Image thresholding

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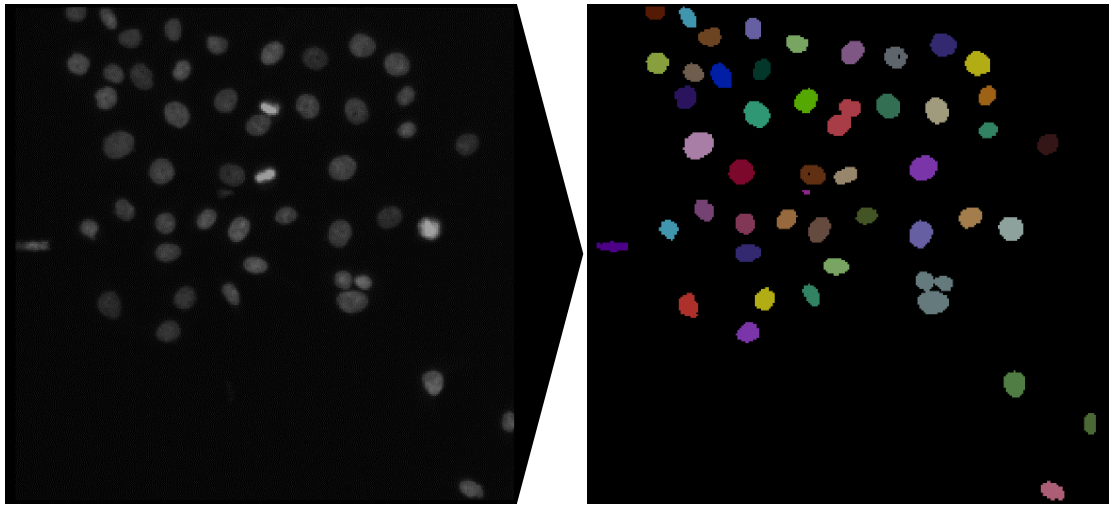
Segmentation

Aim:

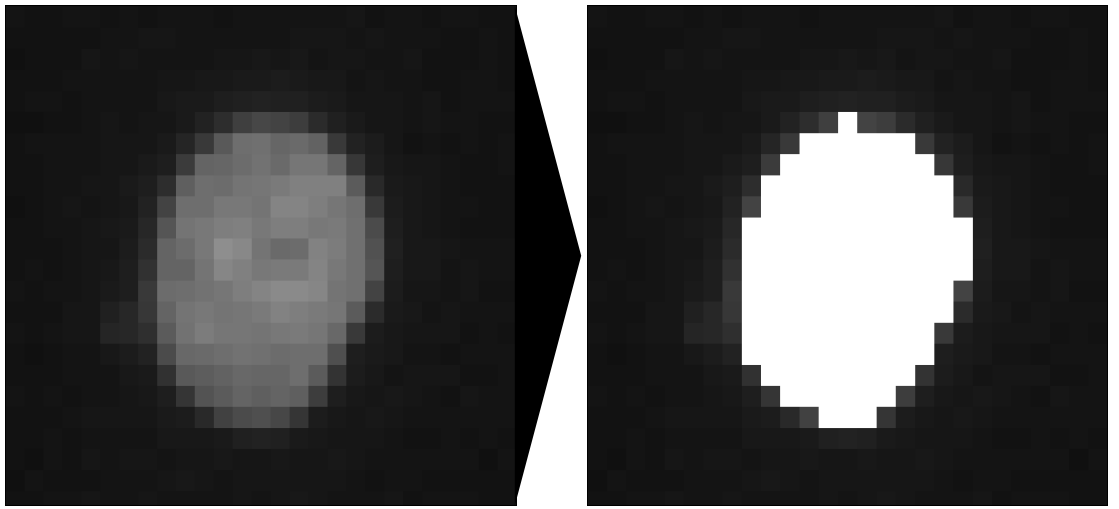
Separate background from foreground

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



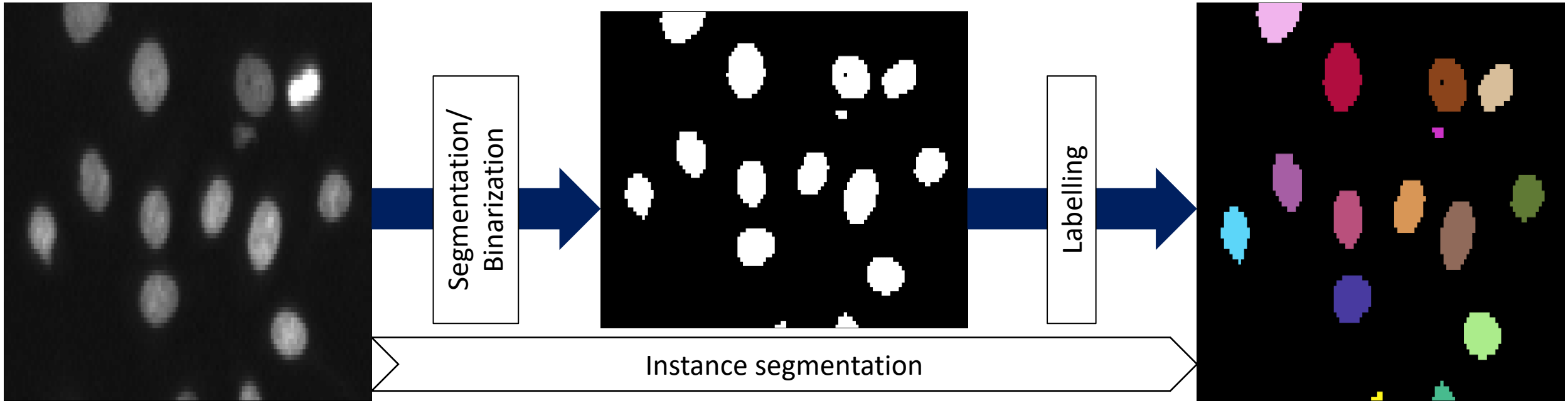
Semantic segmentation

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

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



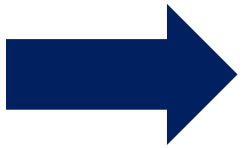
