

# Part 4

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Introduction: Why Spatial resolution?

Part 3: Spatial transcriptomics

- Visium Technology
- Pipeline for Spatial transcriptomics
- Hands-on analysis

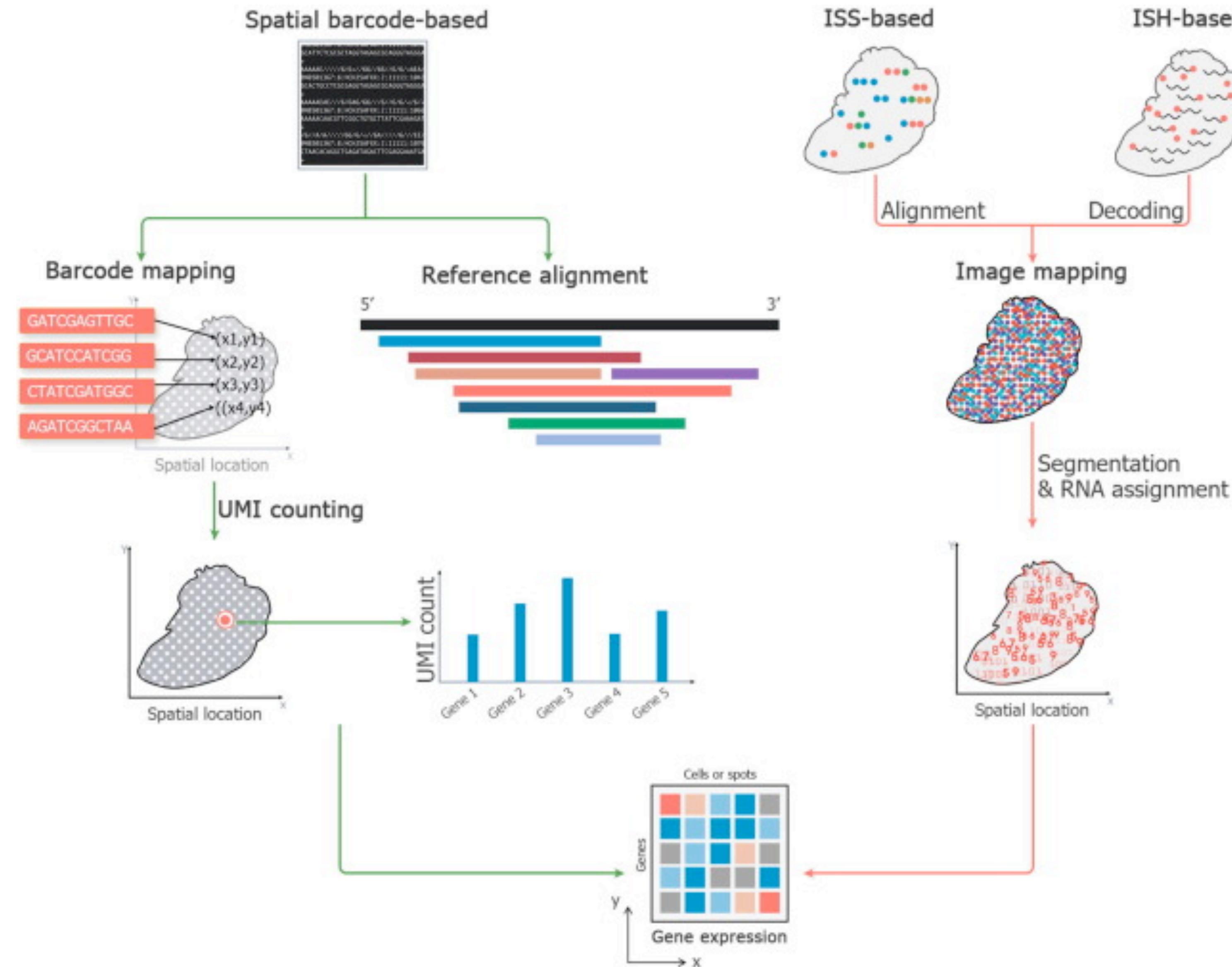
**Part 4: Single-cell Spatial transcriptomics**

- Different technologies
- Pipeline for Single-cell Spatial transcriptomics
- Hands-on analysis

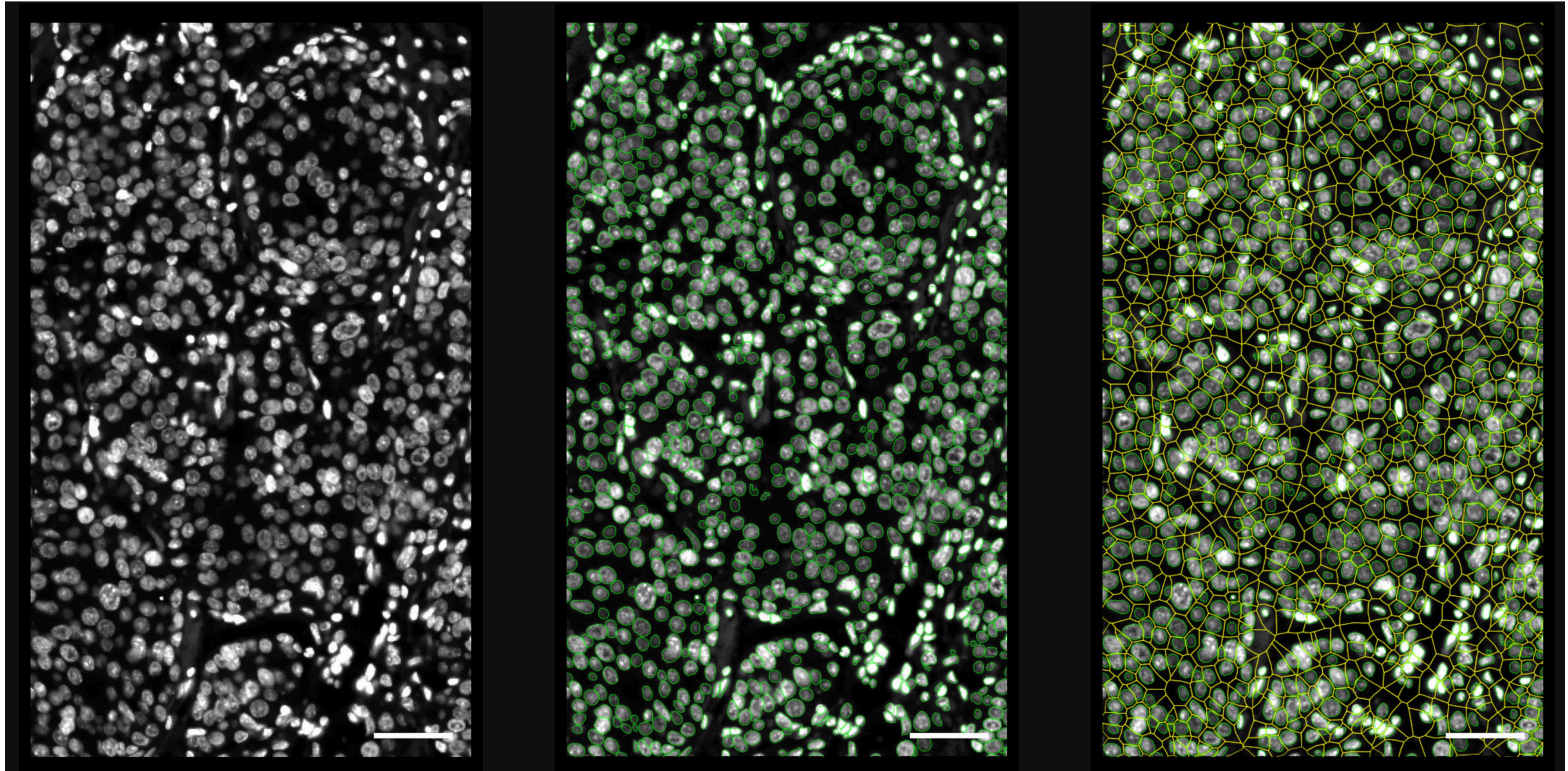
Optional:

- Team-work

# Spatial Transcriptomics technologies - Overview

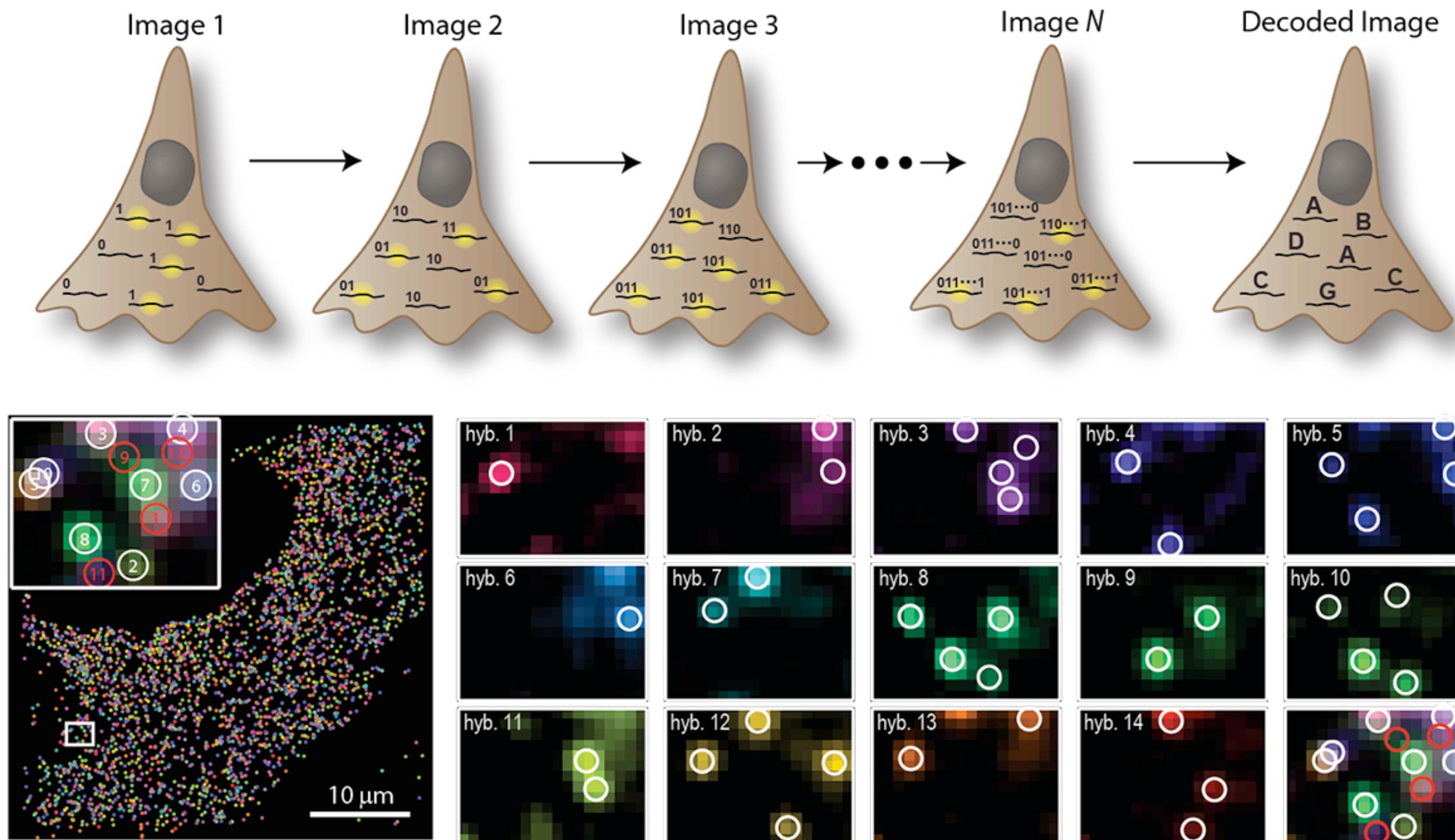


# Cell Segmentation instead of capturing array

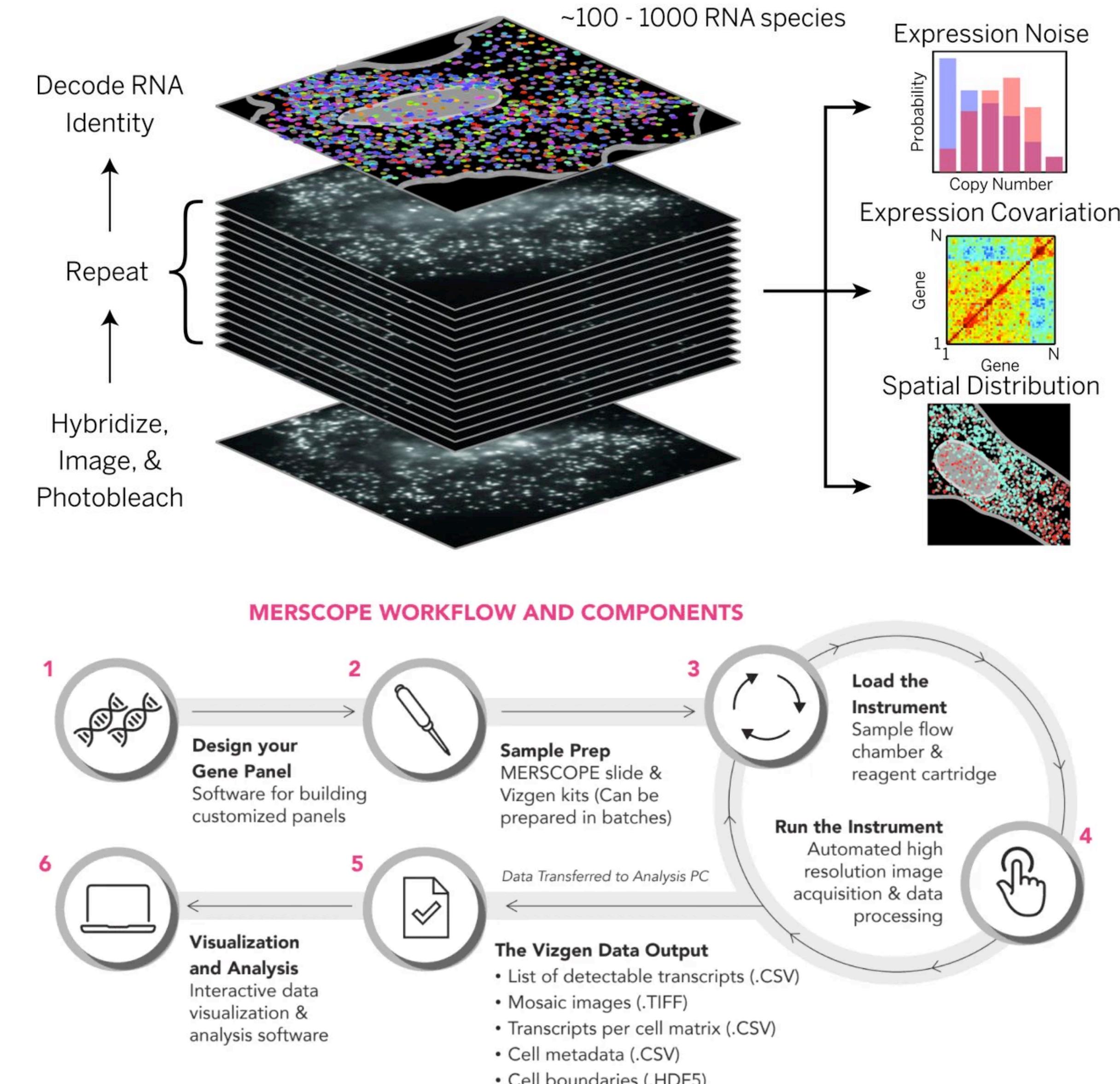


DAPI image with nuclei defined in the middle (green) and cellular boundaries outlines on the right (yellow). Scale bar: 50  $\mu\text{m}$

