Whole-Slide Image Analysis and Quantitative Pathology with QuPath

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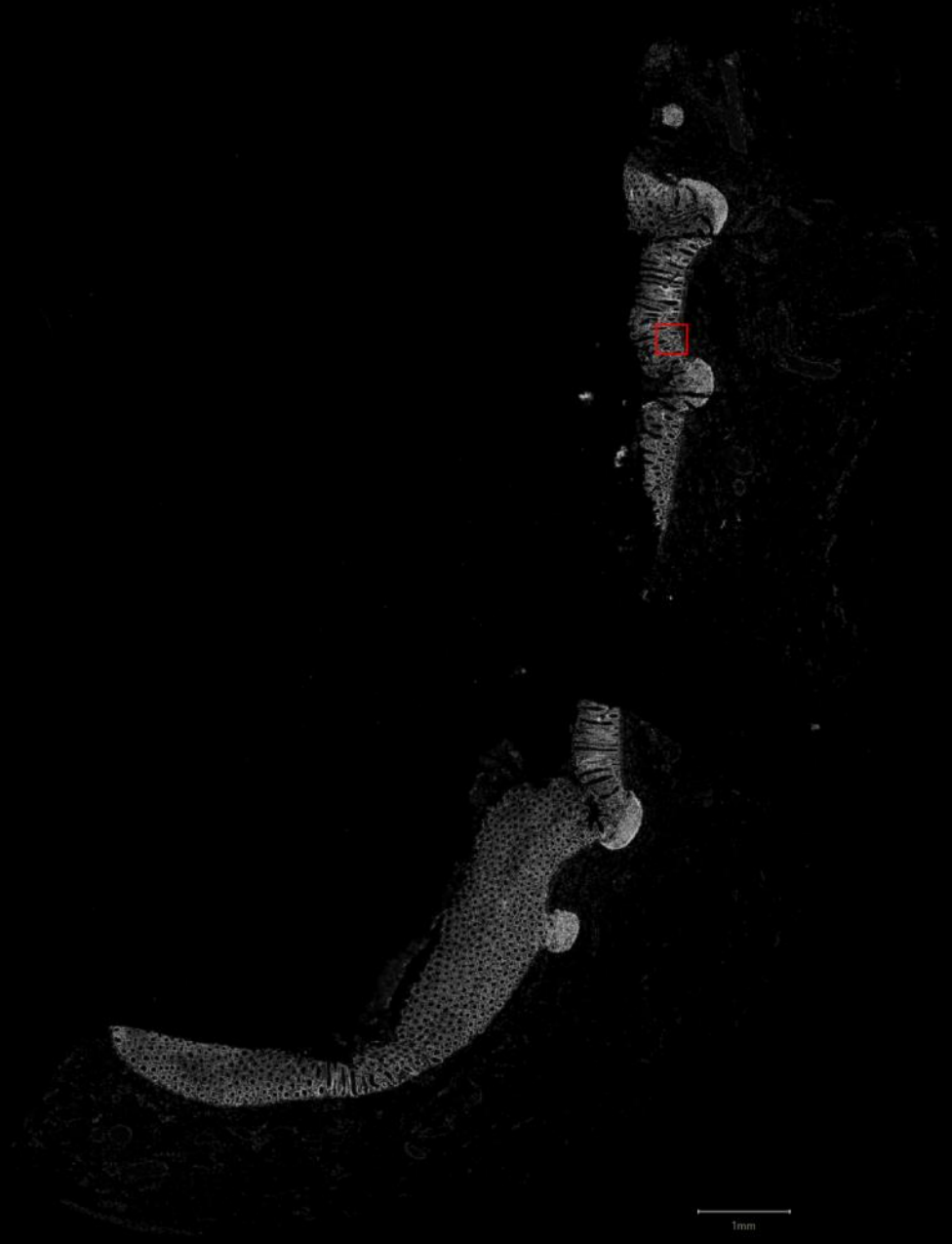
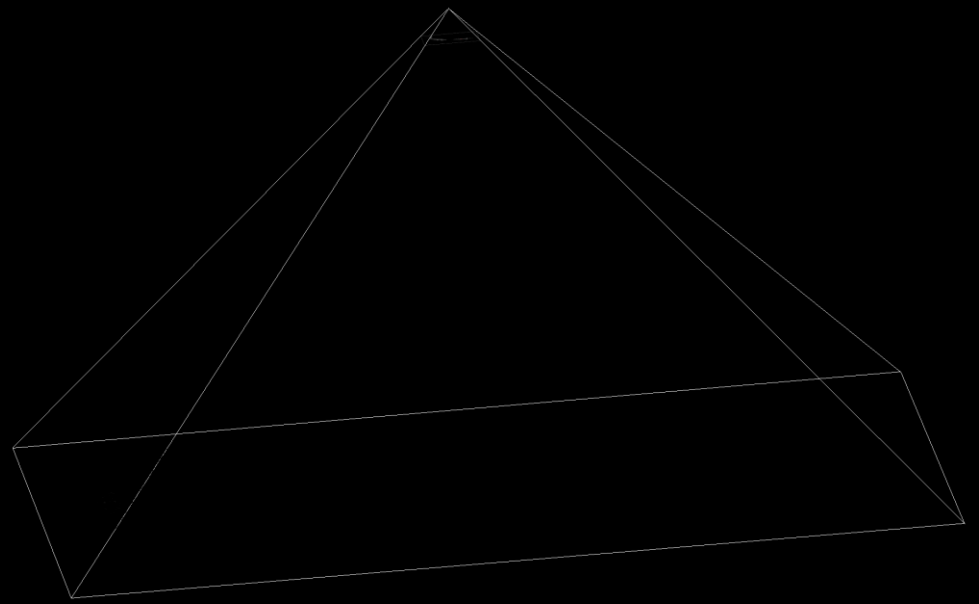
only load the **required information** in the RAM memory:

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

Whole Slide Image – Pyramidal Representation





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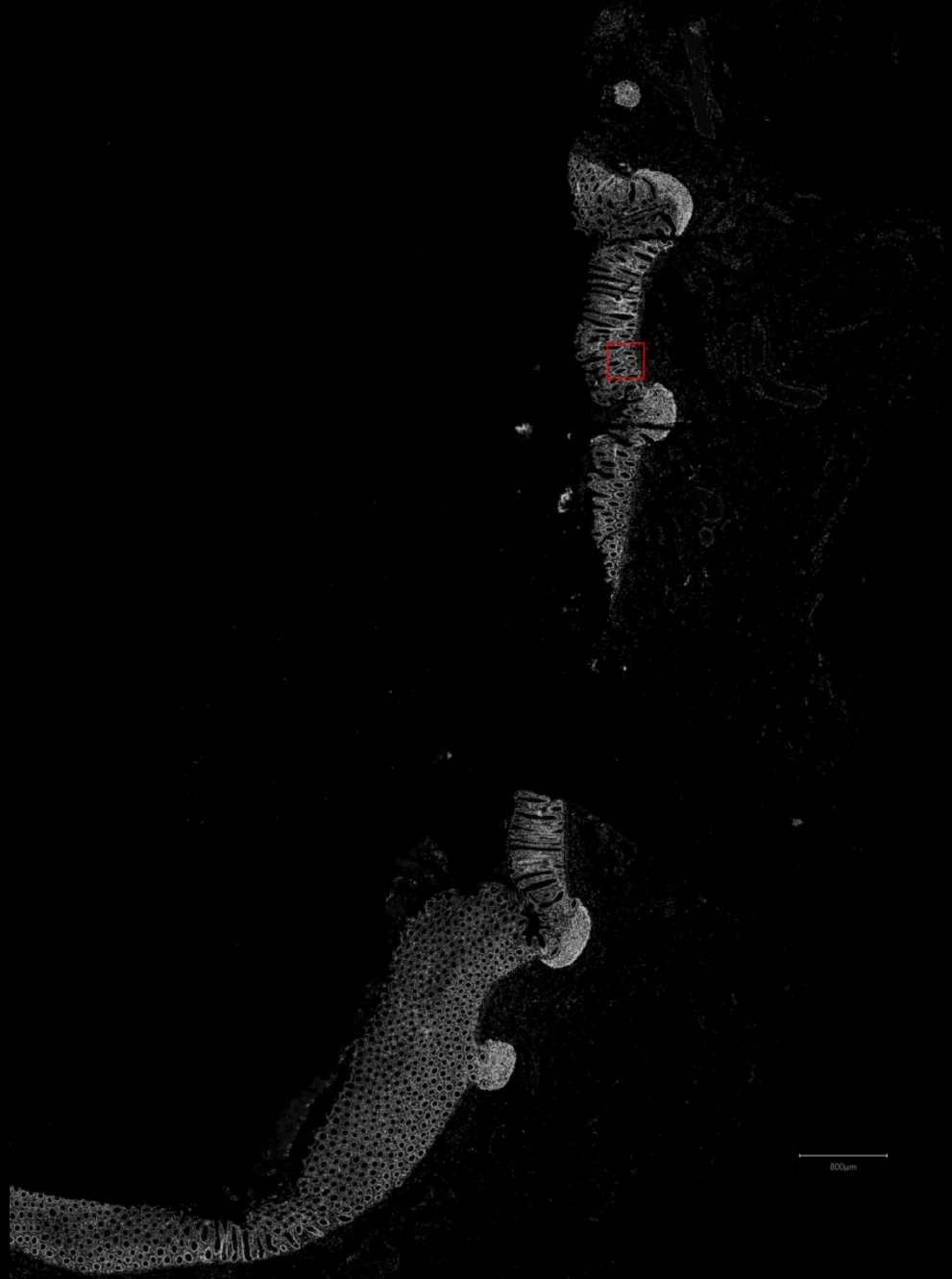

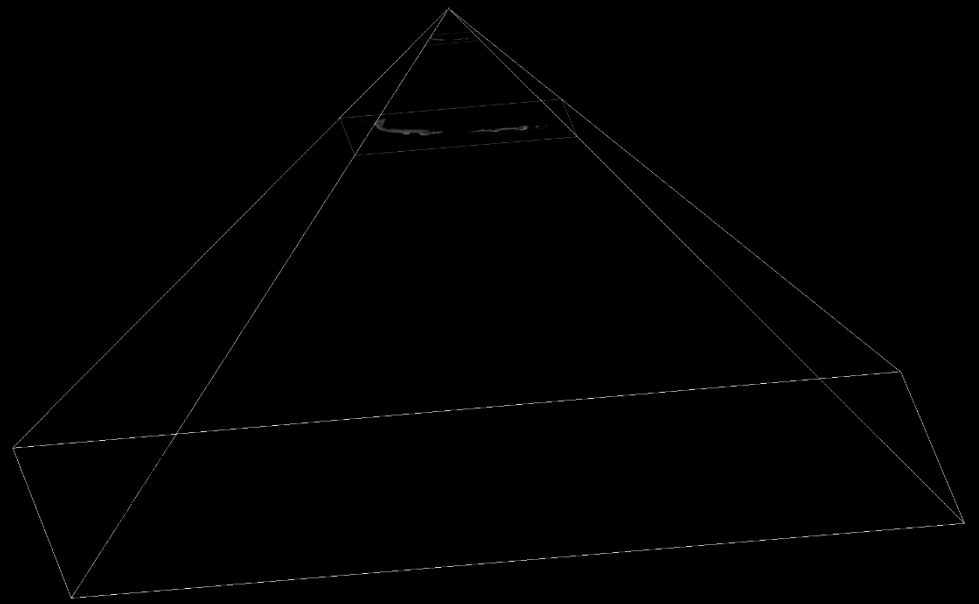

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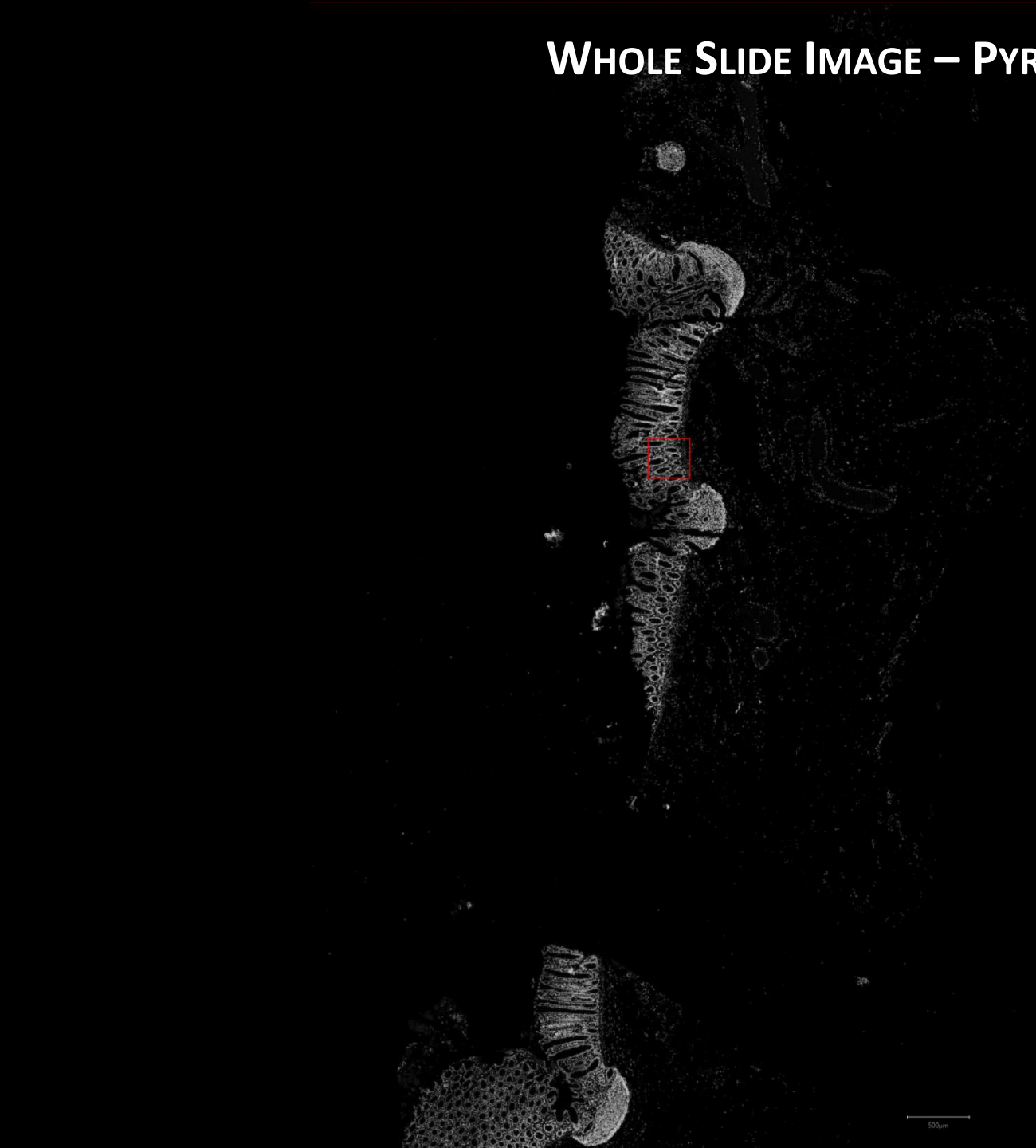

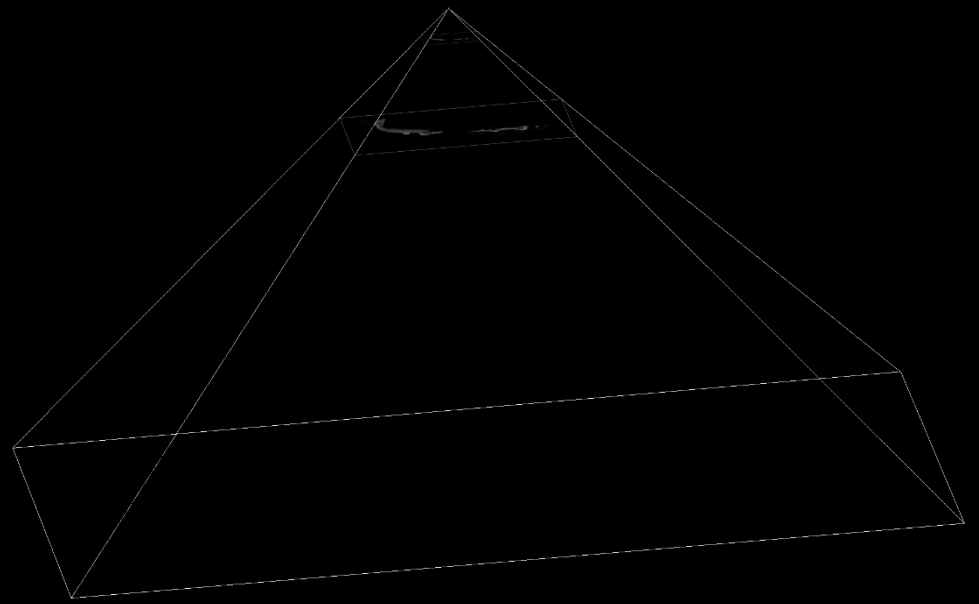

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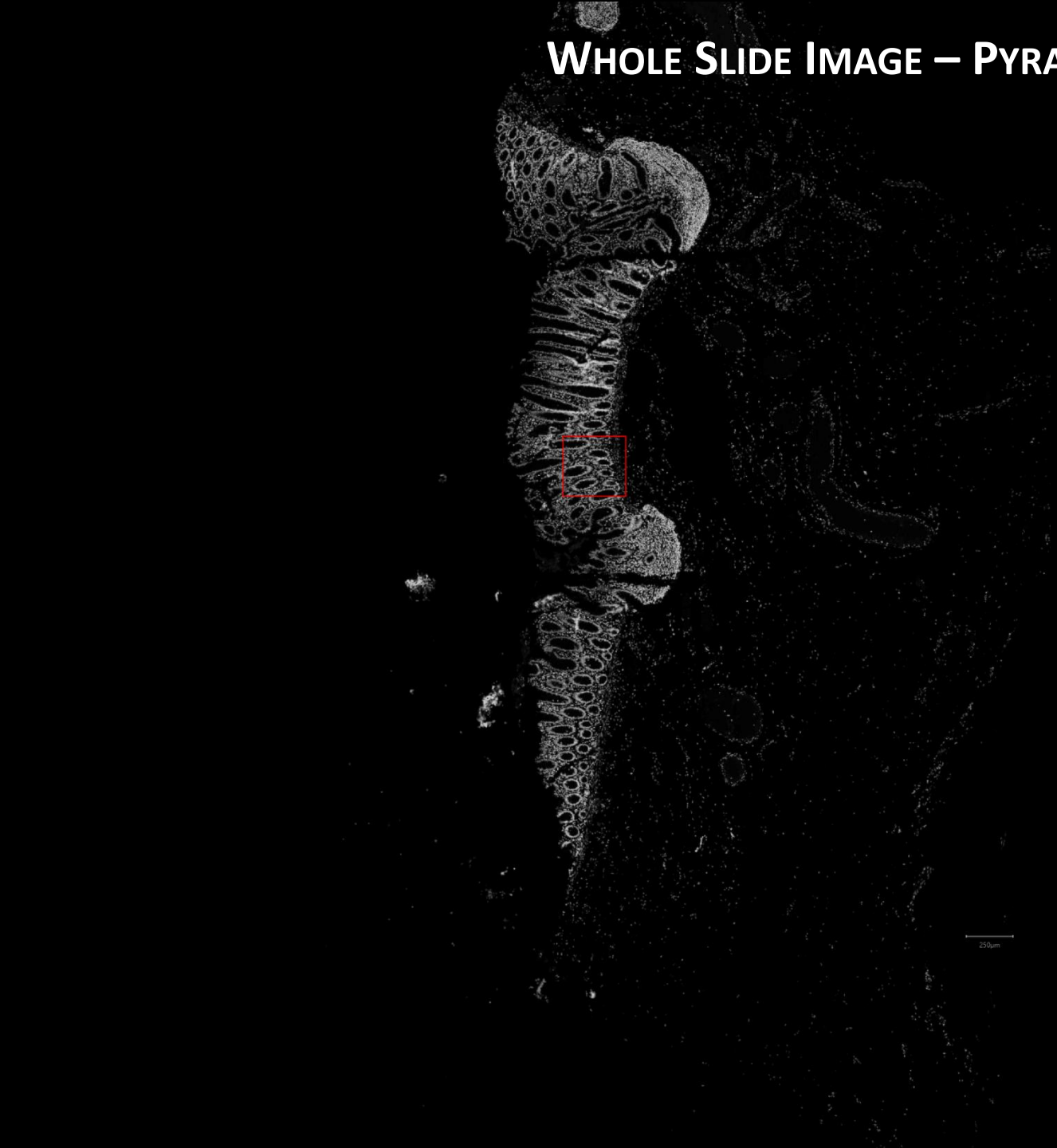

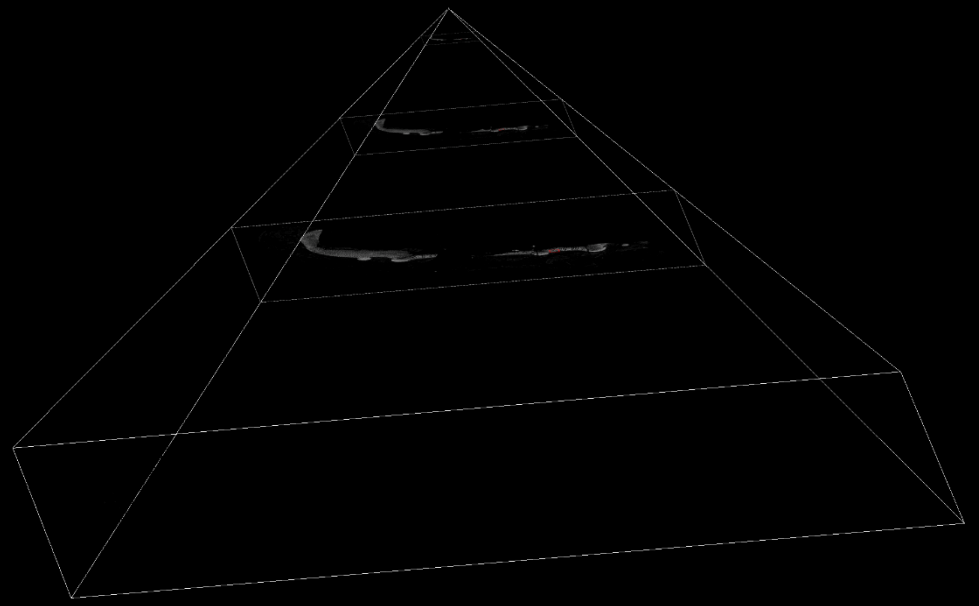

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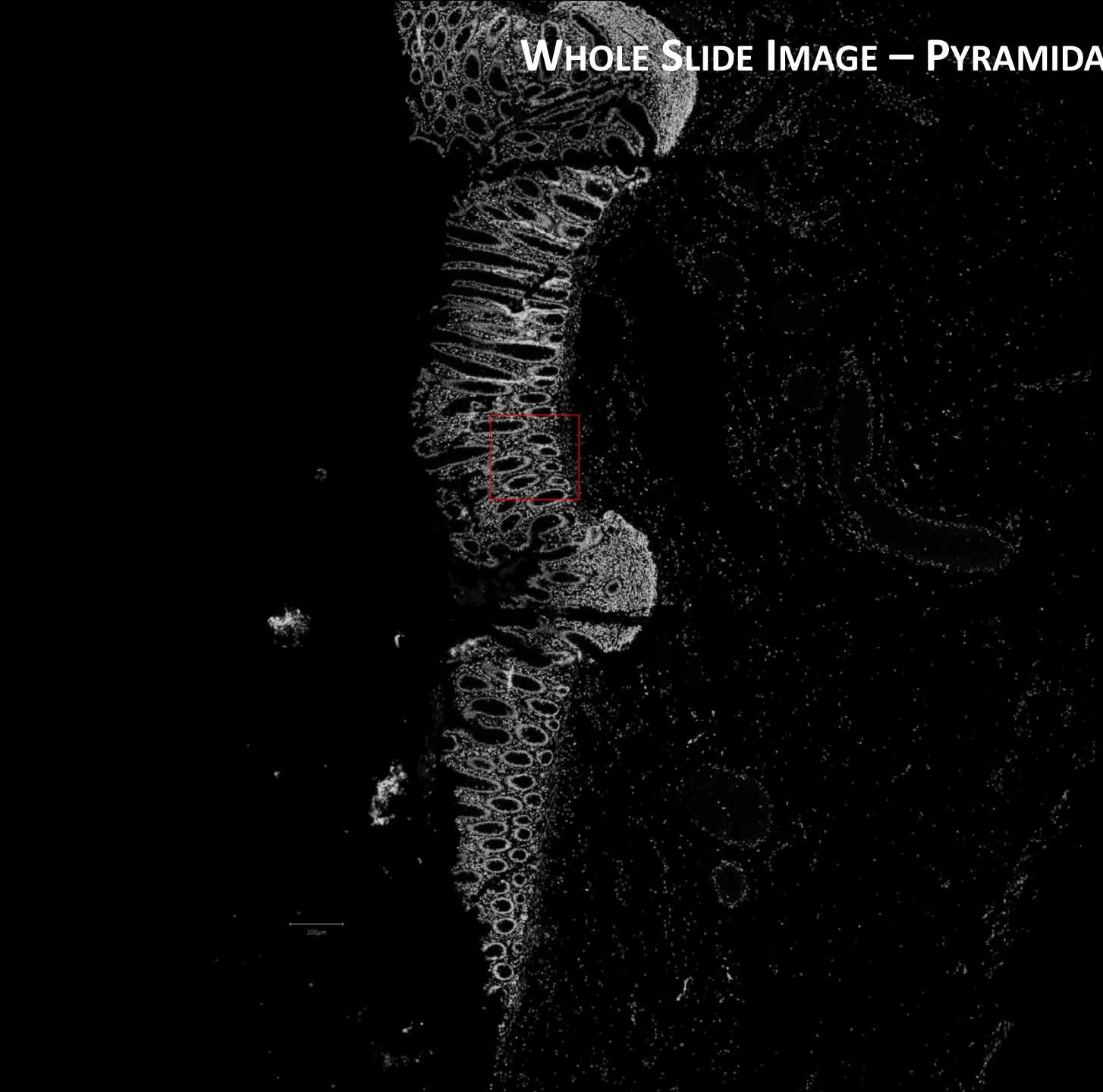

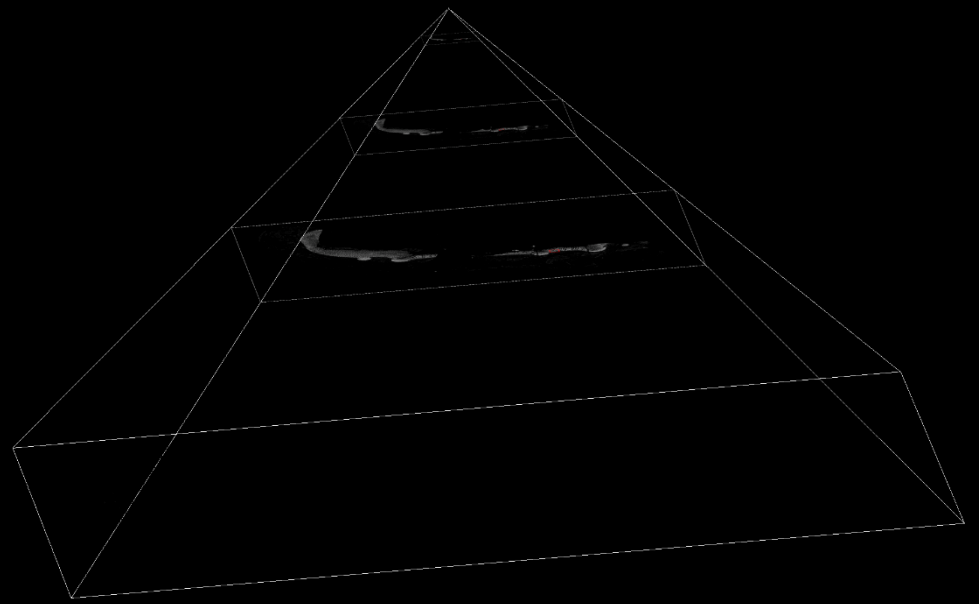

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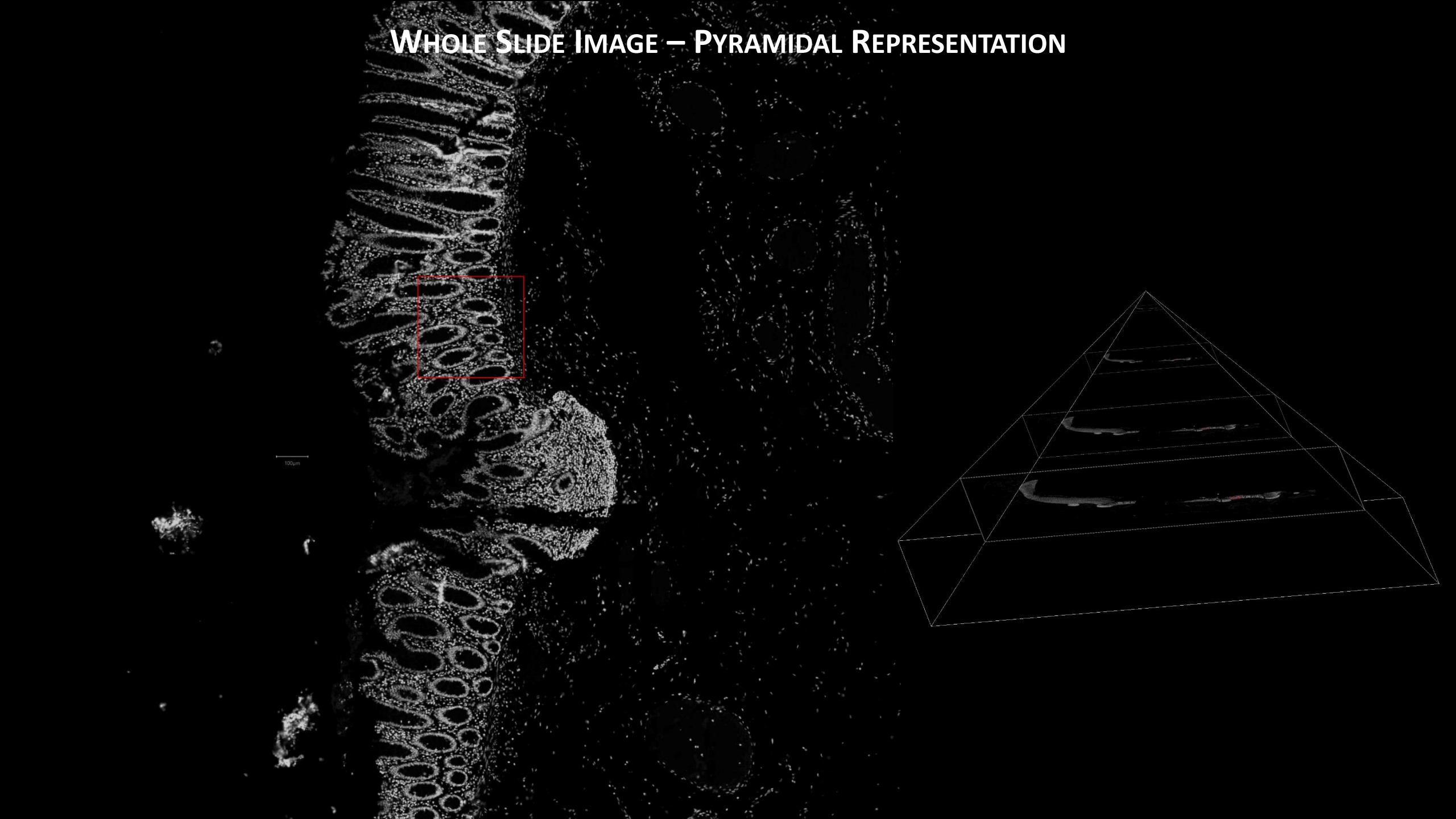

