Bio-image Analysis with Python and Napari

Marcelo Leomil Zoccoler, Johannes Müller

Schedule

13:00 Introduction to Image Analysis with Python

- Bio-Image Analysis
- Napari
- Jupyter Lab

14:00 Python basics

- Basic Python syntax (strings, numbers, put assign variables, indexing)
- Variables and objects (lists, nd-arrays)
- Loops & conditions (for, if, functions)

15:00 Image Analysis

- Images
- Filtering
- Background subtraction
- Morphological operators

16:00 Image Segmentation

17:00 Feature extraction

Short breaks after every session

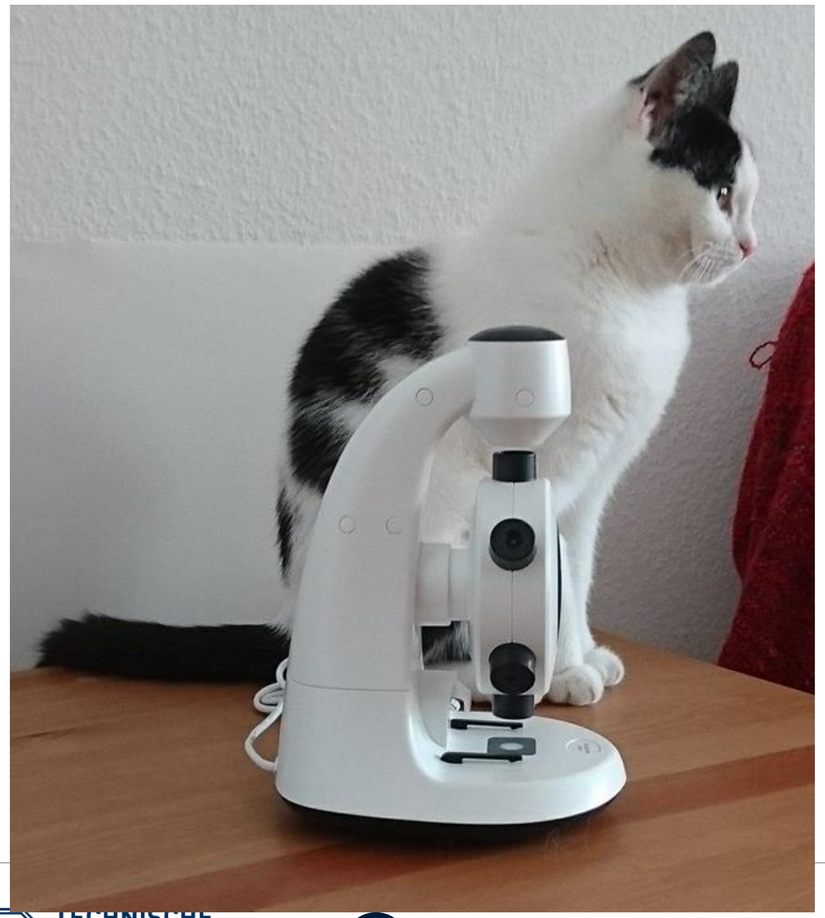
Introduction to Bio-Image Analysis

Marcelo Leomil Zoccoler

With material from:
Robert Haase, TU Dresden and Benoit Lombardot, MPI CBG

Quantitative bio-image analysis

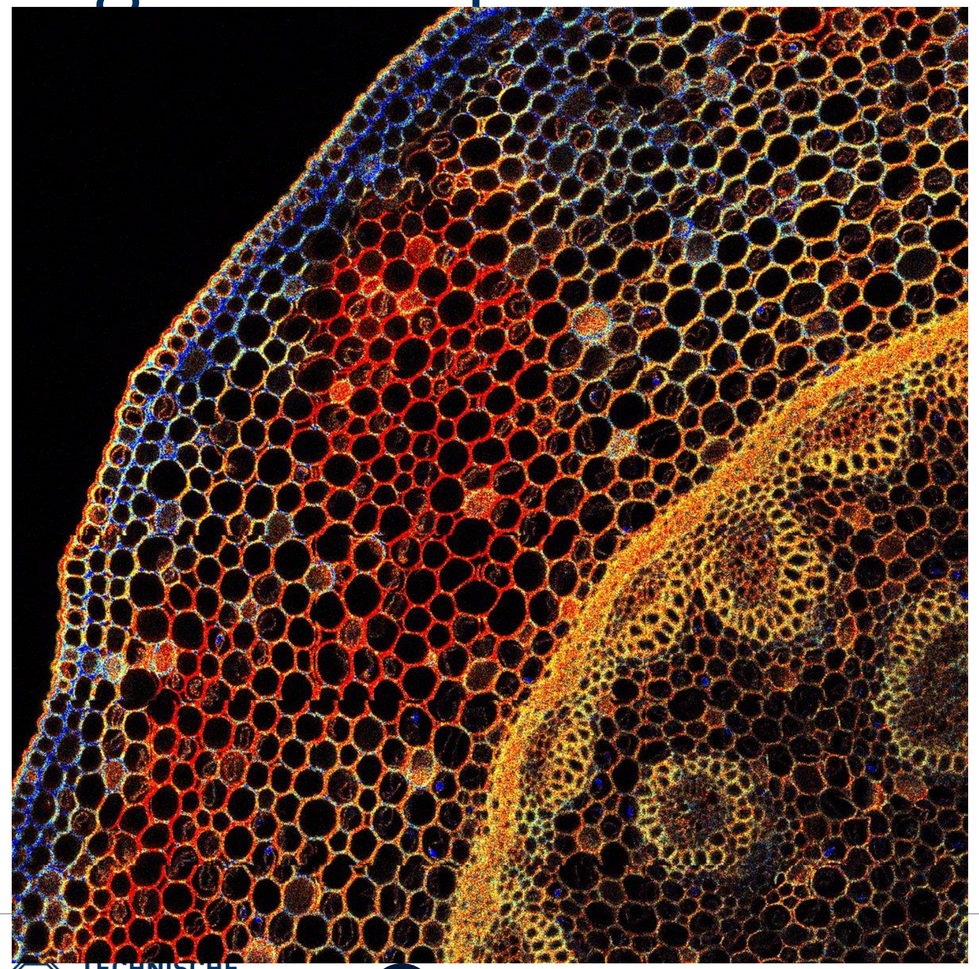
Deriving quantitative information from images of biological samples taken with microscopes



cat height = 1.5 x microscope height

Quantitative bio-image analysis

Deriving quantitative information from images of biological samples taken with microscopes



How many cells are there in the outer ring?

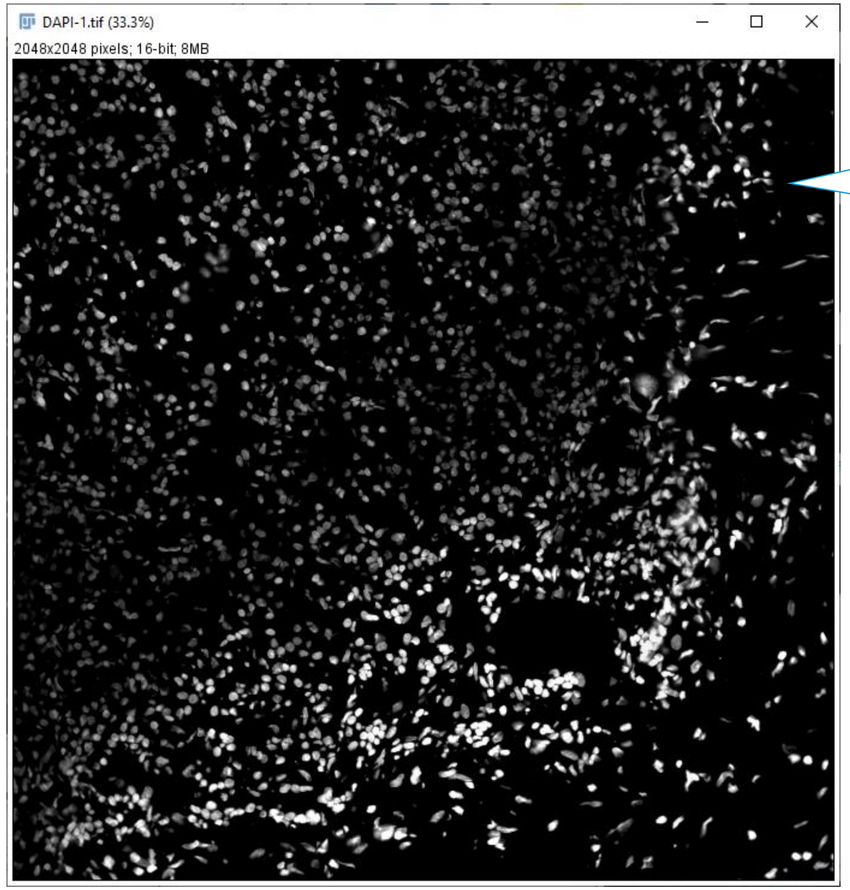
How high is the intensity in the inner ring?

How is the signal in the blue channel distributed?

Image data source: Lorenzo Scipioni, U. California / Irvine @LorenzoScipion3

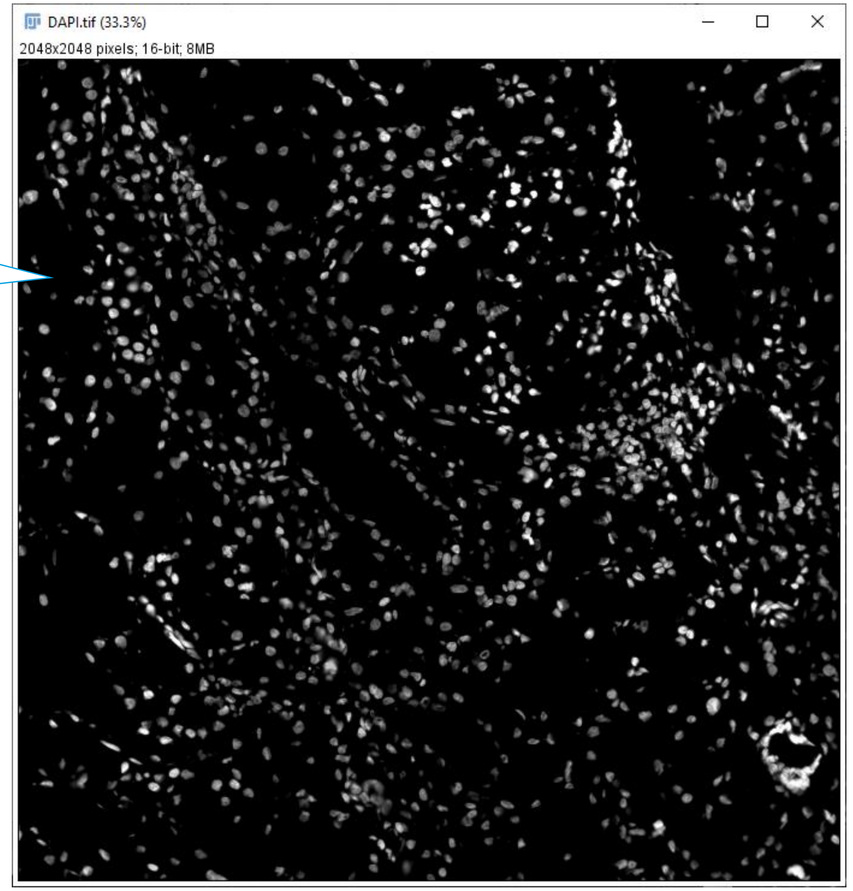
Objective bio-image analysis

Measurements should be objective, not influenced by human interpretation



Nuclei in this image are ... _

... more dense than in this image.



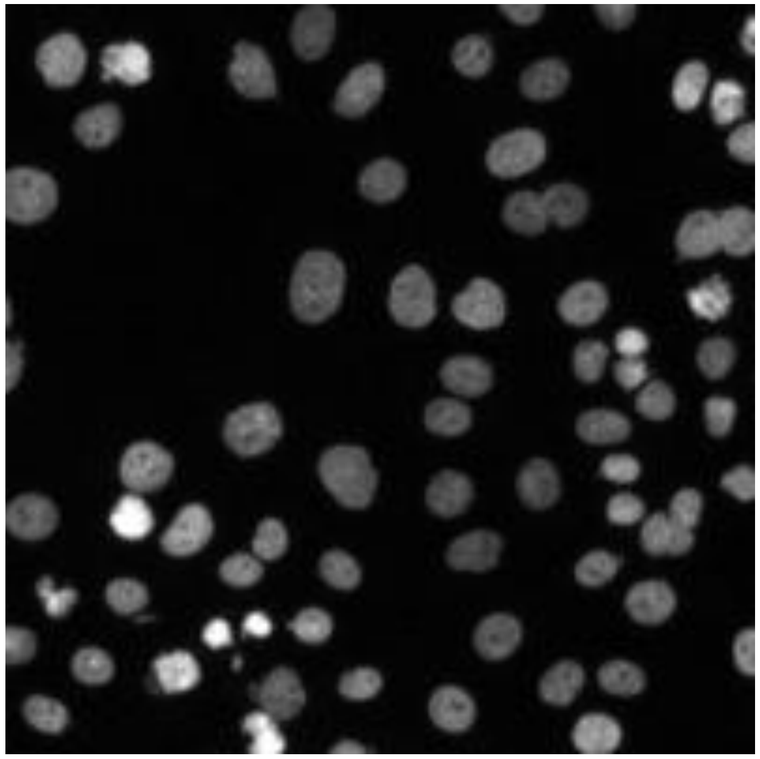
Use automation for less subjective analysis.

Image data source: Pascual-Reguant, Anna. (2021). Immunofluorescence staining of a human kidney (#2, peri-tumor area) obtained by MELC [Data set]. Zenodo. http://doi.org/10.5281/zenodo.4434462 licensed CC-BY 4.0

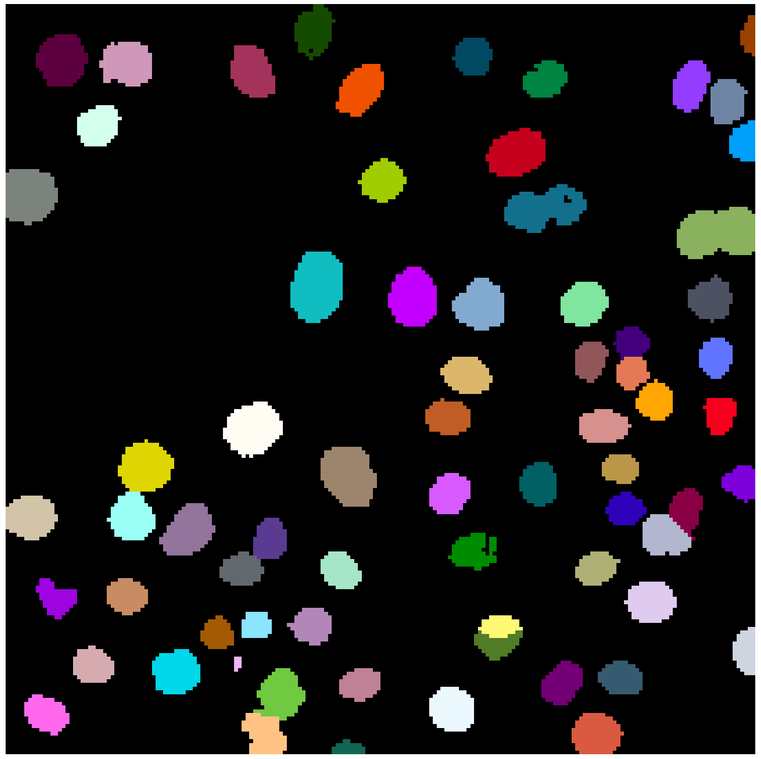
Reliable bio-image analysis

Algorithms must be reliable (trustworthy). Visualization helps gaining trust in automated methods.

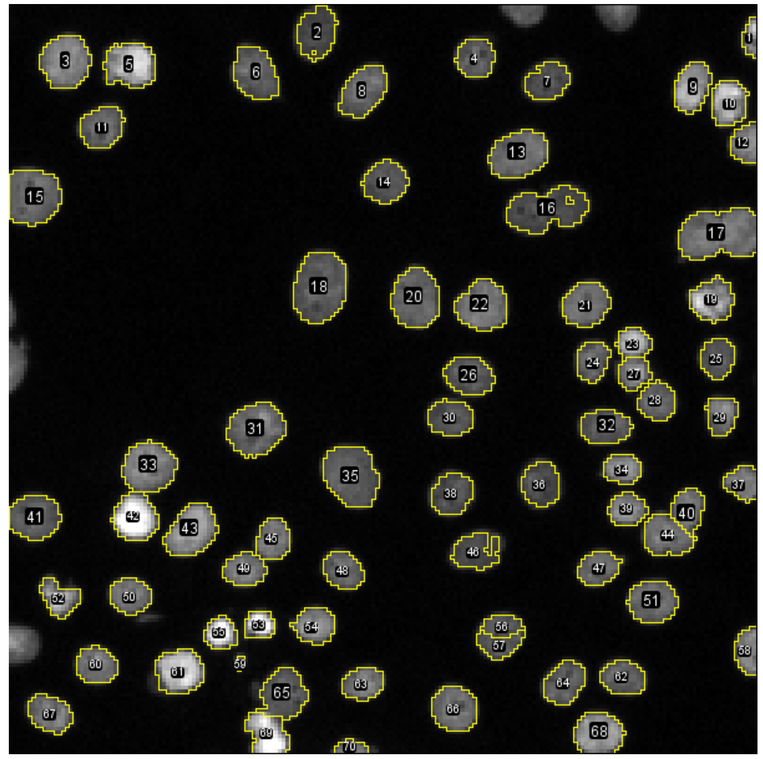
Original image



Label image



Overlay



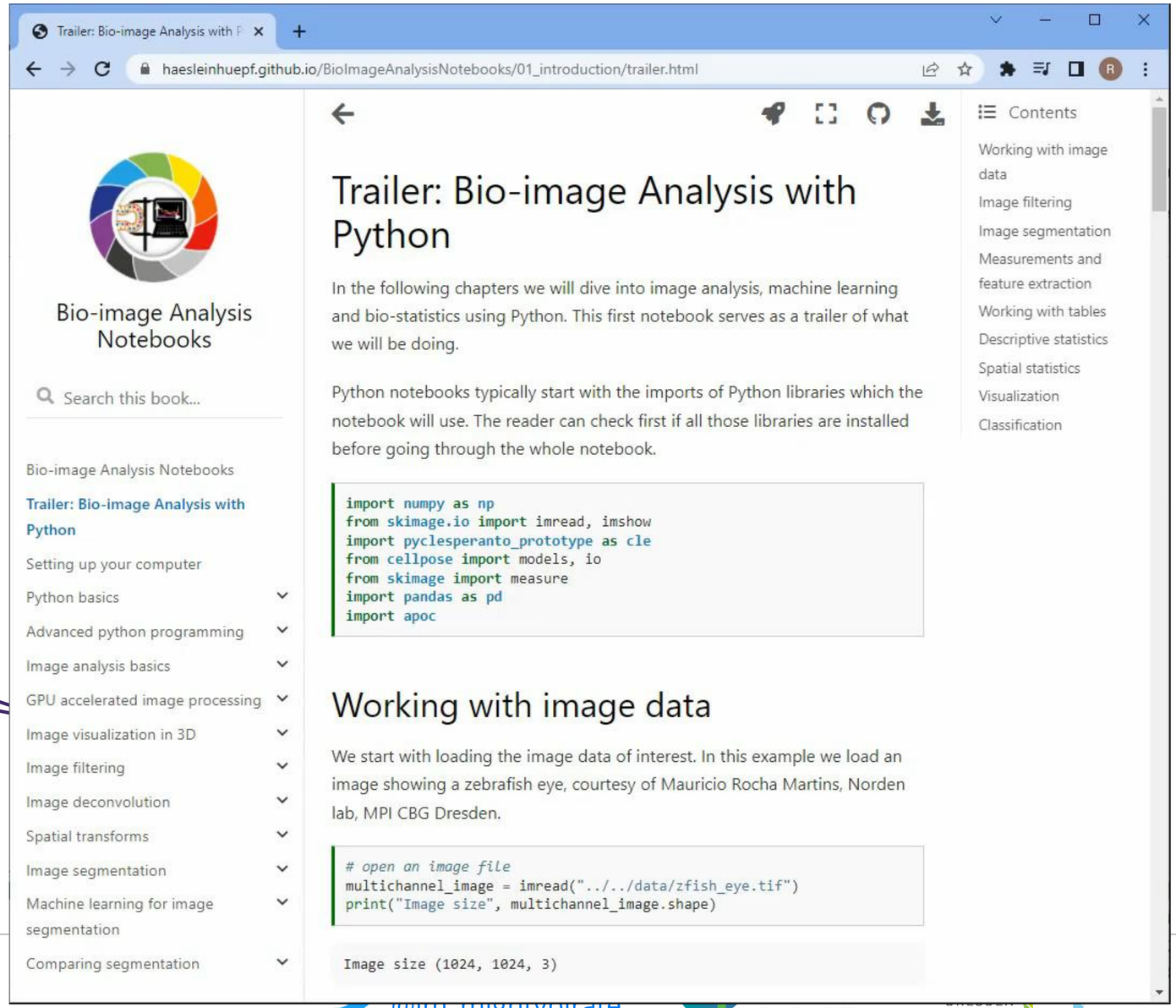
There are 70 nuclei in this image.

Image Data source: BBBC001v1 [Carpenter et al., *Genome Biology*, 2006] from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

Reproducible bio-image analysis

"The image data was analyzed with ImageJ."

Can you reproduce what they did?



Can you reproduce what they did?

Image analysis is part of the experiment

Think about how to analyze your images before starting the experiment.
— Consider adapting your experiment so that quantitative image analysis can be performed easily.

Think about controls, counter-proves, an easy to falsify null-hypothesis.
— Be a lazy scientist. Do simple experiments.

How can you exclude yourself from the experiment?
— Think of blinding yourself or fully automate analysis.

One experiment usually answers just one or less questions.

Image analysis is part of the experiment

Talk to image-analysis / data-science experts early during your project.

Not one week before the submission deadline.

TECHNISCHE UNIVERSITÄT DRESDEN

DIGS-BB

Dresden International Graduate School for Biomedicine and Bioengineering

Napari

Marcelo Leomil Zoccoler

With material from:
Robert Haase, PoL

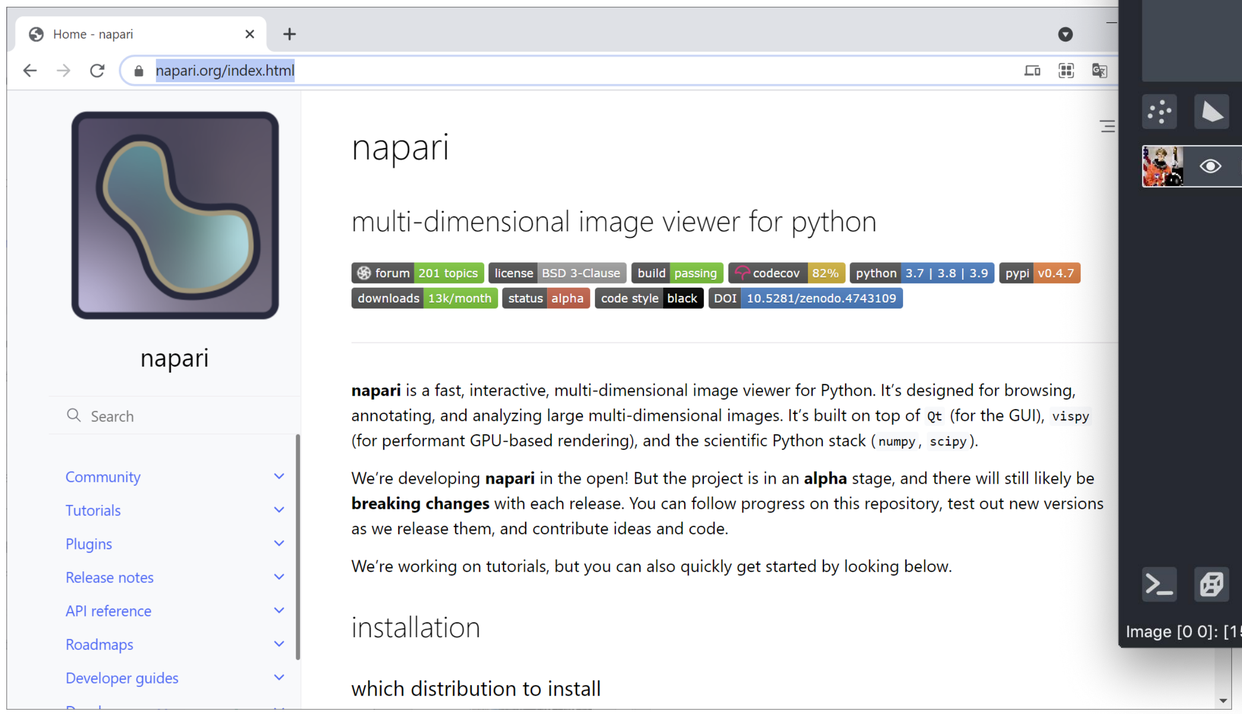
PoL
Physics of Life
TU Dresden

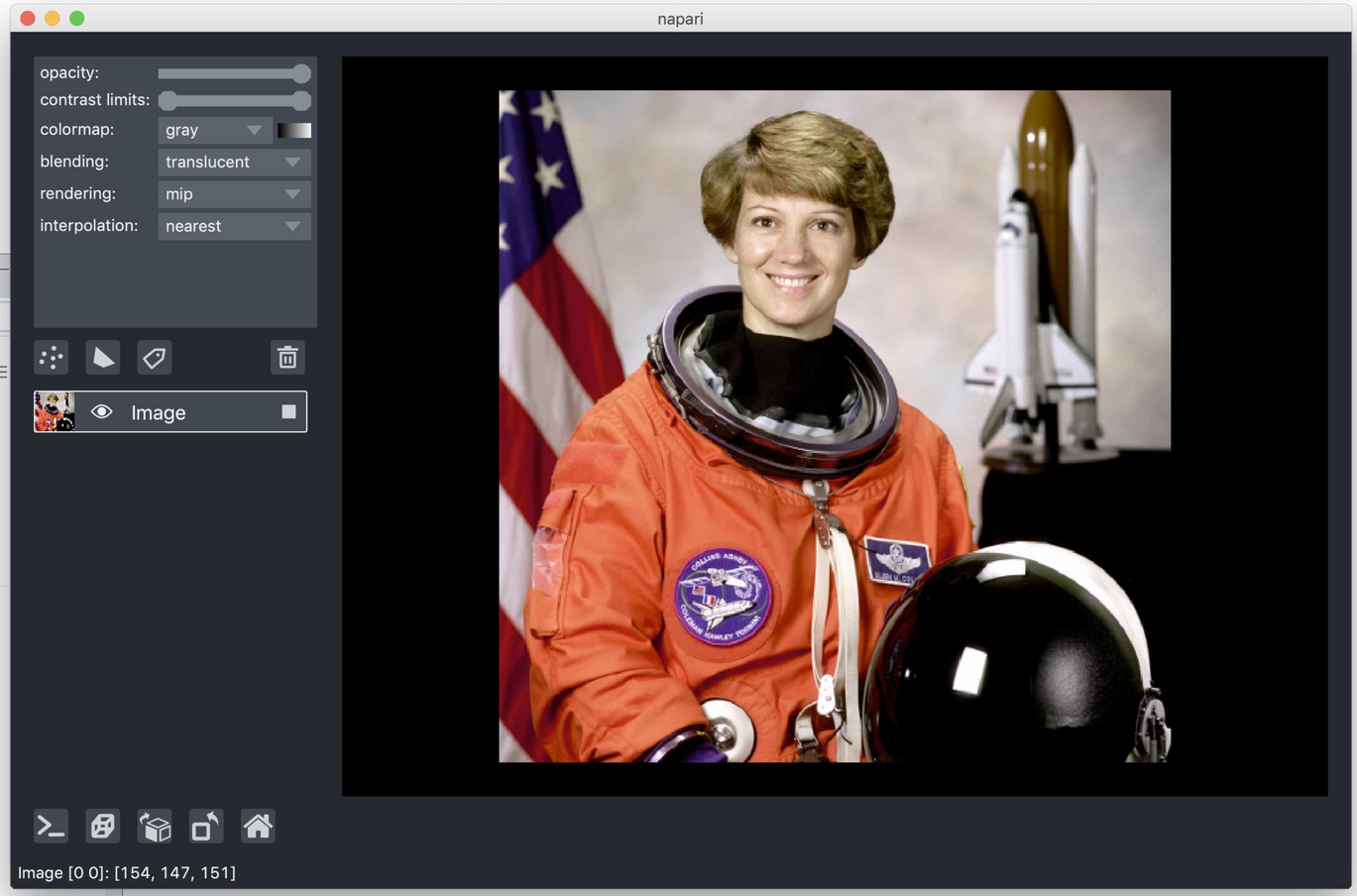
DRESDEN concept

Napari: 3D viewer for Python

Multi-dimensional image viewer in python

https://napari.org/





Napari: 3D viewer for Python

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Napari user interface

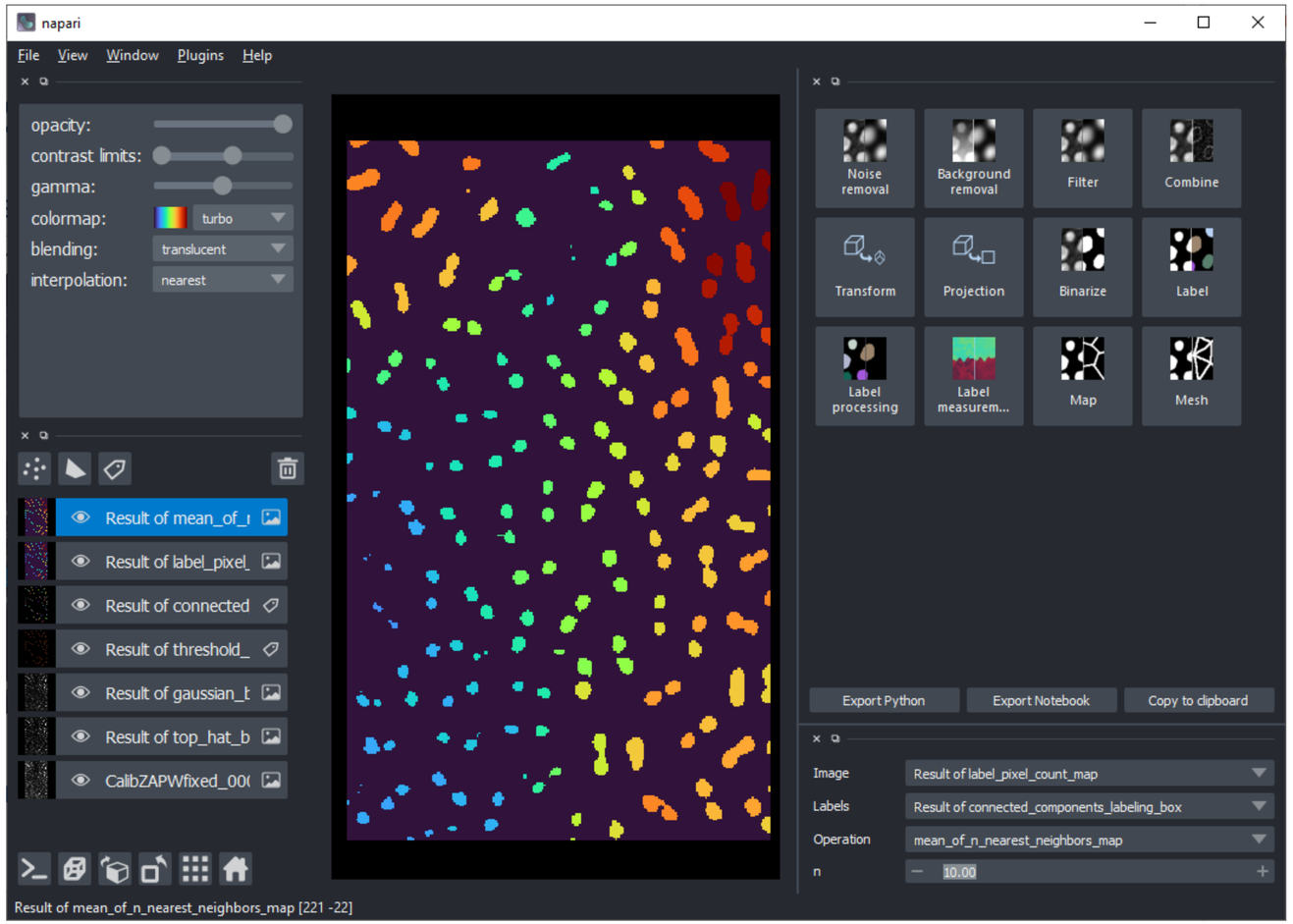
View configuration / tools

```
layer.opacity = 0.5
```

Layers

```
layer.visible = False
```

Viewer controls



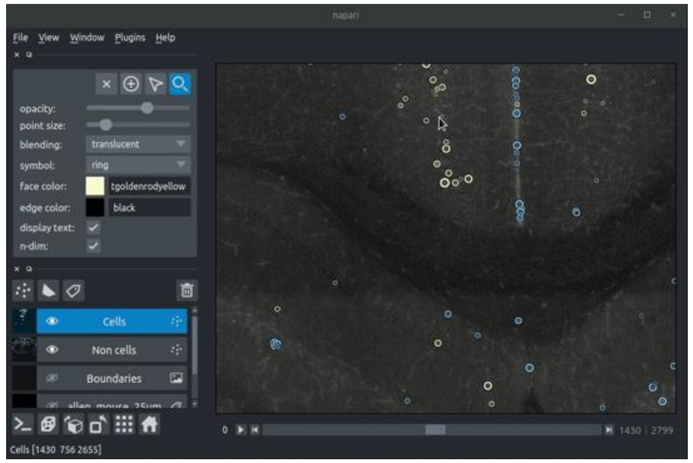
Dock widgets (custom plugins)

Function widgets (custom plugins)

https://napari.org/tutorials/fundamentals/viewer.html

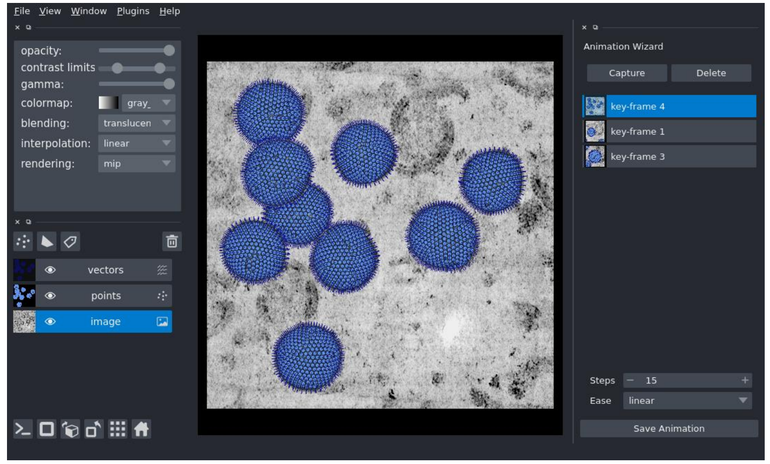
The era of napari plugins has just begun

cellfinder



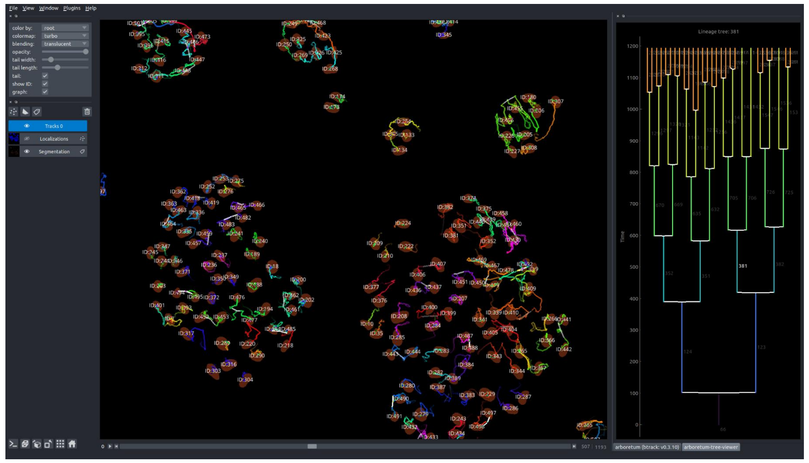
https://github.com/brainglobe/napari-cellfinder

animation



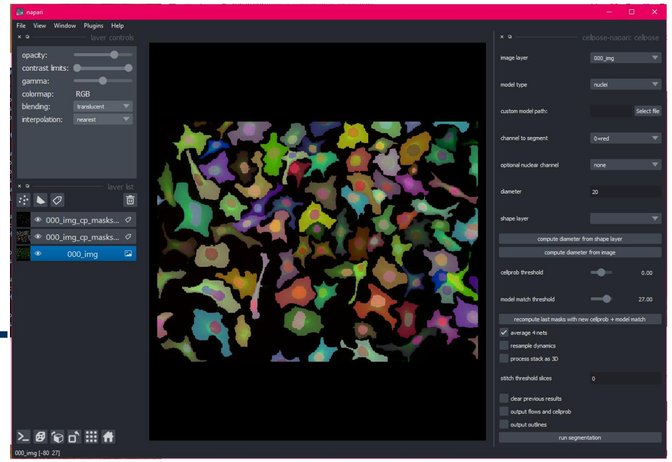
https://github.com/napari/napari-animation

arboretum



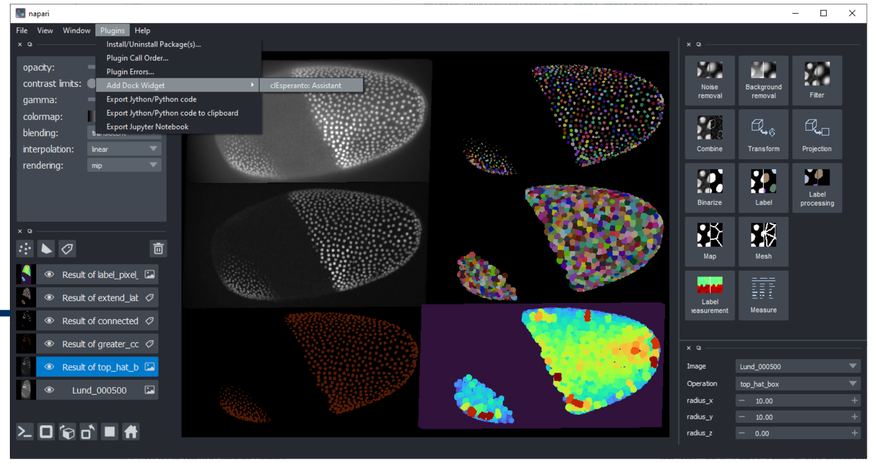
https://github.com/quantumjot/arboretum

cellpose



https://cellpose-napari.readthedocs.io/en/latest/

clesperanto



https://github.com/clEsperanto/napari_pyclesperanto_assistant

In development: https://github.com/topics/napari-plugin
Released: https://pypi.org/search/?q=&o=&c=Framework+%3A%3A+napari

Napari-animation

Making animations – as easy as it gets

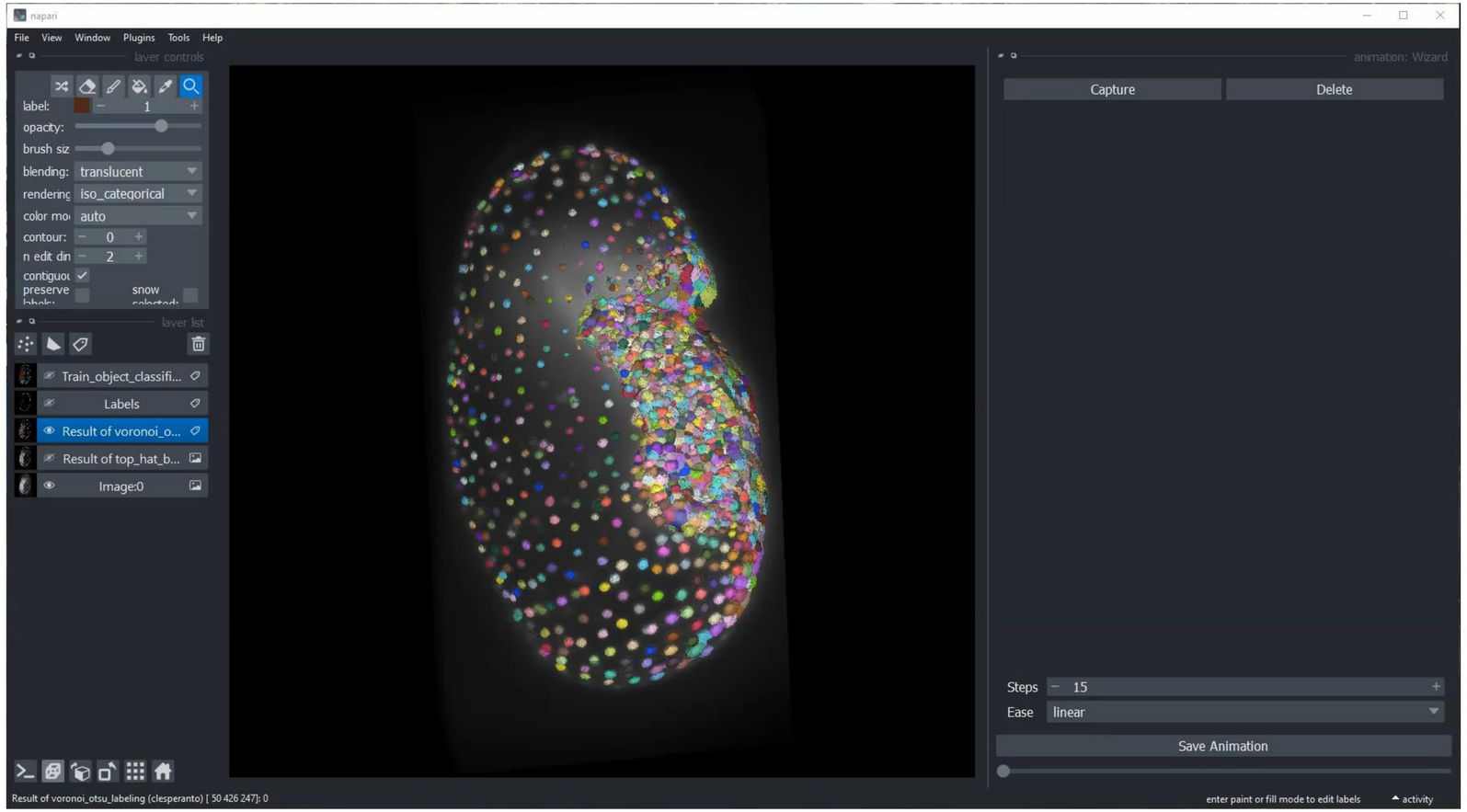
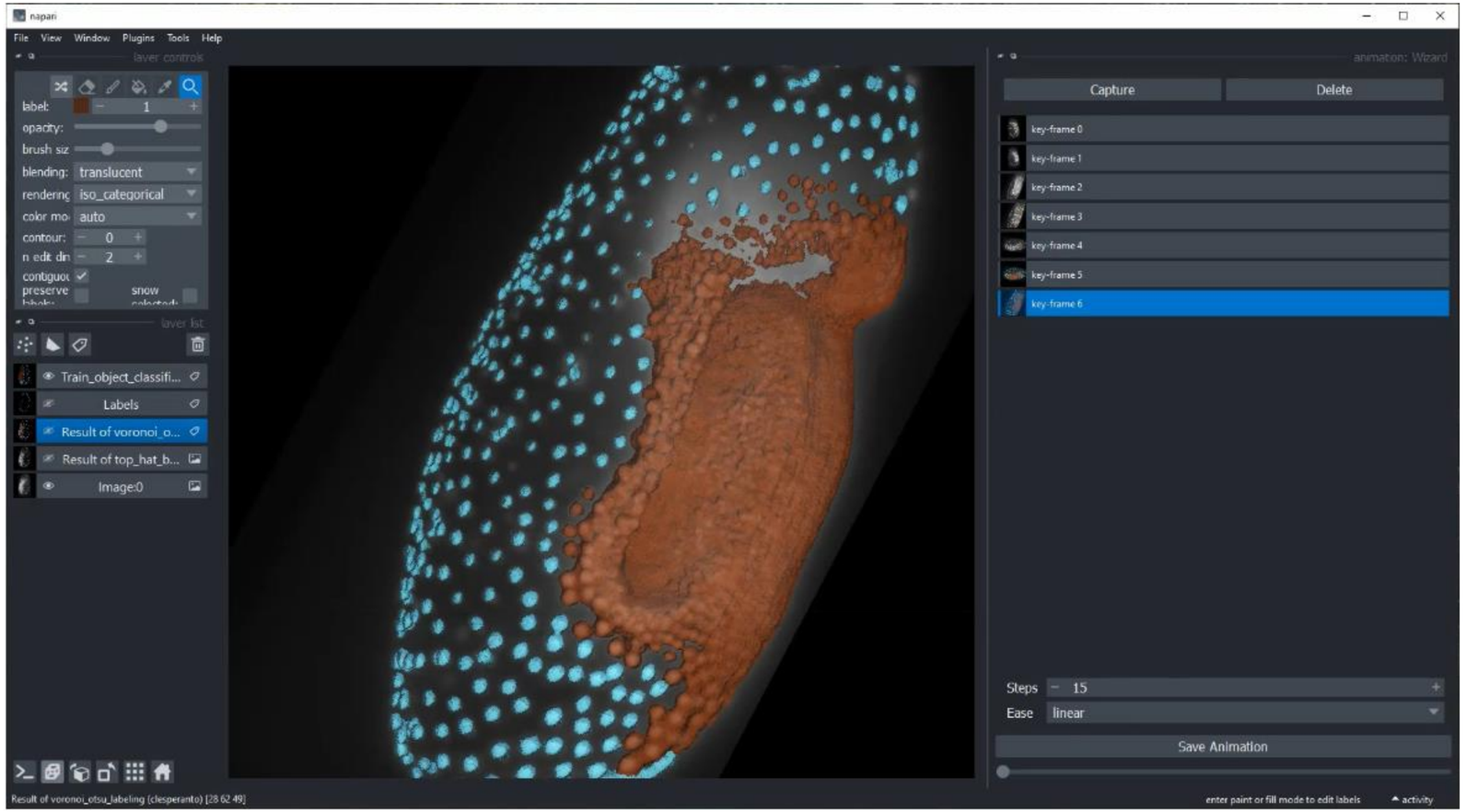


Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Work by Alister Burt, Nicholas Sofroniew, et al (BSD3 licensed)
https://github.com/napari/napari-animation

Napari-animation

Making animations – as easy as it gets



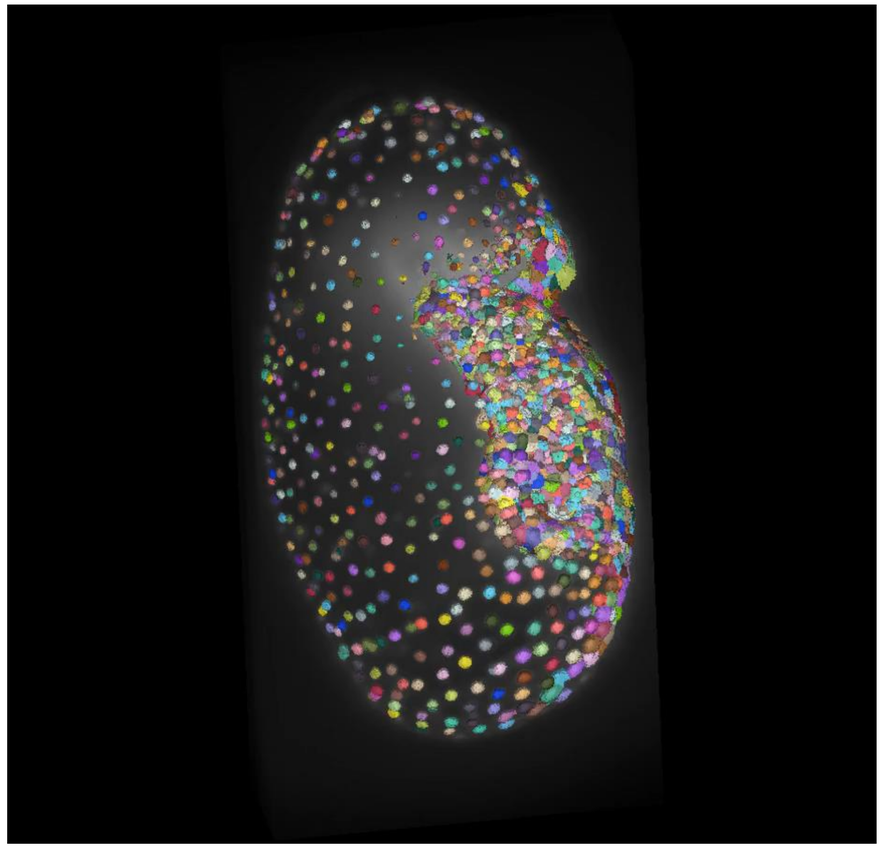


Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Work by Alister Burt, Nicholas Sofroniew, et al
https://github.com/napari/napari-animation

Napari, segment blobs and things with membranes!

