BIDS-Training 2024

ScaDS.AI DRESDEN LEIPZIG

CENTER FOR SCALABLE DATA ANALYTICS AND ARTIFICIAL INTELLIGENCE

Day 2, Session 3: Machine Learning for Pixel and Object Segmentation

SPEAKER: Christian Martin, Anja Neumann

DATE: 14-05-2024

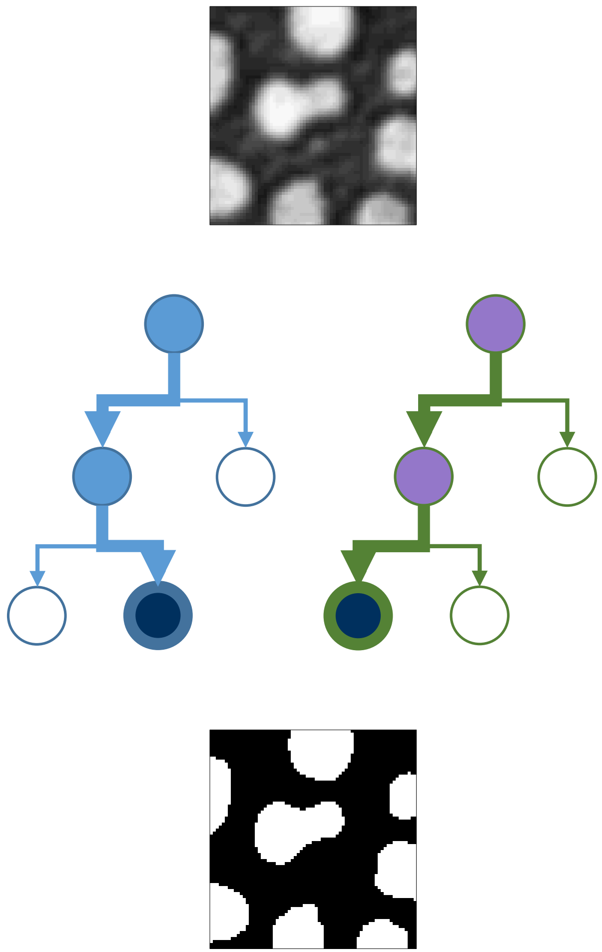
Overview

Machine Learning

- Introduciton
- Decision Tree / Random Forest
- Image Segmentation using thresholding
- Image Segmentation using machine learning
- Object classification
- Segmentation quality
- Model validation
- Outlook

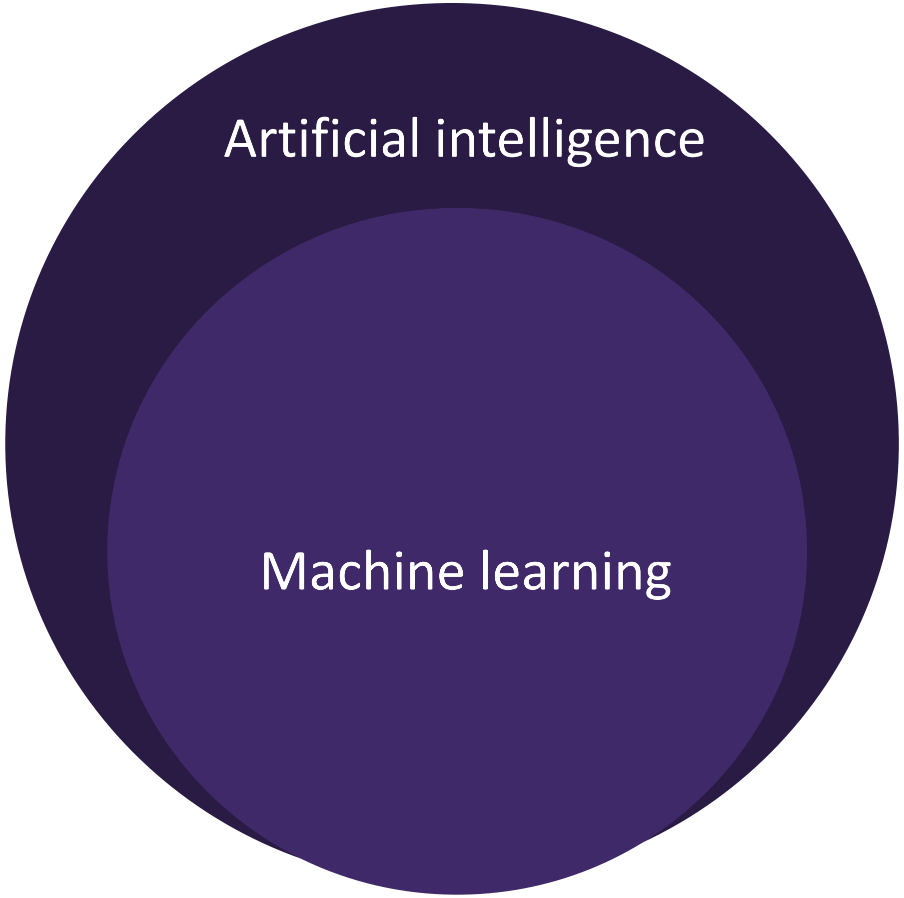
Practical part with Python

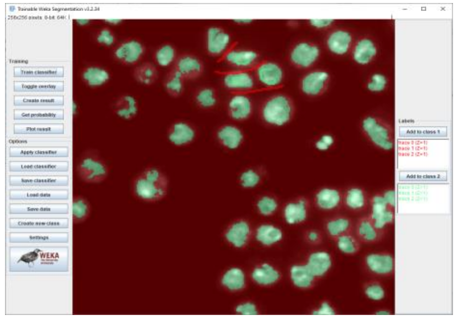
- scikit-learn / napari
- Accelerated pixel and object classification (APOC)



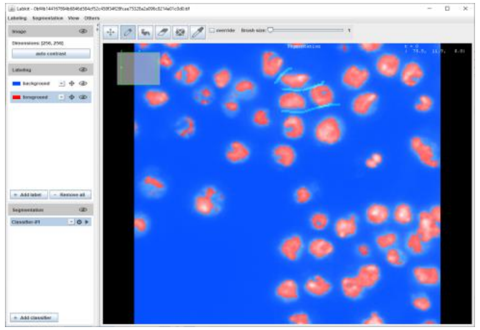
Machine learning

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

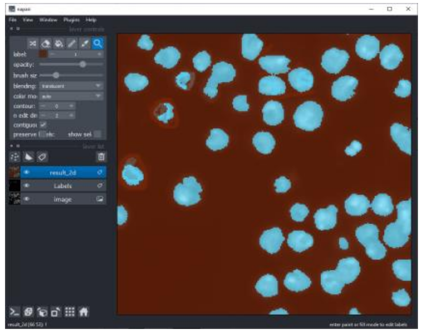




Trainable Weka Segmentation
https://imagej.net/plugins/tws/



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



Python/scikit-learn/napari/apoc

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

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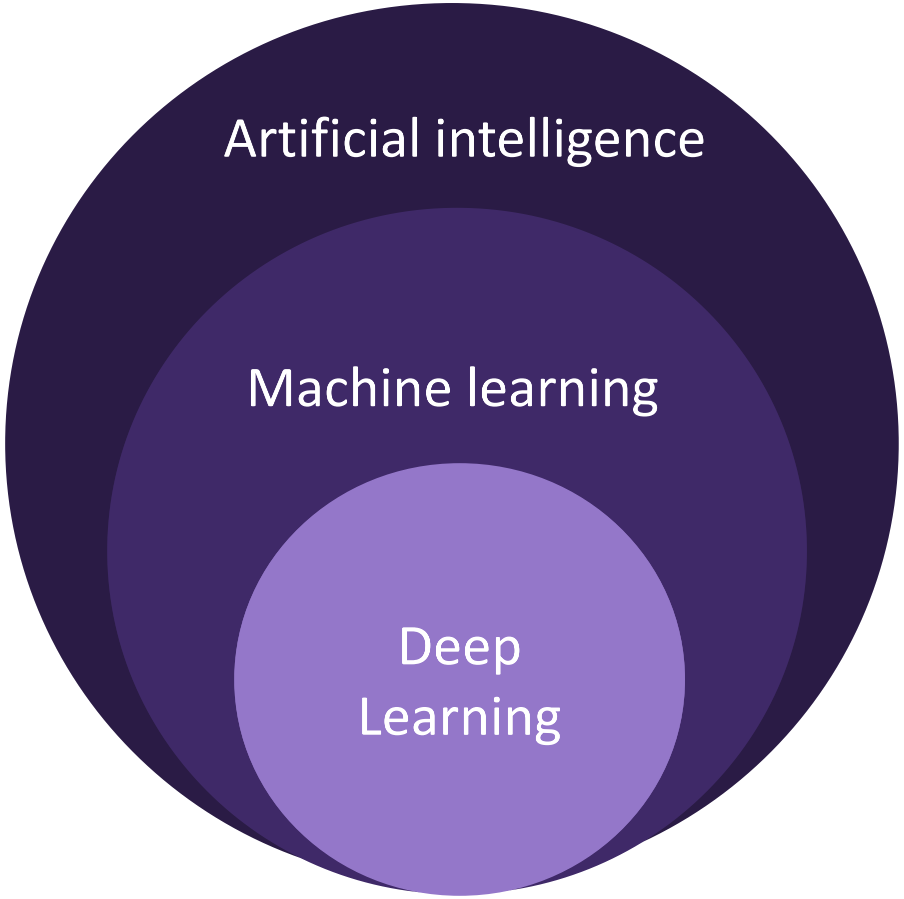
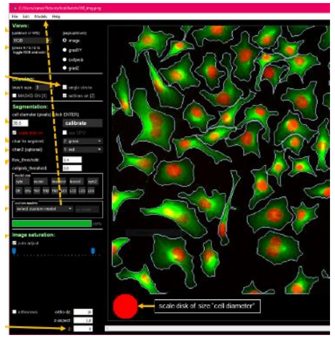
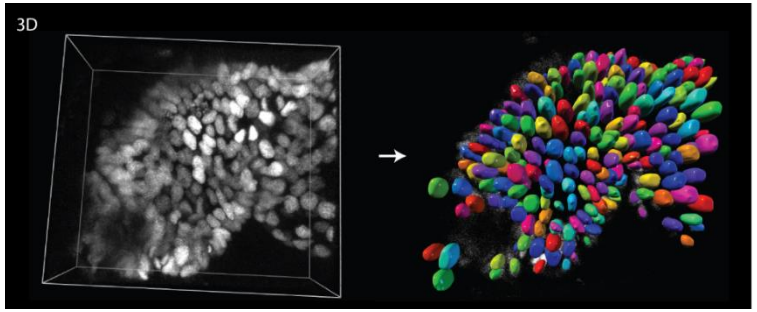
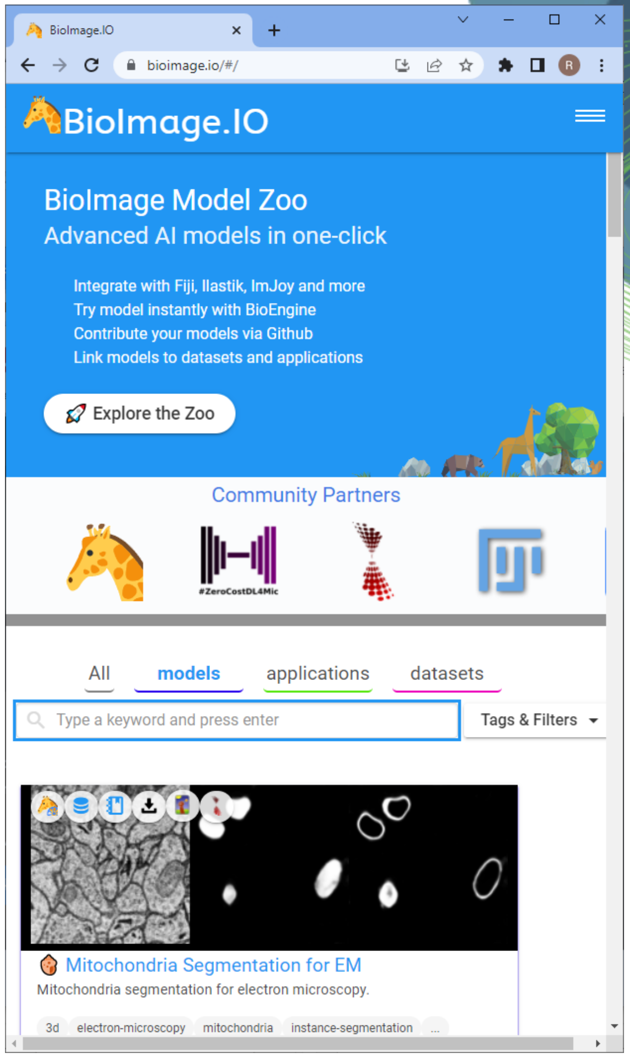




www.cellpose.org/



https://github.com/stardist/stardist



https://bioimage.io/

Logos and screenshots are taken from the github repositories / websites provided under BSD and MIT licenses.

