Machine Learning for Pixel and Object Segmentation

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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)

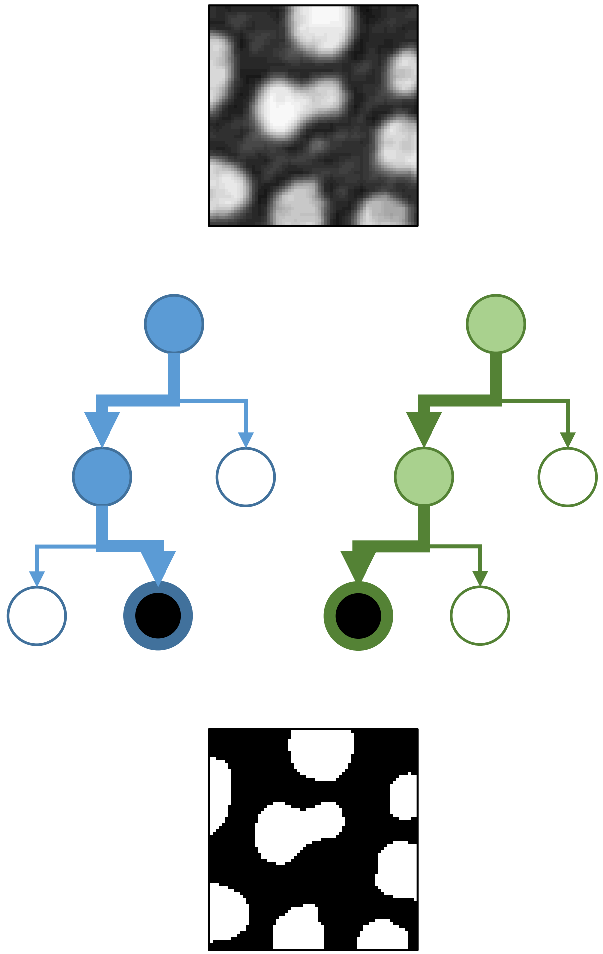


Image segmentation using thresholding

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

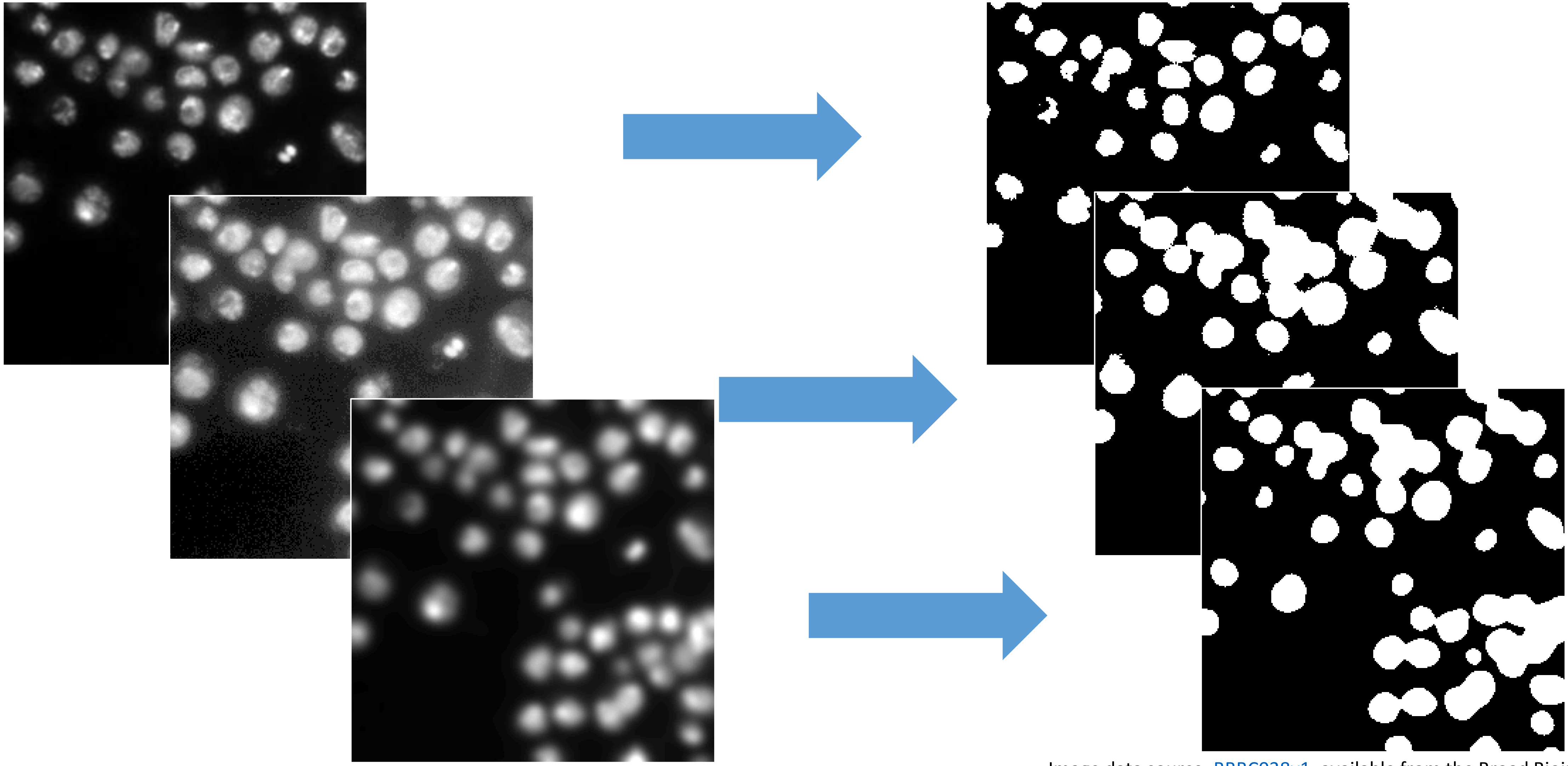


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

Image segmentation using thresholding

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

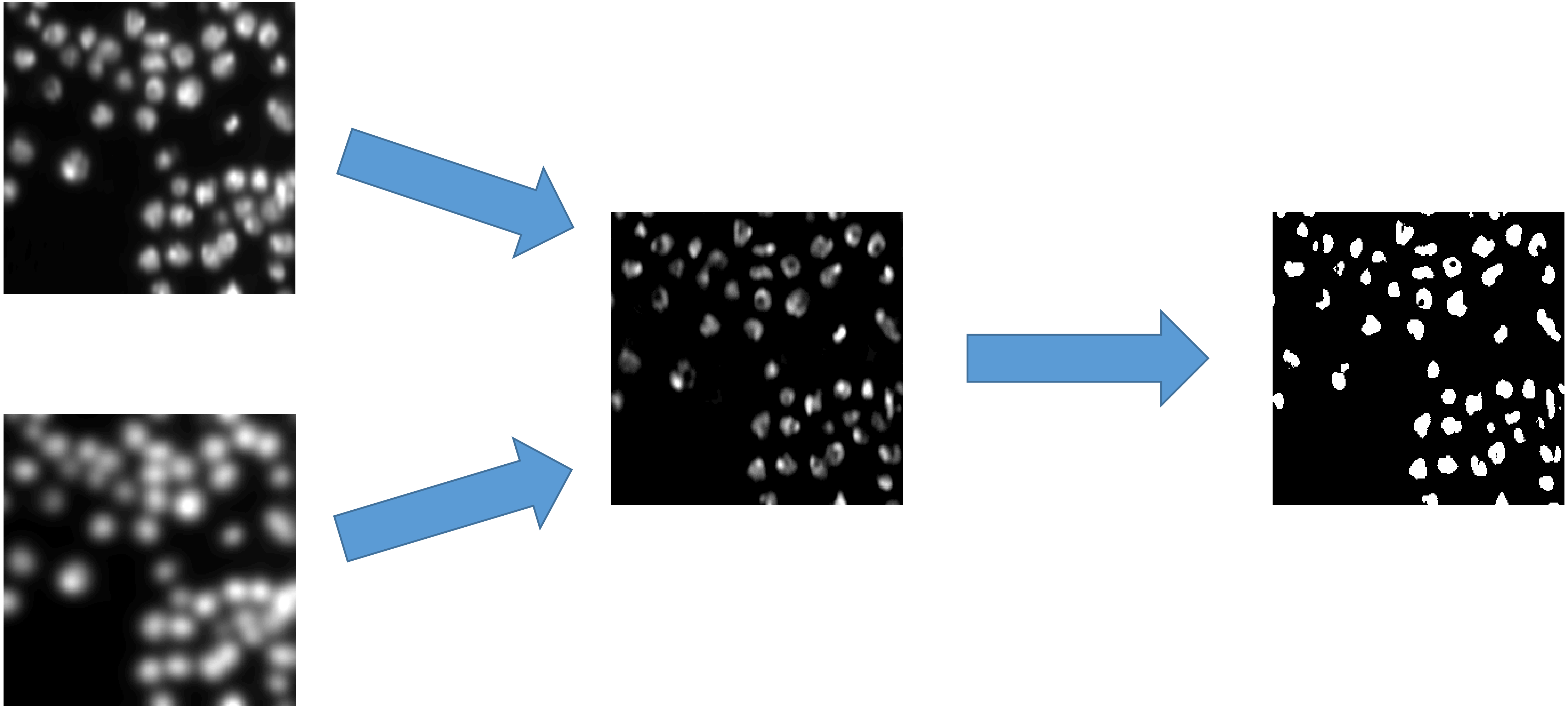
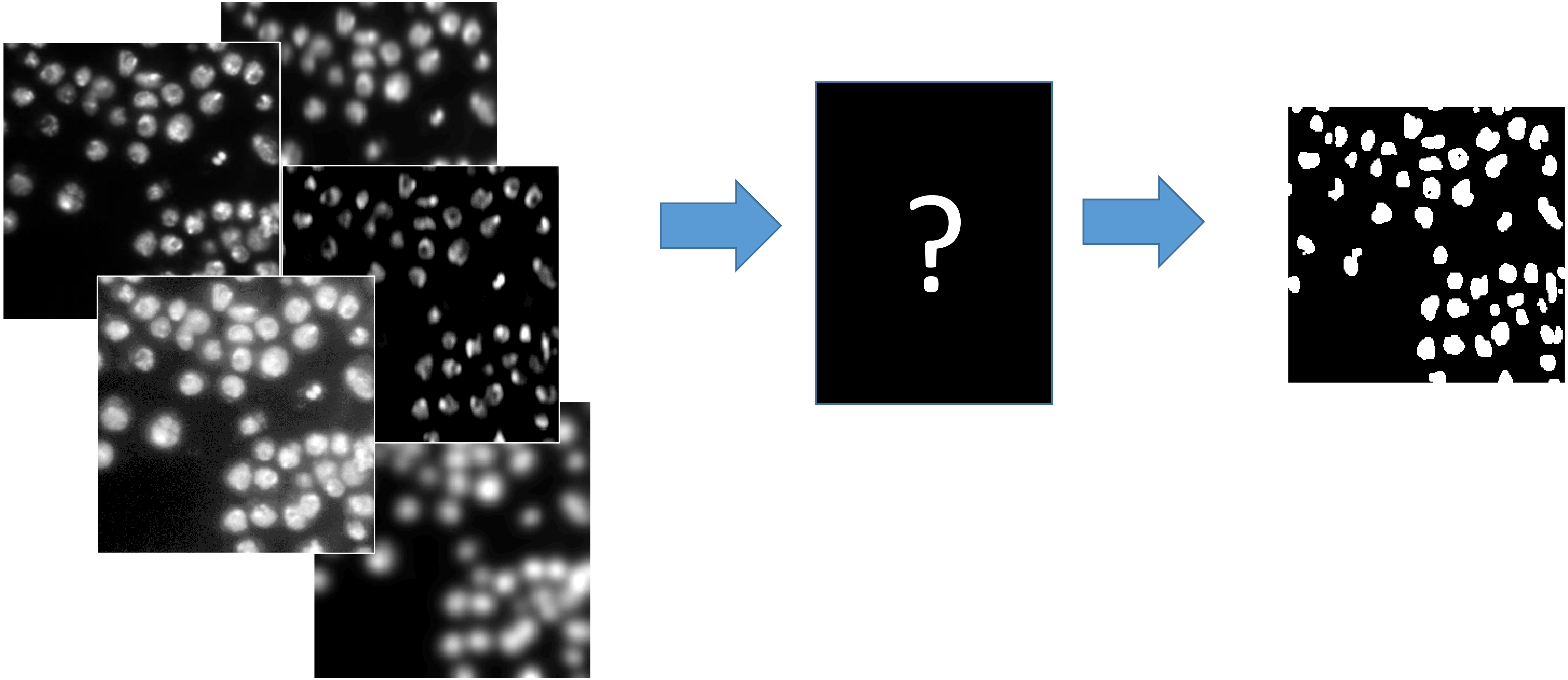


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019].

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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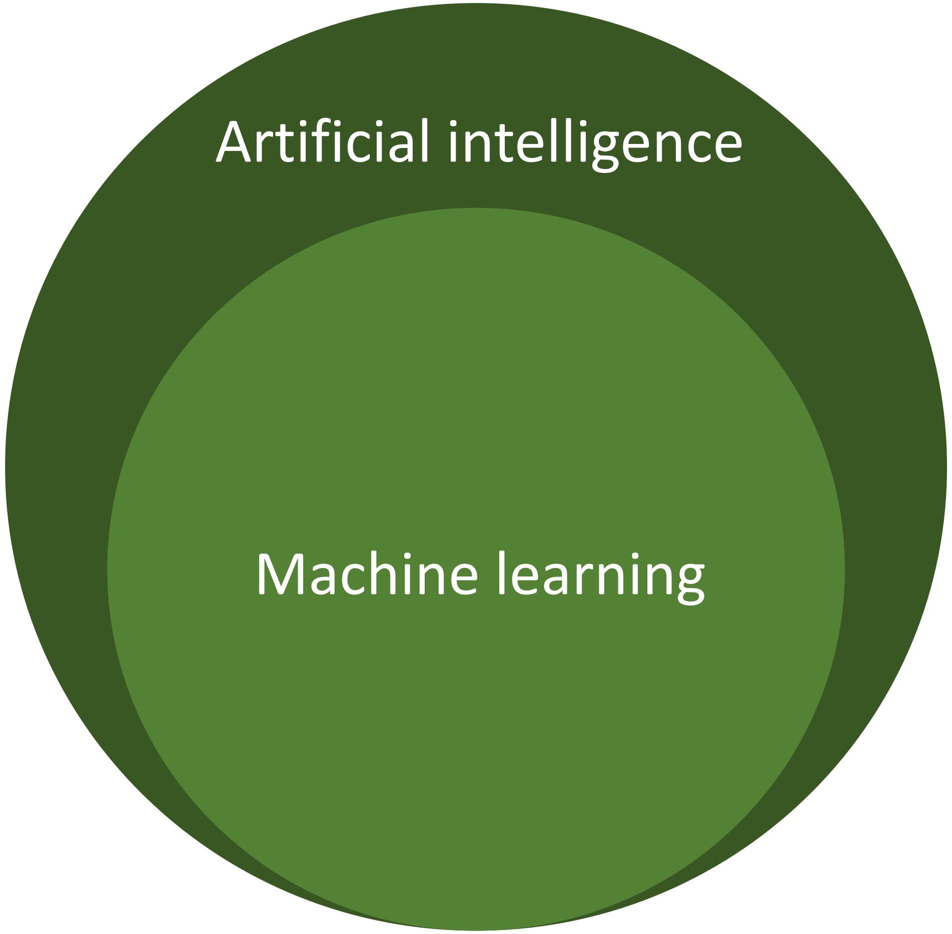
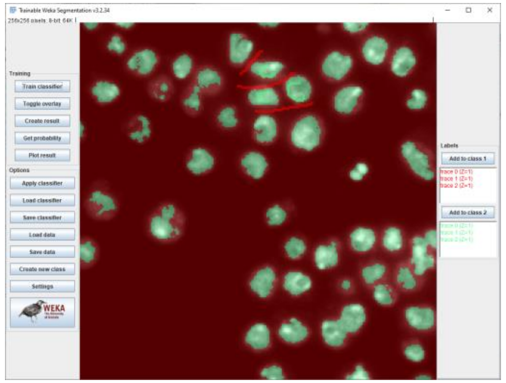
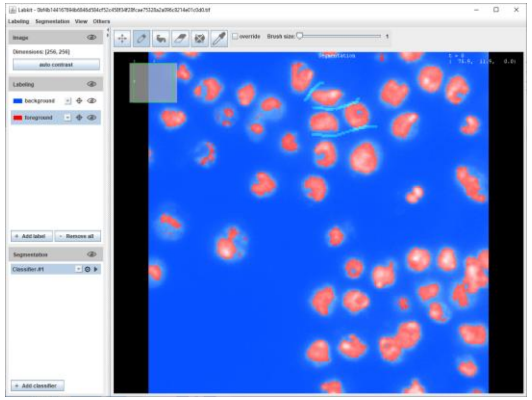
Machine learning

