上課注意事項:

1. 教室內禁止攜帶食物飲料入內, 僅允許“白開水”, 請大家將食物飲料放置於教室外的桌上。
2. 請實體與線上學員掃描以下QR code進行線上簽到。
3. 請線上學員於課程開始前關閉自己的麥克風。
4. 線上學員若有問題, 請先按下“舉手”, 或於聊天室寫下問題, 將於課程結束後在場地時間允許下, 安排QA時間。
5. 現場學員發問時請使用麥克風才可進行收音。

線上簽到

課程材料與資訊連結

IMAGEJ 顯微影像分析與程式設計

生物影像分析概論

Jung-Kun Wen

Assistant Research Specialist

Bio-Imaging Core Facility, Institute of Biological Chemistry, Academia Sinica

East Asia Bioimage Analysts' Society (EABIAS)

https://eabias.github.io/zh-TW/

Outline

- Part I : Introduction of Bioimage & Bioimage analysis
  - Terminology & Image preparation tips
- Part II : Bioimage analysis workflow
  - Processing & Image Operators
- Part II : Open-source tools for Bioimage analysis - (Fiji)

Part of this document is adapted from the following material under CC BY 4.0

Dr. Robert Haase
Center for Scalable Data Analytics and Artificial Intelligence (ScaDS.AI) Dresden/Leipzig, Leipzig University, Germany
https://haesleinhuepf.github.io/

Dr. Wei-Chen Chu, ICOB
Imaging Core Facility Manager (ICOB)

https://www.youtube.com/@icobcorefacilitiesacademia43/featured

Dr. Peter Bankhead
Center for Genomic & Experimental Medicine, University of Edinburgh, UK

Introduction to Bioimage Analysis (2022)
https://bioimagebook.github.io/

BioImage Analysis Workshop
Huang, Cheng-Yu (Kou)
Ph.D. Candicate
University of Cambridge
https://www.youtube.com/watch?v=PSyxeUaw67g
臺大顯微影像核心 **NTU Imaging Core**
https://www.youtube.com/@imagingcore9084

Part I:

Introduction of Bioimage & Bioimage analysis
- Terminology & Image preparation tips

Bioimage

Why Bio-imaging technology is so important to modern scientific research?

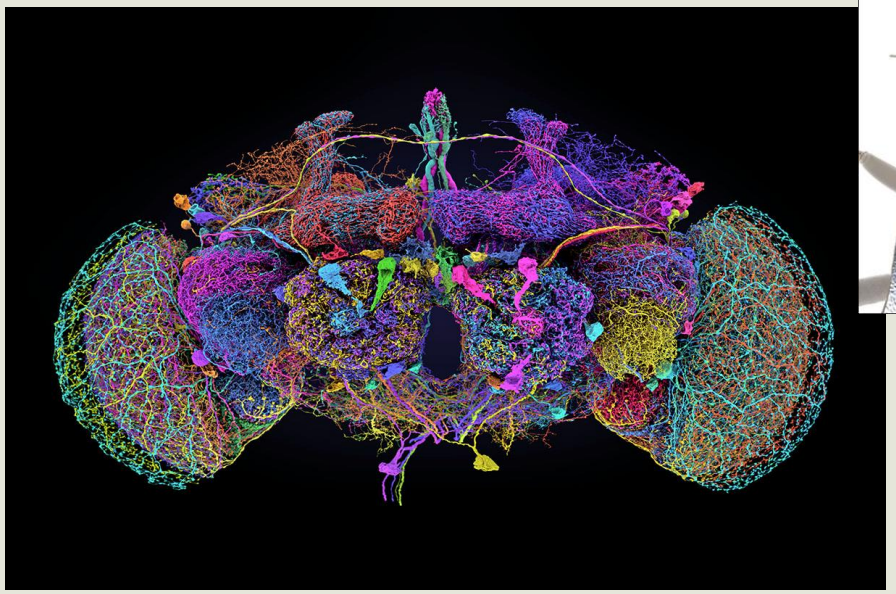
Explain phenomenon ?

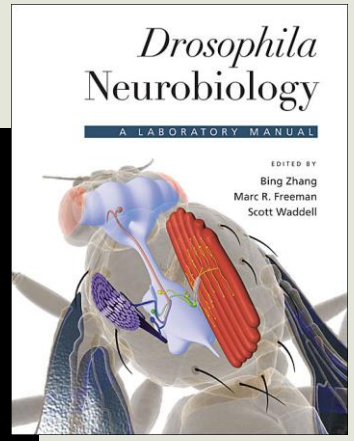
Awesome image?

Make records ?

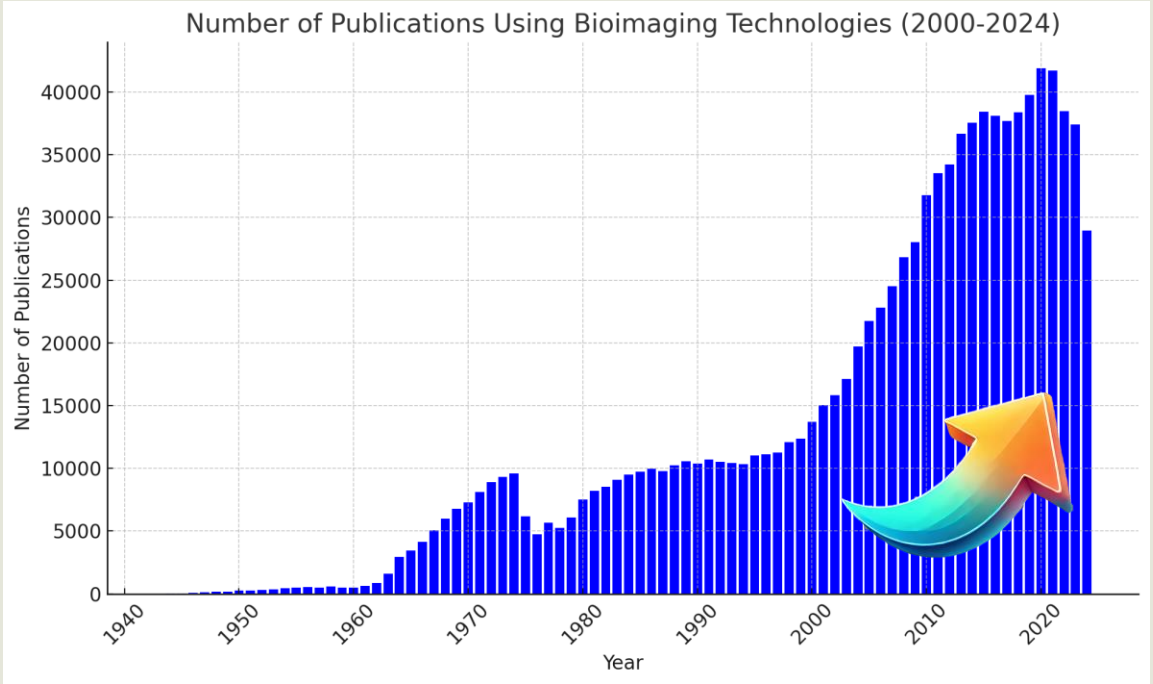
Generate data ?

Visualization and Quantification in Life Sciences





The 50 largest neurons in the fly's brain, Dorkenwald et al., Nature, 2024

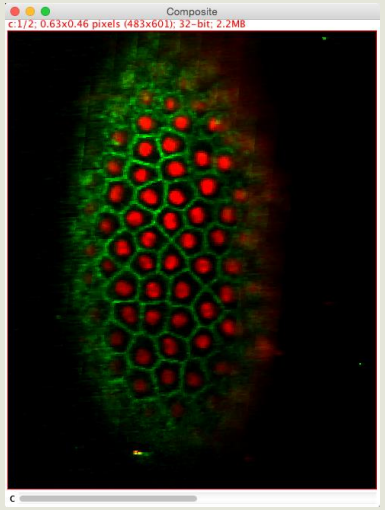


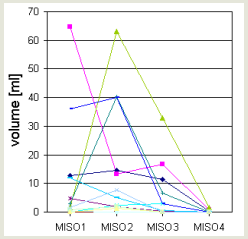
PubMed

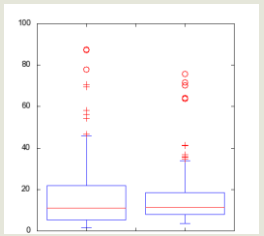
Bioimage analysis

Bioimage Analysis?
Understanding and quantifying microscopy, medical or any other calibrated image data.

Bioimages → Numbers → Knowledge





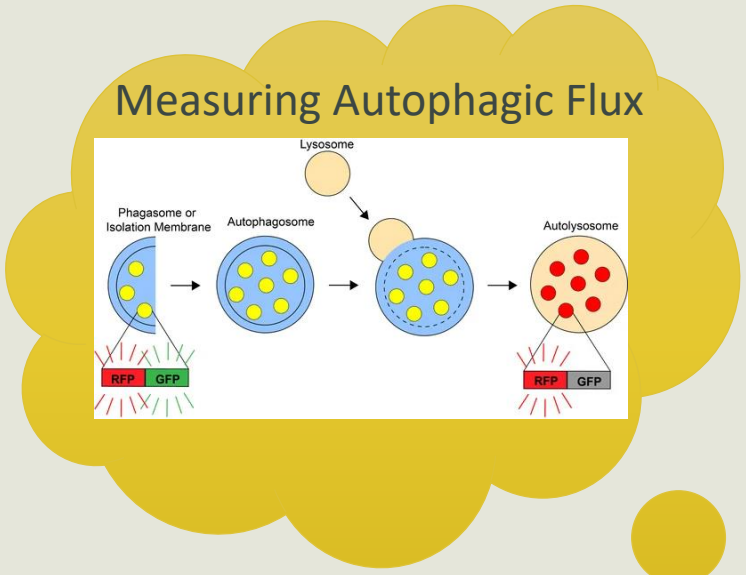


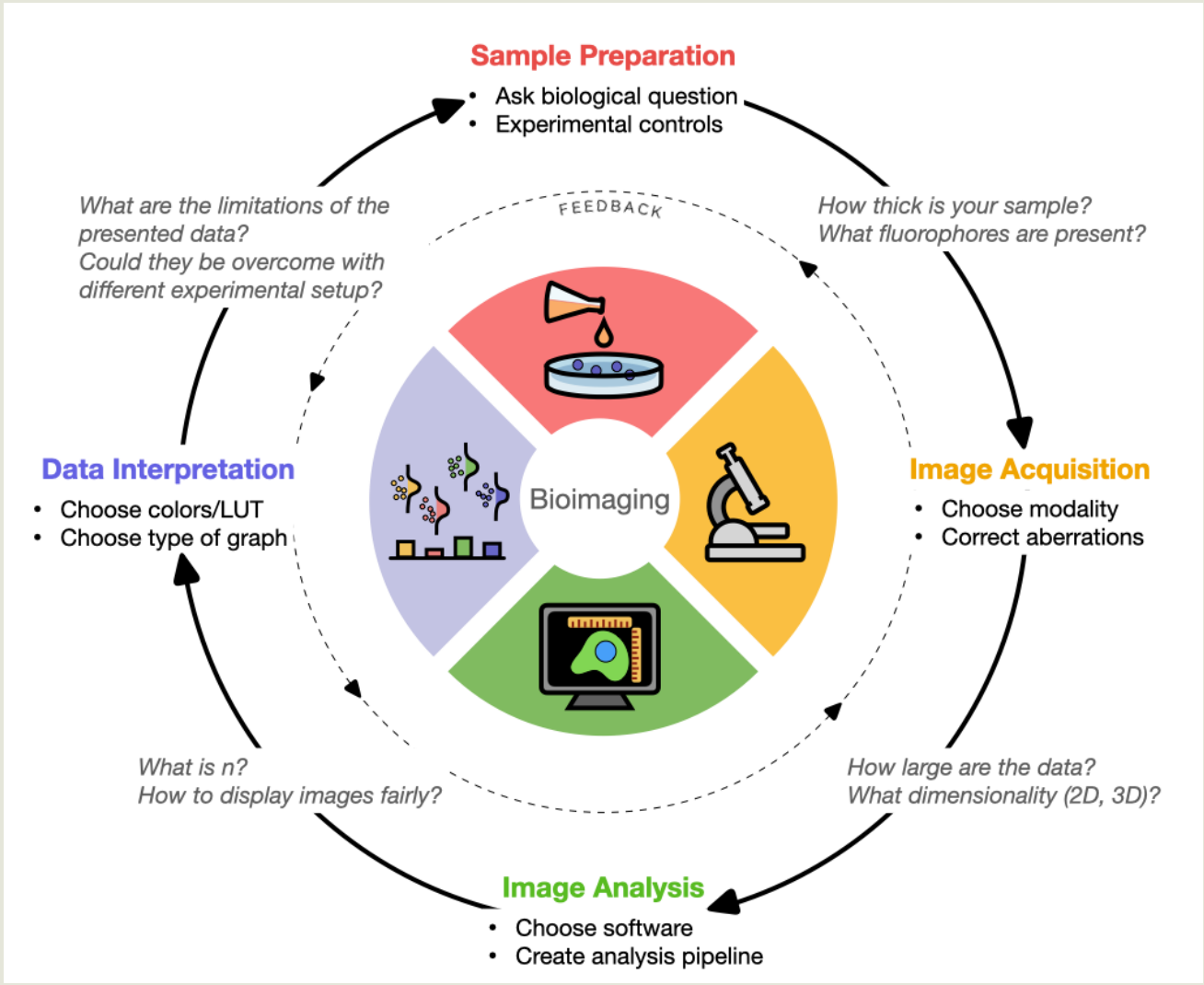
Publications.

Image analysis software universe

Image from https://forum.image.sc/

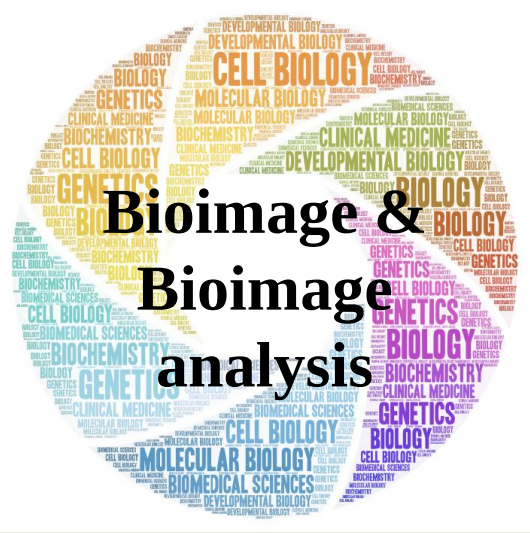
The steps of bioimaging experiment





https://www.bioimagingguide.org/
https://doi.org/10.1371/journal.pbio.3002167

Bioimage & Bioimage analysis



- Tools
- Part of Experiment
- Knowledge

Support your research.

Checklists of Bioimage analysis in publications.

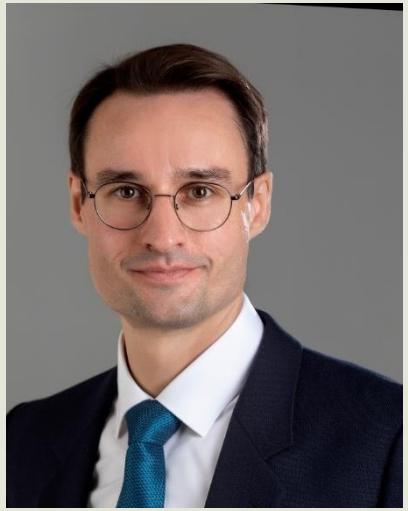
nature methods

Perspective

https://doi.org/10.1038/s41592-023-01987-9

Community-developed checklists for publishing images and image analyses

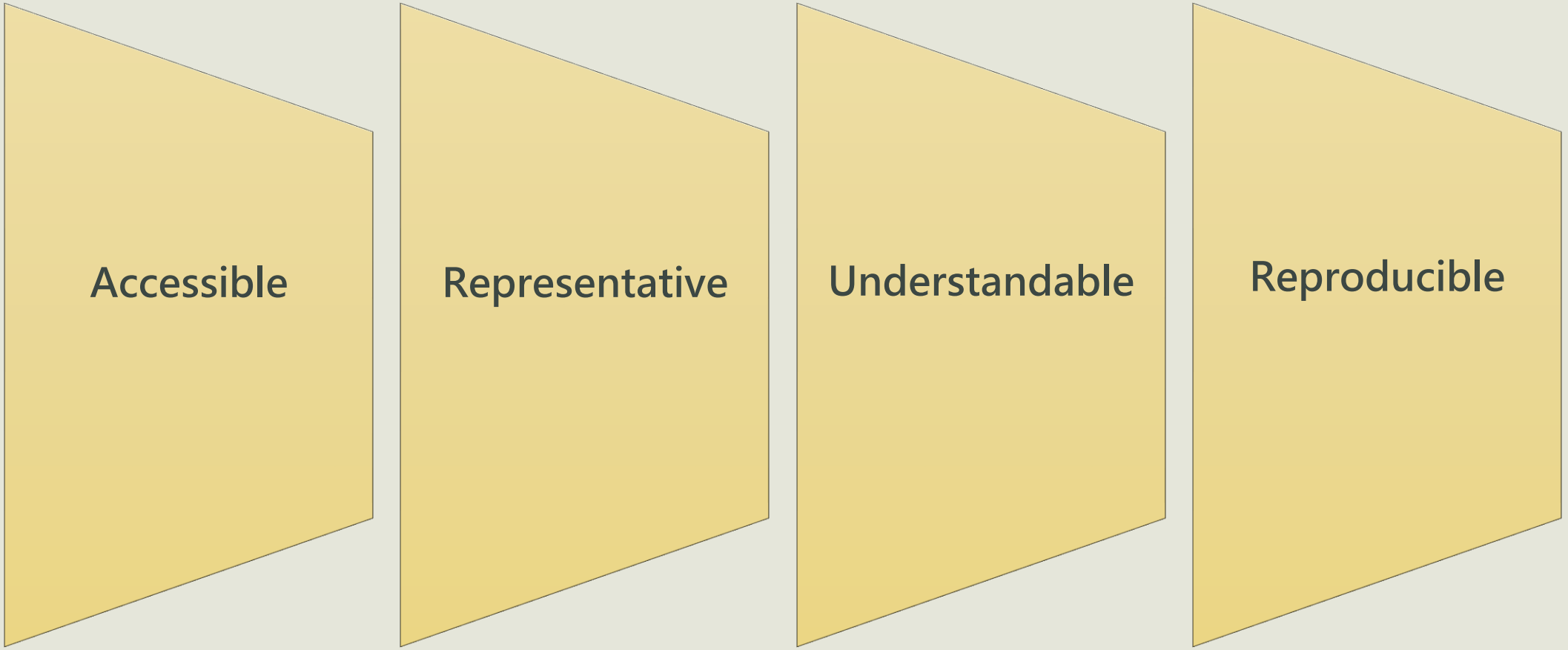
Nature Methods | Volume 21 | February 2024 | 170–181



Christopher Schmied
Senior Research Data Scientist at Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)

Helena Klara Jambor
Mildred Scheel Early Career Center, TU Dresden (Germany)

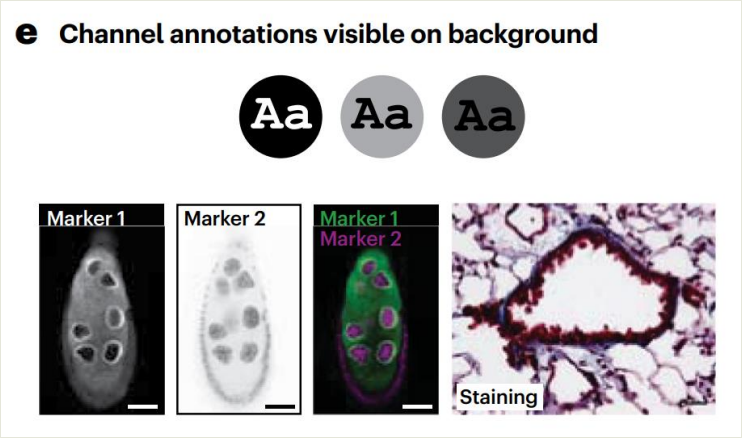
Four elements of Bioimage analysis in publications.



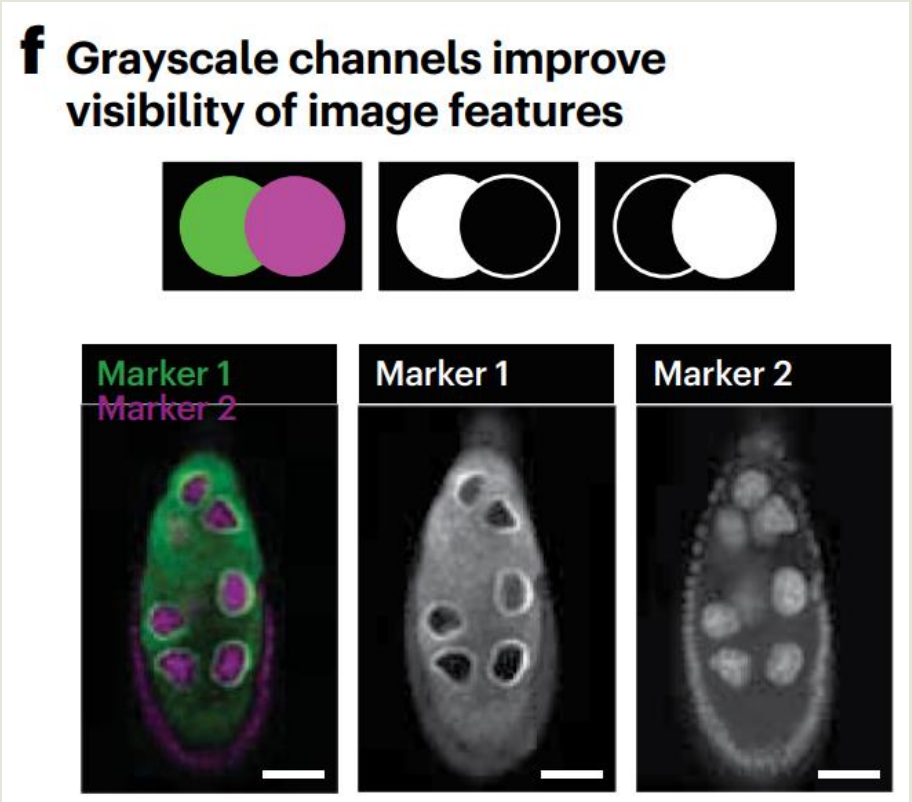
Nature Methods | Volume 21 | February 2024 | 170–181

Four elements of Bioimage analysis in publications.

Accessible



Be friendly to colorblindness

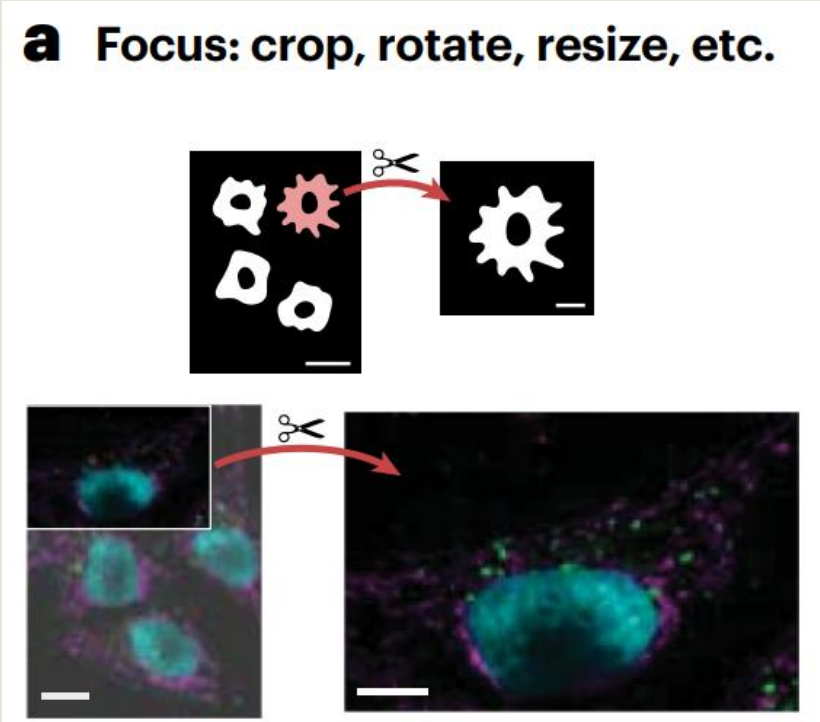


Red/Green only: ➔ Replace Red with Magenta

Nature Methods | Volume 21 | February 2024 | 170–181

Four elements of Bioimage analysis in publications.

