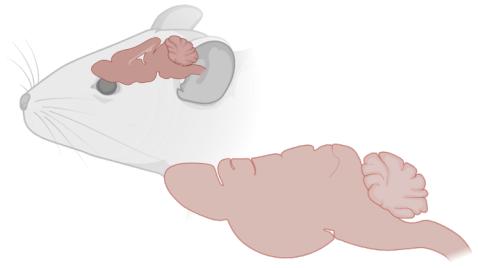
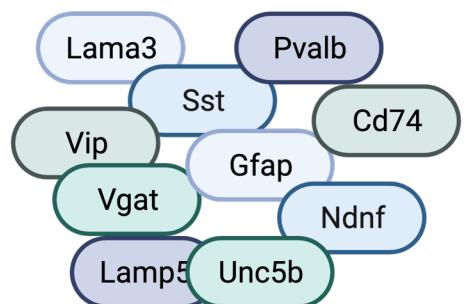


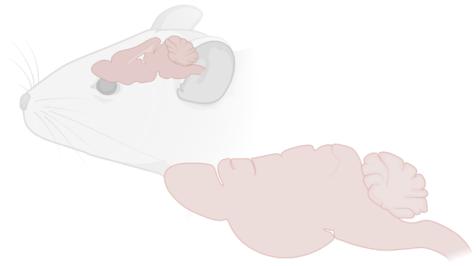
Spatial transcriptomics

Laura Heezen
PhD candidate
Dept. Human Genetics LUMC

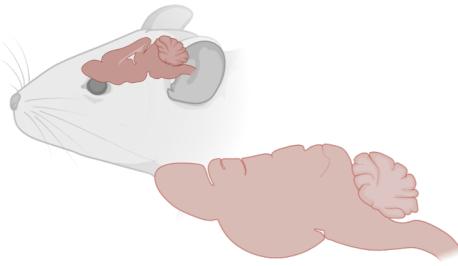


bulk RNAseq

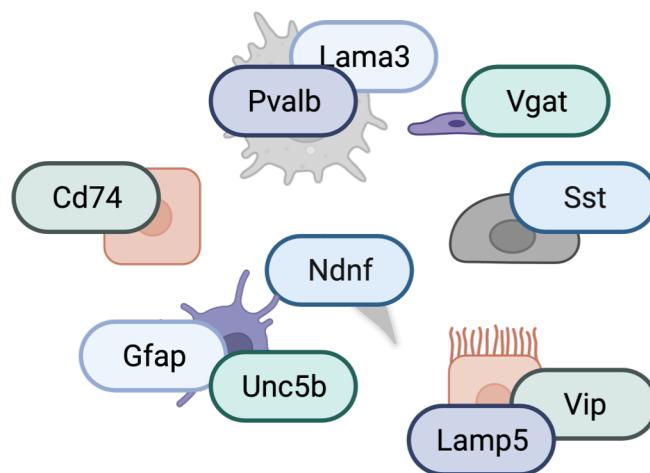
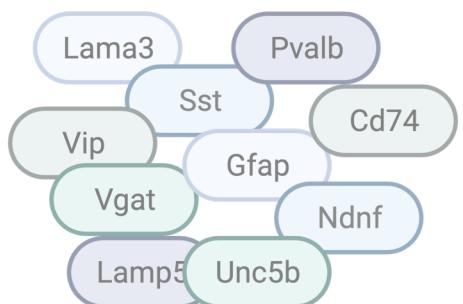
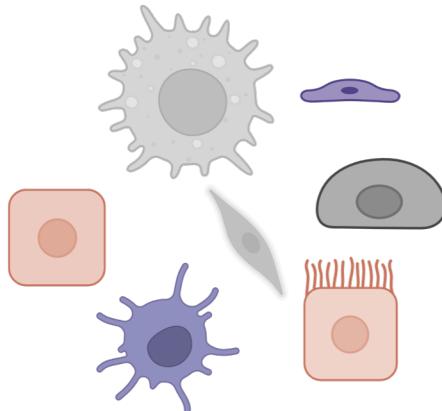


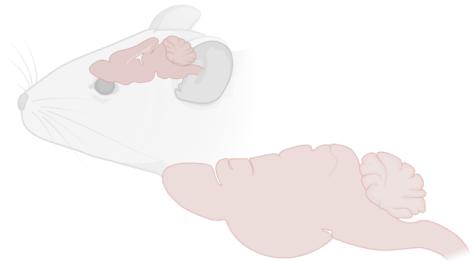


bulk RNAseq

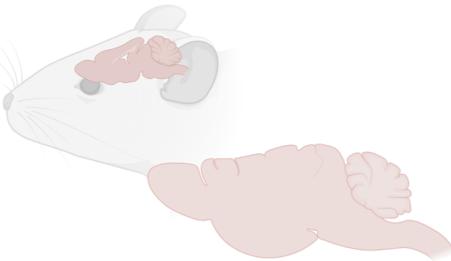
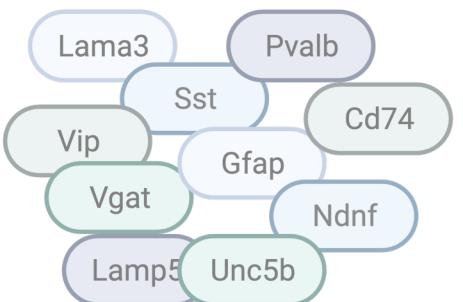


scRNAseq

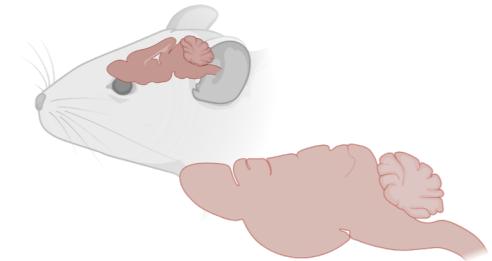
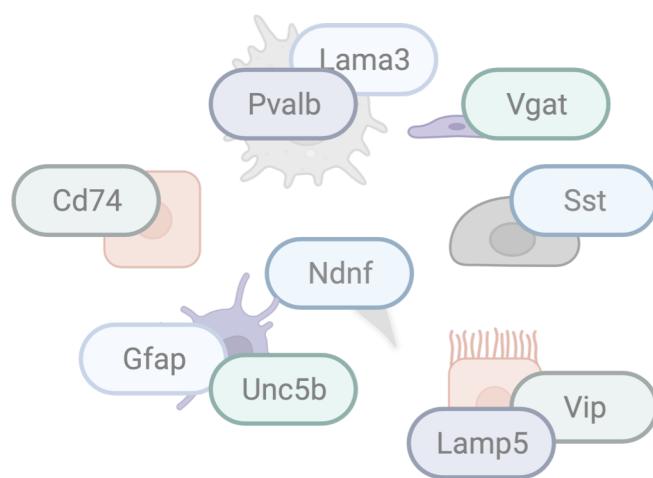
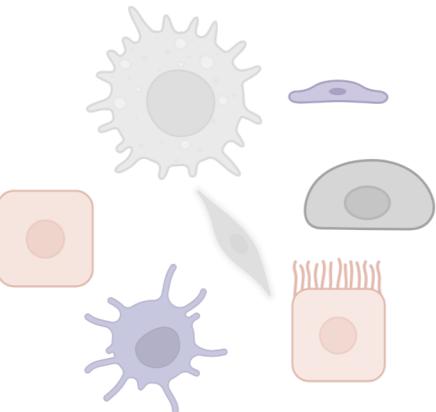




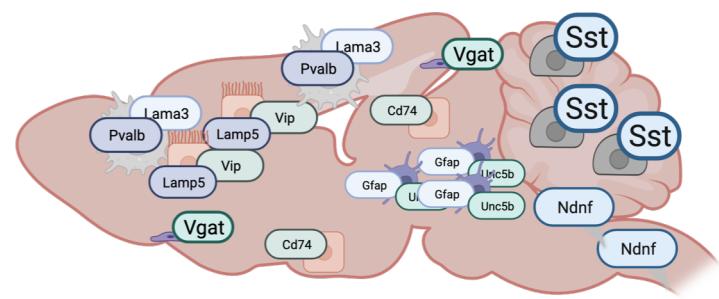
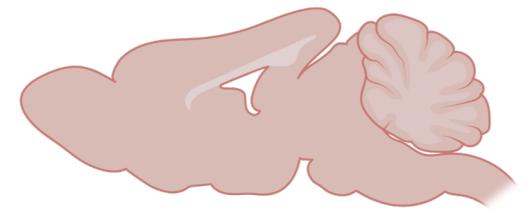
bulk RNAseq

