

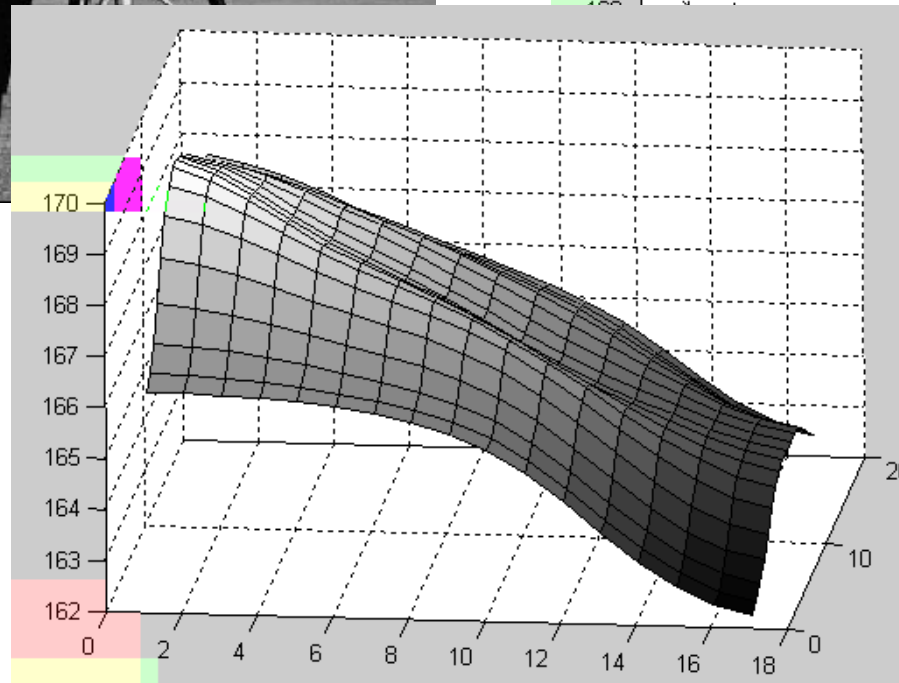
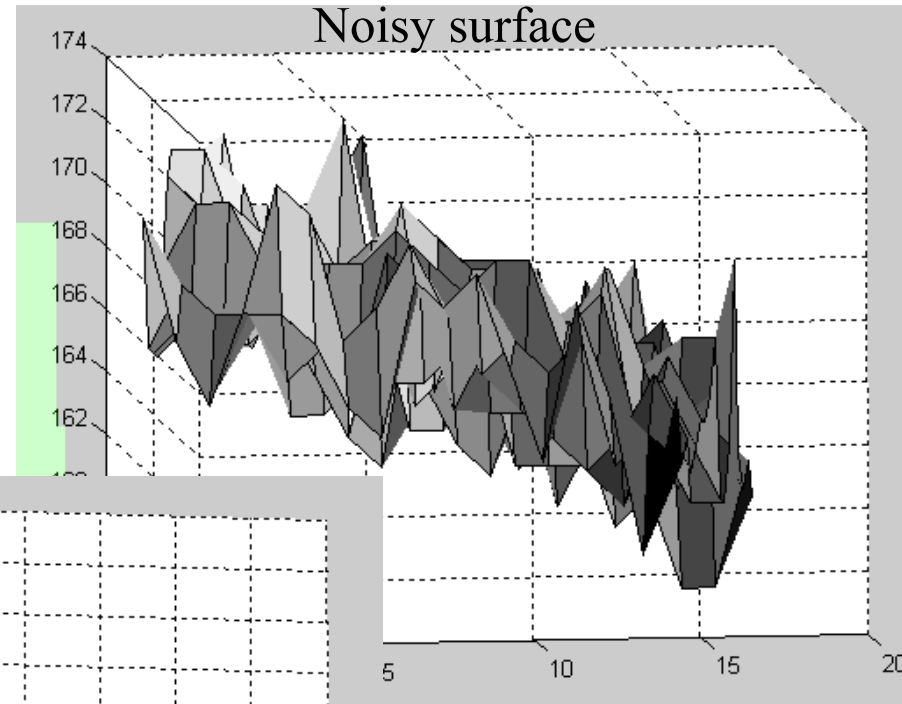
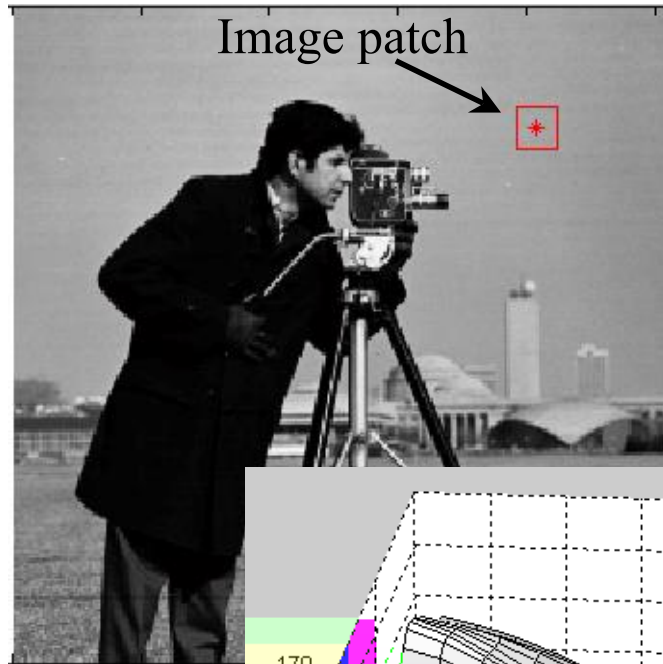
A fluorescence microscopy image of a tissue section, likely a histological slide, showing a dense, granular texture. The image is overlaid with large, stylized, semi-transparent letters in various colors (yellow, blue, purple, red, teal, green) that spell out 'QUANTH'. The text 'Automating tissue identification' is centered over the image in a white, sans-serif font. In the top right corner, there is a small inset image showing a magnified view of a specific region of the tissue, with a red box indicating the area of interest.