Machine Learning

Machine Learning

- subfield of Artificial Intelligence
- Automatic construction of predictive models from given data
- Learning from Data (data-driven approach)
- Input Data: m items of n dimensions
- If available, ground truth for each item
  → classified data

id	dim1	dim 2	…	dim n	class
1	69	23.5	…	4.3	A
2	54	27.4	…	2.7	C
3	81	22.4	…	5.2	B
4	72	31.5	…	1.5	C
5	69	25.4	…	4.8	A
…	…	…	…	…	…
m	78	15.7	…	5.1	C

Main Topics

- Data preprocessing
    - Annotation
    - Missing Values
- Unsupervised Learning
    - Clustering
    - Data Visualization
- Supervised Learning
    - Classification (predict a class)
    - Regression (predict a value)
- Feature Engineering
- Feature Selection
- Dimension Reduction / Embedding

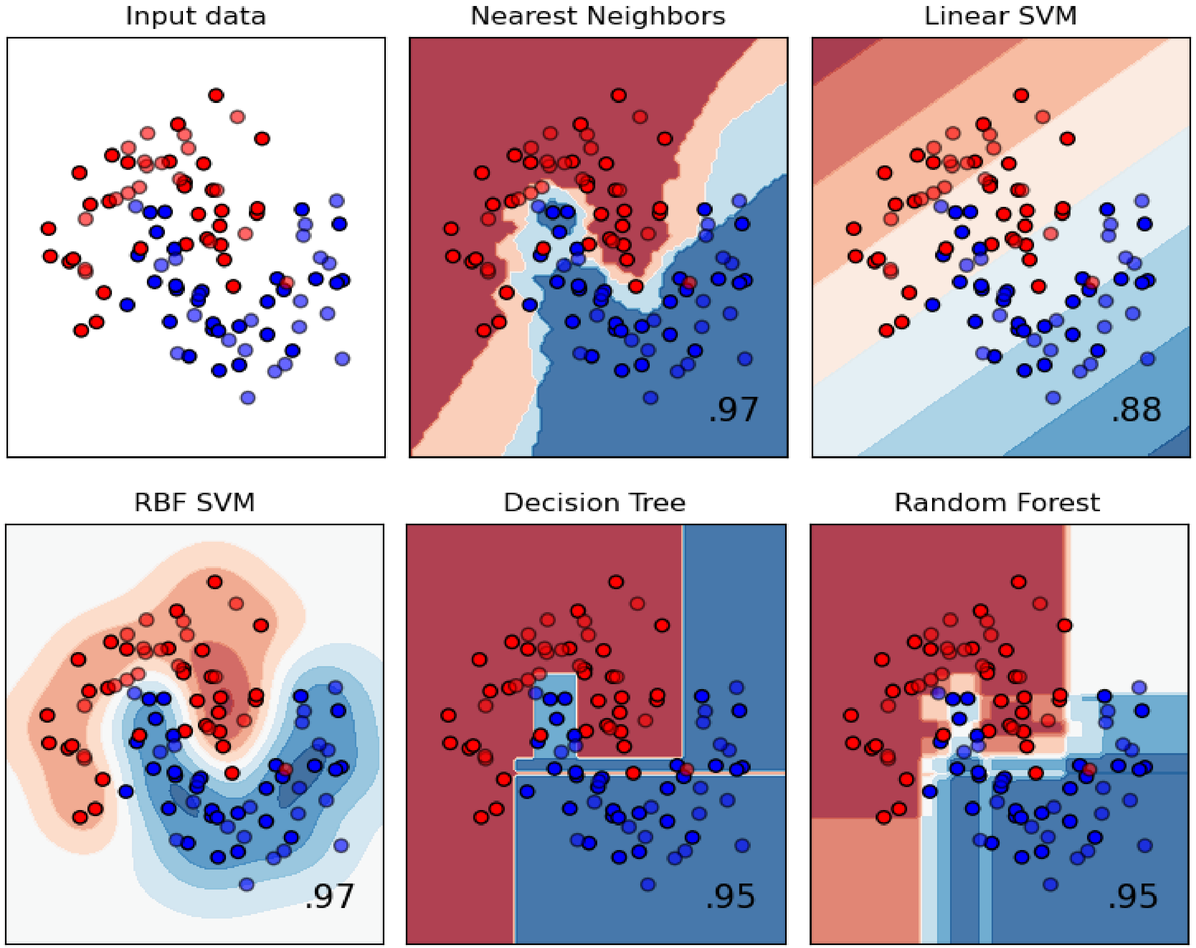
Machine Learning

Supervised Learning

- Train model on training data
  - paint feature space
- Evaluate model on test data
  - estimate class from position of sample in feature space
- Apply model on new data

Supervised Learning Methods

- k-nearest neighbor (knn)
- Linear Regression
- Logistic Regression
- Support Vector Machines (SVM)
- Decision Trees / Random Forests
- Gaussian Process
- Naïve Bayes
- Neural Networks
- …



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

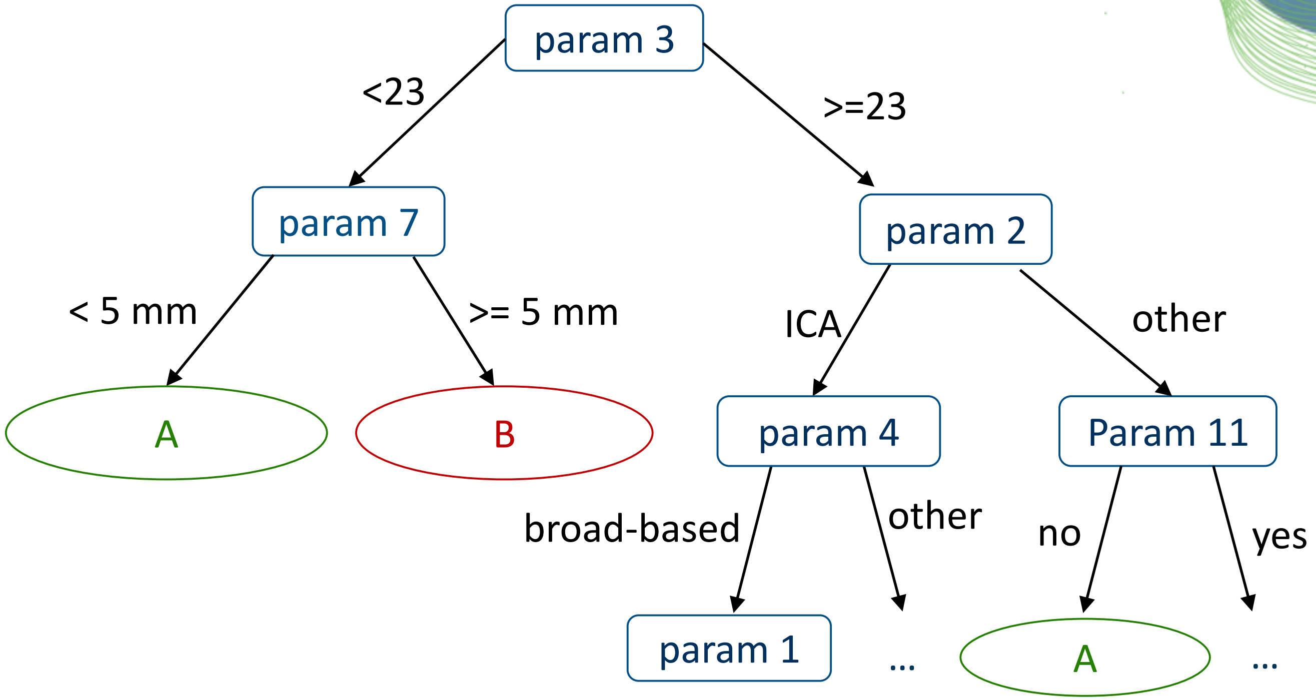
Decision Tree

Introduction

- machine learning algorithm
- data can be interval-scaled, categorical, or mixed
- classification: predict a class
- regression: predict a value
- shows good performance on tabular data
  (5-100 parameters, 50-1000 data points)
- model (tree) is computed based on training data

Preparation

- Divide data in training data / test data
- Use 5-fold cross validation
  - 4/5 of data is training data
  - 1/5 of data is test data
  - Repeat 5 times
- Never train and test trained model on same data!



Decision Tree

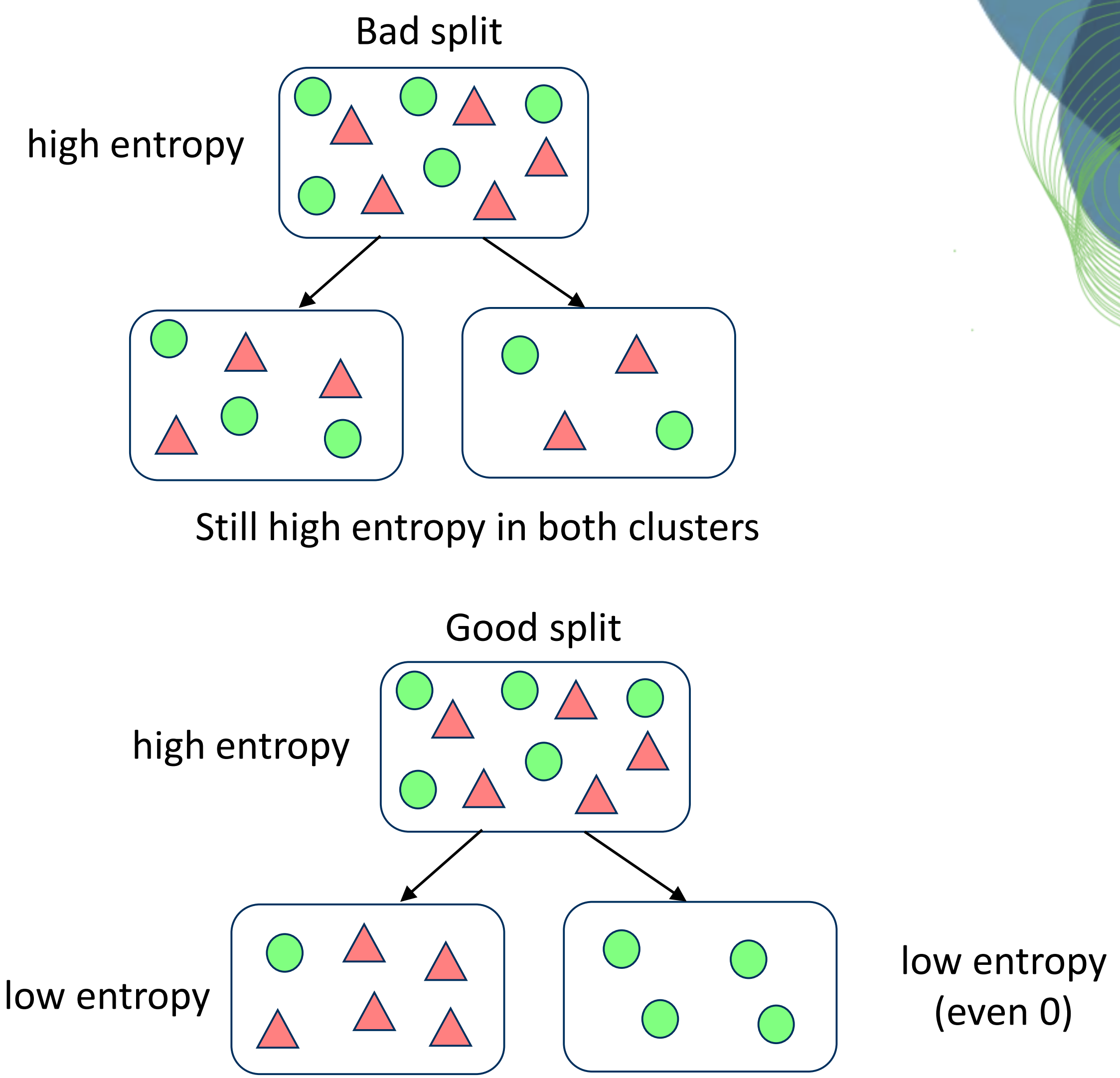
Decision Tree - Training

Training

- Start with complete data of training dataset
- For each step
  - choose parameter and threshold to minimize entropy in remaining clusters (leaves in tree)
  - Split cluster accordingly
- Entropy
  - measure for disorder
- Stopping criteria
  - Maximal depth reached (e.g. 10)
  - Minimal samples in leaf reached (e.g. 5)

Classification / Application

- Apply tree on
  - test data (for testing) or
  - new data (for application)



Decision Trees and Random Forests

Drawback of Decision Trees

- Problem
  - allow few levels → only few parameters are considered
  - allow many levels → overfitting
- Solution: Random Forests

Random Forests

- Idea: train many decision trees with part of the data
- for each tree
  - use only part of the data items
  - use only part of the parameters
- Train n different trees ()
- Result: n slightly different decision trees
- Application: combine results using majority-voting

