 | 23 | 13 | 6 | 6 | 23 | 9 | 19 | 13 | 16 | 66 | 206 | 179 | 13 | 6 | 16 | 13 | 13 | 13 | 16 | 9 | 13 | 9 | 9 | 16 | 13 |
| 13 | 13 | 23 | 16 | 19 | 19 | 6 | 9 | 19 | 13 | 142 | 255 | 103 | 19 | 13 | 6 | 19 | 9 | 16 | 9 | 16 | 9 | 16 | 13 | 23 | 9 |
| 6 | 13 | 23 | 9 | 13 | 16 | 13 | 6 | 9 | 53 | 229 | 246 | 39 | 9 | 13 | 13 | 13 | 13 | 9 | 9 | 19 | 13 | 16 | 13 | 13 | 13 |
| 13 | 19 | 59 | 76 | 26 | 9 | 16 | 16 | 13 | 99 | 249 | 142 | 6 | 19 | 13 | 13 | 13 | 13 | 19 | 4 | 13 | 13 | 6 | 26 | 9 | 13 |
| 16 | 113 | 229 | 219 | 93 | 9 | 26 | 83 | 23 | 159 | 219 | 59 | 9 | 9 | 6 | 13 | 16 | 13 | 16 | 13 | 6 | 9 | 9 | 16 | 23 | 9 |

*this* is your **result**

this just helps to
***communicate*** the result

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# Individual Pixel Values in Fiji

| Prefs | 597 | 598 | 599 | 600 | 601 | 602 | 603 |
|---|---|---|---|---|---|---|---|
| 255 | 1025 | 1059 | 839 | 839 | 1048 | 884 | 1012 |
| 256 | 1093 | 830 | 992 | 1054 | 974 | 992 | 928 |
| 257 | 1019 | 1058 | 932 | 878 | 944 | 948 | 990 |
| 258 | 863 | 959 | 1158 | 1061 | 1011 | 947 | 1042 |
| 259 | 849 | 1012 | 993 | 925 | 957 | 998 | 992 |
| 260 | 882 | 920 | 896 | 1071 | 1023 | 904 | 893 |
| 261 | 1034 | 906 | 960 | 1017 | 940 | 1023 | 1032 |

