A microscopic image of a tissue section, likely stained with hematoxylin and eosin (H&E). The image shows a dense population of cells. The cell nuclei are stained blue, and the cytoplasm and extracellular matrix are stained pink. The image has been processed with a segmentation algorithm. The cell boundaries are outlined in red. Some cells are further outlined in purple, indicating a specific classification. The text "Cell classification" is overlaid in white in the center of the image.

Cell classification

What is cell classification?

explain in one sentence to a toddler what is cell classification in the context of image analysis

< 5/5 >



Cell classification is like sorting different kinds of toys into separate boxes based on how they look.

Thank you, I'm sure students will love being compared to toddlers

< 3/3 >



It's all about finding the right analogy for understanding! If it helps simplify a complex concept, then it's worth it.



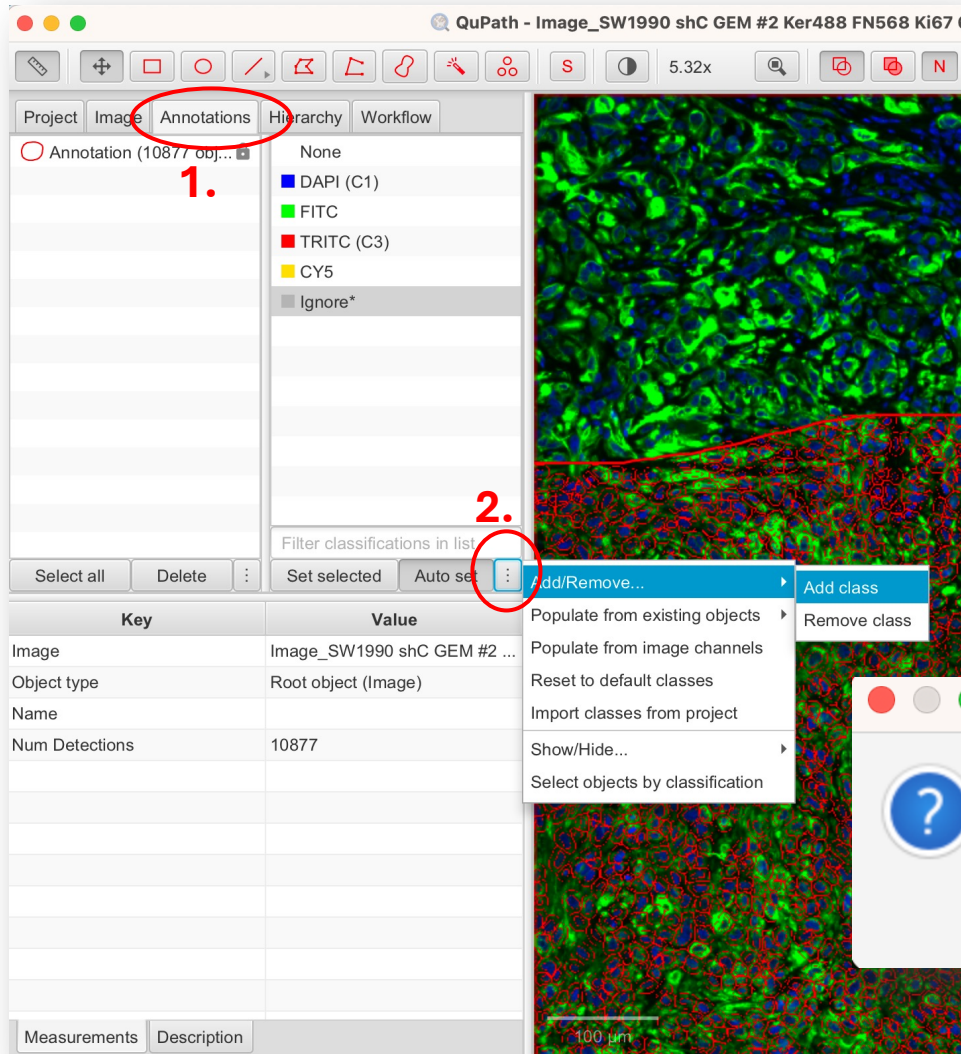
What is cell classification?


- Cell classification in image analysis is sorting different types of cells into groups based on their features or characteristics, such as shape, color, position, neighbors, etc.
- If visually you can't discriminate cells in your images, then your algorithm might be separating cell on very fine differences (careful of *overfitting*)

Cell classification in QuPath

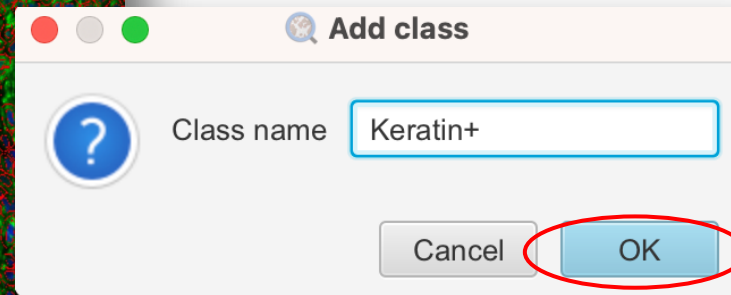
- Single measurement classifier
- Composite thresholder: combine single measurement thresholds together
- Train a machine learning classifier