- Filtering,
- thresholding,
- spot detection,
- seeded watershed segmentation,
- Voronoi-Otsu-labeling

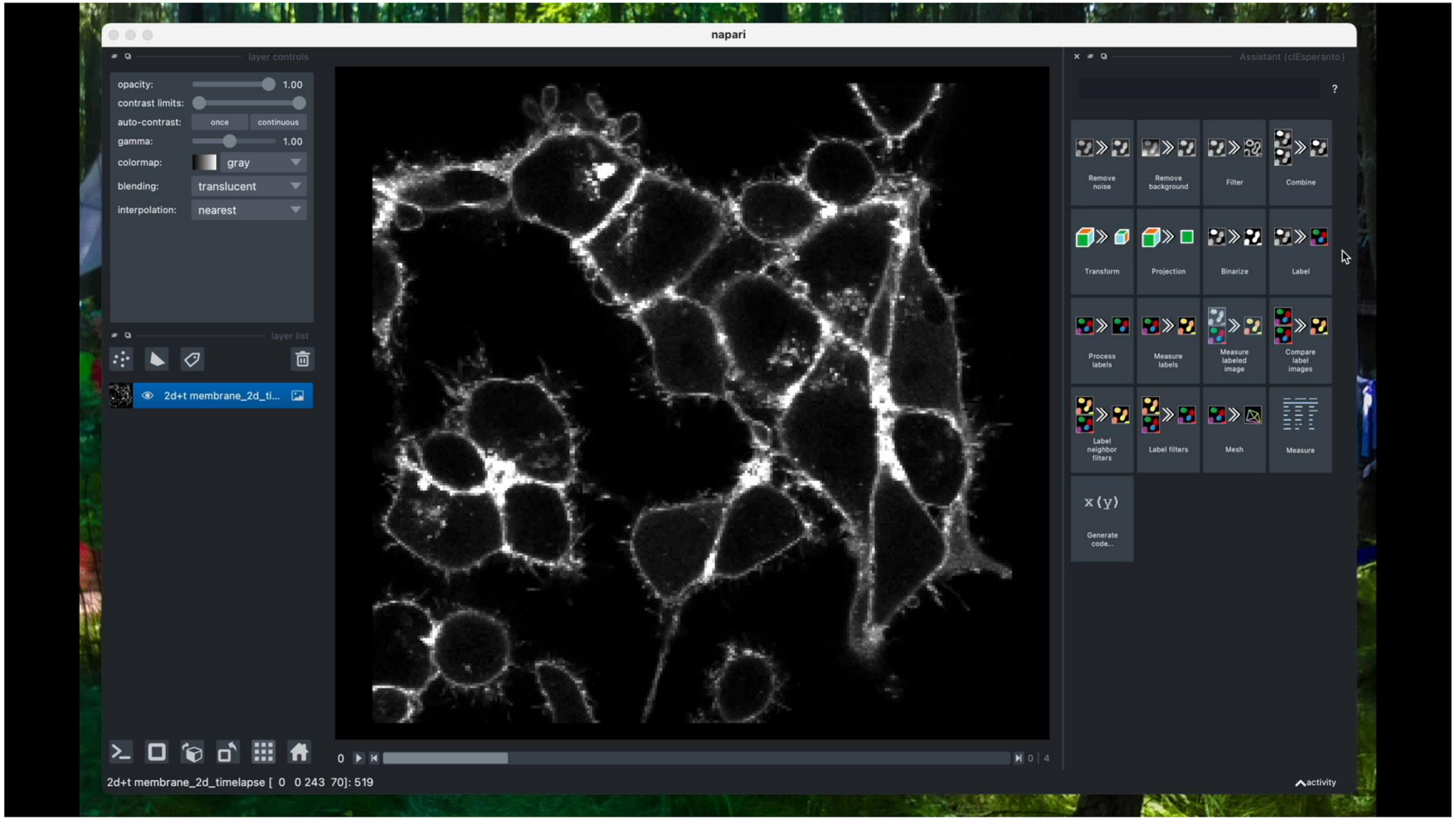
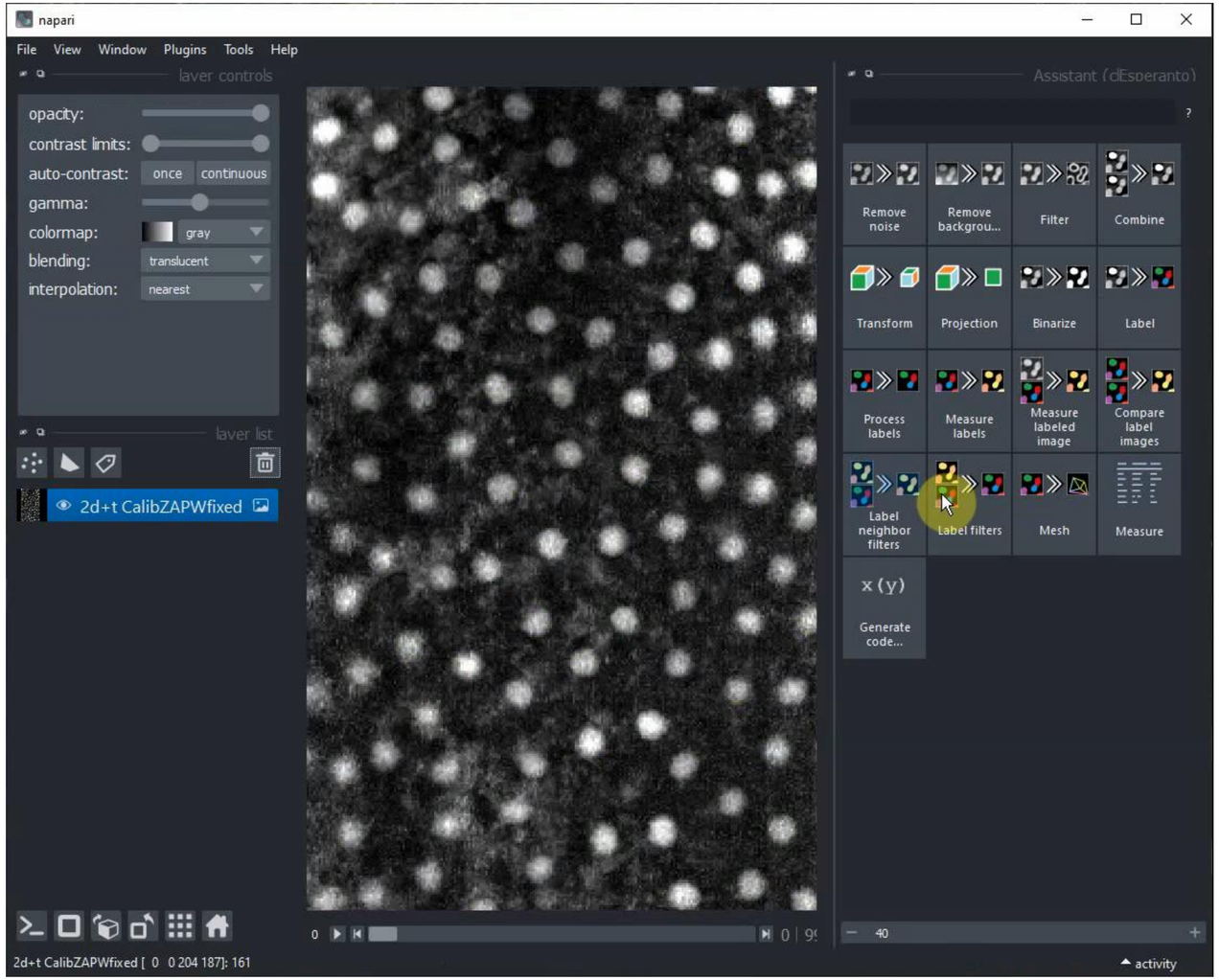


Image data source: Sascha Kuhn, Nadler lab, MPI-CBG
https://www.napari-hub.org/plugins/napari-segment-blobs-and-things-with-membranes

Napari + pyclesperanto + assistant

GPU-accelerated image processing

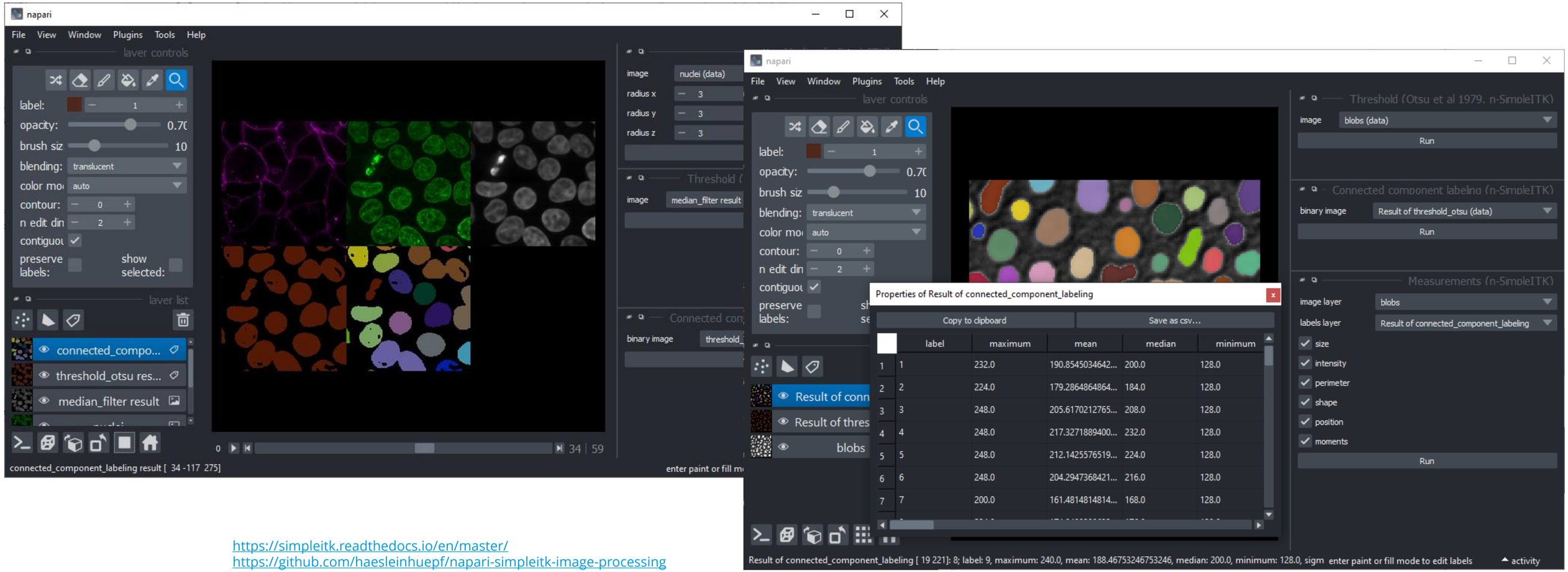
with a pocket-calculator like graphical user interface



https://github.com/clEsperanto/napari_pyclesperanto_assistant

napari-simpleitk-image-processing

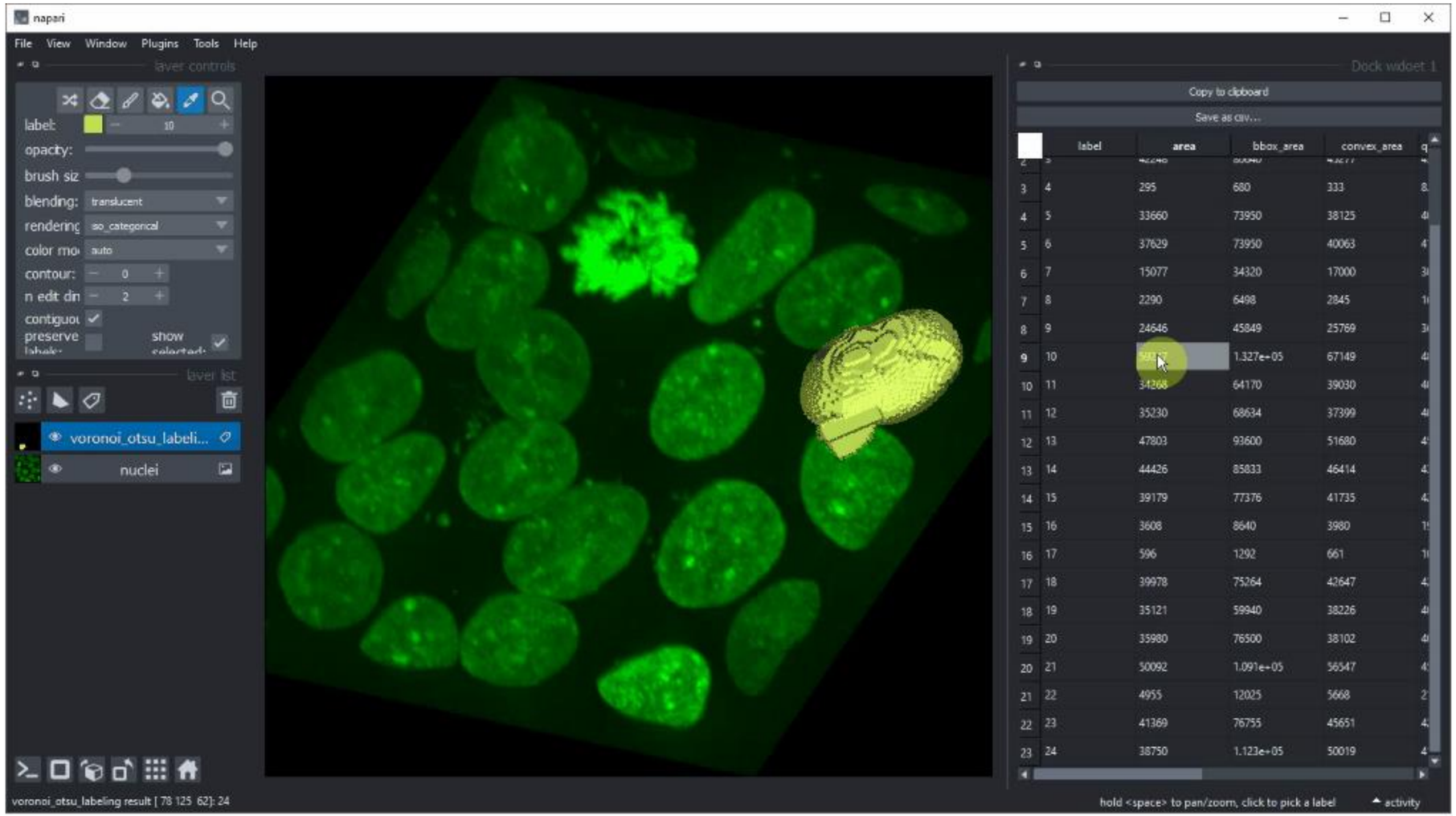
Recommended for 3D-measurements, based on the SimpleITK-project



https://simpleitk.readthedocs.io/en/master/
https://github.com/haesleinhuepf/napari-simpleitk-image-processing

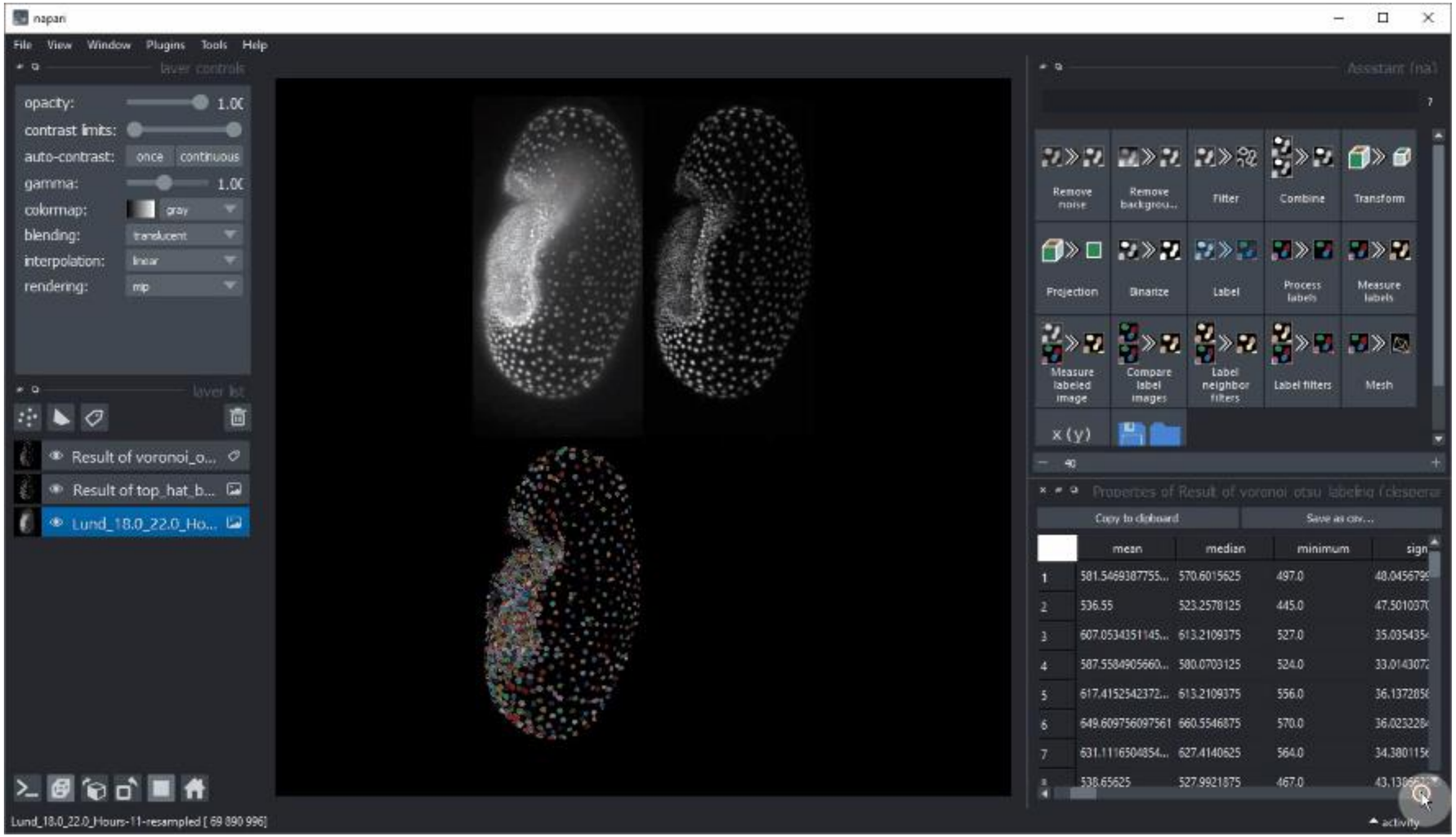
Data exploration

- Click on a cell to view the object the measurement belongs to



Data exploration

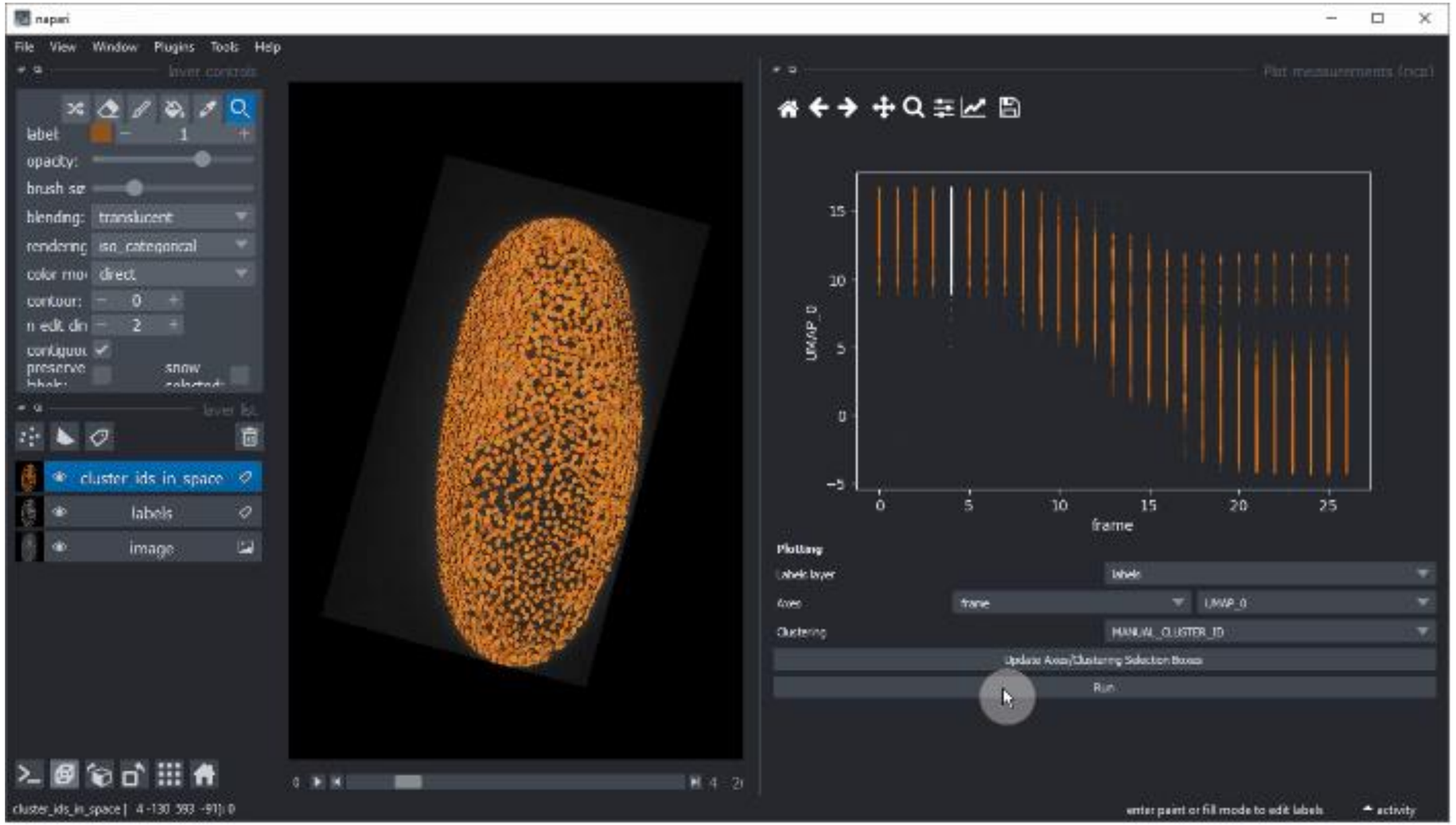
- Double-click on a column of measurements to view a parametric image



https://github.com/haesleinhuepf/napari-skimage-regionprops

Data exploration

- Manual clustering to gain deeper insights in relationships between measured parameters



Laura Žigutytė @zigutyte

Ryan Savill @RyanSavill4

Marcelo Zoccoler @zoccolermarcelo

https://github.com/BiAPoL/napari-clusters-plotter

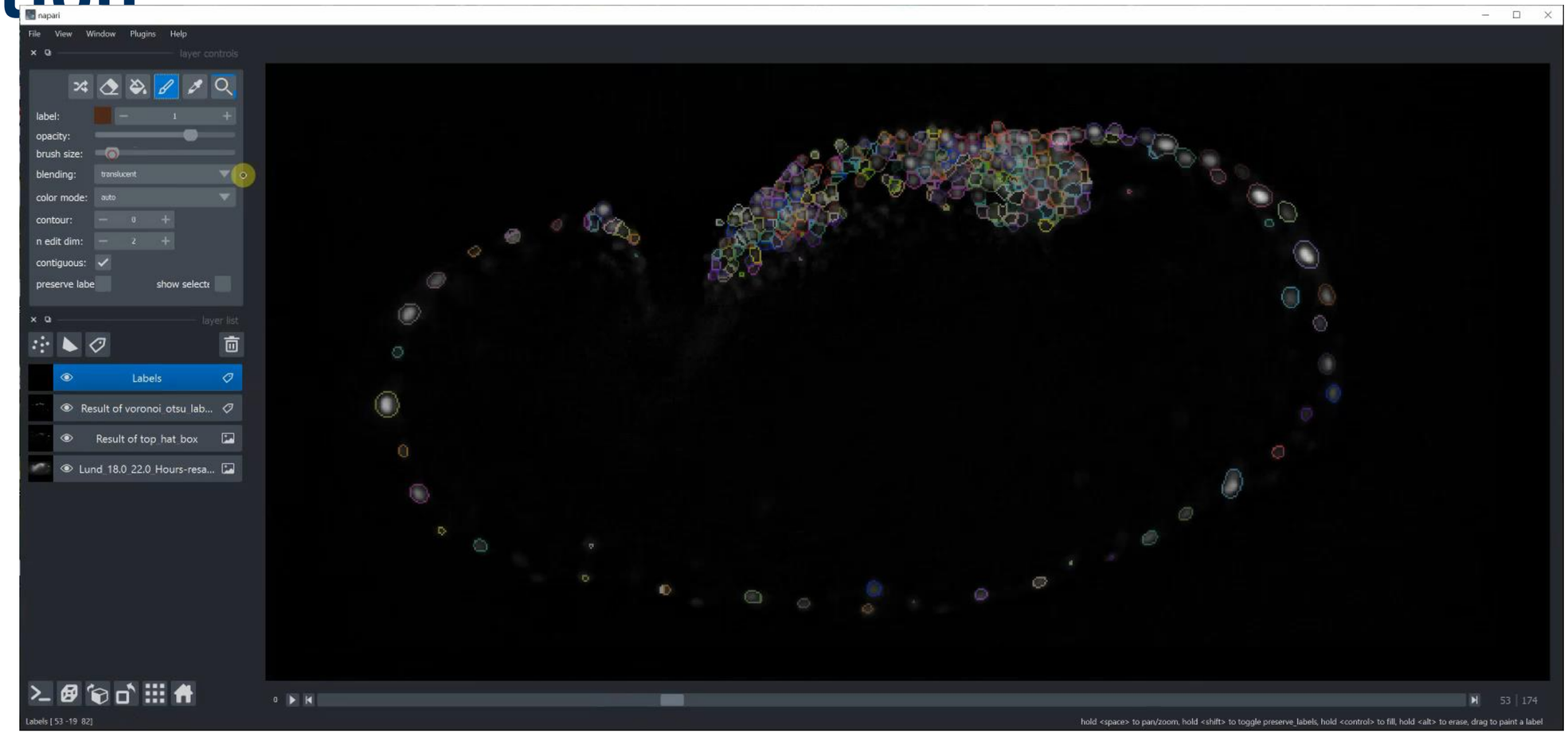
@zoccolermarcelo
@jm_mightypirate

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Supervised machine learning for tissue classification

GPU-accelerated Random Forest Classifiers based on

- scikit-learn and
- clesperanto

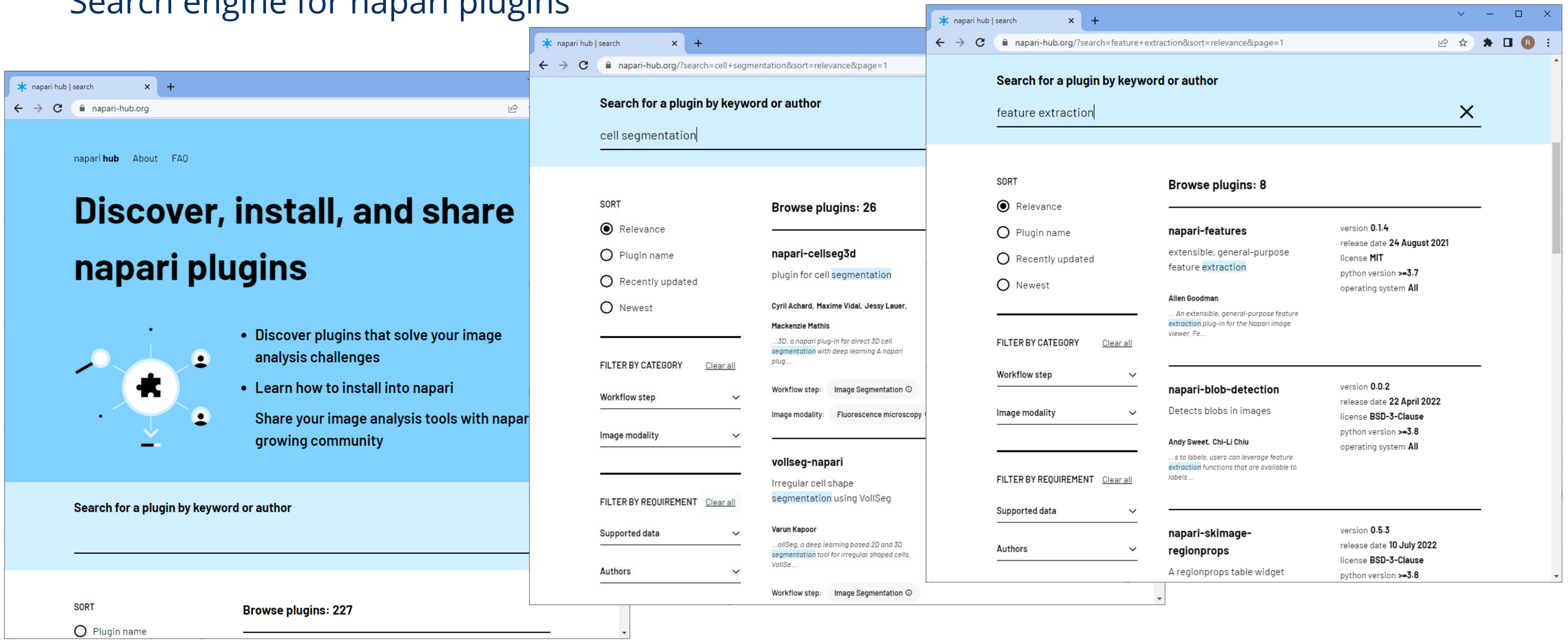


https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

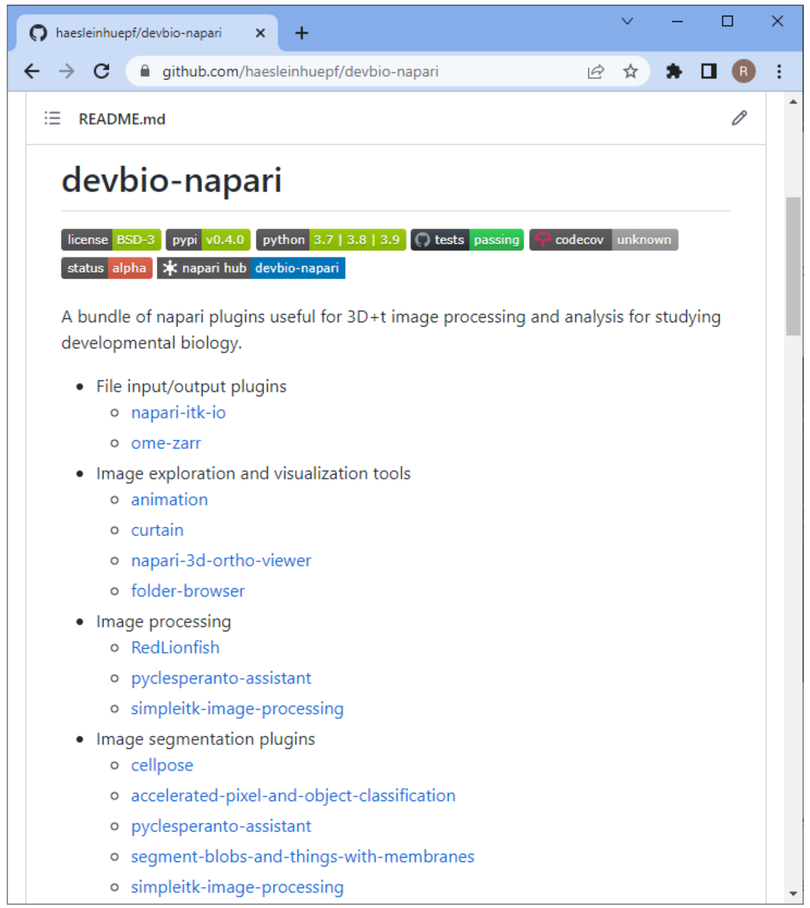
The Napari Hub

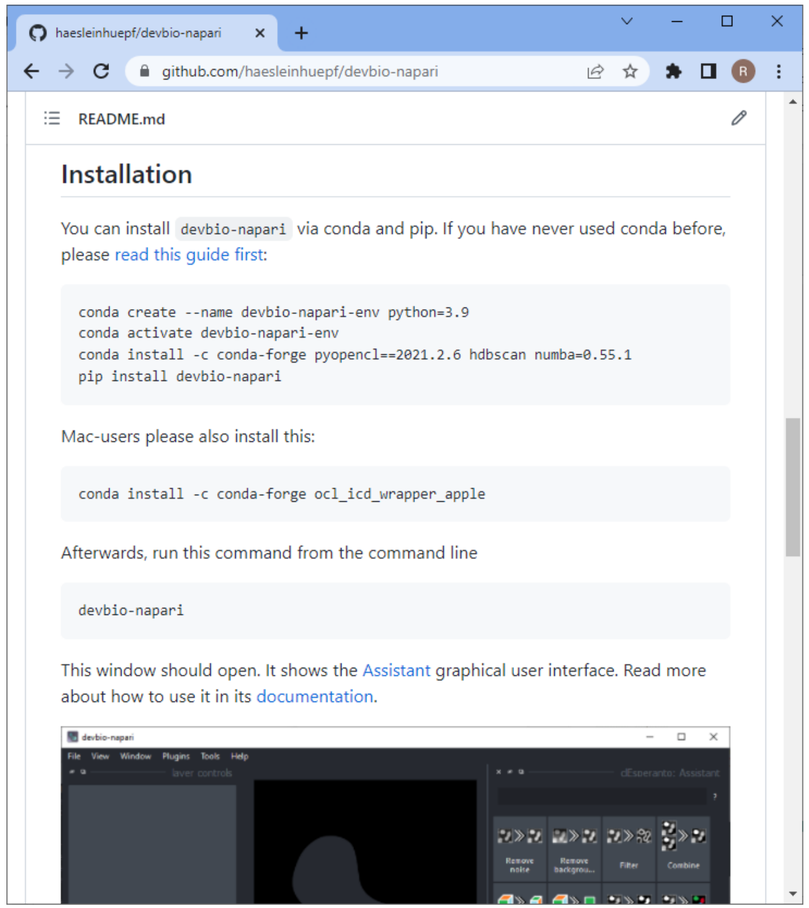
Search engine for napari plugins

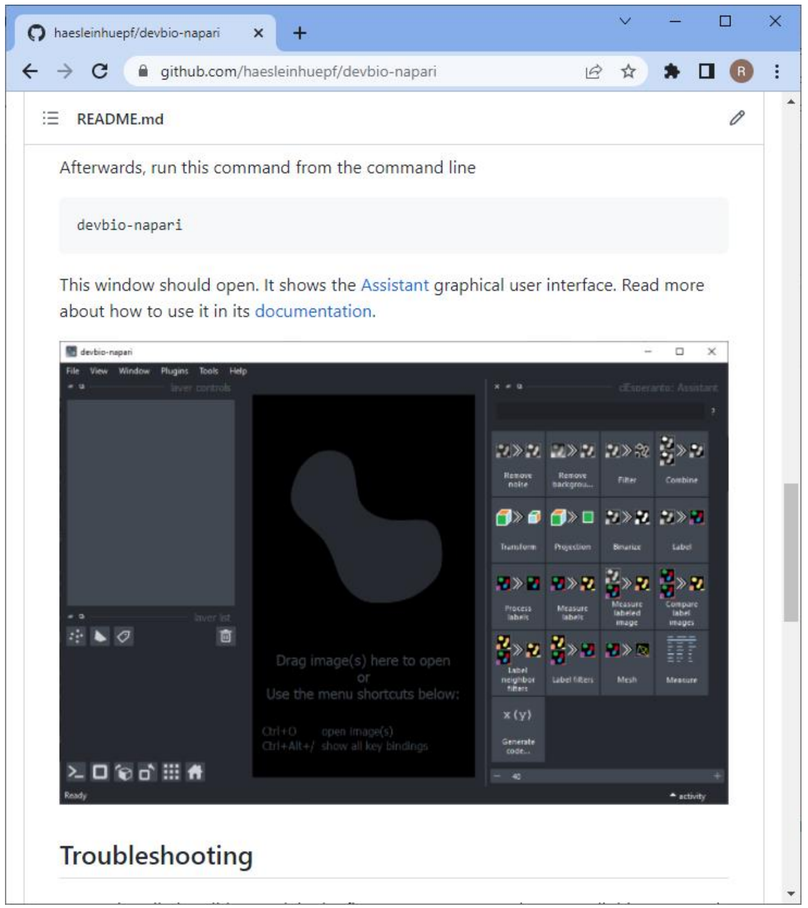


Exercise: Install devbio-napari

A collection of napari plugins for developmental biologists







TECHNISCHE UNIVERSITÄT DRESDEN

DIGS-BB

Dresden International Graduate School for Biomedicine and Bioengineering

Marcelo Leomil Zoccoler

With material from:
Robert Haase

PoL
Physics of Life
TU Dresden

September 2022

DRESDEN concept

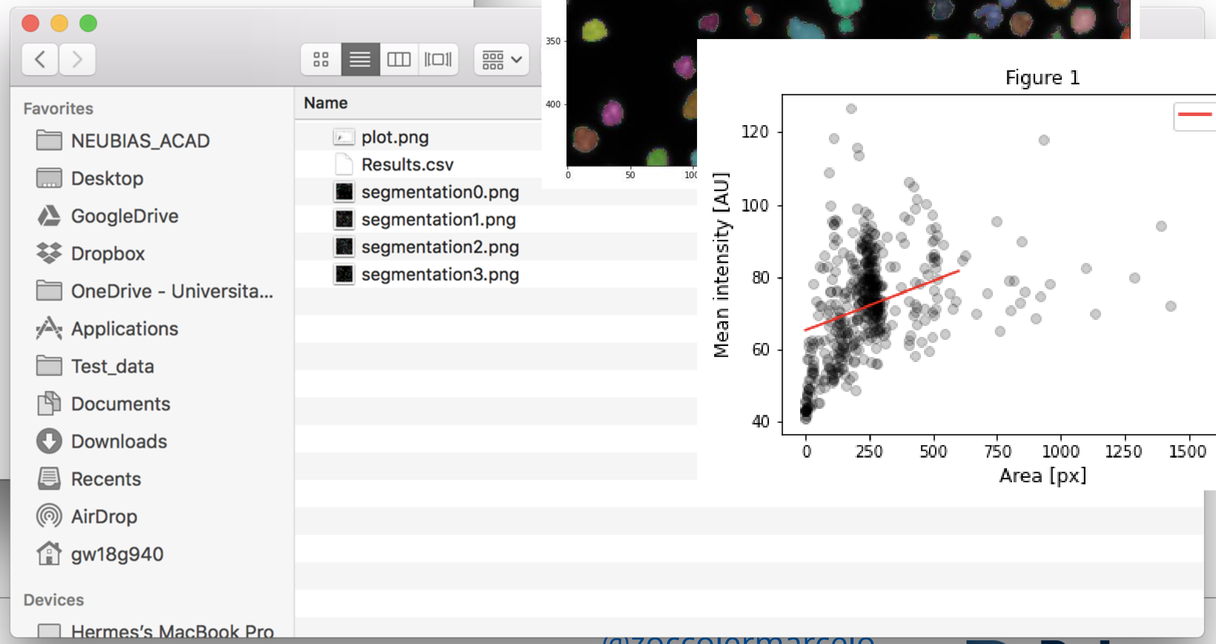
“Classic” software vs. notebooks

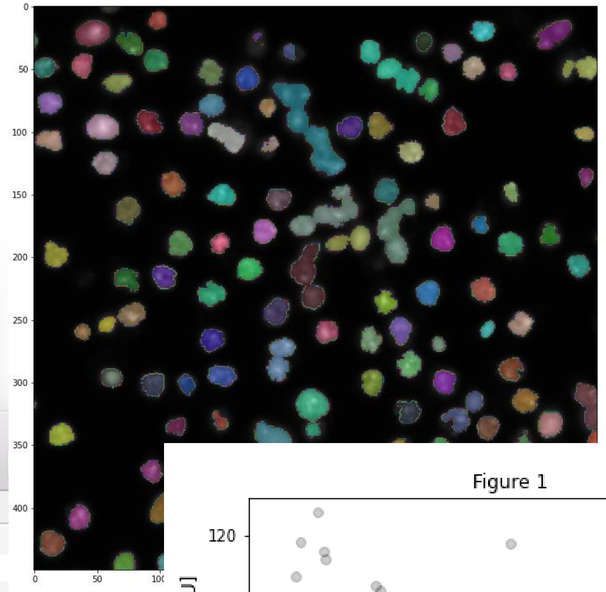
course_functions.py

```
import skimage.morphology
import skimage.filters
import numpy as np
from skimage.measure import label, regionprops_table
import matplotlib

def detect_nuclei(image, si

    # filtering
    image = skimage.filters
    # local thresholding
    image_local_threshold =
    image_local = image > i
    # remove tiny features
    image_local_open = skim
    # label image
    image_labeled = label(i
    # analyze regions
    our_regions = regionpro
    # create a new mask wit
    newimage = np.zeros(ima
    # fill in using region
    for x in range(len(our_
        if our_regions['are
            newimage[our_re

    return newimage
```

gw18g940 — -bash — 72×21

```
Last login: Thu Apr 30 10:41:20 on ttys002
(base) Hermess-MacBook-Pro:~ gw18g940$ python course_functions.py
```





“Classic” software vs. notebooks

Code divided in parts

Dynamic: easy to test

Rich output: processing + visualisation + analysis in one place

Code + formatted text: easy documentation

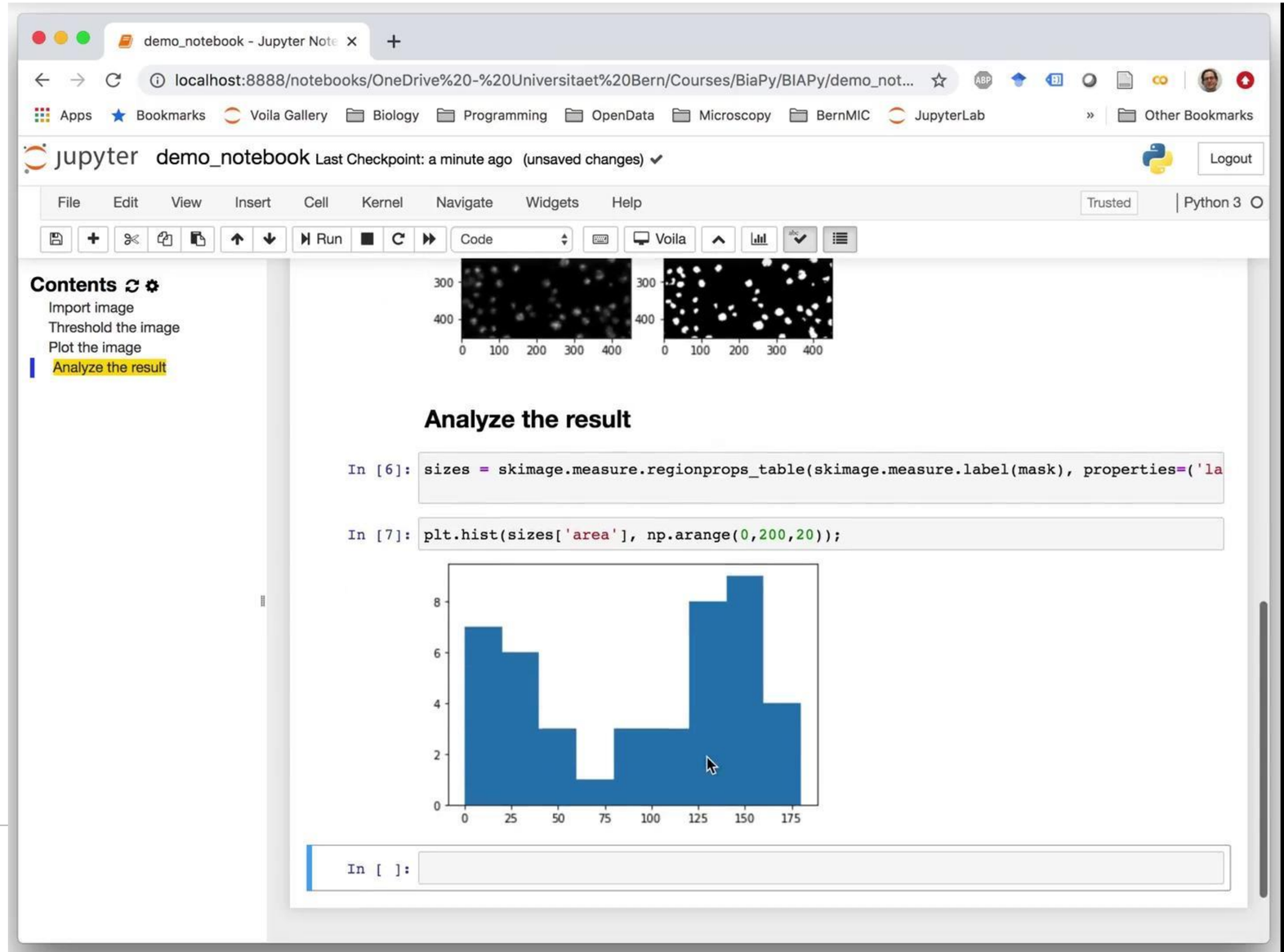


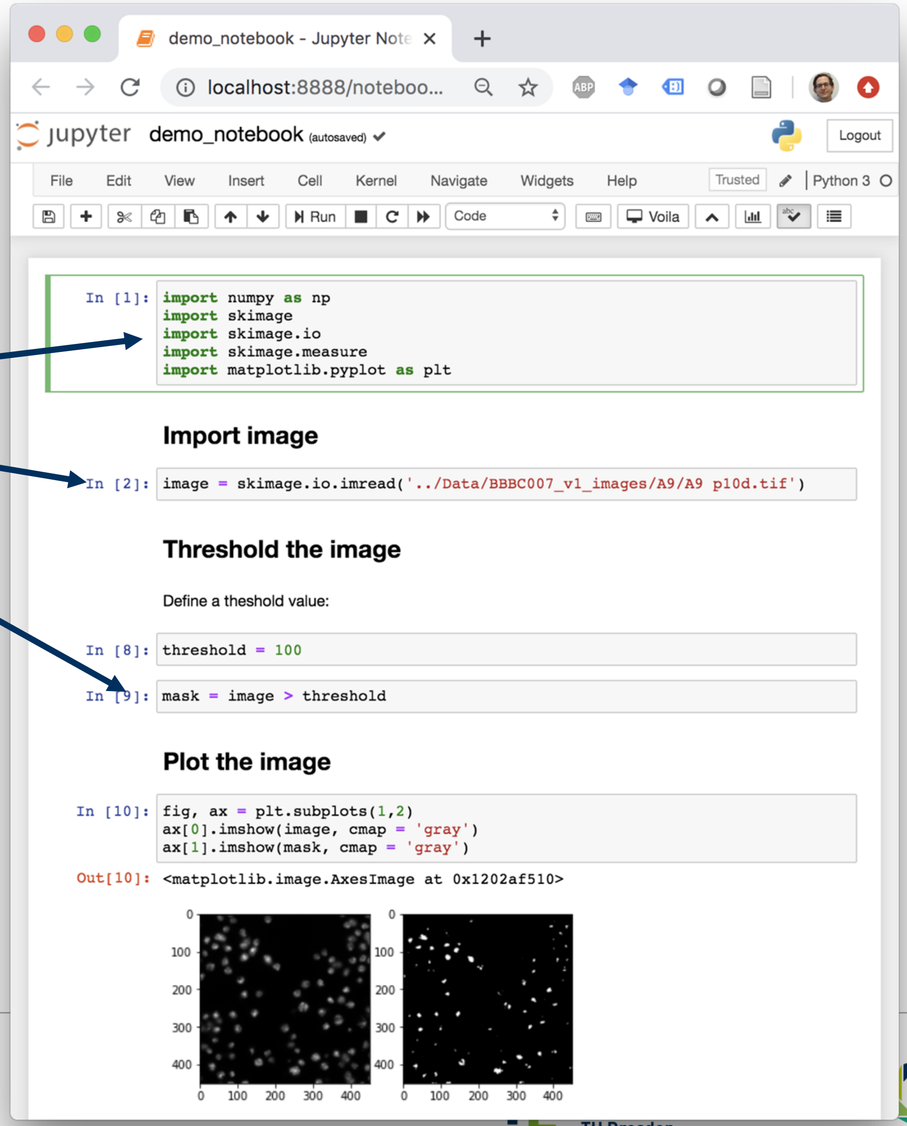
Illustration: load an image, threshold it, analyze object size

What is a jupyter notebook?

A text file (easily sent around)

Rendered by Jupyter in the browser

Split into sections called cells



What is a jupyter notebook?

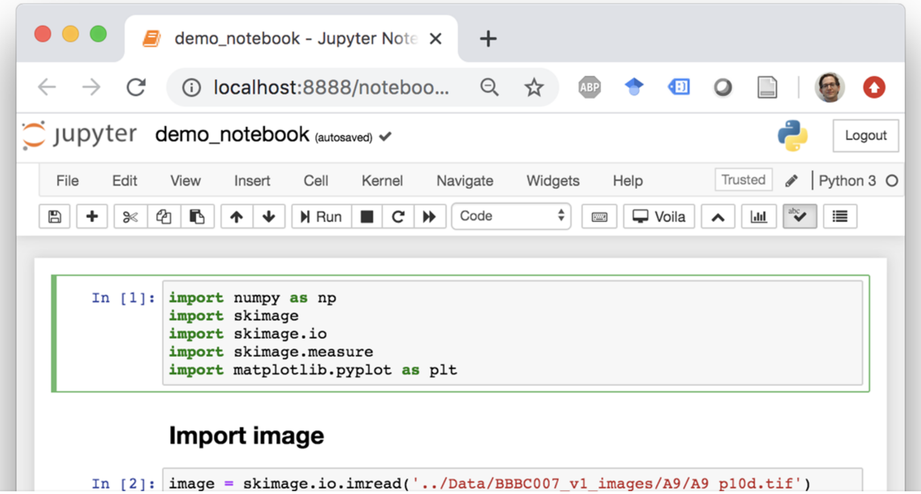
A text file (easily sent around)

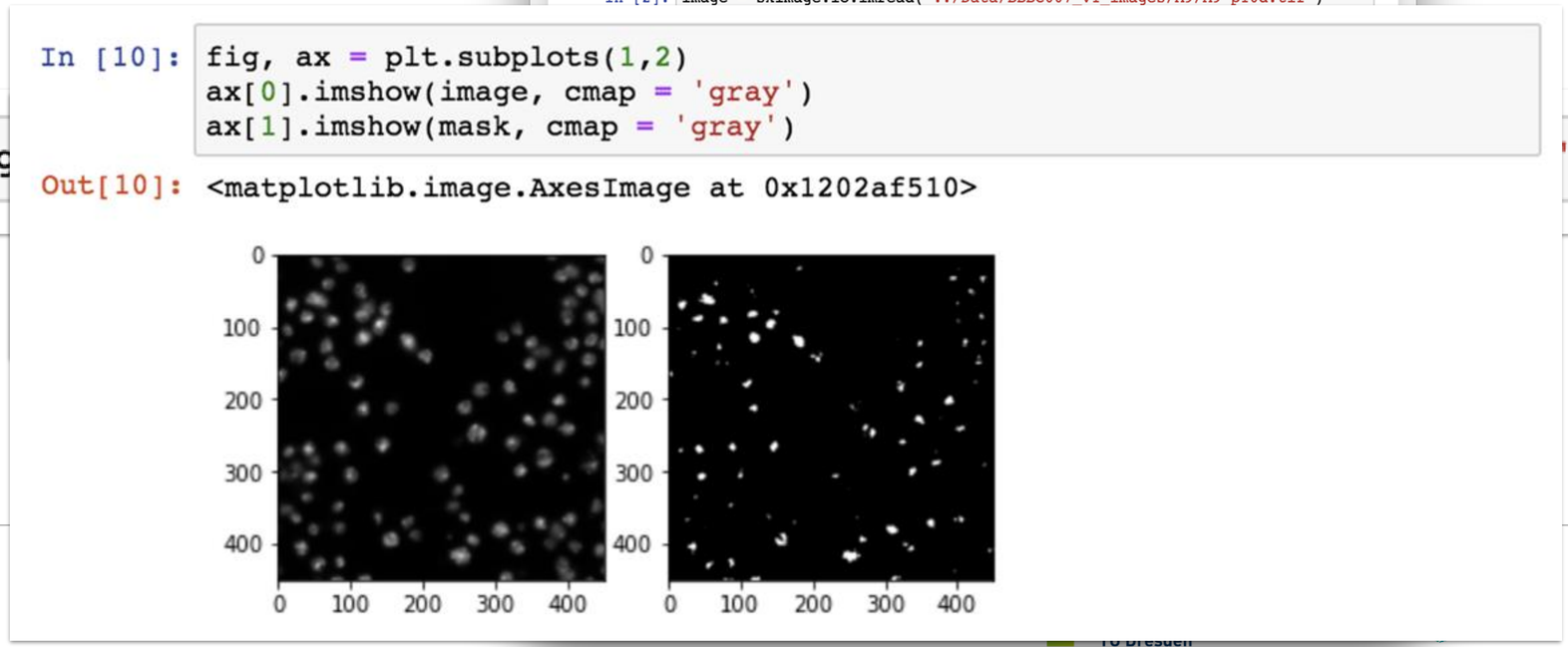
Rendered by Jupyter in the browser

Split into sections called cells

Cells can contain:

- Code
- Formatted text
- Rich output

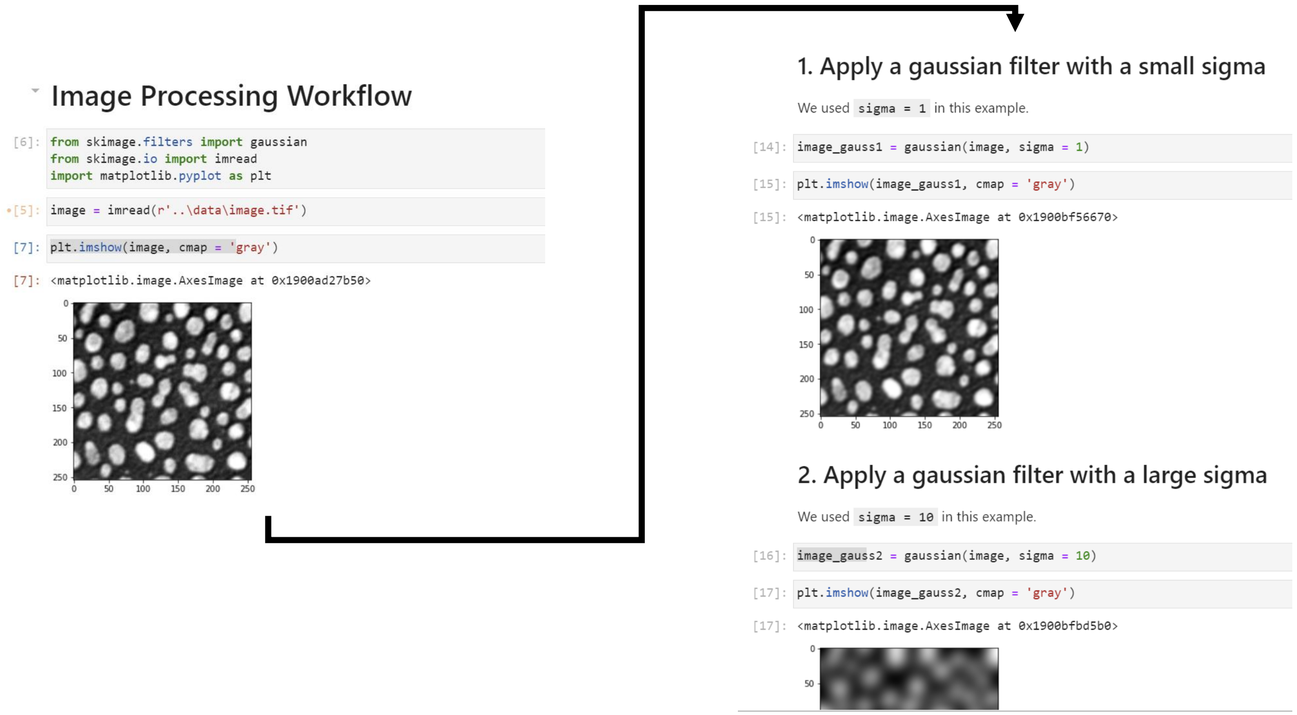




Why using notebooks?

