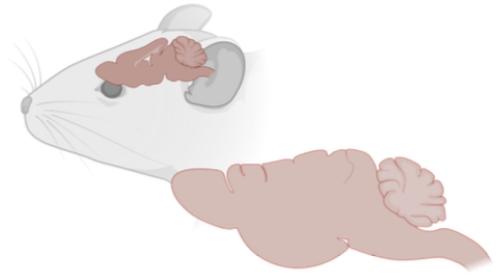
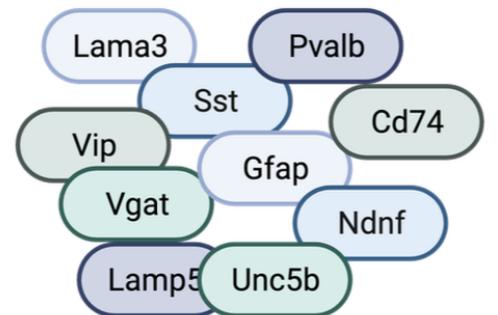


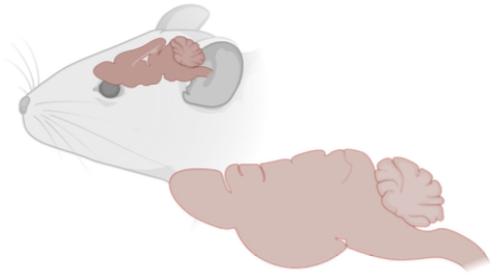
Spatial transcriptomics

Anna Alemany
Dpt. Anatomy & Embryology, LUMC
31/10/2024, MGC course (Rotterdam)

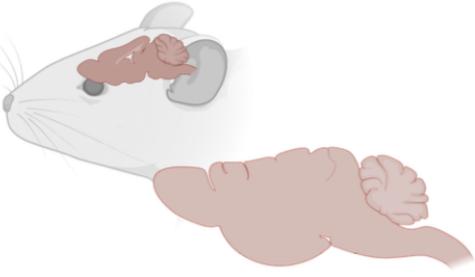
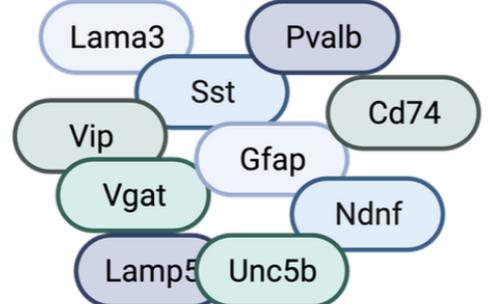


bulk RNAseq

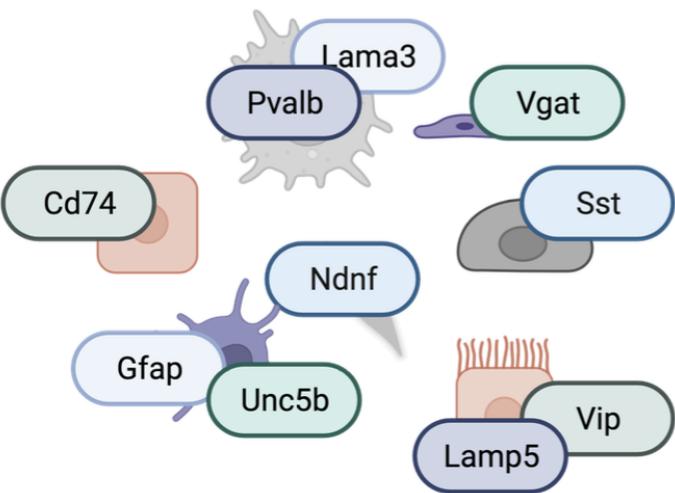
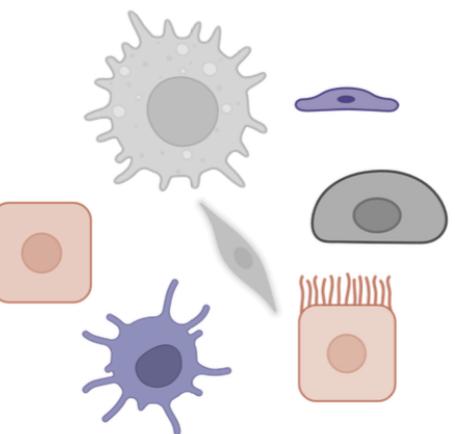


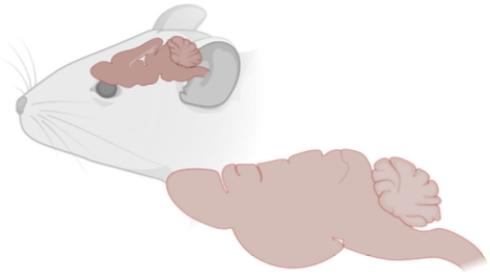


bulk RNAseq

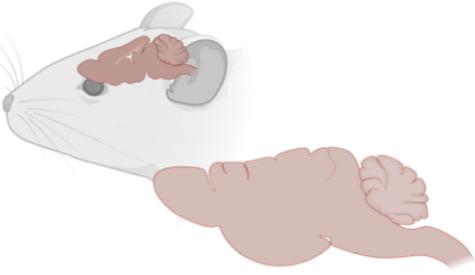
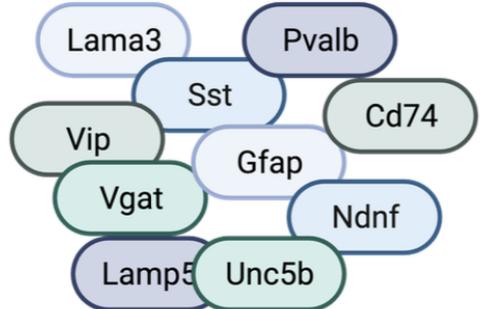


scRNAseq

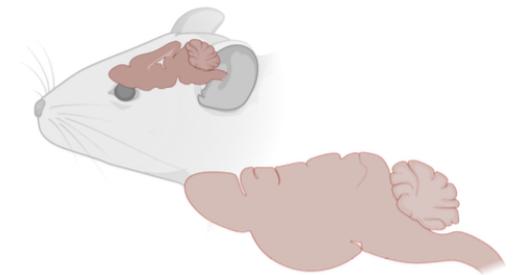
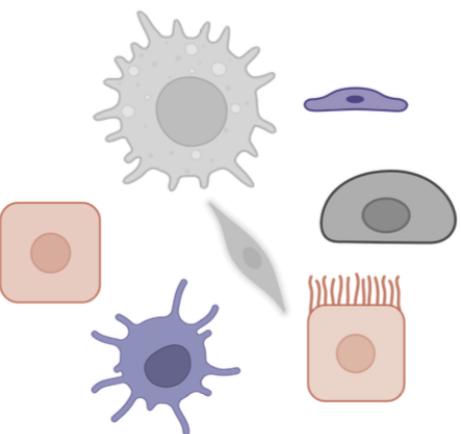




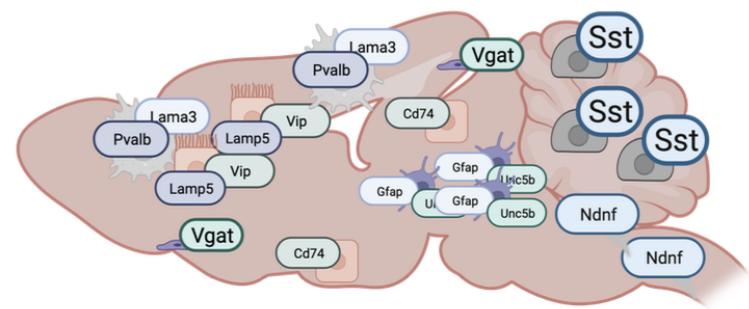
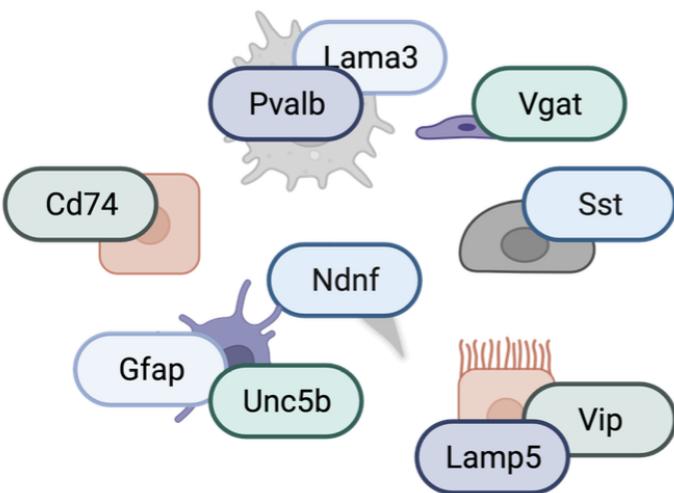
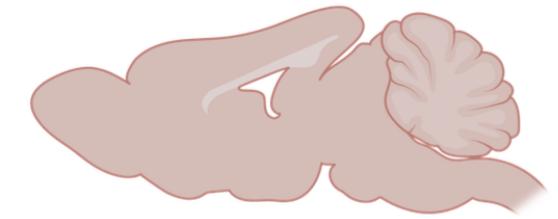
bulk RNAseq



scRNAseq



spatial transcriptomics



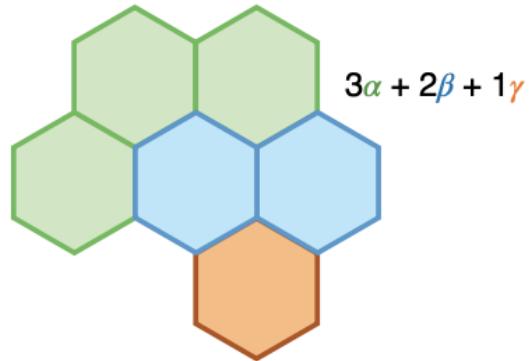
Quantitative advantages of spatial transcriptomics

Quantitative advantages of spatial transcriptomics

Tissues have structure

Quantitative advantages of spatial transcriptomics

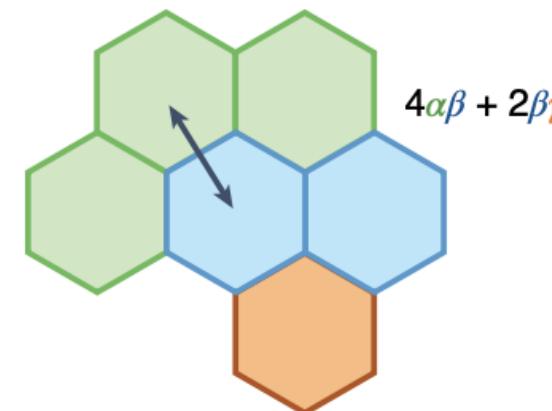
Cell composition



Tissue atlases

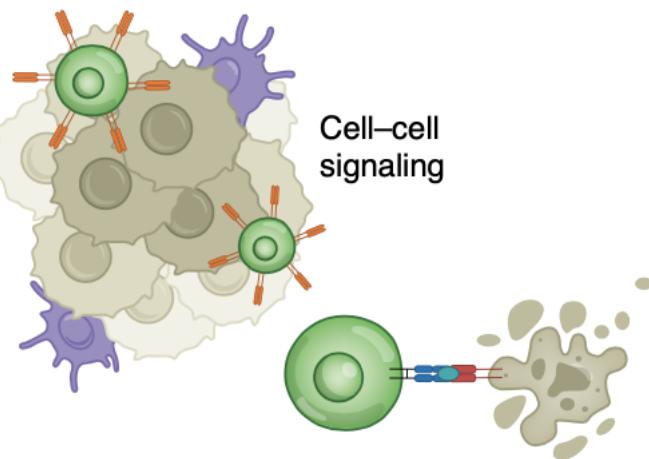
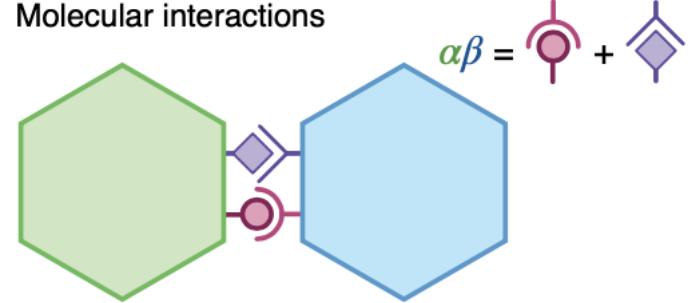
Tissues have structure