https://scikit-image.org/docs/stable/api/skimage.data.html

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]])
```

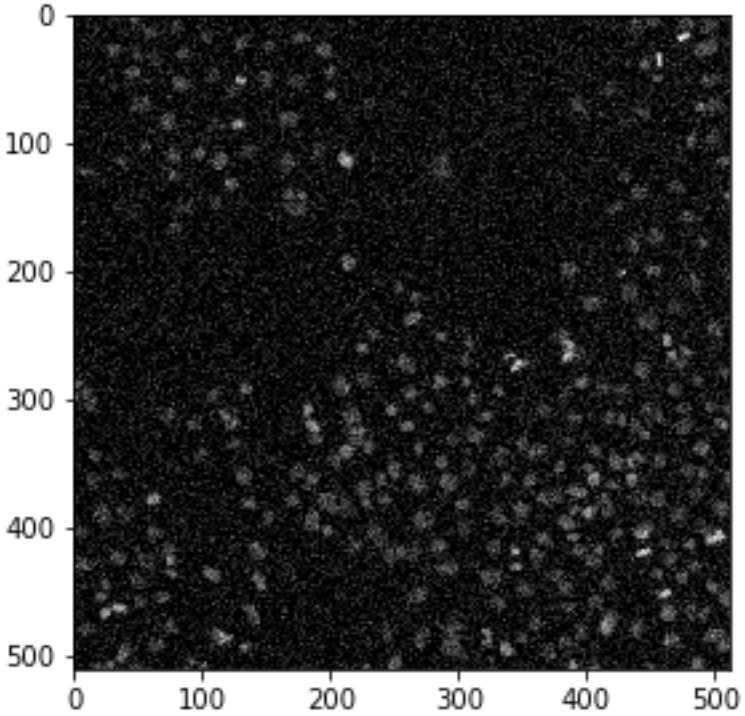
In this case, “image” is a *numpy array* → some operations are automatically applied to every pixel!

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

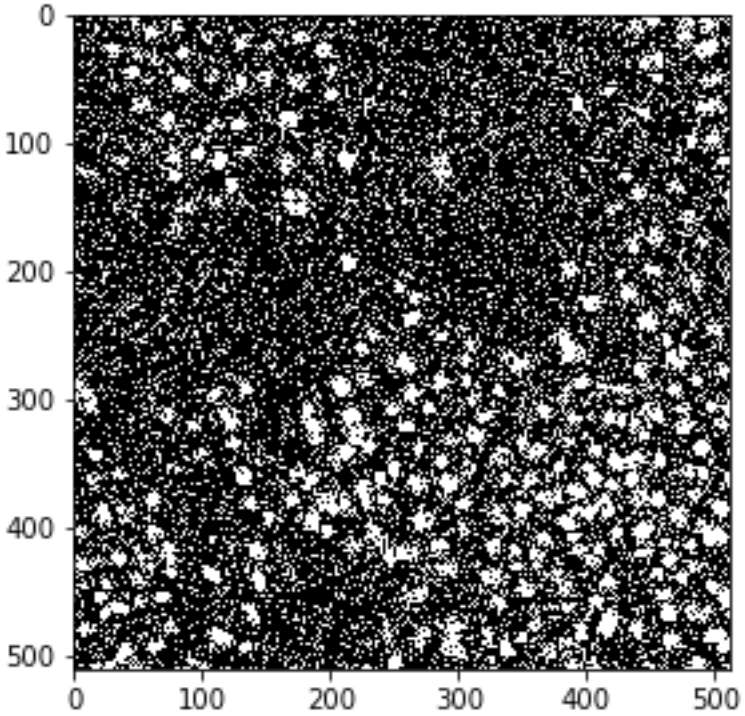
```
binary = image > threshold
```

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!

