

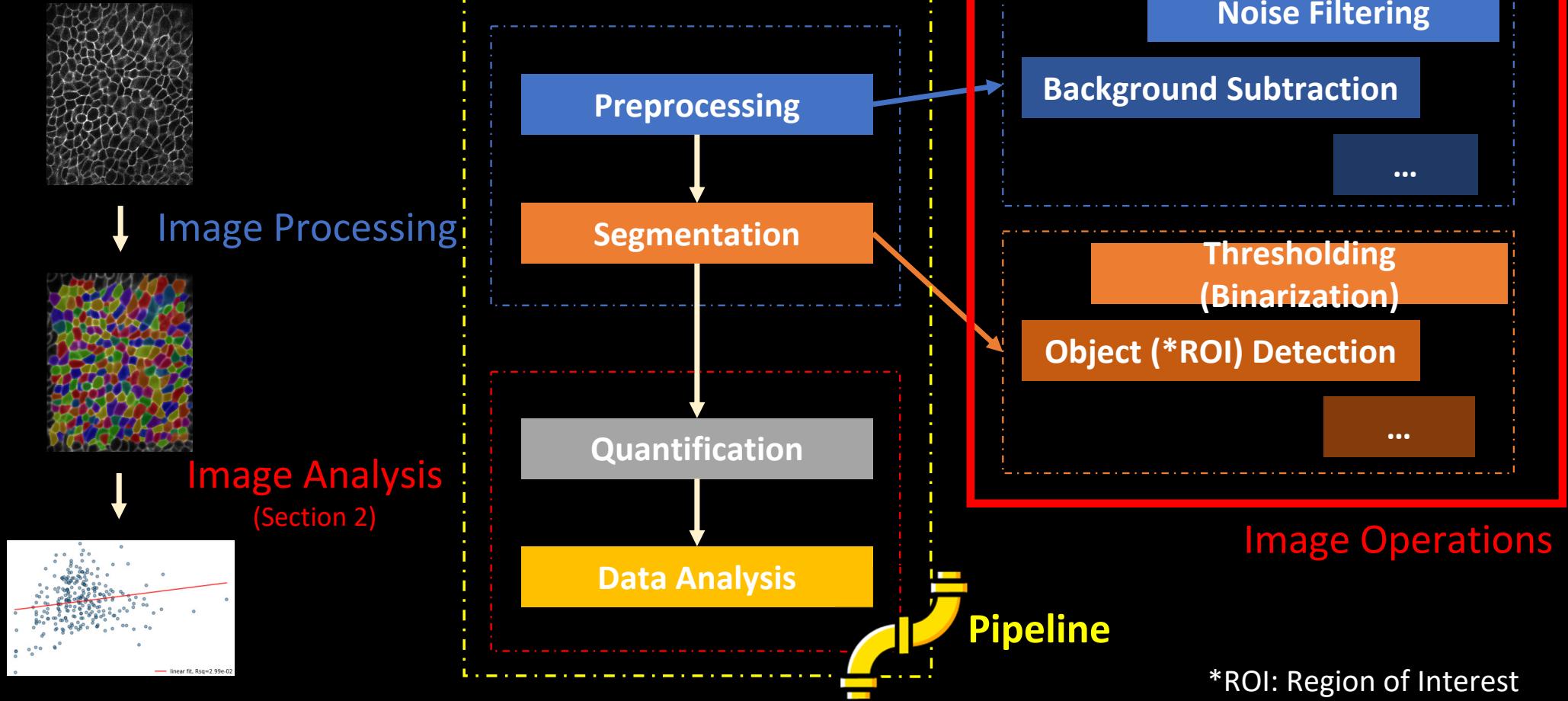
Section 1

(*Getting started with) Bioimage Processing



Most slides and materials were adapted from:
Lecture 2 of Bioimage Analysis 2020 by Robert Haase
Bioimage book by Peter Bankhead
EMBL Image Analysis with Python Course by Jonas Hartmann

A typical image analysis workflow



Agenda

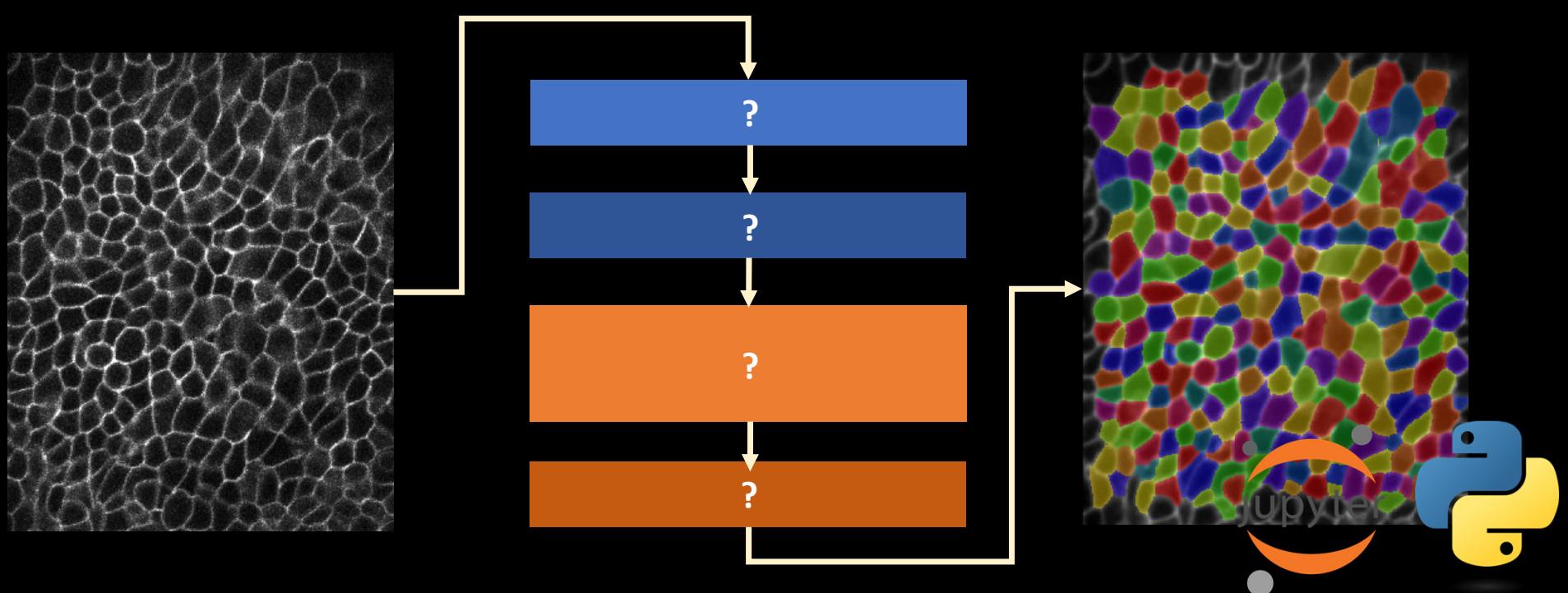
- Introduction to image operators:
 - Thresholding
 - Filters
 - Image Transforms

- Codelab!

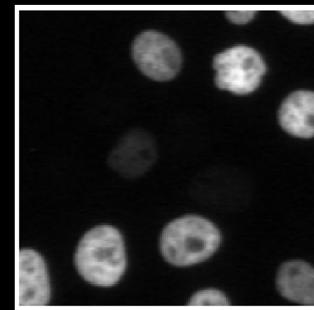


By the end of this section you will know:

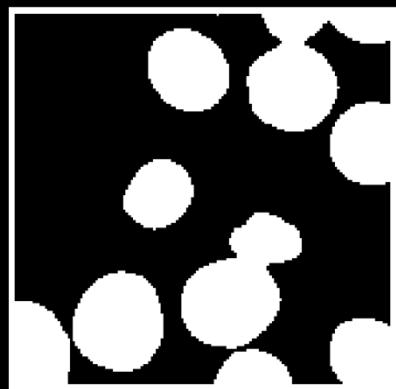
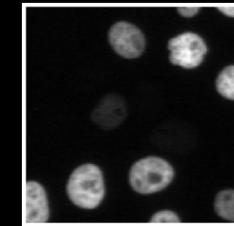
P.S. The workshop material for this section is primarily based on the
Python Bioimage Analysis Tutorial by Jonas Hartmann @ EMBL



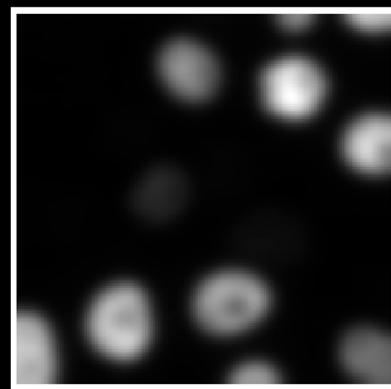
Introduction to Image Operations



Introduction to image operations



Thresholding



Filtering
(or Convolution)

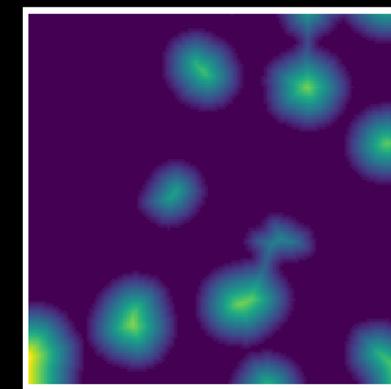
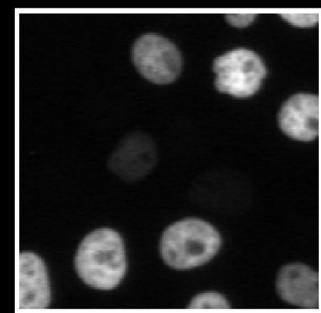


Image Transforms

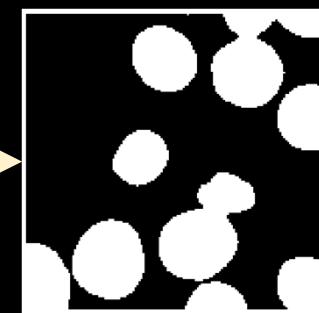
Not covered here:

