

A microscopic image of plant tissue, likely a leaf cross-section, showing a dense network of cells. The cell walls are outlined in red, and the nuclei are highlighted in purple. The cytoplasm and other organelles are visible in shades of blue and green. The text "Cell classification" is overlaid in the center.

# 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?

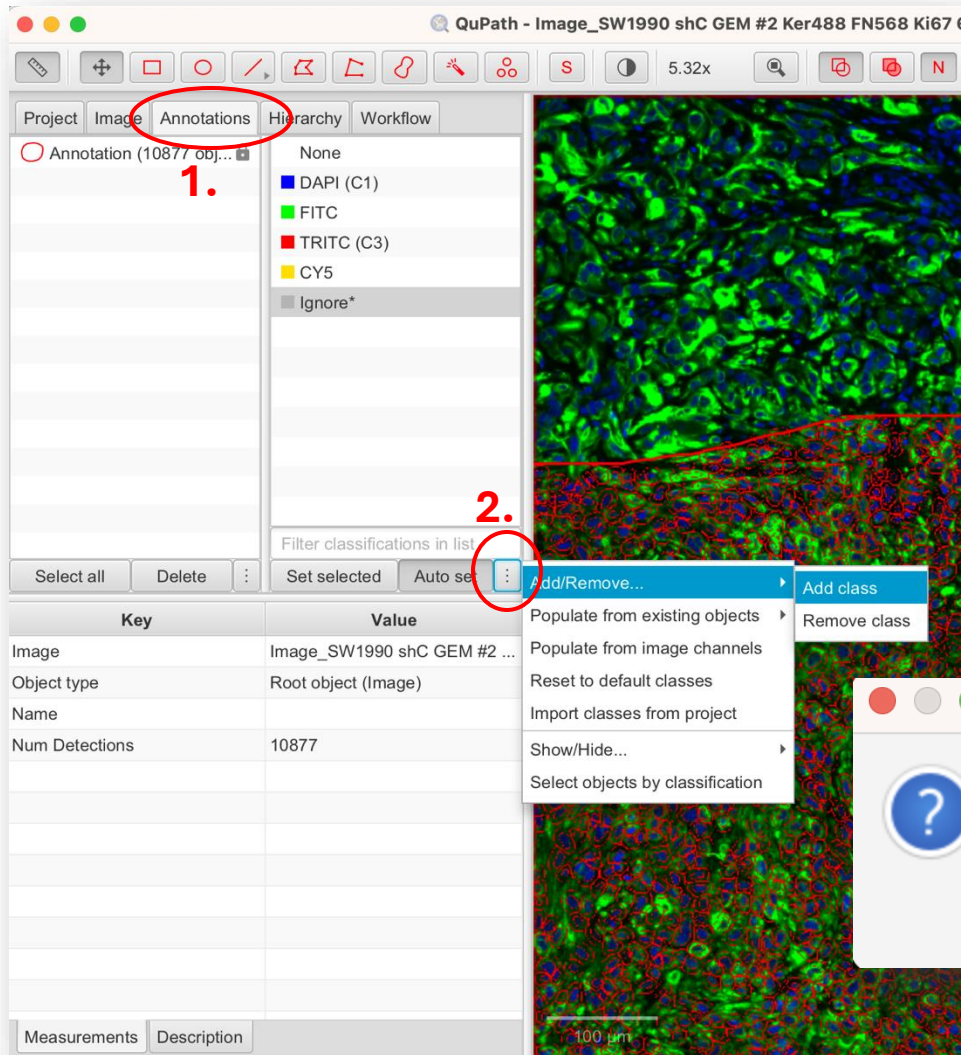
- Cell classification in image analysis is sorting different types of cells into groups based on their features or characteristics, such as shape, color, position, neighbors, etc.
- If visually you can't discriminate cells in your images, then your algorithm might be separating cell on very fine differences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


# Cell classification in QuPath

- Single measurement classifier (Keratin positive vs negative)
- Composite classifier: combine single measurement thresholds together (Keratin positive vs negative; Ki67 positive vs negative)
- Train a machine learning classifier

