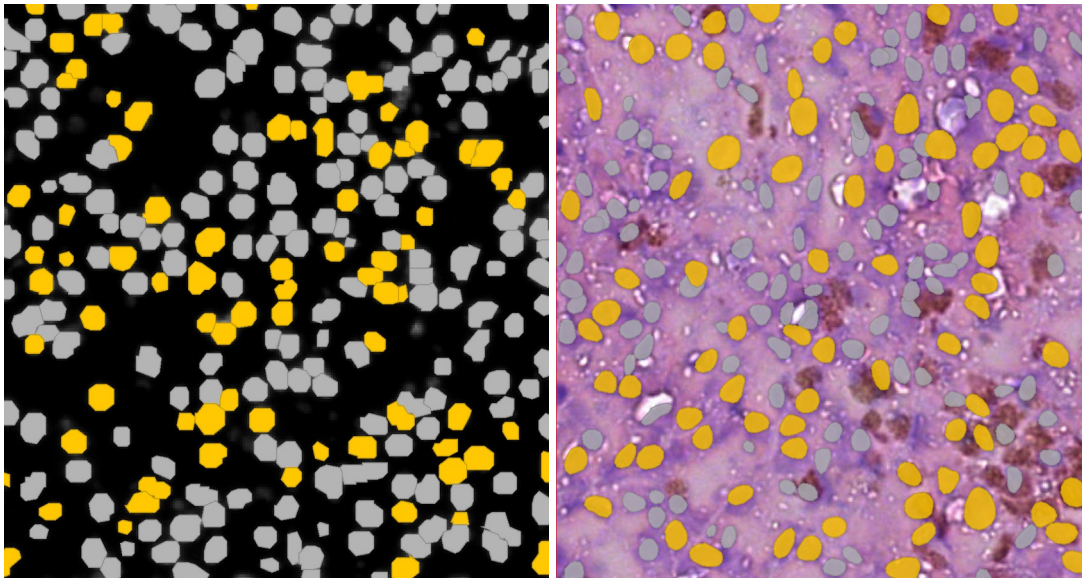
Chapter 6

Cells classification

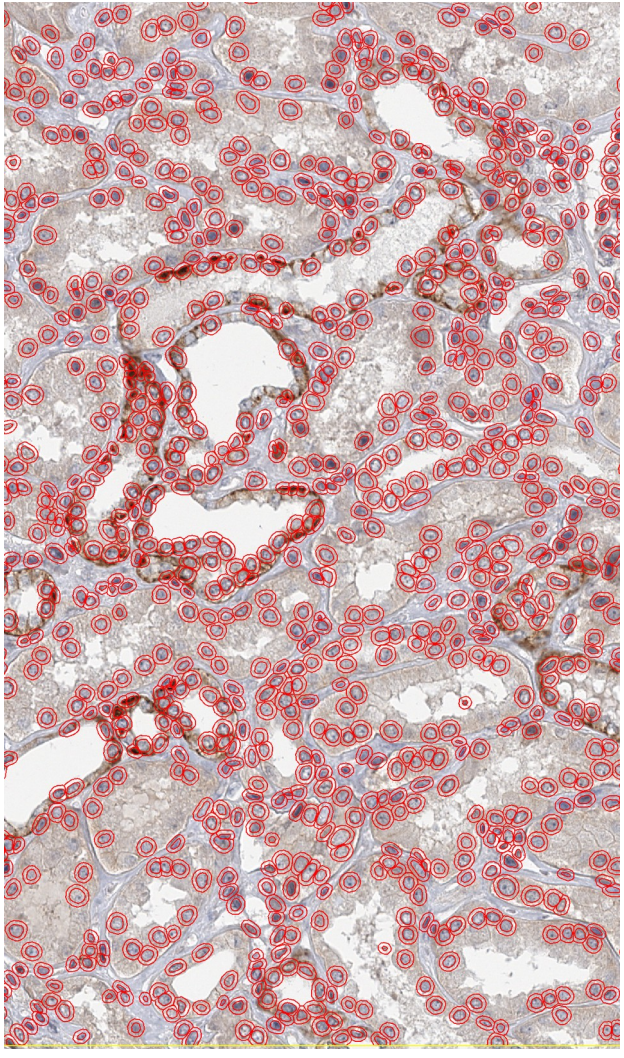


BAECKER Volker
BENEDETTI Clément
HASSEN-KHODJA Cédric

Introduction

Goals:

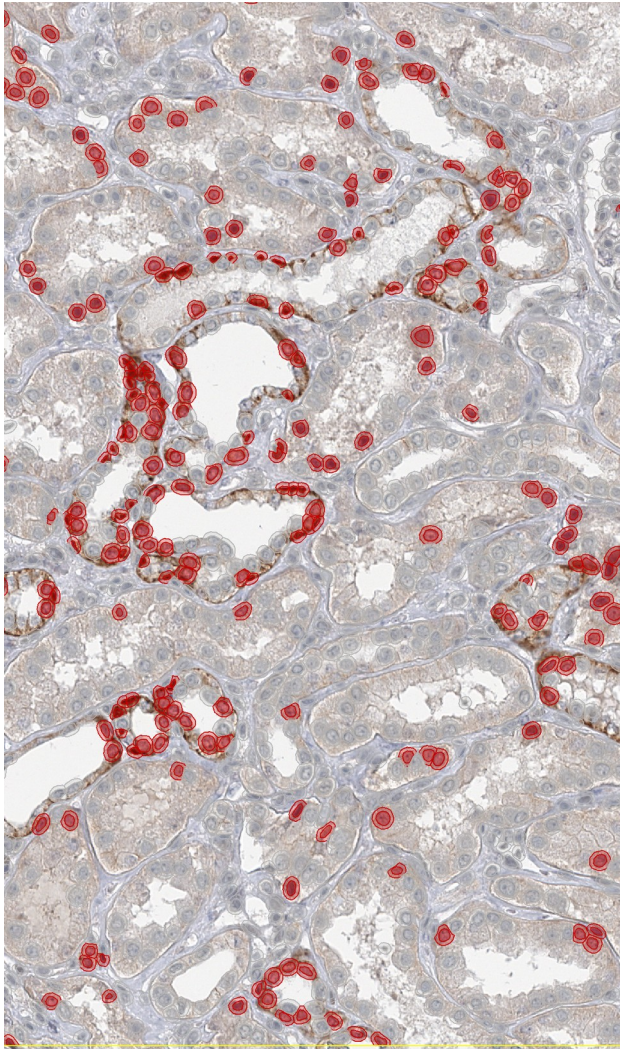
- Start from a set of detections (nuclei, cells, vesicles, …).



Introduction

Goals:

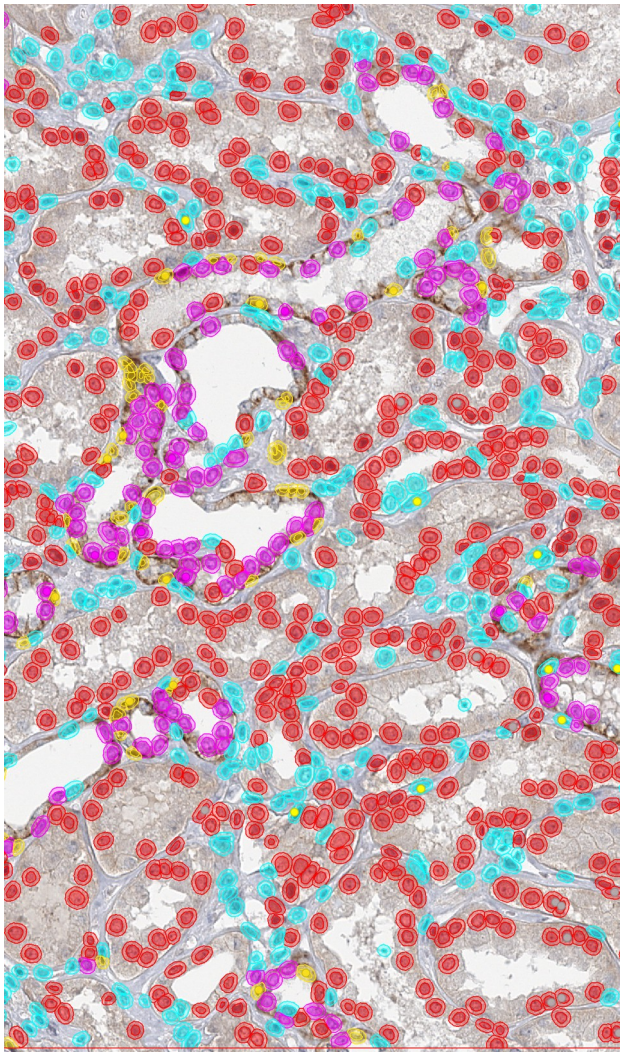
- Start from a set of detections (nuclei, cells, vesicles, …).
- Partition it in **two categories** (classes)



Introduction

Goals:

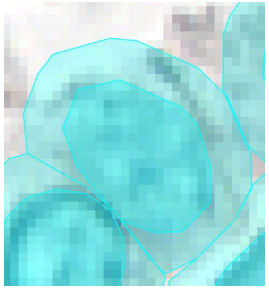
- Start from a set of detections (nuclei, cells, vesicles, …).
- Partition it in **two categories** (classes) or **more**.