- Point Operations
- Multi-dimensional processing

Thresholding



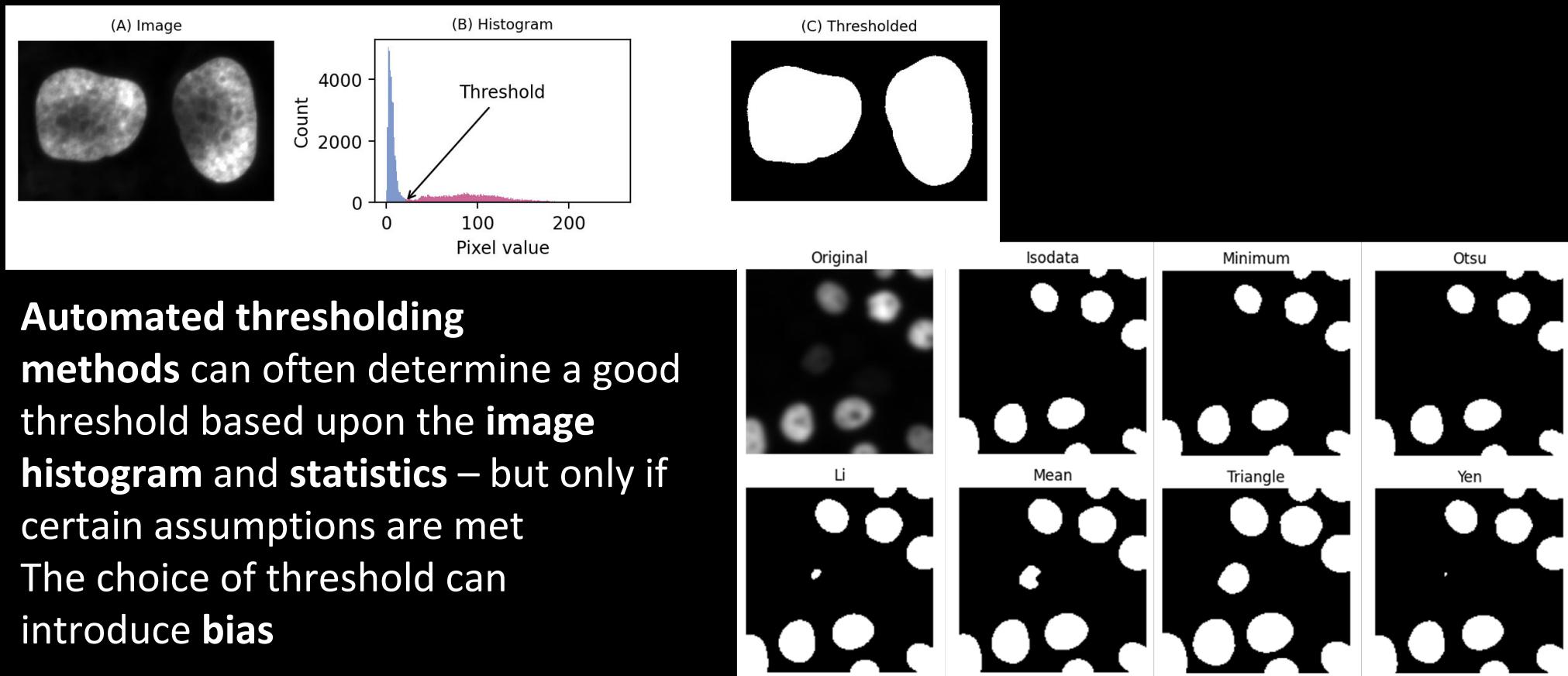
$> 40?$



Global Thresholding
Local Thresholding

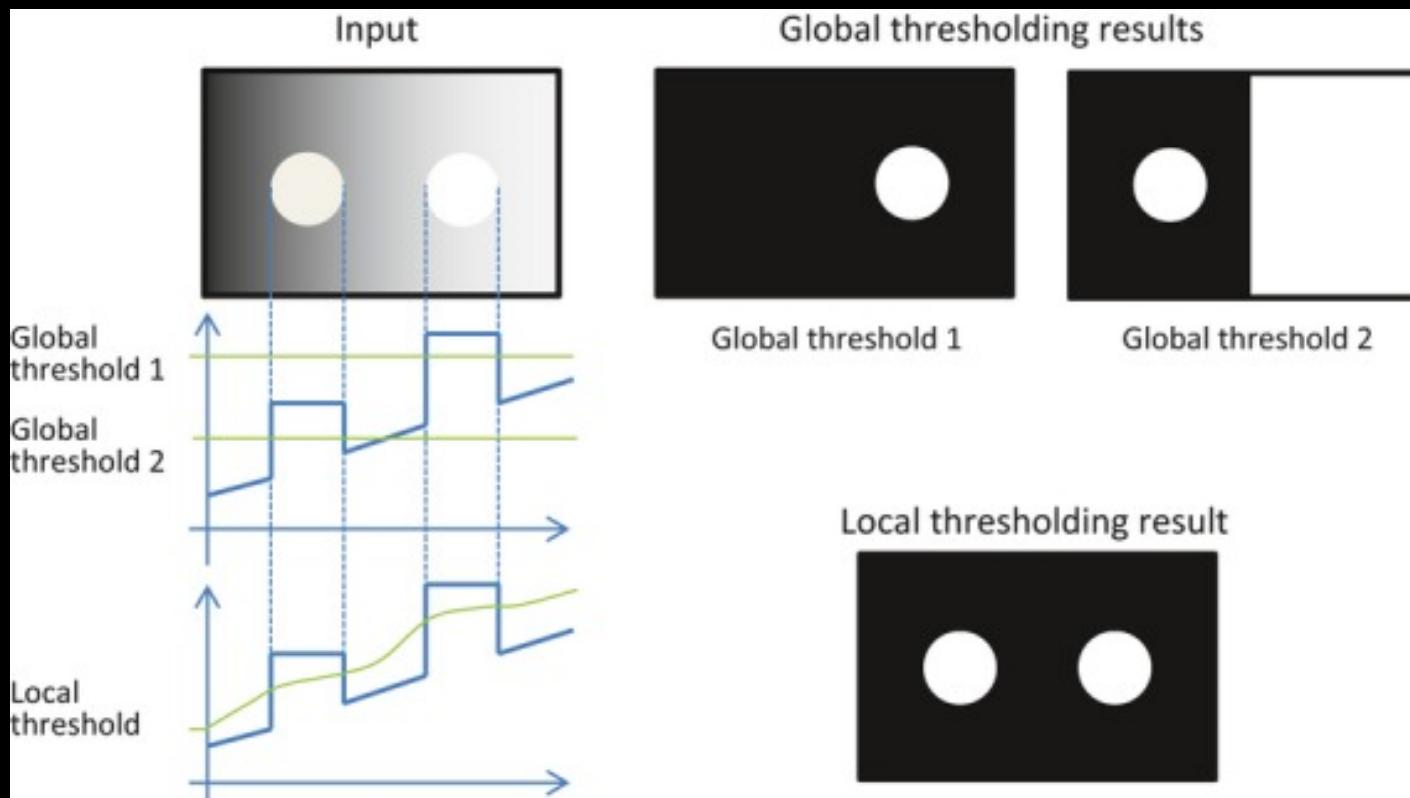
Global thresholding

identifies pixel values above or below a particular threshold

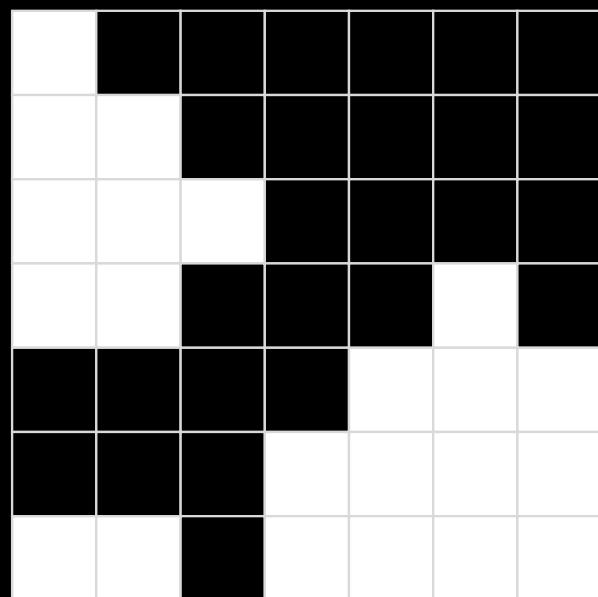


- **Automated thresholding methods** can often determine a good threshold based upon the **image histogram** and **statistics** – but only if certain assumptions are met
- The choice of threshold can introduce **bias**

Thresholding is more powerful when combined with **filtering**
& subtraction (Local thresholding)



Region of Interest (ROI) and Connected Component Labelling/ Analysis

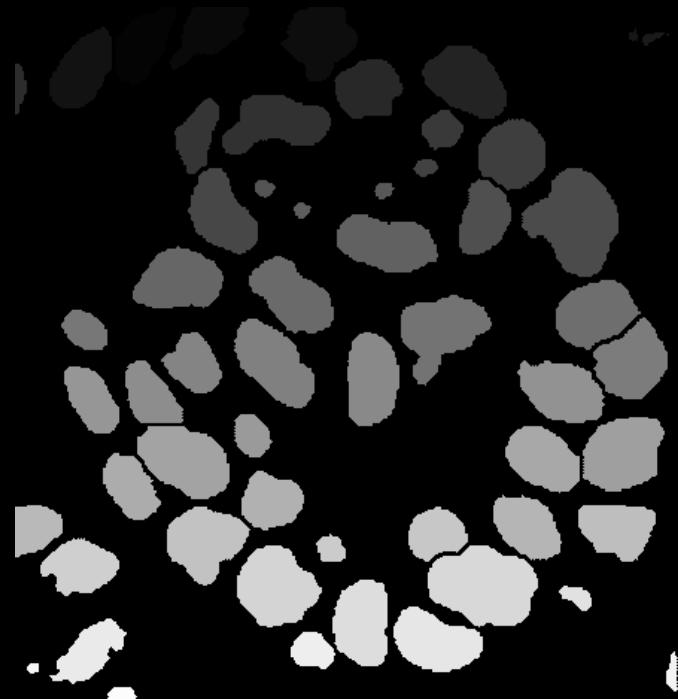
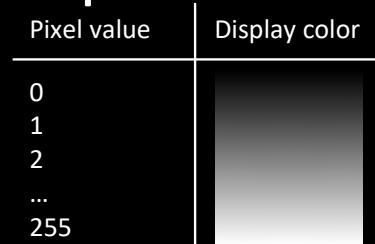


CCL

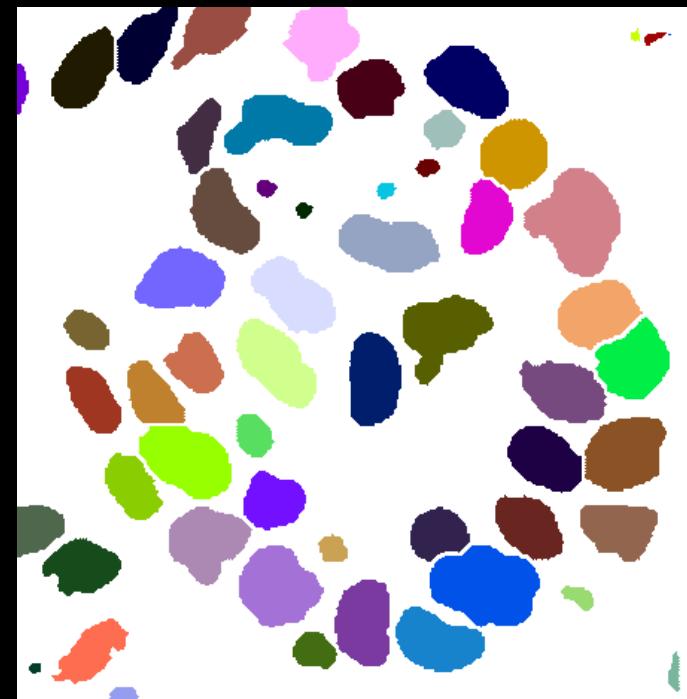
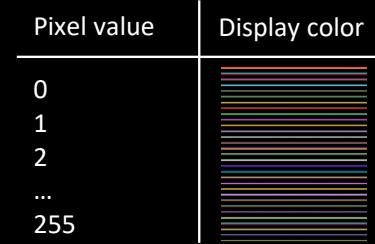
1	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0
1	1	1	0	0	0	0	0
1	1	0	0	0	0	3	0
0	0	0	0	3	3	3	3
0	0	0	3	3	3	3	3
2	2	0	3	3	3	3	3

