

# Whole-Slide Image Analysis and Quantitative Pathology with QuPath

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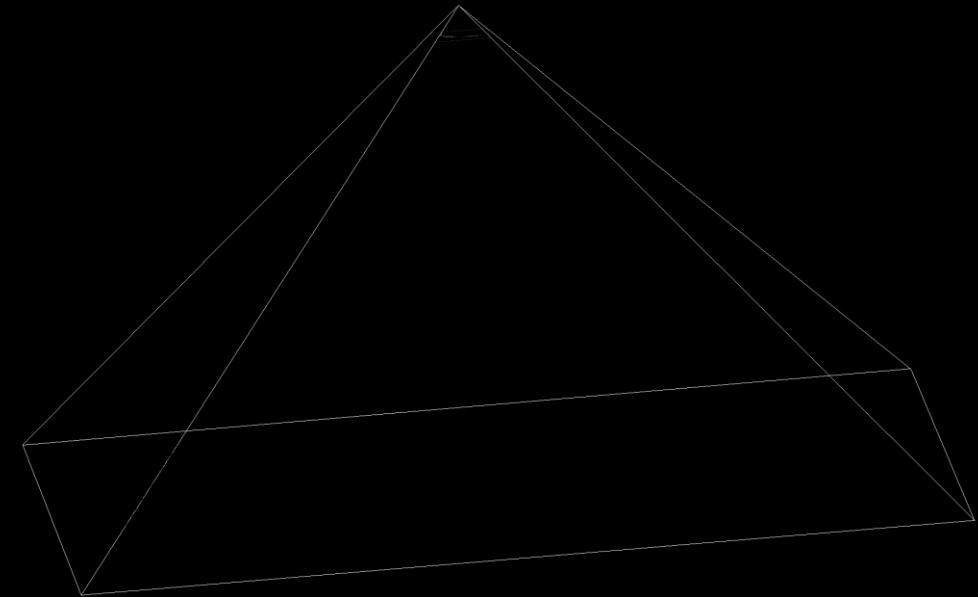
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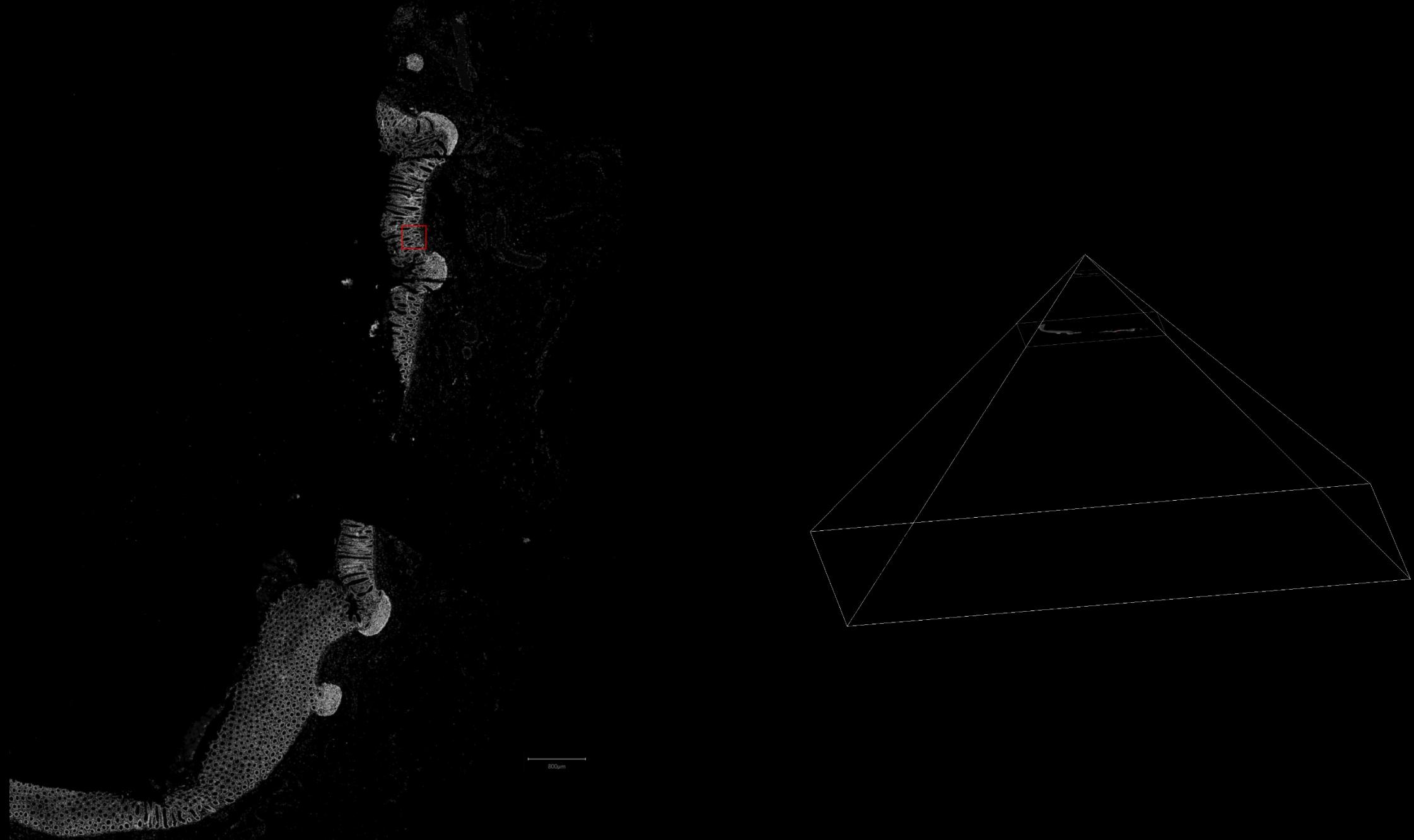
**only** load the **required information** in the RAM memory:

- Define **several resolutions** to create a **pyramidal representation**

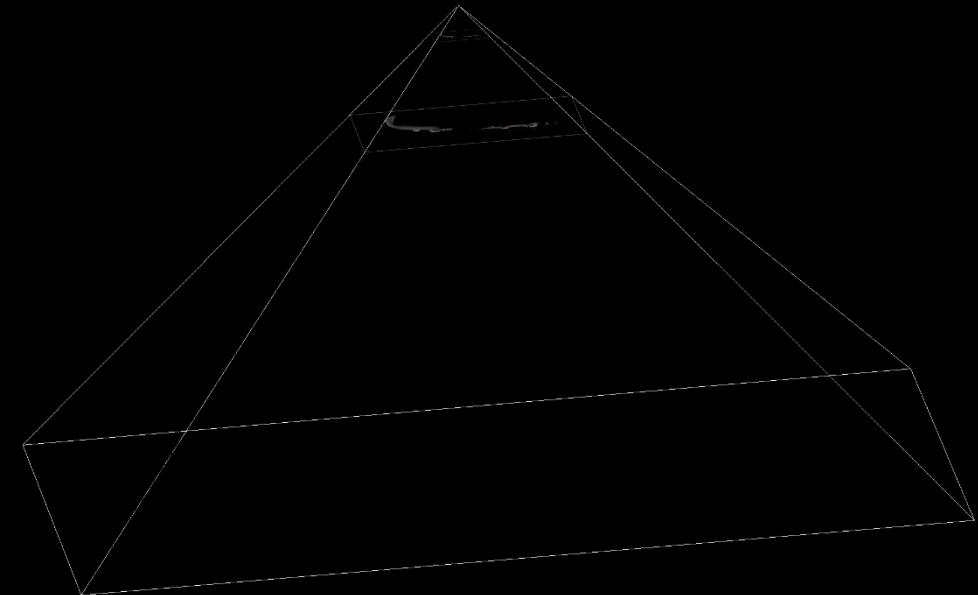
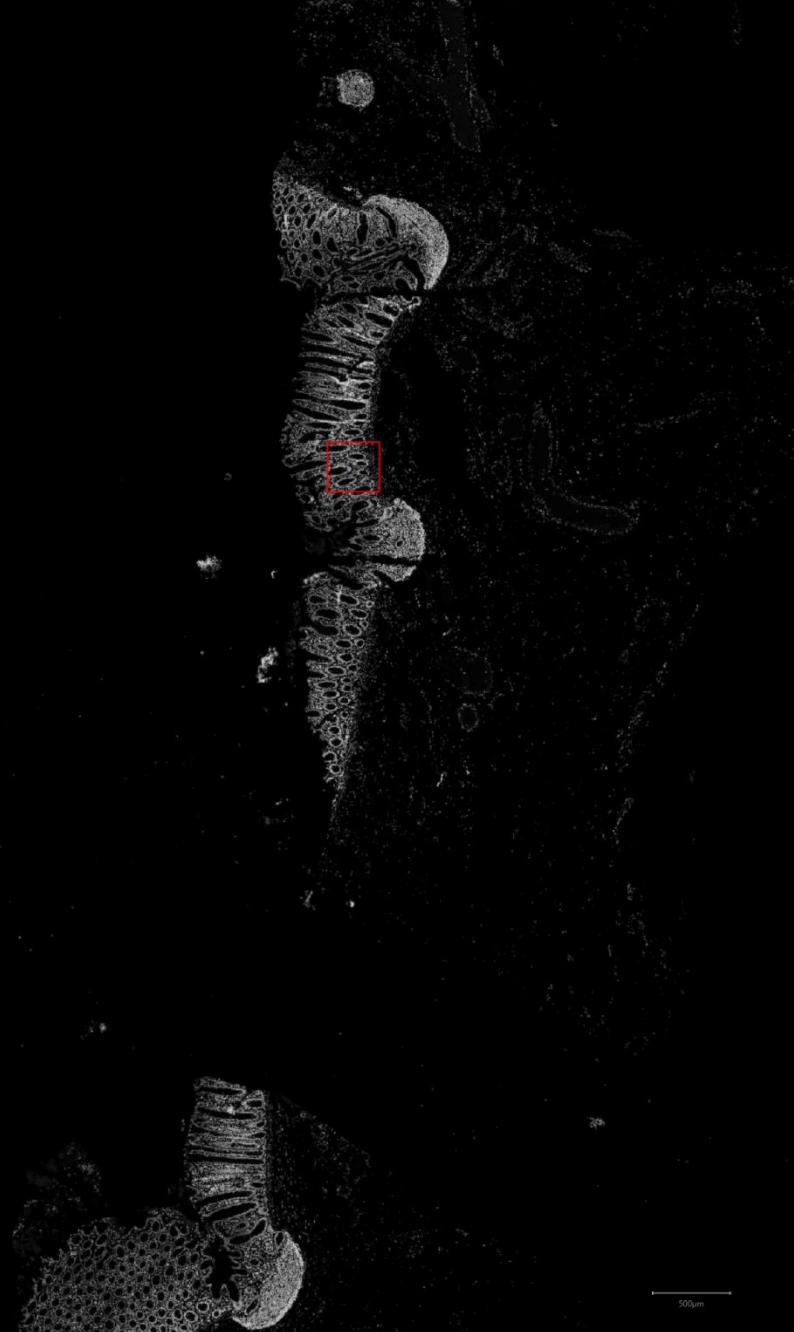
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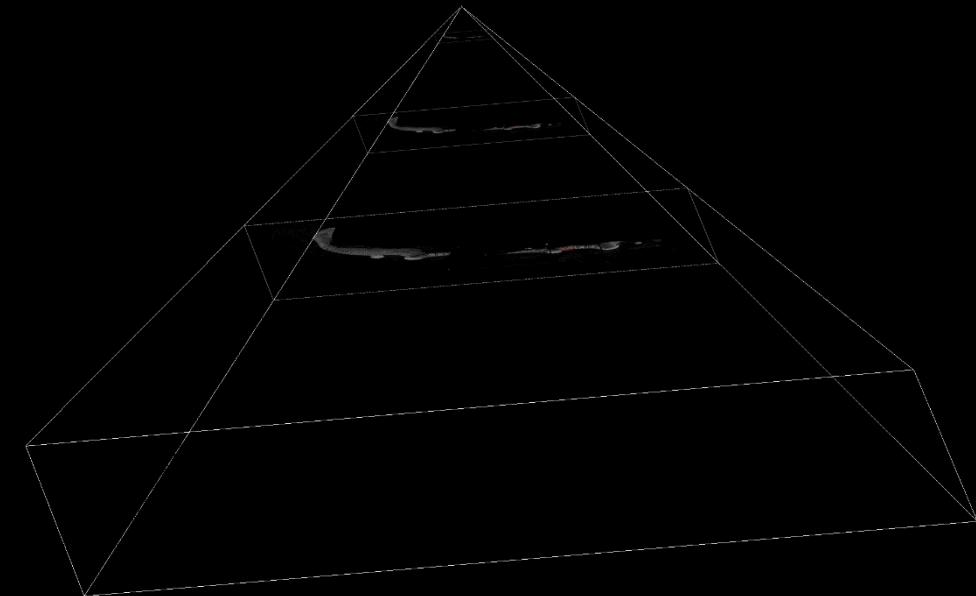
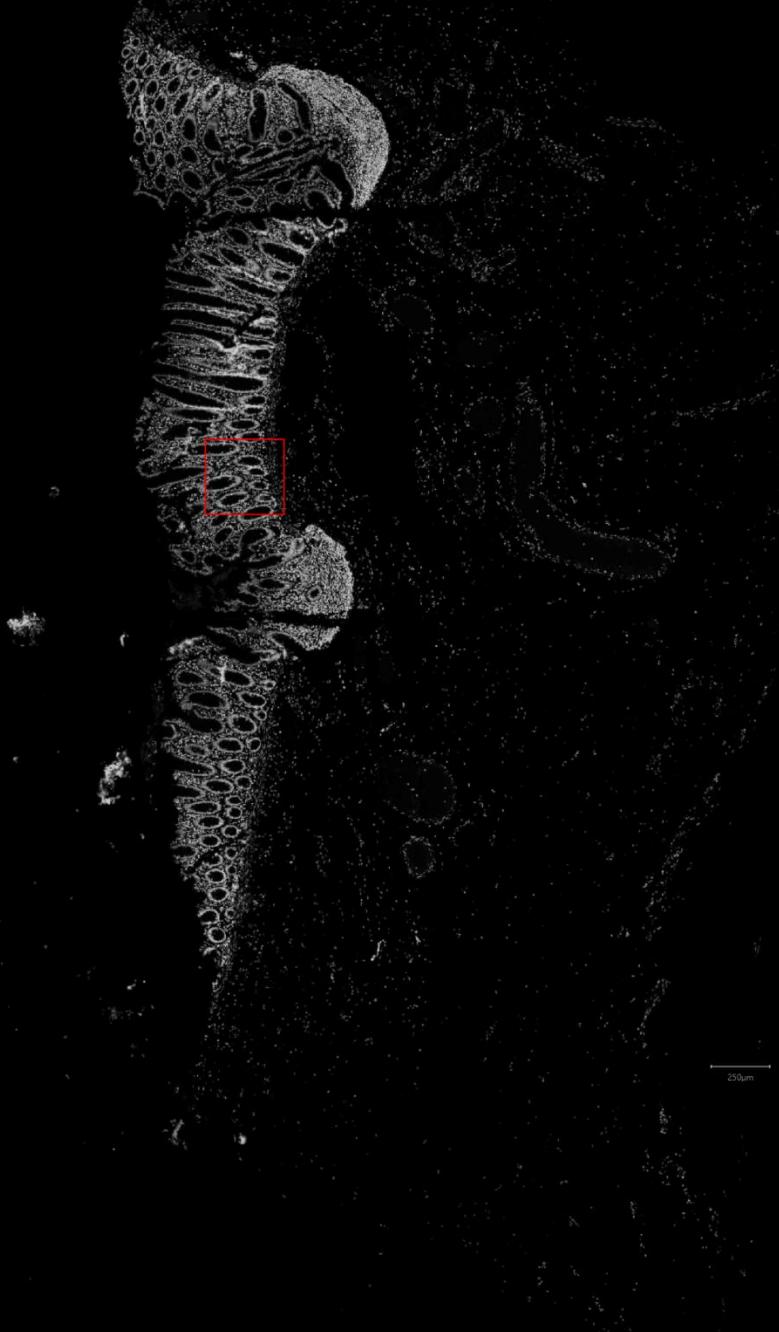


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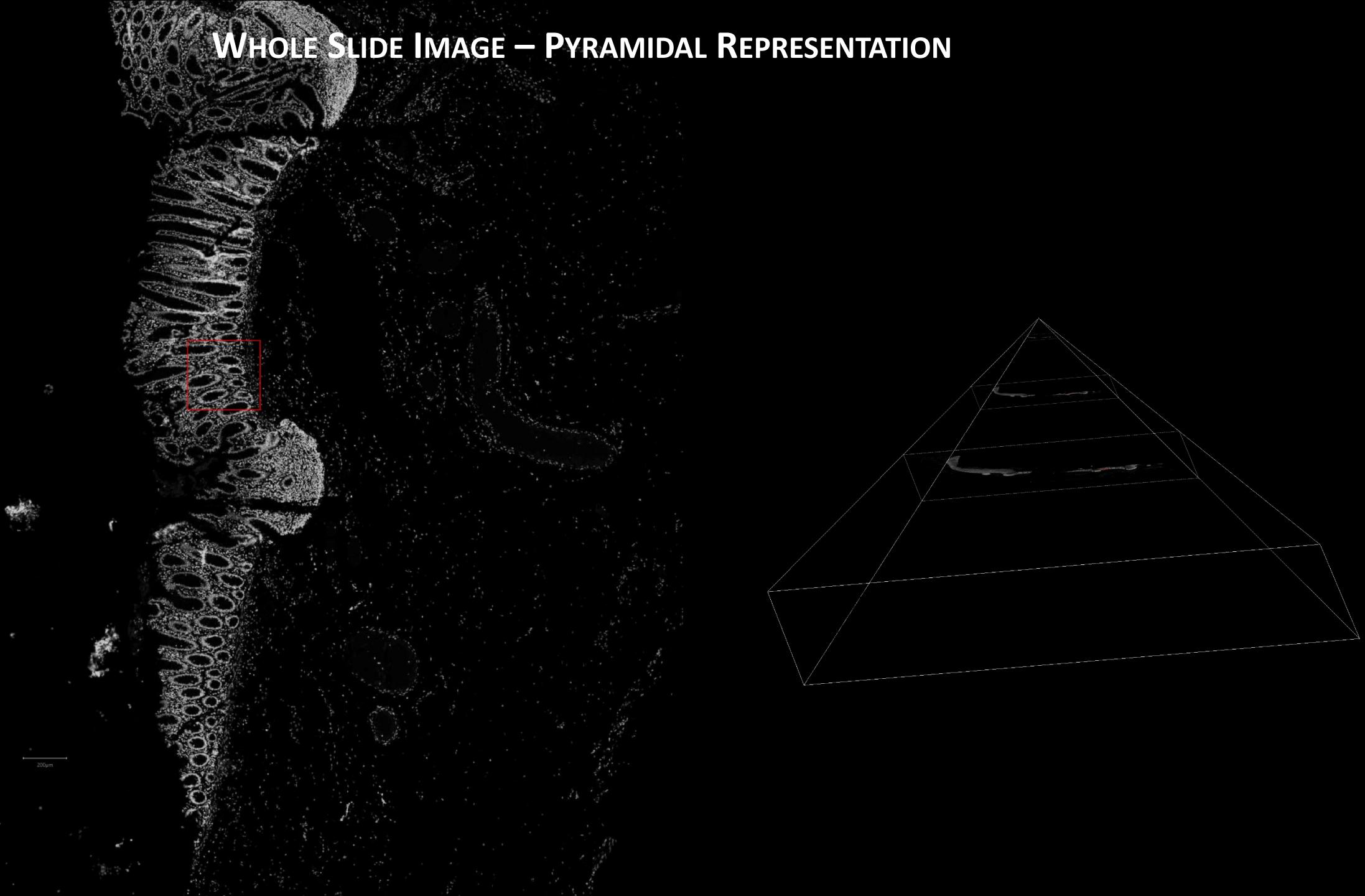