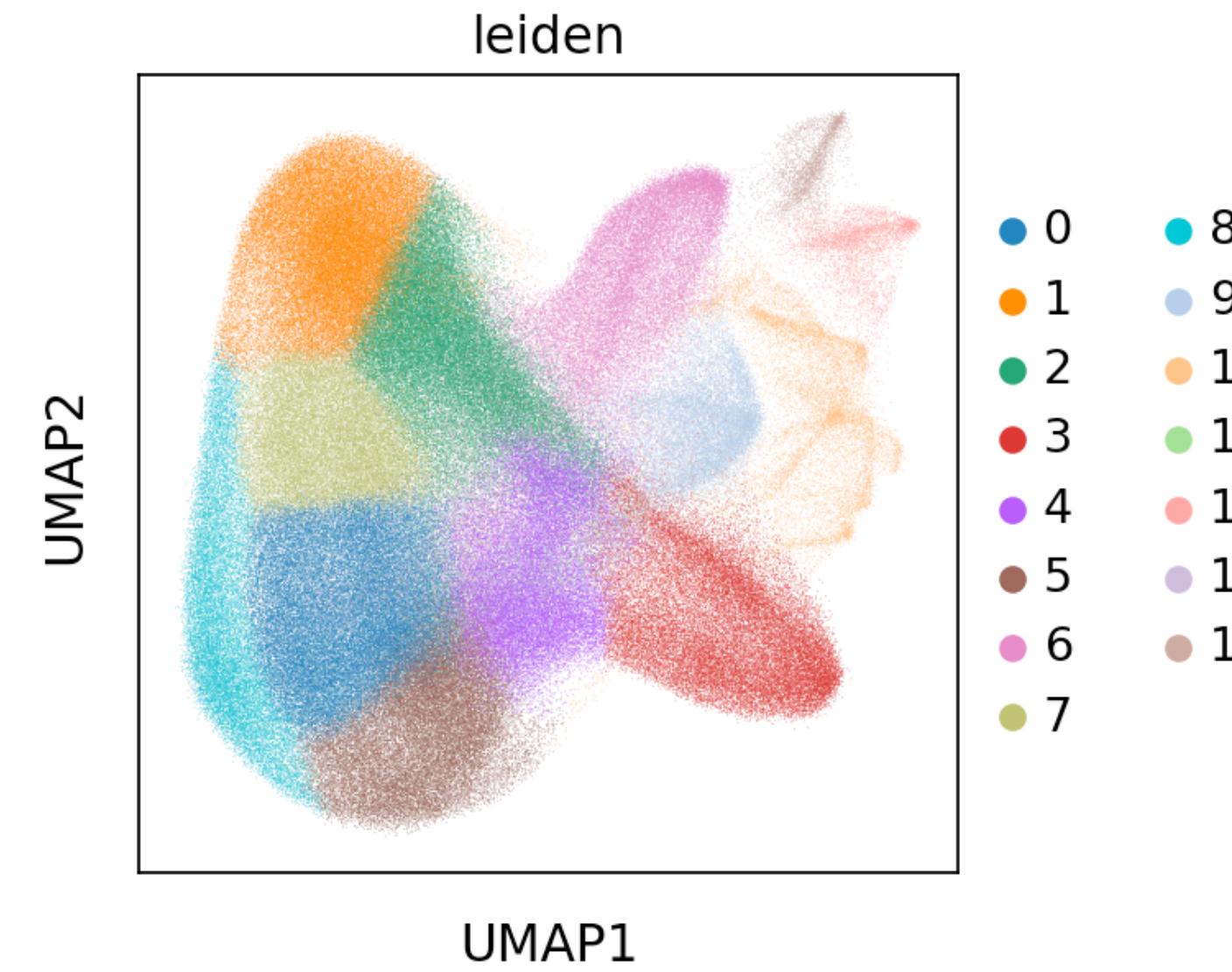
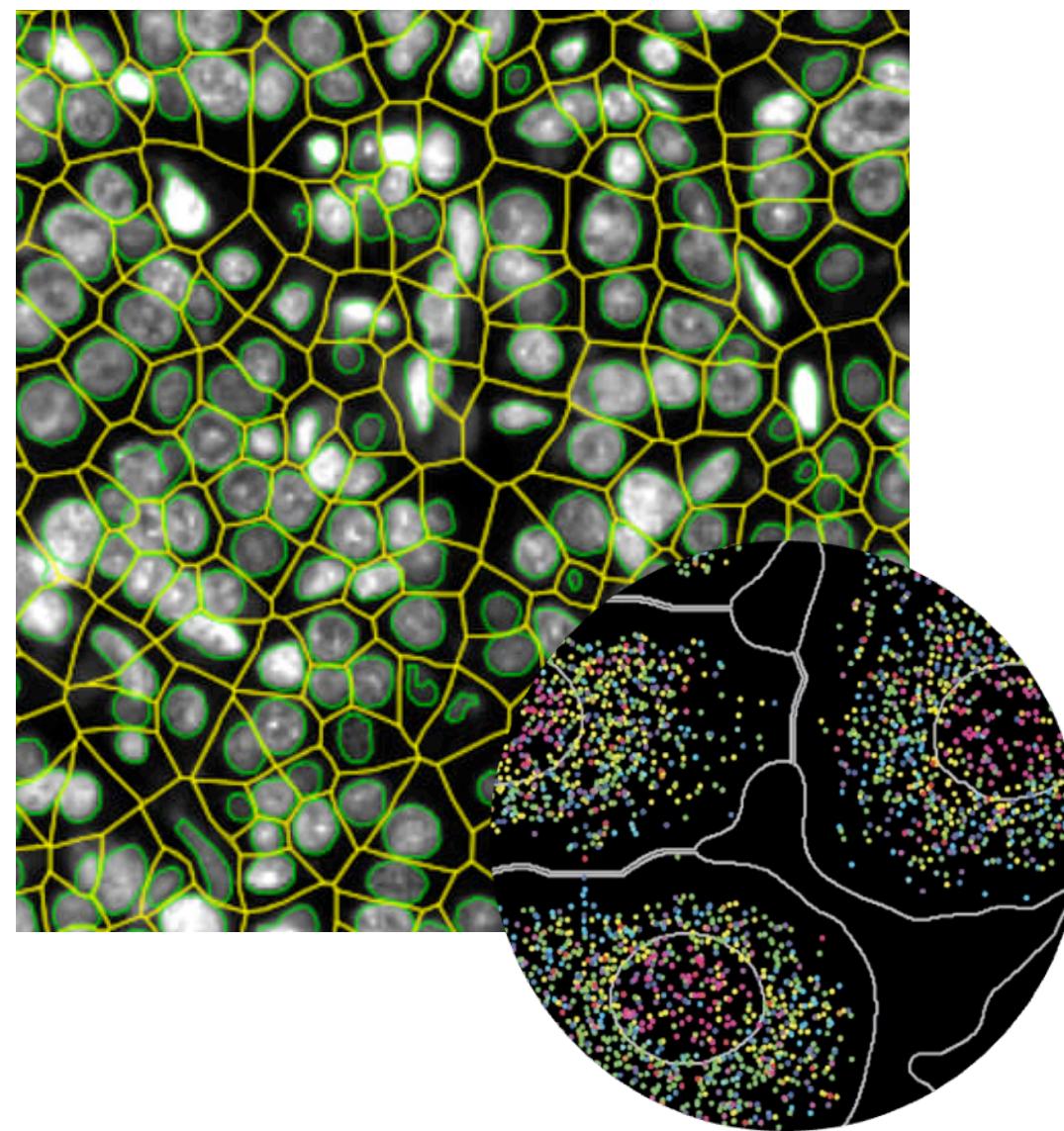
# multiplexed error-robust FISH - MERFISH



Chen et al. (2015): Spatially resolved, highly multiplexed RNA profiling in single cells, *Science*.  
[10.1126/science.aaa6090](https://doi.org/10.1126/science.aaa6090)

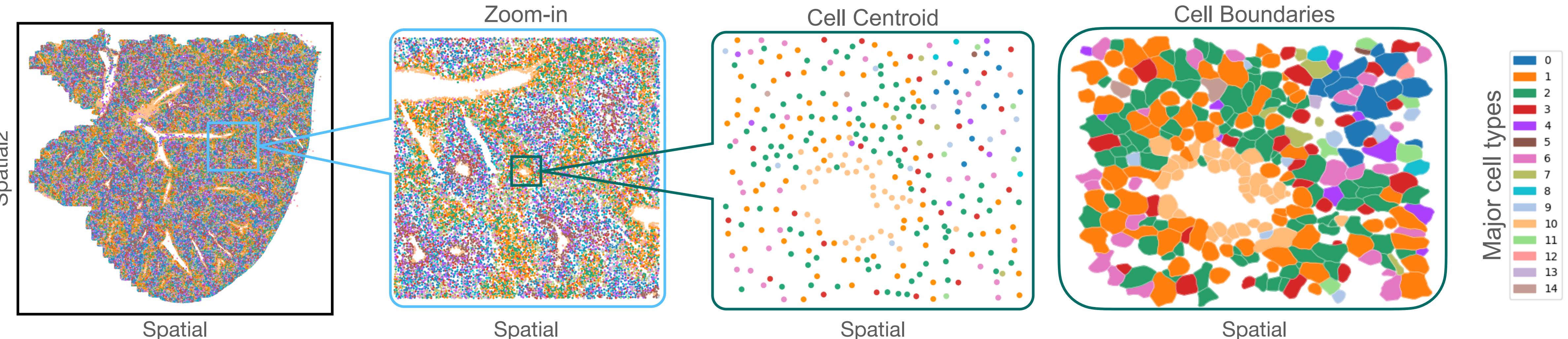


# MERFISH - Murine liver sample



Spatial single-cell data set (MERFISH)  
from murine Liver  
Data: 383 k cells, 385 Transcripts

