Image Processing and Filtering

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Image Visualization

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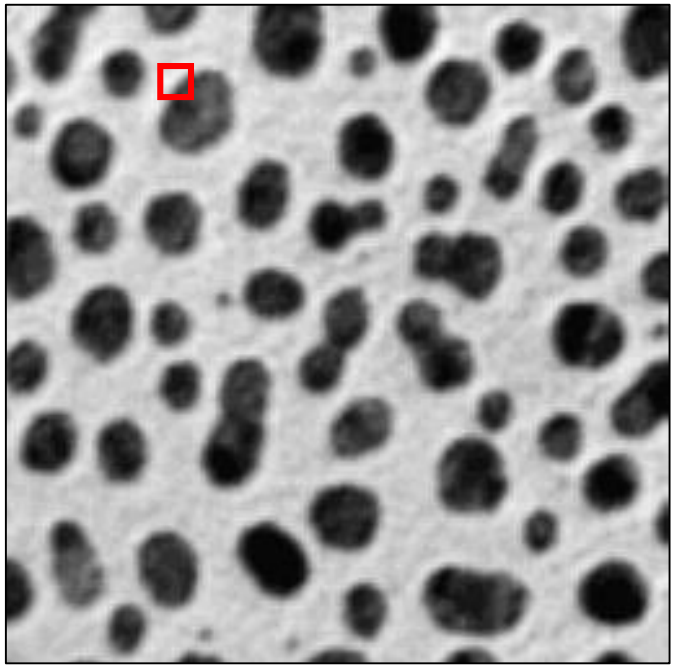
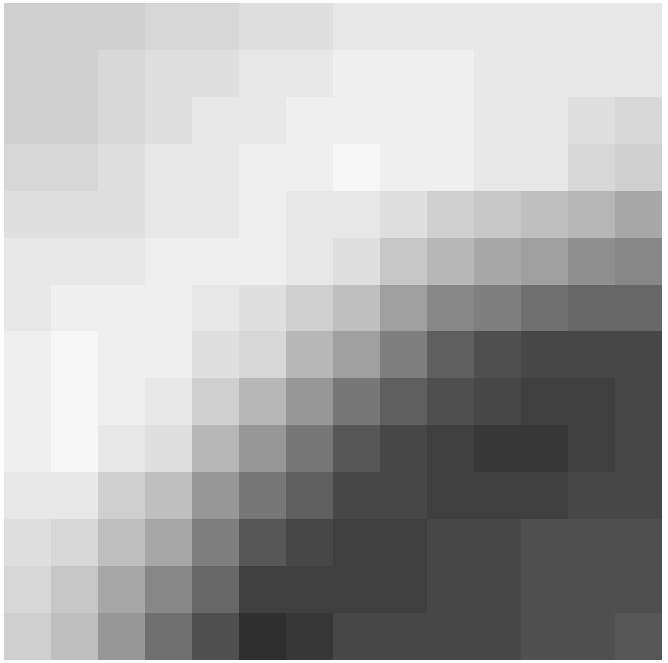
Images and pixels

- An image is just a matrix of numbers
- Pixel: “picture element”
- The edges between pixels are an artefact. In reality, they don’t exist!





48	48	48	40	40	32	32	24	24	24	24	24	24	24
48	48	40	32	32	24	24	16	16	16	24	24	24	24
48	48	40	32	24	24	16	16	16	16	24	24	32	40
40	40	32	24	24	16	16	8	16	16	24	24	40	48
32	32	32	24	24	16	24	24	32	48	56	64	72	88
24	24	24	16	16	16	24	32	56	72	88	96	112	120
24	16	16	16	24	32	48	64	96	120	128	144	152	152
16	8	16	16	32	40	72	96	128	160	176	184	184	184
16	8	16	24	48	72	104	136	160	176	184	192	192	184
16	8	24	32	72	104	136	168	184	192	200	200	192	184
24	24	48	64	104	136	160	184	184	192	192	192	184	184
32	40	64	88	128	168	184	192	192	184	184	176	176	176
40	56	88	120	152	192	192	192	192	184	184	176	176	176
48	64	104	144	176	208	200	184	184	184	184	176	176	168

Histogram

Consider the following image:

60	60	60	60	64	192	192
60	60	60	60	64	192	192
64	64	64	64	64	192	192

How many elements does it have?

Which pixel value occurs more often?

If we may be mistaken in such a small image, imagine for big images!

The histogram answers these questions at a glance!

Histogram: a graph that shows how frequent values are in an array/image

```
from skimage.exposure import histogram

piexl_counts, pixel_values = histogram(array)

plt.bar(pixel_values, piexl_counts)
```

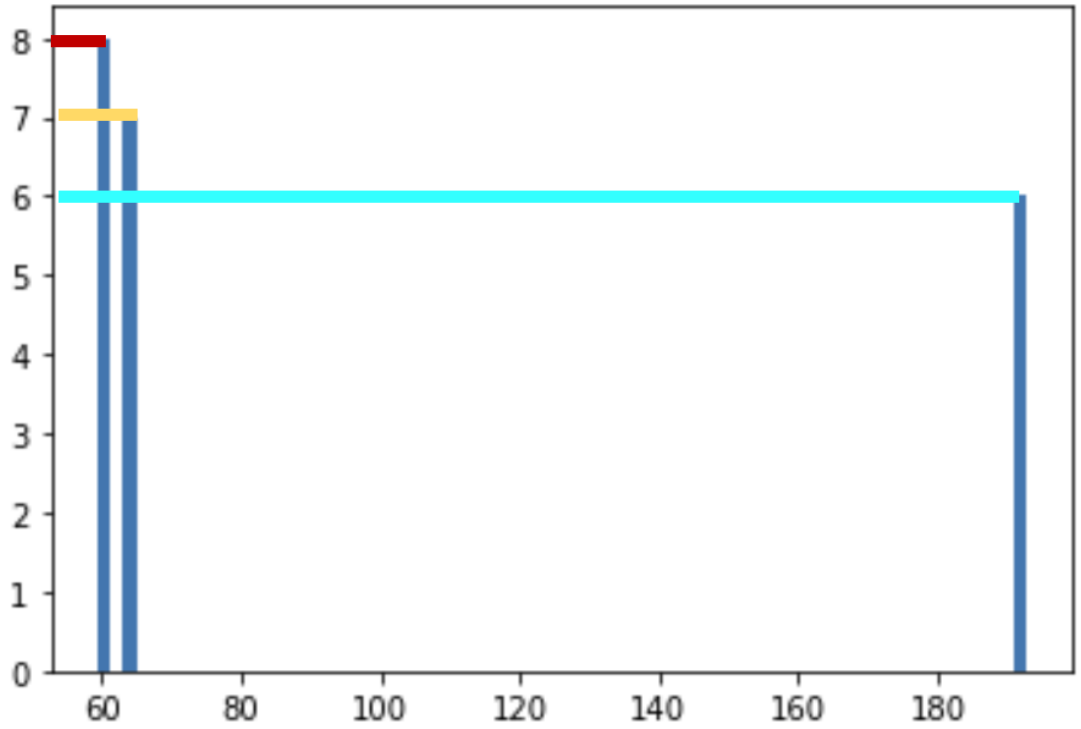
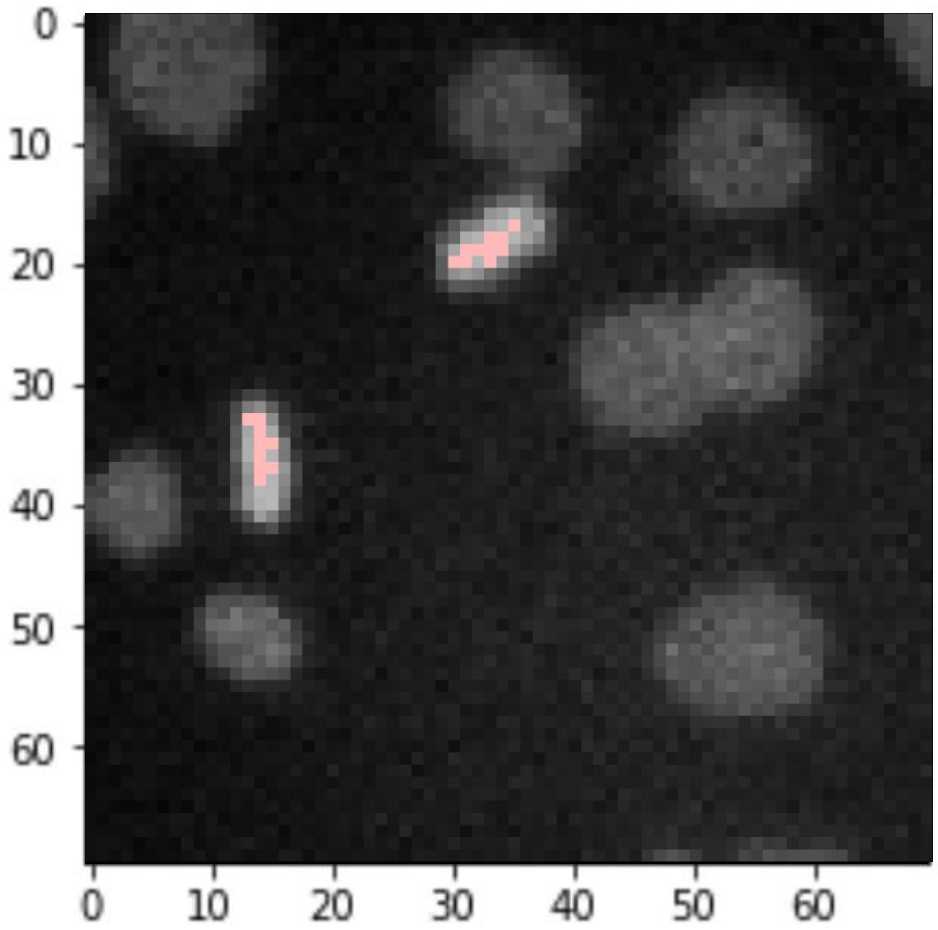
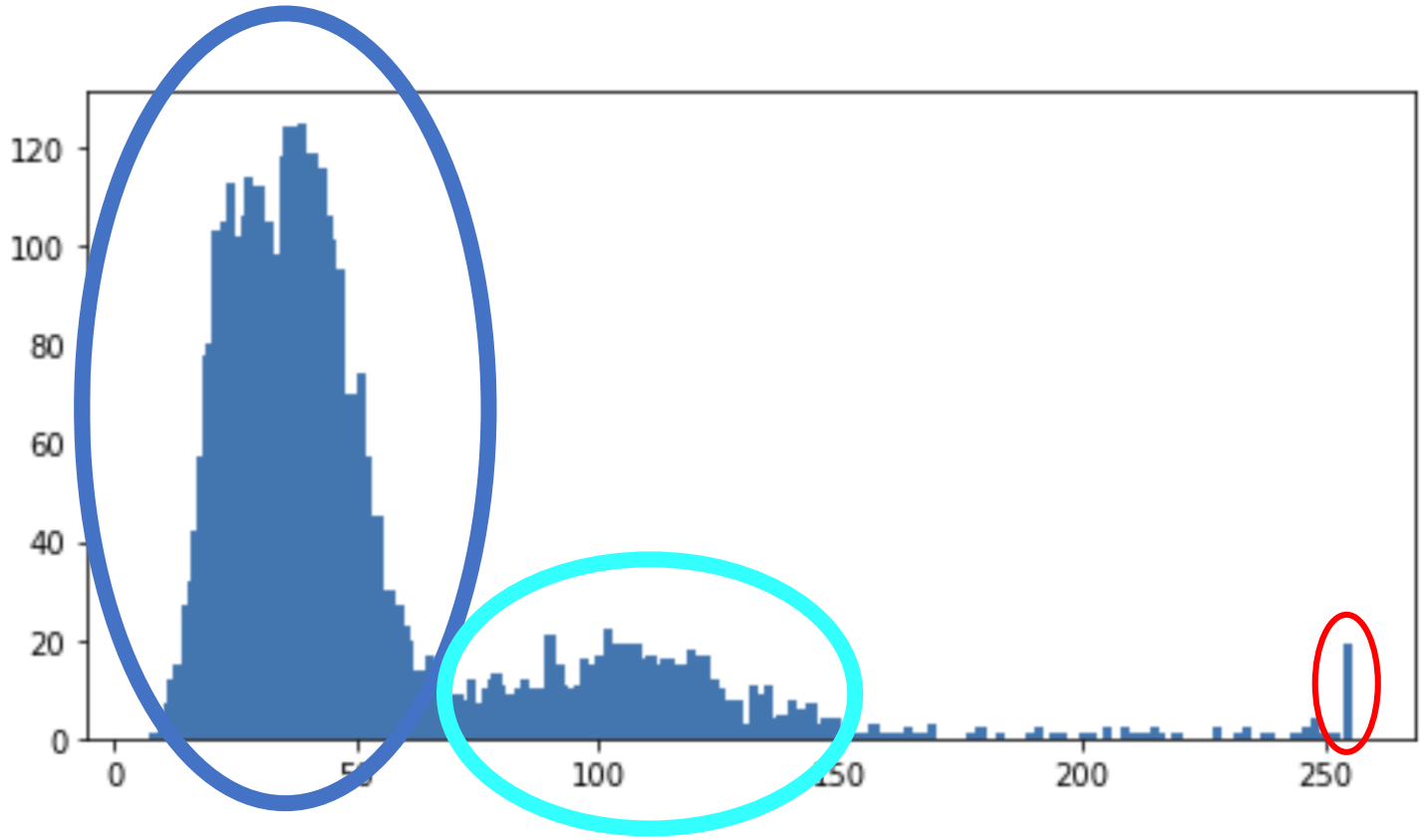