Create a class named 'Keratin+'



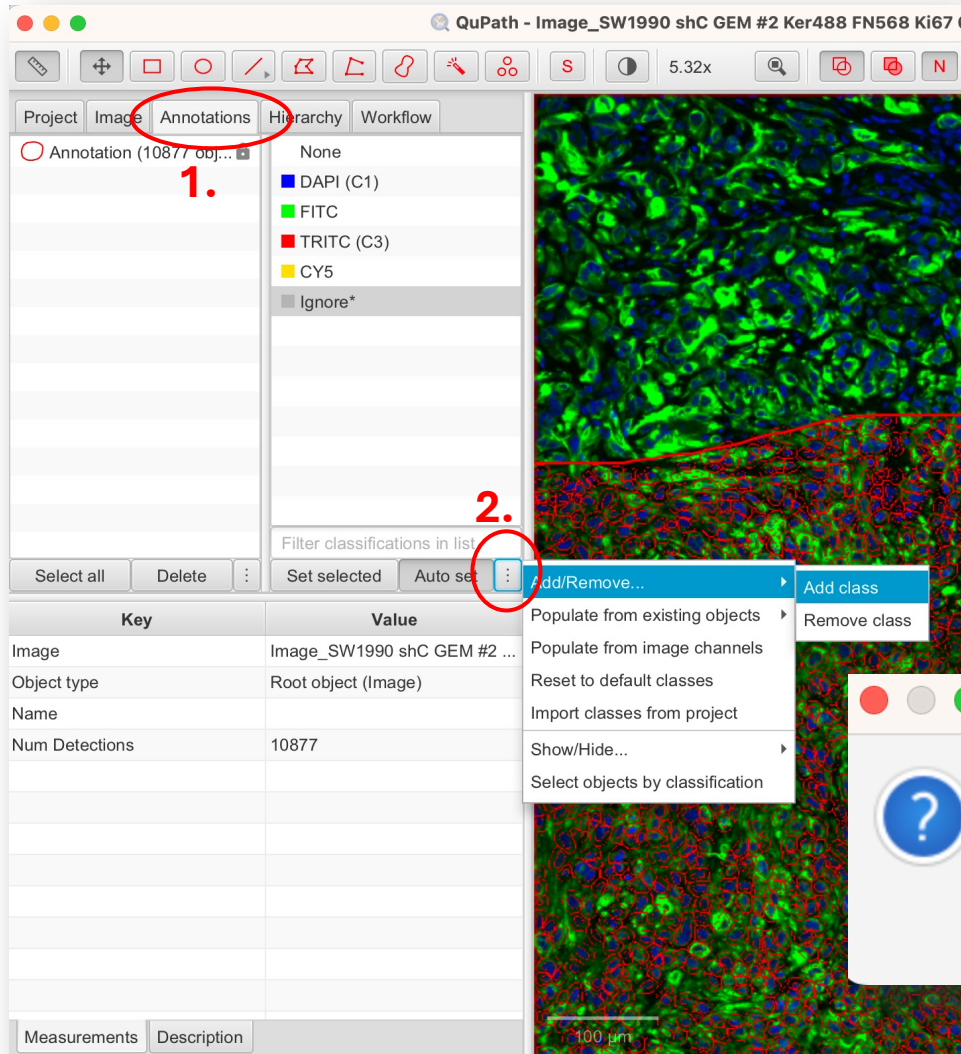
- *Annotations* tab > Classification list >  > Add/Remove... > Add class


**Default classes are channel names.
We'll change that.**



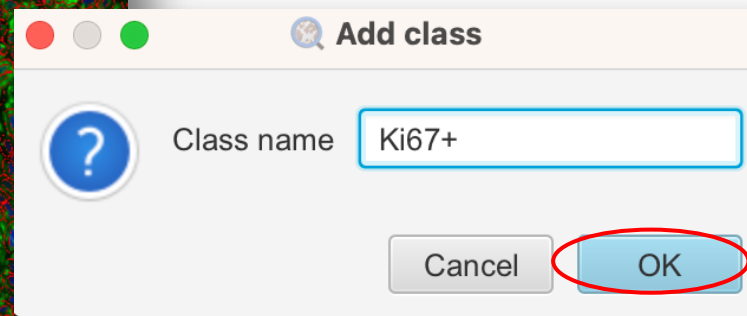
3.