Documenting (for your-(future)-self and for others) and enhanced reproducibility

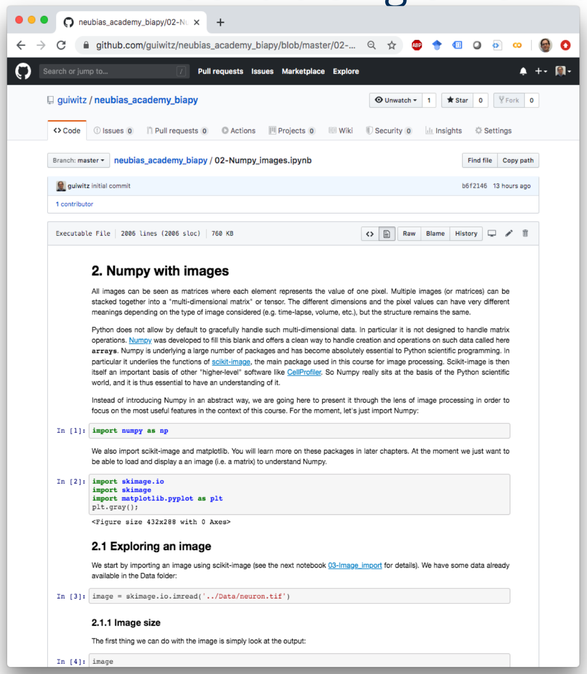
"First, we applied a Gaussian filter from scikit-image with sigma = 1. Then, we applied another Gaussian filter to the original image with sigma = 10. After that, we subtracted the first result from the second...



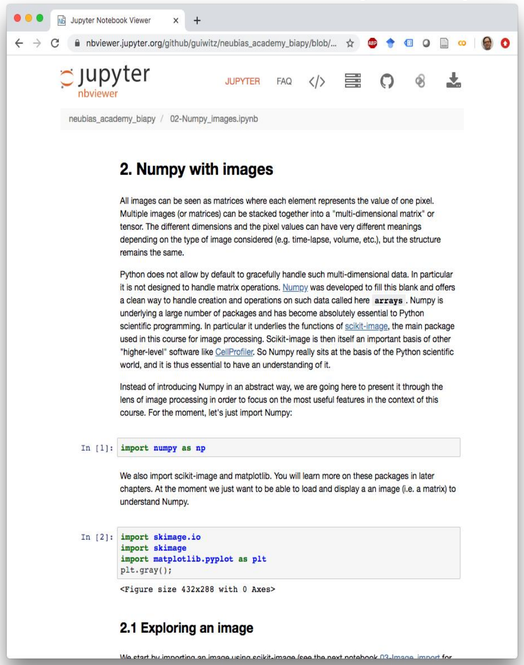
Why using notebooks?

Sharing

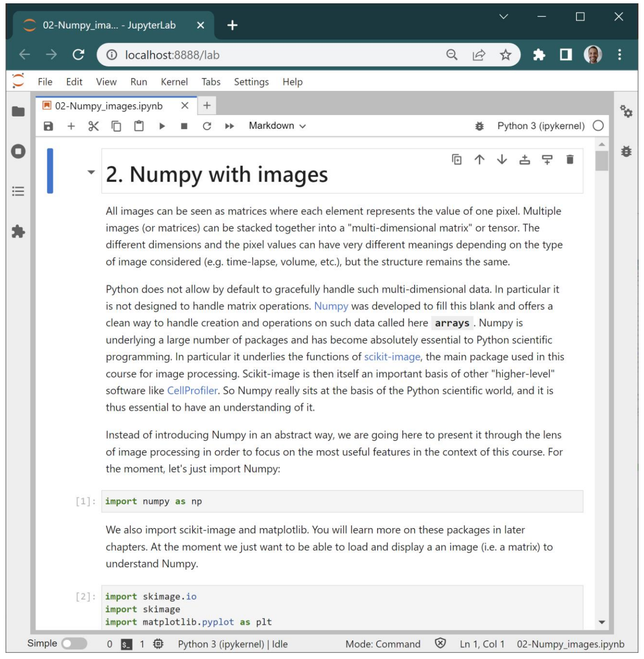
- Send a single file with code, intermediary outputs and rich text explanations



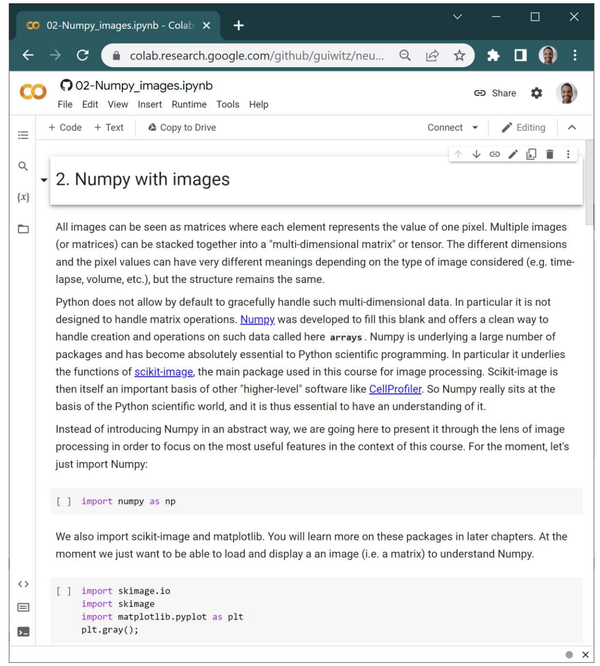
Github rendering



Nbviewer rendering



Jupyter lab (local or Binder)



Google Colab

Why using notebooks?

Keep all the benefits from using code:

- Batch processing

- Running python functions/tools that do not have UI

Why using notebooks with napari?

- Easy data interaction and visualization with napari:
  - Great for visualizing 3D (and more) data
  - Each processing step result can be displayed as a separate layer
- Data annotation

Scripting napari in notebooks

Start napari from a jupyter

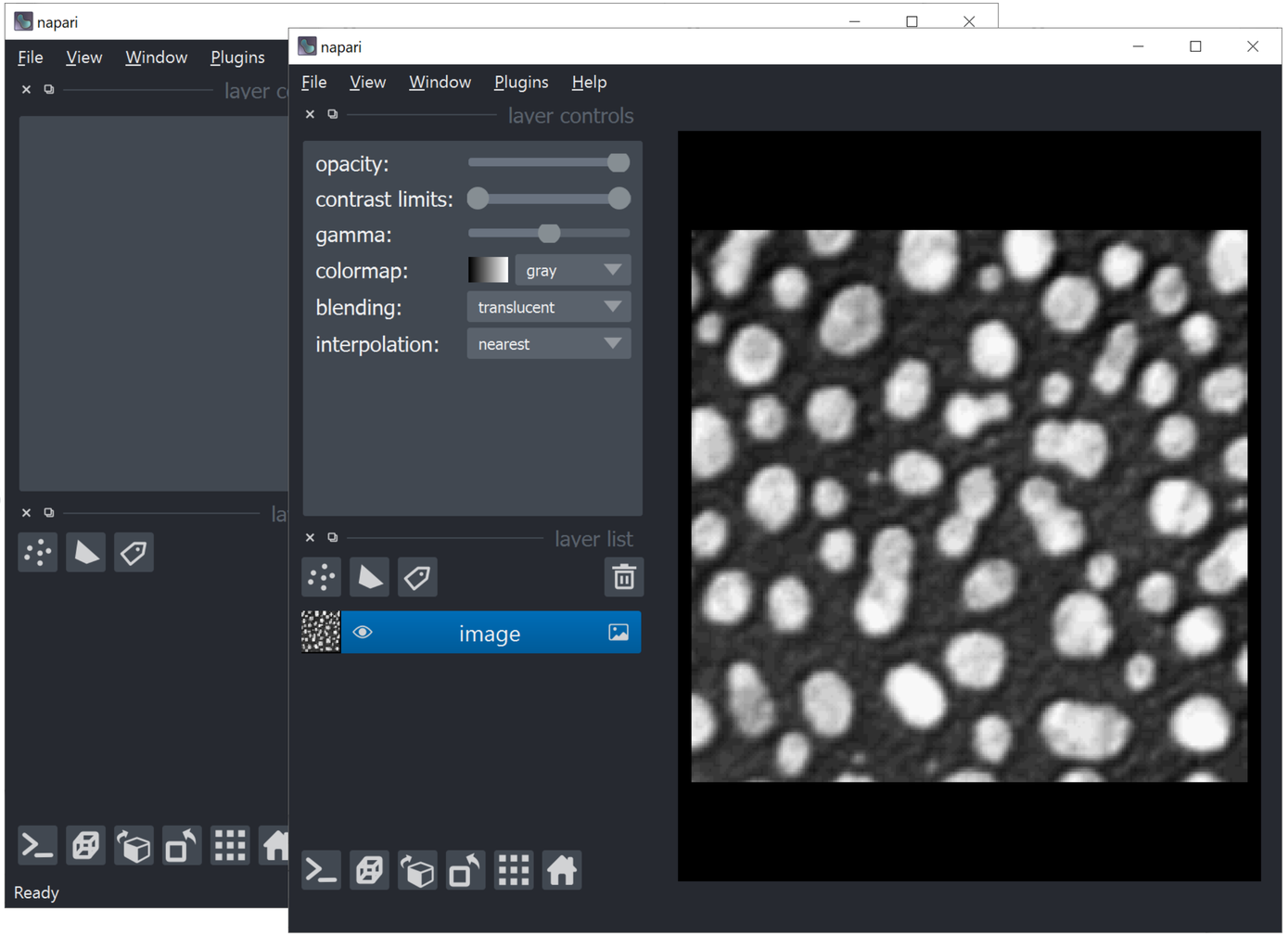
```
import napari

# Create an empty viewer
viewer = napari.Viewer()
```

viewer.add_image(image, name = ‘image’)

Load image from notebook

to napari (and back): flexibility!

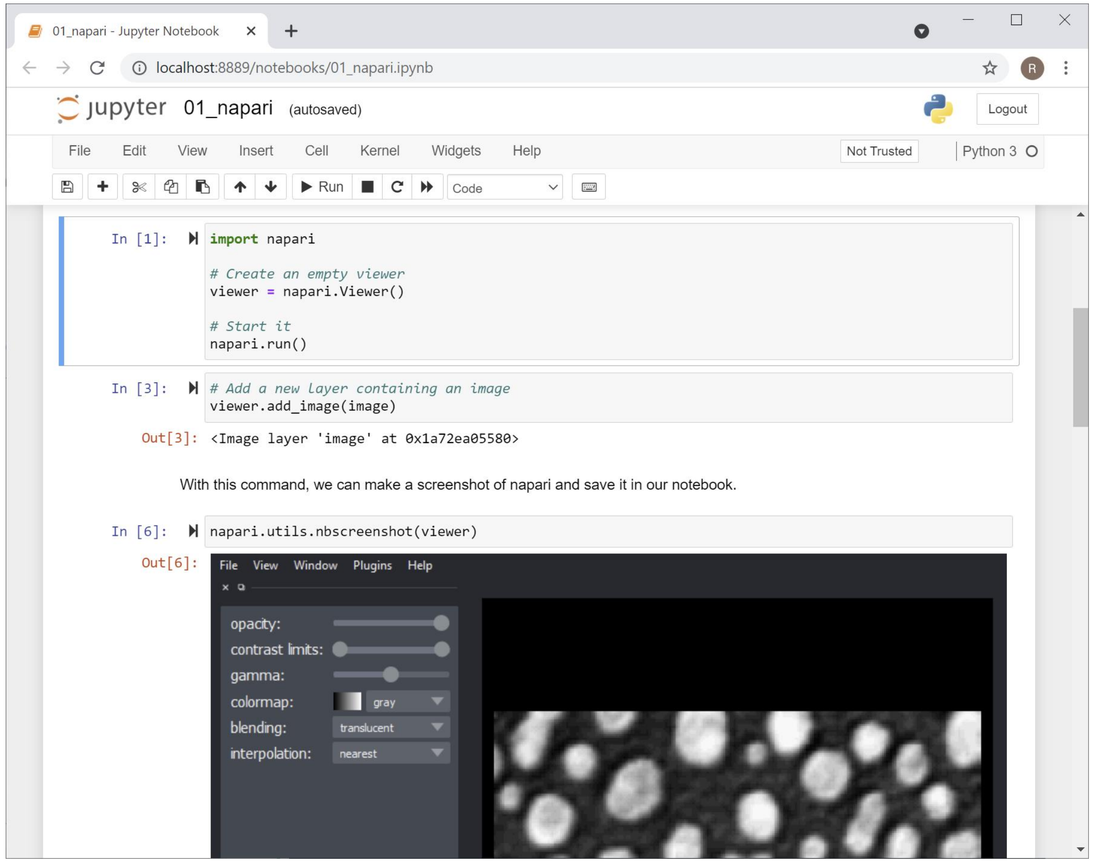
image = viewer.layers[‘image’].data



Scripting napari in notebooks

Make screenshots from napari and put them in your jupter notebook

```
napari.utils.nbscreenshot(viewer)
```



Scripting napari in notebooks

- Add layers to napari to visualize intermediate processing results on top of each other or side by side.
- Change layer visualization within napari...

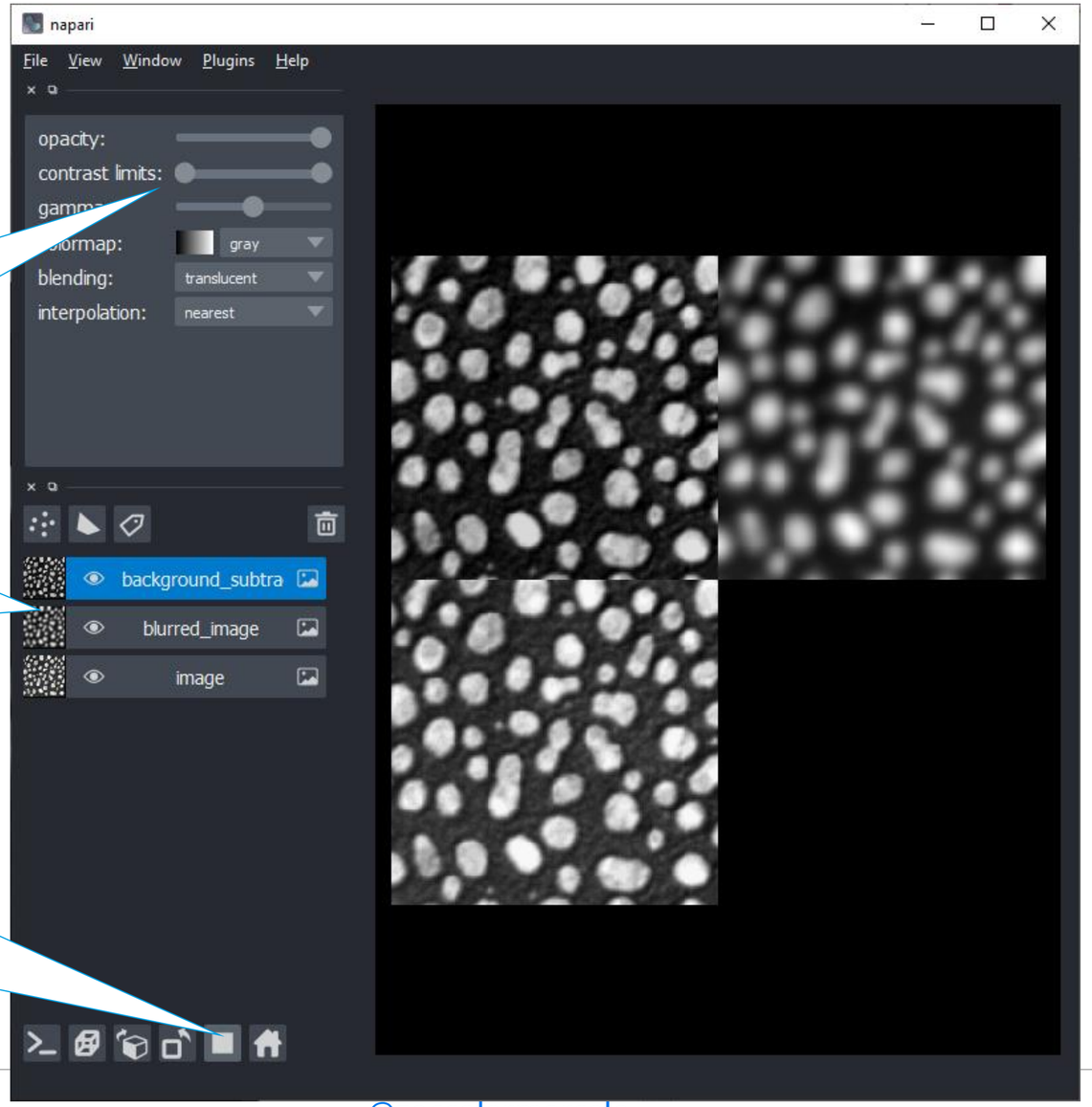
... or via code in a jupyter notebook:

```
viewer.layers[0].contrast_limits
```

```
[0, 255]
```

1. Access the viewer
2. Access the layers
3. Choose a layer (by index or name)
4. “Press TAB” and check out available properties

```
viewer.layers[0].contrast_limits = [30,170]
```



Change Brightness and contrast here

Toggle visualization of layers here

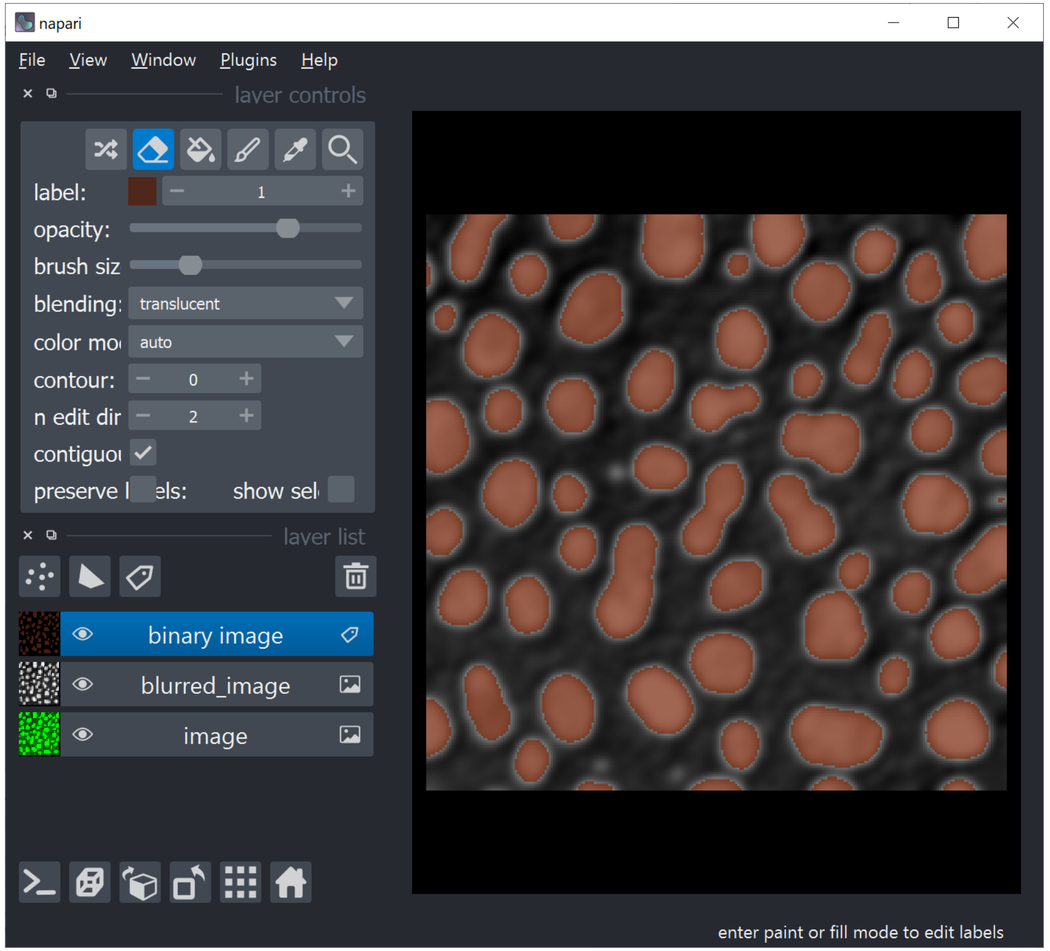
Gallery view

Visualizing image segmentation

Binary images and label images visualized as label layers

```
from skimage.filters import threshold_otsu

threshold = threshold_otsu(blurred_image)

binary_image = blurred_image > threshold


# Add a new labels layer containing an image

viewer.add_labels(binary_image,

                  name="binary image")
```

Name your layers to keep track of what they contain



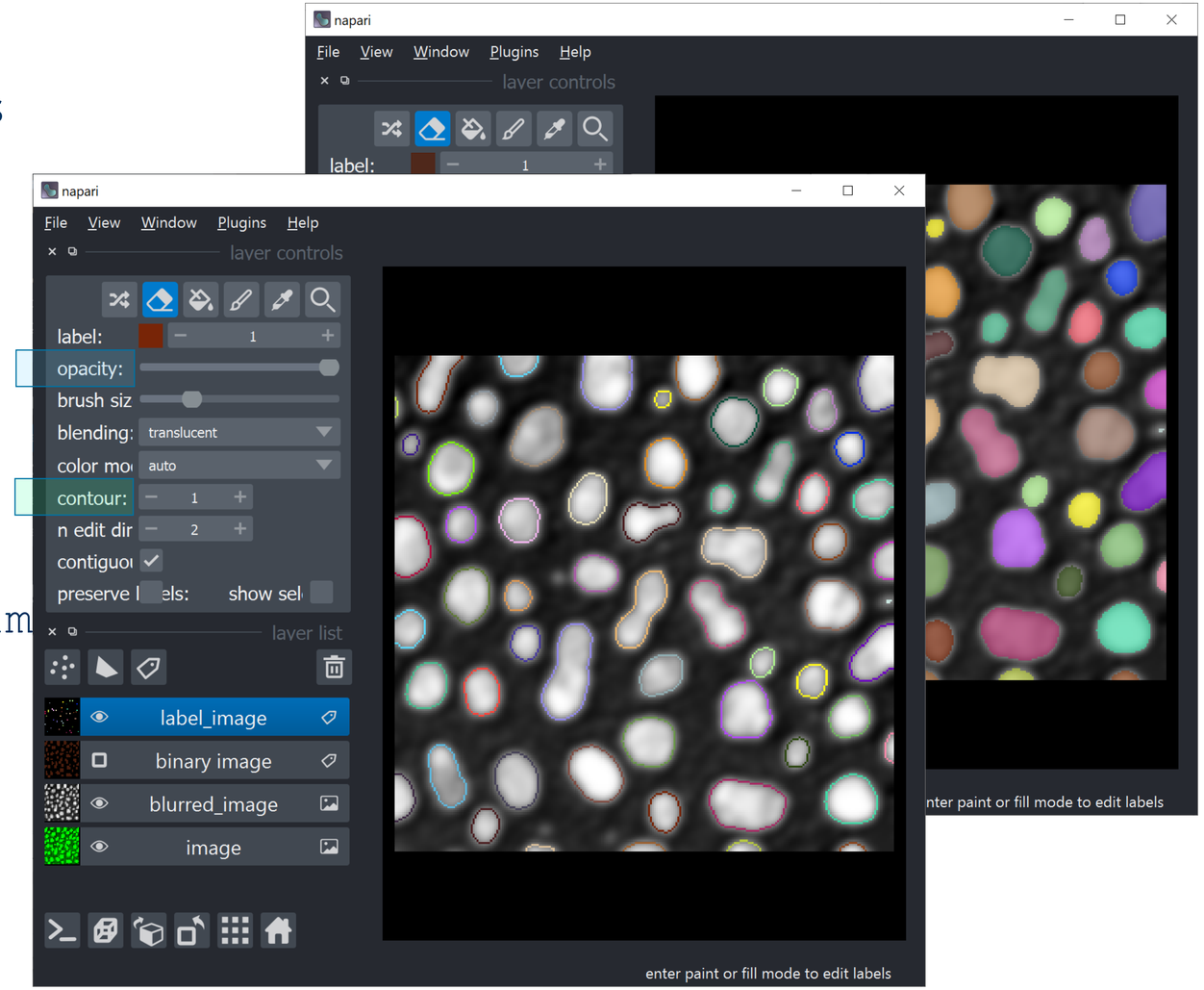
Visualizing image segmentation

Binary images and label images visualized as label layers

```
from skimage.measure import label

label_image = label(binary_image)


# add labels to viewer

label_layer = viewer.add_labels(label_im
```

Visualize contours instead of the overlay

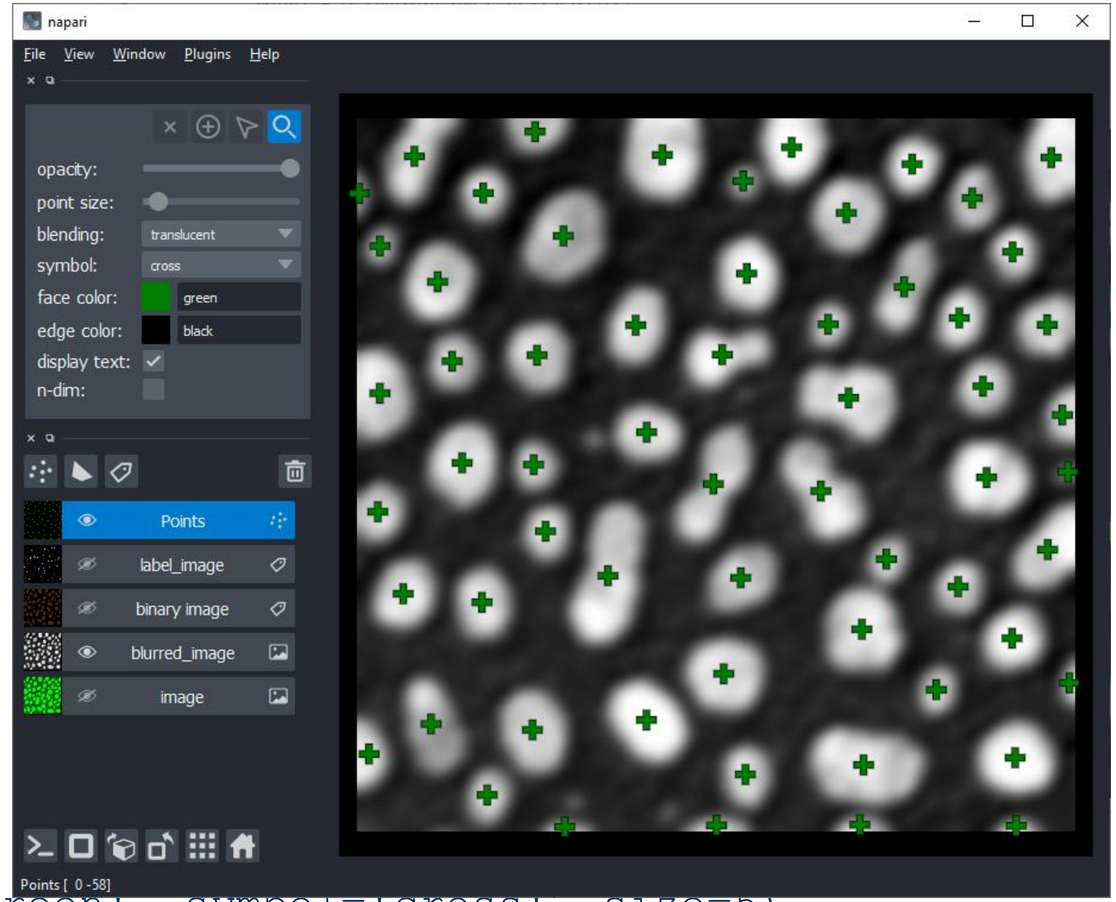
```
label_layer.contour = 1

label_layer.opacity = 1
```



Points layers

There is also other layer types

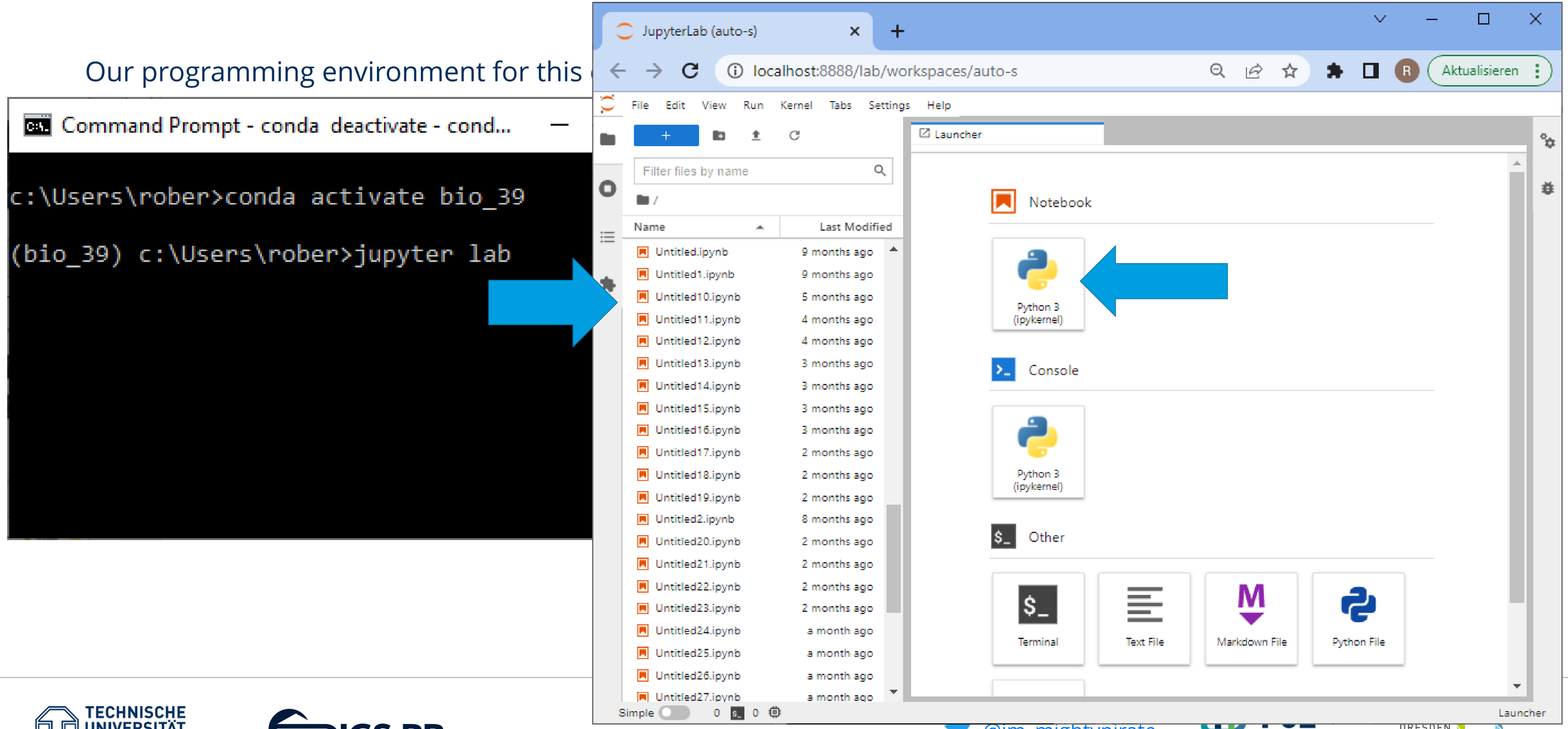
- Shapes
- Points
- Surfaces
- Tracks
- Vectors




```
from skimage.measure import regionprops

statistics = regionprops(label_image)

points = [s.centroid for s in statistics]

# add points to viewer
label_layer = viewer.add_points(points, face_color='green', symbol='cross', size=5)
```

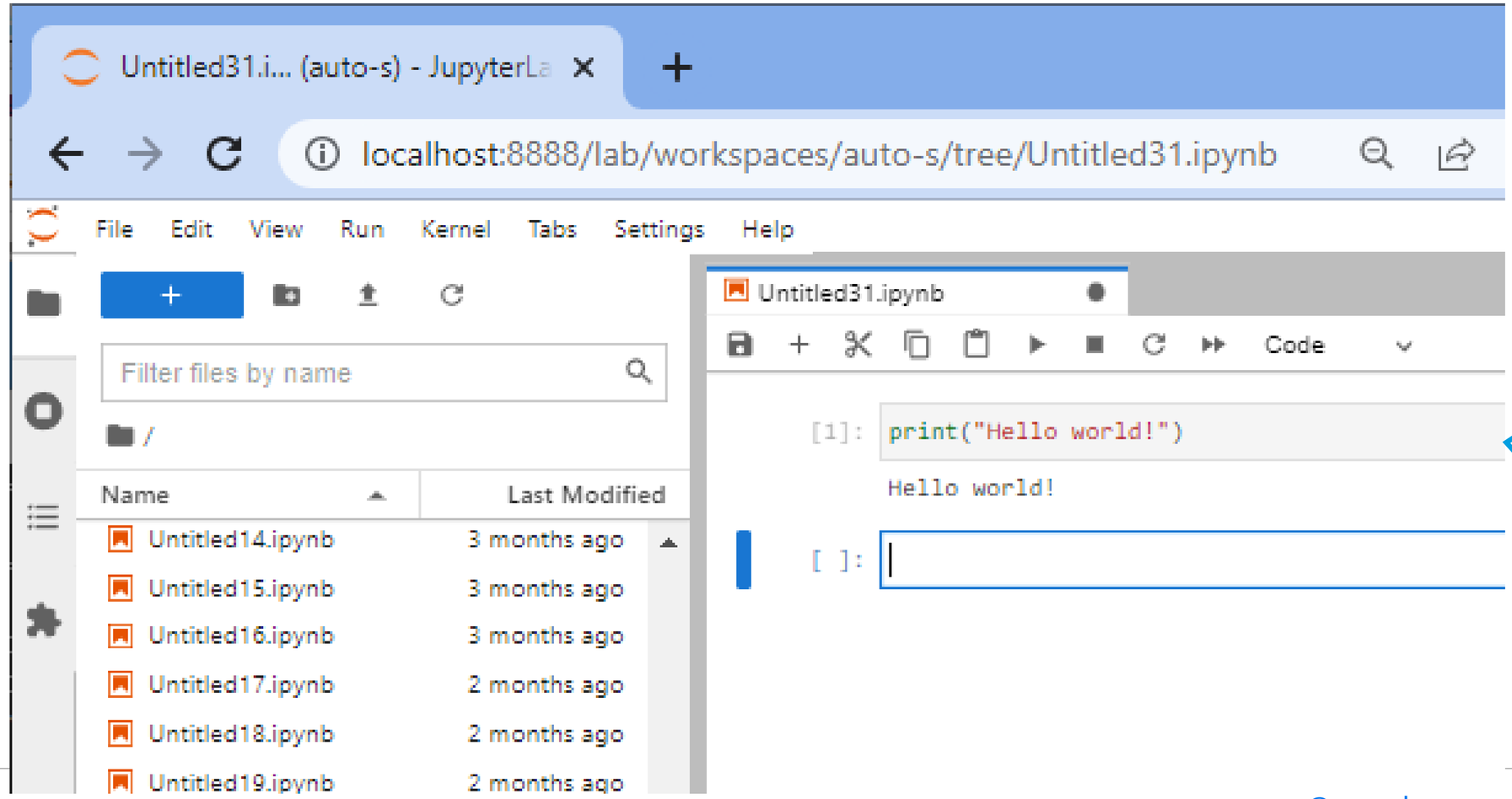
Jupyter lab

Our programming environment for this



Jupyter lab

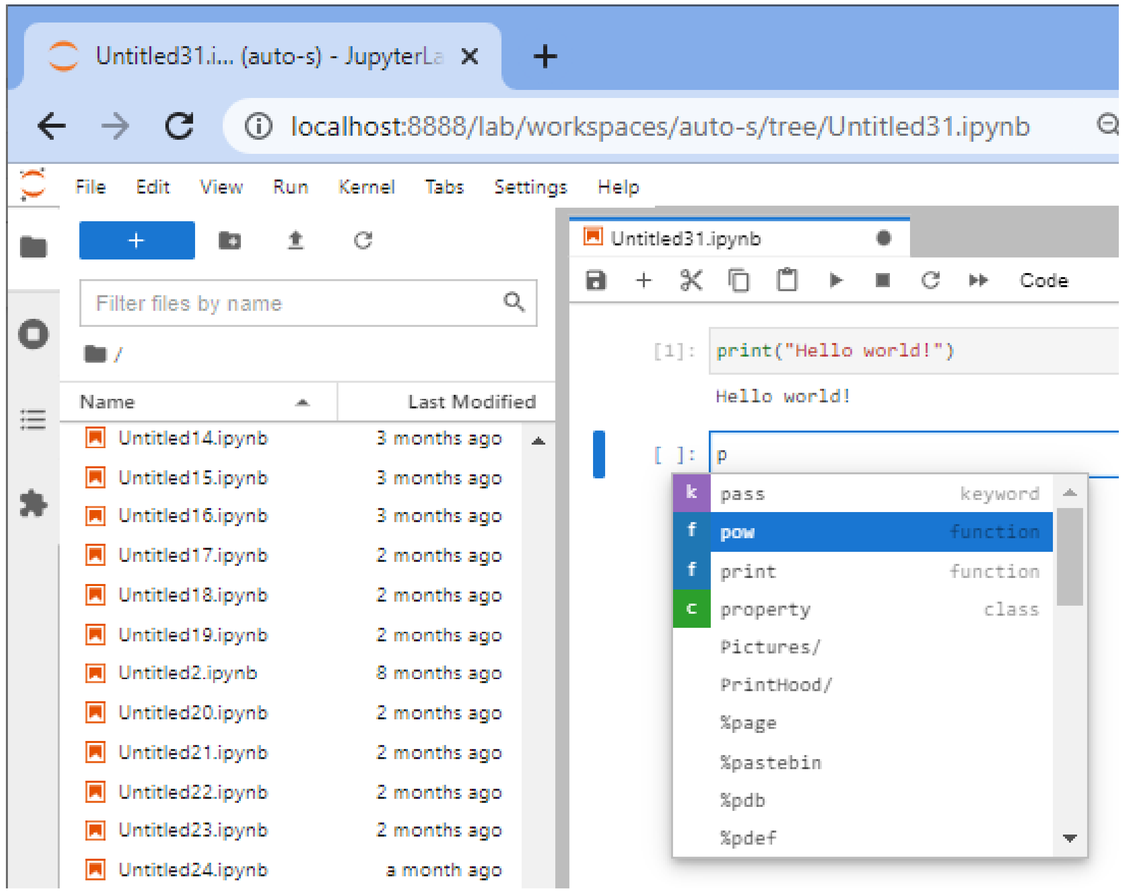
Execute code cell-by-cell and see results instantaneously



SHIFT + ENTER to execute a code cell

Jupyter lab

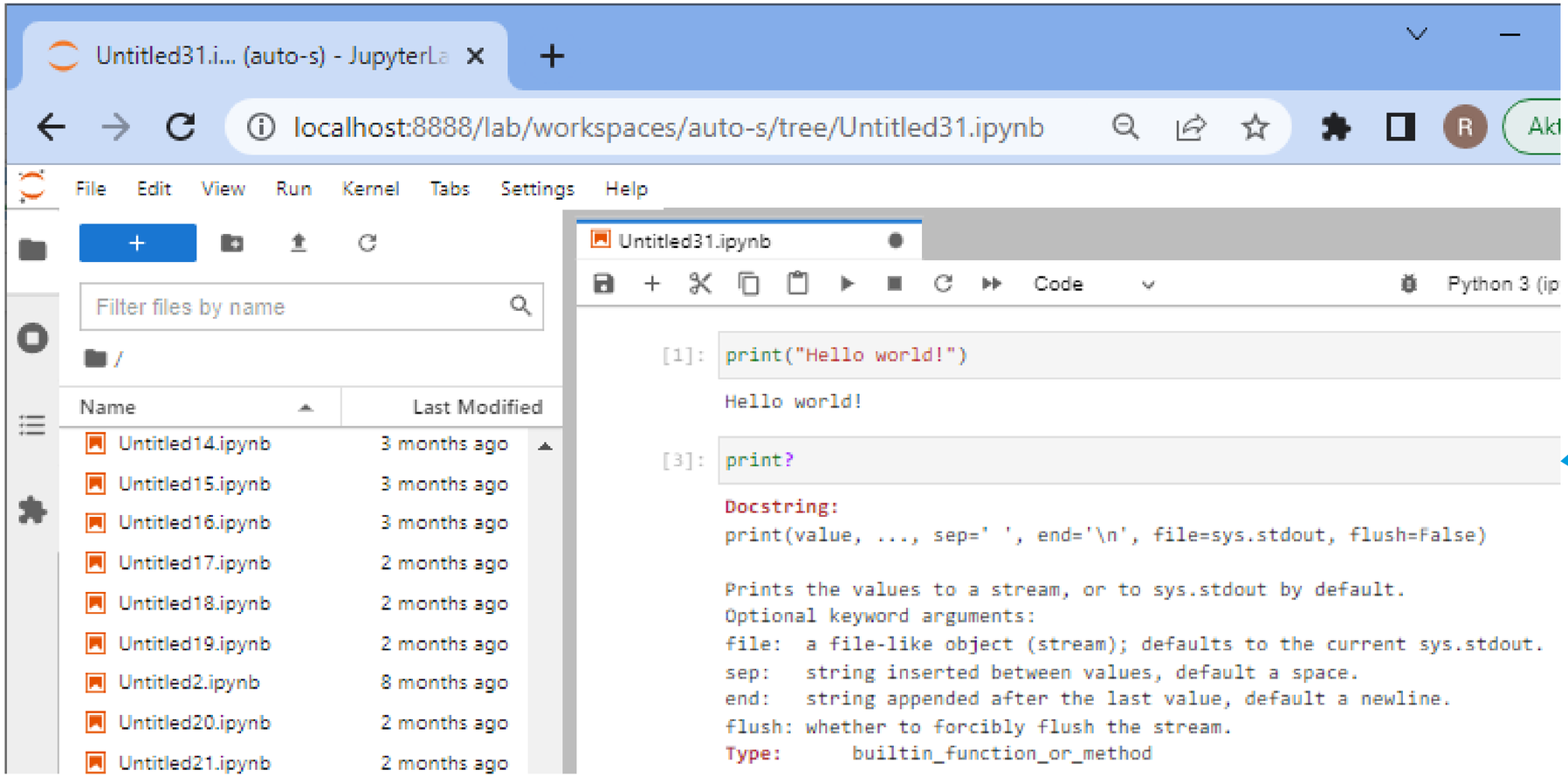
Context-specific help, auto-completion



TAB
to open
auto-
completion

Jupyter lab

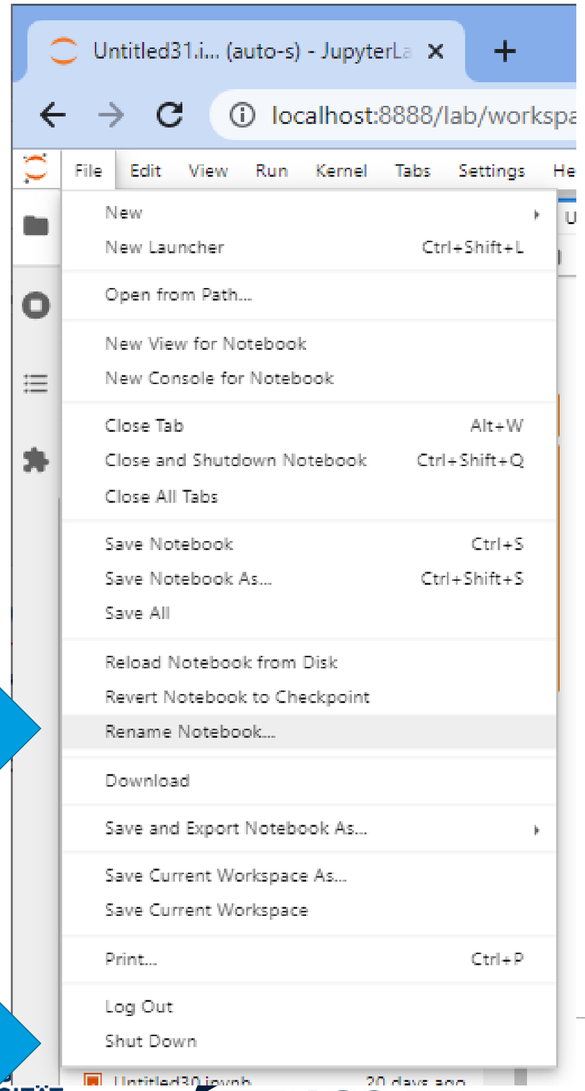
Help / “docstrings”

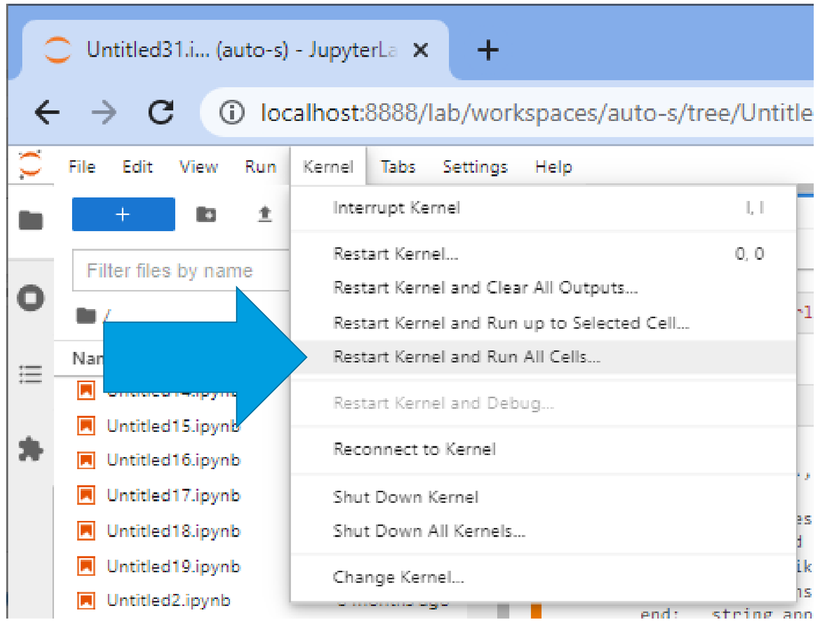


?
to read what a function does

Jupyter lab

Saving / renaming / closing





Enforcing a “clean” execution state is important for ensuring reproducibility and repeatability

@zoccolermarcelo
@jm_mightypirate

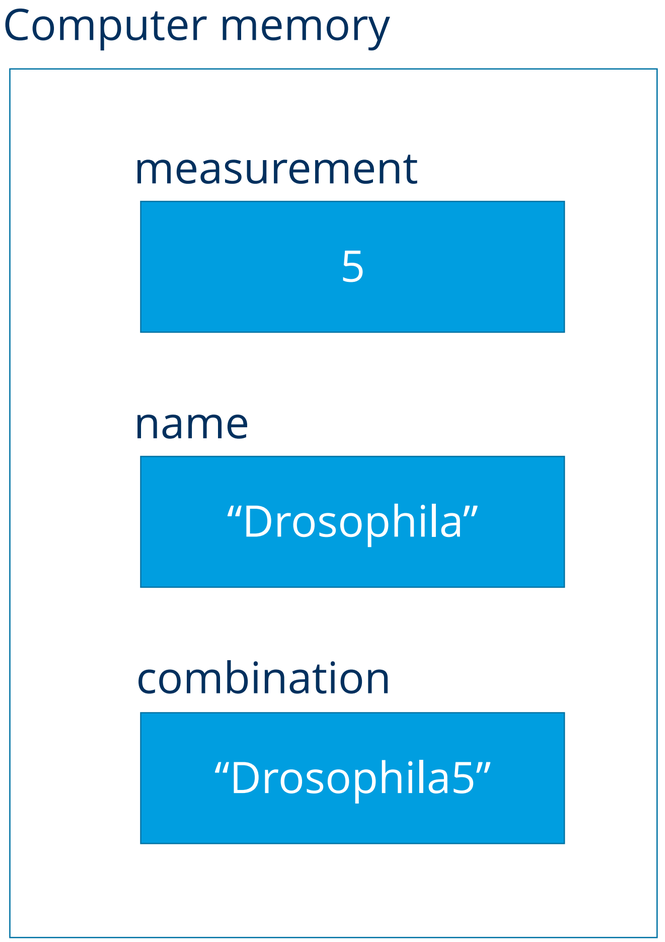
Python
Data structures

Variables

Variables are memory blocks where you can store stuff

```
measurement = 5

name = “Drosophila”

combination = name + str(measurement)
```

Function(argument)



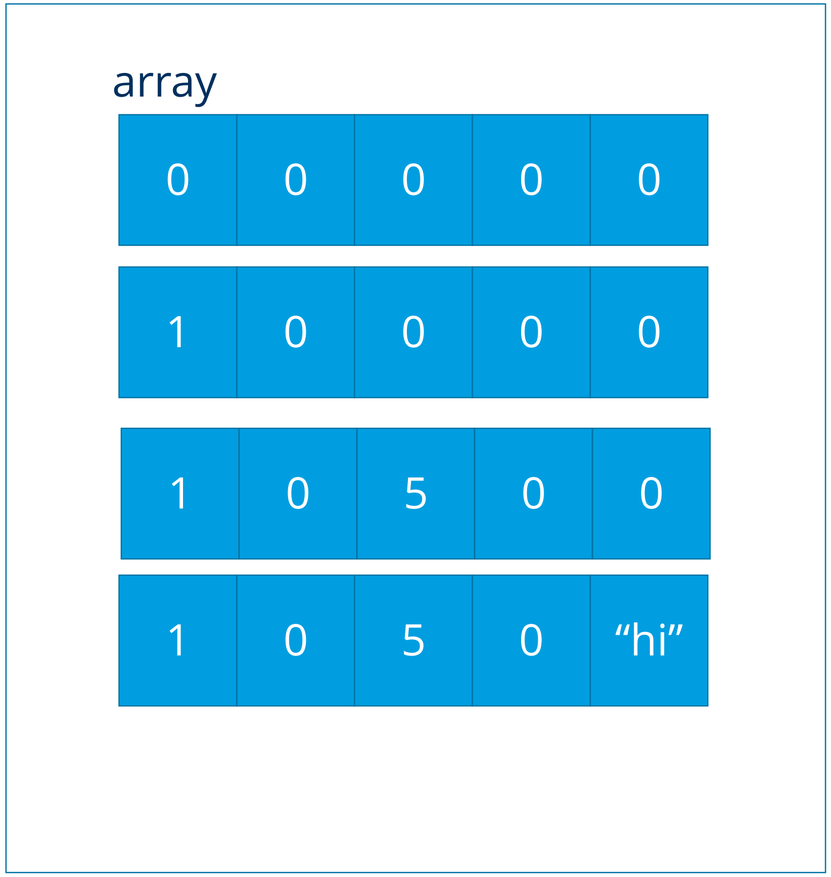
Arrays

Arrays are variables, where you can store multiple values

Give me a “0”, five times!

```
array = [0] * 5

array[0] = 1

array[2] = 5

array[4] = “hi”
```