Image Analysis Collaboratory

# A digital image is a matrix of numbers!

=

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 13 | 19 | 6 | 19 | 13 | 9 | 19 | 9 | 6 | 9 | 6 | 16 | 16 | 6 | 16 | 13 | 132 | 229 | 103 | 19 | 16 | 13 | 23 | 9 | 9 |
| 19 | 19 | 6 | 13 | 13 | 13 | 13 | 16 | 16 | 19 | 9 | 13 | 9 | 6 | 16 | 16 | 49 | 192 | 216 | 106 | 23 | 13 | 16 | 16 | 23 | 13 |
| 13 | 9 | 4 | 13 | 13 | 16 | 19 | 36 | 66 | 93 | 79 | 26 | 13 | 13 | 6 | 16 | 113 | 209 | 196 | 113 | 29 | 19 | 36 | 49 | 36 | 33 |
| 19 | 13 | 19 | 13 | 16 | 13 | 26 | 89 | 123 | 136 | 152 | 116 | 76 | 33 | 13 | 46 | 159 | 162 | 159 | 126 | 79 | 96 | 189 | 229 | 226 | 212 |
| 16 | 16 | 9 | 6 | 13 | 19 | 26 | 93 | 156 | 179 | 106 | 66 | 79 | 136 | 106 | 152 | 179 | 93 | 29 | 13 | 16 | 23 | 79 | 156 | 123 | 49 |
| 16 | 6 | 13 | 13 | 16 | 13 | 23 | 69 | 103 | 69 | 19 | 16 | 6 | 109 | 209 | 236 | 179 | 43 | 9 | 16 | 9 | 13 | 13 | 19 | 13 | 13 |
| 9 | 9 | 16 | 19 | 13 | 13 | 19 | 13 | 26 | 16 | 16 | 13 | 6 | 103 | 179 | 189 | 132 | 33 | 19 | 16 | 16 | 9 | 9 | 6 | 6 | 6 |
| 13 | 9 | 4 | 13 | 13 | 13 | 16 | 19 | 13 | 23 | 6 | 16 | 23 | 123 | 186 | 192 | 169 | 126 | 26 | 16 | 19 | 13 | 6 | 13 | 16 | 13 |
| 13 | 13 | 9 | 16 | 9 | 6 | 13 | 19 | 16 | 19 | 6 | 19 | 63 | 199 | 192 | 106 | 29 | 149 | 162 | 113 | 119 | 53 | 9 | 13 | 6 | 13 |
| 13 | 9 | 16 | 6 | 6 | 19 | 13 | 9 | 23 | 13 | 9 | 6 | 119 | 182 | 149 | 36 | 6 | 39 | 196 | 196 | 176 | 73 | 16 | 9 | 9 | 9 |
| 6 | 19 | 13 | 9 | 19 | 16 | 13 | 13 | 19 | 9 | 9 | 23 | 142 | 179 | 109 | 13 | 16 | 9 | 39 | 59 | 23 | 19 | 13 | 4 | 9 | 9 |
| 19 | 13 | 9 | 9 | 16 | 16 | 16 | 9 | 9 | 13 | 6 | 66 | 169 | 172 | 43 | 16 | 9 | 9 | 9 | 13 | 13 | 19 | 16 | 16 | 16 | 9 |
| 9 | 9 | 6 | 9 | 13 | 9 | 6 | 13 | 4 | 9 | 19 | 116 | 196 | 89 | 9 | 9 | 16 | 16 | 19 | 19 | 9 | 16 | 6 | 16 | 9 | 9 |
| 13 | 13 | 9 | 23 | 19 | 13 | 9 | 9 | 9 | 6 | 26 | 159 | 219 | 59 | 23 | 9 | 13 | 9 | 6 | 13 | 6 | 19 | 16 | 13 | 16 | 13 |
| 9 | 23 | 13 | 6 | 6 | 23 | 9 | 19 | 13 | 16 | 66 | 206 | 179 | 13 | 6 | 16 | 13 | 13 | 13 | 16 | 9 | 13 | 9 | 9 | 16 | 13 |
| 13 | 13 | 23 | 16 | 19 | 19 | 6 | 9 | 19 | 13 | 142 | 255 | 103 | 19 | 13 | 6 | 19 | 9 | 16 | 9 | 16 | 9 | 16 | 13 | 23 | 9 |
| 6 | 13 | 23 | 9 | 13 | 16 | 13 | 6 | 9 | 53 | 229 | 246 | 39 | 9 | 13 | 13 | 13 | 13 | 9 | 9 | 19 | 13 | 16 | 13 | 13 | 13 |
| 13 | 19 | 59 | 76 | 26 | 9 | 16 | 16 | 13 | 99 | 249 | 142 | 6 | 19 | 13 | 13 | 13 | 13 | 19 | 4 | 13 | 13 | 6 | 26 | 9 | 13 |
| 16 | 113 | 229 | 219 | 93 | 9 | 26 | 83 | 23 | 159 | 219 | 59 | 9 | 9 | 6 | 13 | 16 | 13 | 16 | 13 | 6 | 9 | 9 | 16 | 23 | 9 |

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| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

## Where do these number come from?

# Bit depth

# Detectors in Fluorescence Microscopy

The detectors used in fluorescence microscopy are **monochromatic**.

Cameras or PMTs are not able to distinguish between different wavelengths (they just collect photons), you need fluorescence filters to separate your fluorophores.

The detector converts photons in digital numbers (linear relation).

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| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

Each pixel in the digital image has **one digital value** that **depends on** the **intensity** of the signal emitted by the **sample**.

**Digital Values = Pixel Intensity Value**

The **range** of possible **digital values** is defined by the **bit depth**.

# Bit Depth

The **bit depth** defines the range of possible **digital values** that each pixel can have, usually **8**, **12** or **16 bit**.

The **bit depth** is expressed in **grey values**.

