



臺大醫學院研發分處 第一共同研究室顯微影像核心

# IMAGEJ顯微影像分析 與程式設計

零基礎的學生也能掌握基本顯微影像分析能力



海報網址

2025 3.3-4.28 周一 13:30-14:30 共7堂  
影像前處理、AI應用、自動化分析

課程資訊 及 授課教師

2025/3/3(一) 【生物影像分析概論】  
溫榮崑 中央研究院 生化所 生物影像核心設施  
研究助理師

2025/3/10(一) 【生物影像流程與小組討論編組】  
許紹君 臺灣大學分子影像重點技術平台  
助研究專家

2025/3/17(一) 【影像分析自動化】  
張仁乾 日本理化學研究所  
專門技術員

2025/3/24(一) 【互動式影像分析流程建立】  
朱韋臣 中央研究院 細生所 公共儀器室影像組  
專案研發學者

2025/3/31(一) 【物件追蹤分析】  
黃紀穎 中央研究院 植微所 細胞核心實驗室光學顯微鏡組  
專案研究人員

2025/4/7(一) 【AI: 機器學習與深度學習工具介紹】  
羅安琦 臺灣大學分子影像重點技術平台  
副技師

2025/4/28(一) 小組發表  
許紹君 臺灣大學分子影像重點技術平台 助研究專家  
朱韋臣 中央研究院 細生所共儀影像組 專案研發學者

主辦單位：臺大醫學院研發分處 第一共同研究室顯微影像核心  
協辦單位：中央研究院 生物化學研究所  
地點：基醫大樓講堂區 5 樓 未來教室 (原508教室)

課程簡介  
本課程將介紹生物影像的基本元素、如何利用FIJI進行影像前處理、影像切割、特徵萃取、程式設計與編程、互動式影像分析流程與GPU加速、AI(機器學習與深度學習工具)、物件追蹤、常用的資料庫以及如何分享自己的作品。將視報名人數進行小組發表與討論，利用工作中學習的方式提升課程效果。

## 課程目標

希望零基礎的學生參與課程後，都能具備基本分析顯微影像的能力。

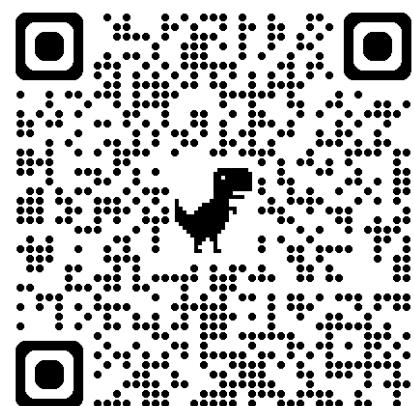
## 上課須知

- 即日起開放報名，報名方式如下：
  - 提供姓名、EMAIL、任職/就學單位、實驗室主持人姓名。
  - 以一張A4篇幅文字說明實驗目的與欲解決的問題，並以一張投影片頁面作為輔助材料。
- 優先錄取自備影像分析問題並想透過課程學會如何自己解決問題者。
- 課程會同步紀錄影音並於課後上傳至教學影音平台。
- 每堂課皆會點名，上課出勤不得缺課超過一堂。
- 需自備筆電。

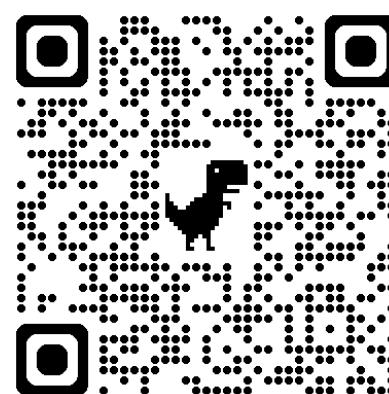
招生人數：實體招收24人、線上30人。  
報名截止日：額滿為止，恕不開放現場候補。  
聯絡人：第一共同研顯微影像核心 林惠廷 szuting@ntu.edu.tw

## 上課注意事項：

- 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。
- 請實體與線上學員掃描以下QR code進行線上簽到。
- 請線上學員於課程開始前關閉自己的麥克風。
- 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。
- 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



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# East Asia Bioimage Analysts' Society (EABIAS)

臺大醫學院研發分處 第一共同研究室顯微影像核心

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聯絡人：第一共同研究室顯微影像核心 林思廷 [szuting@ntu.edu.tw](mailto:szuting@ntu.edu.tw)

National Taiwan University College of Medicine

National Science and Technology Council

NSC

IBS

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

EABIAS (zh-TW)

Home About EABIAS Activities Resources

Home

## Home

歡迎來到 EABIAS ( 東亞生物影像分析協會 )

EABIAS 是一個開放且包容的社群，歡迎所有對生物影像分析感興趣的人士加入。

目標：

社群建立：聯繫對生物影像分析充滿熱情的科學家、工程師和開發者。

知識分享：提供資源、教程和活動，以促進生物影像社群的發展。

工具開發：推動開源工具的創建和改進，應對生物影像分析中的挑戰。

我們誠邀個人及機構加入我們，共同推進生物影像分析領域的發展。貢獻您的專業知識，向他人學習，並成為致力於創新和發現的成長型社群的一部分。

請瀏覽我們的網站，了解更多資訊以及即將舉行的活動更新。

目錄

GloBIAS 2025 in Kobe, Japan

ImageJ Workshop in Taipei (2025)

# 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)

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**Dr. Robert Haase**

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



**Dr. Peter Bankhead**

Center for Genomic & Experimental Medicine, University of Edinburgh, UK  
  
[Introduction to Bioimage Analysis \(2022\)](#)  
<https://bioimagebook.github.io/>



**Dr. Wei-Chen Chu, ICOB**

Imaging Core Facility Manager (ICOB)

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

### BioImage Analysis Workshop

**Huang, Cheng-Yu (Kou)**

Ph.D. Candidate

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

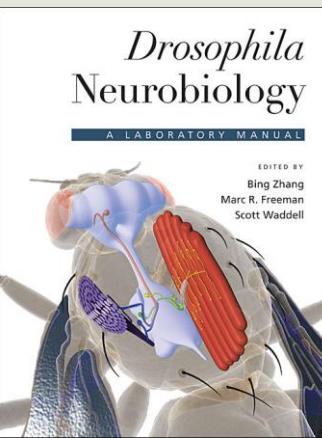
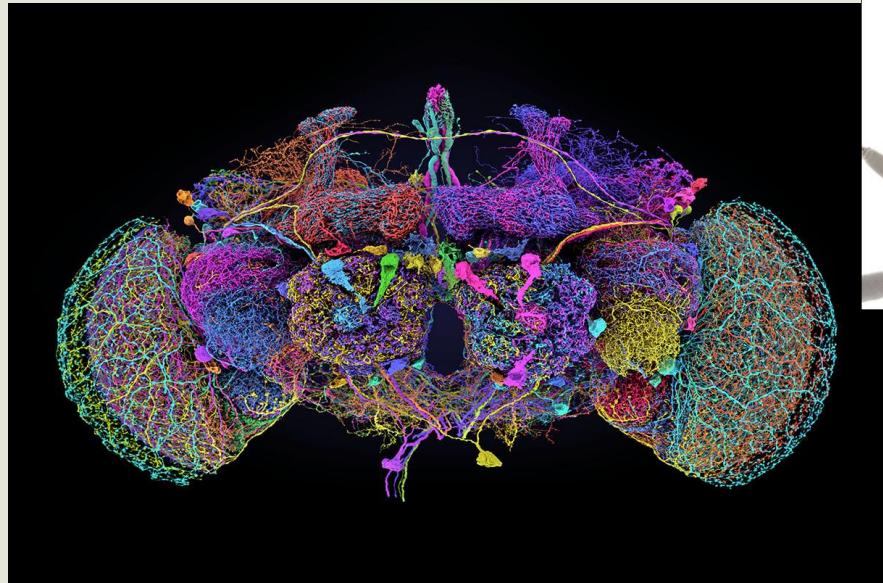
## Why Bio-imaging technology is so important to modern scientific research?

Explain phenomenon?  
Awesome image?

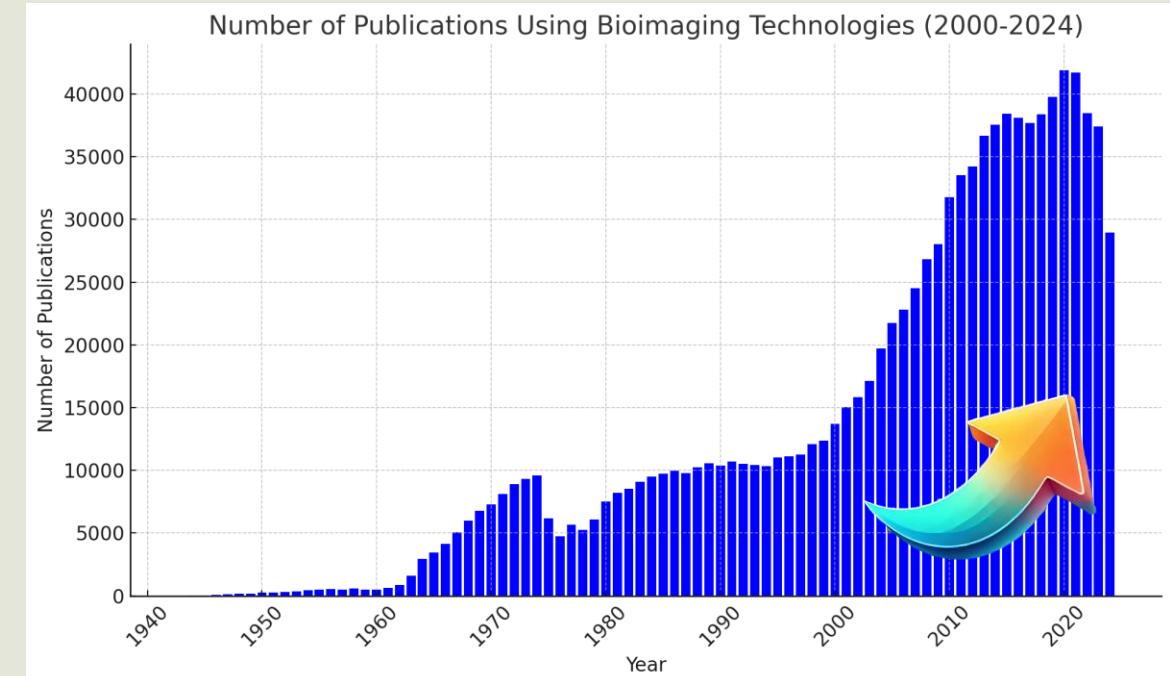


Make records ?  
Generate data ?

# Visualization and Quantification in Life Sciences



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



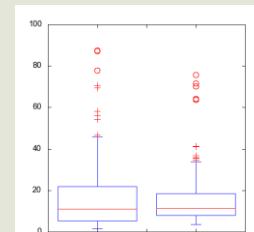
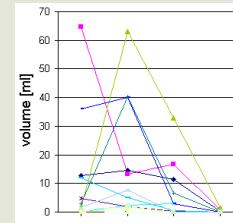
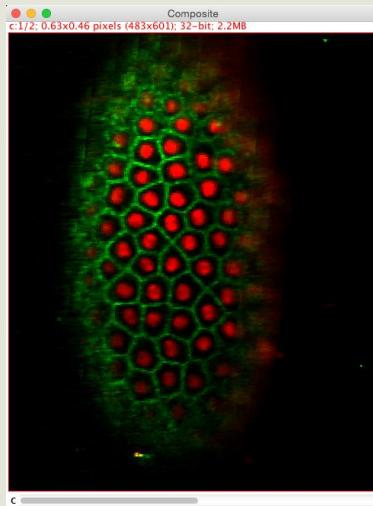
PubMed

# Bioimage analysis

## Bioimage Analysis?

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

Bioimages → Numbers → Knowledge



Publications.

# Image analysis software universe

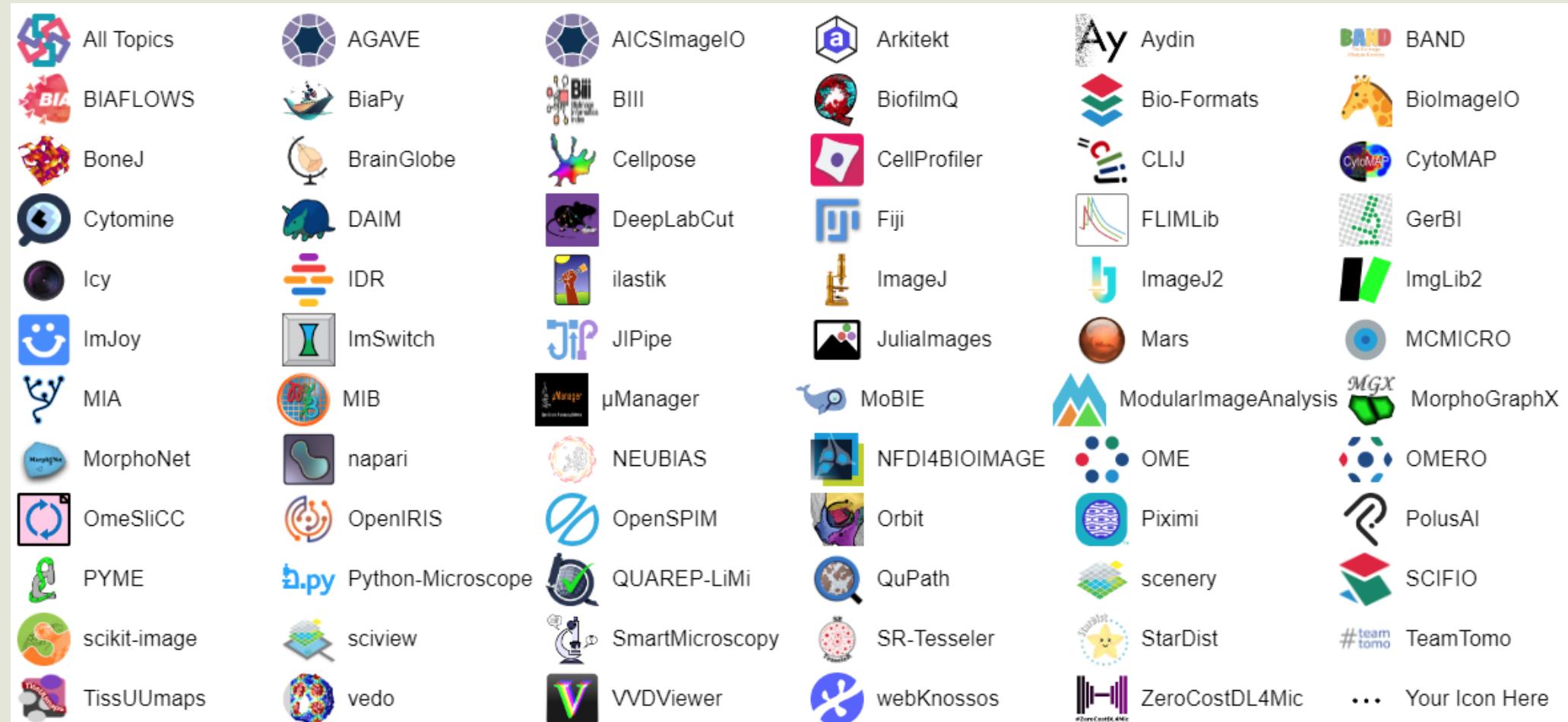
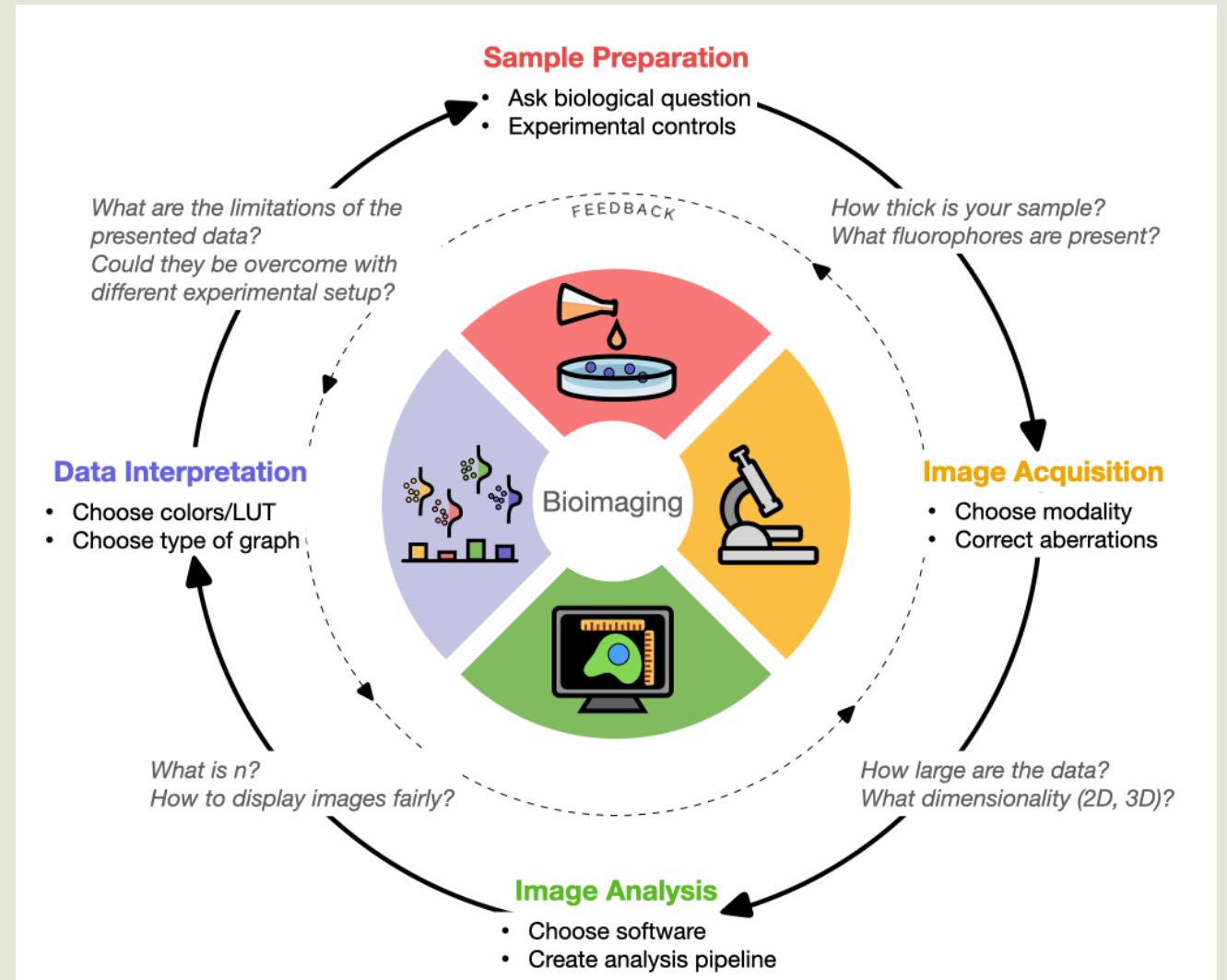
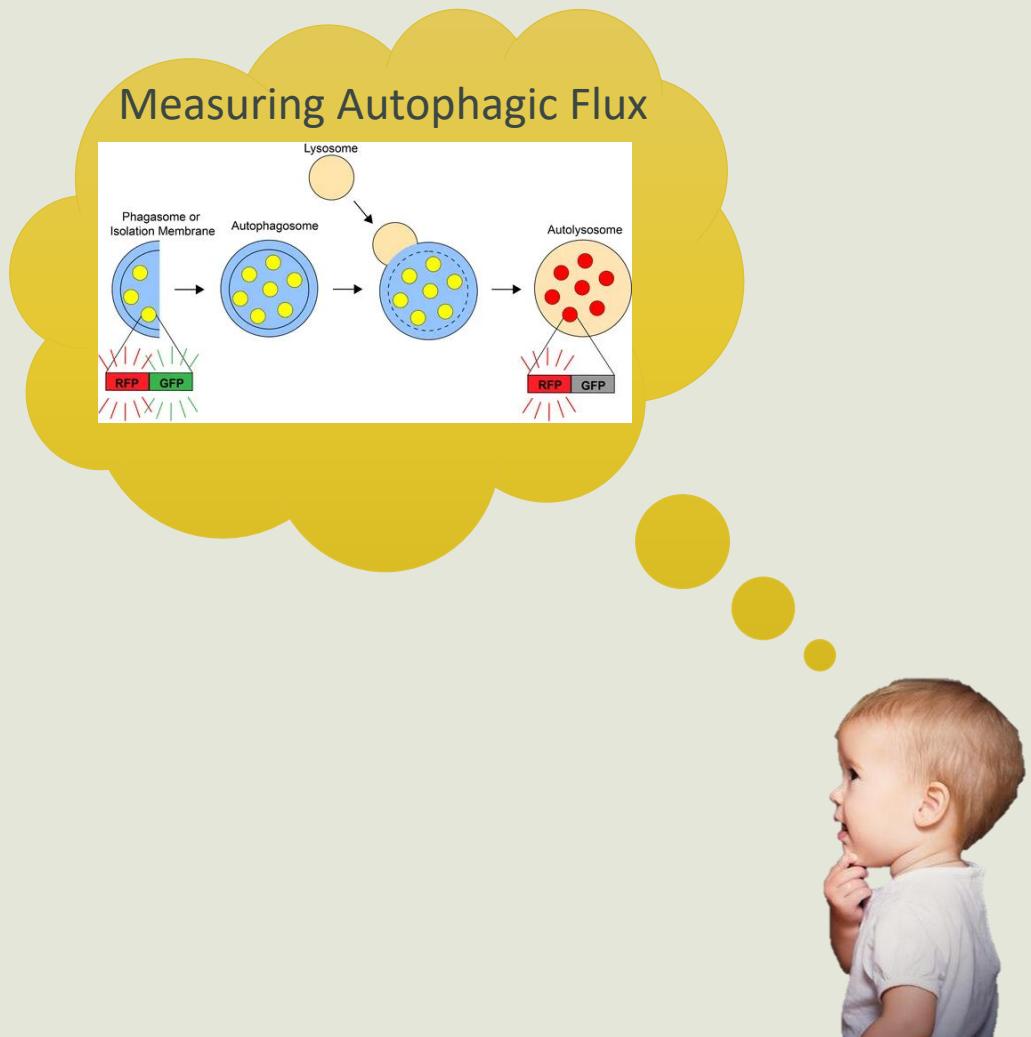
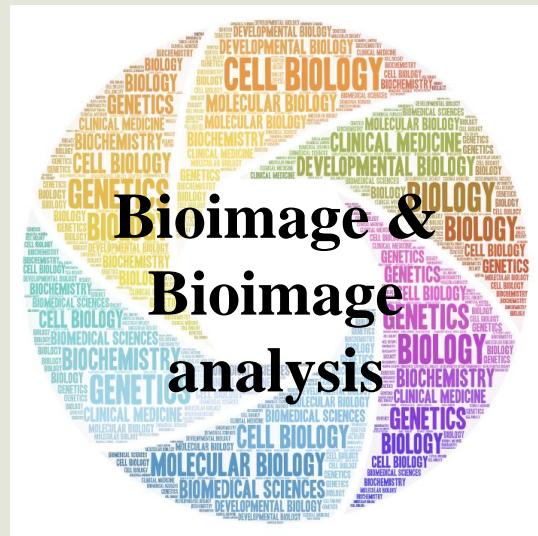