Computer memory

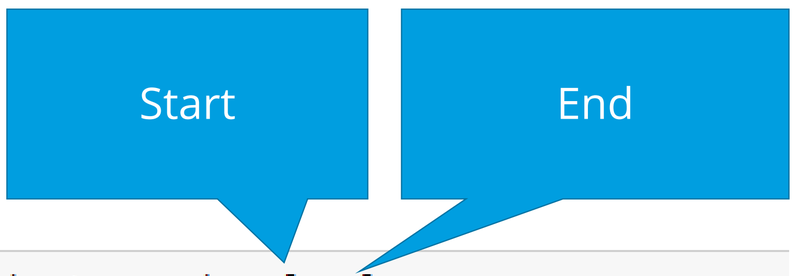


Subsets

```
# Arrays
numbers = [0, 1, 2, 3, 4, 5, 6, 7, 8, 9]
print(numbers)
```

```
[0, 1, 2, 3, 4, 5, 6, 7, 8, 9]
```

- Creating subsets of arrays




```
subset = numbers[2:4]
print(subset)
```

```
[2, 3]
```


```
subset_with_gaps = arr[1:8:2]
print(subset_with_gaps)
```

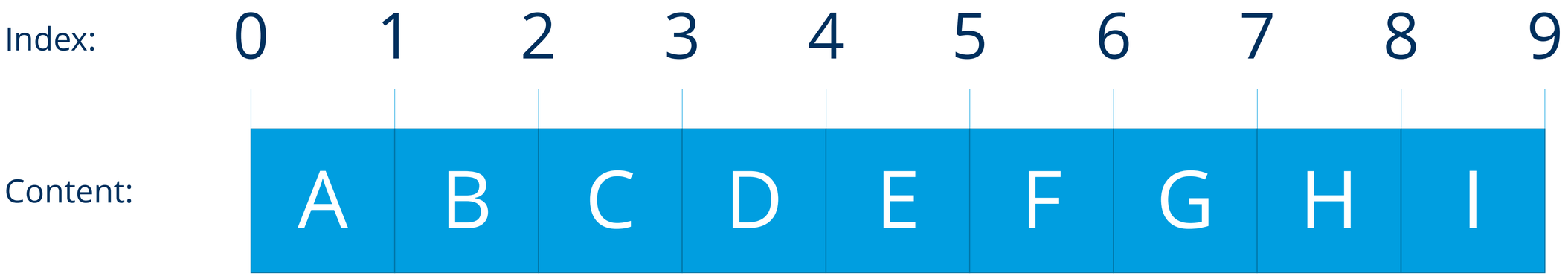
```
[1, 3, 5, 7]
```

`data[start:stop:step]`

Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

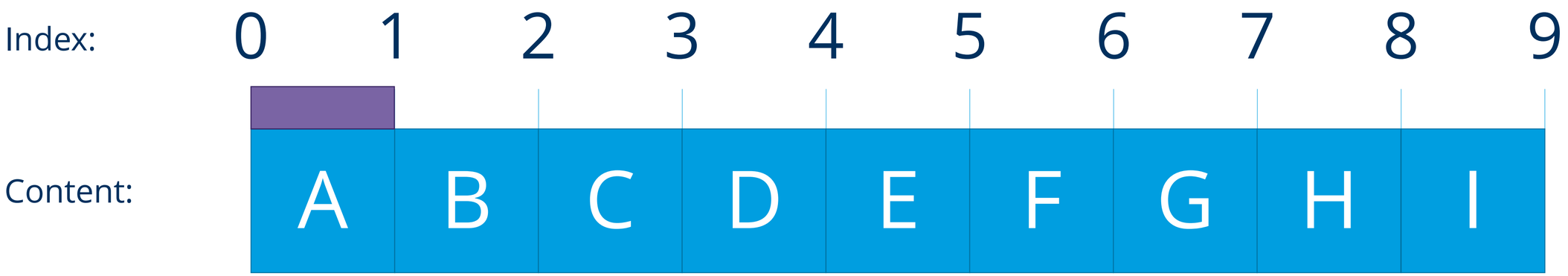
```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



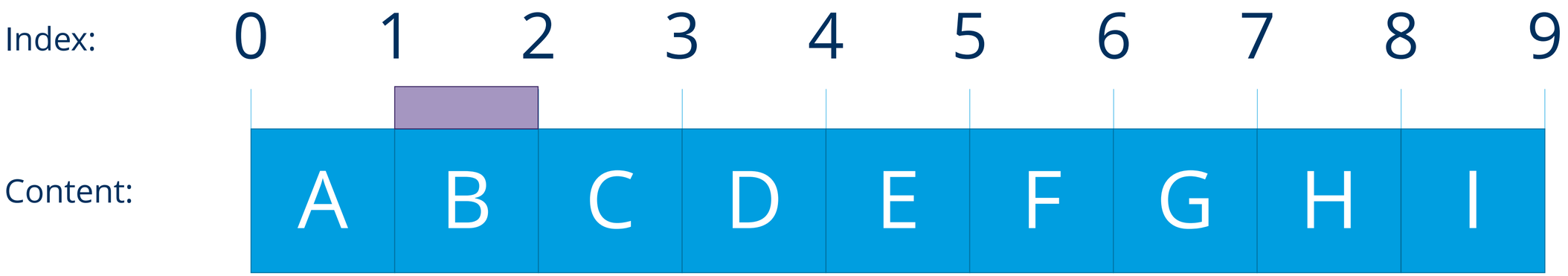

```
data[0]
```

```
'A'
```

Indexing, cropping, subsets

"Indexing" is addressing certain elements in arrays. The first element is "0" away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0]
```

```
'A'
```

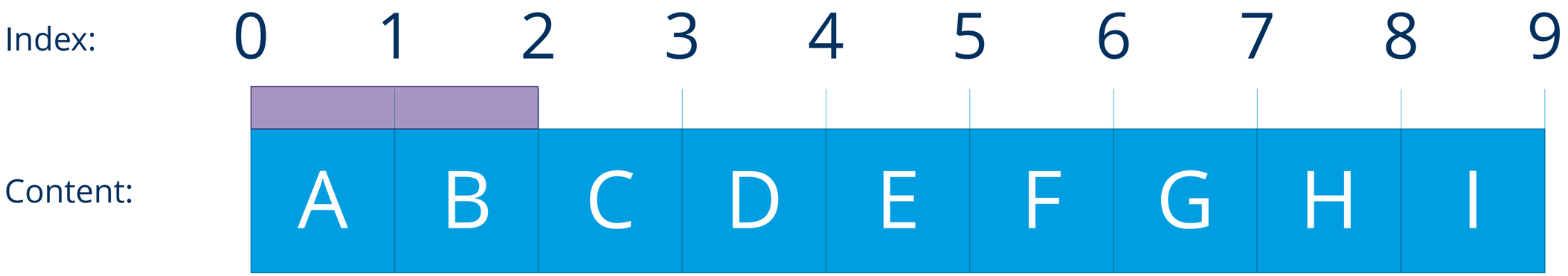
```
data[1]
```

```
'B'
```

Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0]
```

```
'A'
```

```
data[1]
```

```
'B'
```

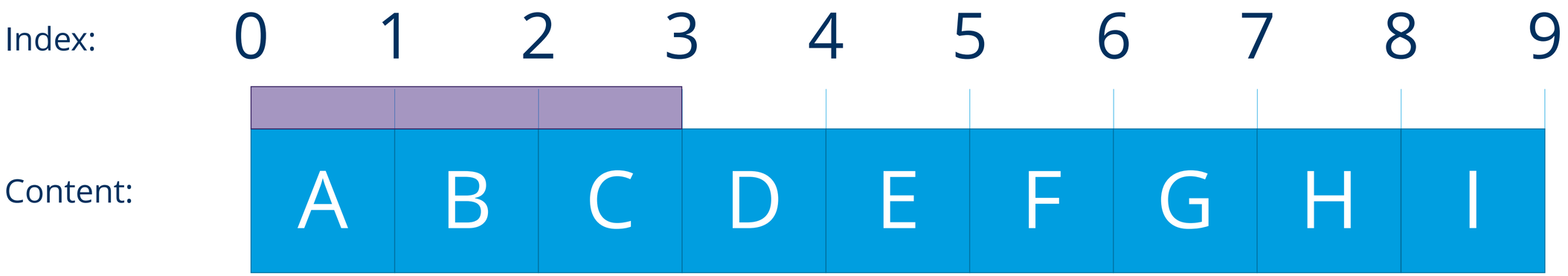
```
data[0:2]
```

```
['A', 'B']
```

Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0]
```
'A'

```
data[1]
```
'B'

```
data[0:2]
```
['A', 'B']

```
data[0:3]
```
['A', 'B', 'C']

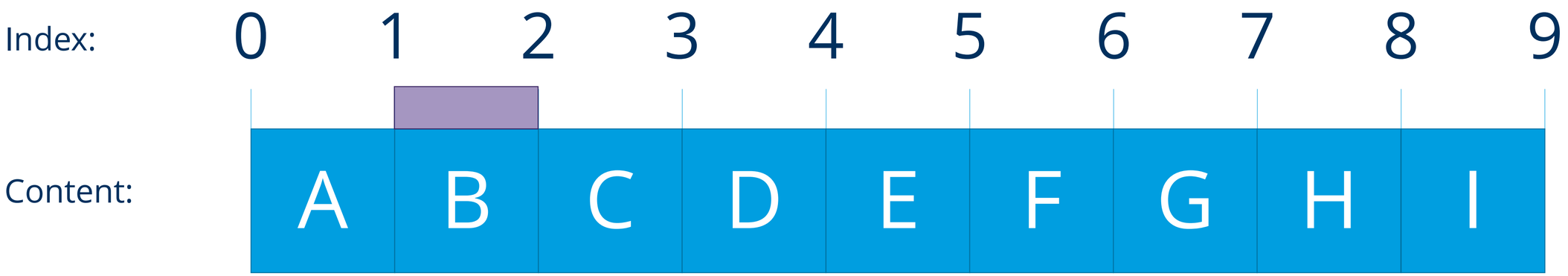
```
data[1:2]
```
['B']

```
len(data)
```
9

Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0]
```
```
'A'
```

```
data[1]
```
```
'B'
```

```
data[0:2]
```
```
['A', 'B']
```

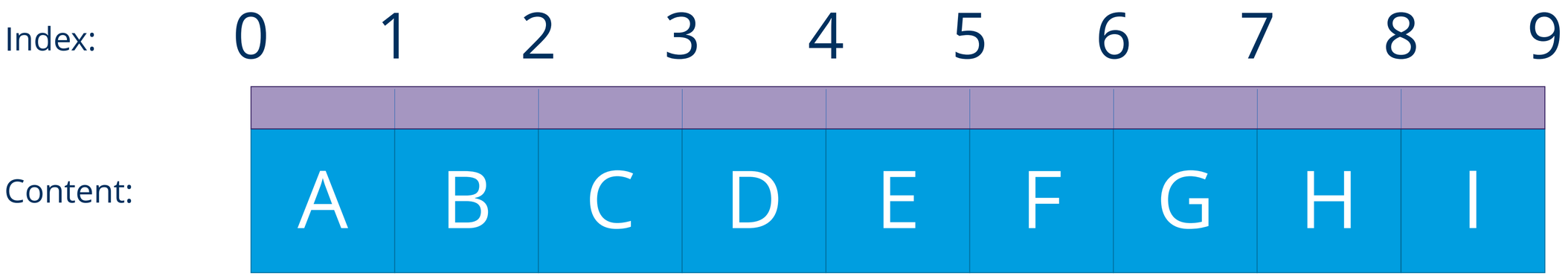
```
data[0:3]
```
```
['A', 'B', 'C']
```

```
data[1:2]
```
```
['B']
```

Indexing, cropping, subsets

“Indexing” is addressing certain elements in arrays. The first element is “0” away from the start.

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```

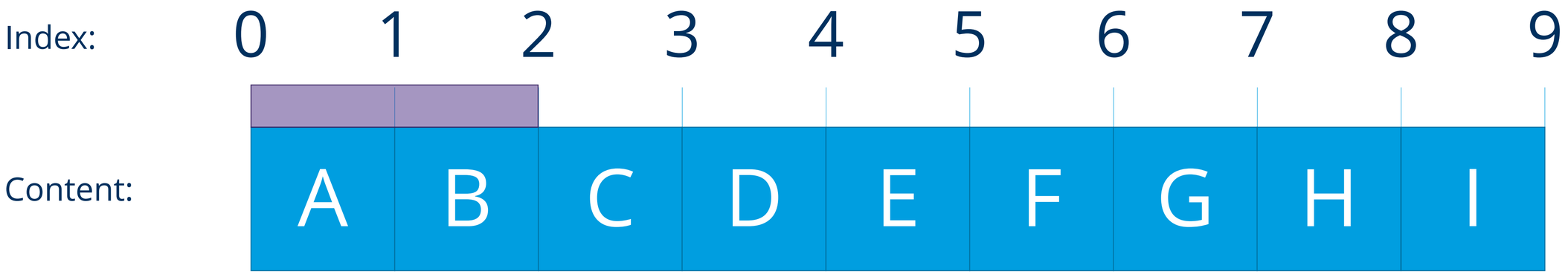


`data[0]`	`data[1]`	`data[0:2]`	`data[0:3]`	`data[1:2]`	`len(data)`
`'A'`	`'B'`	`['A', 'B']`	`['A', 'B', 'C']`	`['B']`	`9`

Indexing, cropping, subsets

You can leave start and end out when specifying index ranges

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



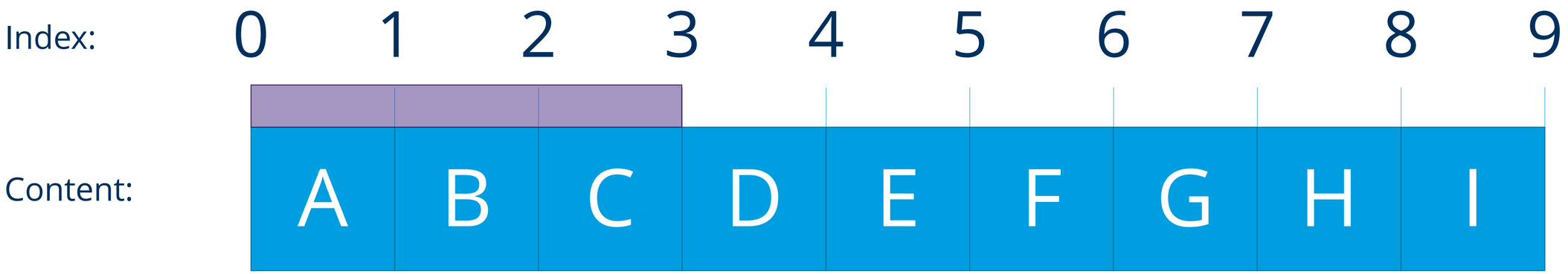

```
data[:2]
```

```
['A', 'B']
```

Indexing, cropping, subsets

You can leave start and end out when specifying index ranges

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[:2]
```

```
['A', 'B']
```

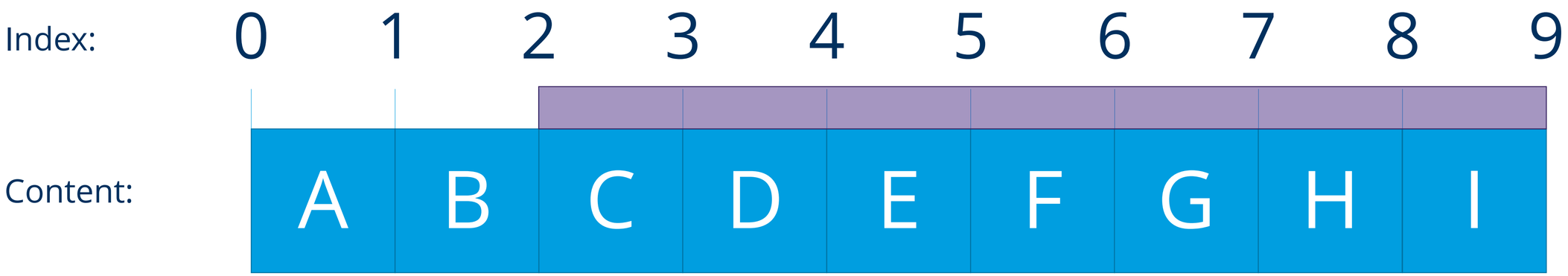
```
data[:3]
```

```
['A', 'B', 'C']
```

Indexing, cropping, subsets

You can leave start and end out when specifying index ranges

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[:2]
```

```
['A', 'B']
```

```
data[:3]
```

```
['A', 'B', 'C']
```

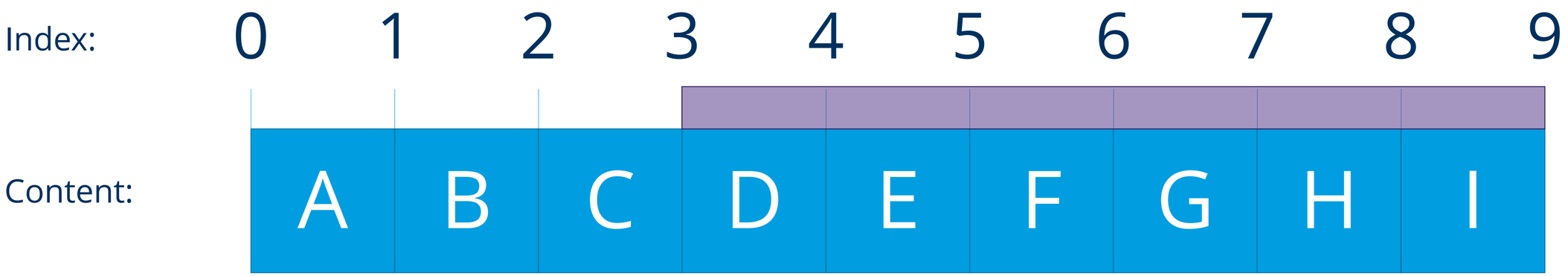
```
data[2:]
```

```
['C', 'D', 'E', 'F', 'G', 'H', 'I']
```

Indexing, cropping, subsets

You can leave start and end out when specifying index ranges

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[:2]
```

```
['A', 'B']
```

```
data[:3]
```

```
['A', 'B', 'C']
```

```
data[2:]
```

```
['C', 'D', 'E', 'F', 'G', 'H', 'I']
```

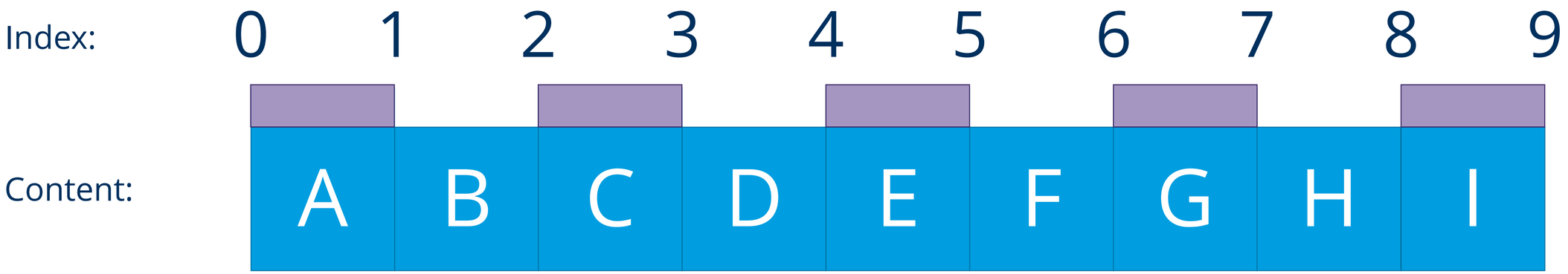
```
data[3:]
```

```
['D', 'E', 'F', 'G', 'H', 'I']
```

Indexing, cropping, subsets

The step-size allows skipping elements

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



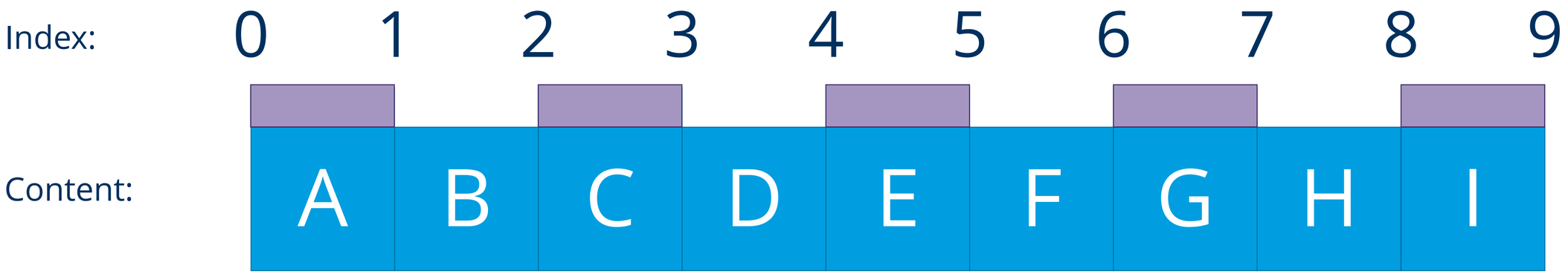

```
data[0:10:2]
```

```
['A', 'C', 'E', 'G', 'I']
```

Indexing, cropping, subsets

The step-size allows skipping elements

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0:10:2]
```

```
['A', 'C', 'E', 'G', 'I']
```

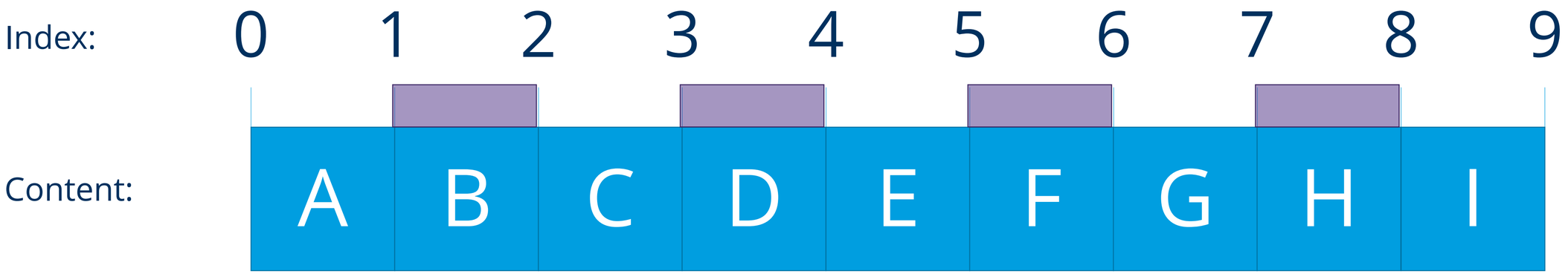
```
data[::2]
```

```
['A', 'C', 'E', 'G', 'I']
```

Indexing, cropping, subsets

The step-size allows skipping elements

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[0:10:2]
```

```
['A', 'C', 'E', 'G', 'I']
```

```
data[::2]
```

```
['A', 'C', 'E', 'G', 'I']
```

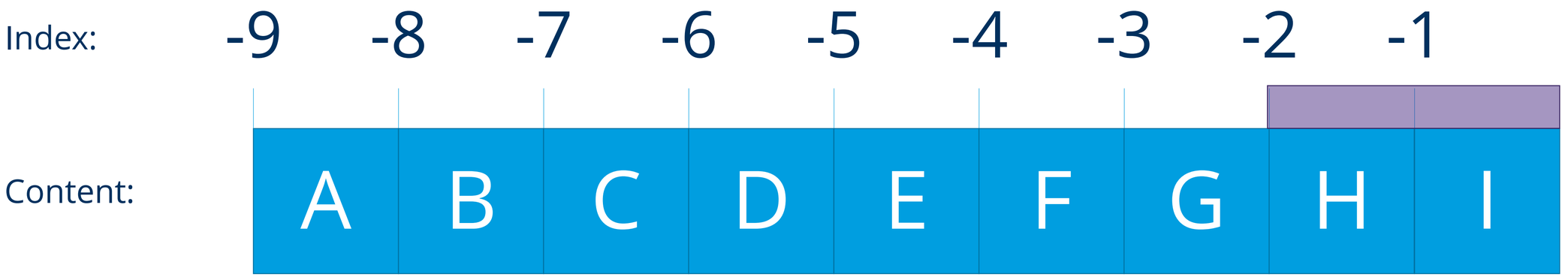
```
data[1::2]
```

```
['B', 'D', 'F', 'H']
```

Indexing, cropping, subsets

Indexing also works with negative indices

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



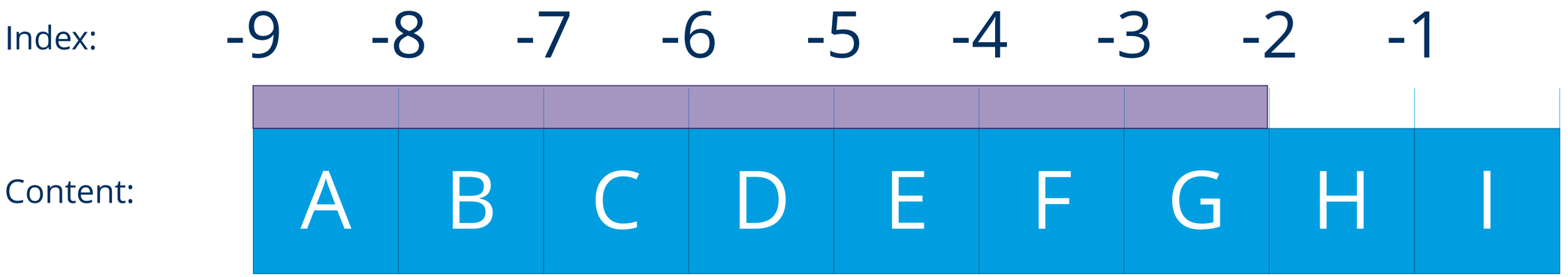

```
data[-2:]
```

```
['H', 'I']
```

Indexing, cropping, subsets

Indexing also works with negative indices

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[-2:]
```

```
['H', 'I']
```

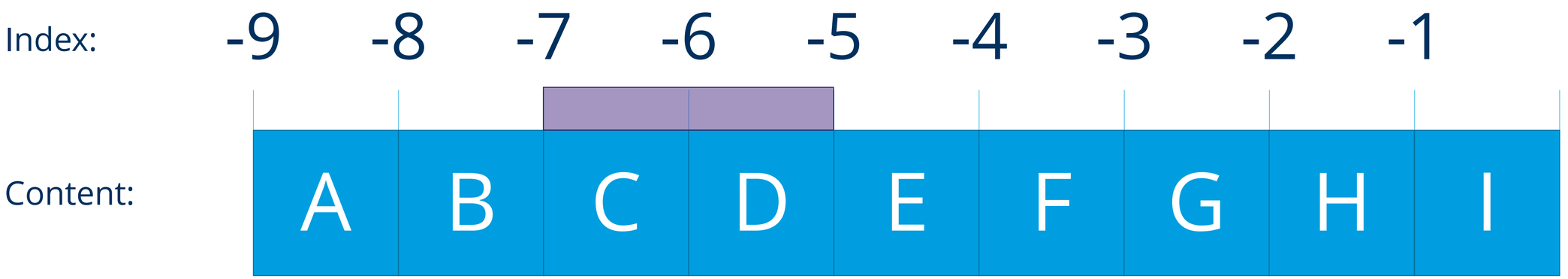
```
data[:-2]
```

```
['A', 'B', 'C', 'D', 'E', 'F', 'G']
```

Indexing, cropping, subsets

Indexing also works with negative indices

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[-2:]
```

```
['H', 'I']
```

```
data[:-2]
```

```
['A', 'B', 'C', 'D', 'E', 'F', 'G']
```

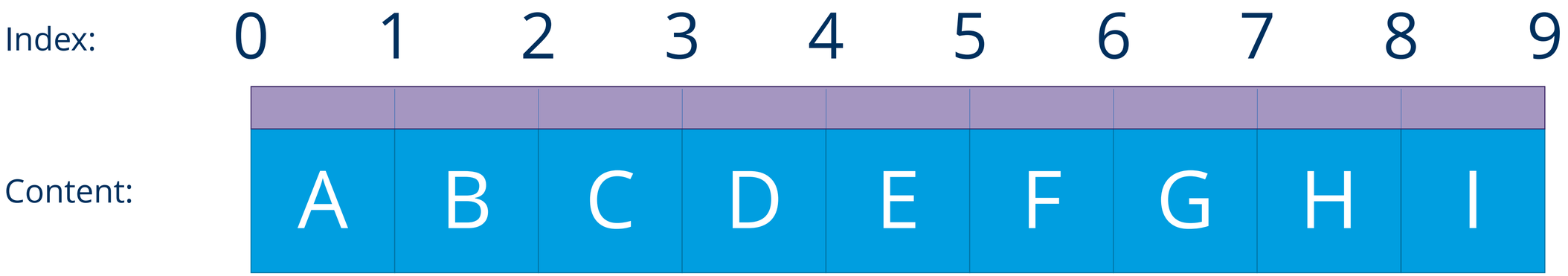
```
data[-7:-5]
```

```
['C', 'D']
```

Indexing, cropping, subsets

Negative stepping also works

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```



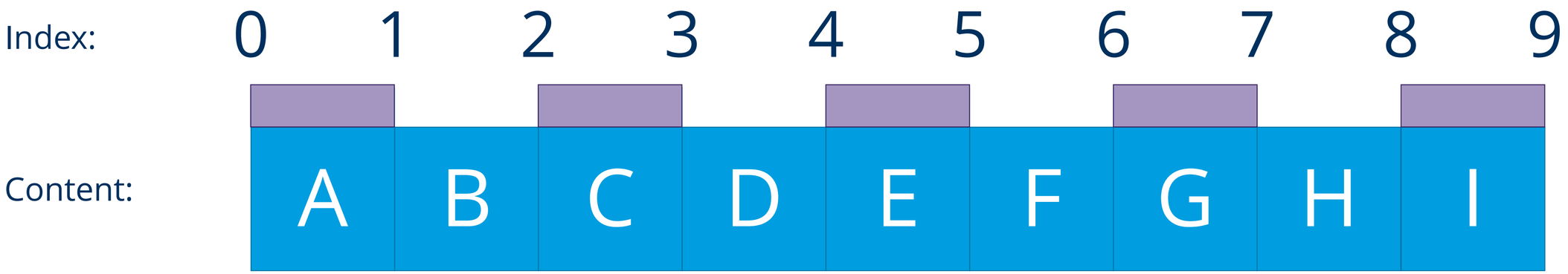

```
data[::-1]
```

```
['I', 'H', 'G', 'F', 'E', 'D', 'C', 'B', 'A']
```

Indexing, cropping, subsets

Negative stepping also works

```
data = ['A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I']
```




```
data[::-1]
```

```
['I', 'H', 'G', 'F', 'E', 'D', 'C', 'B', 'A']
```

```
data[::-2]
```

```
['I', 'G', 'E', 'C', 'A']
```

Arrays in Python

Modifying array elements

```
numbers = [0, 1, 2, 3, 4]

# write in one array element
numbers[1] = 5

print(numbers)
```

```
[0, 5, 2, 3, 4]
```

Note: The first element has index 0!

- Creating arrays of defined size

What?

How many?

```
zeros = [0] * 10
print(zeros)
```

```
[0, 0, 0, 0, 0, 0, 0, 0, 0, 0]
```

- Concatenating arrays

```
ones = [1, 1, 1]
twos = [2, 2, 2, 2]

# concatenate arrays
numbers = ones + twos

print(numbers)
```

```
[1, 1, 1, 2, 2, 2, 2]
```

+ means appending

Arrays: Lists versus Tuples

Lists can be modified

```
measurements = [5.5, 6.3, 7.2, 8.0, 8.8]
```
Create

```
measurements.append(10.2)
```
Add values

```
measurements[1] = 25
```
Replace values

```
measurements
```
Inspect content

```
[5.5, 25, 7.2, 8.0, 8.8, 10.2]
```

Tuples not

Note: round brackets

```
immutable = (4, 3, 7.8)
```
Create

```
immutable[1] = 5
```

```
---------------------------------------------------------------------------
TypeError                                 Traceback (most recent call last)
<ipython-input-49-a01b13633c23> in <module>
----> 1 immutable[1] = 5

TypeError: 'tuple' object does not support item assignment
```

Check the data *type* if uncertain:

```
type(measurements)
```

```
list
```

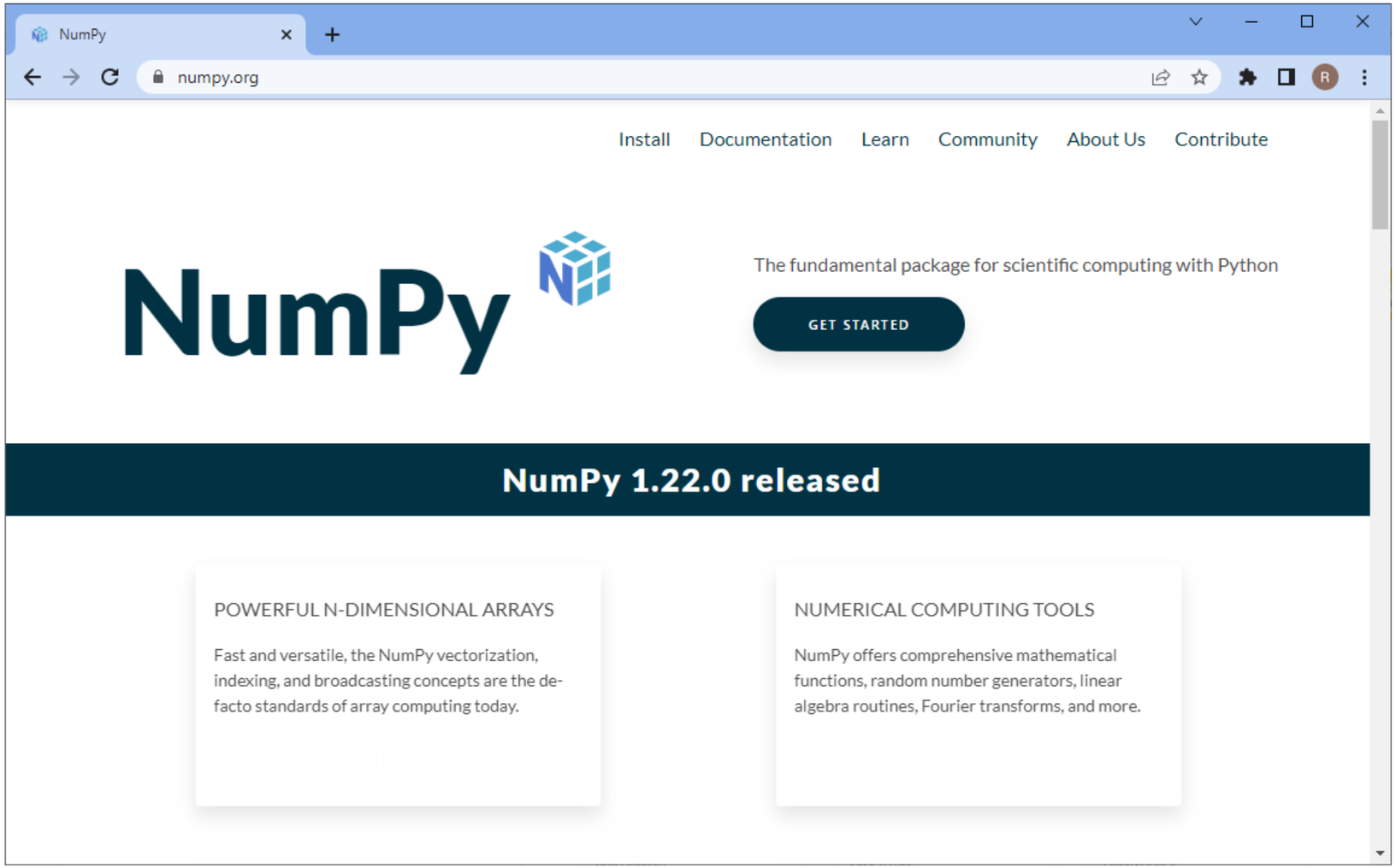
```
type(immutable)
```

```
tuple
```

numpy

The fundamental package for scientific computing with python.

```
conda install numpy
```



https://numpy.org/

numpy

Simplifying mathematical operations on n-dimensional arrays

- Python arrays of arrays (lists of lists)

```
# multidimensional arrays
matrix = [
    [1, 2, 3],
    [2, 3, 4],
    [3, 4, 5]
]

print(matrix)
```

```
[[1, 2, 3], [2, 3, 4], [3, 4, 5]]
```

```
result = matrix * 2
print(result)
```

```
[[1, 2, 3], [2, 3, 4], [3, 4, 5], [1, 2, 3], [2, 3, 4], [3, 4, 5]]
```

- numpy arrays

Tell python that you want to use a library called numpy

```
import numpy as np

np_matrix = np.asarray(matrix)

print(np_matrix)
```

If “numpy” is to long, you can give an alias “np”

```
[[1 2 3]
 [2 3 4]
 [3 4 5]]
```

```
np_result = np_matrix * 2
print(np_result)
```

```
[[ 2  4  6]
 [ 4  6  8]
 [ 6  8 10]]
```

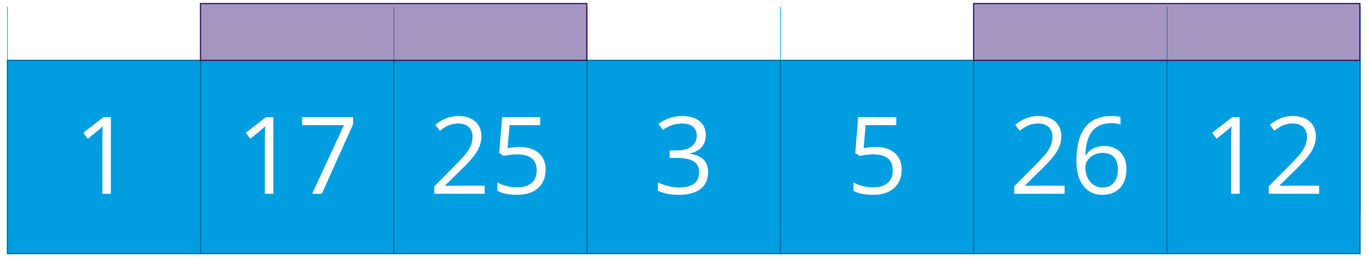
Masking (a.k.a. logical indexing)

“Masking” is addressing certain elements in numpy arrays, e.g. depending on their content

```
import numpy
measurements = numpy.asarray([1, 17, 25, 3, 5, 26, 12])
measurements
```

```
array([ 1, 17, 25,  3,  5, 26, 12])
```

Content:




```
mask = measurements > 10
mask
```

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

```
measurements[mask]
```

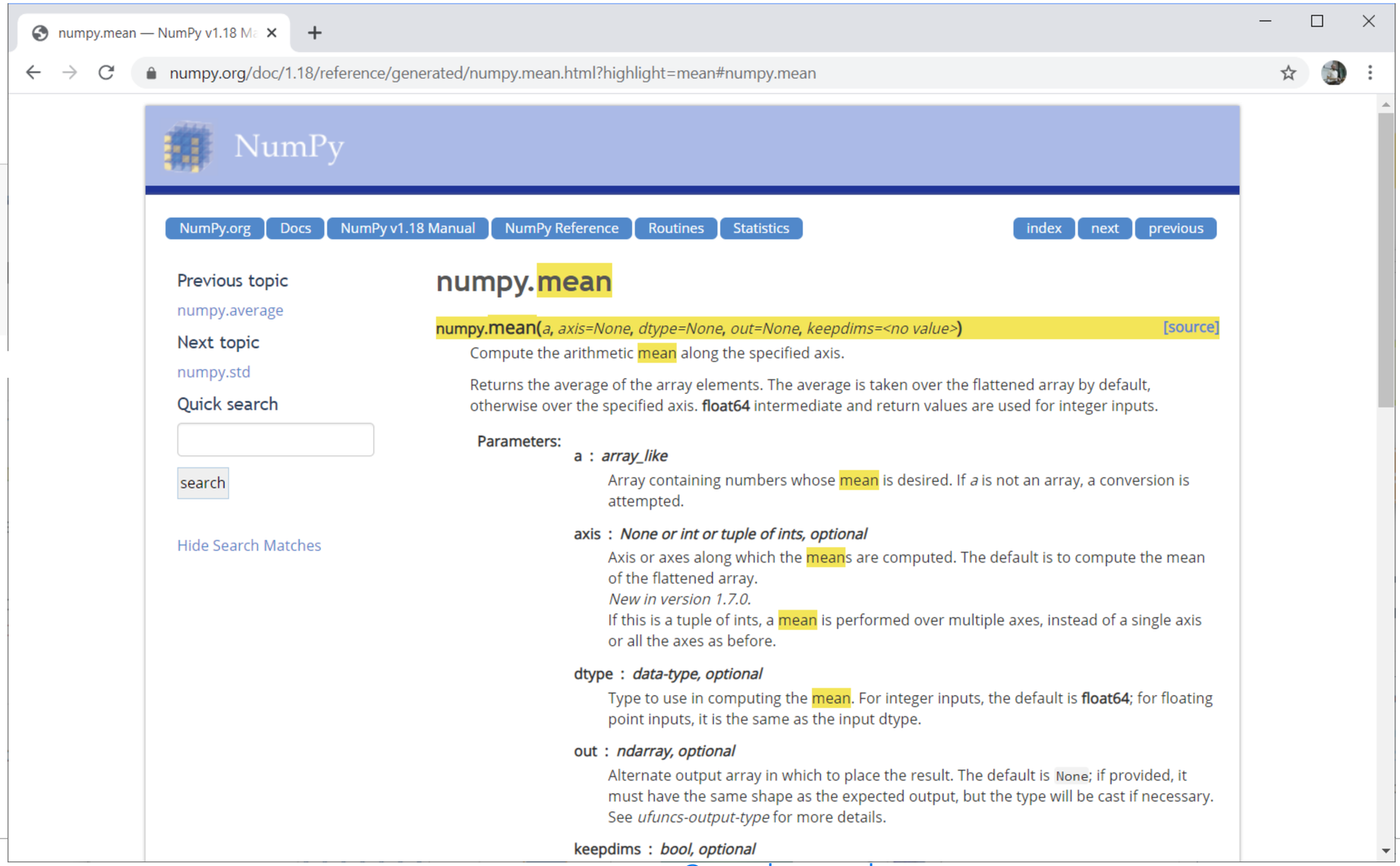
```
array([17, 25, 26, 12])
```

Basic descriptive statistics using numpy

Basic descriptive statistics

```
import numpy as np

measurements = [1, 4, 6, 7, 2]

mean = np.mean(measurements)
print("Mean: " + str(mean))
```

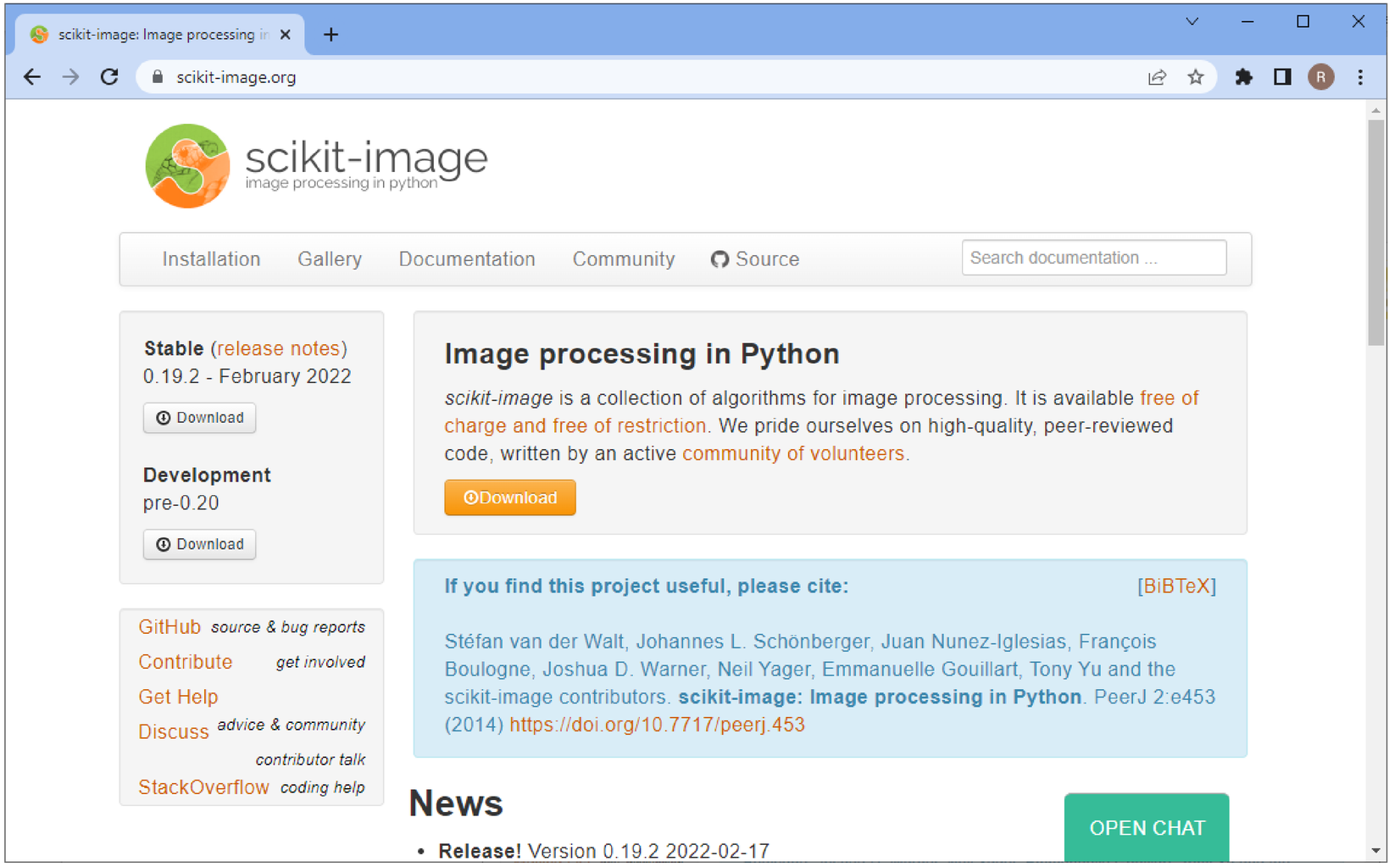
```
Mean: 4.0
```



scikit-image

scikit-image is a collection of algorithms for image processing.

```
conda install scikit-image
```



https://scikit-image.org/

Working with images in python

Open images

```
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”.

Working with images in python

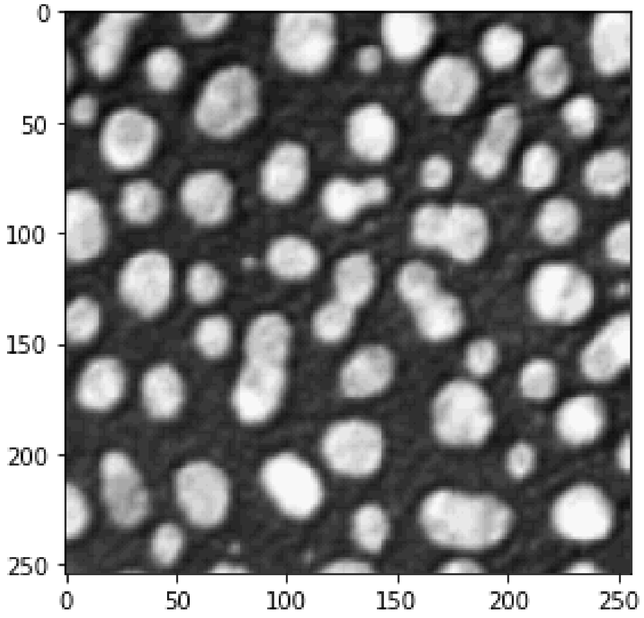
Open images

```
from skimage.io import imread

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

•Visualize images

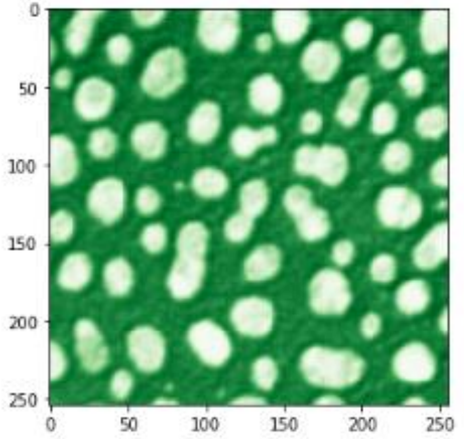
```
from skimage.io import imshow

imshow(image)
```

```
<matplotlib.image.AxesImage at 0x245e7
```



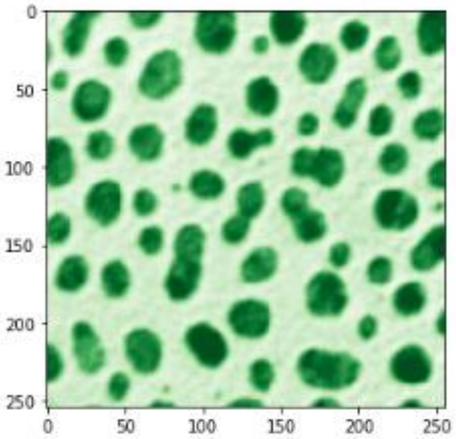

```
imshow(image, cmap="Greens_r")
```

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



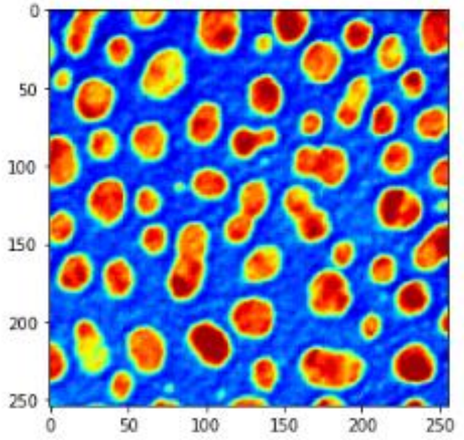

```
imshow(image, cmap="Greens")
```

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




```
imshow(image, cmap="jet")
```

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



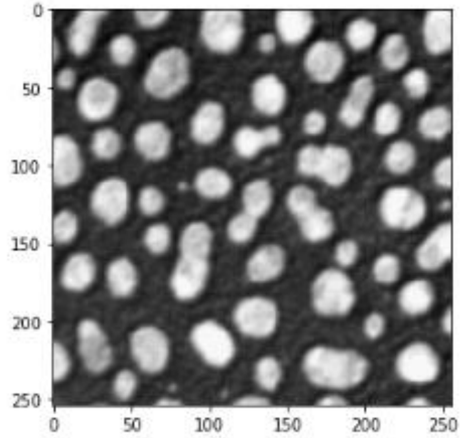
This does not modify the image data. The images are just shown with different colors representing the same values.