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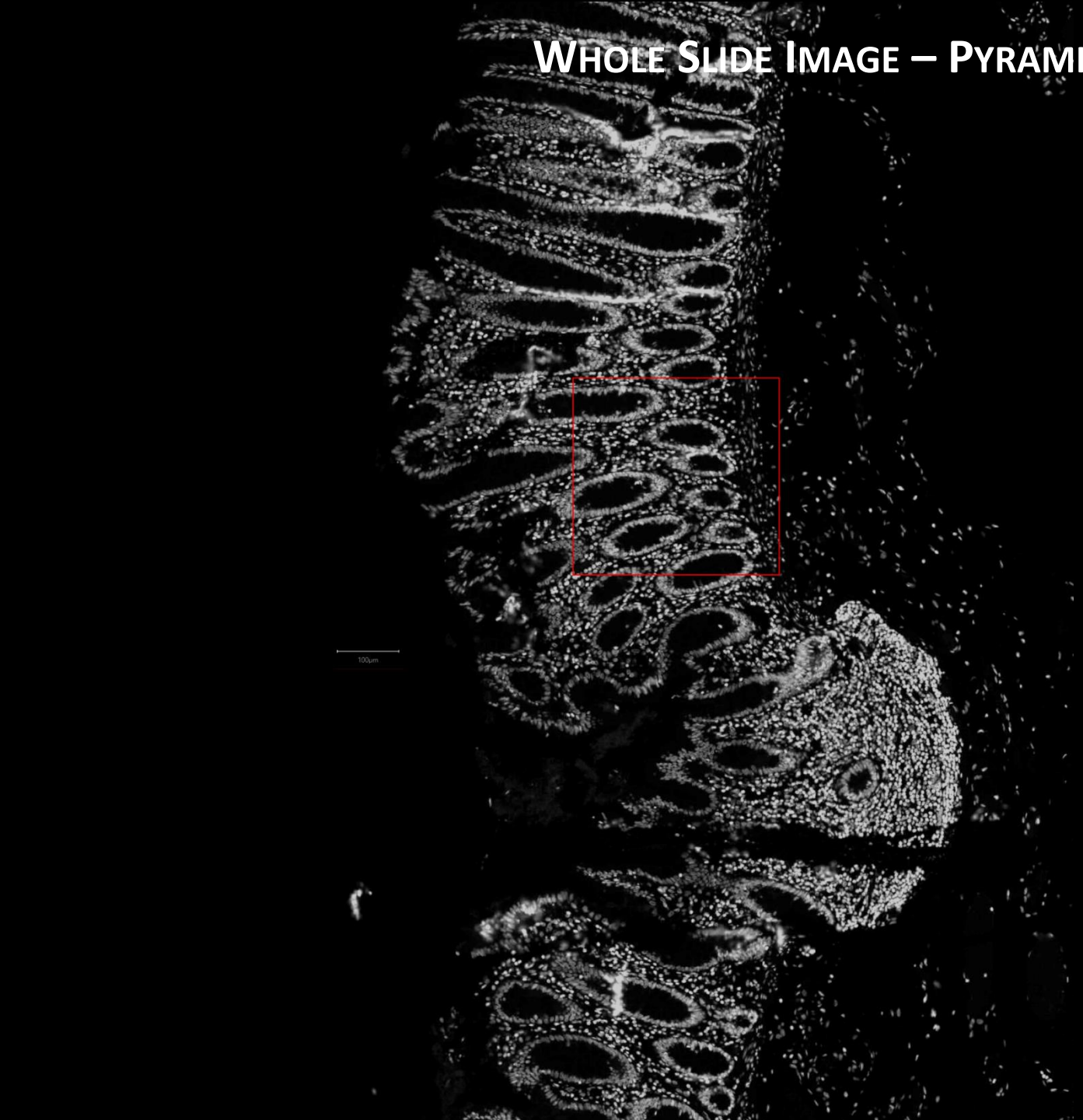

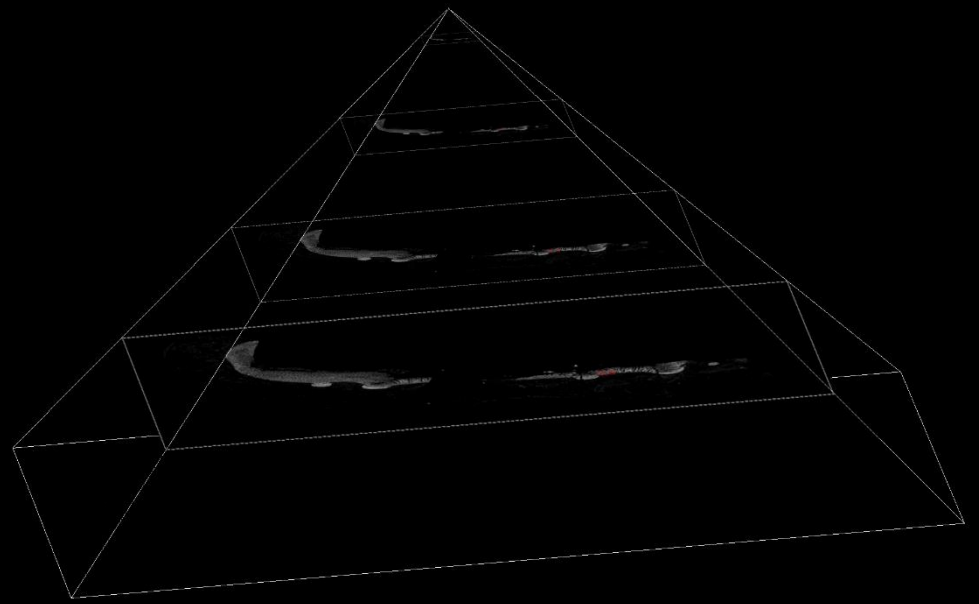

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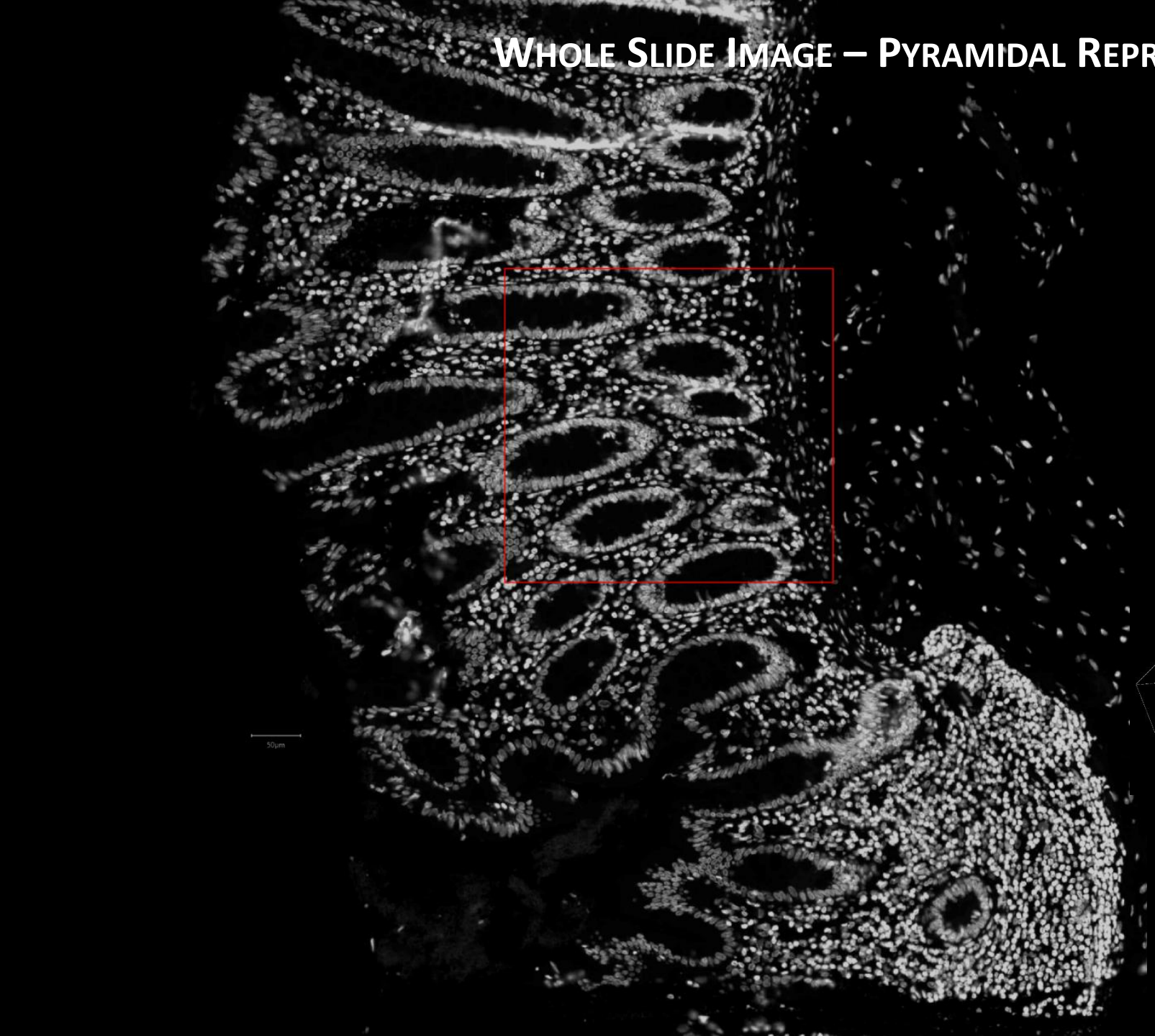

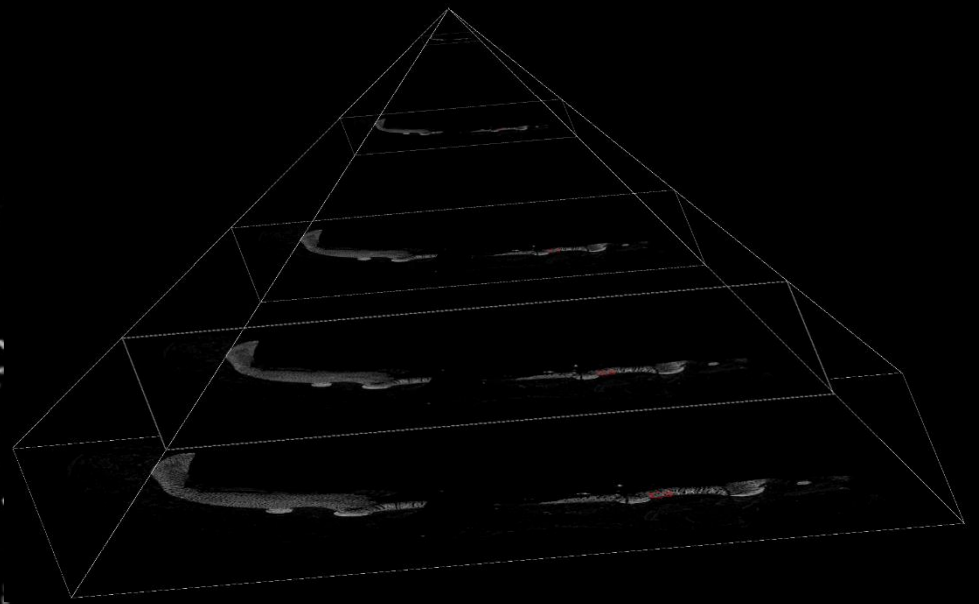

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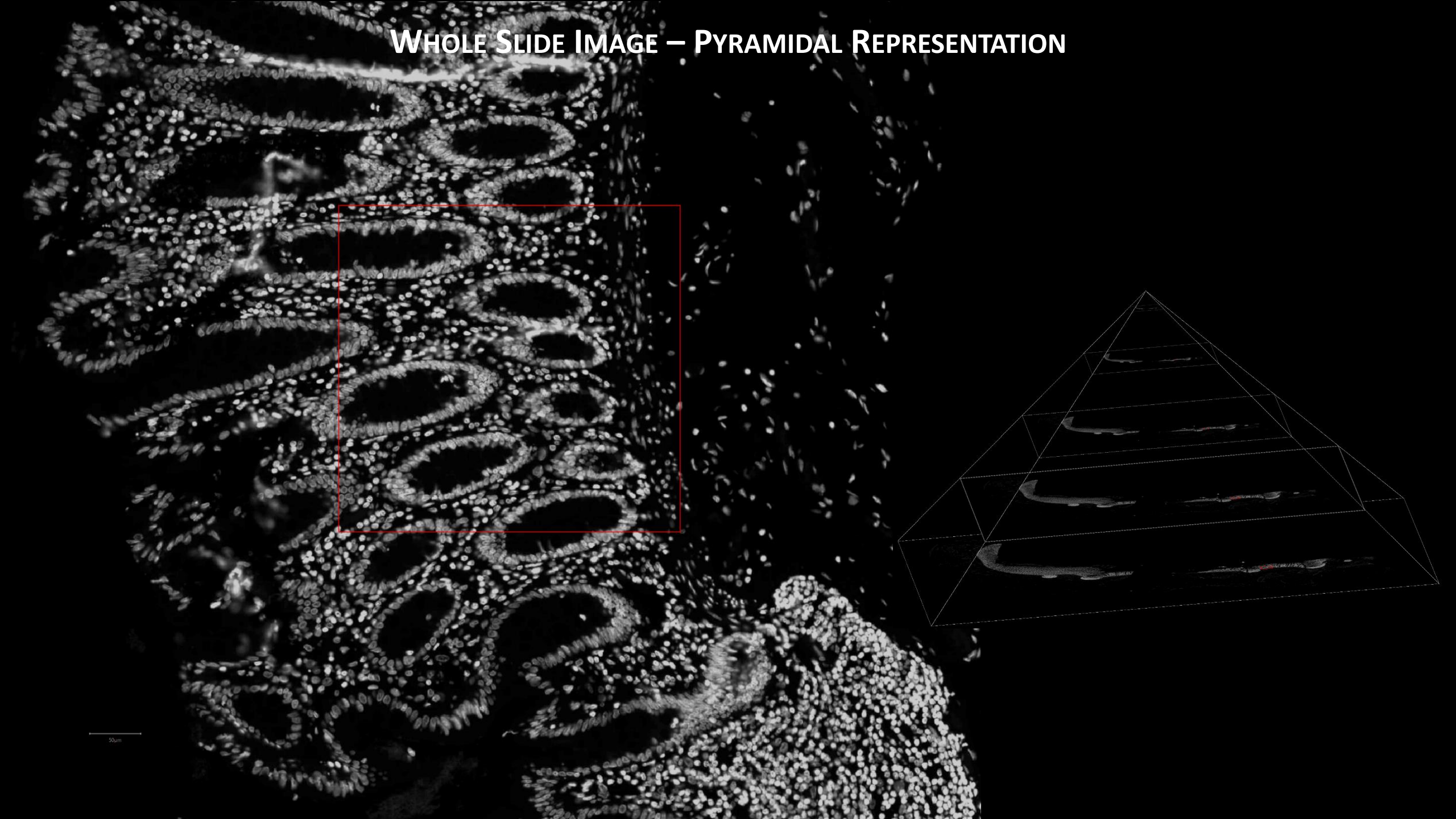

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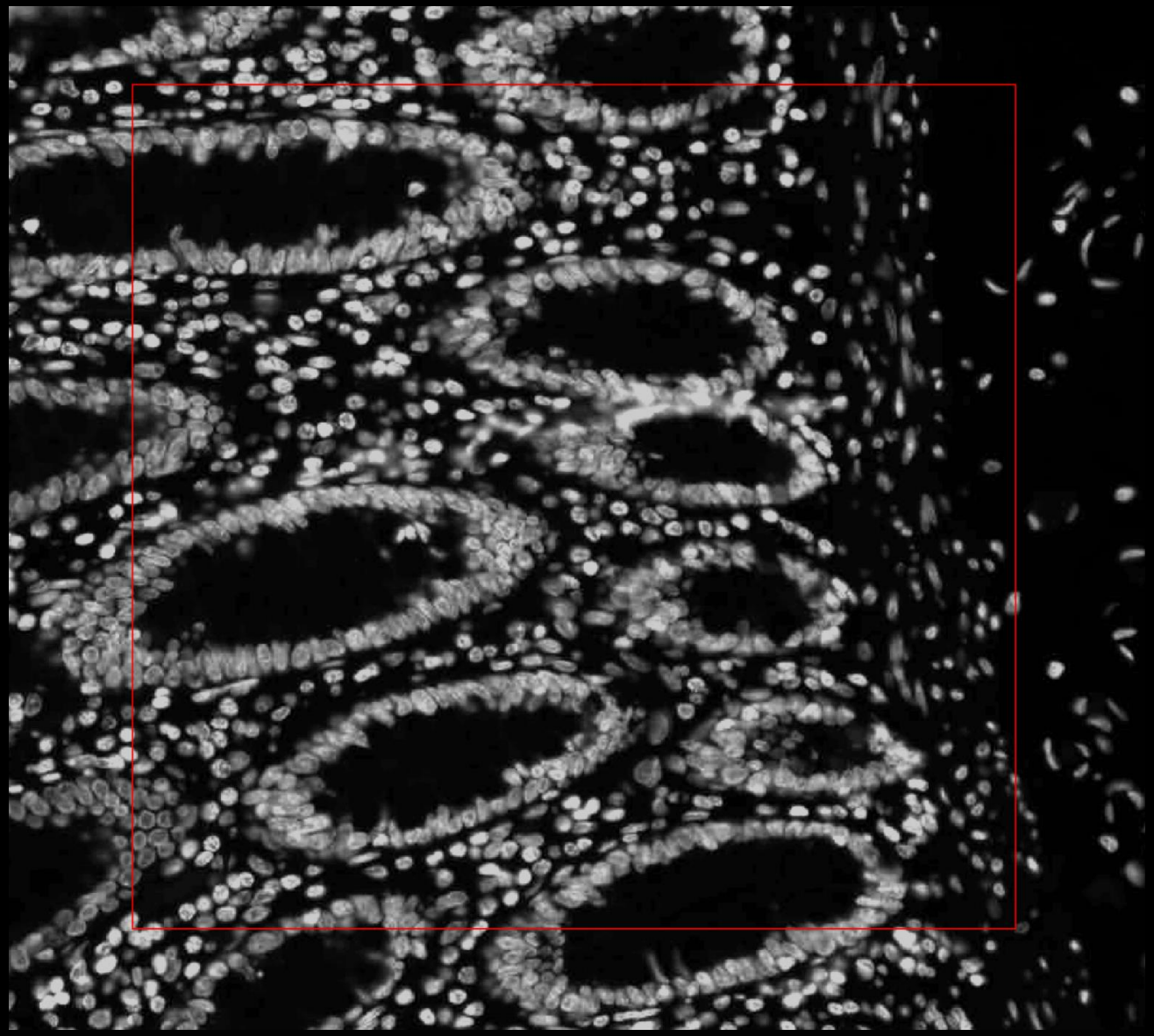

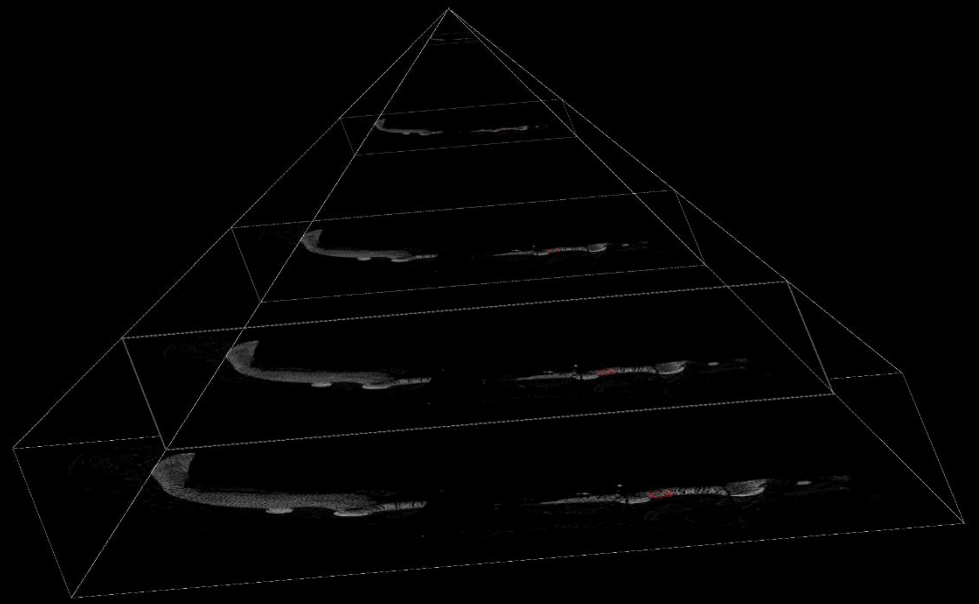

Whole-Slide Image Size

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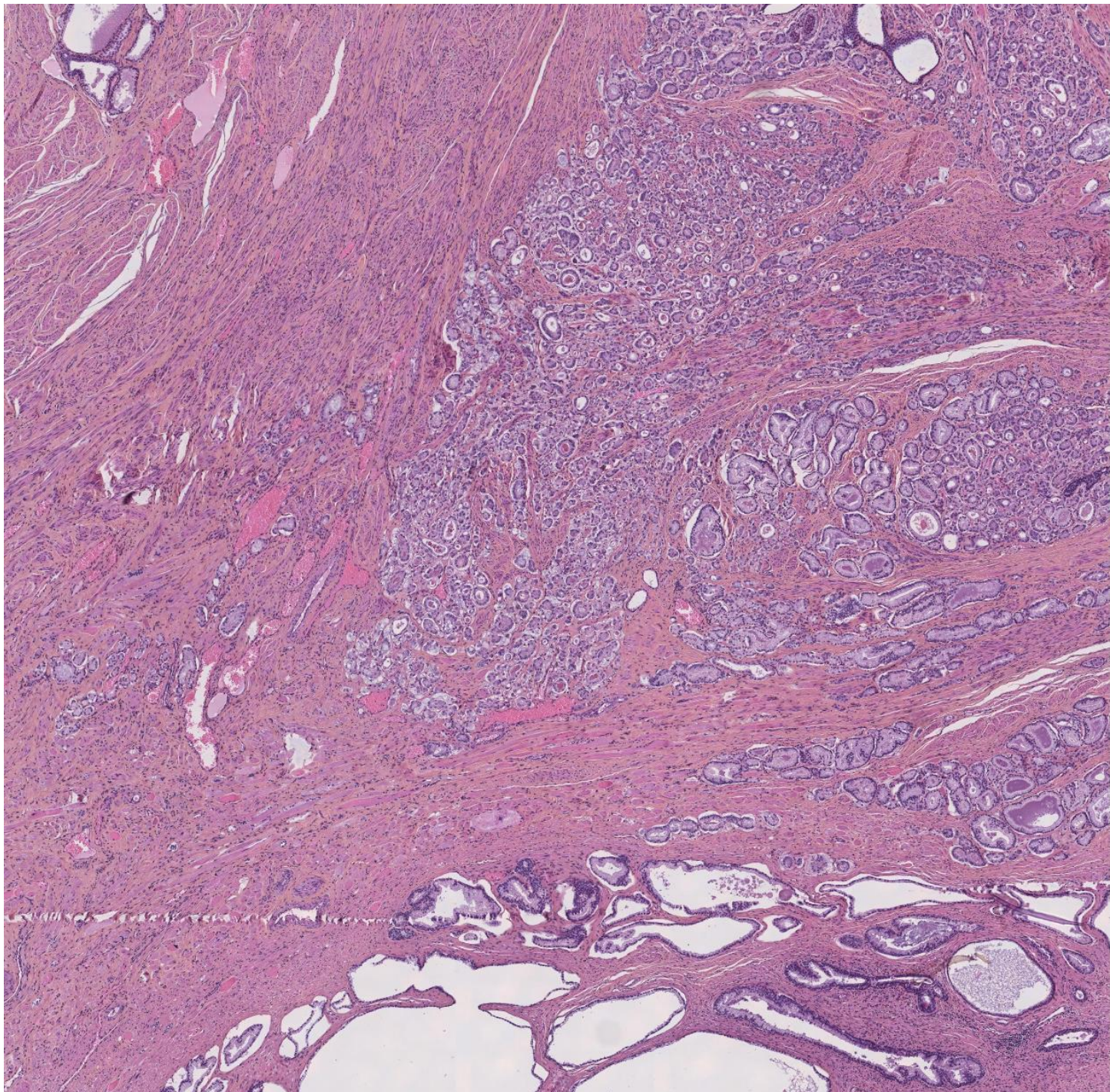

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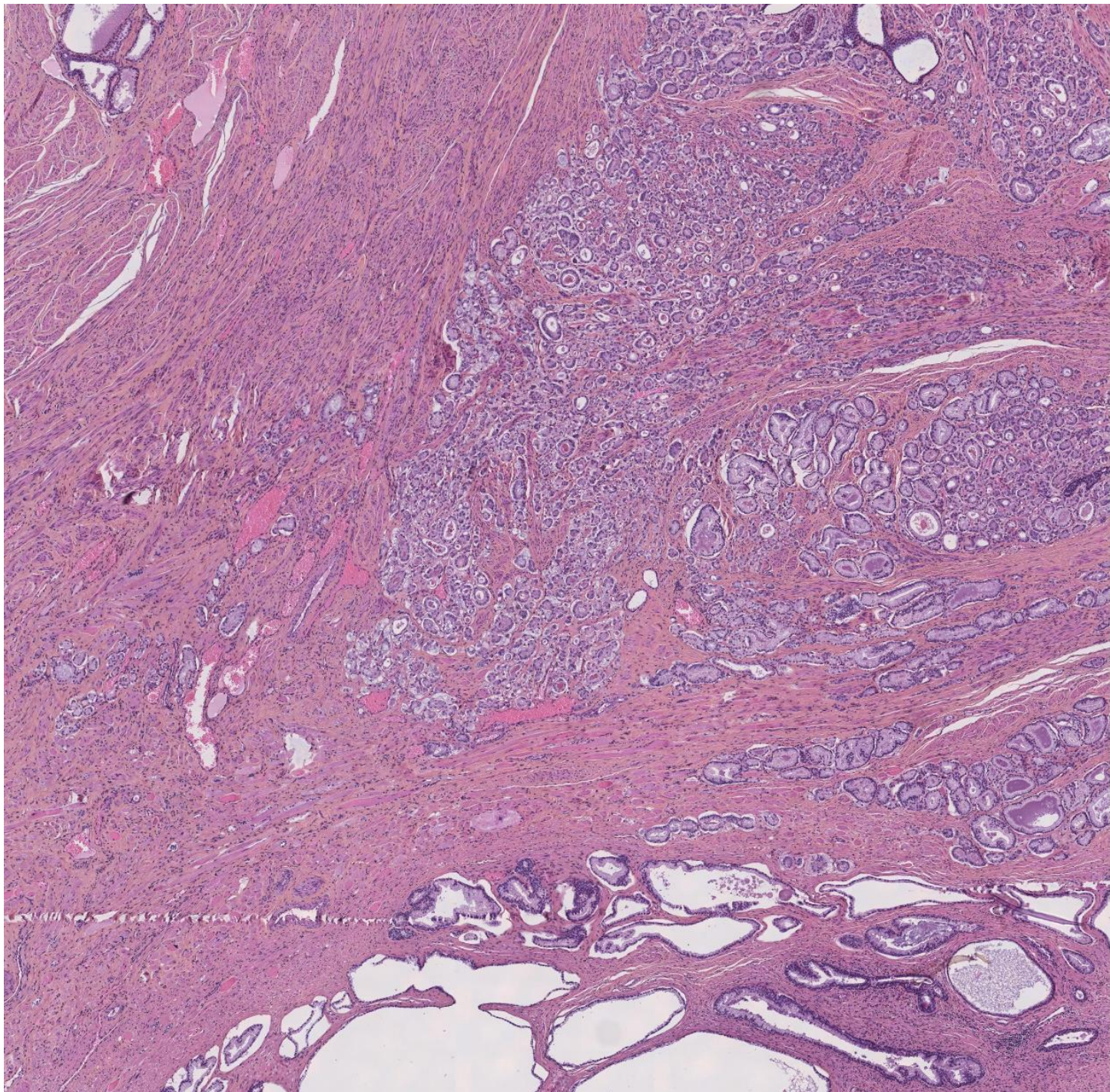
- **Open** Prostate1.ome.tif
- Create **different types** of annotations
- Play with **resolution**
- Look at the **measurements** for each **type of annotation**



Stain Estimation

- **Hematoxylin** stains **nuclei** in purple/blue
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- **Automatic** stain estimation in **QuPath** is based on:

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

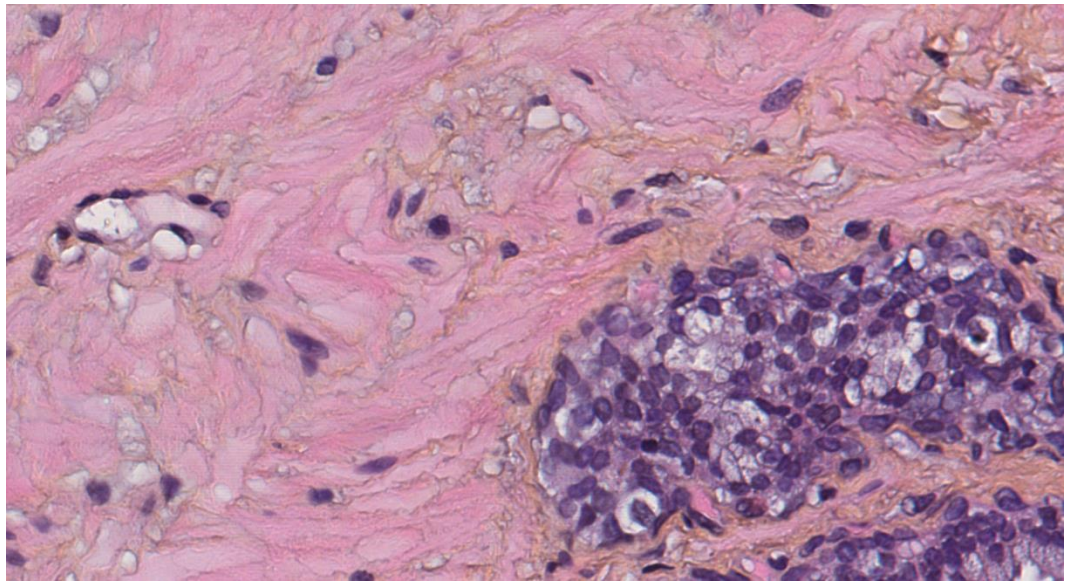
Quantification of histochemical staining by color deconvolution

A C Ruifrok [1], D A Johnston

Affiliations + expand

PMID: 11531144

H&E Stain Estimation



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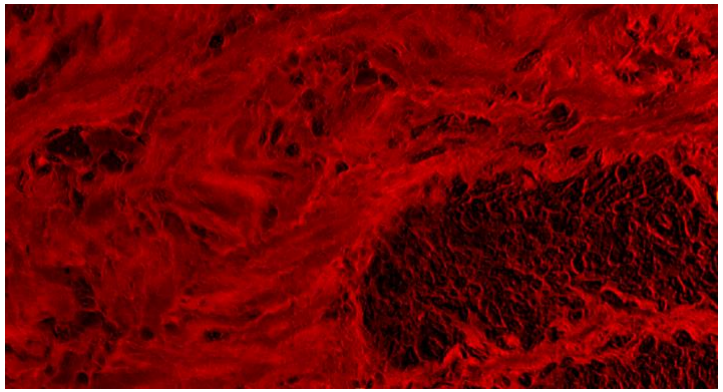
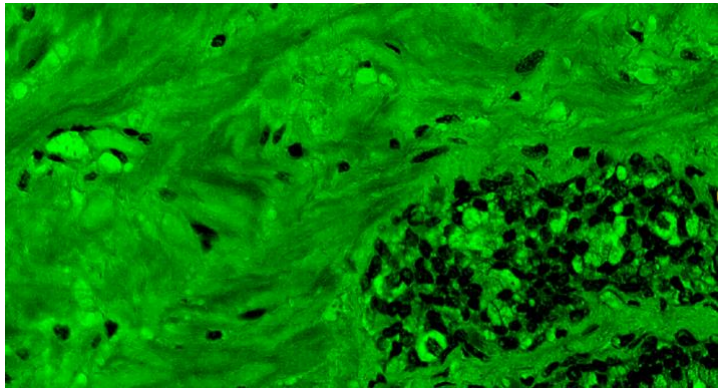
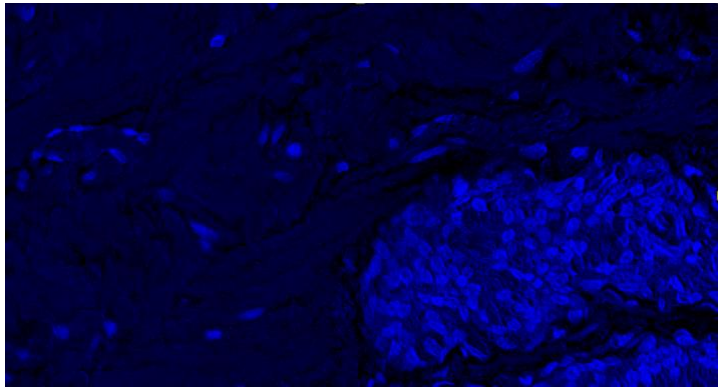
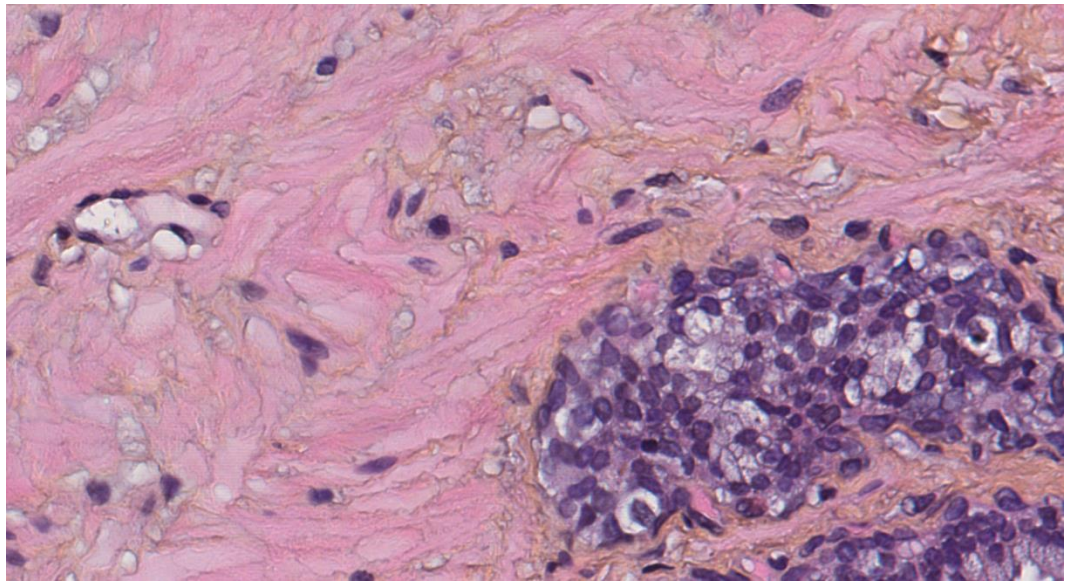






