Machine Learning for Pixel and Object Segmentation

Robert Haase

With Material from

Deborah Schmidt, Jug Lab, MPI CBG

Uwe Schmidt, Myers Lab, MPI CBG

Martin Weigert, EPFL

Ignacio Arganda-Carreras, Universidad del Pais Vasco

Carsen Stringer, HHMI Janelia

Wei Ouyang, KTH Royal Institute of Technology, Stockholm and

The Scikit-Learn community

@haesleinhuepf

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Lecture overview

Overview

- Machine learning for Pixel and Object Classification
  - Random Forest Classifiers
- Python
  - scikit-learn / napari
  - Accelerated pixel and object classification (APOC)

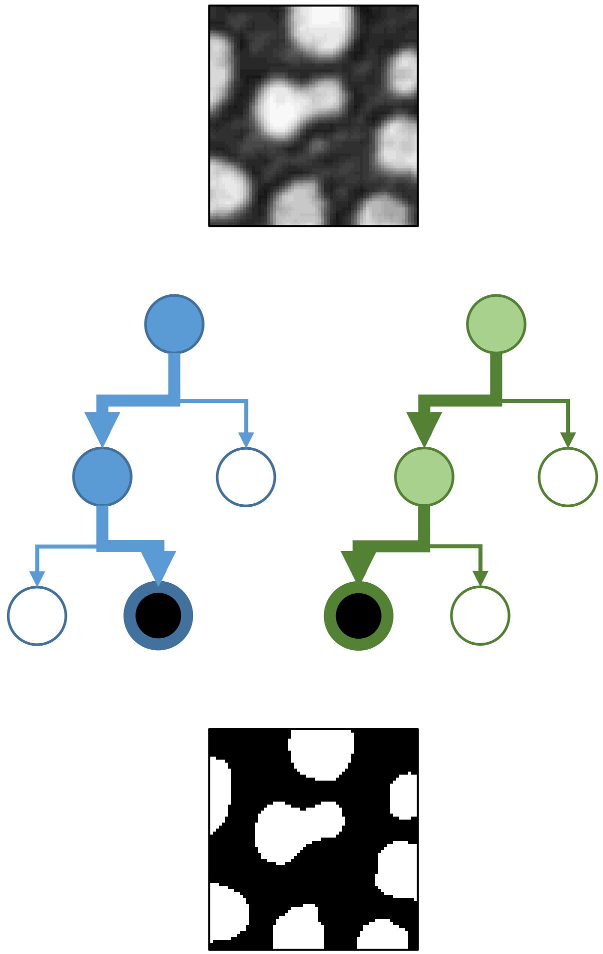


Image segmentation using thresholding

- Recap: Finding the right workflow towards a good segmentation takes time

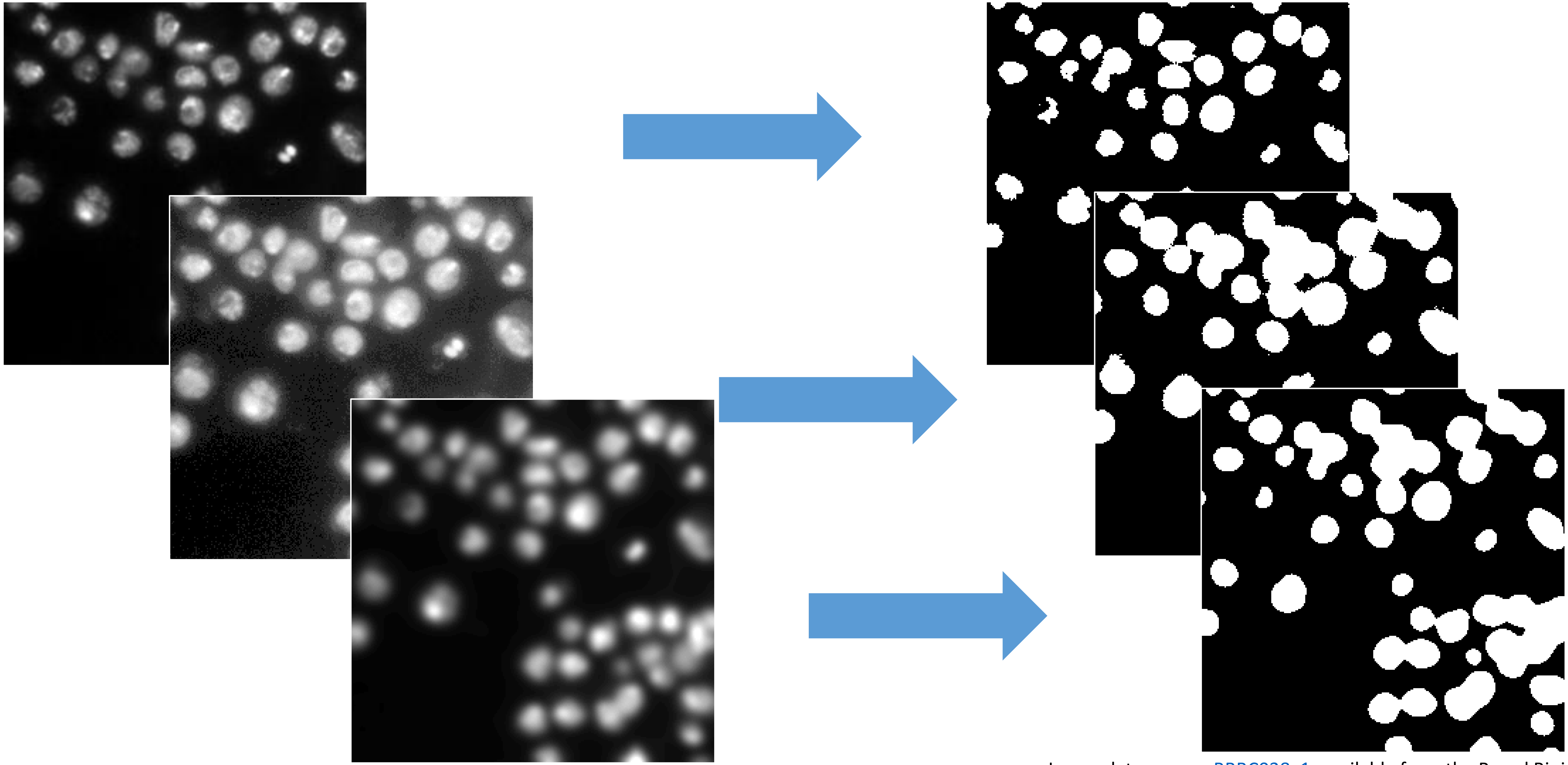


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

- Recap: Combining images, e.g. using Difference of Gaussian (DoG)

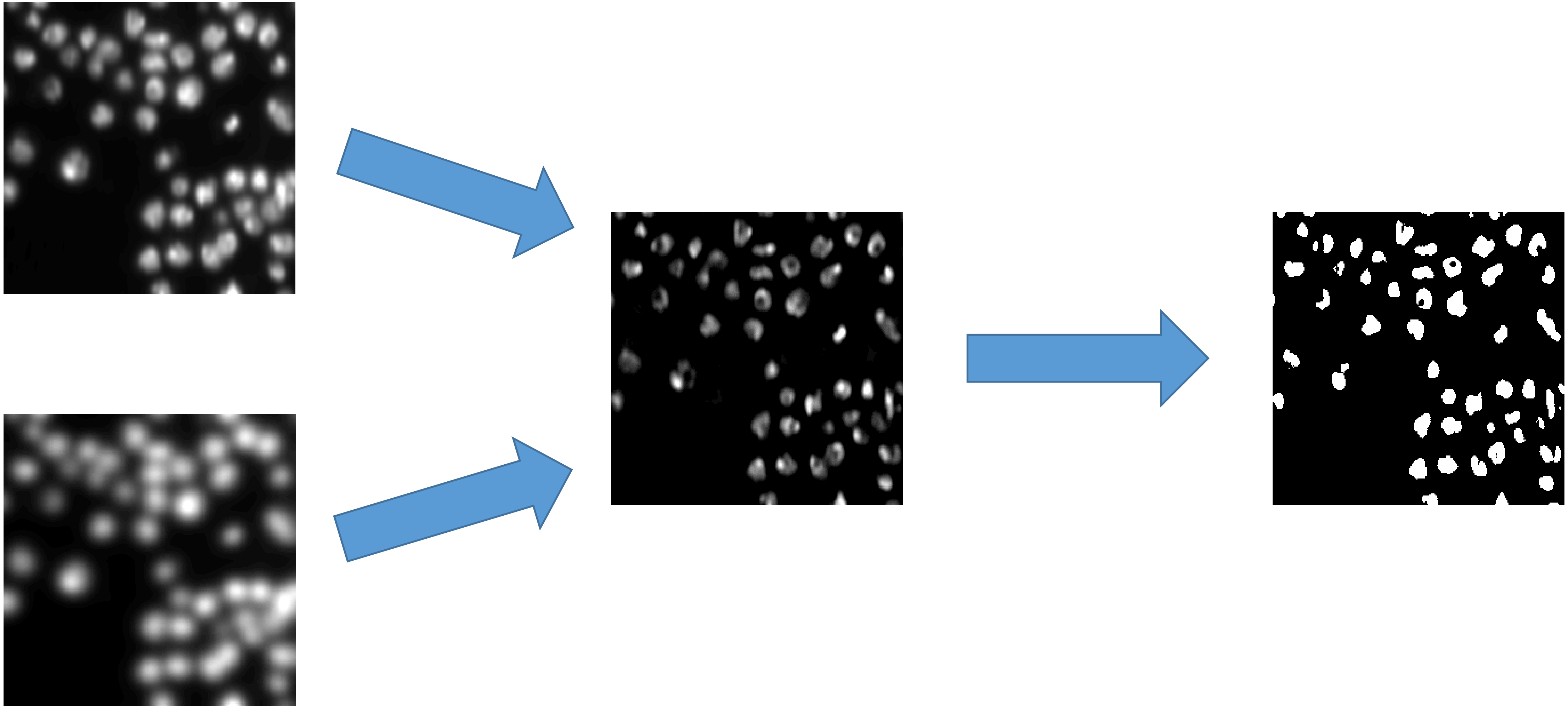
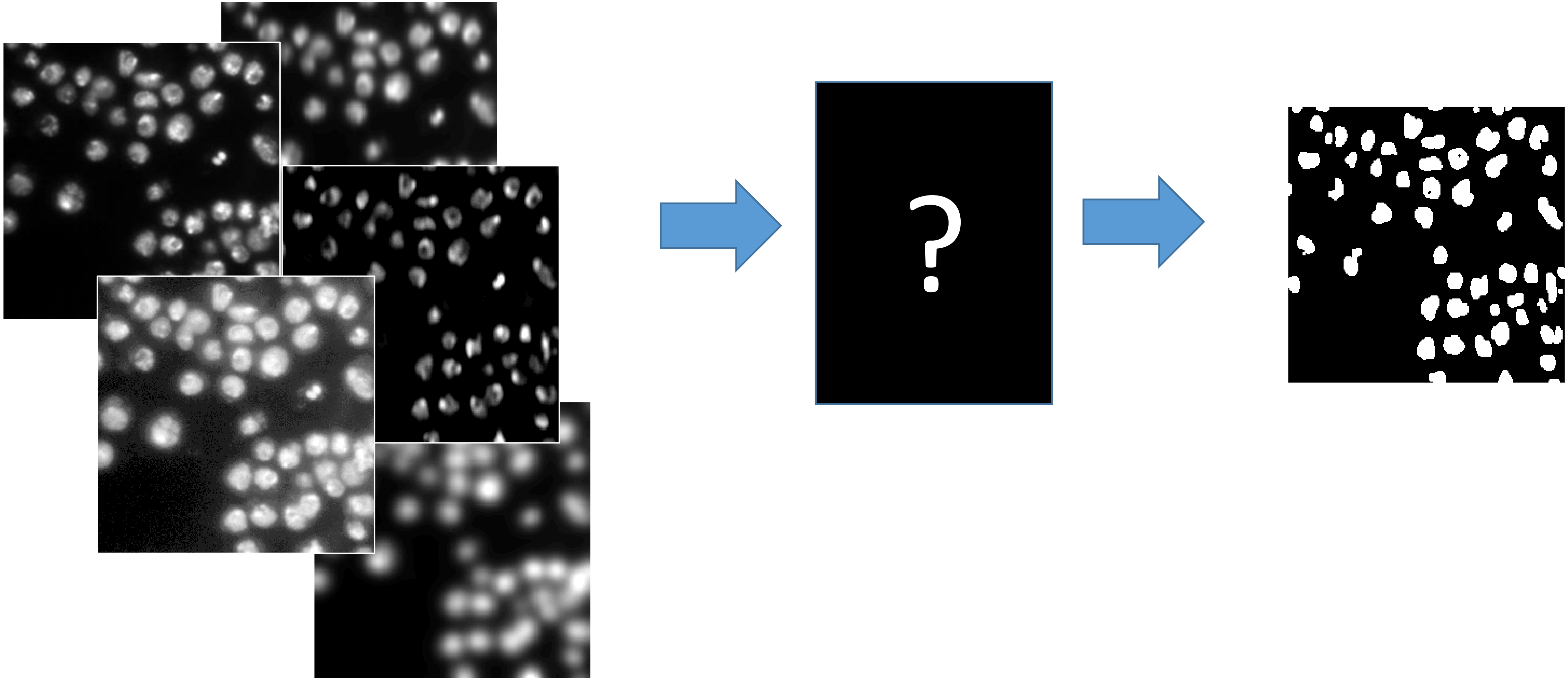


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

- Might there be a technology for optimization which combination of images can be used to get the best segmentation result?



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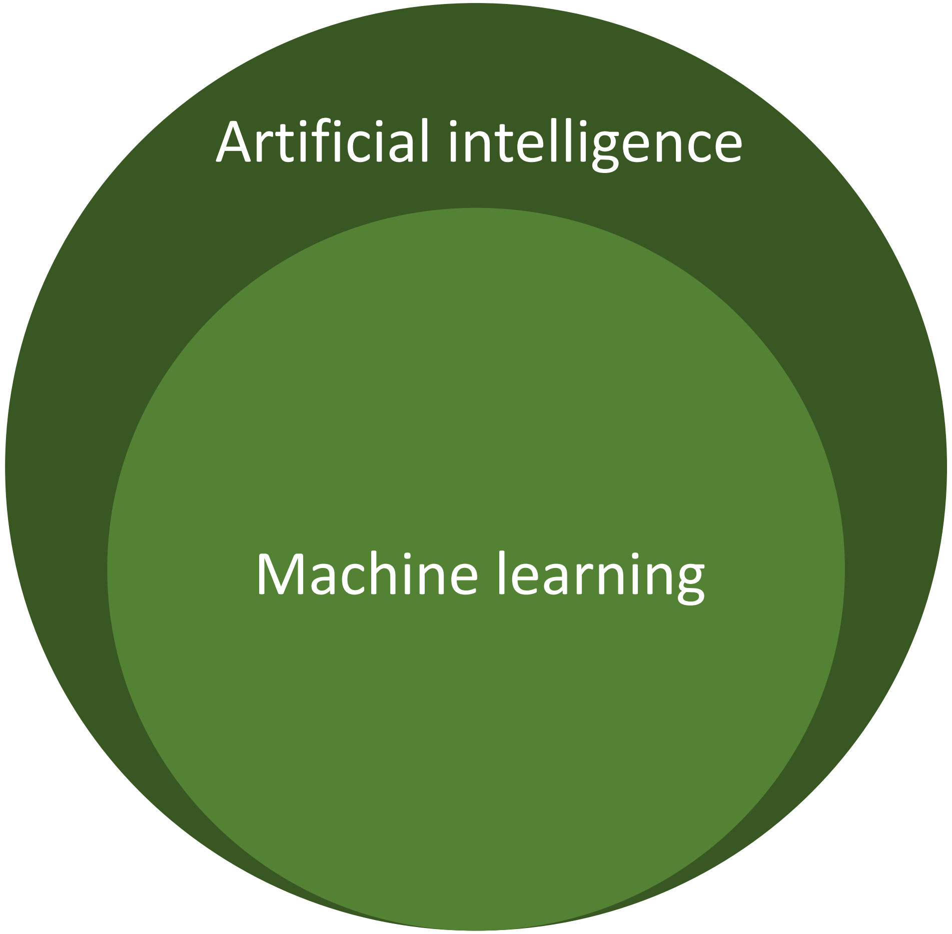
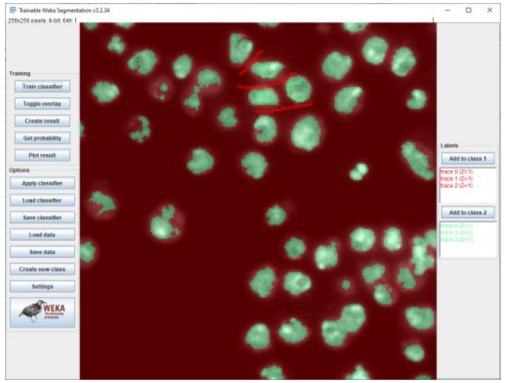
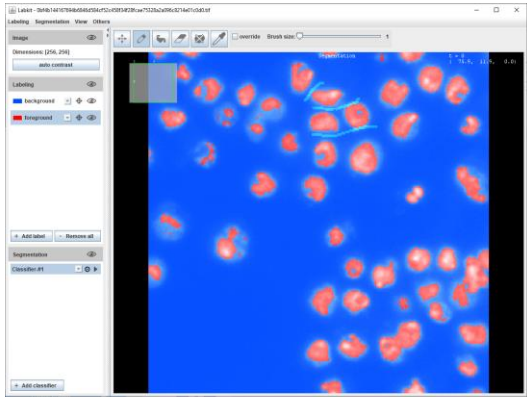
Machine learning

- A research field in computer science
- Finds more and more applications, also in life sciences.



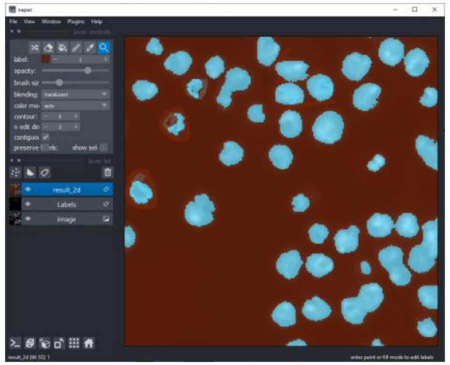


Trainable Weka Segmentation
https://imagej.net/plugins/tws/



LabKit
https://imagej.net/plugins/labkit/

Python / scikit-learn / napari / apoc



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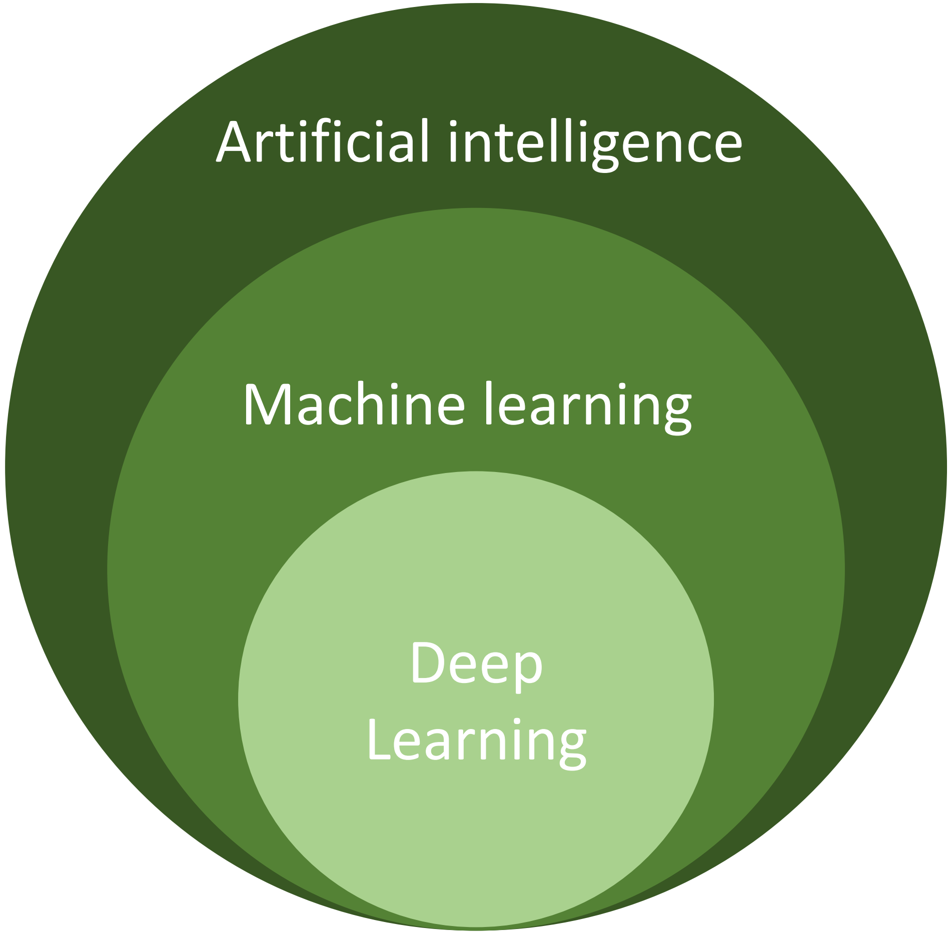
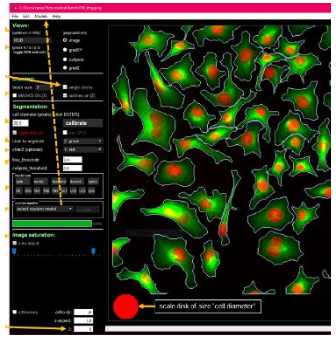
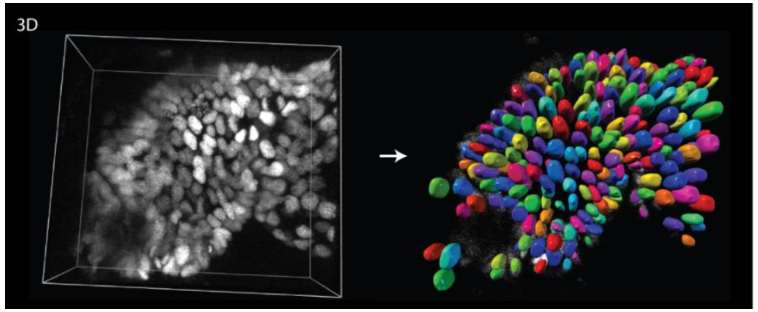
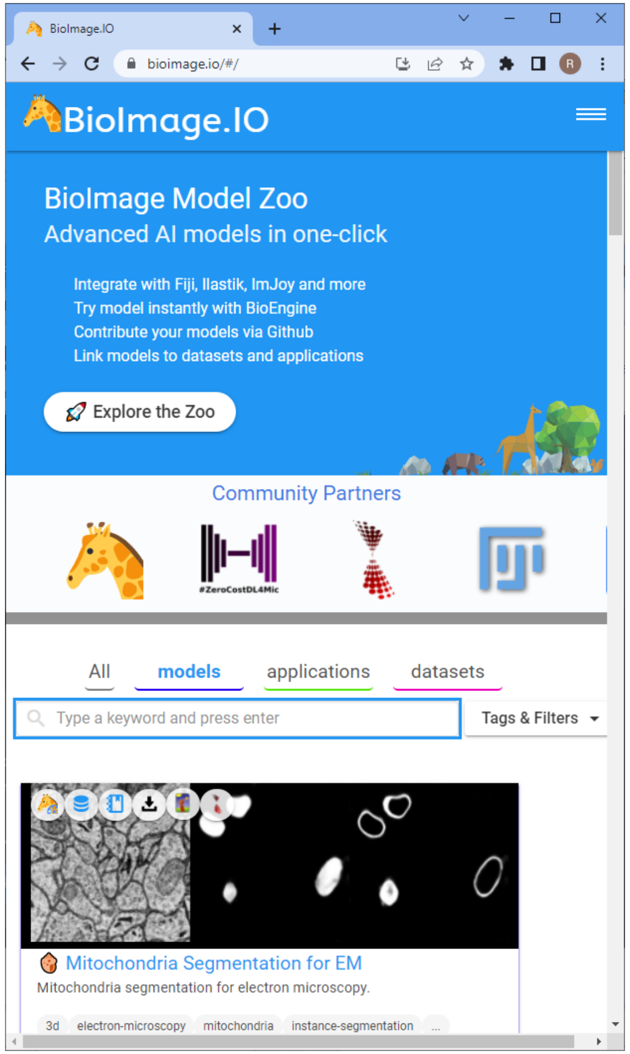




www.cellpose.org/



https://github.com/stardist/stardist

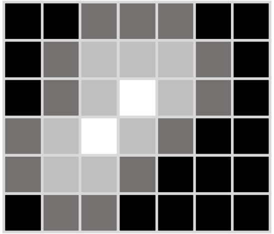
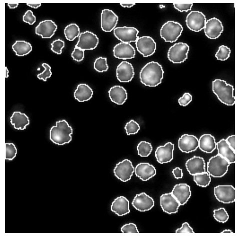