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

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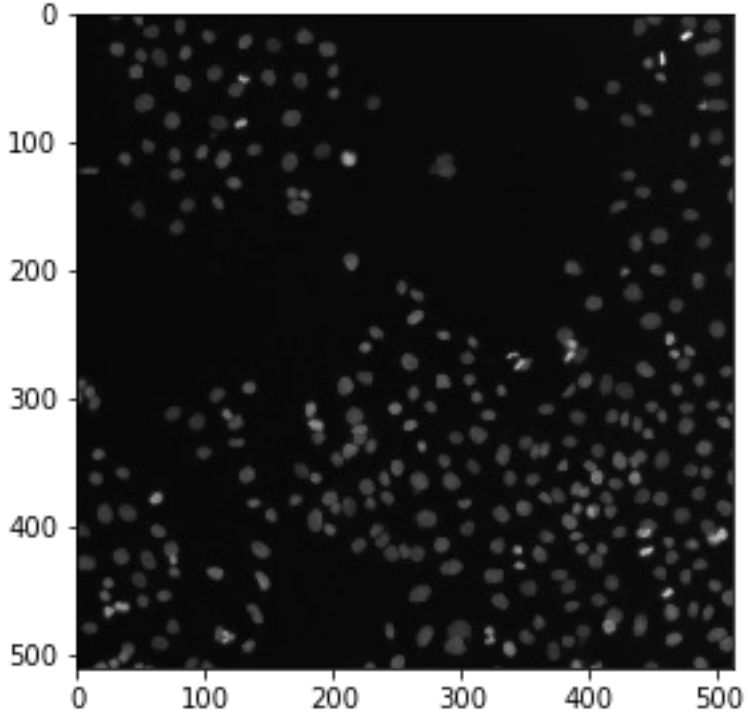
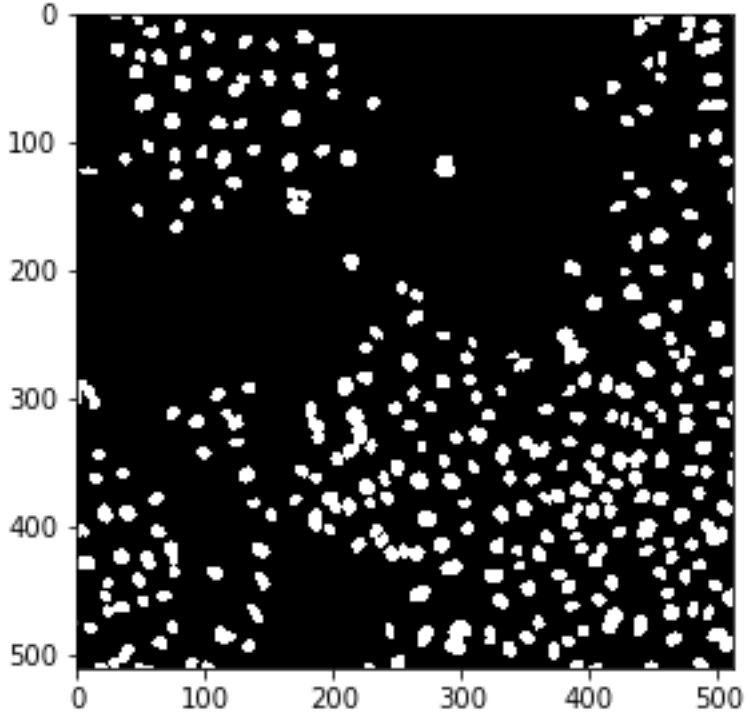


Filtered image



Thresholded image

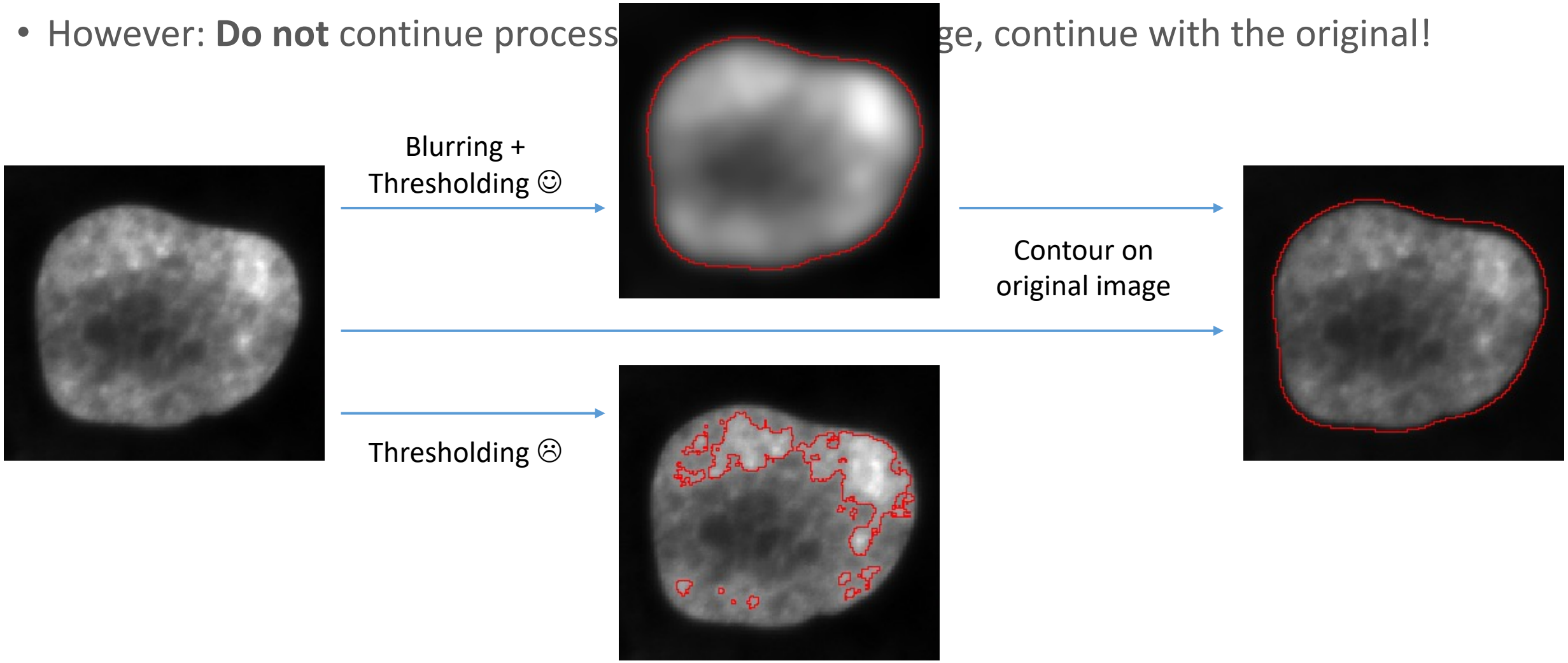
```
filtered = filters.median(image)
