

Introduction to image analysis using ImageJ/Fiji



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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)





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

- **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



• Support and collaboration: for image and data analysis projects

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



Who we are, currently





Antoine A. Ruzette

Associate

Ranit Karmakar

Specialist Postdoc









Research Associate





Backgrounds in

Physics Biology Microscopy Bioengineering **Computer Engineering**

Maria Theiss

Specialist Postdoc

Simon F. Nørrelykke

Director, Lecturer



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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



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!





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



none





Acknowledgements

- Szymon Stoma, ETH Zurich, Switzerland
 - slides and workflows
- - slides and graphics



Peter Bankhead, Edinburgh University, Scotland



Self-introductions

- 1. My name is <u>Donald Duck</u>
- 2. My **position** is as a 1<u>0th year postdoc</u> 3. My lab is <u>Mad City Labs</u>
- 4. My model **system** is <u>blue whales</u>
- 5. I have data from <u>confocal microscopy</u>





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?





http://www.brainbashers.com



Our size estimate is strongly influenced by the local neighbourhood



Identical central discs?



Yes, the discs are identical



Is the dot half-way up?







Our sense of distance depends on neighbourhood



Is the dot half-way up?



http://www.brainbashers.com

Yes it is!

Which car is bigger?









Which car is bigger?





Which car is bigger?



All cars are same size (Erroneous, application of subjective/perceptual constancy algorithm by your brain)

Are A and B equally grey?



http://www.brainbashers.com



Intensity perception depends strongly on neighbourhood



Are A and B equally grey?





http://www.brainbashers.com

Yes they are!

Spot the animal

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

Pattern Recognition



Yes, that is a cow! http://www.brainbashers.com





Pattern Recognition





Yes, that is a cow! http://www.brainbashers.com



Using Prior Knowledge



http://www.brainbashers.com



Keyword: Dalmatian dog!



Using Prior Knowledge



http://www.brainbashers.com



Keyword: Dalmatian dog!



A brief history of image analysis

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 Imaged 1.x: written in Java and also compatible with Windows PC
- Since then, ImageJ has been maintained and upgraded (e.g. ImageJ2, Fiji, ...)





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.







Fiji stands for Fiji Is Just ImageJ It is a "batteries included" distribution of <u>ImageJ2</u> Easy to install ("portable" application) Include a collection of Plugins Documentation





What is Fiji?



- Easy to update (<u>https://imagei.net/list-of-update-sites/</u>)



12 years ago...

<u>AlexNet</u> is published (2012)

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















4 years ago...

CellPose is out (2020)

- "Cellpose: a generalist algorithm for cellular segmentation" Ο
- Trained on highly varied images of cells, over 70,000 segmented objects \bigcirc
- Cells don't have to be star-shaped \bigcirc
- Web-platform and Jupyter notebooks \bigcirc
- https://www.nature.com/articles/s41592-020-01018-x \bigcirc
- ZeroCostDL4Mic available
 - Paper still a year in the future \bigcirc
 - Implementation of common DL technologies to microscopy imaging \bigcirc
 - Relies on GPUs and other infrastructure provided by Google Colab. \bigcirc
 - https://github.com/HenriguesLab/ZeroCostDL4Mic/wiki \bigcirc
- Vision Transformers (2020)
 - \bigcirc
 - https://arxiv.org/abs/2010.11929







"An Image is Worth 16x16 Words: Transformers for Image Recognition at Scale", Dosovitskiy et al.







Today...

Image Analysis Software





forum.image.sc



History of Segmentation

Segmentation Paradigm Over the Past Half Century

Heuristic methods

Optimization/model -based methods

1980s

Thresholding **Region Growing**

...

Variational Models (e.g., Snakes, GAC,...)



 $S^* = \arg\min_{S} E(S)$

Ma et al. SIAM-IS, 2021

More data

More powerful computing









English as the next programming language

LLMs, chatbots and foundation models for bio image analysis

Create a workout plan

 \Box

for resistance training

Explain why popcorn pops

to a kid who loves watching it in the microwave

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/

ChatGPT

Design a database schema

for an online merch store

Write an email

to request a quote from local plumbers



Using ChatGPT for Image Analysis







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




GPT-4, May 2024



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



GPT-40, Oct. 2024



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



GPT-4, May 2024



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-40, Oct. 2024

Segmented Nuclei







GPT-4, May 2024

```
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-40, Oct. 2024

