Whole-Slide Image Analysis with open-source software QuPath

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Whole-Slide Image Size

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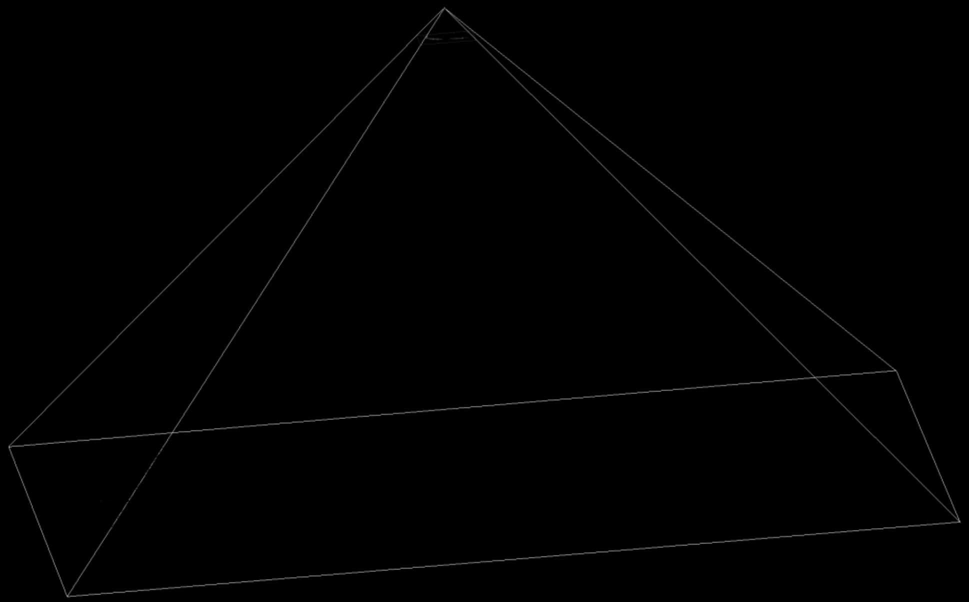
💡 **only** load the **required information** in the RAM memory:

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

Whole Slide Image – Pyramidal Representation



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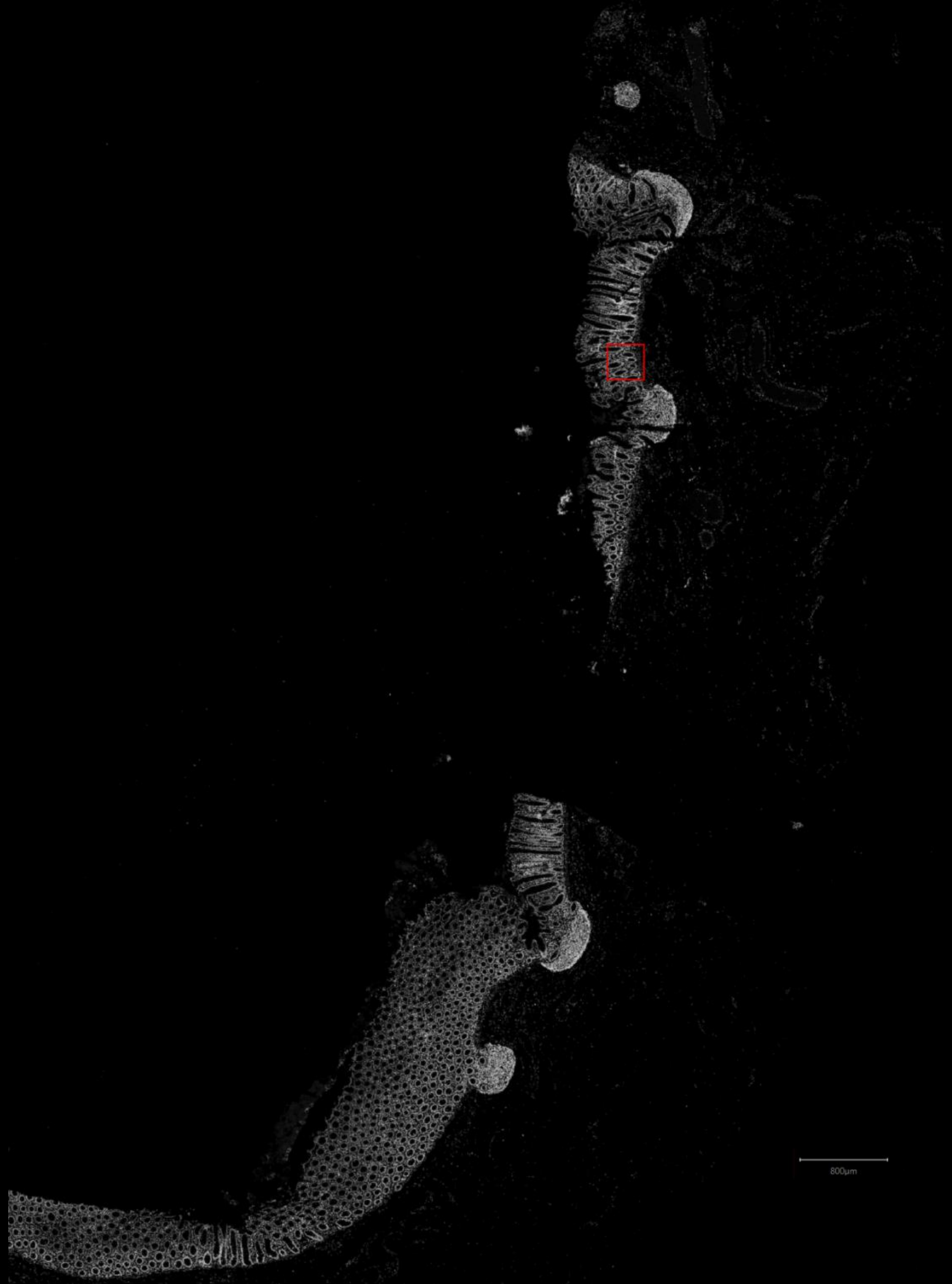

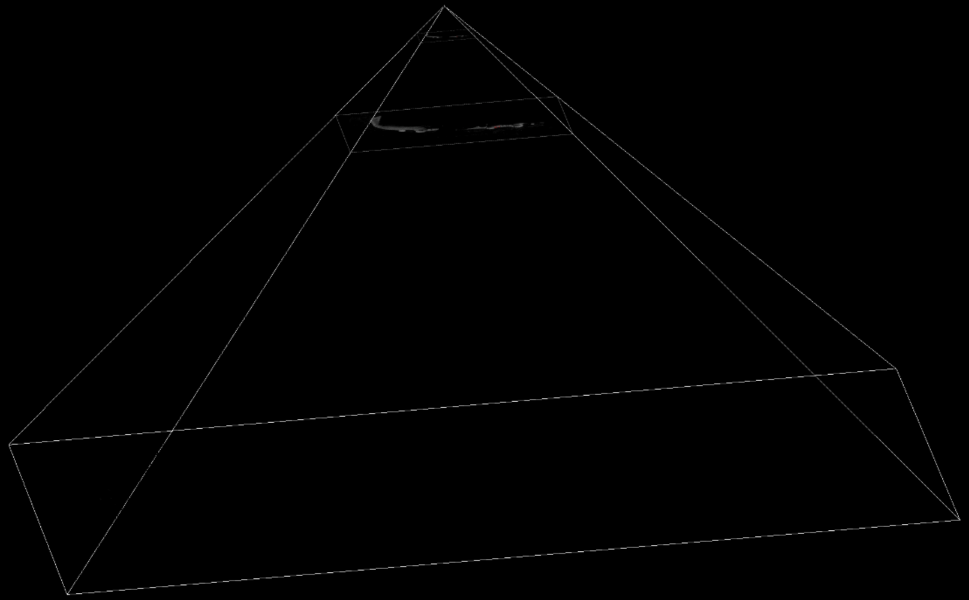

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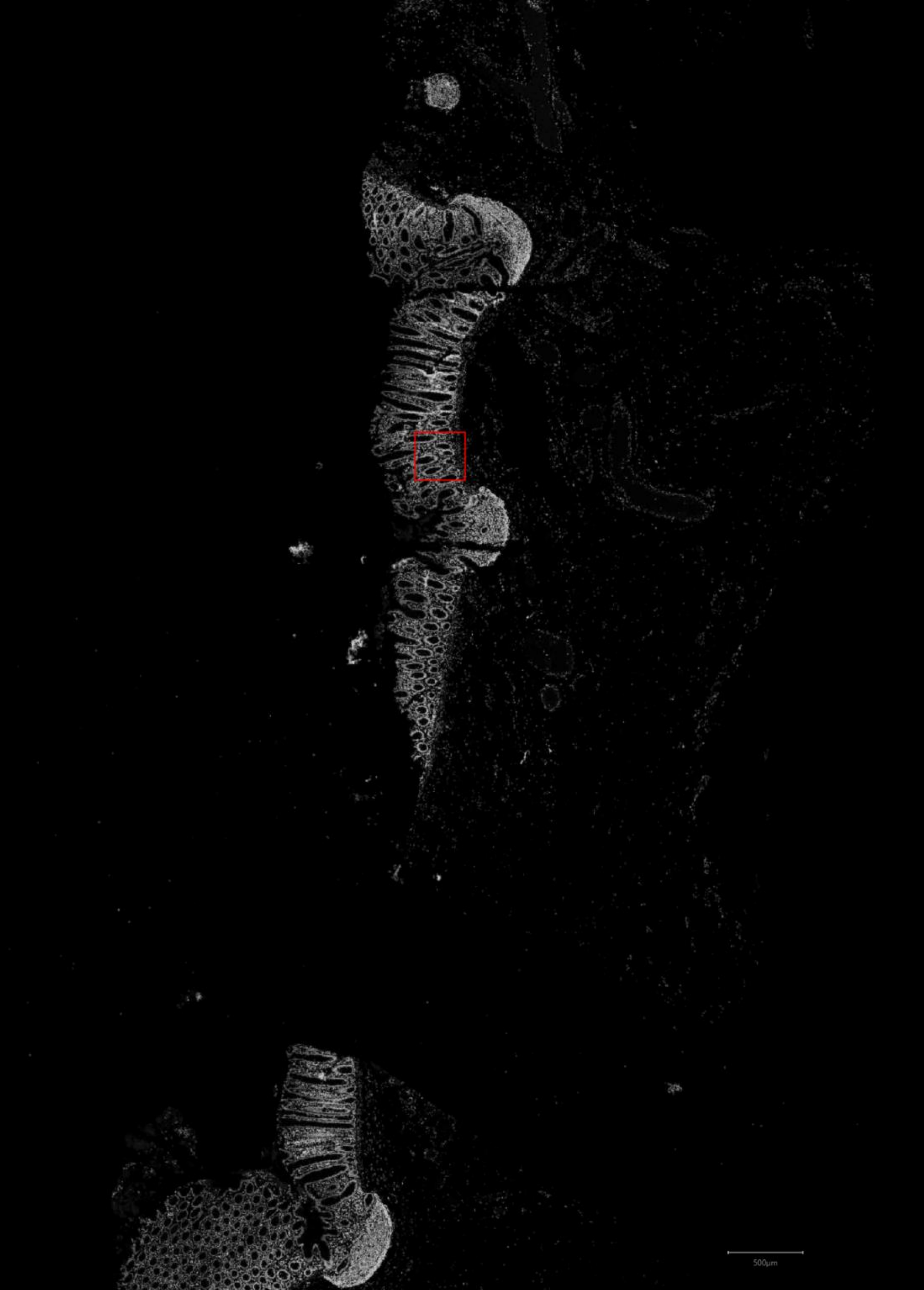

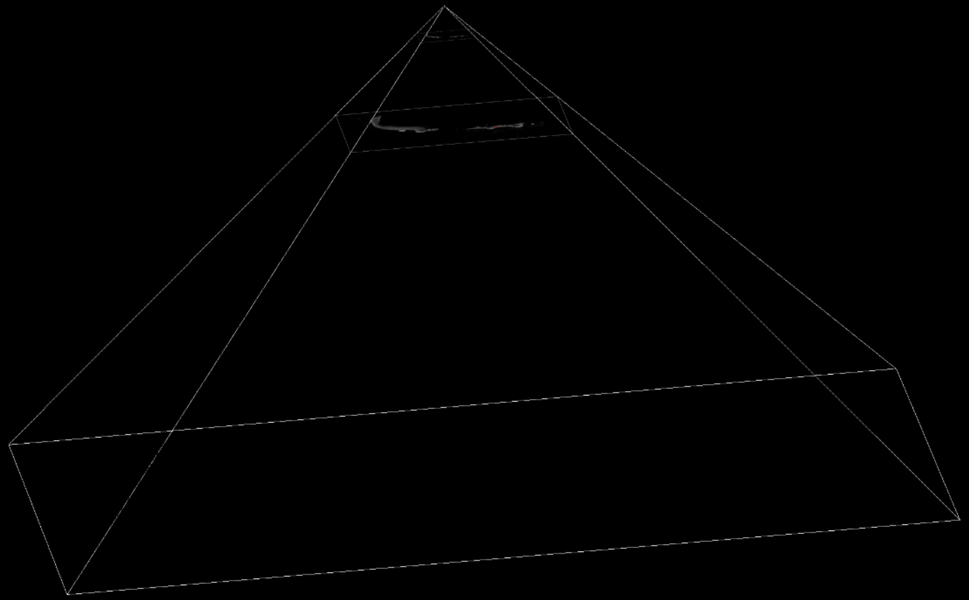

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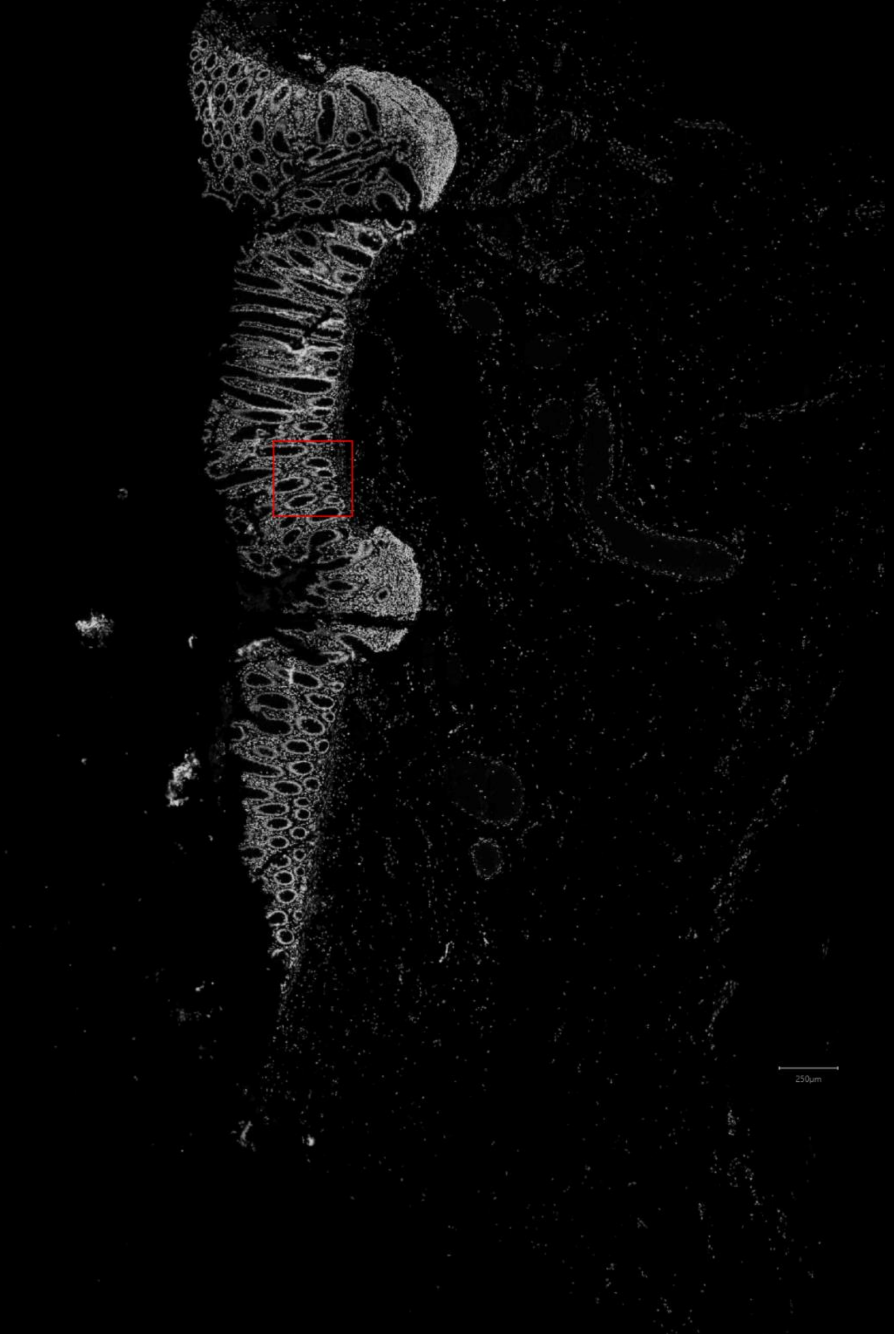

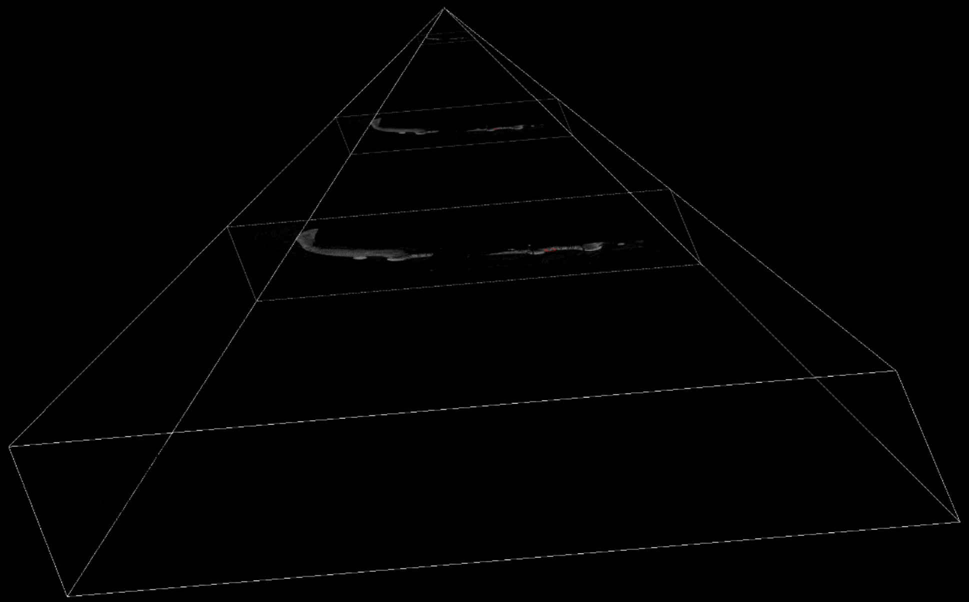

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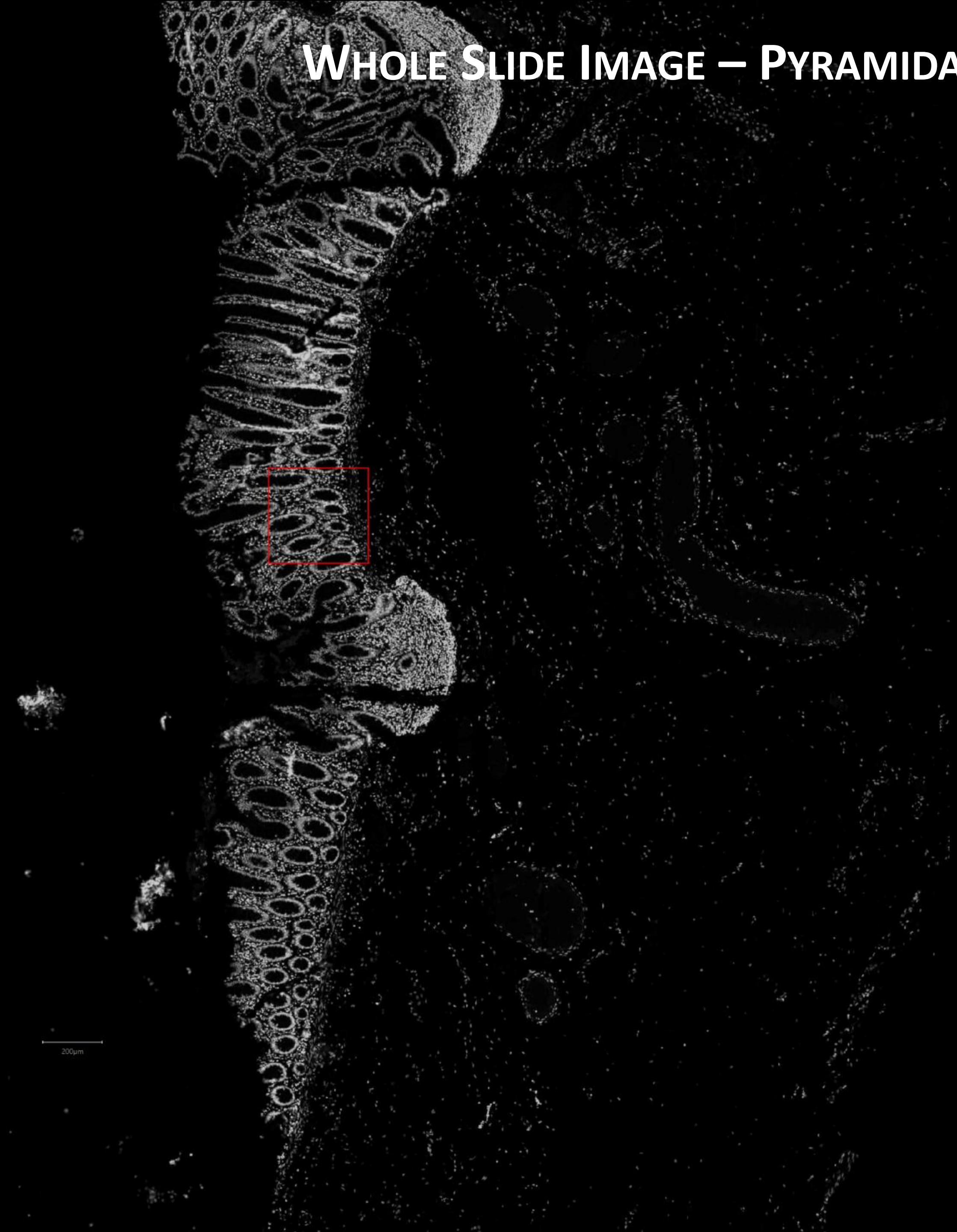

