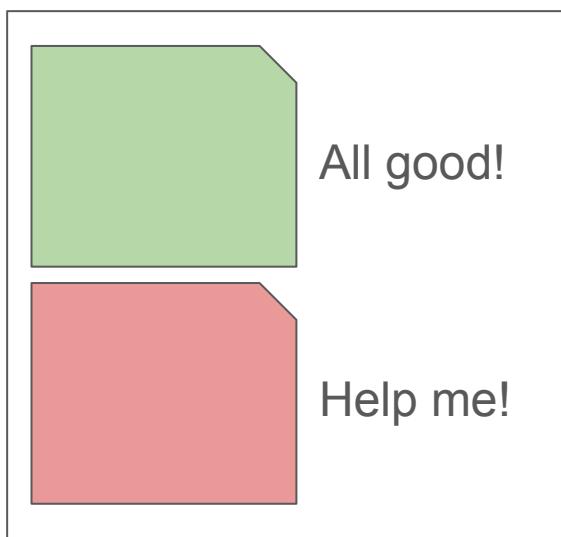


Required dataset:

- QuPath dataset: CMU-1, OS-2 images
- TPecot dataset: Prostate-serie, Polip_fluo
- Pasteur dataset: Uterine_mucosa, Necker_cells_BF



https://github.com/jpylvanainen/Image_analysis_course_24

Bioimage Analysis
for Quantitative Microscopy

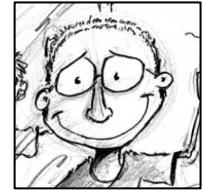
Image Analysis course for bioscientists by the Jacquemet lab

Mon Sep 30th - Fri Oct 4th 2024, Turku, Finland
9-17 every day

QuPath : Framework for large image analysis

Stéphane Rigaud - Image Analysis Hub - Institut Pasteur, Paris
(Course include material from *Thierry Pécot* and *Gaëlle Letort*)

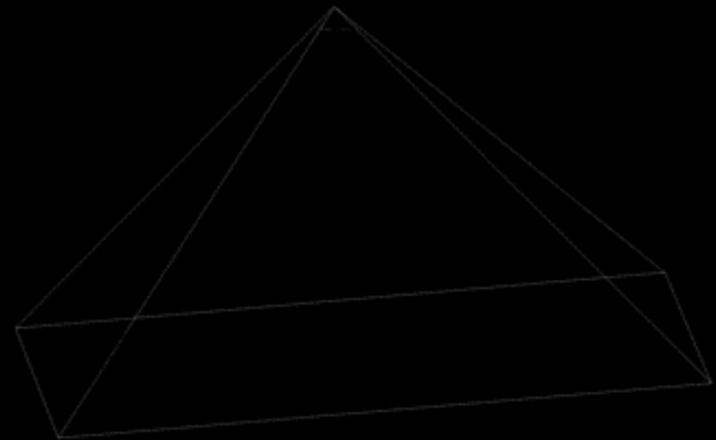
What is QuPath

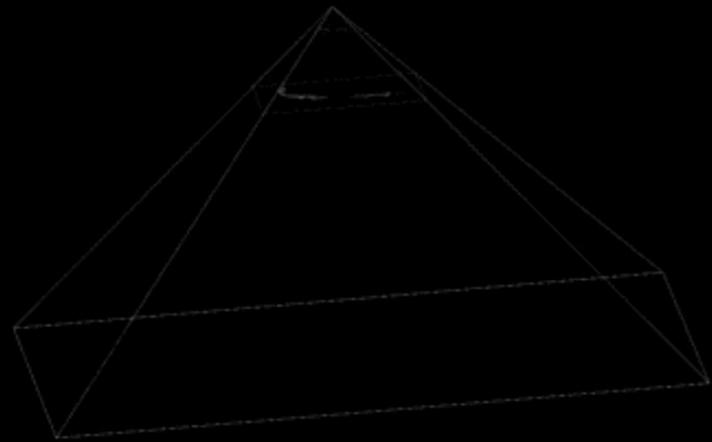
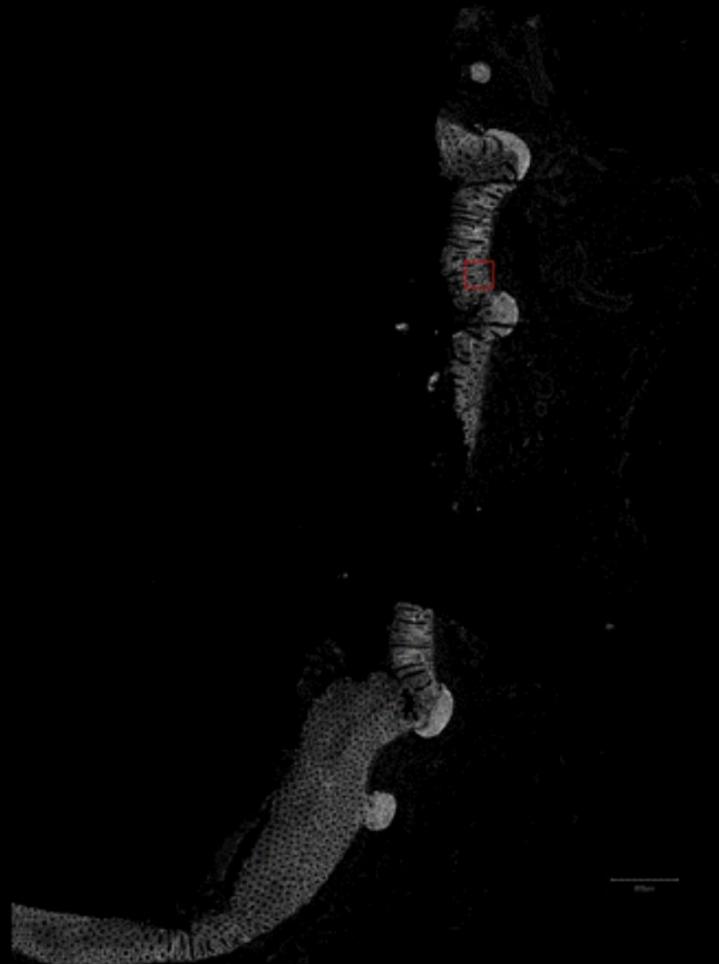


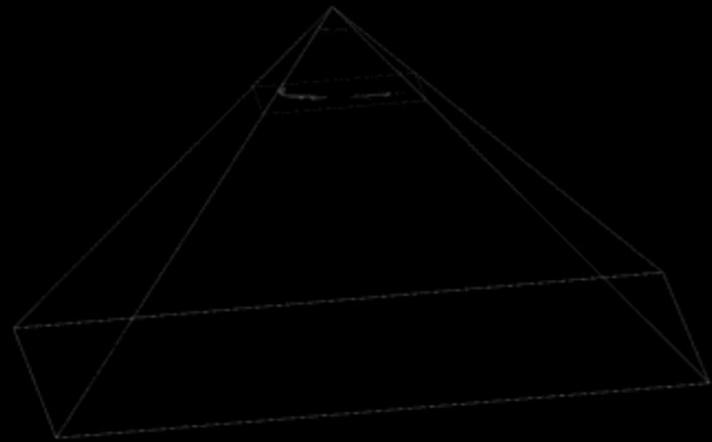
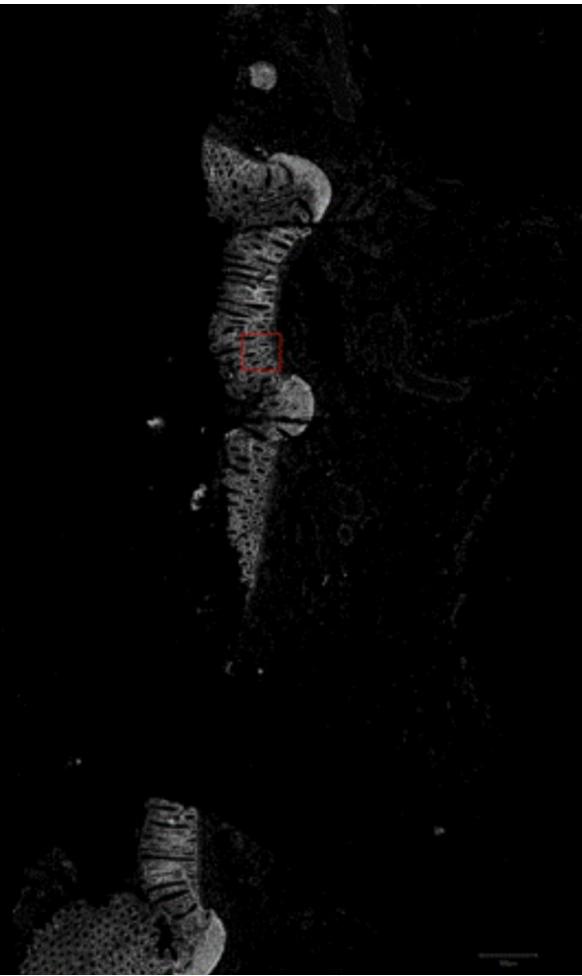
- Open-source software
- Bankhead *et.al.* **QuPath: Open source software for digital pathology image analysis**, Science Report, 2017

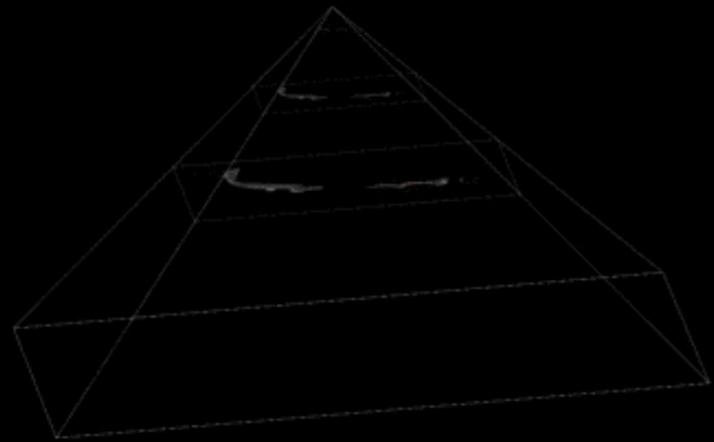
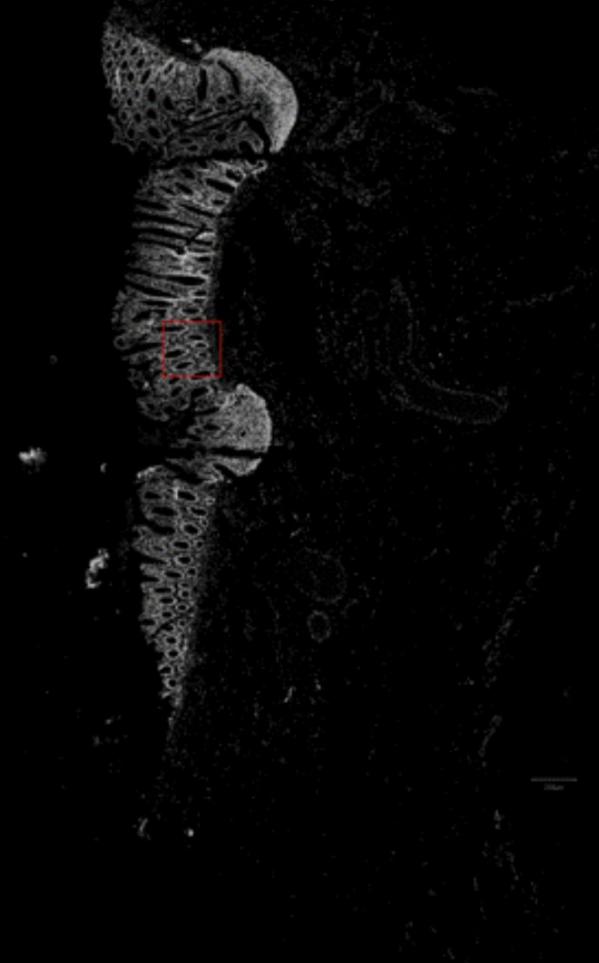
Digital pathology whole slide

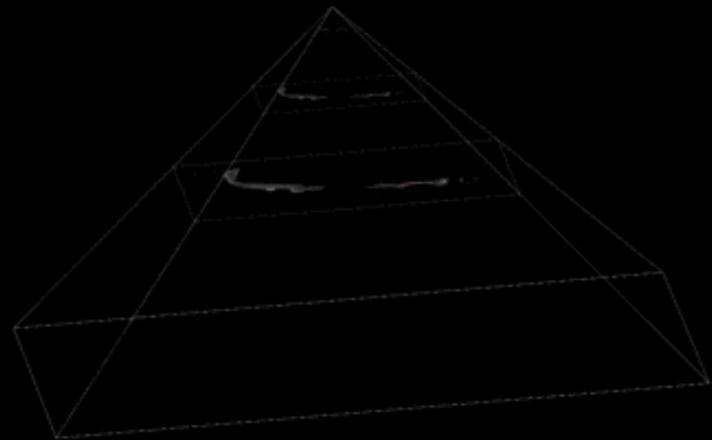
- An average slide size is **~25 mm x 75 mm**
- **20x** magnification gives **0.45 um** of pixel width
- Whole-slide:
 - $(25e-3 * 75e-3) / (0.45e-6)^2 = 9\ 259\ 259\ 259$ pixels