Image Histogram

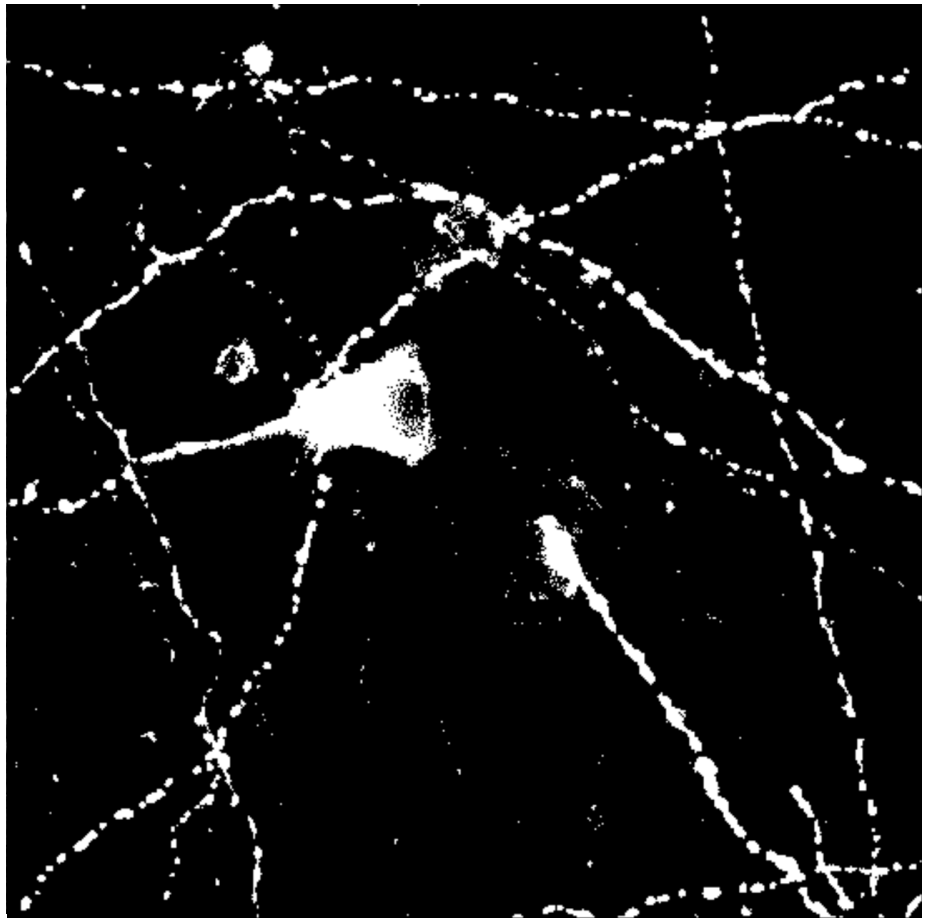
Example with a slightly larger image:

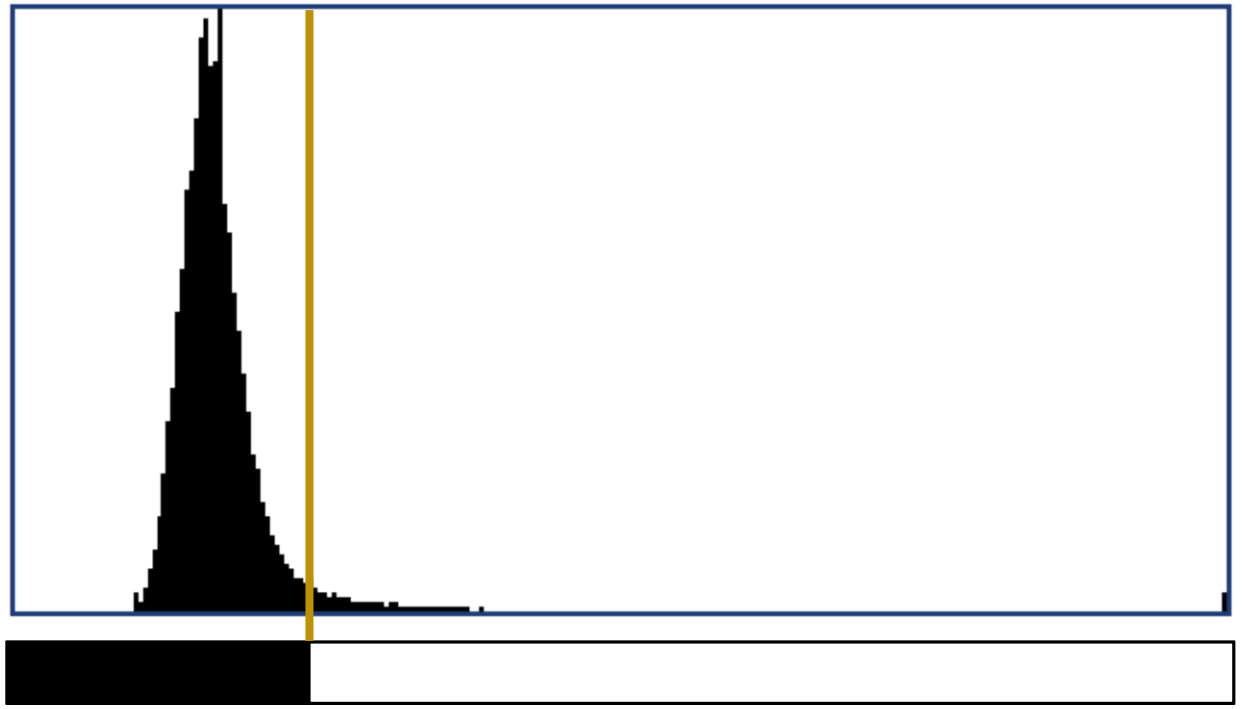




Binarization: a quick peek

Values above a threshold value are replaced by 1 (or True).
Values below or equal threshold are replaced by 0 (or False)





```
image_binary = image > value
```

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Image Processing: Filters

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Filters

- An image processing filter is an operation on an image.
- It takes an image and produces a new image out of it.
- Filters change pixel values.