```
D 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)
```





GPT-4, May 2024

You

please use the blue channel for segmenting the nuclei



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-40, Oct. 2024







Using ChatGPT-40 for Image Analysis GPT-40, Oct. 2024 - prompted to close larger holes

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









Using ChatGPT-40 for Image Analysis GPT-40, 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





Biolmage Chatbot





https://arxiv.org/abs/2310.18351

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





napari-omega interface **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 Supplementary Video 2. Omega can segment nuclei in a 3D image. This video shows how Omega segments the nuclei in a 3D within the editor, and the Python code repair module. The architecture employs a cascaded design, with a central dialog image displayed in the napari viewer. Omega uses a specialized tool for cell and nuclei segmentation and employs a 'classic' loop orchestrated by a primary language model. This model coordinates a suite of specialized tools, each powered by a approach that combines single thresholding, specifically Otsu, with watershed splitting to prevent under-segmentation. After 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 <u>Stardist</u>. 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

●●● E · < > ①

127.0.0.1



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.









Segment Anything

Research by Meta Al





https://segment-anything.com/



Segment Anything

Research by Meta Al





https://segment-anything.com/



Segment Anything in ImageJ/Fiji

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

\circ \circ	SAMJ Annotator	
EfficientSAN	A 💿 EfficientViTSAM-I0 🔘 EfficientViTSAM-I1 🔘 EfficientViTSAM-I2 🔵 EfficientViTSAM-xI0 🔵 EfficientV	/itsam-:
	Install Uninstall	
EfficientViT-S/ Weights size Speed: 1st ou Performance GitHub Repo	AM smallest version (L0) 3: 139.4 MB ut of 6 3: 6th out of 6 5: 6th out of 6 5: https://github.com/mit-han-lab/efficientvit	
Paper: <u>Efficie</u>	ntViT-SAM: Accelerated Segment Anything Model Without Performance Loss	
Paper: <u>Efficie</u> Go!	blobs.gif	
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Paper: Efficie Go!	blobs.gif Export as Label Coming soon Return all ROIs V Add to ROI Manager	



Example of point annotations



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



Image Analysis Workflow

• There are typically *five* steps in an image analysis



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

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

Often a good idea to structure work along these lines before starting





- 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)









Pre-processing

Processing

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

• • •









- Size, shape, intensity
- Texture, location, neighbours
- Binary morphological operations

. . .

• Hit-or-miss transform





• Filtering detected objects based on

- Open/close
- Skeletonise









• Object measures

Measuring

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





- 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

. . .





Processing vs. Analysis

Image Formation

object in \rightarrow image out



Image A	Analy	/sis	
image in →	• feat	tures of	ut
	Obj	Area	Pe
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		3	487.1	1
		4	226.3	
		5	531.8	1
		6	649.5	2
and and		7	582.6	1
		8	498.0	1
		9	543.2	1

Computer Vision image in \rightarrow 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.







Computer Graphics numbers in \rightarrow image out

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8	.5	
С	.3	
9	.2	
7	. 8	
7	. 6	
3	.1	
6	. 4	
2	.9	
5	.1	

х	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



Visualization image in \rightarrow representation out







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 <u>https://forum.image.sc/</u>







Install and Update Fiji





Download Fiji

https://fiji.sc/



Fiji is distributed o which means that you Just download (zij

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.







(Fiji Is Just) ImageJ Search Search ImageJ Website *Rectangle*, rounded rect or rotated rect (right click to switch) Click Click Click Dev. Resources Plugins Macros Plugins Macros Macros Macros Macros Macros Macros Plugins Macros Macros	Ű	Fiji	File	Edit	Image	Process	Analyze	Plugins	Wi	ndow	Help	
ImageJ Website *Rectangle*, rounded rect or rotated rect (right click to switch) Click ImageJ Website ImageJ News Documentation Installation Mailing List Dev. Resources Plugins Macros Macro Functions Examples Update ImageJ About Plugins About Plugins About ImageJ Report a Bug Help on Menu Item Switch to Modern Mode Update		•	1			(Fiji Is Ju	ıst) ImageJ		1			Search
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Report a Bug Help on Menu Item Switch to Modern Mode Update Upload Sample Image												About ImageJ
Help on Menu Item Switch to Modern Mode Update Upload Sample Image												Report a Bug
Switch to Modern Mode Update Upload Sample Image												Help on Menu Item
Update Upload Sample Image												Switch to Modern Mode
Upload Sample Image												Update
												Upload Sample Image

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)

🖆 Fiji File Edit Image Proc	ess Analyze Plugi	ins Wi	indow H	elp
🔴 😑 🍘 (Fij	i Is Just) ImageJ	_		
$\Box \bigcirc \Box \heartsuit / \checkmark \ddagger \land A$	Q m Dev	Stk LUT	0 8	\$
(Fiji Is Just) ImageJ 2.1.0/1.53c / Java 1.8.	0_66 (64-bit)		line width	n l
	Quick Search			
Commands Line Width / Edit/Options/Line Width	<u>Line Width</u>			
✓ Ops	Menu path Edit > Options > Line Y	Width		
Script templates	Identifier legacy:ij.plugin.Optio	ons		
Image.sc Forum	Location jars/ij-1.53c.jar			
Classes				
		Run	· · · · · · ·	
	Help		Sou	rce



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.



🗯 Finder File E	dit View Go Window	Help
	(Fiji Is Just) Imag	eJ
	Ĺ ‡‡ţ × A Q ℓ吶 🗖	Dev Stk LUT 🖉 🔏 🗞 ≫
Drag and Drop>>		Click here to search
	Stack single tif	
		Stack_single_tif
	Stack_single_tif	
Name	 Date Modified 	Stack_single_tif
RCN0000.tif	Today at 10:55	