Introduction

Goals:

- Start from a set of detections (nuclei, cells, vesicles, …).
- Partition it in **two categories** (classes) or **more**.
- Based on **individual** detection **measurements**:
  - Shape
  - Intensities
  - Spatial
  - User defined

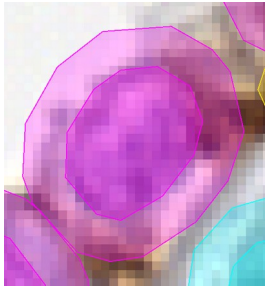


Nucleus: Area px^2	154.7193
Nucleus: Length px	45.1179
Nucleus: Circularity	0.9551
Nucleus: Solidity	1
Nucleus: Max diameter px	15.2497
Nucleus: Min diameter px	13.5935
Cell: Area px^2	388.5916
Cell: Length px	72.9091
Cell: Circularity	0.9186
Cell: Solidity	0.9915
Cell: Max diameter px	24.8691
Cell: Min diameter px	20.2542
Nucleus/Cell area ratio	0.3982
Nucleus: Hematoxylin: Mean	0.236
Nucleus: Hematoxylin: Median	0.217
Nucleus: Hematoxylin: Min	-0.0448
Nucleus: Hematoxylin: Max	0.5774
Nucleus: Hematoxylin: Std.Dev.	0.094
Nucleus: DAB: Mean	0.1228
Nucleus: DAB: Median	0.1117
Nucleus: DAB: Min	0.0362
Nucleus: DAB: Max	0.436
Nucleus: DAB: Std.Dev.	0.0575
Cytoplasm: Hematoxylin: Mean	0.1295
Cytoplasm: Hematoxylin: Median	0.1129
Cytoplasm: Hematoxylin: Min	-0.0448
Cytoplasm: Hematoxylin: Max	0.5892
Cytoplasm: Hematoxylin: Std.Dev.	0.1088
Cytoplasm: DAB: Mean	0.2062
Cytoplasm: DAB: Median	0.1579
Cytoplasm: DAB: Min	0
Cytoplasm: DAB: Max	0.7948
Cytoplasm: DAB: Std.Dev.	0.1489

Introduction

Goals:

- Start from a set of detections (nuclei, cells, vesicles, …).
- Partition it in **two categories** (classes) or **more**.
- Based on **individual** detection **measurements**:
  - Shape
  - Intensities
  - Spatial
  - User defined



Nucleus: Area px^2	221.1687
Nucleus: Length px	55.17
Nucleus: Circularity	0.9131
Nucleus: Solidity	0.9996
Nucleus: Max diameter px	20.8229
Nucleus: Min diameter px	14.103
Cell: Area px^2	477.2087
Cell: Length px	83.1192
Cell: Circularity	0.868
Cell: Solidity	0.9606
Cell: Max diameter px	29.4922
Cell: Min diameter px	20.747
Nucleus/Cell area ratio	0.4635
Nucleus: Hematoxylin: Mean	0.1328
Nucleus: Hematoxylin: Median	0.1288
Nucleus: Hematoxylin: Min	0.0281
Nucleus: Hematoxylin: Max	0.3196
Nucleus: Hematoxylin: Std.Dev.	0.051
Nucleus: DAB: Mean	0.0999
Nucleus: DAB: Median	0.0968
Nucleus: DAB: Min	0.0319
Nucleus: DAB: Max	0.2002
Nucleus: DAB: Std.Dev.	0.0285
Cytoplasm: Hematoxylin: Mean	0.0733
Cytoplasm: Hematoxylin: Median	0.0667
Cytoplasm: Hematoxylin: Min	-0.0164
Cytoplasm: Hematoxylin: Max	0.285
Cytoplasm: Hematoxylin: Std.Dev.	0.0548
Cytoplasm: DAB: Mean	0.0826
Cytoplasm: DAB: Median	0.0764
Cytoplasm: DAB: Min	-0.0031
Cytoplasm: DAB: Max	0.1997
Cytoplasm: DAB: Std.Dev.	0.0456

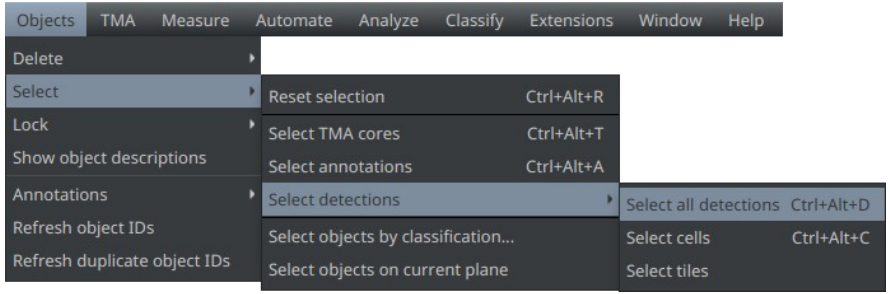
Measurements

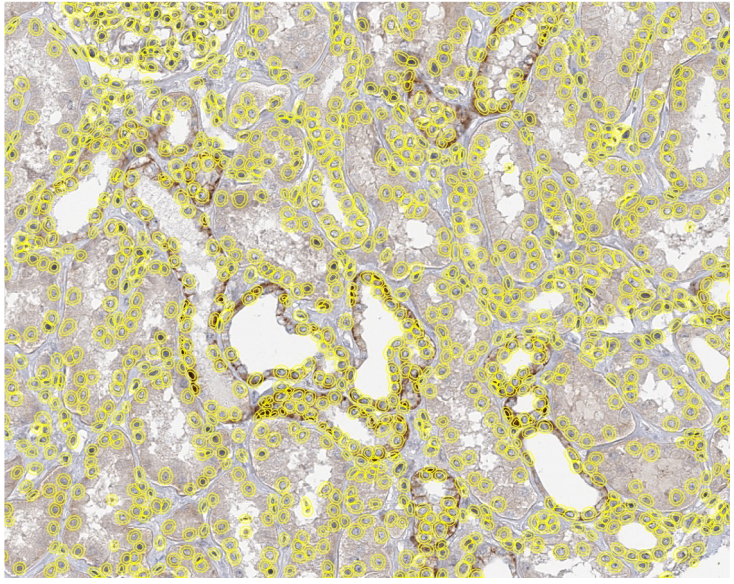
- All detections can have measurements.
  - Automatically processed when using StarDist, CellPose, …
  - What if they are missing?

Measurements

<u>In QuPath:</u> 1. Select detections

- Processed only on selected detections.

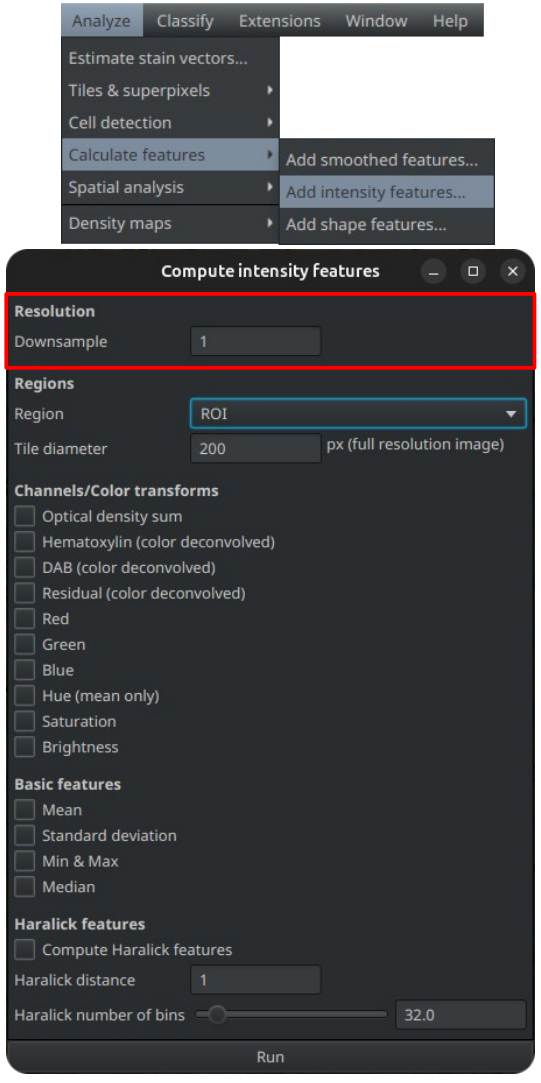




Measurements

In QuPath: 2.a. Intensity measurements

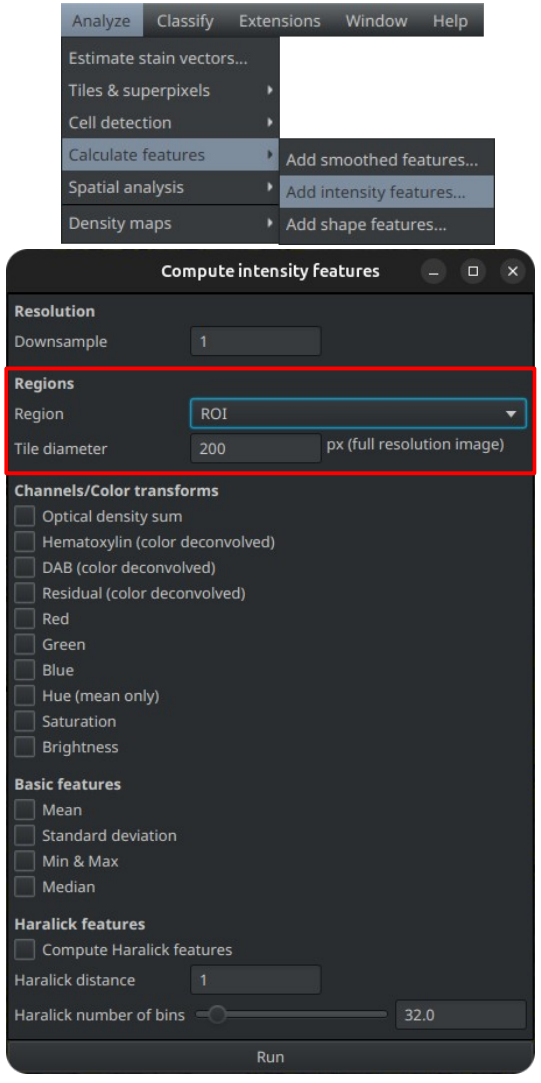
- Requested resolution for measurements.



