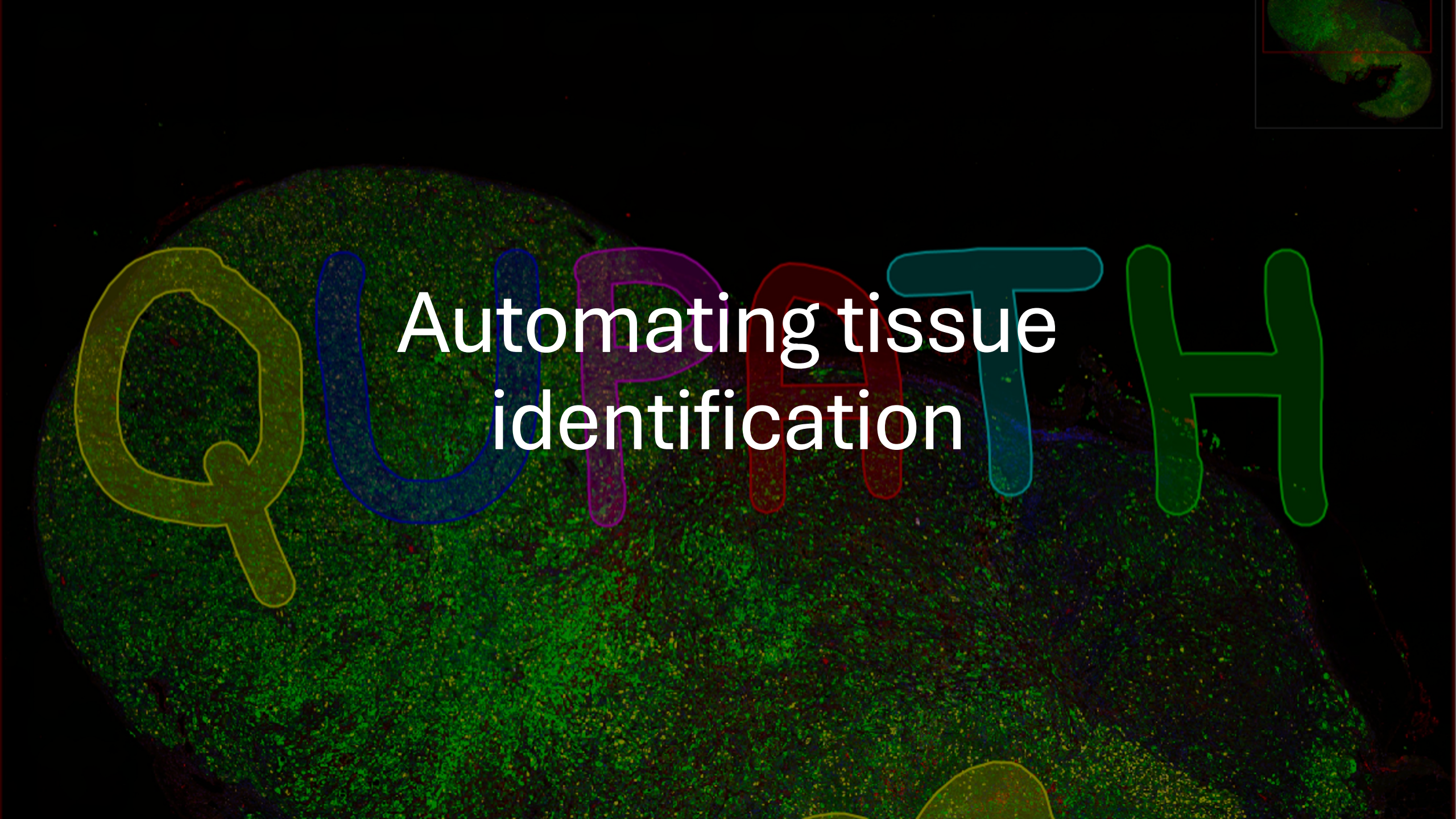