500µm

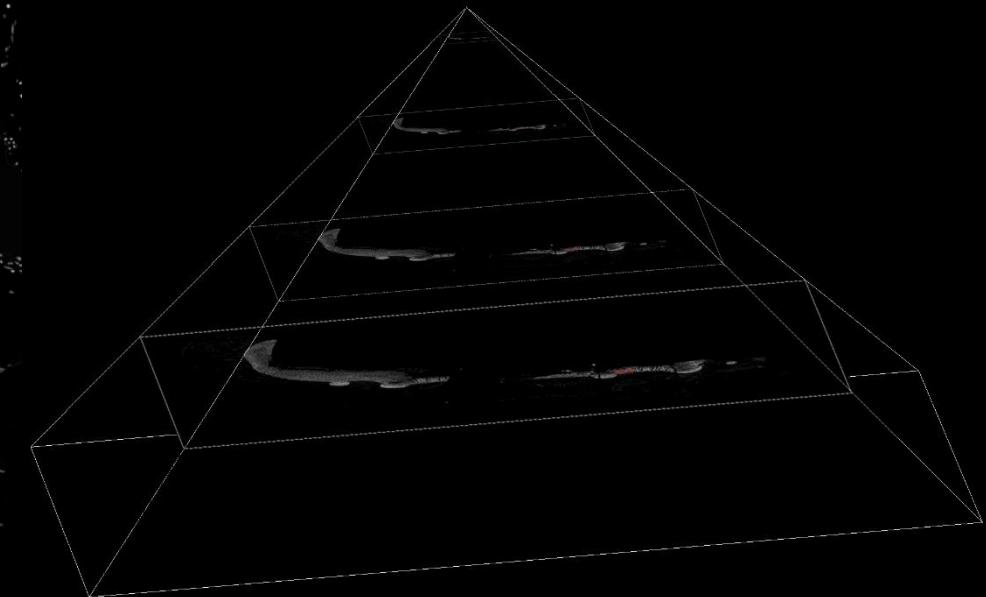
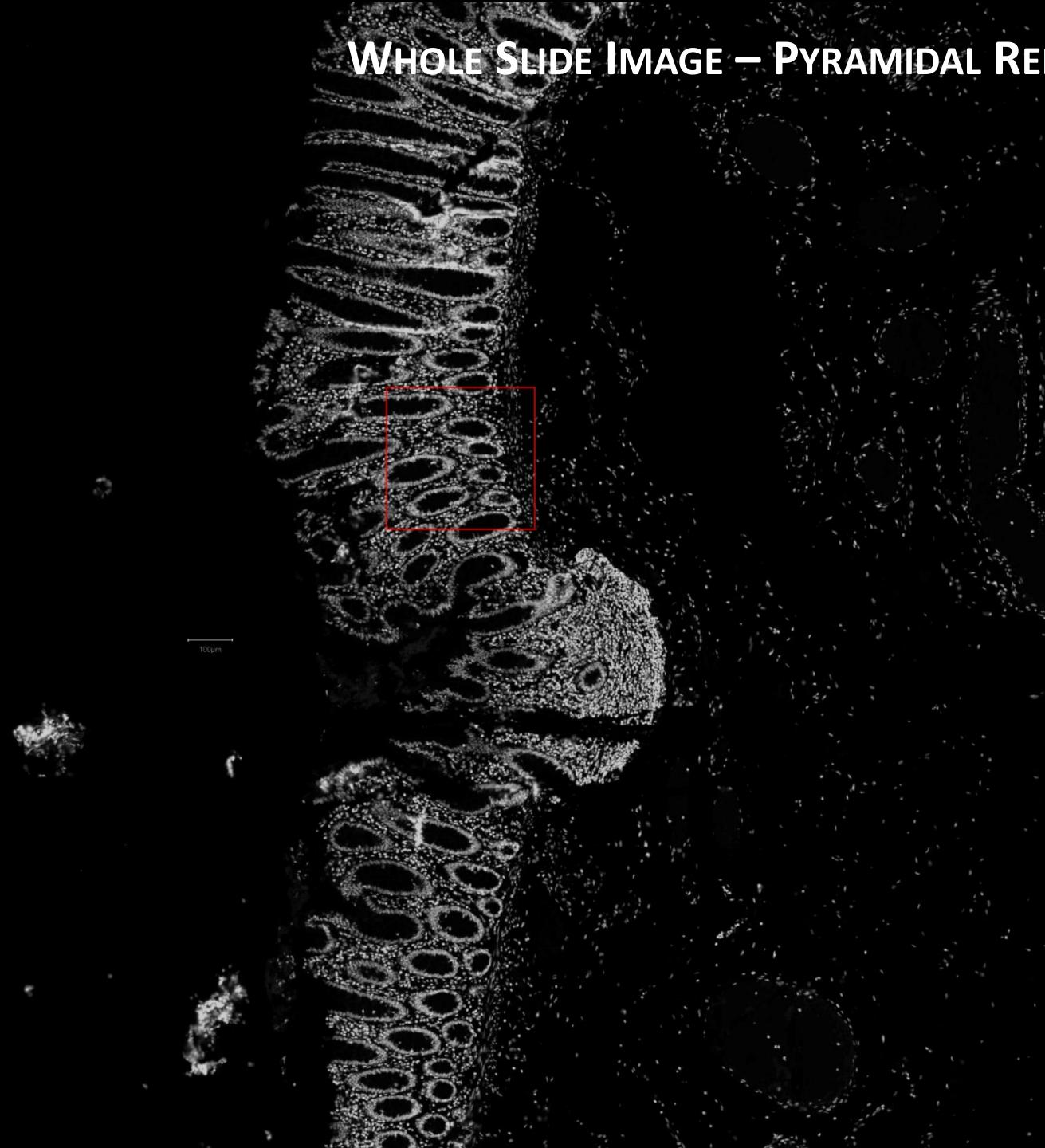
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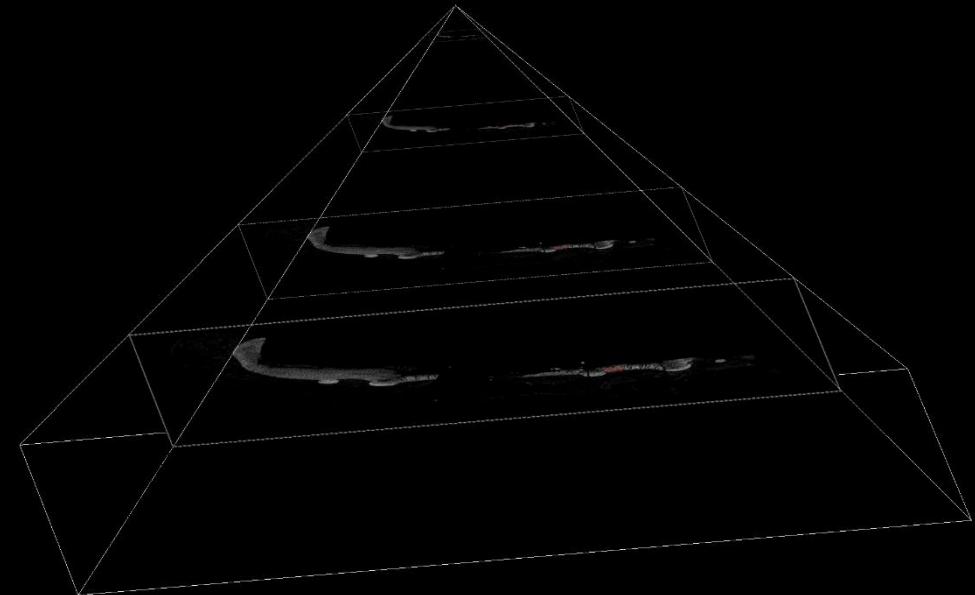
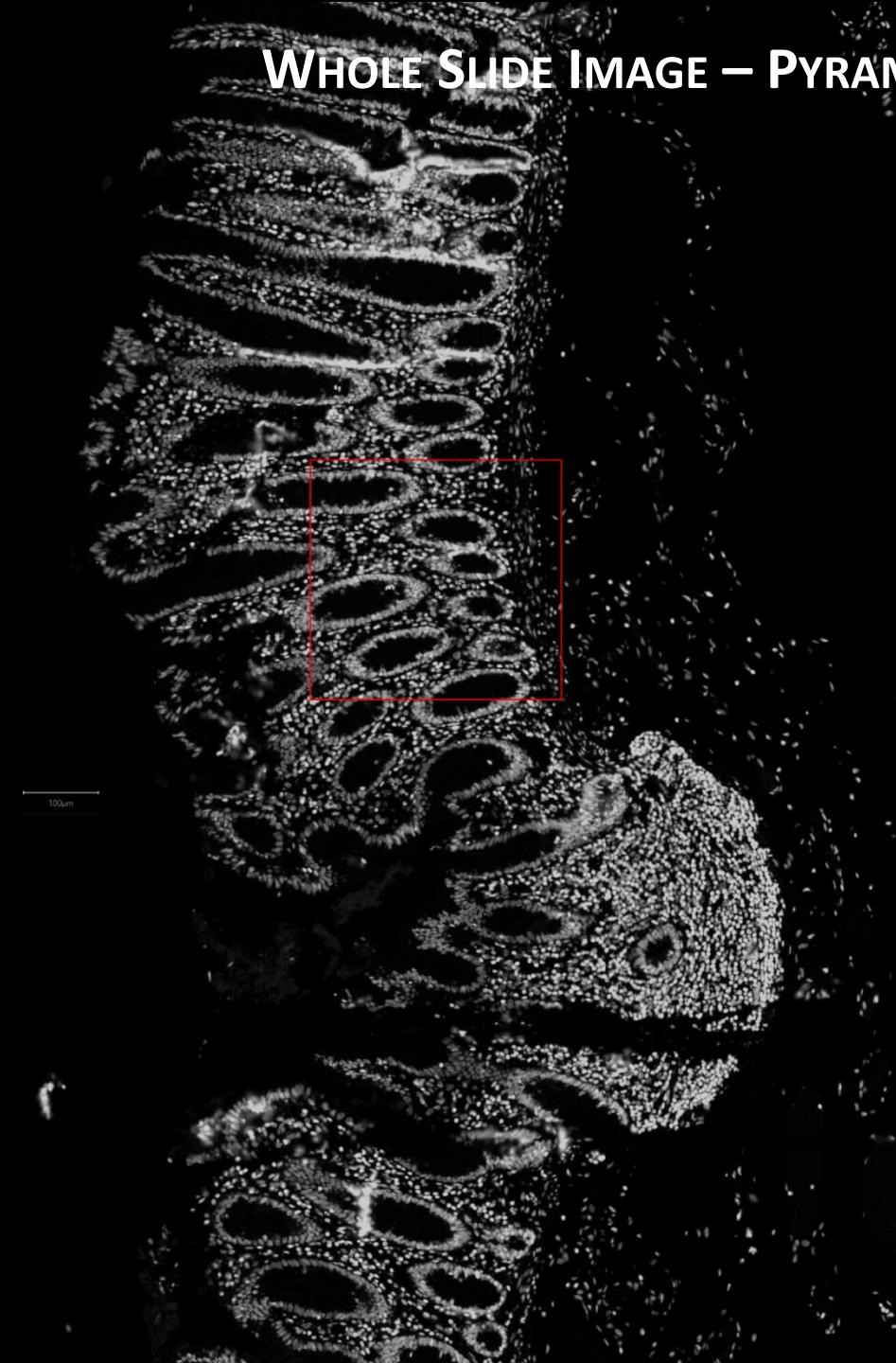
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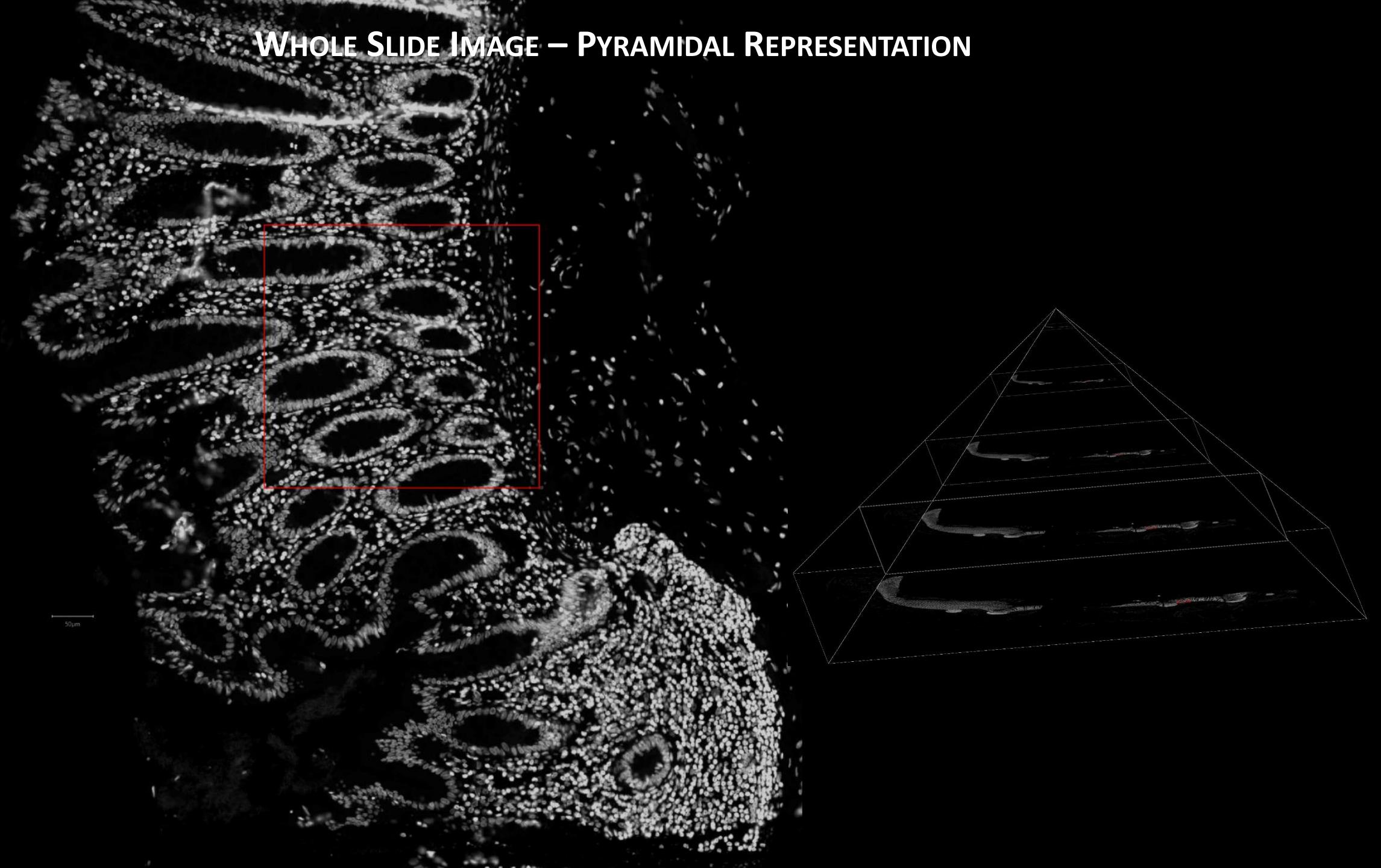
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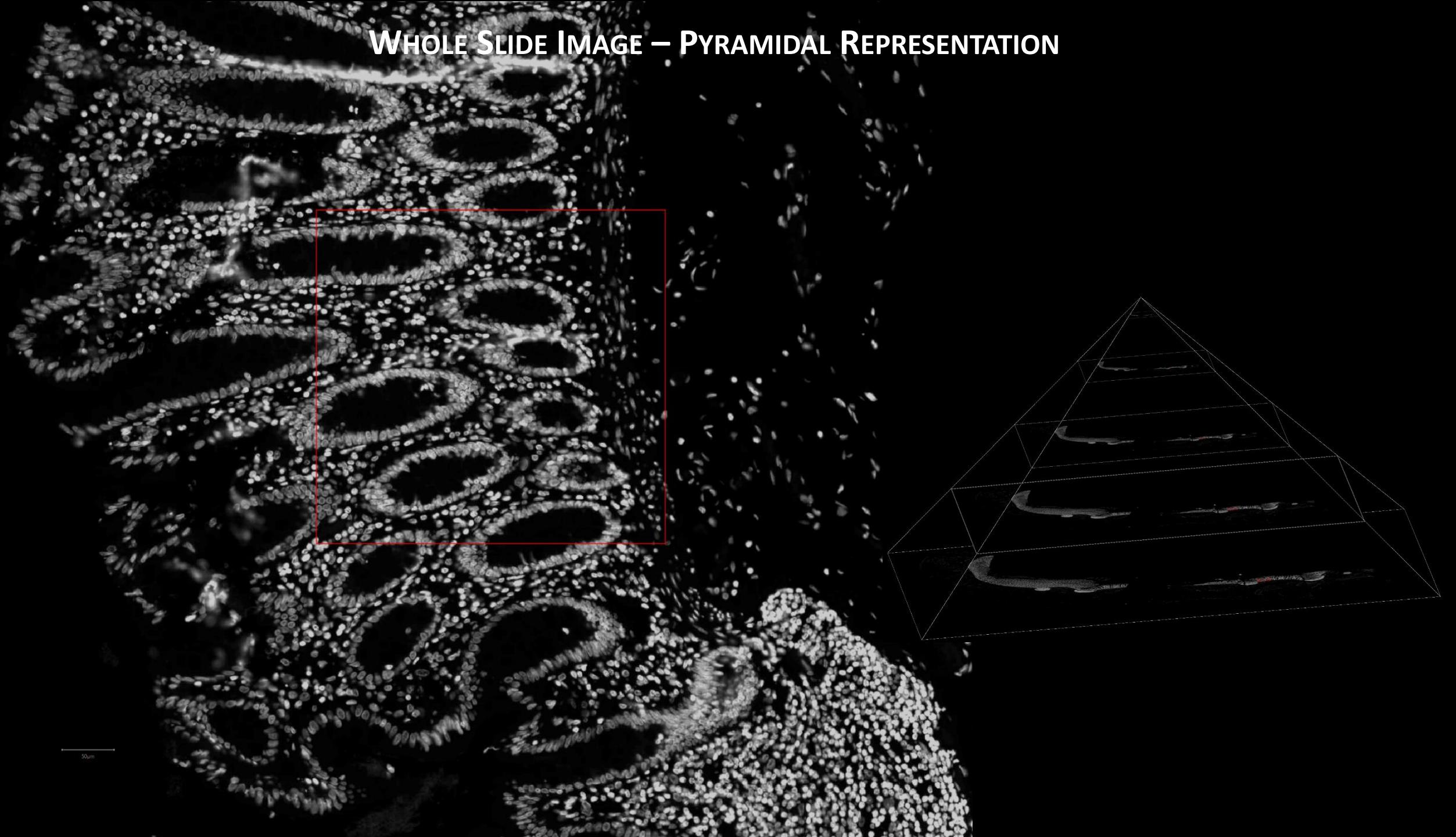
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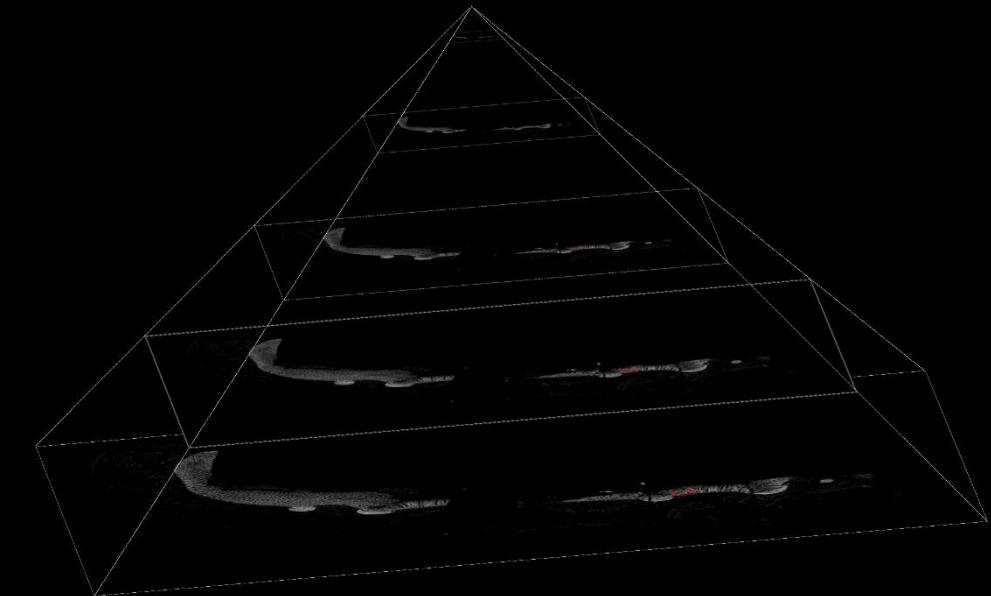
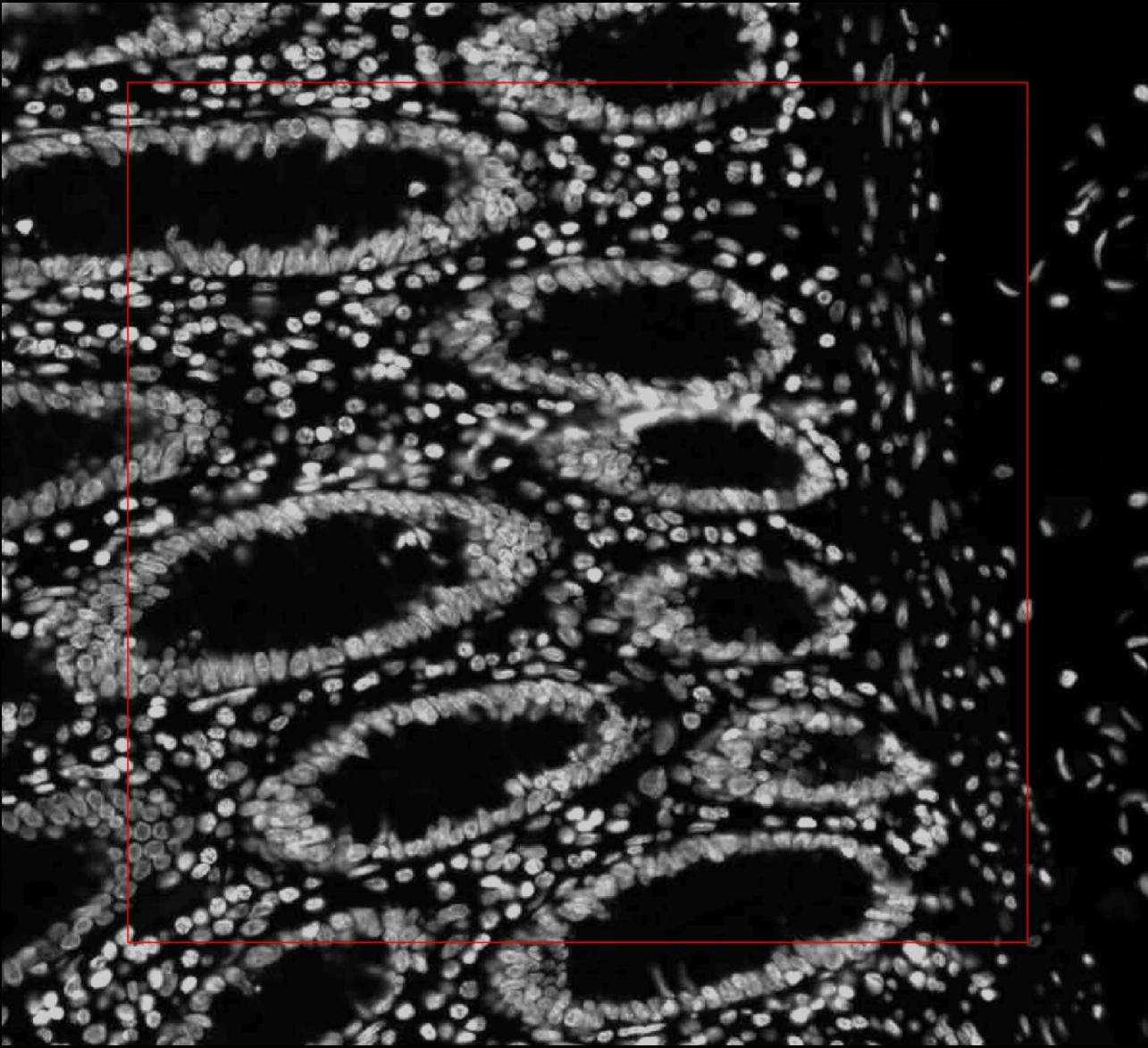


# WHOLE SLIDE IMAGE – PYRAMIDAL REPRESENTATION



50µm

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## WHOLE-SLIDE IMAGE SIZE

- A **full H&E(S)** whole-slide image would be:
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{27.78 \text{ GB}}$  for resolution 1
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{6.94 \text{ GB}}$  for resolution 2
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{1.74 \text{ GB}}$  for resolution 4
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{434 \text{ MB}}$  for resolution 8
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{109 \text{ MB}}$  for resolution 16
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{27 \text{ MB}}$  for resolution 32
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{7 \text{ MB}}$  for resolution 64
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{1.7 \text{ MB}}$  for resolution 128
  - $9\ 259\ 259\ 259 * 1 \text{ byte} * 3 = \mathbf{424 \text{ KB}}$  for resolution 256



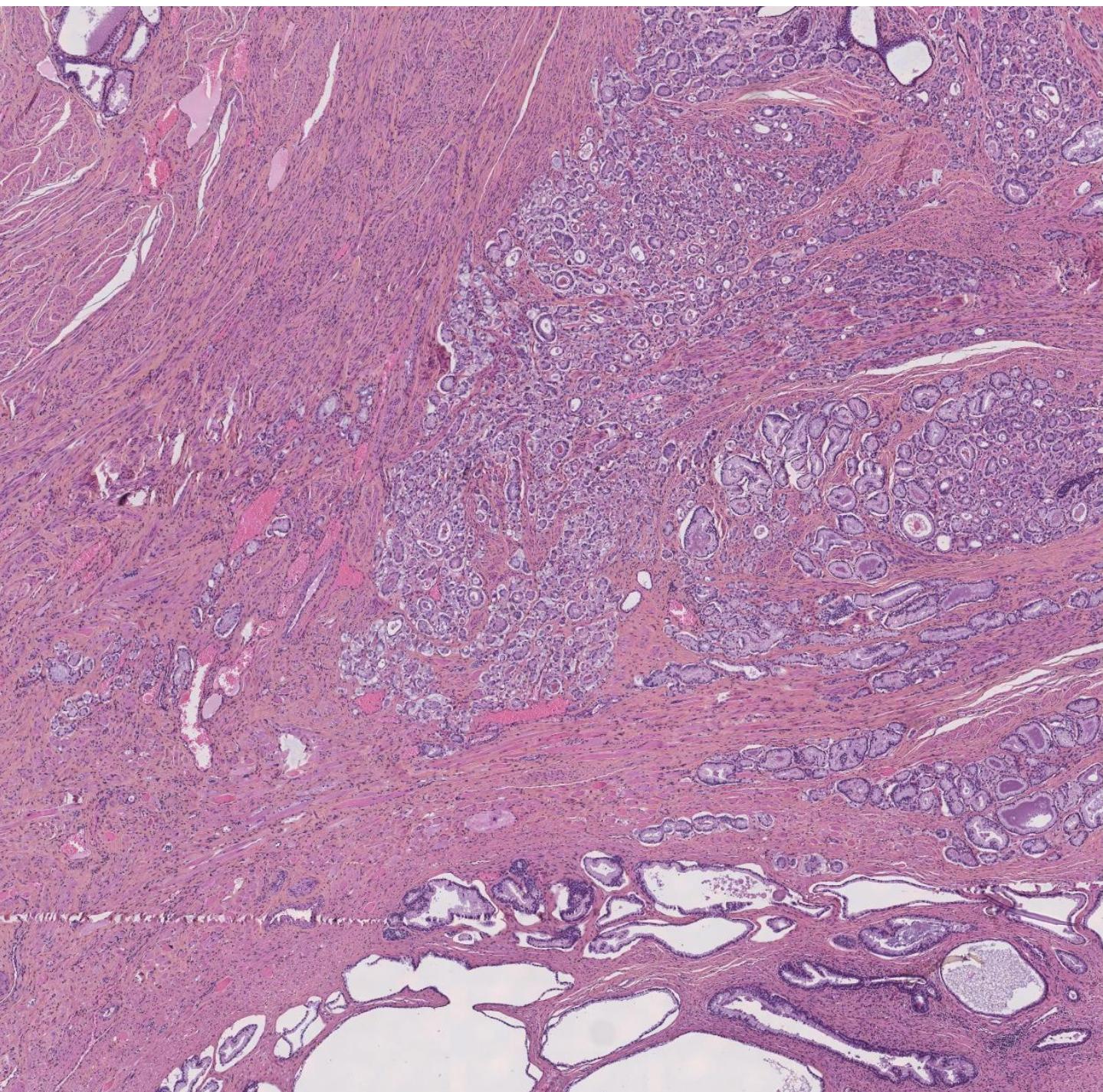
Total of **37 GB** uncompressed data

## ANNOTATIONS

- Allow to **add information** to specific regions or entire images
- Lots of **features/measurements** can then be extracted from these regions
- **Powerful and storage-efficient** way to process images
- Can be **manually** defined or **automatically** estimated
- Can be enriched by **adding classes**

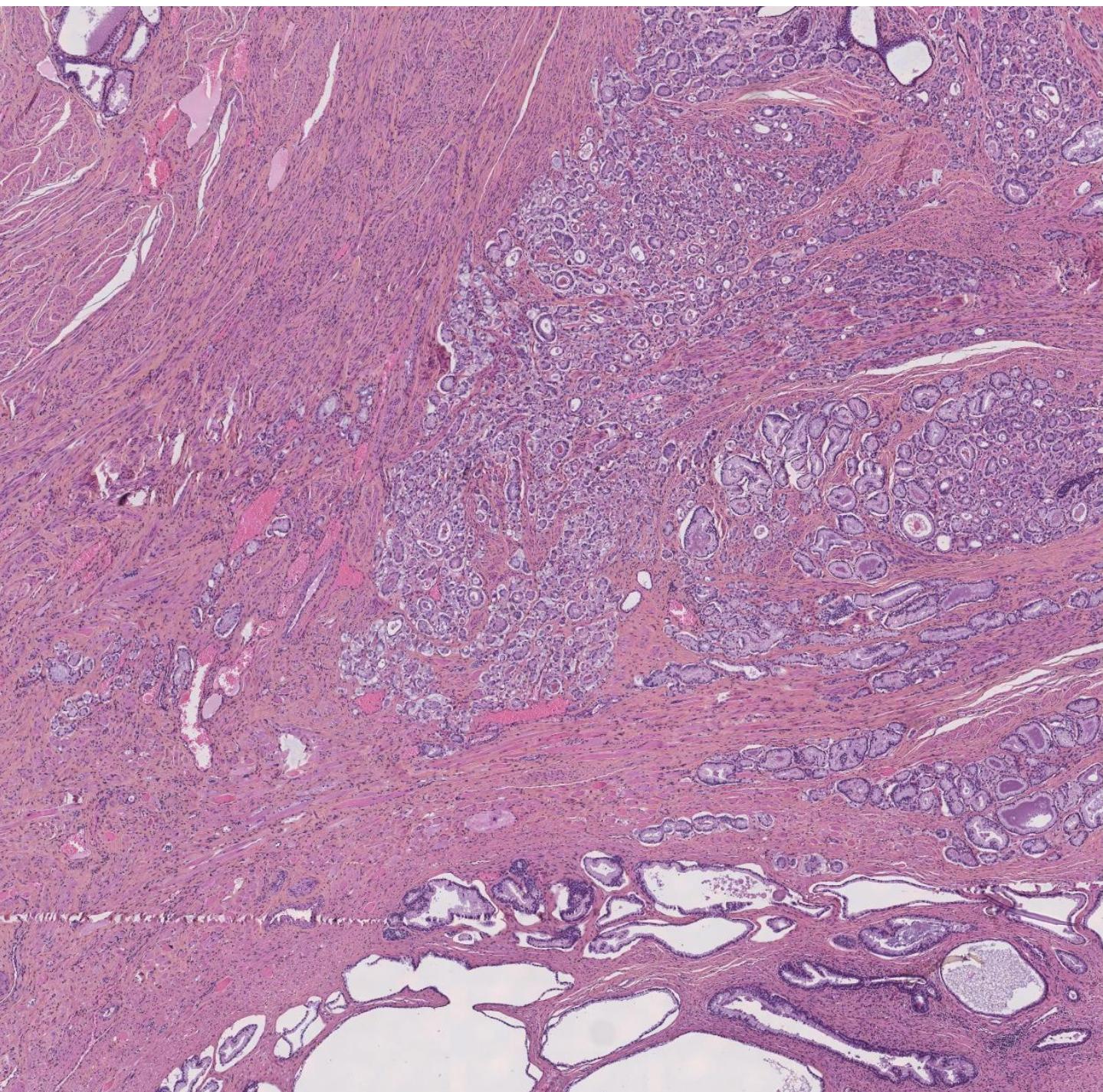
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- **Open Prostate1.ome.tif**
- Create **different types** of annotations
- Play with **resolution**
- Look at the **measurements** for each **type of annotation**



## STAIN ESTIMATION

- **Hematoxylin** stains **nuclei** in purple/blue
- **Eosin** stains **extracellular matrix** and **cytoplasm** in pink
- **DAB** is used to stain **antigens** in brown

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- **Stain estimation** consists in transforming Red-Green-Blue channels to **Hematoxylin-Eosin/DAB-Residue** channels
- It greatly facilitates the **nuclei segmentation** in the **hematoxylin** component, the **DAB** region **characterization** in the **DAB** component, ...

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- It greatly facilitates the **nuclei segmentation** in the **hematoxylin** component, the **DAB** region **characterization** in the DAB component, ...
- **Automatic stain estimation in QuPath** is based on:

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

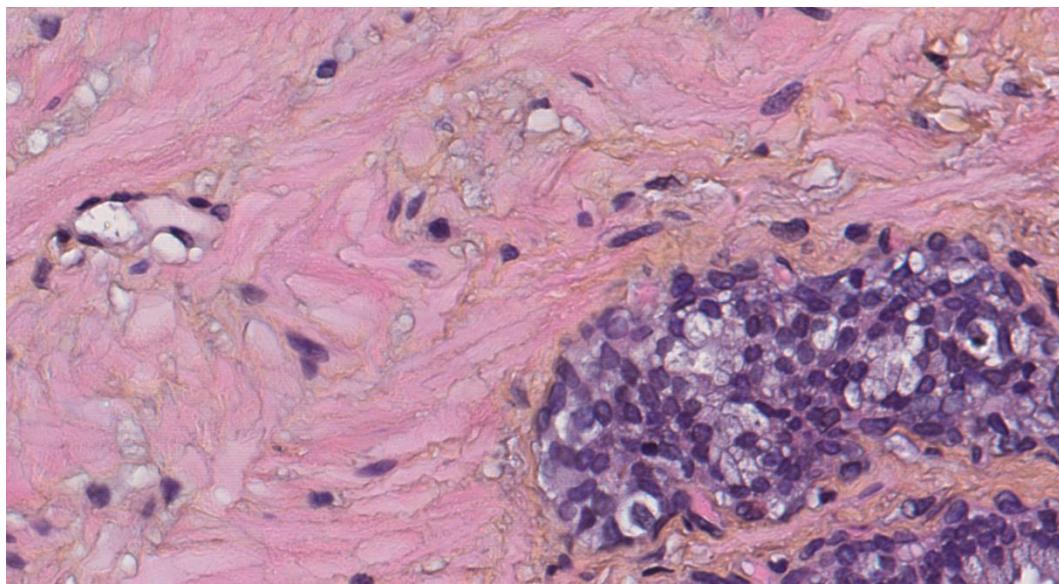
### Quantification of histochemical staining by color deconvolution

A C Ruifrok <sup>1</sup>, D A Johnston

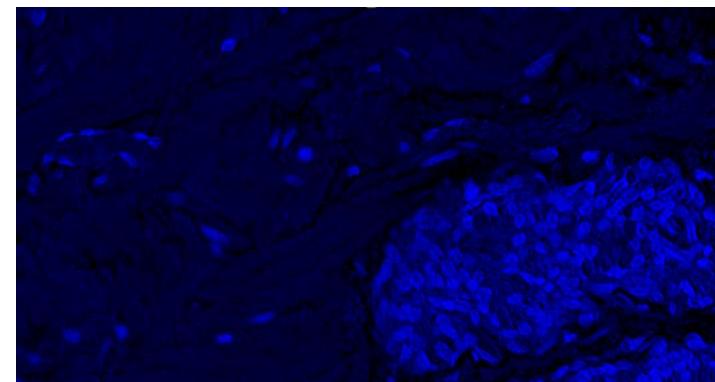
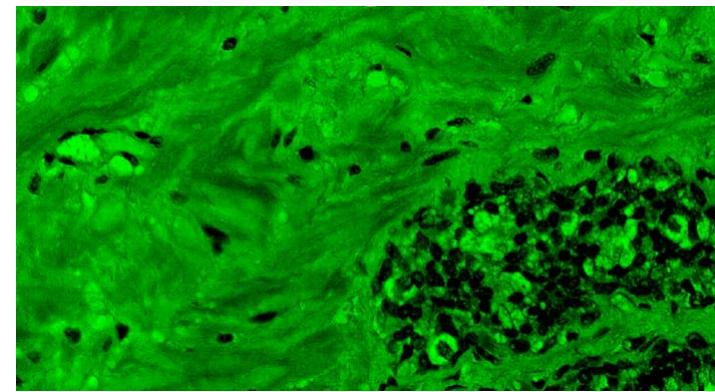
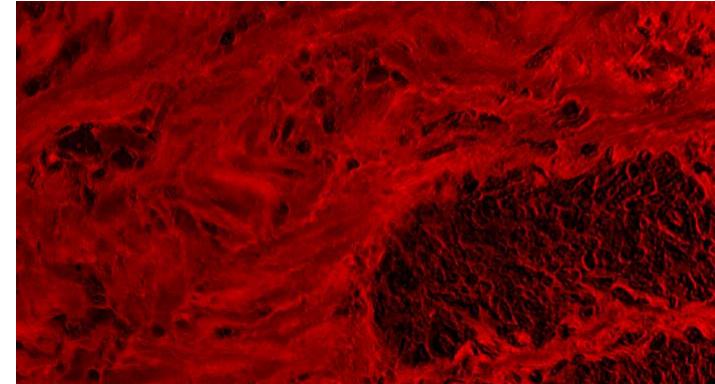
Affiliations + expand