Automating tissue identification

But first, let's talk smoothing

Intermezzo aperto

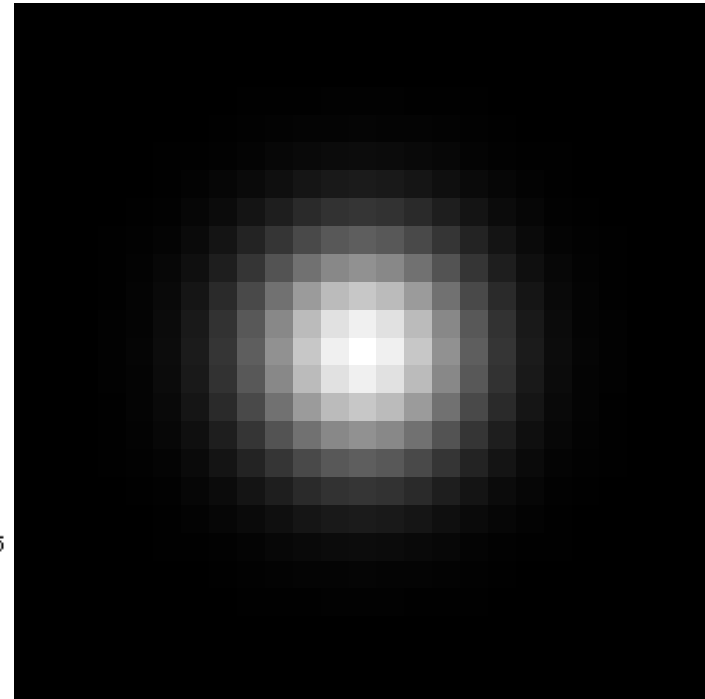
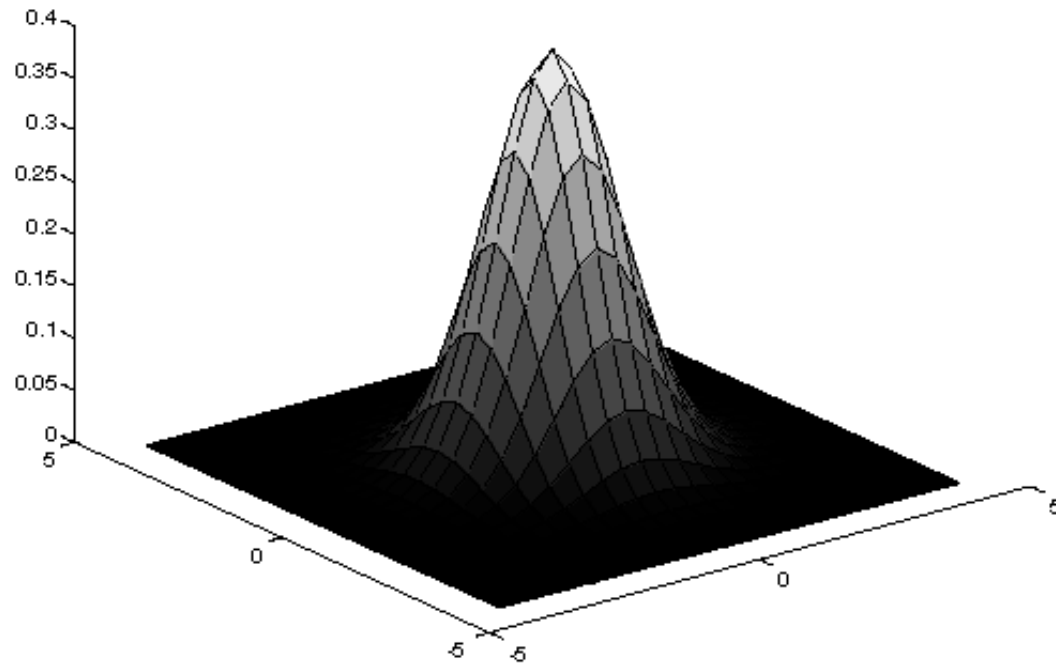
Today: Smoothing Reduces Noise



