Image thresholding

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Segmentation

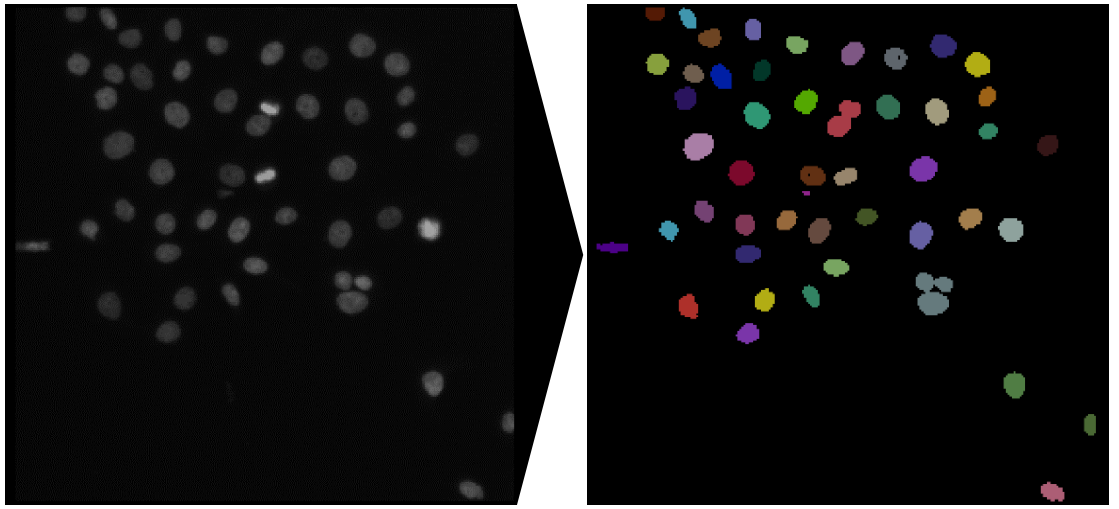
https://scikit-image.org/docs/stable/api/skimage.data.html

Aim:

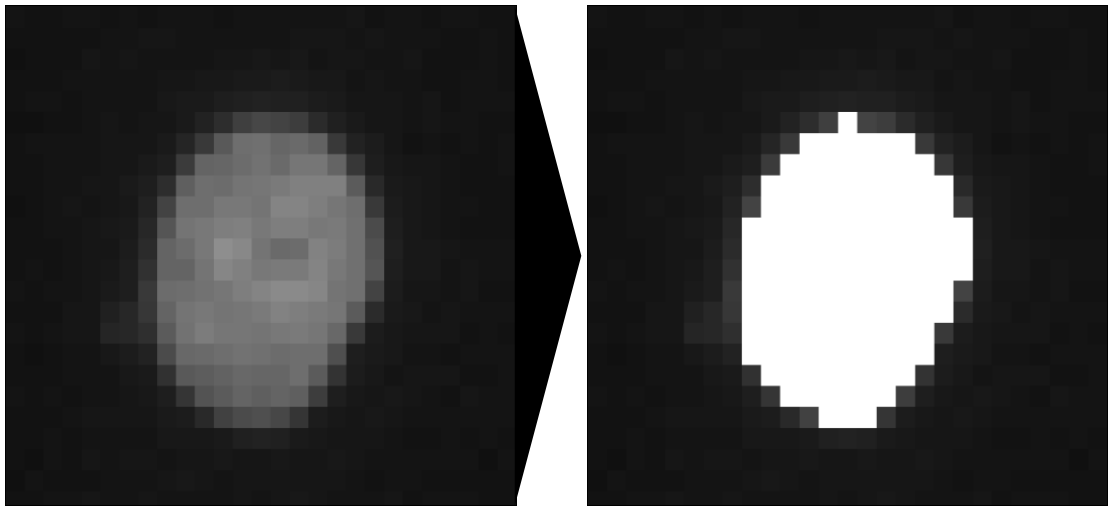
Separate background from foreground

Vocabulary:

- **Segmentation:**
  → Assigning a meaningful *label* to each pixel
  → Segmentation is a *classification* problem
- **Semantic segmentation:**
  Differentiate pixels into multiple *classes* (e.g., membrane, nucleus, cytosol, etc.)
- **Instance segmentation:**
  Differentiate multiple occurrences of the same class into separate instances of this class (e.g., separate *label* for each cell in image)



Instance segmentation

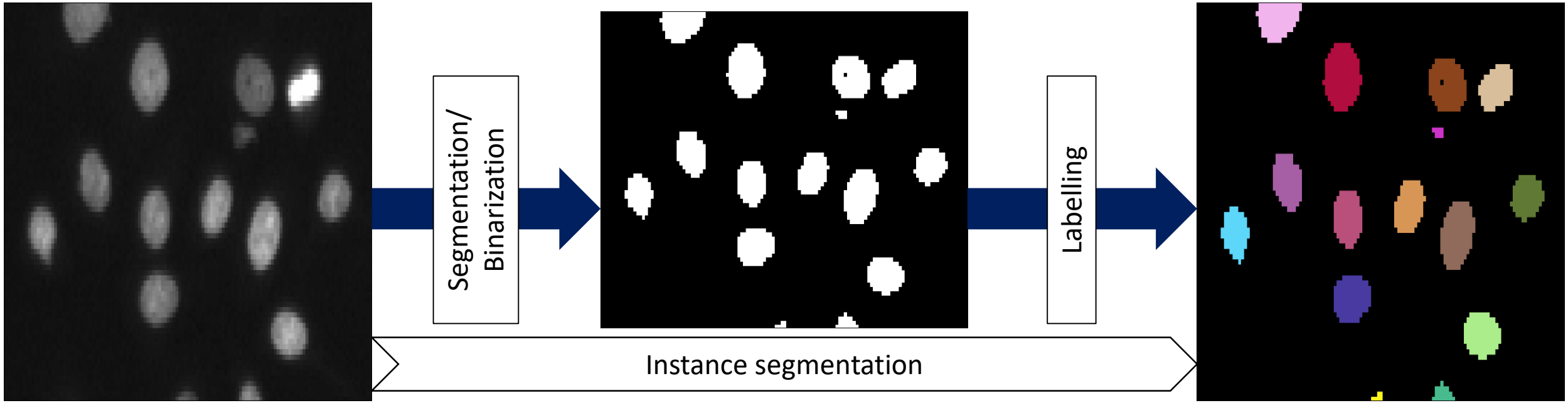


Semantic segmentation

Segmentation and labelling

Analyzing properties (*features*) of individual objects in images requires instance segmentation