Cropping, sampling and flipping images

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

```
imshow(image)
```

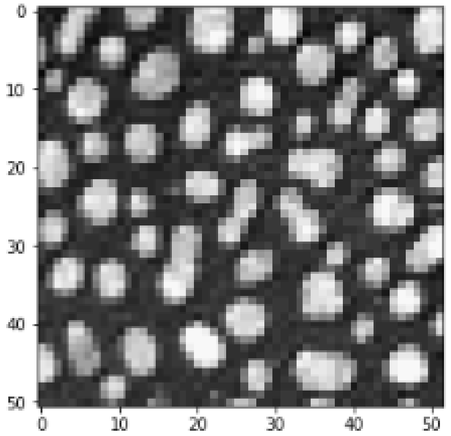
```
<matplotlib.image.AxesImage at 0
```



Original image

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

imshow(sampled_image)
```

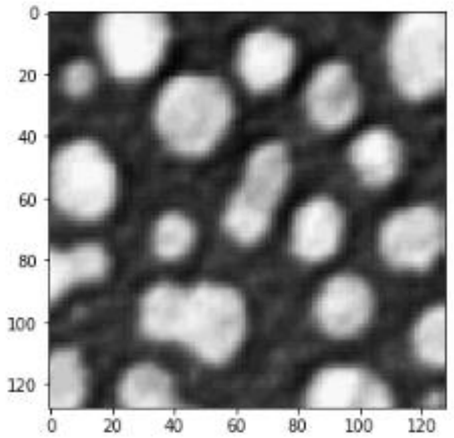
```
<matplotlib.image.AxesImage at 0
```



Sub-sampled image

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

imshow(cropped_image2)
```

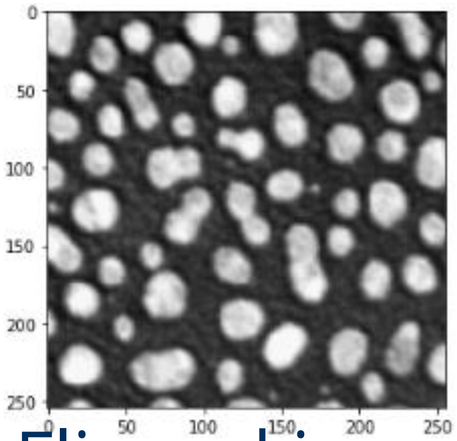
```
<matplotlib.image.AxesImage at 0x29e
```



Cropped image

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

imshow(flipped_image)
```

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



Flipped image

Troubleshooting

If your program throws error messages:

- Don't panic.
- *"There are two ways to write error-free programs; only the third one works."*

  Alan J. Perlis, Yale University
- Read where the error happened.
  - You may see your fault immediately, when looking at the right point.
- Read what appears to be wrong.
  - If you know, what's missing, you may see it, even if it's missing in a slightly different place.
  - Sometimes, something related is missing

```
print(round(4.5)
```

```
  File "<ipython-input-15-09a9be4a90c5>", line 1
    print(round(4.5)
                   ^
SyntaxError: unexpected EOF while parsing
```

Summary

Take home messages

Arrays can be accessed like this:

```
data[start:stop:step]
```

Strings are arrays

Lists are arrays

Tuples are arrays

Dictionaries are arrays with named elements

Columns in tables are arrays

Images are multi-dimensional arrays

Learning how to deal with arrays in Python is key.

Coming up next

- Loops
- Conditions
- Functions
- Libraries

```
animal_set = ["Cat", "Dog", "Mouse"]

for animal in animal_set:
    print(animal)
```

```
Cat
Dog
Mouse
```

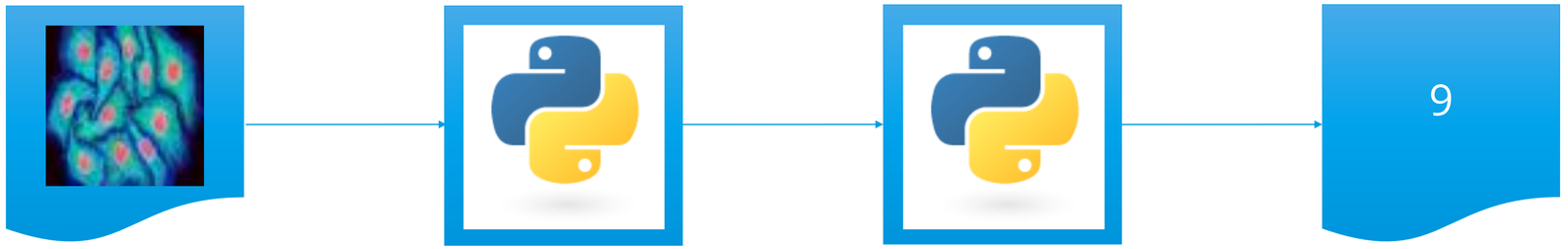
Python Algorithms
Conditions, loops, functions and custom libraries

Johannes Müller

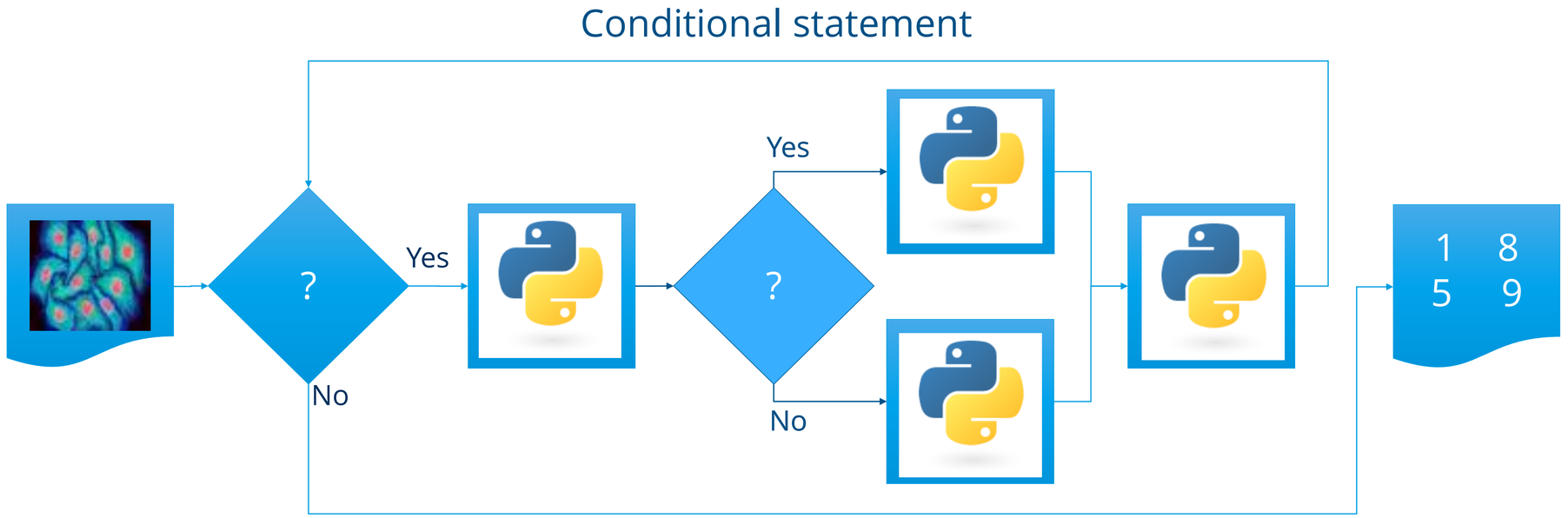
Using material from Robert Haase & Benoit Lombardot,
Scientific Computing Facility, MPI CBG

Conditions

Data science workflows *rarely* look like this:



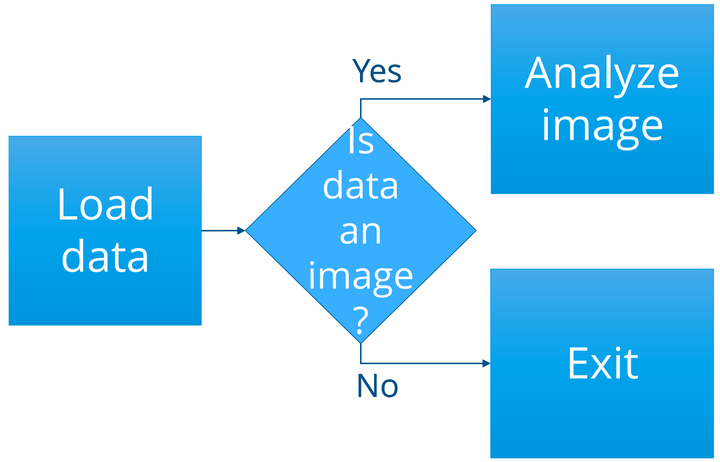
Data science workflows *rather* look like this:



Conditions

Conditional statements can be used to

- Check if pre-requisites are met
- Check if data has the right format
- Check if processing results are within an expected range
- Check for errors



If-statement

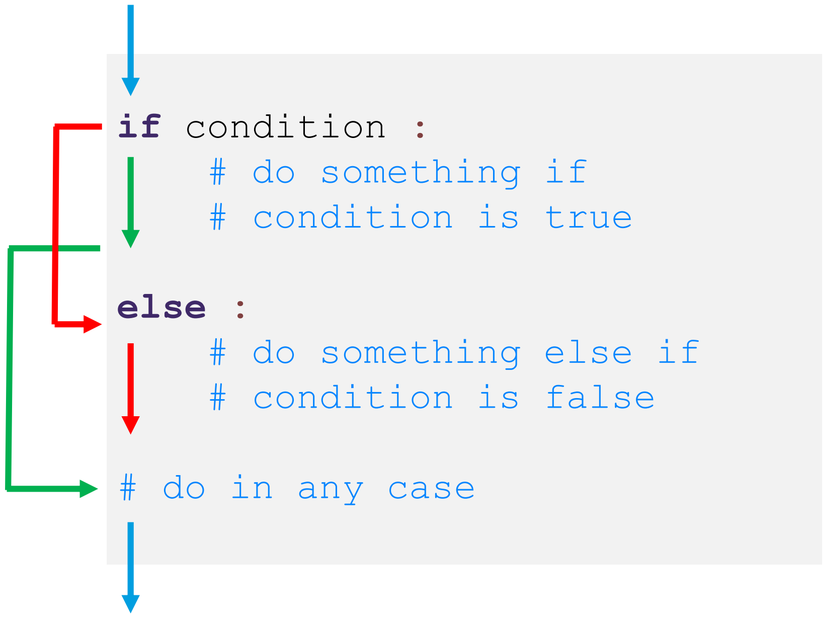
Depending on a condition, some lines of code are executed or not.

```
# do something

if condition:
    # do something only if condition is true

# proceed
```

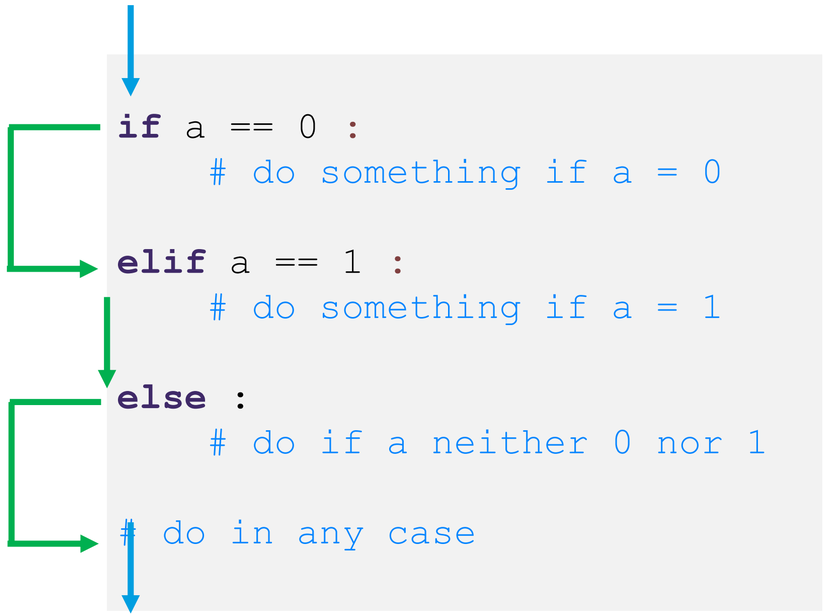
```
# do something

if condition:
    # do something only if condition is true

# proceed
```

If-statement

The **if** / **elif** / **else** statement allows to program alternatives.
Depending on conditions, only one block is computed
Indentation is used to mark where a block starts and ends.
Indentation helps reading blocks,



```
if condition :
    # do something if
    # condition is true

else :
    # do something else if
    # condition is false

# do in any case
```



```
if a == 0 :
    # do something if a = 0

elif a == 1 :
    # do something if a = 1

else :
    # do if a neither 0 nor 1

# do in any case
```

If-statement

Comparison operators always have True (1) or False (0) as results.

```
# initialise program
quality = 99.5

# evaluate result
if quality > 99.9 :
    print("Everything is fine.")
else :
    print("We need to improve!")
```

```
In [1]: a = 4

        if a = 5:
            print("Hello world")

  File "<ipython-input-1-13fb587c9332>", line 3
    if a = 5:
         ^
SyntaxError: invalid syntax
```

Note: These are two equal signs!

Operator	Description	Example
<, <=	smaller than, smaller or equal	a < b

Combined conditions

Logic operators always take conditions as operands and result in a condition.

- and
- or
- not

Also combined conditions can be either True (1) or False (0).

```
# initialise program
quality = 99.9
age = 3

if quality >= 99.9 and age > 5 :
    print("The item is ok.")
```

```
# initialise program
quality = 99.9


if not quality < 99.9 :
    print("The item is ok.")
```

Conditions with arrays

Checking contents of lists can be done intuitively using the `in` statement

```
# initialise program
my_list = [1, 5, 7, 8]
item = 3

if item in my_list :
    print("The item is in the list.")
else :
    print("There is no”, item, "in", my_list )
```

Works for strings, too!

```
cellline = 'PDX_10X_gH2AX_M23'
'PDX' in cellline
```

```
True
```

```
# initialise program
my_list = [1, 5, 7, 8]
item = 3

if item not in my_list :
    print("There is no”, item, "in", my_list )
else :
    print("The item is in the list.")
```

Readable code

Rules for readable code

- Every command belongs on its own line

- Insert empty lines to separate important processing steps

- Put spaces between operators and operands, because:

  This is easier to read thanthat,orisnt'it?

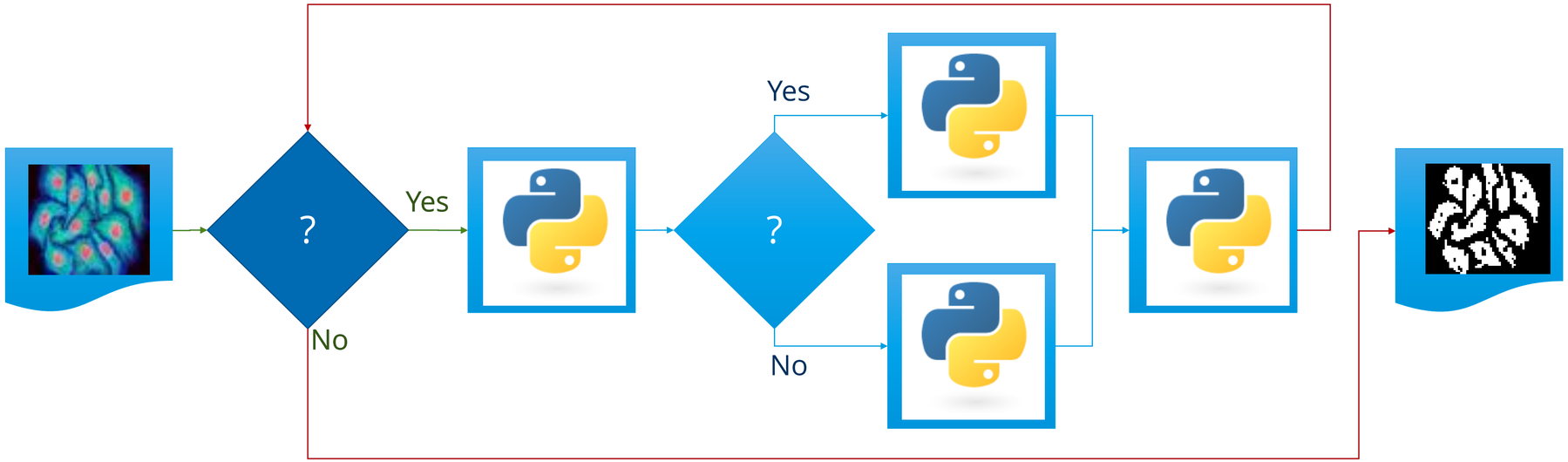
- Indent every conditional block (if/else) using the TAB key

```
# initialise program
a = 5
b = 3
c = 8

# execute algorithm
d = (a + b) / c

# evaluate result
```

```
if a == 5 :
    print("Yin")
else :
    print("Yang")
```

Loops

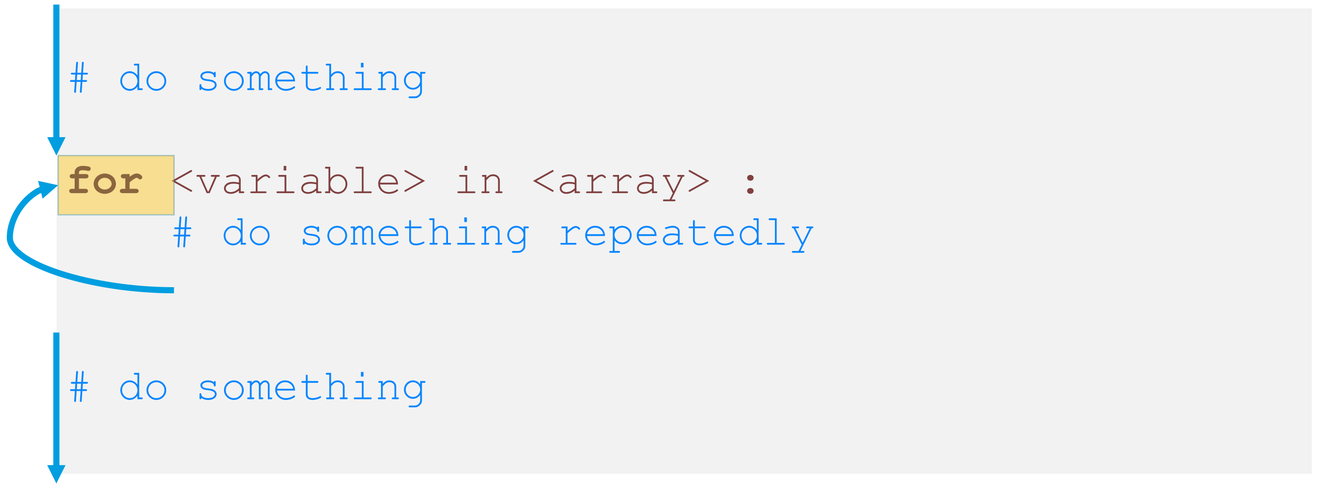
To repeat actions, you run code in loops



Loop statement

Loops

The `for` statement allows us to execute some lines of code *for* several times, typically for all items in an array-like thing (lists, tuples, images)



Example list : `range(start, stop, step)`

```
# for loops
for i in range(0, 5):
    print(i)
```

```
0
1
2
3
4
```

```
animal_set = ["Cat", "Dog", "Mouse"]

for animal in animal_set:
    print(animal)
```

```
Cat
Dog
Mouse
```

For-loops

- Indentation *means* combining operations to a block

```
# for loops
for i in range(0, 5):
print(i)
```

```
  File "<ipython-input-15-59c457ae0ac9>", line 3
    print(i)
        ^
IndentationError: expected an indented block
```

Don’t forget to indent!

- Colon necessary

Don’t forget the colon!

```
# for loops
for i in range(0, 5)
    print(i)
```

```
  File "<ipython-input-13-23157c0ed137>", line 2
    for i in range(0, 5)
                       ^
SyntaxError: invalid syntax
```

Generating arrays within for-loops

Also a combination with the if-statement is possible

```
# we start with an empty list
numbers = []

# and add elements
for i in range(0, 5):
    # check if the number is odd
    if i % 2:
        numbers.append(i * 2)

print(numbers)
```

```
[2, 6]
```

While-loops

While loops keep executing indented code as long as a condition is met:

```
number = 1024

while (number > 1):
    number = number / 2
    print(number)
```

```
512.0
256.0
128.0
64.0
32.0
16.0
8.0
4.0
2.0
1.0
```

Using the break statement, you can leave a loop

```
number = 1024

while (True):
    number = number / 2
    print(number)

    if number < 1:
        break
```

```
512.0
256.0
128.0
64.0
32.0
16.0
8.0
4.0
2.0
1.0
0.5
```

The continue statement allows to skip iterations

```
for i in range(0, 10):
    if i >= 3 and i <= 6:
        continue
    print(i)
```

```
0
1
2
7
8
9
```

Functions

In case repetitive tasks appear that cannot be handled in a loop, *functions* help:

- re-use code in different contexts
- Indentation is crucial
- Functions must be *defined* before called

Definition

```
def sum_numbers(a, b):
    result = a + b
    return result
```

function_name (parameters)

body commands

return statement (optional)

Call

```
c = sum_numbers(4, 5)
print(c)
```

```
9
```

```
sum_numbers(5, 6)
```

```
11
```

```
sum_numbers(3, 4)
```

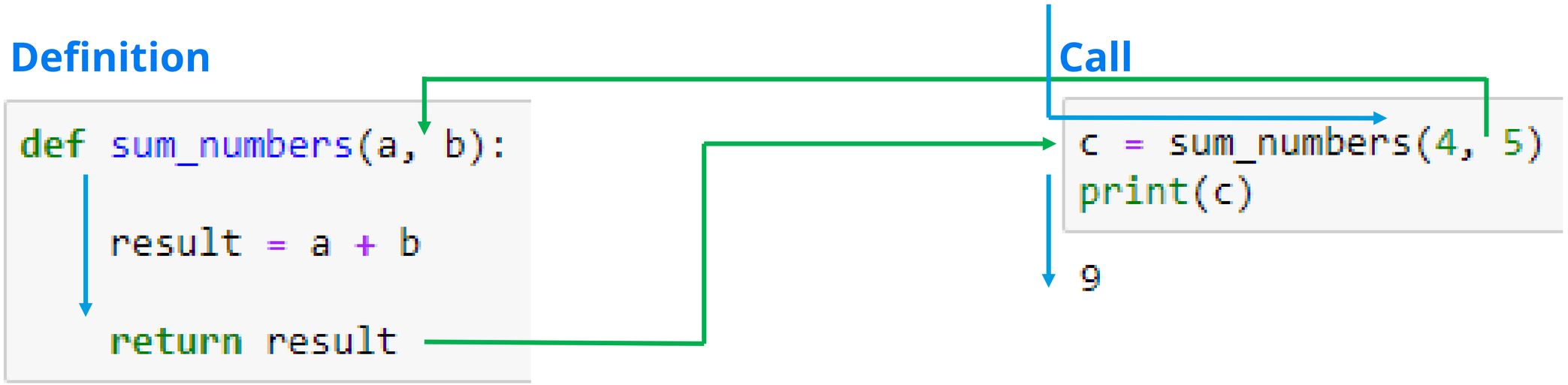
```
7
```

Note: Variables defined inside a function are unknown outside of the function!

Functions

In case repetitive tasks appear that cannot be handled in a loop, *functions* help:

- re-use code in different contexts
- Indentation is crucial
- Functions must be *defined* before called



Functions

Document your functions to keep track of what they do.

Describe what the functions does and what the parameters are meant to be

```
def square(number):
    '''
    Squares a number by multiplying it with itself  and returns its result.
    '''

    return number * number
```

- You can then later print the *documentation* if you can't recall how a function works.

```
square?
```

```
Signature: square(number)
Docstring: Squares a number by multiplying it with itself and returns its
result.
```

TECHNISCHE UNIVERSITÄT DRESDEN

DIGS-BB

Dresden International Graduate School for Biomedicine and Bioengineering

Image Processing and Filtering

Marcelo Leomil Zoccoler

With material from
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Mauricio Rocha Martins, Norden lab, MPI CBG
Dominic Waithe, Oxford University
Nuno P Martins, IGC Lisbon
Paulo Aguiar, INEB, Porto
Sebastian Tosi, IRB Barcelona
Benoit Lombardot, Scientific Computing Facility, MPI CBG
Alex Bird, Dan White, MPI CBG

neubias
network of european bioimage analysts

PoL
Physics of Life
TU Dresden

September 2022

DRESDEN concept

TECHNISCHE UNIVERSITÄT DRESDEN

DIGS-BB

Dresden International Graduate School for Biomedicine and Bioengineering

Image Visualization

Marcelo Leomil Zoccoler

With material from:

Robert Haase, PoL

PoL
Physics of Life
TU Dresden

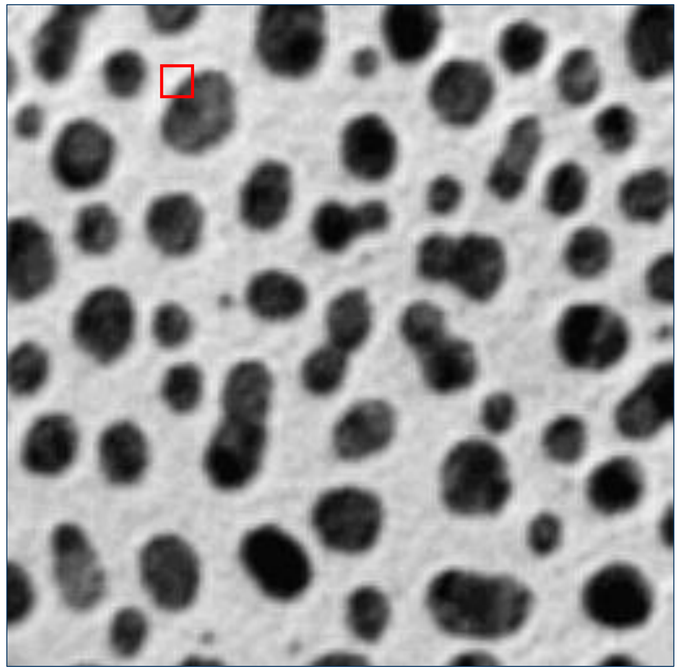
DRESDEN concept

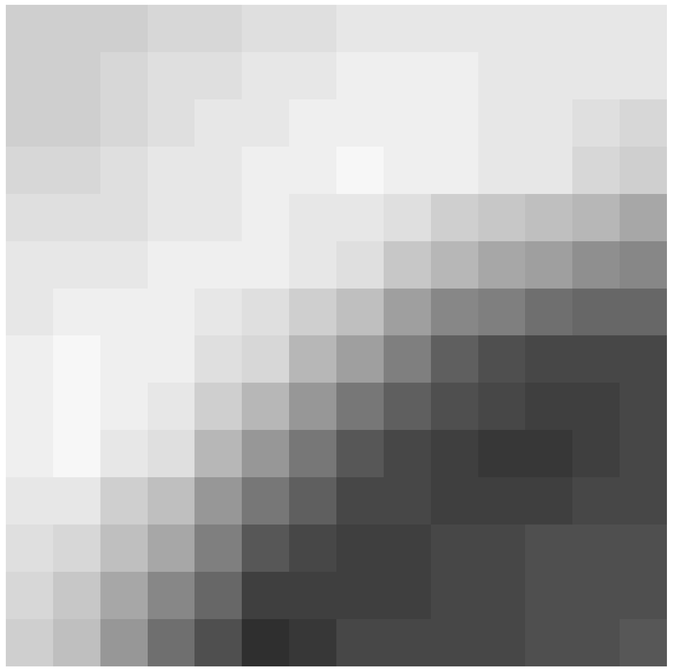
Images and pixels

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

0 255

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

```
from skimage.exposure import histogram

piexl_counts, pixel_values = histogram(array)

plt.bar(pixel_values, piexl_counts)
```

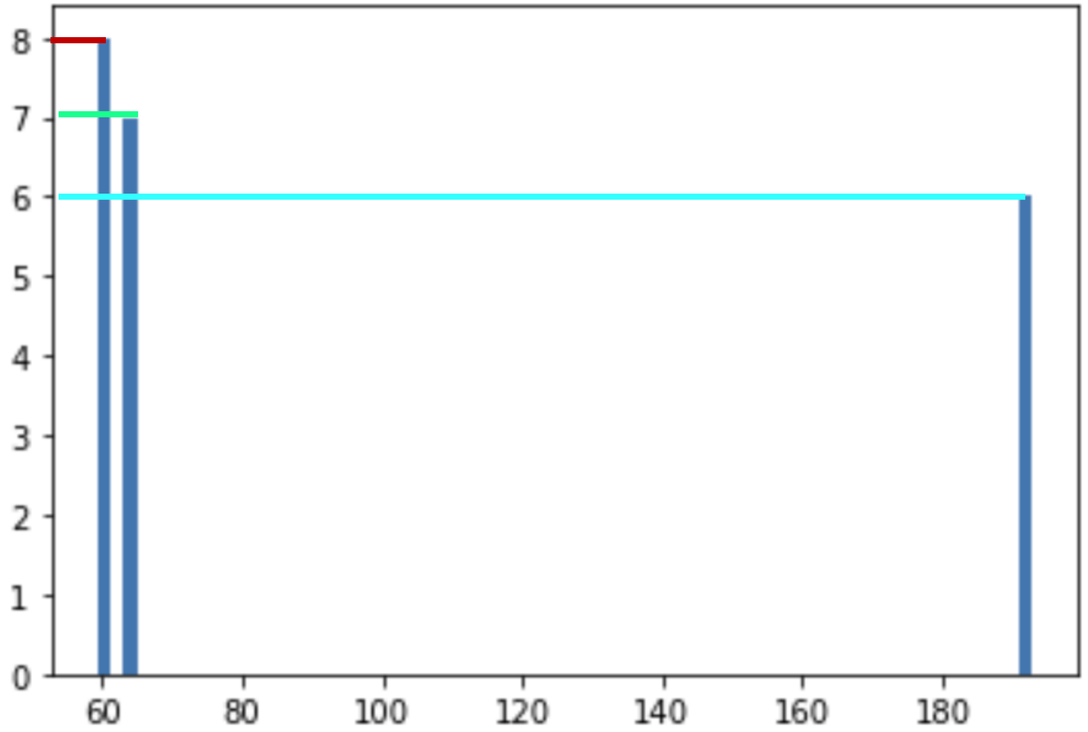
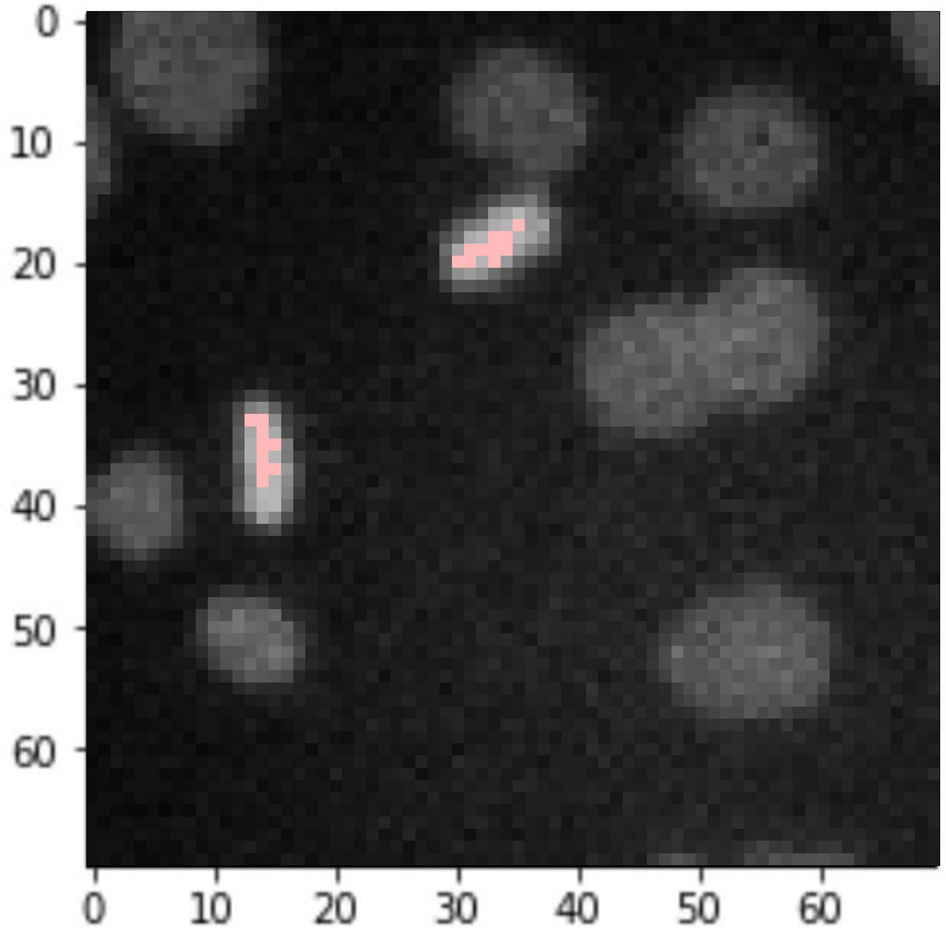
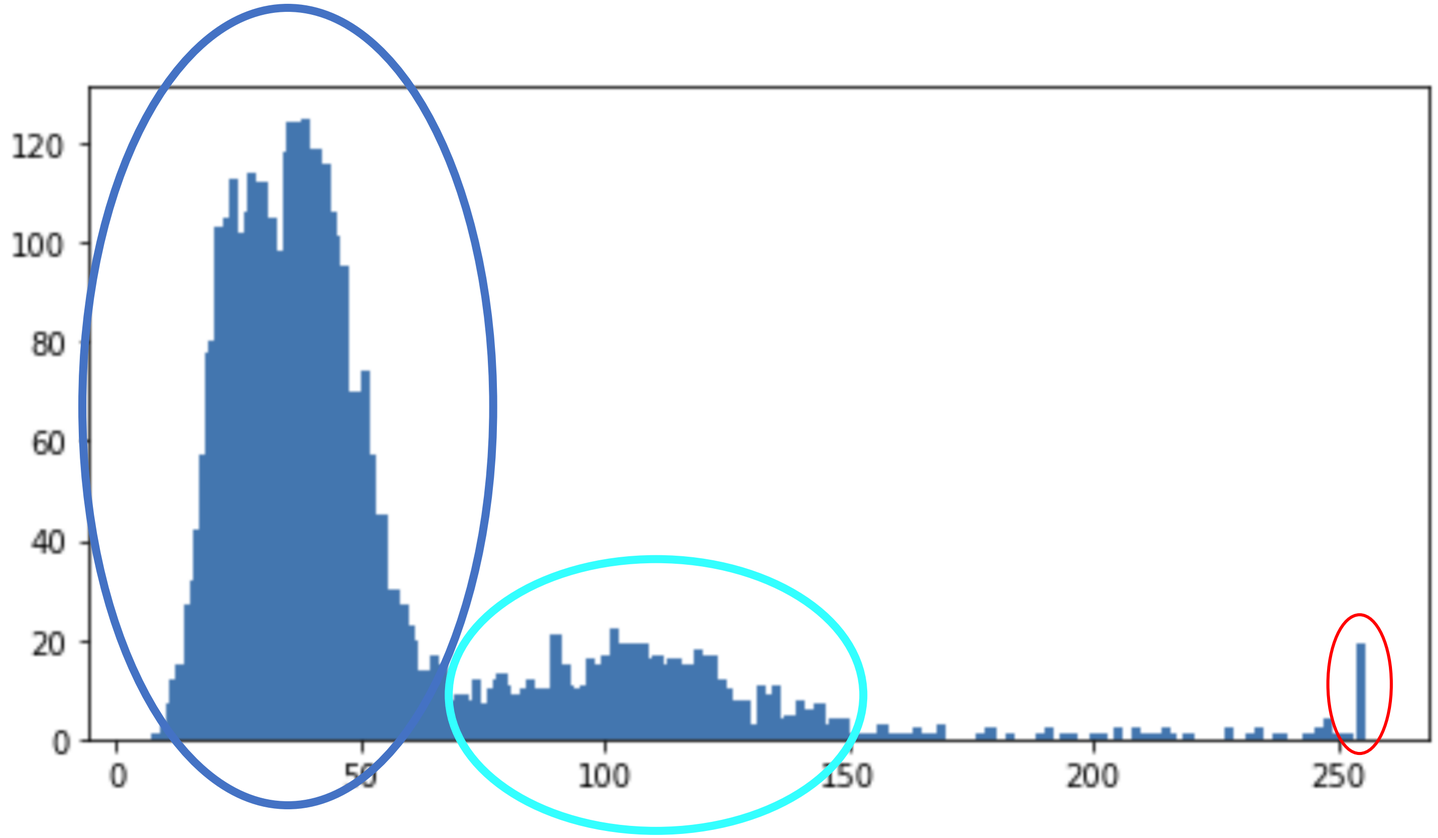


Image Histogram

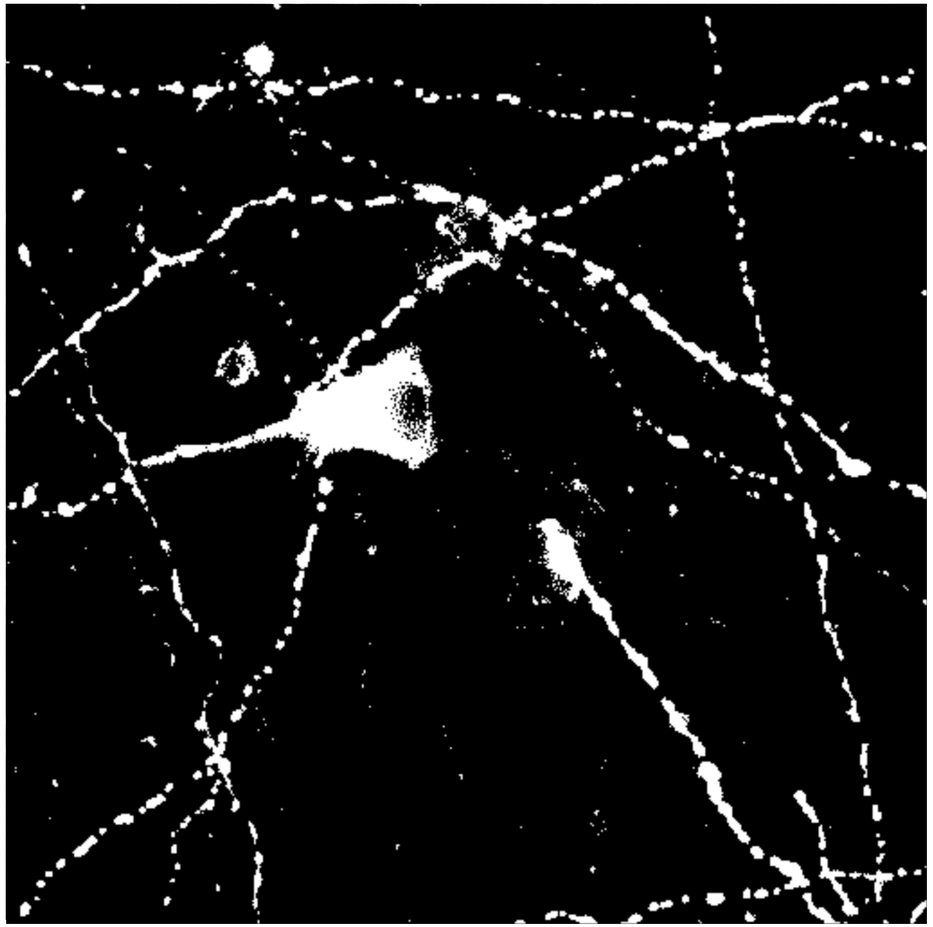
Example with a slightly larger image:

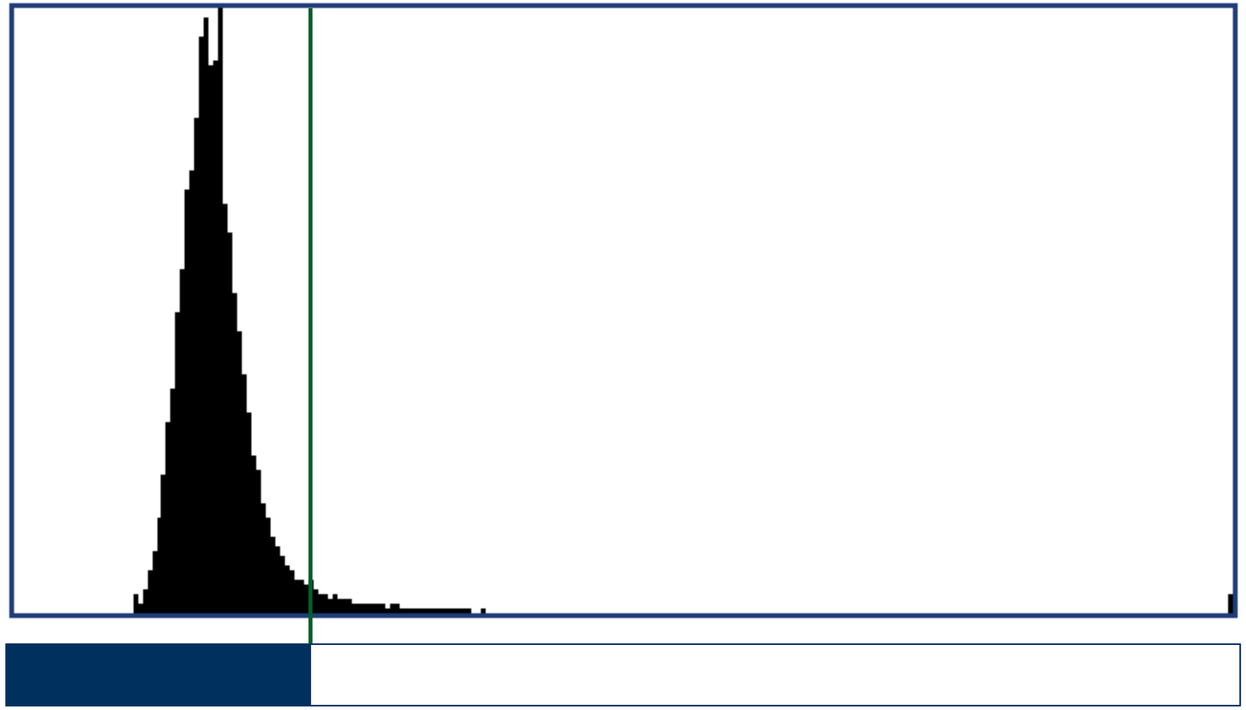




Binarization: a quick peek

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
```

