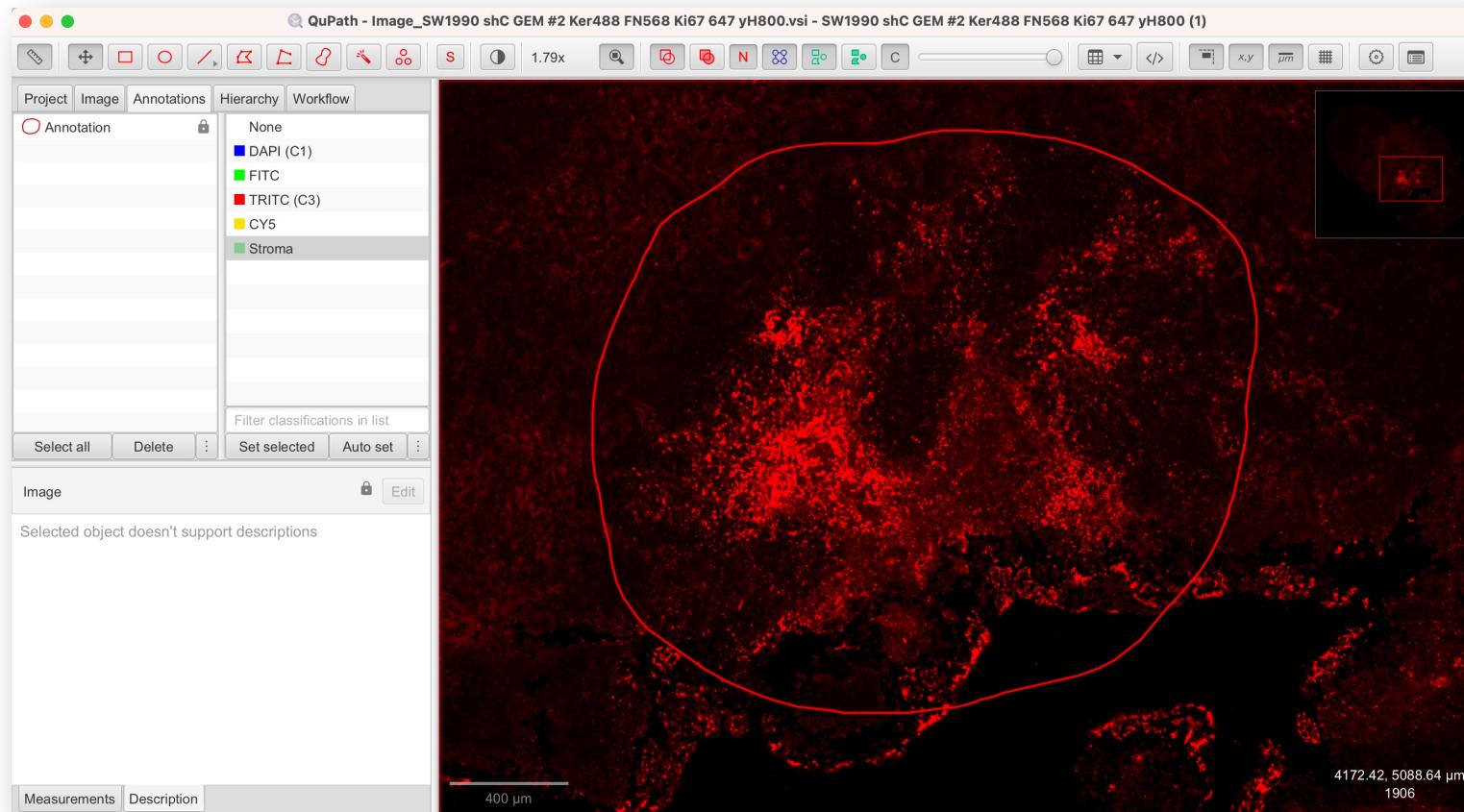


Automating tissue
identification

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:

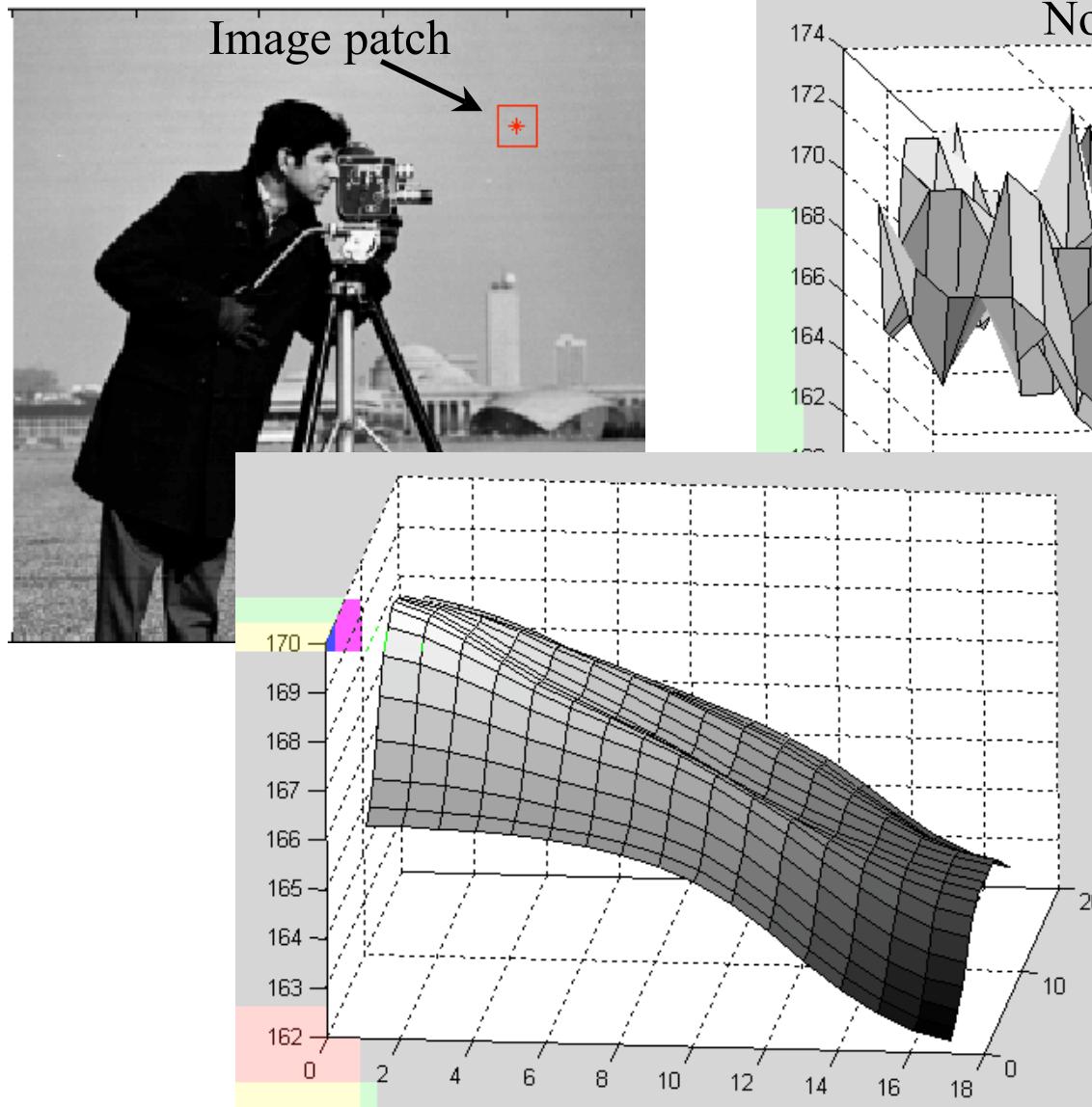
Right-click in the viewer
> Annotations > Lock