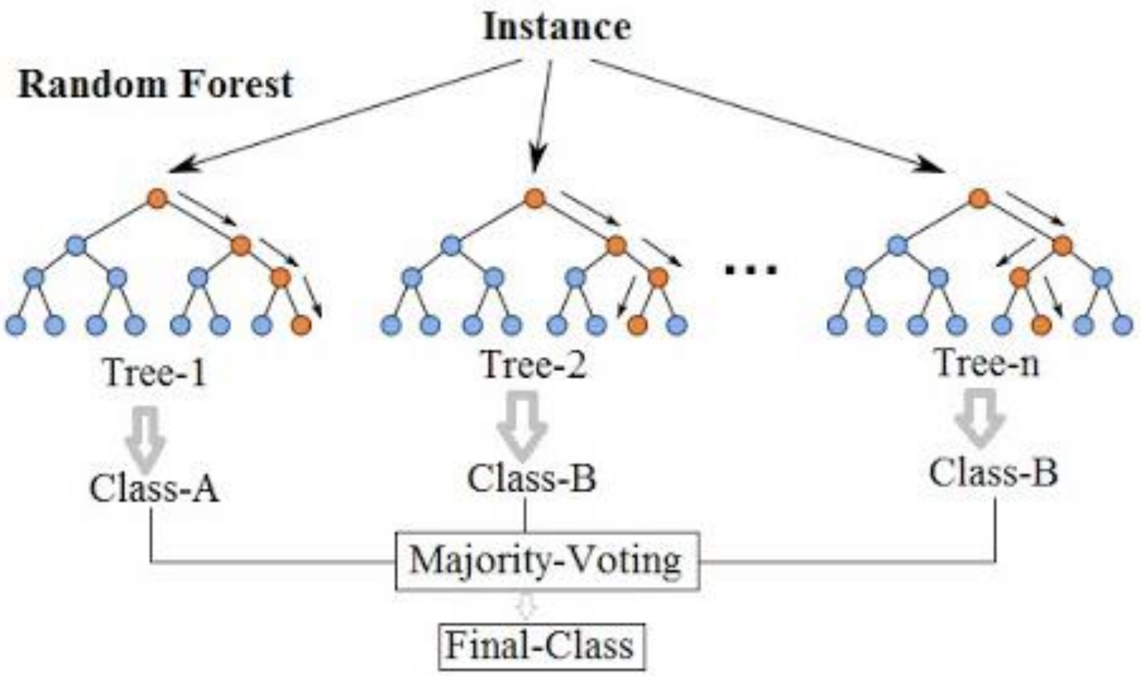


Image Segmentation

Image segmentation using thresholding

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

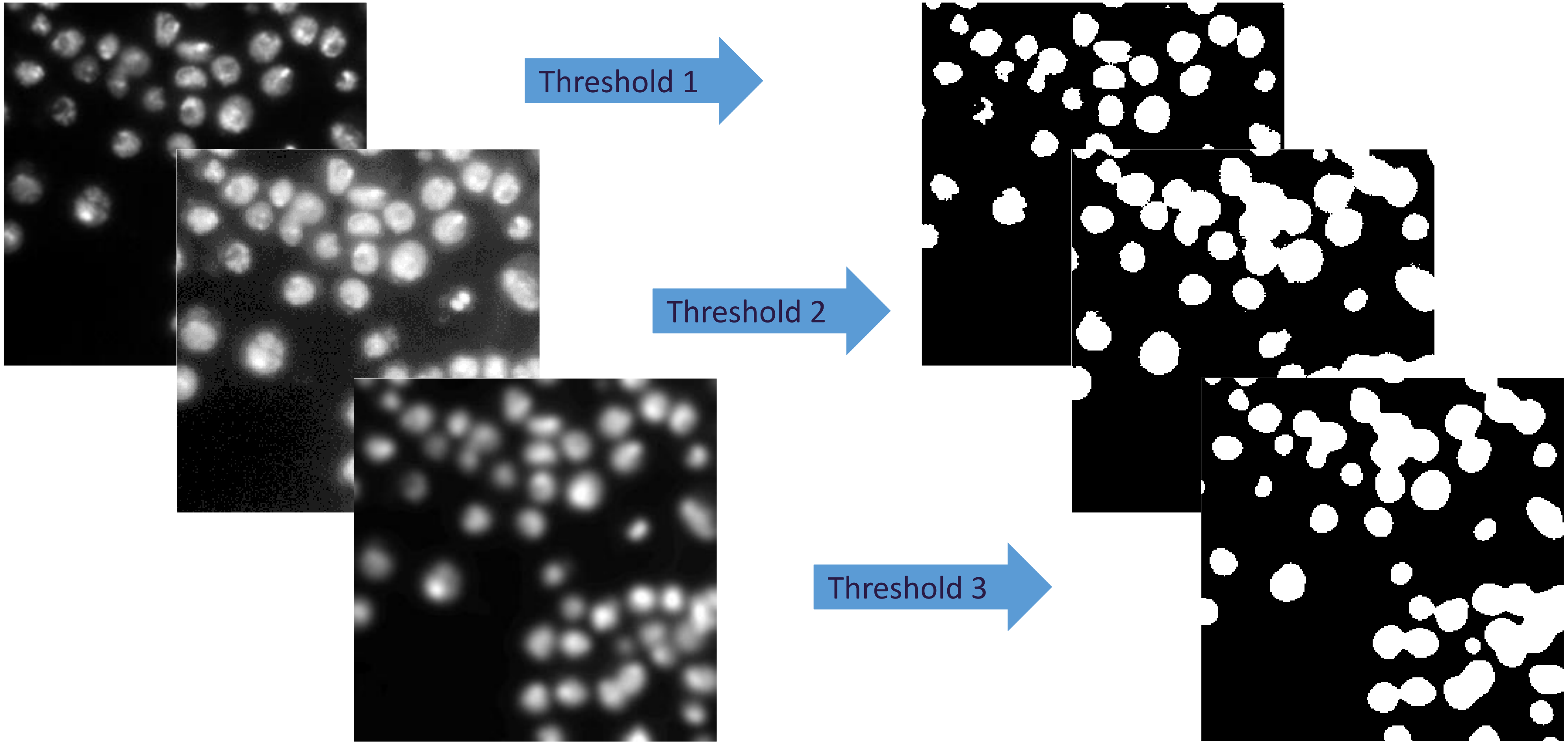


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

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

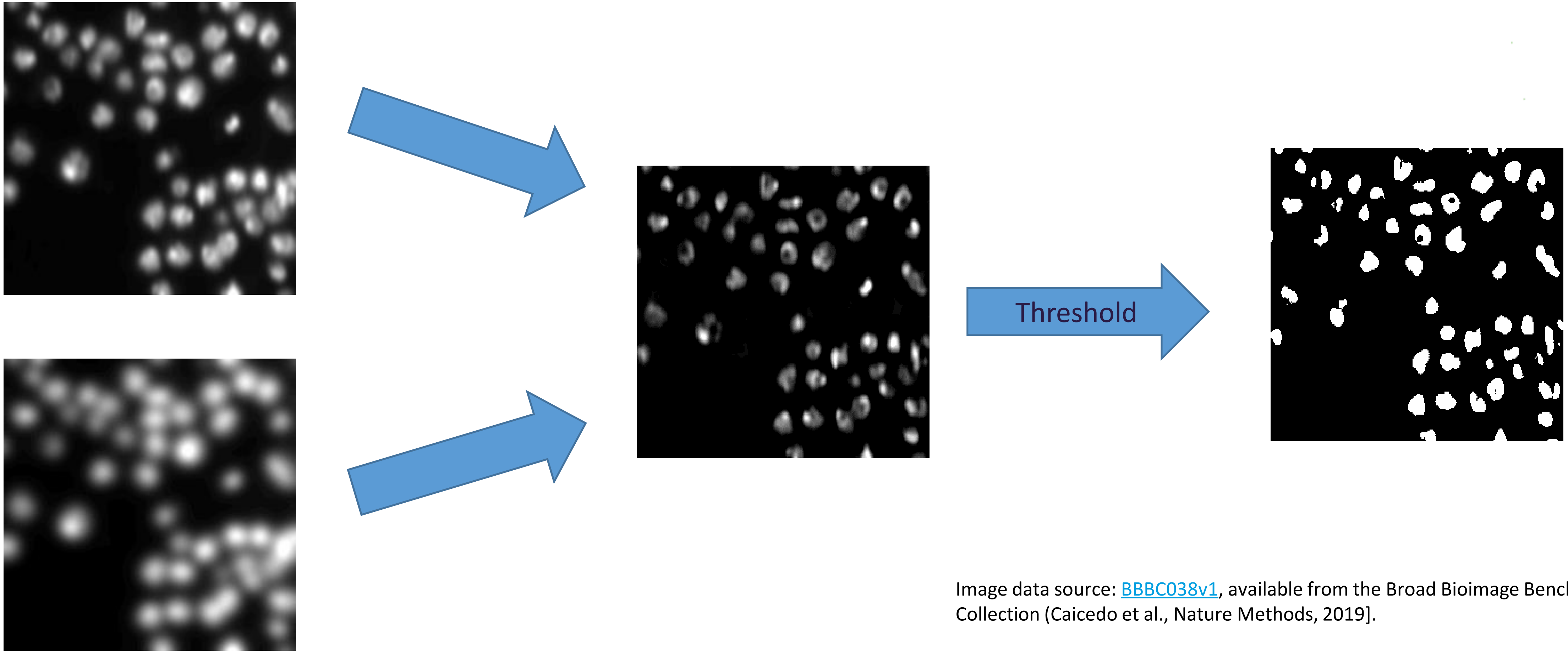
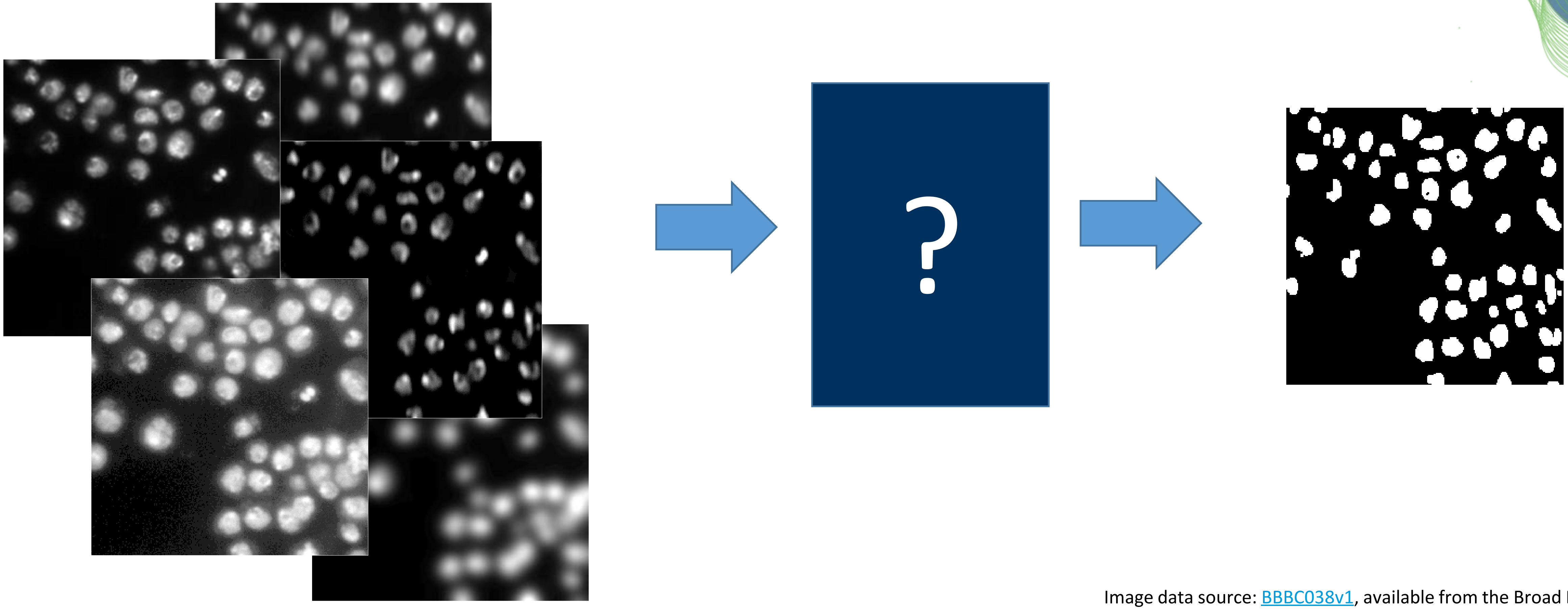


Image data source: [BBBC038v1](), available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

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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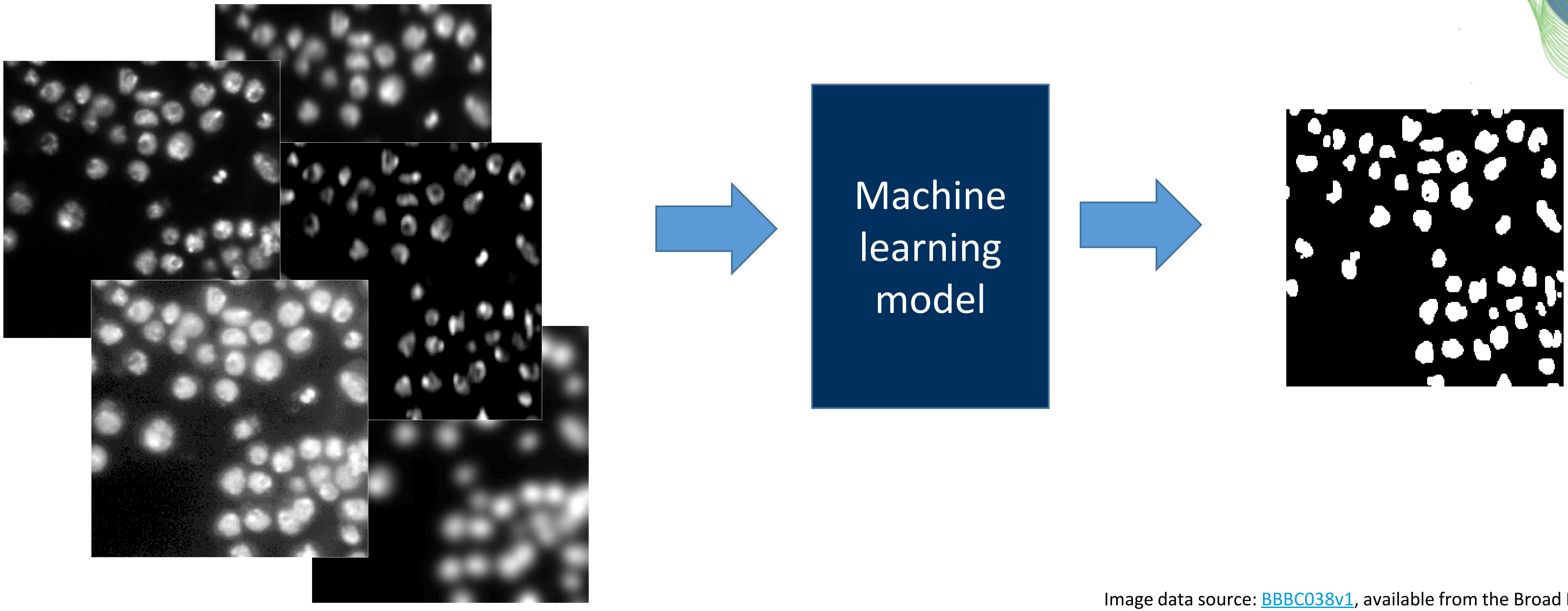


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

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

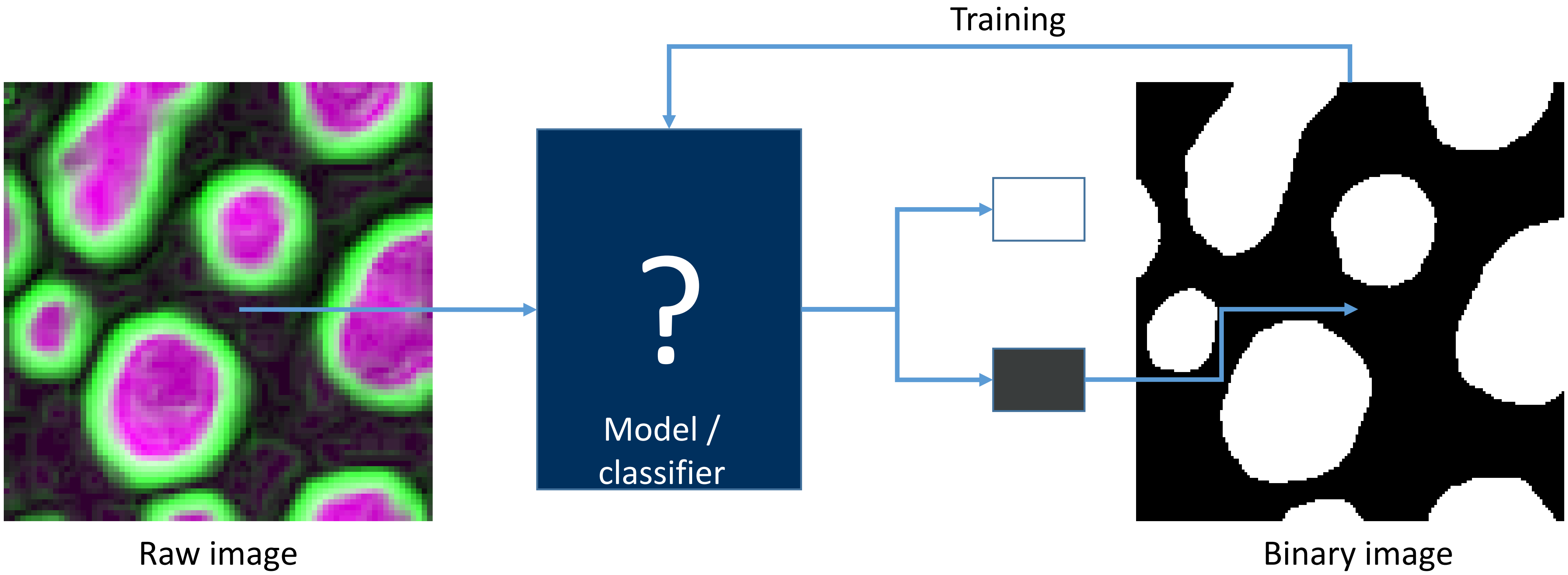
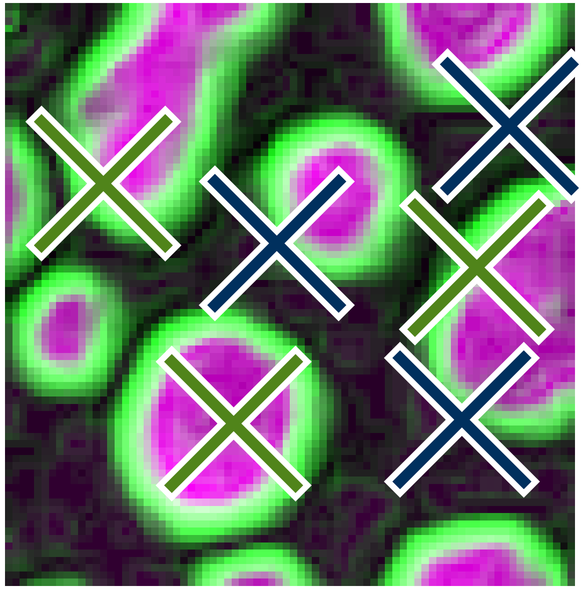
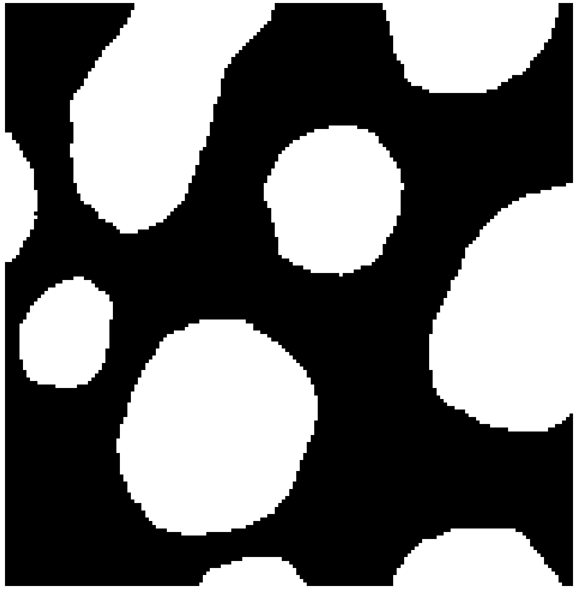