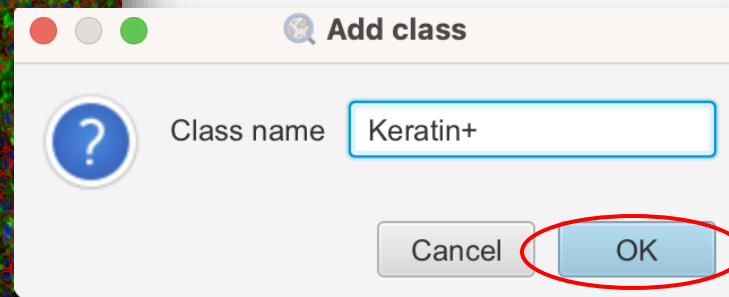


# Create a class named 'Keratin+'



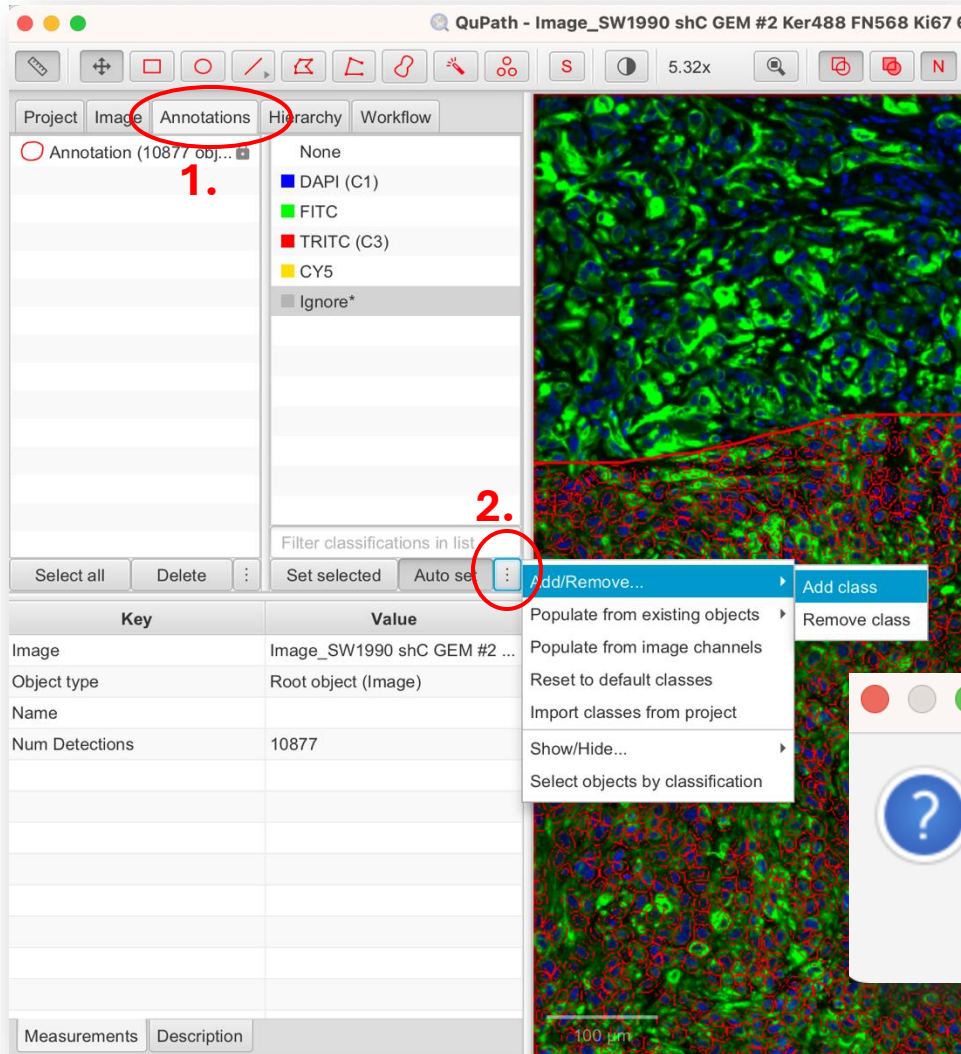
- *Annotations* tab > Classification list >  > Add/Remove... > Add class