- Methods
  - Thresholding + connected components labeling
  - Spot detection + seeded watershed
  - Edge detection based
  - Machine learning

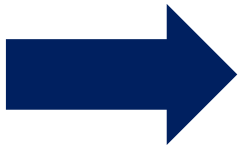


Thresholding in Python

- Applying a threshold to an image requires to compare every pixel to the threshold value
- We can compare values in Python with:

```
a = 5
b = 6
print(a > b)
print(a < b)
print(a==b)
```

```
False
True
False
```



```
image > threshold
```

```
array([[False, False, False, ..., False, False, False],
       [False, False, False, ..., False, False, False],
       [False, False, False, ..., False, False, False],
       ...,
       [False, False, False, ..., False, False, False],
       [False, False, False, ..., False, False, False],
       [False, False, False, ..., False, False, False]])
```

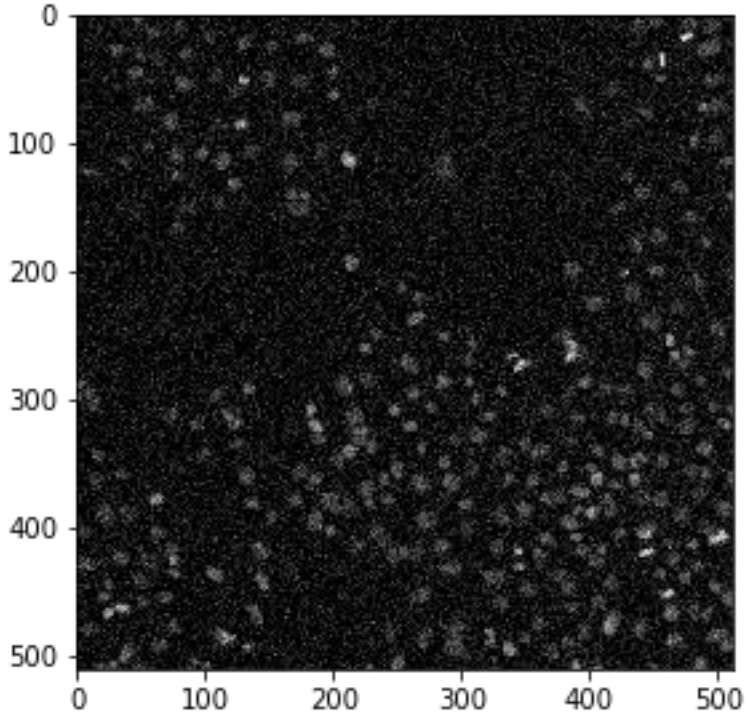
In this case, “image” is a *numpy array* → some operations are automatically applied to every pixel!

- We can then simply store the output of this element-wise comparison in a new variable:

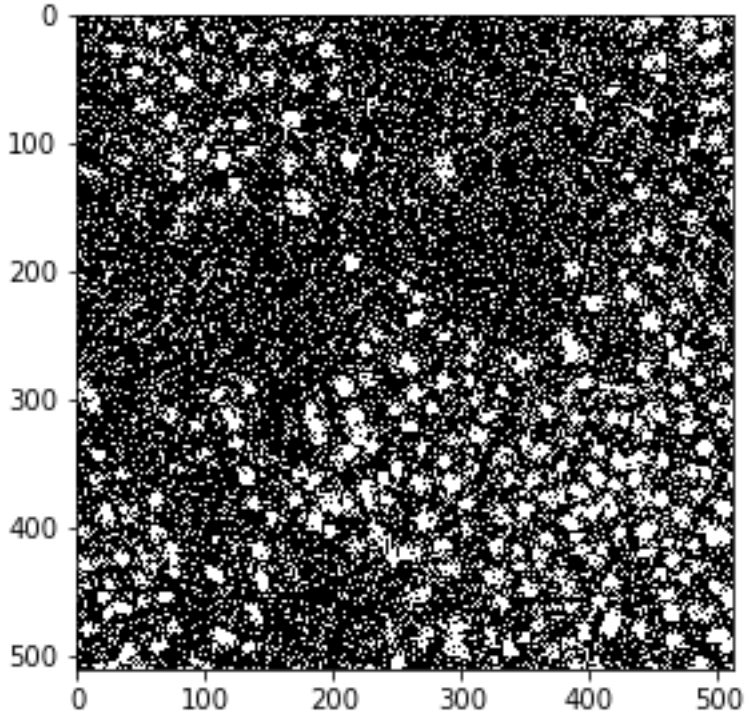
```
binary = image > threshold
```

Reminder: pre-processing!

- Before we can create masks, we need to pre-process images:
  - Noise removal
  - Background subtraction



Noisy image



Thresholded image

```
filtered = filters.median(image)
```

Image filtering *filters* relevant information for subsequent operations from the image!

Reminder: pre-processing!