PMID: 11531144

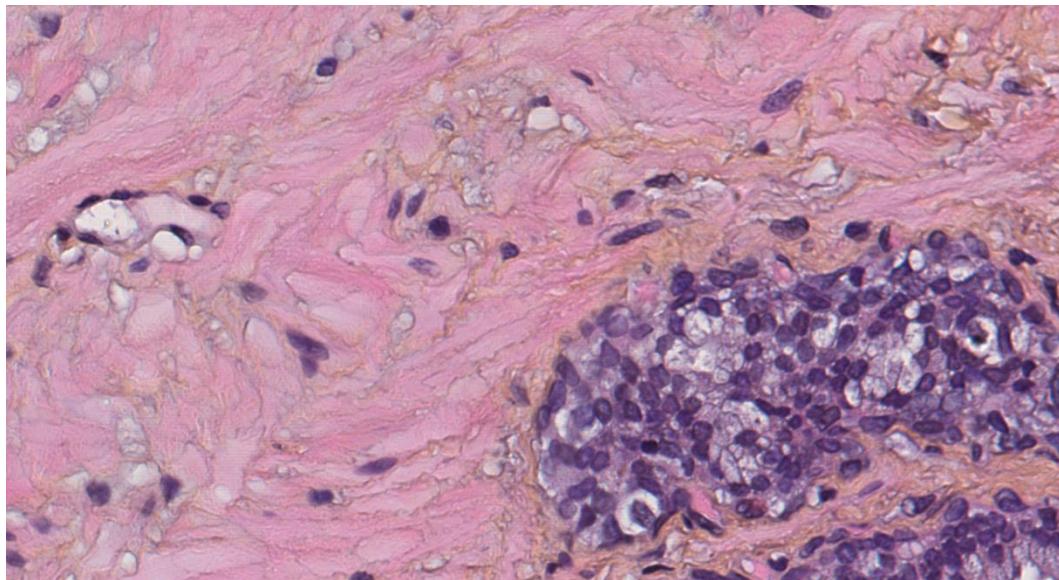
## H&E STAIN ESTIMATION



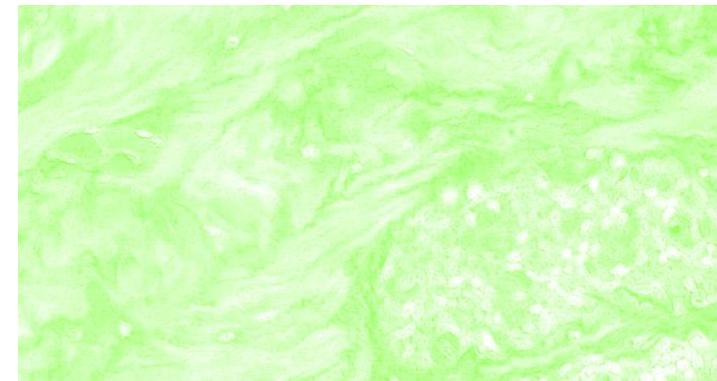
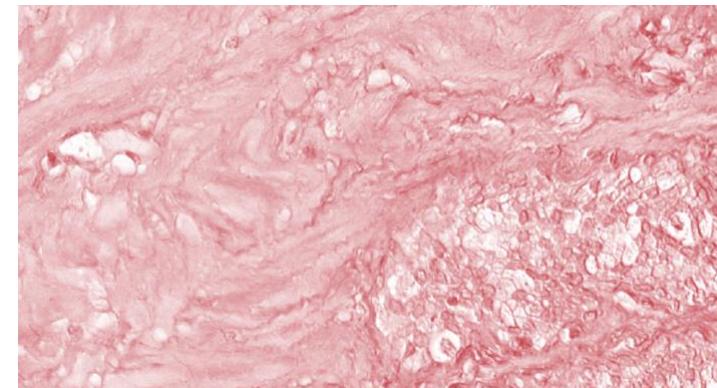
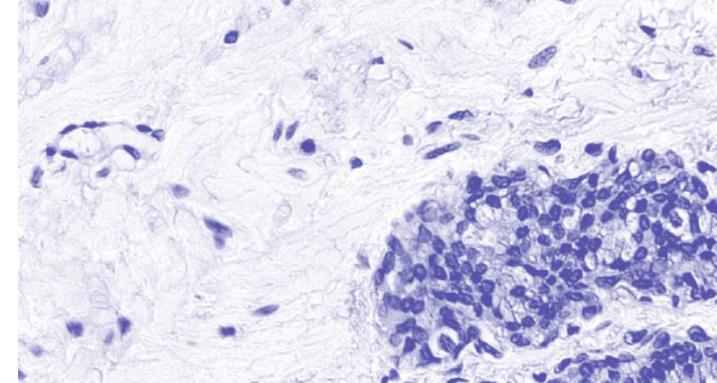
=



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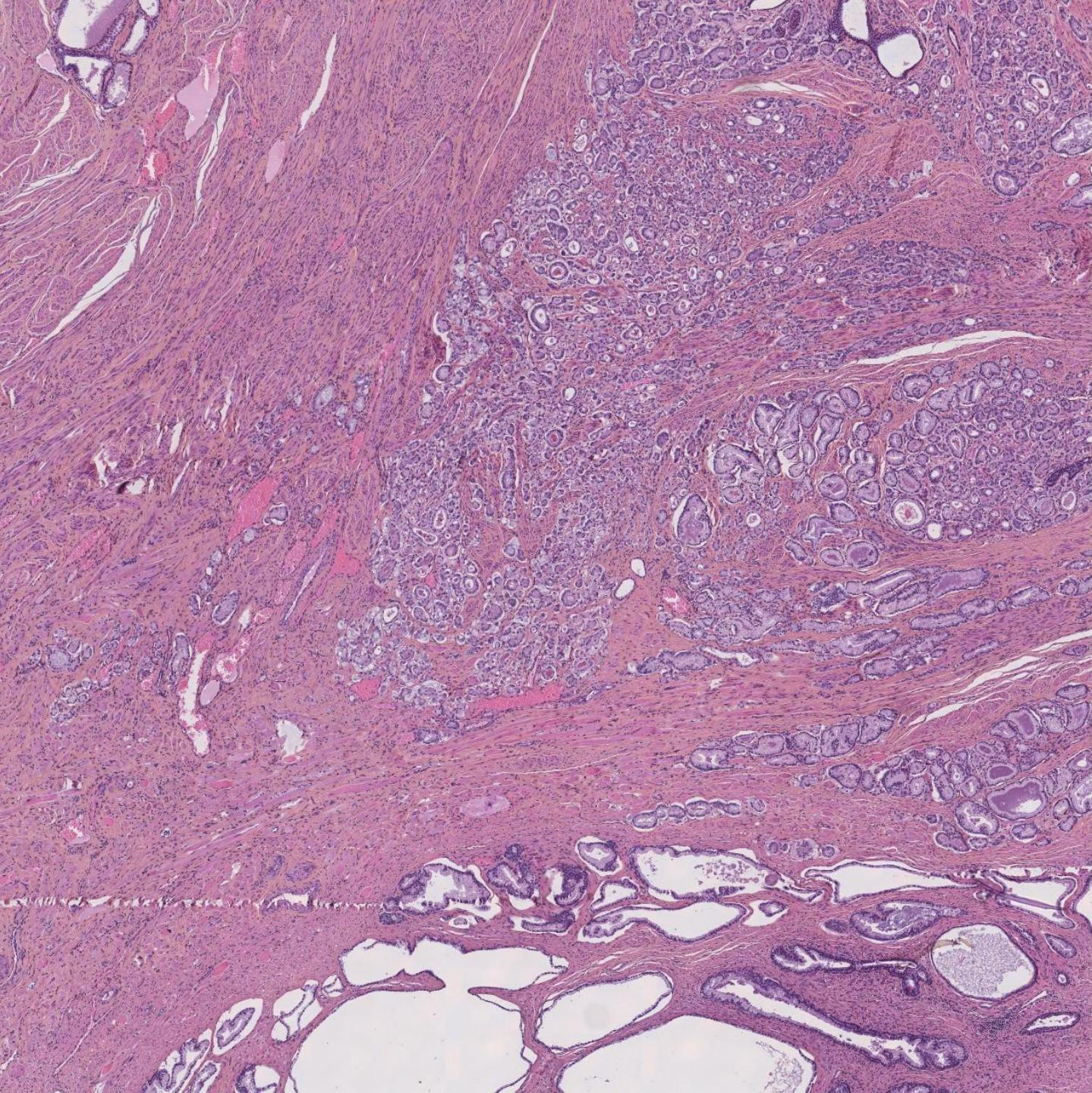


=



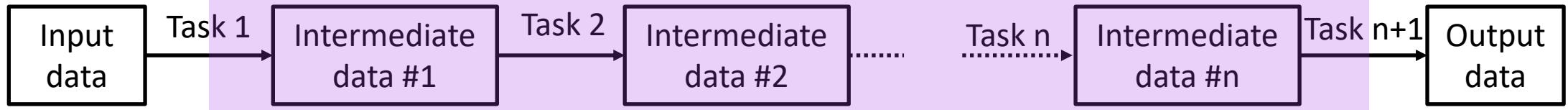
## H&E STAIN ESTIMATION

- **Open Prostate\_1.ome.tif**
- Create a small rectangle annotation and **estimate stain vectors**
- **Manually define Hematoxylin and Eosin components**
- **Visualize the differences**



# EXPLICIT PROGRAMMING

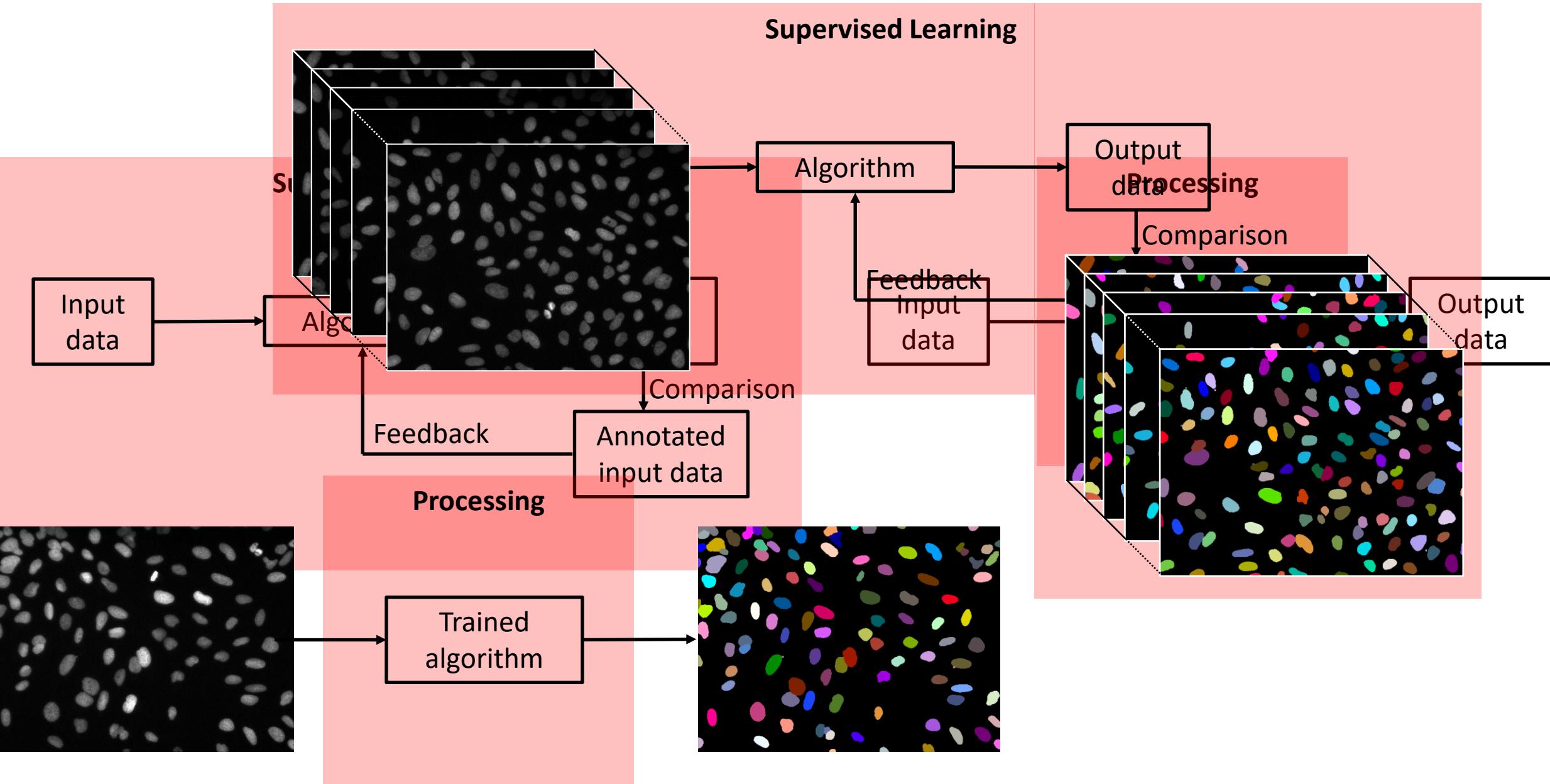
## Image processing workflow



Input image



# SUPERVISED MACHINE LEARNING

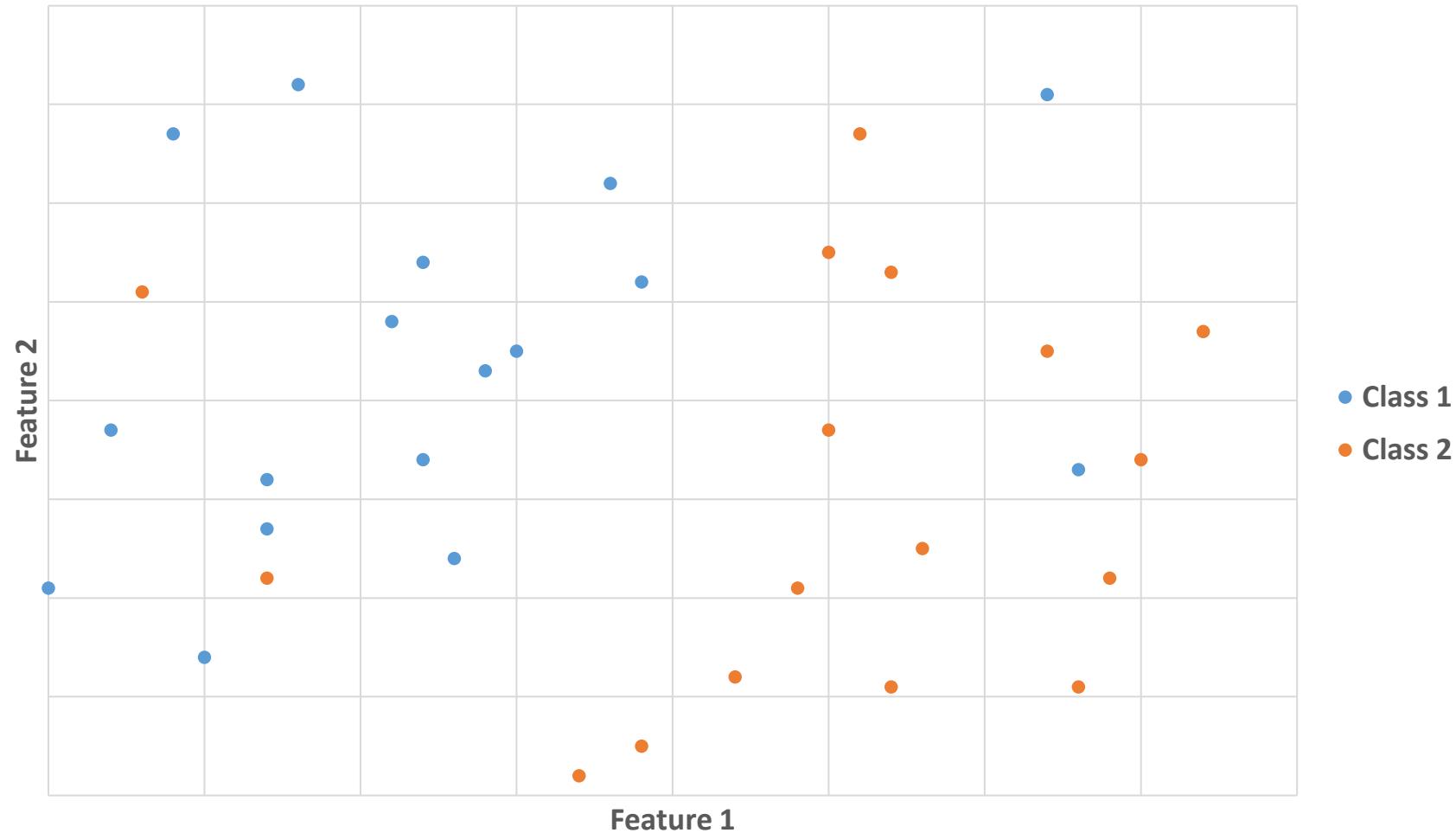


# SHALLOW MACHINE LEARNING FOR PIXEL CLASSIFICATION

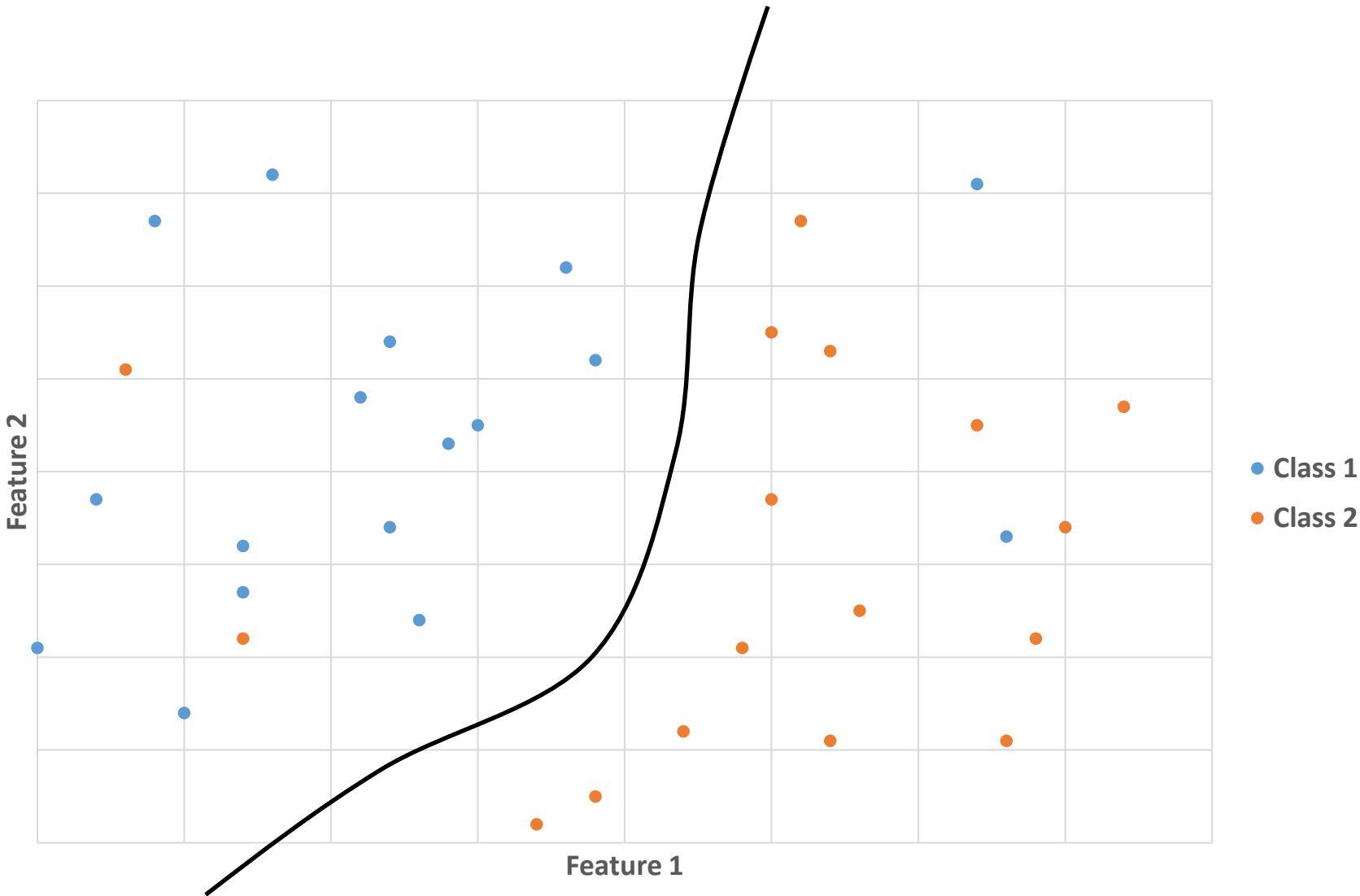
## Supervised classification:

- Examples of classes are **manually** defined by the user
- A **classifier** is **trained** by using **defined features** with these examples
- Data is then **automatically classified** by using the trained classifier

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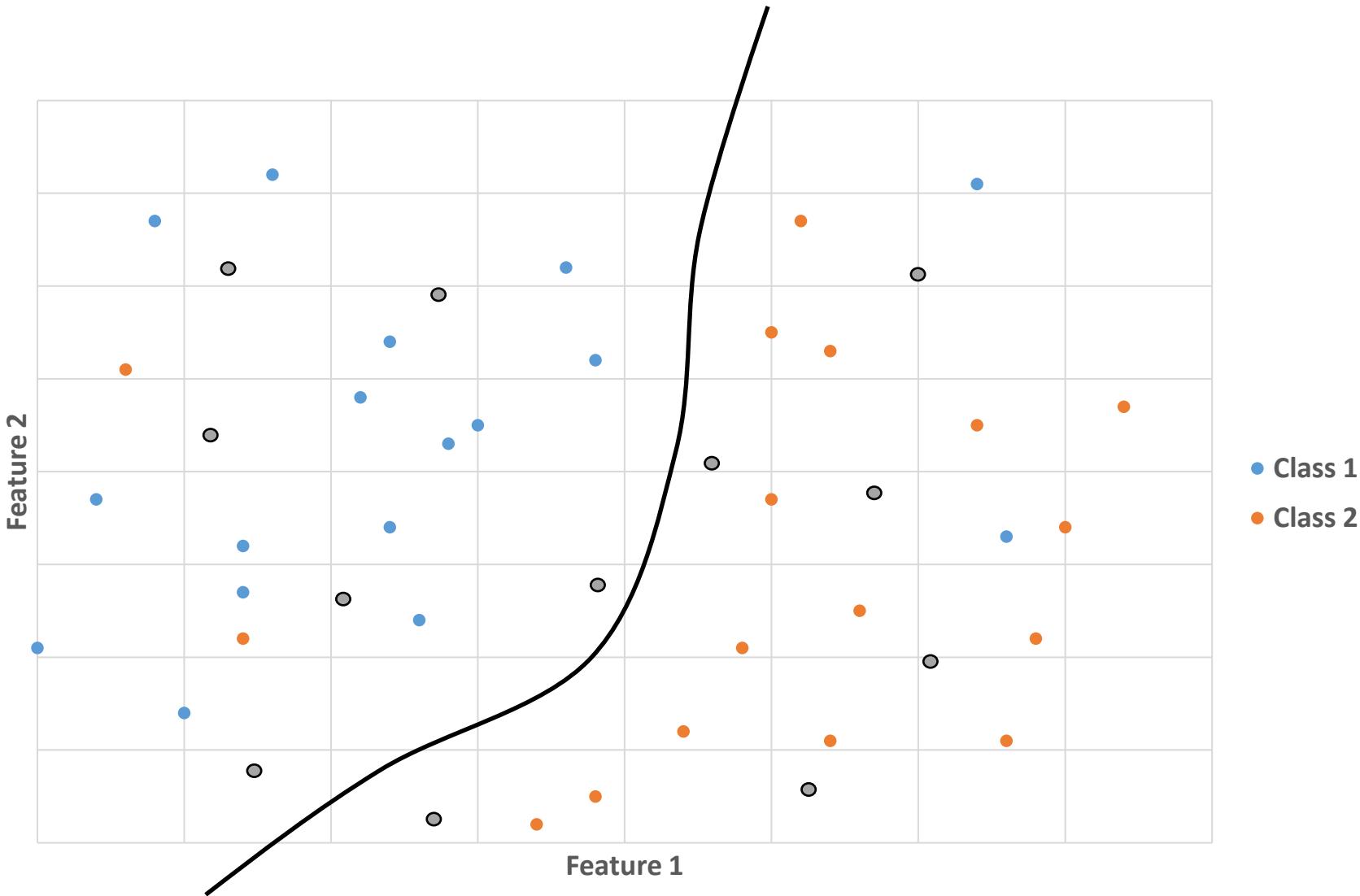


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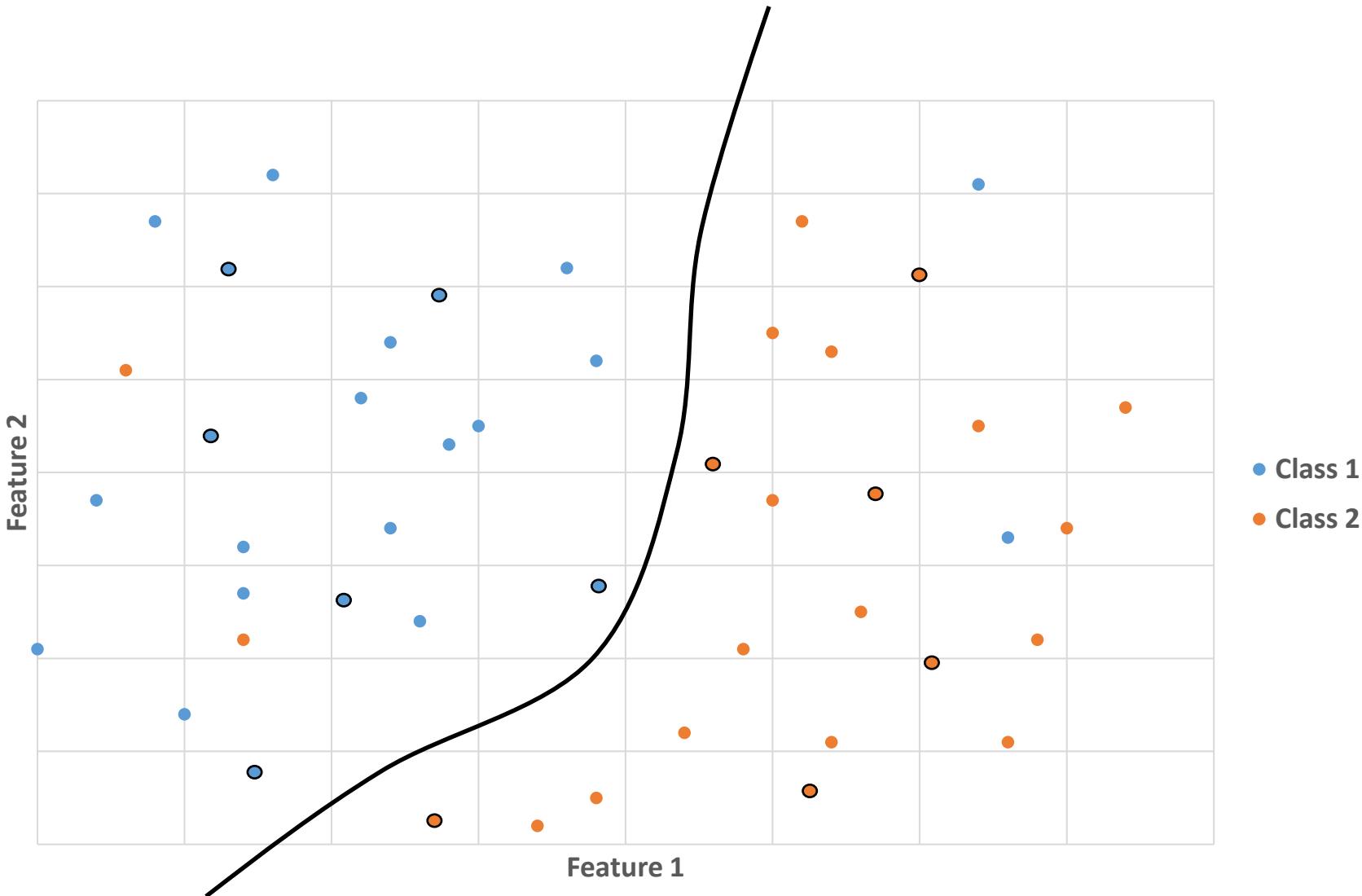
Training a classifier consists in estimating the "best" separation between classes

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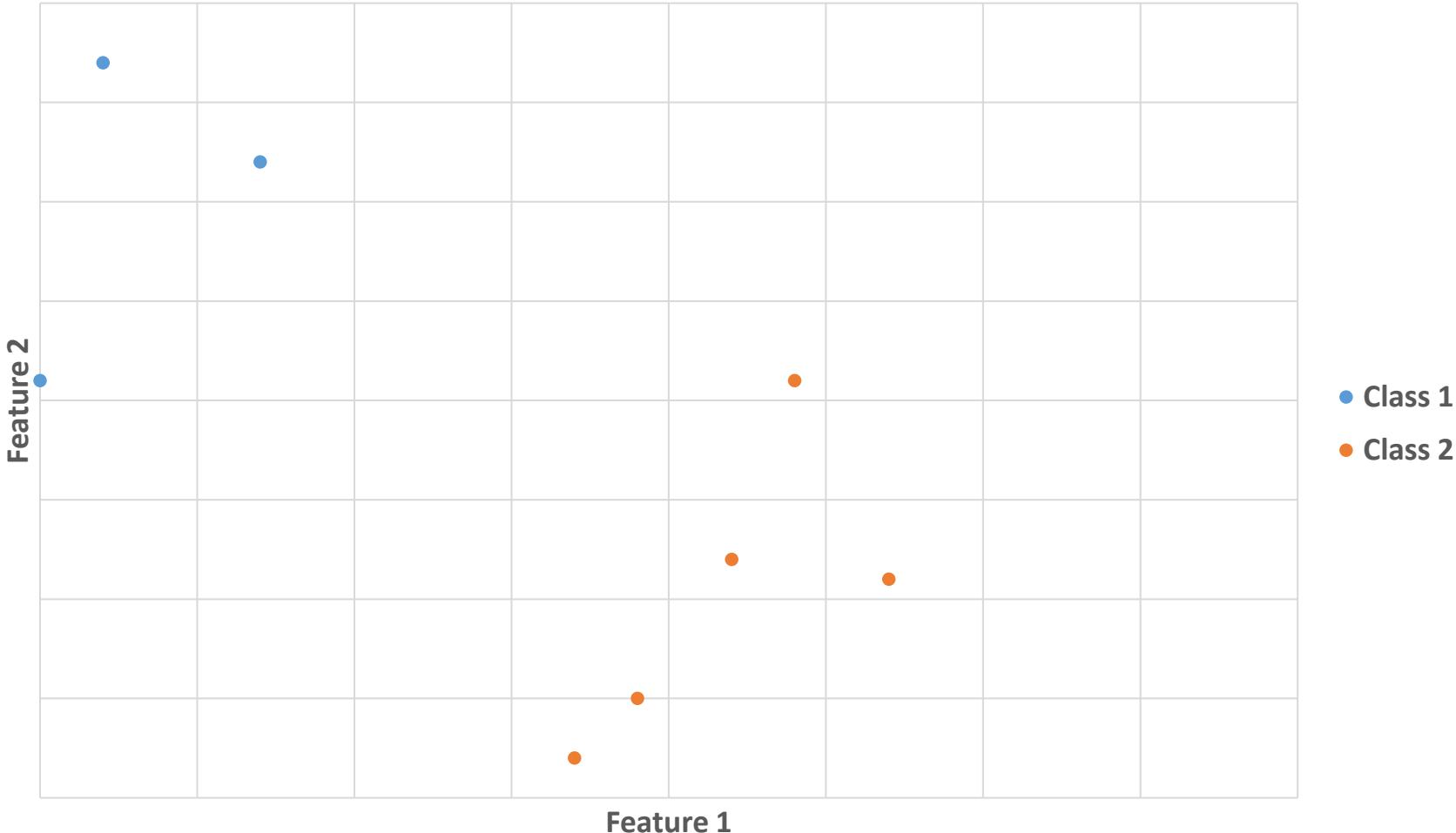
The **estimated class** for new data will be given by the **trained classifier**

