ScaDS.AI
DRESDEN LEIPZIG
CENTER FOR SCALABLE DATA ANALYTICS
AND ARTIFICIAL INTELLIGENCE

Microscopy Image Processing

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Reusing materials from Mauricio Rocha Martins (Norden lab, MPI CBG); Dominic Waithe (Oxford University); Alex Bird, Dan White (MPI CBG), Marcelo Leomil Zoccoler, TU Dresden

GEFÖRDERT VOM
Bundesministerium
für Bildung
und Forschung

SACHSEN

Diese Maßnahme wird gefördert durch die Bundesregierung aufgrund eines Beschlusses des Deutschen Bundestages.
Diese Maßnahme wird mitfinanziert durch Steuermittel auf der Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.

Recap quiz

- We write good documentation to enabling others to do an experiment. This is good for …

Repeatability

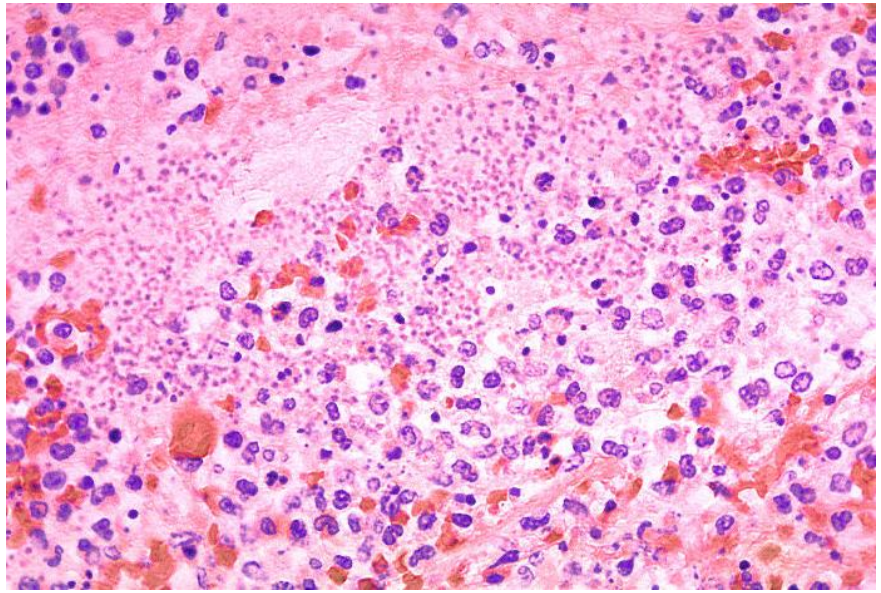
Reproducibility

Replicability

Reliability

Recap quiz

- This image is from a …



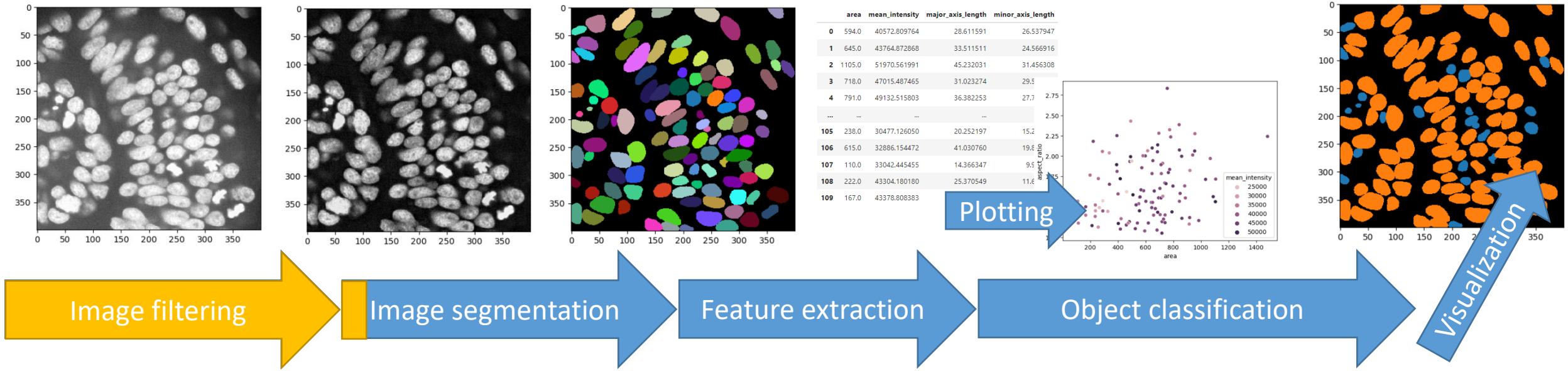
Transmitted light microscope

Fluorescence microscope

Electron microscope

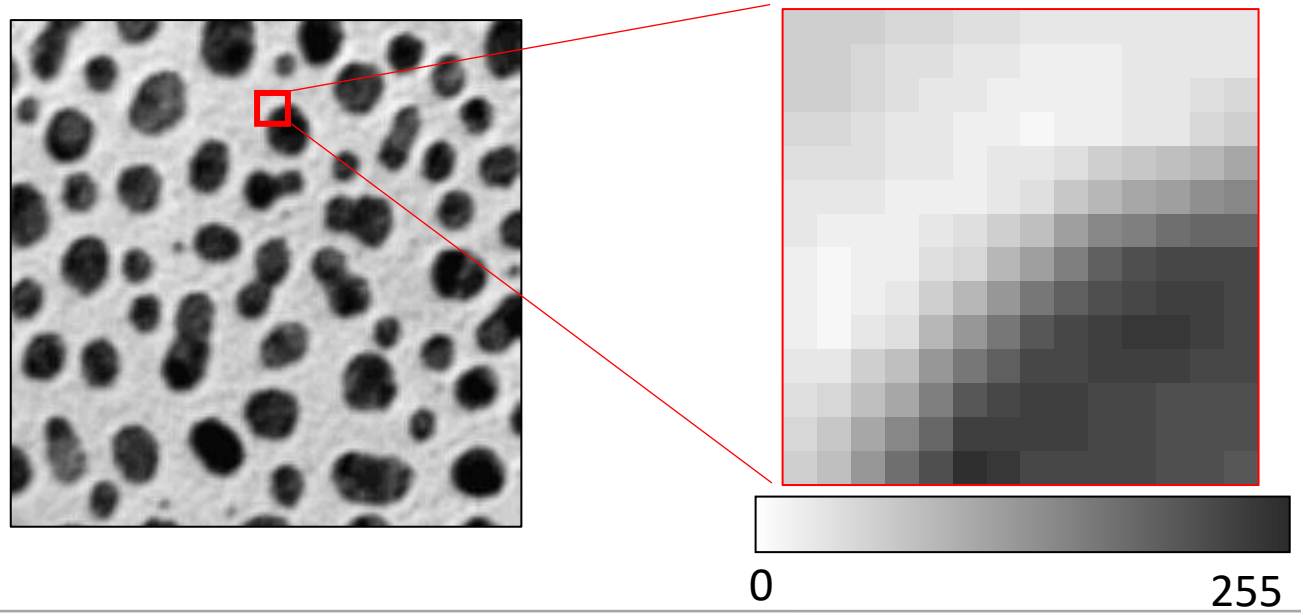
Lecture overview: Bio-image Analysis

- Image Data Analysis workflows
- Goal: **Quantify observations, substantiate conclusions with numbers**



Images and pixels

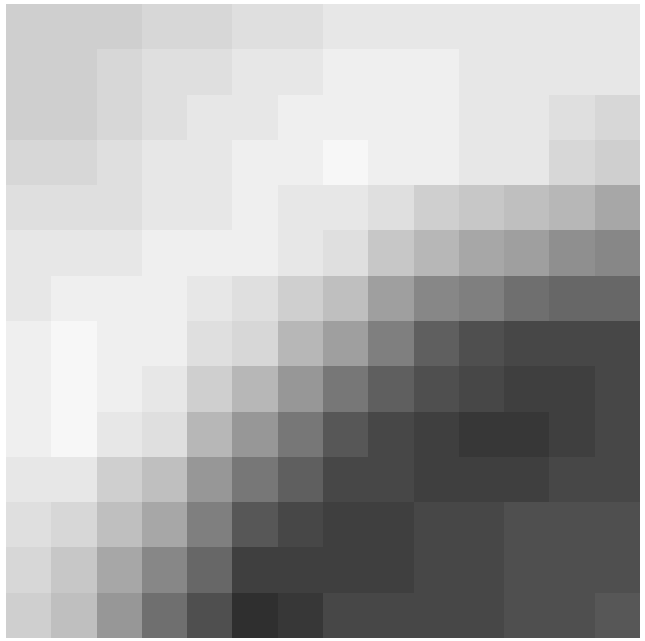
- An image is just a matrix of numbers: pixels: “picture element”
- The edges between pixels are an artefact of the imaging / digitization. They are not real!

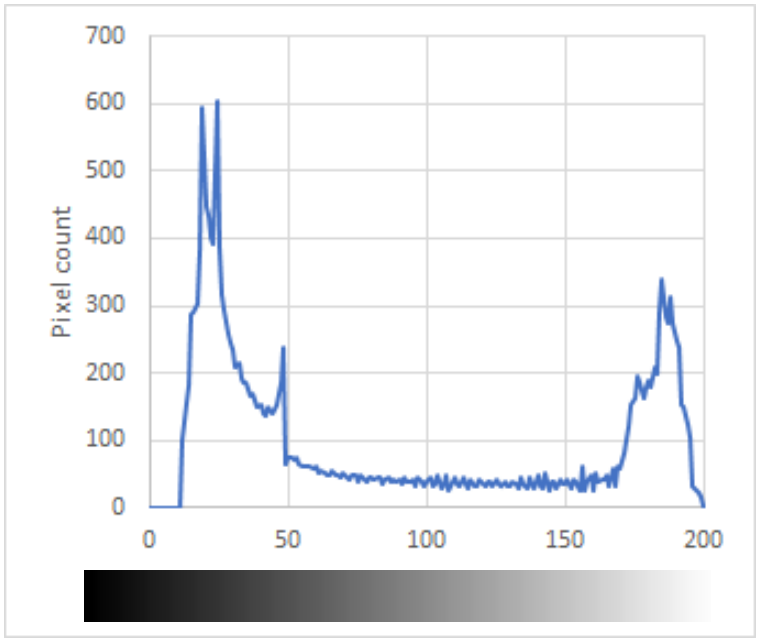


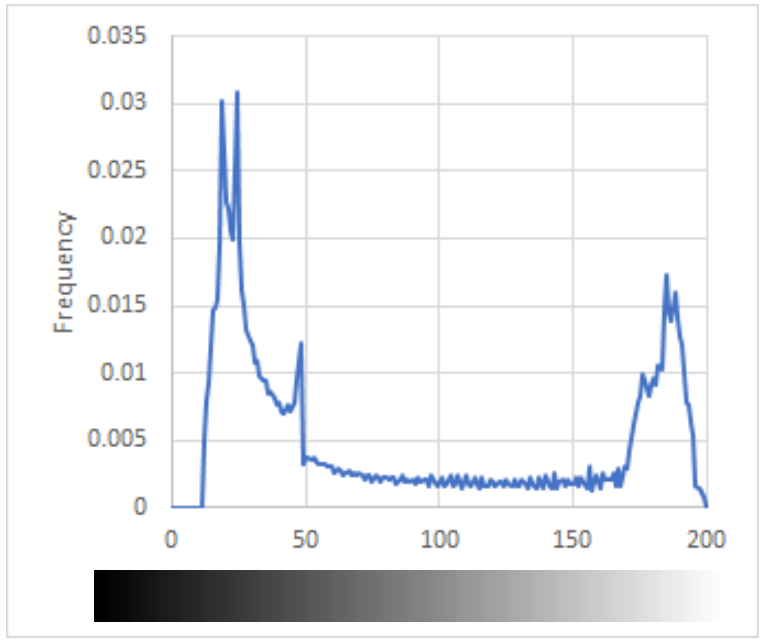
48	48	48	40	40	32	32	24	24	24	24	24	24	24
48	48	40	32	32	24	24	16	16	16	24	24	24	24
48	48	40	32	24	24	16	16	16	16	24	24	32	40
40	40	32	24	24	16	16	8	16	16	24	24	40	48
32	32	32	24	24	16	24	24	32	48	56	64	72	88
24	24	24	16	16	16	24	32	56	72	88	96	112	120
24	16	16	16	24	32	48	64	96	120	128	144	152	152
16	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

Histograms

- A histogram shows the probability distribution of pixel intensities.
- The probability of a pixel having a certain grey value can be measured by counting pixels and calculating the frequency of the given intensity.
- Whenever you see a histogram, try to imagine the lookup-table on the X-axis

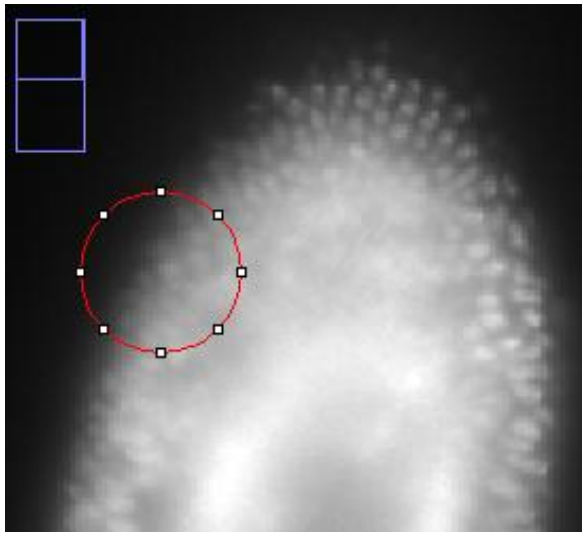


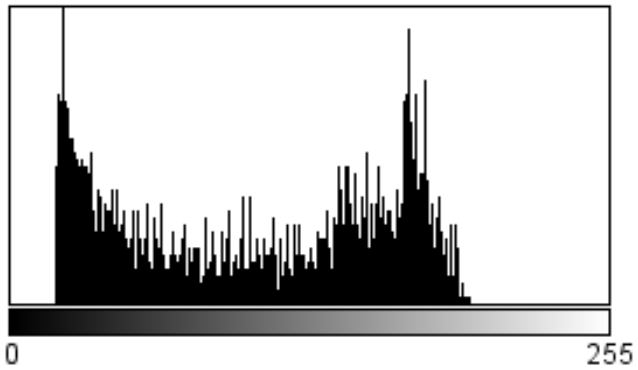




Histograms

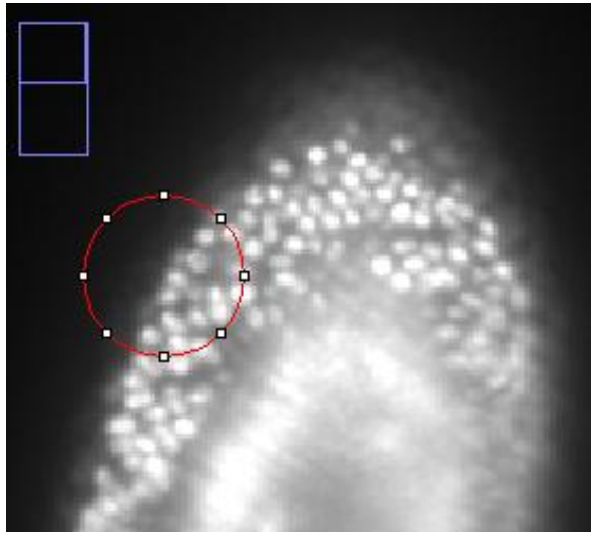
- Histograms are summaries of images
- Tell stories, e.g. about image quality

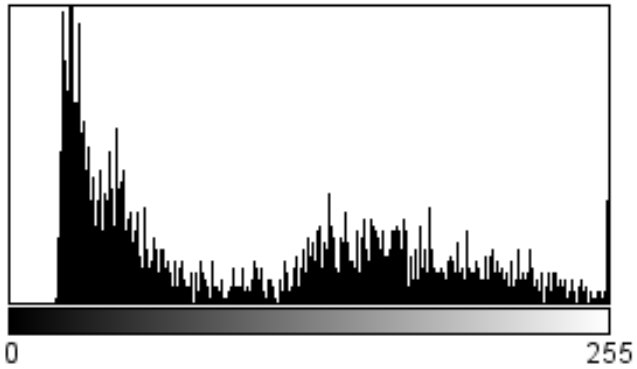




Count: 2053
Mean: 103.818
StdDev: 57.093

Min: 19
Max: 196
Mode: 22 (41)



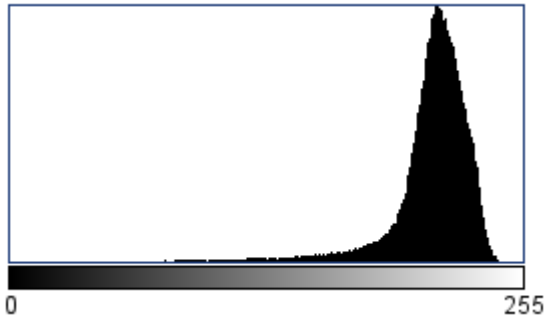


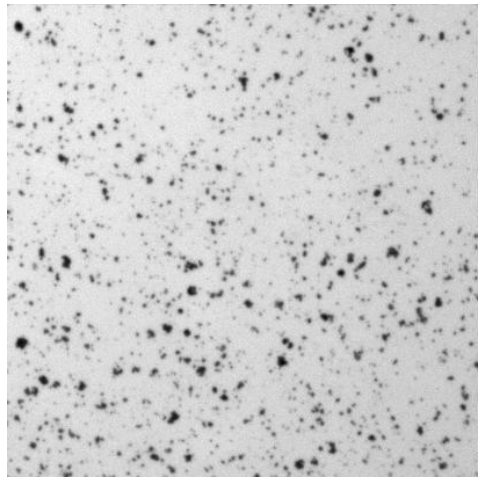
Count: 2053
Mean: 103.370
StdDev: 70.260

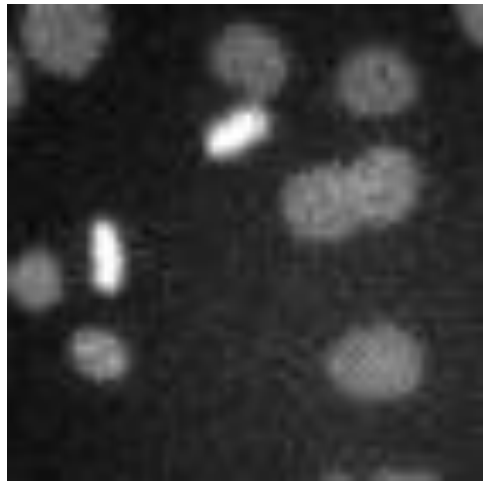
Min: 19
Max: 255
Mode: 25 (49)

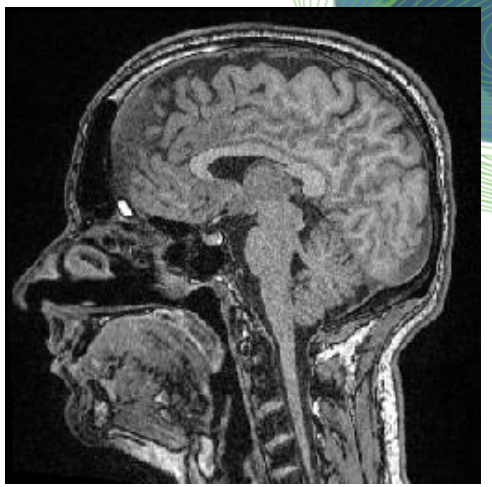
Histograms

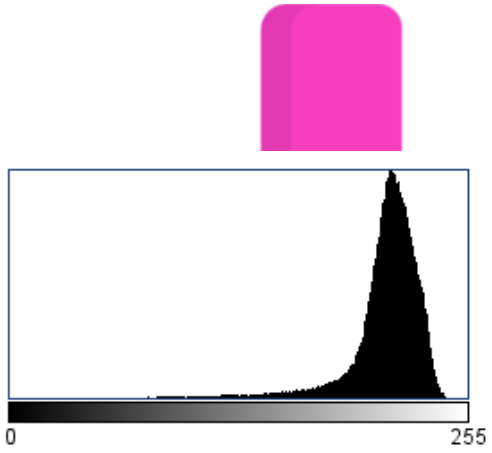
- To which of the three images does this histogram belong to?