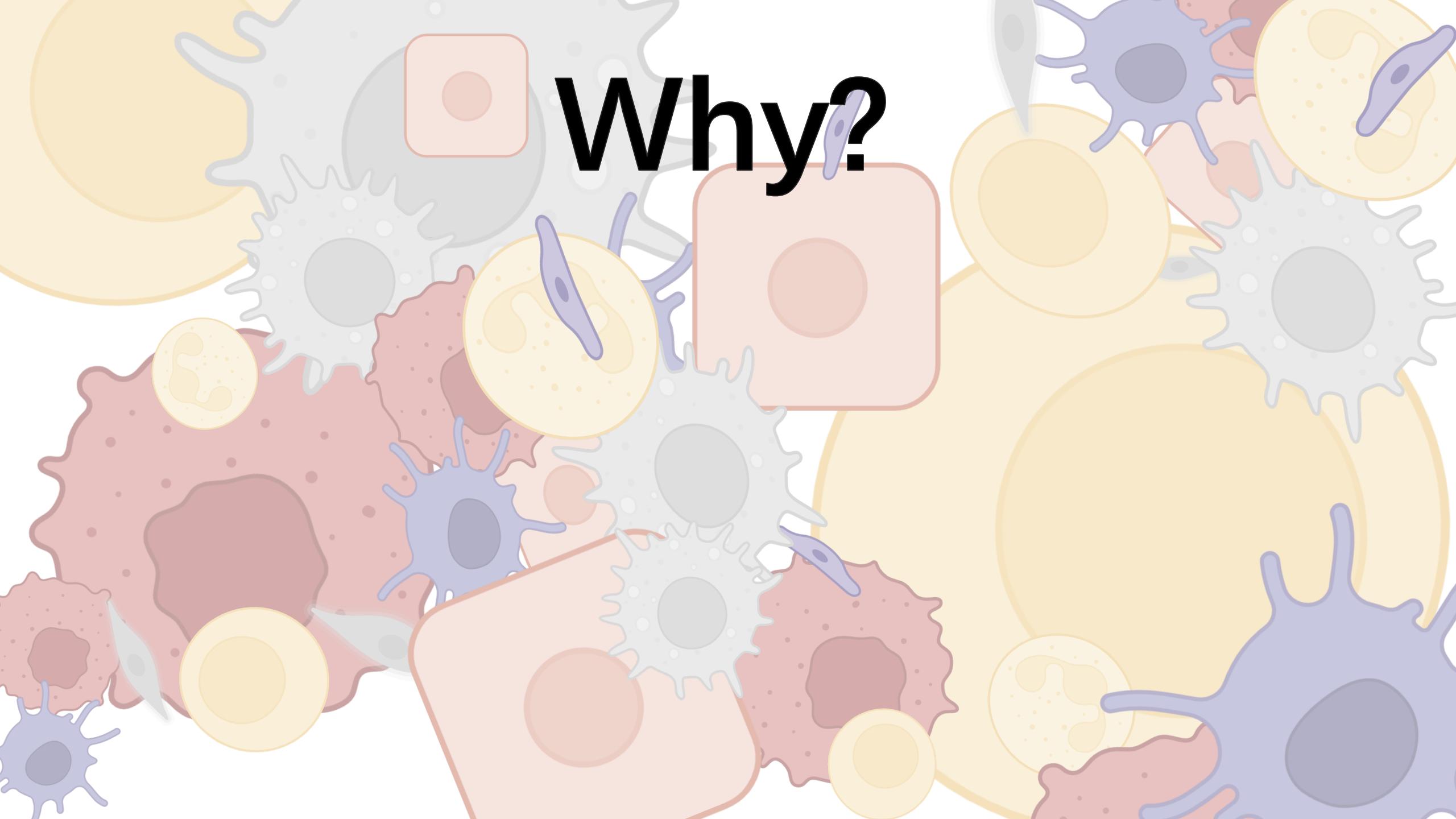


scRNAseq

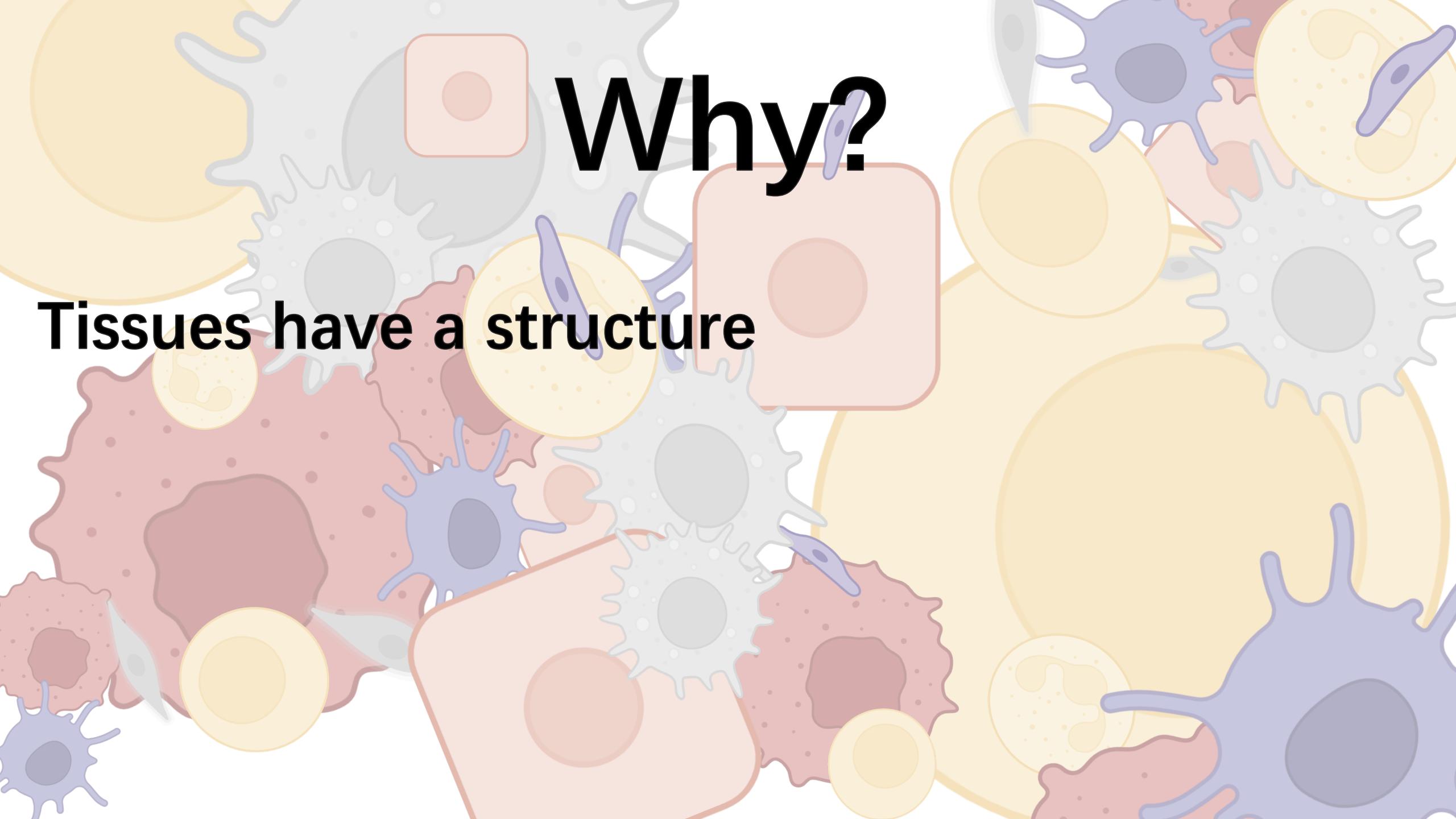


spatial transcriptomics



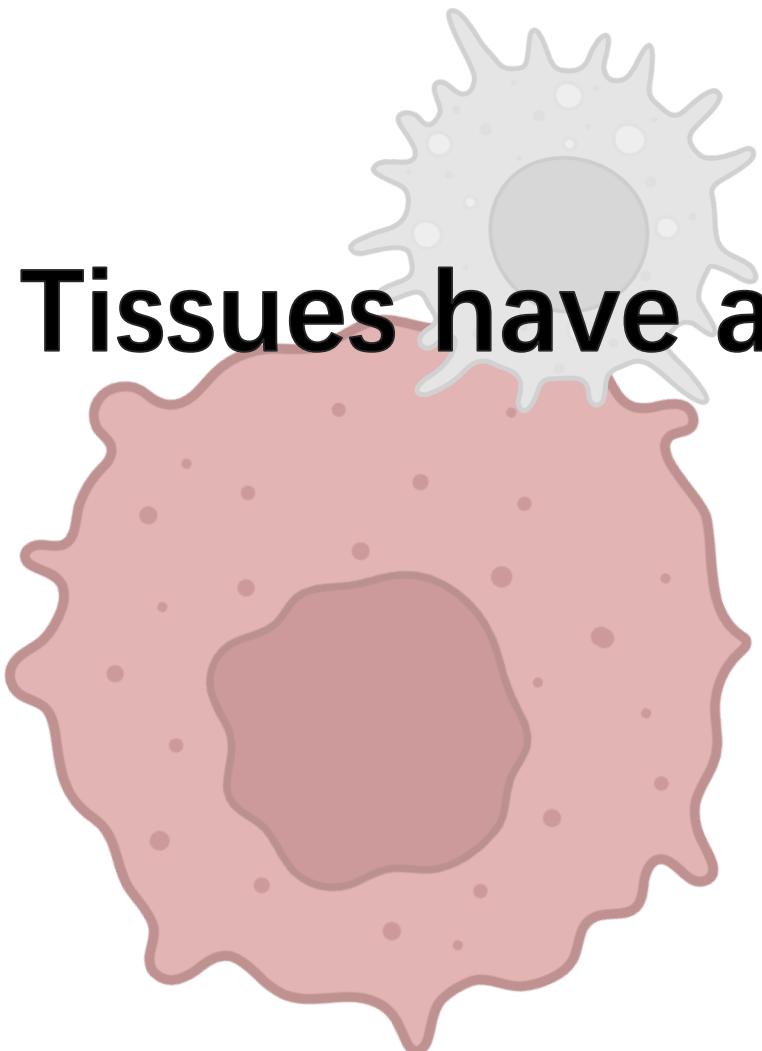


Why?

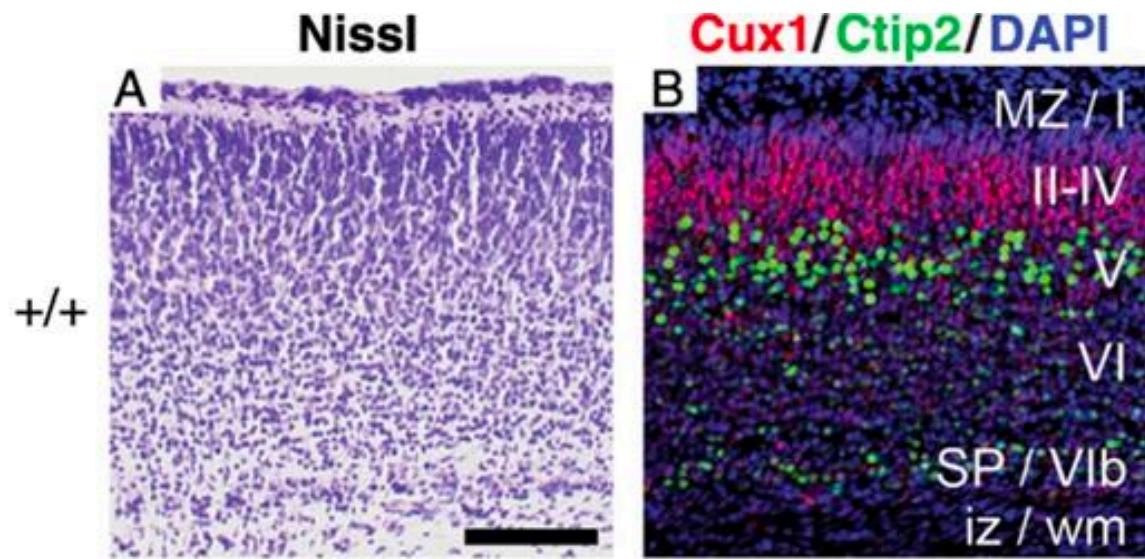


Why?

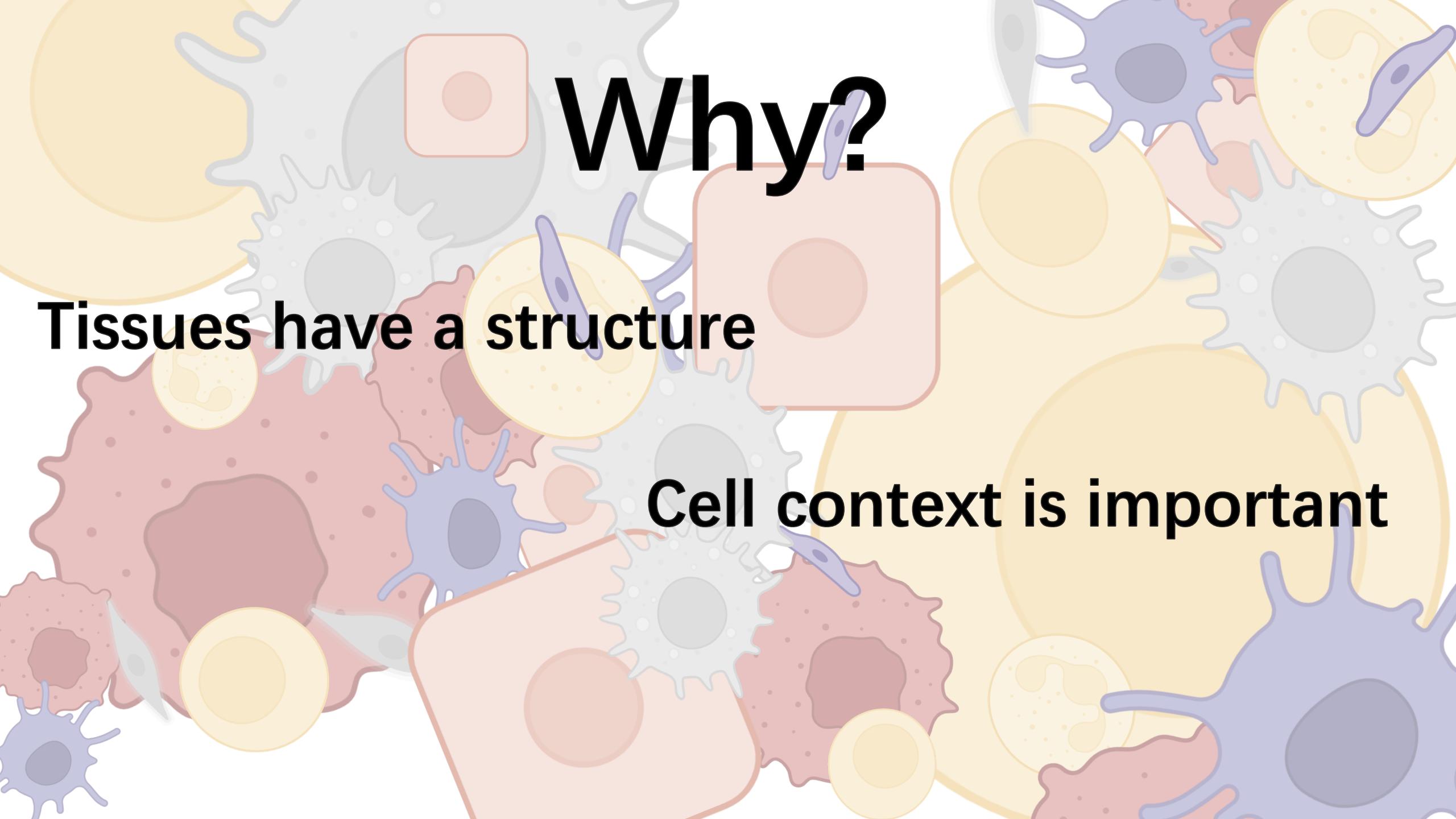
Tissues have a structure



Tissues have a structure



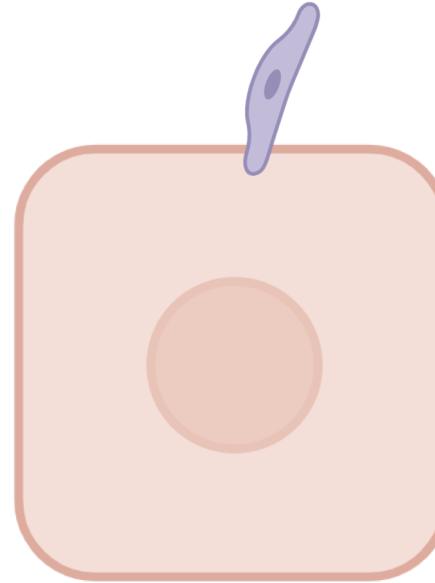
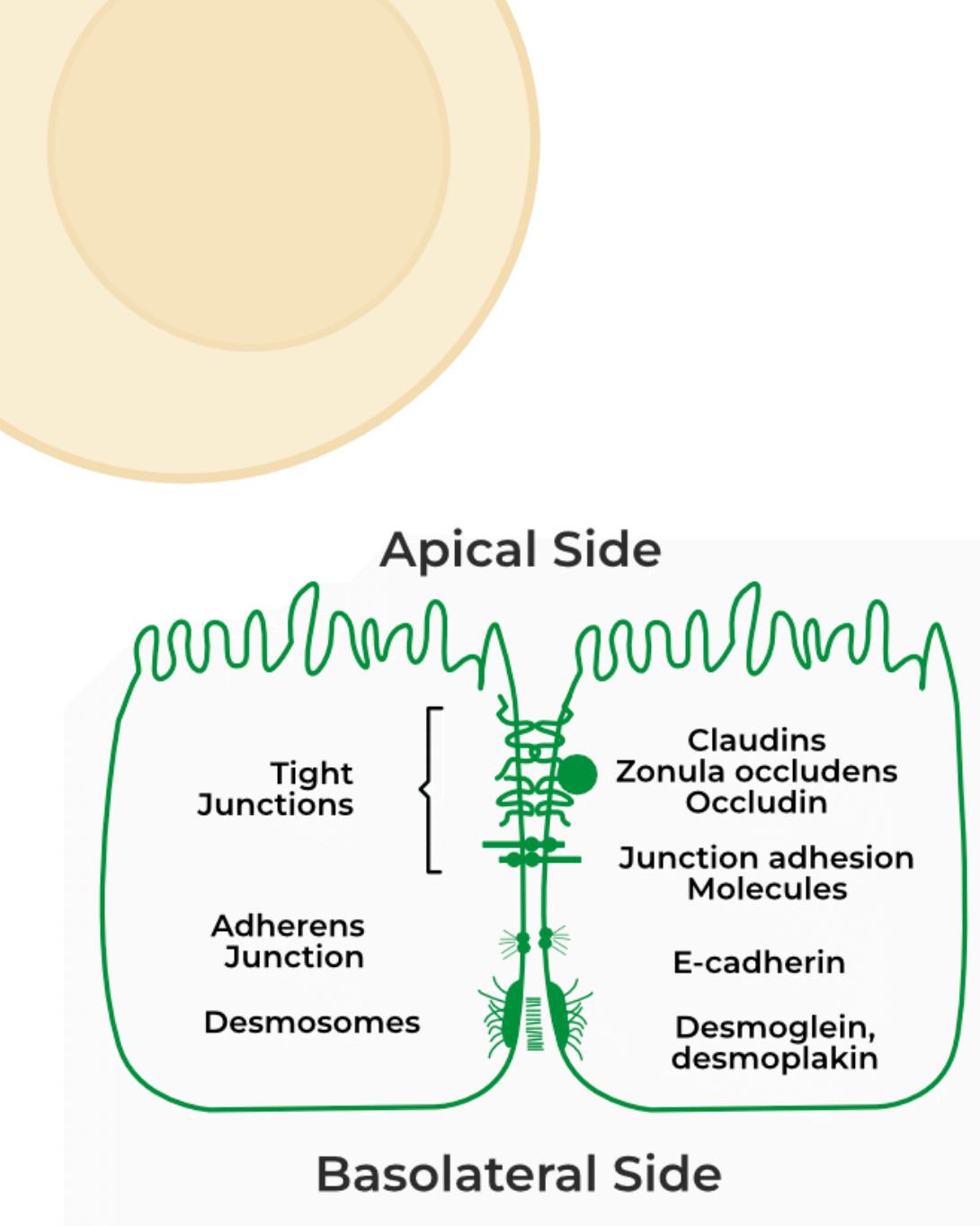
Cortical layers of the brain
Specific genes expressed in specific locations to manifest
specific function(s)



Why?