RCN0001.tif	Today at 10:55	
RCN0002.tif	Today at 10:55	
RCN0003.tif	Today at 10:55	
RCN0004.tif	Today at 10:55	
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0 C Image Analysis Collaboratory

Bio-Format Plugin import a file - options

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	(Fiji Is	Just) ImageJ													
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		Bio-Formats Import Options													
Stack viewing	Metadata only Standard ImageJ	Metadata viewing													
View stack with:	✓ Hyperstack	Display metadata													
Stack order:	View5D	Display OME-XML metadata													
		Display ROIs													
		ROIs Import Mode: ROI manager 🗘													
Dataset organiza	ation	Memory management													
🗹 Group files w	vith similar names	Use virtual stack													
Open files in	dividually	Specify range for each series													
Swap dimens	ions	Crop on import													
Open all serie	es														
Concatenate	series when compatible	Split into separate windows													
Stitch tiles		Split channels													
Color options		Split focal planes													
Color mode:	Default 🗘	Split timepoints													
Autoscale															

https://imagej.net/Bio-Formats



Information

View stack with - The type of image viewer to use when displaying the dataset.

Possible choices are:

- Metadata only Display no pixels, only metadata.
- Standard ImageJ This option is deprecated (i.e. intended for use by old macros only). Please use *Hyperstack* instead.
- Hyperstack Display the pixels in ImageJ's built-in 5D viewer.
- Data Browser Display the pixels in the multidimensional Data Browser viewer. The Data Browser has some additional features on top of the normal ImageJ hyperstack.
- Image5D Display the pixels in Joachim Walter's Image5D viewer. Requires the Image5D plugin.
- View5D Display the pixels in Rainer Heintzmann's View5D viewer. Requires the View5D plugin.

Cancel

ОК

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



https://imagej.net/Bio-Formats

Image Analysis Collaboratory

Bio-Format Plugin import a file - options





https://imagej.net/Bio-Formats

Bio-Format Plugin import a file

Drag and Drop

File > Import > Bio-Formats

or

Plugins > Bio-Formats > Bio-Formats Importer



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



Cancel OK





what is an image?





A digital image is a matrix of numbers!



Pixel = Picture Element

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
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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
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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





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









Individual Pixel Values in Fiji









A digital image is a matrix of numbers!



Where do these number come from?

i i i						i i				i	i	i	i	i			i								
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

109	209
103	179





Bit depth





Detectors in Fluorescence Microscopy

The detectors used in <u>fluorescence microscopy</u> 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).





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

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





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 2⁸ 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

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

		C2·	-hela_	cells.tif	(50%	%)
34.60x	24.30	µm (6	72x4	72); 16	–bit;	620K
[Image	Process	Analyze	Plugins	Wind	
	Тур			8-bit		
	Adjus Show	t Info	► ₩1	✓ 16-bit 32-bit	-	





display your images



Mapping Image Intensity to Monitor Intensity (LookUp Tables)



LUT = how the grey values are <u>displayed</u>



and a start	lmage (12 bit)	Displayed color
	0	
223	1	<u>k</u>
	•••	
The Long	2000	5
THE F	•••	
	4095	

<u>LUTs do not change the pixel values</u>







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).







Analyze > Histogram



background

foreground



Pixel Count



jaehyuk-lee: https://jaehyuk-lee.com/animatedimage-histogram/



Pixel Values





Analyze > Histogram



Fiji auto-adjust the range (default option)

Histogram

h

		Histogram	n of DAPL14	
300	x246 piz	xels; RGB;	288K	
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	N: 6400	00	MIN: 1	
	N: 6400 Mean: 13	00 328.815	Min: 1 Max: 14039	
	N: 6400 Mean: 1: StdDev:	00 328.815 1273.577	Min: 1 Max: 14039 Mode: 1015	(212550)
	N: 6400 Mean: 1: StdDev: Bins: 250	00 328.815 1273.577 6	Min: 1 Max: 14039 Mode: 1015 Bin Width: 25	(212550) 55.996
	N: 6400 Mean: 1: StdDev: Bins: 250 Value:	00 328.815 1273.577 5 	Min: 1 Max: 14039 Mode: 1015 Bin Width: 23 Count:	(212550) 55.996
	N: 6400 Mean: 1: StdDev: Bins: 250 Value:	00 328.815 1273.577 5	Min: 1 Max: 14039 Mode: 1015 Bin Width: 25 Count:	(212550) 55.996

Bit depth





Which image has more fluorescence?



Mean:	4803
Display range:	188- 16828

Mean:	4803	
Display range:	188- 16828	



4803

188-16828





Which image has more fluorescence?



Mean:	4803
Display range:	188- 19540

Mean:	4803
Display range:	188-1 9540









188-19540

Do NOT trust Your eyes, Do NOT trust Your eyes, rely on numbers!





"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 Condition 1	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

LUT = how the grey values are <u>displayed</u>

<u>LUTs do not change the pixel values</u>

the second is	[[lma (8 b	ge it)	Displayed color	
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1 5	1	25	5		
136	106	152	179		

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

Lookup Tables (LUTs)



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





Images and Colors Lookup Tables (LUTs)



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





Images and Colors in Fiji Choose the right LUT



The human eye evaluates intensity best in grayscale

If you are imaging for example a blue fluorophore, you are <u>NOT FORCED</u> to display it in blue!

Which is brighter?





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"





Color blind people don't distinguish some colors





Images and Colors in Fiji

Choose the right LUT

Protanope (no red)

Tritanope (no blue)

