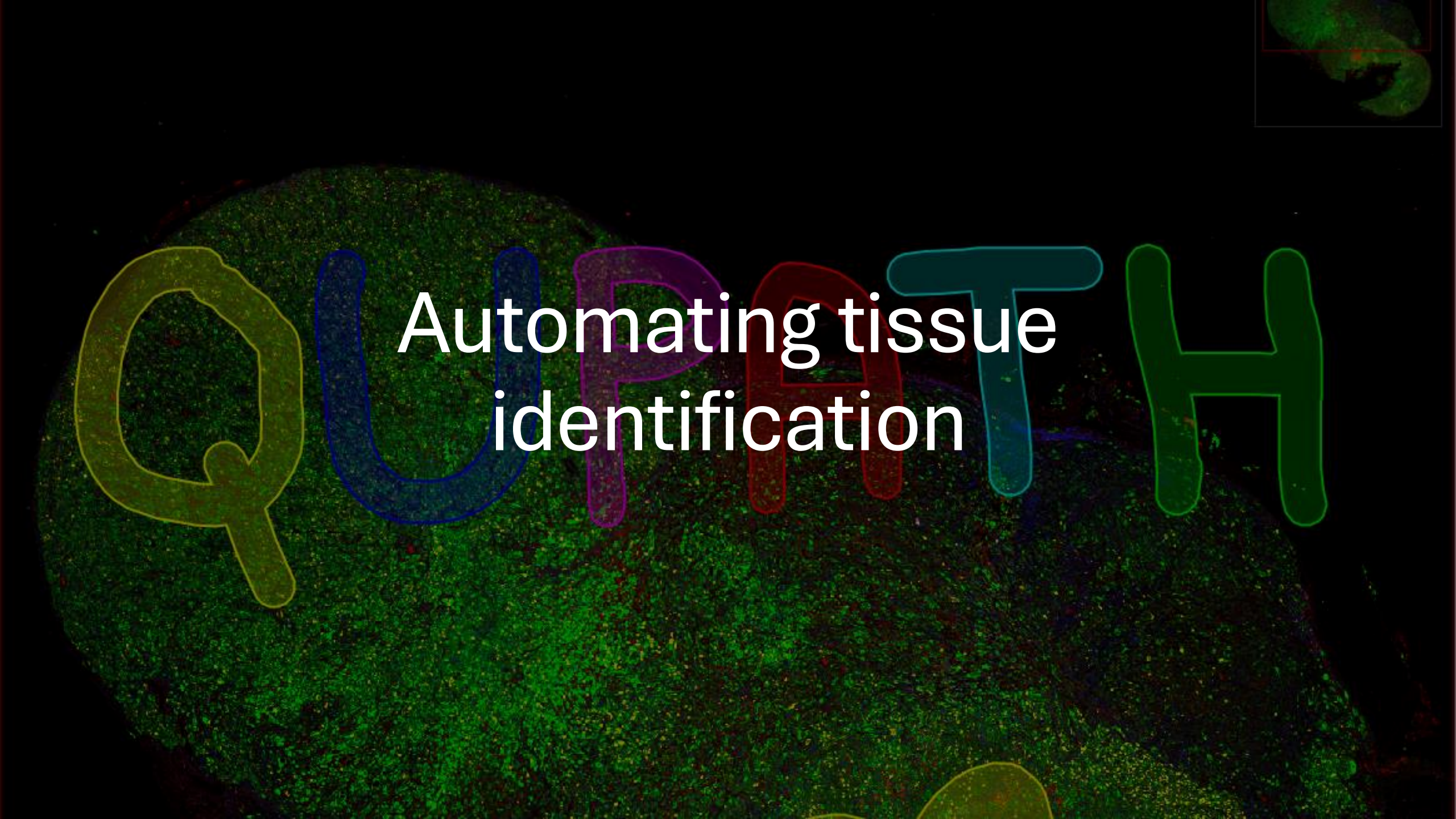

Automating tissue identification

But first, let’s talk smoothing

Intermezzo aperto

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