H&E Stain Estimation



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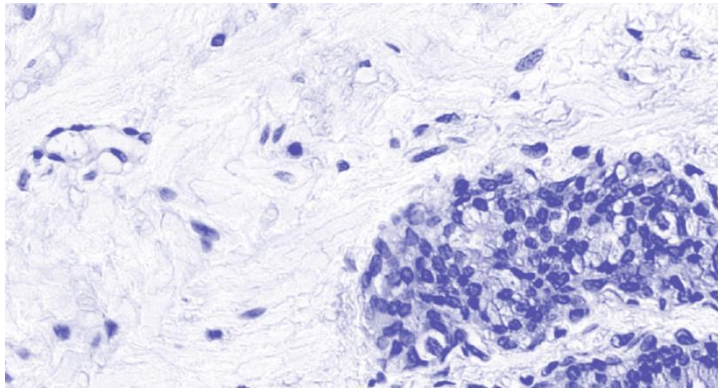
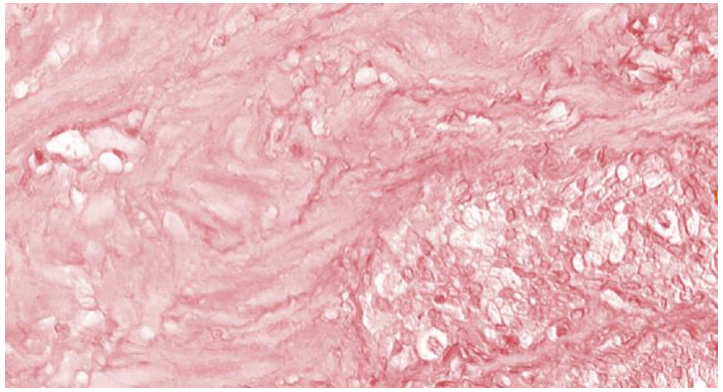
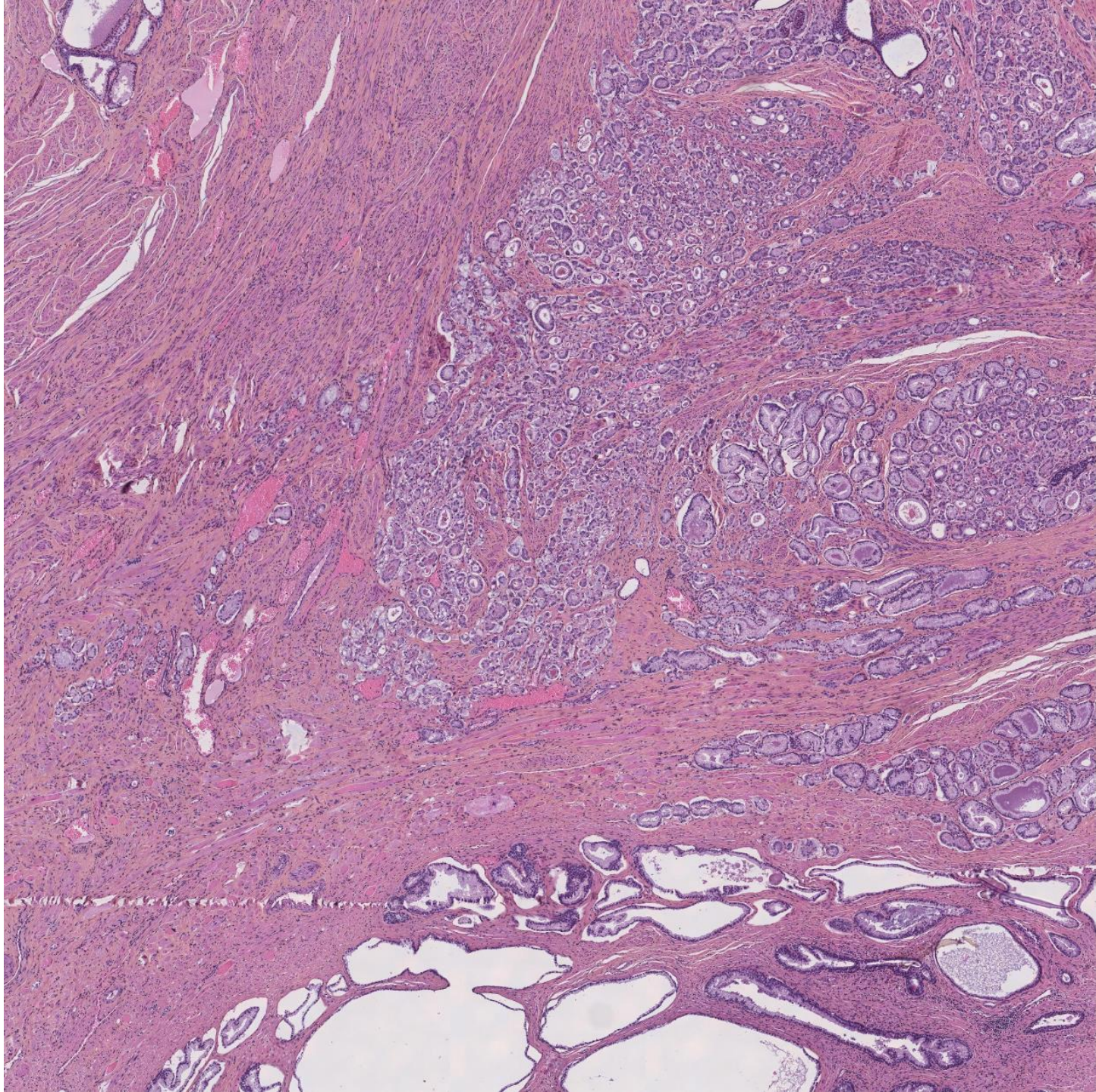


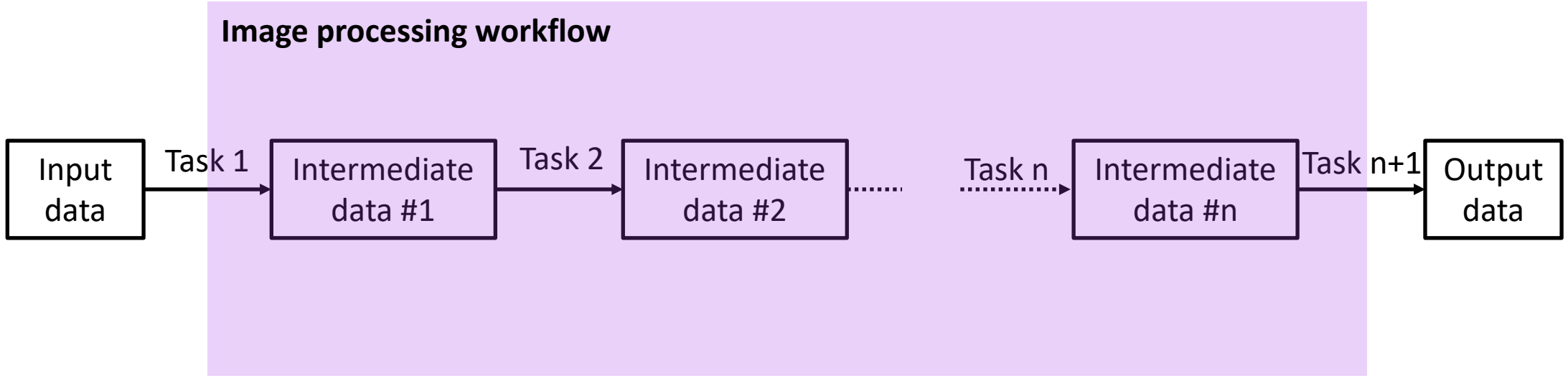


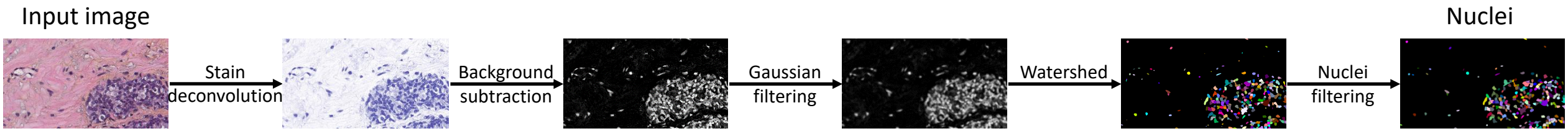
H&E Stain Estimation

- **Open** Prostate_1.ome.tif
- Create a small rectangle annotation and **estimate stain vectors**
- **Manually define Hematoxylin** and **Eosin** components
- **Visualize** the differences

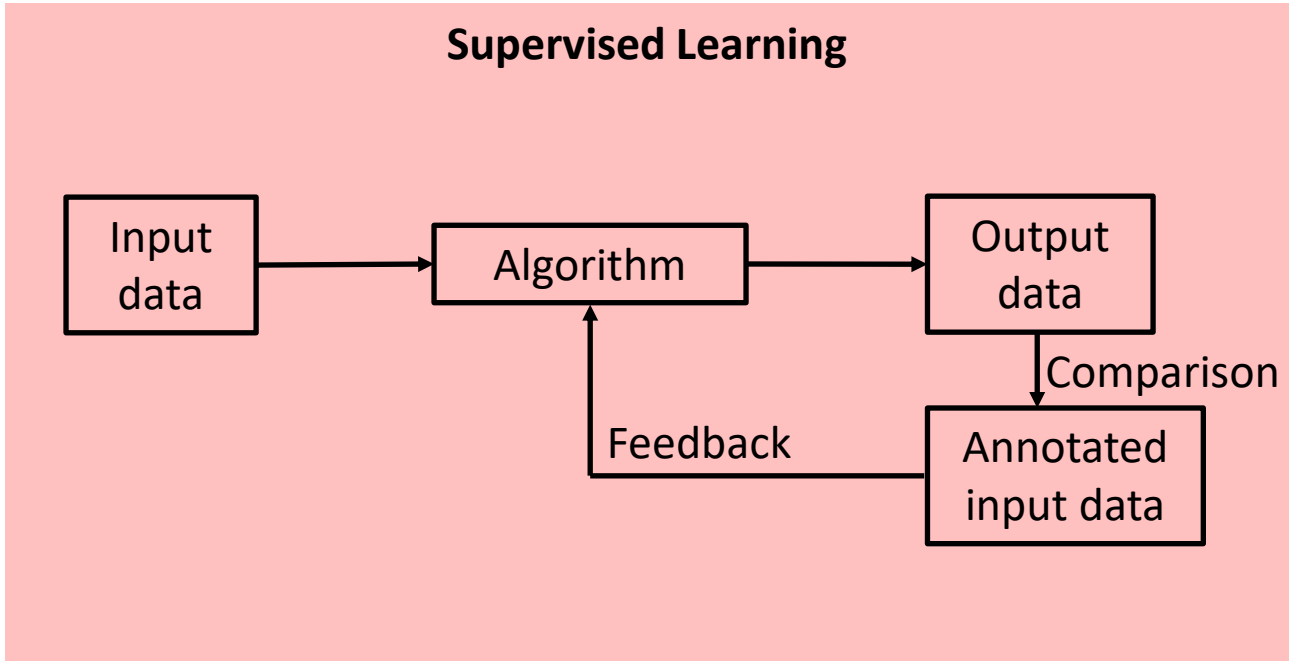


EXPLICIT PROGRAMMING





Supervised Machine Learning



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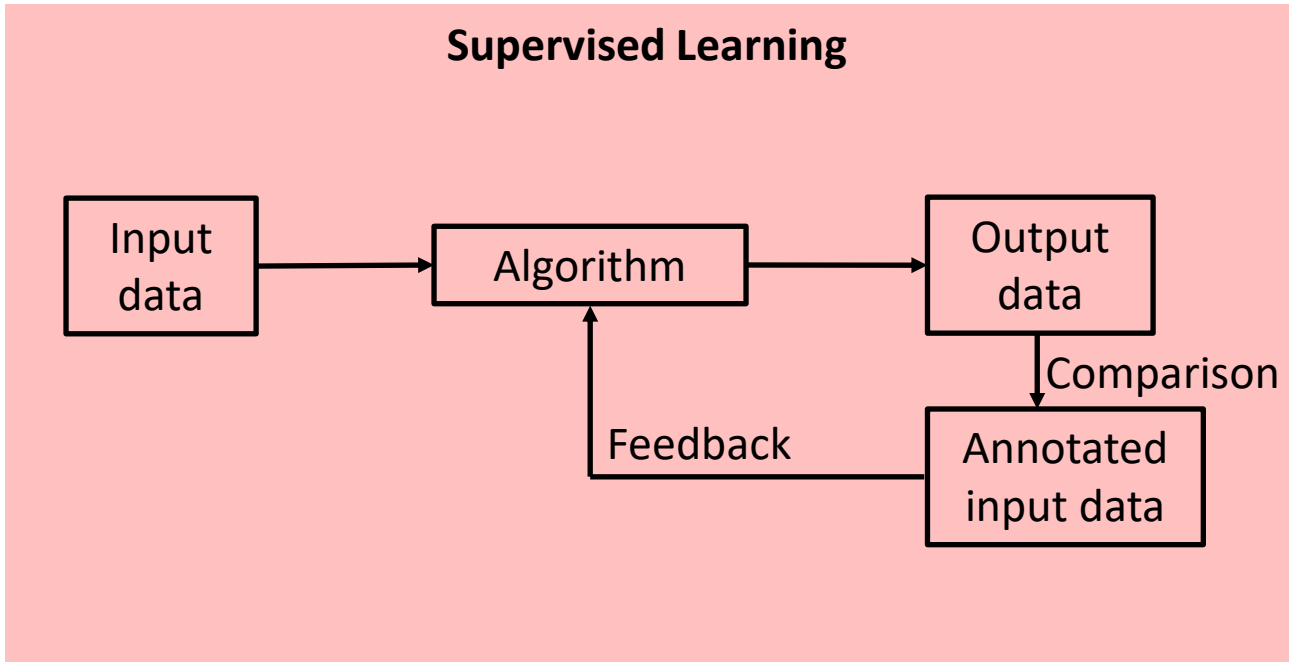

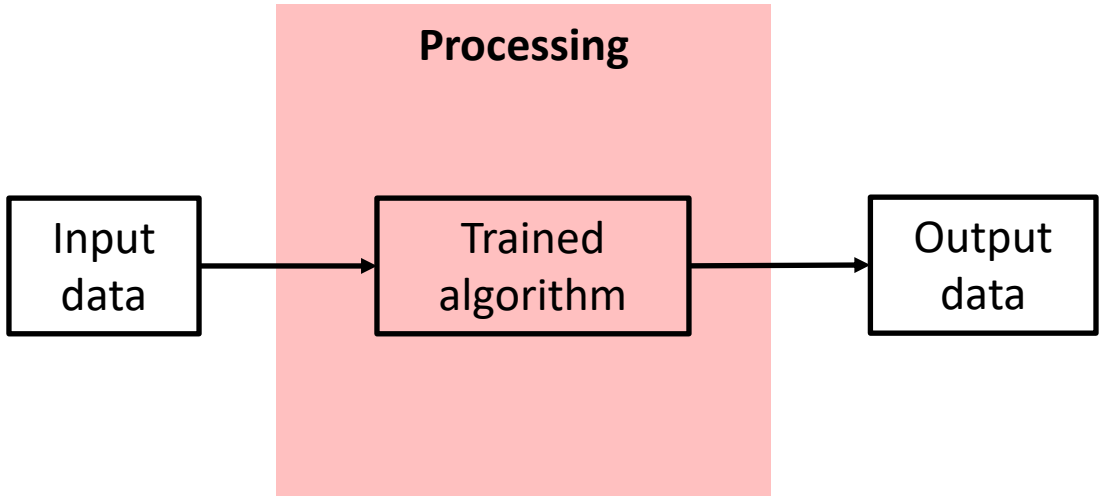

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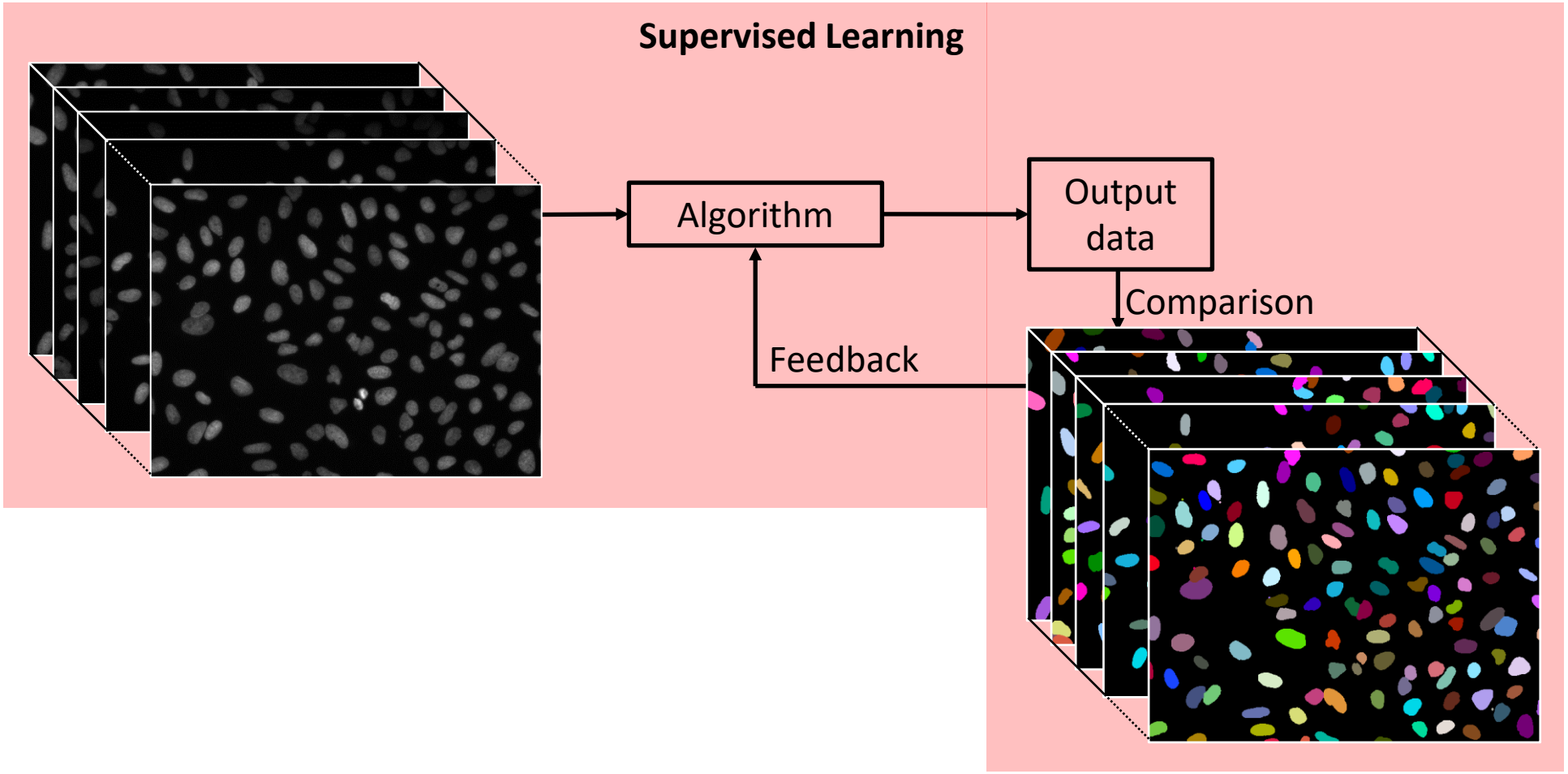

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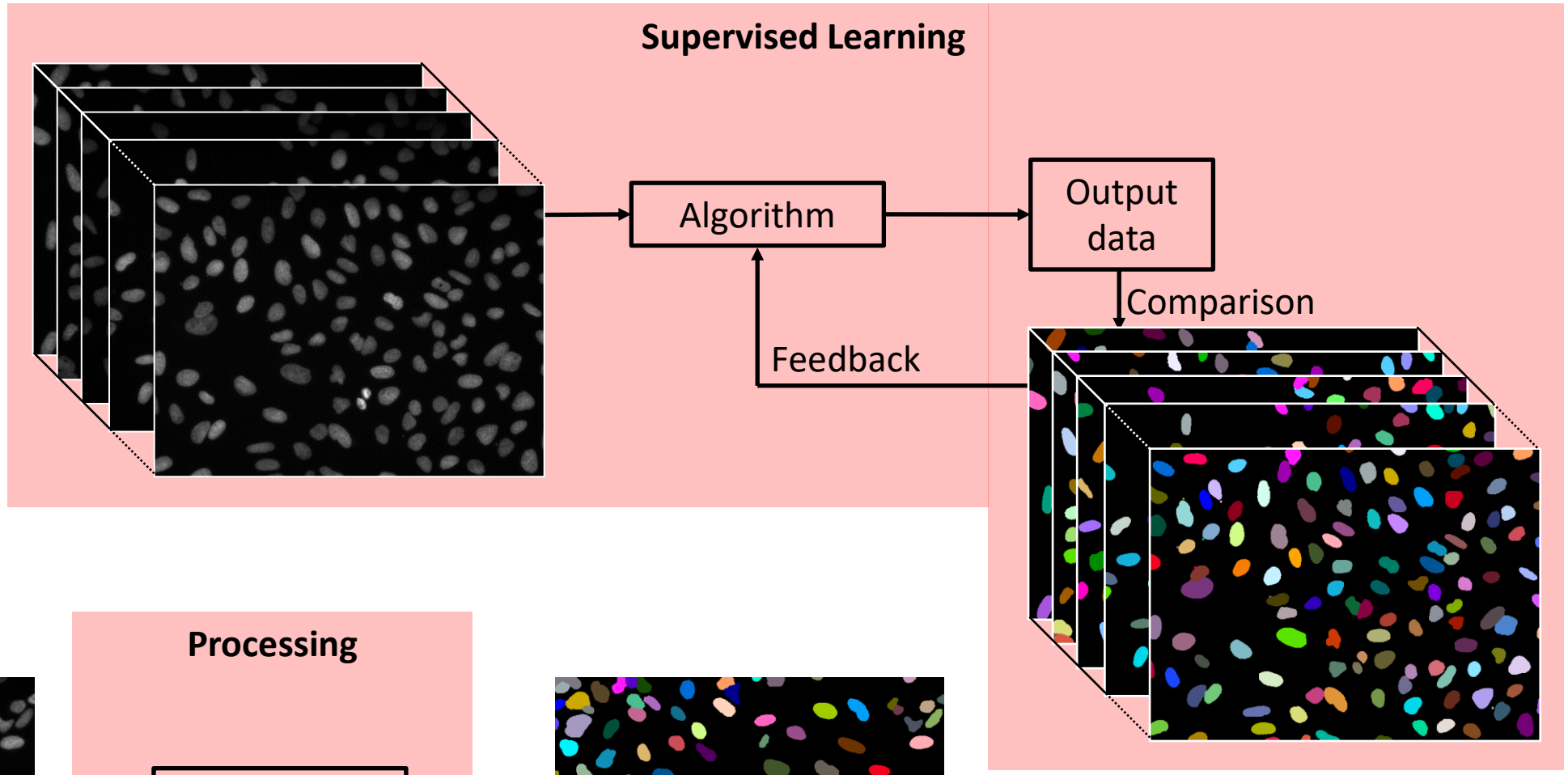
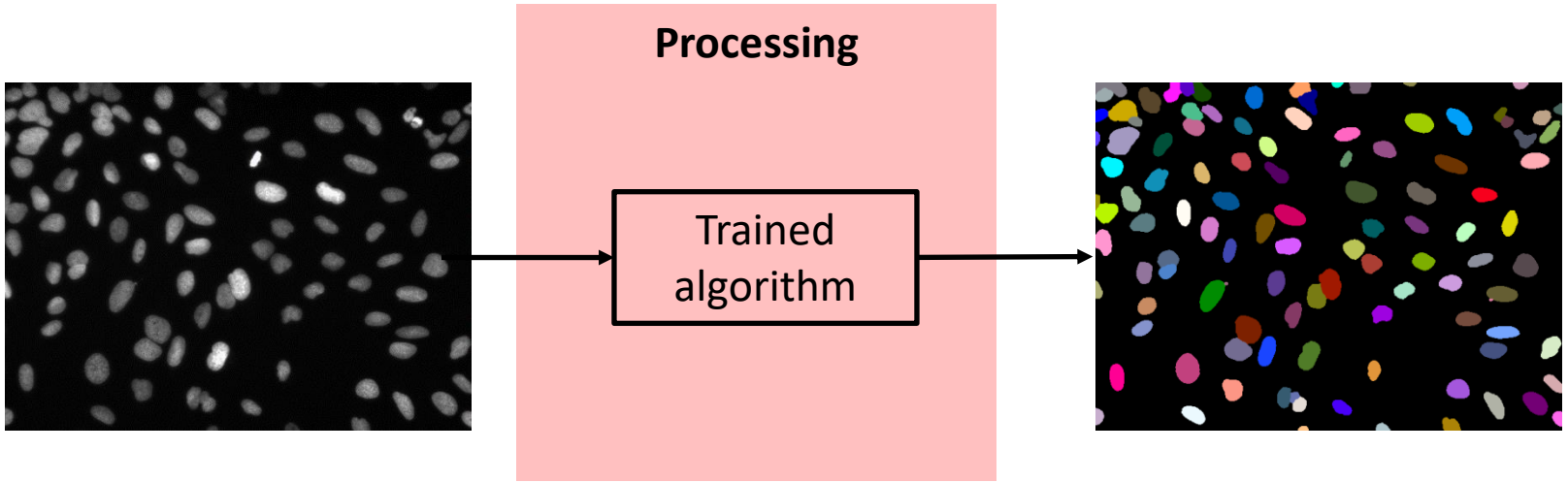


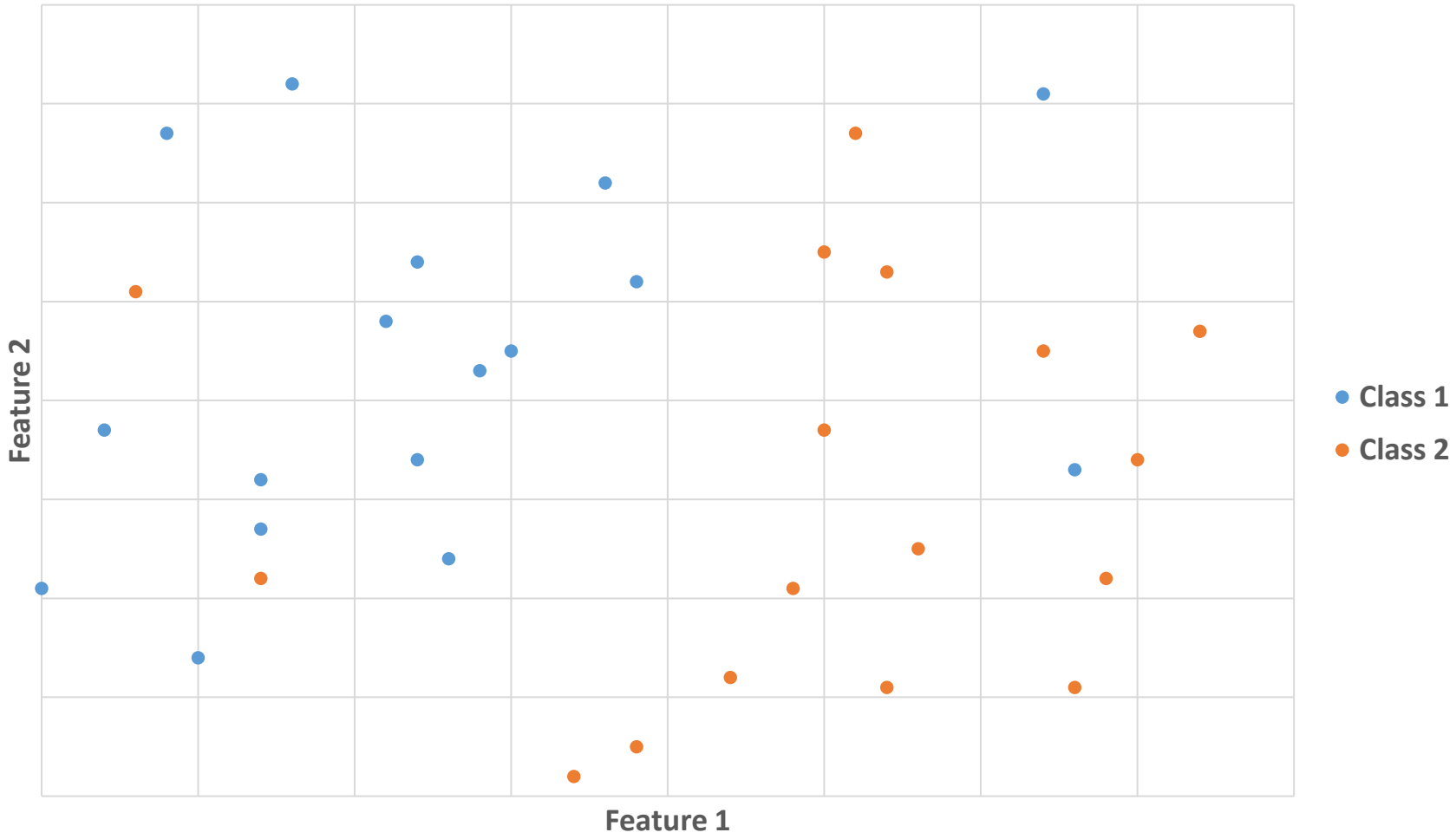


Shallow Machine Learning for Pixel Classification

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

Shallow Machine Learning for Pixel Classification



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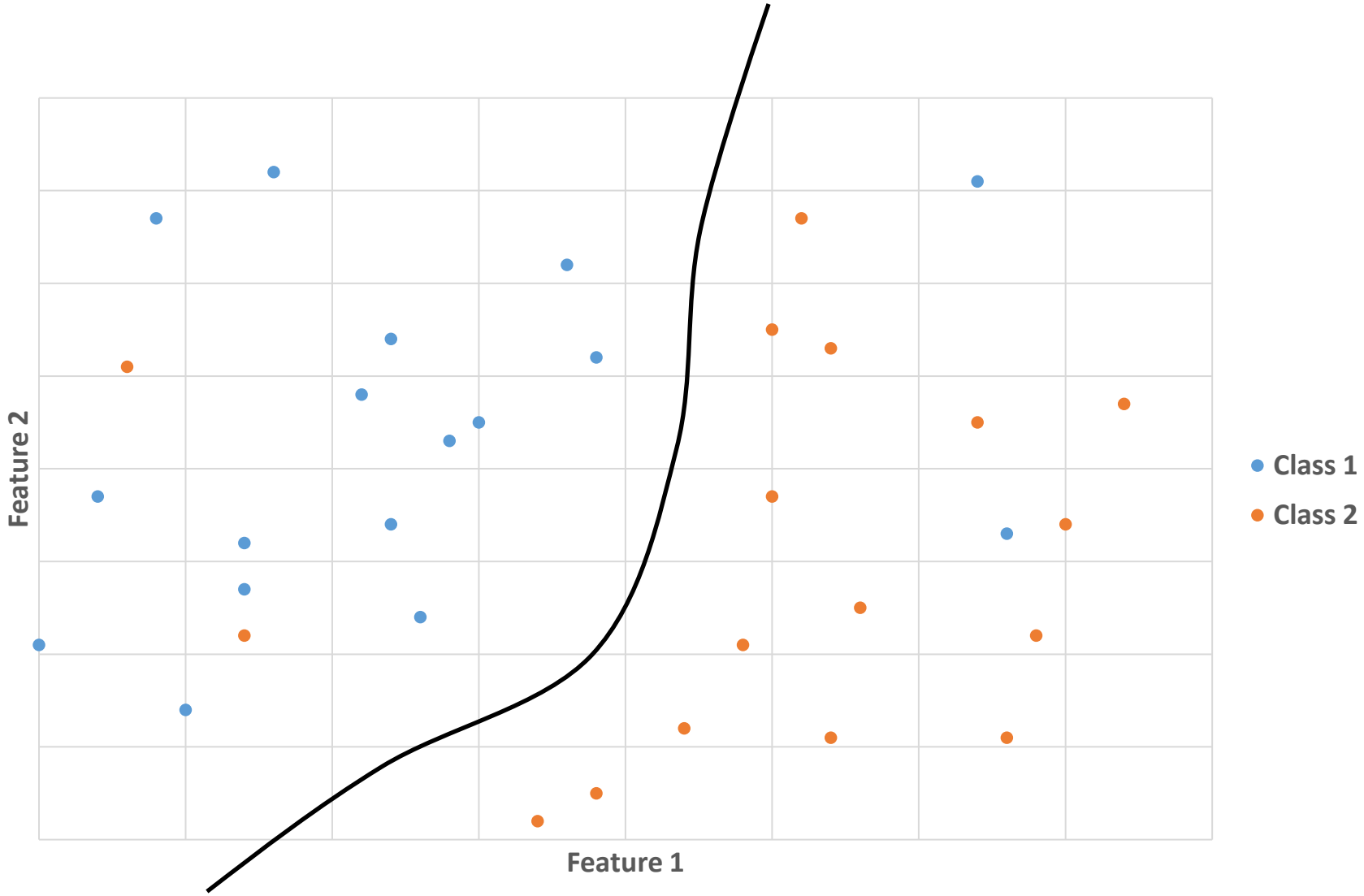
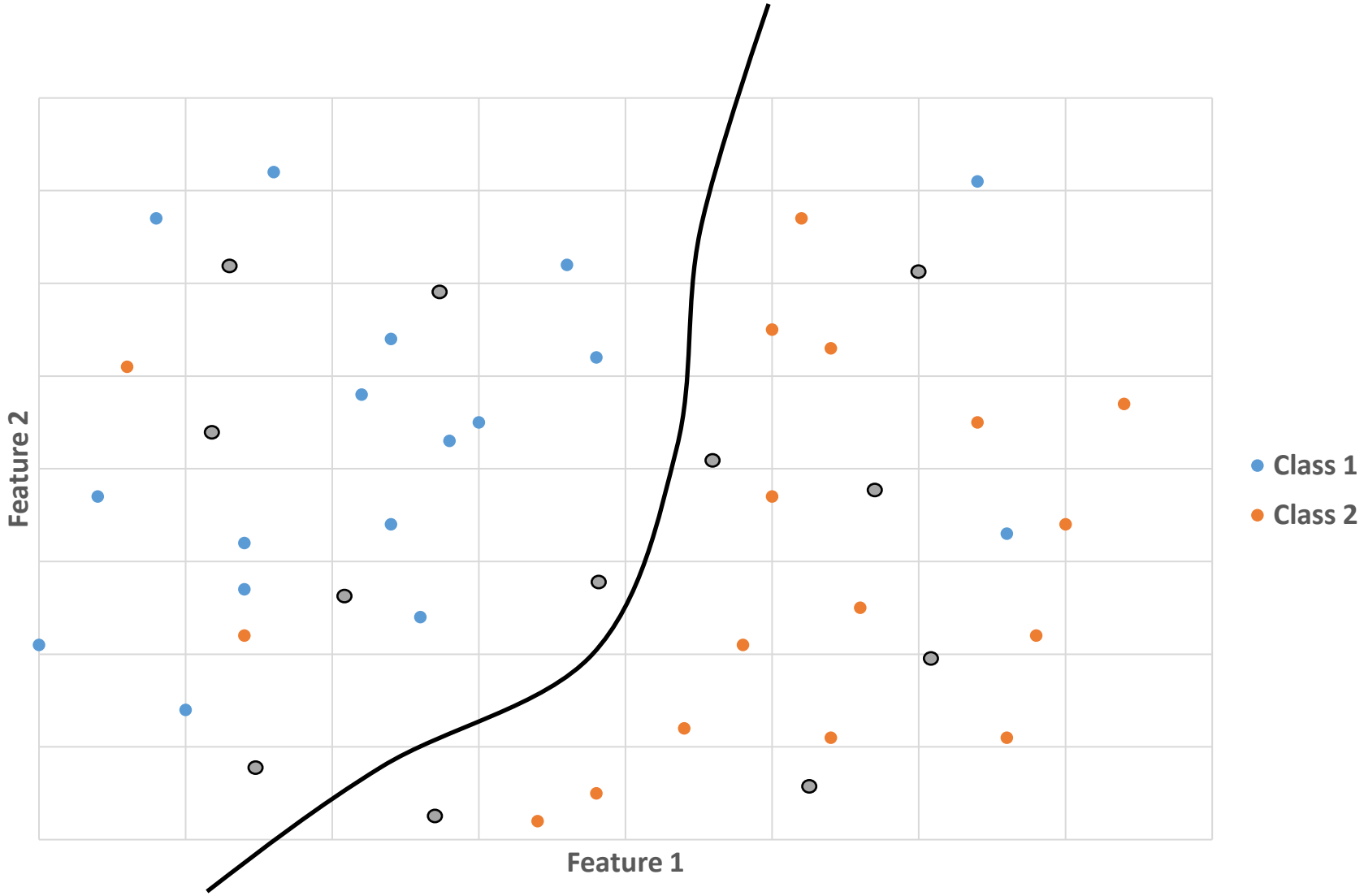