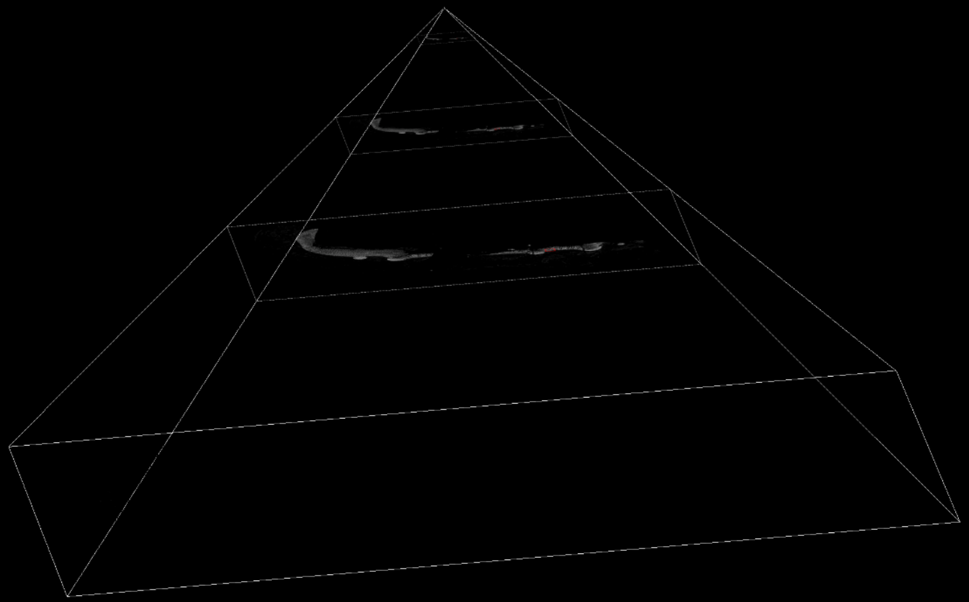

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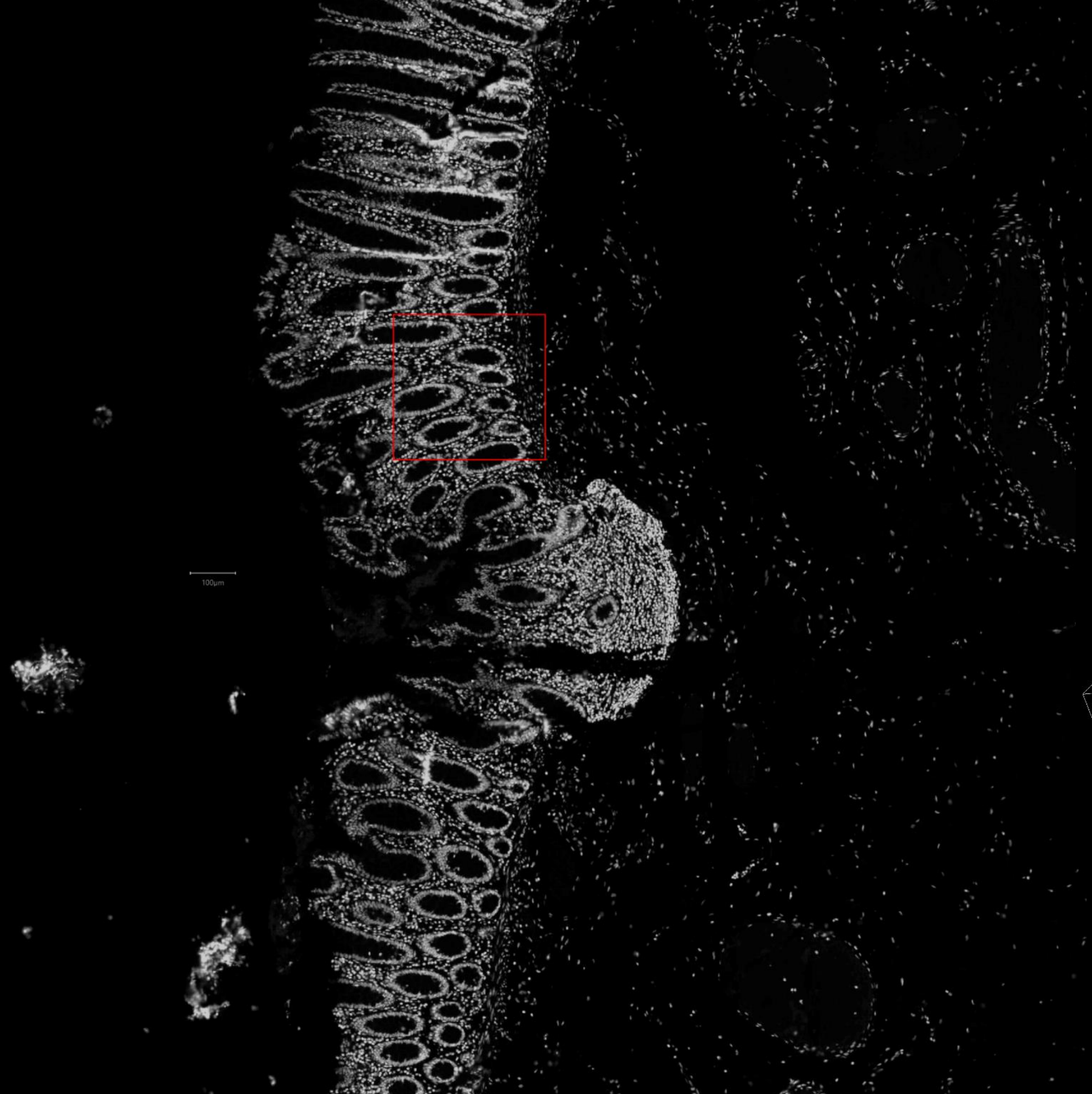
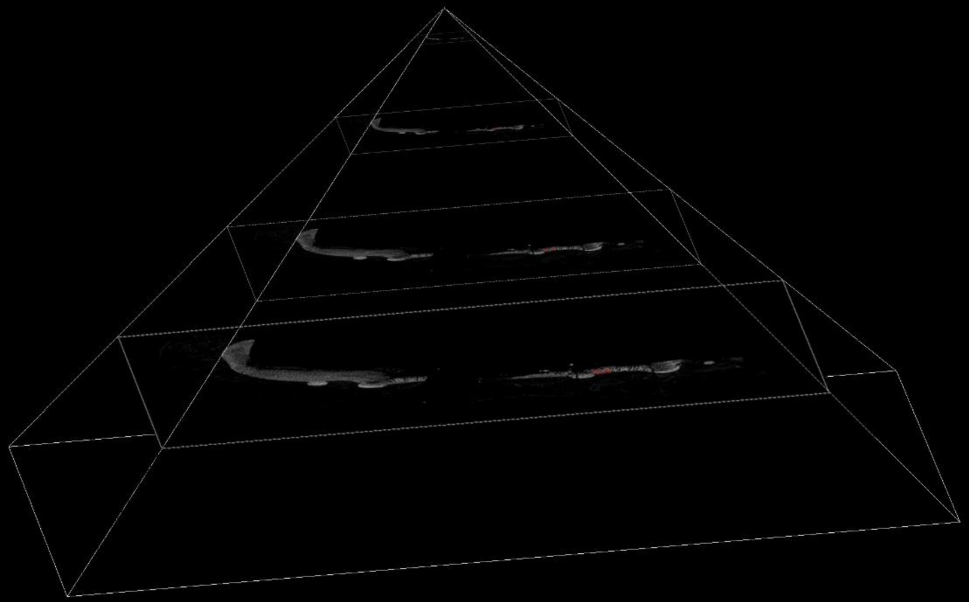
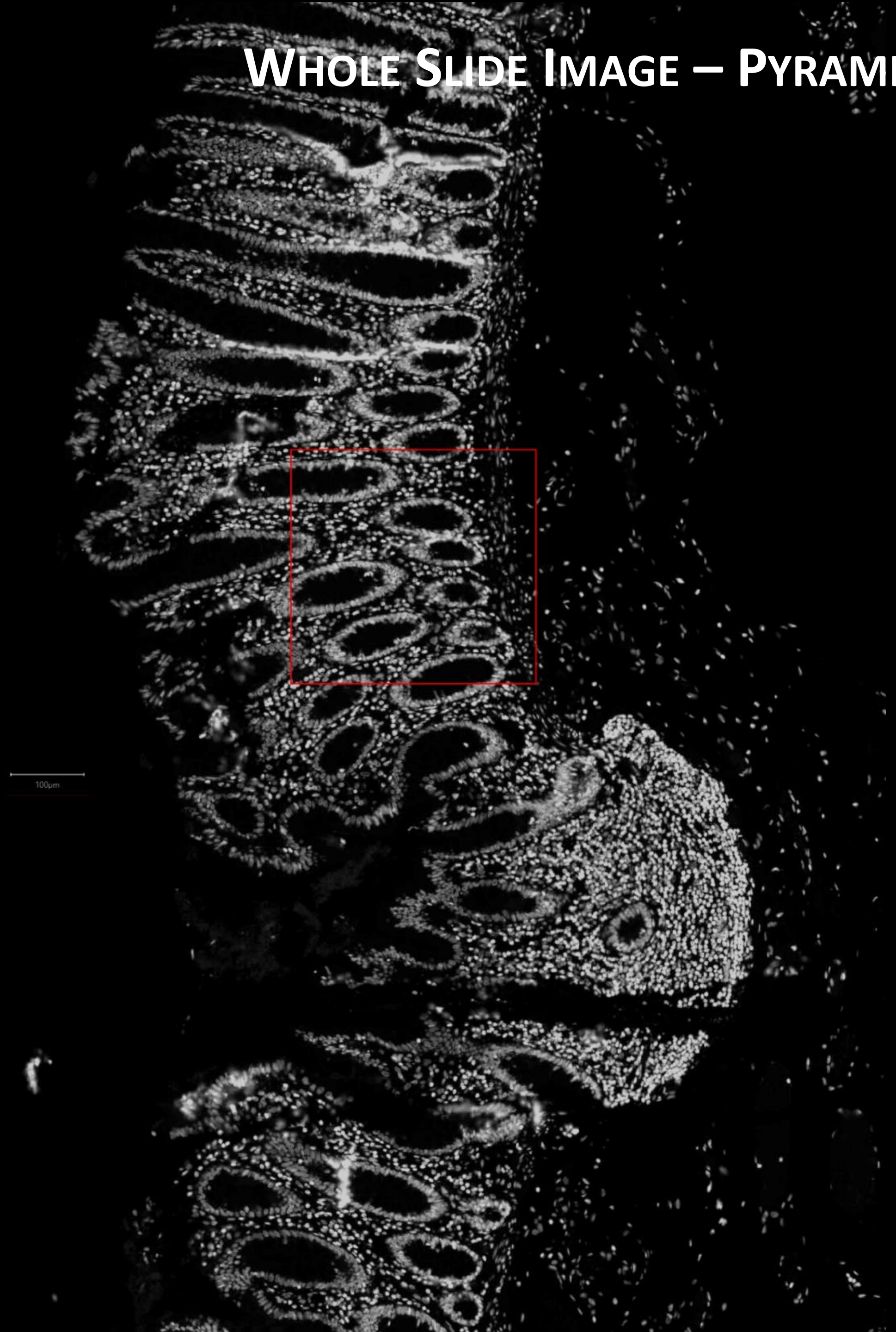
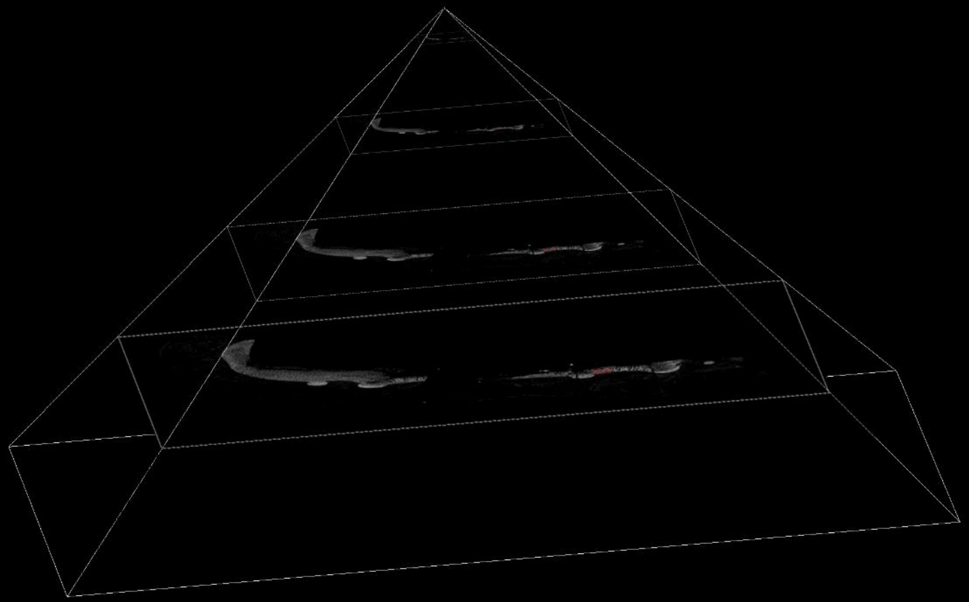




WHOLE SLIDE IMAGE – PYRAMIDAL REPRESENTATION





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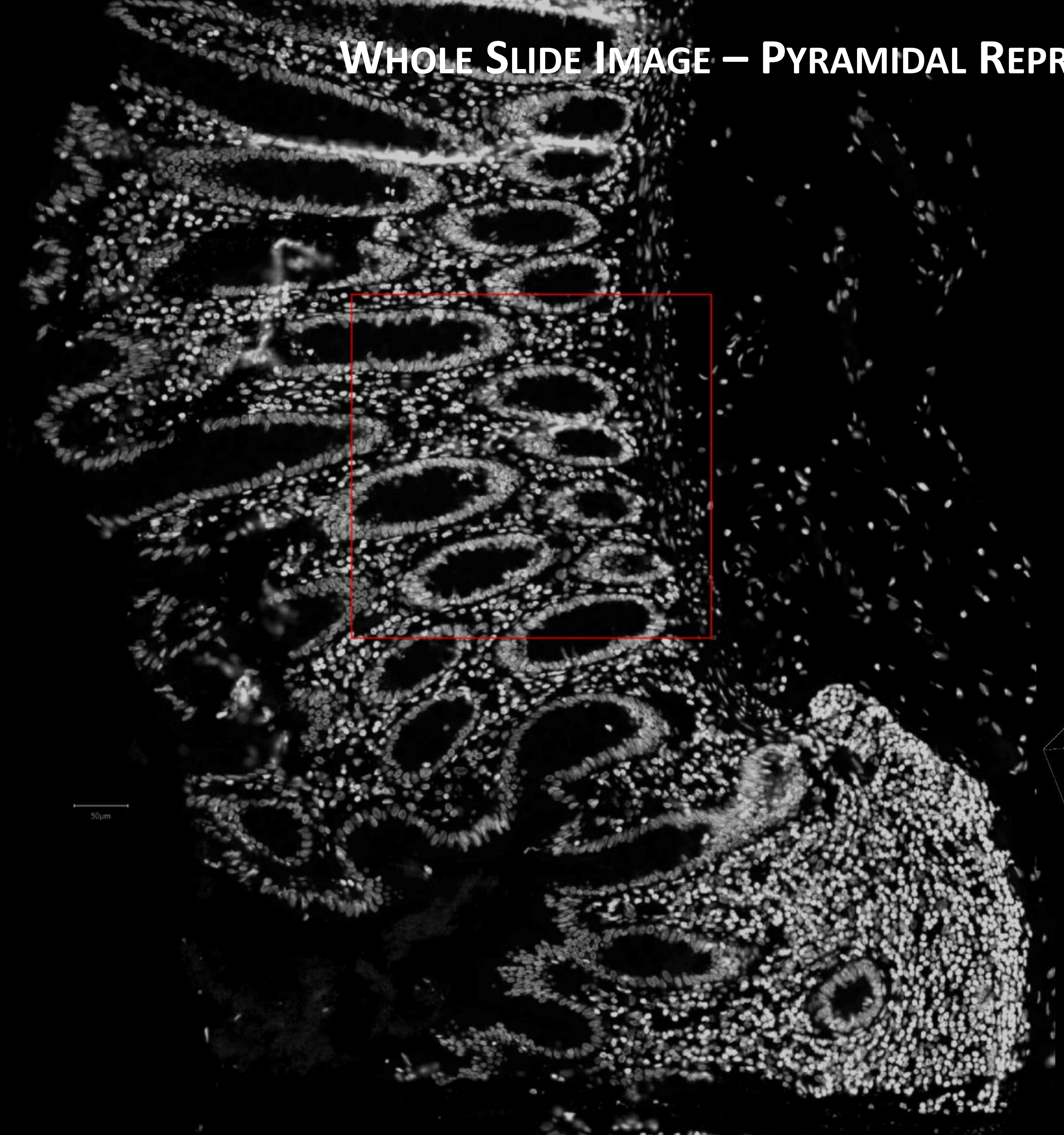

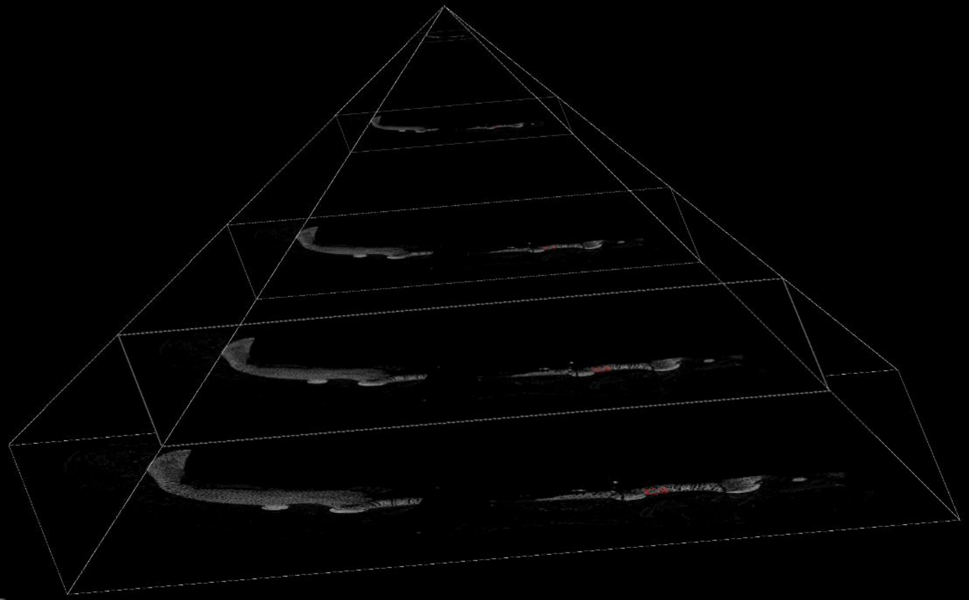

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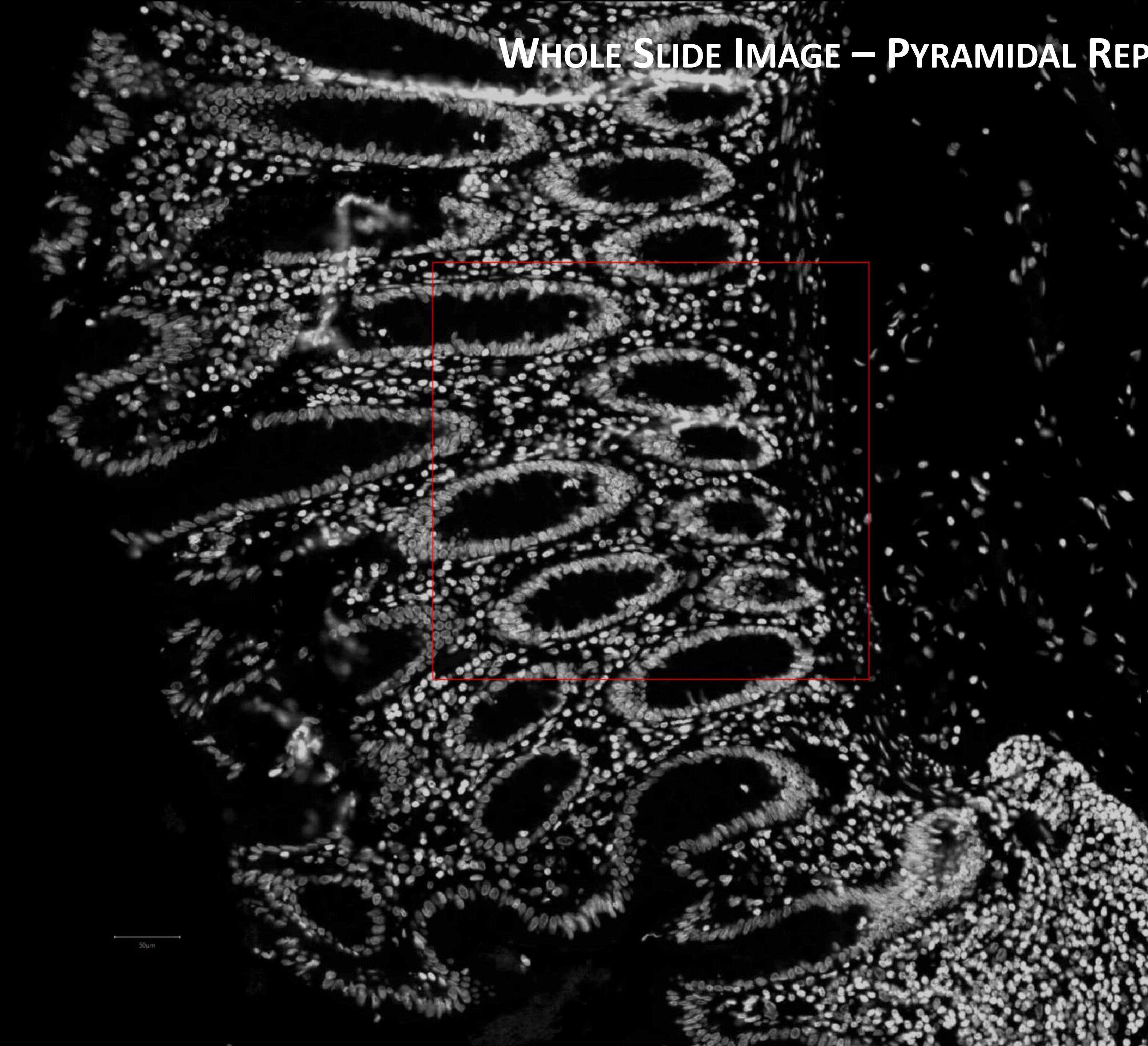