Tissues have a structure

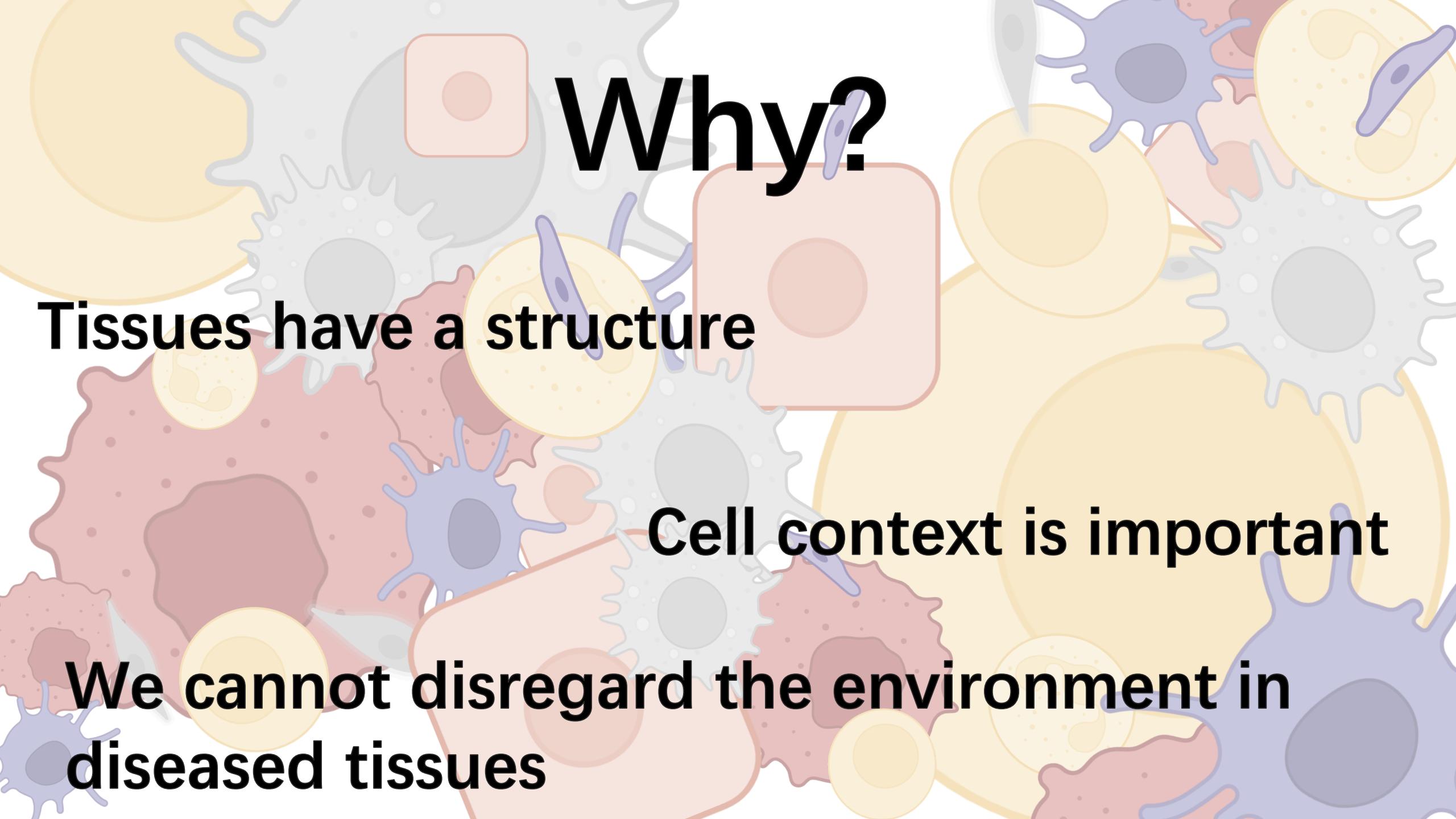
Cell context is important



Cell context is important

Cells interact, isolating them and then imputing their interactions is a way, but removes/ignores some important information

Also think about tissue development (patterning of morphogens)

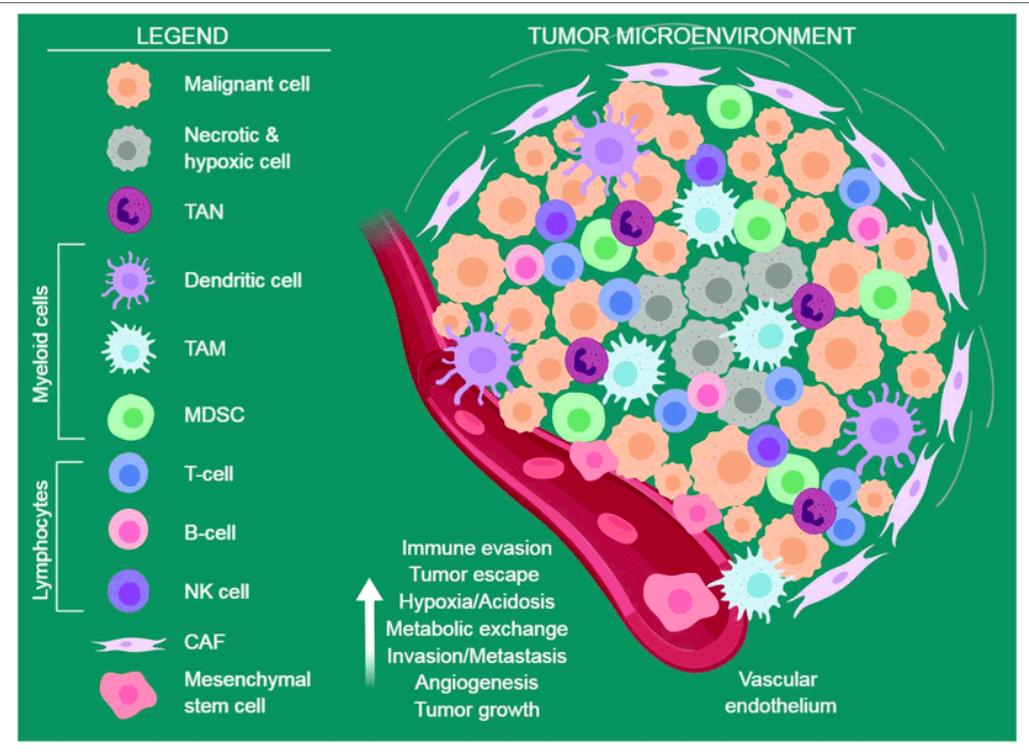


Why?

Tissues have a structure

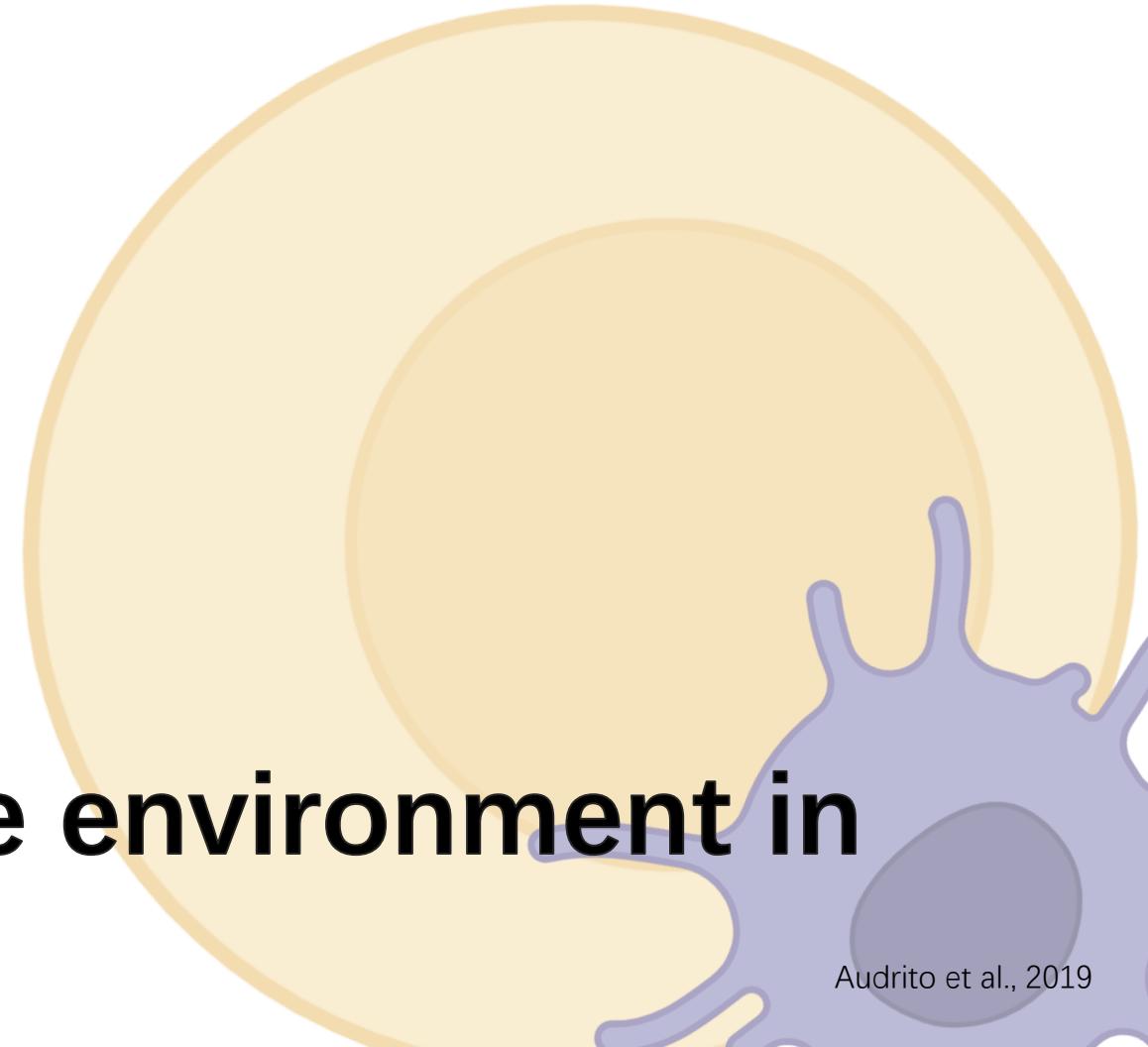
Cell context is important

We cannot disregard the environment in
diseased tissues



Tumor microenvironment
All different cell types contributing to the disease state and/or progression

