

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

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

2025/3/31(一) 【生物影像分析概論】
溫榮峯 中央研究院 生化所 生物影像核心設施
研究助師

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：第一共同研究室顯微影像核心 林思廷 szuting@ntu.edu.tw

國立臺灣大學 National Taiwan University

台大醫學院 College of Medicine

NSTC 國家科學及技術委員會 National Science and Technology Council

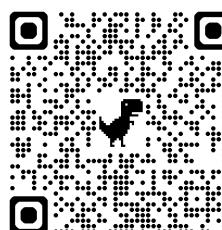
IBI

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。

第二堂課的提醒：

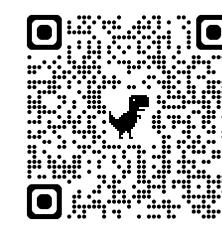
- 請先在電腦中開啟Fiji並將課程材料中下載Demo影像的資料夾開啟，放在一側方便上課過程中直接拖曳檔案練習。
- 來不及跟上實作的同學，可於線上或課後詢問，線上會有其他講師可回答問題，現場的同學可於課後留下來討論問題。
- 第二堂課內容較長，若要提早離開的人，請先完成課程回饋調查再離開，謝謝！
- 我們正在準備明年Python的課程，如果您也想成為我們的講師群，歡迎與我們聯絡！



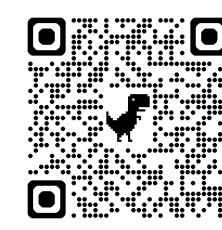
線上簽到



課程材料與相關連結



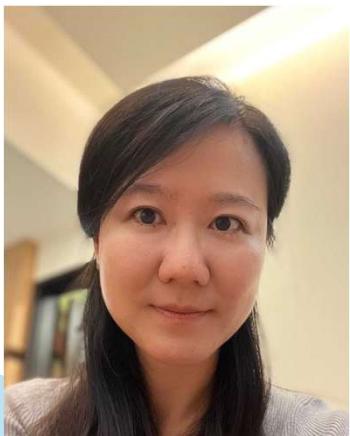
課後意見調查



EABIAS Lecturer Recruiting

IMAGEJ顯微影像分析與程式設計

生物影像分析流程



許紹君

Shao-Chun Hsu (Peggy)

Assistant Research Specialist

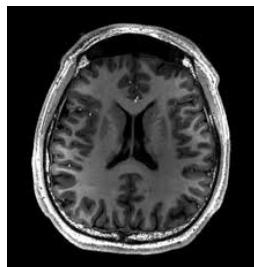
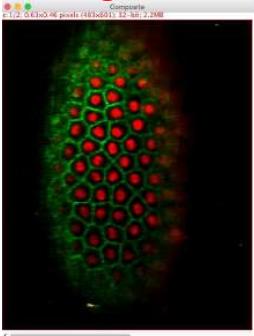
NTUCM Imaging Core/

NTU Consortium of Molecular Imaging Key Technology

2025-3-10

Aim of Bioimage Analysis

Reliable and quantifiable
image data

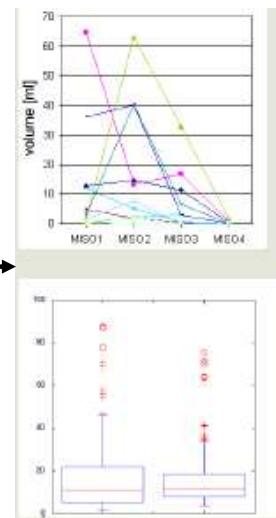


Aim: Understanding and quantifying microscopic, medical or any calibrated image data.

Quantification

	A	B	C	D	E
1		Area	Mean	IntDen	RawIntDen
2	1	4.79	387.39	1855.67	268074
3	2	3.3	171.52	566.33	81813
4	3	2.87	0	0	0
5	4	13.89	340.27	4725	682582
6	5	1.25	0	0	0
7	6	1.79	391.36	698.94	100970
8	7	6.67	281.61	1877.26	271193
9	8	1.27	0	0	0
10	9	3.38	0	0	0
11	10	10.33	0	0	0
12	11	4.79	387.39	1855.67	268074
13	12	3.3	171.52	566.33	81813
14	13	2.87	0	0	0
15	14	13.89	340.27	4725	682582
16	15	1.25	0	0	0
17	16	1.79	391.36	698.94	100970
18	17	6.67	281.61	1877.26	271193
19	18	1.27	0	0	0
20	19	3.38	0	0	0
21	20	10.33	0	0	0
22	21	4.79	387.39	1855.67	268074
23	22	3.3	171.52	566.33	81813
24	23	2.87	0	0	0
25	24	13.89	340.27	4725	682582
26	25	1.25	0	0	0

Statistics



Report
biological
insight



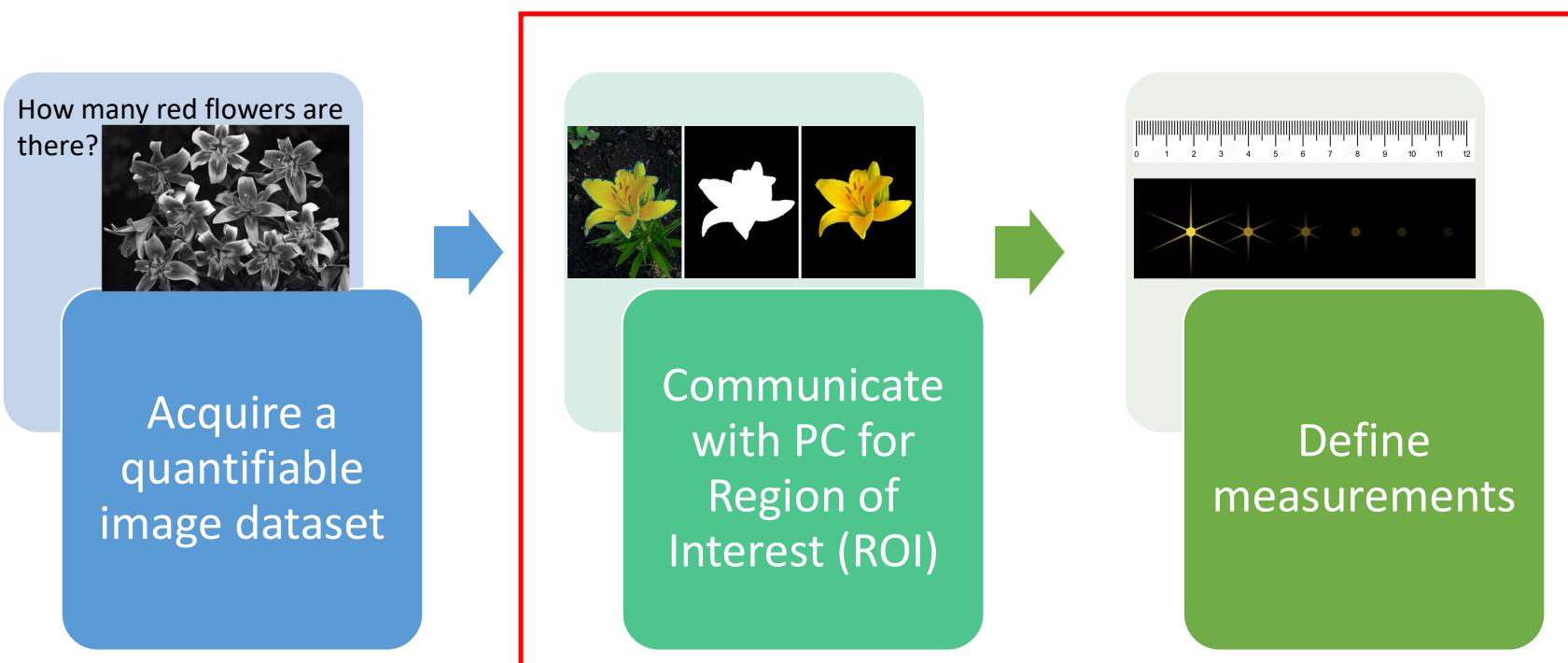
臺大顯微影像核心 NTU Imaging Core
@imagingcore004 · 337 位訂閱者 · 67 部影片
彰基核心計畫委員會99年10月：辦理台灣院區第一公共研究室核心實驗室之一，主...
rd.mc.ntu.edu.tw/bomrd/imgcore/index.asp



GloBIAS blog series

Outline of the second lecture

- Bioimage analysis workflow



<https://www.thelilygarden.com/>

<https://www.mathworks.com/help/images/specify-roi-as-binary-mask.html>

<https://stock.adobe.com/hk/images/ruler-is-isolated-on-white-12-inch-measuring-tool-png/552996567>
https://en.wikipedia.org/wiki/Magnitude_%28astronomy%29

The way to tell PC what the region of interest (ROI) is— Image Segmentation

Most of the segmentation algorithms are based on one of the two basic properties of image intensity values: **discontinuity** and **similarity**.
Segmentation

Biological Question:

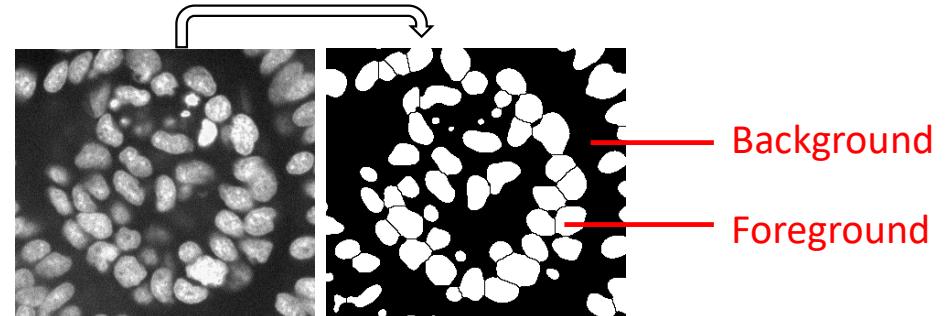
I want to count the cell number in this image.

Presumption:

Each nucleus represents the location of a cell.

Bioimage Analysis:

Define each nucleus as a ROI. And then count the
ROI number.



This is a **raw** image. This is a **mask** image.

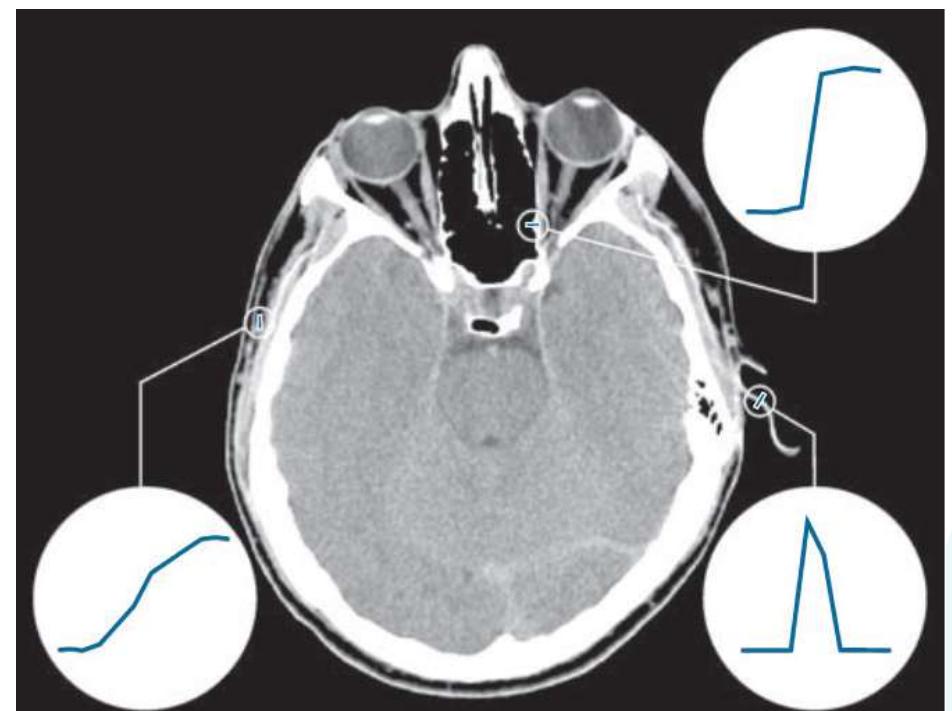
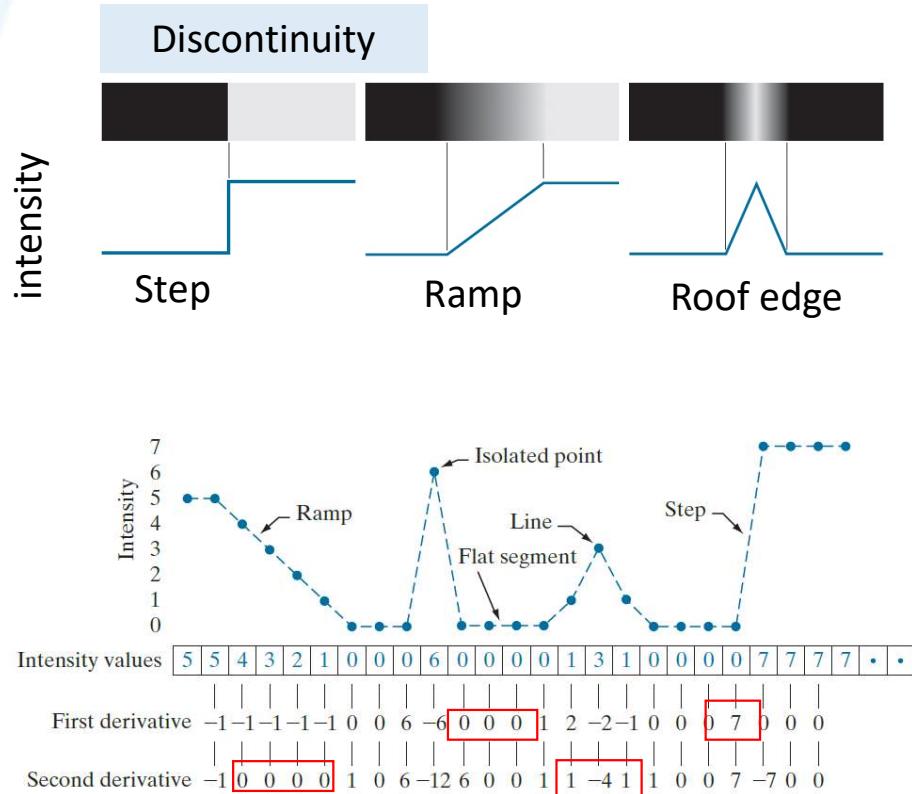
The raw image is **binarized**, which means **two intensity values (0,1 or 0,255 pairs)** left in the mask image.

Similarity														
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	12	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			

$$\begin{aligned}f(0,0) &= 53 & f(1,0) &= 191 \dots \text{Gray value}, \\f(0,1) &= 0 & & \text{Intensity} \\f(0,2) &= 71 & & \text{Coordinate} \\&\dots\end{aligned}$$

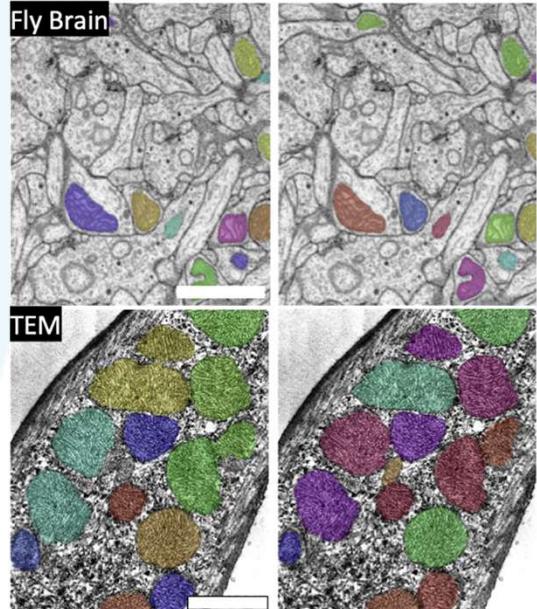
$$R = \begin{cases} 1 & \text{if } f(x,y) > T \text{ Threshold} \\ 0 & \text{if } f(x,y) \leq T \end{cases}$$

Segmentation algorithms



Other tools uncovered in this series

Prediction



Ground Truth

MONAI

HOME FRAMEWORKS MODEL ZOO WORKING GROUPS COMMUNITY

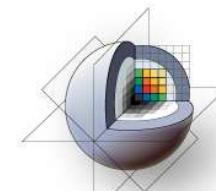
Spleen CT segmentation MONAI team A pre-trained model for volumetric (3D) segmentation of the spleen from CT image v0.5.9 View Details Download	Pancreas CT DiNTS segmentation MONAI team Searched architectures for volumetric (3D) segmentation of the pancreas from CT image v0.5.0 View Details Download	BraTS MRI segmentation MONAI team A pre-trained model for volumetric (3D) segmentation of brain tumor subregions from multimodal MRIs based on BraTS 2018 data v0.5.2 View Details Download
Spleen DeepEdit annotation MONAI team This is a pre-trained model for 3D segmentation of the spleen organ from CT images using DeepEdit. v0.5.6 View Details Download	Swin UNETR BTCV segmentation MONAI team A pre-trained model for volumetric (3D) multi-organ segmentation from CT image v0.5.6 View Details Download	Ventricular short axis 3 label segmentation Eric Kerfoot This network segments full cycle short axis images of the ventricles, labelling LV pool separate from myocardium and RV pool. v0.3.3 View Details Download

For radiology app: 3D Slicer and Open Health Imaging Foundation (OHIF)
 For pathology app: Digital Slide Archive (DSA) and QuPath
 For Endoscopy app: CVAT

<https://monai.io/model-zoo.html>



Monai



3DSlicer



QuPath

QuPath - PhenoCycler-Fusion without saturation protection.qptiff - resolution #1

File Edit Tools View Objects TMA Measure Automate Analyze Classify Extensions Help

Installed extensions

- ImageJ
- SAM
- StarDist

Community Partners

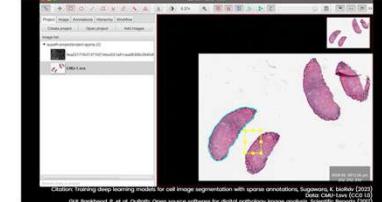
QuPath extension Segment Anything Model (SAM)

Ko Sugawara Isugawara Jun 2023

Overview

I released a QuPath [extension](#) for SAM [\[1\]](#). This is a part of the following preprint.