**bit depth** of the **image** = **bit depth** of the **detector**
(Unless you change that during acquisition)

***x bit = a range of $2^x$ grey values***

**8 bit** image = **each pixel** can have **$2^8$ grey values** = 256 grey values = **range 0-255**

**12 bit** image = **each pixel** can have **$2^{12}$ grey values** = 4096 grey values = **range 0-4095**

**16 bit** image = **each pixel** can have **$2^{16}$ grey values** = 65536 grey values = **range 0-65535**

=

| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

**Digital Value = Pixel Intensity Value = Grey Value**

# Image Metadata

**Image > Show Info…**

**(cmd) + i**

***Show the Metadata stored with the file***

***e.g. bit depth, camera bit depth***

# display your images

# Mapping Image Intensity to Monitor Intensity (LookUp Tables)

*LUT = how the grey values are displayed*

*LUTs do not change the pixel values*

| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

| Image (12 bit) | Displayed color |
|---|---|
| 0 | grey LUT |
| 1 | |
| … | |
| 2000 | |
| … | |
| 4095 | |

# Display a file: Brightness & Contrast

**Image > Adjust > Brightness/Contrast**

***Interactively change the <u>displayed</u> brightness and contrast of the <u>active</u> image.***

**(cmd) + shift + c**

***You are NOT changing the pixels values, you are just changing how the image is displayed (unless you click on the "Apply" button).***

# Histogram

**Analyze > Histogram**

**h**

**jaehyuk-lee**: https://jaehyuk-lee.com/animated-image-histogram/

# Histogram

**Analyze > Histogram**

**h**

*Fiji auto-adjust the range (default option)*

*Bit depth*

# Display a file: Brightness & Contrast

Which image has more fluorescence?

| | | |
|---|---|---|
| *Mean:* | **4803** | **4803** |
| *Display range:* | 188-**16828** | 188-**45514** |

| | | |
|---|---|---|
| *Mean:* | **4803** | **4803** |
| *Display range:* | 188-**16828** | 188-**16828** |

# Display a file: Brightness & Contrast

Which image has more fluorescence?

| | | |
|---|---|---|
| *Mean:* | **4803** | **2074** |
| *Display range:* | 188-**19540** | 112-**7768** |

| | | |
|---|---|---|
| *Mean:* | **4803** | **2074** |
| *Display range:* | 188-1**9540** | 188-**19540** |

**Do NOT trust your eyes, rely on numbers!**

# Display a file: Brightness & Contrast

## *"Set" button - visually compare images*

*Use the same acquisition parameters*
***(per channel) if you want to compare images!!!***
***(e.g. same exposure time, Illumination intensity,…)***

| *Example* | *Exposure time Condition1* | *Exposure time Condition2* |
|---|---|---|
| *Channel 1* | *100 ms* | *100 ms* |
| *Channel 2* | *200 ms* | *200 ms* |

***For a meaningful comparison, you have to extract numbers from your images (analysis). Use the images to support your results.***

***If you save the images as jpeg/png/tiff, they will maintain the display range you set.***

1.1 image inspection

1.2 adjust brightness/contrast of all open images

# Images and Colors

## *Lookup Tables (LUTs)*

***LUT = how the grey values are displayed***

***LUTs do not change the pixel values***

| Image (8 bit) | Displayed color |
|---|---|
| 0 | grey LUT |
| 1 | |
| … | |
| 100 | |
| … | |
| 255 | |

=

| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

| Image (8 bit) | Displayed color |
|---|---|
| 0 | green LUT |
| 1 | |
| … | |
| 100 | |
| … | |
| 255 | |

=

| | | | |
|---|---|---|---|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

# Images and Colors

## *Lookup Tables (LUTs)*

**Image > Lookup Tables**

**or**

**"LUT" menu in Startup Tools Bar**

Examples of LUTs

**Image > Color > Display LUTs - to display default LUTs available*

# Images and Colors in Fiji

## *Choose the right LUT*

Which is brighter?

The human eye evaluates intensity best in grayscale

**If you are imaging for example a blue fluorophore, you are NOT FORCED to display it in blue!**

Image Analysis Collaboratory

# Images and Colors in Fiji

***Choose the right LUT***

Color blind people don't distinguish some colors

POINTS OF VIEW

**Color blindness**

NATURE METHODS | VOL.8 NO.6 | JUNE 2011 | 441

"If a submitted manuscript happens to go to three male reviewers of Northern European descent, the chance that at least one will be color blind is 22 percent"