```

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

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

Low-pass filtering to improve thresholding results

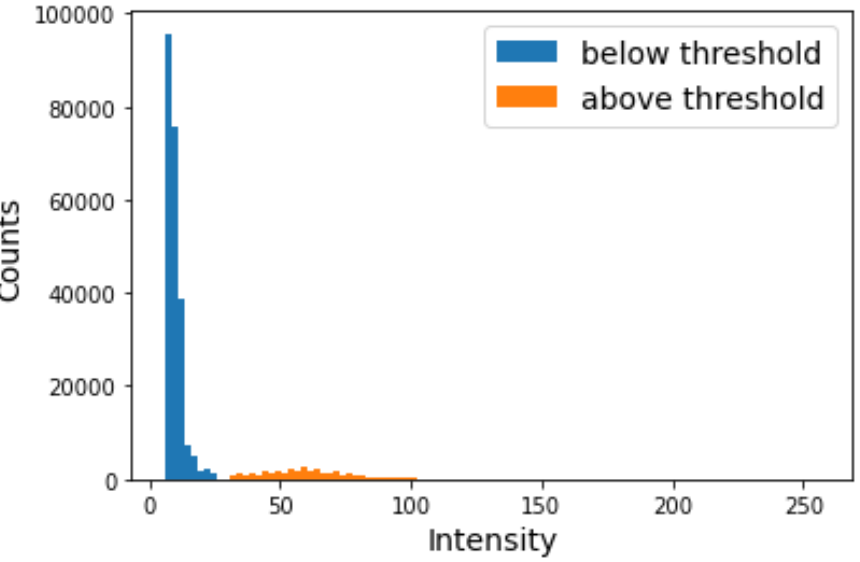
- In case thresholding algorithms outline the wrong structure, blurring in advance may help.