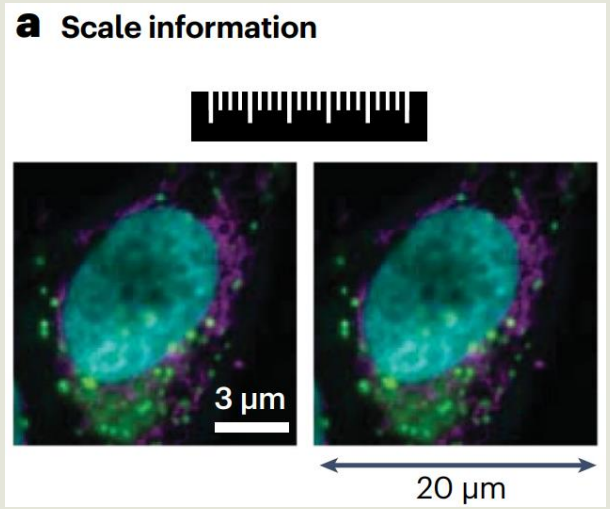
Representative

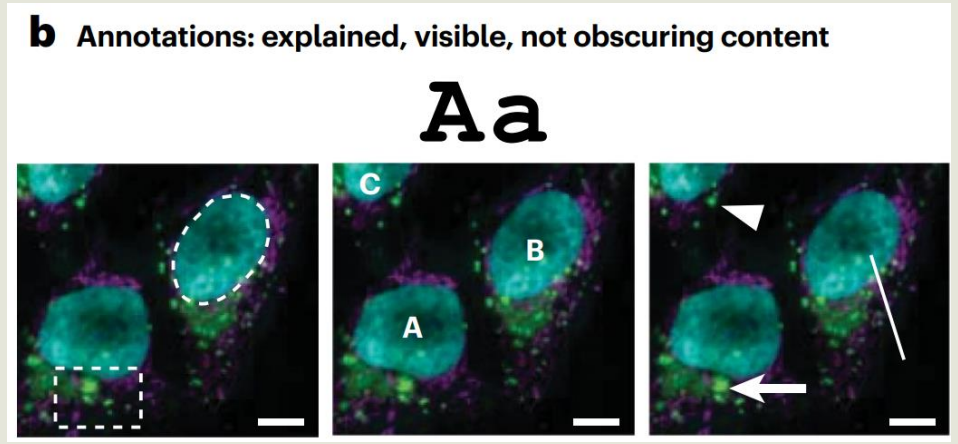


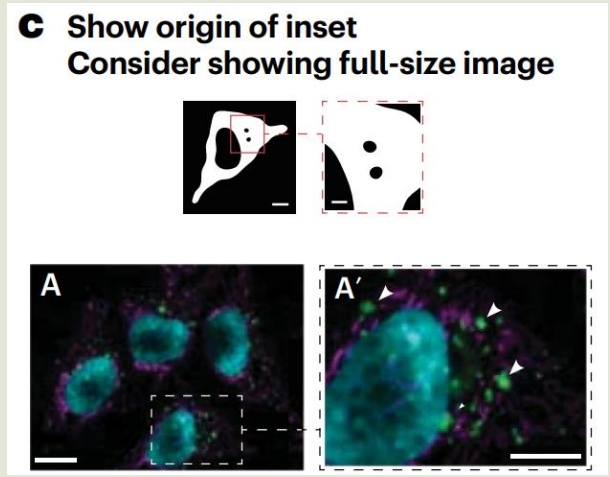
Nature Methods | Volume 21 | February 2024 | 170–181

Four elements of Bioimage analysis in publications.

Understandable





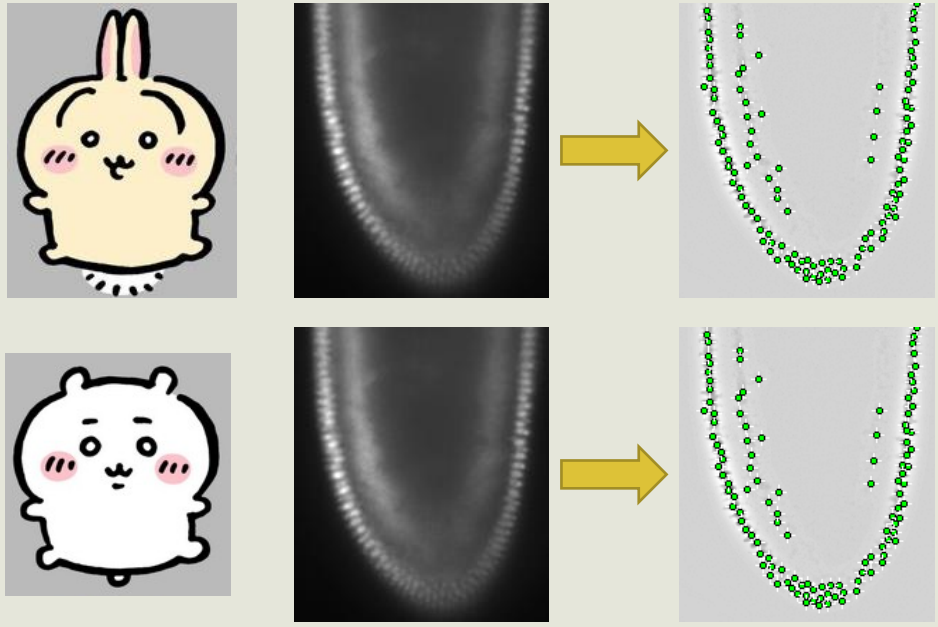


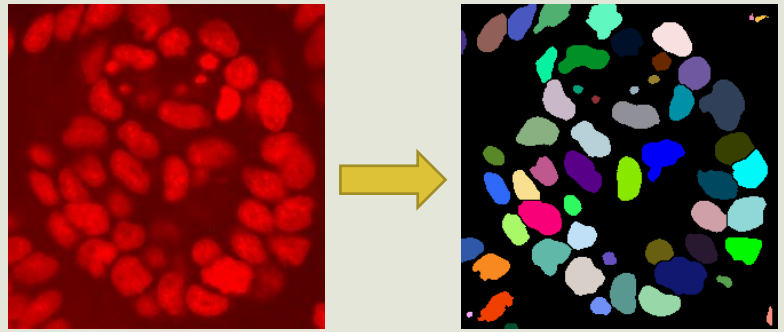
Nature Methods | Volume 21 | February 2024 | 170–181

Four elements of Bioimage analysis in publications.

Reproducible

- Bio-image analysis is supposed to be **reproducible**
  Experiments have to be documented so that others can repeat them.
- Bio-image analysis is supposed to be **repeatable**
  The experiment must be designed so that one can repeat it with the same sample.





Checklists of image analysis in publications.

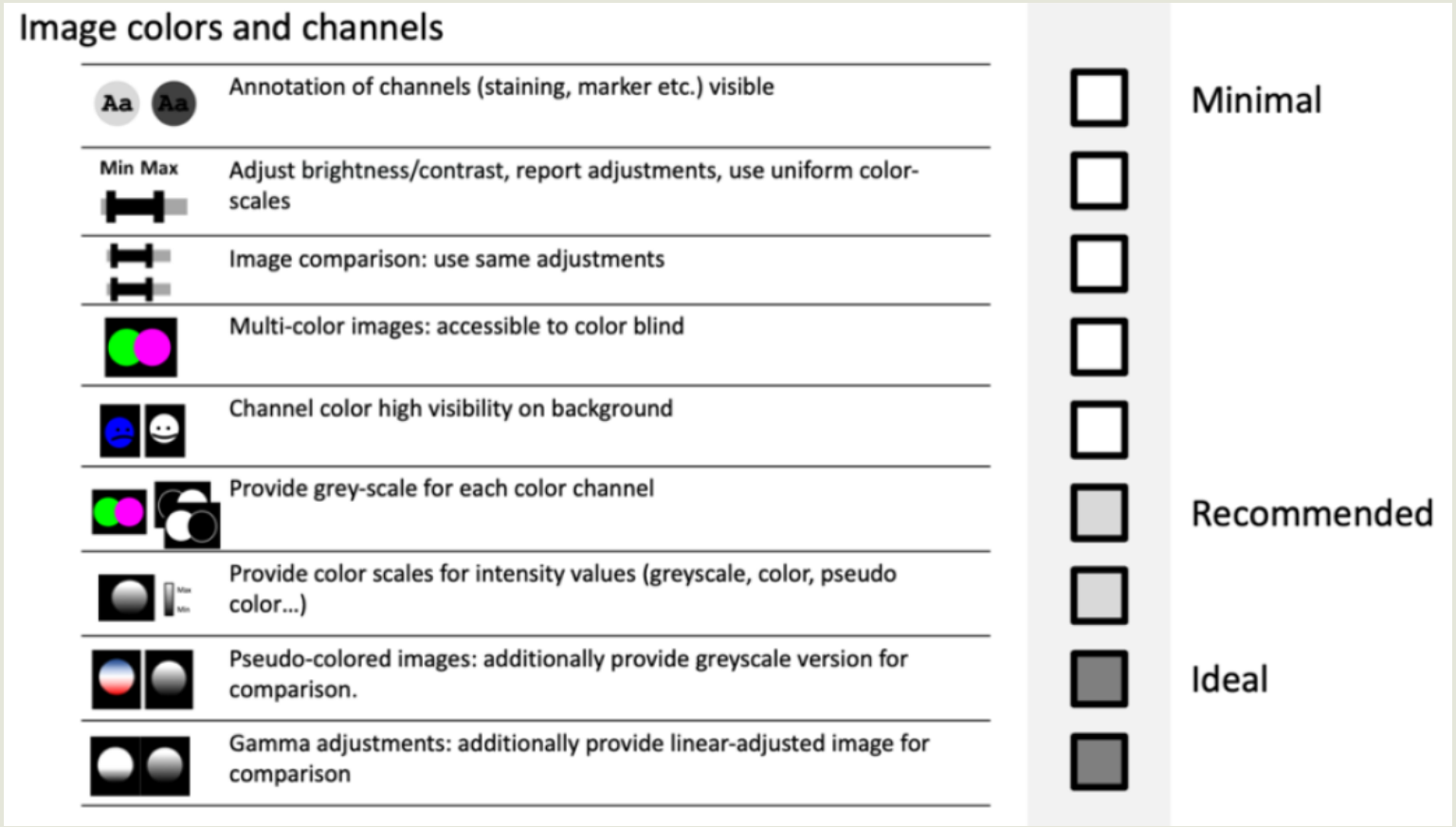
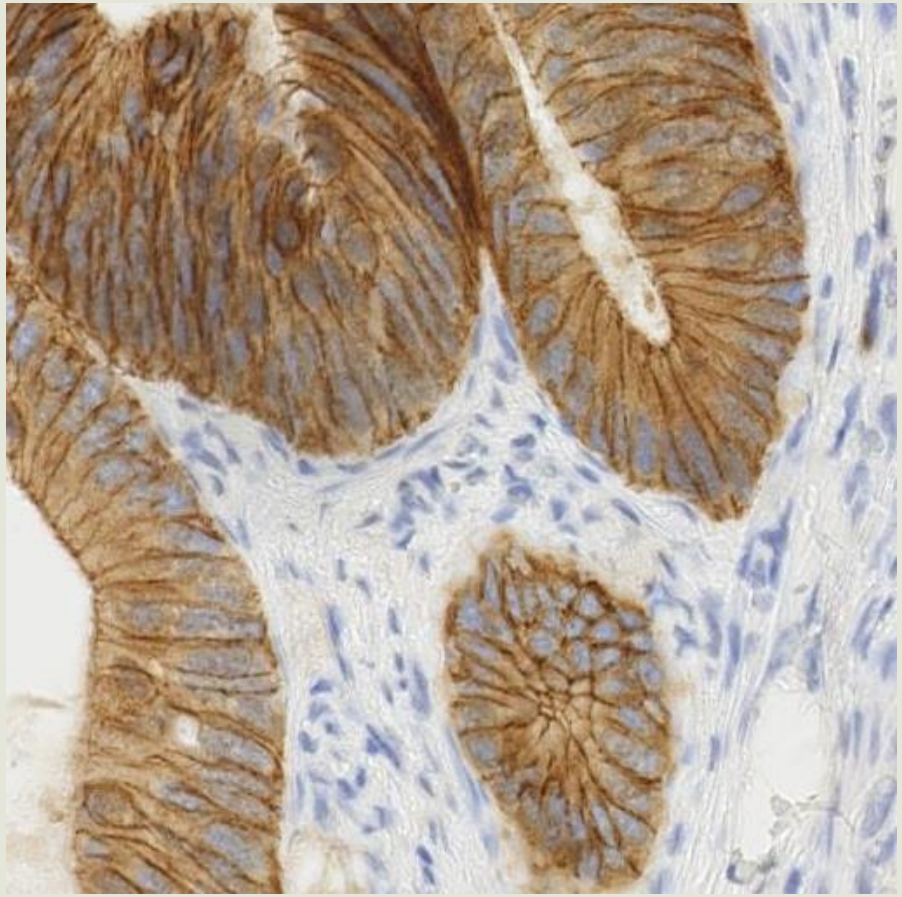


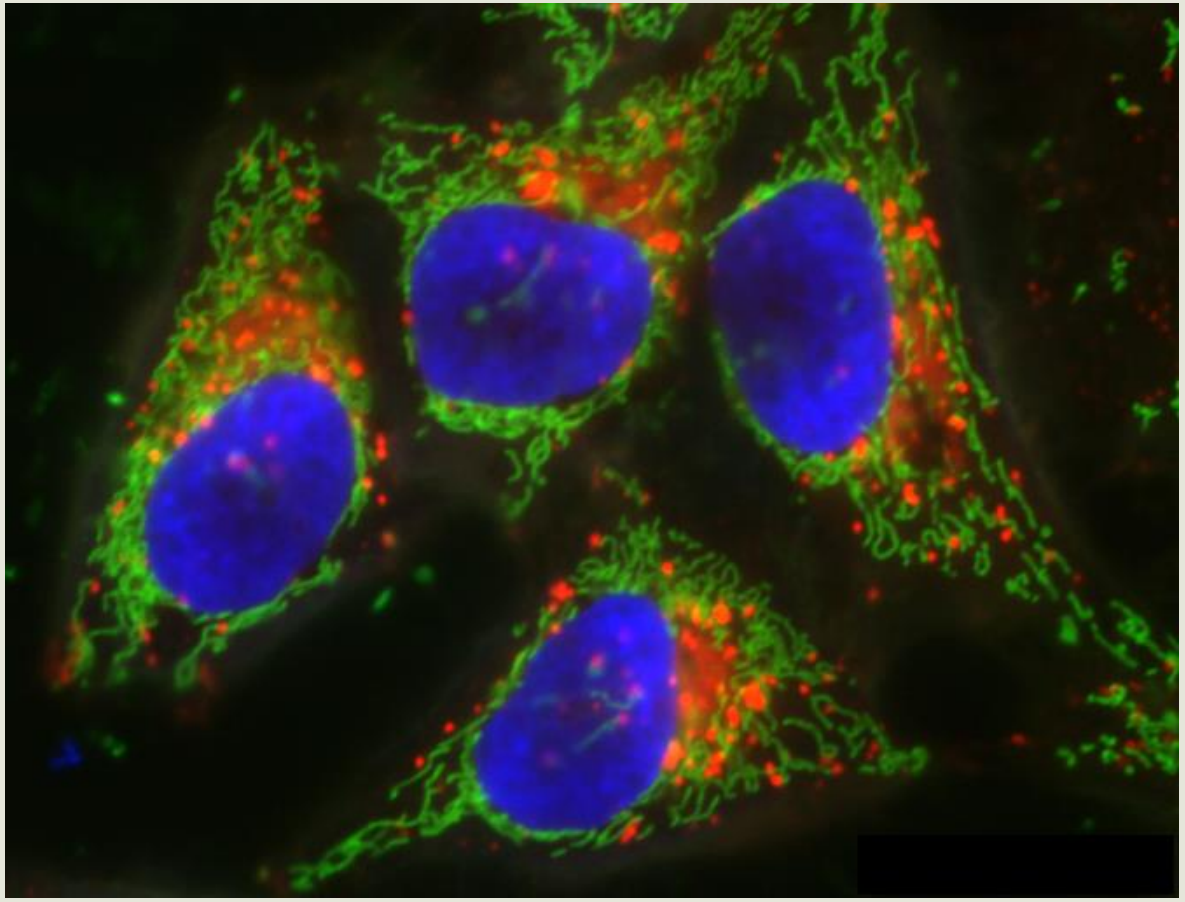
Image source: Schmied et al., (2023) , licensed CC-BY-SA 4.0

Nature Methods | Volume 21 | February 2024 | 170–181

Color Image vs. Intensity-based Image (e.g. fluorescence images)



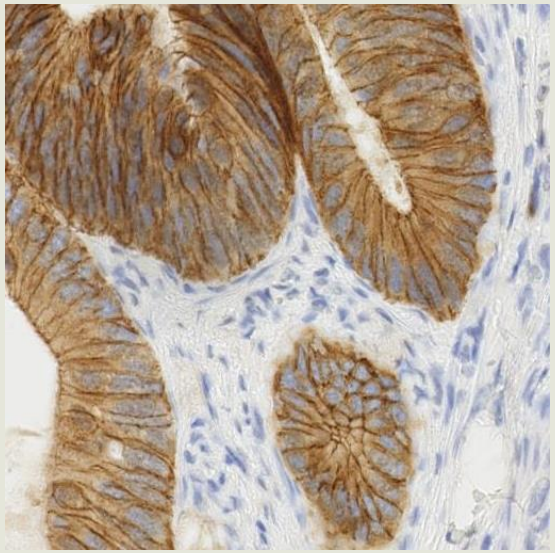
Transmitted light microscope

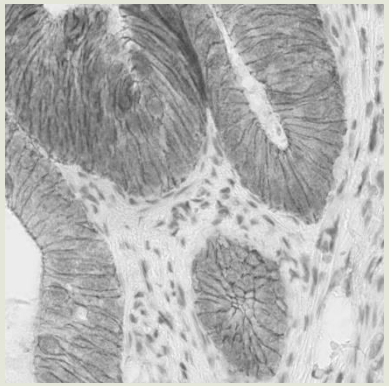


Fluorescence microscope

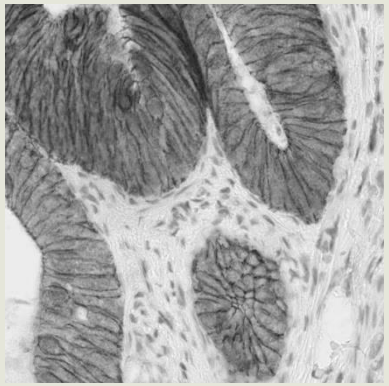
Image Source: Napari sample image (immunohistochemistry), FIJI sample images (Hela cells)

Color Image (RGB format)

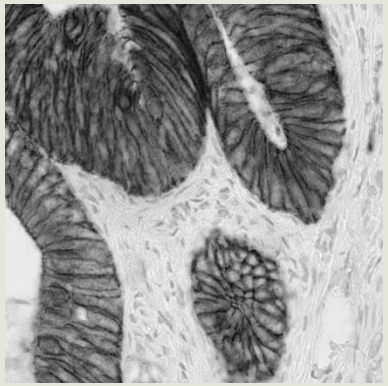




R

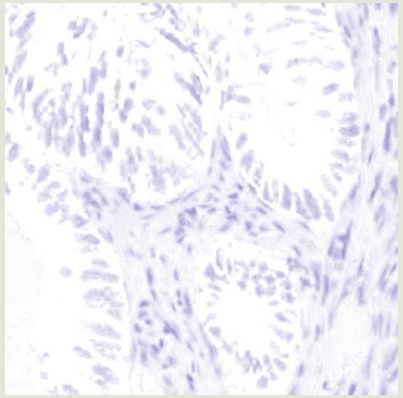


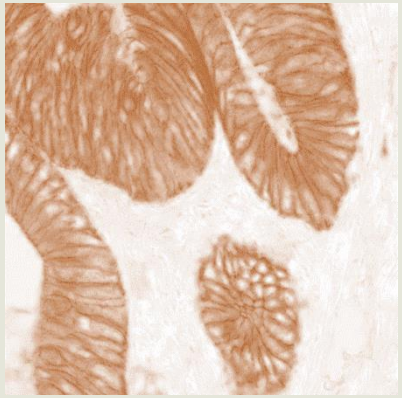
G

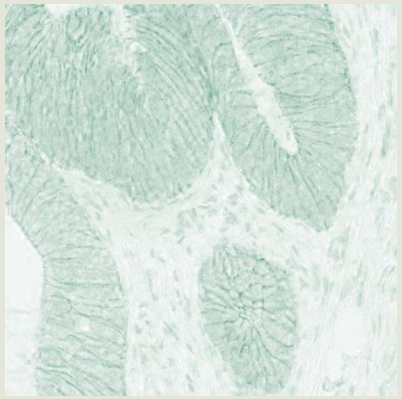


B

Color deconvolution







Source: Napari sample image

Fluorescence images

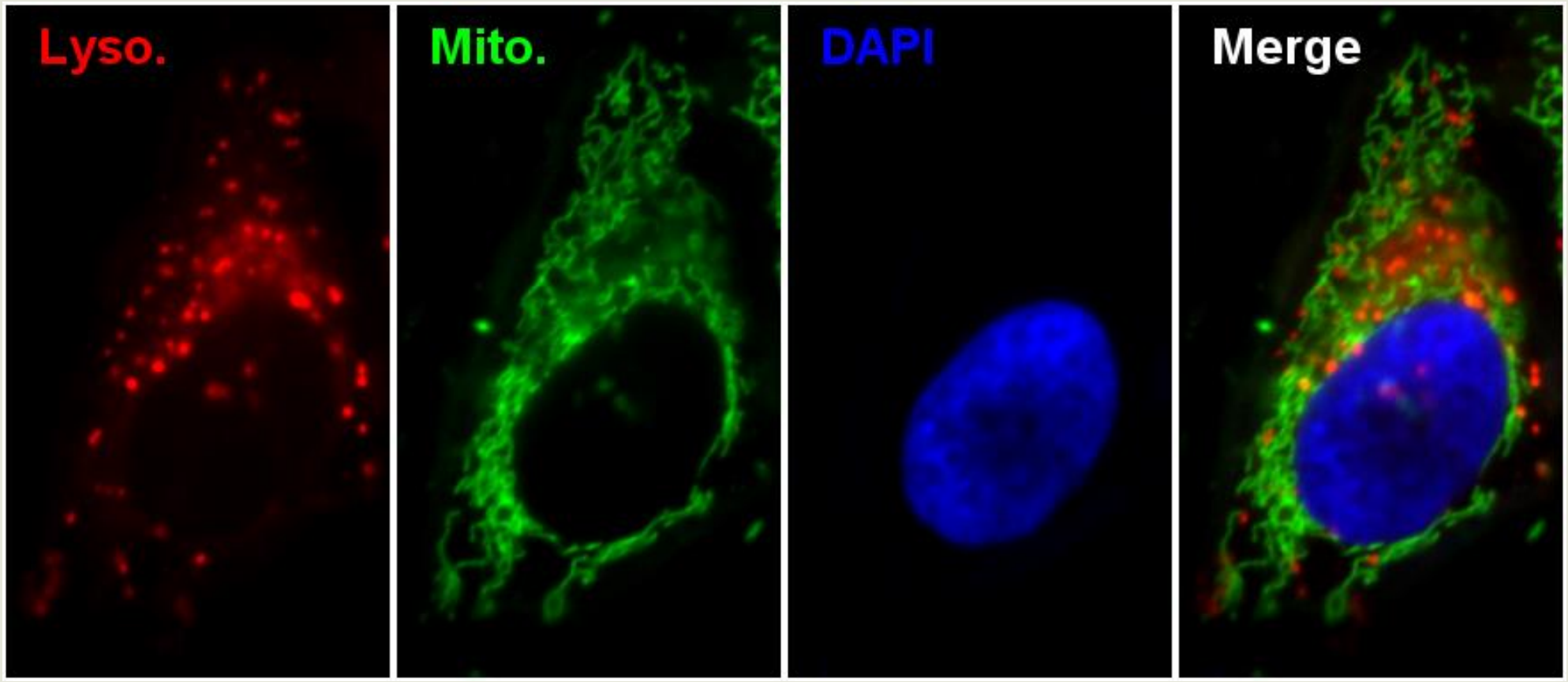


Image source: FIJI sample images (Hela cells, cropped)

Image are composed of pixels (picture + element)

(A) Original image

(B) Enlarged view from (A)

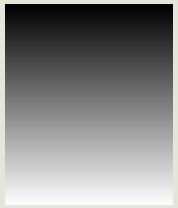
(C) Pixel values from (B)

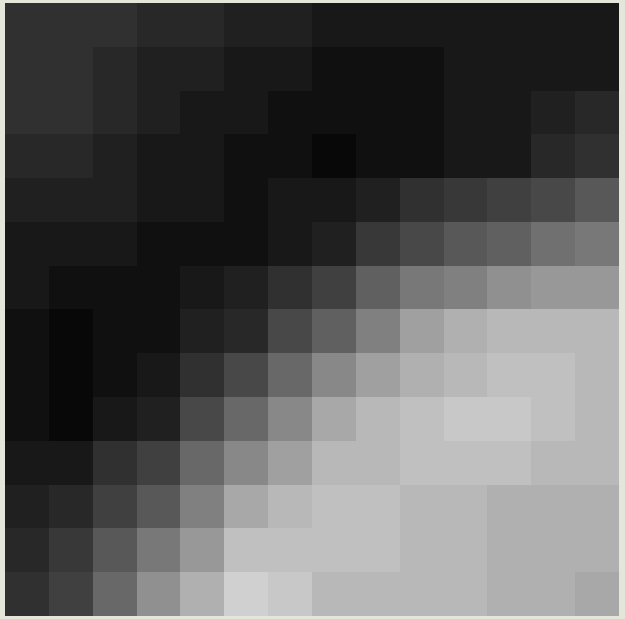
53	191	239	241	255	225	181	111	61	180	255	255
	35	168	244	255	243	210	119	85	176	244	252
71		45	161	246	227	206	99	60	158	255	255
137	26	42	31	143	214	199	138	125	185	255	255
172	99	78	21	72	106	149	153	190	183	252	255
200	129	102	41	64	65	95	166	206	200	255	255
255	153	17		49	31	44	145	187	219	255	255
255	227	145	42	38	58	71	106	91	202	255	255
255	255	242	129	107	48	30	95	57	162	255	255
255	255	255	189	78		17	74	60	119	228	255
255	255	255	246	133	65	73	32	129	136	144	247
255	255	253	229	112	40	23	29	111	175	93	183

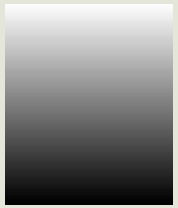
0

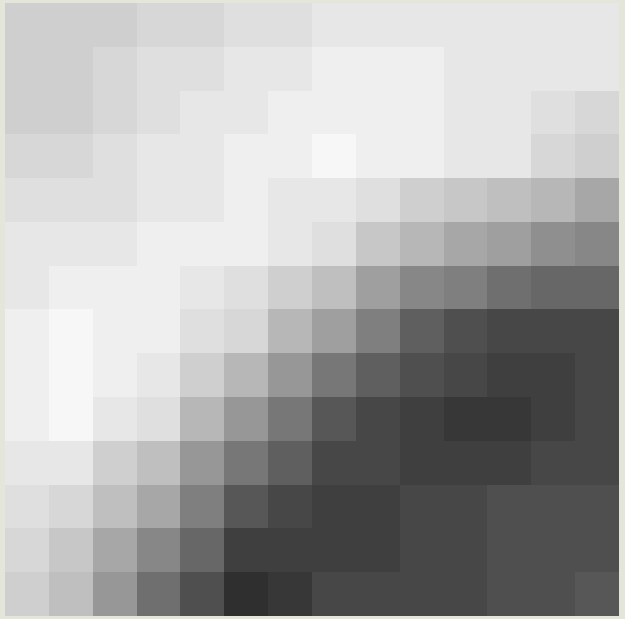
255

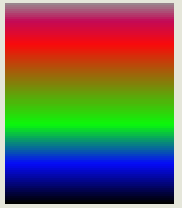
Lookup table (LUT)