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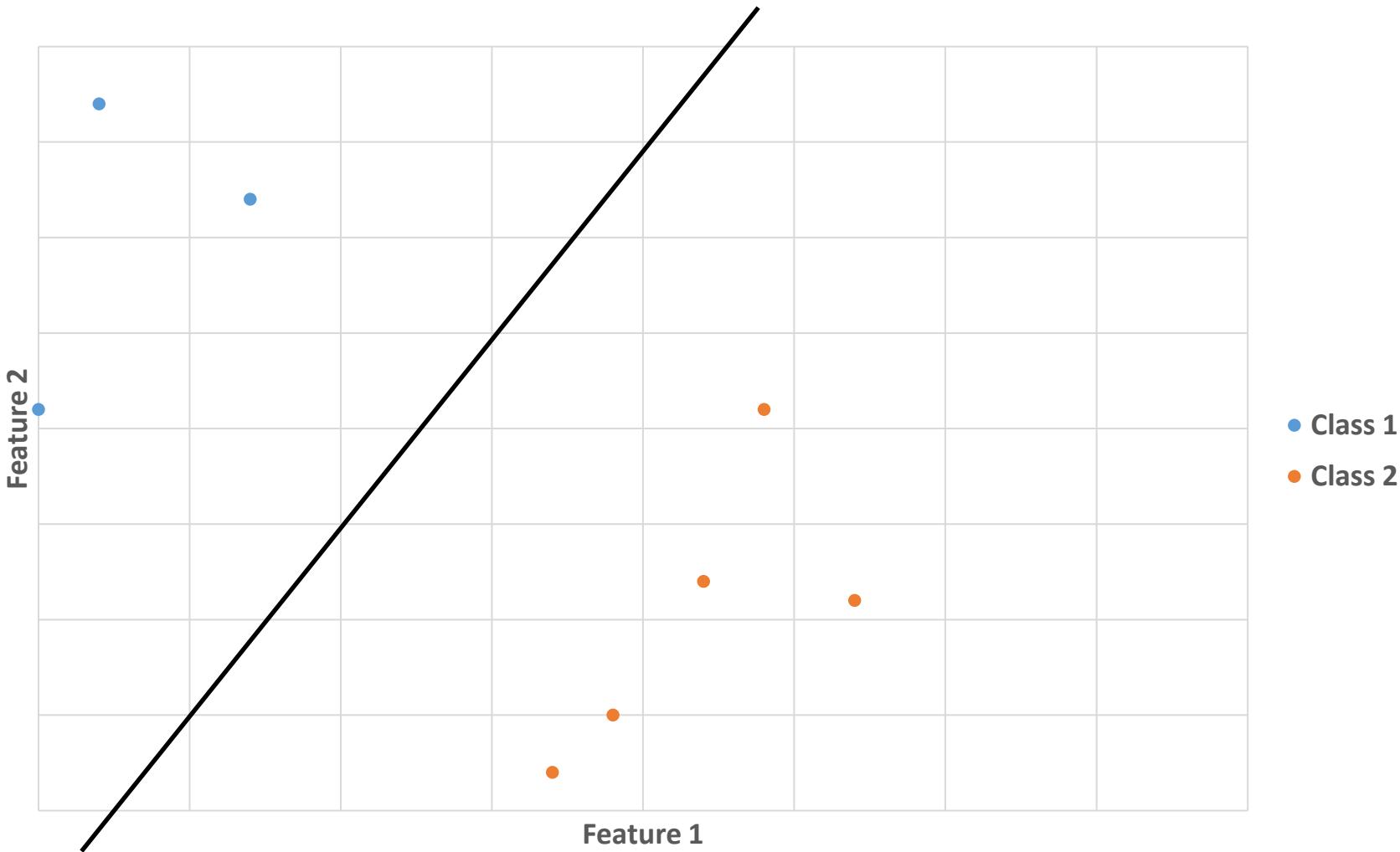
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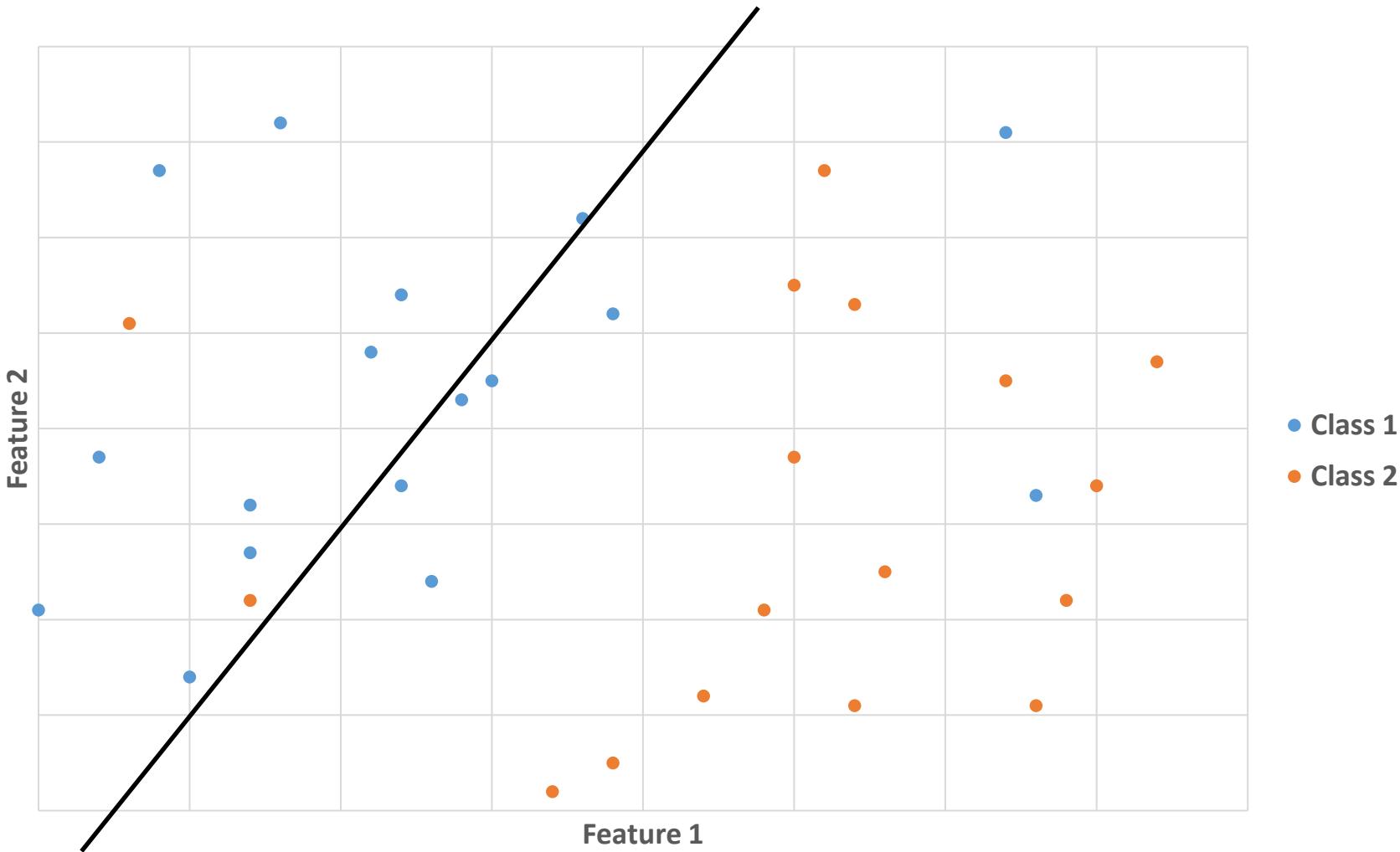
Training will be easier if **enough** and **representative** data is used

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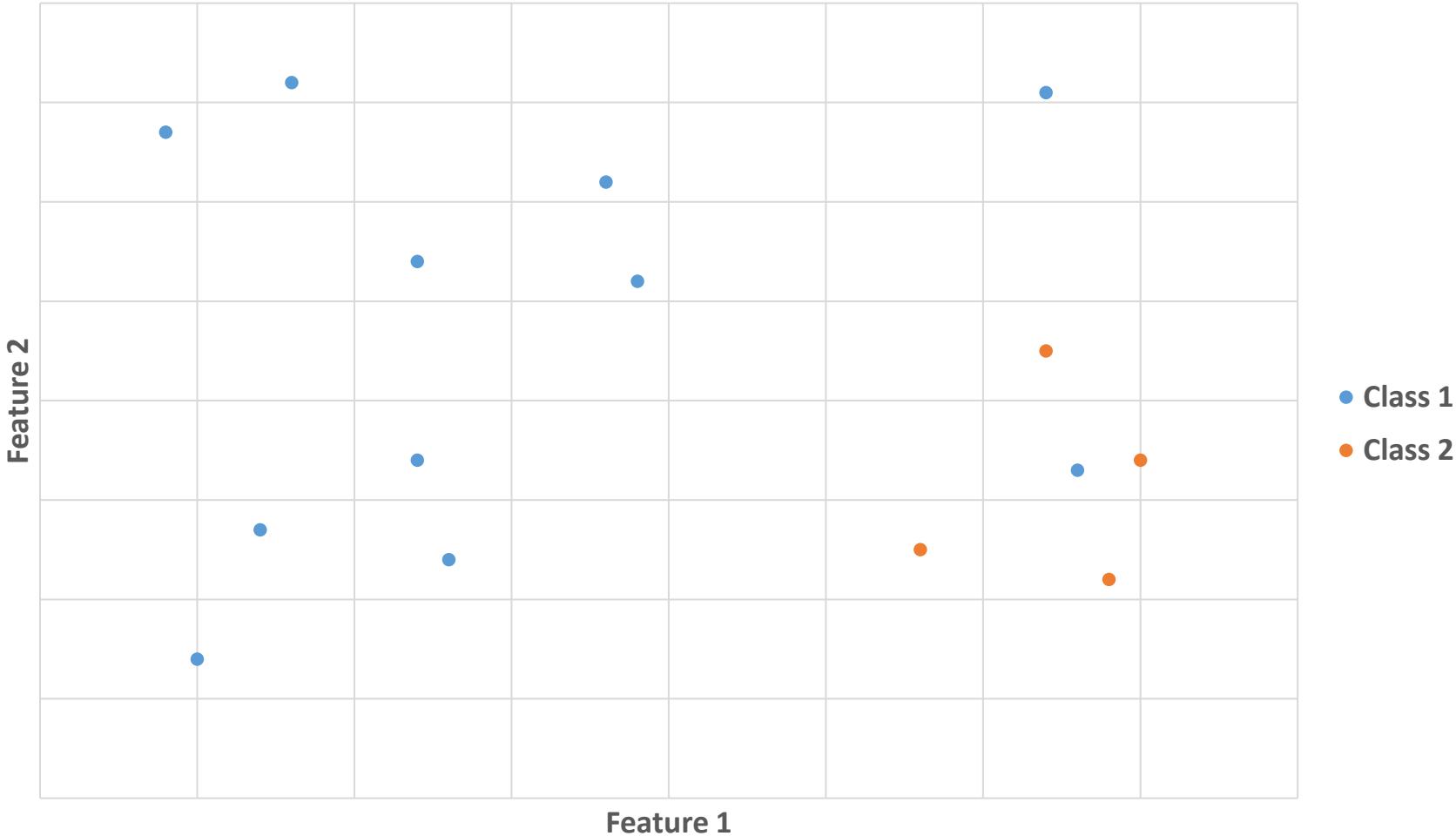
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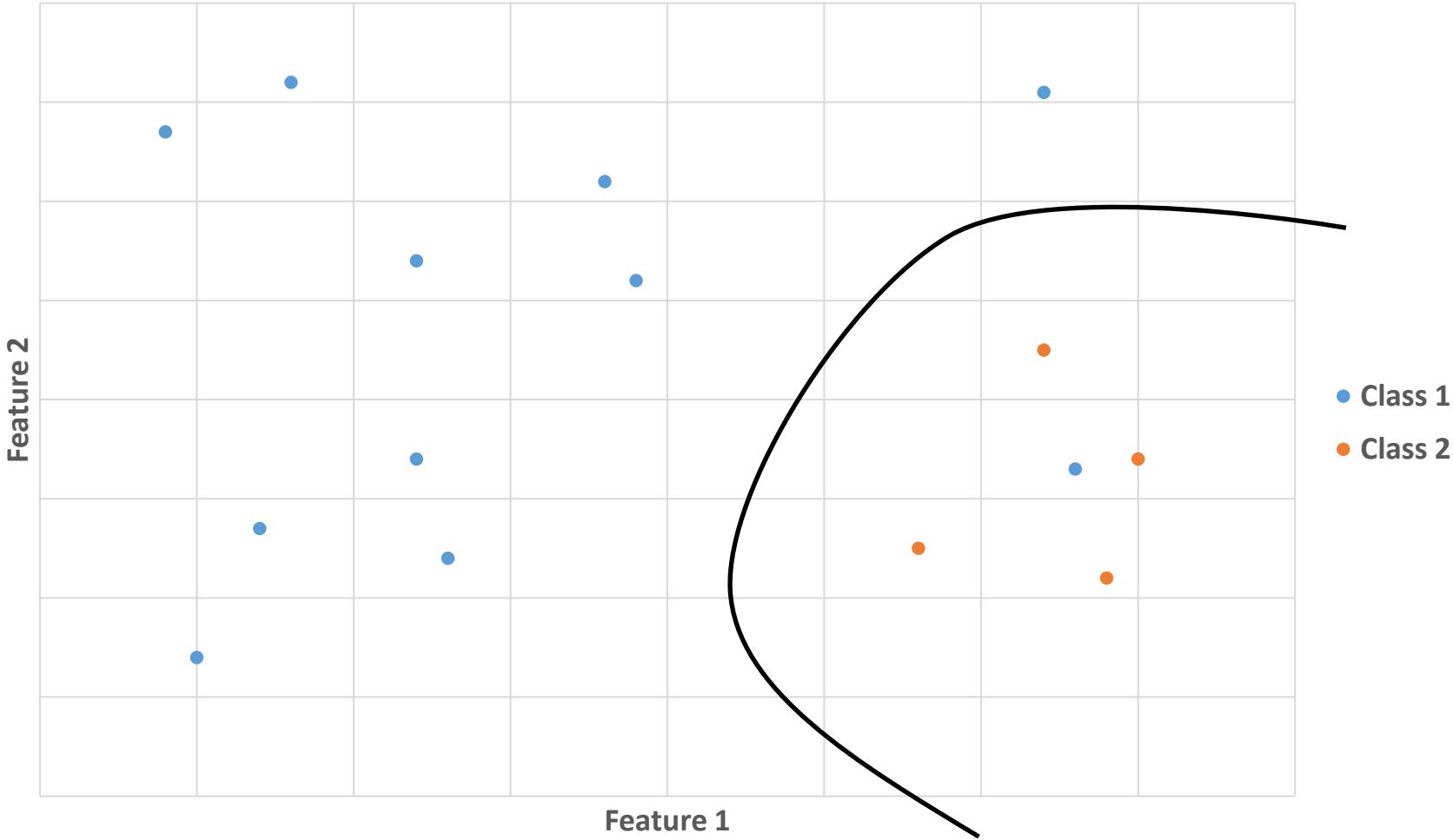
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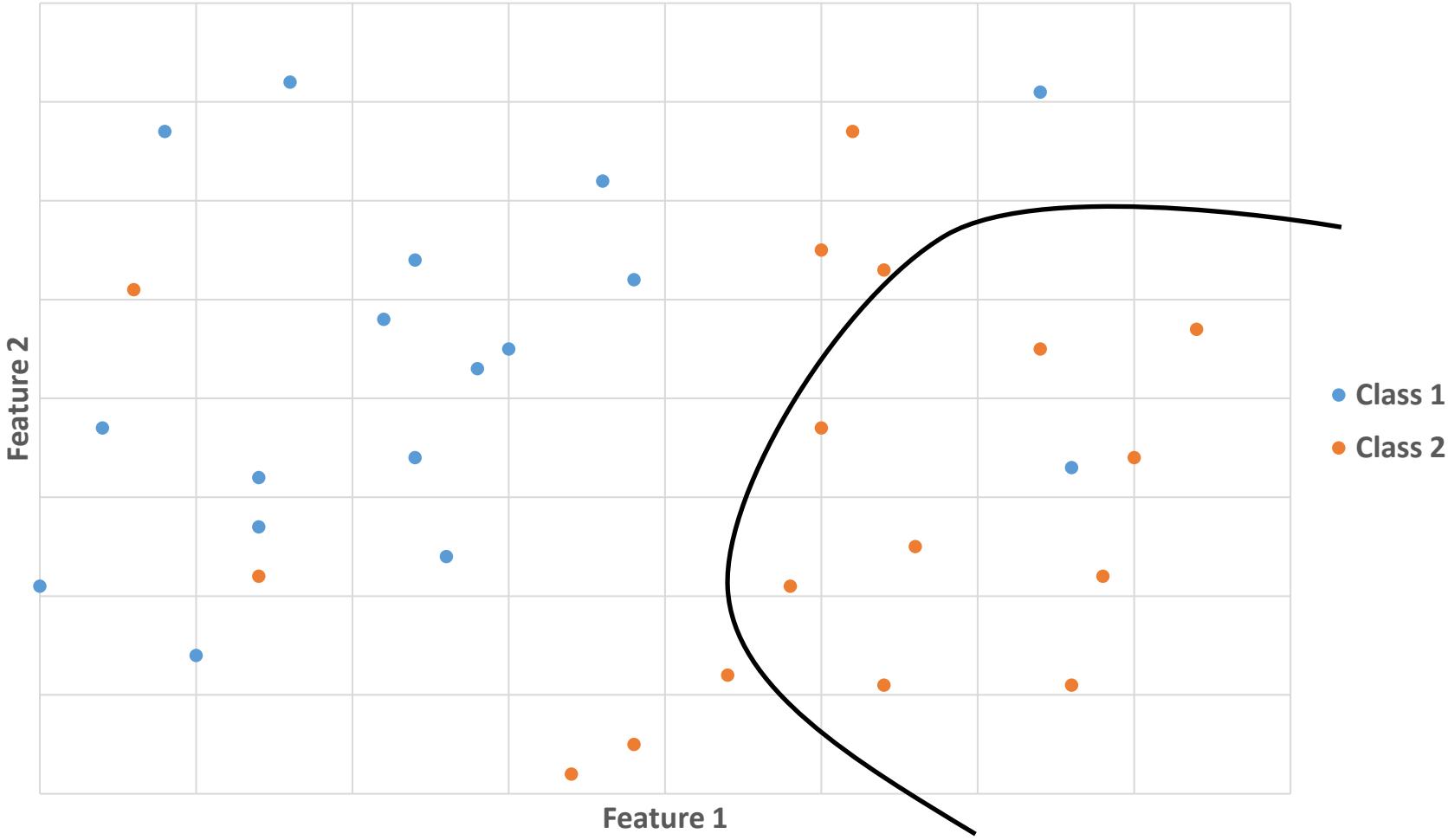
**Class imbalance** makes the training **more difficult**

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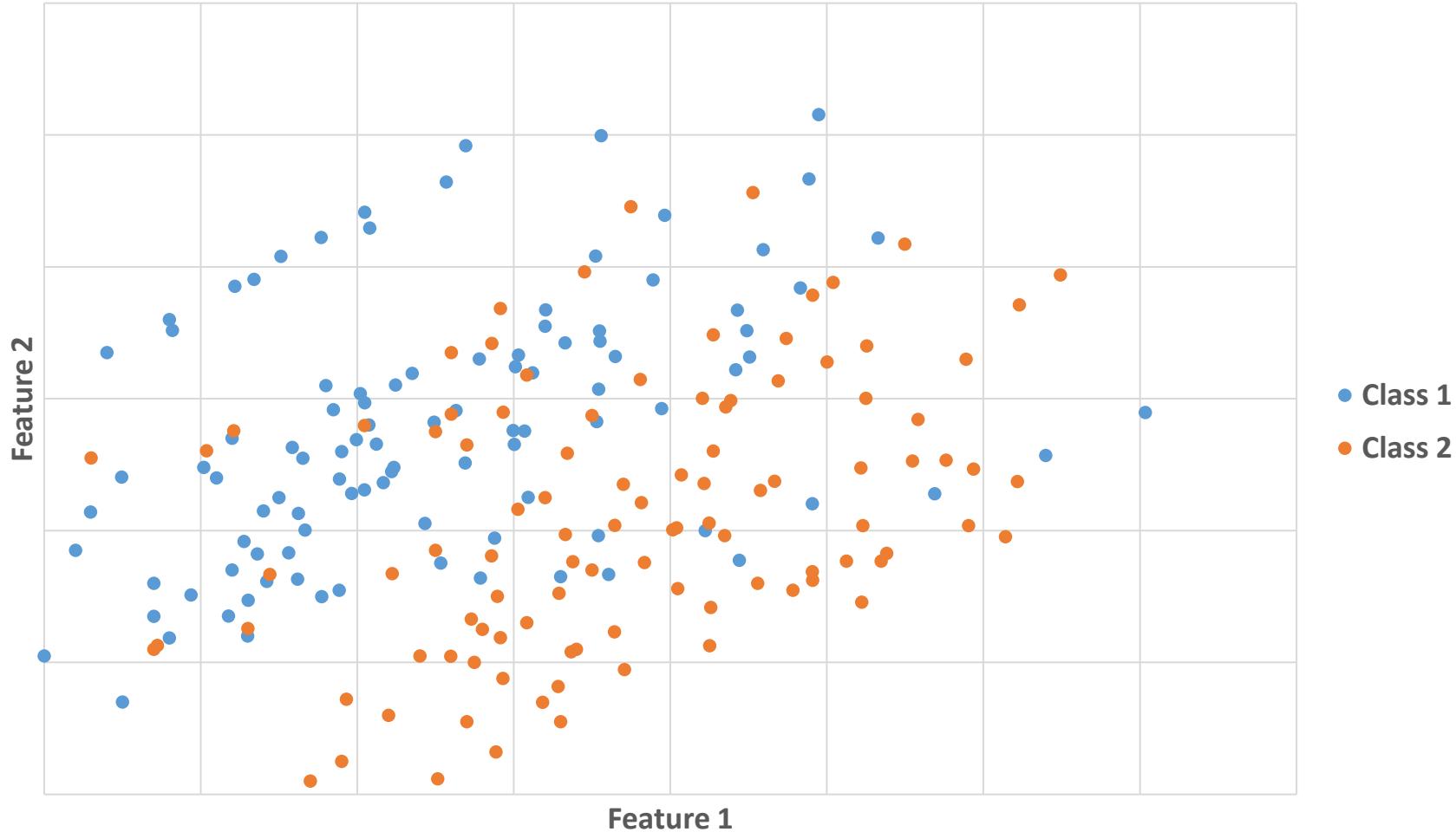
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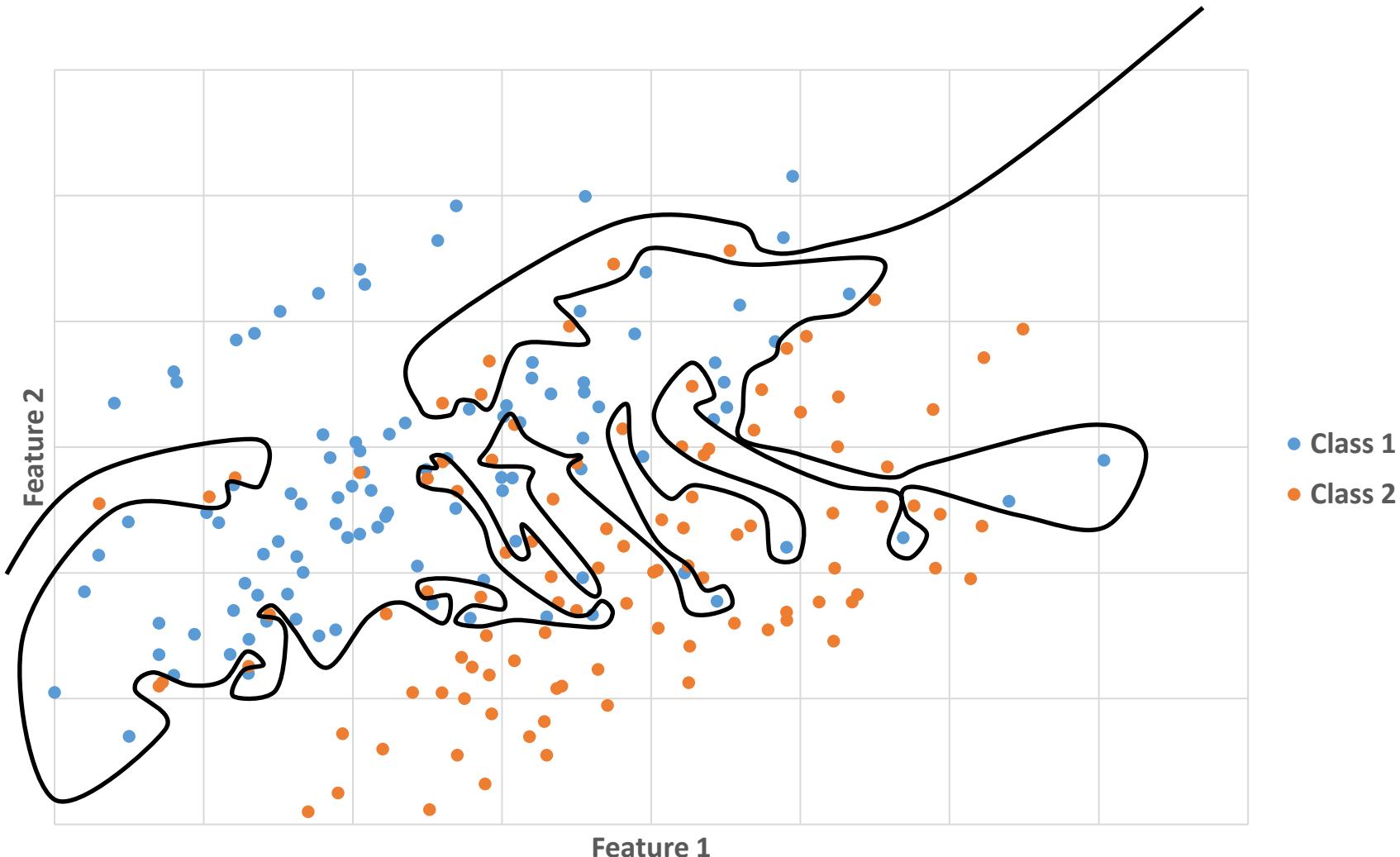
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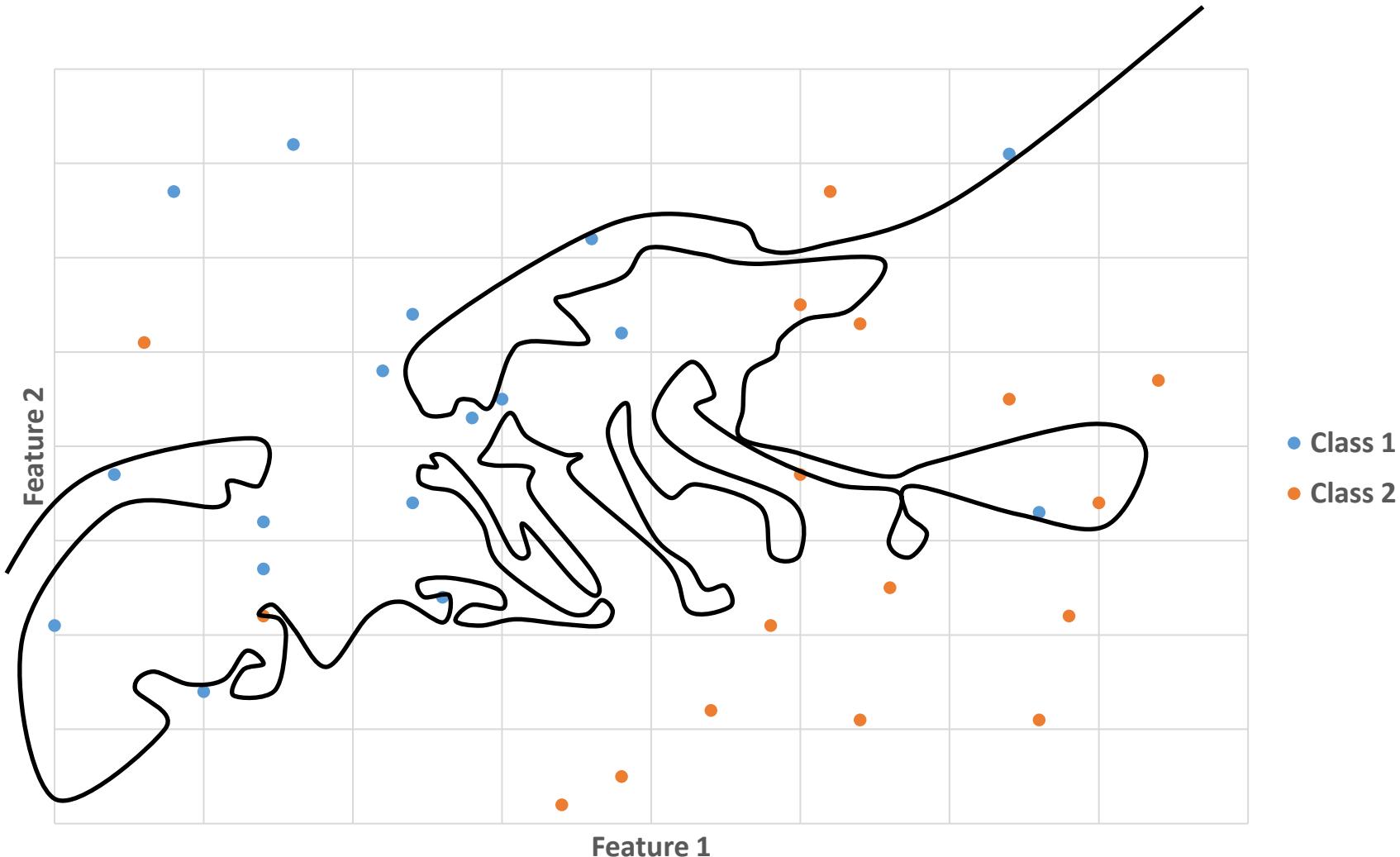
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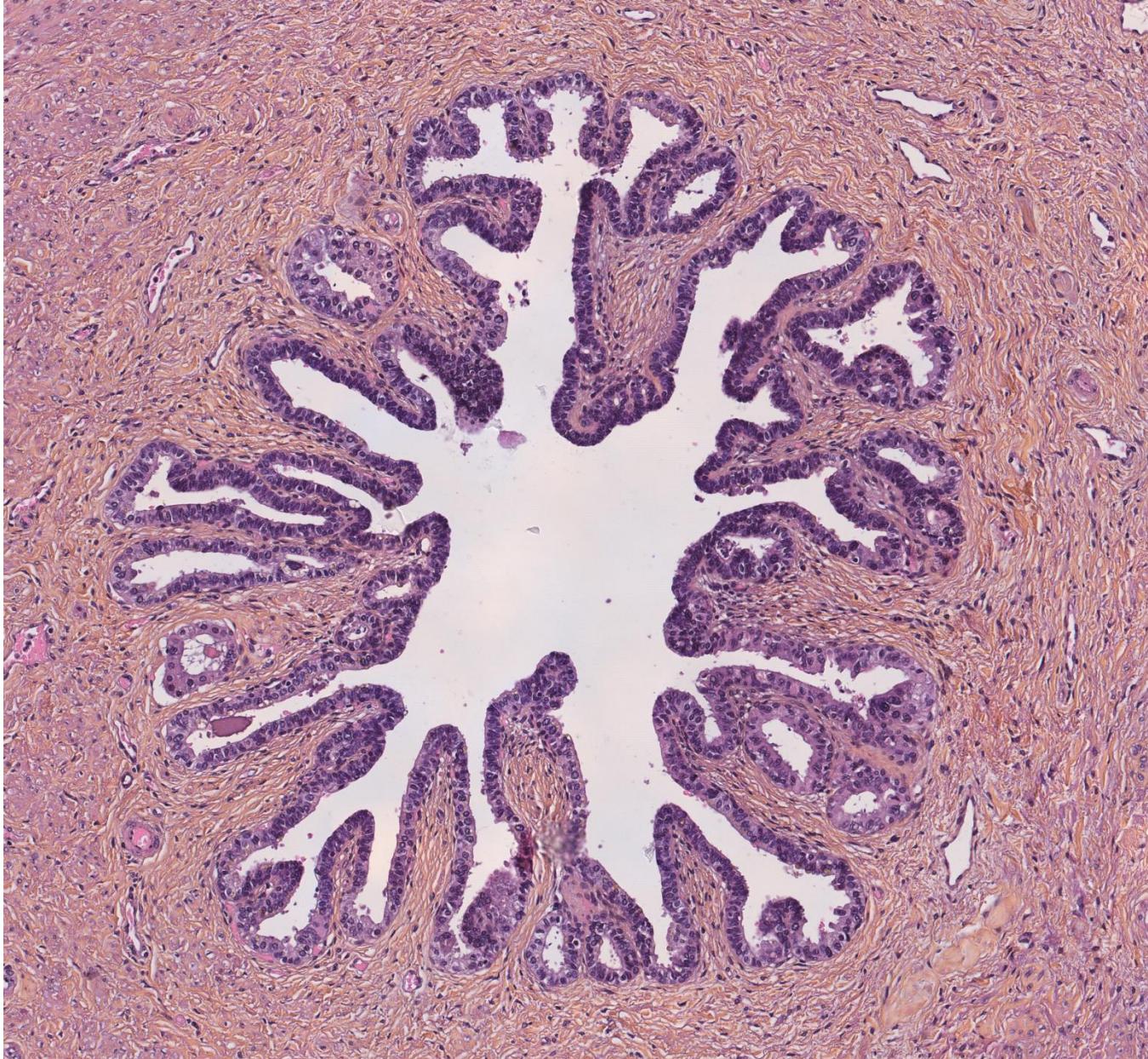
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## SHALLOW MACHINE LEARNING FOR PIXEL CLASSIFICATION