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



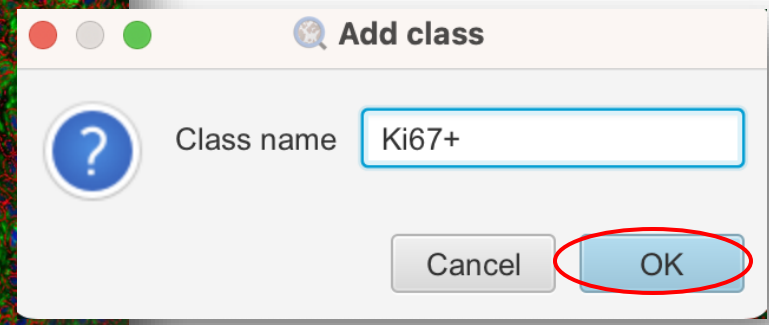
**3.**

# Create a second class named 'Ki67+'



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

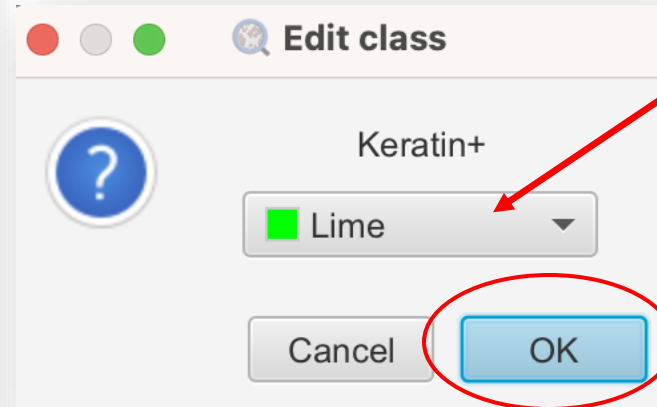
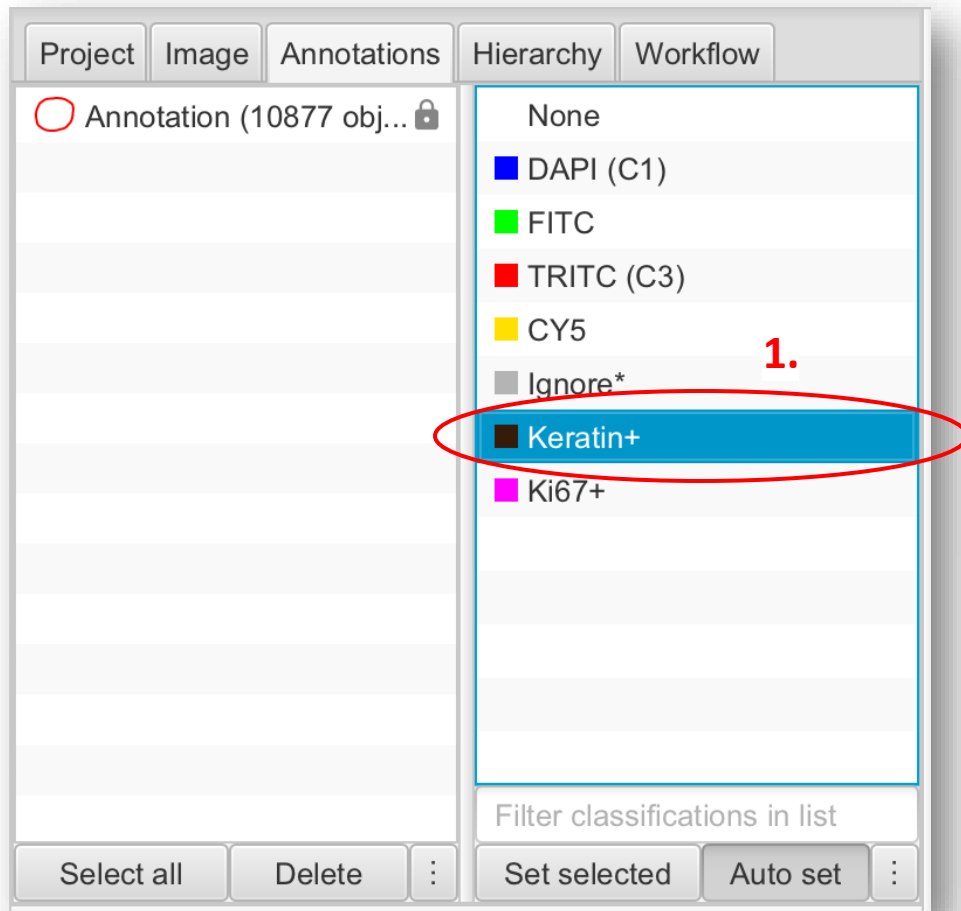
Default classes are channel names.  
We'll change that.



3.