Image segmentation using pixel classification

- Idea: use different features of a pixel to classify it to background or foreground
- Each pixel is considered separately
- Features:
  - Intensity/color of original pixel
  - Gaussian blur image
  - DoG image
  - LoG image
  - Hessian
- Features from different images
- For efficient processing, we randomly *sample* our dataset
- Create a dataset with pixel features vectors that belong to the background and the foreground
- Use machine learning (e.g. Random Forest) to classify each pixel





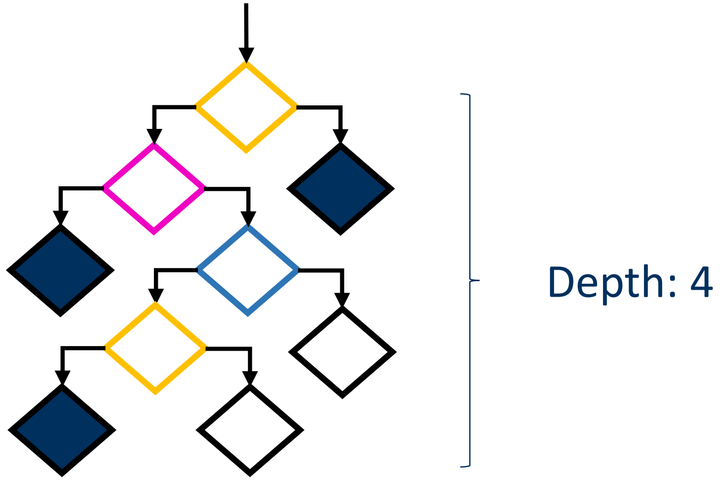
Background

Foreground

Random Forest Pixel Classifier

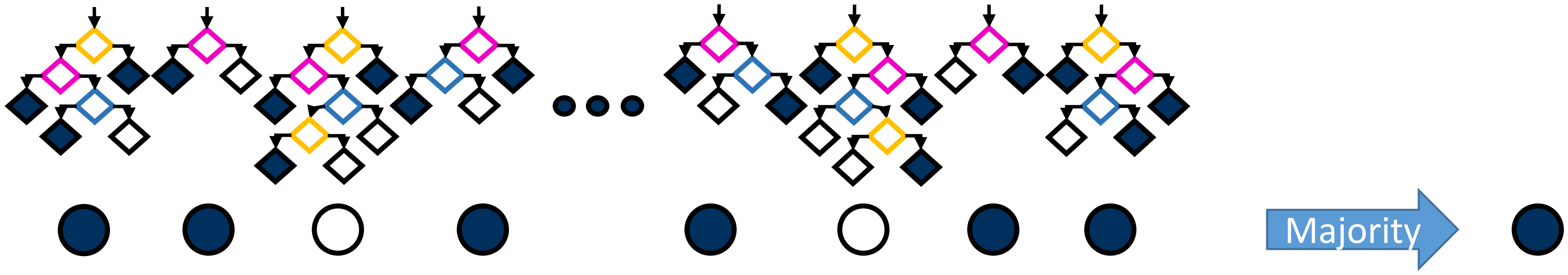
Available features: > 20

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



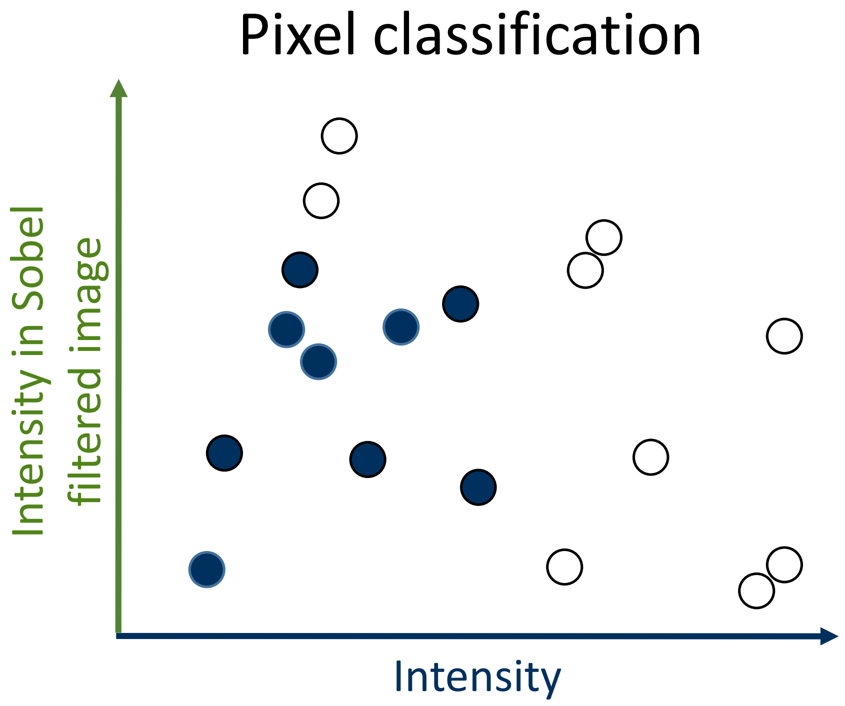
- Train t trees on selected features and sampled pixels -> t different trees
- Combination of different tree decisions by max/mean voting

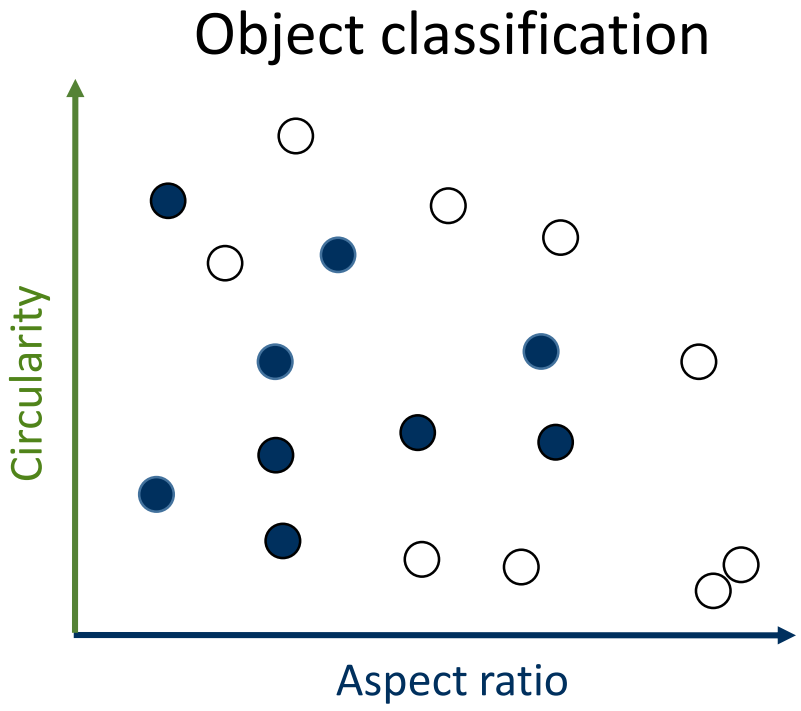
t > 100 trees

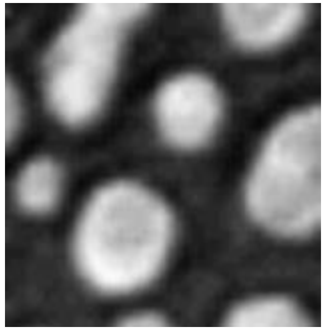


Object classification

- Use object features instead of pixel features (e.g. size, aspect ratio, shape, circularity)
- The algorithms work the same

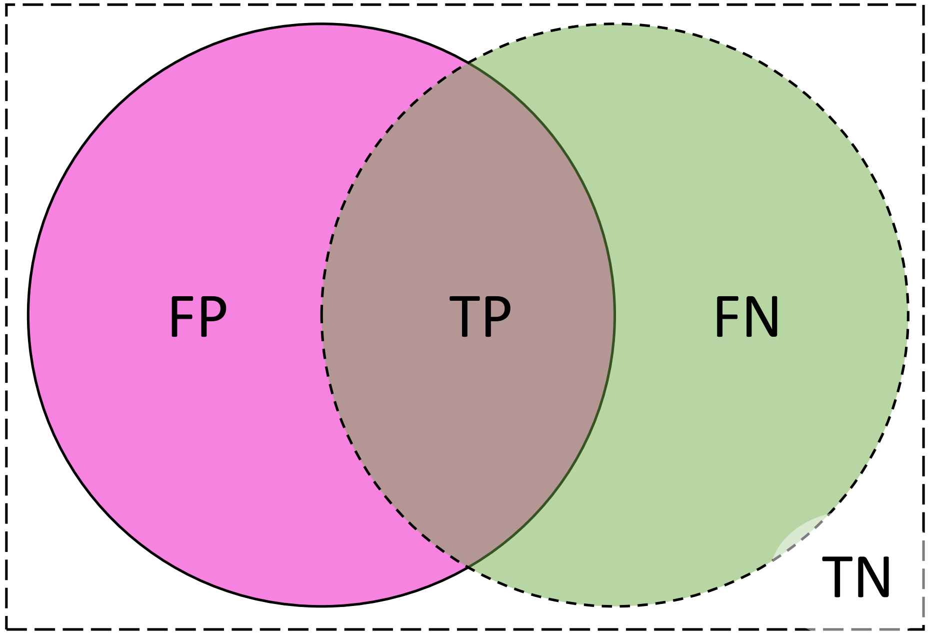






Segmentation quality estimation

- 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 <u>Theory of Sets</u>



A Prediction A

B Reference B (ground truth)

ROI Region of interest

TP True-positive

FN False-negative

FP False-positive

TN True-negative

Overlap (a.k.a. Jaccard index) $\frac{TP}{FP + TP + FN}$ 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?

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 (80% of the available data)
  - Test set (20% of the available data)