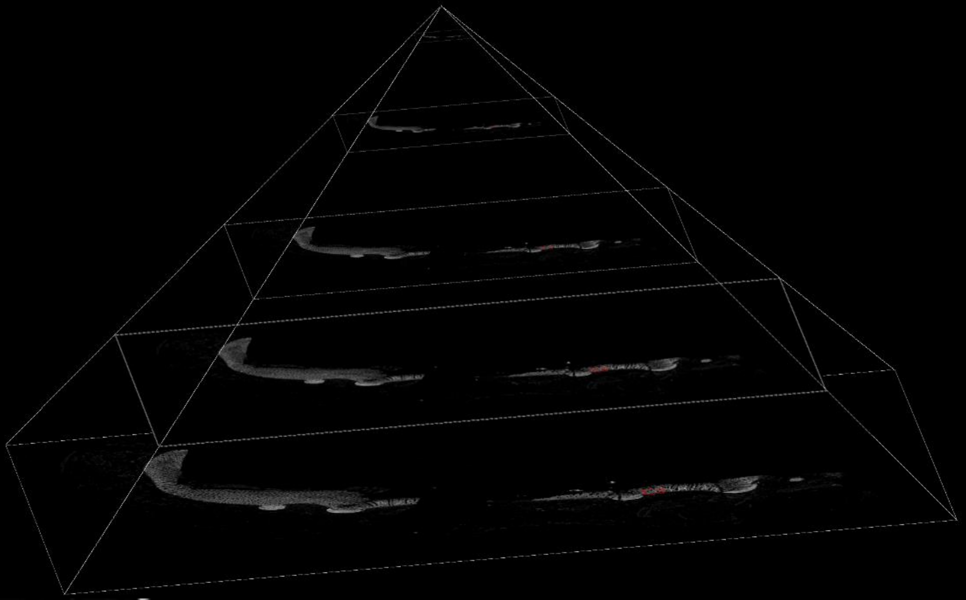

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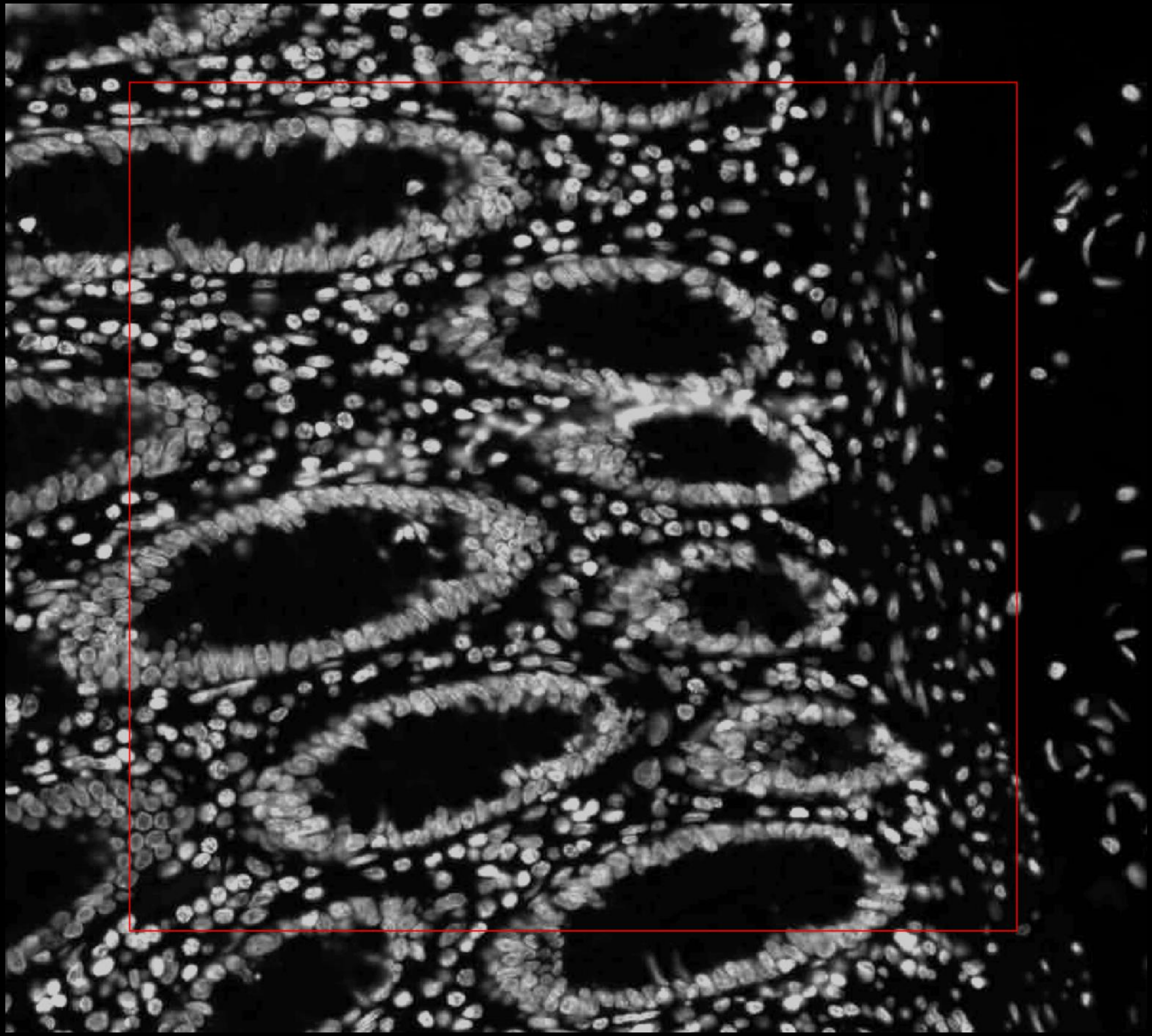
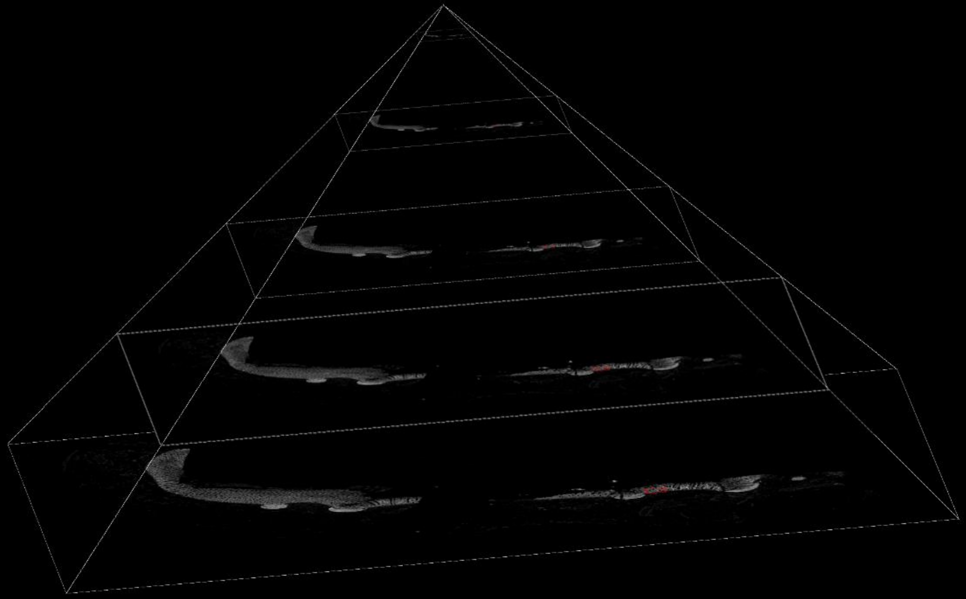
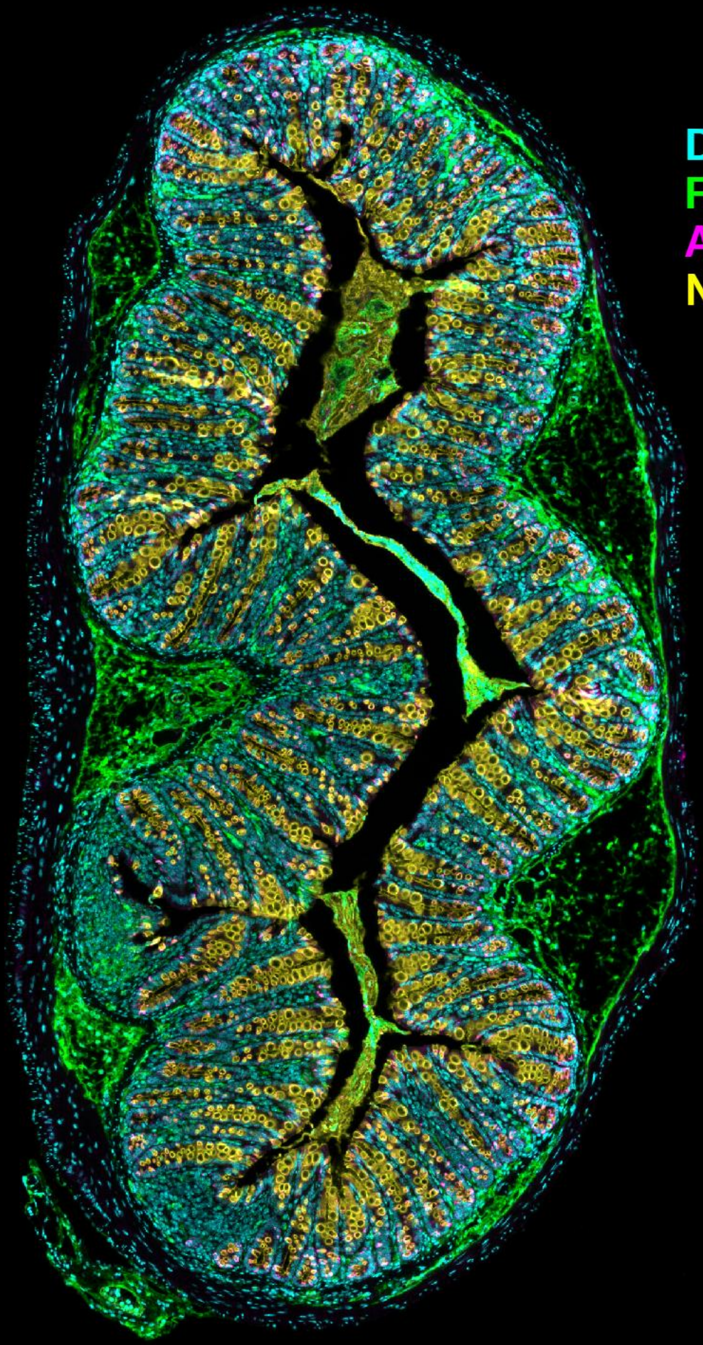




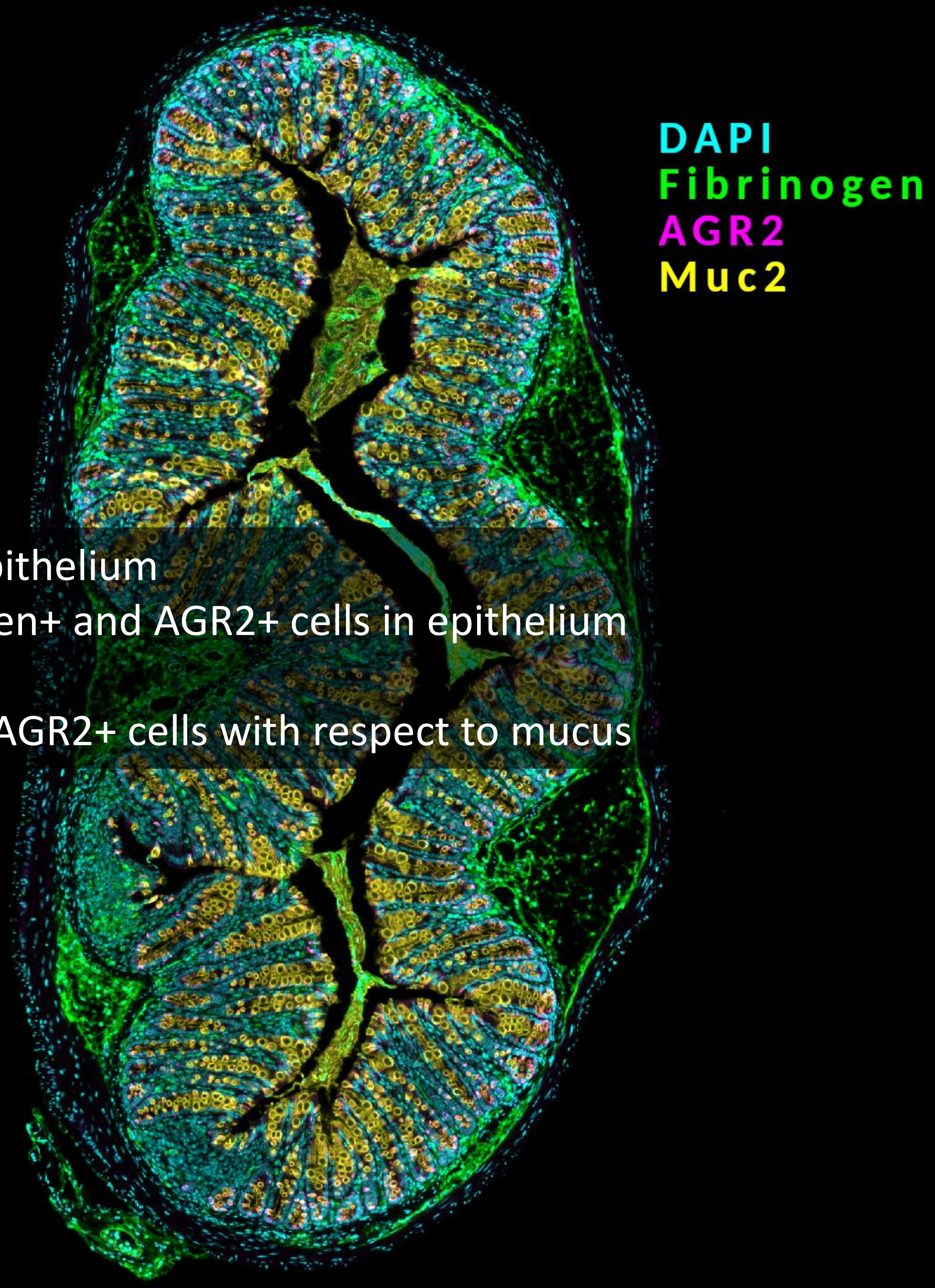
Whole-Slide Image Size

- 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**



Mouse colon, Marine Seffals, H2P2



- Muc2 tissue ratio in epithelium
- Proportion of Fibrinogen+ and AGR2+ cells in epithelium and in the stroma
- Spatial distribution of AGR2+ cells with respect to mucus

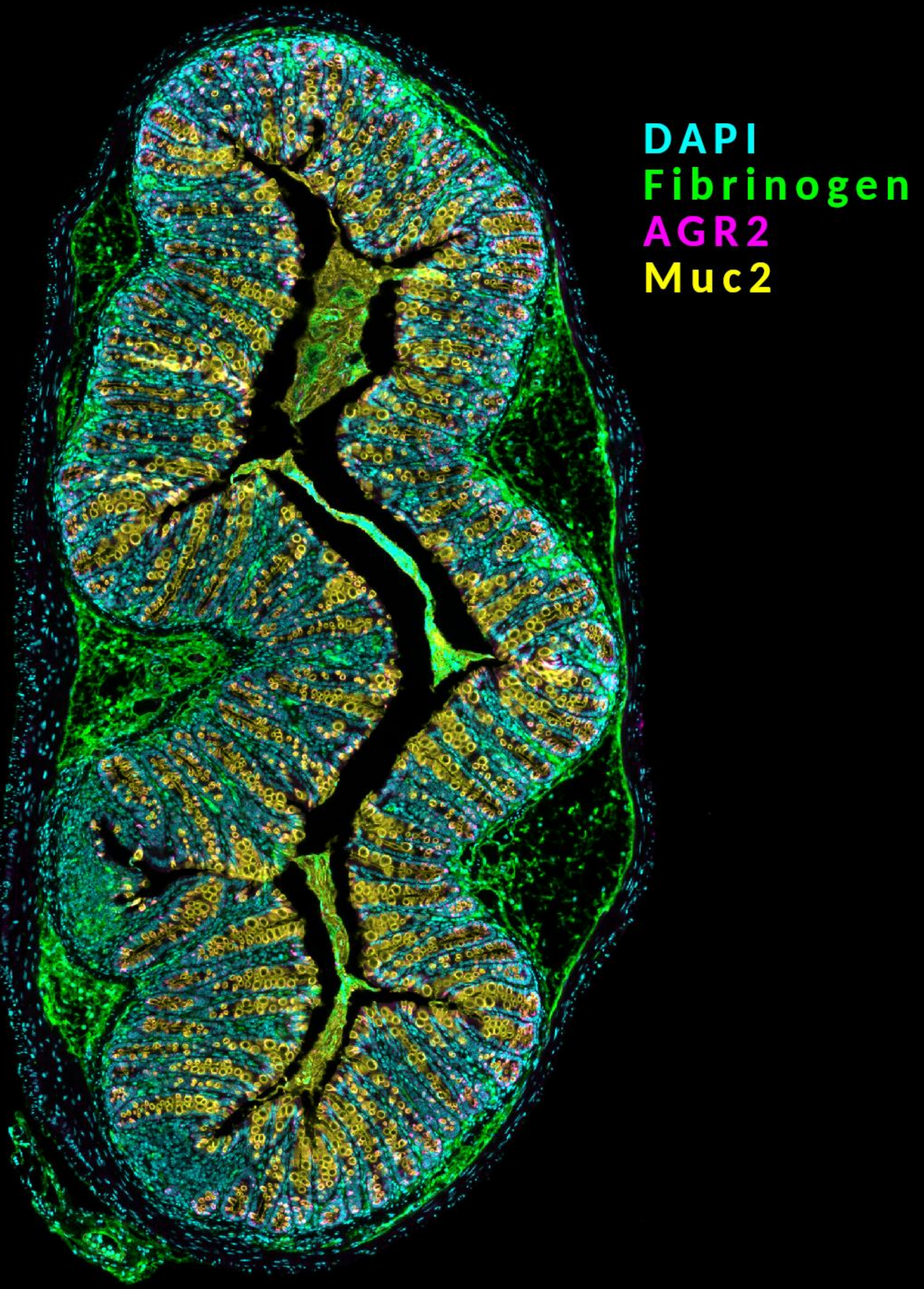
Mouse colon, Marine Seffals, H2P2

ANNOTATIONS

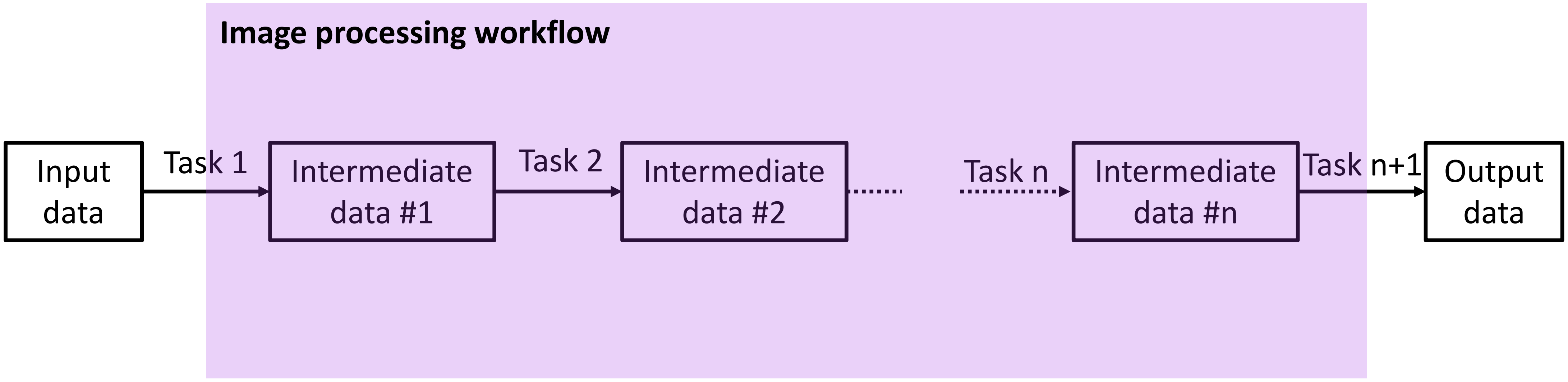
- Allow to **add information** to specific regions or entire images
- Lots of **features/measurements** can then be extracted from these regions
- **Powerful** and **storage-efficient** way to process images
- Can be **manually** defined or **automatically** estimated
- Can be enriched by **adding classes**