- However: **Do not** continue process[illegible]ge, continue with the original!

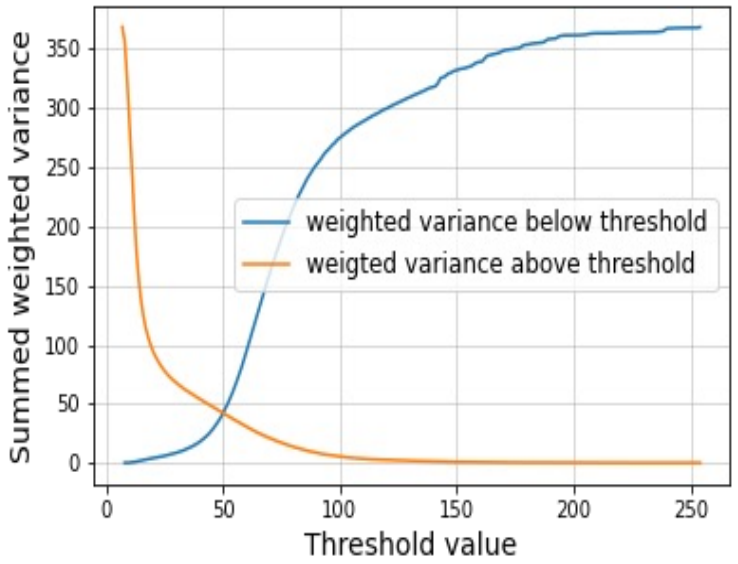


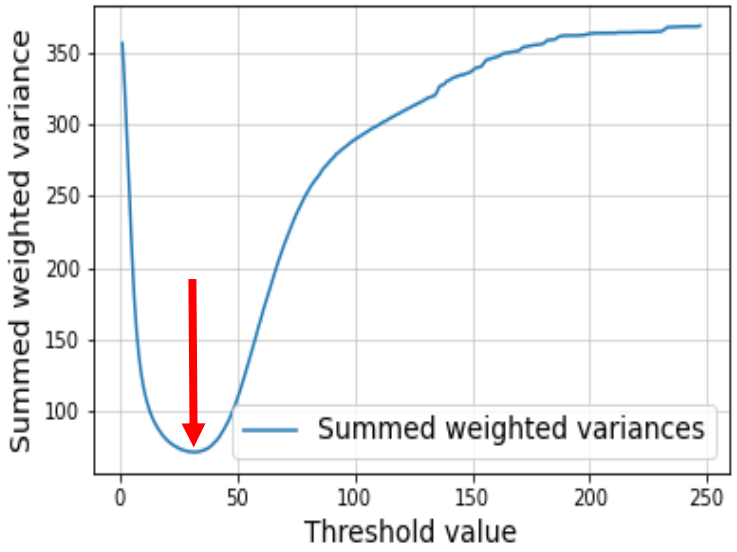
Threshold algorithms

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

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



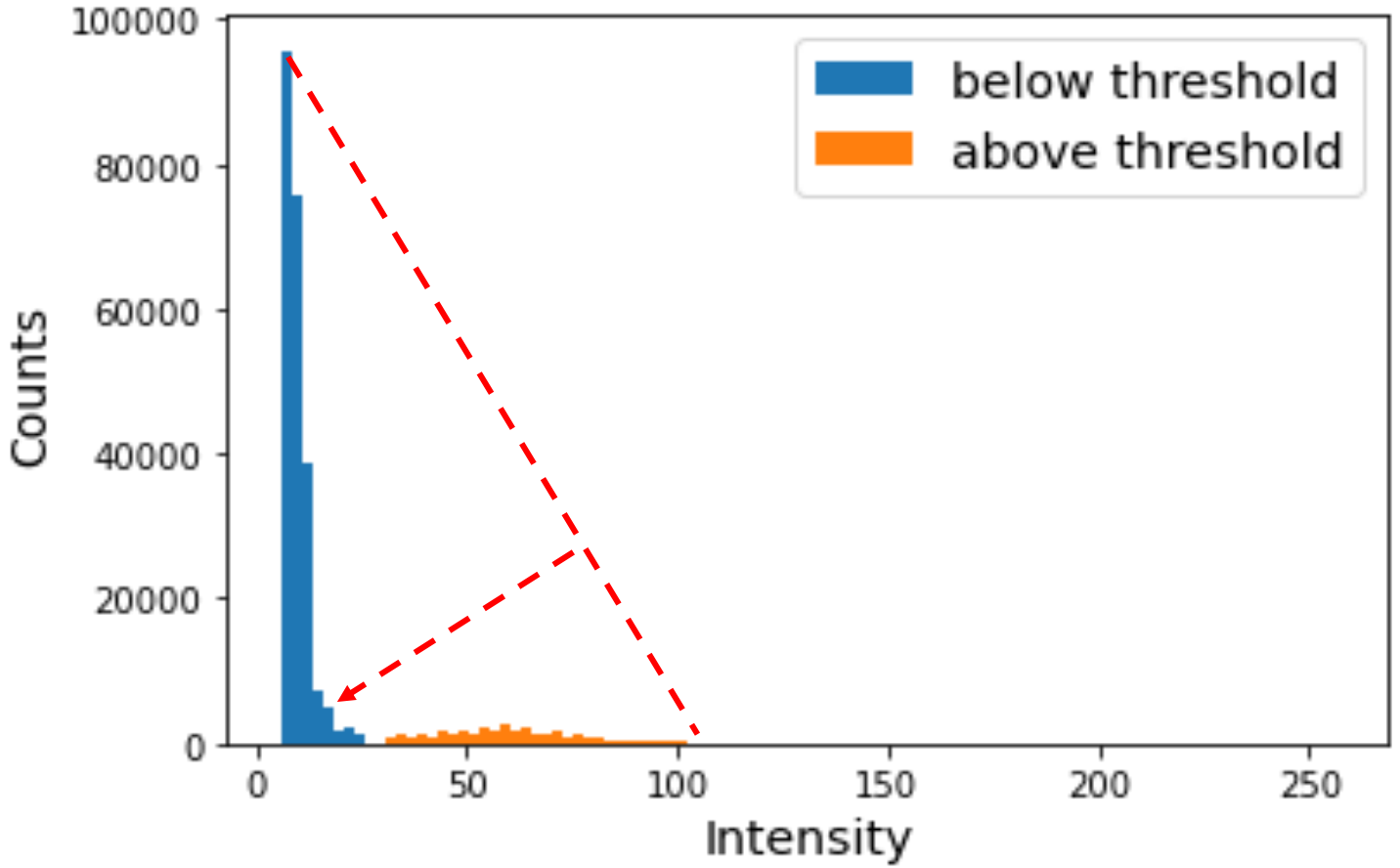