- There is no “best” filter. Which filter fits your needs, depends on the context.
- Filters do not do magic. They can not make things visible which are not in the image.

- Application examples
  - Noise-reduction
  - Artefact-removal
  - Contrast enhancement
  - Correct uneven illumination

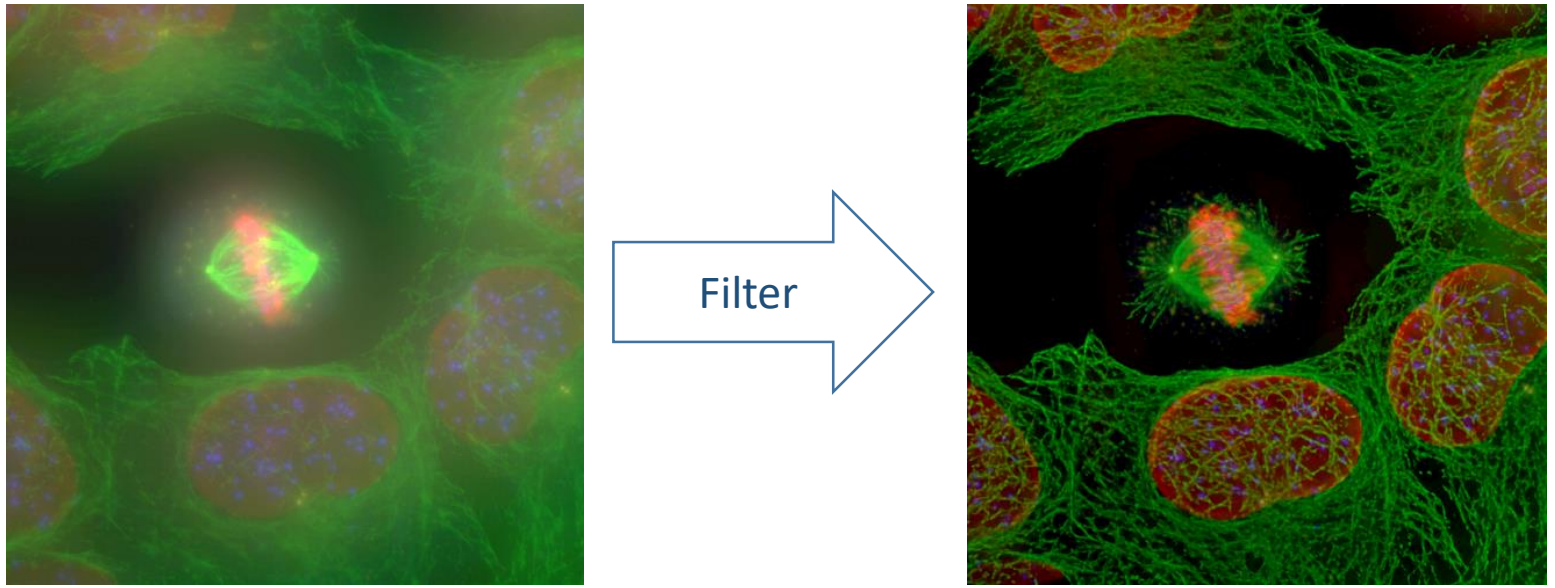


Image source: Alex Bird / Dan White MPI CBG

Effects harming image quality

- Noise is a general term for unwanted modifications that a signal may suffer during capture, storage, transmission, processing, or conversion.
- In microscopy, image quality suffers from
  - shot noise: Statistical variation of the photons arriving at the camera
  - dark noise: Statistical variation of how many electrons are generated if a photon arrives in a camera pixel (temperature dependent).
  - read out noise: introduced by the electronics, especially the analog-digital-converter
  - Physical/optical effects: aberrations, defocus
  - Biological/physiological/structural effects: motion, diffusion

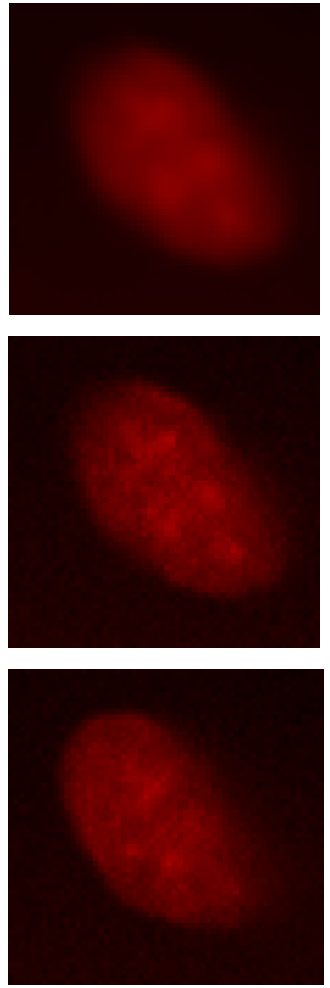


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Noise

- When dealing with noise in microscopic image processing, we mostly mean the noise visible in the images.

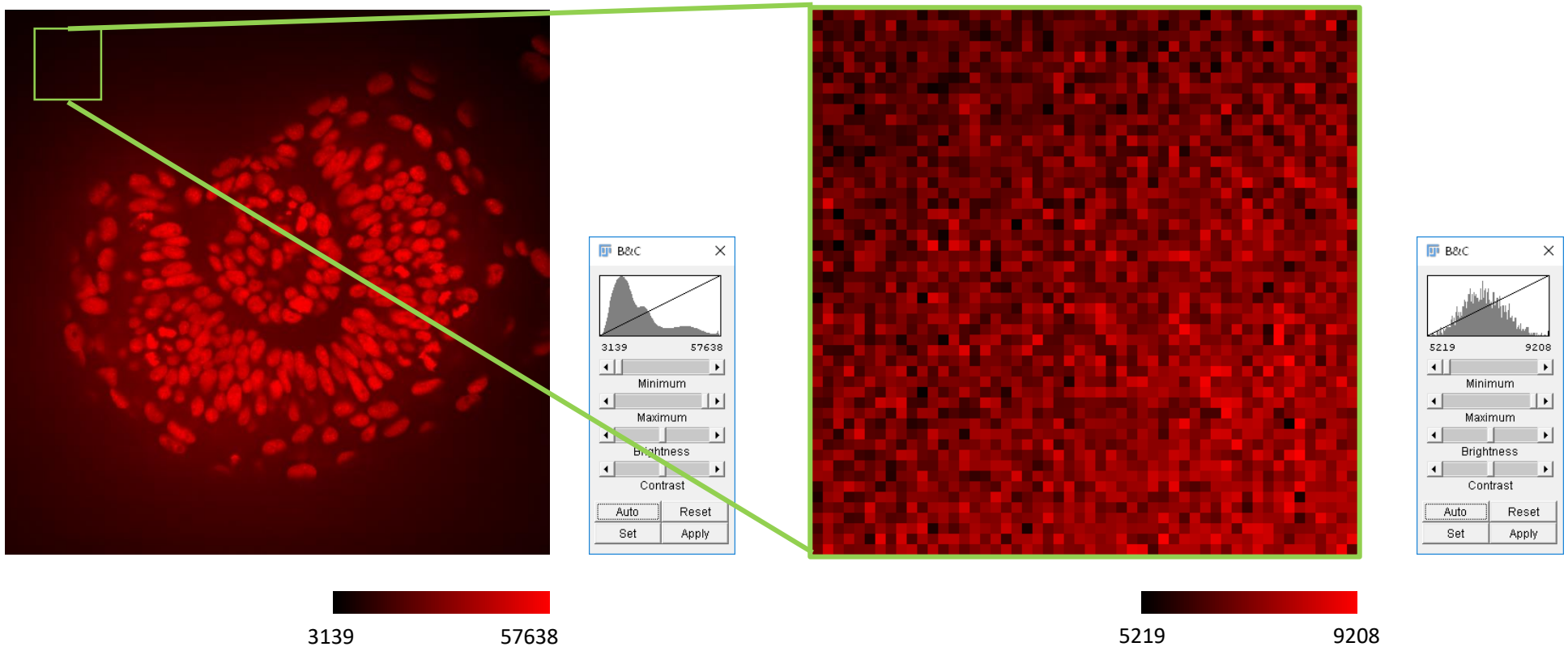


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Image correction / noise removal