# Images and Colors in Fiji

## *Choose the right LUT*

Color blind people don't distinguish some colors

**Protanope (no red)** **Tritanope (no blue)**

***Image > Color > Dichromacy*** *or* ***Image > Color > Simulate Color Blindness***

DAPI
Phalloidin (Alexa 488)
MitoTracker Red

Talley Lambert

# RGB Images (still matrix of numbers)

## LUTs cannot be applied to RGB Images

*RGB image*

=

| | | | |
|---|---|---|---|
| 255<br>0<br>234 | 0<br>255<br>0 | 0<br>186<br>28 | 255<br>255<br>1 |
| 0<br>0<br>255 | 1<br>199<br>255 | 255<br>0<br>0 | 0<br>255<br>111 |
| 255<br>0<br>255 | 255<br>255<br>1 | 255<br>255<br>255 | 0<br>0<br>0 |
| 254<br>105<br>0 | 117<br>117<br>117 | 141<br>137<br>0 | 118<br>0<br>143 |

RGB Color image (e.g. jpeg, png) = **Red + Green + Blue**

RGB Color image = 8 bit Red, 8 bit Green, 8 bit Blue = R (0-255), G (0-255), B (0-255)

**Image > Type > RGB Color** or **Save As…png, jpeg**

# Images and Colors in Fiji

## *The Color menu*

**Image > Color > Split Channels**

**Image > Color > Merge Channels**

***Split* or *Merge* the channels of the active image**

# Images and Colors in Fiji

## *The Color menu*

**Image > Color > Channels tools**

**(cmd) + shift + z**

***Interactive visualization/handling of each image channel.***

***Choose how to display your multicolour image: "Color" mode (single channel) vs "Composite" mode (overlay)***

# Segmentation with pixel based classifier—exercises

2.1 composite images - splitting and merging
2.2 RGB images - Replace Red with Magenta

# Pixel Size

# Scale Bar and Pixel Size

# Pixel Size and Scale Bar

**If you want to make some physical measurements of your sample (length, size, …), you need to know the pixel size of your image.**

**w/ Metadata**

**Image > Show Info…**

**(cmd) + i**

***Show the Metadata stored with the file.***

**Pixel width (Pixel x) = 0.0515 µm**
**Pixel height (Pixel y) = 0.0515 µm**
**Voxel depth (Pixel z) = 0.0515 µm**

**1 µm = 19.4201 pixel (xy)**
**(1 µm / 0.0515 µm)**

# Pixel Size and Scale Bar

**If you want to make some physical measurements of your sample (length, size, …), you need to know the pixel size of your image.**

**Image > Properties…**

**(cmd) + shift + p**

*Open the image properties windows containing pixel size properties (and multi-dimensional properties)*

*You can set/read the image pixel size in xyz.*

# Scale Bar and Pixel Size

**What if the pixel size is not stored in the metadata?**

**If you know the magnification and the camera you used for the acquisition, you can estimate the image pixel size.**

**image pixel size = camera pixel size/magnification**

Example

Magnification = 100x Objective

Camera = Hamamatsu Orca Flash 4

| | |
|---|---|
| Product number | C13440-20CU |
| Imaging device | sCMOS |
| Cell (pixel) Size (μm²) | 6.5×6.5 |
| Pixel Array (horizontal by vertical) | 2048×2048 |
| Effective Area (horizontal by vertical in mm) | 13.312×13.312 |

**pixel width and height:**
**6.5 μm / 100X = 0.065 μm**

**Image > properties**

If a z-series was acquired, "Voxel depth" is the z step size choose for the acquisition (cannot estimate).

# Scale Bar and Pixel Size

**Add pixel information to your images from a reference (same magnification)**

**Analyze > Set Scale…**

***Set the pixel size information starting from an object with a known dimension.***

****Perform multiple measures and average them to be more precise***

# Scale Bar and Pixel Size

## Add scale bar to your images

**Analyze > Tools > Scale Bar…**

- "Overlay" means that the scale bar is not embedded in your image. You can remove it: "*Image >Overlay > Remove Overlay*".
- If you save the image as tiff, the scale bar will be saved as an overlay.
- If you save as jpeg/png, the image will have the scale bar embedded.