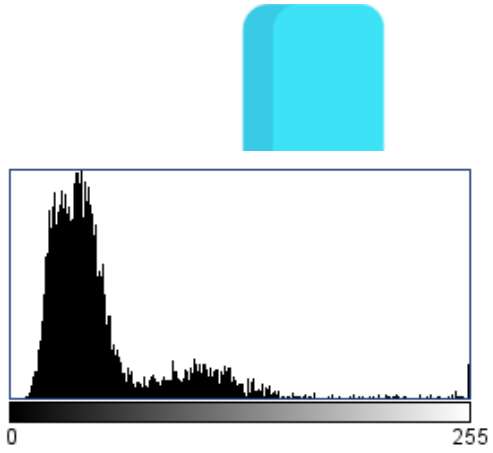












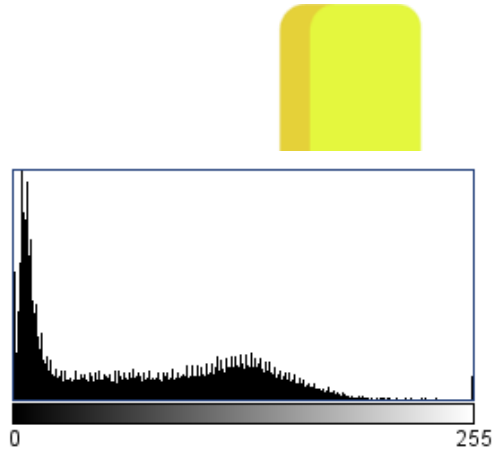
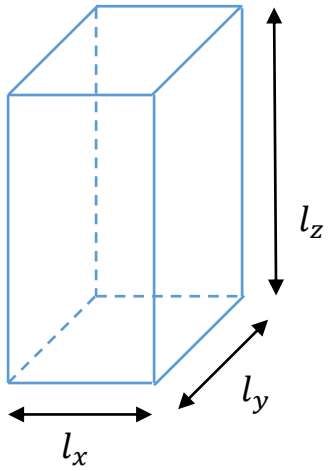
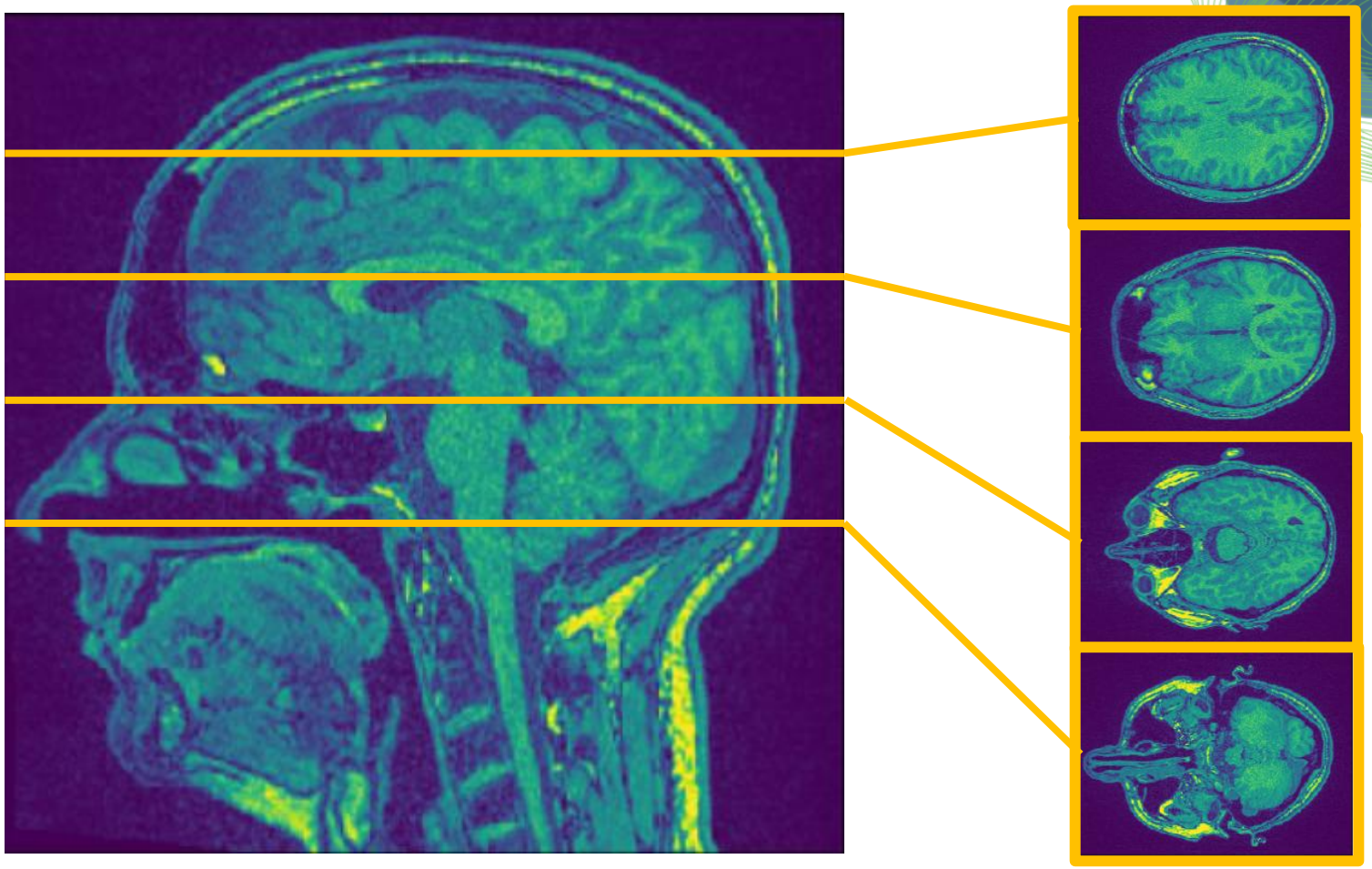


Image stacks and voxels

- 3-dimensional images consisting of voxels
- “Image stack”
- Often *anisotropic* (not equally large in all directions)

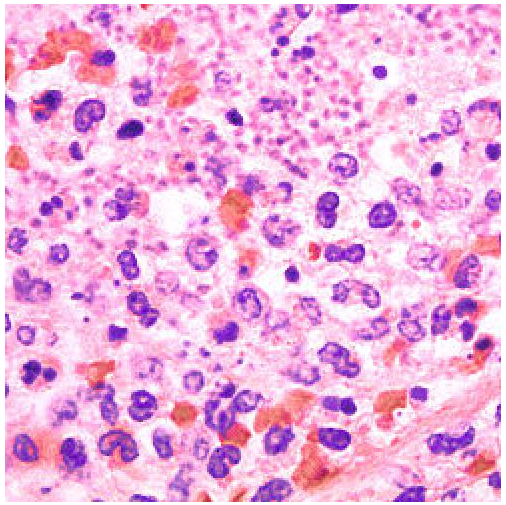




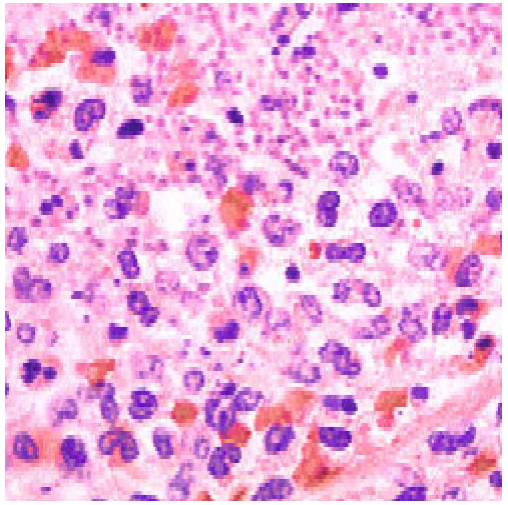
$l_x = l_y \neq l_z$

Anisotropy

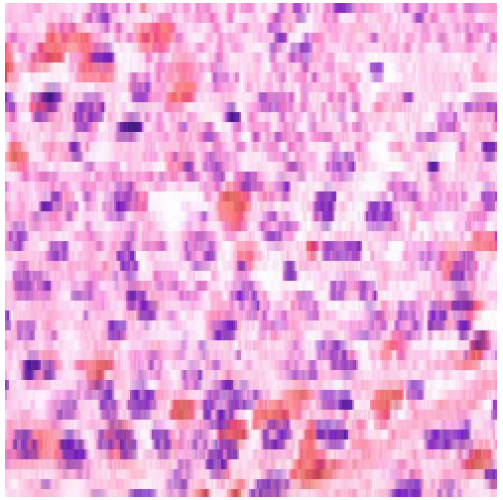
- Voxel size has immediate impact on image quality and thus, on processing / analysis results.



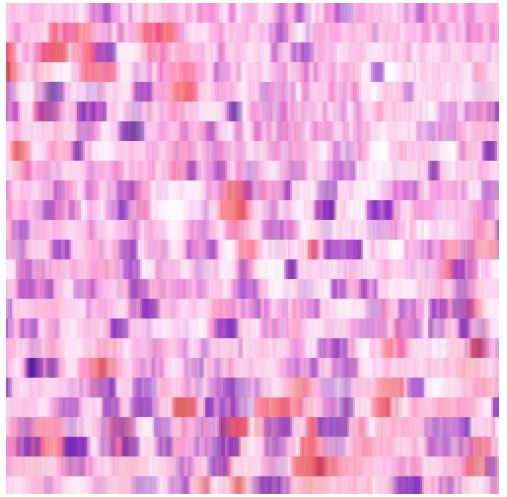
1:1
250 x 250



1:2
250 x 125



1:5
250 x 50

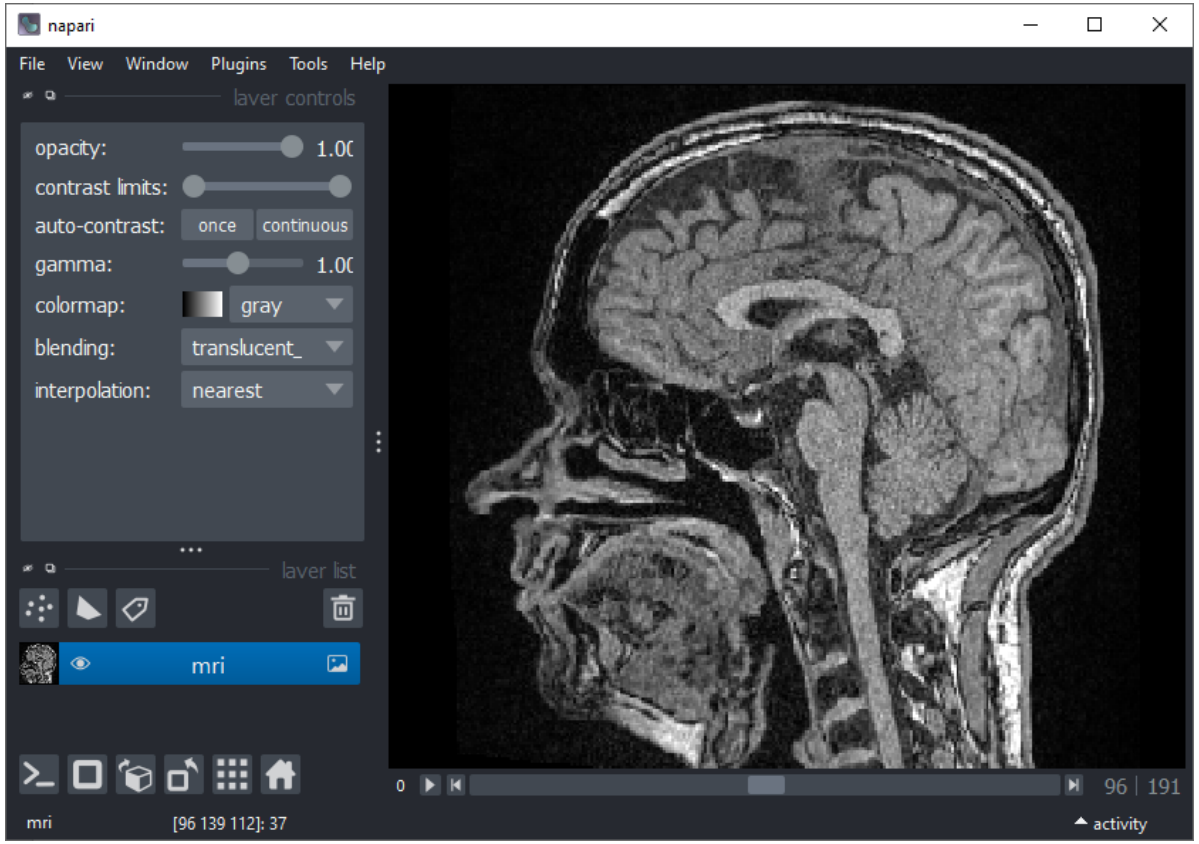


1:10
250 x 25

Image source: cropped from
https://de.m.wikipedia.org/wiki/Datei:Histo_Lungenpest.jpg

3D Image visualization

Interactive tools available



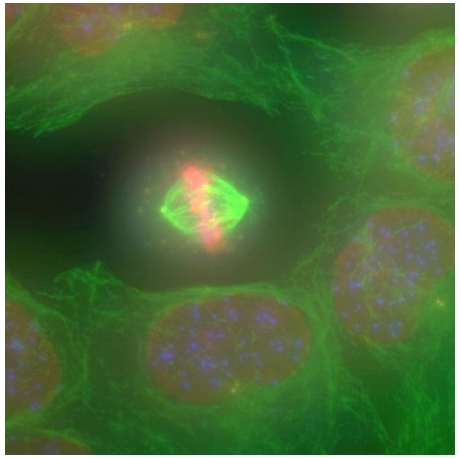

```
import stackview

stackview.slice(mri_image)
```

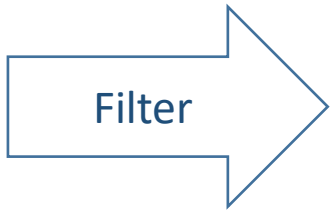
Filters

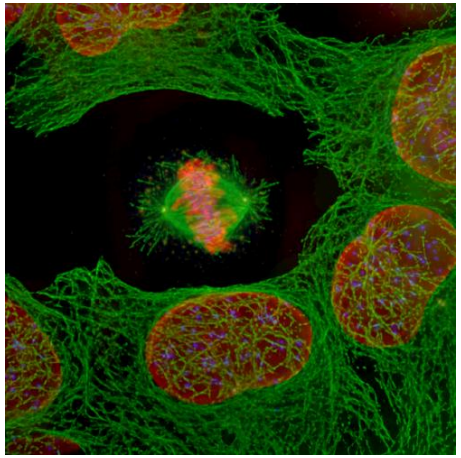
- An image processing filter is an operation on an image.
- It takes an image and produces a new image out of it.

- 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





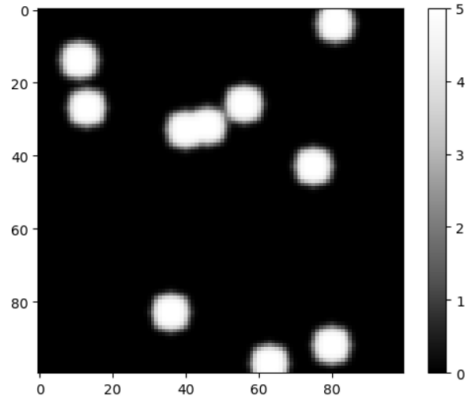


Quiz

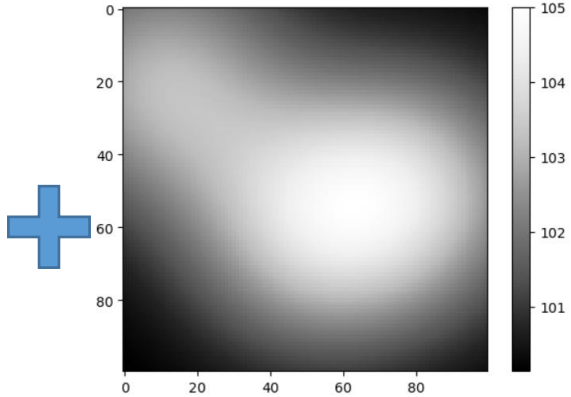
- What is noise?

Effects harming image quality

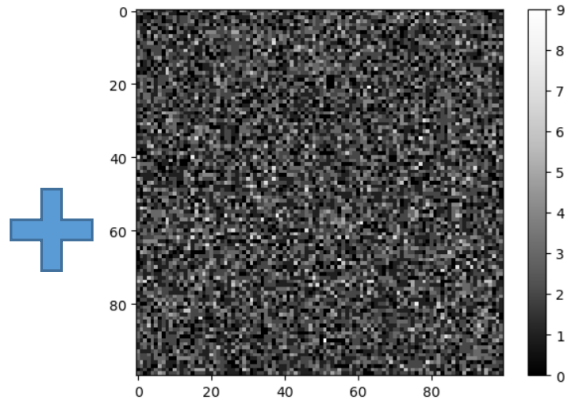
“nuclei”



“background”



“noise”



- Aberrations, defocus
- Motion blur

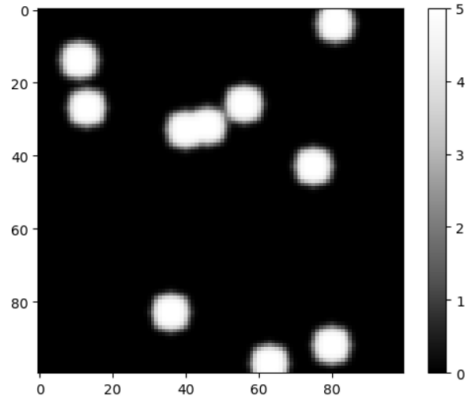
- Light from objects behind and in front of the scene (out-of-focus light)
- Dirt on the object slide
- Camera offset

- Shot noise (arriving photons in the camera from the scene)
- Dark noise (electrons made from photons)
- Read-out-noise (electronics)

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/49a787514a367829c3e0e1832f6cc533e96d549f/03_image_processing/simulated_dataset.ipynb

Effects harming image quality

“nuclei”



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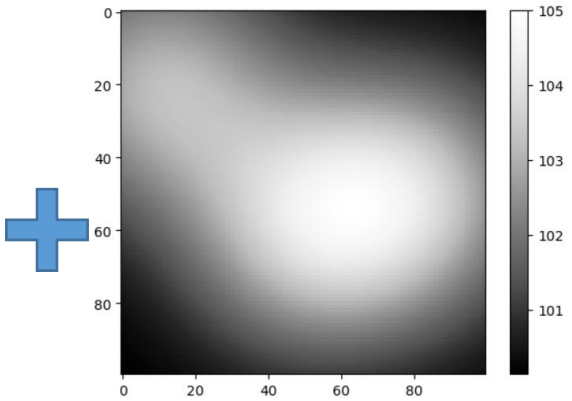
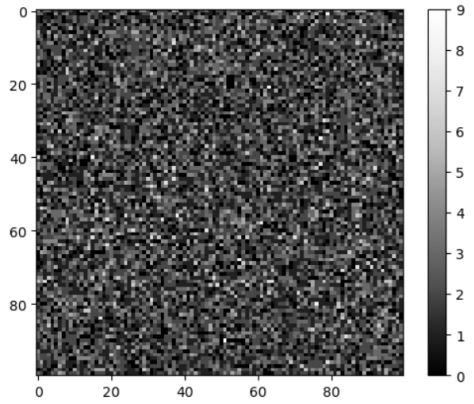
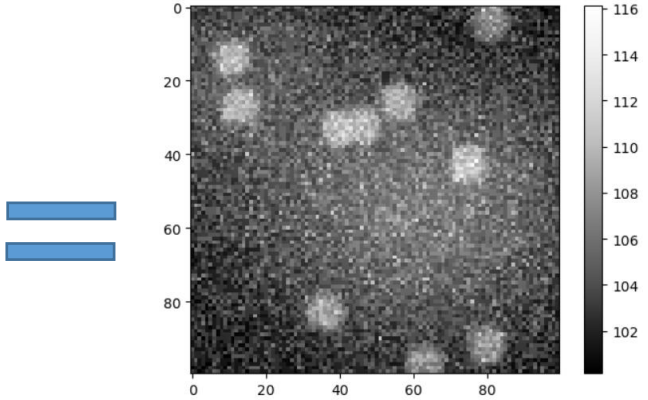
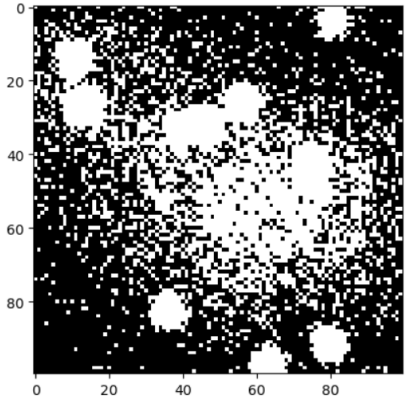


“noise”





Segmentation



https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/49a787514a367829c3e0e1832f6cc533e96d549f/03_image_processing/simulated_dataset.ipynb

Image filtering

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

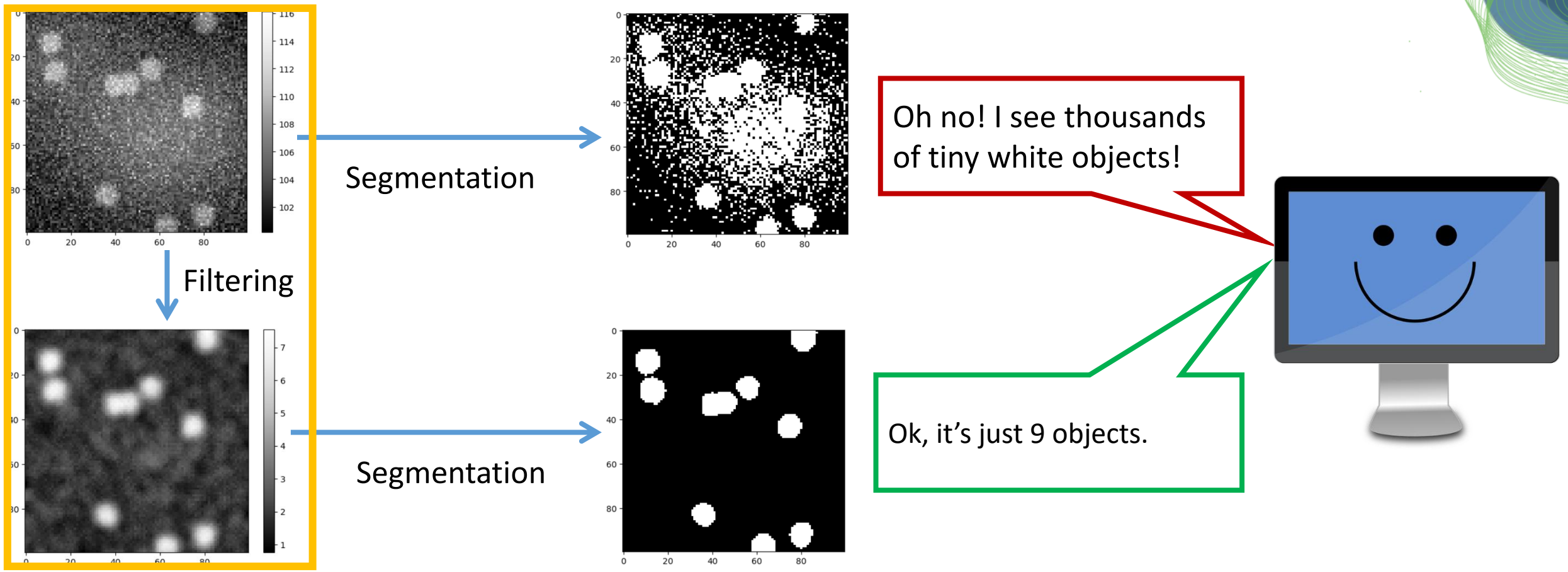
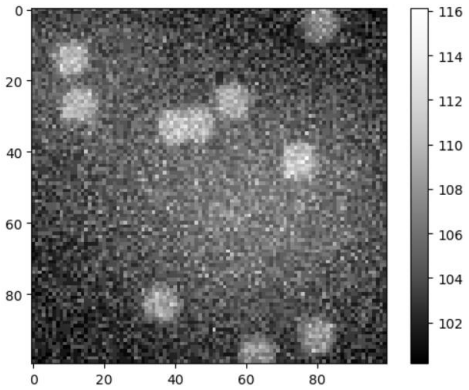