- We need to remove the noise to help the computer *interpreting* the image

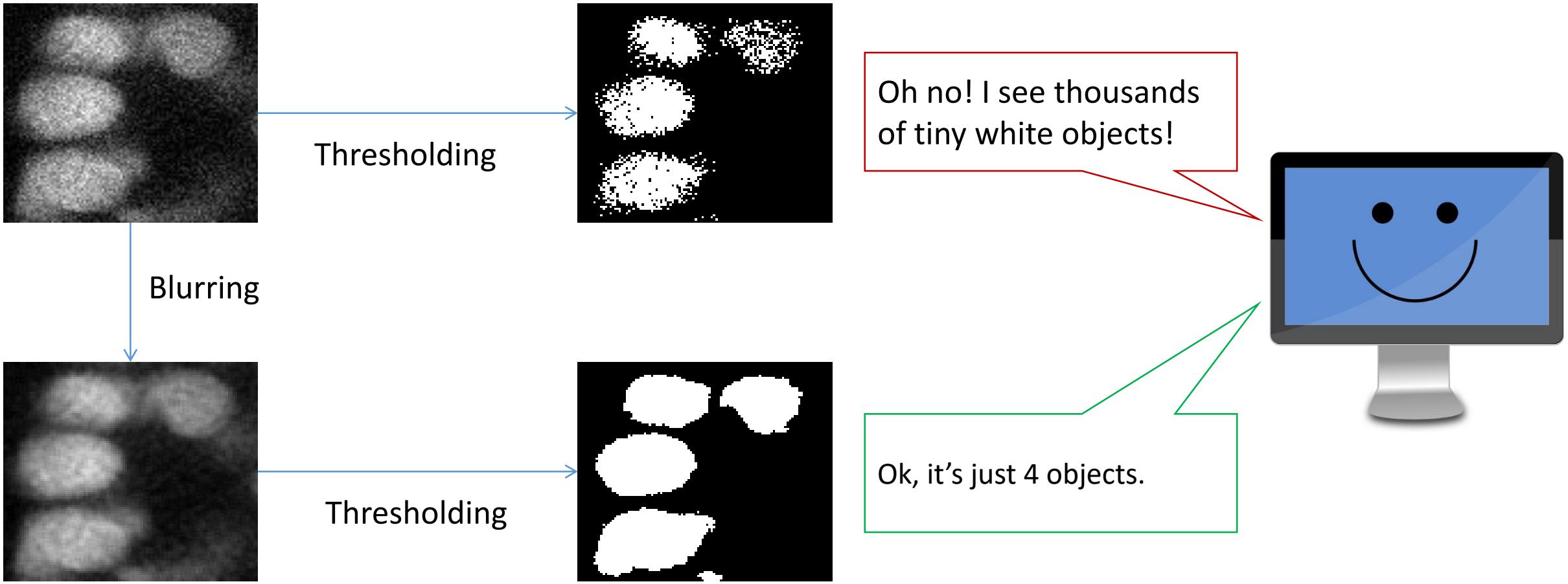
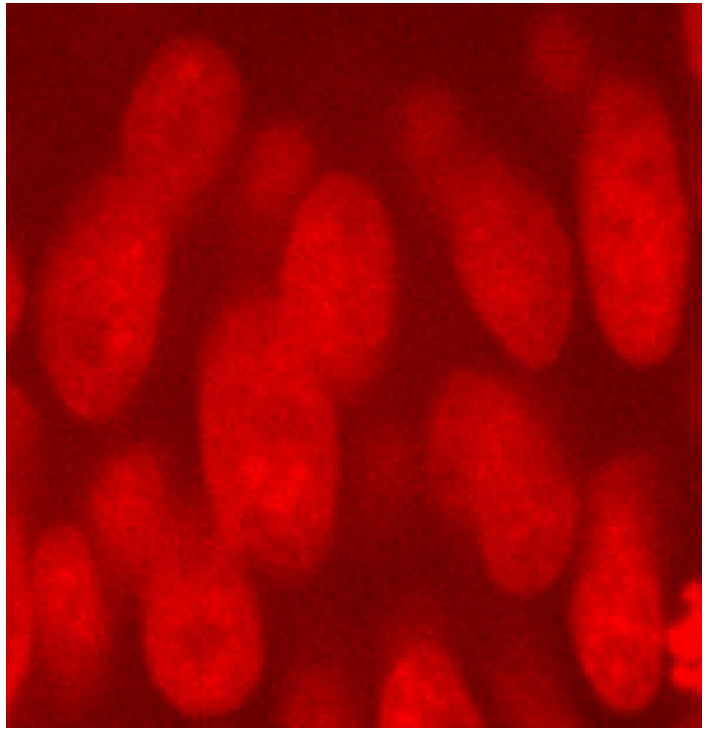
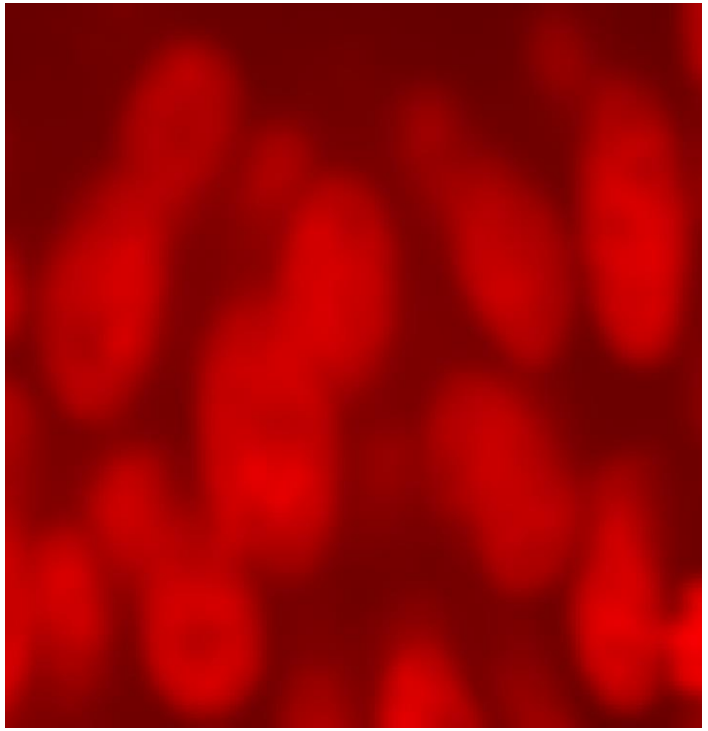


Image correction / noise removal

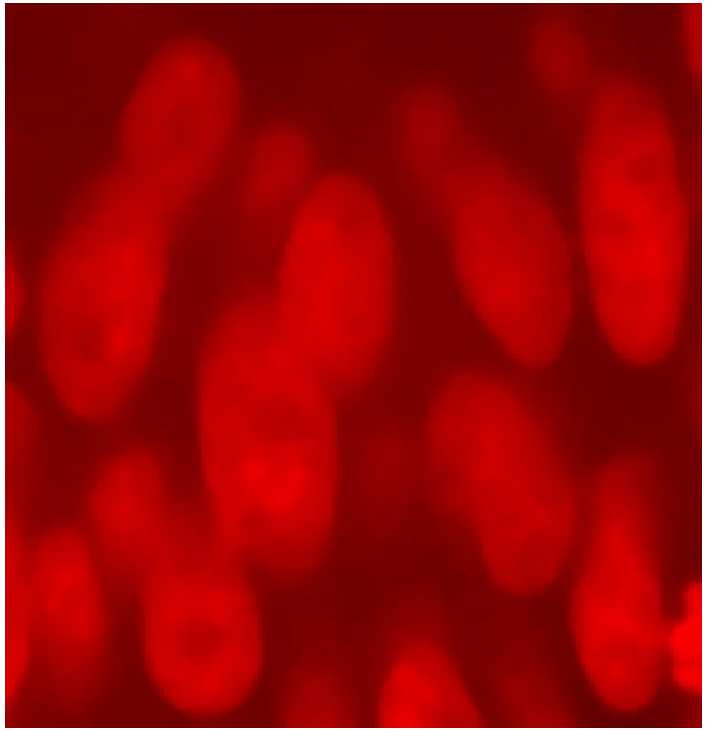
- Noise removal using *filters*



Original image



Gaussian blur filtered

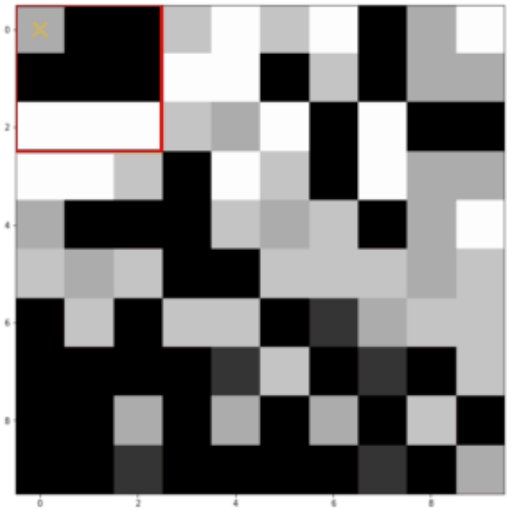


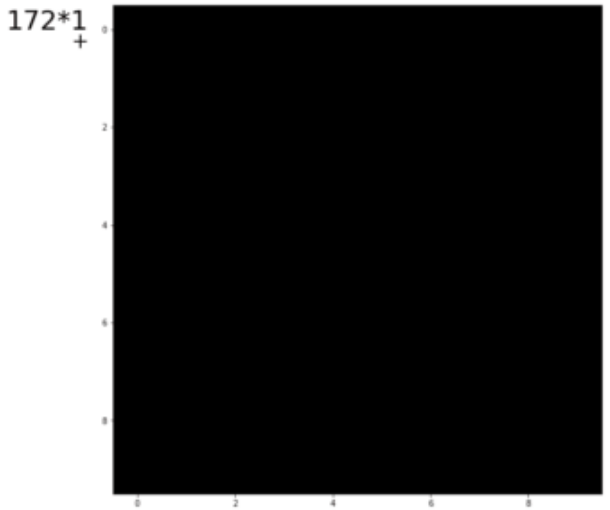
Median filtered

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

Linear Filters

- 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.





