

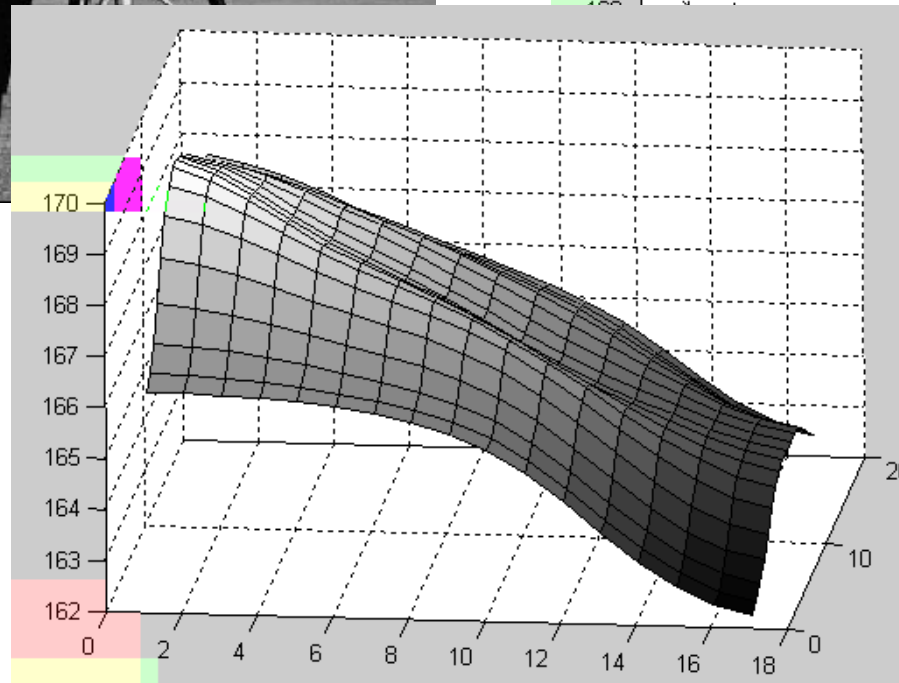
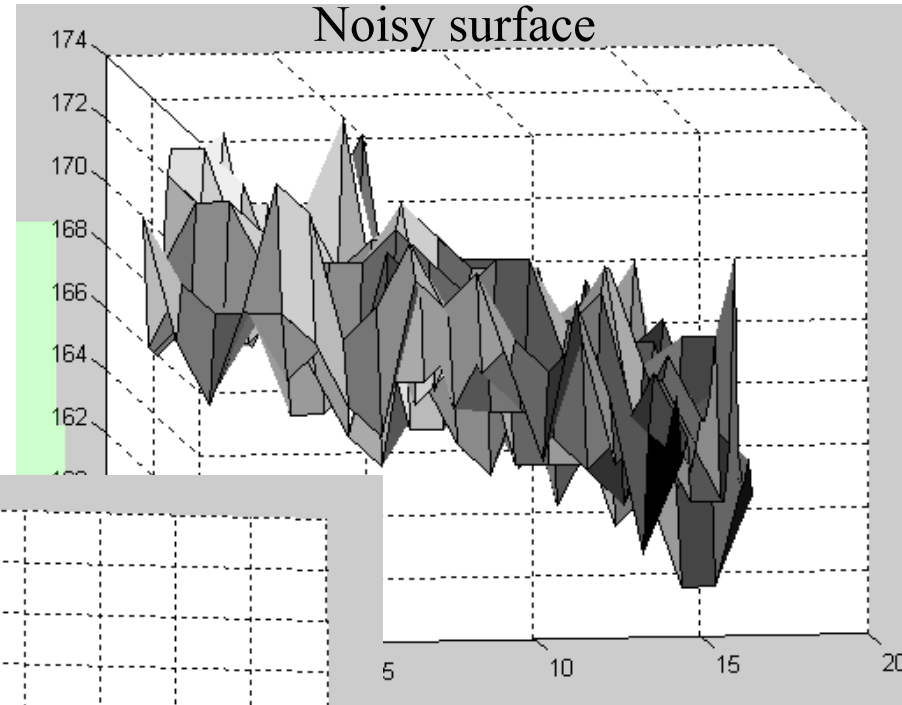
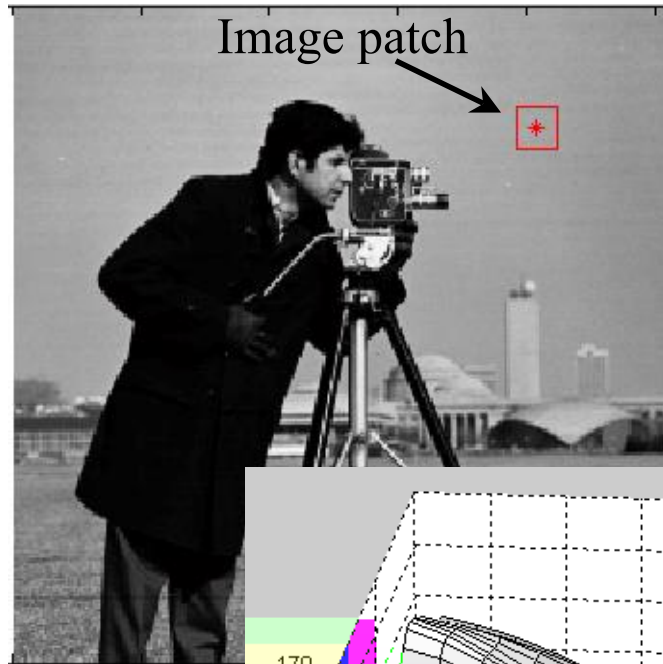
A fluorescence microscopy image of a tissue section, likely a histological slide, showing a dense, granular texture. The image is overlaid with large, stylized, semi-transparent letters in various colors (yellow, blue, purple, red, teal, green) that spell out 'QUANTH'. The text 'Automating tissue identification' 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