We cannot disregard the environment in diseased tissues





Check for updates

Method of the Year 2020: spatially resolved transcriptomics

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

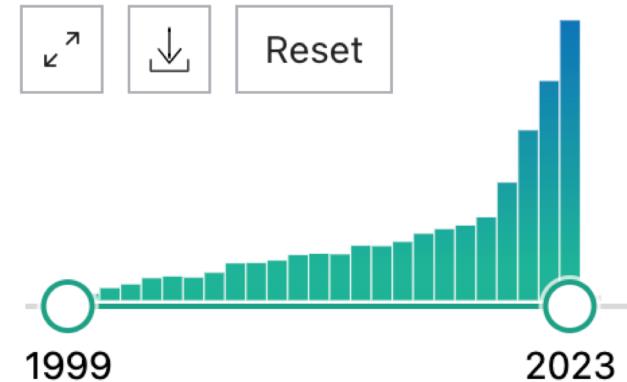


Check for updates

Method of the Year 2020: spatially resolved transcriptomics

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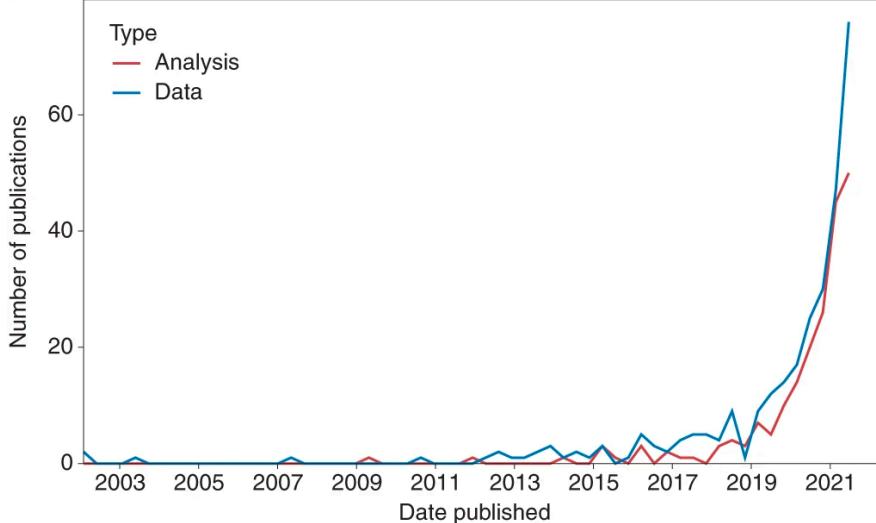
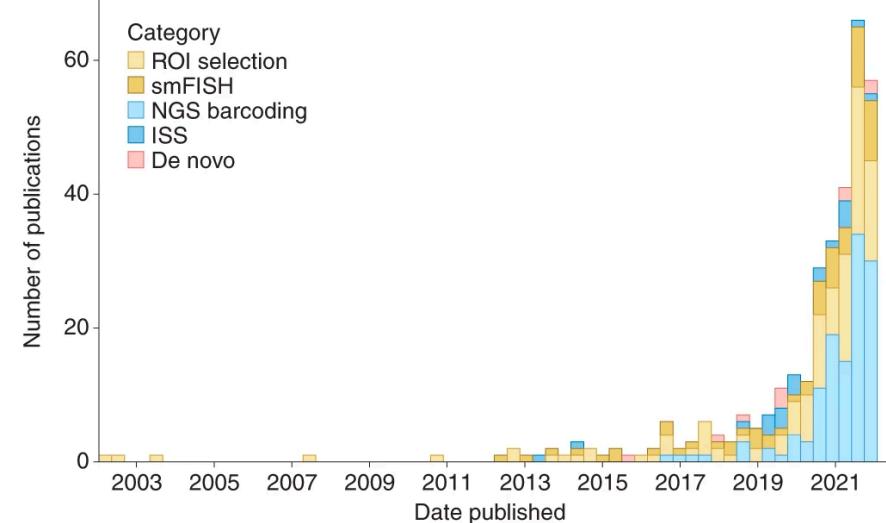
RESULTS BY YEAR





Method of the Year 2020: spatially resolved transcriptomics

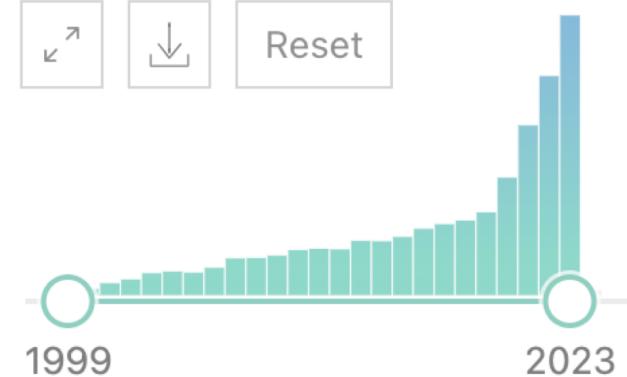
Spatially resolved transcriptomics methods are changing the way we understand complex tissues.

a**b**

RESULTS BY YEAR



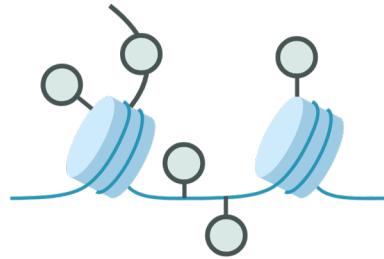
Reset



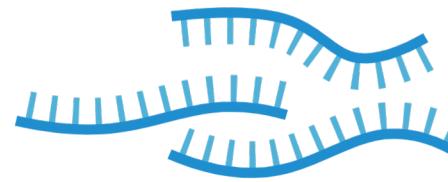
Spatial ‘omic’ technologies



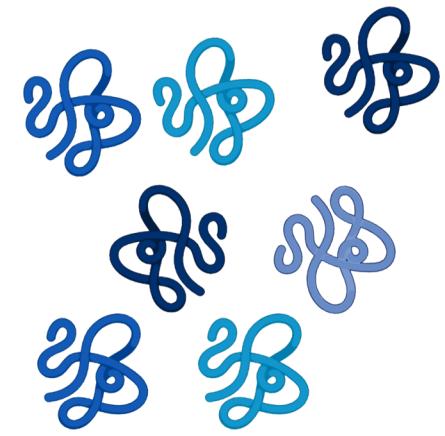
Genome



Epigenome



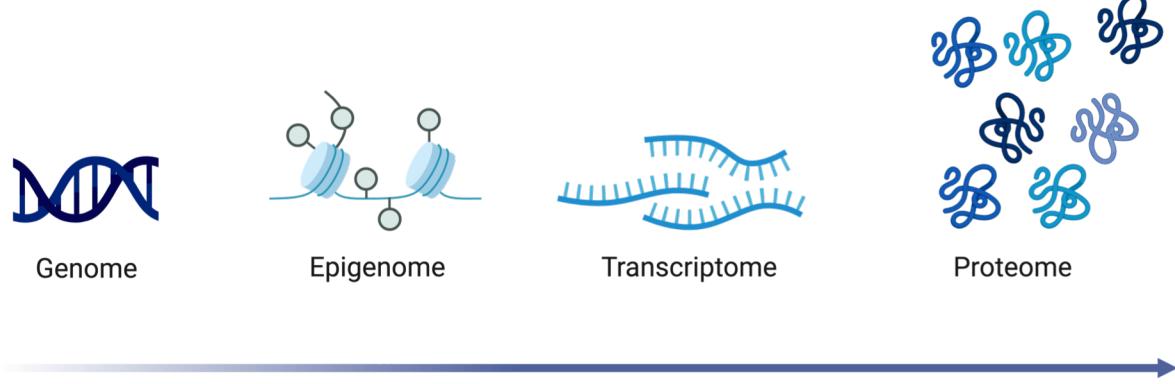
Transcriptome



Proteome

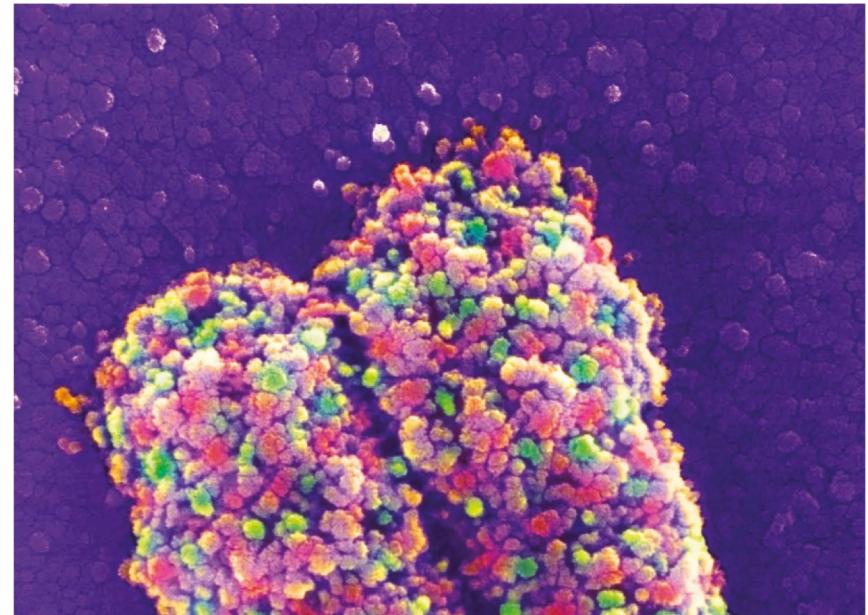


Spatial ‘omic’ technologies



SEVEN TECHNOLOGIES TO WATCH IN 2022

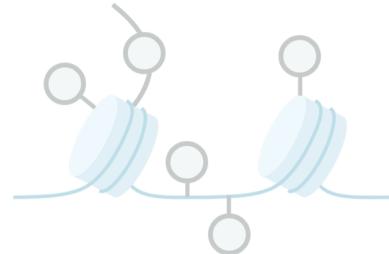
Our fifth annual round-up of the tools that look set to shake up science this year. By Michael Eisenstein



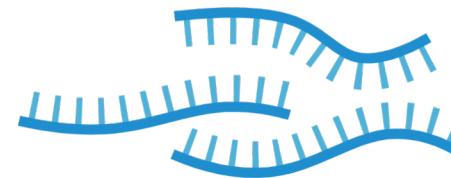
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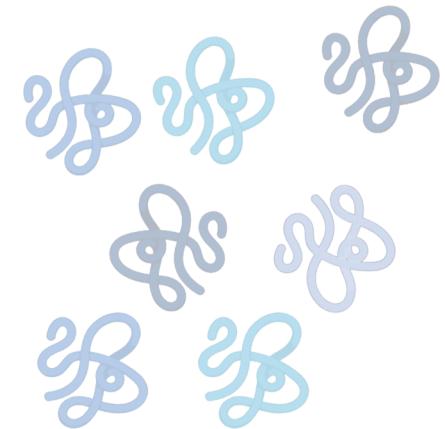
Genome



