

Bio-Image Analysis, Programming, Biostatistics and Machine Learning for Computational Biology

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Programming

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- What is the output of these programs?

```
# we start with an empty list  
numbers = []  
  
# and add elements  
for i in range(0, 5):  
    numbers.append(i * 2)  
  
print(numbers)
```

```
numbers = [i * 2 for i in range(0, 5)]  
print(numbers)
```

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

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

- What do these programs output?

Starting at
(including)

Ending at
(excluding)

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

Step size

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

- What would be good comments in this code?

```
▶ #  
from skimage.io import imread  
blobs = imread("blobs.tif")  
  
#  
from skimage.filters import threshold_otsu  
threshold = threshold_otsu(blobs)  
binary_blobs = blobs > threshold  
  
#  
from skimage.measure import label  
labeled_blobs = label(binary_blobs)  
  
#  
import matplotlib.pyplot as plt  
fig, axs = plt.subplots(1, 3, figsize=(15,15))  
  
axs[0].imshow(blobs)  
axs[1].imshow(binary_blobs)  
axs[2].imshow(labeled_blobs, cmap=label_cmap)
```

Bio-image Analysis

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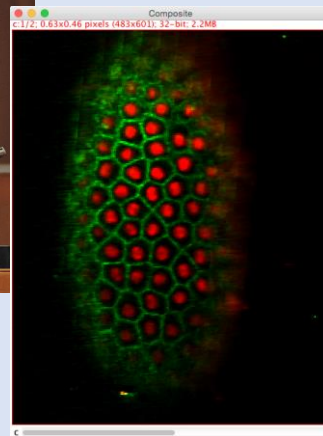
Image analysis is part of the experiment



Observation

$$p_{ij}(t) = \frac{[\tau_{ij}(t)]^\alpha \cdot [\eta_{ij}]^\beta}{\sum_{j=1}^n [\tau_{ij}(t)]^\alpha \cdot [\eta_{ij}]^\beta}$$

Modeling



Imaging

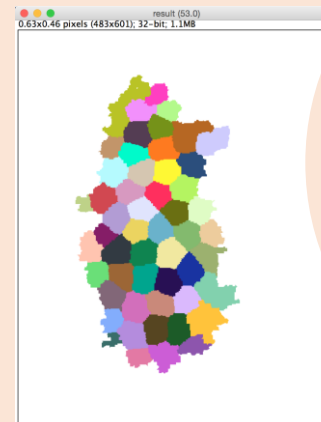


Image processing

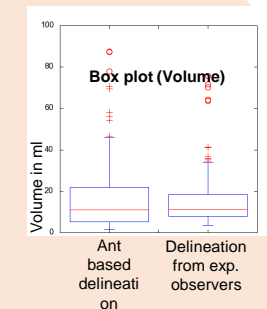
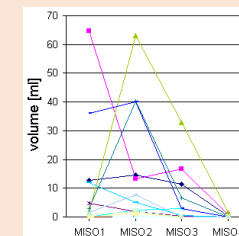
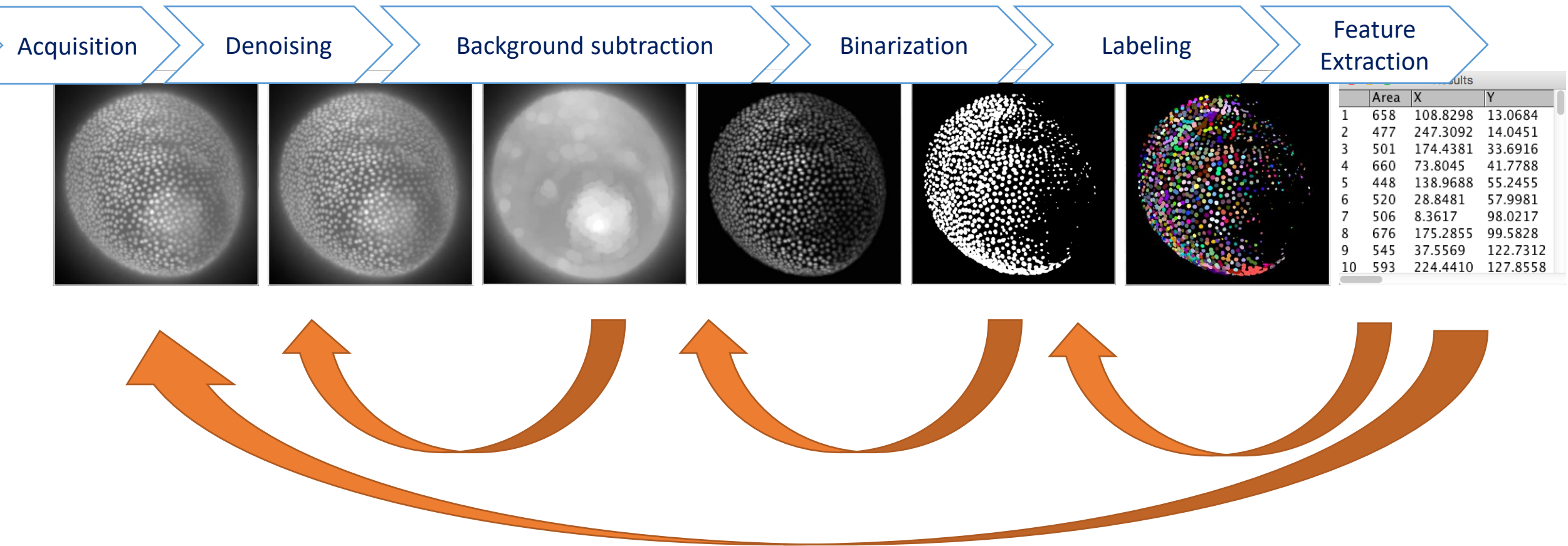


Image analysis
Bio-statistics

Image analysis is part of the experiment

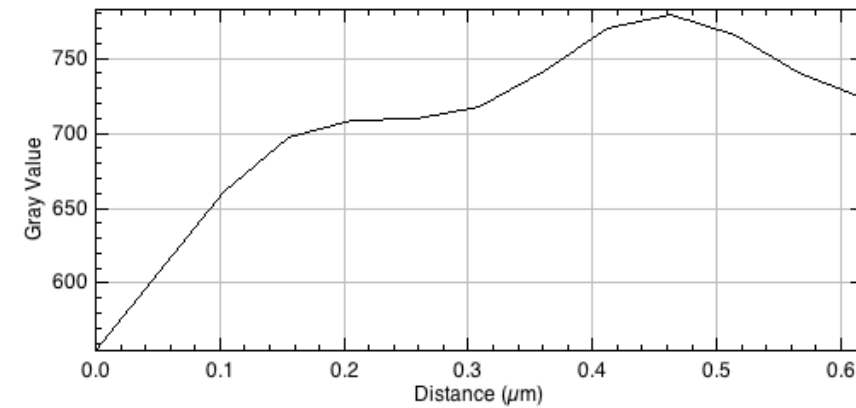
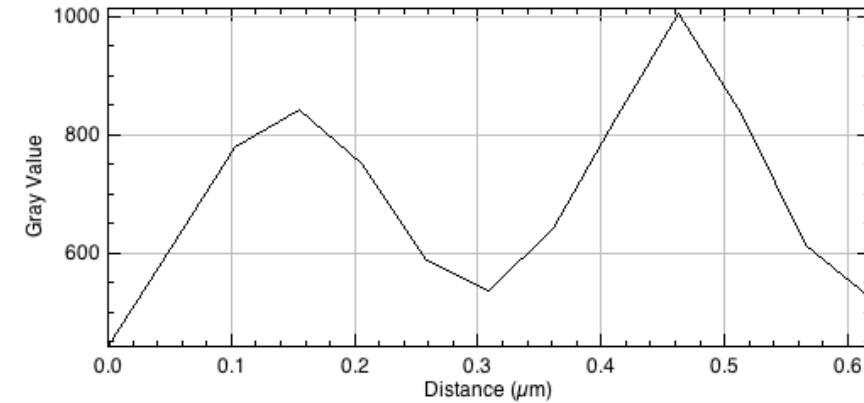
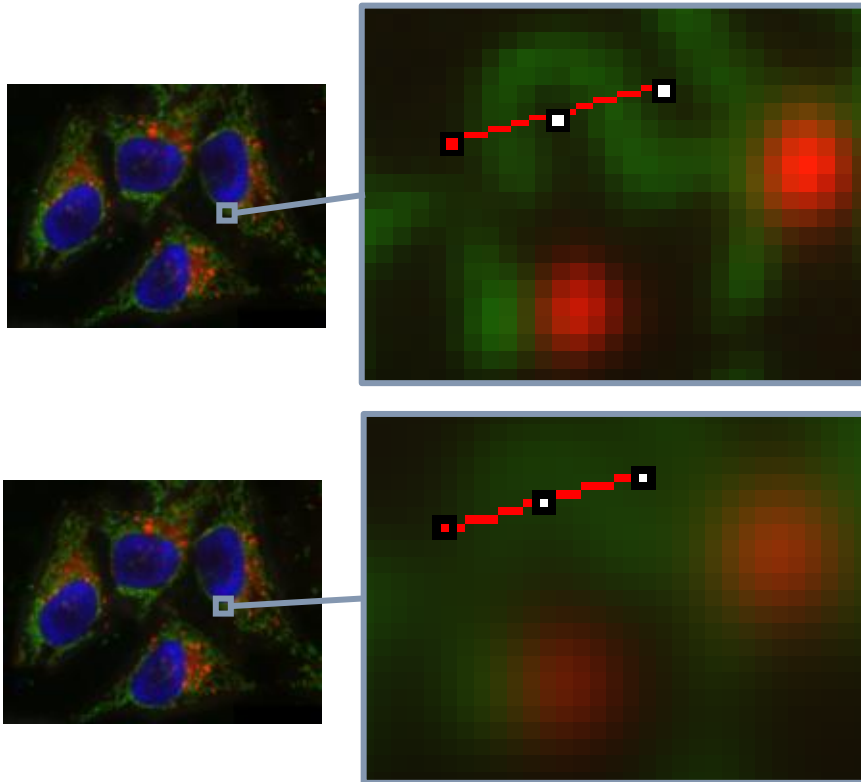
- Going back allows us to improve
- We're not "failing"



- Bio-image analysis is supposed to be
 - **Quantitative**
 - We derive numbers from images which describe physical properties of the observed sample.
 - **Objective**
 - The derived measurement does not depend on who did the measurement. The measurement is free of interpretation.
 - **Reliable / valid / trustworthy**
 - We are confident that the measurement is describing what it is supposed to describe.
 - **Reproducible**
 - Somebody else can do the experiment under *different conditions* and gets similar measurements. For this, documentation is decisive!
 - **Repeatable**
 - We can do the same experiment twice under the *same conditions* and get a similar measurements.