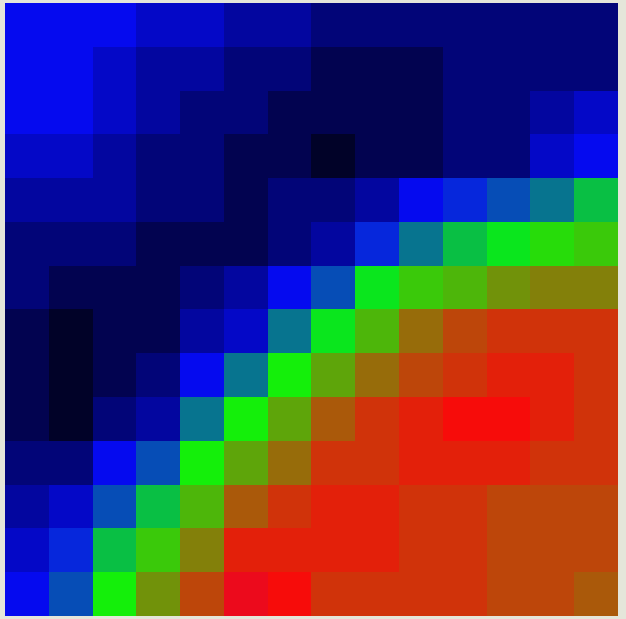
Pixel value	Display color
0 1 2 … 255	



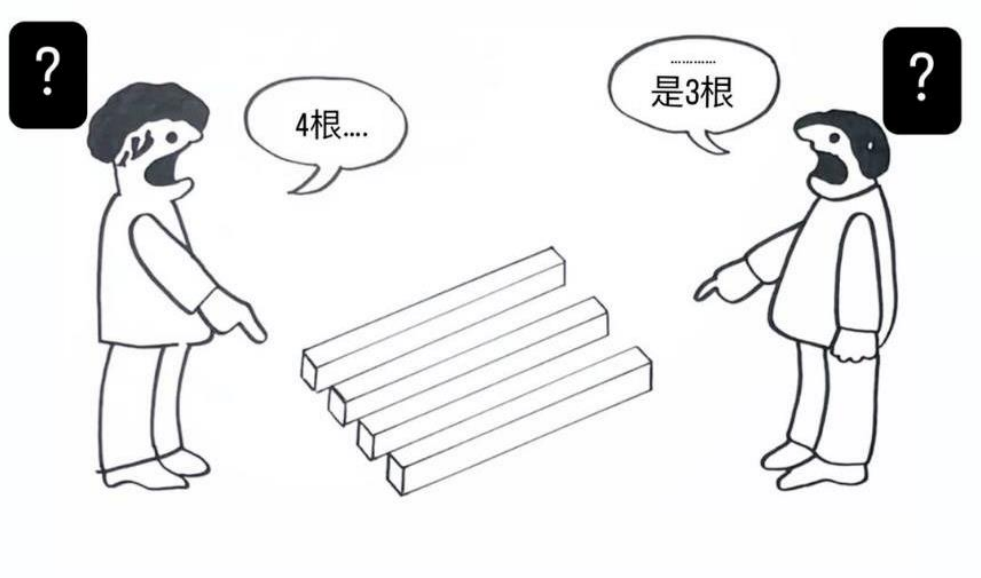
Pixel value	Display color
0 1 2 … 255	

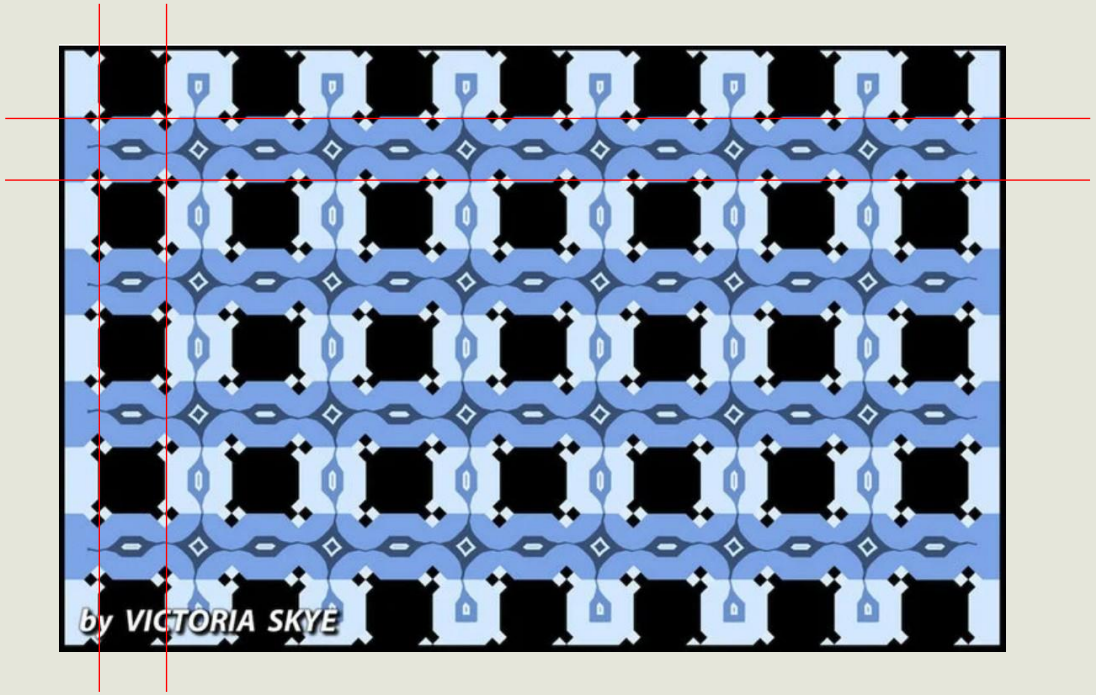


Pixel value	Display color
0 1 2 … 255	



Don't believe your eyes.



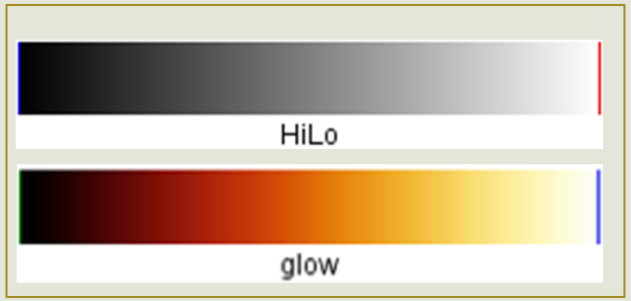


Useful LUT

“Classic”

Color Blindness friendly

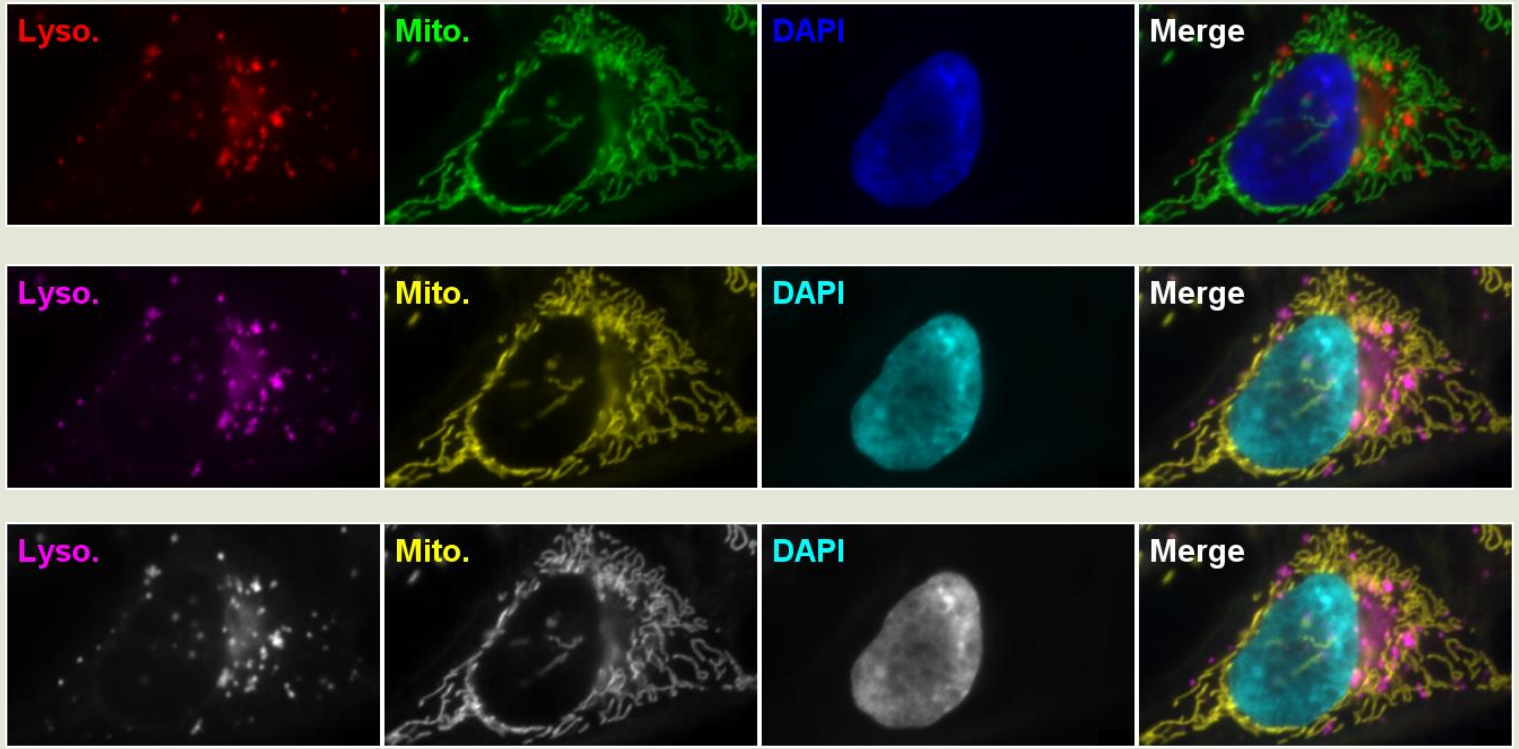
Range indictor



Good for weak signal visualization (nonlinear, calibration bar is necessary)

For labeling/ count mask

Lookup-tables(LUT) / Pseudo-colors / Colormaps



Visibility

Colorblind-friendly

Visibility

Colorblind-friendly

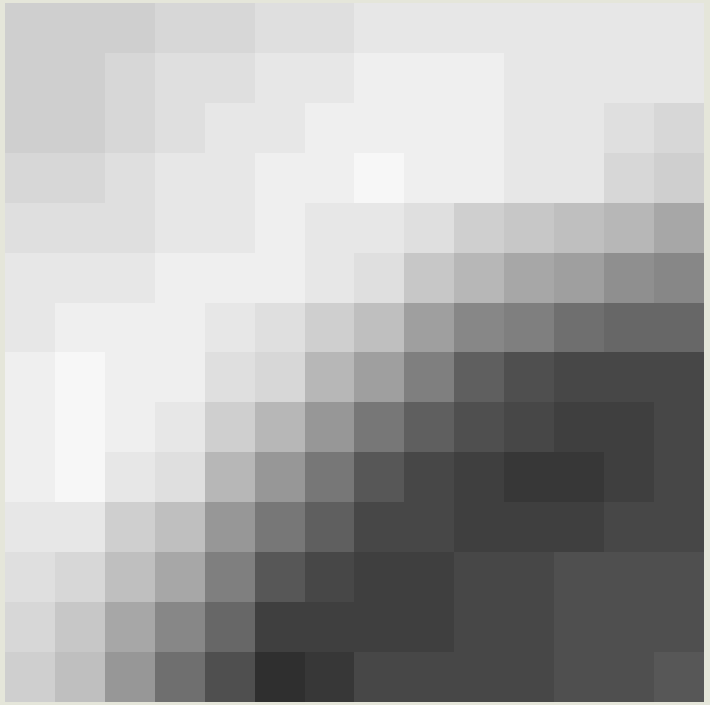
Visibility

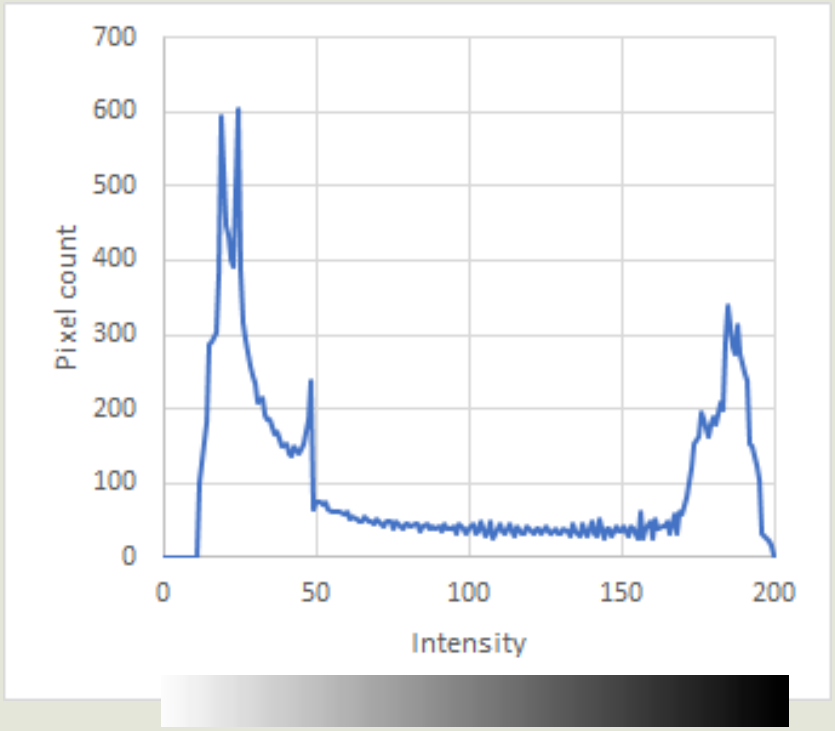
Colorblind-friendly

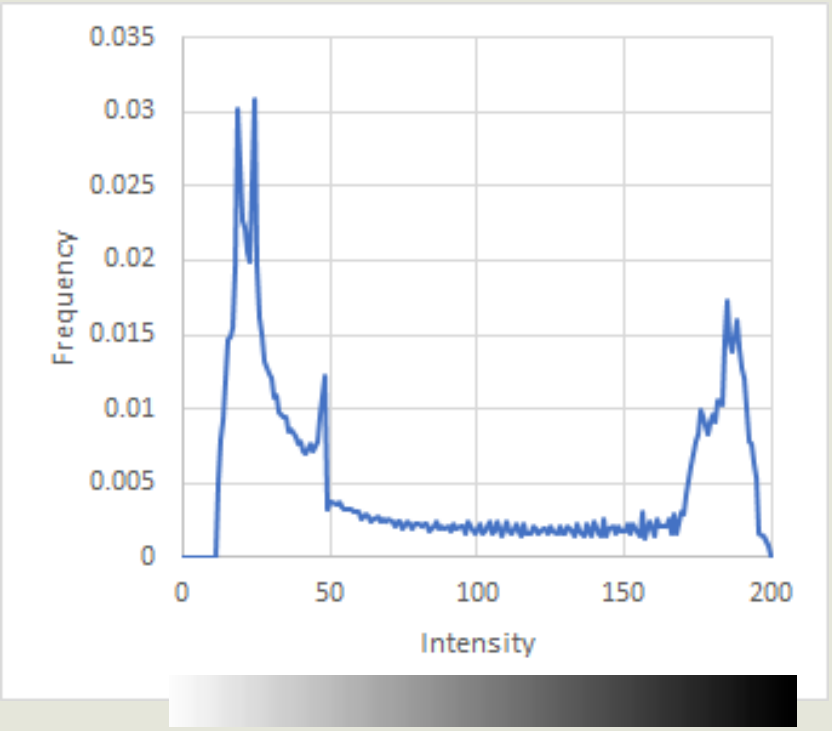
Image source: FIJI sample images (Hela cells, cropped)
See also Figure Making Best Practices (2024) youtube.com

Histograms

A histogram shows the probability distribution of pixel intensities.

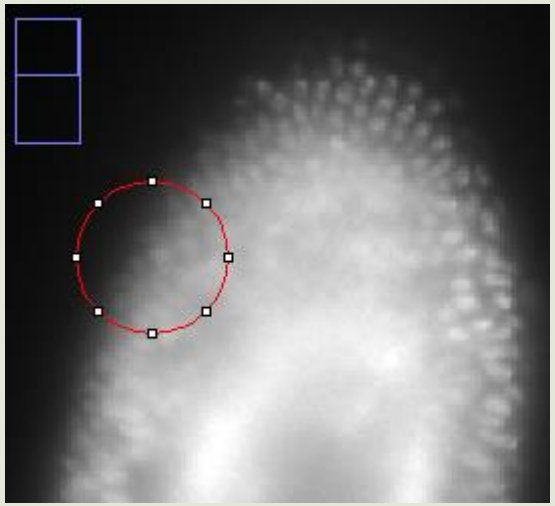


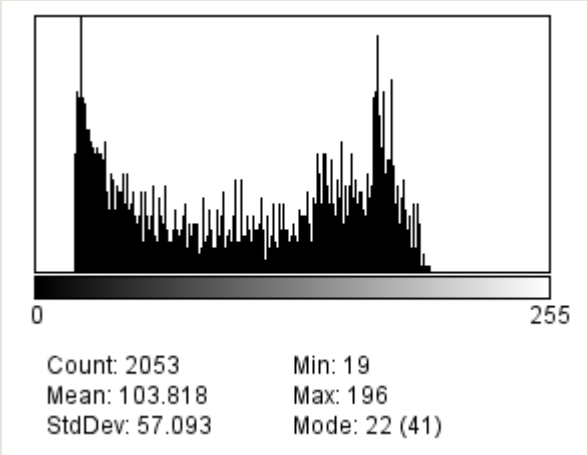


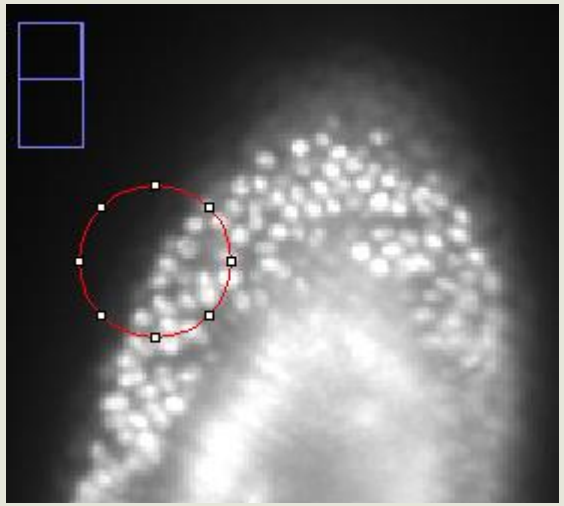


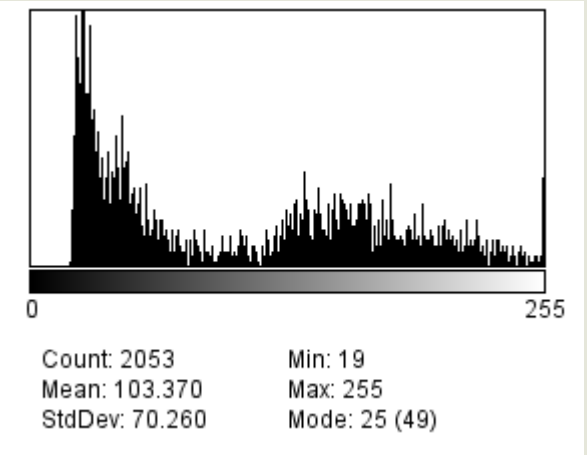
Histograms

Histograms are summaries of images



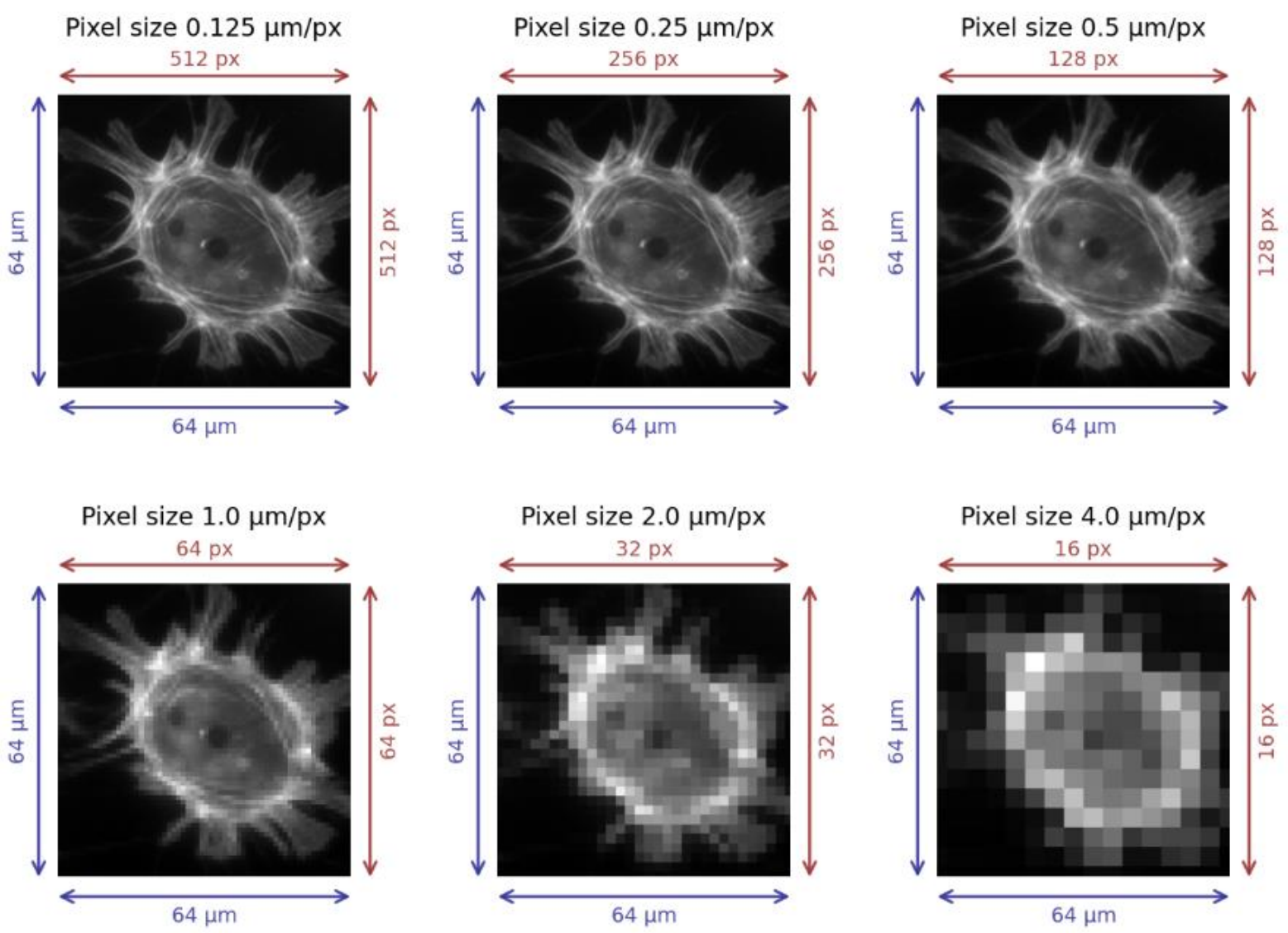






Quickly compare the images.
Selected LUTs.

Pixel Size : How the data is saved



Pixel number:

- Total number of individual pixels that make up that image.
- Note: The pixel number is often referred to as the resolution in cameras.

Pixel size:

- A property of a digital image.
- You configure it during the imaging session at the microscope (Pixel numbers, Objective, Zoom…etc.)