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



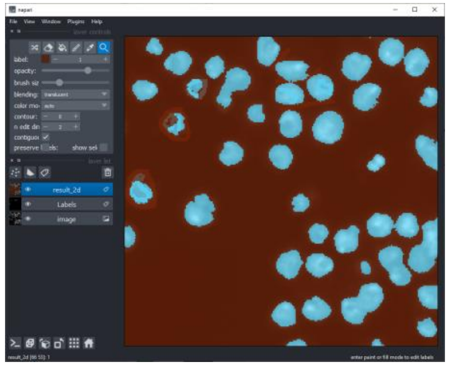


Trainable Weka Segmentation
https://imagej.net/plugins/tws/



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

Python / scikit-learn / napari / apoc



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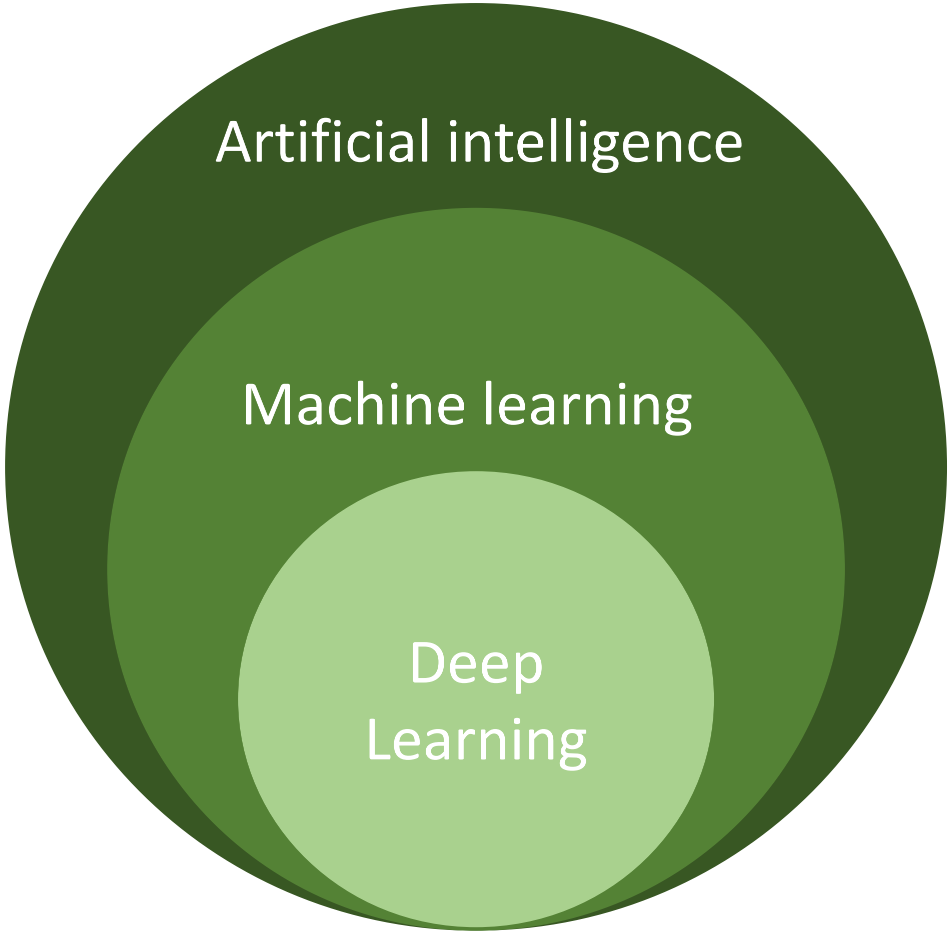
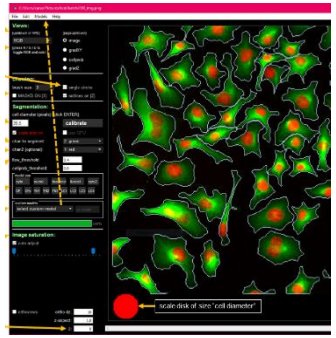
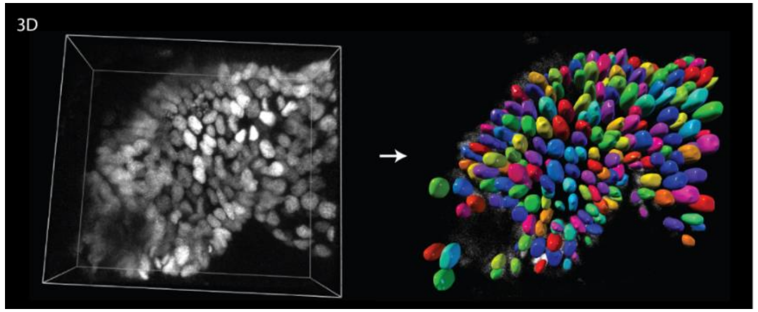
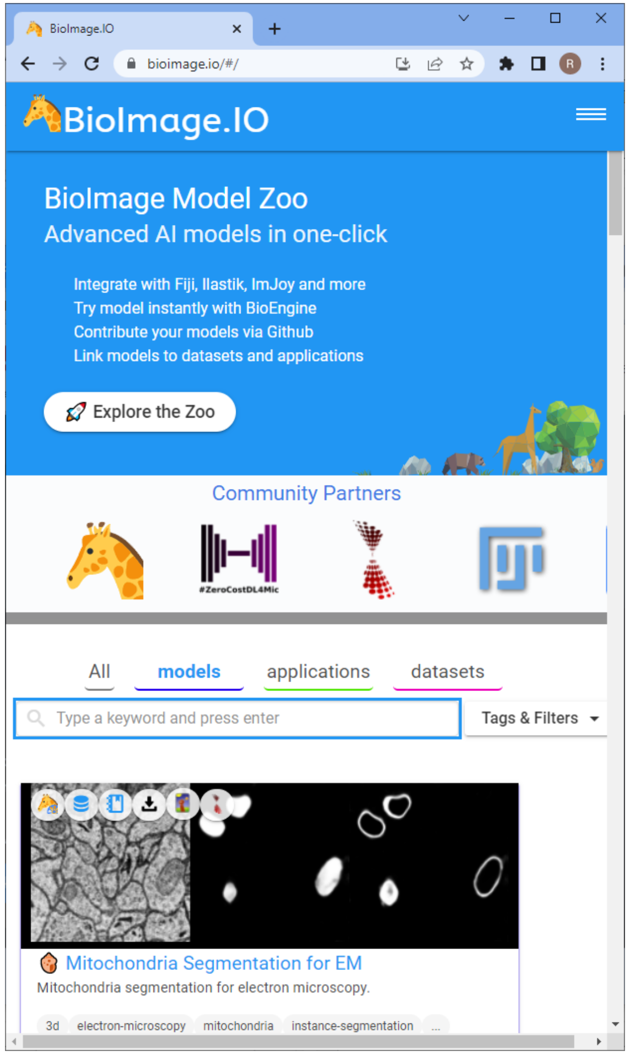




www.cellpose.org/



https://github.com/stardist/stardist



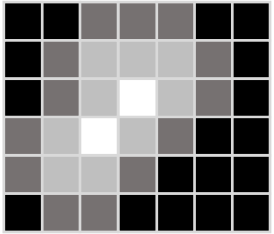
https://bioimage.io/

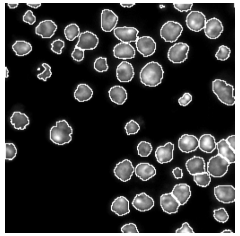
Logos and screenshots are taken from the github repositories / websites provided under BSD and MIT licenses.

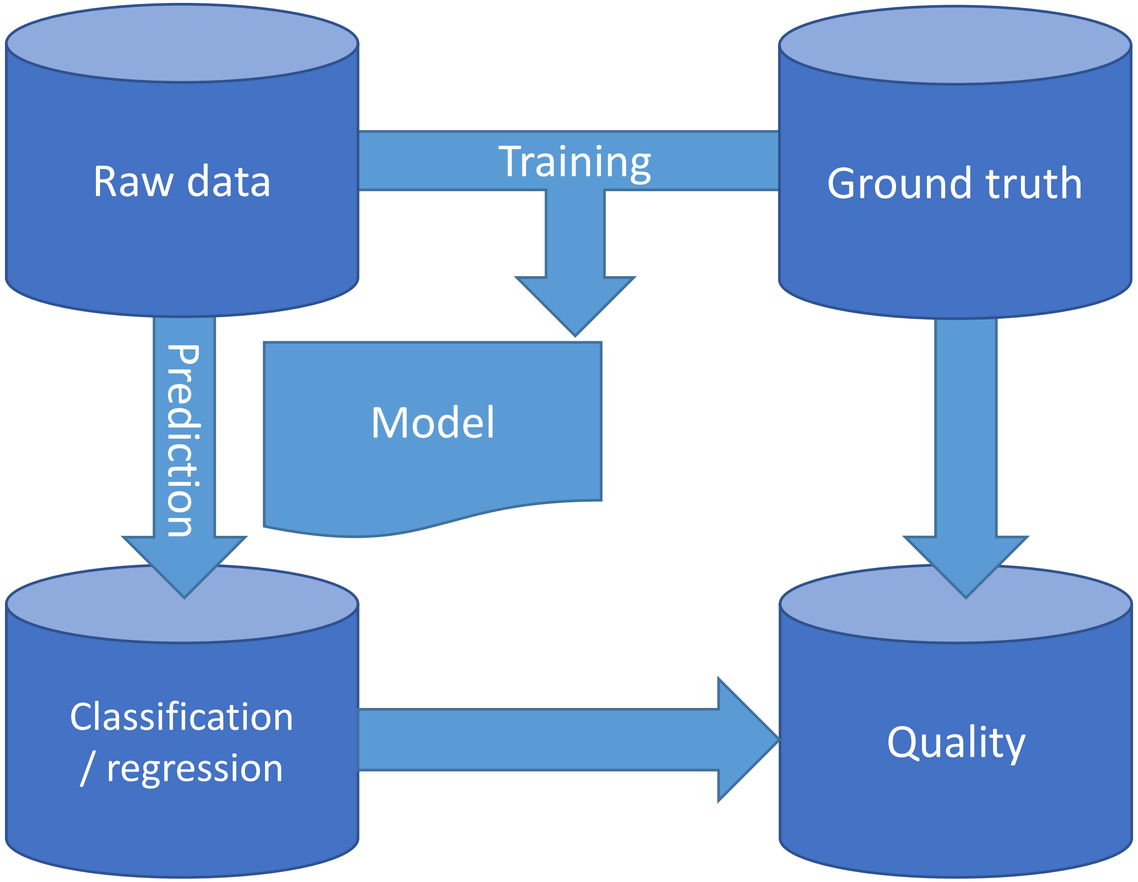
Machine learning

- Automatic construction of predictive models from given data

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



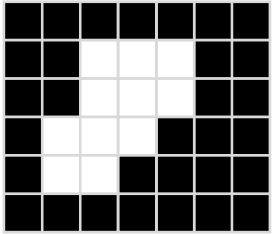




Annotated raw data, usually generated by humans

Precision, Recall

Dense Segmentation / Binarization



Object classification

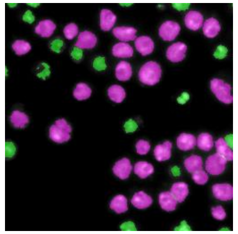
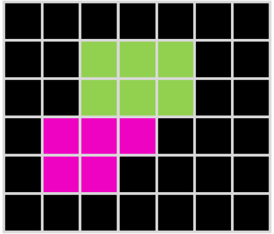


Image classification

"Cat"

Instance segmentation

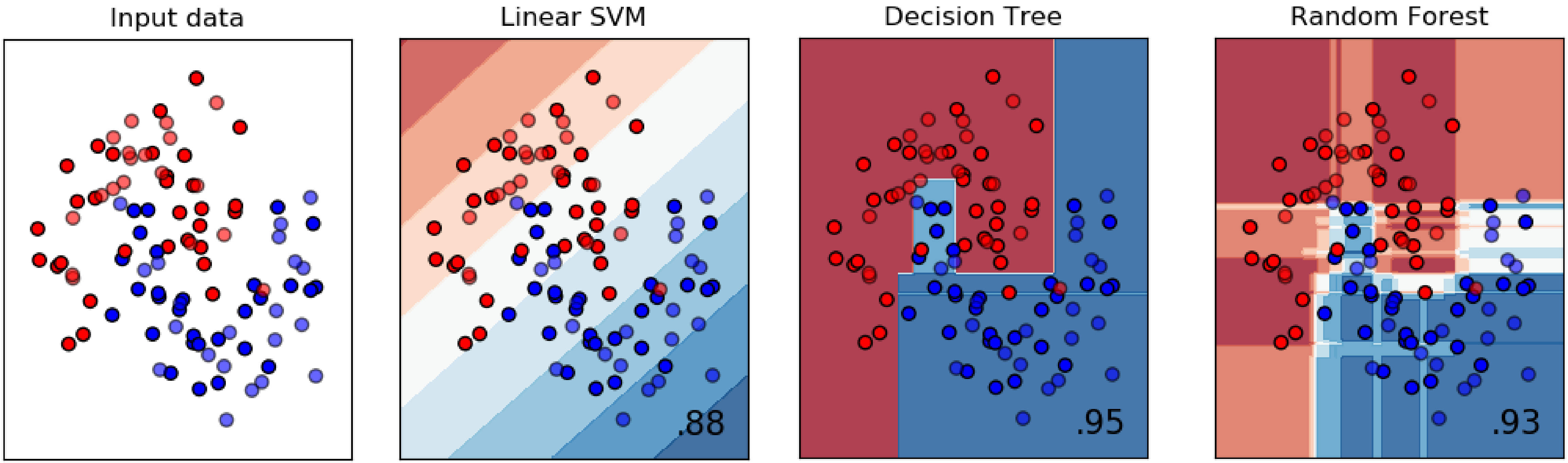


Cont. quantity

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

Goal

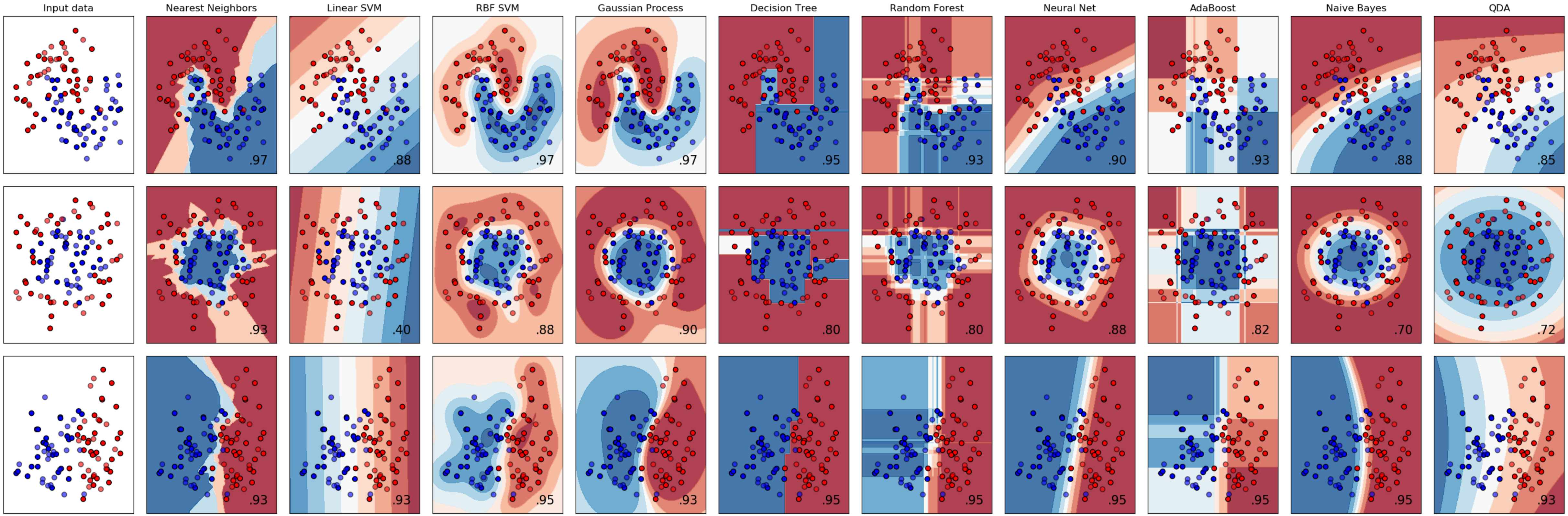
- Guess classification (color) from position of a sample in parameter space.