- Select **image features appropriate** to the classification problem
- Manually annotate regions/objects that are **representative** of what is seen in images
- Use **V** tool for annotations to **avoid over-representation**
- Define roughly the **same amount** of annotations for **each class**
- **Do not** manually annotate an **entire region of slide** to avoid over-fitting

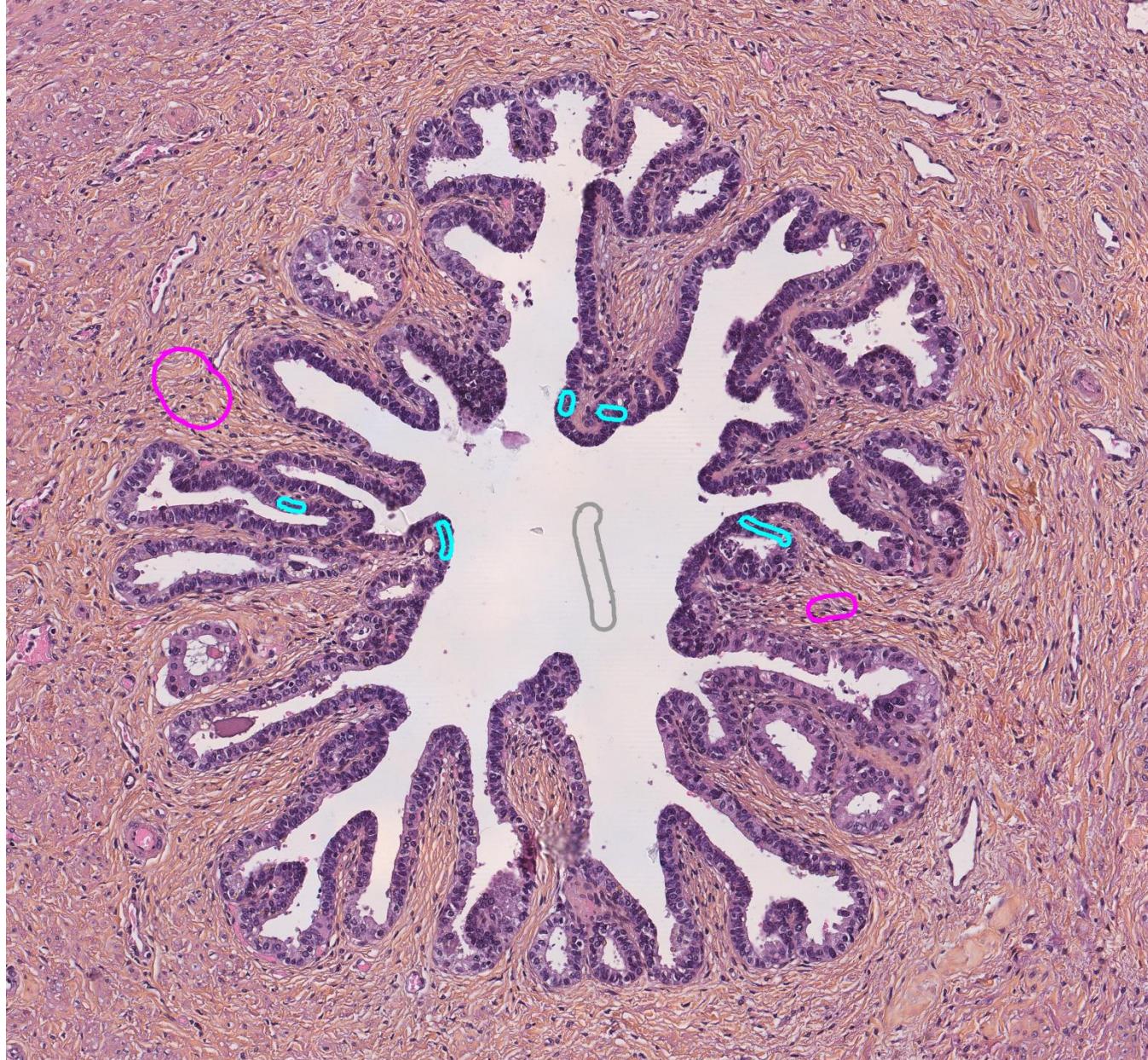
## PIXEL CLASSIFICATION

Find **regions** corresponding to **epithelium**, **stroma** and **background**



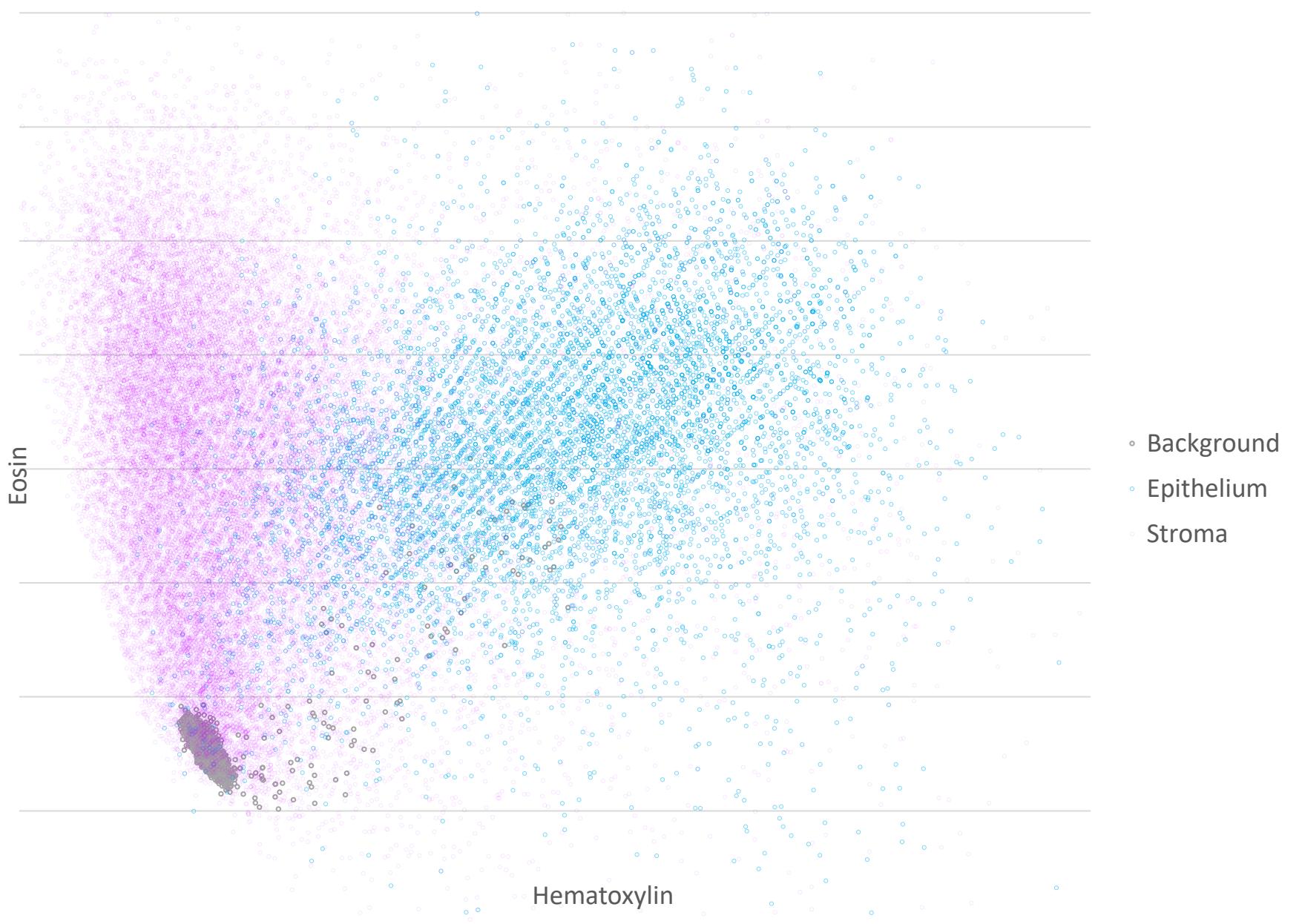
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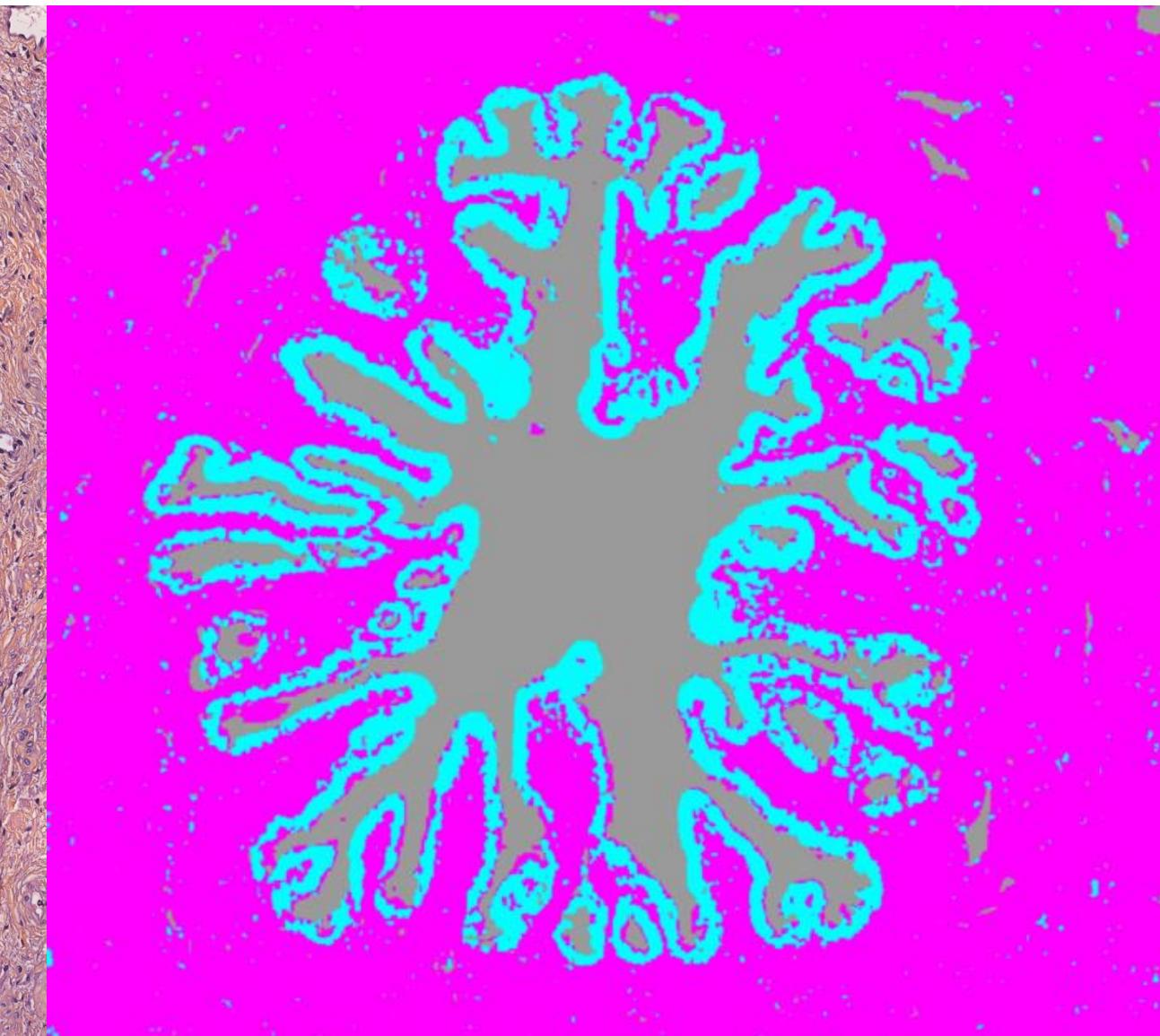
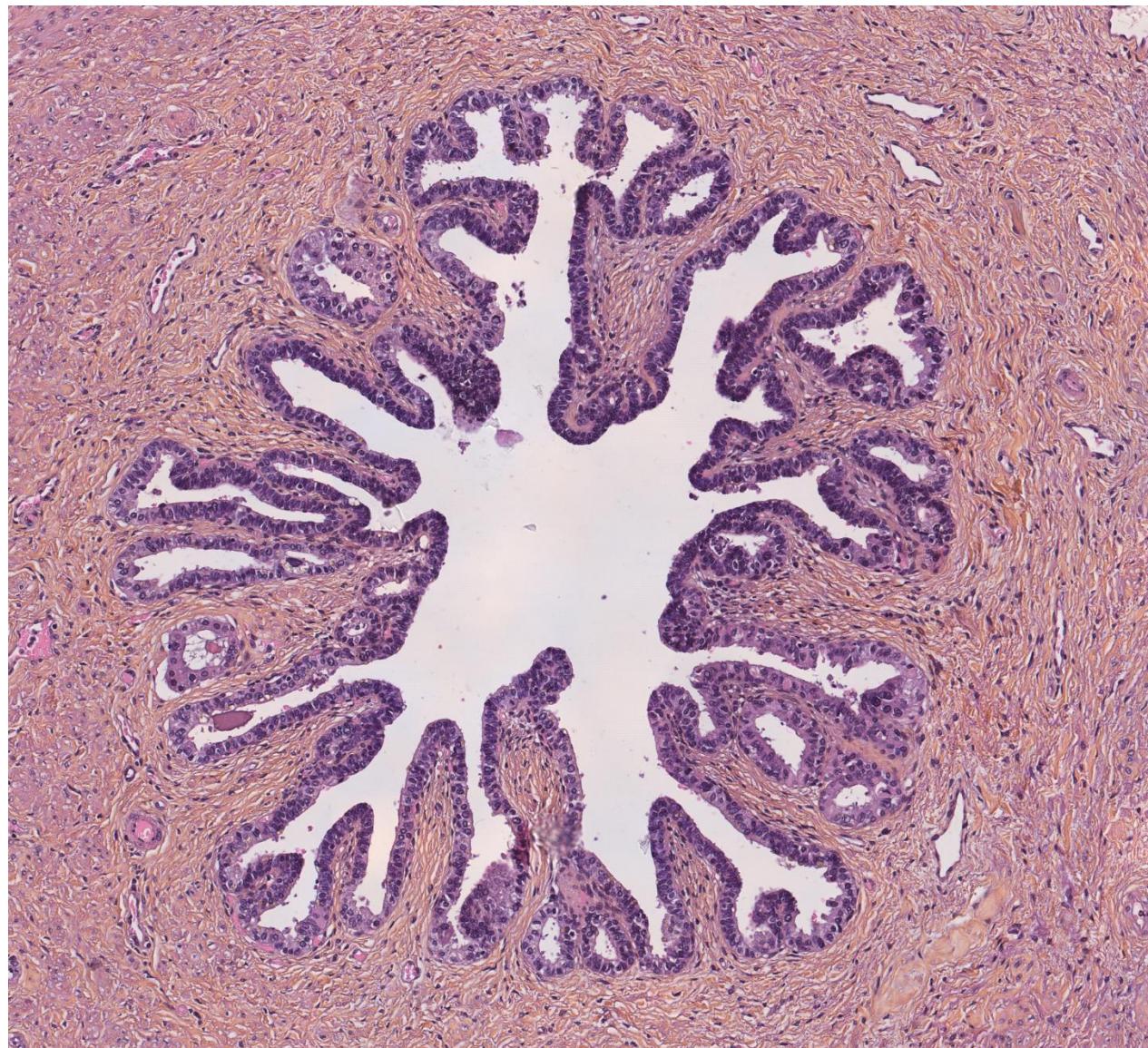


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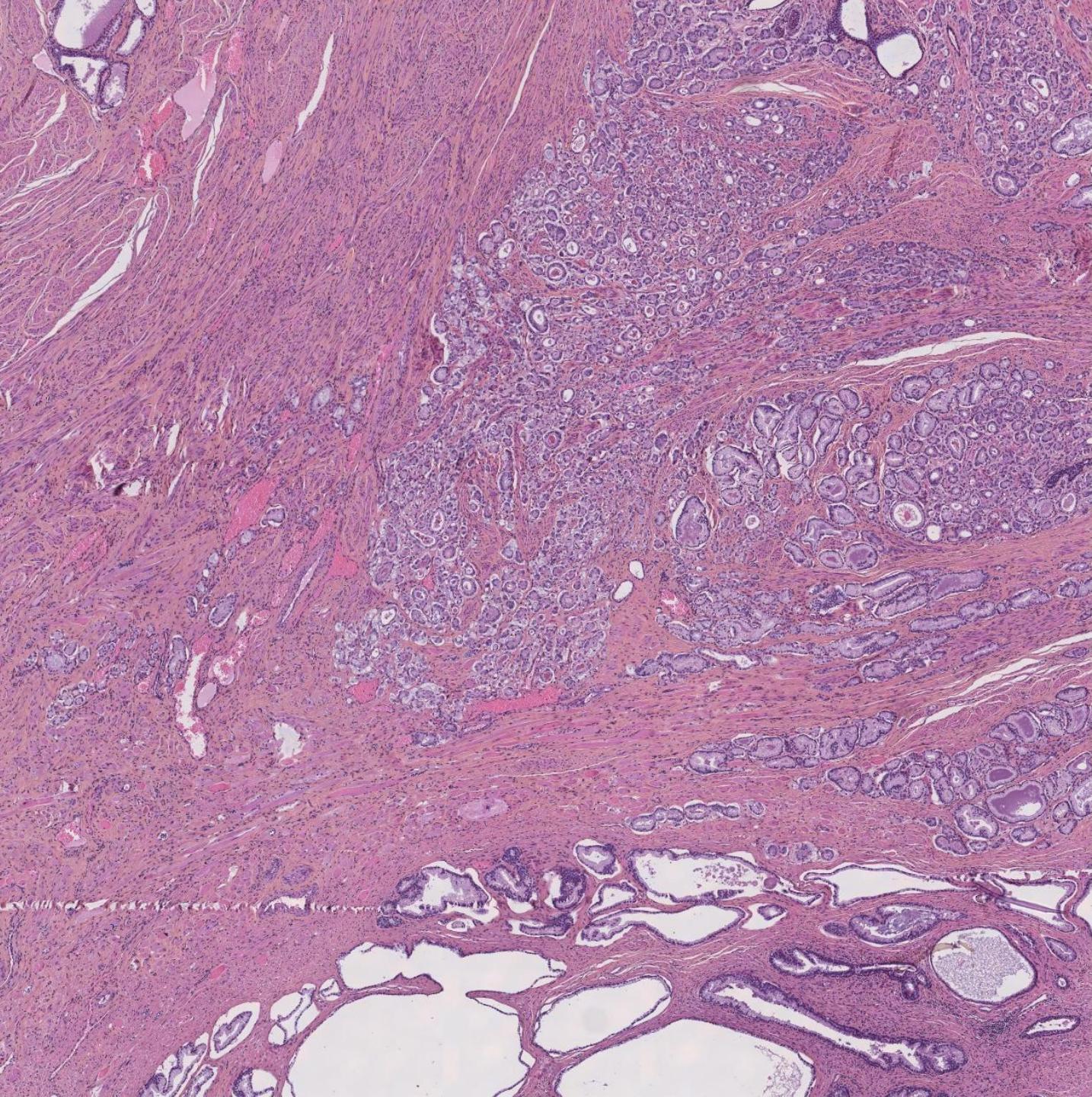


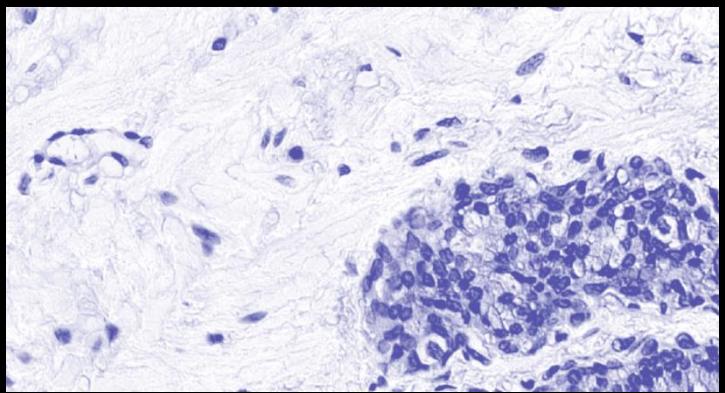
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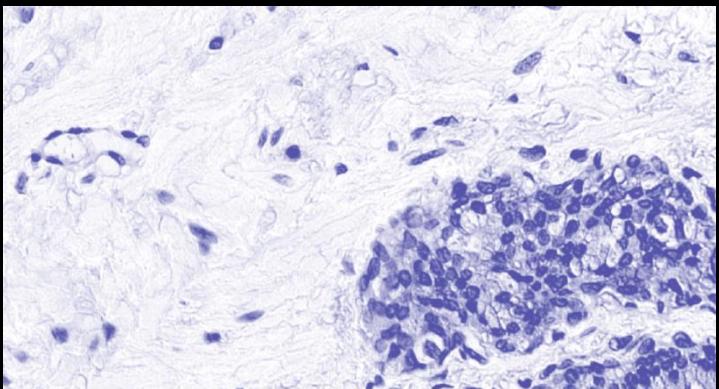
- Open prostate\_1.ome.tif, prostate\_2.ome.tif, prostate\_3.ome.tif, prostate\_4.ome.tif, prostate\_5.ome.tif and prostate\_6.ome.tif
- Create **annotations** in each image that recapitulate the **diversity** of the tissue
- Create **regions annotations**
- Open "Pixel classifier"
- Annotate pixels belonging to **background**, **epithelium** and **stroma**
- Save classifier and apply it to each image with a script (workflow tab)
- Get proportions of tissues



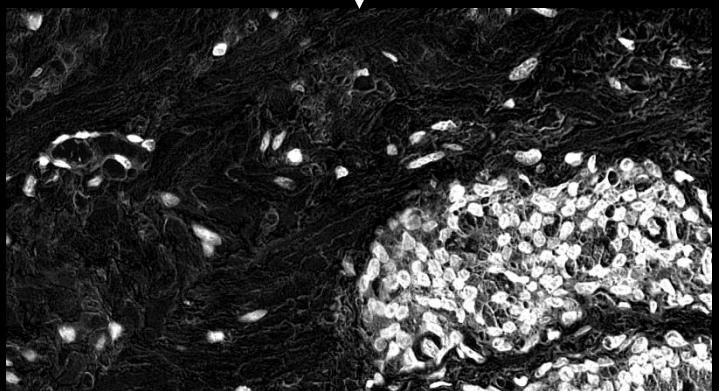


## CELL DETECTION

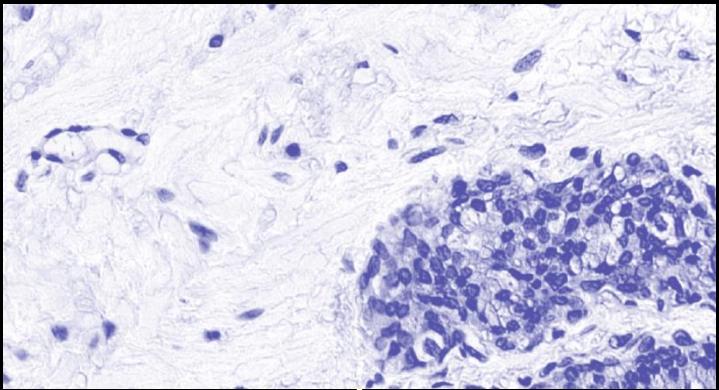
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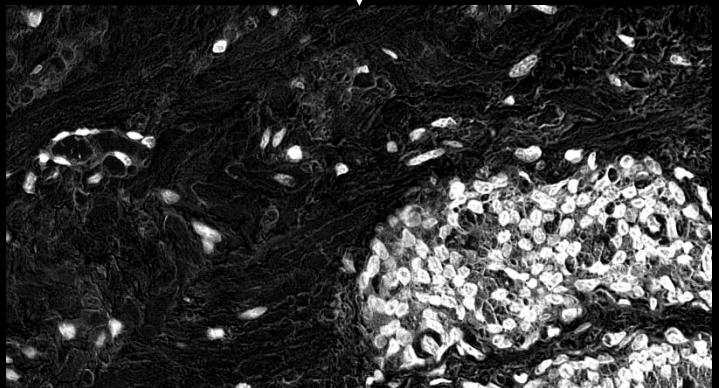
Gray levels



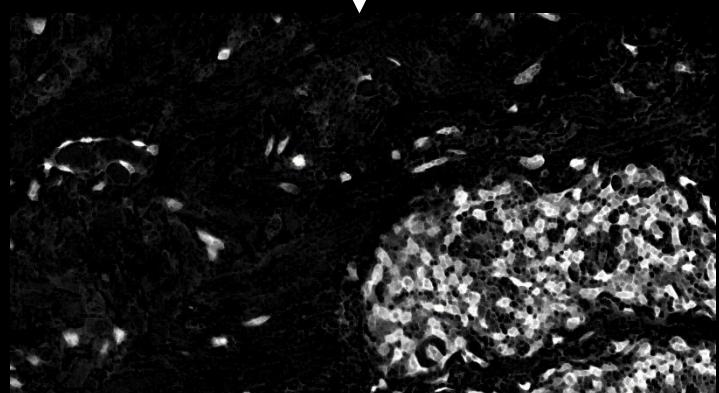
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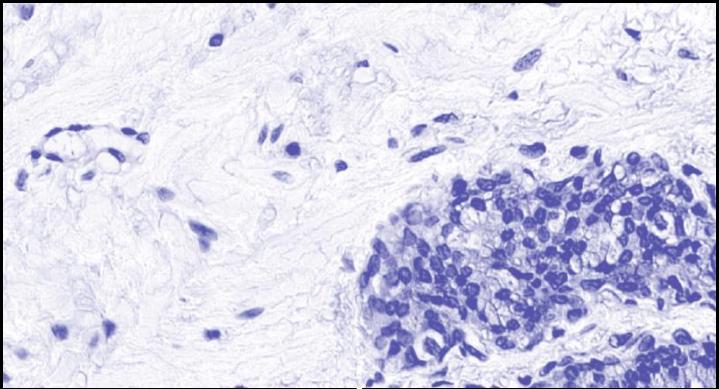
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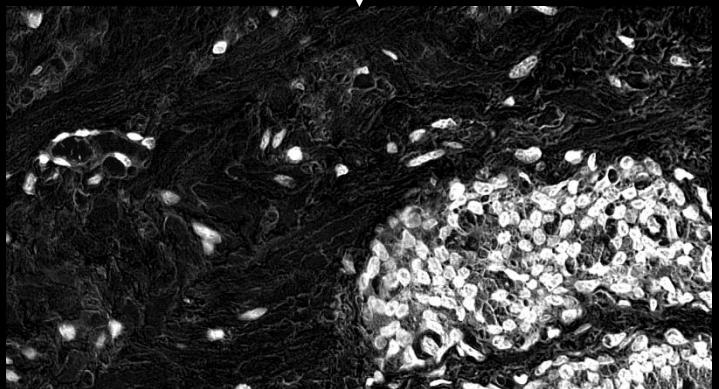
Minimum filtering



