Machine Learning for Pixel and Object Segmentation

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With Material from
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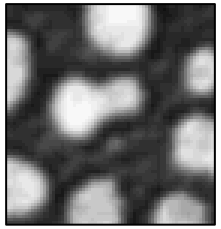
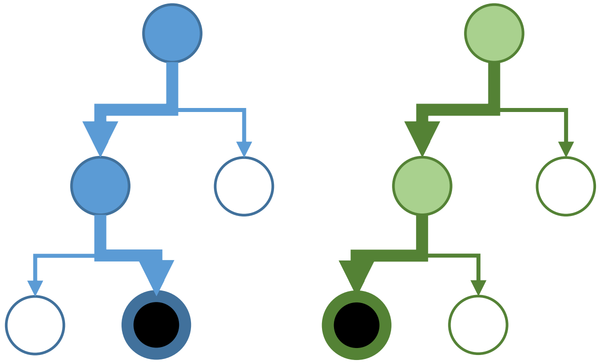
The Scikit-Learn community

Lecture overview

Overview

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





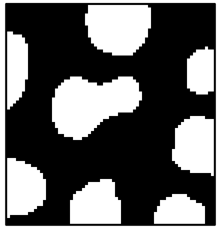


Image segmentation using thresholding

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

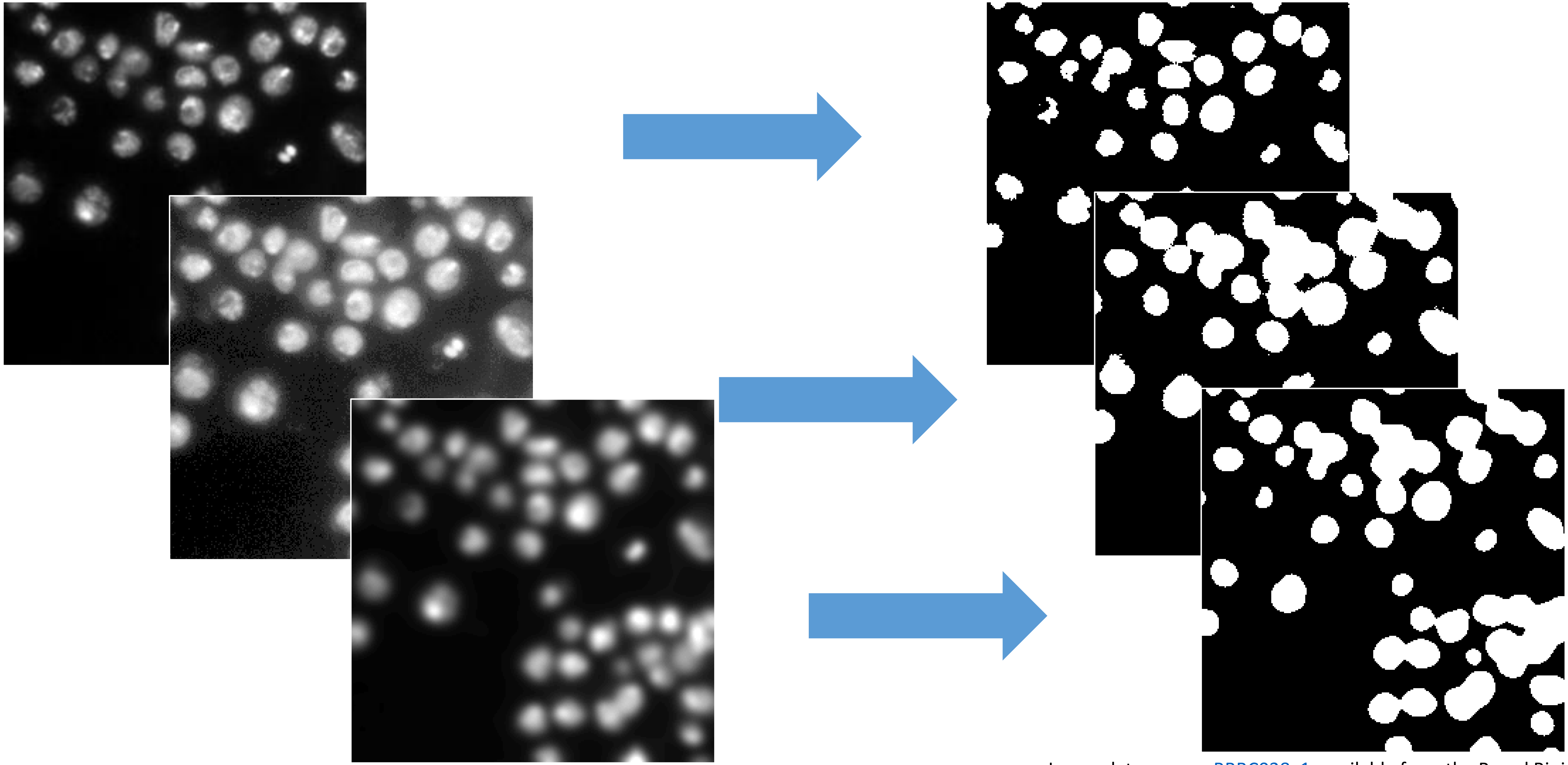


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

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

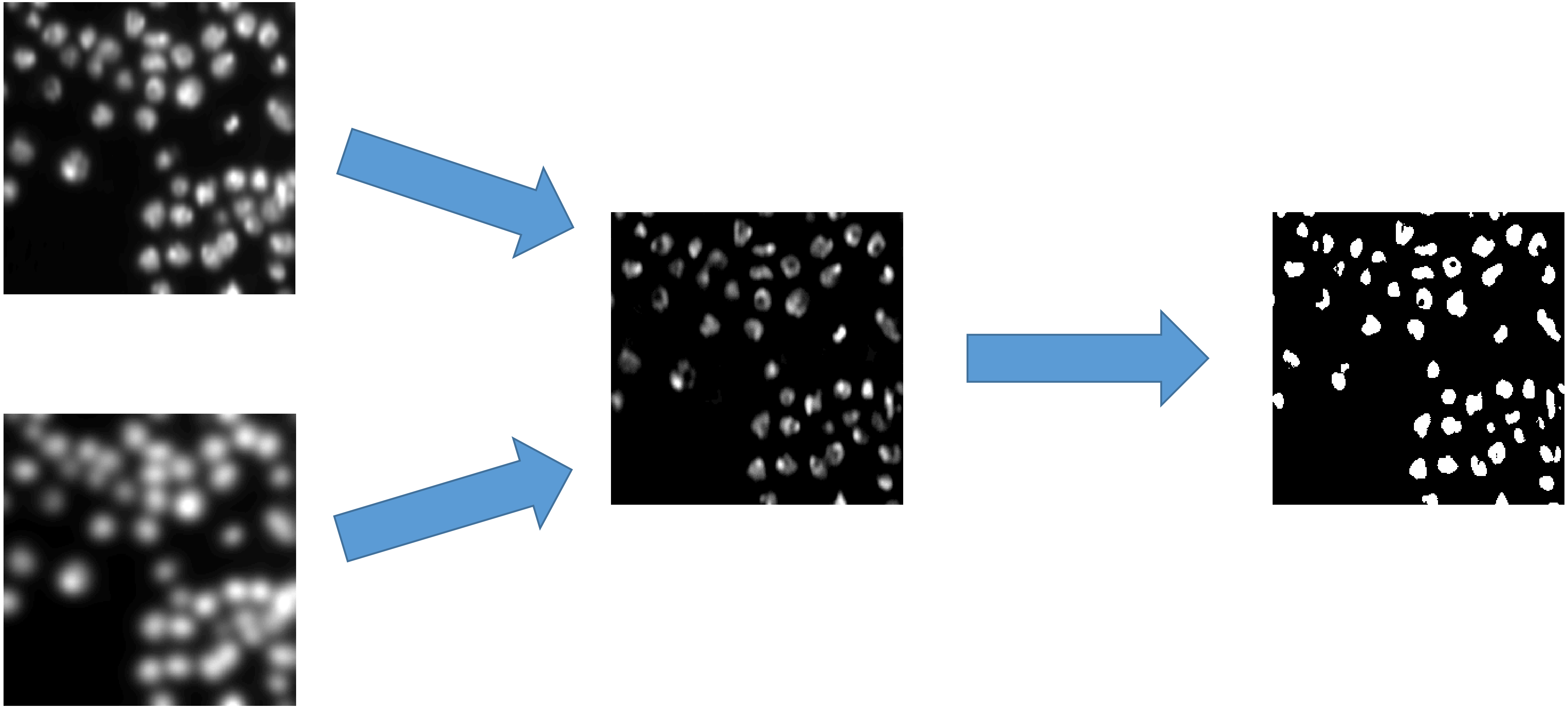


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

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

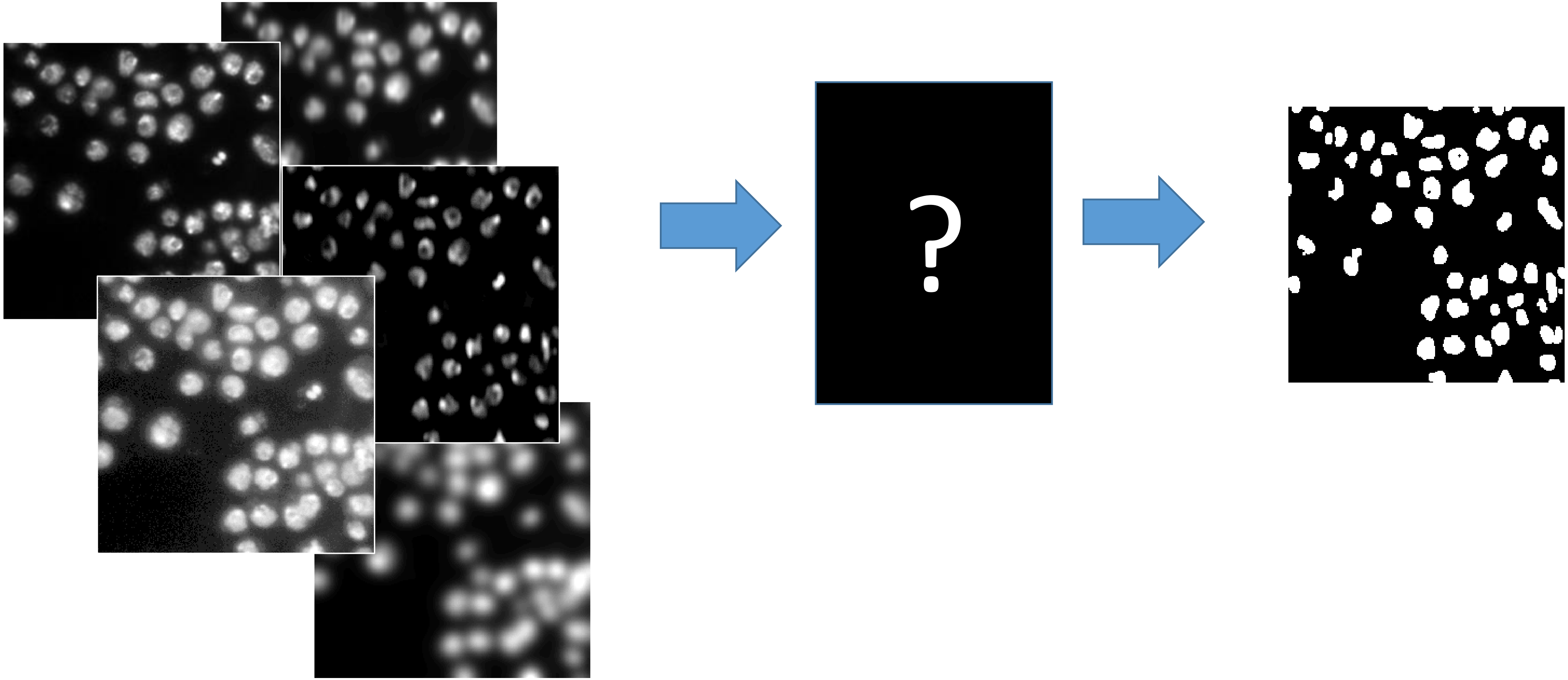
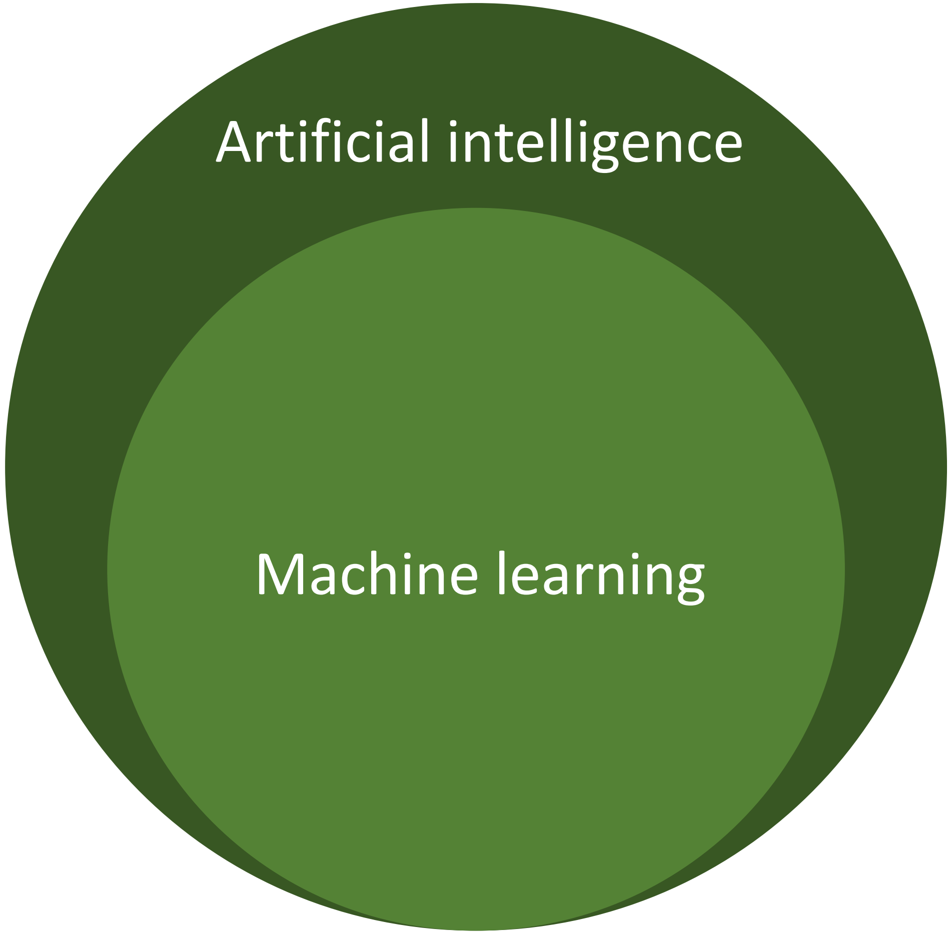
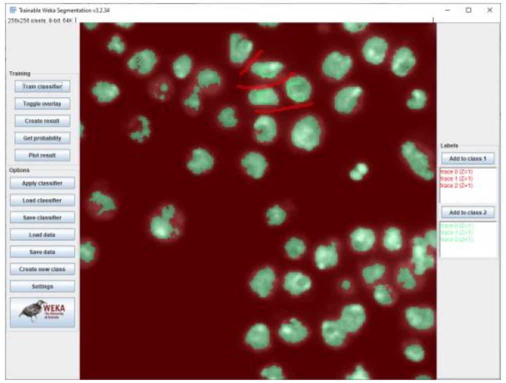