- Kernels are matrices describing a linear filter

Mean filter, 3x3 kernel

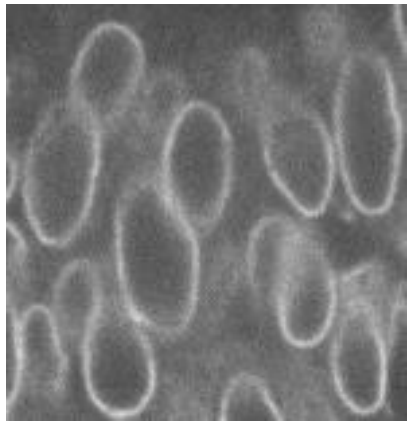
$$\begin{bmatrix} 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \end{bmatrix}$$

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

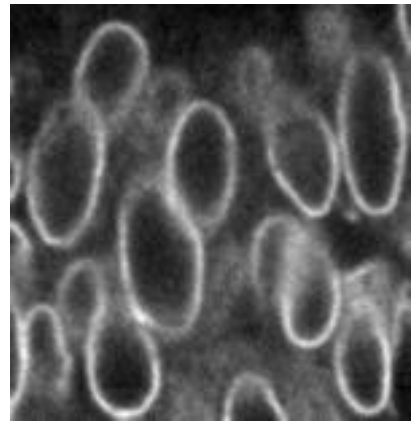
Linear filters

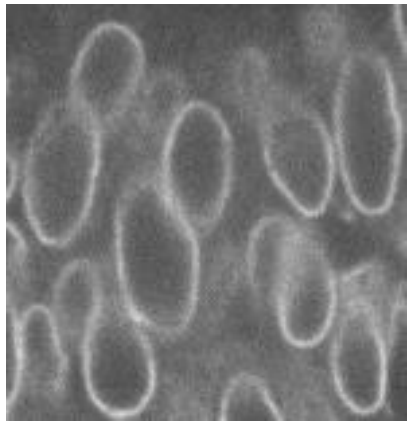
- Terminology:
  - “We convolve an image with a kernel.”
  - Convolution operator: *

- Examples
  - Mean
  - Gaussian blur
  - Sobel-operator
  - Laplace-filter

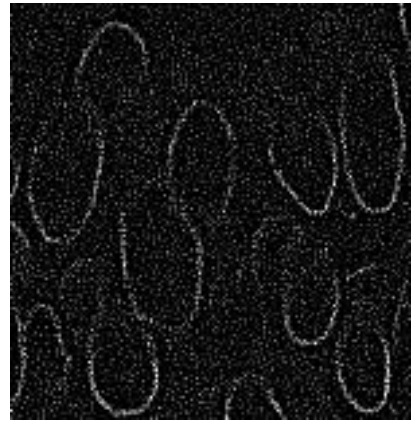


$$*\begin{bmatrix} 1 & 1 & 1 \\ 1 & 8 & 1 \\ 1 & 1 & 1 \end{bmatrix} =$$





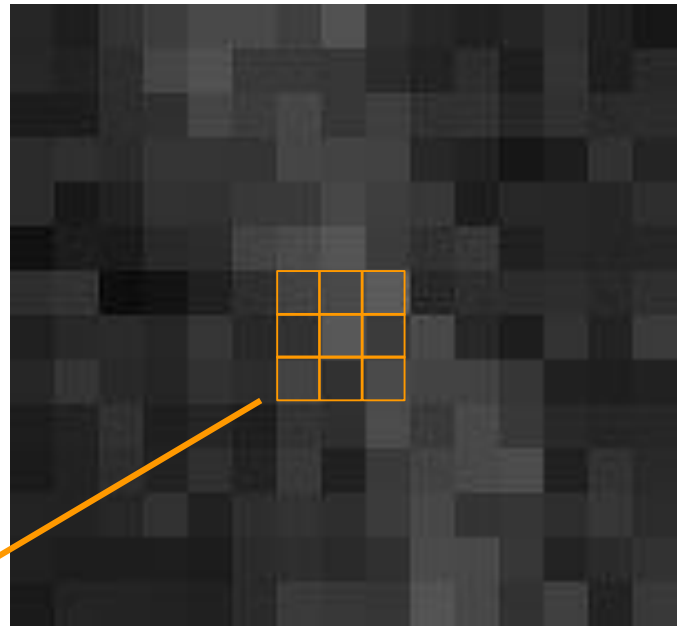
$$*\begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix} =$$

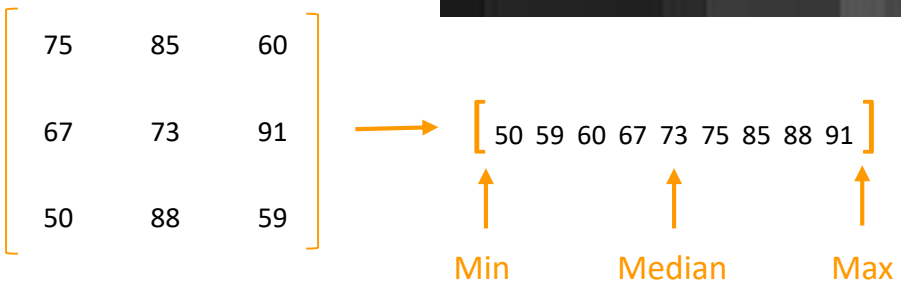


Nonlinear Filters

- Non linear filters also replace pixel value inside as rolling window but in a non linear function.

- Examples: order statistics filters
  - Min
  - Median
  - Max
  - Variance
  - Standard deviation





Filters

In python, for linear filters, we could apply a kernel to an image like this:

```
kernel = np.array(
    [[1/9, 1/9, 1/9],
     [1/9, 1/9, 1/9],
     [1/9, 1/9, 1/9]])
```

```
from scipy.ndimage import convolve

output = convolve(image, kernel)
```

But scikit-image already has several of these filters implemented and optimized.

```
from skimage.filters import gaussian

output = gaussian(image, sigma = 1)
```

Then there is no need to provide a kernel. And you can also directly apply non-linear filters.

```
from skimage.filters import median

output = median(image)
```

Available filters can be searched like this:

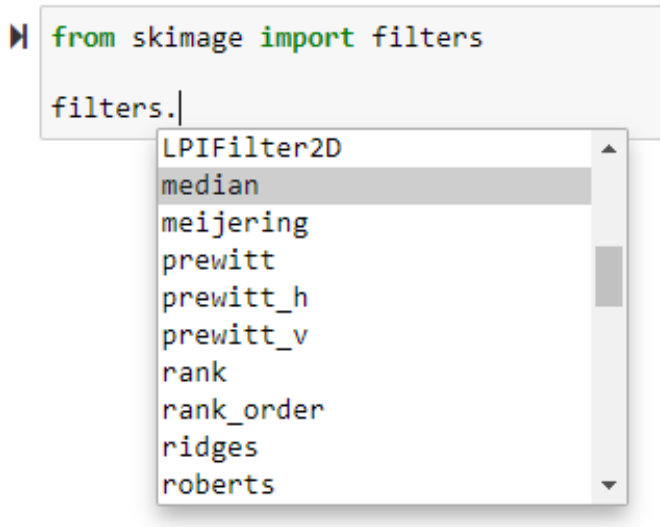


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Image Processing: Morphological Operations

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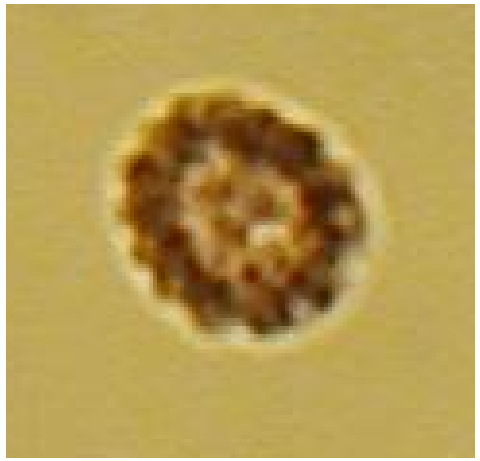
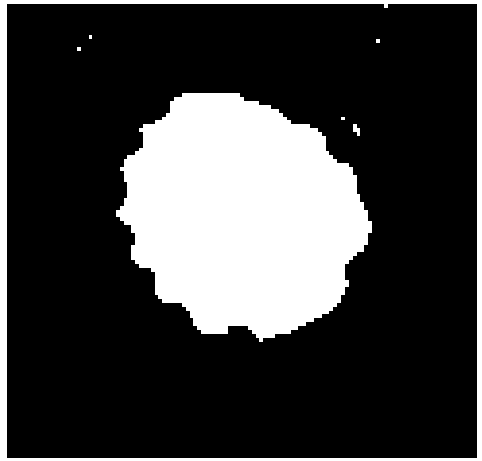
Refining masks

- Binary mask images may not be perfect immediately after thresholding.
- There are ways of refining them

Thresholding → Binary closing (Fill holes) → Binary opening






Background removal

- Differentiating objects is easier if their background intensity is equal.