Measurements

In QuPath: 2.a. Intensity measurements

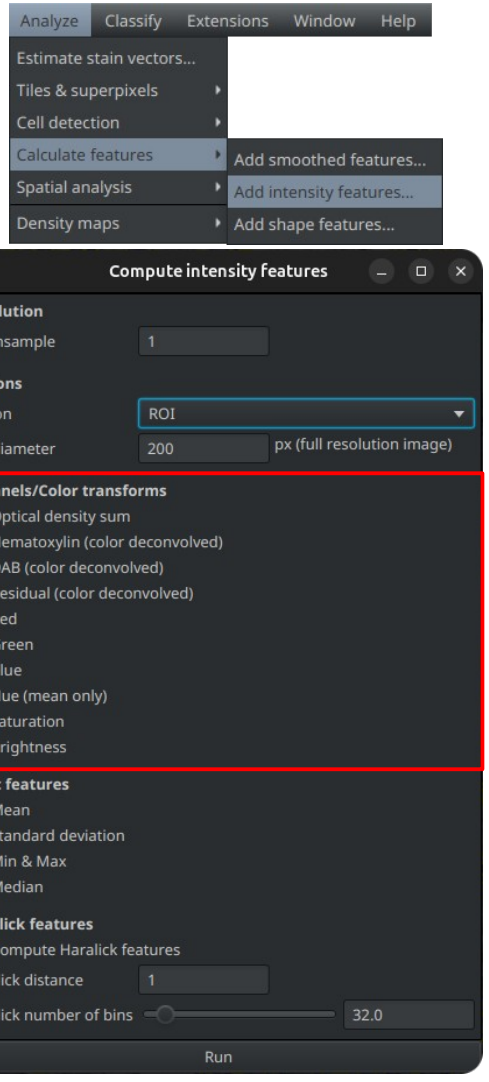
- Where to take values?
  - **ROI**: in the detection's polygon
  - **Square tiles**: Pixels in a square centered on detection
  - **Circular tiles**: Pixels in a circle centered on detection
  - **Cell nucleus**: If cell: only pixels in the nucleus
- Tile diameter: only if square or circular tiles.



Measurements

In QuPath: 2.a. Intensity measurements

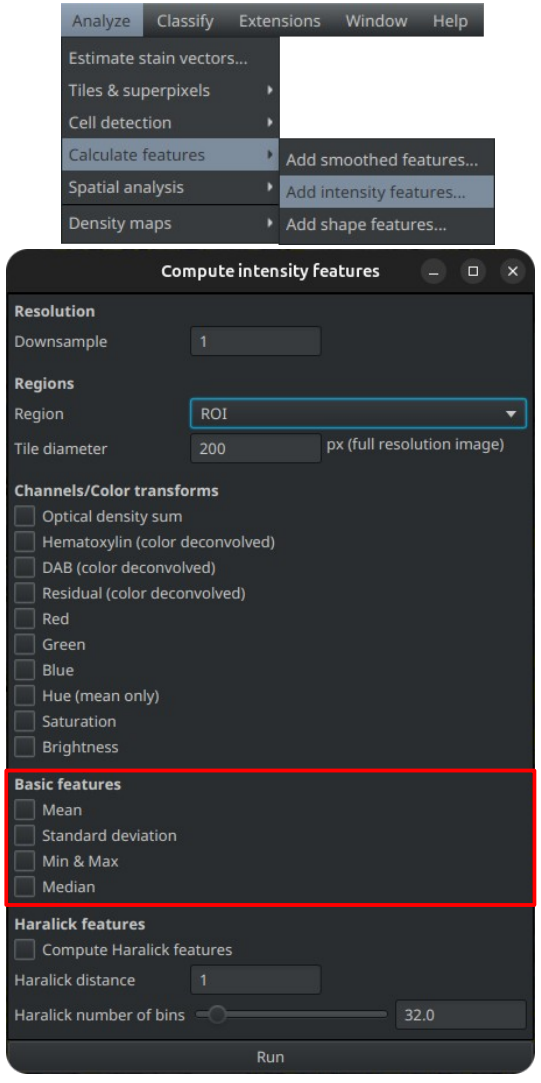
- In which channel(s) make the measurements?
  - **IHC**: RGB or deconvolued channels or HSL
  - **Fluo**: individual channels



Measurements

In QuPath: 2.a. Intensity measurements

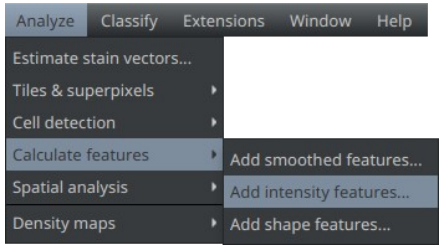
- What to measure
  - in **each** channel
  - for **each** compartment (nucleus, cell, cyto, ...)

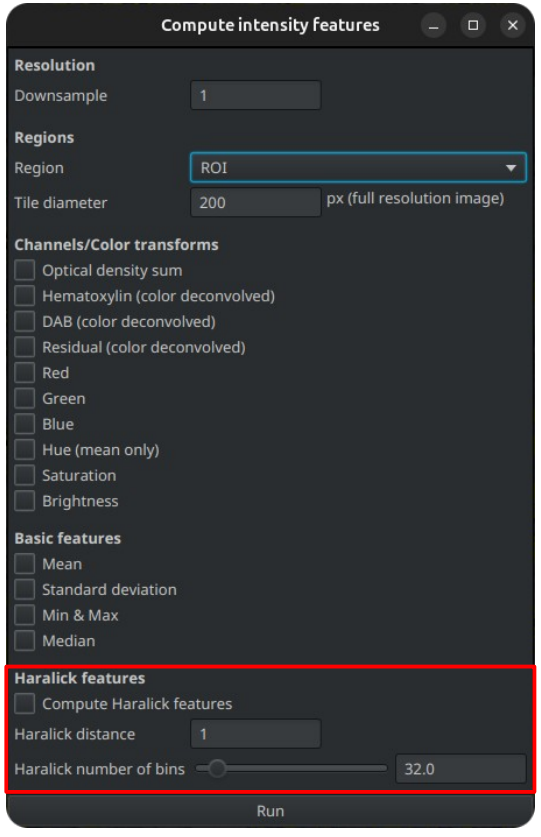


Measurements

In QuPath: 2.a. Intensity measurements

- Haralick features: texture information
  - Local contrast
  - Repeating patterns
  - Direction
  - Granularity
  - ...