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

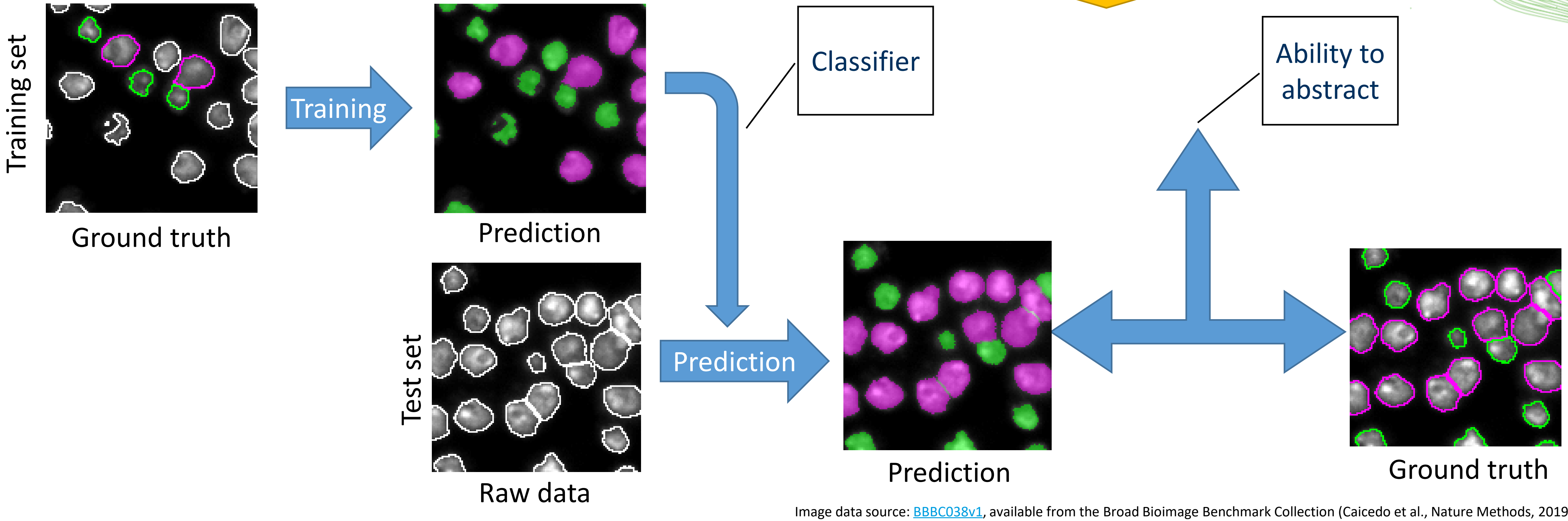


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

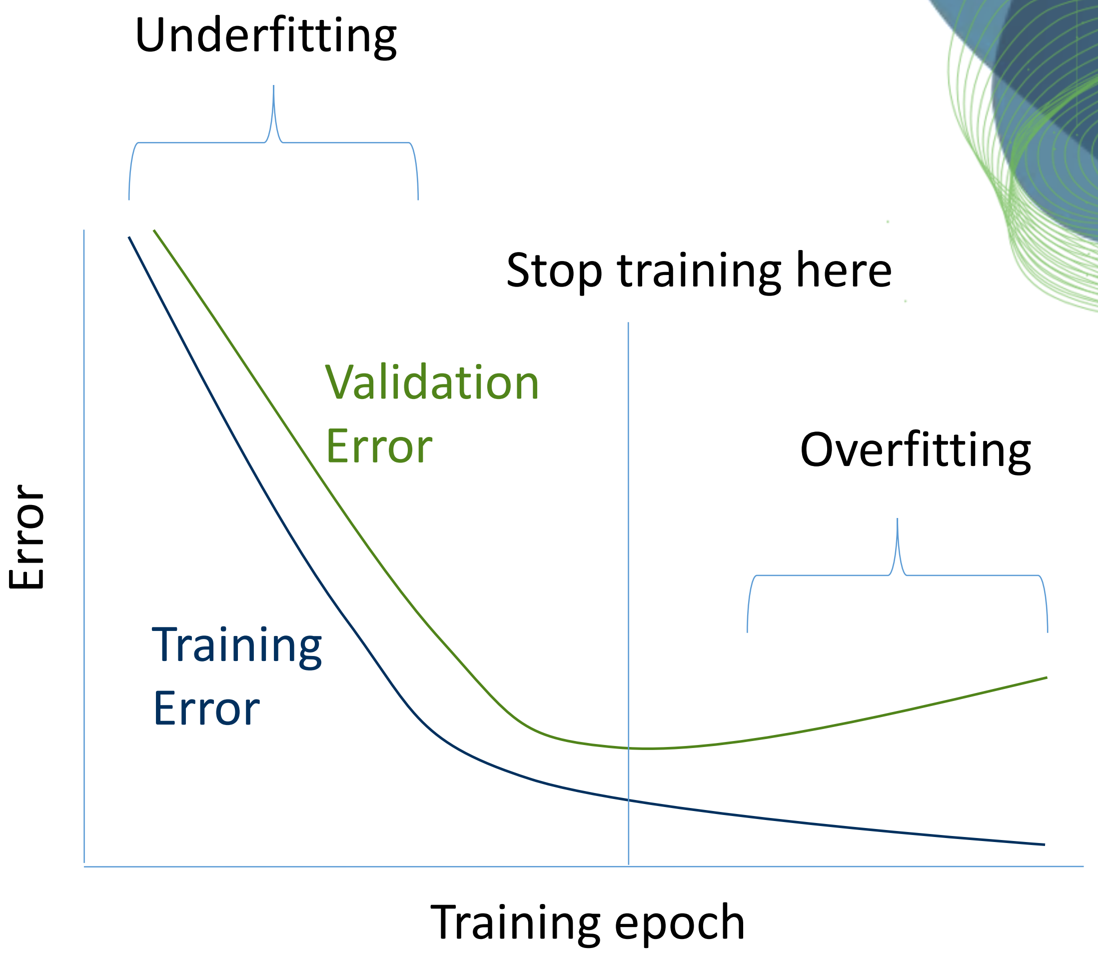
Model validation

Split data in

- Training dataset (80% of the data):
  used for training the model
- Validation dataset (10% of the data):
  after each iteration, see if the model overfits
- Test dataset (10% of the data):
  final evaluation after training is finished

Training

- Find spot with lowest validation error
- Avoid Underfitting: A model that is not trained long enough to capture the structure of the data
- Avoid Overfitting: A model that has been trained too long, has memorized the training data, but is not able to generalize on new data



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

Outlook: Machine learning for image analysis

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

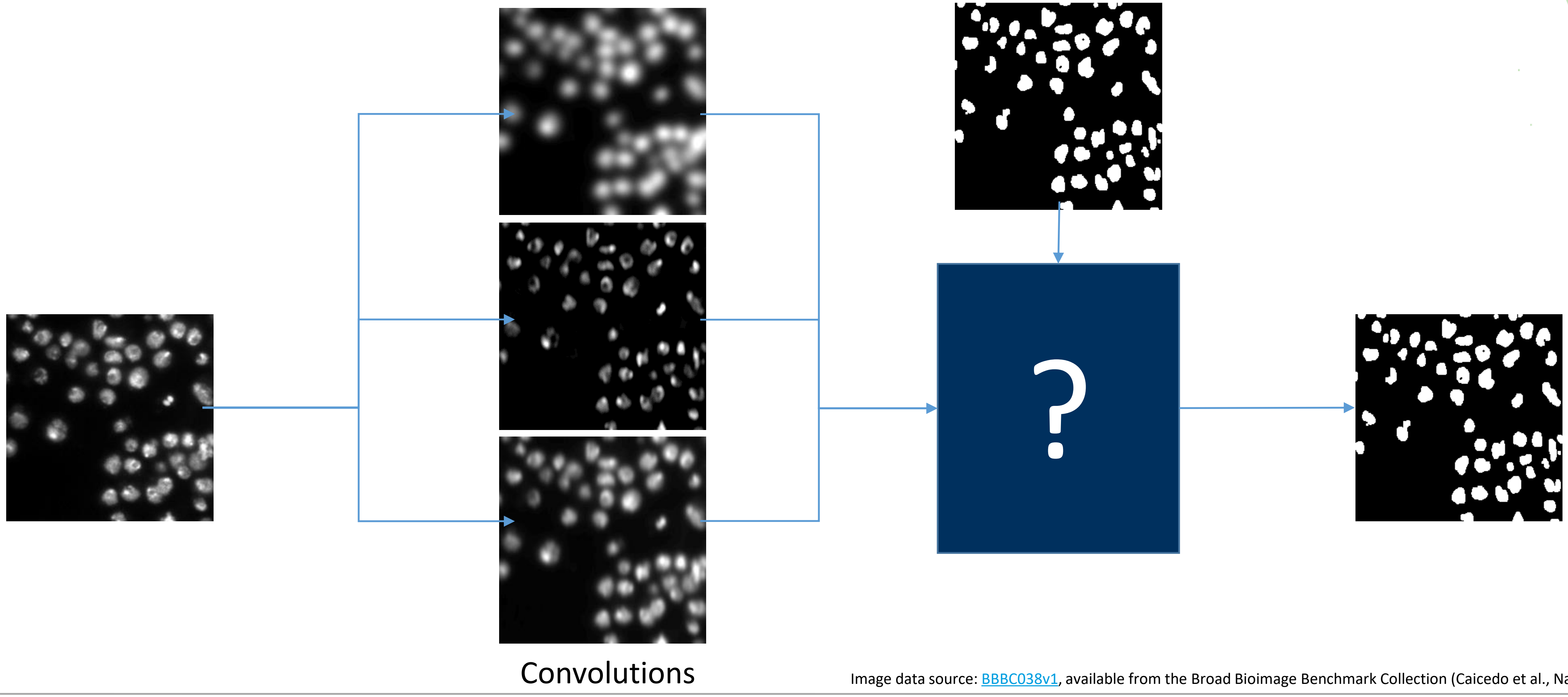
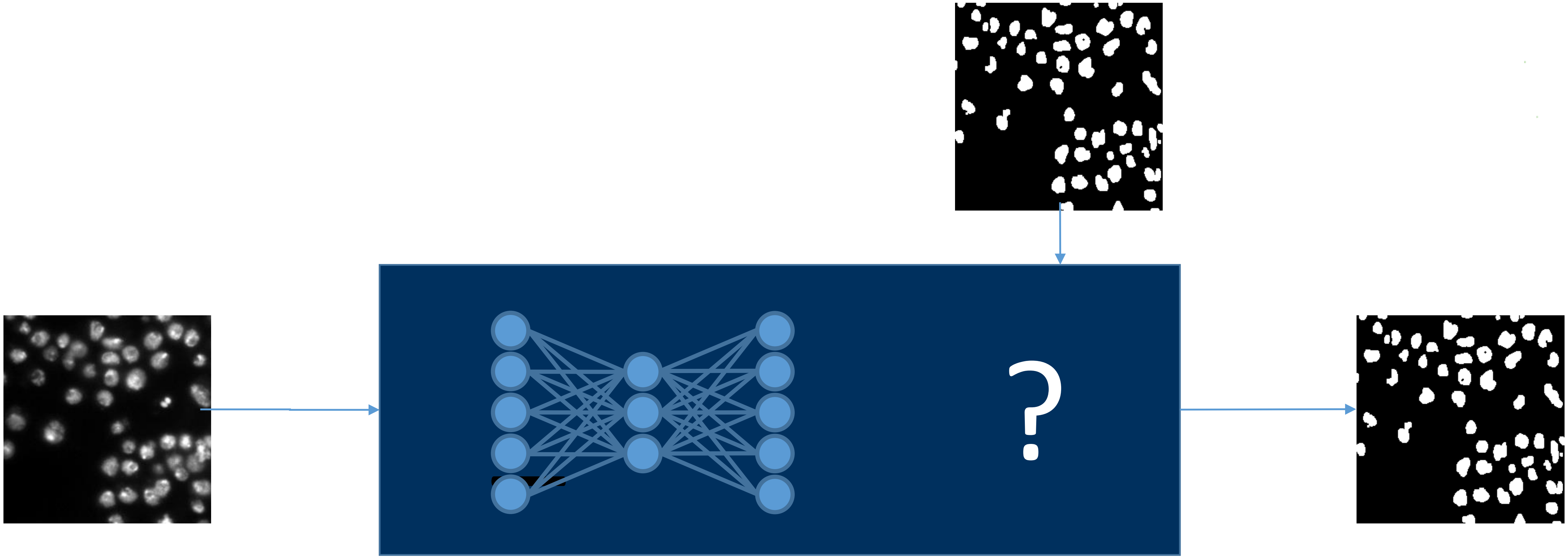


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Outlook: Deep learning for image analysis

- In deep learning, this is done automatically by the neural network



Convolutional neural networks

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

Pixel classification using scikit-learn

With material from

Robert Haase

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

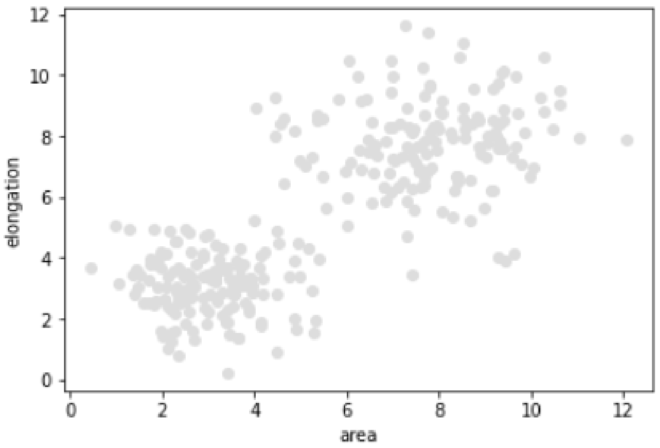
```
annotation = [1, 1, 2, 1, 1, 1, 2, 2,
```

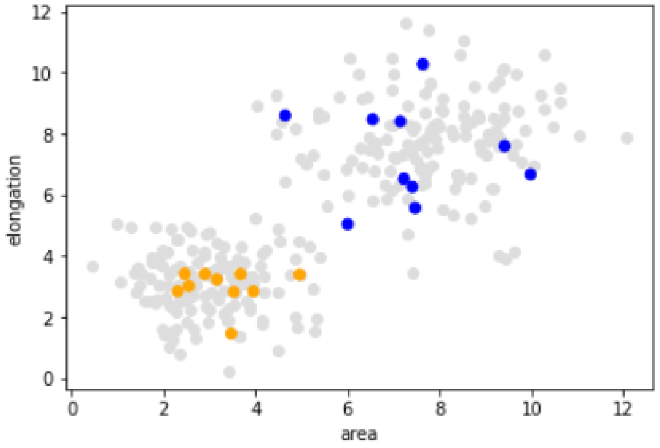
Classifier training

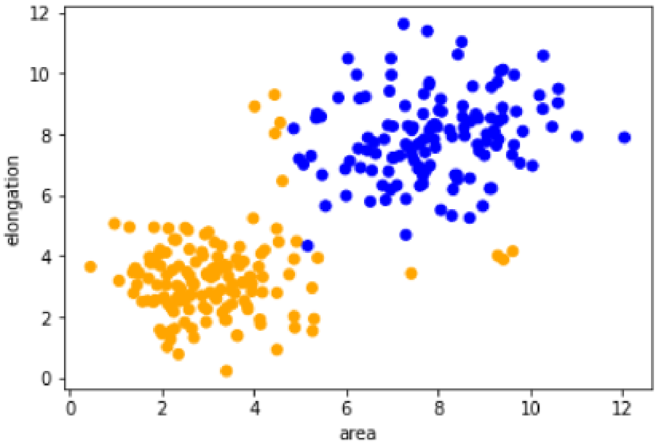
```
classifier = RandomForestClassifier()
classifier.fit(train_data, train_annotation)
```