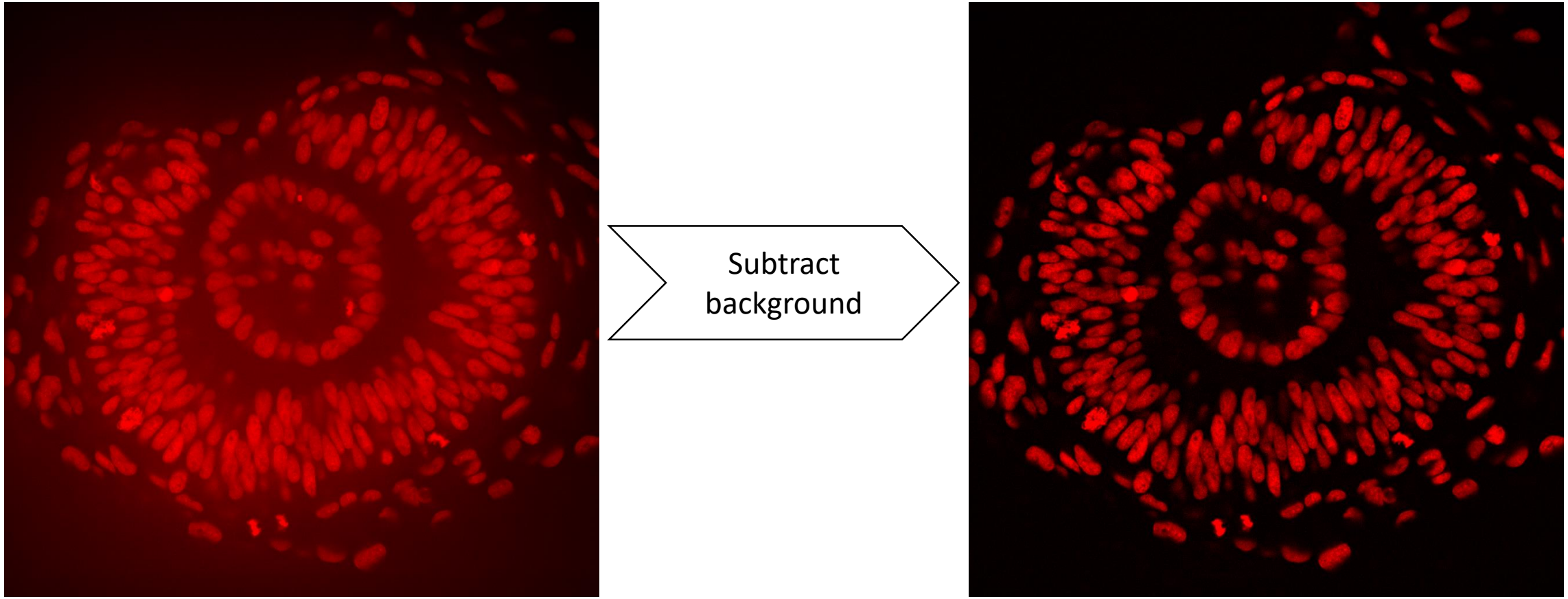
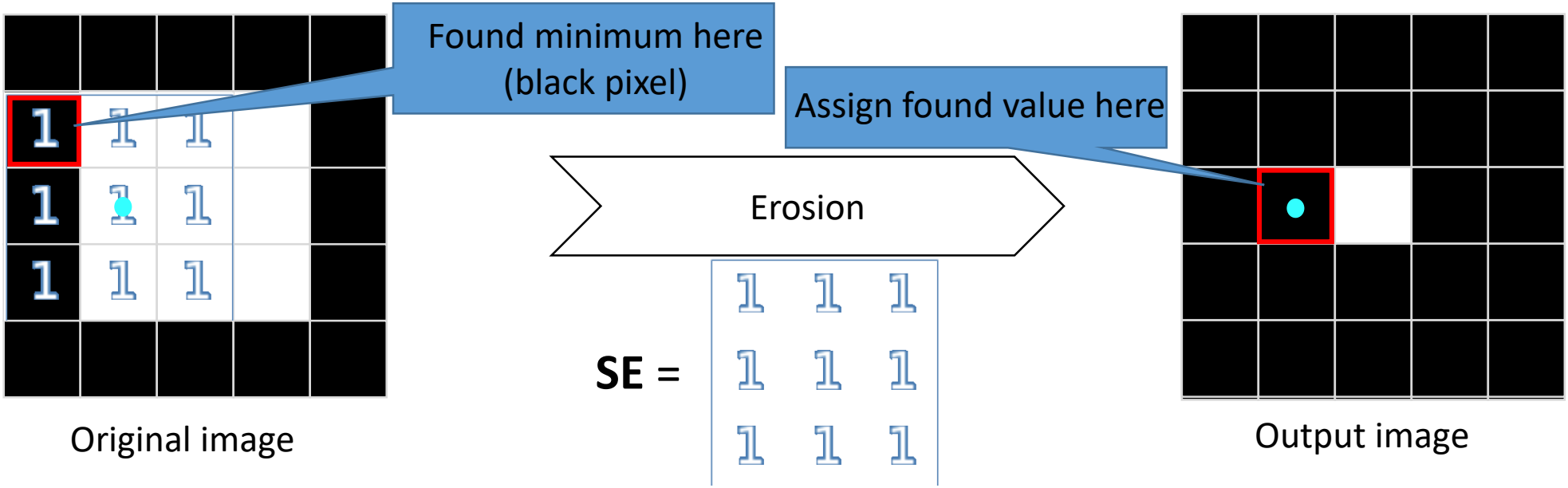


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Refining masks: Erosion

- Erosion: Every pixel with at least one black neighbor becomes black.



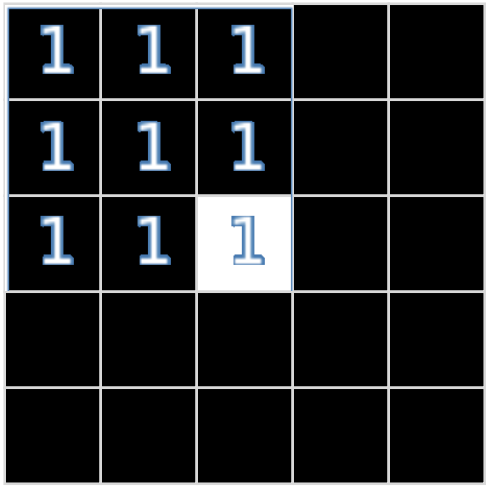
Erosion is essentially a minimum filter whose extent is defined by the kernel (structural element or **SE**) size and shape.

Dilation is essentially a maximum filter whose extent is defined by the kernel (structural element or **SE**) size and shape.

For an example with a grayscale image, check out this gif: https://en.wikipedia.org/wiki/Erosion_(morphology)#/media/File:Grayscale_Morphological_Erosion.gif

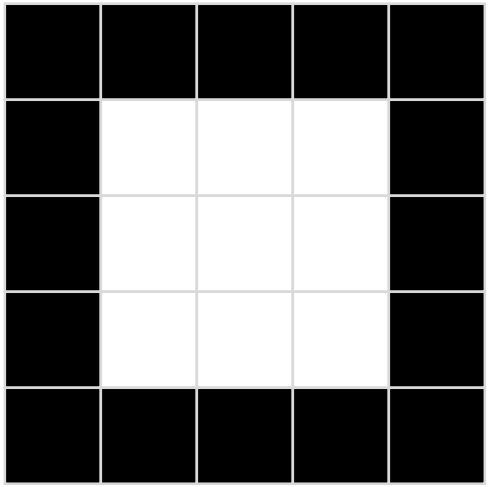
Refining masks: Dilation

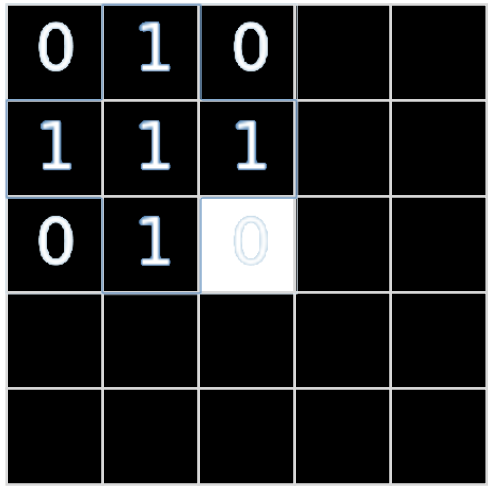
- Dilation: Every pixel with at least one white neighbor becomes white.



Dilation

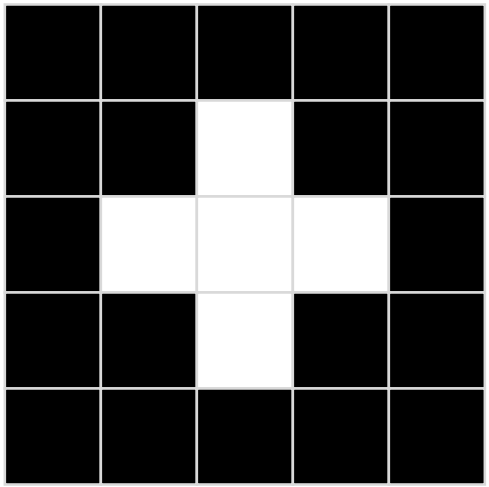
8-connected neighborhood
Moore-Neighborhood





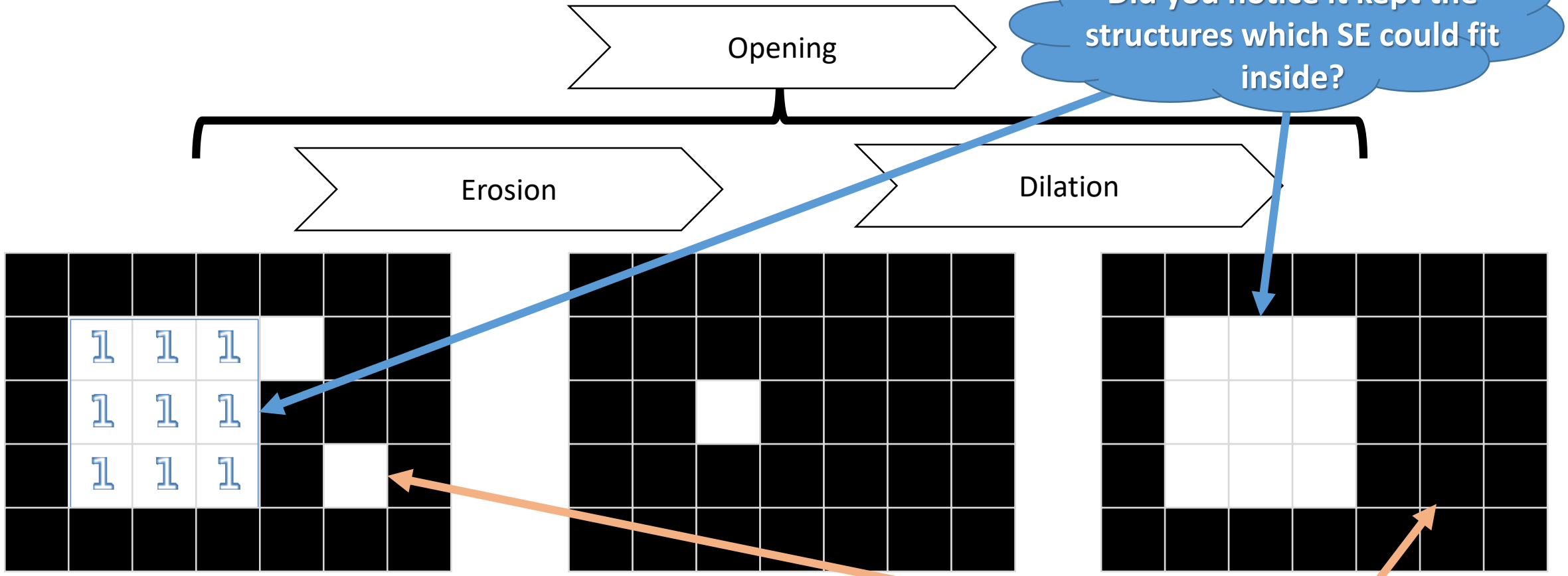
Dilation

4-connected neighborhood
von-Neumann-Neighborhood



Refining masks: Erosion & Dilation

- Erosion and dilation combined allow correcting outlines.