Cell-cell interactions



Cellular neighborhoods

Molecular interactions



Cell-cell
signaling



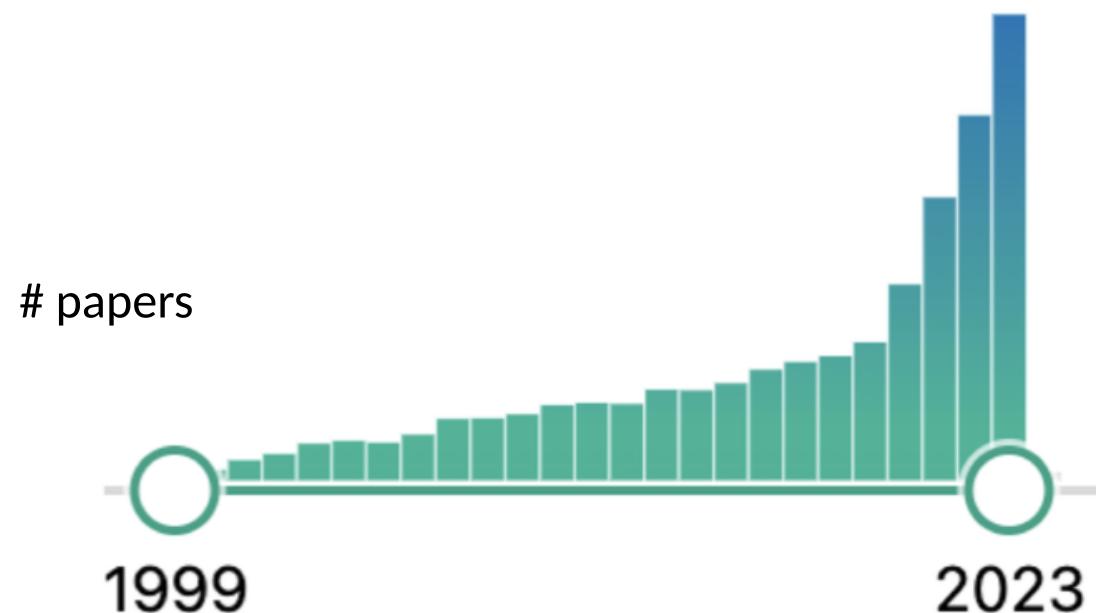
Method of the Year 2020: spatially resolved transcriptomics

Spatially resolved transcriptomics methods are changing the way we understand complex tissues.



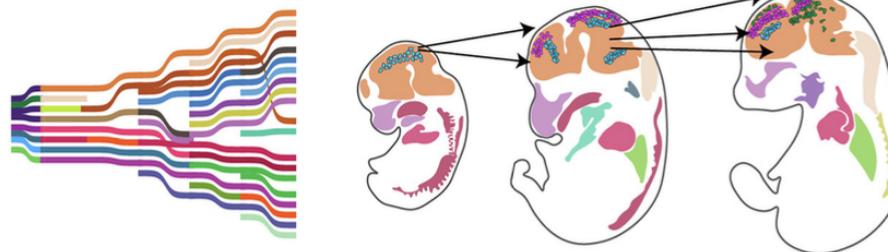
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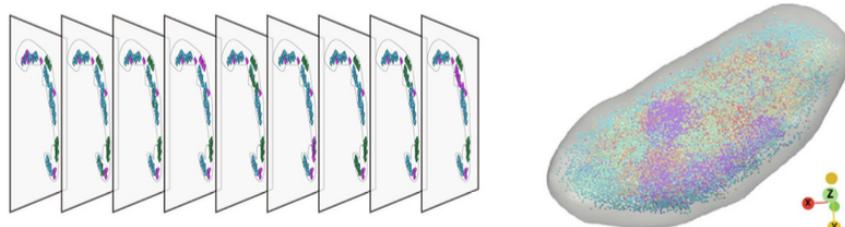