Image from <https://forum.image.sc/>

# The steps of bioimaging experiment



# 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.

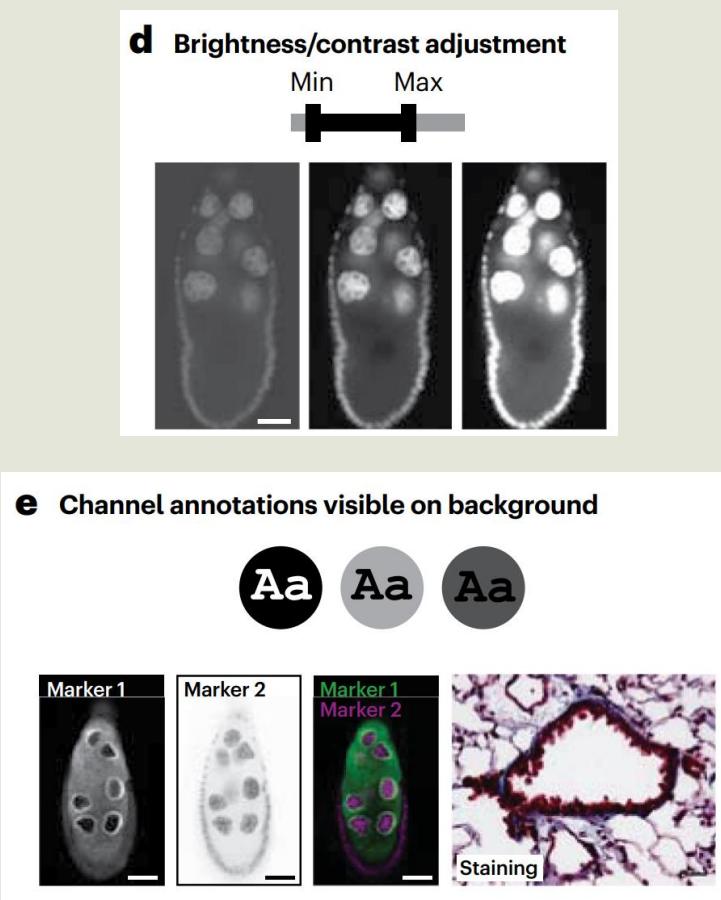
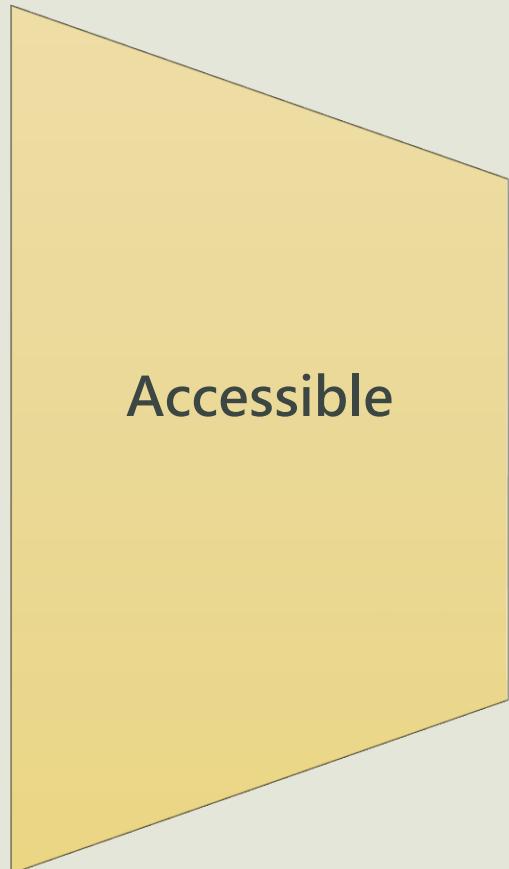
Accessible

Representative

Understandable

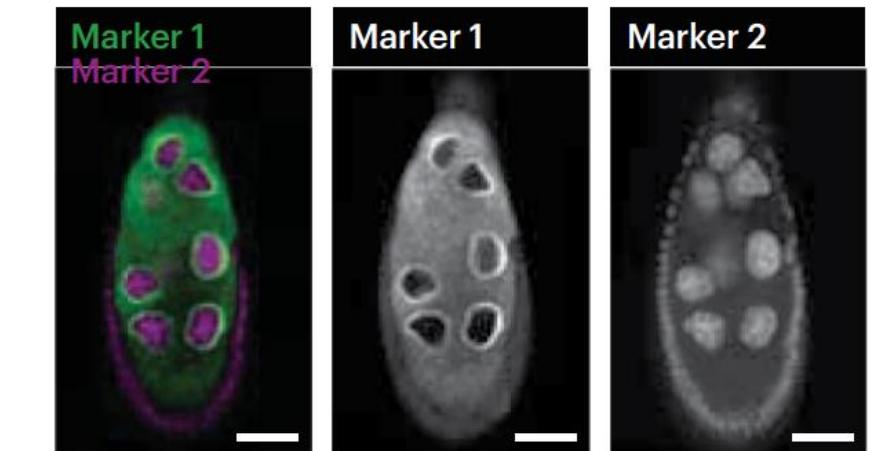
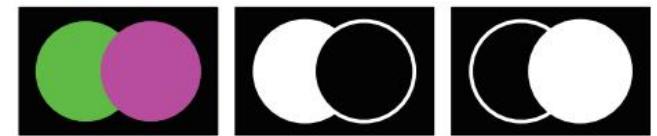
Reproducible

# Four elements of Bioimage analysis in publications.



Be friendly to colorblindness

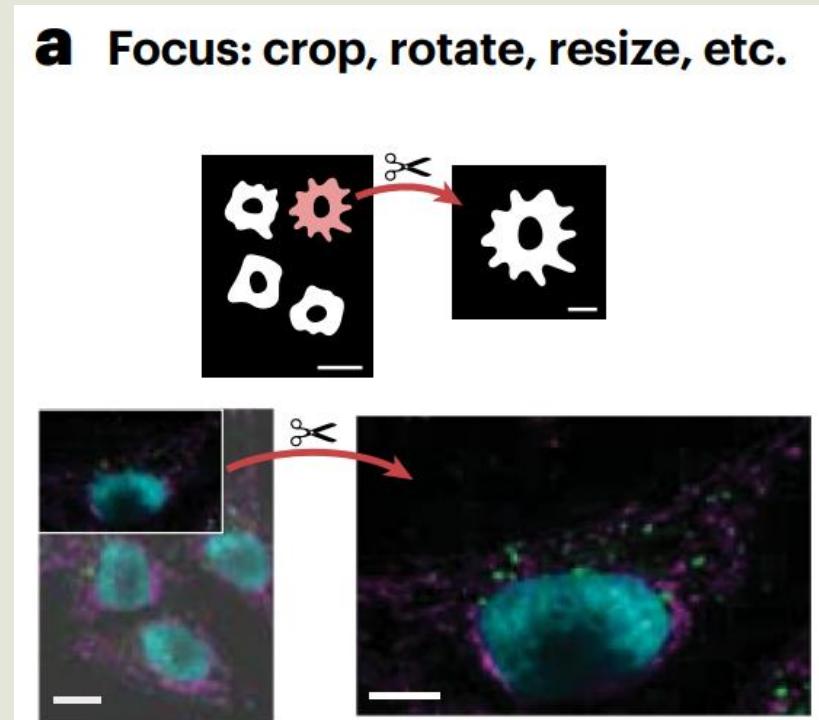
**f Grayscale channels improve visibility of image features**



Red/Green only: → Replace Red with Magenta

# Four elements of Bioimage analysis in publications.

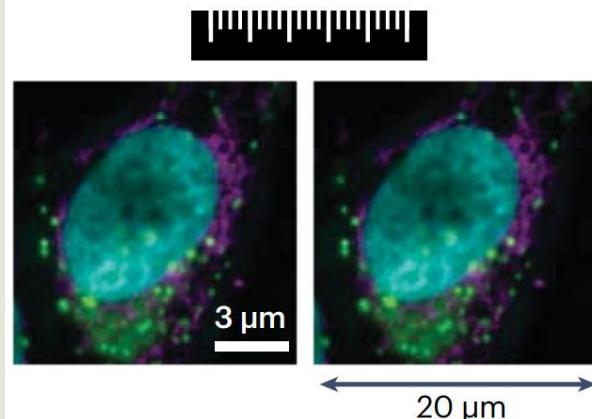
Representative



# Four elements of Bioimage analysis in publications.

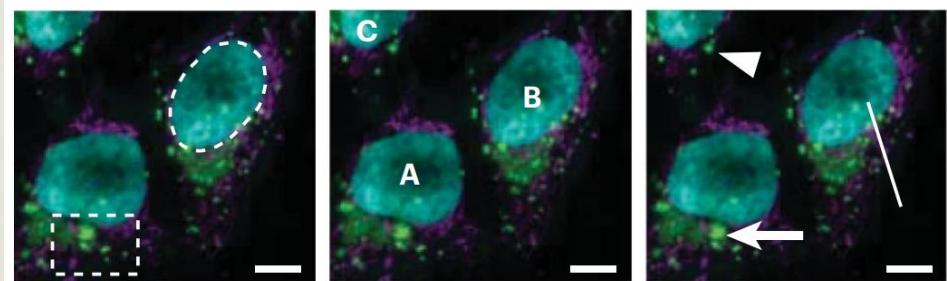
Understandable

## a Scale information

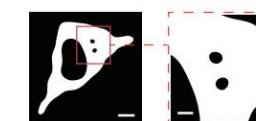


## b Annotations: explained, visible, not obscuring content

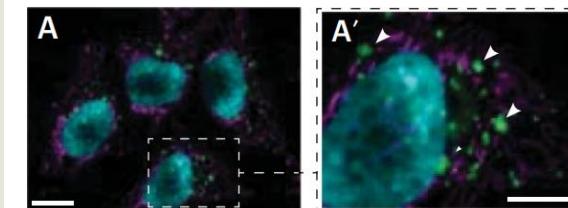
Aa



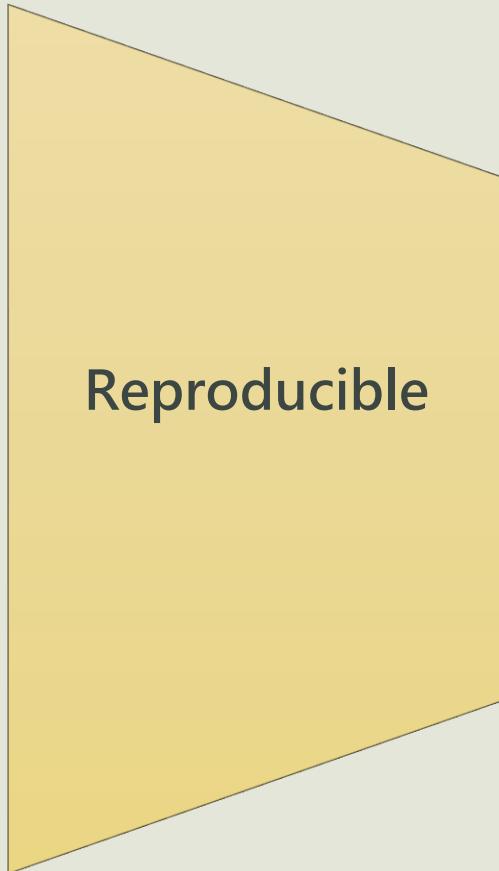
## c Show origin of inset Consider showing full-size image



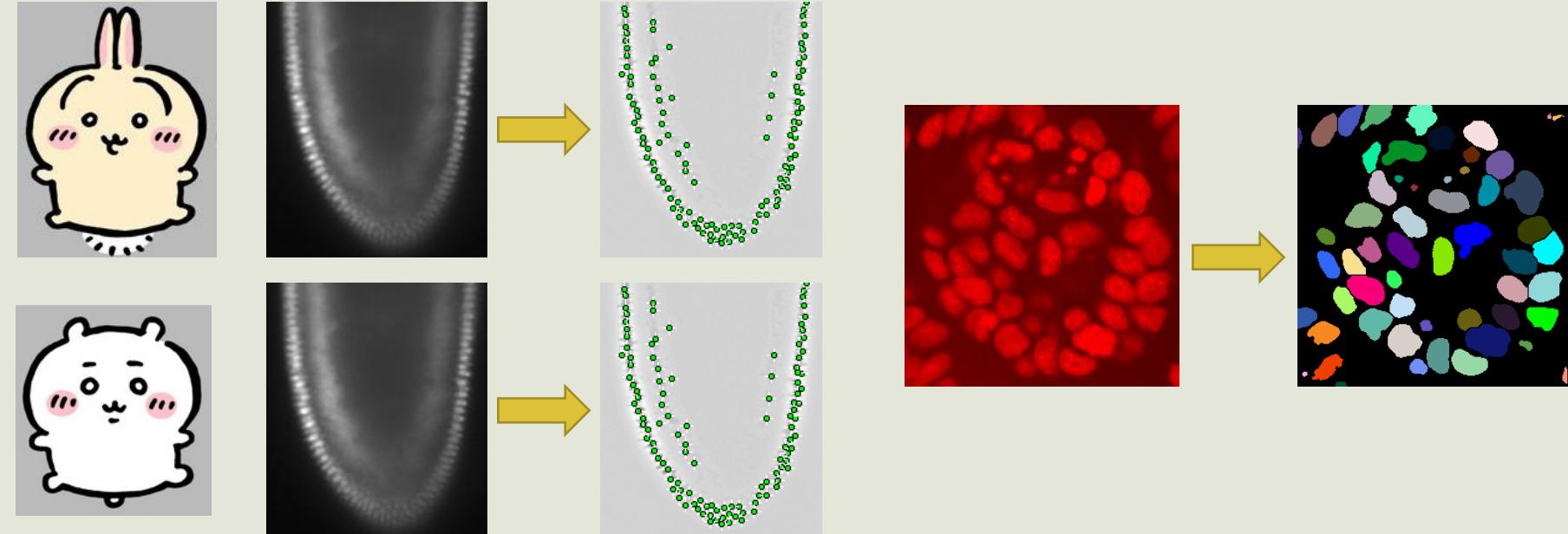
A



# Four elements of Bioimage analysis in publications.



- 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 colors and channels

|  |   |
|--|---|
|  | Annotation of channels (staining, marker etc.) visible                        |
|  | Adjust brightness/contrast, report adjustments, use uniform color-scales      |
|  | Image comparison: use same adjustments  |
|  | Multi-color images: accessible to color blind                                 |
|  | Channel color high visibility on background                                   |
|  | Provide grey-scale for each color channel                                     |
|  | Provide color scales for intensity values (greyscale, color, pseudo color...) |
|  | Pseudo-colored images: additionally provide greyscale version for comparison. |
|  | Gamma adjustments: additionally provide linear-adjusted image for comparison  |



Minimal



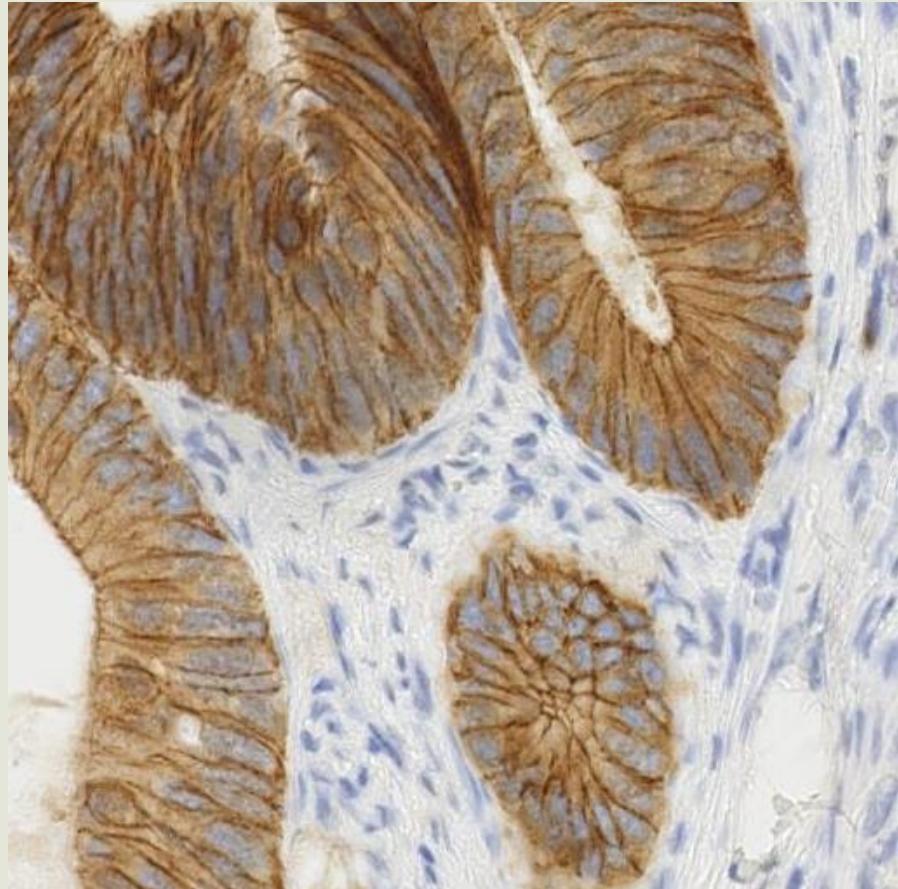
Recommended



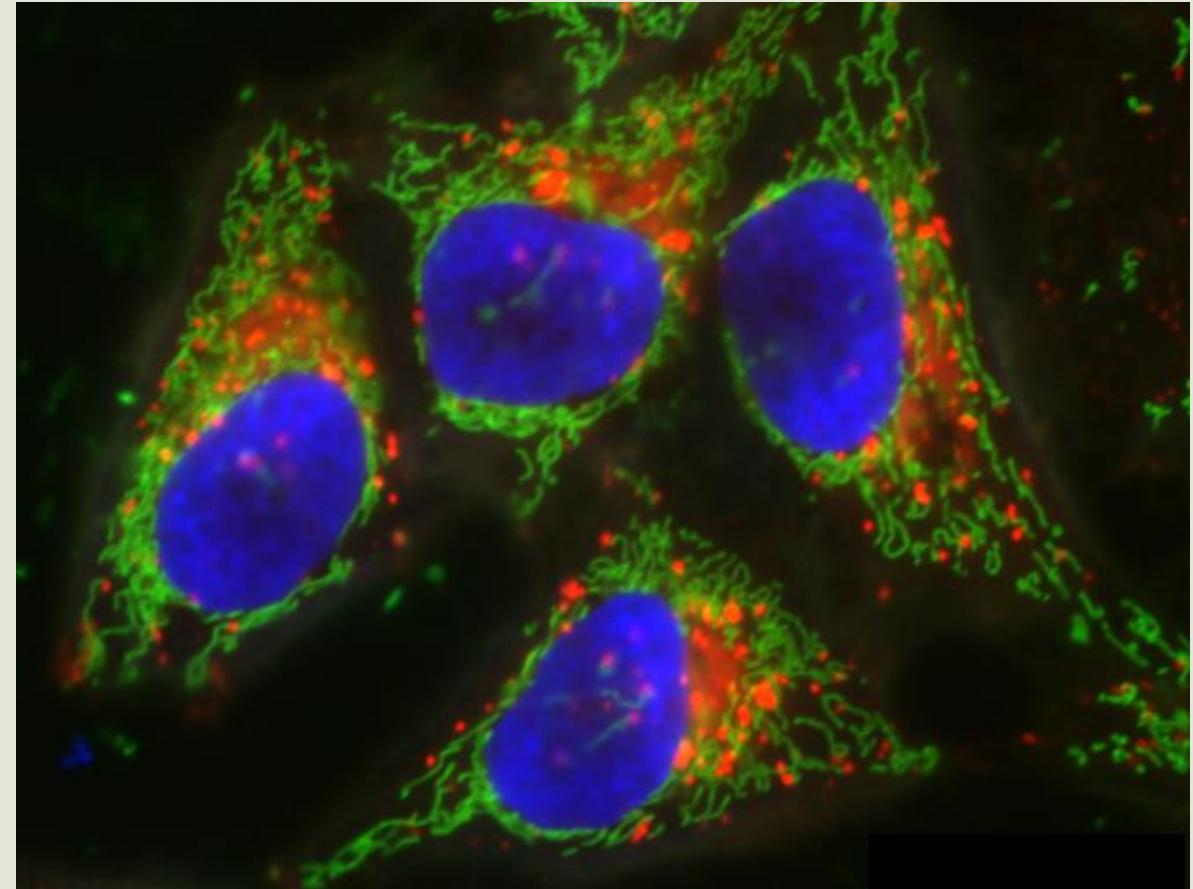
Ideal



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

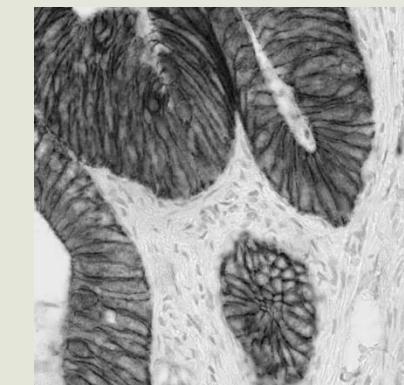
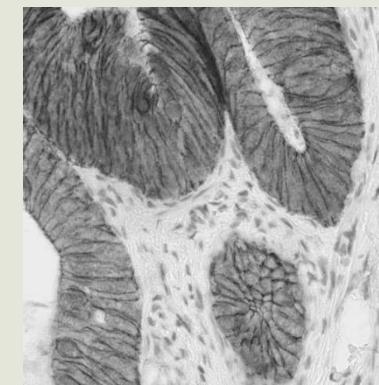
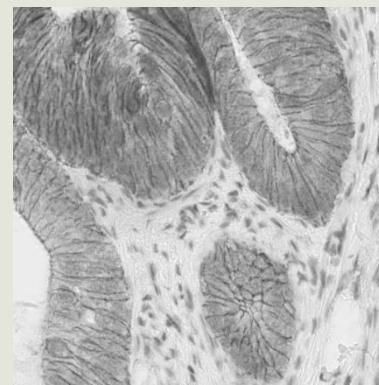
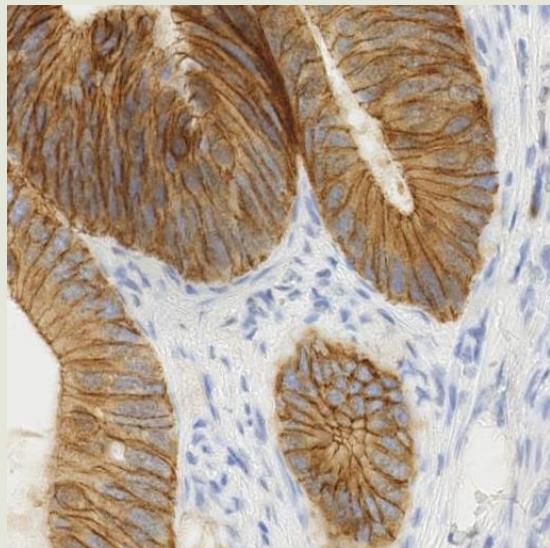


Transmitted light microscope

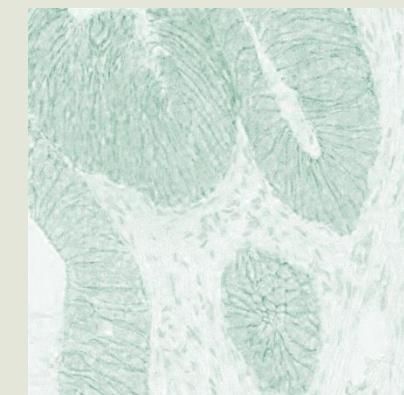
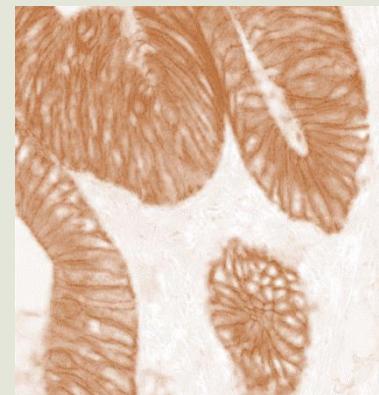
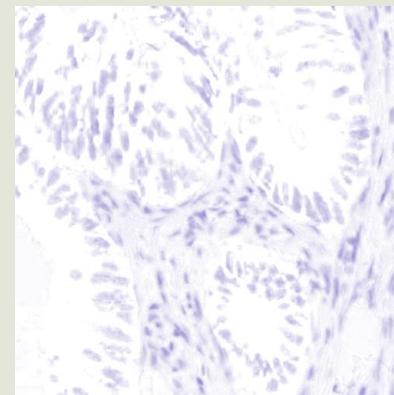


Fluorescence microscope

# Color Image (RGB format)



Color deconvolution



Source: Napari sample image

Slide adapted from Dr. Wei-Chen CHU , Bioimage Analysis with FIJI/ImageJ and Friends, licensed [CC BY 4.0](#)