smoothing reduces noise,
giving us (perhaps) a more
accurate intensity surface.

Gaussian Smoothing Filter

An isotropic (circularly symmetric) Gaussian:



Gaussian Smoothing Example



original



$\sigma = 3$

Robert Collins
CSE486, Penn State

Gaussian Smoothing at Different Scales



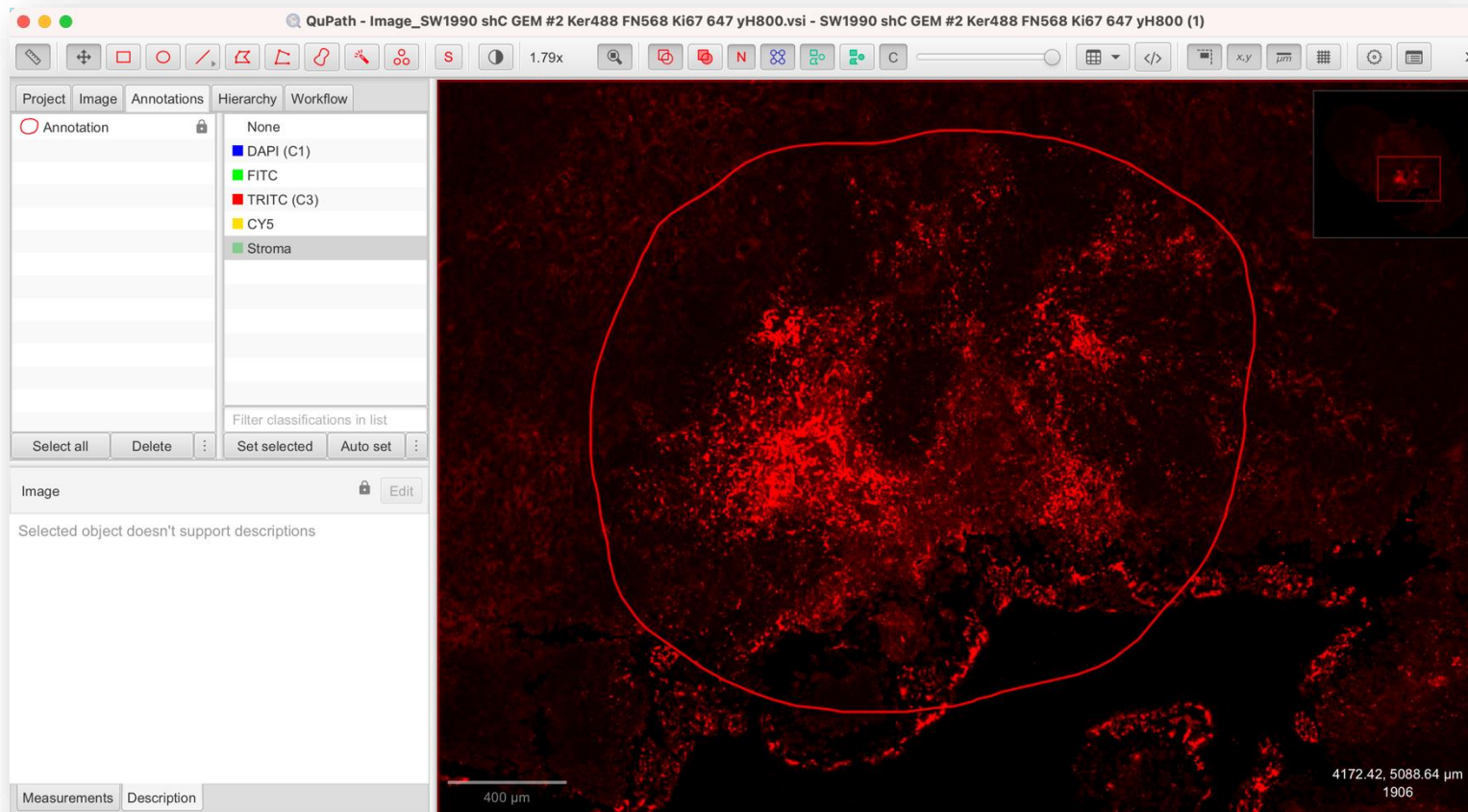
**Balancing act: smooth enough to “clean up”
the noise, but not so much as to remove
important image gradients.**