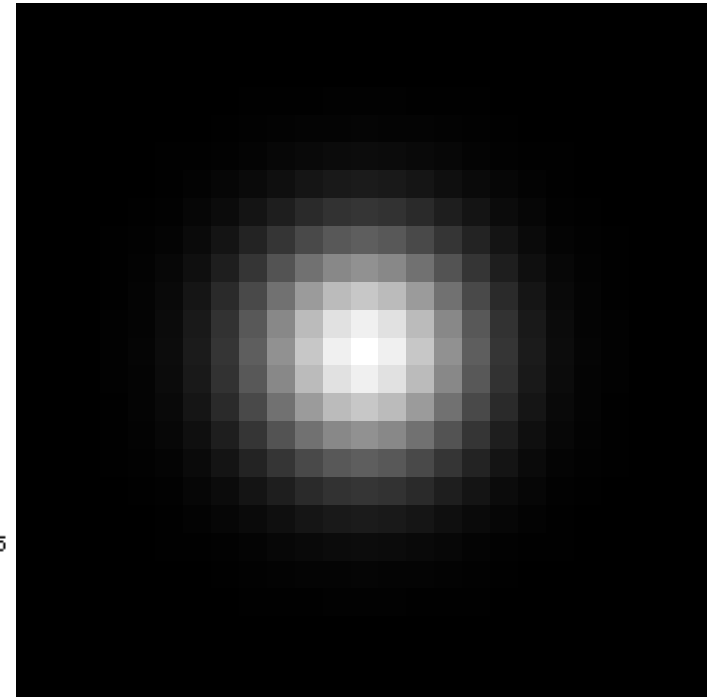
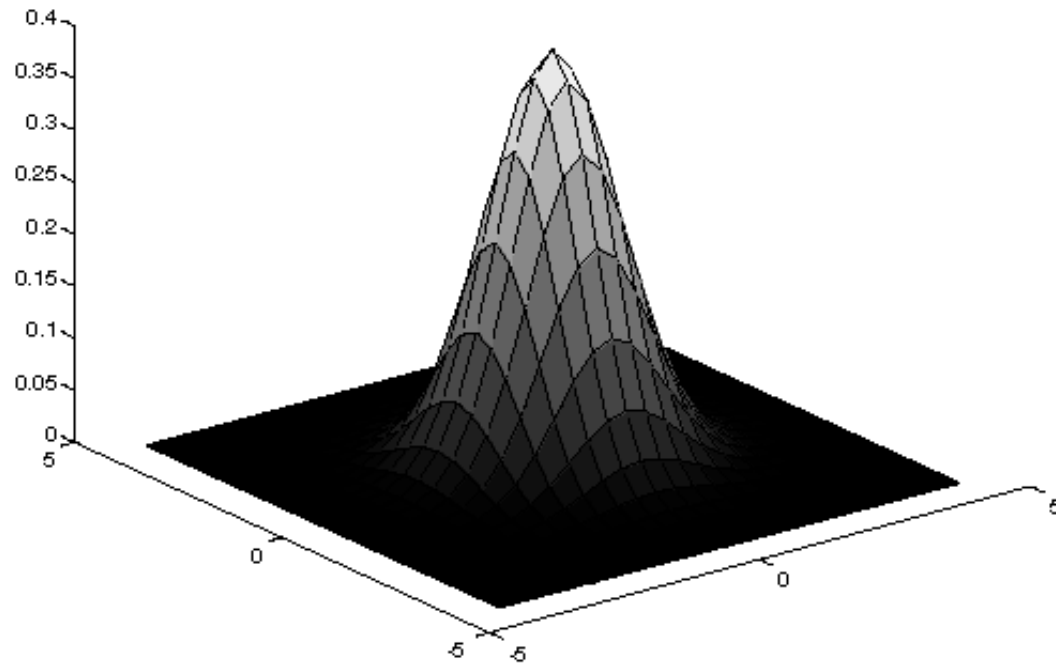
Today: Smoothing Reduces Noise