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

Approaches

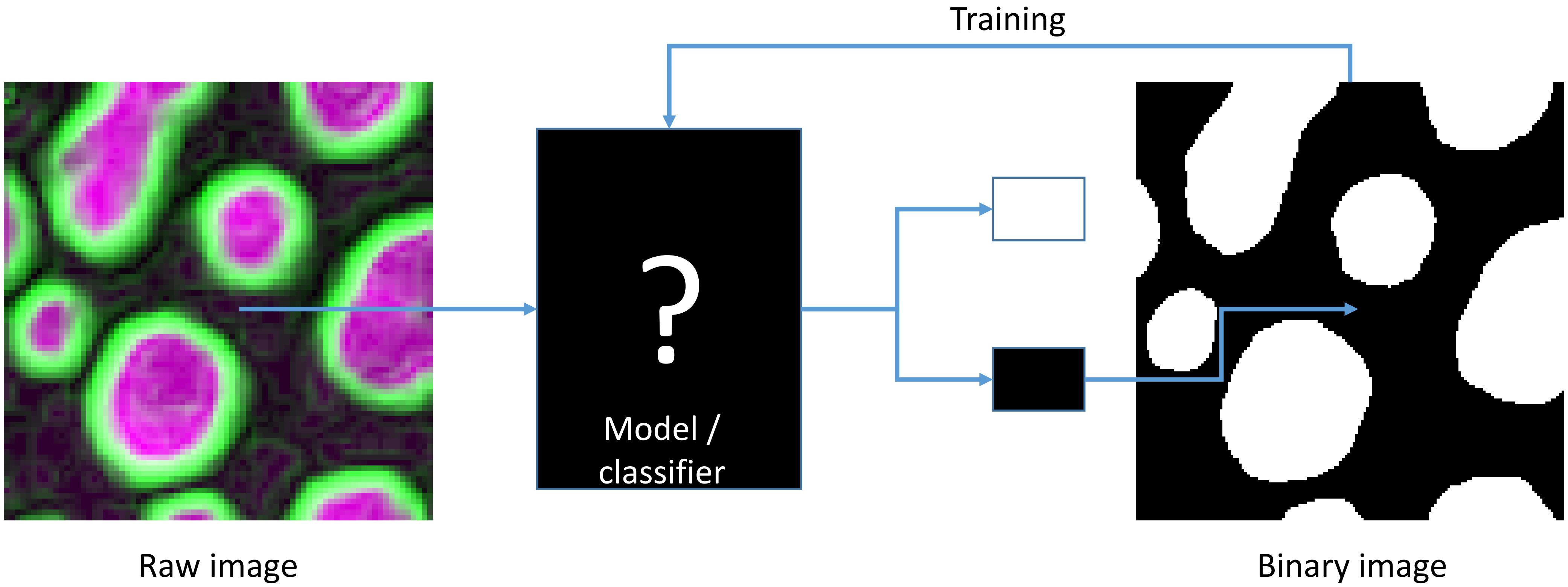
- The right approach depends on data, computational resources and desired quality



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

Machine learning for image segmentation

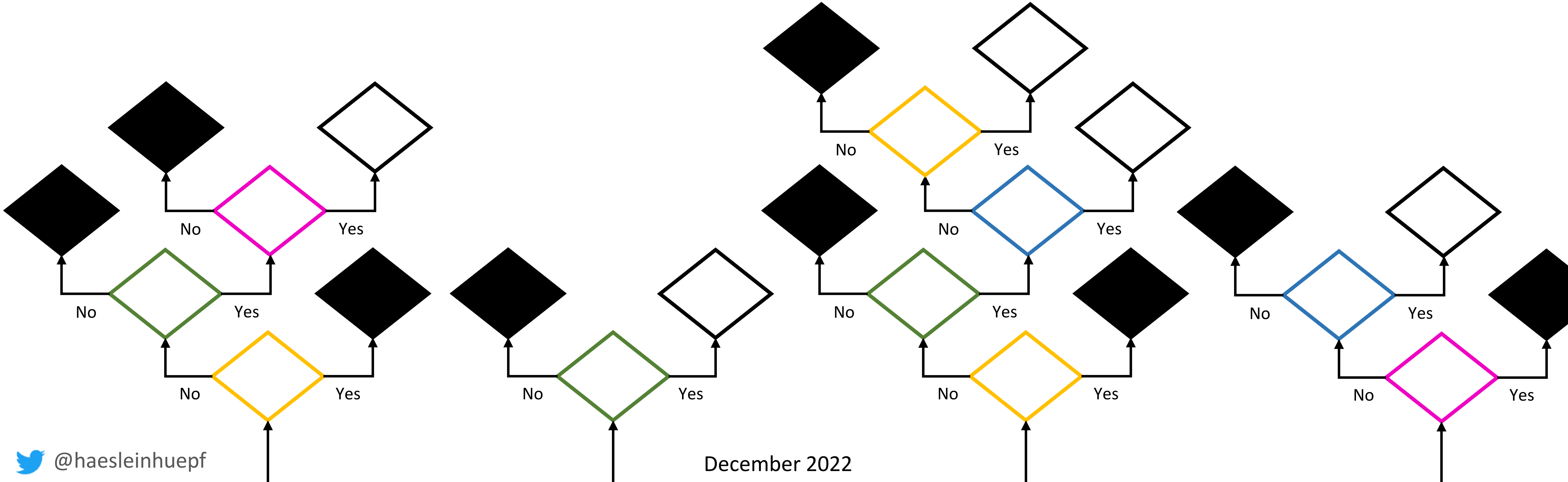
- *Supervised* machine learning: We give the computer some ground truth to learn from
- The computer derives a *model* or a *classifier* which can judge if a pixel should be foreground (white) or background (black)
- Example: Binary classifier



Random forest based image segmentation

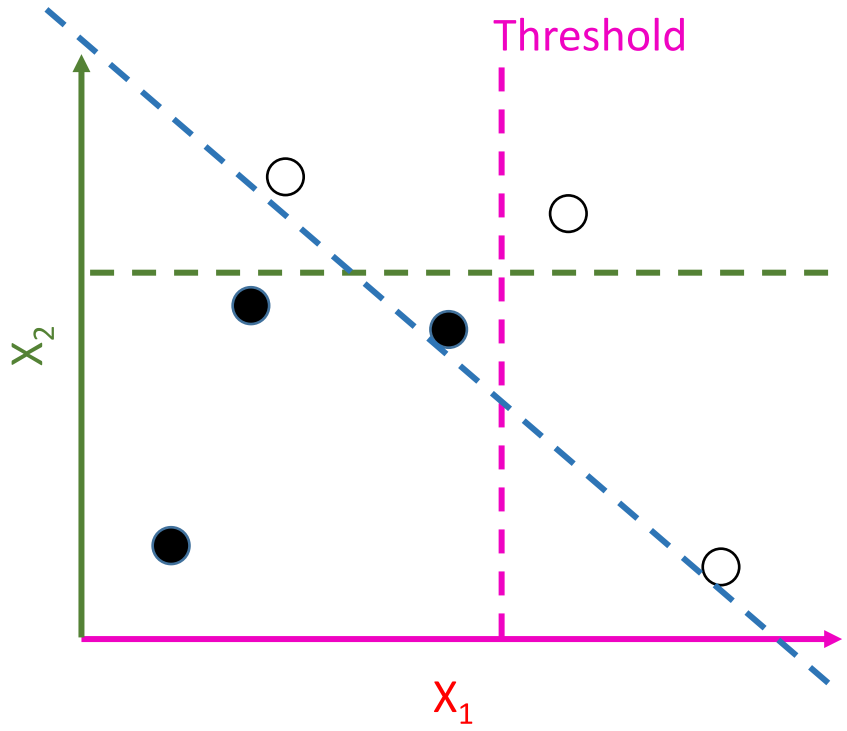
- Decision trees are classifiers, they decide if a pixel should be white or black
- Random decision trees are randomly initialized, afterwards evaluated and selected
- Random forests consist of many random decision trees

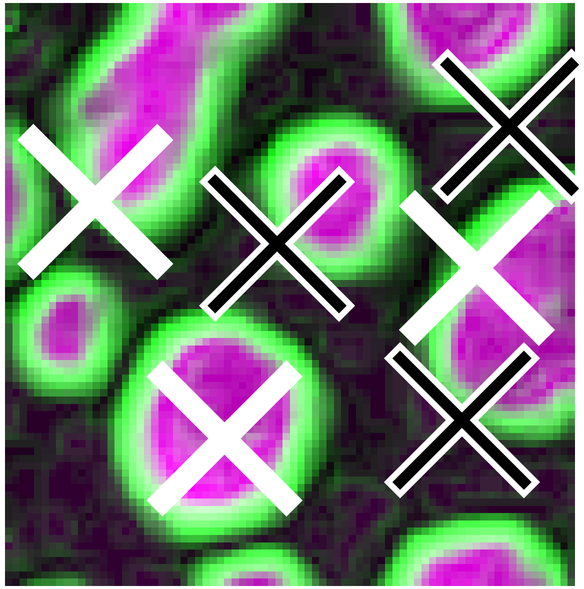
- Example: Random forest of binary decision trees

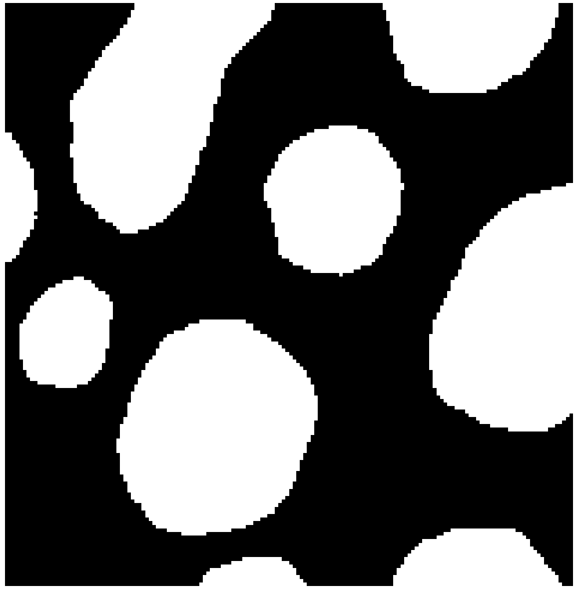


Deriving random decision trees

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



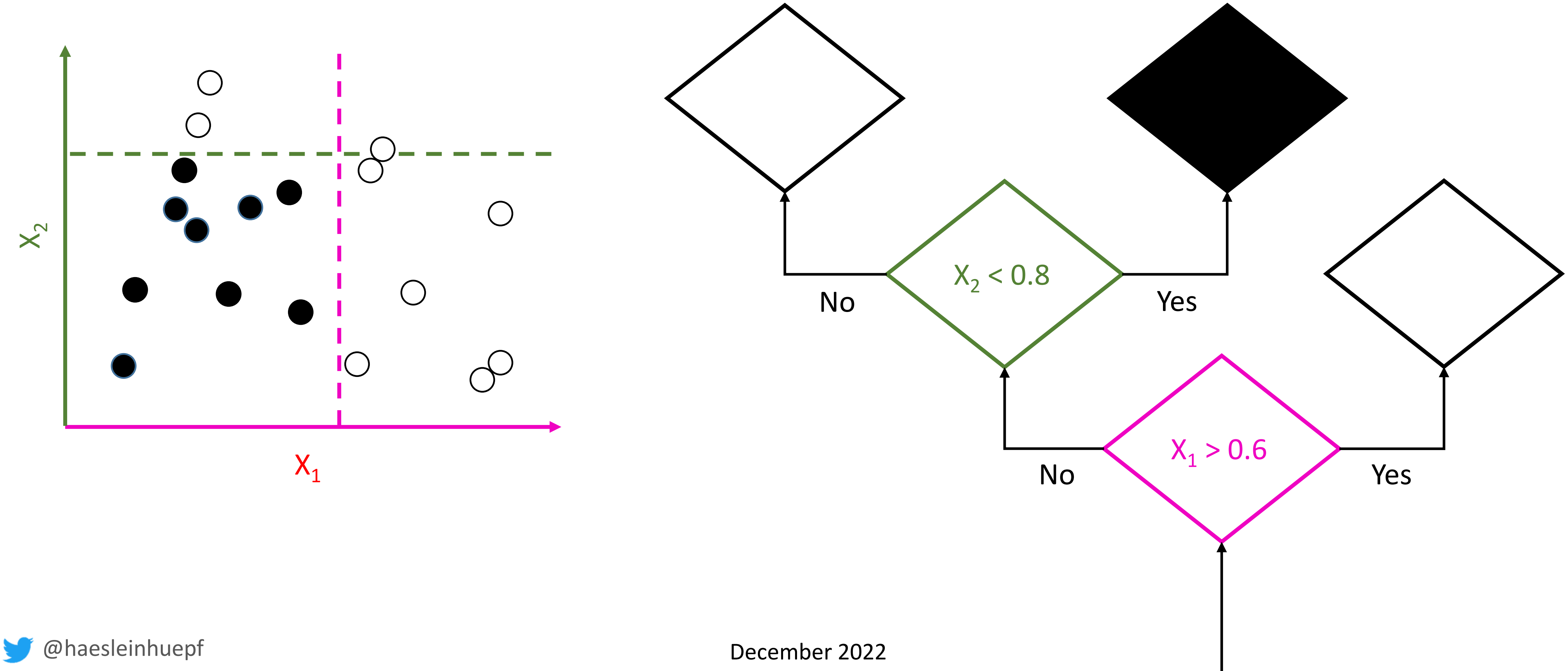




Note: You cannot use a single threshold to make the decision correctly

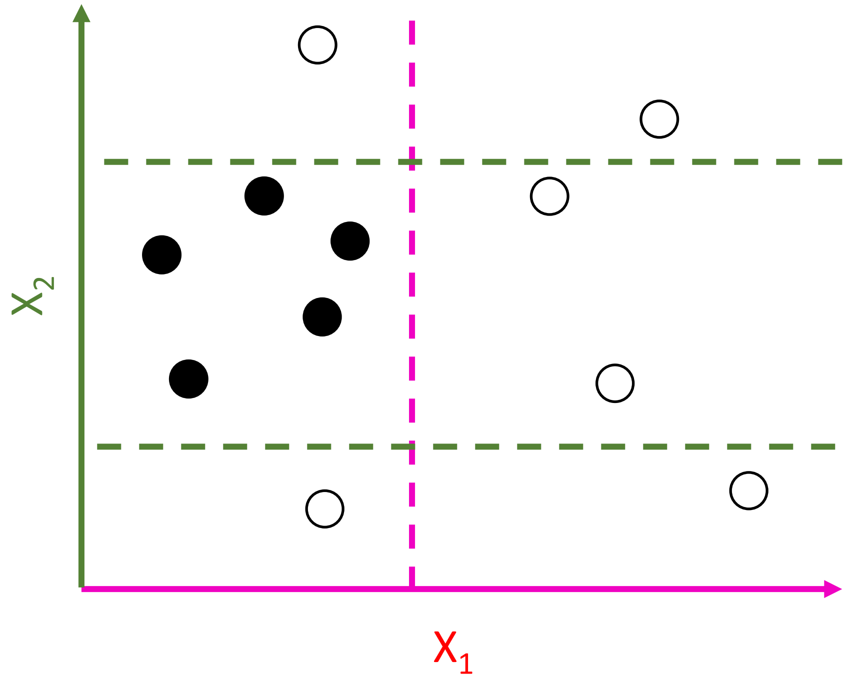
Deriving random decision trees

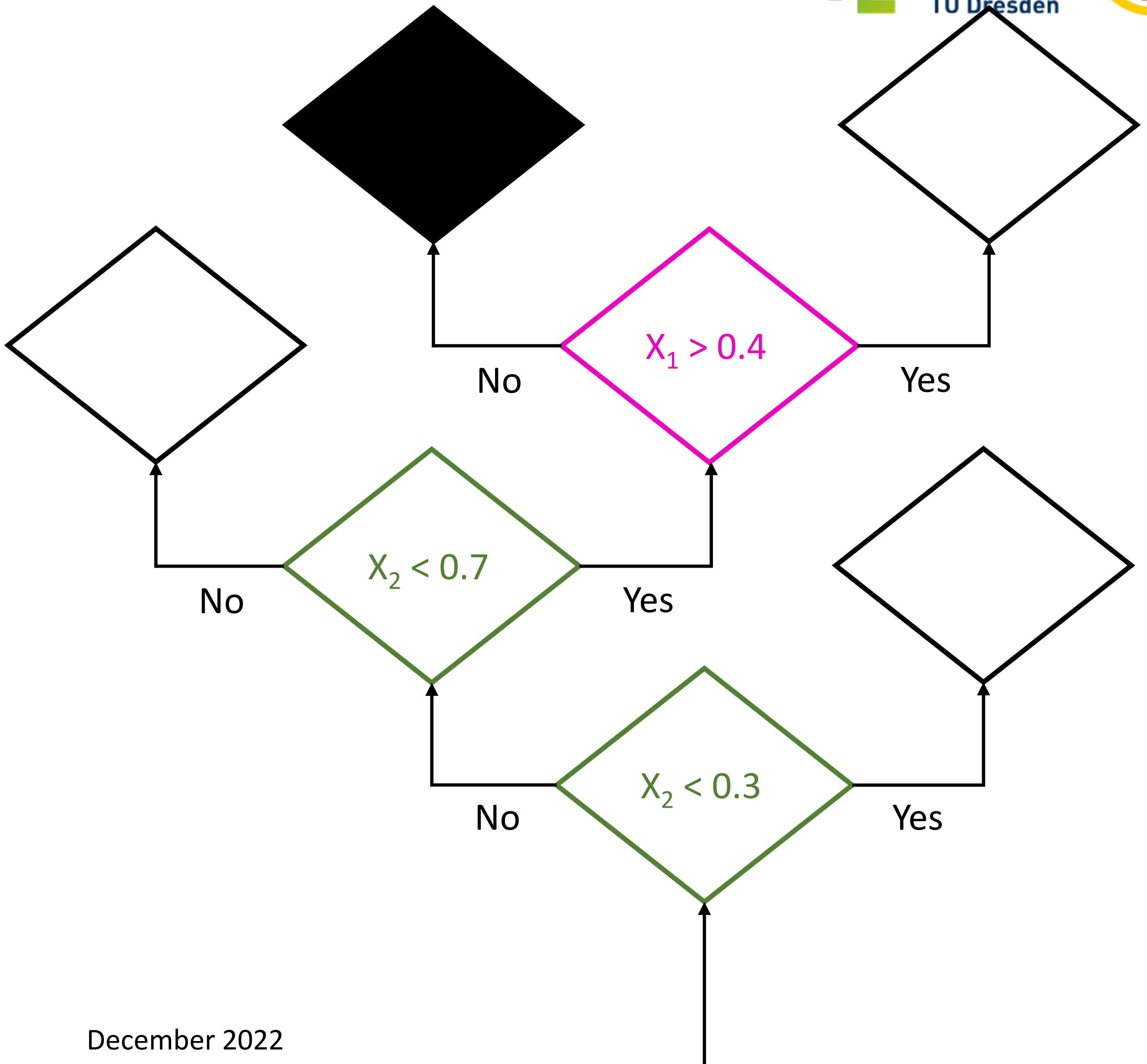
- Decision trees combine several thresholds on several parameters



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.