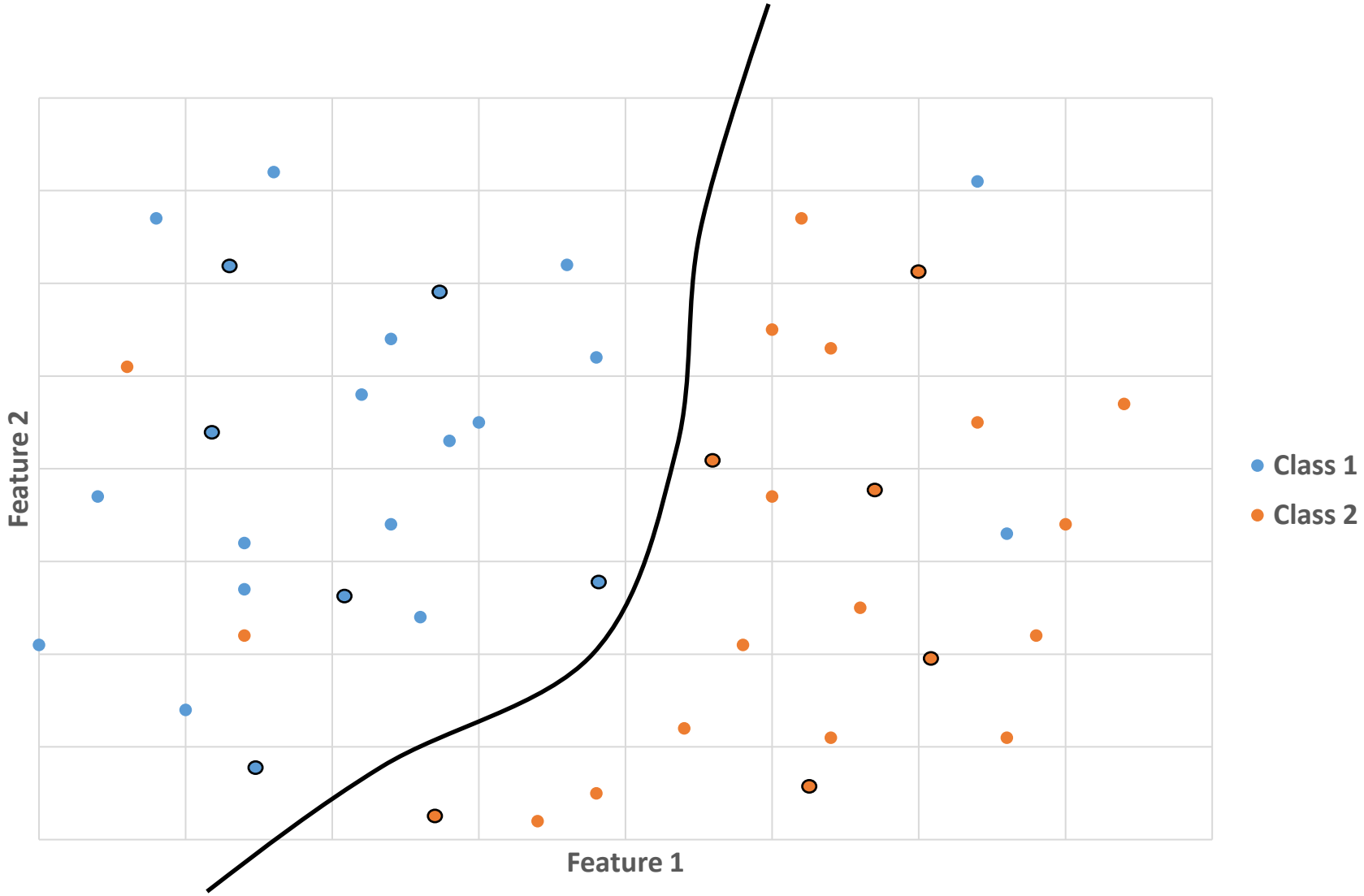
Training a classifier consists in **estimating** the **"best" separation** between classes

Shallow Machine Learning for Pixel Classification



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

Shallow Machine Learning for Pixel Classification



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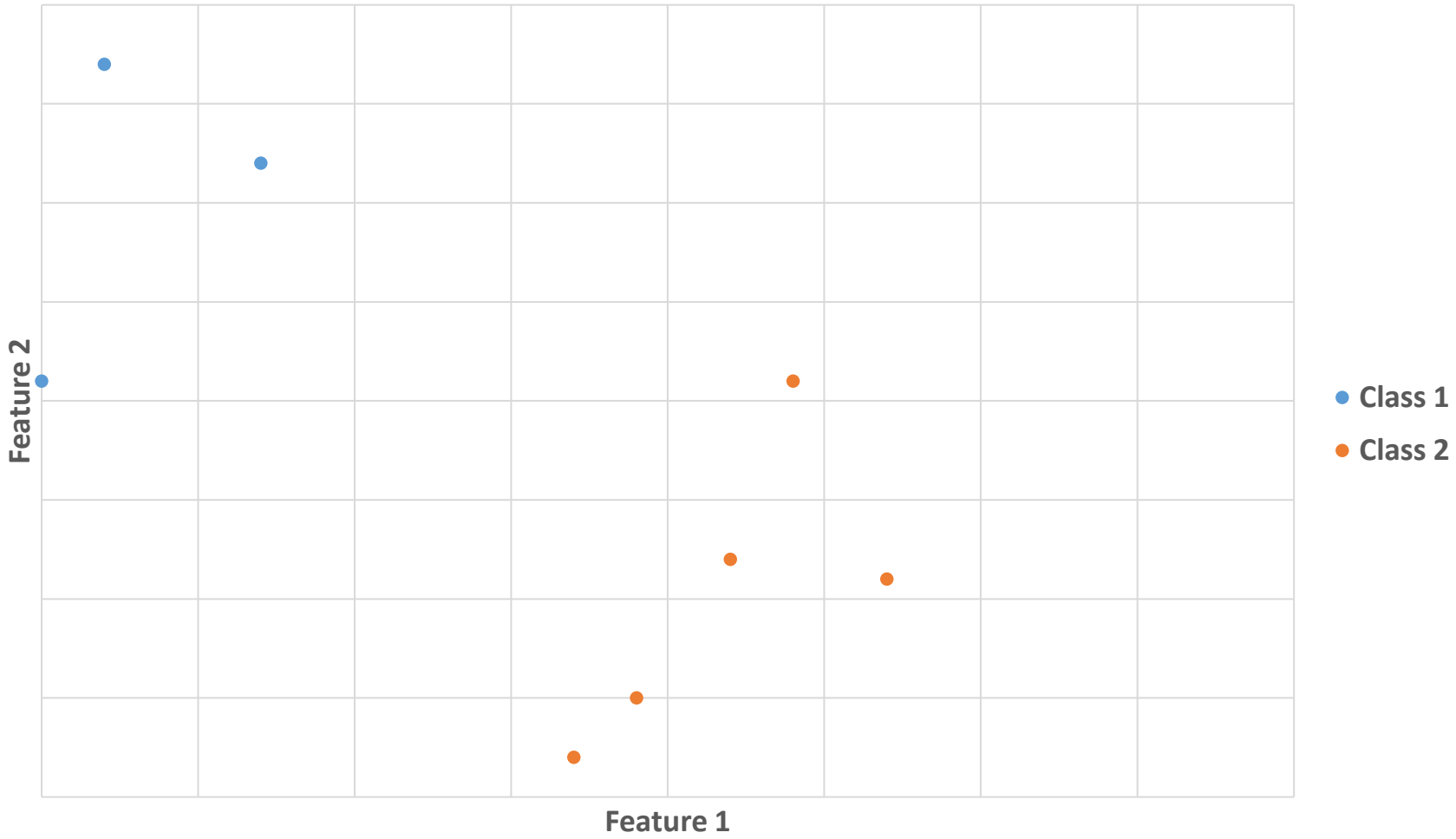
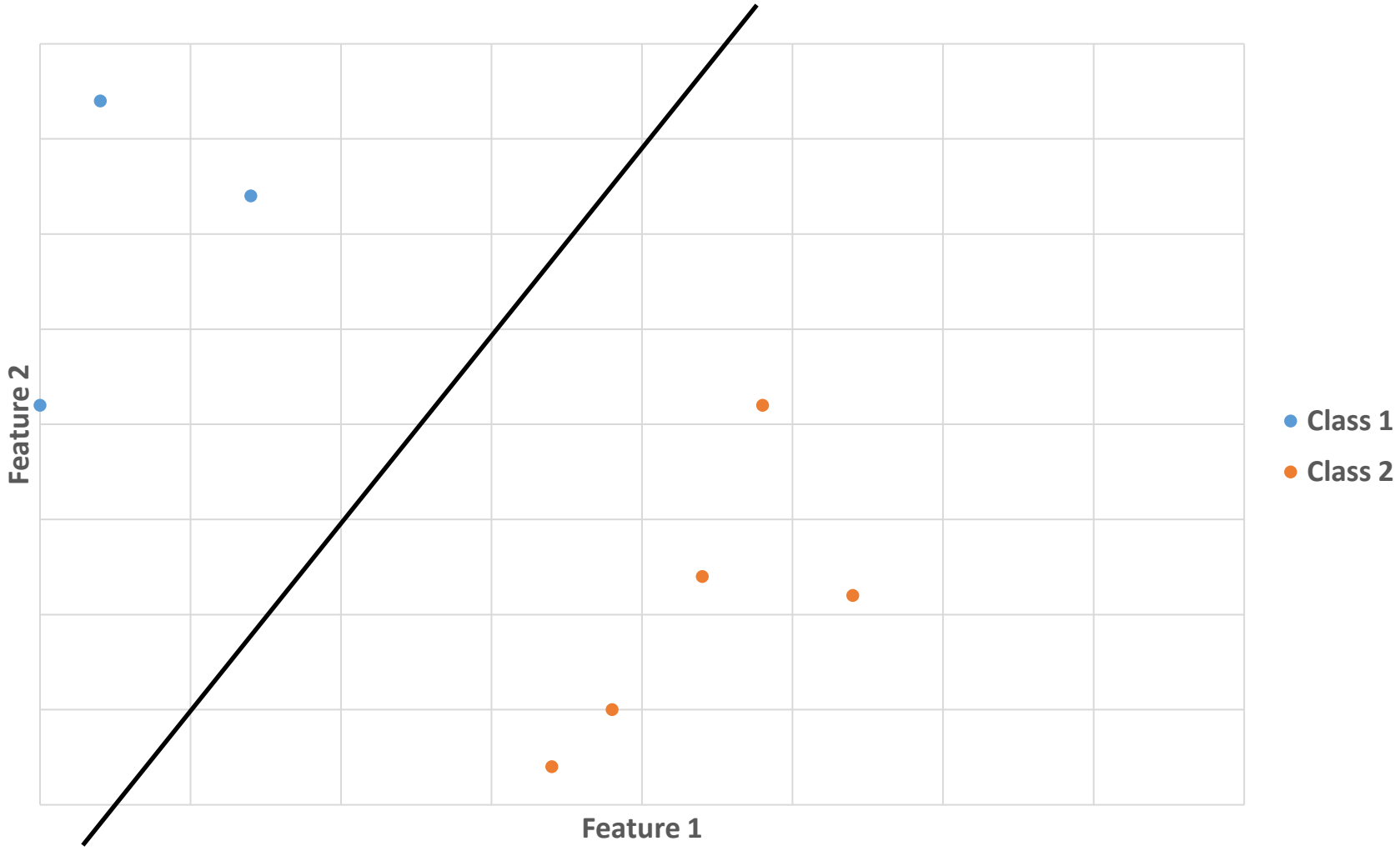
Shallow Machine Learning for Pixel Classification



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

Shallow Machine Learning for Pixel Classification



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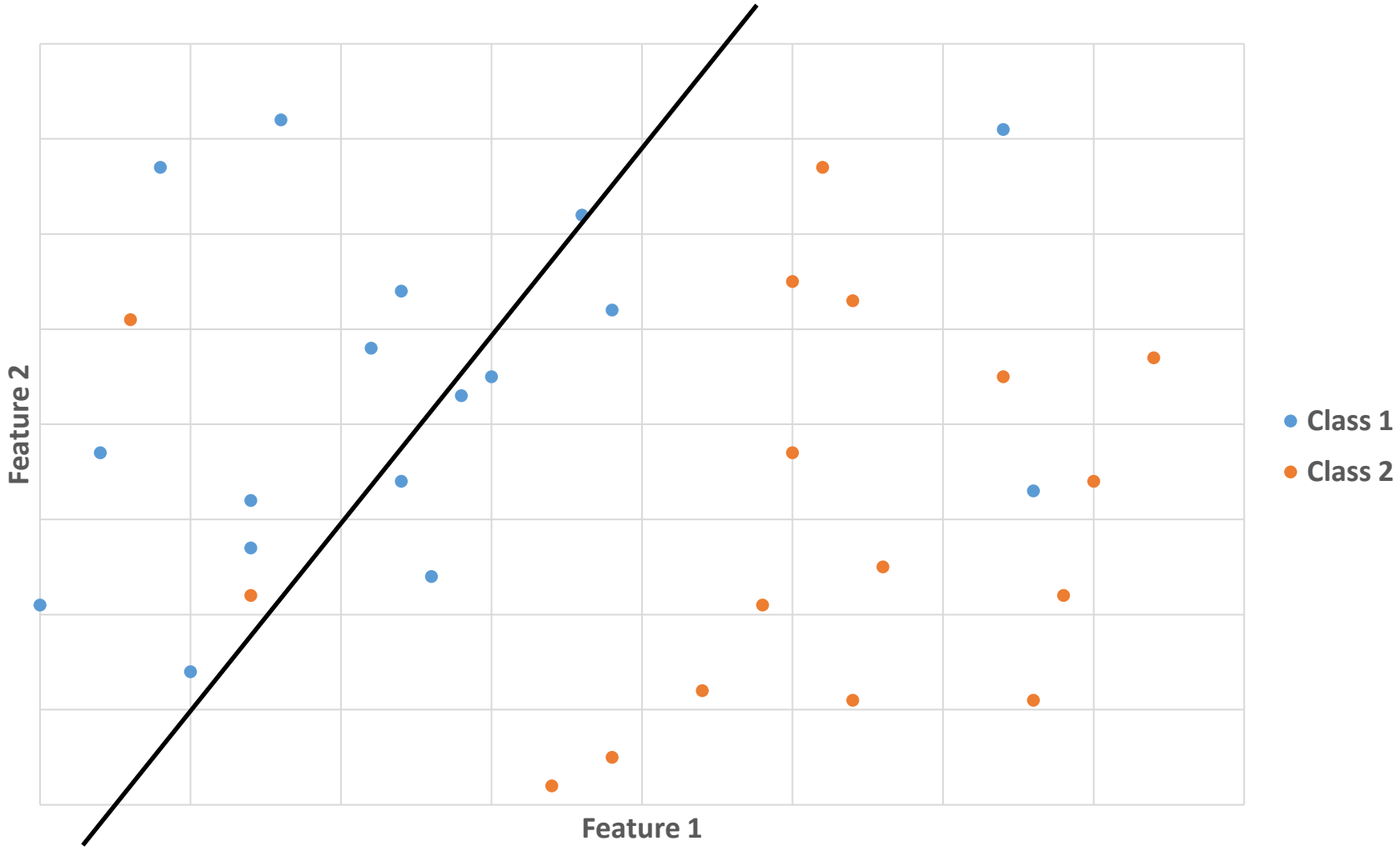
Shallow Machine Learning for Pixel Classification



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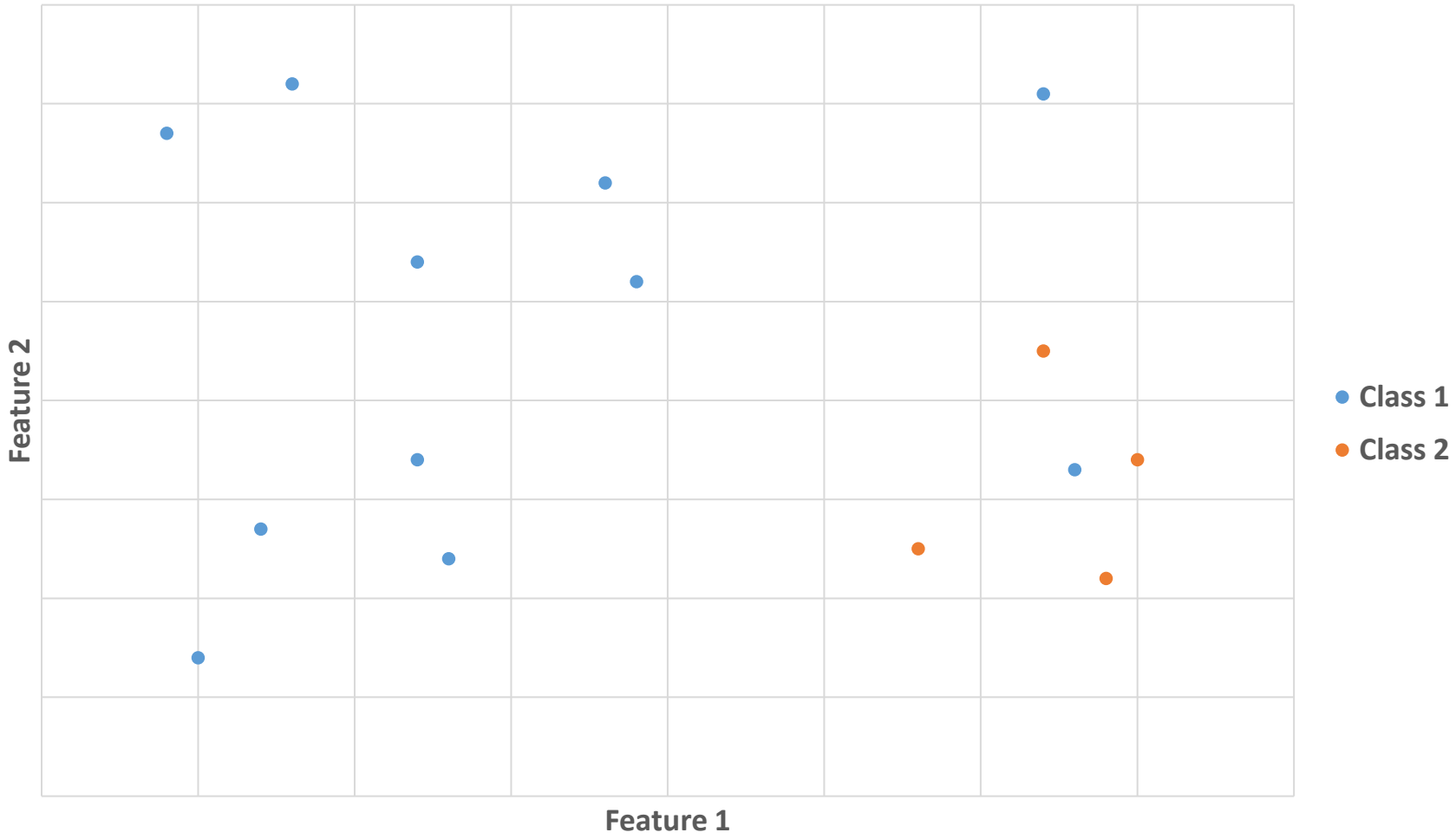
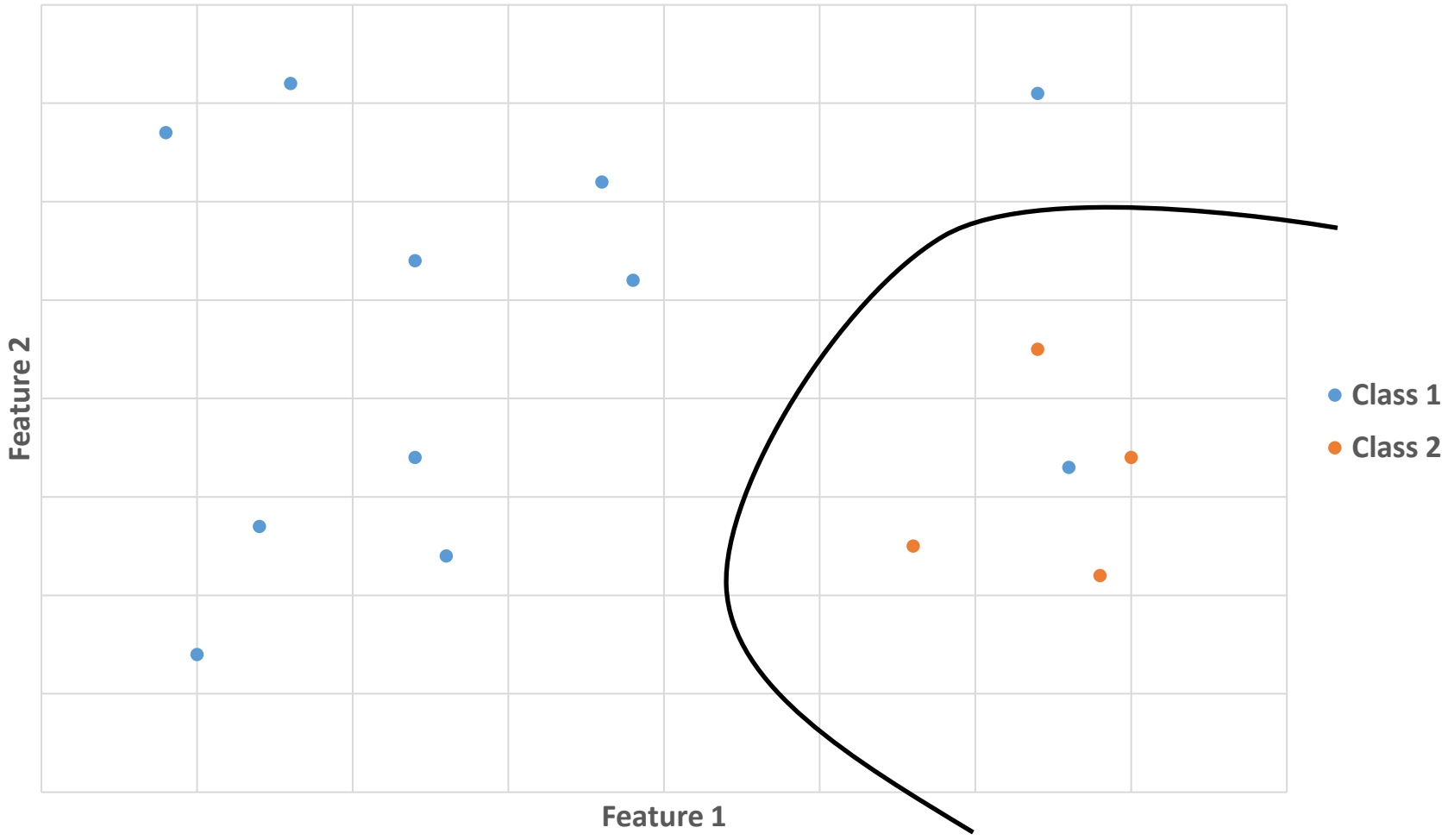
Shallow Machine Learning for Pixel Classification



Class imbalance makes the training **more difficult**

Shallow Machine Learning for Pixel Classification



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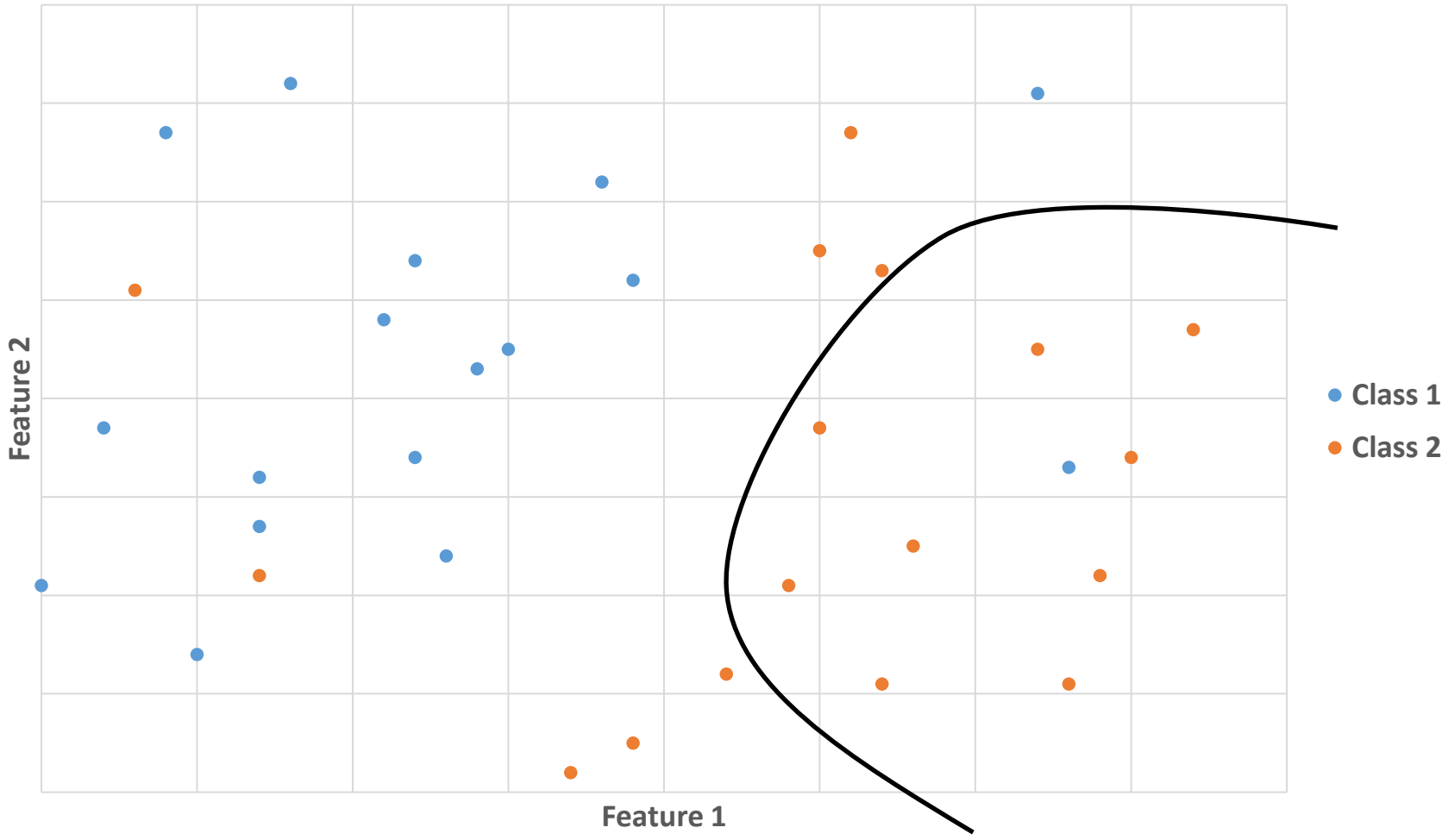
Shallow Machine Learning for Pixel Classification