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

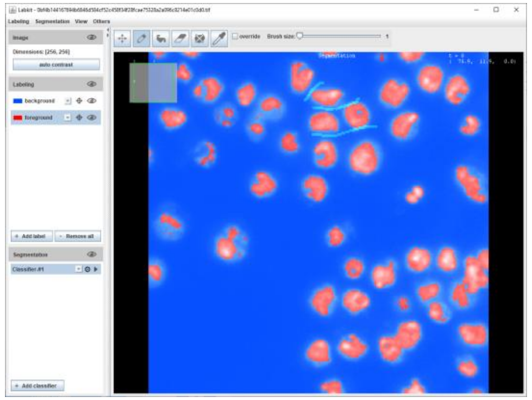
Machine learning

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



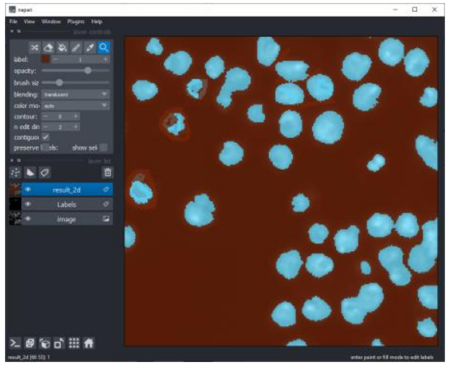


Trainable Weka Segmentation
https://imagej.net/plugins/tws/



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

Python / scikit-learn / napari / apoc



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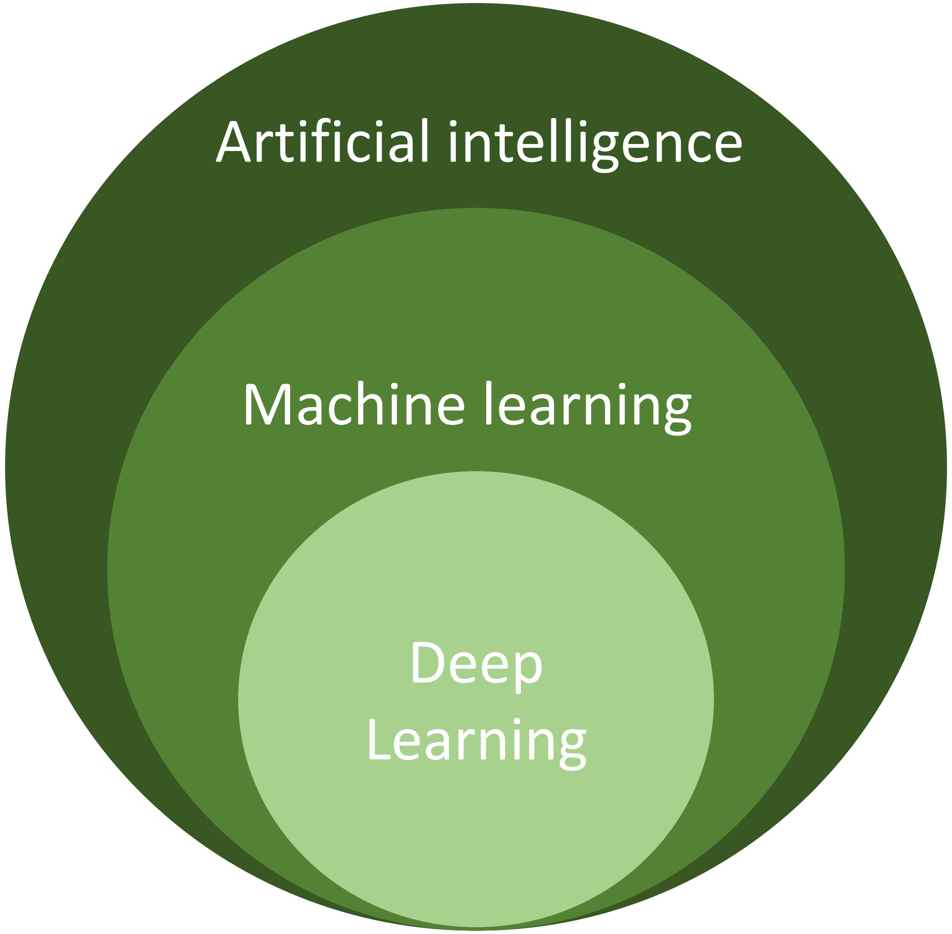
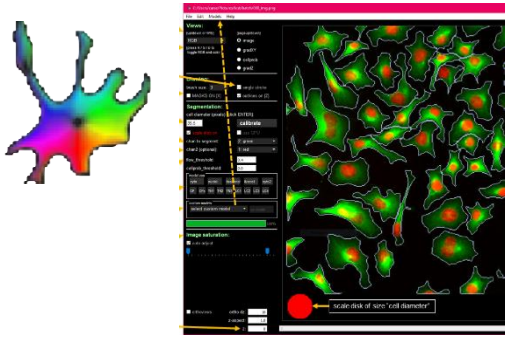
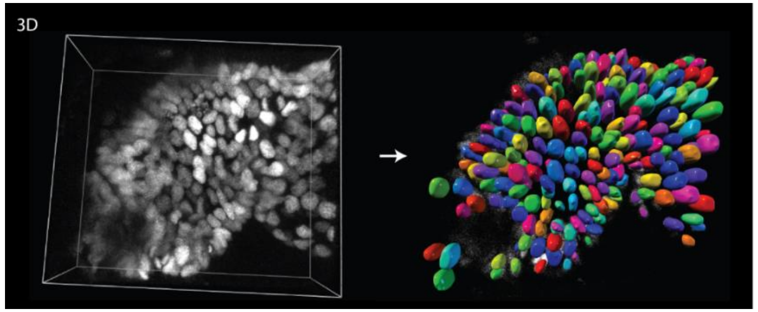
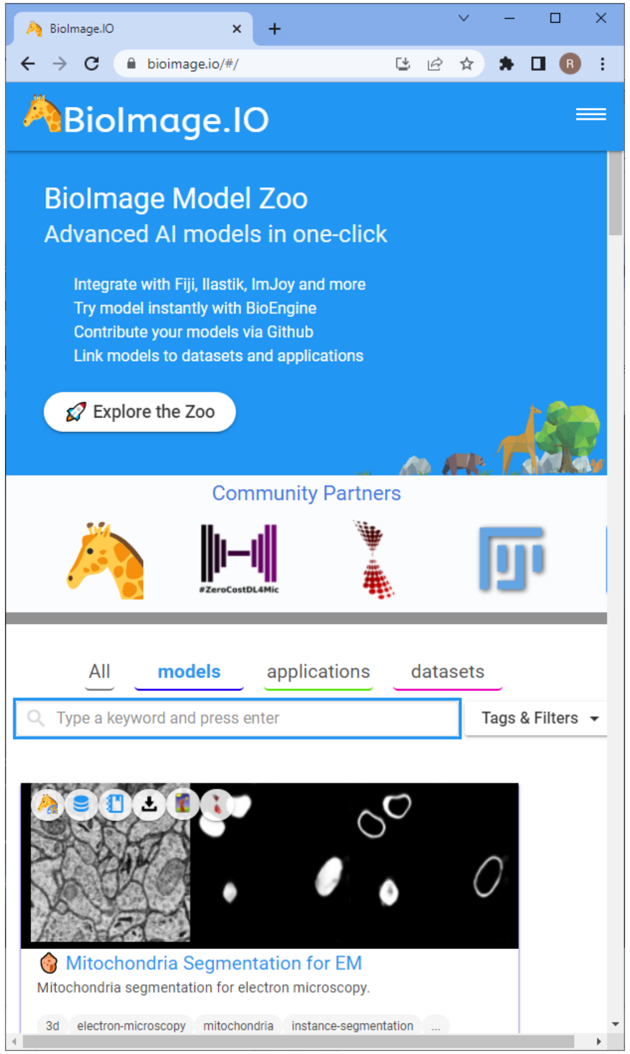




www.cellpose.org/



https://github.com/stardist/stardist

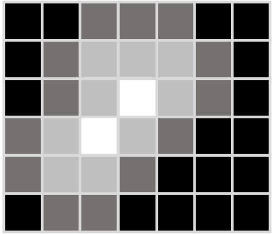


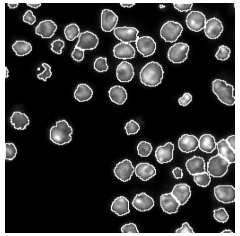
https://bioimage.io/

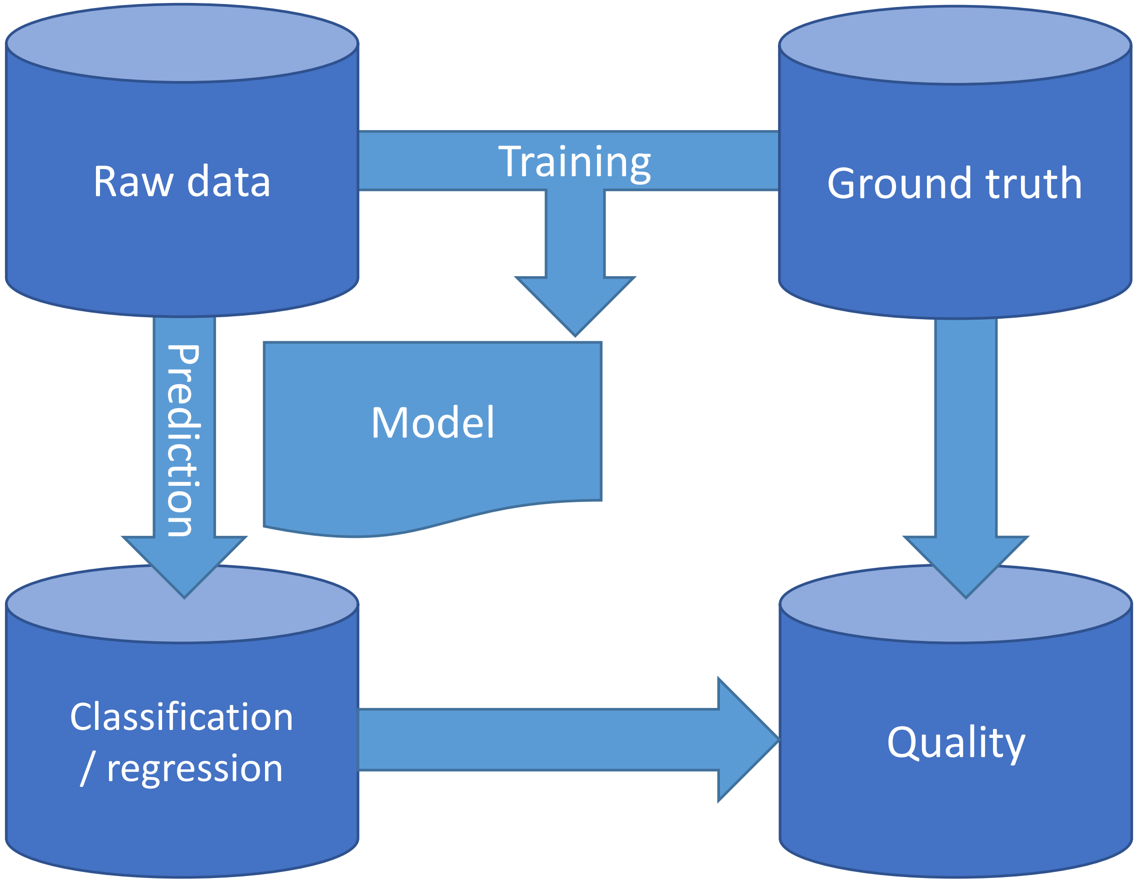
Machine learning

- Automatic construction of predictive models from given data

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



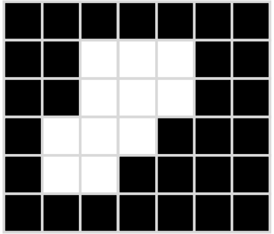




Annotated raw data, usually generated by humans

Precision, Recall

Dense Segmentation / Binarization



Object classification

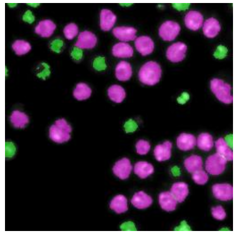
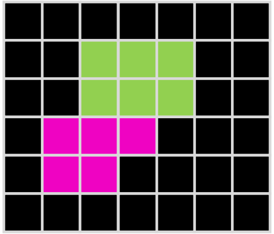


Image classification

"Cat"

Instance segmentation

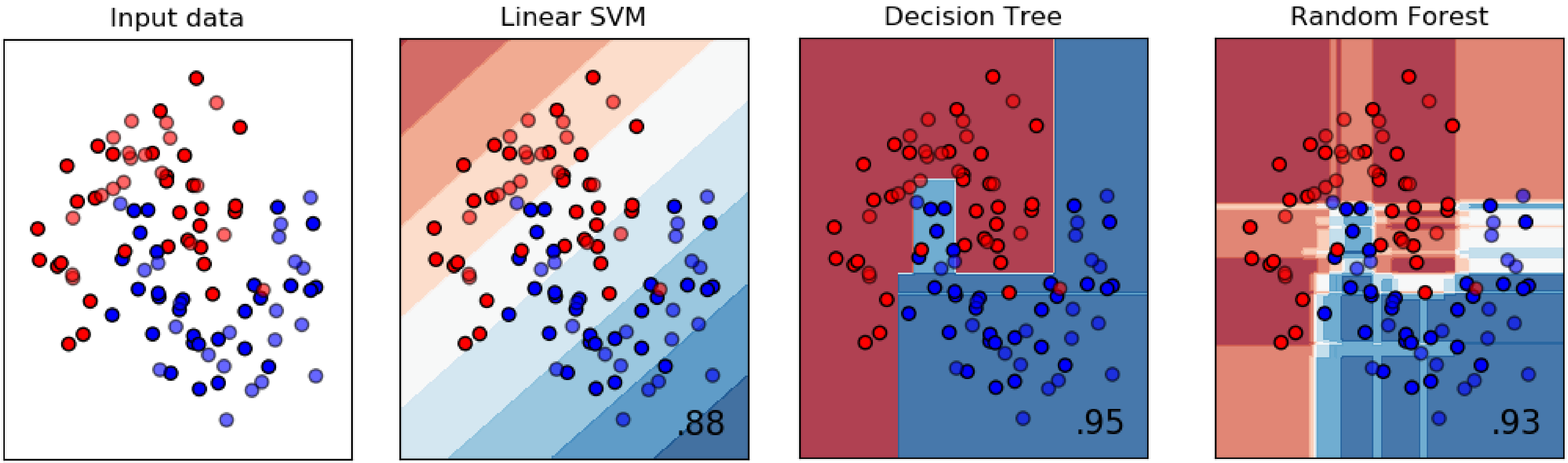


Cont. quantity

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

Goal

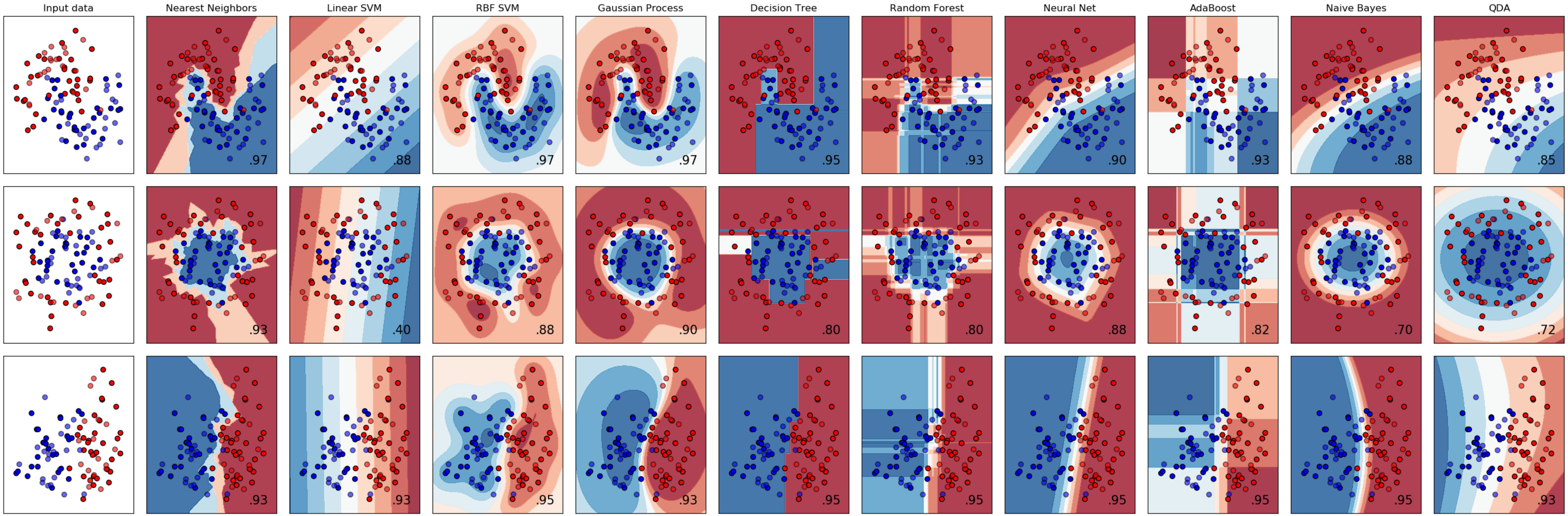
- Guess classification (color) from position of a sample in parameter space.



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

Approaches

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

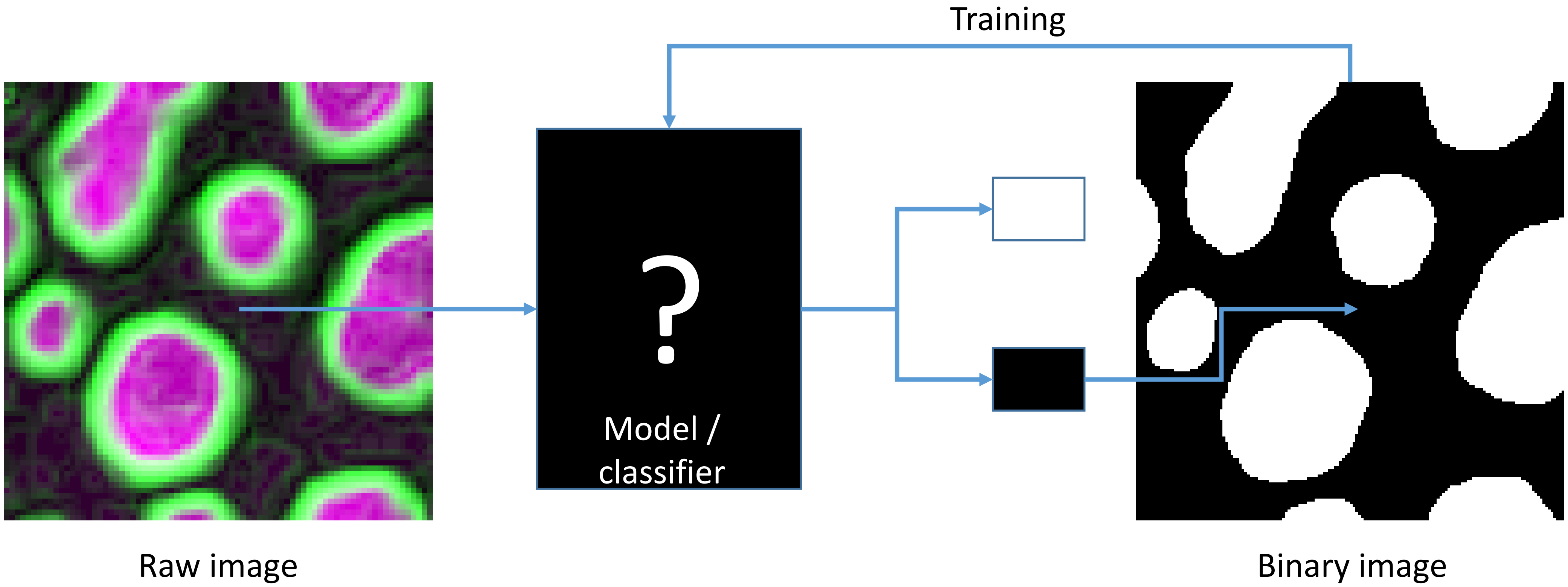


How to select a suitable classifier

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

Machine learning for image segmentation

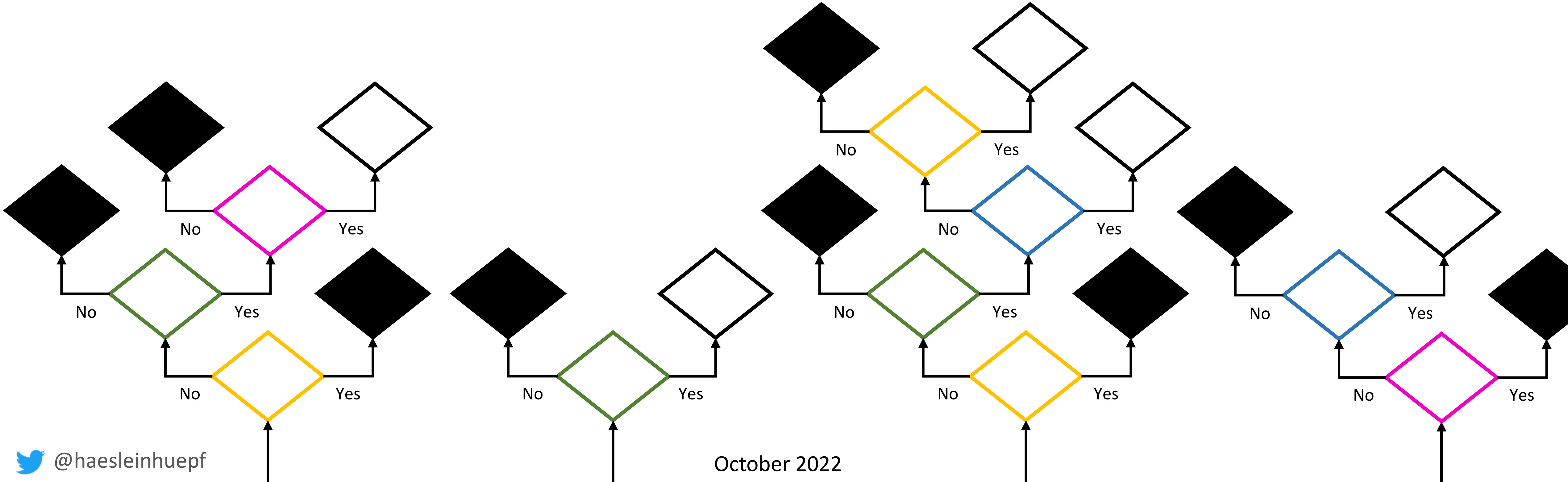
- *Supervised* machine learning: We give the computer some ground truth to learn from
- The computer derives a *model* or a *classifier* which can judge if a pixel should be foreground (white) or background (black)
- Example: Binary classifier