Label map visualization

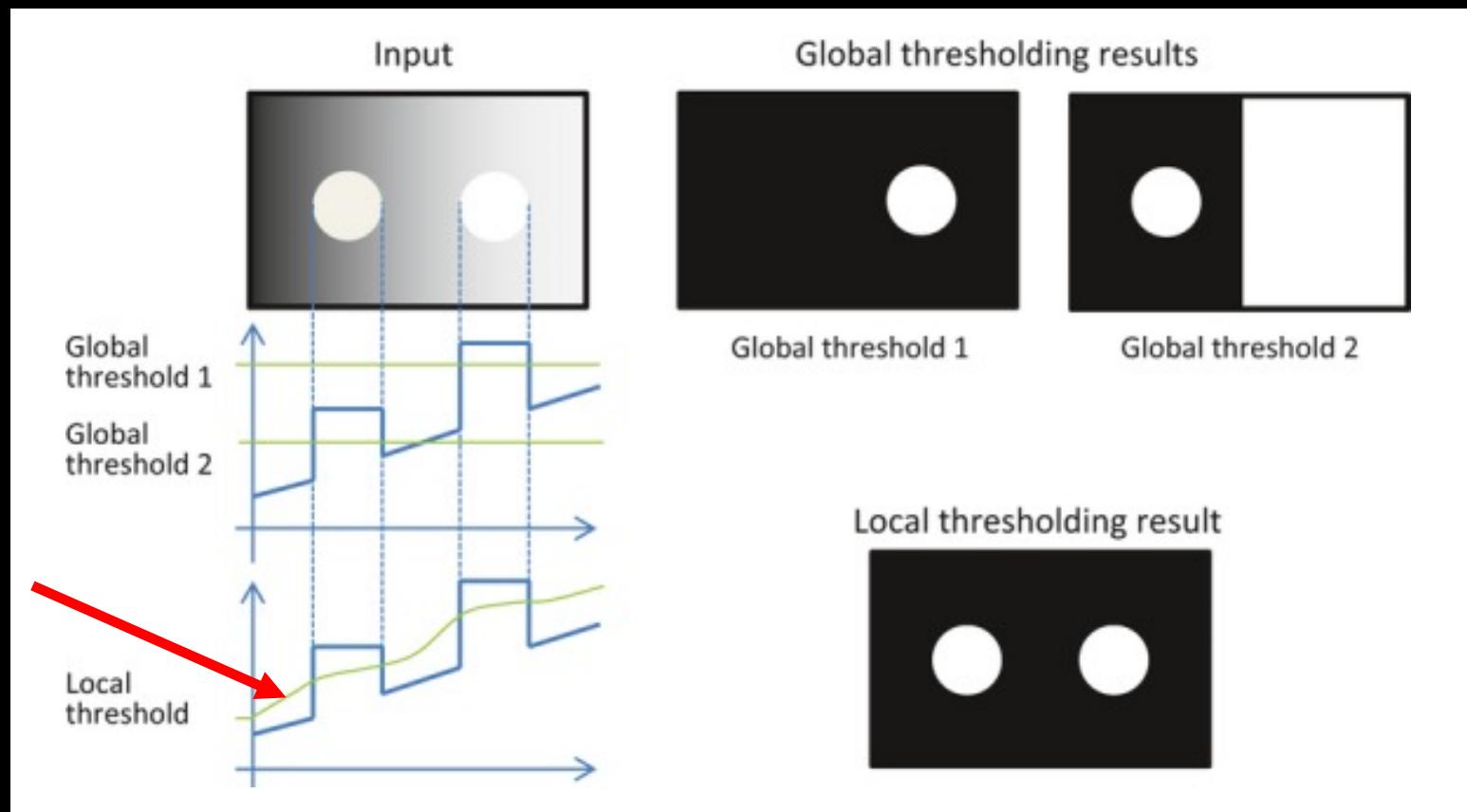
Grey



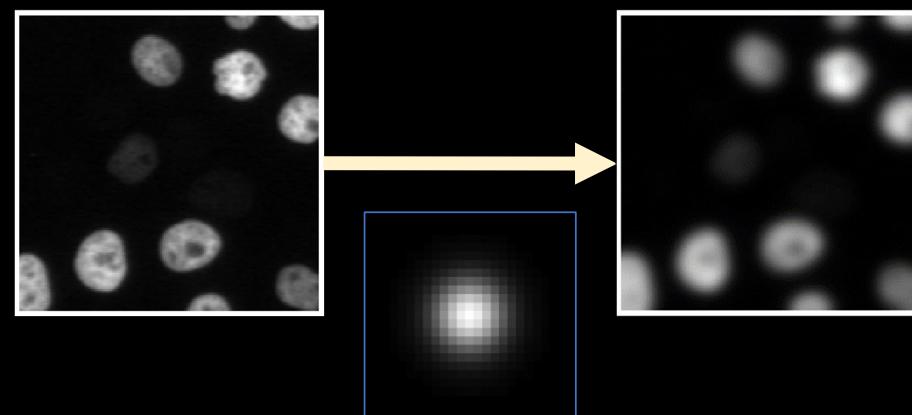
Glasbey/
Spectrum



How to make that???

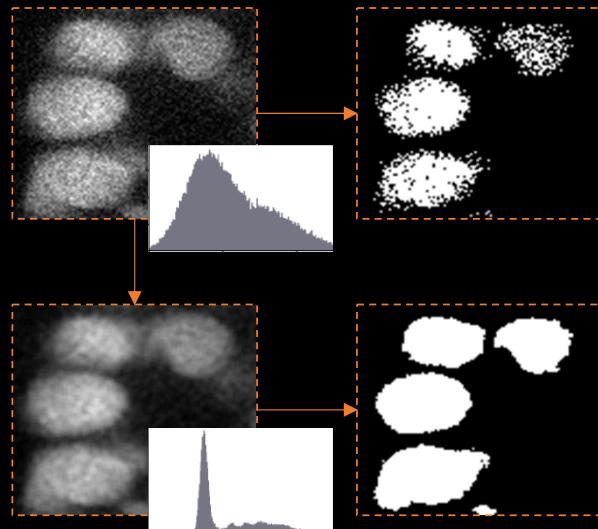


Filtering

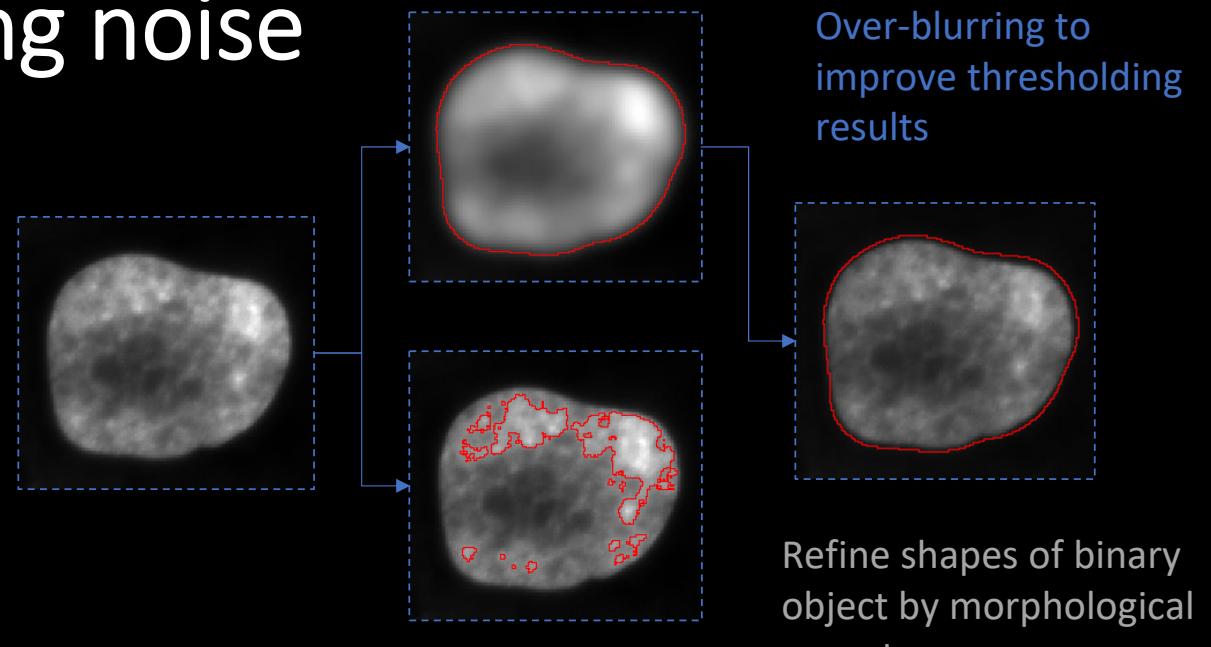
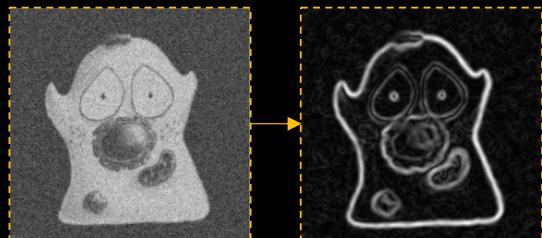


Filtering can make segmentation much easier by enhancing features and reducing noise