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





MitoTracker Red









RGB Images (still matrix of numbers)

LUTs cannot be applied to RGB Images

RGB image



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

Image > Type > RGB Color or Save As...png, jpeg

	255 0 234	0 255 0	0 186 28	255 255 1
	0	1	255	0
	0	199	0	255
	255	255	0	111
1.12	255	255	255	0
	0	255	255	0
	255	1	255	0
	254	117	141	118
	105	117	137	0
	0	117	0	143

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





Images and Colors in Fiji

The Color menu

单 Fiji	File	Edit	Image	Process	Analyze	Plugins	Window	Help		
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						Cat Cala		longth		



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

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C			Video	Editing	•	Replace	Red with	Maq	ent

p	
夠 🛛 ≫	Image > Color > Channels tools
o search	
	(cmd) + shift + z
℃業Z	
	Interactive visualization/handling of each image channel.
<mark>ት</mark> <mark>አ</mark>	
5	Choose how to display your multicolour image: "Color" mode (single channel) vs
nta	"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







Laurence Haren @HarenLaurence

never forget the scale bar! @StearnsLab when biology meets astronomy: cell vs nebula, centrosome vs dying star! @EtienneKlein



Scale Bar and Pixel Size

...





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



Pixel Size and Scale Bar







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.





Scale Bar and Pixel Size

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

Example

Magnification = 100x Objective

Camera = Hamamatsu Orca Flash 4

Product number	C13440-2	20CU
Imaging device	SCMOS	
Cell (pixel) Size (µm²)	6.5×6.5	
Pixel Array (horizontal by vertical)	2048×204	48
Effective Area (horizontal by vertical in mm)	13.312×1	3.312



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

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



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



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



Input





Semantic

Background

Instance



Background Nucleus 1 Nucleus 2 Nucleus 3

. . .





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.





Thresholding method



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



Thresholding method





Select only a range of digital values in the image.

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




Thresholding method

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.











Thresholding method

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**:

0

0

Image Analysis

Collaboratory

Plugins

- > Labkit
- > Open Current Image With Labkit



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in **Fiji**:

0

0

Image Analysis

Collaboratory

Plugins

- > Labkit
- > Open Current Image With Labkit



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Image
Dimensions: [25
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foreground
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+ Add classifi





Image Analysis Labkit Segmentation

in **Fiji**:

Collaboratory

00

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
🗹 gaussi	an blur (for each sigma)
differen	nce of gaussians (for each sigma)
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Cancel

OK



00

Image Analysis









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Image Analysis











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Image Analysis



Segmentation (\mathbf{a}) #3 - Labkit Pixel Clas... - \mathbf{n}





00

Image Analysis







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4.3 DAPI segmentation with Labkit

Breakpoint at 12: Create results





















Where is Waldo?











Filtering

Neighborhood Transformation

Linear Transformation

Smoothing: Mean, Gaussian Edge detection: Sobel, Canny

Non-linear Transformation

Smoothing: Median Cleaning: Morphology





Weights are defined by the kernels Weighted average = Multiply, add, divide

Convolution is weighted average in local neighborhood

One neighborhood at a time

Neighborhood defined by the kernel size

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	рхЗ	px4	px5	px6	px7	px8	px9			
px10	px11	px12	px13	px14	px15	px16	px17	px18		2 8	a ker
px19	px20	px21	px22	px23	px24	px25	px26	px27		.	
px28	px29	px30	px31	px32	px33	px34	px35	px36	convolution	k1	k2
px37	px38	рх39	px40	px41	px42	px43	px44	px45	\bigcirc	k4	k5
px46	px47	px48	px49	px50	px51	px52	px53	px54		k7	k8
px55	px56	px57	px58	px59	px60	px61	px62	px63			
px64	px65	px66	px67	px68	px69	px70	px 7 1	px72			
px73	px74	px75	px76	px77	px78	px79	px80	px81	ksum = k1 + k2	e + k3 +	- k4 + 1







Convolution operation

Image

px1	px2	рхЗ	px4	px5	px6	px7	px8	px9					(px2 * k1 / ksum) +				
px10	px11	px12	px13	px14	px15	px16	px17	px18					(px3 * k2 / ksum) +	c1	?		
px19	px20	px21	px22	px23	px24	px25	px26	px27		3 >	k 3 ker	nei	(px4 * k3 / ksum) +				
px28	px29	px30	px31	px32	px33	px34	px35	px36	convolution	k1	k2	k3	(px11 * k4 / ksum) +				
										LA.	k5	ke	(px12 * k 5 / ksum) +				
px37	px38	px39	px40	px41	px42	px43	px44	px45		K4	ĸə	ĸŎ				 	
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px55	px56	px57	px58	px59	px60	px61	px62	px63					(px21 * <u>k8</u> / ksum) +				
px64	px65	px66	px67	px68	px69	px70	px71	px72					(px22 * k9 / ksum) =				
px73	px74	px75	px76	px77	px78	рх79	px80	px81	$\mathbf{ksum} = \mathbf{k1} + \mathbf{k2}$	2 + k 3 -	+ k4 +]	k5 + k0	c2 + k7 + k8 + k9				





Convolution operation

Image

px1	px2	рхЗ	px4	px5	px6	px7	px8	px9					(px3 * k1 / k	(sum) +					
px10	px11	px12	px13	px14	px15	px16	px17	px18			-		(px4 * <mark>k</mark> 2 / l	ksum) +	c1	c2	?		
px19	px20	px21	px22	px23	px24	px25	px26	px27		3 x	k 3 ker	nel	(px5 * k3 / k	ksum) +					
px28	px29	px30	px31	px32	px33	px34	px35	px36	convolution	k1	k2	k3	(px12 * k4 /	ksum) +					
									6	k4	k5	k6	(px13 * k5 /	ksum) +					
px37	px38	px39	px40	px41	px42	px43	px44	px45					(px14 * <mark>k6</mark> /	ksum) +					
px46	px47	px48	px49	px50	px51	px52	px53	px54		k7	k8	k9	(px21 * <mark>k7</mark> /	ksum) +					
px55	px56	px57	px58	px59	px60	px61	px62	px63					(px22 * <mark>k</mark> 8 /	' ksum) +					