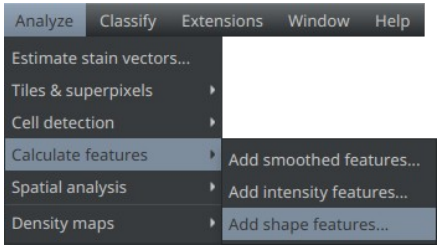


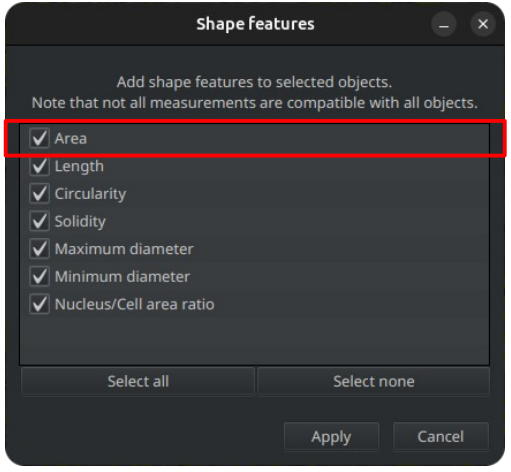


Measurements

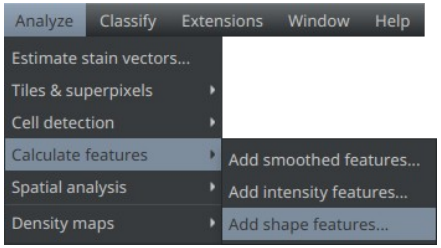
In QuPath: 2.b. Shape measurements

- Area of the detections



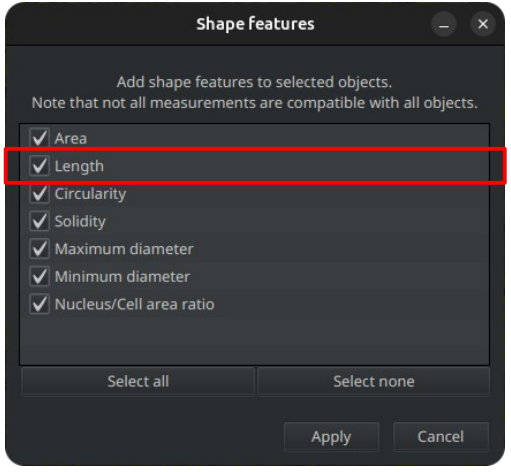


Measurements

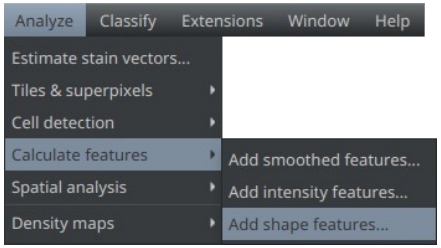


<u>In QuPath:</u> 2.b. Shape measurements

- For polygons: perimeter
- For lines: length

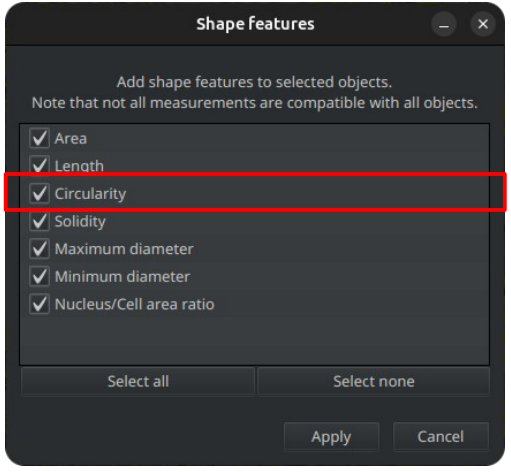


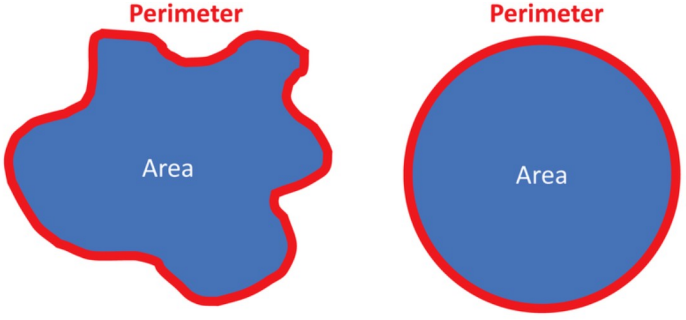
Measurements



In QuPath: 2.b. Shape measurements

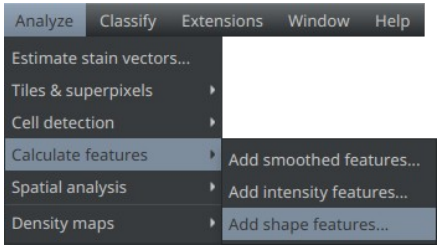
- How far is the object to be a perfect circle?





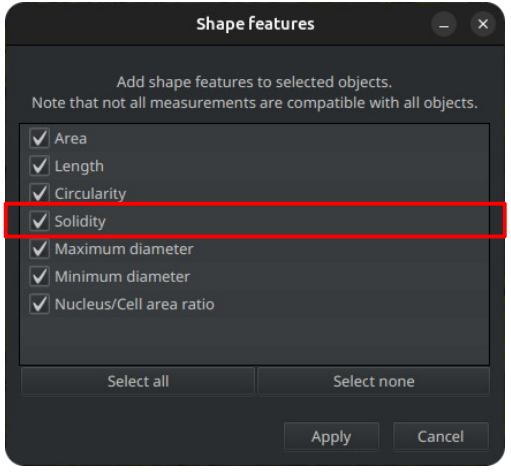
Circularity = 4π*area / perimeter²
Perfect Circle = Circularity of 1

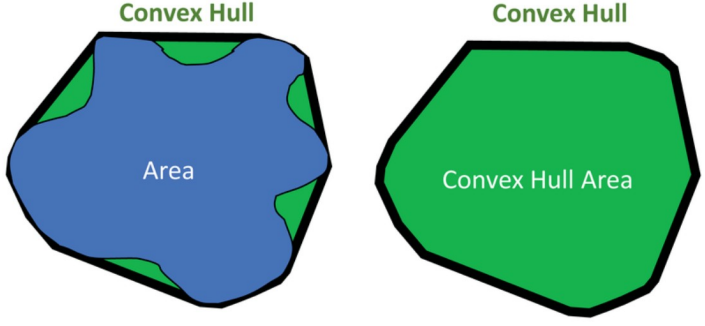
Measurements



In QuPath: 2.b. Shape measurements

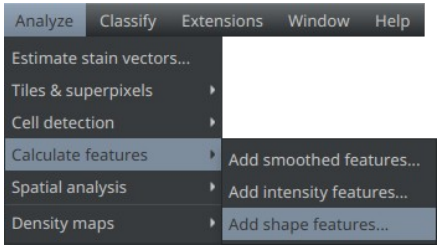
- How far is the object close to be convex?
- Convex hull:
  - Like the object is solid
  - You release an elastic band around it





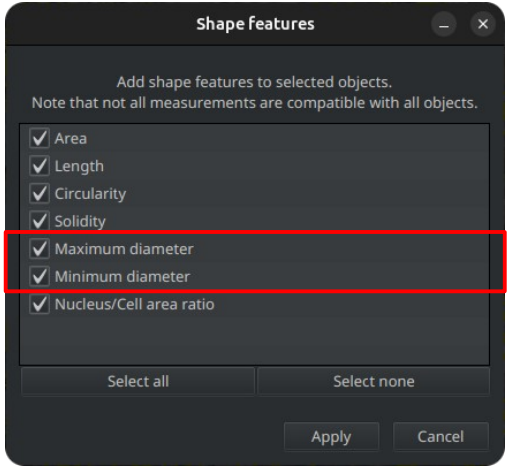
Solidity = Area / Convex Hull Area
Perfect Convex Object = Solidity of 1

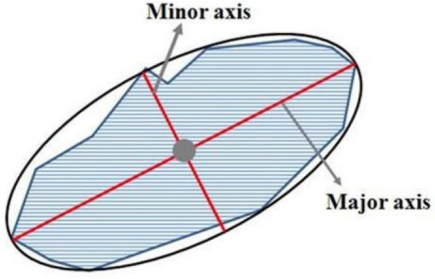
Measurements



<u>In QuPath:</u> 2.b. Shape measurements

- Min & max axes length of a **fitted ellipse**.

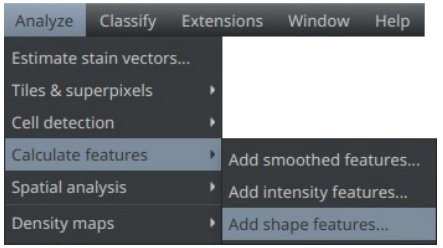


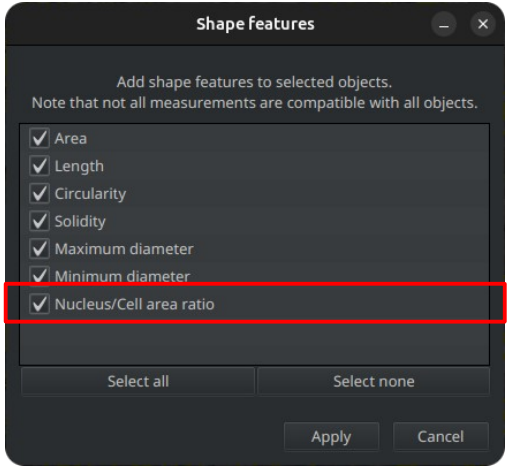


Measurements

In QuPath: 2.b. Shape measurements

- If cell: nucleus/cell ratio

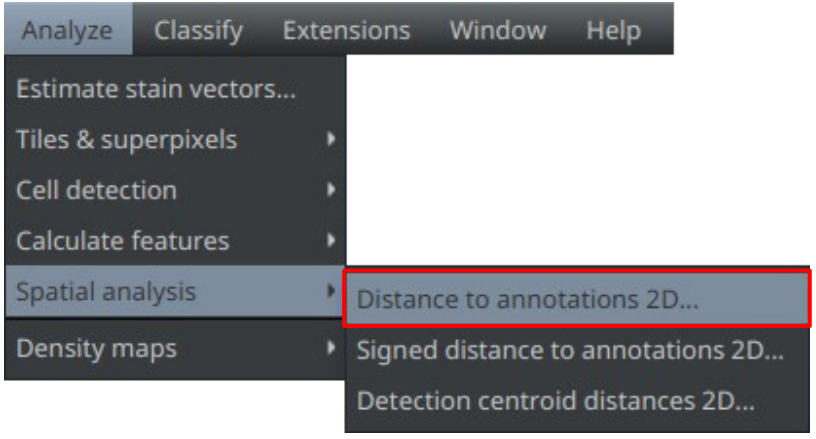




Measurements

In QuPath: 2.c. Spatial measurements

- Shortest distance:
  - **From**: detection centroid
  - **To**: the annotation
- Annotation?
  - Every annotation having a class



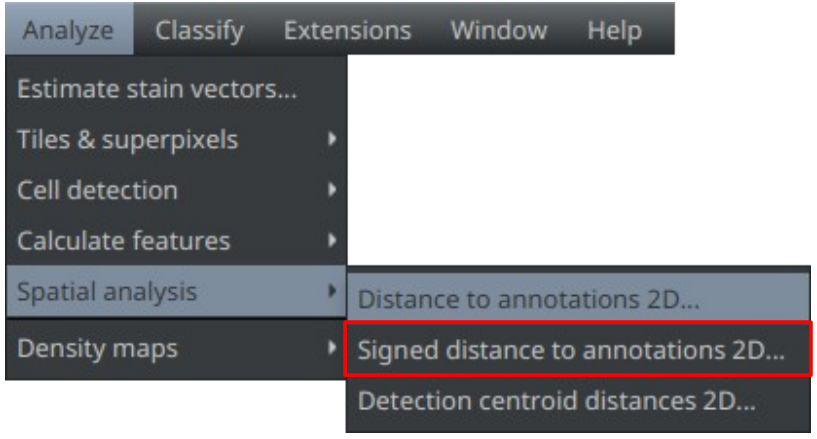
Ex:

- Shortest distance to get to any Tumor annotation
- Independently of how many Tumor annotation there are.