- **Statistical thresholding**: Pixels above statistical parameter of I belong to foreground. (Possibilities: Mean, Median, Quartiles, etc.)

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)
```

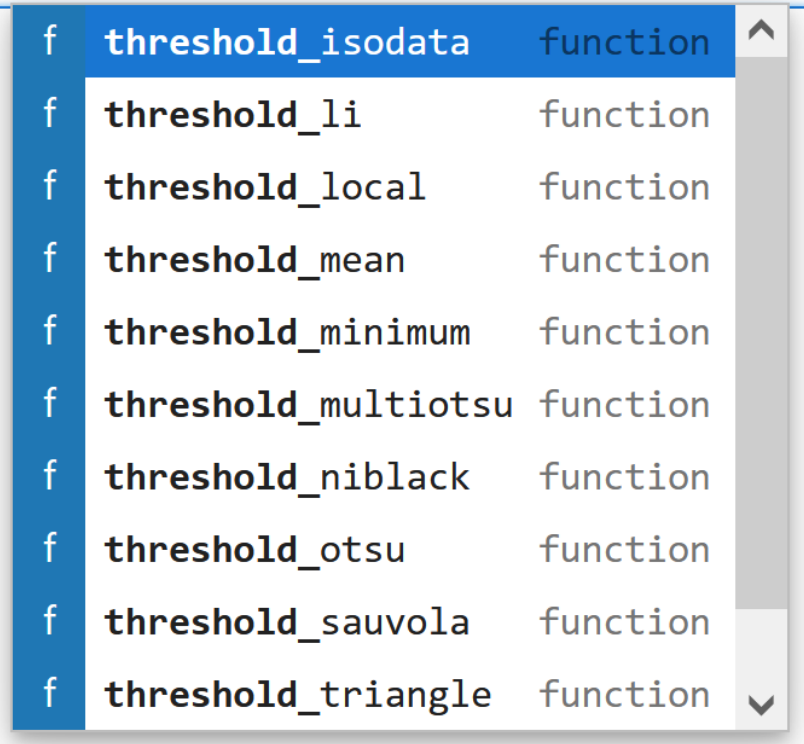
- **Statistical thresholding**: Pixels above statistical parameter of I belong to foreground. (Possibilities: Mean, Median, Quartiles, etc.)