- Before we can create masks, we need to pre-process images.
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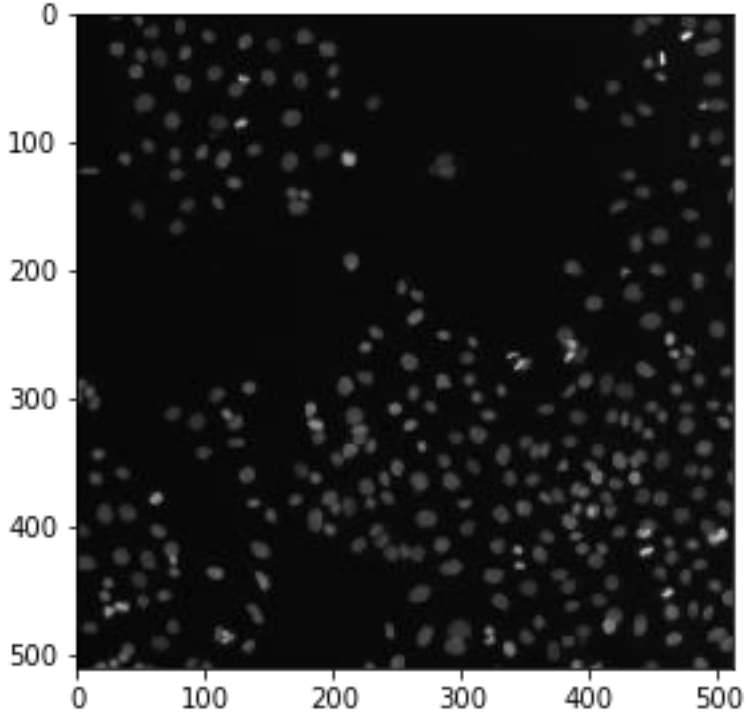
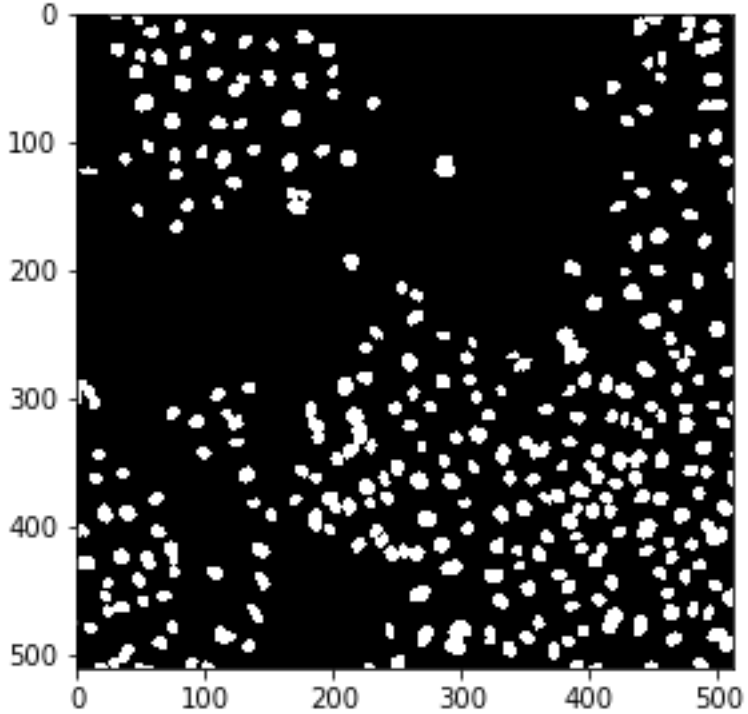
  - Background subtraction



Filtered image



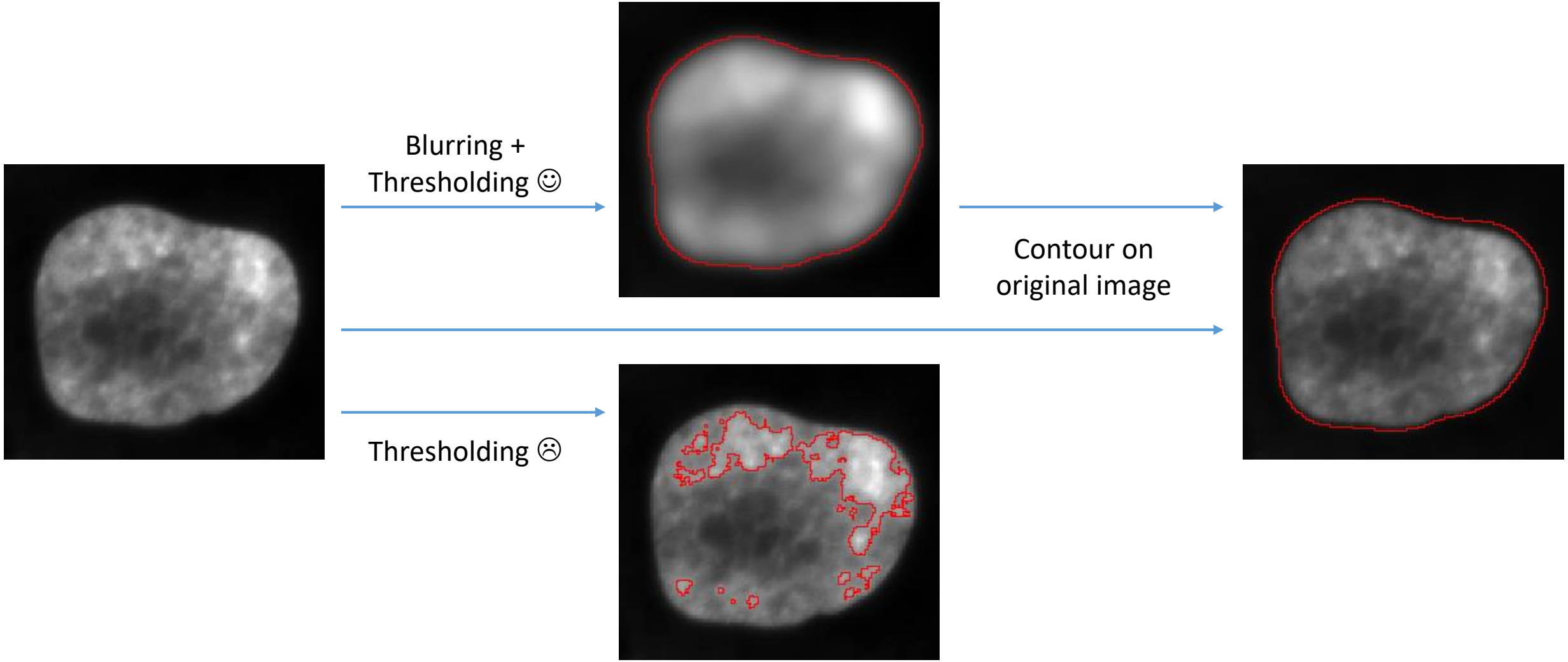
Thresholded image

```
filtered = filters.median(image)
```

Image filtering *filters* relevant information for subsequent operations from the image!