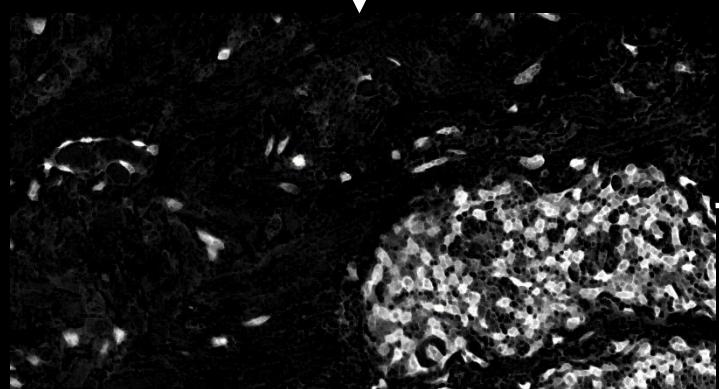
## CELL DETECTION



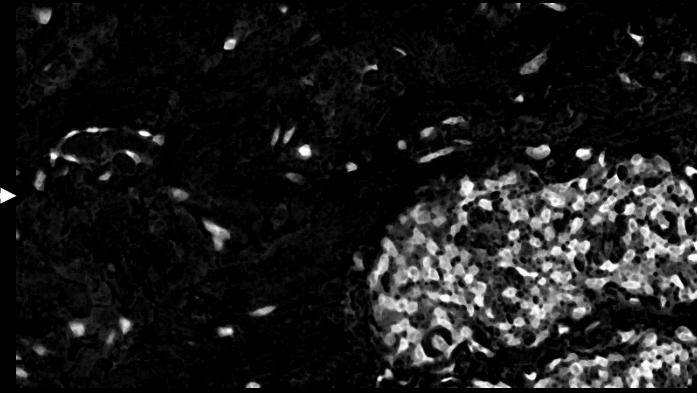
Gray levels

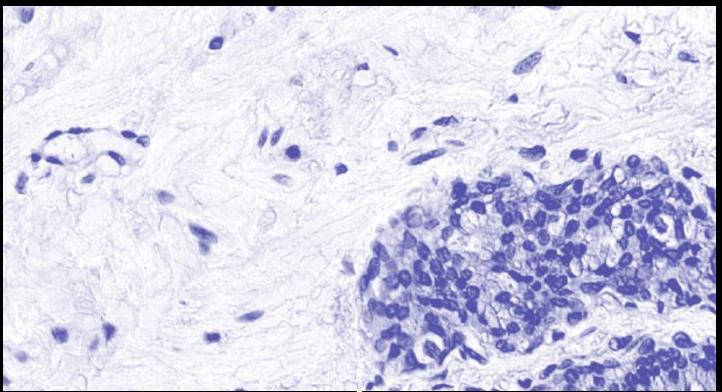


Minimum filtering

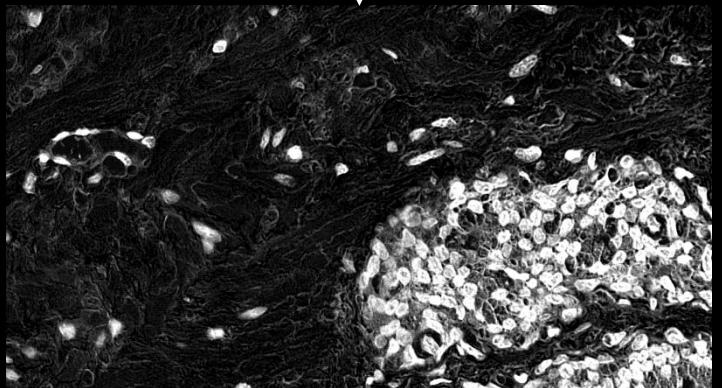


Gaussian blur

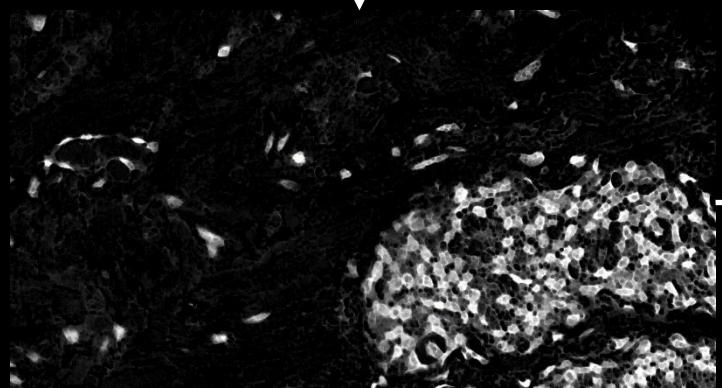




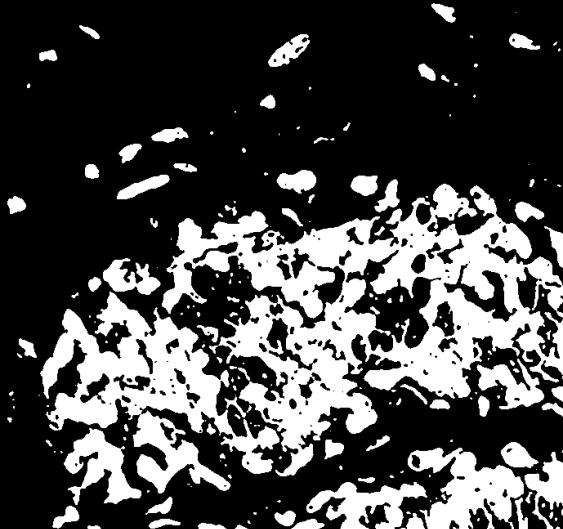
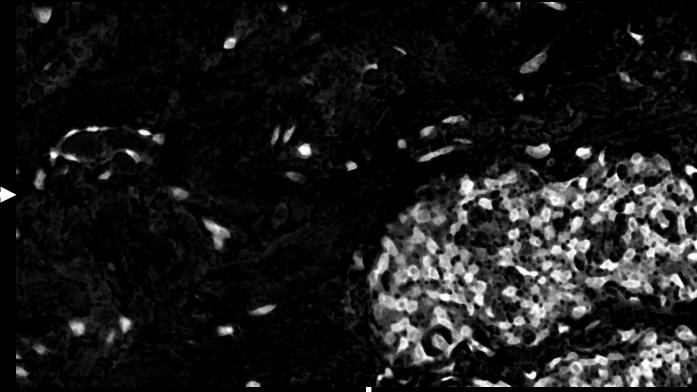
## CELL DETECTION



Gaussian blur

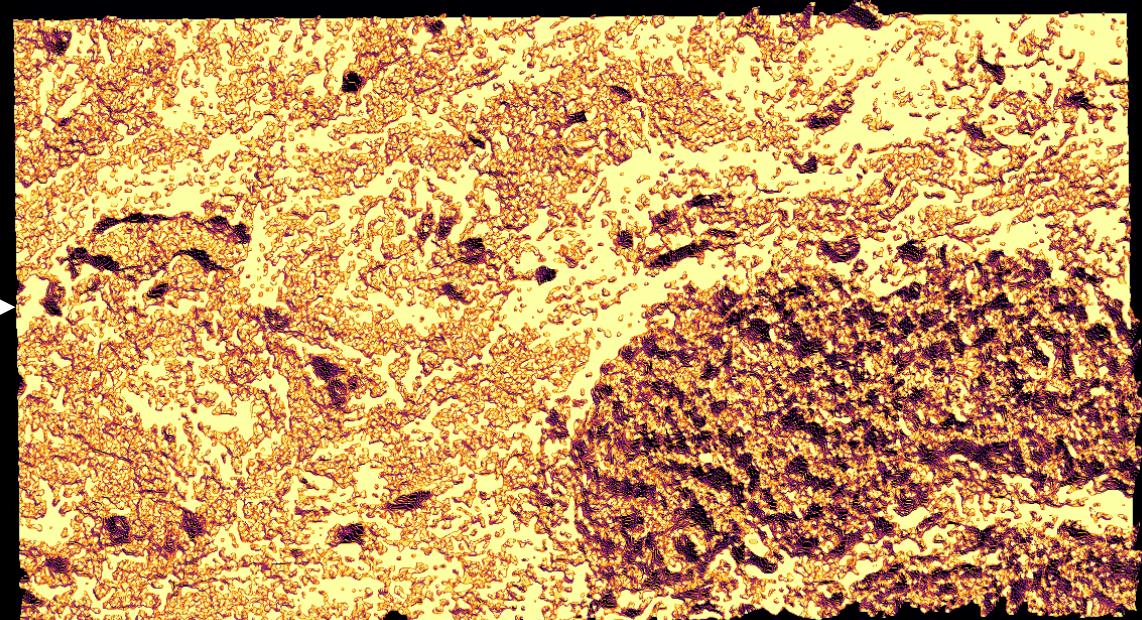
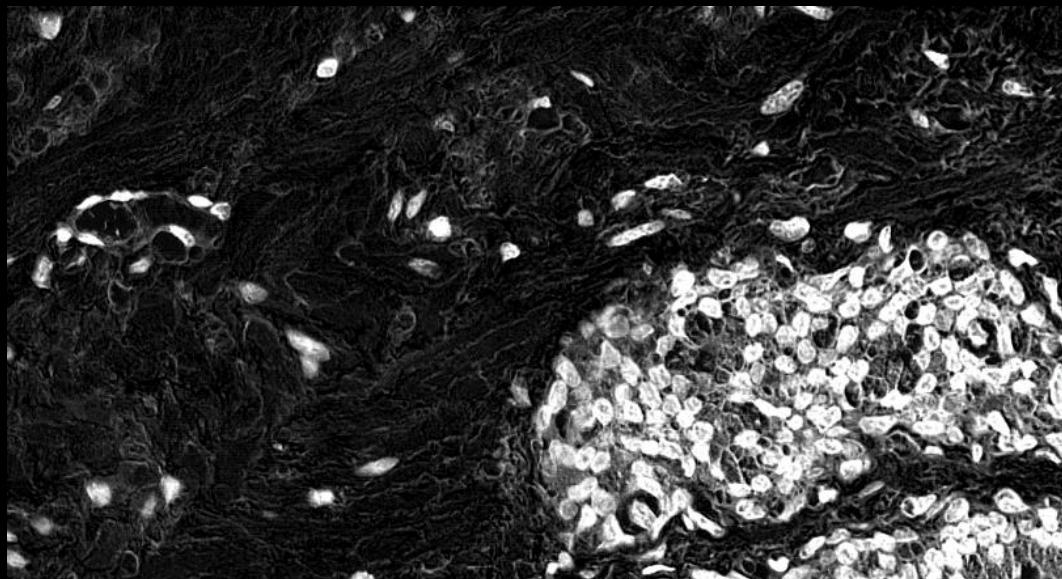


Thresholding



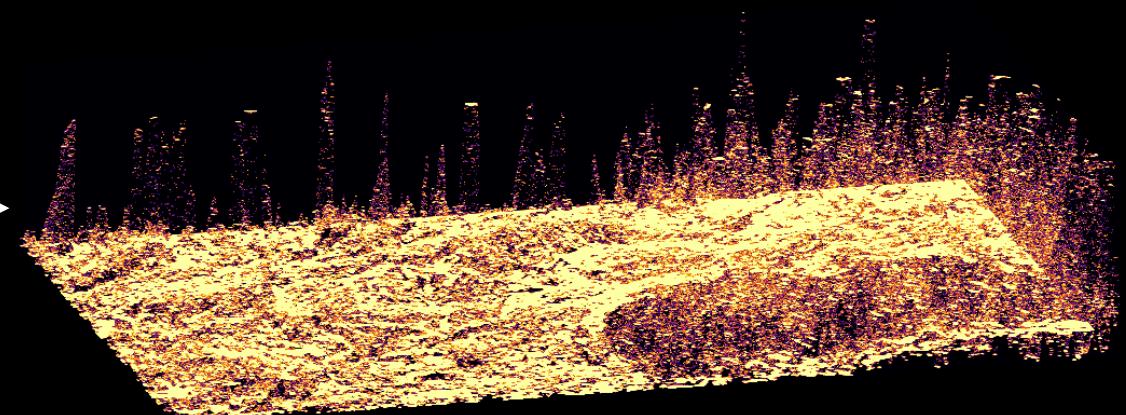
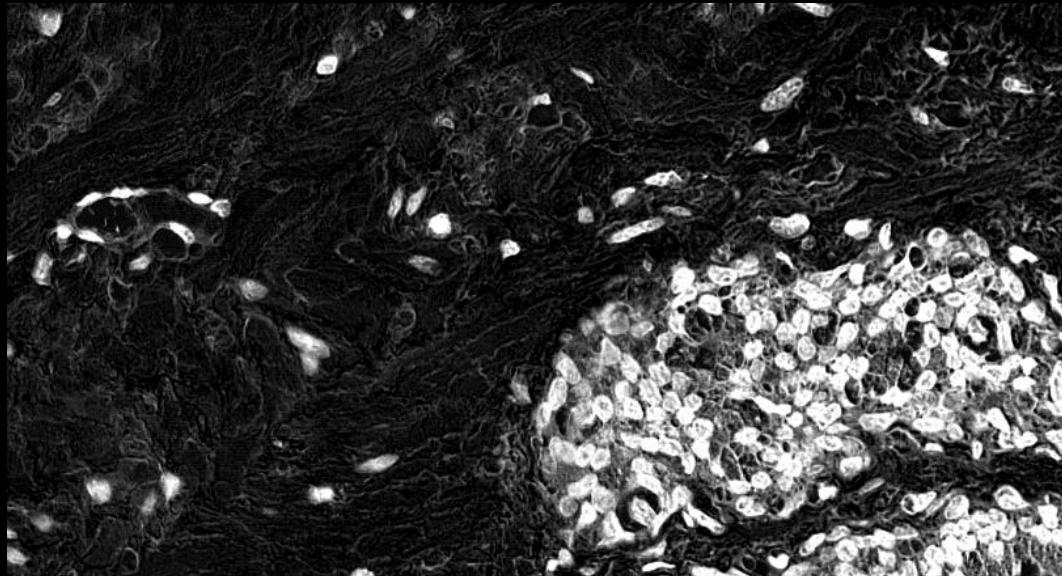
## WATERSHED

Transform image so that intensity becomes 3<sup>rd</sup> dimension



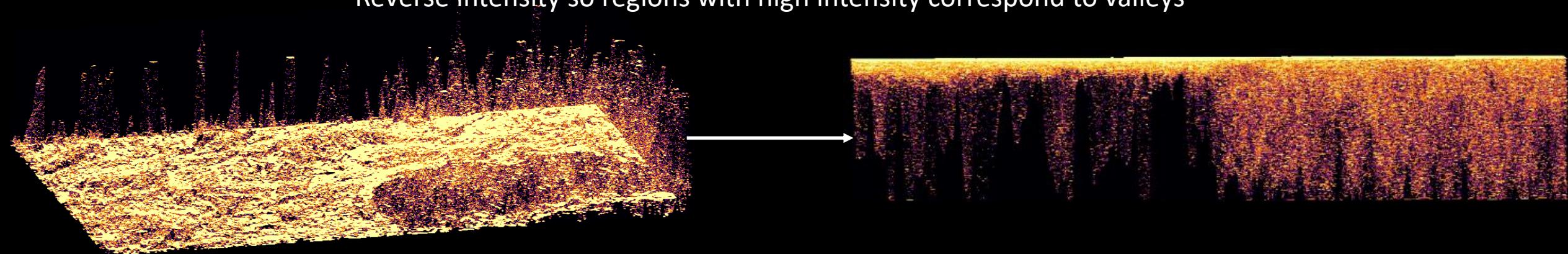
## WATERSHED

Transform image so that intensity becomes 3<sup>rd</sup> dimension

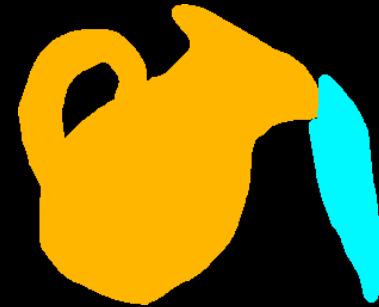


## WATERSHED

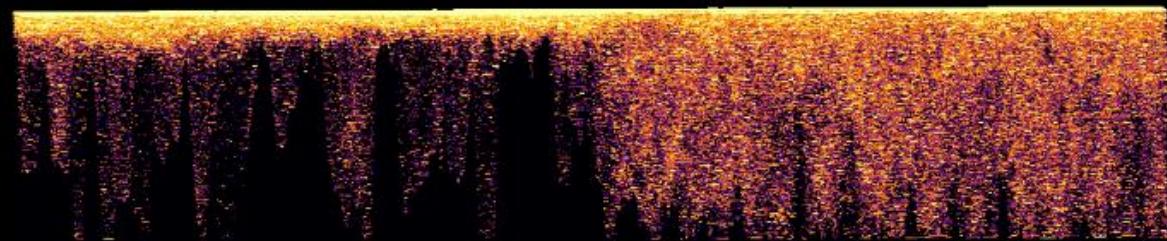
Reverse intensity so regions with high intensity correspond to valleys