Plotting with matplotlib: a quick peek

Use matplotlib to put images side-by-side

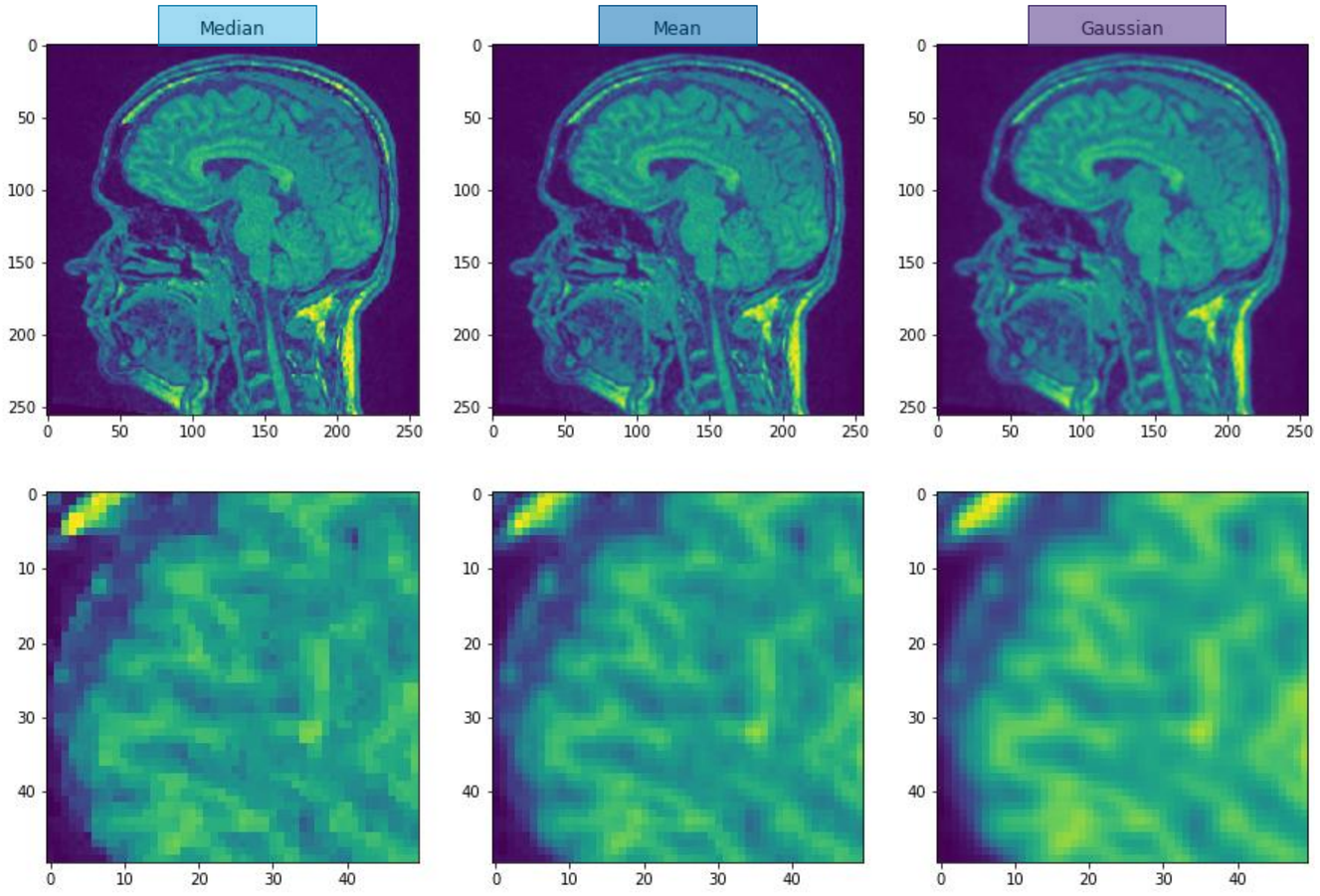
```
median_filtered = filters.median(noisy_mri, disk(1))
mean_filtered = filters.rank.mean(noisy_mri, disk(1))
gaussian_filtered = filters.gaussian(noisy_mri, sigma=1)

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

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Image Processing: Filters

Marcelo Leomil Zoccoler

With material from:

Robert Haase, PoL

Mauricio Rocha Martins, Norden lab, MPI CBG

PoL
Physics of Life
TU Dresden

DRESDEN concept

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.

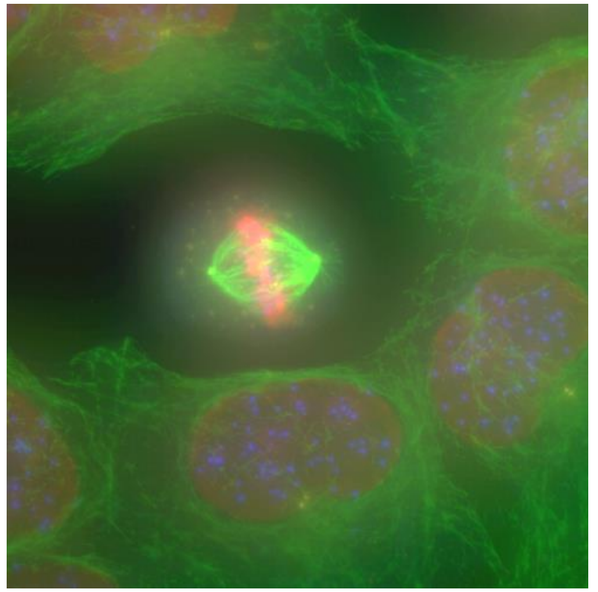
There is no “best” filter. Which filter fits your needs, depends on the context.

Filters do not do magic. They can not make things visible which are not in the image.

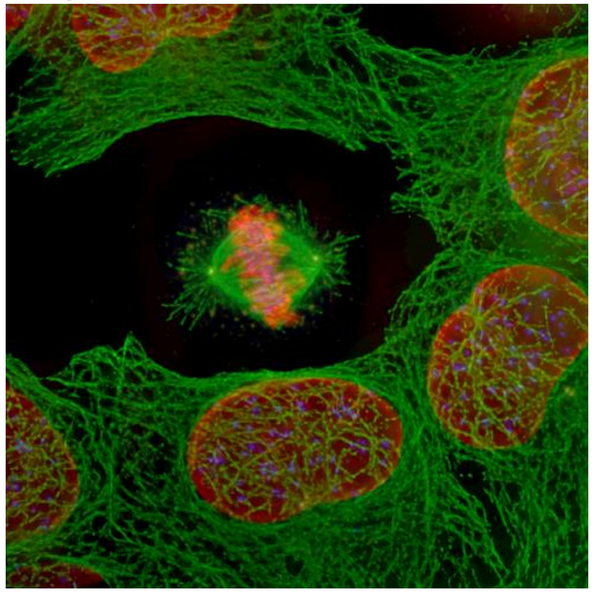
Application examples

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

Image source: Alex Bird / Dan White MPI CBG

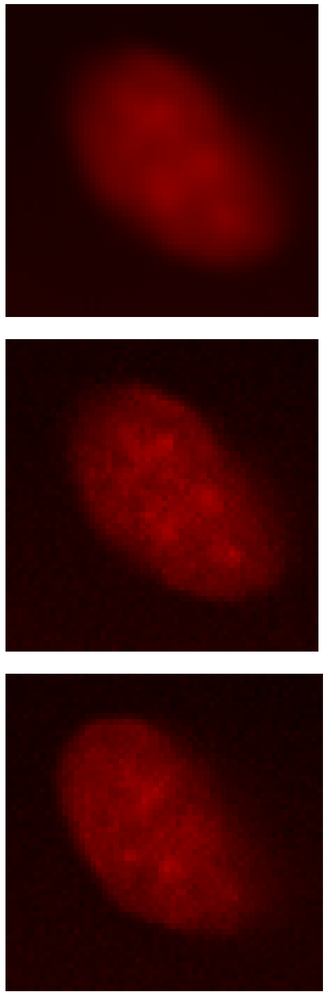


Filter



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

Noise

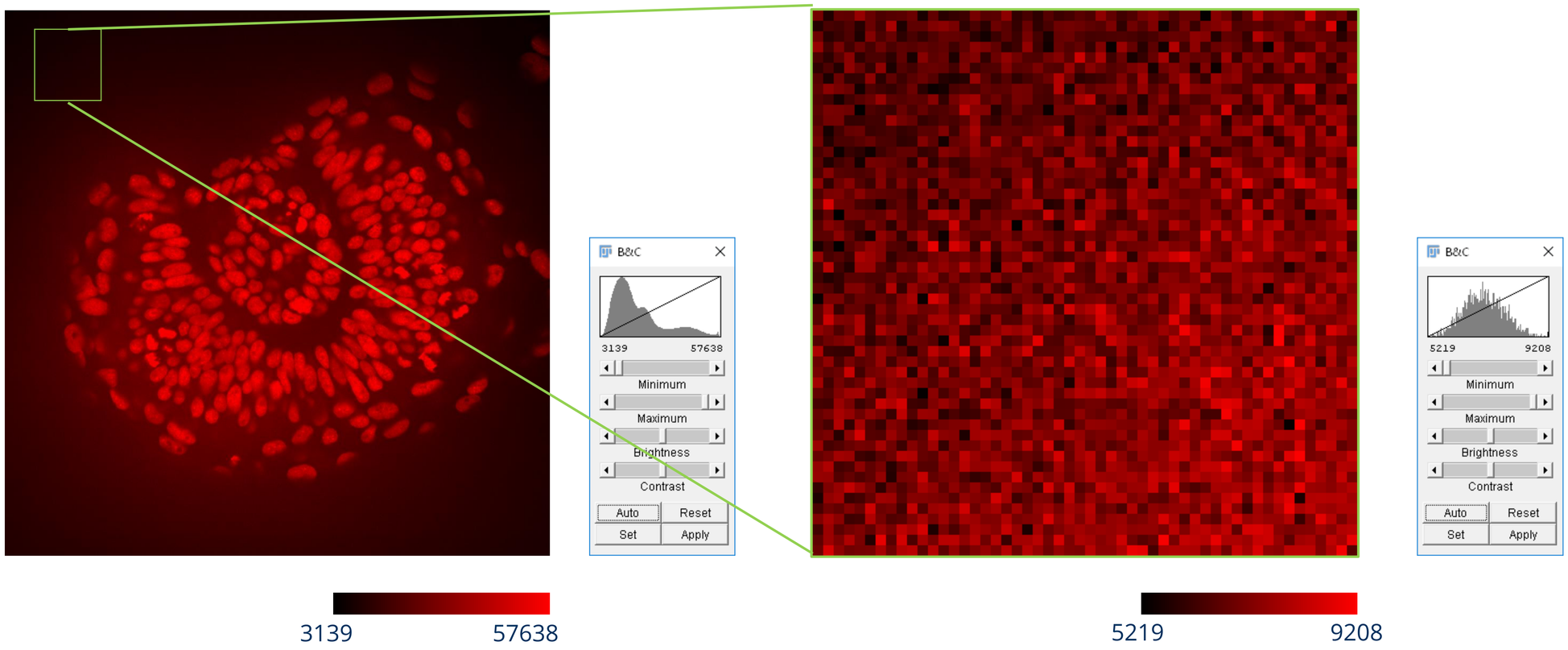
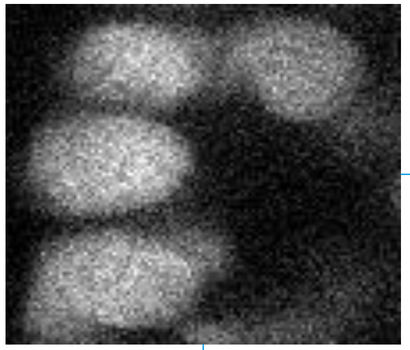


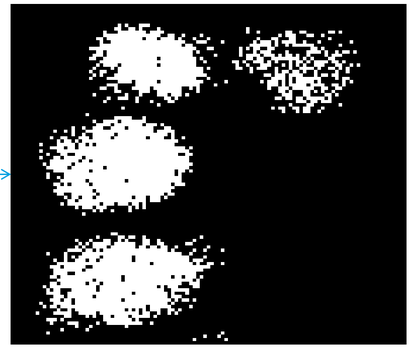
Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Image correction / noise removal

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

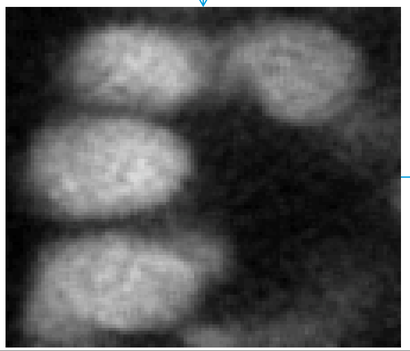


Thresholding

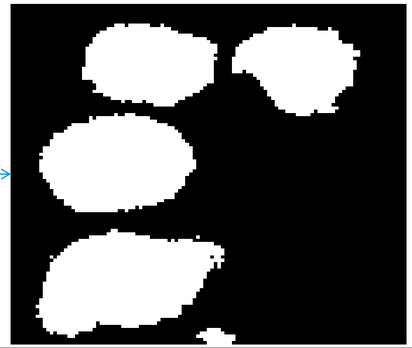


Oh no! I see thousands of tiny white objects!

Blurring



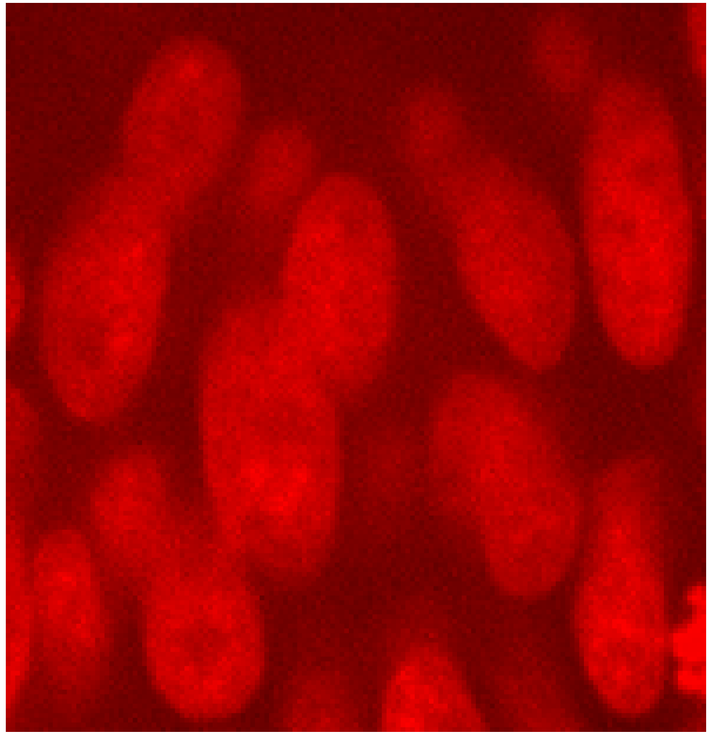
Thresholding



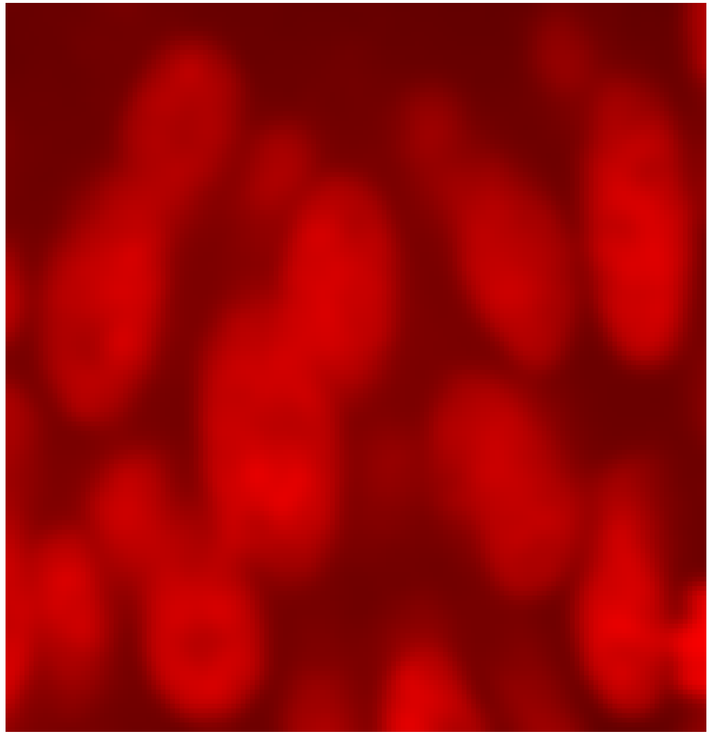
Ok, it’s just 4 objects.

Image correction / noise removal

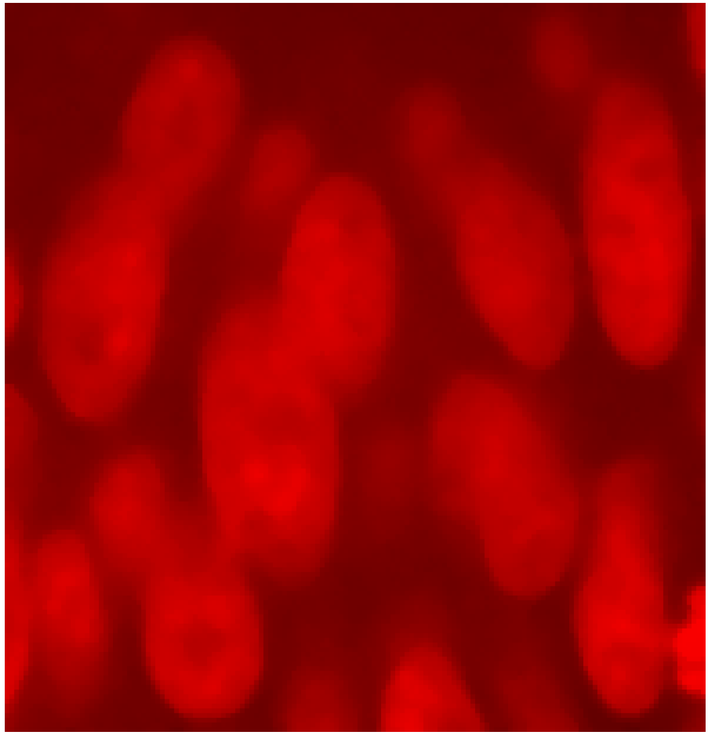
Filters can remove noise



Original image



Gaussian blur filtered



Median filtered

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

Linear Filters

- Linear filters replace each pixel value with a linear combination of surrounding pixels
- Kernels are matrices describing a linear filter

Mean filter 3x3 kernel =

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

Kernel

32	48	64	96	120	128	144	152	152
40	72	96	128	160	176	184	184	184
72	104	136	160	176	184	192	192	184
104	136	168	184	192	200	200	192	184
136	160	184	184	192	192	192	184	184
168	184	192	192	184	184	176	176	176
192	192	192	192	184	184	176	176	176
208	200	184	184	184	184	176	176	168

New_value = (1/9)***32** + (1/9)***48** + (1/9)***64** + (1/9)***40** + (1/9)***72** + (1/9)***96** + (1/9)***72** + (1/9)***104** + (1/9)***136**

New_value = 73.8

What operation is this?

Linear Filters

- Linear filters replace each pixel value with a linear combination of surrounding pixels
- Kernels are matrices describing a linear filter

Mean filter 3x3 kernel =

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

Kernel

32	48	64	96	120	128	144	152	152
40	72	96	128	160	176	184	184	184
72	104	136	160	176	184	192	192	184
104	136	168	184	192	200	200	192	184
136	160	184	184	192	192	192	184	184
168	184	192	192	184	184	176	176	176
192	192	192	192	184	184	176	176	176
208	200	184	184	184	184	176	176	168

New_value = (1/9)***48** + (1/9)***64** + (1/9)***96** + (1/9)***72** + (1/9)***96** + (1/9)***128** + (1/9)***104** + (1/9)***136** + (1/9)***160**

New_value = 100.4

What operation is this?

Linear Filters

- 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 neighborhood

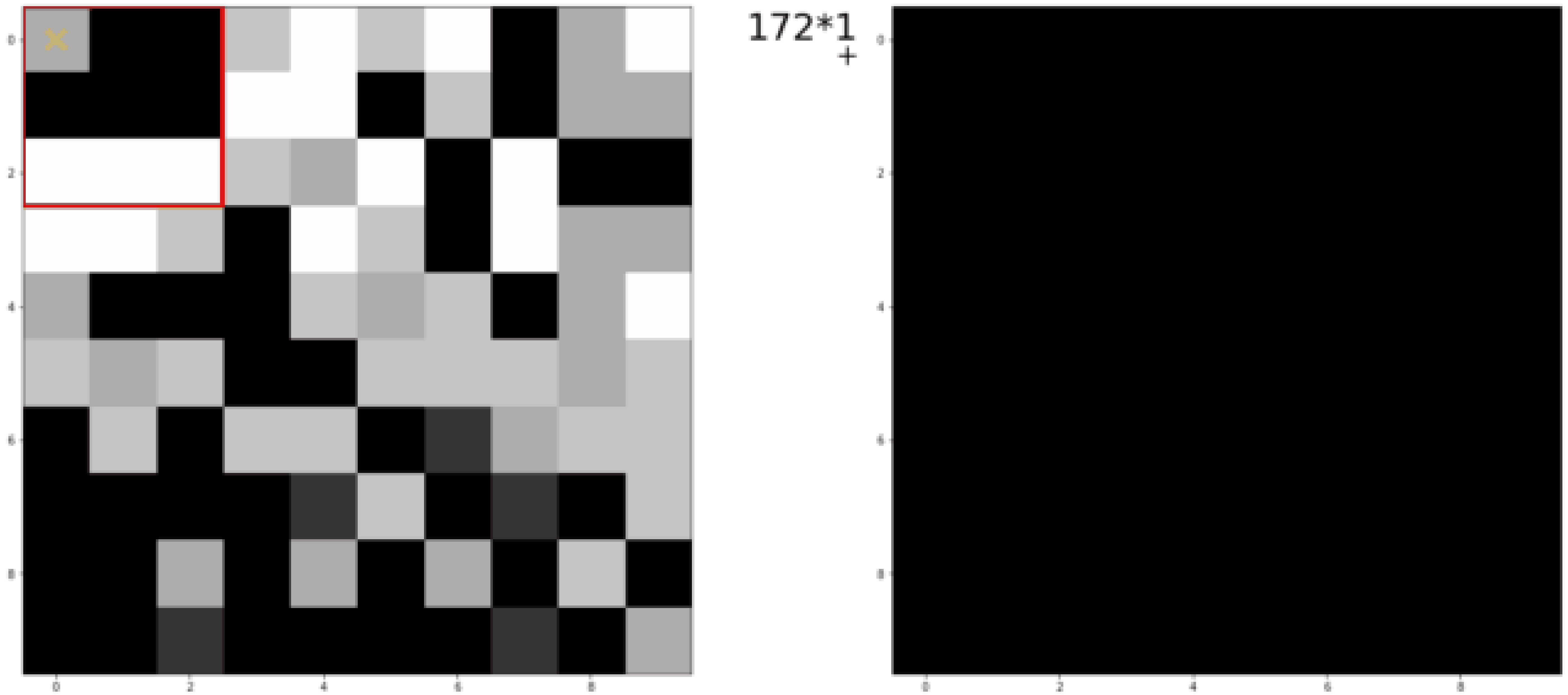


Image Output_image

Terminology:

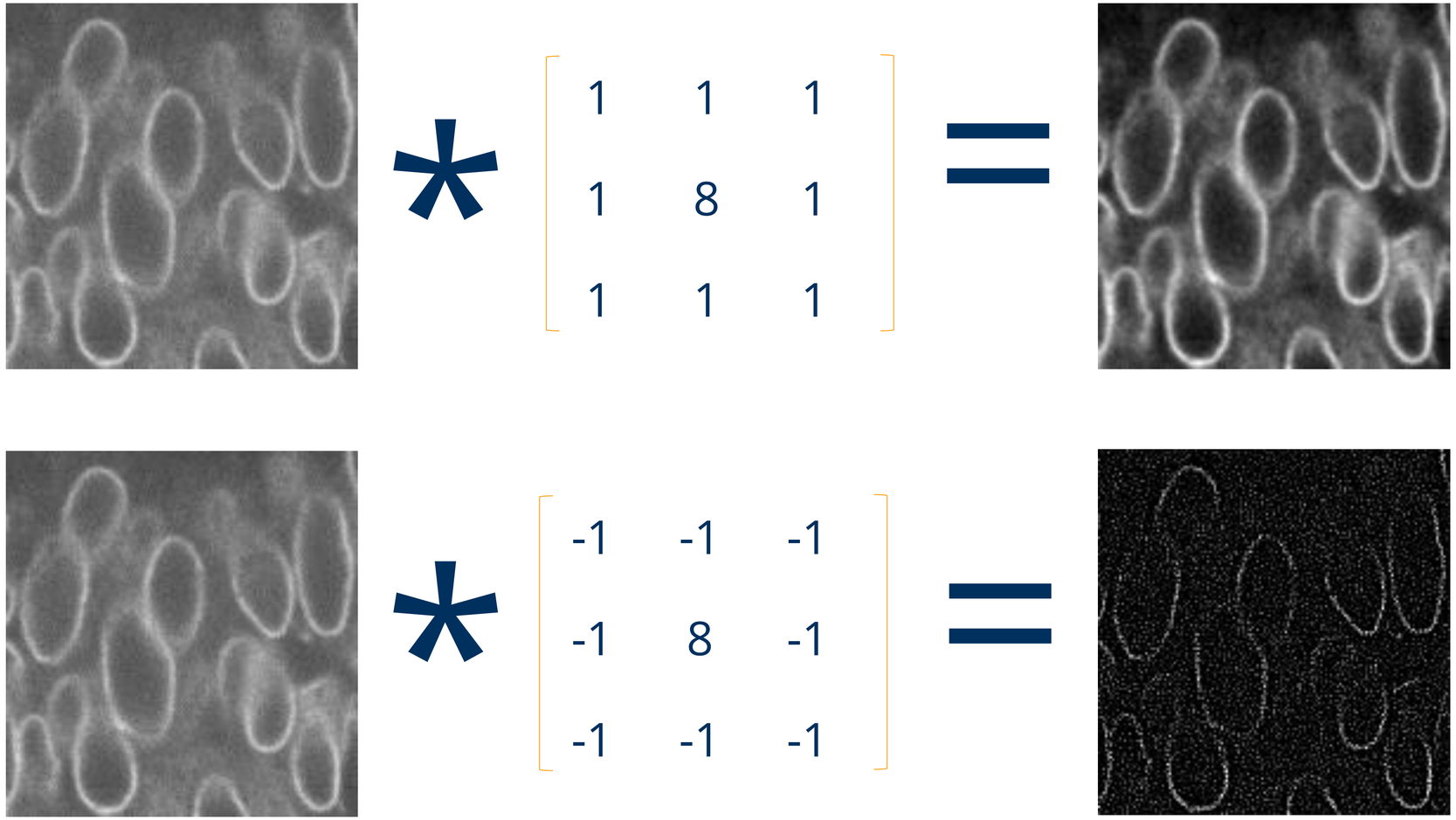
- “We convolve an image with a kernel.”
- Convolution operator: *

Output_image = Image * Kernel

Linear filters

Different linear filters use different kernels

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

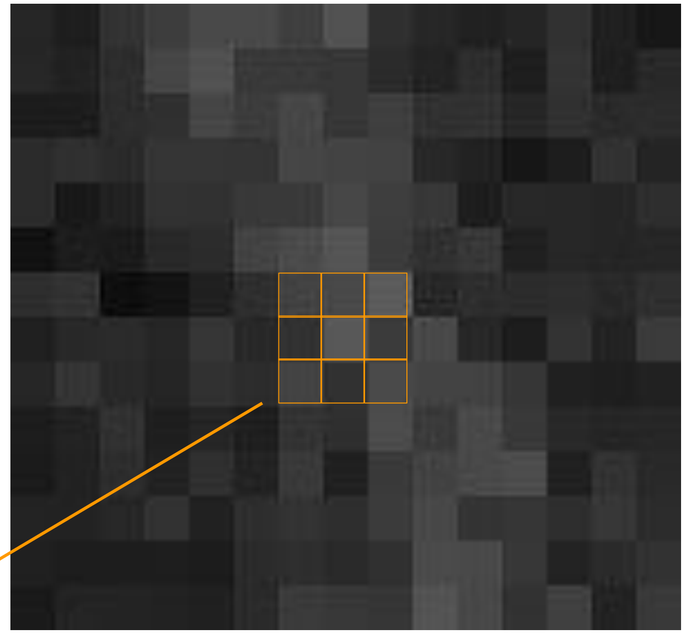


Nonlinear Filters

- Nonlinear 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



75	85	60
67	73	91
50	88	59

50 59 60 67 73 75 85 88 91

Min Median Max

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:

```
from skimage import filters

filters.
```

```
LPIFilter2D
median
meijering
prewitt
prewitt_h
prewitt_v
rank
rank_order
ridges
roberts
```

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Image Processing: Morphological Operations

Marcelo Leomil Zoccoler

With material from

Robert Haase, PoL

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PoL
Physics of Life
TU Dresden

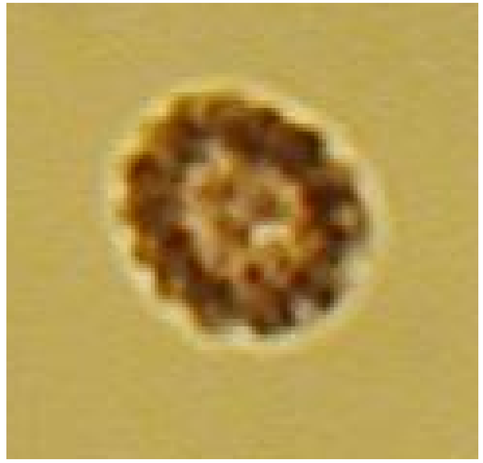
DRESDEN concept

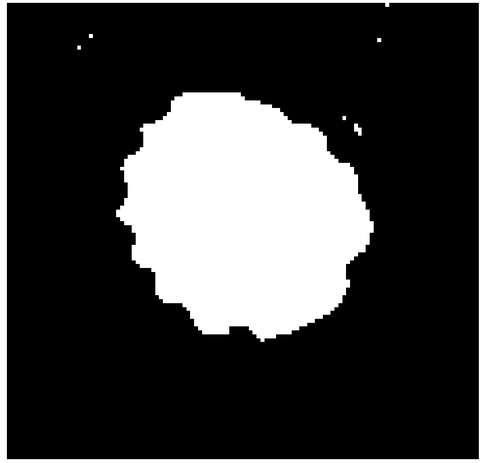
Refining masks

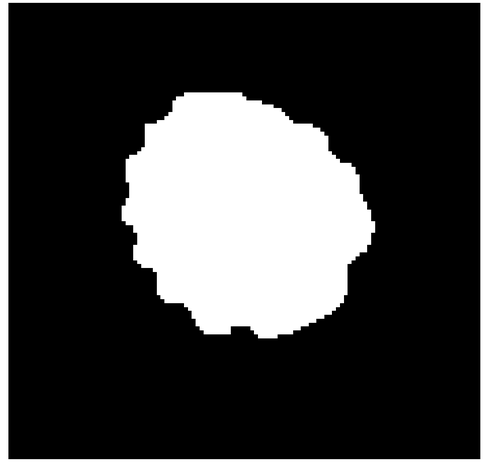
Binary mask images may not be perfect immediately after thresholding.

There are ways of refining them

Thresholding → Binary closing (Fill holes) → Binary opening

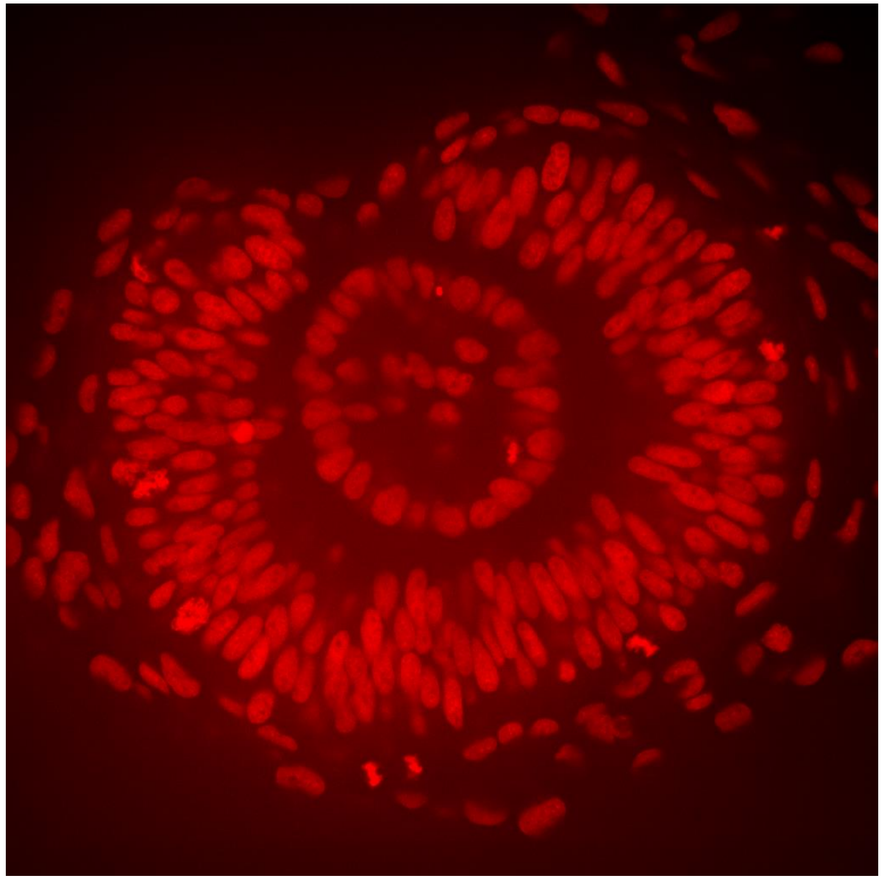






Background removal

Differentiating objects is easier if their background intensity is equal.



Subtract background

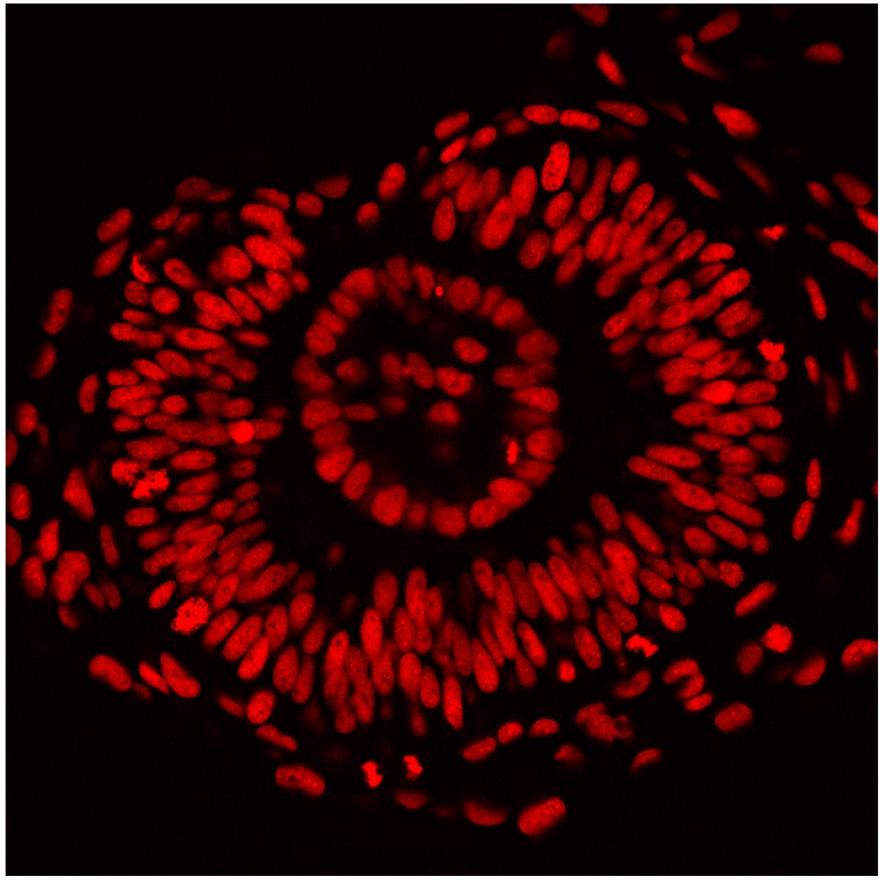
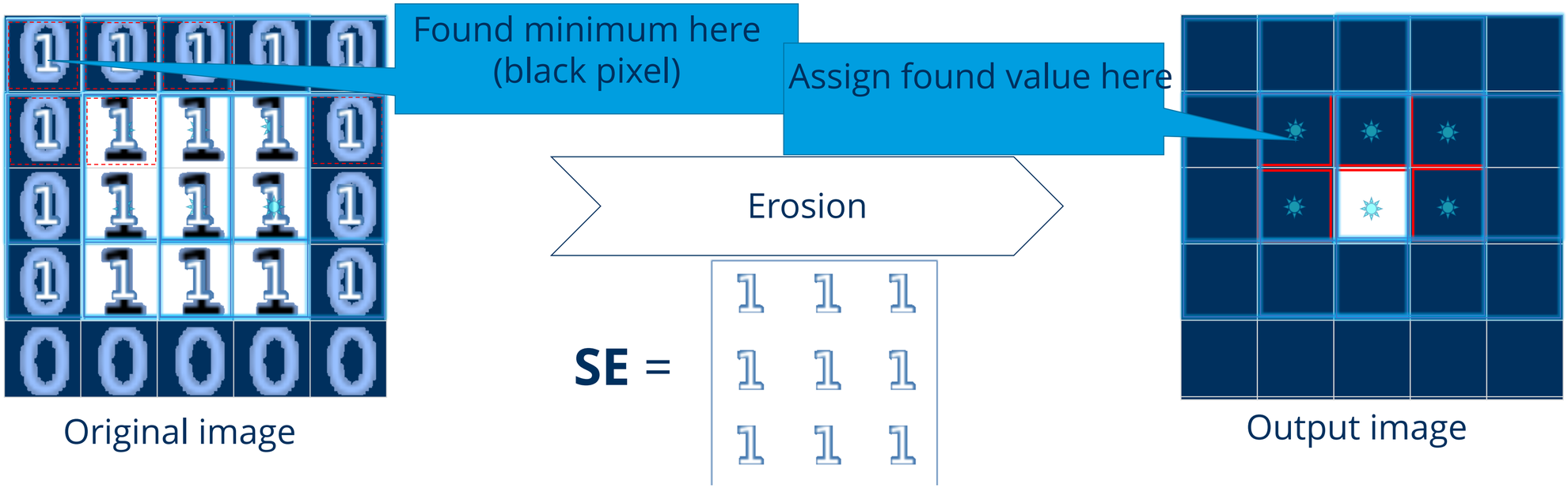


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI

Refining masks: Erosion

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

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

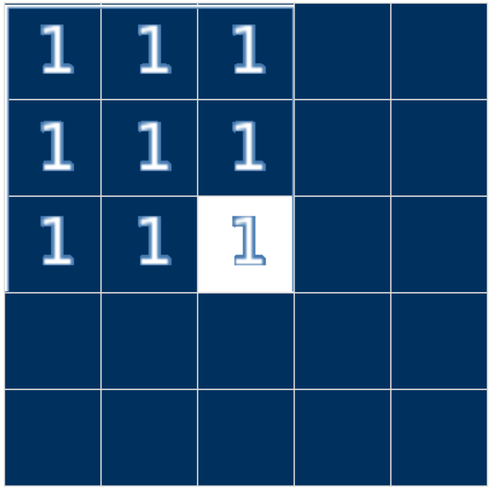


Erosion is essentially a minimum filter whose extent is defined by the kernel (structural element or **SE**) size and shape.

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

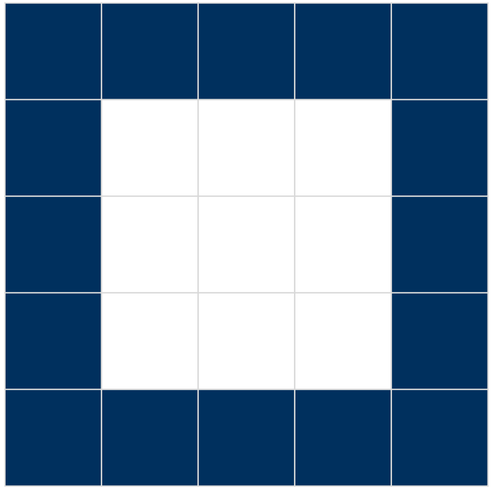
Refining masks: Dilation

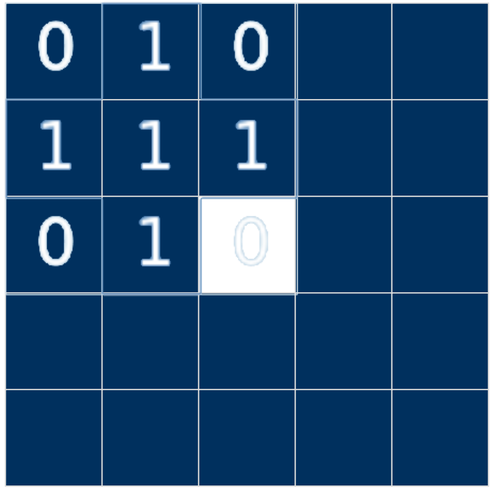
Dilation: Every pixel with at least one white neighbor becomes white.



Dilation

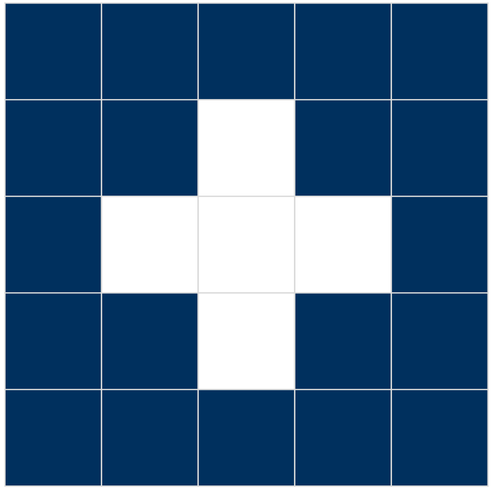
8-connected neighborhood
Moore-Neighborhood





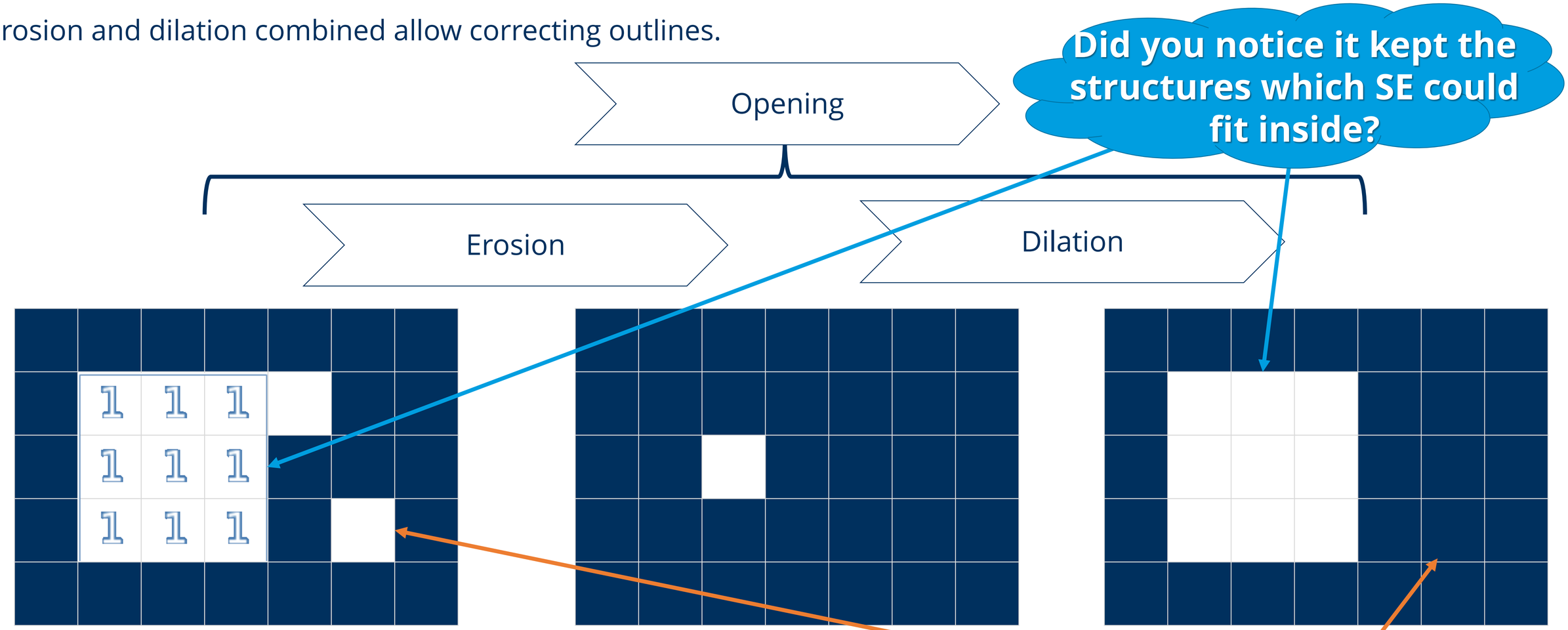
Dilation

4-connected neighborhood
von-Neumann-Neighborhood



Refining masks: Erosion & Dilation

Erosion and dilation combined allow correcting outlines.

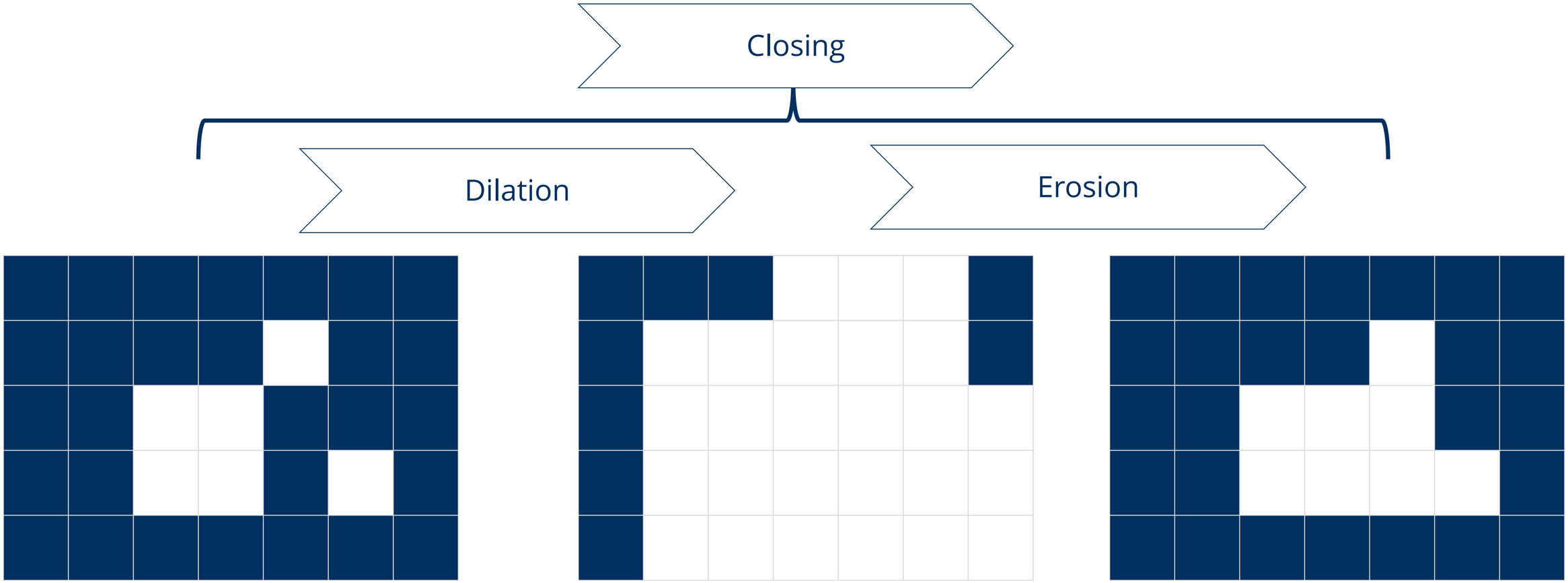


Did you notice it kept the structures which SE could fit inside?

- 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?

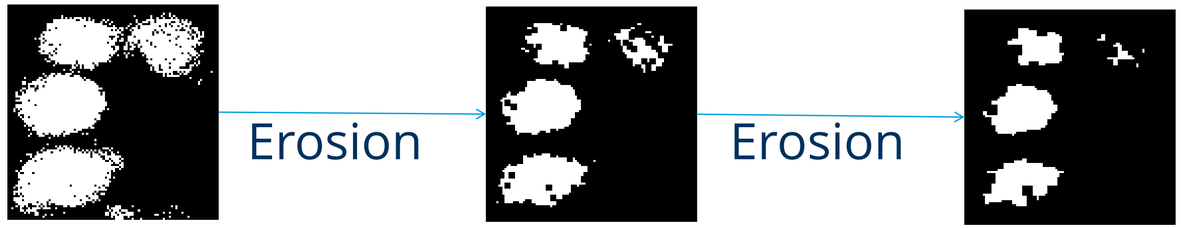
Refining masks: Erosion & Dilation



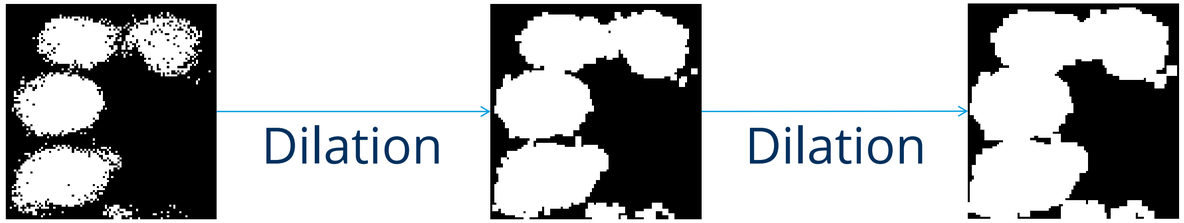
- It can connect white (high intensity) structures that are nearby
- It may close small holes inside structures
- It tends to better preserve area of structures

Refining masks: Erosion / dilation

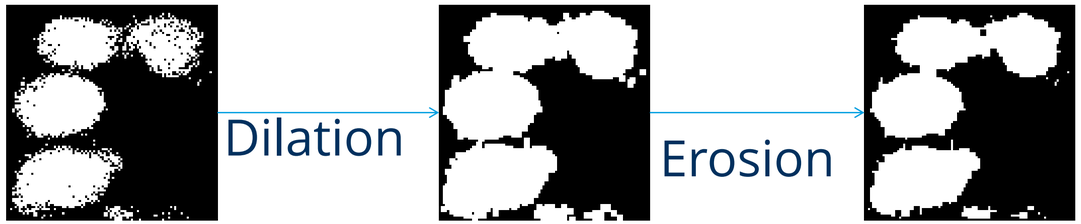
Dilation: Set all pixels to white which have at least one white neighbor.



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



Closing: Dilation + Erosion



Opening: Erosion + Dilation

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)
```

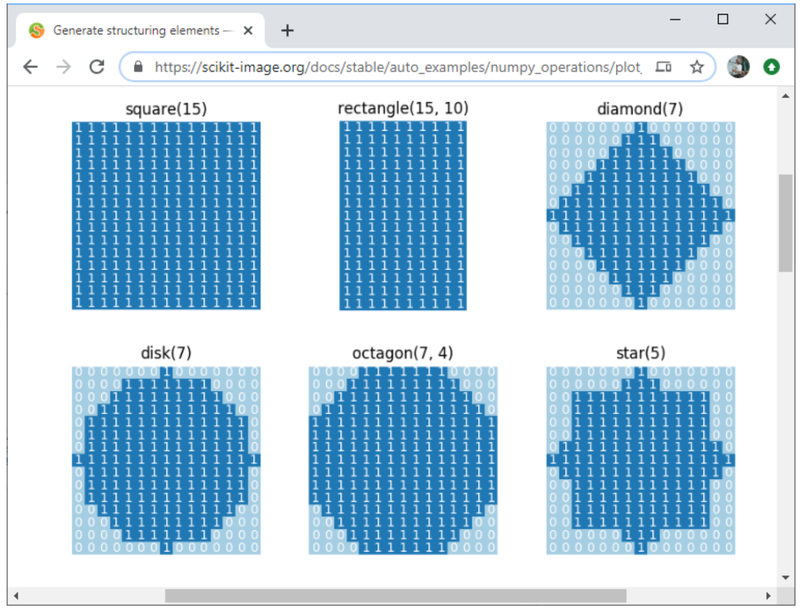
For a binary image, you apply it like this:

```
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

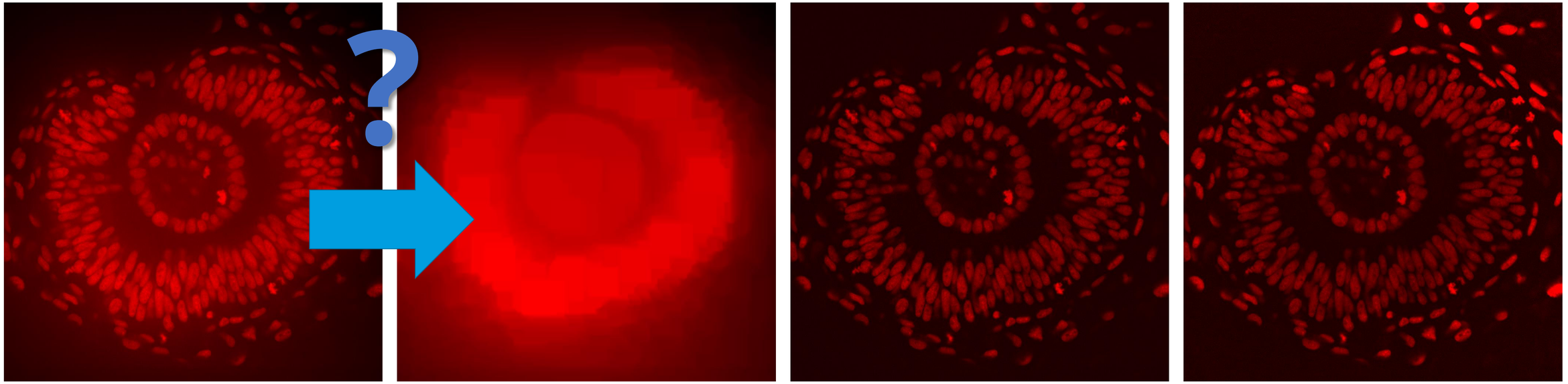
Background removal

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?

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

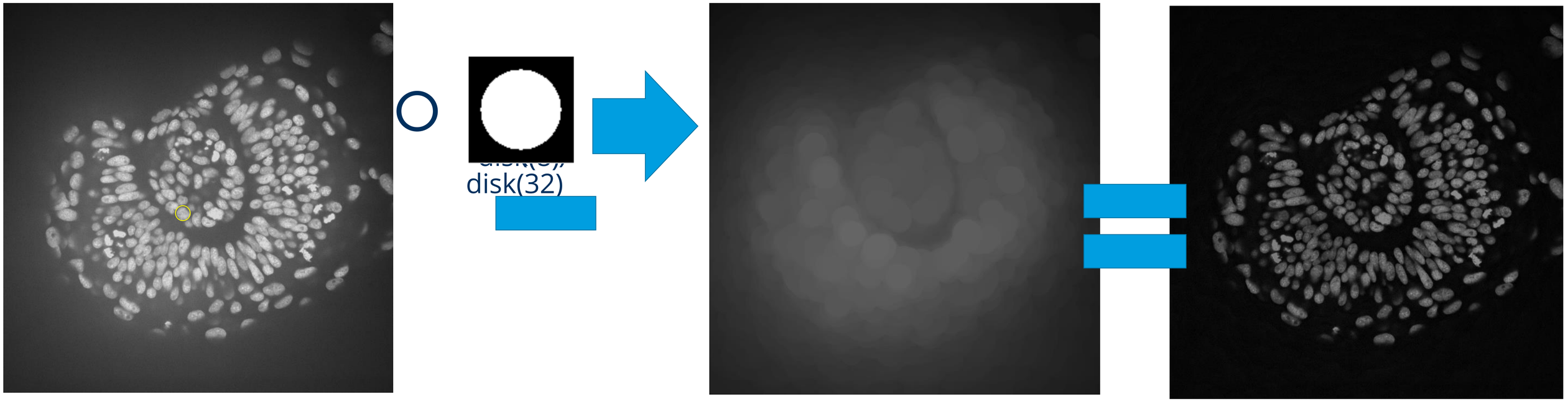


Original image | Extracted background | Subtracted background | Image divided by background

Background removal

Opening

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



Original image

This is a good estimation of the background

Strucutres have a radius ≈ 12

You have just learned the white tophat filter!

Exercises

Image thresholding and feature extraction

Johannes Müller

With material from
Robert Haase, BiaPoL, PoL TU Dresden
Marcelo Zoccoler, BiaPol, PoL, TU Dresden
Benoit Lombardot, Scientific Computing Facility, MPI CBG

Lecture overview

Today's content:

Complete workflow from raw images toward extracting quantitative features from image data

Creating binary masks from intensity images (**Segmentation**)

→ Thresholding

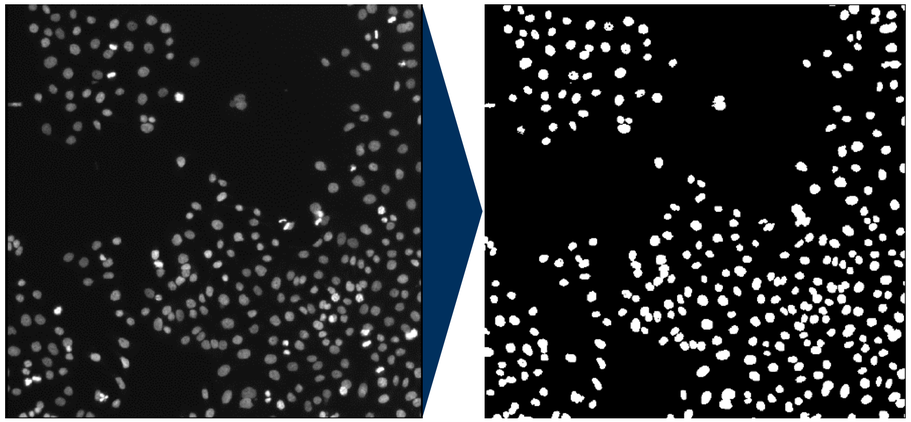
Create label images from binary masks

→ Connected-component analysis

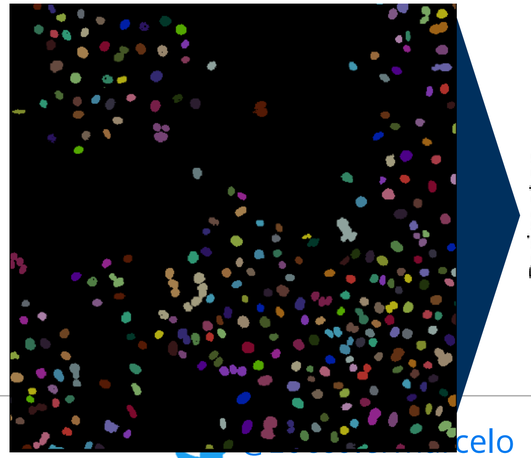
Measure features from label images

→ Shape, Intensity, etc.

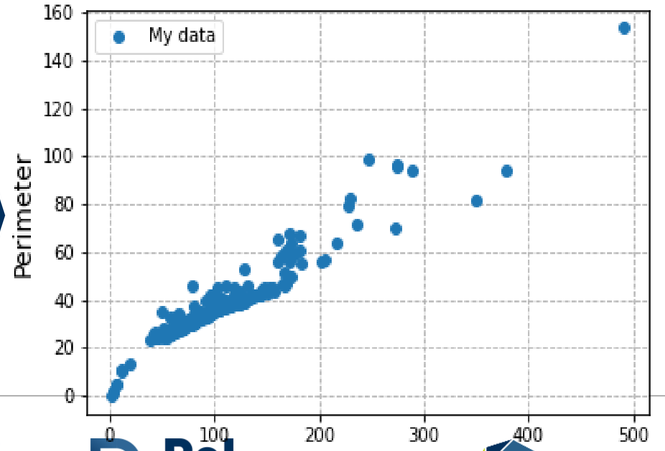
→ Visualize measured features



Labelled image



Measurement



Segmentation

Aim:

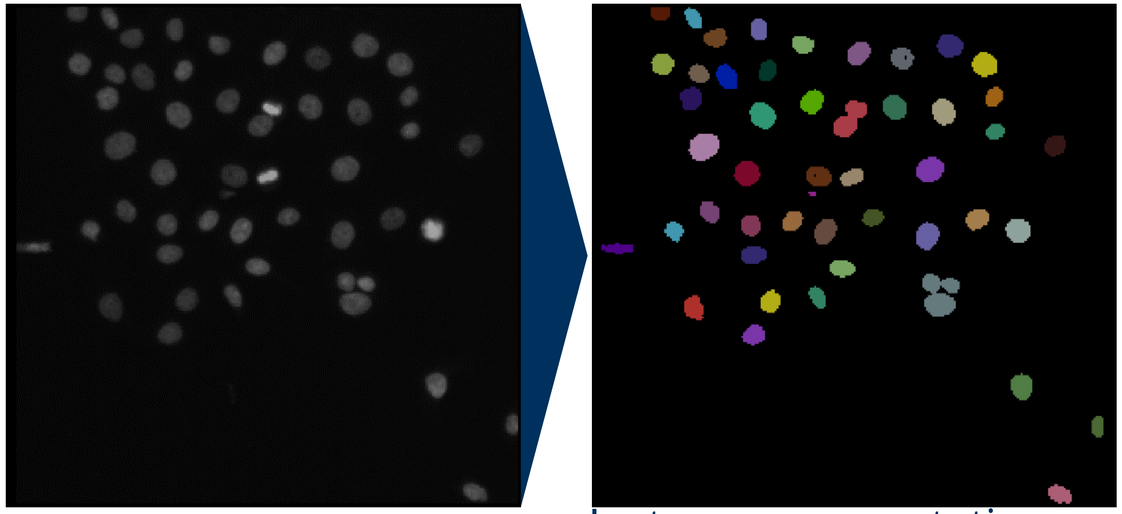
Separate background from foreground

Vocabulary:

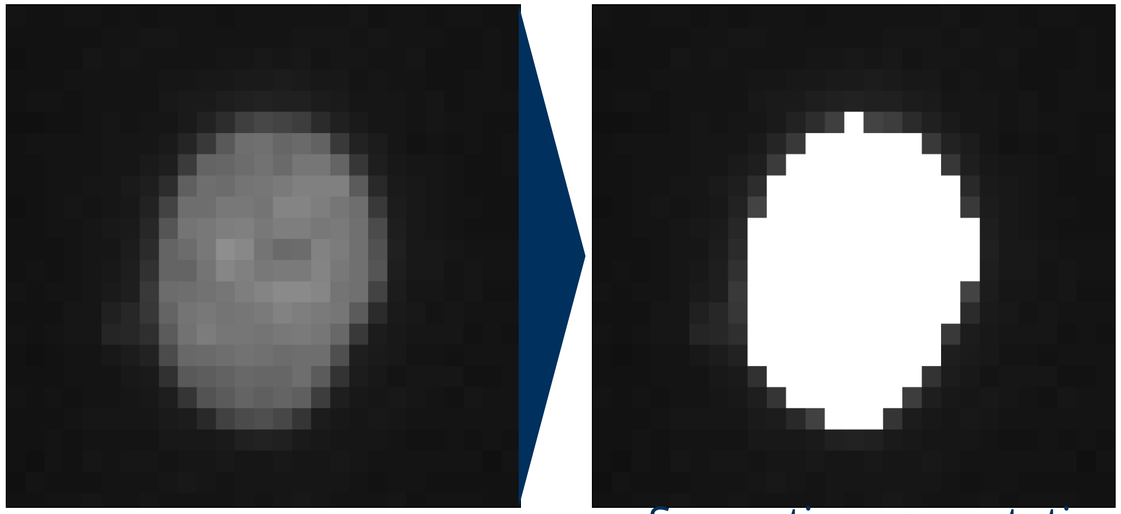
-**Segmentation:**
Assigning a meaningful *label* to each pixel

-**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



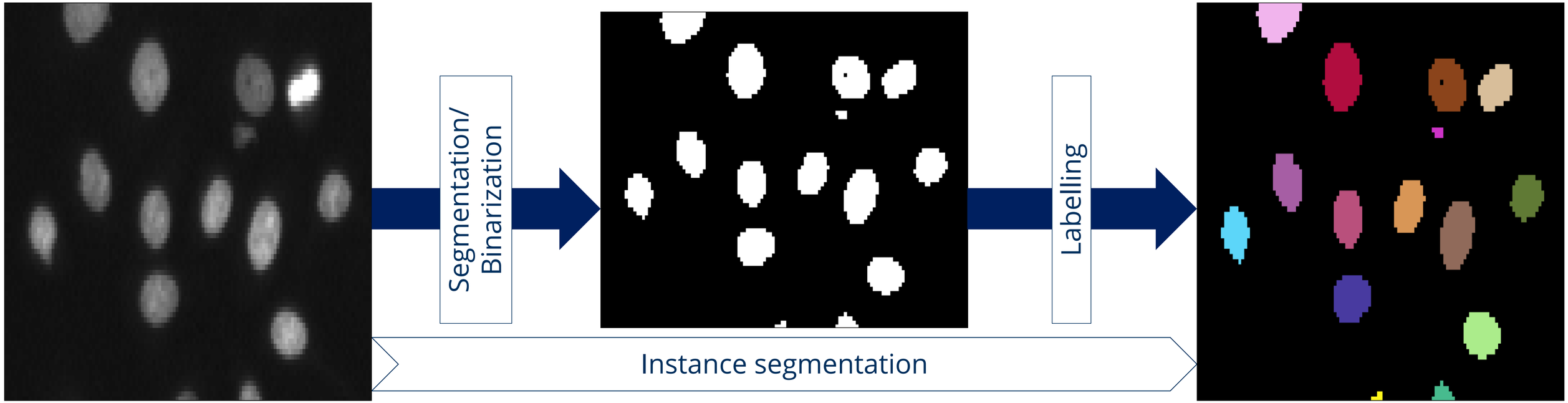
Semantic segmentation

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



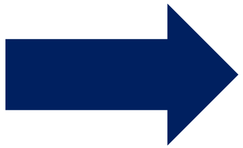
Thresholding in Python

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]])
```

se, “image” is a applied to every pixel!