Back to QuPath

Intermezzo chiuso

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?**

The screenshot shows the QuPath software interface. The main window displays a histology image with a red overlay. The 'Create threshold' dialog box is open, showing settings for Resolution (Moderate (2.60 µm/px)), Channel (TRITC (C3)), Prefilter (Gaussian), Smoothing sigma (5), Threshold (3500), Above threshold (Stroma), Below threshold (Unclassified), Region (Any annotation ROI), and Classifier name (stroma_classifier). The 'Save' button is highlighted with a red circle. The 'Classify' menu is also visible, with the 'Create threshold' option highlighted with a red circle.

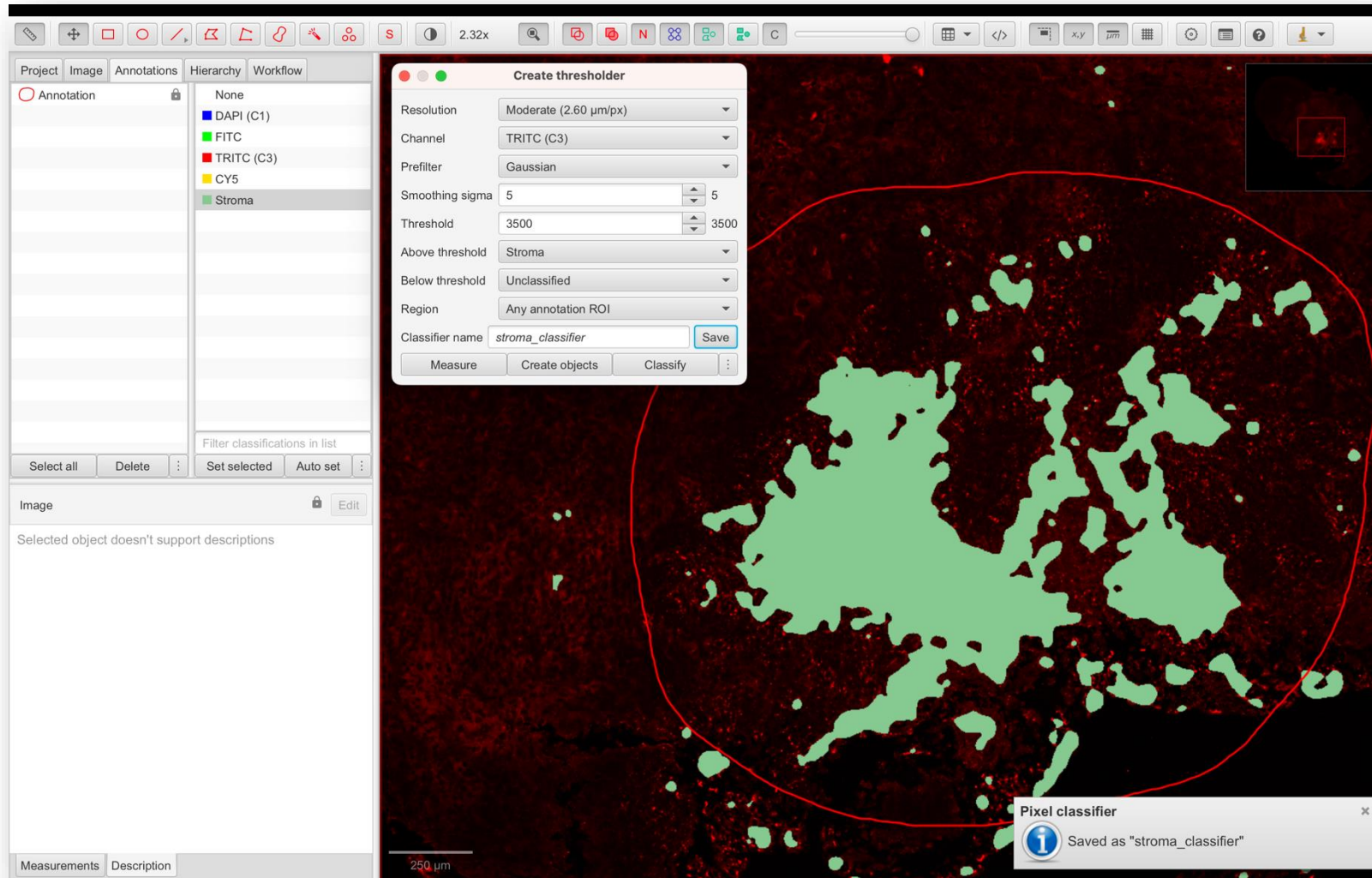
Resolution: trade-off between details and computational cost