Random forest based image segmentation

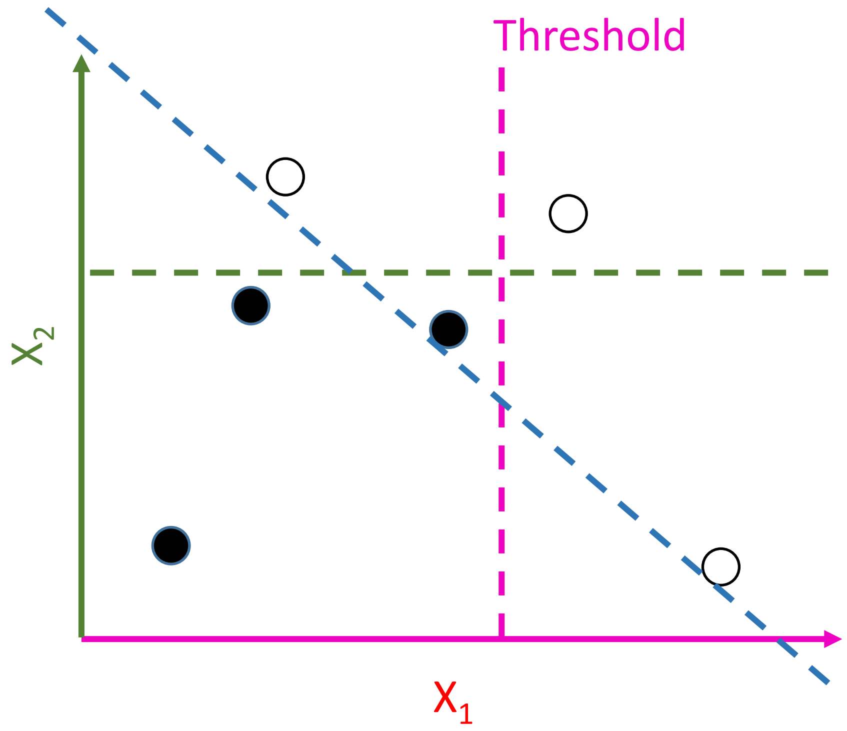
- Decision trees are classifiers, they decide if a pixel should be white or black
- Random decision trees are randomly initialized, afterwards evaluated and selected
- Random forests consist of many random decision trees

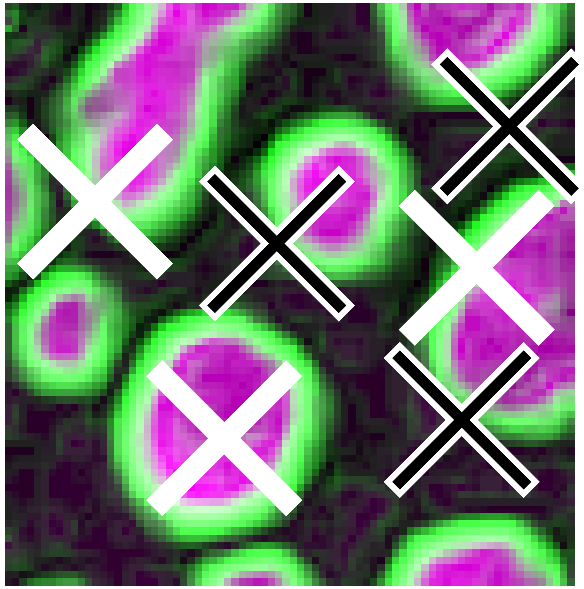
- Example: Random forest of binary decision trees

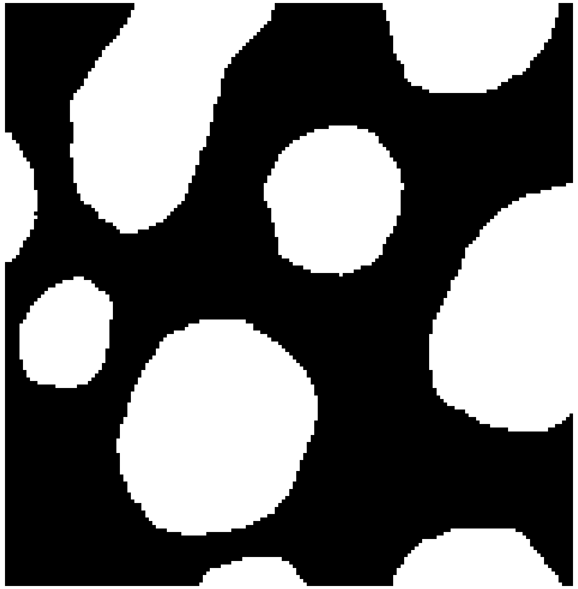


Deriving random decision trees

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



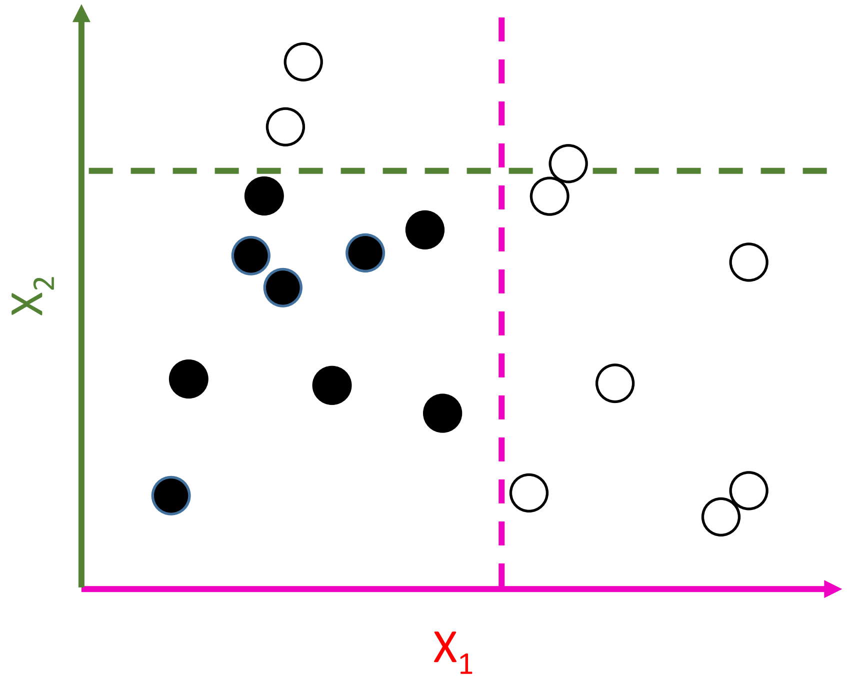


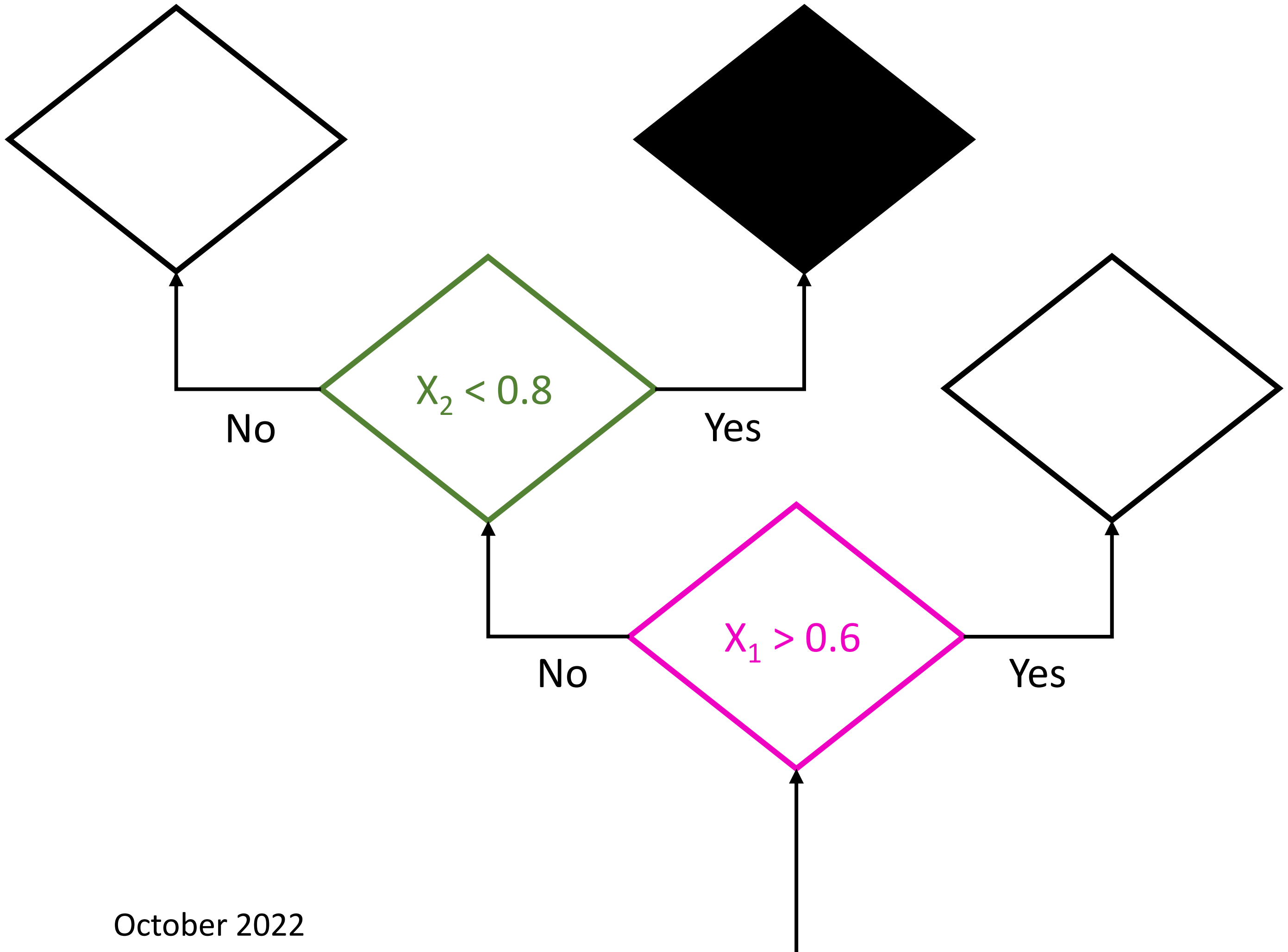


Note: You cannot use a single threshold to make the decision correctly

Deriving random decision trees

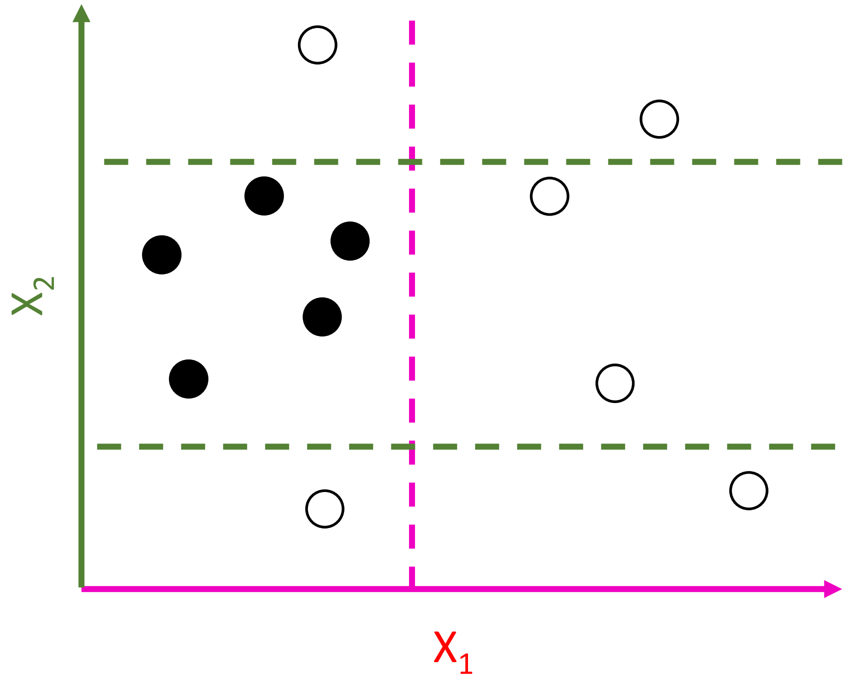
- Decision trees combine several thresholds on several parameters

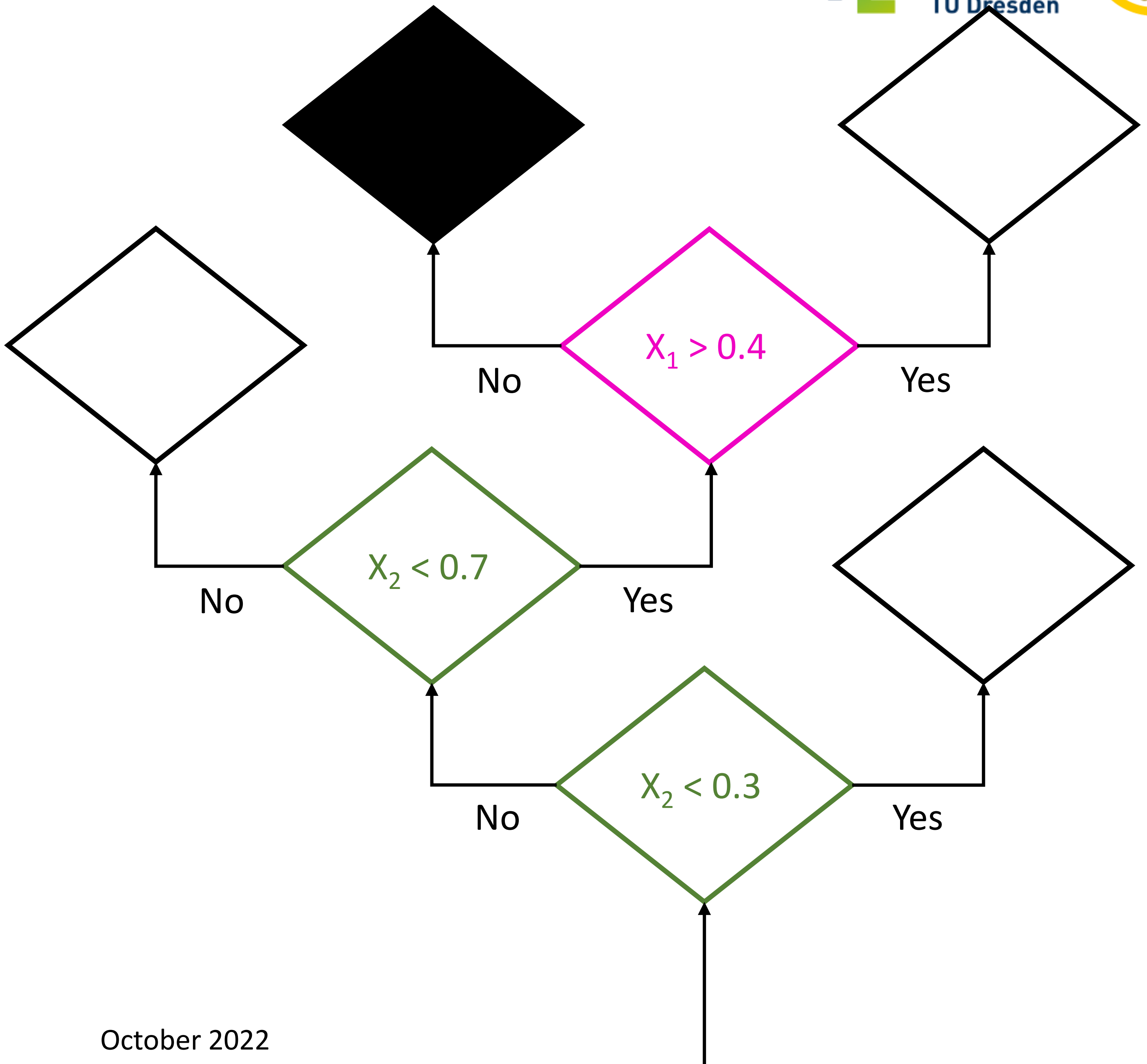




Deriving random decision trees

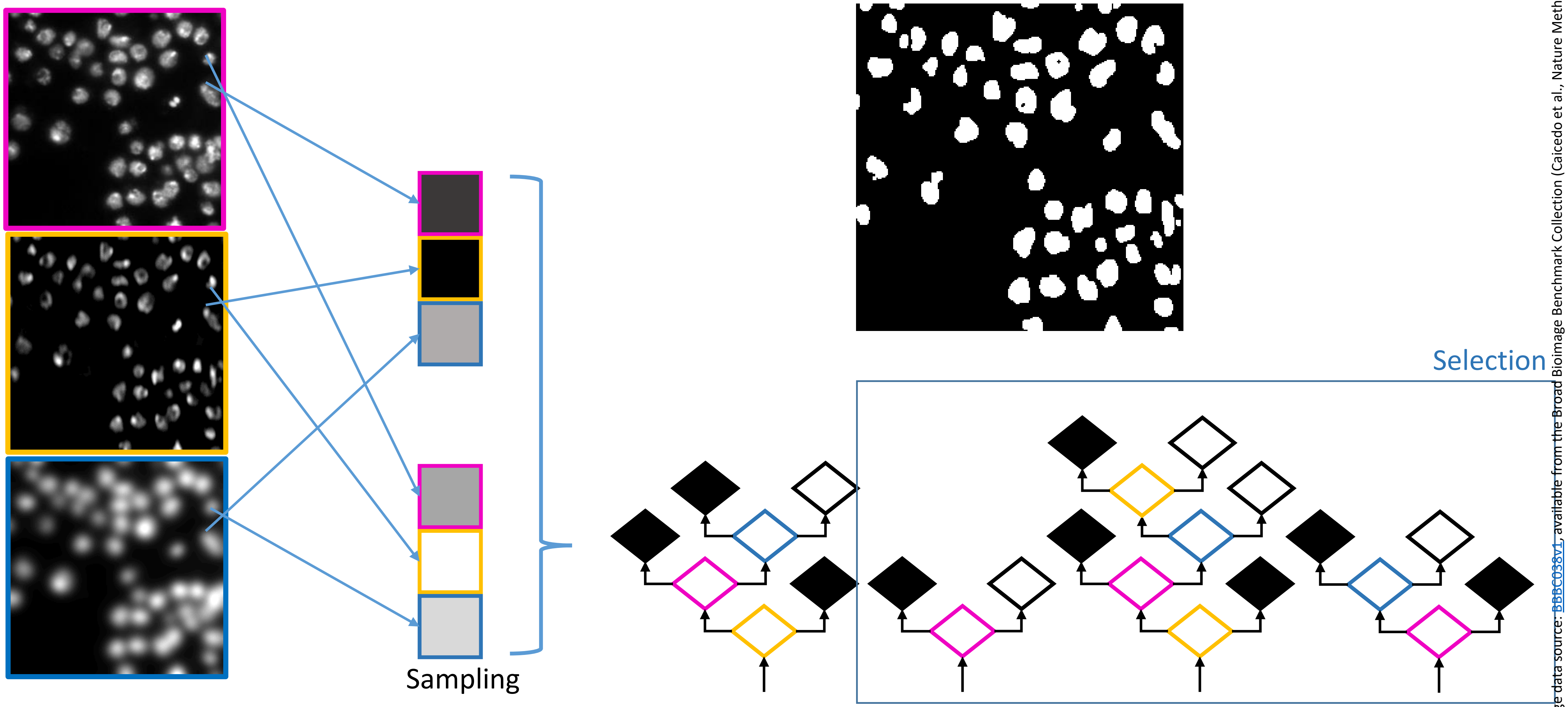
- Depending on sampling, the decision trees are different