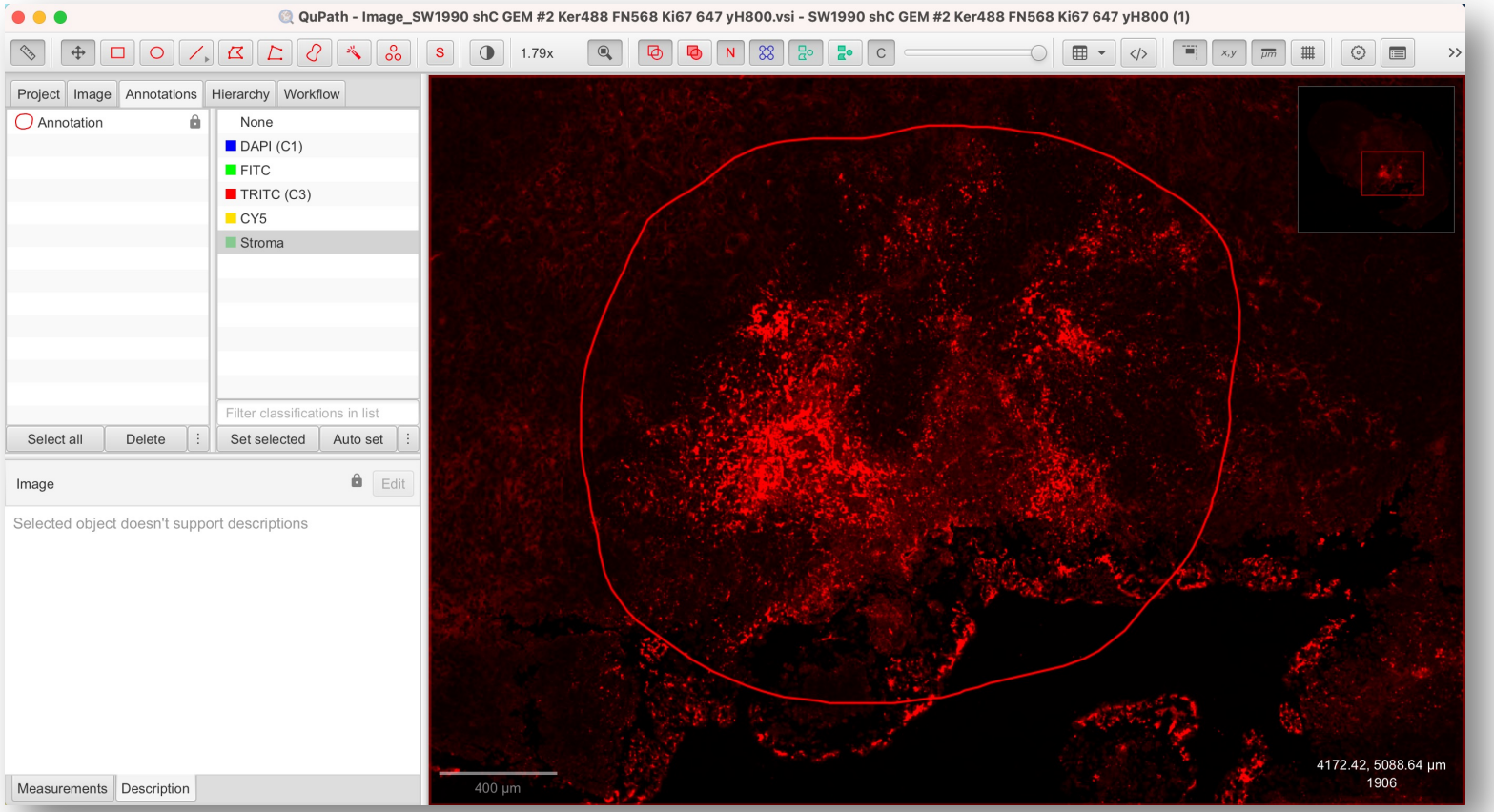