Image filtering

- Attempt to invert / “undo” processes disturbing image quality



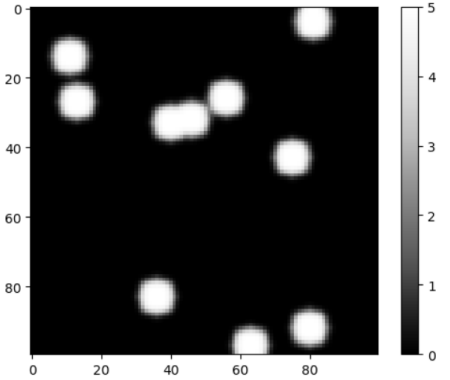
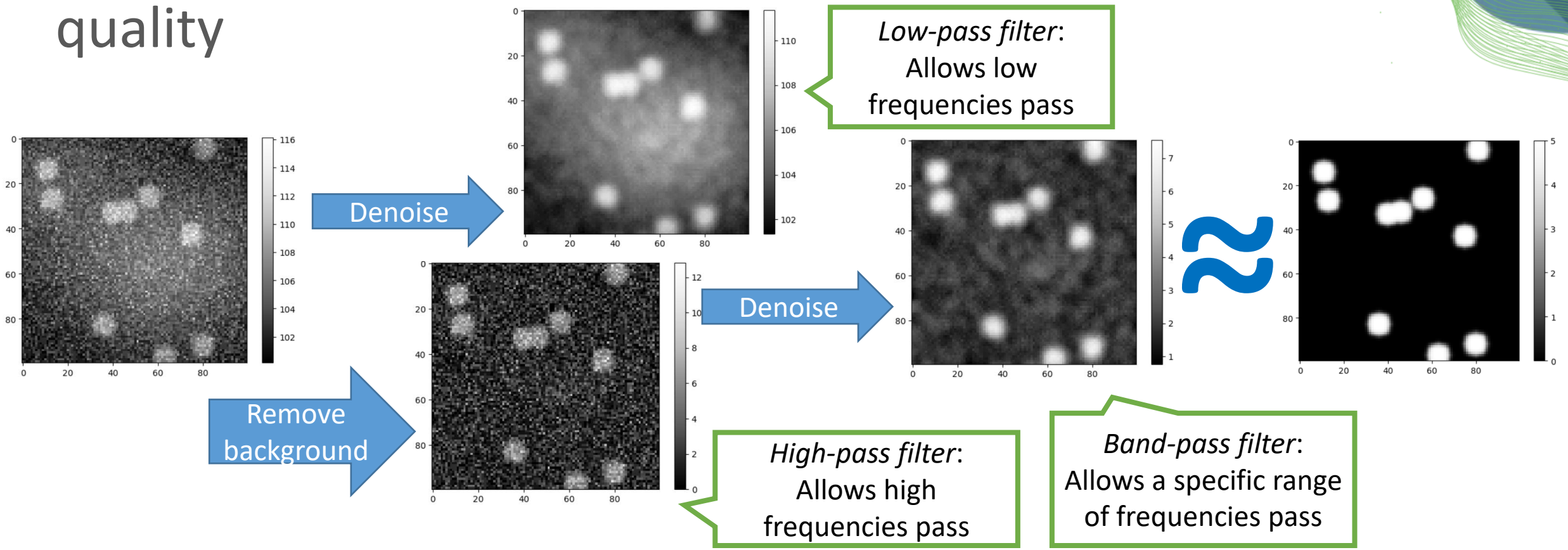


Image filtering

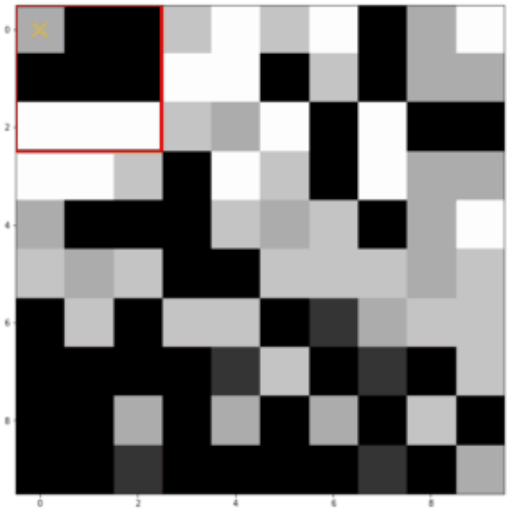
- Attempt to invert / “undo” processes disturbing image quality

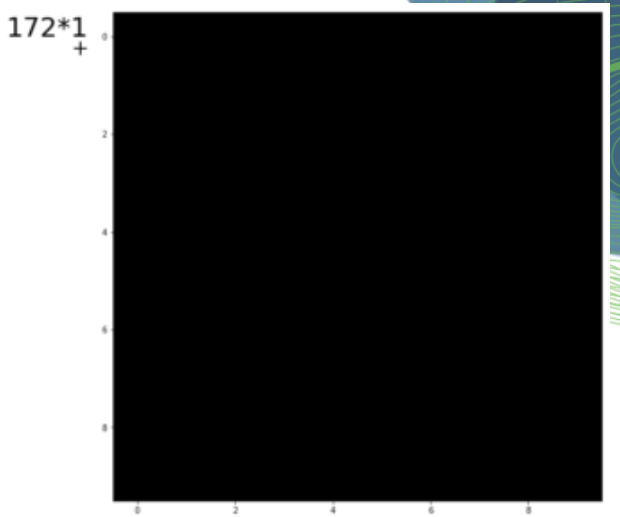


https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/49a787514a367829c3e0e1832f6cc533e96d549f/03_image_processing/simulated_dataset.ipynb

Linear filters

- *Linear filters* replace each pixel value with a weighted linear combination of surrounding pixels
- Filter *kernels* are matrices describing a linear filter
- This multiplication of surrounding pixels according to a matrix is called *convolution*





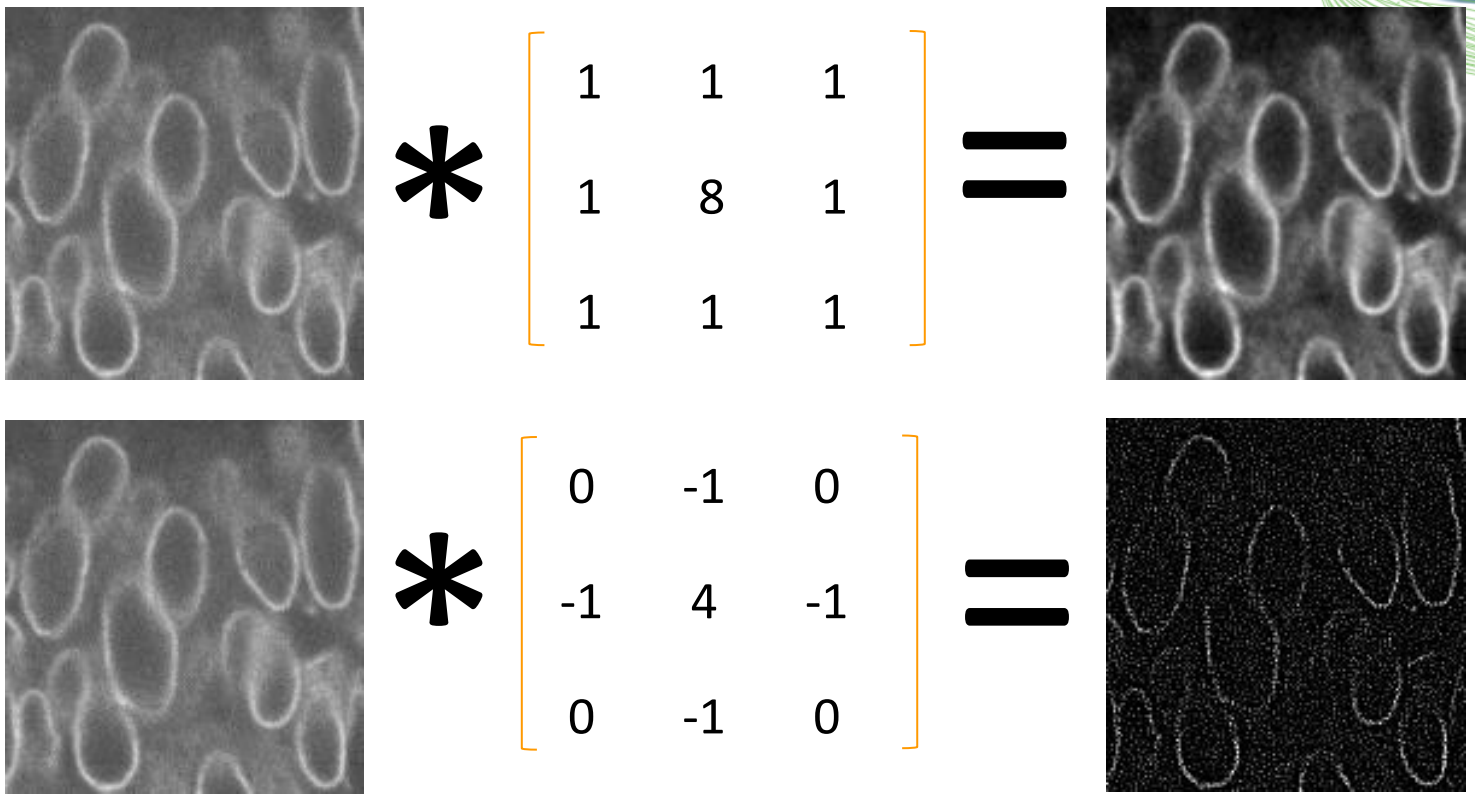
Mean filter, 3x3 kernel:

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

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

Linear filters

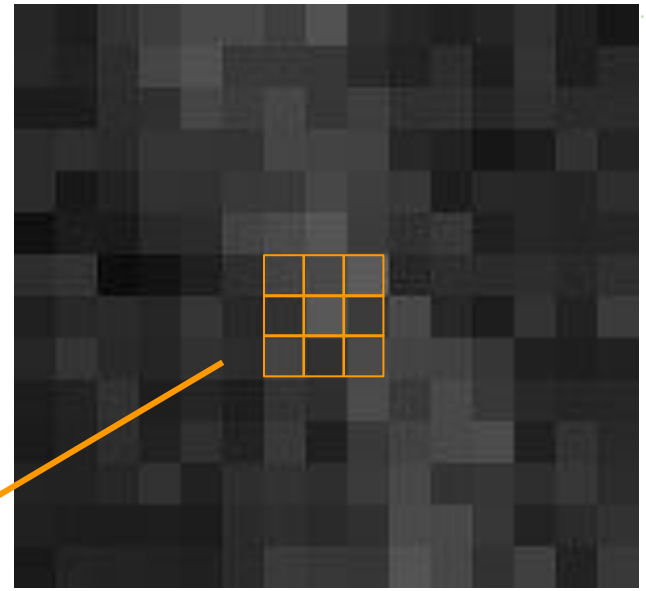
- Terminology:
  - “We convolve an image with a kernel.”
  - Convolution operator: *
  - Examples
    - Mean
    - Gaussian blur
    - Sobel
    - Laplace

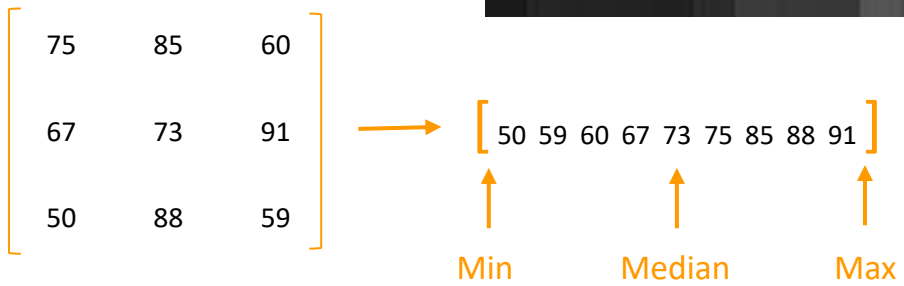


Non-linear Filters

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

- Examples: descriptive statistics filters
  - Minimum
  - Median
  - Maximum
  - Variance
  - Standard deviation





Noise removal

- Gaussian filter
- Median filter (computationally expensive)
- Deep learning (even more expensive, but state-of-the-art)

"Edge preserving"

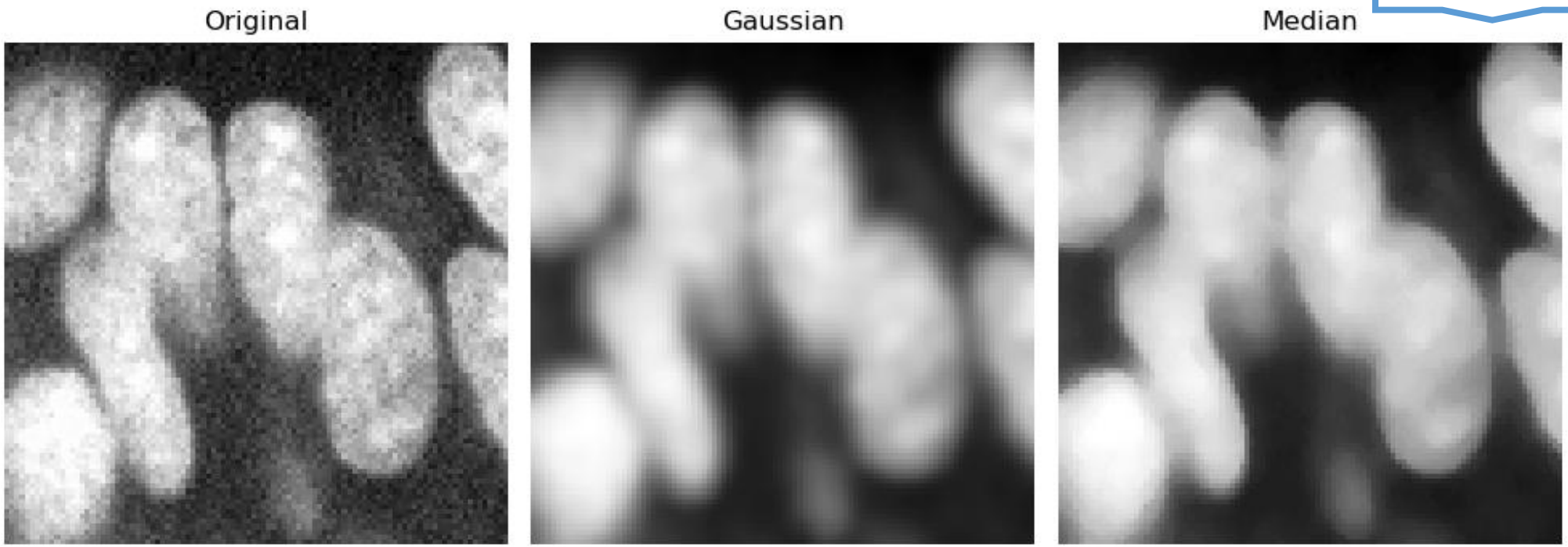
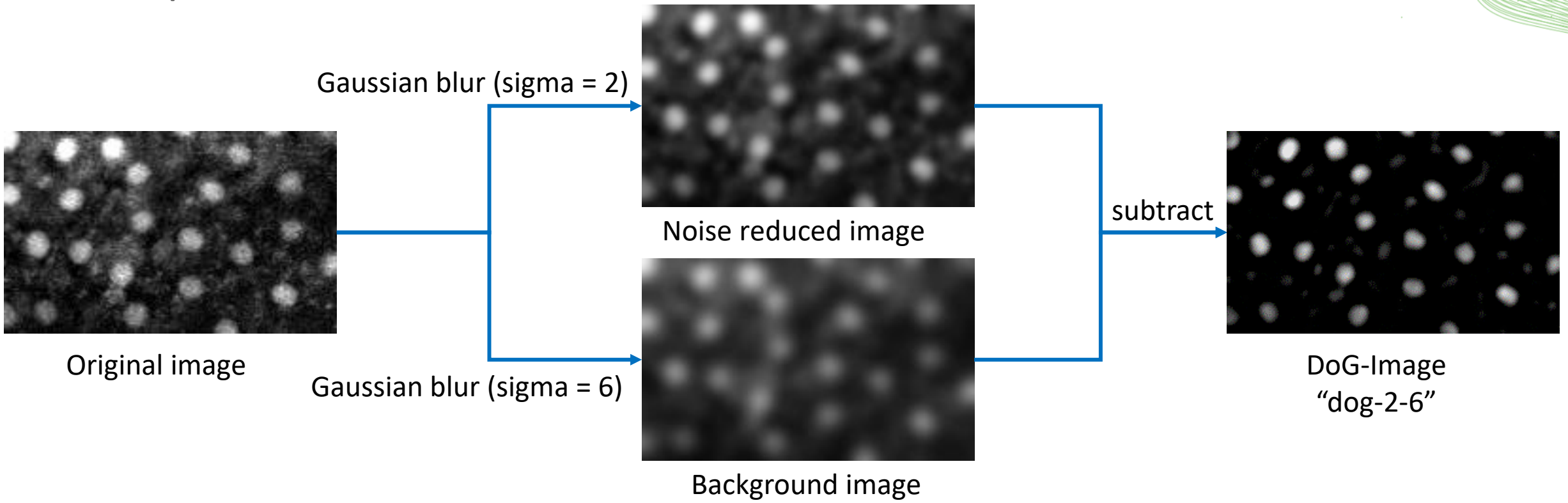


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

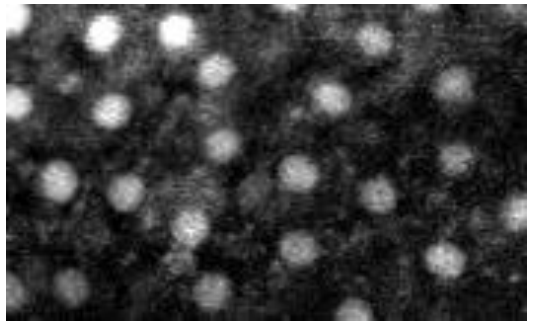
Difference-of-Gaussian (DoG)

- Improve image in order to detect bright objects.
- Band-pass filter

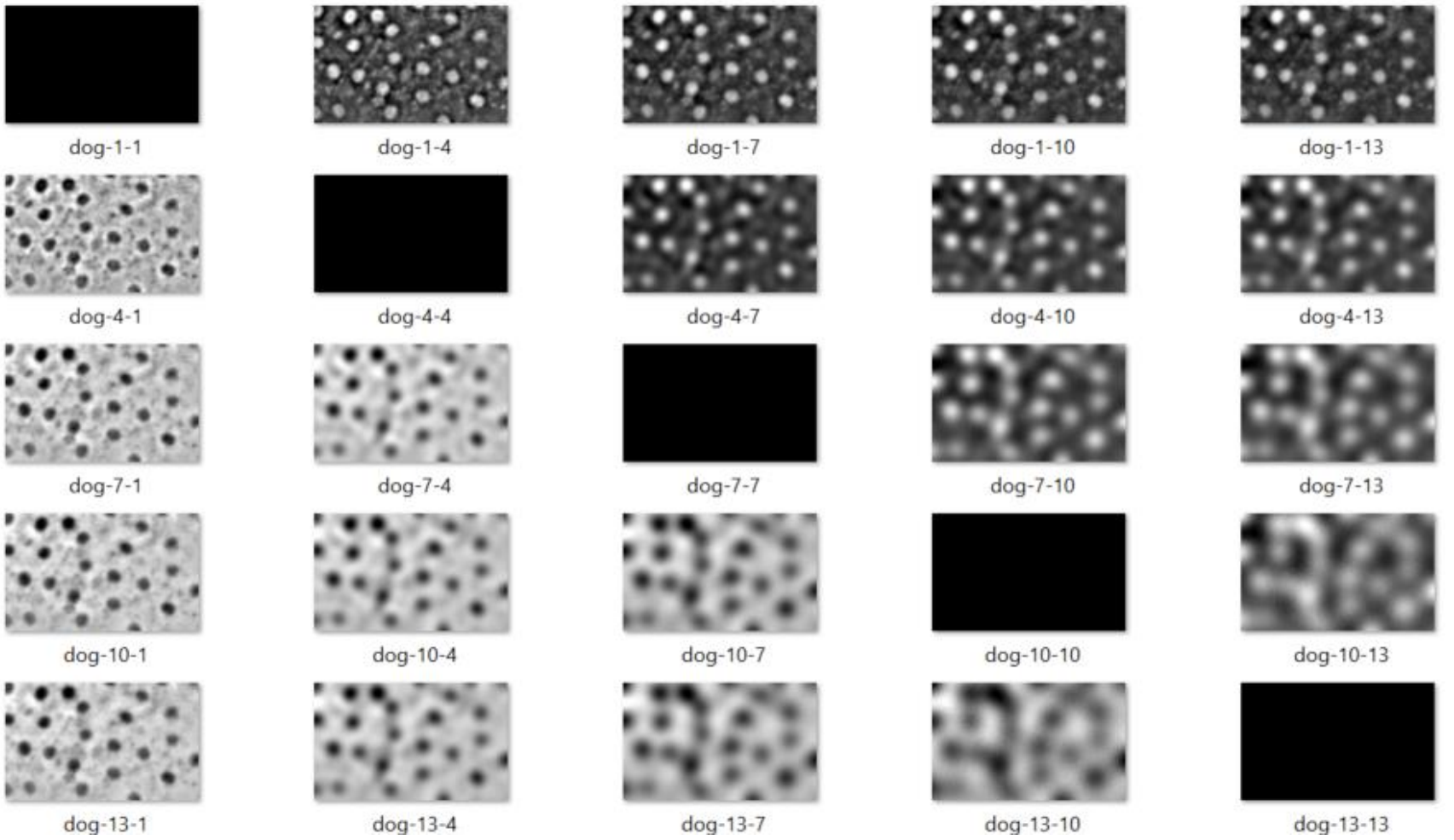


Difference-of-Gaussian (DoG)