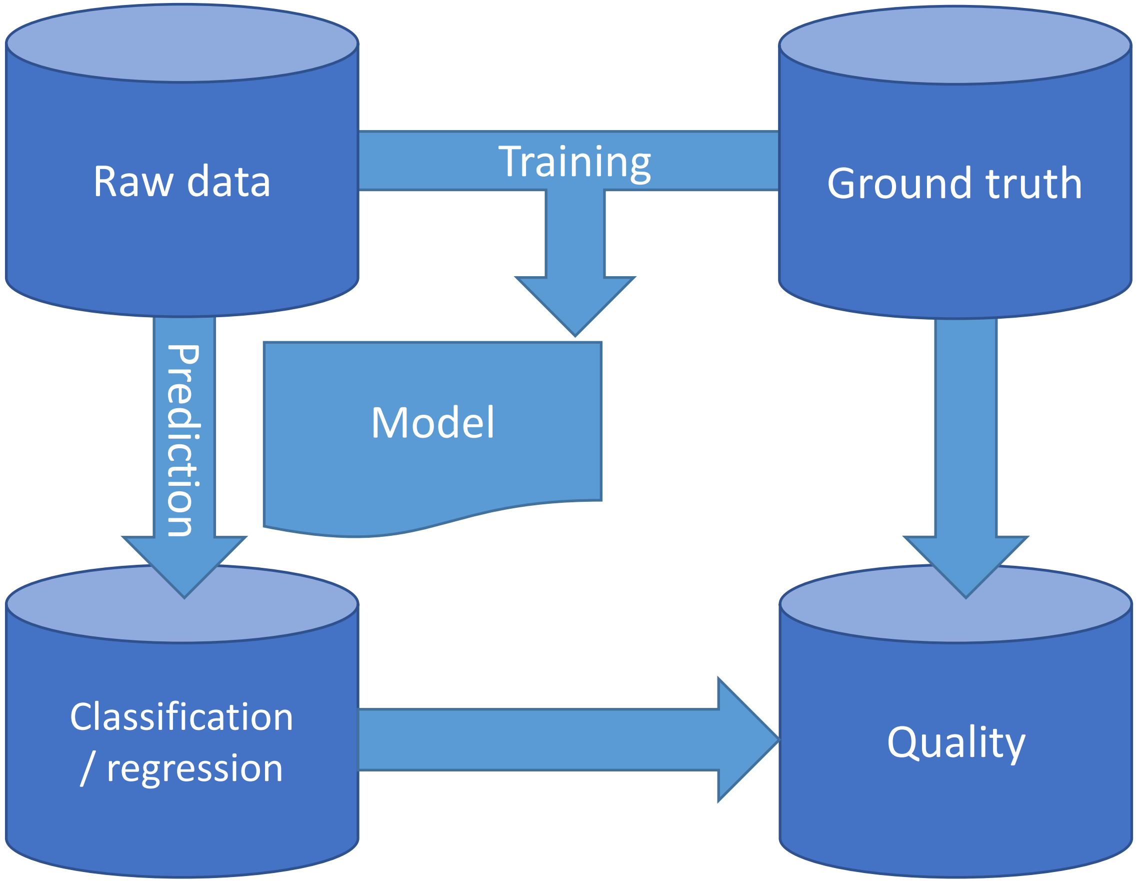
https://bioimage.io/

Machine learning

- Automatic construction of predictive models from given data

Pixels, Objects, Images, Audio, Text, Measurements, …

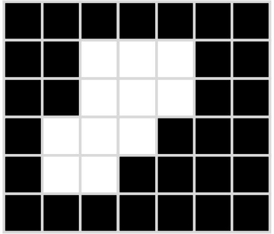



Annotated raw data, usually generated by humans

Precision, Recall

Dense Segmentation / Binarization



Object classification

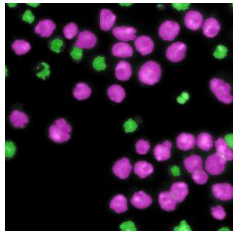
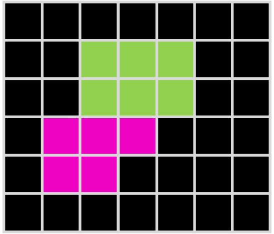


Image classification

"Cat"

Instance segmentation

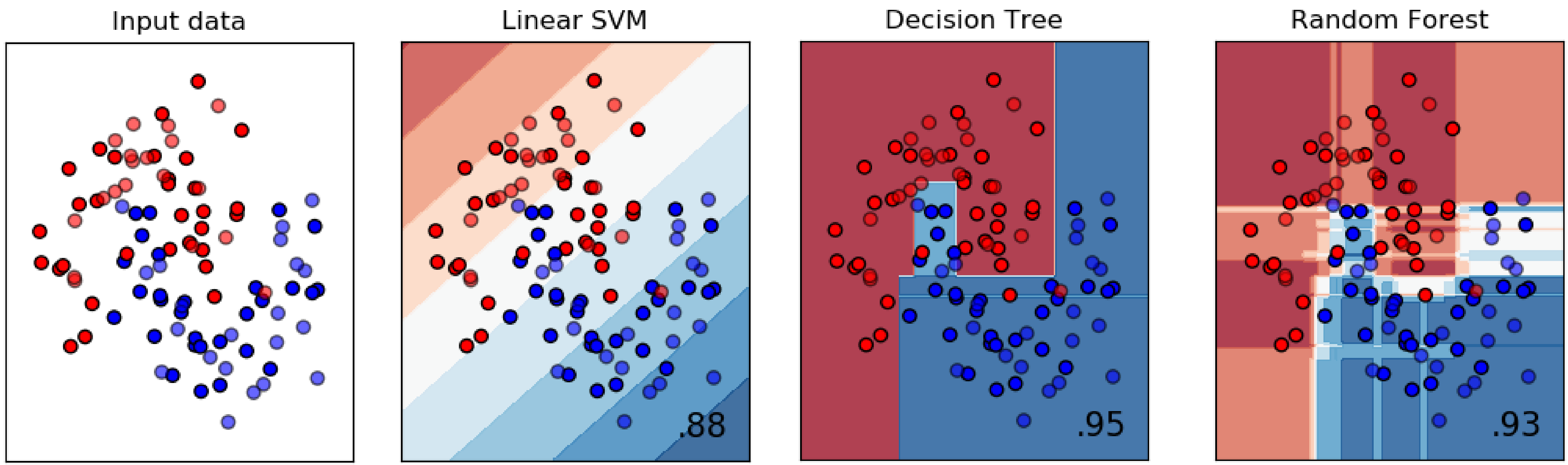


Cont. quantity

$P_{Cat} = 0.5$
$P_{Microscope} = 0.4$
Height = 80 cm

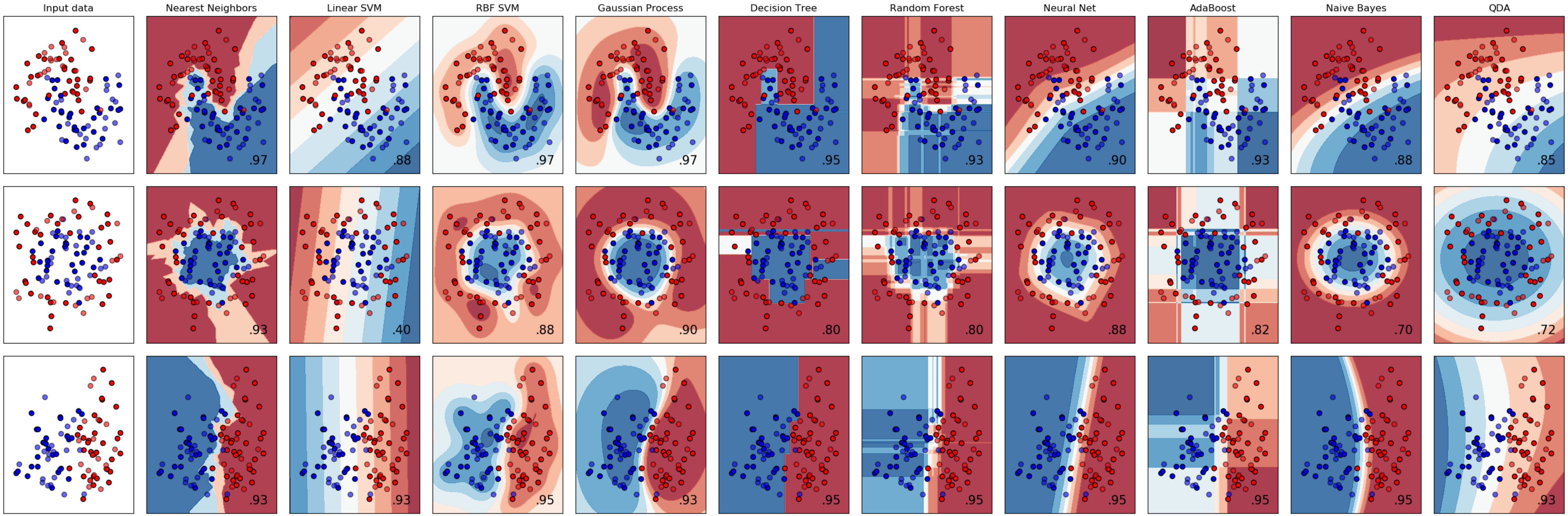
Goal

- Guess classification (color) from position of a sample in parameter space.



Approaches

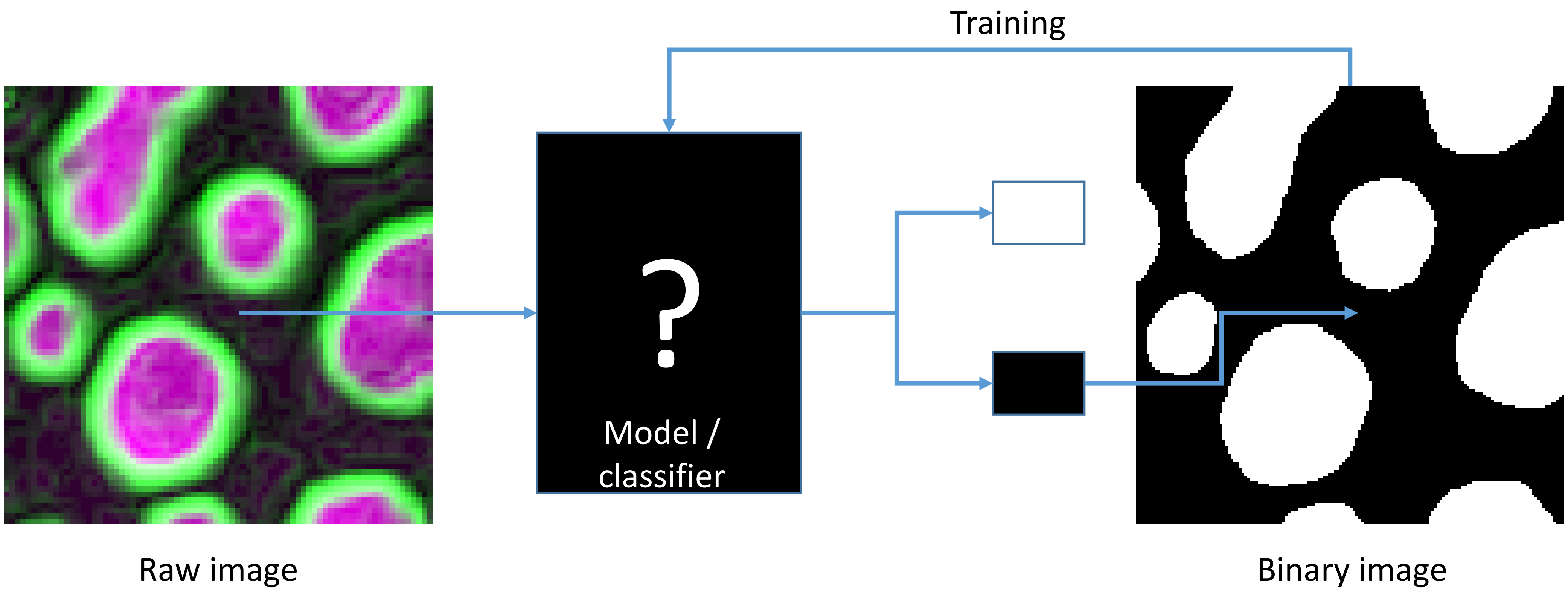
- The right approach depends on data, computational resources and desired quality



Adapted from https://scikit-learn.org/stable/auto_examples/classification/plot_classifier_comparison.html

Machine learning for image segmentation

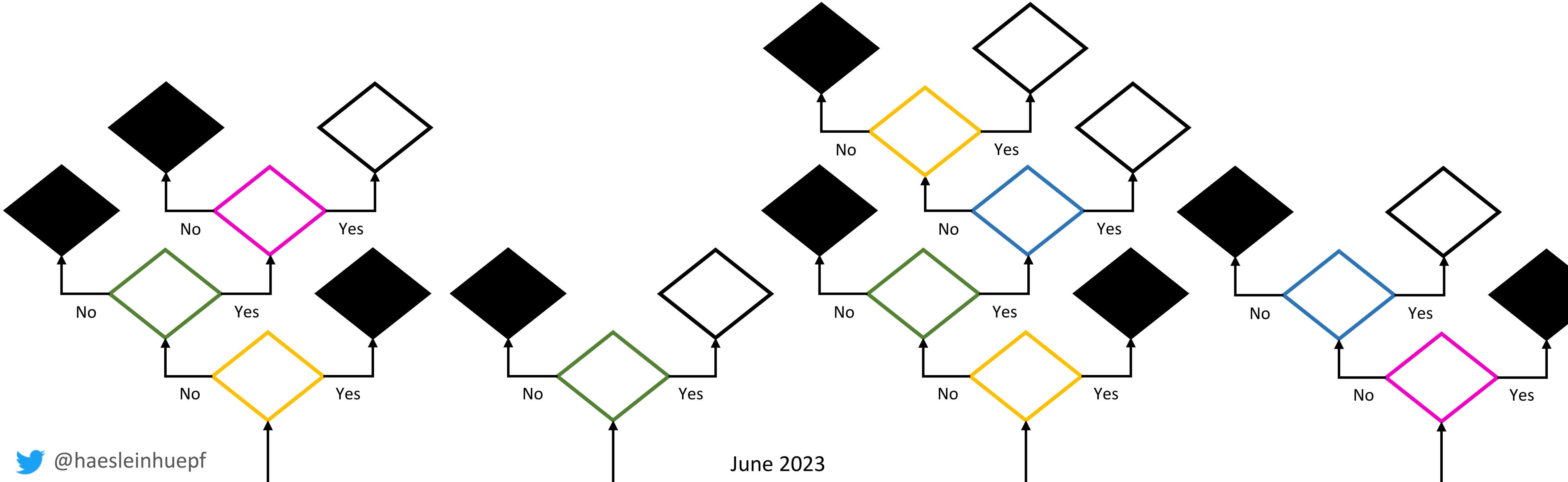
- *Supervised* machine learning: We give the computer some ground truth to learn from
- The computer derives a *model* or a *classifier* which can judge if a pixel should be foreground (white) or background (black)
- Example: Binary classifier



Random forest based image segmentation

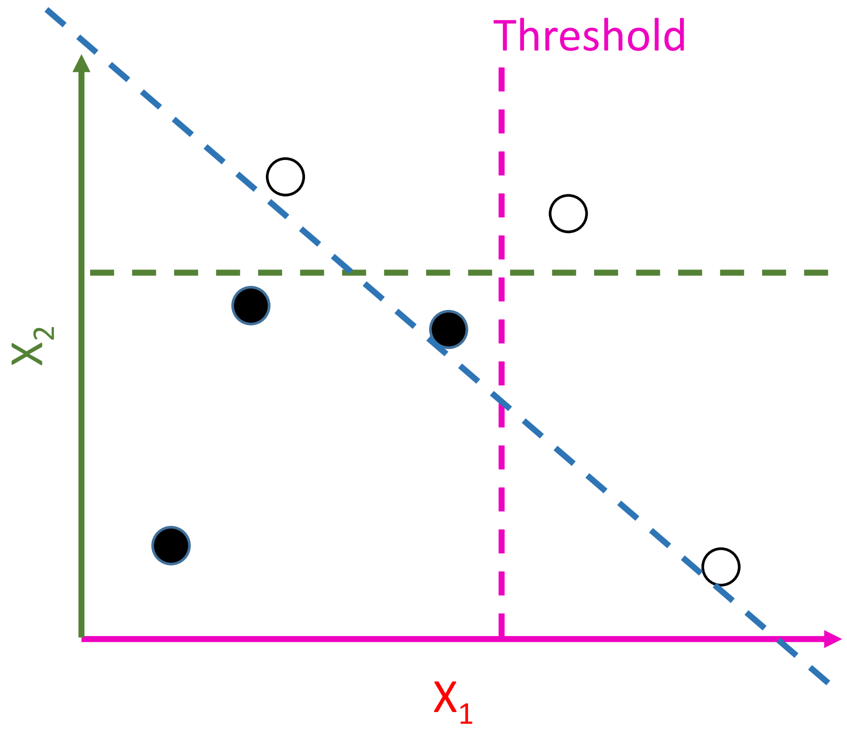
- Decision trees are classifiers, they decide if a pixel should be white or black
- Random decision trees are randomly initialized, afterwards evaluated and selected
- Random forests consist of many random decision trees

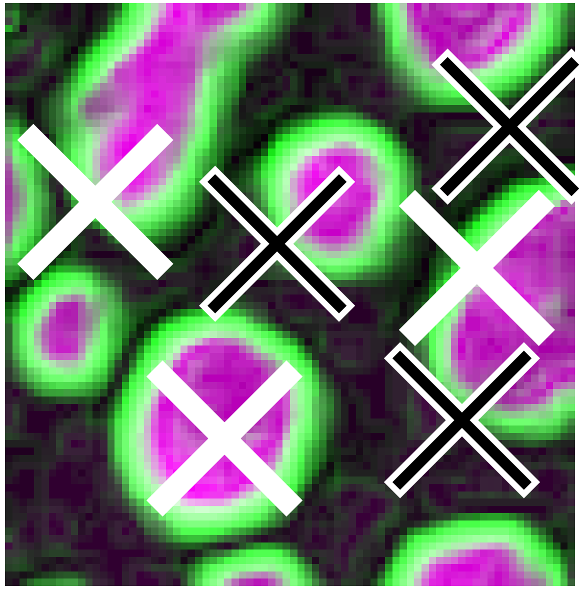
- Example: Random forest of binary decision trees

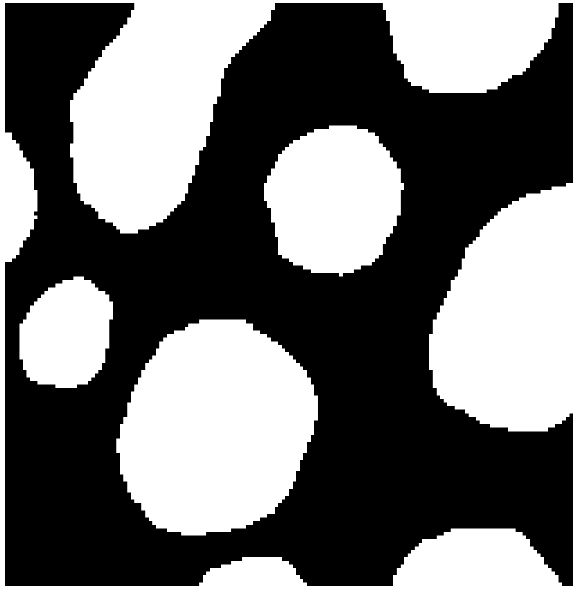


Deriving random decision trees

- For efficient processing, we randomly *sample* our data set
  - Individual pixels, their intensity and their classification



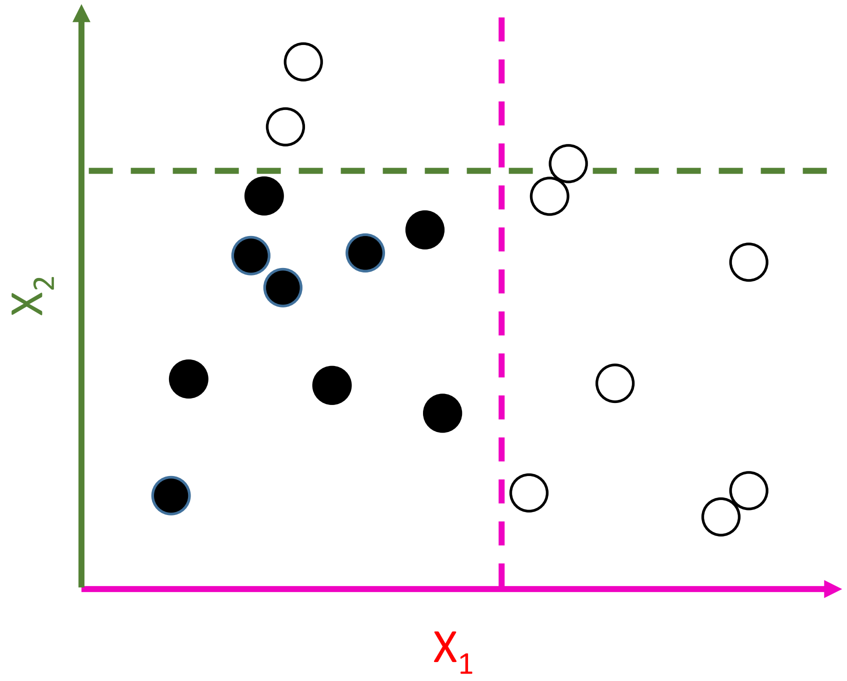


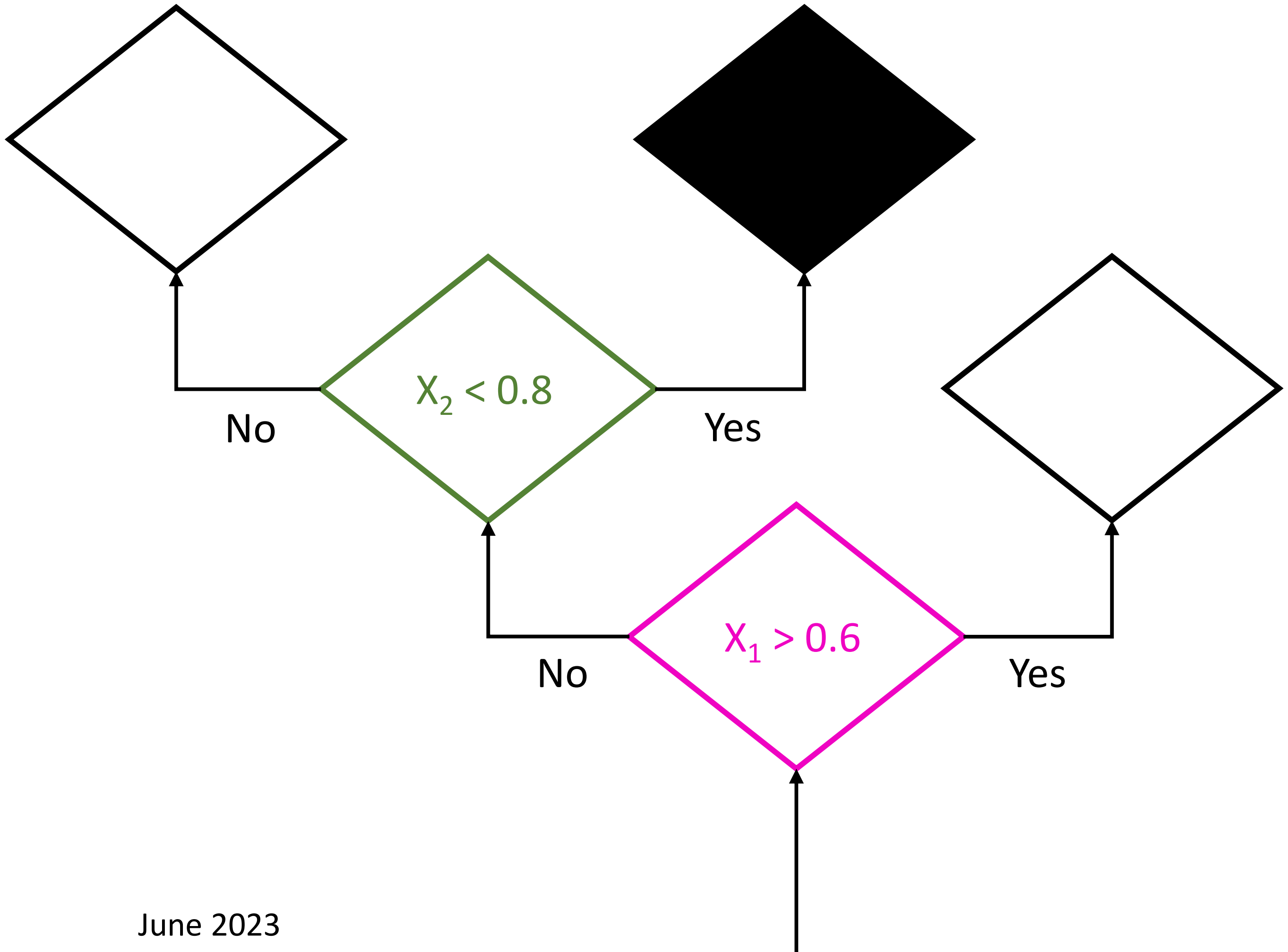


Note: You cannot use a single threshold to make the decision correctly

Deriving random decision trees

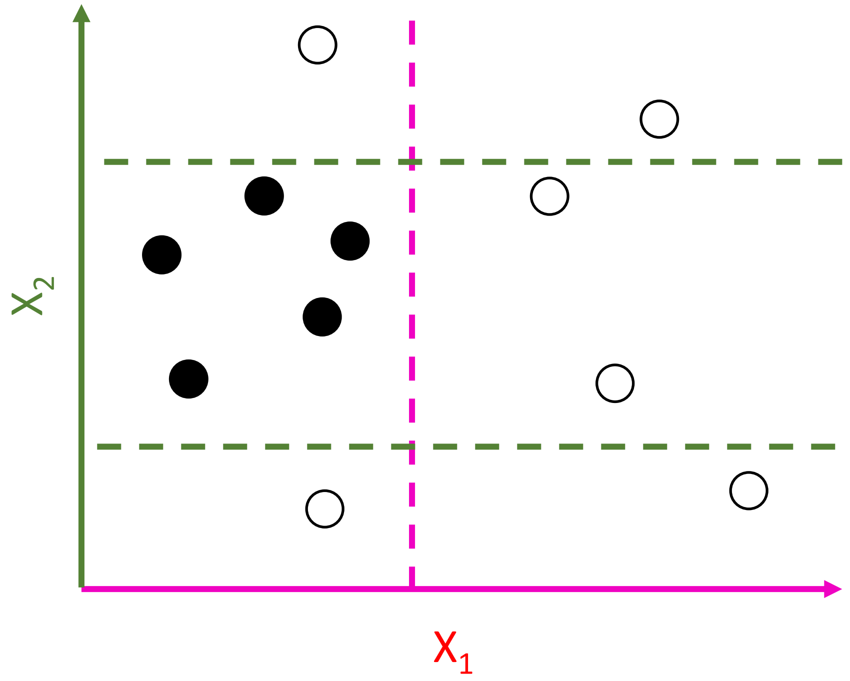
- Decision trees combine several thresholds on several parameters