Create a second class named 'Ki67+'



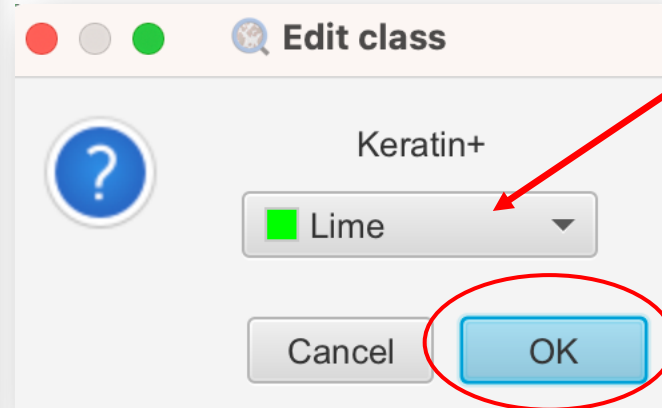
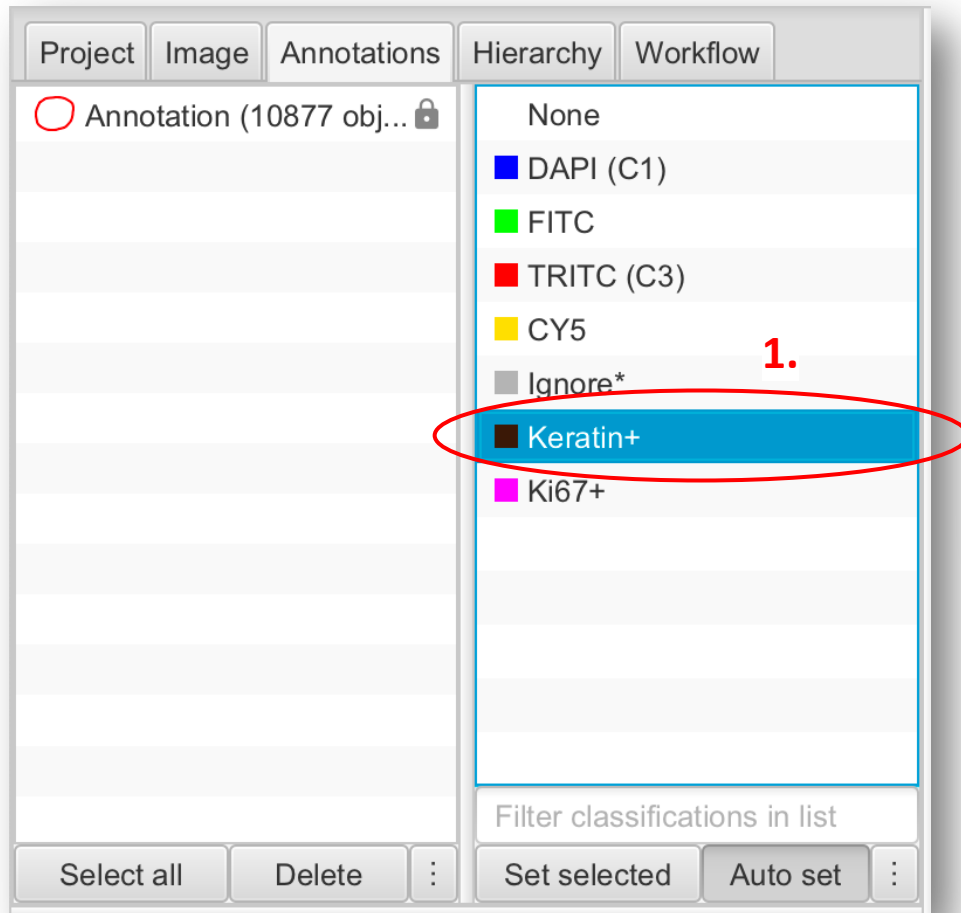
- Annotations tab > Classification list >  > Add/Remove... > Add class

Default classes are channel names.
We'll change that.