Epigenome



Transcriptome



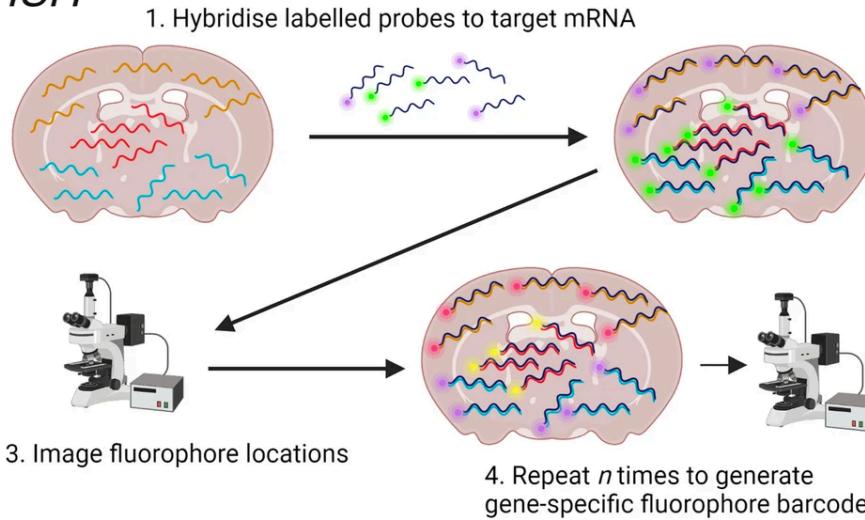
Proteome



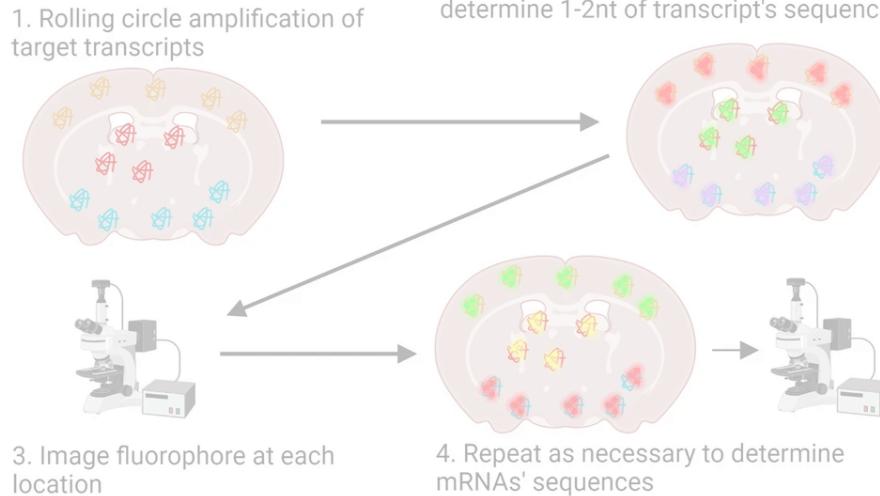
Spatial transcriptomics

Imaging methods

ISH

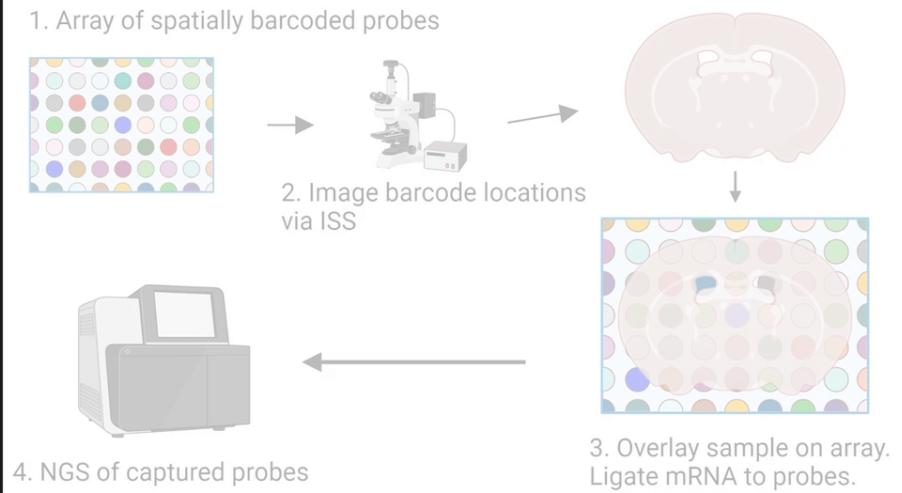


ISS

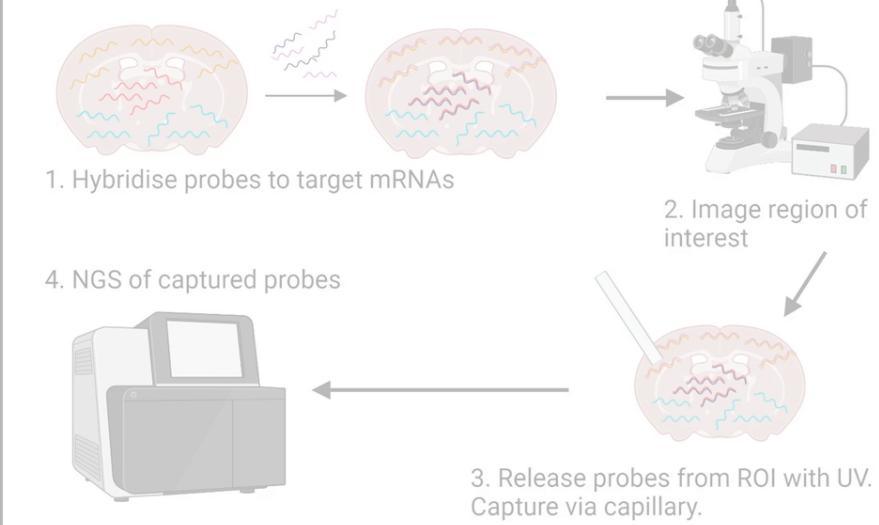


Sequencing methods

Arrays



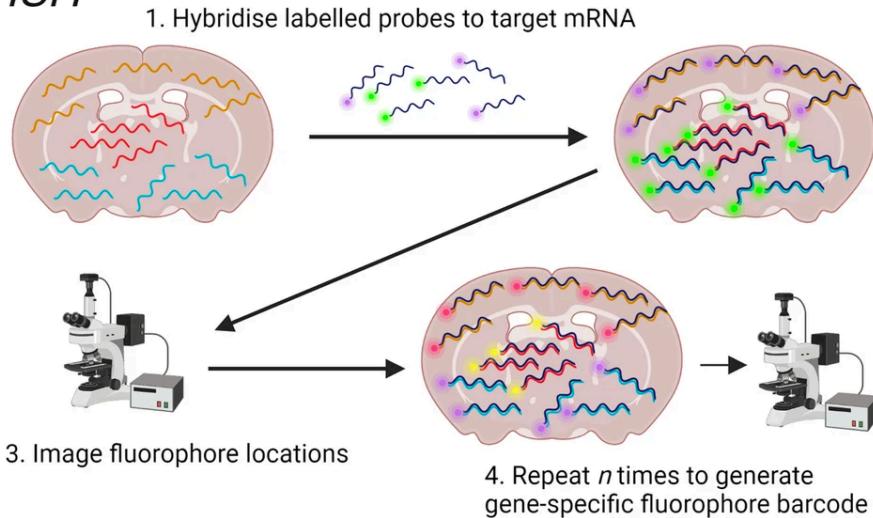
Microdissection



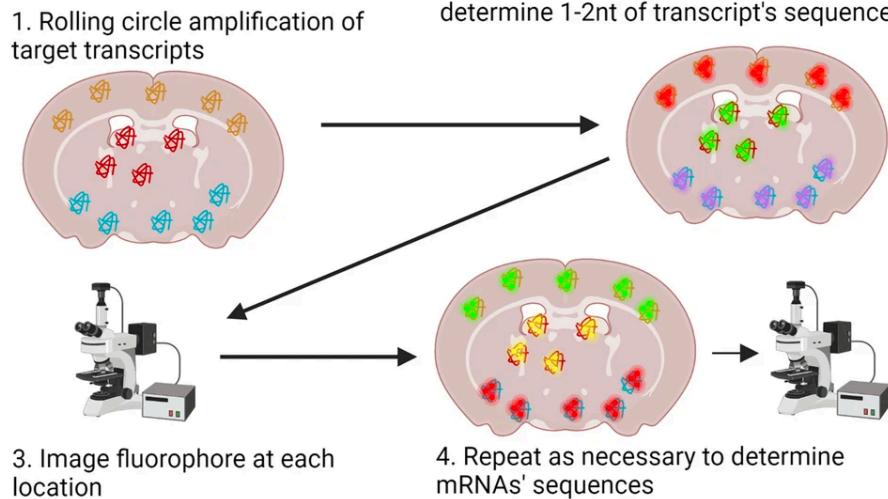
Spatial transcriptomics

Imaging methods

ISH

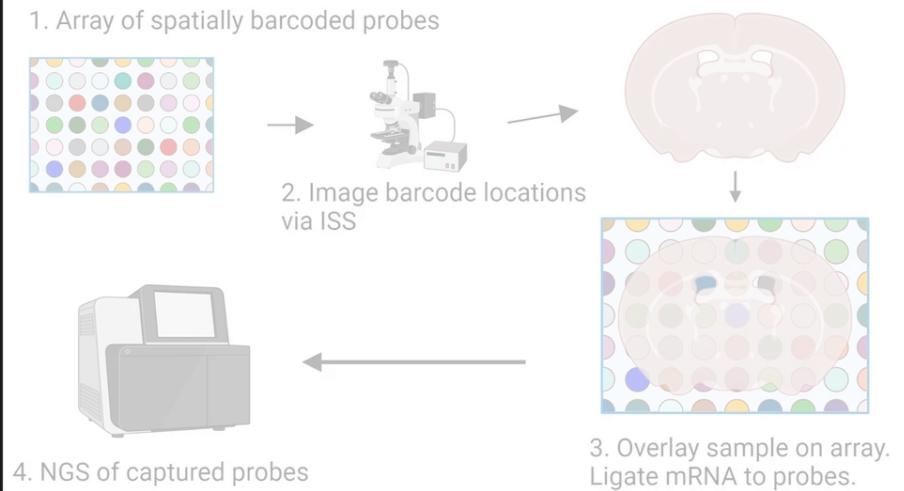


ISS

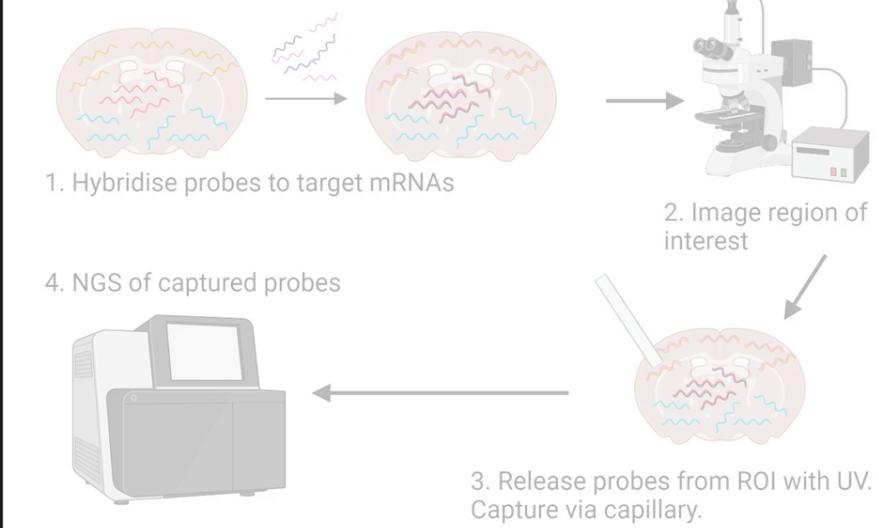


Sequencing methods

Arrays



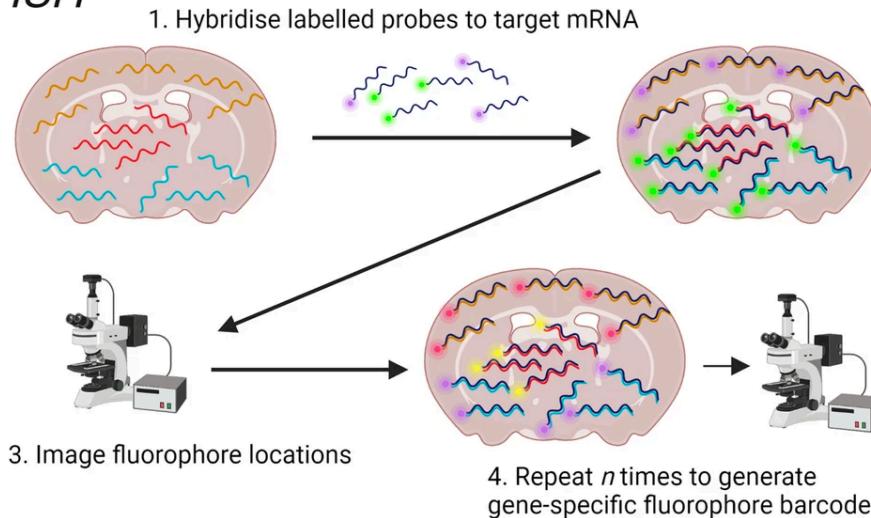
Microdissection



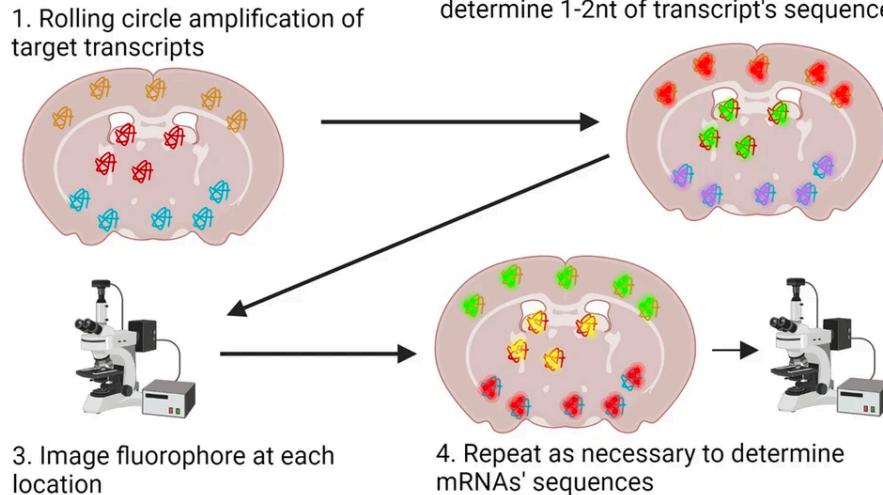
Spatial transcriptomics

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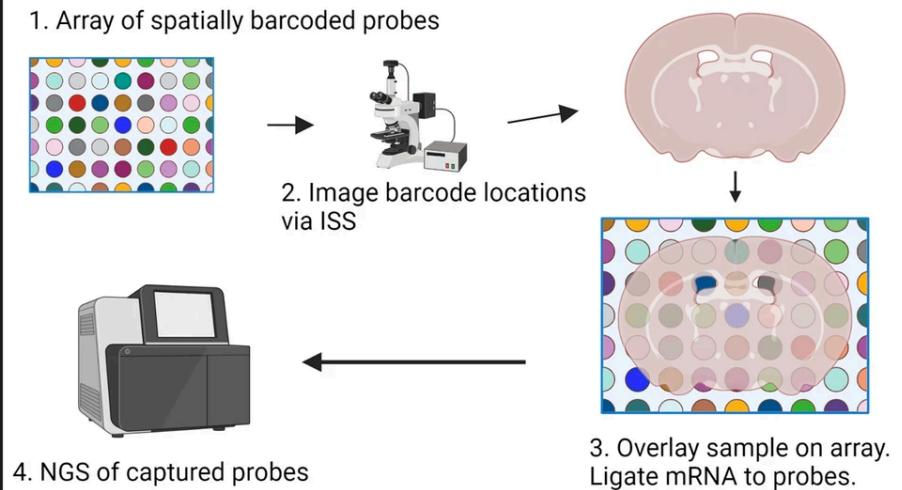


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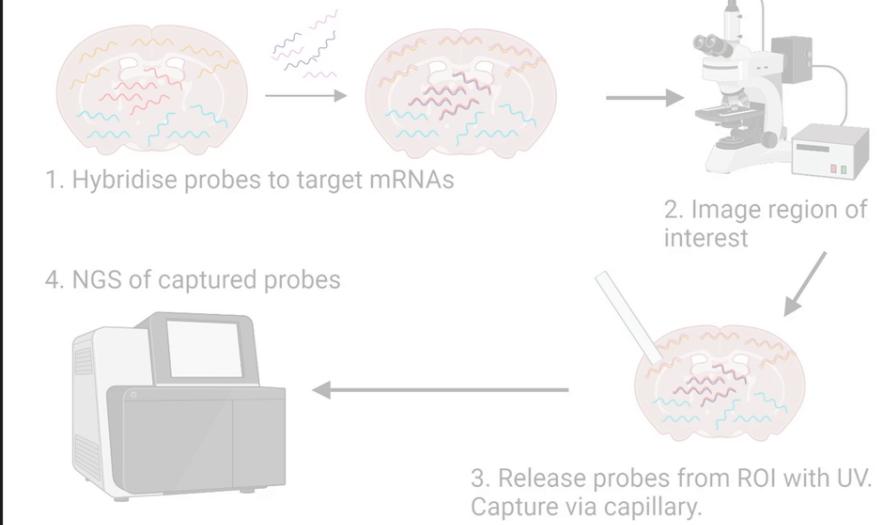