Low-pass filtering to improve thresholding results

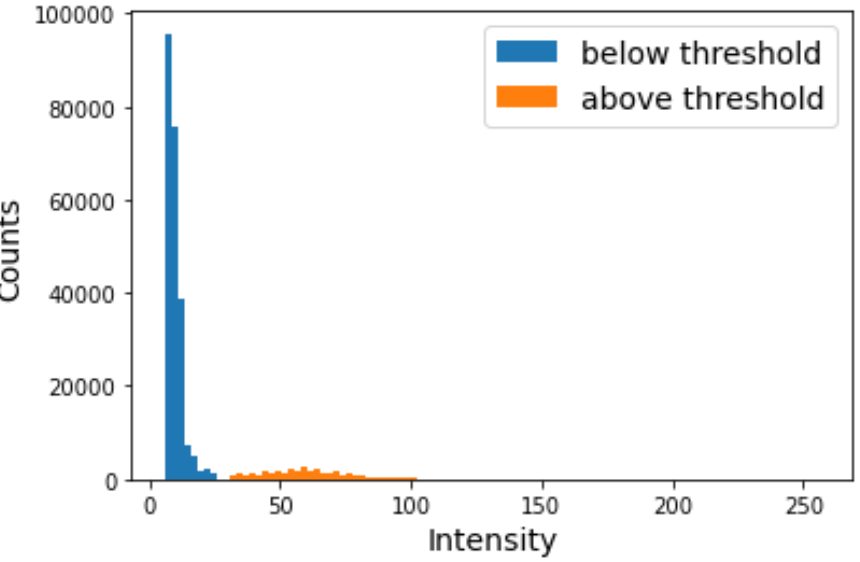
- In case thresholding algorithms outline the wrong structure, blurring in advance may help.
- However: **Do not** continue processing the blurred image, continue with the original!

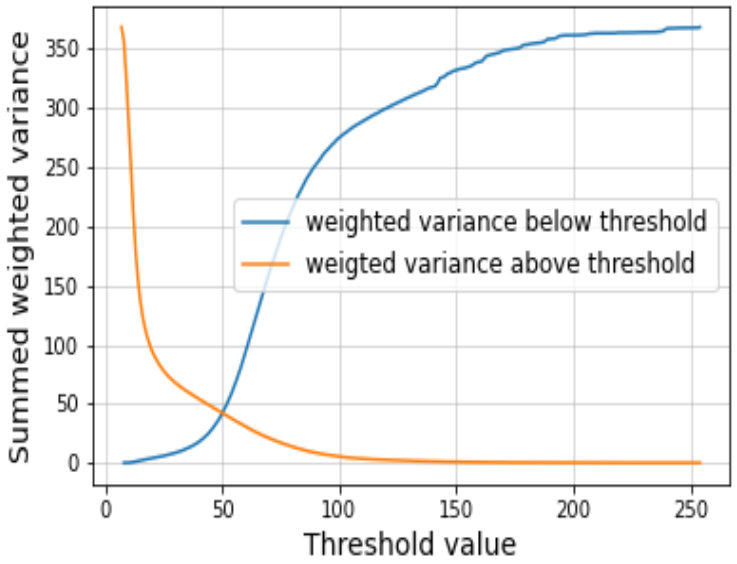


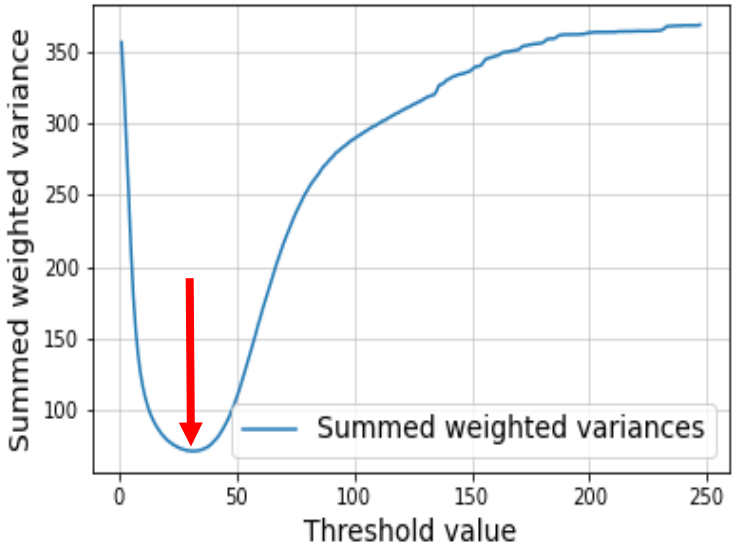
Threshold algorithms

- **Otsu-thresholding** (Otsu et Al. 1979): Find threshold so that the summed, weighted variance $Var_{w,sum}$ becomes minimal:

$$Var(I) = \frac{1}{n_I}\sum\left(I - mean(I)\right)^2 \quad \rightarrow \quad Var_{w,sum} = \frac{n_A}{n_I} \cdot Var(A) + \frac{n_B}{n_I} \cdot Var(B)$$