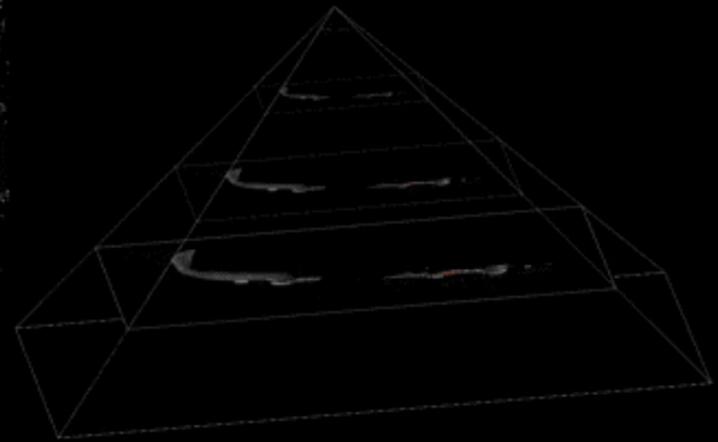
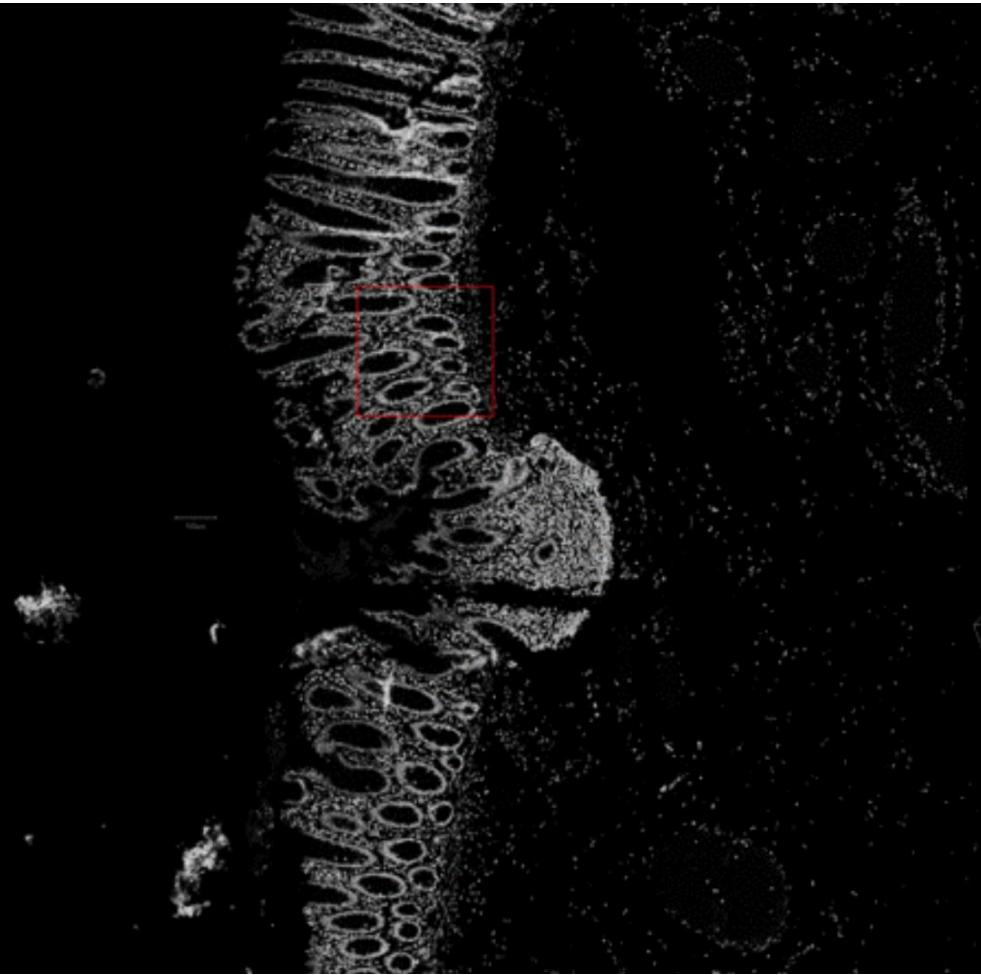


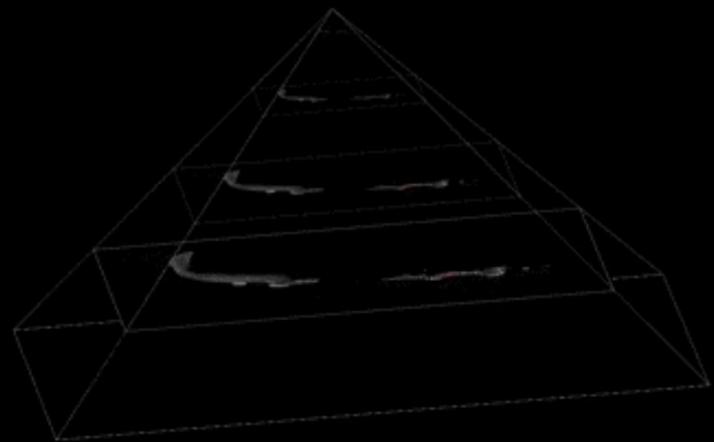
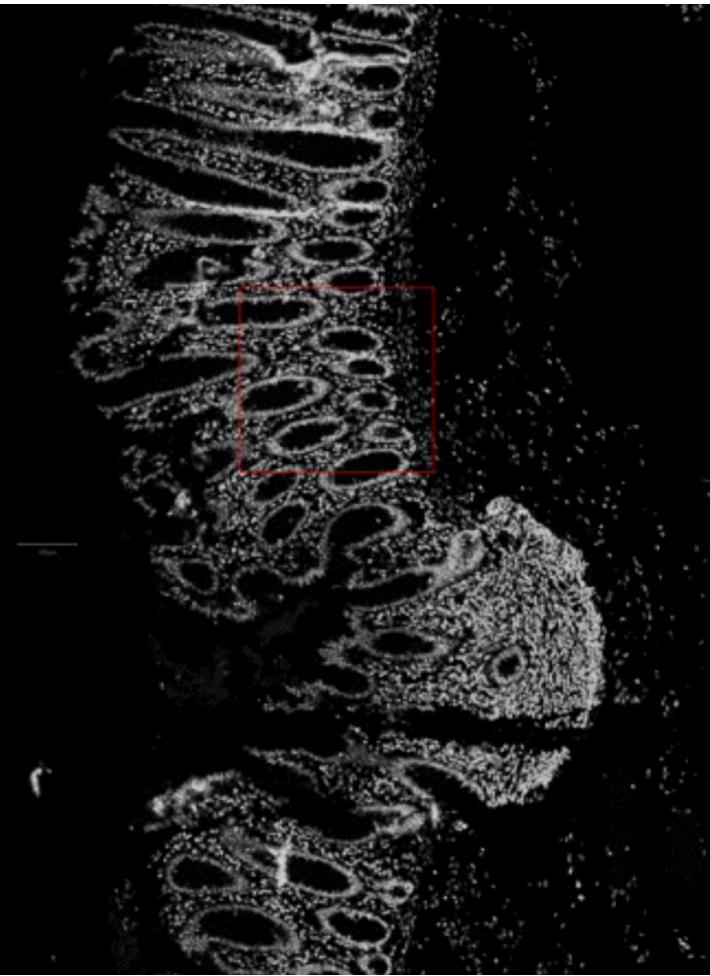


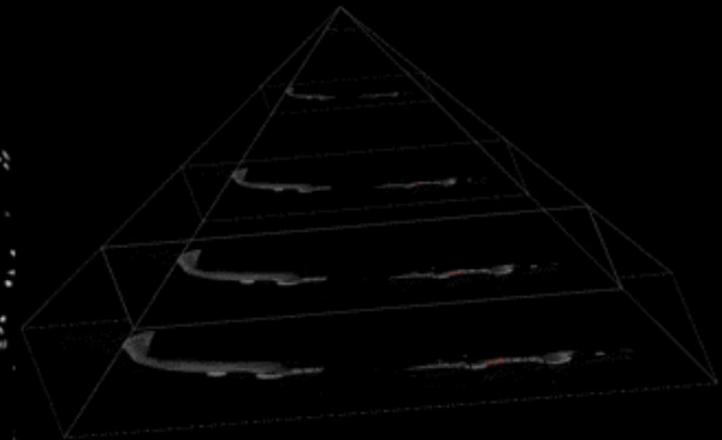
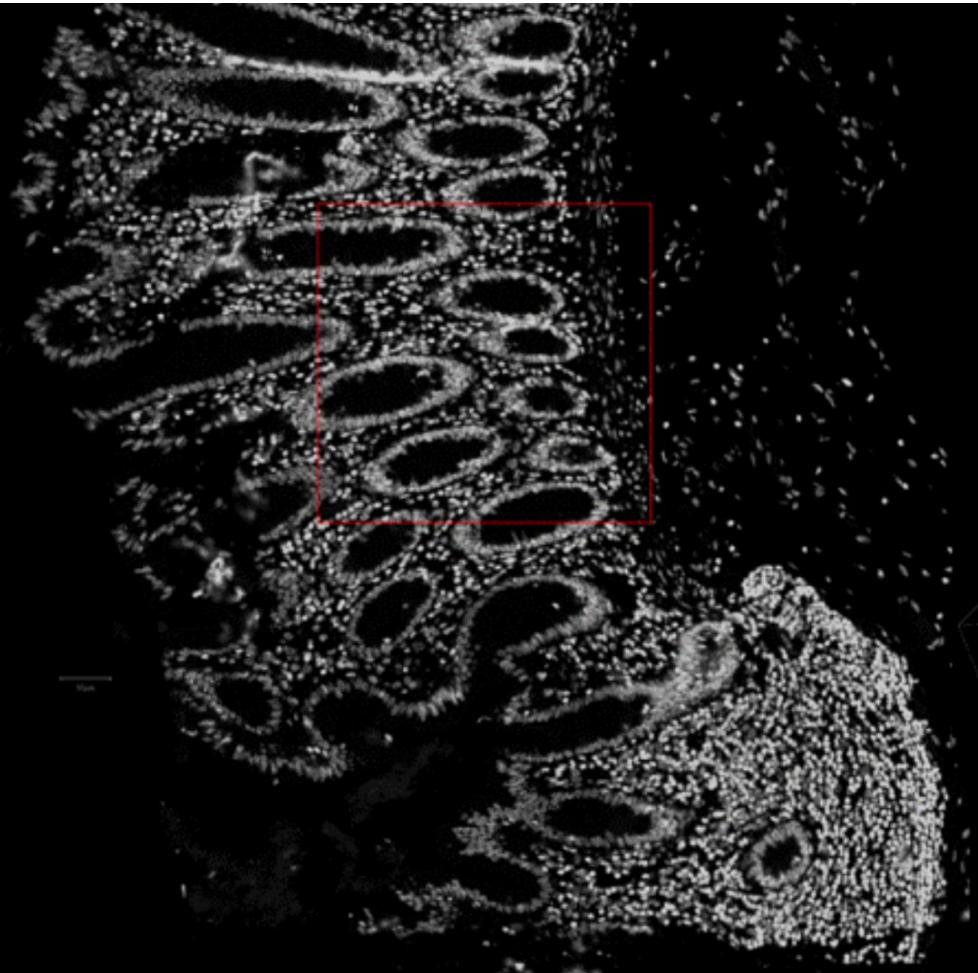