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 threshold to use it!

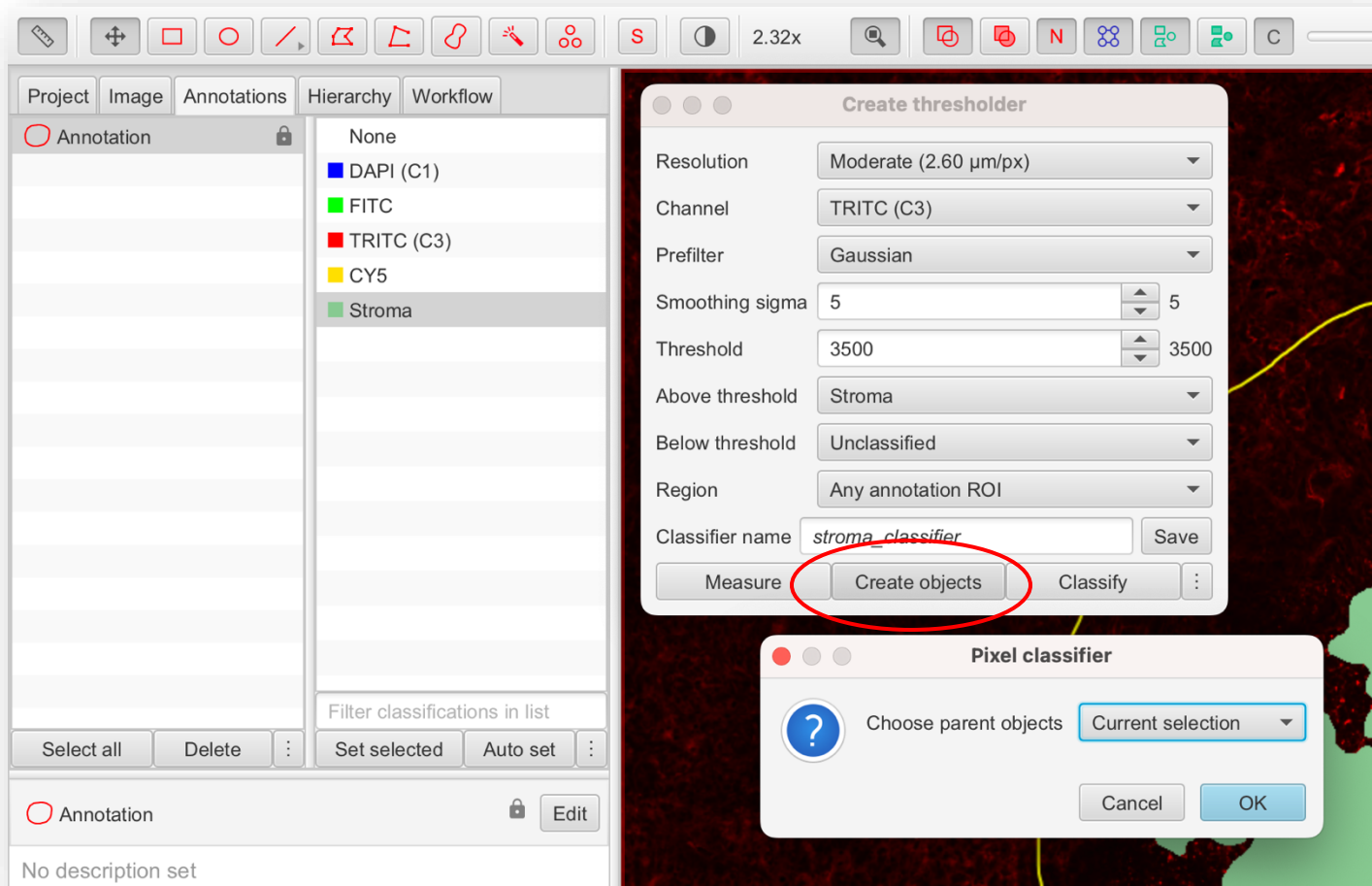
Interactive visualization of thresholding results