- Example DoG images

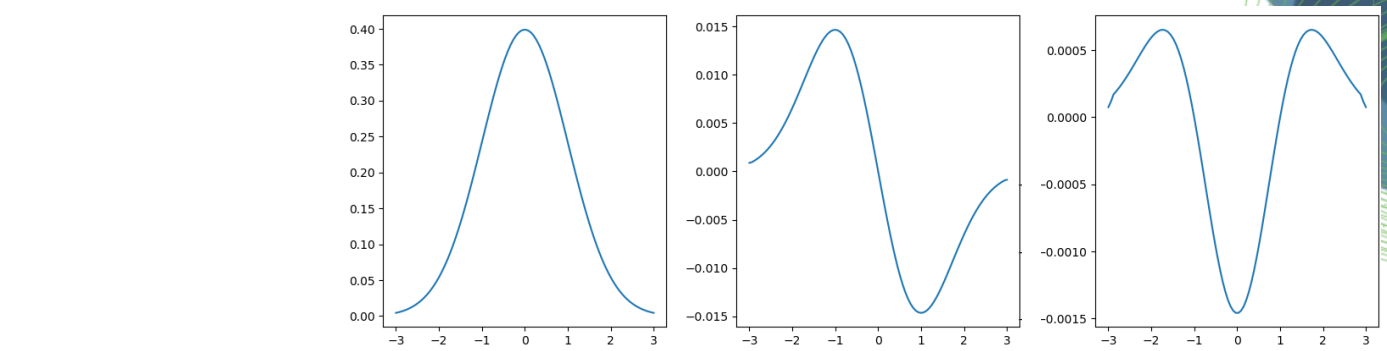


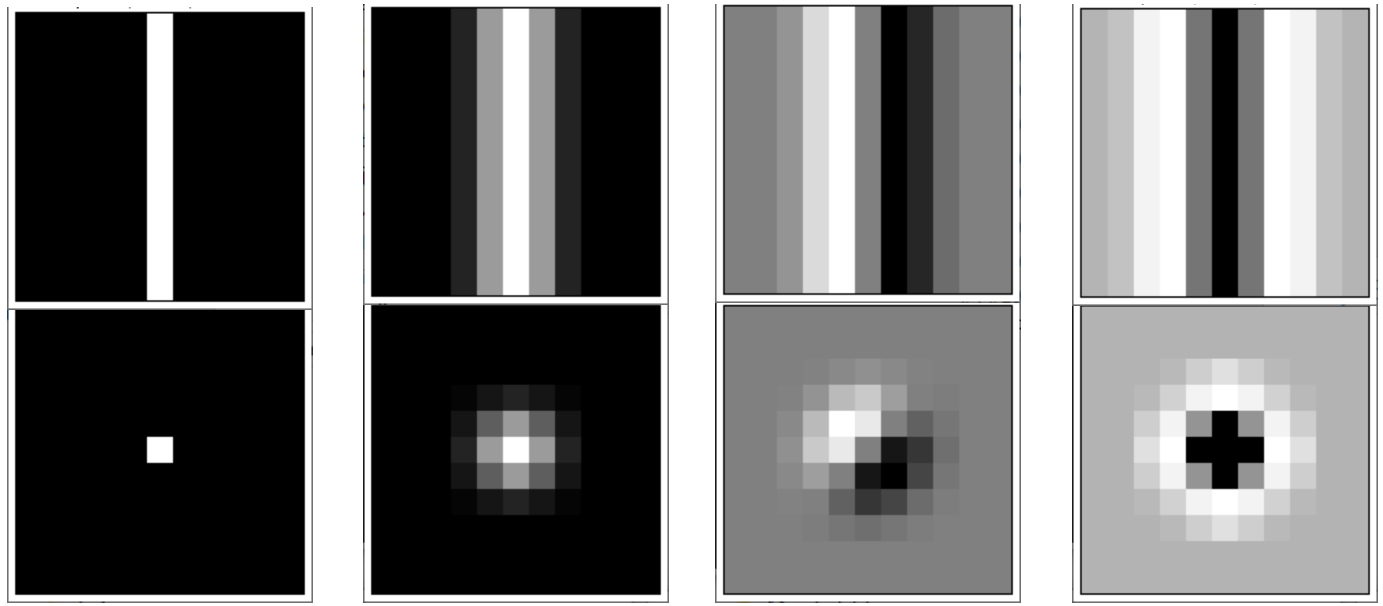
Original image



Laplace-filter

- *Second derivative of a Gaussian filter*
- Used for edge-detection and edge enhancement
- Also known as the *“Mexican-hat-filter”*

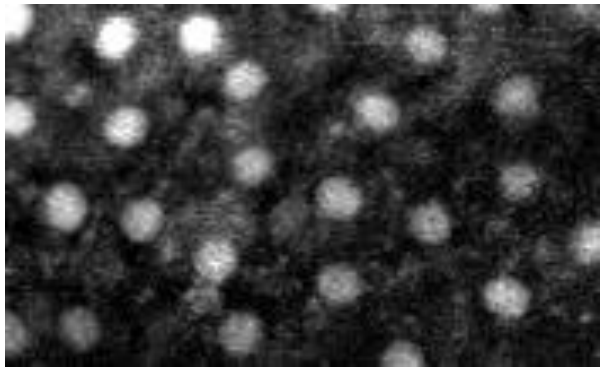




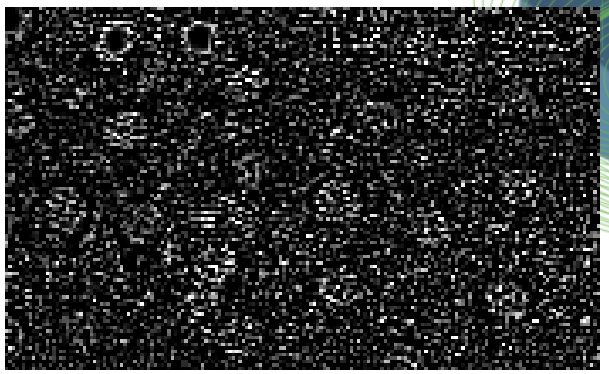
Ground truth | image | 1st derivative | 2nd derivative

Laplacian-of-Gaussian (LoG)

Laplace filter



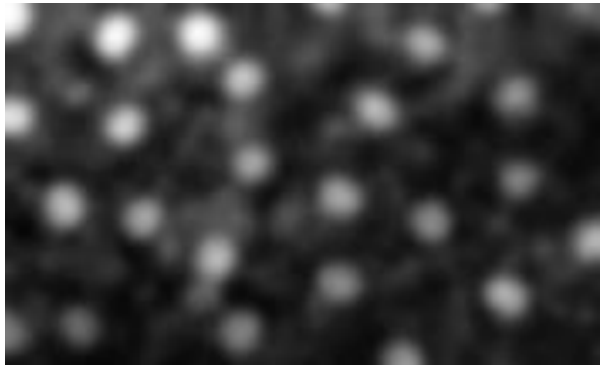
$$* \begin{bmatrix} 0 & -1 & 0 \\ -1 & 4 & -1 \\ 0 & -1 & 0 \end{bmatrix} =$$



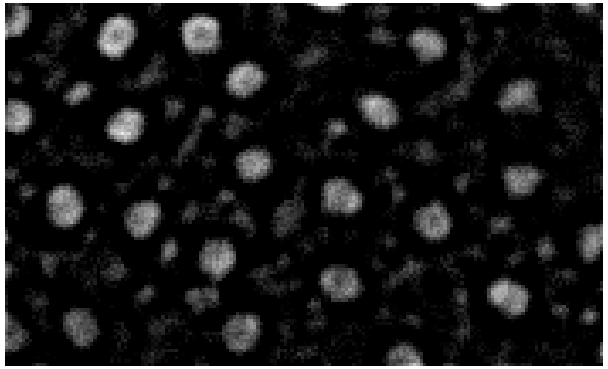
Laplace filtered image

Gaussian filter

Laplacian of Gaussian filter



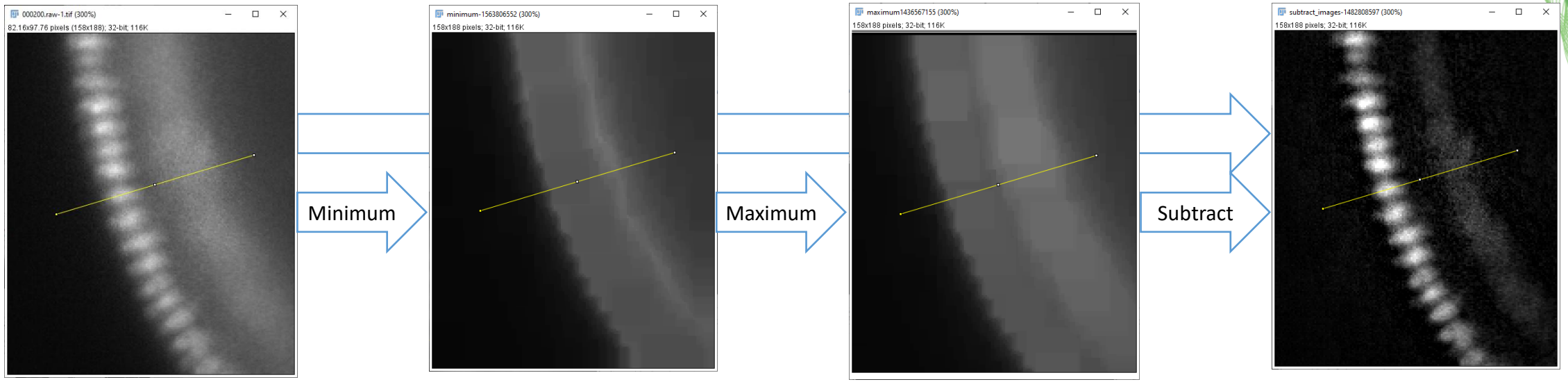
$$* \begin{bmatrix} 0 & -1 & 0 \\ -1 & 4 & -1 \\ 0 & -1 & 0 \end{bmatrix} =$$



LoG image

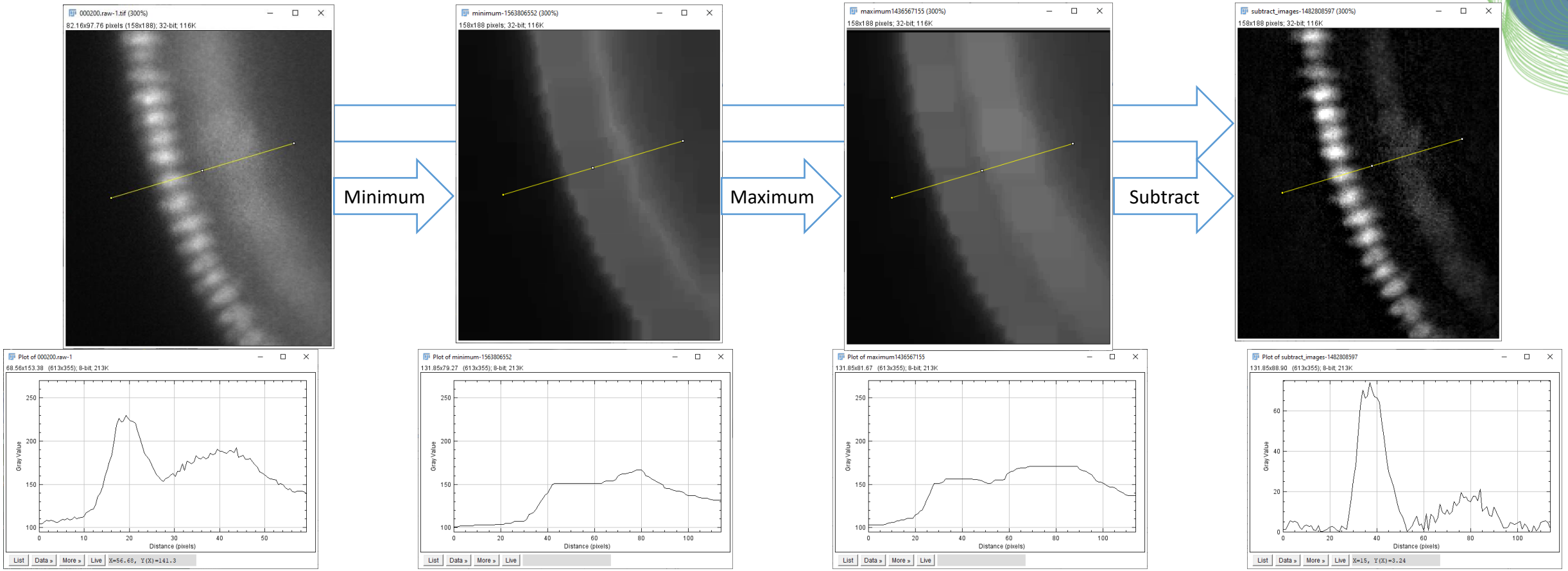
Top-hat filter

- Background subtraction



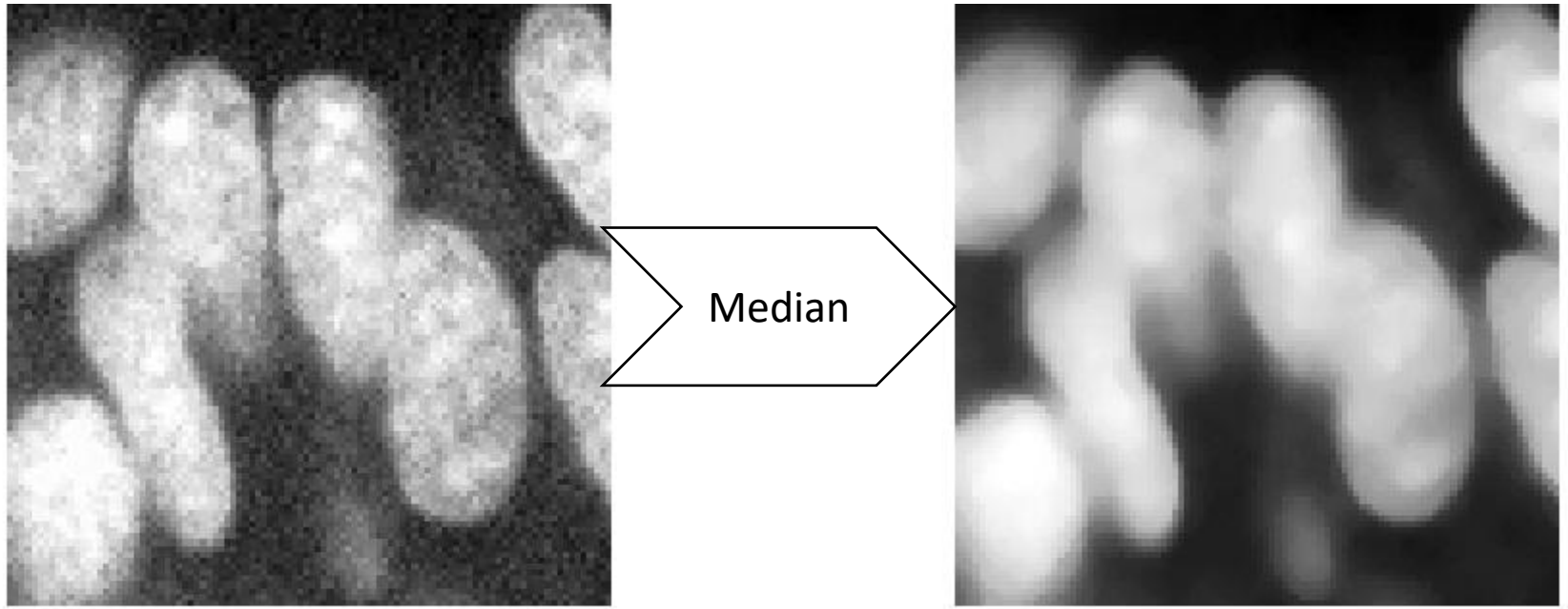
Top-hat filter

- Background subtraction



Quiz: Noise removal

- The median filter is a …

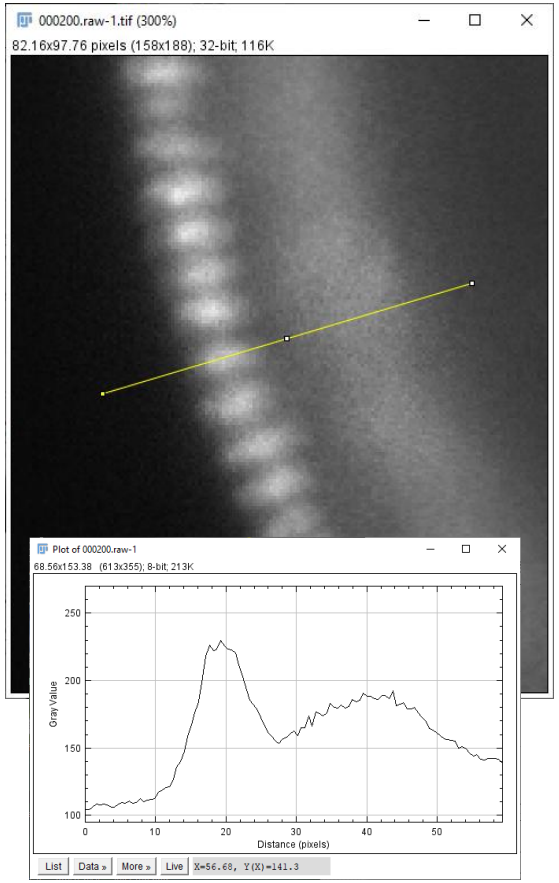


Linear filter

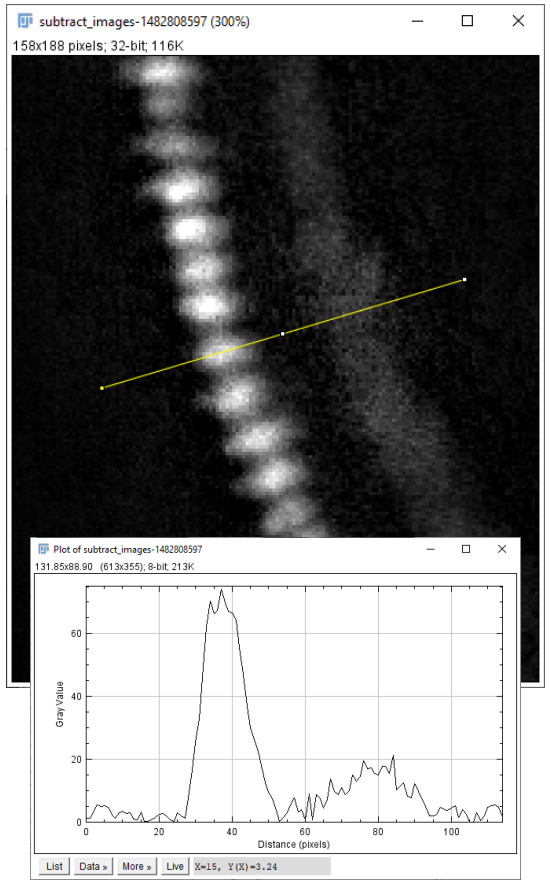
Non-linear filter

Background removal

- Removing background from an image is a … ?



Top-hat



Low-pass filter

High-pass filter

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

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Short detour: Image segmentation

Robert Haase

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für Bildung
und Forschung

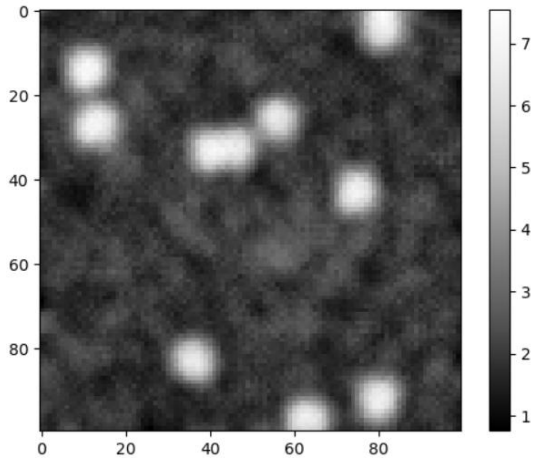
SACHSEN
Diese Maßnahme wird gefördert durch die Bundesregierung aufgrund eines Beschlusses des Deutschen Bundestages.
Diese Maßnahme wird mitfinanziert durch Steuermittel auf der Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.

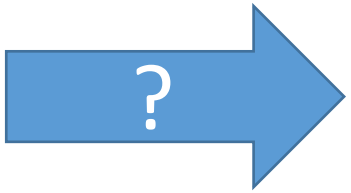
Short detour: Segmentation

Binarization, e.g. via thresholding

- Very basic and yet efficient segmentation technique
- Histogram based, to determine an intensity threshold above which pixels become white
- Not state-of-the-art in many fields anymore

Intensity image





Binary image

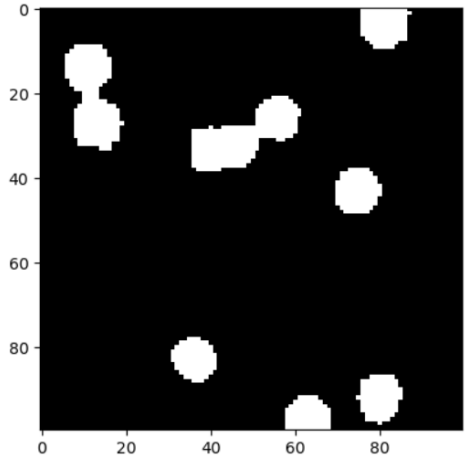


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Image Processing: Morphological Operations

Robert Haase

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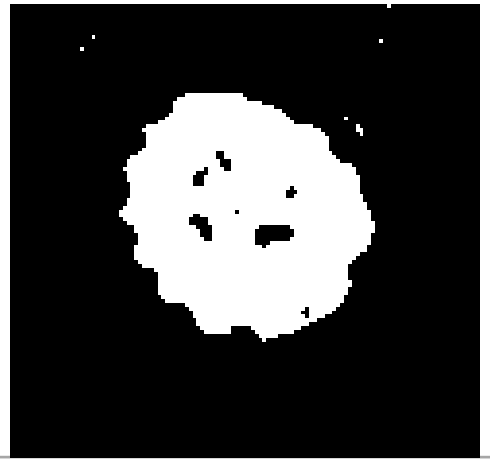
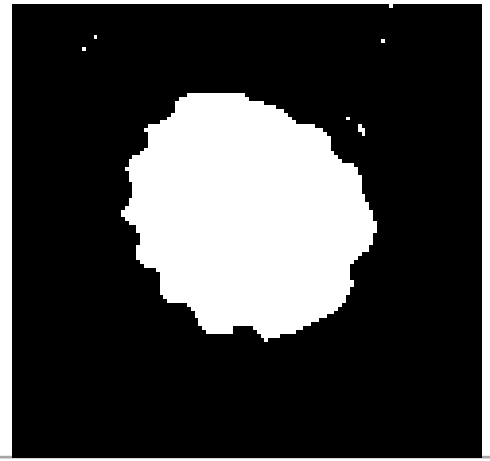
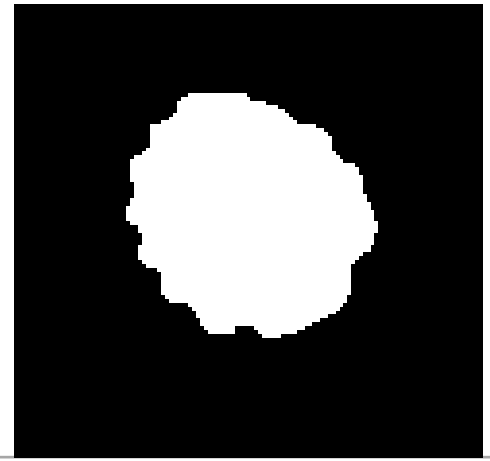
With material from