Gaussian Filtering for noise reduction

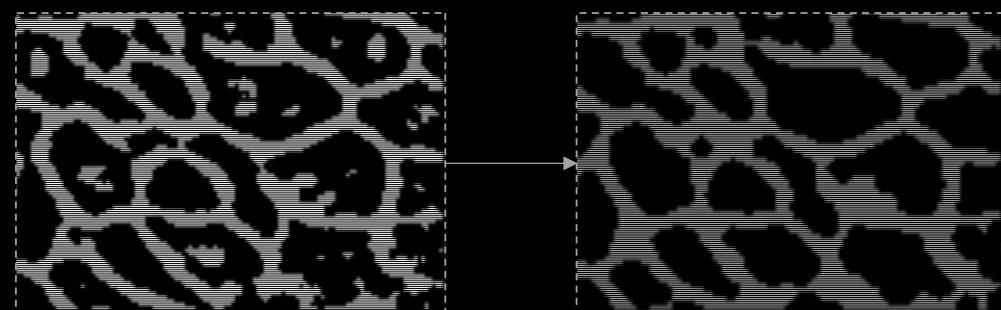


Edge detection by gradient filter



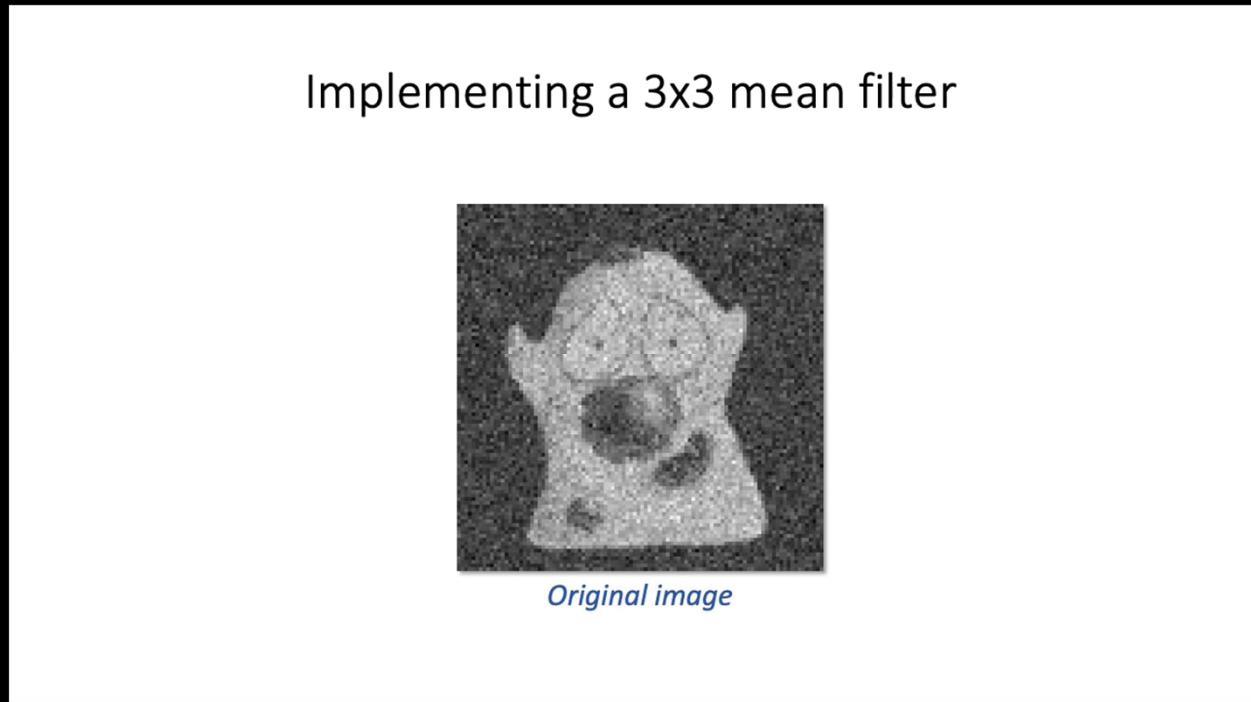
Over-blurring to improve thresholding results

Refine shapes of binary object by morphological operators

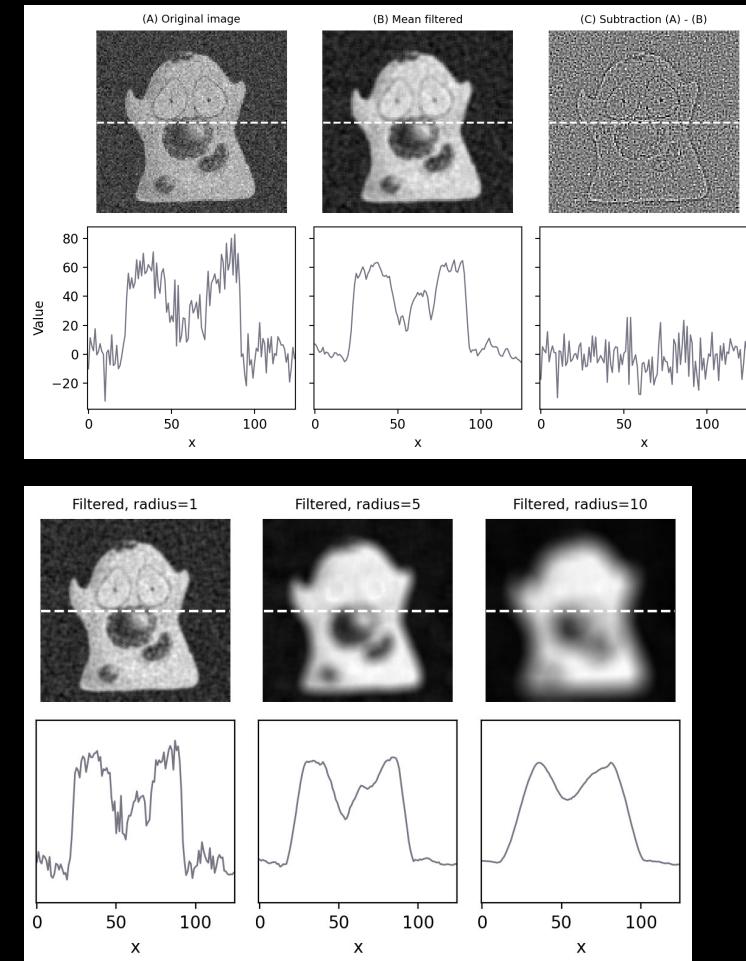


Linear filters

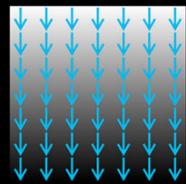
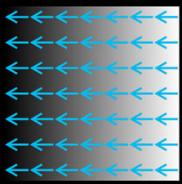
replace each pixel by a weighted sum of surrounding pixels



3x3 square	Circular, radius=1.5	Circular, radius=2.5	5x5 square
0 0 0 0 0	0 0 $\frac{1}{13}$ 0 0	0 $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ 0	$\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$
0 $\frac{1}{9}$ $\frac{1}{9}$ $\frac{1}{9}$ 0 0	0 $\frac{1}{13}$ $\frac{1}{13}$ $\frac{1}{13}$ 0	$\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$	$\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$
0 $\frac{1}{9}$ $\frac{1}{9}$ $\frac{1}{9}$ 0 0	$\frac{1}{13}$ $\frac{1}{13}$ $\frac{1}{13}$ $\frac{1}{13}$ 0	$\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$	$\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$
0 $\frac{1}{9}$ $\frac{1}{9}$ $\frac{1}{9}$ 0 0	0 $\frac{1}{13}$ $\frac{1}{13}$ $\frac{1}{13}$ 0 0	$\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$	$\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$
0 0 0 0 0	0 0 $\frac{1}{13}$ 0 0	0 $\frac{1}{21}$ $\frac{1}{21}$ $\frac{1}{21}$ 0	$\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$ $\frac{1}{25}$



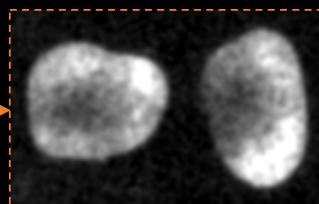
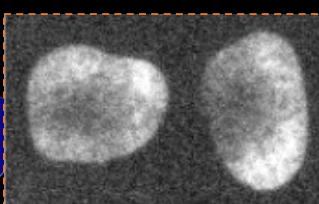
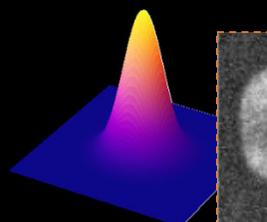
Linear filters (kernels) of different shapes and patterns



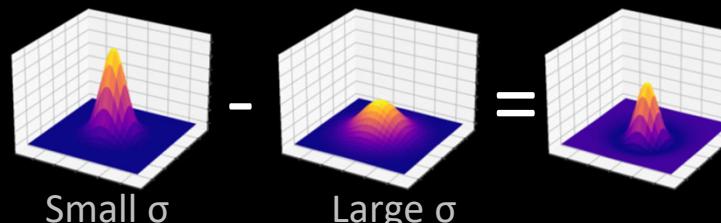
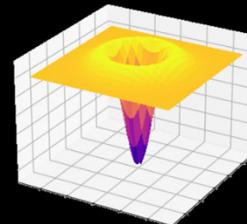
**Gradient Filter
or Sobel Filter**



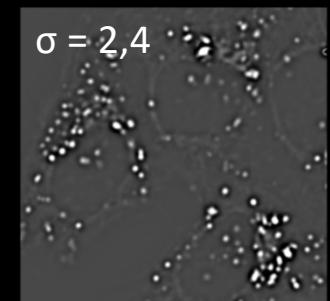
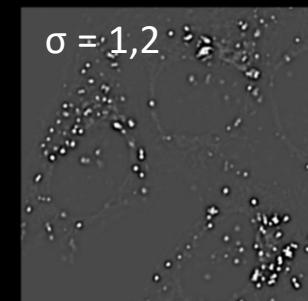
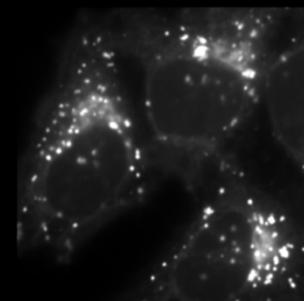
Gaussian Filter



Laplacian (of Gaussian) Filter (LoG)



**Difference of
Gaussian (DoG)
Filter**



Final note on Linear Filtering

- Terminologies:
 - Linear Filtering == Convolution
 - Filter Kernel == Filter Mask

Nonlinear filters replace each pixel with the result of another computation using surrounding pixels

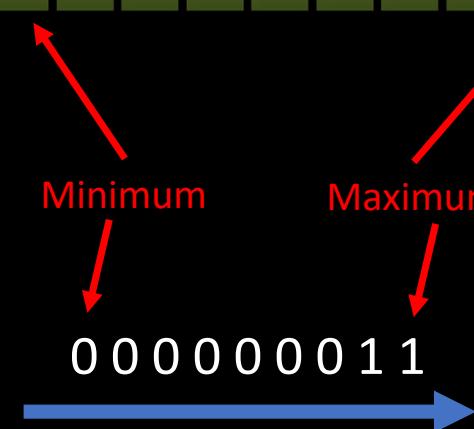
4	3	6	7	8	6	4	6	7
8	9	6	12	6	9	5	5	4
2	5	11	12	15	11	4	5	3
5	8	15	19	17	11	6	3	6
8	9	15	14	17	12	7	5	6
6	8	9	10	11	8	6	6	3
6	7	7	7	9	6	4	4	6
5	5	6	6	4	6	5	5	7
5	2	6	4	7	7	7	4	5
7	3	4	5	4	1	5	5	4

3x3 Structural Element

Minimum

Maximum

000000011



4	3	6	7	8	6	4	6	7	5
8	9	6	12	6	9	5	5	4	1
2	5	1	11	5	11	4	5	3	6
5	8	1	11	7	11	6	3	6	4
8	9	15	14	17	12	7	5	6	1
6	8	9	10	11	8	6	6	3	4
6	7	7	7	9	6	4	4	6	5
5	5	6	6	4	6	5	5	5	7
5	2	6	4	7	7	7	4	5	6
7	3	4	5	4	1	5	5	4	2