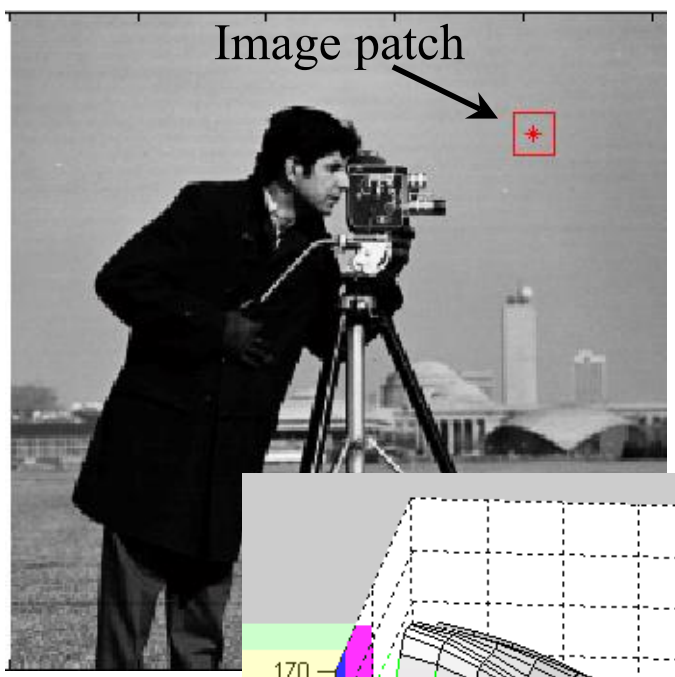
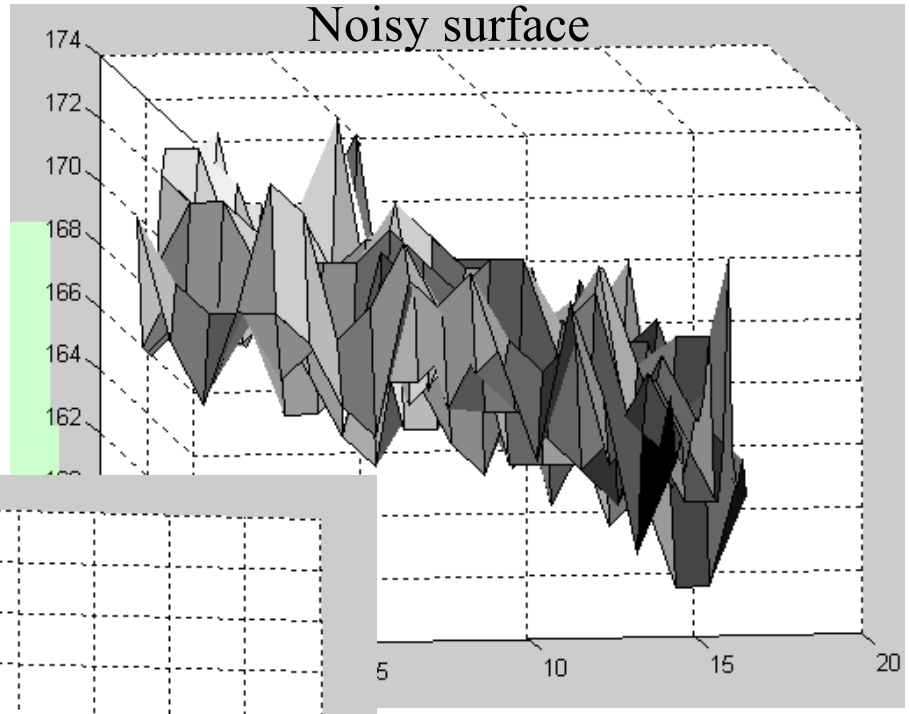
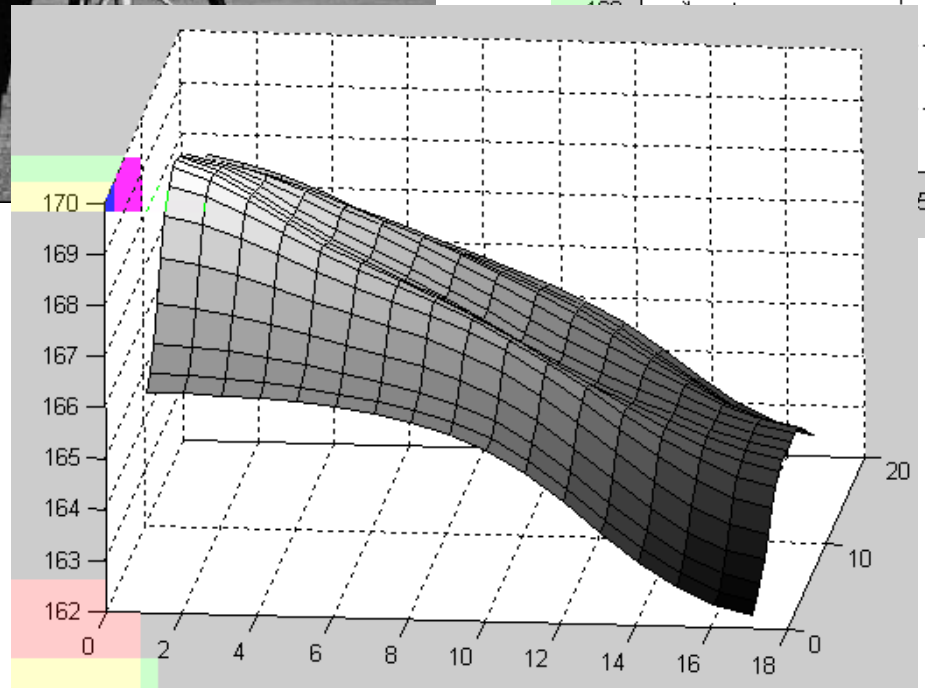
CSE486, Penn State