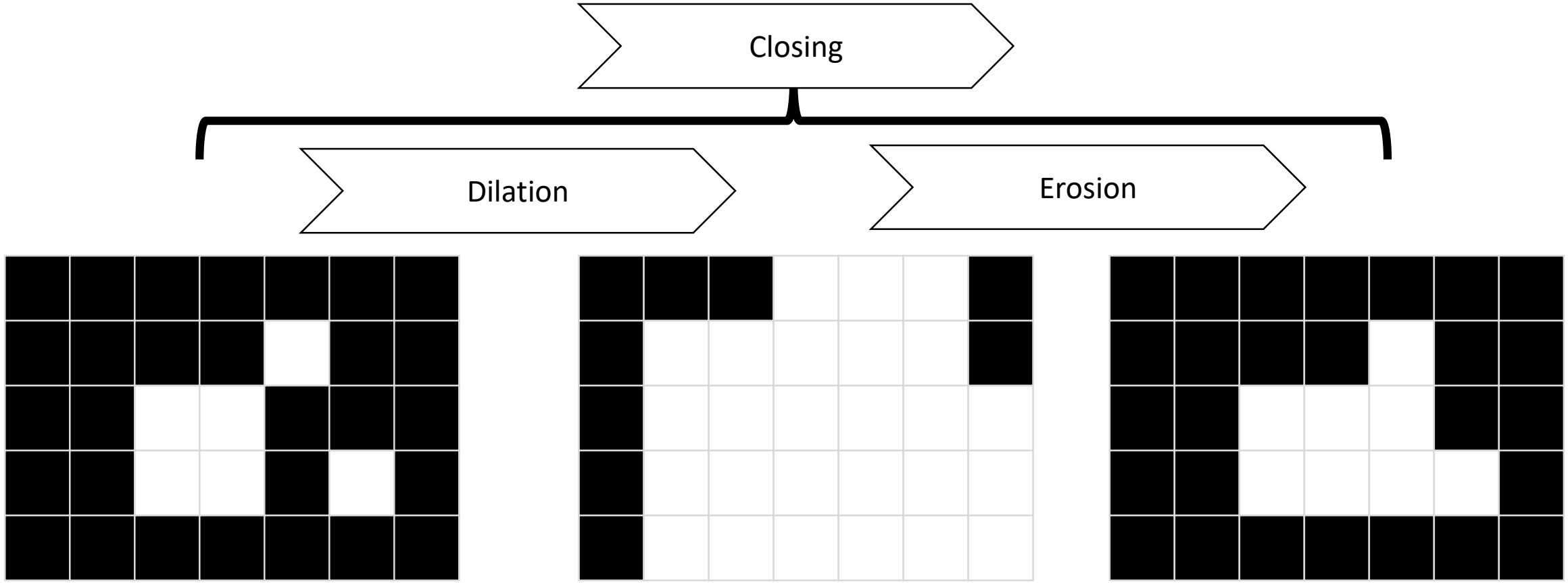


Did you notice it kept the structures which SE could fit inside?

- It can separate white (high intensity) structures that are weakly connected
- It may erase small white structures
- It tends to better preserve area of structures

And deletes structures smaller than SE?

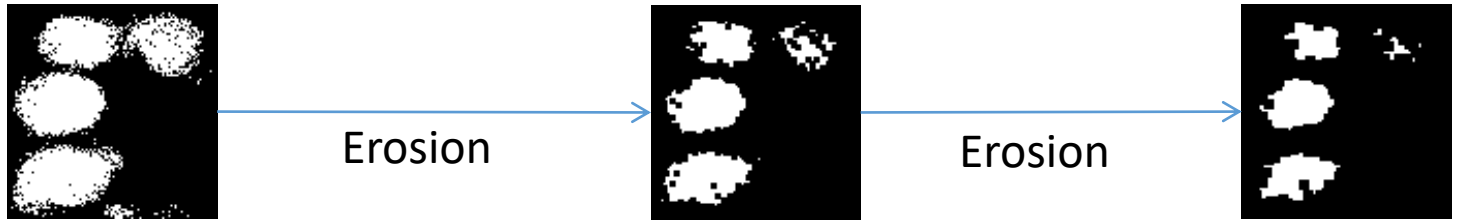
Refining masks: Erosion & Dilation



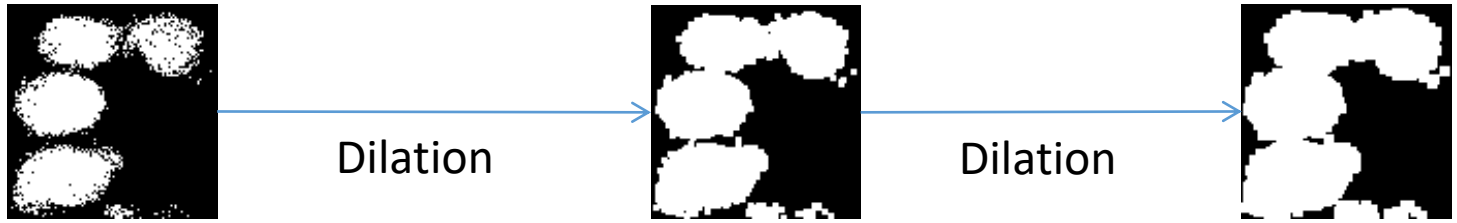
- It can connect white (high intensity) structures that are nearby
- It may close small holes inside structures
- It tends to better preserve area of structures

Refining masks: Erosion / dilation

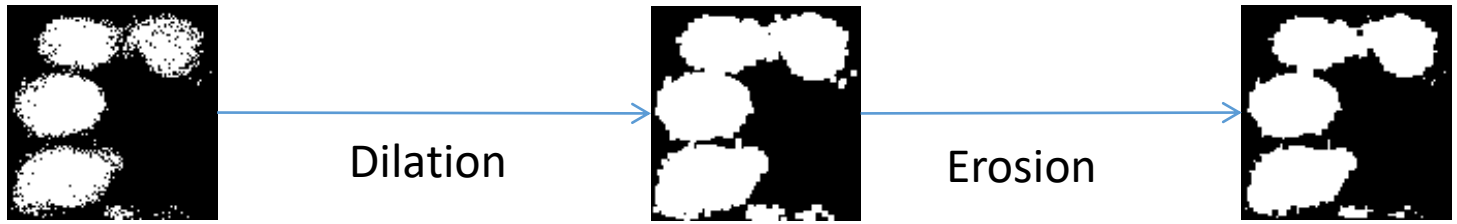
- Erosion: Set all pixels to black which have at least one black neighbor.



- Dilation: Set all pixels to white which have at least one white neighbor.



- Closing: Dilation + Erosion



- Opening: Erosion + Dilation

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Morphological Operations in Python

Scikit-image has a sub-package called morphology

```
from skimage import morphology
```

You must define a SE first (also called footprint):

```
SE = morphology.square(3)
SE
```

```
array([[1, 1, 1],
       [1, 1, 1],
       [1, 1, 1]], dtype=uint8)
```

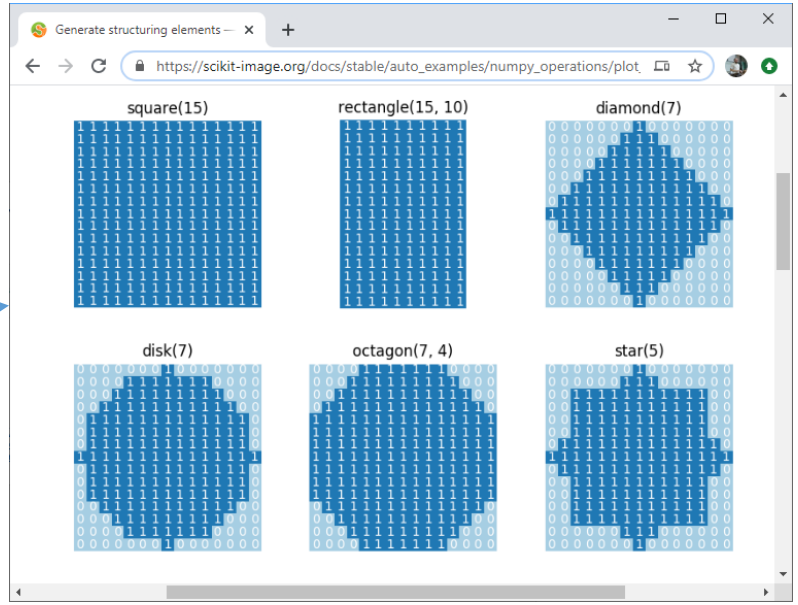
For a binary image, you apply it like this:

```
output = morphology.binary_dilation(binary_image, SE)
```