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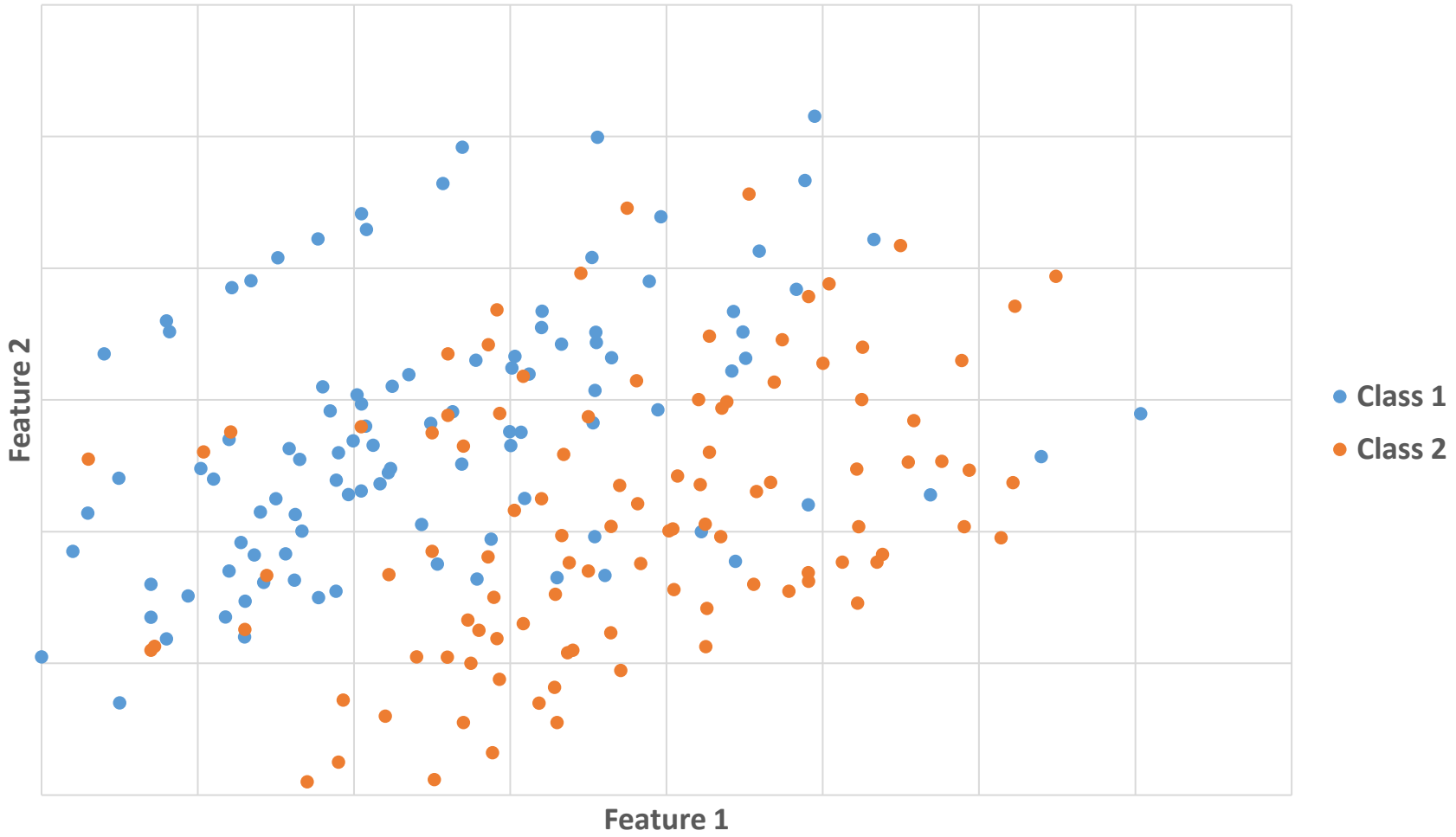
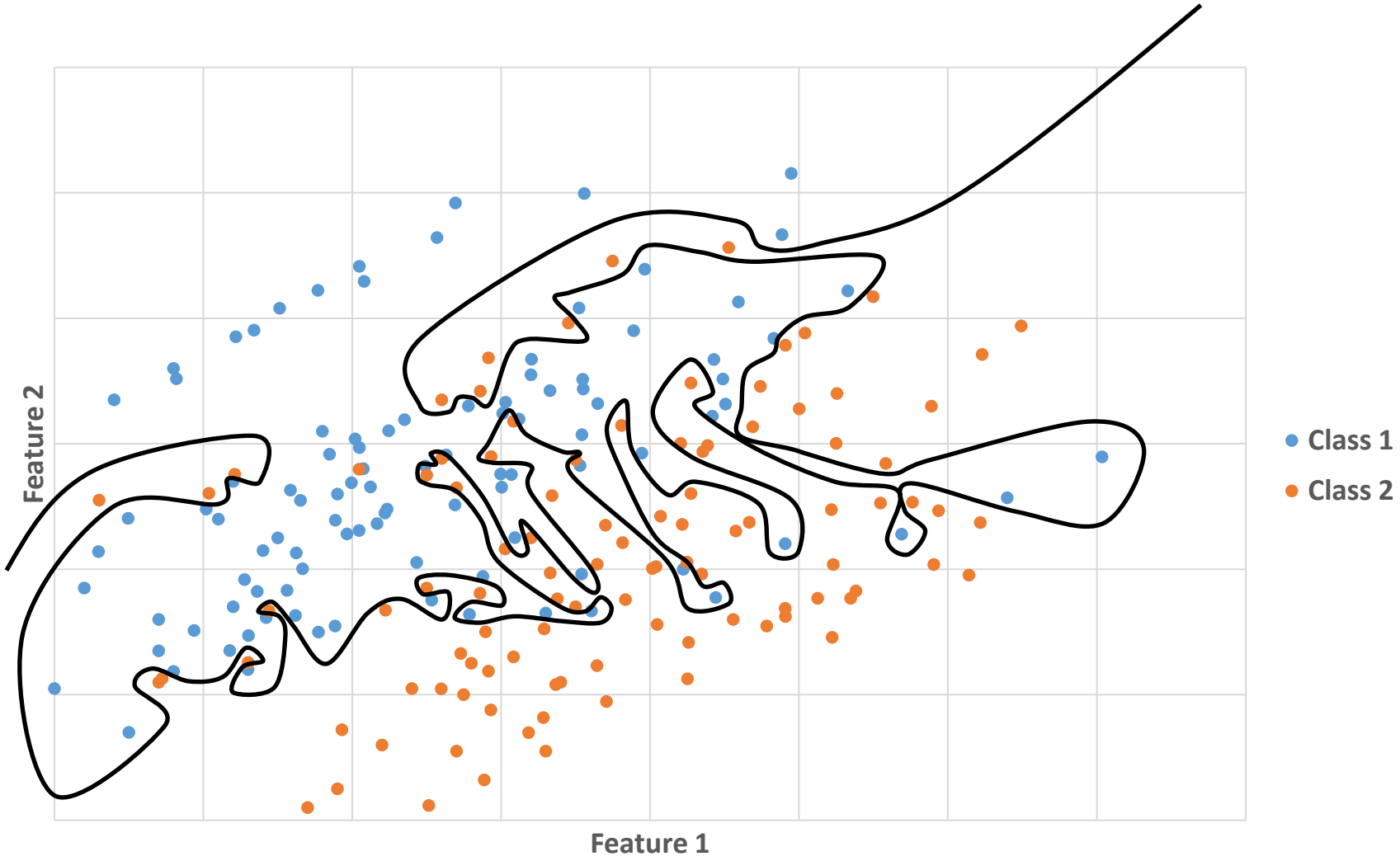


Too many annotations can lead to **over-fitting**: works great on training data but poorly on new data

Shallow Machine Learning for Pixel Classification



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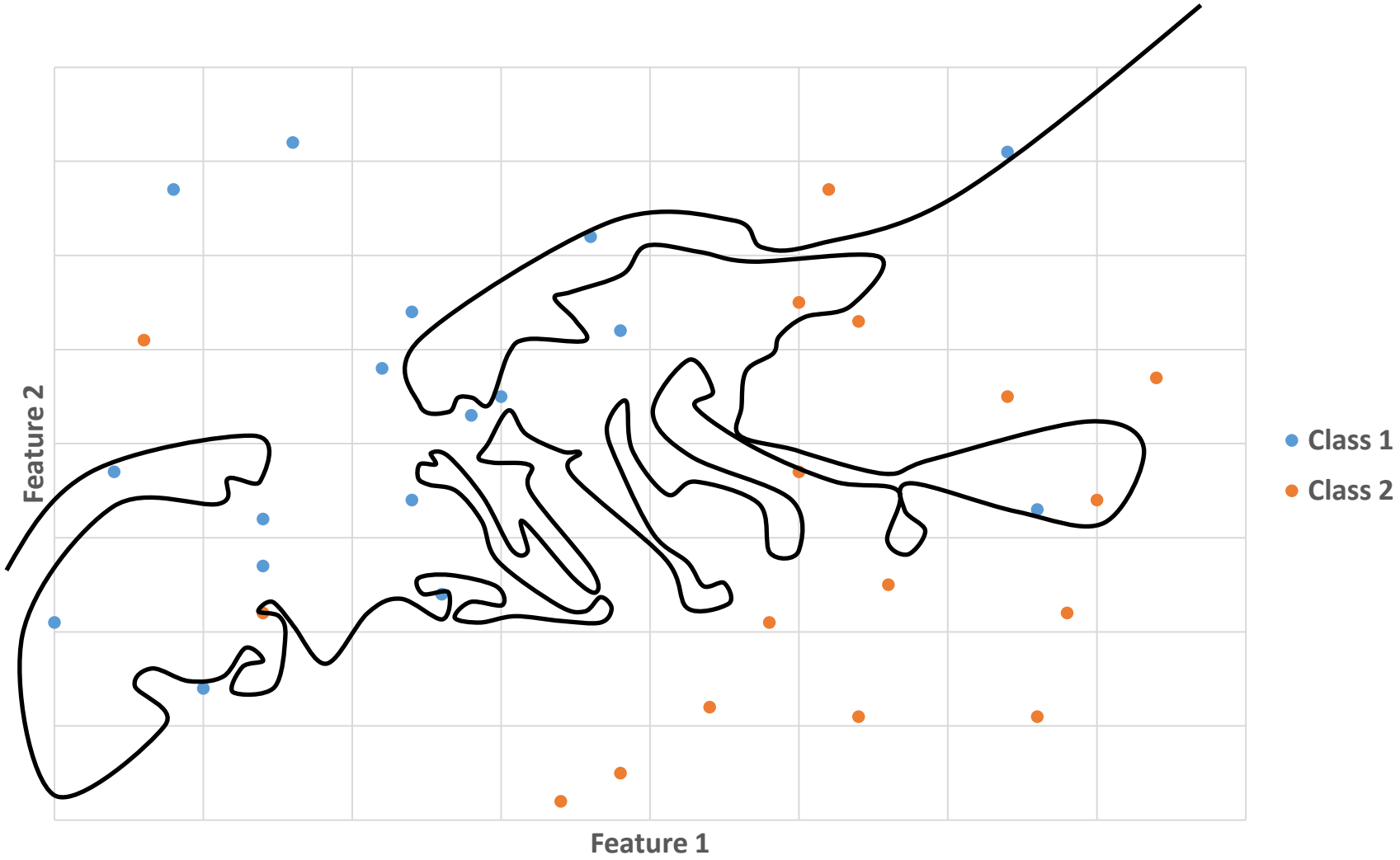
Shallow Machine Learning for Pixel Classification



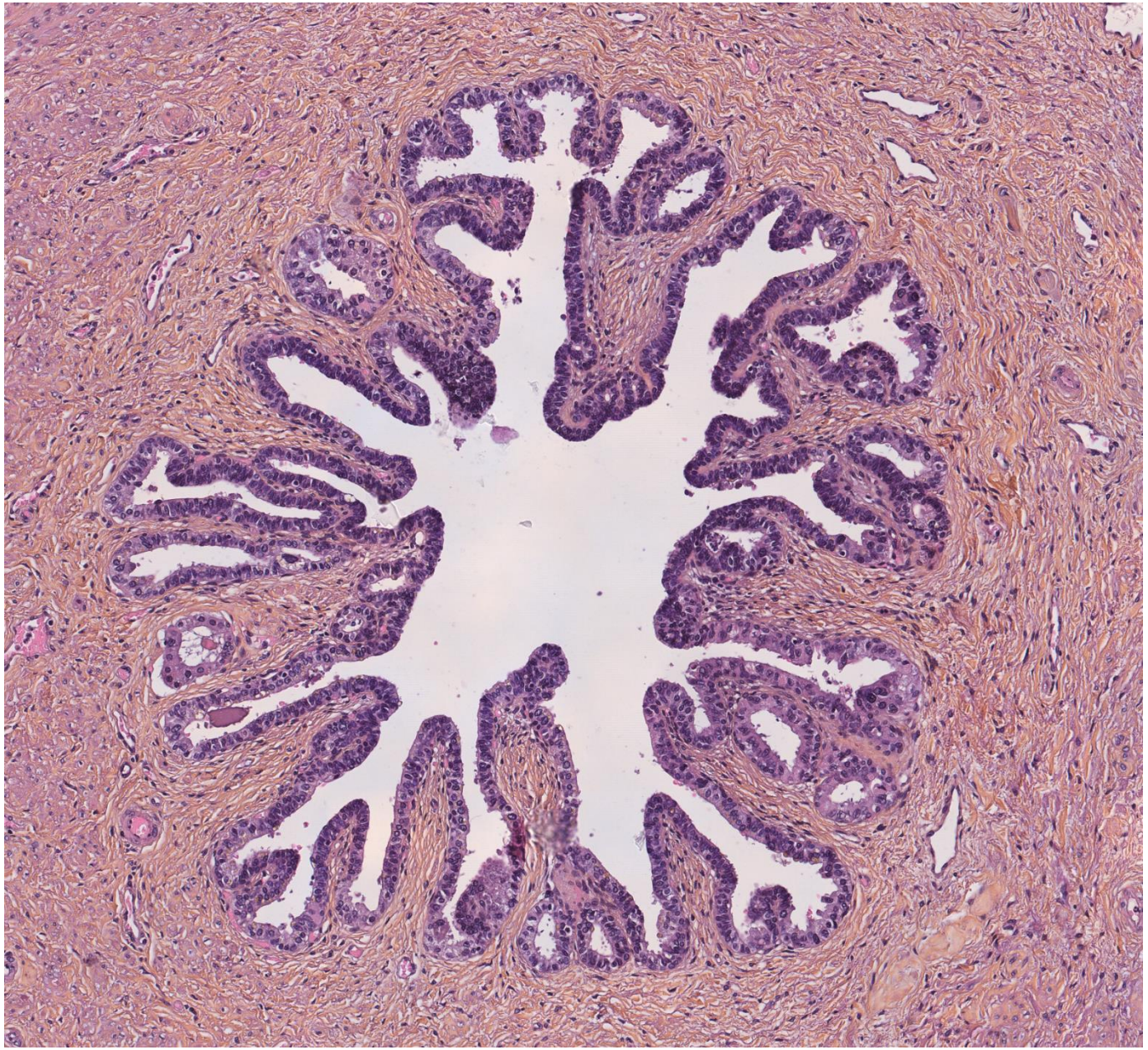
Too many annotations can lead to **over-fitting**: works great on training data but poorly on new data

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**

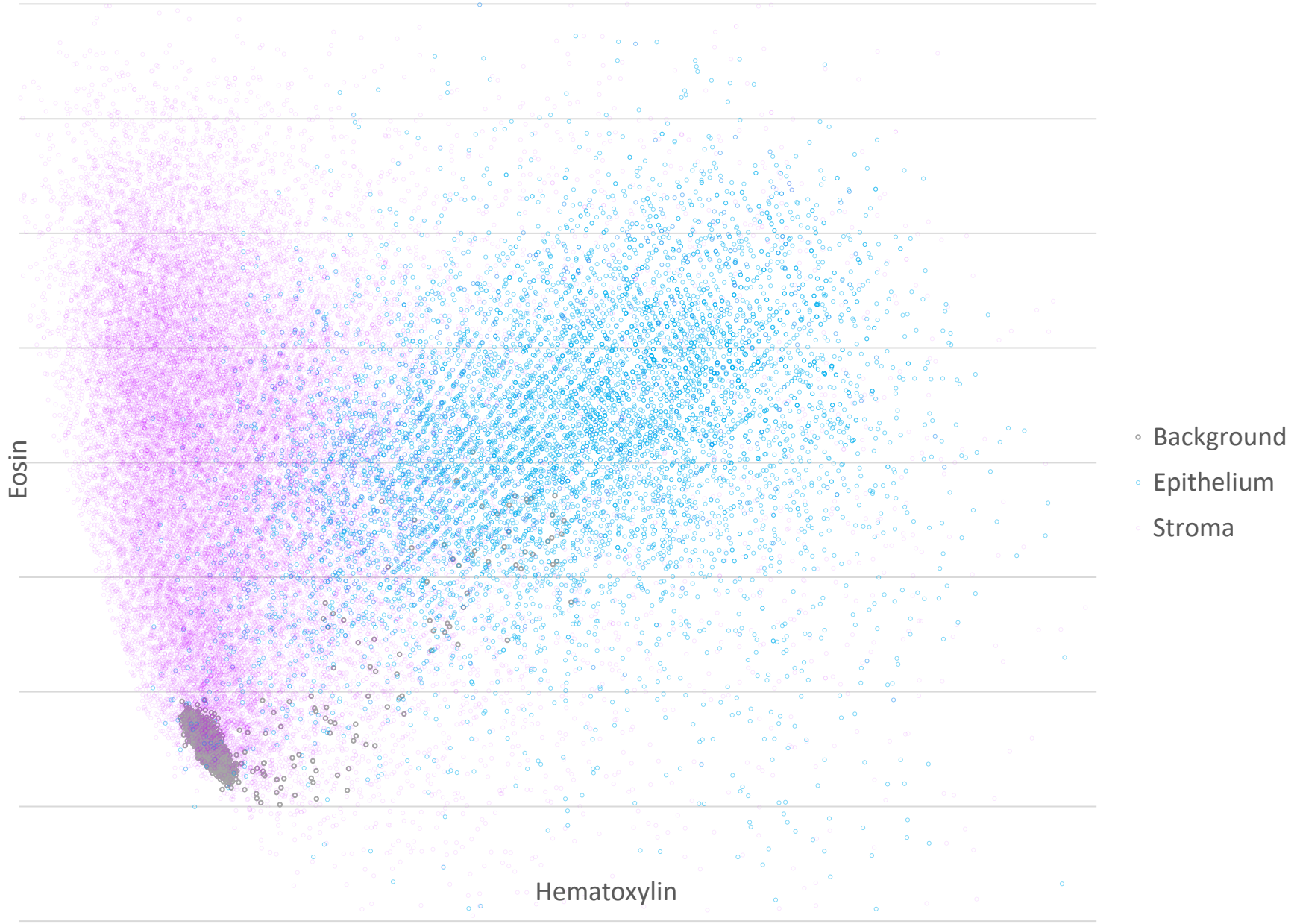


Pixel Classification

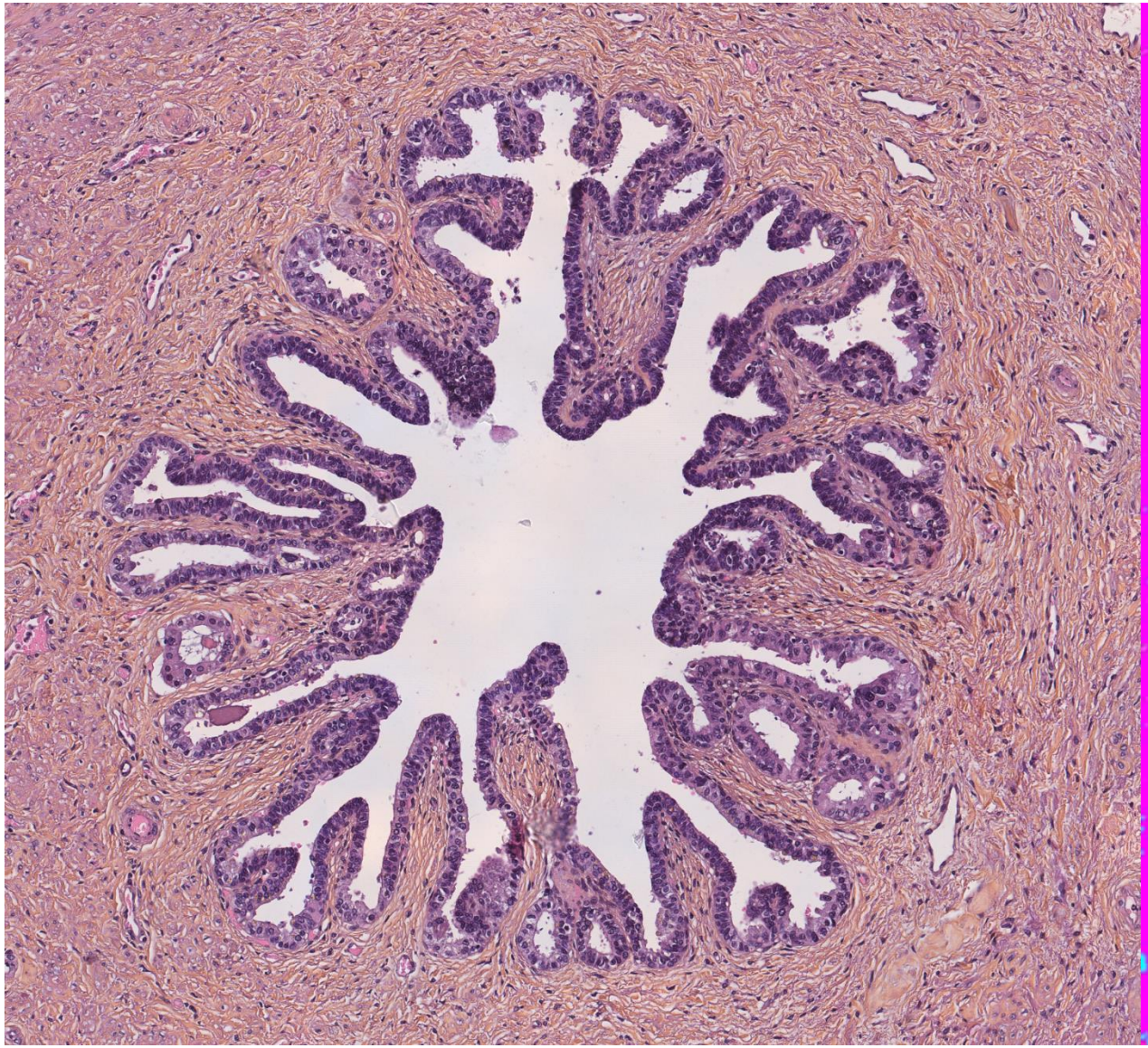
Find **regions** corresponding to **epithelium**, **stroma** and **background**

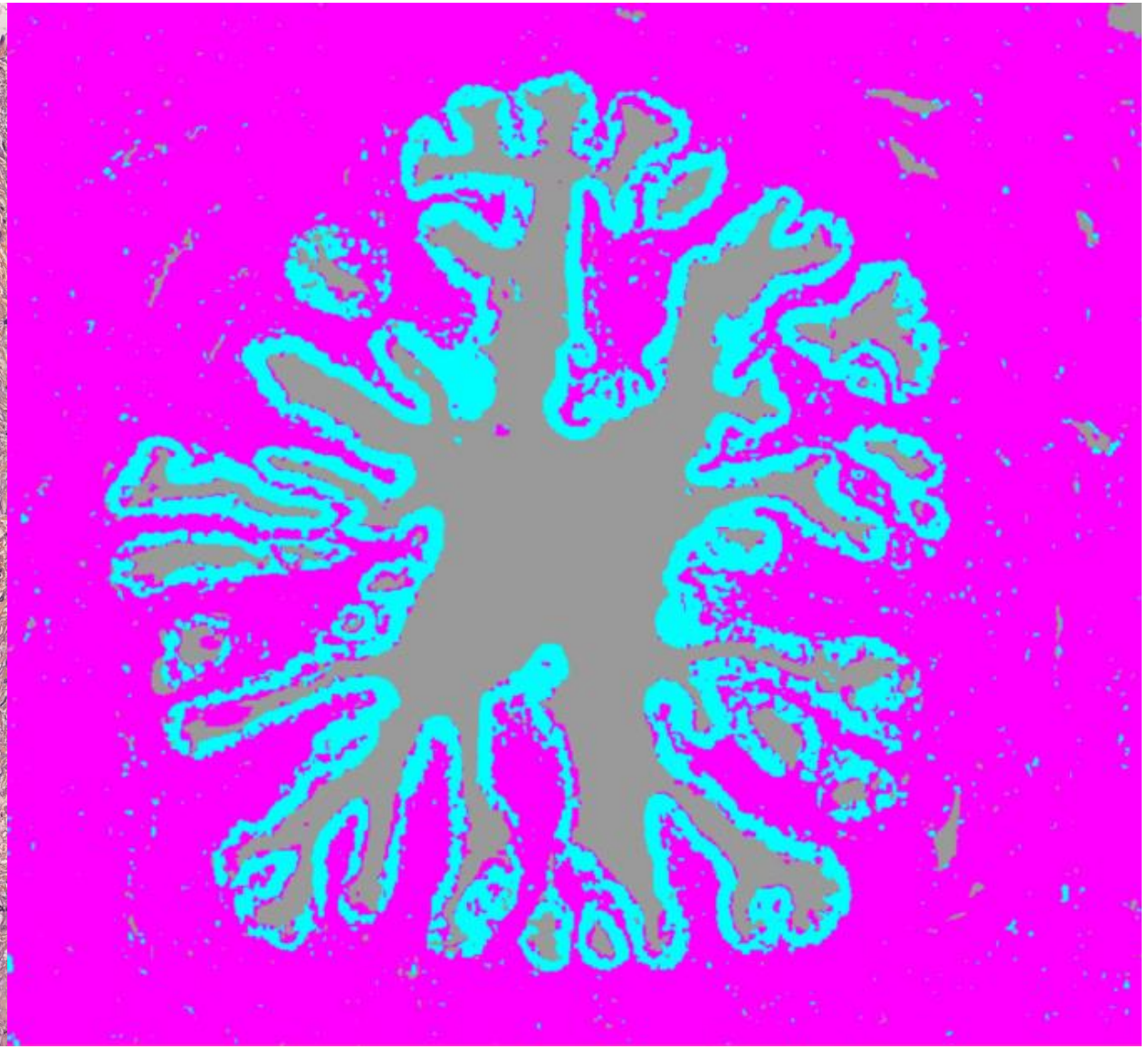
Pixel Classification

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



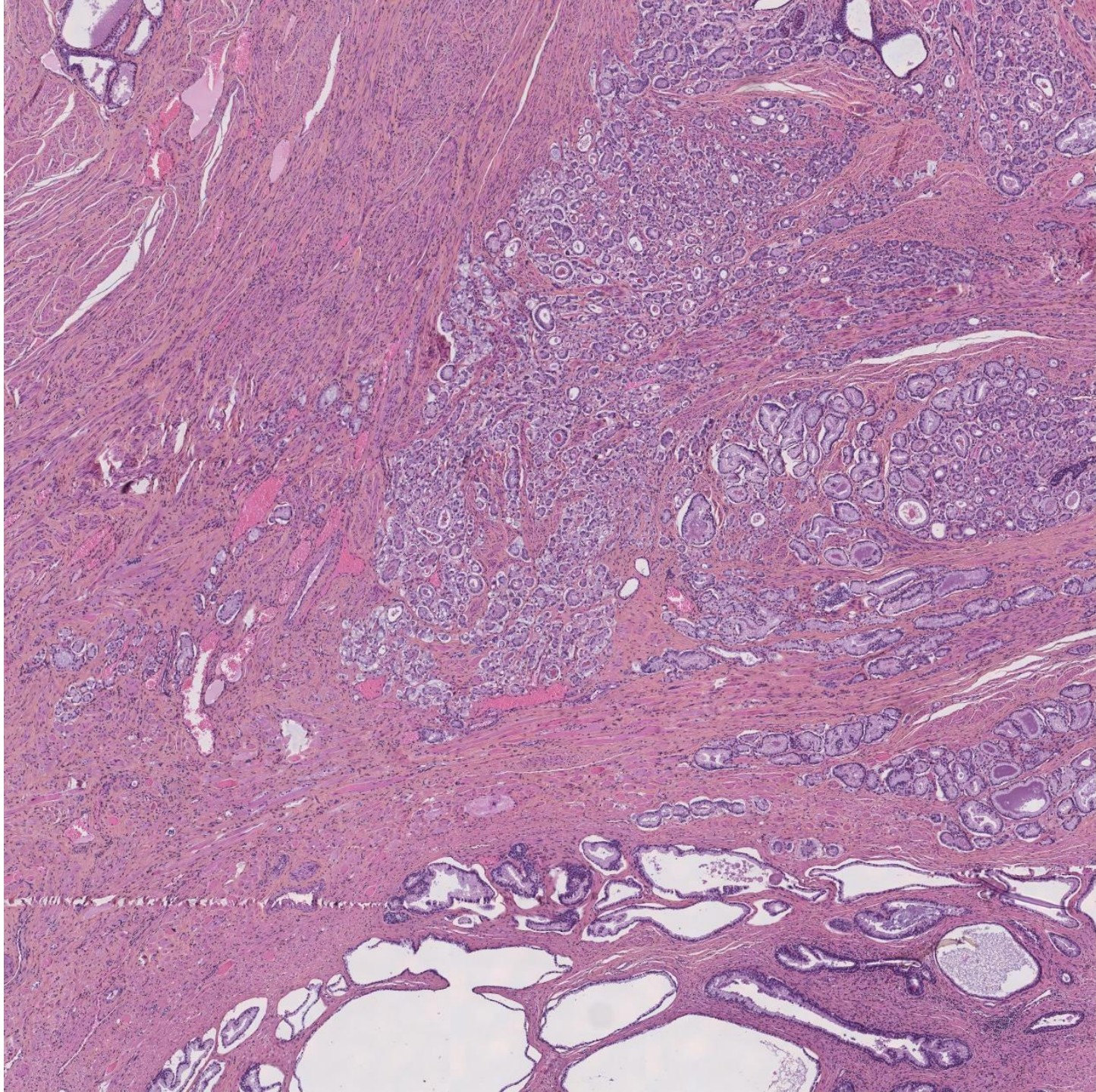
Pixel Classification

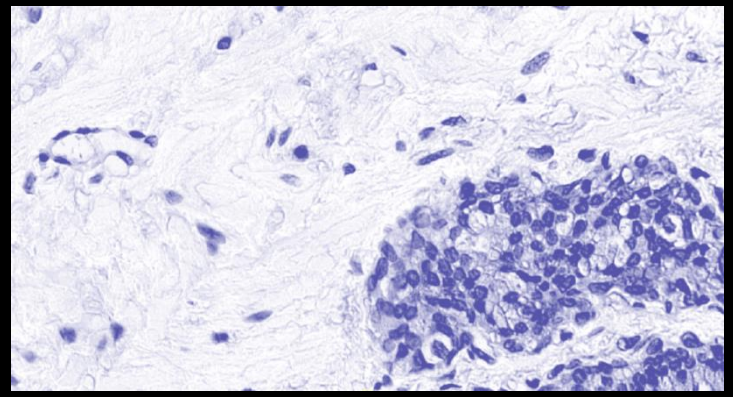




Pixel Classification

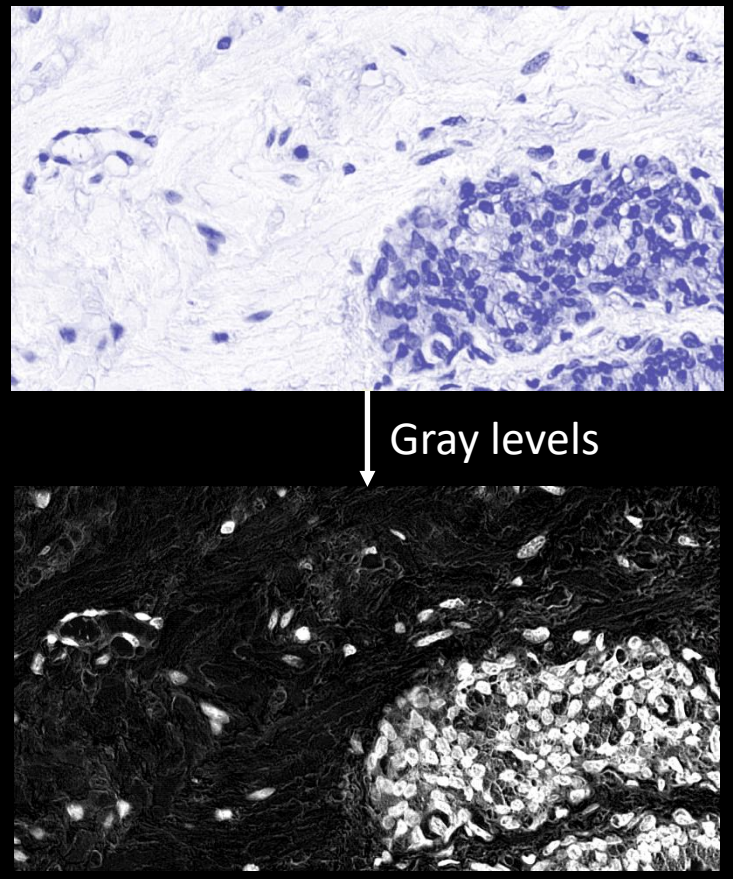
- **Open** prostate_1.ome.tif, prostate_2.ome.tif, prostate_3.ome.tif, prostate_4.ome.tif, prostate_5.ome.tif and prostate_6.ome.tif
- Create **annotations** in each image that recapitulate the **diversity** of the tissue
- Create **regions annotations**
- Open **"Pixel classifier"**
- **Annotate** pixels belonging to **background, epithelium** and **stroma**
- **Save classifier** and apply it to **each image** with a **script** (workflow tab)
- Get **proportions** of **tissues**