Pixel size versus resolution

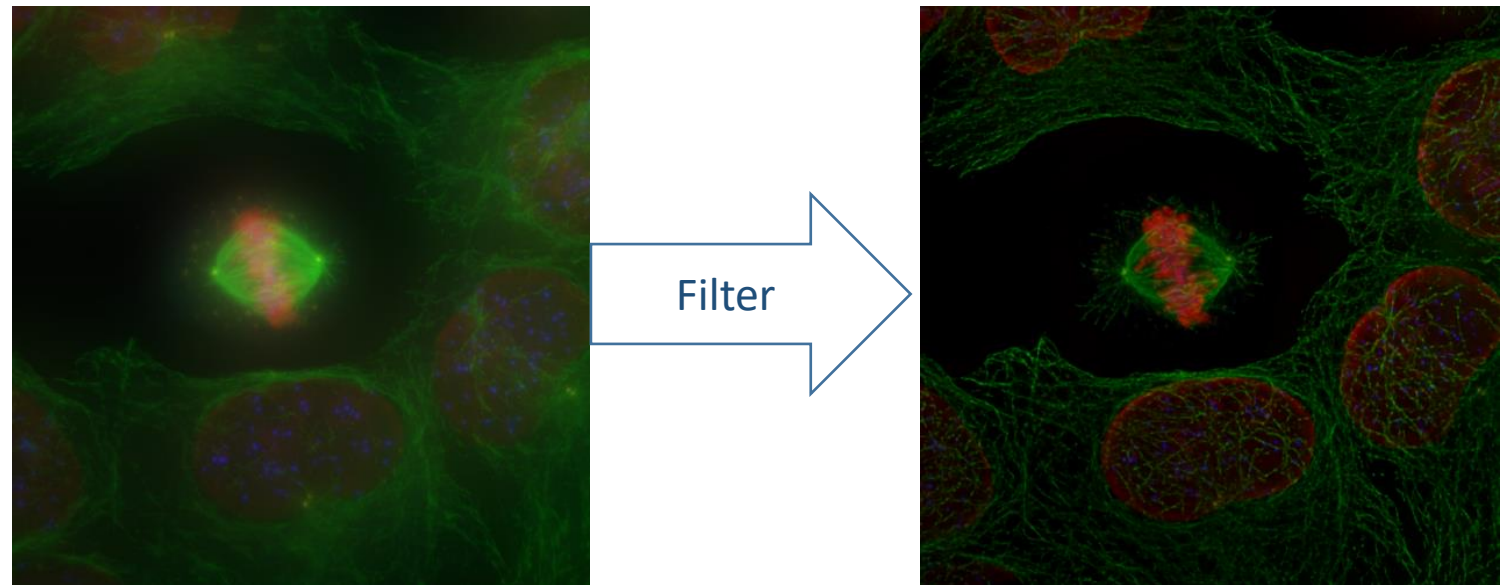
- How is the resolution of an imaging system defined?
- How is the pixel size of an image defined?



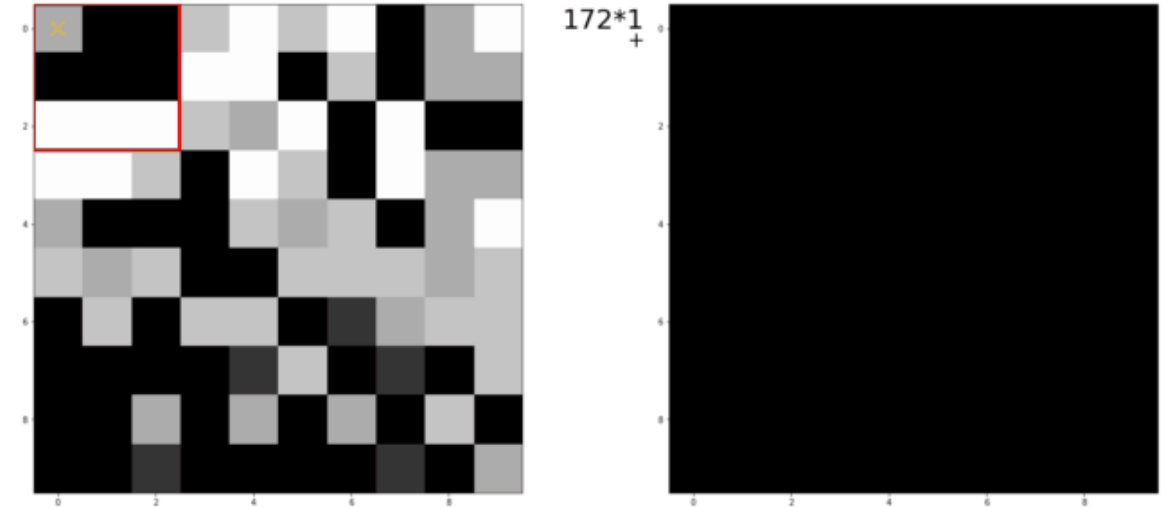
Filters don't do magic

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- 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
 - Background removal
 - Artefact-removal
 - Contrast enhancement
 - Correct uneven illumination



- What is an image processing filter?
- What is a filter kernel?
- How does convolution work?

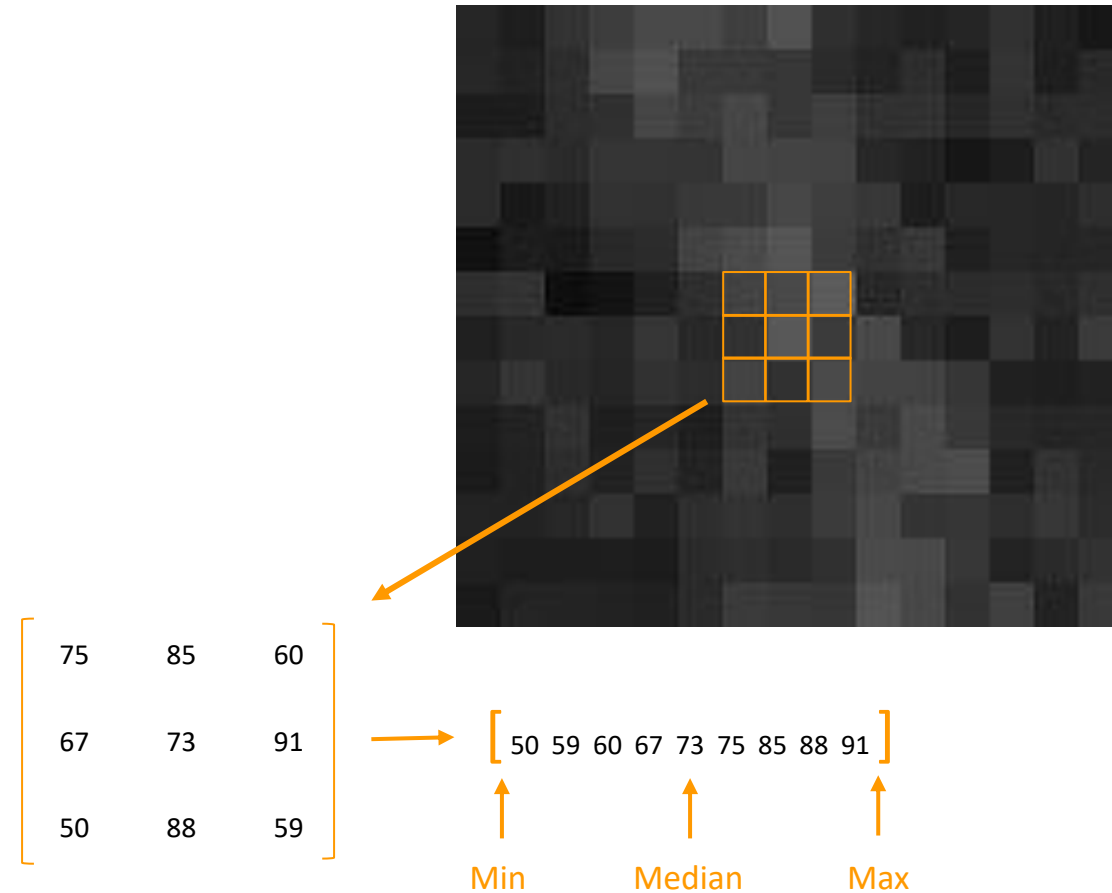


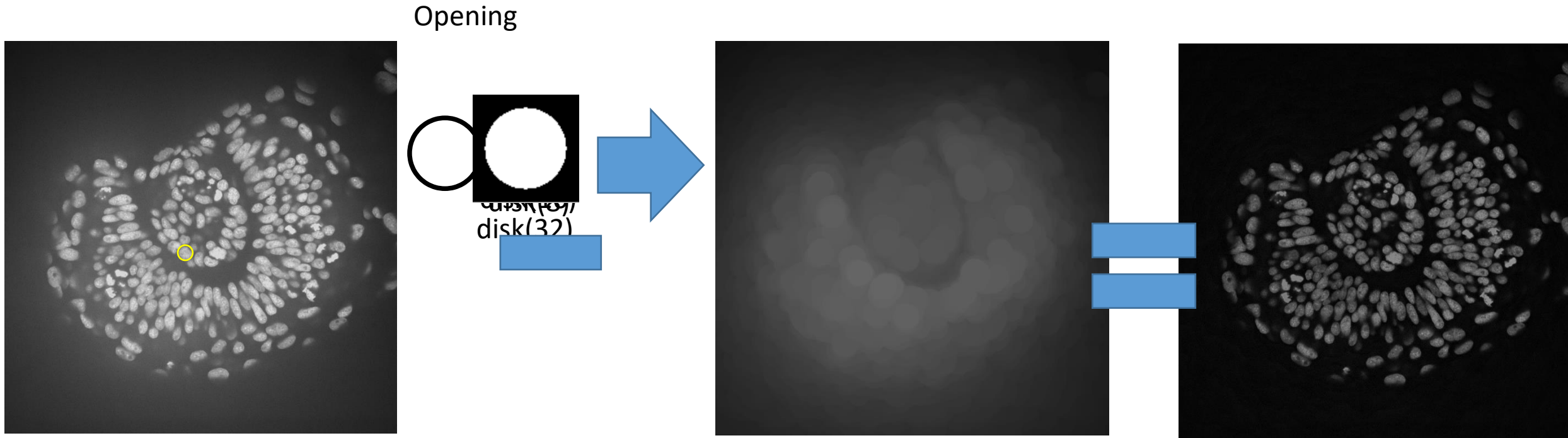
Mean filter

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

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

- What differentiates linear and non-linear filters?





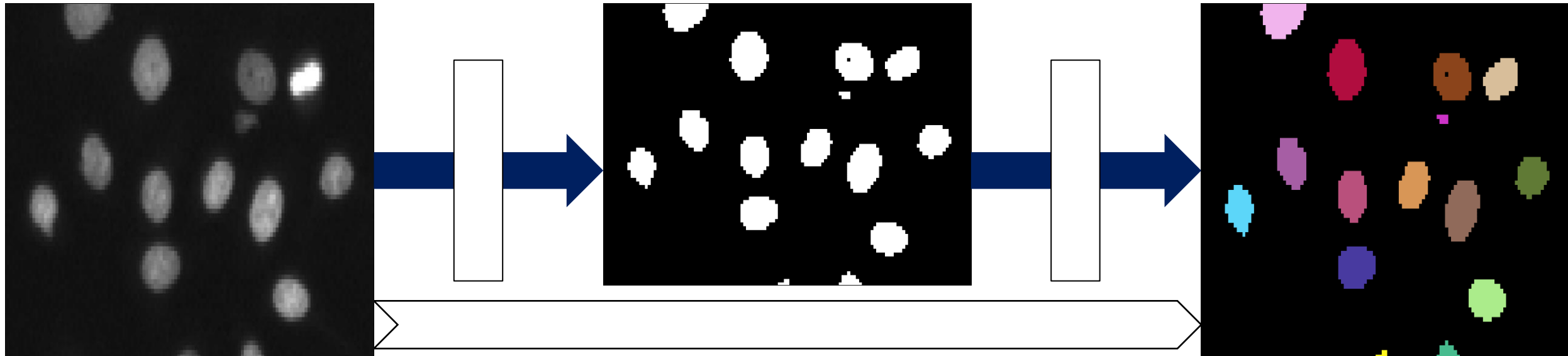
Structures have a radius ≈ 12

You have just learned the white tophat filter!