Rank Filter

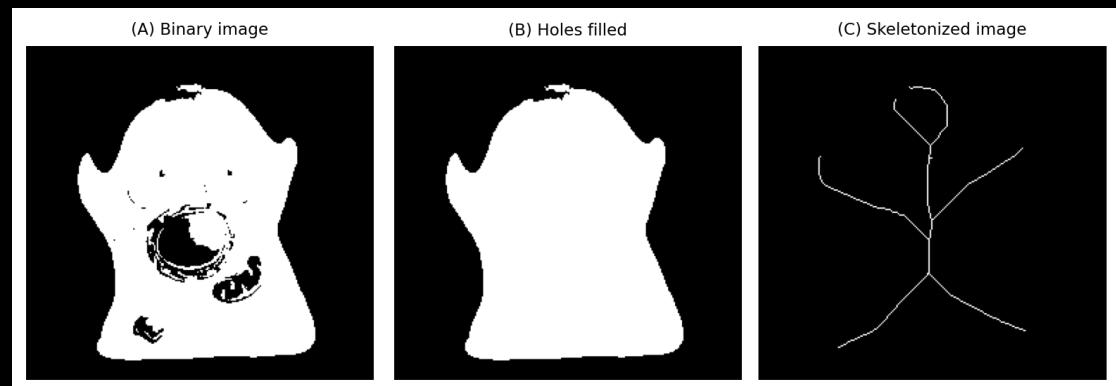
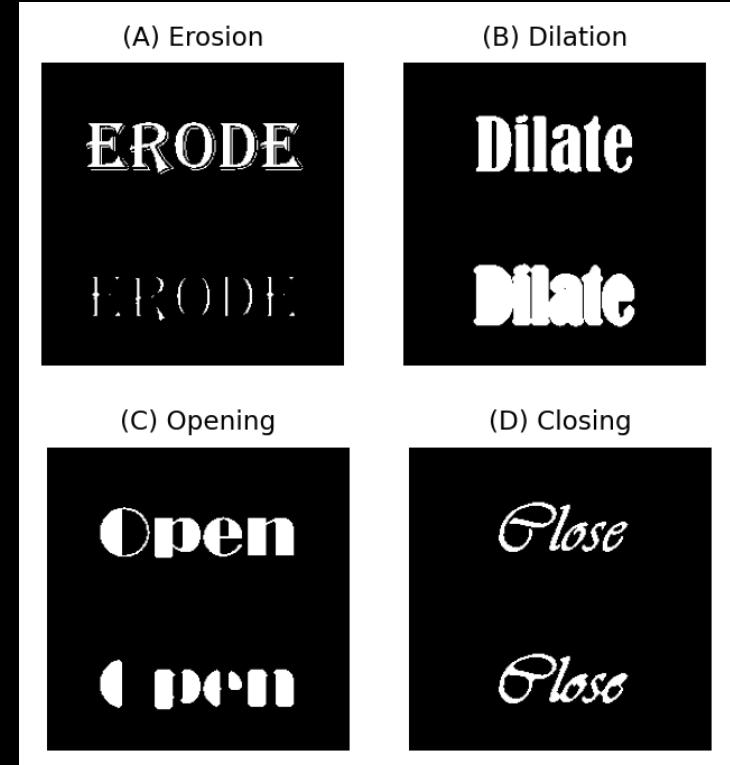
A 10x10 binary matrix where most cells contain the value 1. The matrix features several blue-colored rectangular blocks of varying sizes. A specific cell at row 4, column 5 is highlighted with a red square border and contains the value 0.

Morphological Operators

(3x3 dilation)

Morphological Operators

- **Morphological operations** can be used to refine or modify the shapes of objects in binary images
- Erosion/ Dilation
- Opening = Erosion -> Dilation
- Closing = Dilation -> Erosion
- Fill holes
- Skeletonization



Morphological Operators

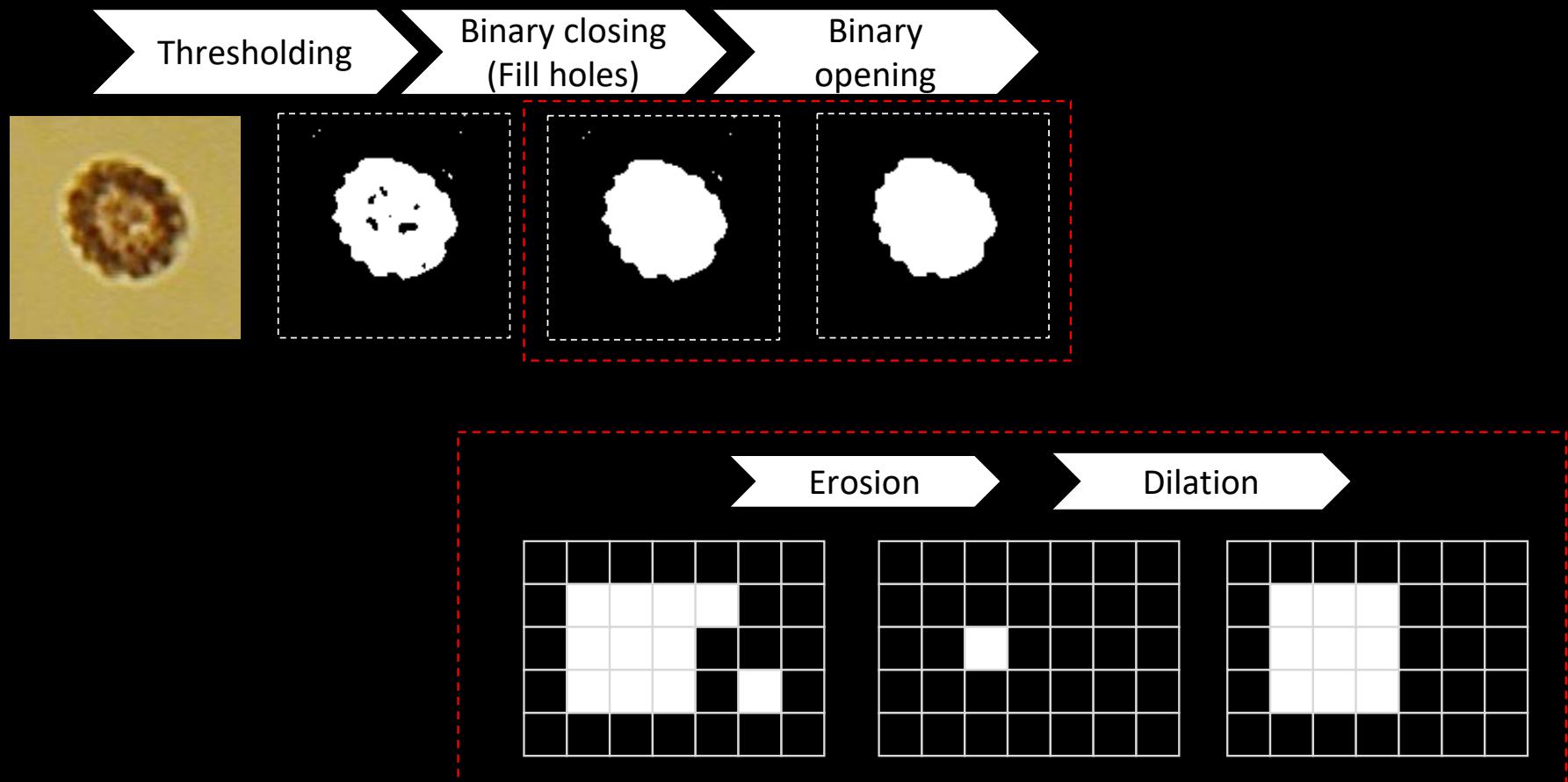
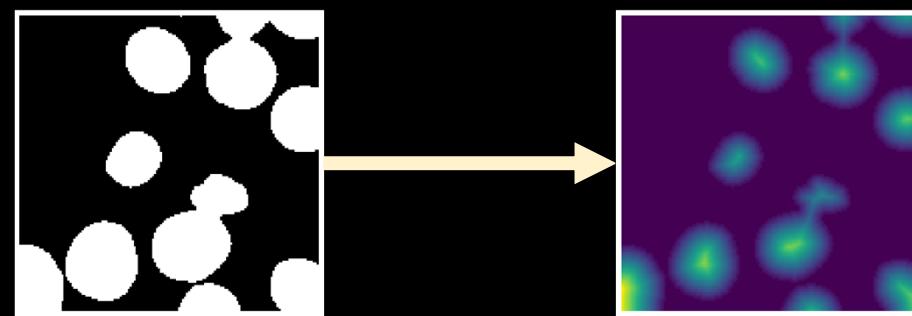
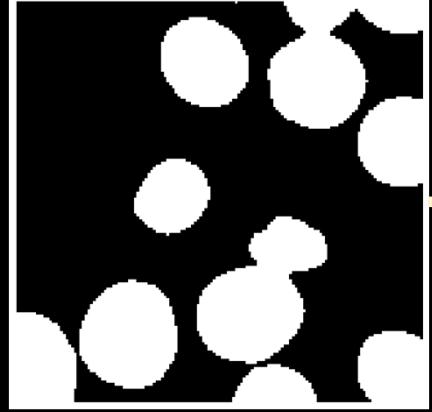


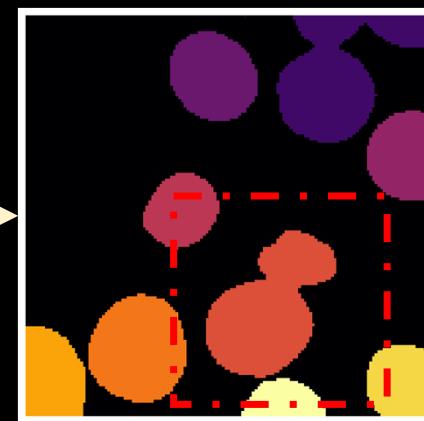
Image Transforms

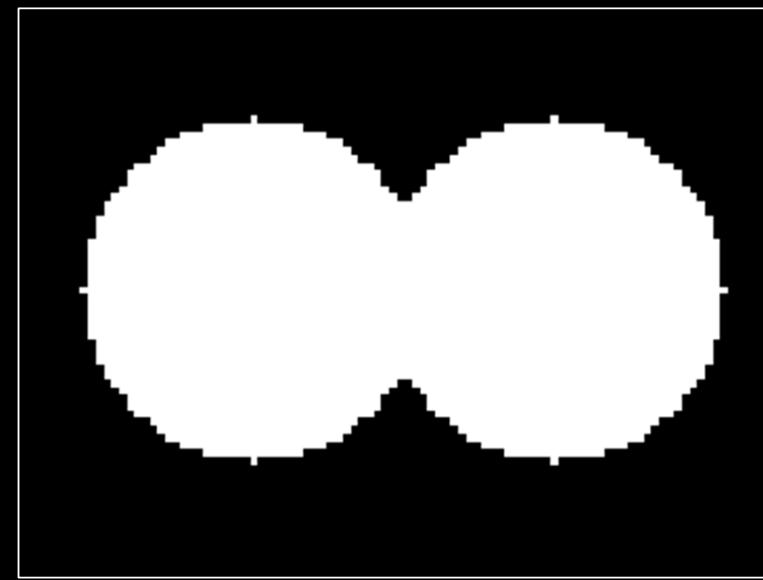




1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

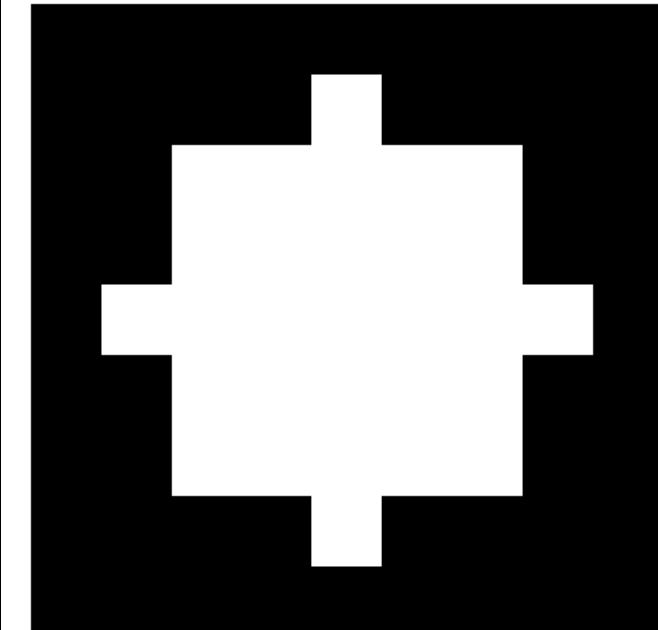
Connected
Components
Labelling (CCL)