Sequencing methods

Arrays



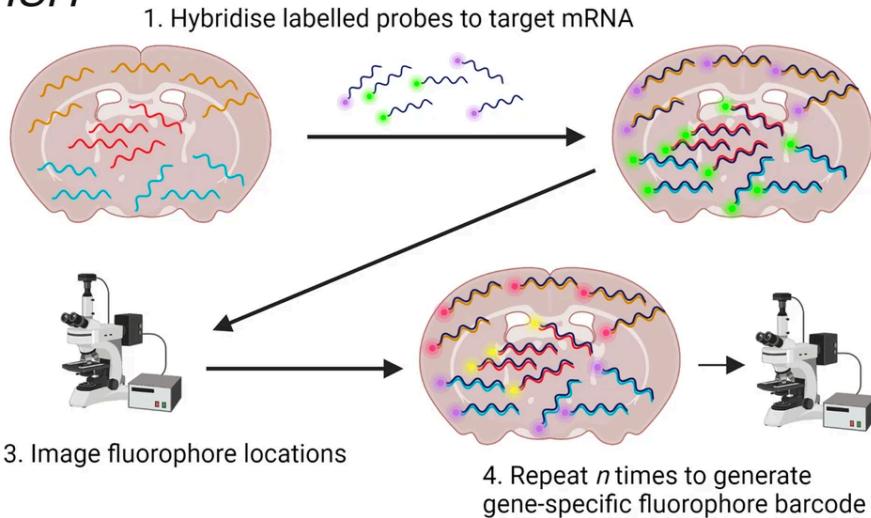
Microdissection



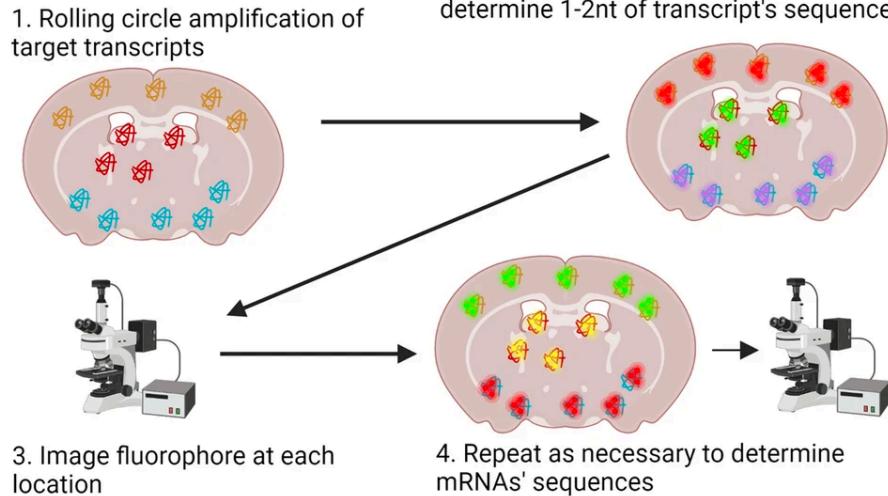
Spatial transcriptomics

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ISH

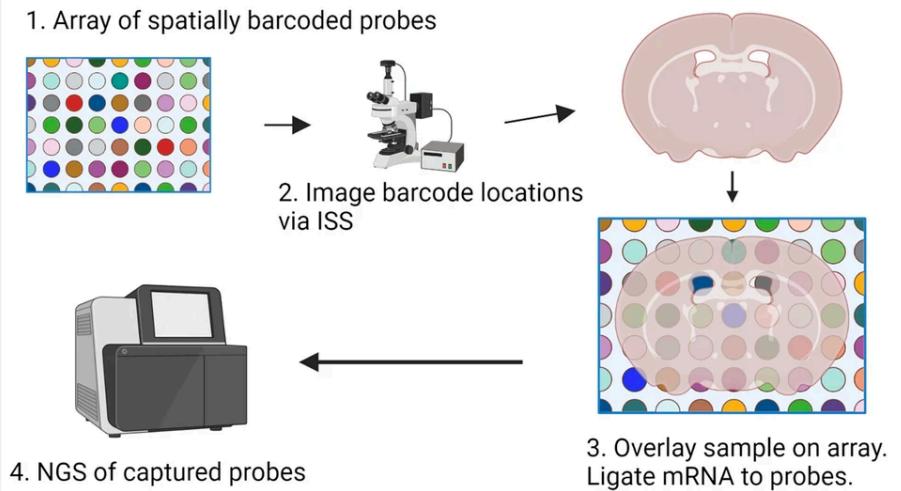


ISS

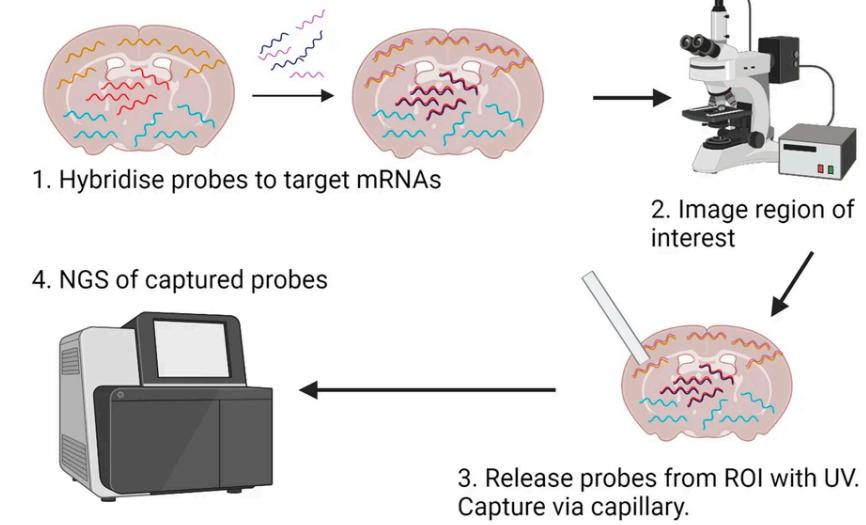


Sequencing methods

Arrays



Microdissection



Differences sequencing/imaging based

- Targeted vs. untargeted
 - ‘Biased’ vs. ‘unbiased’ exploration
 - Sequencing based is whole transcriptome
 - Imaging based mostly uses a panel: rapidly increasing numbers here though

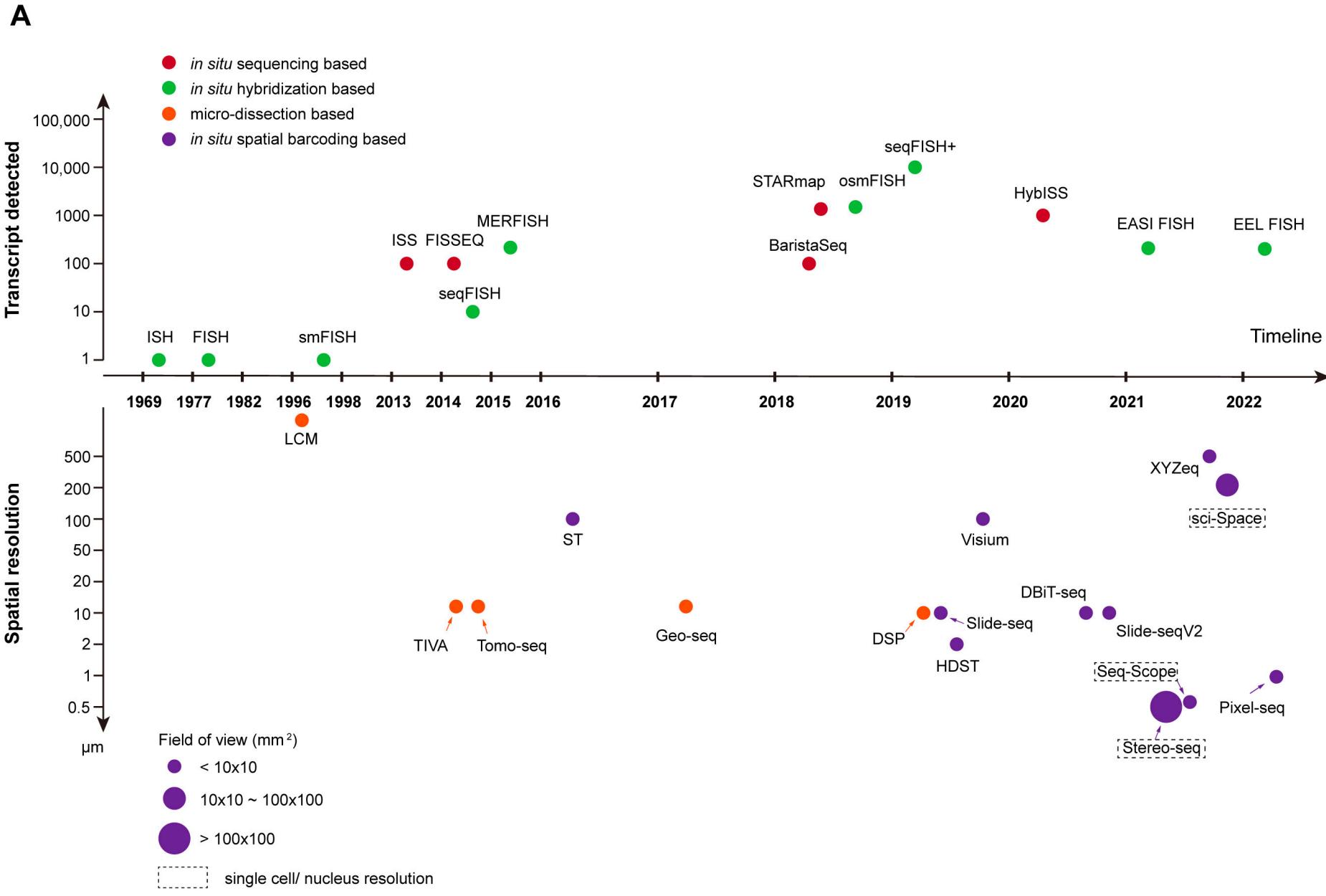
Differences sequencing/imaging based

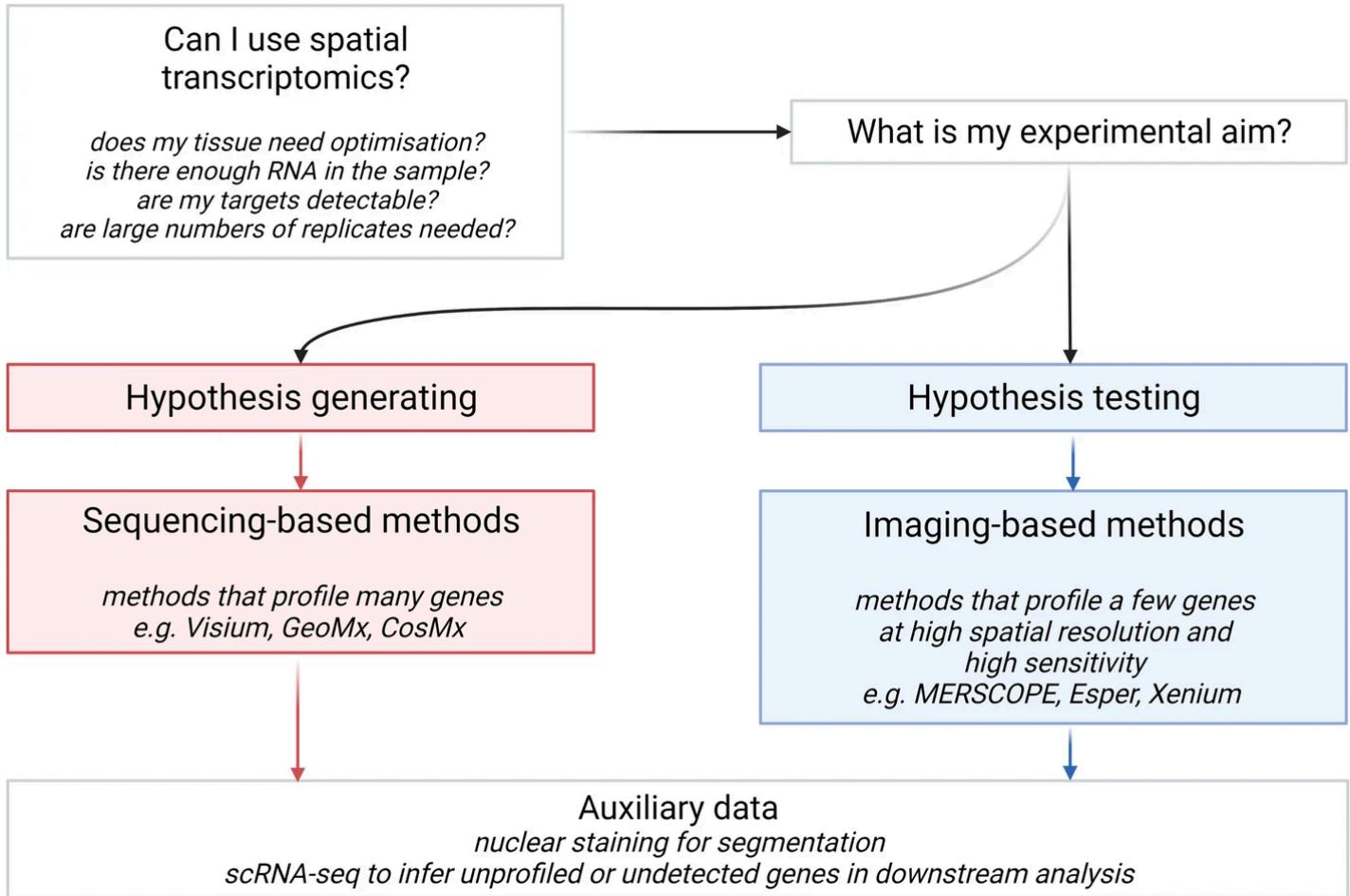
- Targeted vs. untargeted
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 - Sequencing based is whole transcriptome
 - Imaging based mostly uses a panel: rapidly increasing numbers here though
- Sequencing based: often grid type of profiling, not at single cell level
 - Also more development here, smaller grids going down to single cell level
 - Computational tools to deconvolute the transcriptome signal from spots