Change the color of a class

- Double click on the class > Edit class > Choose a new color > OK

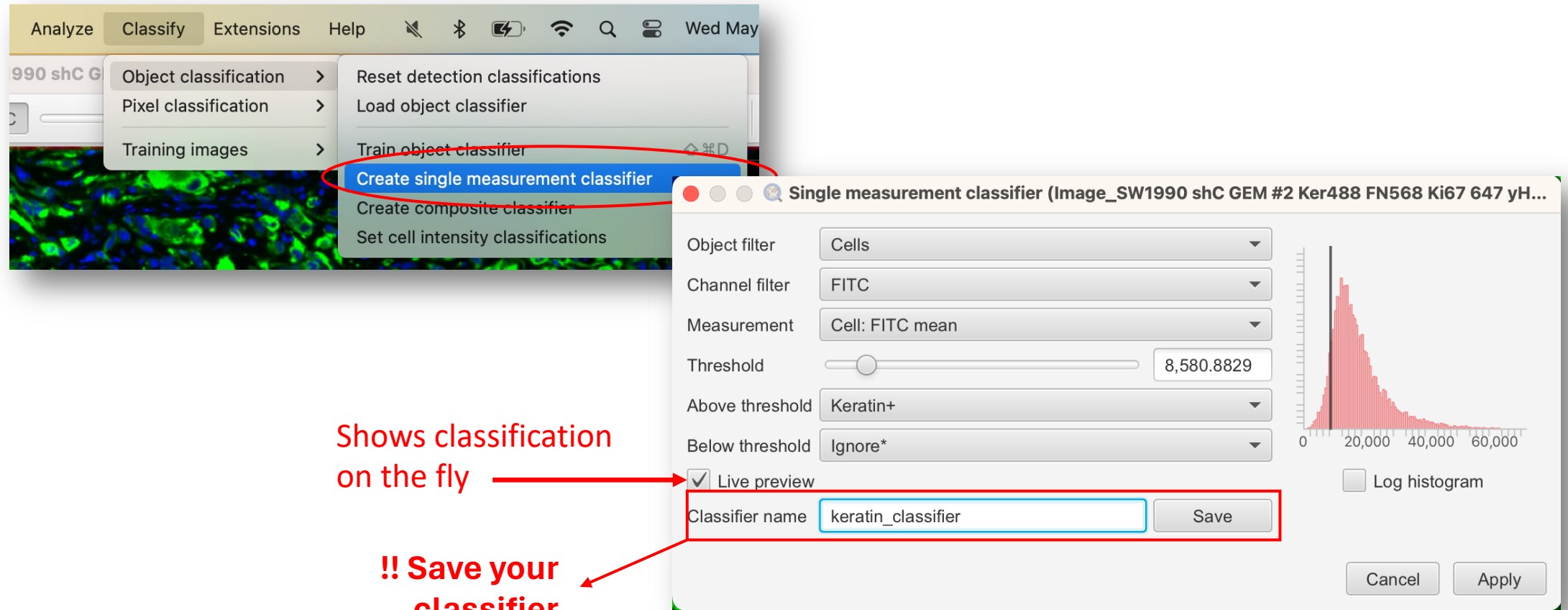


Your favorite color.

By default, classes are populated by image channels.

Simple measurement classifier on Keratin signal intensity (FITC channel)

- *Classify > Object classification > Create single measurement classifier*



The screenshot shows the Fiji software interface. The 'Classify' menu is open, and the 'Create single measurement classifier' option is highlighted. The dialog box for 'Single measurement classifier' is open, showing the following settings:

- Object filter: Cells
- Channel filter: FITC
- Measurement: Cell: FITC mean
- Threshold: 8,580.8829
- Above threshold: Keratin+
- Below threshold: Ignore*
- Live preview
- Classifier name: keratin_classifier
- Buttons: Save, Cancel, Apply

A histogram on the right side of the dialog box shows the distribution of FITC mean values for the selected objects. The x-axis ranges from 0 to 60,000, and the y-axis represents frequency. The distribution is unimodal and slightly right-skewed, peaking around 10,000.

Red arrows point from the text annotations to the 'Live preview' checkbox and the 'Classifier name' field.

Shows classification on the fly

!! Save your classifier

Simple measurement classifier on Ki67 signal intensity (CY5 channel)

- *Classify > Object classification > Create single measurement classifier*

The image shows a software interface with a 'Classify' menu open. The menu items are: Object classification, Pixel classification, Training images, Reset detection classifications, Load object classifier, Train object classifier, **Create single measurement classifier** (highlighted with a red circle), Create composite classifier, and Set cell intensity classifications. Below the menu is a preview of a cell image with blue and yellow regions.

The 'Single measurement classifier' dialog box is open, showing the following settings:

- Object filter: Cells
- Channel filter: CY5
- Measurement: Nucleus: CY5 mean
- Threshold: 1500
- Above threshold: Ki67+
- Below threshold: Ignore*
- Live preview
- Classifier name: CY5

On the right side of the dialog, there is a histogram showing the distribution of signal intensity. The x-axis ranges from 0 to 30,000. A checkbox for 'Log histogram' is present and unchecked. At the bottom right, there are 'Save', 'Cancel', and 'Apply' buttons.

Exercise 4.a: single-measurement classifier

Combine single measurement classifiers into a composite classifier

- *Classify > Object classification > Create composite classifier*

Analyze Classify Extensions Help Wed May

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Object classification > Reset detection classifications
Pixel classification > Load object classifier
Training images > Train object classifier
Create single measurement classifier
Create composite classifier
Set cell intensity classifications

Create composite classifier

Move individual classifiers to the column on the right to be included in the composite classifier. Note that the order of classifiers in the list determines the order in which they will be applied.