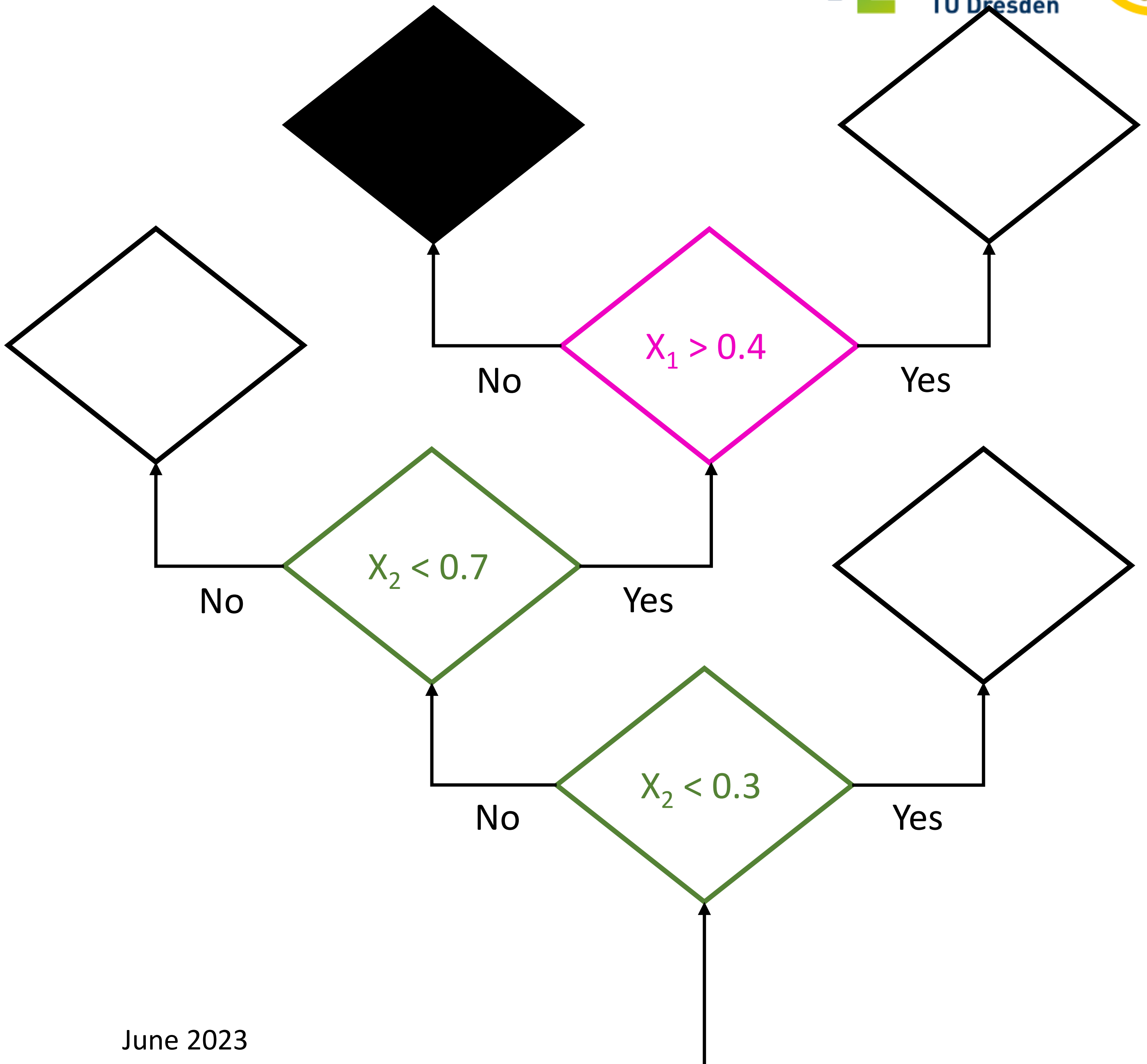




Deriving random decision trees

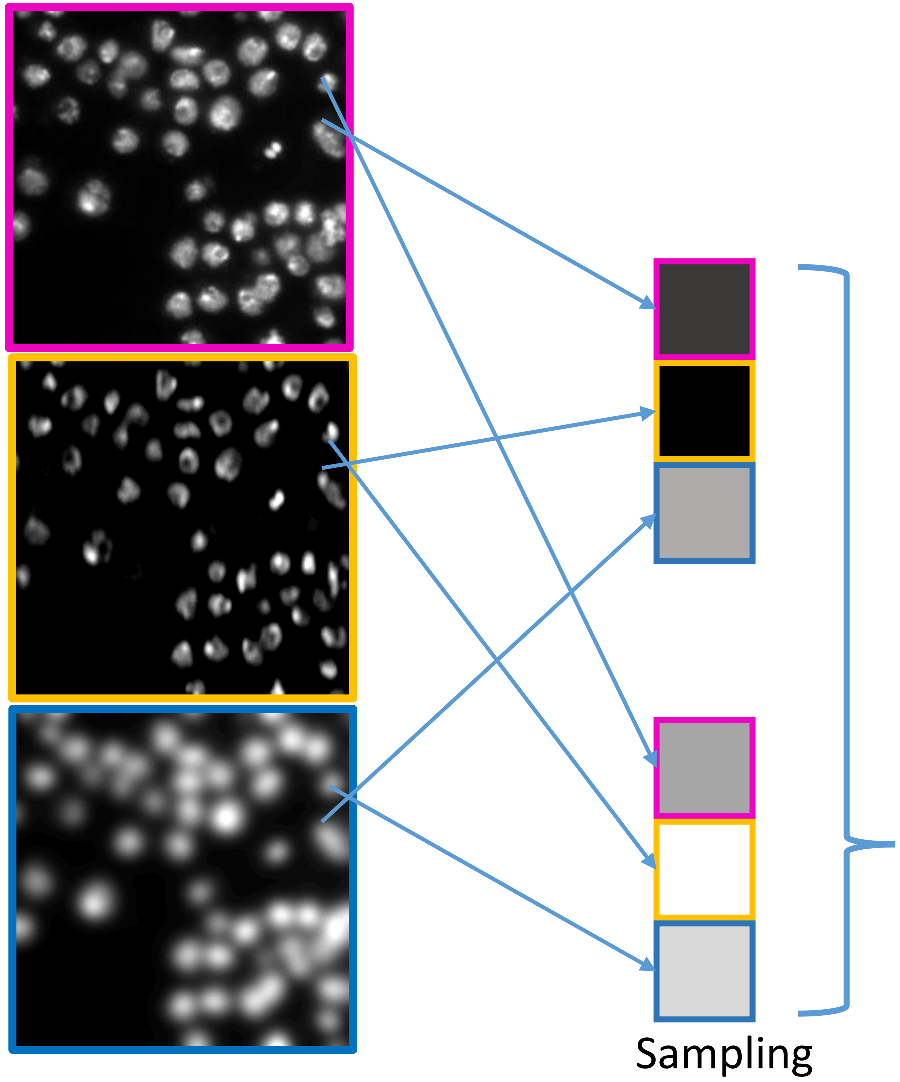
- Depending on sampling, the decision trees are different

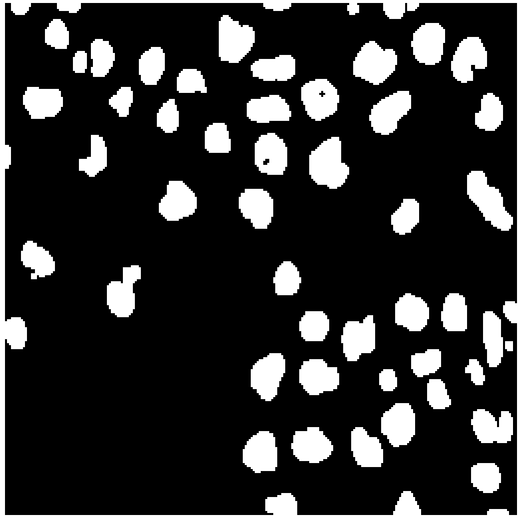




Random Forest Pixel Classifiers

- By comparing performance of individual decision trees, good ones can be selected, bad ones excluded.





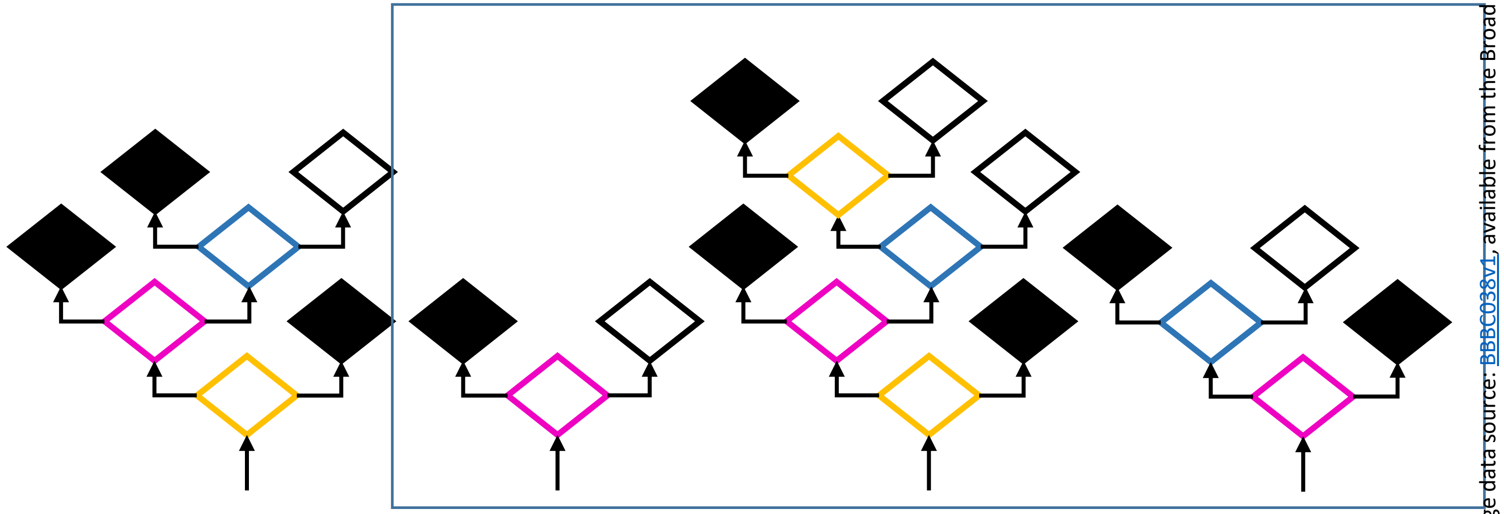
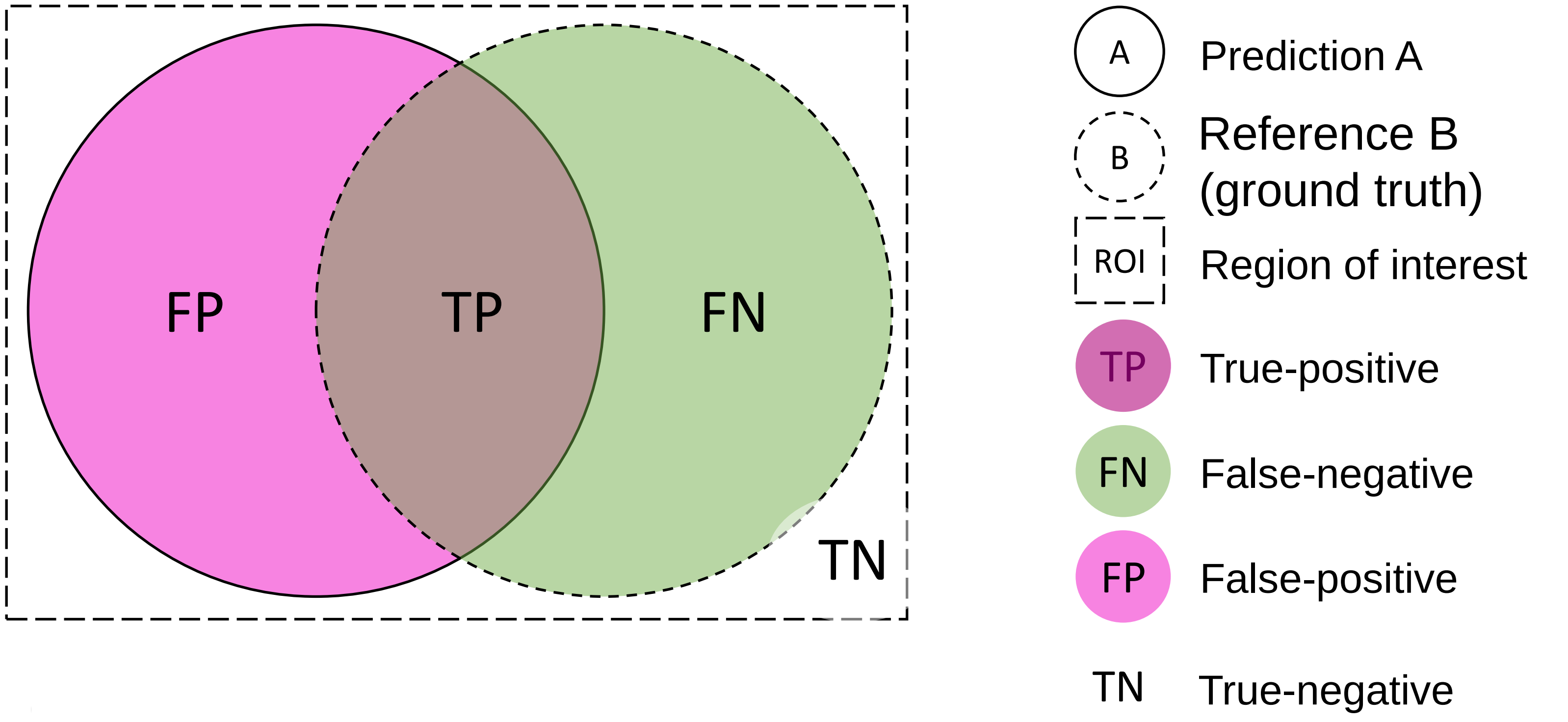


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Segmentation quality estimation

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

  - Welcome to the Theory of Sets



Overlap (a.k.a. Jaccard index) $\frac{TP}{TP + FN + FP}$ How much do A and B overlap?

Precision $\frac{TP}{TP + FP}$ What fraction of points that were predicted as positives were really positive?

Recall (a.k.a. sensitivity) $\frac{TP}{TP + FN}$ What fraction of positives points were predicted as positives?

Random Forest Pixel Classifiers

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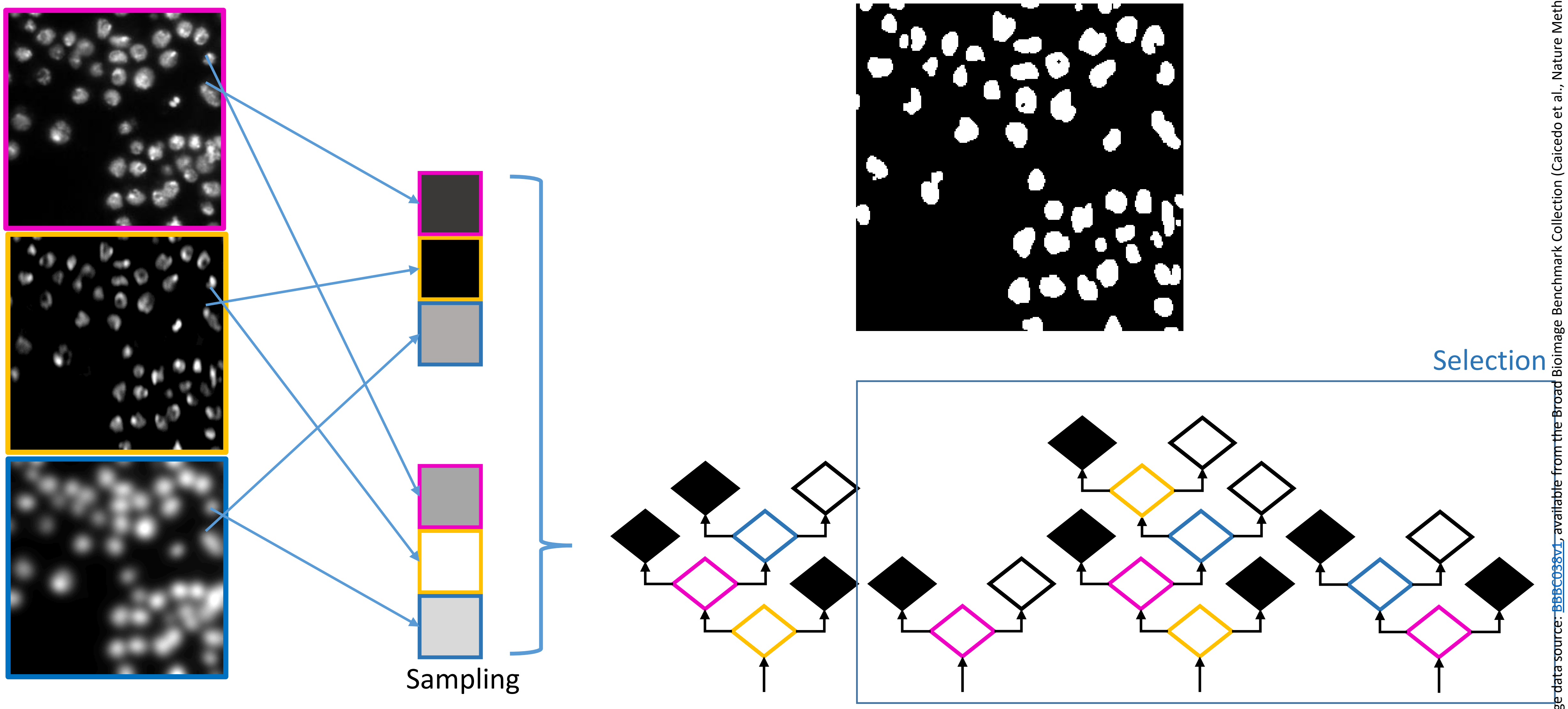
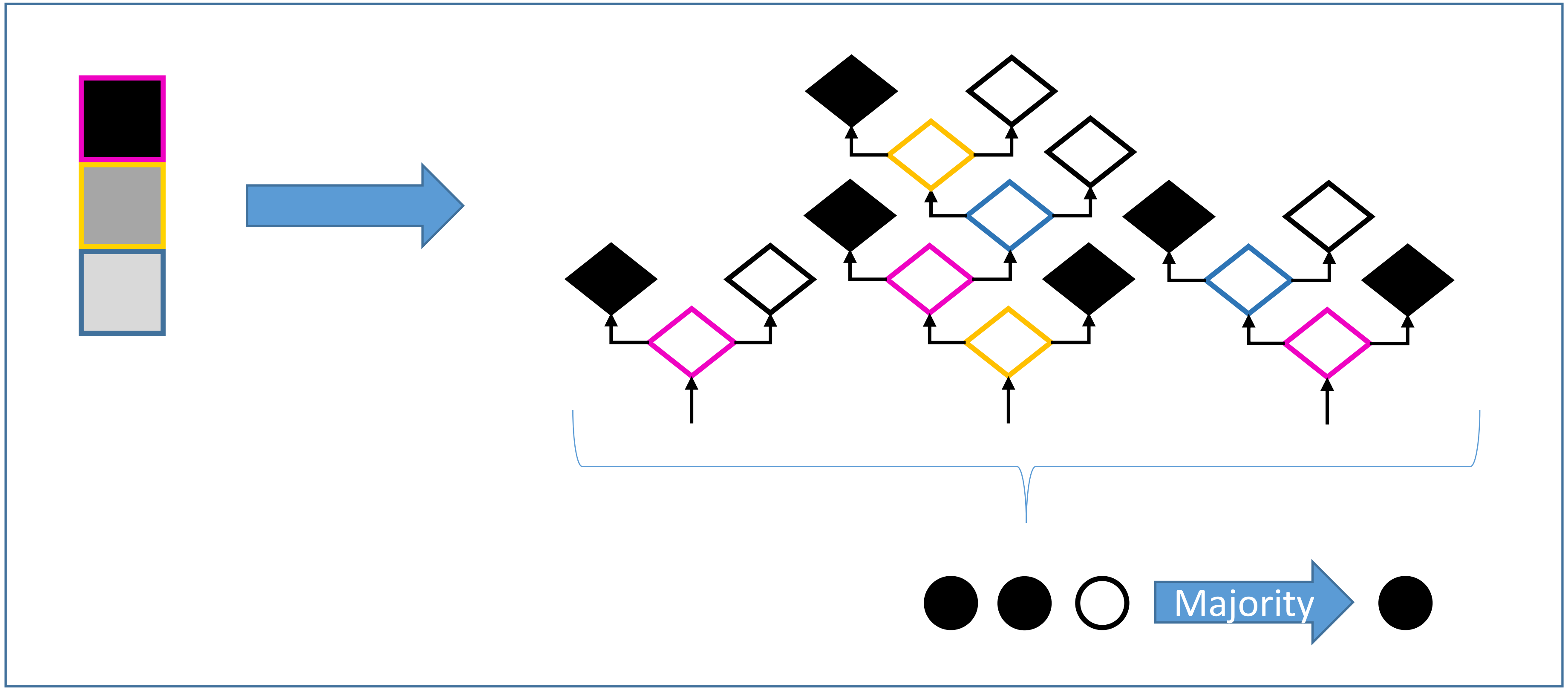


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection [Caicedo et al., Nature Methods, 2019].

Random Forest Pixel Classifiers

- Combination of individual tree decisions by voting or max / mean

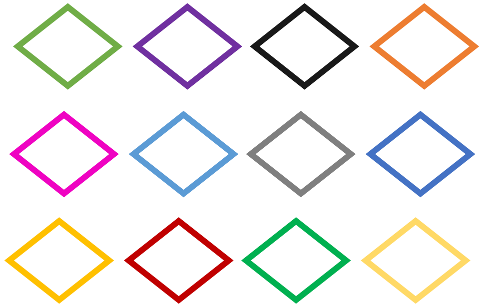
Prediction



Random Forest Pixel Classifiers

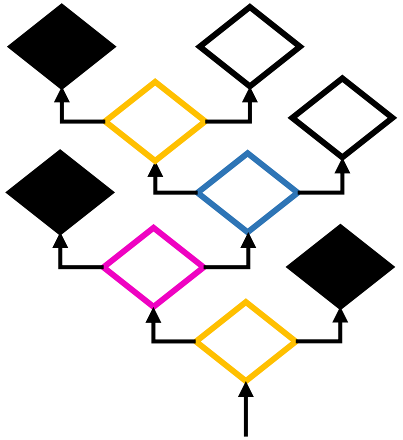
- Typical numbers for pixel classifiers in microscopy

Available features: > 20



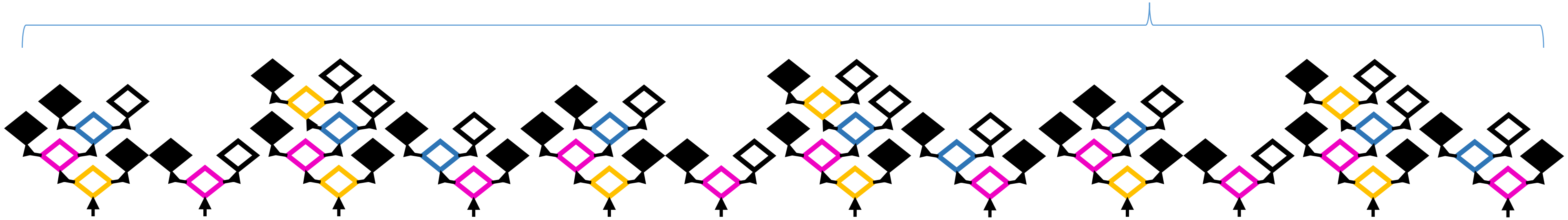
- Gaussian blur image
- DoG image
- LoG image
- Hessian
- ....