```
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:

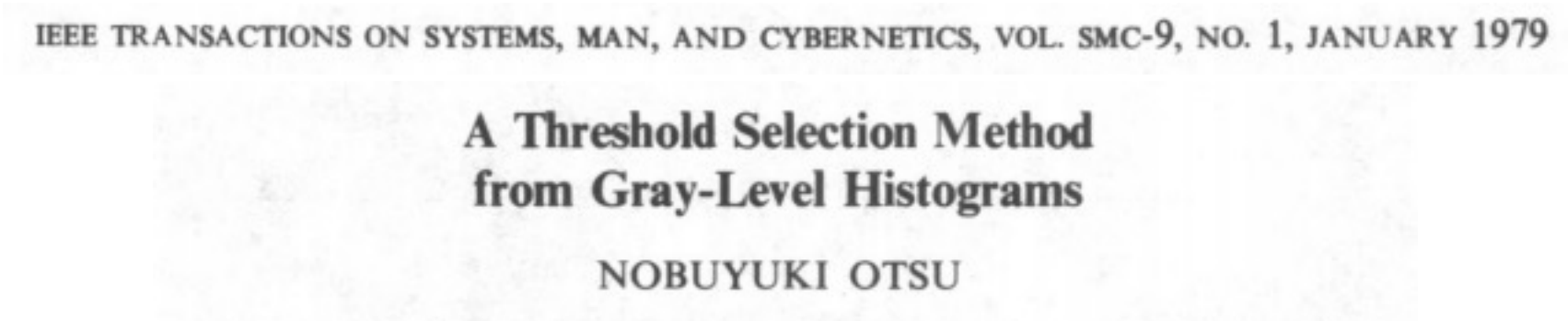
```
from skimage import filters

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

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)
```

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

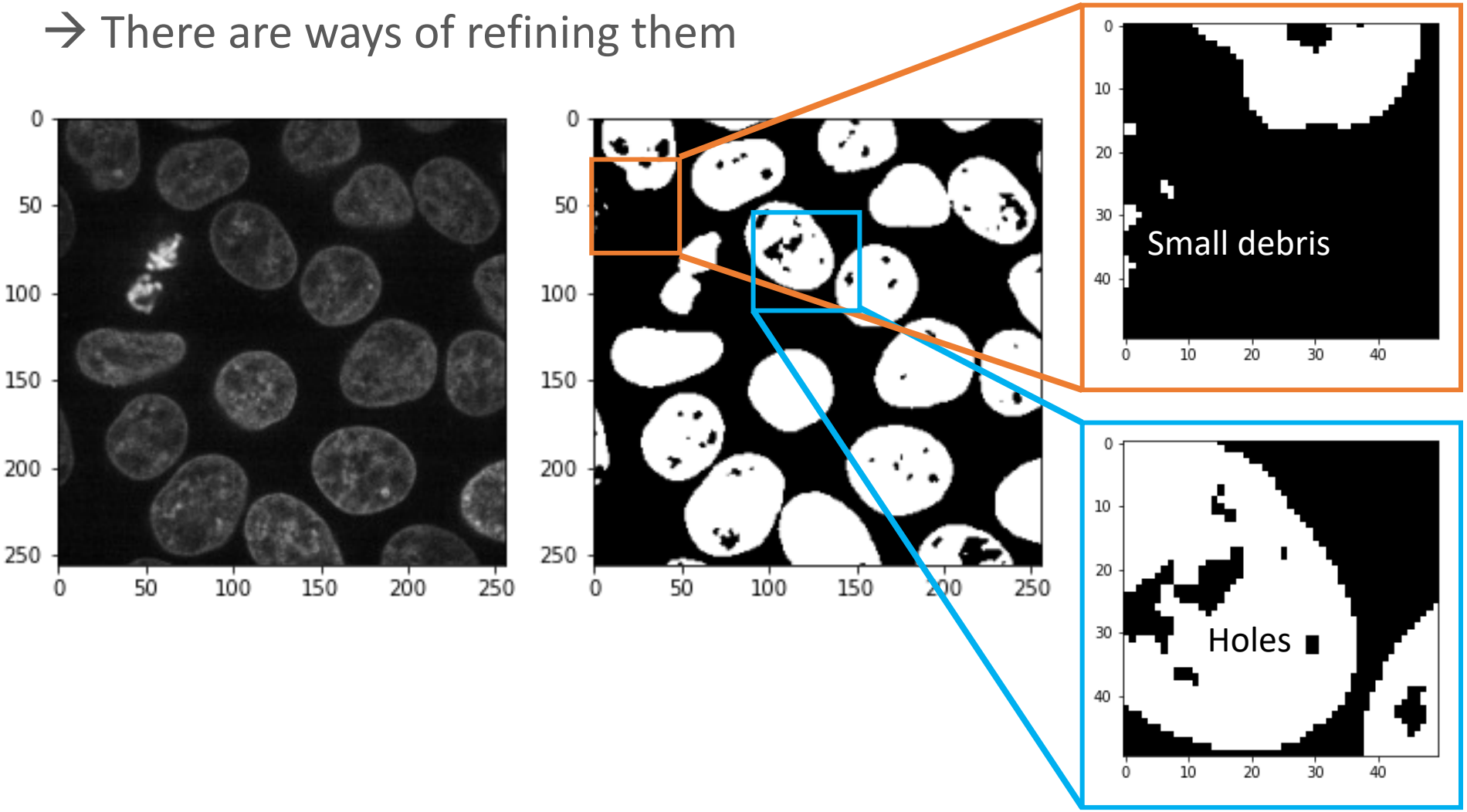
Do not over-engineer

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



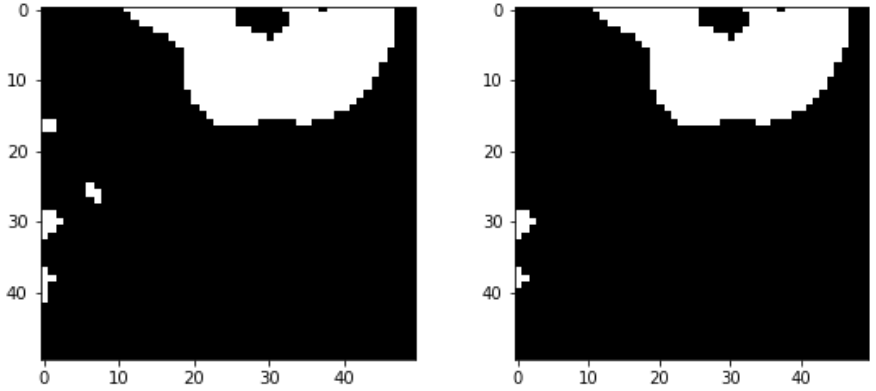