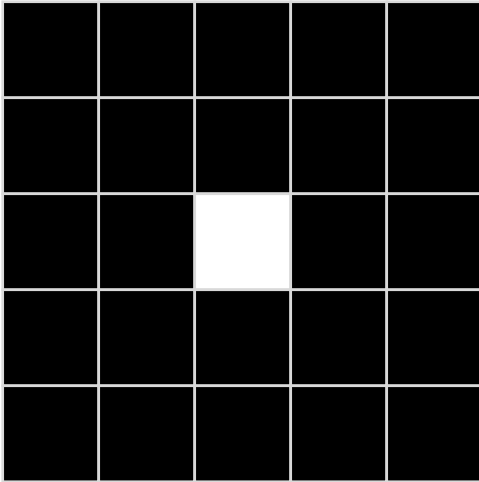
Image Segmentation

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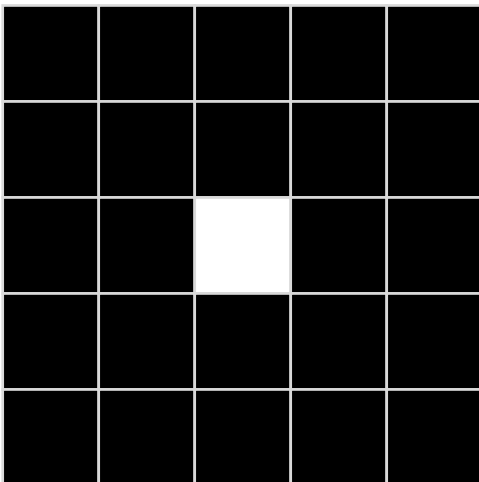
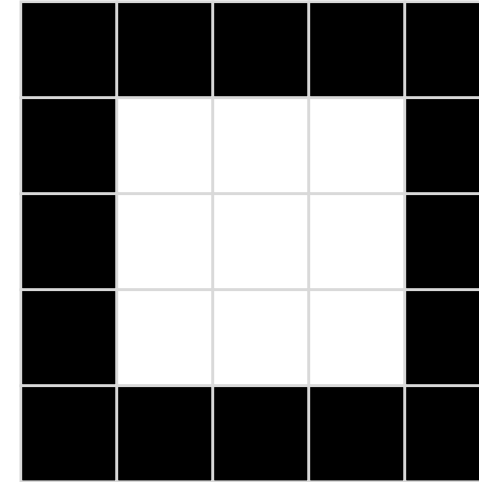
What does “instance segmentation” mean?



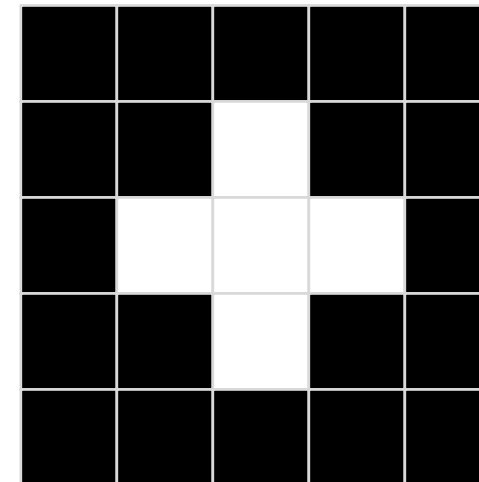
- Dilation: Every pixel with at least one white neighbor becomes white.



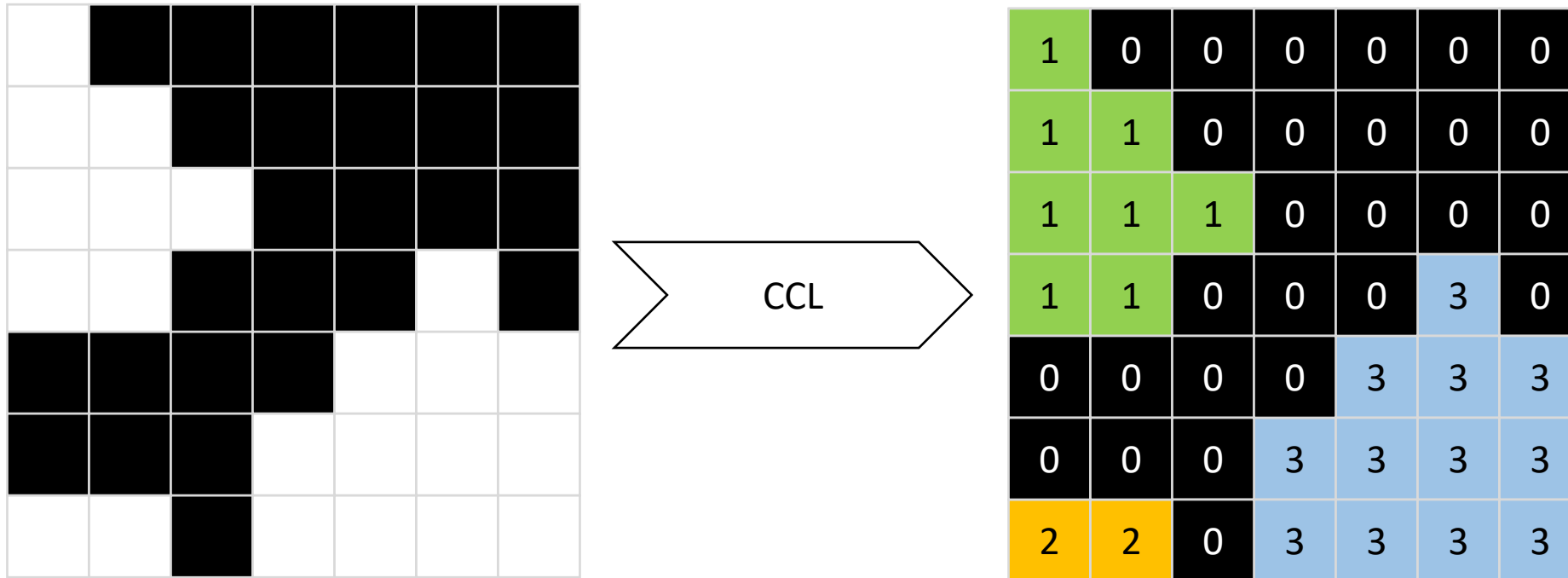
8-connected neighborhood
Moore-Neighborhood



4-connected neighborhood
von-Neumann-Neighborhood



- 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.