- Cell segmentation and transcript quantification already done
- Hands-on: Dimensional reduction, Clustering, Spatial analysis



# Resolve Bioscience

## Molecular Cartography™ chemistry

Assay and imaging

1 Cryosectioning

2 Hybridization of gene-specific probes

Patented Process

3 Colorization of probes

4 Imaging

5 Decolorization of probes

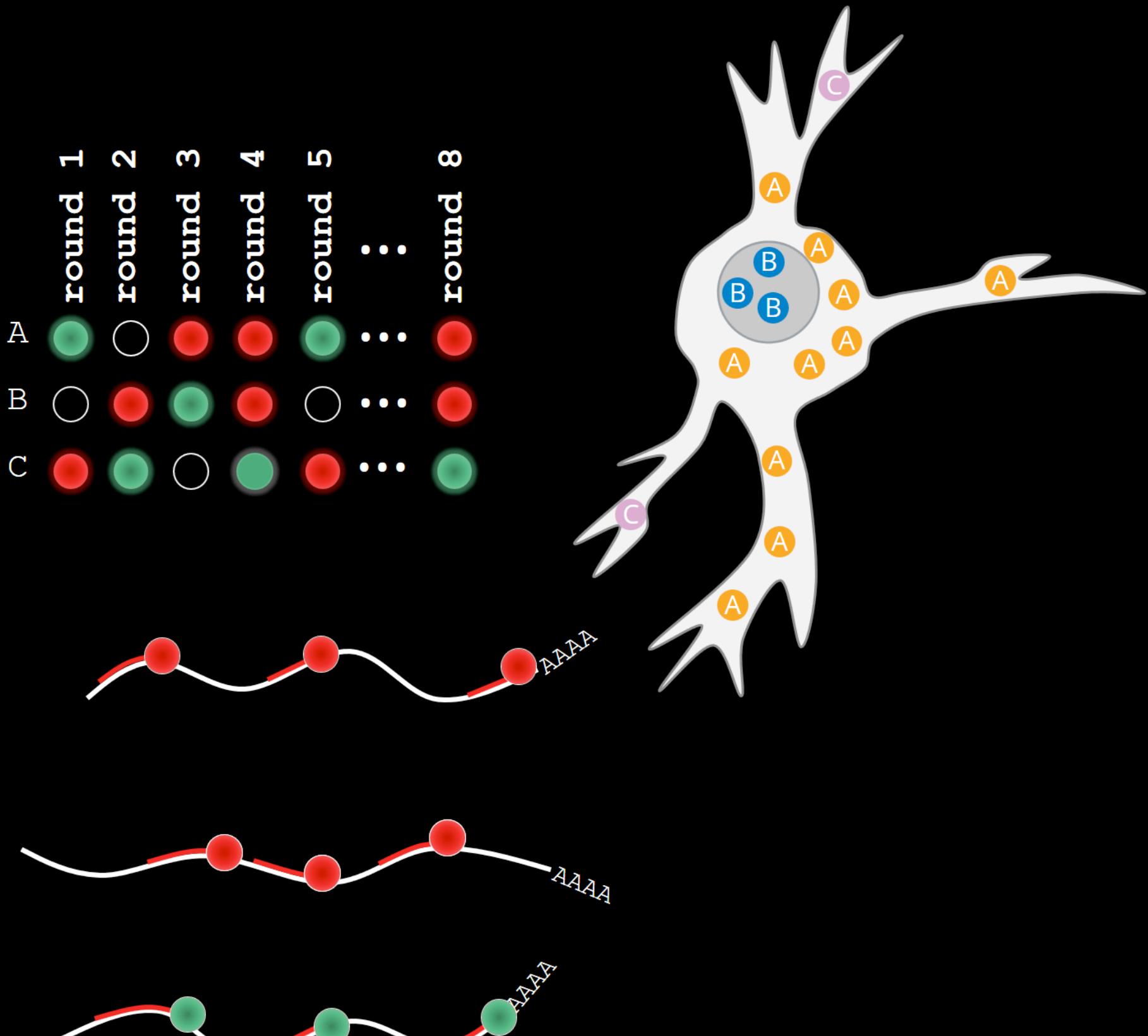
Code:

	round 1	round 2	round 3	round 4	round 5	...	round 8
Transcript A	●	○	●	●	●	...	●
Transcript B	○	●	●	○	○	...	●
Transcript C	●	●	○	●	●	...	●

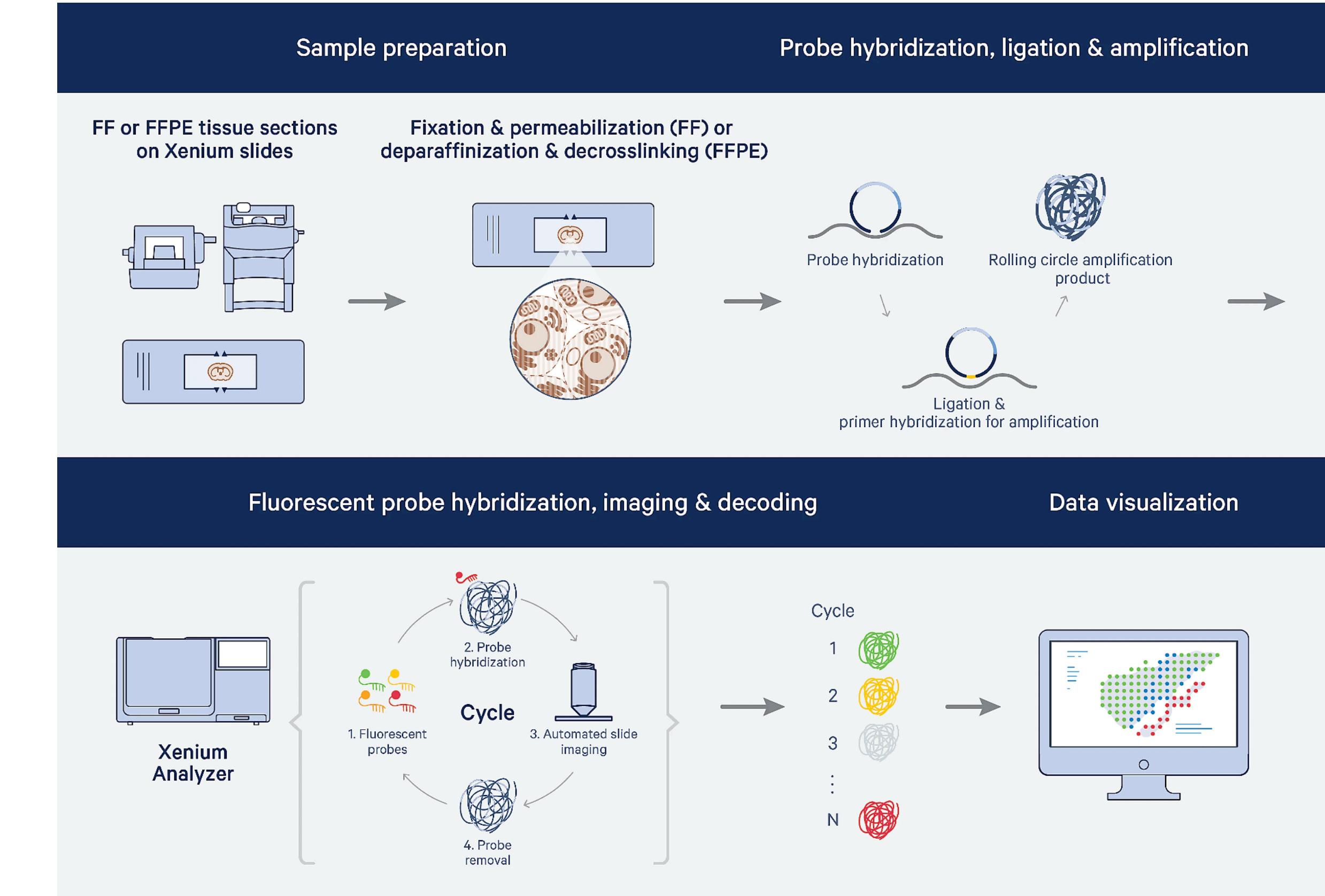
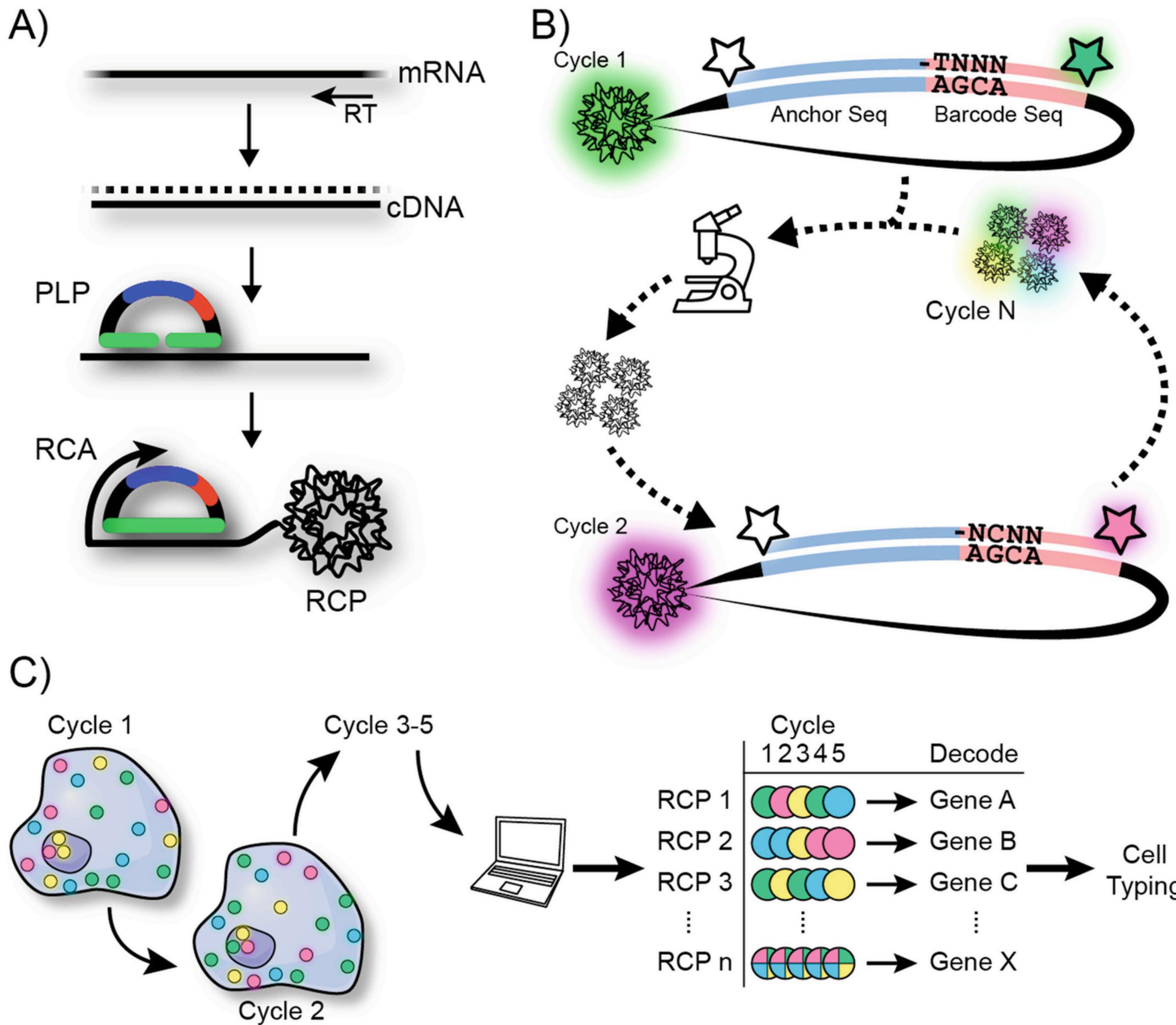
Transcript A