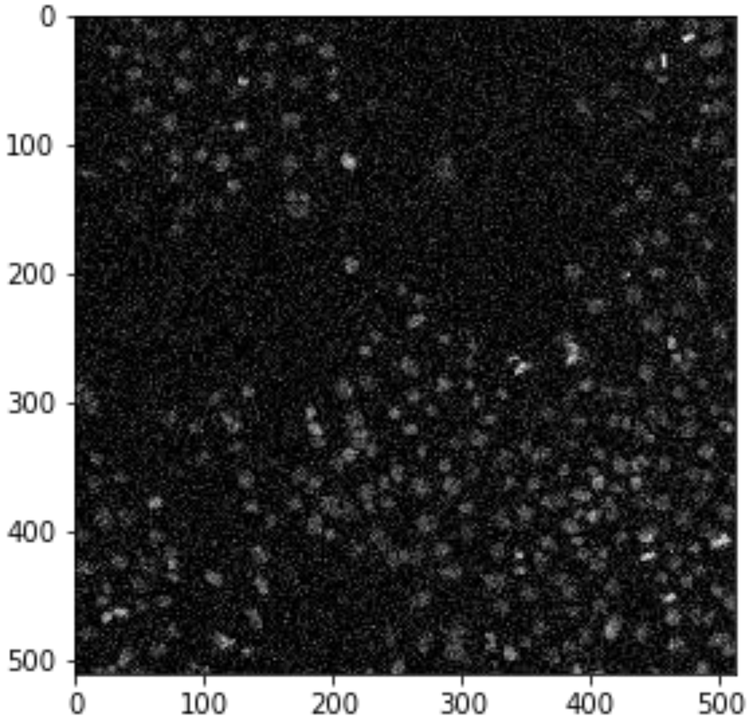
We can then simply store the output of this element-wise comparison in a new variable:

```
binary = image > threshold
```

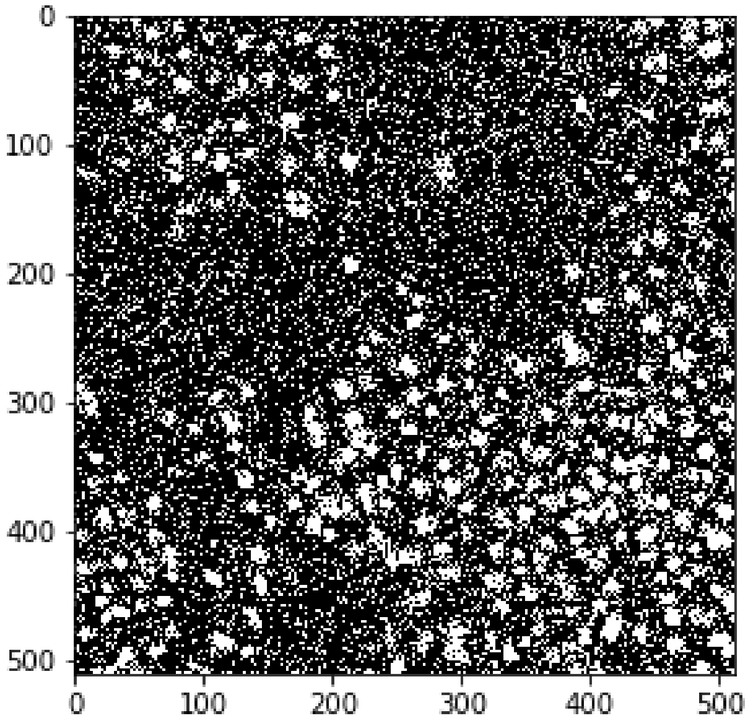
Reminder: pre-processing!

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

— Noise removal
— Background subtraction



Noisy image



Thresholded image

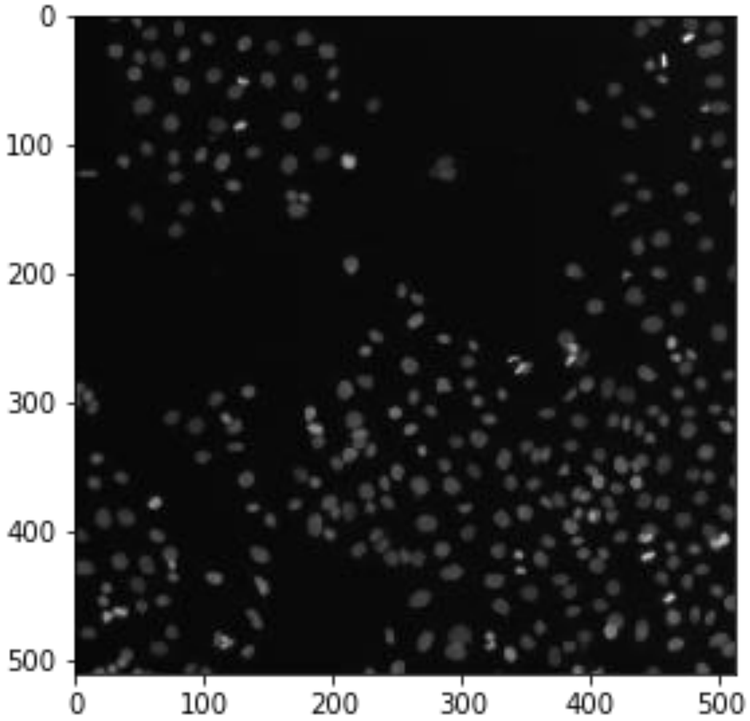
```
filtered = filters.median(image)
```

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

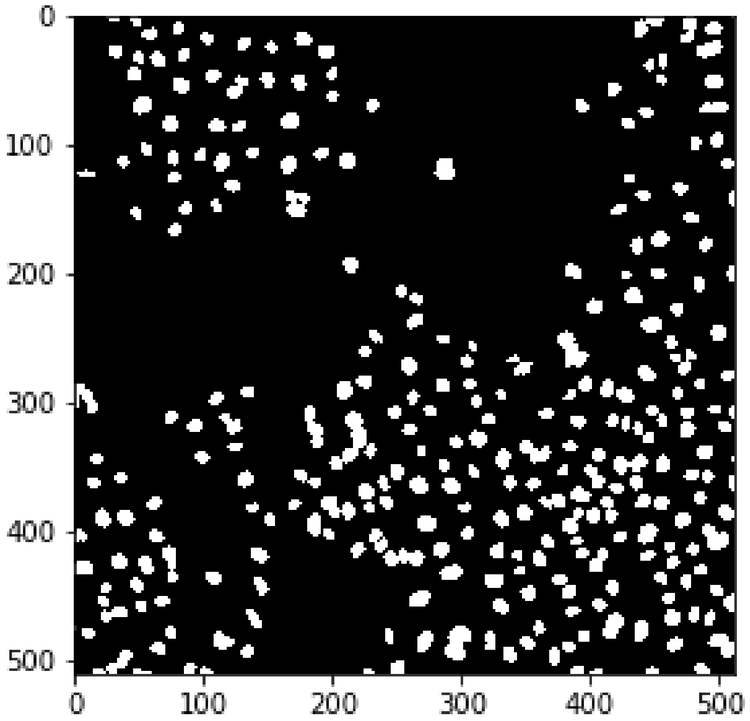
Reminder: pre-processing!

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

— Noise removal
— Background subtraction



Filtered image



Thresholded image

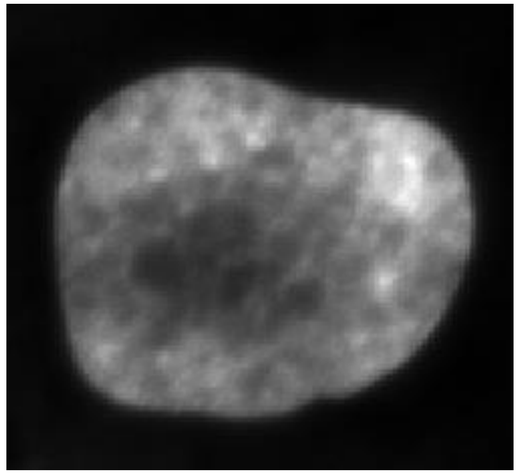
```
filtered = filters.median(image)
```

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

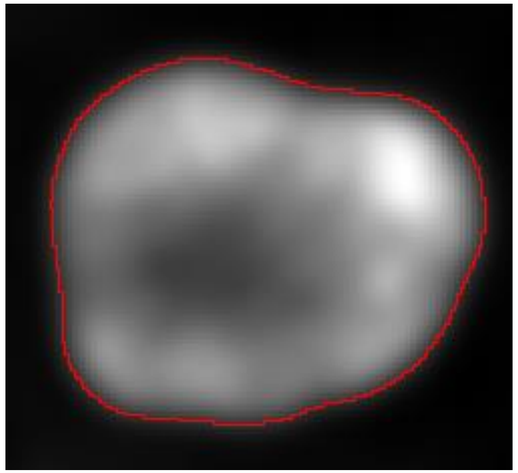
Low-pass filtering to improve thresholding results

In case thresholding algorithms outline the wrong structure, blurring in advance may help.

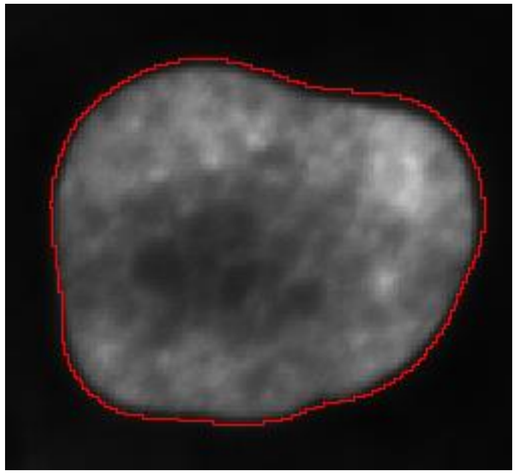
However: **Do not** continue processing the blurred image, continue with the original!



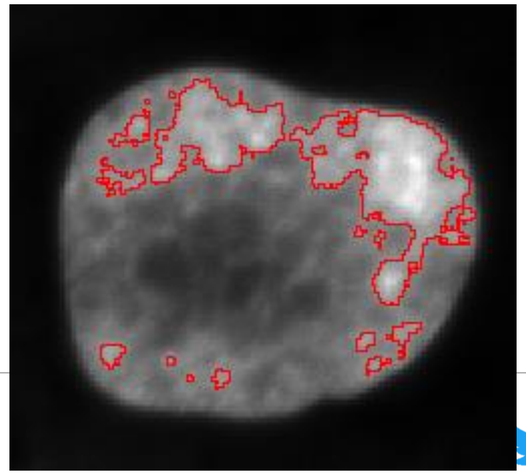
Blurring +
Thresholding ☺



Contour on
original image



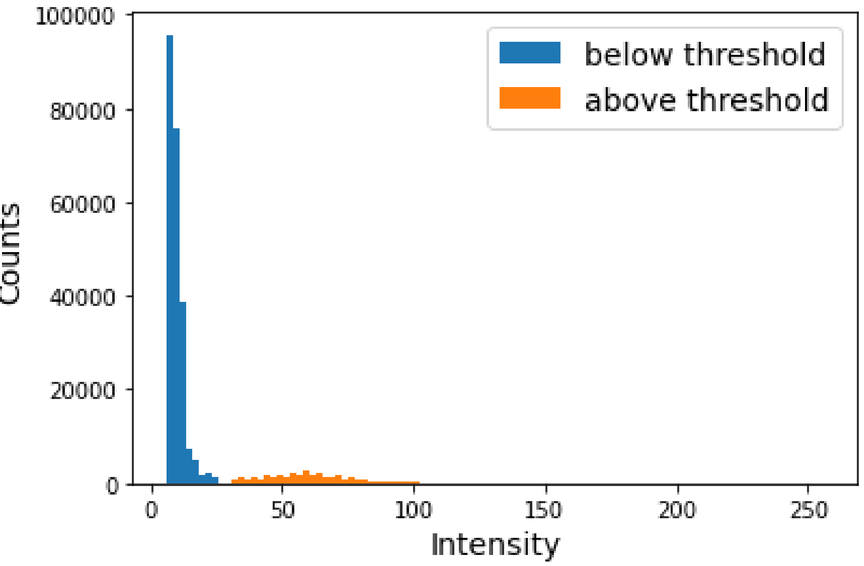
Thresholding ☹

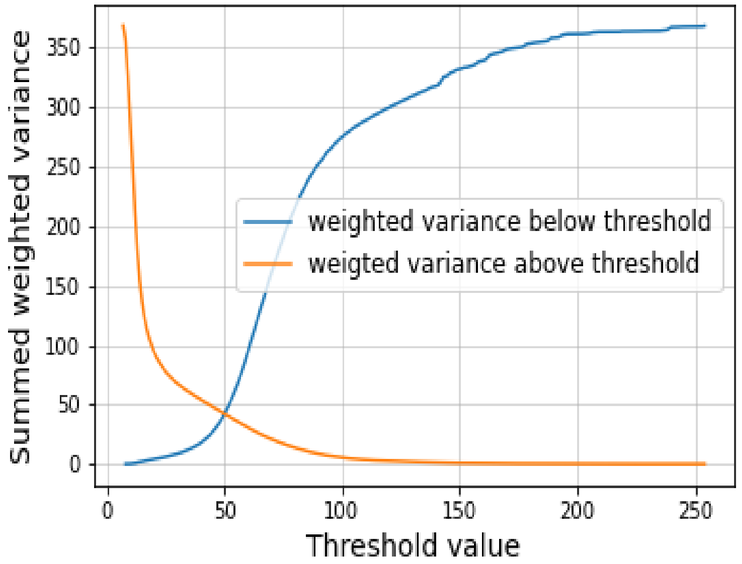


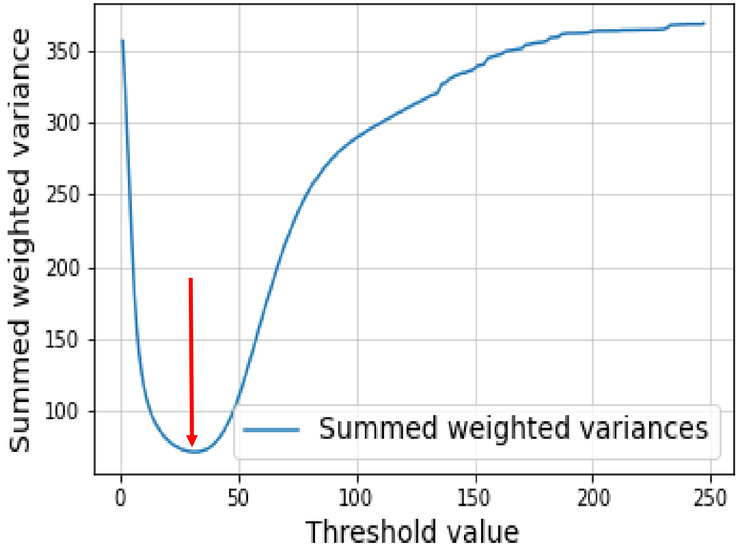
Threshold algorithms

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

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



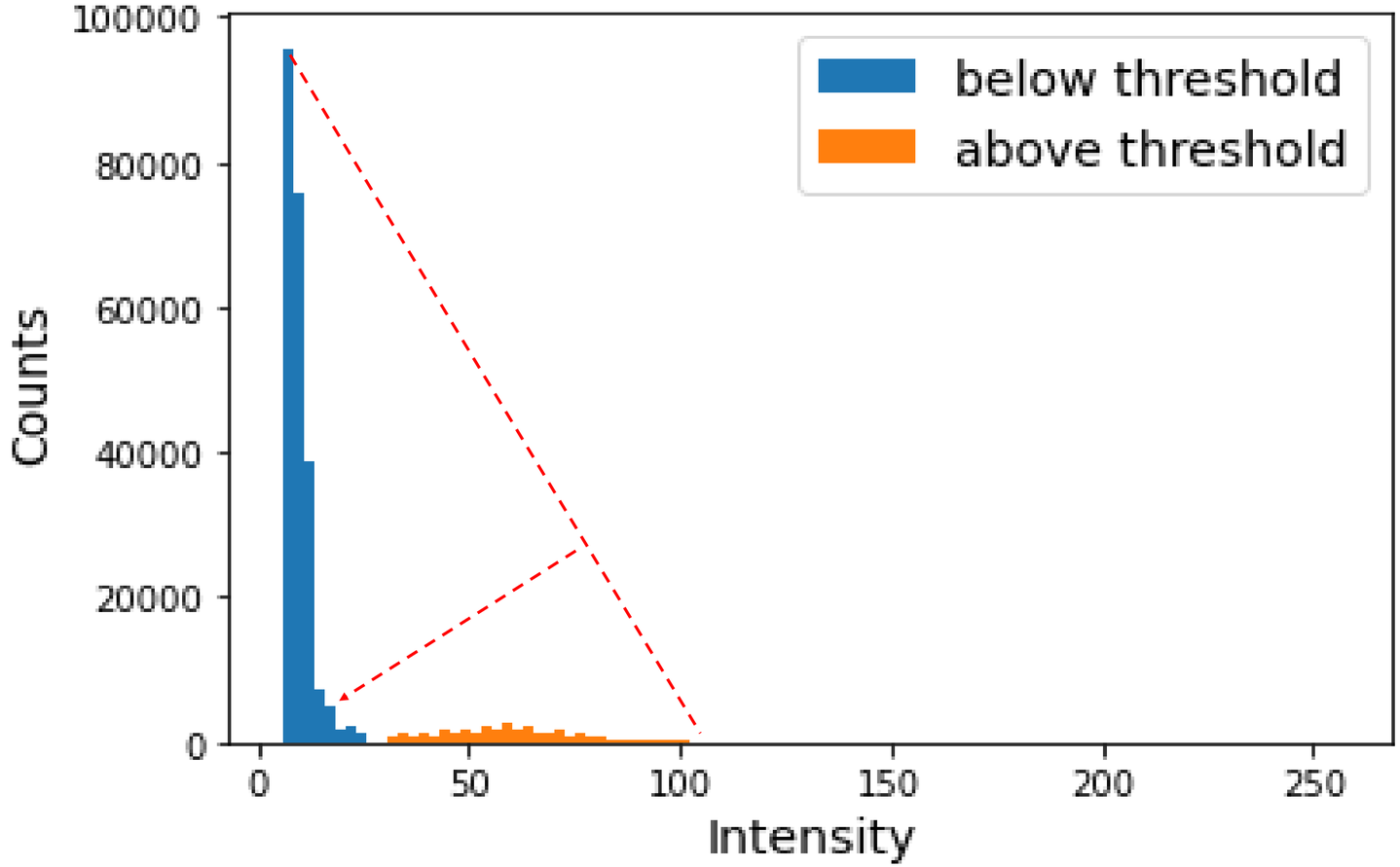




Statistical thresholding: Pixels above statistical parameter of I belong to foreground. (Possibilities: Mean, Median, Quartiles, etc.)

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)
```

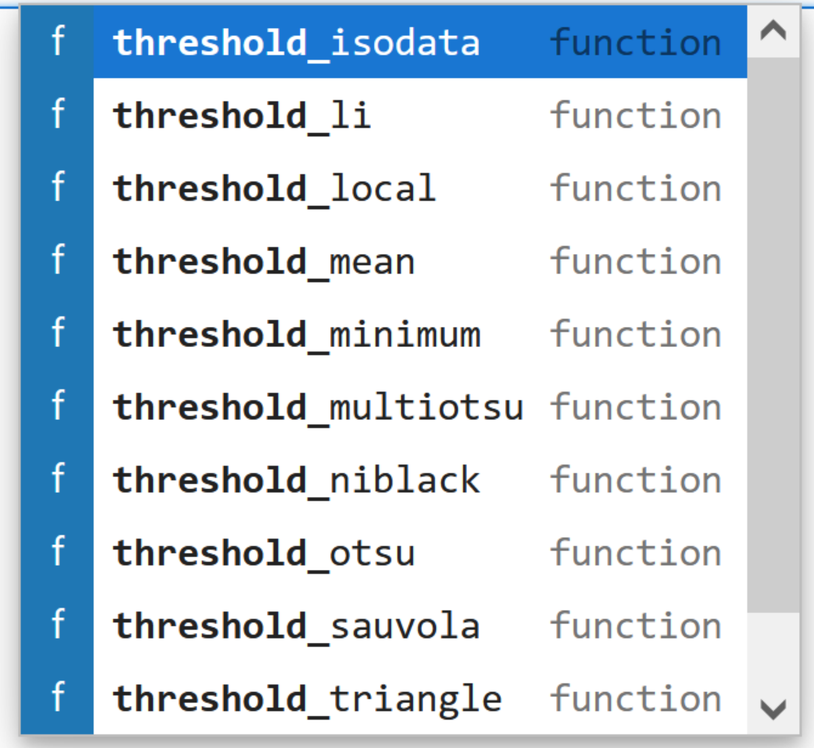
Statistical thresholding: Pixels above statistical parameter of I belong to foreground.
(Possibilities: Mean, Median, Quartiles, etc.)

```
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:

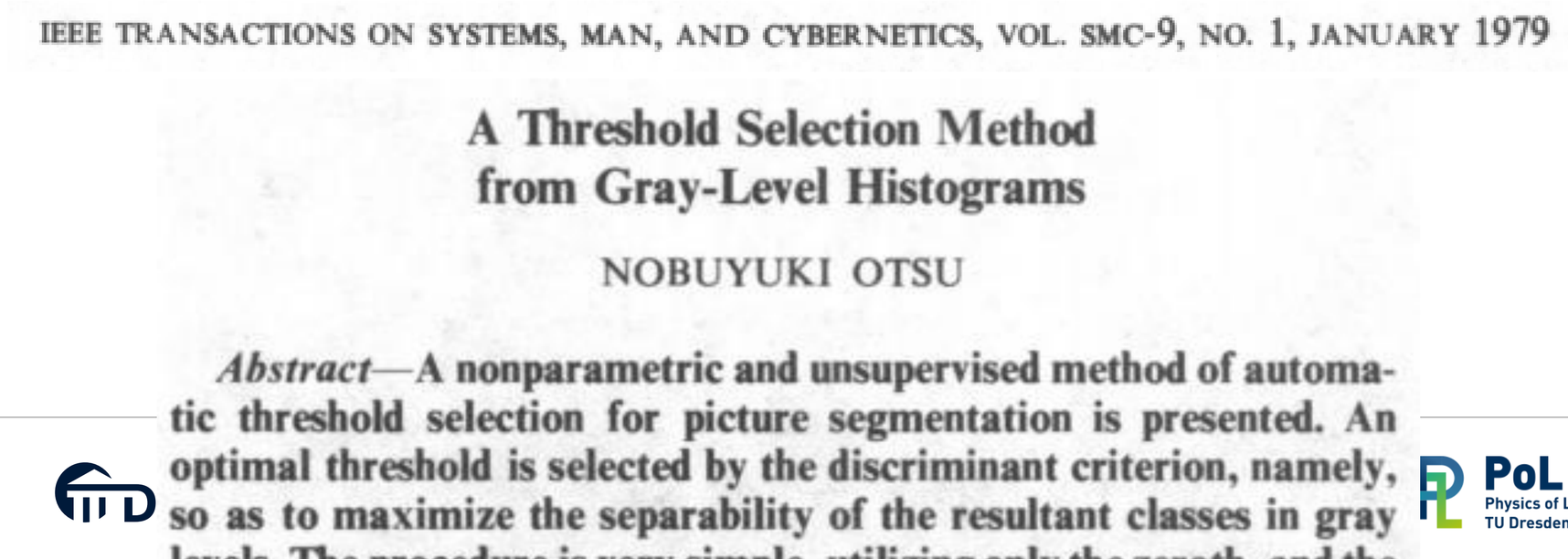
```
from skimage import filters

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

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!

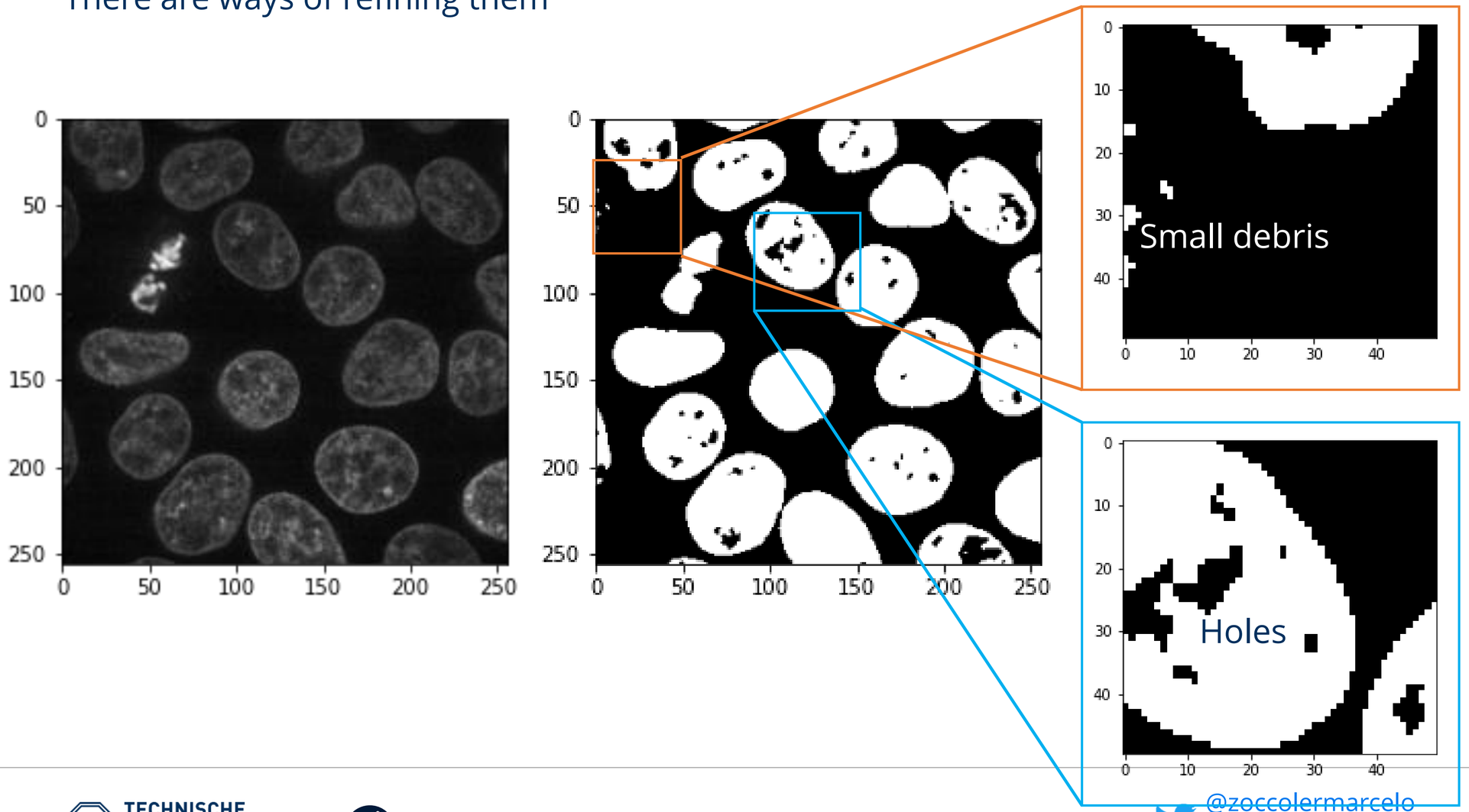
Do not over-engineer

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

Refining masks

Binary mask images may not be perfect immediately after thresholding.

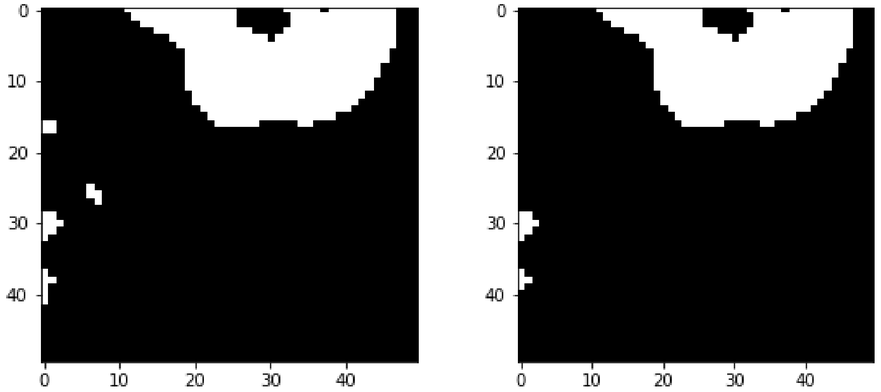
There are ways of refining them



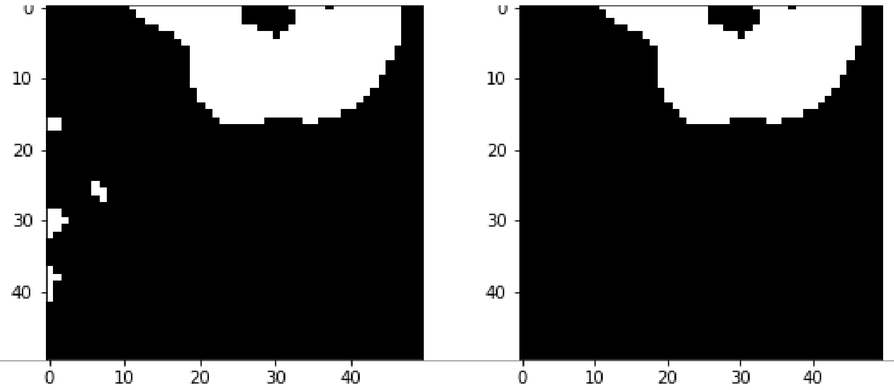
Refining masks: Opening & Closing

```
from skimage import morphology
```

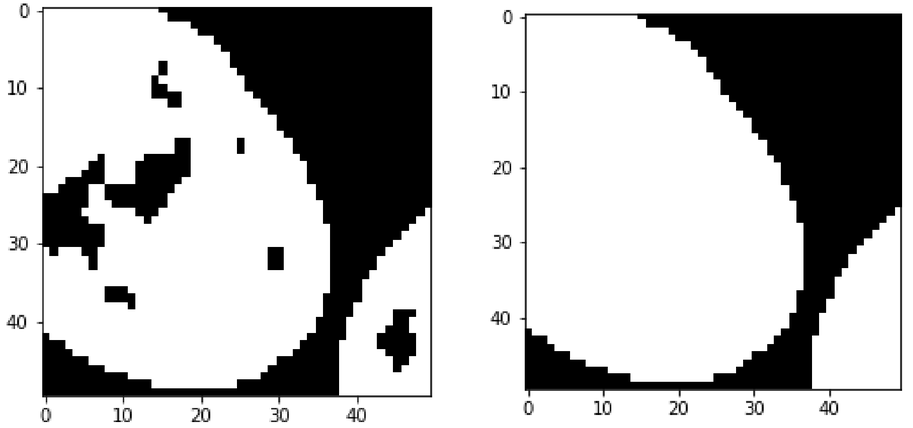
```
opened = morphology.binary_opening(binary)
```



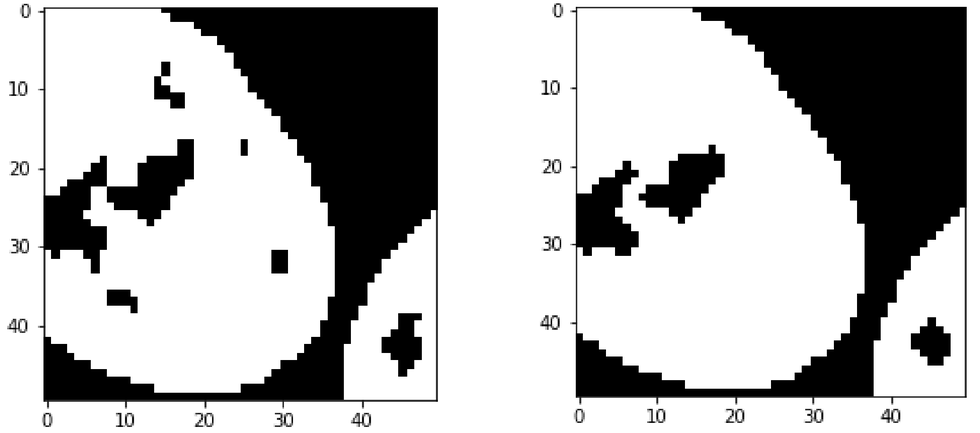
```
opened = morphology.area_opening(binary, area_threshold=20)
```



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



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

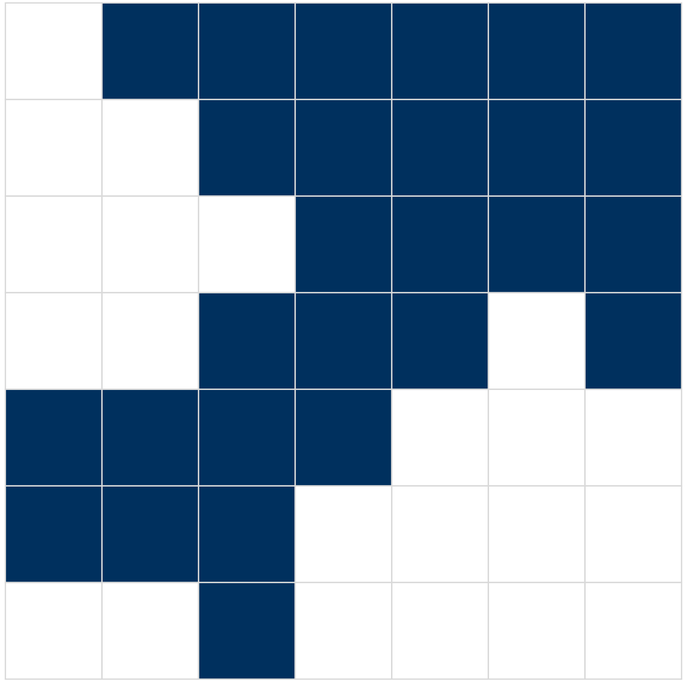


Connected components labelling

In order to allow the computer differentiating objects, connected components analysis (CCA) is used to mark pixels belonging to different objects with different numbers

Background pixels are marked with 0.

The maximum intensity of a labelled map corresponds to the number of objects.

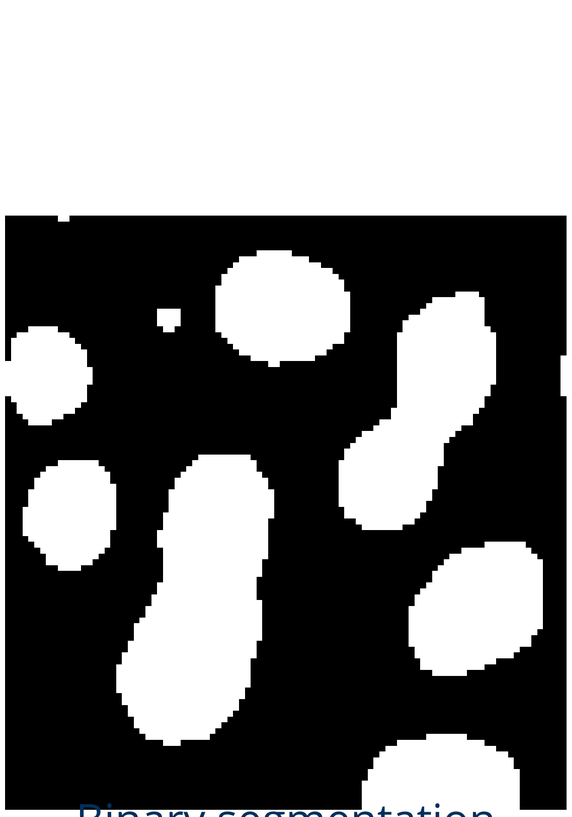


CCA

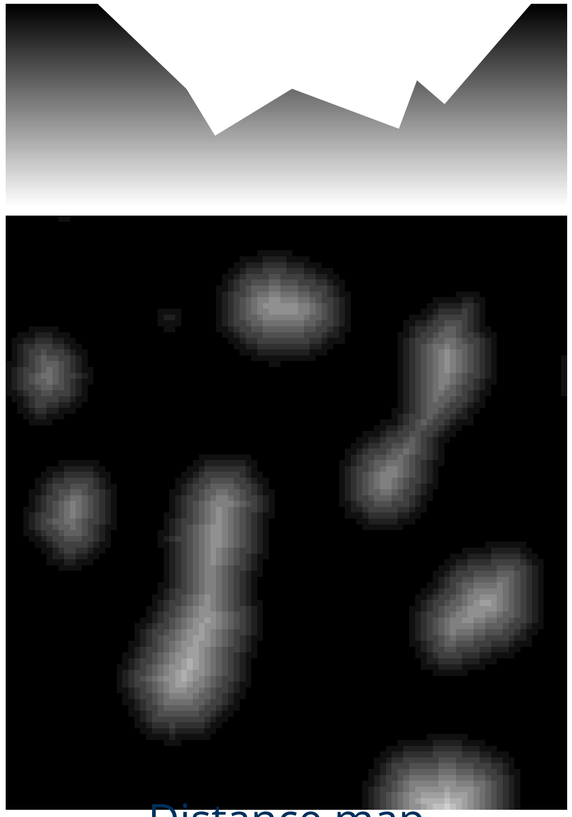
1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

Watershed

The watershed algorithm for binary images allows cutting one object into tow where it’s reasonable.



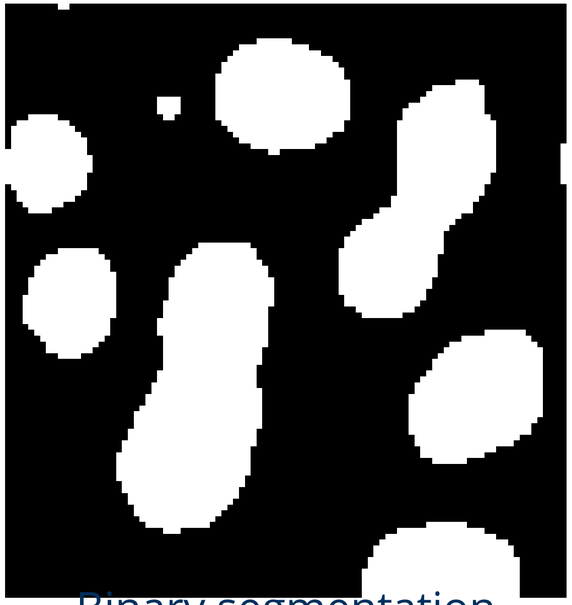
Binary segmentation



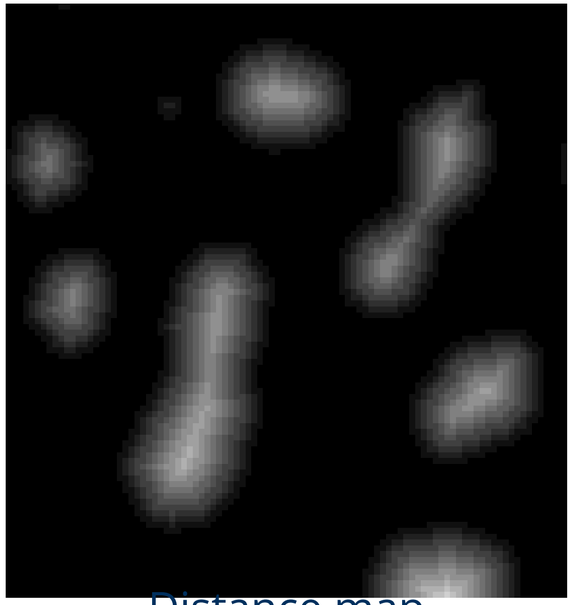
Distance map

Watershed

The watershed algorithm for binary images allows cutting one object into tow where it’s reasonable.



Binary segmentation

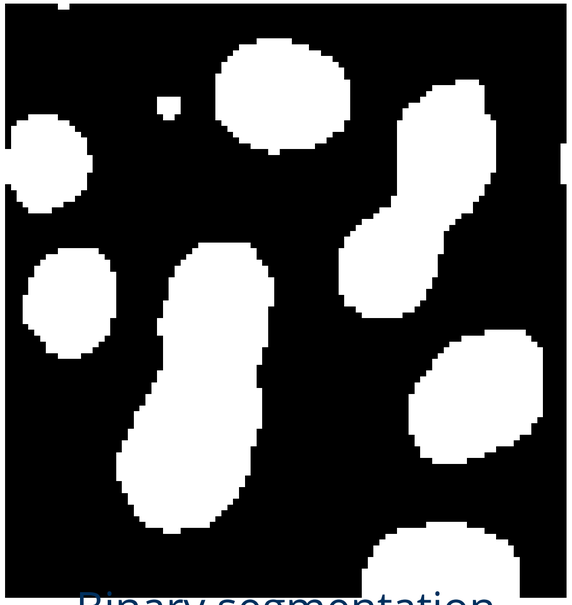


Distance map

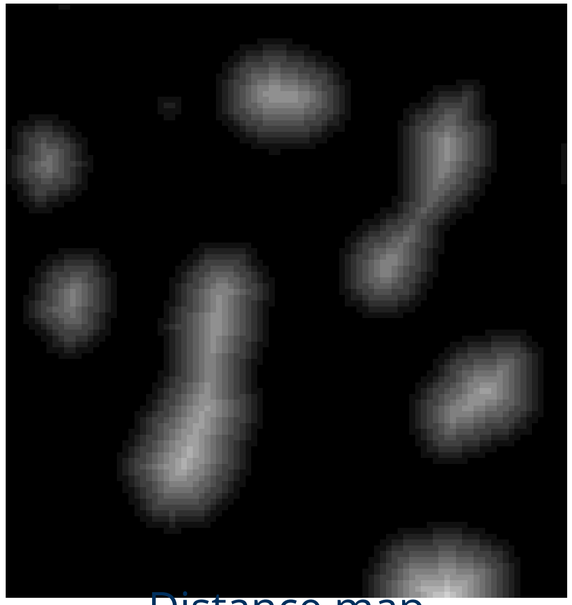
Watershed

The watershed algorithm for binary images allows cutting one object into tow where it’s reasonable.

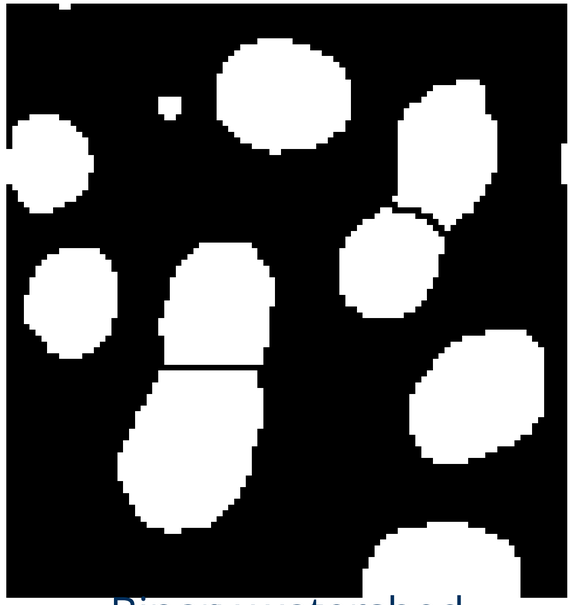
The watersheds are made from binary images. The algorithm does not take the original image into account!



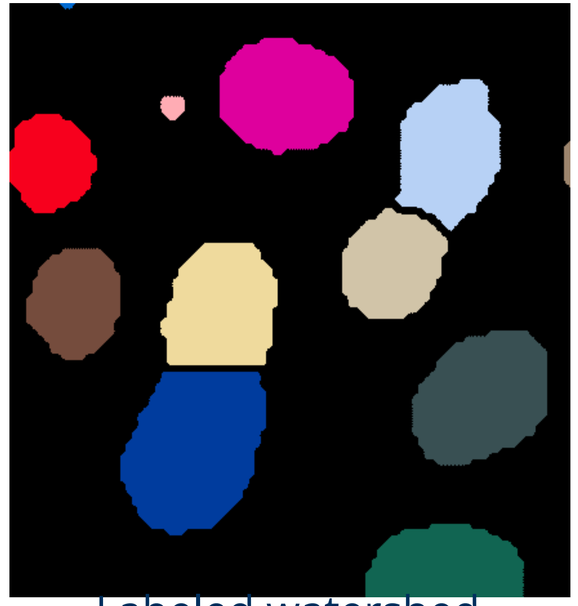
Binary segmentation



Distance map



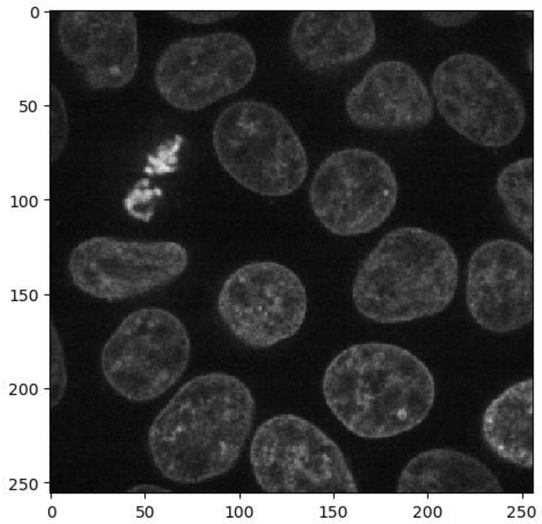
Binary watershed

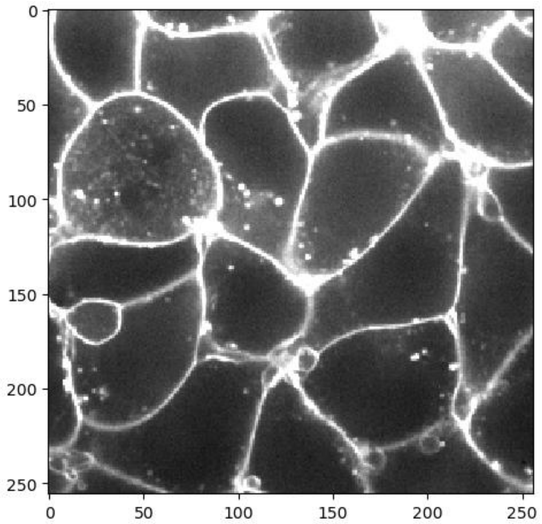


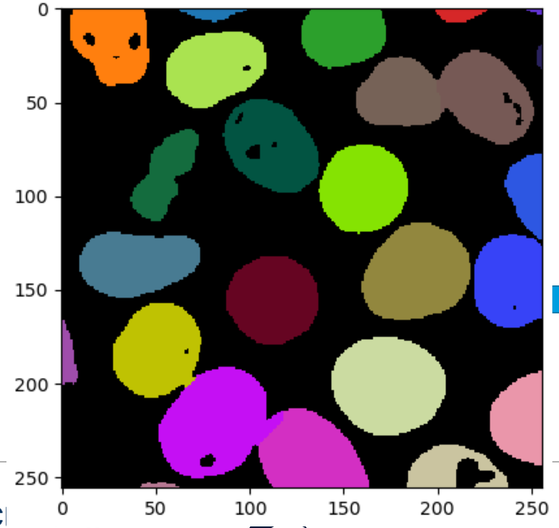
Labeled watershed

Watershed

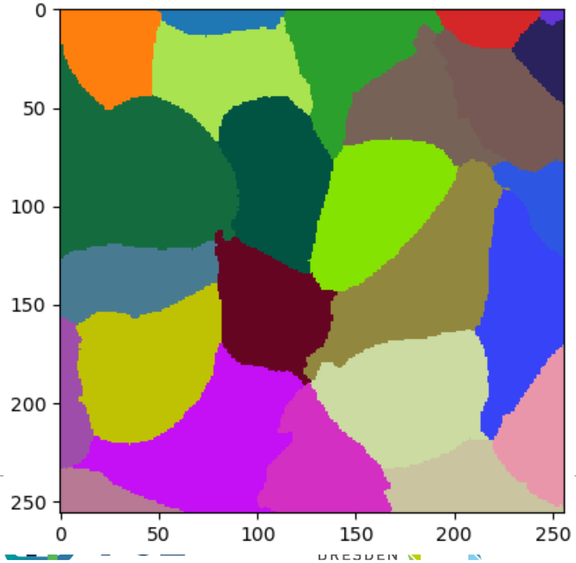
... in Python practice