Add multiple images as layers

- 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]
```

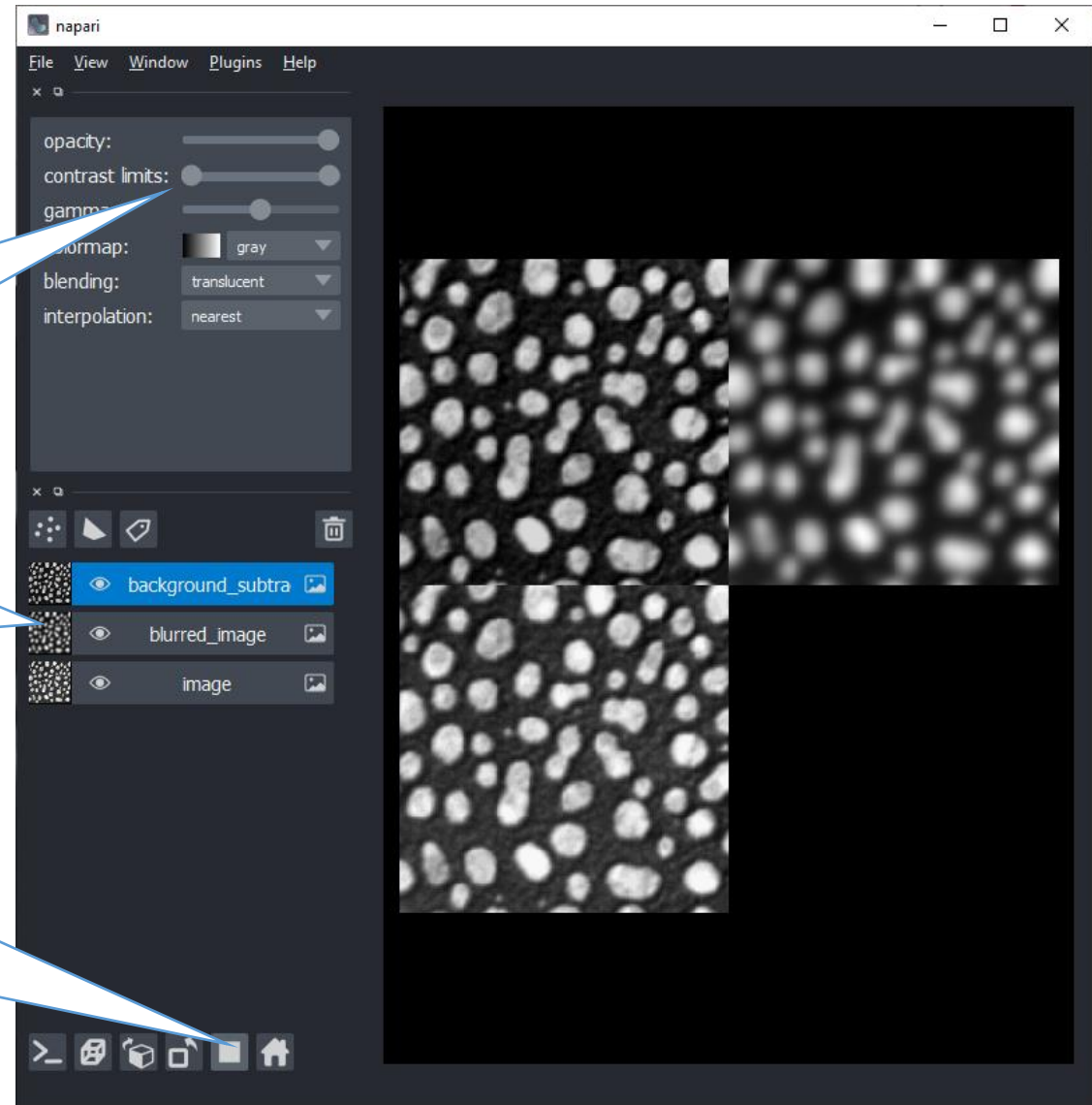
1. Access the viewer
2. Access the layers
3. Choose a layer (by index or name)
4. "Press TAB" and check out available properties

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

Change
Brightness
and contrast
here

Toggle
visualization
of layers
here

Gallery view



Find more image layer attributes here:

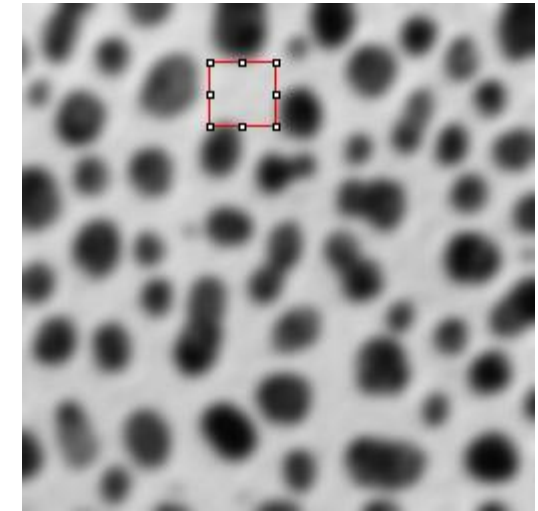
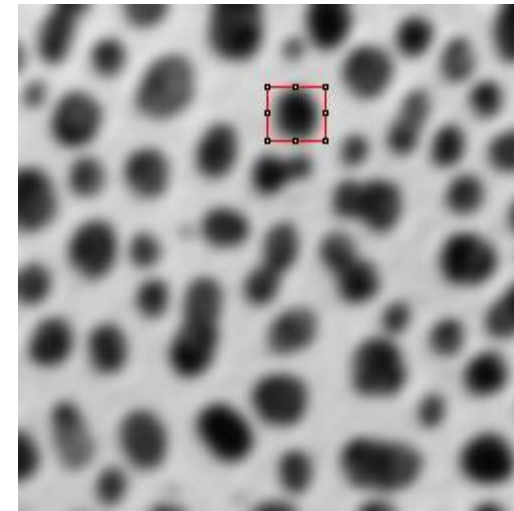
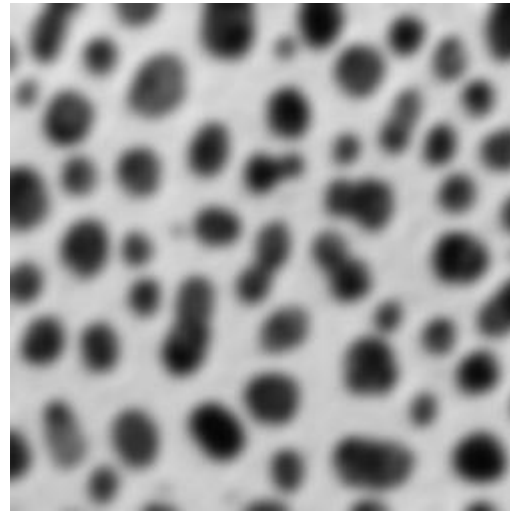
<https://napari.org/api/napari.layers.Image.html#napari.layers.Image>

Feature extraction

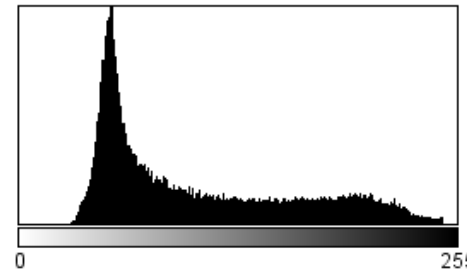
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- A *feature* is a countable or measurable property of an image or object.
- Goal of feature extraction is finding a minimal set of features to describe an object well enough to differentiate it from other objects.
- Intensity based features
 - Mean intensity
 - Standard deviation
 - Total intensity
 - Textures
 - ...
- Shape based / spatial features
 - Area / Volume
 - Roundness
 - Solidity
 - Circularity / Sphericity
 - Elongation
 - Centroid
 - Bounding box
 - ...
- Spatio-temporal features
 - Displacement,
 - Speed,
 - Acceleration,
 - ...
- Others
 - Overlap
 - Colocalisation
 - Network-analysis
 - ...
- Mixed features
 - Center of mass
 - Local minima / maxima
 - ...

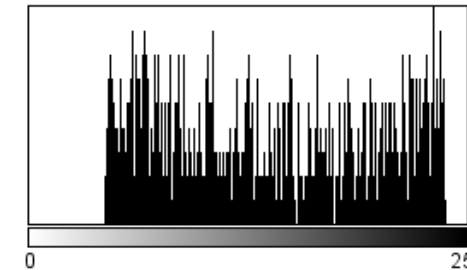
- Min / max
- Median
- Mean
- Mode
- Variance
- Standard deviation



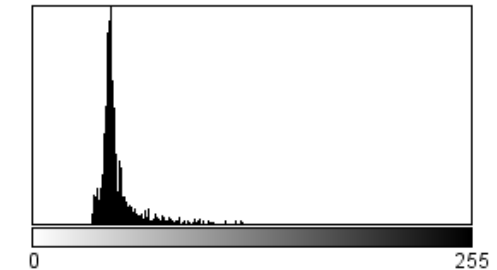
- Can be derived from pixel values
- Don't take spatial relationship of pixels into account
- See also:
 - descriptive statistics
 - histogram



Count: 65024 Min: 29
Mean: 103.301 Max: 248
StdDev: 57.991 Mode: 53 (1663)

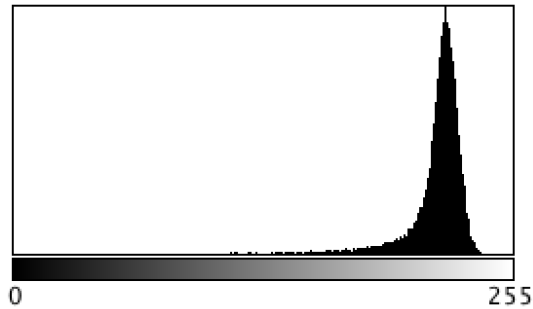


Count: 783 Min: 44
Mean: 141.308 Max: 243
StdDev: 61.876 Mode: 236 (9)

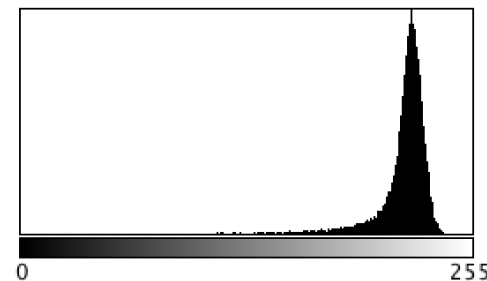
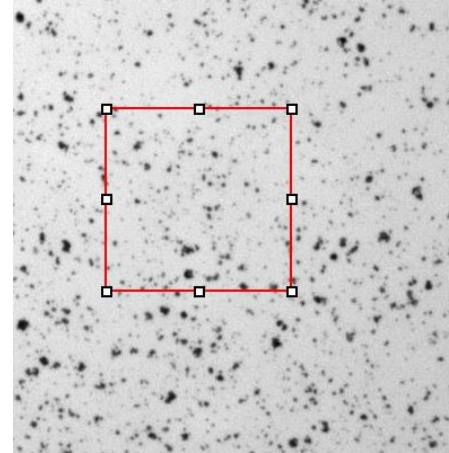


Count: 1056 Min: 34
Mean: 49.016 Max: 122
StdDev: 12.685 Mode: 45 (120)

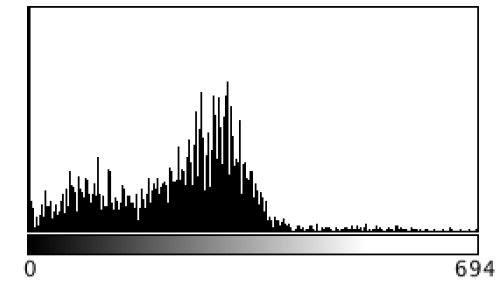
- To which of the two selections / images does this histogram belong to?



Count: 26568
Mean: 213.678
StdDev: 22.598
Min: 18
Max: 244
Mode: 221 (1552)

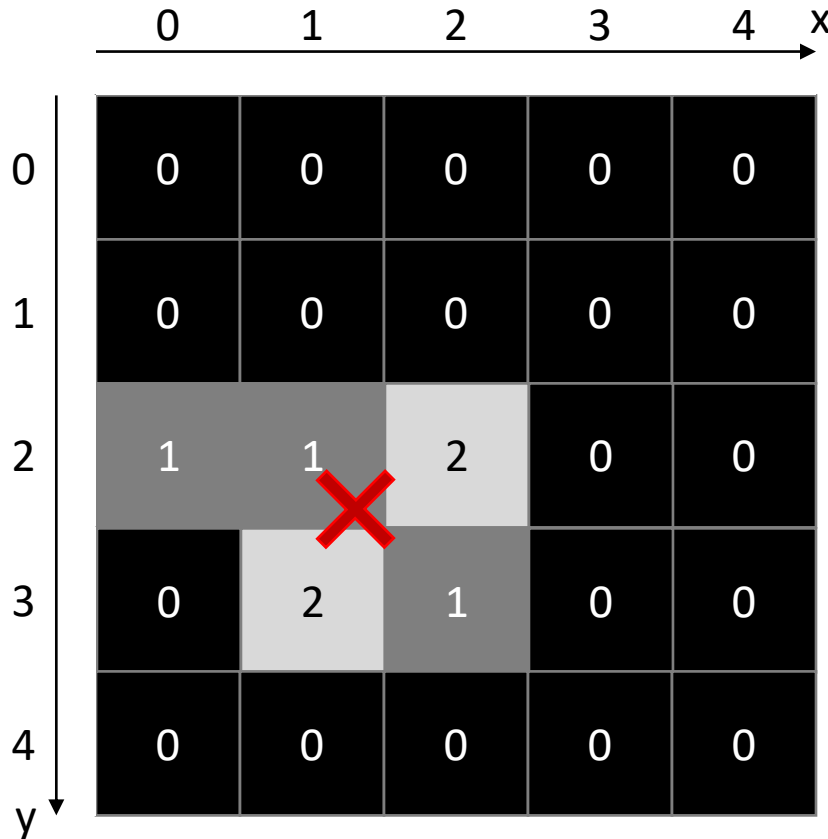


Count: 26568
Mean: 213.678
StdDev: 22.598
Min: 18
Max: 244
Mode: 221 (1552)

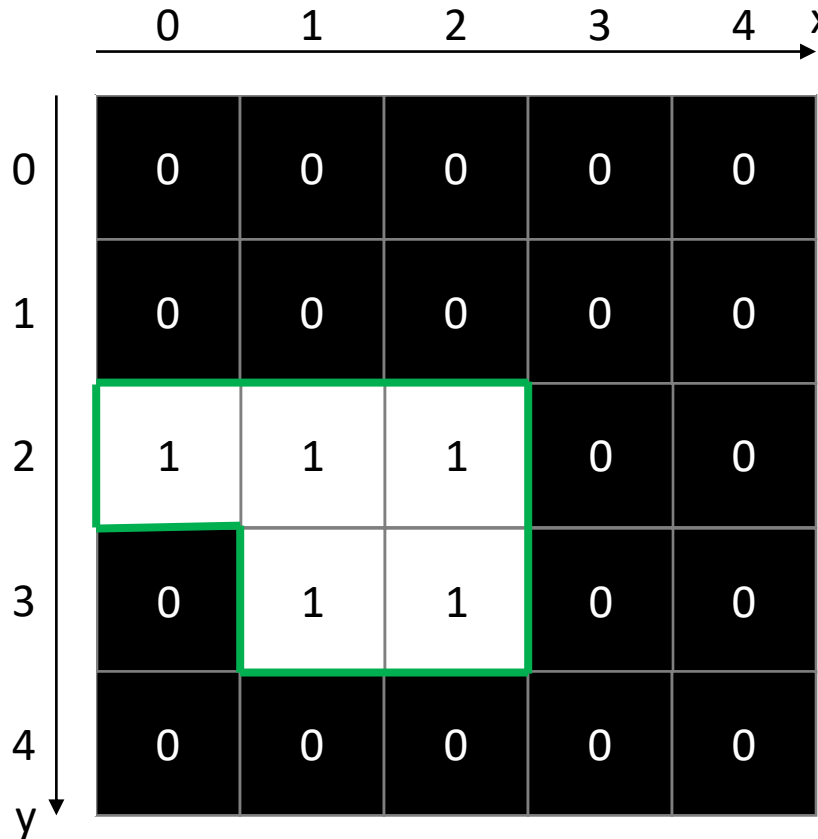


Count: 5846
Mean: 158.761
StdDev: 139.792
Bins: 256
Min: 0
Max: 694
Mode: 0 (1854)
Bin Width: 2.711

- Where and how large are given objects?



- Center of mass / centroid



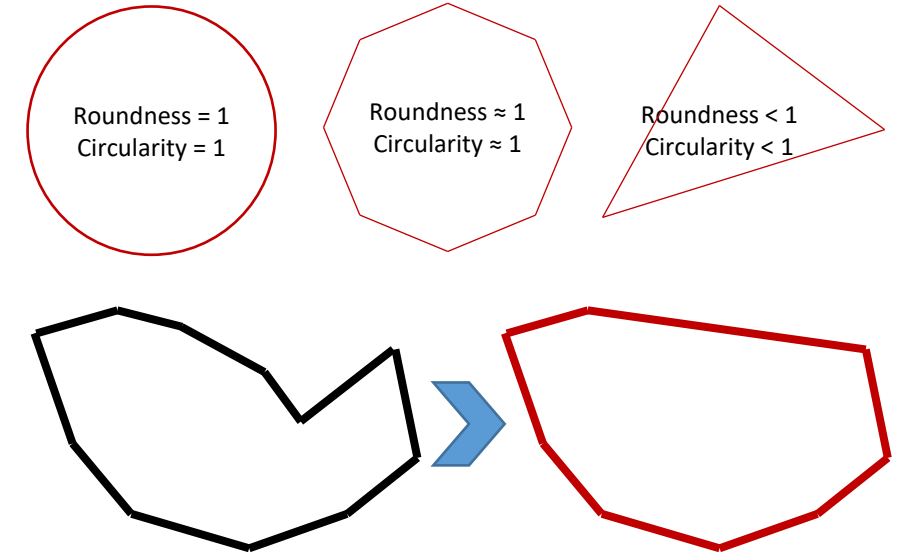
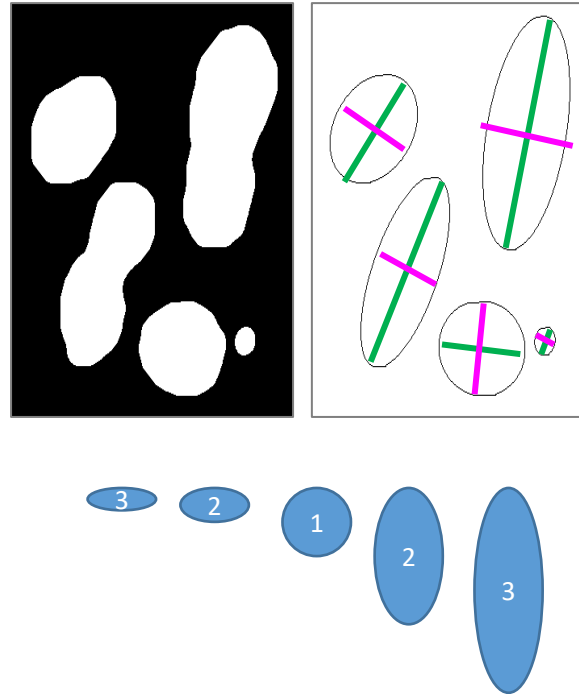
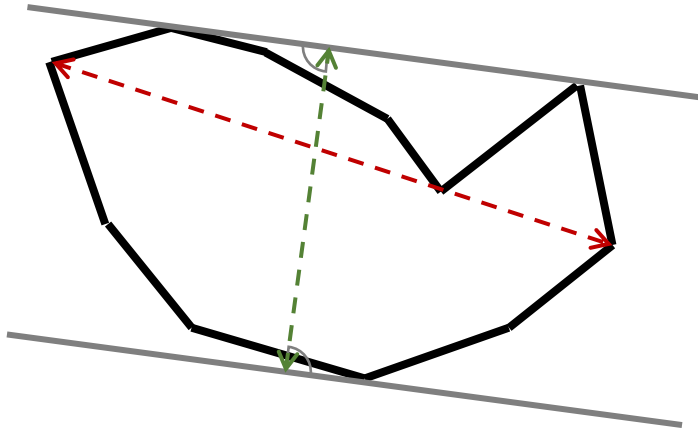
- Area, perimeter

variable	value
x_b	0
y_b	2
w_b	3
h_b	2

- Bounding box

If the center of mass is a mixed feature, why is not the centroid (it in shape based/spatial features) if it's the center of mass for binary pictures? Thank you :)

- How are objects shaped?

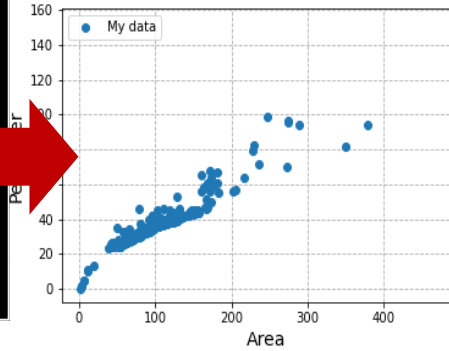
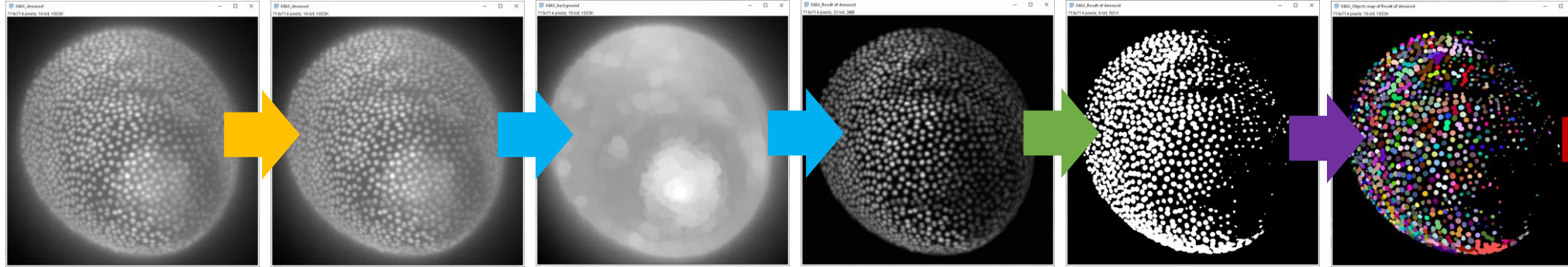


- **Feret's diameter**
- The **minimum caliper** ("Minimum Feret")

- Fit ellipse
 - Major axis ... long diameter
 - Minor axis ... short diameter
- Aspect ratio

- Roundness
- Circularity
- Solidity

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)
```