Transcript B

Transcript C



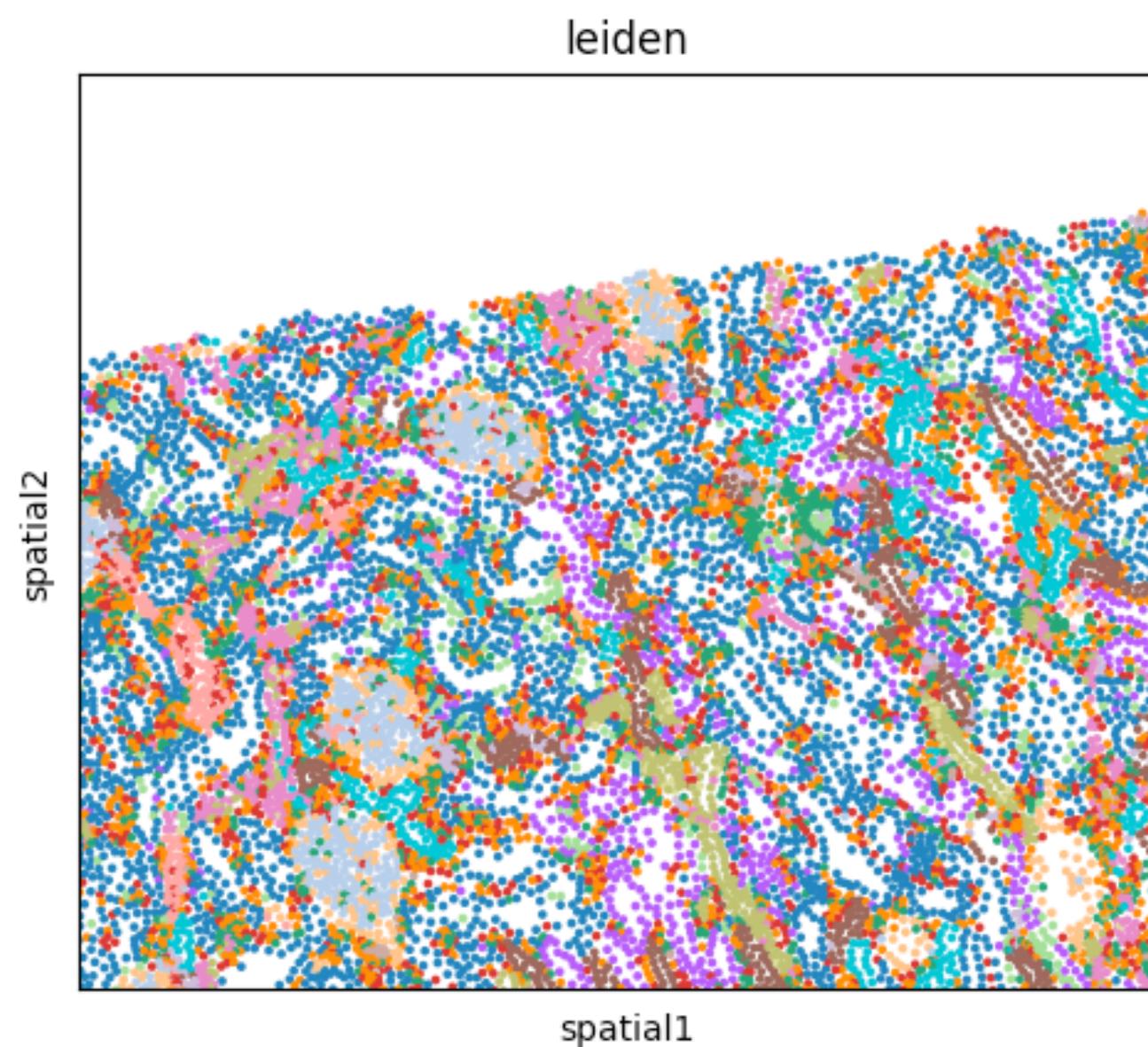
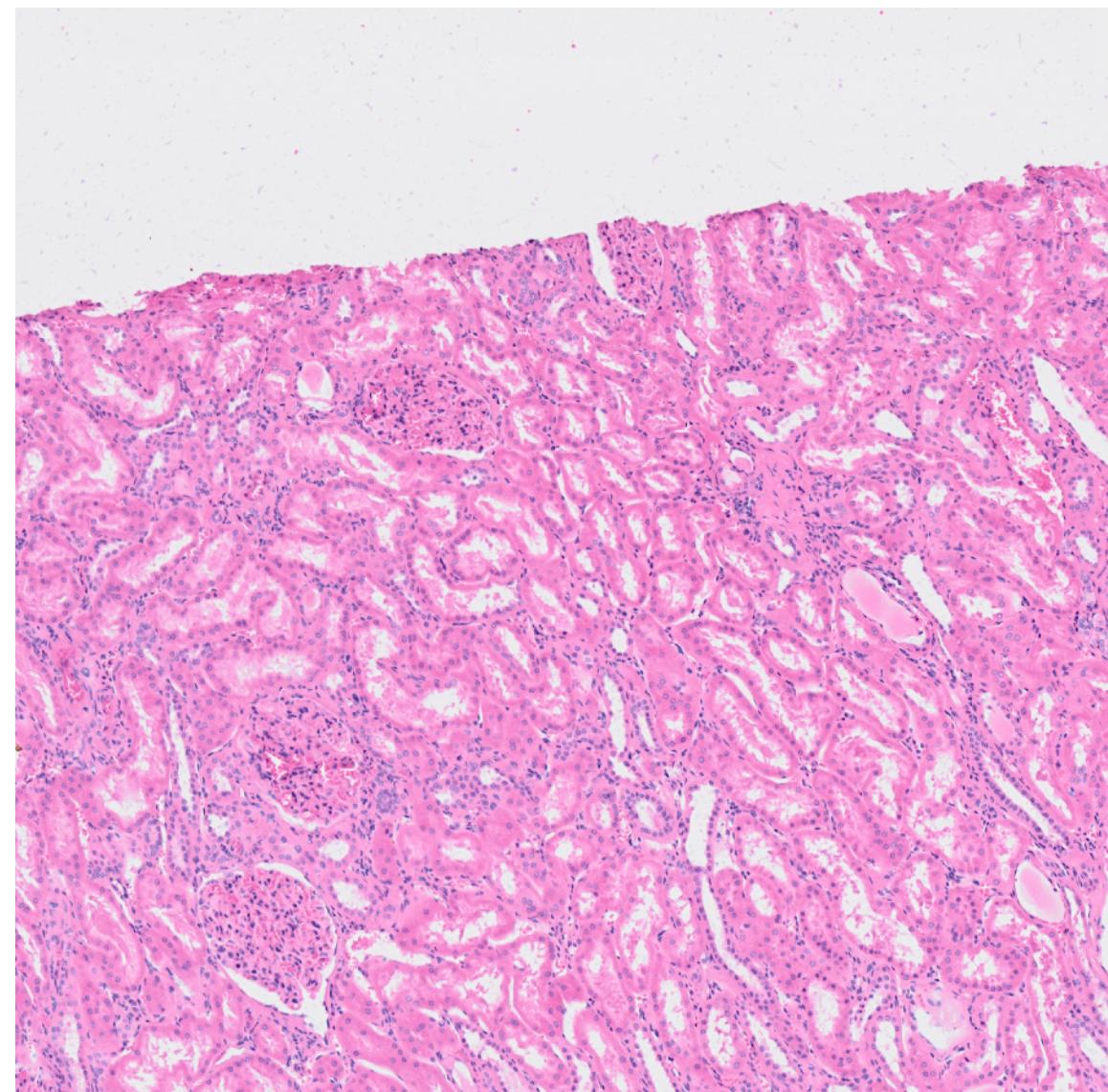
# In situ sequencing (ISS) - Xenium