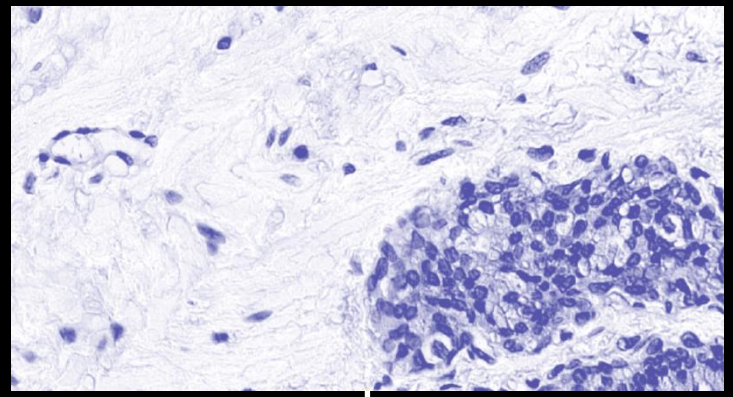


Cell Detection

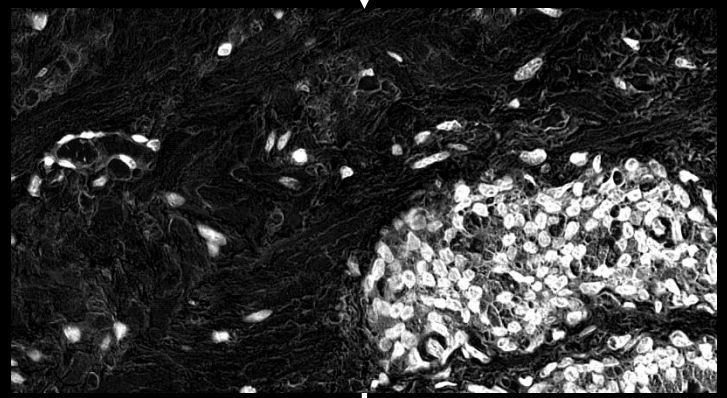
Cell Detection



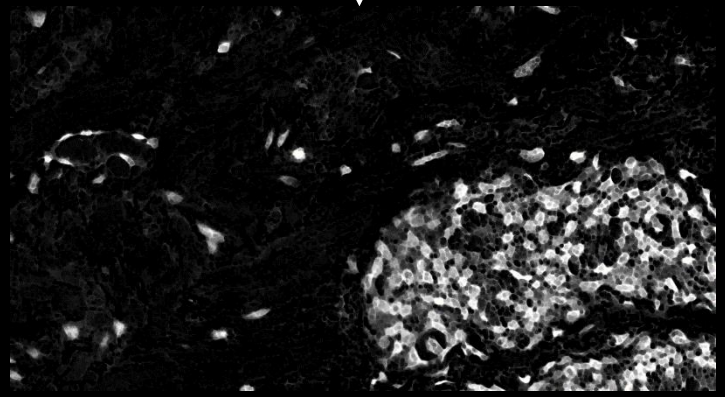
Cell Detection



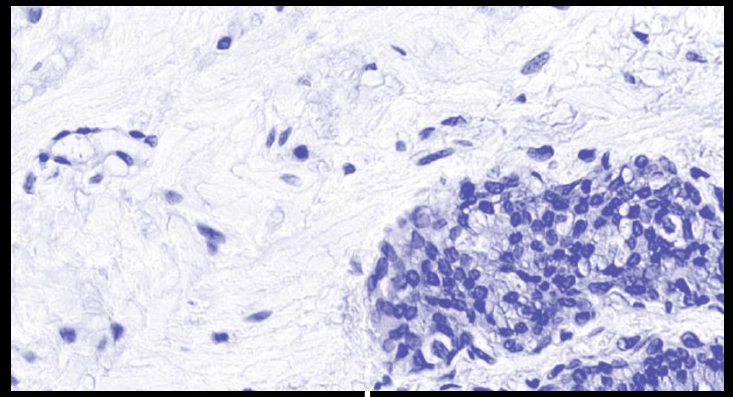
Gray levels



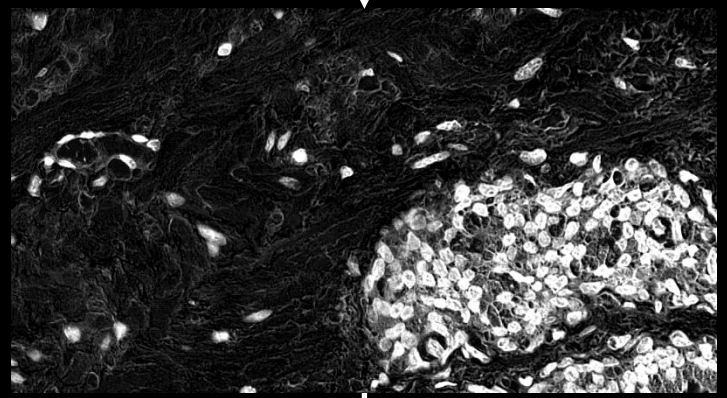
Minimum filtering



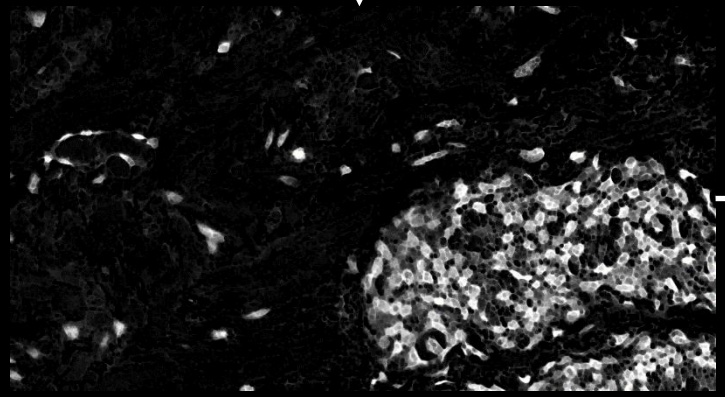
Cell Detection



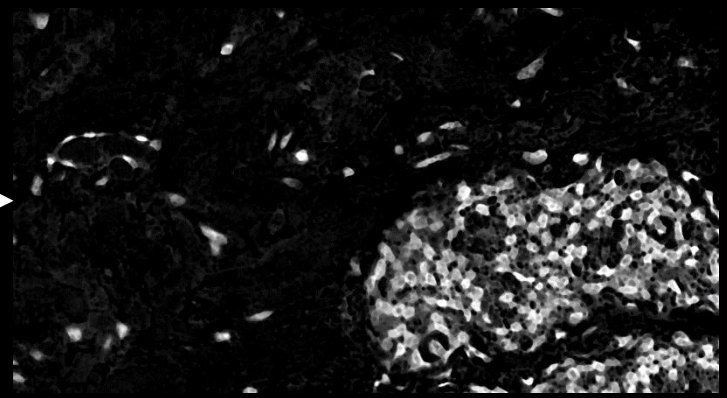
Gray levels



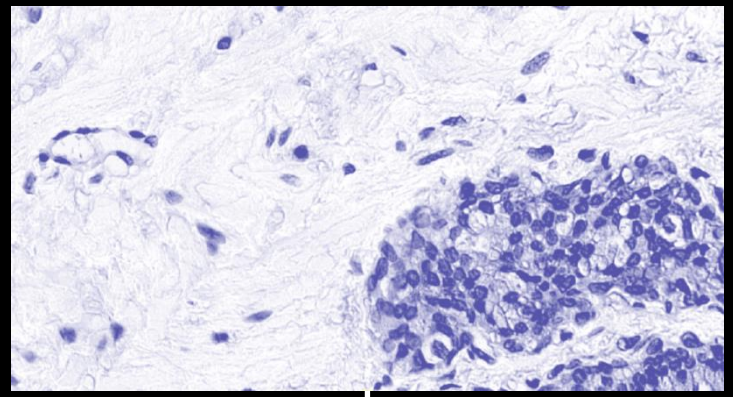
Minimum filtering



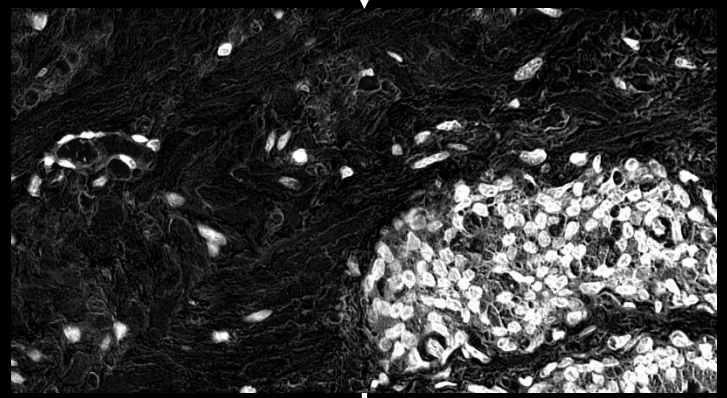
Gaussian blur



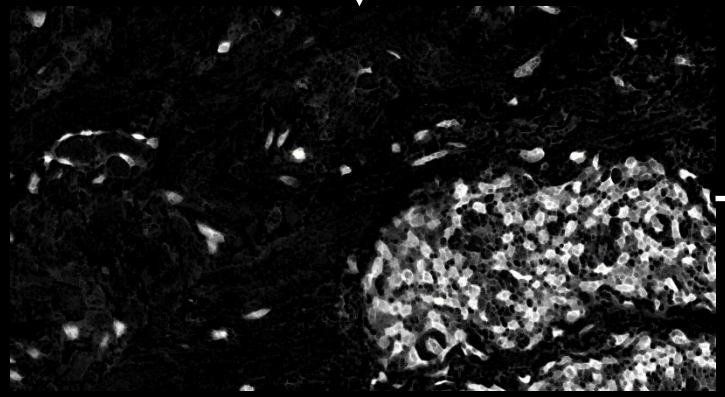
Cell Detection



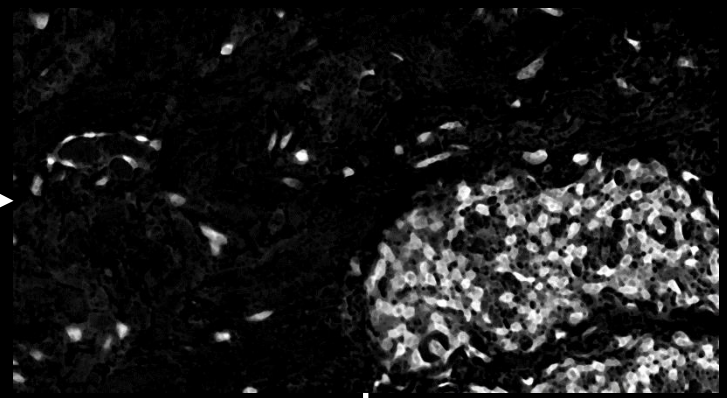
Gray levels



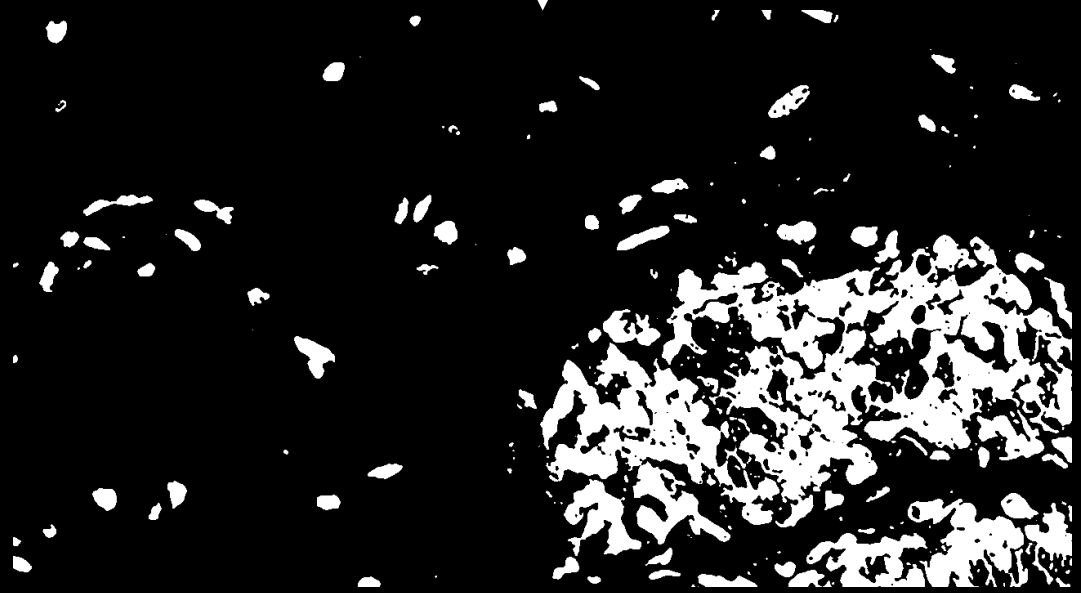
Minimum filtering



Gaussian blur

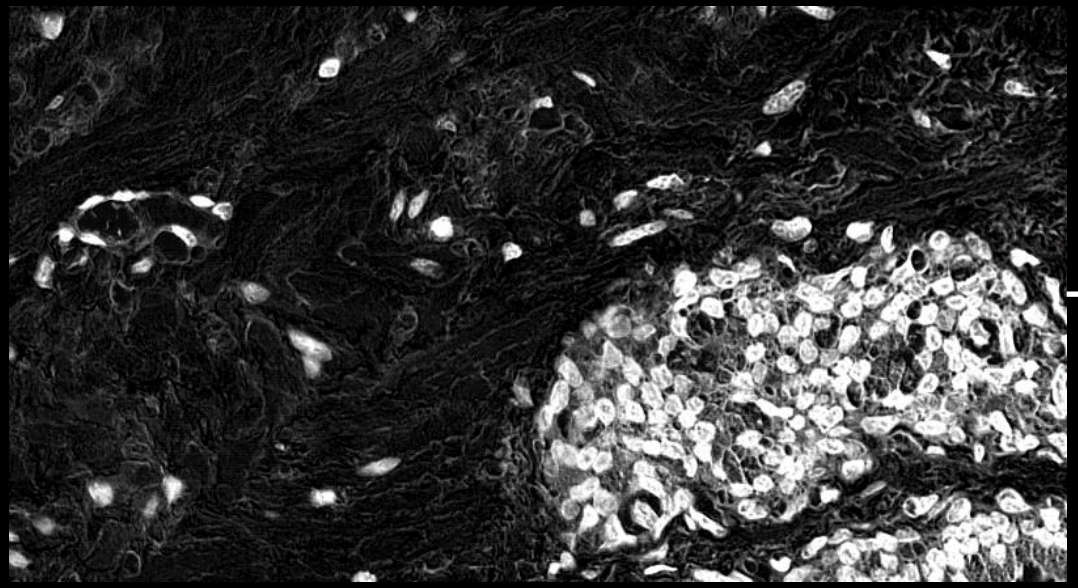


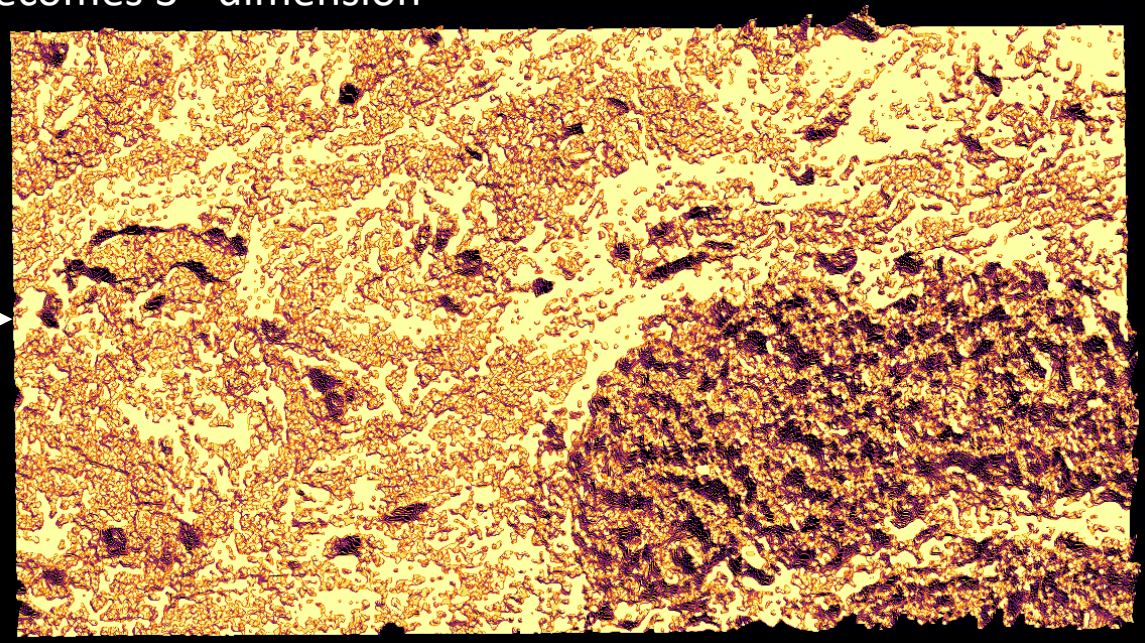
Thresholding



WATERSHED

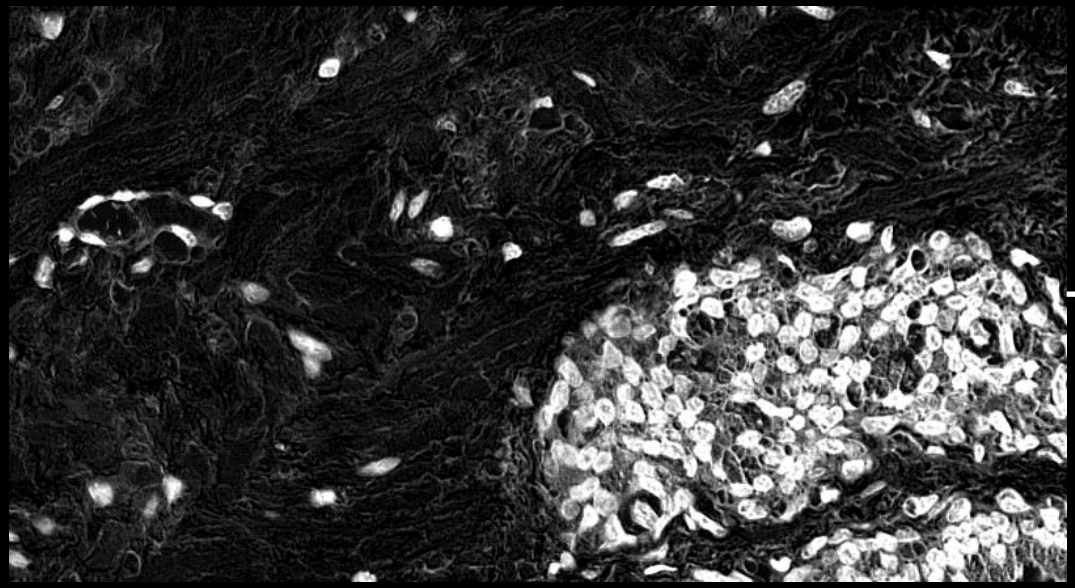
Transform image so that intensity becomes 3rd dimension

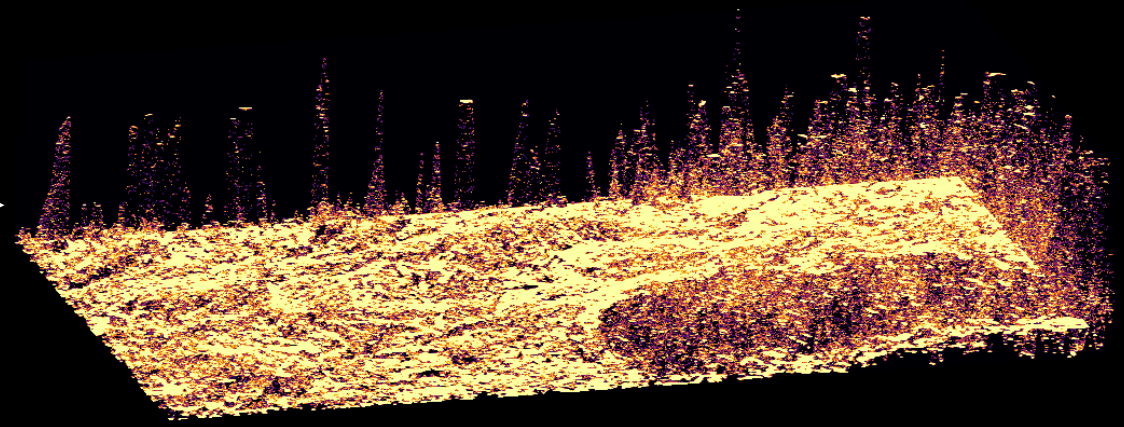




WATERSHED

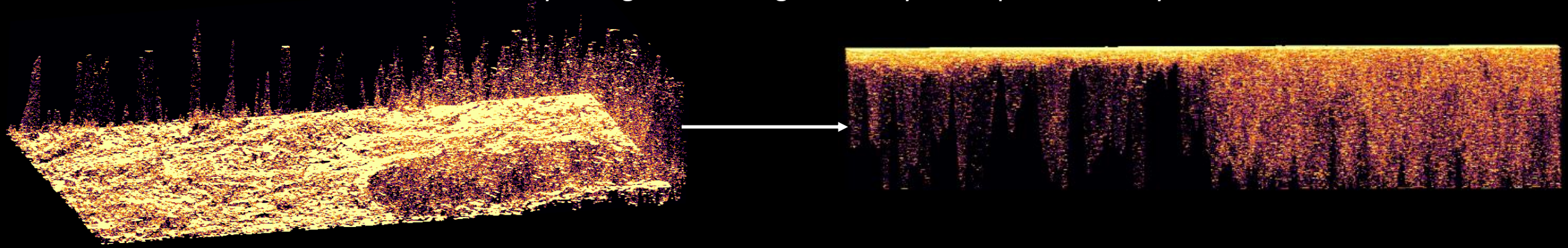
Transform image so that intensity becomes 3rd dimension