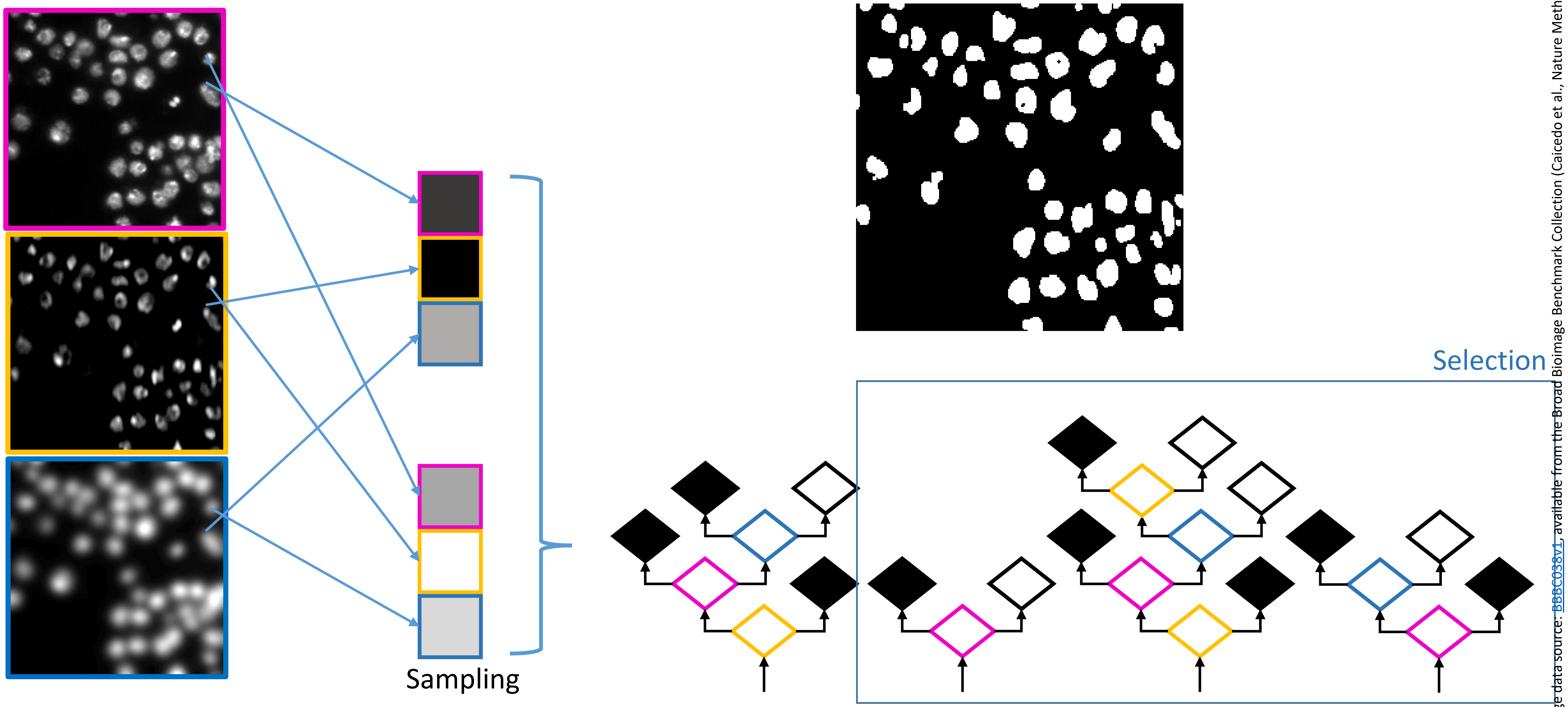
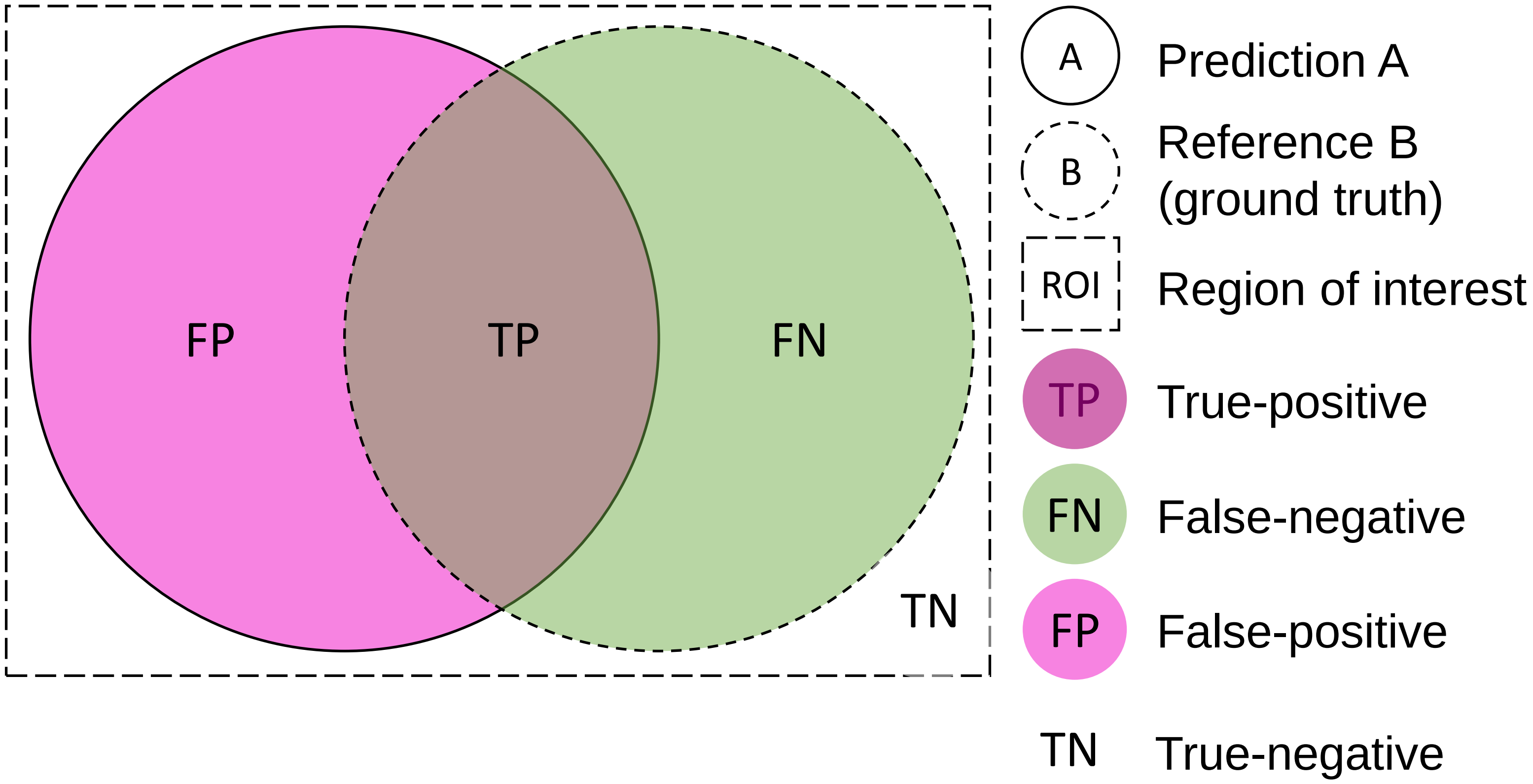


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

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 <u>Theory of Sets</u>

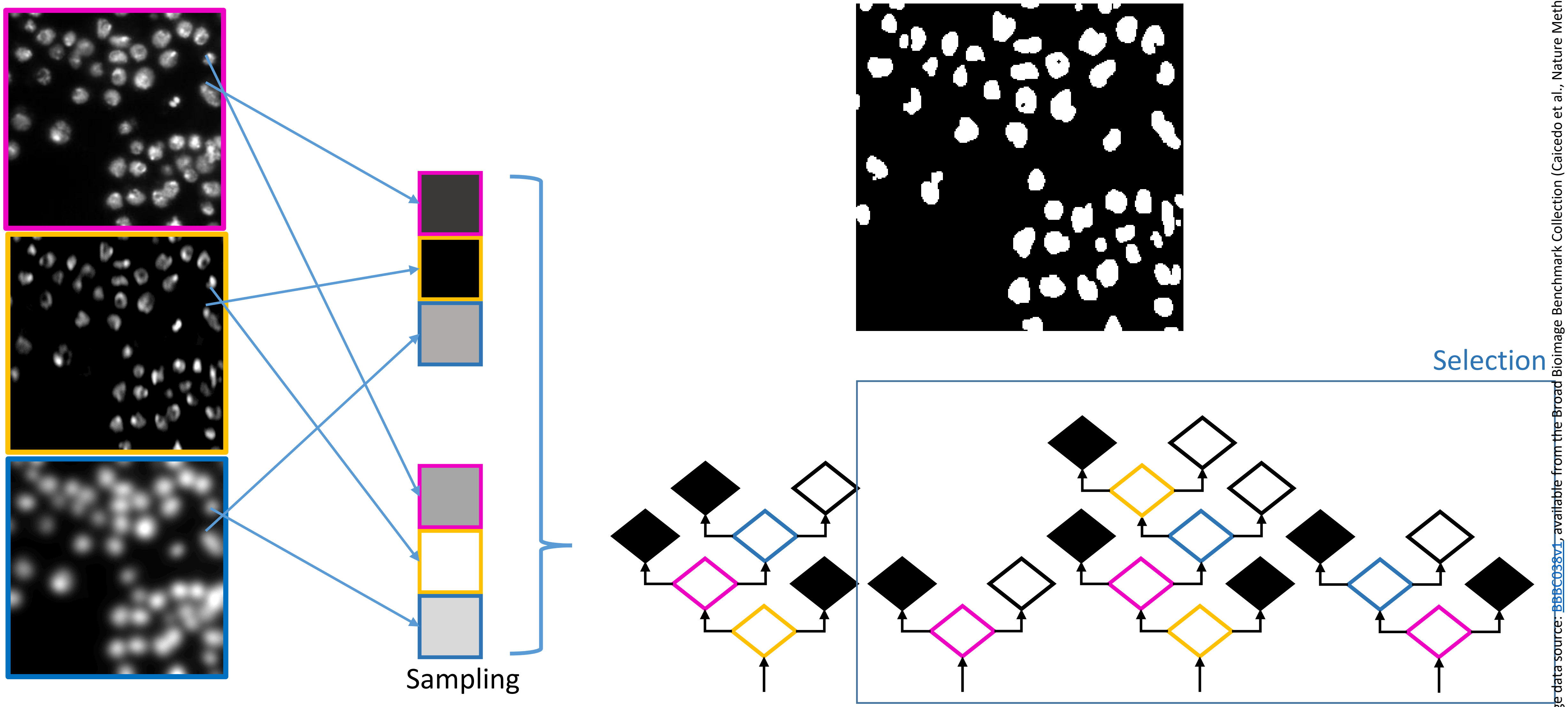


Precision $$\frac{TP}{TP+FP}$$ What fraction of points that were predicted as positives were really positive?

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

Random Forest Pixel Classifiers

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



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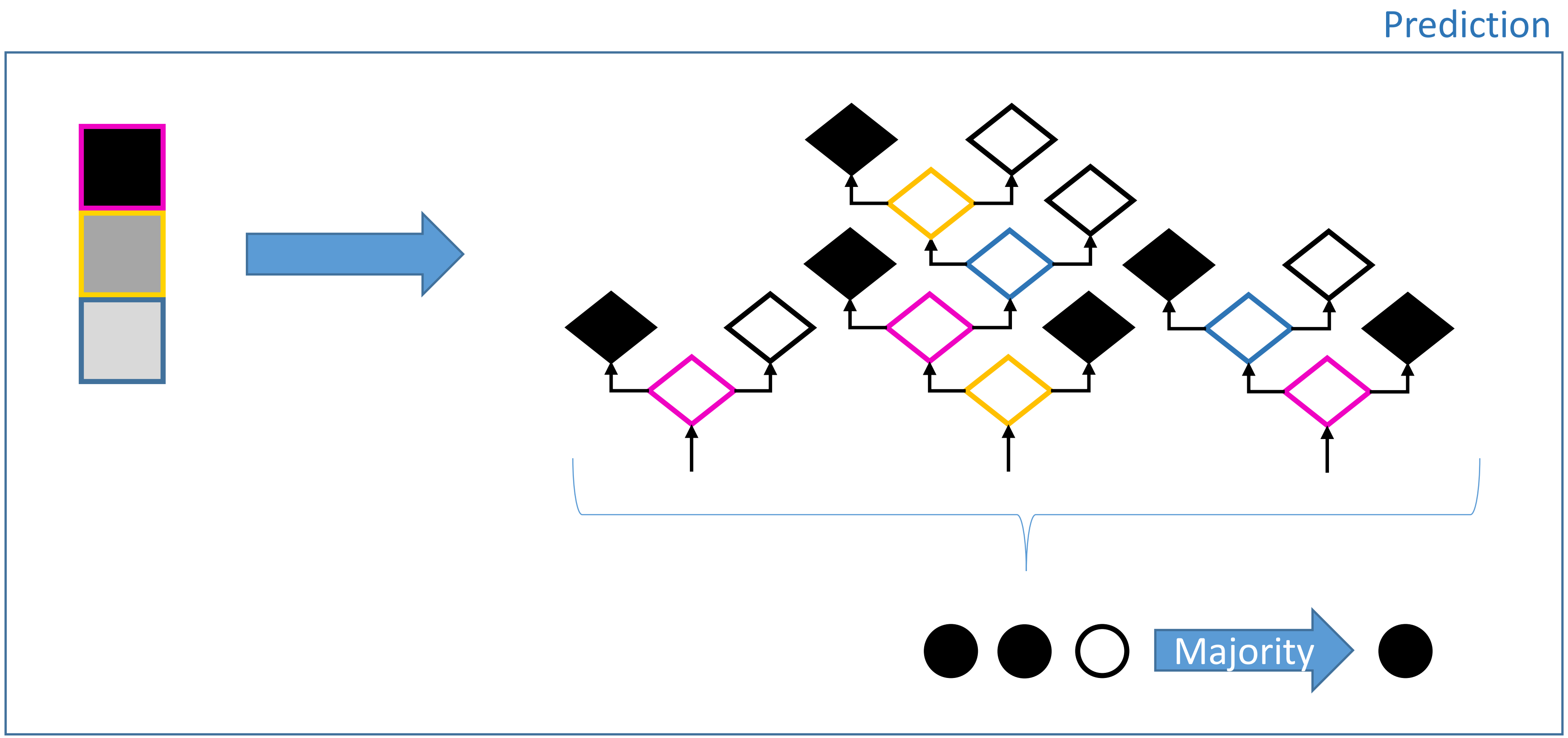
Random Forest Pixel Classifiers

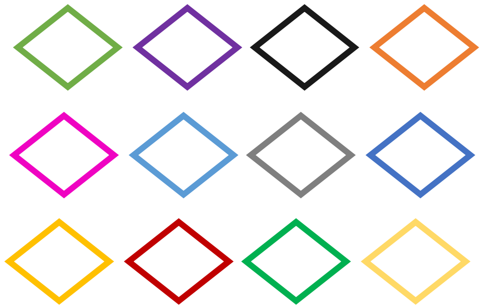
- Combination of individual tree decisions by voting or max / mean



Random Forest Pixel Classifiers

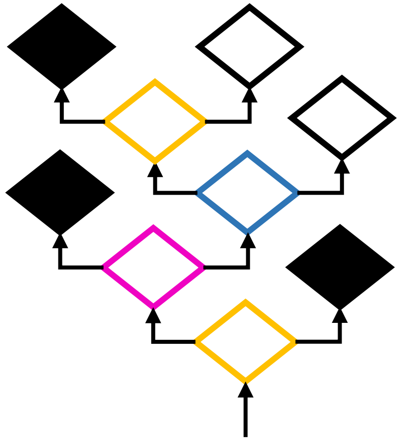
- Typical numbers for pixel classifiers in microscopy

Available features: > 20



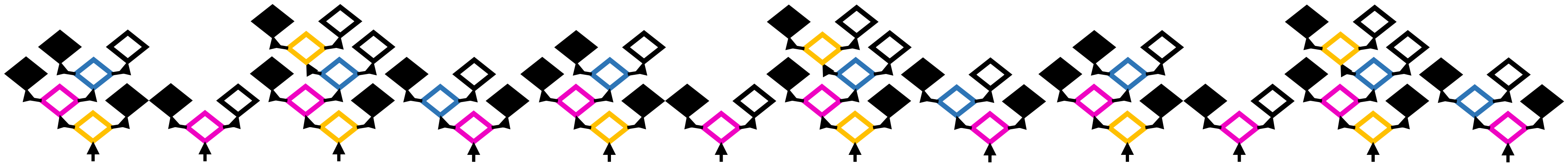
- Gaussian blur image
- DoG image
- LoG image
- Hessian
- ....