- Sugawara, K. *Training deep learning models for cell image segmentation with sparse annotations*. [bioRxiv](#) 2023. doi:10.1101/2023.06.13.544786



Citation: Training deep learning models for cell image segmentation with sparse annotations, Sugawara, K. *bioRxiv* (2023). doi:10.1101/2023.06.13.544786

It implements client and server architecture, and you can set up the server either locally or remotely.

@petebankhead has made the server compatible with Apple's Metal Performance Shaders (MPS) on macOS.

Thanks to QuPath [\[2\]](#), this implementation supports running SAM in multiple resolutions.

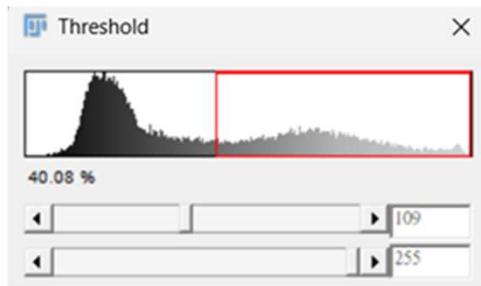
Types of thresholding

1. Global thresholding Use a single (global) threshold applicable over the entire image.

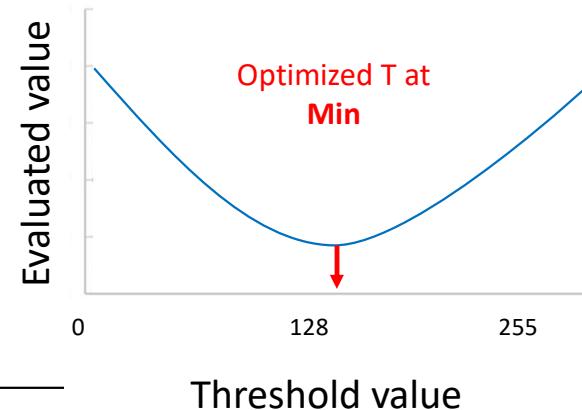
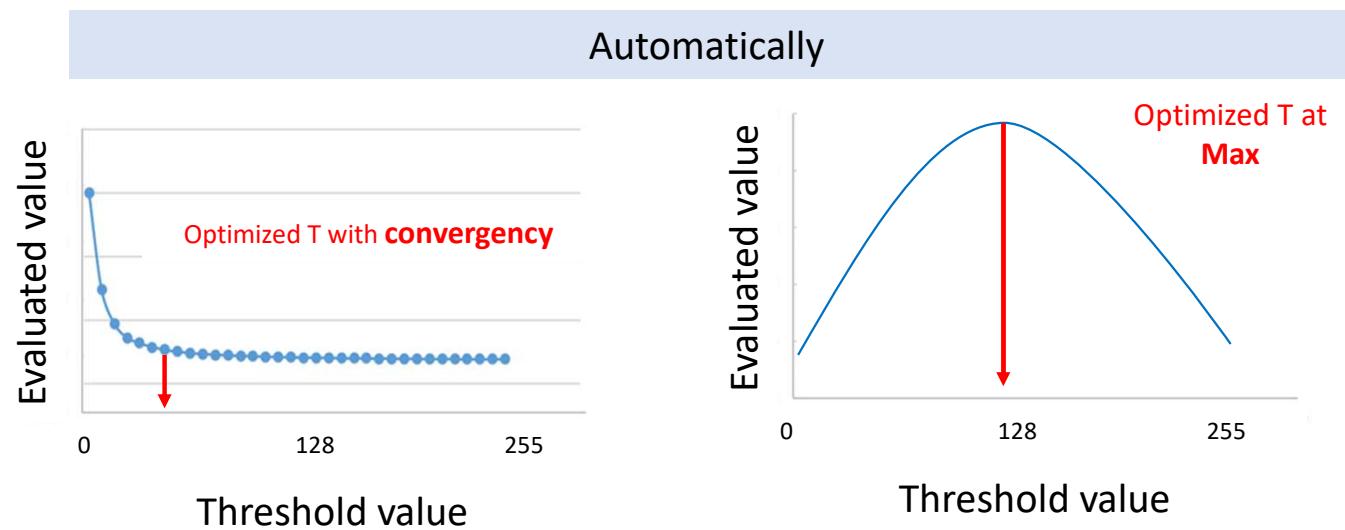
2. Variable thresholding Compute a threshold at every point, (x,y) , in the image based on one
(adaptive thresholding) or more specified properties in a neighborhood of (x,y) .
(local thresholding)
(regional thresholding)
(dynamic thresholding)

Manual thresholding vs Auto thresholding

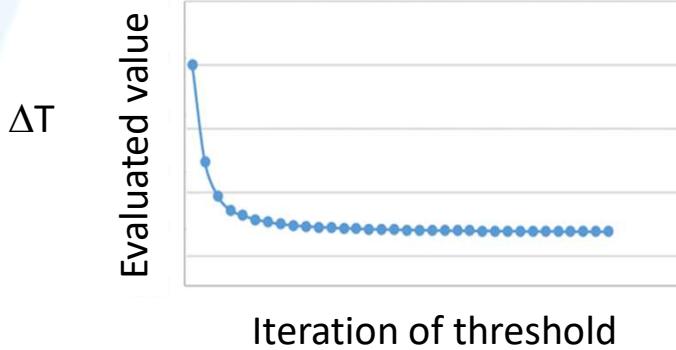
Manually



Automatically



How is automation actually implemented? Default method



T_{new}

1. Initial threshold $T_i = 0$

2. $T = T_i \quad \text{or} \quad T_{\text{new}}$

$$G_{\text{raw}} = \begin{cases} 1 & \text{if } f(x, y) > T \\ 0 & \text{if } f(x, y) \leq T \end{cases}$$

G_1 Foreground
 G_2 Background

3. Get

mean_1 from G_1 Foreground

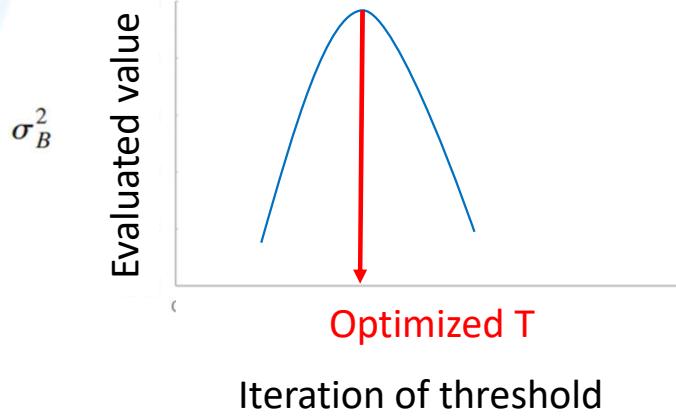
mean_2 from G_2 Background

4. $T_{\text{new}} = (\text{mean}_1 + \text{mean}_2)/2$

5. Calculate $\Delta T = T_{\text{new}} - T_i$

Until $\Delta T = T_n - T_{n-1} <$ Predefined value

How is automation actually implemented? Otsu method



1. Initial threshold $T_i = 0$

2.

$$G_{\text{raw}} = \begin{cases} 1 & \text{if } f(x, y) > T \\ 0 & \text{if } f(x, y) \leq T \end{cases}$$

G_1 Foreground

G_2 Background

3. Get mean_{raw} from G_{raw}
 mean_1 from $G_1 = m_1$ Foreground
 mean_2 from $G_2 = m_2$ Background

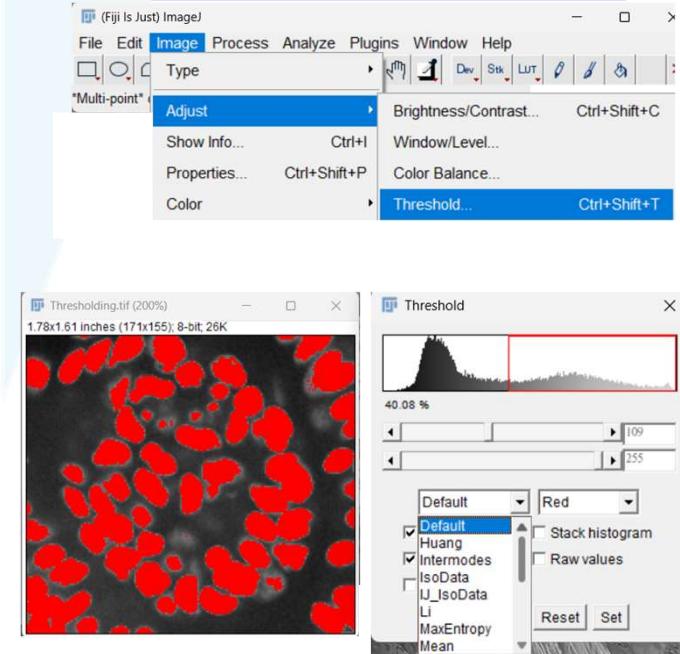
$$4. \sigma_B^2 = \frac{P_1(m_1 - m_G)^2}{A} + \frac{P_2(m_2 - m_G)^2}{B}$$

利用變異數(variance)評估二值化(binariization)後
前景與原始檔案是否有最大的不同(項次A)
背景與原始檔案是否有最大的不同(項次B)
當項次A與B總和達到最大值時,定義此灰階度為最理想的threshold

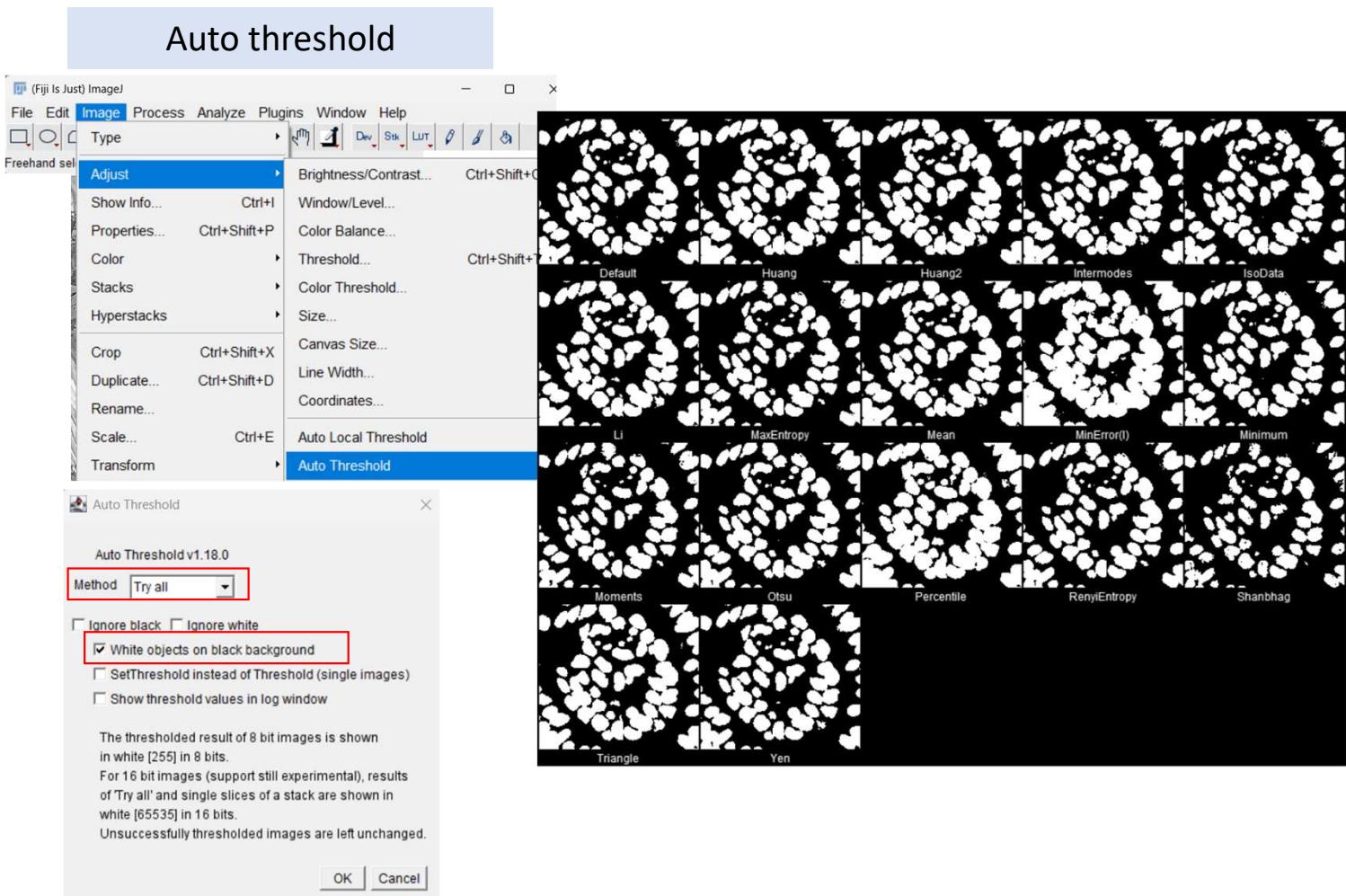
https://github.com/fiji/Auto_Threshold/blob/master/src/main/java/fiji/threshold/Auto_Threshold.java

Inspect Histogram and test for thresholding

Manually adjustment

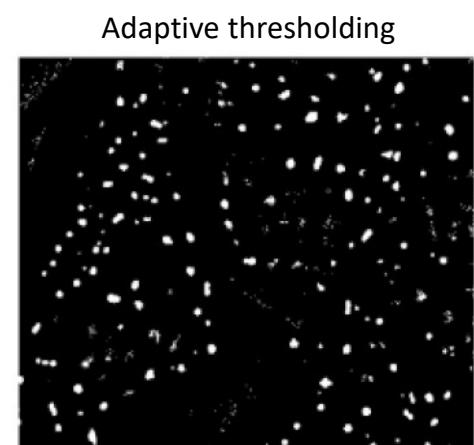
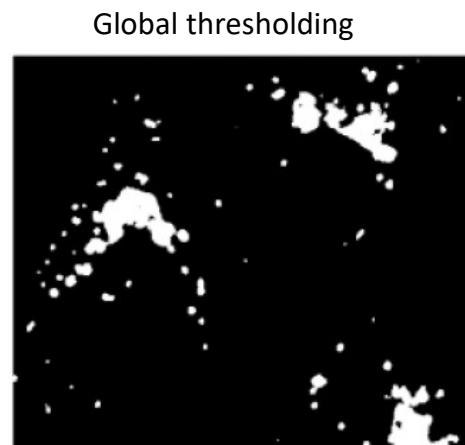
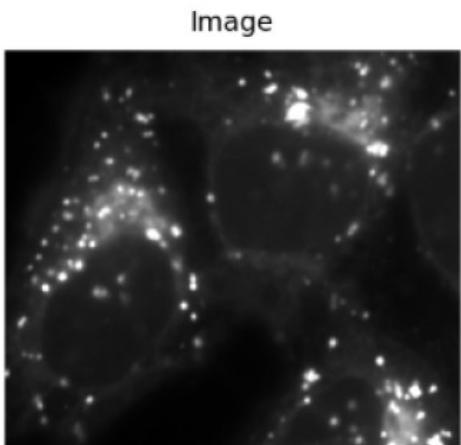


Auto threshold



Types of thresholding

1. Global thresholding Use a single (global) threshold applicable over the entire image.
2. Variable thresholding Compute a threshold at every point, (x,y) , in the image based on one
(adaptive thresholding) or more specified properties in a neighborhood of (x,y) .
(local thresholding)
(regional thresholding)
(dynamic thresholding)



Local thresholding

Bernsen

Implements Bernsen's thresholding method. Note that this implementation uses circular windows instead of rectangular in the original.

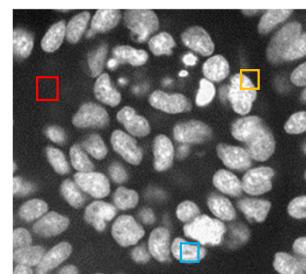
Parameter 1: is the *contrast threshold*. The default value is 15. Any number different than 0 will change the default value.

Parameter 2: not used, ignored.