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



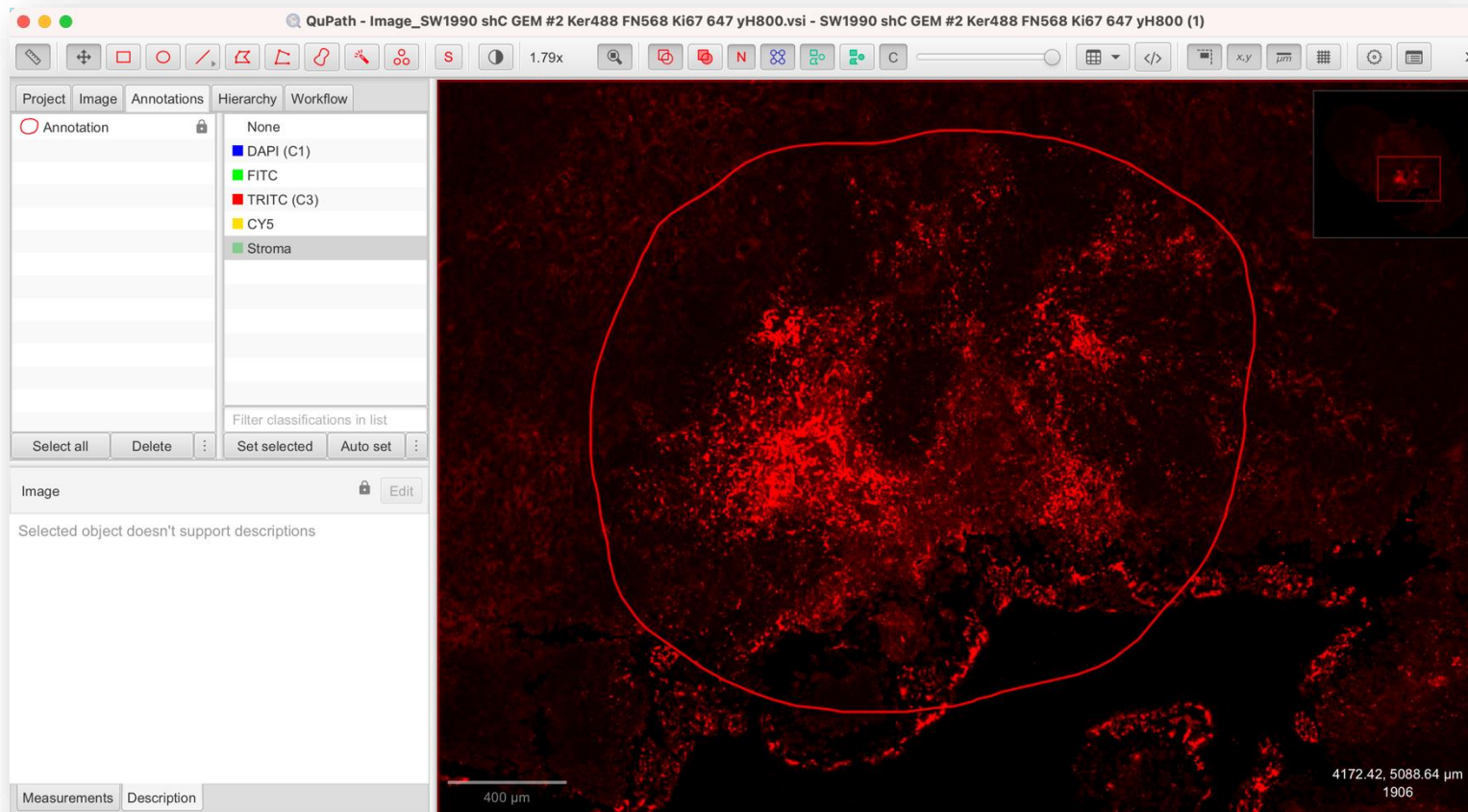
**Balancing act: smooth enough to “clean up”
the noise, but not so much as to remove
important image gradients.**