Selected features: <= depth



Depth: 4

Number of trees: > 100



Model validation

- A good classifier is trained on a hand full of datasets and works on thousands similarly well.
- In order to assess that, we split the ground truth into two set
  - Training set (50%-90% of the available data)
  - Test set (10%-50% of the available data)

Typically done with hundreds or thousands of cells / images / objects / whatever.

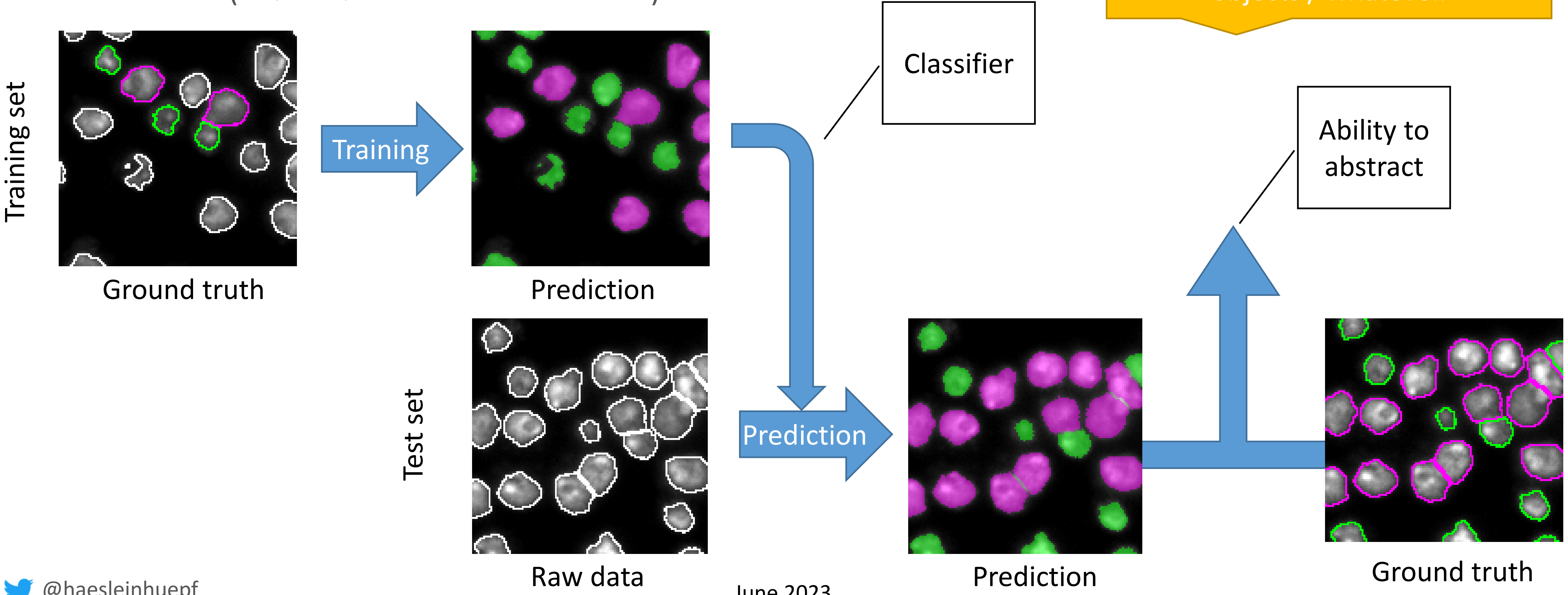


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Model validation

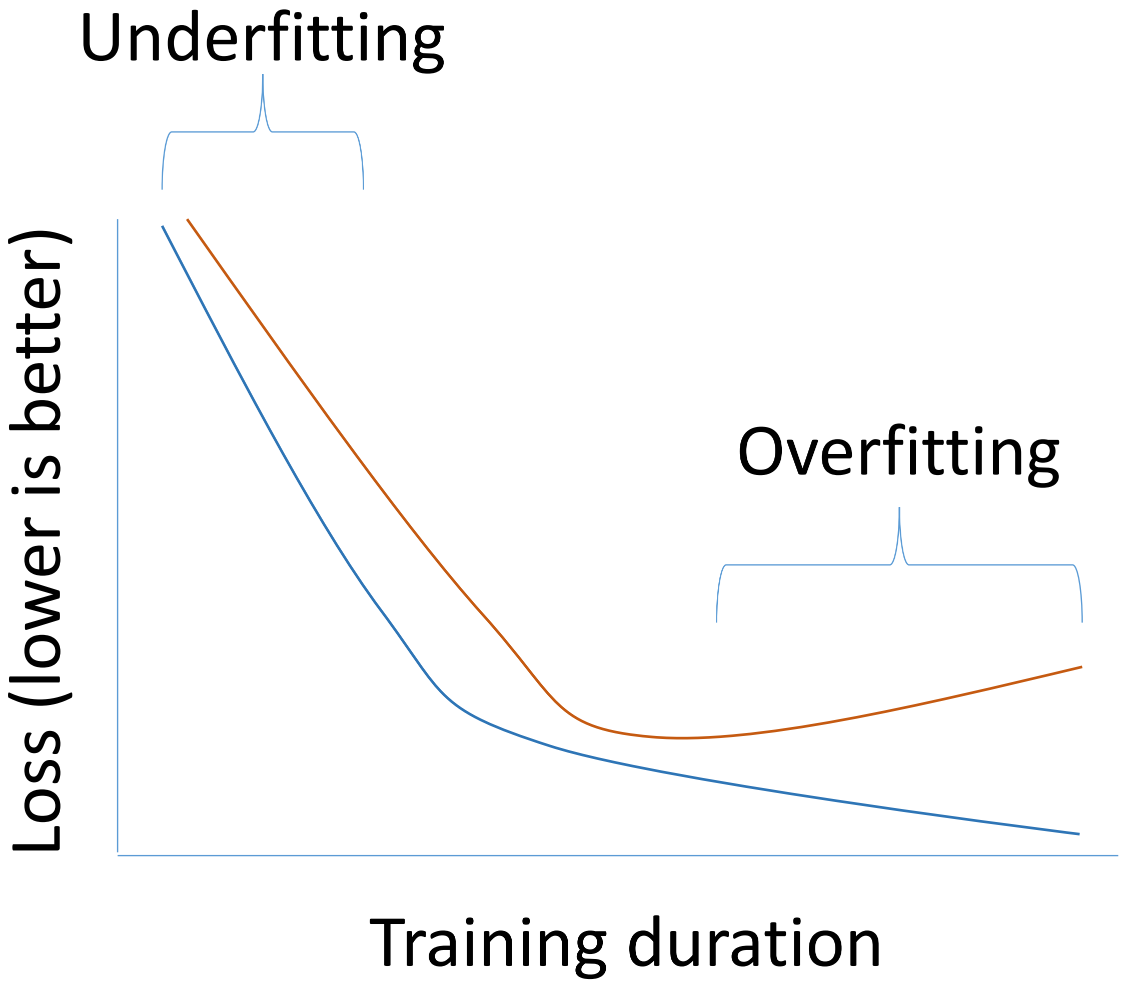
Train dataset (e.g. 80% of the data)
- Used for training directly

Validation dataset (10% of the data)
- After every iteration see if the model overfits

Test dataset (10% of the data)
- Final evaluation after training is finished (once)

Underfitting
- A trained model that is not even able to properly process the data it was trained on

Overfitting
- A model that is able to process data it was trained on well
- It processes other data poorly

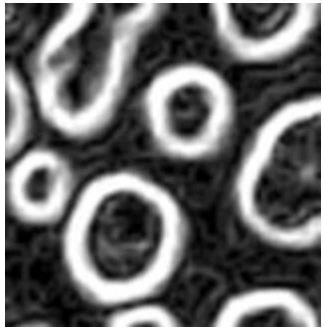


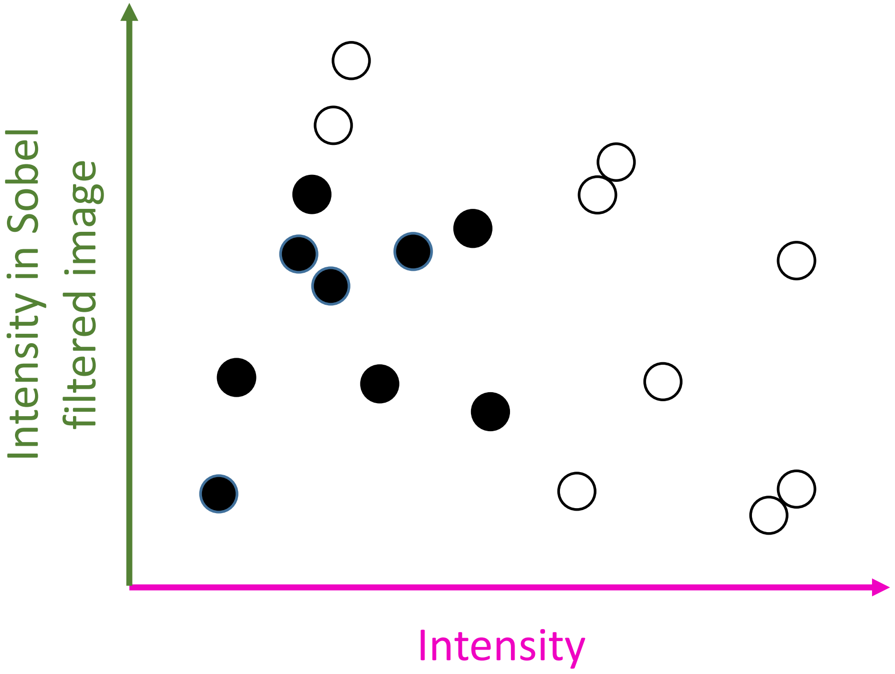
https://towardsdatascience.com/how-to-split-data-into-three-sets-train-validation-and-test-and-why-e50d22d3e54c

Object classification

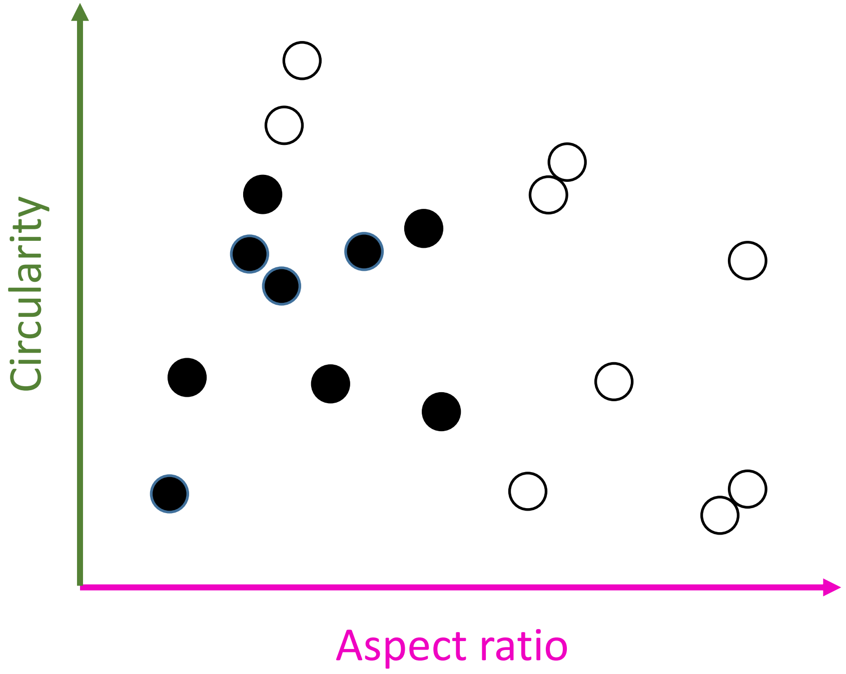
- What if we exchange pixel features with object features?

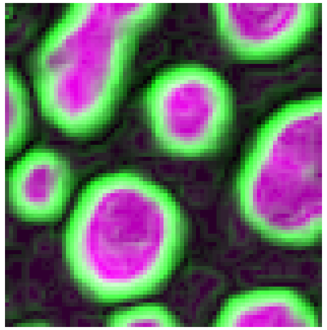
Pixel classification

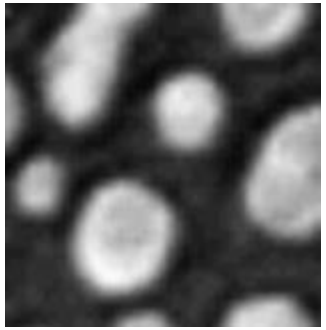




Object classification



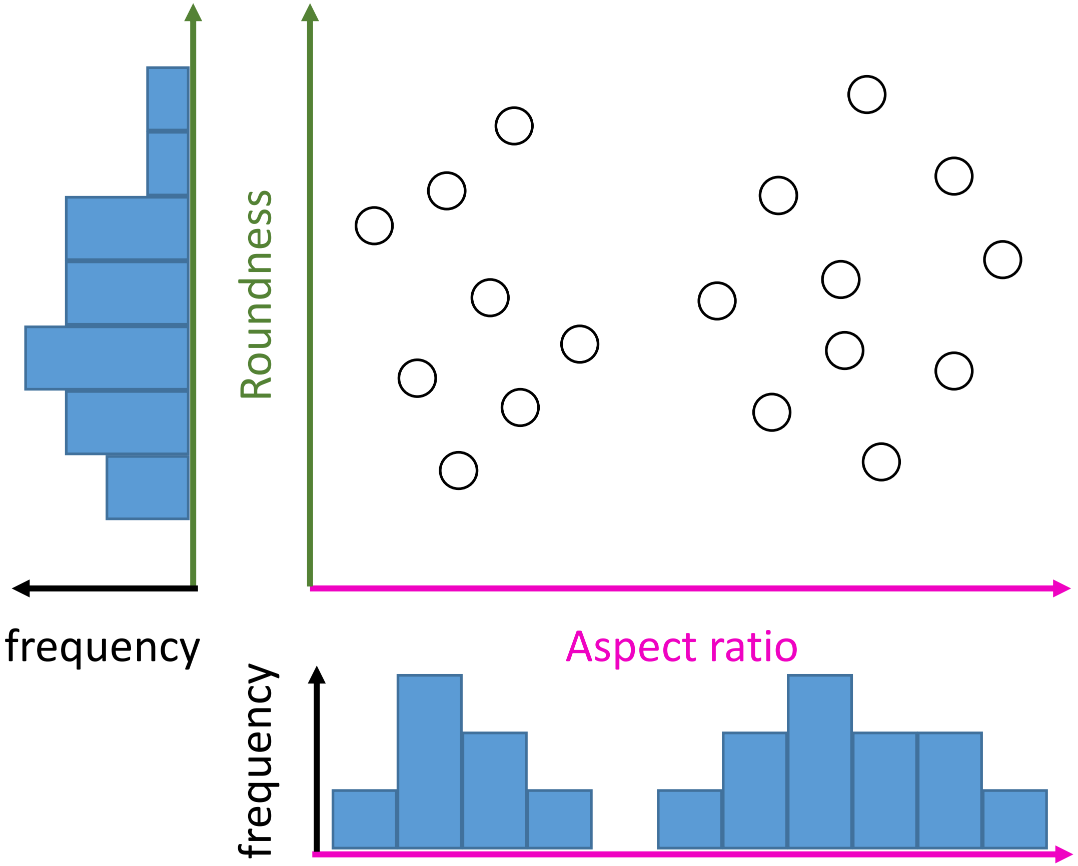




- The algorithms work the same but with different
  - Features
  - Number of features
  - Tree / forest parameters
  - Selection criteria

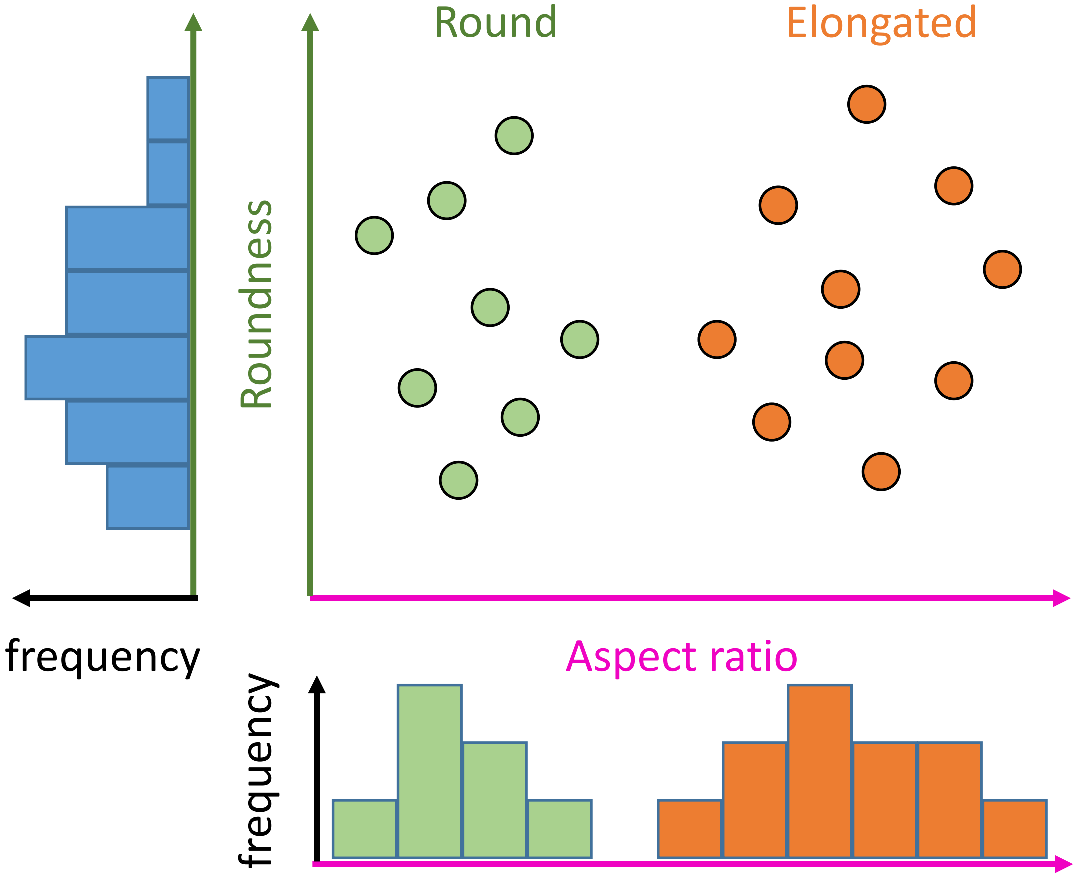
Unsupervised machine learning

- If you don't provide ground truth, the algorithm is *unsupervised*.



Unsupervised machine learning

- If you don't provide ground truth, the algorithm is unsupervised.
- Nevertheless, algorithms can tell us something about the data



Feature extraction

Robert Haase

With material from

Johannes Soltwedel, BiaPoL, PoL TU Dresden

Marcelo Zoccoler, BiaPol, PoL, TU Dresden

June 2023

@haesleinhuepf

Feature extraction

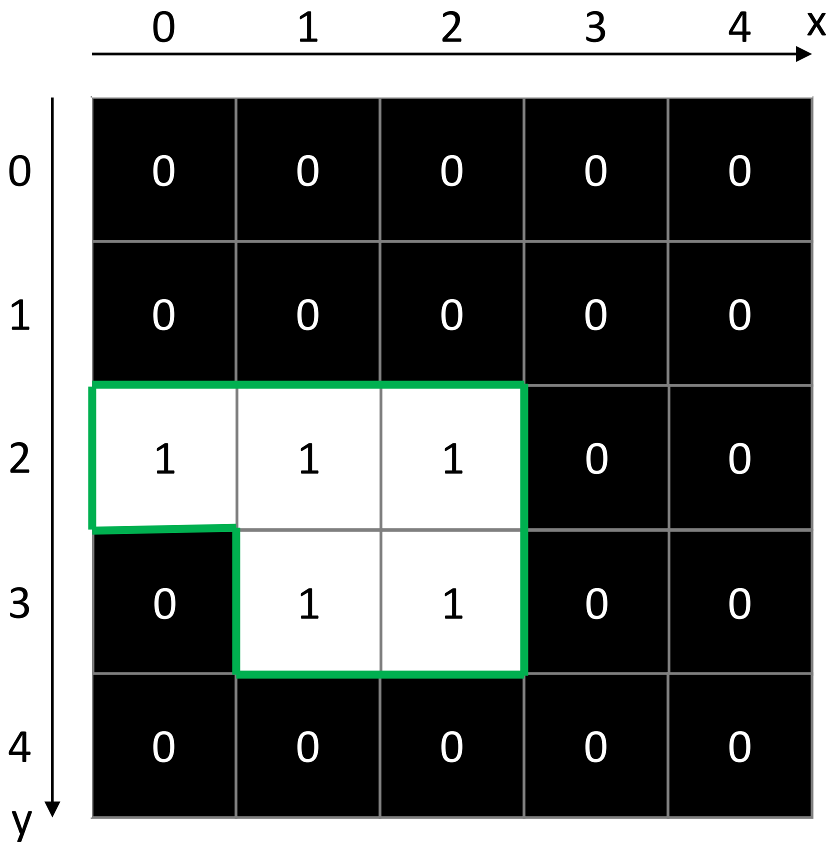
- A *feature* is a countable or measurable property of an image or object.
- Goal of feature extraction is finding a minimal set of features to describe an object well enough to differentiate it from other objects.

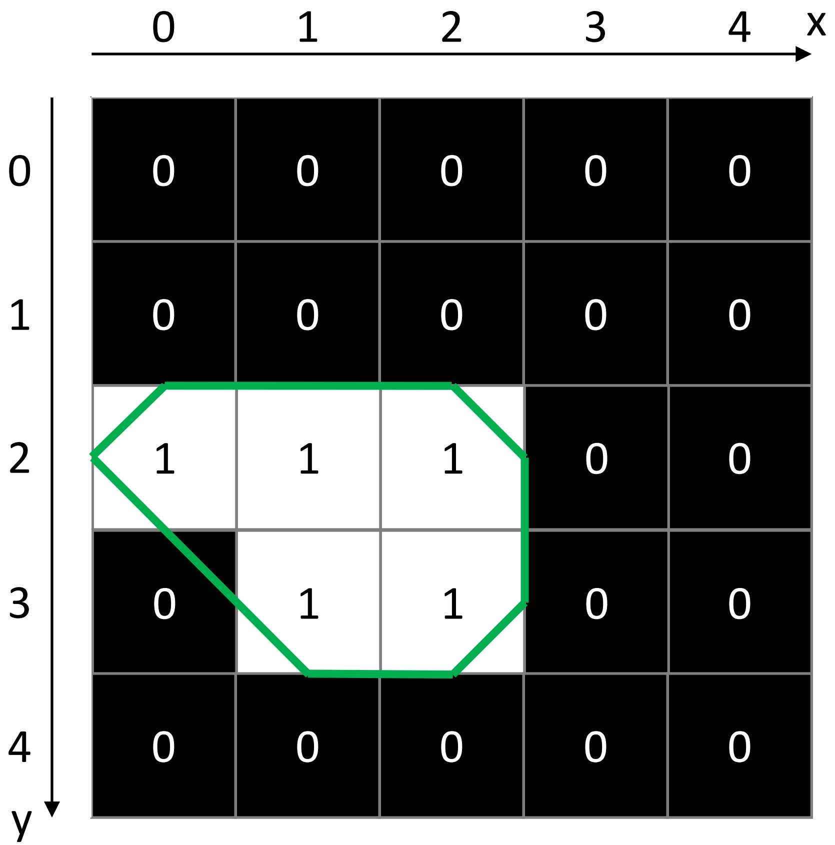
- **Intensity based**
  - Mean intensity
  - Standard deviation
  - Total intensity
  - Textures
- **Shape based /spatial**
  - Area / Volume
  - Roundness
  - Solidity
  - Circularity / Sphericity
  - Elongation
  - Centroid
  - Bounding box
- **Spatio-temporal**
  - Displacement,
  - Speed,
  - Acceleration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