The method uses a user-provided *contrast threshold*. If the *local contrast* ($\text{max}-\text{min}$) is above or equal to the *contrast threshold*, the *threshold* is set at the *local midgrey value* (the mean of the minimum and maximum grey values in the local window). If the *local contrast* is below the *contrast threshold* the neighbourhood is considered to consist only of one class and the pixel is set to object or background depending on the value of the midgrey.

```
if ( local_contrast < contrast_threshold )
    pixel = ( mid_gray >= 128 ) ? object : background
else
    pixel = ( pixel >= mid_gray ) ? object : background
```

max-min = local contrast <15,
Mid_gray <128 => background



max-min = local contrast <15,
Mid_gray >128 => foreground

max-min = local contrast >15,
Mid_gray >128 => foreground
Mid_gray <128 => background

Local window Output

255	5	15		
55	16	100		0
1	6	20		

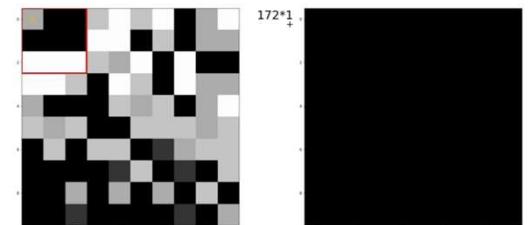
Local window.max = max = 255

Local window.min = min = 1

Local contrast = max – min = 254

Local window.mean = Mid_gray = 52.5

∴ Local contrast = max – min = 254 > 15,
Λ Mid_gray = 52.5 < 128,
∴ Classify as background
Center px value = 0



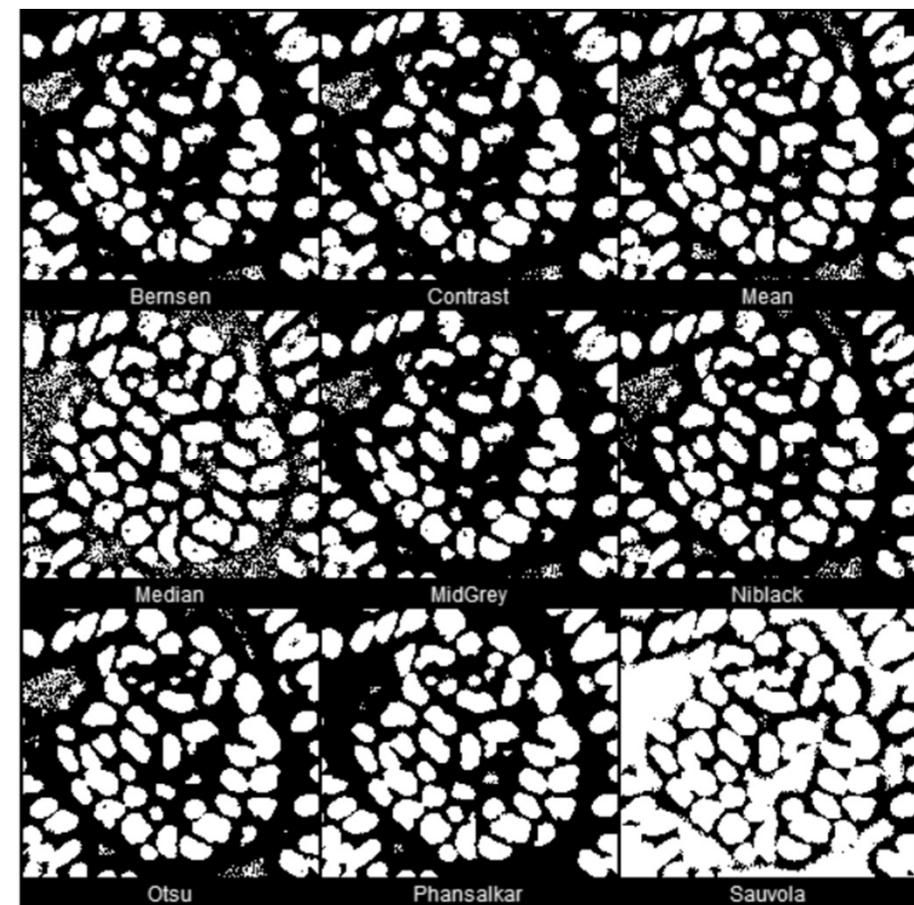
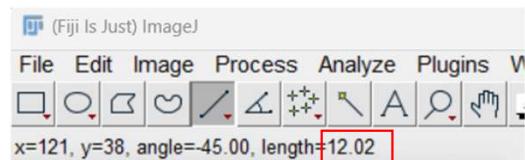
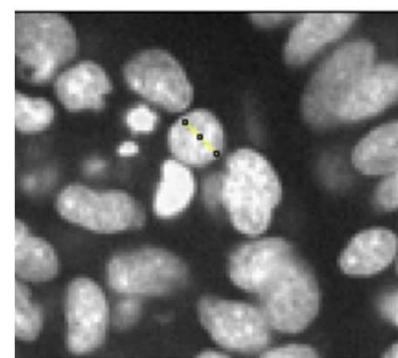
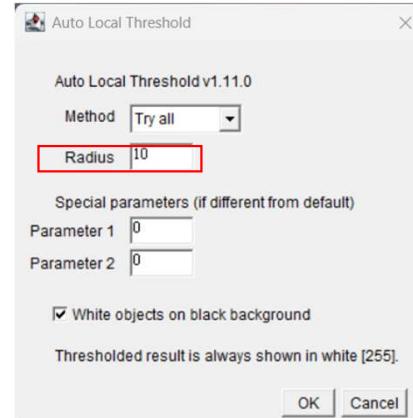
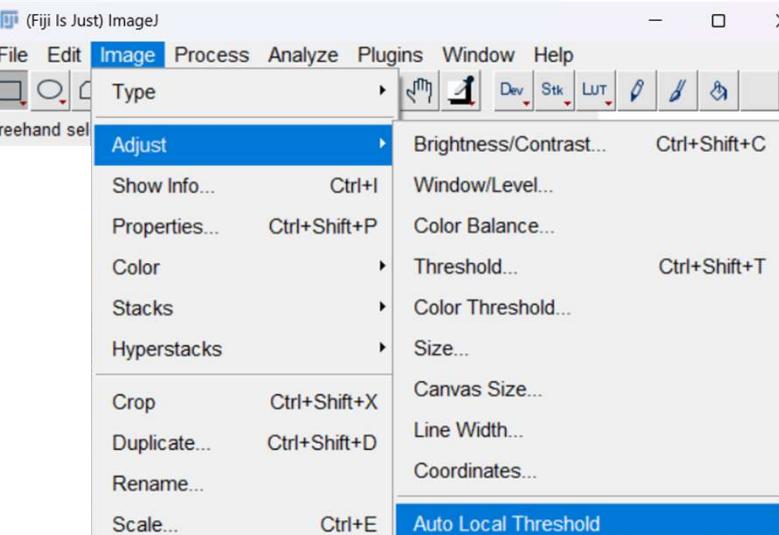
Mean filter

<https://imagej.net/plugins/auto-local-threshold>

Animation source: Dominic Waithé, Oxford University

https://github.com/dwaithé/generalMacros/tree/master/convolution_ani

Auto local threshold



Adaptive thresholding from local background

2 Count Nuclei Objects ▼ X

Source Nucleus

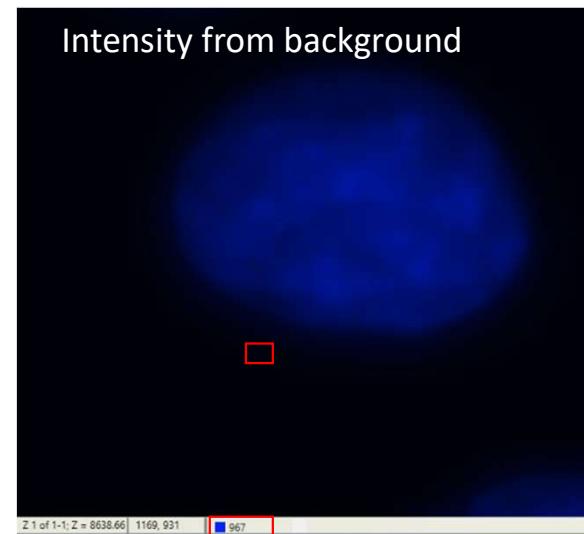
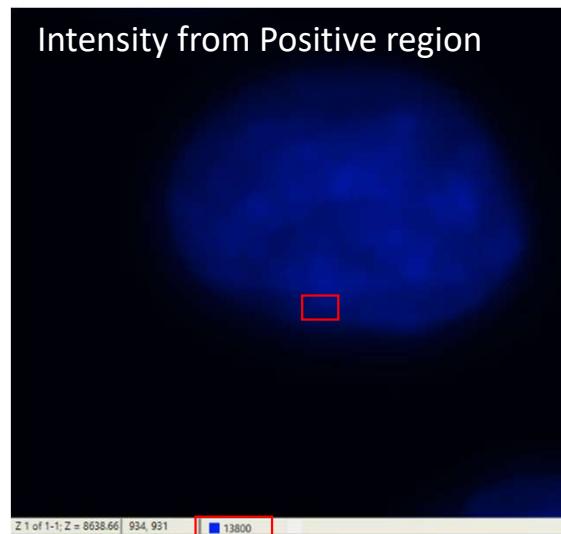
Parameters

Approximate Minimum Width (μm) 3

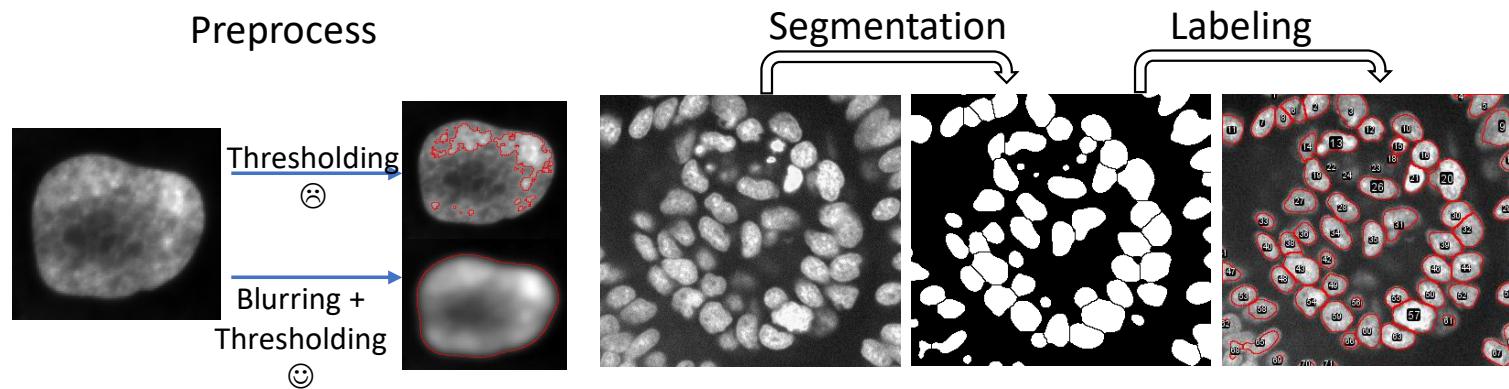
Approximate Maximum Width (μm) 20

Intensity Above Local Background 1000

$$13800 - 967 = 12833$$



Why preprocess and postprocess?



Preprocess—

Generate masks more easily and better fit our expectations.

- ✓ Noise reduction
 - Median filter
 - Gaussian filter
- ✓ Correct background
 - Background subtraction

Postprocess—

Modify masks

- ✓ Fill holes in the ROI
- ✓ Merge objects
- ✓ Get outline
- ✓ Split

Refining masks

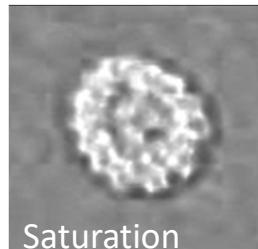
Split color image by different spaces



Raw

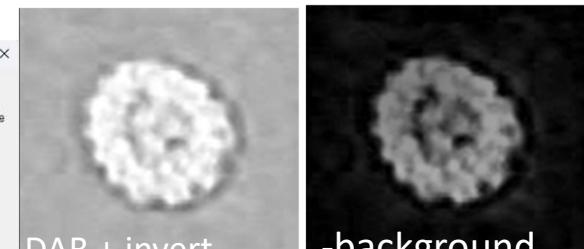
Fiji interface showing 'Image' > 'Type' > 'HSB (3-bit)' selected.

Saturation



Fiji interface showing 'Image' > 'Color' > 'Colour Deconvolution 1.7' > 'H&E DAB' selected.

Split color image by color deconvolution



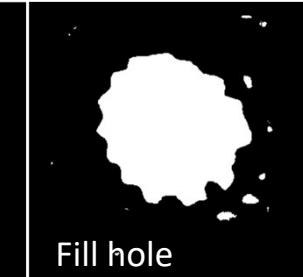
DAB + invert

-background

Threshold



Fill hole



Erode

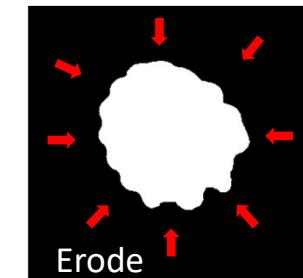


Image Calculator...

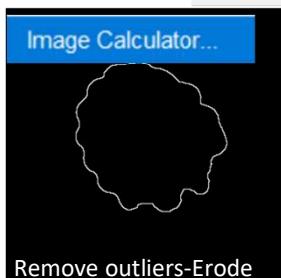
Binary Options

- Iterations (1-100): 1
- Count (1-8): 1
- Black background
- Pad edges when eroding

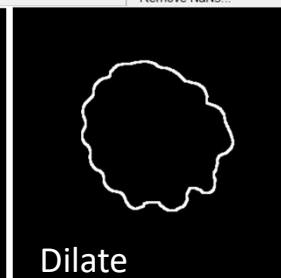
Remove outliers



Remove outliers-Erode



Dilate



Watershed

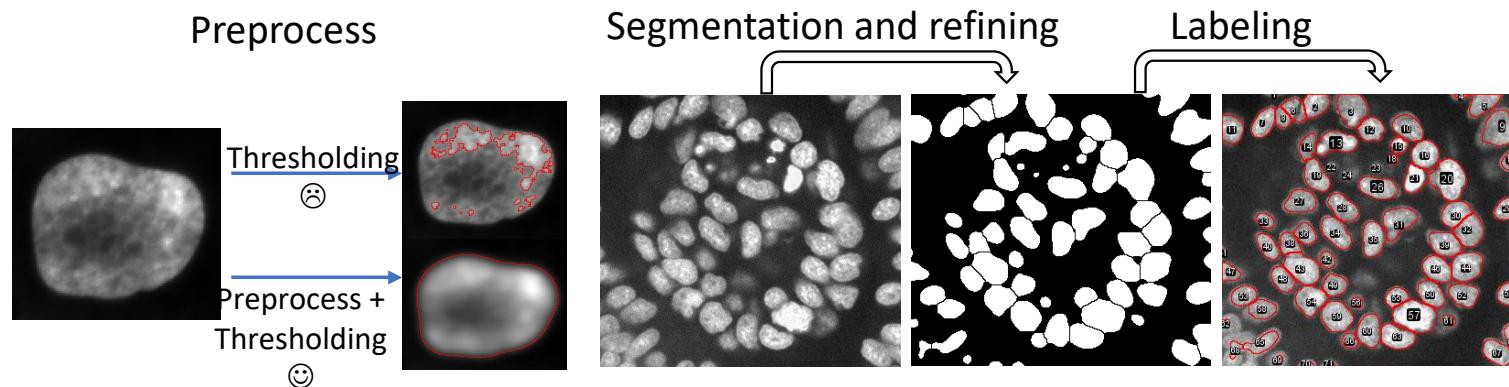


Fiji interface showing 'Process' > 'Binary' > 'Image Calculator...' selected.

Fiji interface showing 'Process' > 'Binary' > 'Fill Holes' selected.

Fiji interface showing 'Process' > 'Noise' > 'Remove Outliers...' selected.

Why preprocess and postprocess?



Preprocess—

Generate masks more easily and better fit our expectations.

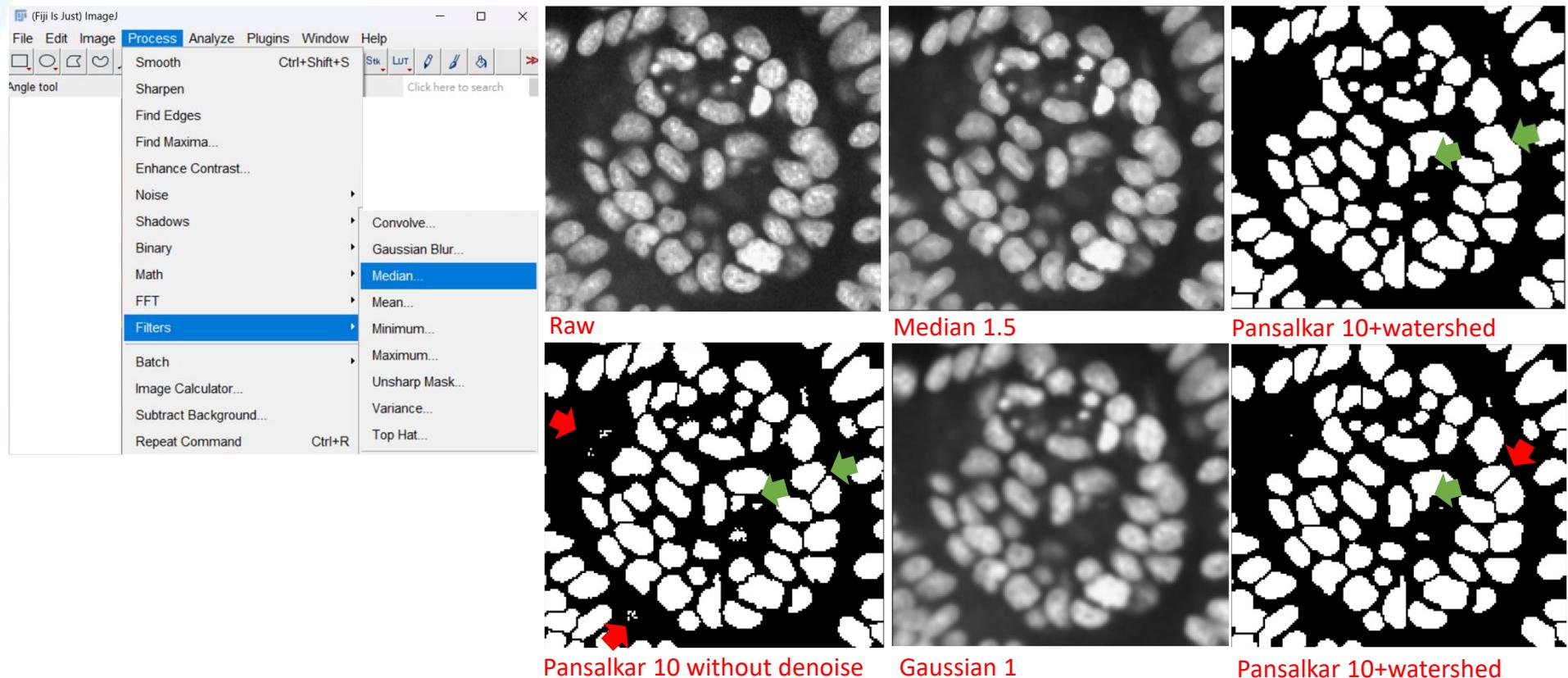
- ✓ Noise reduction
 - Median filter
 - Gaussian filter
- ✓ Correct background
 - Background subtraction