px64	px65	px66	px67	px68	px69	px70	px71	px72					(px23 * k9 /	' ksum) =					
px73	px74	px75	px 7 6	px77	px78	px 7 9	px80	px81	ksum = k1 + k 2	2 + k3 -	+ k4 +]	k5 + k6	+ k7 + k8 + k9	c3					





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



Different Types of Kernel

0	0	0	0	0	0	0	0	0	
0	-1	0	1	0	0	-1	-1	-1	
0	-1	0	1	Ο	0	0	0	0	
0	-1	0	1	Ο	0	1	1	1	
0	0	0	0	0	0	0	0	0	

1D Prewitt Filter



2D Prewitt Filter



3D Prewitt Filter





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







Horizontal Prewitt Operator



Horizontal Edges



Vertical Prewitt Operator







Different Filters in Action



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





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
		T		



Mean Filter



Gaussian Filter







1 1 1

0	0	0	0	0	0
0	0	-1	0	1	0
0	0	-1	0	1	0
0	0	-1	0	1	0
0	0	0	0	0	0









Sharpening Filter

Prewitt 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







Interactive tools based on classic machine learning



Annotate



Predict





Standalone: Ilastik



For histopathology



https://www.ilastik.org/

https://qupath.github.io/

As a Fiji plugin: Labikit



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







Plugins

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- > Labkit
- > Open Current Image With Labkit



0	•
Im	age
Dir	mensions:
	auto contra
La	beling
	backgro
	foregrou
+	Add label
æ	Add label
Se	gmentation
A	dd segmer






+ Add classifier



Image Analysis Labkit Segmentation

in **Fiji**:

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Collaboratory

Plugins

- > Labkit
- > Open Current Image With Labkit



Use GPU accelerat	tion: (experimental, requires CLU2 and NVIDIA GP	ມ
Dimensions:	2D V	-,
List of sigmas:	1.0; 2.0; 4.0; 8.0	
✓ Basic Filt	ers	
🗹 original	image	
🗹 gaussia	an blur (for each sigma)	
differer	nce of gaussians (for each sigma)	
🗹 gaussia	an gradient magnitude (for each sigma)	
🗹 laplacia	an of gaussian (for each sigma)	
🗹 hessiar	n eigenvalues (for each sigma)	
structu	re tensor eigenvalues (for each sigma)	
in filte	ers (for each sigma)	
max filt	ters (for each sigma)	
mean f	ilters (for each sigma)	
varianc	e filters (for each sigma)	
> Customiz	able Filters	
> Deprecate	ed Filters	
	Canc	el OK



Image Analysis Labkit Segmentation

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Image Analysis Labkit Segmentation

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Collaboratory











Labkit Segmentation

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Image Analysis



Segmentation



#3 - Labkit Pixel Clas... 💌 😳 🕨







Labkit Segmentation

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Image Analysis



Segmentation



#3 - Labkit Pixel Clas... 💌 😳 🕨



















4.3 DAPI segmentation with Labkit

Breakpoint at 12: Create results



Image	Filter	Threshold	Binary Mask	
	No Filter	Labit		Erosion
	Mean 1 1 1 1 1 1 1 1 1	Labrit		Erosion
	Gaussian Blur	Labeit		Erosion
	Median	Labrit		Erosion









Summary







10 //clear the Log at every execution print("\\Clear"); 11 12

13 //get list of files in the input_folder 14 file_list = getFileList(input_folder); 15

16 for (f = 0; f < file_list.length; f++) {</pre> 17

> //get file name filename = file list[f];

18

19

Attps://iac.hms.harvard.edu/

The Image Analysis Collaboratory is organizing two workshops!

Introduction to Image Analysis using ImageJ/Fiji

- January 22nd & January 24th, 1pm-5:30pm
- Search Application Deadline: January 17th
- In Person @ HMS Registration Required
- Mattheway Matter Mat



Introduction to Macro writing in ImageJ/Fiji

- January 28th, 10am-5:30pm
- Application Deadline: January 24th
- In Person @ HMS Registration Required
- <u>https://tinyurl.com/iac-fiji-macro-20250128</u>











Morphological Filters





Two Primary Morphological Operations

Erosion





Dilation





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	о	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	1	1 1	1 1	1	1 1	1 1	1 1	0 1	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 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	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 1	1 1 1	1 1 1	1 1 1	1 1 1 1	1 1 1	1 1 1	1 1 1	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 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	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 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	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 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	1 1 1 1 1 1 0	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1 2	1 1 1 1 1 1 0	0 1 1 1 1 0 0	0 0 0 0 0 0



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0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
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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
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0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0
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0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
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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	0	0	0	0	0	0	0	0	0	0	0	0
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0 0	0 0	0	0 0	0 0	0 0	0 0	1 1	1	1 1	1 1	1 1	1 1	1 1	0 1	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 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	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 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	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 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	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 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	1 1 1 1 1	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 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	1 1 1 1 1 1 0	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1	0 1 1 1 1 0	0 0 0 0 0 0





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0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
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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
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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