# Fluorescence images

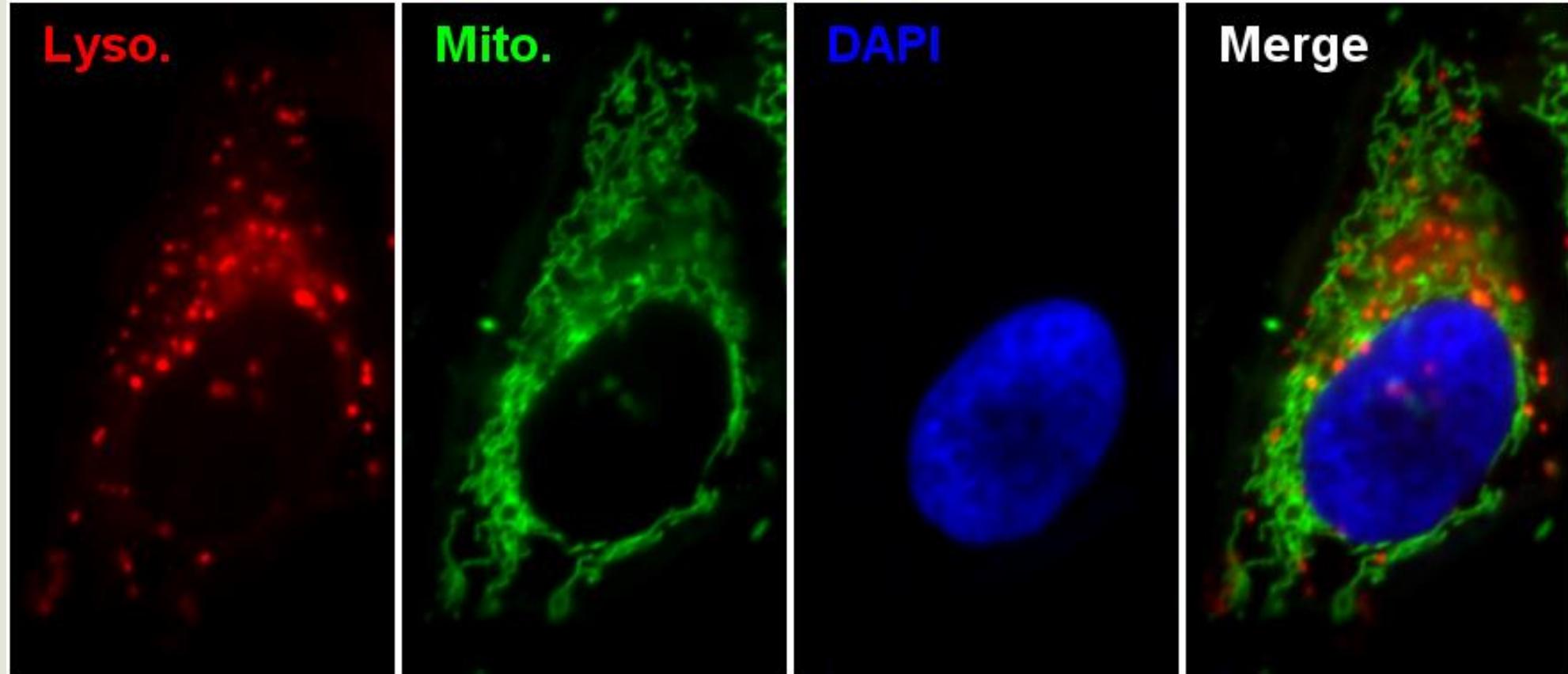


Image source: FIJI sample images (Hela cells, cropped)

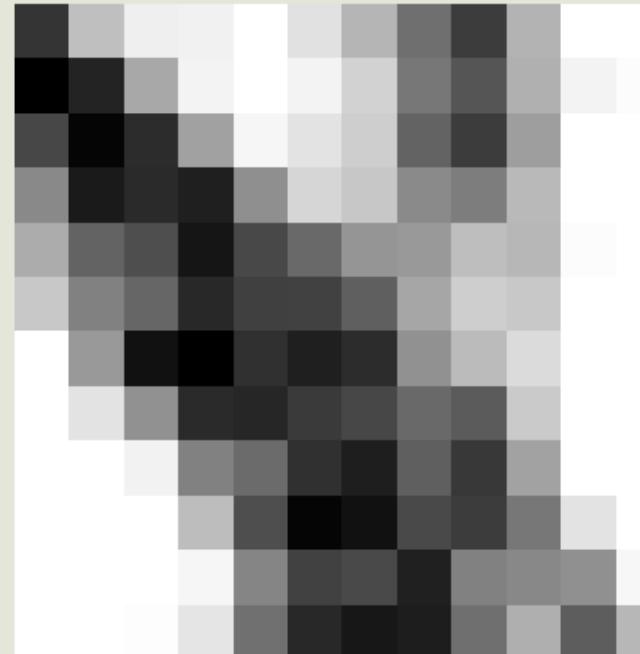
Slide adapted from Dr. Wei-Chen CHU , Bioimage Analysis with FIJI/ImageJ and Friends, licensed [CC BY 4.0](#)

# Image are composed of pixels (picture + element)

(A) Original image



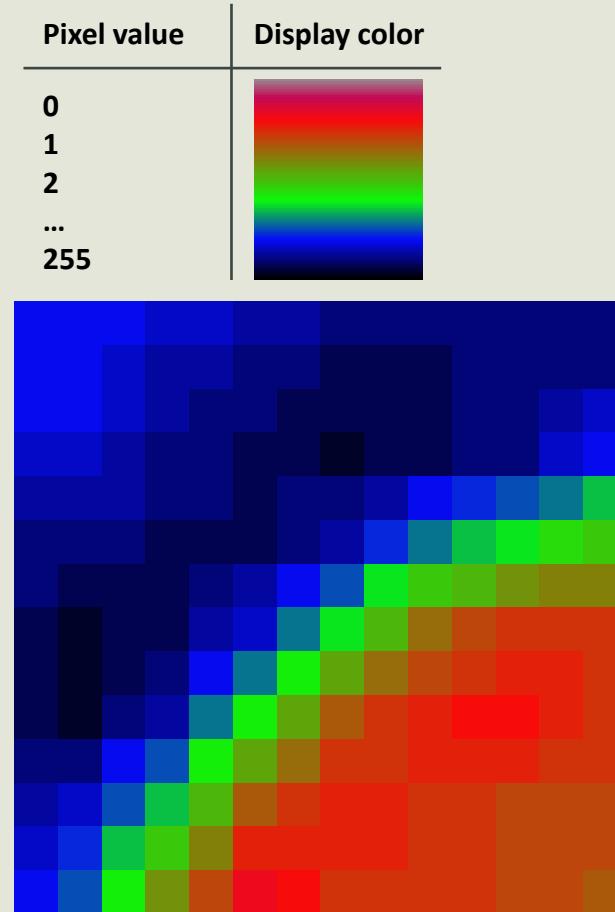
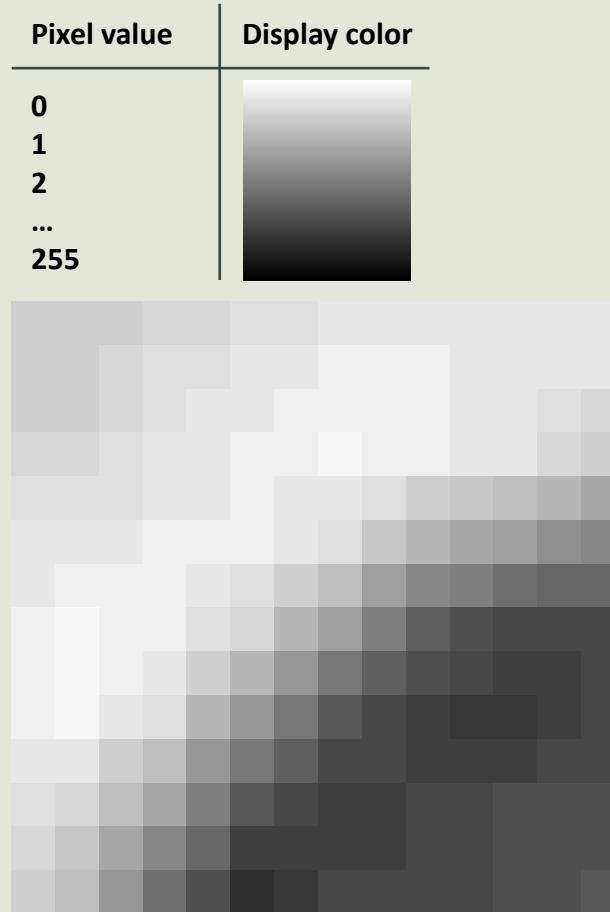
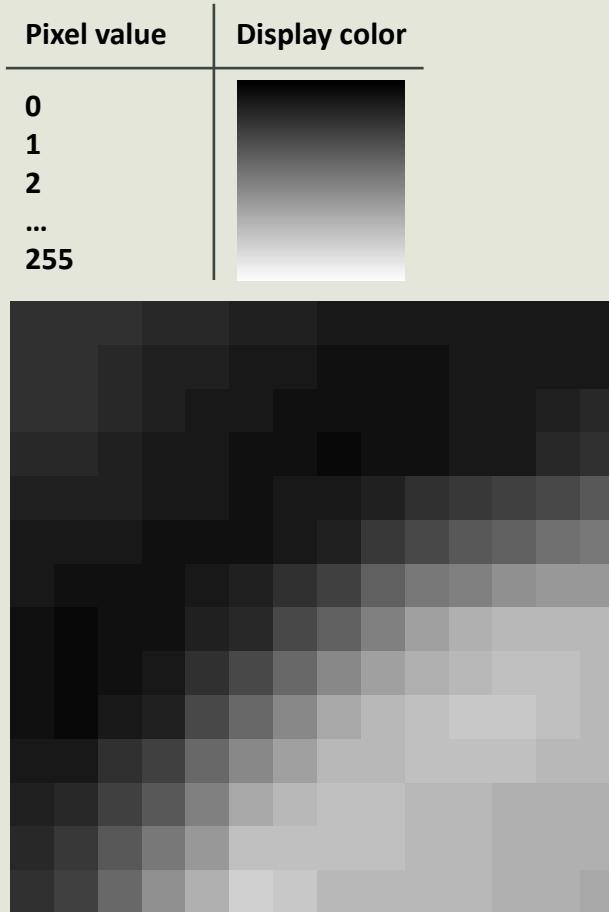
(B) Enlarged view from (A)



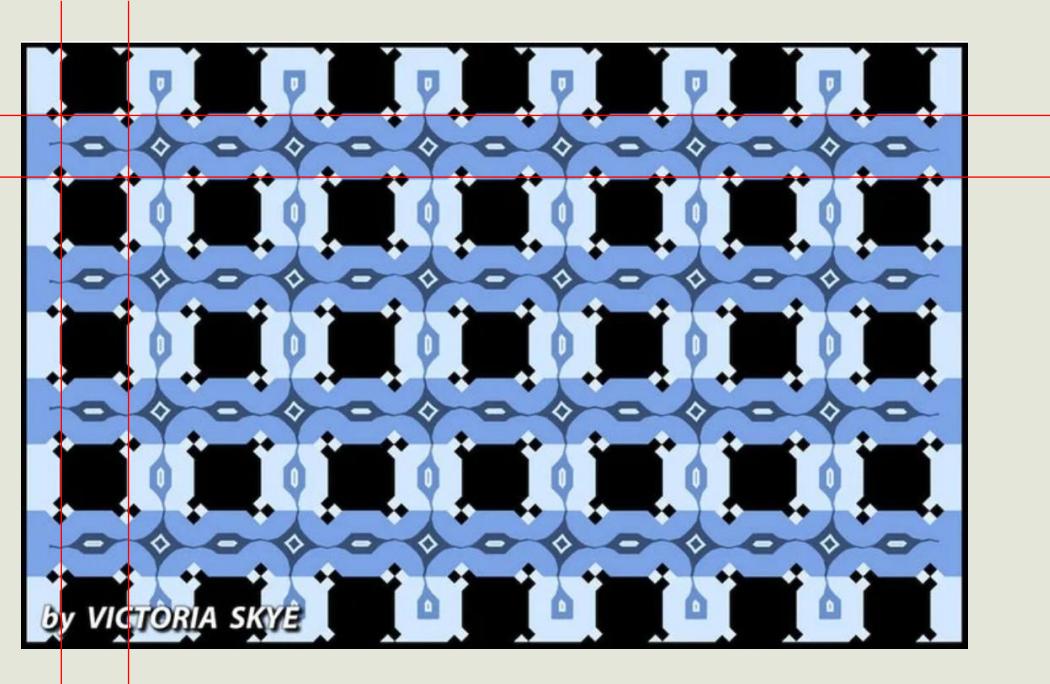
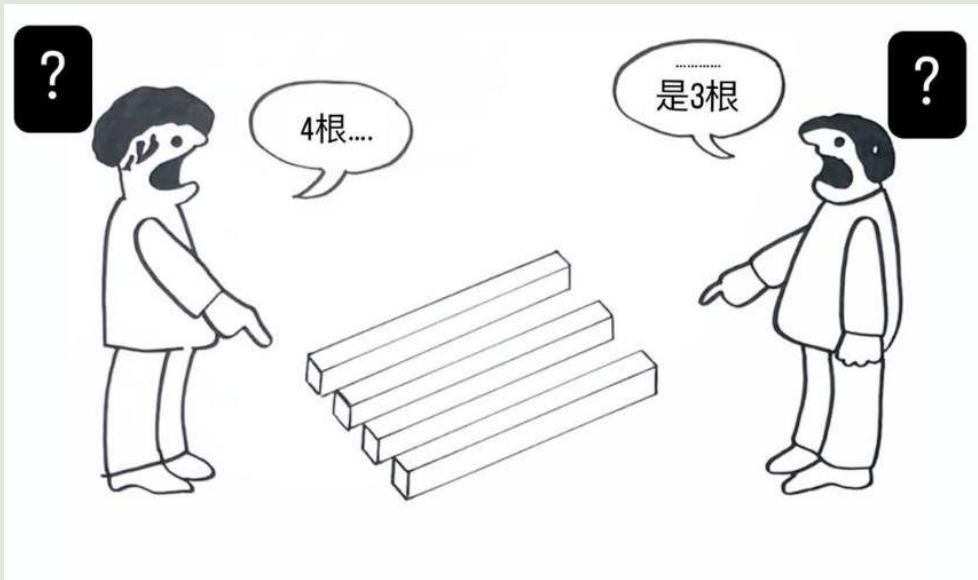
(C) Pixel values from (B)

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 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 |

# Lookup table (LUT)

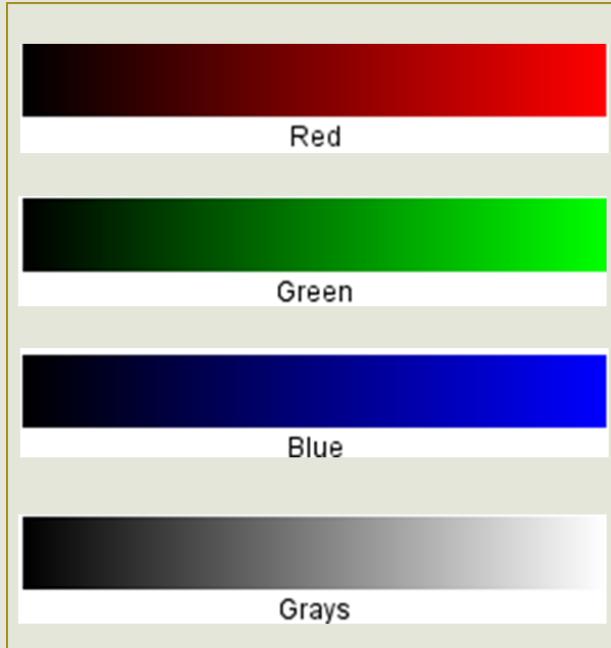


# Don't believe your eyes.



# Useful LUT

“Classic”



Color Blindness friendly



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



For labeling/ count mask



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

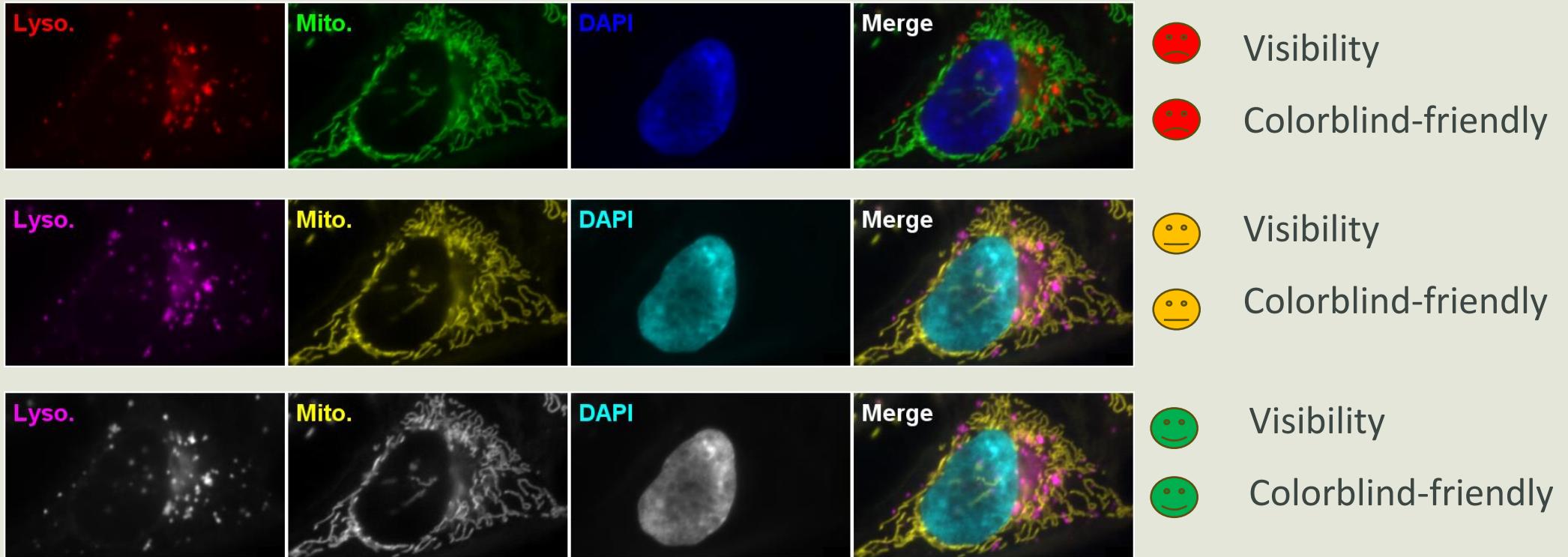


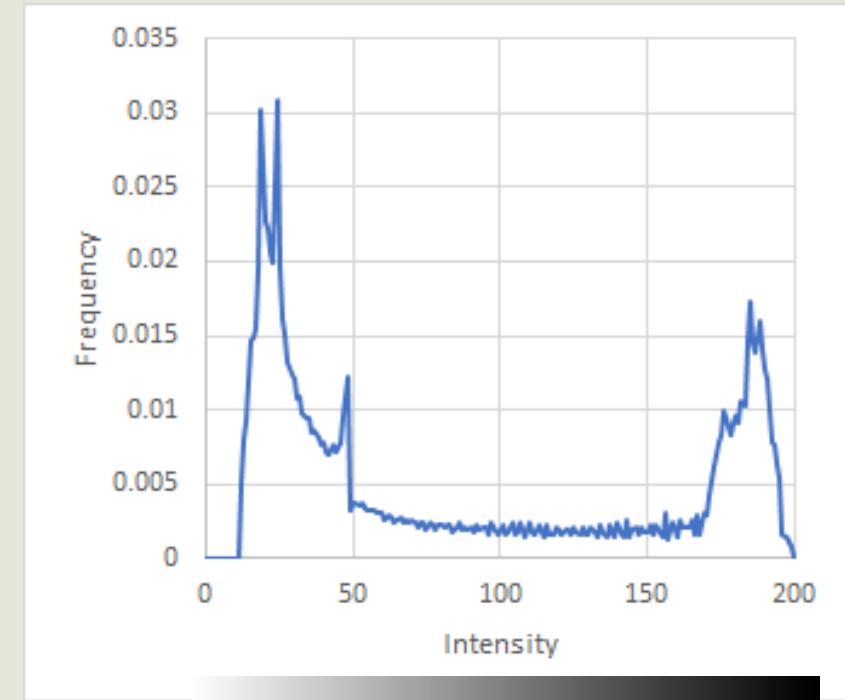
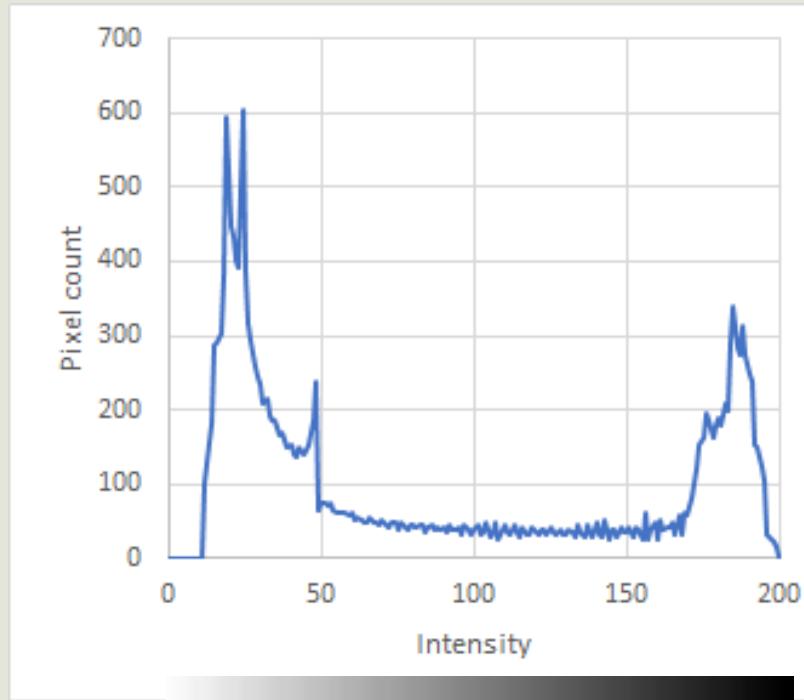
Image source: FIJI sample images (HeLa cells, cropped)

See also Figure Making Best Practices (2024) youtube.com

Slide adapted from Dr. Wei-Chen CHU , Bioimage Analysis with FIJI/ImageJ and Friends, licensed [CC BY 4.0](#)

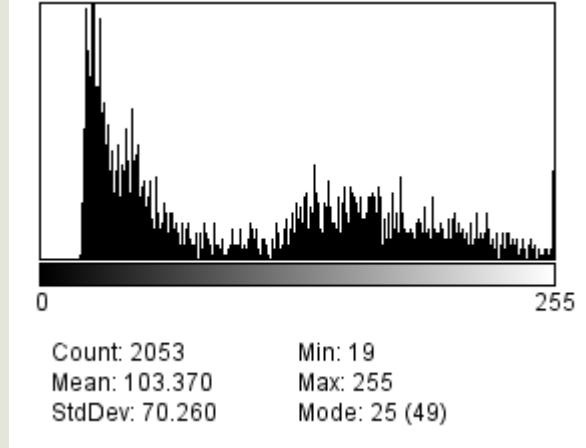
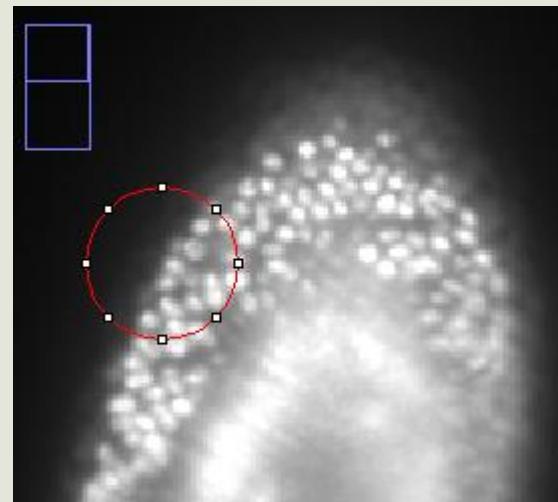
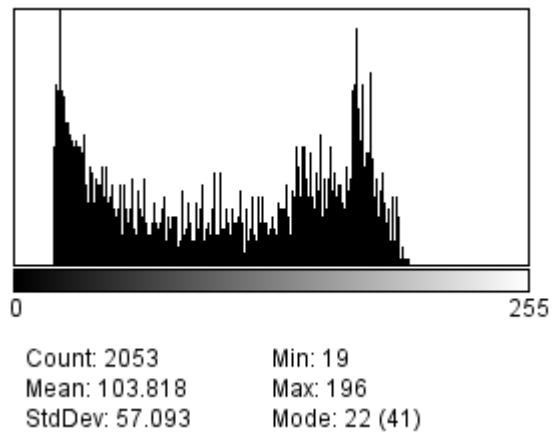
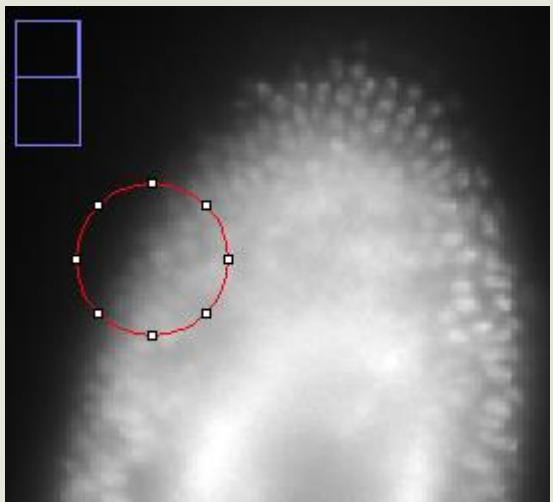
# Histograms

A histogram shows the probability distribution of pixel intensities.