ANNOTATIONS

- **Open** Colon_1.ome.tif
- Create **different types** of annotations
- Play with **resolution**
- Look at the **measurements** for each **type of annotation**



EXPLICIT PROGRAMMING



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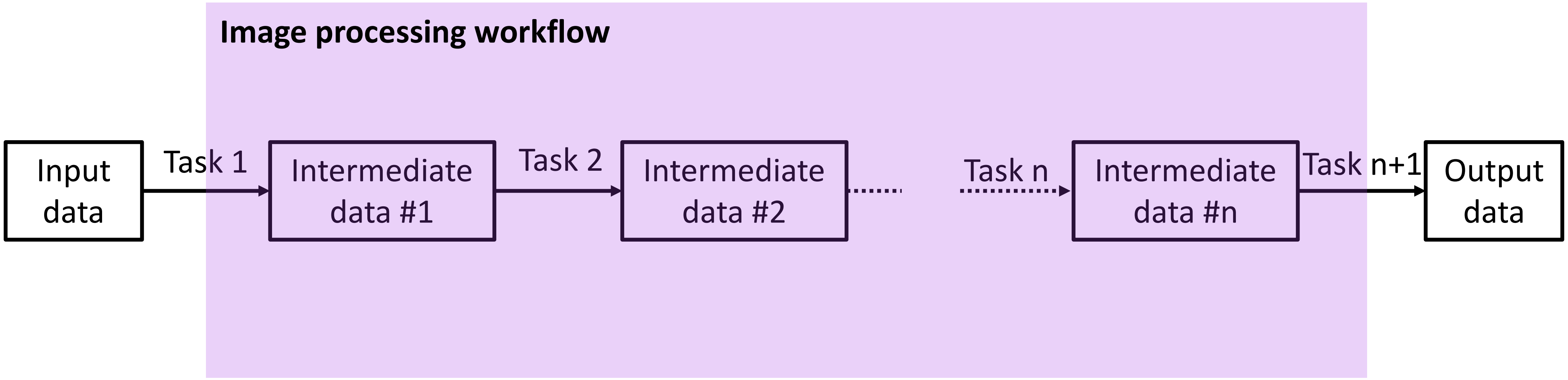
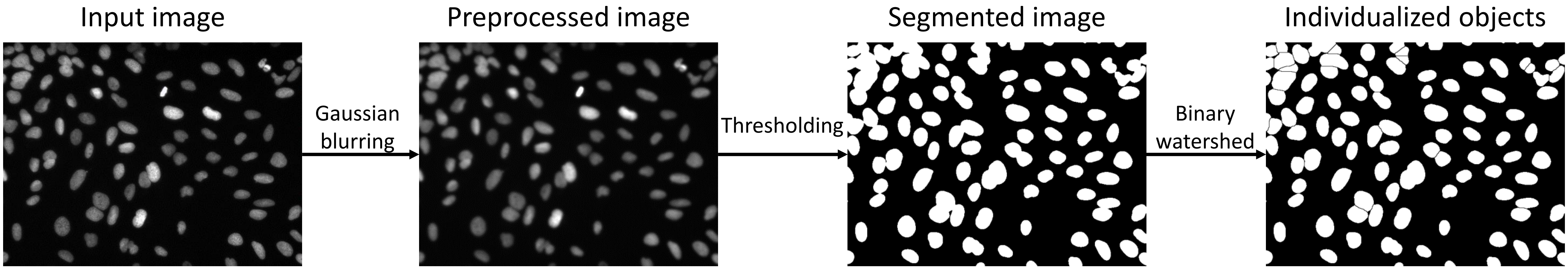
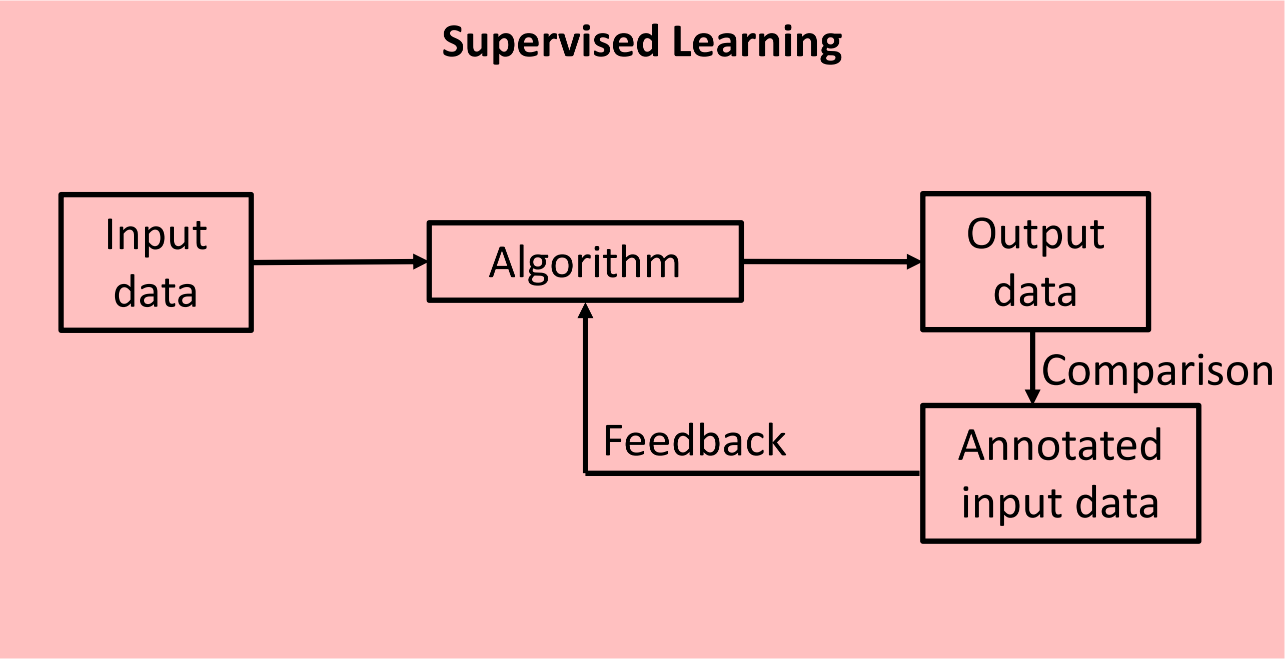




SUPERVISED MACHINE LEARNING



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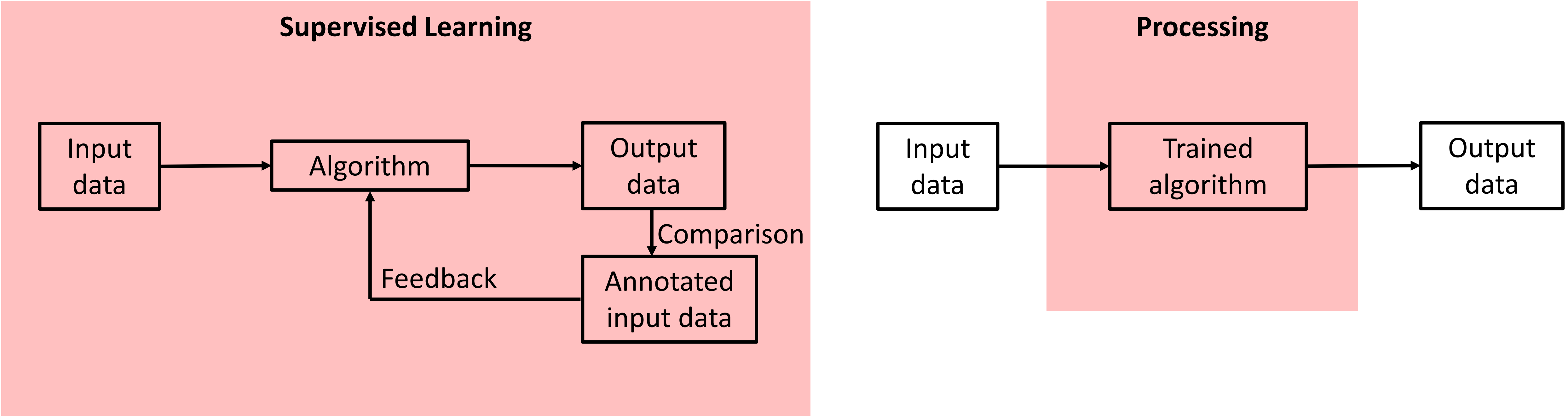

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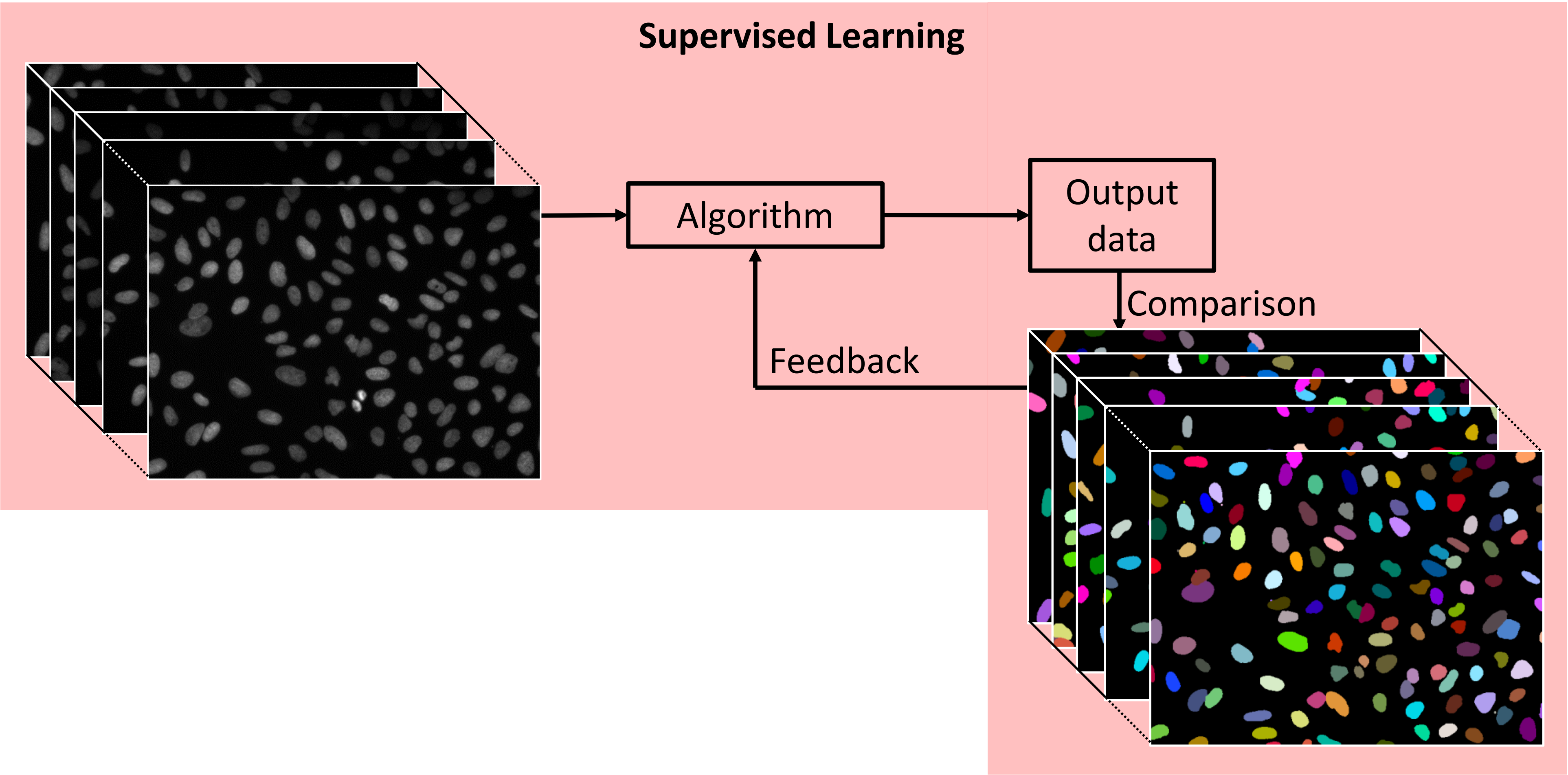