0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
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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
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						-	Т	Т	T	1	1	1	1	1	0
0	0	0	0	0	0	1	1	1	1	1	1	1	1 1	1	0
0 0	0 0	0 0	0 0	0 0	0	1	1	1 1	1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	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	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	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	1 1 1 0 0	1 1 1 1	1 1 1 0	0 0 0 0						







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0	1	1	1	1	1	1	1	1	1	о	0	0	0	0	0
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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	1 1	1 1	1 1	1 1	1 1	1 1	1 1	0 1	0 0						
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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 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	0 1 1 1	0 0 0 0
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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
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0









Erosion - The Movie







Dilation Operation

 \bullet

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0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
0	0	0	1	1	1	0	0	0	0	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	1	0
0	0	0	0	0	0	1	1	1	0	1	0	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	0	0	0	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







Dilation Operation















Image Analysis Collaboratory

Dilation Operation











0	0	0
0	0	0
0	0	0



Dilation Operation











Dilation - The Movie









But wait, there is a problem.





Together We Stand

Main image



Dilated image



Eroded image



Main image



Closed 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







Circular structuring element



Ο





 \rightarrow









Structuring Element Shape







Circular structuring element



Ο





 \rightarrow







BE CAREFUL!











Morphology in Fiji

Process Analyze Plugins	Window	Help
Smooth	☆雑S	
Sharpen		
Find Edges		
Find Maxima		
Enhance Contrast		
Noise	>	
Shadows	>	
Binary	>	Make Binary
Math	>	Convert to Mask
FFT	>	Frode
Filters	>	Dilate
Batch	>	Open
Image Calculator		Close-
Subtract Background		Median
Repeat Command	96 R	Outline
Calculator Plus		Fill Holes
Morphology	>	Skeletonize
Image Expression Parser		Distance Man
Image Expression Parser (Macro)	Ultimate Dainte
Multiple Image Processor		Watershad
Enhance Local Contrast (CLAHE)	Voronoi
		Options

	Process Analyze
	Smooth
	Sharpen
	Find Edges
	Find Maxima
	Enhance Contrast
	Noise
	Shadows
	Binary
	Math
	FFT
	Filters
	Batch
	Image Calculator
	Subtract Background.
	Repeat Command
	Calculator Plus
1	Morphology
	Image Expression Pars
	Image Expression Pars
	Multiple Image Proces
	Enhance Local Contra
-	



• • •	Parameters		
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Tvr	e of structure element	circle ×	1
.,,		enere	l.
Show mask	:		_
	Operator	erode ~	
		erode	OK
		onen	OK
		close	
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		rast open	
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This plugin p Radius of the stru Typ	Parameters erforms morphology op octure element (pixels): be of structure element Operator	fast close fast close erators on grav 1.0 circle diamond square hor line ver line 2p h 2p v	ylevel imag







Morphology — exercises



3.4 - Morphological filters - Binary3.5 - Morphological filters - Gray



ROI Manager





Annotation Tools *see also Edit > Selection

Region Of Interest (ROI)







Secondary/Right Click for more options

ess	Analyze	Plugins	Window	Help
(Fiji Is	s Just) Imag	leJ		
Q	٩ ښ	Dev_	nnota	tion Tools
0_66	(64-bit)			

S	Analyze	Plugins	Window	Help		
Ju	st) ImageJ					
2	<n alig<="" aligned.com="" http:="" td="" www.aligned.com=""><td>Dev Stk</td><td>Lut 👌</td><td>1 3</td><td>≫</td><td></td></n>	Dev Stk	Lut 👌	1 3	≫	
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° «)	1.2.					
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	3.4.1.8					



Double Click to set/change properties



"Analyze" menu



ROI Manager

Fiji File Edit Image Process Image Image Image Image Image Image	Analyze Plugins Measure	Window H	lelp	Analyze >	> Tools >	ROI Ma	nager
(Fiji Is Just) ImageJ 2.1.0/1.53c; Java 1.8.0_66 [C2-hela-cells.tif (50%) "Green"; 34.60x25.03 μm (672x486); 16-bit; 6	Analyze Particles Summarize Distribution Label Clear Results Set Measurements Set Scale	here	te to search	The ROI Mo with m	t anager is	s a tool for lections (F	* working
Jer Strate	Calibrate Histogram Plot Profile Surface Plot Gels	ж К >			ROI Mar 0249-0127 0220-0513 0159-0291 0393-0346	Add [t] Update Delete	(C 15 <u>)</u> .
	Tools 3D Objects Counter 3D OC Options Skeleton Colocalization Color Histogram Directionality	> Sav Fra An Cu RO Sca Ca Syr	actal Box Count actal Box Count alyze Line Graph urve Fitting OI Manager cale Bar alibration Bar ynchronize Windows			Rename Measure Deselect Properties Flatten [F] More » Show All Labels	

*(*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.


"Analyze" menu





ROI Manager

Add new ROI (†).

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.



"Analyze" menu





ROI Manager





"Analyze" menu

"Edit" menu





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







"Image" menu



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

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



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

Set ROI(s) label options

🗯 Fiji	
(Fiji Is Just) ImageJ	
	0 1 3 >
(Fiji Is Just) ImageJ 2.1.0/1.53c; Java 1.8.0_66 [64-bit]; C	lick here to search
C3-hela-cells.tif (50%)	lanager
34.60x26.36 μm (672x512); 16-bit; 672K ROI_1	Add [t]
ROI_2 ROI_3	Update
ROI_4	Delete
	Rename
ROI_2 ROI_1	Measure
Color: yellow 📀	Deselect
ROI 3 Font size: 24 😒	Properties
	Flatten [F]
ROL 4 Show labels	More »
Use names as labels	Show All
✓ Draw backgrounds	✓ Labels
Bold	
Cancel OK	





Live Demo of ROI manager



And

Segmentation Measurements









Export and Measure





X







Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