# Change the color of a class

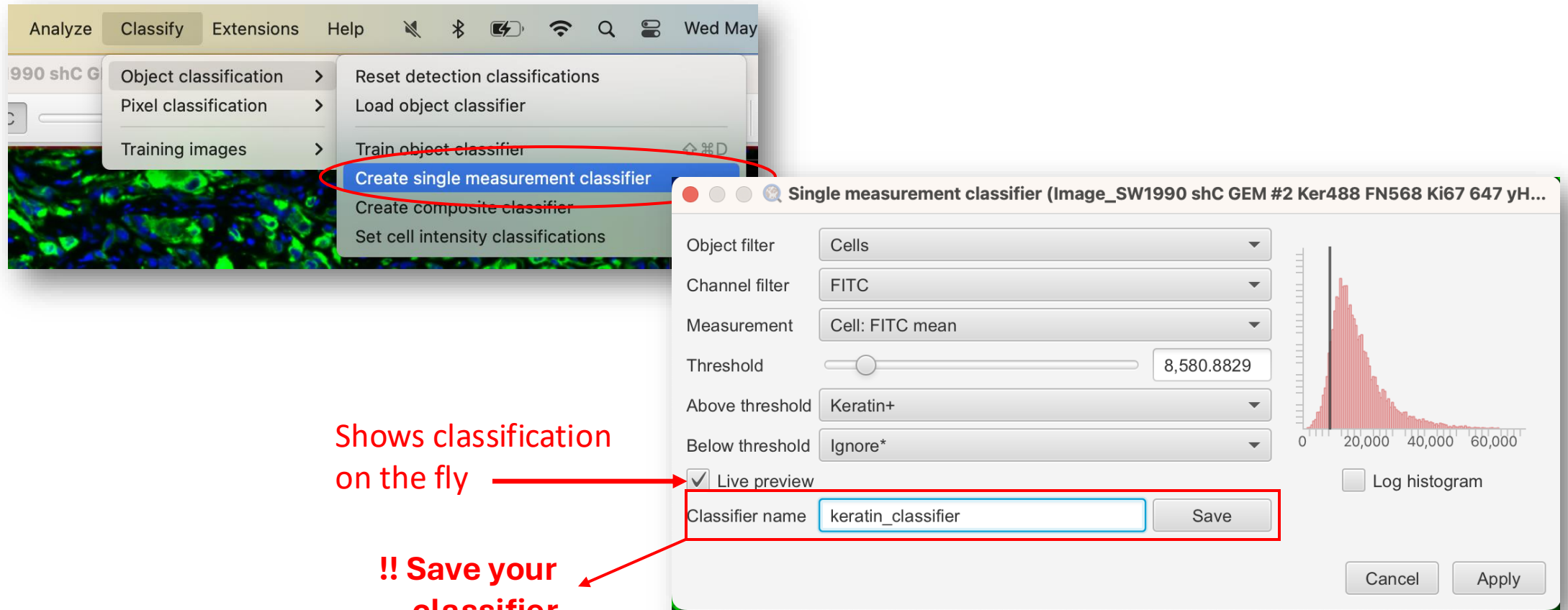
- Double click on the class > Edit class > Choose a new color > OK



**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 image shows a screenshot of the Fiji software interface. The 'Classify' menu is open, and the 'Create single measurement classifier' option is highlighted with a red circle. Below the menu, a red arrow points to a fly image with the text 'Shows classification on the fly'. The 'Single measurement classifier' dialog box is open, showing various settings: Object filter (Cells), Channel filter (FITC), Measurement (Cell: FITC mean), Threshold (8,580.8829), Above threshold (Keratin+), Below threshold (Ignore\*), and a checked 'Live preview' checkbox. A red box highlights the 'Classifier name' field containing 'keratin\_classifier' and the 'Save' button. A red arrow points to this box with the text '!! Save your classifier'. A histogram is visible on the right side of the dialog box.