Application

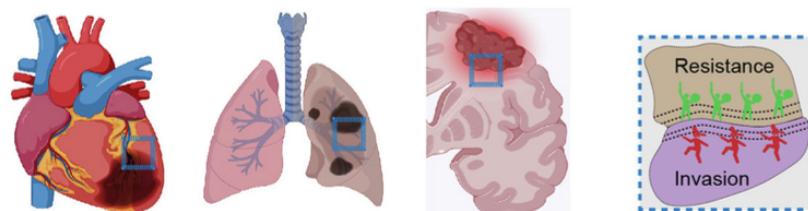
● Development



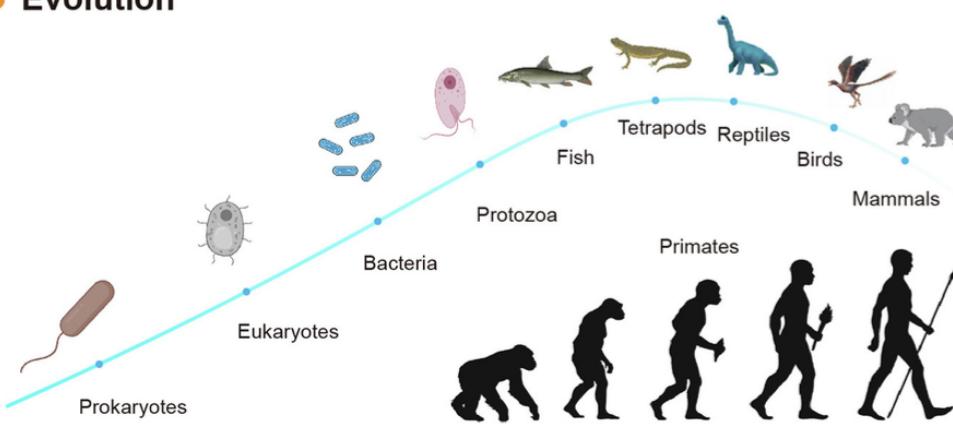
● Spatial/ 3D Atlas



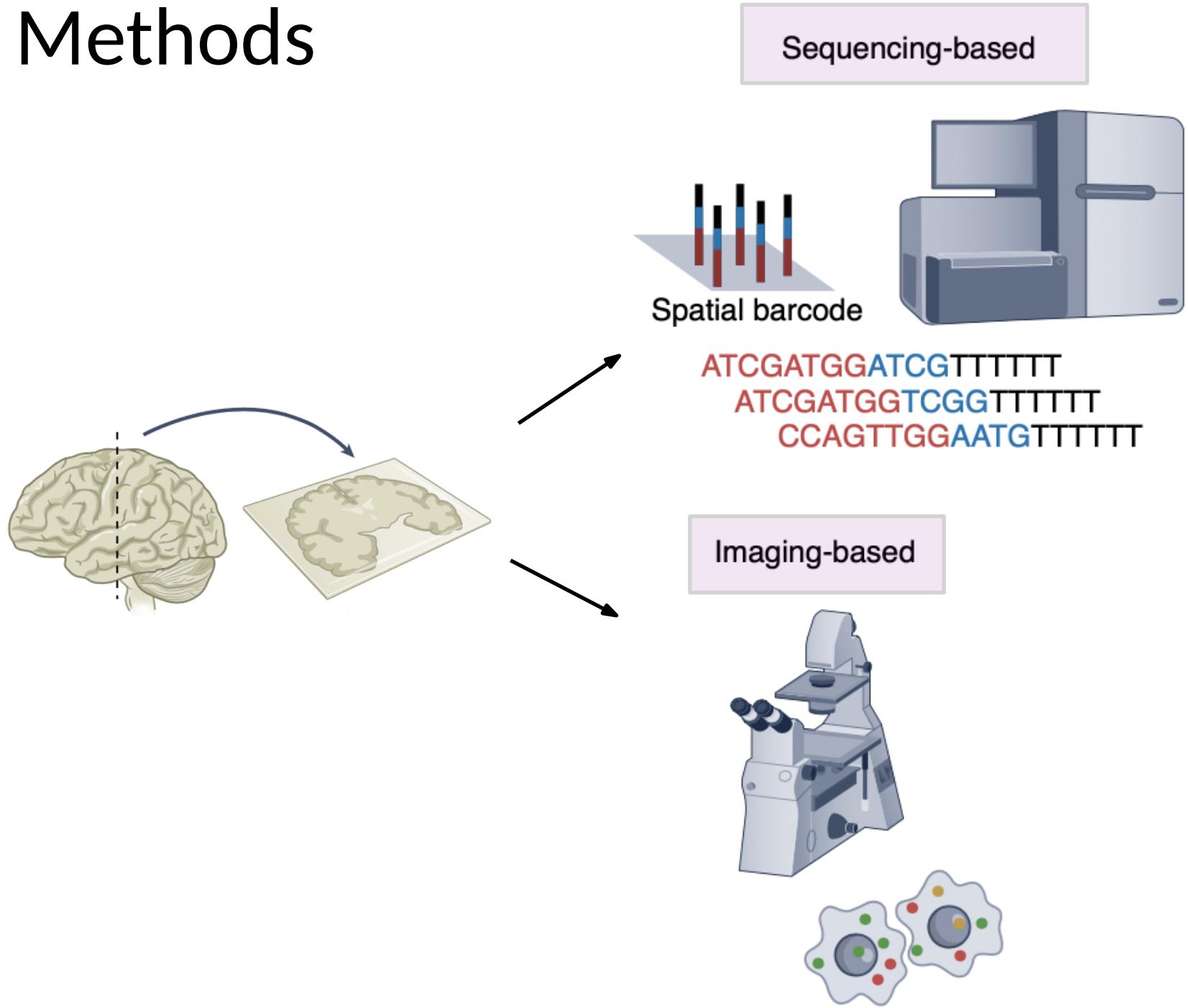
● Clinical Diseases



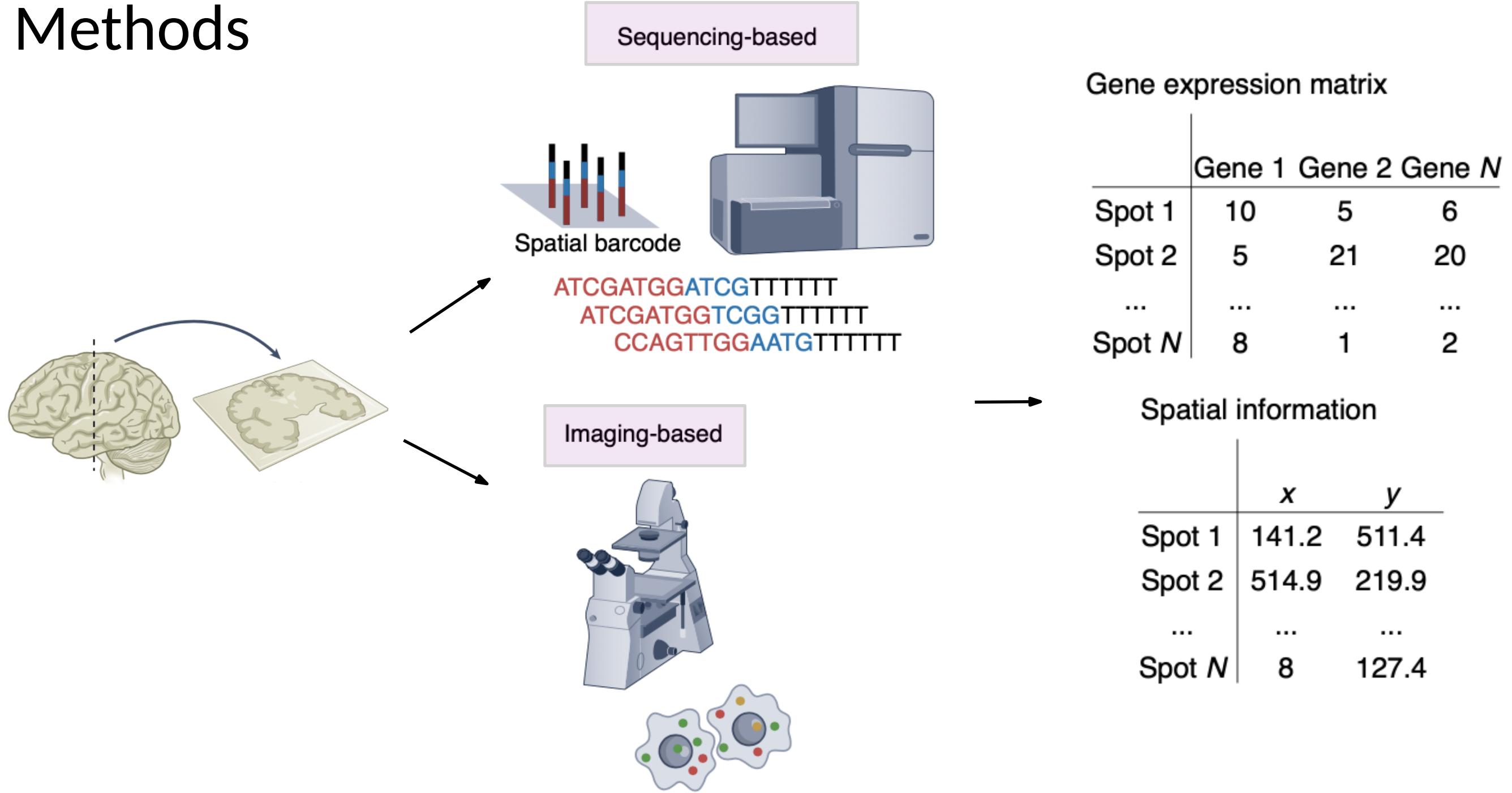
● Evolution



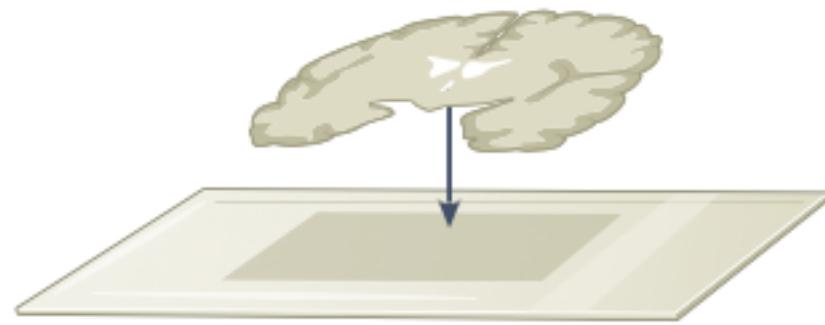
Methods



Methods



Sequencing-based spatial transcriptomics

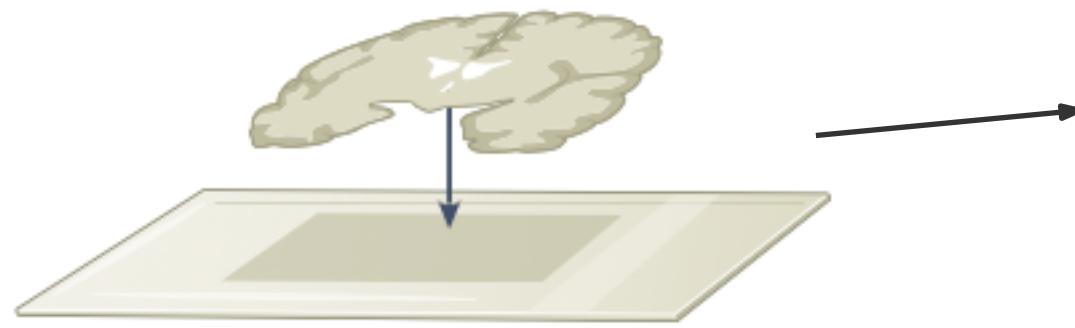


Spatially-resolved indexed surface

polyT-barcoded primers

pixel \leftrightarrow barcode

Sequencing-based spatial transcriptomics



Spatially-resolved indexed surface

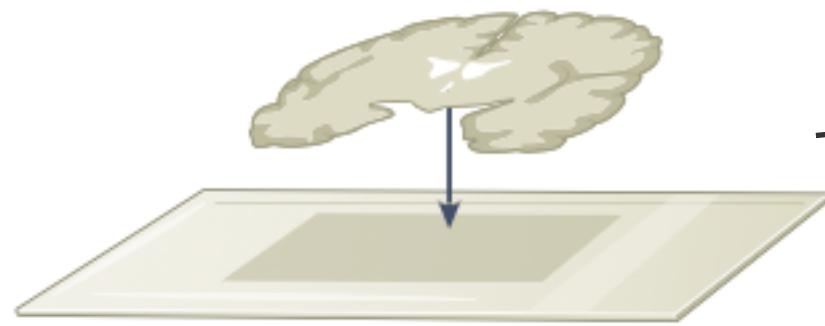
- tissue to surface
- surface to tissue

Spatially label
tissue's mRNA

polyT-barcoded primers

pixel \leftrightarrow barcode

Sequencing-based spatial transcriptomics



Spatially-resolved indexed surface

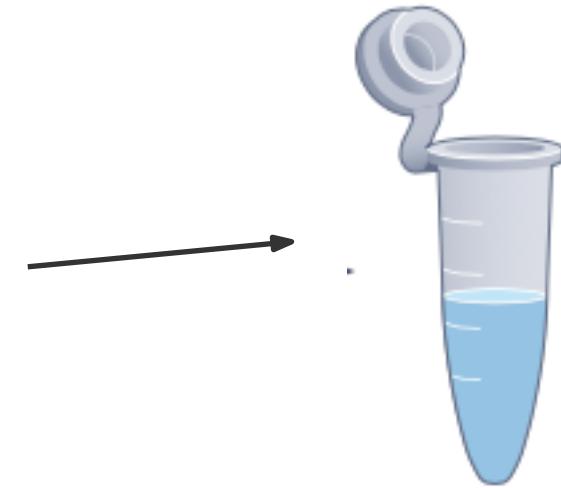
polyT-barcoded primers

pixel \leftrightarrow barcode



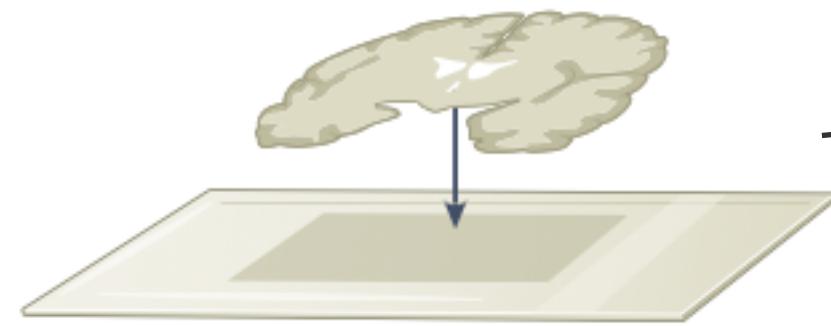
- tissue to surface
- surface to tissue

Spatially label
tissue's mRNA



library prep
sequencing

Sequencing-based spatial transcriptomics



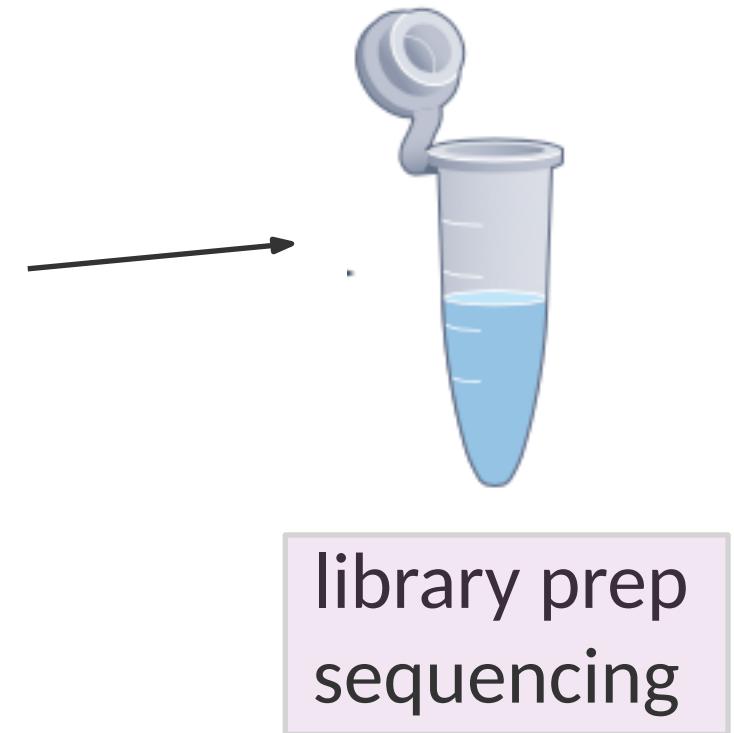
Spatially-resolved indexed surface

polyT-barcoded primers

pixel↔barcode

- tissue to surface
- surface to tissue

Spatially label
tissue's mRNA



Visium, slide-seq, stereo-seq,
seq-scope, DBiT-seq...

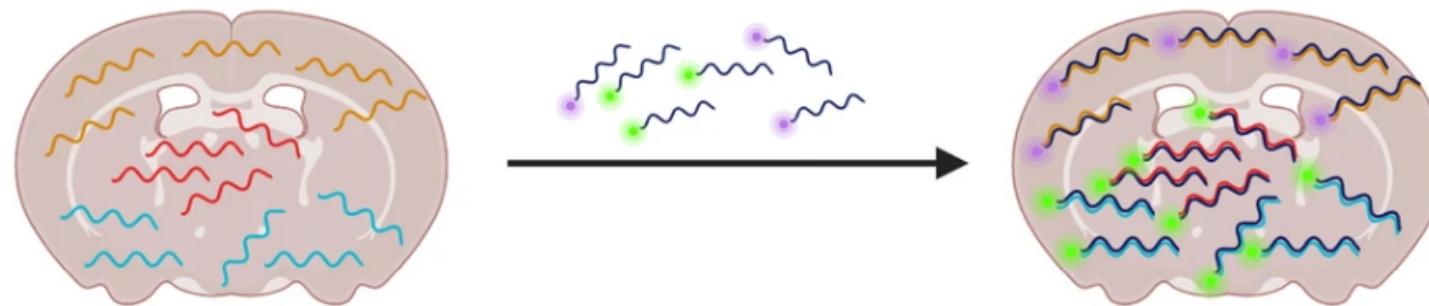
Imaging-based spatial transcriptomics

Tissue fixation followed by...

Imaging-based spatial transcriptomics

Tissue fixation followed by...

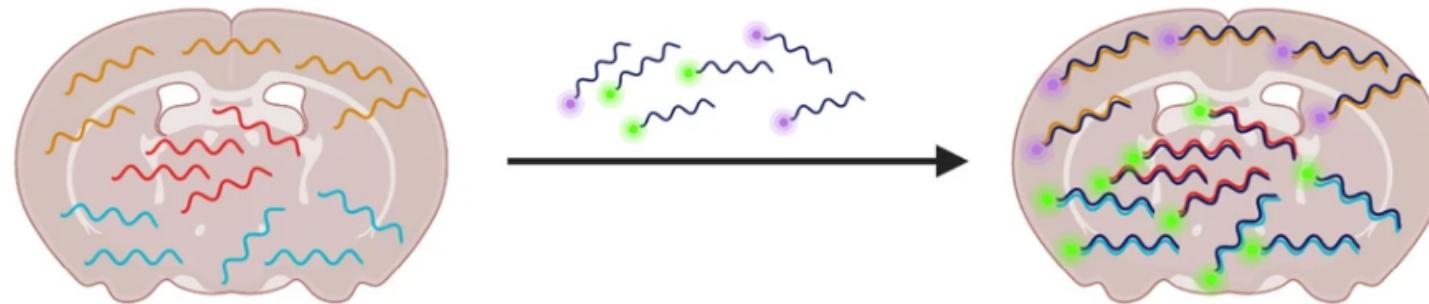
- 1) Hybridise labelled probes to target mRNA



Imaging-based spatial transcriptomics

Tissue fixation followed by...