Available		Selected
	>	ki67_classifier
	>>	keratin_classifier
	<	
	<<	

Classifier name Save

Cancel **Save & apply**

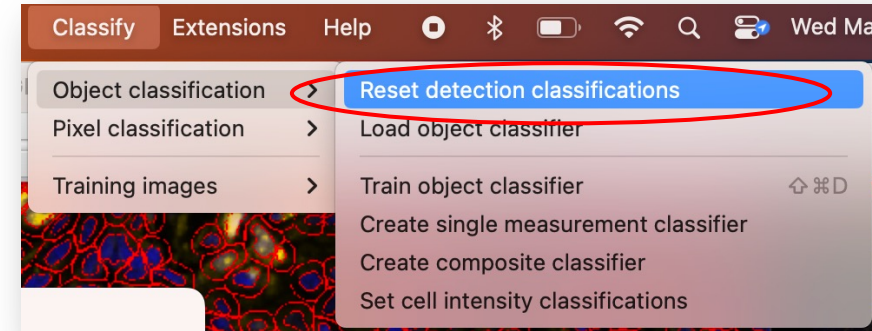
Select a classifier by moving it onto the 'Selected' list.

A name is required to 'Save & apply'

Exercise 4.b: composite classifier

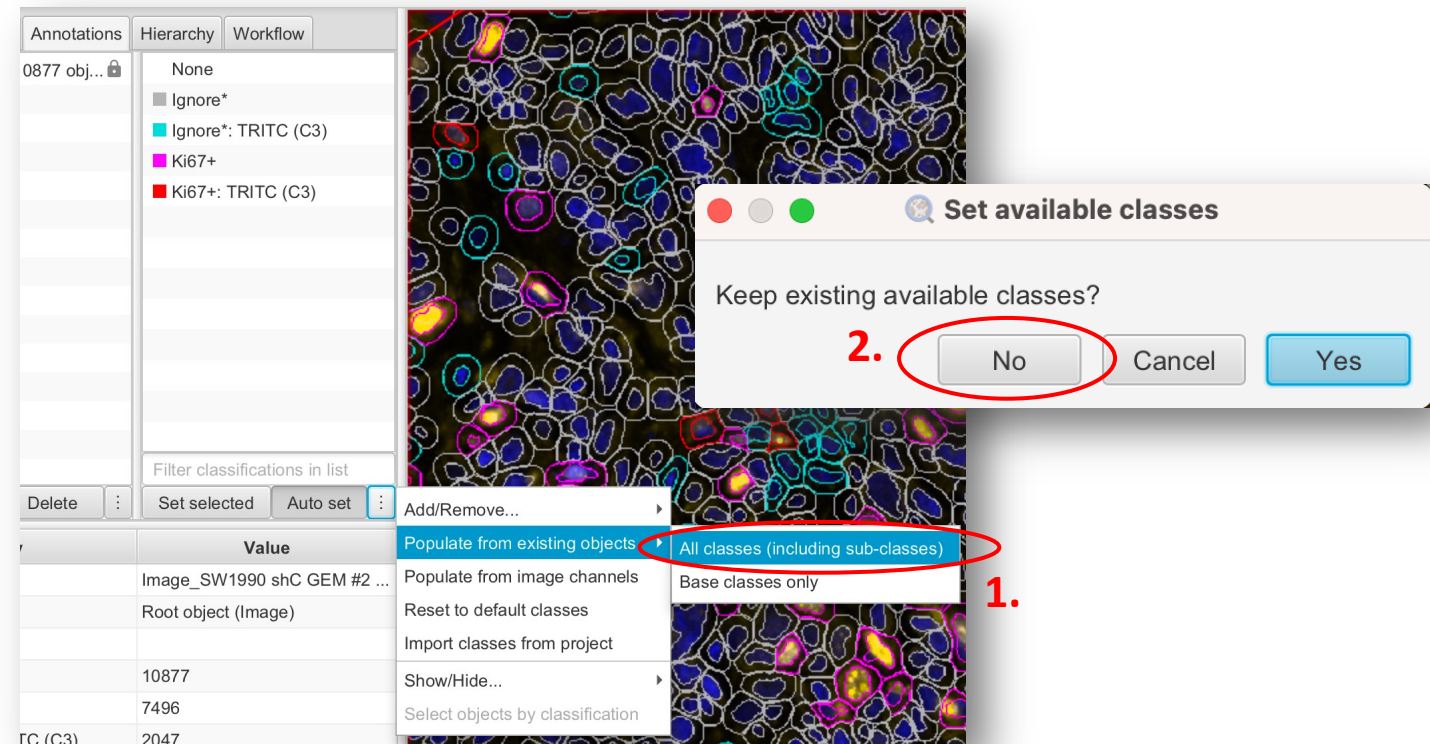
Reset detection classes

- *Classify > Object classification > Reset detection classifications*



Populate classes in the classification panel

- *Annotations tab > Classification list > [Menu Icon] > Populate from existing objects > All classes (including sub-classes)*