https://scikit-image.org/docs/stable/api/skimage.data.html

Refining masks: Opening & Closing

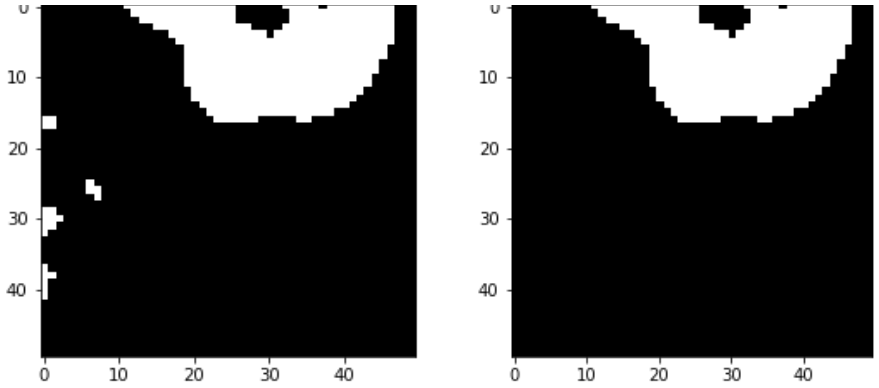
PoL Physics of Life TU Dresden

```
from skimage import morphology
```

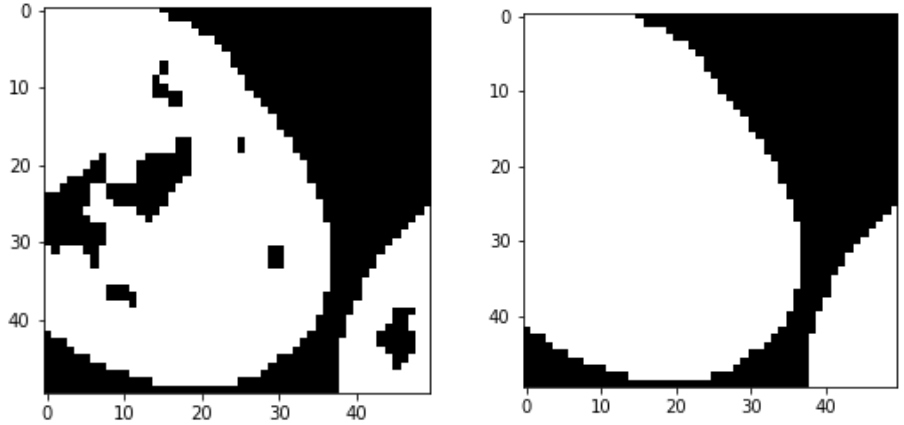
```
opened = morphology.binary_opening(binary)
```



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



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



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