Automating tissue identification

Creating a region of interest

In the TRITC channel (fibronectin), create a region of interest that enclose high-fibronectin content regions aka stromal regions



Once you have finished your annotation, **lock** it:

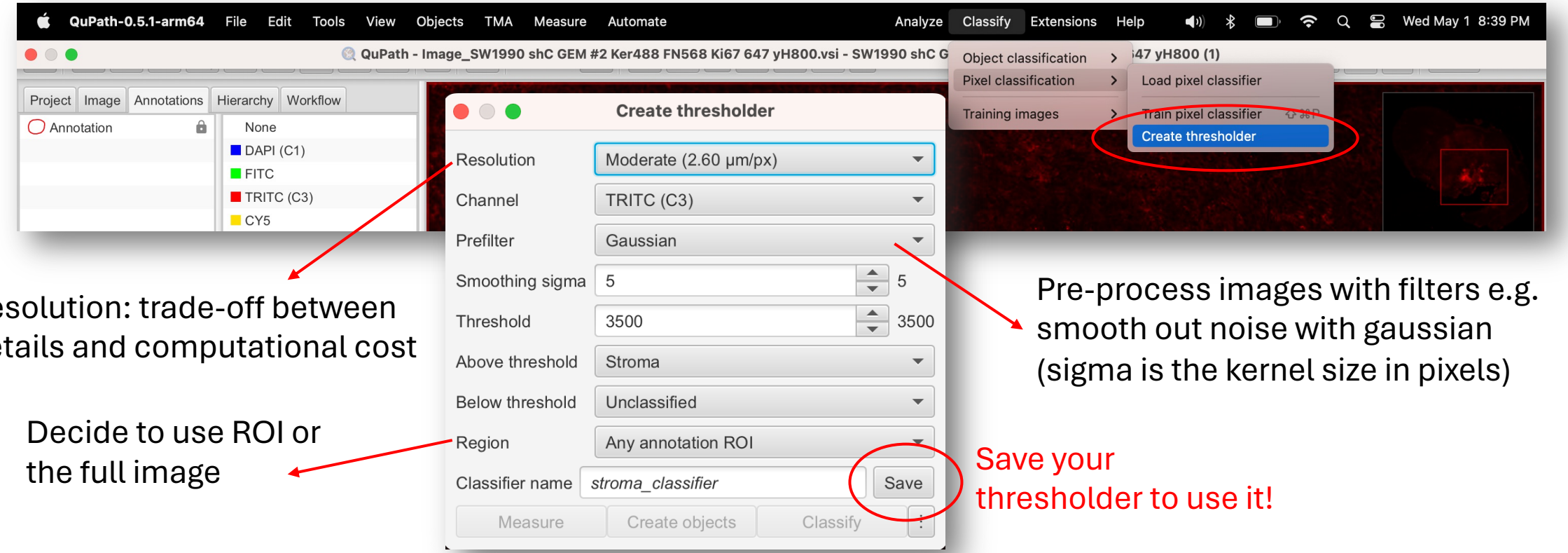
Right-click in the viewer > *Annotations* > *Lock*

or

Right-click on the annotation in the analysis panel > *Lock*

Pixel-based tissue annotation

- Simplest case of annotation: every pixel get assigned a class based on its intensity value – **or is a given pixel above or below a certain numeric value?**