Random Forest Pixel Classifiers

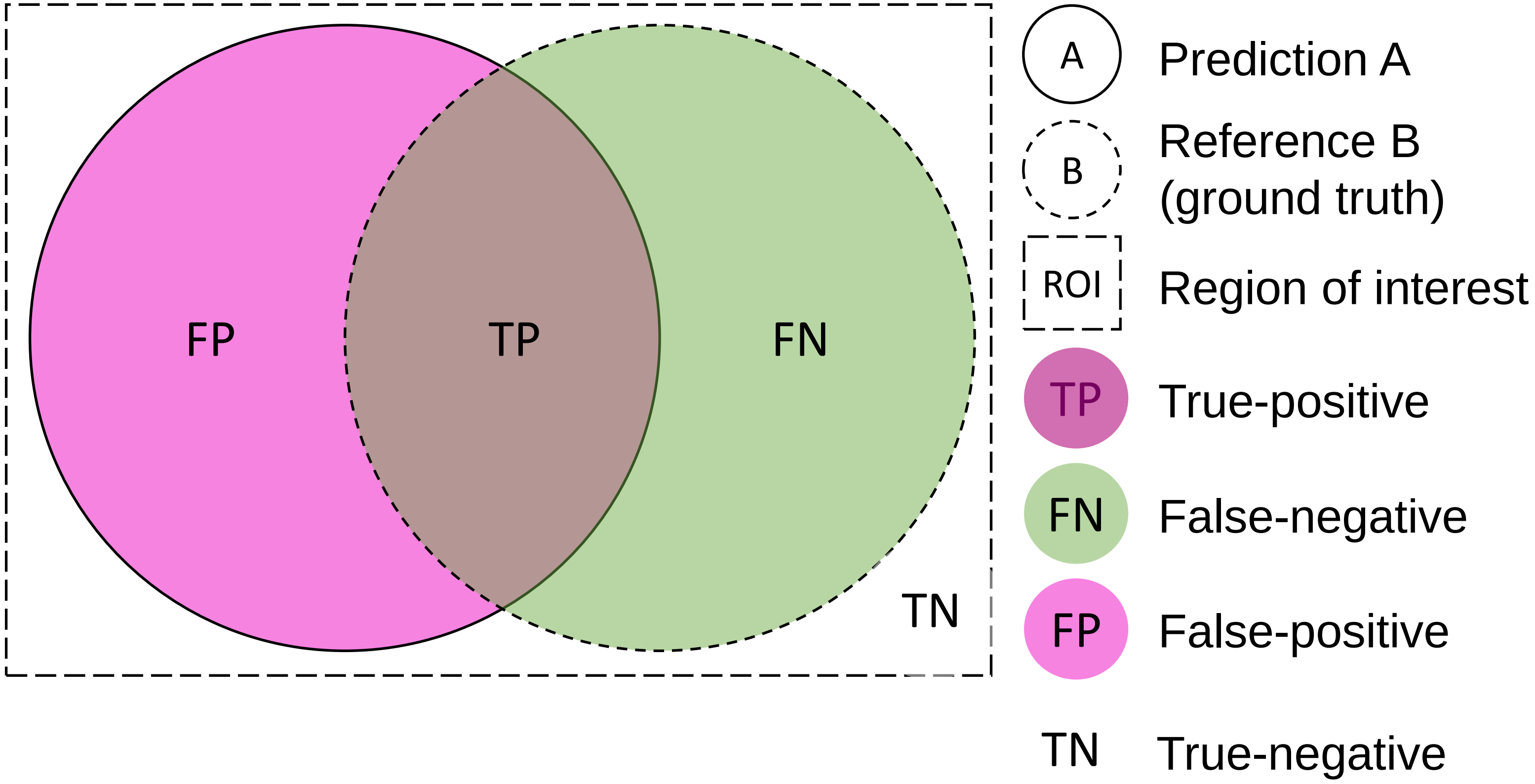
- By comparing performance of individual decision trees, good ones can be selected, bad ones excluded.



Recap: Algorithm evaluation

- 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



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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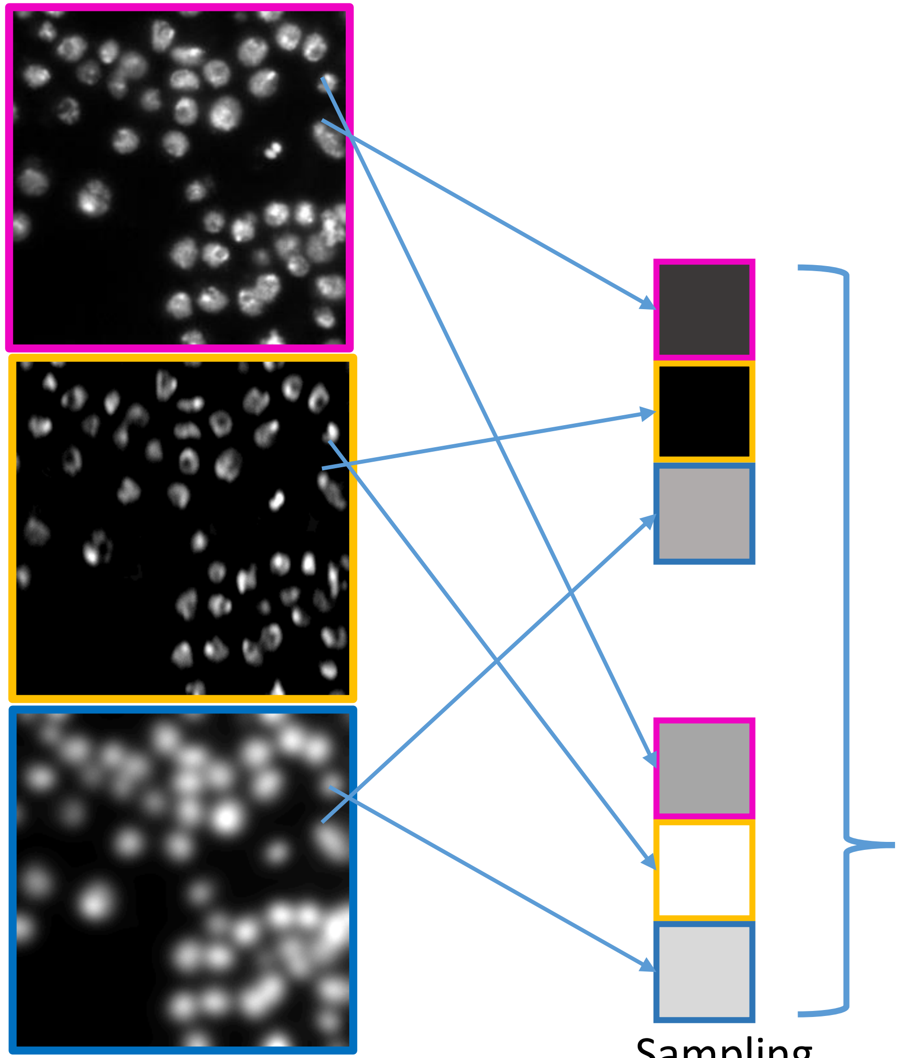
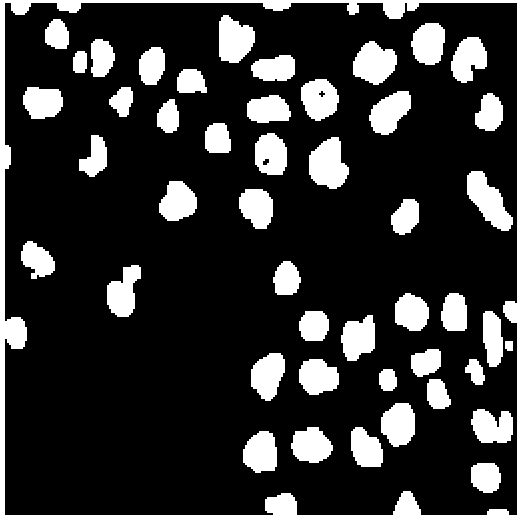




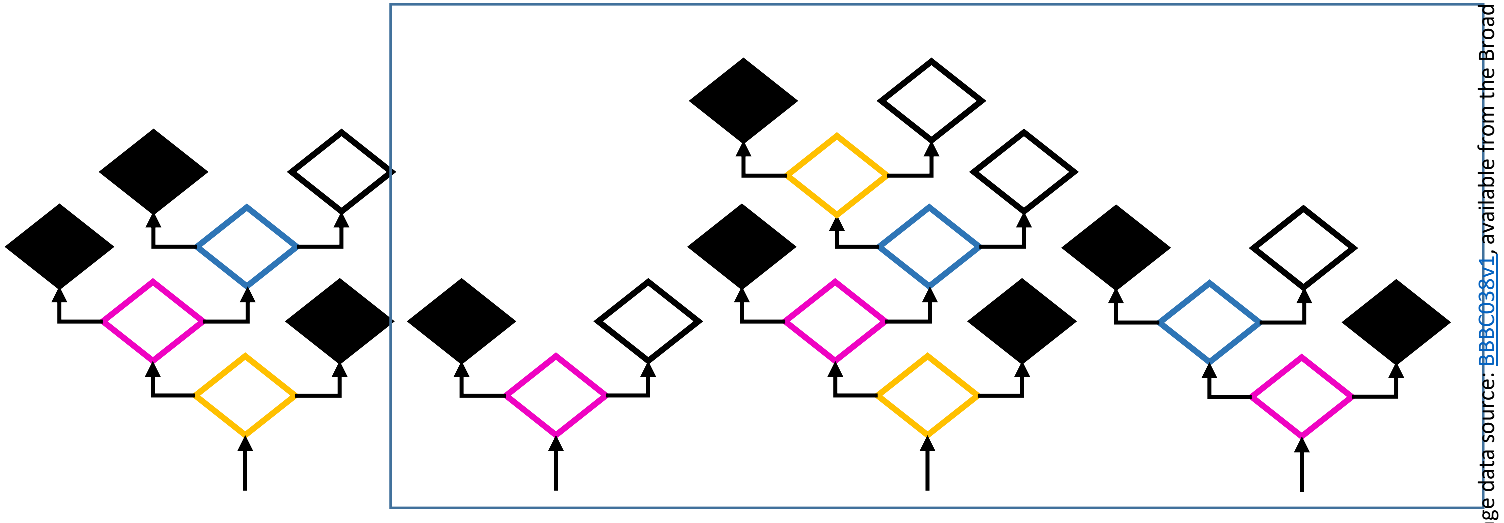
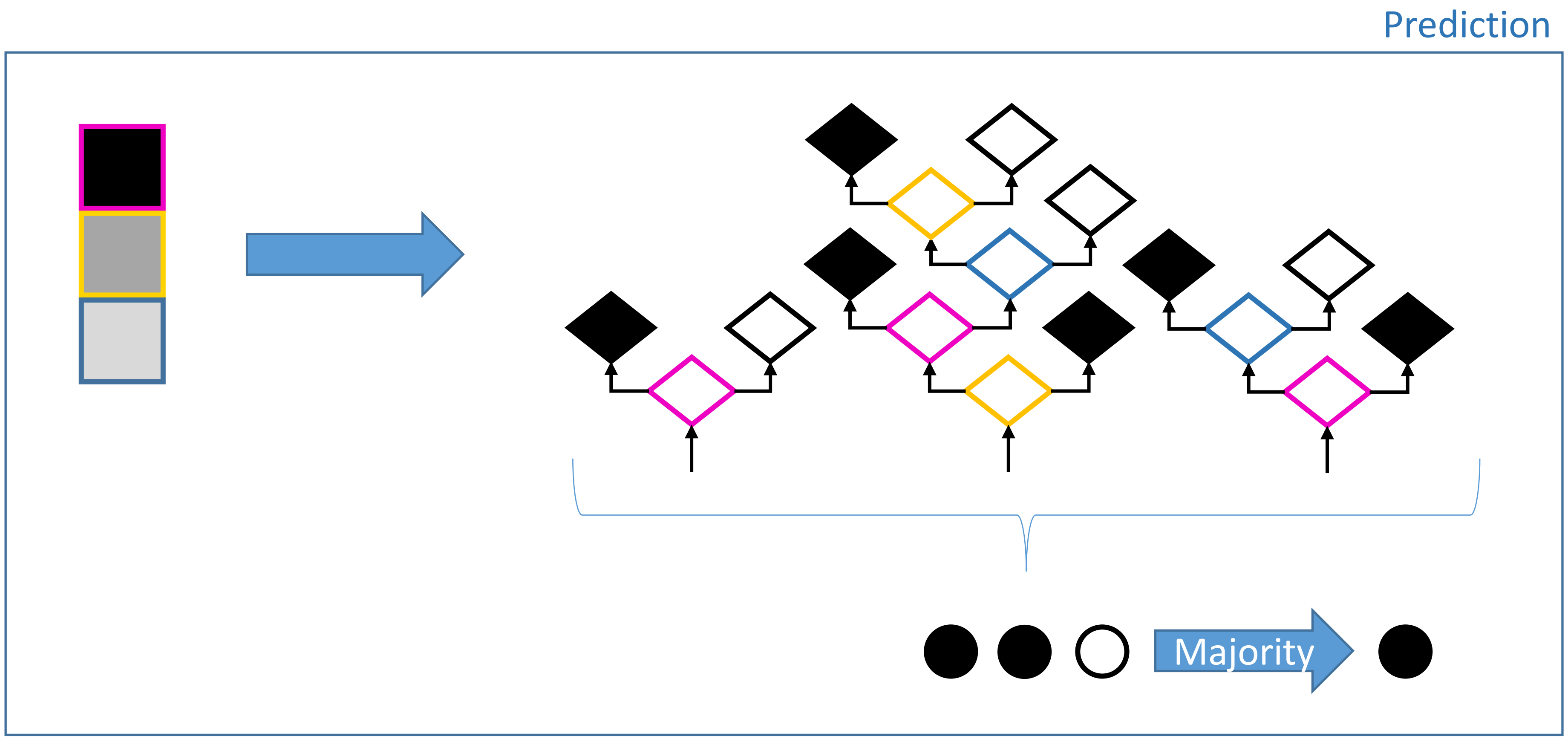


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Random Forest Pixel Classifiers

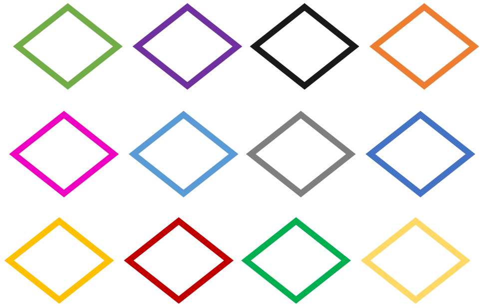
- Combination of individual tree decisions by voting or max / mean



Random Forest Pixel Classifiers

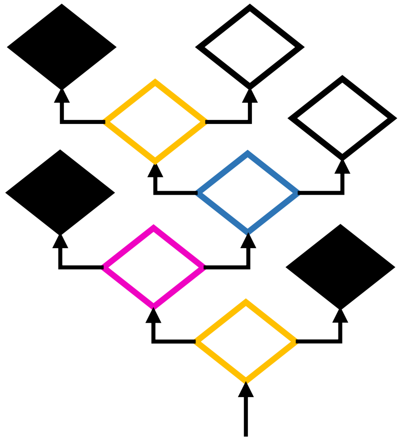
- Typical numbers for pixel classifiers in microscopy

Available features: > 20



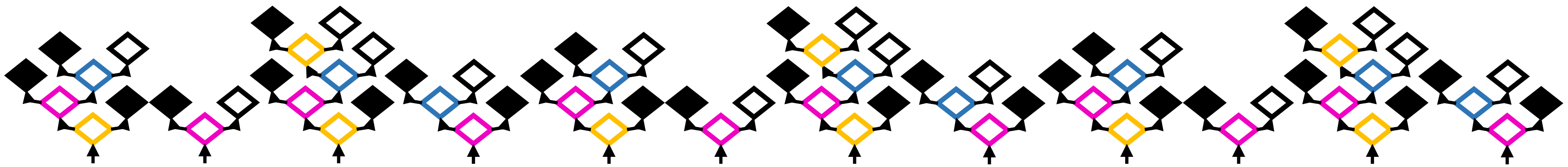
- Gaussian blur image
- DoG image
- LoG image
- Hessian
- ….