# Segmentation with pixel based classifier—exercises

1.3 file handling and non-invasive editing

1.4  file handling and invasive editing

# 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**.

# 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***

***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

# Examples

## Standalone: Ilastik

https://www.ilastik.org/

## For histopathology

https://qupath.github.io/

## As a Fiji plugin: Labikit

Labkit

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

# Tips

## *4.3 DAPI segmentation with Labkit*

## *Breakpoint at 12: Create results*

# Filters

Image Analysis Collaboratory

# Where is Waldo?

# Where is Waldo?

Image Analysis Collaboratory

# Where is Waldo?

# Filtering

## Neighborhood Transformation

### Linear Transformation

Smoothing: Mean, Gaussian

Edge detection: Sobel, Canny

### Non-linear Transformation

Smoothing: Median

Cleaning: Morphology

Image Analysis Collaboratory

Weights are defined by the kernels

Weighted average = Multiply, add, divide

One neighborhood at a time

Neighborhood defined by the kernel size

# Convolution is weighted average in local neighborhood

**Terminologies**

A **kernel** is a small matrix used in image processing for convolution.

A **filter** is a broader term that refers to any technique that modifies an image.

# Convolution operation

**Image**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| px1 | px2 | px3 | px4 | px5 | px6 | px7 | px8 | px9 |
| px10 | px11 | px12 | px13 | px14 | px15 | px16 | px17 | px18 |
| px19 | px20 | px21 | px22 | px23 | px24 | px25 | px26 | px27 |
| px28 | px29 | px30 | px31 | px32 | px33 | px34 | px35 | px36 |
| px37 | px38 | px39 | px40 | px41 | px42 | px43 | px44 | px45 |
| px46 | px47 | px48 | px49 | px50 | px51 | px52 | px53 | px54 |
| px55 | px56 | px57 | px58 | px59 | px60 | px61 | px62 | px63 |
| px64 | px65 | px66 | px67 | px68 | px69 | px70 | px71 | px72 |
| px73 | px74 | px75 | px76 | px77 | px78 | px79 | px80 | px81 |

convolution ✪

**3 x 3 kernel**

| | | |
|---|---|---|
| k1 | k2 | k3 |
| k4 | k5 | k6 |
| k7 | k8 | k9 |

=

(px1 * k1 / ksum) +

(px2 * k2 / ksum) +

(px3 * k3 / ksum) +

(px10 * k4 / ksum) +

(px11 * k5 / ksum) +

(px12 * k6 / ksum) +

(px19 * k7 / ksum) +

(px20 * k8 / ksum) +

(px21 * k9 / ksum) =

c1

ksum = k1 + k2 + k3 + k4 + k5 + k6 + k7 + k8 + k9

*Adapted from Federico M. Gasparoli*

# Convolution operation

**Image**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| px1 | px2 | px3 | px4 | px5 | px6 | px7 | px8 | px9 |
| px10 | px11 | px12 | px13 | px14 | px15 | px16 | px17 | px18 |
| px19 | px20 | px21 | px22 | px23 | px24 | px25 | px26 | px27 |
| px28 | px29 | px30 | px31 | px32 | px33 | px34 | px35 | px36 |
| px37 | px38 | px39 | px40 | px41 | px42 | px43 | px44 | px45 |
| px46 | px47 | px48 | px49 | px50 | px51 | px52 | px53 | px54 |
| px55 | px56 | px57 | px58 | px59 | px60 | px61 | px62 | px63 |
| px64 | px65 | px66 | px67 | px68 | px69 | px70 | px71 | px72 |
| px73 | px74 | px75 | px76 | px77 | px78 | px79 | px80 | px81 |

convolution ✪

**3 x 3 kernel**

| | | |
|---|---|---|
| k1 | k2 | k3 |
| k4 | k5 | k6 |
| k7 | k8 | k9 |

=

(px2 * k1 / ksum) +

(px3 * k2 / ksum) +

(px4 * k3 / ksum) +

(px11 * k4 / ksum) +

(px12 * k5 / ksum) +

(px13 * k6 / ksum) +

(px20 * k7 / ksum) +

(px21 * k8 / ksum) +

(px22 * k9 / ksum) =

c2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | c1 | ? | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