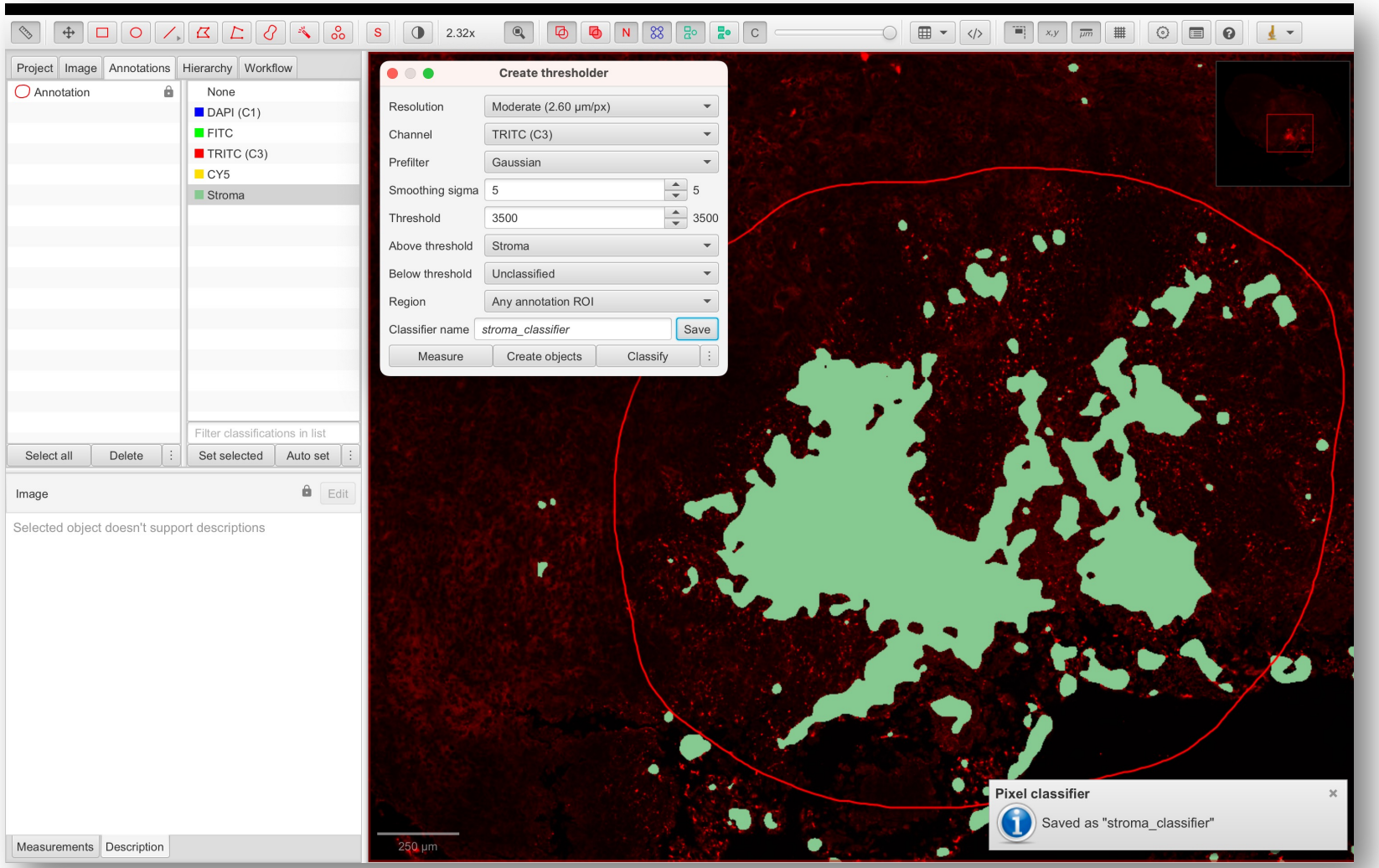
Resolution: trade-off between details and computational cost

Decide to use ROI or the full image

Pre-process images with filters e.g. smooth out noise with gaussian (sigma is the kernel size in pixels)

Save your thresholder to use it!