Selected features: <= depth



Depth: 4

Number of trees: > 100



Model validation

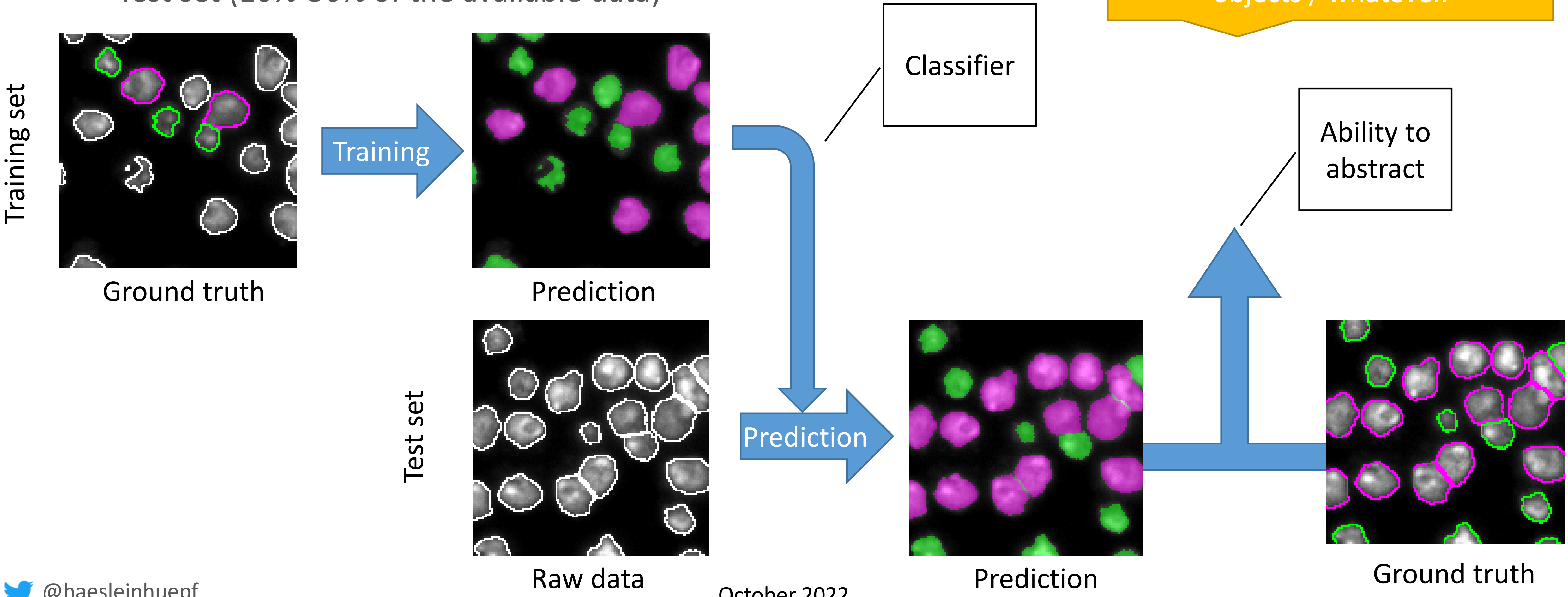
- Underfitting
  - A trained model that is not even able to properly process the data it was trained on

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

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.



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Pixel classification using scikit-learn

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October 2022

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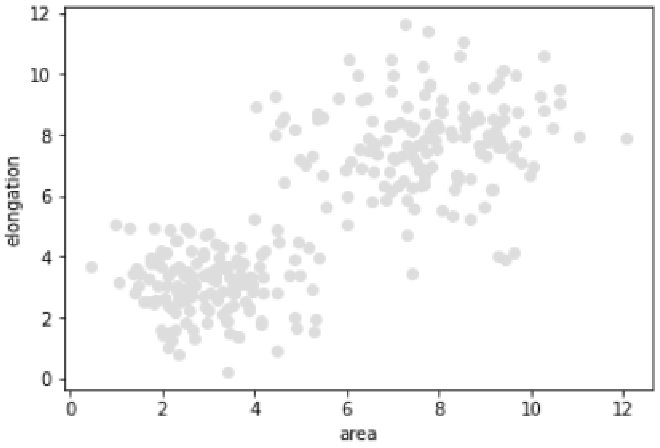
Tabular object classification

- Classify objects starting from feature vectors (table columns)

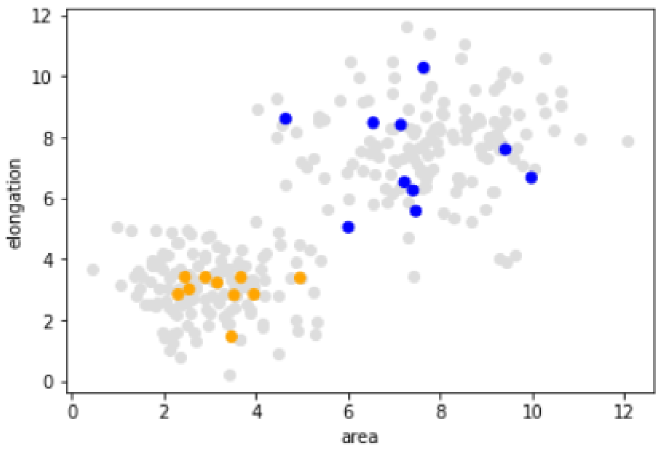
Raw data

	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,
```

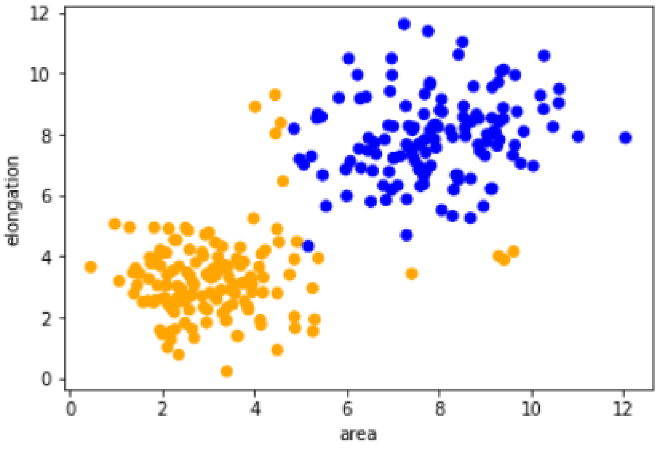


Classifier training

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

Classifier prediction

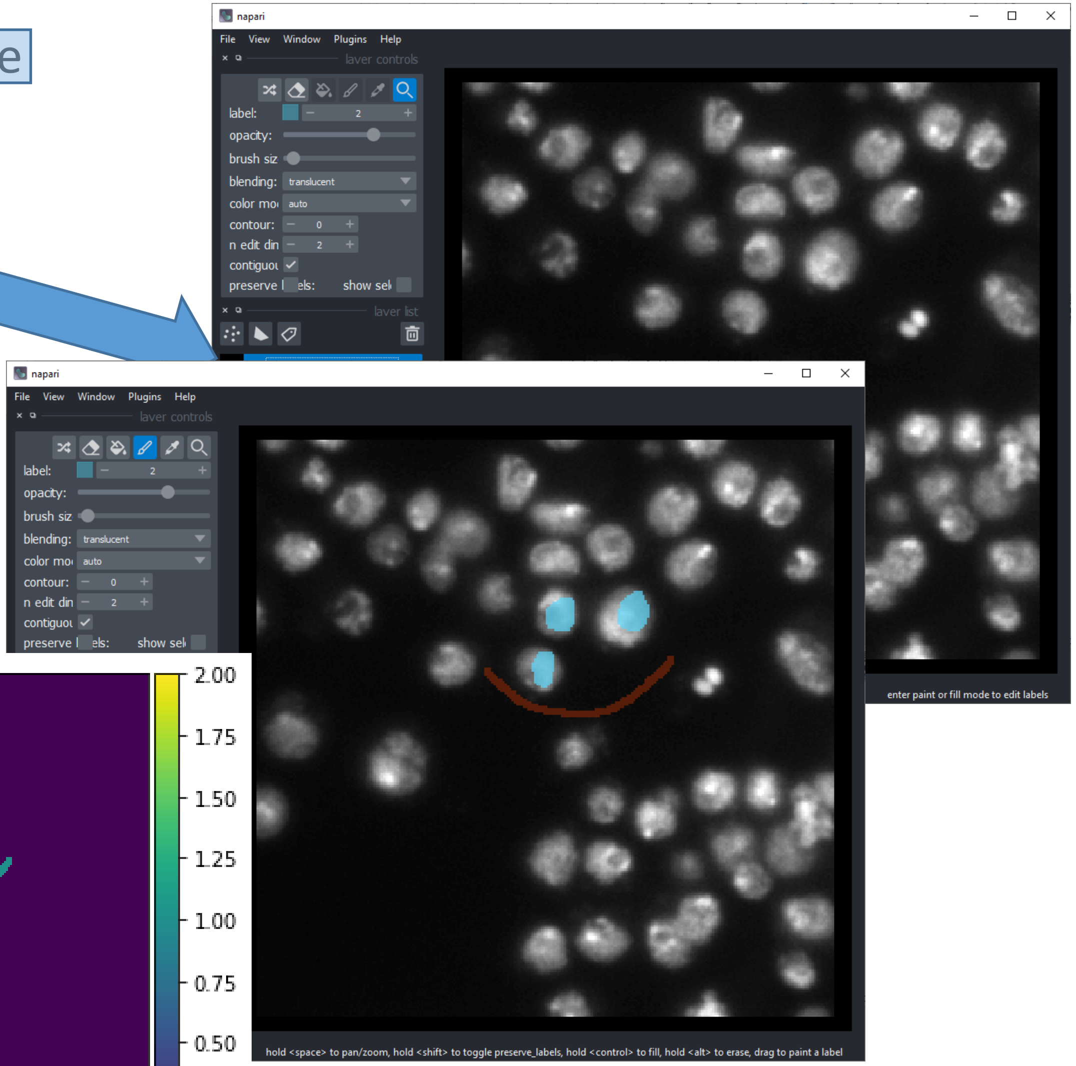
```
result = classifier.predict(validation_data)
```



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
```

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

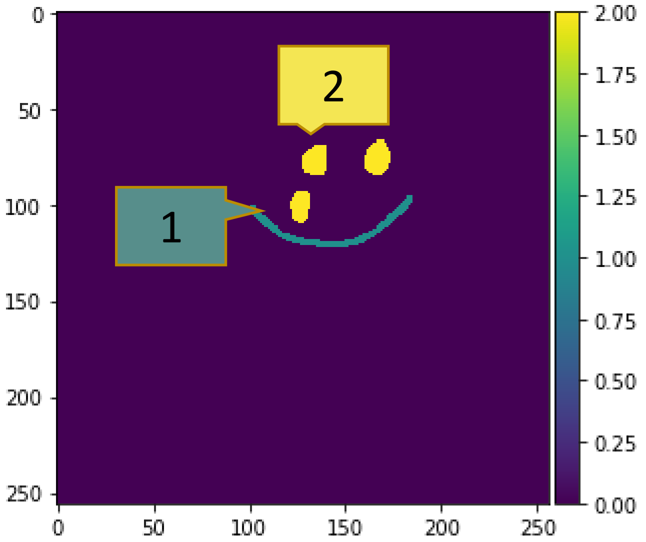


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Interactive pixel classification

PoL
Physics of Life
TU Dresden

- 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

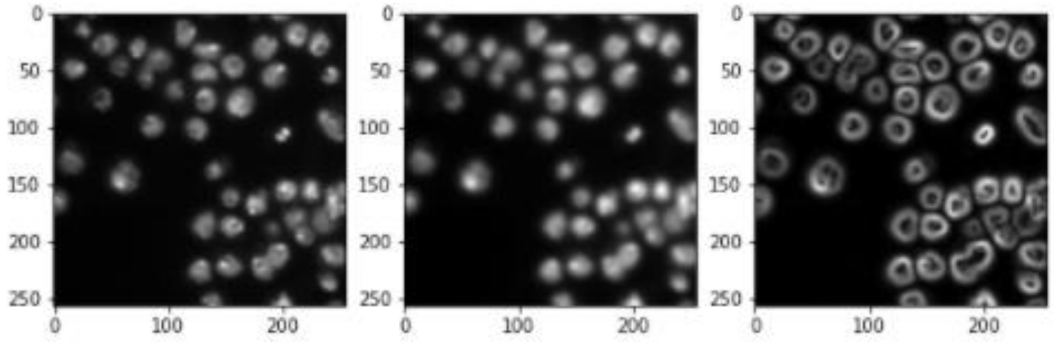
```
y_ = classifier.predict(X)
```

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)
```

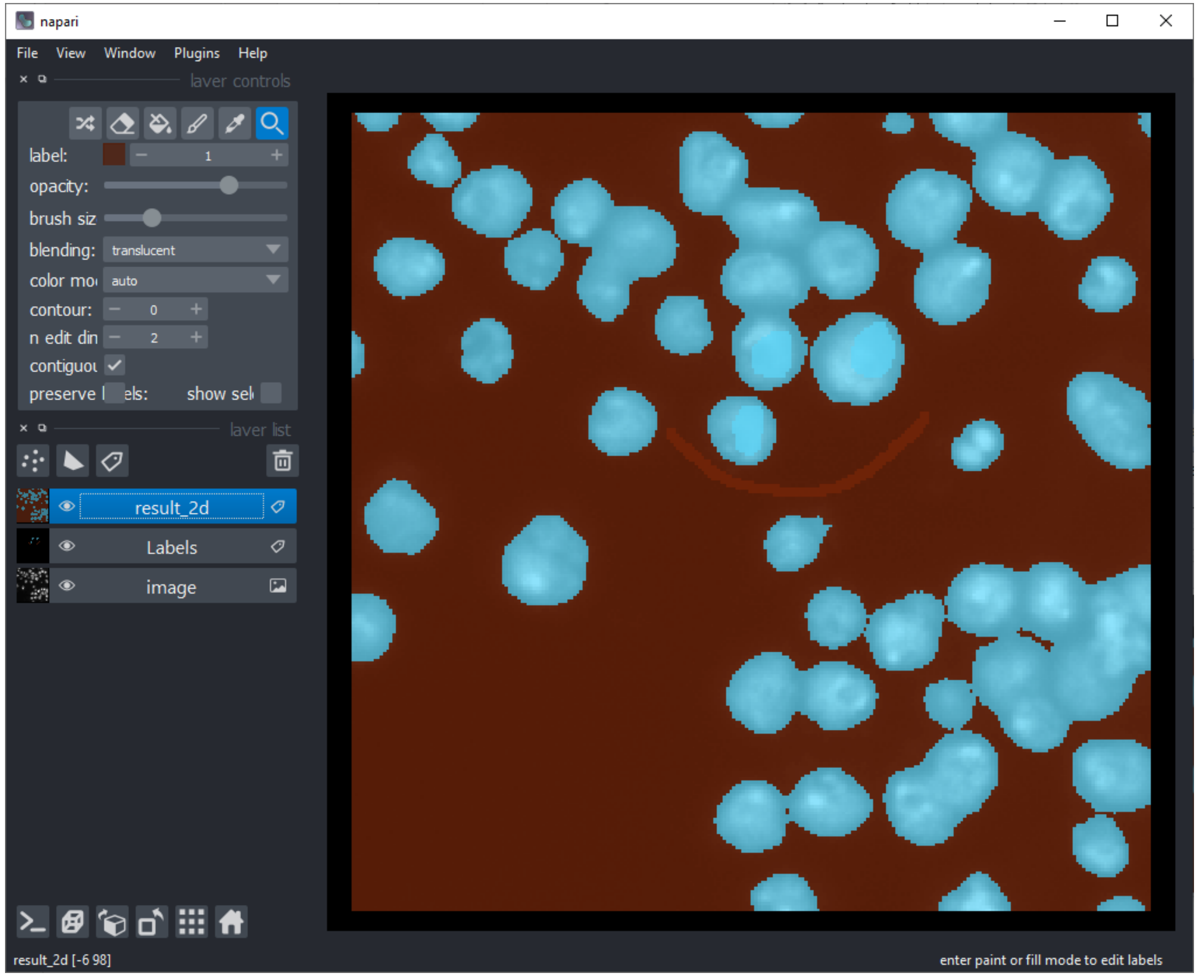
Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection [Caicedo et al., Nature Methods, 2019].

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)
```



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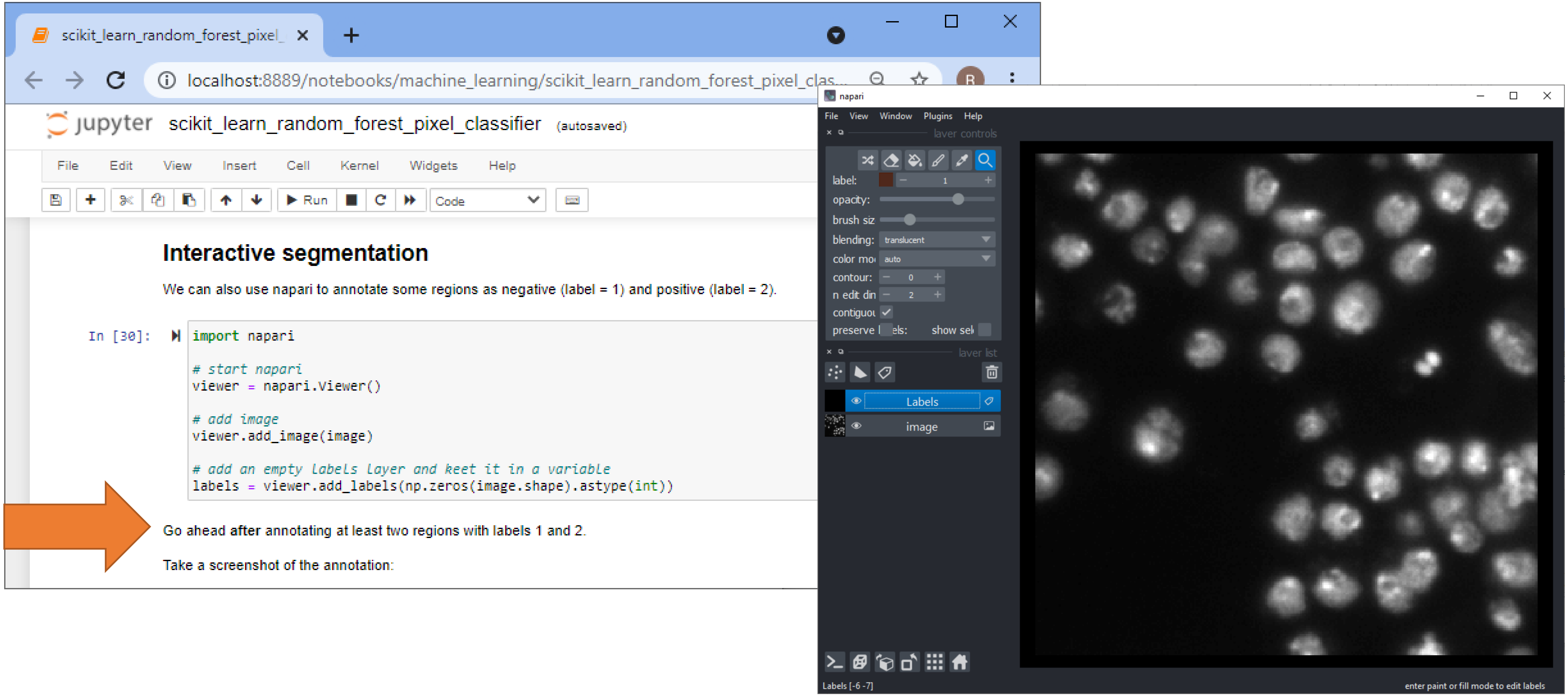
Interactive pixel classification