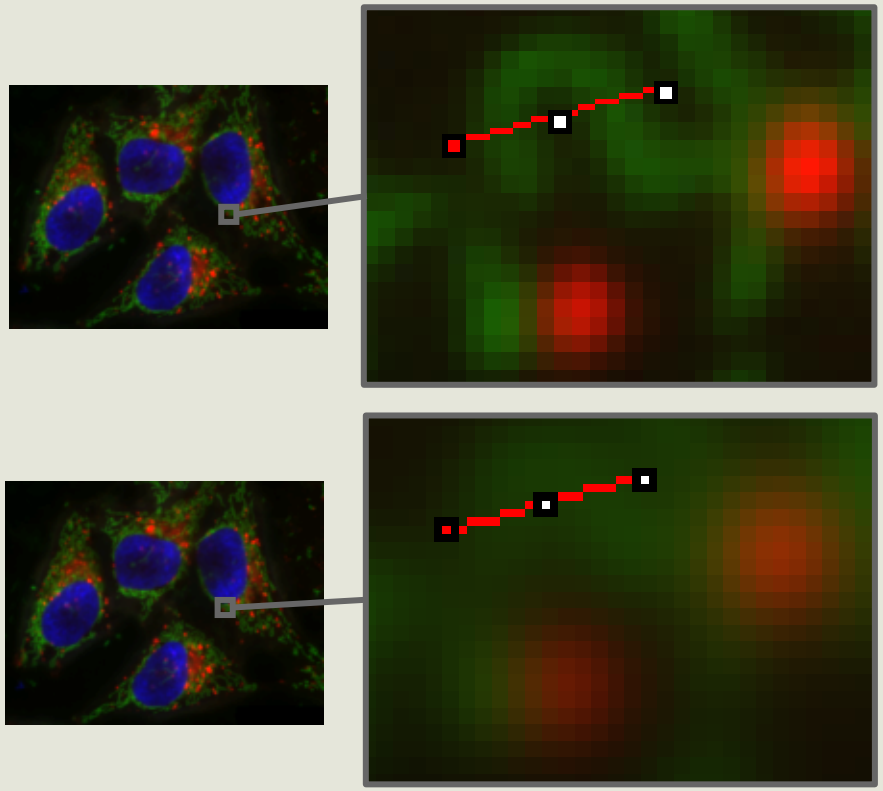
Resolution:

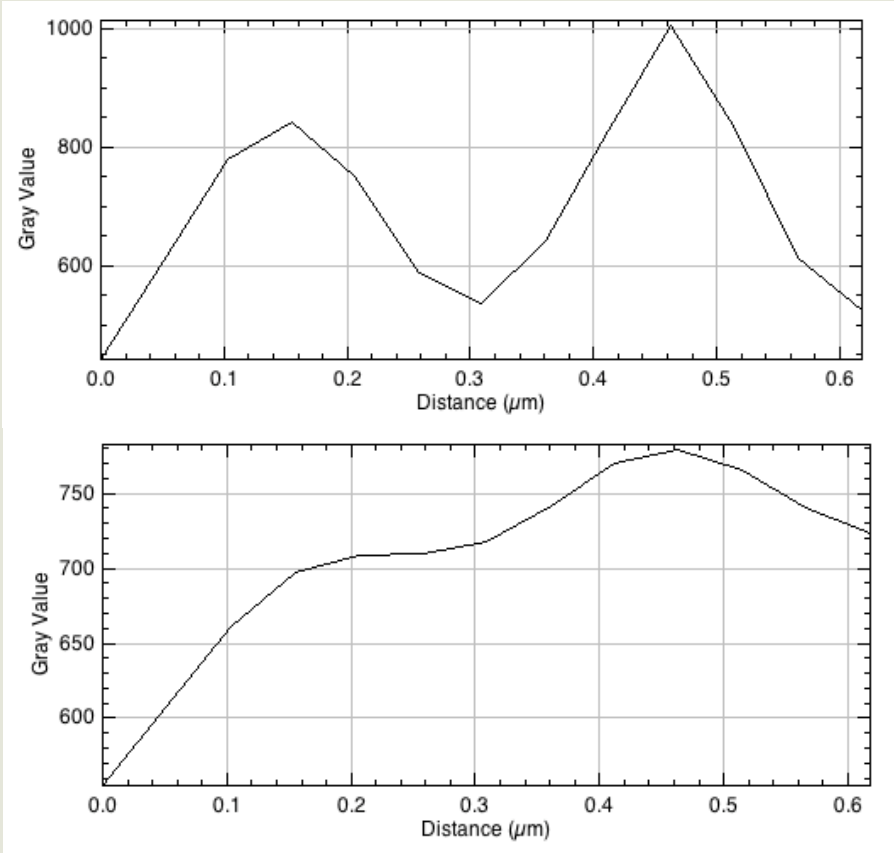
- A property of your imaging system.
- **How small can objects be, to be still differentiable?**

Introduction to Bioimage Analysis (2022)
https://bioimagebook.github.io/

Resolution

- Resolution is a property of your imaging system.
- The measure of how close object can be in an image while still being differentiable, is called spatial resolution.





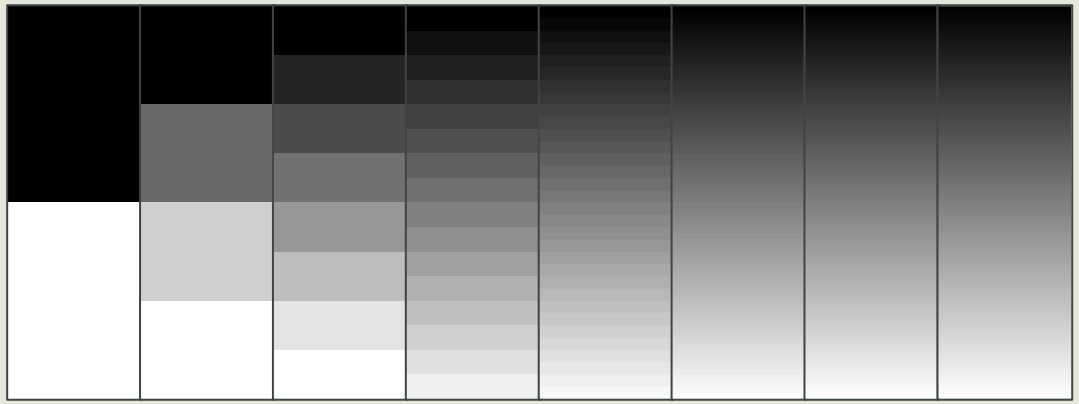
Representing an image using different bit-depths

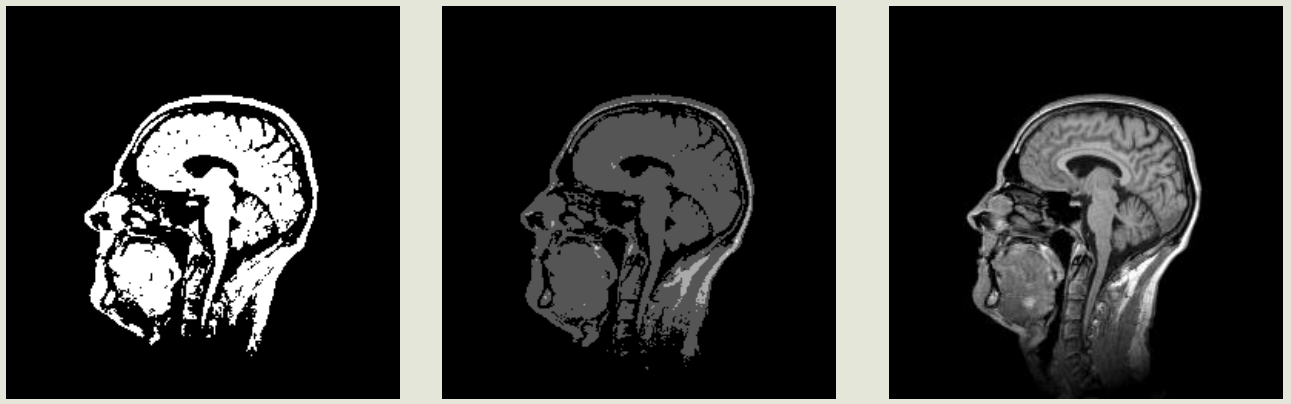
- A bits is the smallest memory unit in computers.
- The bit-depth *n* enumerates how many different intensity values are present in an image:
  - 2^n grey values Ex : 8 bits $2^8 = 256$
- In microscopy, images are usually stored as 8, 12 or 16-bit images.

Bit = "0" or "1"
1 Bytes = 8 bits -> basic store unit

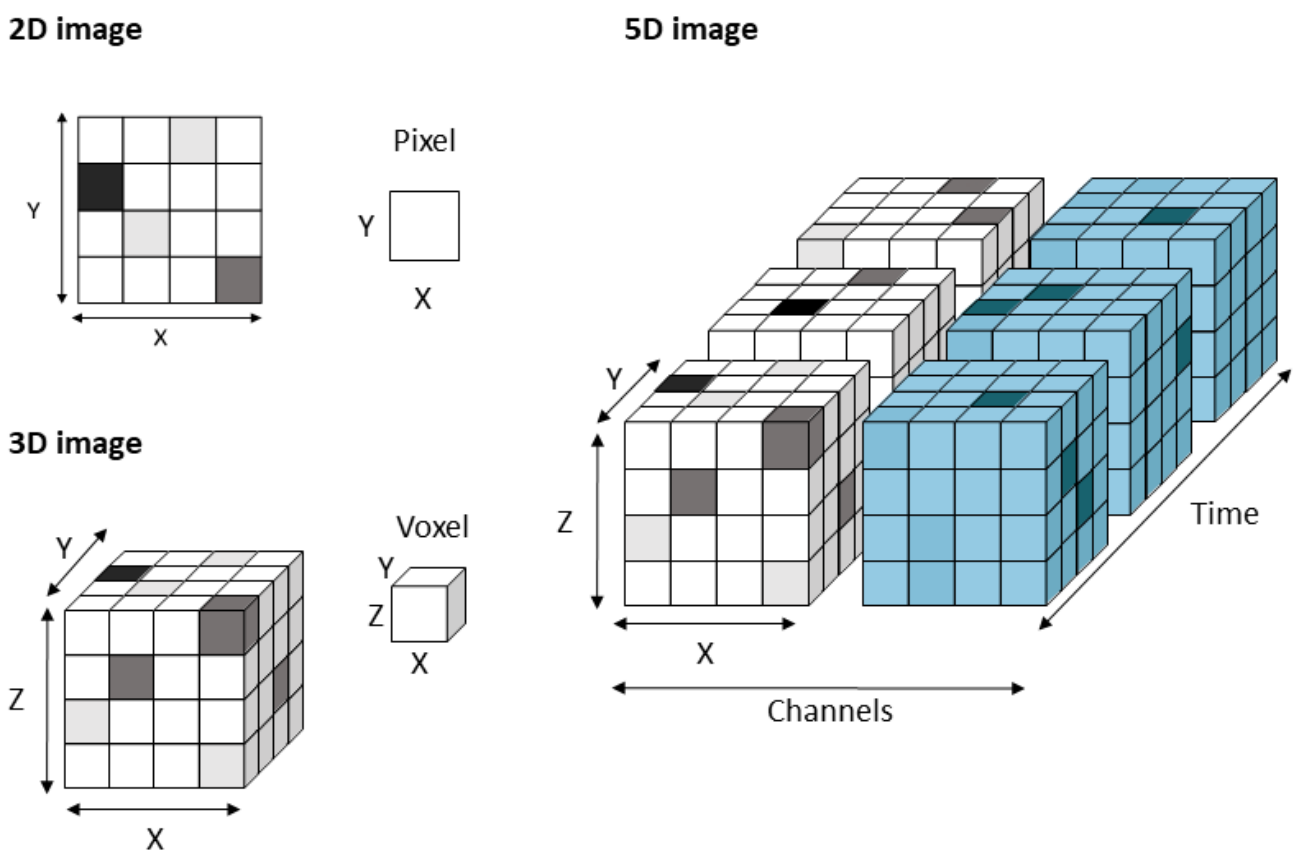
8 bits (1 byte) = 0 ~ 255
16 bits (2 bytes) = 0 ~ 65535

12 bits (2 bytes!) = 0 ~ 4095
14 bits (2 bytes!) = 0 ~ 16383
32 bits (4 bytes, floating-point)





N-dimensional images



- Most fluorescence image:
  (n color -> n Channels)
- Data from color camera:
  RGB format
  (n color -> R/G/B Value, 24 bits)

Files & file formats

Image files consist of **pixel values** and **metadata**

- **Pixel values** – the 'raw numbers' of the image
- **Metadata** – additional information, such as dimensions, image type, bit-depth, pixel sizes and microscope settings ('data about data')

Format	Extensions	Main use	Compression	Comment
TIFF	.tif, .tiff	Analysis, display (print)	None, lossless, lossy	Very general image format
OME-TIFF	.ome.tif, .ome.tiff	Analysis, Display (print)	None, lossless, lossy	TIFF, with standardized metadata for microscopy
Zarr	.zarr	Analysis	None, lossless, lossy	Emerging format, great for big datasets – but limited support currently
PNG	.png	Display (web, print)	Lossless	Small(ish) file sizes without compression artefacts
JPEG	.jpg, .jpeg	Display (web)	Lossy (usually)	Small file sizes, but visible artefacts

Introduction to Bioimage Analysis (2022)
https://bioimagebook.github.io/

Part II :

Bioimage analysis workflow - Processing & Image Operators

Bioimage analysis workflow - Processing

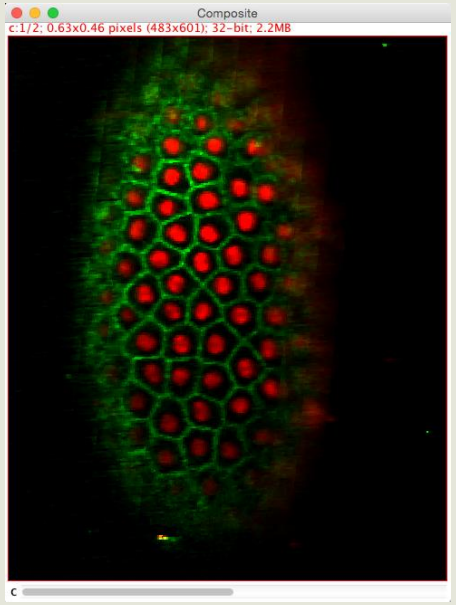


Image Processing

Preprocessing

Segmentation

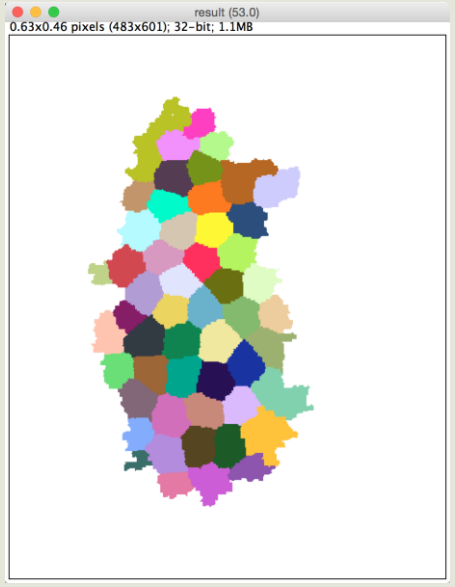
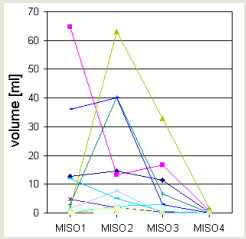
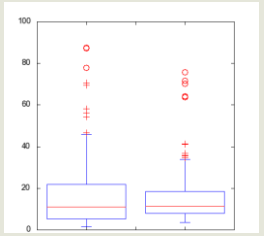


Image Analysis

Quantification

Data Analysis





Bioimage analysis workflow – Image Operators

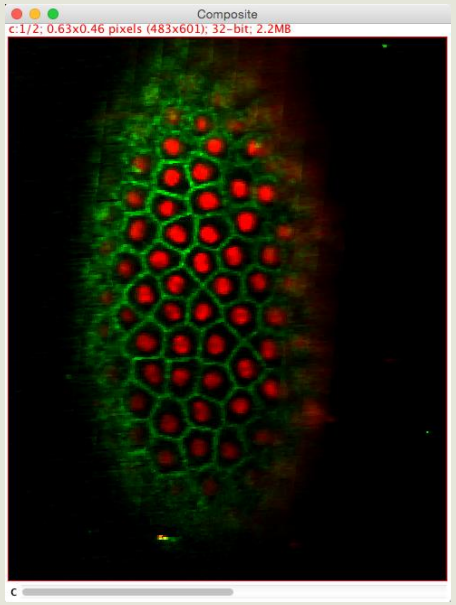


Image Processing

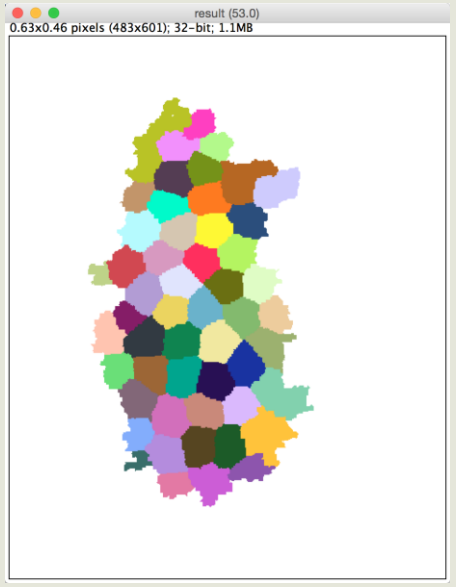
Preprocessing

Segmentation

- Point operations
- Background Subtraction
- Filtering
- …

- Thresholding
- Connected Component Labelling
- …

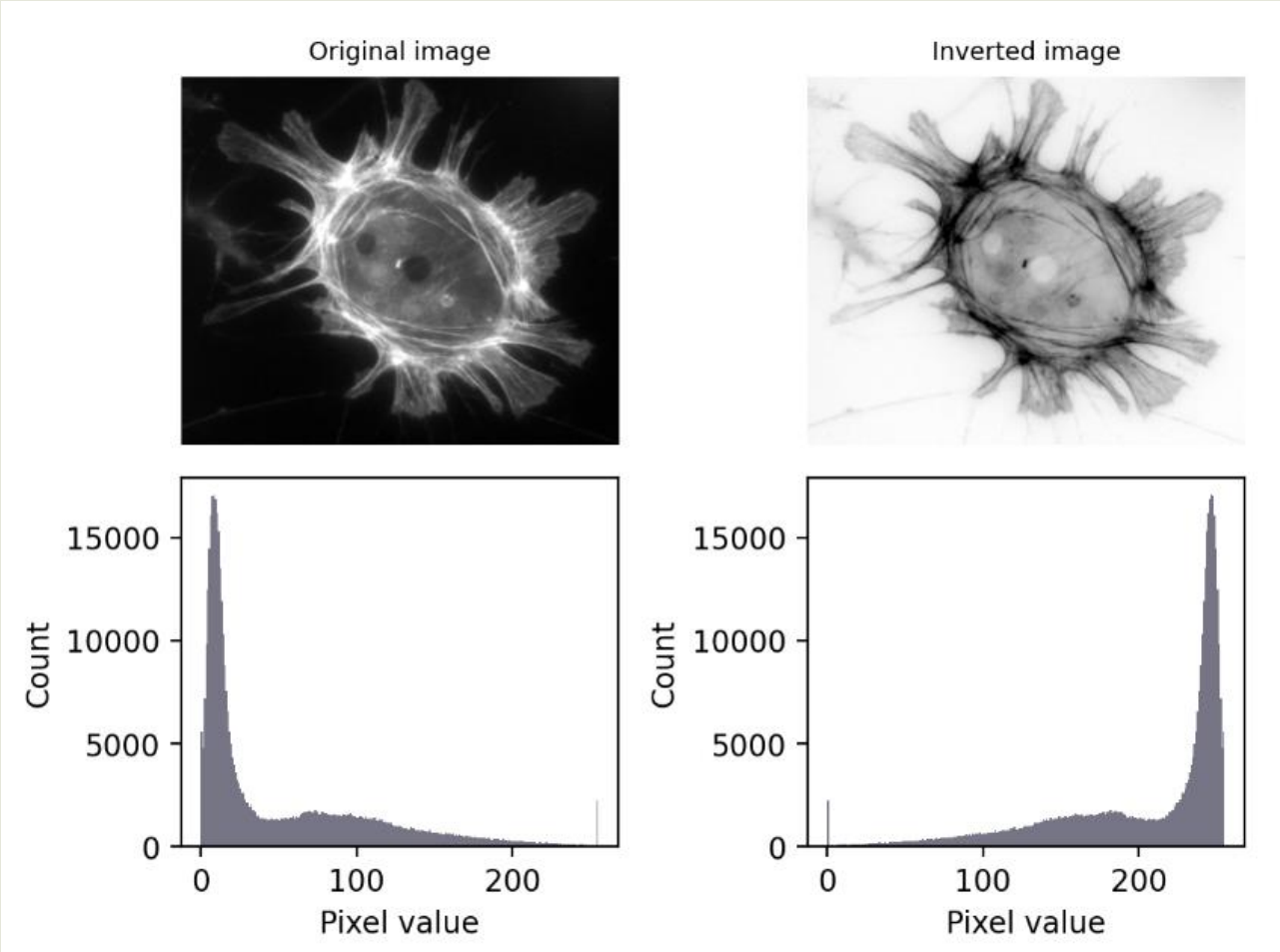
Image Operators



Point operations

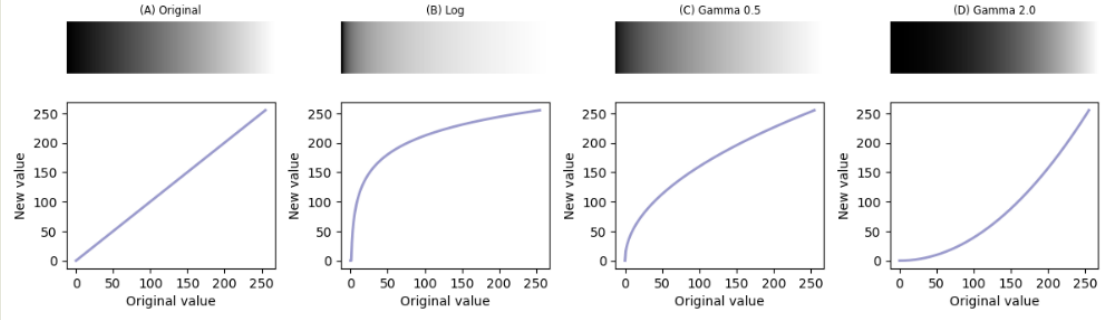
Point operations

Pixel values are just numbers

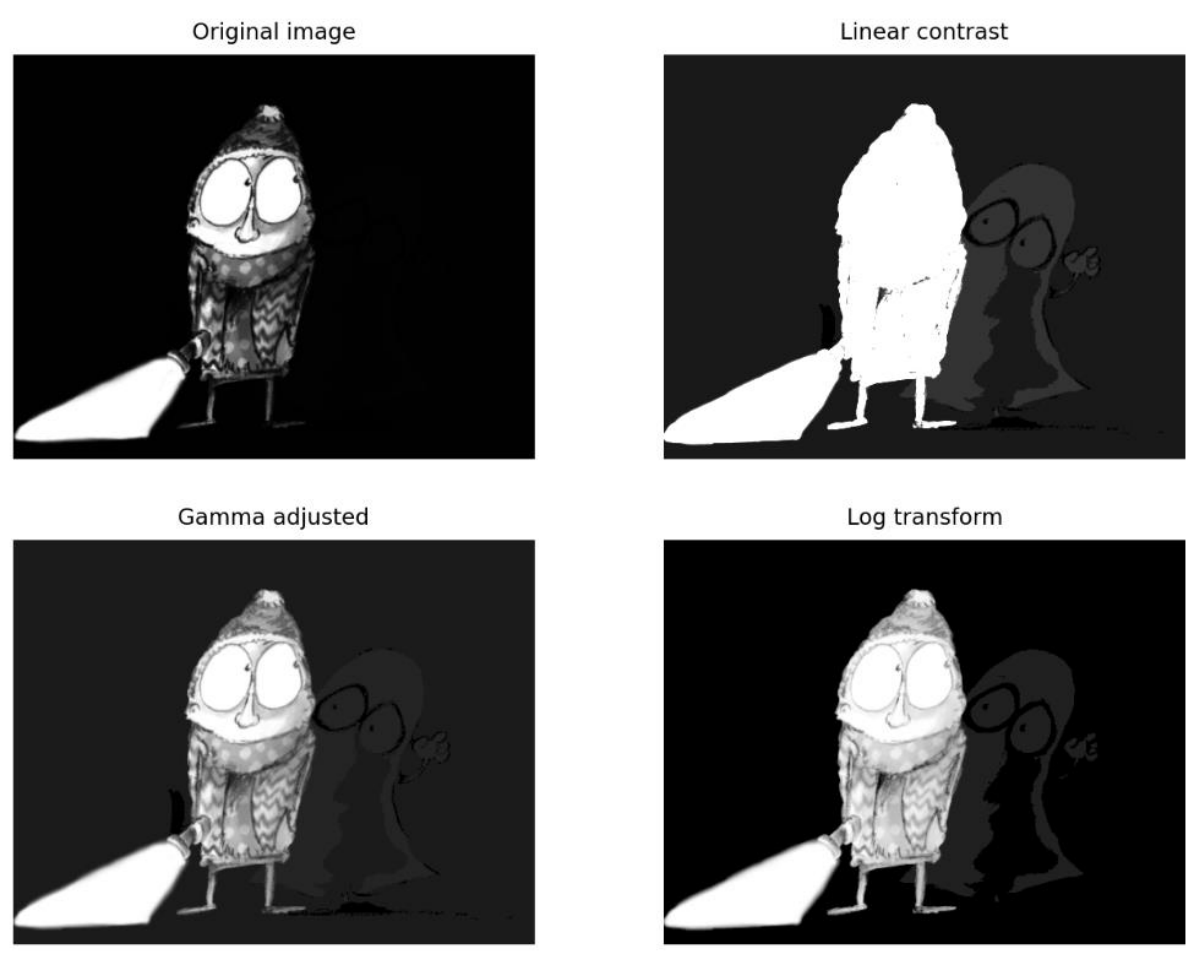


Nonlinear contrast enhancement

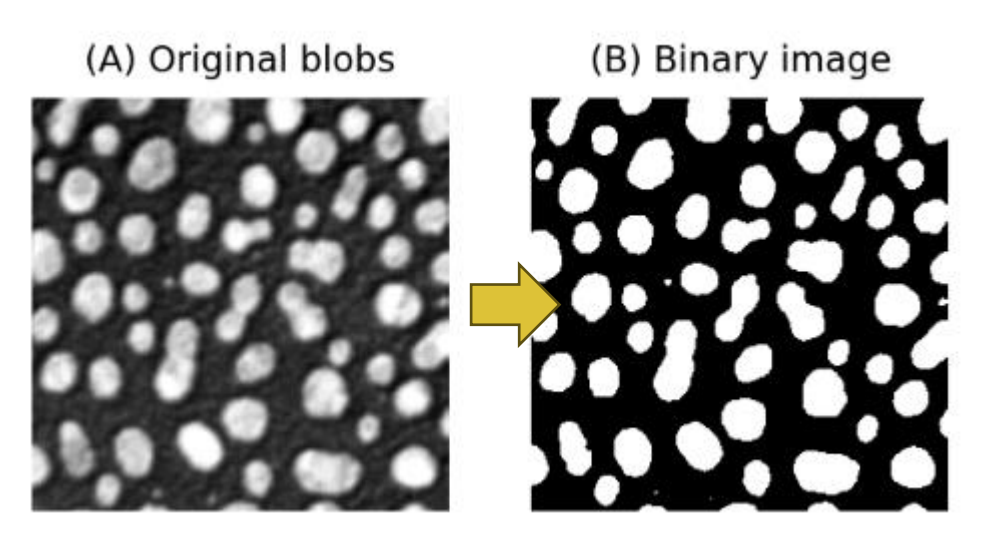
Nonlinear contrast enhancement



should be used with caution



Thresholding

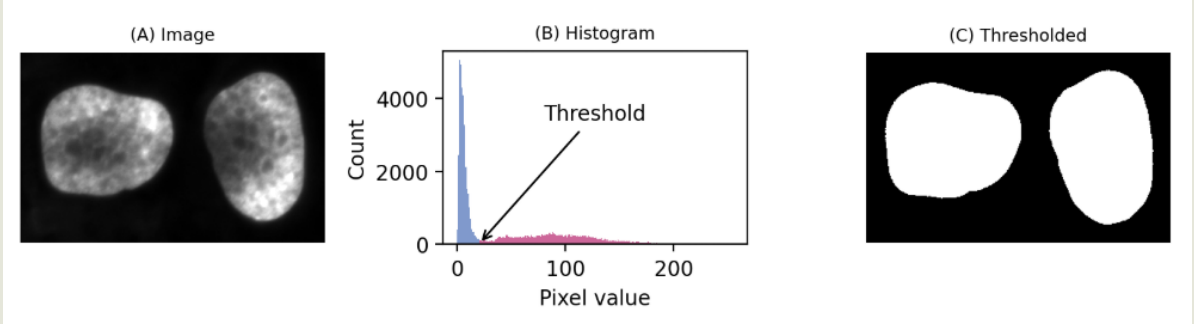


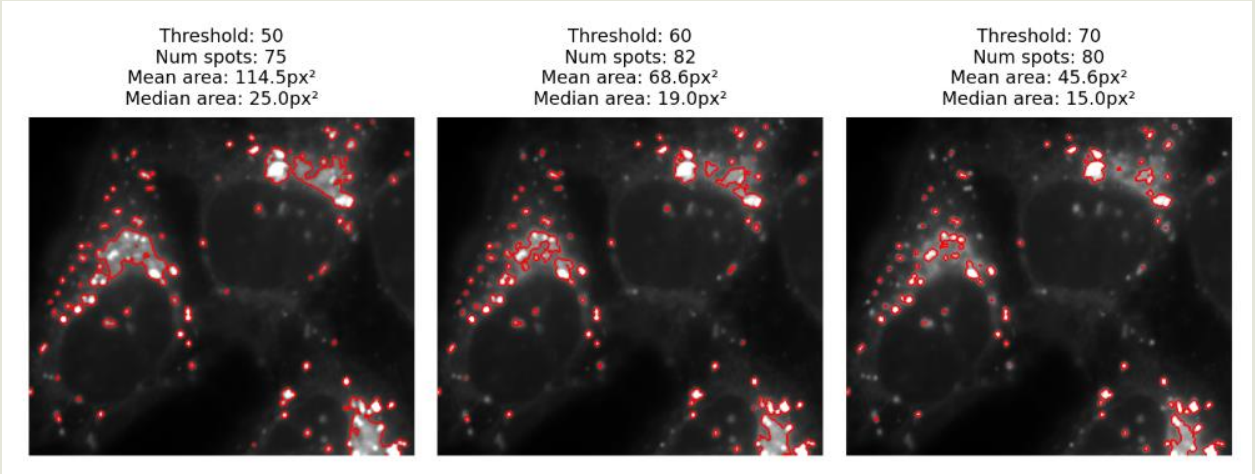
Background

Foreground

Avoid manual adjustments if possible.