Marcelo Leomil Zoccoler, Physic of Life, TU Dresden

Refining masks

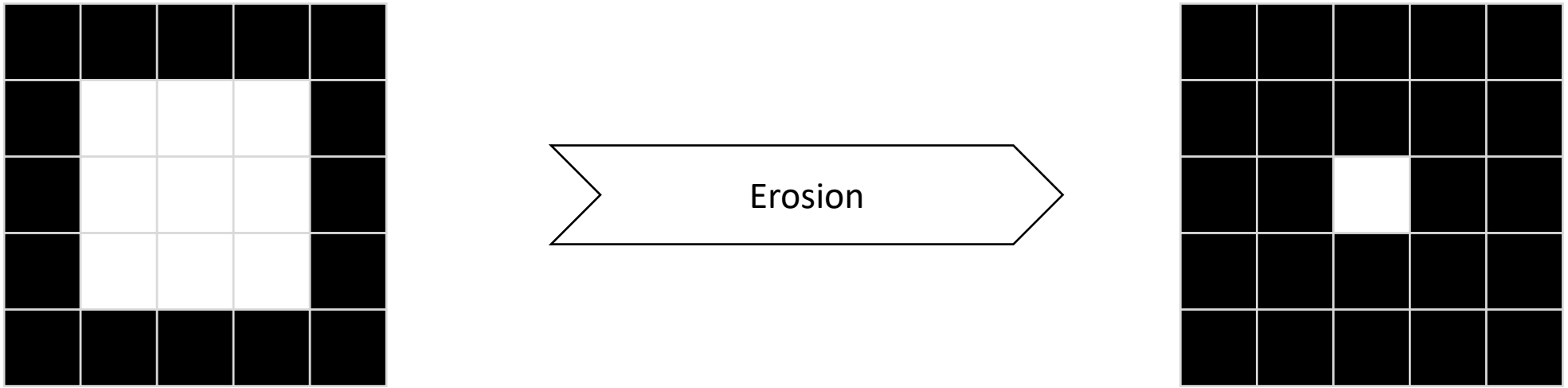
- Binary mask images may not be perfect immediately after thresholding.
- There are ways of refining them

Thresholding → Closing → Opening

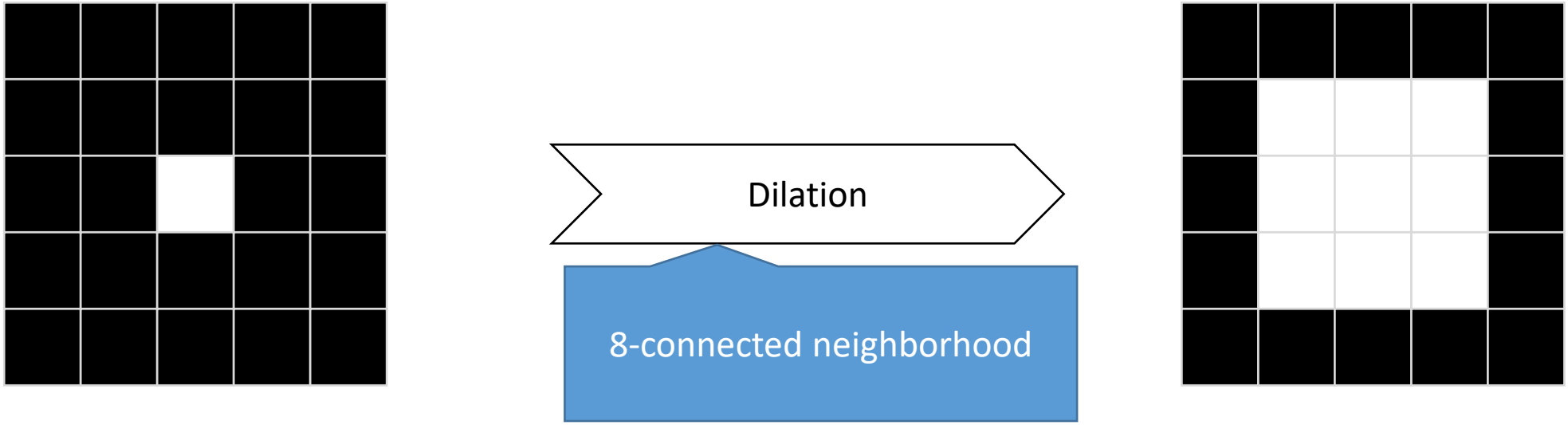
Erosion

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



Dilation

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



Quiz

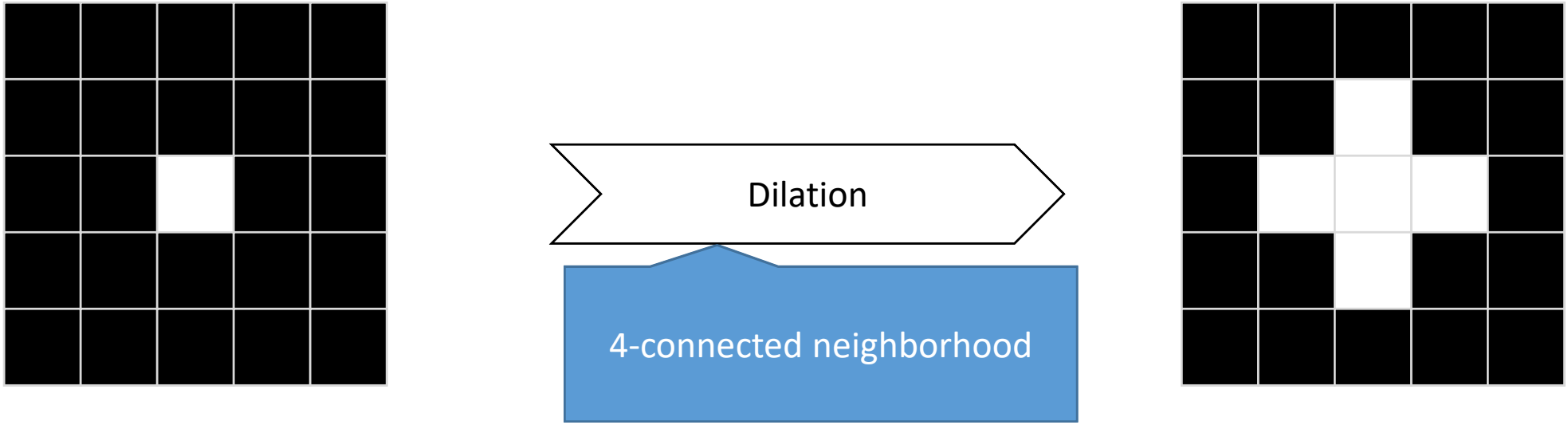
- What would be the equivalent image processing filter you learned before? Hint: assume black=0 and white=1.

0	0	0	0	0
0	0	0	0	0
0	0	1	0	0
0	0	0	0	0
0	0	0	0	0

0	0	0	0	0
0	1	1	1	0
0	1	1	1	0
0	1	1	1	0
0	0	0	0	0

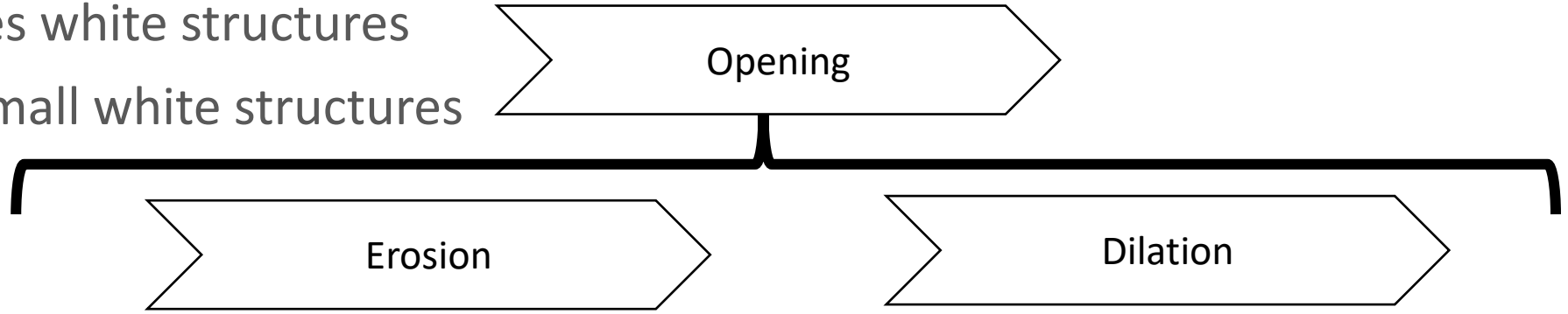
Dilation

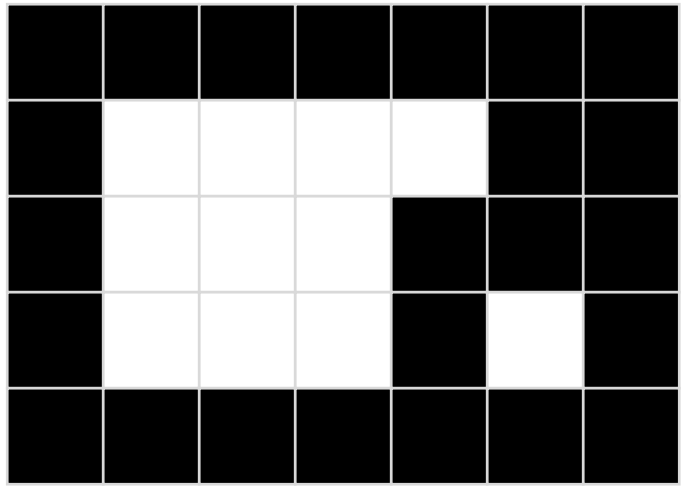
- Dilation: Every pixel with at least one white neighbor becomes white.

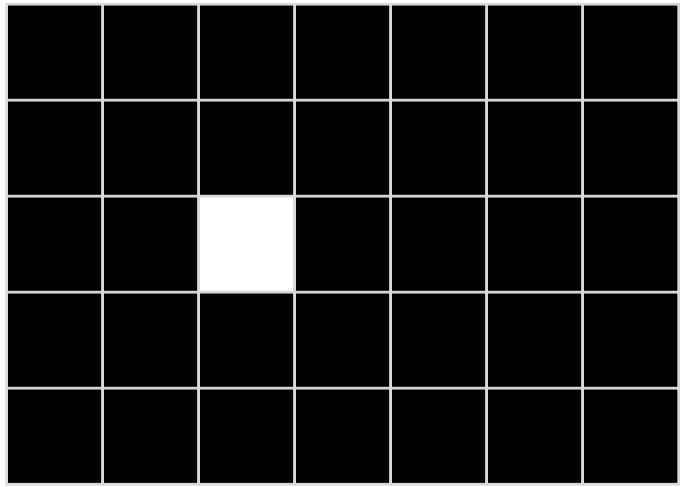


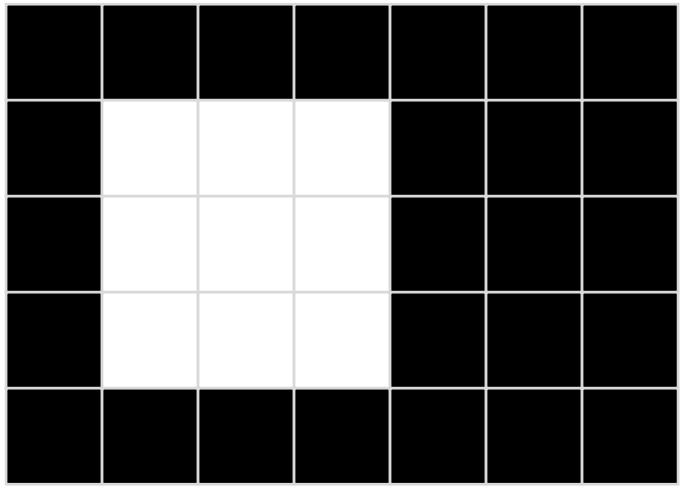
Opening

- Erosion and dilation combined allow correcting outlines.
- Separates white structures
- Erases small white structures



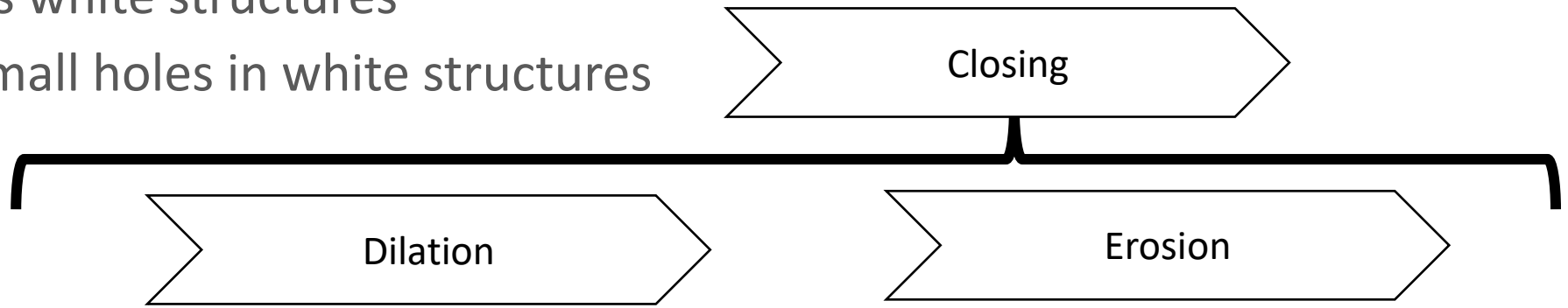


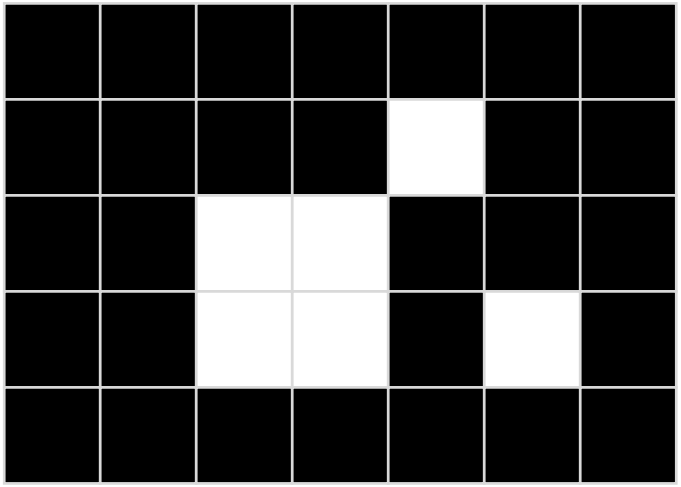


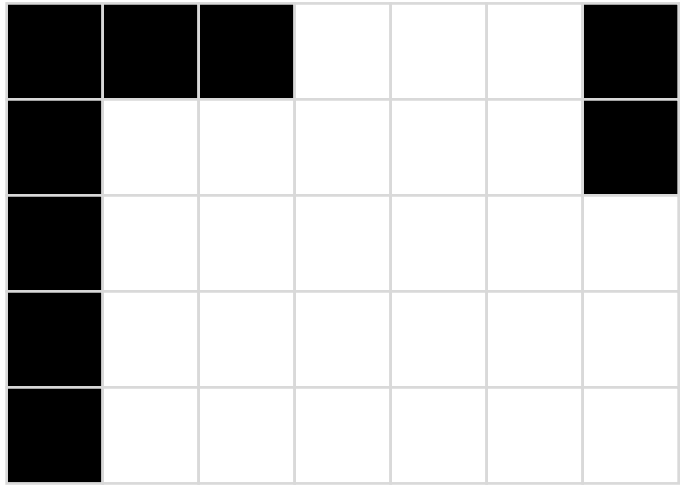


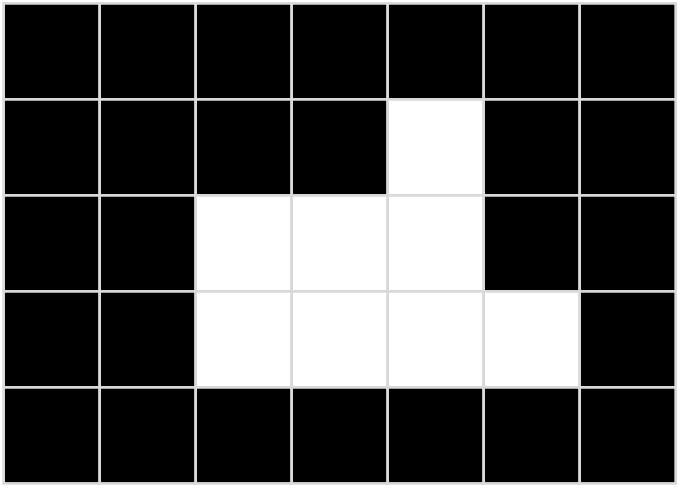
Closing

- Erosion and dilation combined allow correcting outlines.
- Connects white structures
- Closes small holes in white structures



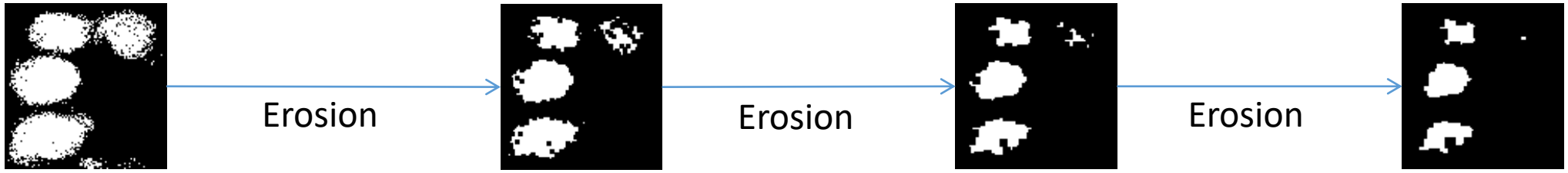




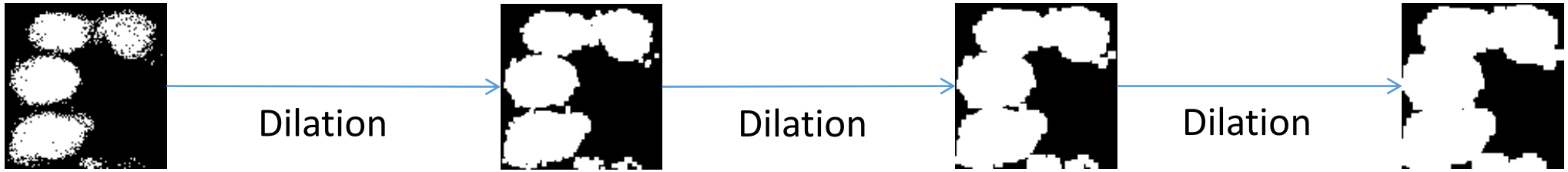


Chaining erosion and dilation

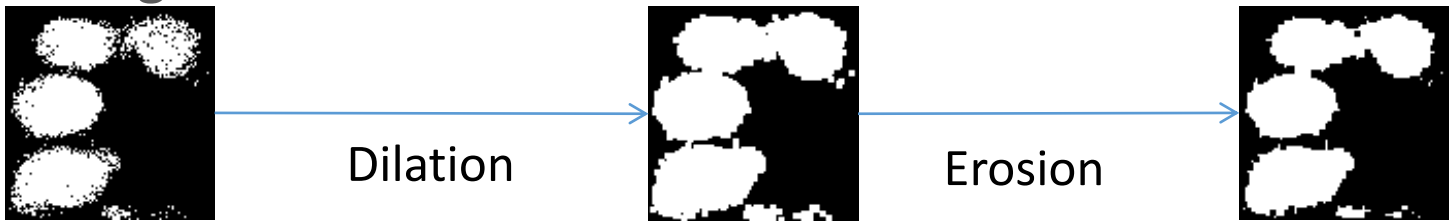
- Erosion: Set all pixels to black which have at least one black neighbor.



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



- Closing: Dilation + Erosion



- Opening: Erosion + Dilation

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Microscopy Image Processing in Python

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Working with images in python

- Open images, e.g. using scikit-image

```
from skimage.io import imread

image = imread("blobs.tif")
```

```
image
```

```
array([[ 40,  32,  24, ..., 216, 200, 200],
       [ 56,  40,  24, ..., 232, 216, 216],
       [ 64,  48,  24, ..., 240, 232, 232],
       ...,
       [ 72,  80,  80, ...,  48,  48,  48],
       [ 80,  80,  80, ...,  48,  48,  48],
       [ 96,  88,  80, ...,  48,  48,  48]], dtype=uint8)
```

Images are *just* multi-dimensional arrays or "arrays of arrays".