Object classification using machine learning

- Detections (and annotations) can be classified into classes using a ML classifier
- Classification requires measurements!
- Object classifiers are trained using manual annotations of 2 or more classes
 - Need to create some training data points
- **Live demo of object classification using ML**

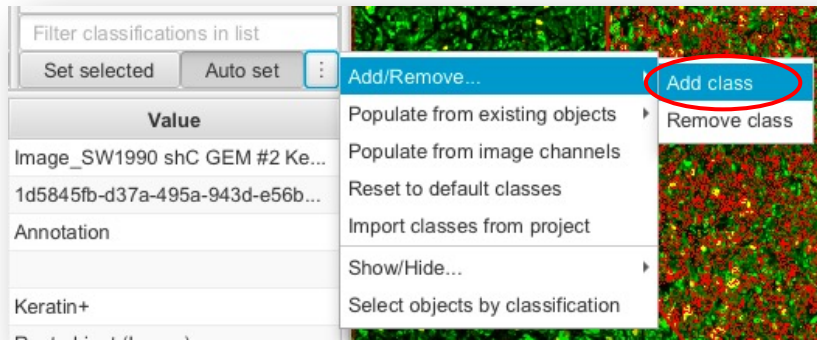
Reset your detection classes!



Adobe Stock | #32445303

Train an object classifier: create classes

- *Annotations* tab > Classification list >  > Add/Remove... > Add class



- Create 4 classes:

- Keratin+
- Keratin-
- Ki67+
- Ki67-

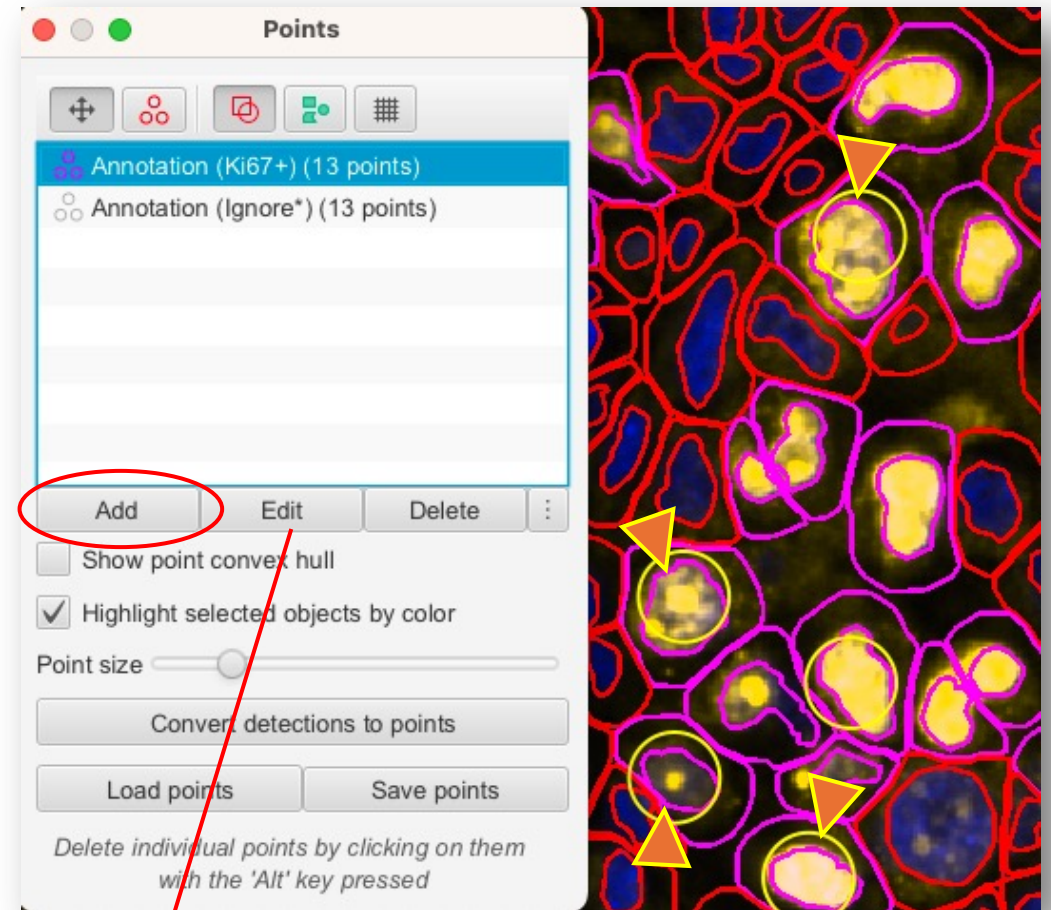


Train an object classifier: training data points

-  > Add > Label ~10 for each class

To remove a single point:
Option + click (Mac) or left-click

- Assign each training data set a class:
 - Select the annotation set
 - Select the class



Click edit to change color

 Training data

Train an object classifier: training data points

- Assign each training data set a class in the *Annotations* tab

1. Annotation (13 points)

Annotation (13243 objects) Lock

Annotation (Keratin+) (13 p...