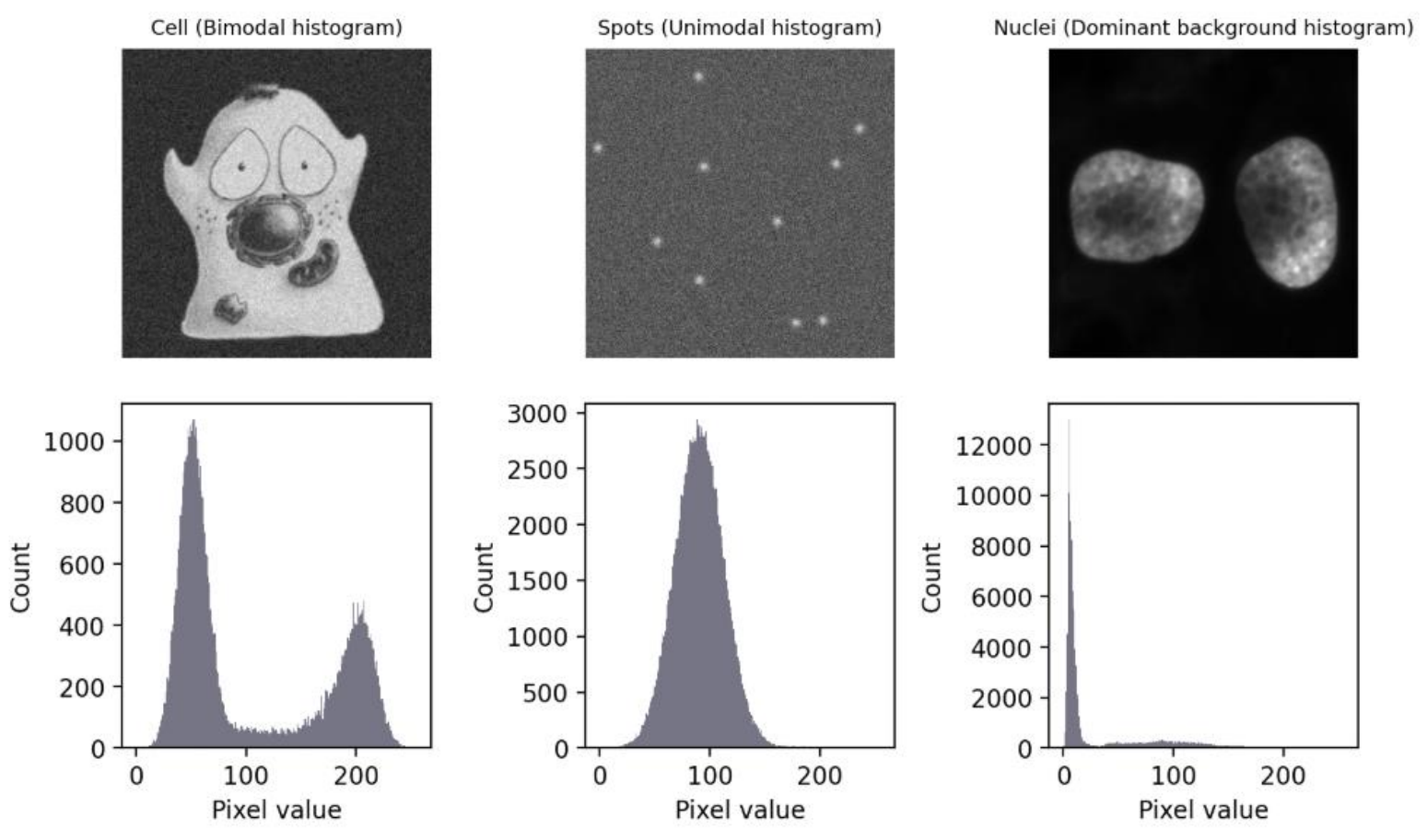
Global thresholding





Thresholding

Automated thresholds

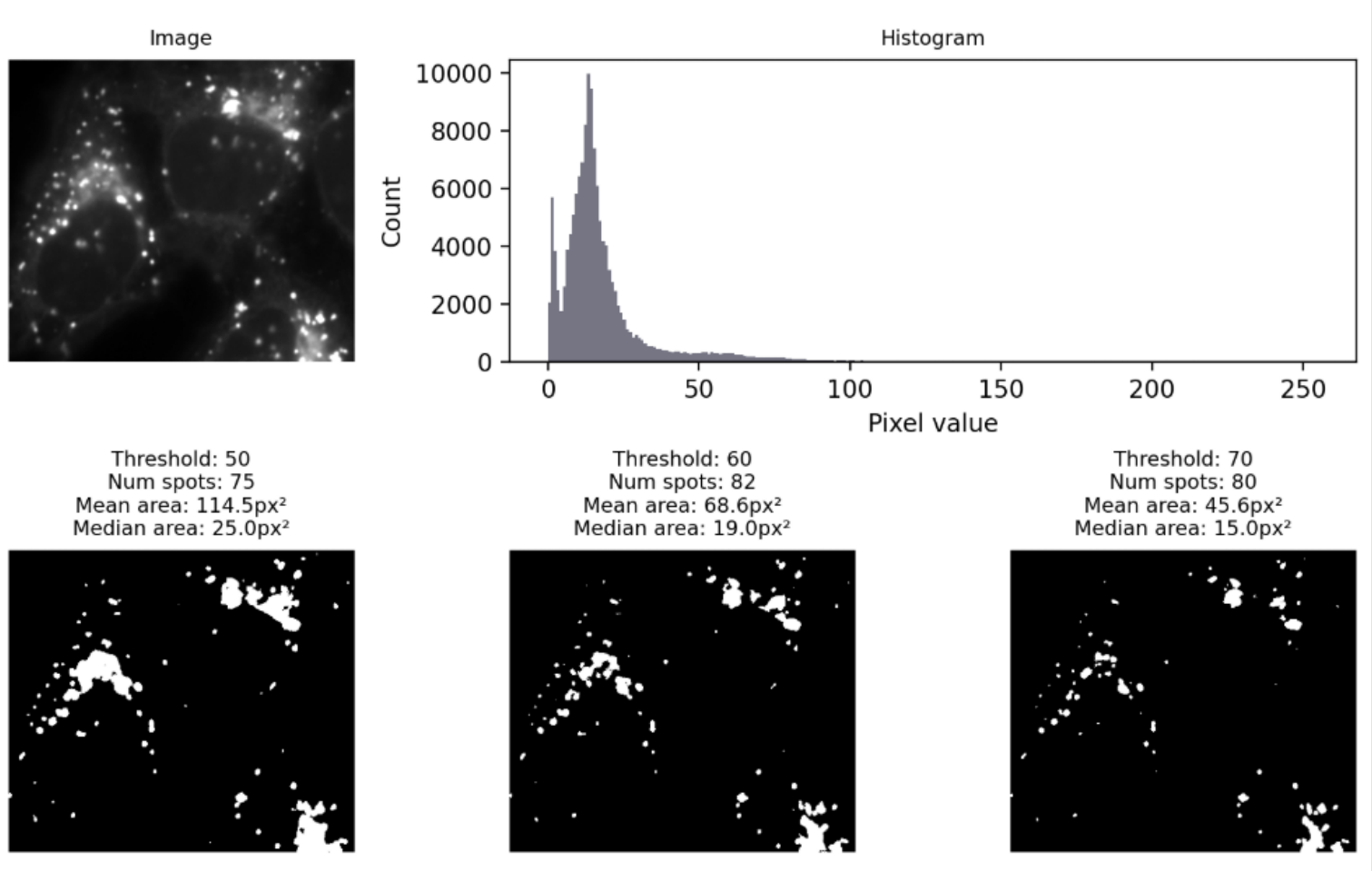


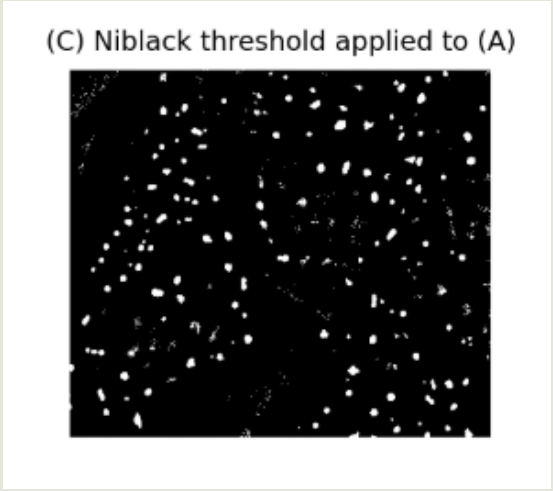
- **Otsu's method**
- **Minimum method**
- **Triangle method**
- **Mean method**
- **Mean & Standard deviation**
- **Median & Median Absolute Deviation**
- **…**

Reduce bias !

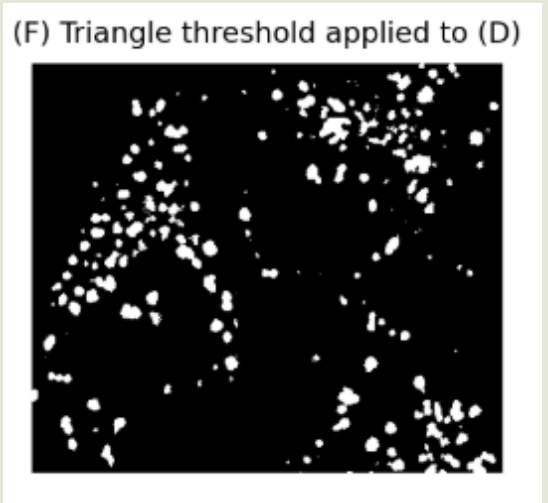
Thresholding

Local thresholding





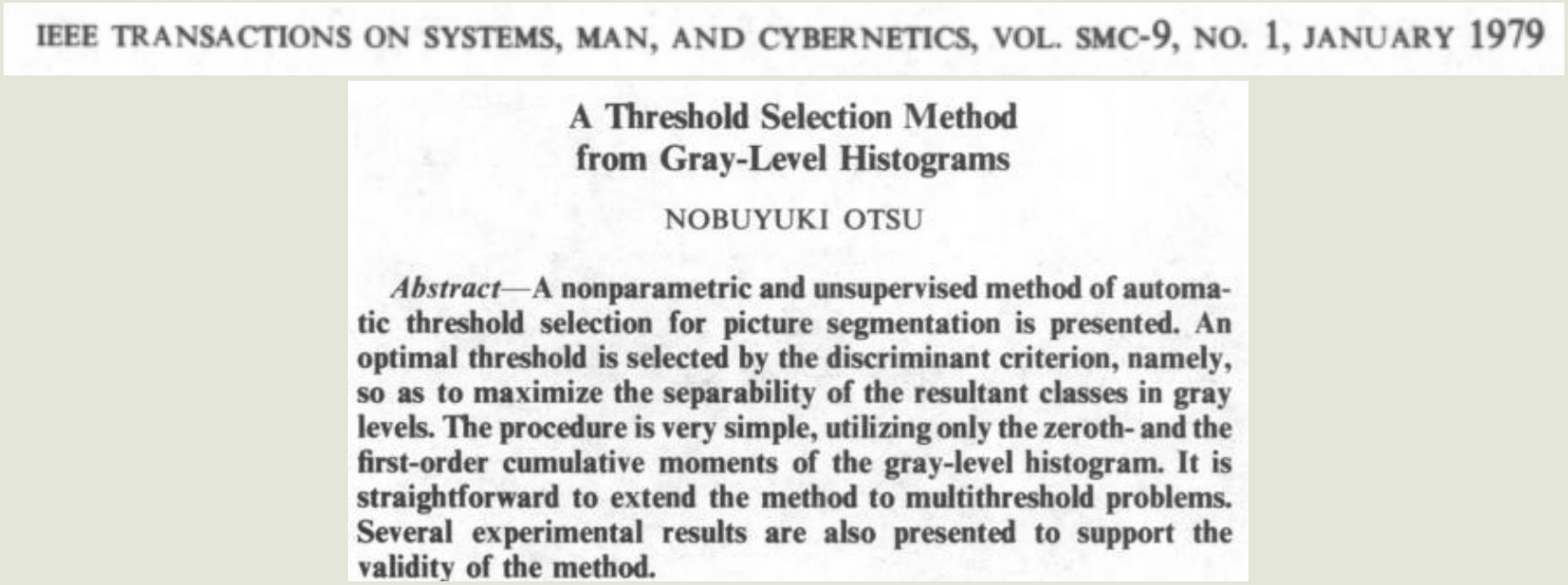
Or Background subtracted



Citing the thresholding algorithm

- Cite the thresholding method of your choice properly

"We segmented the cell nuclei in the images using Otsu's thresholding method (Otsu et al. 1979) implemented in Fiji (Schindelin et al. 2012)."



IEEE TRANSACTIONS ON SYSTEMS, MAN, AND CYBERNETICS, VOL. SMC-9, NO. 1, JANUARY 1979

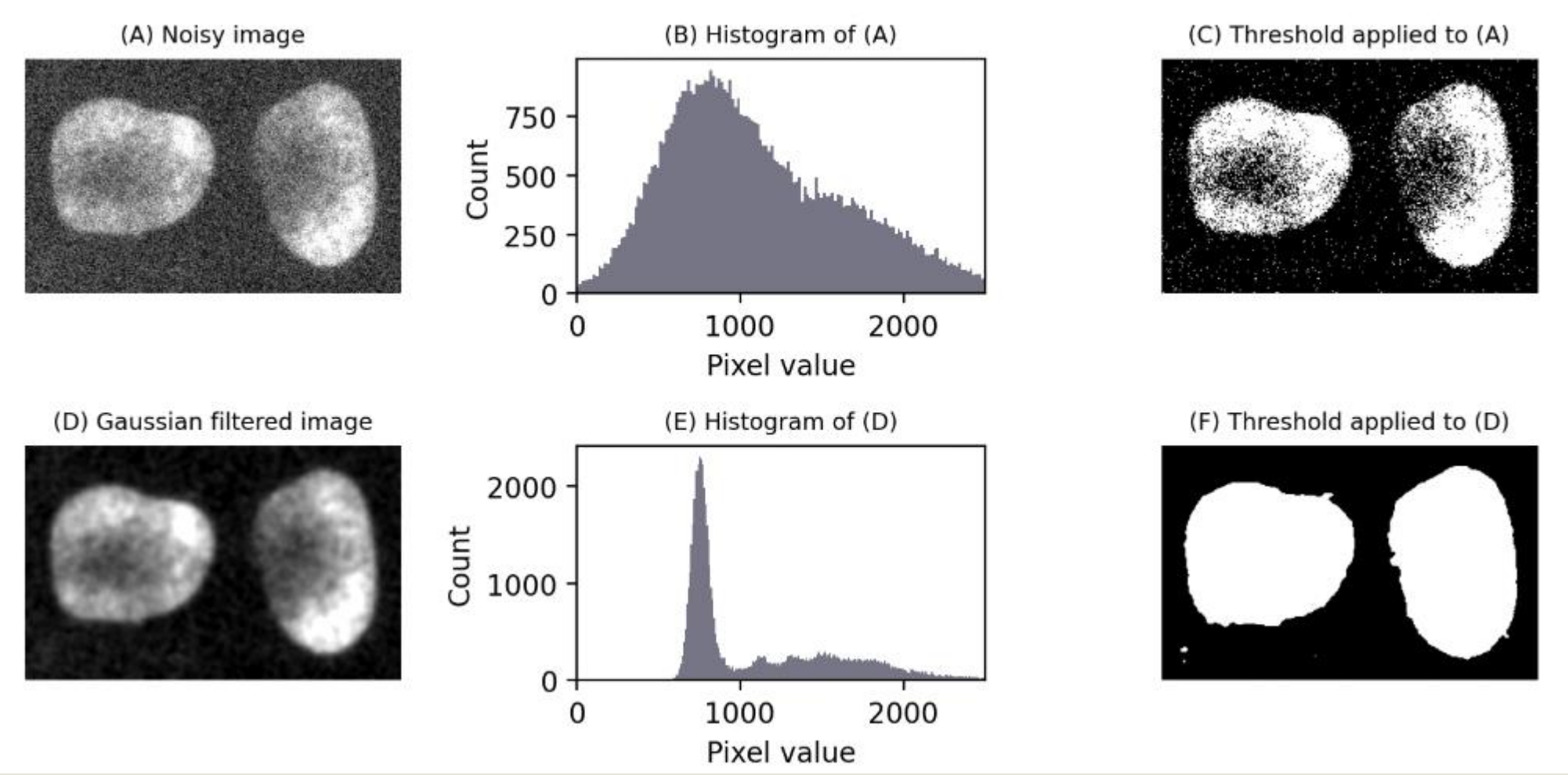
A Threshold Selection Method from Gray-Level Histograms

NOBUYUKI OTSU

Abstract—**A nonparametric and unsupervised method of automatic threshold selection for picture segmentation is presented. An optimal threshold is selected by the discriminant criterion, namely, so as to maximize the separability of the resultant classes in gray levels. The procedure is very simple, utilizing only the zeroth- and the first-order cumulative moments of the gray-level histogram. It is straightforward to extend the method to multithreshold problems. Several experimental results are also presented to support the validity of the method.**

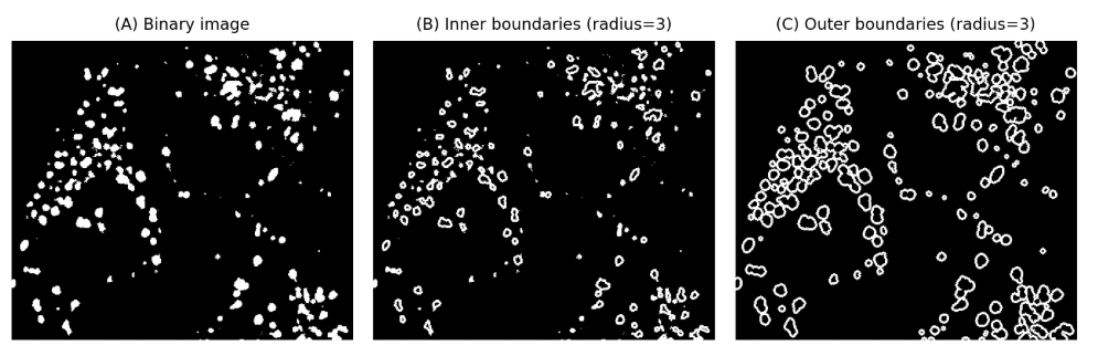
Filters - improving thresholding results

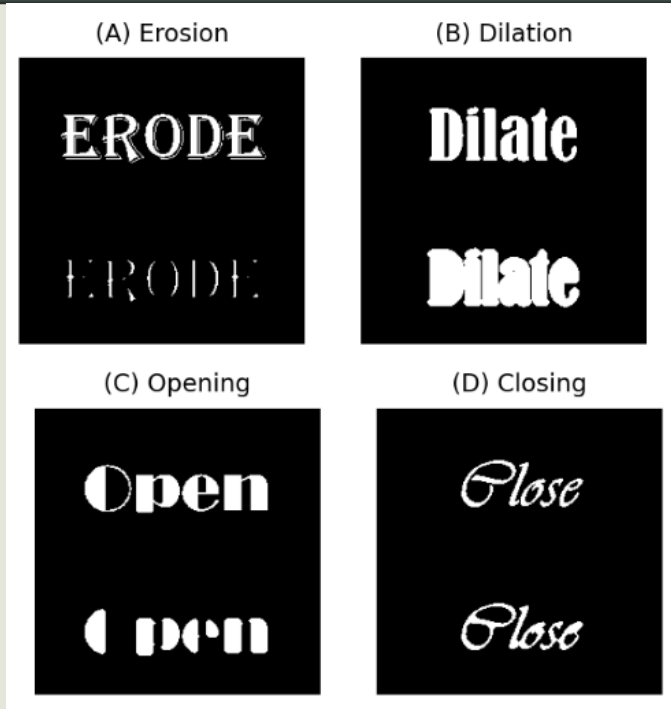
Filtering can make segmentation much easier by **enhancing features** and **reducing noise**



Morphological Operators

- **Morphological operations** can be used to refine or modify the shapes of objects in images.
- Erosion, dilation, opening & closing
- Boundaries & outlines
- Filling holes
- Thinning & skeletonization





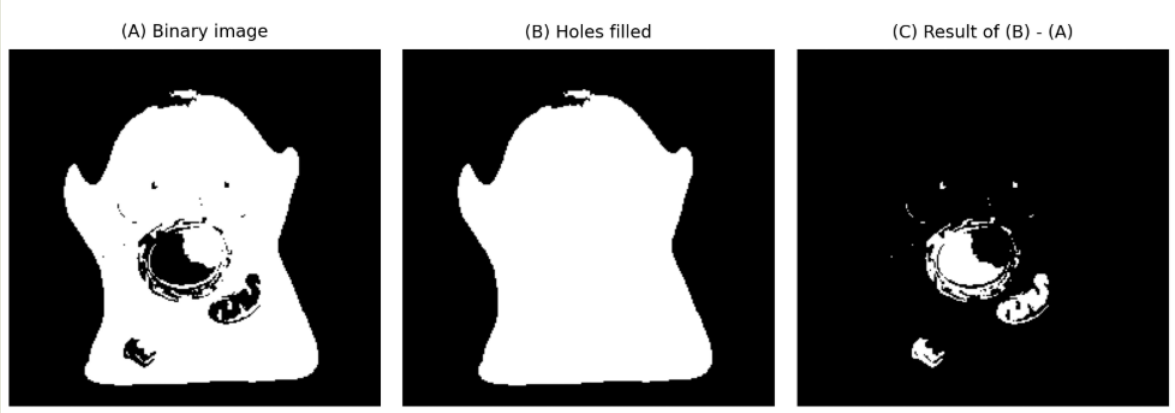
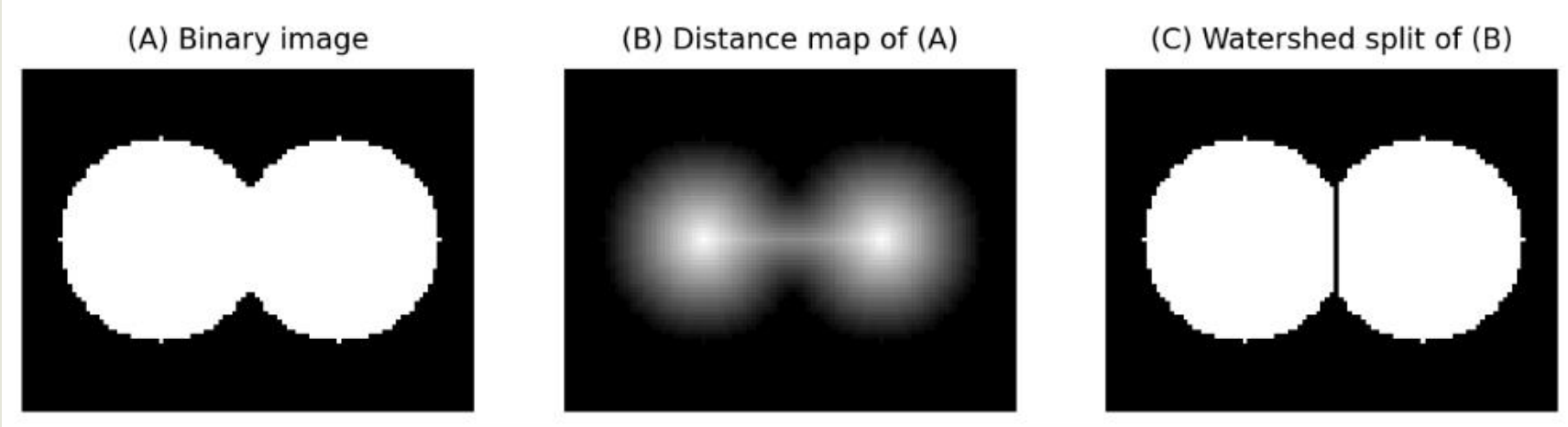