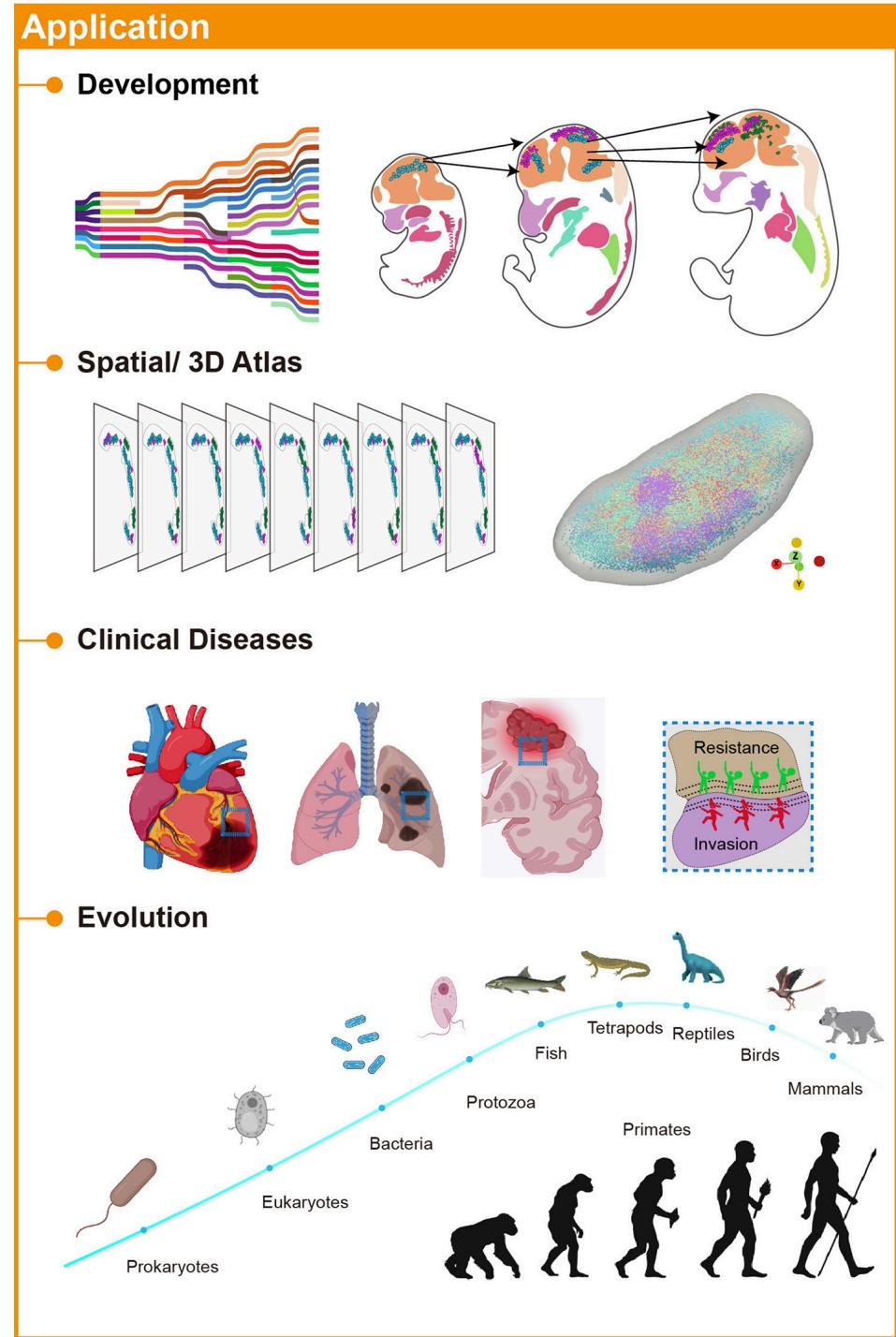
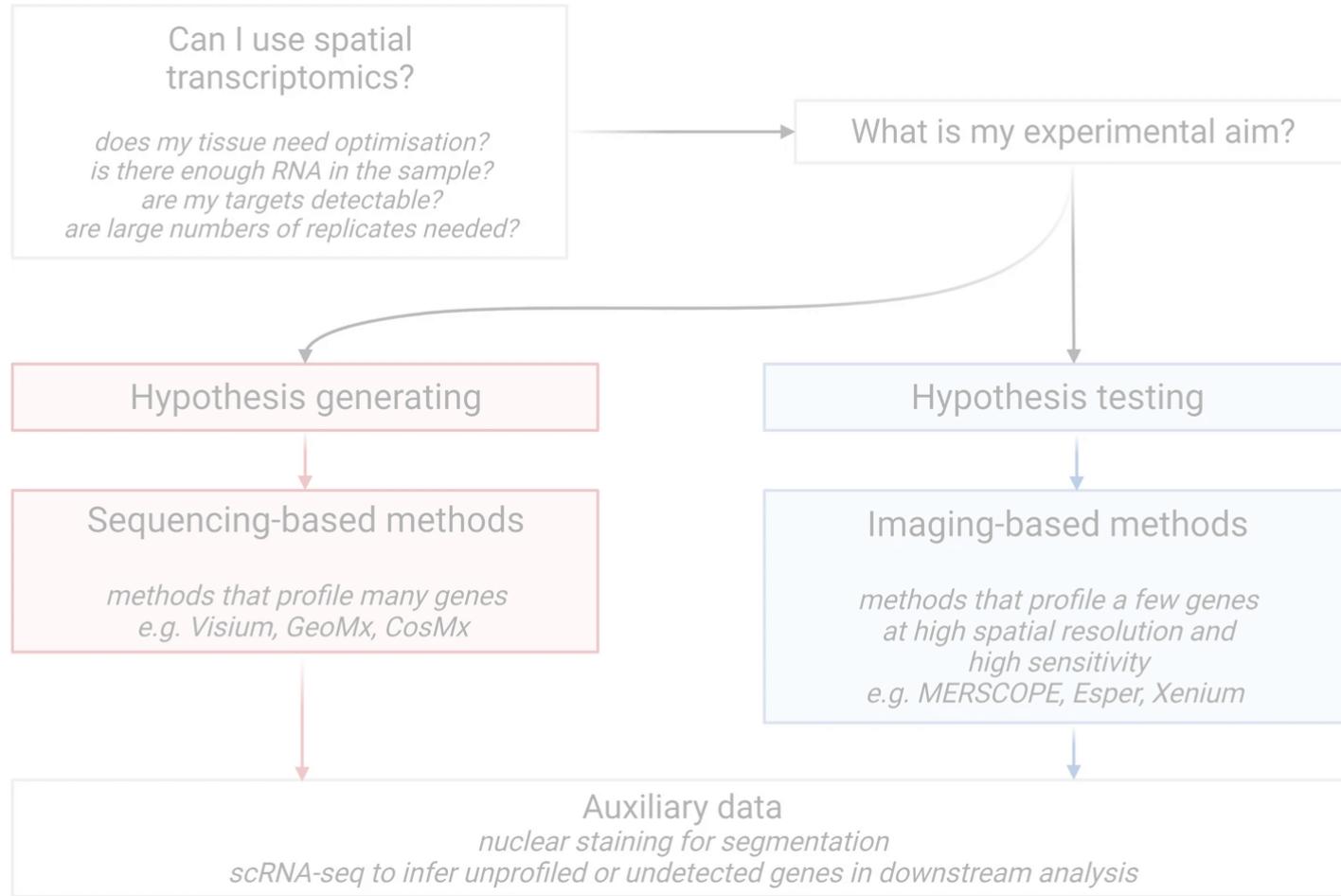
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 - Also more development here, smaller grids going down to single cell level
 - Computational tools to deconvolute the transcriptome signal from spots
- Some sequencing based protocols are easier to implement, often do not need a fancy set-up/microscope
 - Now also commercially available imaging-based platforms such as MERFISH and Xenium

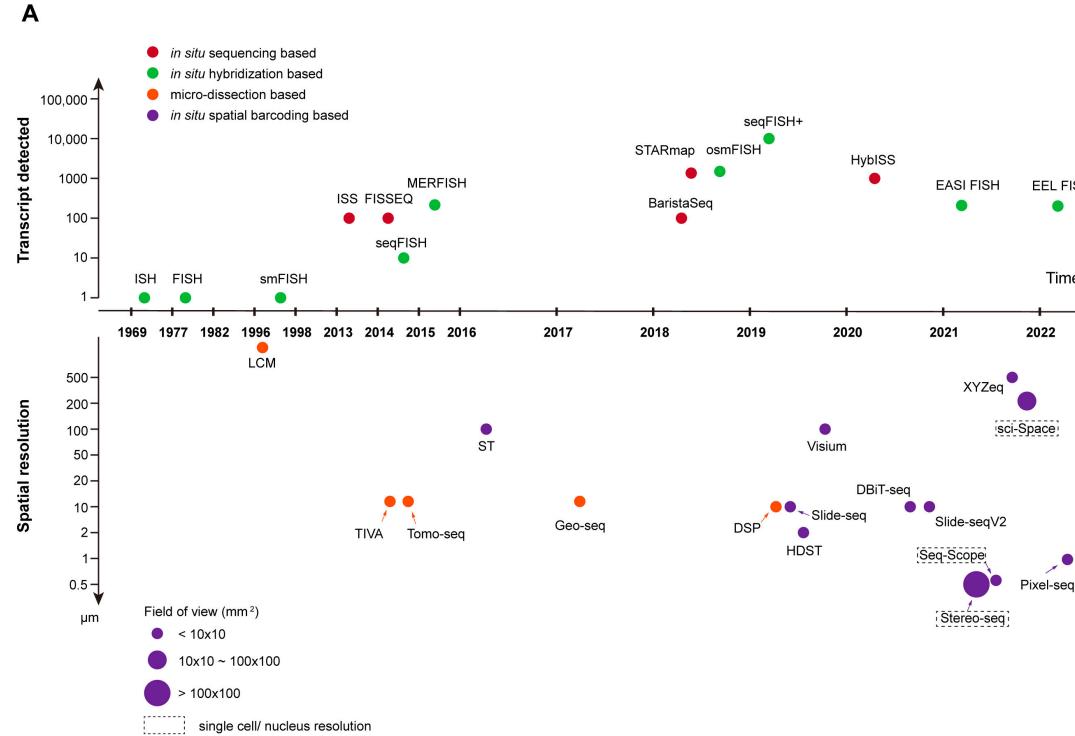
Spatial transcriptomics







Highlight of ST technologies

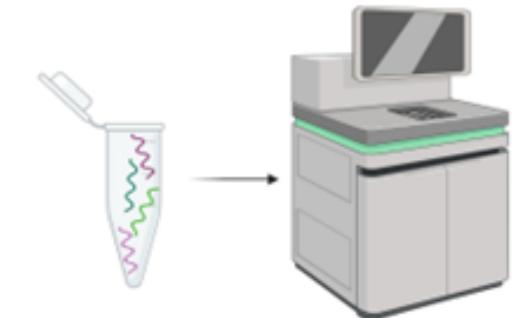
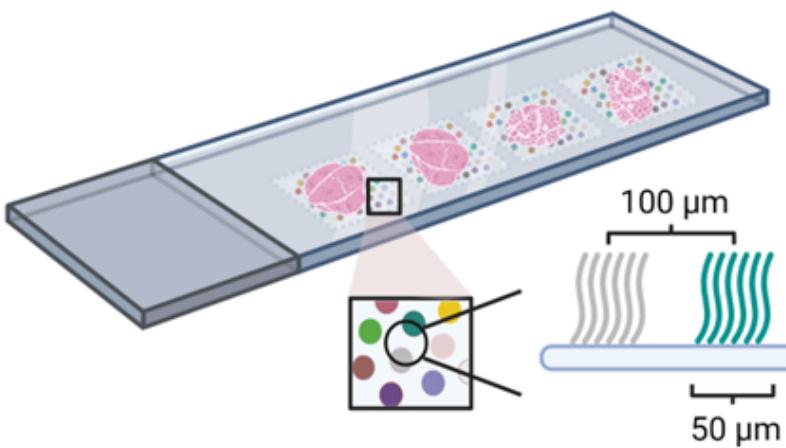


Easiest comparison is one within 1 company:

1. Visium sequencing based
2. Xenium imaging based

Visium (10x Genomics)

- Sequencing based
- Resolution: 55um
- Unbiased discovery (whole transcriptome)
- Throughput: 5000 spots on 6*6mm capture area
- Tissue type: FFPE and fresh frozen



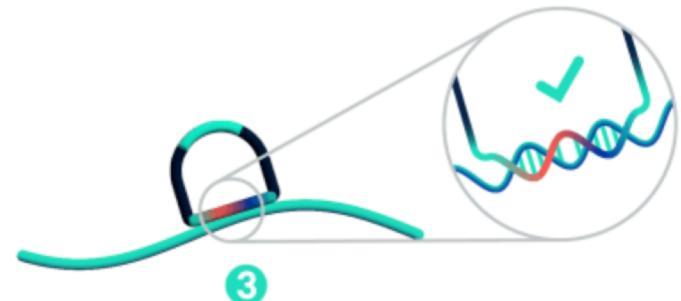
Xenium (10x Genomics)