For a grayscale image, you apply it like this:

```
output = morphology.dilation(image, SE)
```

It can have other shapes/sizes:



https://scikit-image.org/docs/stable/auto_examples/numpy_operations/plot_structuring_elements.html#sphx-glr-auto-examples-numpy-operations-plot-structuring-elements-py

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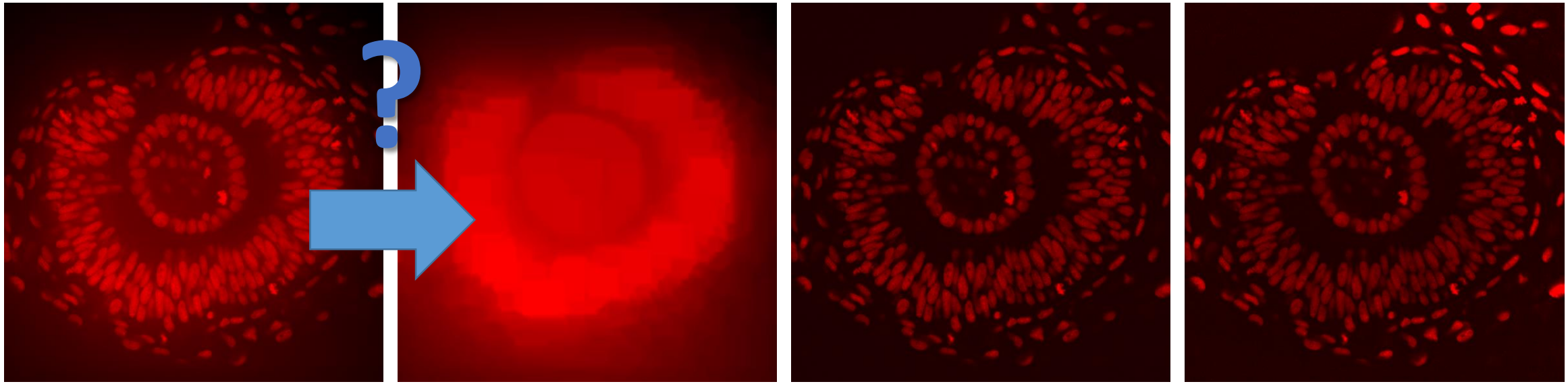
Background removal

- Depending on the effect we want to correct for, it might make sense to divide an image by its background.

Do you remember that opening kept the structures which the SE could fit inside?

And do you remember that opening deletes structures smaller than SE?

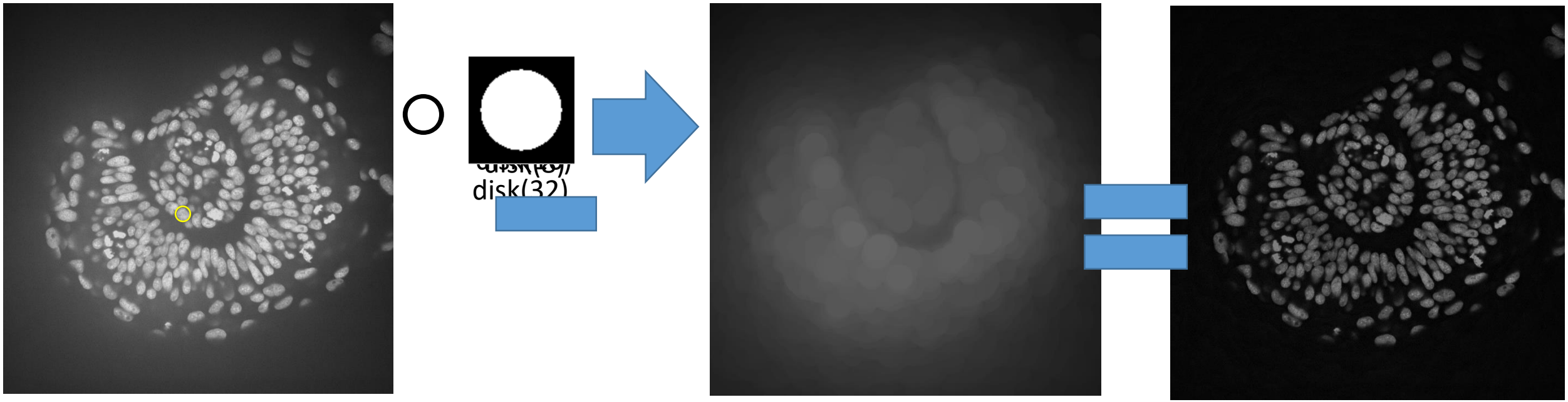


Original image | Extracted background | Subtracted background | Image divided by background

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

Background removal

Opening



Original image

This is a good estimation of the background

Strucutres have a radius ≈ 12

You have just learned the white tophat filter!

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

Plotting with matplotlib: a quick peek

PoL Physics of Life TU Dresden

- Use matplotlib to put images side-by-side

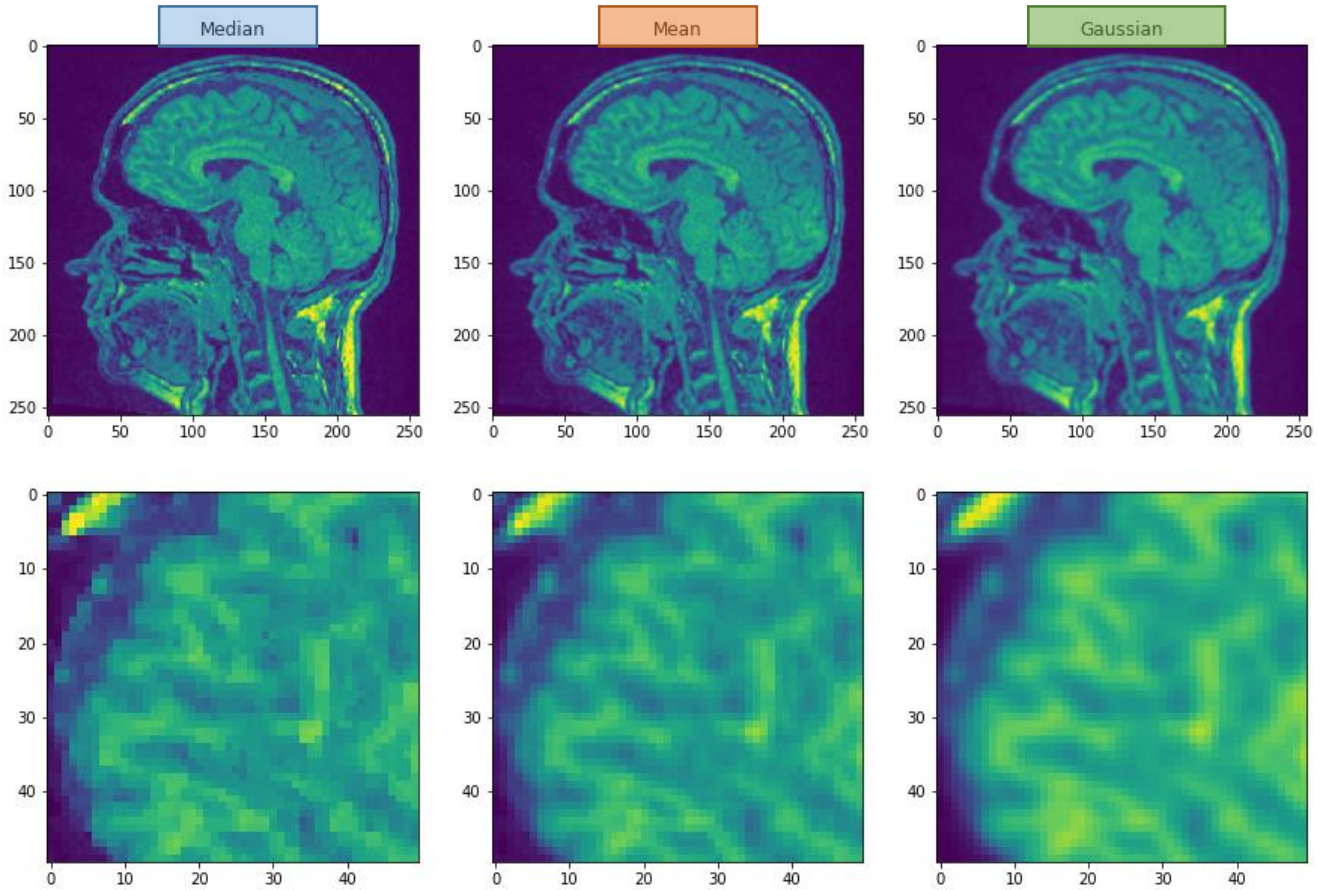
```
median_filtered = filters.median(noisy_mri, disk(1))
mean_filtered = filters.rank.mean(noisy_mri, disk(1))
gaussian_filtered = filters.gaussian(noisy_mri, sigma=1)

fig, axs = plt.subplots(2, 3, figsize=(15,10))

# first row
axs[0, 0].imshow(median_filtered)
axs[0, 0].set_title("Median")
axs[0, 1].imshow(mean_filtered)
axs[0, 1].set_title("Mean")
axs[0, 2].imshow(gaussian_filtered)
axs[0, 2].set_title("Gaussian")

# second row
axs[1, 0].imshow(median_filtered[50:100, 50:100])
axs[1, 1].imshow(mean_filtered[50:100, 50:100])
axs[1, 2].imshow(gaussian_filtered[50:100, 50:100])
```

rows

columns

row

column



https://matplotlib.org/stable/api/_as_gen/matplotlib.pyplot.subplots.html

Exercises

1. Plot an image histogram

2. Make a binary image

3. Apply custom filters to images

4. Apply morphological operations to images