Measurements

In QuPath: 2.c. Spatial measurements

- Same as “Distance to annotation 2D…”
- **BUT**
- Signed:
  - Negative: The detection is inside the annotation
  - Positive: The detection is outside the annotation



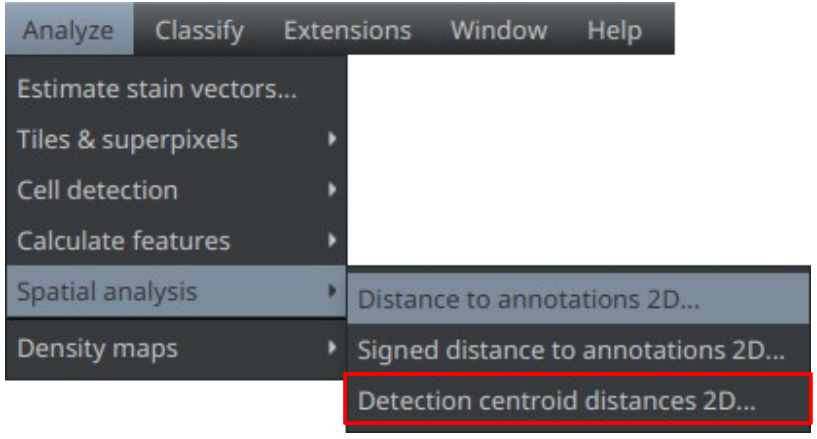
Measurements

<u>In QuPath:</u> 2.c. Spatial measurements

- !!! Only if detections are already classified !!!
- Shortest distance to reach a classified detection

Ex:

- Shortest distance to get a DAB+
- Shortest distance to get to a Size+



Measurements

In QuPath: Visualize measurements

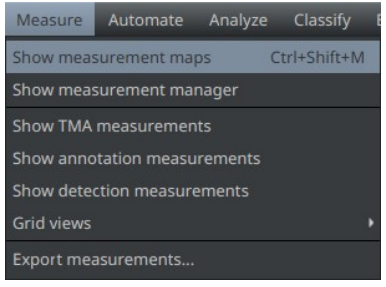
- Click on every individual object !?

Cell: DAB: Mean	0.1174
Cell: DAB: Mean	0.2024
Cell: DAB: Mean	0.1895
Cell: DAB: Mean	0.5263

Measurements

In QuPath: Visualize measurements

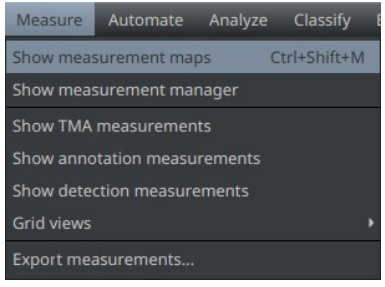
- Click on every individual object !?
- Activate the filling of detections 
- Activate the “measurement maps” tool

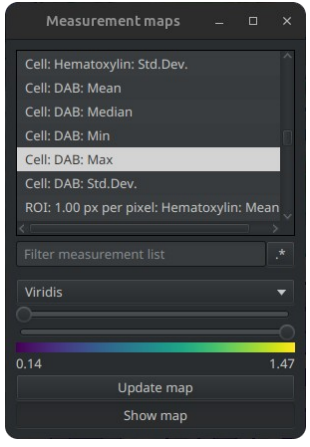


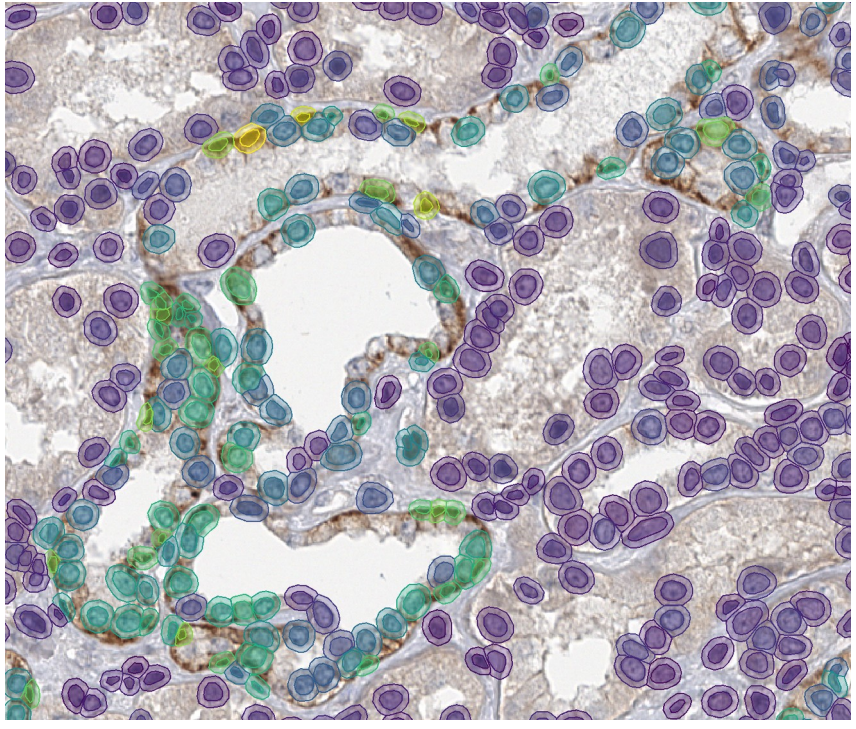
Measurements

In QuPath: Visualize measurements

- Click on every individual object !?
- Activate the filling of detections 
- Activate the “measurement maps” tool
- Choose which measurement to visualize and a LUT







Threshold-based classification

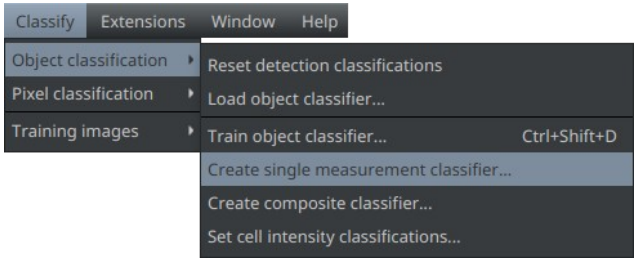
Principle:

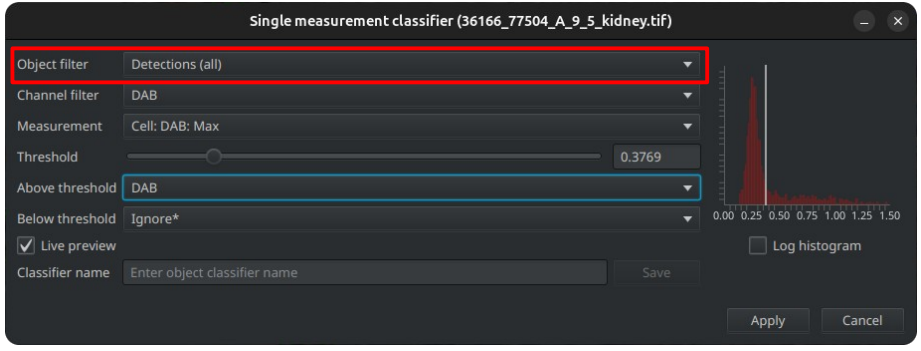
- Pick a measurement in your list (ex: “Cell: DAB mean”)
- Provide a threshold value:
  - Cells above: **DAB+**
  - Cells below: **Ignore***

Threshold-based classification

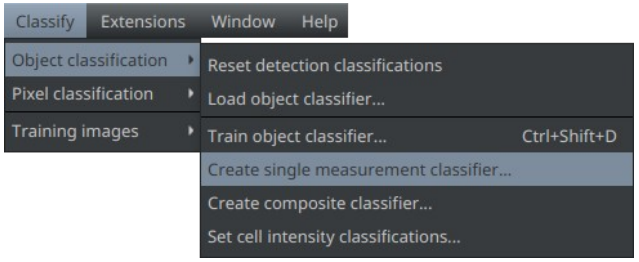
In QuPath:

- Which objects should be classified



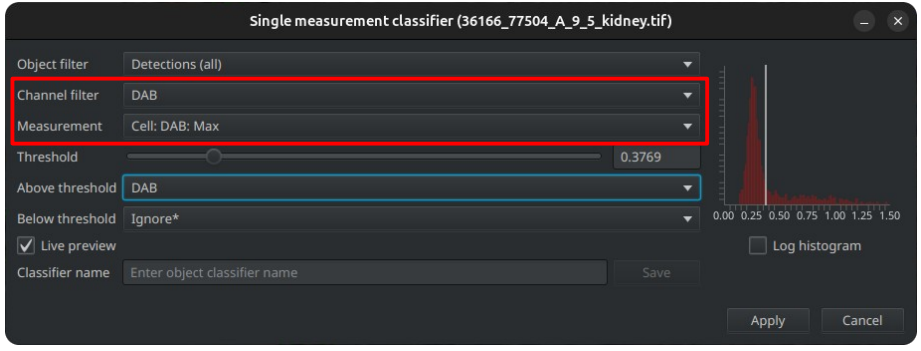


Threshold-based classification



In QuPath:

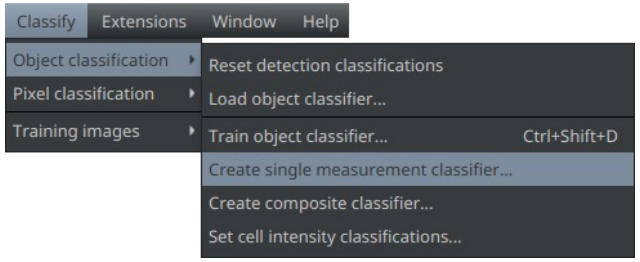
- Convenience filter for the measurements list below.
- On which measurement base the classification?

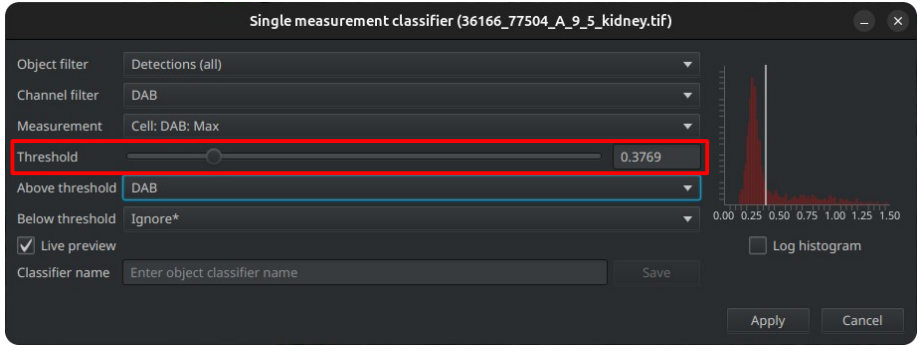


Threshold-based classification

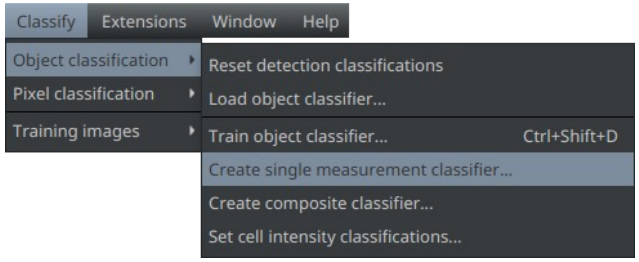
In QuPath:

- Threshold for the measurement.



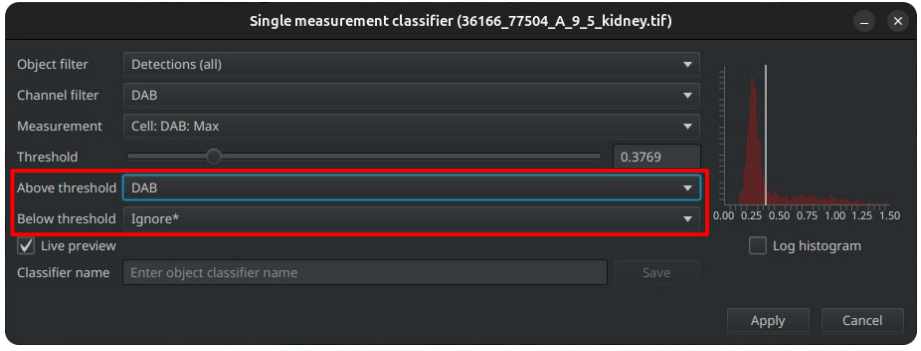


Threshold-based classification

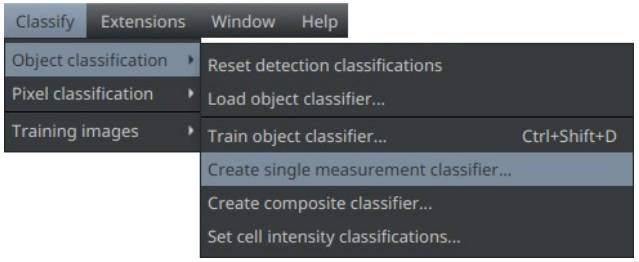


In QuPath:

- Class to give to the objects

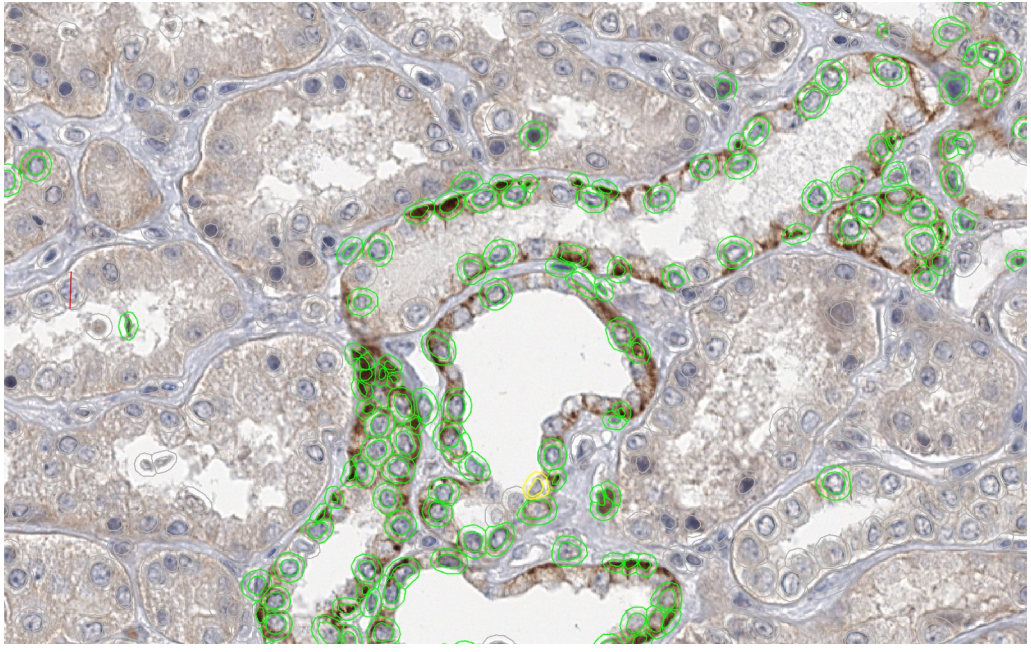


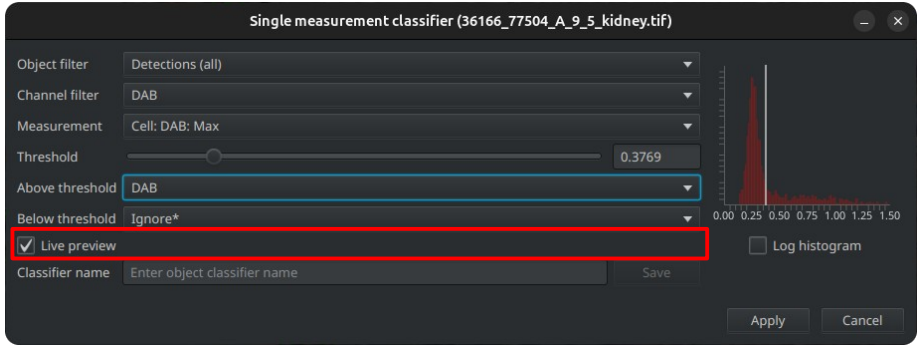
Threshold-based classification



In QuPath:

- See in real time the threshold effect.





→ Exercise 6.1: Using a threshold object classifier

Threshold-based classification

Problems:

- Same as threshold with pixel classifier:
  - Intensities may shift (and so positivity).
  - We are limited to 2 classes.