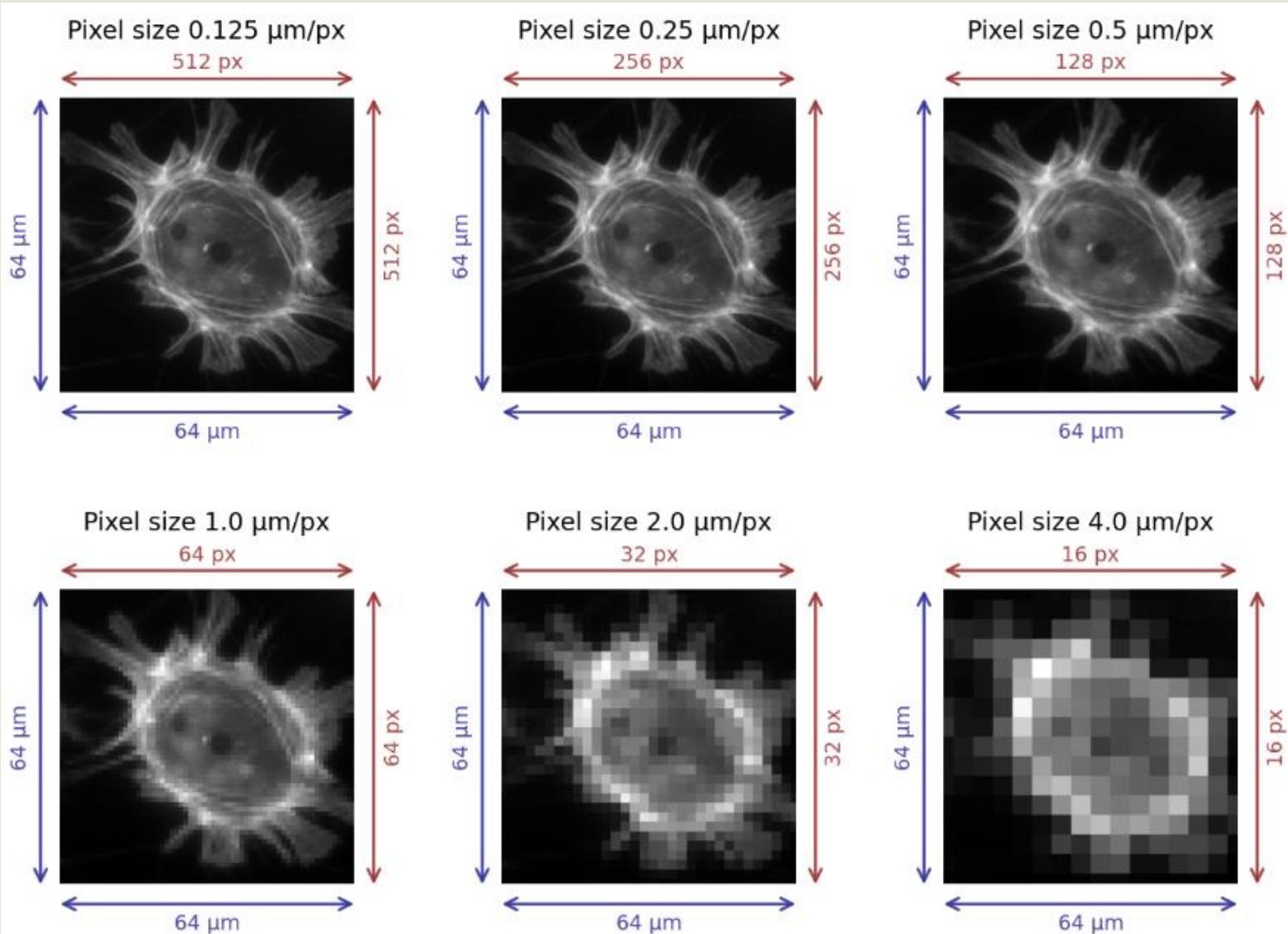
# Histograms

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:

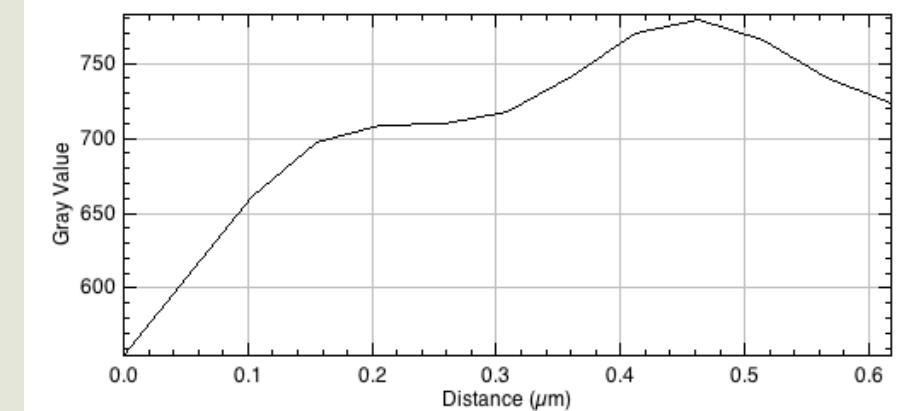
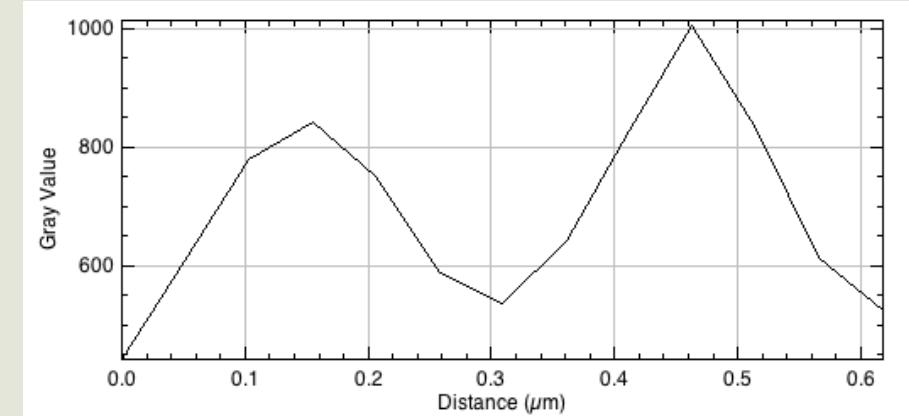
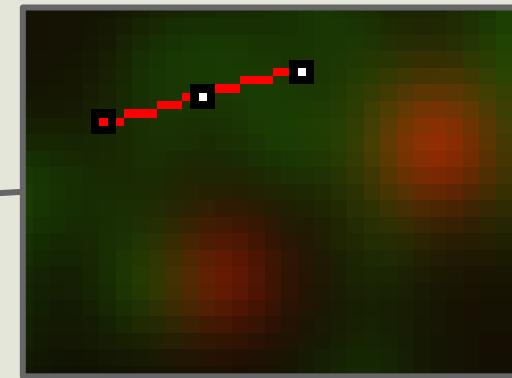
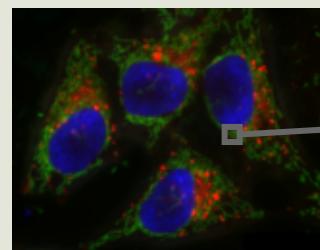
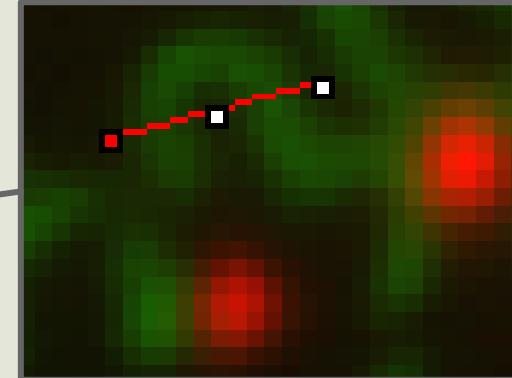
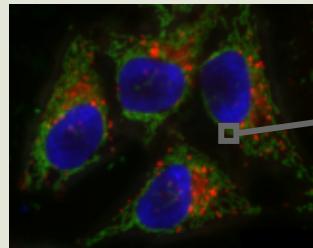
- A property of a digital image.
- You configure it during the imaging session at the microscope (Pixel numbers, Objective, Zoom...etc.)

## Resolution:

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

# 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 bit 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

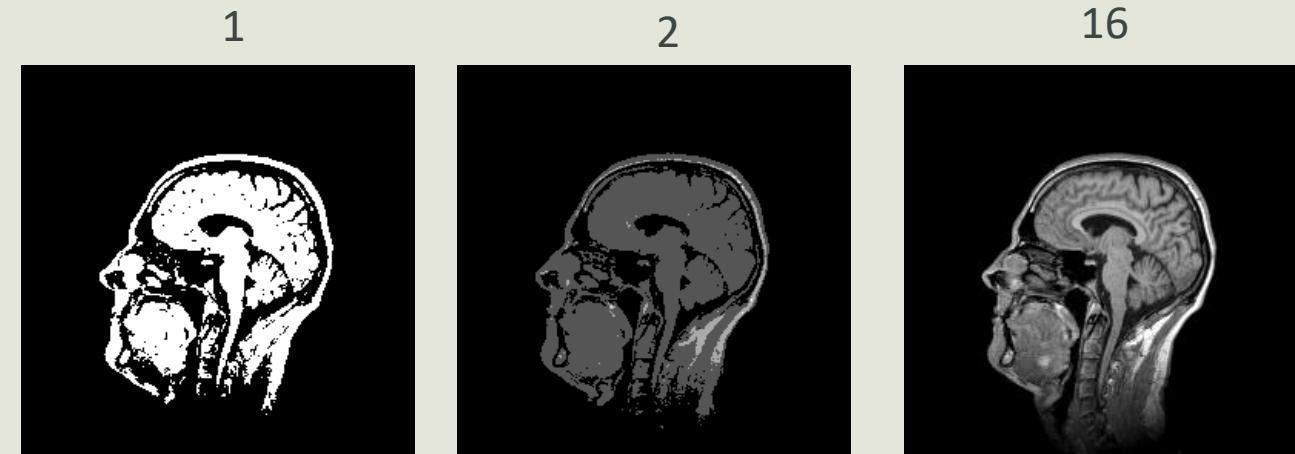
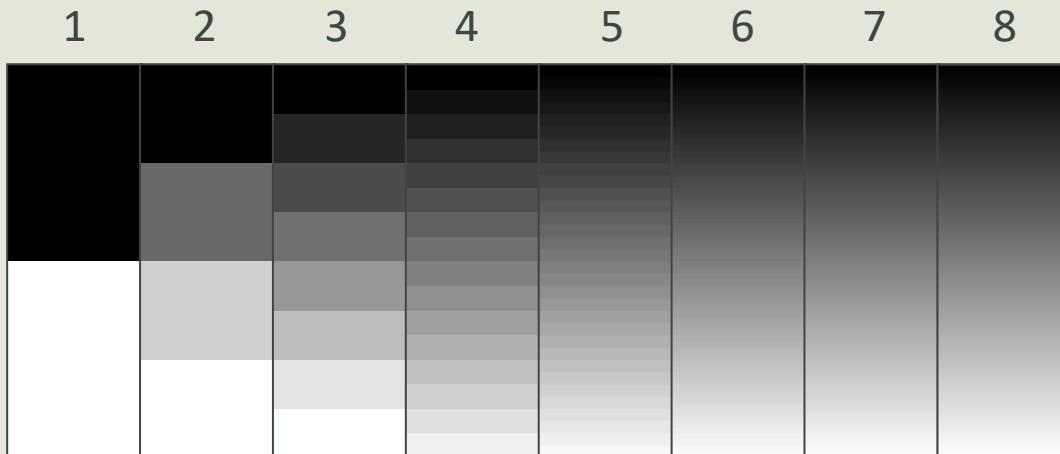
8 bits (1 byte) = 0 ~ 255

16 bits (2 bytes) = 0 ~ 65535

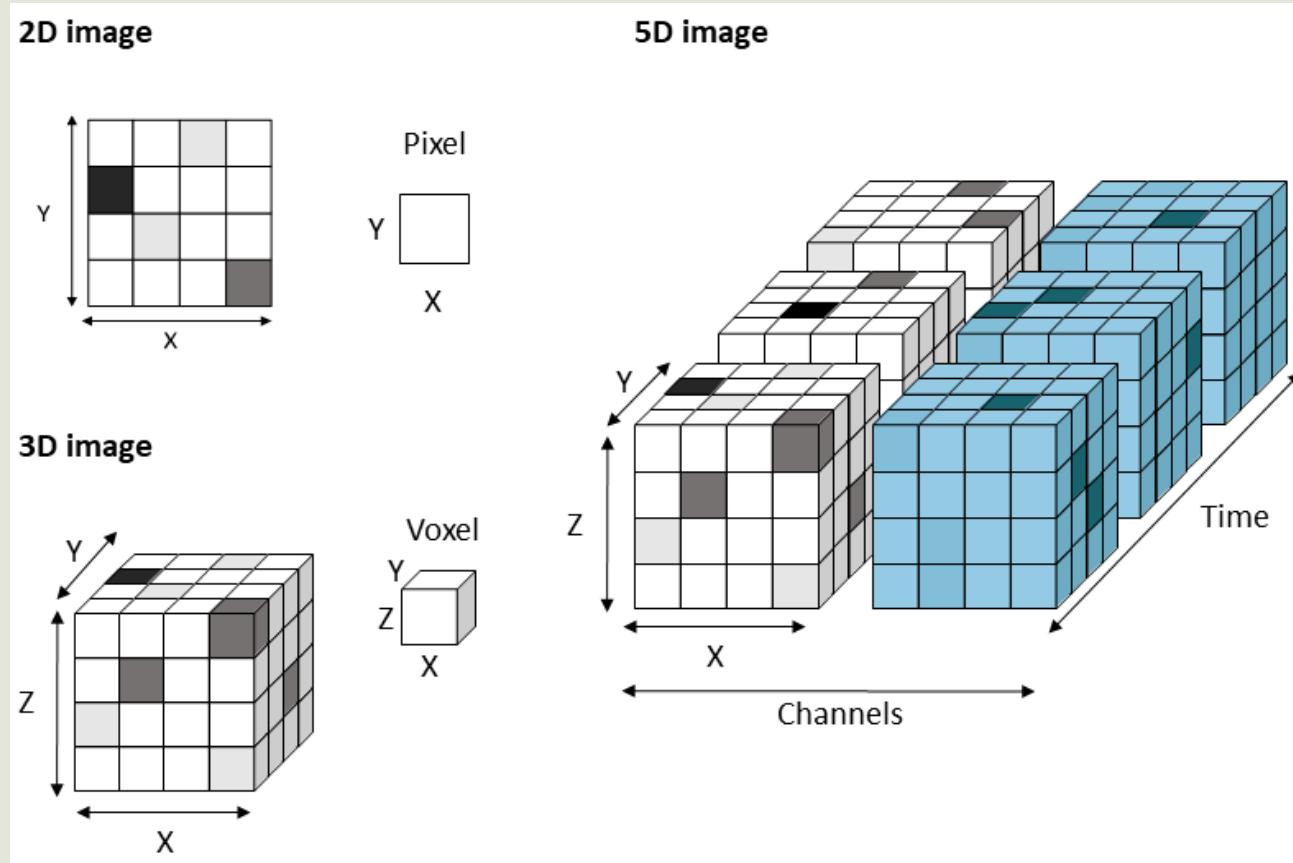
12 bits (2 bytes!) = 0 ~ 4095

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

# Part II :

Bioimage analysis workflow - Processing & Image Operators

# Bioimage analysis workflow - Processing

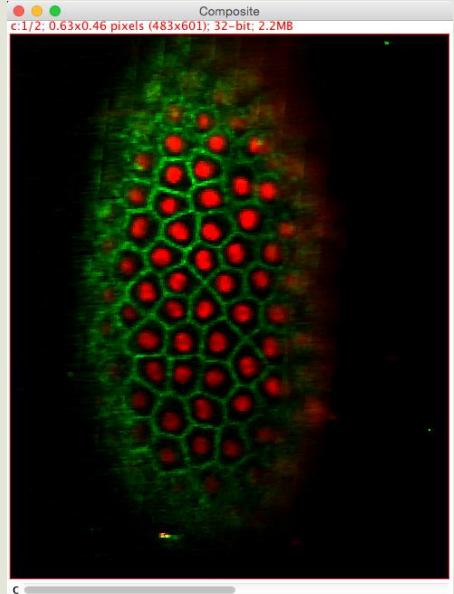


Image Processing

Preprocessing



Segmentation

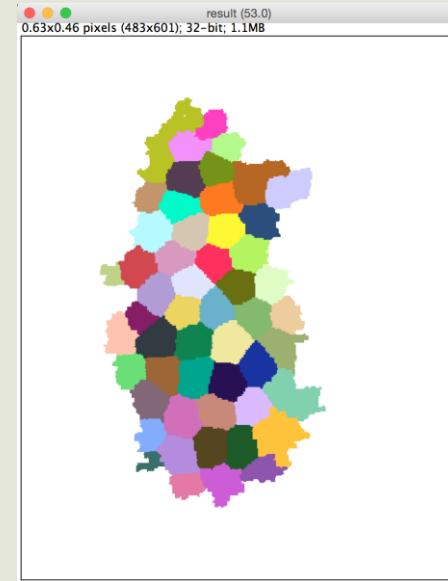
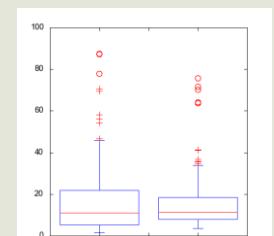
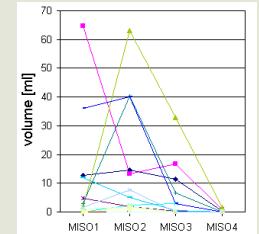


Image Analysis

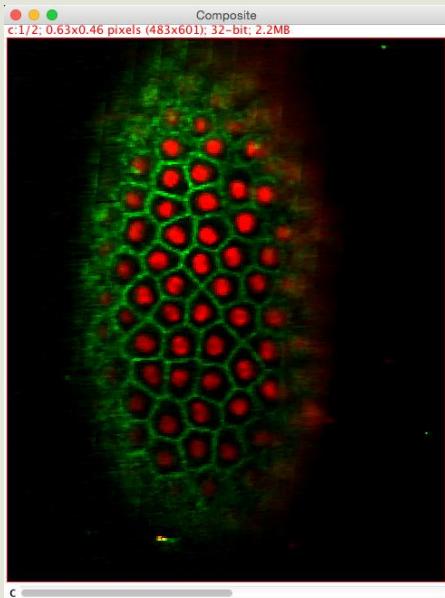
Quantification



Data Analysis



# Bioimage analysis workflow – Image Operators



Preprocessing



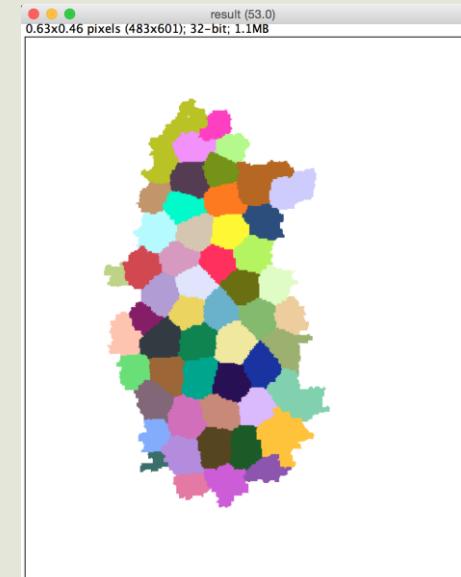
Segmentation

## Image Processing

- Point operations
- Background Subtraction
- Filtering
- ...

- Thresholding
- Connected Component Labelling
- ...