Today: Smoothing Reduces Noise







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

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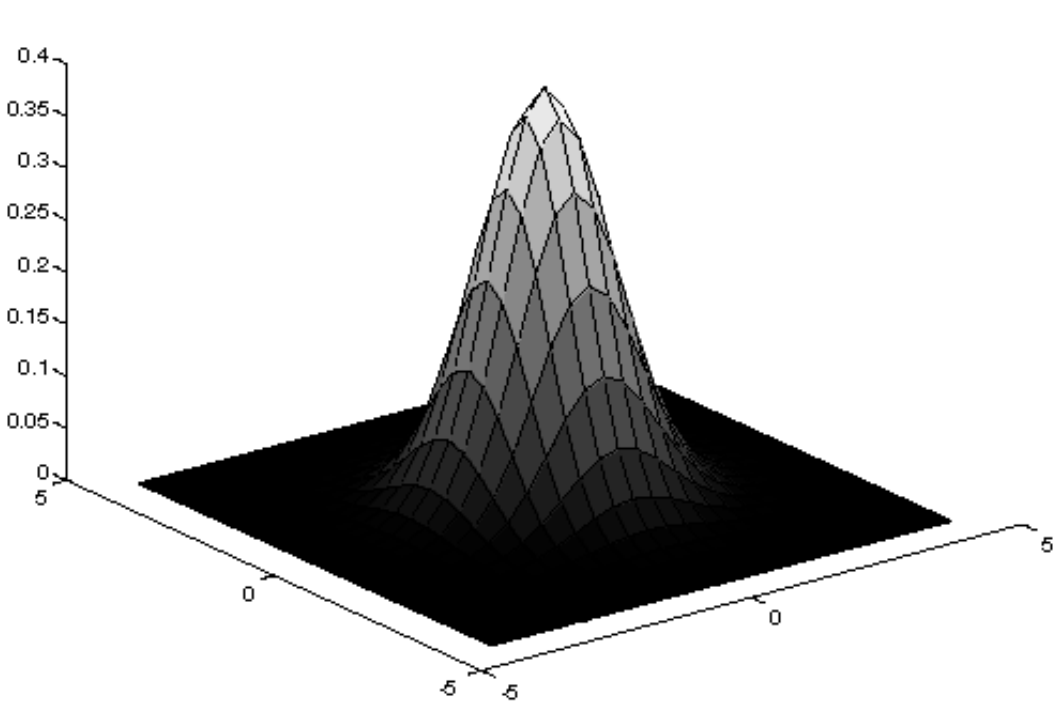
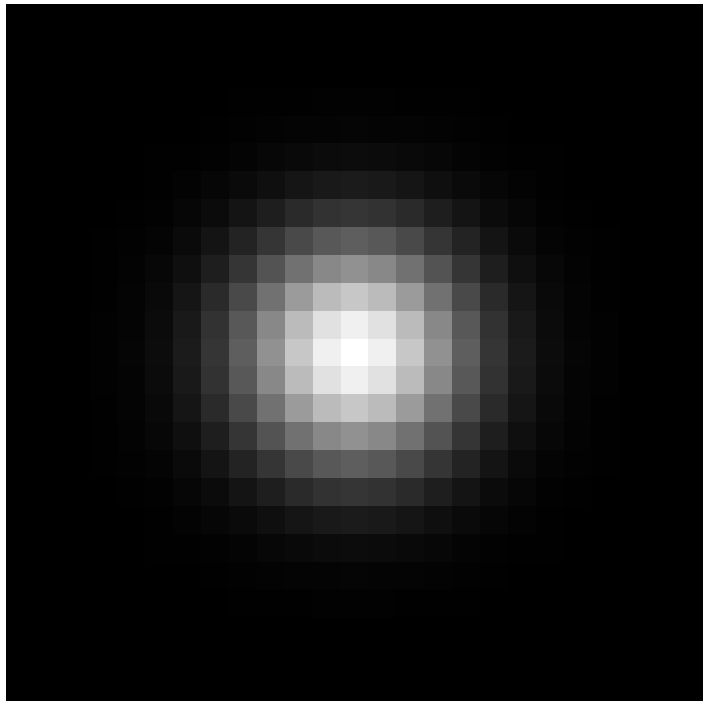
Gaussian Smoothing Filter

An isotropic (circularly symmetric) Gaussian:





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CSE486, Penn State

Gaussian Smoothing Example

original

sigma = 3

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Gaussian Smoothing at Different Scales