or

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

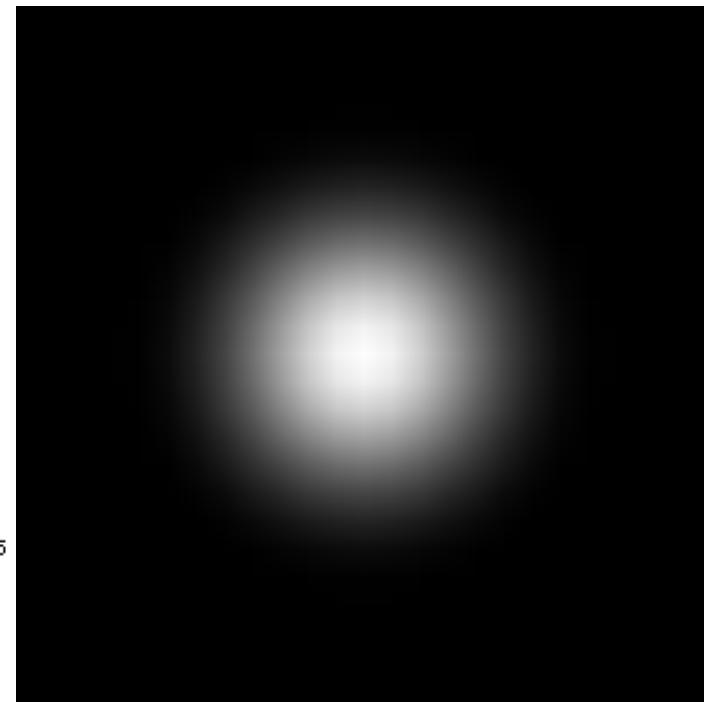
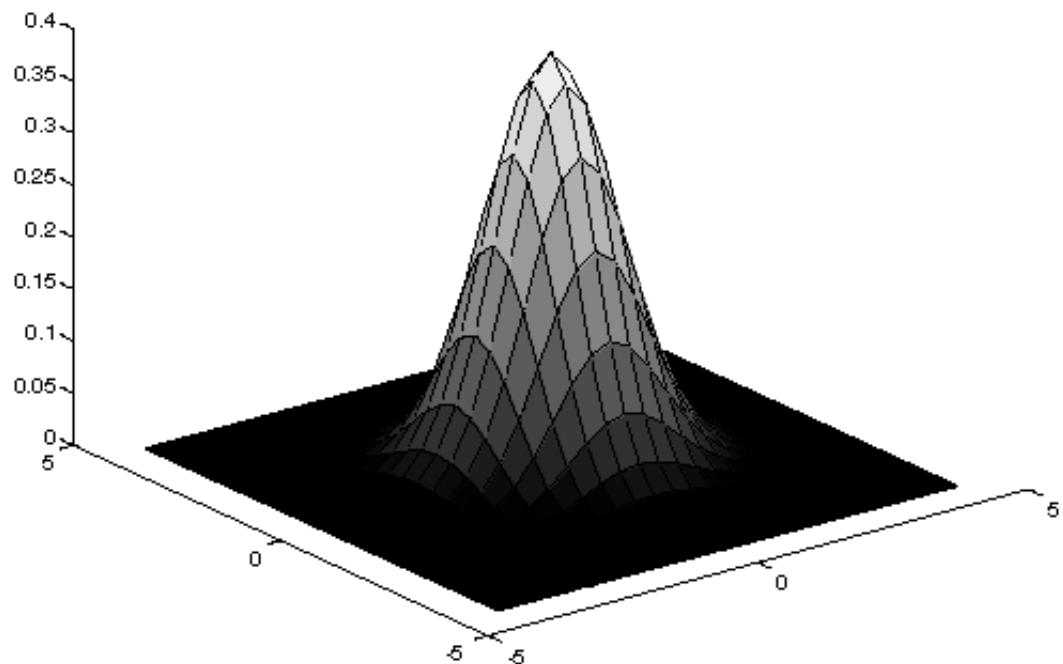
Notes on smoothing

Today: Smoothing Reduces Noise



Gaussian Smoothing Filter

An isotropic (circularly symmetric) Gaussian:



Gaussian Smoothing Example



original



$\sigma = 3$

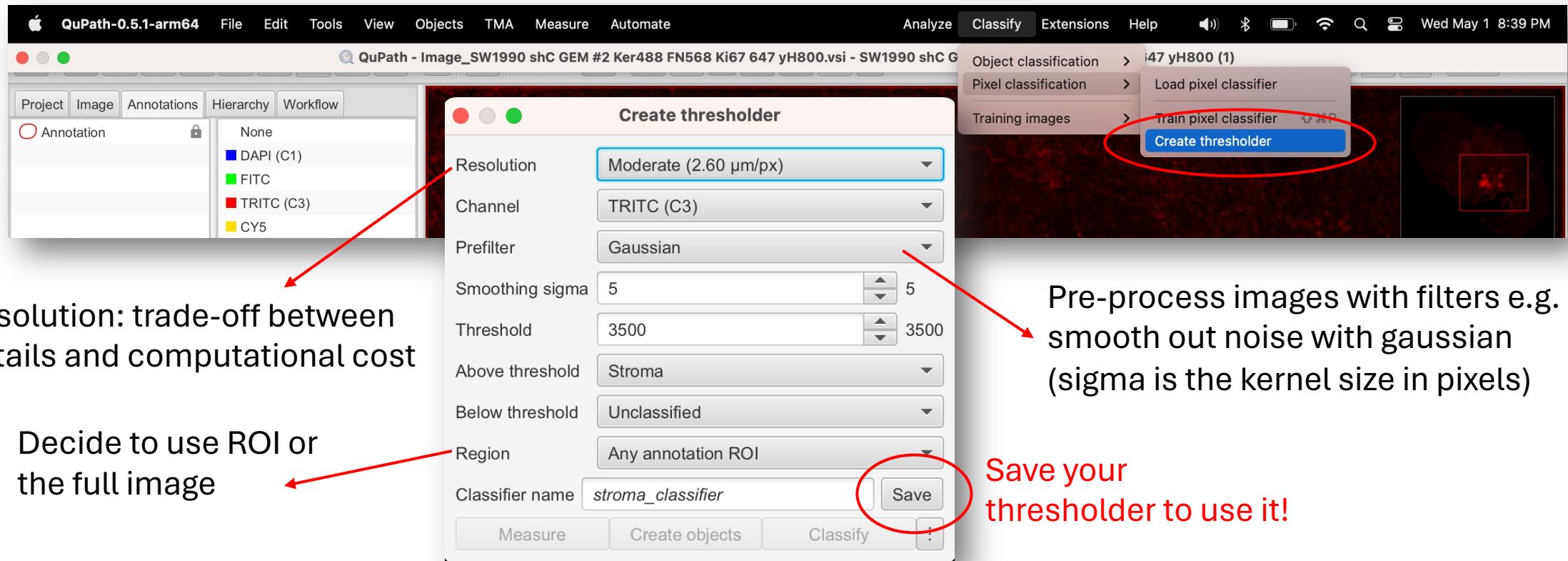
Gaussian Smoothing at Different Scales