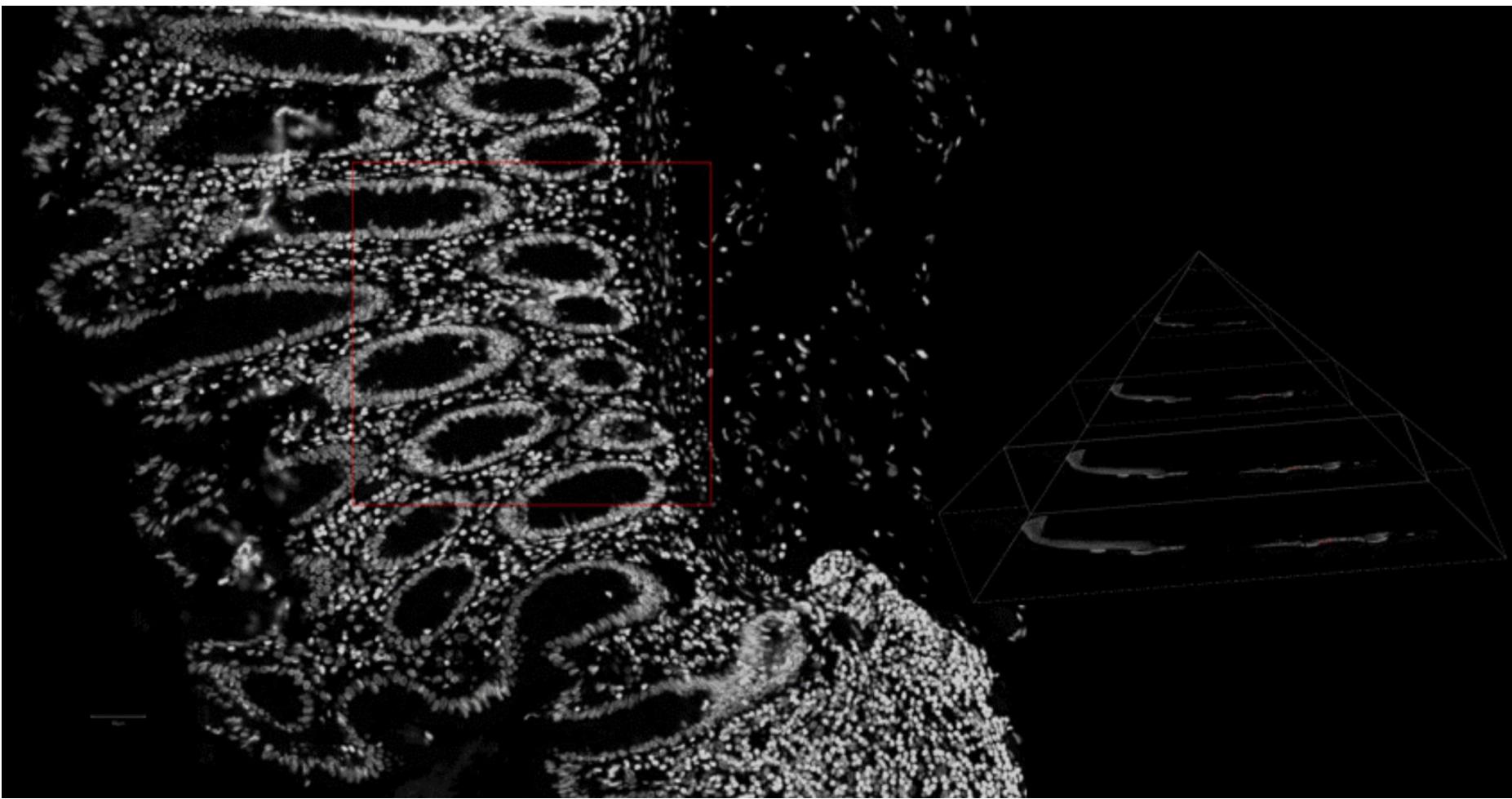


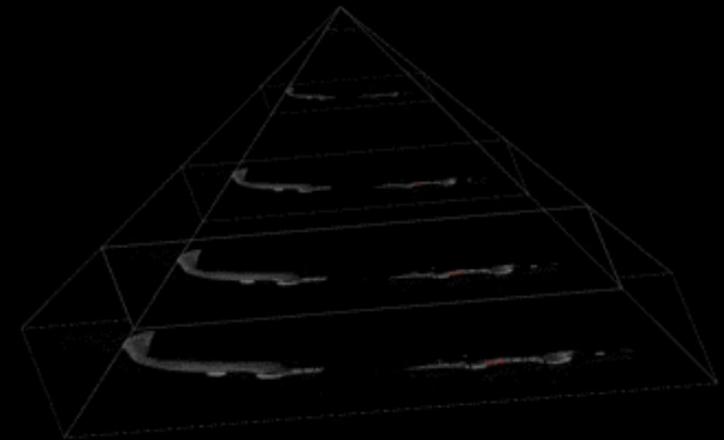
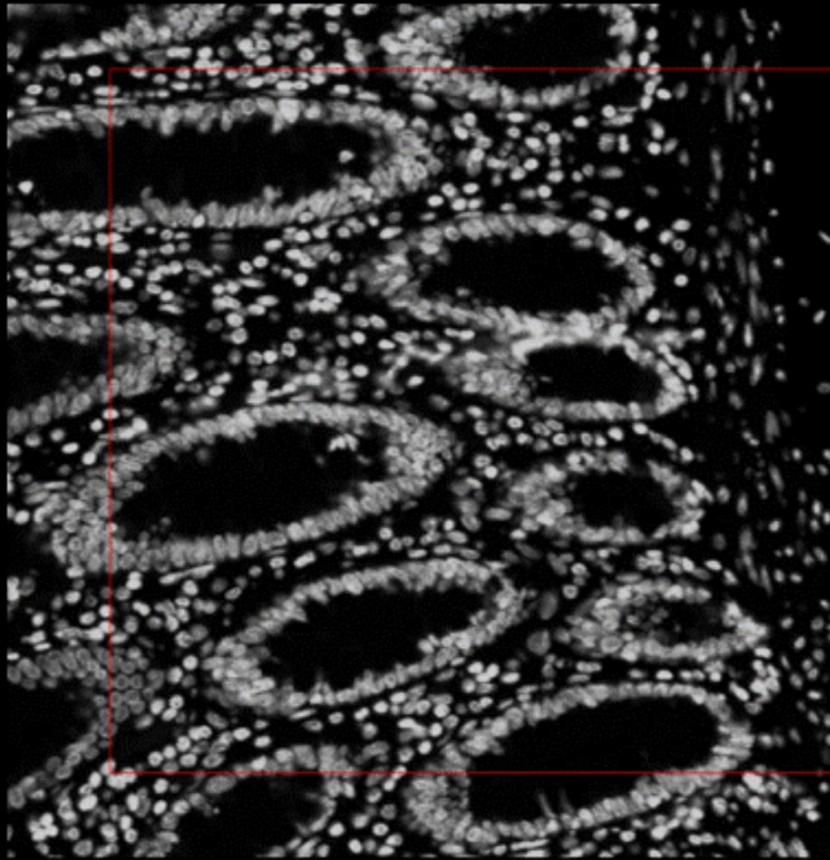












Digital pathology whole slide

Pyramidal storage:

- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 27.78 \text{ Gb}$ for resolution 1
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 6.94 \text{ Gb}$ for resolution 2
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 1.74 \text{ Gb}$ for resolution 4
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 434 \text{ Mb}$ for resolution 8
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 109 \text{ Mb}$ for resolution 16
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 27 \text{ Mb}$ for resolution 32
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 7 \text{ Mb}$ for resolution 64
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 1.7 \text{ Mb}$ for resolution 128
- $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = 424 \text{ Kb}$ for resolution 256

Total = 37 Gb uncompressed data, we display only what we need.

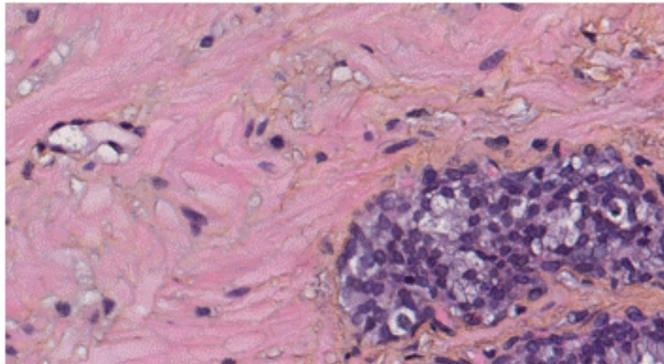
Staining and Stain estimation for brightfield image

- **Hematoxylin** stains **nuclei** in purple/blue
- **Eosin** stains **extracellular matrix & cytoplasm** in pink
- **DAB** is used for **antigens** in brown

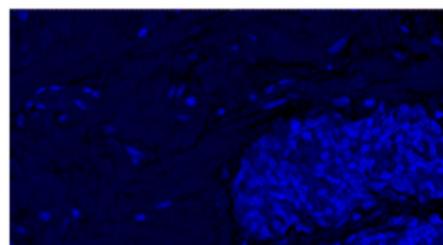
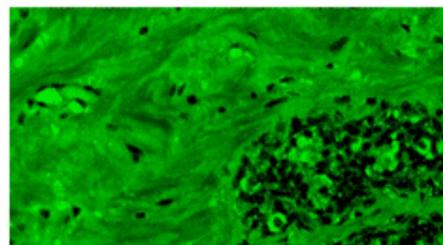
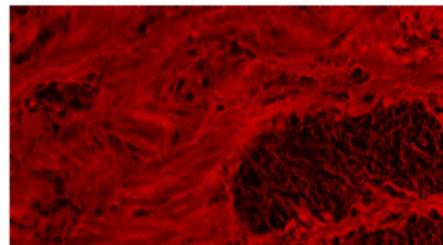
Brightfield scanner images in 3 components **Red Green Blue (RGB)**

RGB Brightfield \neq Fluorescence

Stain estimation for brightfield image



=



Stain estimation for brightfield image

- **Hematoxylin** stains **nuclei** in purple/blue
- **Eosin** stains **extracellular matrix & cytoplasm** in pink
- **DAB** is used for **antigens** in brown

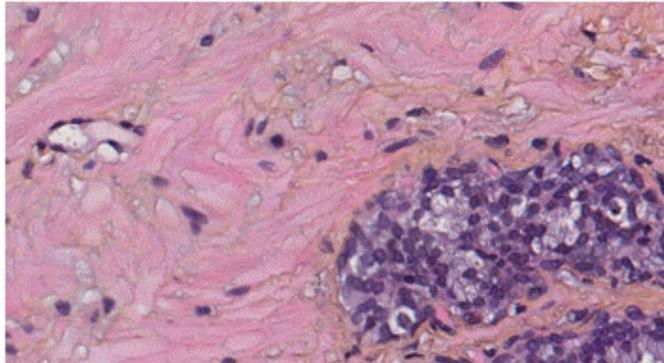
Brightfield scanner images in 3 components **Red Green Blue (RGB)**

Comparative Study > *Anal Quant Cytol Histol.* 2001 Aug;23(4):291-9.

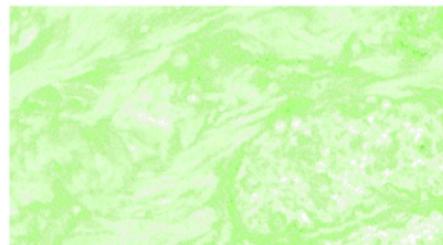
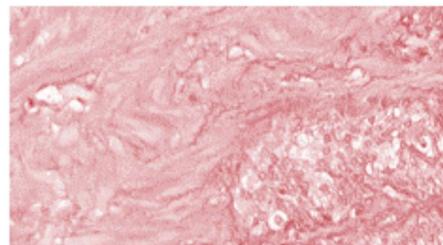
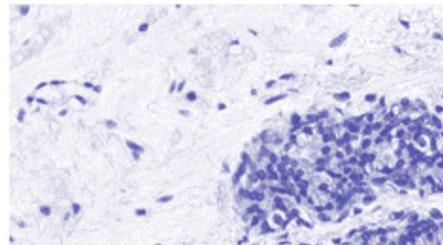
Quantification of histochemical staining by color deconvolution