Annotation (Ki67+) (13 points)

None

Ki67+ (1)

Ignore*

Keratin+ (1)

Ki67-

keratin-

2.

3.

Set selected

Auto set

Annotation (13243 objects) Lock

Annotation (Keratin+) (13 p...

Annotation (Ki67+) (13 points)

Annotation (Ki67-) (13 points)

Annotation (keratin-) (13 poi...

None

Ki67+ (1)

Ignore*

Keratin+ (1)

Ki67- (1)

keratin- (1)

Filter classifications in list

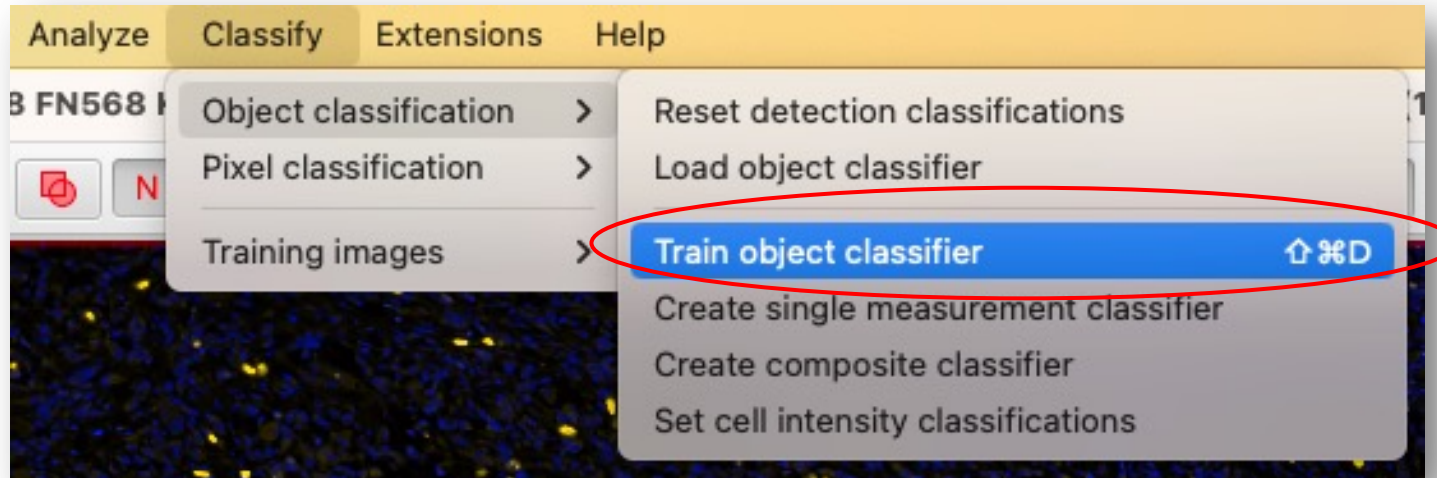
Set selected

Auto set

Make sure to lock your annotation: Ctrl+click > Lock

Train an object classifier

- *Classify > Object classification > Train object classifier*



Train an object classifier

- *Classify > Object classification > Train object classifier*

Model type (RT, ANN, k-NN)

Features: choose Selected measurements and click Select to restrict the feature space

Name	Selected
Keratin+	<input type="checkbox"/>
Ki67+	<input checked="" type="checkbox"/>
Ki67-	<input checked="" type="checkbox"/>
keratin-	<input type="checkbox"/>

Train object classifier

Object filter: Detections (all)

Classifier: Random trees (RTrees) [Edit]

Features: All measurements [Select]

Classes: Selected classes [Select]

Training: Points only

Load training | Advanced options

Live update

Training data

Ki67+ (pink)