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. A 'Create threshold' dialog box is open in the center, with fields 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 circled in red. To the right, the 'Classify' menu is open, showing options like 'Load pixel classifier', 'Train pixel classifier', and 'Create threshold', with the latter circled in red. On the left, the 'Annotations' panel shows a list of channels: DAPI (C1), FITC, TRITC (C3), and CY5. Red arrows point from text annotations to specific parts of the interface: one to the 'Resolution' field, one to the 'Region' dropdown, one to the 'Smoothing sigma' field, and one to the 'Create threshold' button in the menu.

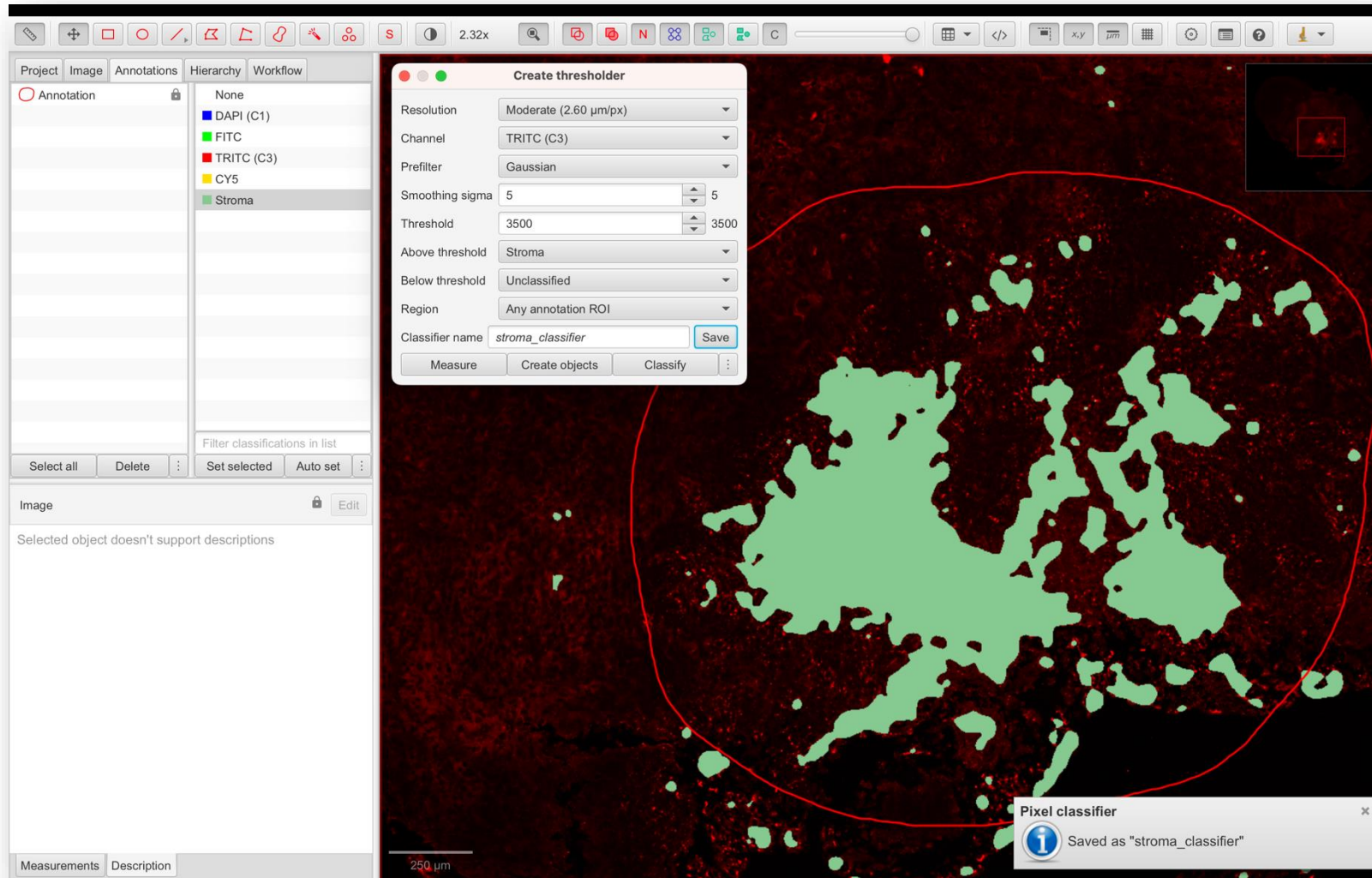
Resolution: trade-off between details and computational cost