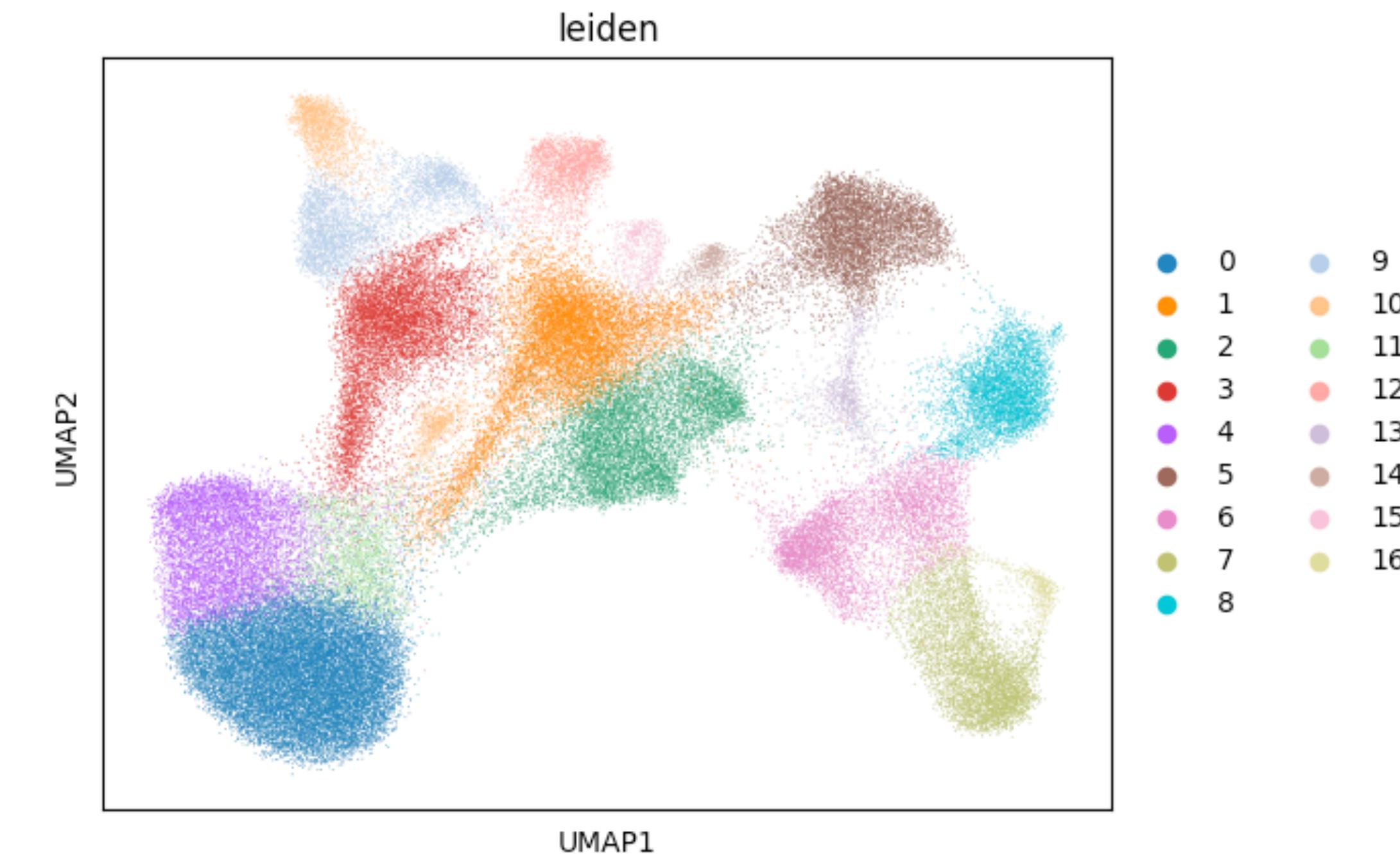
**Qian et al. (2020):** Probabilistic cell typing enables fine mapping of closely related cell types in situ, *Nature methods*.

[10.1038/s41592-019-0631-4](https://doi.org/10.1038/s41592-019-0631-4)