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

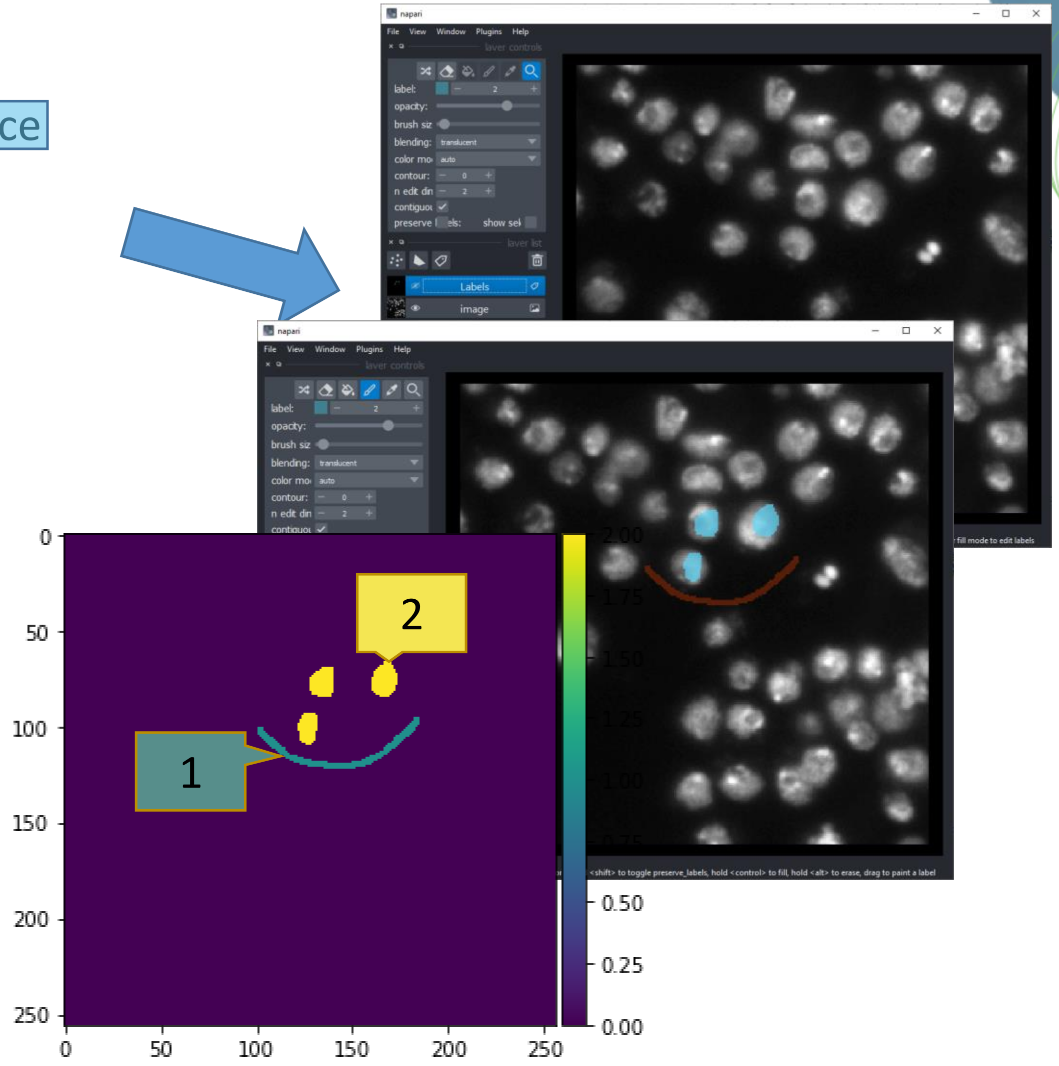
- Prepare an empty layer for annotations and keep a reference

```
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)
```



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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

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

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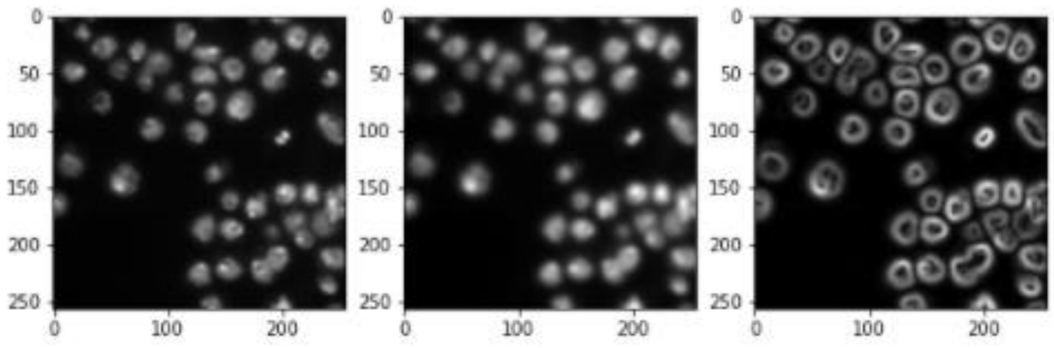


```
# for training, we need to generate features
feature_stack = generate_feature_stack(image)
X, y = format_data(feature_stack, manual_annotations)

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

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Interactive pixel classification

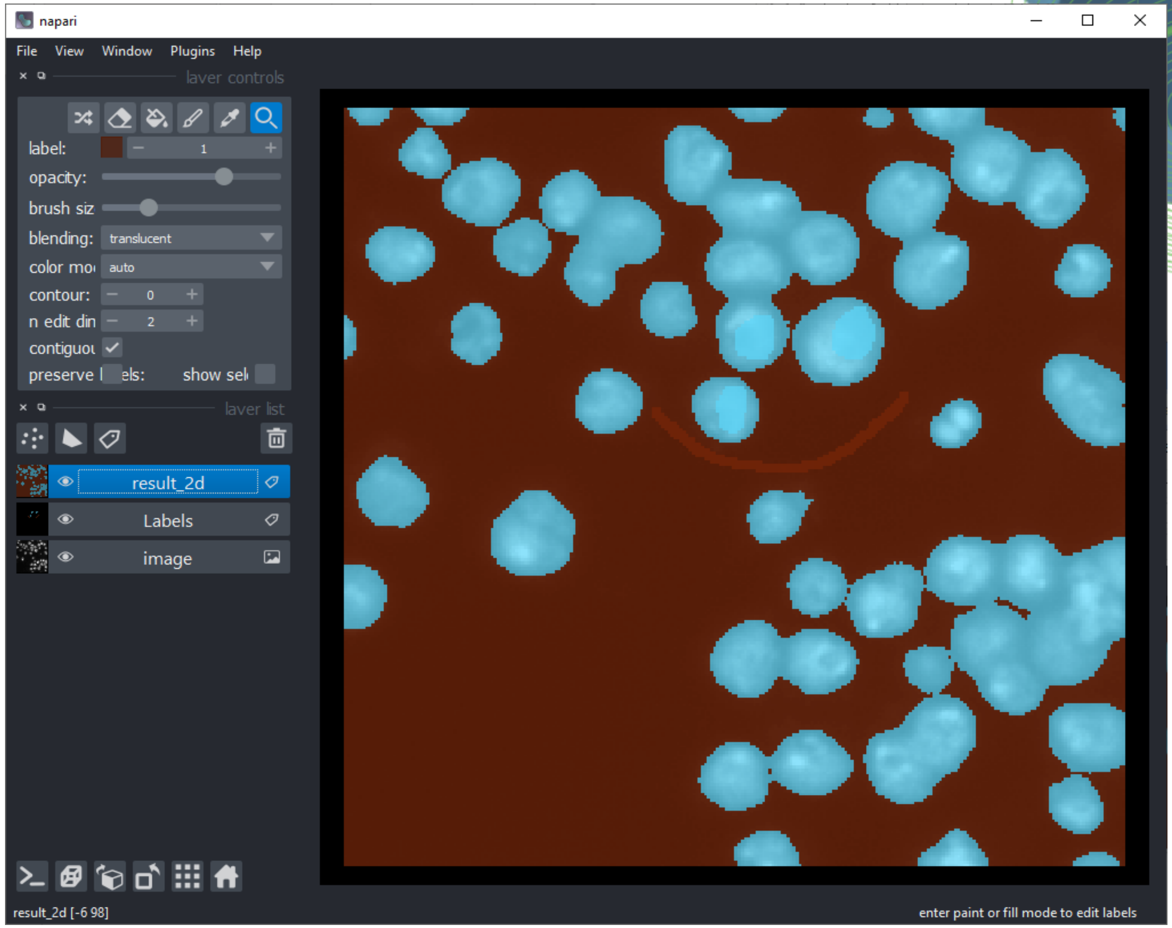
- Pixel classification using scikit-learn

```
# 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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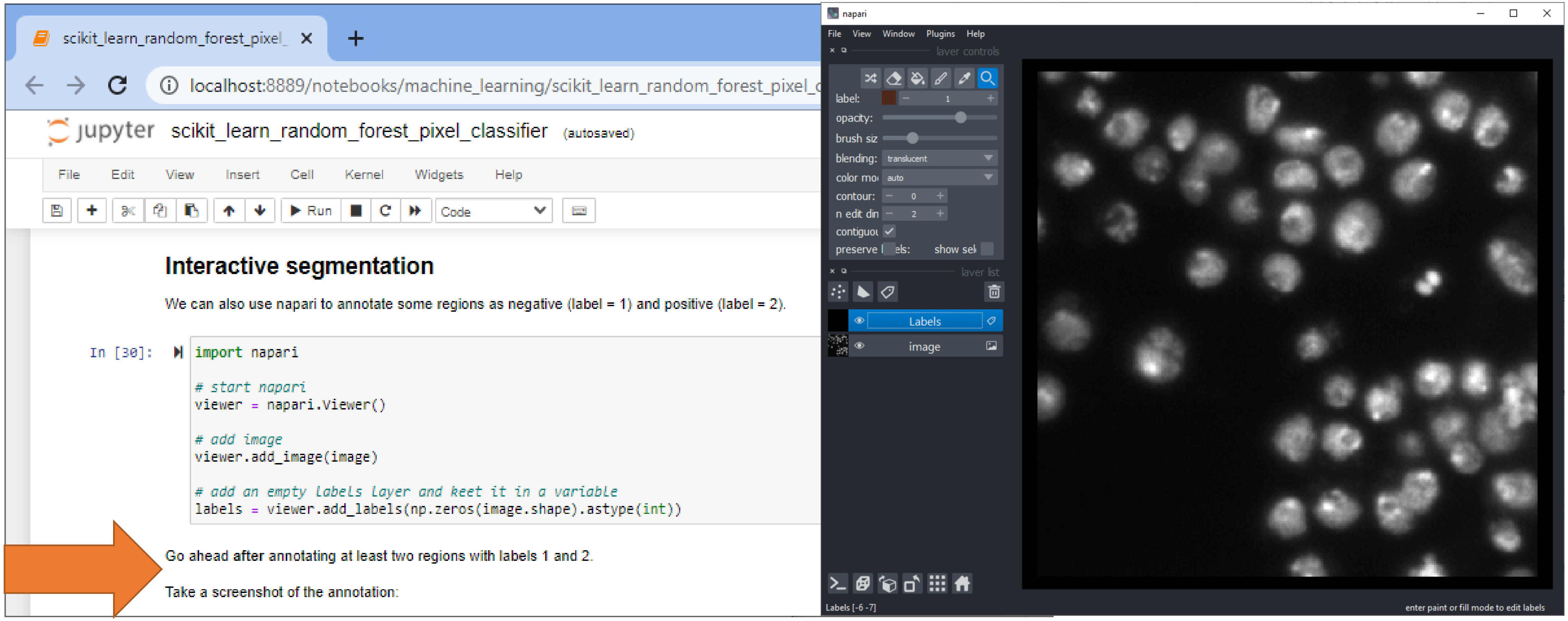
Interactive pixel classification

- Jupyter notebooks and napari side-by-side



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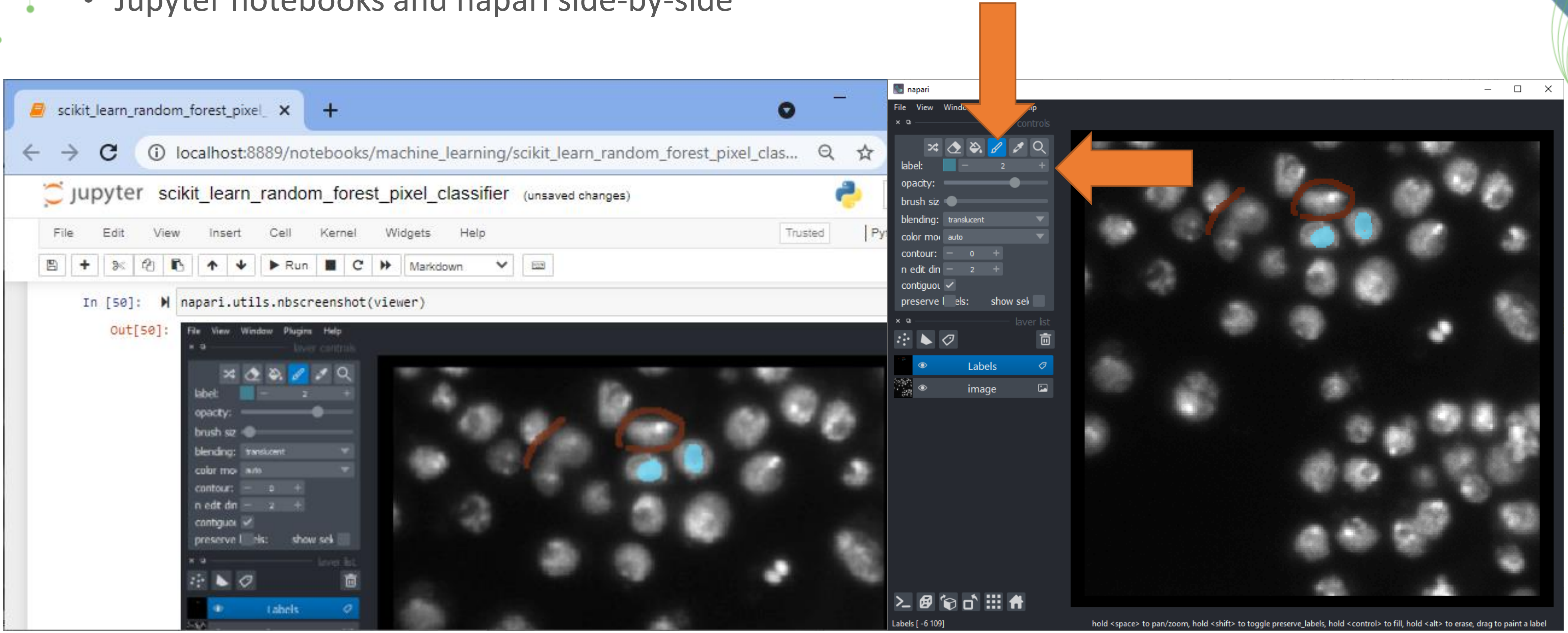