Random-trees-based classification

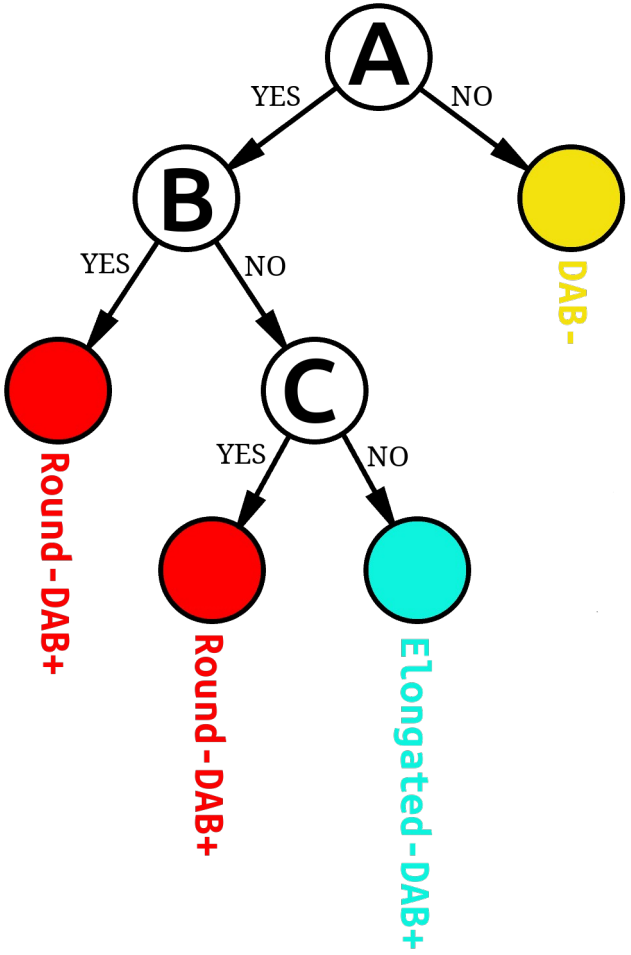
Principle:

- Features → already processed per object.
- Example of decision tree:

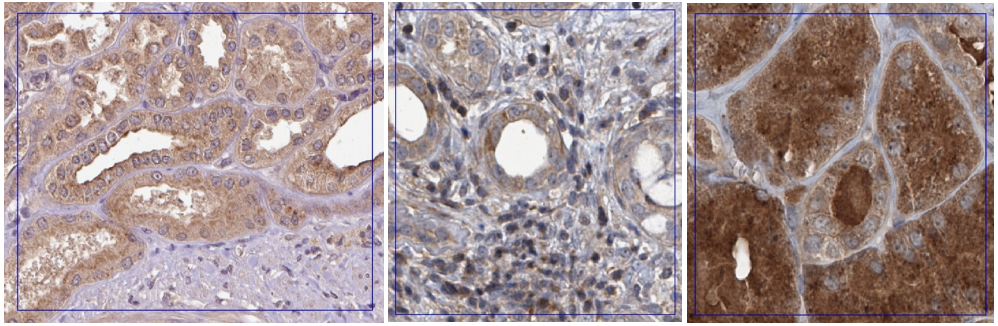
  A) Is “Mean DAB” > 0.55?

  B) Is “Cell circularity” > 0.7?

  C) Is “Max diameter” > 15?

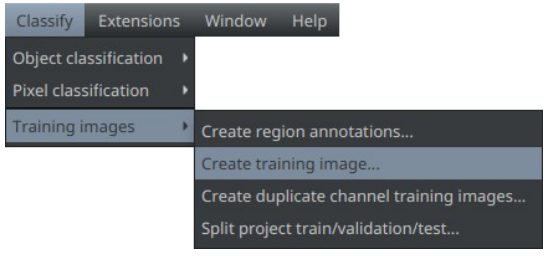


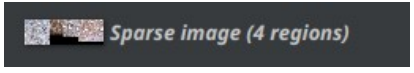
Random-trees-based classification



In QuPath: 1. Prepare a training image

- Aggregate a representative set of patches.
- Same instructions as for the pixel classifier.

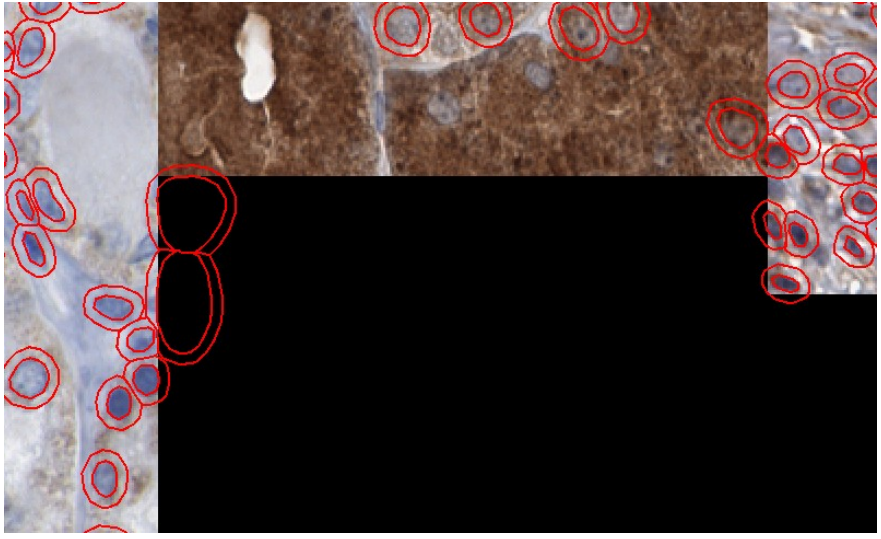




Random-trees-based classification

In QuPath: 2. Find objects

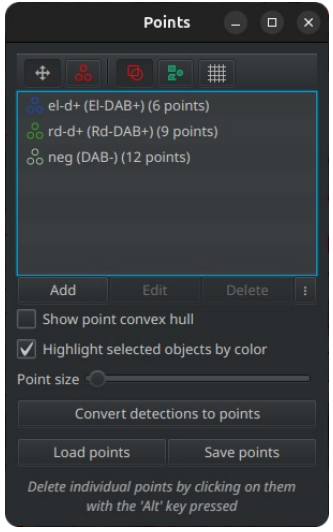
- IHC → don’t forget color deconvolution.
- Use same method as for other images.
- Don’t use:
  - Hallucinations
  - Cells overlapping with background



Random-trees-based classification

In QuPath: 3. Place example points

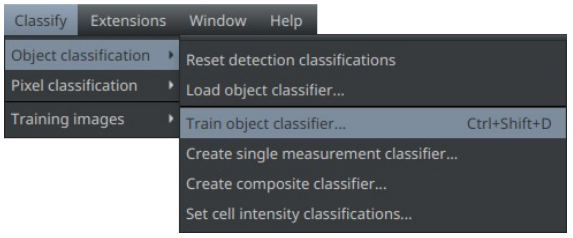
- Points designate objects, not pixels → location doesn't matter.
- Ex:
  - DAB-
  - Elongated DAB+
  - Round DAB+

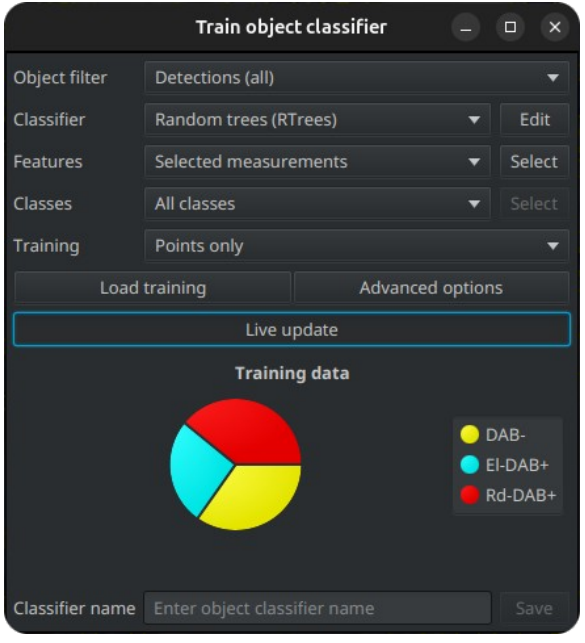


Random-trees-based classification

In QuPath: 4. Adjust settings

- Open the training settings.

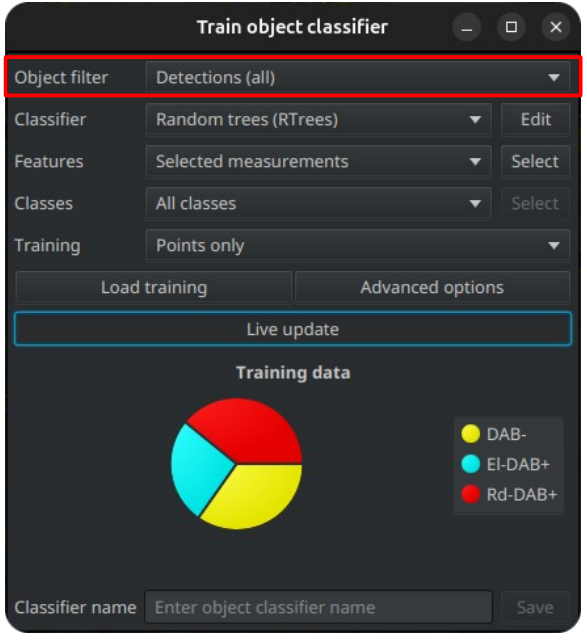




Random-trees-based classification

In QuPath: 4. Adjust settings

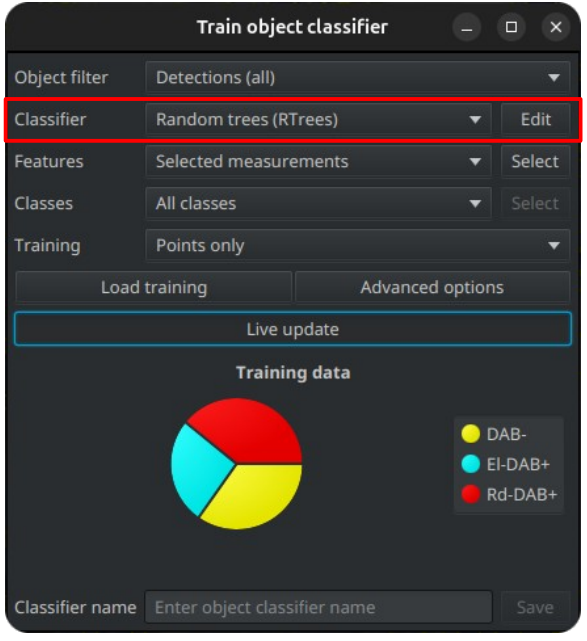
- Which objects should be classified?