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!

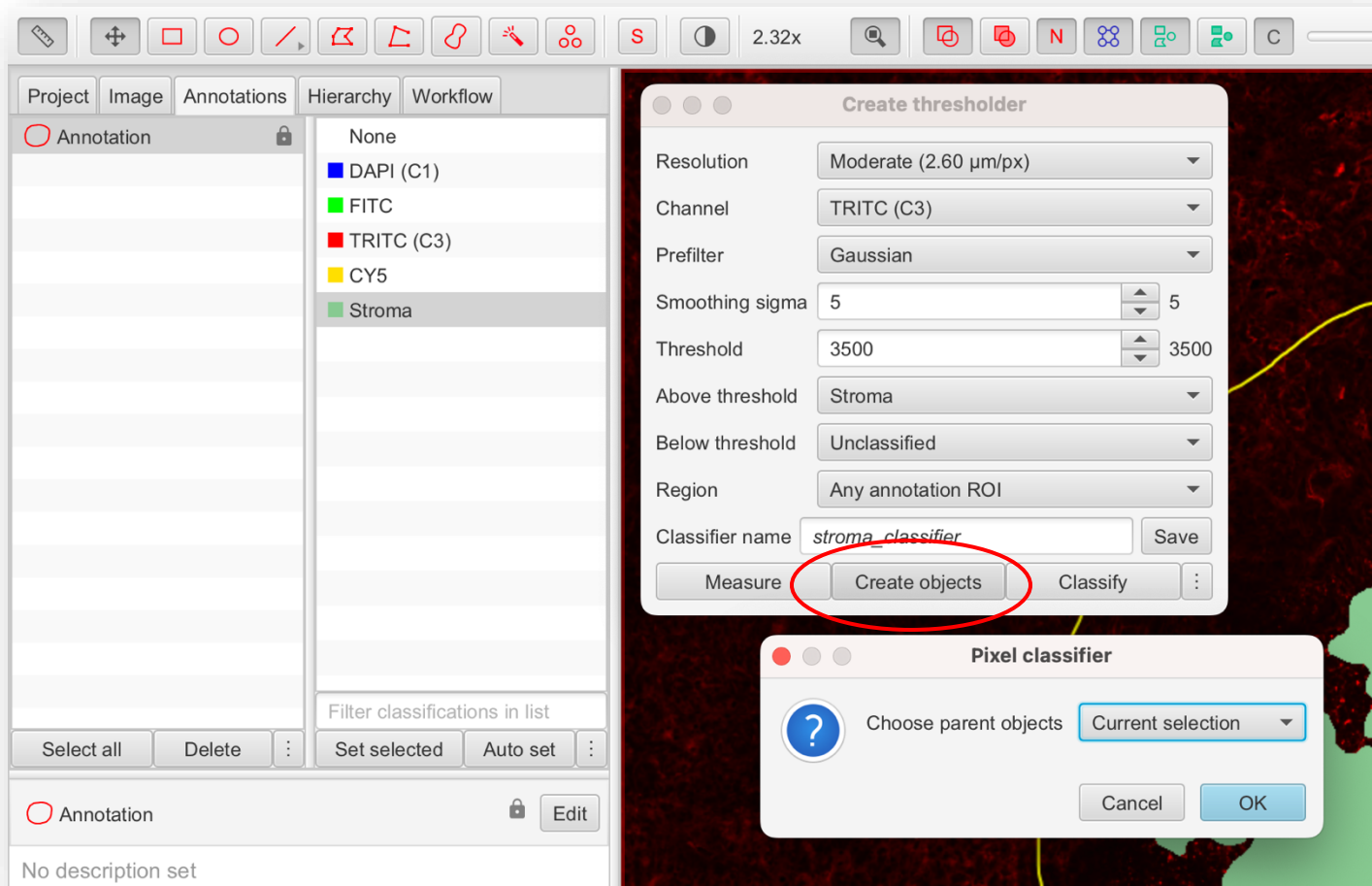
Interactive visualization of thresholding results