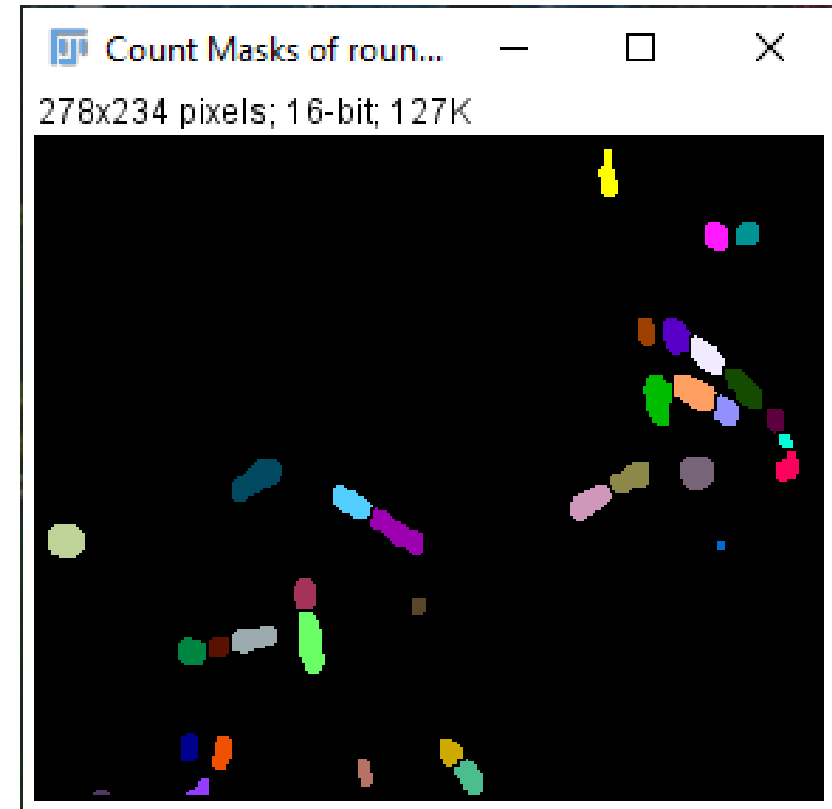
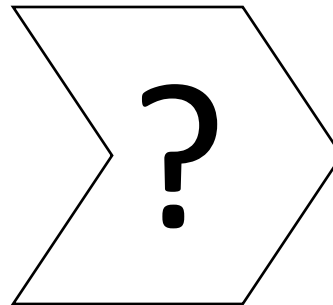
Connected-component labeling groups pixels to objects

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

Feature extraction allows descriptive statistics

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

- What's the name of the operation applied here?