Random-trees-based classification

In QuPath: 4. Adjust settings

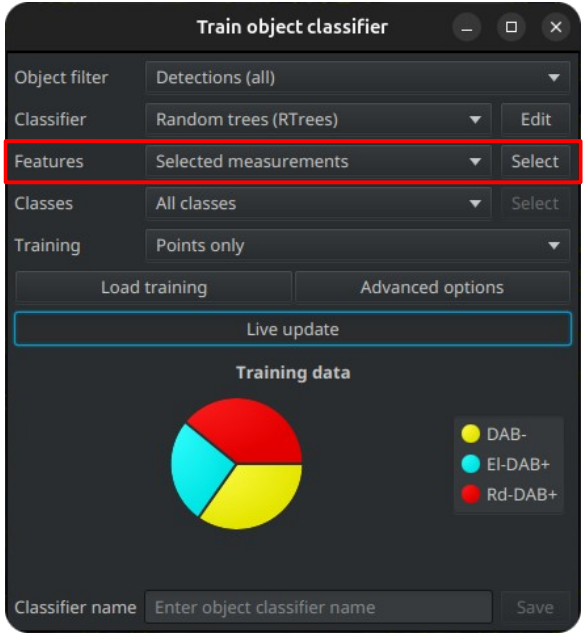
- Algorithm to use.
- Keep the random trees.



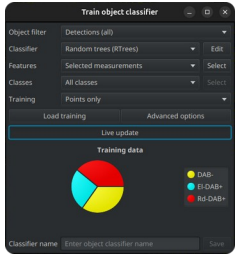
Random-trees-based classification

In QuPath: 4. Adjust settings

- Switch to “Selected measurements”

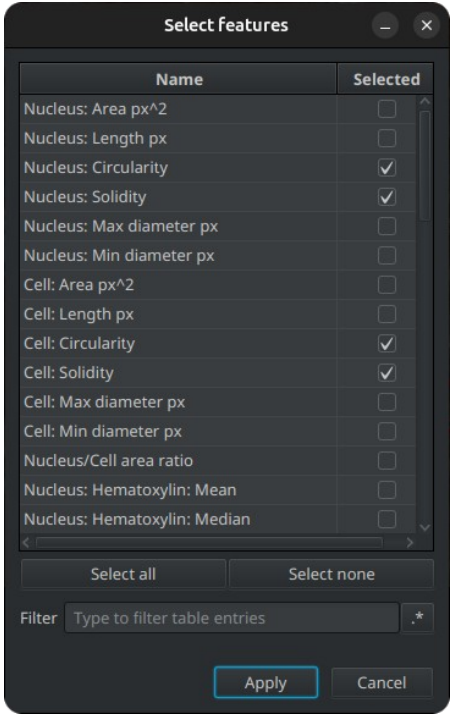


Random-trees-based classification



In QuPath: 4. Adjust settings

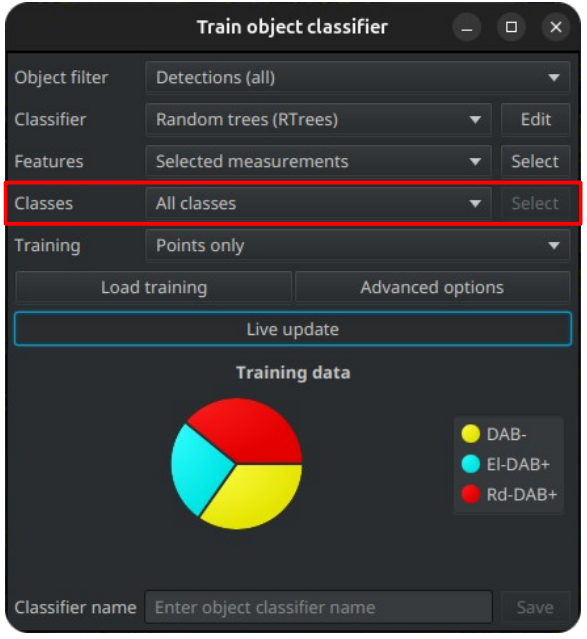
- Switch to “Selected measurements”
- Select the required items in the list
- Like for the pixel classifier, all items are not useful.



Random-trees-based classification

In QuPath: 4. Adjust settings

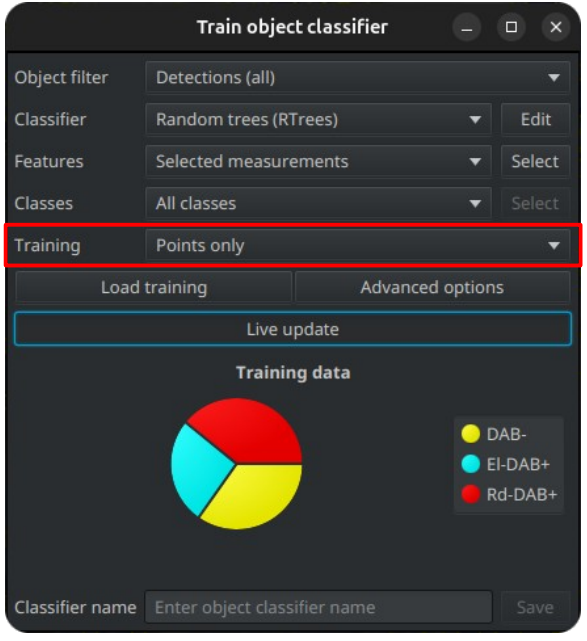
- Which classes to use
- Possible to ignore annotations



Random-trees-based classification

In QuPath: 4. Adjust settings

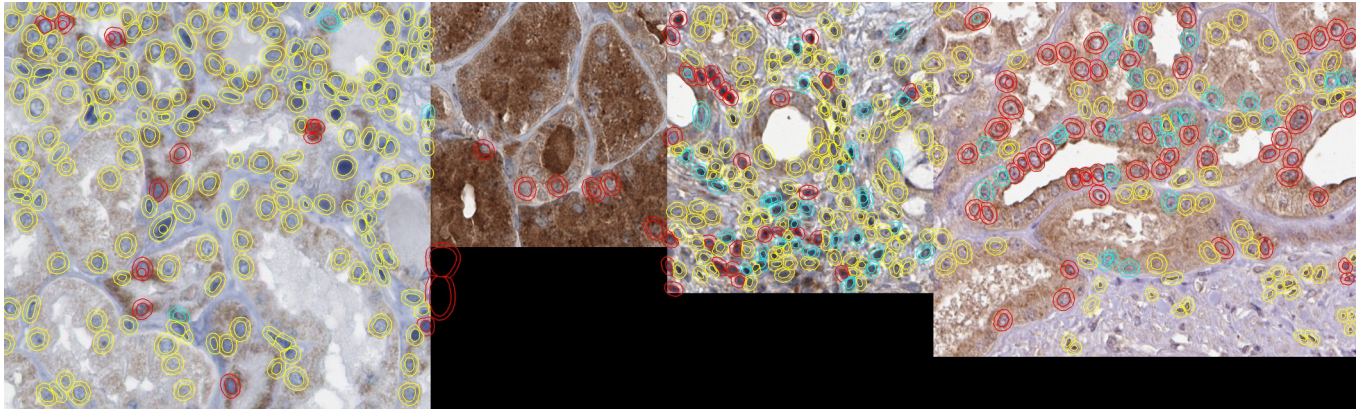
- Which annotations designate examples.
- Use only points.

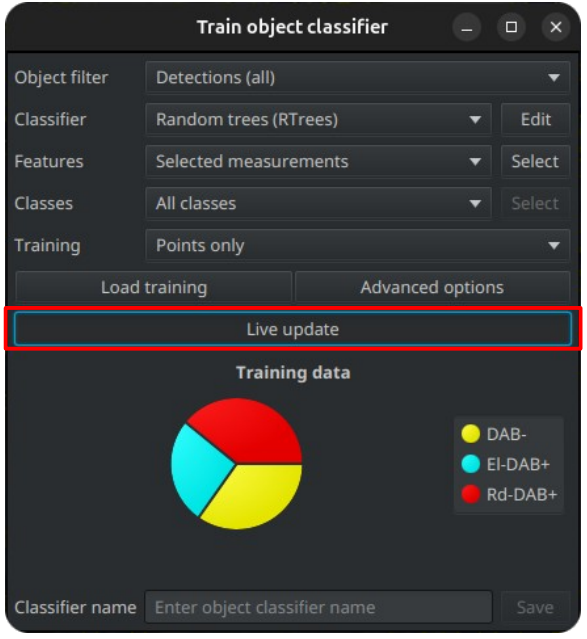


Random-trees-based classification

In QuPath: 4. Adjust settings

- Activate the live update
- Add points where the classifier makes mistakes





→ Exercise 6.2: Using a N-classes object classifier