Postprocess—

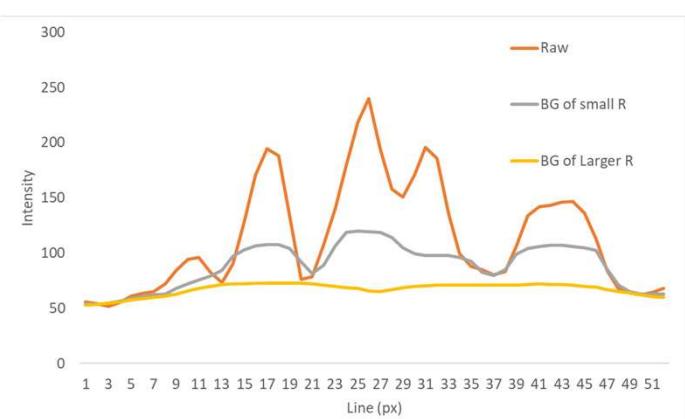
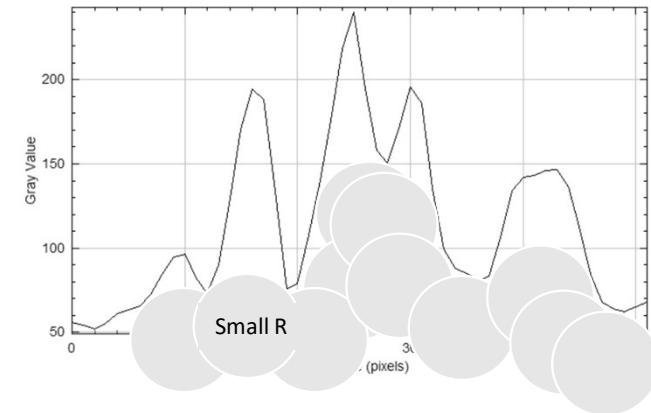
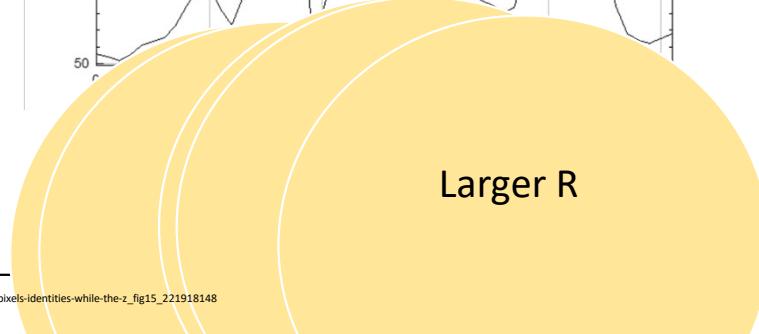
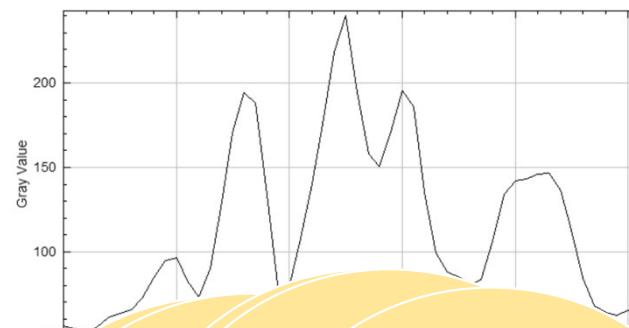
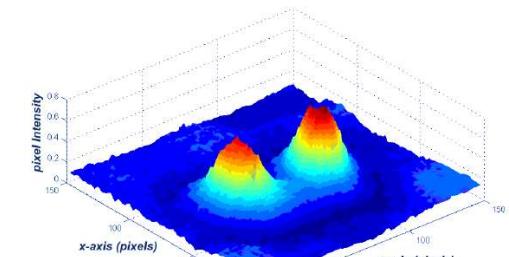
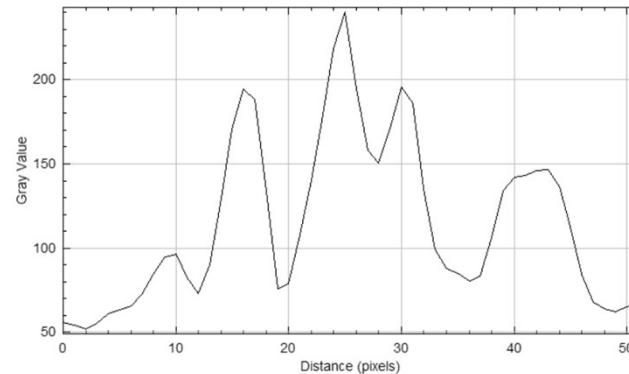
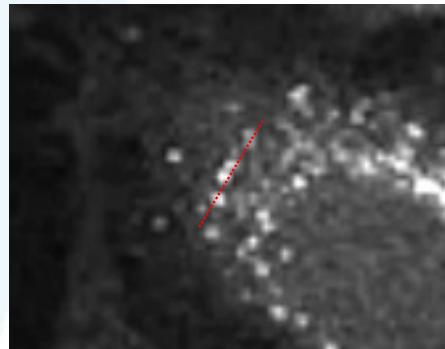
Modify masks

- ✓ Fill holes in the ROI
- ✓ Merge objects
- ✓ Get outline
- ✓ Split

Noise reduction

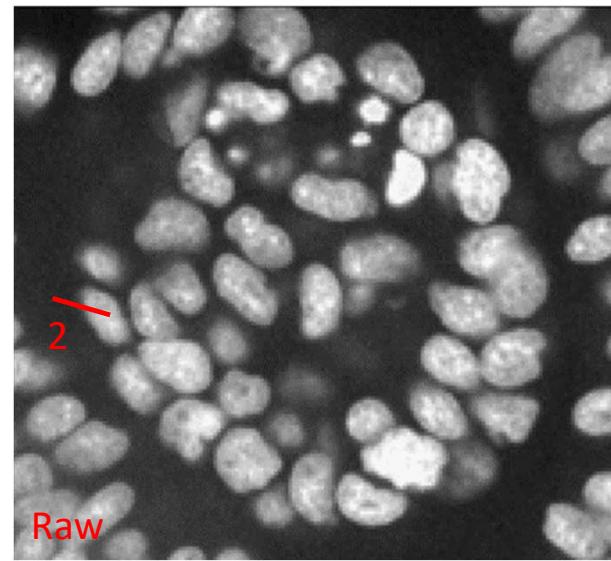
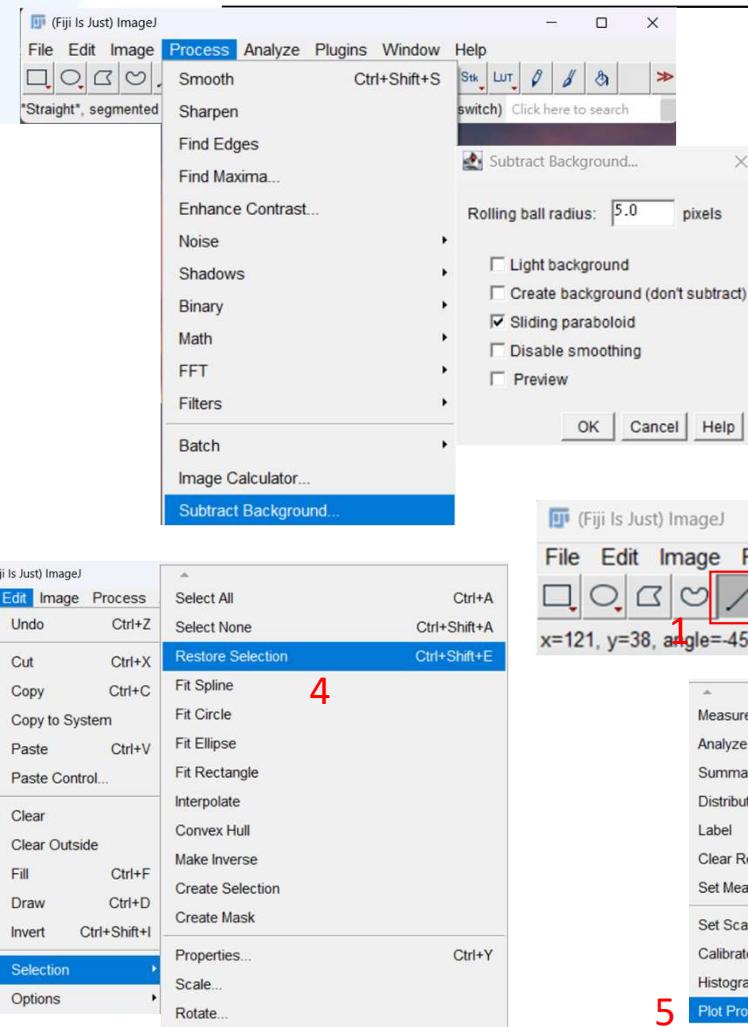


Background subtraction--Rolling ball

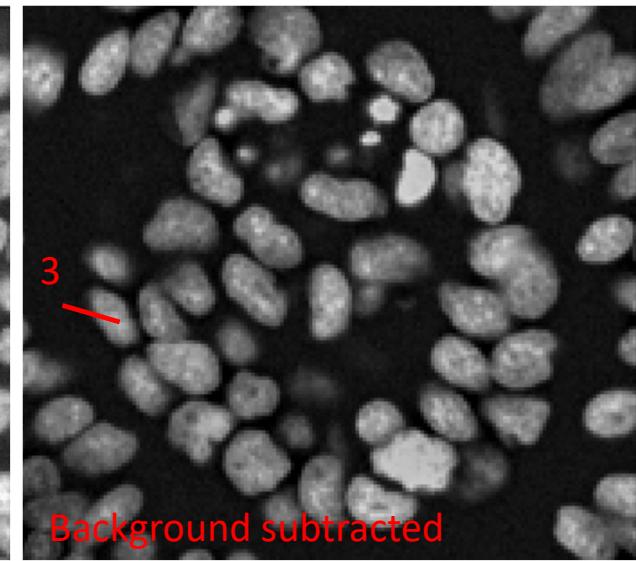


https://www.researchgate.net/figure/Surface-Plot-of-pixel-intensity-x-and-y-axes-represent-the-pixels-identities-while-the-z_fig15_221918148

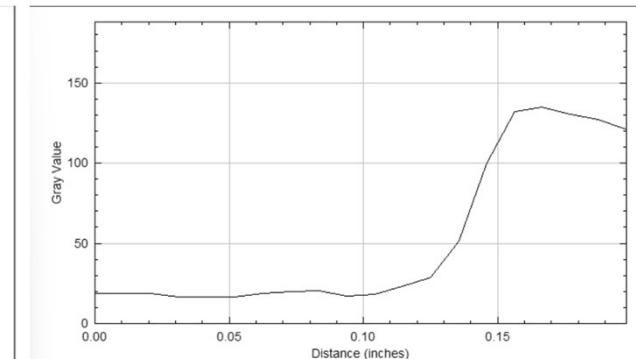
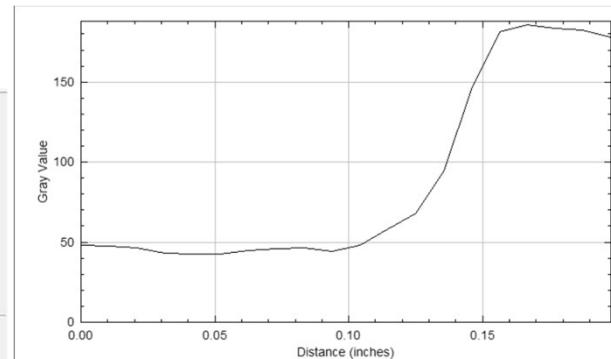
Background subtraction



Raw



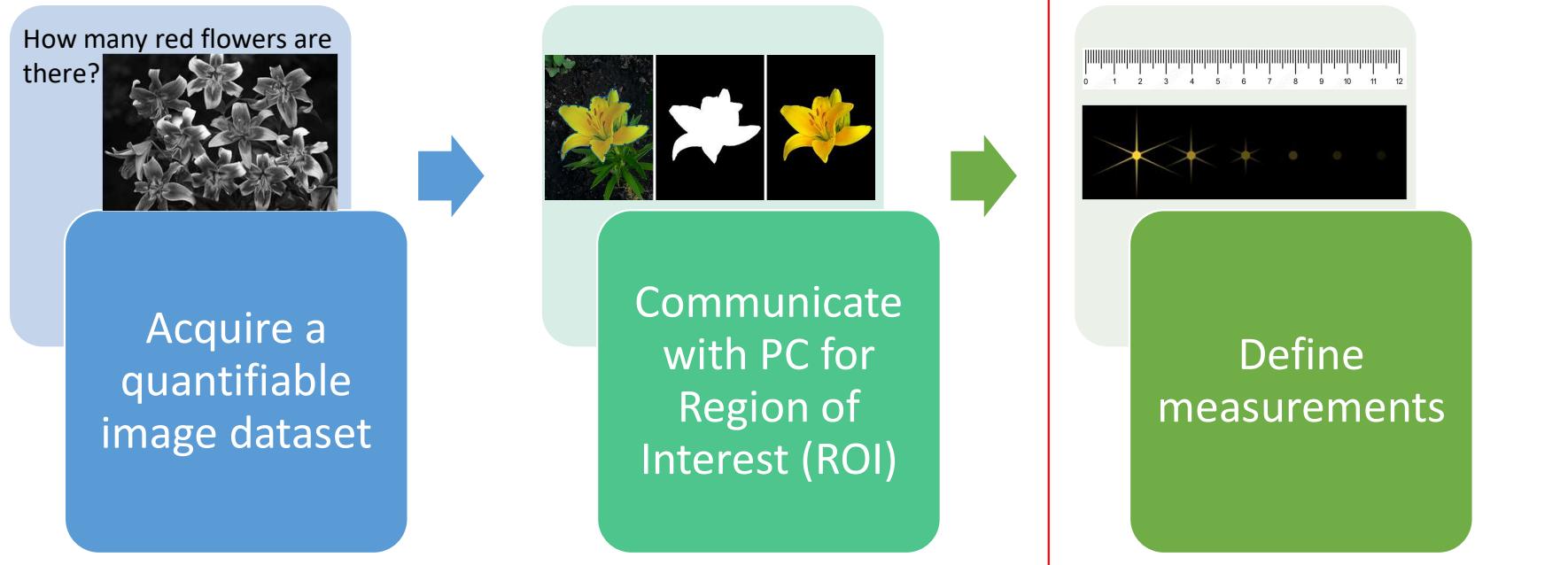
Background subtracted



5

Plot Profile Ctrl+K

Bioimage analysis workflow



<https://www.thelilygarden.com/>

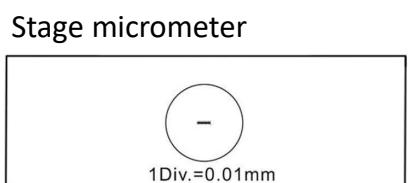
<https://www.mathworks.com/help/images/specify-roi-as-binary-mask.html>

<https://stock.adobe.com/hk/images/ruler-is-isolated-on-white-12-inch-measuring-tool-png/552996567>
https://en.wikipedia.org/wiki/Magnitude_%28astronomy%29

- ✓ Set scale
- ✓ Analyze particle
- ✓ Remove blurred image in a big dataset
 - 32bit\Find edge\Measure the standard deviation

Scale information

- Scale setting in microscopes



100 um

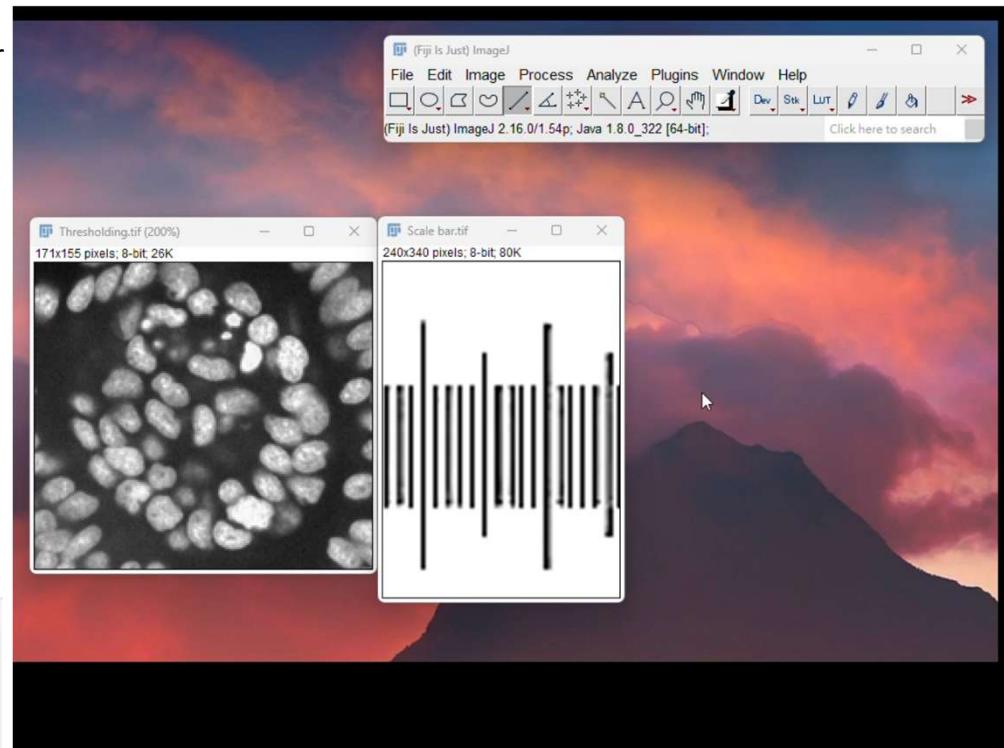
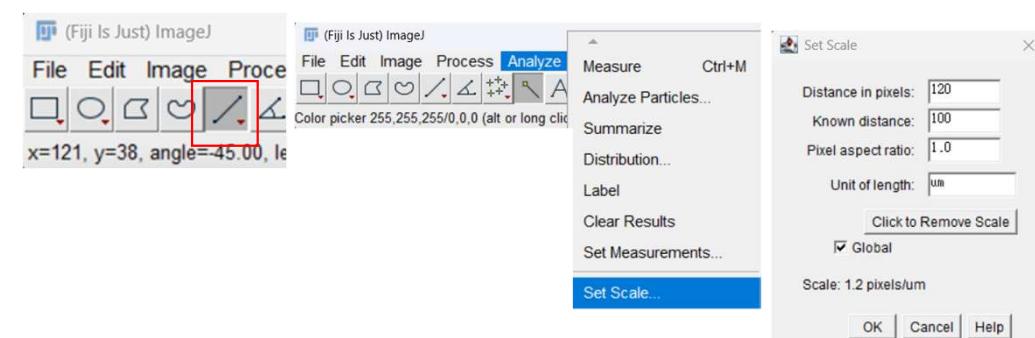
Image captured by the camera/detector