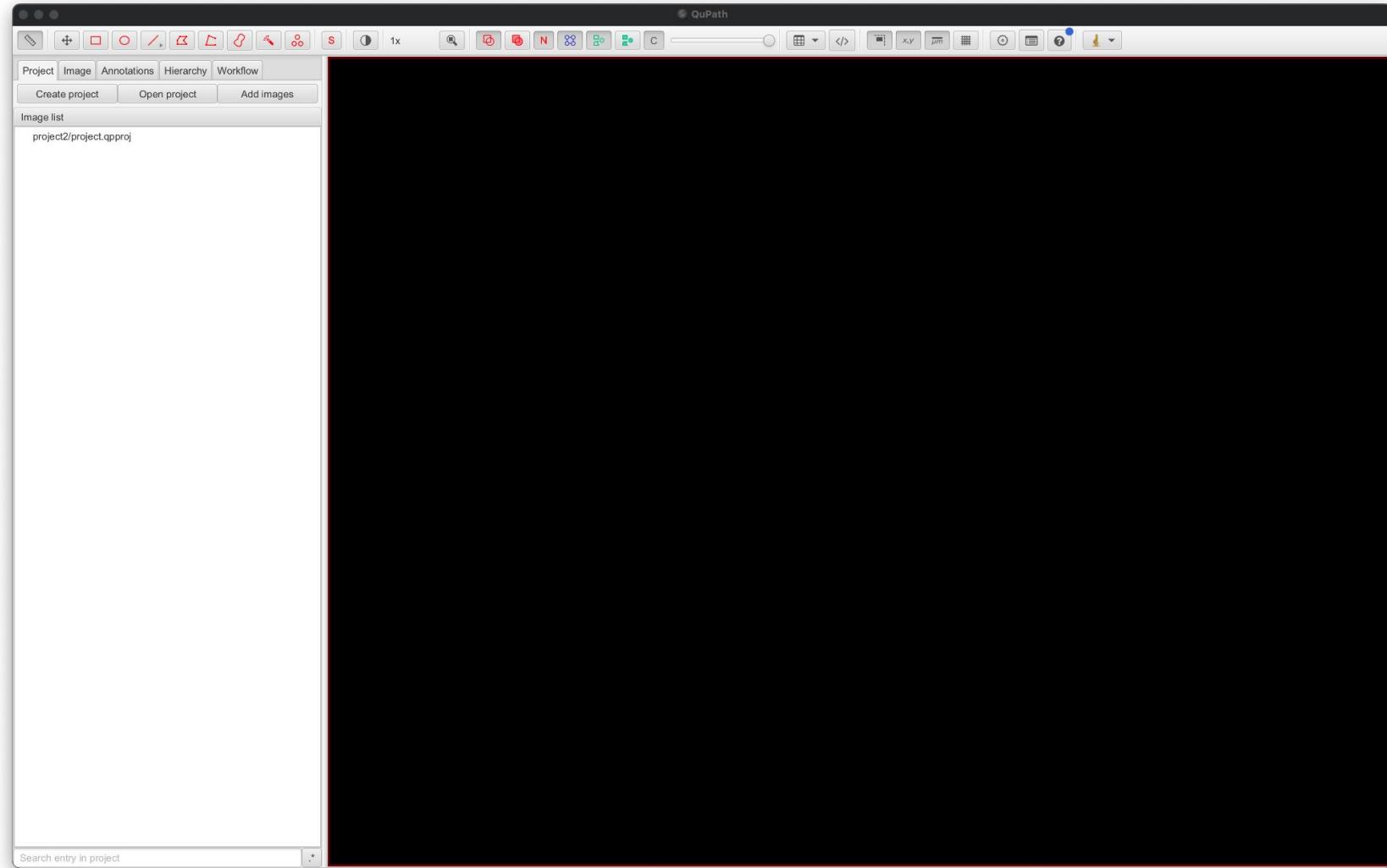
A C Ruifrok ¹, D A Johnston

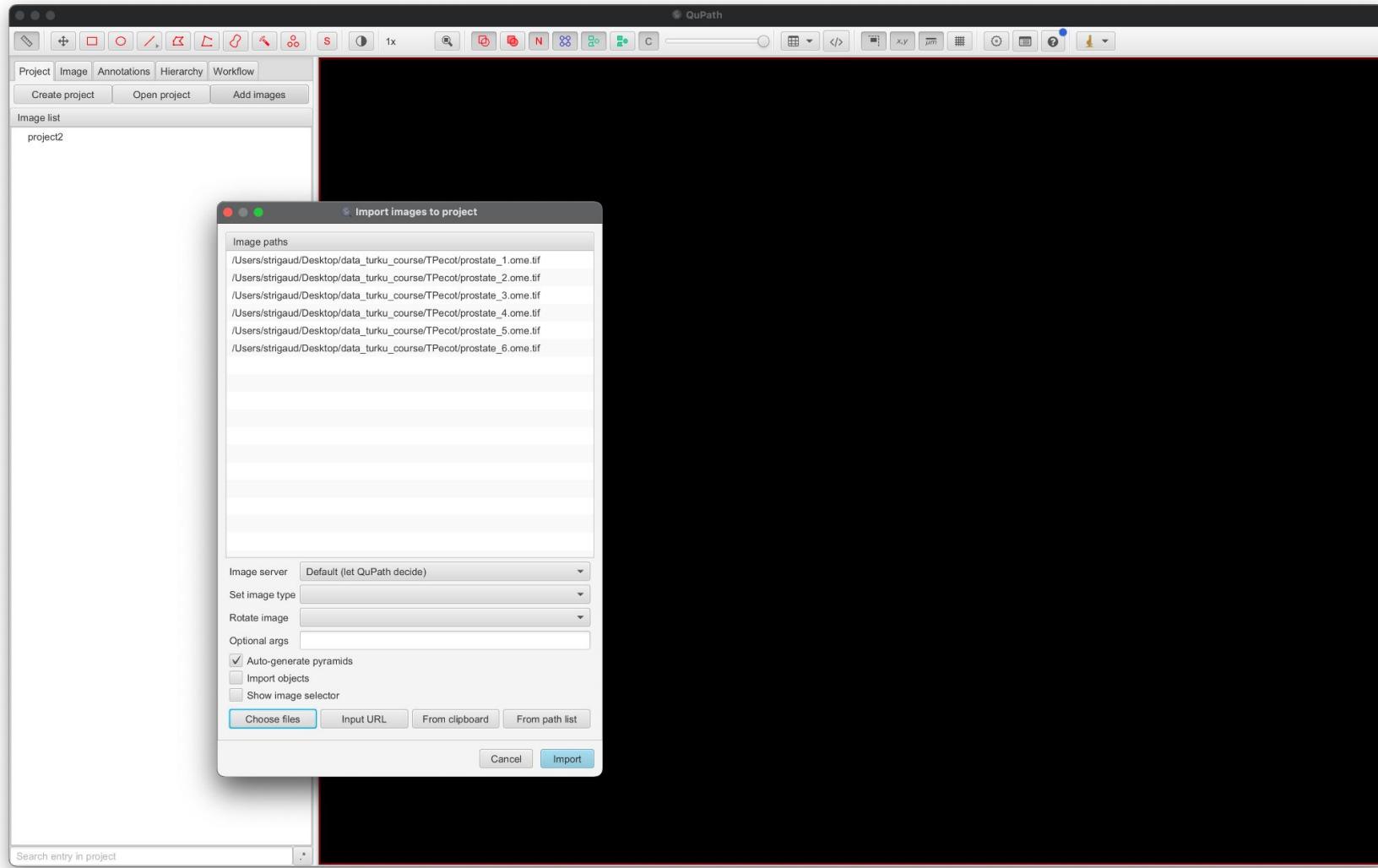
Stain estimation for brightfield image

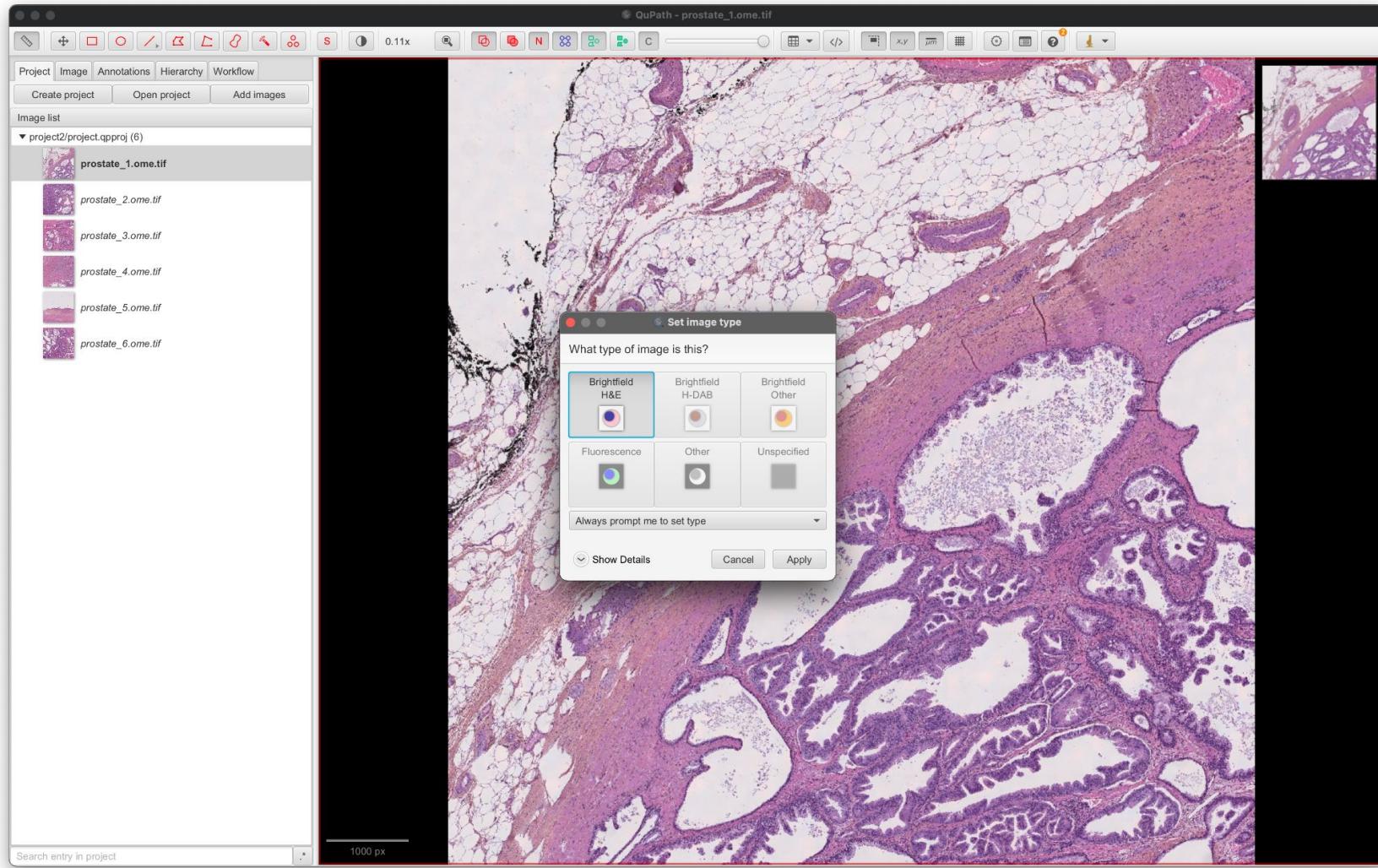


=

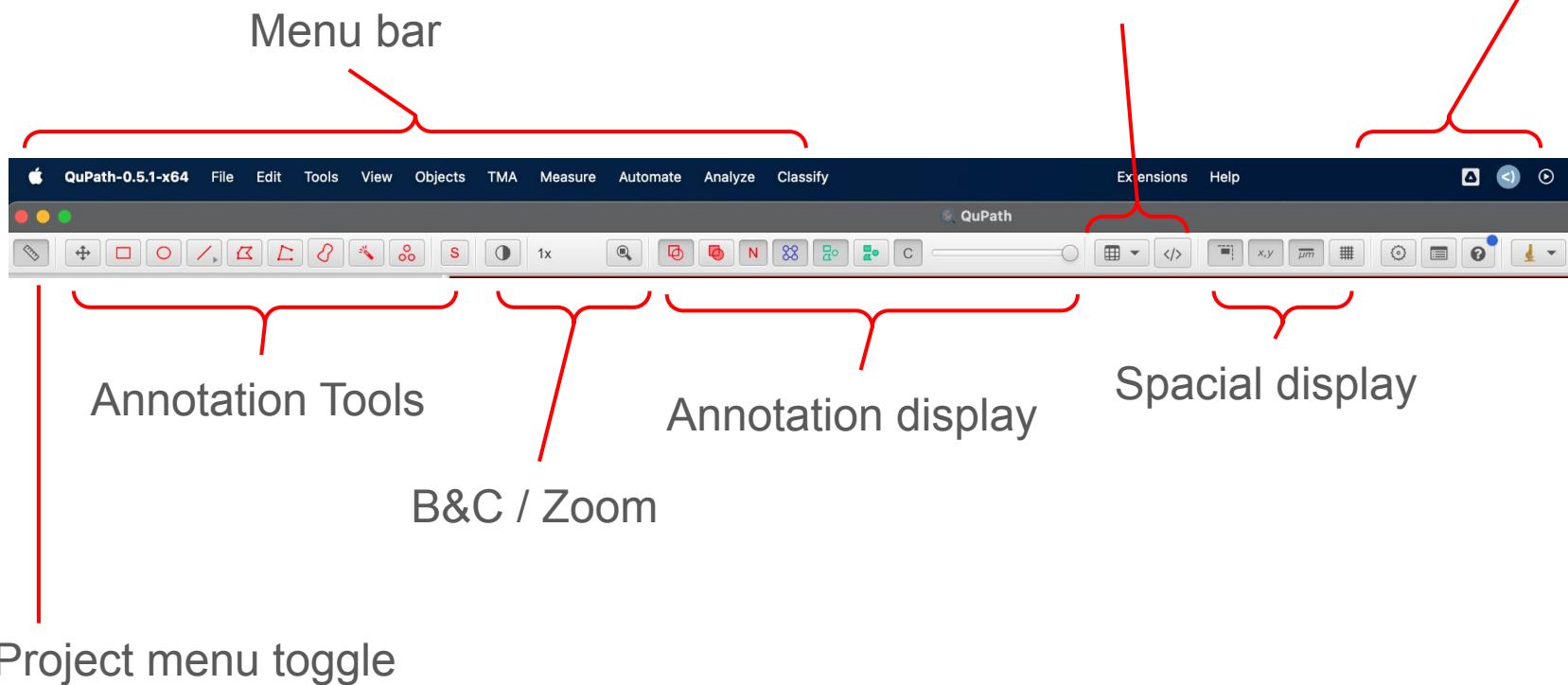








Let's discover the interface



Projects and Images



- Create a project
- Import the following images from the QuPath dataset
 - CMU-1
 - OS-2
- Import the following images from the TPecot dataset
 - Prostate serie

What are these image types?

Image navigation & visualisation

>> Zoom in/out of the image: Mouse wheel, +/-, Left click menu ( reset / lock viewer)

>> Pan navigation: Hold right click

Image navigation & visualisation

>> Zoom in/out of the image: Mouse wheel, +/-, Left click menu ( reset / lock viewer)

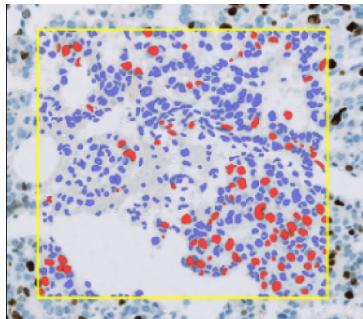
>> Pan navigation: Hold right click

>> Brightness & contrast: View > B&C or shift+C, or 

QuPath objects

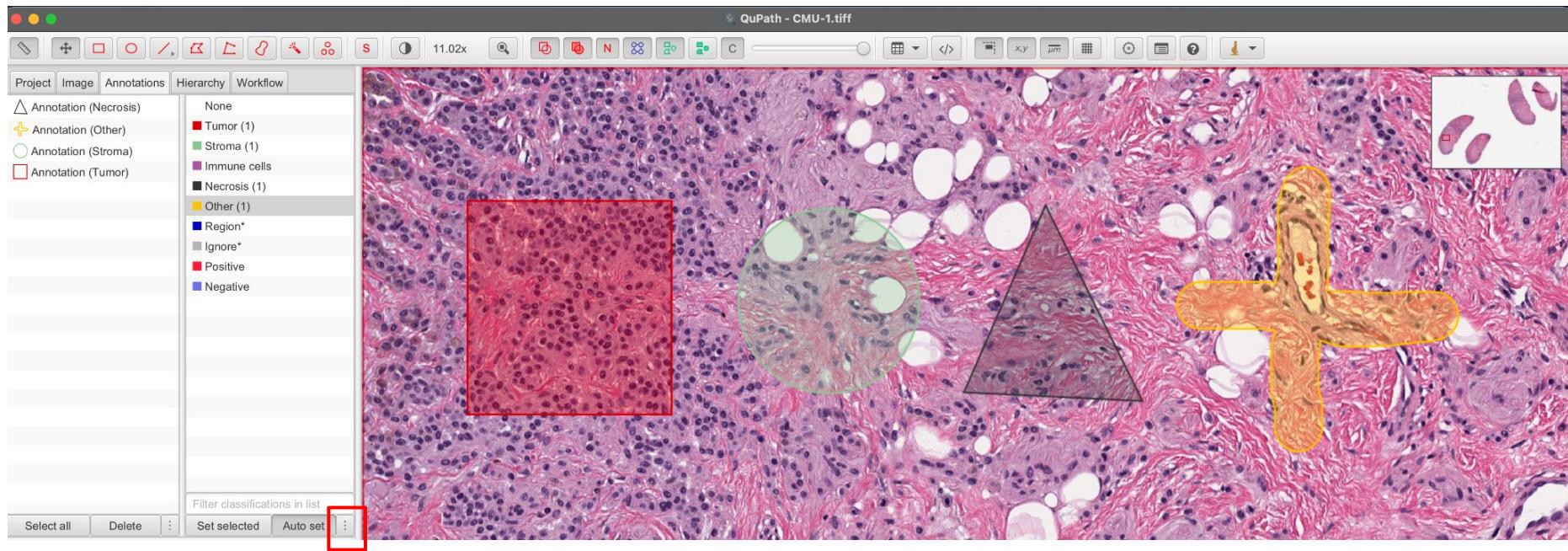
Annotations & Detection are hierarchical organisation of objects:

- Annotations: a specific region in the image (a tissue, a gland, an area)
 - Editable, multi-shaped, manual or automatic, limited in number per images
- Detections: small objects (nucleus, cells) created from a processing step
 - Non-editable, not manually generated, can have millions per images
- Classes: class type associated to objects
 - Default classes, can be add/remove, **classes with * are special**



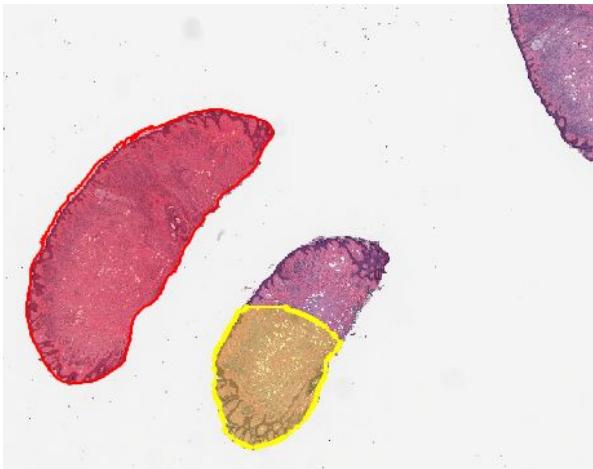
1 annotation incl. ~200 detections, split into 2 classes