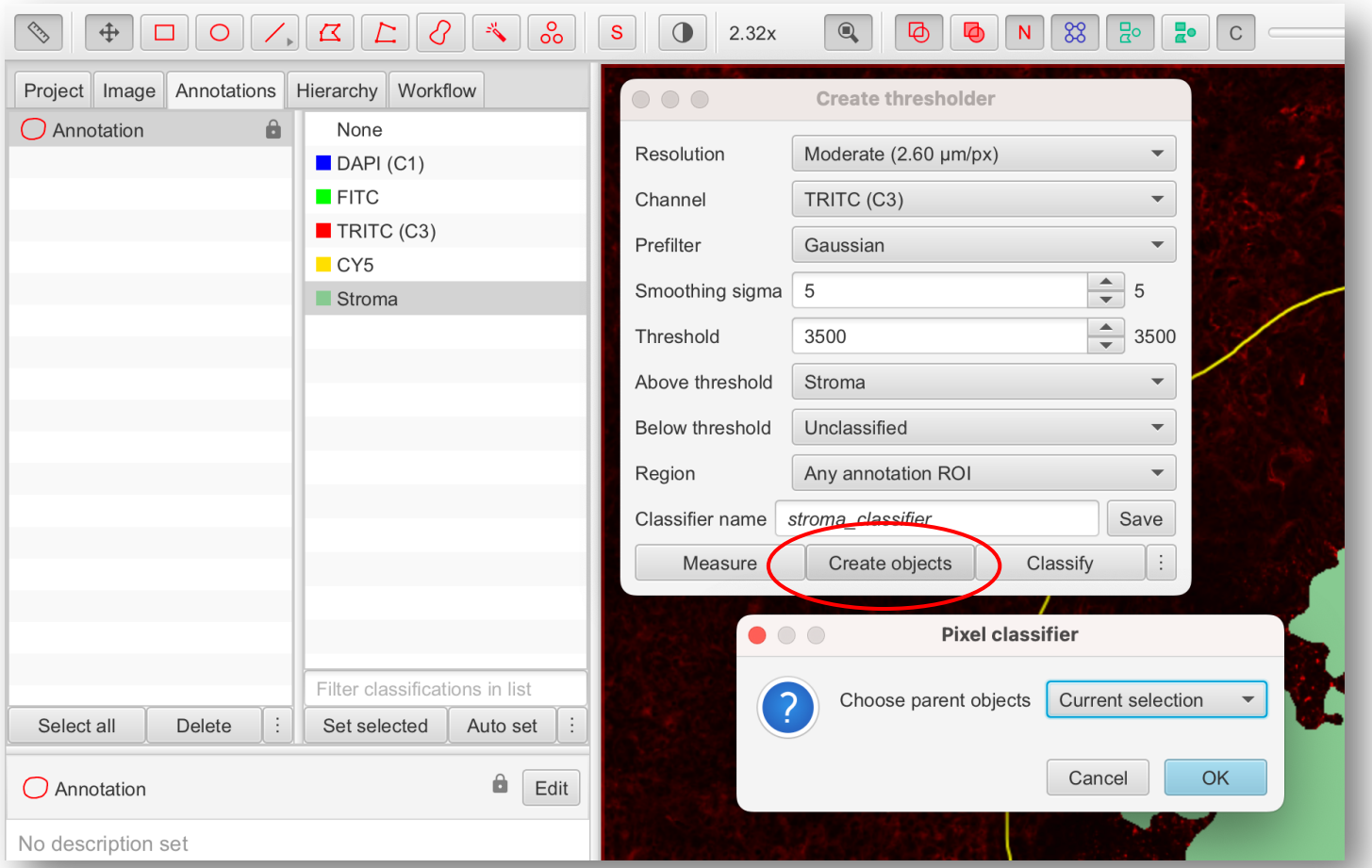
Interactive visualization of thresholding results



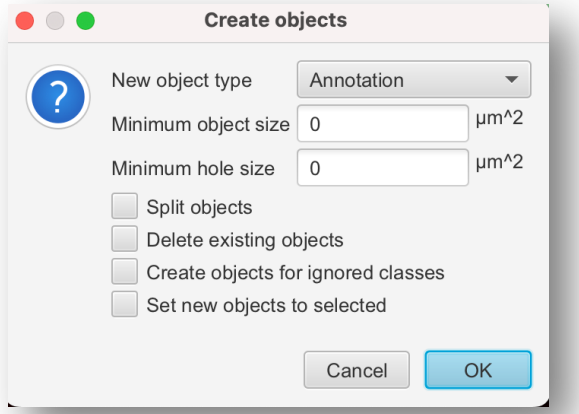
Create a class ‘Stroma’

Try varying the value of the different parameters!