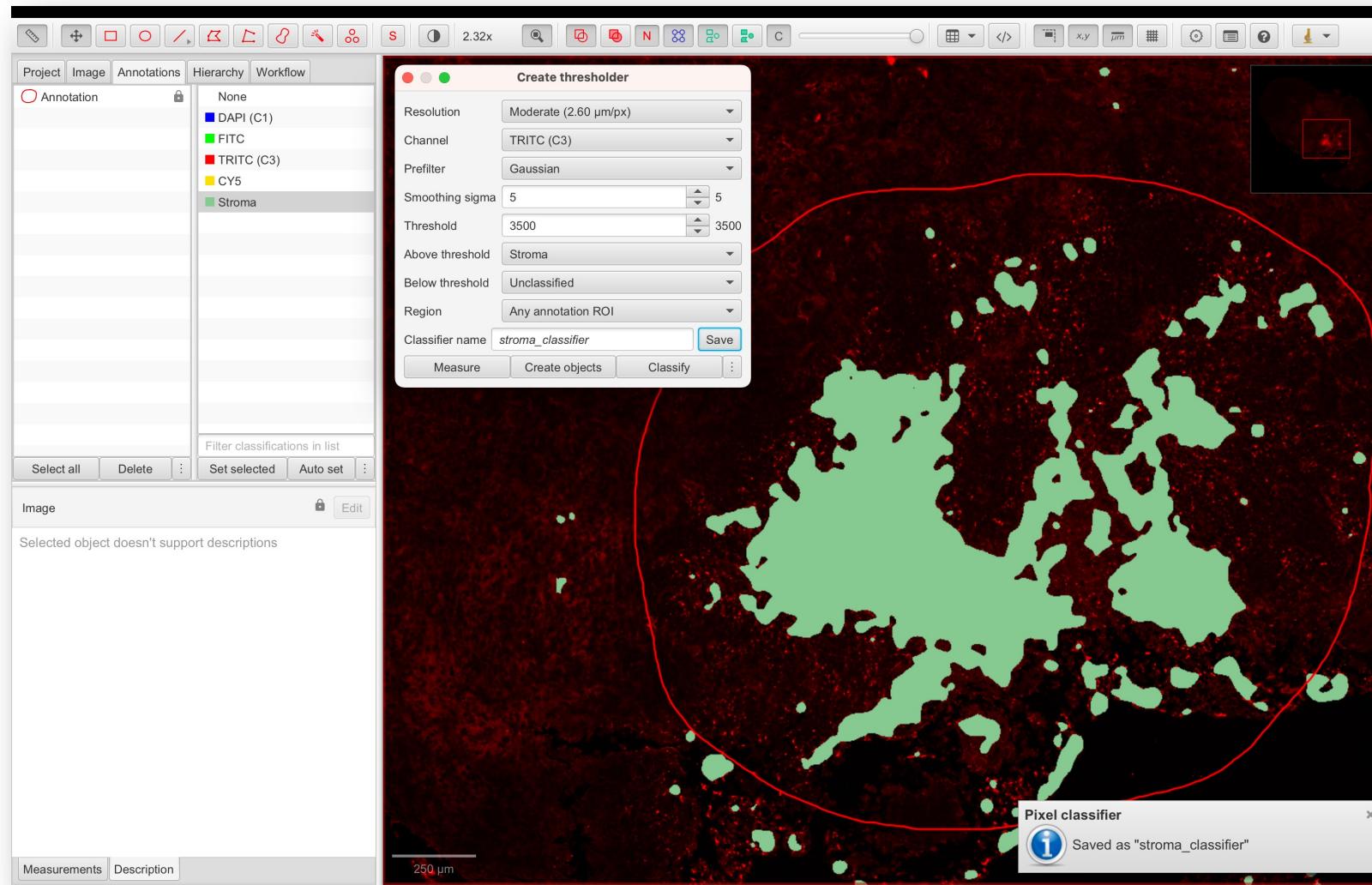
**Balancing act: smooth enough to “clean up”
the noise, but not so much as to remove
important image gradients.**