**ksum** = k1 + k2 + k3 + k4 + k5 + k6 + k7 + k8 + k9

*Adapted from Federico M. Gasparoli*

# Convolution operation

**Image**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| px1 | px2 | px3 | px4 | px5 | px6 | px7 | px8 | px9 |
| px10 | px11 | px12 | px13 | px14 | px15 | px16 | px17 | px18 |
| px19 | px20 | px21 | px22 | px23 | px24 | px25 | px26 | px27 |
| px28 | px29 | px30 | px31 | px32 | px33 | px34 | px35 | px36 |
| px37 | px38 | px39 | px40 | px41 | px42 | px43 | px44 | px45 |
| px46 | px47 | px48 | px49 | px50 | px51 | px52 | px53 | px54 |
| px55 | px56 | px57 | px58 | px59 | px60 | px61 | px62 | px63 |
| px64 | px65 | px66 | px67 | px68 | px69 | px70 | px71 | px72 |
| px73 | px74 | px75 | px76 | px77 | px78 | px79 | px80 | px81 |

convolution ✪

**3 x 3 kernel**

| | | |
|---|---|---|
| k1 | k2 | k3 |
| k4 | k5 | k6 |
| k7 | k8 | k9 |

=

(px3 * k1 / ksum) +

(px4 * k2 / ksum) +

(px5 * k3 / ksum) +

(px12 * k4 / ksum) +

(px13 * k5 / ksum) +

(px14 * k6 / ksum) +

(px21 * k7 / ksum) +

(px22 * k8 / ksum) +

(px23 * k9 / ksum) =

c3

ksum = k1 + k2 + k3 + k4 + k5 + k6 + k7 + k8 + k9

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | c1 | c2 | ? | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

*Adapted from Federico M. Gasparoli*

# Different Types of Kernel

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

1D Mean Filter

2D Mean Filter

3D Mean Filter

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 2 | 1 | 0 |
| 0 | 2 | 5 | 2 | 0 |
| 0 | 1 | 2 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

1D Gaussian Kernel

2D Gaussian Kernel

3D Gaussian Kernel

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | -1 | 0 | 1 | 0 |
| 0 | -1 | 0 | 1 | 0 |
| 0 | -1 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | -1 | -1 | -1 | 0 |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

1D Prewitt Filter

2D Prewitt Filter

3D Prewitt Filter

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | -1 | 1 | 0 |
| 0 | -1 | 2 | -1 | 0 |
| 0 | 1 | -1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

1D Sharpening Filter

2D Sharpening Filter

3D Sharpening Filter

# Mean Filter

# Gaussian Filter

# Effects of Different Gaussian Kernel

Kernel

Blurred image

# Edge Filters

## Prewitt Operator

# Different Filters in Action

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

**Mean Filter**

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 2 & 1 & 0 \\ 0 & 2 & 5 & 2 & 0 \\ 0 & 1 & 2 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

**Gaussian Filter**

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} \quad \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 1 & 0 \\ 0 & -1 & 0 & 1 & 0 \\ 0 & -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

**Prewitt Filter**

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 1 & 0 \\ 0 & -1 & 2 & -1 & 0 \\ 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

**Sharpening Filter**

# Filtering — exercises

3.1 edge filters - vertical stripes
3.2 edge filters - horizontal stripes
3.3 edge filters - checkerboard

# Filtering — exercises

4.2 DAPI segmentation with filters and thresholding

# Morphological Filters

# Two Primary Morphological Operations

Erosion

Dilation

# Erosion Operation

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| 1 | 1 | 1 |
| 1 | 1 | 1 |
| 1 | 1 | 1 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# Erosion Operation

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| 1 | 1 | 1 |
| 1 | 1 | 1 |
| 1 | 1 | 1 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 0 | 0 |
| 0 | 0 | 1 |

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| | | |
|---|---|---|
| 1 | 1 | 1 |
| 1 | 1 | 1 |
| 1 | 1 | 1 |

→

| |
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| 0 |

# Erosion Operation

# Erosion Operation

# Erosion Operation

# Erosion Operation

Image Analysis Collaboratory

# Erosion Operation

# Erosion - The Movie

# Dilation Operation

# Dilation Operation

# Dilation Operation

# Dilation Operation

# Dilation - The Movie

Image Analysis Collaboratory

# But wait, there is a problem.

# Together We Stand

Main image

Dilated image

Closed image

Main image

Eroded image

Opened image

**Closing:**

Erosion of the dilation.

**Opening:**

Dilation of the erosion.