Loading images

- Proprietary file formats, e.g. using *aicsimageio* or *bioio*

```
[3]: aics_image = AICSImage("data/PupalWing.czi")
     aics_image

[3]: <AICSImage [Reader: CziReader, Image-is-in-Memory: False]>

[4]: aics_image.shape

[4]: (1, 1, 80, 520, 692)

[5]: aics_image.dims

[5]: <Dimensions [T: 1, C: 1, Z: 80, Y: 520, X: 692]>

[6]: aics_image.dims.order

[6]: 'TCZYX'
```

bioio is the successor of aicsimageio, but seems not to work yet

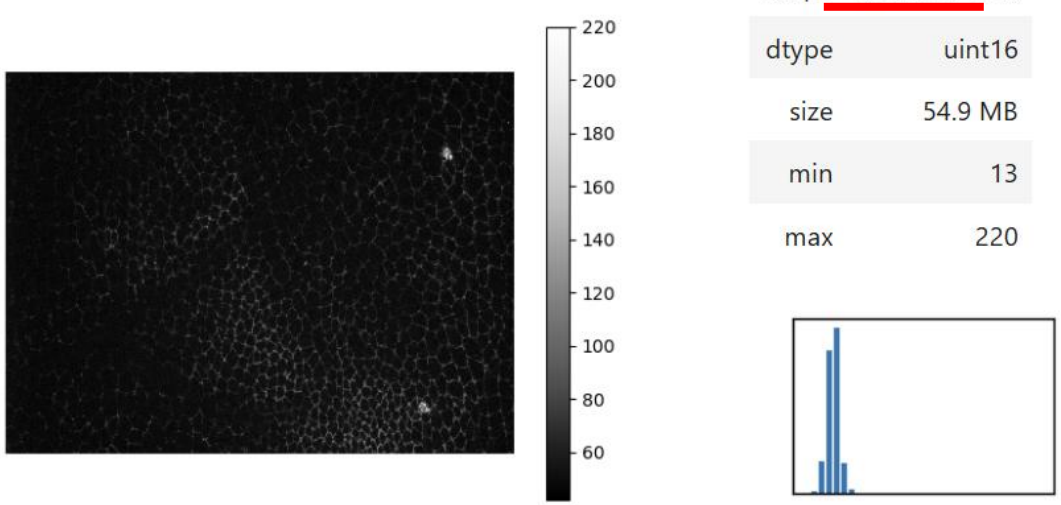
X and Y: 2D image size
Z: Number of images in 3D stack
T: time-points
C: channels
S: scenes

Loading images

- Before you can work with such images, you need to extract a numpy-array first

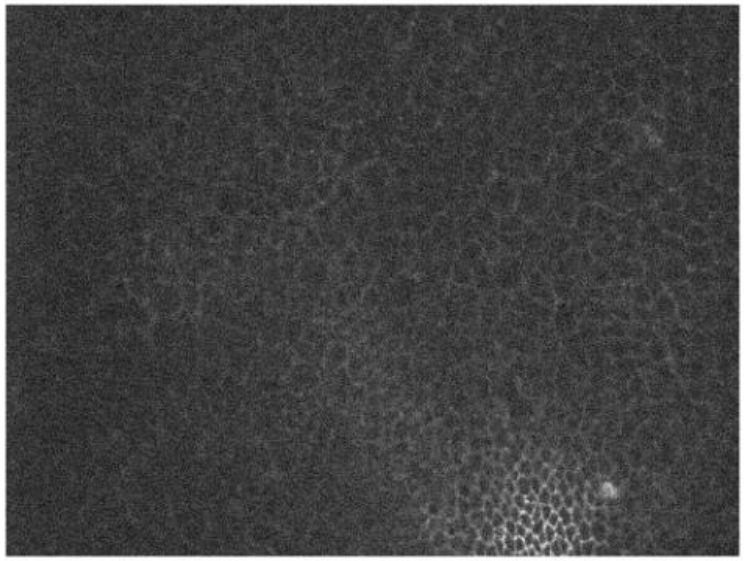
```
[6]: np_image = aics_image.get_image_data("ZYX", T=0)
     np_image.shape

[6]: (80, 520, 692)

[7]: stackview.insight(np_image)
```

[7]:



Select a 2D plane from a 3D image stack

```
[9]: stackview.imshow(np_image[39])
```



Visualizing images

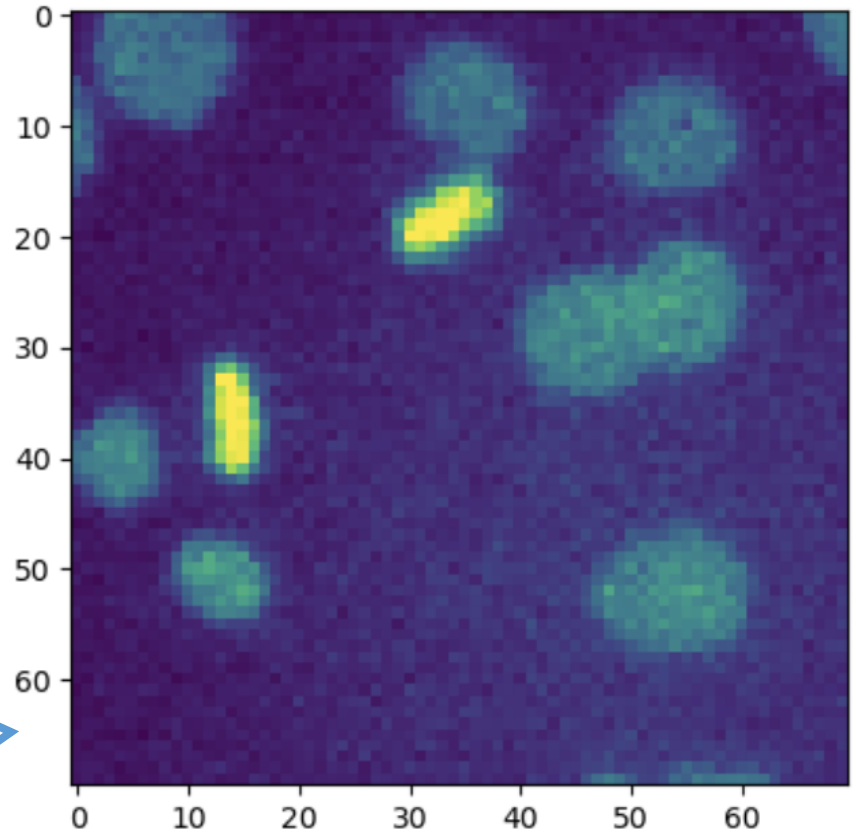
```
[3]: from skimage.io import imread
     from matplotlib.pyplot import imshow
     import stackview

     image = imread("data/mitosis_mod.tif")
     image
```

```
[3]: array([[ 19,  29,  44, ...,  88, 115, 113],
            [ 19,  29,  55, ...,  87, 101, 112],
            [ 25,  36,  61, ...,  90,  90, 106],
            ...,
            [ 20,  21,  20, ...,  57,  33,  40],
            [ 22,  25,  25, ...,  41,  34,  40],
            [ 20,  25,  18, ...,  41,  35,  39]], dtype=uint8)
```

Suboptimal image display

```
[2]: imshow(image)
```

```
[2]: <matplotlib.image.AxesImage at 0x2cd0e7cad90>
```



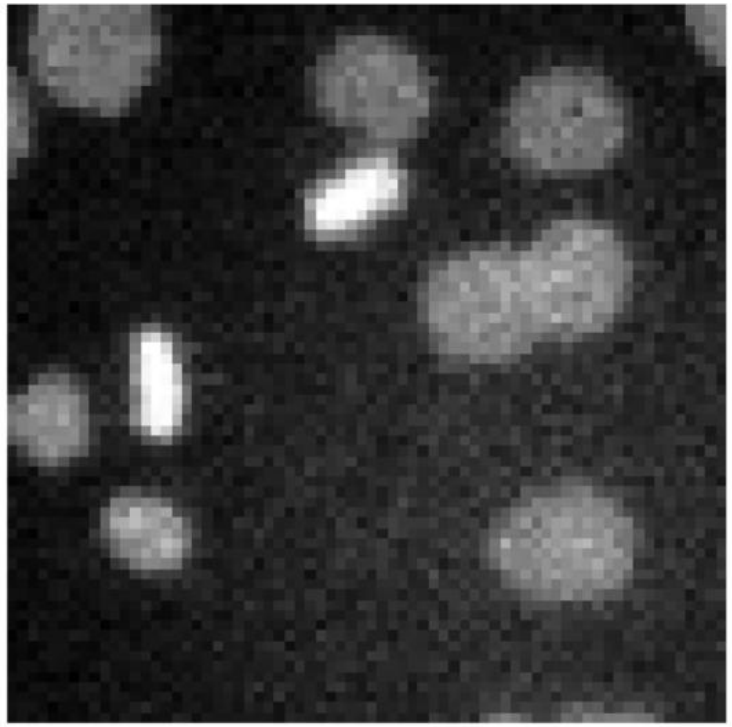
The default colormap has been criticized many times.

Visualizing images

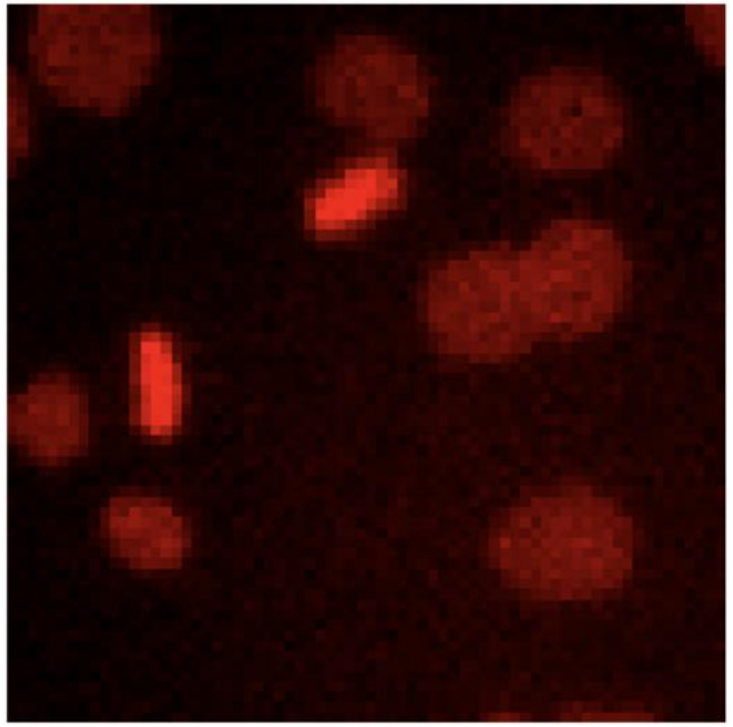
- Alternative: stackview

Disclaimer: I maintain this

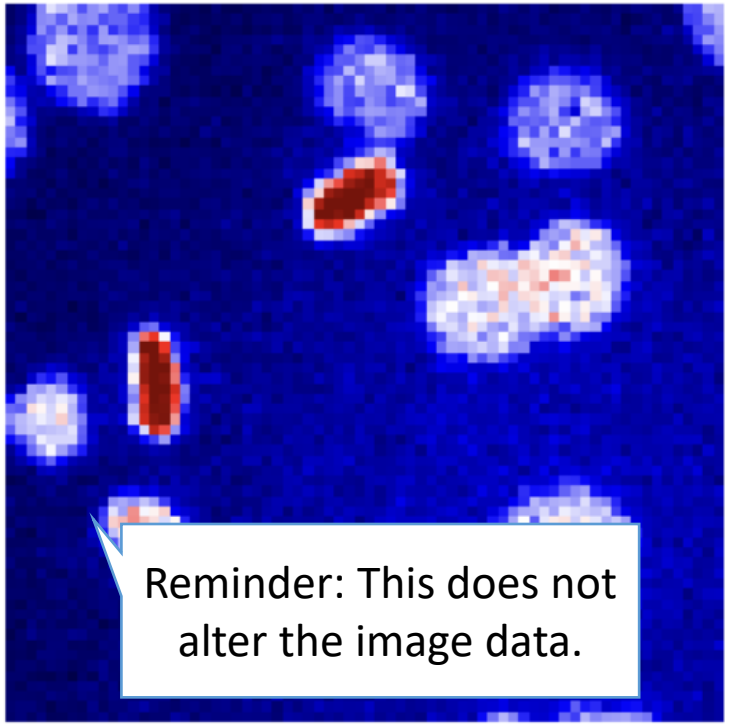
```
[3]: import stackview
     stackview.imshow(image)
```



```
[5]: stackview.imshow(image, colormap="pure_red")
```



```
[6]: stackview.imshow(image, colormap="seismic")
```



Visualizing images

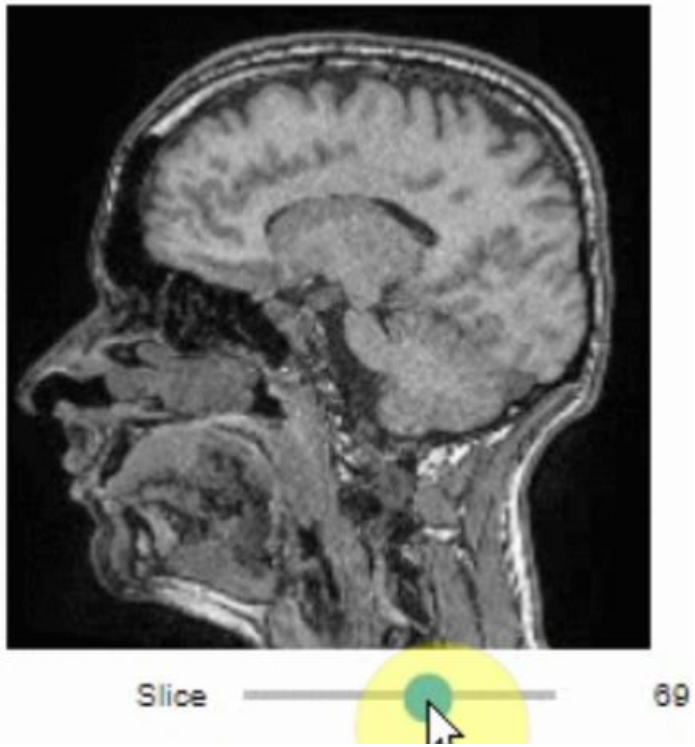
- Alternative: stackview

```
[6]: stackview.slice(mri_image)
```

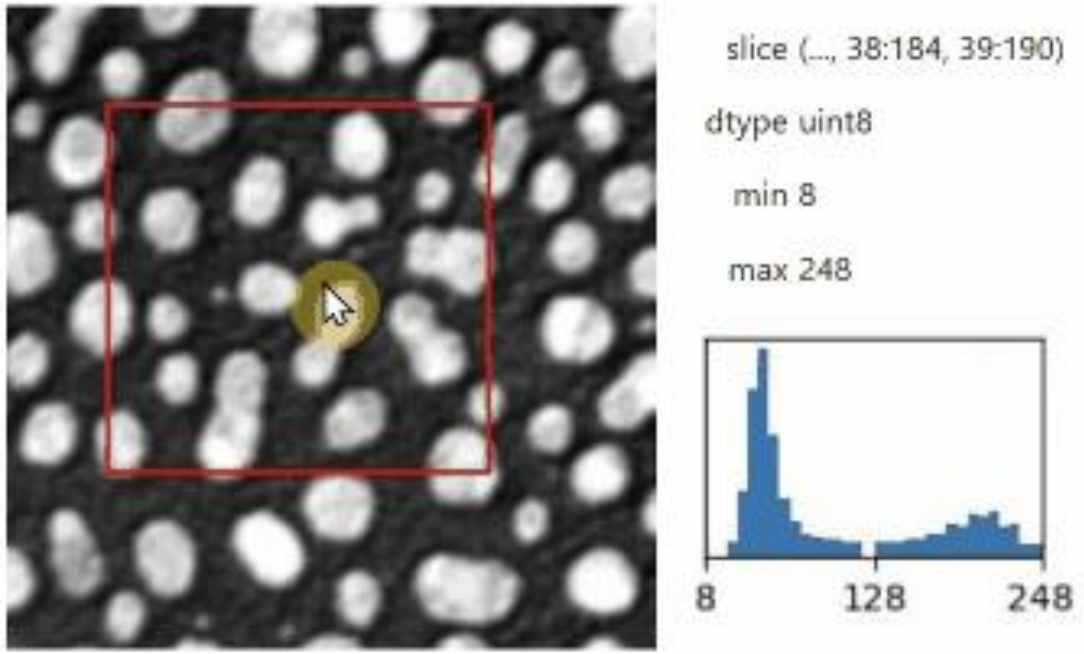
Visualizing images

- Alternative: stackview

```
[6]: stackview.slice(mri_image)
```

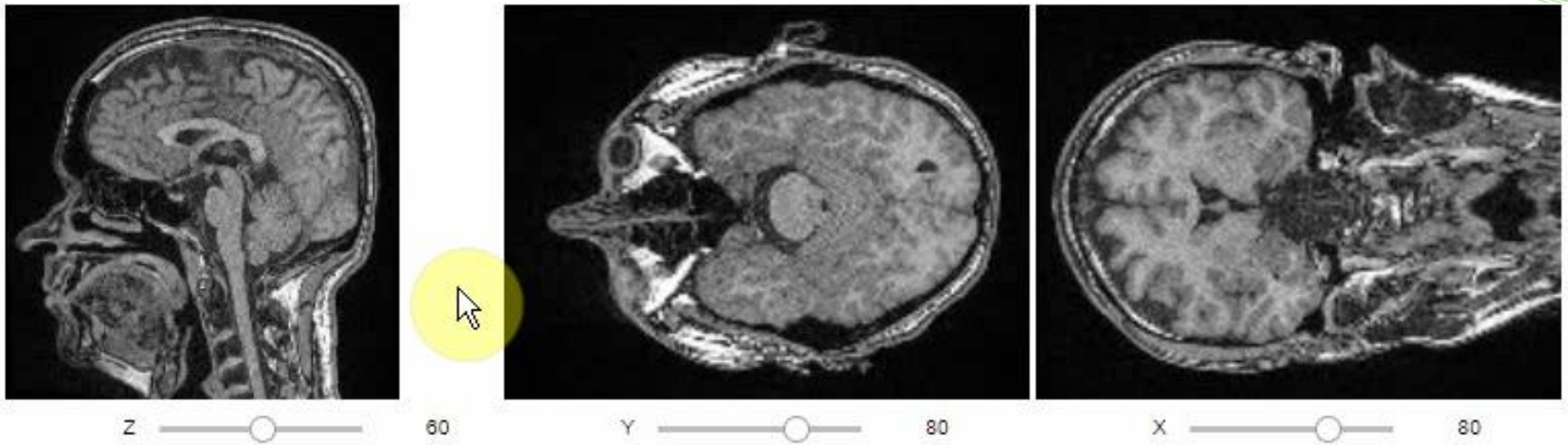



```
[8]: stackview.histogram(image)
```



Orthogonal planes

```
[10]: stackview.orthogonal(mri_image, crosshairs=False)
```



Orthogonal planes

Quiz: What does this T stand for?

[3]:
```
xy_plane = mri_image[100]

stackview.imshow(xy_plane)
```

[4]:
```
zx_plane = mri_image[:,128,:]

stackview.imshow(zx_plane)
```

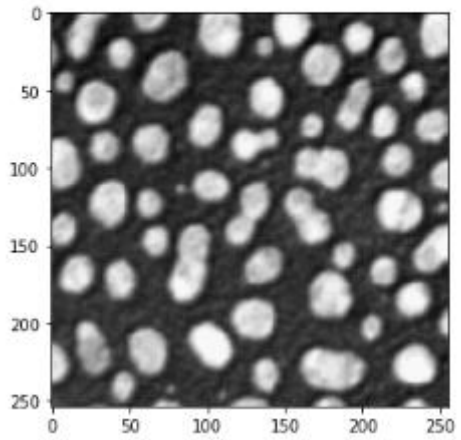
[5]:
```
stackview.imshow(zx_plane.T)
```

Cropping and resampling images

- Indexing and cropping *numpy-arrays* works like with python arrays.

```
imshow(image)
```

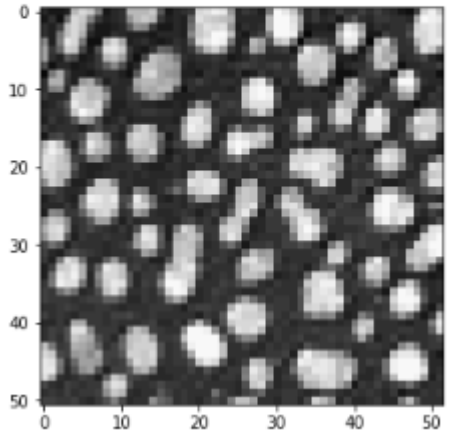
```
<matplotlib.image.AxesImage at 0
```



Original image

```
sampled_image = image[::5, ::5]

imshow(sampled_image)
```

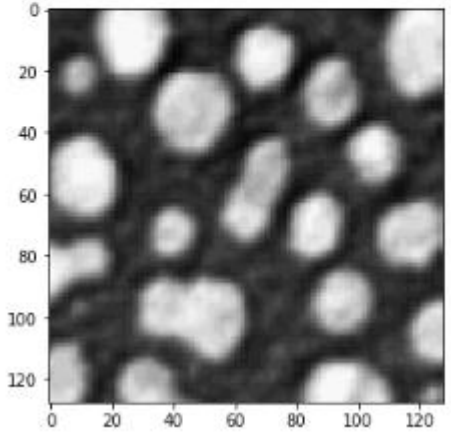
```
<matplotlib.image.AxesImage at 0
```



Sub-sampled image

```
cropped_image2 = image[0:128, 128:]

imshow(cropped_image2)
```

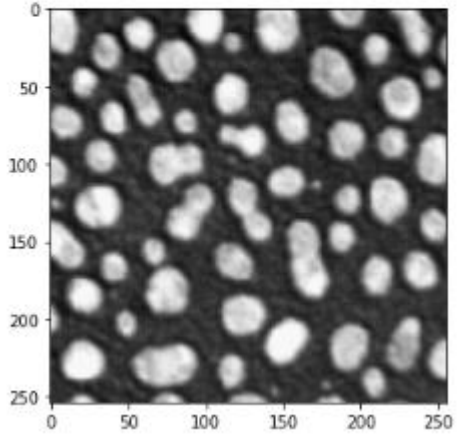
```
<matplotlib.image.AxesImage at 0x29e
```



Cropped image

```
flipped_image = image[:, ::-1]

imshow(flipped_image)
```

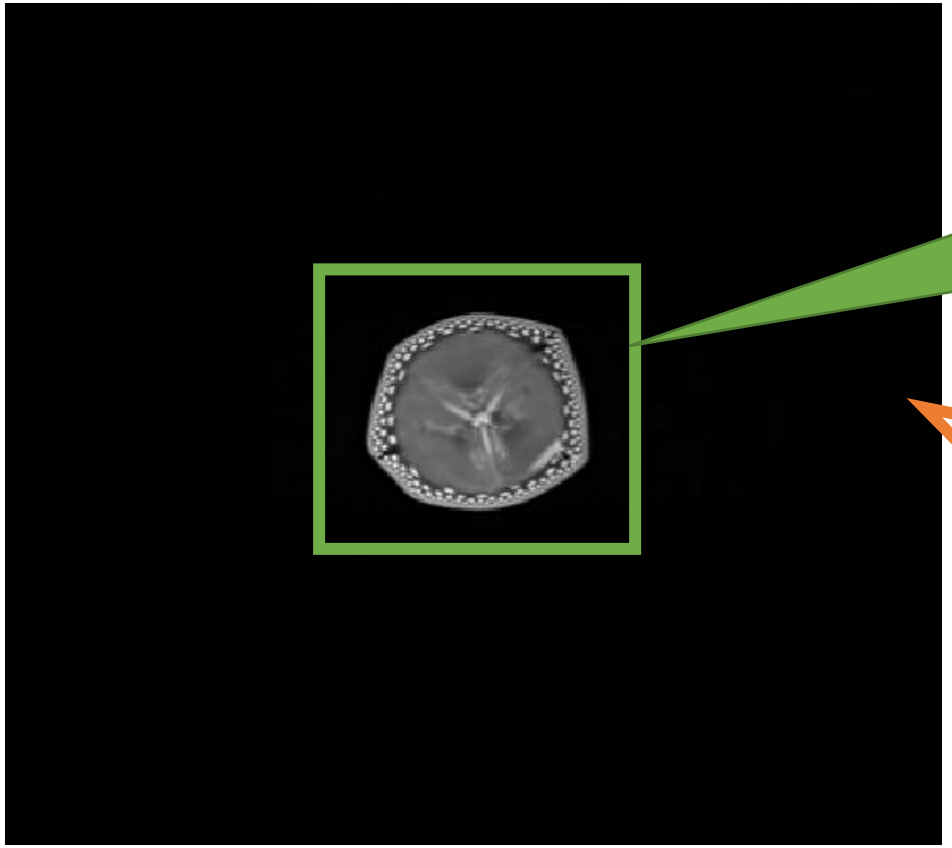
```
<matplotlib.image.AxesImage at 0x
```