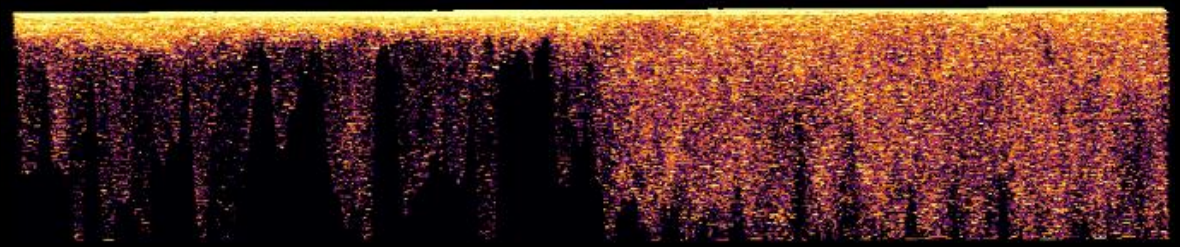
WATERSHED

Reverse intensity so regions with high intensity correspond to valleys



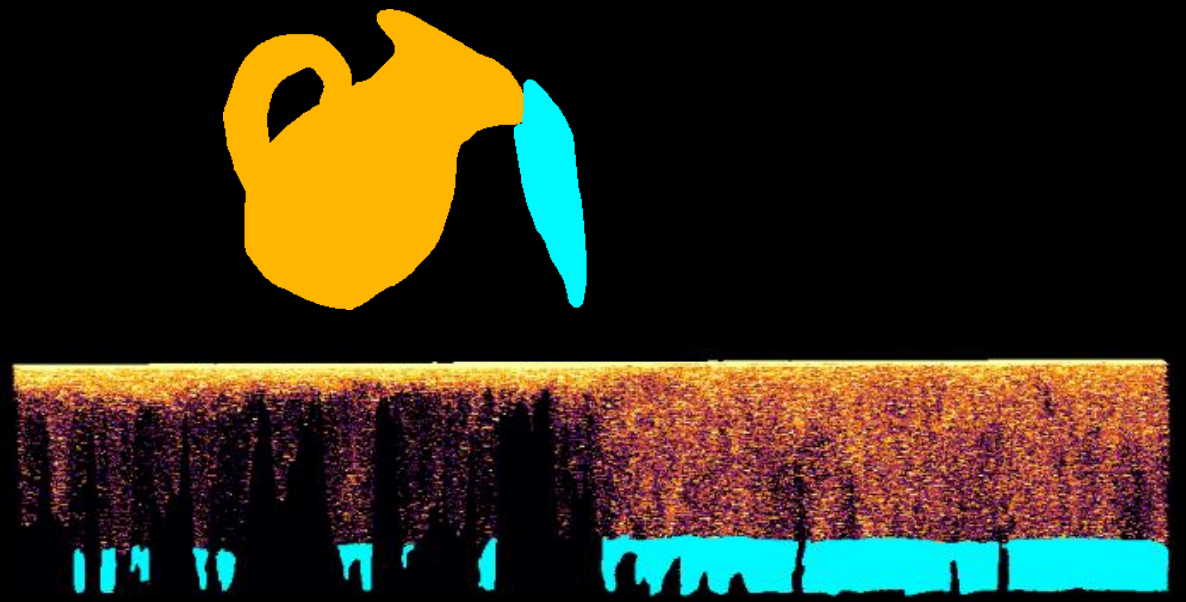
WATERSHED

Pour water into valleys



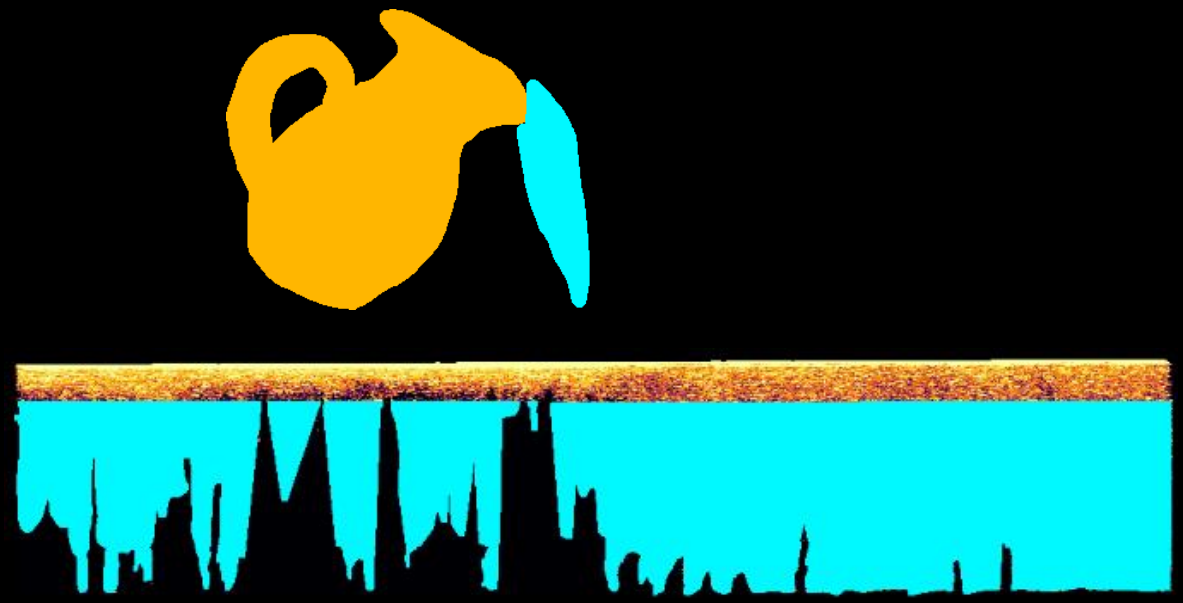
WATERSHED

Pour water into valleys



WATERSHED

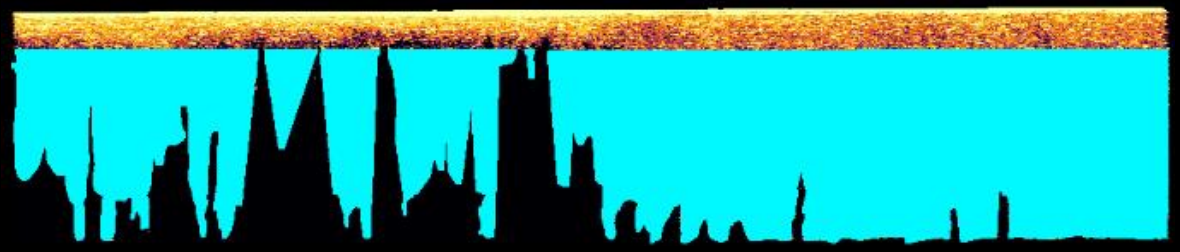
Pour water into valleys



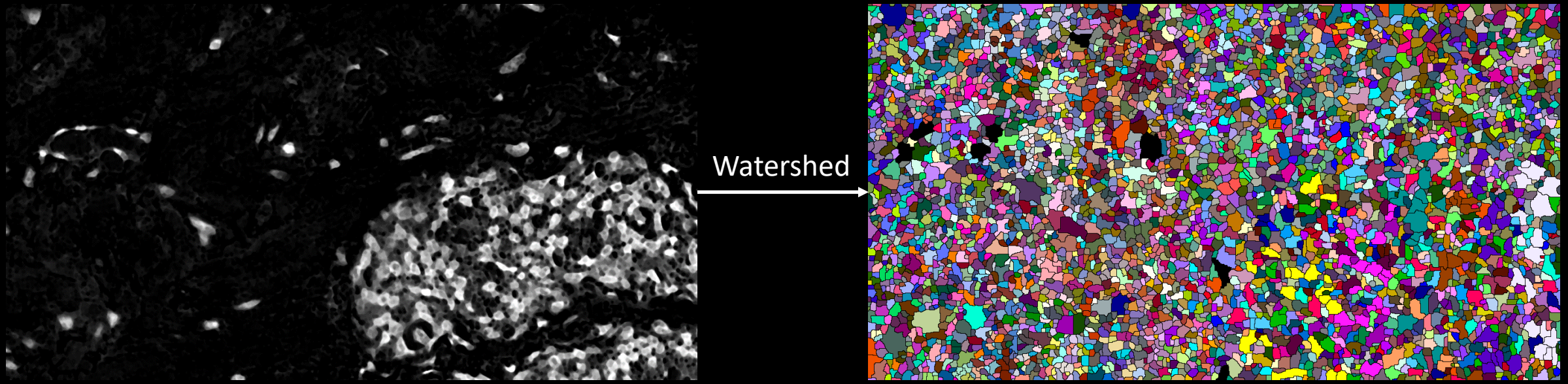
WATERSHED

Pour water into valleys

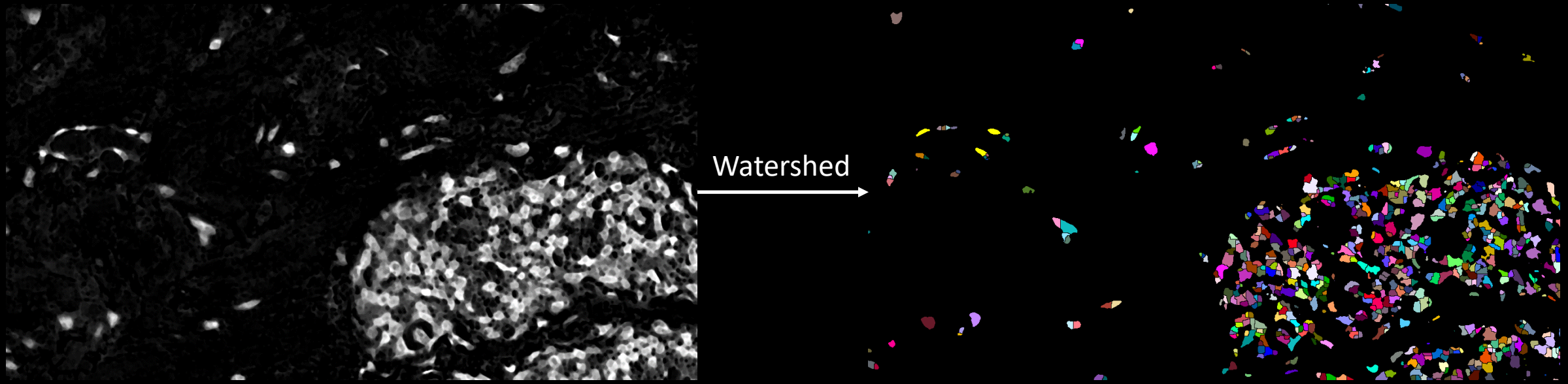
Threshold



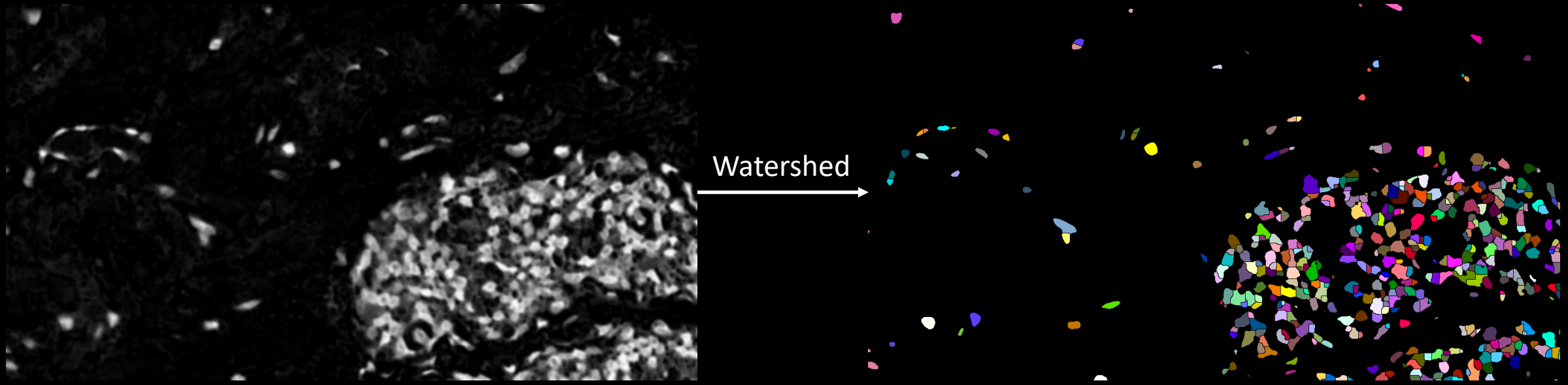
WATERSHED



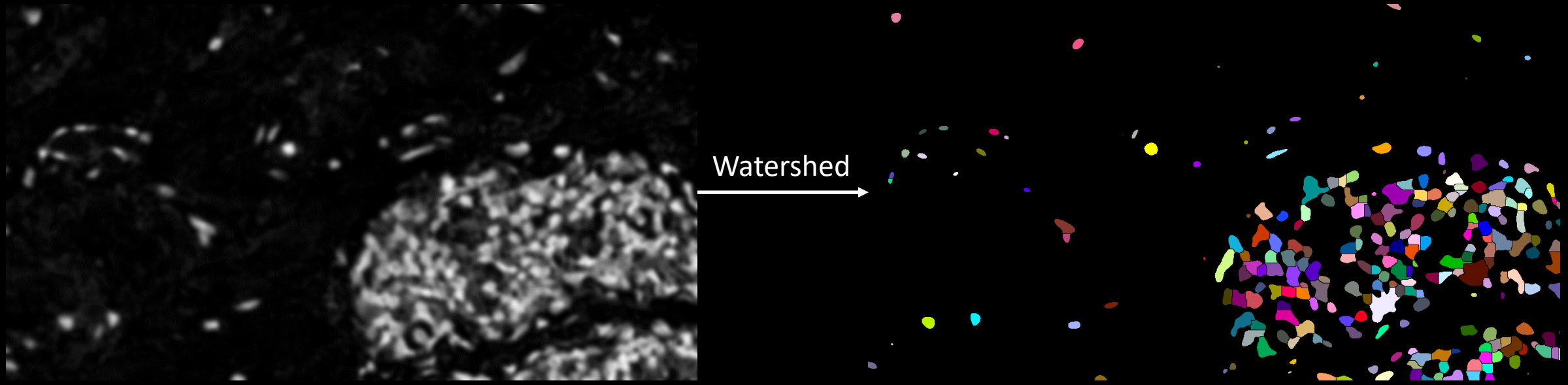
WATERSHED



WATERSHED



WATERSHED



Cell Detection Tool in QuPath

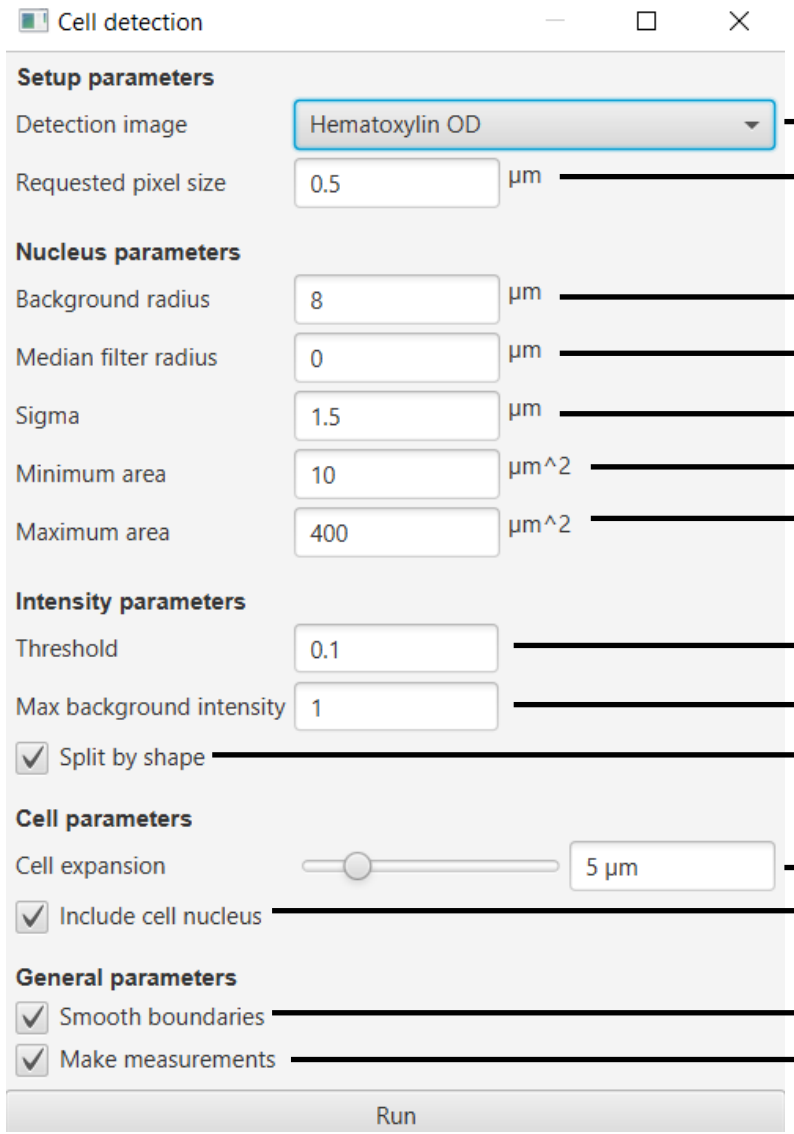


Image **component** used for cell detection

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**

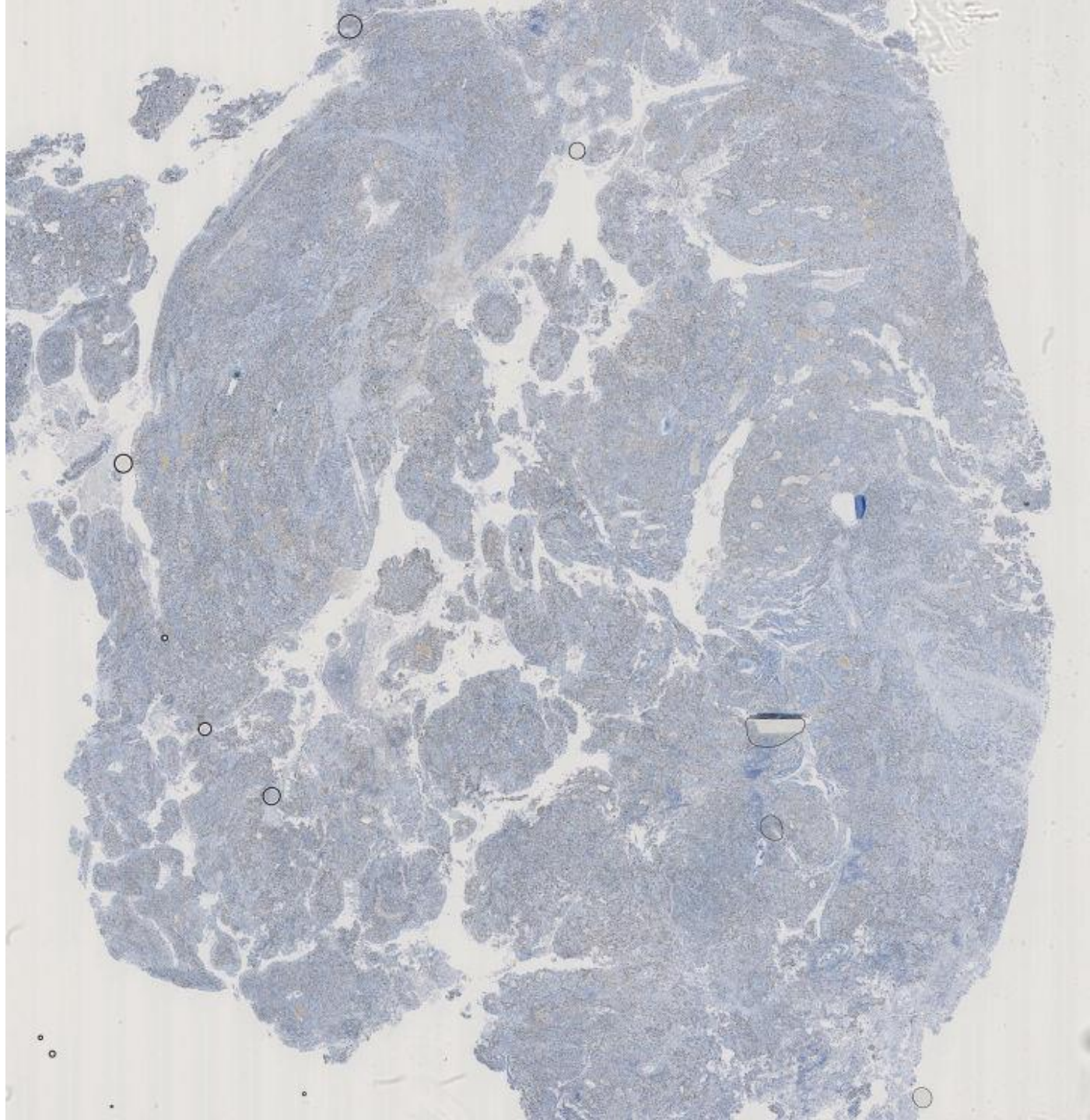
Define **nuclei and cytoplasm** areas or only cell areas

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 thresholder**
- **Apply** "Positive cell detection" on **the tissue**
- Get **number of nuclei** and **proportion** of **positive cells**



Cell Detection with Star-convex Polygons

Uwe Schmidt[1,⋆], Martin Weigert[1,⋆], Coleman Broaddus[1], and Gene Myers[1,2]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
Center for Systems Biology Dresden, Germany
[2] Faculty of Computer Science, Technical University Dresden, Germany

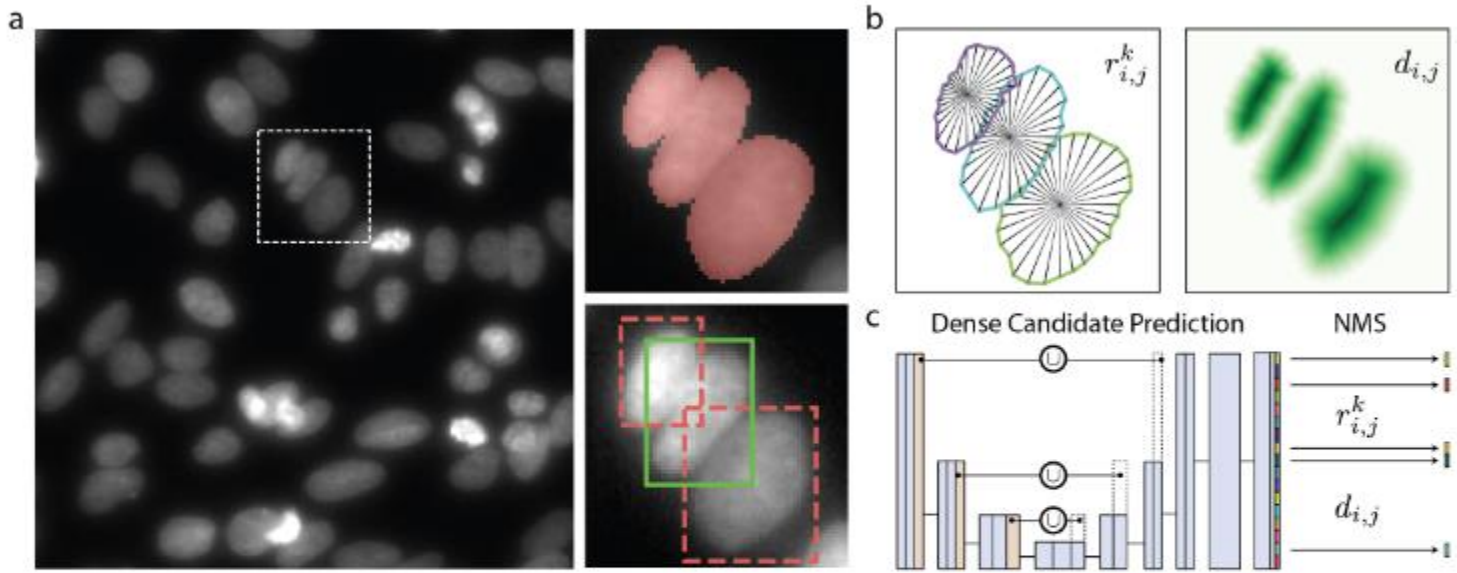


Fig. 1: *(a)* Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). *(b)* The proposed StarDist method predicts object probabilities $d_{i,j}$ and star-convex polygons parameterized by the radial distances $r^k_{i,j}$. *(c)* We densely predict $r^k_{i,j}$ 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[1,*], Martin Weigert[1,*], Coleman Broaddus[1], and Gene Myers[1,2]

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Center for Systems Biology Dresden, Germany
[2] 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).*

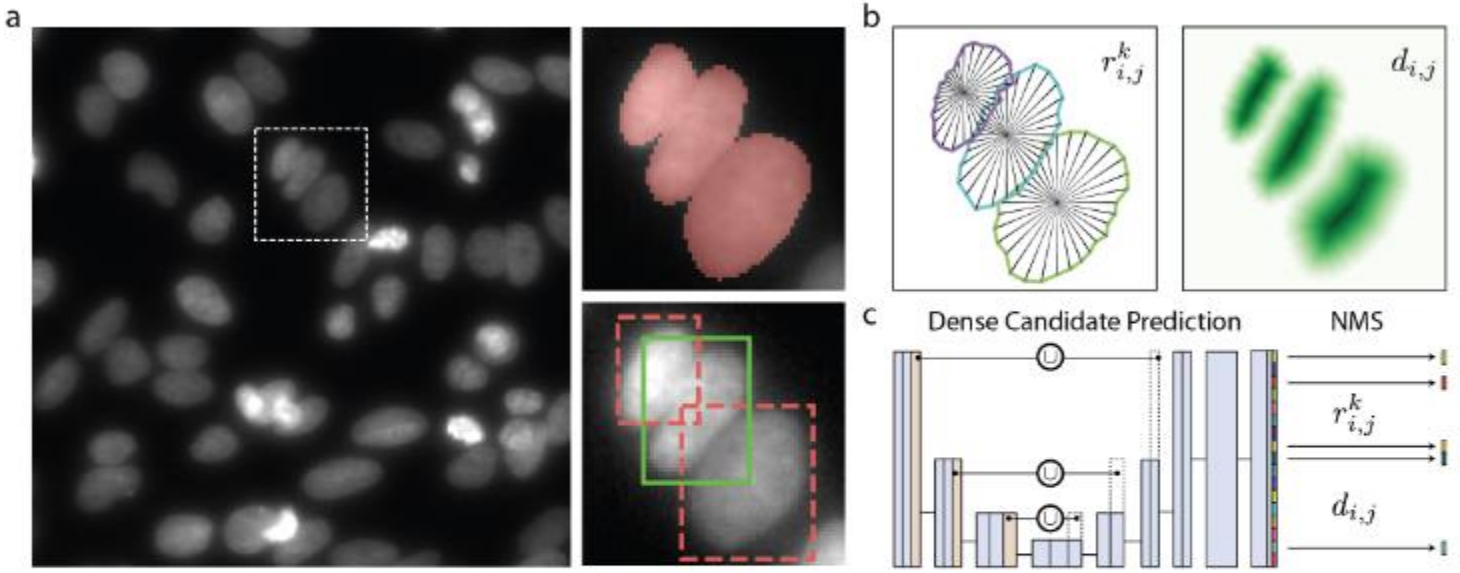
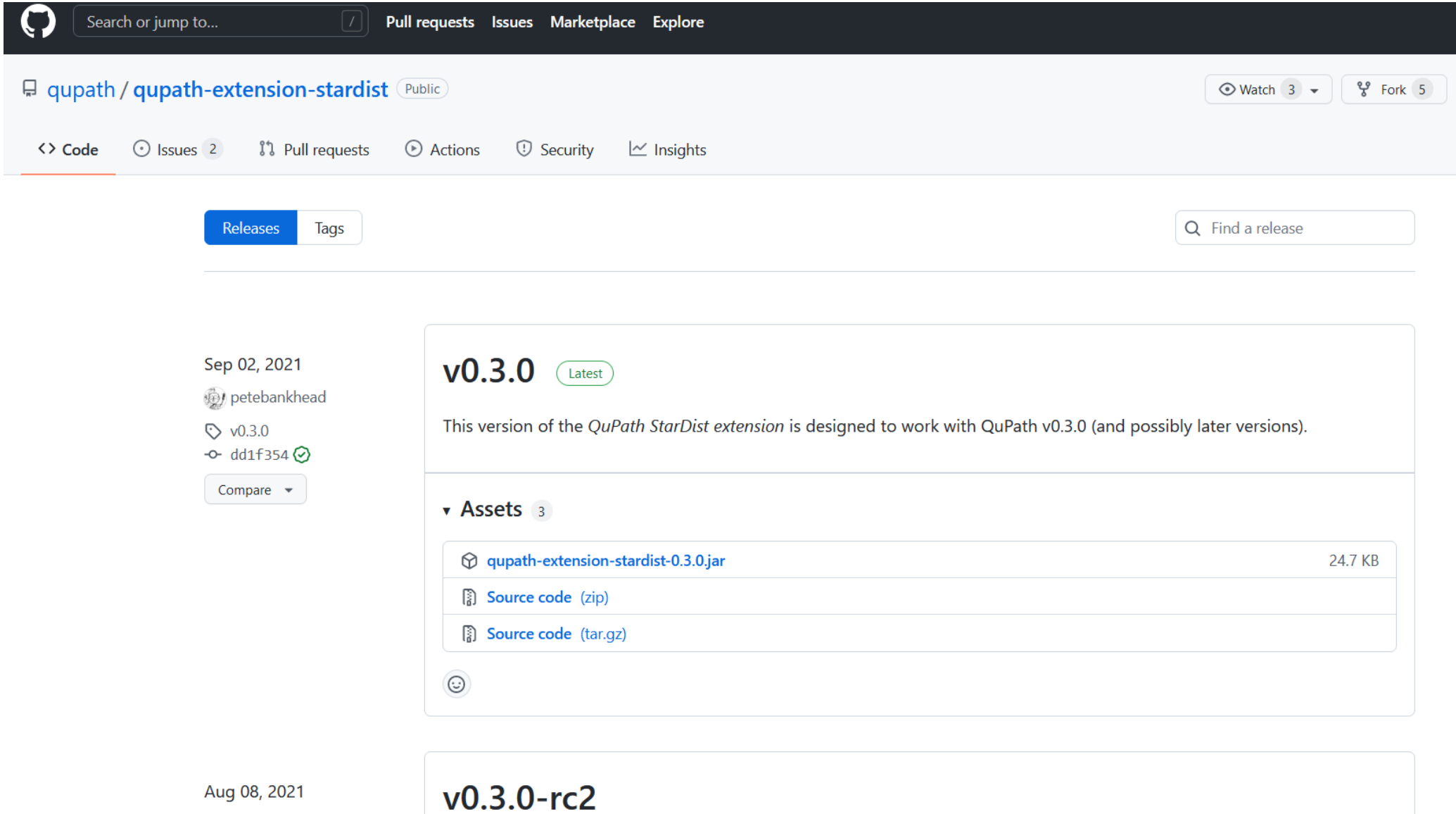


Fig. 1: *(a)* Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). *(b)* The proposed StarDist method predicts object probabilities $d_{i,j}$ and star-convex polygons parameterized by the radial distances $r^k_{i,j}$. *(c)* We densely predict $r^k_{i,j}$ 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**



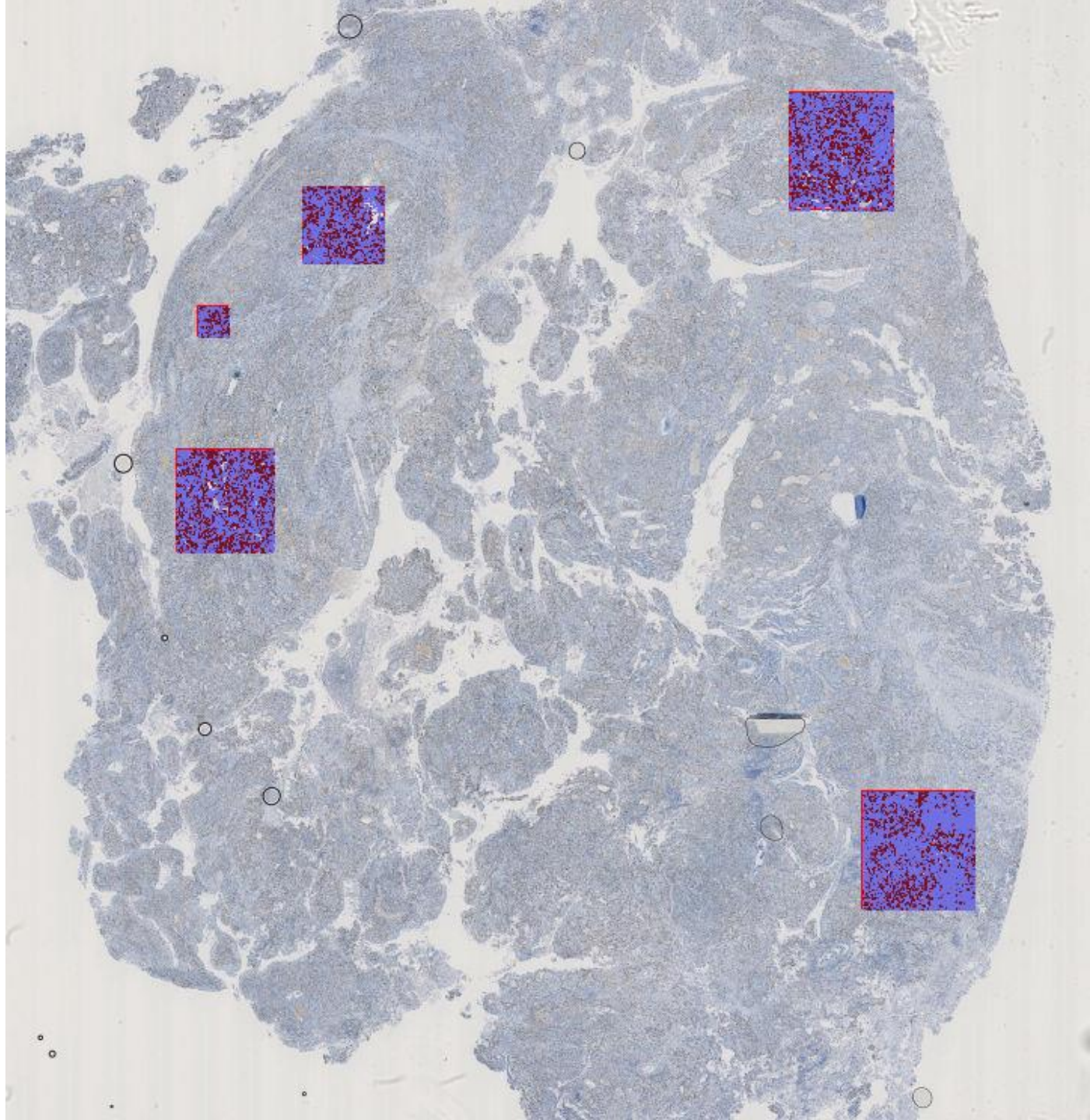
Segmentation with Stardist

Open **Scrip editor**, then open nucleus_detection_hematoxylin_dab.groovy

```groovy
import qupath.ext.stardist.StarDist2D
import qupath.lib.images.servers.ColorTransforms
import qupath.imagej.gui.ImageJMacroRunner

min_nuclei_area = 15

// Specify the model directory (you will need to change this!)
def pathModel = "C:/Work/QuPath/scripts/StardistModels/TissueNet_all.pb"

def stardist_segmentation = StarDist2D.builder(pathModel)
        .threshold(0.5)              // Prediction threshold
        .normalizePercentiles(1, 99.8) // Percentile normalization
        .pixelSize(0.5)              // Resolution for detection
        .channels(
            ColorTransforms.createColorDeconvolvedChannel(getCurrentImageData().getColorDeconvolutionStains(), 1),
            ColorTransforms.createColorDeconvolvedChannel(getCurrentImageData().getColorDeconvolutionStains(), 2)
            )
        .cellExpansion(5.0)          // Approximate cells based upon nucleus expansion
        .cellConstrainScale(1.5)     // Constrain cell expansion using nucleus size
        .measureShape()              // Add shape measurements
        .measureIntensity()          // Add cell measurements (in all compartments)
        .build()


def imageData = getCurrentImageData()
def hierarchy = imageData.getHierarchy()
def annotations = hierarchy.getAnnotationObjects()

// Run detection for the selected objects
stardist_segmentation.detectObjects(imageData, annotations)

//def toDelete = getDetectionObjects().findAll {measurement(it, 'Circularity') < 0.9}
def toDelete = getDetectionObjects().findAll {measurement(it, 'Area µm^2') < min_nuclei_area}
removeObjects(toDelete, true)


println 'Done!'
```

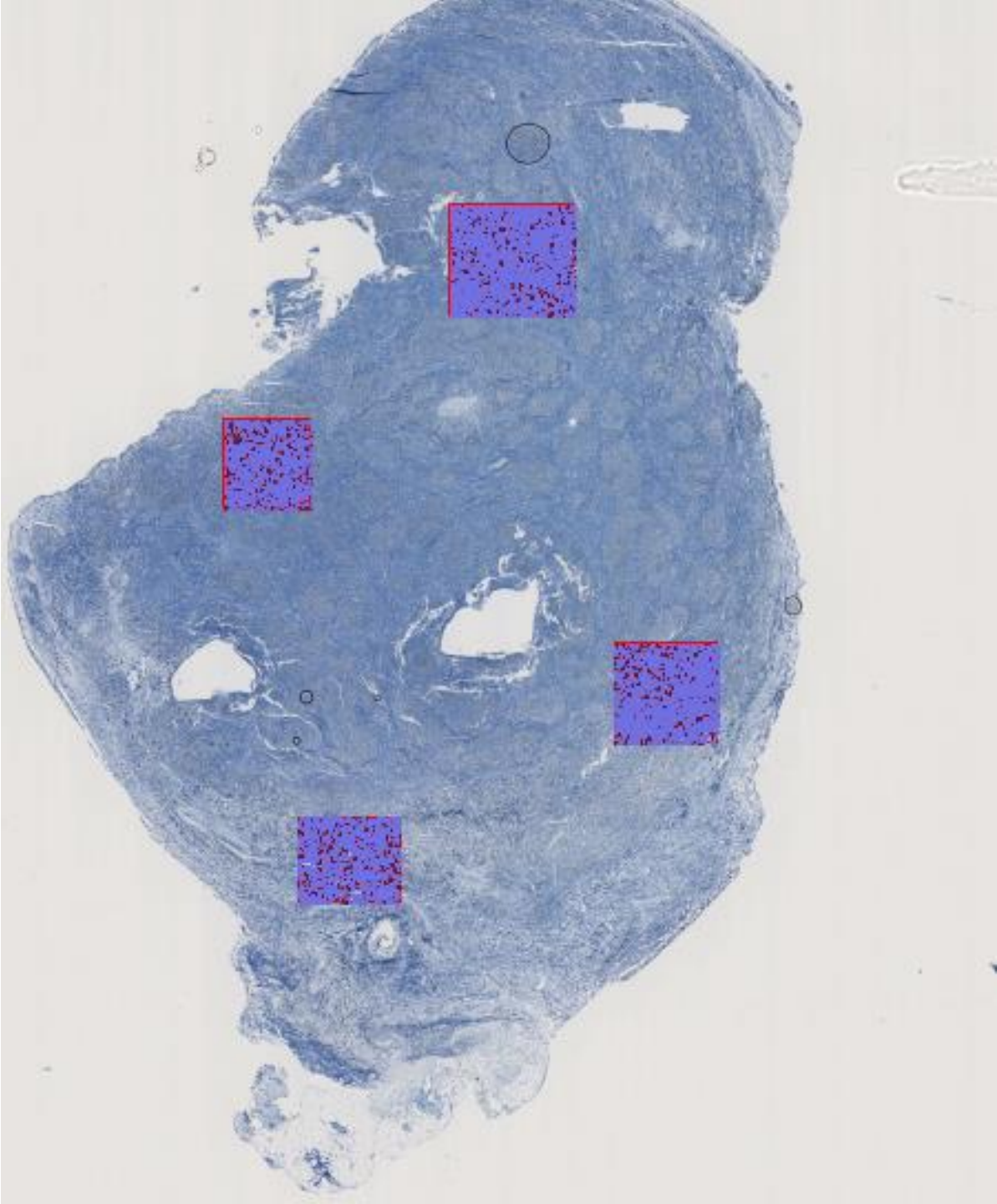
Segmentation with Stardist

- **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

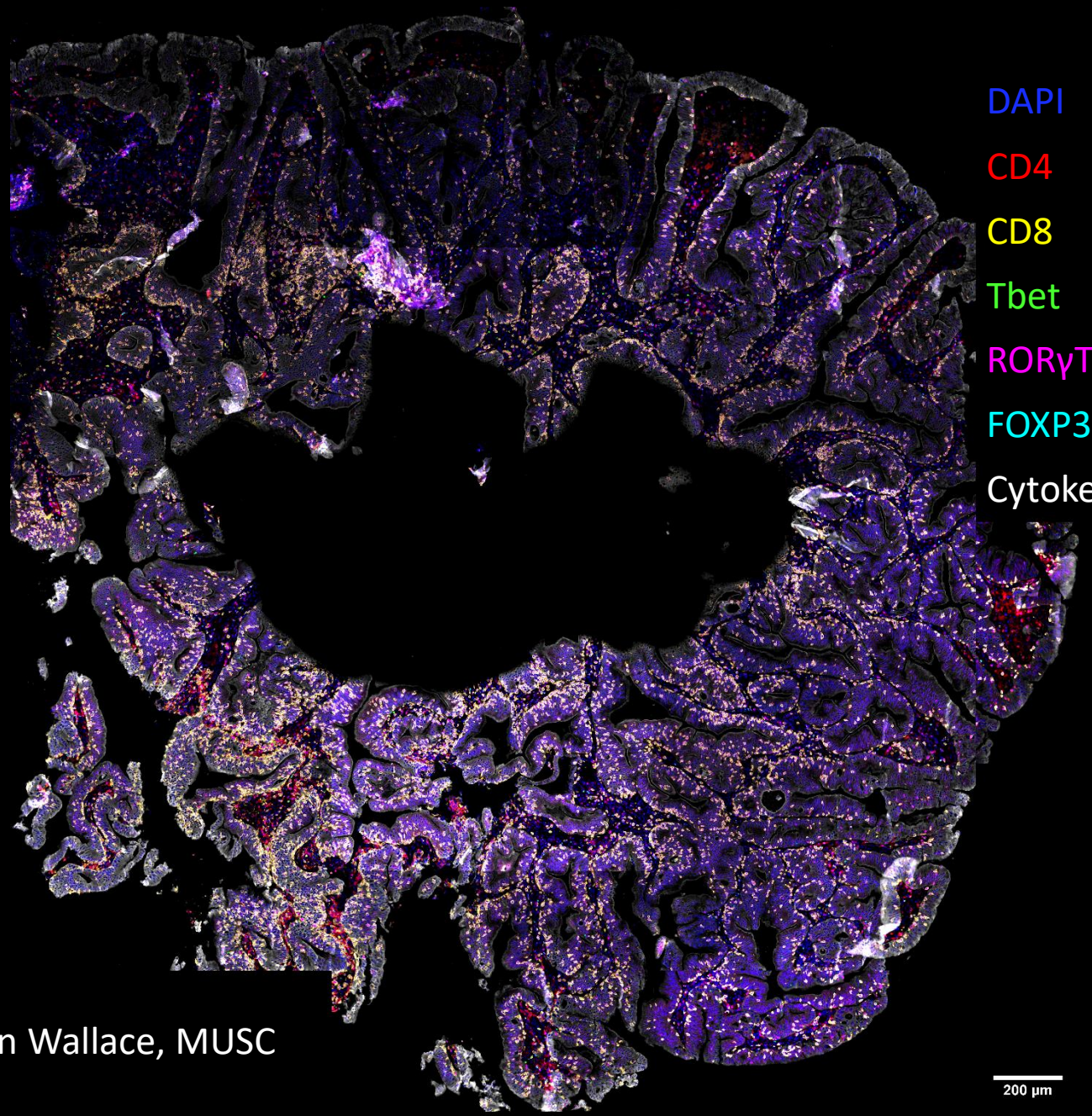


Segmentation with Stardist

- **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 ?