B C C C C C C C C C C	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.
	24 20 33 27	ED	













Image Analysis Solution: Watershed

in Fiji: Process > Binary > Watershed

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

Image





Process and segment

Binary Mask



Watershed





Solution: Watershed

Binary mask





0





Process and segment

Distance transform











From binary image to instance segmentation



Binary mask



Instance segmentation





in Fiji: Analyze > Analyze Particles...







in **Fiji**: Analyze

> Analyze Particles...







in Fiji: Analyze

> Analyze Particles...









in **Fiji**: **Analyze**

> Analyze Particles...







in **Fiji**: **Analyze**

> Analyze Particles...







in **Fiji**: **Analyze**

> Analyze Particles...







Analyze particles: Select for morphology

in **Fiji**: **Analyze** > **Analyze Particles...**



ize (pixel^2):	0-In	finity		
Circularity:	0.00	-1.00		
Show:	Noth	ing	\sim	
Display res	ults	Excl	ude on ed	ges
Clear result	5	Inclu	ude holes	
Summarize		Over	rlay	

Process and segment



Circularity







Image Analysis Analyze particles: Size

in Fiji: Analyze > Analyze Particles...

ize (pixel^2):	0–Infinity
Circularity:	0.00-1.00
Show:	Nothing ~
Display res Clear result	ults Seclude on edges

Size range of the particles that you want to detect.









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







Analyze particles: Circularity

in Fiji: Analyze > Analyze Particles...

Size (pixel^2):	0–Infinity
Circularity:	0.00-1.00
Show:	Nothing ~
Display rest	ults 🗹 Exclude on edges
Clear result	s Include holes
Summarize	Overlay
Add to Man	ager Composite POIs



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





Analysis Analyze particles: Circularity











Image Analysis Collaboratory Analysis















Analysis Analyze particles: Circularity

















Analyze particles: Exclude on Edges

in Fiji: Analyze > Analyze Particles...

ize (pixel^2):	0-In	finity		
Circularity:	0.00	-1.00		
Show:	Noth	ning	Ý	
Display res	ults	Exc	lude o	n edge
Clear result	5	Inc	lude ho	oles
Summarize		Ov	erlay	
		Dr.		- DOI-

Process and segment

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













Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 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.
	24 20 33 37	63) 650	







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

Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
⁸ 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.
	24 20 33 37	E D	







Analyze particles: Save the segmented image

in Fiji: Analyze > Analyze Particles...

Anal	lyze Particles	
Size (micron^2):	0–Infinity nits	
Circularity:	0.00-1.00	
Show:	Count Masks	
 Display result Clear results Summarize Add to Manage 	Nothing Overlay Overlay Masks Outlines Bare Outlines Ellipses	edges es ROIs
Help	Masks	ik
	Count Masks	

Export



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





Analyze particles: Add to ROI Manager

in Fiji: Analyze > Analyze Particles...

Size (micron^2): 0-In	finity
Pixel units	
Circularity: 0.00	-1.00
Show: No	othing
Display results	Z Exclude on edges
Clear results	include holes
Summarize	Overlay
Add to Manager	Composite ROIs

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

ROI M	anager
	Add [t]
	Update
	Delete
	Rename
	Measure
	Deselect
	Properties
	Flatten [F]
	More »
	Show All
	Labels





Analyze particles: Add to ROI Manager

in Fiji: Analyze > Analyze Particles...

Size (micron^2): 0-1	nfinity
Pixel units	
Circularity: 0.0	0-1.00
Show: N	othing 😂
Display results	Exclude on edges
Clear results	Include holes
Summarize	Overlay







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

Export and Measure

	Results		
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
B C C C C C C C C C C	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.
	24 20 33 37	63) 65)	





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

Export and Measure

	Results		
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
B C C C C C C C C C C	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.
	24 20 33 37	63) 65)	





0

Collaboratory

Analyze > Set Measurements...

	aromonto
🗹 Area	🗹 Mean gray value
Standard deviation	Modal gray value
Min & max gray value	Centroid
Center of mass	Perimeter
Bounding rectangle	Fit ellipse
Shape descriptors	Feret's diameter
Integrated density	Median
Skewness	Kurtosis
Area fraction	Stack position
 Limit to threshold Invert Y coordinates Add to overlay 	 Display label Scientific notation NaN empty cells
Redirect to: D/	API_14.tif 🗸
Decimal places (0-9): 9	
Help	Cancel OK

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

Ú	Fiji					
	@	(Fiji	Is Just) ImageJ			
ļ			Q. ₹™ ₫ Dev	Stk LUT	0 8	ð 🔍
=109	0.20 (336), y=15	.60 (48), value=0			Click here to	search
6 1	DAPI 14	tif (33 3%)		osek (22	20/1	
'- D	API.nd2 (series	14)"; 260.00x260.00) mi "– DAPI.nd2 (se	eries 14)";	260.00x26	50.00 mi
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	•		9			
	1 N I I I		•	0	9	
				17 L		
					9	21
				9		
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			•			•
			B	14		
					G	
9			4 2			
100	lister	h Ause	results	A.C.		
1		Area	Mean	MIN 2270	Max 7102	
2	DAPI 14 Hif	142 805007140	6100 802004092	3275	0/195	
2	DAPI 14 tif	173 964383608	8027 766848816	2965	12624	
4	DAPI 14 tif	143 438757172	5482 965390280	2305	7660	





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, ...)

• • •	Set Me	easur	ements	
	Redirect to:	DAP	l_14.tif ∨	
Decimal	places (0-9):	9		
	Help	, 11	Cancel	ОК

Export





Manage Analysis Side note: other kinds of measurements

Analyze > Plot Profile

00

Collaboratory

(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

Set Meas	urements
📝 Area	🗹 Mean gray value
Standard deviation	Modal gray value
Min & max gray value	Centroid
Center of mass	Perimeter
Bounding rectangle	Fit ellipse
Shape descriptors	Feret's diameter
Integrated density	Median
Skewness	Kurtosis
Area fraction	Stack position
Limit to threshold	Display label
Invert Y coordinates	Scientific notation
Add to overlay	NaN empty cells
Redirect to: DA	API_14.tif 🗸
Decimal places (0-9): 9	
Help	Cancel OK

e e Ana	lyze Particles