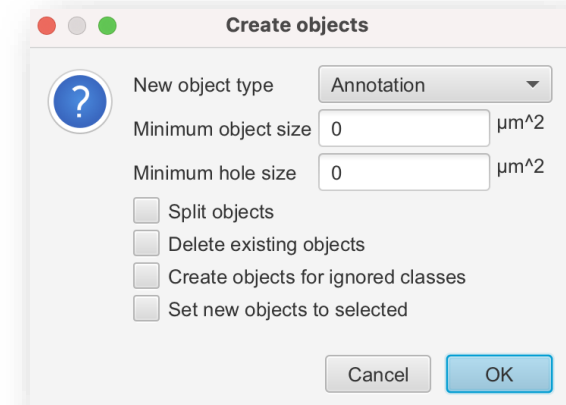
Create a class
'Stroma'

Try varying the value
of the different
parameters!

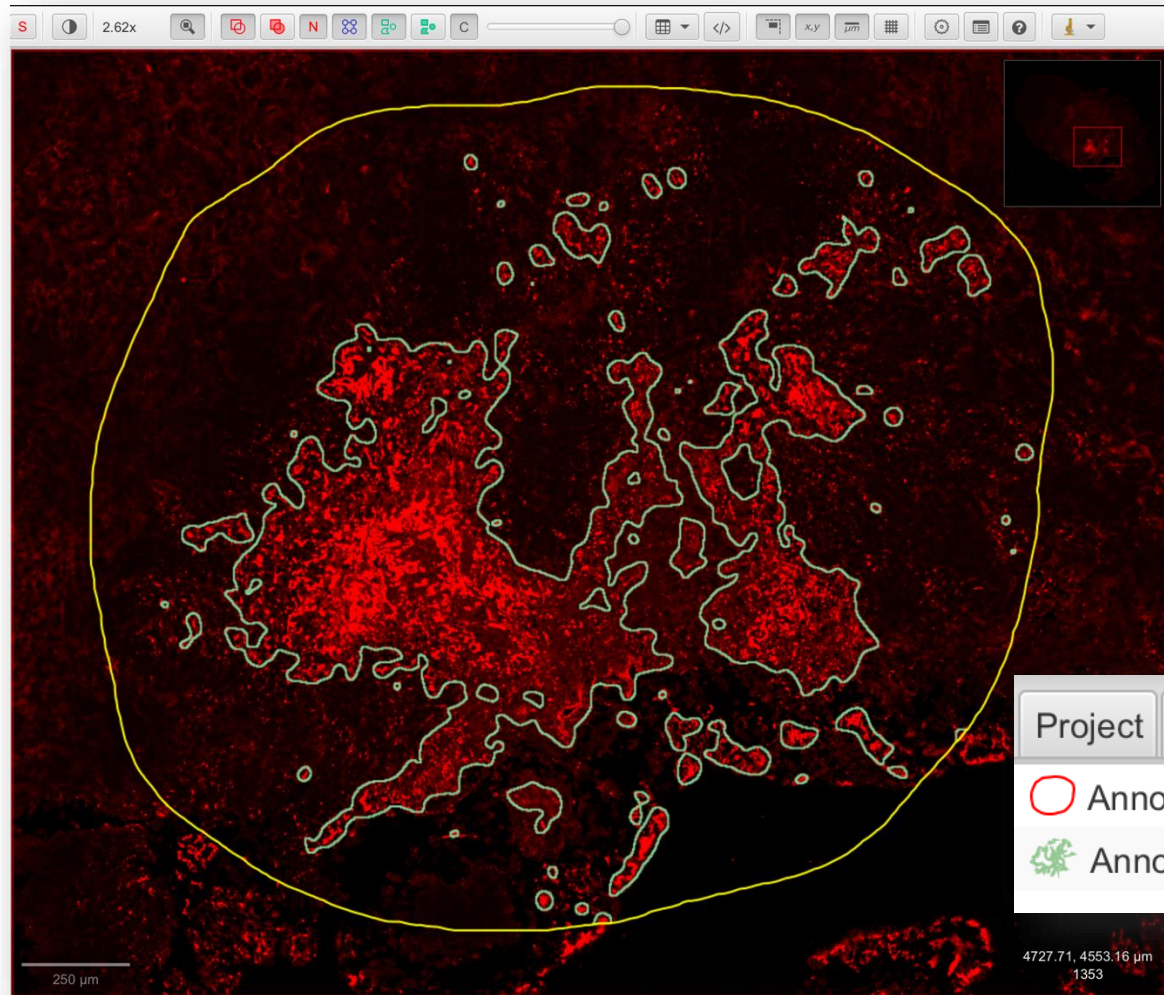
Create annotations from pixel classifier