Annotations, Detections, and classes



More classes options here

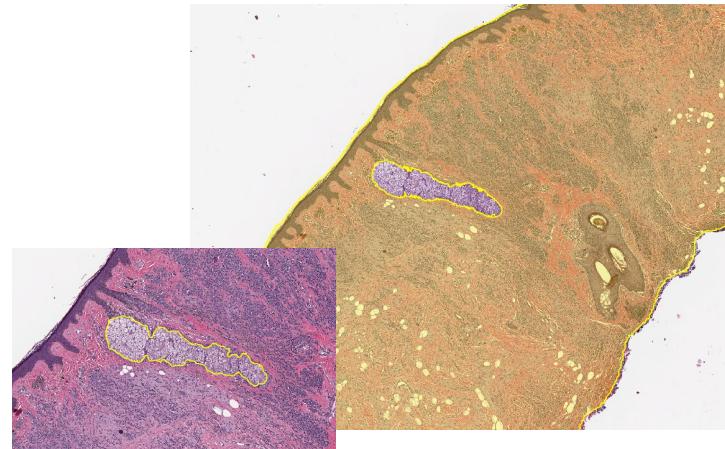
Annotations, Detections, and classes



Magic Wand (shortcut : **W**)

- Quick manual annotations
- Directly linked to the zoom scale
- Snap to object / structure
- Holding shift for eroding

>> Zoom in and annotate some sub-tissue structures
>> Lock the annotation (right-click menu)
>> Zoom out and annotate the whole tissue
>> Select both annotation and subtract them
(tips: right click on both selected annotations)



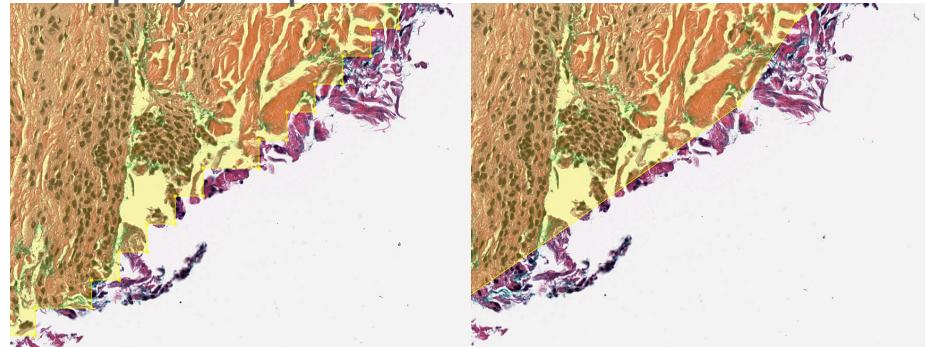
Annotation post-processing

Logical operations between objects

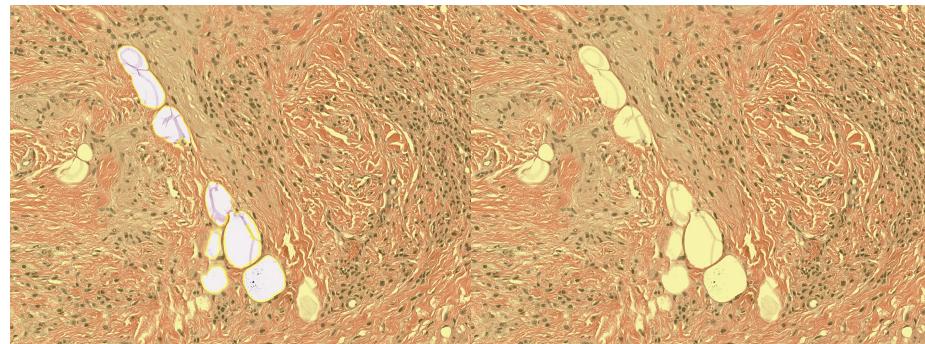
- Subtract
- Merge
- Split
- Expend
- Fill
- Remove
- Duplicate
- Transfert
- ...

Menu > Objects > Annotations ...
Right click on selected Annotations ...

Simplify shape

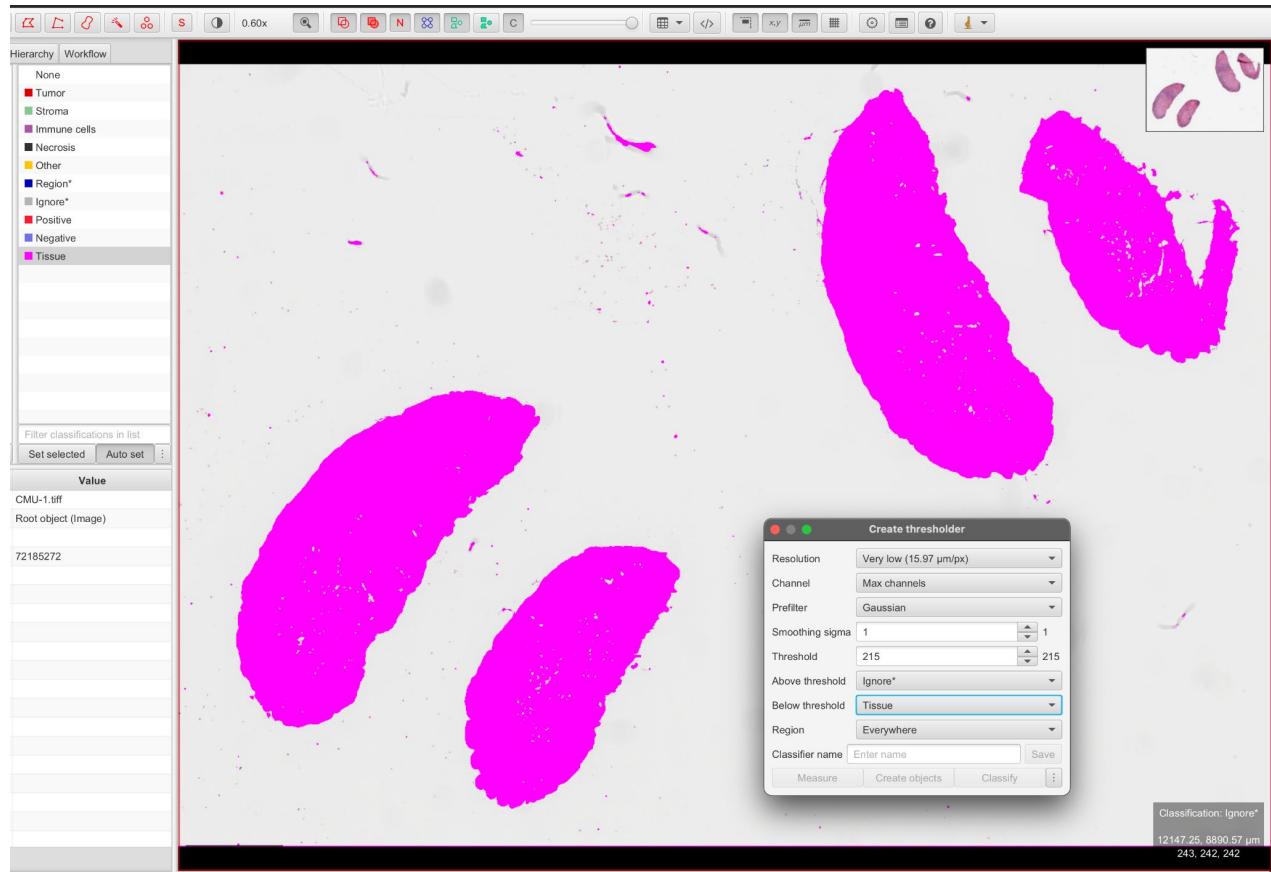


Fill holes



Tissue annotation: Thresher

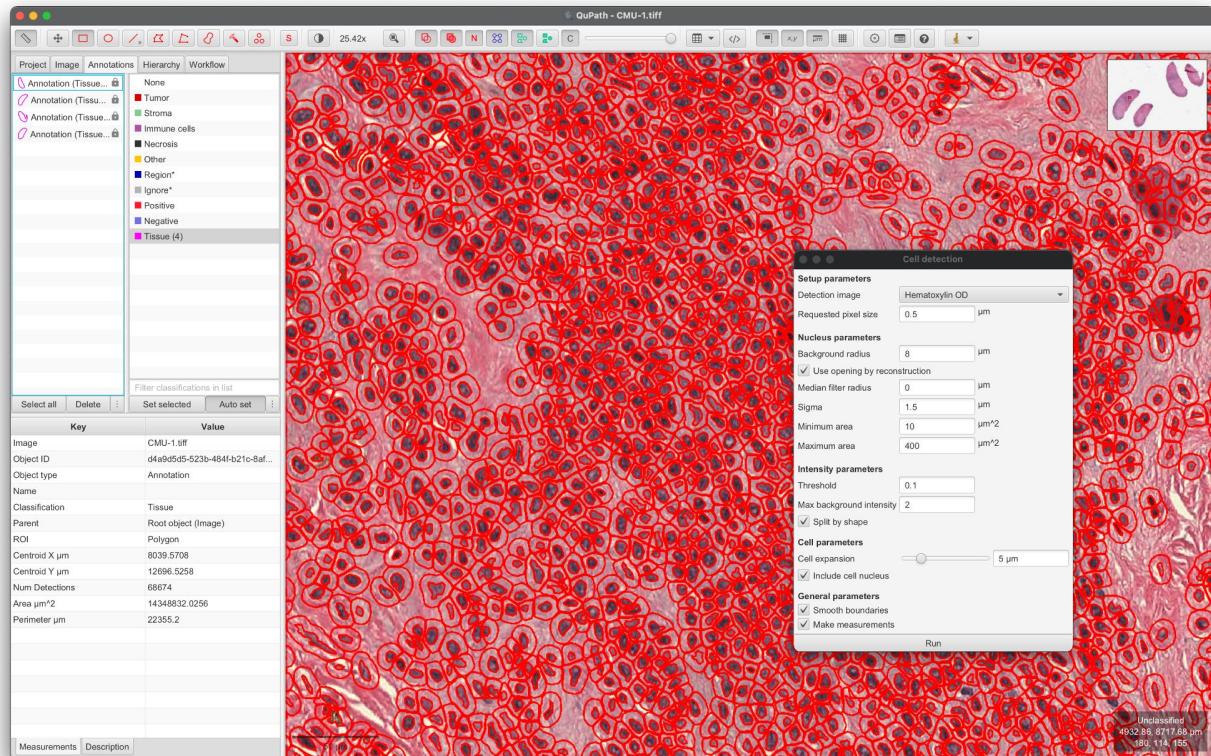
Classify >
Pixel Classification >
Create threshold



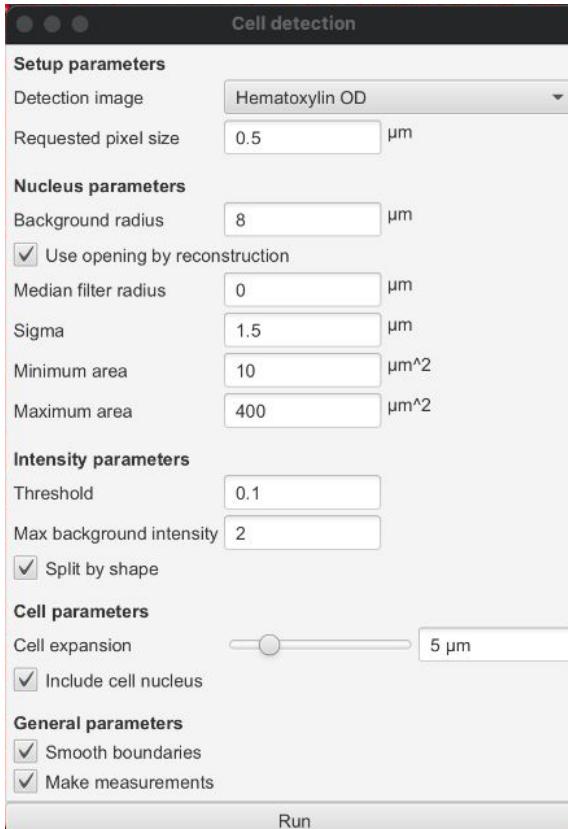
- >> Annotate the 4 tissues
- >> Clean up annotations
- >> Lock annotations

Cell detection in an annotation

- Detect based on a stain / channel
- Composed of
 - Nuclei
 - Nuclei expansion
- Detection cannot be edited!
- Menu > Analyze > Cell detection