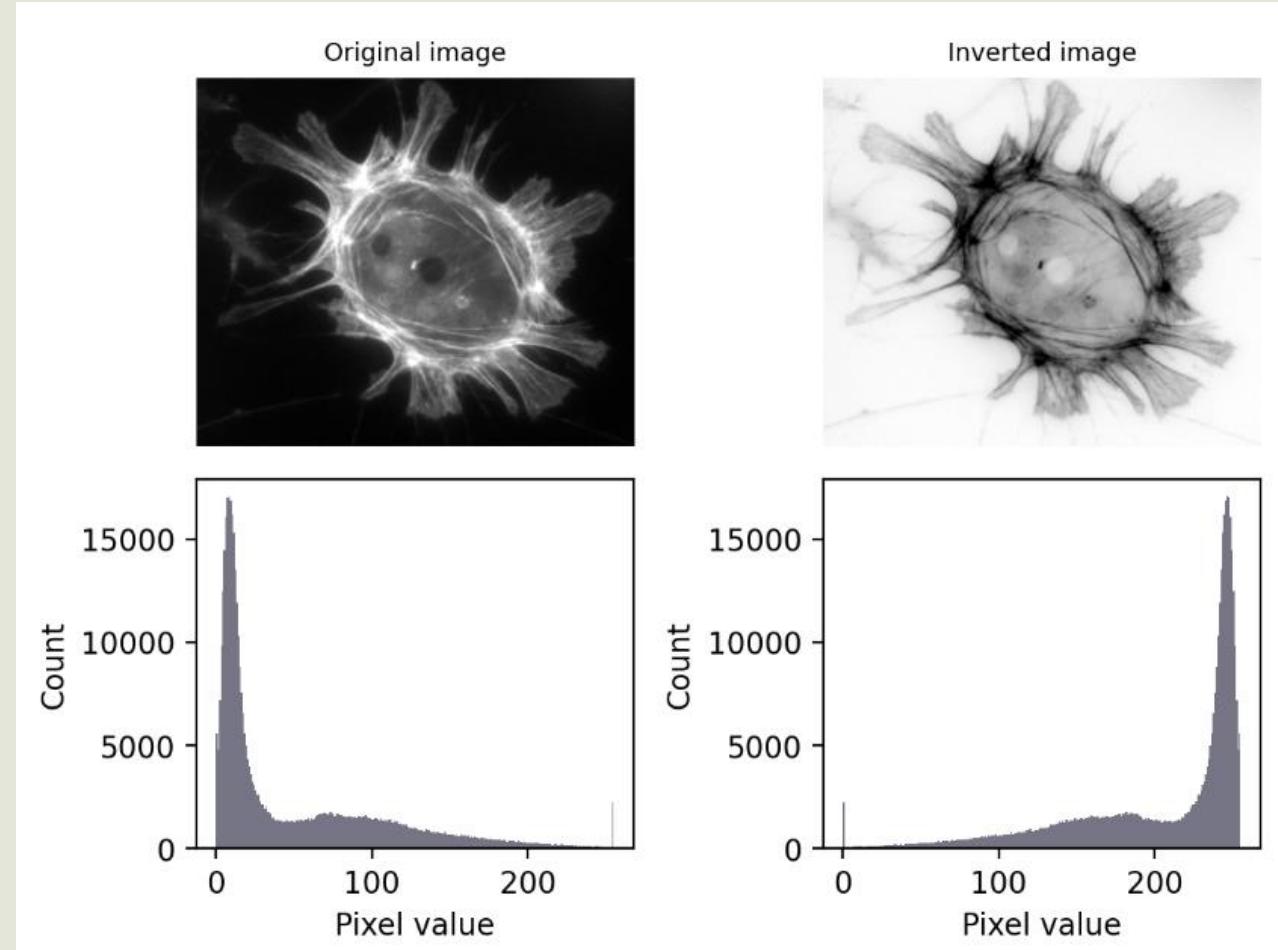
## Image Operators



# Point operations

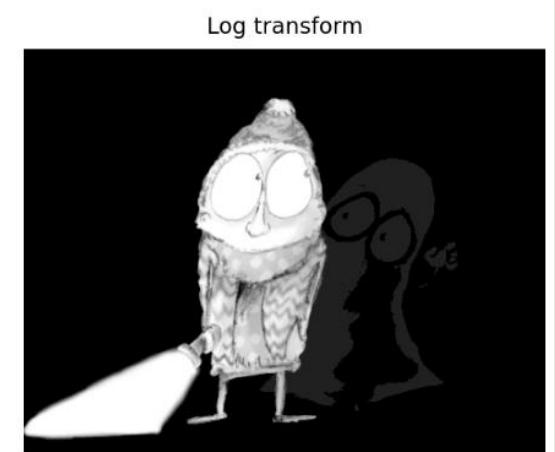
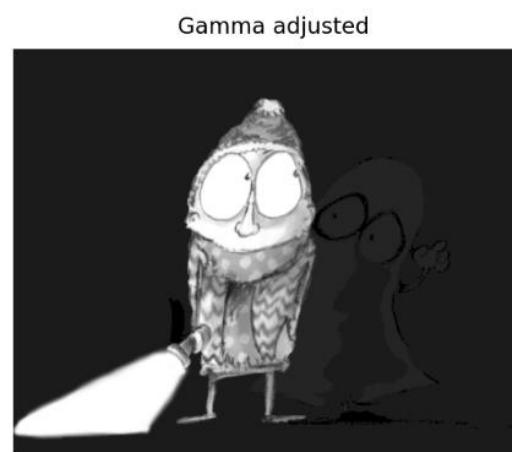
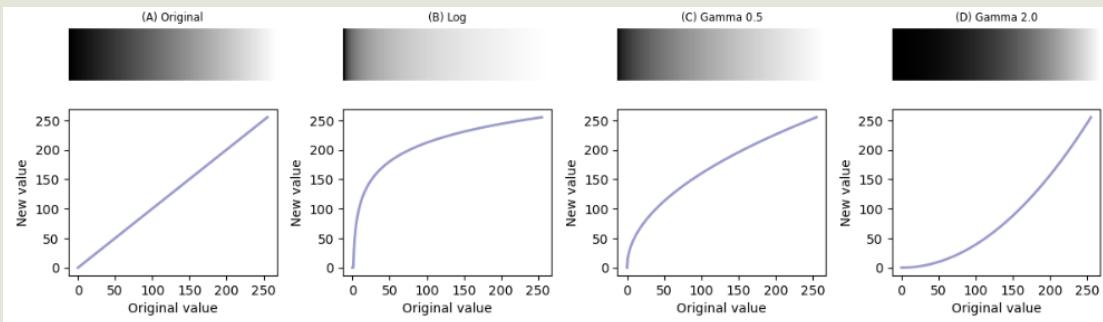
Point operations

Pixel values are just numbers



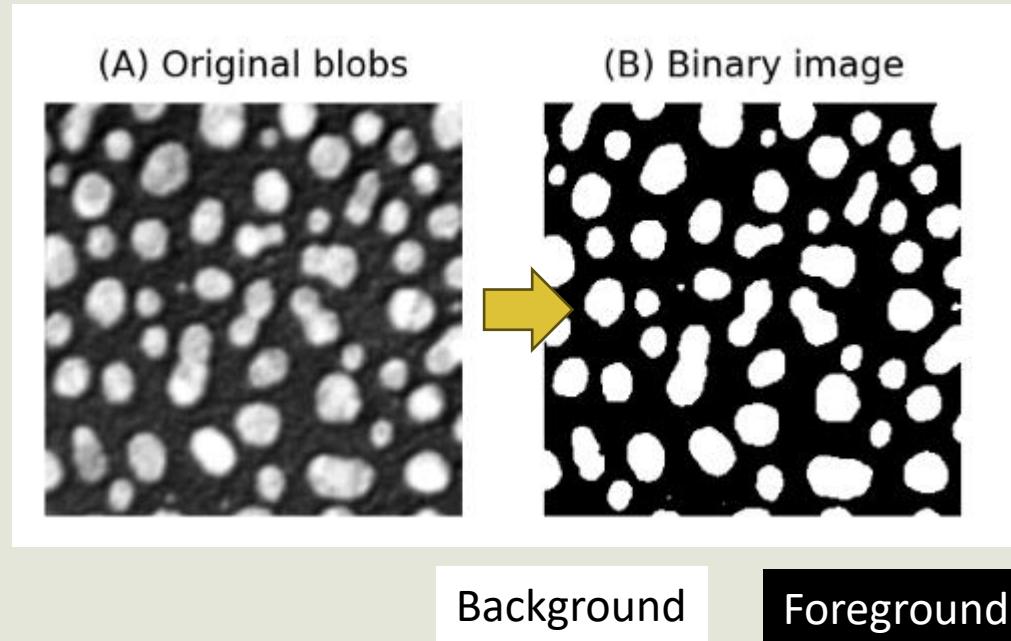
# Nonlinear contrast enhancement

## Nonlinear contrast enhancement



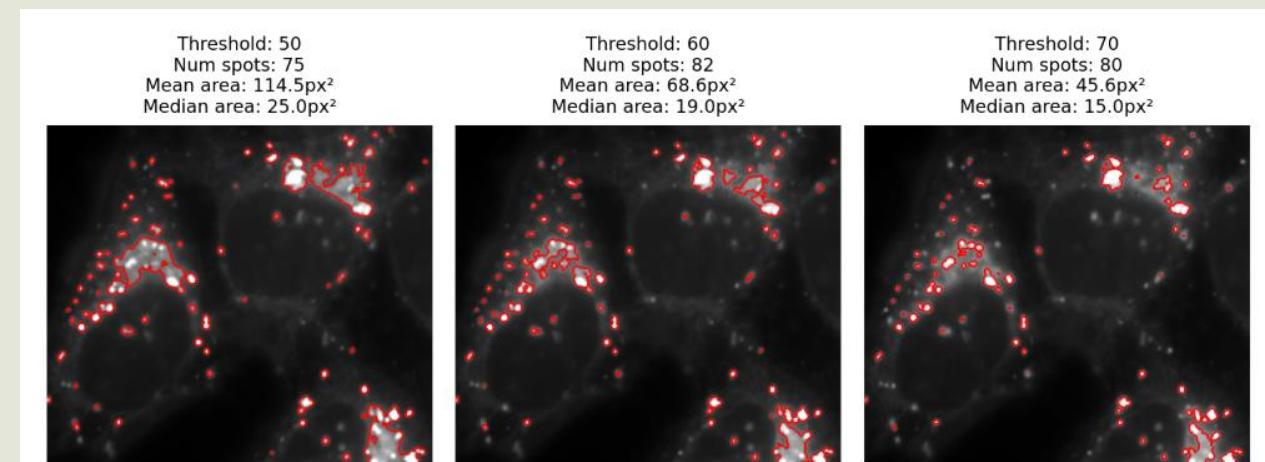
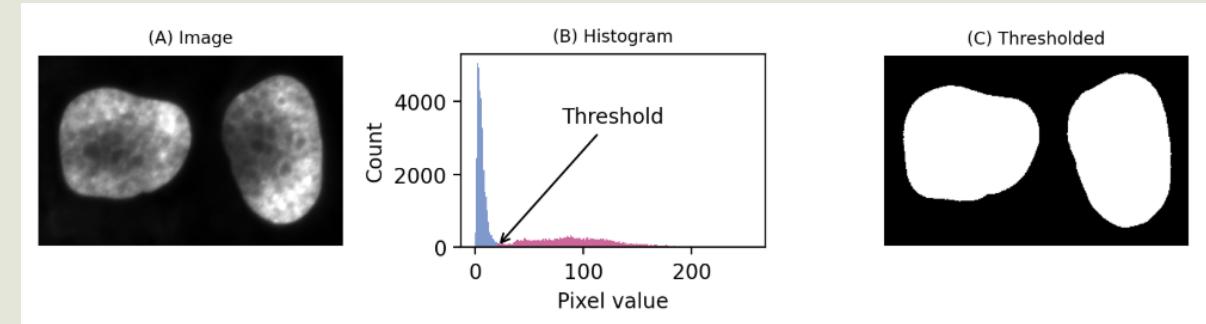
should be used with caution

# Thresholding



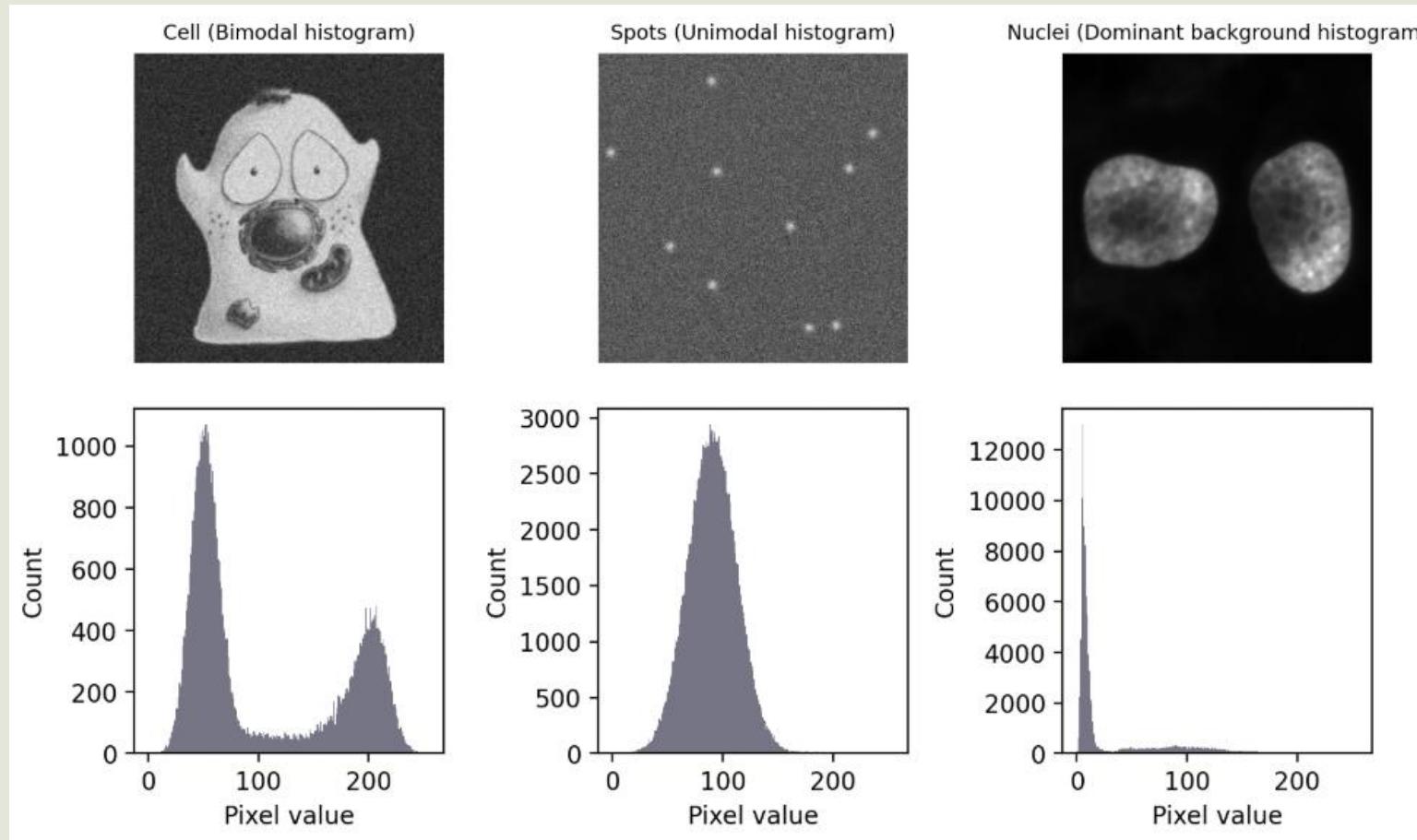
Avoid manual adjustments if possible.

## Global thresholding



# Thresholding

## Automated thresholds

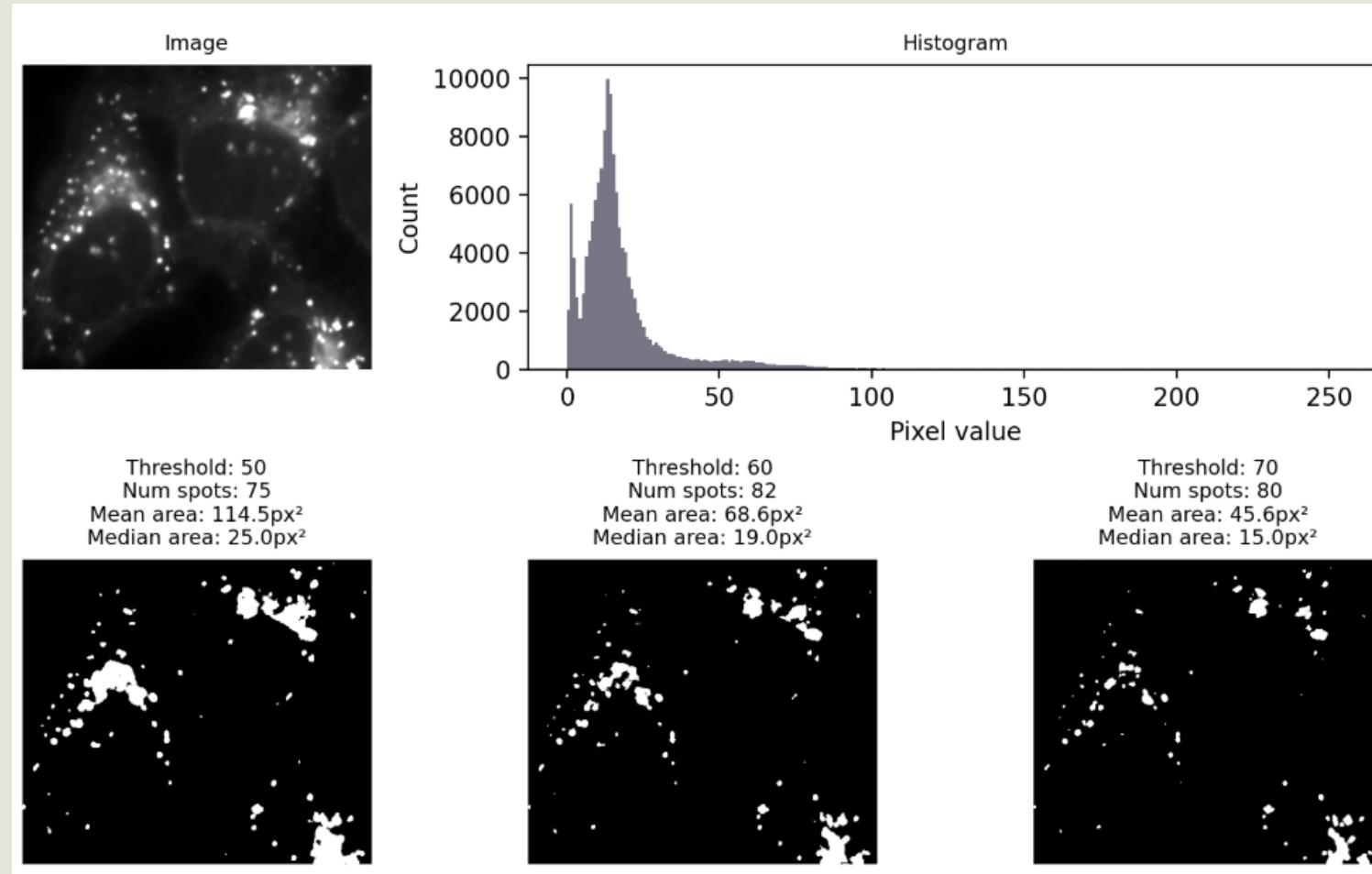


- **Otsu's method**
- **Minimum method**
- **Triangle method**
- **Mean method**
- **Mean & Standard deviation**
- **Median & Median Absolute Deviation**
- ...

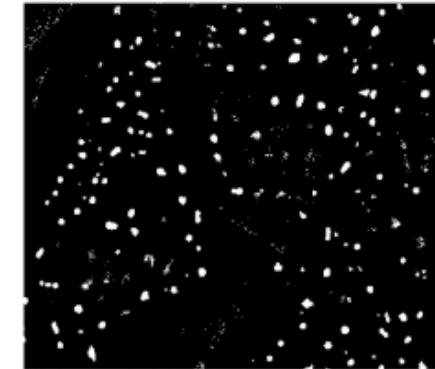
Reduce bias !

# Thresholding

## Local thresholding

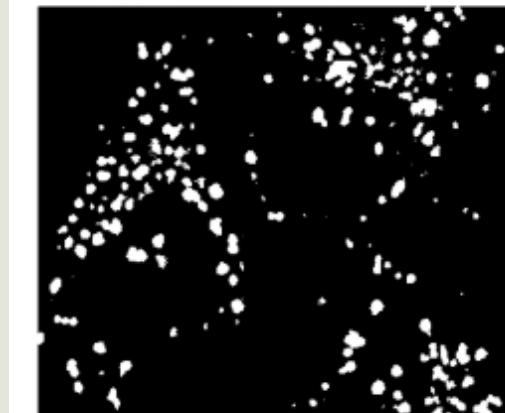


(C) Niblack threshold applied to (A)



Or Background subtracted

(F) Triangle threshold applied to (D)



# 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

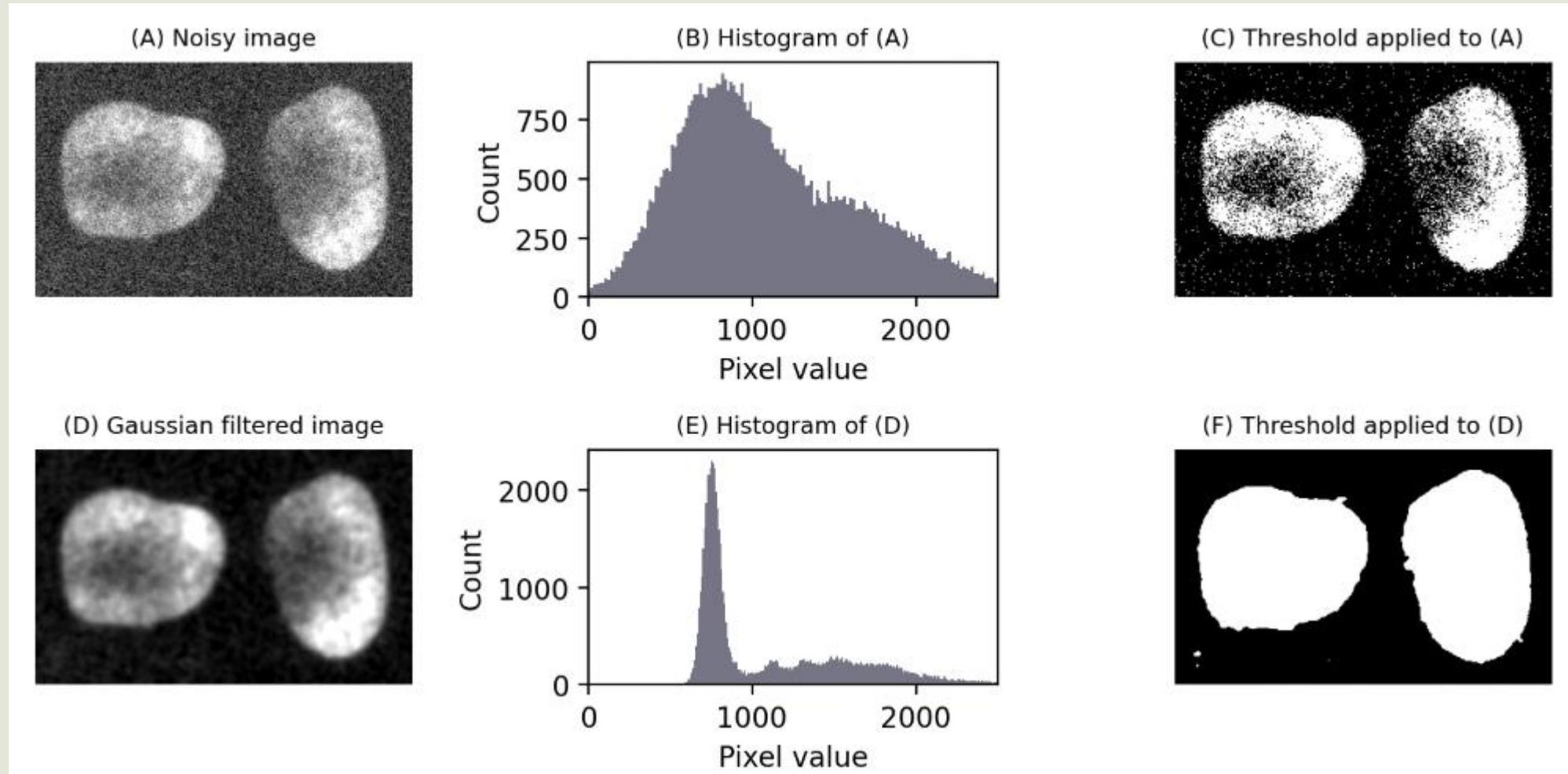
## A Threshold Selection Method from Gray-Level Histograms

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.