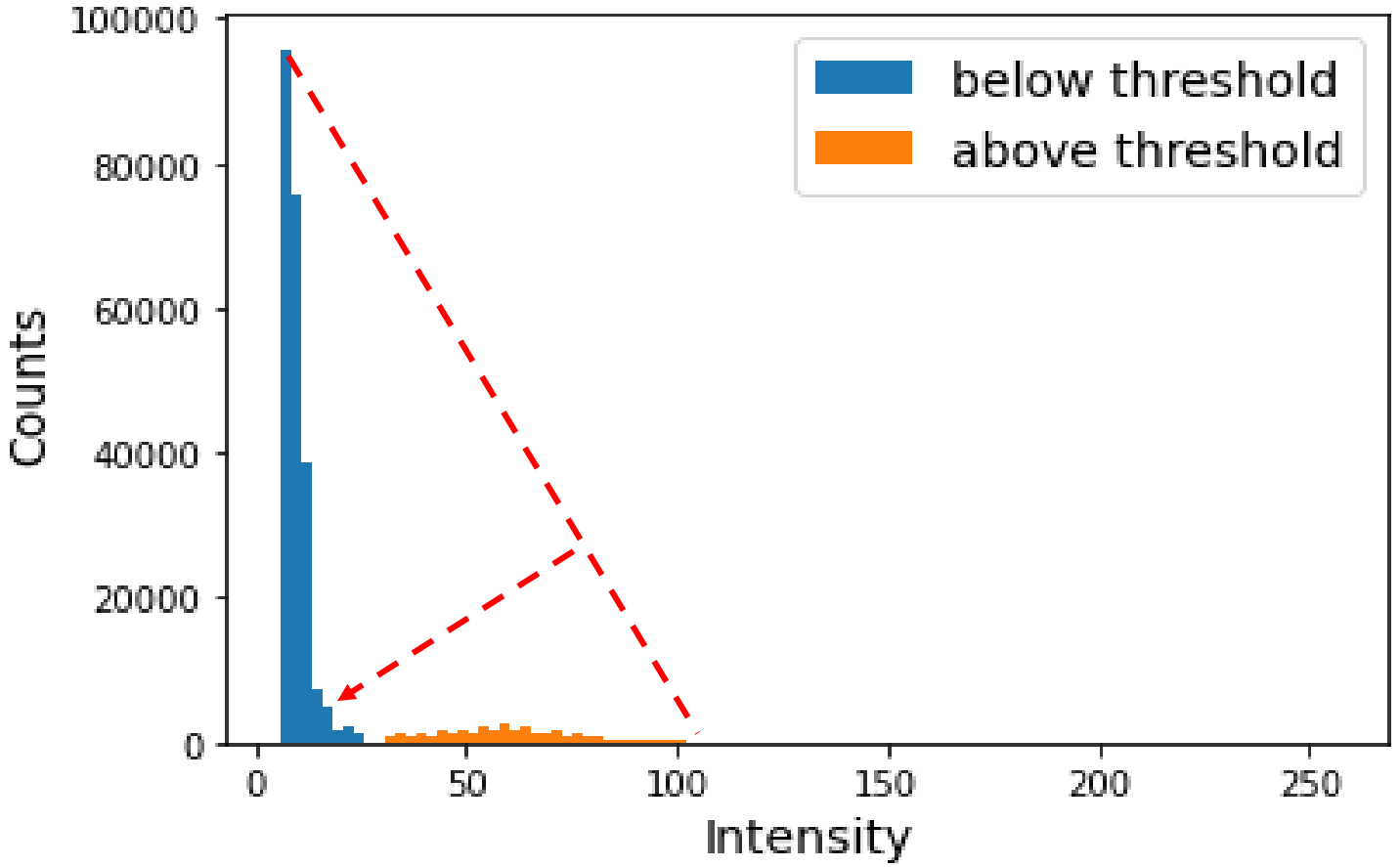




- **Statistical thresholding**: Pixels above statistical parameter of I belong to foreground. (Possibilities: Mean, Median, Quartiles, etc.)

Threshold algorithms

- **Triangle thresholding**: Draw a line between histogram point with max. counts and max. intensity and find point in histogram with maximal distance to this line (----)



Thresholding with scikit-image

```
threshold = filters.threshold_otsu(image)
```

- **Otsu-thresholding** (Otsu et Al. 1979): Find threshold so that the summed, weighted variance $Var_{w,sum}$ becomes minimal.

```
threshold = filters.threshold_mean(image)
```

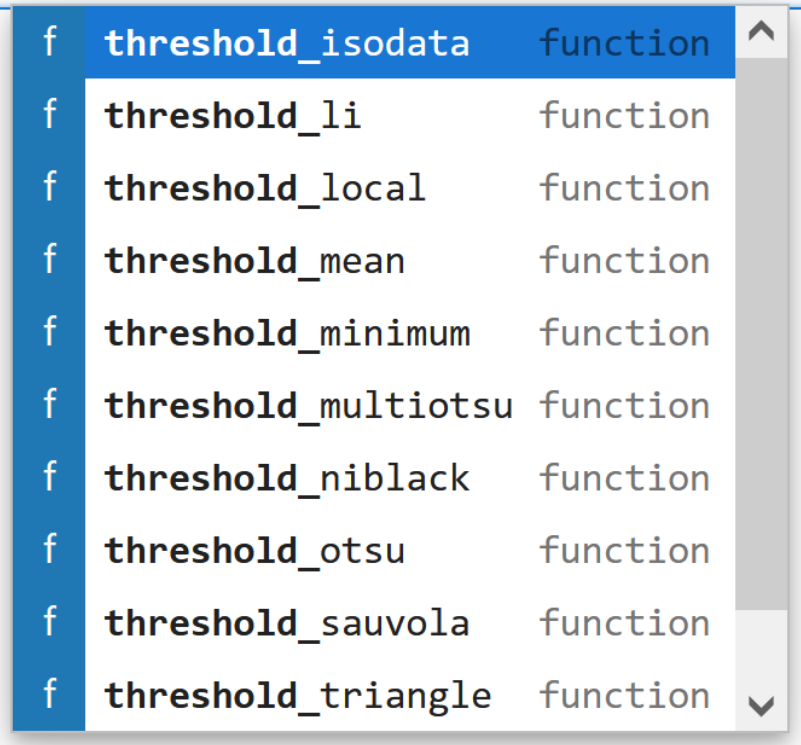
- **Statistical thresholding**: Pixels above statistical

```
threshold = filters.threshold_triangle(image)
```

- **Triangle thresholding**: Draw a line between histogram point with max. counts and max. intensity and find point in histogram with maximal distance to this line.