Cell detection in a tissue



Channel of interest and pixel size

Background subtraction

Denoising and min/max size filtering

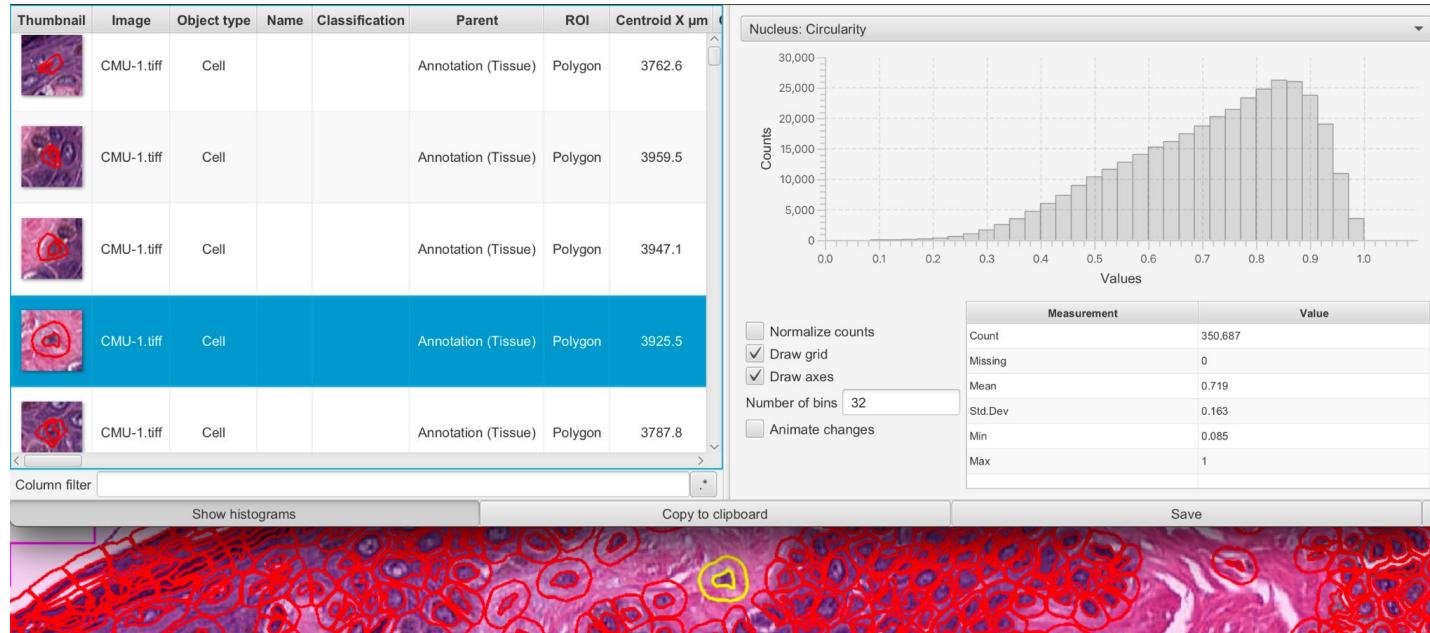
Nuclei intensity threshold

Cell extension area

Extra processing

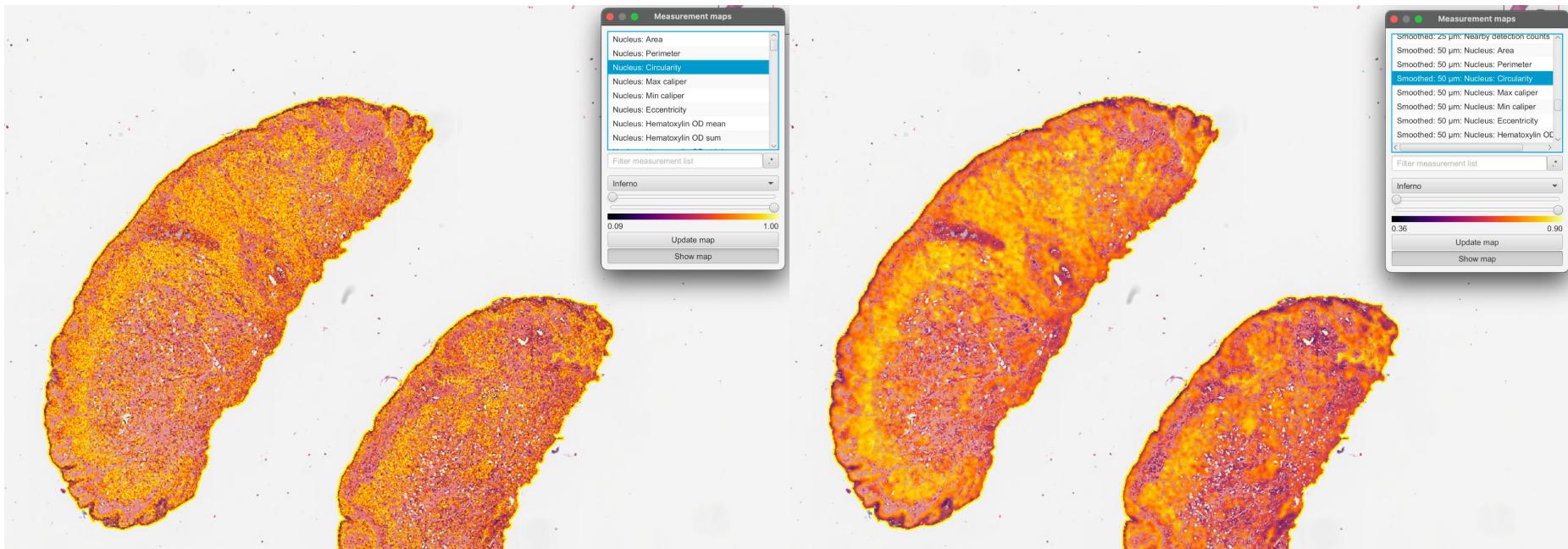
Measurement of Objects and Analysis

- Objects (annotations & detections) can have measurement associated (features)
- Not automatically computed
 - Analyse > Calculate features > ...



Measurement and analysis

- Objects (annotations & detections) can have measurement associated (features)
- Not automatically computed
 - Analyse > Calculate features > ...



Measurement and analysis

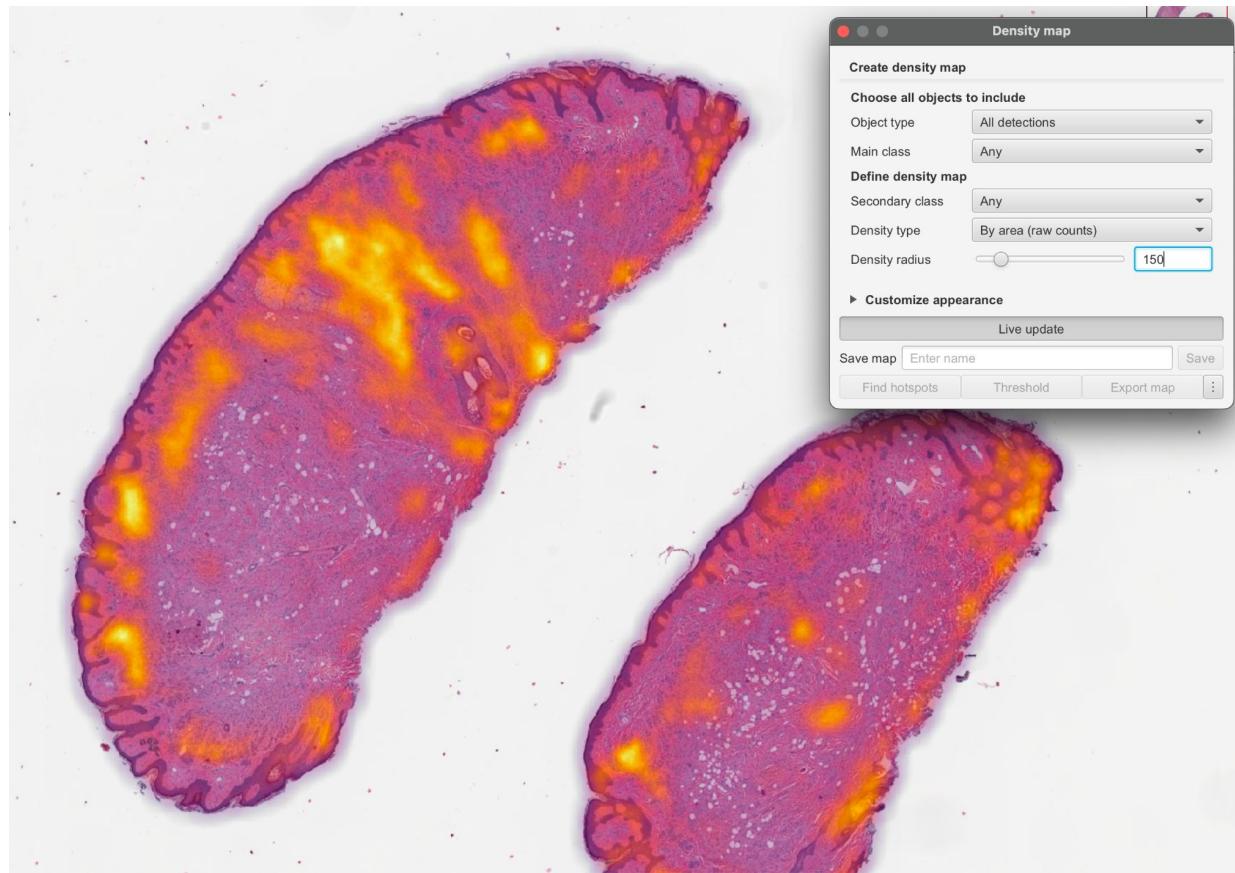
Analyze > Calculate features >

...

Measure > show
annotations/detection
measurements

Measure > show measurement
maps

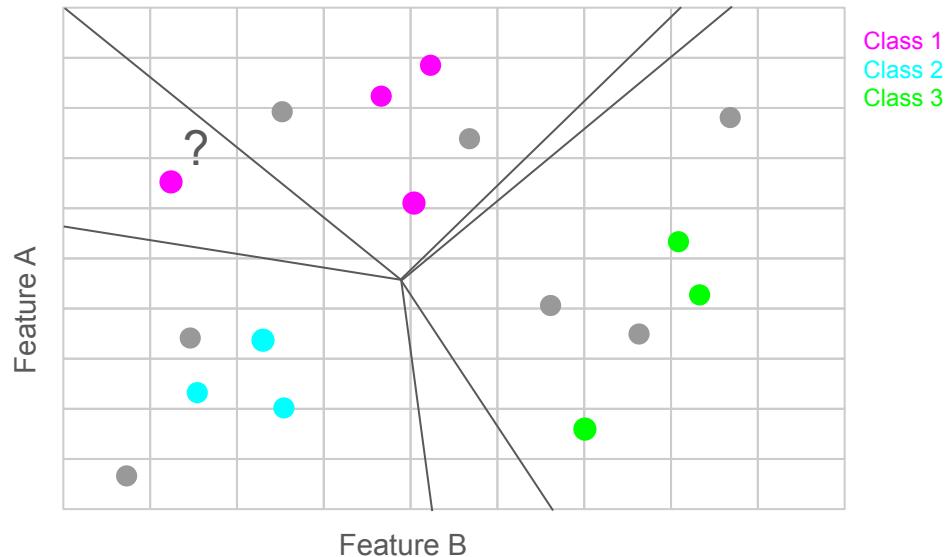
Analyze > Density maps > ...



Are you still with me?

Classification

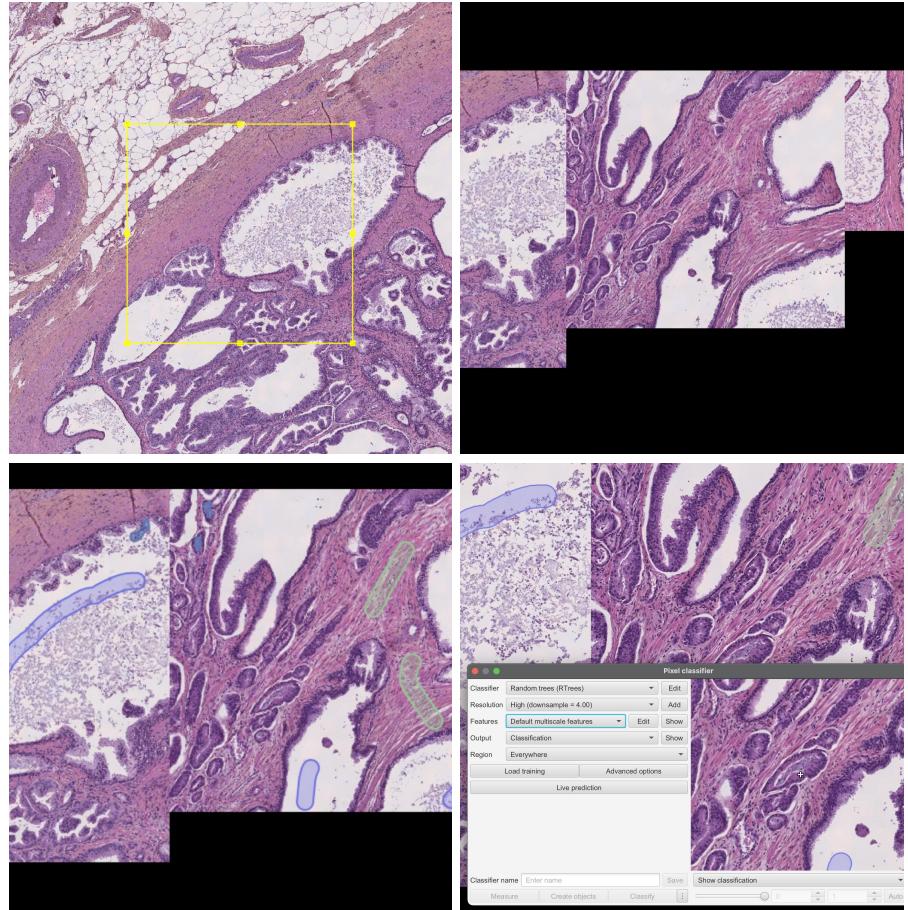
- Describes classes with a set of features
 - Learn separation between classes in the feature space
 - Classify new input using previously learned description
-
- Pixel can be classified
 - Intensity description
 - Texture description
 - Applied to a region (sigma σ)
-
- Object can be classified
 - Intensity description
 - Shape description



Sparse interactive classification

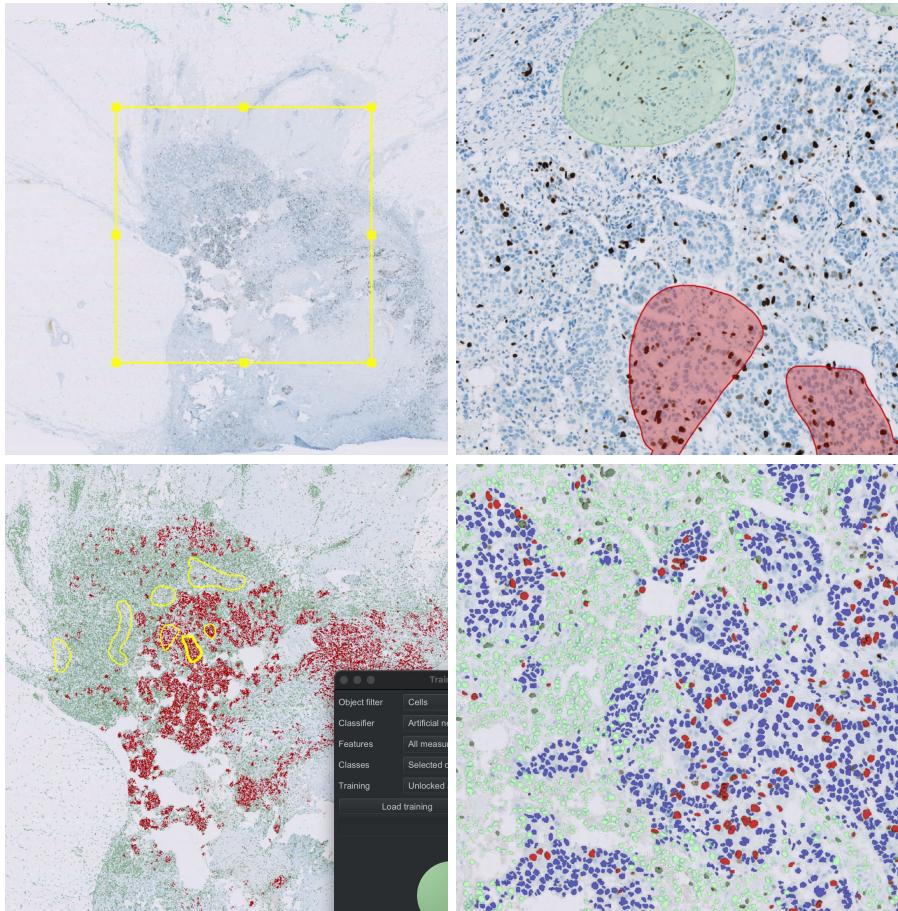
Pixels classification

- Load the prostate dataset
- Create **region*** annotations using Rectangle in the dataset
- Generate a **Training image** using the **region*** annotations
- Train a pixel classifier to identify different tissues using annotations (**Brush**)
 - Stroma
 - Epithelium
 - Background / **ignore***
- Curate the classifier with some additional annotations
- Save and apply it to the dataset



Object classification

- Load the OS-2 image
- Annotate a region around the tumor (**Rectangle**)
- Detect the cells inside the region:
 - Compute object features (shape, intensity)
 - Smooth object features for more information
- Annotate the cells (brush)
 - Belonging to Tumor
 - Belonging to Stroma
- Train a cell classifier using the annotations
- Extend classification with Intensity Classify
 - Classify > Object ... > Set cell intensity ...
 - Use the DAB staining
- Make density map of DAB positive cell in Tumor region



Lunch break, or whatever ...