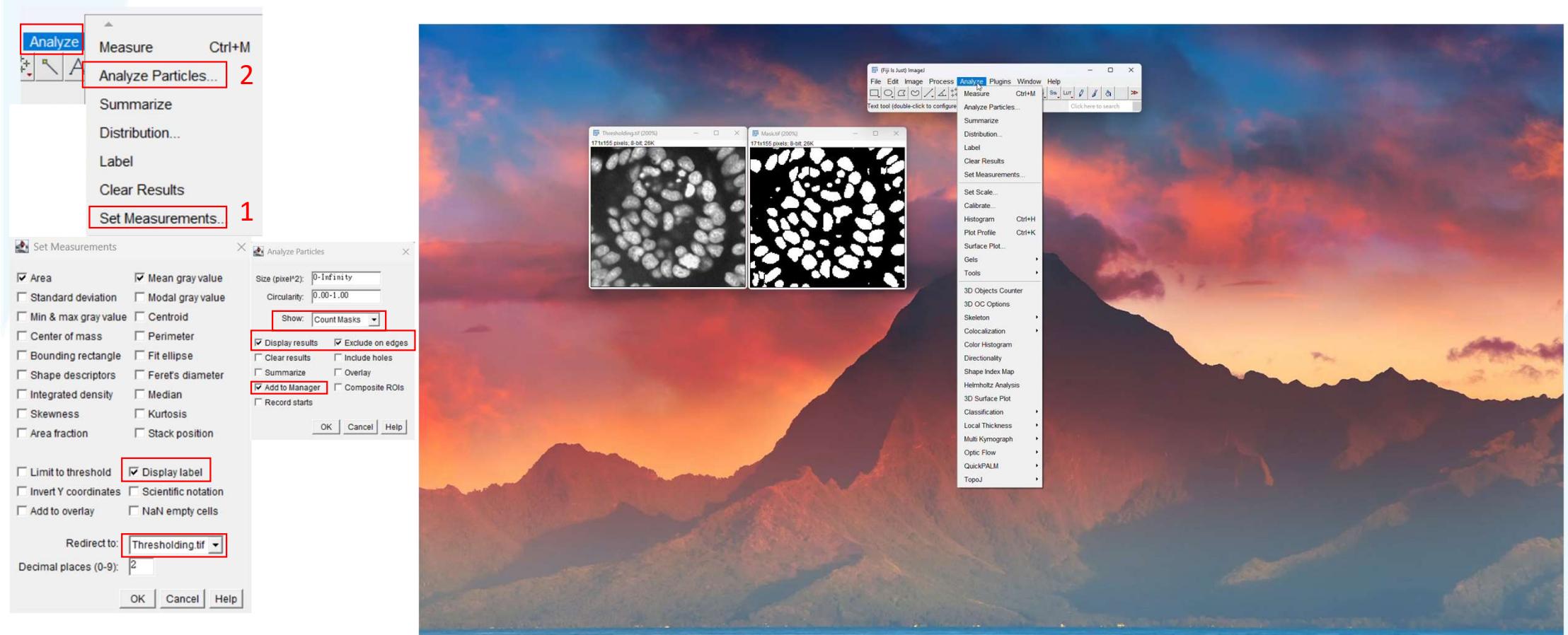
100 um= How many pixels in this image

Here 100 um = 120 pixel

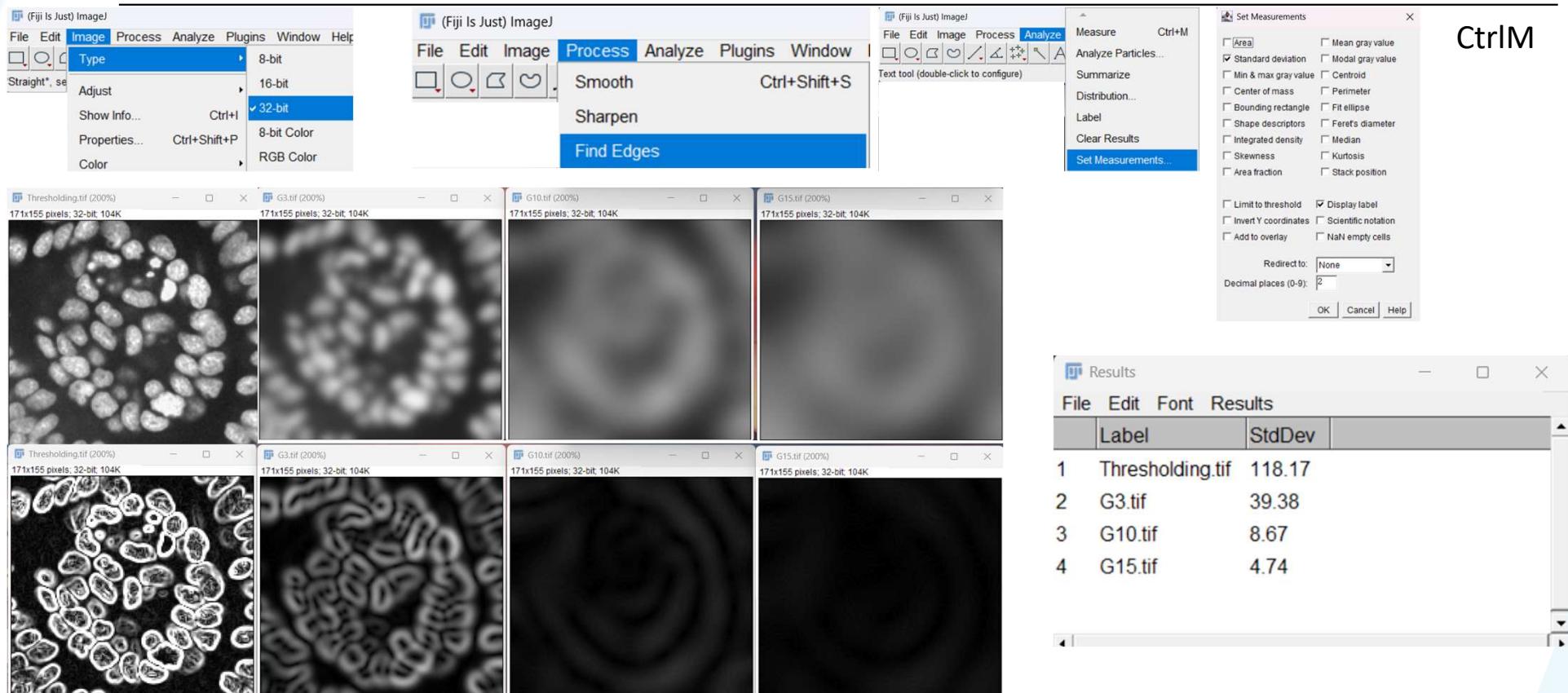
$$0.83 \text{ um/pixel} = 1.2 \text{ pixels/um}$$



Analyze particle



Remove the blurred image from a big dataset

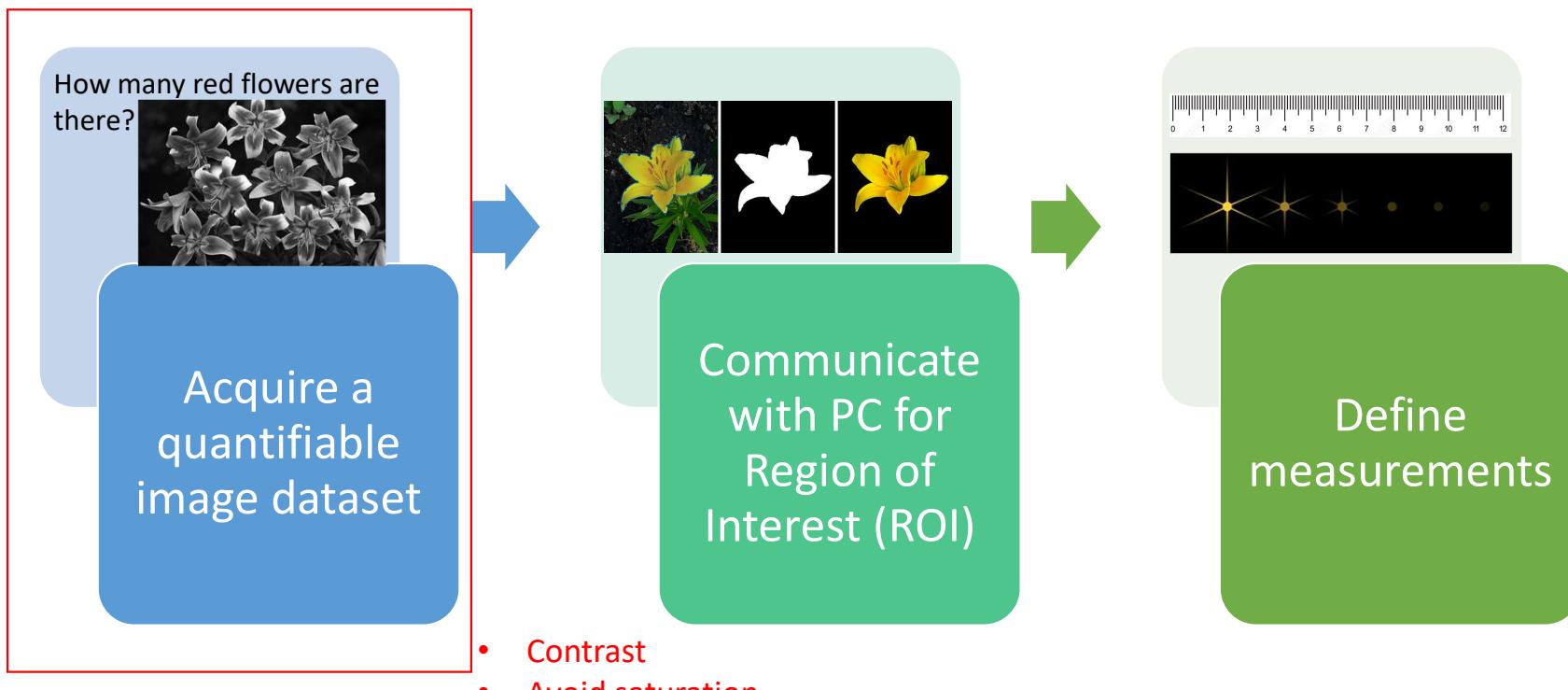


CtrlM

The floating calculation is only supported in the 32-bit type.

Outline of the second lecture

• Bioimage analysis workflow



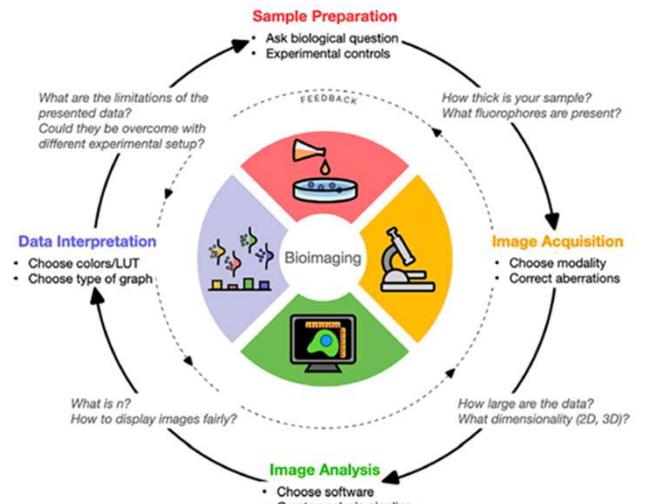
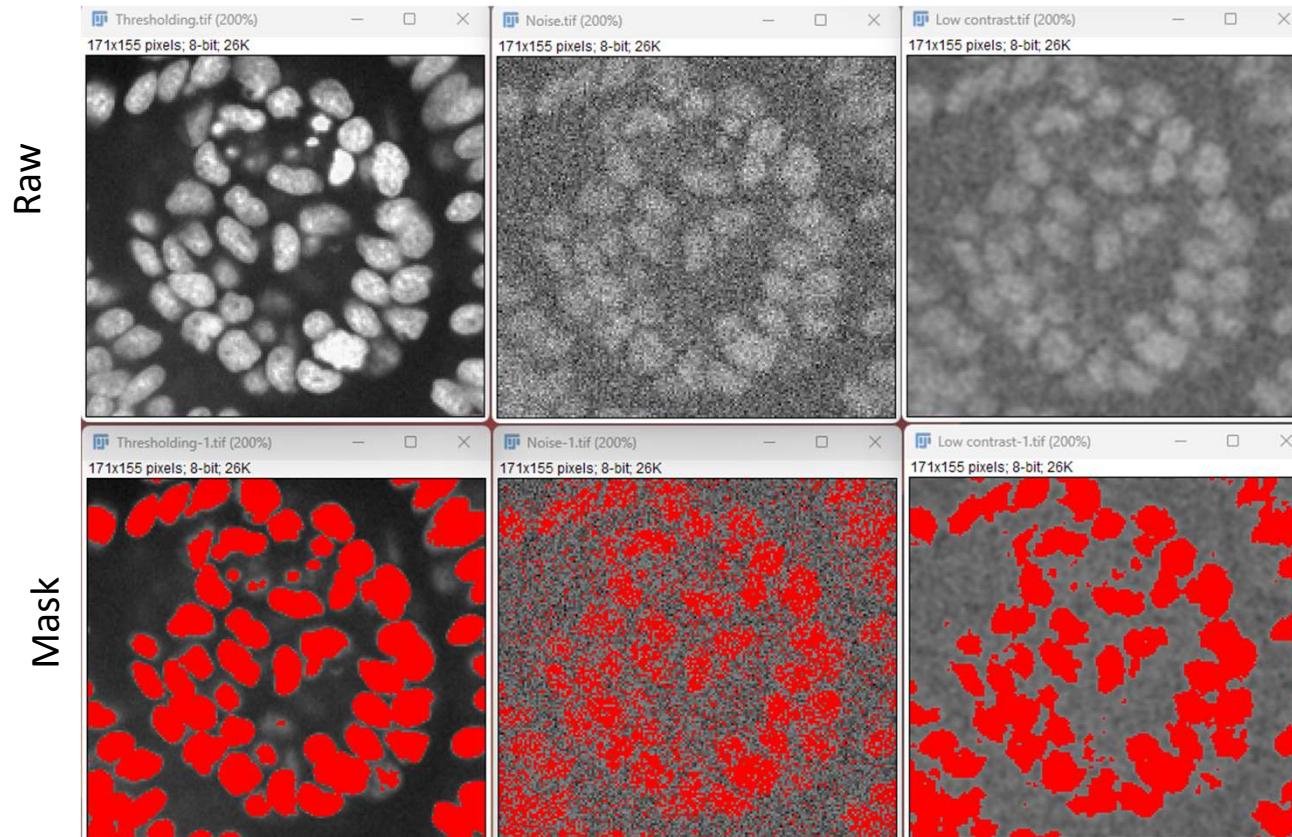
<https://www.thelilygarden.com/>

<https://www.mathworks.com/help/images/specify-roi-as-binary-mask.html>

<https://stock.adobe.com/hk/images/ruler-is-isolated-on-white-12-inch-measuring-tool-png/55299657>
https://en.wikipedia.org/wiki/Magnitude_%28astronomy%29

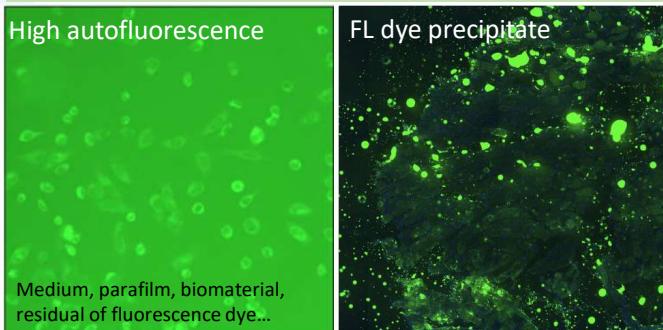


Contrast and noise affect image segmentation.



Intensity-based concern—Is the signal true? Does the image carry the reliable information?

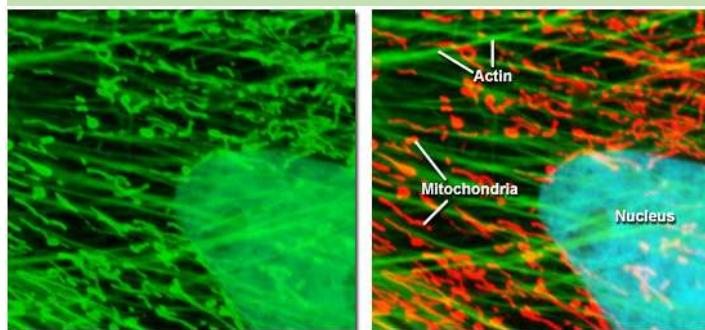
1. Remove the unpleasant **background** or materials from at the beginning



<https://www.cellsignal.com/products/buffers-dyes/backdrop-green-background-suppressor/12388>

<https://www.researchgate.net/topic/Immunohistology>

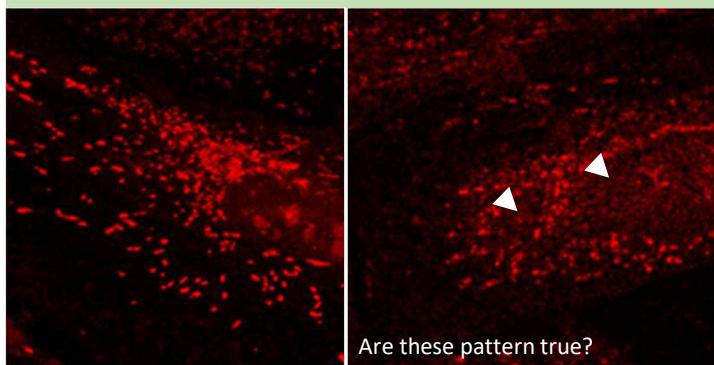
2. Avoid the **crosstalk** by adjusting labeling methods, sequential scan, spectral imaging, and FLIM



<https://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/introduction.html>

- From Ab cross recognition?
- The emission ranges of the second Ab are too closed?
- From inappropriate imaging setting?