Supervised Learning

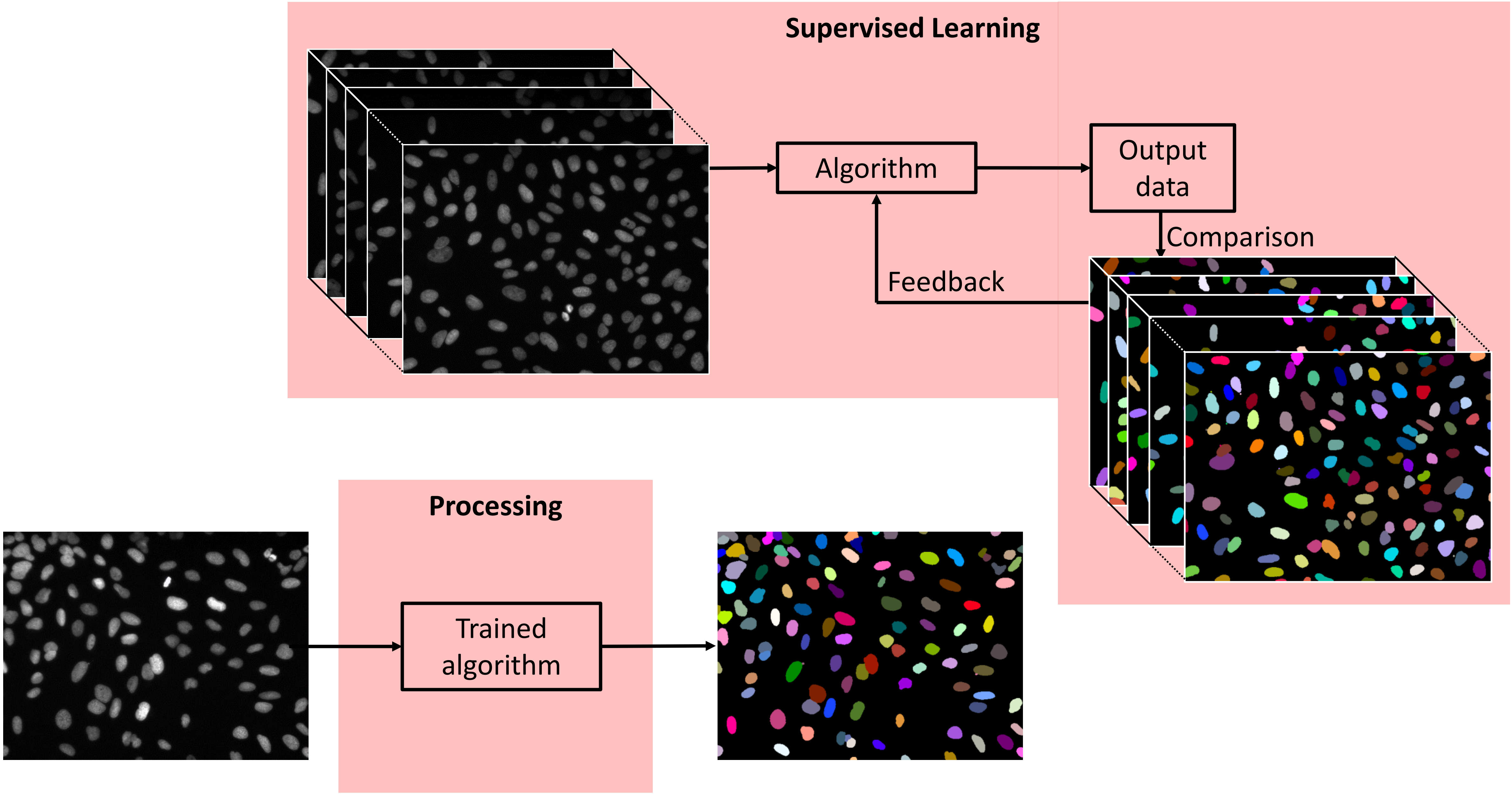
Algorithm

Output data

Comparison

Feedback

Supervised Machine Learning

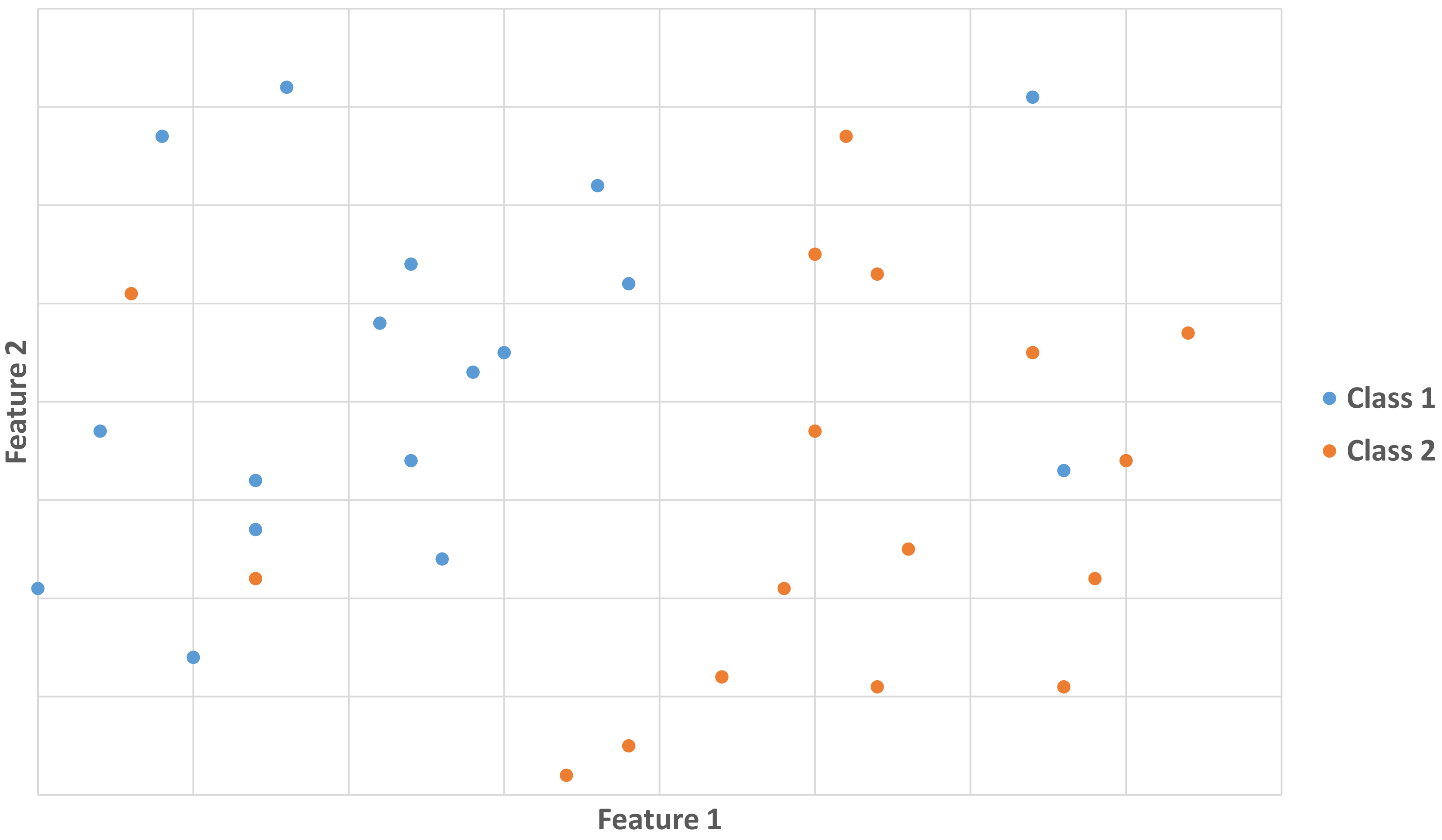


Shallow Machine Learning for Pixel Classification

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

Shallow Machine Learning for Pixel Classification



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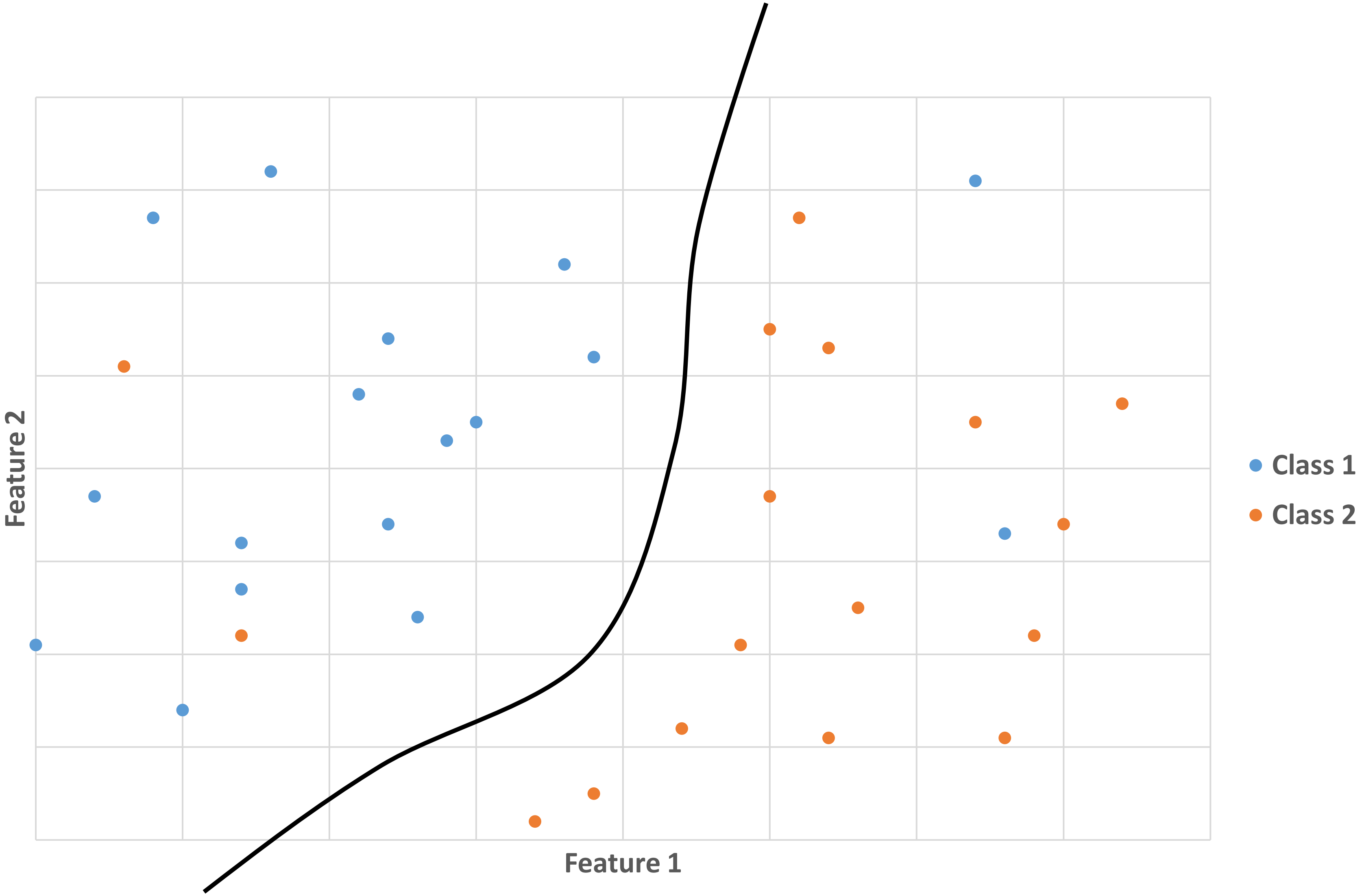
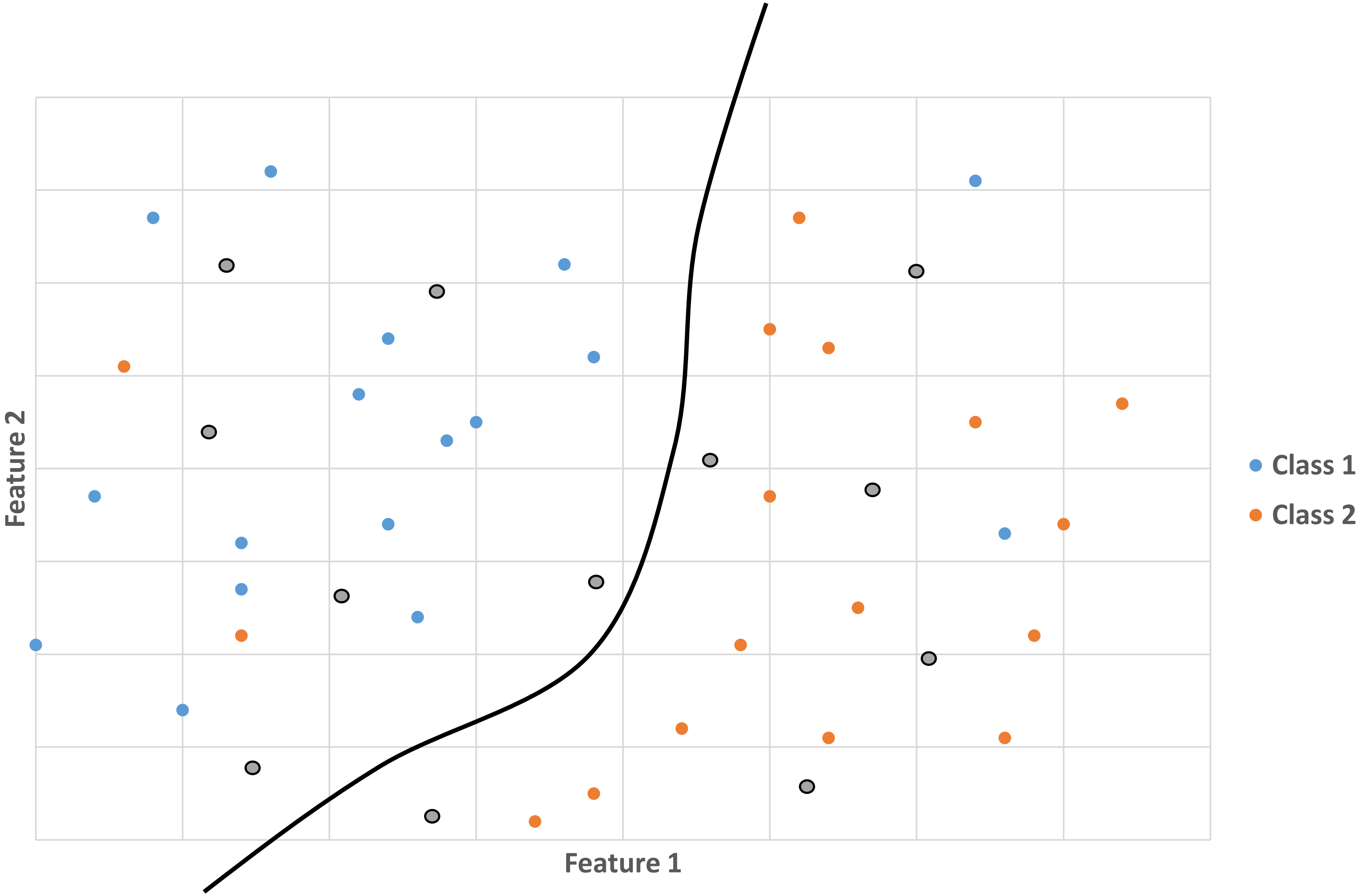


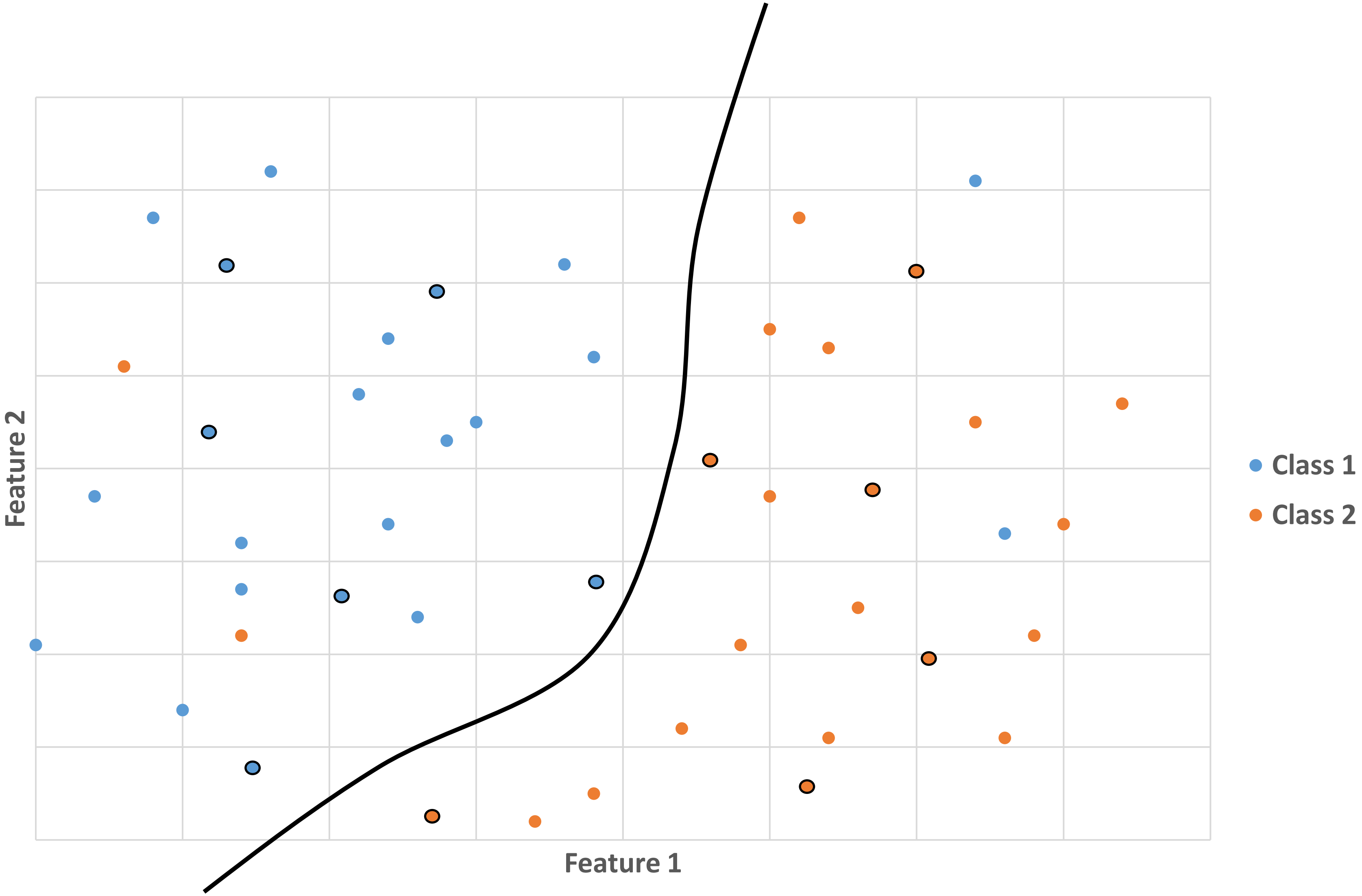
Training a classifier consists in **estimating** the **"best" separation** between classes

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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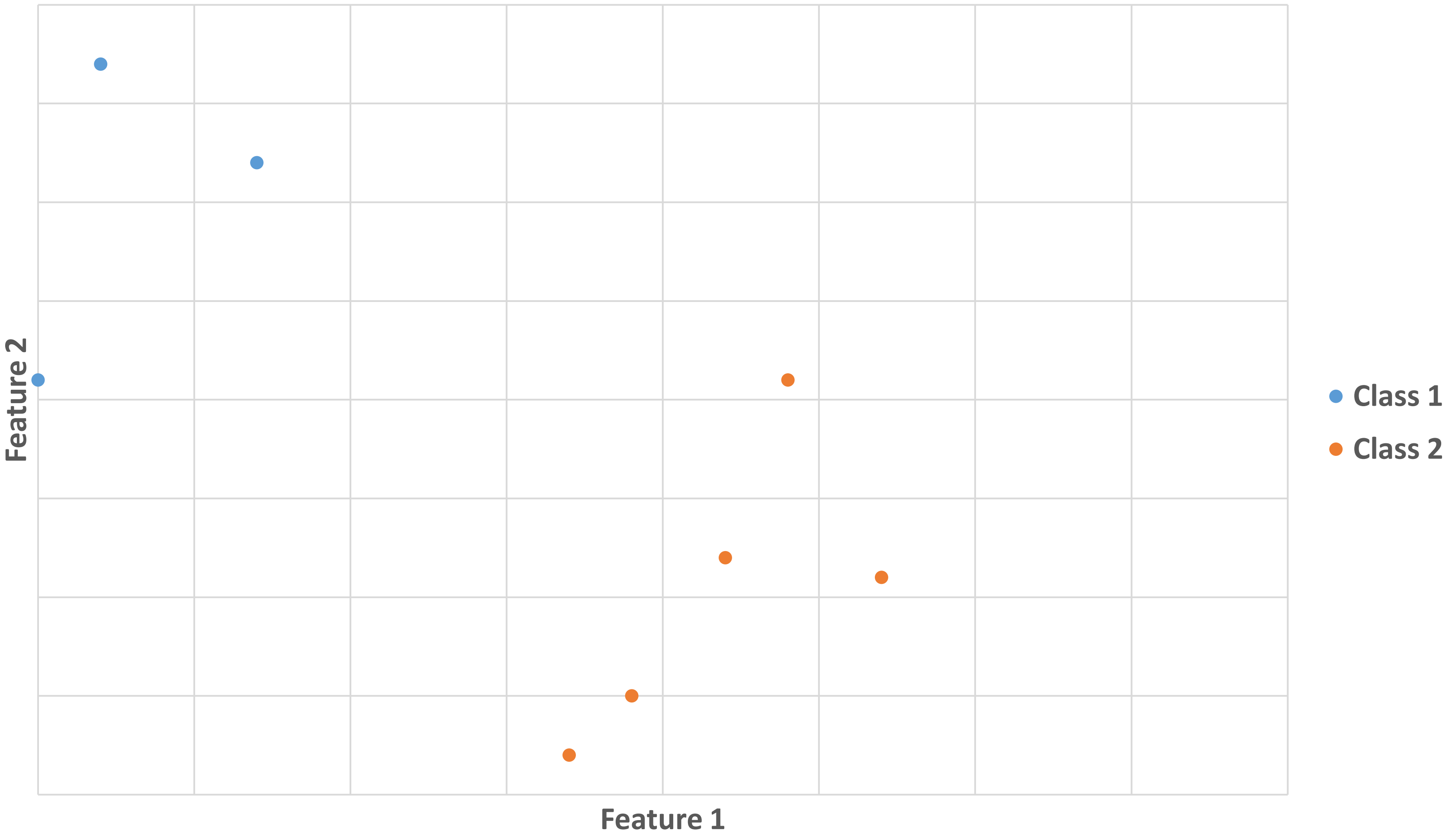
Shallow Machine Learning for Pixel Classification



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

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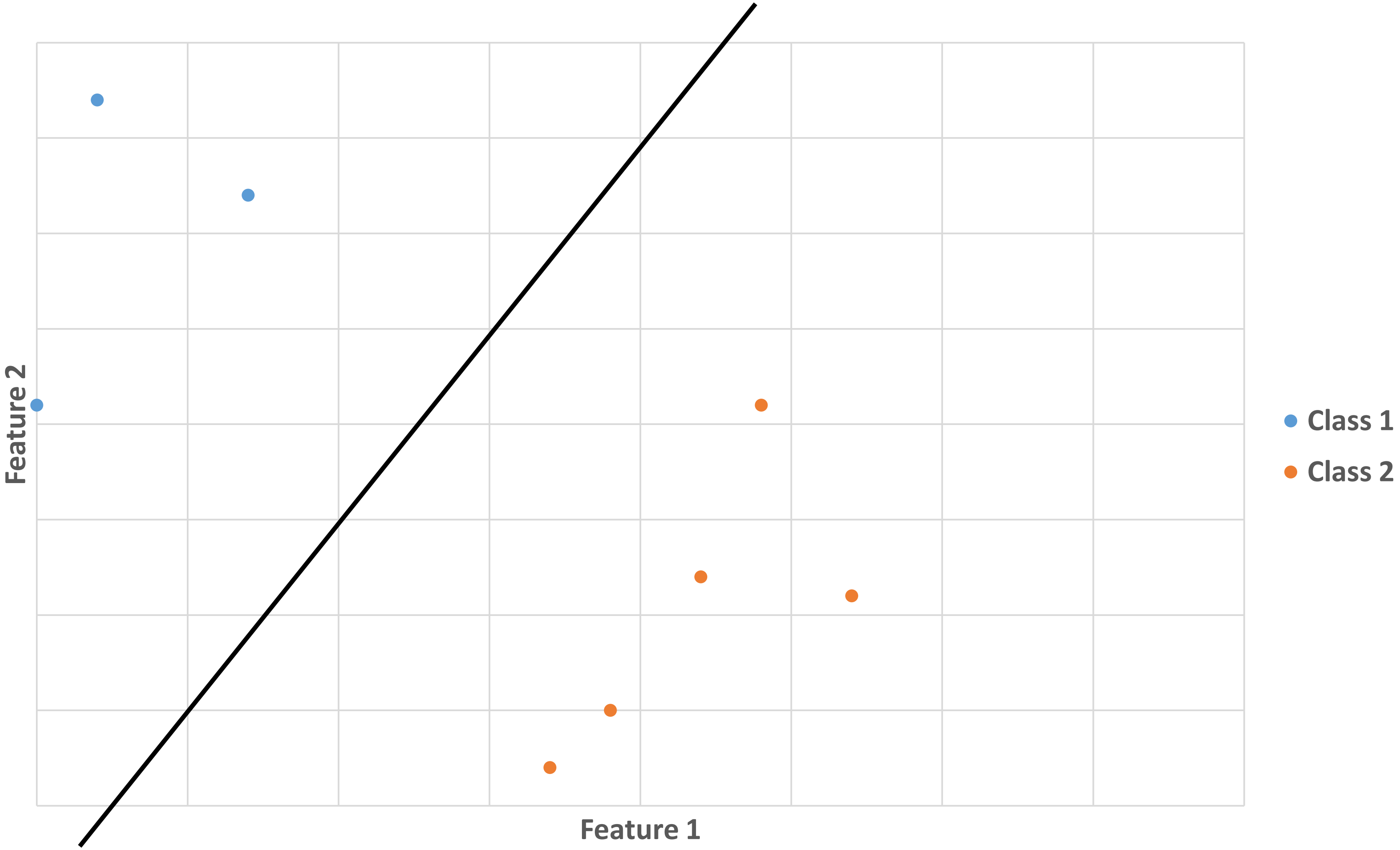

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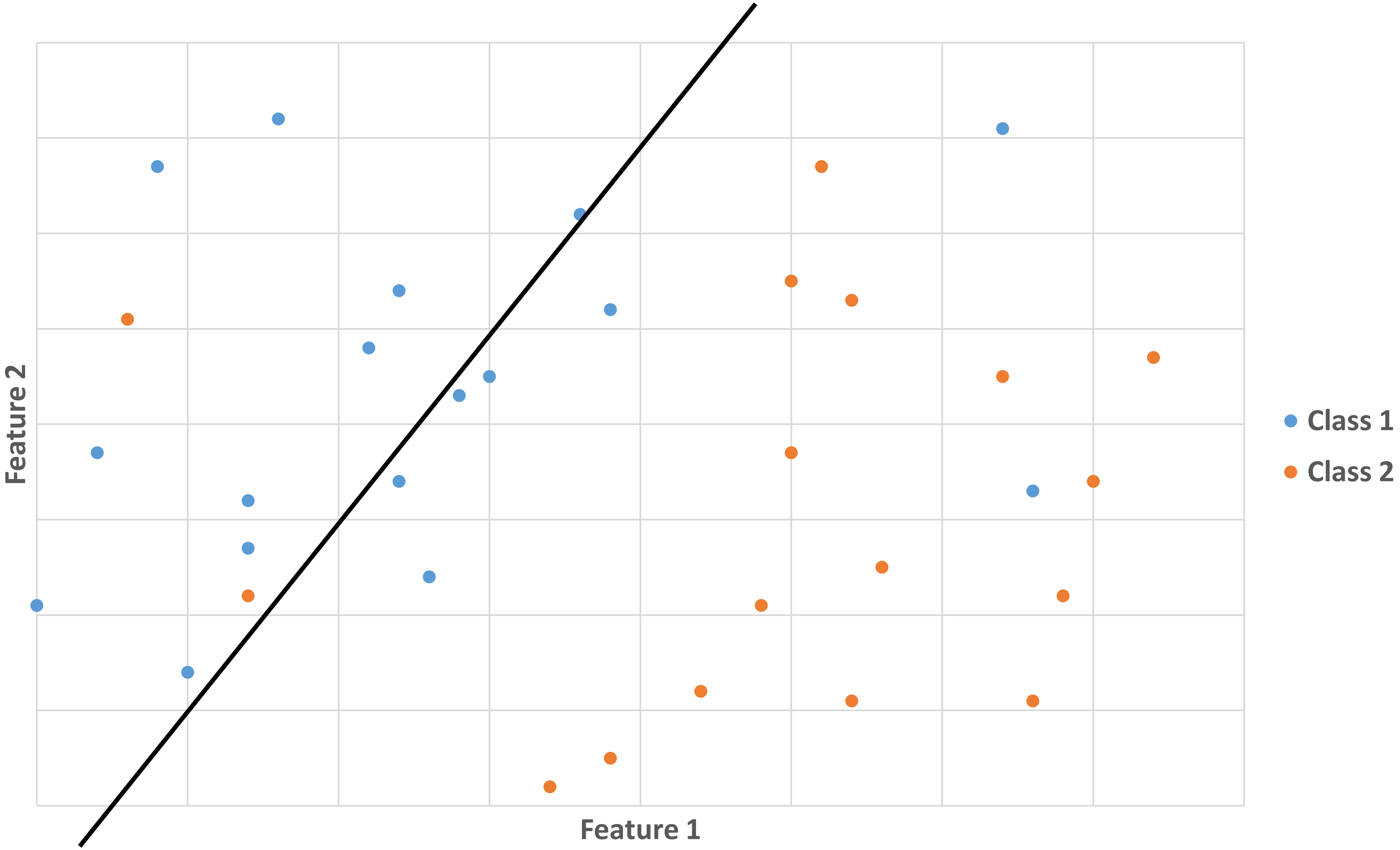