QuPath extension and Python environement

If you do not have **conda** or **mamba** already installed!

See github repository:

https://github.com/jpylvanainen/Image_analysis_course_24/tree/main/Installation_instruction

conda-forge/
miniforge



A conda-forge distribution.

40
Contributors

115
Issues

17
Discussions

6k
Stars

321
Forks



Plugin and scripts

- Similar to Fiji, QuPath can be extended with third-party solution
- Work as a **drag & drop** a **.jar** file inside QuPath
 - Possible additional work depending on the tool you want to use
- A lot of extension can be found there:
 - Github > QuPath : <https://github.com/qupath>
 - Github > BIOP : <https://github.com/BIOP>
 - Others

WARNING: not all extension you can find are maintained !

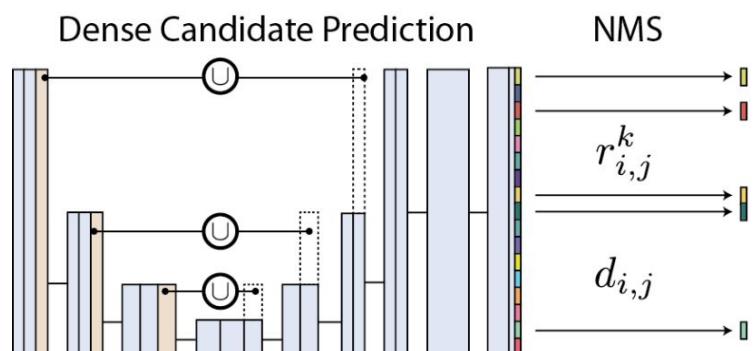
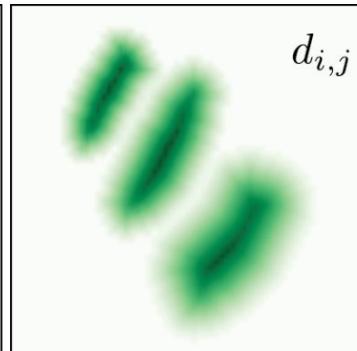
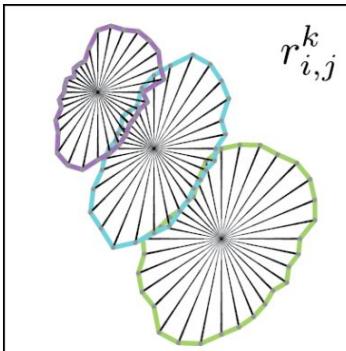
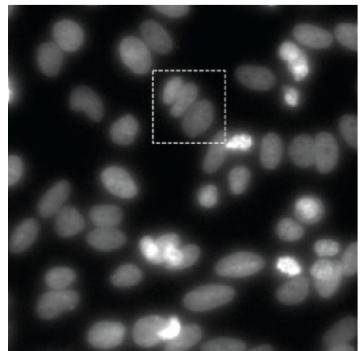
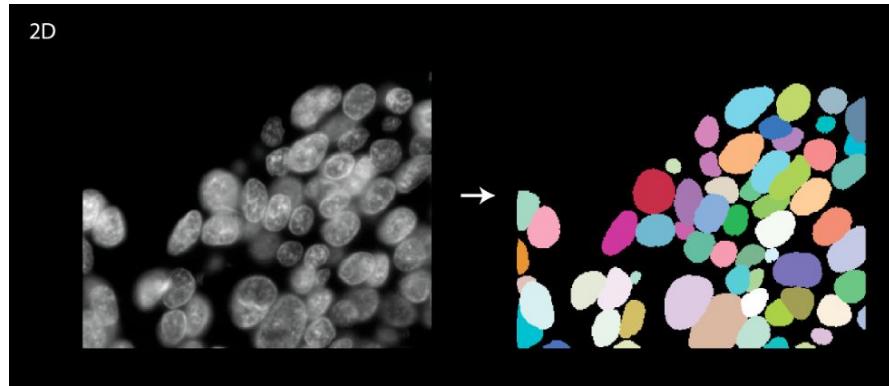
Stardist

NUCLEI INSTANCE SEGMENTATION AND CLASSIFICATION IN HISTOPATHOLOGY IMAGES WITH STARDIST

Martin Weigert¹, Uwe Schmidt²

¹Institute of Bioengineering, School of Life Sciences, EPFL, Switzerland

²Independent Researcher, Dresden, Germany



Stardist and MultiFlex images

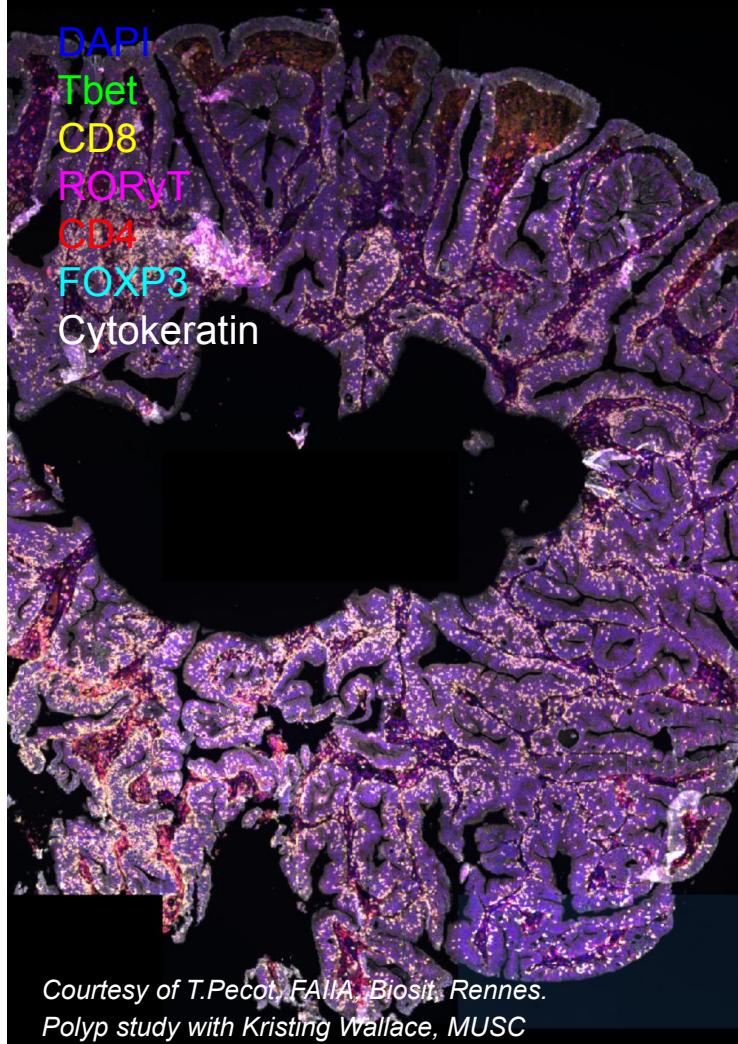
- Load `polip_fluo` image and update channel information
- Update channels information (name & lut)
- Define an epithelium annotation based on Cytokeratin
 - Pixel classifier is a good option!
- Start Stardist `detection script` from extension
- Update script information
 - Select a model -> `path to dbs_heavy_extended`
 - Select channel -> `DAPI`
 - Pixel size -> fetch in image information tab
 - Uncomment other options if needed -> remove `//`
 - (optional) Tests parameters on smaller annotations
- Run the script (update parameters if needed)
- Compute measurements
 - Analyse >
 - Shape, intensity
 - Smooth measurements



Stardist and MultiFlex images

- Create training images for different cell type
 - Classify > Training images > Create ...
 - Populate classes using channels information
- For each channels, build a classifier
 - Use brush or points to mark cell types
 - Use the **ignore*** class for negatives
 - E.g. CD4 classifier uses 2 classes (CD4 & ignore*)
 - Save classifier (for later)
- Build a multi-object classifier and apply it on the original image

1. **CD4** -> Th (T cell helper)
2. **CD4/Tbet** -> Th1
3. **CD4/RORgt** -> Th17
4. **CD4/FOXP3** -> Treg
5. **CD4/RORgt/FOXP3** -> IL-17
6. **CD8** -> Tc (Cytotoxic T cells)
7. **CD8/Tbet** -> Tc1
8. **CD8/RORgt** -> Tc17
9. **CD8/RORgt/Tbet** -> Tc17/1 (impaired)
10. **FOXP3** -> Macrophages

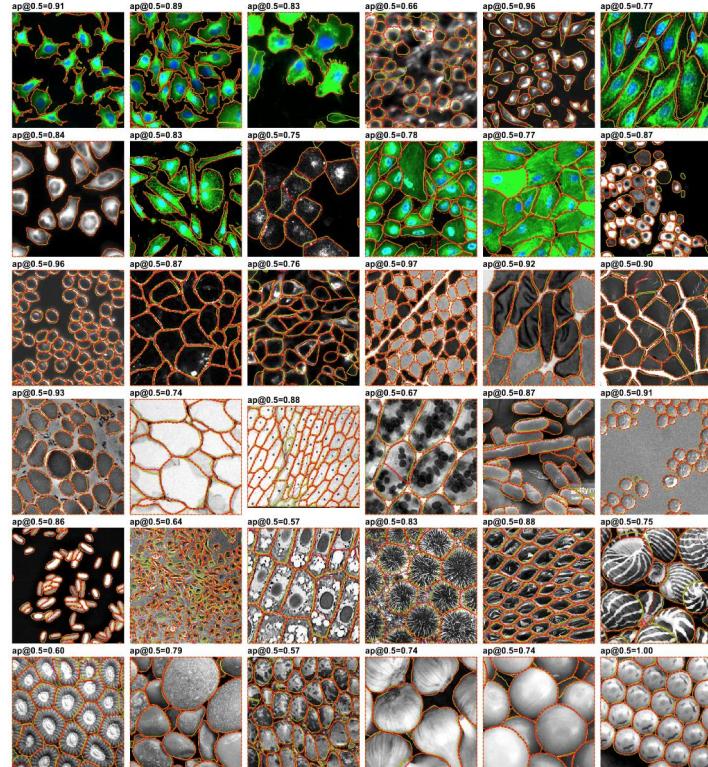
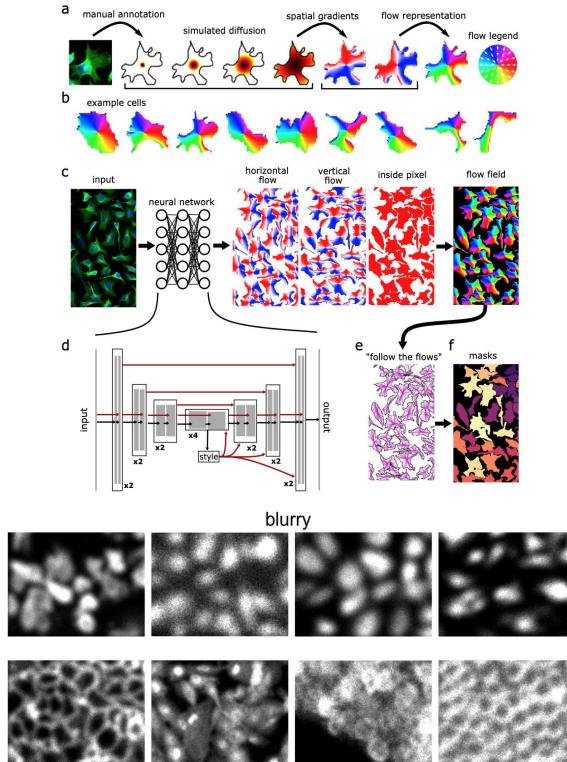


Cellpose3: one-click image restoration for improved cellular segmentation

Carsen Stringer[†], Marius Pachitariu[†]