Explore more threshold options in scikit-image with:

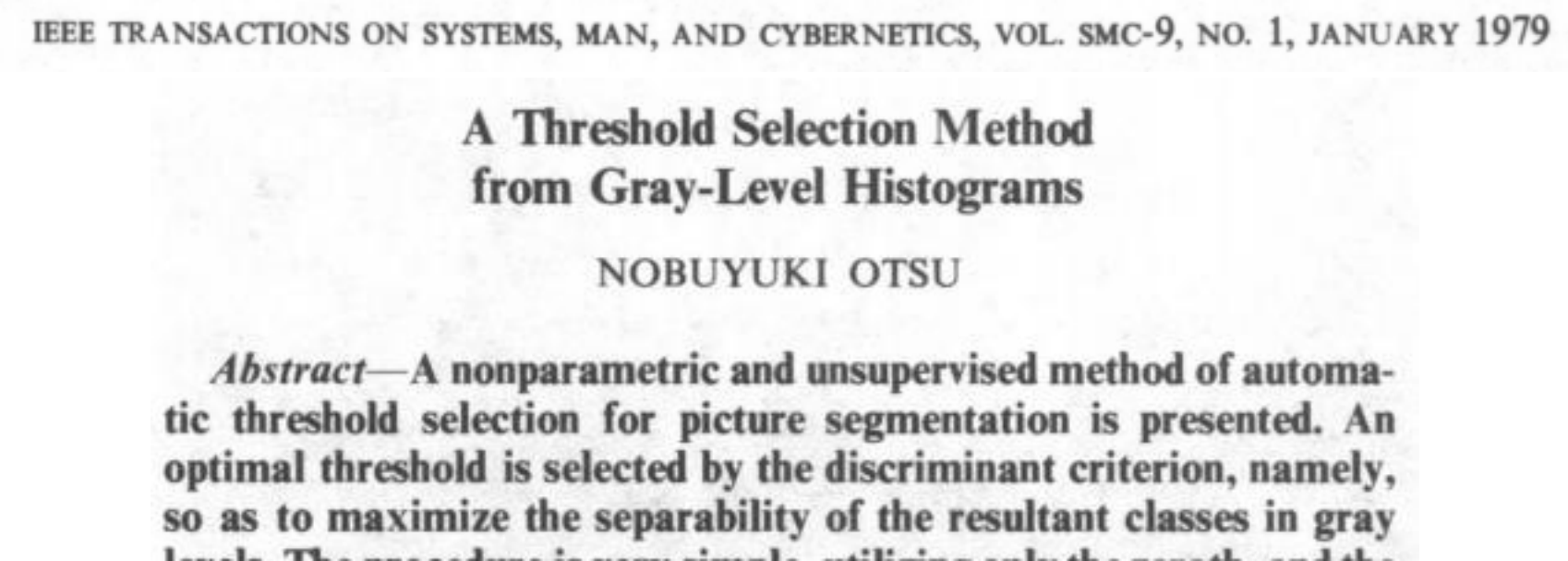
```
from skimage import filters

threshold = filters.threshold_
```



Thresholding: Citing

- Cite the thresholding method of your choice properly

> *We segmented the cell nuclei in the images using the Otsu thresholding method (Otsu et Al. 1979) implemented in scikit-image (van der Walt et Al. 2014).*



IEEE TRANSACTIONS ON SYSTEMS, MAN, AND CYBERNETICS, VOL. SMC-9, NO. 1, JANUARY 1979

A Threshold Selection Method from Gray-Level Histograms

NOBUYUKI OTSU

Abstract—**A nonparametric and unsupervised method of automatic threshold selection for picture segmentation is presented. An optimal threshold is selected by the discriminant criterion, namely, so as to maximize the separability of the resultant classes in gray**

Thresholding: Pitfalls

```
binary = image > a_good_threshold_value_of_my_choice
```

Inter-observer variability

Never use manual thresholding!

- Different observers come to different results when selecting a “good” threshold value
- ➢ You may come to different results when selecting a threshold value repeatedly

```
binary = image > threshold
intensities = some_function_to_measure_intensities(binary, image)
```

Intra-observer variability

Avoid thresholding an image and afterwards measure intensities in the same image

- You would measure the threshold you entered

```
binary_1 = image_1 > threshold_1(image_1)
binary_2 = image_2 > threshold_2(image_2)
```

Chose one threshold algorithm:

…and stick to it for the whole study. Using a new method for every image impairs reproducibility!