- Jupyter notebooks and napari side-by-side



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Interactive pixel classification

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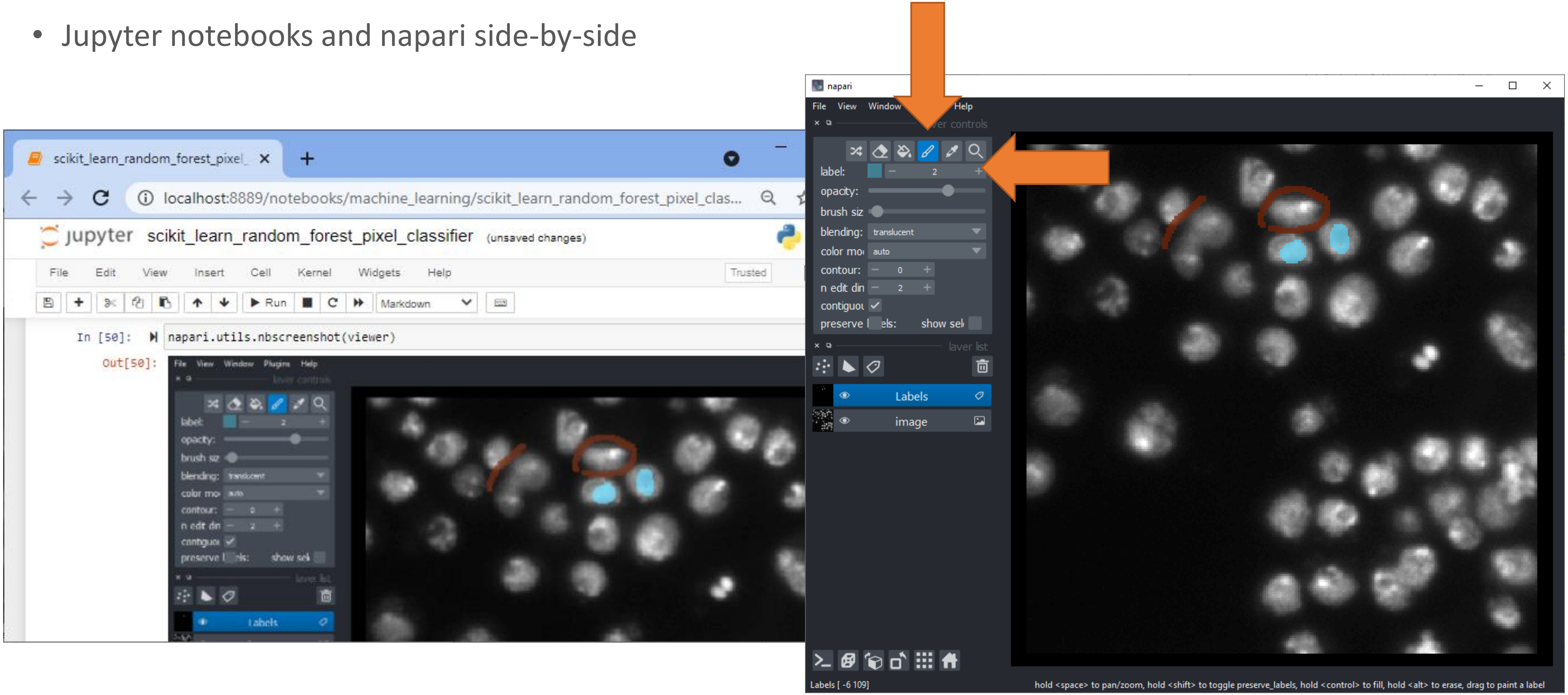

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Interactive pixel classification

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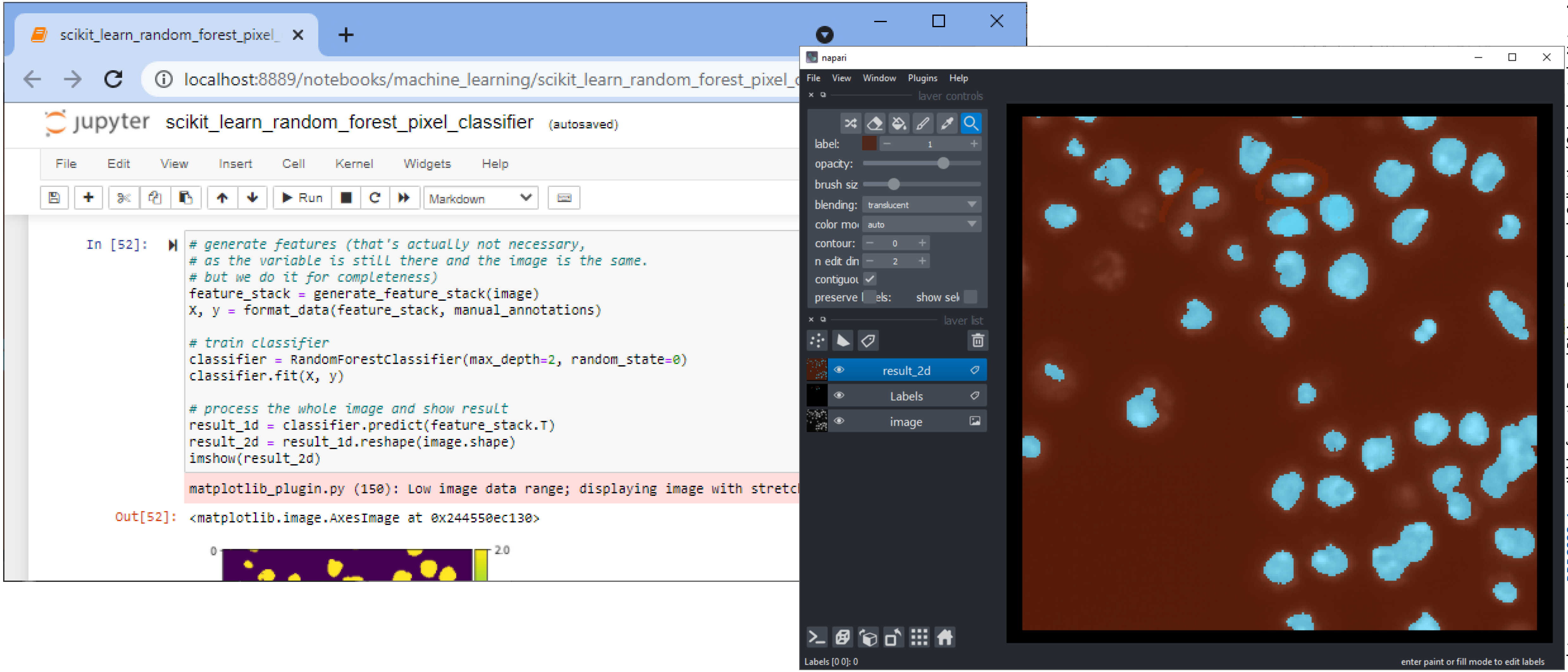

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Accelerated pixel and object classification (APOC)

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October 2022

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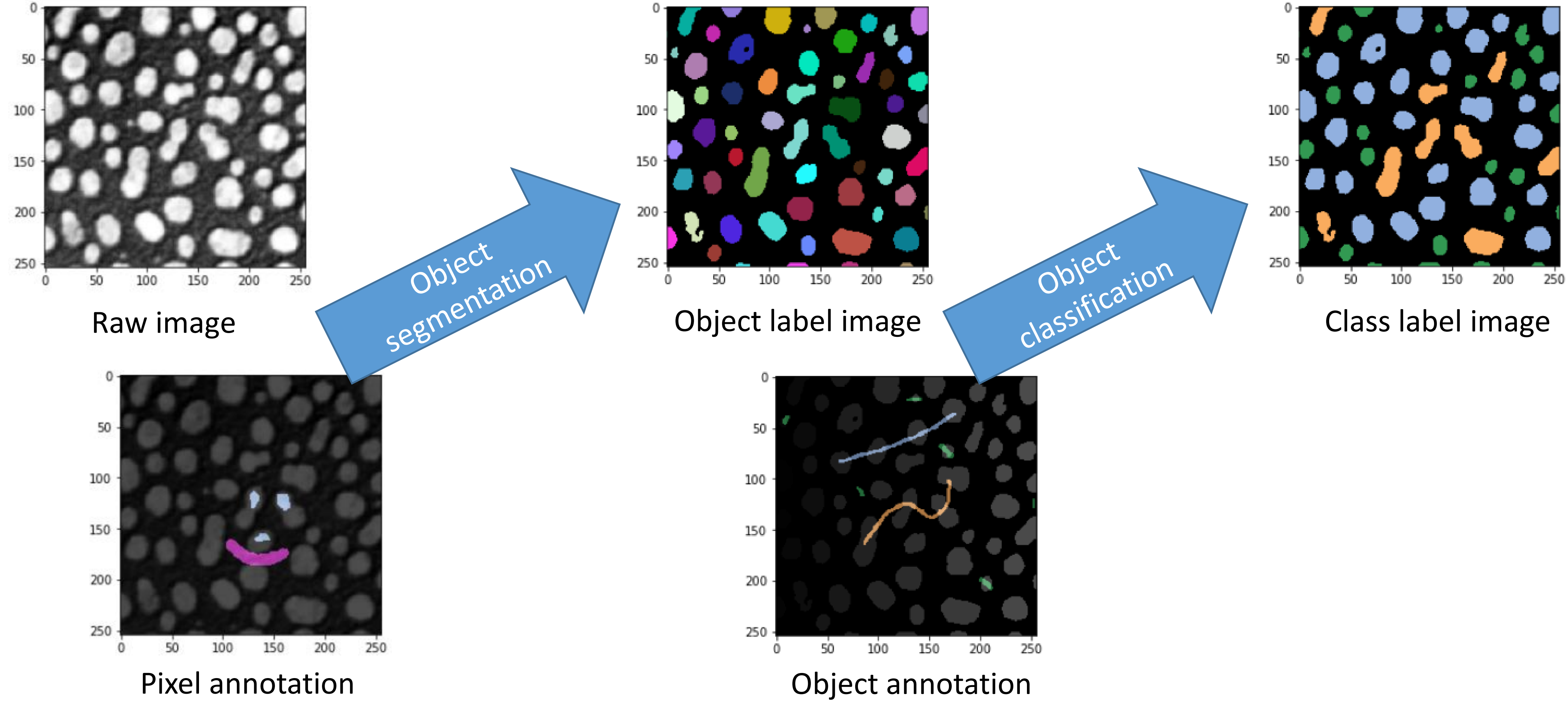
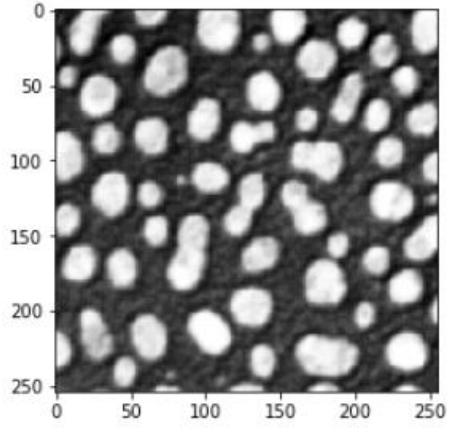
Accelerated pixel and object classification

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

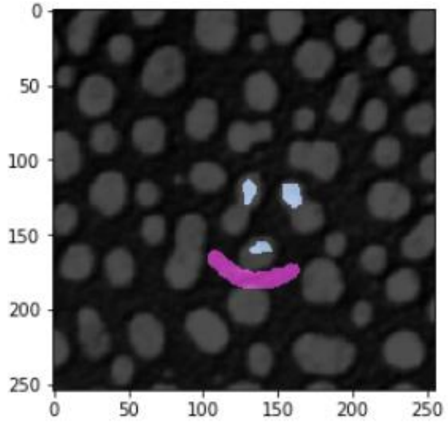


Object segmentation

- Pixel classification + connected component labeling



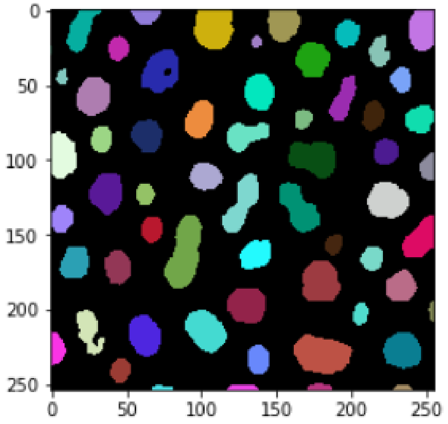
Raw image



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)
```

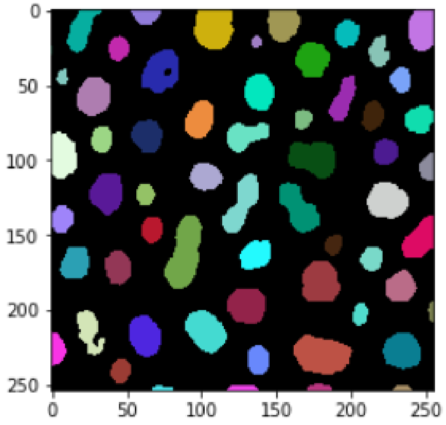
Object segmentation



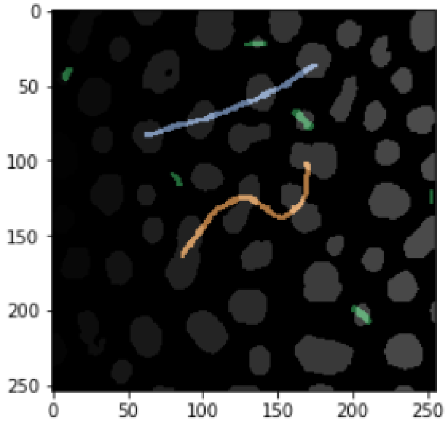
Object label image

Object classification

- Feature extraction + tabular classification



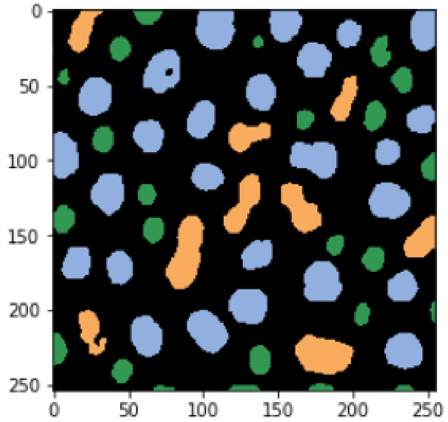
Object label image



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)
```