Selected features: <= depth



Depth: 4

Number of trees: > 100



Model validation

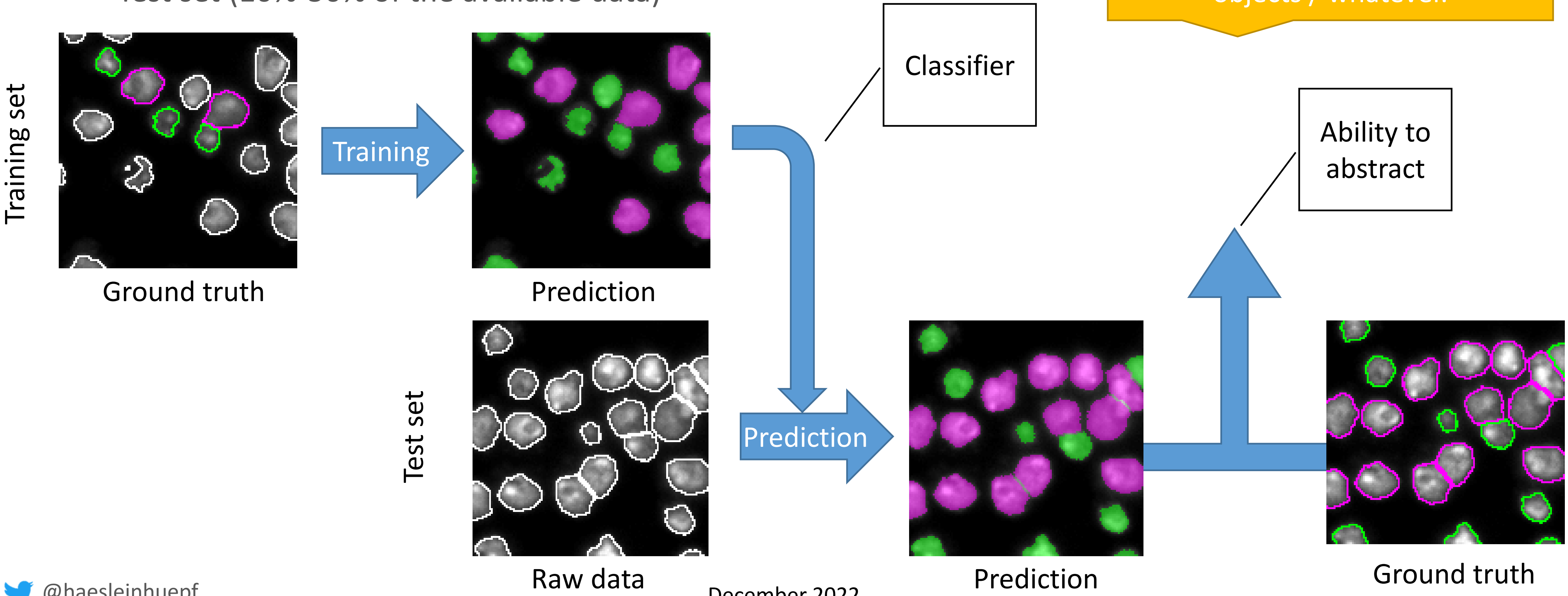
- Underfitting
  - A trained model that is not even able to properly process the data it was trained on

- 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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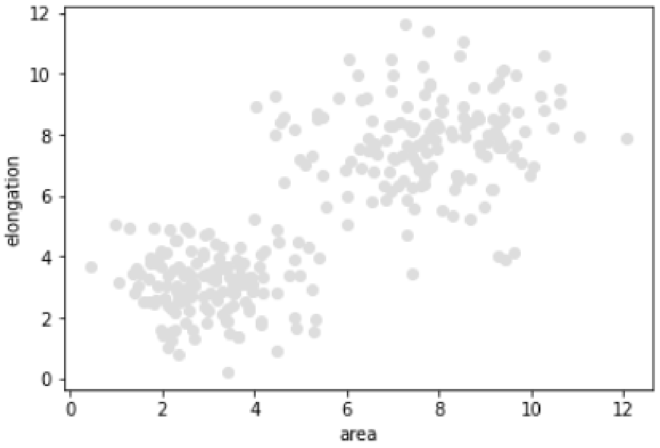
Tabular object classification

- Classify objects starting from feature vectors (table columns)

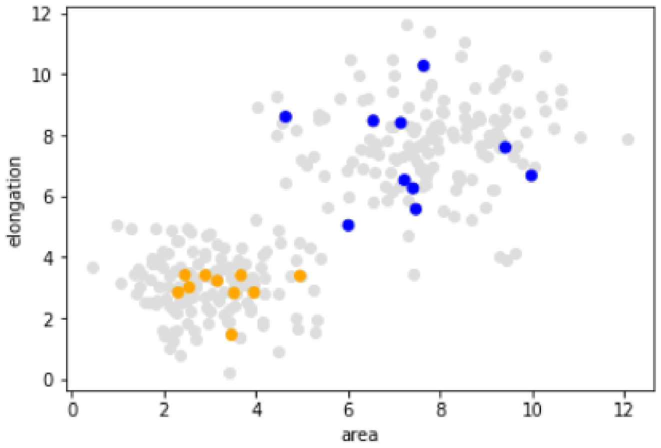
Raw data

	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,
```

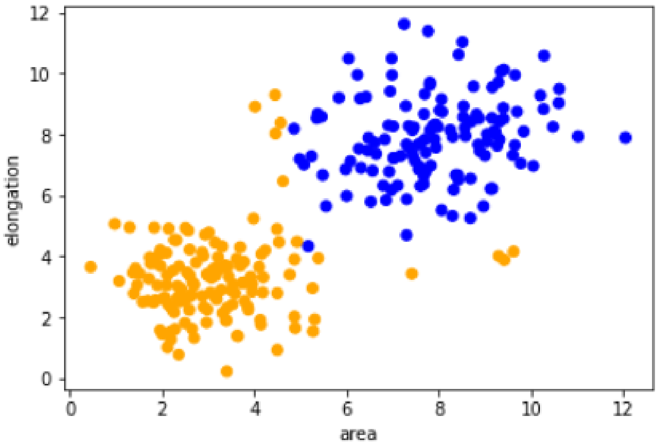


Classifier training

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

Classifier prediction

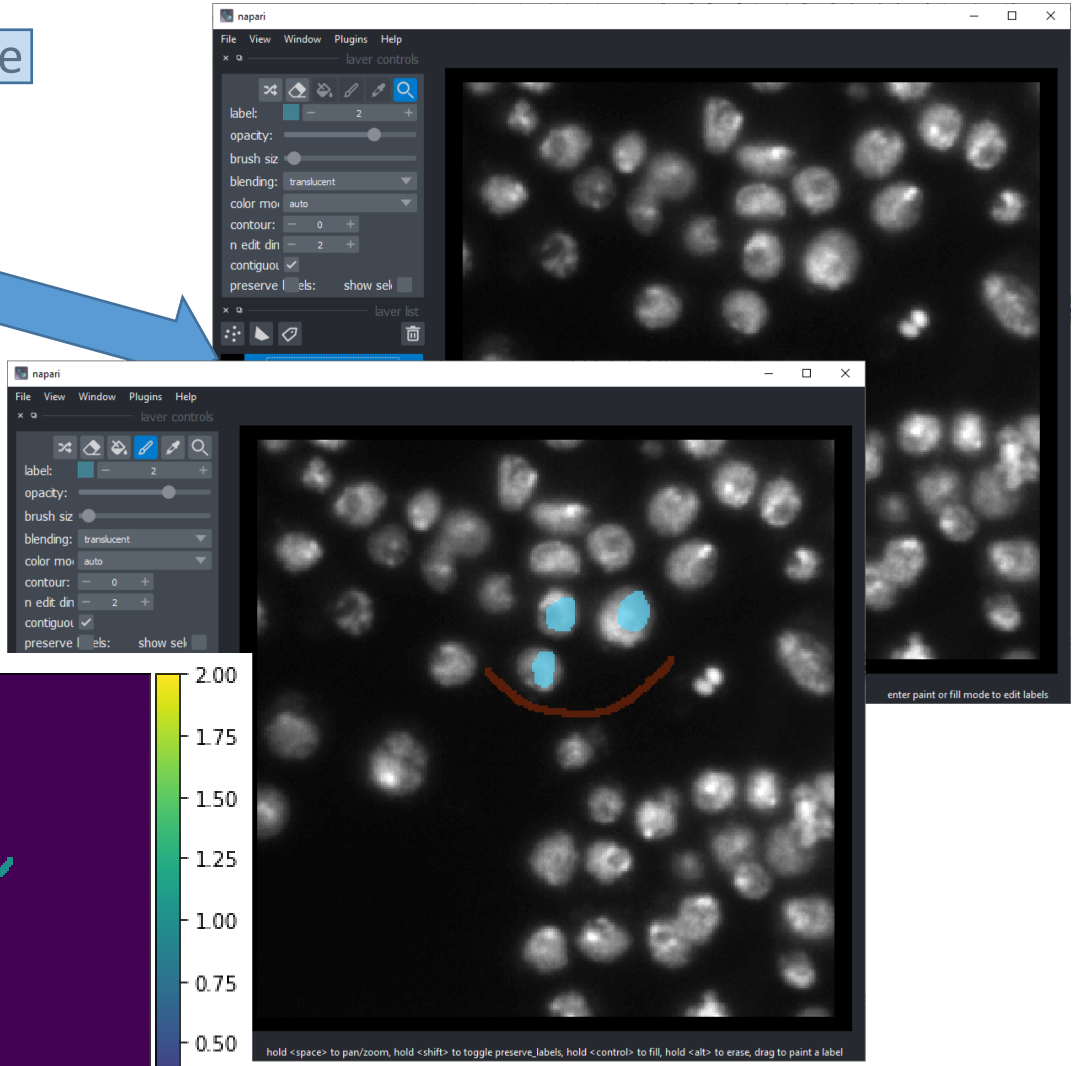
```
result = classifier.predict(validation_data)
```



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
```

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

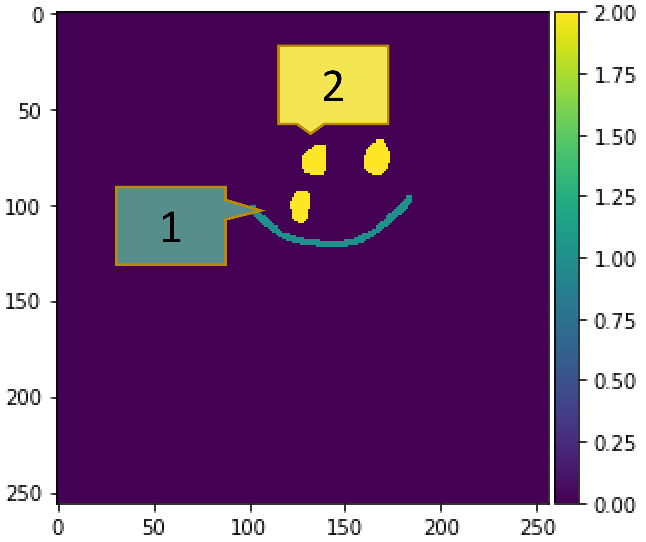


Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Interactive pixel classification