# WATERSHED

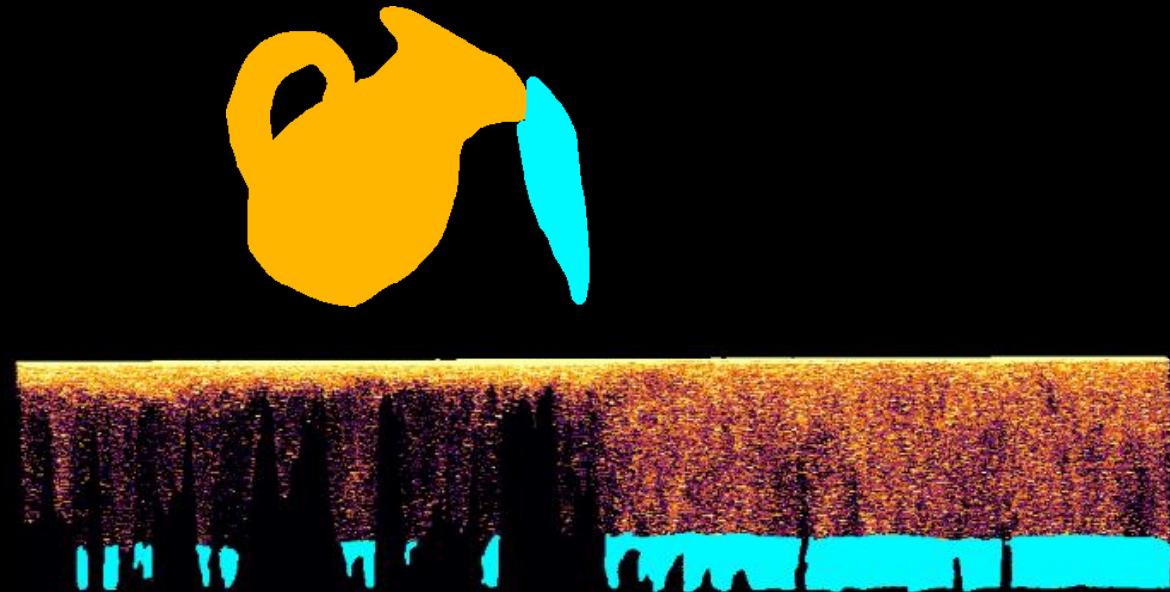


Pour water into valleys



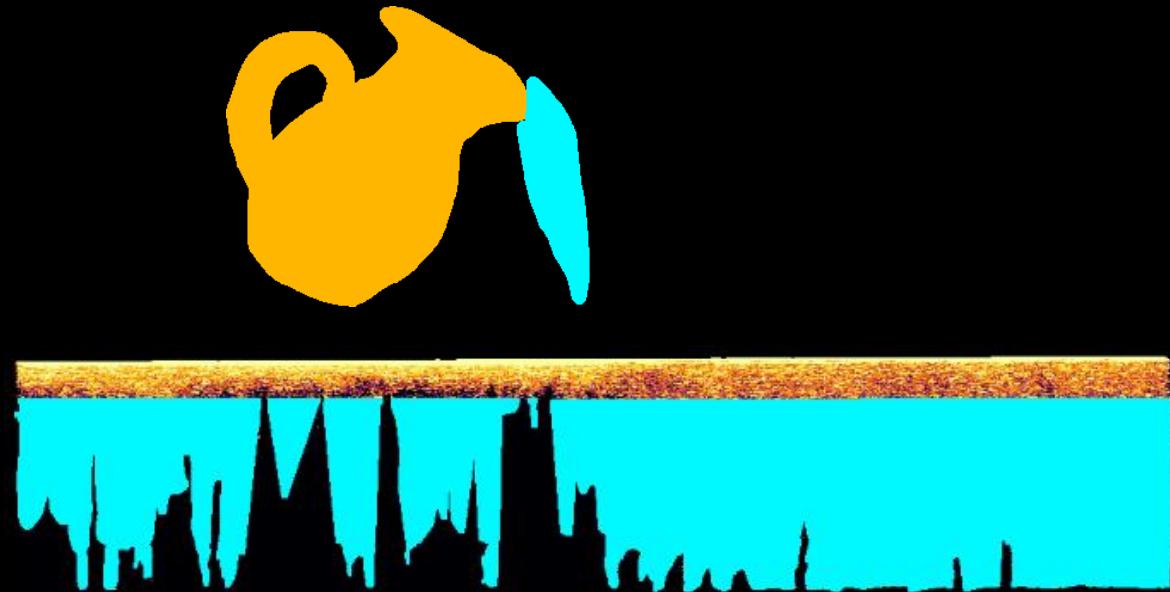
# WATERSHED

Pour water into valleys



# WATERSHED

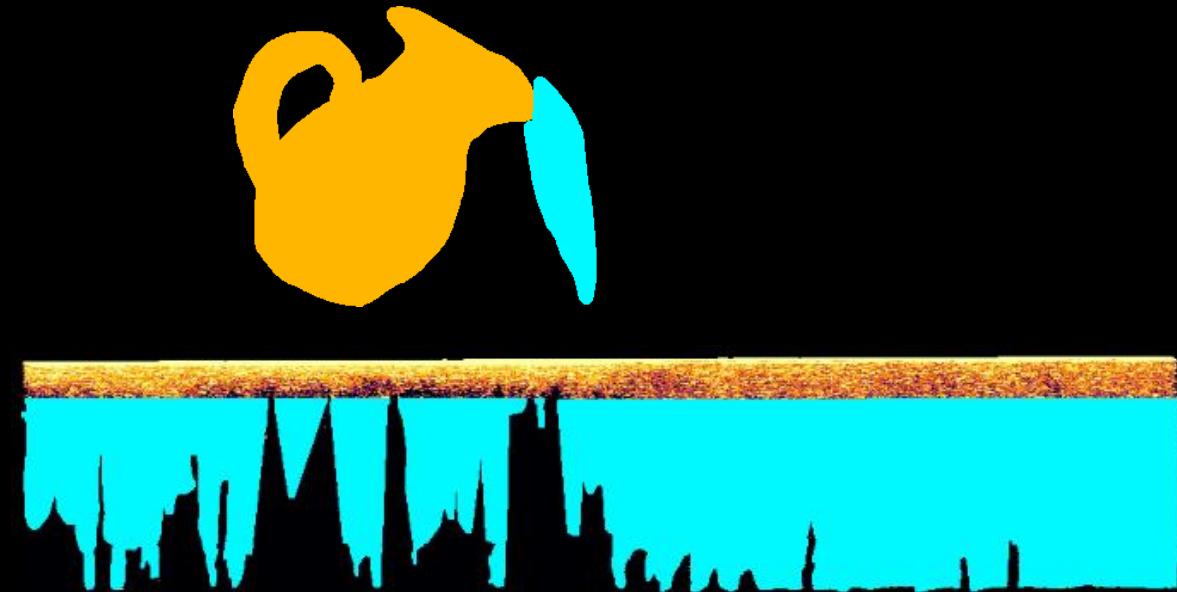
Pour water into valleys



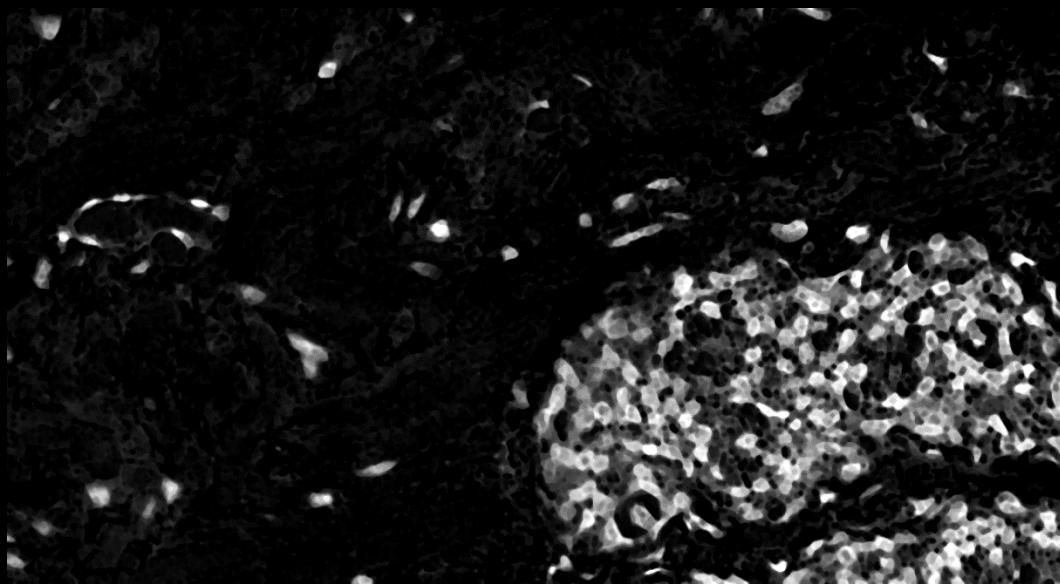
# WATERSHED

Pour water into valleys

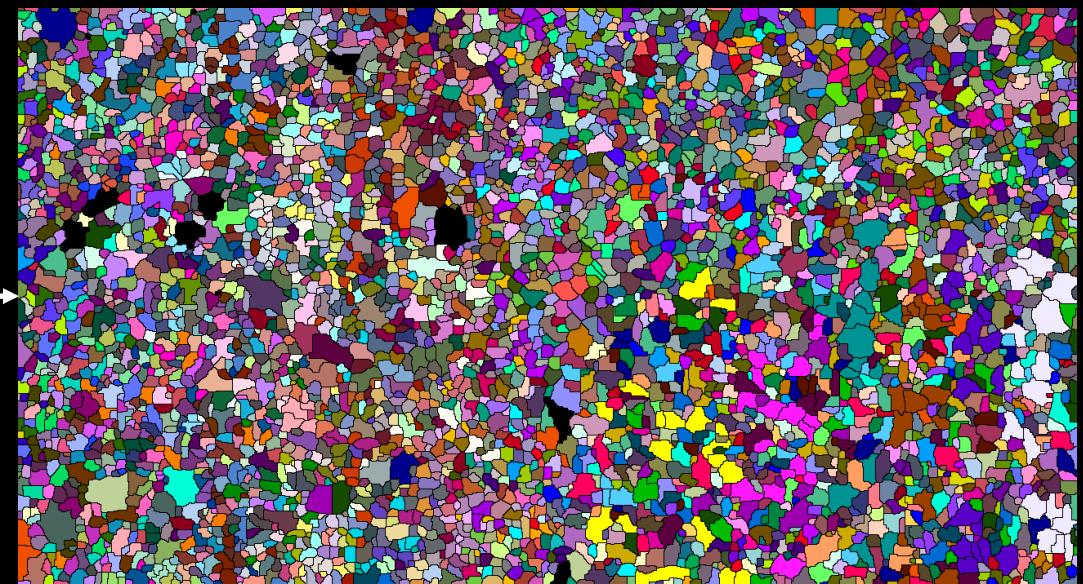
Threshold



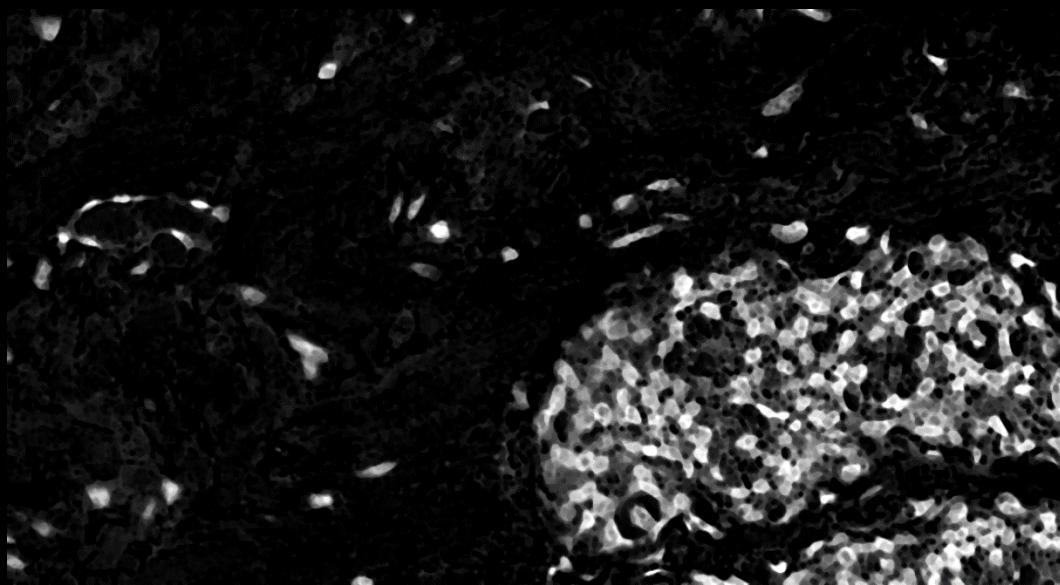
# WATERSHED



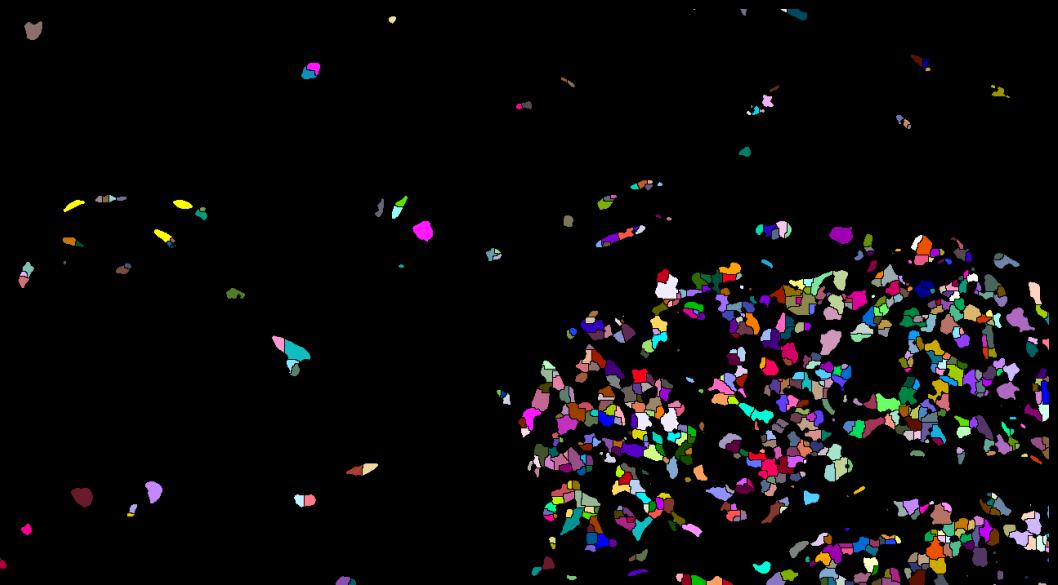
Watershed



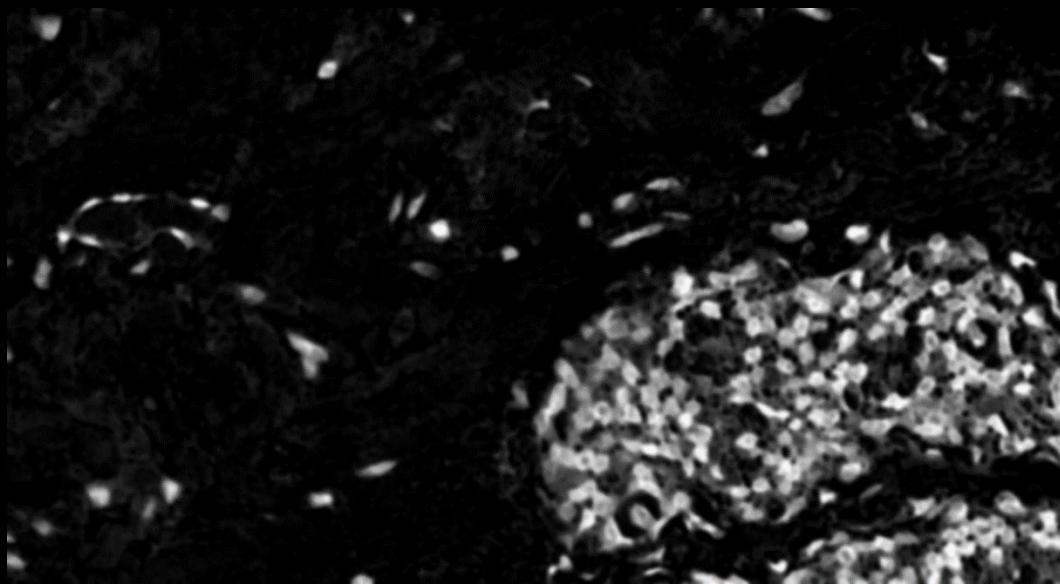
# WATERSHED



Watershed



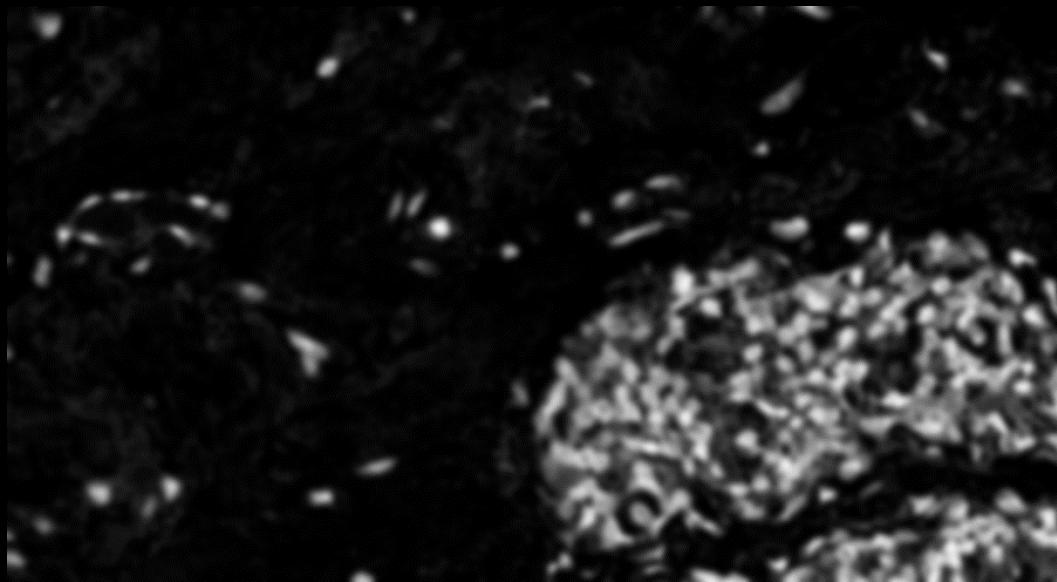
# WATERSHED



Watershed



# WATERSHED



Watershed



# CELL DETECTION TOOL IN QuPATH

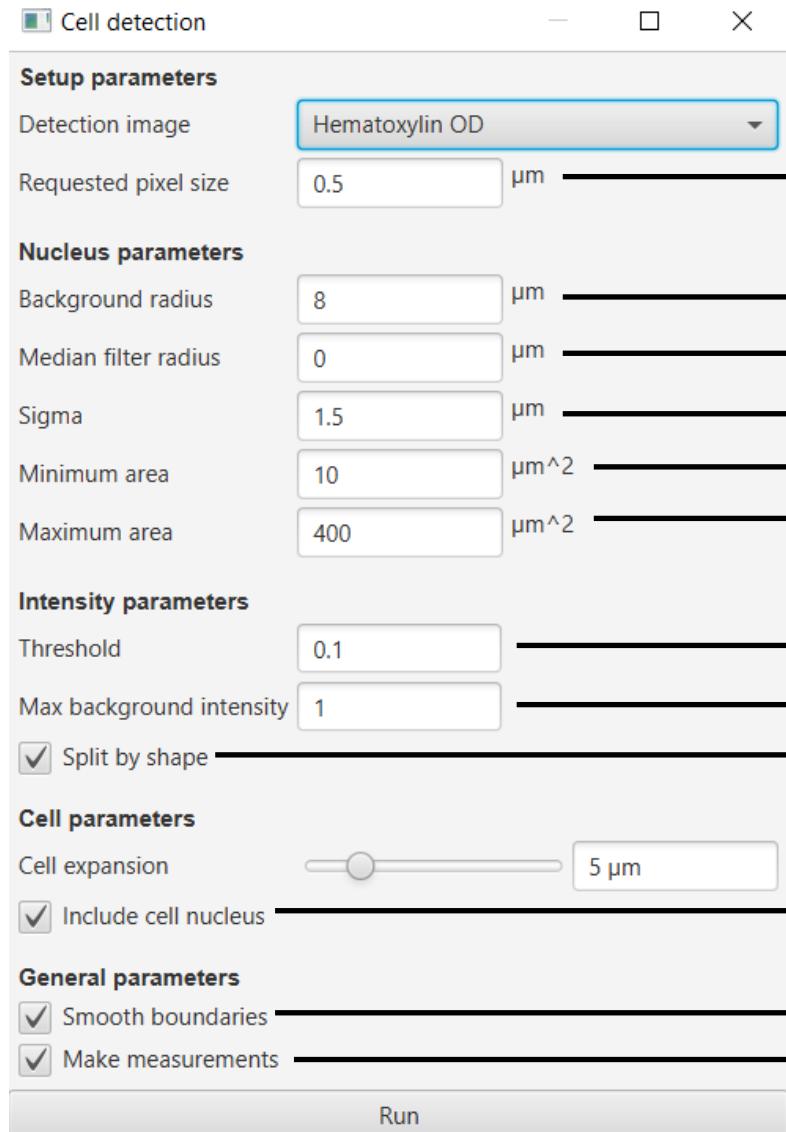


Image **component** used for cell detection

Image **resolution** used for cell detection

**Radius** used for **minimum filtering**

**Radius** for **median filtering**

**Kernel size** used for **Gaussian blur** before watershed

Nuclei with **area inferior** to this value are **filterd out**

Nuclei with **area superior** to this value are **filterd out**

**Threshold** used for **watershed**

**Threshold** used for **minimum filtering**

Separate nuclei based on shape (**binary watershed**)

**Size** used for cell expansion to define **cytoplasm area**

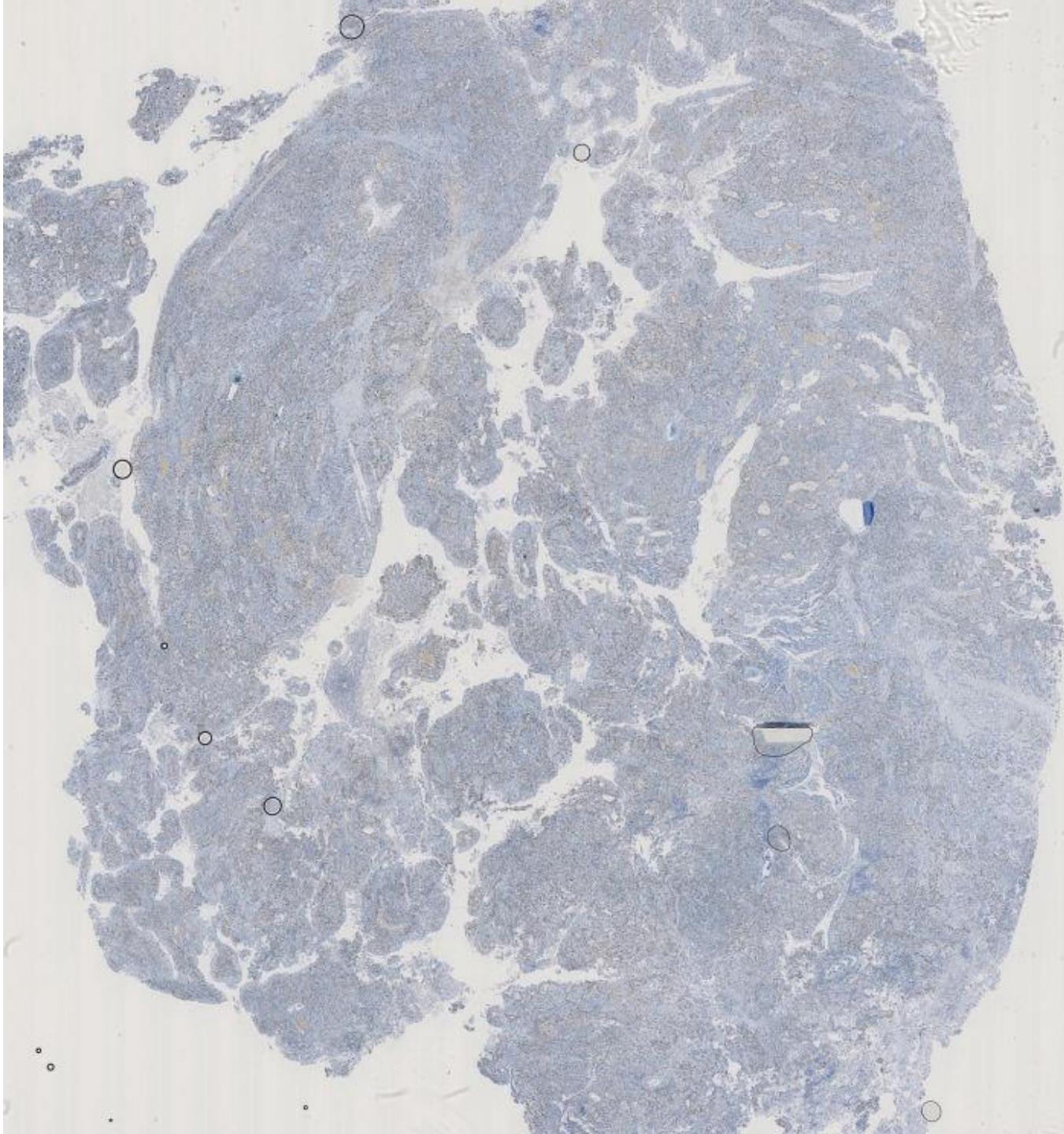
Define **nuclei** and **cytoplasm** areas or only cell areas

Define **smooth cell boundaries**

Get **measurements** associated with nuclei

## NUCLEI SEGMENTATION

- Open KI67\_lung.ndpi
- Open **Positive cell detection**
- Create an **annotation**
- In the annotation, **detect positive** and **negative cells** by defining **one threshold**
- Identify tissue with **Create thresher**
- **Apply "Positive cell detection" on the tissue**
- **Get number of nuclei and proportion of positive cells**



# Cell Detection with Star-convex Polygons

Uwe Schmidt<sup>1,\*</sup>, Martin Weigert<sup>1,\*</sup>, Coleman Broaddus<sup>1</sup>, and Gene Myers<sup>1,2</sup>

<sup>1</sup> Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany  
Center for Systems Biology Dresden, Germany  
<sup>2</sup> Faculty of Computer Science, Technical University Dresden, Germany

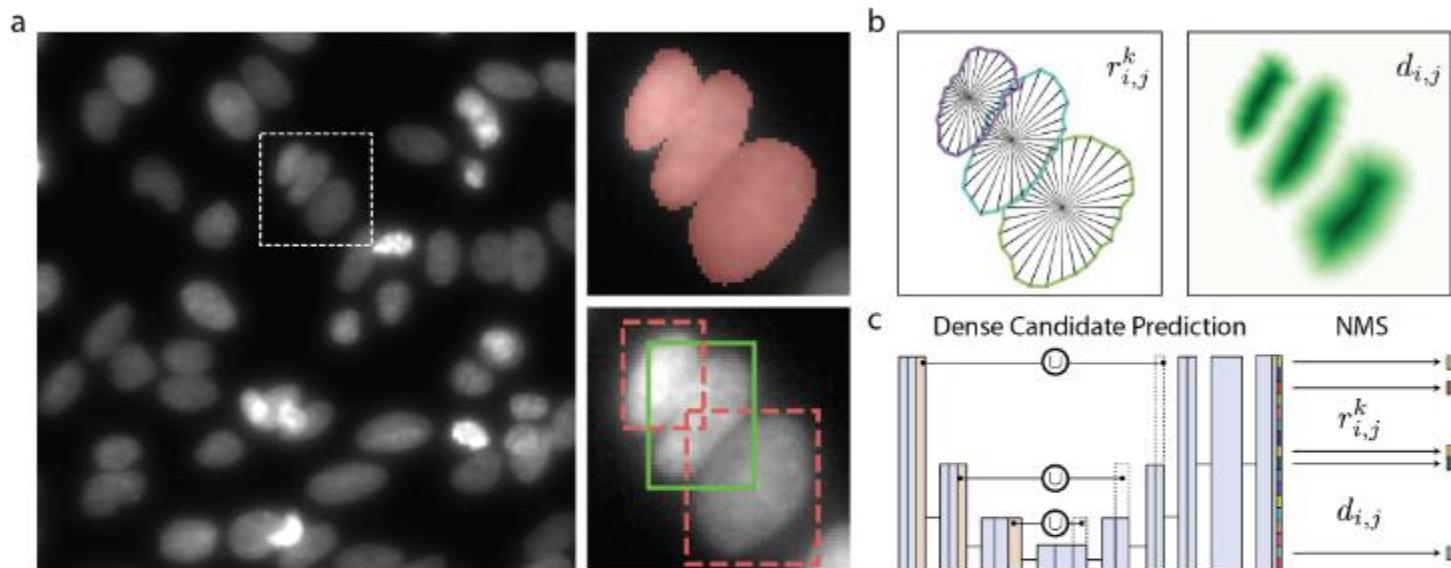


Fig. 1: (a) Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). (b) The proposed STAR DIST method predicts object probabilities  $d_{i,j}$  and star-convex polygons parameterized by the radial distances  $r_{i,j}^k$ . (c) We densely predict  $r_{i,j}^k$  and  $d_{i,j}$  using a simple U-Net architecture [15] and then select the final instances via non-maximum suppression (NMS).

# Cell Detection with Star-convex Polygons

Uwe Schmidt<sup>1,\*</sup>, Martin Weigert<sup>1,\*</sup>, Coleman Broaddus<sup>1</sup>, and Gene Myers<sup>1,2</sup>

<sup>1</sup> Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany  
Center for Systems Biology Dresden, Germany  
<sup>2</sup> Faculty of Computer Science, Technical University Dresden, Germany

For the workshop, a Stardist model was trained with data coming from 3 articles:

- **Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning.** *Nature Biotechnology* (2022).
- **A deep learning segmentation strategy that minimizes the amount of manually annotated images.** *F1000 Research* (2022).
- **Deep learning tools and modeling to estimate the temporal expression of cell cycle proteins from 2D still images.** *PLOS Computational Biology* (2022).

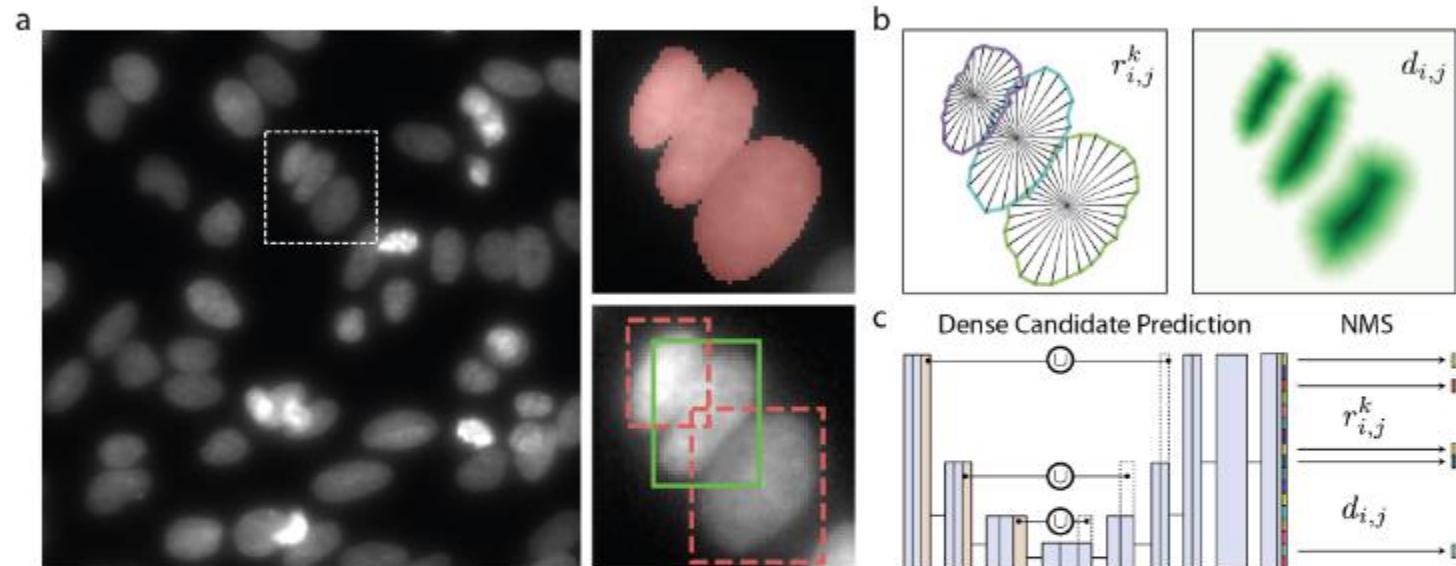


Fig. 1: (a) Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). (b) The proposed STARDIST method predicts object probabilities  $d_{i,j}$  and star-convex polygons parameterized by the radial distances  $r_{i,j}^k$ . (c) We densely predict  $r_{i,j}^k$  and  $d_{i,j}$  using a simple U-Net architecture [15] and then select the final instances via non-maximum suppression (NMS).

# SEGMENTATION WITH STARDIST

**Download the latest  
Stardist extension for  
QuPath and drag it into  
QuPath**

The screenshot shows a GitHub repository page for 'qupath/qupath-extension-stardist'. The repository is public and has 3 watches and 5 forks. The 'Code' tab is selected. Below the tabs, there are 'Issues' (2), 'Pull requests', 'Actions', 'Security', and 'Insights' buttons. A search bar at the top right says 'Find a release'. The main content area shows the 'v0.3.0' release, which was published on Sep 02, 2021, by petebankhead. It includes a download link for 'qupath-extension-stardist-0.3.0.jar' (24.7 KB) and source code links for 'zip' and 'tar.gz'. There are also three other assets listed under 'Assets'. At the bottom of the release page, there is a smiley face icon.

Aug 08, 2021

v0.3.0-rc2

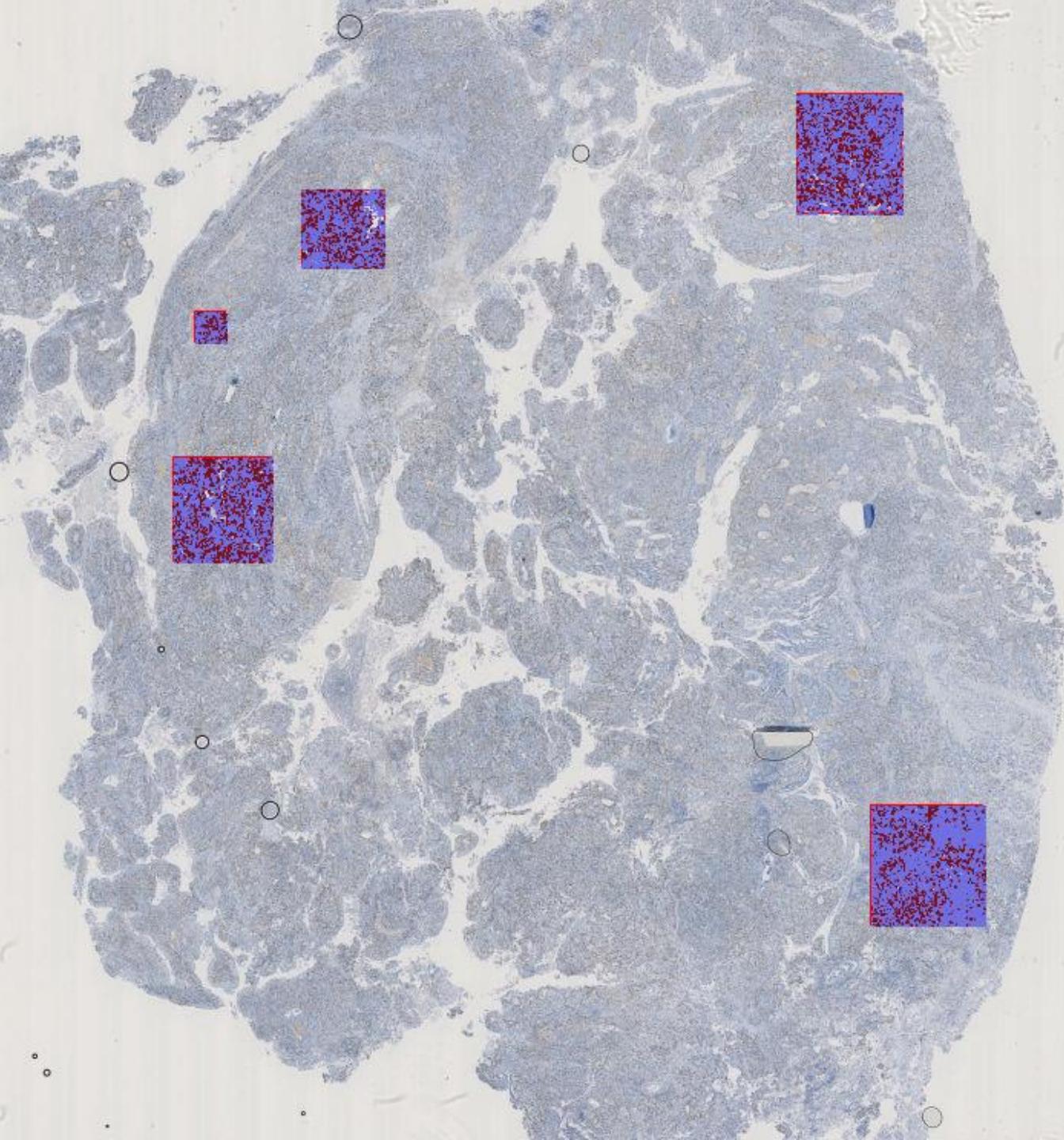
# SEGMENTATION WITH STARDIST

Open **Scrip editor**, then open  
nucleus\_detection\_hematoxylin\_da  
b.groovy

```
1 import qupath.ext.stardist.StarDist2D
2 import qupath.lib.images.servers.ColorTransforms
3 import qupath.imagej.gui.ImageJMacroRunner
4
5 min_nuclei_area = 15
6
7 // Specify the model directory (you will need to change this!)
8 def pathModel = "C:/Work/QuPath/scripts/StardistModels/TissueNet_all.pb"
9
10 def stardist_segmentation = StarDist2D.builder(pathModel)
11     .threshold(0.5)                      // Prediction threshold
12     .normalizePercentiles(1, 99.8)        // Percentile normalization
13     .pixelSize(0.5)                     // Resolution for detection
14     .channels(
15         ColorTransforms.createColorDeconvolvedChannel(getCurrentImageData().getColorDeconvolutionStains(), 1),
16         ColorTransforms.createColorDeconvolvedChannel(getCurrentImageData().getColorDeconvolutionStains(), 2)
17     )
18     .cellExpansion(5.0)                  // Approximate cells based upon nucleus expansion
19     .cellConstrainScale(1.5)            // Constrain cell expansion using nucleus size
20     .measureShape()                   // Add shape measurements
21     .measureIntensity()              // Add cell measurements (in all compartments)
22     .build()
23
24
25 def imageData = getCurrentImageData()
26 def hierarchy = imageData.getHierarchy()
27 def annotations = hierarchy.getAnnotationObjects()
28
29 // Run detection for the selected objects
30 stardist_segmentation.detectObjects(imageData, annotations)
31
32 //def toDelete = getDetectionObjects().findAll {measurement(it, 'Circularity') < 0.9}
33 def toDelete = getDetectionObjects().findAll {measurement(it, 'Area µm^2') < min_nuclei_area}
34 removeObjects(toDelete, true)
35
36
37 println 'Done!'
```

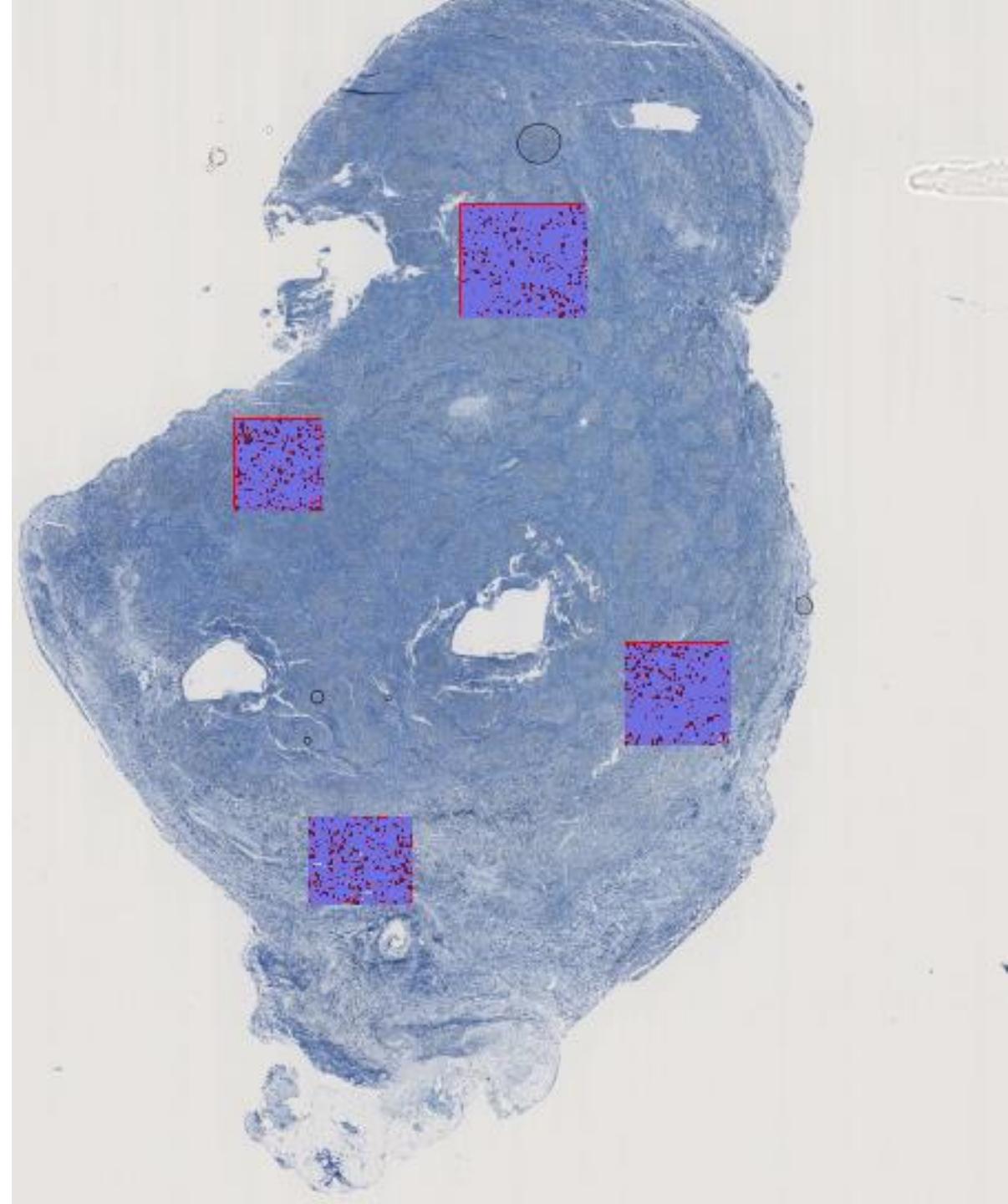
## SEGMENTATION WITH STARDIST

- Open KI67\_lung.ndpi
- Define a **small rectangle annotation** to test the **Stardist parameters**
- Open **Create single measurement classifier** and define threshold to identify DAB+ cells
- Run **stardist** on a small number of annotations and identify DAB+ cells
- Get the **proportion of DAB+ cells**
- **Compare** with the results obtained with the **watershed-based approach**