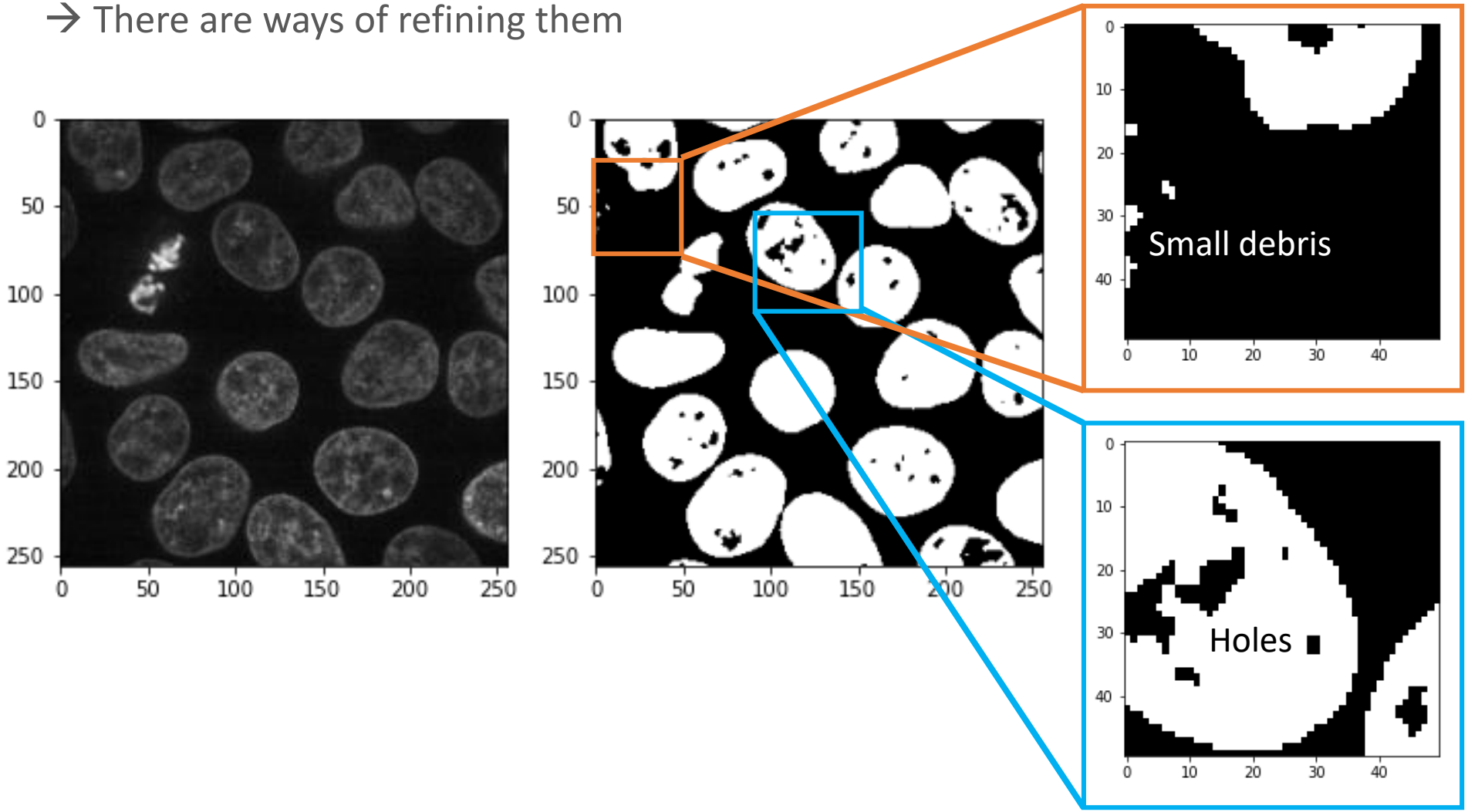
Do not over-engineer

There will be always images where thresholding fails – better report the errors!

Refining masks

Binary mask images may not be perfect immediately after thresholding.

→ There are ways of refining them

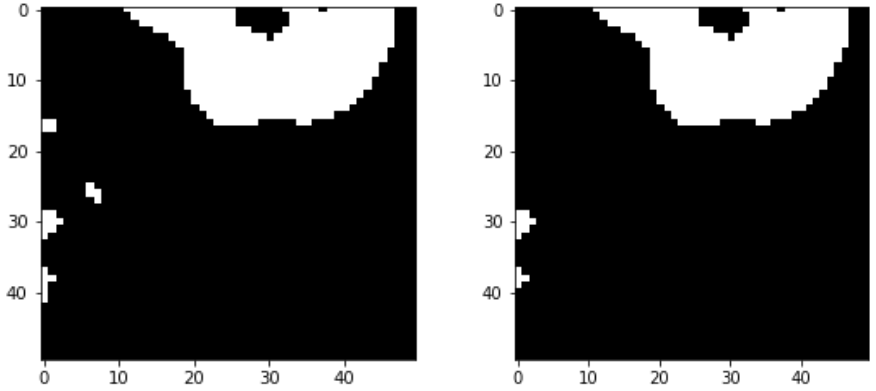


Refining masks: Opening & Closing

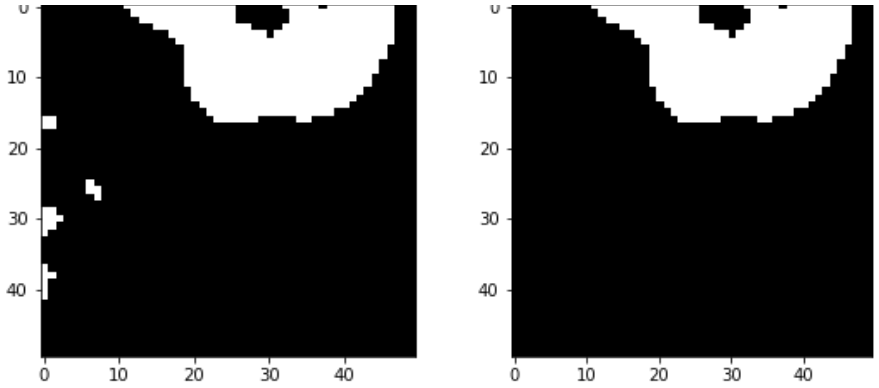
PoL Physics of Life TU Dresden

```
from skimage import morphology
```

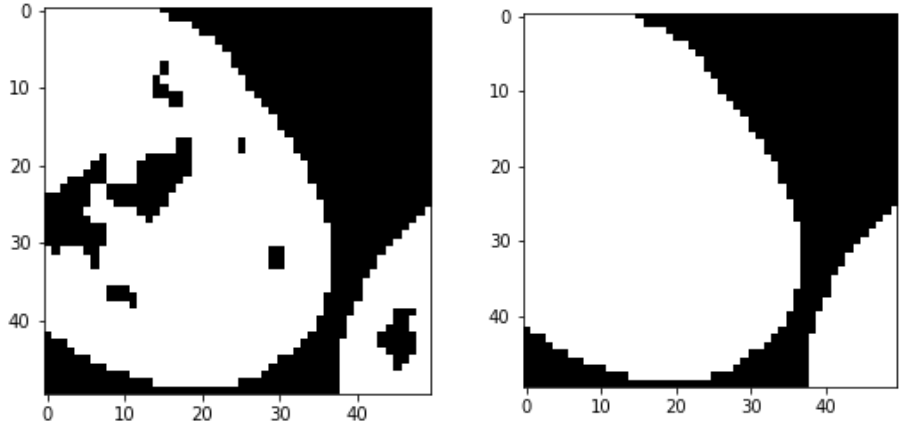
```
opened = morphology.binary_opening(binary)
```



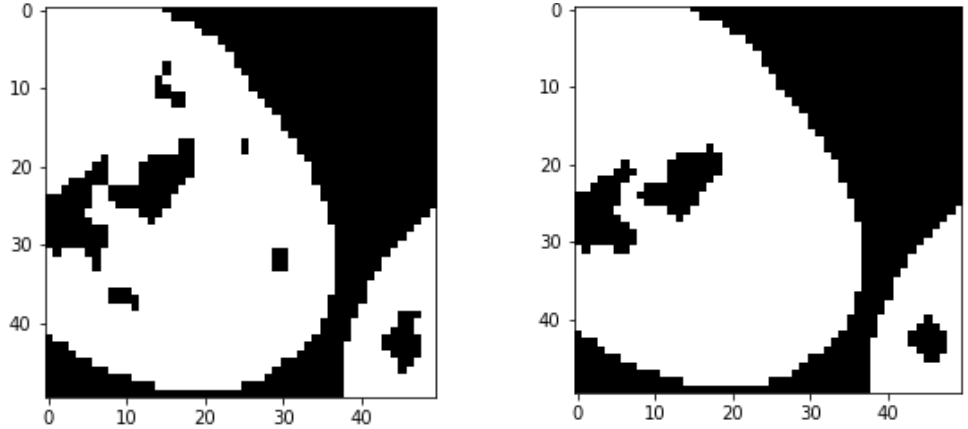
```
opened = morphology.area_opening(binary, area_threshold=20)
```



```
closed = morphology.area_closing(binary, area_threshold=100)
```



```
closed = morphology.binary_closing(binary)
```