- 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

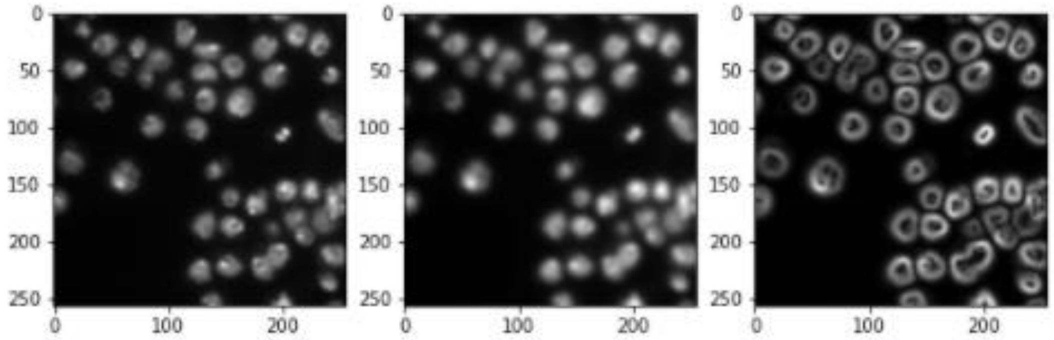
```
y_ = classifier.predict(X)
```

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)
```

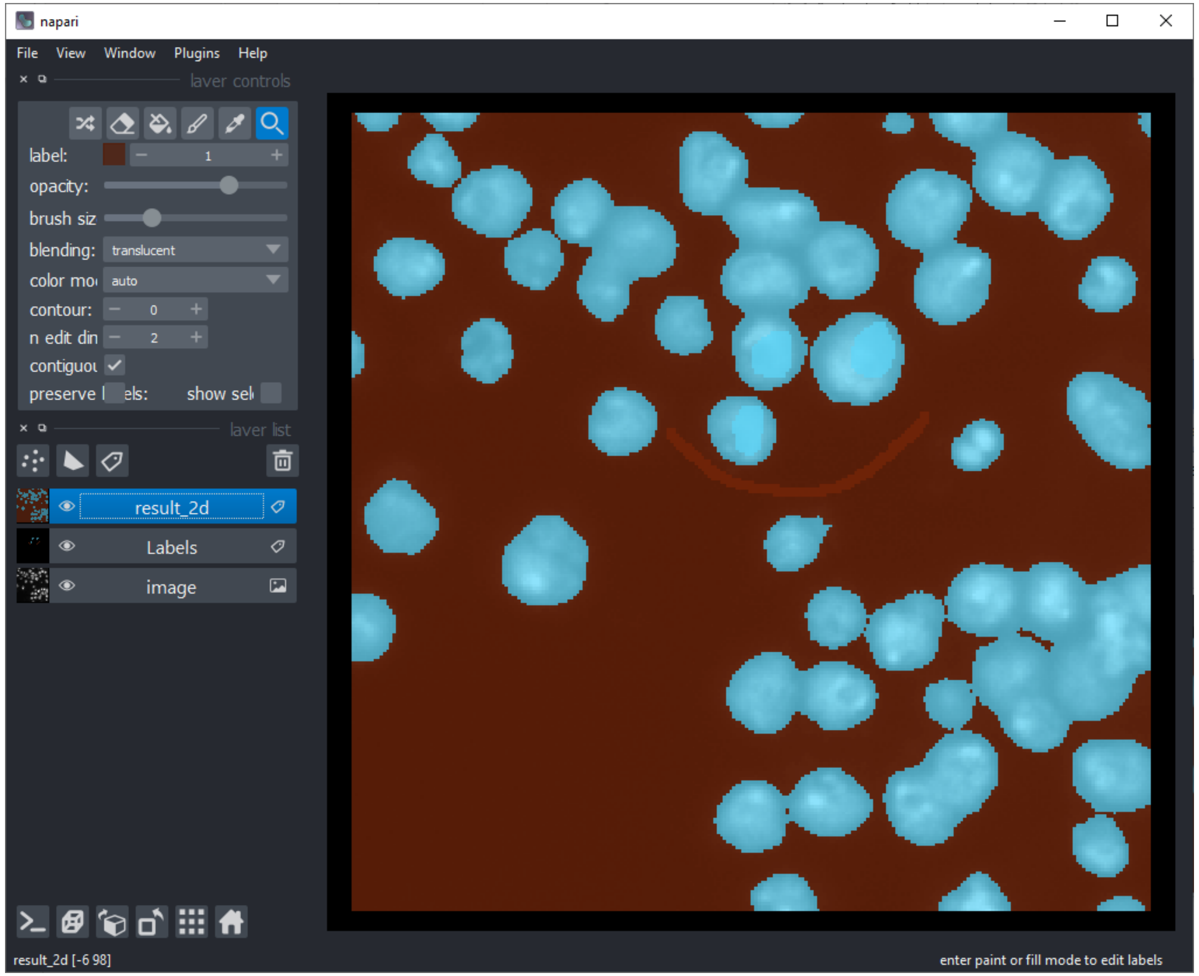
Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

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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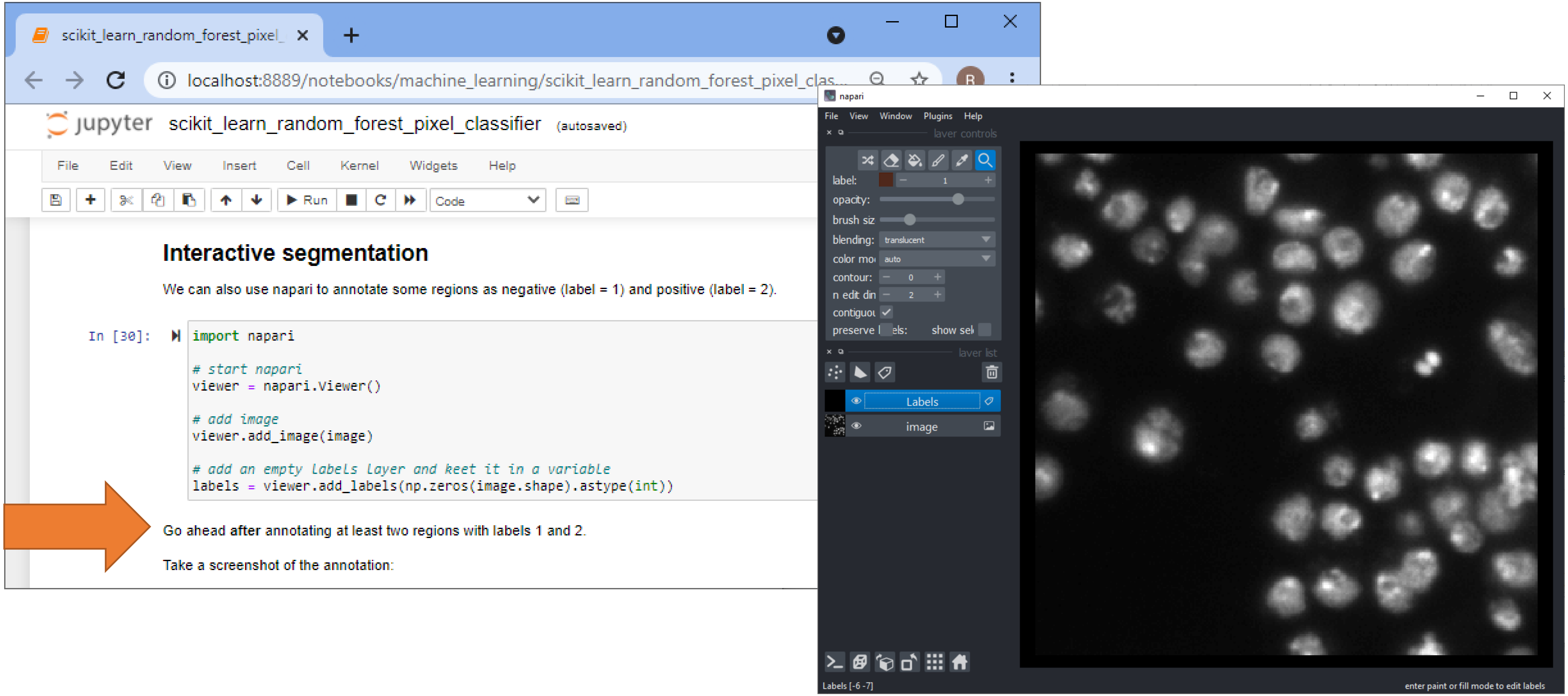
Interactive pixel classification

- Jupyter notebooks and napari side-by-side



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Interactive pixel classification

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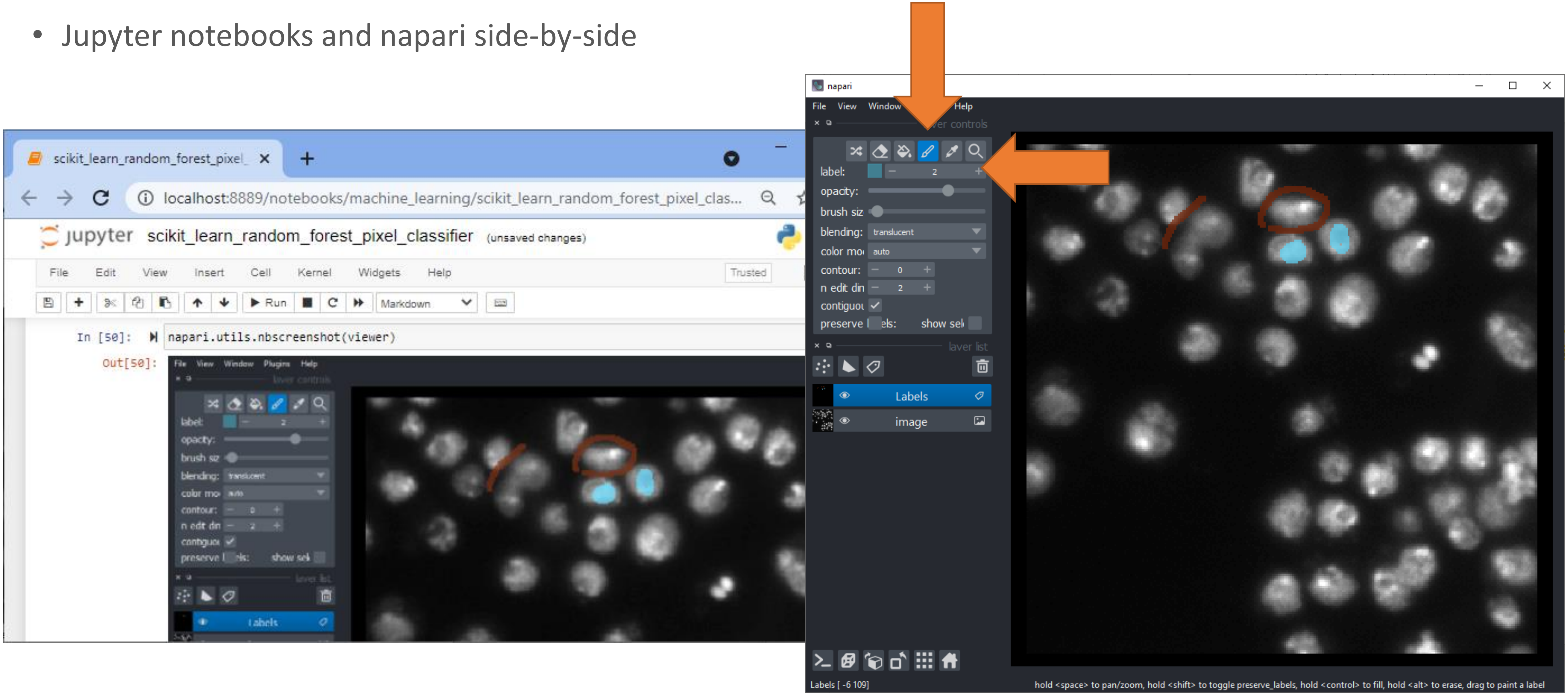

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Interactive pixel classification

- Jupyter notebooks and napari side-by-side

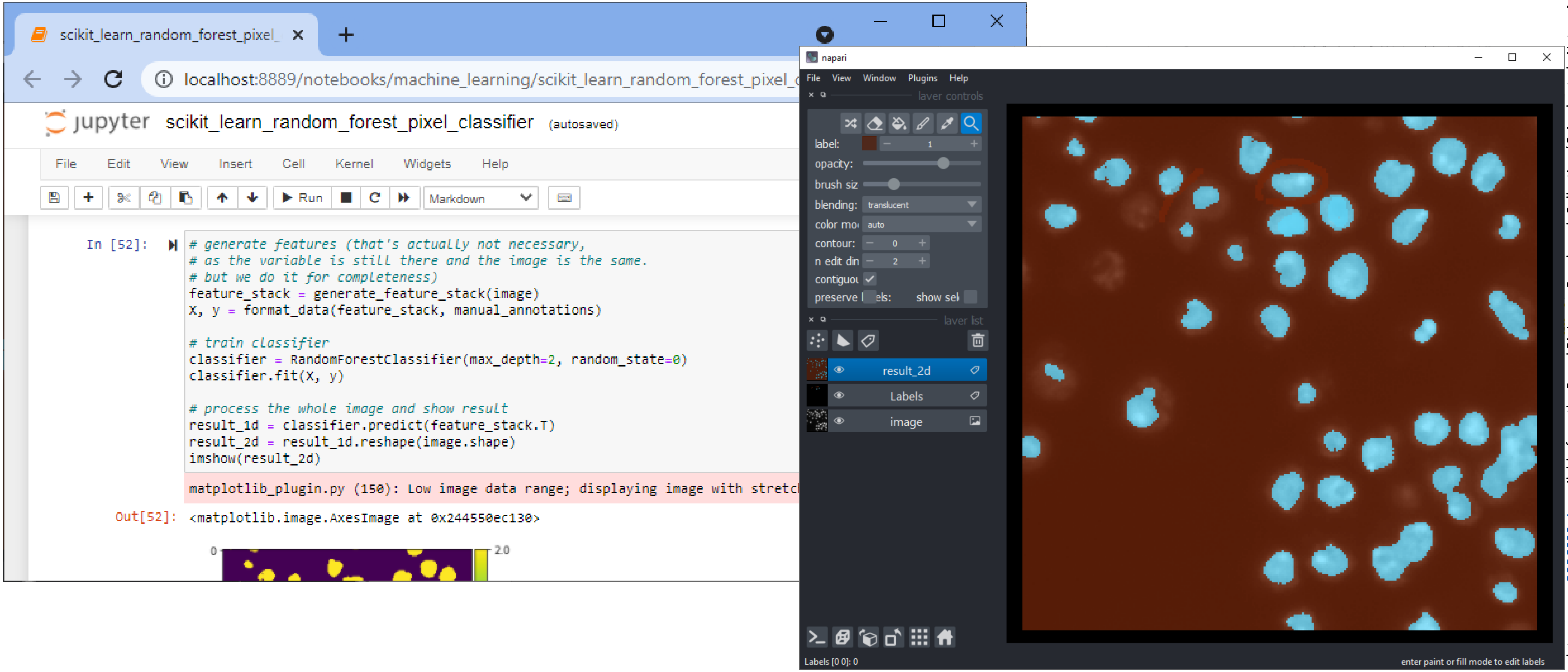

Image data source: BBBC038v1, available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019).

Accelerated pixel and object classification (APOC)

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December 2022

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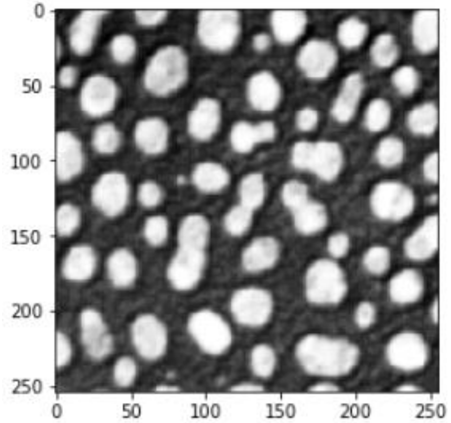
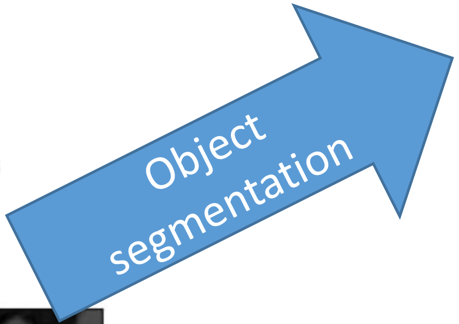
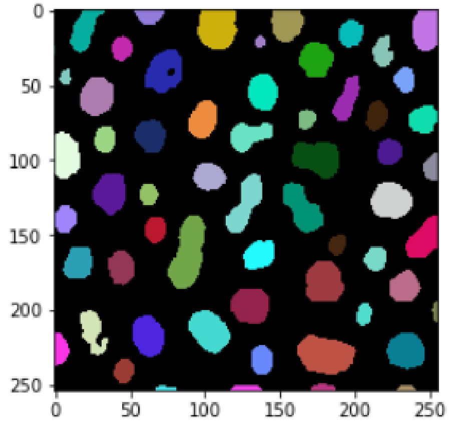
Accelerated pixel and object classification

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

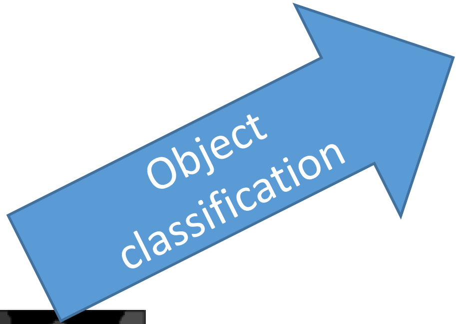


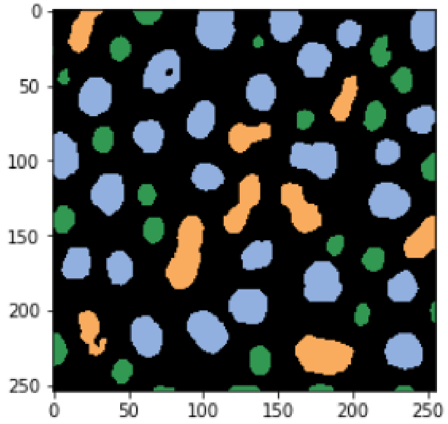
Raw image



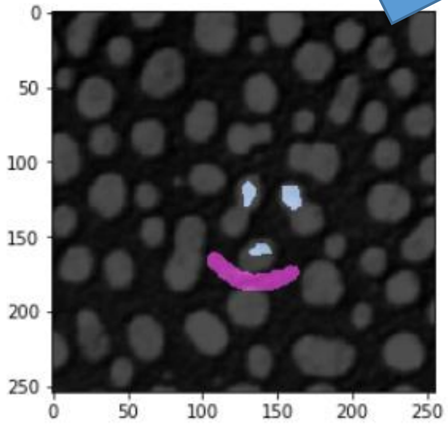


Object label image

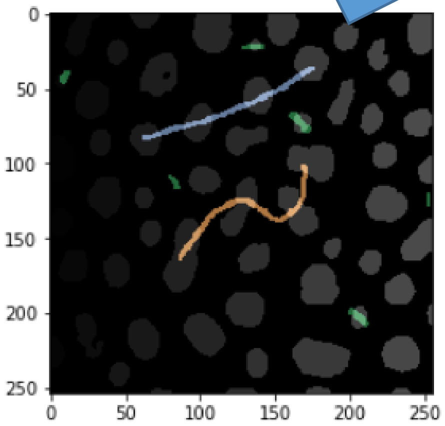




Class label image



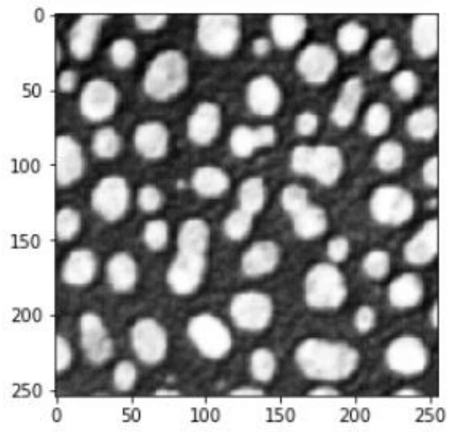
Pixel annotation



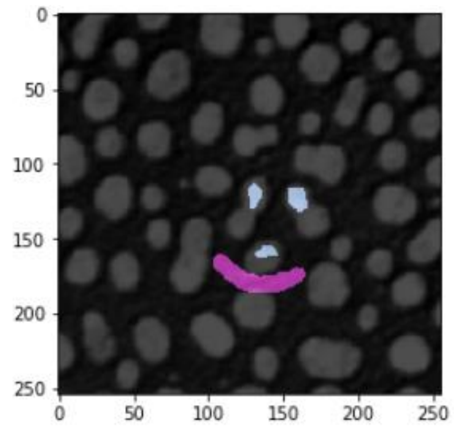
Object annotation

Object segmentation

- Pixel classification + connected component labeling



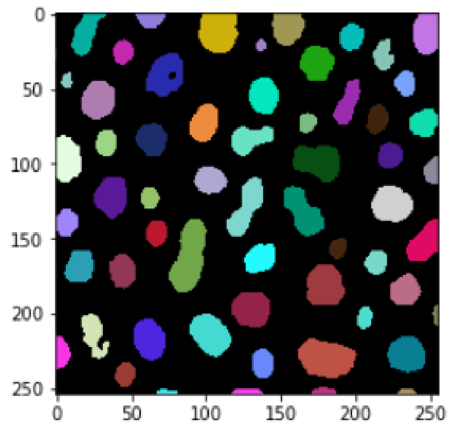
Raw image



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)
```