# In situ sequencing (ISS) - Xenium

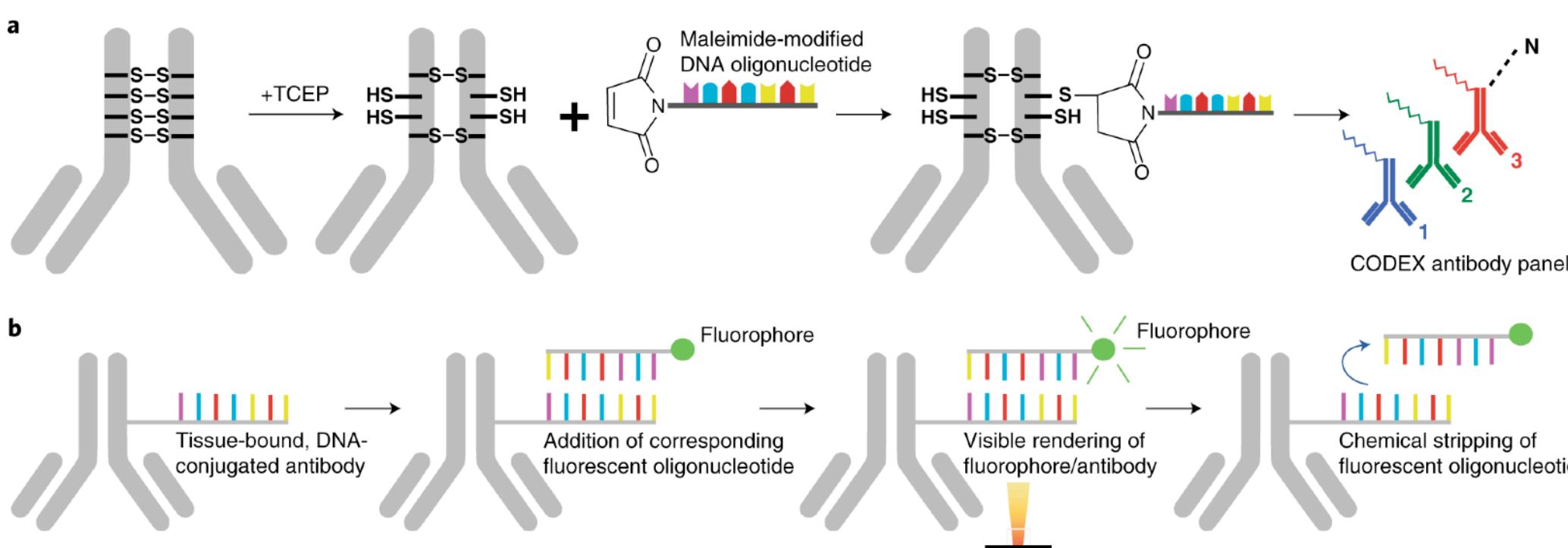


- Spatial single-cell data set (Xenium) from human Kidney
- Data: 97 k cells, 377 Transcripts
  - Cell segmentation and transcript quantification already done
  - Hands-on: Dimensional reduction, Clustering, Spatial analysis

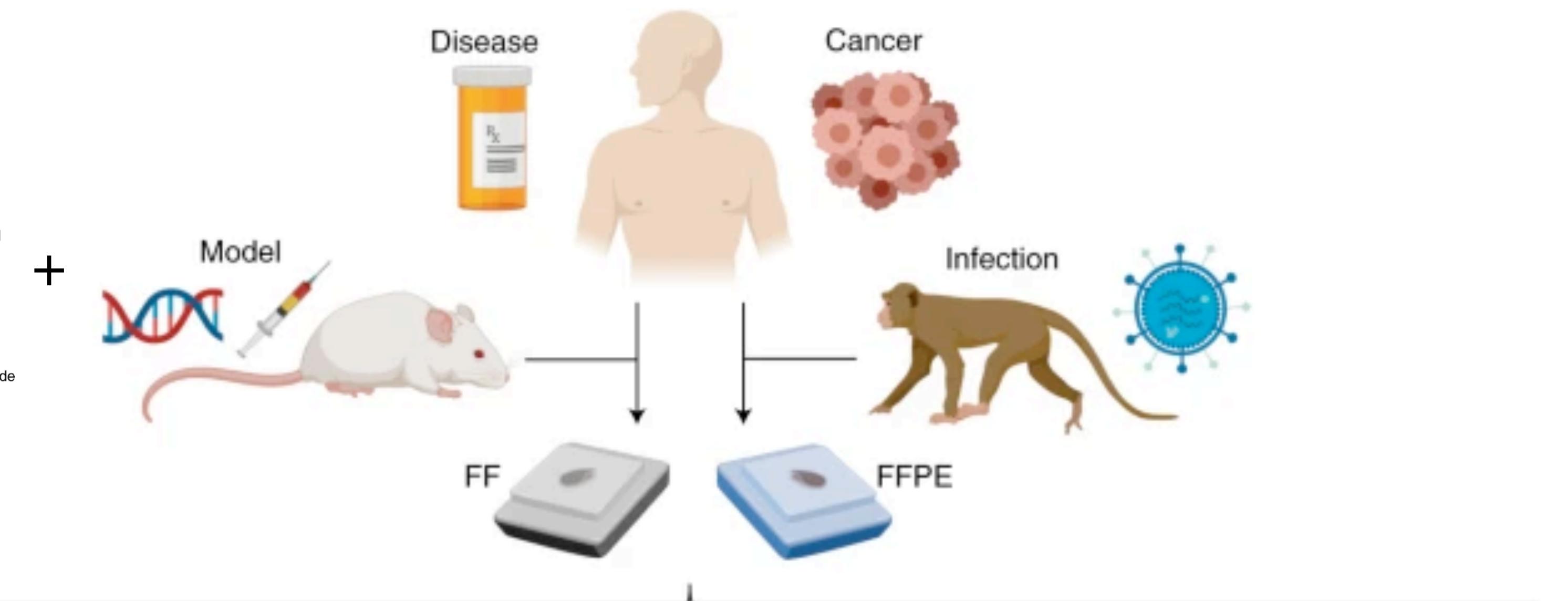


# Co-detection by indexing - CODEX

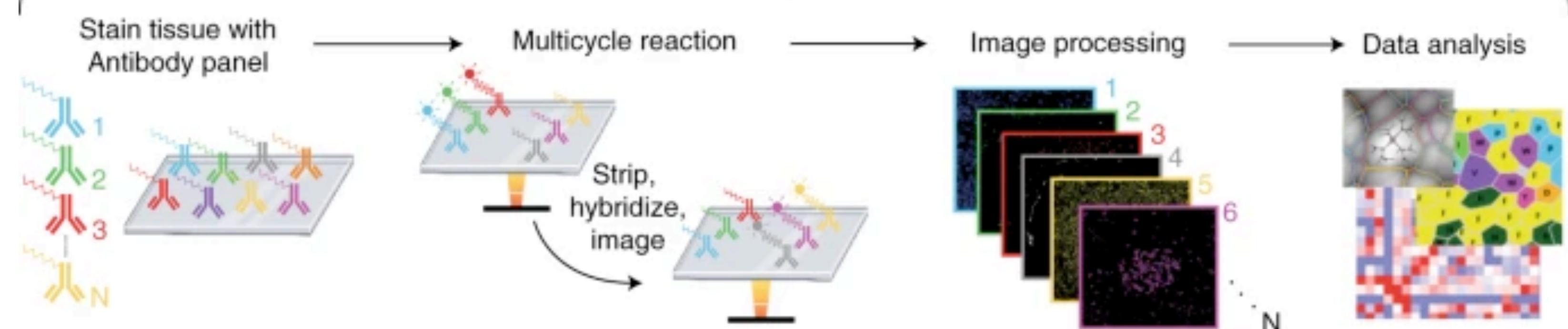
## Antibody-DNA Conjugations



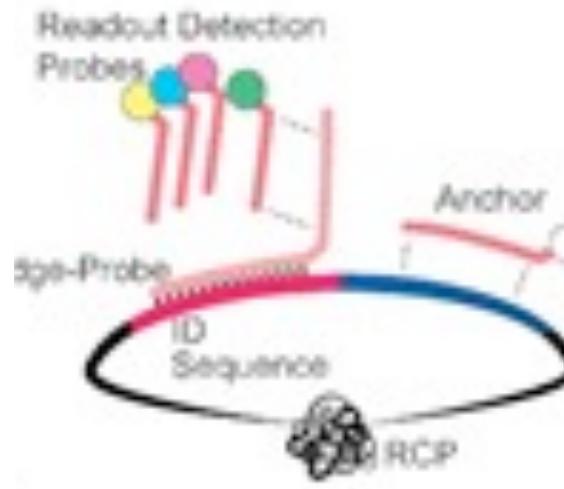
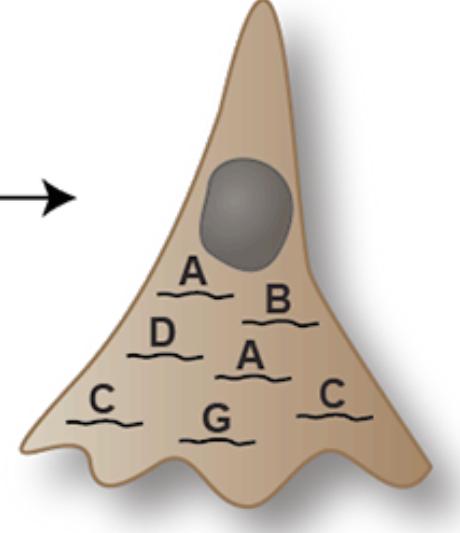
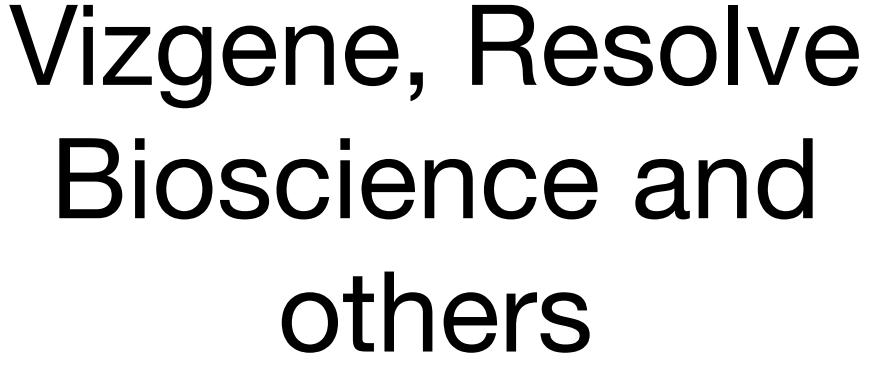
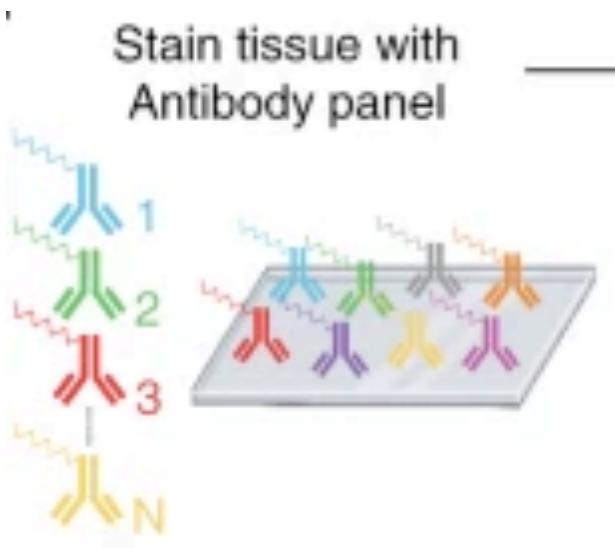
## Tissue preparation and imaging