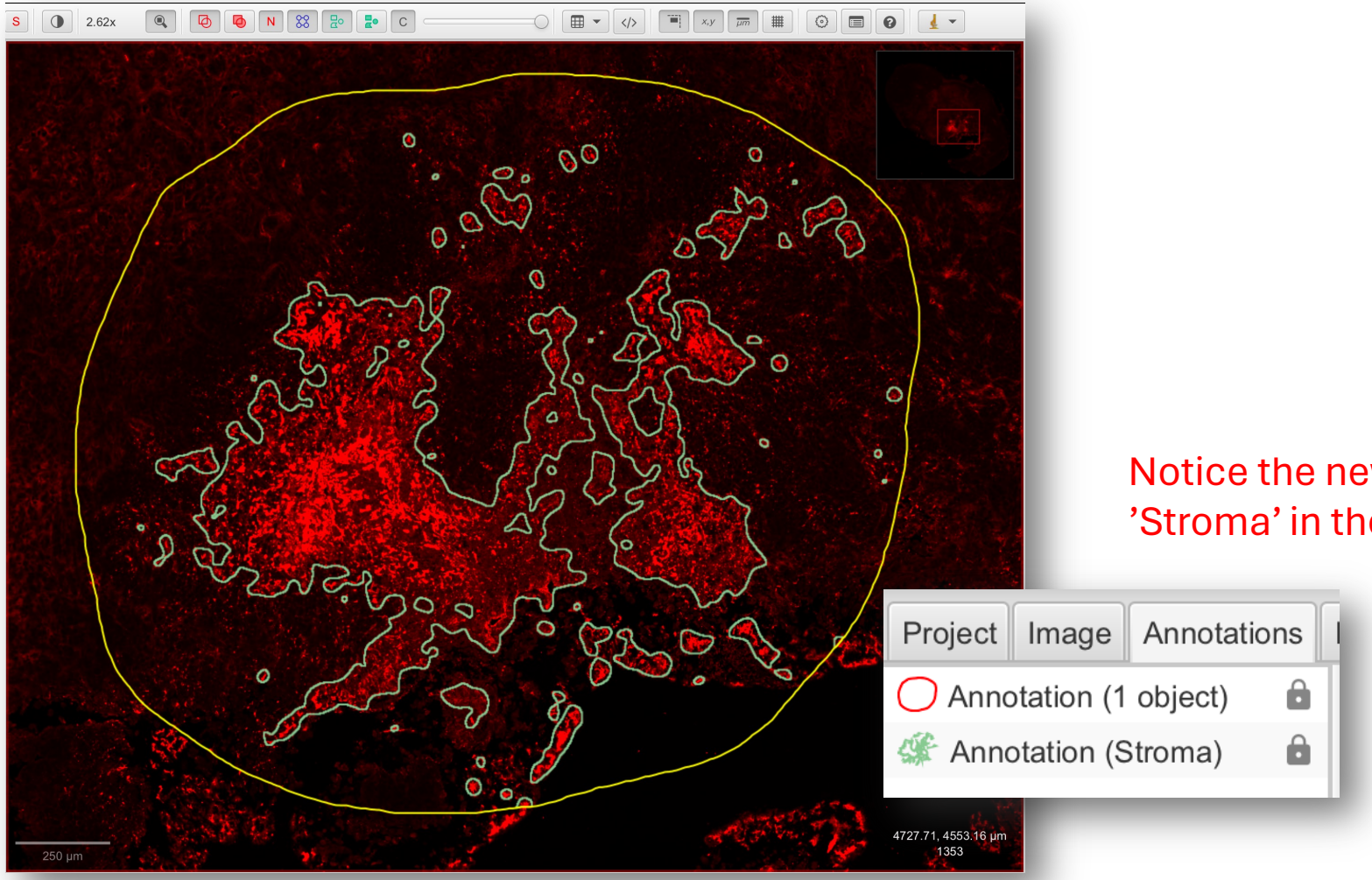
Create annotations from pixel classifier