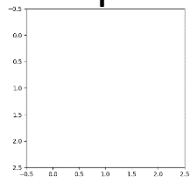


Pitfalls

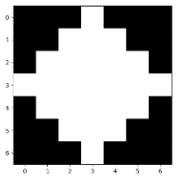
- Eroding/dilating binary images with structural element tends to reproduce the structural element

```
n_iters = 2
for i in range(n_iters):
    disk = morphology.erosion(disk, footprint=SE)

for i in range(n_iters):
    disk = morphology.dilation(disk, footprint=SE)
```

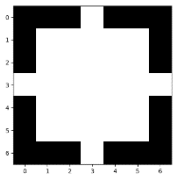
square

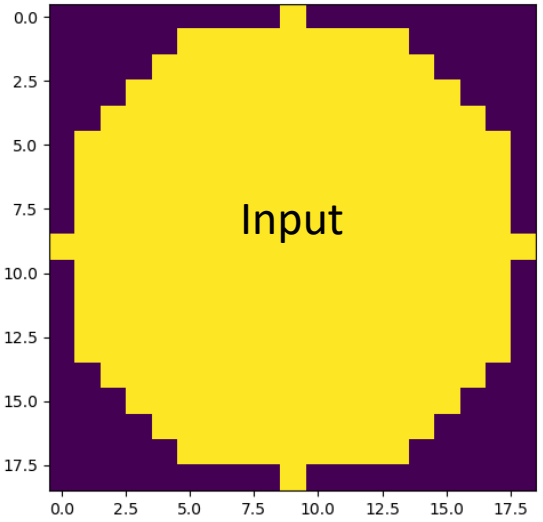


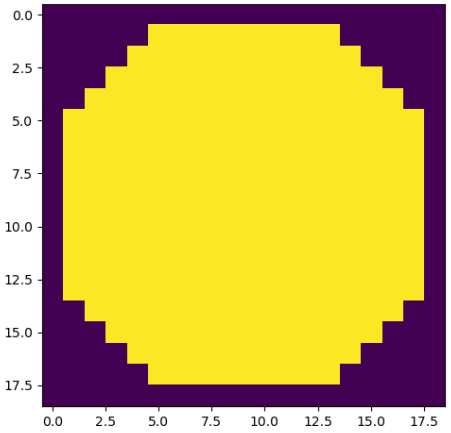
diamond

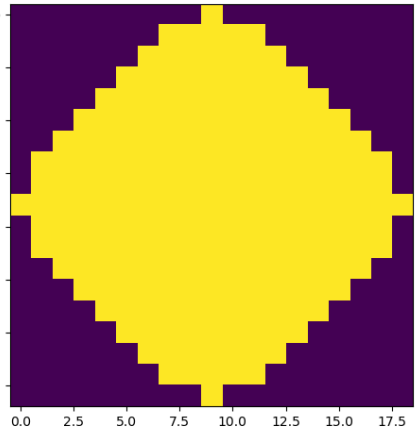


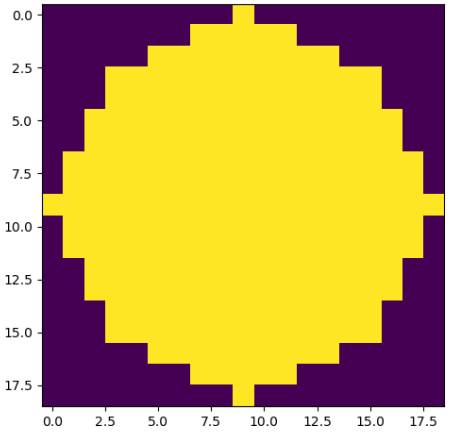
disk









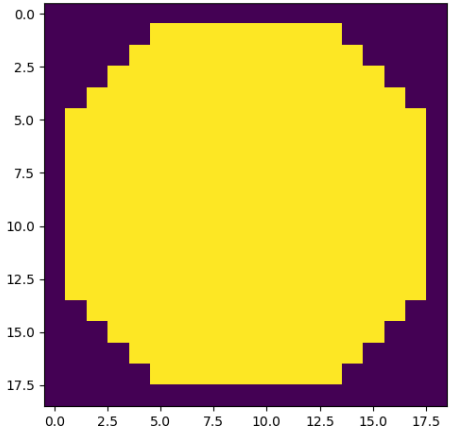


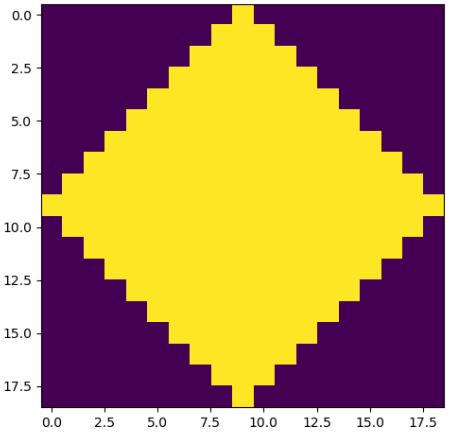
n_iters=2

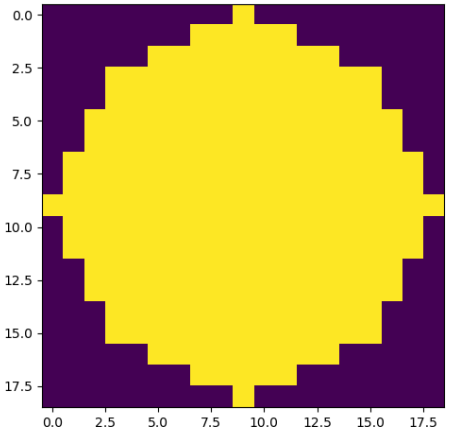
square

diamond

disk







n_iters=3