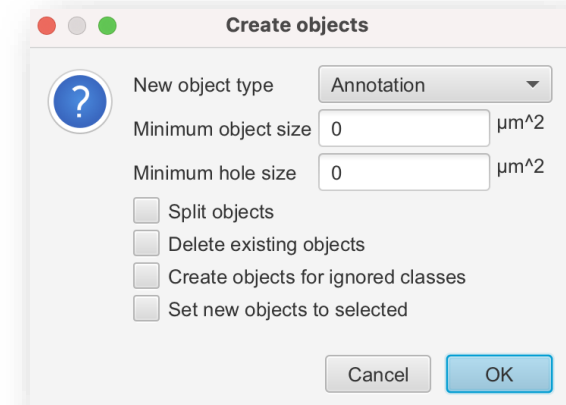
Create a class
'Stroma'

Try varying the value
of the different
parameters!

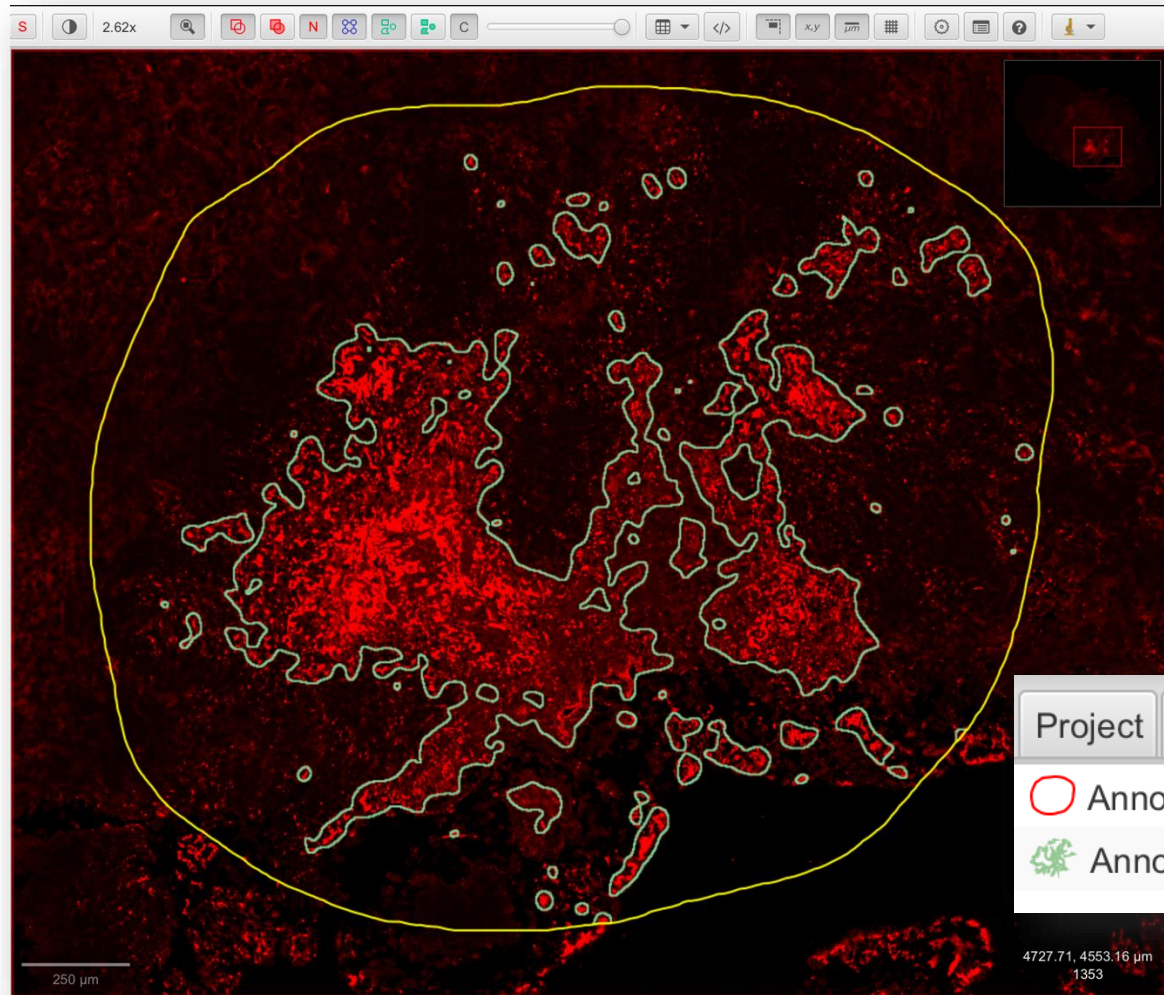
Create annotations from pixel classifier