Size (micron^2):	0–Infinity hits
Circularity:	0.00-1.00
Show:	Overlay Masks 🖂
 Display result Clear results Summarize Add to Manage 	er Composite ROIs
Help	Cancel OK

Export





Image Analysis Collaboratory

Measure — Option 3: Using the ROI manager



- 1. Select the "ORIGINAL" image.
- 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 <u>"Analyze Particle" step</u> form <u>ONE</u> of the workflow exercises below.

4.1 DAPI segmentation with thresholding

<u>OR</u>

4.3 - DAPI segmentation with Labkit



Spot Detection (Point tool and Find Maxima)



"Edit" and "Analyze" menu



Point and Multi-Point tool

Select and Measure multiple points

Mouse right-click

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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







IAC Website - iac.hms.harvard.edu



About - Si

Support • Research •

Resources - Co

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Welcome to the Image Analysis Collaboratory

at Harvard Medical School

We research, develop, and apply algorithms to analyze scientific images. We also offer workshops, consultations, and project support in matters quantitative bioimage analysis. Funded by the Foundry, we collaborate with any department of the school (though mainly Quad-based pre-clinical) and work closely with the local microscopy facilities.

Announcements

- To Workshop: Introduction to Image Analysis using ImageJ/Fiji
- Stress Workshop: Introduction to Macro writing in ImageJ/Fiji
- Contraction to Bioimage Analysis using QuPath



We are here to help you consult regarding your data and data analysis needs.



Collaborate with Us

Come talk to us about how we can work together on a project.

By Collaboratory we mean,

"center without walls, in which [...] researchers can perform their research without regard to physical location, interacting with colleagues, accessing instrumentation, sharing data and computational resources, [and] accessing information in digital libraries." - (Wulf, 1989)

People

Principal Investigator



Researchers







Aaria Theiss Specialist Postdoc





*		Image Analysis Collaboratory	*	^	About 🝷	Support •	Research -	& Resources ▼	Contact	
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Image Analysis Consultation

IAC offers two tailored consultation options for individuals seeking assistance with image analysis. Depending on your needs and affiliation, choose the service that best aligns with your goals.

Image Clinics: Comprehensive, hour-long sessions focused on addressing complex image analysis challenges with one or more IAC experts.

* Walk-ins: Quick, 20-minute sessions designed to resolve straightforward issues such as script troubleshooting or software installation.

Example 1: I have a set of time-lapse images and need to track each cell to determine the length of the cell cycle. As a beginner in bio-image analysis, I am unsure where to start. --> Book an Image Clinic.

Example 2: I built an image analysis pipeline in Fiji but need help writing a macro to apply the same pipeline to all images. --> Visit a Walk-in.

More details about these services are provided below. These consultations are intended to guide you through your image analysis needs and introduce you to the field of bio-image analysis. While they may not completely solve your issues, they serve as an excellent starting point. For those seeking in-depth analysis or collaboration on long-term projects, please visit our <u>collaborate</u> page.

III Image Clinics

Purpose: These clinics are ideal for users who require significant guidance on designing workflows, troubleshooting advanced analysis, or identifying the right tools and techniques for their projects. They are particularly suited for those working on intricate datasets or novel research questions.

Preparation: Participants are expected to prepare a concise presentation outlining their project, key challenges, and goals. Additionally, representative images or datasets should be brought to the session.

Data: Please bring sample data that reflects your project's scope, even if it is not your own. This helps us provide practical and actionable recommendations.

Length: Usually an hour or longer, depending on the complexity of the problem.

Available for: Open to everyone (HMS, Harvard affiliates, and industry partners).

Fees: Varying* (based on affiliation and session requirements).

Book an Image Clinic

🖈 Image Analysis Walk-ins

Purpose: Walk-ins are ideal for resolving straightforward questions, such as setting up software, troubleshooting scripts, or addressing specific image analysis workflows. They are also suitable for users who need assistance with technical challenges like writing Fiji macros or using Python-based tools.

Preparation: Usually, no extensive preparation is needed. However, if it is your first visit, we recommend preparing a brief overview of your problem to make the session more effective.

Data: Bringing representative example data can help us better understand your problem and provide actionable guidance.

Length: Each session is ~20 minutes, allowing for focused and efficient problemsolving.

Available for: HMS Quad A (only).

When: Every Monday to Thursday from 4pm to 5pm

Where: LHRRB Room 105

Fees: Free*

*Note: Thanks to the generous funding provided by the <u>Harvard Medical School Foundry</u> award program, we are able to provide some of these services for free for all <u>HMS Quad A</u> researchers.





Further Learning (<u>https://iac.hms.harvard.edu/resources/</u>)





https://forum.image.sc/ \bigcirc

https://bioimagebook.github.io/ \bigcirc

Online training: NEUBIAS Academy

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

Fiji manual from Monash University

https://bridges.monash.edu/articles/educational resource/Fiji Training Manual v6 4 /20033513 \bigcirc

Biolmaging North America (BINA)

https://www.bioimagingnorthamerica.org/







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







Feedback Form - Thanks!

Every course is shaped by the ones before it. We ask you to pay it forward by telling us frankly what did and didn't work this time-it will help the next students.

Please take a few minutes to fill out the feedback form.