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?**



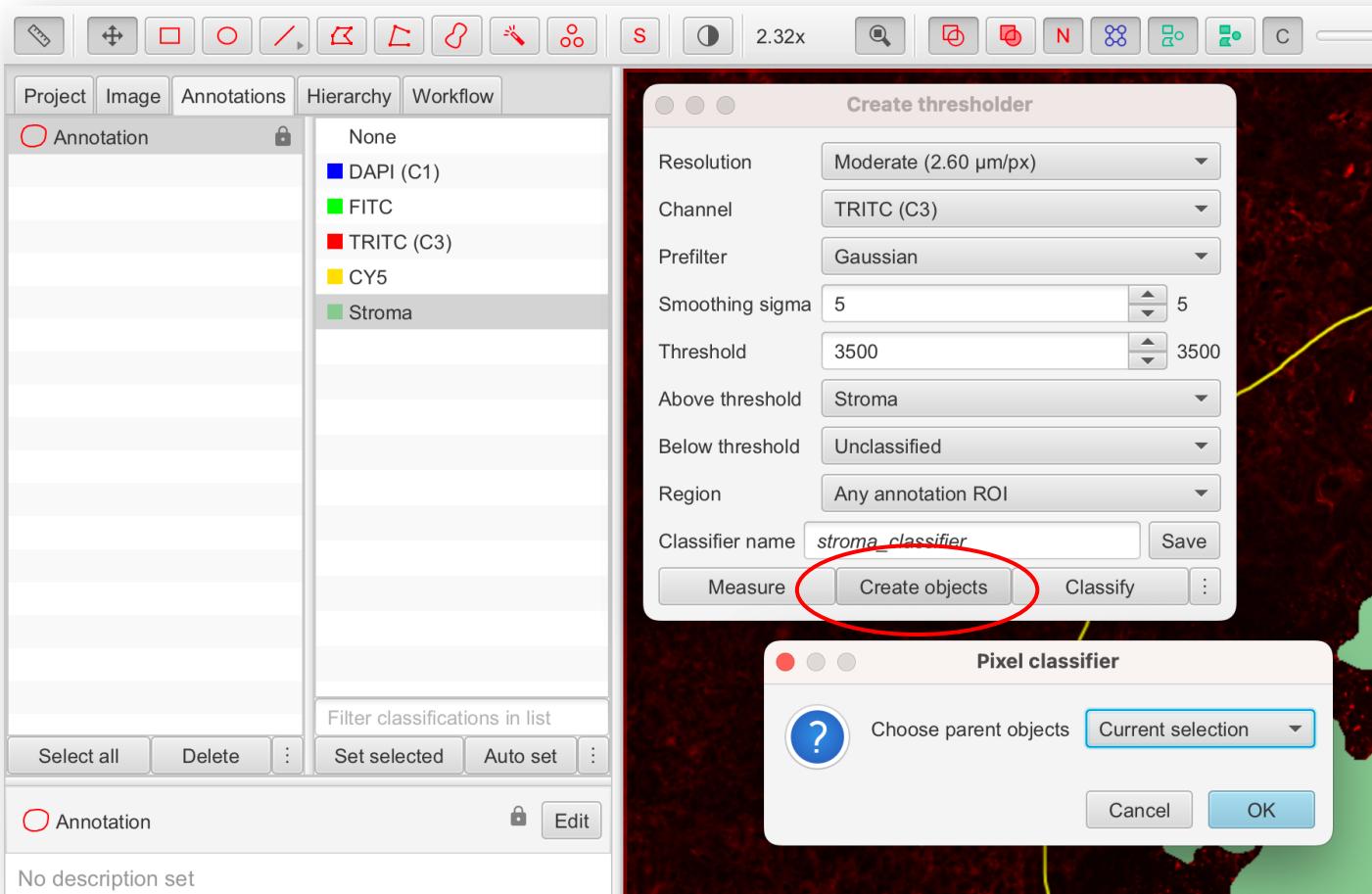
Interactive visualization of thresholding results