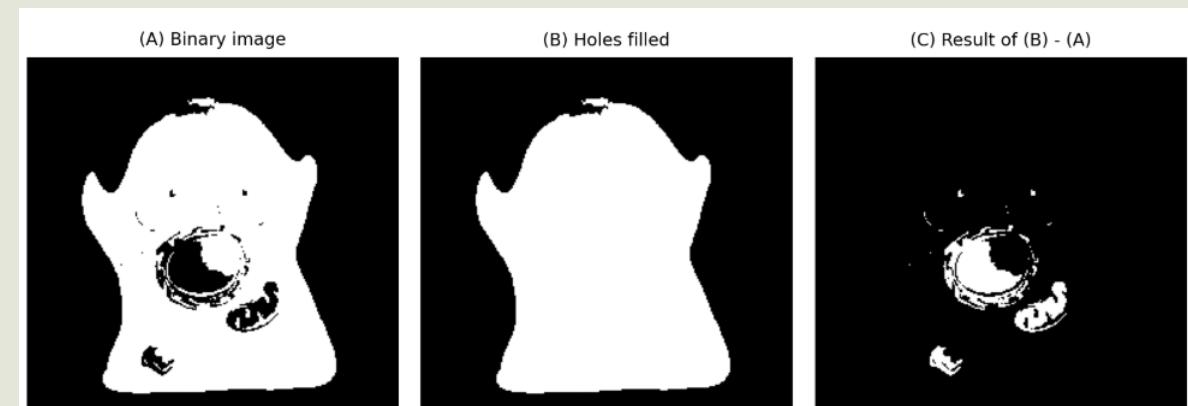
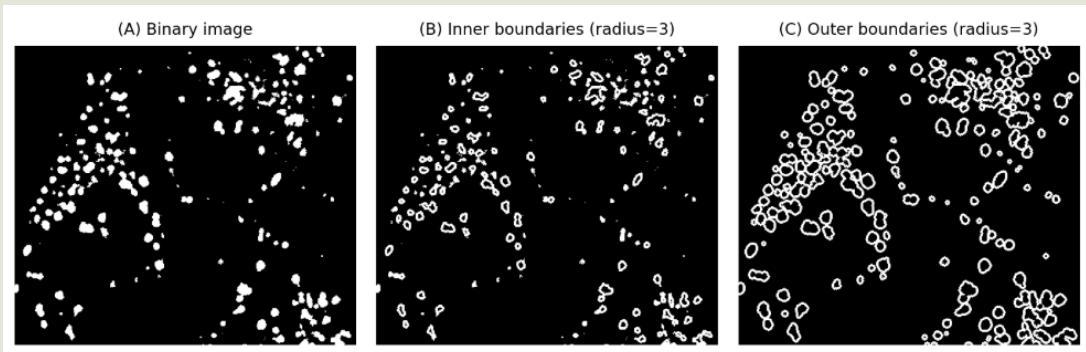
# Filters - improving thresholding results

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

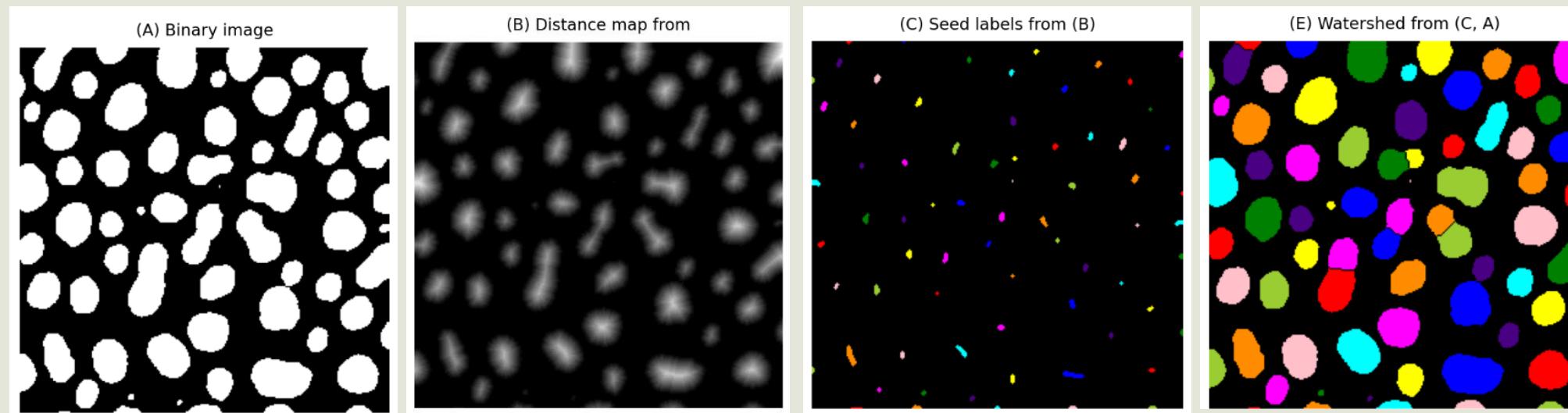
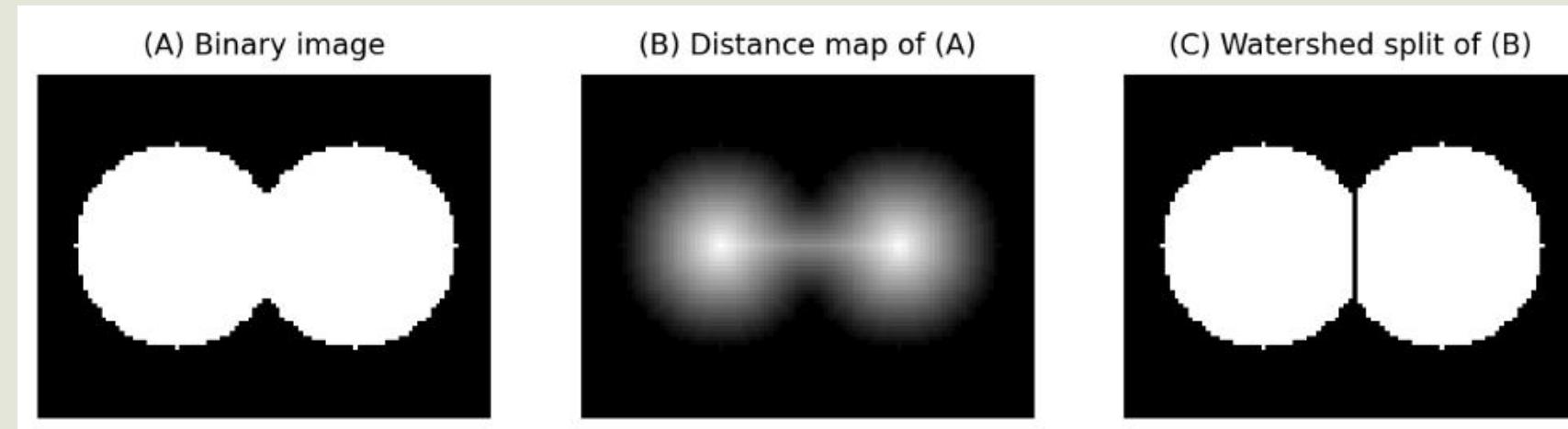


# Morphological Operators

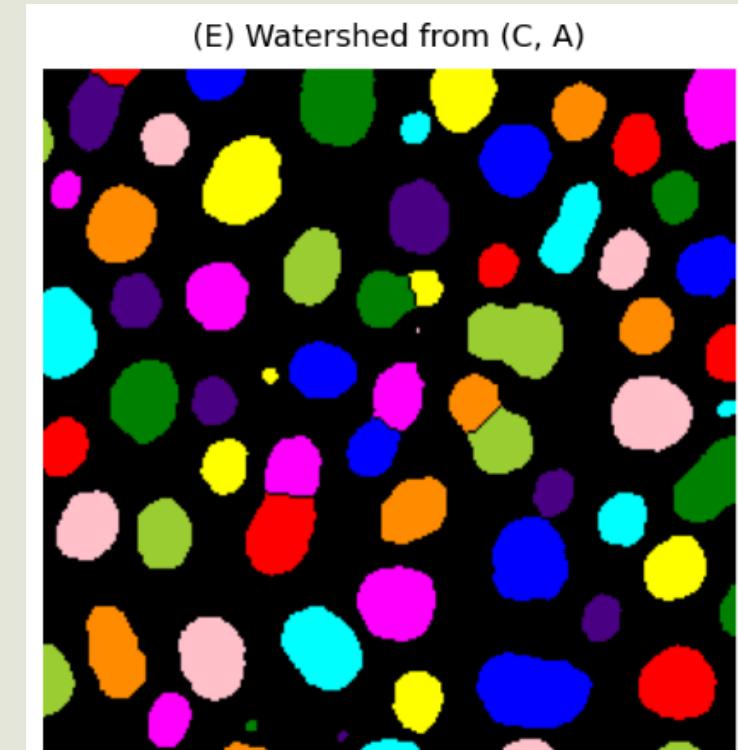
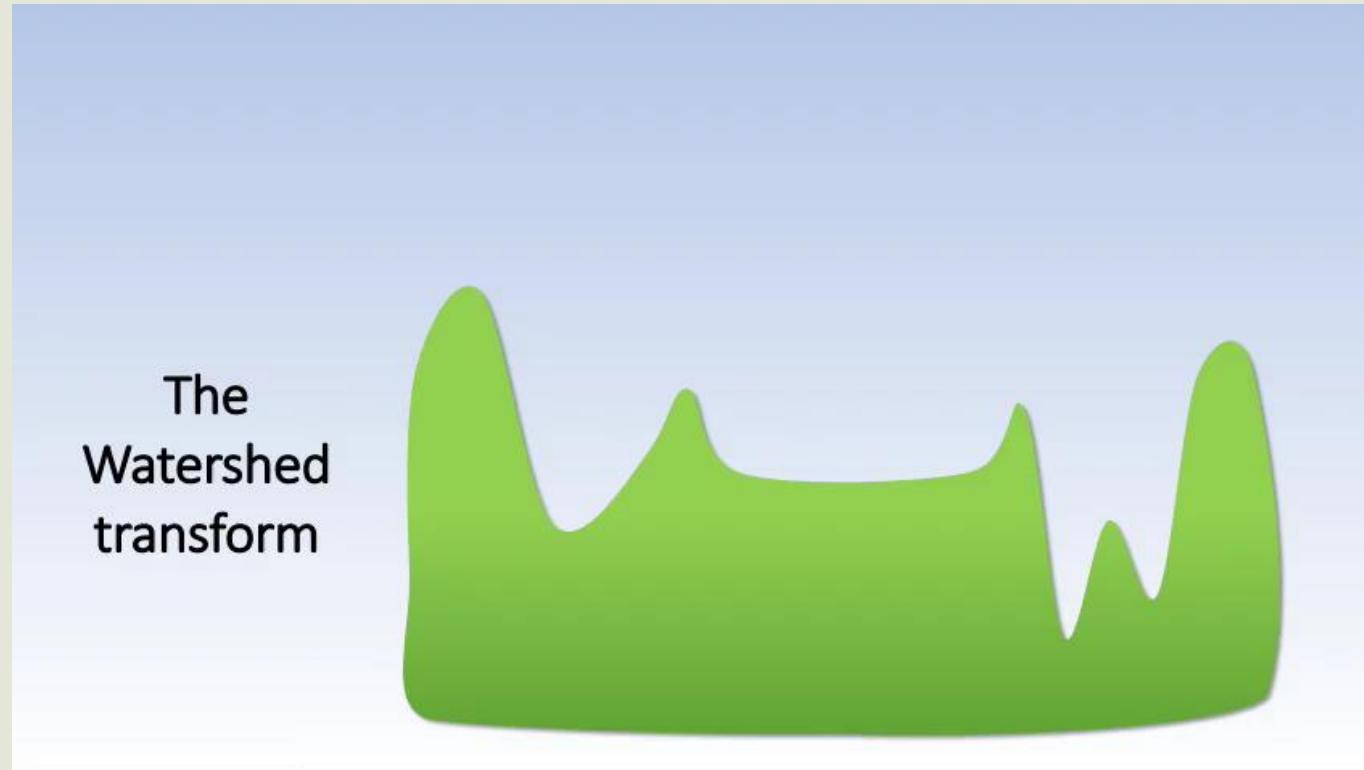
- **Morphological operations** can be used to refine or modify the shapes of objects in images.
- Erosion, dilation, opening & closing
- Boundaries & outlines
- Filling holes
- Thinning & skeletonization



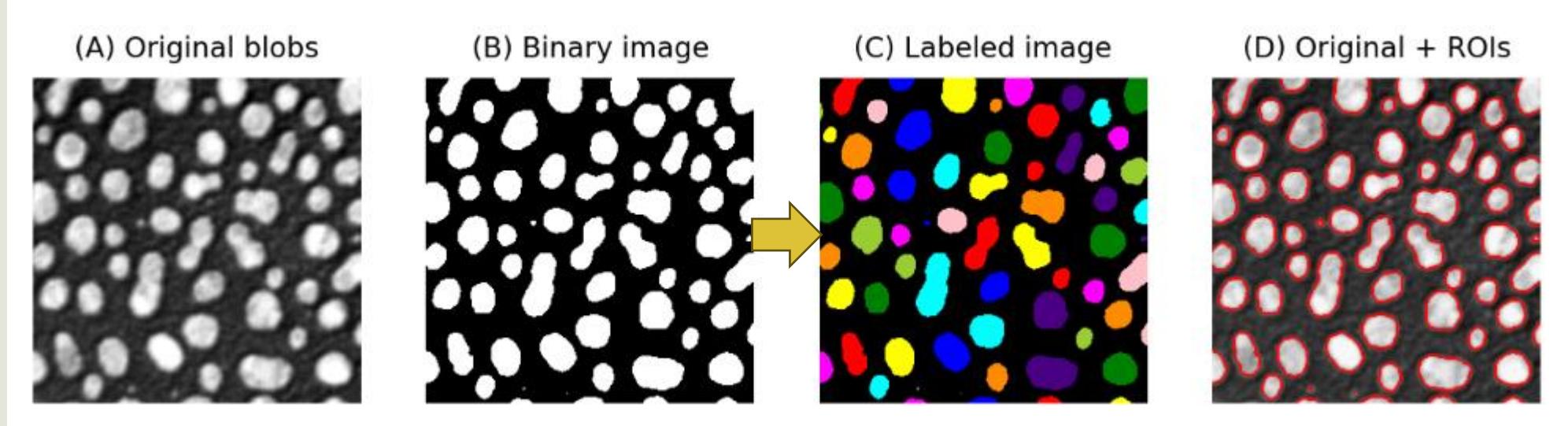
# Image transforms (The watershed transform)



# Image transforms (The watershed transform)

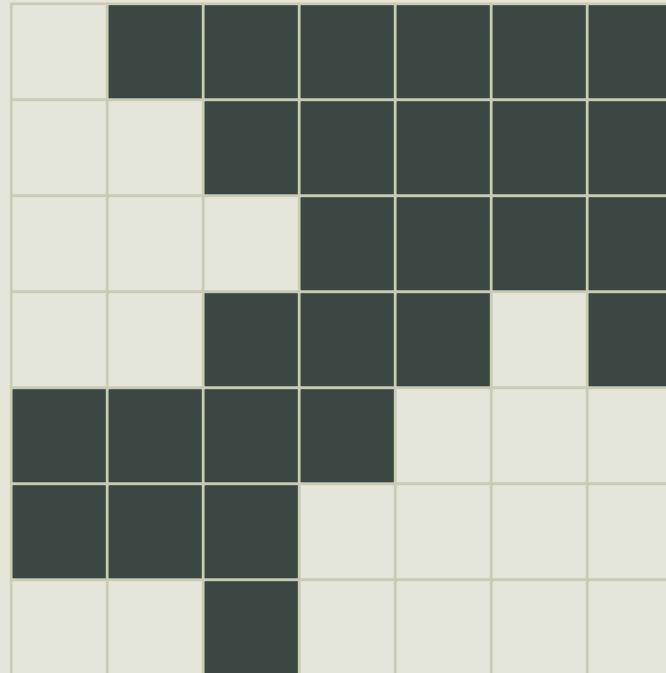


# Connected Component Labelling & Region of Interest (ROI)



# 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.



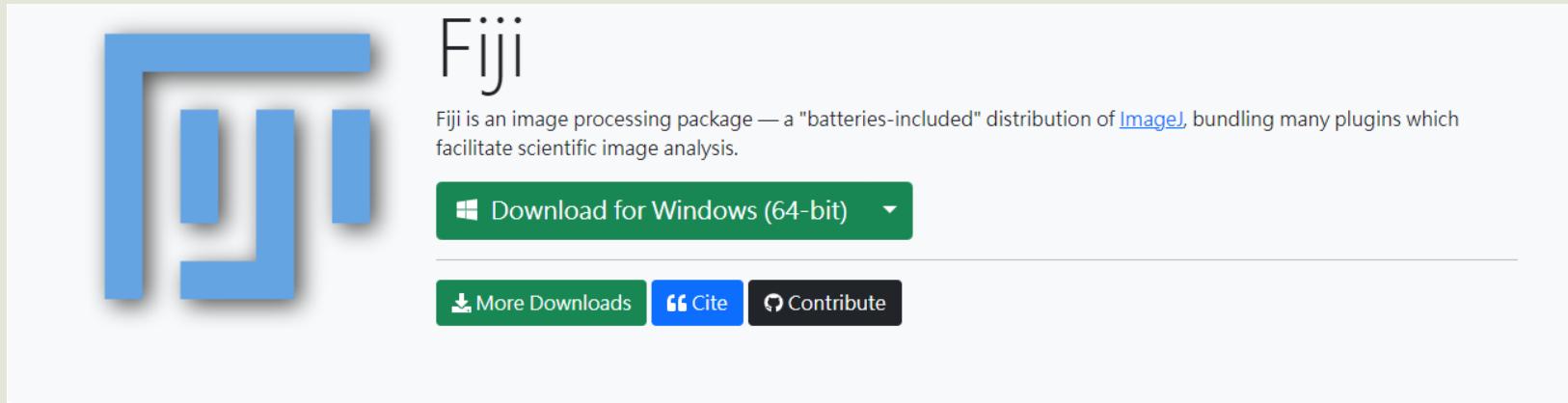
CCA

|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 3 | 0 | 0 |
| 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 |
| 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 |
| 2 | 2 | 0 | 3 | 3 | 3 | 3 | 3 |

# Part III:

Open-source tools for Bioimage analysis -  
(Fiji)

# (Fiji is just) imageJ



The screenshot shows the official Fiji project website. At the top left is the Fiji logo, which consists of a stylized blue 'f' shape. To its right is the word "Fiji". Below the logo is a brief description: "Fiji is an image processing package — a "batteries-included" distribution of [ImageJ](#), bundling many plugins which facilitate scientific image analysis." Below this text are several buttons: a green "Download for Windows (64-bit)" button with a white icon, a green "More Downloads" button with a white icon, a blue "Cite" button with a white icon, and a black "Contribute" button with a white icon. The main content area features three sections: "Why Fiji?" with icons for "Easy to Use" (green checkmark), "Powerful" (blue gears), and "Free & Open Source" (red heart). Each section includes a brief explanatory paragraph. At the bottom right of the main content area is a blue link: <https://fiji.sc/>.

## Why Fiji?

 **Easy to Use**  
Fiji is easy to use and install - in one-click, Fiji installs all of its plugins, features an automatic updater, and offers comprehensive documentation.

 **Powerful**  
Fiji bundles together many popular and useful ImageJ plugins for image analysis into one installation, and automatically manages their dependencies and updating.

 **Free & Open Source**  
Like ImageJ itself, Fiji is an [open source](#) project hosted on [GitHub](#), developed and written by the community.

# 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](https://doi.org/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](https://doi.org/10.1186/s12859-017-1934-z)



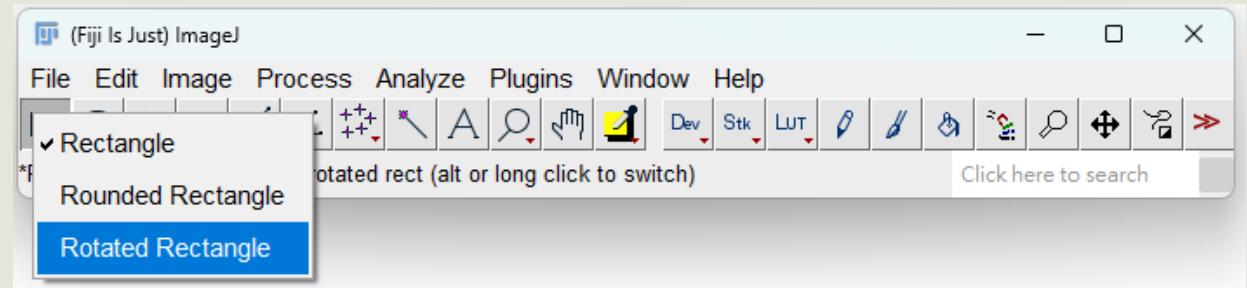
## FIJI Citation:

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](https://doi.org/10.1038/nmeth.2019)

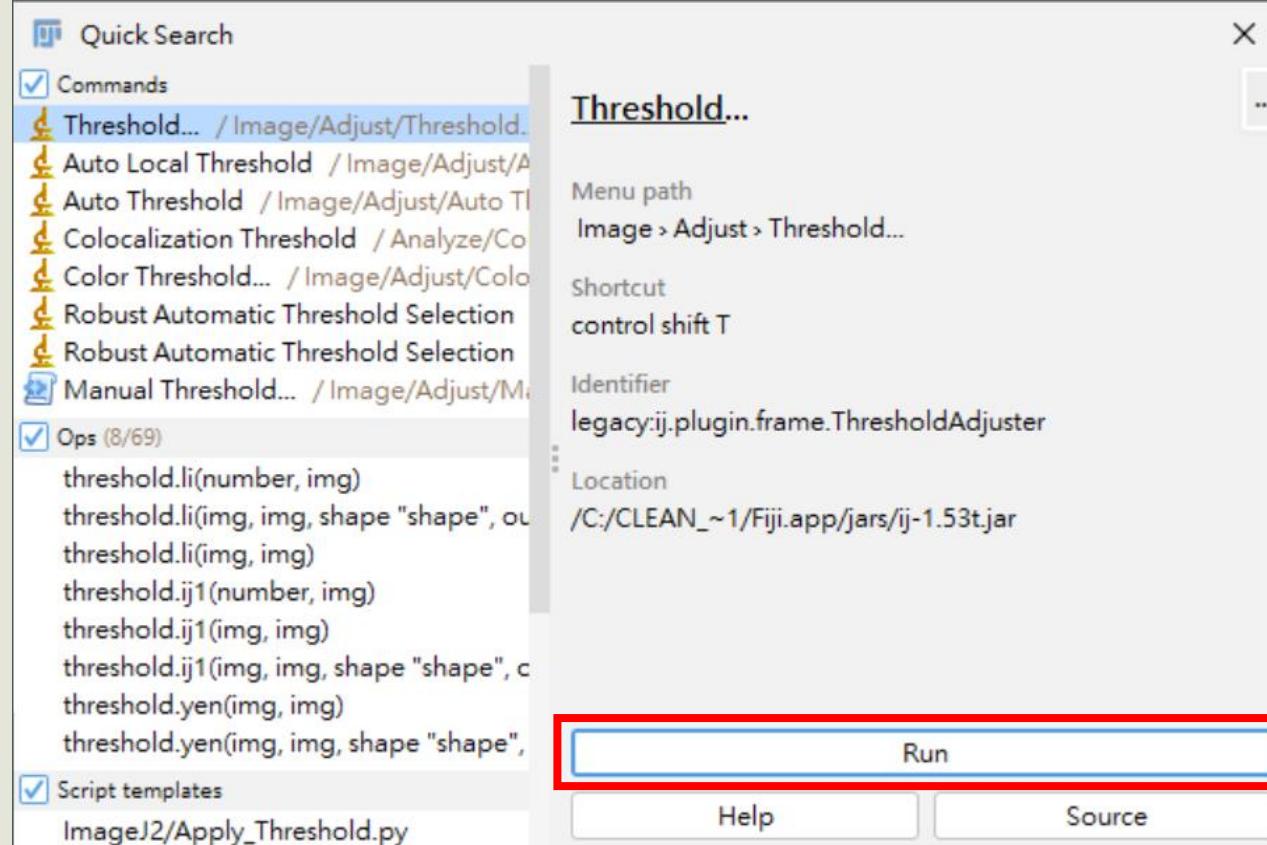
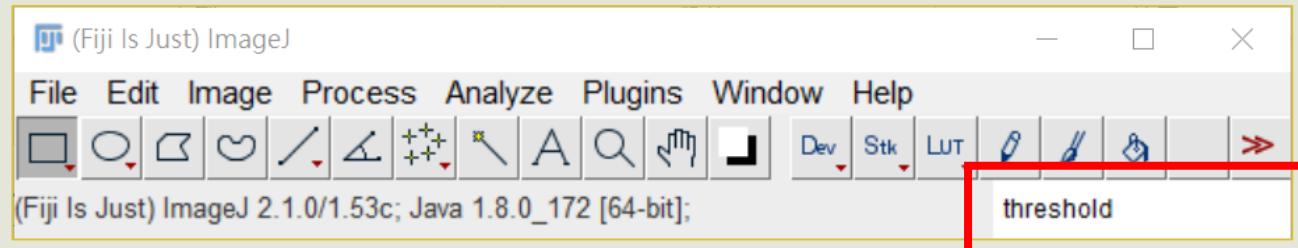
**Also, cite the plugins and the algorithm used in your research.**