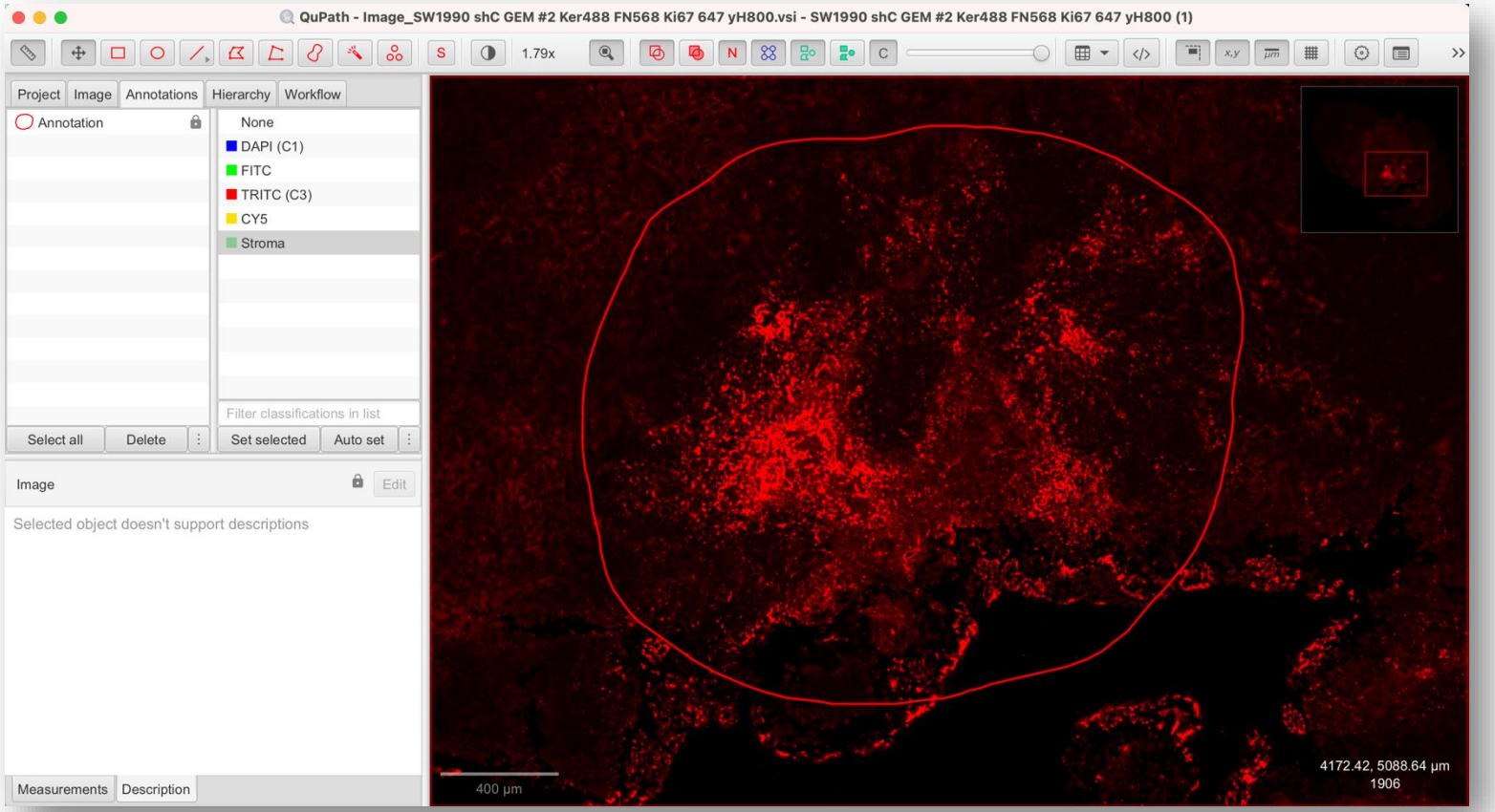
Balancing act: smooth enough to “clean up” the noise, but not so much as to remove important image gradients.

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:

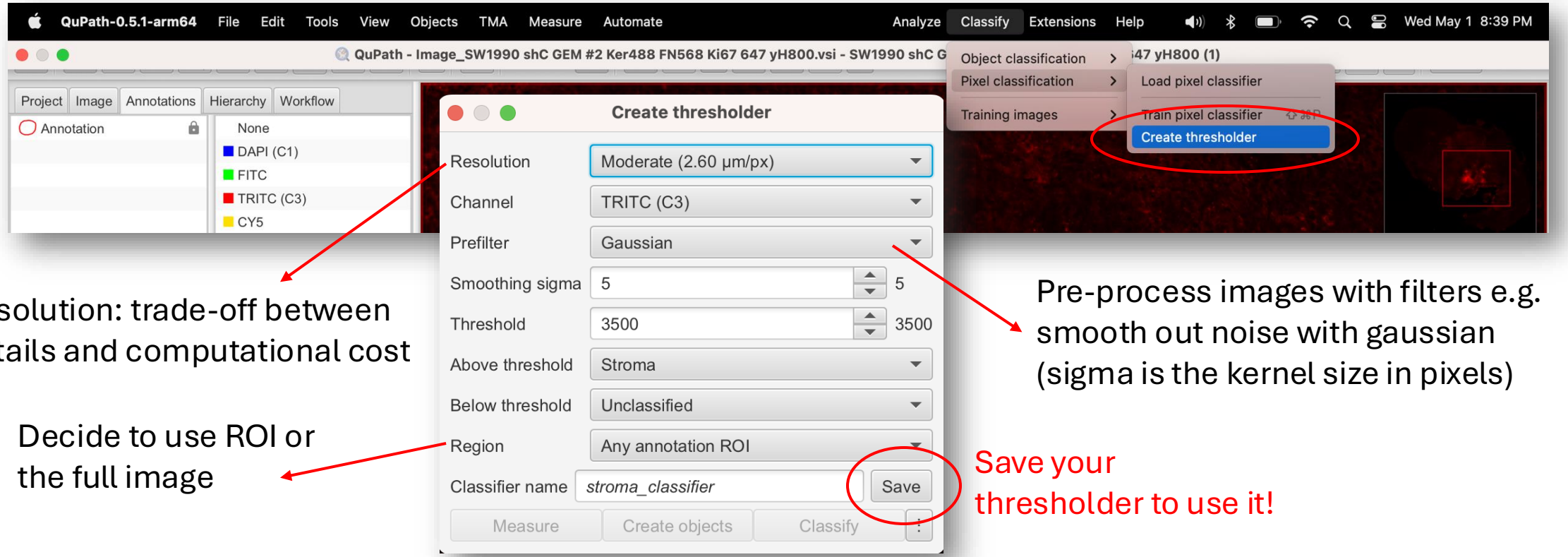
Right-click in the viewer > *Annotations* > *Lock*