```
labeled_cells = seeded_watershed(membrane_channel, labeled_nuclei)

labeled_cells
```

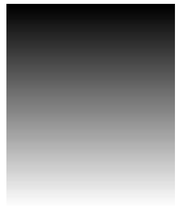


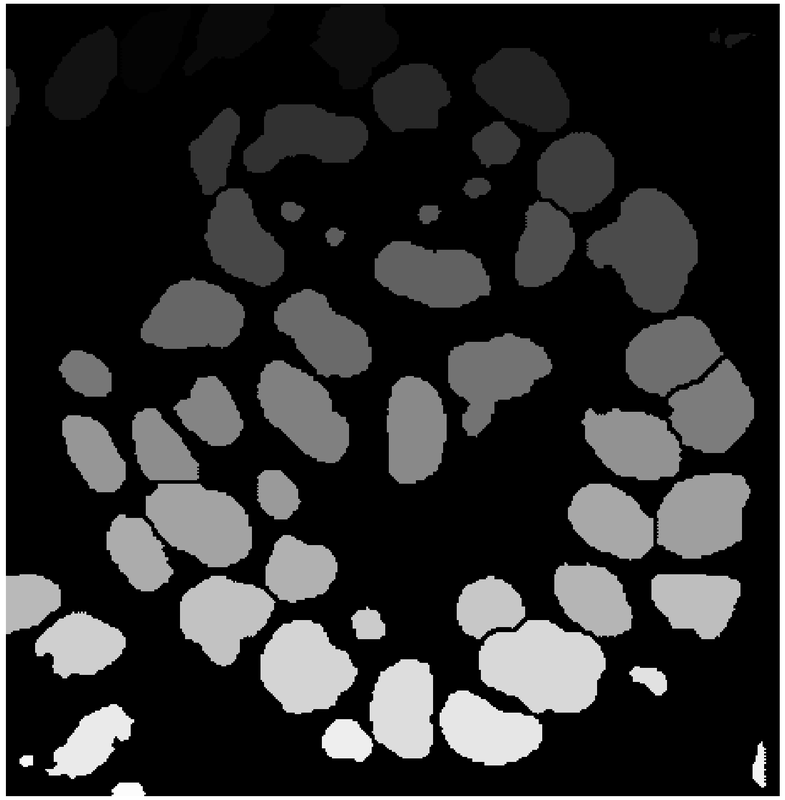
https://www.napari-hub.org/plugins/napari-segment-blobs-and-things-with-membranes#seeded-watershed

Label map visualisation

Label maps can be nicely visualized with the right lookup table

Grey

Pixel value	Display color
0 1 2 ... 255	



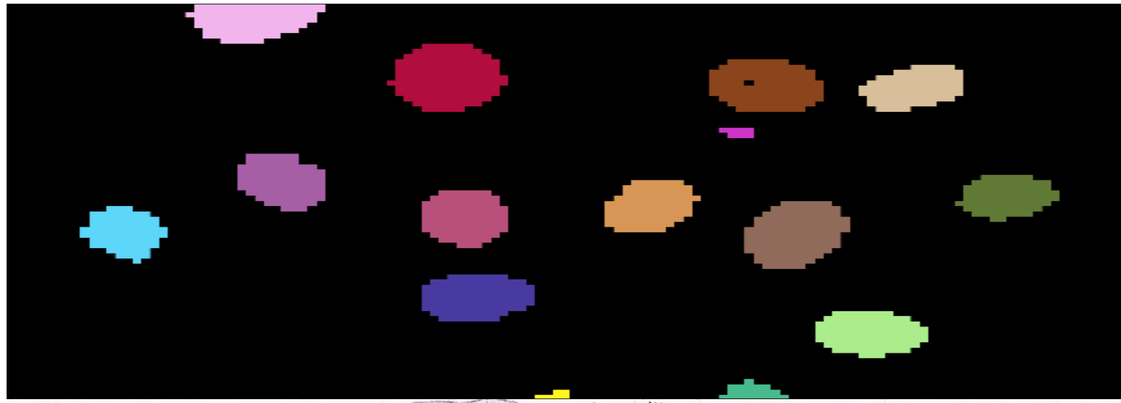
Glasbey

Pixel value	Display color
0 1 2 ... 255	

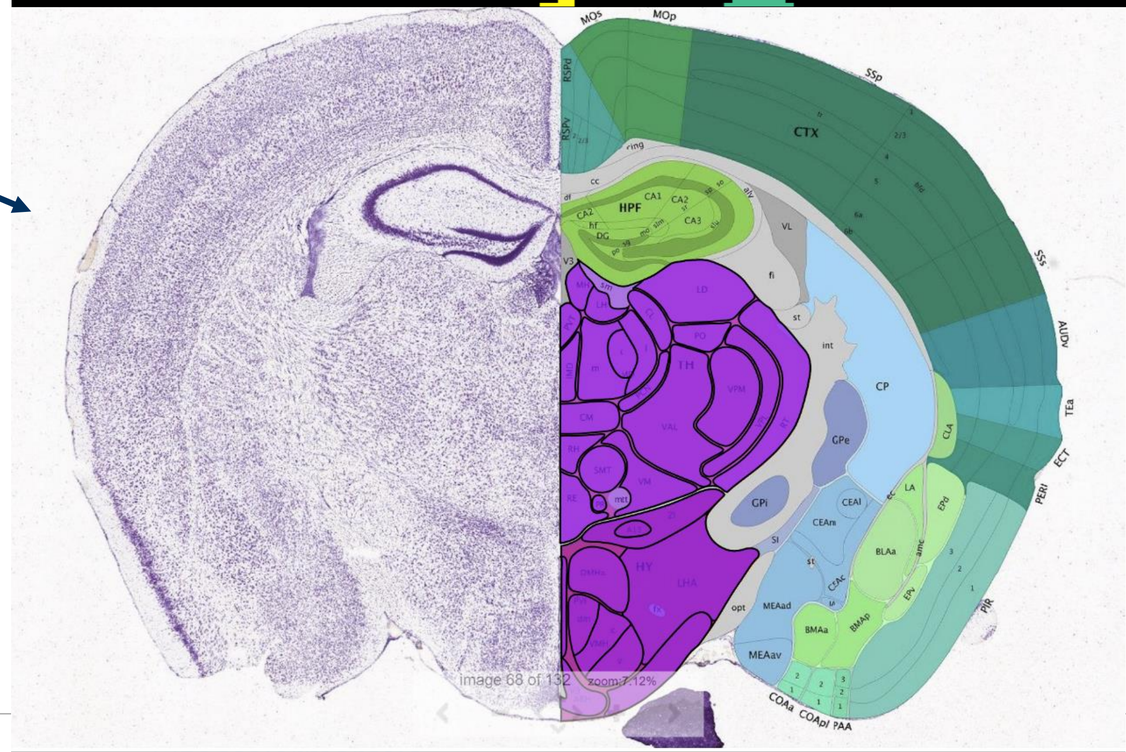
Reminder: Vocabulary

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

Instance segmentation
Label 1 = Nucleus #1
Label 2 = Nucleus #2
Label 3 = Nucleus #3



Semantic segmentation
Label 1 = Cortex
Label 2 = Cerebellum
Label 3 = Hippocampus



Allen adult mouse atlases,
https://atlas.brain-map.org

Feature extraction

A *feature* is a countable or measurable property of an image or object.

Goal of feature extraction is finding a minimal set of features to describe an object well enough to differentiate it from other objects.

- **Intensity based**
  - Mean intensity
  - Standard deviation
  - Total intensity
  - Textures
- **Shape based /spatial**
  - Area / Volume
  - Roundness
  - Solidity
  - Circularity / Sphericity
  - Elongation
  - Centroid
  - Bounding box
- **Spatio-temporal**
  - Displacement,
  - Speed,
  - Acceleration
- **Others**
  - Overlap
  - Colocalization
  - Neighborhood
- **Mixed features**
  - Center of mass
  - Local minima / maxima

Intensity based features

Min / max

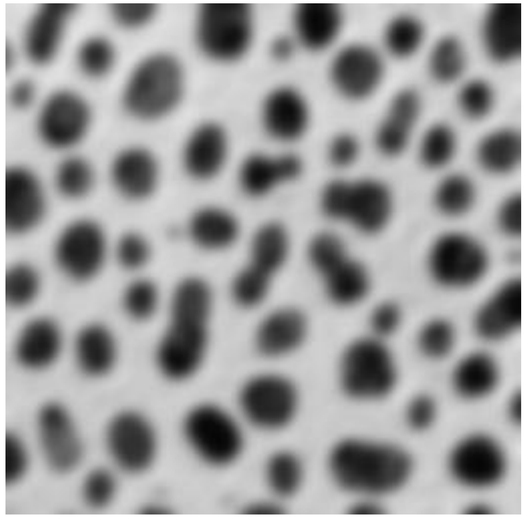
Median

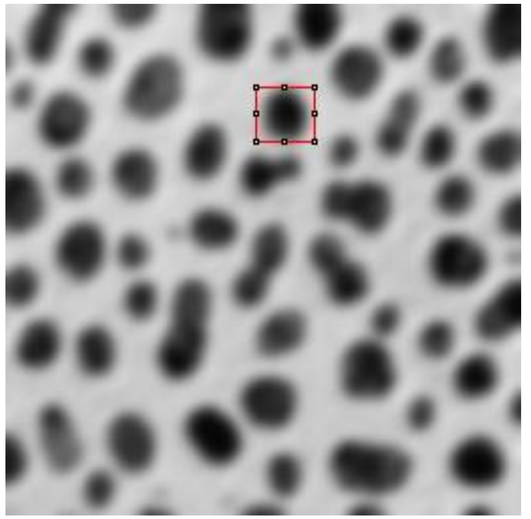
Mean

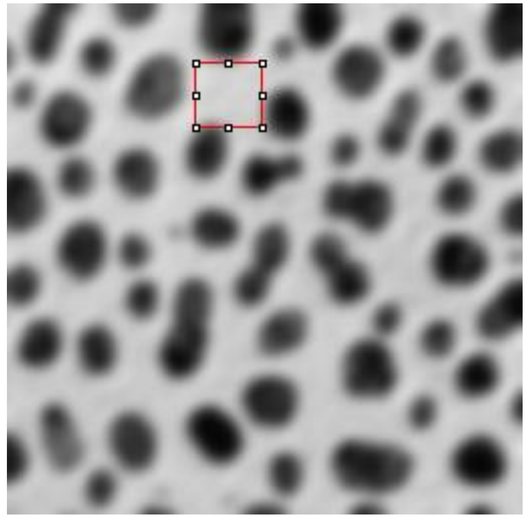
Mode

Variance

Standard deviation





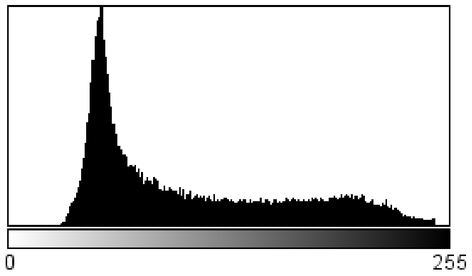


Can be derived from pixel values

Don’t take spatial relationship of pixels into account

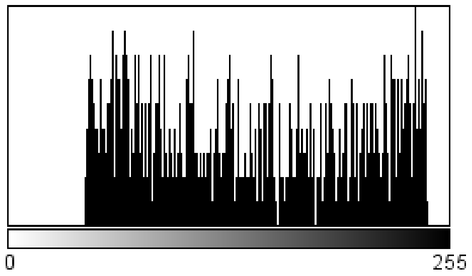
See also:

- descriptive statistics
- histogram



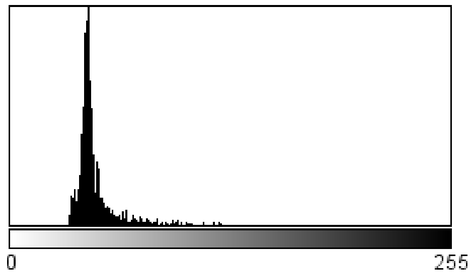
Count: 65024
Mean: 103.301
StdDev: 57.991

Min: 29
Max: 248
Mode: 53 (1663)



Count: 783
Mean: 141.308
StdDev: 61.876

Min: 44
Max: 243
Mode: 236 (9)



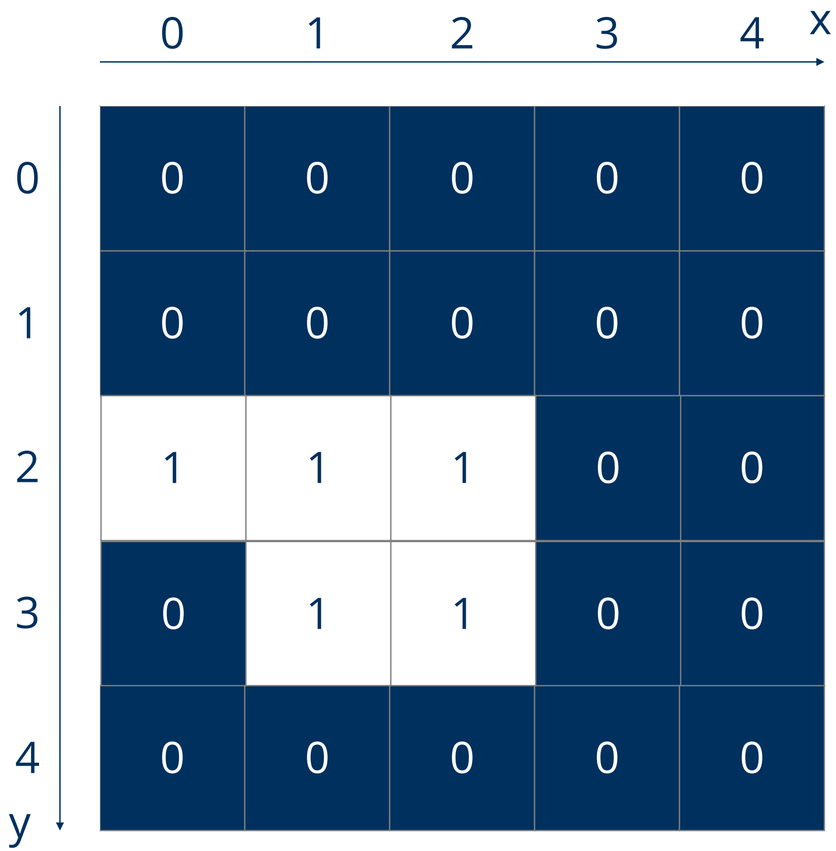
Count: 1056
Mean: 49.016
StdDev: 12.685

Min: 34
Max: 122
Mode: 45 (120)

Bounding rectangle / bounding box

Position and size of the smallest rectangle containing all pixels of an object

- x_b, y_b … position of the bounding box
- w_b … width of the bounding box
- h_b … height of the bounding box



variable	value
x_b	0
y_b	2
w_b	3

Center of mass

Relative position in an image weighted by pixel intensities

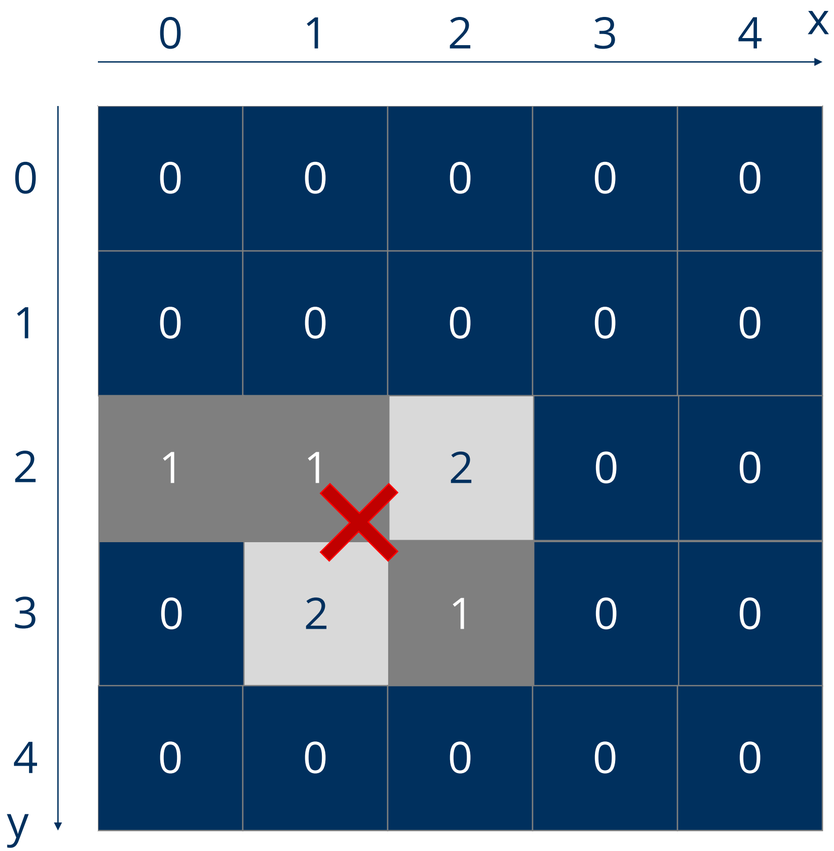
- x, y … pixel coordinates
- w … image width
- h … image height
- μ … mean intensity
- $g_{x,y}$ … pixel grey value
- x_m, y_m … center of mass coordinates

$$\mu = \frac{1}{wh} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} g_{x,y}$$

$$x_m = \frac{1}{wh\mu} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} x \, g_{x,y}$$

"sum intensity"
"total intensity"

$$y_m = \frac{1}{wh\mu} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} y \, g_{x,y}$$



x_m = 1/7 (1·0 + 1·1 + 2·2 + 2·1 + 1·2) = 1.3

y_m = 1/7 (1·2 + 1·2 + 2·3 + 2·2 + 1·3) = 2.4

Center of geometry / centroid

Relative position in an image weighted by pixel intensities

Special case of center of mass for binary images

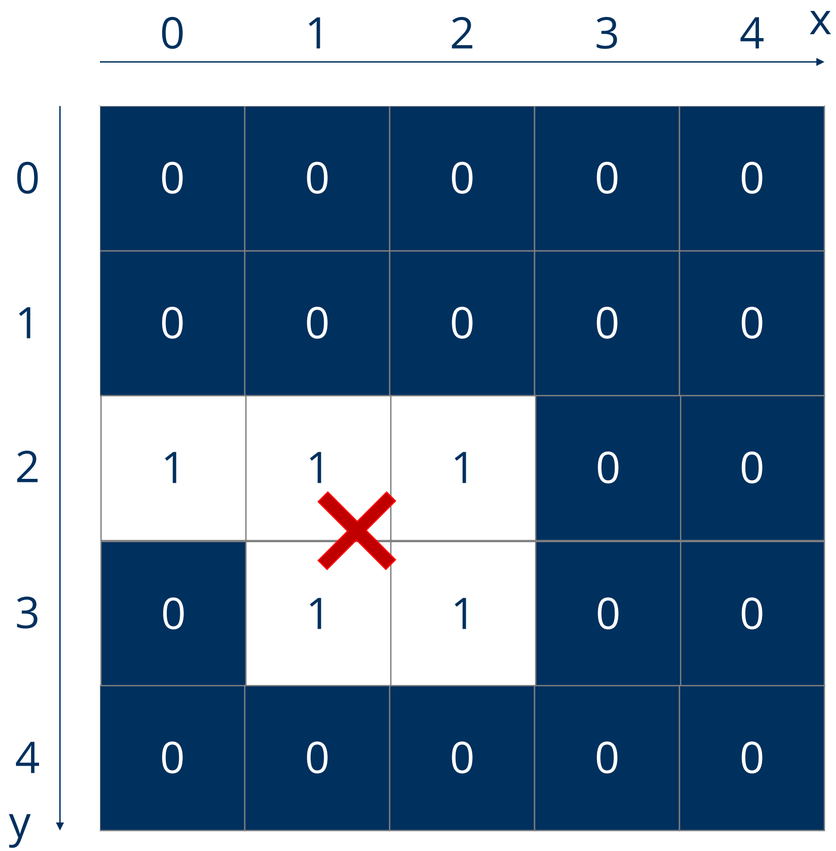
- x, y … pixel coordinates
- w … image width
- h … image height
- μ … mean intensity
- $g_{x,y}$ … pixel grey value, integer in range [0;1]
- x_m, y_m … center of mass coordinates

$$\mu = \frac{1}{wh} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} g_{x,y}$$

$$x_m = \frac{1}{wh\mu} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} x \, g_{x,y}$$

Number of white pixels

$$y_m = \frac{1}{wh\mu} \sum_{y=0}^{h-1} \sum_{x=0}^{w-1} y \, g_{x,y}$$



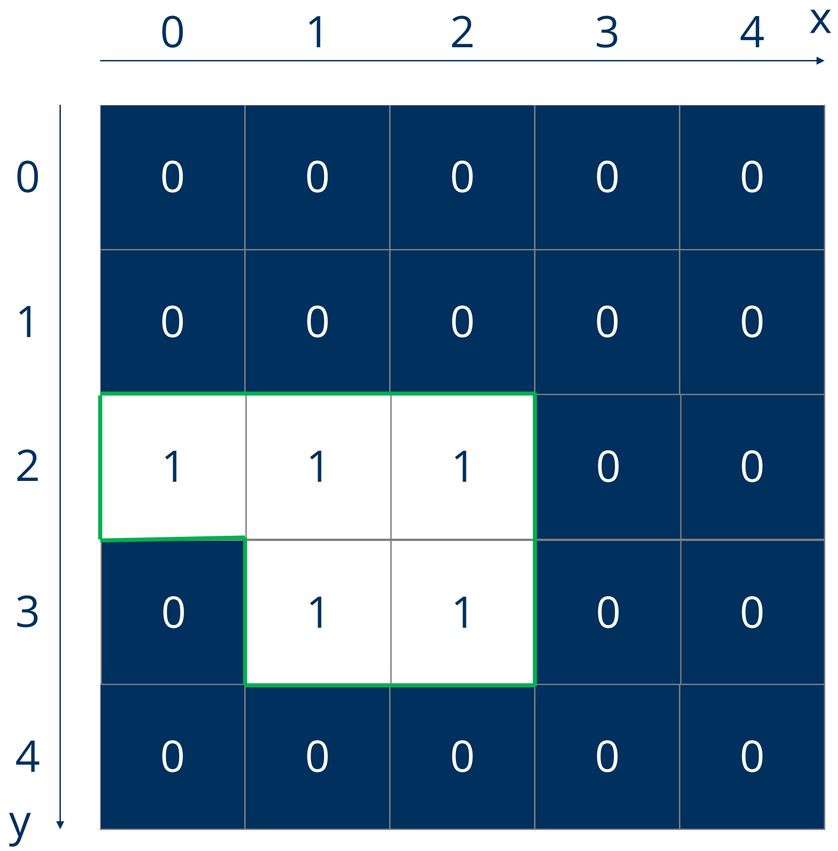
x_m = 1/5 (1·0 + 1·1 + 1·2 + 1·1 + 1·2) = 1.2

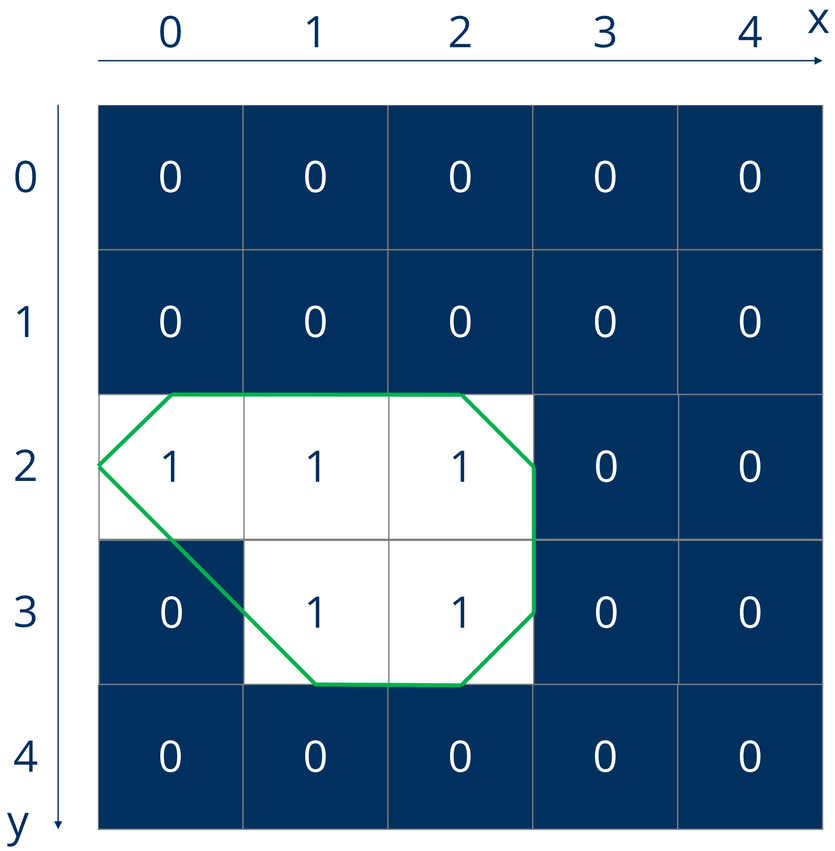
y_m = 1/5 (1·2 + 1·2 + 1·3 + 1·2 + 1·3) = 2.4

Perimeter

Length of the outline around an object

Depends on the actual implementation





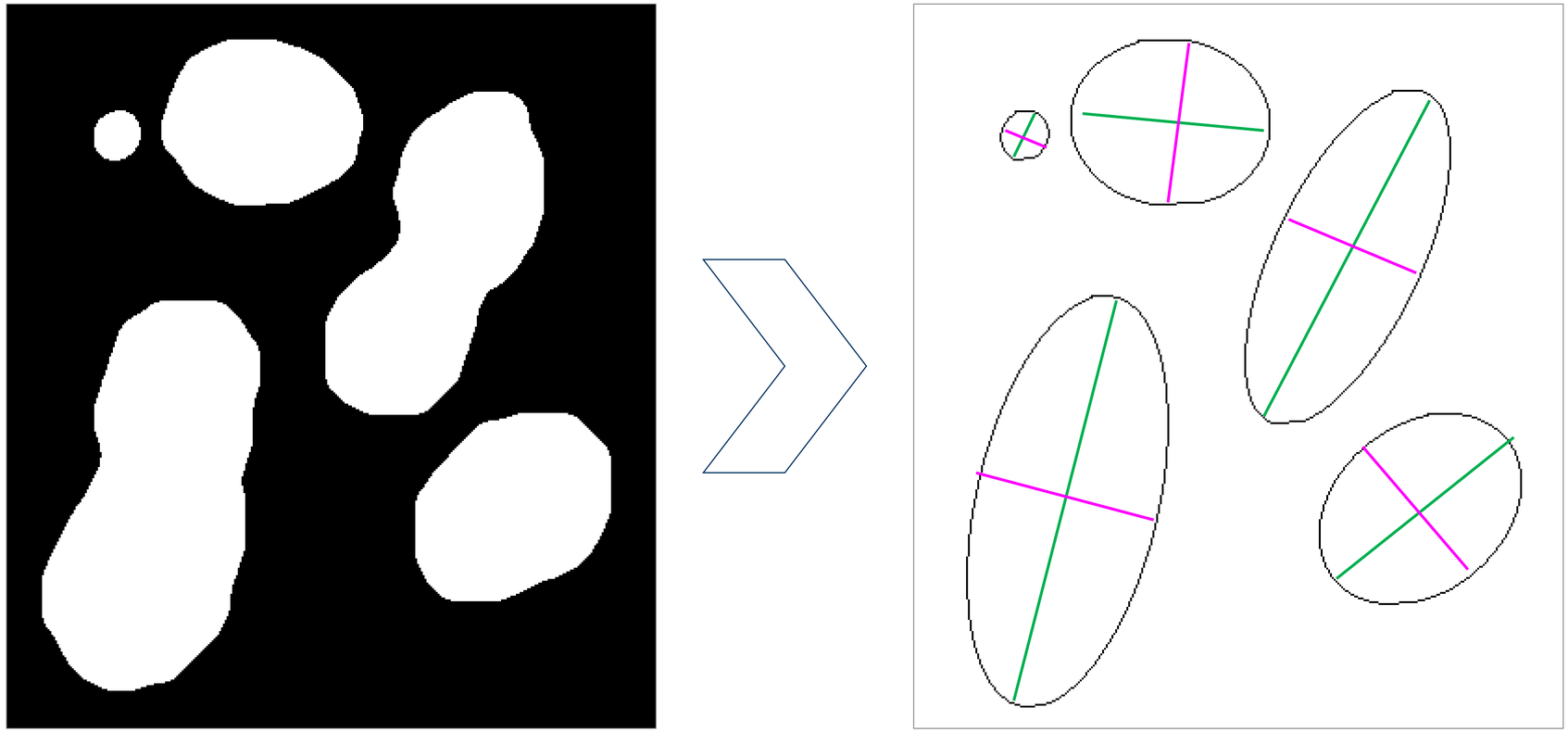
Fit ellipse

For every object, find the optimal ellipse simplifying the object.

Major axis ... long diameter

Minor axis ... short diameter

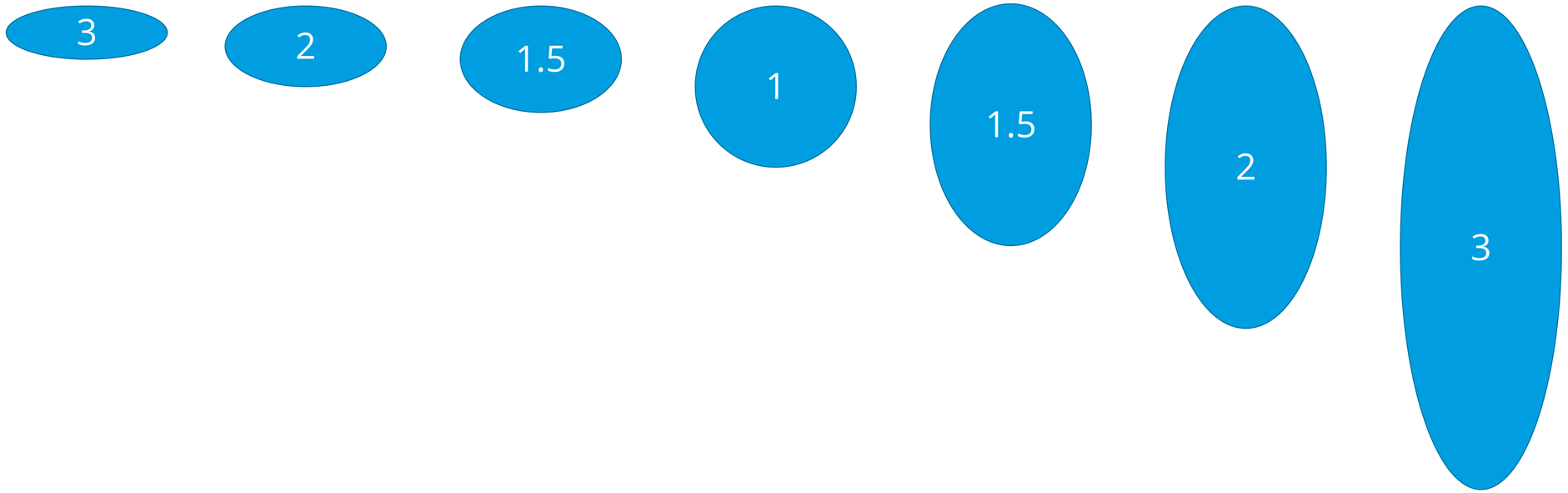
Major and minor axis are perpendicular to each other



Aspect ratio

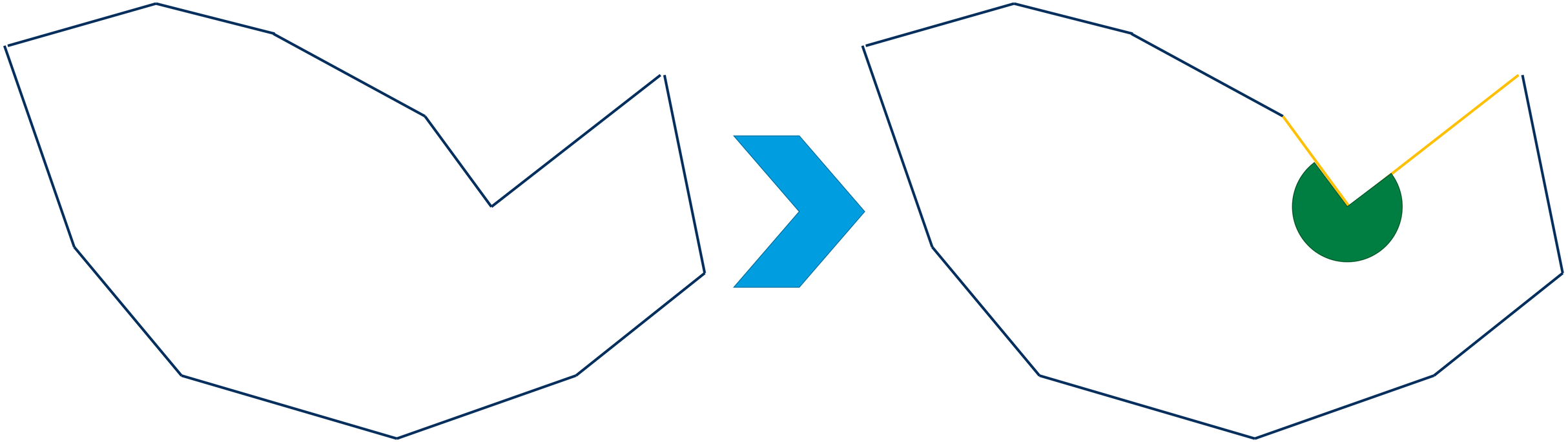
The aspect ratio describes the elongation of an object.

AR = major / minor



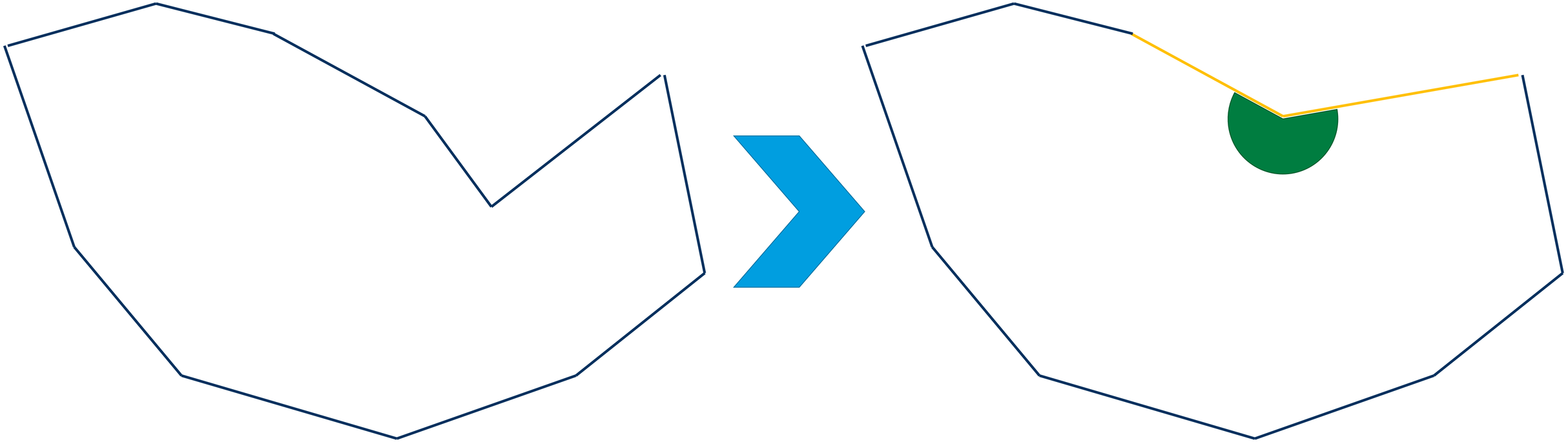
Convex hull

By removing all concave corners of an object, we retrieve its convex hull.



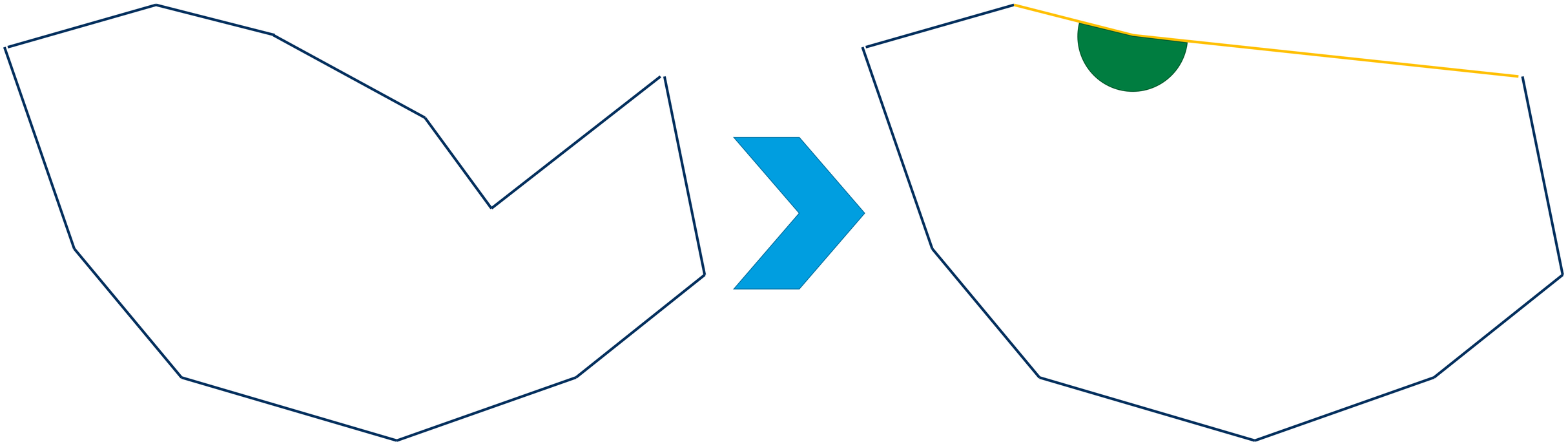
Convex hull

By removing all concave corners of an object, we retrieve its convex hull.



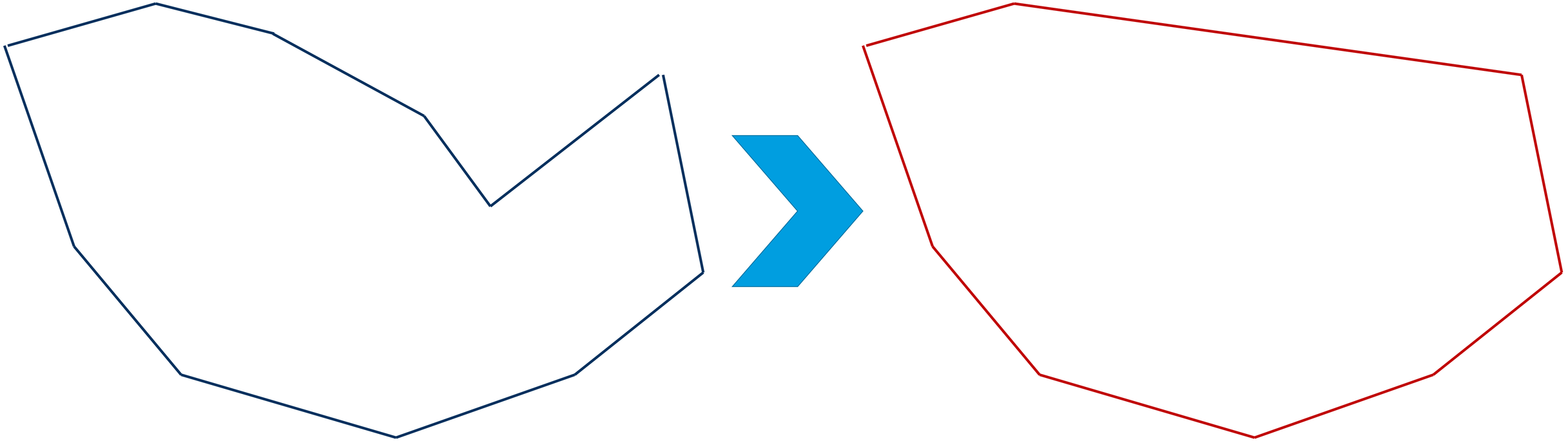
Convex hull

By removing all concave corners of an object, we retrieve its convex hull.



Convex hull

By removing all concave corners of an object, we retrieve its convex hull.



$$solidity = \frac{A}{A_{convexHull}}$$

Roundness and circularity

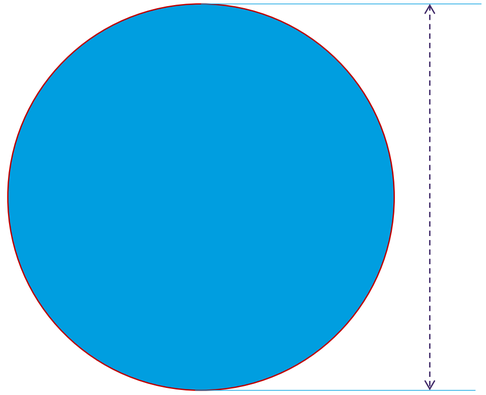
The definition of a circle leads us to measurements of circularity and roundness.

In case you use these measures, define them correctly. They are not standardized!

Diameter d

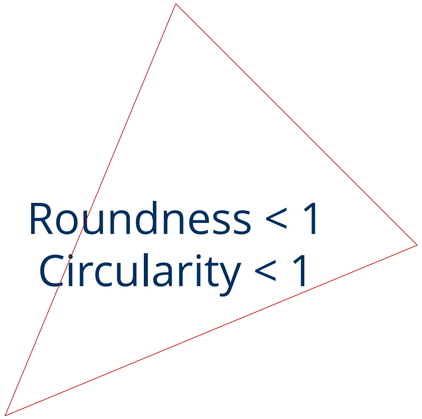
Circumference $C = \pi d$

Area $A = \frac{\pi d^2}{4}$

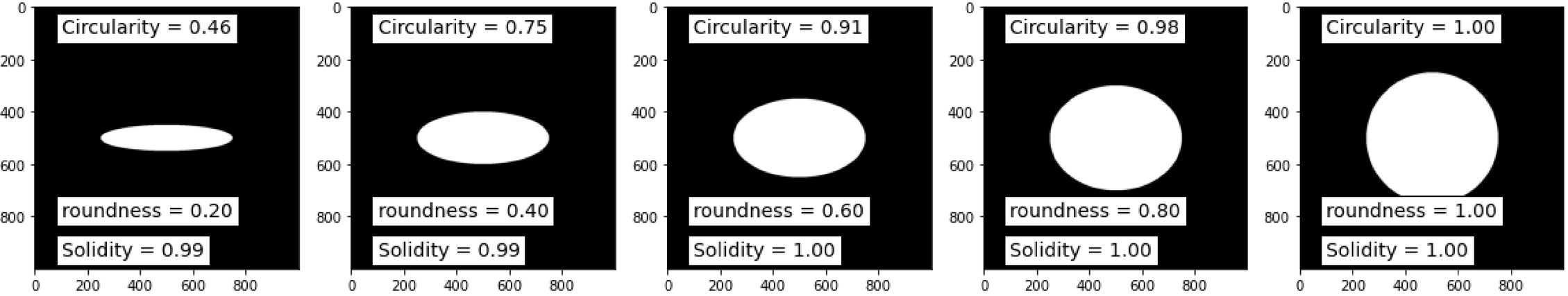


$$roundness = \frac{4 * A}{\pi\ major^2}$$

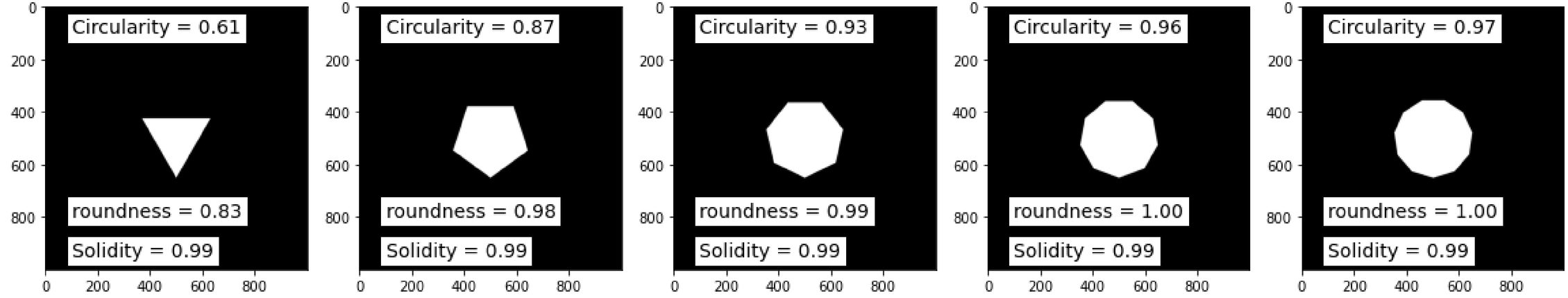
$$circularity = \frac{4\pi * A}{perimeter^2}$$



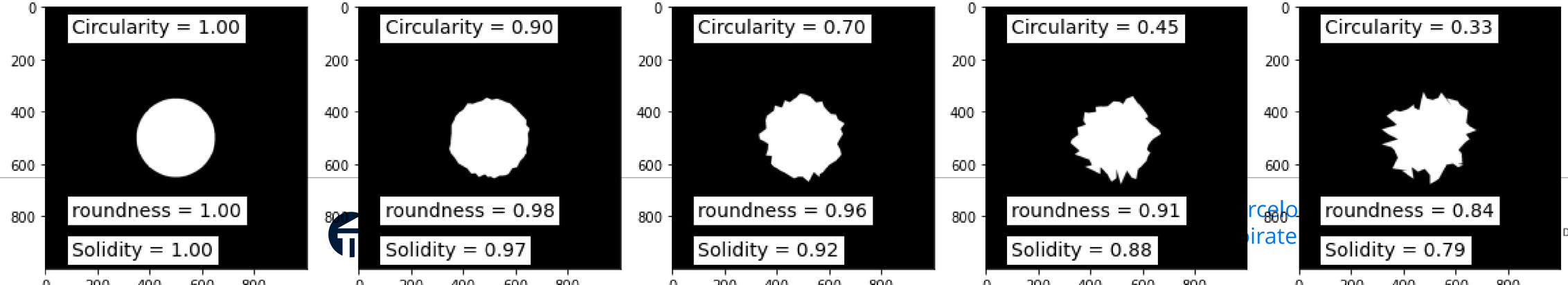
Roundness and circularity



$$roundness = \frac{4 * A}{\pi\, major^2}$$



$$circularity = \frac{4\pi * A}{perimeter^2}$$



$$solidity = \frac{A}{A_{convexHull}}$$

Feature extraction in Python

In Fiji: *Analyze > Analyze Particles…*

In Python:

```
from skimage import measure
```

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

`skimage.measure.blur_effect` (image[, h_size, …])	Compute a metric that indicates the strength of blur in an image (0 for no blur, 1 for maximal blur).
`skimage.measure.euler_number` (image[, …])	Calculate the Euler characteristic in binary image.
`skimage.measure.find_contours` (image[, …])	Find iso-valued contours in a 2D array for a given level value.
`skimage.measure.grid_points_in_poly` (shape, verts)	Test whether points on a specified grid are inside a polygon.
`skimage.measure.inertia_tensor` (image[, mu])	Compute the inertia tensor of the input image.
`skimage.measure.inertia_tensor_eigvals` (image)	Compute the eigenvalues of the inertia tensor of the image.
`skimage.measure.label` (label_image[, …])	Label connected regions of an integer array.
`skimage.measure.regionprops` (label_image[, …])	Measure properties of labeled image regions.
`skimage.measure.regionprops_table` (label_image)	Compute image properties and return them as a pandas-compatible table.

area : int

Number of pixels of the region.

area_bbox : int

Number of pixels of bounding box.

area_convex : int

Number of pixels of convex hull image, which is the smallest convex polygon that e

area_filled : int

Number of pixels of the region will all the holes filled in. Describes the area of the i

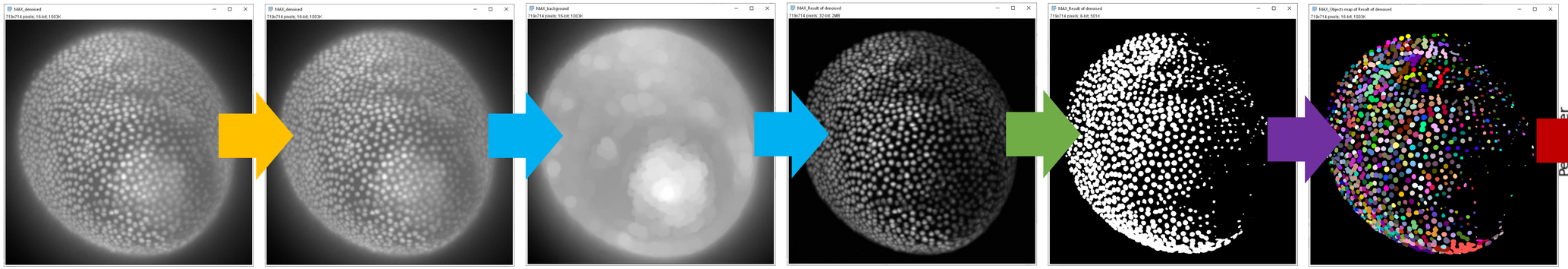
axis_major_length : float

The length of the major axis of the ellipse that has the same normalized second ce the region.

axis_minor_length : float

The length of the minor axis of the ellipse that has the same normalized second ce the region.

Summary




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

```
filtered = filters.gaussian(image, sigma=5)
```

Filtering the image reduces pixel noise

```
bg_subtracted = morphology.white_tophat(image, footprint=footprint)
```

Top-hat filtering removes the background

Thresholding binarizes the image

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

Connected-components analysis groups pixels to objects

```
labels = measure.label(binary)
```

Feature extraction allows descriptive statistics

```
measurements = measure.regionprops_table(labels, properties=properties)
```

Image data source: Daniela Vorkel, Myers lab, MPI CBG