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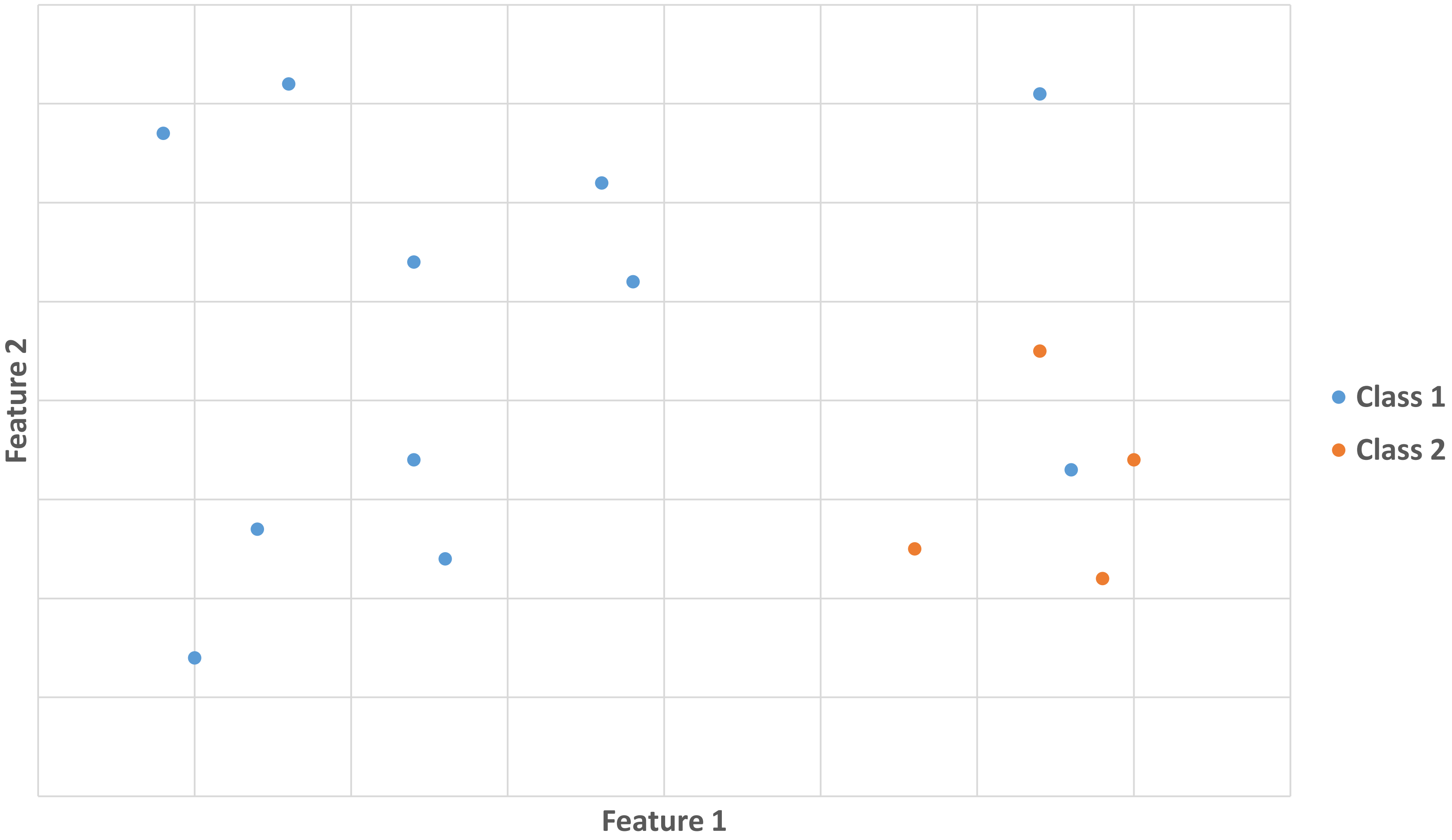
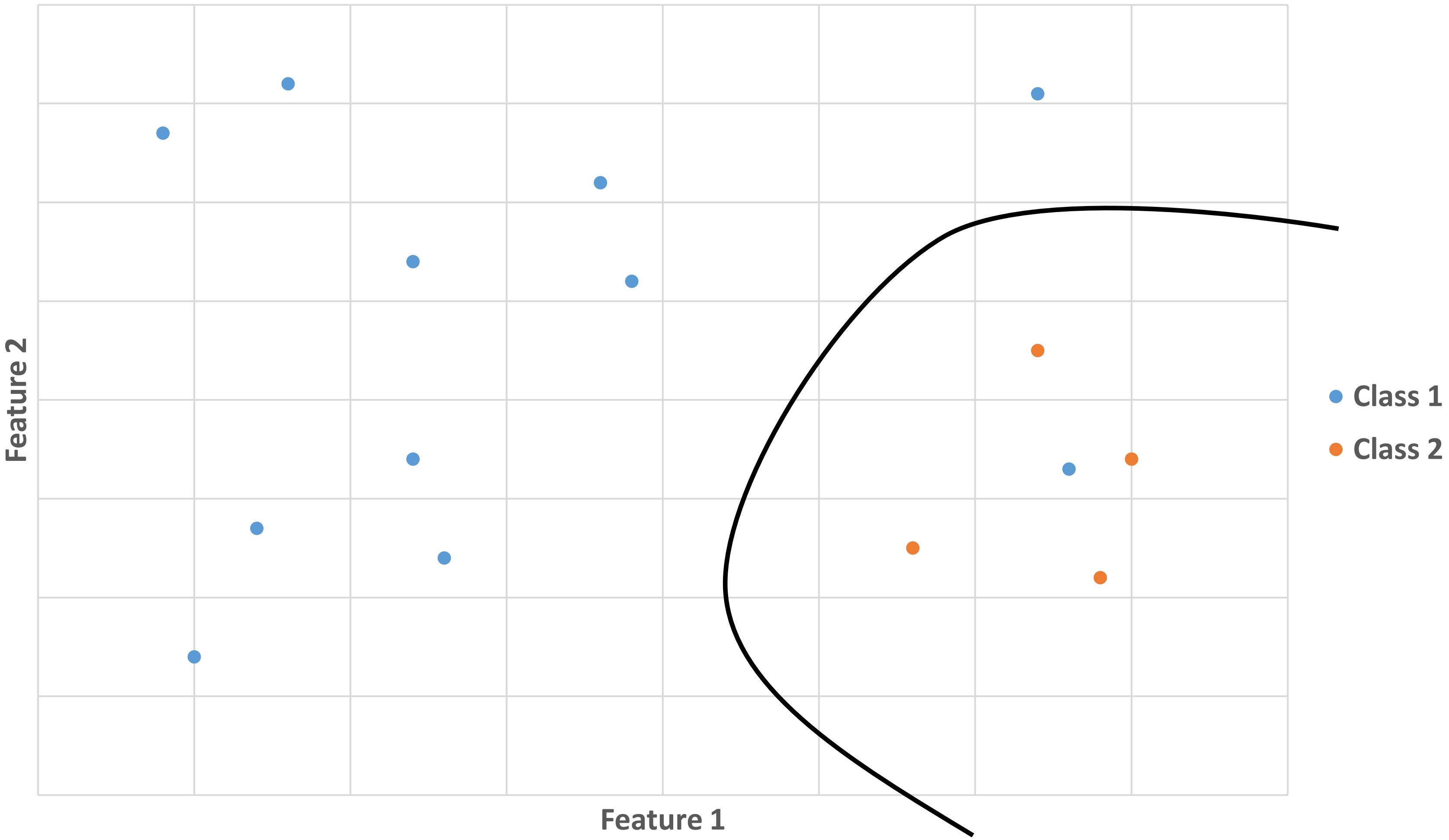


Class imbalance makes the training **more difficult**

Shallow Machine Learning for Pixel Classification



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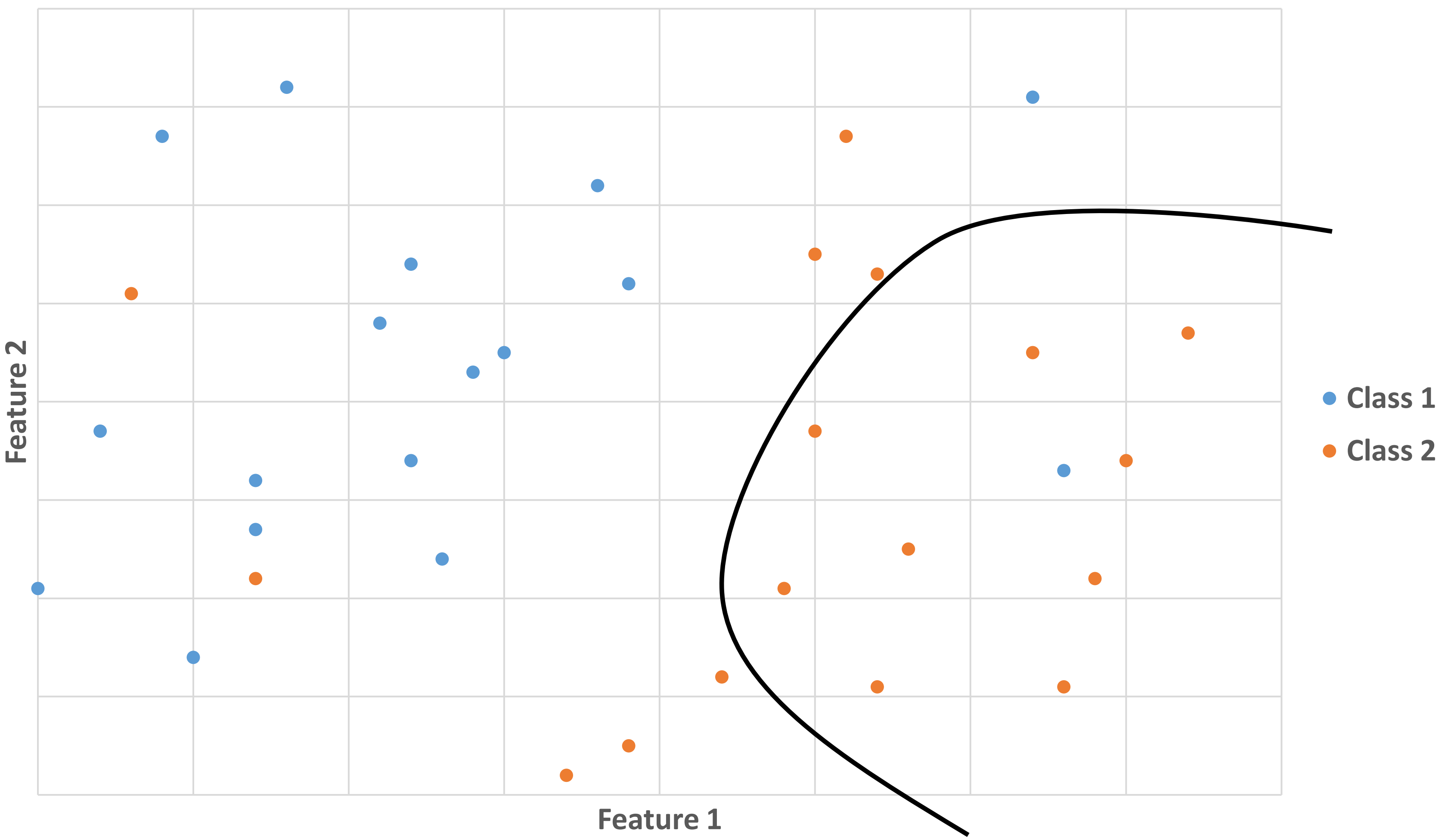

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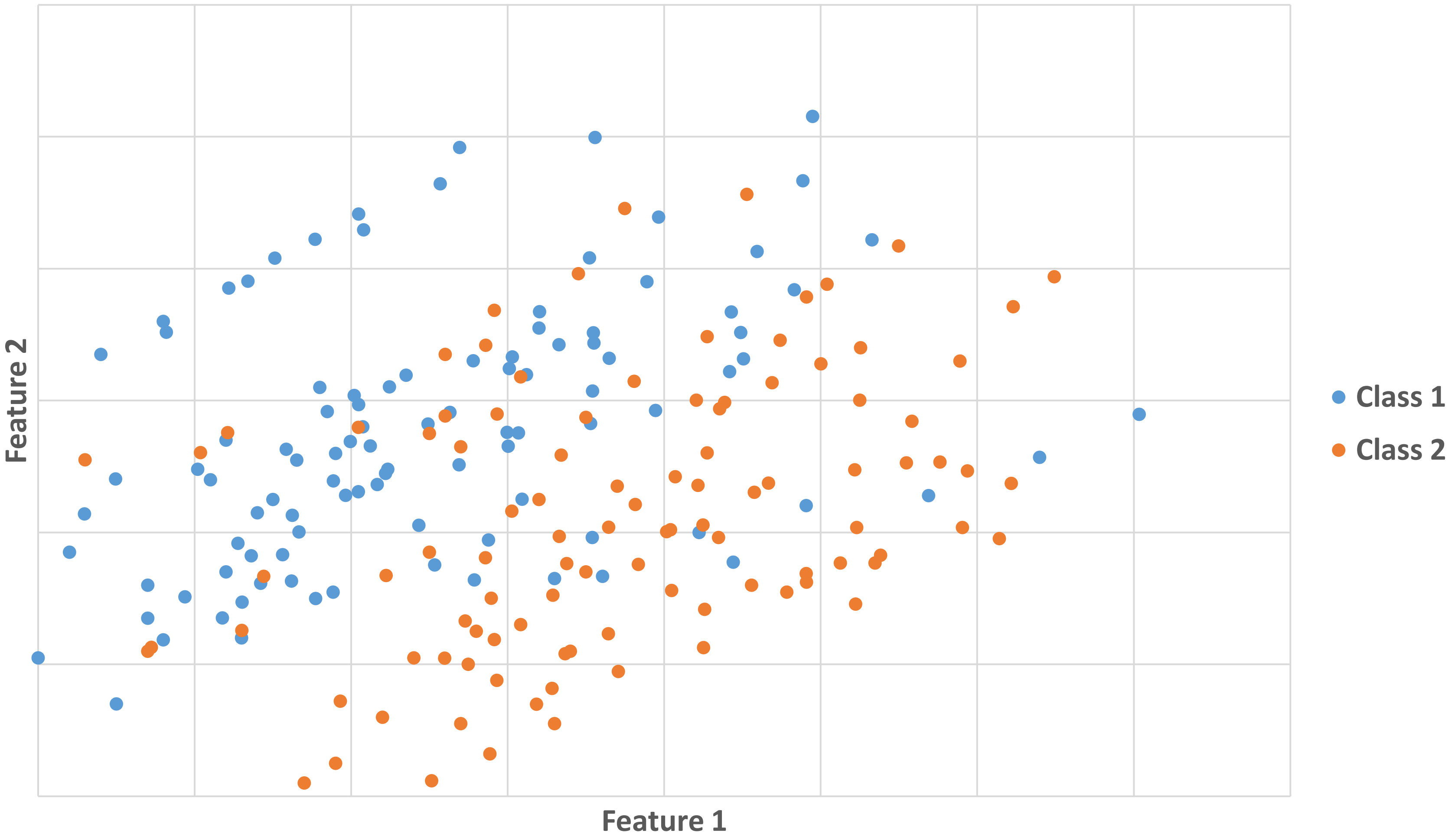

Too many annotations can lead to **over-fitting**: works great on training data but poorly on new data

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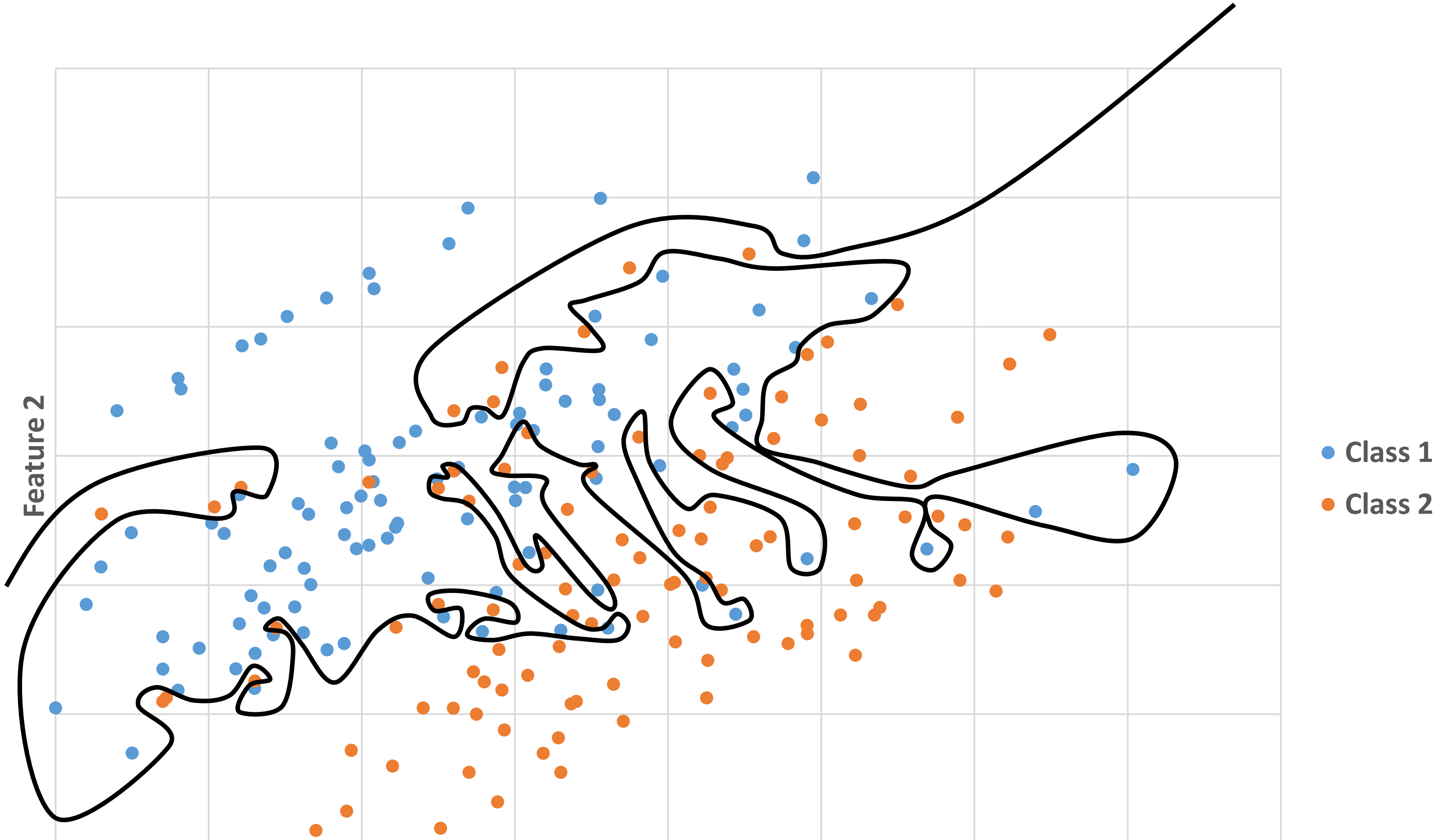