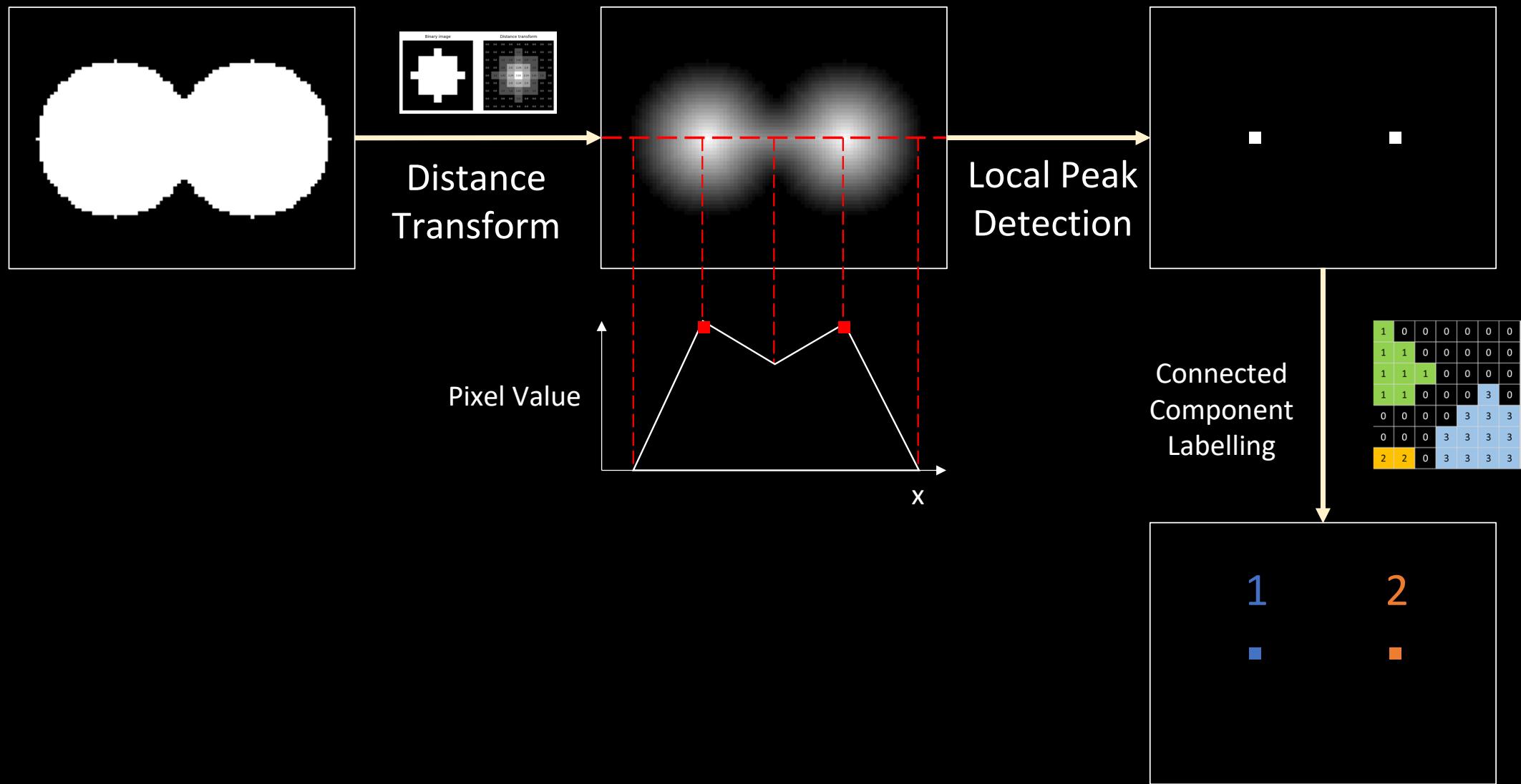


The **distance transform** calculates the distance between foreground and background pixels in a binary image

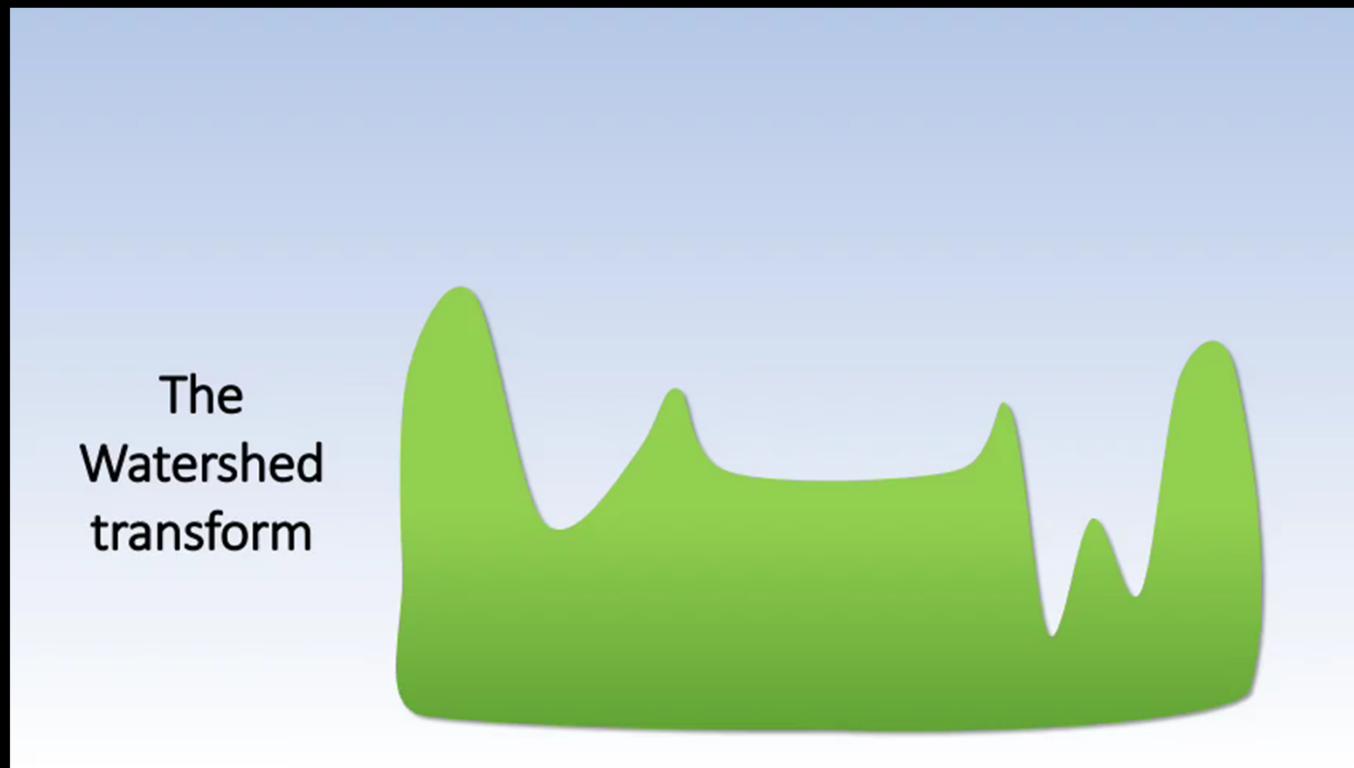
Binary image

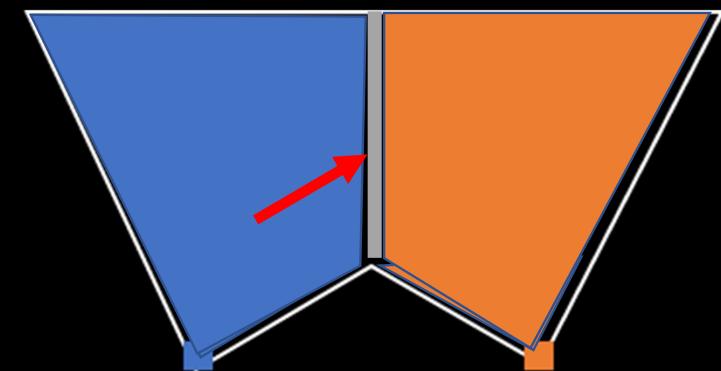
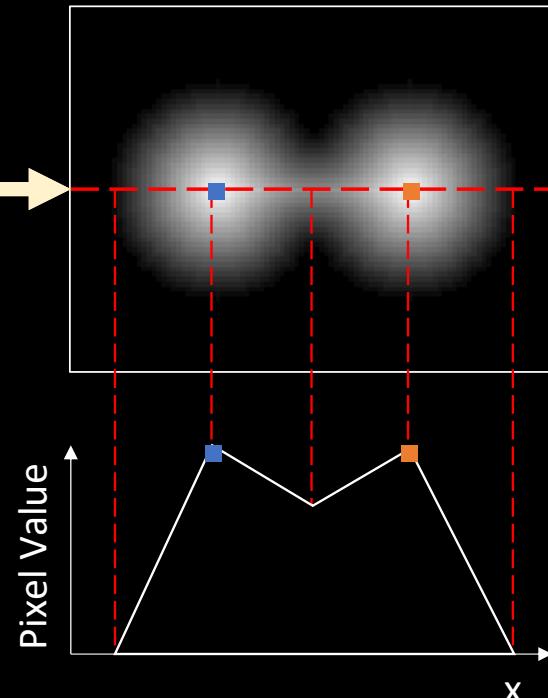
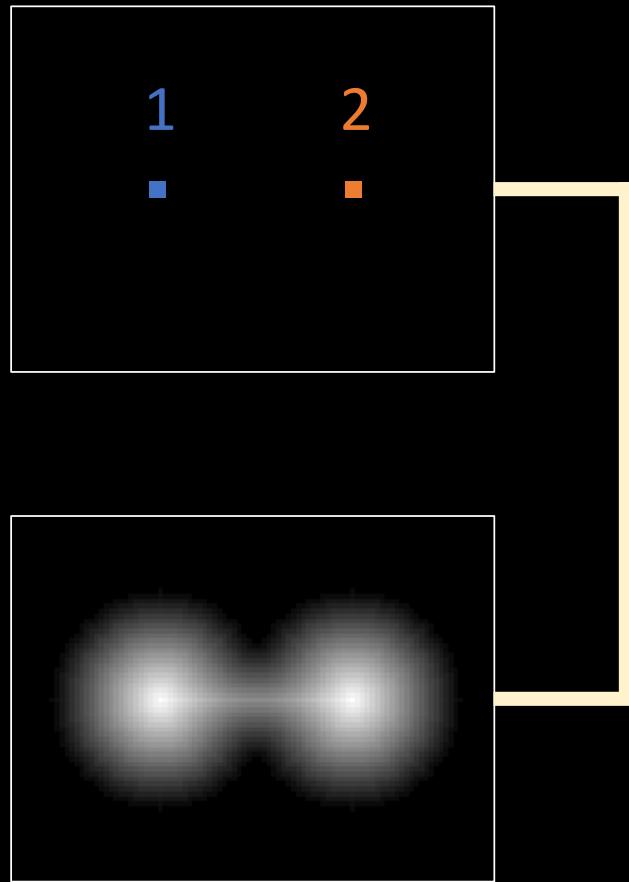