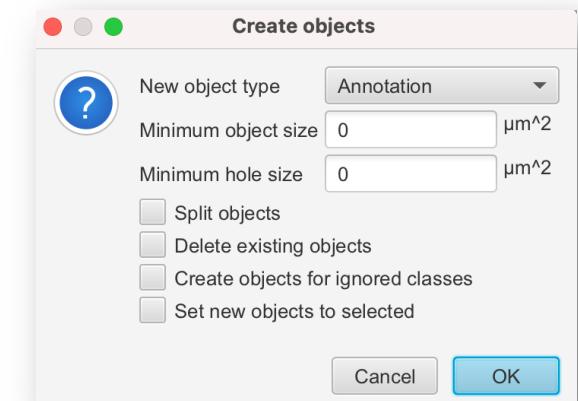
Create a class
‘Stroma’

Try varying the value
of the different
parameters!

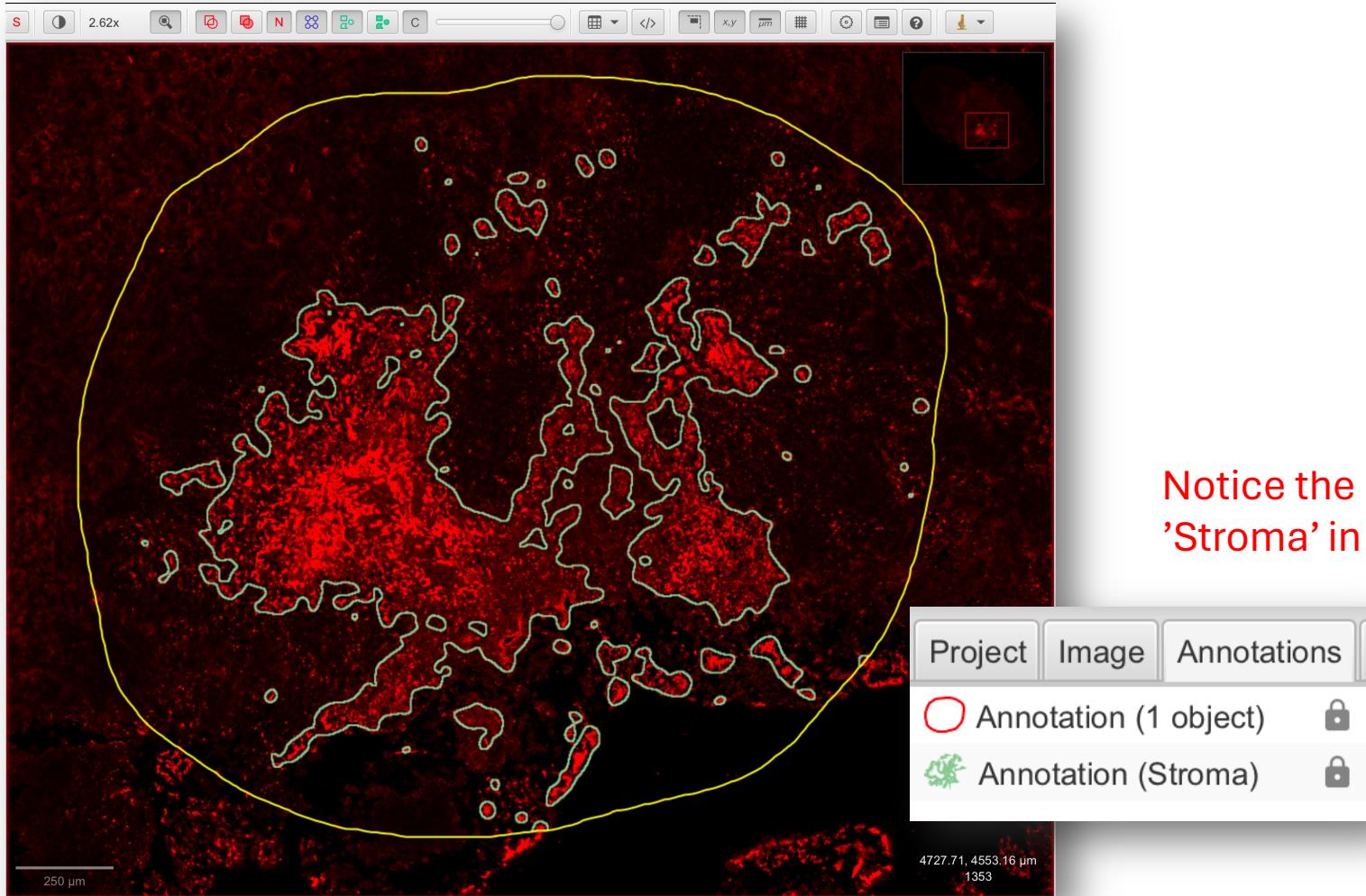
Create annotations from pixel classifier