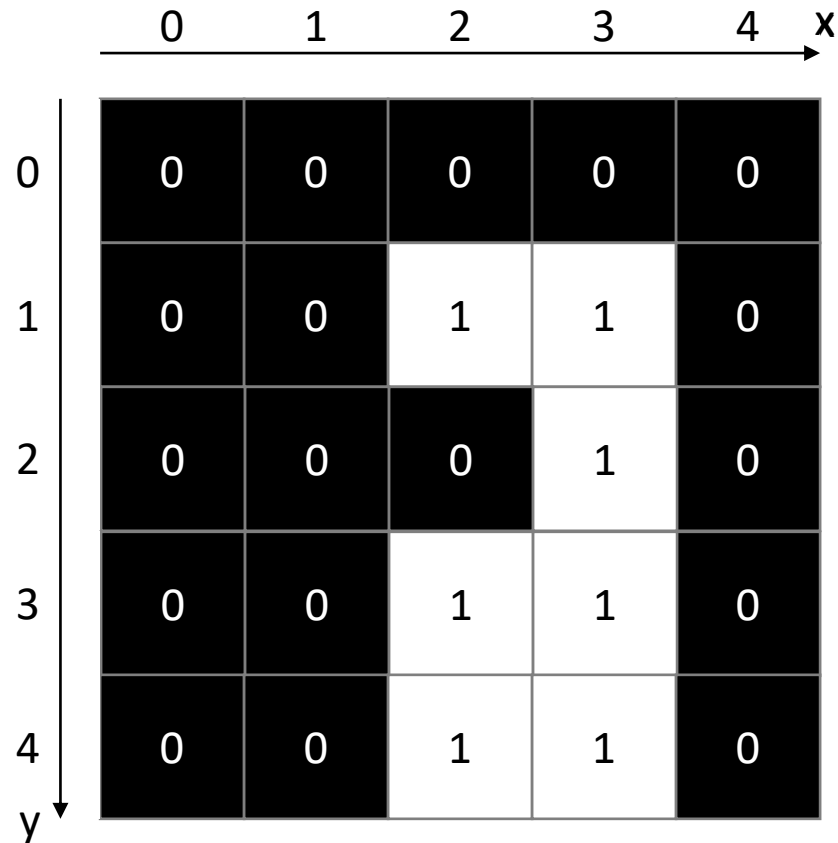
Thresholding

Binary closing

Connected components
analysis

Watershed

- What is the solidity of the white object in this image?
 - Hint: Area of the convex hull



0.875

1

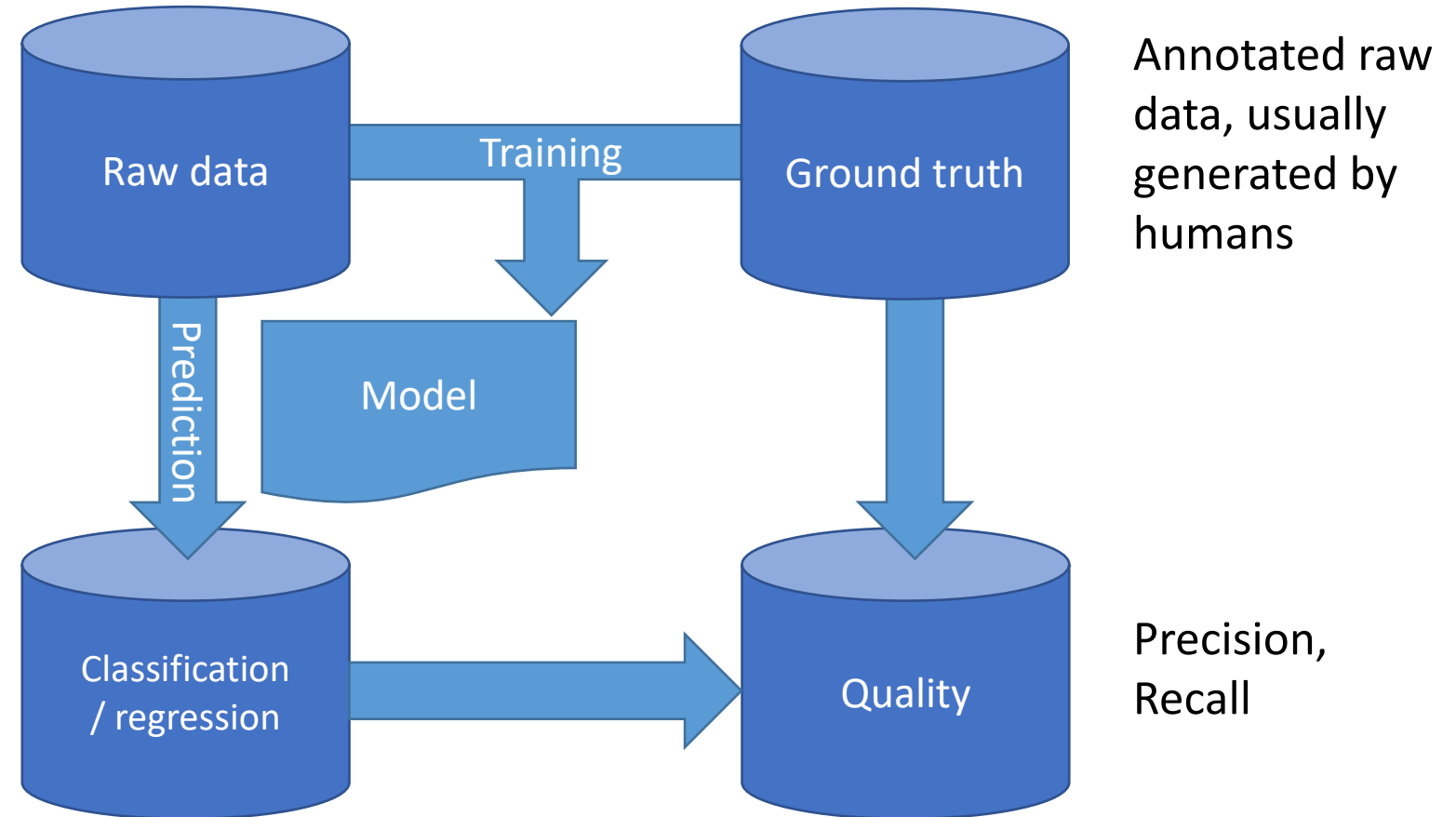
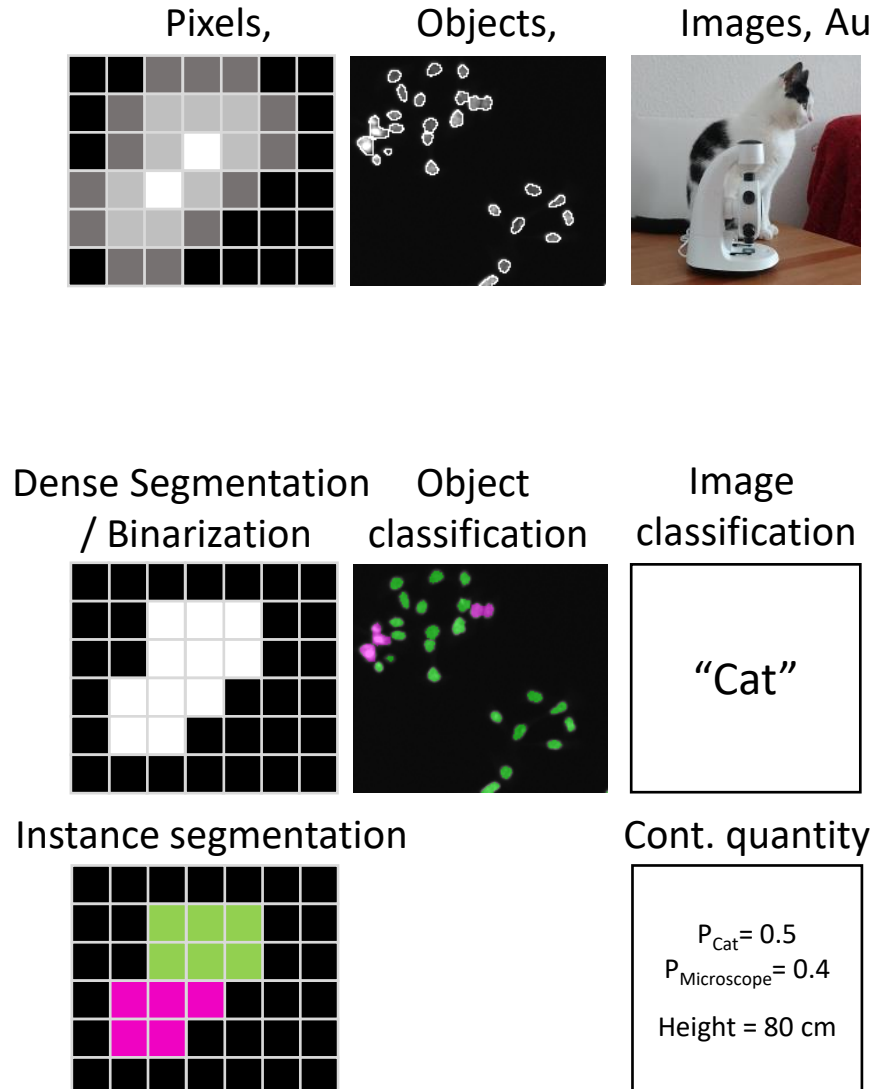
5

1.2

Machine learning

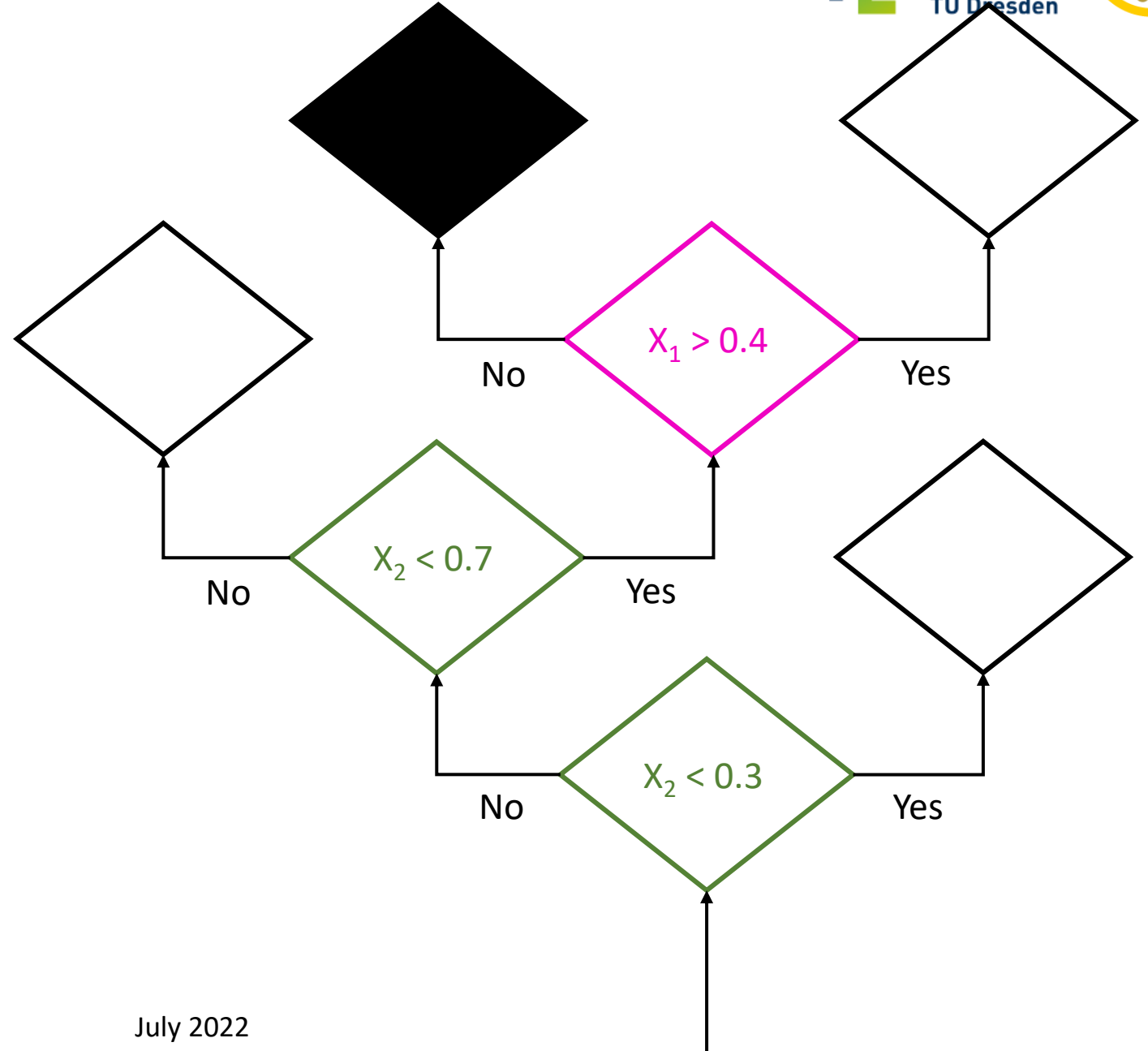
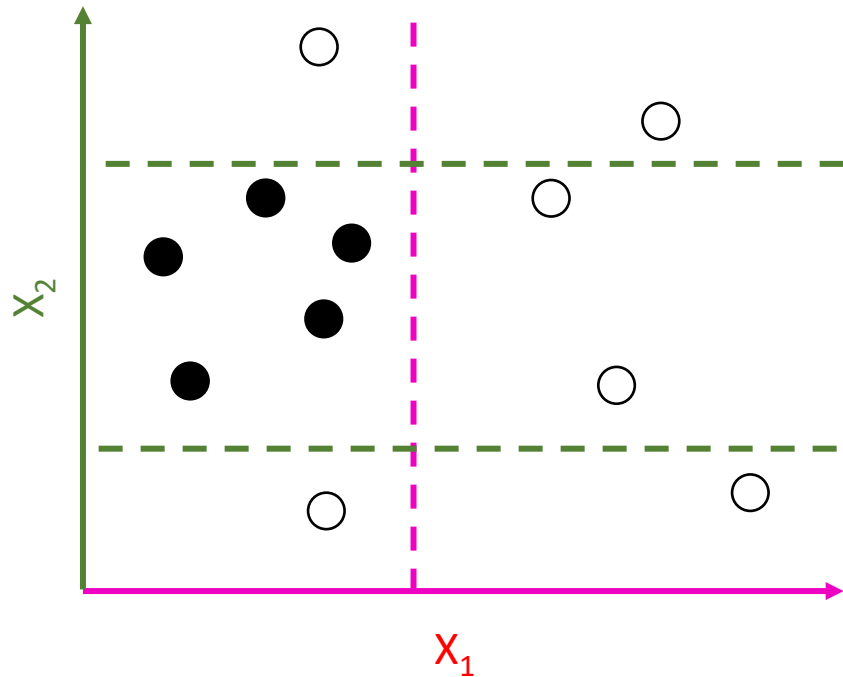
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- Automatic construction of predictive models from given data