# Bonus Example

**Main image**

**Inner edge**

Erode more, dilate less, then subtract

**Outer edge**

Dilate more, erode less, then subtract

# Structuring Element Size

# Structuring Element Shape

Image Analysis Collaboratory

# Structuring Element Shape

**BE CAREFUL!**

Image Analysis Collaboratory

# Morphology in Fiji

# Morphology — exercises

3.4 - Morphological filters - Binary
3.5 - Morphological filters - Gray

# ROI Manager

Image Analysis Collaboratory

Annotation Tools
*see also Edit > Selection

# Region Of Interest (ROI)

Secondary/Right Click for more options

Double Click to set/change properties

# ROI Manager

**Analyze > Tools > ROI Manager…**

**t***

***The ROI Manager is a tool for working with multiple selections (ROIs).***

****(cmd) + t* can be used to both to open the ROI Manager and/or add a new ROI to the Manager.**

****<u>shift + e</u> can be used to draw the last ROI.**

# ROI Manager

Add new ROI (t).

After modifying a ROI, you can use Update to save the changes.

Delete selected ROI. If none is selected, delete all.

Rename selected ROI.

Deselect one or more selected ROI.

# ROI Manager

Change the properties of the selected ROI(s)

**Rectangle...**

**Text**

**Line**

**Dot**

Image Analysis Collaboratory

"Analyze" menu

"Edit" menu

# ROI Manager

Open a saved ROI.zip file (drag & drop works as well)

Save ROI(s) as a .zip file

Image Analysis Collaboratory

"Analyze" menu

"Image" menu

# ROI Manager

Open a saved ROI.zip file (drag & drop works as well)

Save ROI(s) as a .zip file

Set ROI(s) label options

*Labels option can also be found under "Image > Overlay"

*"Use names as labels" can be also found under "More>Options..."

# Live Demo of ROI manager

# Segmentation
# And
# Measurements

Image Analysis Collaboratory

Binary mask → Process and segment → Export and Measure

Results

| | Area | Mean | X |
|---|---|---|---|
| 0016 | 285 | 204.29474 | 197. |
| 0028 | 278 | 174.84892 | 219. |
| 0027 | 231 | 188.46753 | 45.0 |
| 0034 | 501 | 189.14172 | 174. |
| 0041 | 660 | 171.69697 | 73.8 |
| 0048 | 228 | 195.89474 | 233. |
| 0055 | 448 | 209.03571 | 138. |

Image Analysis Collaboratory

Binary mask

Process and segment

Export and Measure

Results

| | Area | Mean | X |
|---|---|---|---|
| 0016 | 285 | 204.29474 | 197. |
| 0028 | 278 | 174.84892 | 219. |
| 0027 | 231 | 188.46753 | 45.0 |
| 0034 | 501 | 189.14172 | 174. |
| 0041 | 660 | 171.69697 | 73.8 |
| 0048 | 228 | 195.89474 | 233. |
| 0055 | 448 | 209.03571 | 138. |

# Do you see an issue here?

# Solution: Watershed



in **Fiji**: **Process** > **Binary** > **Watershed**

**Watershed** is a useful algorithm to try to **separate touching objects**.

**Image**

**Binary Mask**

**Watershed**

# Solution: Watershed



Binary mask

Distance transform

# From binary image to instance segmentation

Binary mask

Instance segmentation

# Analyze particles



in Fiji: **Analyze** > **Analyze Particles…**

# Analyze particles — underlying principles



in Fiji:
**Analyze**
> **Analyze Particles…**

# Analyze particles — underlying principles



in Fiji:
**Analyze**
> **Analyze Particles…**

# Analyze particles — underlying principles



in Fiji:
**Analyze**
> **Analyze Particles…**

# Analyze particles — underlying principles



in Fiji:
**Analyze**
> **Analyze Particles…**

# Analyze particles — underlying principles



in Fiji:
**Analyze**
> **Analyze Particles…**

# Analyze particles: Select for morphology



in Fiji:
**Analyze**
> **Analyze Particles…**

Size

Circularity

Fill

# Analyze particles: Size



in Fiji: **Analyze** > **Analyze Particles…**

**Size range** of the **particles** that you want to detect.

**particle**

# Analyze particles: Size

the **size range** of the **particles** that you want to detect.

# Analyze particles: Circularity



in Fiji: **Analyze** > **Analyze Particles…**

**Circularity** of the **particles** that you want to detect.

# Analyze particles: Circularity



$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$

# Analyze particles: Circularity



$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$

# Analyze particles: Circularity



$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} < 1$$

$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$

# Analyze particles: Exclude on Edges



in Fiji: **Analyze** > **Analyze Particles…**

"**Exclude on edges**" excludes objects that are touching the borders of the image.