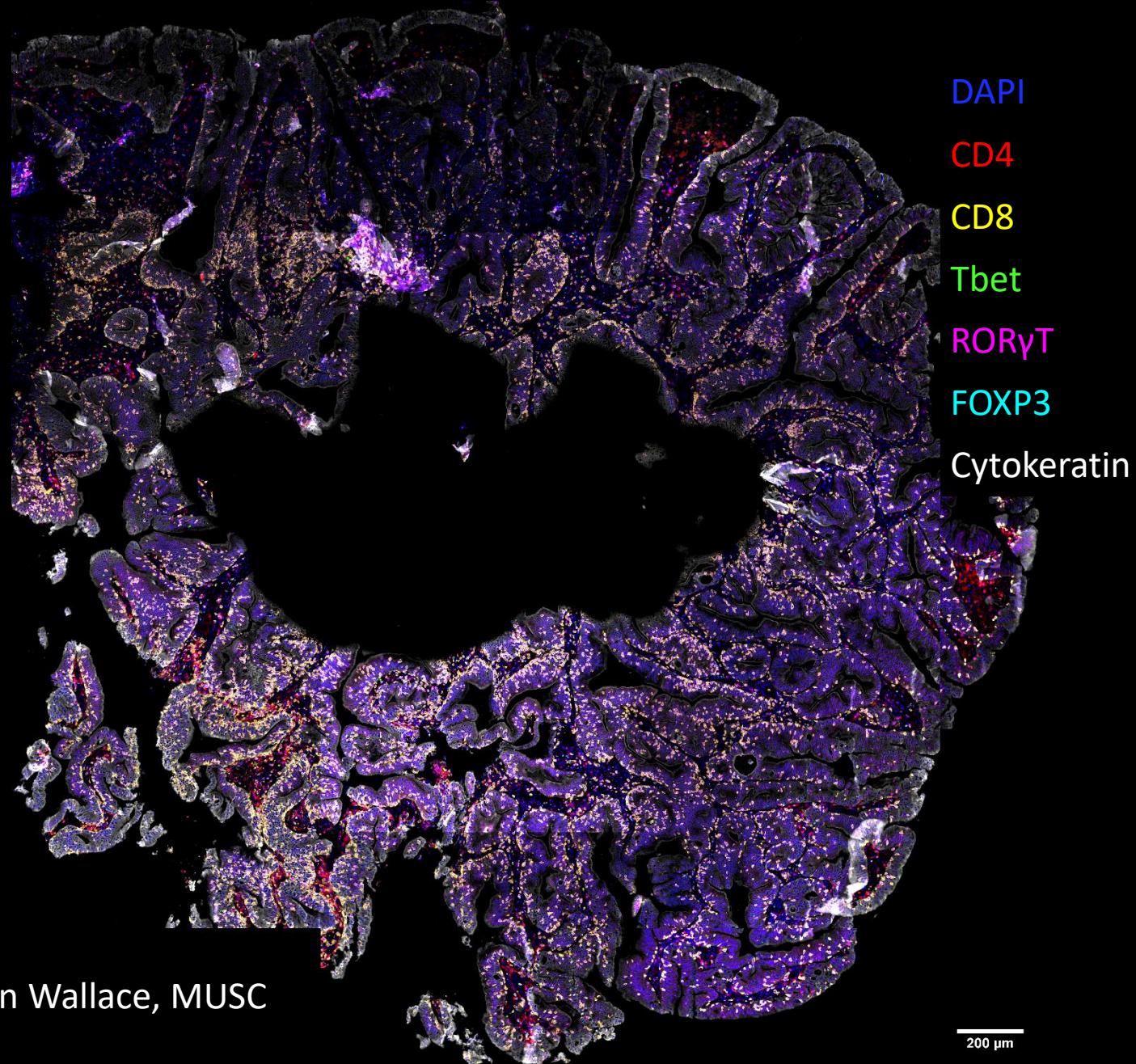


## SEGMENTATION WITH STARDIST

- Open KI67\_lymphoma.ndpi
- Define **3 or 4 ROIs**
- Modify script to do **both segmentation and thresholding** (workflow tab)
- Is it a **good way to quantify** this image ?



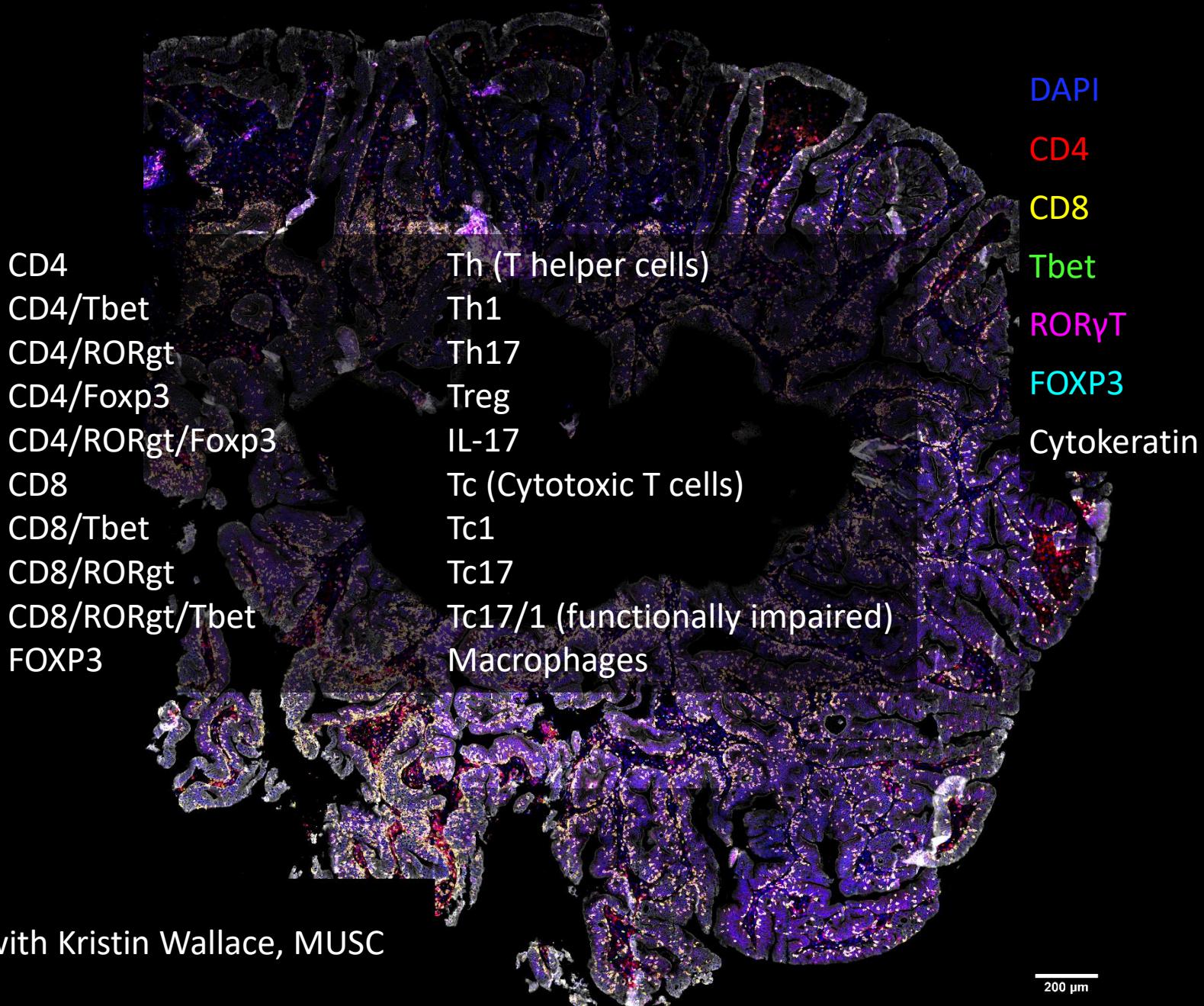
# MULTI/HYPER-PLEXED IMAGES



Polyp study with Kristin Wallace, MUSC

200  $\mu$ m

# MULTI/HYPER-PLEXED IMAGES



## SHALLOW MACHINE LEARNING FOR OBJECT CLASSIFICATION

**As for pixel classification:**

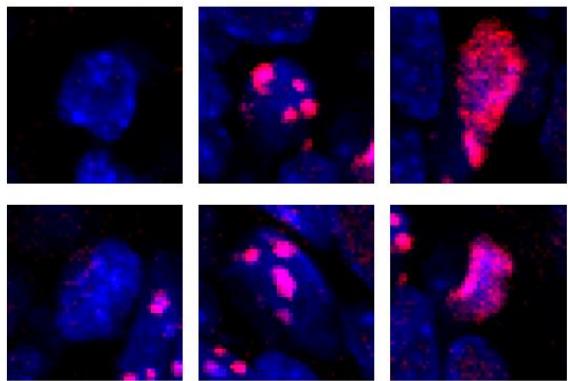
- **Examples** of classes are **manually** defined by the user
- A **classifier** is **trained** with these examples
- Data is then **automatically classified** by using the trained classifier

But this time, features are **measurements associated to detections** (most often cells or nuclei) such as:

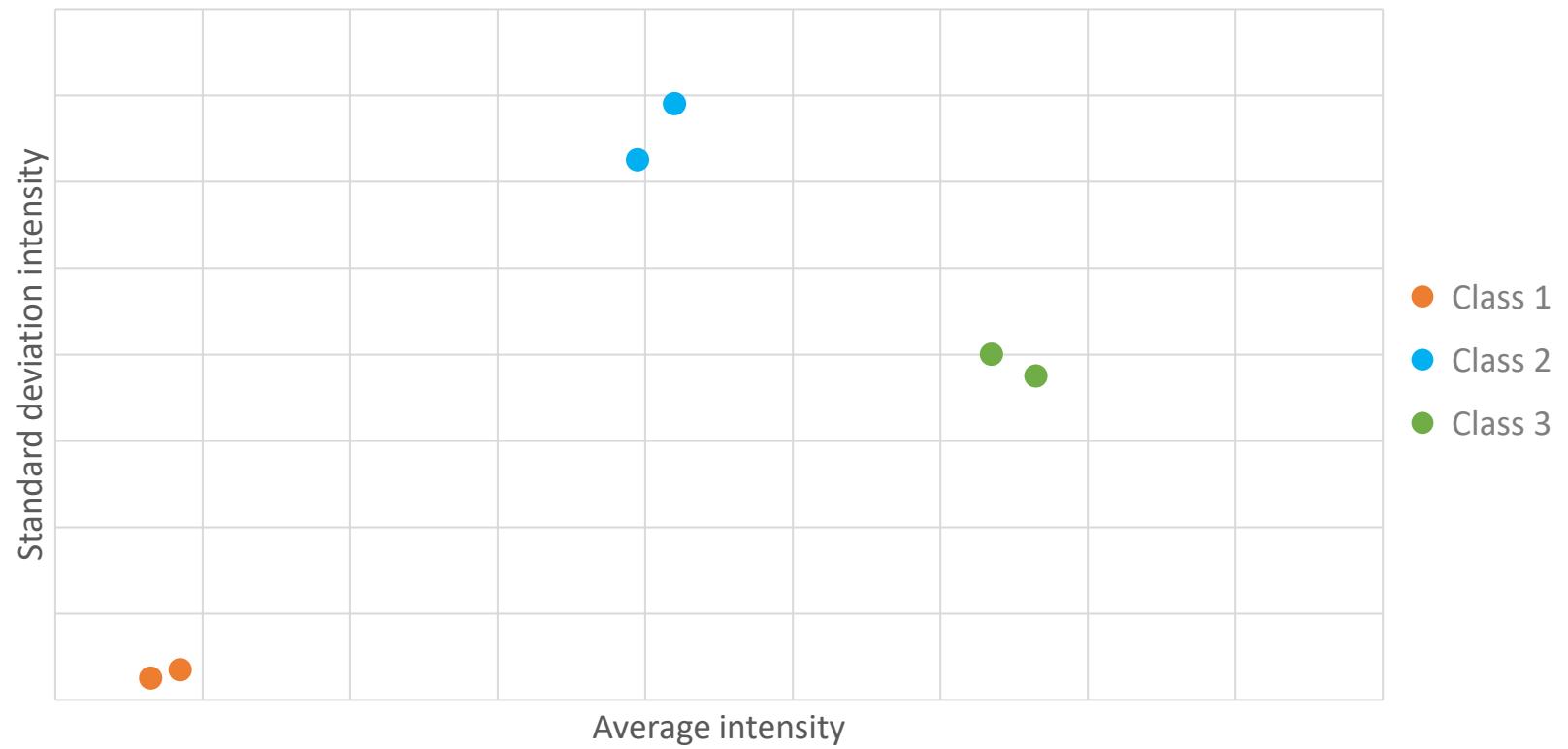
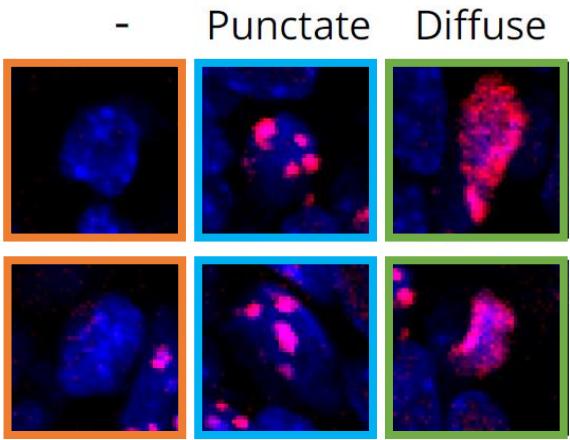
- **Average** intensity
- **Median** intensity
- **Standard deviation** of intensity
- **Minimum/Maximum** intensity
- **Object area**
- **Object Circularity**
- ...

# SHALLOW MACHINE LEARNING FOR OBJECT CLASSIFICATION

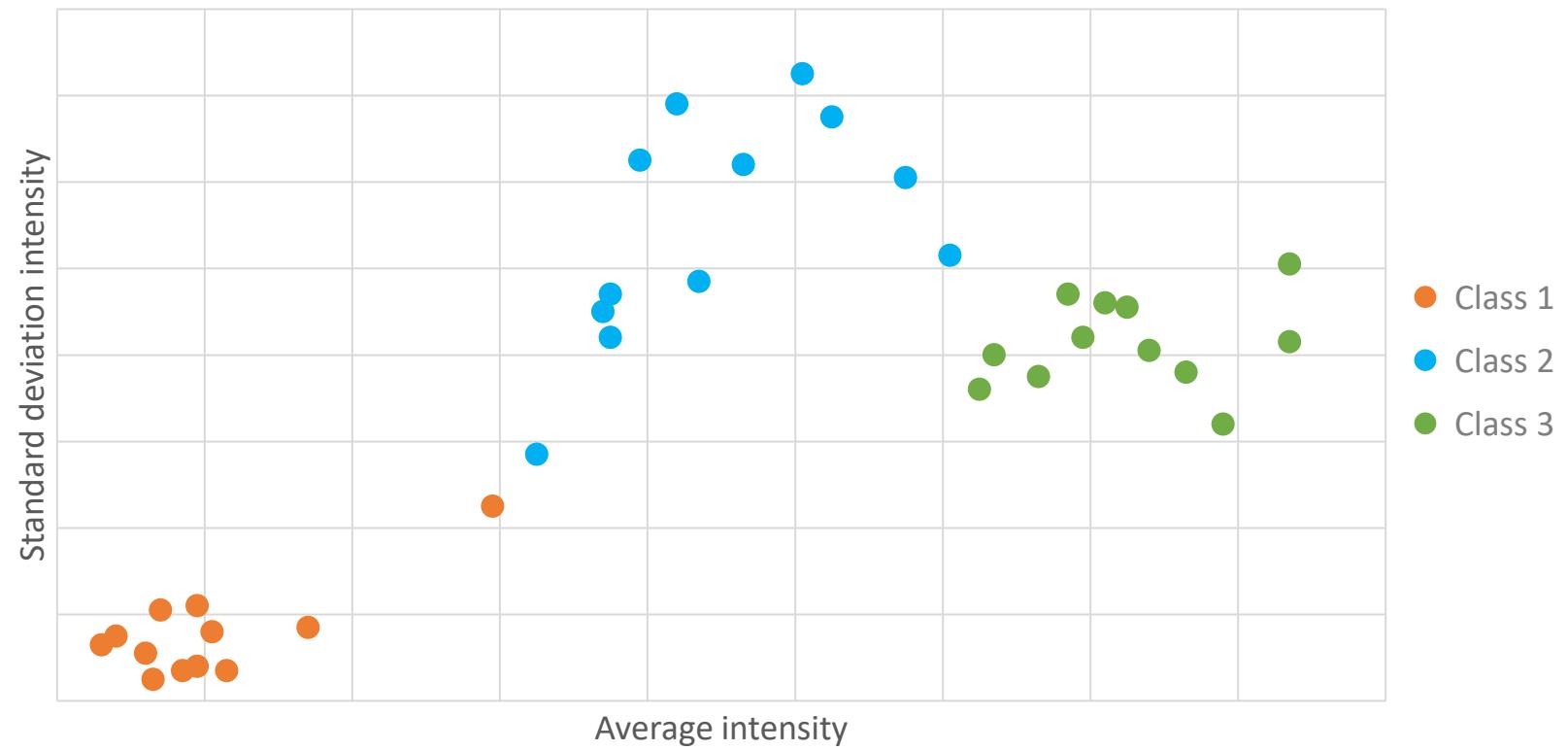
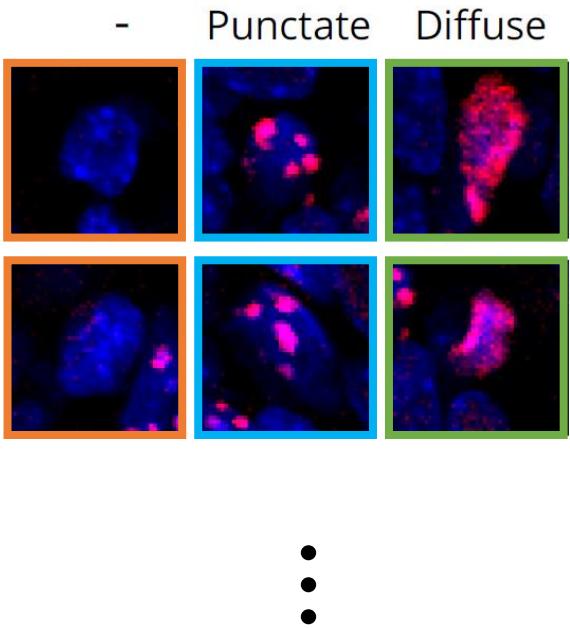
- Punctate Diffuse



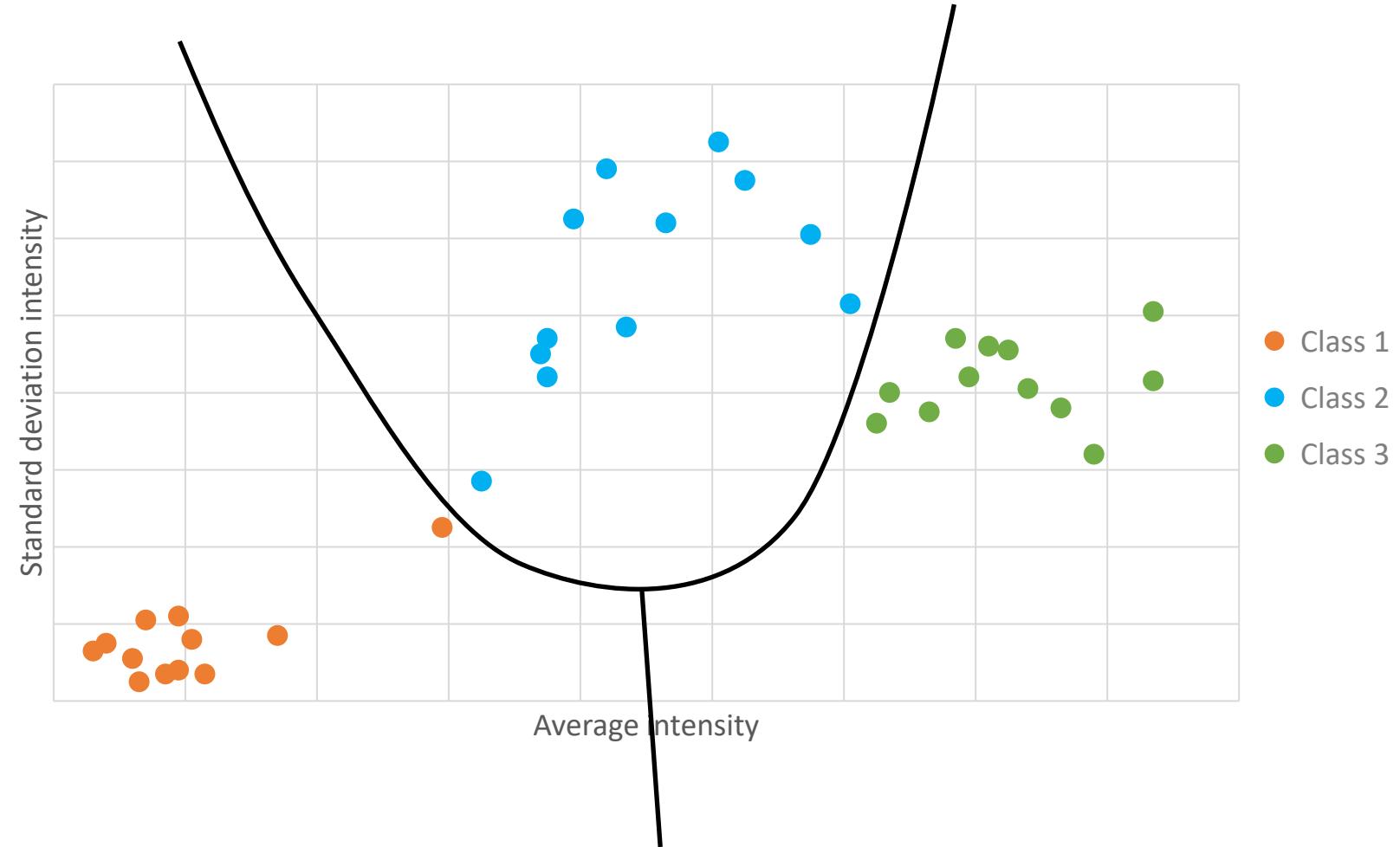
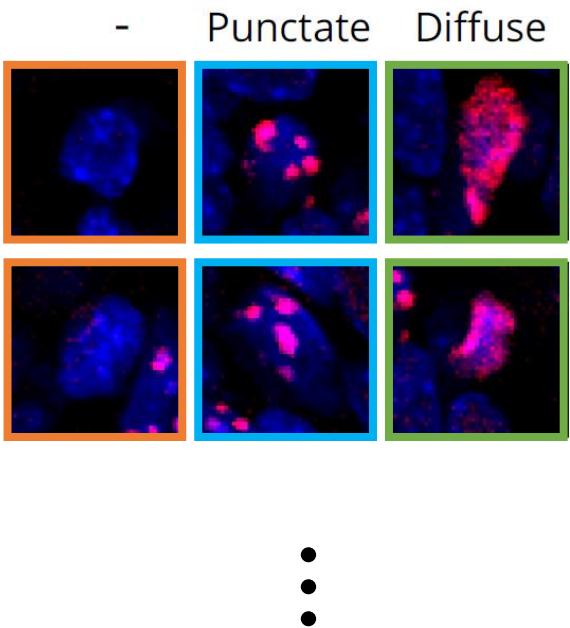
# SHALLOW MACHINE LEARNING FOR OBJECT CLASSIFICATION



# SHALLOW MACHINE LEARNING FOR OBJECT CLASSIFICATION

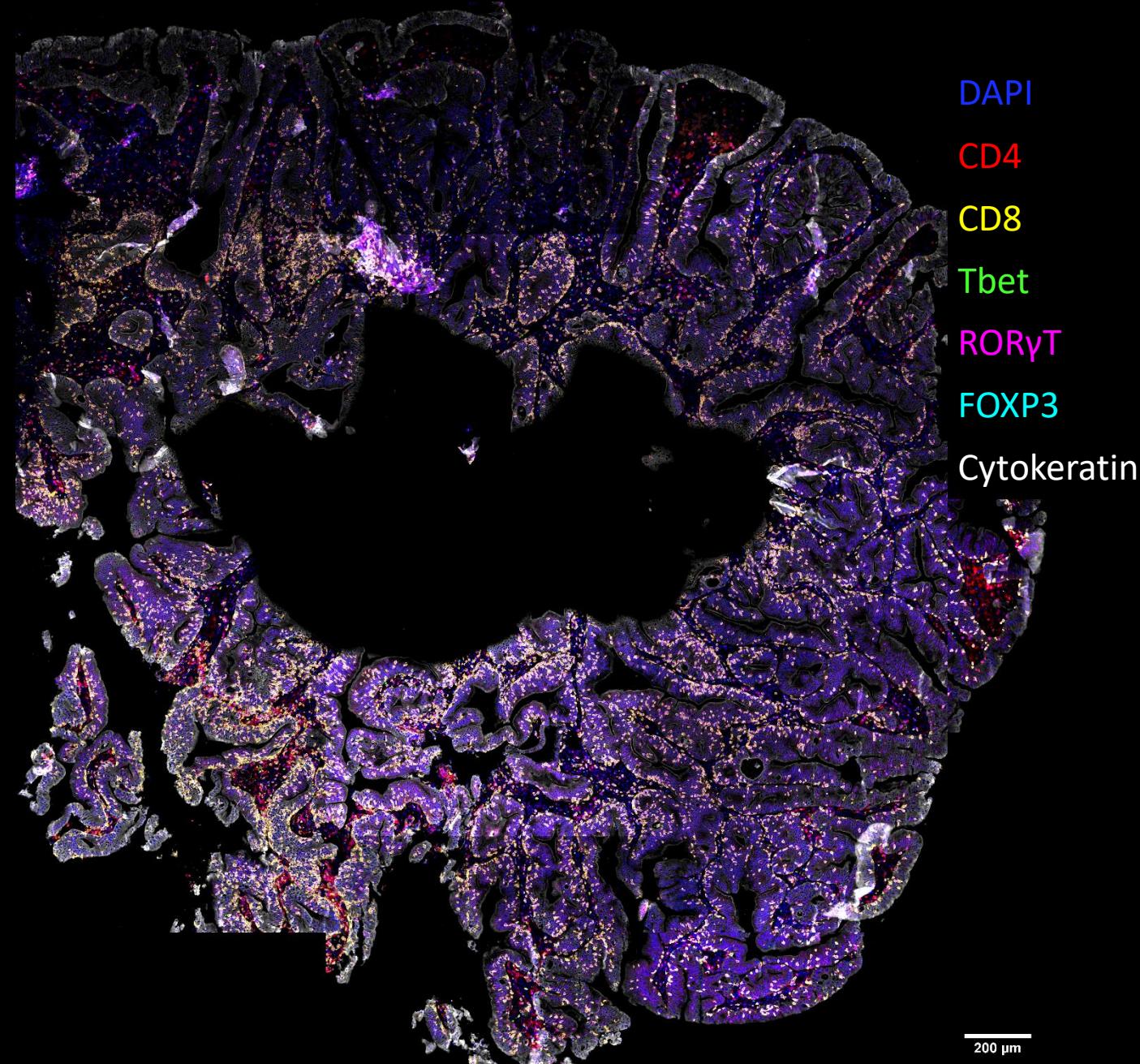


# SHALLOW MACHINE LEARNING FOR OBJECT CLASSIFICATION



## MULTI/HYPER-PLEXED IMAGES

- Use a pixel classifier to segment epithelium and stroma, save it and run it
- Define a small annotation and run Stardist to optimize parameters for nuclei segmentation
- Apply Stardist to the entire image
- Train an object classifier to identify positive cells for each marker
- Compute distances to tissues and between cell types
- Export measurements



## CITATIONS

- P. Bankhead *et al.* **QuPath: Open source software for digital pathology image analysis.** *Scientific Reports* (2017). <https://doi.org/10.1038/s41598-017-17204-5>
- 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>
- N.F. Greenwald *et al.* **Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning.** *Nature Biotechnology* (2021). <https://doi.org/10.1038/s41587-021-01094-0>
- T. Péicot *et al.* **A deep learning segmentation strategy that minimizes the amount of manually annotated images.** *F1000 Research* (2022) <https://doi.org/10.12688/f1000research.52026.2>
- T. Péicot *et al.* **Deep learning tools and modeling to estimate the temporal expression of cell cycle proteins from 2D still images.** *PLOS Computational Biology* (2022)

## VIDEO TUTORIALS

- [QuPath installation, data and script downloading](#)
- [Project creation and annotations](#)
- [Stain deconvolution](#)
- [Pixel classification \(epithelium/stroma for H&E images\)](#)
- [Nuclei segmentation \(watershed\) and DAB positive cells](#)
- [Nuclei segmentation \(stardist\) and DAB positive cells \(thresholding\)](#)
- [Visualization of fluorescence images](#)
- [Pixel classification \(epithelium/stroma for fluorescence images\)](#)
- [Nuclei segmentation \(stardist\)](#)
- [Object classification for marker identification](#)