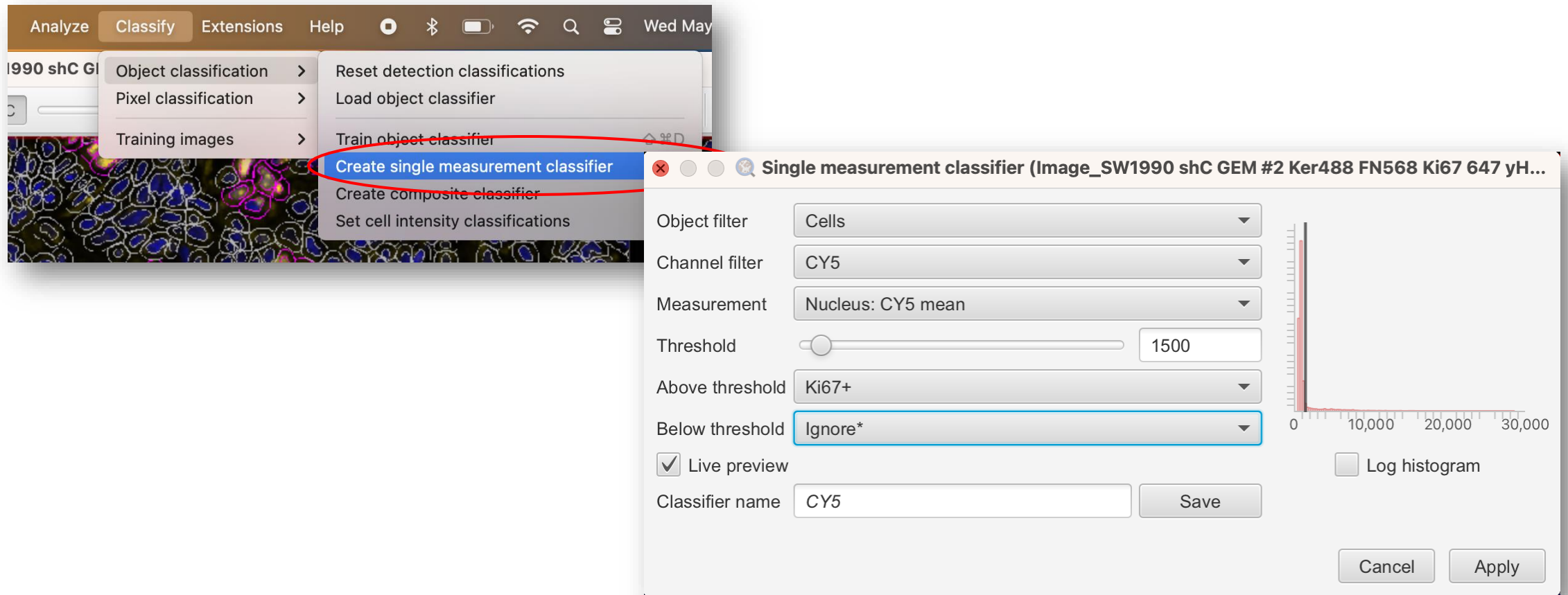
Shows classification on the fly

!! 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 yellow and red outlines. To the right is a dialog box titled 'Single measurement classifier (Image\_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH...)' with 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 box, 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 of the dialog box are 'Save', 'Cancel', and 'Apply' buttons.

*Practice time*

Exercise 4.a: single-measurement classifier

# Combine single measurement classifiers into a composite classifier

- *Classify > Object classification > Create composite classifier*

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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'*