- **Mixed features**
  - Center of mass
  - Local minima / maxima

Perimeter

- Length of the outline around an object
- Depends on the actual implementation



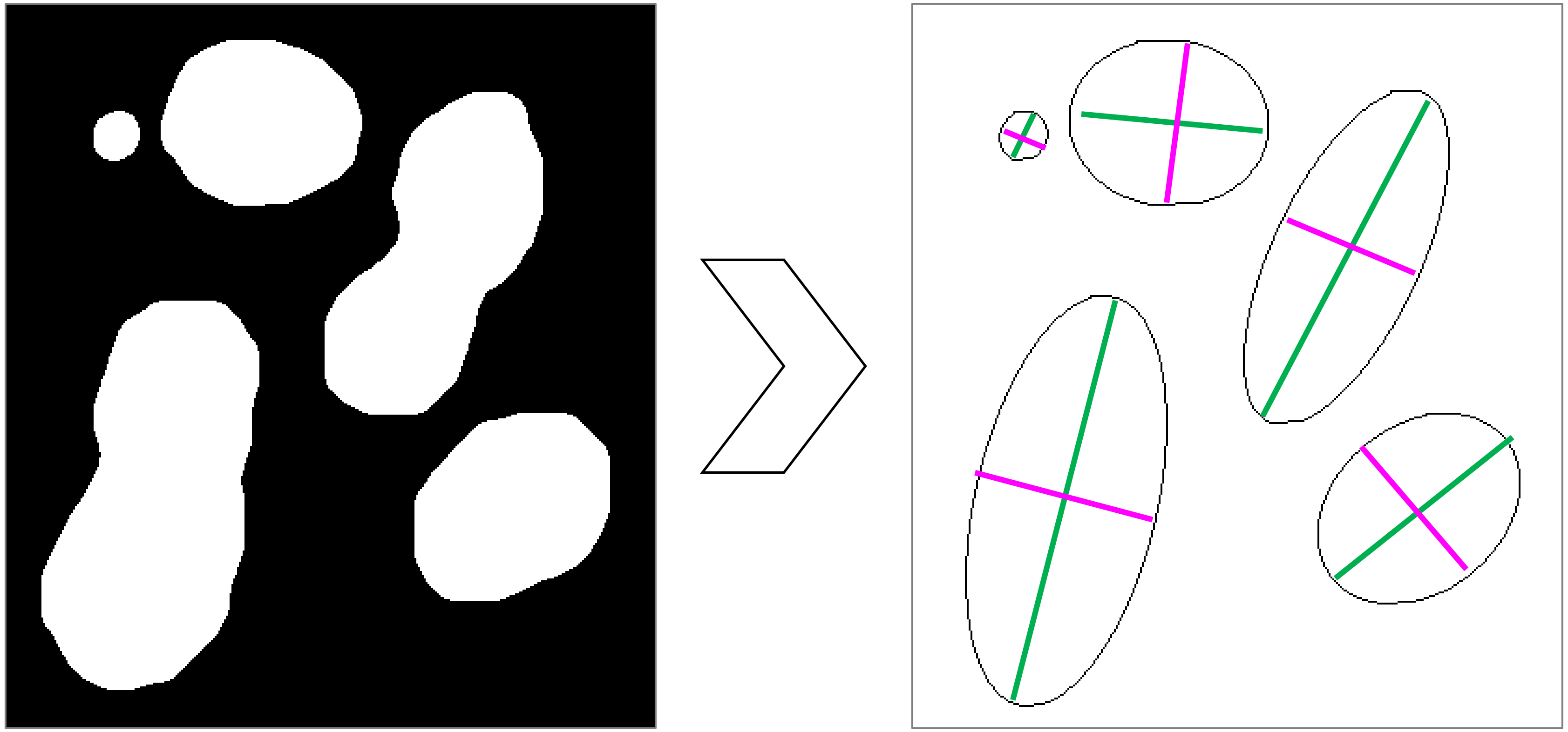


Fit ellipse

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

- Major axis … long diameter
- Minor axis … short diameter

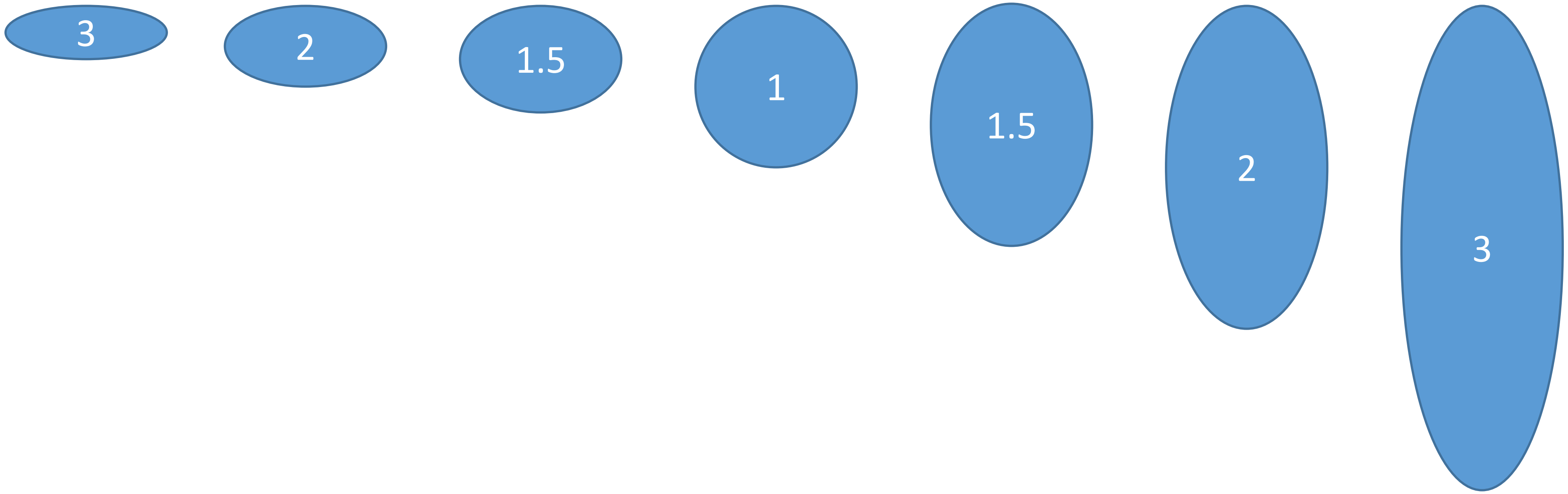
- Major and minor axis are perpendicular to each other



Aspect ratio

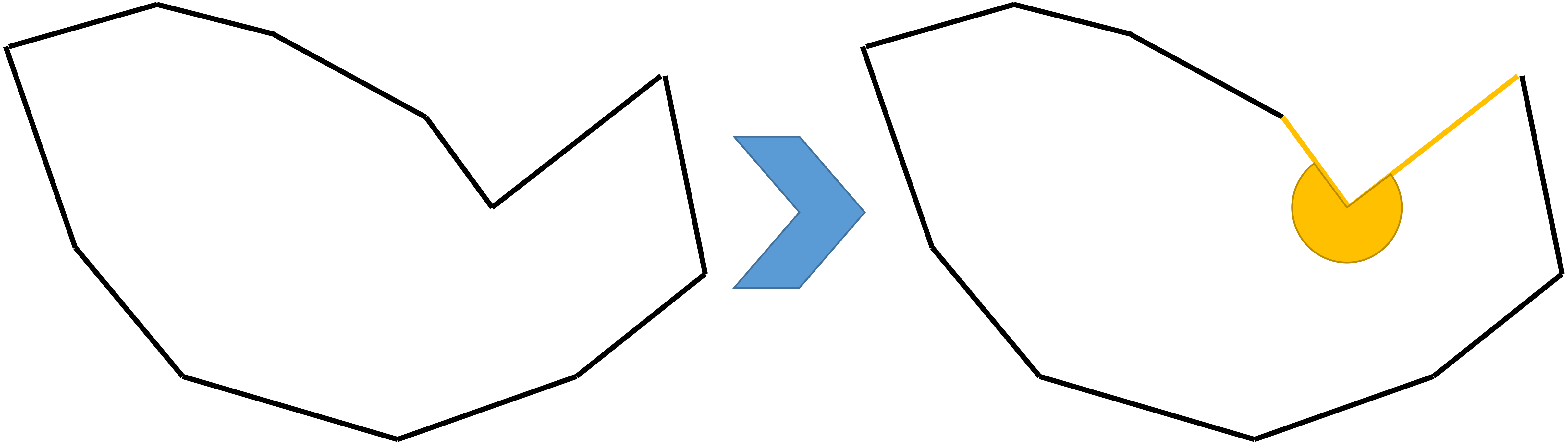
- The aspect ratio describes the elongation of an object.

AR = major / minor



Convex hull

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



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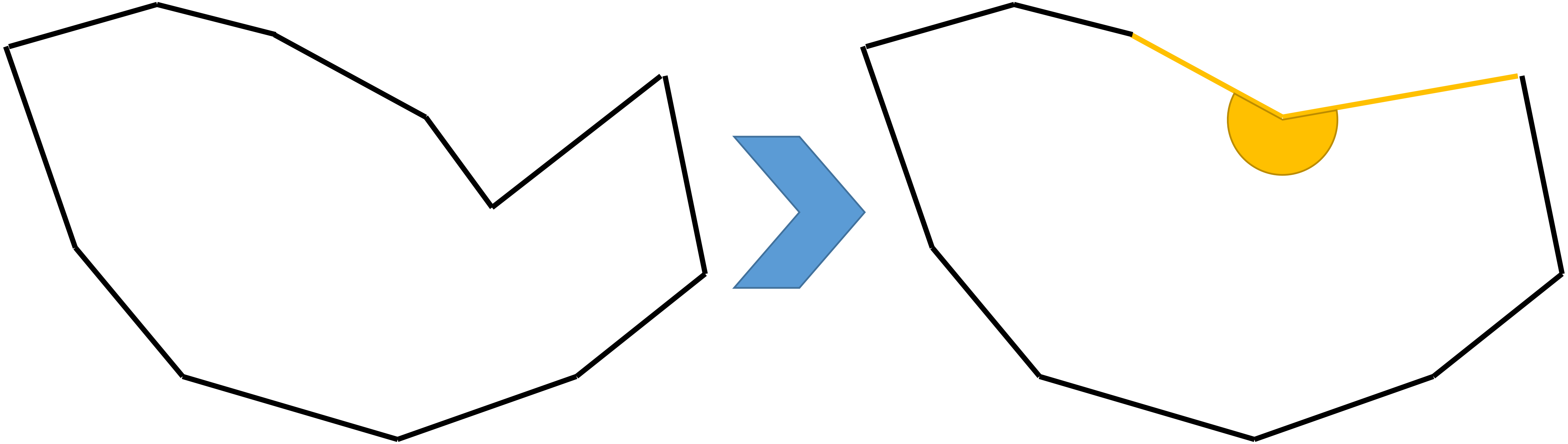

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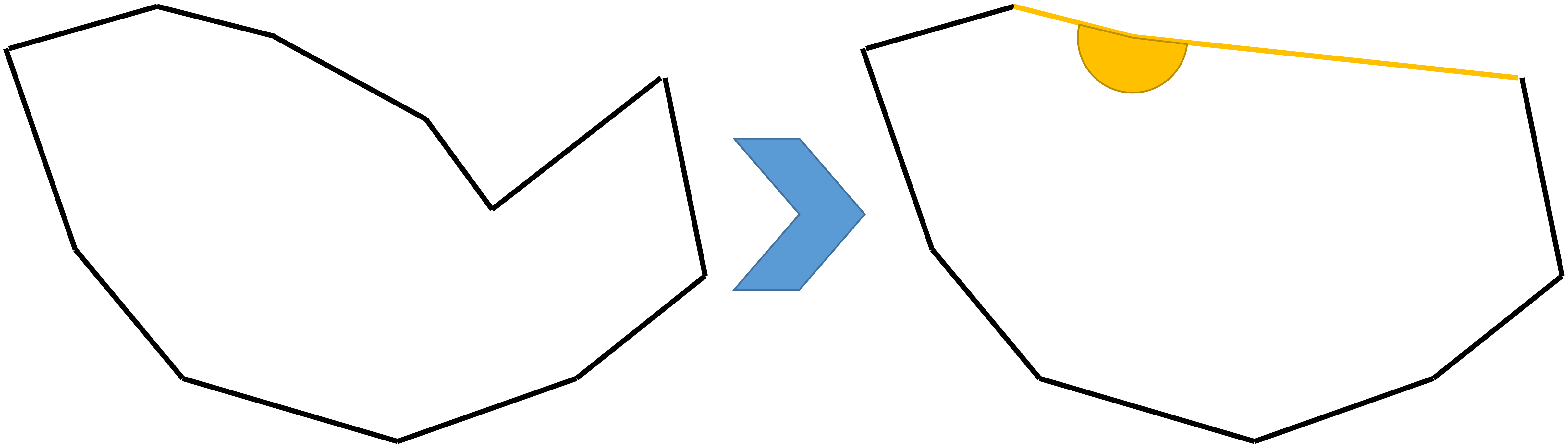

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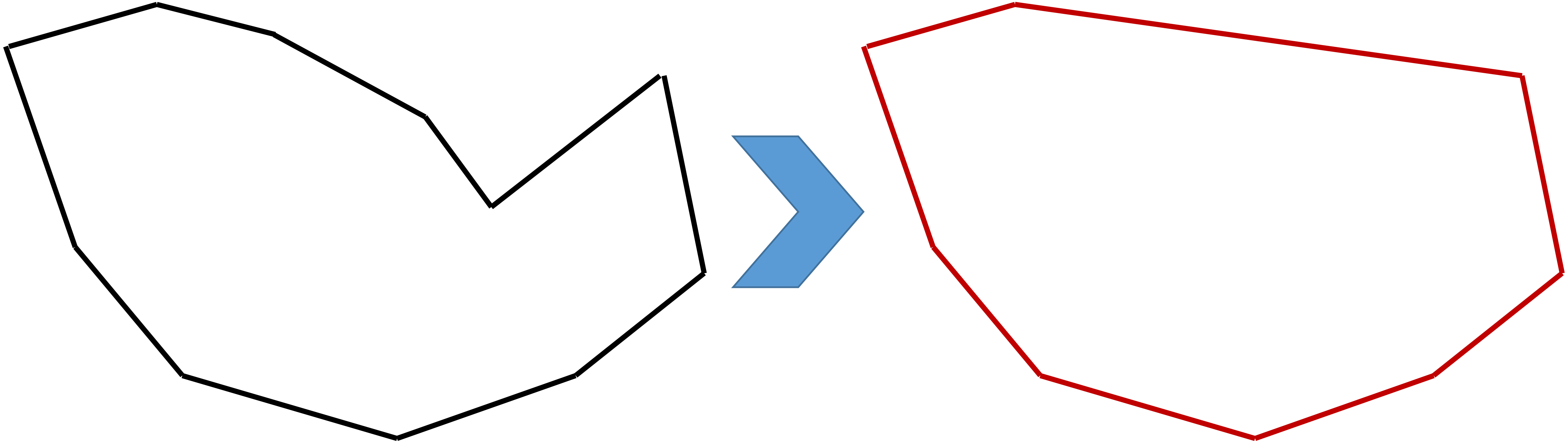
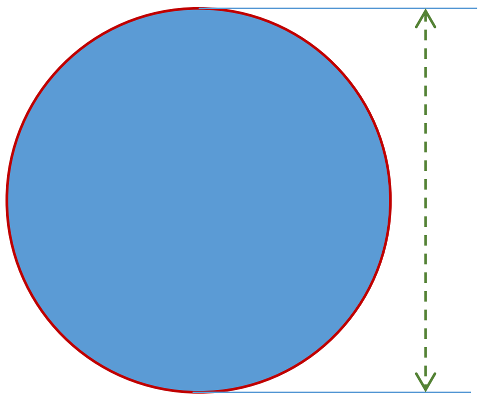


$$solidity = \frac{A}{A_{convexHull}}$$

Roundness and circularity

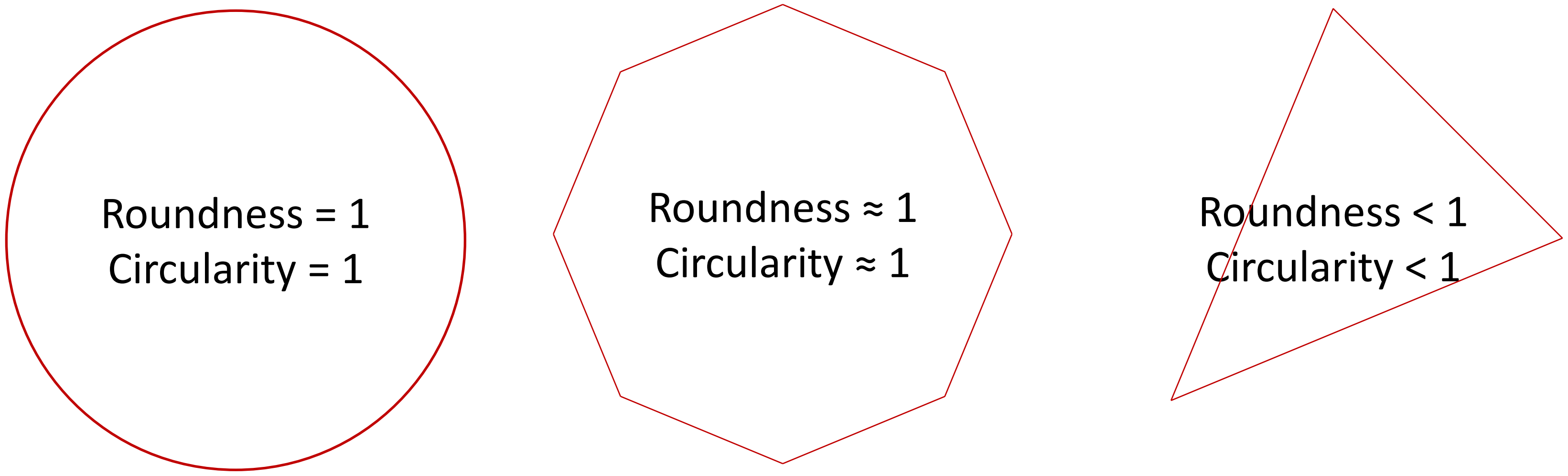
- The definition of a circle leads us to measurements of circularity and roundness.
- In case you use these measures, define them correctly. They are not standardized!

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



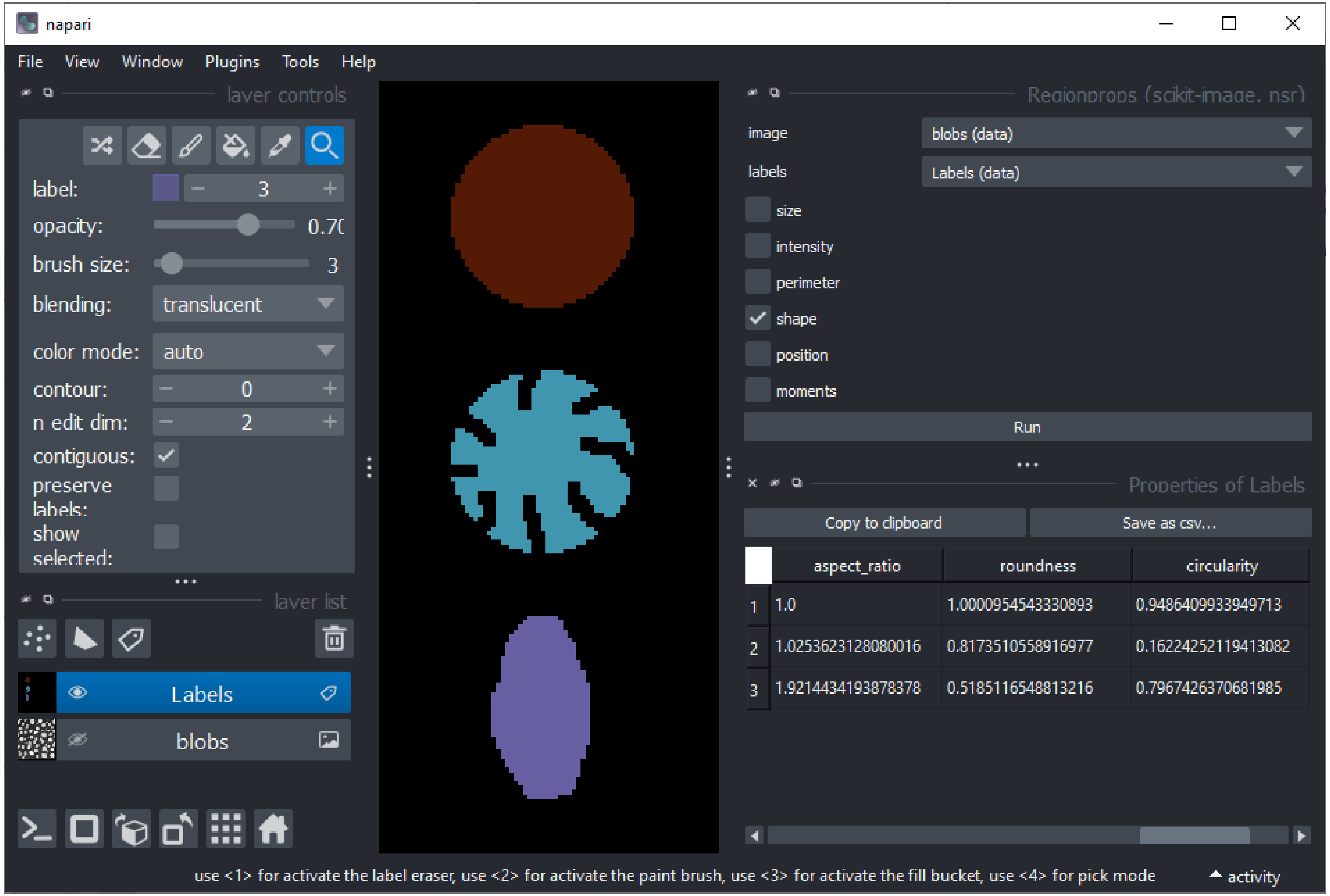
$$roundness = \frac{4 * A}{\pi \ major^2}$$

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



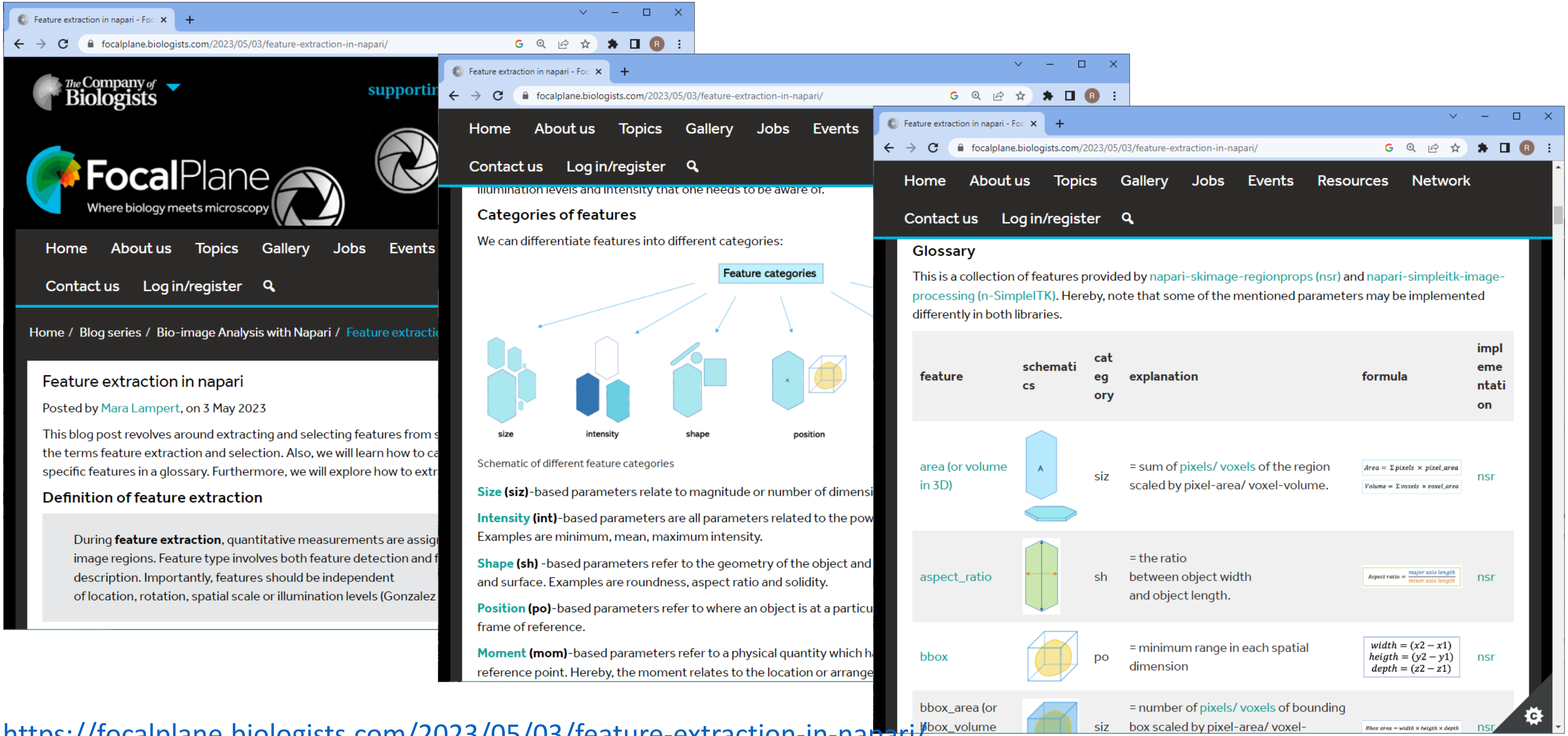
What’s the difference between circularity and roundness?