Image transforms (The watershed transform)



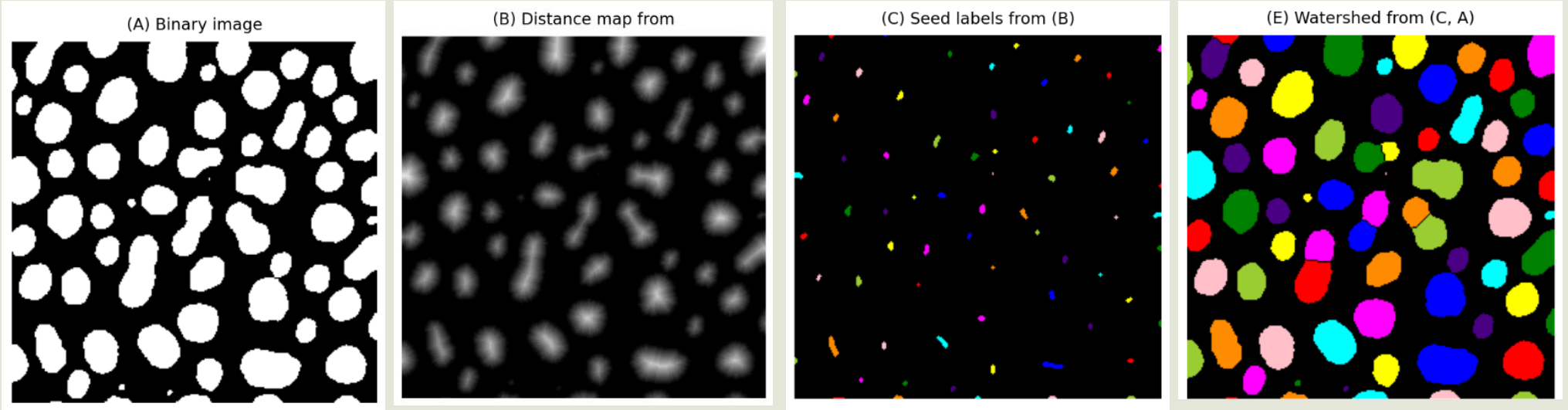
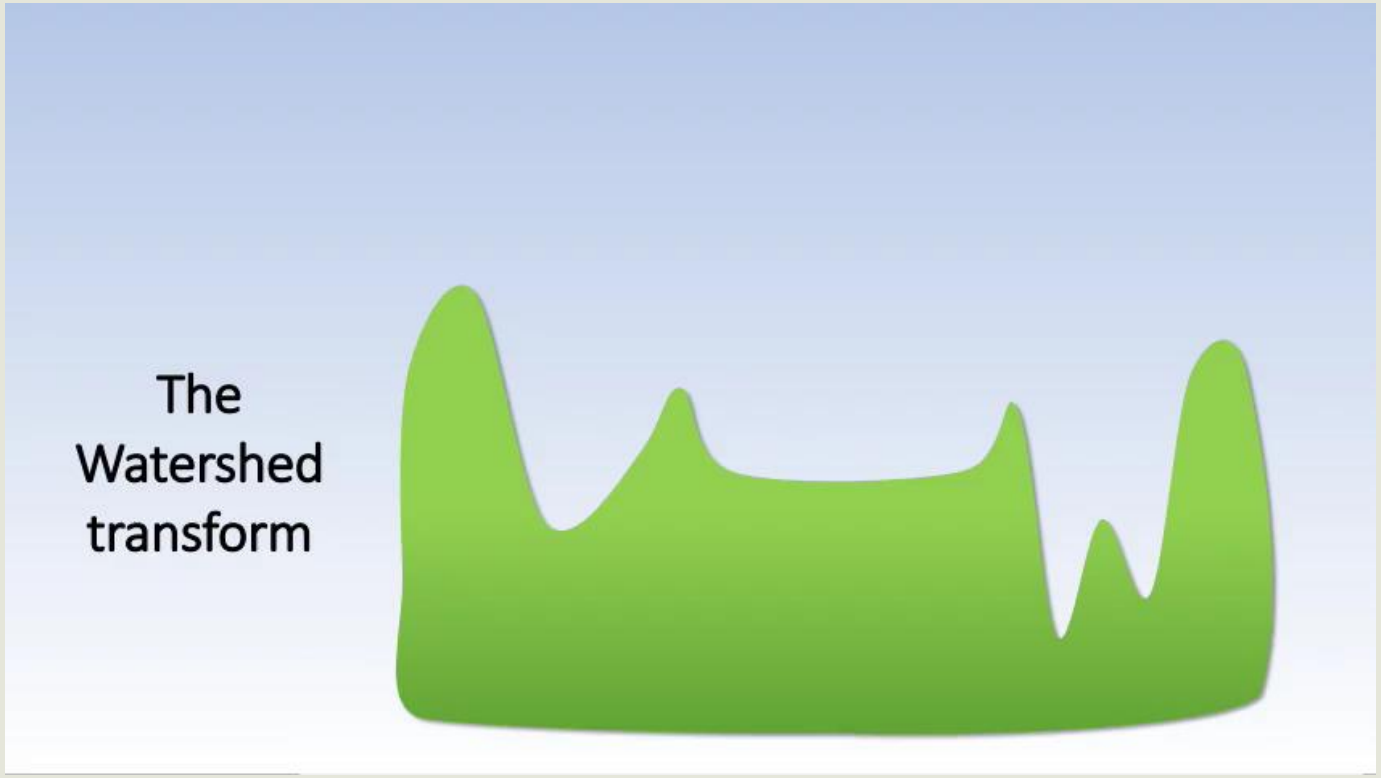
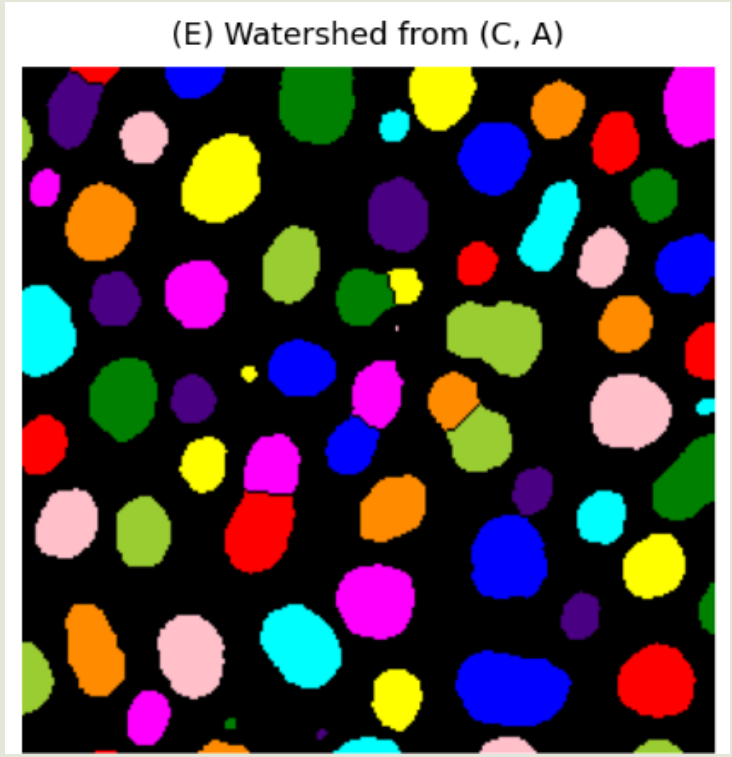
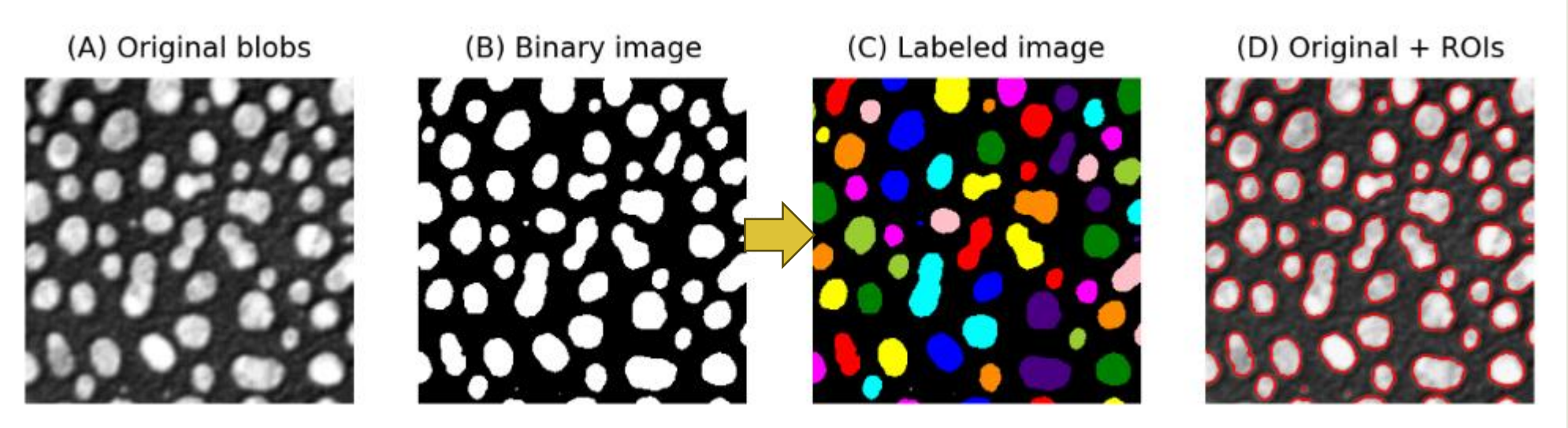


Image transforms (The watershed transform)



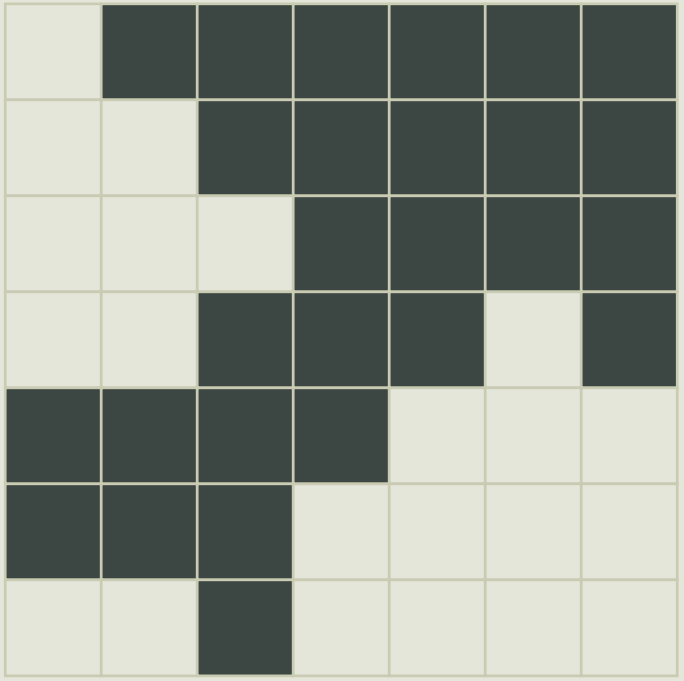


Connected Component Labelling & Region of Interest (ROI)



Connected Component Labelling & Region of Interest (ROI)

- In order to allow the computer differentiating objects, connected component analysis (CCA) is used to mark pixels belonging to different objects with different numbers
- Background pixels are marked with 0.
- The maximum intensity of a labelled map corresponds to the number of objects.



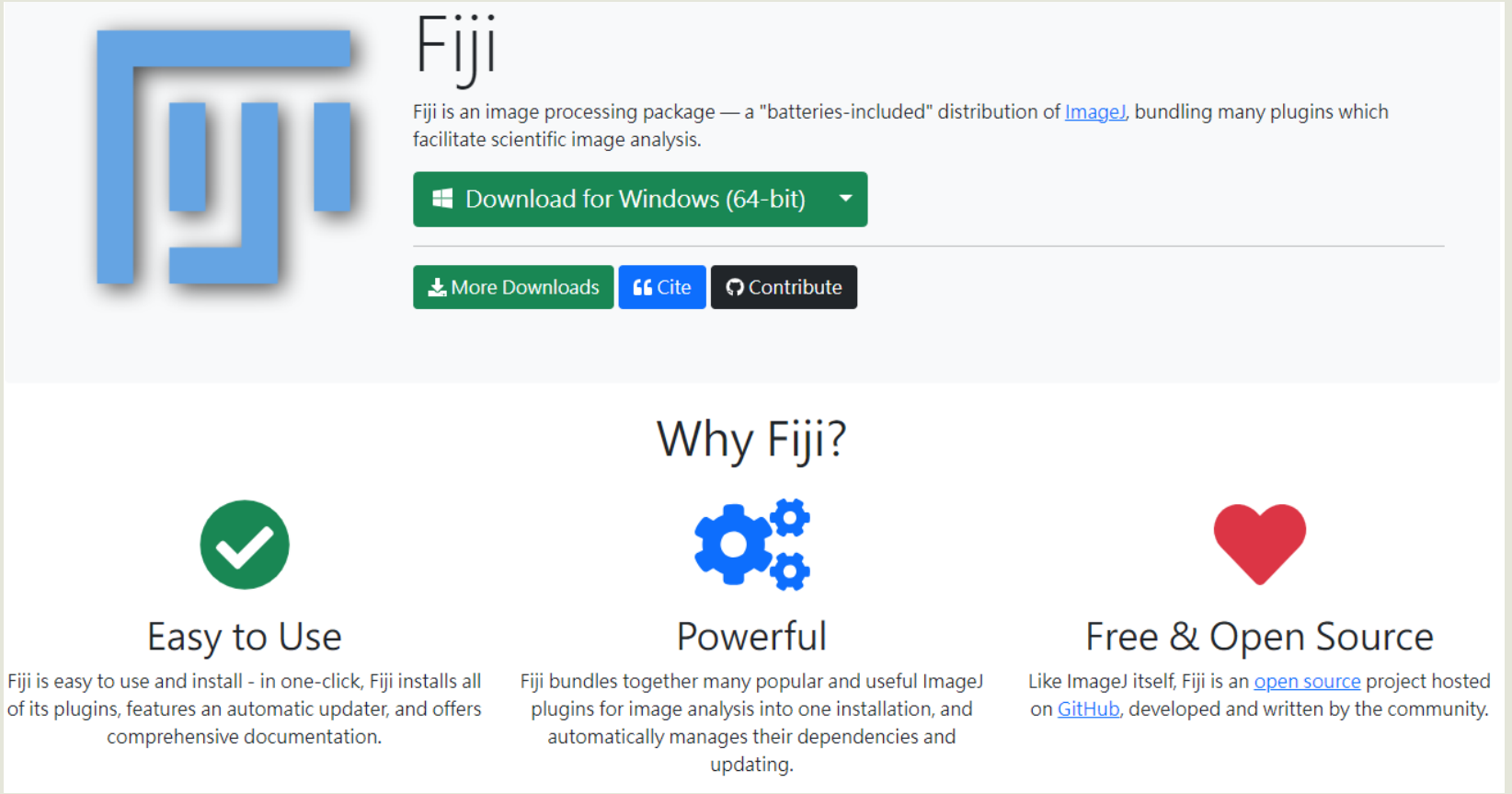
CCA

1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

Part III:

Open-source tools for Bioimage analysis - (Fiji)

(Fiji is just) imageJ



https://fiji.sc/

ImageJ, ImageJ2 and FIJI

ImageJ Citation:
Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012).
NIH Image to ImageJ: 25 years of image analysis.
Nature Methods, *9*(7), 671–675. doi:10.1038/nmeth.2089

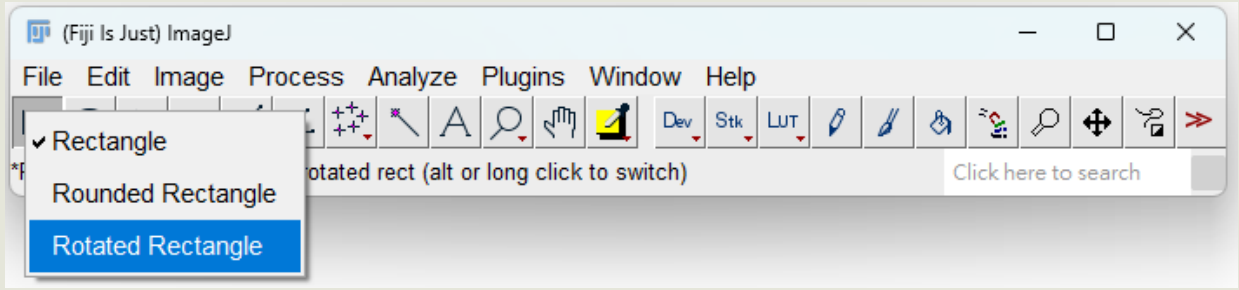
ImageJ2 Citation:
Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017).
ImageJ2: ImageJ for the next generation of scientific image data.
BMC Bioinformatics, *18*(1). doi:10.1186/s12859-017-1934-z

FIJI Citation:
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012).
Fiji: an open-source platform for biological-image analysis.
Nature Methods, *9*(7), 676–682. doi:10.1038/nmeth.2019

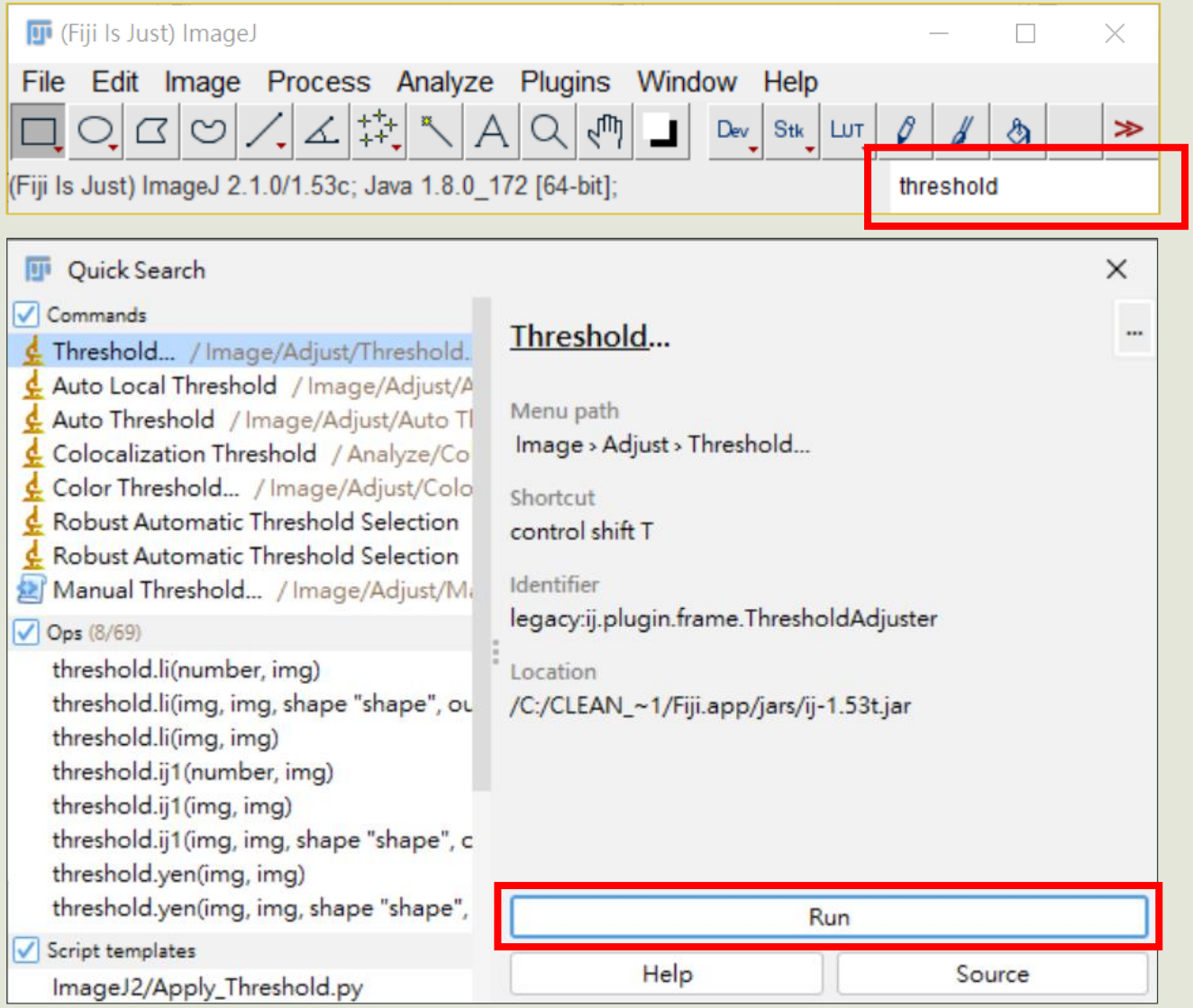
Also, cite the plugins and the algorithm used in your research.

Fijis user interface

- There are more tools in the toolbar than expected…
- Use the right click or double click to discover them!