Deriving random decision trees

- Depending on sampling, the decision trees are different



- In general
 - Define what's positive and what's negative.
 - Compare with a reference to figure out what was true and false

Overlap
(a.k.a. Jaccard index)

$$\frac{TP}{TP + FN + FP}$$

Precision

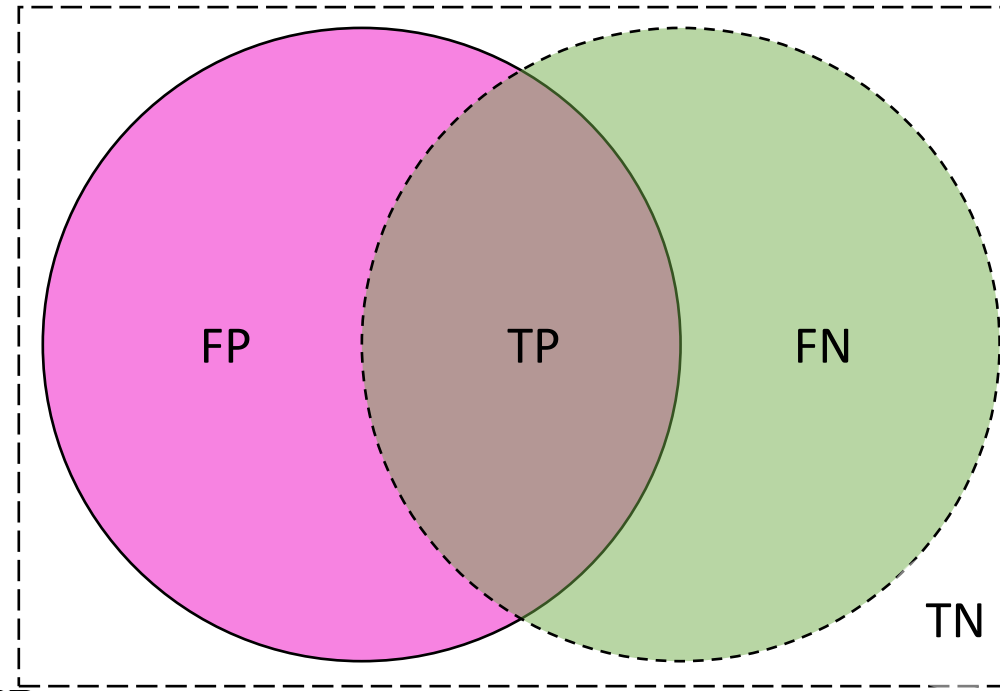
$$\frac{TP}{TP + FP}$$



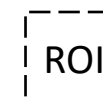



Recall
(a.k.a. sensitivity)

$$\frac{TP}{TP + FN}$$

What fraction of points that were predicted as positives were really positive?

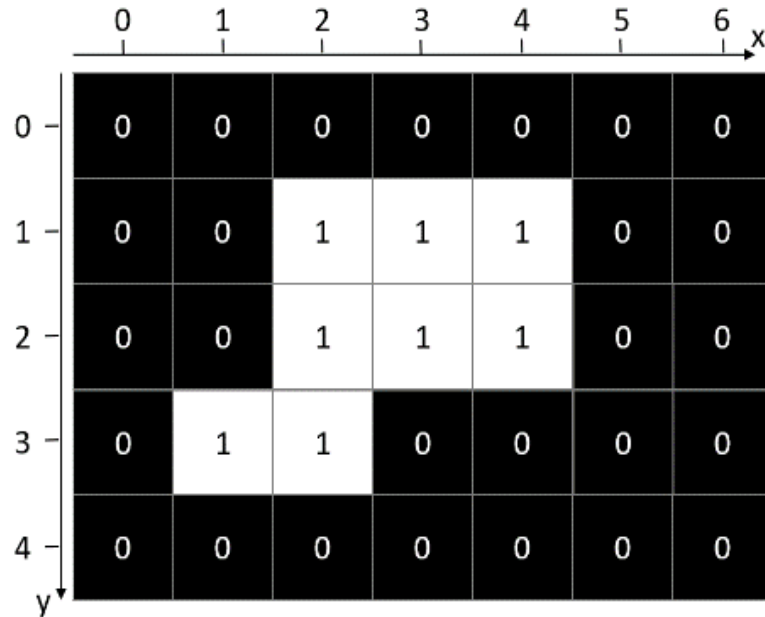
What fraction of positives points were predicted as positives?



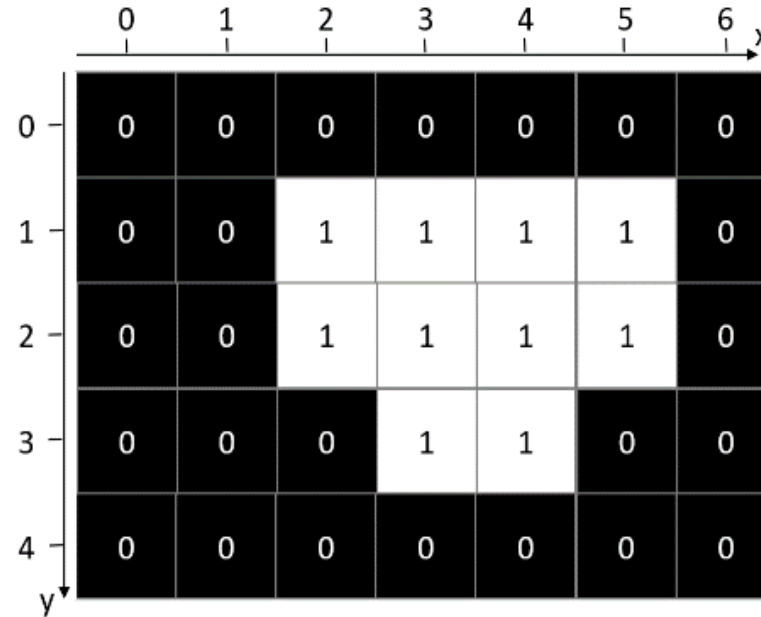
-  A Prediction A
-  B Reference B (ground truth)
-  ROI Region of interest
-  TP True-positive
-  FN False-negative
-  FP False-positive
- TN True-negative

- Assume you are evaluating a binary segmentation algorithm by comparing its result to a given ground

Segmentation result



Ground truth



Jaccard index

$$\frac{TP}{TP + FN + FP}$$

Precision

$$\frac{TP}{TP + FP}$$

Recall

$$\frac{TP}{TP + FN}$$

$$TP = 6$$

$$FP = 2$$

$$FN = 4$$

$$J = 6 / 12$$

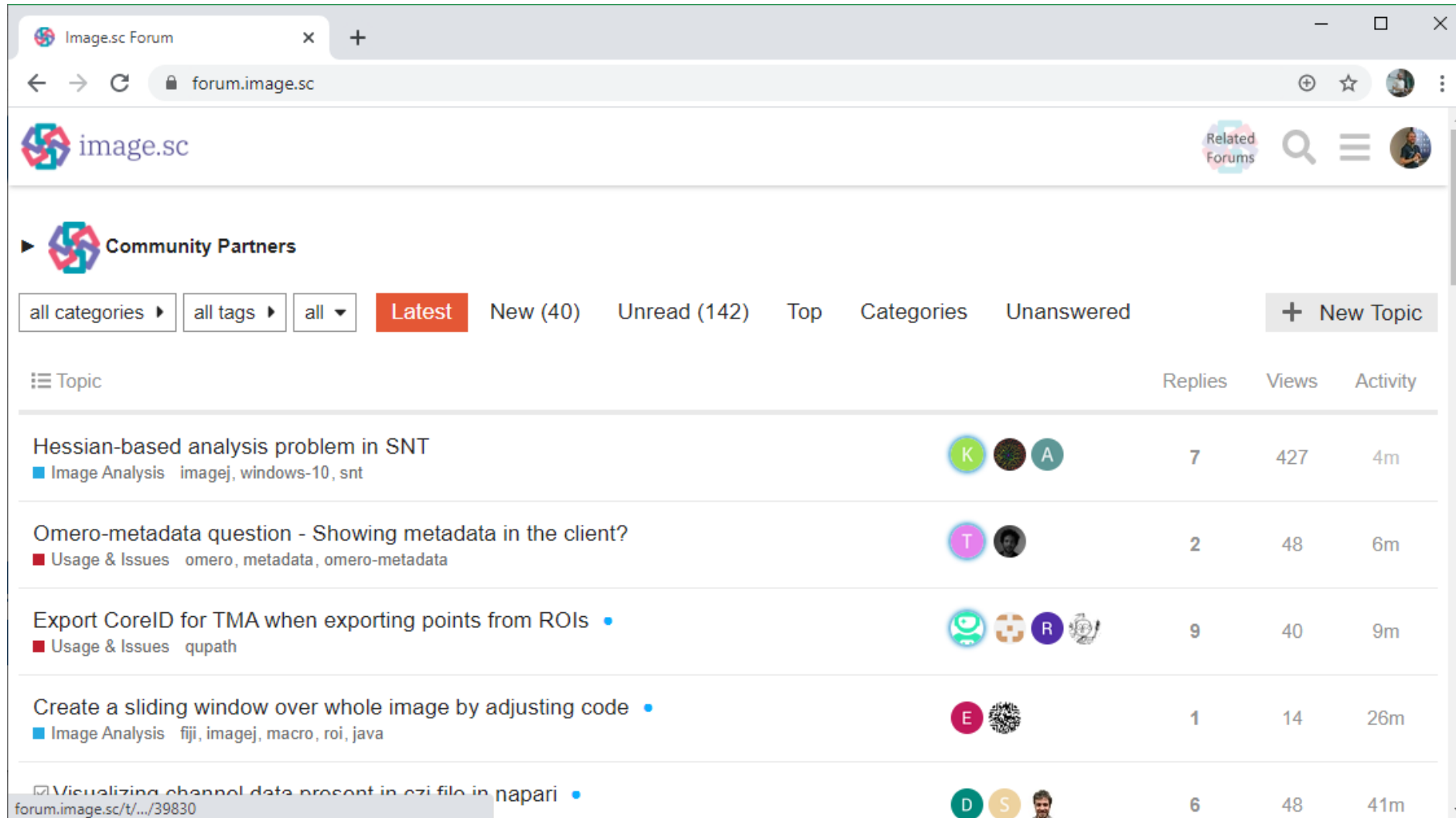
$$P = 6 / 8$$

$$R = 6 / 10$$

Final remarks

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- Visit <http://forum.image.sc> !