Black et al. (2020): CODEX multiplexed tissue imaging with DNA-conjugated antibodies, *Nature protocols*.  
[10.1038/s41596-021-00556-8](https://doi.org/10.1038/s41596-021-00556-8)

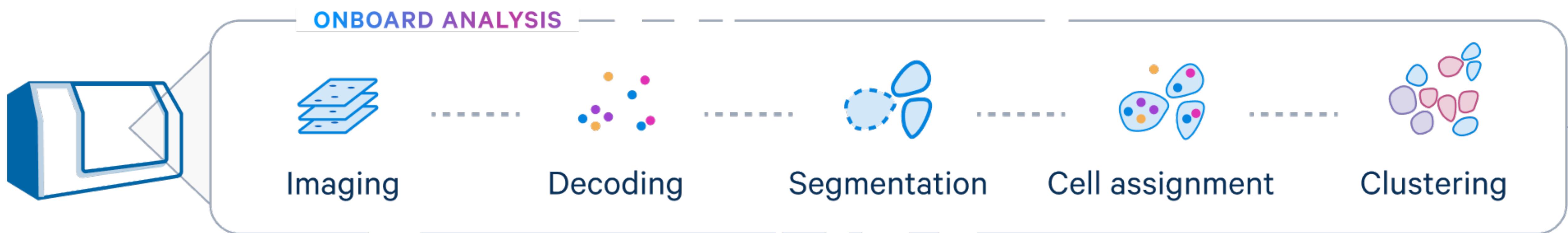


# Overview single-cell spatial transcriptomics

Methods	Target probes	Targets	Other staining	Commercial Available	
1 - <i>In situ</i> sequencing (ISS) / Xenium			RNA		10X Genomics
2. FISH-based (MERFISH / Molecular Cartography)			RNA		Vizgene, Resolve Bioscience and others
3. Co-detection by indexing (CODEX)			Protein		Akoya Bioscience

# Spatial single-cell - Data Processing

- NO sequencing submission is required
- HUGE data files are generated!
- First preprocessing is part of the technology and fully automated (takes several days)
- No spot deconvolution BUT cell segmentation is required.
- Cell segmentation is a complex computational problem and several approaches exists



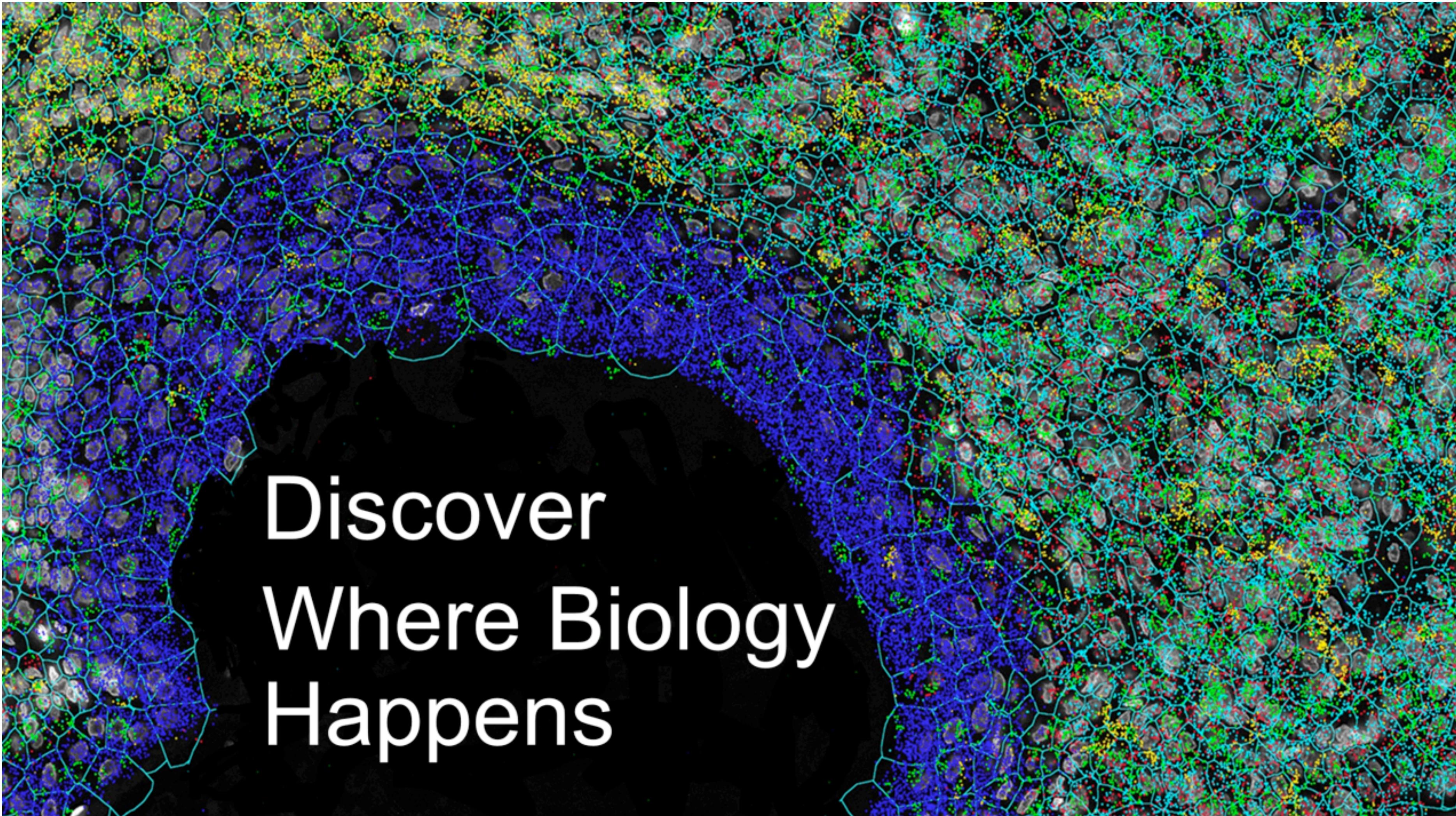
## REQUIRED FILES:

- Cell Centroids file (CSV with x and Y coordinates from cell segmentation)
- Probe expression matrix (h5 format, similar to single-cell RNA-seq)

## OPTIONAL:

- Cell Boundaries file (csv with Polygons from cell segmentation)
- Additional staining and images of the slides
- Other additional processed data

# Spatial single-cell - Hands-on



Discover  
Where Biology  
Happens

# Spatial single-cell - Recap

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How does single-cell transcriptomics (Xenium, Vizgen)  
compare to  
spatial transcriptomics (Visium)?

Advantages

Disadvantages

Future perspectives

# Hands-on: Team work

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Visium: Define the cell types of a spot and for the spatial clusters.  
How do you interpret your results?

Gene expression : How could you find spatially differentially expressed genes?

Structure: How could you find out the neighboring cells/spots of a given cell cluster?

Advanced : How could you modify the function to load also the tissue image or the cell boundaries?

# Closure and Feedback

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