- 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

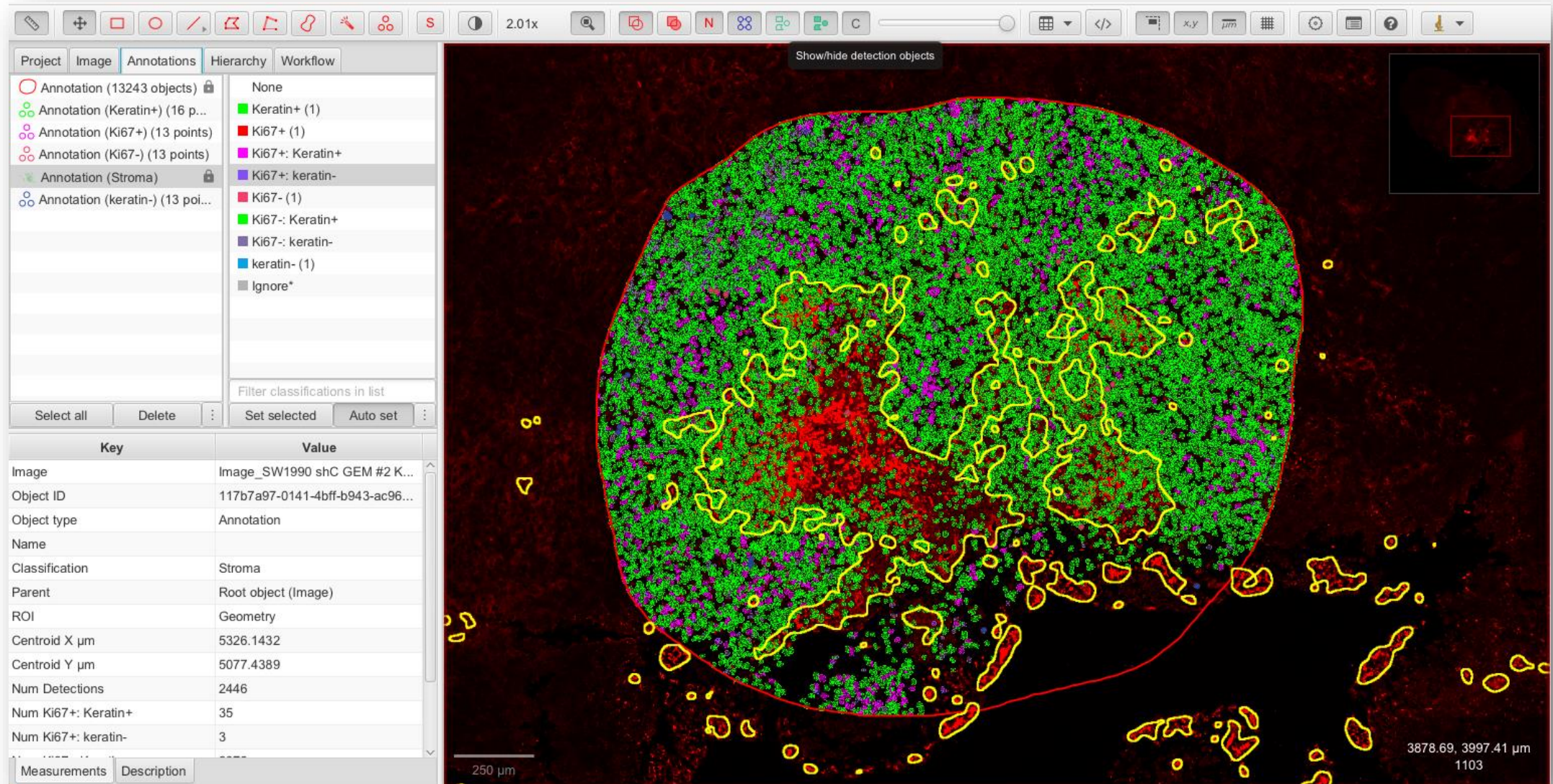


Create annotations from pixel classifier



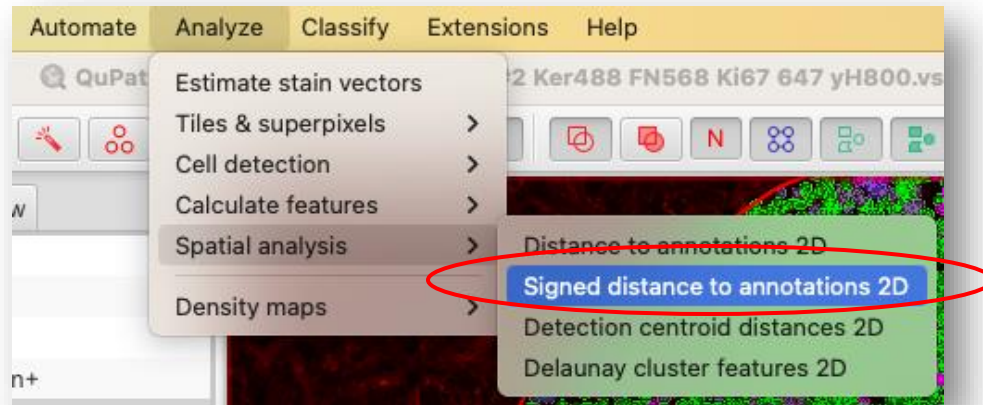
Notice the new annotation named
'Stroma' in the *Annotations* list