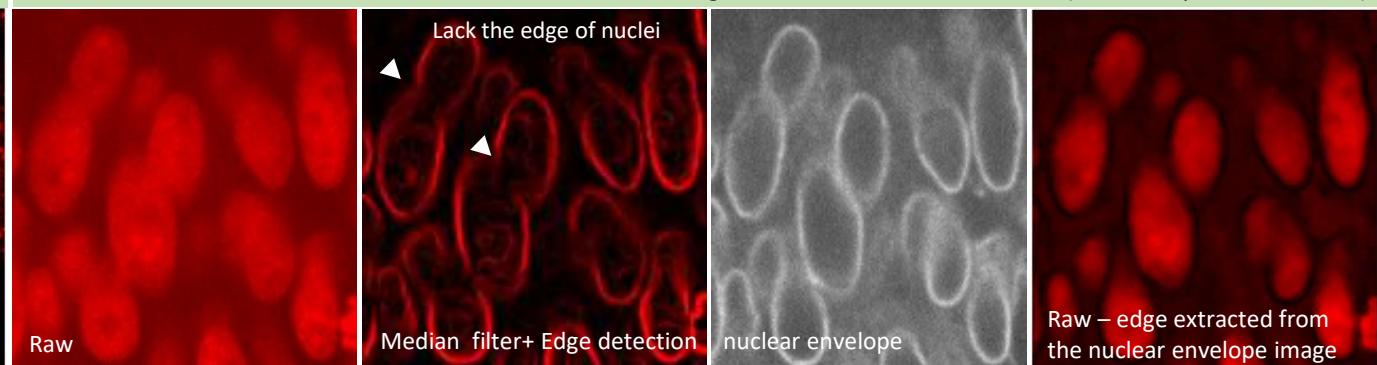
2. Enhance the signal-to-noise ratio (by **labeling**, **genetic modification**, adjusting **excitation power**, **exposure time**, **gain.....**)



Are these pattern true?

4. Sometimes additional **experimental design** is required for reliable analysis.

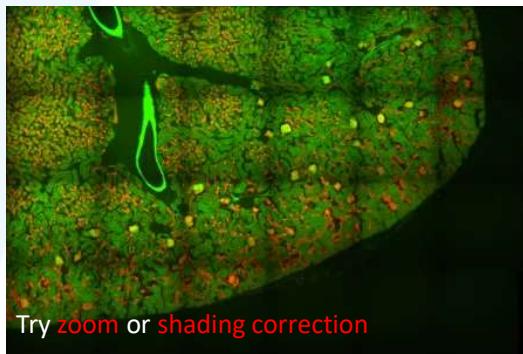
Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)



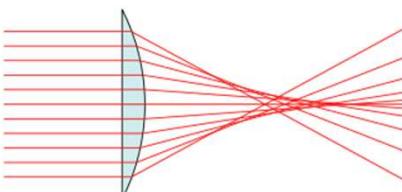
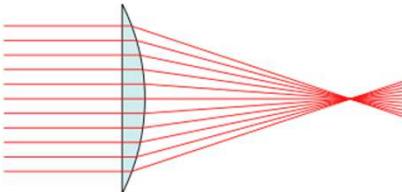
Raw – edge extracted from the nuclear envelope image

Intensity-based concern—Homogeneous illumination

1. Spherical aberration

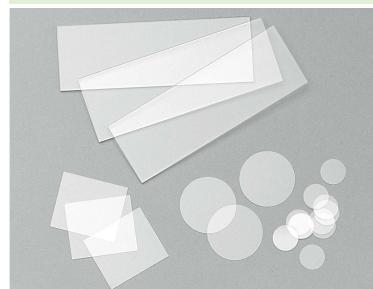


<https://imb.uq.edu.au/research/facilities/microscopy/training-manuals/microscopy-online-resources/image-capture/shading-corrections>

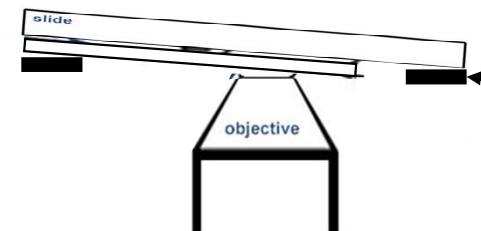


https://en.wikipedia.org/wiki/Spherical_aberration

2. The imaging plane is not perpendicular to the illumination plane.



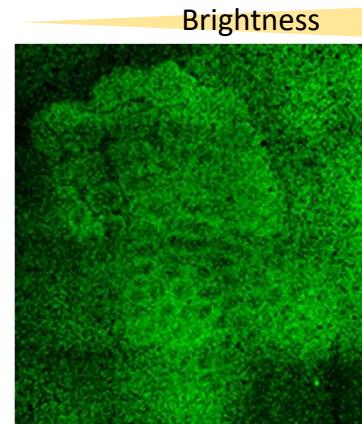
<https://imb.uq.edu.au/research/facilities/microscopy/training-manuals/microscopy-online-resources/sample-prep/coverslip-selection>
<https://www.hurondigitalpathology.com/>
<https://www.microscopeworld.com/p-5316-prior-scientific-universal-specimen-holder-for-h117-stages.aspx>