Distance transform



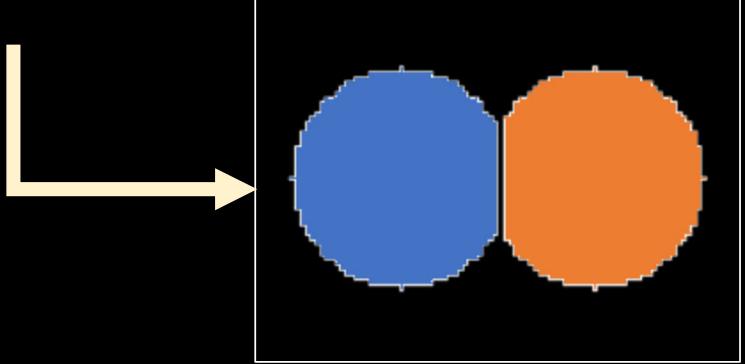
The **watershed transform** can be used to split structures using intensity values

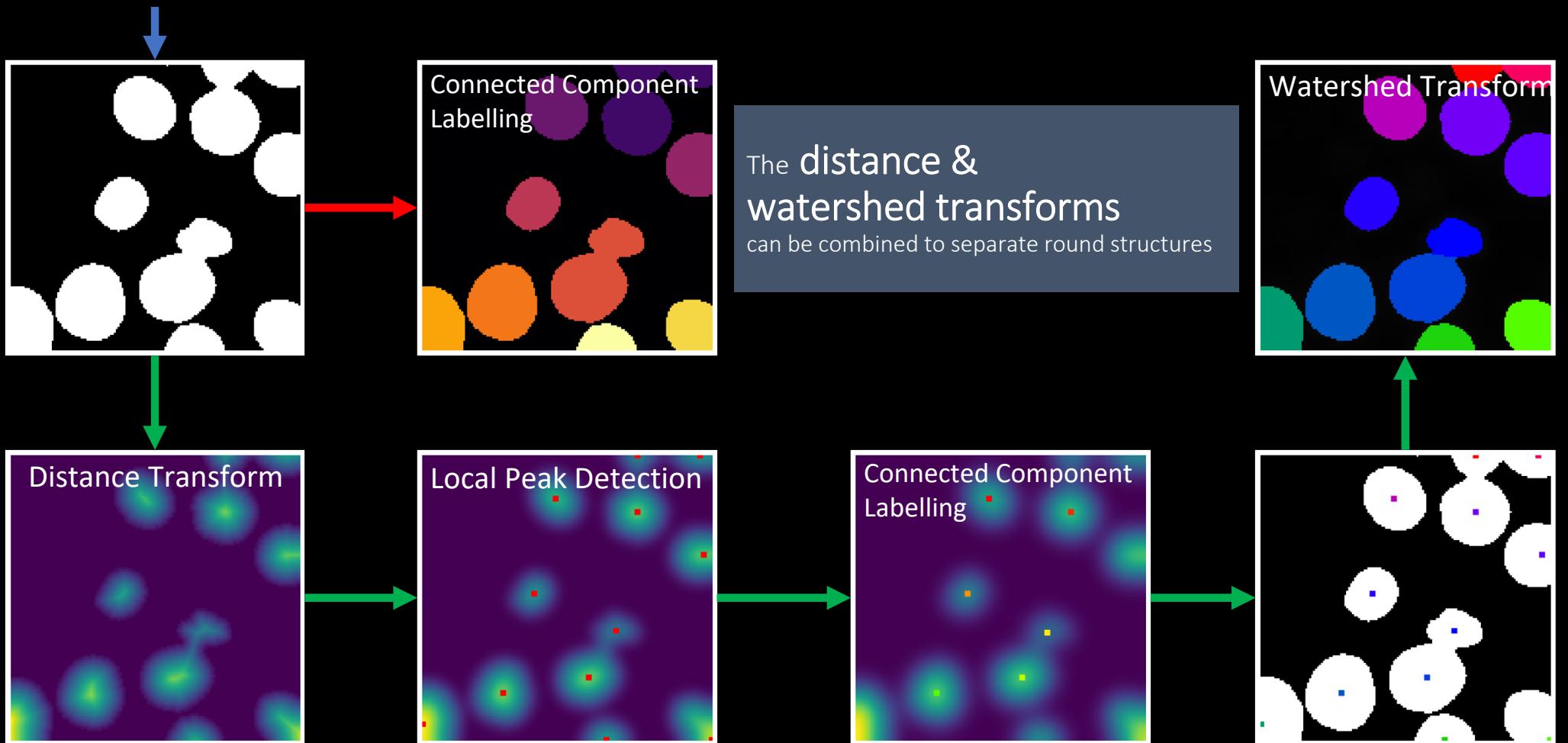




Watershed
分水嶺

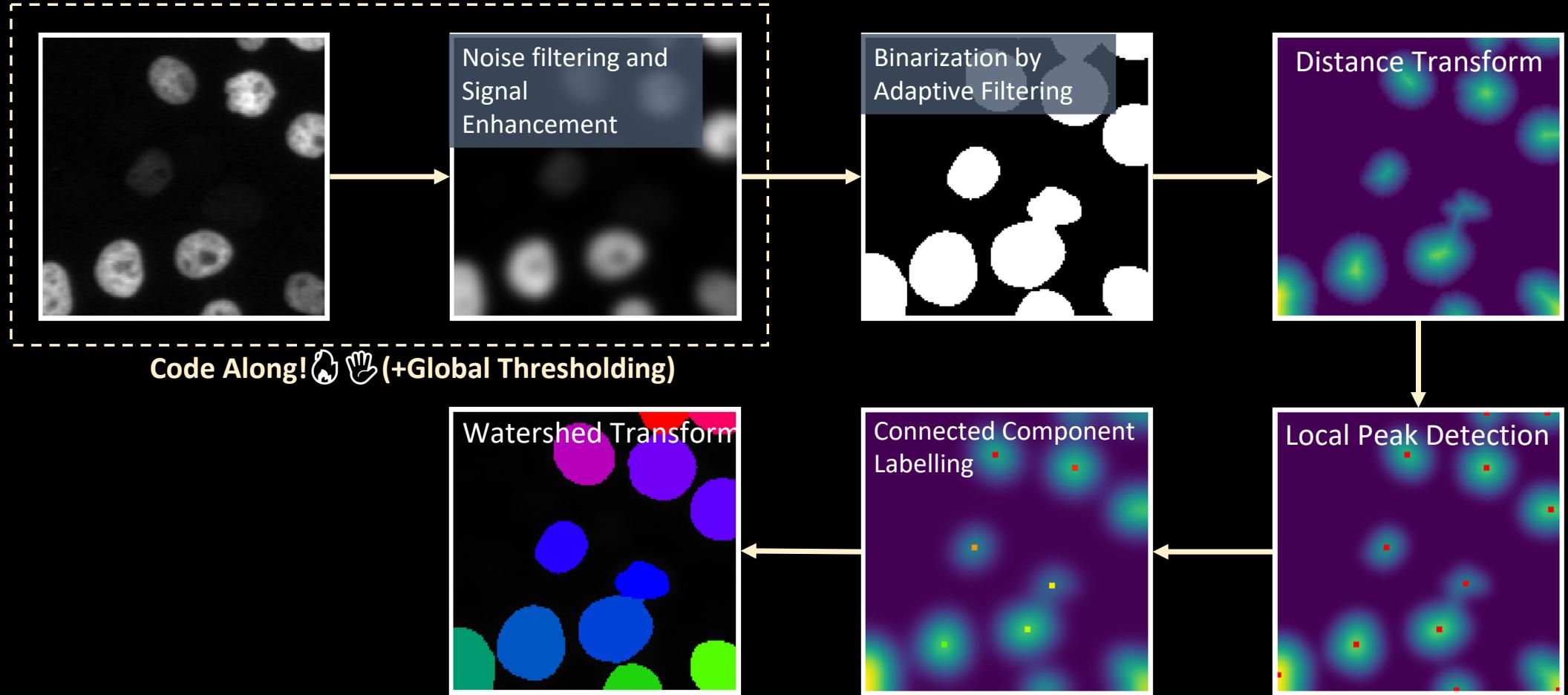
“The **watershed transform** can be used to split structures using intensity values”



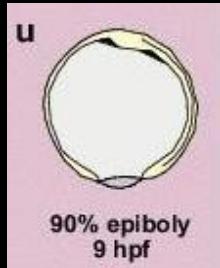


Codelab

Overall pipeline of this section

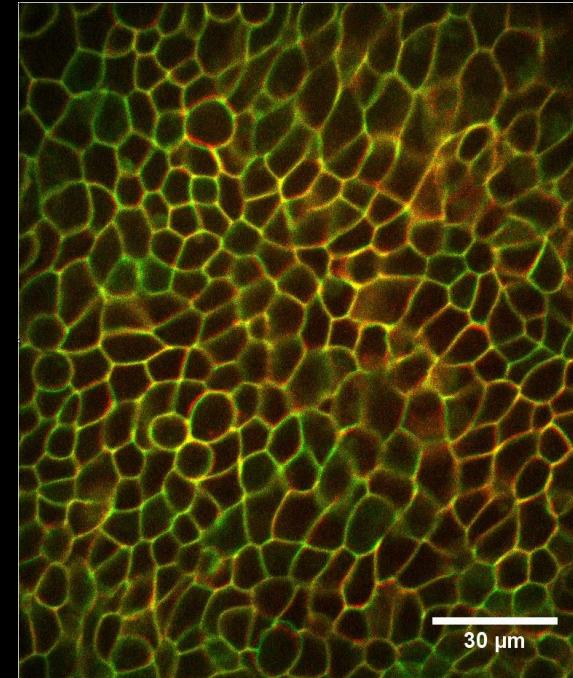


The sample image



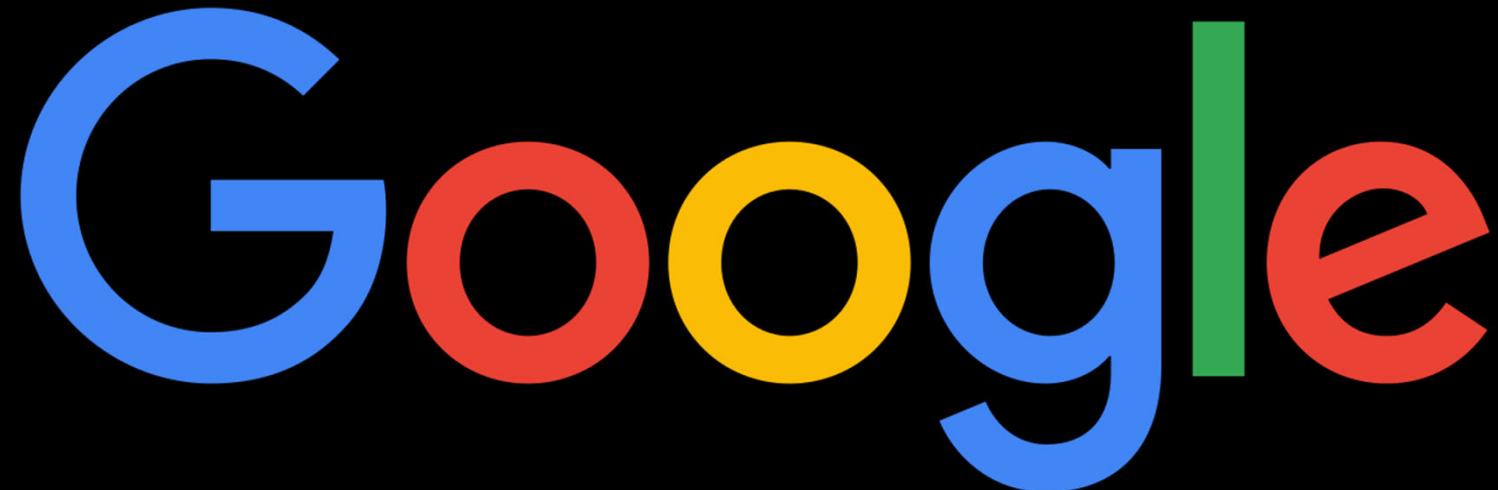
- Early zebrafish embryo
- An “in vivo cell culture”
- Observable: division, migration, differentiation, morphogenesis