or

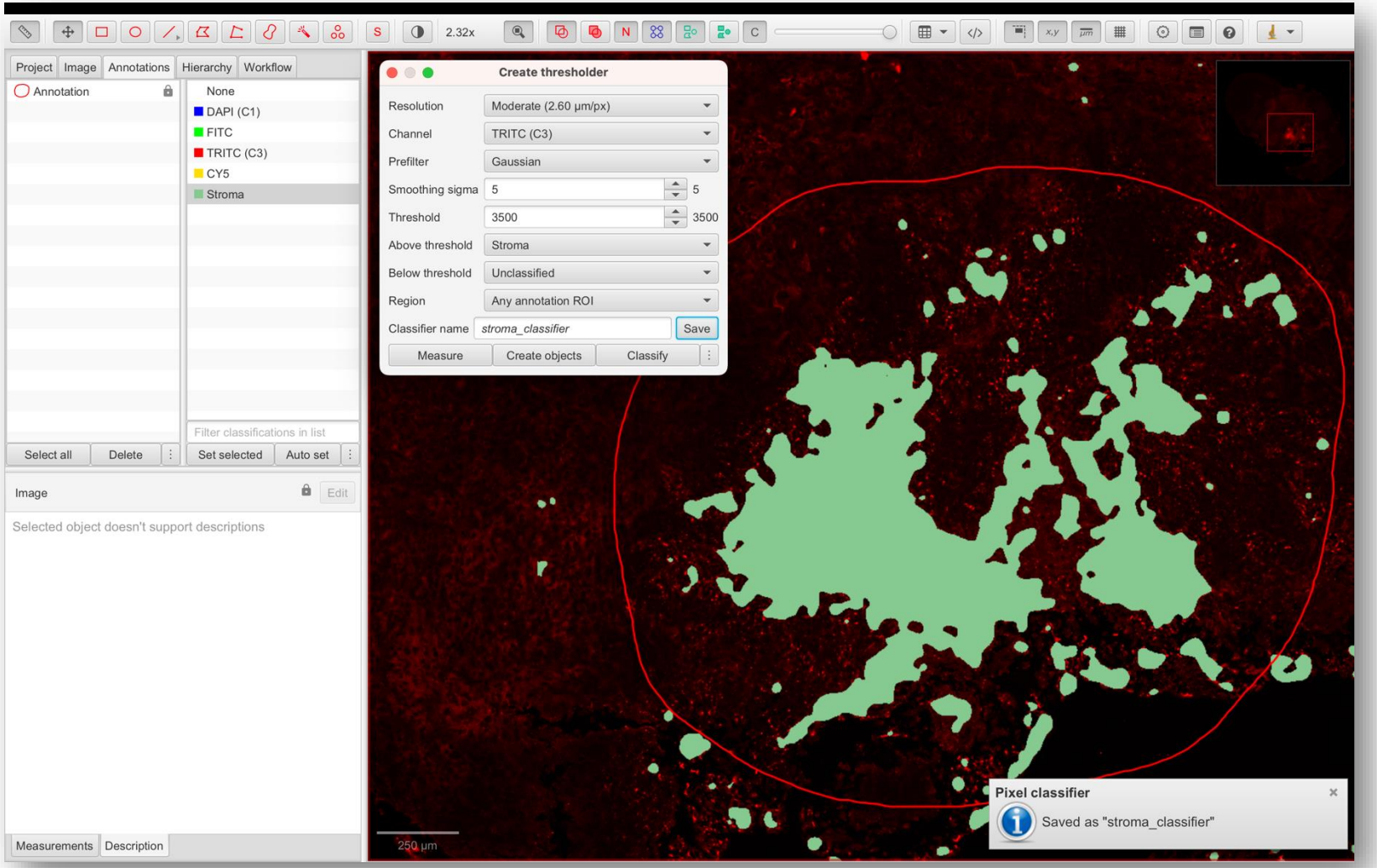
Right-click on the annotation in the analysis panel > *Lock*