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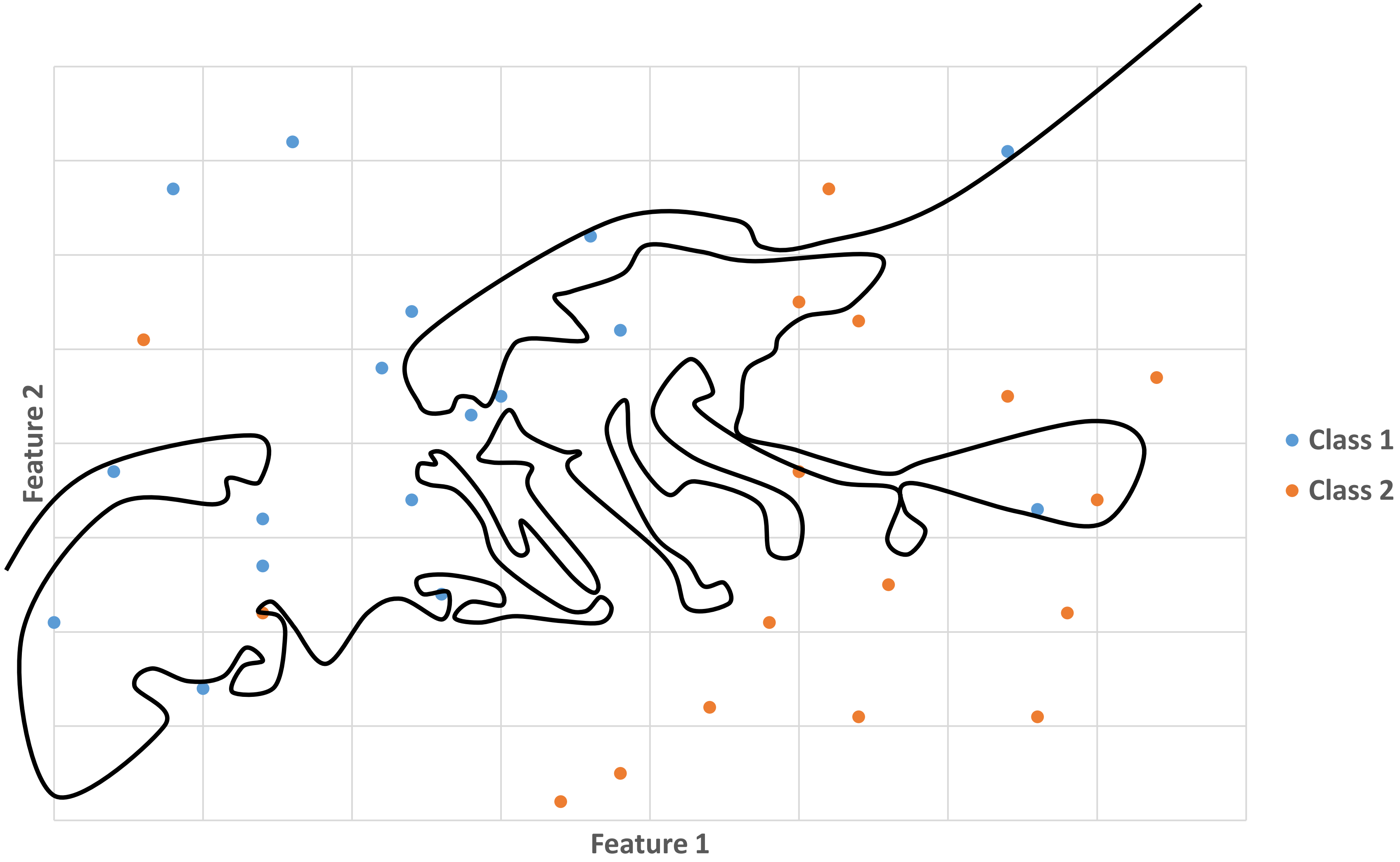

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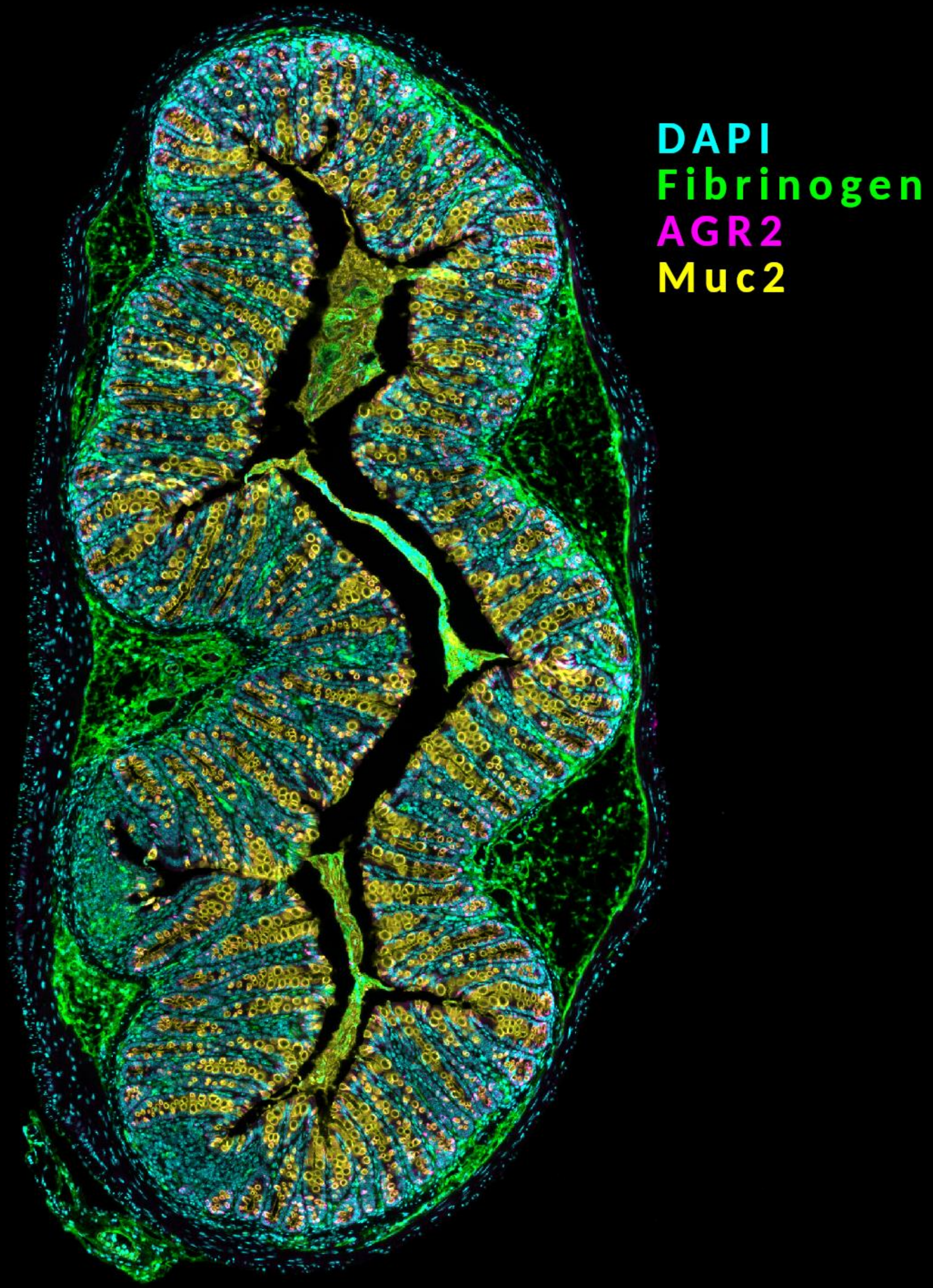
Shallow Machine Learning for Pixel Classification

- 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

- **Open** Colon_1.ome.tif and Colon_2.ome.tif
- Create **annotations** in each image that recapitulate the **diversity** of the tissue
- Create **regions annotations**
- Open **"Pixel classifier"**
- **Annotate** pixels belonging to **Mucus, Epithelium without Mucus** and **everything else**
- **Save classifier** and apply it to **each image** with a **script** (workflow tab)
- Get **proportions** of **tissues**



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

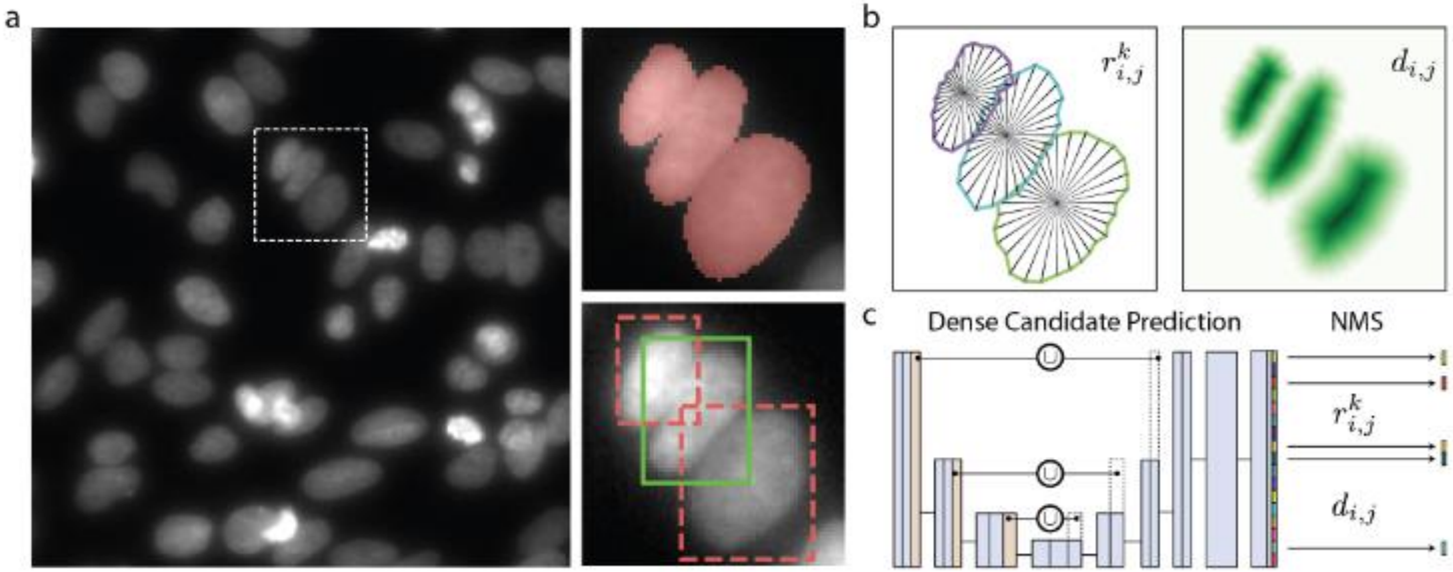
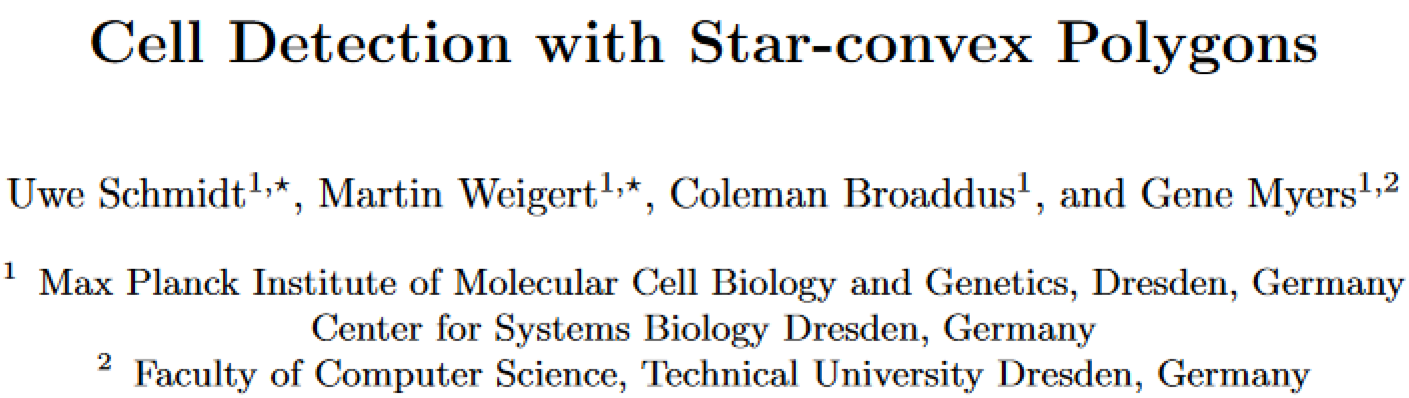


Fig. 1: *(a)* Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). *(b)* The proposed StarDist method predicts object probabilities $d_{i,j}$ and star-convex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



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[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).*

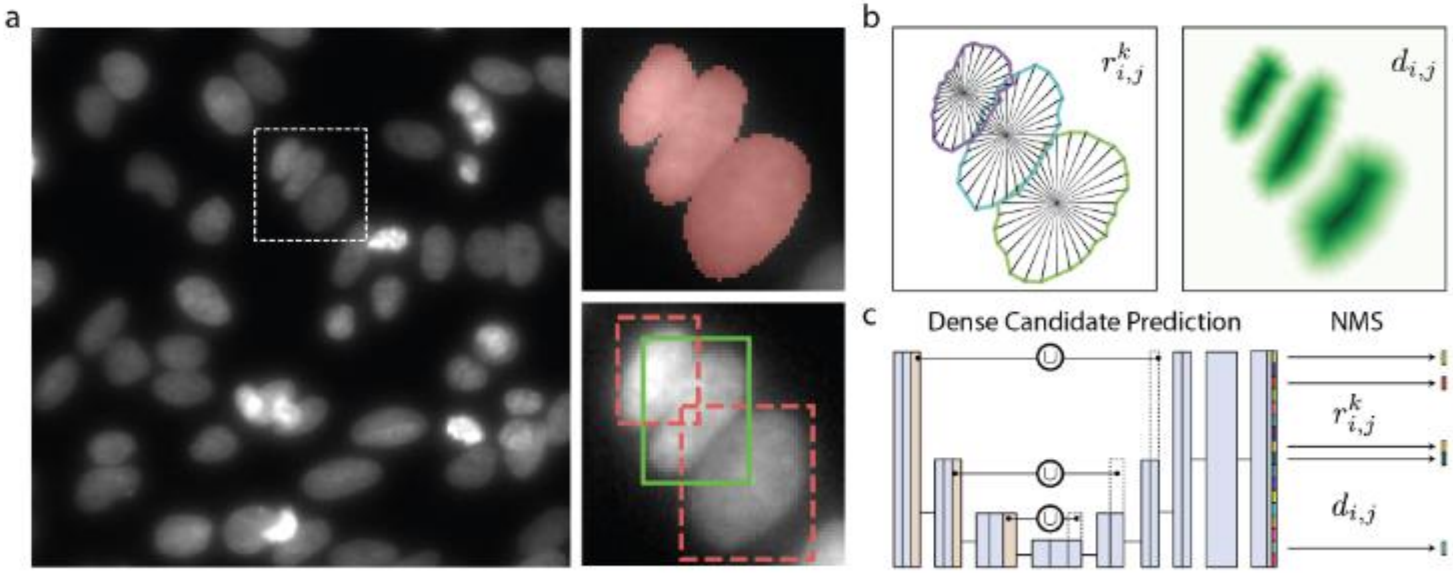
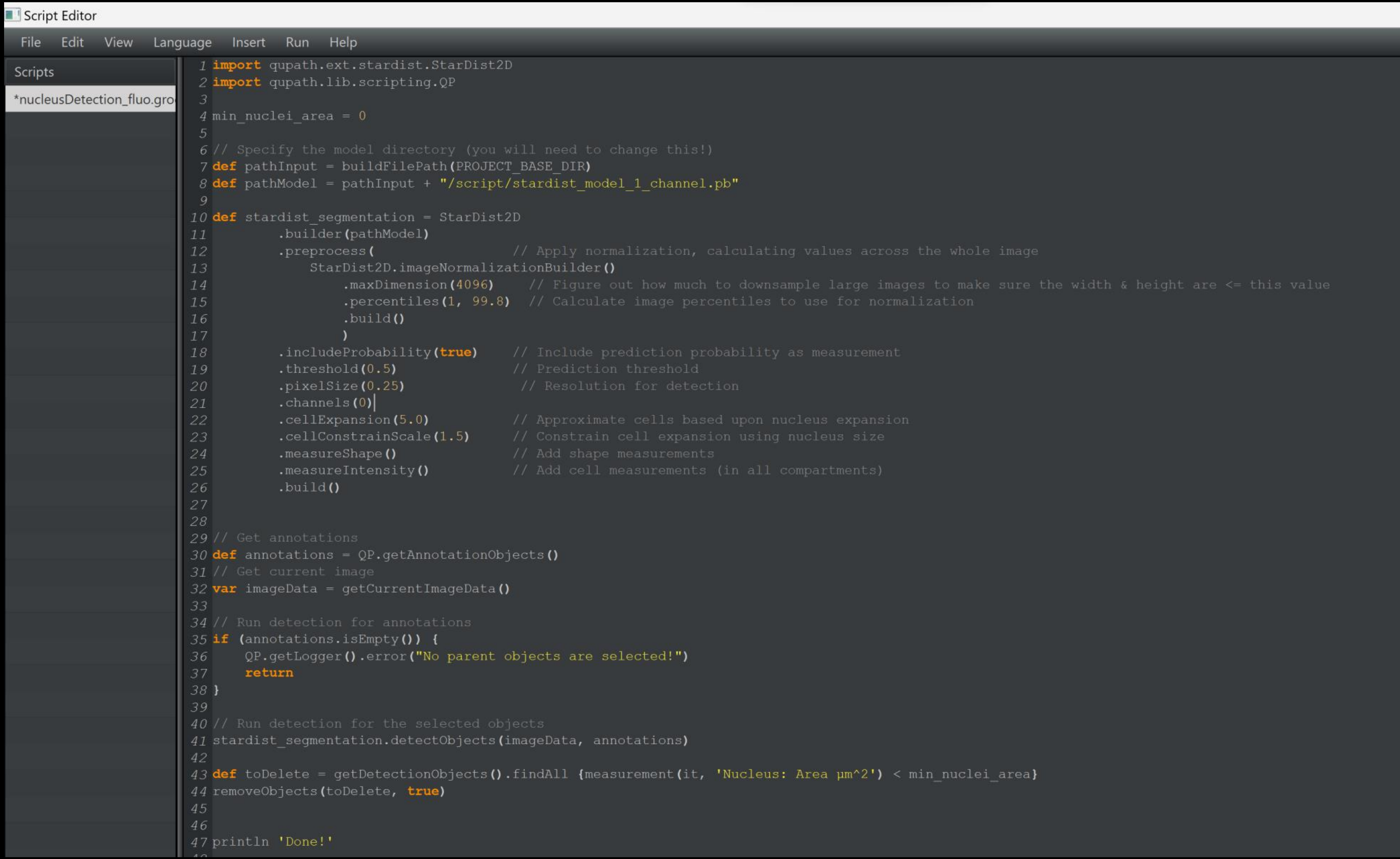


Fig. 1: *(a)* Potential segmentation errors for images with crowded nuclei: Merging of touching cells (upper right) or suppression of valid cell instances due to large overlap of bounding box localization (lower right). *(b)* The proposed StarDist method predicts object probabilities $d_{i,j}$ and star-convex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

- **Copy** script folder into QuPath project
- **Open** nucleusDetection_fluo.groovy



```
import qupath.ext.stardist.StarDist2D
import qupath.lib.scripting.QP

min_nuclei_area = 0

// Specify the model directory (you will need to change this!)
def pathInput = buildFilePath(PROJECT_BASE_DIR)
def pathModel = pathInput + "/script/stardist_model_1_channel.pb"

def stardist_segmentation = StarDist2D
        .builder(pathModel)
        .preprocess(                 // Apply normalization, calculating values across the whole image
            StarDist2D.imageNormalizationBuilder()
                .maxDimension(4096)    // Figure out how much to downsample large images to make sure the width & height are <= this value
                .percentiles(1, 99.8)  // Calculate image percentiles to use for normalization
                .build()
                )
        .includeProbability(true)    // Include prediction probability as measurement
        .threshold(0.5)              // Prediction threshold
        .pixelSize(0.25)              // Resolution for detection
        .channels(0)
        .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()


// Get annotations
def annotations = QP.getAnnotationObjects()
// Get current image
var imageData = getCurrentImageData()

// Run detection for annotations
if (annotations.isEmpty()) {
    QP.getLogger().error("No parent objects are selected!")
    return
}

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

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


println 'Done!'
```