- 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

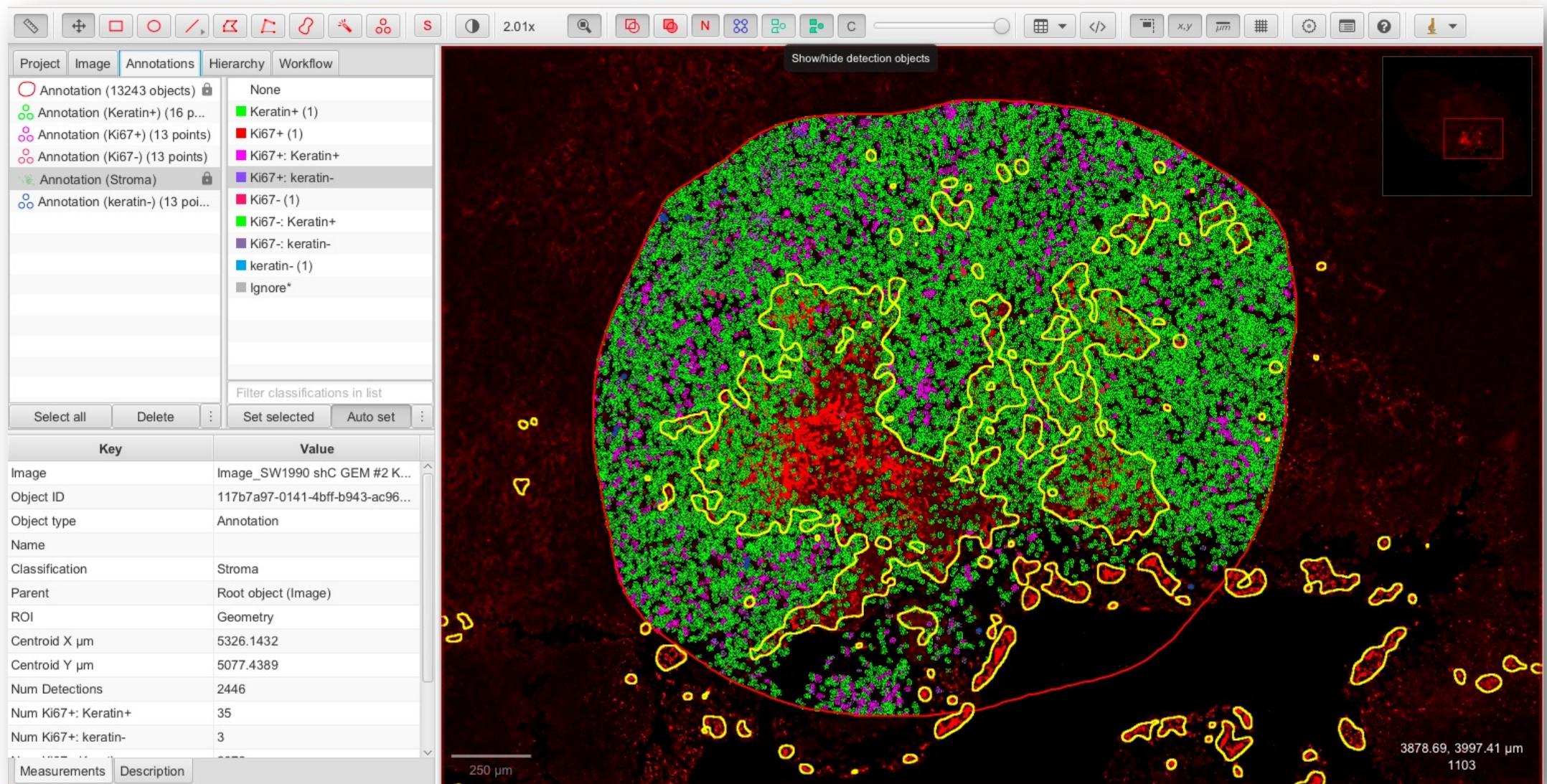


Create annotations from pixel classifier



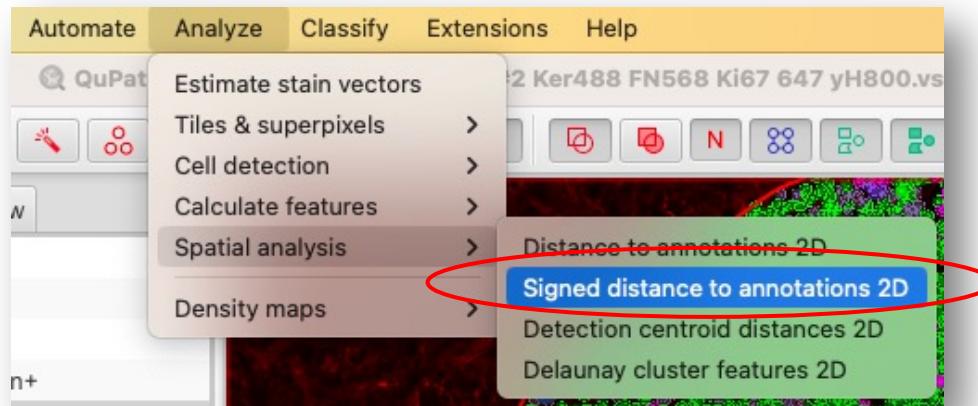
Notice the new annotation named
'Stroma' in the *Annotations* list

Fully annotated image



Spatial information: signed distance

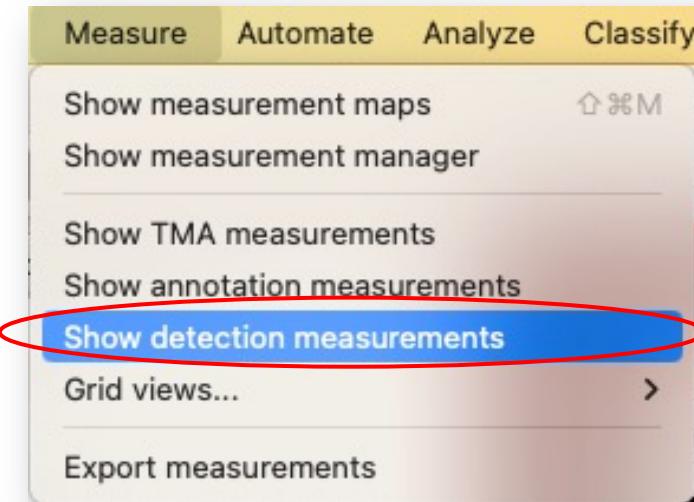
- *Analyze > Spatial analysis > Signed distance to annotations 2D*



- 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