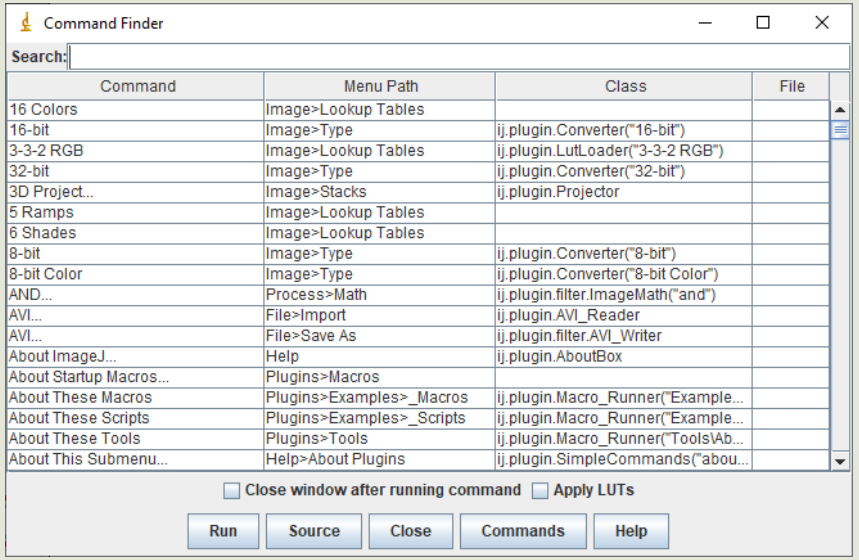


Search Bar

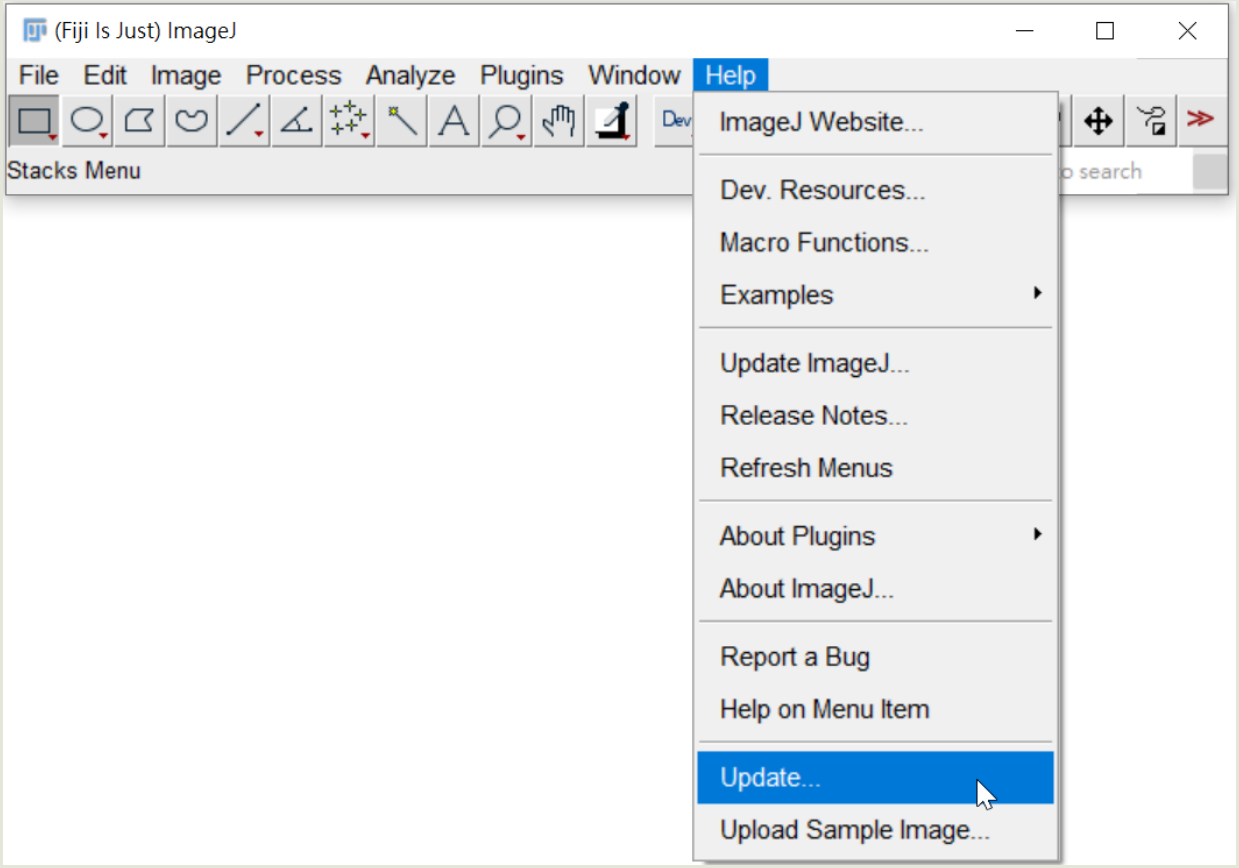


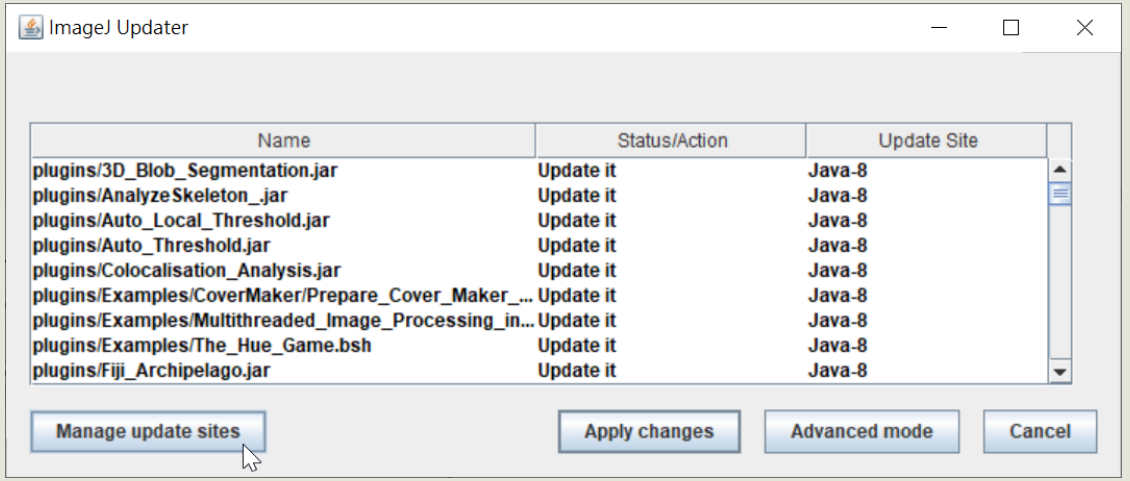
- It shows you where the plugin is located
- You can run it from here (press enter)

ImageJ : command finder (Hotkey “L”)



FIJI updater





- Keep a backup of working version before you update.

http://imagej.net/List_of_update_sites

Loading data and the image formats

Drag to FIJI and release

- Generic image format
- Folder (Import Image Sequence)
- ROI (.roi, .zip)
- Macro files (.ijm)

Bio-Format Importer

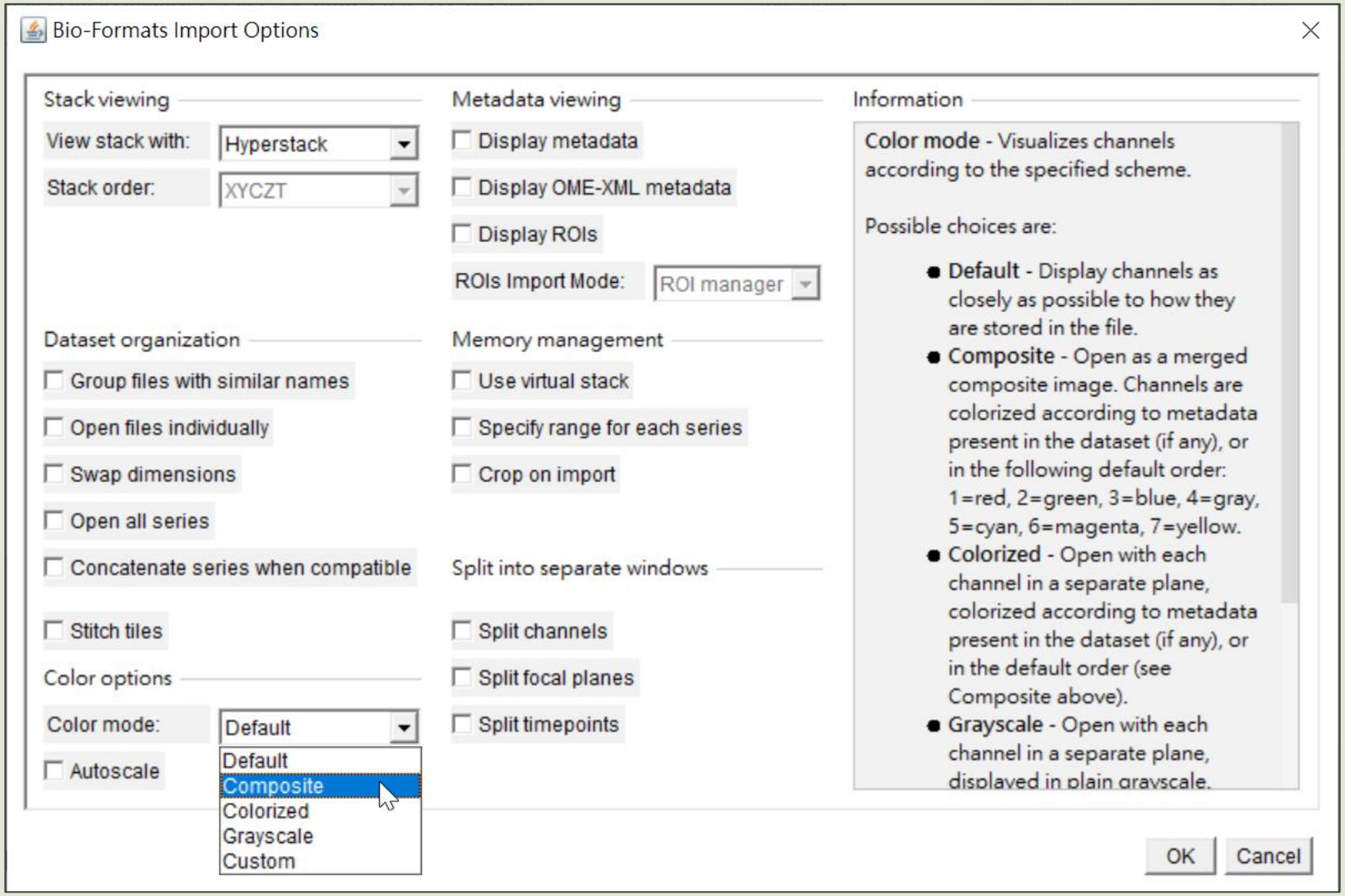
- Vender specific image format(.czi, .lif, .oir…etc)
- Huygens deconvoluted image ICS2 format (.ics)
- Big Data (Use Virtual Stack)
- Grouped generic image format
- More details in the measurement “label” (Channel, Z, T, ROI)

Generic image format

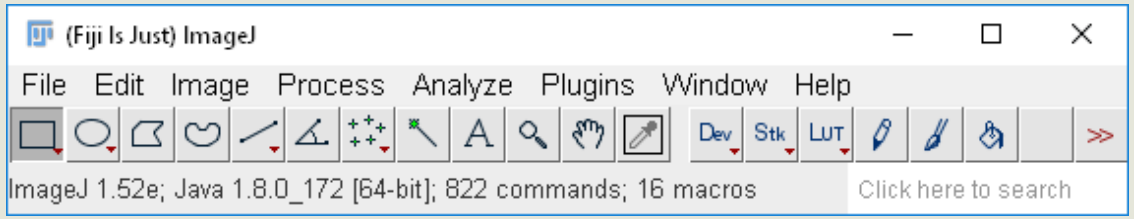
- Tiff/ Tif: 8 bits, 16 bits, 32 bits per channel
  24 bits RGB
  Can keep part of metadata (e.g. Pixel Size)
- Png: RGB mode only, metadata loss,
  (no stacks possible)
- Jpg: lossy compression
- Zip: compressed Tiff (lossless)

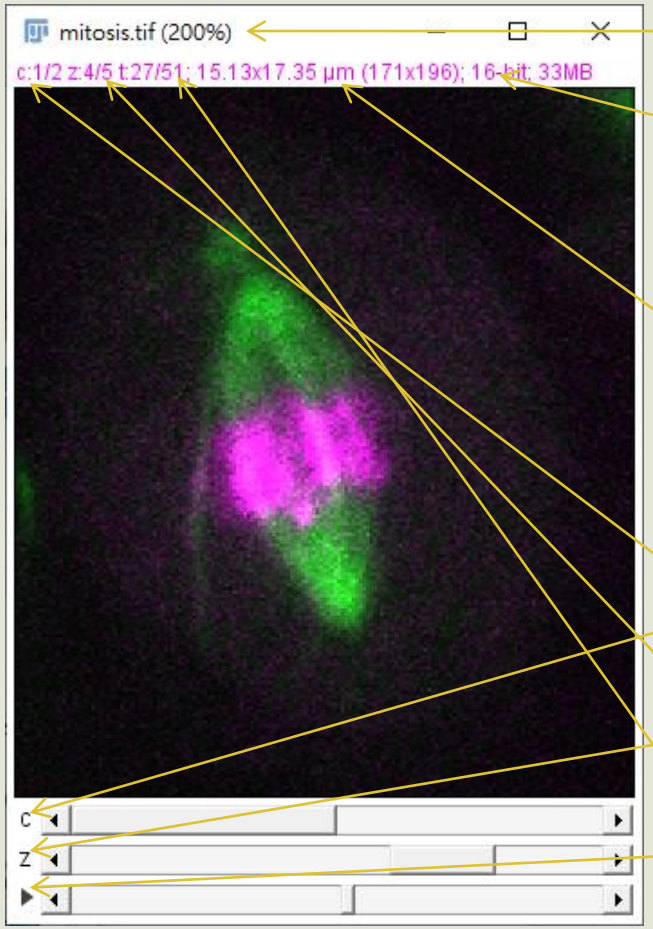
Bio-formats importer

- **Composite** is useful for multi-color imaging
- **Autoscale** -> similar to auto contrast
- **Display OME-XML metadata**: easier to read image acquisition settings.
- **Virtual Stack** -> useful for large image
(Lazy loading)



Fiji user interface





Zoom factor

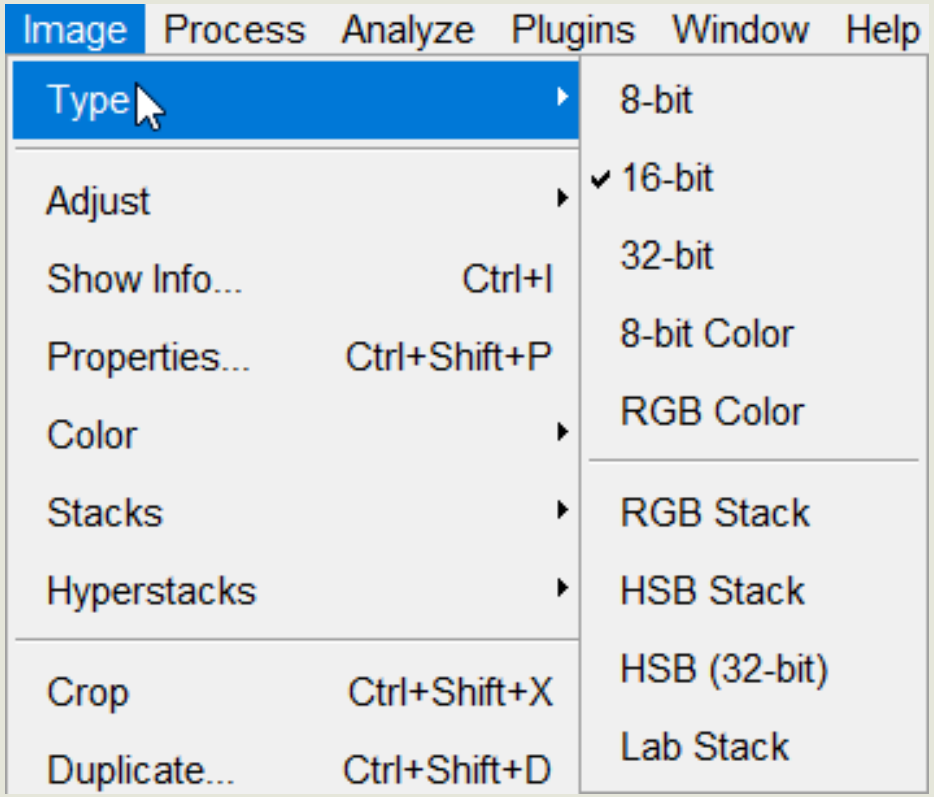
File Type

Image size in physical units (and in pixels)

Current color channel

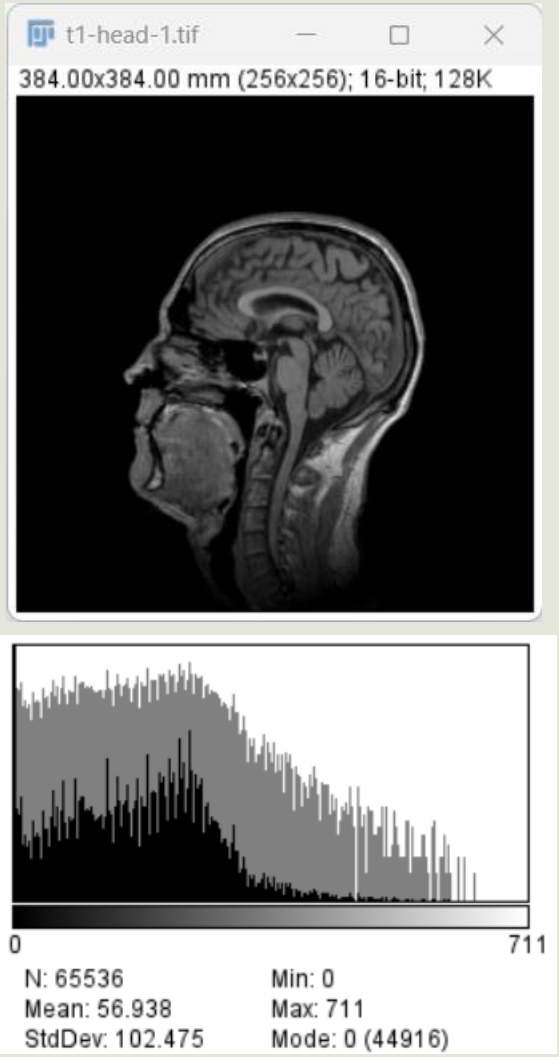
Current slice-position in space

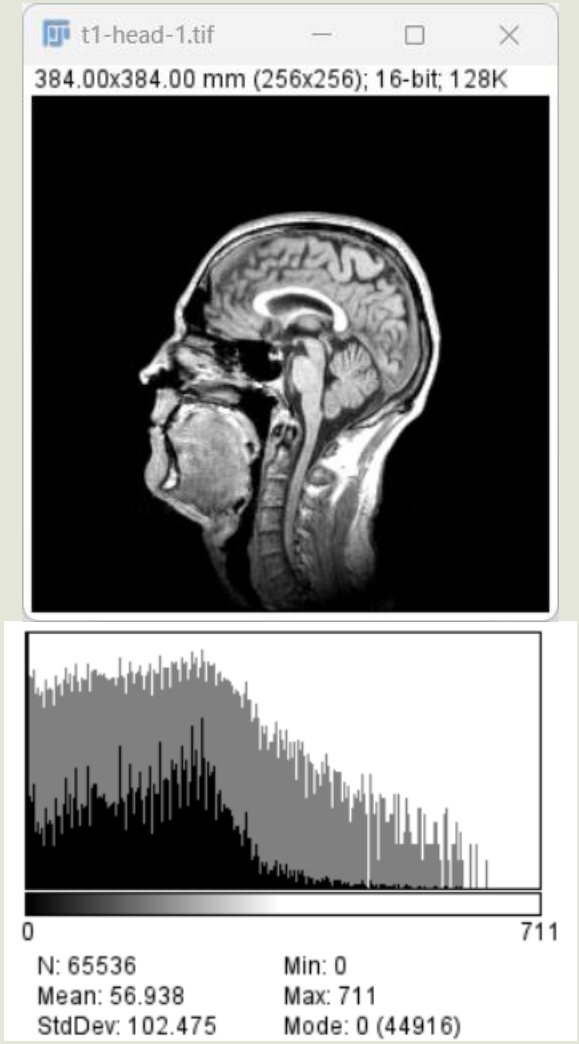
Current position in time (frame number)

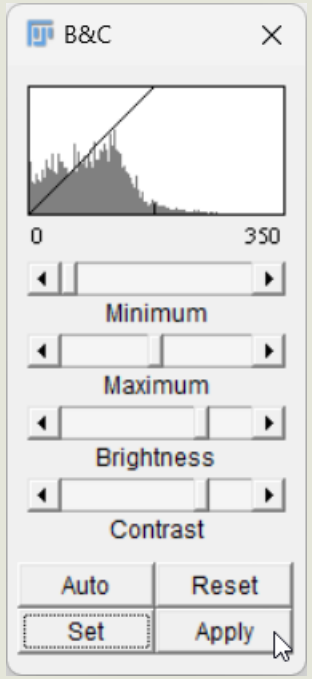


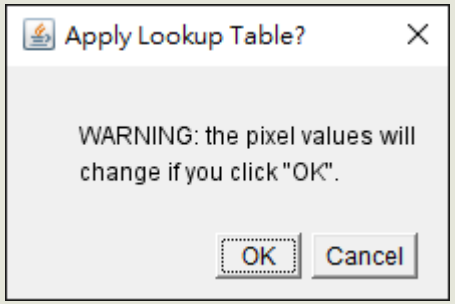
Only 1 active window
Only 1 activate channel

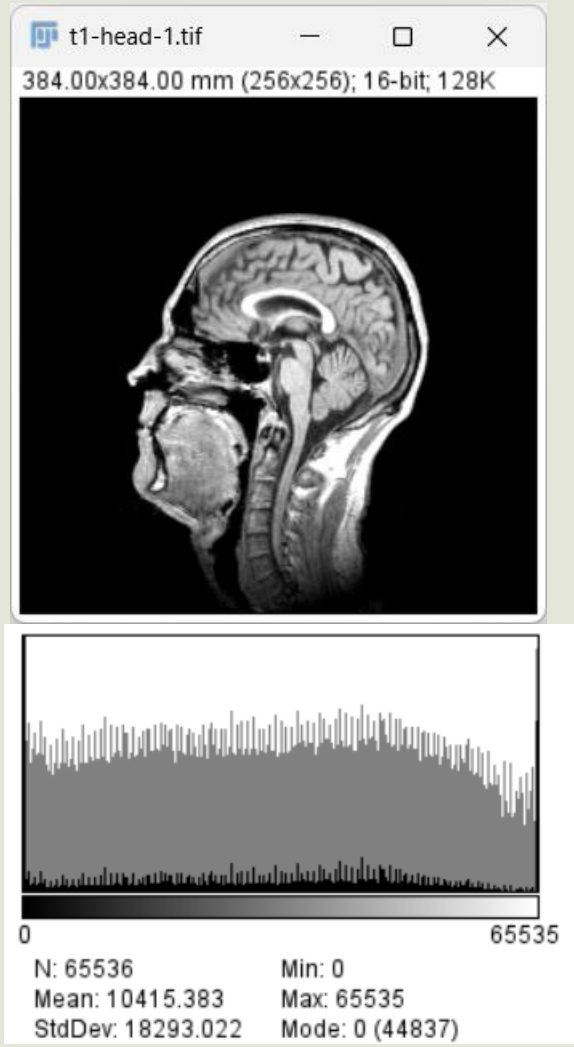
Don’t “Apply” when you adjust the Brightness & Contrast