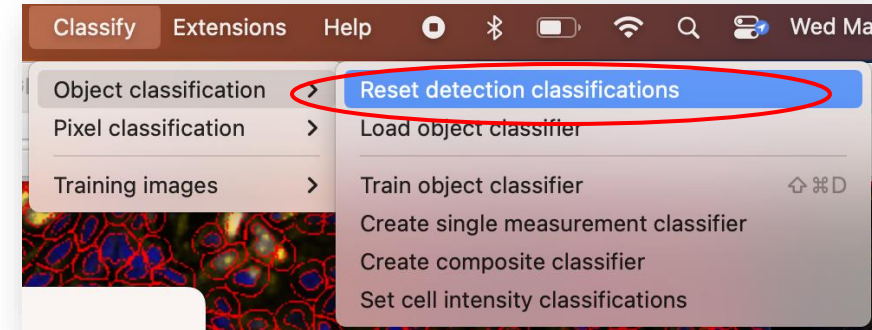
*Practice time*

Exercise 4.b: composite classifier



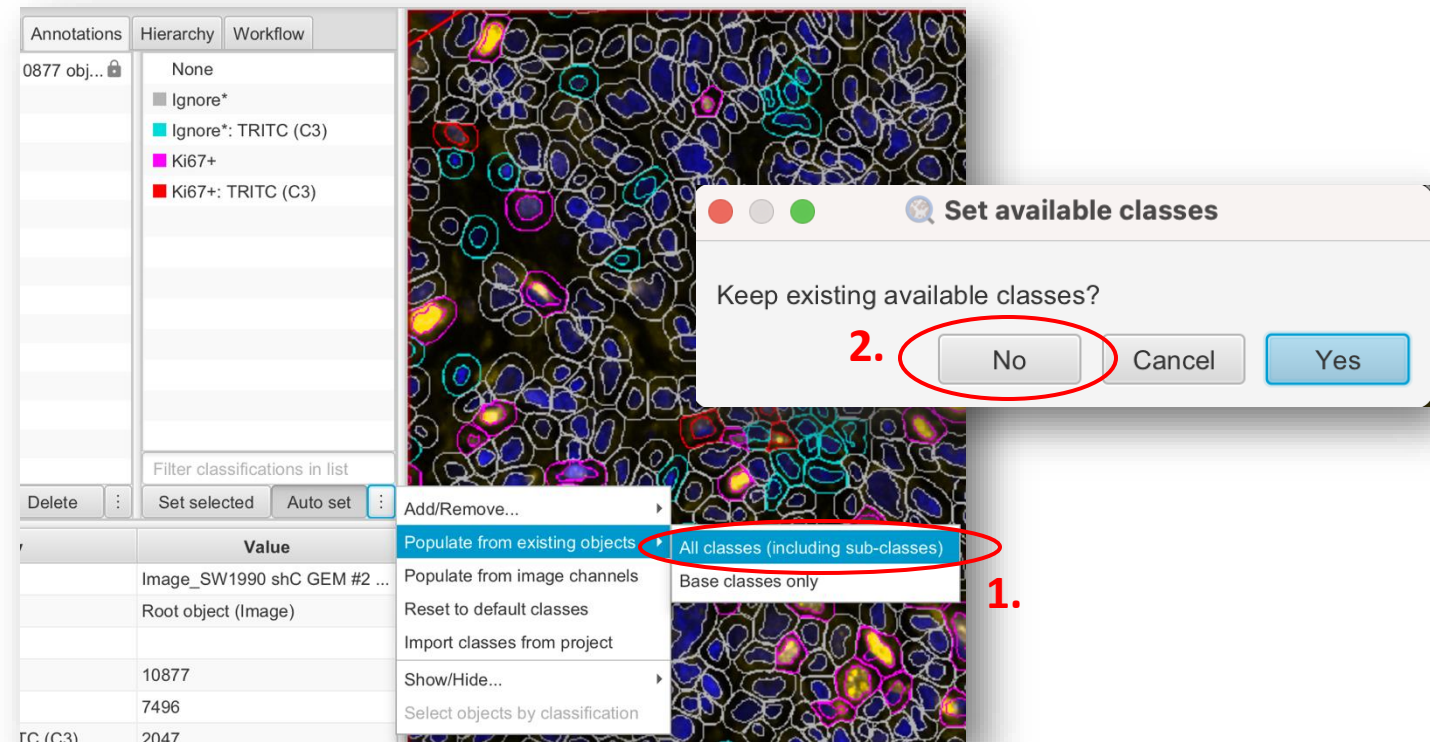
# Reset detection classes

- *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**

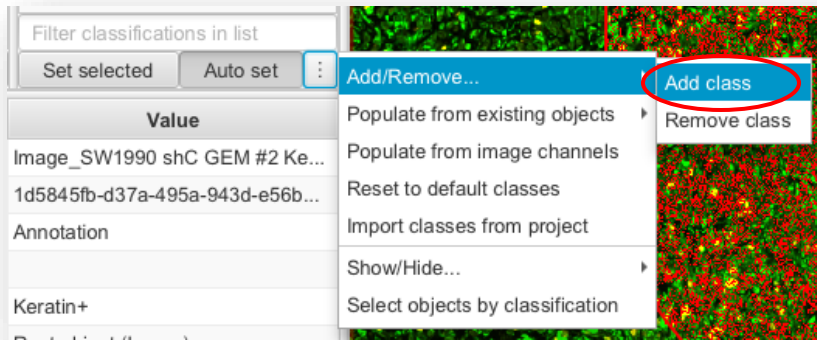
Reset your detection classes!



Adobe Stock | #32445303

# Train an object classifier: create classes

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



- Create 4 classes:

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



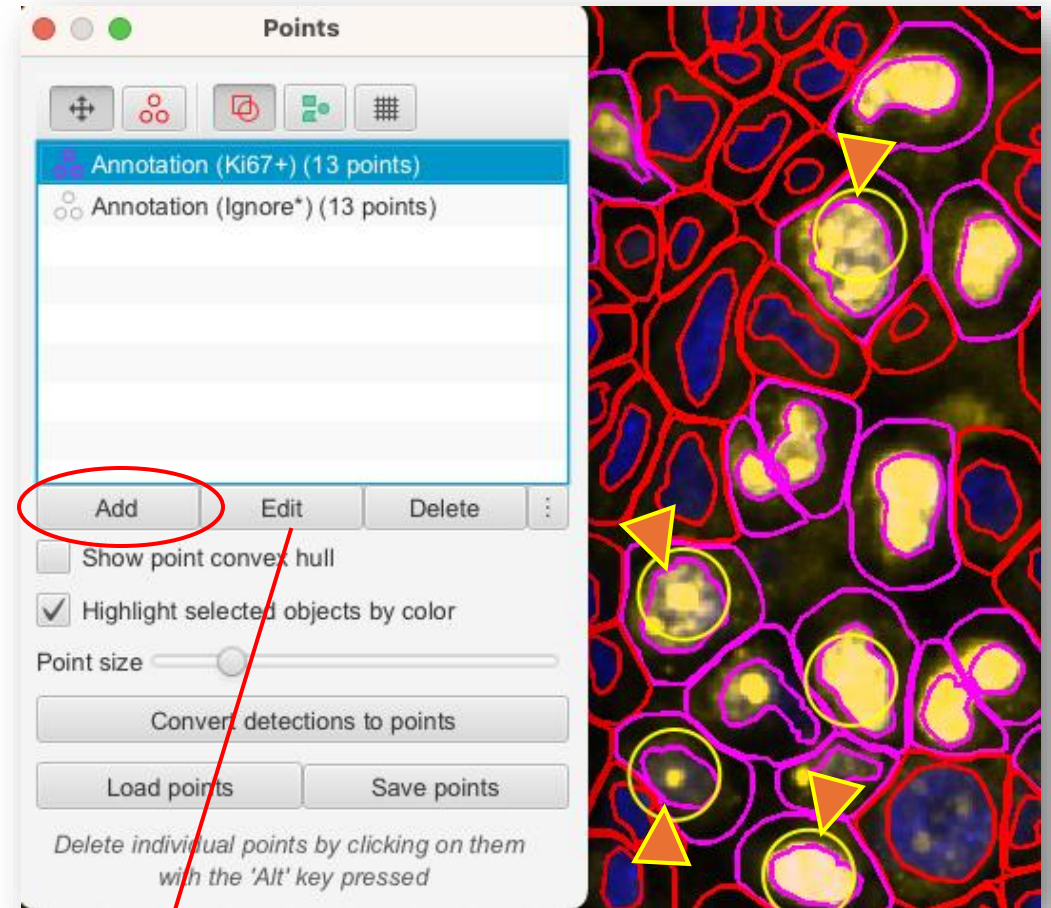


# Train an object classifier: training data points

-  > 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

The image shows two screenshots of a software interface, likely a microscopy analysis tool, illustrating the process of assigning a class to an annotation. The interface has tabs for Project, Image, Annotations, Hierarchy, and Workflow.

**Left Screenshot (Step 1):** The 'Annotations' tab is active. A list of annotations is shown on the left, with the first one, 'Annotation (13 points)', circled in red and labeled '1.'. A dropdown menu is open, showing classification options: None, Ki67+ (1), Ignore\*, Keratin+ (1), Ki67-, and keratin-. The 'keratin-' option is highlighted in blue and circled in red, labeled '2.'. At the bottom, the 'Set selected' button is circled in red and labeled '3.'. Below the screenshots, the text '3.' is also present.

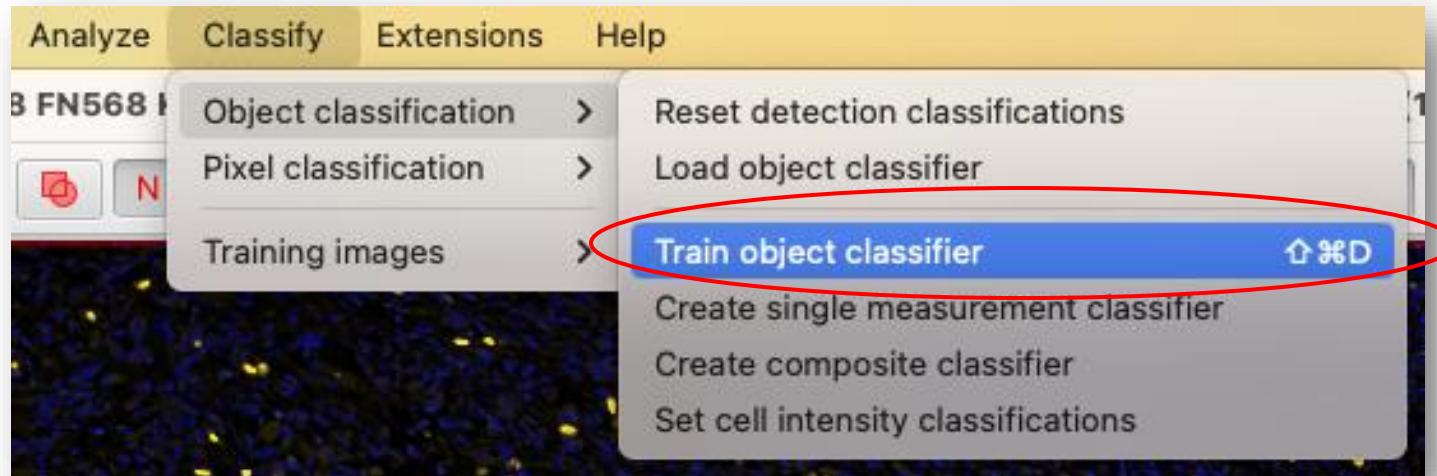
**Right Screenshot (Step 2):** The 'Annotations' tab is active. The list of annotations is updated, and the 'keratin-' class is now assigned to the first annotation, 'Annotation (13243 objects)'. The 'keratin-' class is also listed in the classification list on the right.

A red arrow points from the left screenshot to the right screenshot, indicating the transition from selecting the class to applying it to the annotation.