1) Hybridise labelled probes to target mRNA

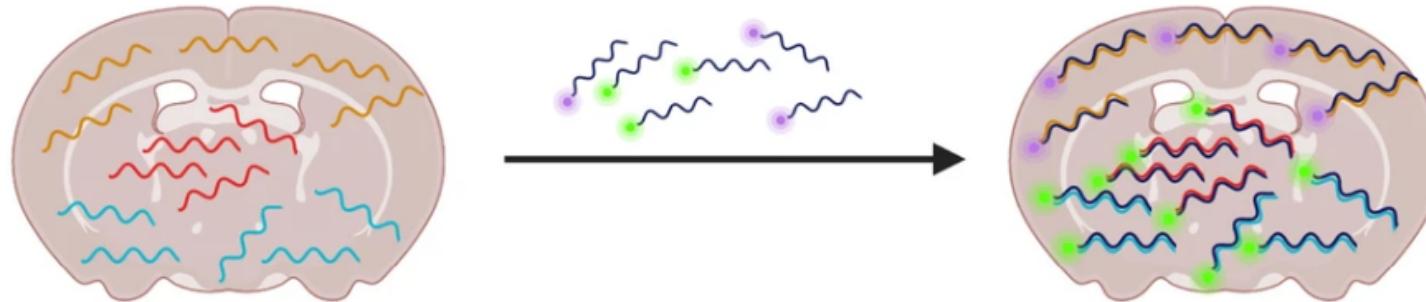


- direct probe-based detection
- enzymatically-assisted probe detection
- enzymatic sequencing

Imaging-based spatial transcriptomics

Tissue fixation followed by...

1) Hybridise labelled probes to target mRNA



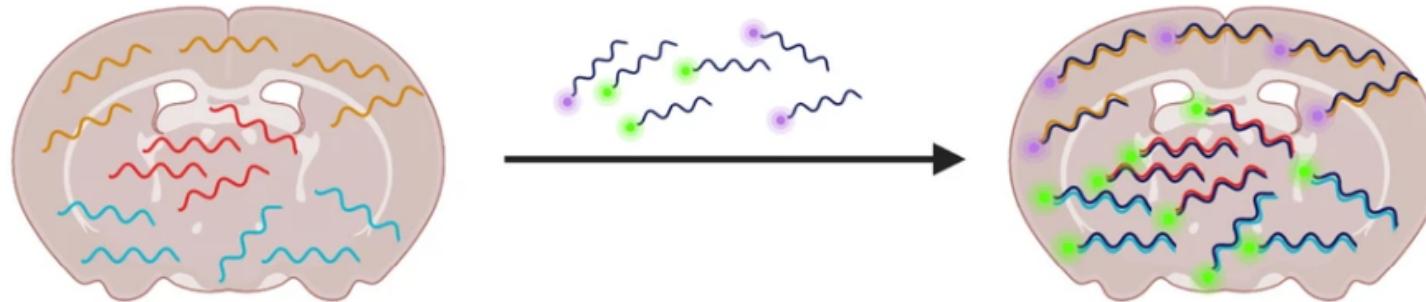
- direct probe-based detection
- enzymatically-assisted probe detection
- enzymatic sequencing

2) Image fluorophore locations

Imaging-based spatial transcriptomics

Tissue fixation followed by...

1) Hybridise labelled probes to target mRNA



- direct probe-based detection
- enzymatically-assisted probe detection
- enzymatic sequencing

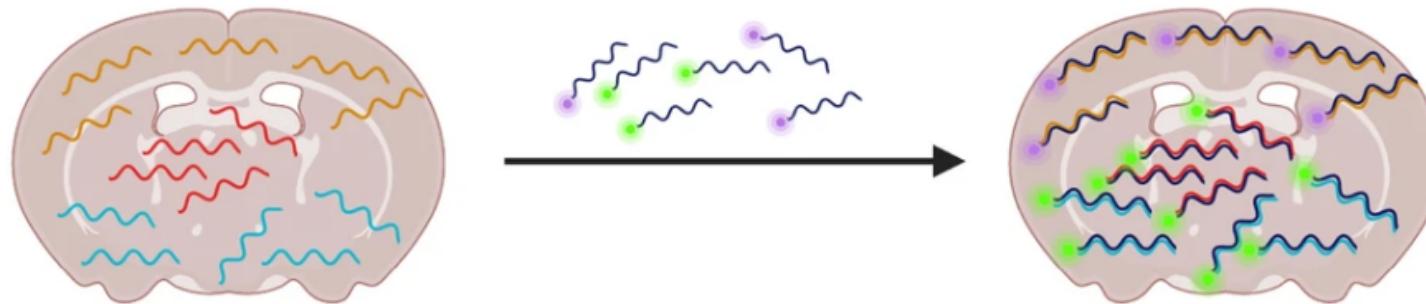
2) Image fluorophore locations

Limitations in spectral bandwidth

Imaging-based spatial transcriptomics

Tissue fixation followed by...

1) Hybridise labelled probes to target mRNA



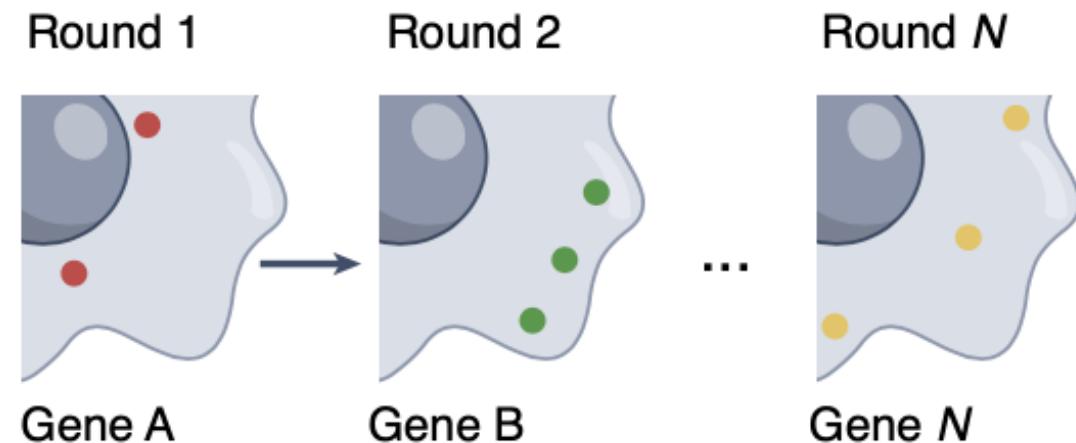
- direct probe-based detection
- enzymatically-assisted probe detection
- enzymatic sequencing

2) Image fluorophore locations

Limitations in spectral bandwidth

3) Multiple imaging rounds

Encoding



Imaging- **versus** sequencing-based spatial transcriptomics



Imaging- **versus** sequencing-based spatial transcriptomics

Targeted vs. untargeted

- ⇒ Sequencing-based is **whole transcriptome**
- ⇒ Imaging-based uses a **panel** (rapidly increasing numbers)

Different resolution levels

- ⇒ Sequencing-based offers **grid-type profiling**
- ⇒ Imaging-based has **subcellular resolution**

Imaging- **versus** sequencing-based spatial transcriptomics

Targeted vs. untargeted

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- ⇒ Imaging-based uses a **panel** (rapidly increasing numbers)

Different resolution levels

- ⇒ Sequencing-based offers **grid-type profiling**
- ⇒ Imaging-based has **subcellular resolution**

Easiest comparison is one within 1 company:

1. Visium sequencing based
2. Xenium imaging based

Visium versus Xenium

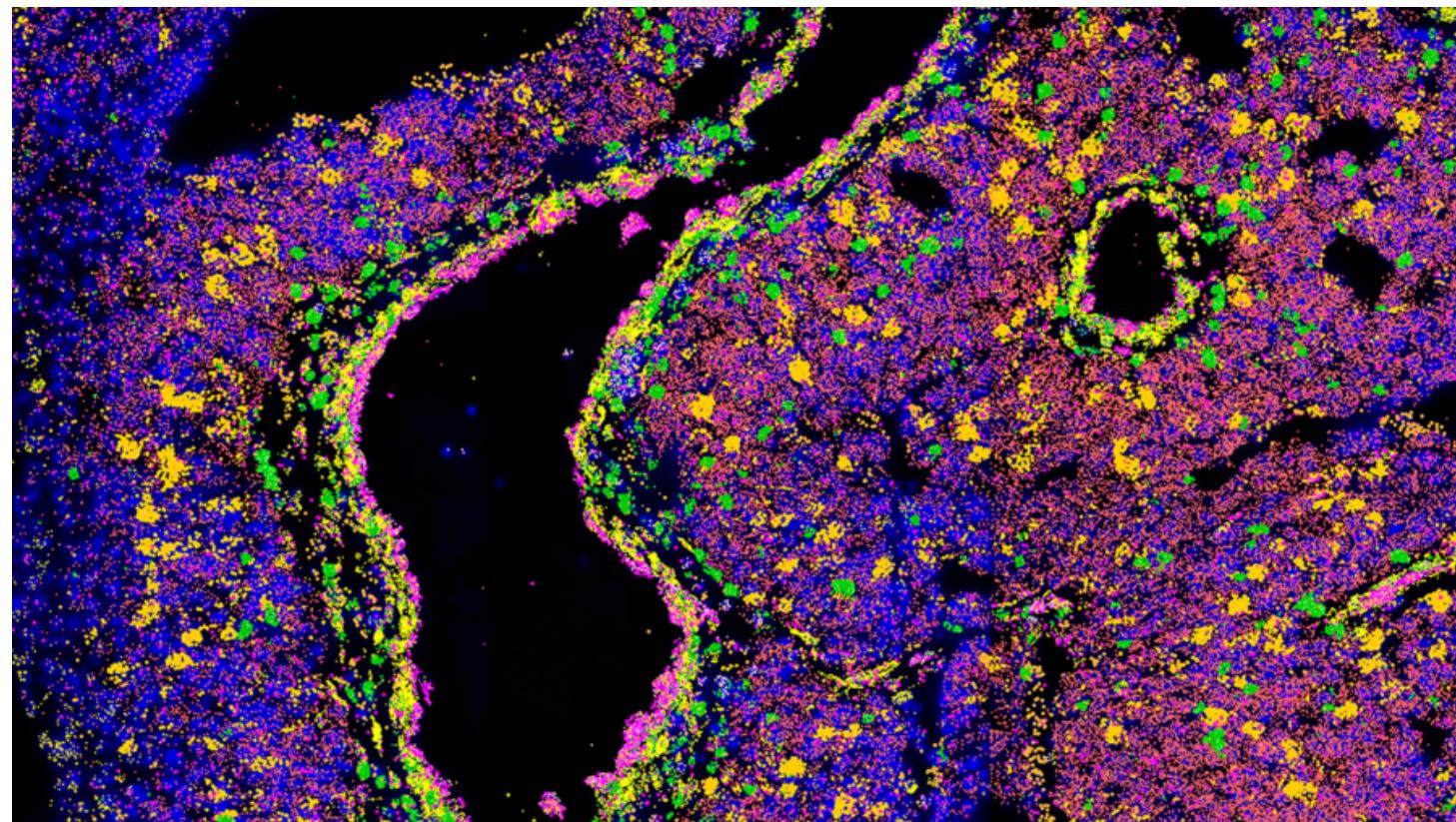
Visium

- Sequencing-based
- Resolution: 55um
- Whole transcriptome
- 5000 spots on 6*6mm capture area
- FFPE and fresh frozen fixation

Xenium

- Imaging-based
- Subcellular resolution ($\pm 0.2\text{um/pixel}$)
- Targeted discovery
- 2 slides per run ($2 \times 1\text{ cm}^2$)
- FFPE and fresh frozen fixation

We have the data... now what?