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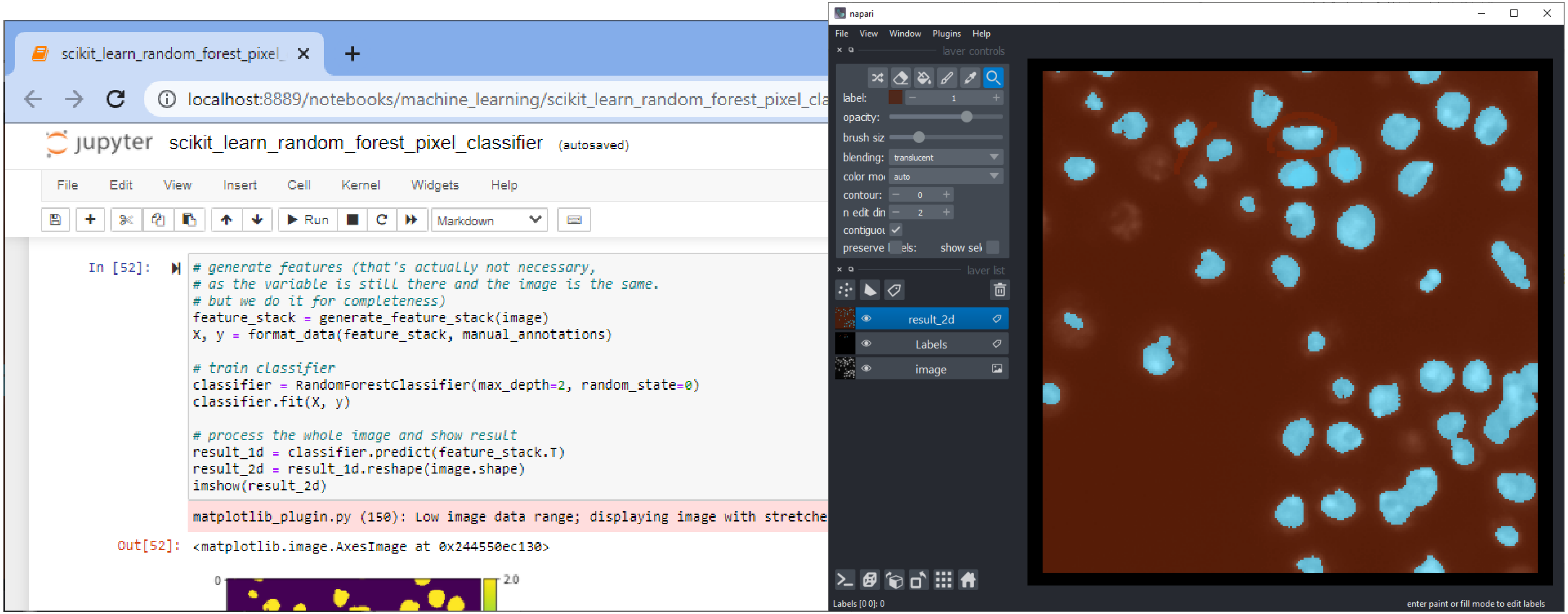

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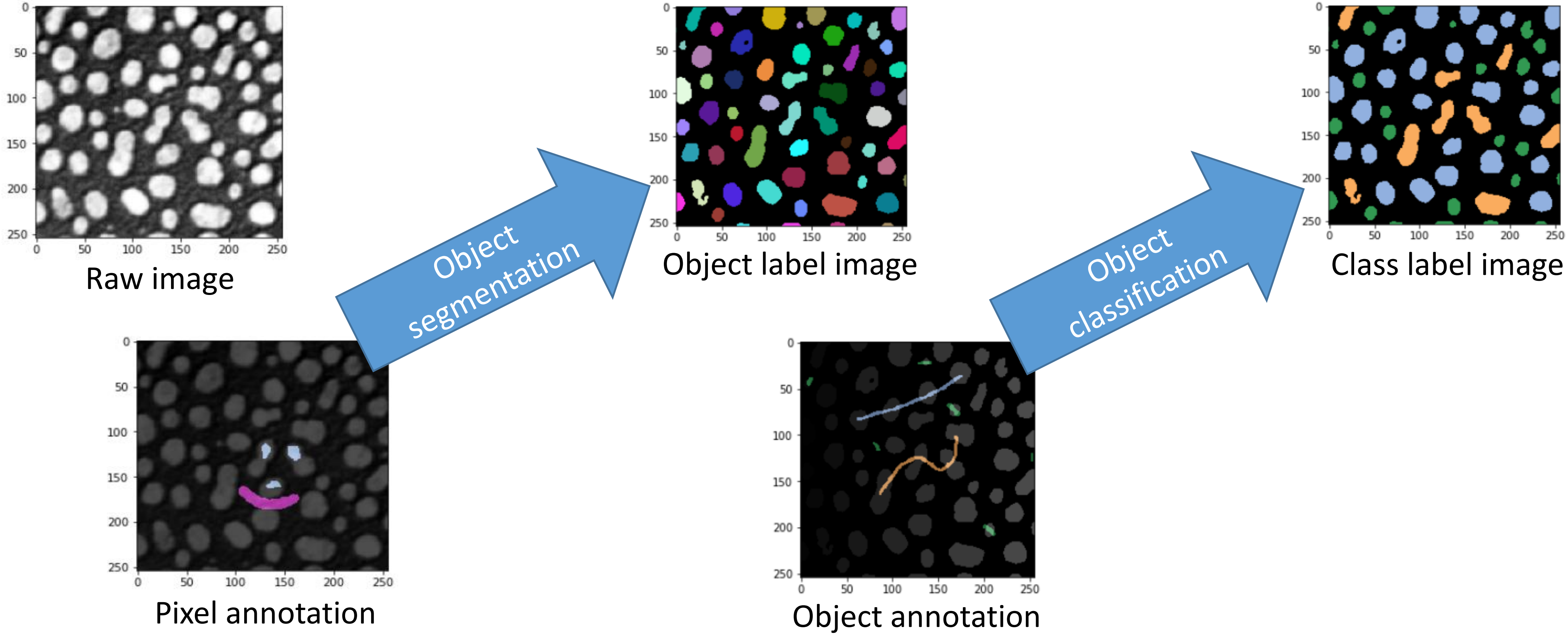
Accelerated pixel and object classification (APOC)

With material from

Robert Haase

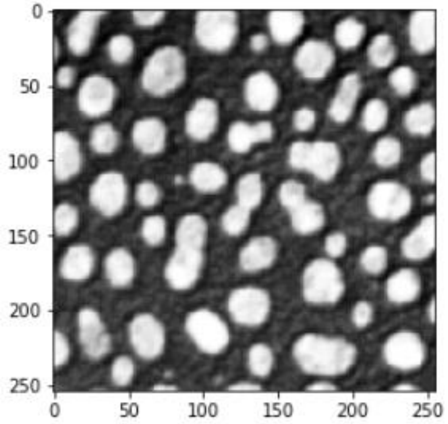
Accelerated pixel and object classification

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

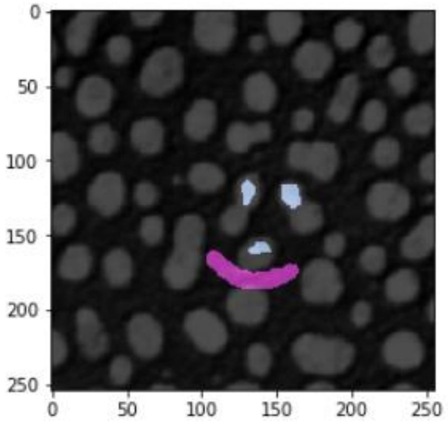


Object segmentation

- Pixel classification + connected component labeling



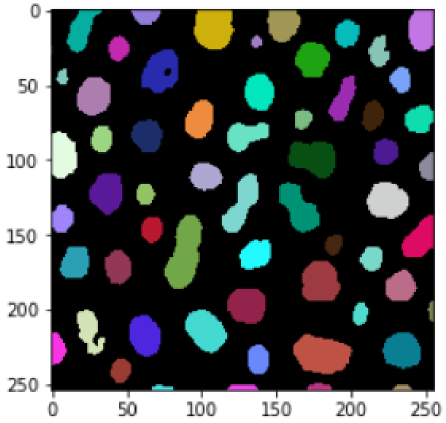
Raw image



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

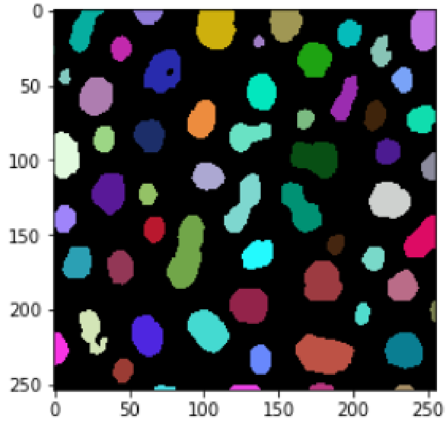


Object label image

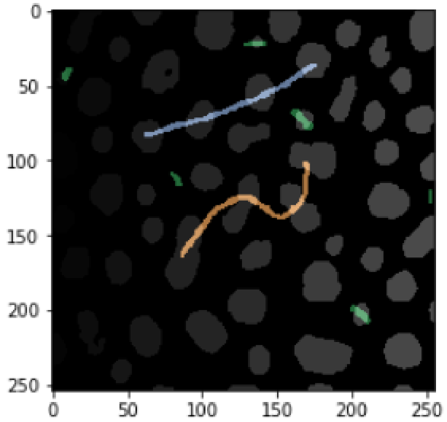
https://github.com/BiAPoL/Bio-image_Analysis_with_Python/blob/main/09_machine_learning/03_apoc_object_segmenter.ipynb