Fully annotated image



Spatial information: signed distance

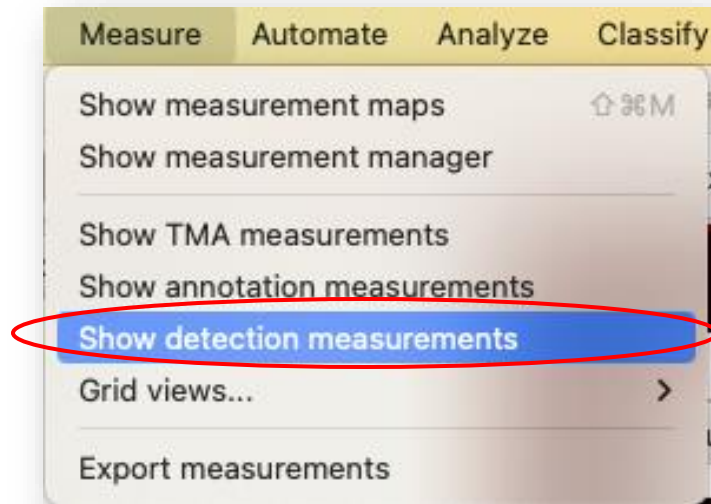
- *Analyze > Spatial analysis > Signed distance to annotations 2D*



- 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**