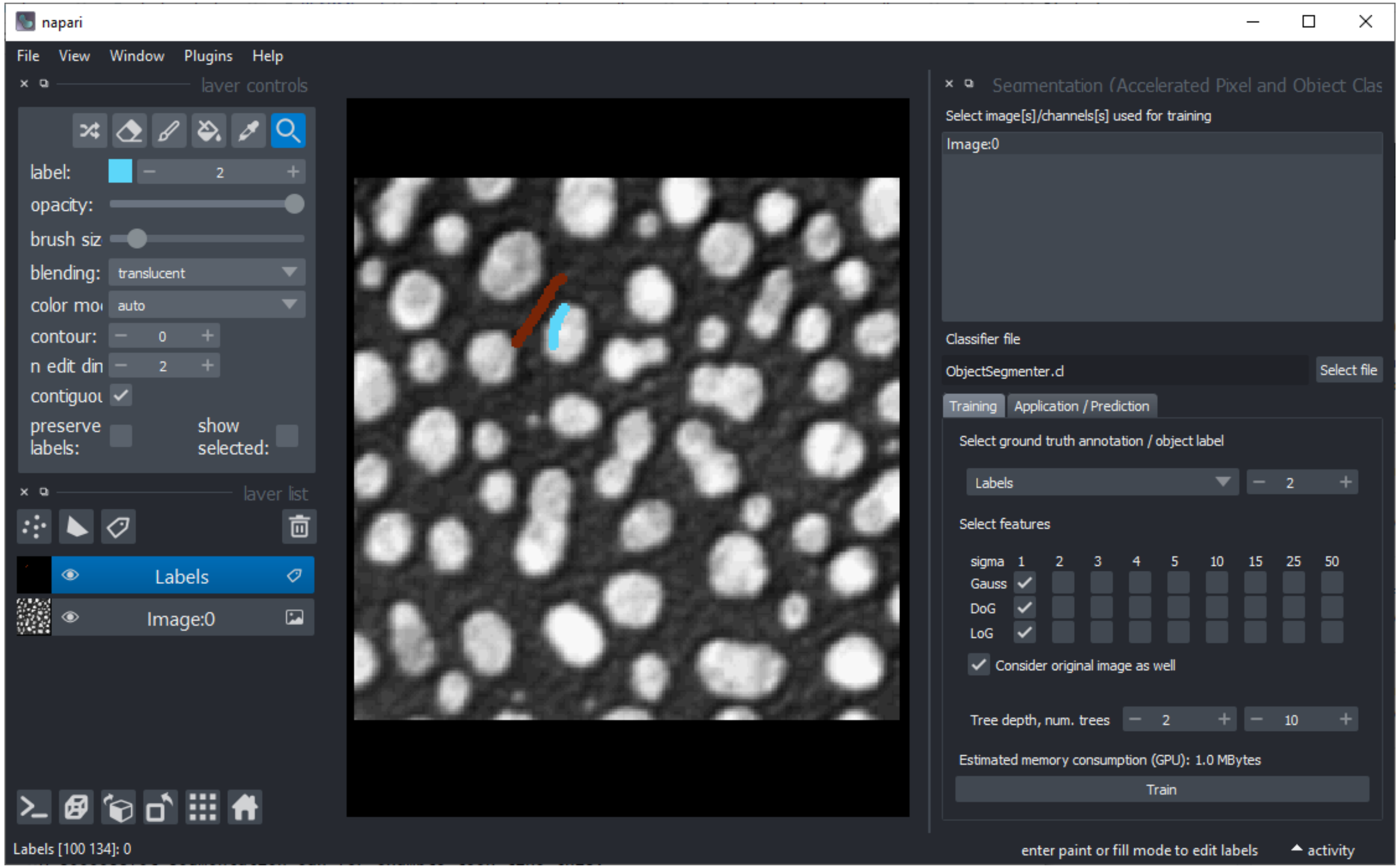
Object classification



Class label image

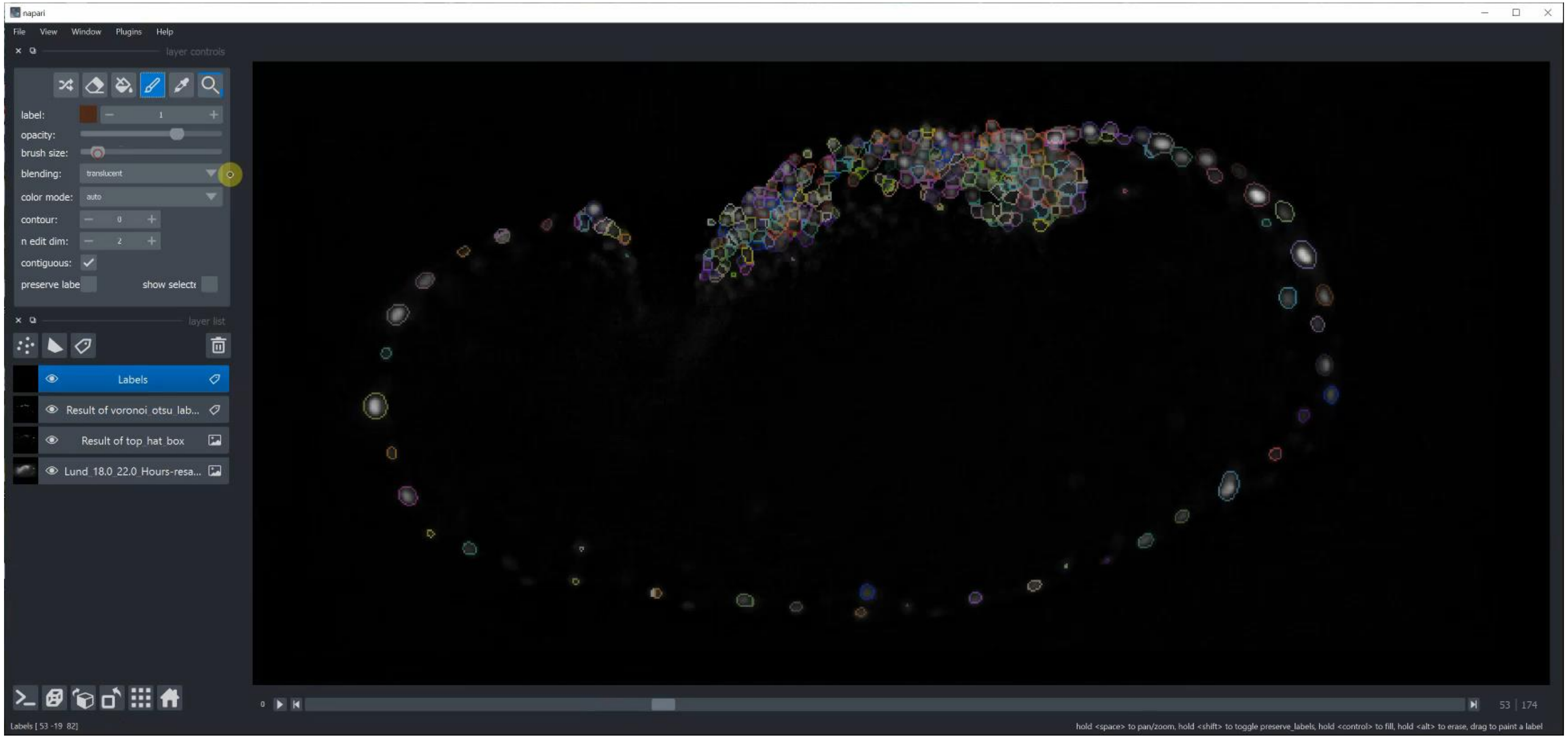
Graphical user interface

- 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



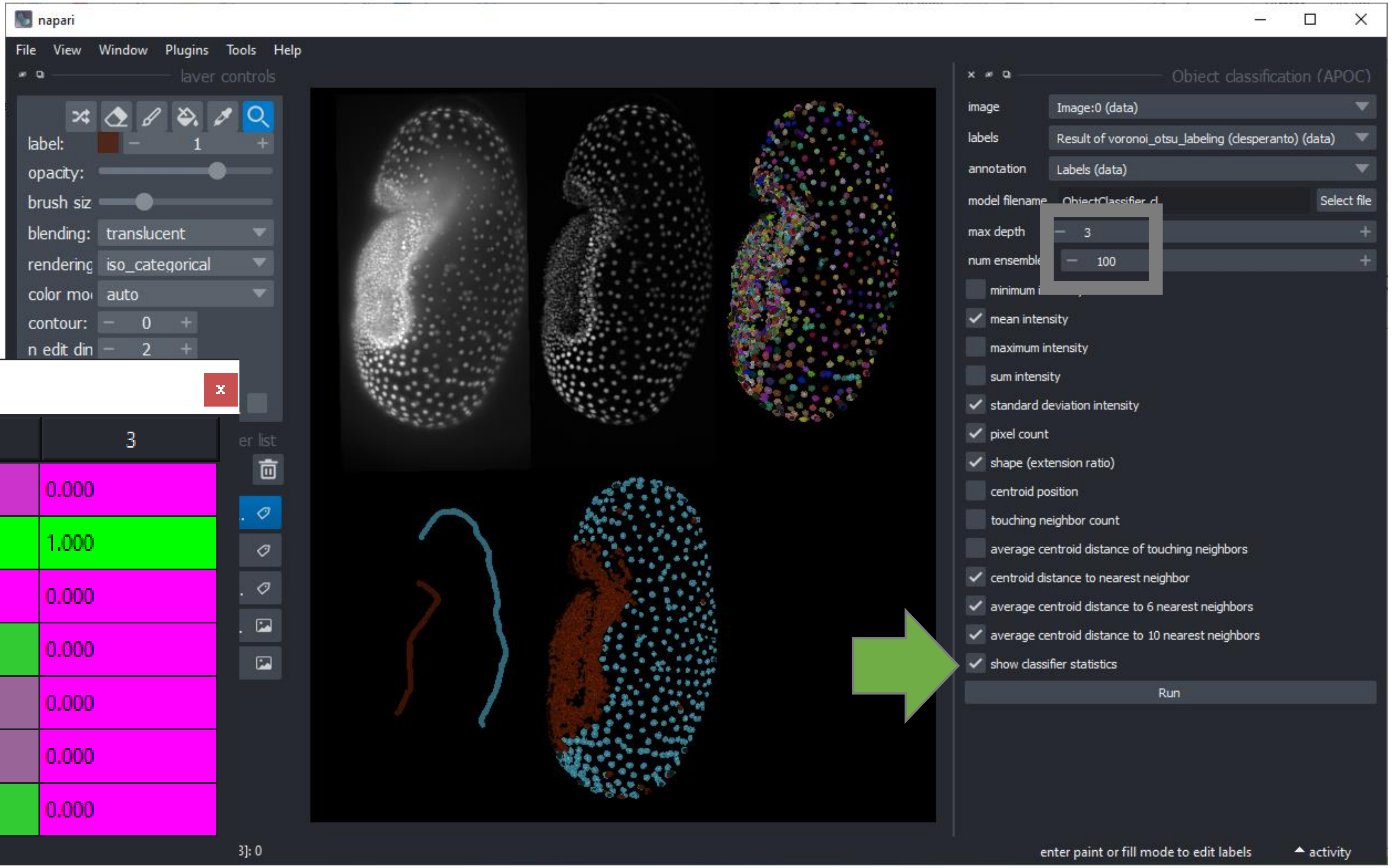
https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification

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

Data exploration / supervised machine learning

PoL Physics of Life TU Dresden

- 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

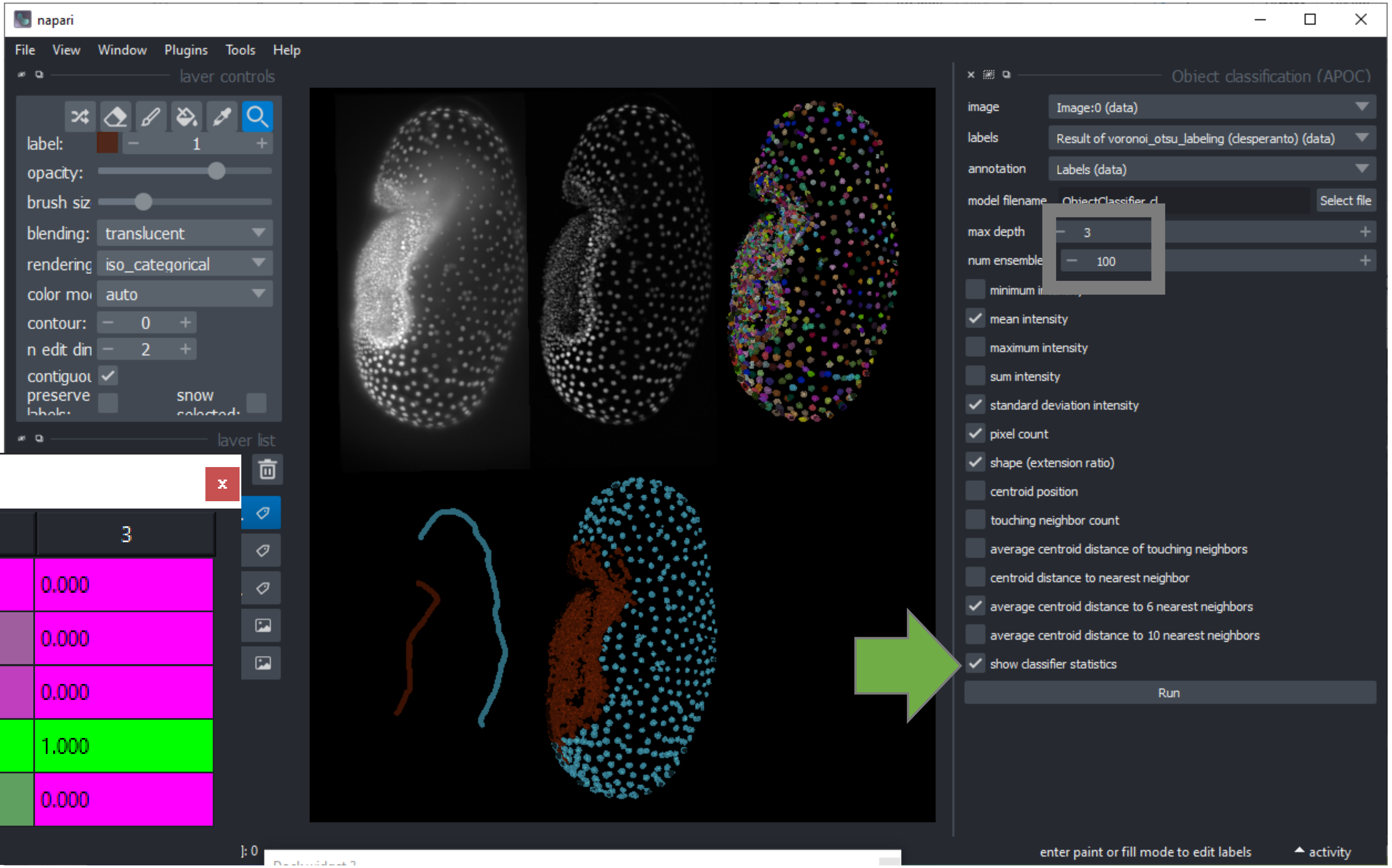
https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification

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 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

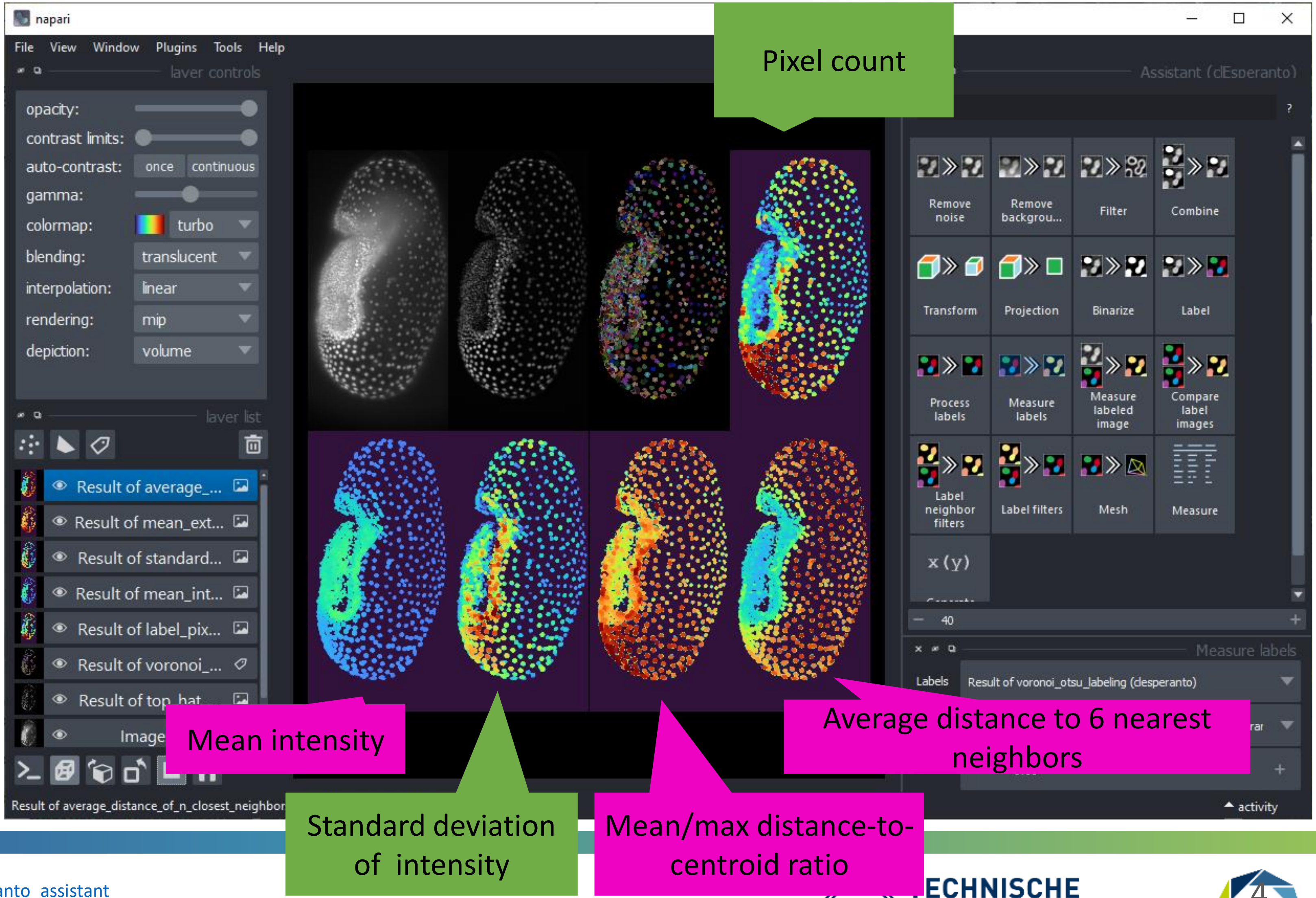
Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Data exploration / supervised machine learning