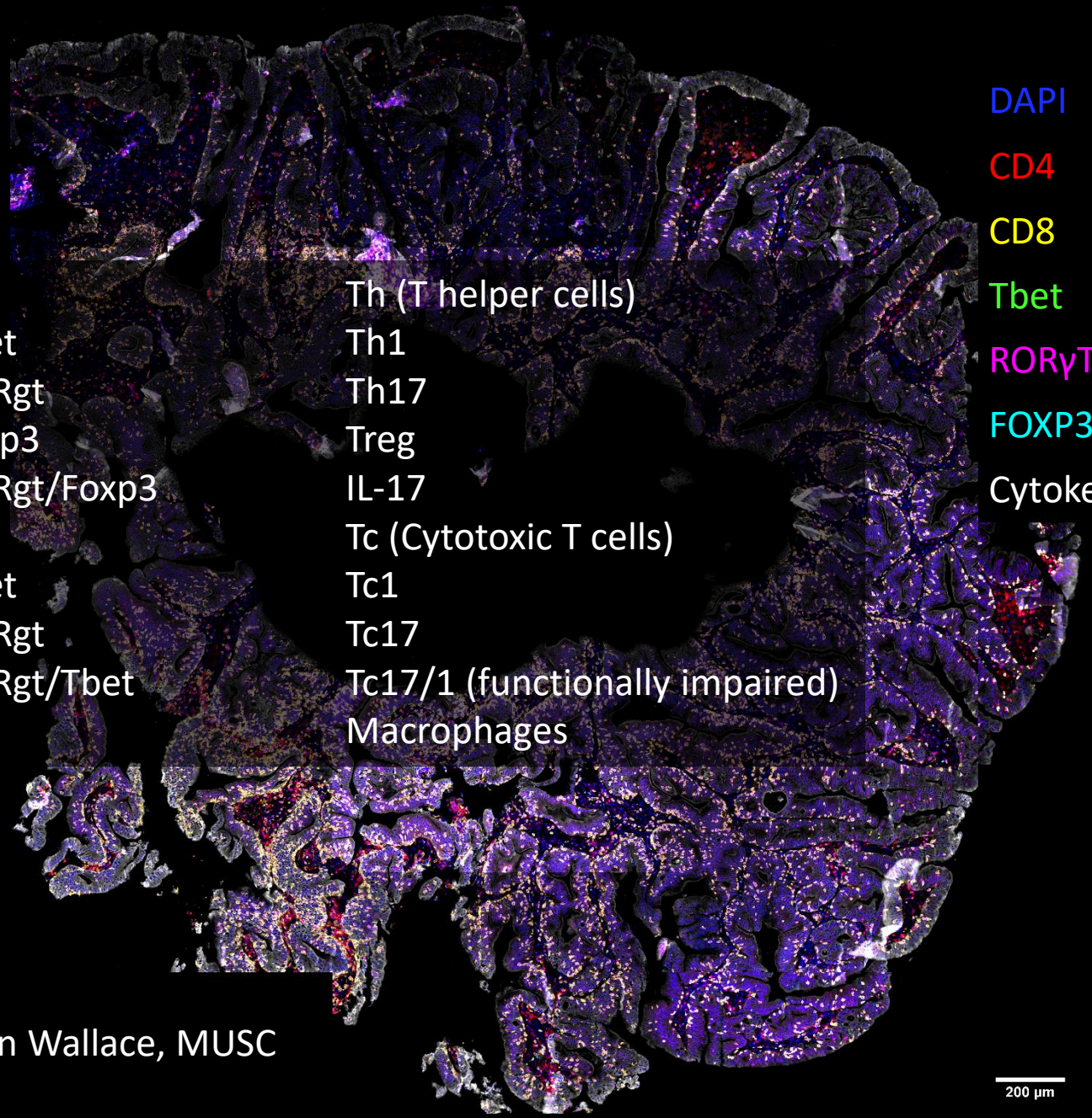


Multi/Hyper-plexed Images



Polyp study with Kristin Wallace, MUSC

Multi/Hyper-plexed Images

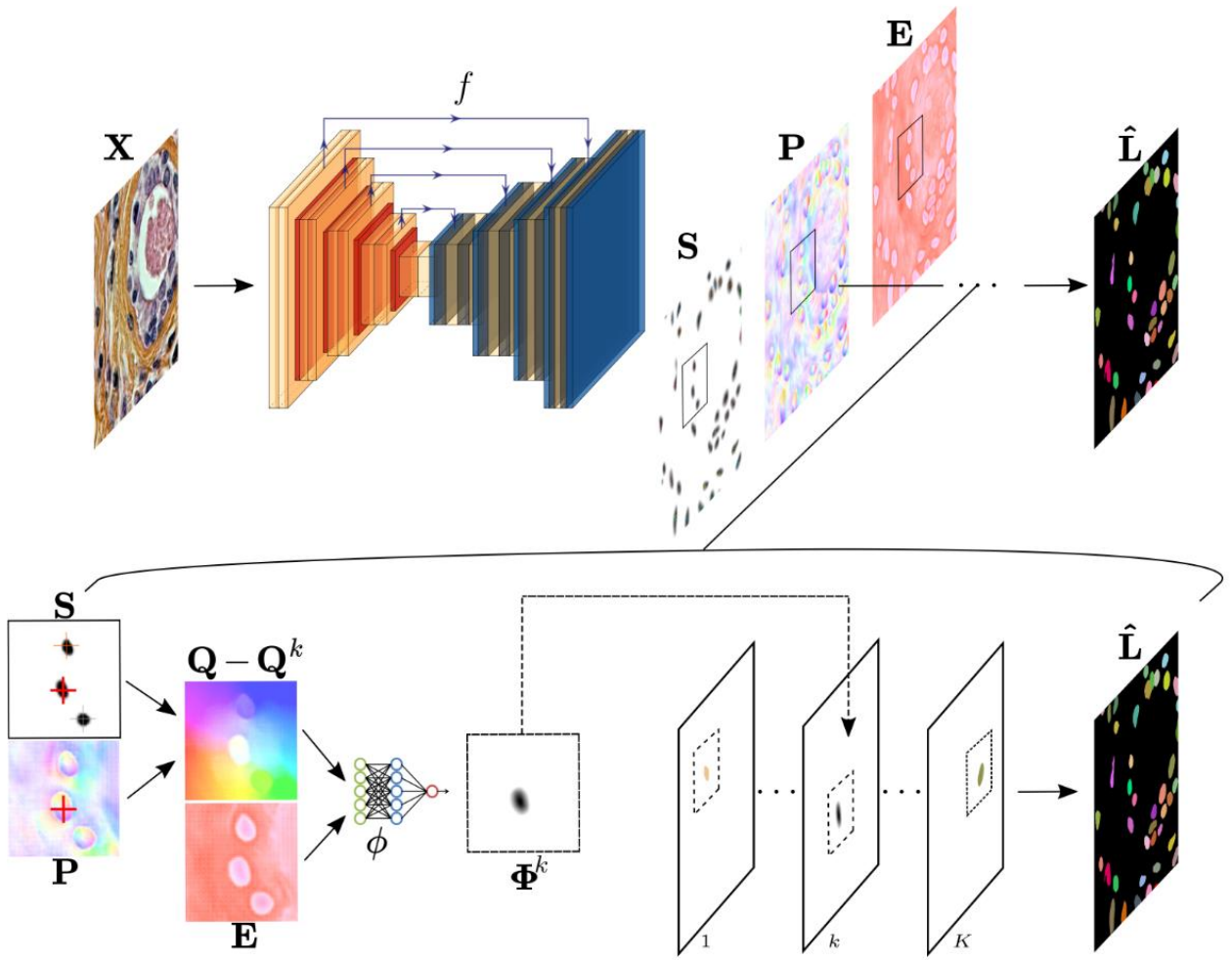


CD4	Th (T helper cells)
CD4/Tbet	Th1
CD4/RORgt	Th17
CD4/Foxp3	Treg
CD4/RORgt/Foxp3	IL-17
CD8	Tc (Cytotoxic T cells)
CD8/Tbet	Tc1
CD8/RORgt	Tc17
CD8/RORgt/Tbet	Tc17/1 (functionally impaired)
FOXP3	Macrophages

Polyp study with Kristin Wallace, MUSC

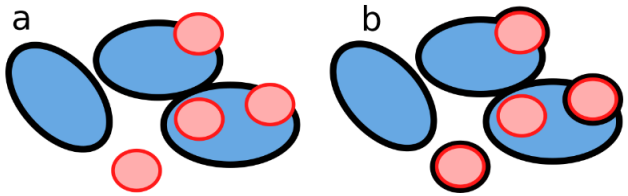
INSTANSEG: AN EMBEDDING-BASED INSTANCE SEGMENTATION ALGORITHM OPTIMIZED FOR ACCURATE, EFFICIENT AND PORTABLE CELL SEGMENTATION

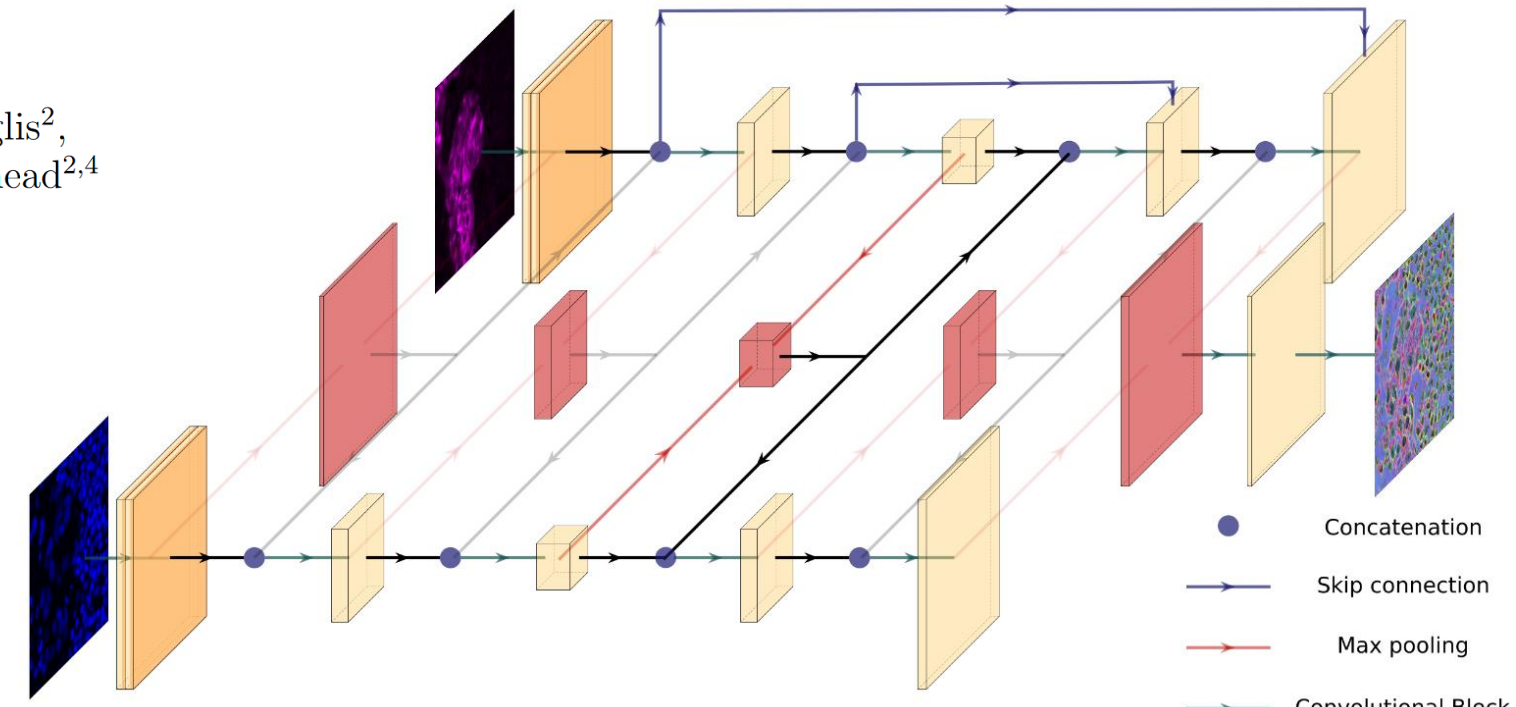
Thibaut Goldsborough[1], Ben Philps[1], Alan O'Callaghan[2], Fiona Inglis[2], Leo Leplat[2], Andrew Filby[3], Hakan Bilen[1], and Peter Bankhead[2,4]



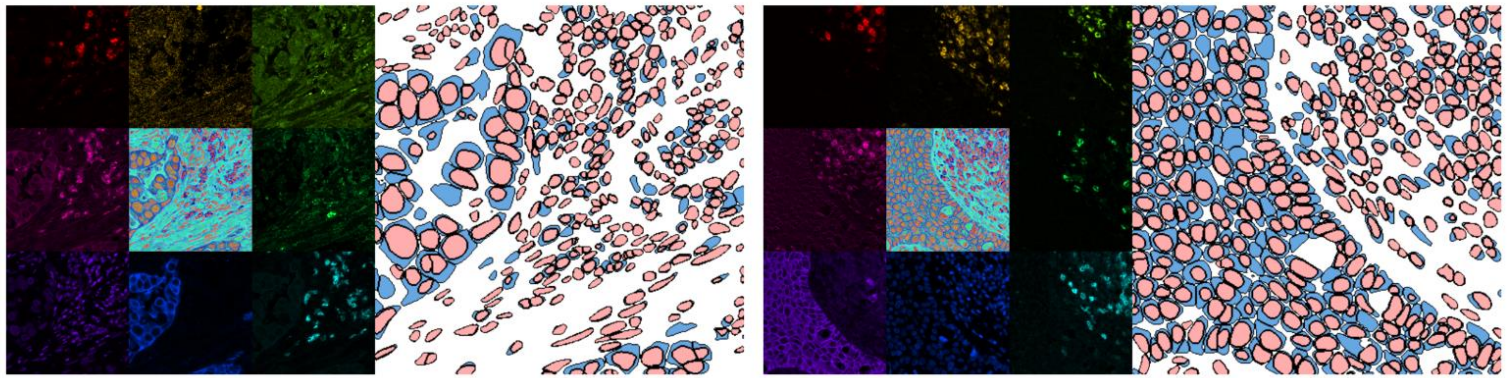
A novel channel invariant architecture for the segmentation of cells and nuclei in multiplexed images using InstanSeg

Thibaut Goldsborough[1], Alan O'Callaghan[2], Fiona Inglis[2], Léo Leplat[2], Andrew Filby[3], Hakan Bilen[1], Peter Bankhead[2,4]





b



Shallow Machine Learning for Object Classification

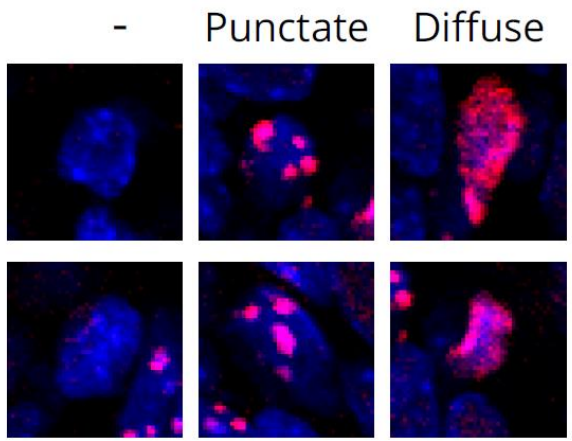
As for pixel classification:

- **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

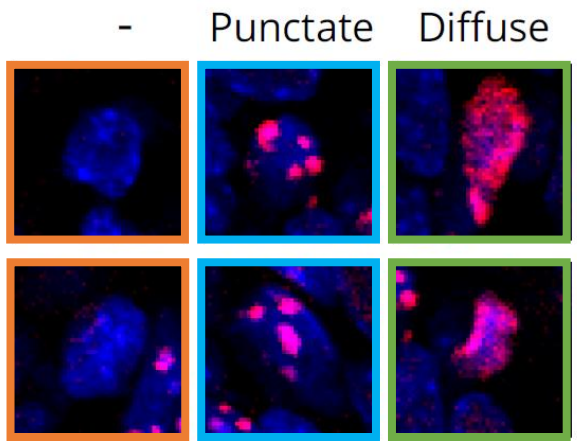
But this time, features are **measurements associated to detections** (most often cells or nuclei) such as:

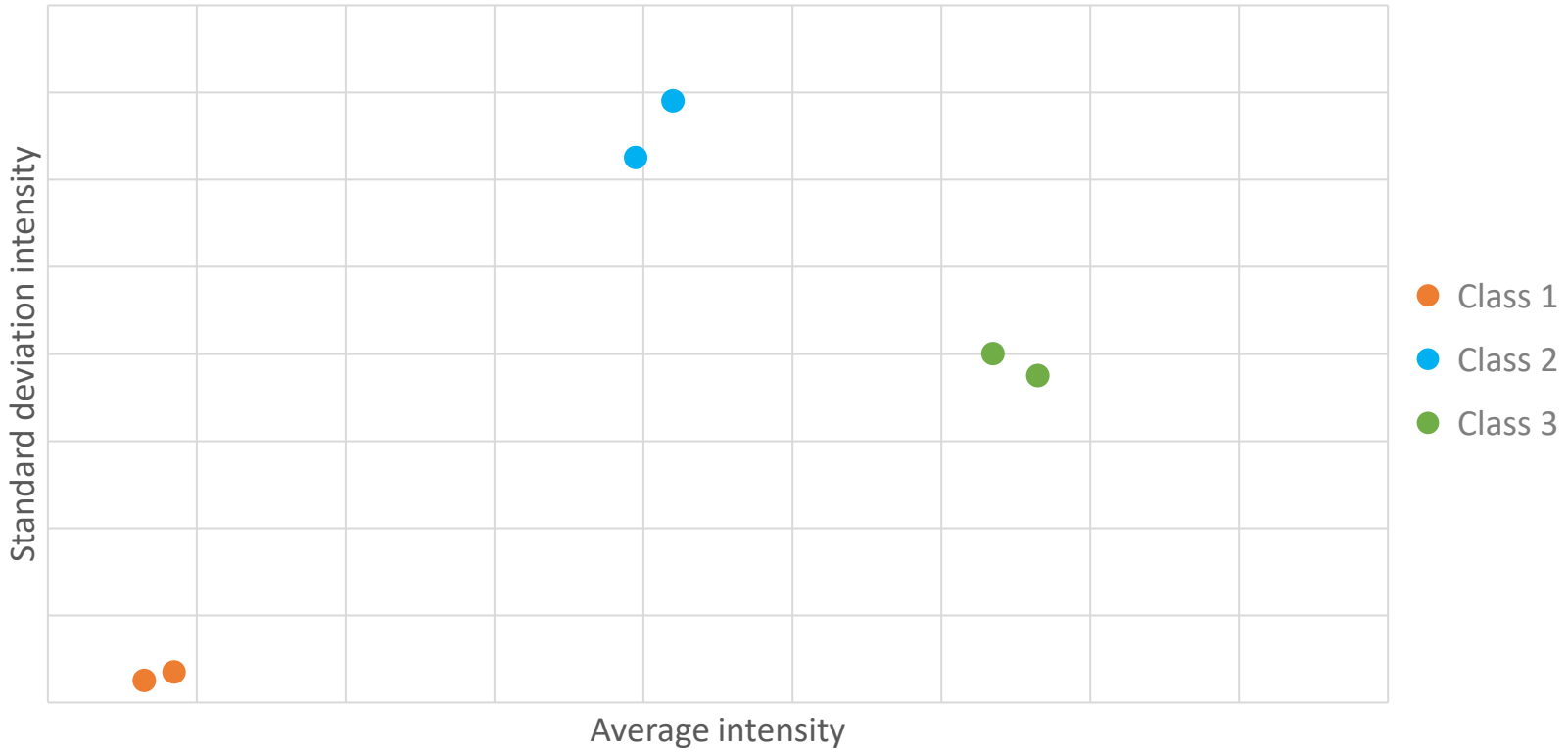
- **Average** intensity
- **Median** intensity
- **Standard deviation** of intensity
- **Minimum/Maximum** intensity
- Object **area**
- Object **Circularity**
- …

Shallow Machine Learning for Object Classification

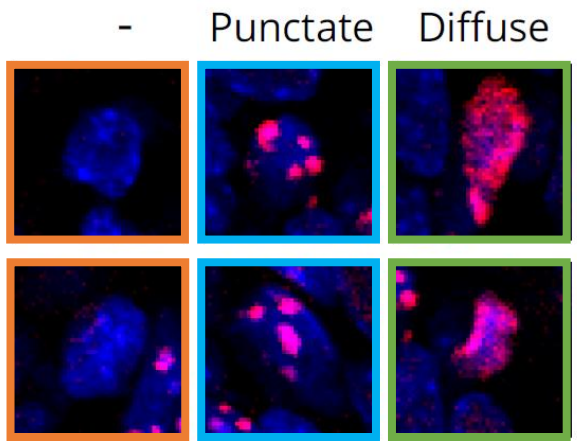


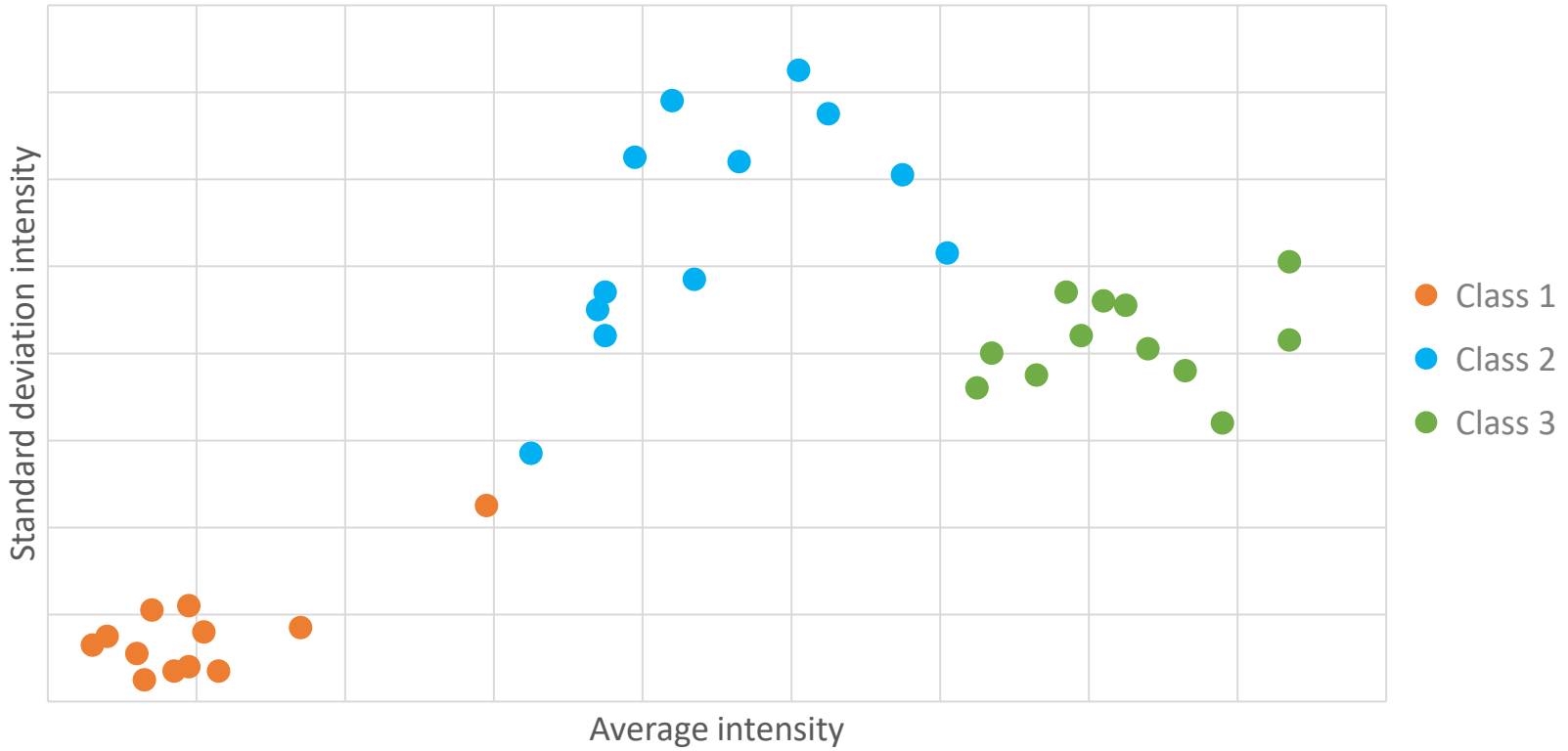
Shallow Machine Learning for Object Classification



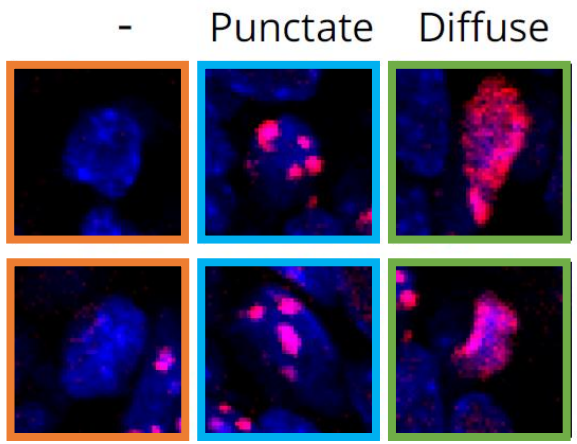


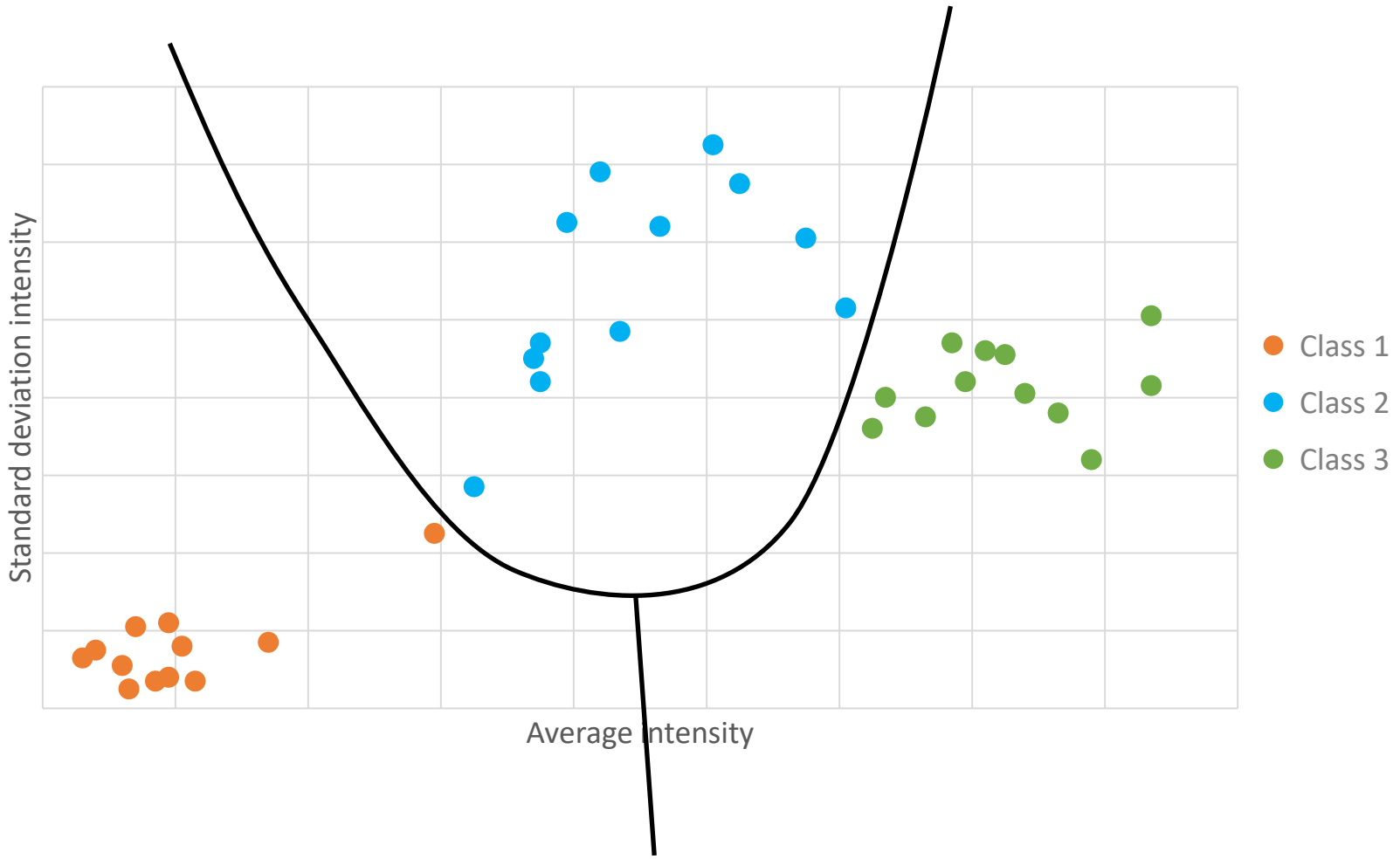
Shallow Machine Learning for Object Classification





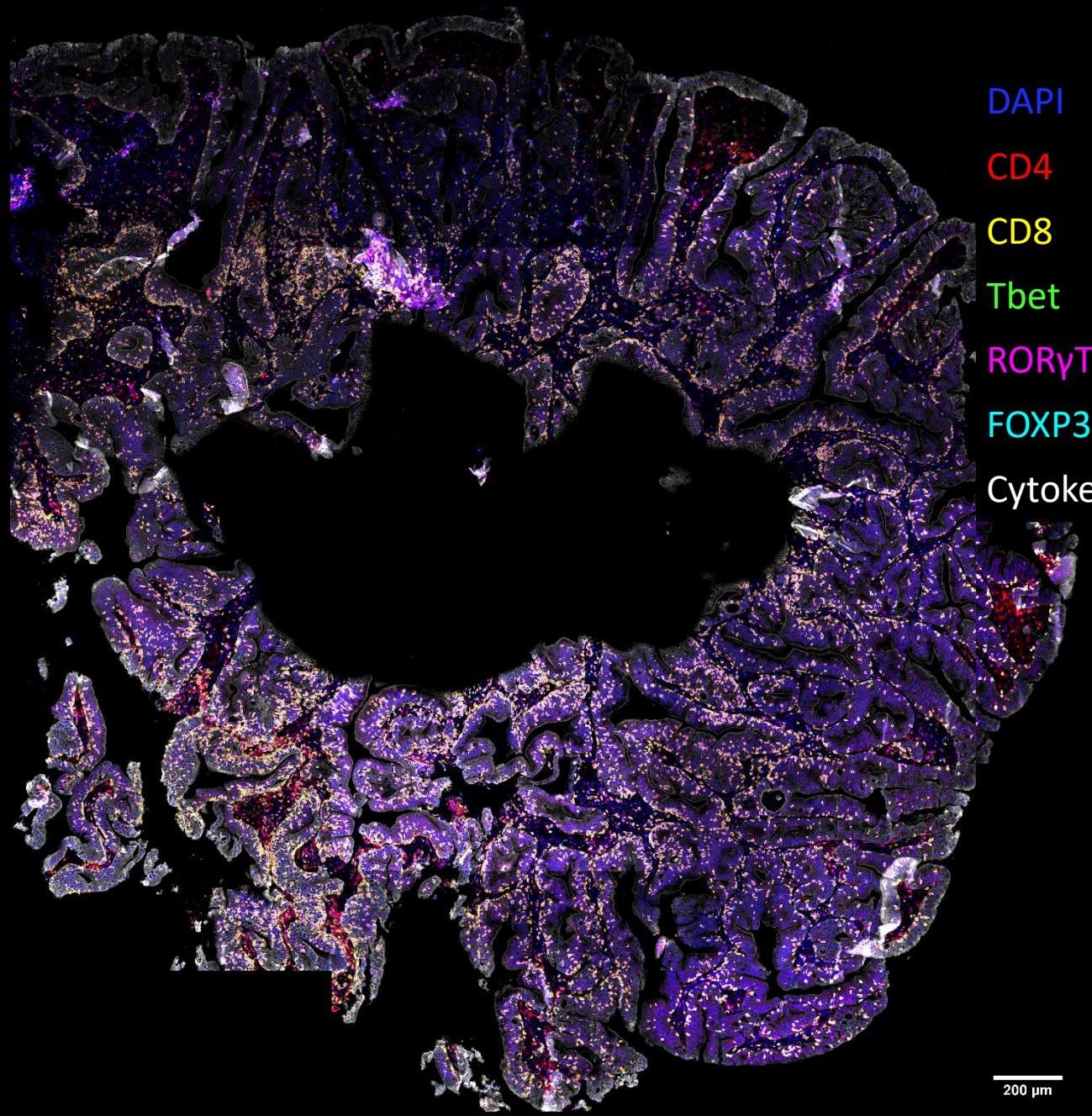
Shallow Machine Learning for Object Classification





Multi/Hyper-plexed Images

- Use a pixel classifier to segment epithelium and stroma, save it and run it
- Define a small annotation and run InstanSeg to optimize parameters for nuclei and cell segmentation
- Apply InstanSeg 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. Pécot *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. Pécot *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