Binary mask → Process and segment → Export and Measure

Results

| | Area | Mean | X |
|---|---|---|---|
| 0016 | 285 | 204.29474 | 197. |
| 0028 | 278 | 174.84892 | 219. |
| 0027 | 231 | 188.46753 | 45.0 |
| 0034 | 501 | 189.14172 | 174. |
| 0041 | 660 | 171.69697 | 73.8 |
| 0048 | 228 | 195.89474 | 233. |
| 0055 | 448 | 209.03571 | 138. |

- Save the segmented image
- Add to ROI manager
- Generate and export measurements

## Export and Measure

# Analyze particles: Save the segmented image



in Fiji: **Analyze** > **Analyze Particles…**

"**Count Masks**" encodes object identity as gray value.

# Analyze particles: Add to ROI Manager



in Fiji: **Analyze** > **Analyze Particles…**

"**Add to Manager**" stores all found objects in the **ROI Manager**.

# Analyze particles: Add to ROI Manager

Export

in Fiji: **Analyze** > **Analyze Particles…**

Image Analysis Collaboratory

- Save the segmented image
- Add to ROI manager
- Generate and export measurements

## Export and Measure

- Save the segmented image
- Add to ROI manager
- Generate and export measurements
  - Select what to measure
  - Measure

## Export and Measure

# Select what to measure: Set Measurements



**Analyze > Set Measurements…**

*Specifies which measurements have to be performed (e.g. area, mean grey value, max and min grey values, …)*

# Measure — Option 1: Manually



**Analyze > Measure**

**(cmd) + m**

*Measures the parameters chosen under "Analyze > Set Measurements…" in relation to the selected ROI.*

*Results are displayed in a Result Table (which can be saved as .csv, .xlsx, …)*

# Side note: other kinds of measurements

**Analyze > Plot Profile**

**(cmd) + k**

*Plots can be saved as .csv file ("Save Data…") and also as images (e.g. "File > Save AS > PNG")

# Measure — Option 2: from Analyze particles

Export

# Measure — Option 3: Using the ROI manager

Export

1. Select the "ORIGINAL" image.

2. To measure <u>all</u> the ROIs stored in the "ROI Manager", **no ROI has to be selected** in the"ROI Manager". Use the "Deselect" button.

3. Click on the "**Measure**" button.

4. A "**Results**" **table** will appear.

# Segmentation with pixel based classifier—exercises

Continue with the *“Analyze Particle” step* form *ONE* of the workflow exercises below.

4.1 DAPI segmentation with thresholding

***OR***

4.3 - DAPI segmentation with Labkit

# Spot Detection
# (Point tool and Find Maxima)

# Point and Multi-Point tool

***Select and Measure multiple points***

### *Mouse right-click*

### *Mouse double-click*

***to clear all points:***

**Edit > Selection > Select None ***

**(cmd) + shift + a**

***to measure all points:***

**Analyze > Measure ****

**(cmd) + m**

***to add point selection to ROI Manager:***

**t**

**you can also toggle the "Show all" checkbox in the ROI Manager.* ***you can also use the "Measure" button in the ROI Manager.*

# Point and Multi-Point tool

***Select and Measure multiple points***

**Process > Find Maxima...**

# Segmentation with pixel based classifier—exercises

*5.1 - Manual spot detection with the Multi-point Tool*

*5.2 Algorithmic spot detection with Find Maxima*

*5.3 Automatic spot segmentation with thresholding*

*5.4. Spot detection with noise*

*5.5. Spot detection with variable background*

# Further Learning (https://iac.hms.harvard.edu/resources/)

**Forum**: Knowledge exchange and support

- https://forum.image.sc/

**Online book with code**: Introduction to Bioimage Analysis

- https://bioimagebook.github.io/

**Online training**: NEUBIAS Academy

- https://eubias.org/NEUBIAS/training-schools/neubias-academy-home/
- https://www.youtube.com/c/NEUBIAS

**Fiji manual** from Monash University

- https://bridges.monash.edu/articles/educational_resource/Fiji_Training_Manual_v6_4_/20033513

**BioImaging North America (BINA)**

- https://www.bioimagingnorthamerica.org/