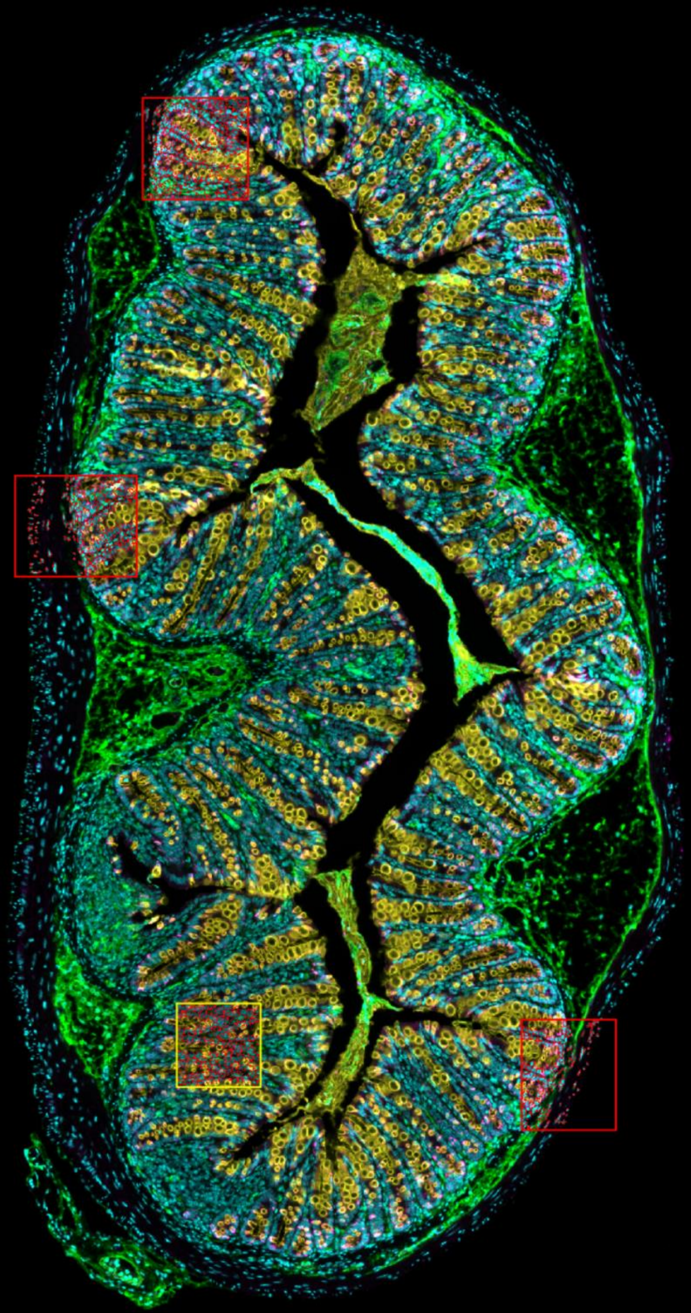
SEGMENTATION WITH STARDIST

- **Copy** script folder into QuPath project

- **Open** nucleusDetection_fluo.groovy

- Define a **small rectangle annotation** to test the **Stardist parameters**

- **Test** on **few small other** rectangle annotations



Shallow Machine Learning for Object Classification

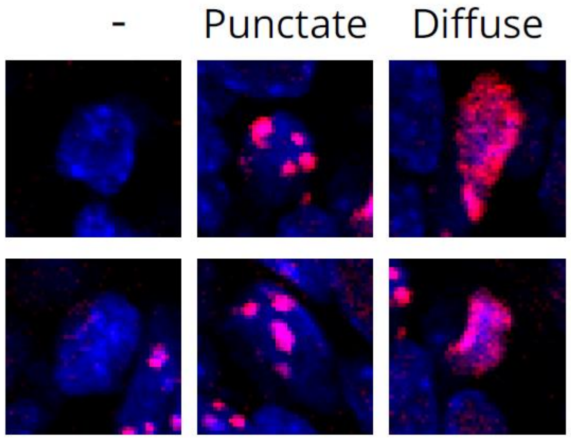
As for pixel classification:

- **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

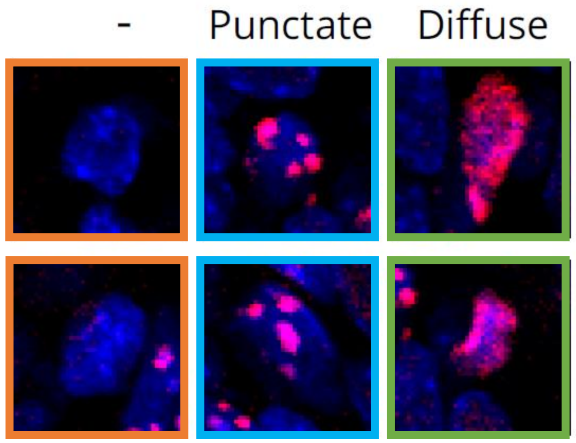
But this time, features are **measurements associated to detections** (most often cells or nuclei) such as:

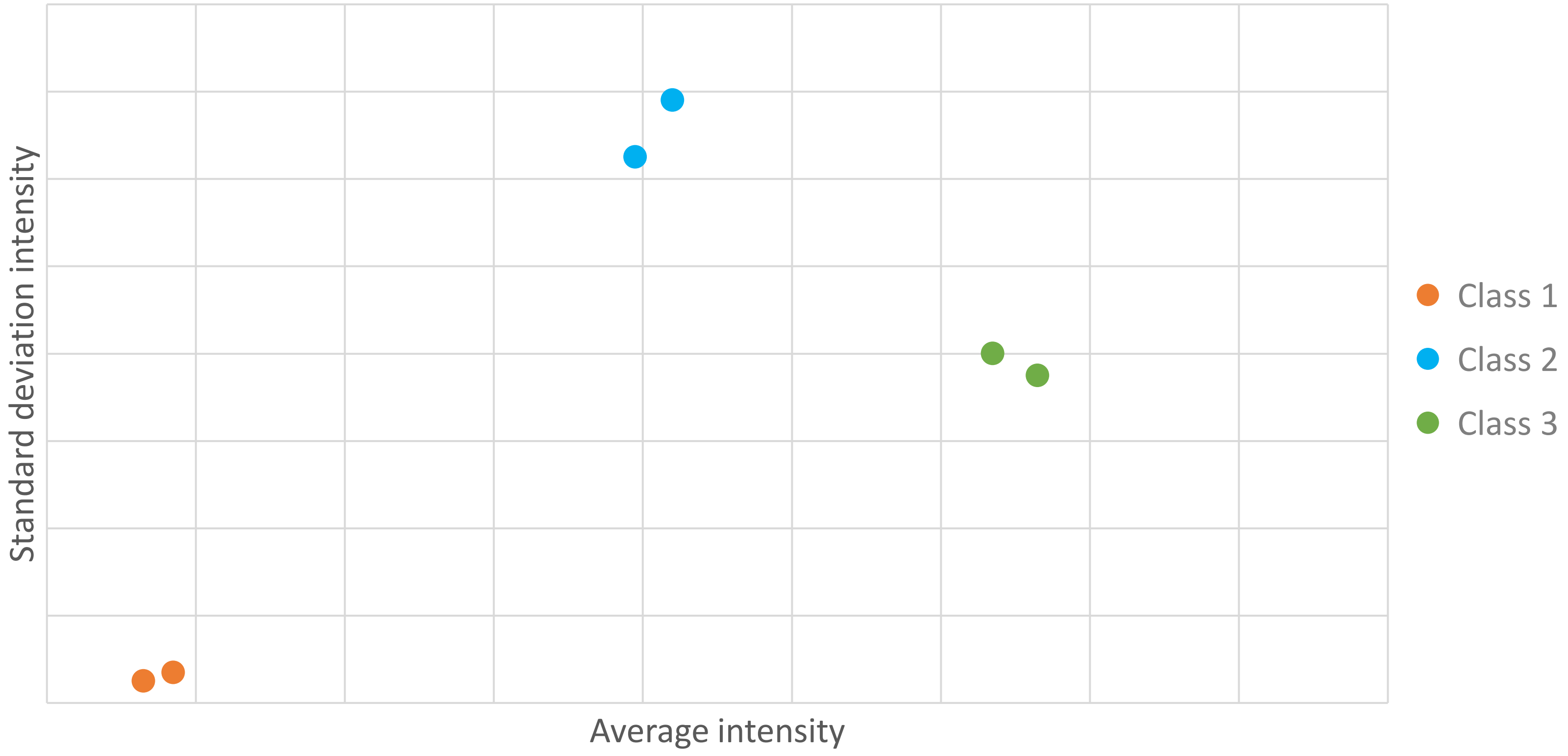
- **Average** intensity
- **Median** intensity
- **Standard deviation** of intensity
- **Minimum/Maximum** intensity
- Object **area**
- Object **Circularity**
- …

Shallow Machine Learning for Object Classification

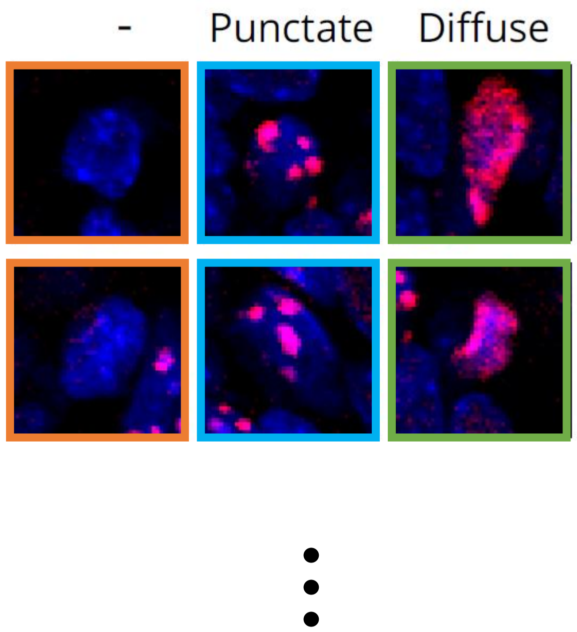


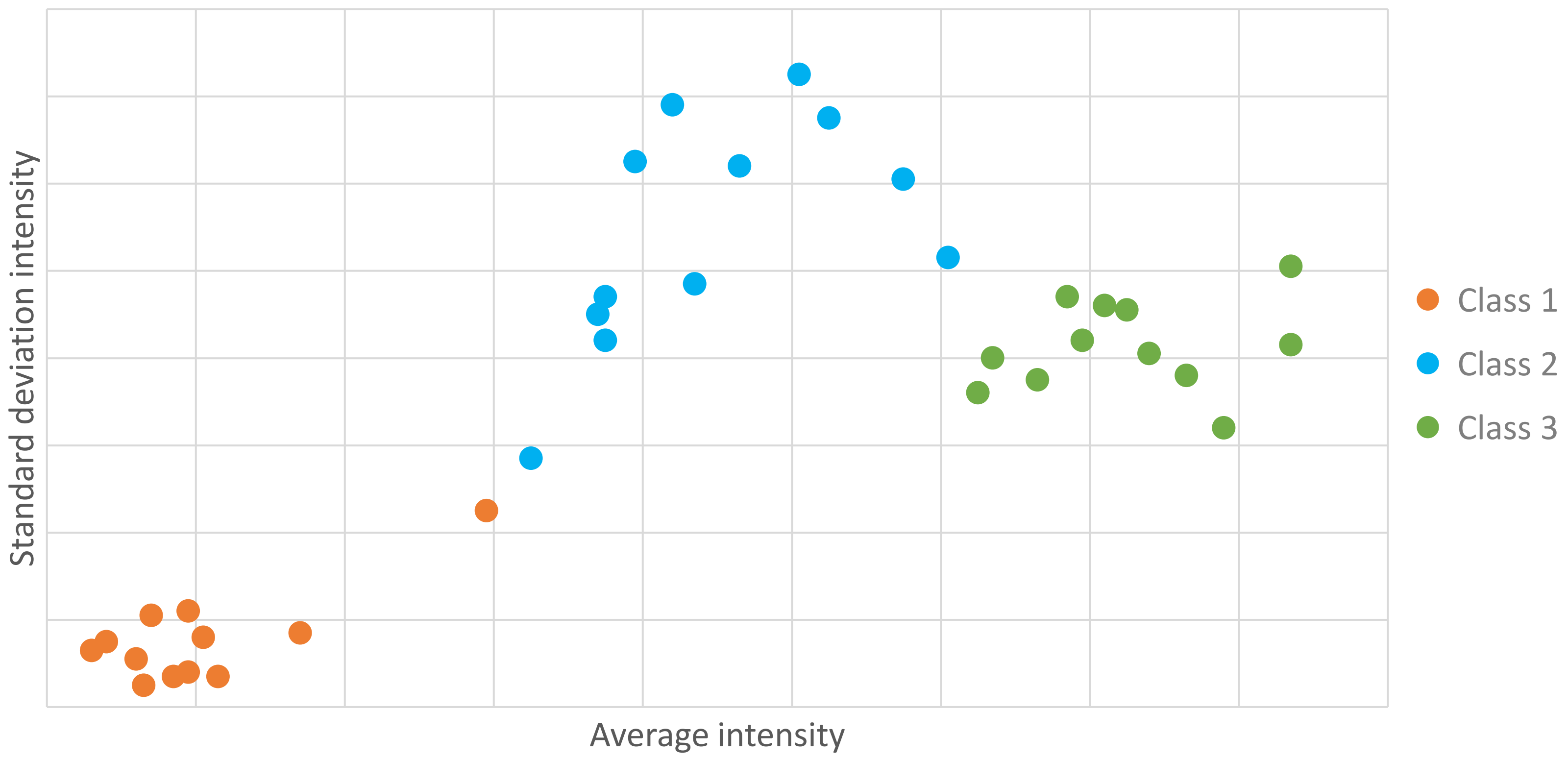
Shallow Machine Learning for Object Classification



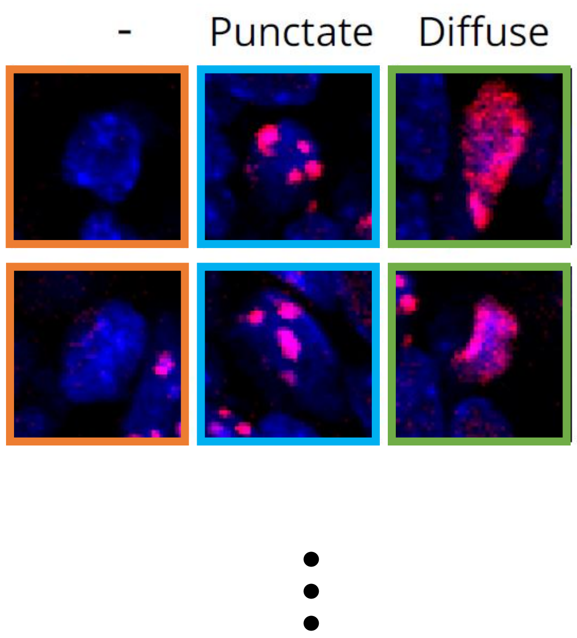


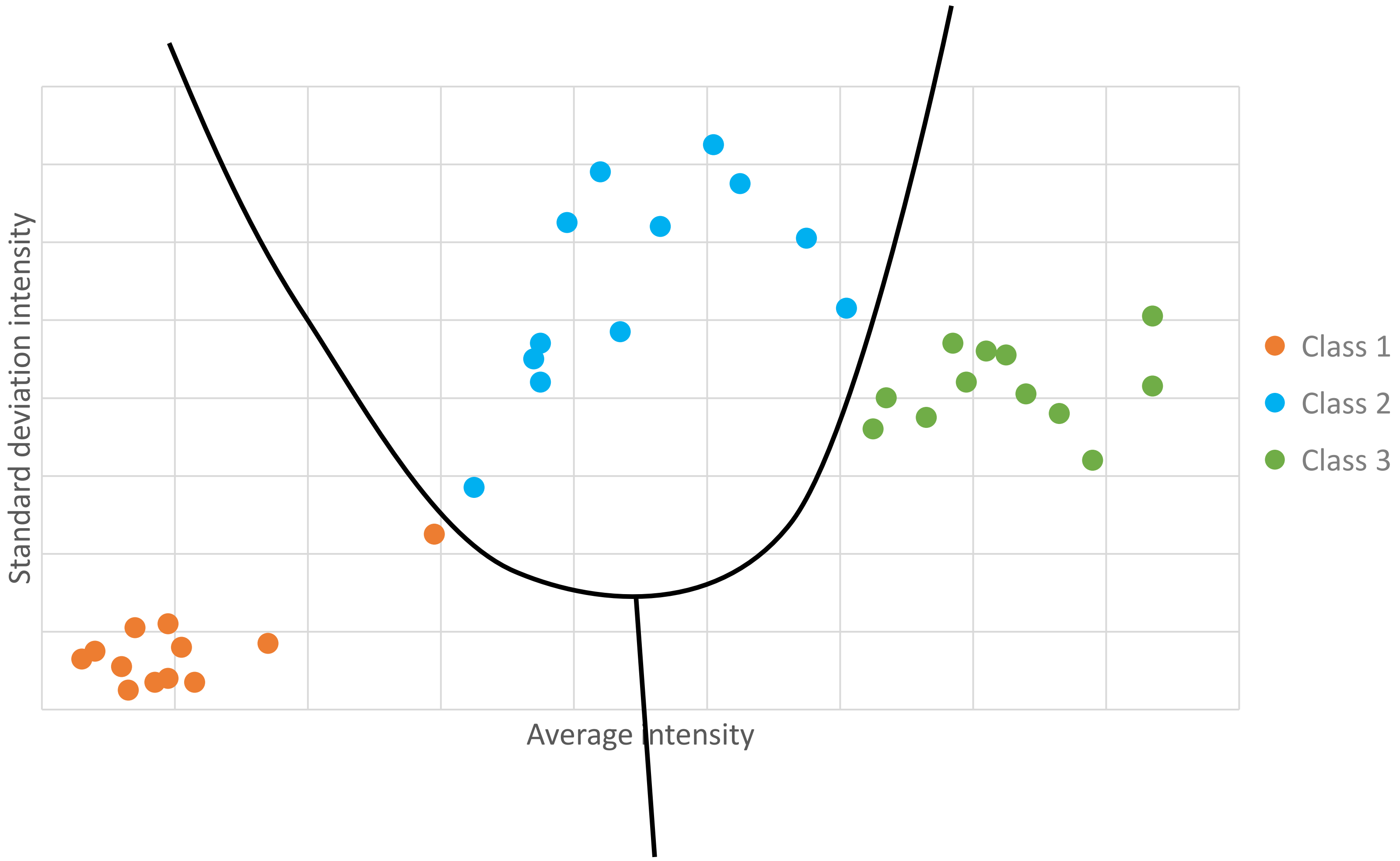
Shallow Machine Learning for Object Classification





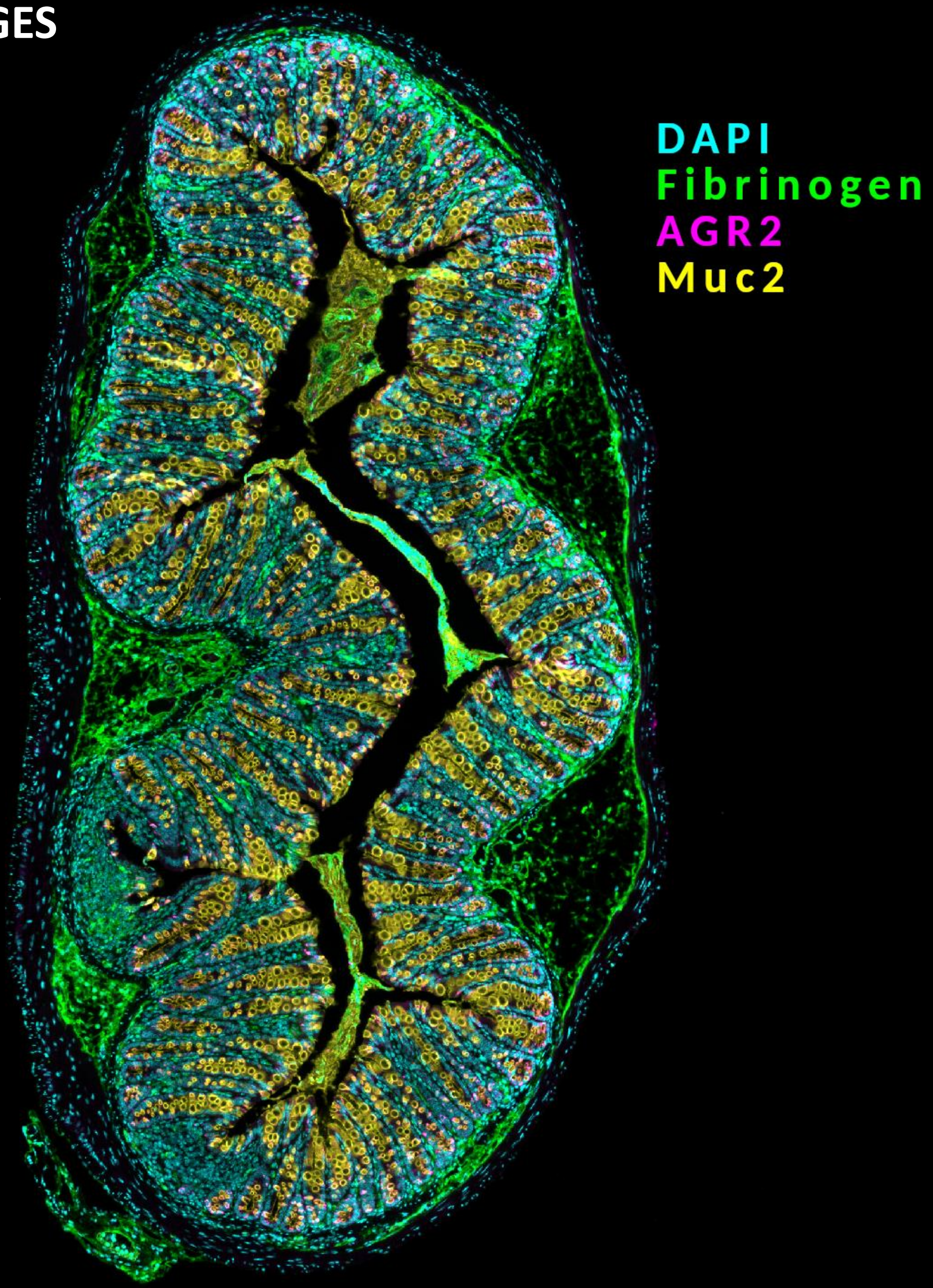
Shallow Machine Learning for Object Classification





Multi/Hyper-plexed Images

- Create **annotations** in each image that recapitulate the **diversity** of the tissue
- Create **regions annotations**
- Apply **Stardist**
- **Train** an **object** classifier to identify positive cells for Fibrinogen and AGR2
- Apply **Stardist** on the Colon_1, **classify cells** and apply **pixel classifier**
- Use the **workflow tab** to create a **script** that applies these **3 tasks** to Colon_2
- Compute **distances** between AGR2 positive cells and mucus
- **Export measurements** for both images



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