Flipped image

Cropping and resampling images

- Crop out the region you're interested in



In this case
you can spare
8/9 compute time and
memory for subsequent
processing steps

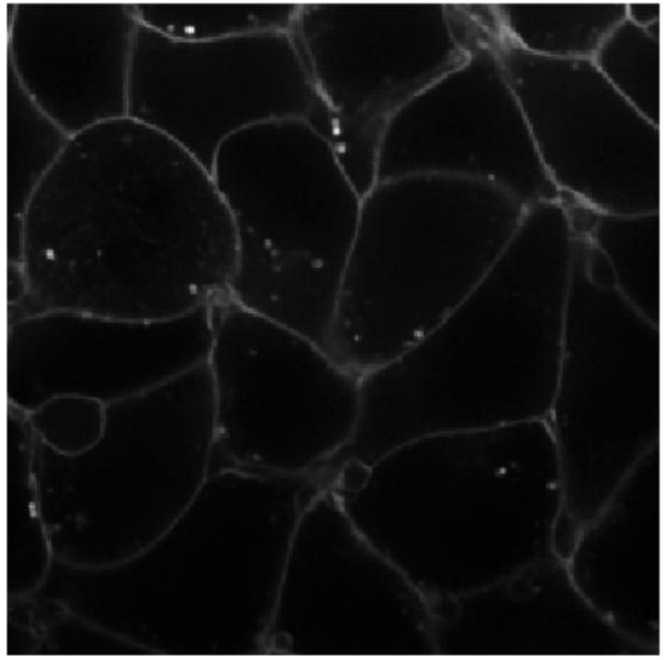
Image data source: Nasreddin Abolmaali, TU Dresden

https://github.com/ScaDS/BIDS-lecture-2025/blob/main/02a_image_processing/05_Cropping_images.ipynb

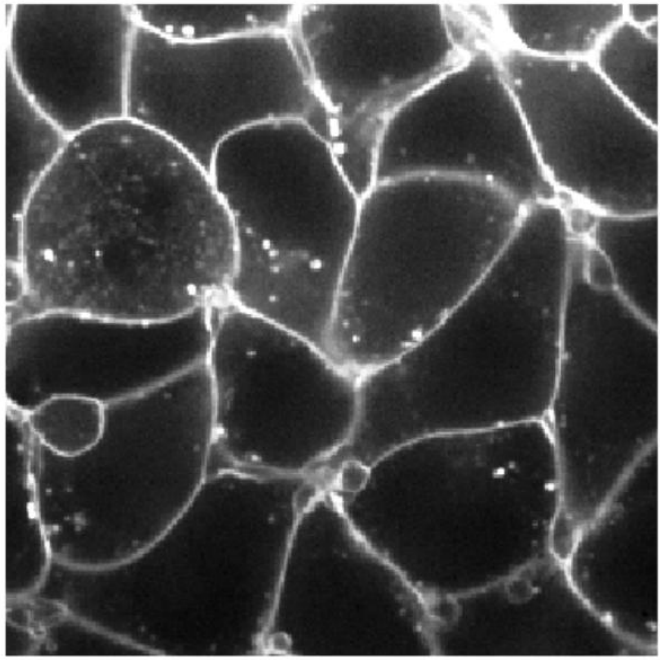
Brightness, contrast, display-range

- After loading data, make sure you can see the structure you're interested in

```
[5]: stackview.imshow(image)
```

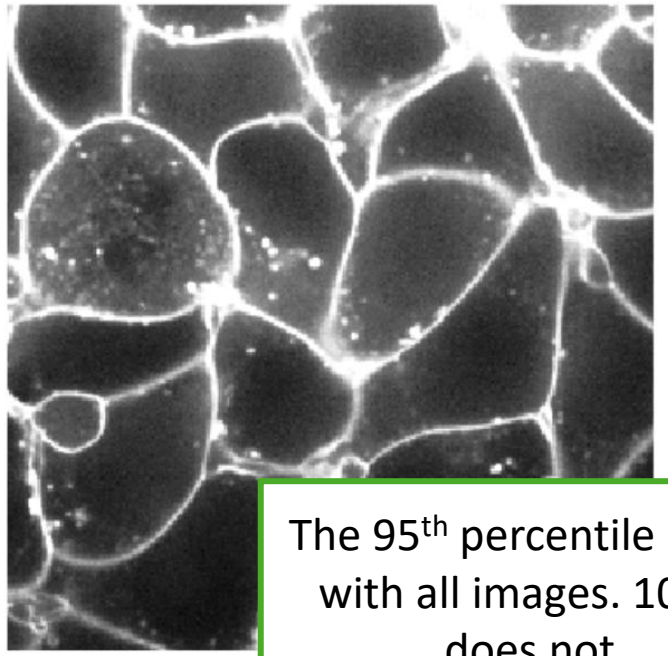


```
[9]: stackview.imshow(image, max_display_intensity=10000)
```



```
[6]: upper_limit = np.percentile(image, 95)
     upper_limit

[6]: 6580.0
```

```
[7]: stackview.imshow(image, max_display_intensity=upper_limit)
```



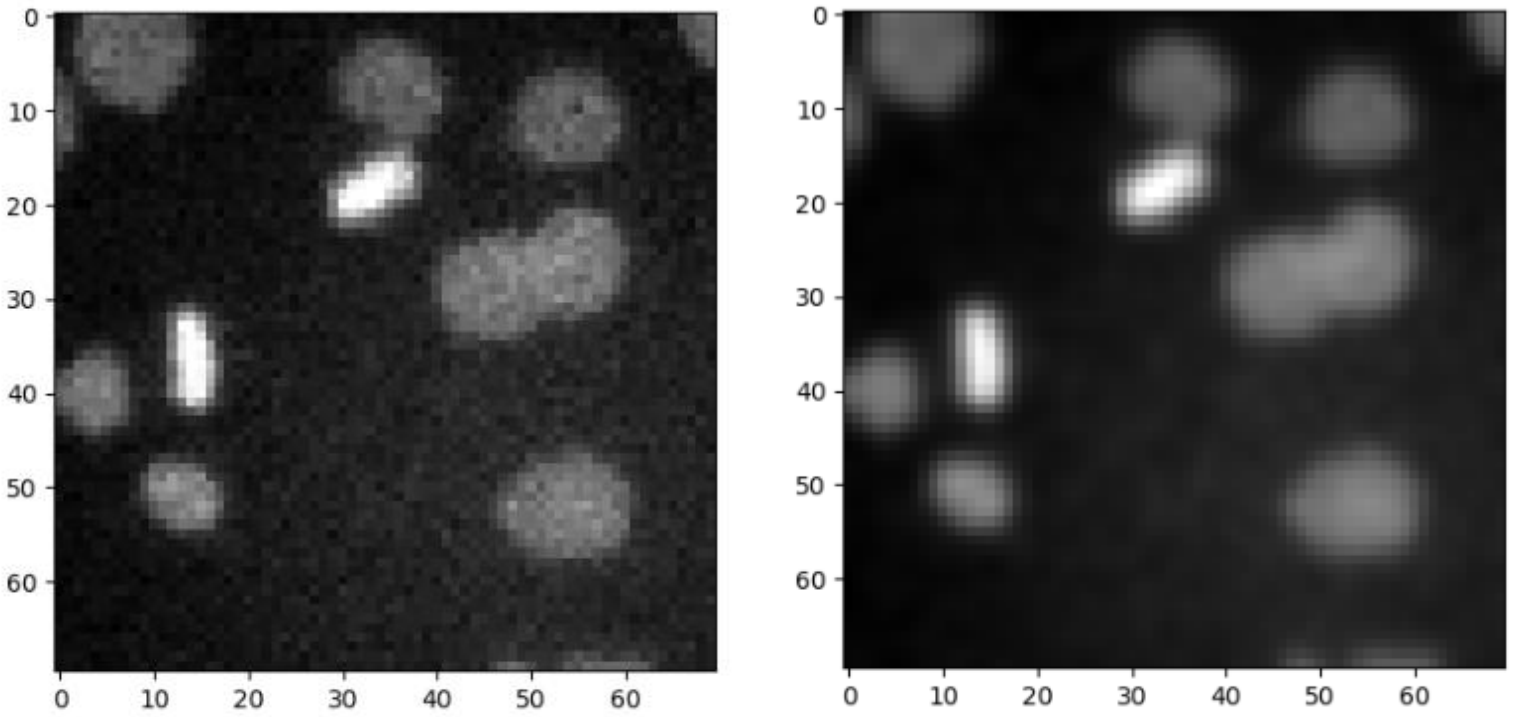
The 95th percentile works with all images. 10000 does not.

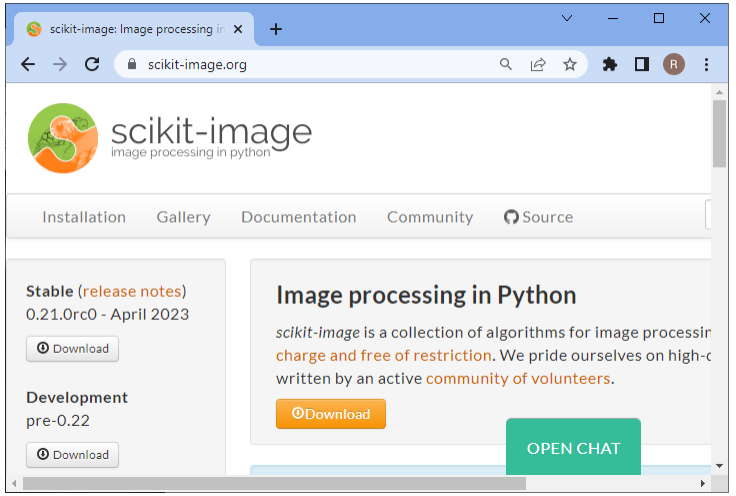
Filters

… are just functions

```
denoised_gaussian = filters.gaussian(image3, sigma=1)

plt.imshow(denoised_gaussian, cmap='gray')
```

```
<matplotlib.image.AxesImage at 0x283aab3ba90>
```



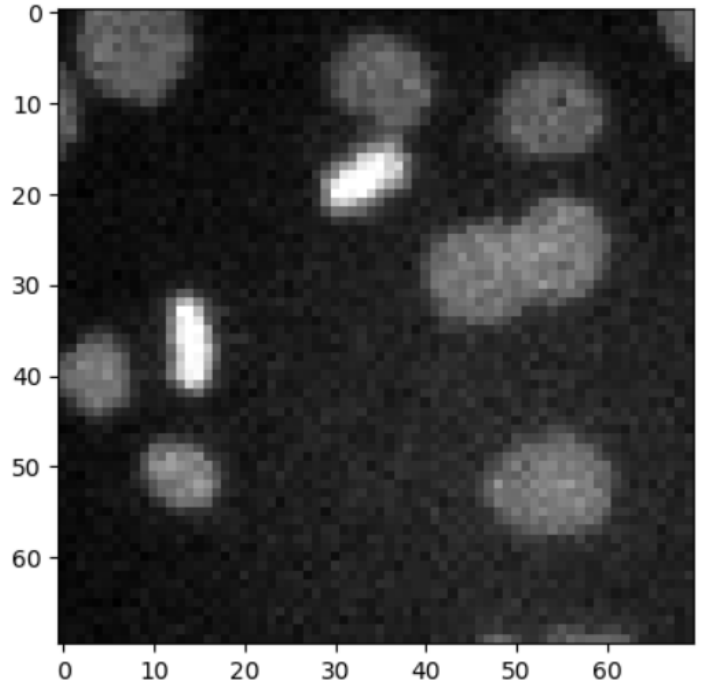


Filters

- Use every opportunity and play with filter parameters to get an idea what they do.

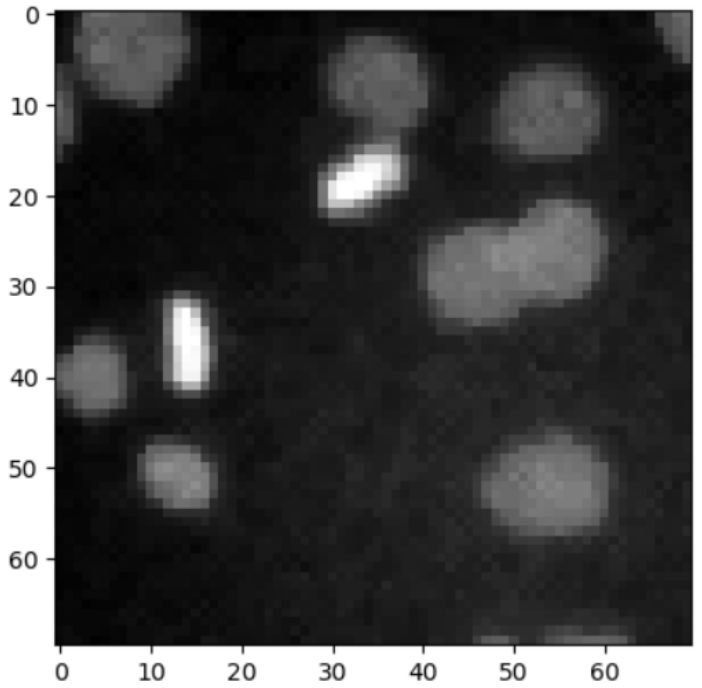
```
plt.imshow(image3, cmap='gray')
```

```
<matplotlib.image.AxesImage at 0x1d86893b6d0>
```



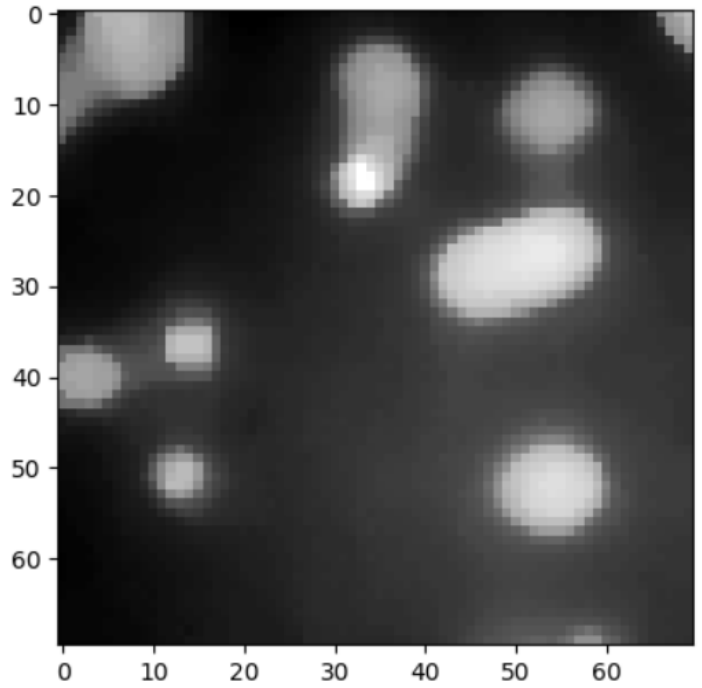

```
denoised_median = filters.median(image3, morphology.disk(1))

plt.imshow(denoised_median, cmap='gray')
```

```
<matplotlib.image.AxesImage at 0x1d868a189d0>
```




```
denoised_median2 = filters.median(image3, morphology.disk(5))

plt.imshow(denoised_median2, cmap='gray')
```

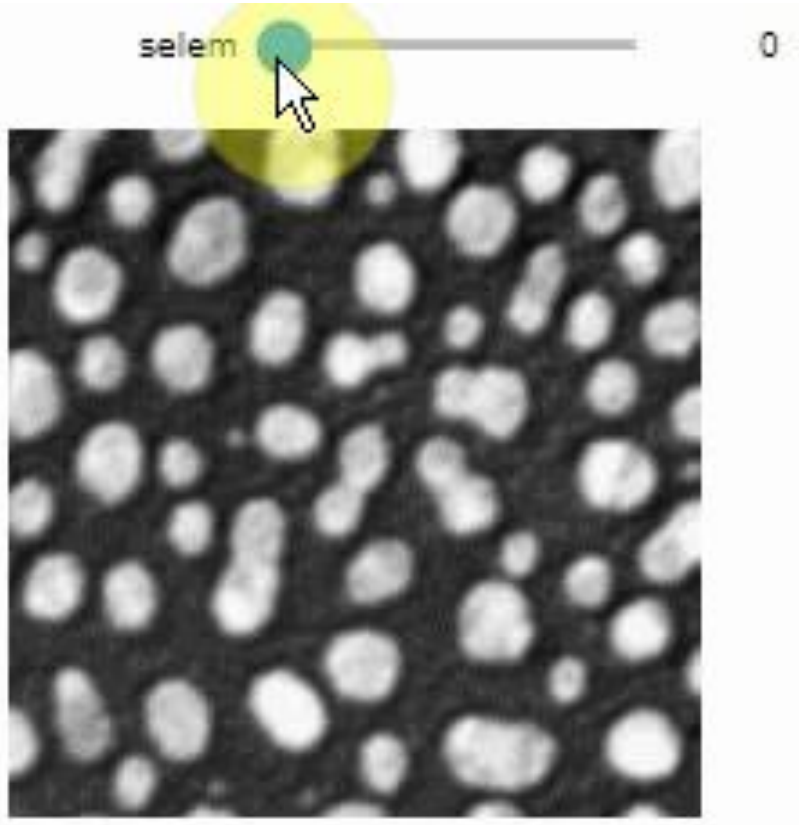
```
<matplotlib.image.AxesImage at 0x1d868ca7af0>
```



Filters

- Use every opportunity and play with filter parameters to get an idea what they do.

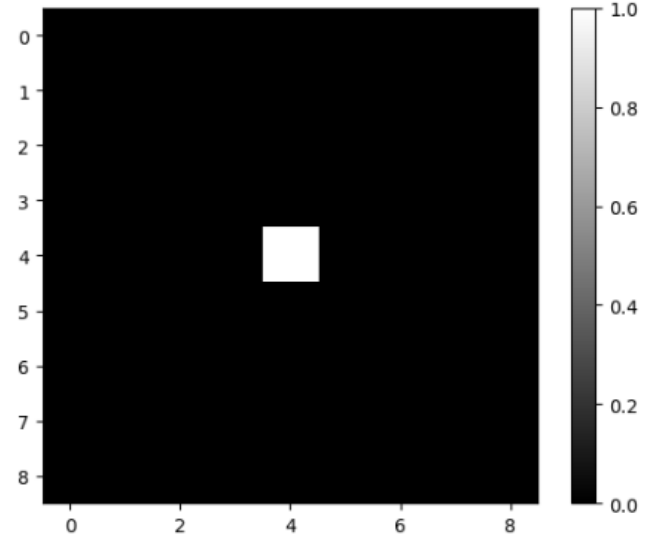
```
from skimage.filters.rank import maximum
stackview.interact(maximum, slice_image)
```



Filters

... may be custom functions

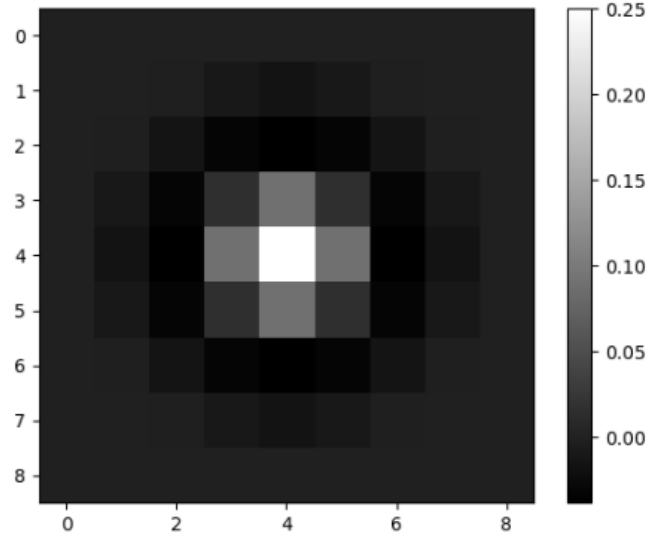
Recommendation: Apply custom filters to super simple images to see if they do the right thing.



```python
def laplacian_of_gaussian(image, sigma):
    """
    Applies a Gaussian kernel to an image and the Laplacian afterwards.
    """

    # blur the image using a Gaussian kernel
    intermediate_result = filters.gaussian(image, sigma)

    # apply the mexican hat filter (Laplacian)
    result = filters.laplace(intermediate_result)

    return result
```

```python
log_image1 = laplacian_of_gaussian(image2, sigma=1)

plt.imshow(log_image1, cmap='gray')
plt.colorbar()
```

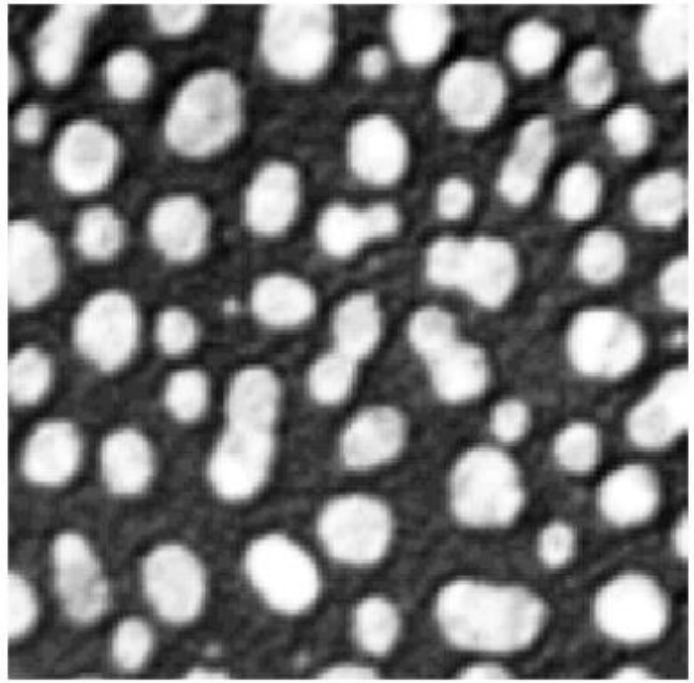
```
<matplotlib.colorbar.Colorbar at 0x283a9679430>
```