- *Draw* representative shapes and study measurements



Further reading

Physics of Life
TU Dresden



https://focalplane.biologists.com/2023/05/03/feature-extraction-in-napari/

Pixel classification using scikit-learn

Robert Haase

June 2023

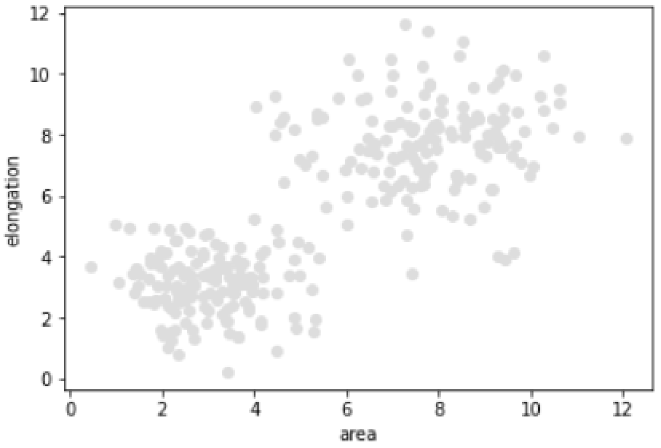
@haesleinhuepf

Tabular object classification

- Classify objects starting from feature vectors (table columns)

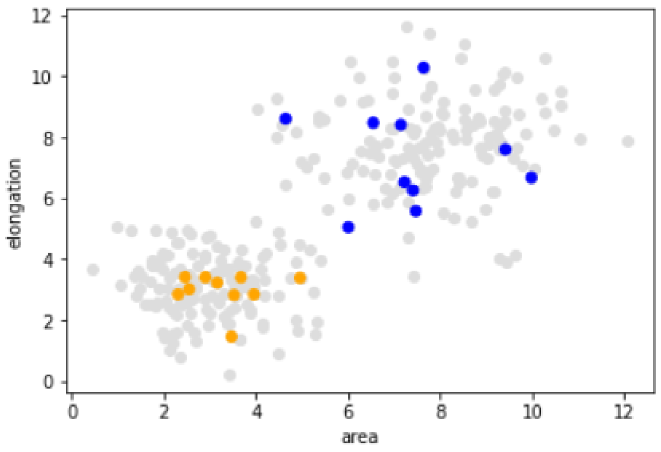
Raw data

	area	elongation
0	3.950088	2.848643
1	4.955912	3.390093
2	7.469852	5.575289
3	2.544467	3.017479
4	3.465662	1.463756
5	3.156507	3.232181
6	9.978705	6.676372
7	6.001683	5.047063
8	2.457139	3.416050
9	3.672295	3.407462
10	9.413702	7.598608



“Ground truth” annotation

```
annotation = [1, 1, 2, 1, 1, 1, 2, 2,
```

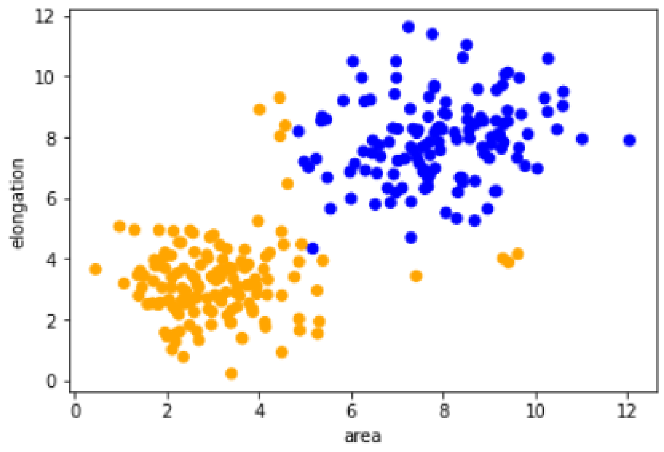


Classifier training

```
classifier = RandomForestClassifier()
classifier.fit(train_data, train_annotation)
```

Classifier prediction

```
result = classifier.predict(validation_data)
```

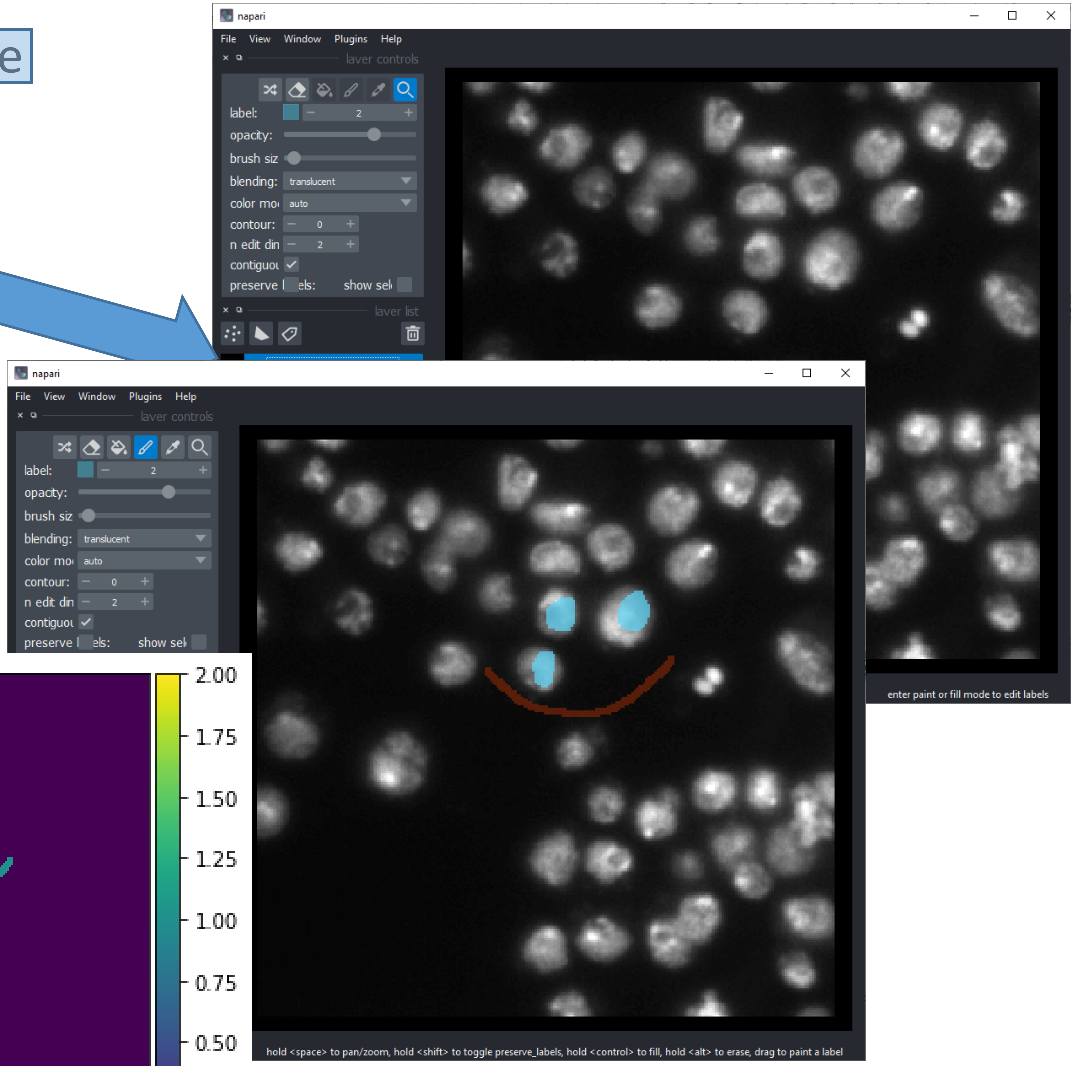


https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/01_supervised_machine_learning.ipynb

Interactive pixel classification

- Prepare an empty layer for annotations and keep a reference

```
labels = viewer.add_labels(
    np.zeros(image.shape).astype(int))
```



- Read annotations

```
manual_annotations = labels.data
```

```
from skimage.io import imshow
imshow(manual_annotations,
             vmin=0, vmax=2)
```

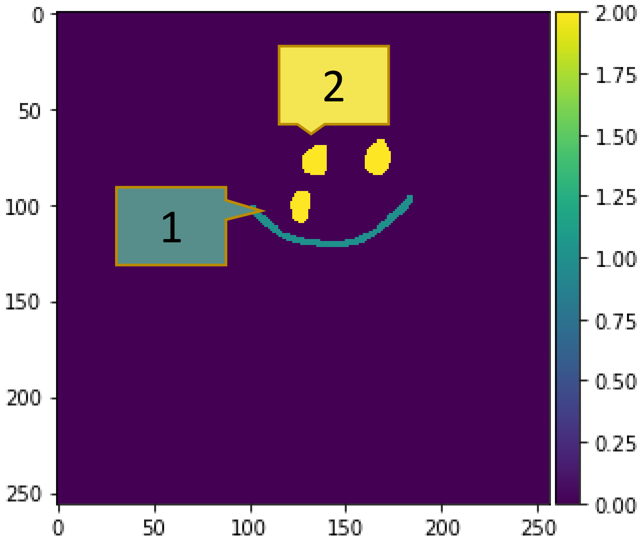


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

@haesleinhuepf

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Interactive pixel classification

- Pixel classification using scikit-learn
  - Expects one-dimensional arrays for
    - every feature individually
    - ground truth

```
# train classifier
from sklearn.ensemble import RandomForestClassifier
classifier = RandomForestClassifier(max_depth=2, random_state=0)
classifier.fit(X, y)
```

Image data

Ground truth / annotation

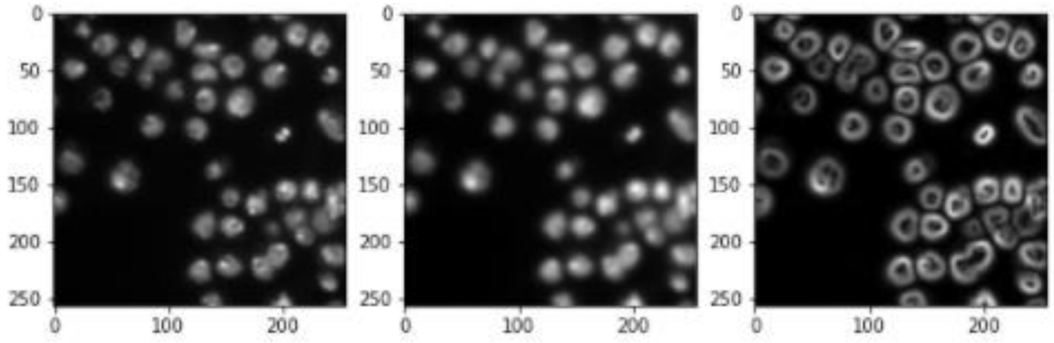
```
y_ = classifier.predict(X)
```

Image data

prediction

Interactive pixel classification

- Pixel classification using scikit-learn
  - Expects one-dimensional arrays for
    - every feature individually
    - ground truth




```
# for training, we need to generate features
feature_stack = generate_feature_stack(image)
X, y = format_data(feature_stack, manual_annotations)

# train classifier
from sklearn.ensemble import RandomForestClassifier
classifier = RandomForestClassifier(max_depth=2, random_state=0)
classifier.fit(X, y)
```

Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection [Caicedo et al., Nature Methods, 2019].

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Interactive pixel classification

- Pixel classification using scikit-learn

```
# process the whole image and show result
result_1d = classifier.predict(feature_stack.T)
result_2d = result_1d.reshape(image.shape)

viewer.add_labels(result_2d)
```

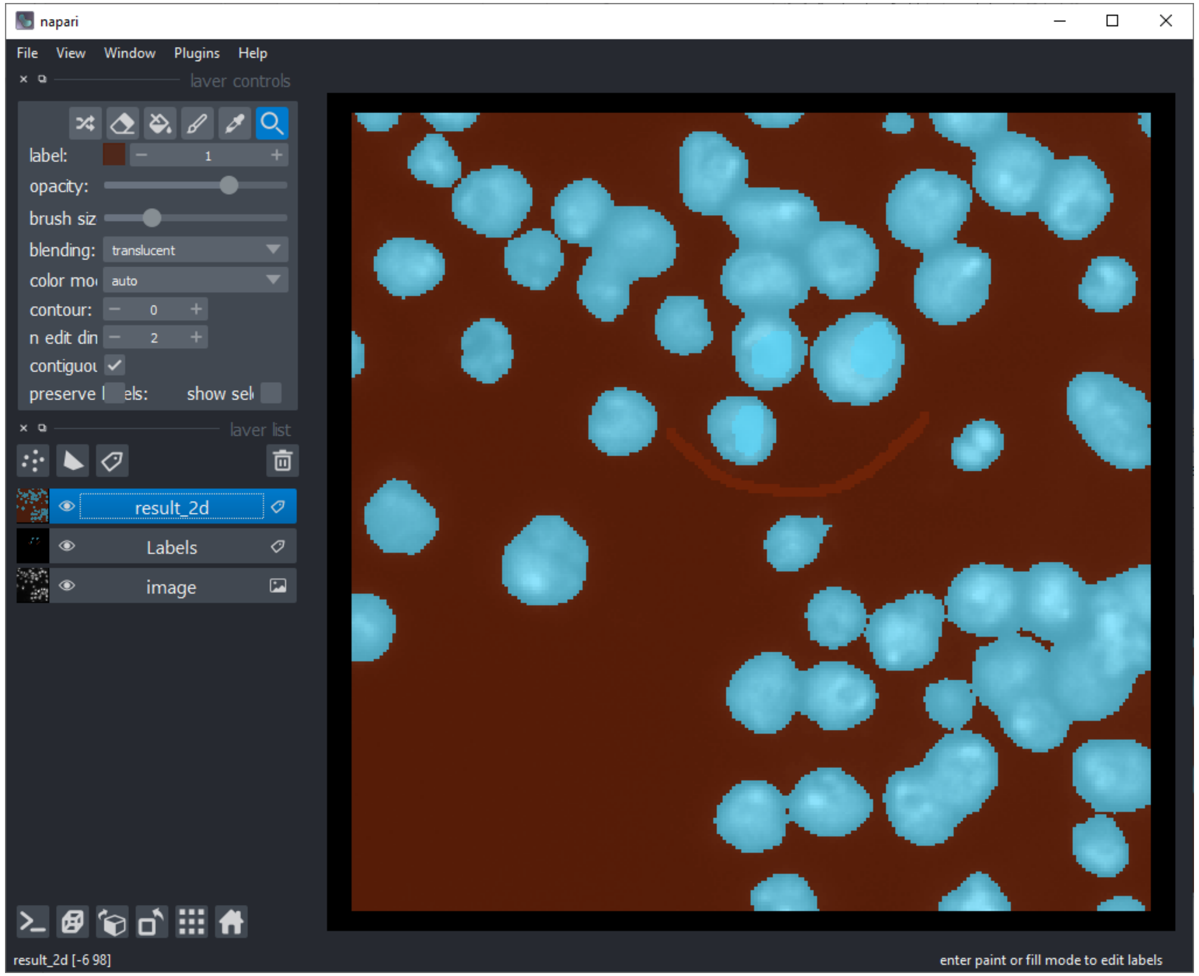


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

@haesleinhuepf

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Interactive pixel classification

- Jupyter notebooks and napari side-by-side

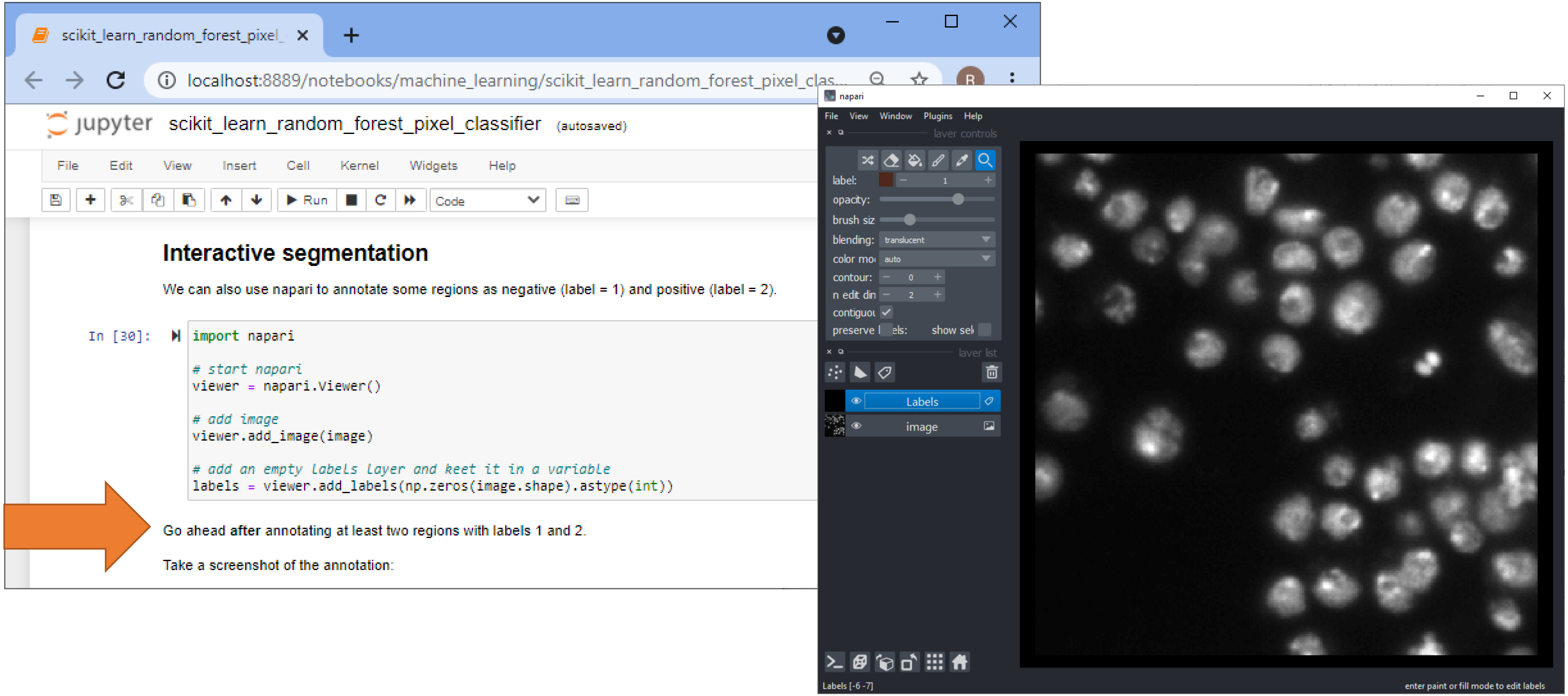


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Interactive pixel classification

- Jupyter notebooks and napari side-by-side

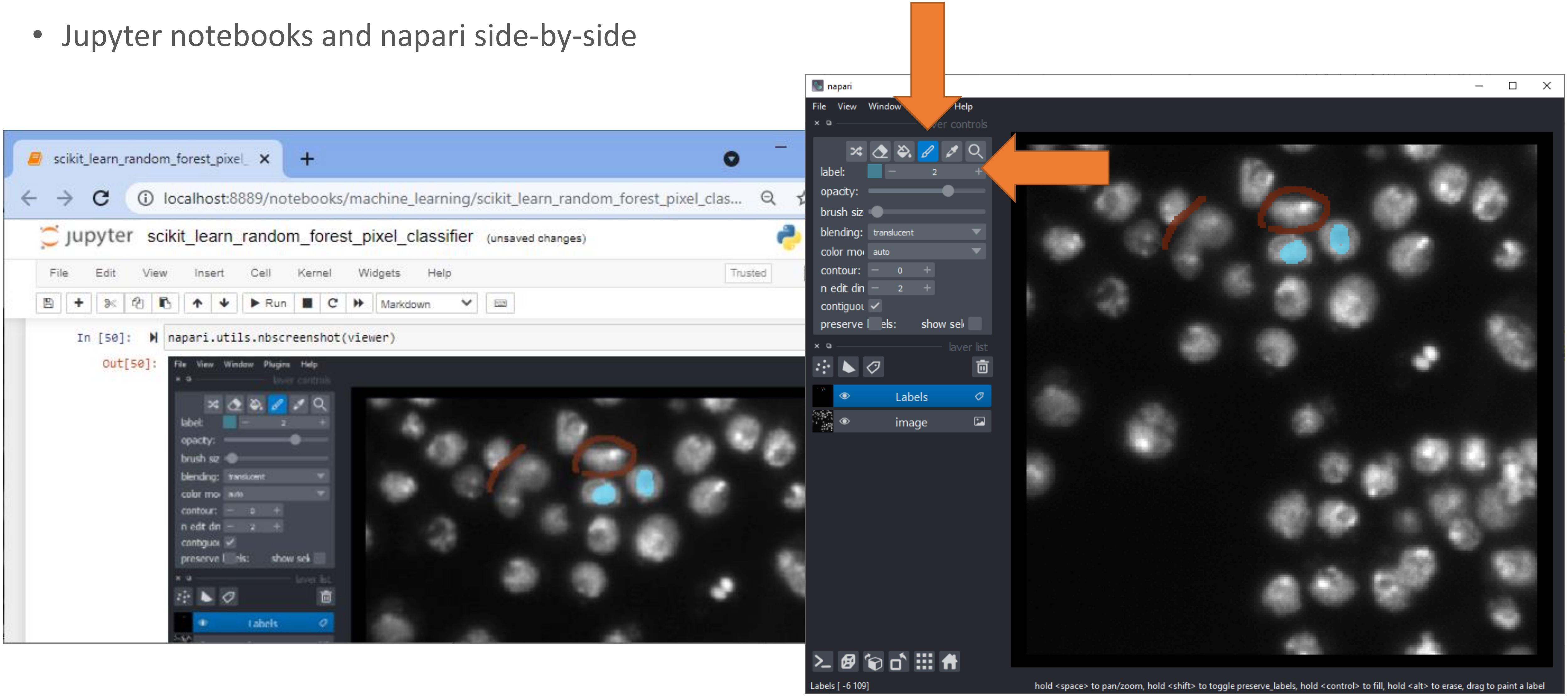


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

@haesleinhuepf

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Interactive pixel classification

- Jupyter notebooks and napari side-by-side

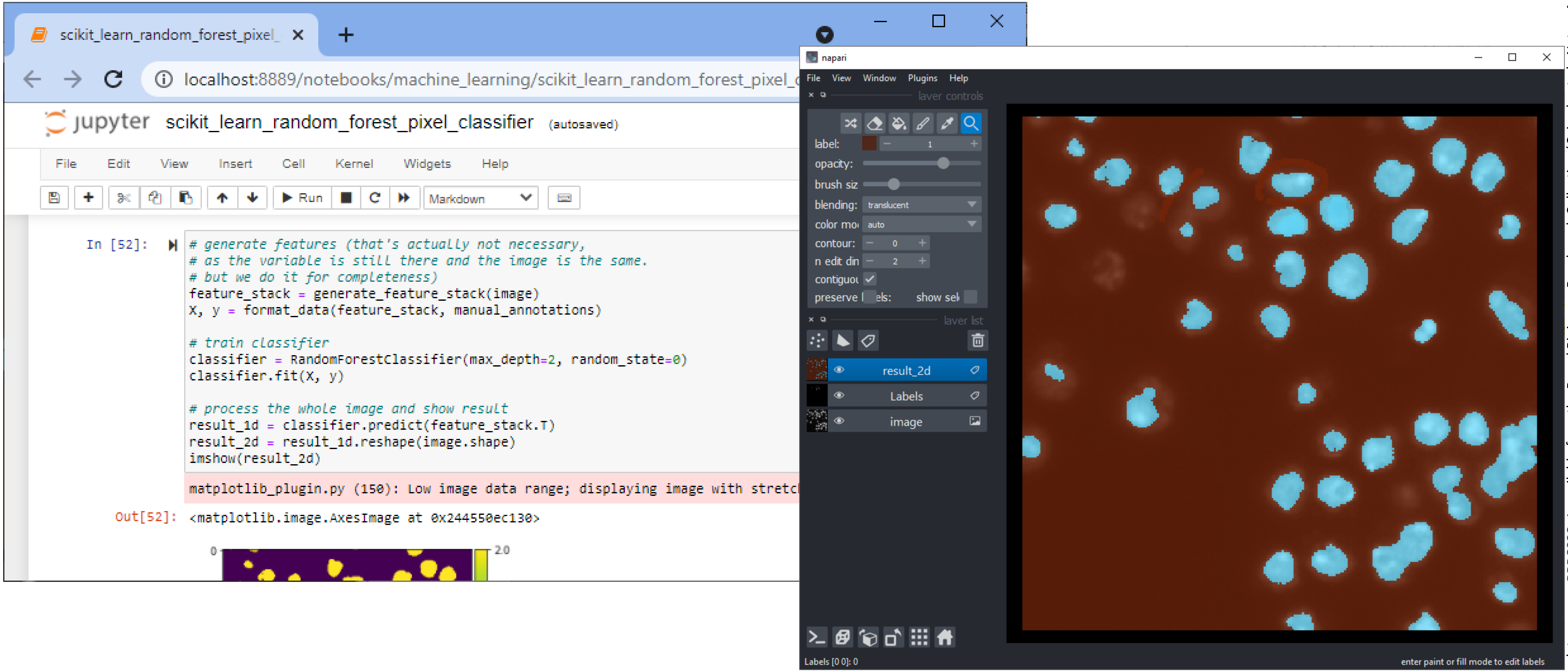


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/02_scikit_learn_random_forest_pixel_classifier.ipynb

Accelerated pixel and object classification (APOC)