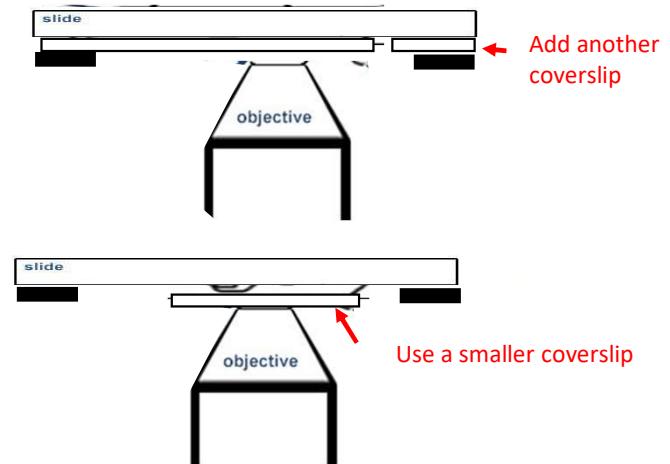
Crescent arm of the holder

<https://microscopynotes.com/coverslips/>

Too much mounting solution



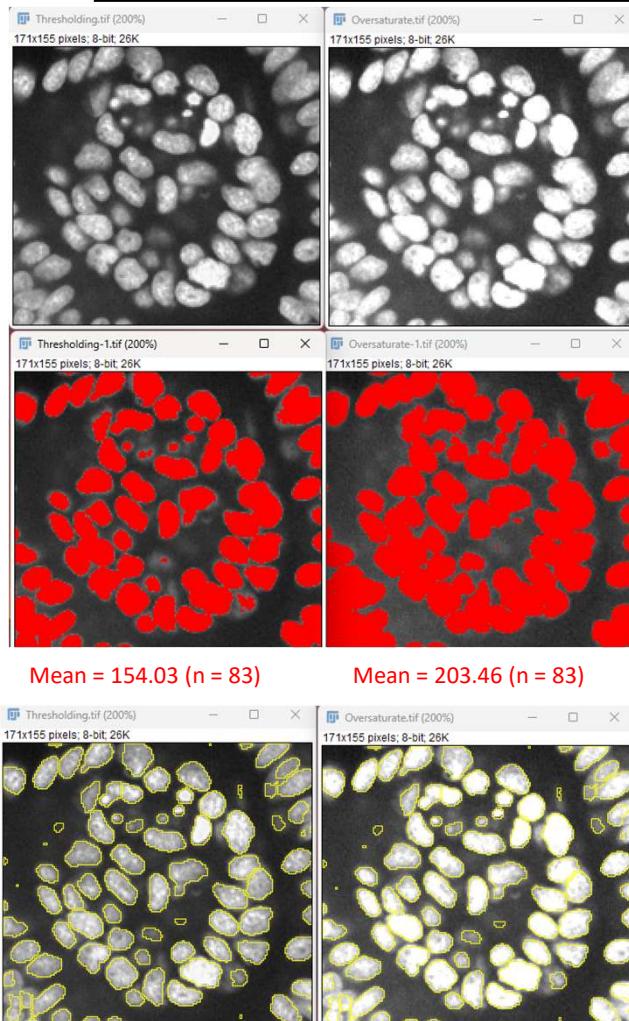
Brightness



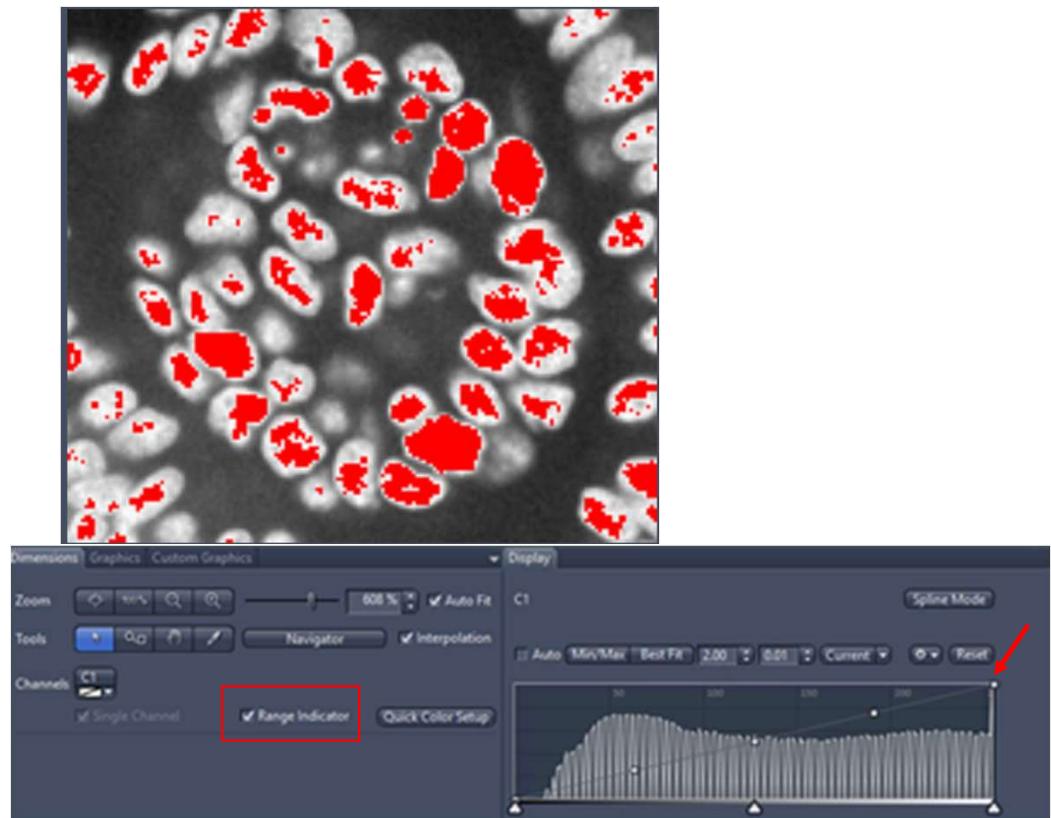
Add another coverslip

Use a smaller coverslip

Avoid pixel saturation if you want to measure the intensity

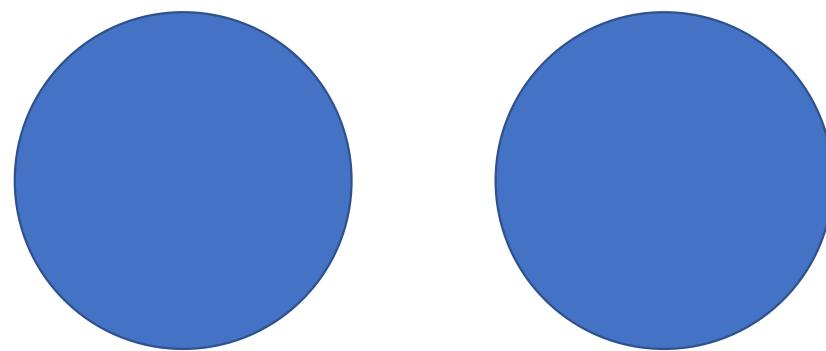


1. Cause the difficulty in segmentation.
2. Mislead the quantitative results.
=> Aid with LUT during imaging.



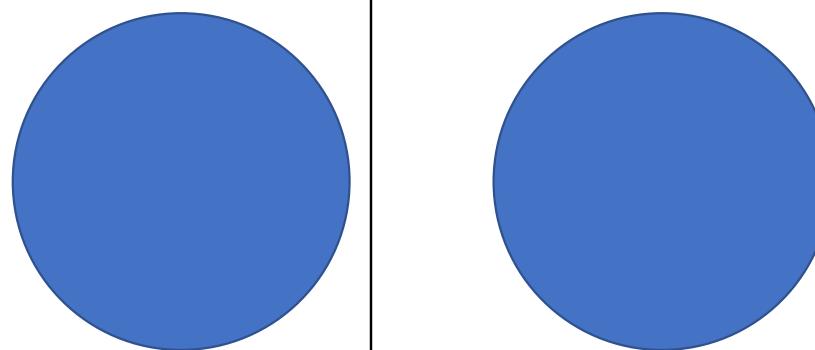


Sampling size



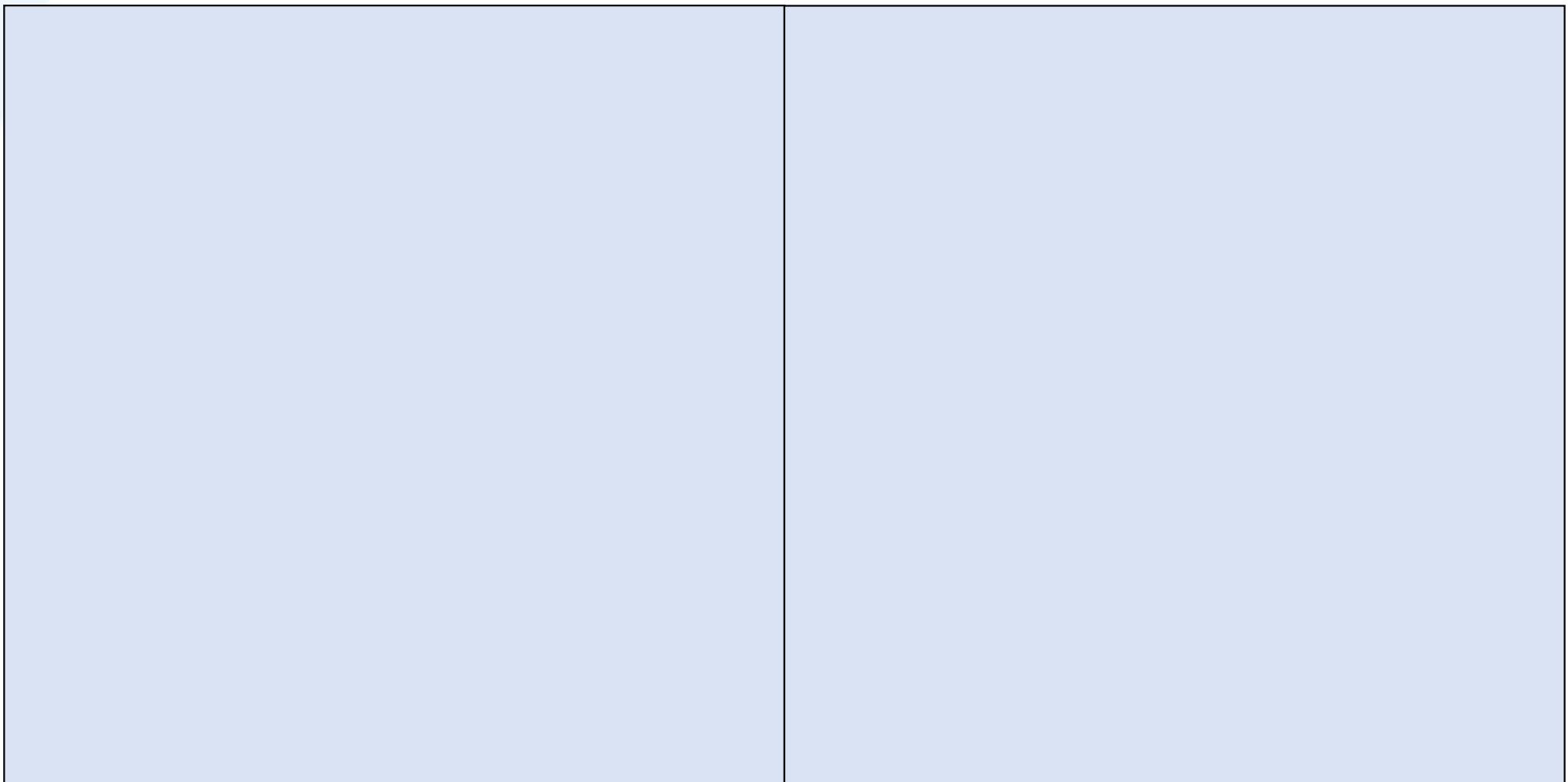


Two pixels with big size



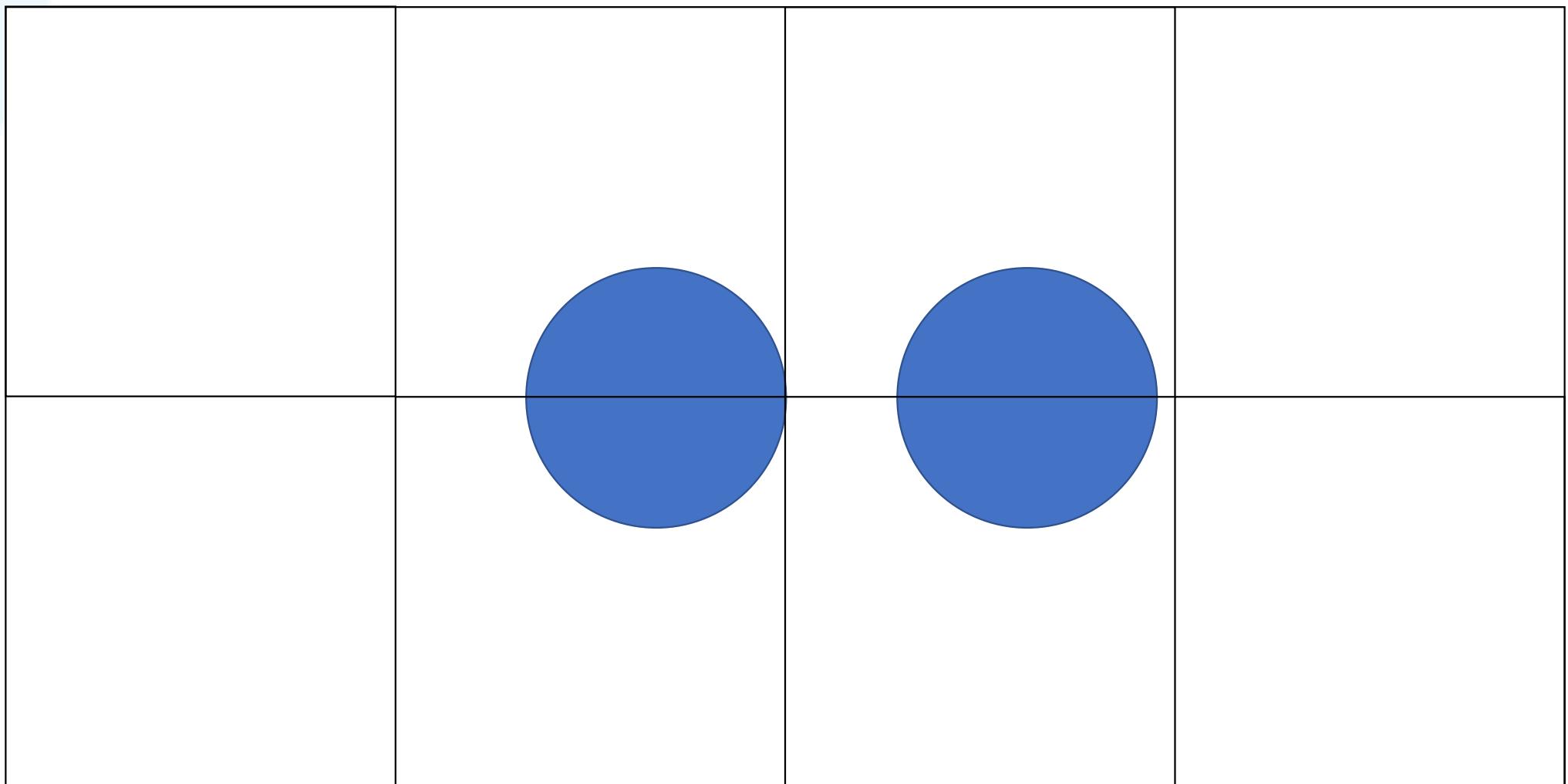


Two pixels with big size



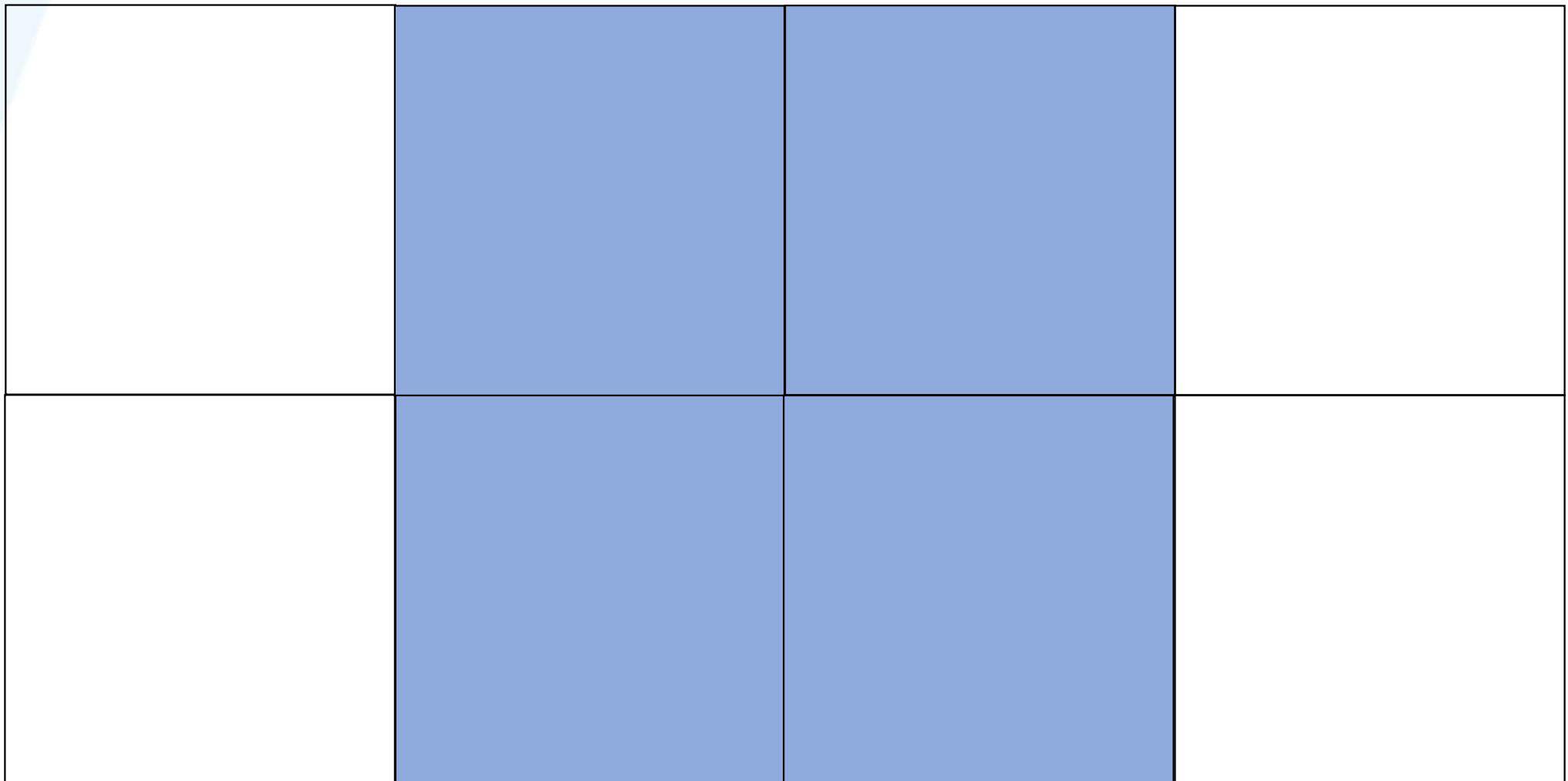


8 pixels with smaller size



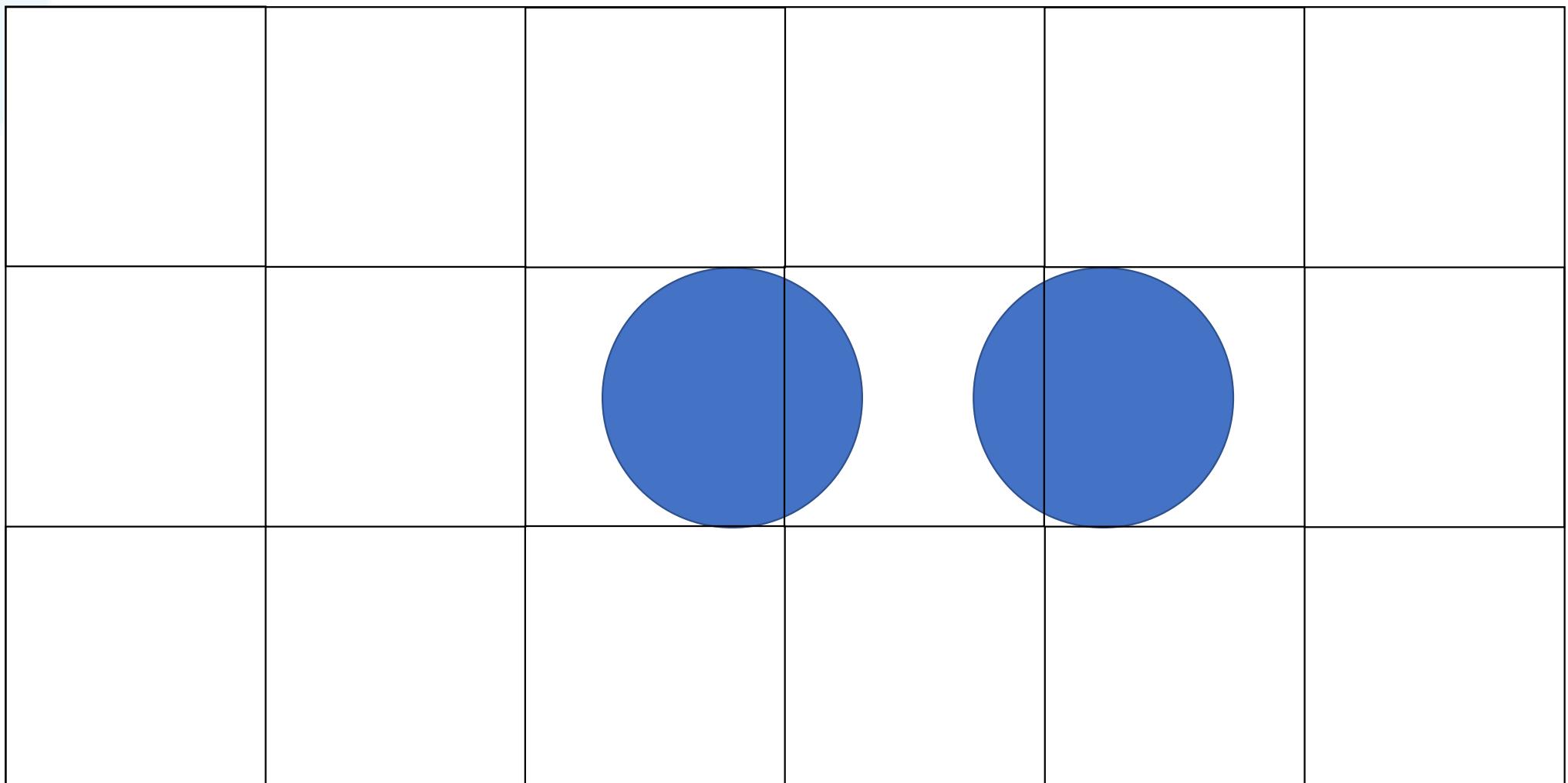


8 pixels with smaller size





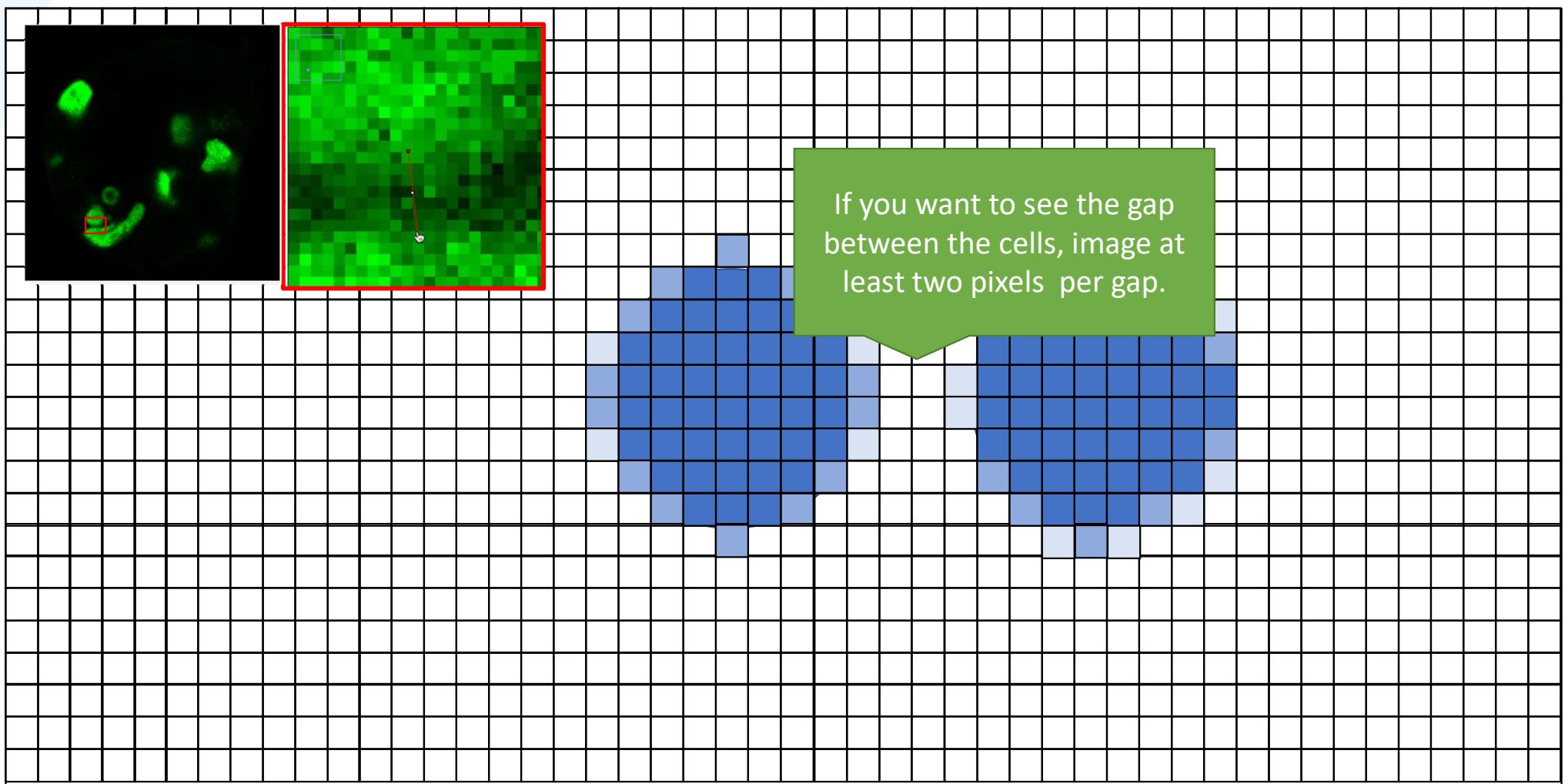
18 pixels with even smaller size



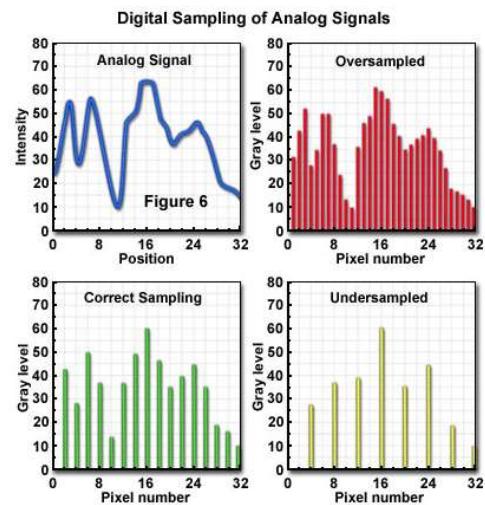


Geometry-based concern-- Sampling size

Geometry-based concern-- Sampling size



Geometry-based concern-- Sampling size

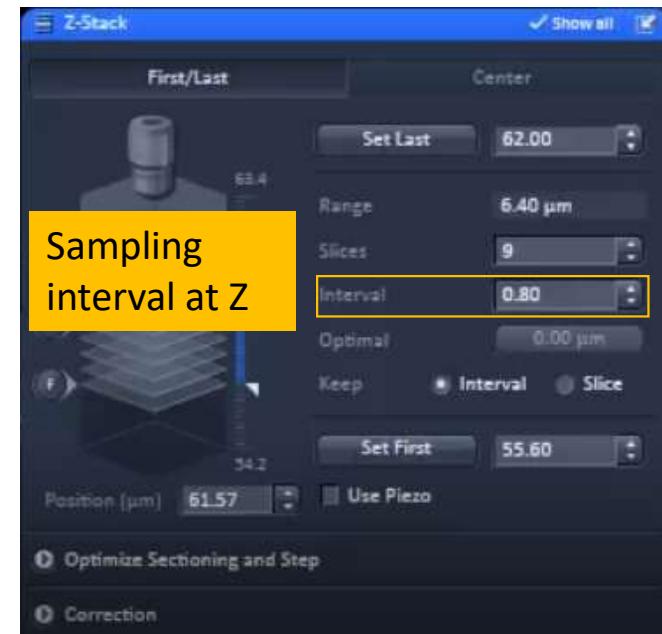


$$d_{xy} = \frac{\lambda}{2 * \text{N.A.}} = \frac{500 \text{ nm}}{2 * 1.46} = 171 \text{ nm}$$

$$\text{xy sampling size} = 171 \text{ nm} / 2 = 85.6 \text{ nm} \\ = 0.09 \mu\text{m}$$

$$d_z = 2 * d_{xy} = 171 \text{ nm} * 2 = 342 \text{ nm}$$

$$\text{z sampling size} = 342 \text{ nm} / 2 = 171 \text{ nm}$$



Sampling
interval at Z

Sampling
interval at
X and Y

Factors affect
sampling
interval at X
and Y

<https://bioimage.io/chat/>



Please help me count the number of cells
in the mounted file.

Could you try using Cellpose? Some of the
regions need to be further split.

Cellpose gave the correct result. Please
compile a Python script to achieve a
similar outcome without using Cellpose.

Please apply the script to the mounted
image and show the result.

Please show me the script.

Please convert the above Python script to
a Fiji macro.

Image analysis AI agency



Reference

- Chapter 10 Image segmentation, Digital image processing. 4th Edition.
- Autothresholding https://github.com/fiji/Auto_Threshold/blob/master/src/main/java/fiji/threshold/Auto_Threshold.java
- Auto local thresholding <https://imagej.net/plugins/auto-local-threshold>
- Filter and Segmentation https://git.mpi-cbg.de/rhaase/lecture_applied_bioimage_analysis_2020/-/blob/master/02_Image_Filtering_and_Segmentation/02a_Filtering_Segmentation.pptx?ref_type=heads
- Filter and Segmentation https://git.mpi-cbg.de/rhaase/lecture_applied_bioimage_analysis_2020/-/blob/master/02_Image_Filtering_and_Segmentation/02b_Filtering_Segmentation_Fiji.pptx?ref_type=heads
- Volumetric image data https://git.mpi-cbg.de/rhaase/lecture_applied_bioimage_analysis_2020/-/tree/master/12_Volumetric_image_data?ref_type=heads
- Filter and Segmentation <https://www.youtube.com/watch?v=LT8L3vSLQ2Q>
- Filter and Segmentation <https://www.youtube.com/watch?v=08SZnZB14IM>
- Volumetric image data <https://www.youtube.com/watch?v=GoaGsrTerA0>
- LUT <https://www.youtube.com/watch?v=kNGhKnMGx-Q&t=479s>
- Tips for image acquisition <https://www.youtube.com/watch?v=SEfARbJv4HE&t=31s>
- Image Scaling <https://www.youtube.com/watch?v=n8XczFF6WMQ>
- Legesse, F.B. et al., *Seamless stitching of tile scan microscope images*. *Journal of Microscopy*, 2015. 258(3):p.223.
- Senft, R.A., et al., *A biologist's guide to planning and performing quantitative bioimaging experiments*. *PLoS Biol*, 2023. 21(6): p. e3002167.
- Color space <https://www.datacolor.com/business-solutions/blog/what-is-cielab/>
- EMpanada Conrad, R., Narayan, K. *Instance segmentation of mitochondria in electron microscopy images with a generalist deep learning model trained on a diverse dataset*. *Cell Systems*, 14(1), 58-7.e5.
- SAM Sugawara Ko. *Training deep learning models for cell image segmentation with sparse annotations*. *bioRxiv*, 2023. 06.13.544786.
- BioImage.IO Chatbot Lei et al. *BioImage.IO Chatbot: a community-driven AI assistant for integrative computational bioimaging*. *Nature Methods*, 2024, 21, 1368.