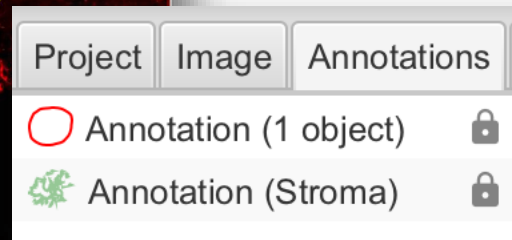
- Real-time visualization of results, once happy with it:
 1. Save your thresholder
 2. Select ROI
 3. Click *Create objects*
 4. Keep default parameters > OK



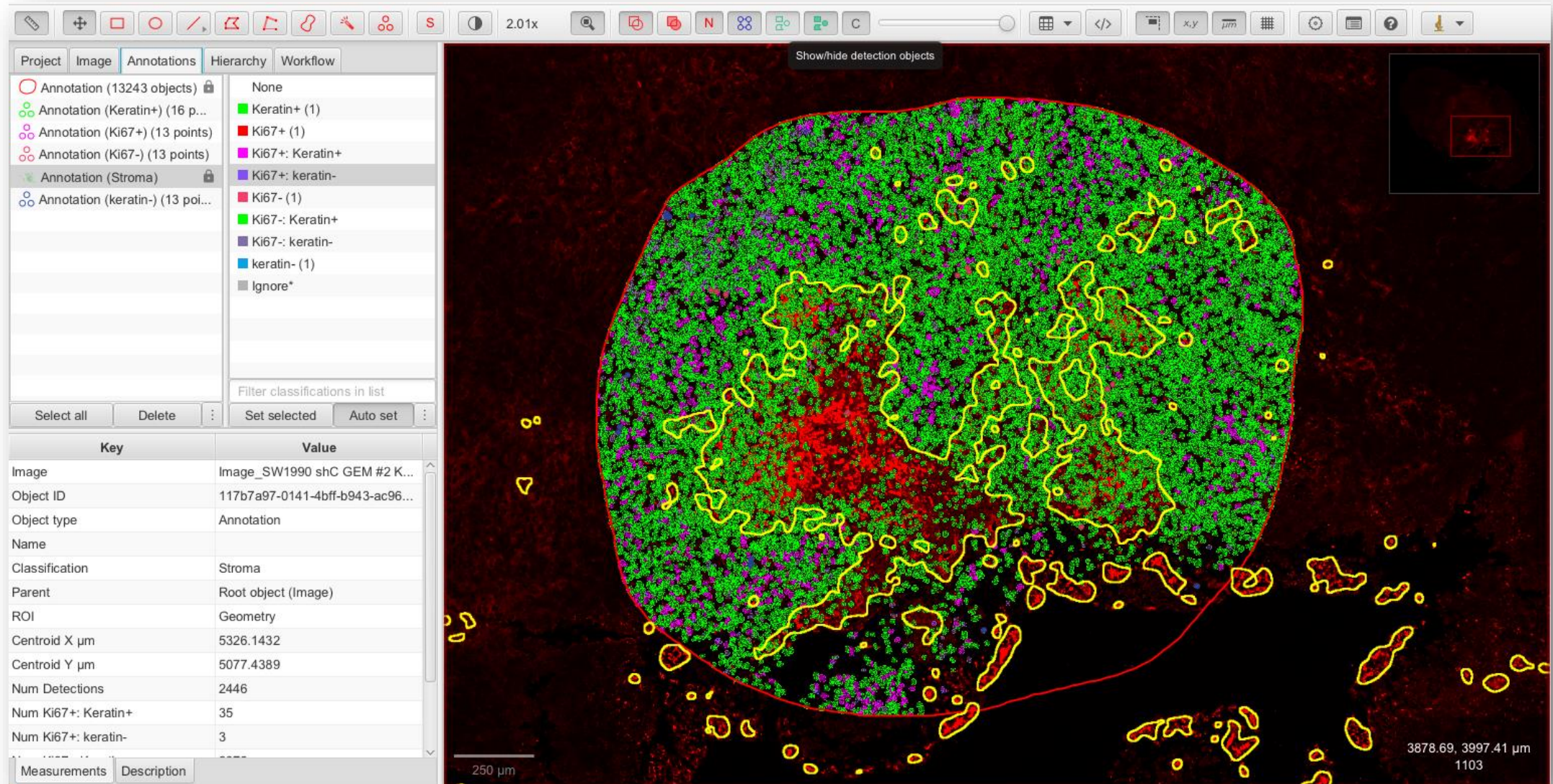
Create annotations from pixel classifier