**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+ (purple)

Ki67- (red)

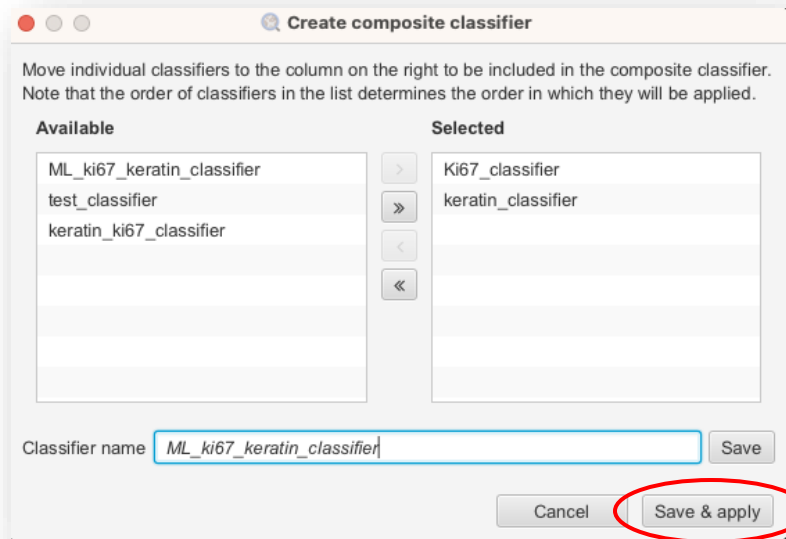
Classifier name: Ki67\_classifier [Save]

**Name it to save it.**

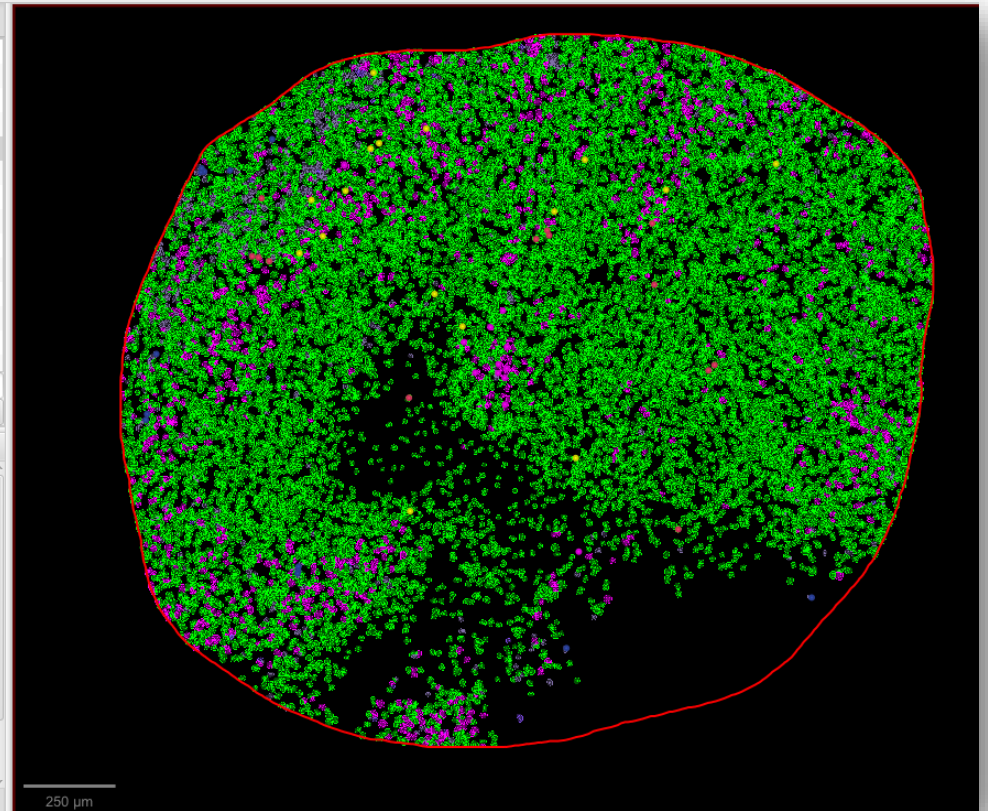


# Combine multiple ML classifiers together

- *Classify > Object classification > Create composite classifier*



Key	Value
Image	Image_SW1990 shC GEM #2 K...
Object ID	1d5845fb-d37a-495a-943d-e56...
Object type	Annotation
Name	
Classification	Keratin+
Parent	Root object (Image)
ROI	Points
Centroid X $\mu\text{m}$	5173.7928
Centroid Y $\mu\text{m}$	4524.1271
Num Detections	0
Num Ki67+: Keratin+	0
Num Ki67+: keratin-	0
Measurements	Description



**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