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?**



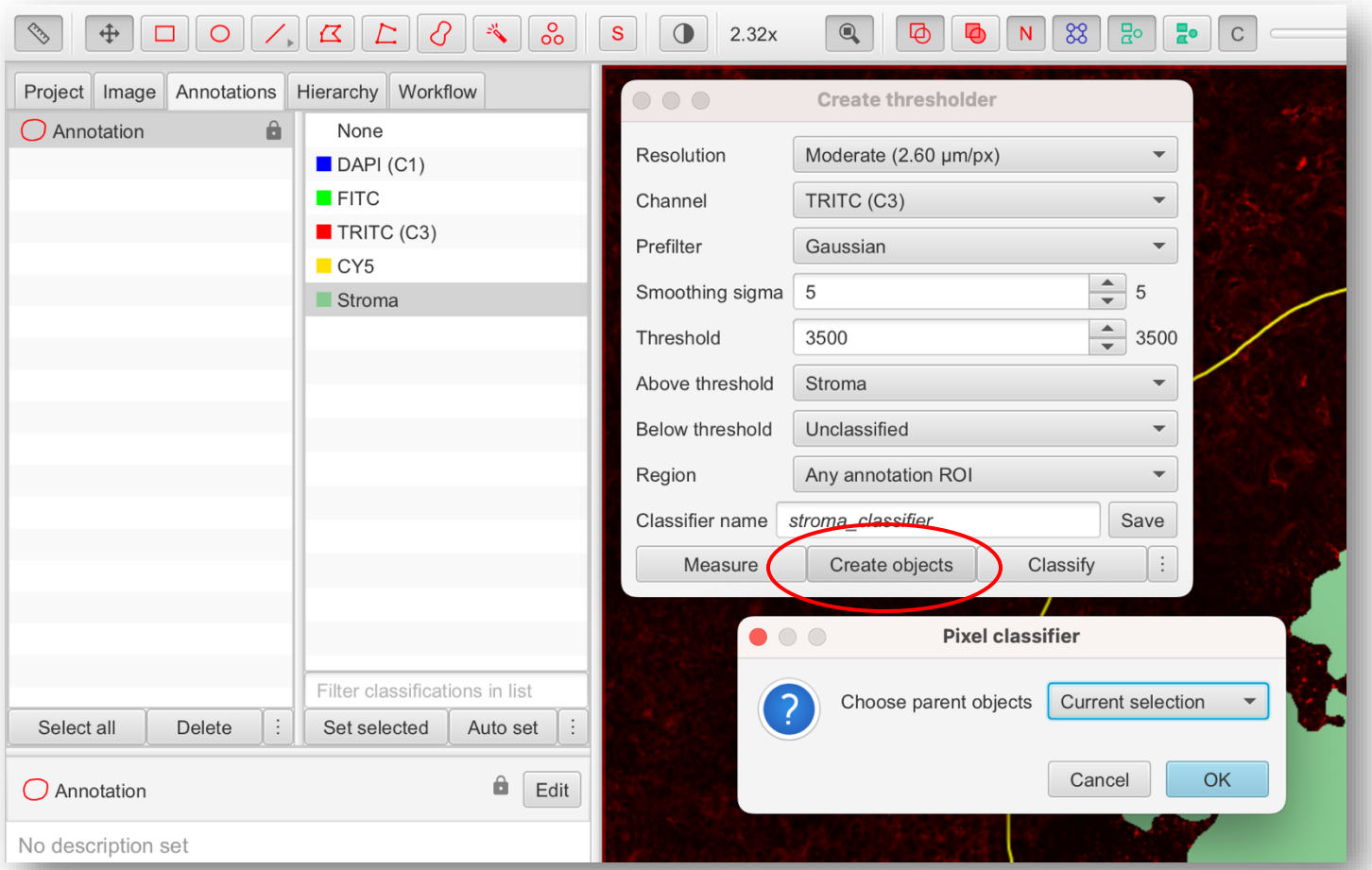
Interactive visualization of thresholding results



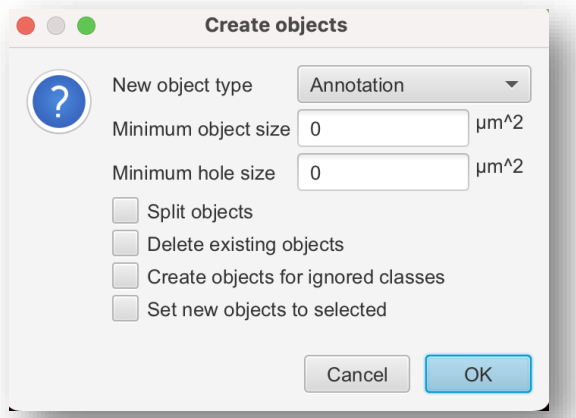
Create a class ‘Stroma’

Try varying the value of the different parameters!