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

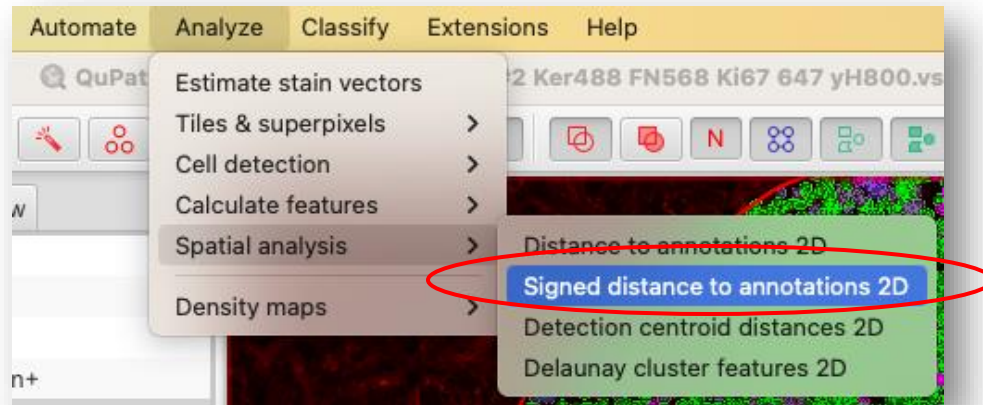


Fully annotated image



Spatial information: signed distance

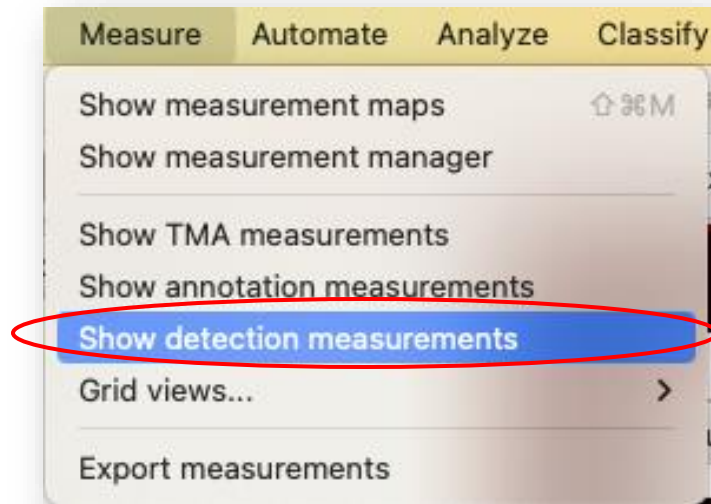
- *Analyze > Spatial analysis > Signed distance to annotations 2D*



- 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**