# Fiji's user interface

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



# Search Bar

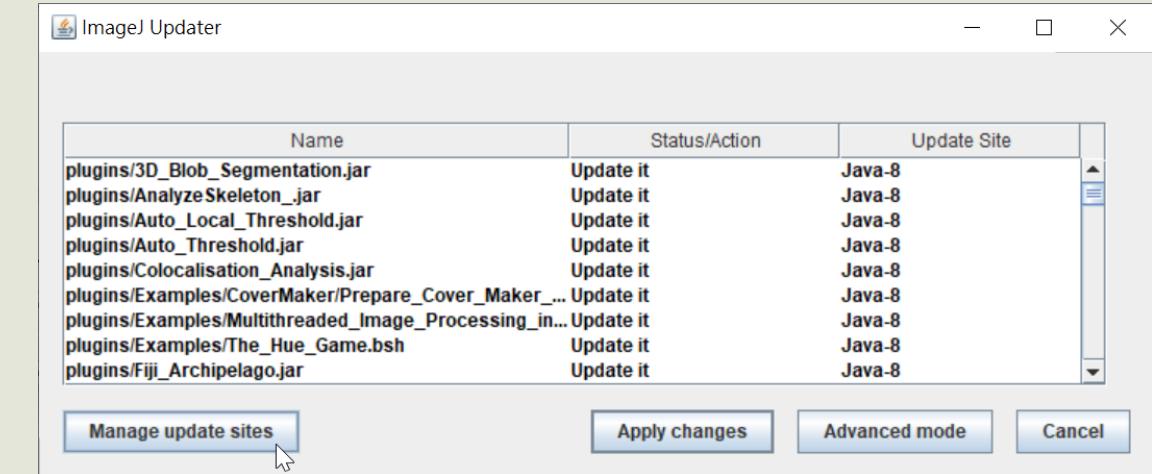
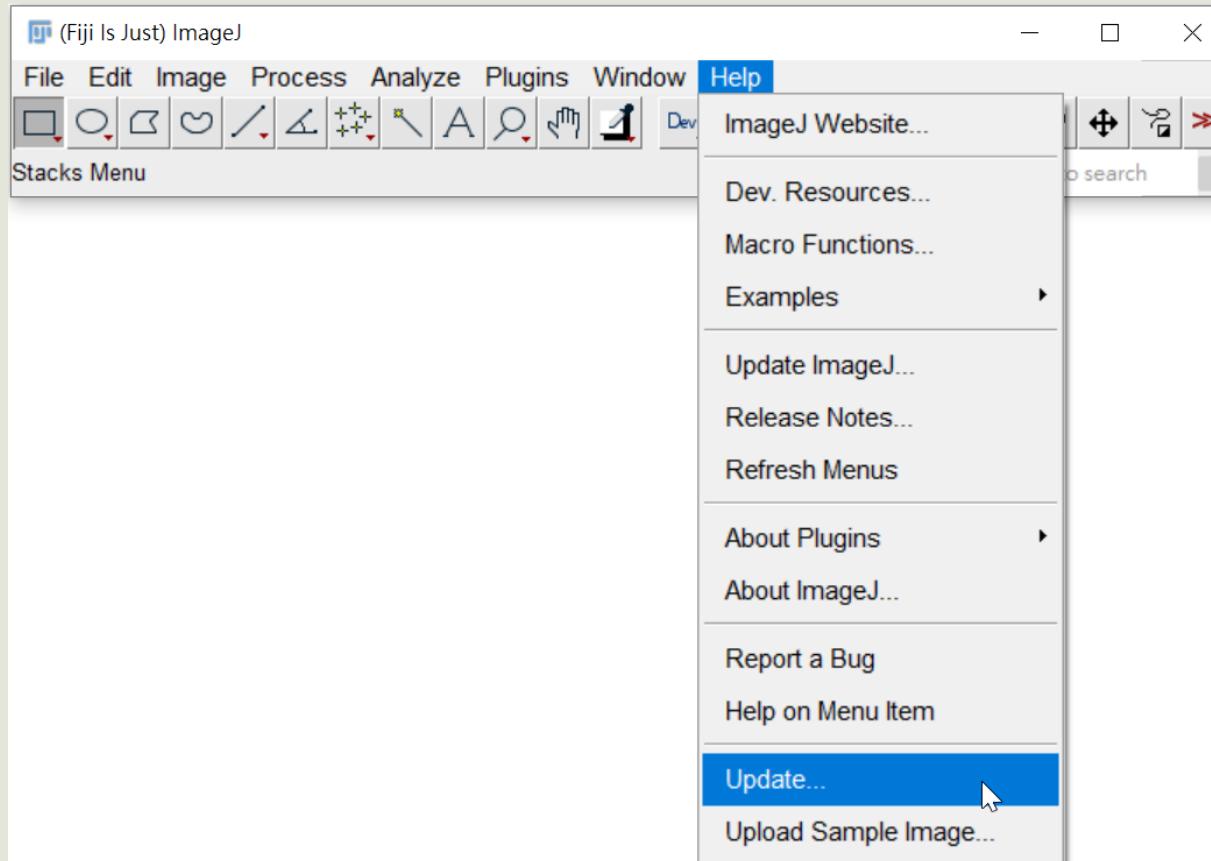


- It shows you where the plugin is located
- You can run it from here (press enter)

ImageJ : command finder (Hotkey “L”)

| Command                 | Menu Path                 | Class                                | File |
|-------------------------|---------------------------|--------------------------------------|------|
| 16 Colors               | Image>Lookup Tables       |                                      |      |
| 16-bit                  | Image>Type                | ij.plugin.Converter("16-bit")        |      |
| 3-2 RGB                 | Image>Lookup Tables       | ij.plugin.LutLoader("3-2 RGB")       |      |
| 32-bit                  | Image>Type                | ij.plugin.Converter("32-bit")        |      |
| 3D Project...           | Image>Stacks              | ij.plugin.Projector                  |      |
| 5 Ramps                 | Image>Lookup Tables       |                                      |      |
| 6 Shades                | Image>Lookup Tables       |                                      |      |
| 8-bit                   | Image>Type                | ij.plugin.Converter("8-bit")         |      |
| 8-bit Color             | Image>Type                | ij.plugin.Converter("8-bit Color")   |      |
| AND...                  | Process>Math              | ij.plugin.filter.ImageMath("and")    |      |
| AVI...                  | File>Import               | ij.plugin.AVI_Reader                 |      |
| AVI...                  | File>Save As              | ij.plugin.filter.AVI_Writer          |      |
| About ImageJ...         | Help                      | ij.plugin.AboutBox                   |      |
| About Startup Macros... | Plugins>Macros            |                                      |      |
| About These Macros      | Plugins>Examples>_Macros  | ij.plugin.Macro_Runner("Example...") |      |
| About These Scripts     | Plugins>Examples>_Scripts | ij.plugin.Macro_Runner("Example...") |      |
| About These Tools       | Plugins>Tools             | ij.plugin.Macro_Runner("Tools")      |      |
| About This Submenu...   | Help>About Plugins        | ij.plugin.SimpleCommands("about")    |      |

# FIJI updater



- Keep a backup of working version before you update.

[http://imagej.net>List\\_of\\_update\\_sites](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

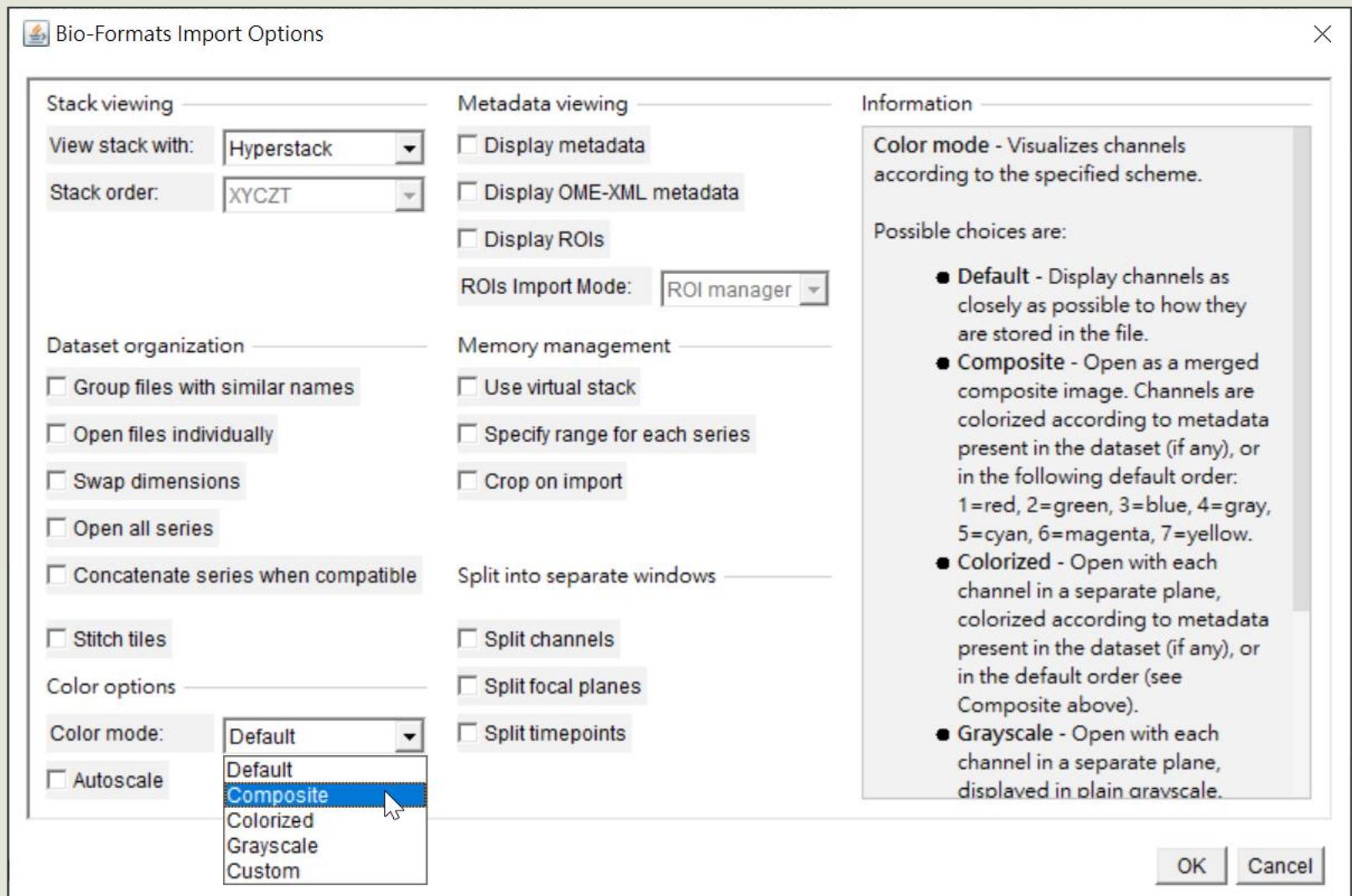
- Vendor specific image format (.czi, .lif, .oir...etc)
- Huygens deconvoluted image ICS2 format (.ics)
- Big Data (Use Virtual Stack)
- Grouped generic image format
- More details in the measurement “label”  
(Channel, Z, T, ROI)

## 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

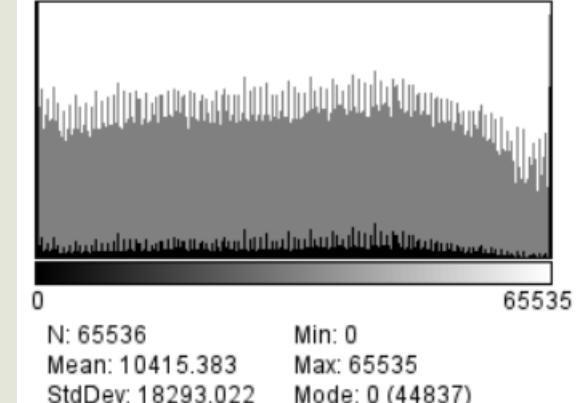
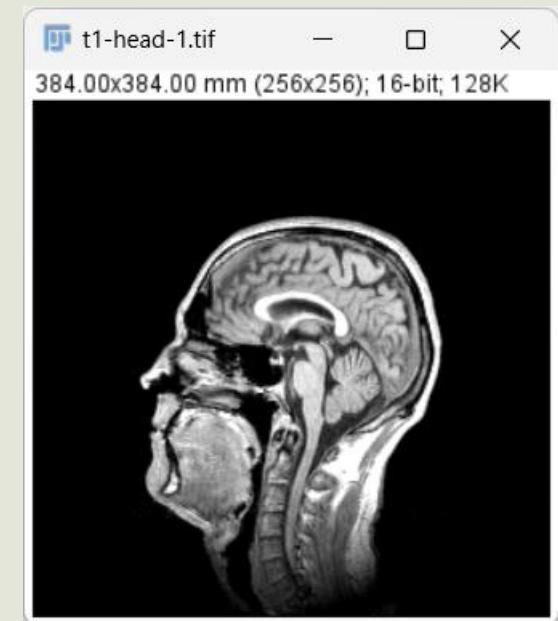
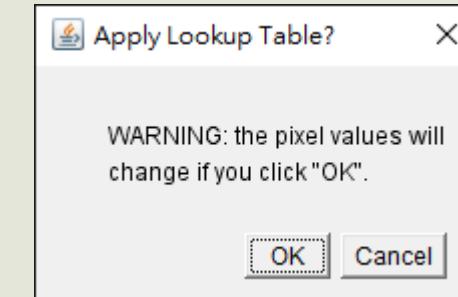
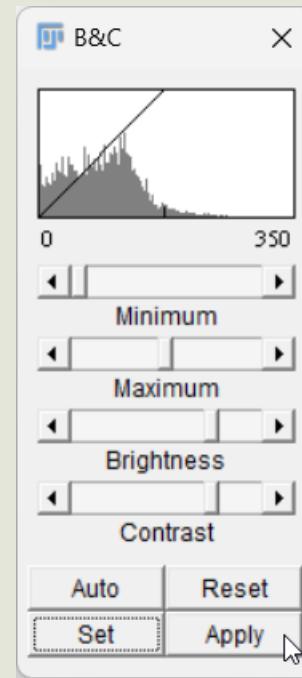
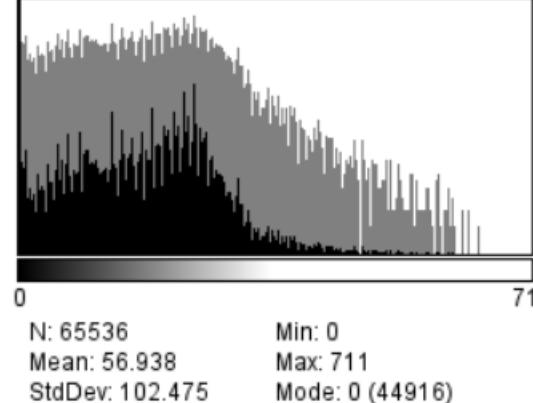
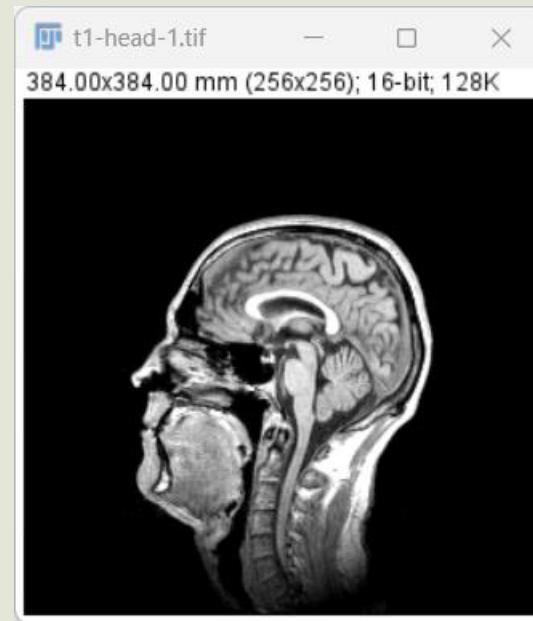
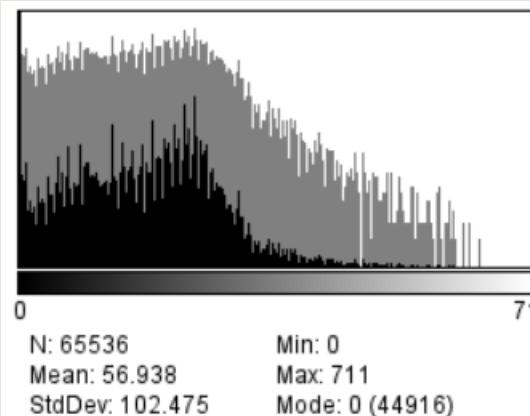
The image shows the Fiji user interface. On the left is the main workspace displaying a fluorescence microscopy image of a mitosis event. The image is labeled "mitosis.tif (200%)". Below the image are three numerical sliders for "c", "z", and "t". A status bar at the bottom provides image metadata: "c:1/2 z:4/5 t:27/51; 15.13x17.35 µm (171x196); 16-bit; 33MB". Yellow arrows point from various labels to specific UI elements:

- Zoom factor: Points to the zoom level in the top status bar.
- File Type: Points to the file type in the top status bar.
- Image size in physical units (and in pixels): Points to the image dimensions in the top status bar.
- Current color channel: Points to the current color channel selection in the bottom status bar.
- Current slice-position in space: Points to the current z-slice position in the bottom status bar.
- Current position in time (frame number): Points to the current frame number in the bottom status bar.

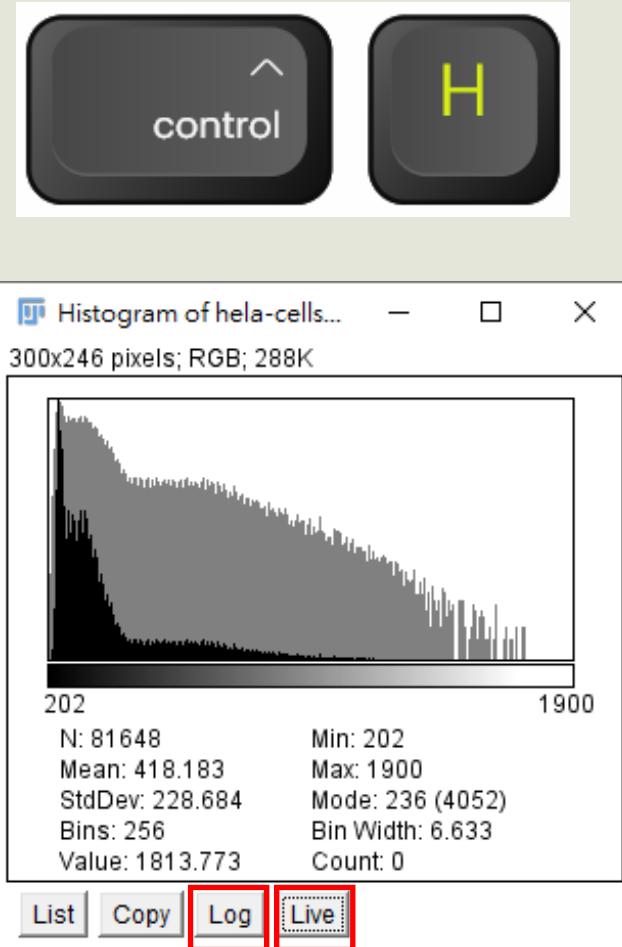
On the right is a screenshot of the "Image" menu. The "Type" option is highlighted. A submenu is open, listing various image types with their corresponding bit depths and color spaces. The "16-bit" option is checked.

**Only 1 active window  
Only 1 activate channel**

# Don't "Apply" when you adjust the Brightness & Contrast



# Histogram



- log scale
- normal view

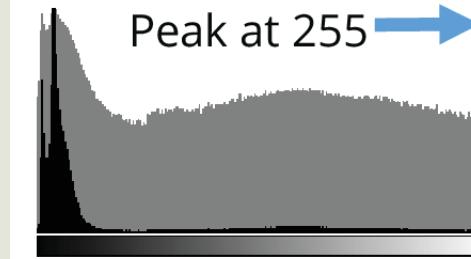
## Image histogram examples

Offset, gap to 0



Properly sampled raw images have background!

Peak at 255



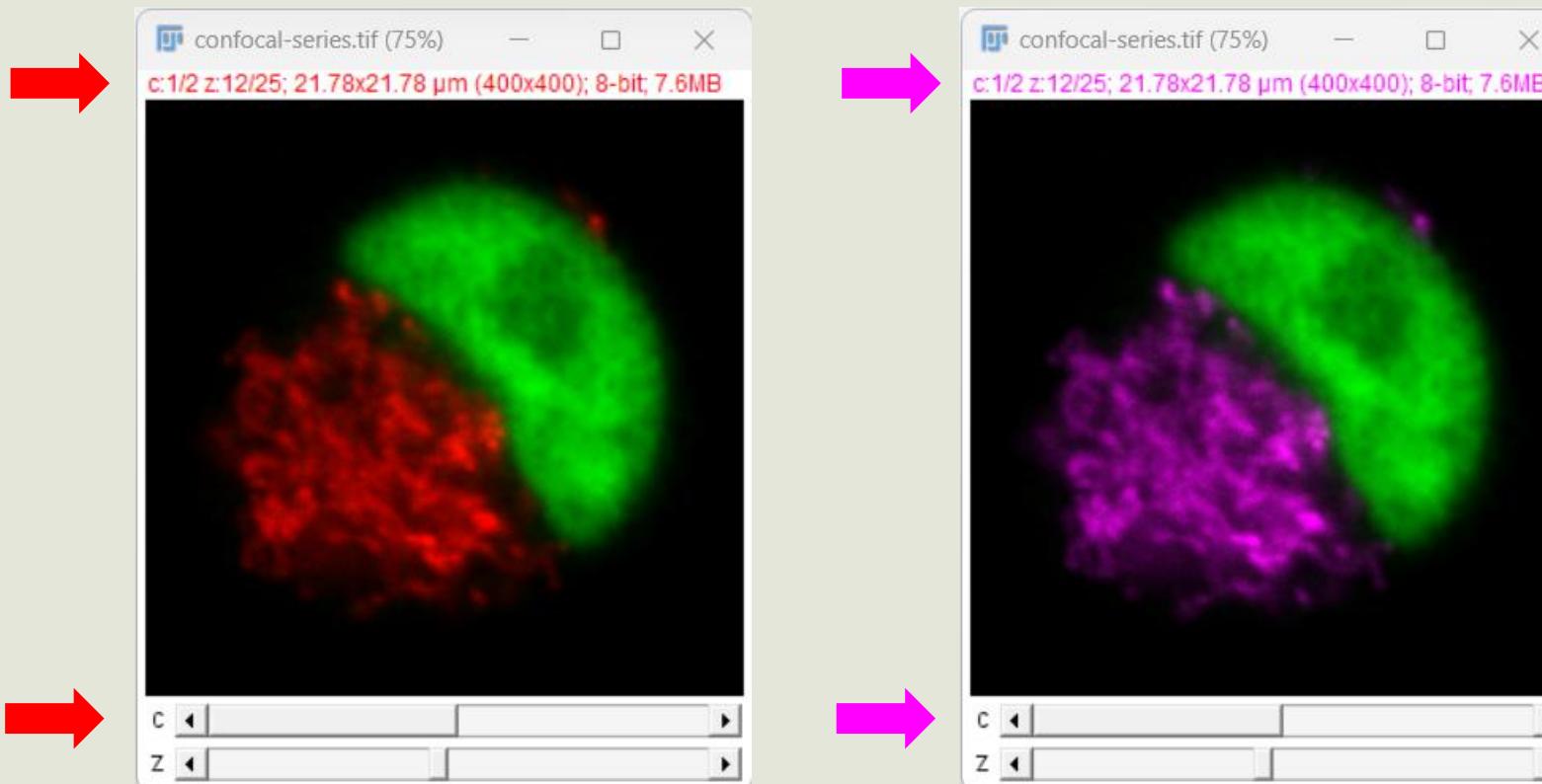
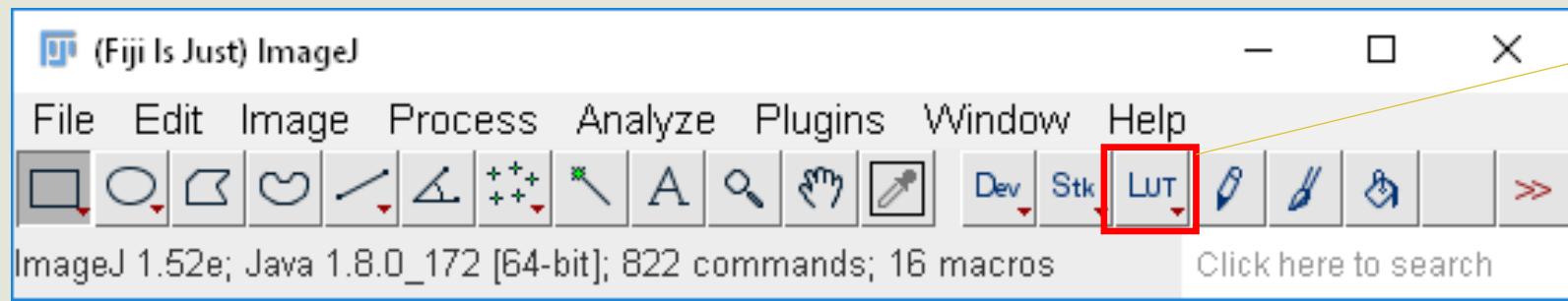
High values clipped  
No background