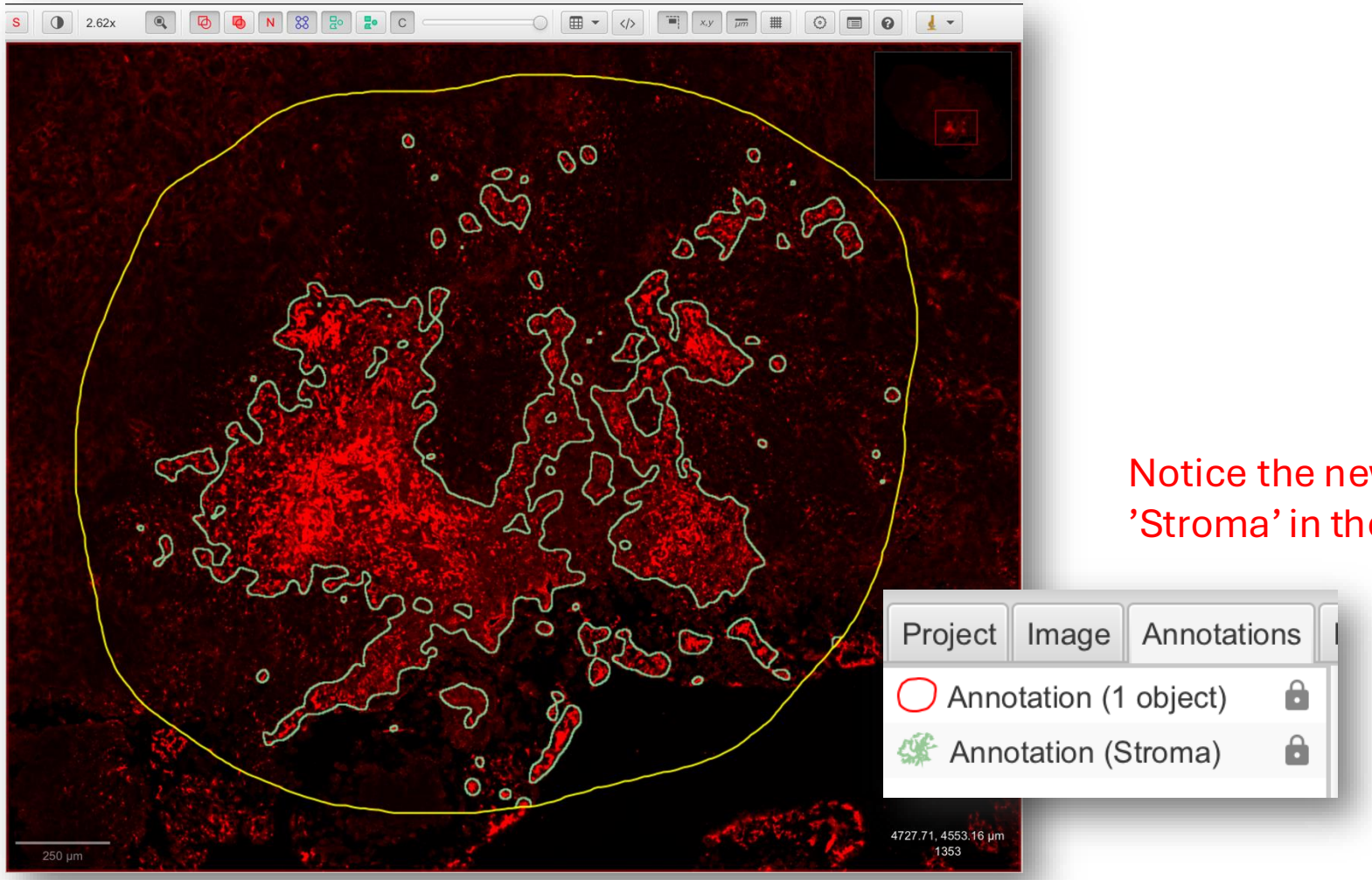
Create annotations from pixel classifier