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

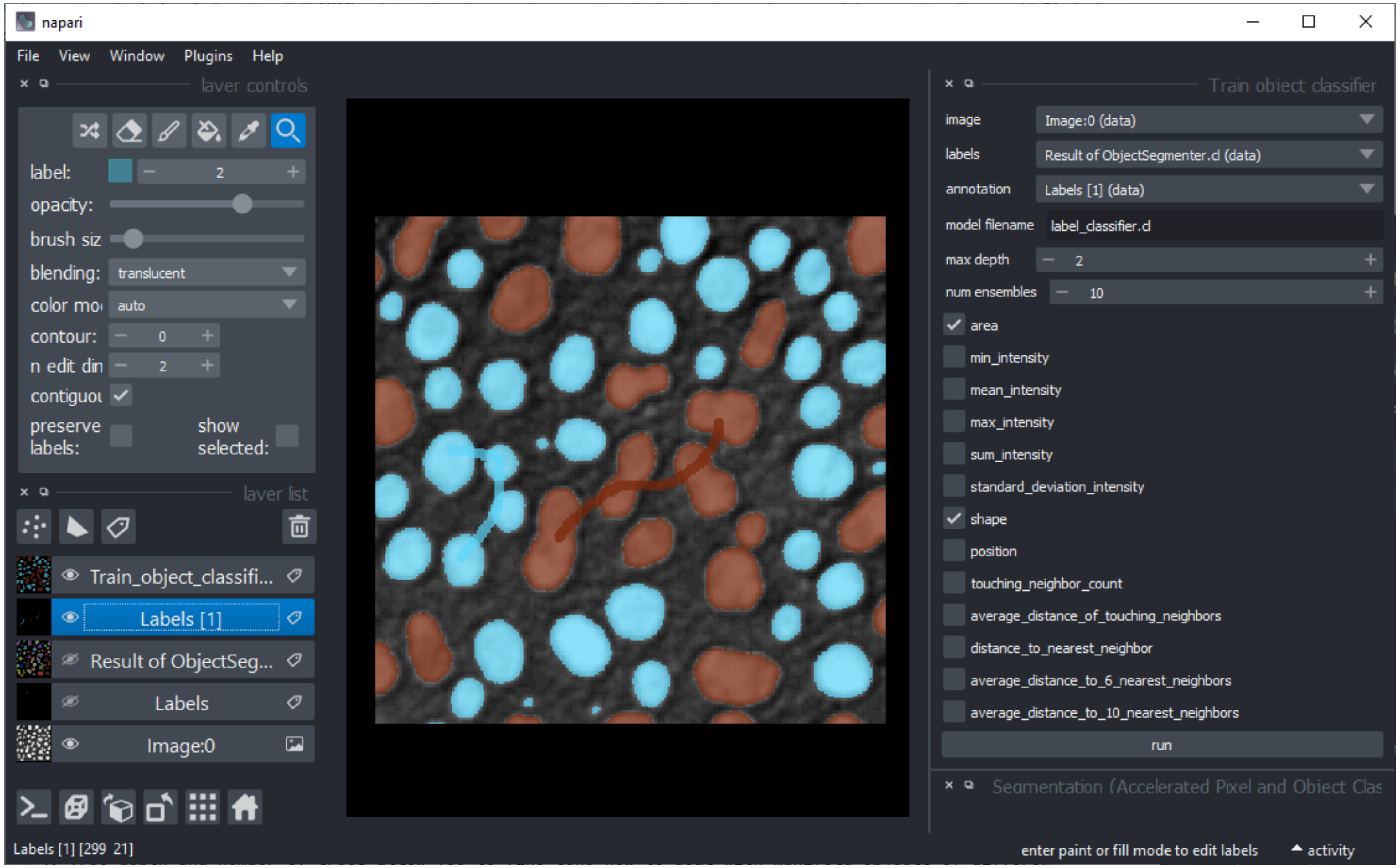
Dock widget 2

	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



Graphical user interface

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



Summary & outlook

Machine learning for image analysis

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

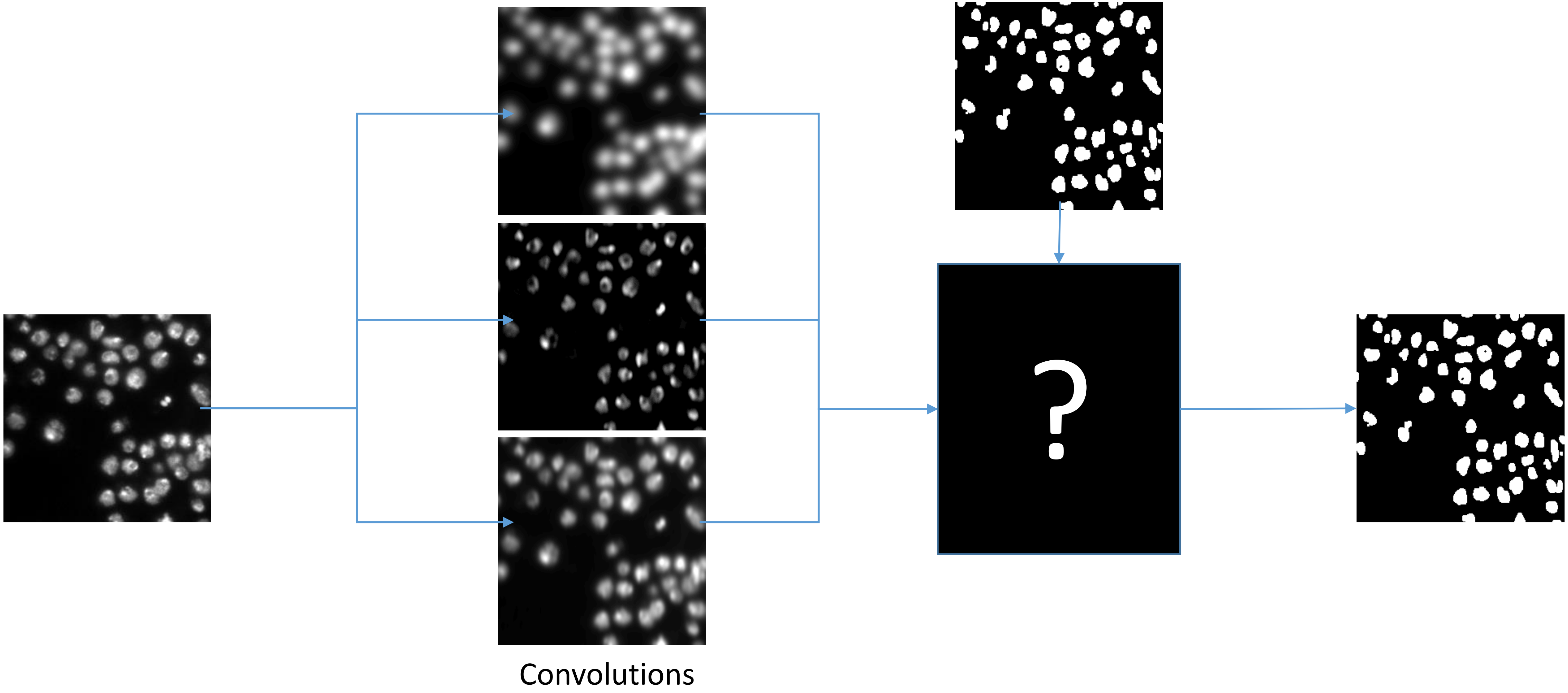
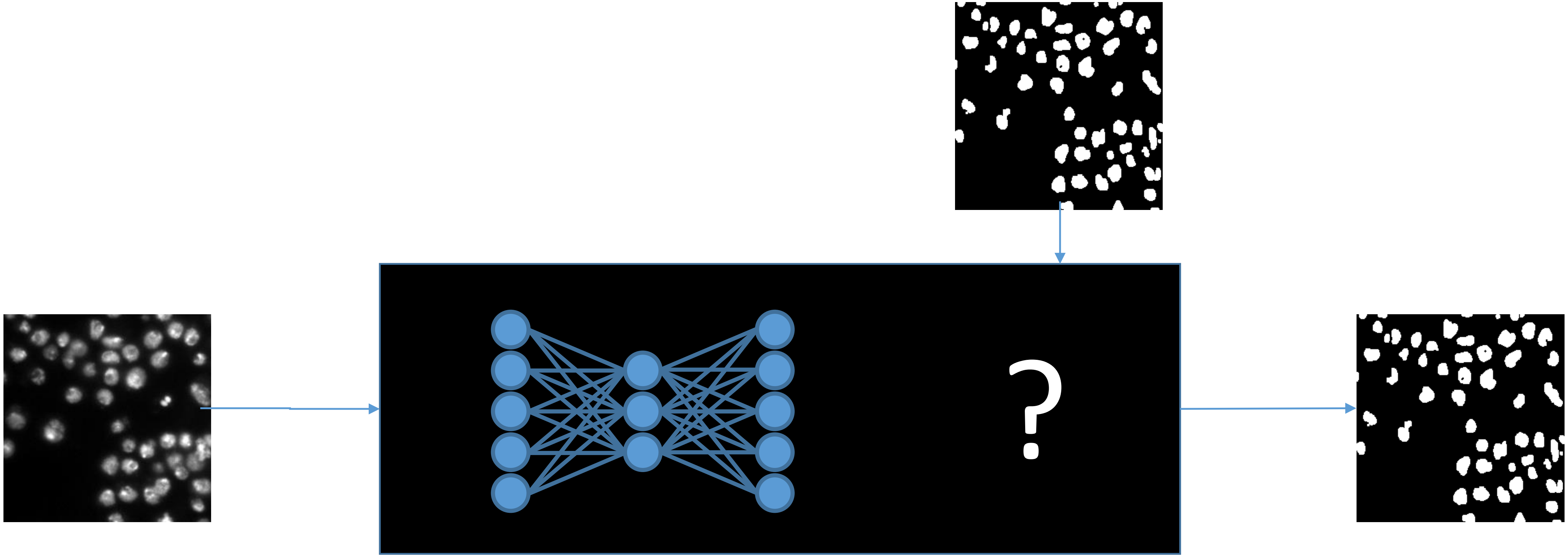


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

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).

Manual workflow design versus machine learning versus deep learning

- Manual workflow design
- Machine learning
- Deep learning

Explicit definition of what's analysed

Semi-automatic feature selection

Fully automatic feature generation

We specify features and how to combine them

We need to check carefully that the algorithm doesn't learn misleading features

Workload on human side is high

Training can take long time

We can inspect code / models to understand how a decision is made

Allows us to analyze data we cannot manage manually

Uses neural networks

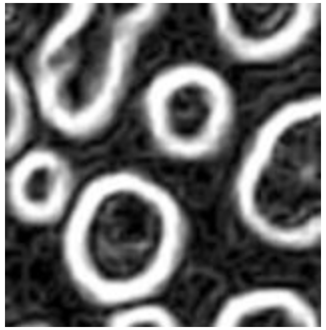
Uses artificial neural networks

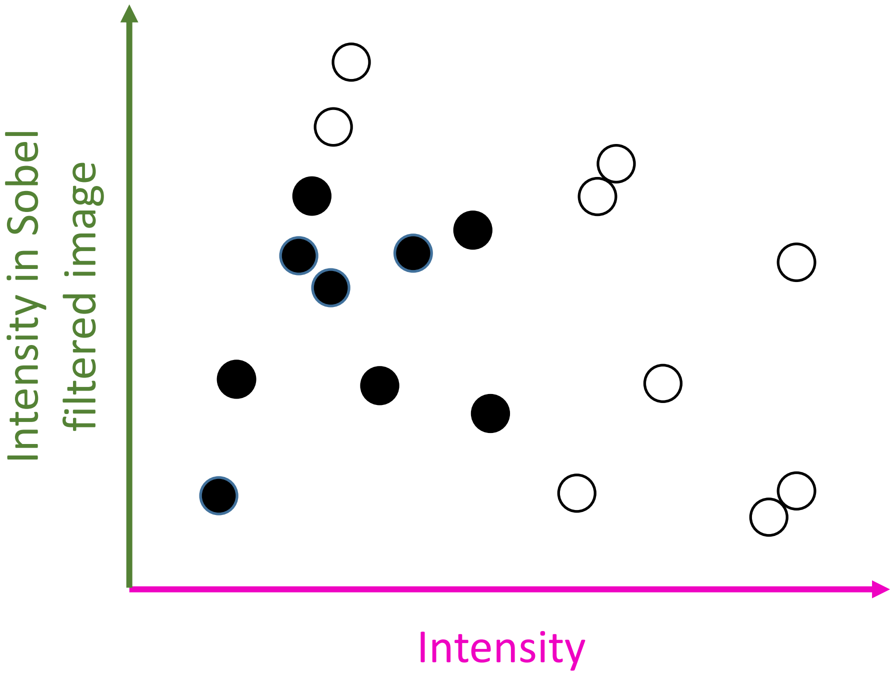
Needs training

Object classification

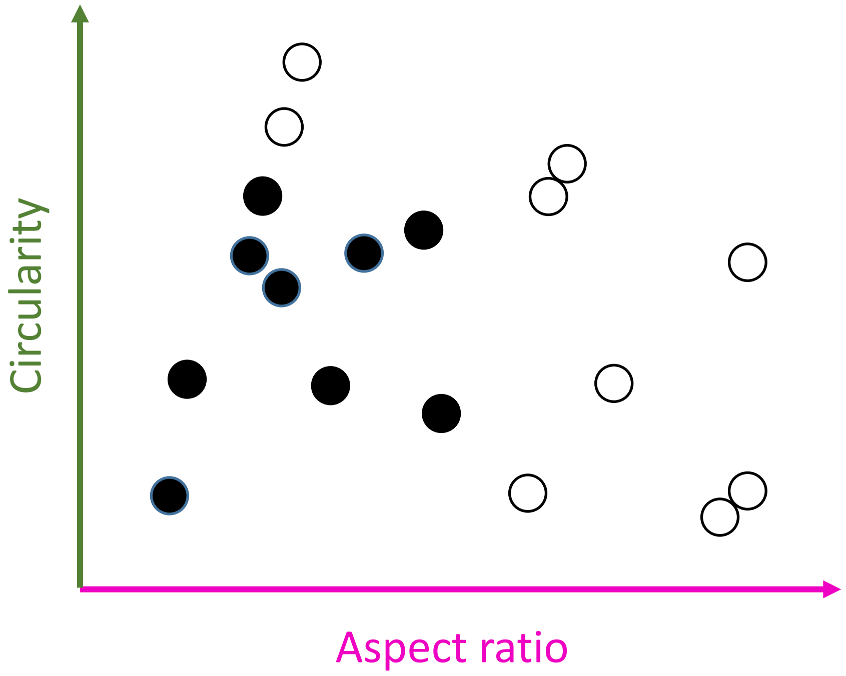
- What if we exchange pixel features with object features?

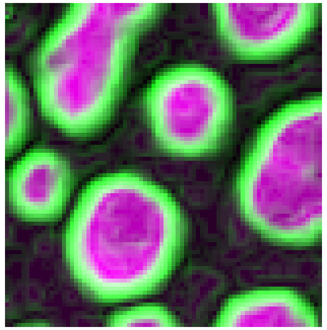
Pixel classification

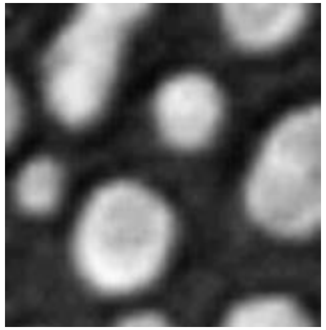




Object classification



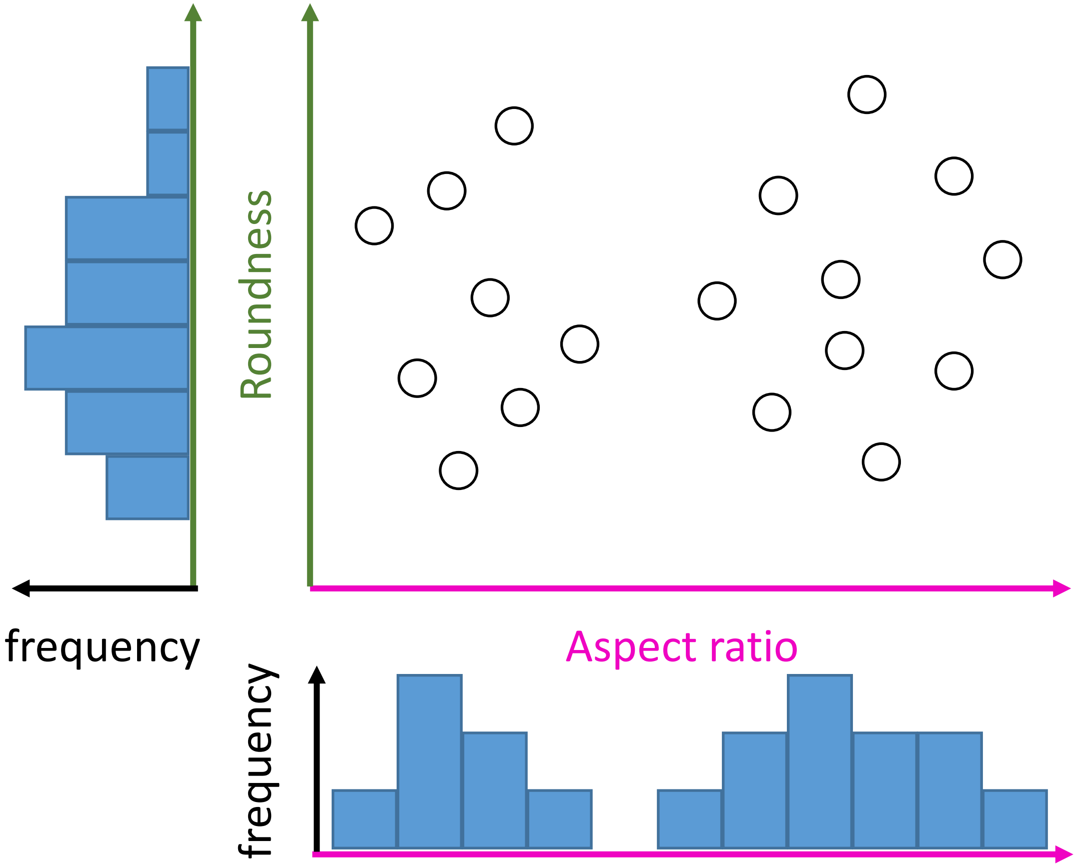




- The algorithms work the same but with different
  - Features
  - Number of features
  - Tree / forest parameters
  - Selection criteria

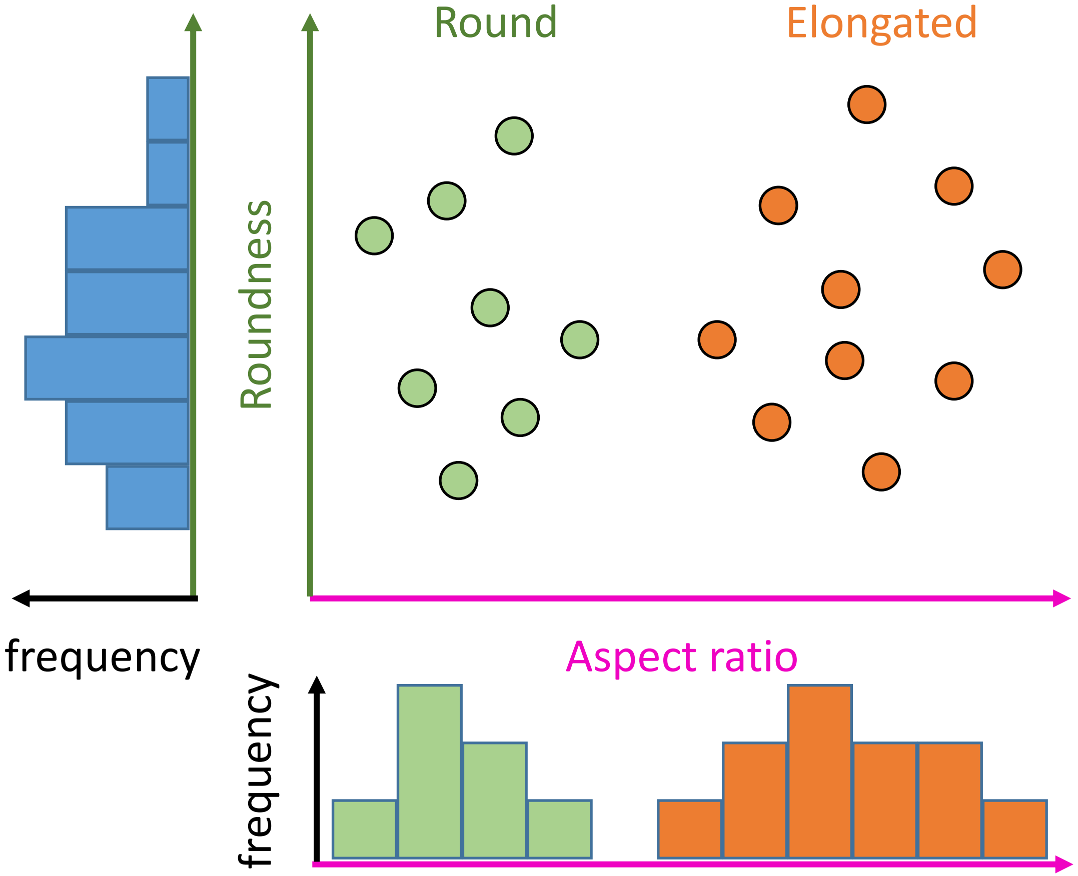
Unsupervised machine learning

- 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

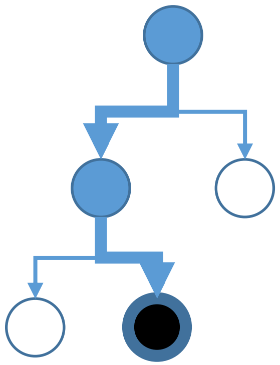


Further reading:
- Principal component analysis
- Cluster analysis

Summary

Today, you learned

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



Coming up next:

- Deep learning