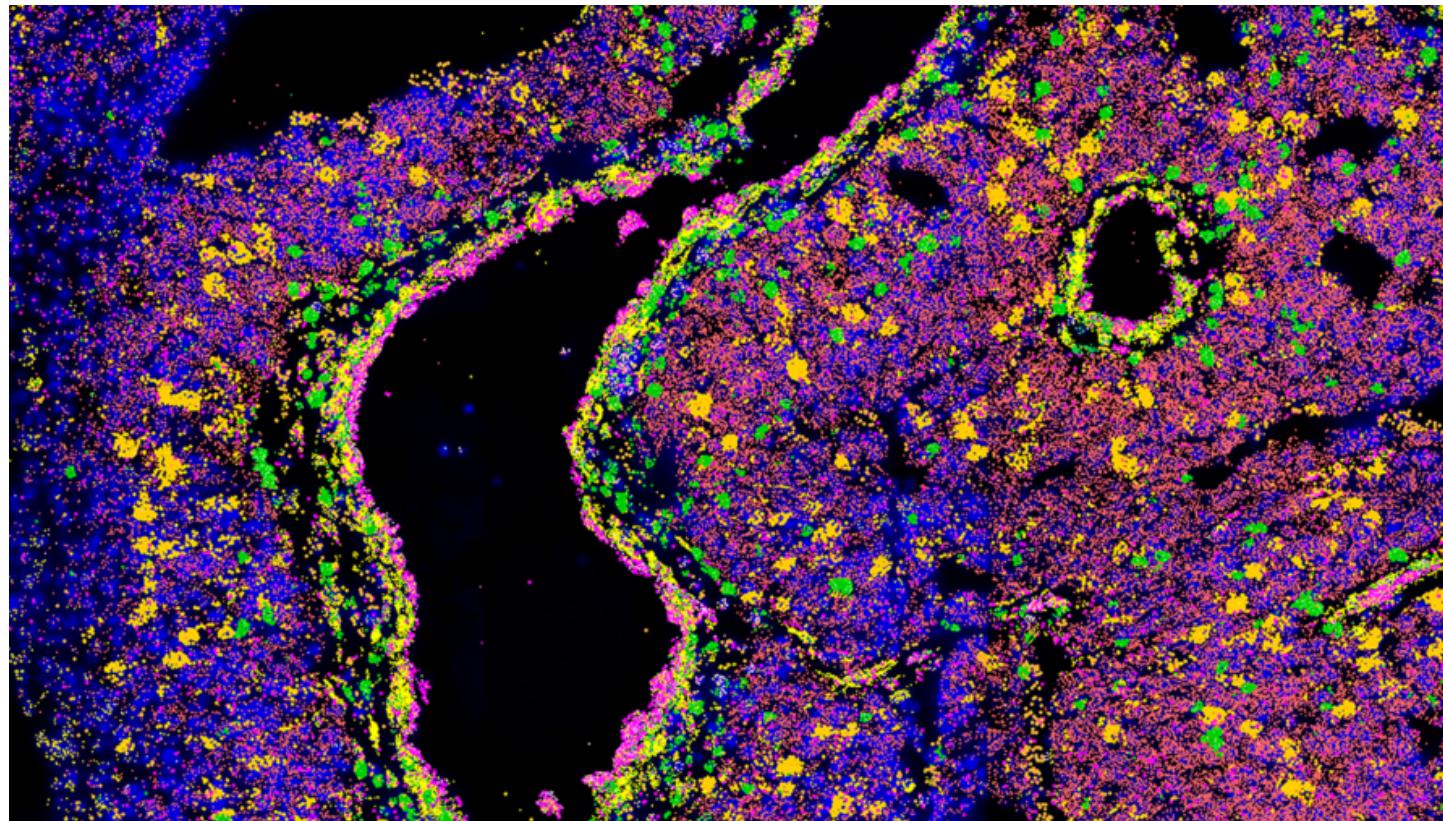
Gene expression matrix

	Gene 1	Gene 2	Gene N
Spot 1	10	5	6
Spot 2	5	21	20
...
Spot N	8	1	2

Spatial information

	x	y
Spot 1	141.2	511.4
Spot 2	514.9	219.9
...
Spot N	8	127.4

We have the data... now what?



- Data normalization
- Batch effect correction
- Dimensionality reduction
- Clustering
- Spatially variable genes
- Cell type identification
- Deconvolution
- Spatial trajectory inference
- Cell-cell communication analysis
- Integration with other omics
- ...

Comprehensive analysis toolkits

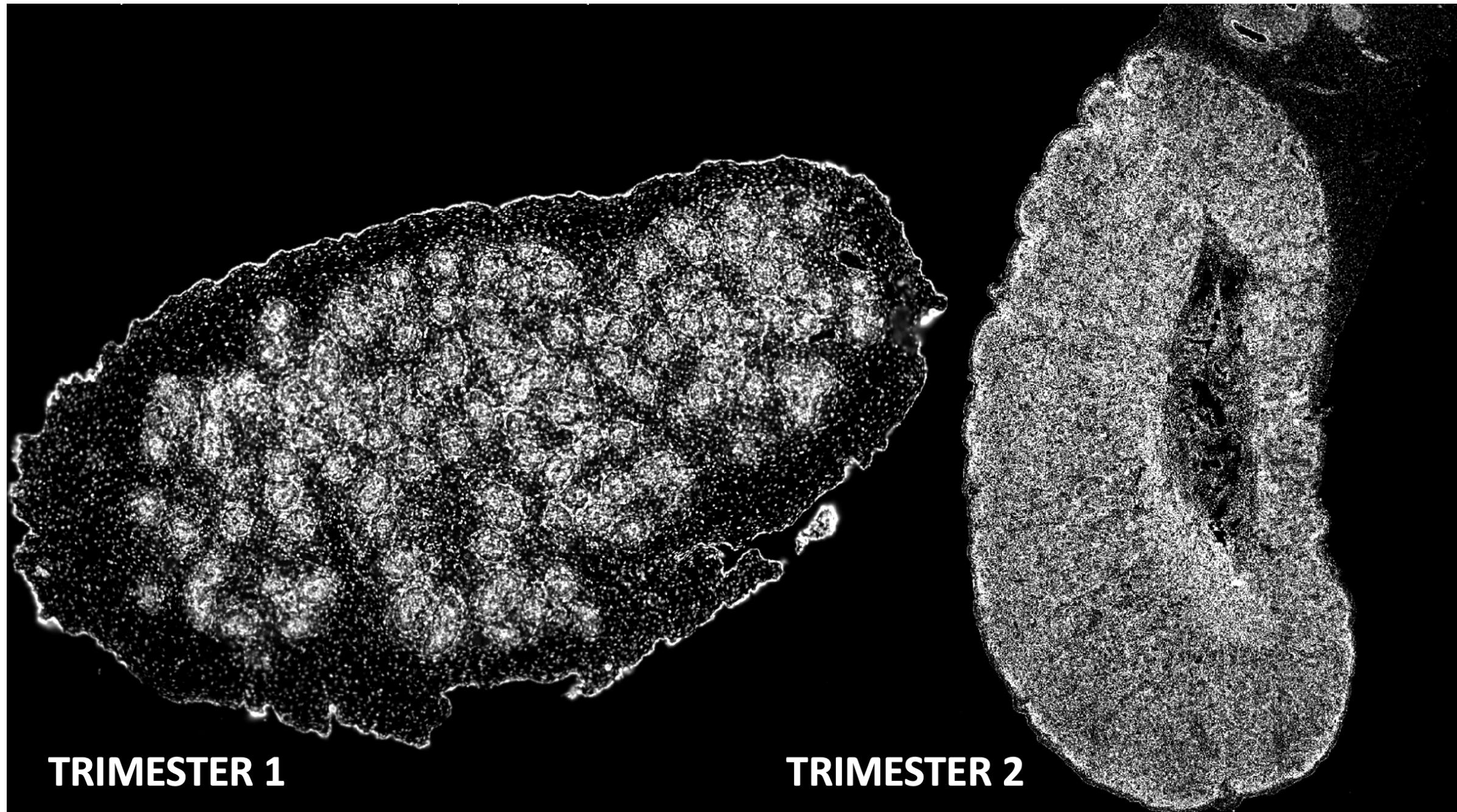
Table 2 List of representative spatial transcriptomics bioinformatics tools

Tool	function	language	Description	Website
space ranger	data pre-processing(10X Visium)	shell	Space Ranger is a set of analysis pipelines that process 10x Genomics Visium data with brightfield or fluorescence microscope images, allowing users to map the whole transcriptome in a variety of tissues.	https://www.10xgenomics.com/support/software/space-ranger/latest
SAW starfish	data pre-processing(Stereo-seq) data pre-processing(ISS/ISH)	shell python	Workflow for analyzing Stereo-seq transcriptomics data It is a scalable pipelines for image-based transcriptomics, can be utilized to localize and quantify RNA transcripts in image data	https://github.com/STOmics/SAW https://spacex-starfish.readthedocs.io/en/latest/index.html
Seurat	Comprehensive tool	R	Seurat is a toolbox for scRNA-seq and ST data analysis in R	https://github.com/satijalab/seurat
Scanpy	Comprehensive tool	Python	Scanpy is a toolbox for scRNA-seq and ST data analysis in python	https://scanpy.readthedocs.io/en/stable/
Giotto	Comprehensive tool	R/Python	Gitto provides tools for processing, analyzing, and visualizing spatial multiomics data at various scales and multiple resolutions	https://github.com/RubD/Giotto
STUtility	Comprehensive tool	R	A versatile toolkit for spatially resolved transcriptomic analysis and visualization	https://github.com/jbergenstrahle/STUtility
Squidpy	Comprehensive tool	Python	It brings together tools from omics and image analysis to enable scalable description of spatial molecular data, such as transcriptome or multivariate proteins	https://github.com/scverse/squidpy
BayesSpace	Dimensionality reduction and clustering	R; C++	It's a fully Bayesian statistical method that uses the information from spatial neighborhoods for resolution enhancement of spatial transcriptomics data and for clustering analysis.	https://github.com/edward130603/BayesSpace
SC-MEB	Dimensionality reduction and clustering	R; C++	spatial clustering with hidden Markov random field using empirical Bayes	https://github.com/Shufeyangyi2015310117/SC.MEB
SpaGCN	Dimensionality reduction and clustering	Python	SpaGCN is a graph convolutional network to integrate gene expression and histology to identify spatial domains and spatially variable genes.	https://github.com/jianhuupenn/SpaGCN
DeepST	Spatial domain identification	Python	DeepST is a method based on deep learning to identify spatial domains and enhance gene expression data	https://github.com/JiangBioLab/DeepST
STAGATE	Spatial domain identification	Python	STAGATE utilizes GAEs and GNNs to integrate and propagate spatial information from spatial transcriptomics data, enabling the effective identification of both local and global spatial domains.	https://github.com/QIFEIDKN/STAGATE
SiGra	Spatial domain identification	Python	SiGra uses single-cell spatial maps and graph transformation models to identify spatial domains based on imaging data	https://github.com/QSong-github/SiGra
trendsseek	Find HVGs	R	Trendsseek is a method based on marked point processes that identifies genes with statistically significant spatial expression trends.	https://github.com/edsgard/trendsseek