Scaling artefact



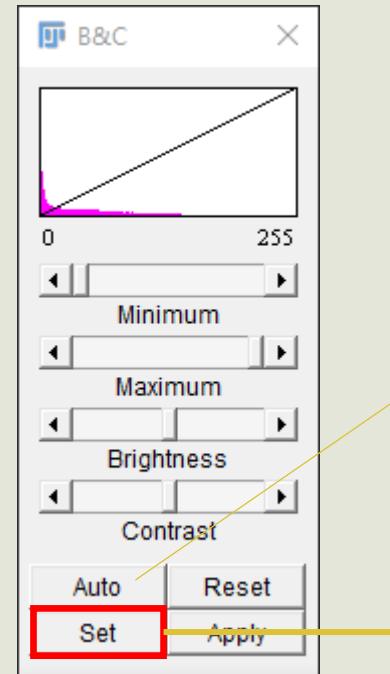
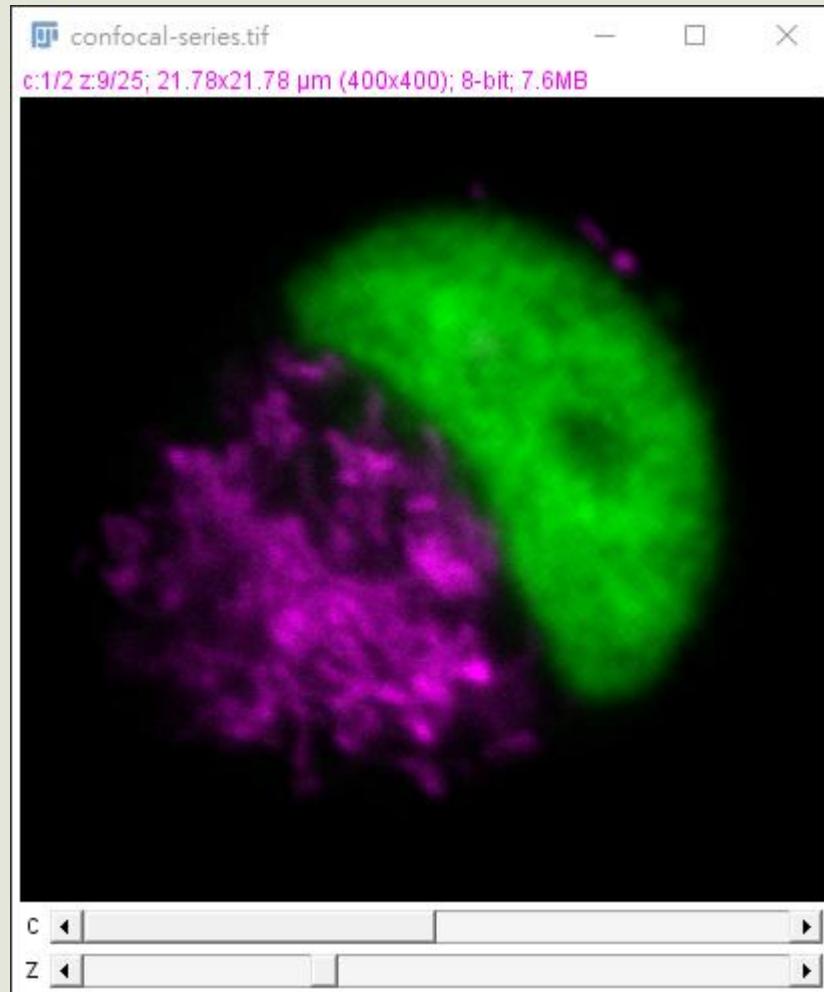
# Change color (LUT /Pseudo-colors / Colormaps)



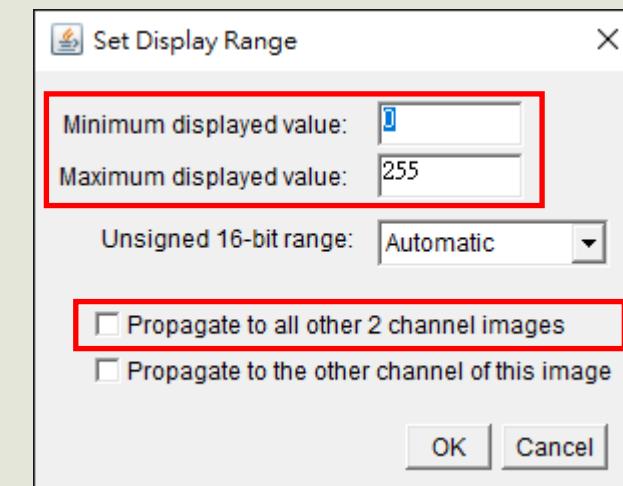
- **LUT button**
- **Image -> Lookup tables**



# Use the same display range for the comparable images

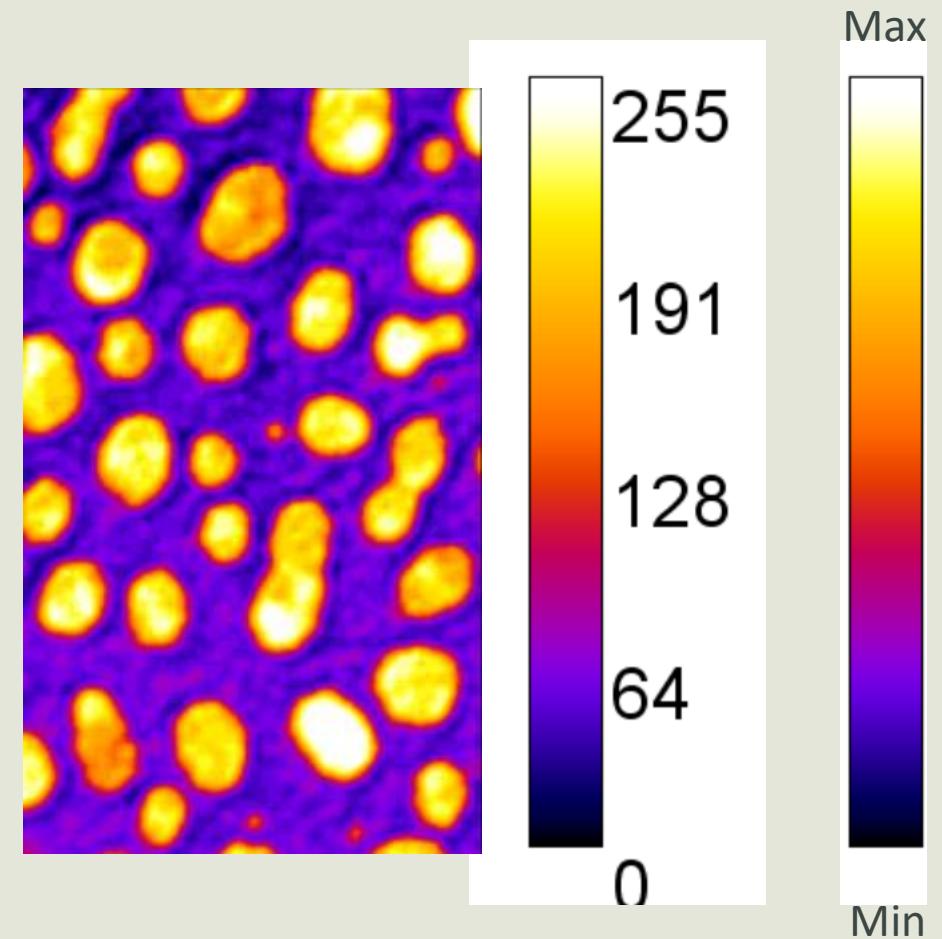
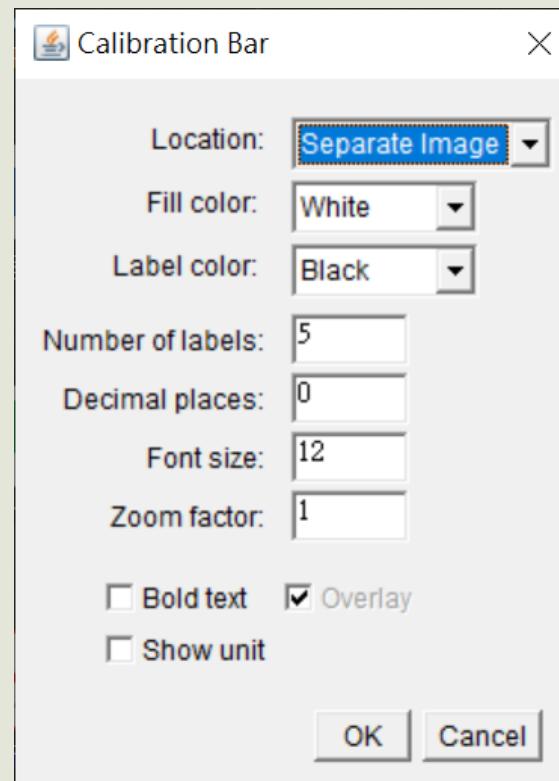


**Don't use auto function independently on each images!**

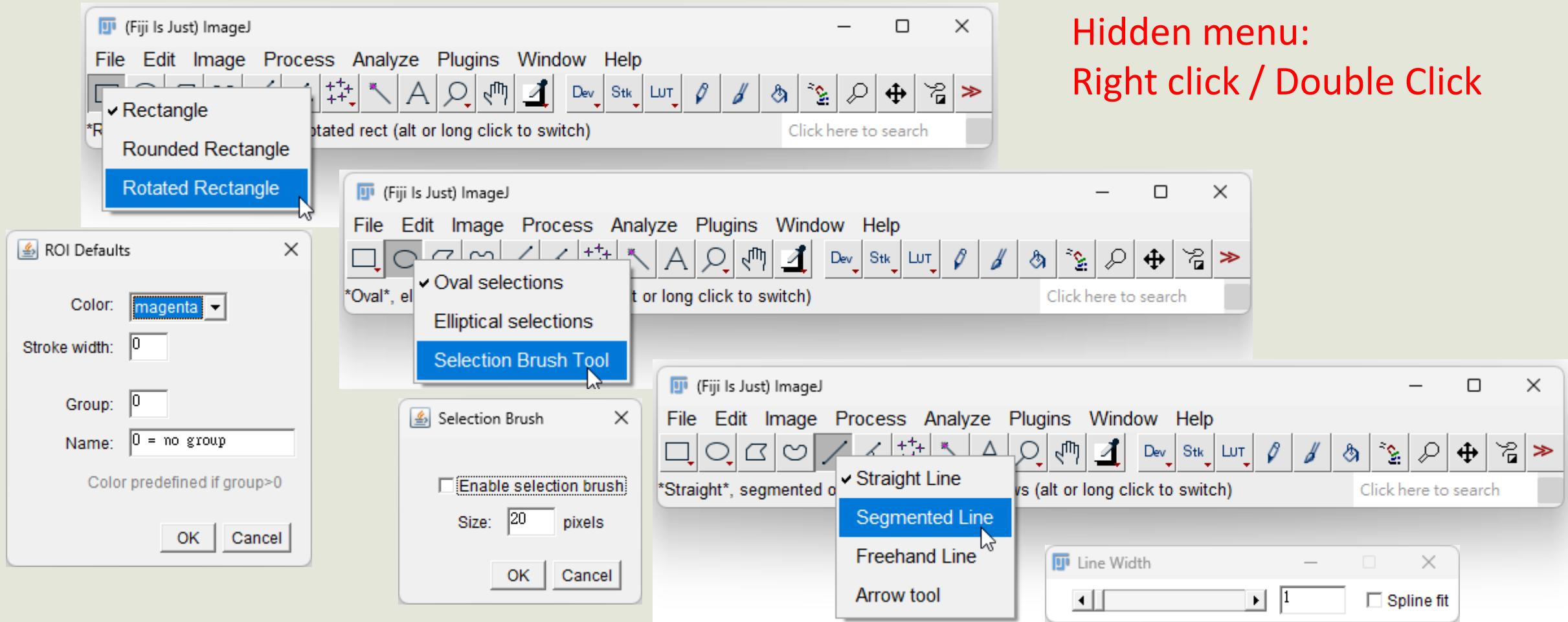


# Calibration Bar (Color scale)

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



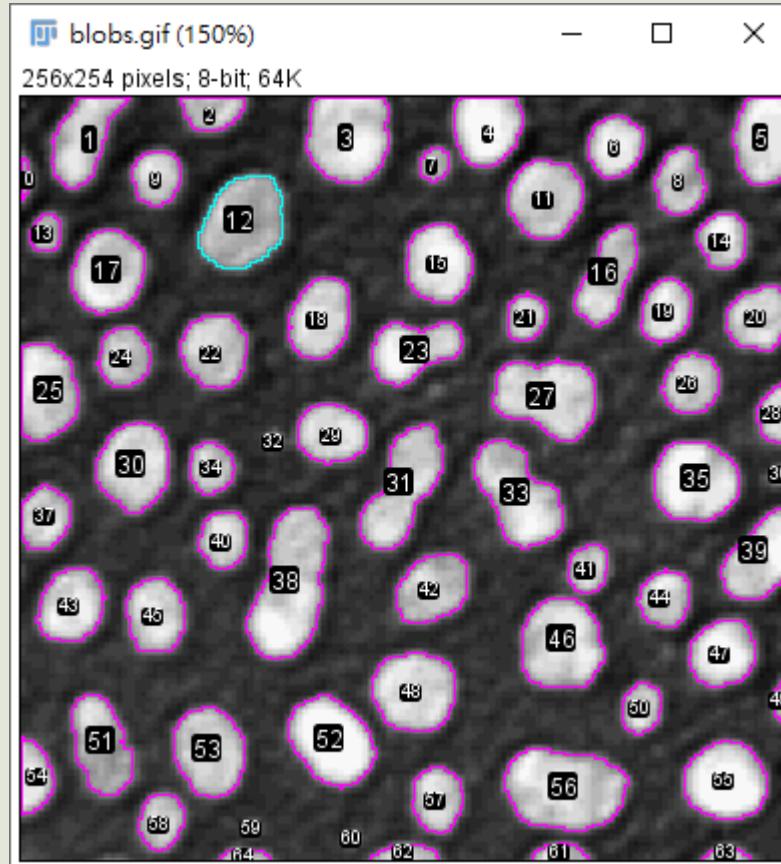
# Region of Interest (ROI) Tools



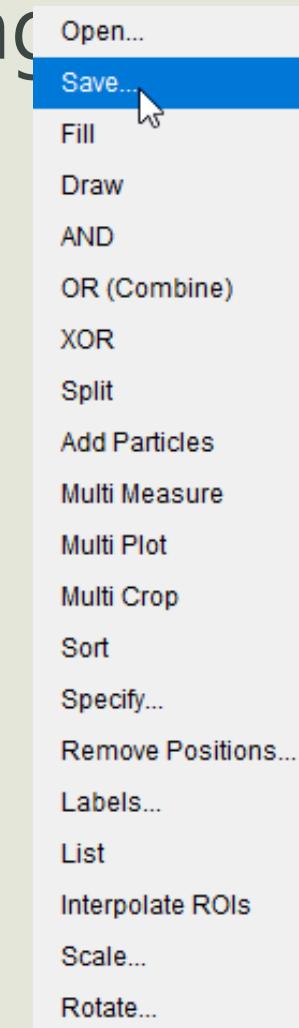
Hidden menu:  
Right click / Double Click

# ROI manager

## ■ Analysis -> Tools -> ROI Manager

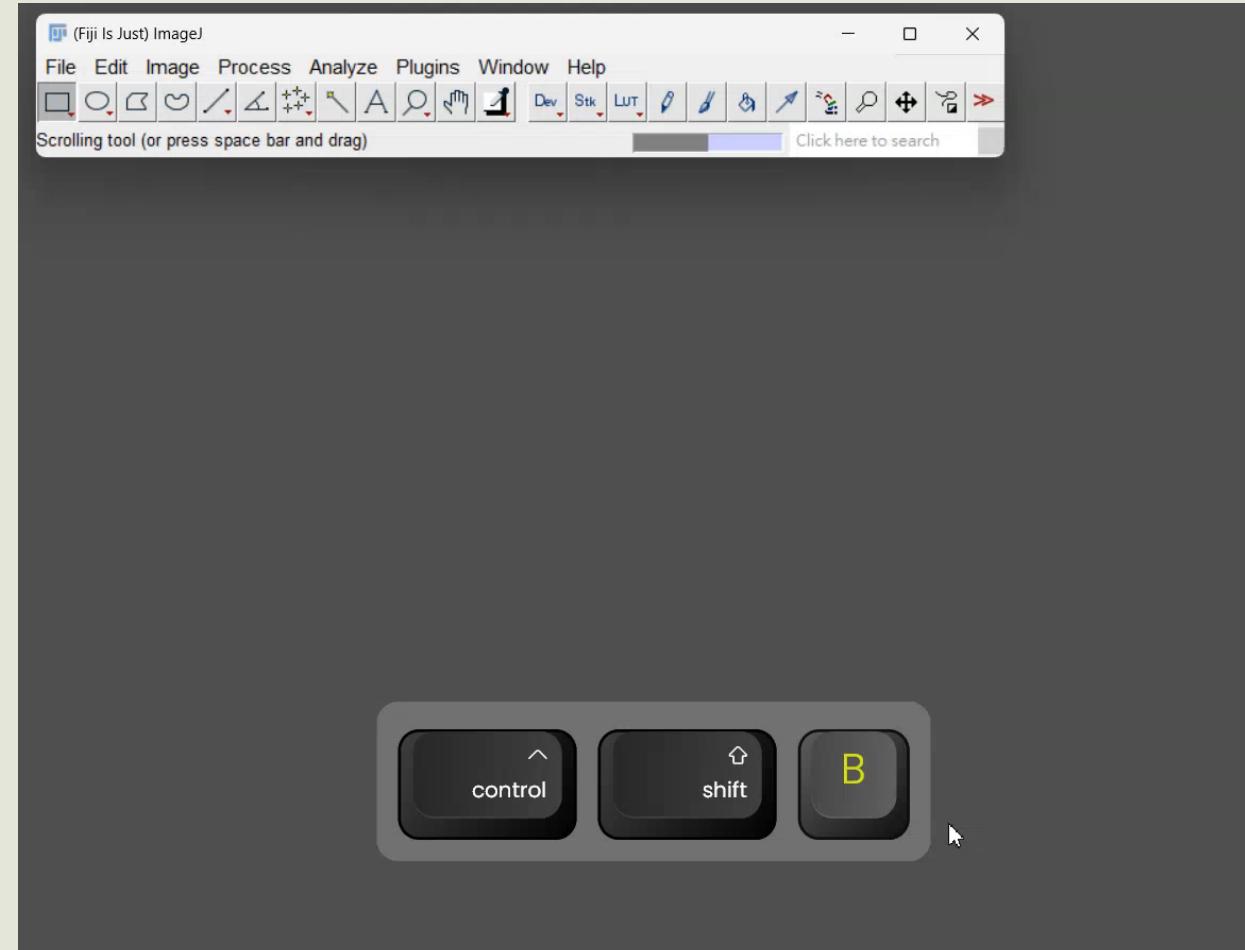
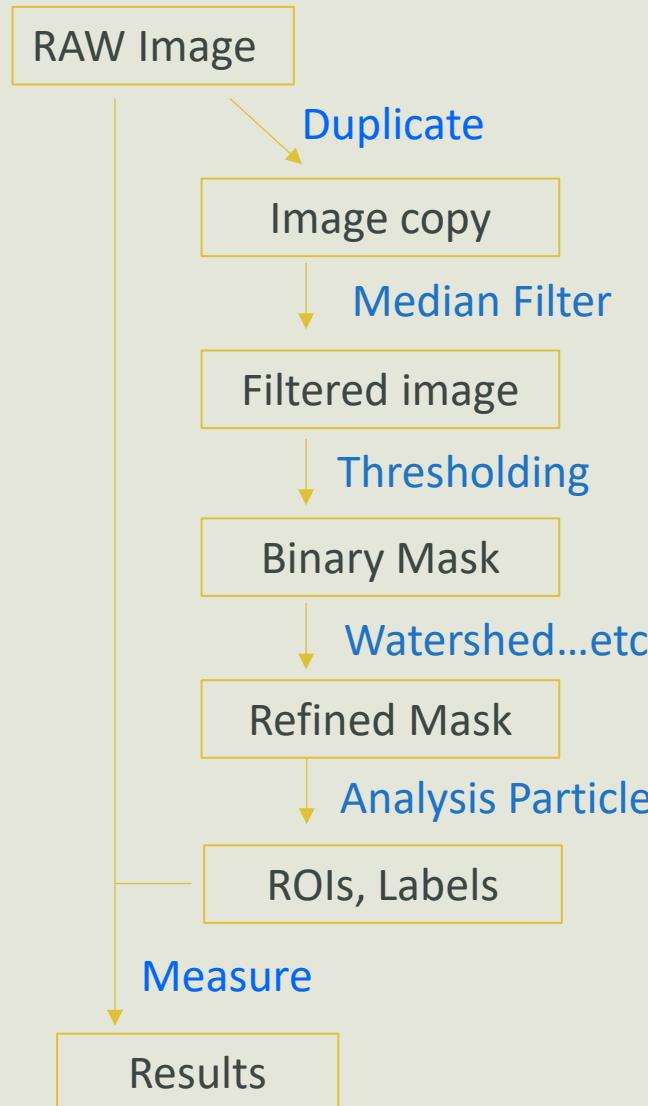


| ROI ...   |
|-----------|
| 0001-0015 |
| 0002-0005 |
| 0003-0014 |
| 0004-0011 |
| 0005-0014 |
| 0006-0016 |
| 0007-0022 |
| 0008-0028 |
| 0009-0027 |
| 0010-0027 |
| 0011-0034 |
| 0012-0041 |
| 0013-0045 |



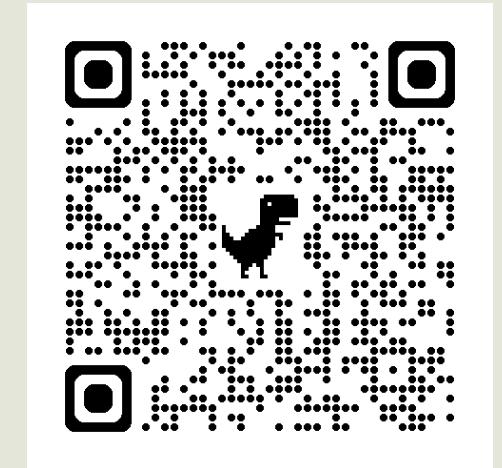
- Save the ROI files is important!
- Single ROI: \*.roi
- Multiple ROI: \*.zip

# Segmentation Sample



# Acknowledgements

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



課後意見調查