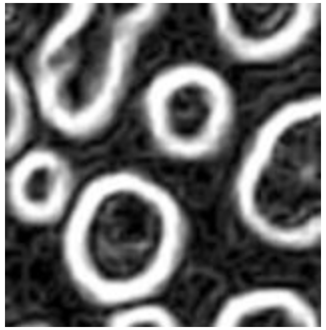
Object segmentation



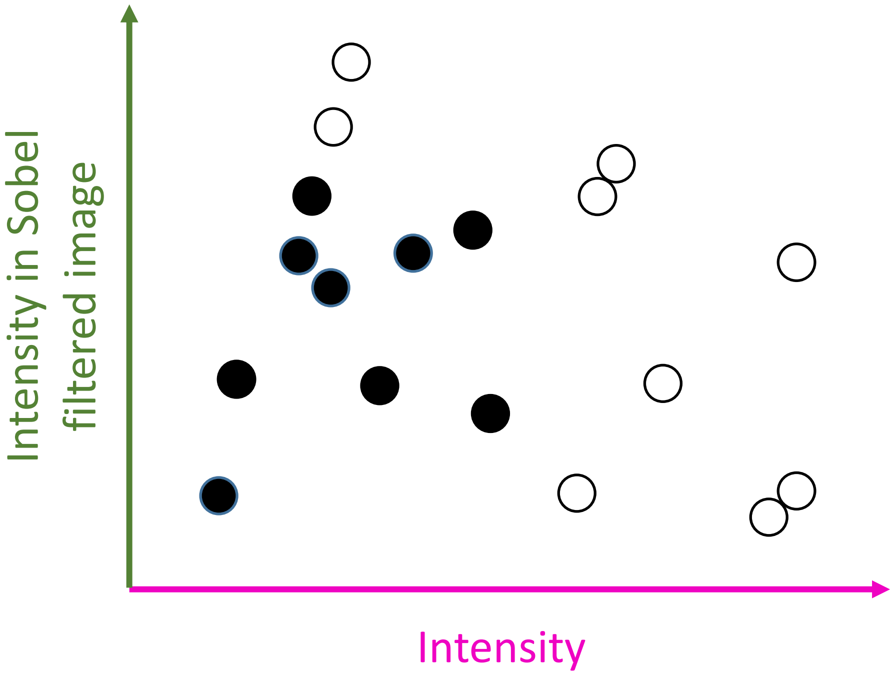
Object label image

Object classification

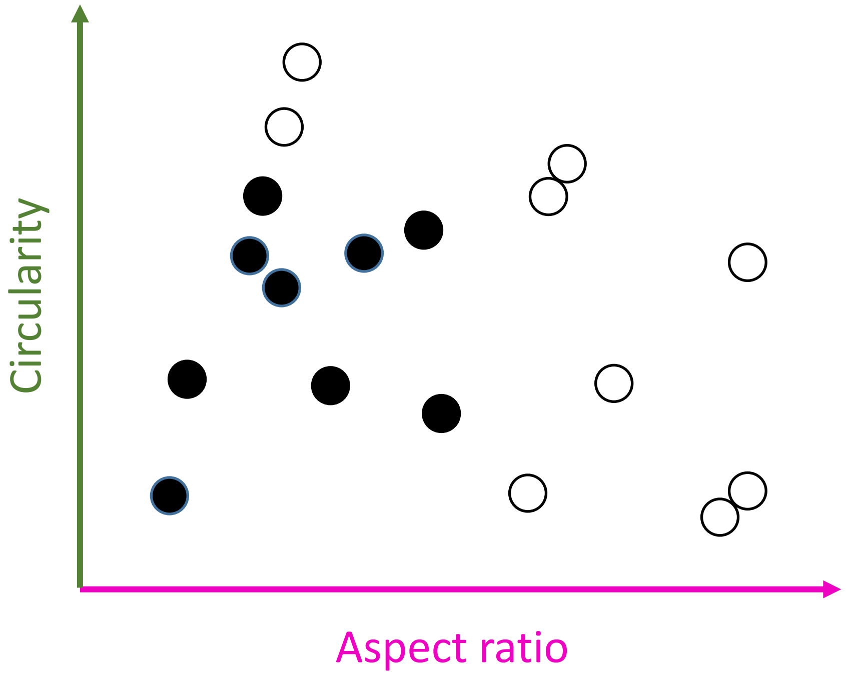
- What if we exchange pixel features with object features?

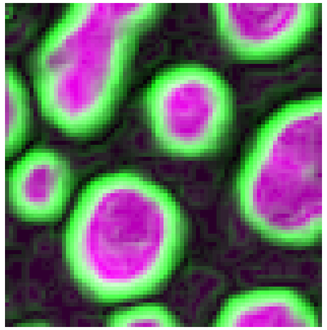


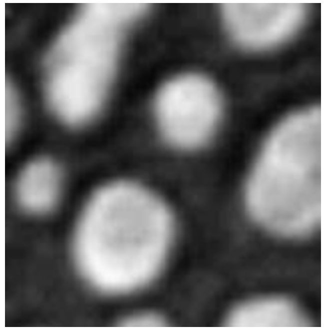
Pixel classification



Object classification



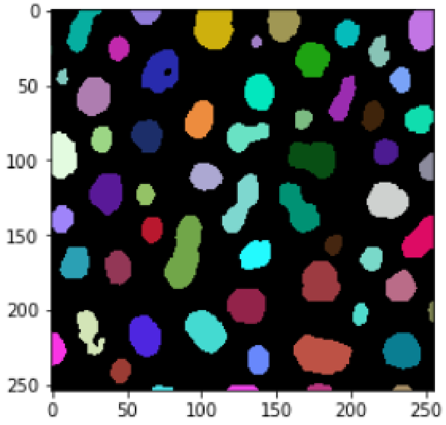




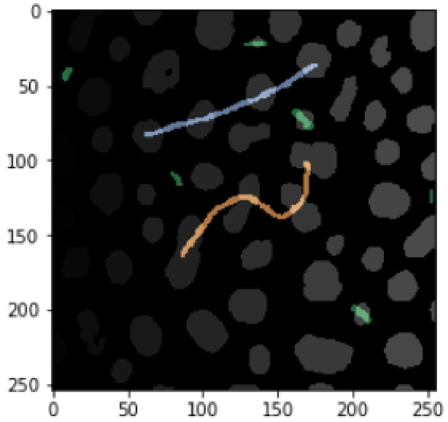
- The algorithms work the same but with different
  - Features
  - Number of features
  - Tree / forest parameters
  - Selection criteria