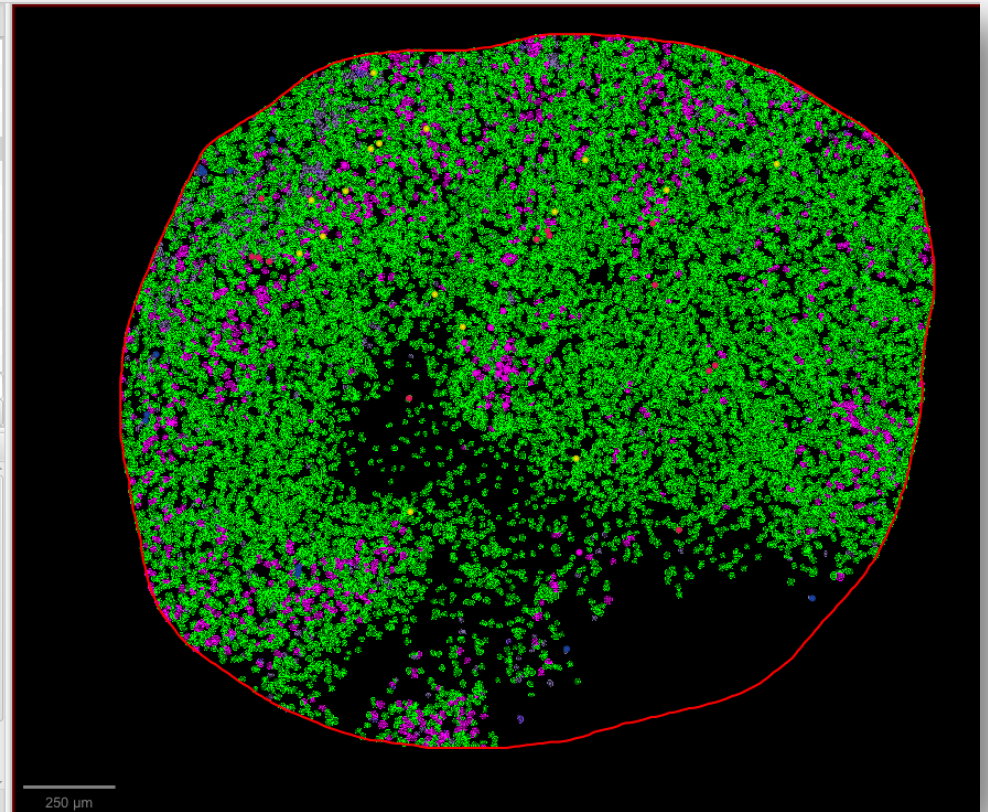
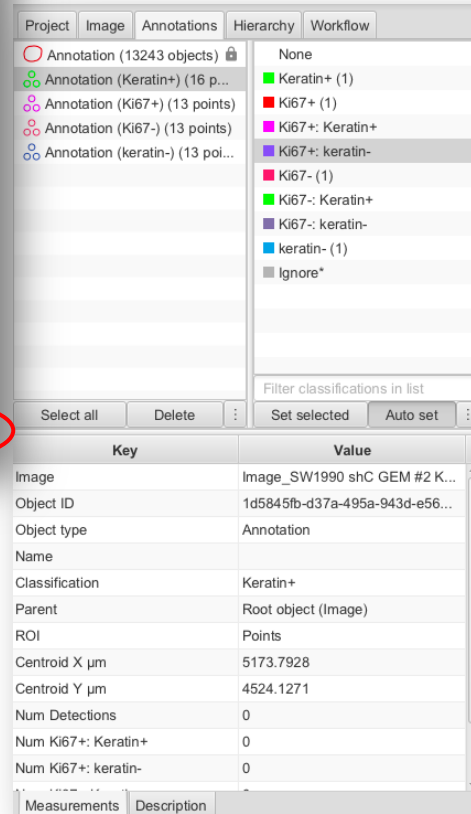
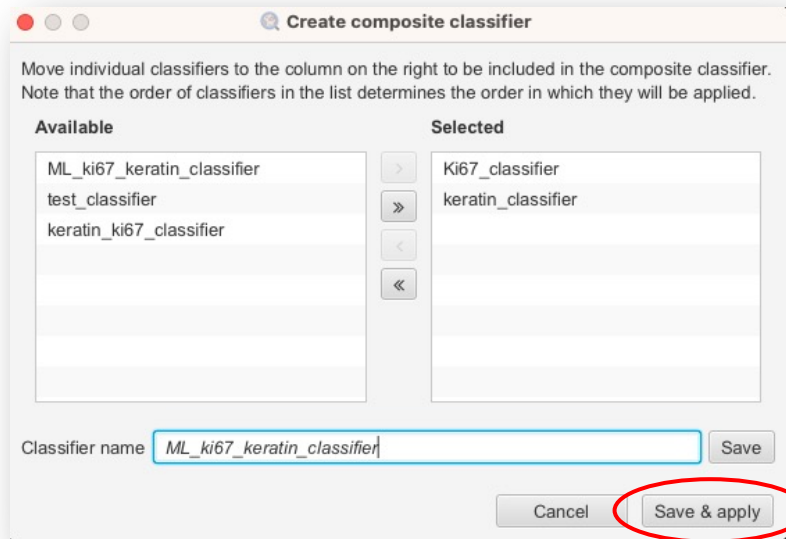
Ki67- (magenta)

Classifier name: Ki67_classifier [Save]

Name it to save it.

Combine multiple ML classifiers together

- *Classify > Object classification > Create composite classifier*



8 resulting classes!
+ ignore*

Refine your classifier

- Add more training data points
 - Classification results will change in real time if 'Live update' option is enabled
- Typically, *fewer*, but *well-chosen* features provides more robust results

Visualizing results using density maps

- Analyze > Density maps > Create density maps