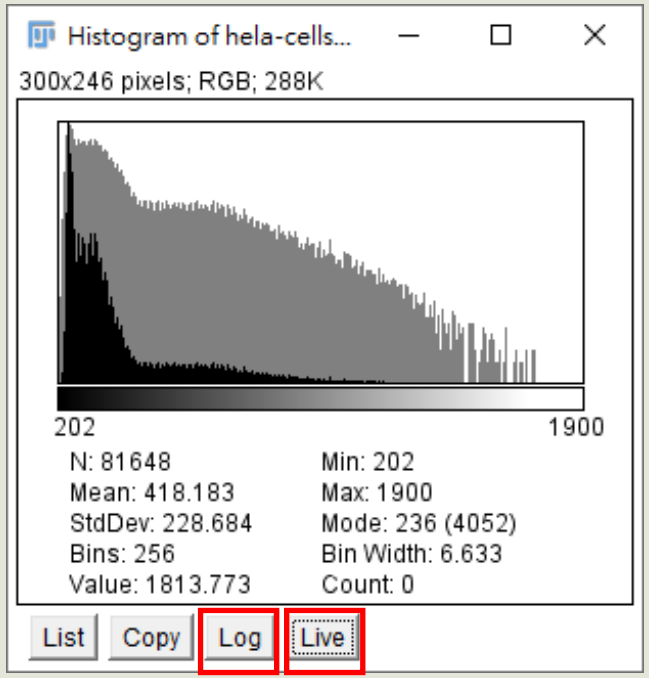






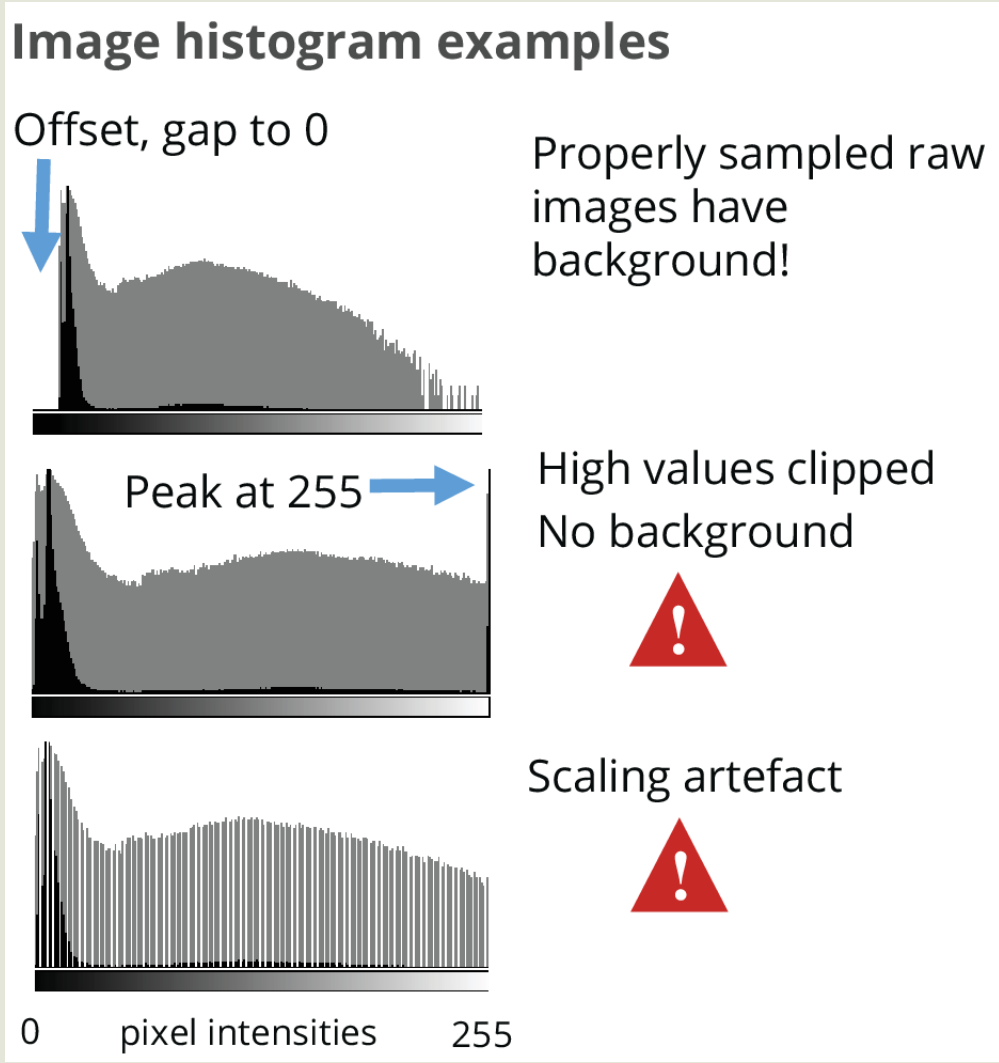


Histogram

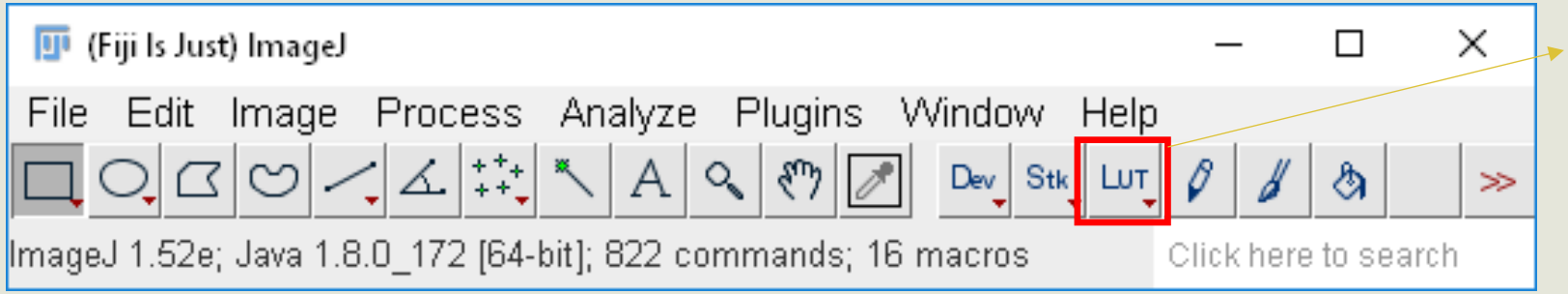


log scale

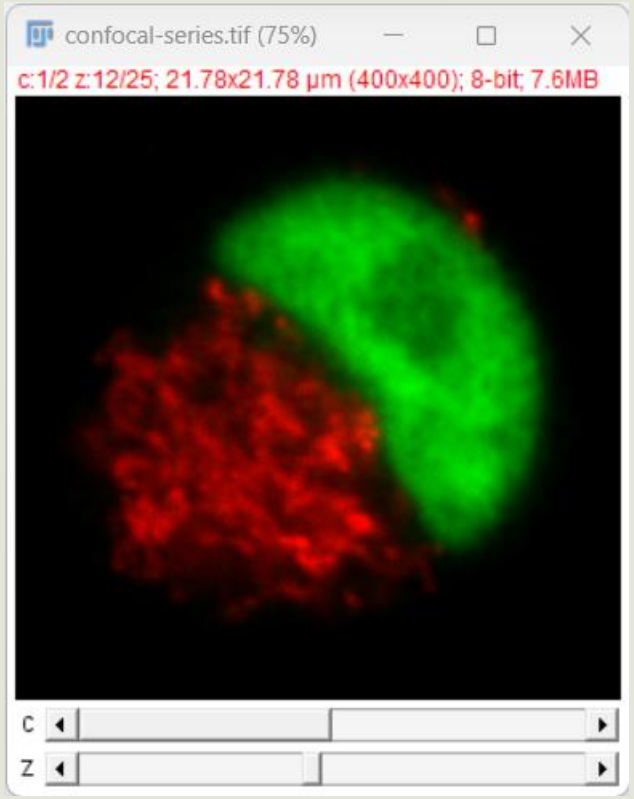
normal view

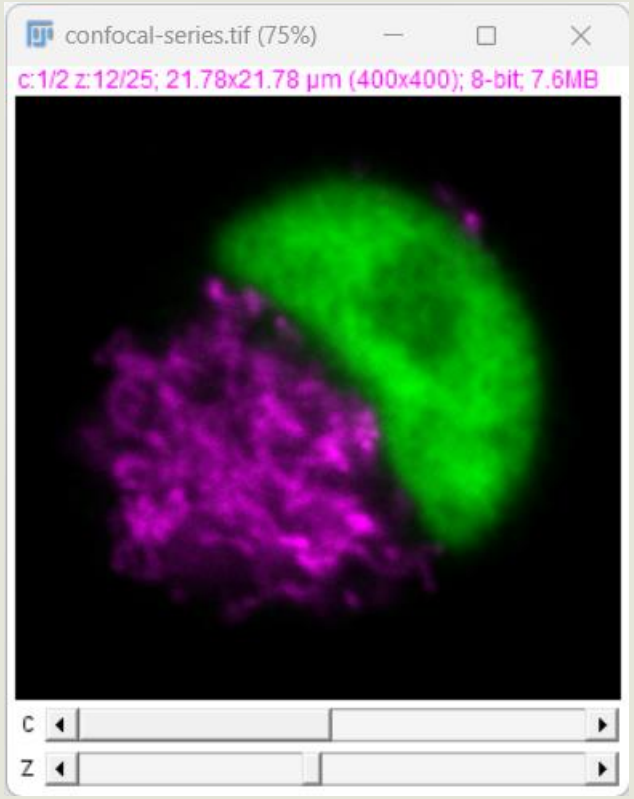


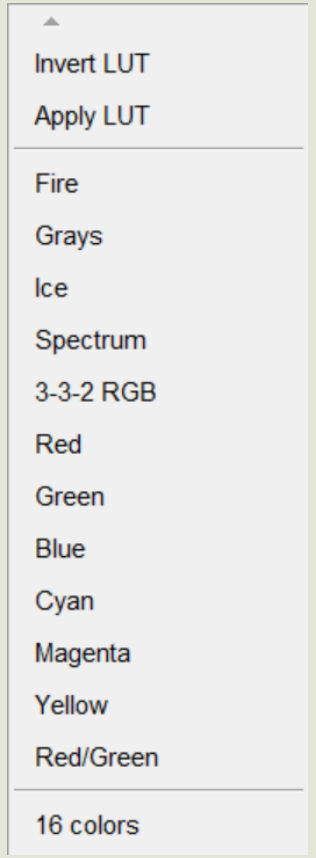
Change color (LUT /Pseudo-colors / Colormaps)



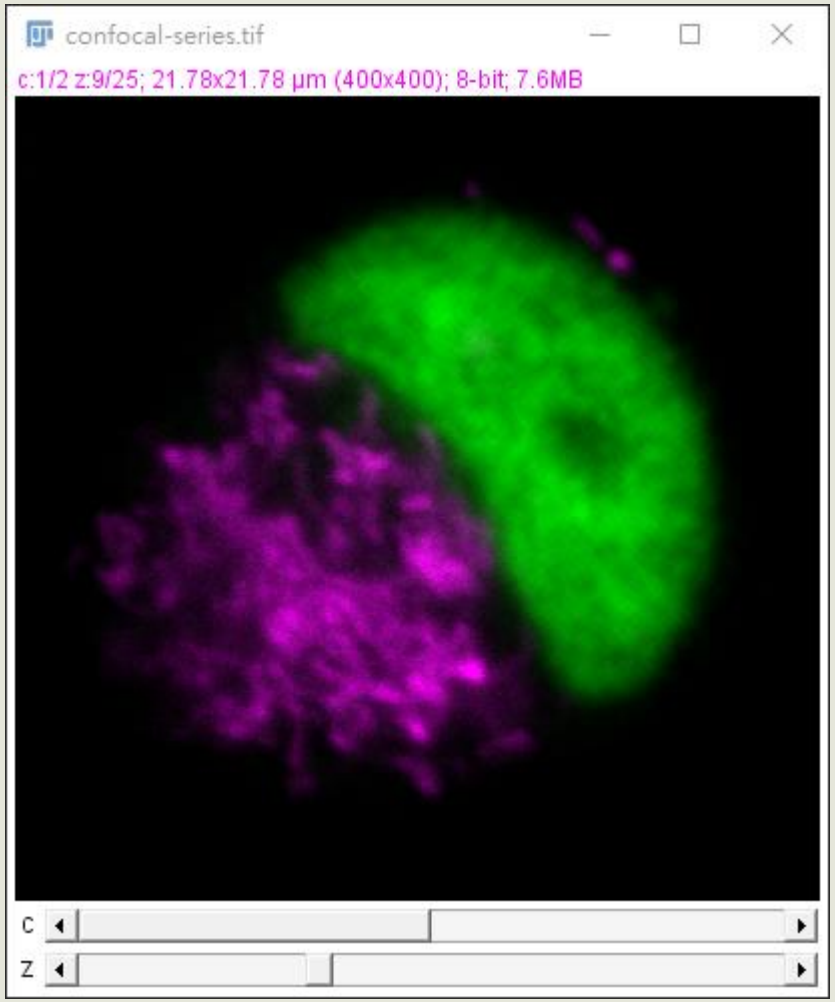
- **LUT button**
- **Image -> Lookup tables**

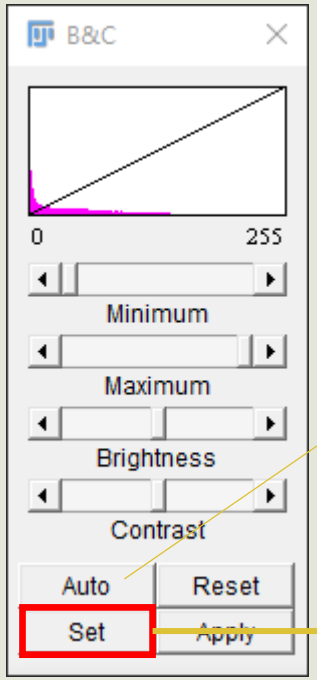




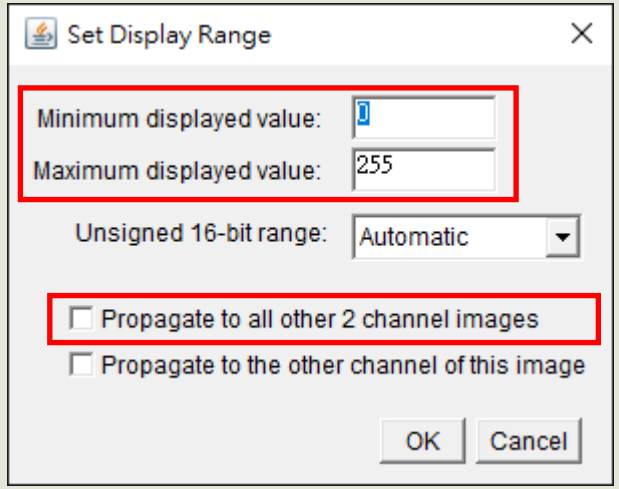


Use the same display range for the comparable images



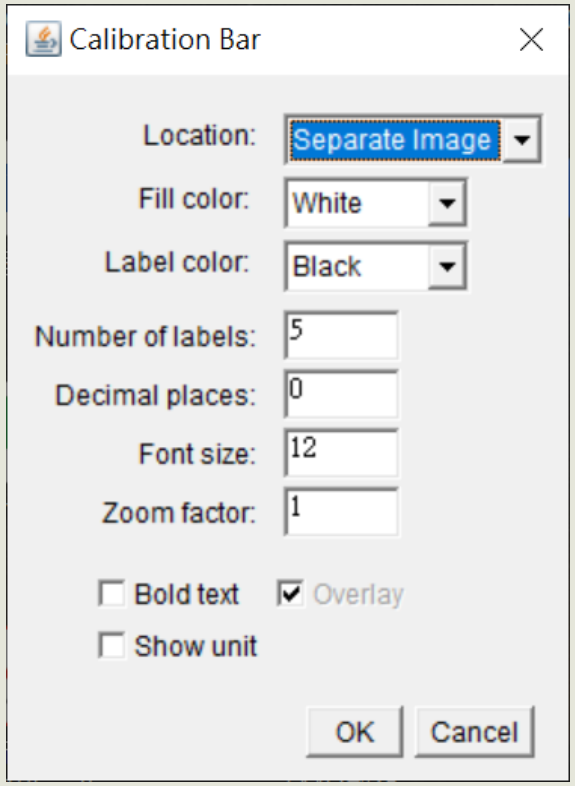


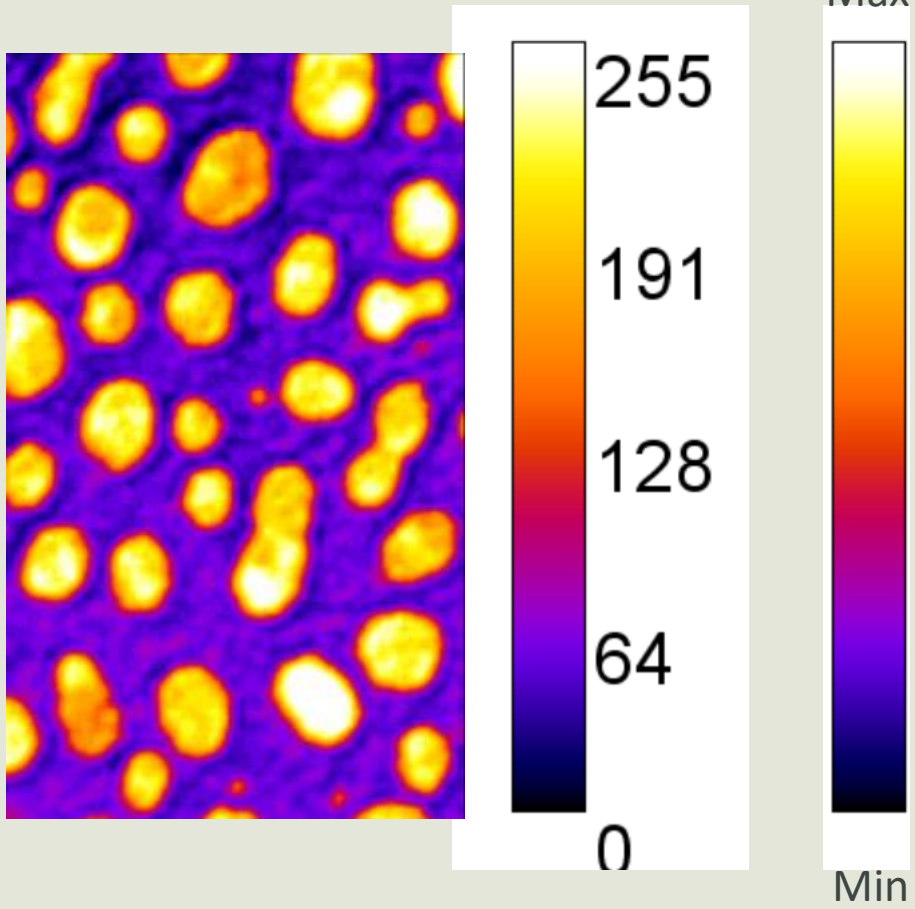
Don’t use auto function independently on each images!



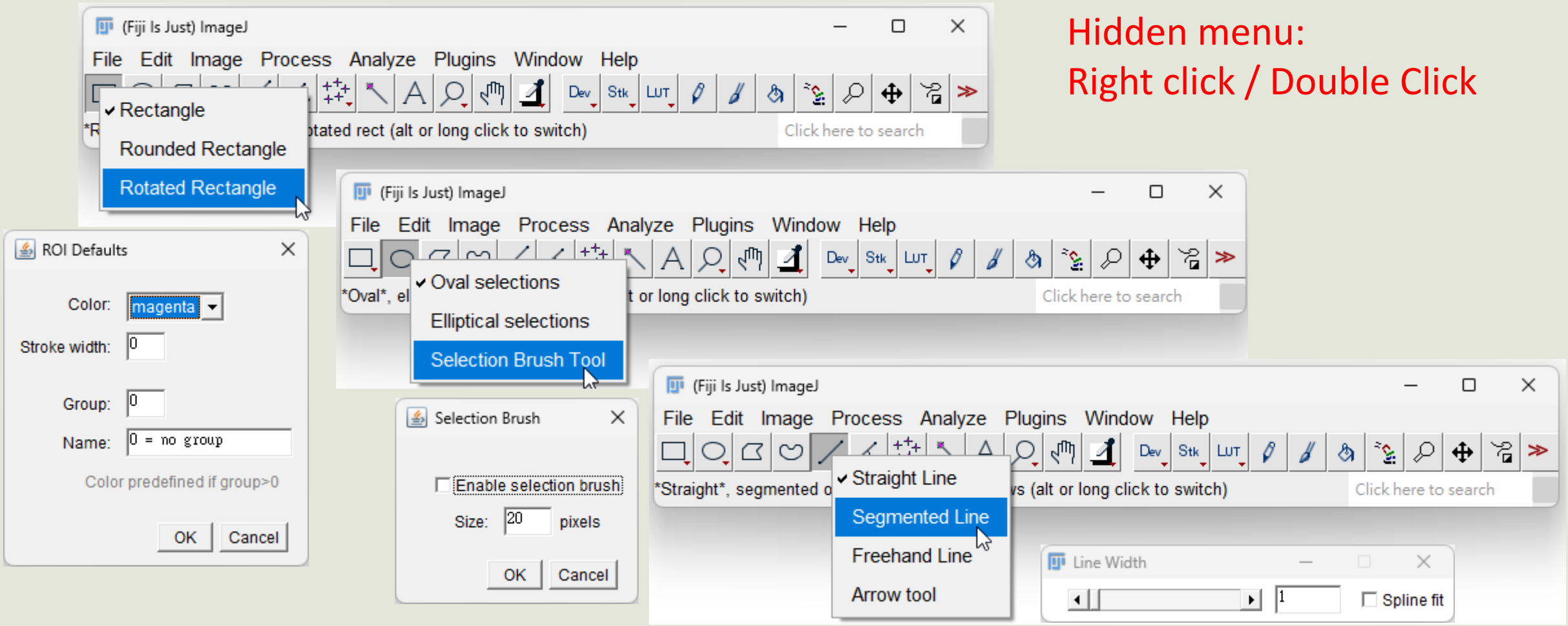
Calibration Bar (Color scale)

- Analysis -> Tools -> Calibration Bar
- Only work for single channel image
- Brightness and Contrast setting affect the Calibration Bar!





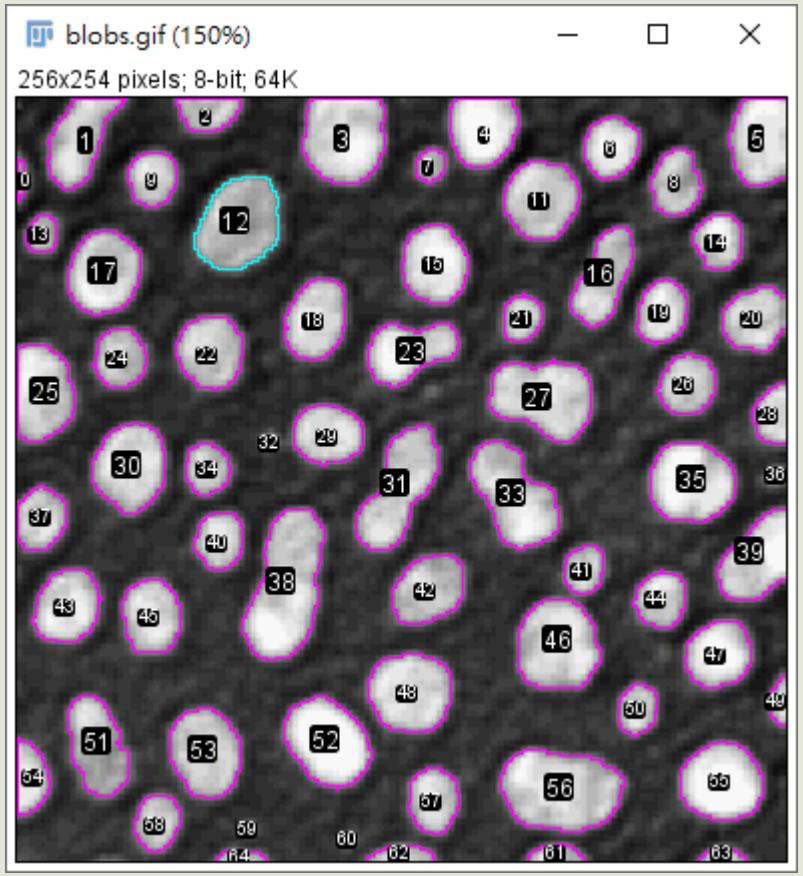
Region of Interest (ROI) Tools

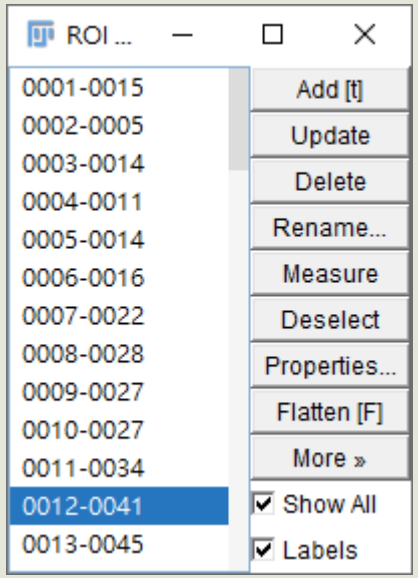


Hidden menu:
Right click / Double Click

ROI manager

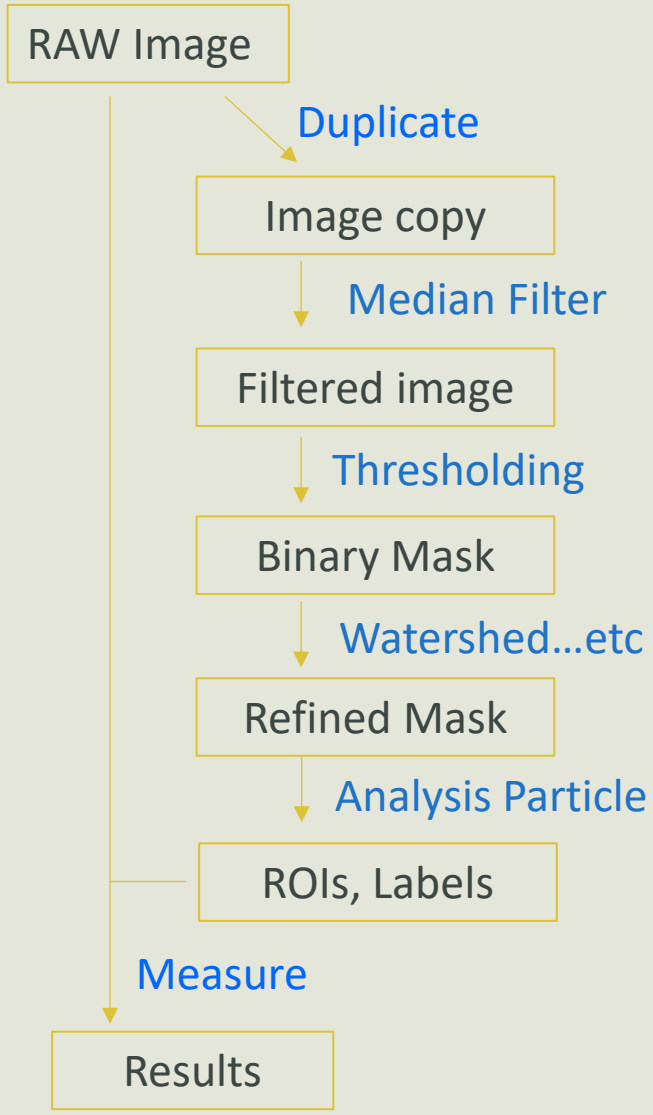
- Analysis -> Tools -> ROI Manag

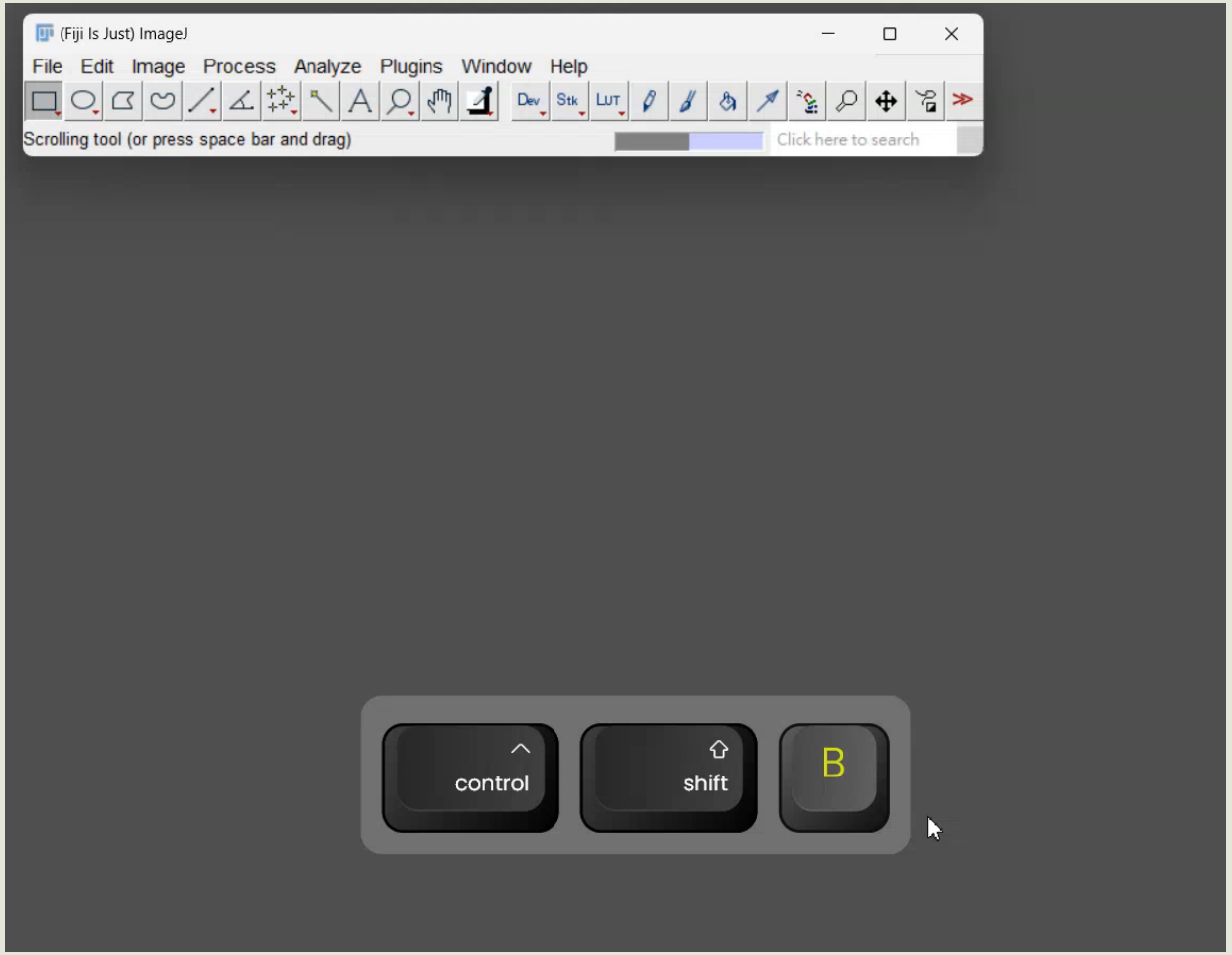




- Save the ROI files is important!
- Single ROI: *.roi
- Multiple ROI: *.zip

Segmentation Sample





Acknowledgements

The bioimage community (**EABIAS)** inspires this workshop. https://eabias.github.io/

課後意見調查

國立臺灣大學 National Taiwan University　臺大醫學院 National Taiwan University College of Medicine　NSTC 國家科學及技術委員會 National Science and Technology Council　中央研究院 Academia Sinica　IBC