HHMI Janelia Research Campus, Ashburn, VA, USA

[†] correspondence to (stringerc, pachitarium) @ janelia.hhmi.org

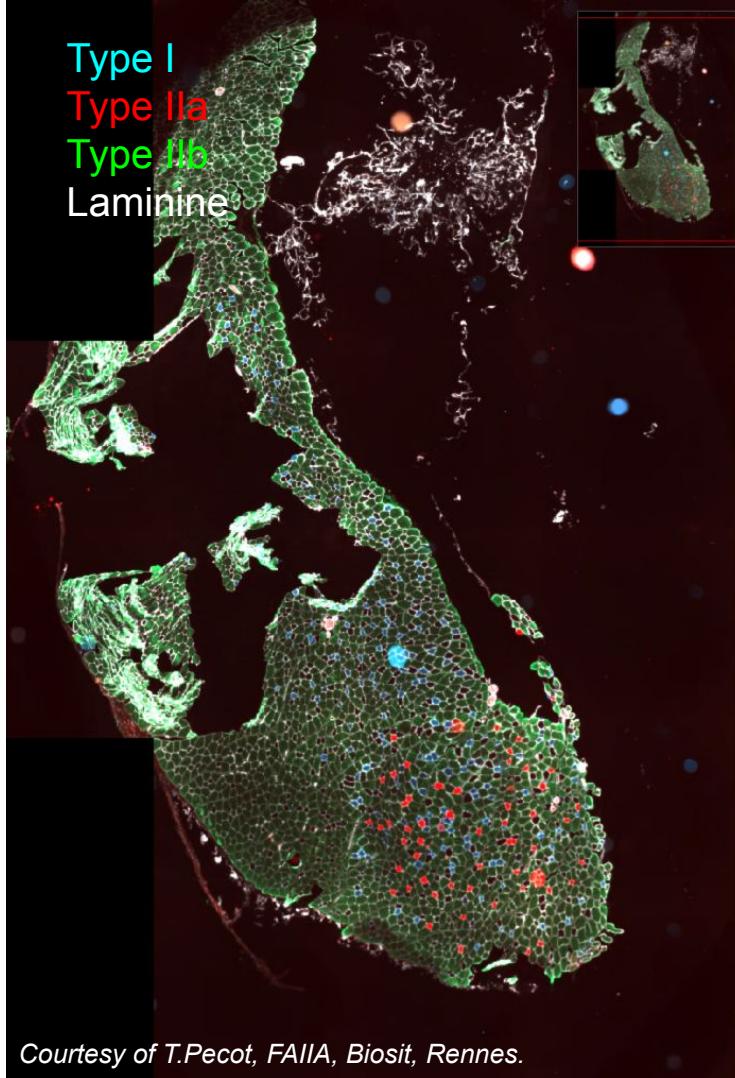


Cellpose and Flex images

- Load `muscle_fluo` and update channel information
- Update channels information (name & lut)
- Use a pixel classifier to identify the tissue
- Start cellpose `detection script` from extension
- Update script information
 - Select a model -> `cyto3, nuclei, ...`
 - Select channel -> `DAPI`
 - Pixel size -> fetch in image information tab
 - Uncomment other options if needed -> remove `//`
 - Test different values for:
 - `.preprocess(...)`
 - `.diameter(...)`
 - (optional) Add `.addParameter("gpu_device", "mps")`
- Run the script (and cross your fingers!)

Cellpose and Flex images

- Load XXXX and update channel information
- Update channels information (name & lut)
- Use a pixel classifier to identify the tissue
- Start cellpose **detection script** from extension
- Update script information
 - Select a model -> **cyto3, nuclei, ...**
 - Select channel -> **DAPI**
 - Pixel size -> fetch in image information tab
 - Uncomment other options if needed -> remove **//**
 - Test different values for:
 - **.preprocess(...)**
 - **.diameter(...)**
 - (optional) Add **.addParameter("gpu_device", "mps")**
- Run the script (and cross your fingers!)
- Use point annotations and cell classifier to detect cell types
- Reuse Cellpose and the cell classifier on the second image



Courtesy of T.Pecot, FAIIA, Biosit, Rennes.

Segment Anything Model

Segment Anything

Alexander Kirillov^{1,2,4} Eric Mintun² Nikhil Ravi^{1,2} Hanzi Mao² Chloe Rolland³ Laura Gustafson³

Tete Xiao³

Spencer Whitehead

Alexander C. Berg

Wan-Yen Lo

Piotr Dollár⁴ Ross Girshick⁴

¹project lead ²joint first author ³equal contribution

⁴directional lead

Meta AI Research, FAIR

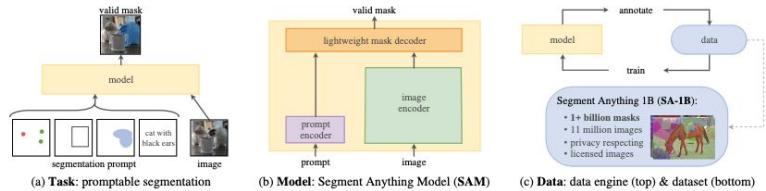
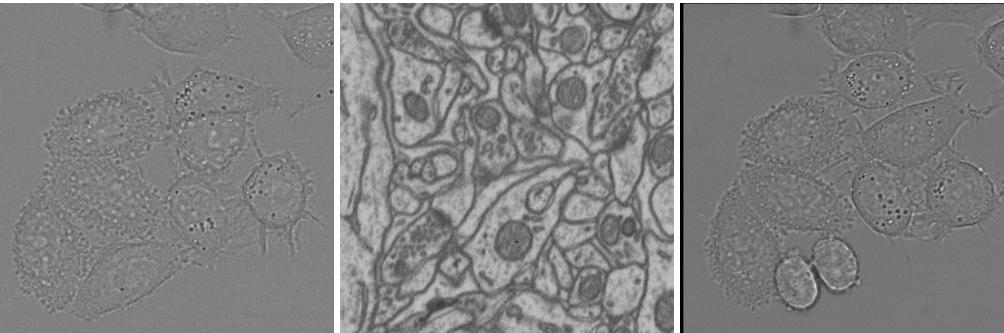


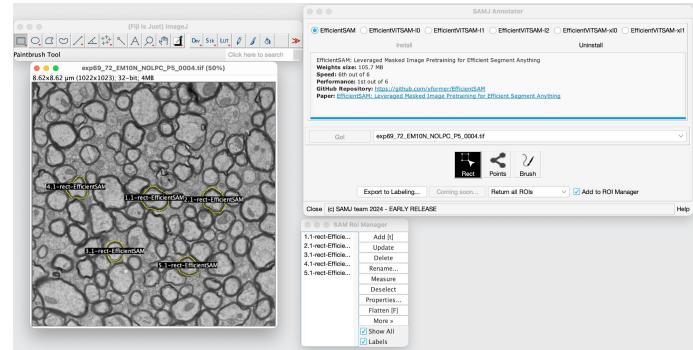
Figure 1: We aim to build a foundation model for segmentation by introducing three interconnected components: a promptable segmentation task, a segmentation model (SAM) that powers data annotation and enables zero-shot transfer to a range of tasks via prompt engineering, and a data engine for collecting SA-1B, our dataset of over 1 billion masks.



Napari Micro-SAM (Archit et.al. bioRxiv)



Fiji SAM-IJ



SAM install and conda environment

Sam extension:

Download the **.jar** and drop it into QuPath: <https://github.com/ksugar/qupath-extension-sam/releases>

Open a Terminal:

```
mamba create -n samapi -y python=3.10 && mamba activate samapi  
python -m pip install "torch>=2.3.1,<2.4" torchvision --index-urlhttps://download.pytorch.org/whl/cu118  
python -m pip install git+https://github.com/ksugar/samapi.git
```

Start the server:

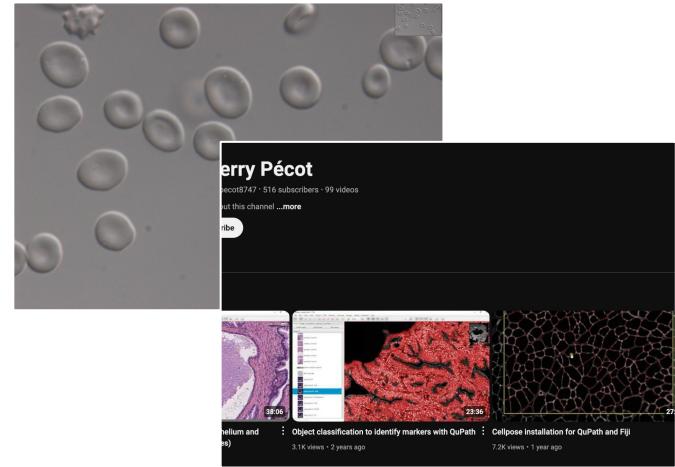
```
export PYTORCH_ENABLE_MPS_FALLBACK=1 (MAC Silicon only)  
uvicorn samapi.main:app --workers 2
```

First run will take time as it download the models

SAM semi-automatic annotations

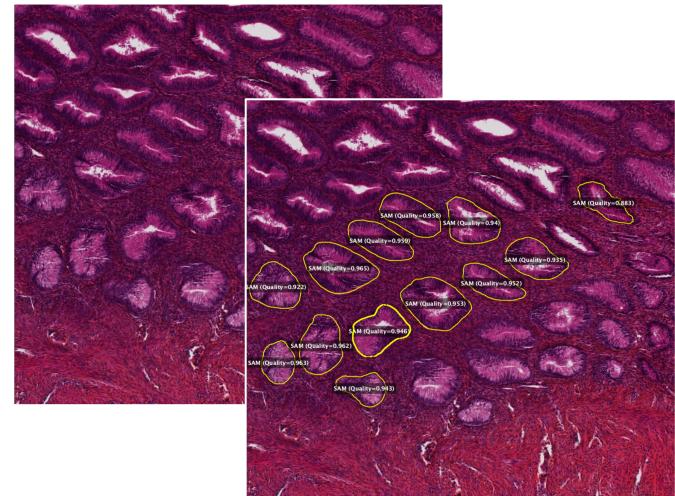
- Load XXXX and select an image to process
- Start the SAM server (in a terminal) and the SAM extension
- Annotates the red cells using Rectangle

Courtesy of BioTiGR Teams, BIGR Unite, Necker Hospital, Paris.

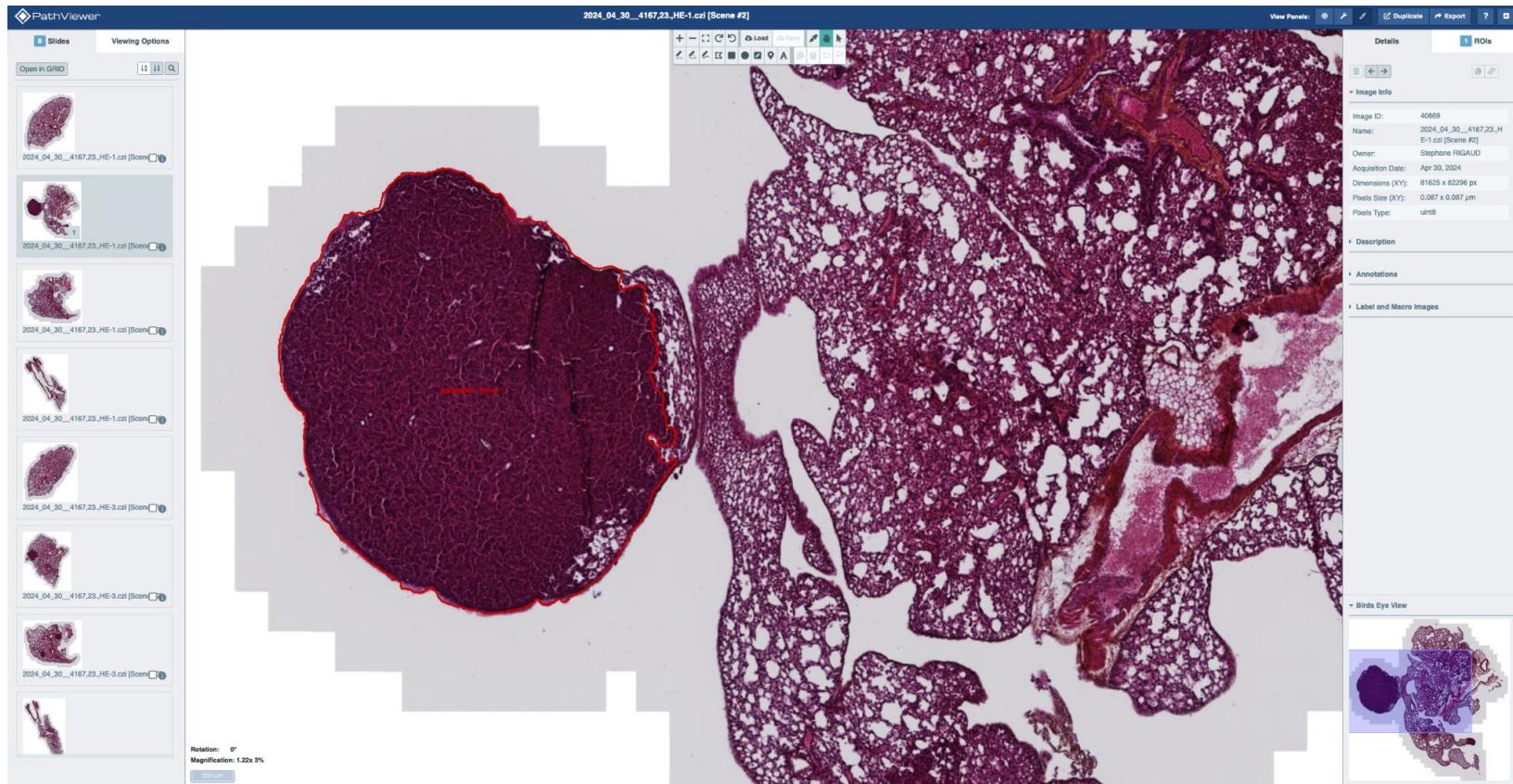


-
- Load XXXX and select an image to process
 - Start the SAM server (in a terminal) and the SAM extension
 - Annotates the secretion glands using Rectangle

Courtesy of E.Liorzou, Comparative Functional Genomics, Institut Pasteur, Paris.



Omero + PathViewer



Additional material - go have a look

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QuPath

Welcome to QuPath!

[Edit on GitHub](#)

0.5

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- Introduction
- Getting started



Acknowledge

Bankhead, P., Loughrey, M.B., Fernández, J.A. *et al.* QuPath: Open source software for digital pathology image analysis. *Sci Rep* **7**, 16878 (2017). <https://doi.org/10.1038/s41598-017-17204-5>

Pécot Thierry. (2022, March 28). Whole-Slide Image Analysis and Quantitative Pathology with QuPath. Zenodo. <https://doi.org/10.5281/zenodo.6391629>

U. Schmidt et al. Cell Detection with Star-convex Polygons. International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI) (2018). <https://arxiv.org/abs/1806.03535>