- 40X spinning disc confocal slice
- Green: **mNG:Gy9 (G-protein)**
 - *The important channel for us!*
- Red: **Cxcr4b:tagRFP-T (GPCR)**
- “Real-World Data”



Before you start... the philosophy

- The arts of Googling



^ Your friend today

- Debug debug debug...





File Edit View Insert Cell Kernel Widgets Help Not Trusted Python 3 (ipykernel) ○



Can you find the error???

```
..._3]: # (iv) BONUS: Show the raw and smoothed images side by side using 'plt.s
```

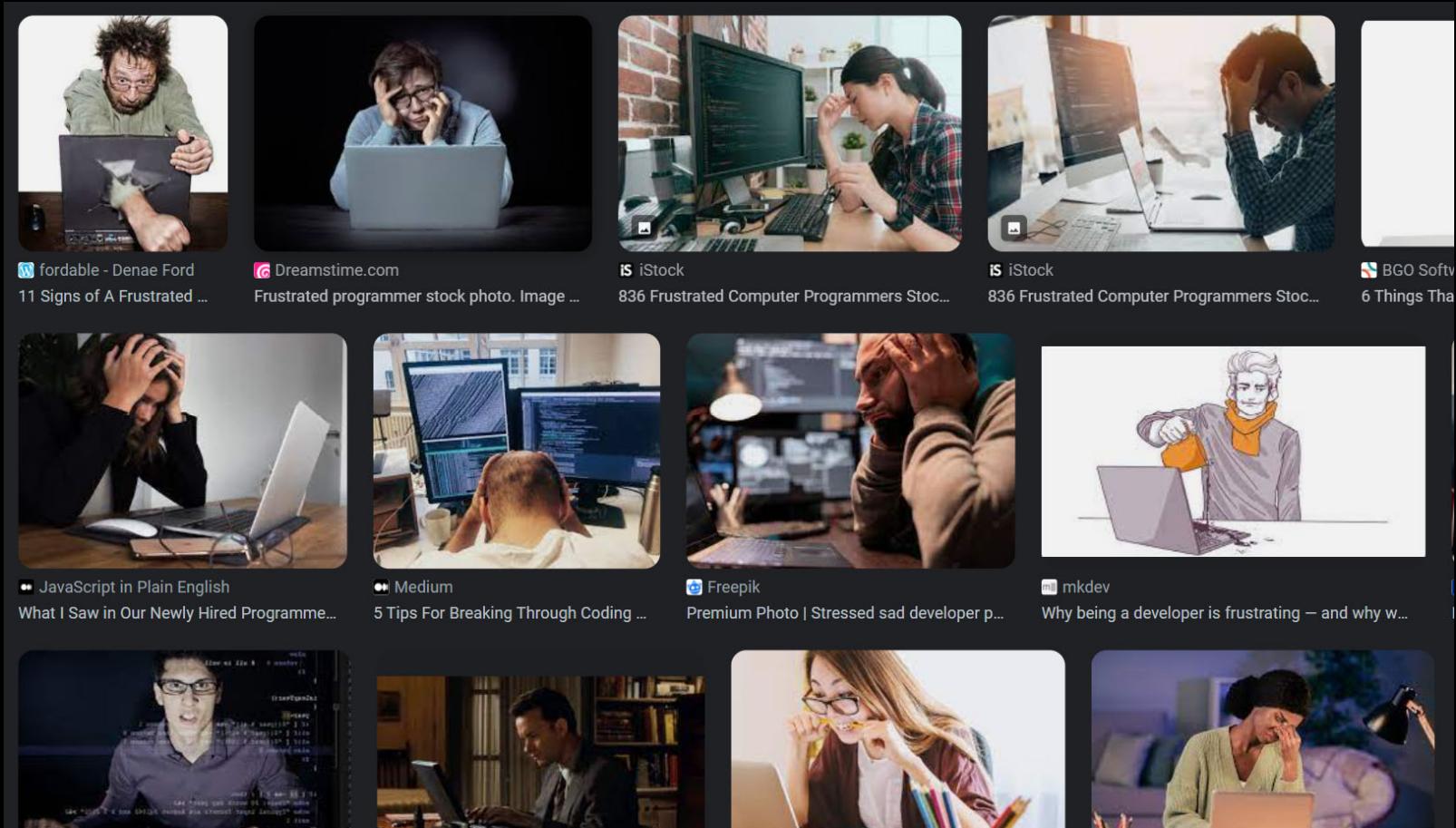
```
### YOUR CODE HERE!
fig, ax = plt.subplots(1, 2, figsize=(10,7))
ax[0].imshow(img, interpolation='none', cmap='gray')
ax[1].imshow(img_smooth, interpolation='none', cmap='gary')
ax[0].set_title('Raw Image')
ax[1].set_title('Smoothed Image')
plt.show()
```

```
dised by
    740 # the internal usage of this (ValueError vs KeyError)
--> 741 _api.check_in_list(sorted(_colormaps), cmap=cmap_name)
    742 return mpl.colormaps[cmap_name]
```

```
File ~/opt/anaconda3/envs/bioimage-analysis/lib/python3.8/site-packages
/matplotlib/_api/__init__.py:131, in check_in_list(_values, _print_supported_values, **kwargs)
    129 if _print_supported_values:
    130     msg += f"; supported values are {', '.join(map(repr, values
))}"
--> 131 raise ValueError(msg)
```

```
ValueError: 'gary' is not a valid value for cmap; supported values are
'Accent' 'Accent_r', 'Blues', 'Blues_r', 'BrBG', 'BrBG_r', 'BuGn', 'Bu
Gn_r', 'BuPu', 'BuPu_r', 'CMRmap', 'CMRmap_r', 'Dark2', 'Dark2_r', 'Gnb
u', 'GnBu_r', 'Greens', 'Greens_r', 'Greys', 'Greys_r', 'OrRd', 'OrRd_r
', 'Oranges', 'Oranges_r', 'PRGn', 'PRGn_r', 'Paired', 'Paired_r', 'Pas
tel1', 'Pastel1_r', 'Pastel2', 'Pastel2_r', 'PiYG', 'PiYG_r', 'PuBu', 'Pu
BuGn', 'PuBuGn_r', 'PuBu_r', 'PuOr', 'PuOr_r', 'PuRd', 'PuRd_r', 'Pur
```

- Expect *frustration*



First part of the notebook: code along

- Code Along! 🙌 🖐️ 😊

```
In [10]: # (i) Create a variable  
        ### YOUR CODE HERE!  
        # After implementing the
```

```
from skimage.feature import  
seeds_ = peak_local_max(dis  
  
# BONUS: You will see a "Fu  
# Look for peak_local_max a  
seeds = peak_local_max(dis  
seeds[seeds > 0] = 1
```

Code Along

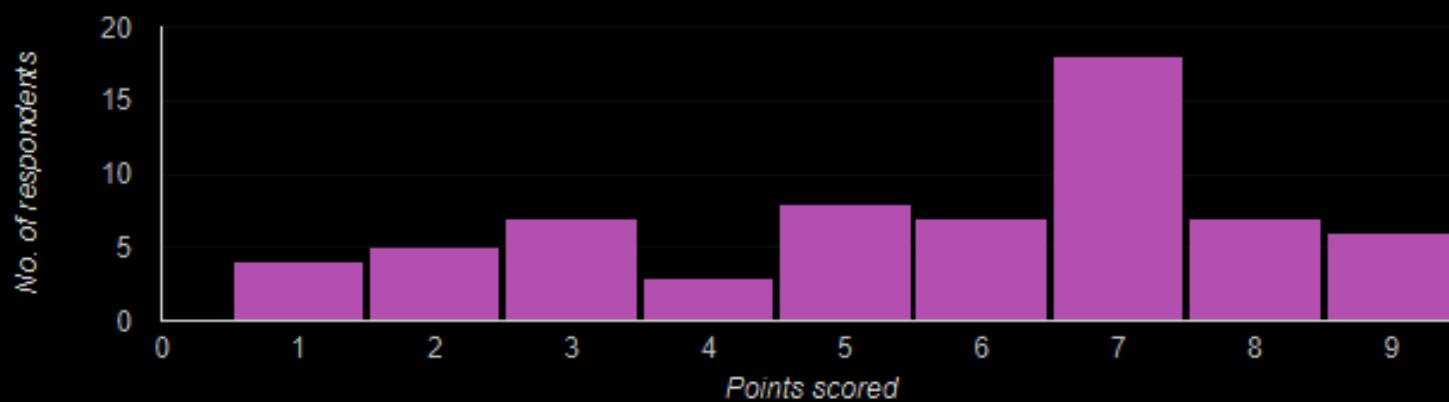
Code with your
Codemate! (BONUS)

+ BONUS! 🎉

Help others 😊



Total points distribution



DEMO: Start Jupyterlab
Open the **CodeAlong** and **Tutorial** file and start coding!



The image shows a Windows desktop environment. On the left, there is a vertical column of icons for various software applications, including Microsoft Edge, LINE, Google Chrome, Adobe Acrobat DC, TeamViewer, Blender 3.2, KiCad, MATLAB R2022b, XPG Prime (beta), FreeCAD 0.19, and OBS Studio. The desktop background features a scenic image of a person running on a beach at sunset. On the right side of the screen, a web browser window is open, displaying a GitHub repository page for "Koushouu/Bioimage-Analysis". The repository has 1 star and 0 forks. The "Code" tab is selected, showing a list of files and folders:

- README_images
- codelab
- Basics in Python.ipynb
- Cellpose_2.0_in_colab_part_1_v2.ipynb
- Cellpose_2.0_in_colab_part_2_v2.ipynb
- LICENSE
- README.md

The browser also shows a download progress bar for "Bioimage-Analysi...zip". At the bottom of the screen, the taskbar displays various pinned icons and system status indicators.