- 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

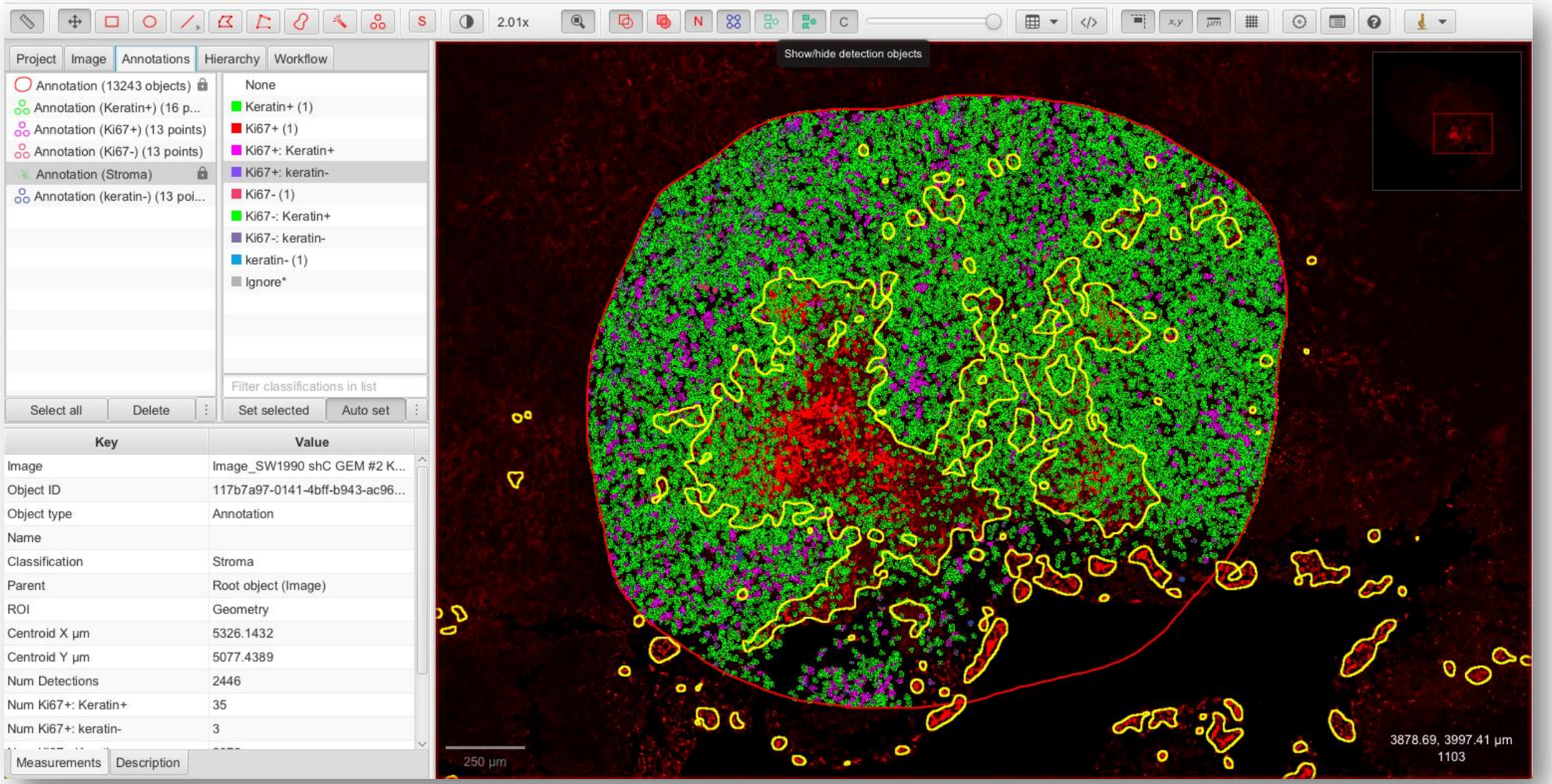


Create annotations from pixel classifier



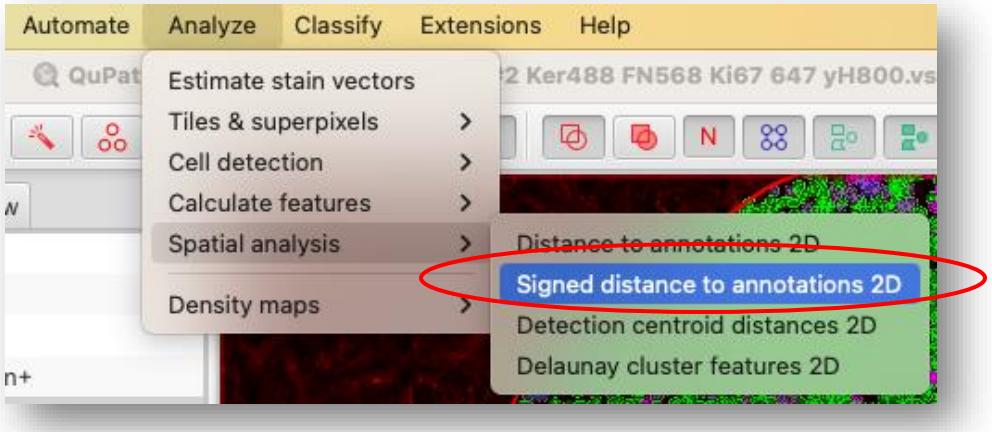
Notice the new annotation named 'Stroma' in the *Annotations* list

Fully annotated image



Spatial information: signed distance

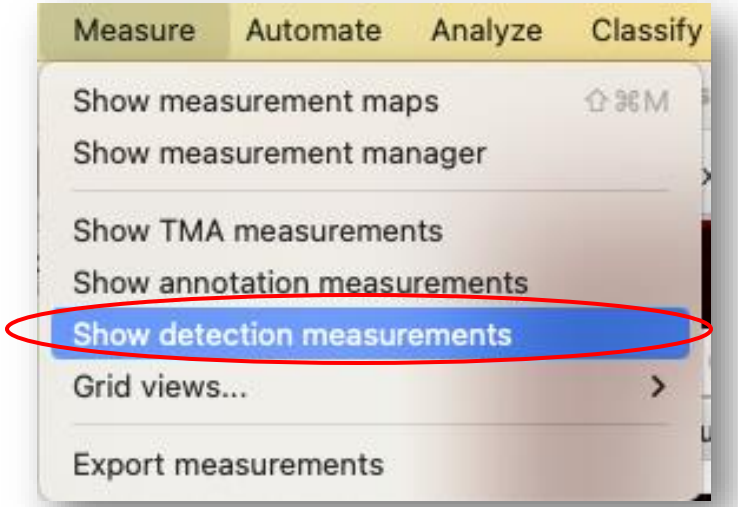
- *Analyze > Spatial analysis > Signed distance to annotations 2D*



- 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