Object classification

- Feature extraction + tabular classification



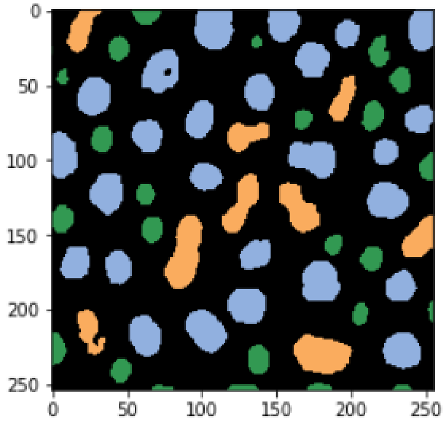
Object label image



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

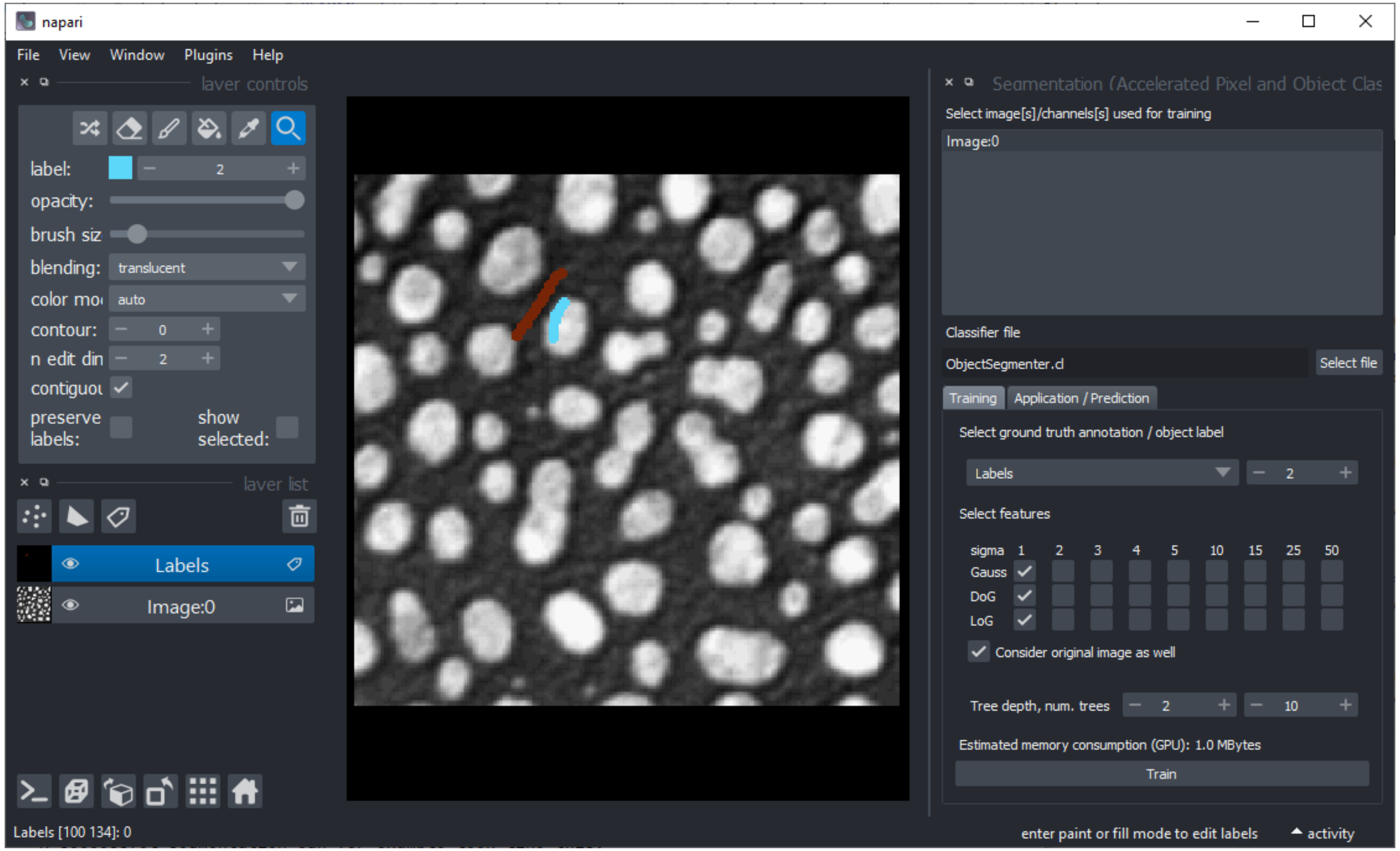


Class label image

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

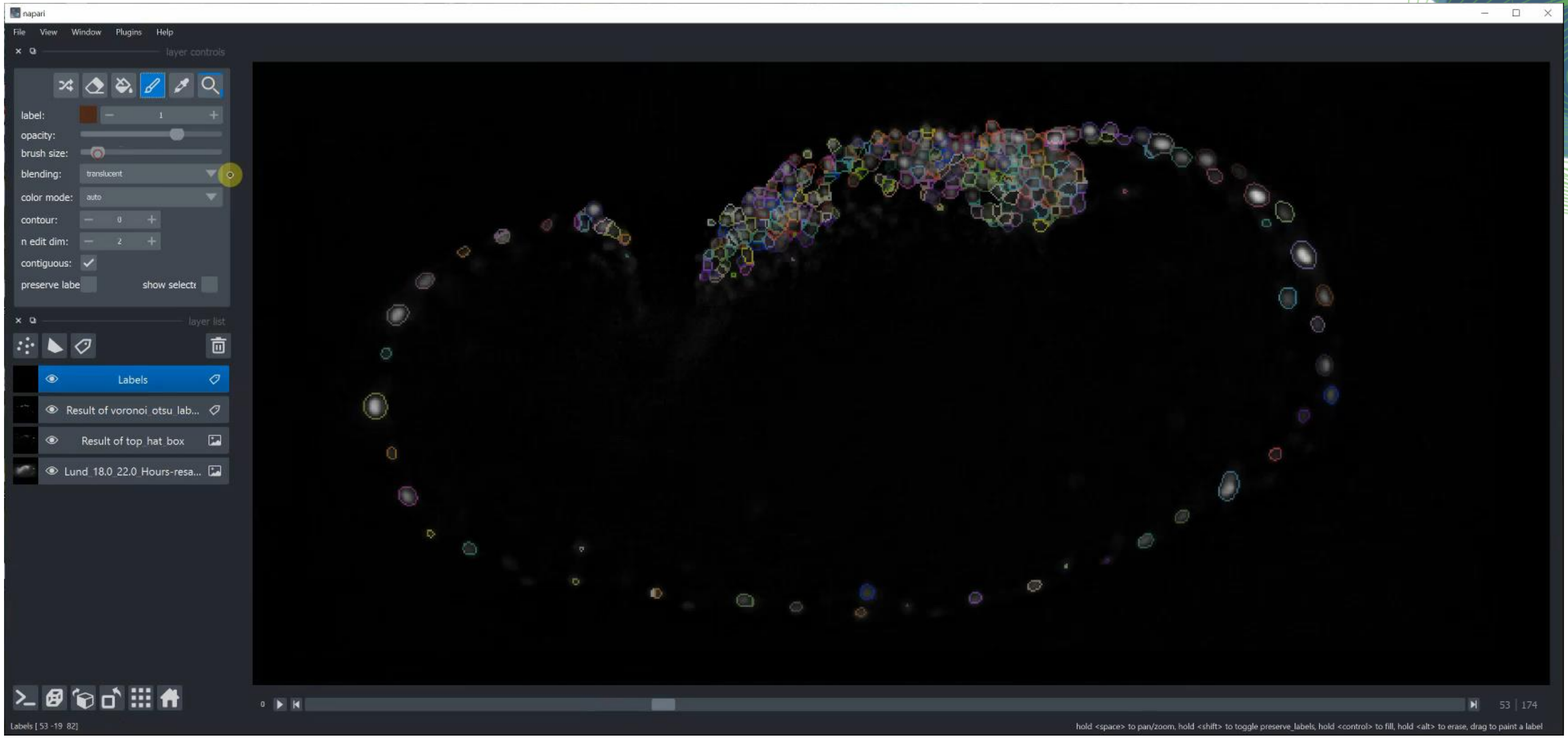
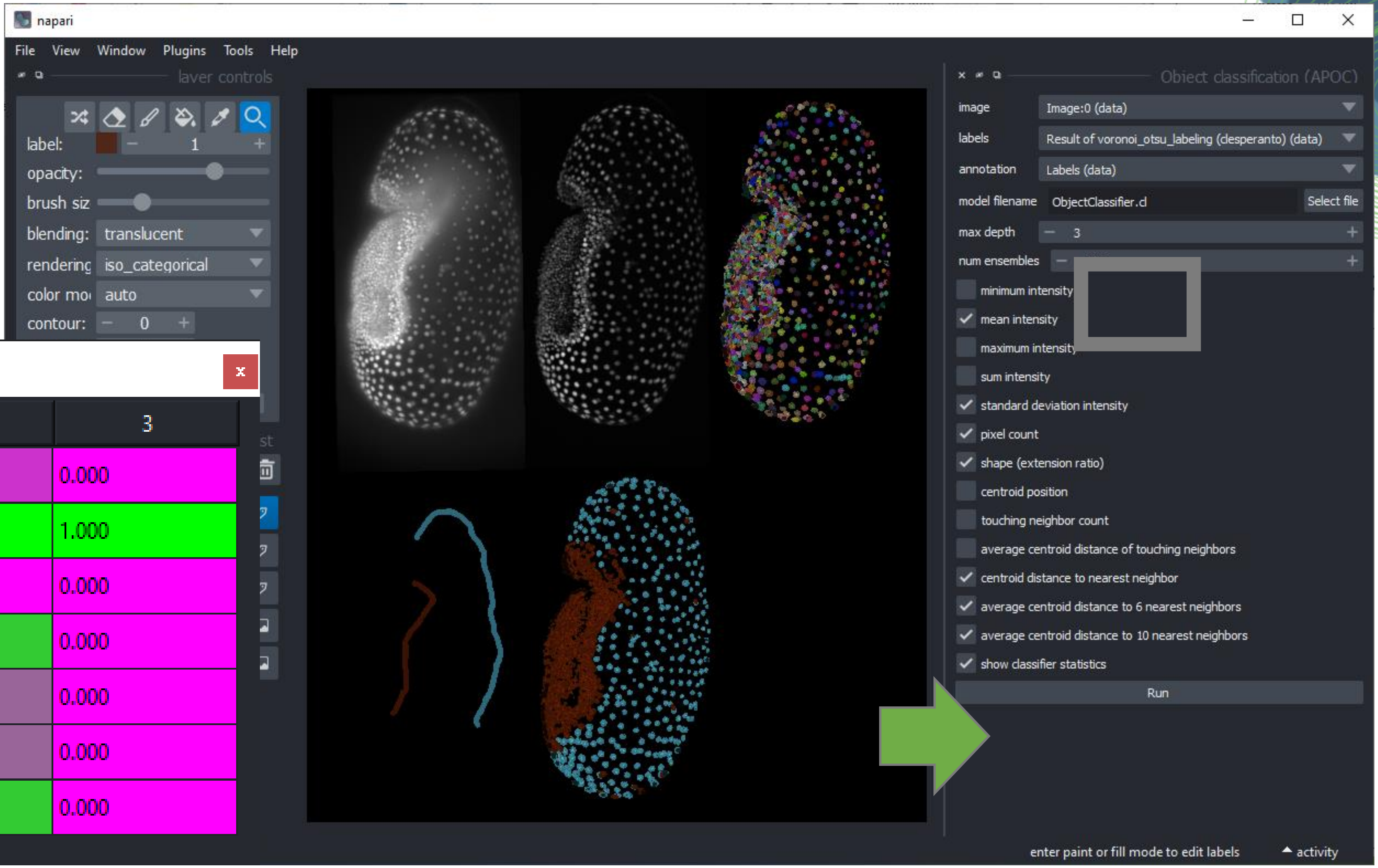


Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD
https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification

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

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD
https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification

Data exploration / supervised machine learning

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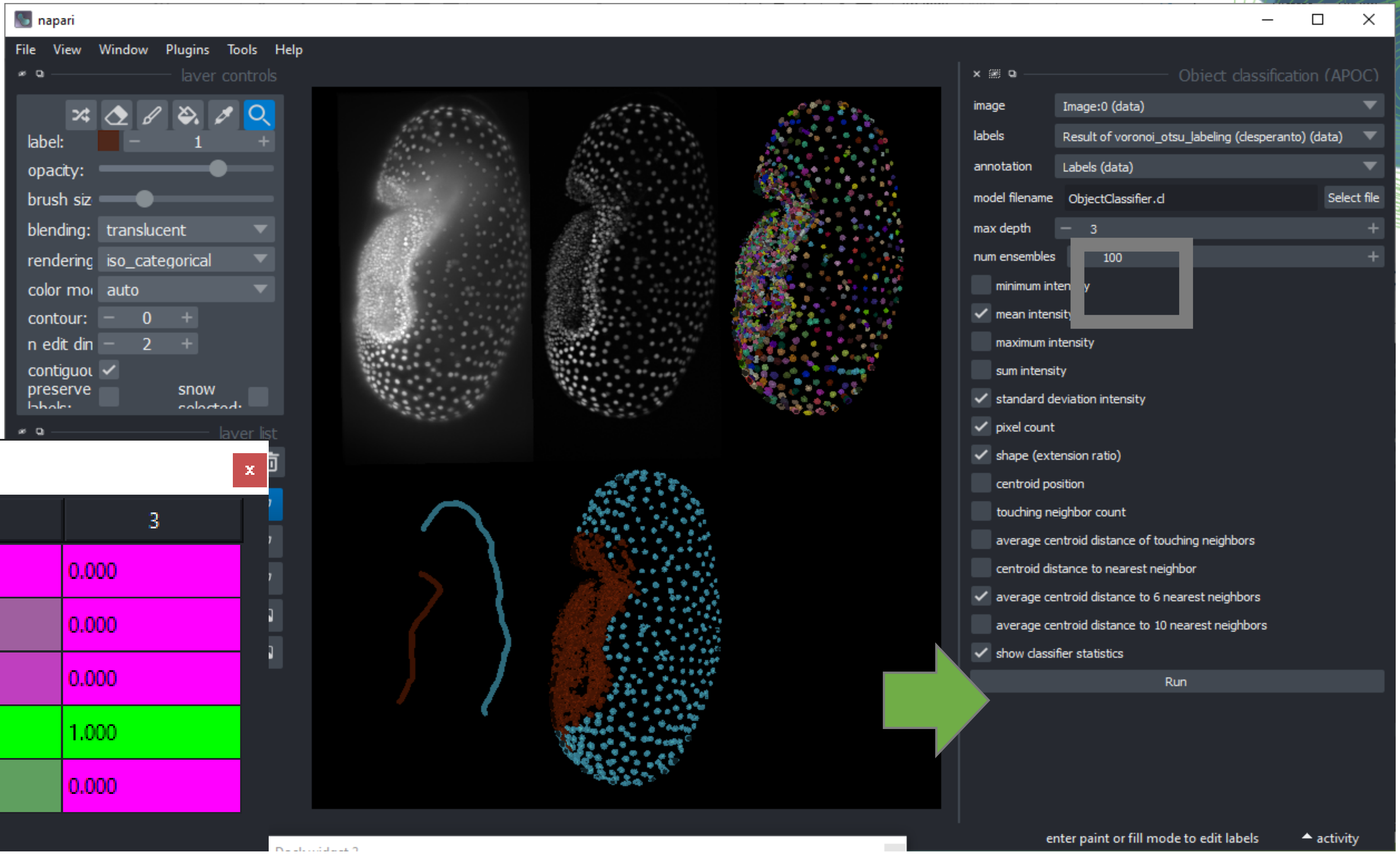


Dock widget 2

	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

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

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

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

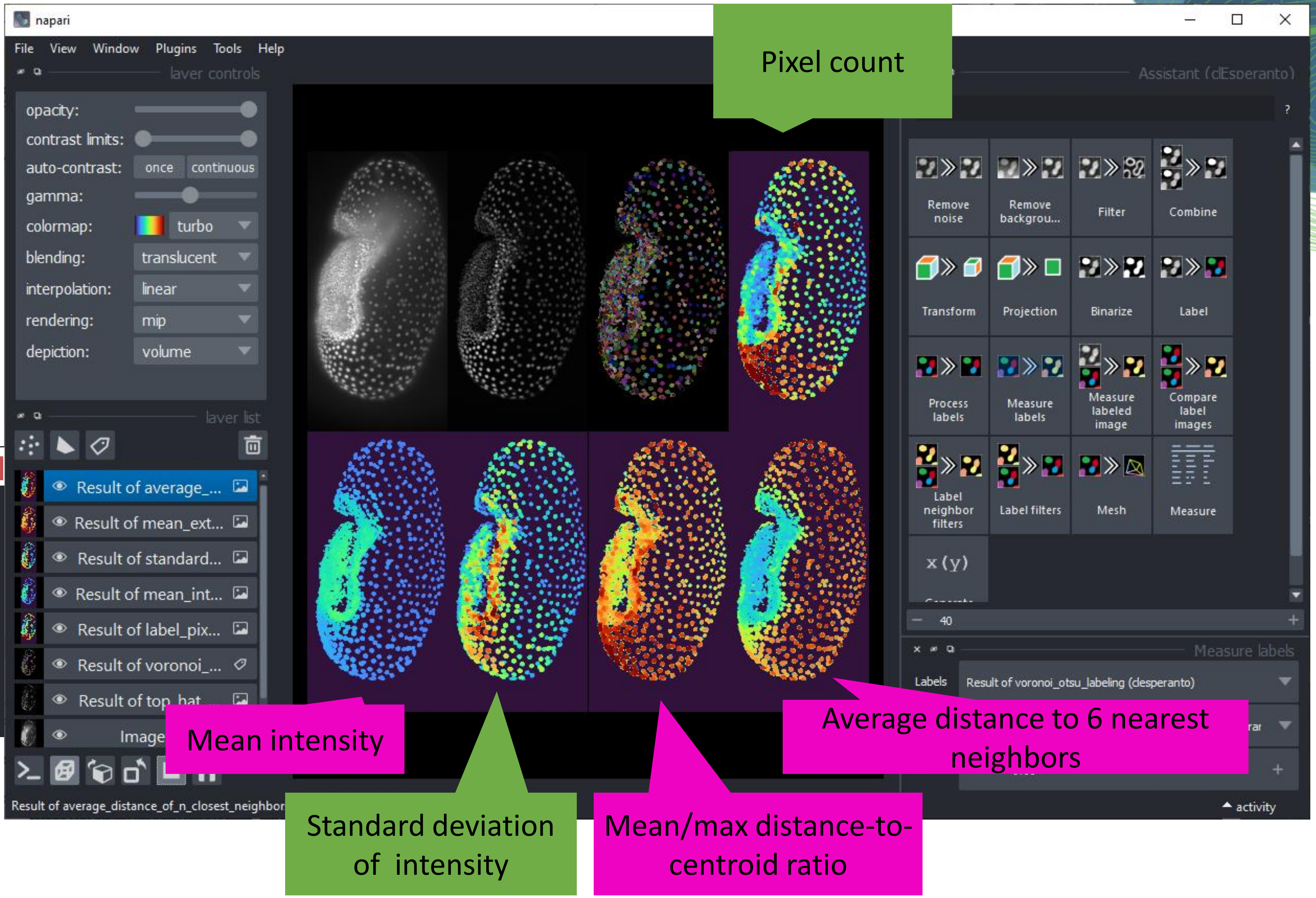
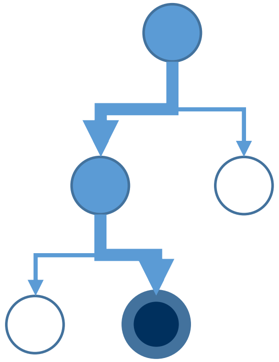
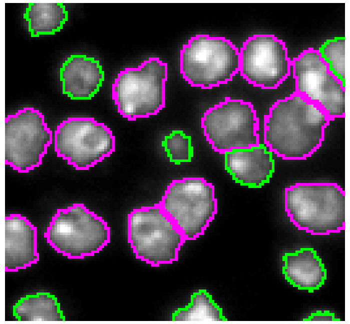


Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD
https://github.com/clEsperanto/napari_pyclesperanto_assistant

Thank you for your attention!





with material from

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The Scikit-Learn community