Group assignments

- **Topics**

- Group 1:** Tracking
- Group 2:** AI Segmentation
- Group 3:** Colocalization

- **Tasks**

1. **Demo image Survey** – Find an appropriate dataset from the **IDR or SSBD archives** based on the assigned topic or use your own image dataset which could be published in the tutorial video.
2. **Analysis Implementation** – Apply the skills learned in class to start your analysis.
3. **Automation** – Use the **Macro Recorder** and edit a script for automatic analysis.
4. **Workflow Visualization** – Create a **workflow diagram** illustrating your analysis pipeline.

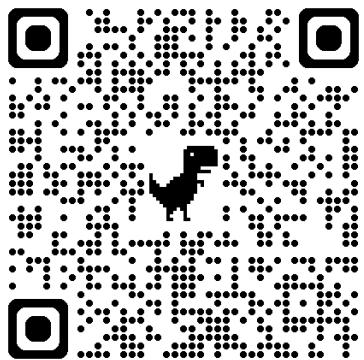
- **Data resource**

1. IDR <https://idr.openmicroscopy.org/>
2. SSBD <https://ssbd.riken.jp/database/>
3. Self-generated Please make sure that your dataset is open for sharing on the education video.

- **Presentation Format:**

- Demonstrate the group work in **10 minutes** for each group.
- The presentation should cover:
 1. **Workflow Diagram** – Explain the aim and the pipeline of this analysis script.
 2. **Work demonstration** – Showcase the workflow of this script.
 3. **Discussion** – Share insights, challenges, and key takeaways from developing this workflow.

- **Role of the group leader** Ensure your group will present the result at the last course.



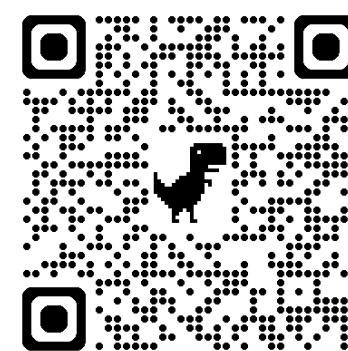
線上簽到



課程材料與資訊連結



課後意見調查



EABIAS Lecturer
Recruiting

IJ Default

```
public static int IJDefault(int [] data ) {  
    // Original IJ implementation for compatibility.  
    int level;  
    int maxValue = data.length - 1;  
    double result, sum1, sum2, sum3, sum4;  
  
    int min = 0;  
    while ((data[min]==0) && (min<maxValue))  
        min++;  
    int max = maxValue;  
    while ((data[max]==0) && (max>0))  
        max--;  
    if (min>=max) {  
        level = data.length/2;  
        return level;  
    }  
  
    int movingIndex = min;  
    int inc = Math.max(max/40, 1);  
    do {  
        sum1=sum2=sum3=sum4=0.0;  
        for (int i=min; i<=movingIndex; i++) {  
            sum1 += i*data[i];  
            sum2 += data[i];  
        }  
        for (int i=(movingIndex+1); i<=max; i++) {  
            sum3 += i*data[i];  
            sum4 += data[i];  
        }  
        result = (sum1/sum2 + sum3/sum4)/2.0; 取前景與背景灰階均值的平均值作為新的閾值。  
        movingIndex++;  
    } while ((movingIndex+1)<=result && movingIndex<max-1); 當 movingIndex + 1 > result 時，表示 movingIndex 已經足夠接近 result，可以停止迴圈。  
  
    //.showProgress(1.0);  
    level = (int)Math.round(result);  
    return level;  
}
```

https://github.com/fiji/Auto_Threshold/blob/master/src/main/java/fiji/threshold/Auto_Threshold.java

Set measurements



1. 幾何測量 (Geometrical Measurements)

- Area (面積): 計算選取區域的像素數量，轉換為實際單位。
- Perimeter (周長): 計算選取區域的邊界長度。
- Feret's Diameter (費雷直徑): 物件的最大距離，即兩點之間的最大歐幾里得距離。
- Bounding Box (邊界框): 計算能完整包圍物件的最小矩形框，其長寬可用於形狀分析。
- Shape Descriptors (形狀描述): 包括 Circularity (圓形度), Aspect Ratio (長寬比), Roundness (圓度)，用於量化形狀。

2. 強度與灰階測量 (Intensity & Gray Value Measurements)

- Mean Gray Value (平均灰階值): 區域內所有像素的灰階值平均。
- Min/Max Gray Value (最小/最大灰階值): 區域內像素的最低與最高強度。
- Integrated Density (總灰階密度): 平均灰階值乘以面積，適用於螢光影像分析。
- Skewness (偏斜度) & Kurtosis (峰度): 量化灰度分布的形狀，如是否偏斜或有尖峰。
 - Skewness (偏斜度): 衡量數據分布的對稱性，正偏斜 (右偏) 表示數據集中在左側，負偏斜 (左偏) 則表示數據集中在右側。
 - Kurtosis (峰度): 衡量數據分布的尖銳程度，高峰度表示數據較集中且尾部較重，而低峰度則表示數據分布較平坦。

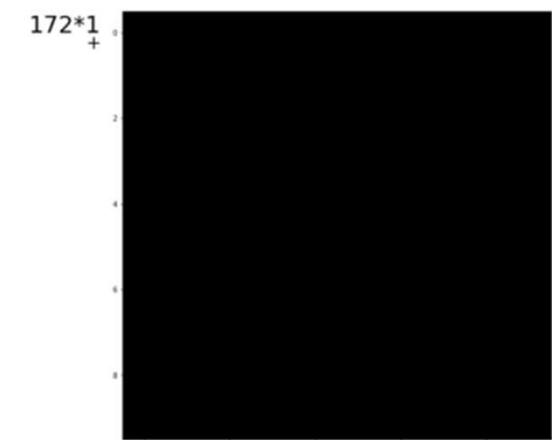
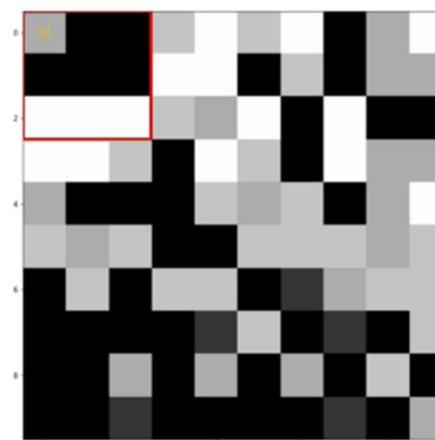
3. 位置測量 (Locational Measurements)

- Centroid (質心): 計算選取區域的幾何中心 (X, Y 座標)。
- Center of Mass (質量中心): 根據像素強度計算的重心，比幾何中心更適用於亮度變化大的影像。

4. 其他測量 (Miscellaneous Measurements)

- Limit to Threshold (限制於閾值內): 只測量選取範圍內符合閾值的像素。
- Stack Position (堆疊位置): 在影像堆疊中標記當前切片。

- Linear filters replace each pixel value with a linear combination of surrounding pixels
 - Basically, linear filtering is a Convolution
 - It needs a kernel (weight template)
 - Result: new image where each pixel is replaced by the weighted sum of pixel values in the neighbourhood.



Linear Filters

- Kernels are matrices describing a linear filter

Mean filter, 3x3 kernel

$$\begin{bmatrix} 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \end{bmatrix}$$

Animation source: Dominic Waithe, Oxford University
https://github.com/dwaithe/generalMacros/tree/master/convolution_ani

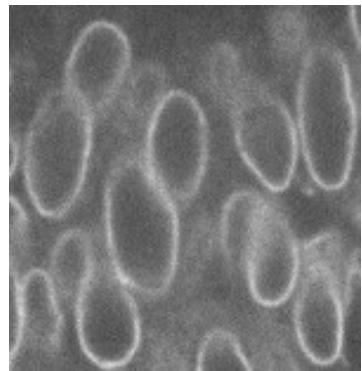
Linear filters

- Terminology:

- “We convolve an image with a kernel.”
- Convolution operator: *

- Examples

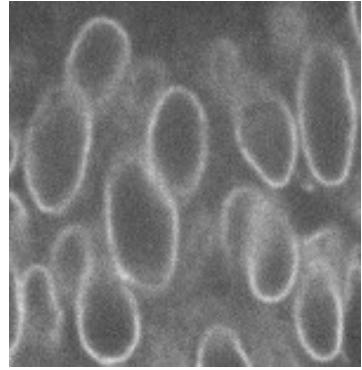
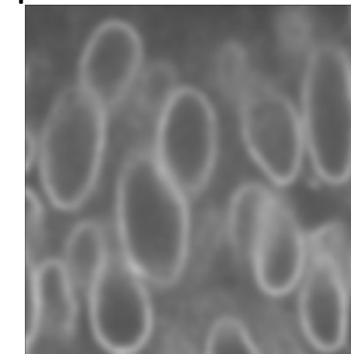
- Mean
- Gaussian blur
- Sobel-operator



*

$$\begin{bmatrix} 2.19e-6 & 5.63e-6 & 1.30e-5 & \dots & 5.63e-6 & 2.19e-6 \\ 5.63e-6 & 1.45e-5 & 3.33e-5 & \dots & 1.45e-5 & 5.63e-6 \\ 1.30e-5 & 3.33e-5 & 7.66e-5 & \dots & 3.33e-5 & 1.30e-5 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 5.63e-6 & 1.45e-5 & 3.33e-5 & \dots & 1.45e-5 & 5.63e-6 \\ 2.19e-6 & 5.63e-6 & 1.30e-5 & \dots & 5.63e-6 & 2.19e-6 \end{bmatrix}$$

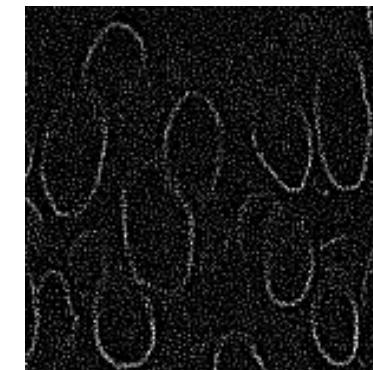
=



*

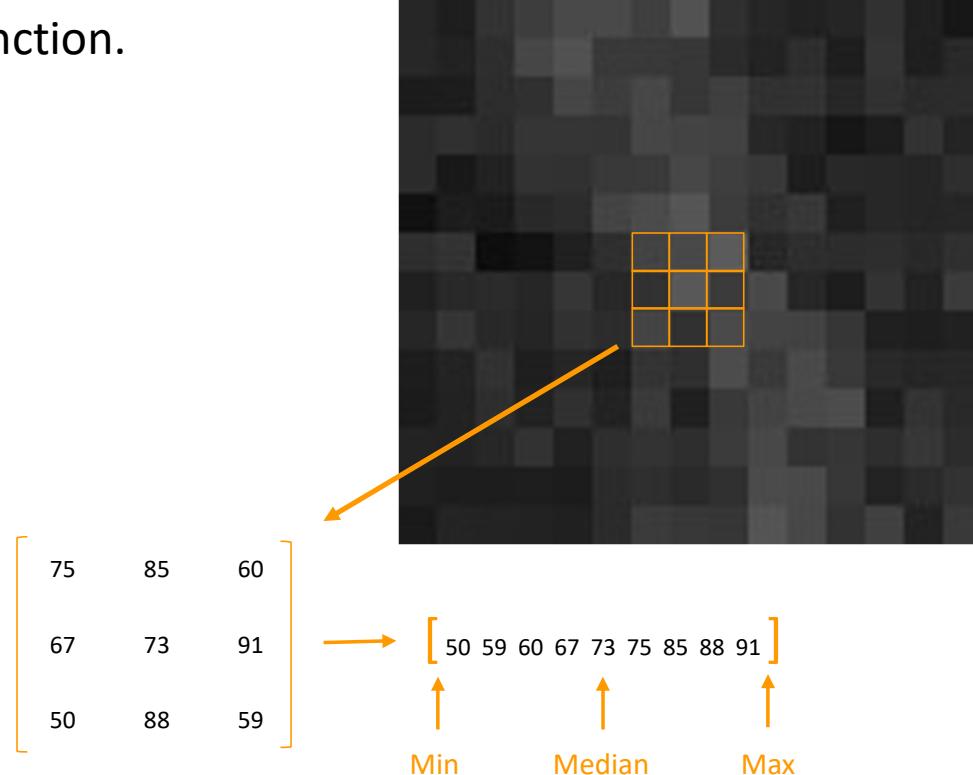
$$\begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix}$$

=



Nonlinear Filters

- Non linear filters also replace pixel value inside a rolling window but in a non linear function.
- Examples: order statistics filters
 - Min
 - Median
 - Max
 - Variance
 - Standard deviation



Setting of the particle analyzer window

The figure illustrates the configuration of the Analyze Particles window and its associated results and visualization.

Analyze Particles Window:

- Size (μm^2):** 0–Infinity (Pixel units checked)
- Circularity:** 0.00–1.00
- Show:** Count Masks
- Display results** (checked)
- Clear results** (checked)
- Summarize** (checked)
- Add to Manager** (checked)
- Exclude on edges** (unchecked)
- Include holes** (unchecked)
- Record starts** (unchecked)
- In situ Show** (unchecked)
- Help**, **Cancel**, **OK** buttons

Results Table:

	Area	Mean	Min	Max
1	3.533	255	255	255
2	6.075	255	255	255
3	1.077	255	255	255
4	1.206	255	255	255
5	1.594	255	255	255
6	3.145	255	255	255
7	39.551	255	255	255
8	3.662	255	255	255
9	22.791	255	255	255
10	0.646	255	255	255

Summary Table:

Slice	Count	Total Area	Avg
ch4_watershed.tif	65	845.176	13

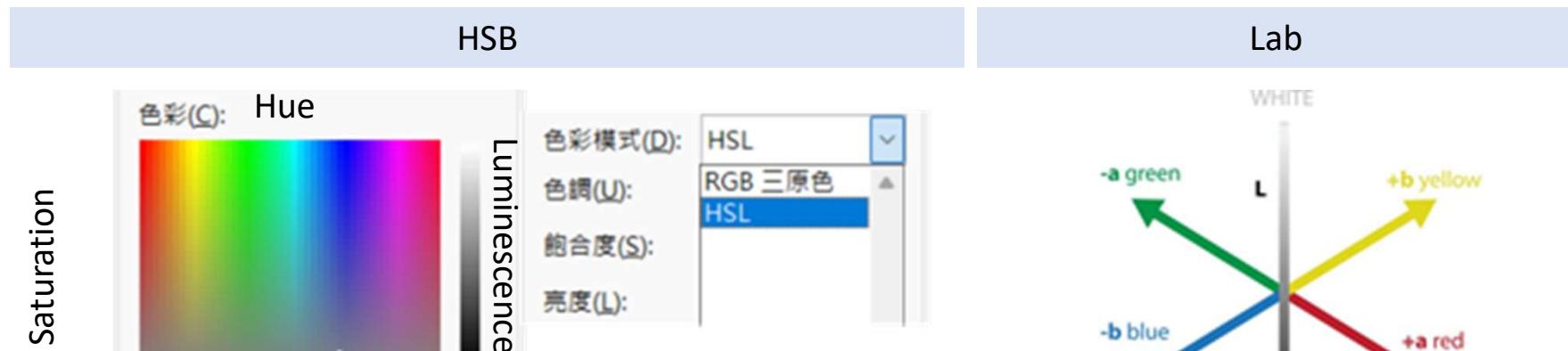
ROI Manager:

- Entries: 0001-0004, 0002-0006, 0003-0005, 0004-0023, 0005-0026, 0006-0033, 0007-0051, 0008-0031, 0009-0047, 0010-0041, 0011-0051, 0012-0059, 0013-0061
- Add [t]**, **Update**, **Delete**, **Rename...**, **Measure**, **Deselect**, **Properties...** buttons

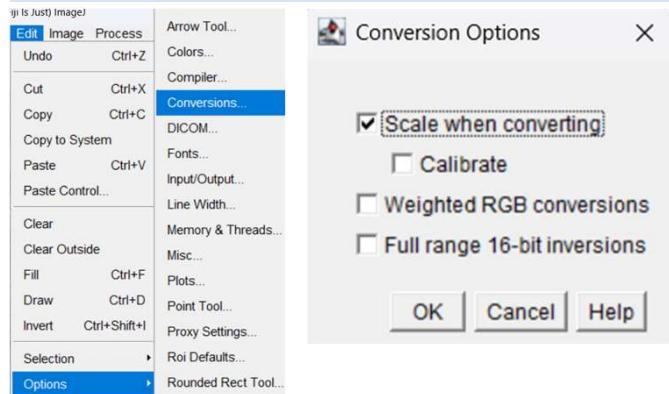
Measurement filter:

Color Map Selection:

Color space



RGB or weighted RGB



If *Weighted RGB to Grayscale Conversion* is checked, the formula $gray=0.299*red+0.587*green+0.114*blue$ is used to convert RGB images

If it is not checked, the formula $gray=(red+green+blue)/3$ is

課程連結

- 線上簽到 <https://forms.gle/bawaeRZpkjhpWFmq7>
- 課程意見調查 <https://forms.gle/fNC8jm1ey9VoaGeW8>
- 課程教材 <https://eabias.github.io/activities/>
- 課程錄影連結 <https://www.youtube.com/@EABIAS>