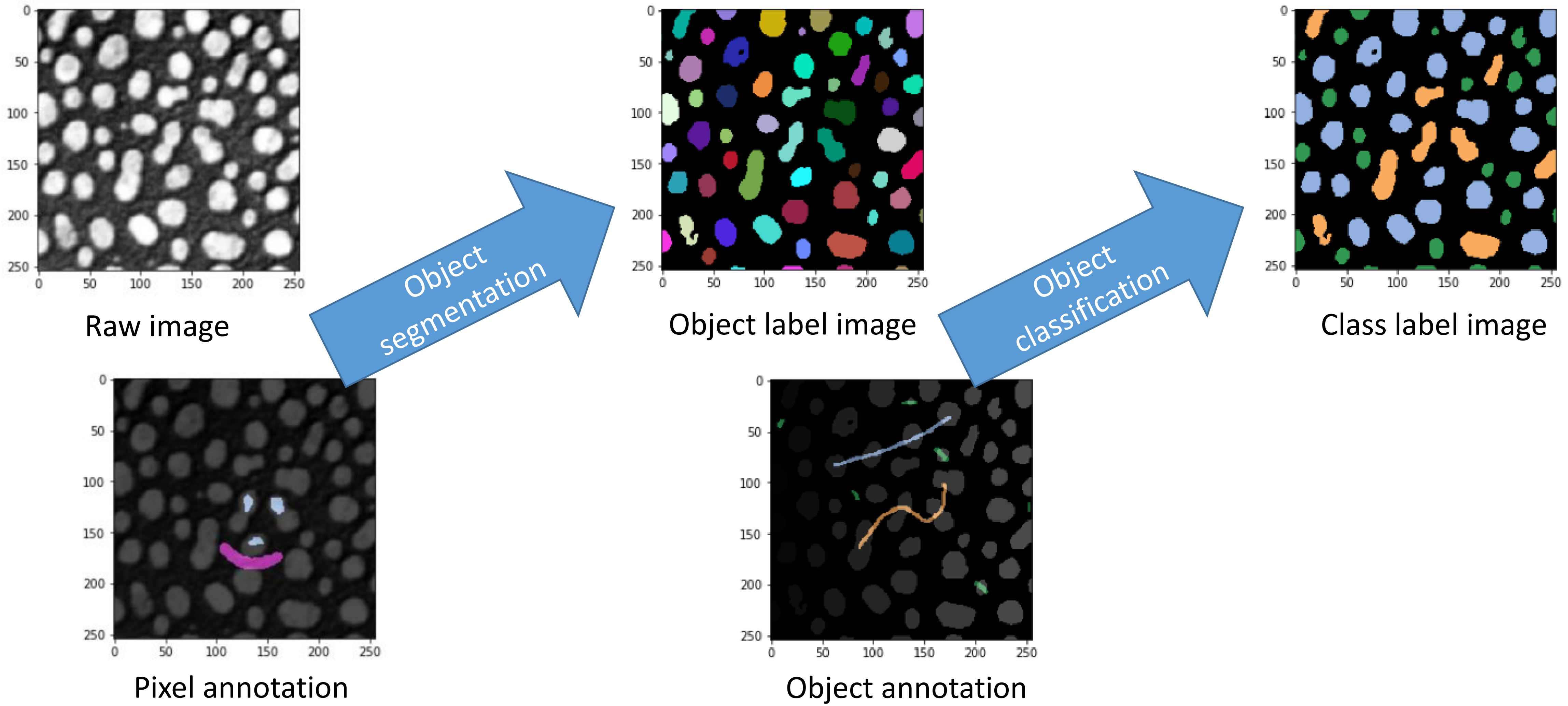
Robert Haase

October 2022

@haesleinhuepf

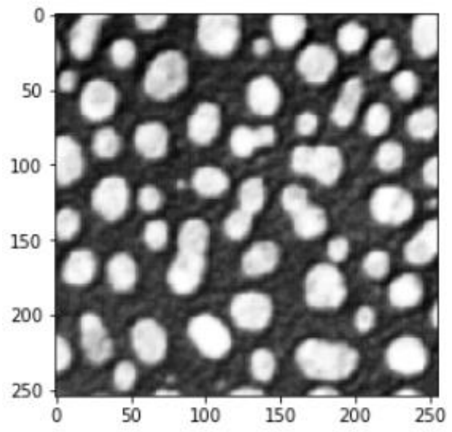
Accelerated pixel and object classification

- APOC is a python library that makes use of OpenCL-compatible Graphics Cards to accelerate pixel and object classification

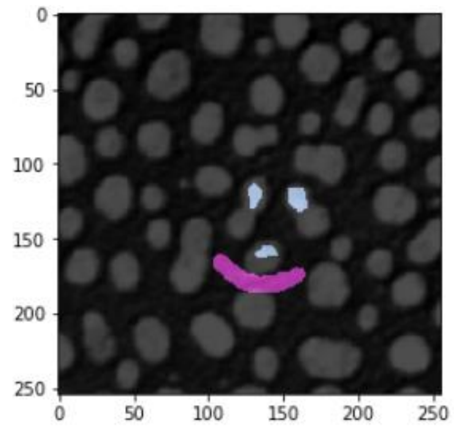


Object segmentation

- Pixel classification + connected component labeling



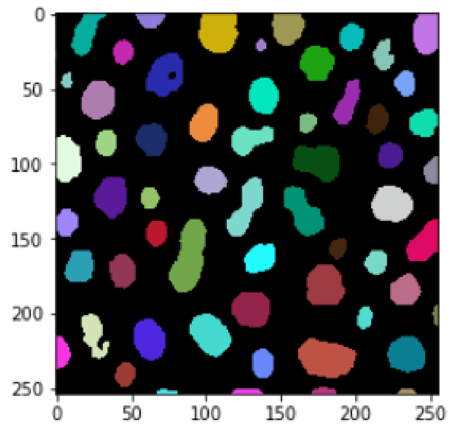
Raw image



Pixel annotation

```
# define features
features = "gaussian_blur=1 gaussian_blur=5 sobel_of_gaussian_blur=1"

# this is where the model will be saved
cl_filename = 'my_object_segmenter.cl'

# delete classifier in case the file exists already
apoc.erase_classifier(cl_filename)

# train classifier
clf = apoc.ObjectSegmenter(opencl_filename=cl_filename, positive_class_identifier=2)
clf.train(features, manual_annotations, image)

segmentation_result = clf.predict(features=features, image=image)
cle.imshow(segmentation_result, labels=True)
```

Object segmentation

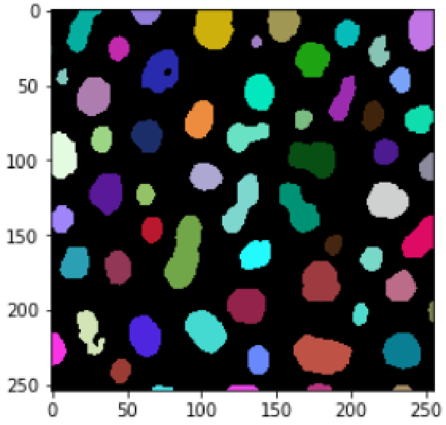


Object label image

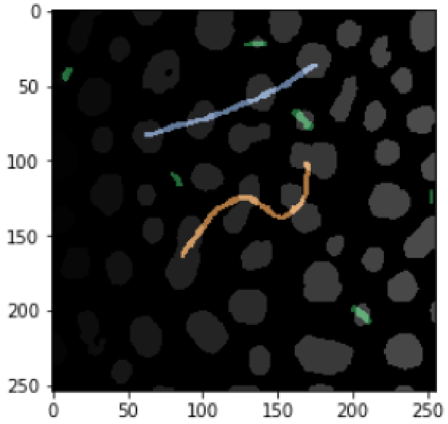
https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/03_apoc_object_segmenter.ipynb

Object classification

- Feature extraction + tabular classification



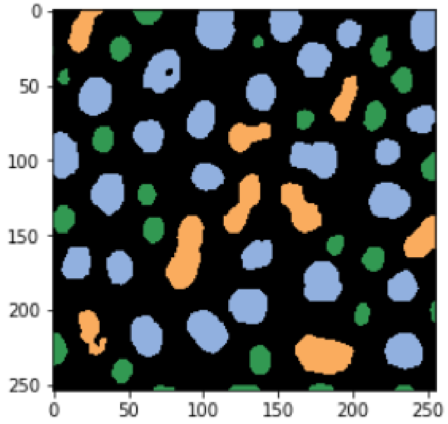
Object label image



Object annotation

```python
# for the classification we define size and shape as criteria
features = 'area mean_max_distance_to_centroid_ratio'

# This is where the model will be saved
cl_filename_object_classifier = "my_object_classifier.cl"

# delete classifier in case the file exists already
apoc.erase_classifier(cl_filename_object_classifier)

# train the classifier
classifier = apoc.ObjectClassifier(cl_filename_object_classifier)
classifier.train(features, segmentation_result, annotation, image)

# determine object classification
classification_result = classifier.predict(segmentation_result, image)
cle.imshow(classification_result, labels=True)
```

Object classification

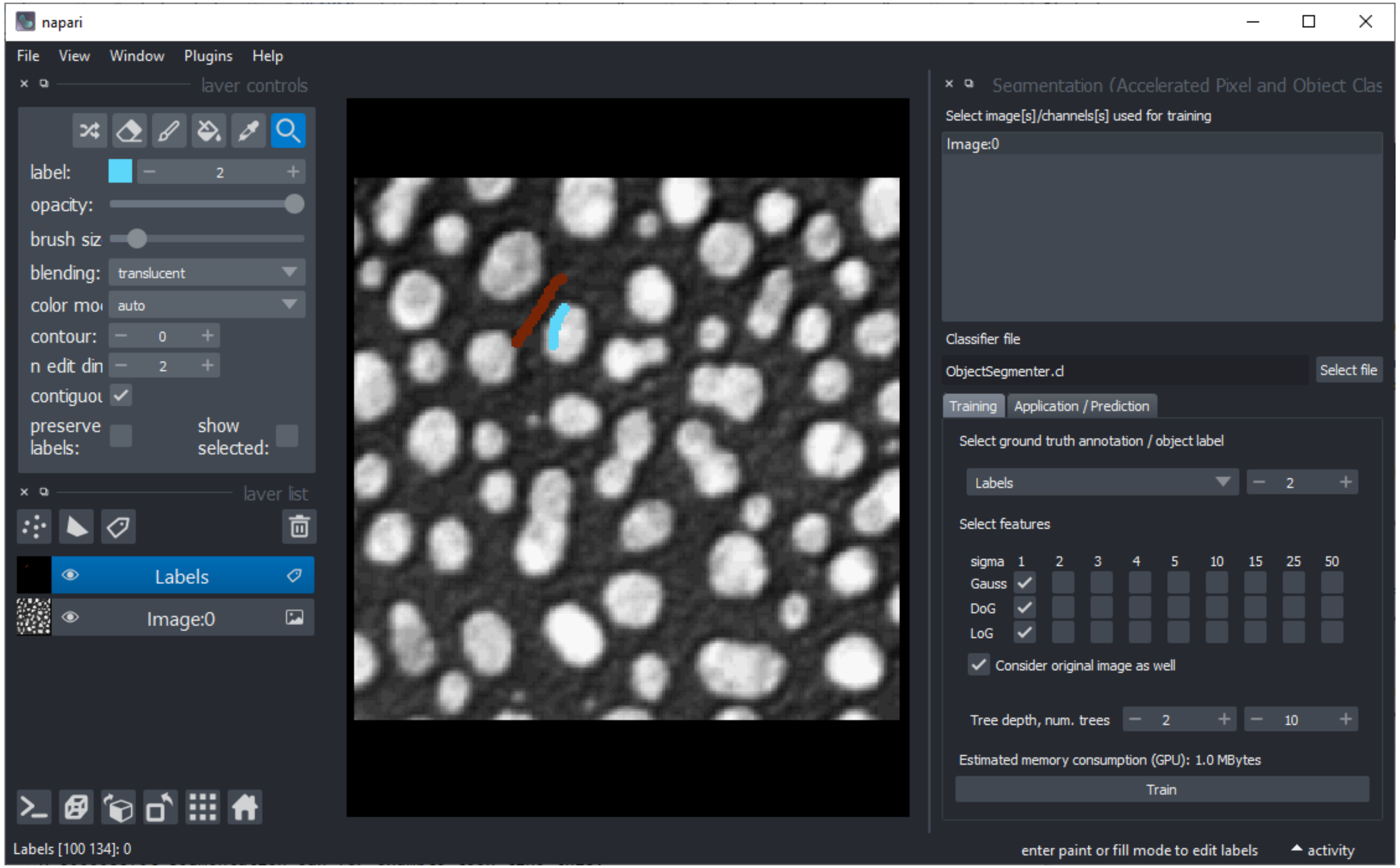


Class label image

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/03_apoc_object_segmenter.ipynb

Graphical user interface

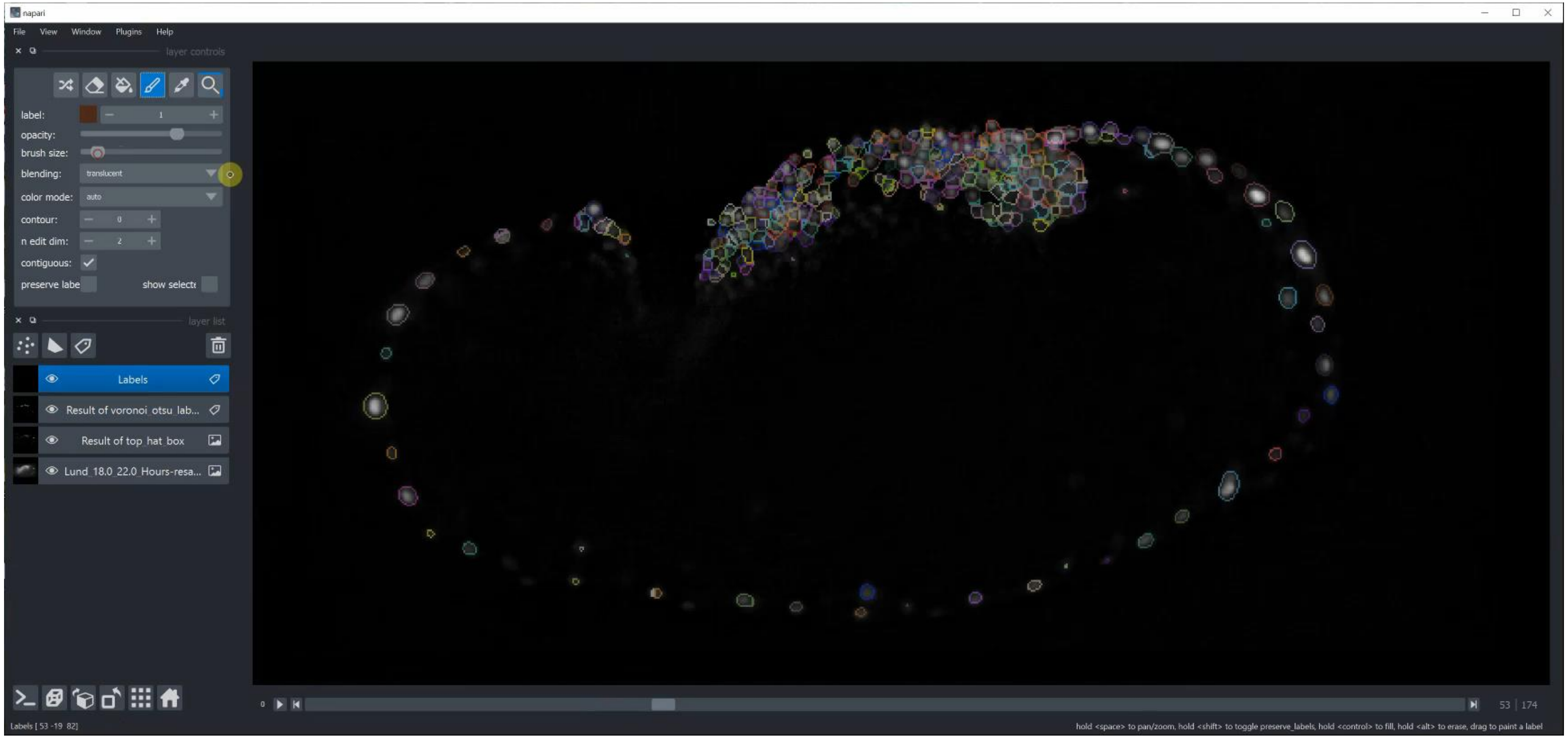
- Object segmentation
- https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification#object-and-semantic-segmentation



Supervised machine learning for tissue classification

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

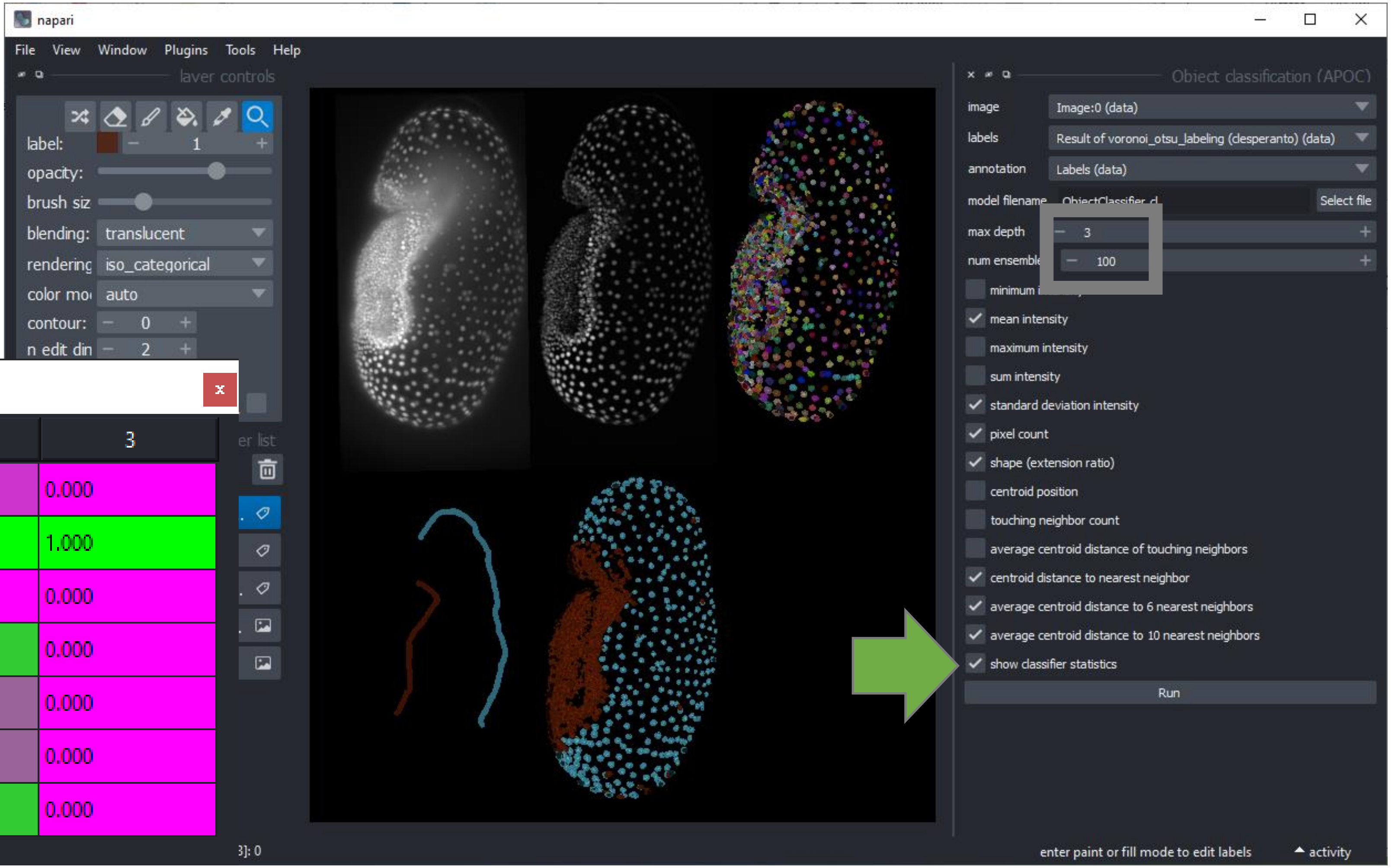
Data exploration / supervised machine learning

- Inspect how the random forest classifier makes decisions

- Note: Beware of correlated parameters!

Dock widget 1

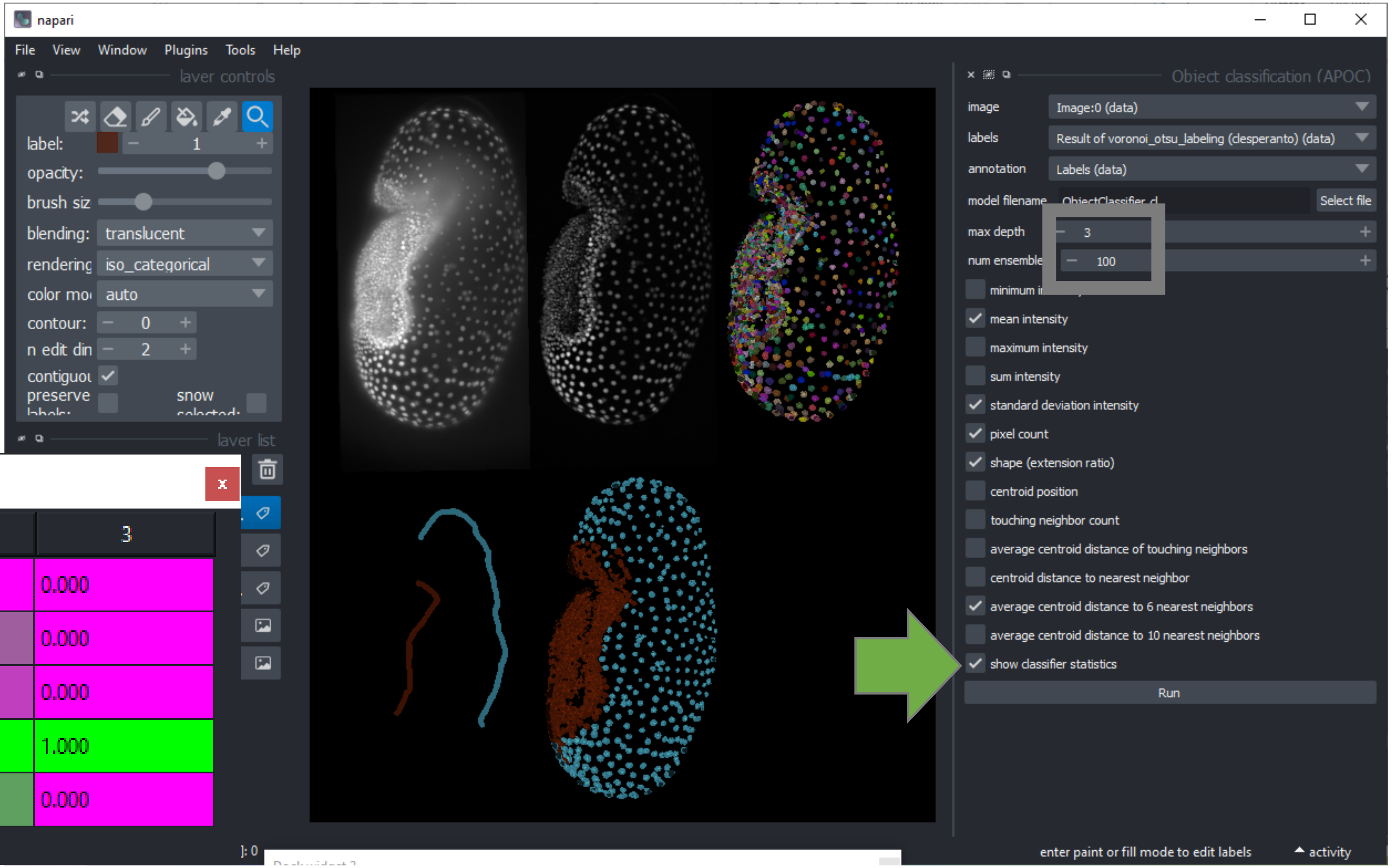
	1	2	3
area	0.010	0.056	0.000
mean_intensity	0.200	0.278	1.000
standard_deviation_intensity	0.030	0.000	0.000
mean_max_distance_to_centroid_ratio	0.270	0.222	0.000
average_distance_of_n_nearest_neighbors=1	0.120	0.111	0.000
average_distance_of_n_nearest_neighbors=6	0.170	0.111	0.000
average_distance_of_n_nearest_neighbors=10	0.200	0.222	0.000



Data exploration / supervised machine learning

- Inspect how the random forest classifier makes decisions

- Note: Beware of correlated parameters!