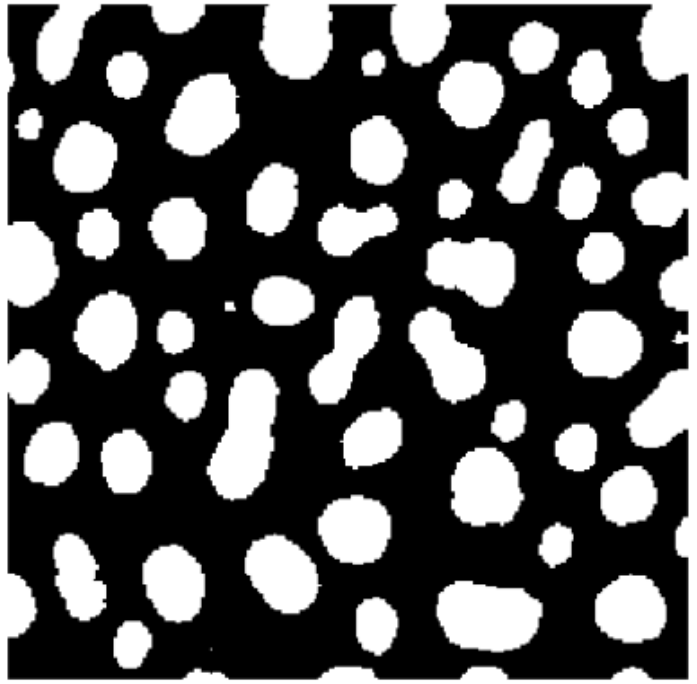
Binarization / Thresholding

- Turn images into binary images (most basic form of segmentation)
- Basic math operation thanks to numpy

```
[2]: image = imread("data/blobs.tif")
     stackview.imshow(image)
```

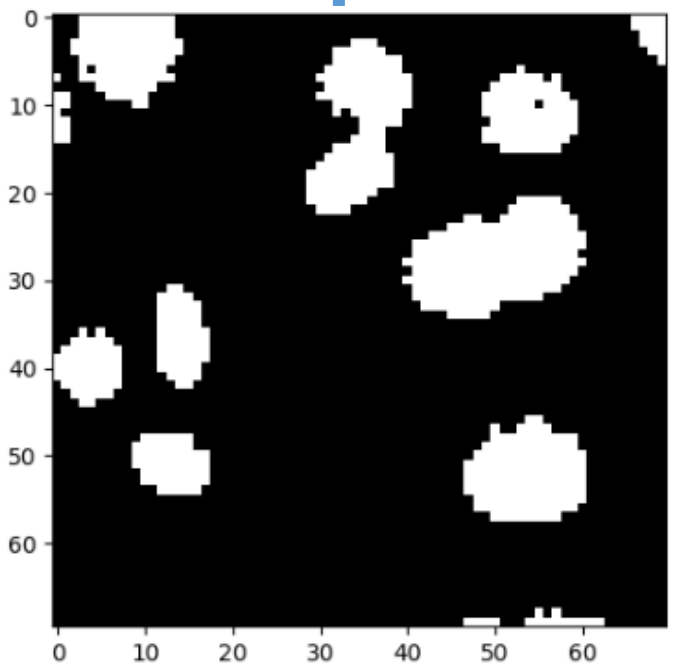


```
[3]: binary_image = image > 128

     stackview.imshow(binary_image)
```



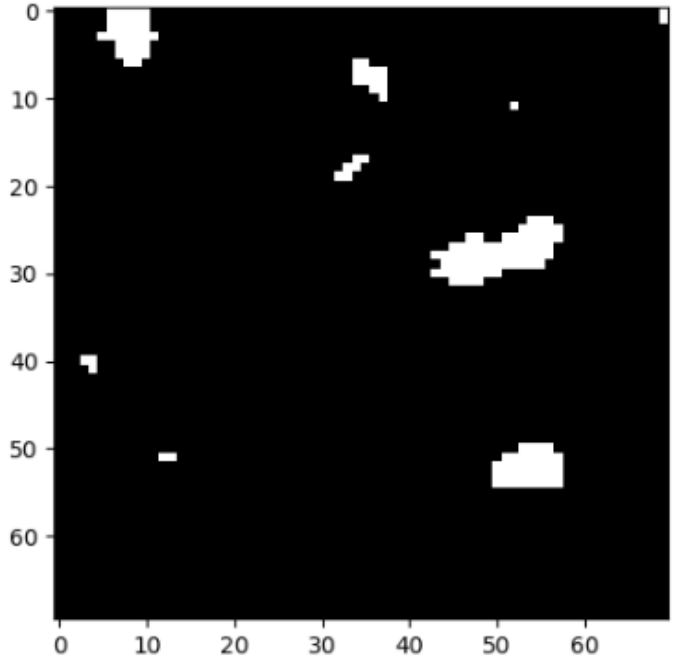
Morphological operations

- To *morph* objects in binary images



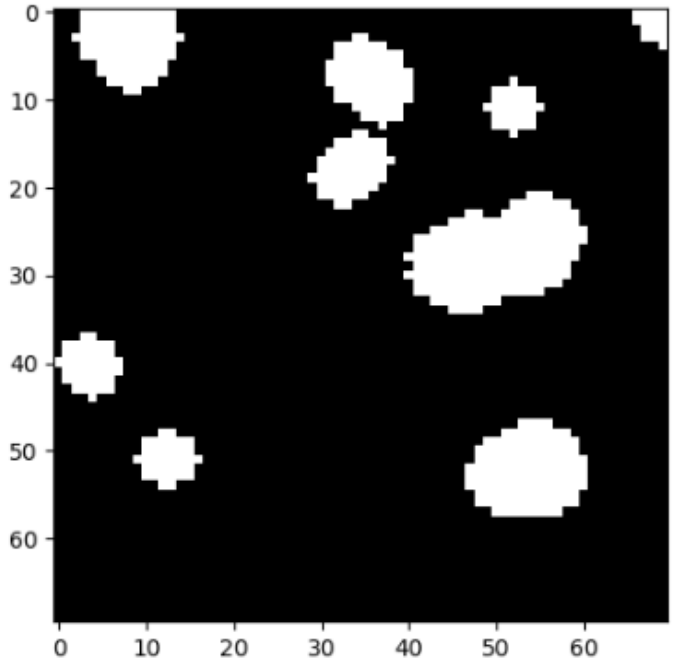

```
eroded = morphology.binary_erosion(image_binary, disk)

plt.imshow(eroded, cmap='gray')
```

```
<matplotlib.image.AxesImage at 0x15288661520>
```



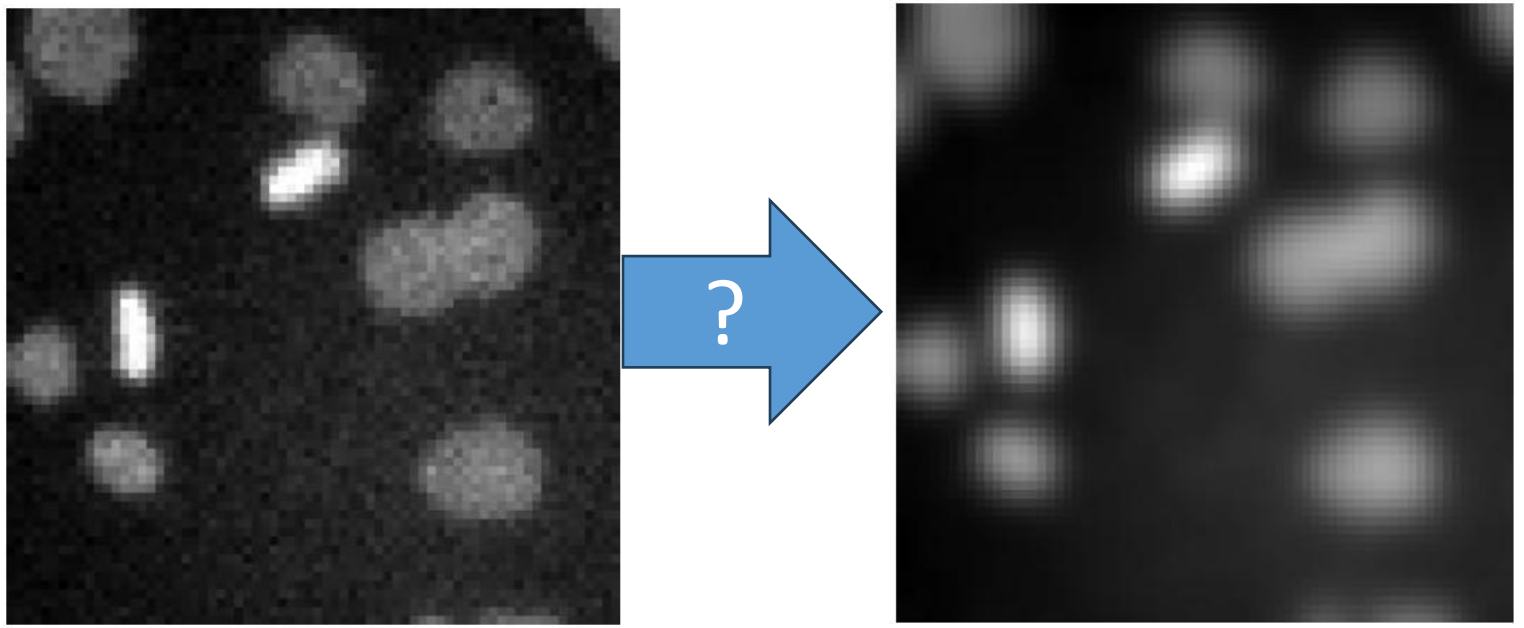

```
eroded_dilated = morphology.binary_dilation(eroded, disk)

plt.imshow(eroded_dilated, cmap='gray')
```

```
<matplotlib.image.AxesImage at 0x1528893ffd0>
```



Quiz: Image processing

Which kind of operation is applied here?



Denoising

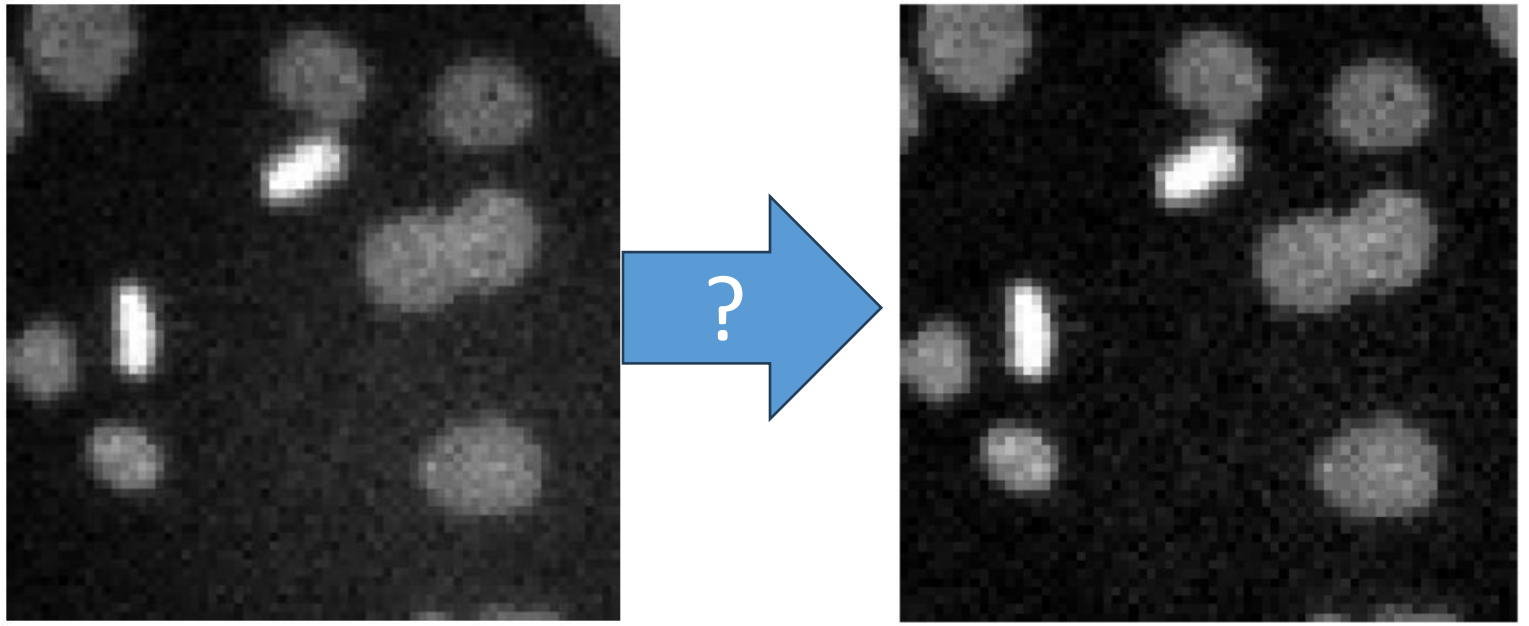
Background removal

Binarization

Morphological operation

Quiz: Image processing

Which kind of operation is applied here?



Denoising

Background removal

Binarization

Morphological operation

ScaDS.AI
DRESDEN LEIPZIG
CENTER FOR SCALABLE DATA ANALYTICS
AND ARTIFICIAL INTELLIGENCE

Exercises

Robert Haase

GEFÖRDERT VOM
Bundesministerium
für Bildung
und Forschung

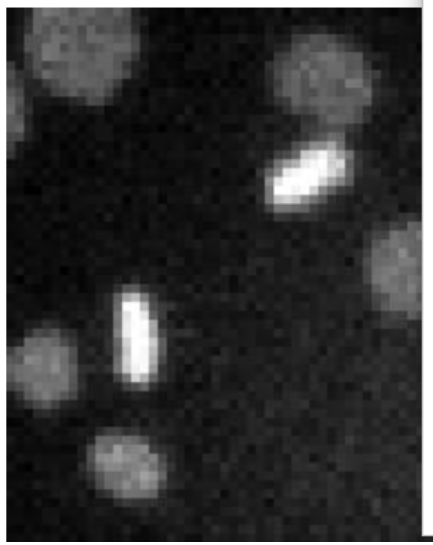
SACHSEN
Diese Maßnahme wird gefördert durch die Bundesregierung aufgrund eines Beschlusses des Deutschen Bundestages.
Diese Maßnahme wird mitfinanziert durch Steuermittel auf der Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.

Hint: Jupyter code hints

- Press SHIFT-Tab within brackets behind a function to read its documentation.

```
[10]: def custom_median(image, radius:int):
          return filters.median(image, morphology.disk(radius))

      stackview.interact(custom_
```

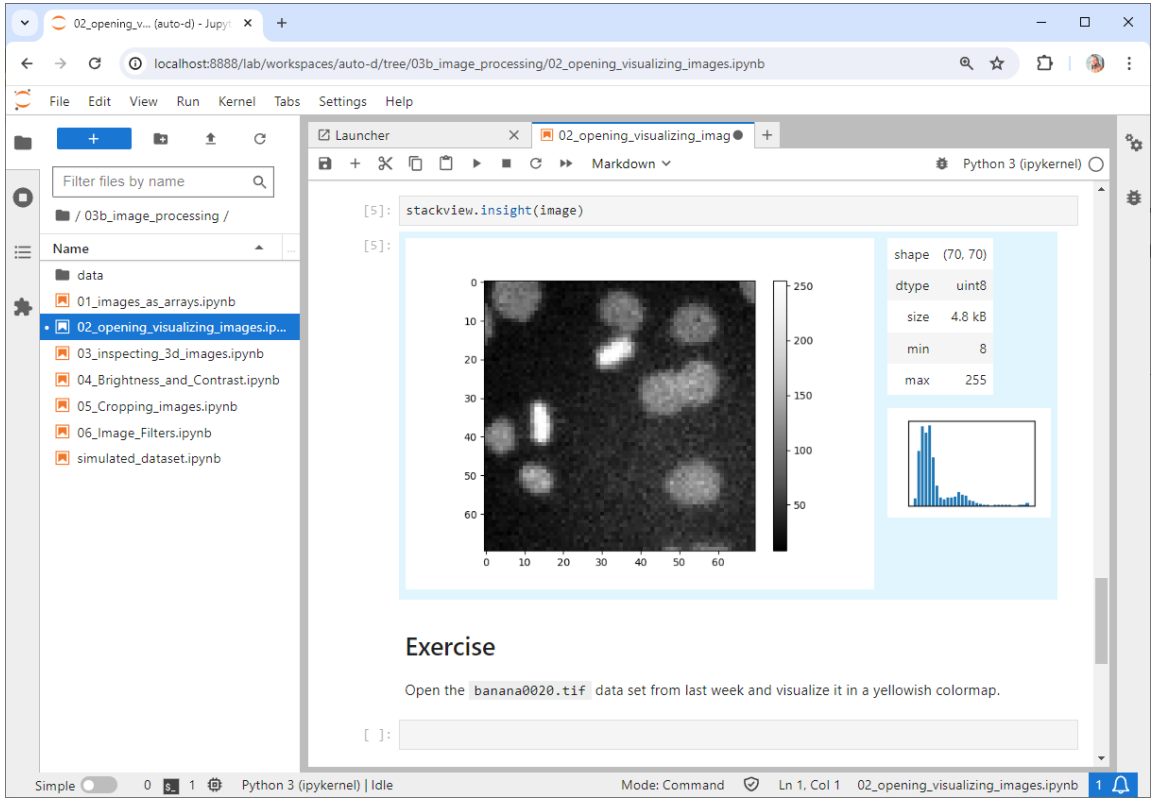
```
[10]: radius

custom_median(..., radiu
```

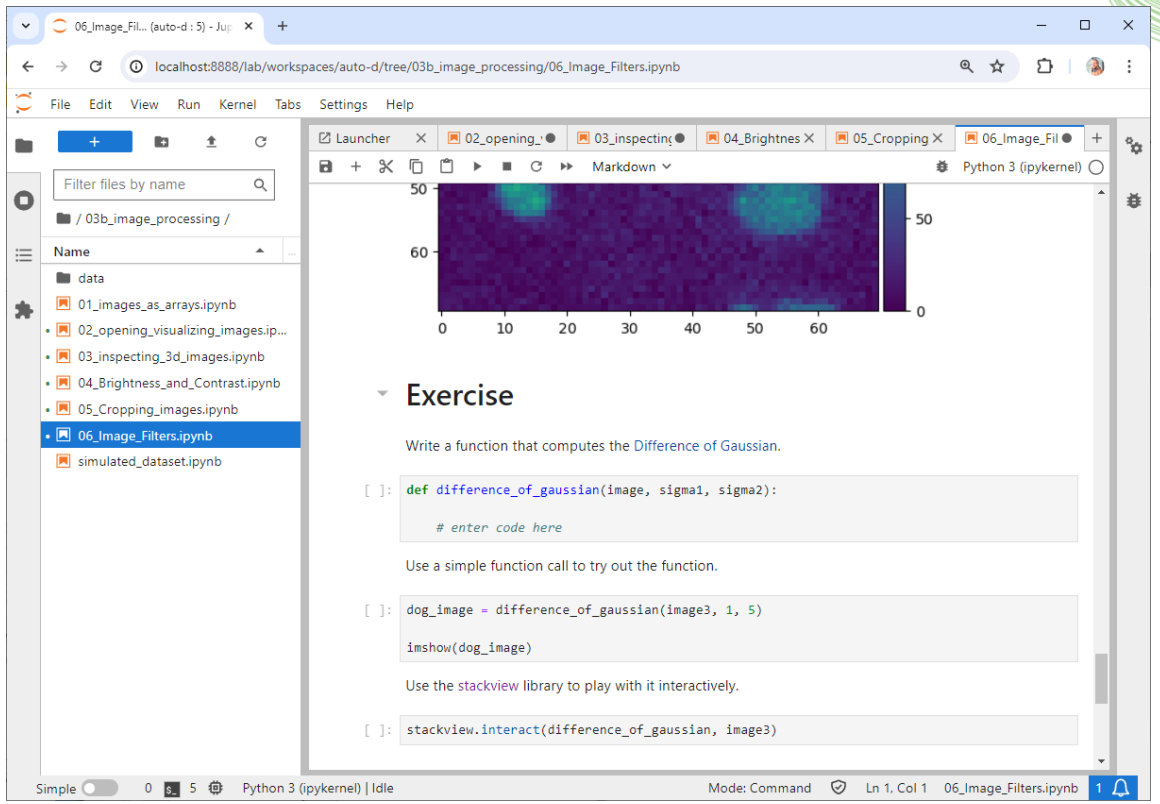


```
Signature:
filters.median(
    image,
    footprint=None,
    out=None,
    mode='nearest',
    cval=0.0,
    behavior='ndimage',
)
Docstring:
Return local median of an image.

Parameters
----------
image : array-like
    Input image.
footprint : ndarray, optional
    If ``behavior=='rank'``, ``footprint`` is a 2-D array of 1's and 0's.
    If ``behavior=='ndimage'``, ``footprint`` is a N-D array of 1's and 0's
    with the same number of dimension than ``image``.
```

Exercise: image processing

- Get started with loading, viewing, cropping and processing images

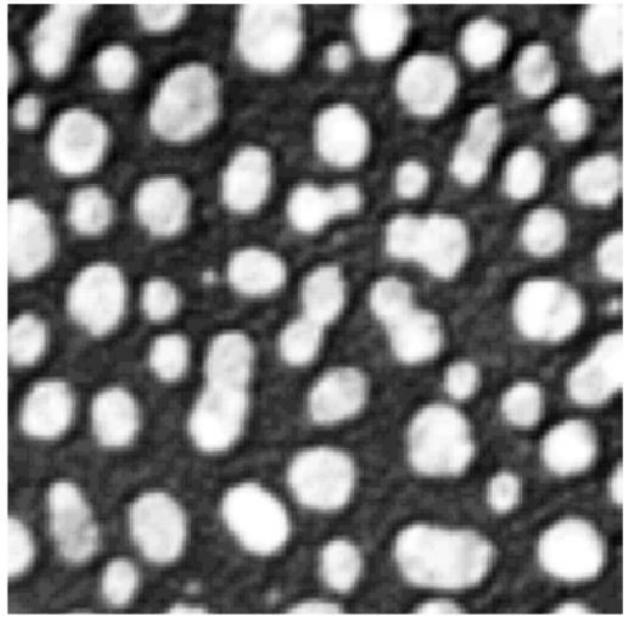




Exercise: dependencies

- There is a Jupyter Notebook which doesn't work (anymore). Find out why.
- Fix it in two ways:
  - A) by changing the code
  - B) by *not* changing the code

```
[2]: image = imread("data/blobs.tif")
     stackview.imshow(image)
```



```
[3]: filtered = median(image, selem=np.ones((7,7)))

     stackview.imshow(filtered)
```