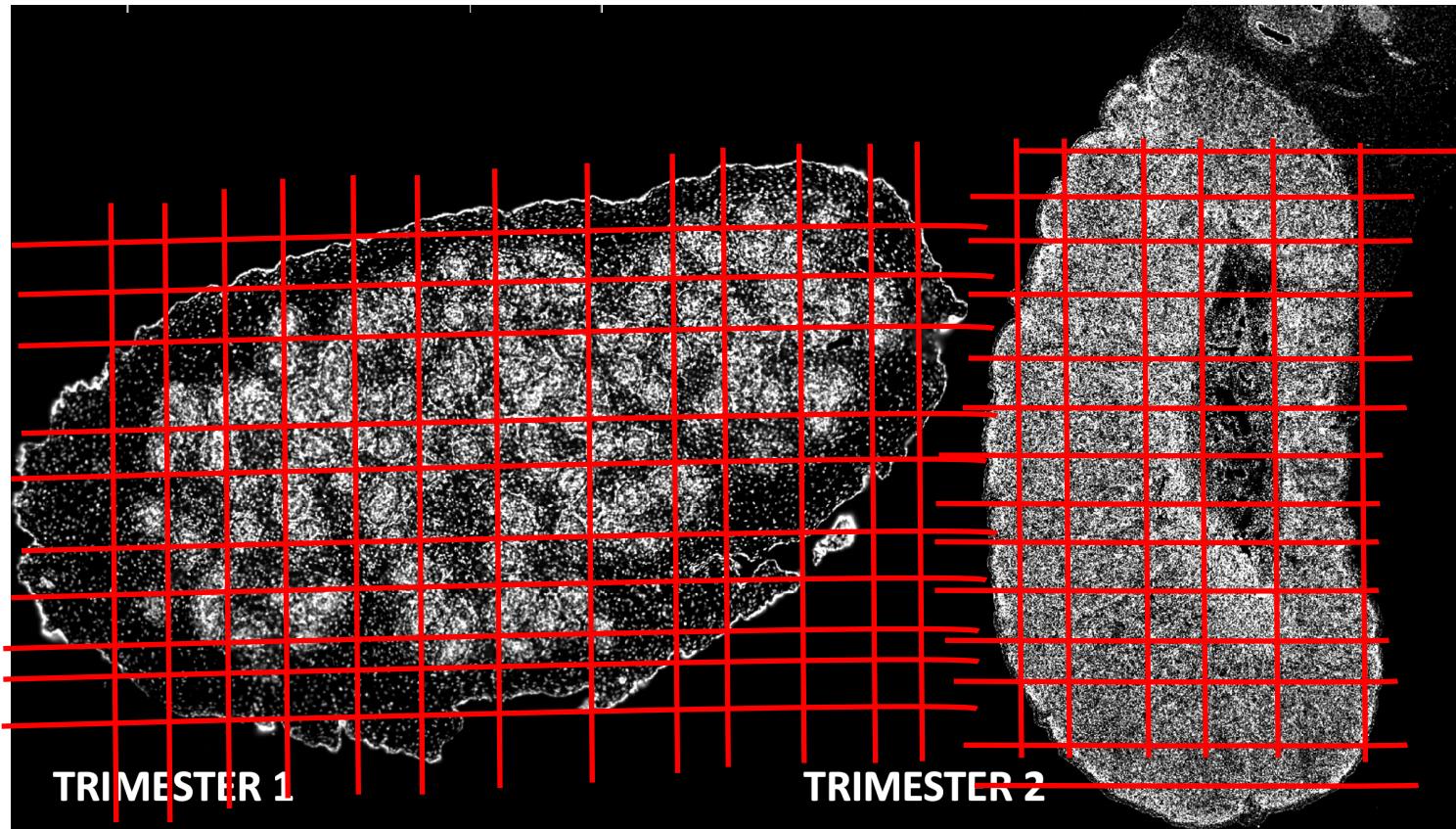
Limitations and challenges of spatial transcriptomics

- Abundance of mRNAs captured is limited
- Probe capture efficiency limits depth and coverage
- Depth-resolution trade-off
- Laborious sample preparation (slicing, permeabilization, labelling, dissociation...)
- Huge demands on computational power and storage capacity
- **Build specialized databases** to summarize and integrate data from different studies and different platforms