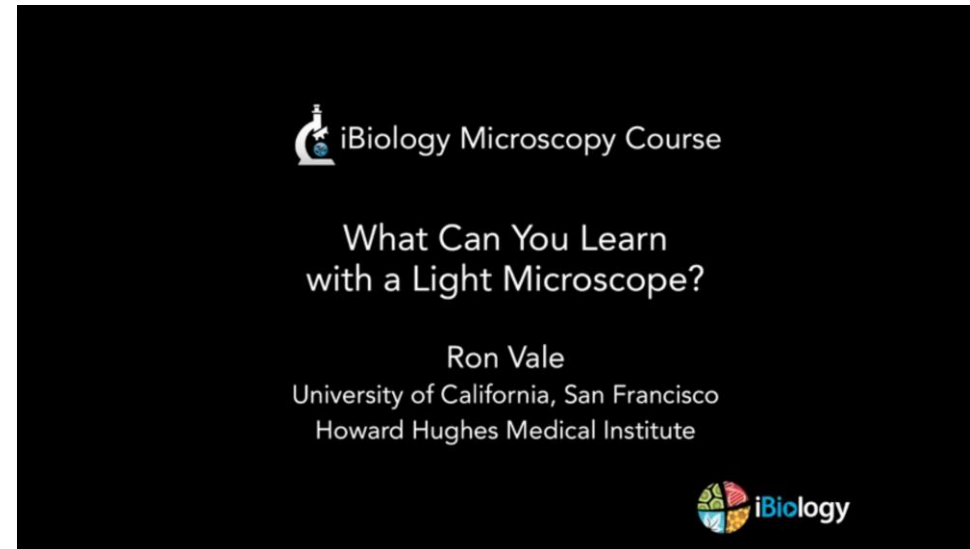
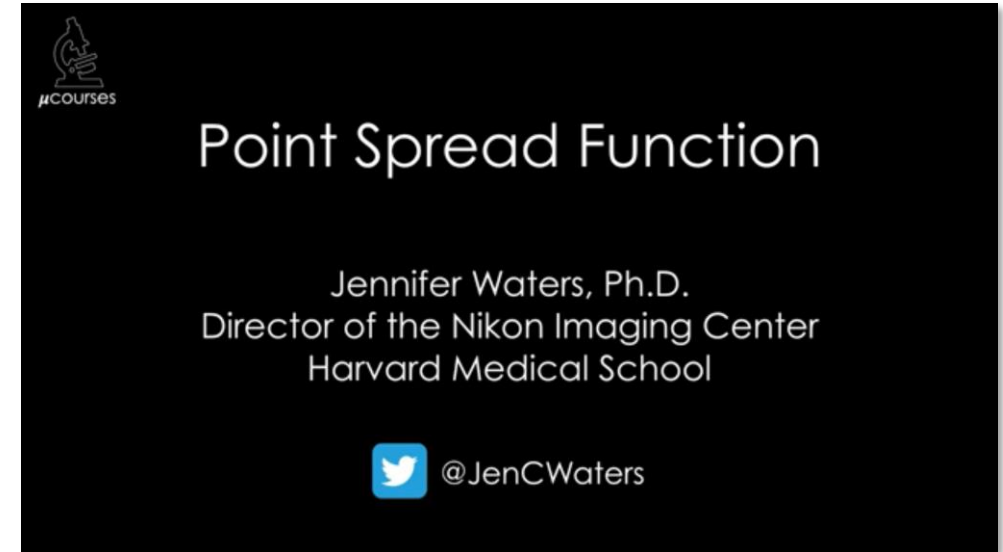
The screenshot shows the Image.sc Forum homepage. At the top, there's a navigation bar with the Image.sc logo, a search icon, and a user profile icon. Below the navigation bar, there's a section for "Community Partners". The main content area features a list of forum topics with columns for "Topic", "Replies", "Views", and "Activity". The topics listed are:

Topic	Replies	Views	Activity
Hessian-based analysis problem in SNT Image Analysis imagej, windows-10, snt	7	427	4m
Omero-metadata question - Showing metadata in the client? Usage & Issues omero, metadata, omero-metadata	2	48	6m
Export CoreID for TMA when exporting points from ROIs Usage & Issues qupath	9	40	9m
Create a sliding window over whole image by adjusting code Image Analysis fiji, imagej, macro, roi, java	1	14	26m
Visualizing channel data present in ozi file in napari forum.image.sc/t/.../39830	6	48	41m

What's next?

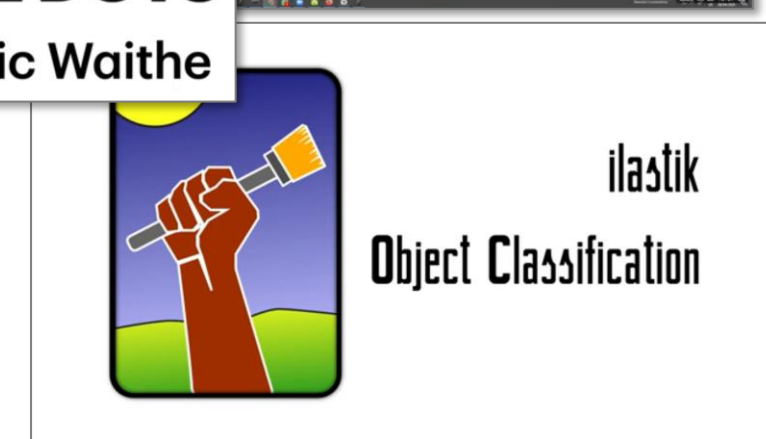
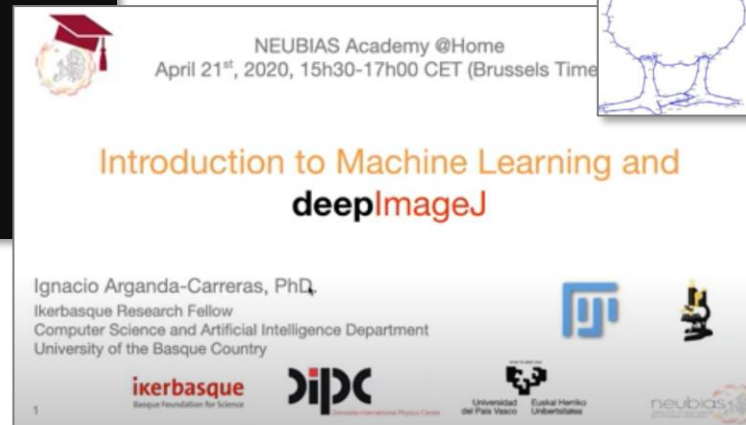
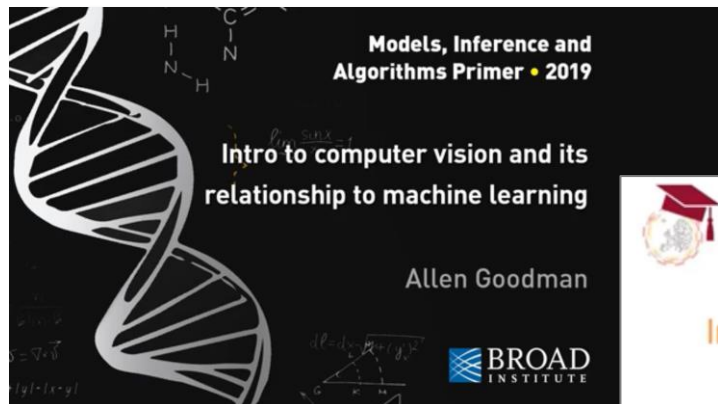
Microscopy

- BioDIP Dresden Light Microscopy Course: https://youtu.be/60_jgZtyR6U
- Microcourses: https://youtu.be/Tkc_GOCjx7E
- iBiology Microscopy Course: <https://youtu.be/4c5ILWQmqRY>





What's next?

- More machine learning for Bio-Image analysis?
 - Computer Vision / machine learning: <https://youtu.be/Kzb5vTpvDBM>
 - Computer vision: <https://youtu.be/Smw3suzynho>
 - DeepImageJ: <https://youtu.be/0vTbsO8Vnuo>
 - CSBDeep: <https://youtu.be/ipp0mx fjhwY>
 - StarDist: https://youtu.be/Amn_eHRGX5M
 - ilastik: <https://www.youtube.com/ilastikTeam>




What's next?

- Image Analysis with Python
 - Python & Jupyter
 - <https://youtu.be/2KF8vBrp3Zw>
 - <https://youtu.be/Y3pB3wnOivE>
 - Scikit-image
 - https://youtu.be/pZATswy_IsQ
 - <https://youtu.be/d1CIV9irQAY>
 - Napari
 - <https://youtu.be/VgvDSq5aCDQ>




NEUBIAS Academy @Home:
**Interactive Bioimage Analysis
with Python and Jupyter**

Guillaume Witz
Microscopy Imaging Center, Science IT Support
Bern University



ScITS
Science IT Support

Image Analysis in Python with SciPy and scikit-image | SciPy 2018 Tutorial | Stefan van der Walt



SciPy 2018

Scientific Computing with Python
Austin, Texas • July 9-15, 2018

Stéfan van der Walt, Juan Nunez-Iglesias, Joshua Warner

ENTHOUGHT

Image Analysis in Python with SciPy and scikit-image

0:01 / 2:19:05

Scroll for details



napari
a multi-dimensional image viewer for Python

June 4th 2020 - NEUBIAS Academy @Home
Nicholas Sofroniew

Chan
Zuckerberg
Initiative



cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

What's next?

More general

- Python for Microscopists + beyond: <https://youtube.com/digitalsreeni>
- Image data integrity: https://youtu.be/c_Oi2HKom_Y
- Coloc: <https://youtu.be/cOrCz4qc8DI>
- Automated microscopy: <https://youtu.be/w0ERCrKx4gk>