- Real-time visualization of results, once happy with it:
  1. Save your thresholder
  2. Select ROI
  3. Click *Create objects*
  4. Keep default parameters > OK

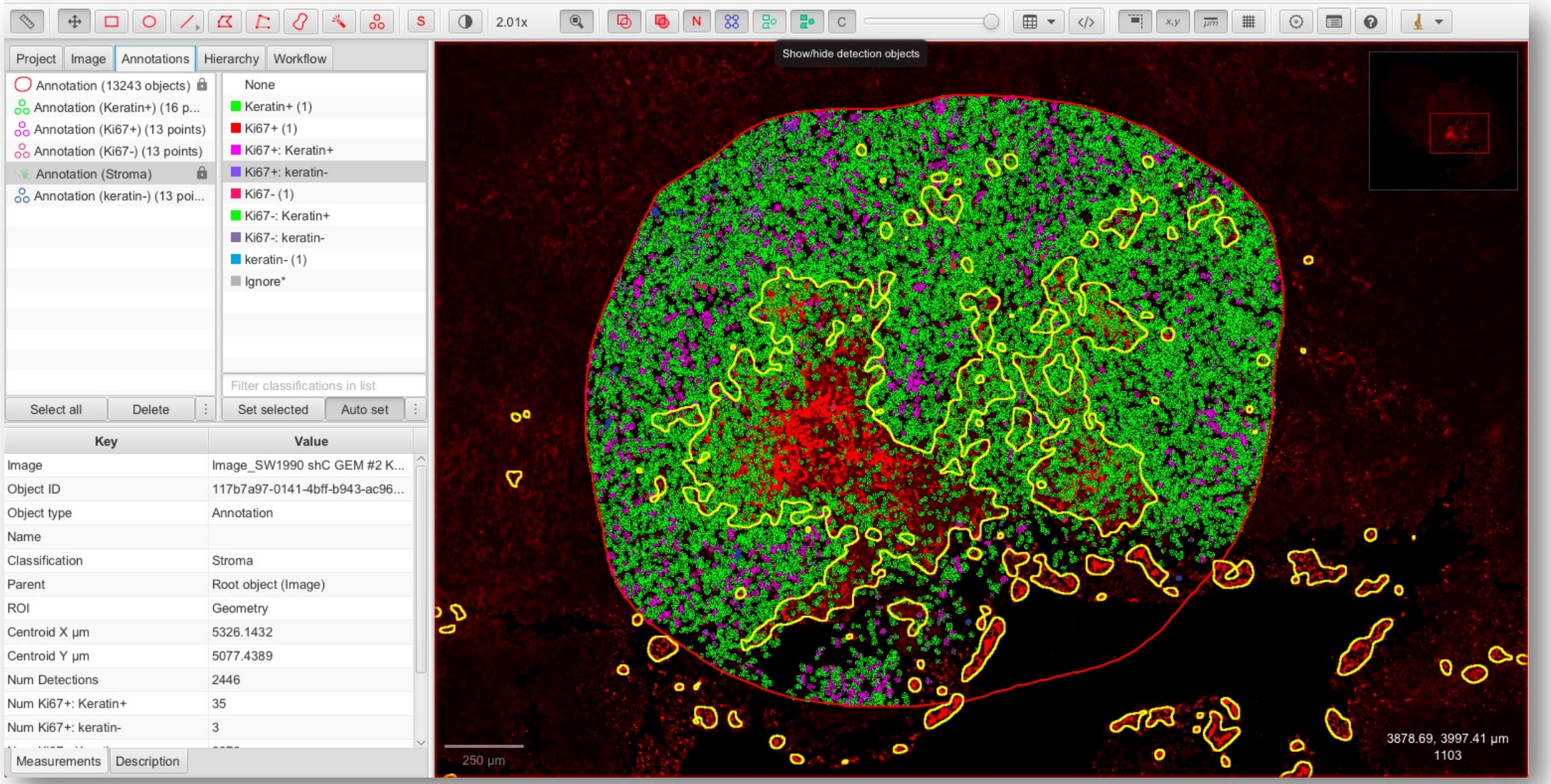


Create annotations from pixel classifier



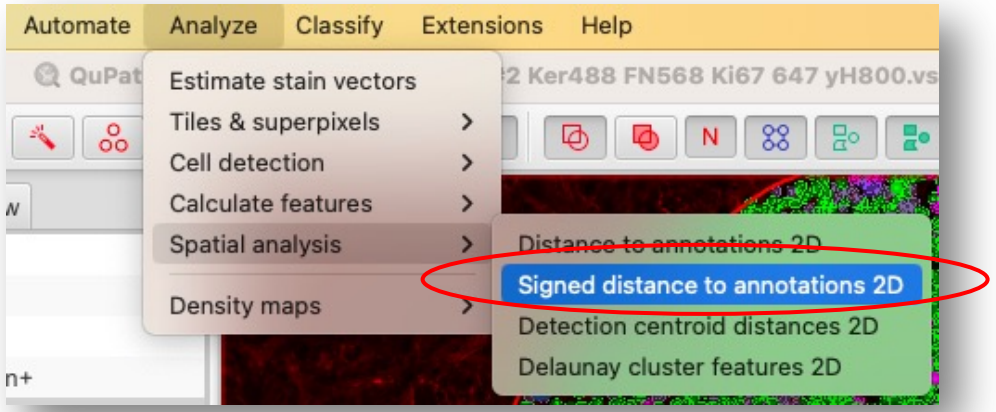
Notice the new annotation named ’Stroma’ in the *Annotations* list

Fully annotated image



Spatial information: signed distance

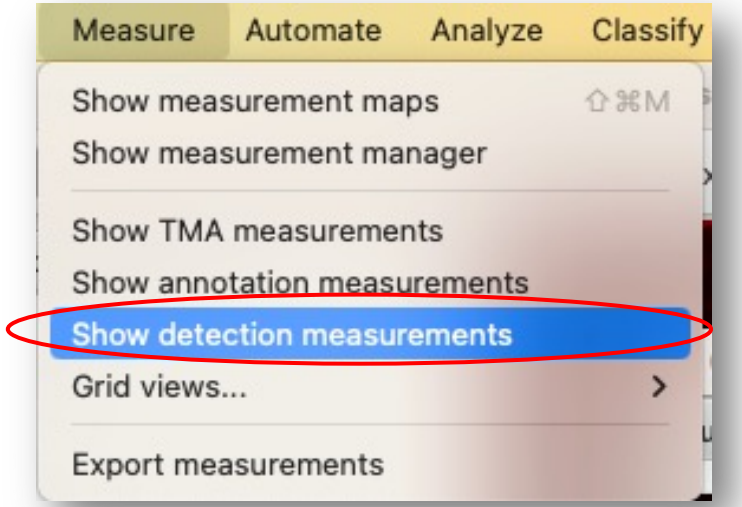
- *Analyze > Spatial analysis > Signed distance to annotations 2D*



- Calculates the signed distance (2D euclidian) between cells and annotations
  - If a cell lies inside the annotation: negative distance
  - If a cell lies outside the annotation: positive distance

Spatial information: signed distance

- *Measure > Show detection measurements*



Export measurements table and use Python/R for visualization based on classes