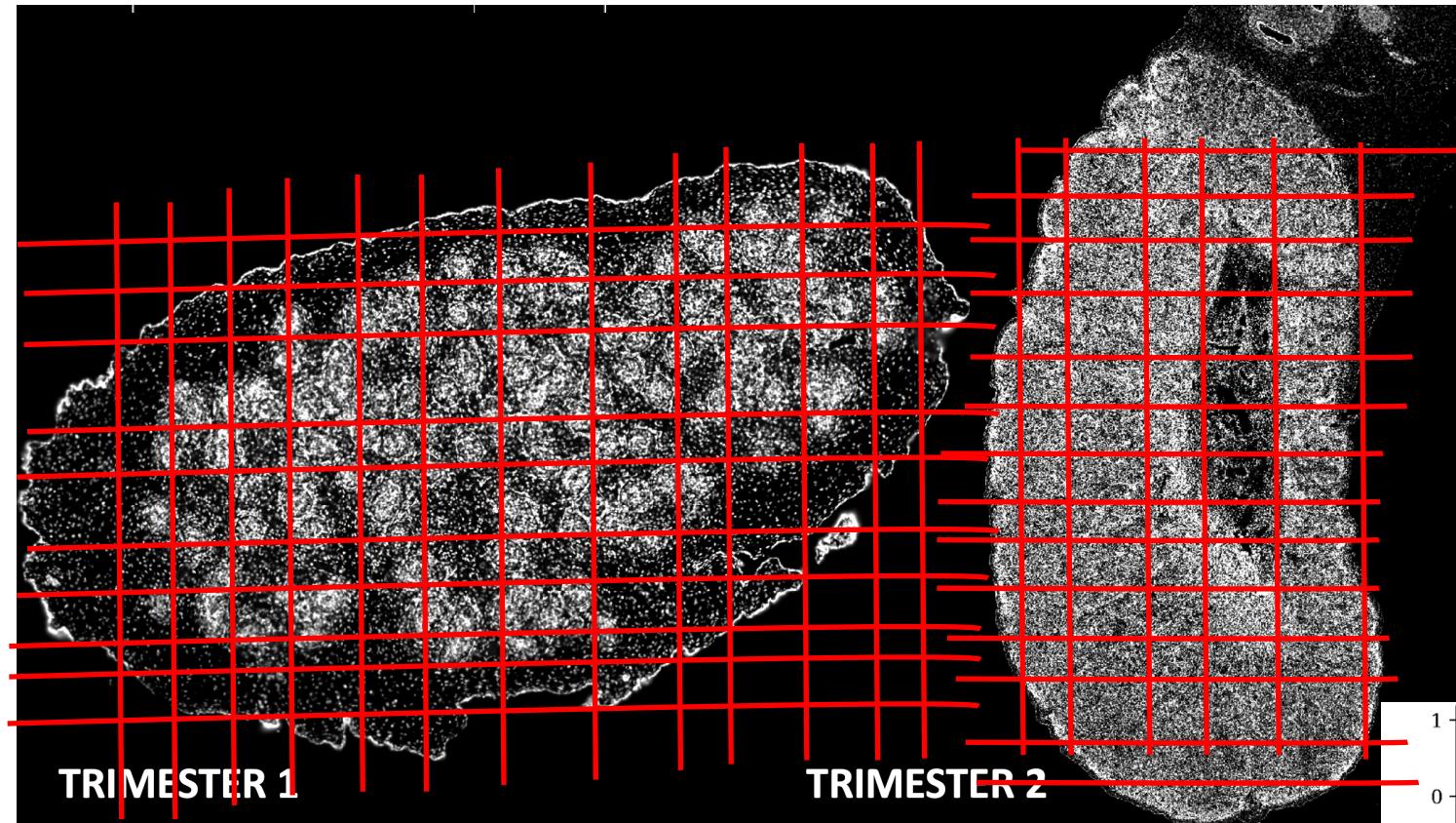
Case study: human embryonic testis



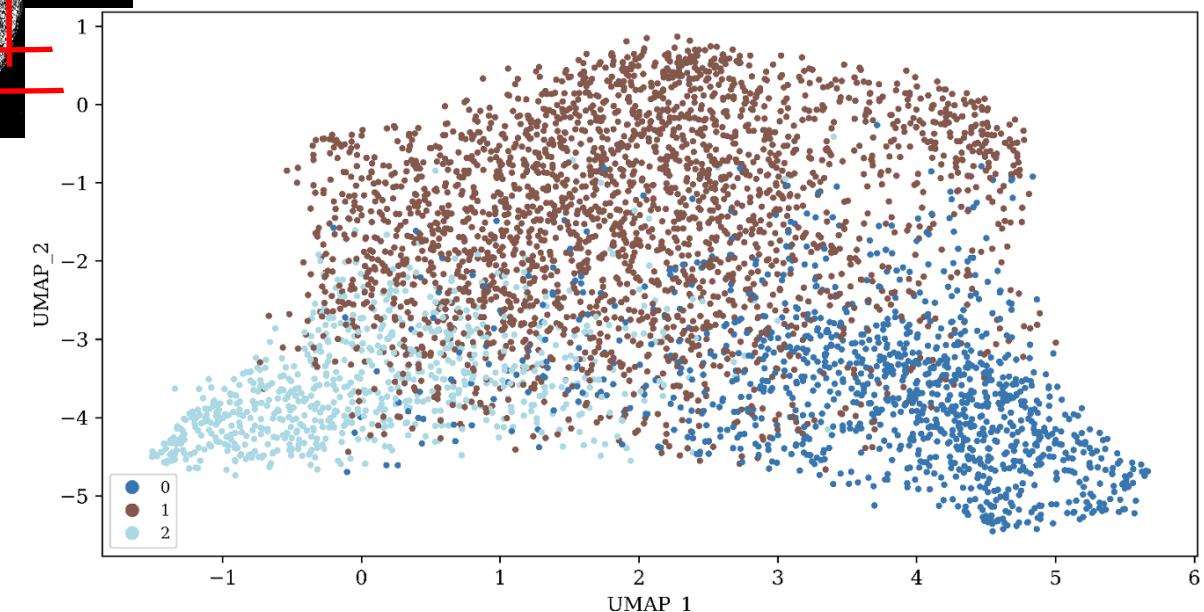
Grid-based segmentation



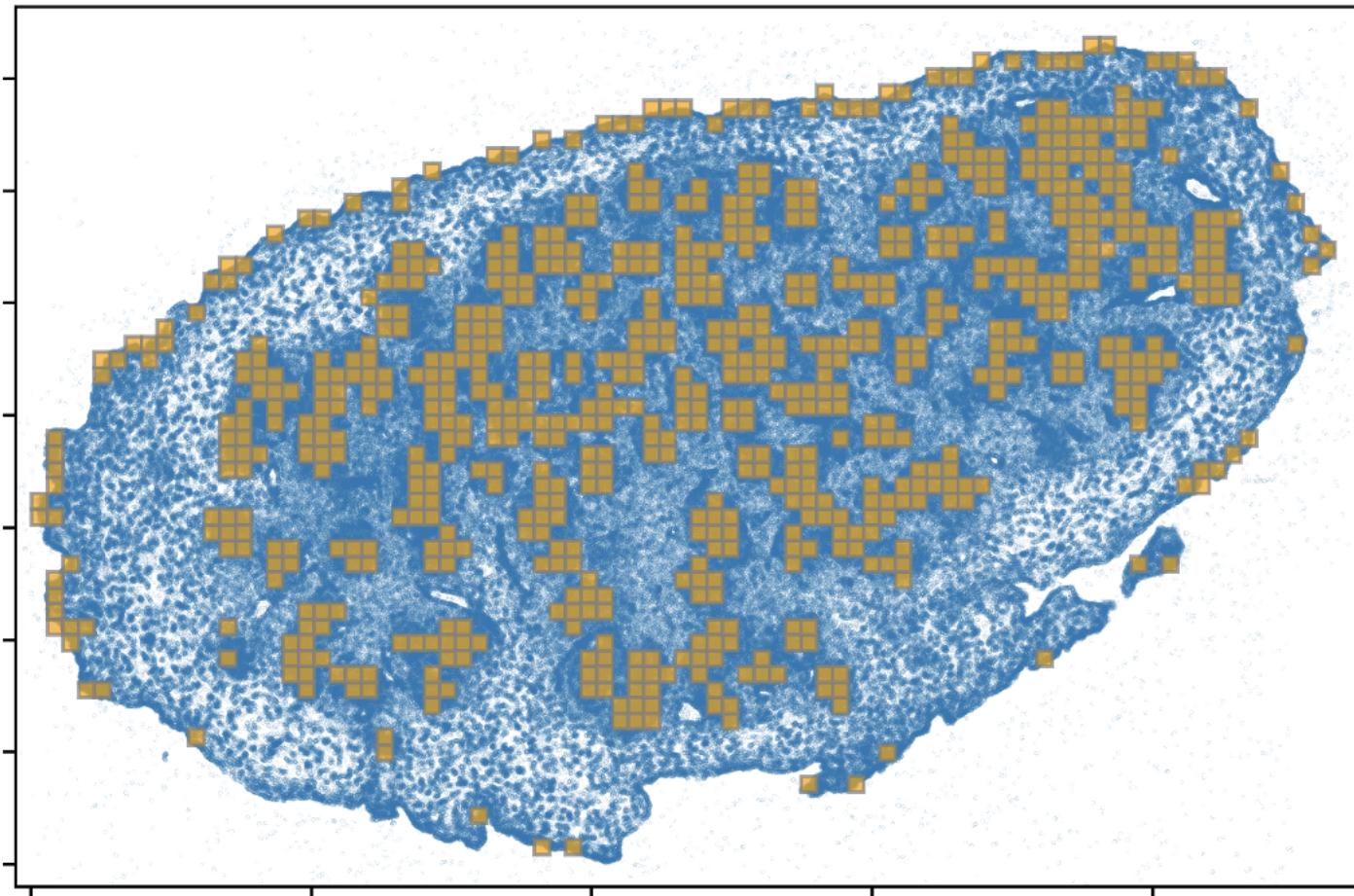
Grid-based segmentation



treat each box
as a single cell



Cluster ID projection into tissue



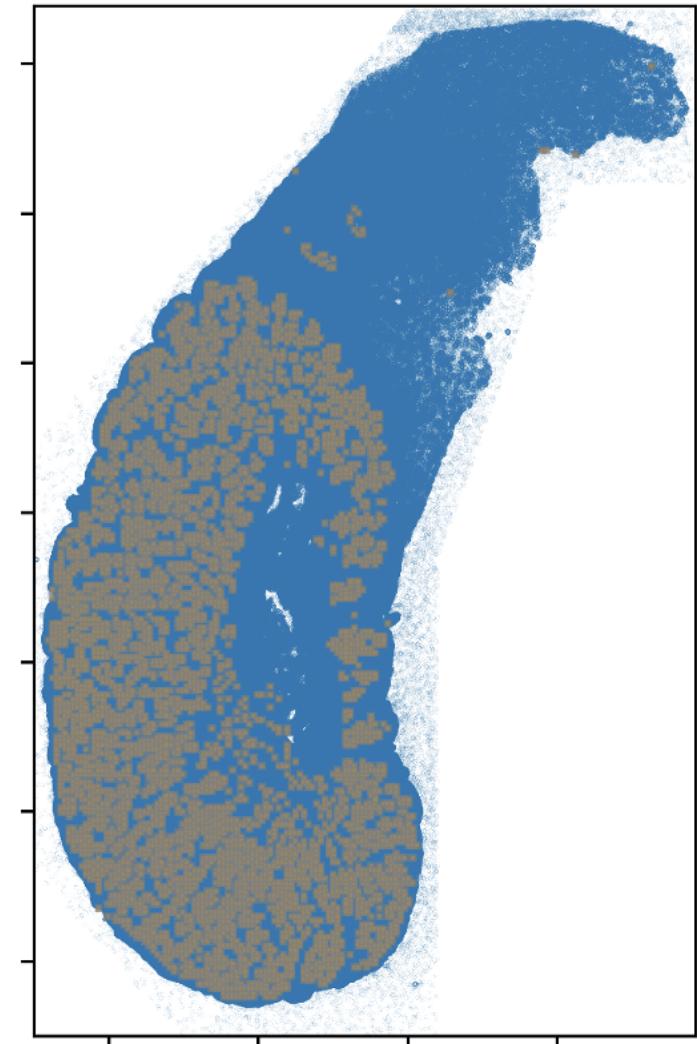
Trimester 1

Upregulated:

GATM
PTGDS
NTN4
TMC5
TENT5C

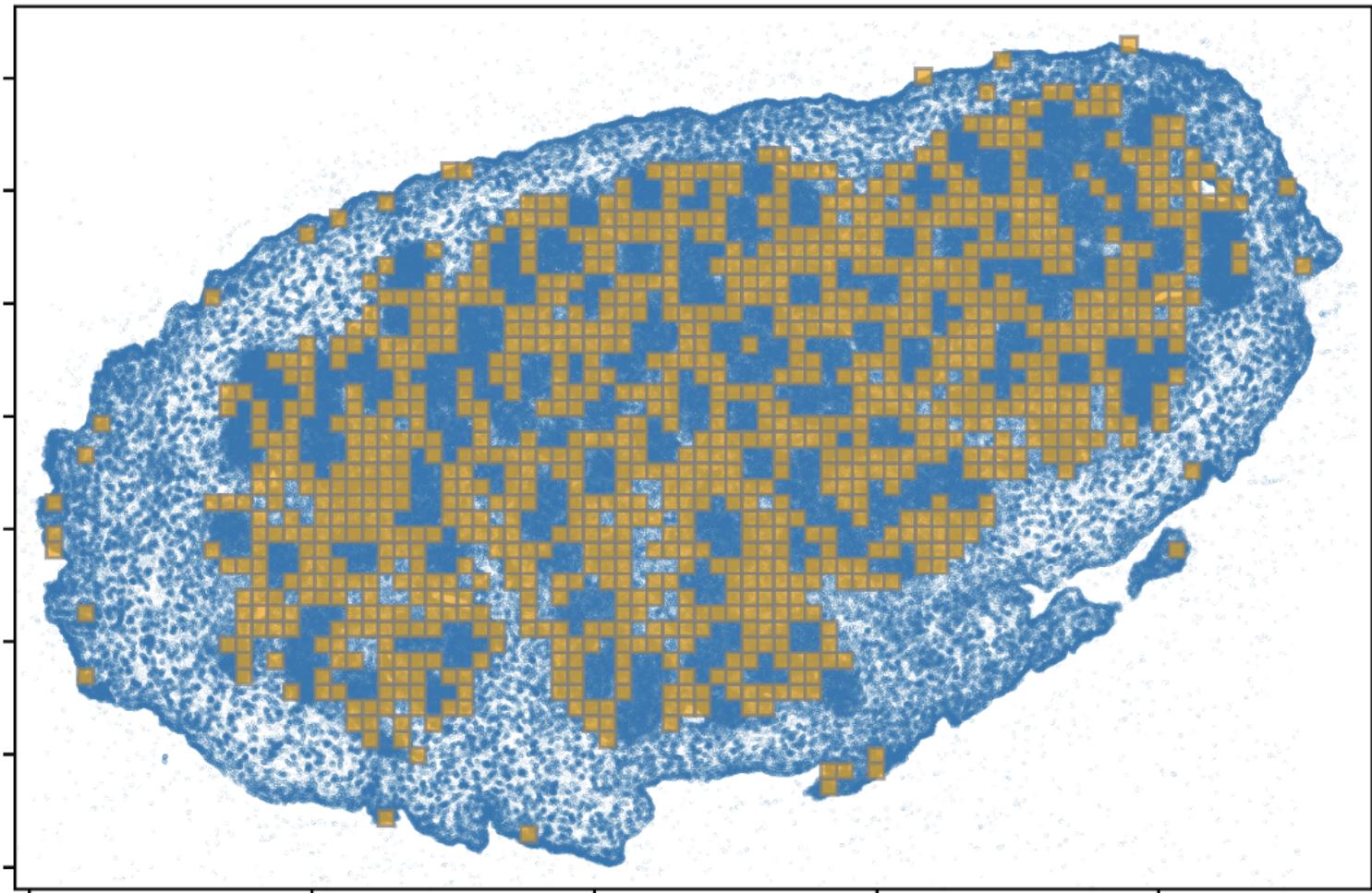
Downregulated:

PDPN
EPCAM
PPP1R1A
FSTL3
GPC1



Trimester 2

Cluster ID projection into tissue



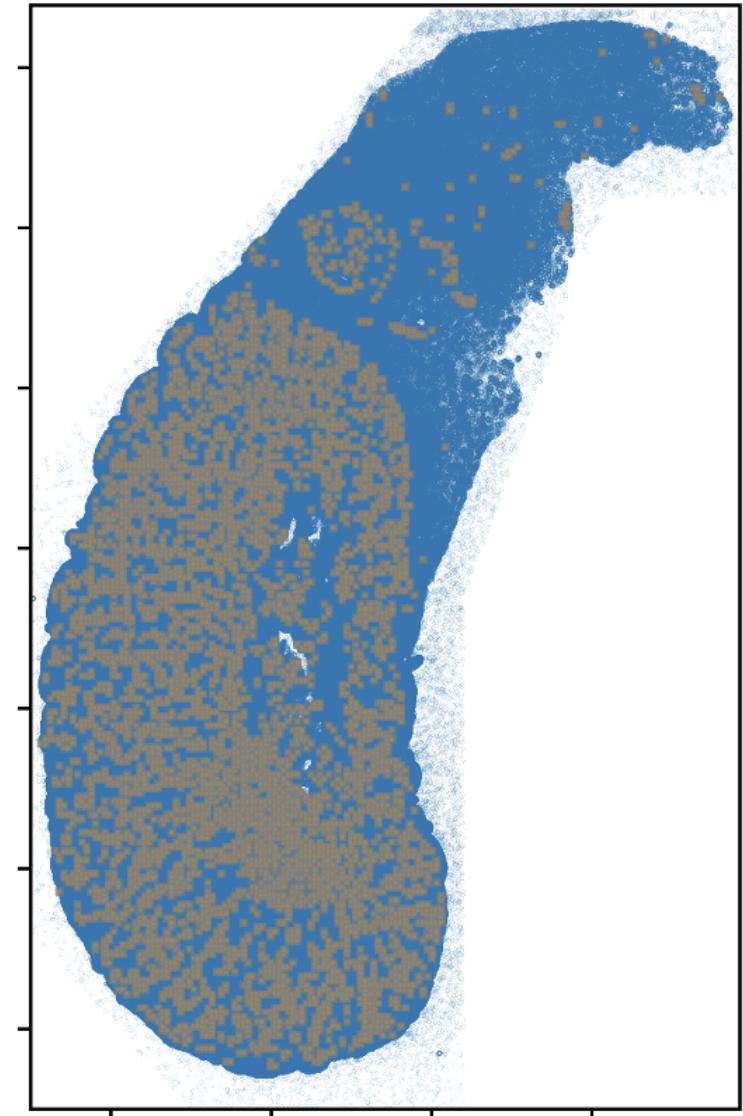
Trimester 1

Upregulated:

SNCG
TCIM
AQP2
VWF
KCNK3

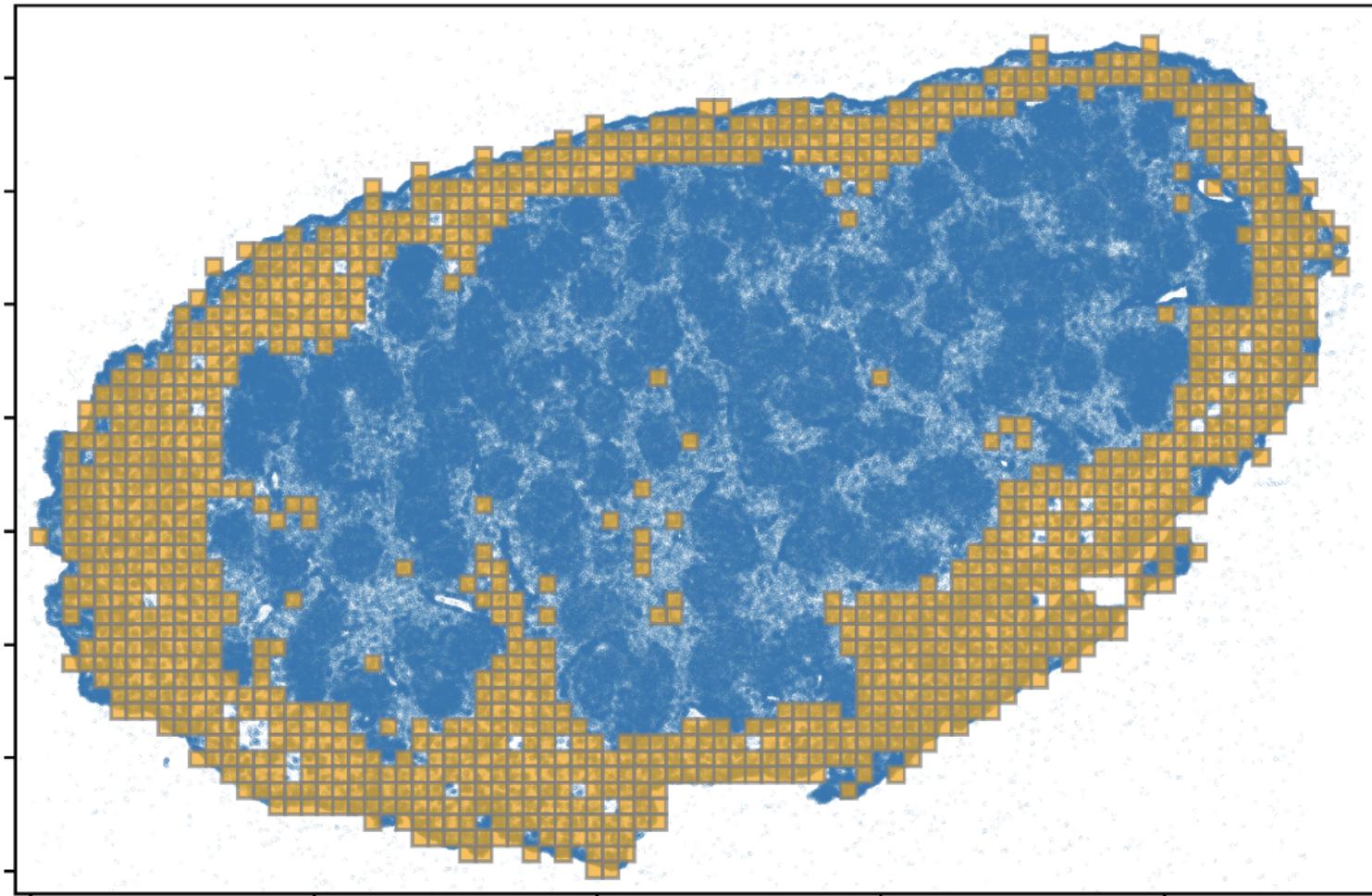
Downregulated:

CNN1
LTBP2
FBLN1
ASPN
SFRP2



Trimester 2

Cluster ID projection into tissue



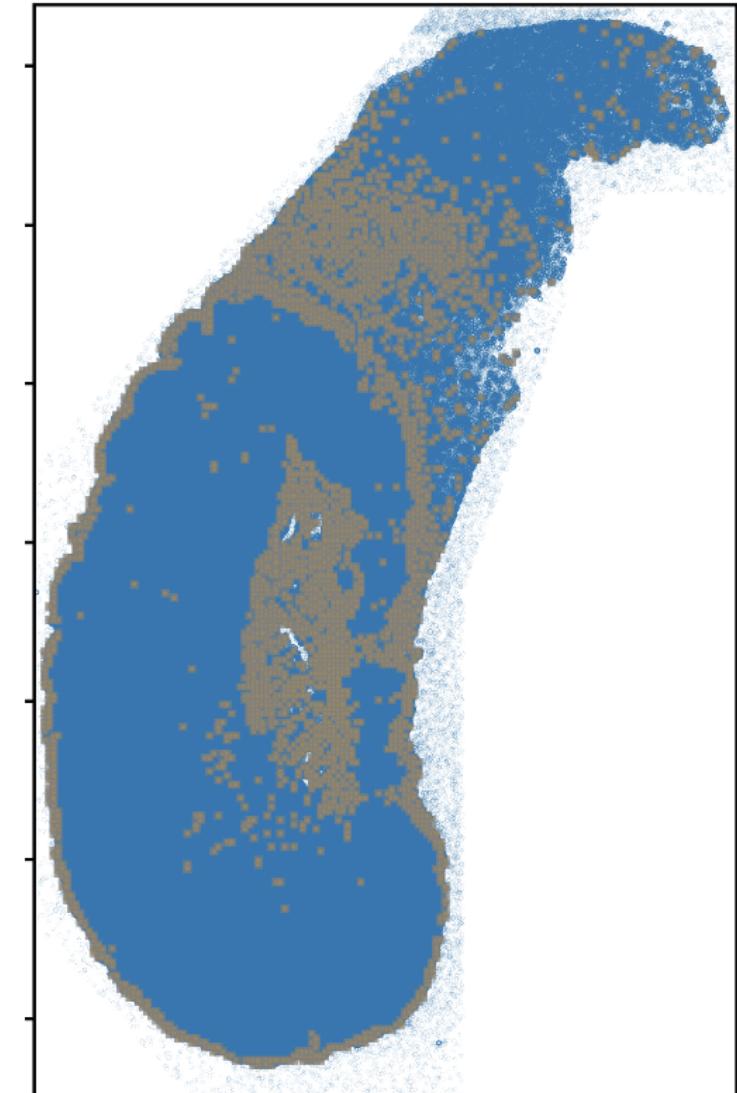
Trimester 1

Upregulated:

GPC3
THBS2
OGN
ASPN
PCOLCE

Downregulated:

NTN4
EPCAM
TMC5
GATM
PTGDS

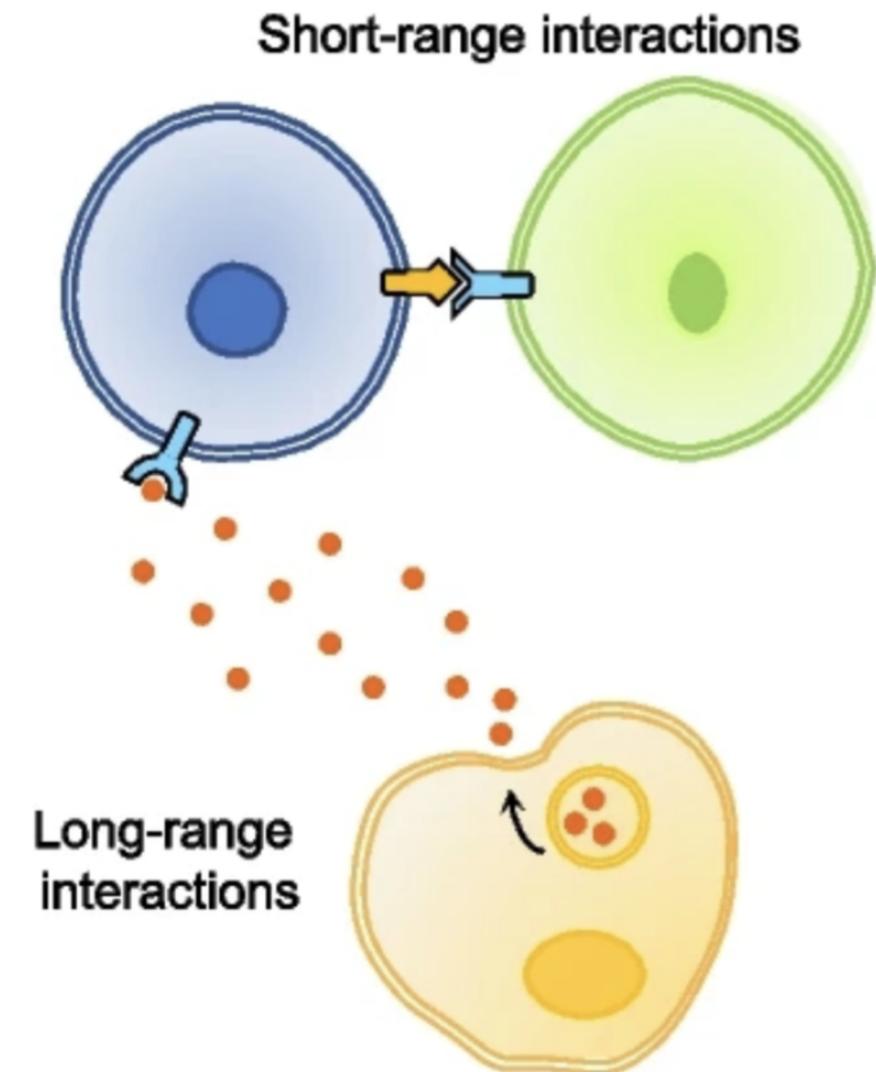


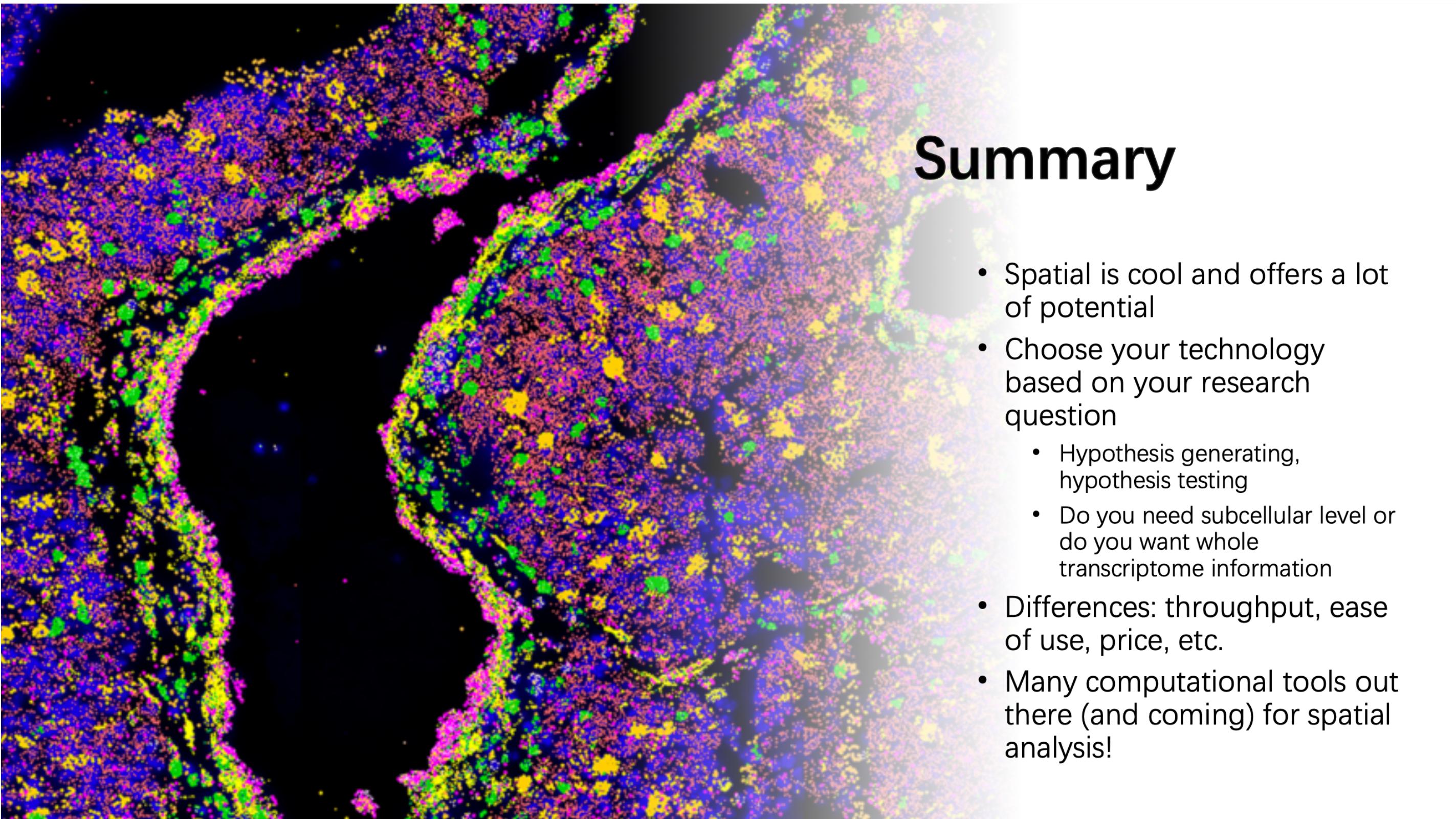
Trimester 2

Tissue organisation and cell-cell communication

Tools:

- COMMOT
- CellChat
- CellPhoneDB
- NicheNet
- ICELLNET



A grayscale image of a plant root system, likely a Arabidopsis thaliana seedling, with numerous small, colored dots (red, green, blue) scattered across its surface and branches. These dots represent spatial data points, such as gene expression levels or protein localization, mapped onto the physical structure of the root.

Summary

- Spatial is cool and offers a lot of potential
- Choose your technology based on your research question
 - Hypothesis generating, hypothesis testing
 - Do you need subcellular level or do you want whole transcriptome information
- Differences: throughput, ease of use, price, etc.
- Many computational tools out there (and coming) for spatial analysis!





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