Dock widget 2

	1	2	3
area	0.060	0.000	0.000
mean_intensity	0.330	0.167	0.000
standard_deviation_intensity	0.040	0.111	0.000
mean_max_distance_to_centroid_ratio	0.260	0.444	1.000
average_distance_of_n_nearest_neighbors=6	0.310	0.278	0.000

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

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

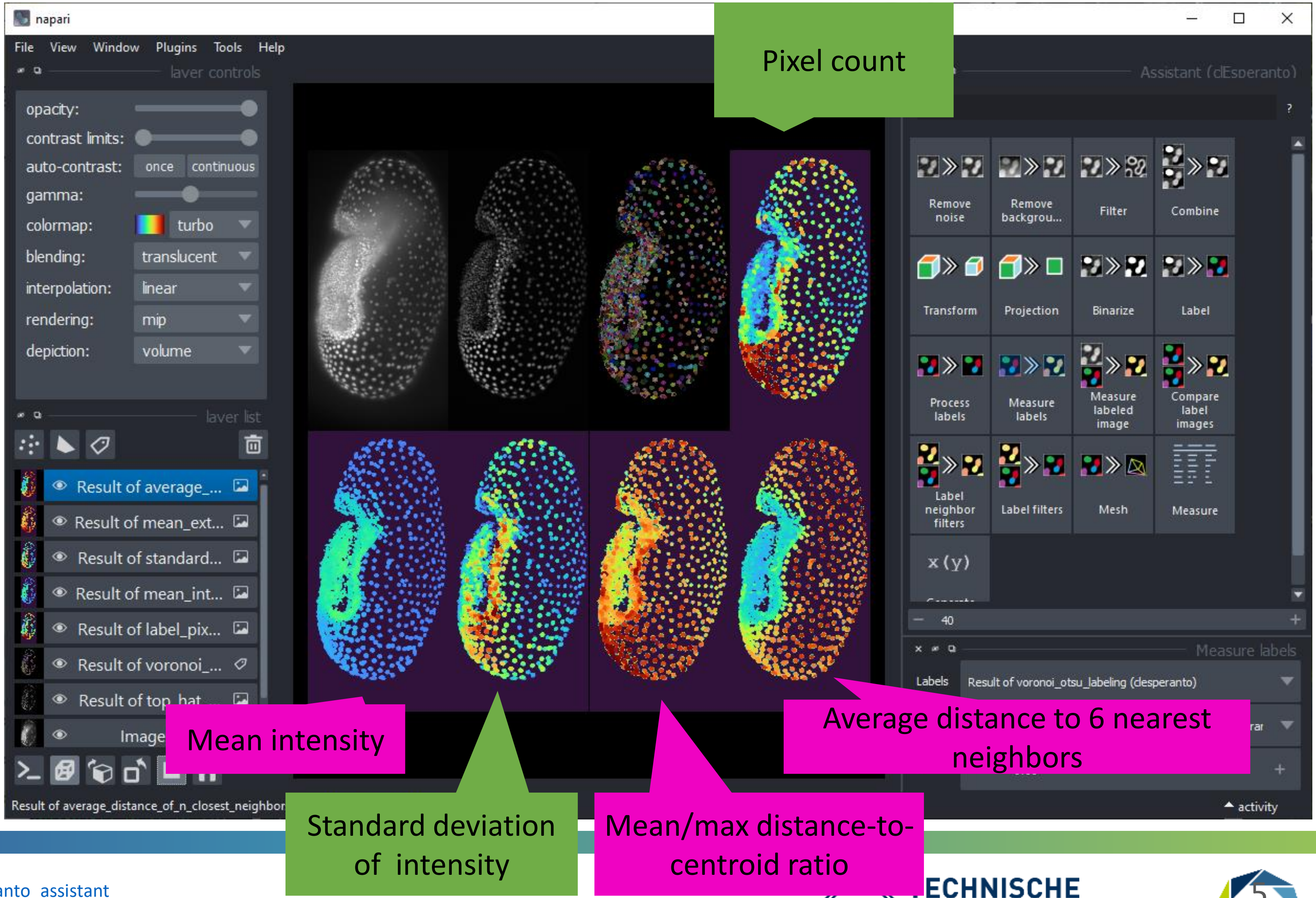
Data exploration / supervised machine learning

PoL Physics of Life TU Dresden

- Inspect how the random forest classifier makes decisions
- Note: Beware of correlated parameters!

Dock widget 2

	1	2	3
area	0.060	0.000	0.000
mean_intensity	0.330	0.167	0.000
standard_deviation_intensity	0.040	0.111	0.000
mean_max_distance_to_centroid_ratio	0.260	0.444	1.000
average_distance_of_n_nearest_neighbors=6	0.310	0.278	0.000



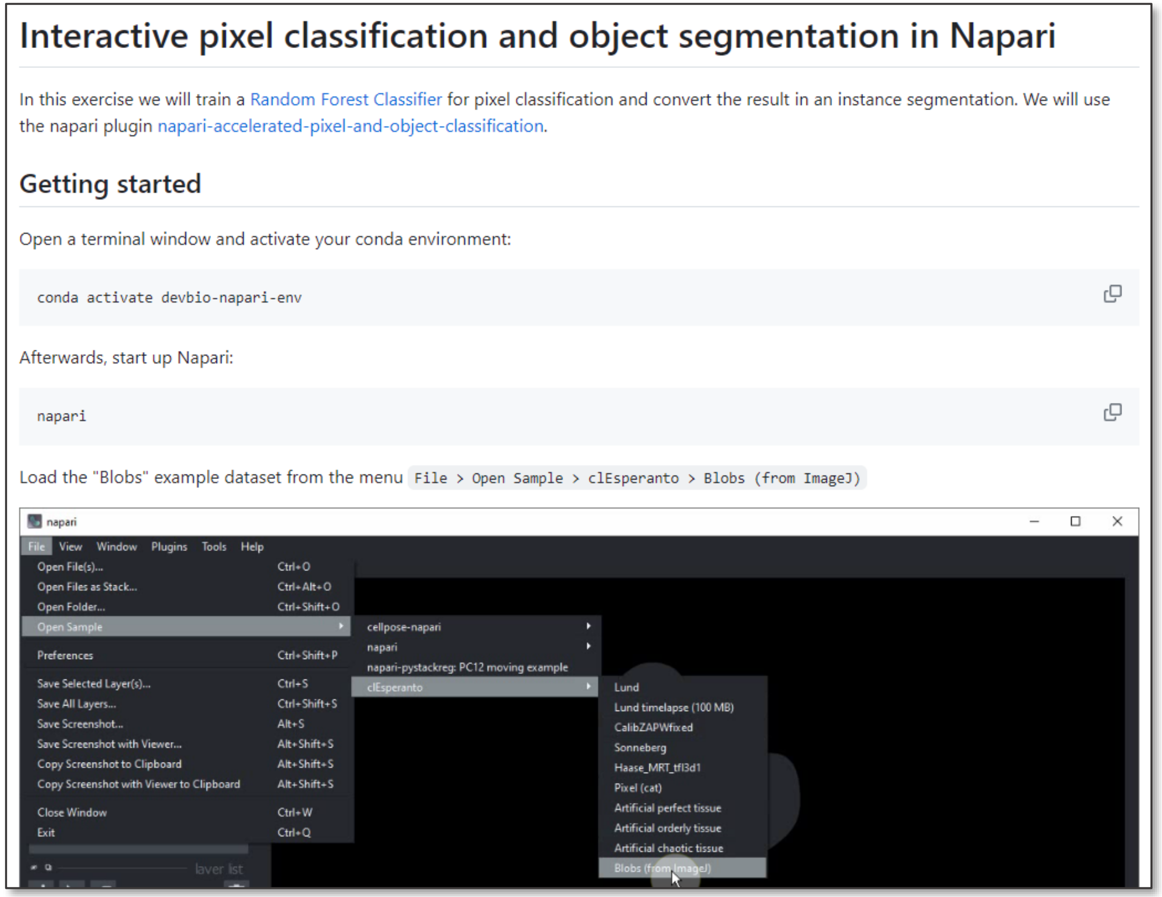
Physics of Life TU Dresden

CENTER FOR SYSTEMS BIOLOGY DRESDEN

Exercises

Pixel classification / object segmentation

- Use Napari to segment objects



Interactive pixel classification and object segmentation in Napari

In this exercise we will train a Random Forest Classifier for pixel classification and convert the result in an instance segmentation. We will use the napari plugin napari-accelerated-pixel-and-object-classification.

Getting started

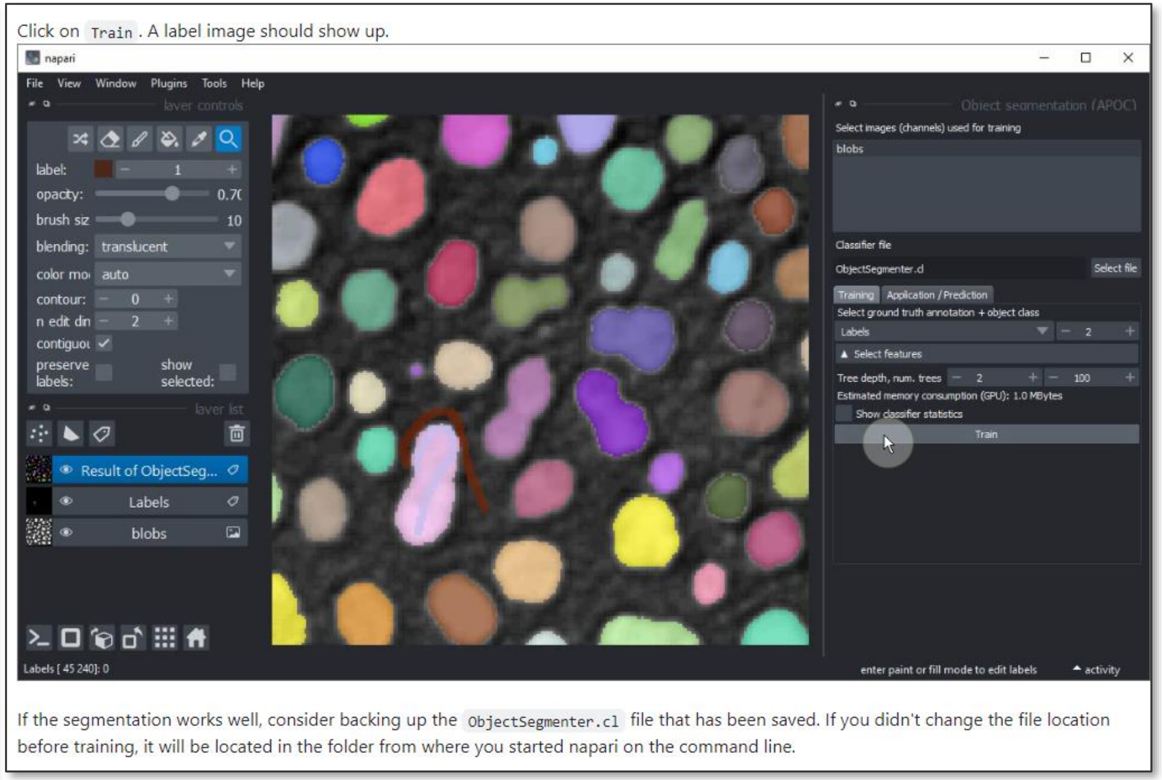
Open a terminal window and activate your conda environment:

```
conda activate devbio-napari-env
```

Afterwards, start up Napari:

```
napari
```

Load the "Blobs" example dataset from the menu `File > Open Sample > clEsperanto > Blobs (from ImageJ)`



Click on `Train`. A label image should show up.

If the segmentation works well, consider backing up the `ObjectSegmenter.cl` file that has been saved. If you didn't change the file location before training, it will be located in the folder from where you started napari on the command line.

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/interactive_pixel_classification/readme.md

Object classification

- Use Napari to group round and elongated objects

Interactive object classification in Napari

In this exercise we will train a Random Forest Classifiers for classifying segmented objects. We will use the napari plugin napari-accelerated-pixel-and-object-classification.

Getting started

Open a terminal window and activate your conda environment:

```
conda activate devbio-napari-env
```

Afterwards, start up Napari:

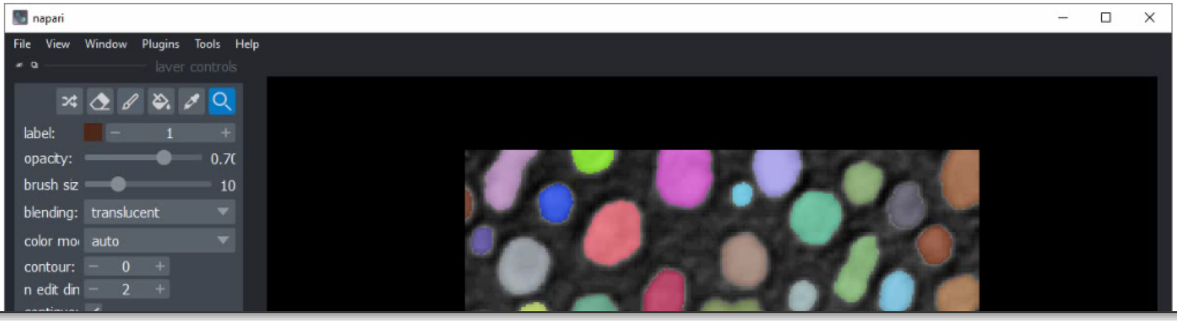
```
napari
```

Load the "Blobs" example dataset from the menu `File > Open Sample > clEsperanto > Blobs (from ImageJ)`

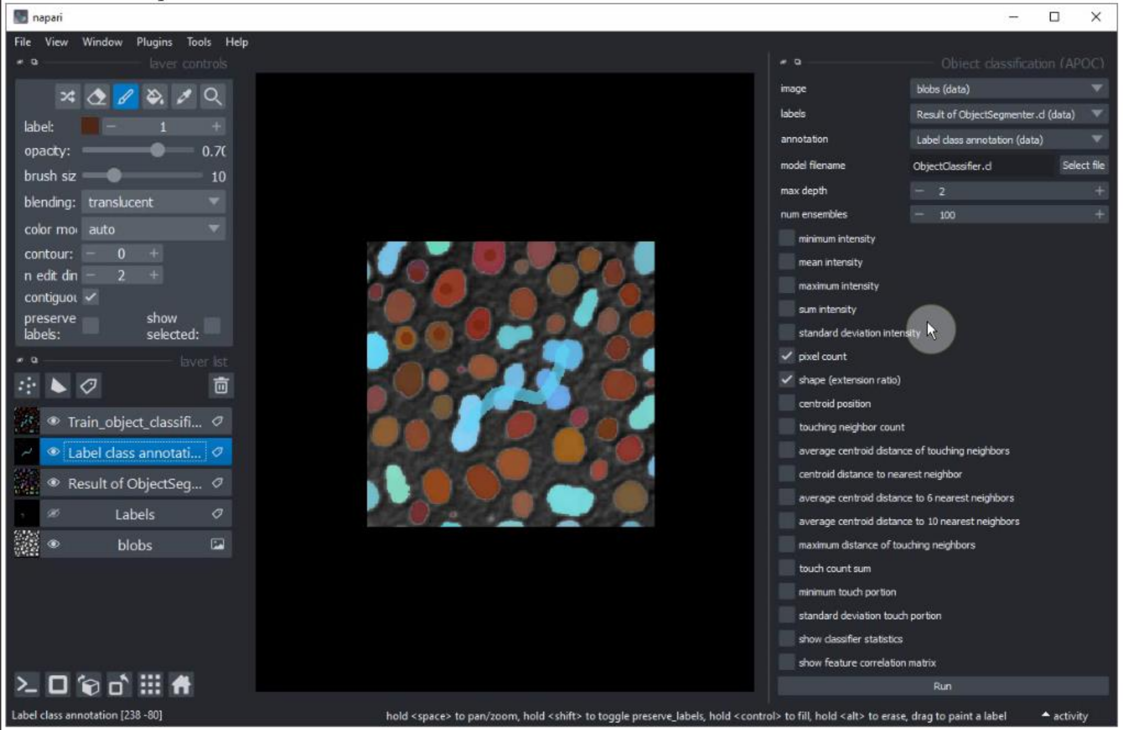
We furthermore need a label image. You can create it using the pixel classifier trained earlier or using the menu `Tools > Segmentation / labeling > Gauss-Otsu Labeling (clesperanto)`.

Object classification

Our starting point is a loaded image and a label image with segmented objects. The following procedure is also shown in this video.



Train the classifier again.



If you are happy with the trained classifier, copy the file to a safe place. When training the next classifier this one might be overwritten.

Extra exercise

Retrain the classifier so that it can differentiate three different classes:

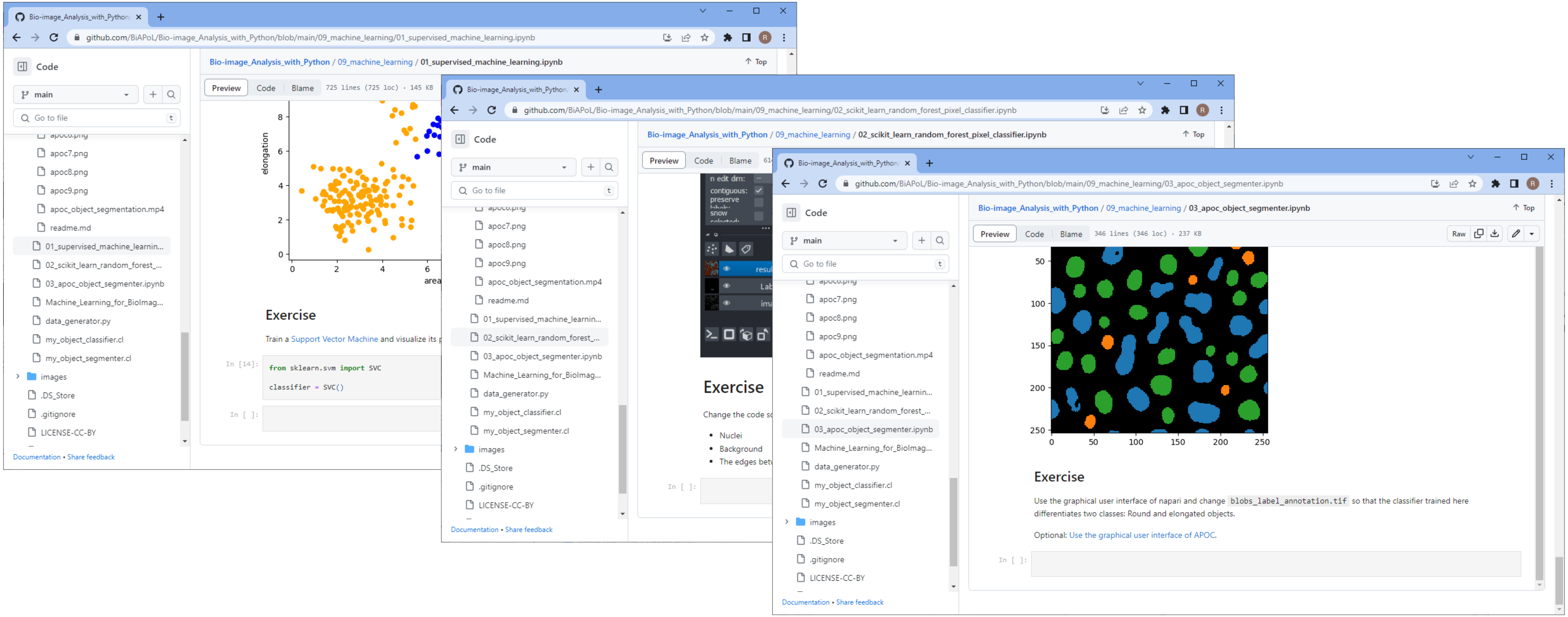
- Small round objects
- Large round objects
- Large elongated objects

https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/interactive_object_classification/readme.md

Machine learning using Python

PoL Physics of Life TU Dresden

- Use scikit-learn and apoc in Jupyter Notebooks to train and apply Random Forest Classifiers and Support Vector Machines



https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/

Summary & outlook

Machine learning for image analysis

- In classical machine learning, we typically select features for training our classifier

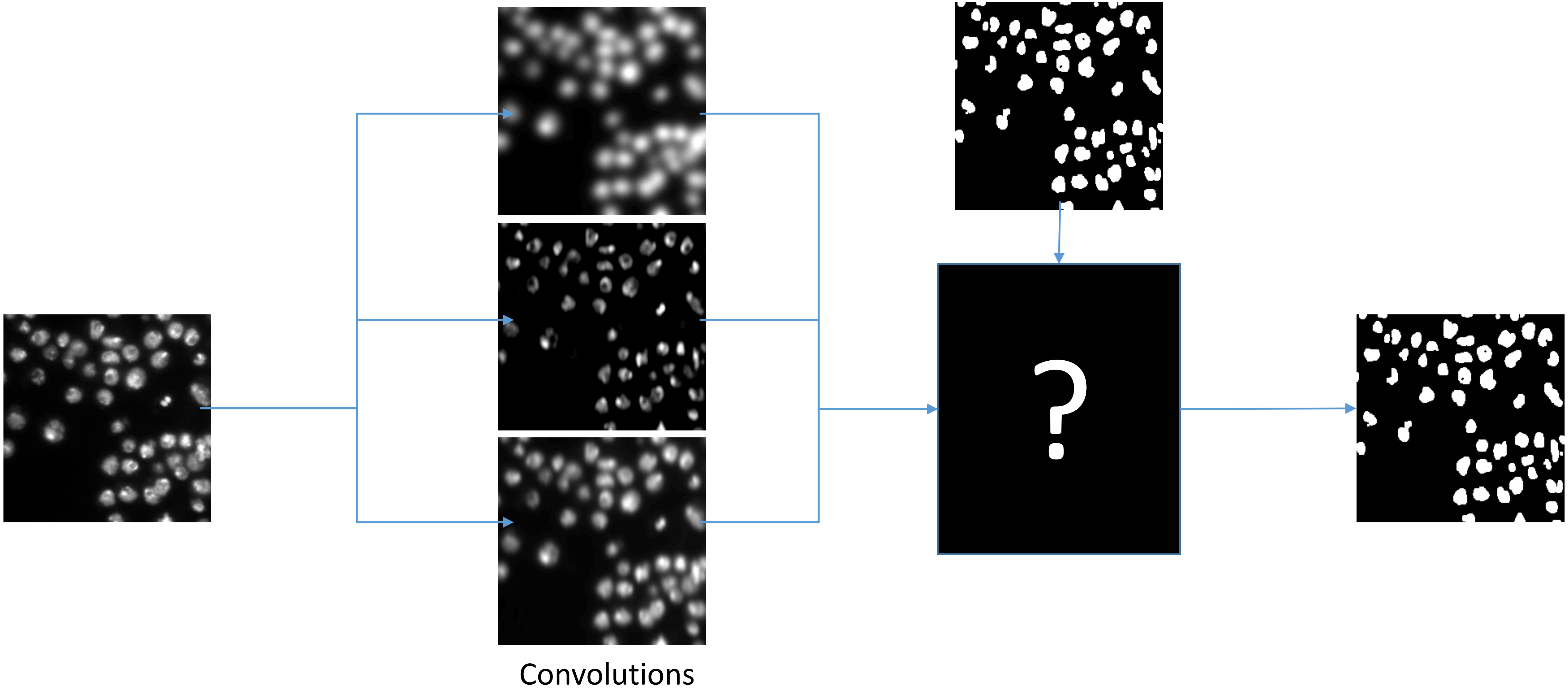
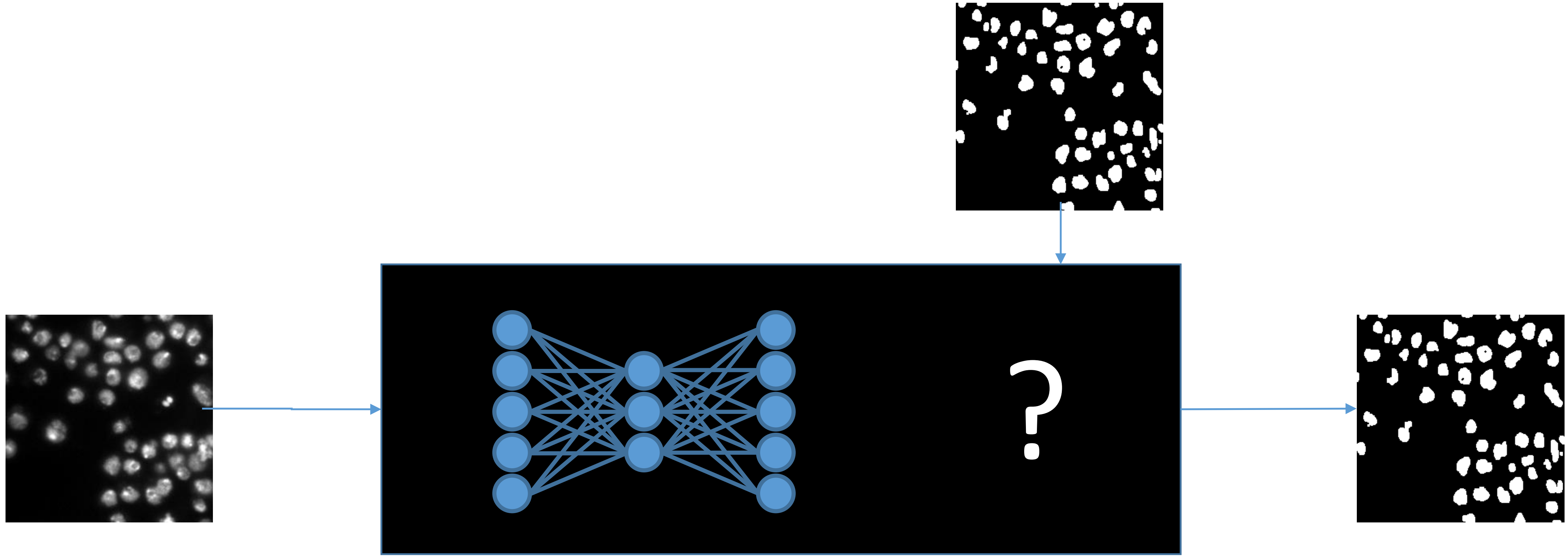


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Outlook: Deep learning for image analysis

- In deep learning, this selection becomes part of the black box



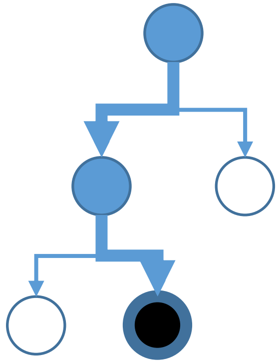
Convolutional neural networks

Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Summary

Today, you learned

- Machine learning for Pixel and Object segmentation
- Python
  - Scikit-learn / napari
  - Accelerated pixel and object classifiers (APOC)



Coming up next:

- Unsupervised machine learning
- Deep learning