Object classification

- Feature extraction + tabular classification



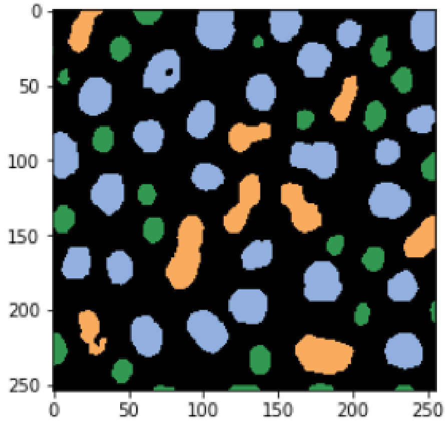
Object label image



Object annotation

```
# 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

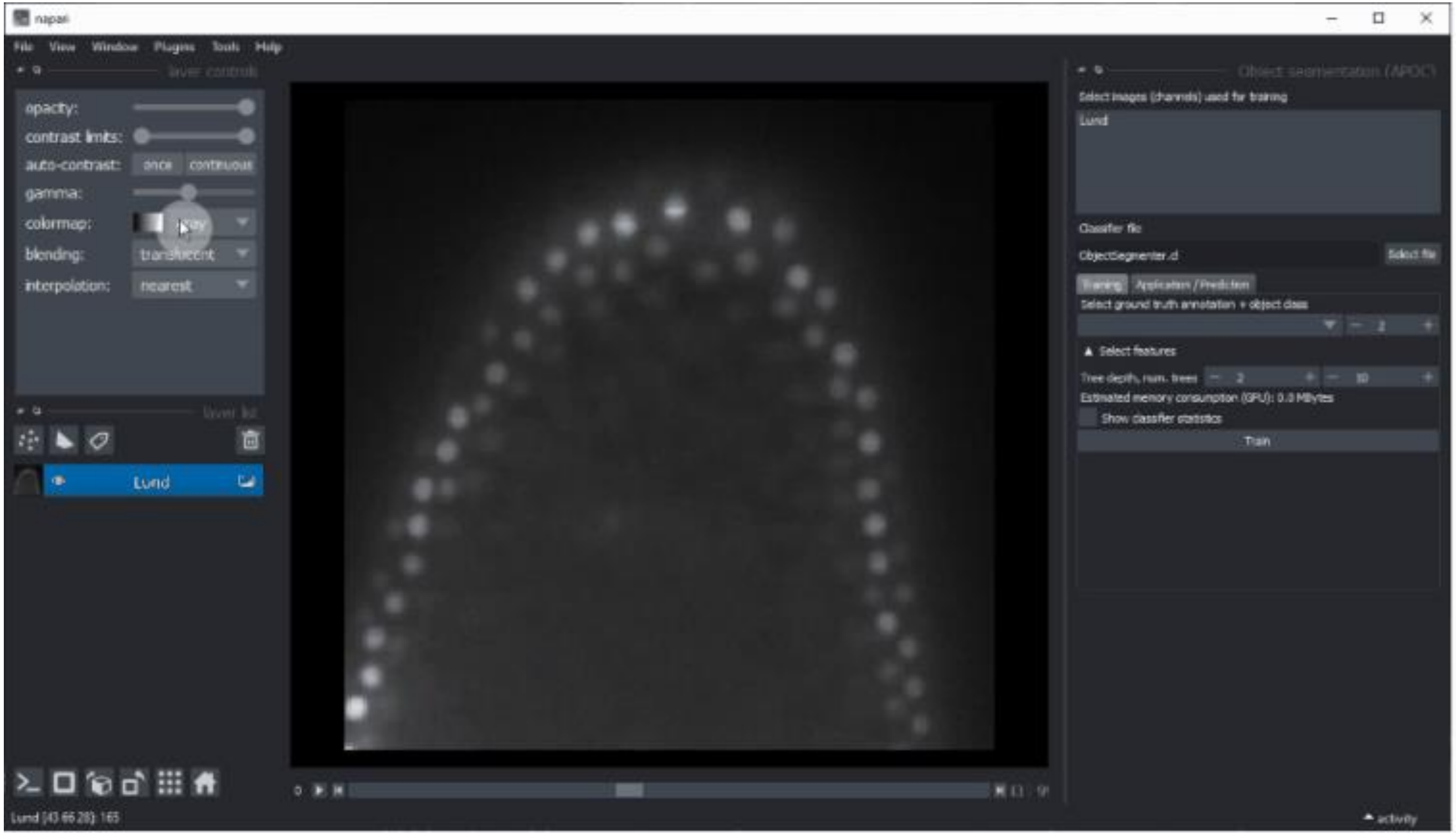


Class label image

Graphical user interface

Object segmentation

- Random Forest Classifiers for Pixel Classification + Connected Component Labeling

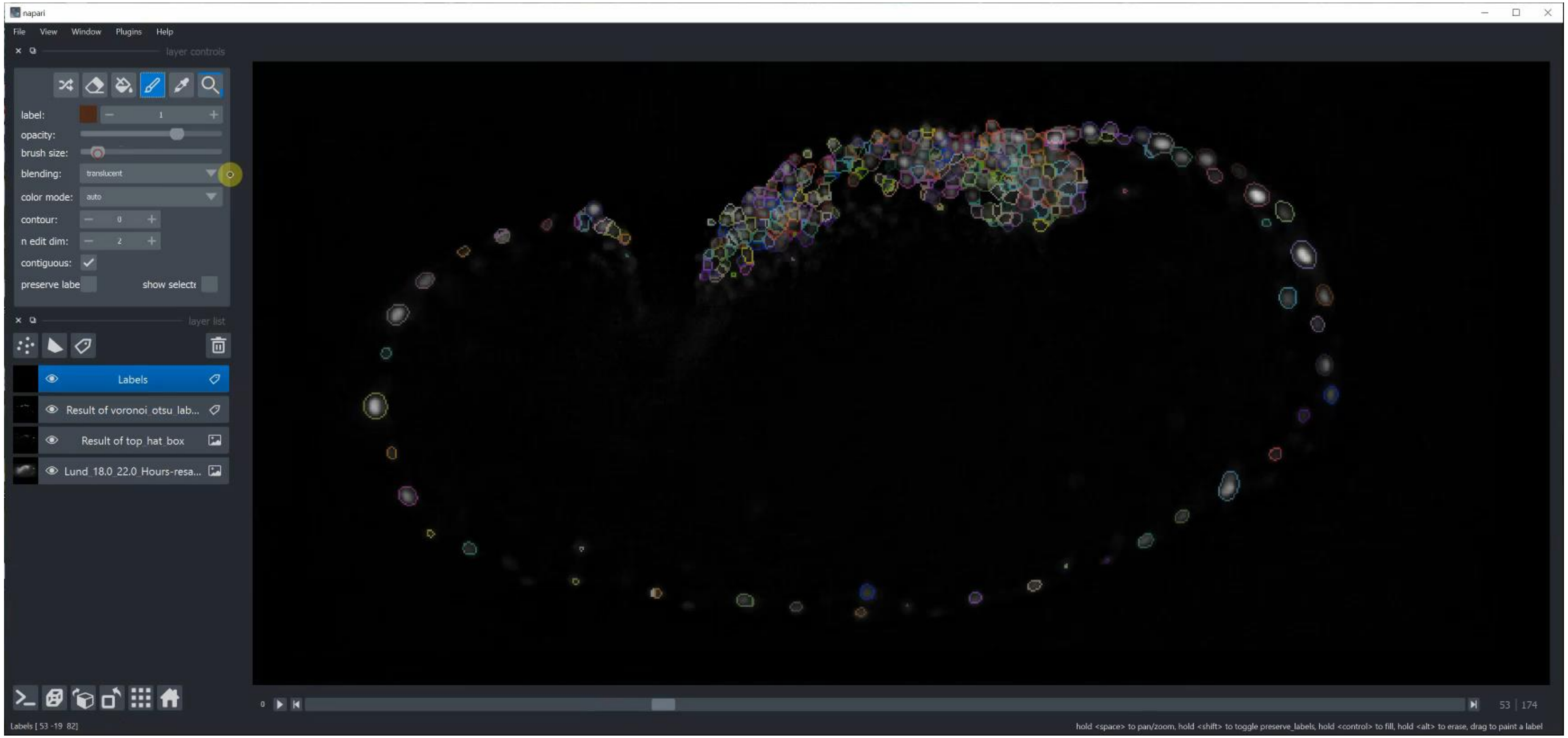


https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification#object-and-semantic-segmentation

Supervised machine learning for tissue classification

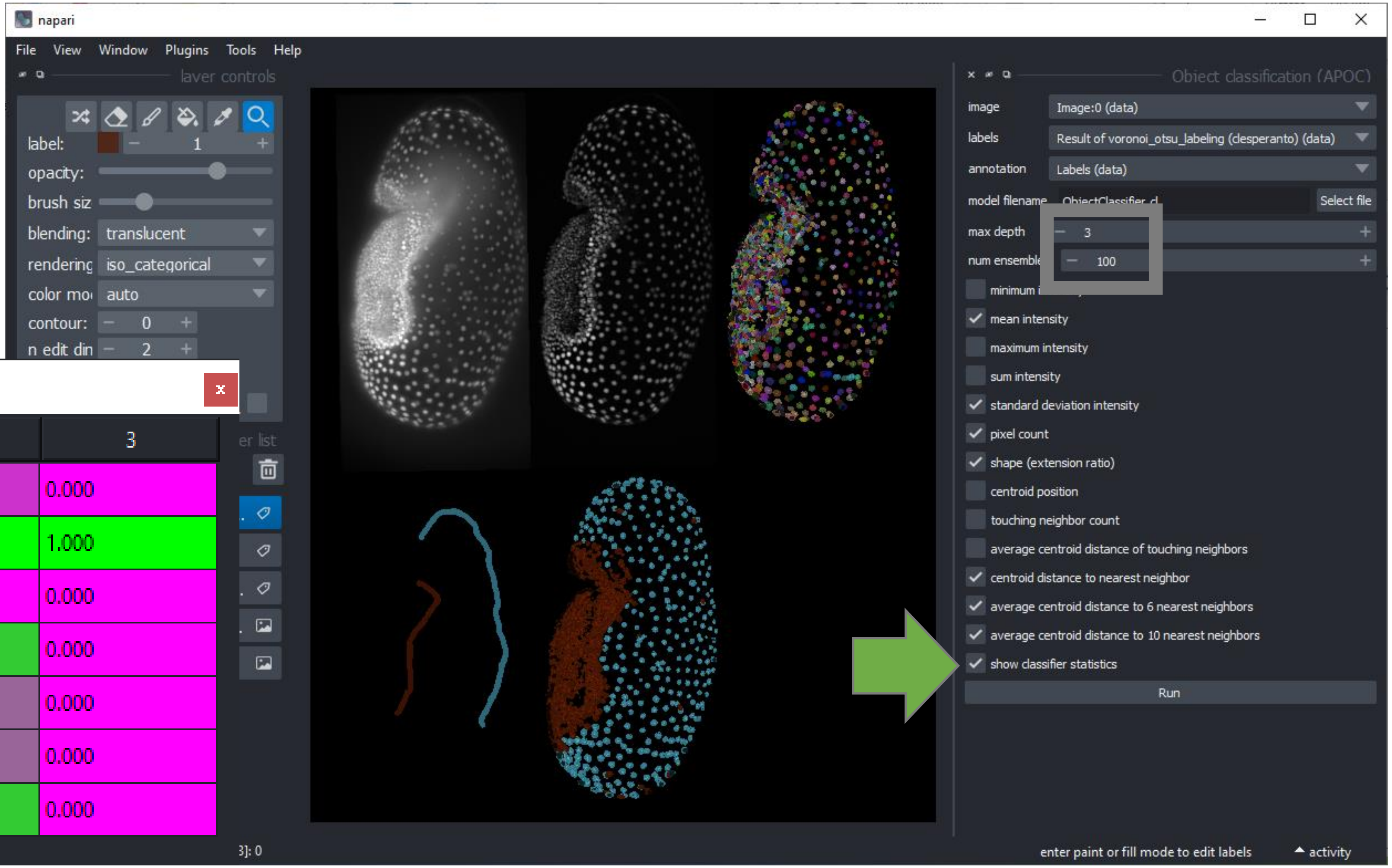
Random Forest Classifiers based on

- scikit-learn and
- clesperanto



Data exploration / supervised machine learning

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

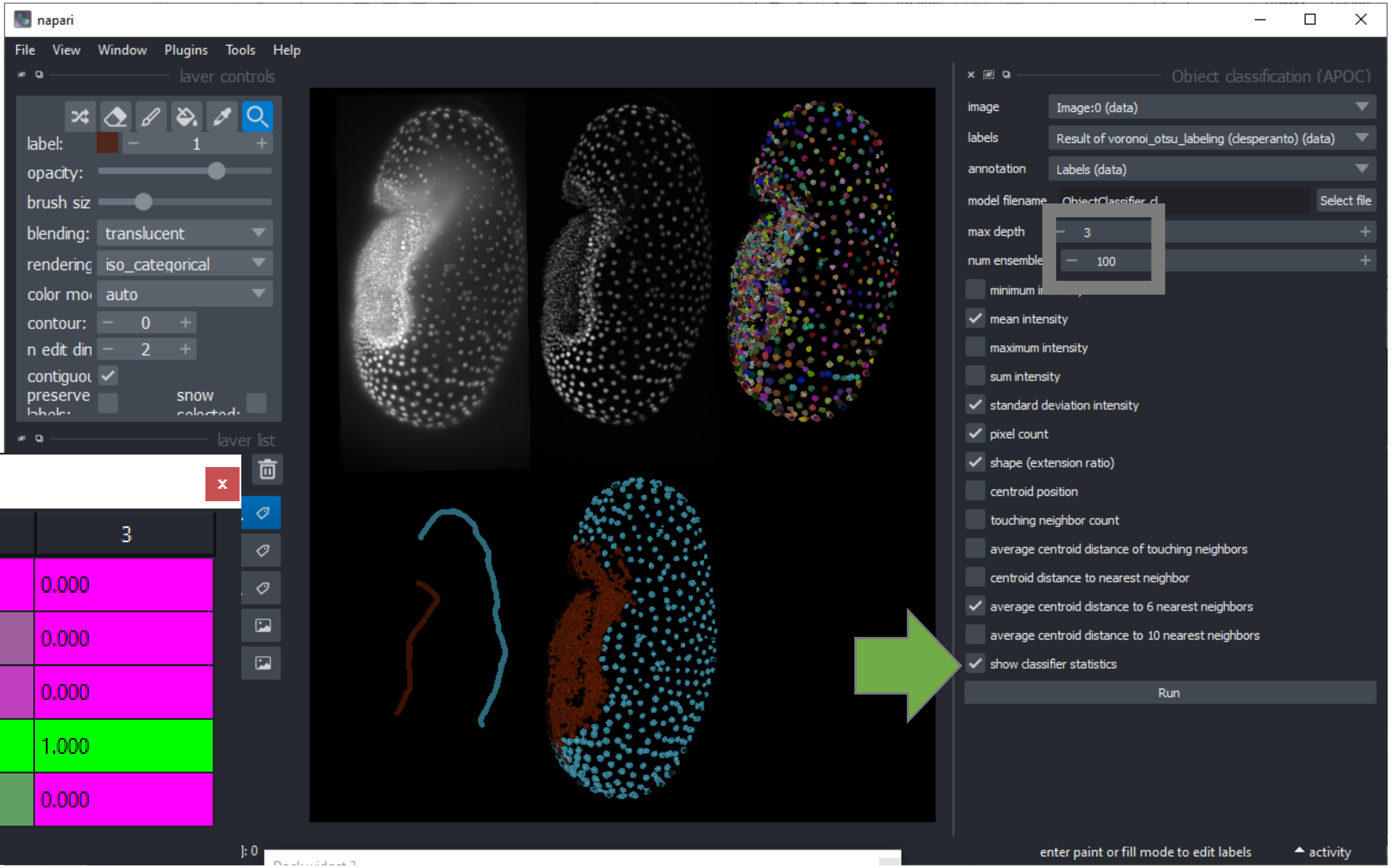


Dock widget 1

	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

Data exploration / supervised machine learning

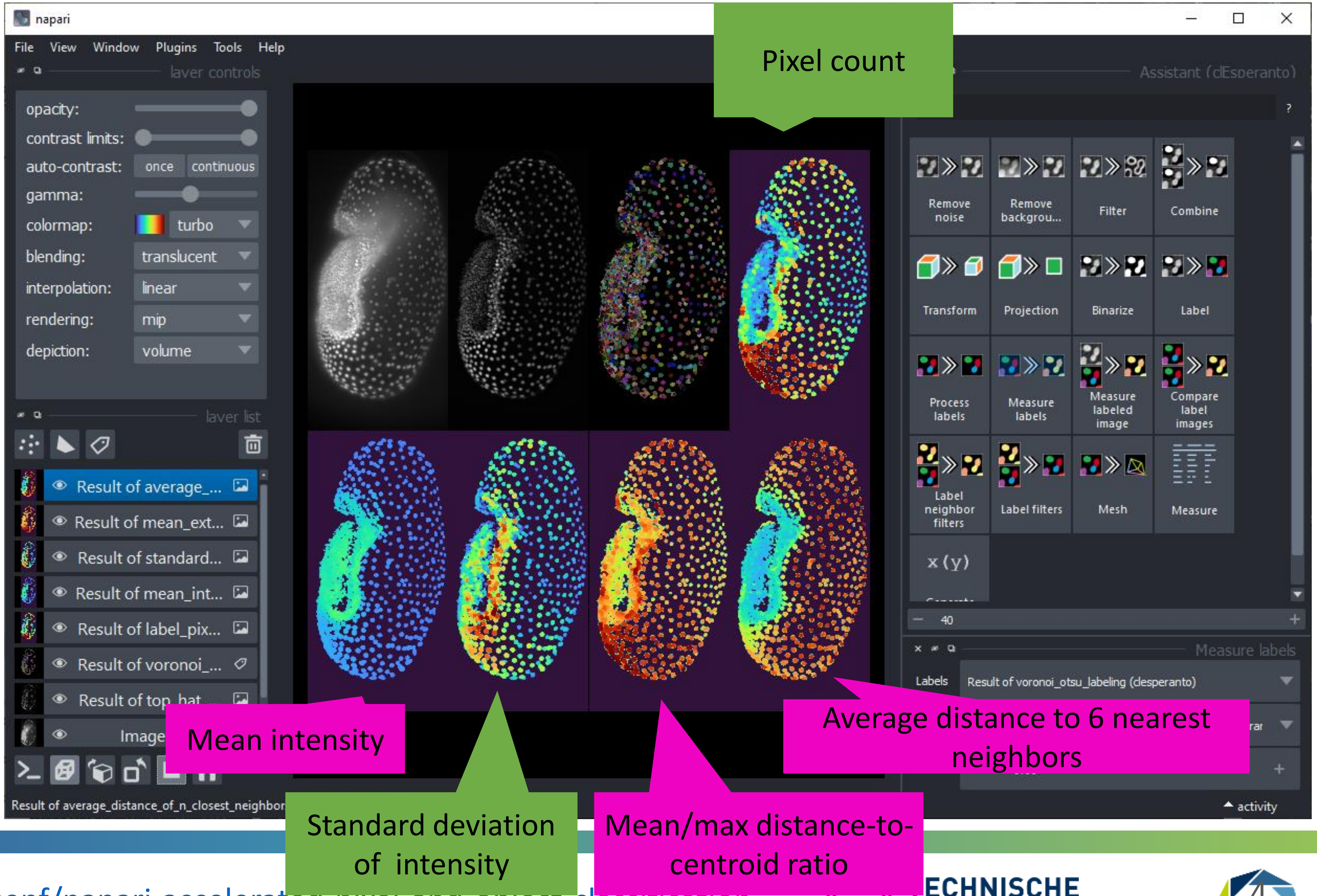
PoL Physics of Life TU Dresden

- Inspect how the random forest classifier makes decisions

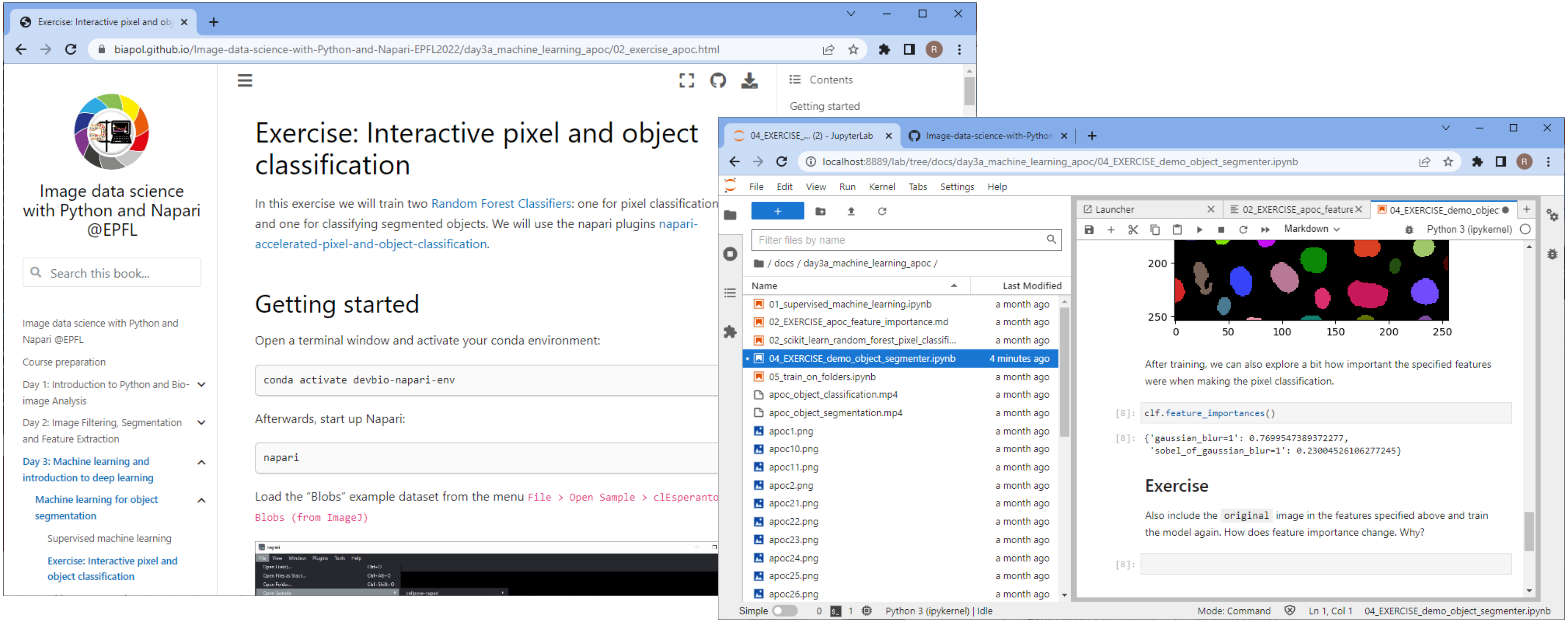
- Note: Beware of correlated parameters!

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



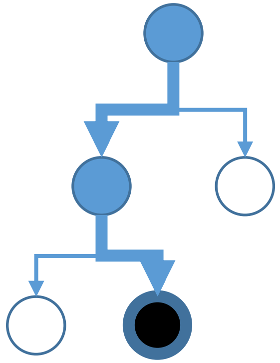
Exercises



Exercise: Interactive pixel and ob
biapol.github.io/Image-data-science-with-Python-and-Napari-EPFL2022/day3a_machine_learning_apoc/02_exercise_apoc.html
Image data science with Python and Napari @EPFL
Search this book...
Image data science with Python and Napari @EPFL
Course preparation
Day 1: Introduction to Python and Bio-image Analysis
Day 2: Image Filtering, Segmentation and Feature Extraction
Day 3: Machine learning and introduction to deep learning
Machine learning for object segmentation
Supervised machine learning
Exercise: Interactive pixel and object classification
Contents
Getting started
Exercise: Interactive pixel and object classification
In this exercise we will train two Random Forest Classifiers: one for pixel classification and one for classifying segmented objects. We will use the napari plugins 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)
04_EXERCISE_... (2) - JupyterLab
Image-data-science-with-Python
localhost:8889/lab/tree/docs/day3a_machine_learning_apoc/04_EXERCISE_demo_object_segmenter.ipynb
File Edit View Run Kernel Tabs Settings Help
Filter files by name
/ docs / day3a_machine_learning_apoc /
Name
Last Modified
01_supervised_machine_learning.ipynb a month ago
02_EXERCISE_apoc_feature_importance.md a month ago
02_scikit_learn_random_forest_pixel_classifi... a month ago
04_EXERCISE_demo_object_segmenter.ipynb 4 minutes ago
05_train_on_folders.ipynb a month ago
apoc_object_classification.mp4 a month ago
apoc_object_segmentation.mp4 a month ago
apoc1.png a month ago
apoc10.png a month ago
apoc11.png a month ago
apoc2.png a month ago
apoc21.png a month ago
apoc22.png a month ago
apoc23.png a month ago
apoc24.png a month ago
apoc25.png a month ago
apoc26.png a month ago
Launcher
02_EXERCISE_apoc_feature
04_EXERCISE_demo_objec
Markdown
Python 3 (ipykernel)
After training, we can also explore a bit how important the specified features were when making the pixel classification.
[8]: clf.feature_importances()
[8]: {'gaussian_blur=1': 0.7699547389372277,
'sobel_of_gaussian_blur=1': 0.23004526106277245}
Exercise
Also include the original image in the features specified above and train the model again. How does feature importance change? Why?
Simple
Python 3 (ipykernel) | Idle
Mode: Command
Ln 1, Col 1
04_EXERCISE_demo_object_segmenter.ipynb

Summary

- Machine learning for Pixel and Object classification
  - Random Forest Classifiers
- Python
  - Scikit-learn + Napari
  - Accelerated pixel and object classifiers (APOC)



Coming up next:

- Deep learning