Off instrument chemistry

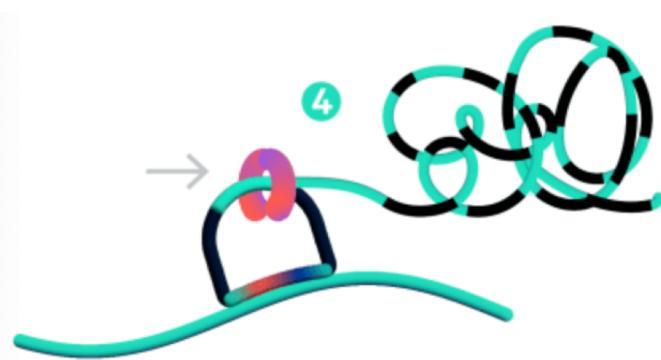
Step 1&2: dual target recognition and hybridization of padlock probes



Step 3: probe ligation (only ligation possible when there is a perfect match of the probe arms; sensitive)



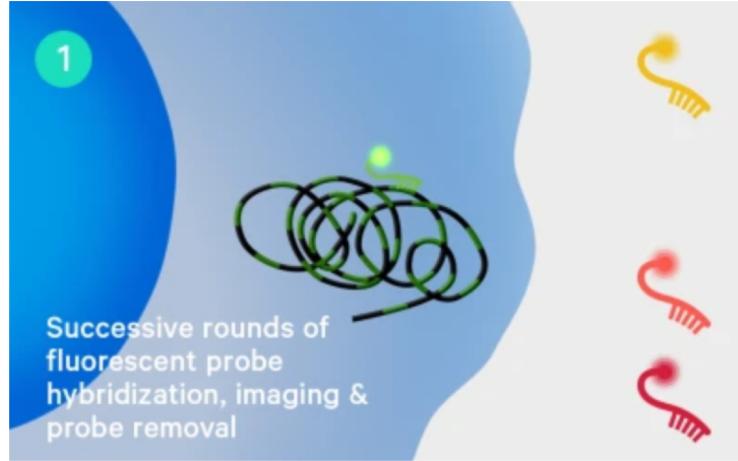
Step 4: rolling circle amplification (RCA) on the ligated probes, reducing off-target events



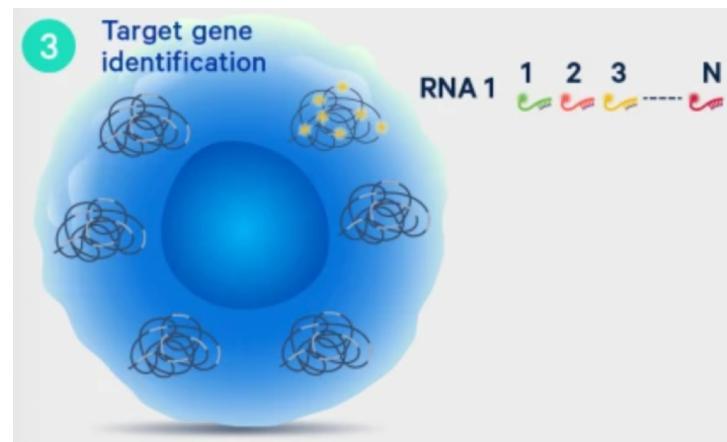
Xenium (10x Genomics)

On instrument detection

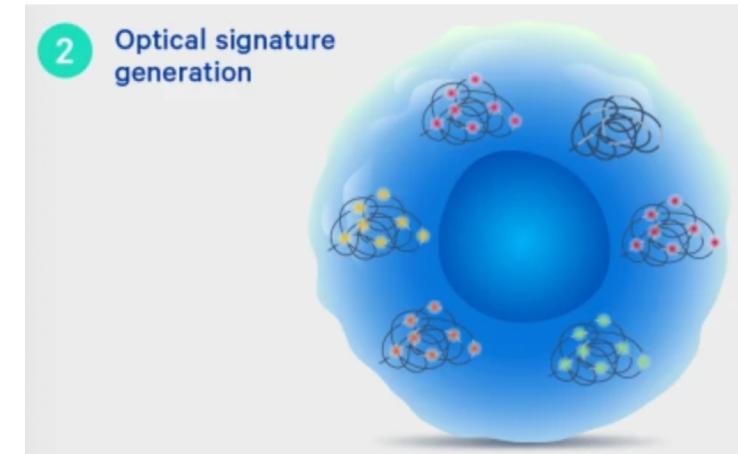
Rounds of fluorescent probe hybridization, imaging and probe removal



Target gene identification

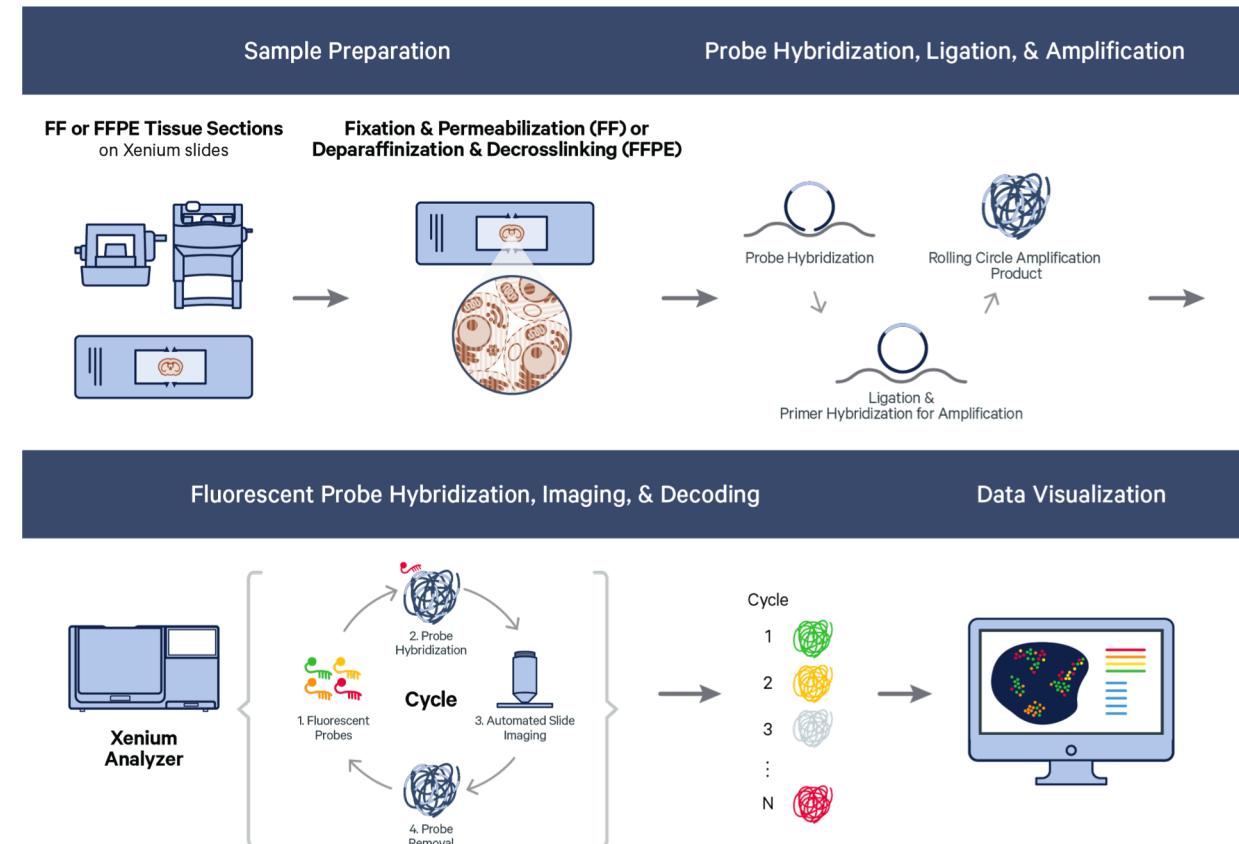


Optical signature generation

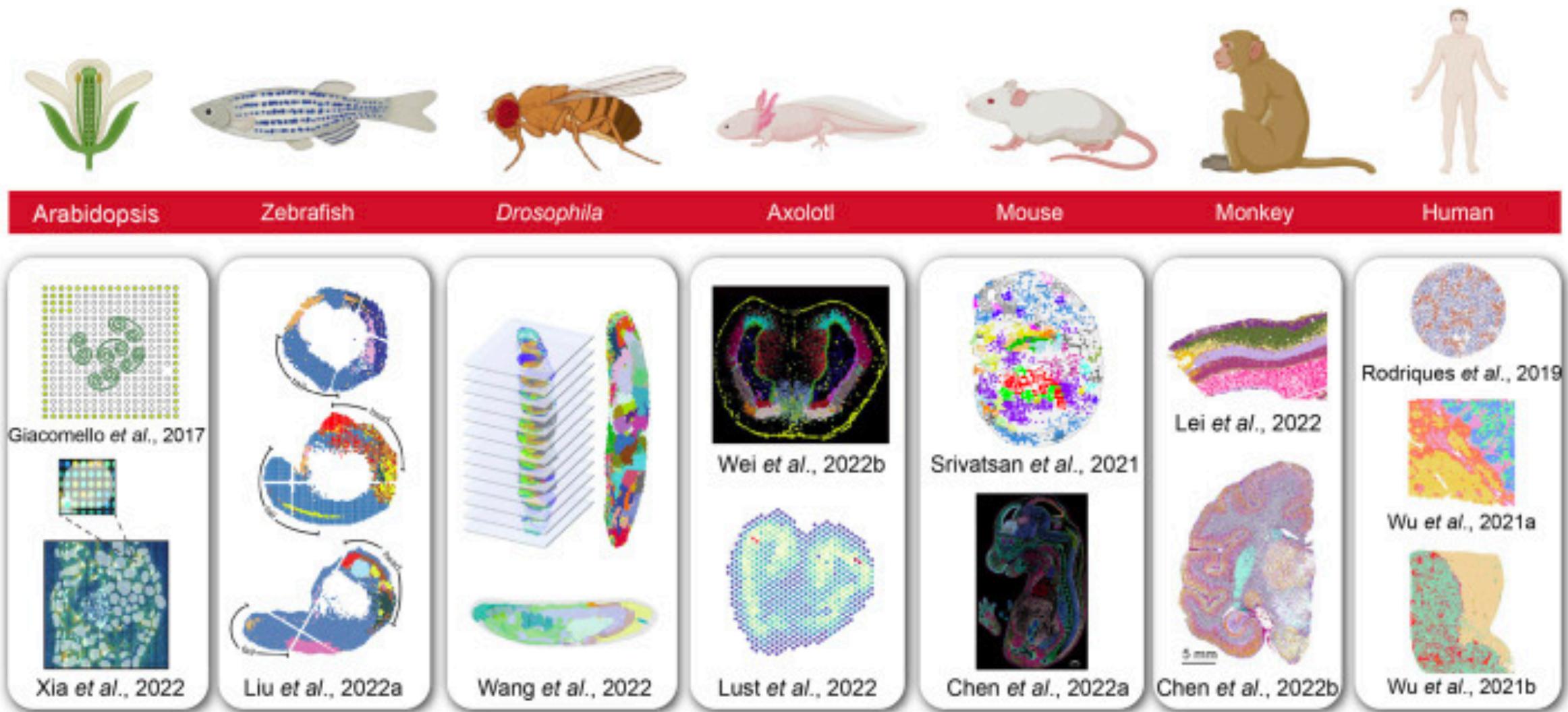


Xenium (10x Genomics)

- Imaging based (builds on *in situ* hybridization)
- Subcellular resolution (+-0.2um/pixel)
- Targeted discovery: work with panels
 - Rapid development here
 - Standard panels and custom panels
 - Custom up to 480plex genes currently
- Throughput: 2 slides per run
 - 2-3 days sample prep
 - 400mm² in <50h
- Tissue type: FFPE and fresh frozen



Now you have your beautiful dataset



What is usually done?

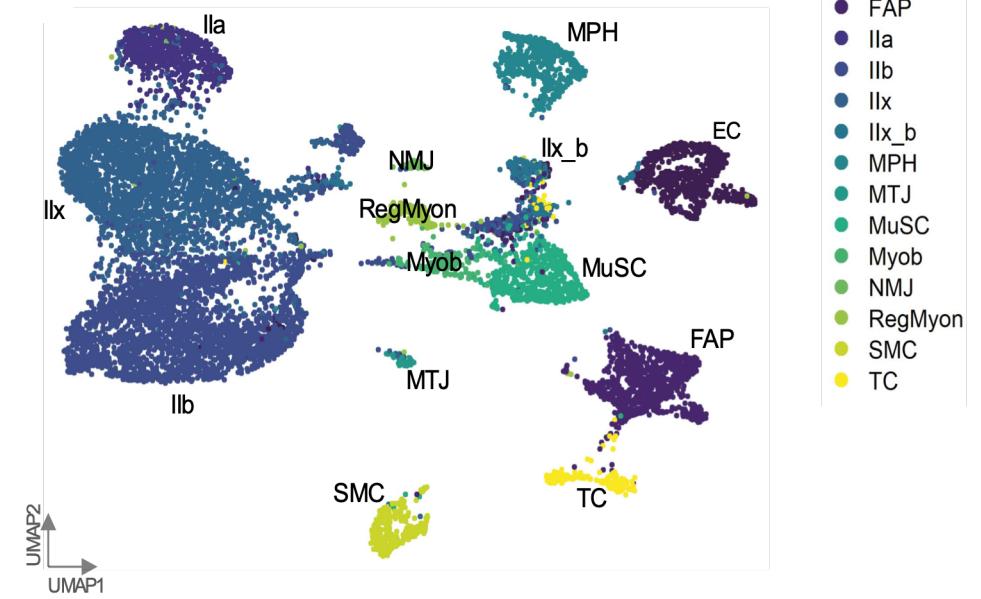
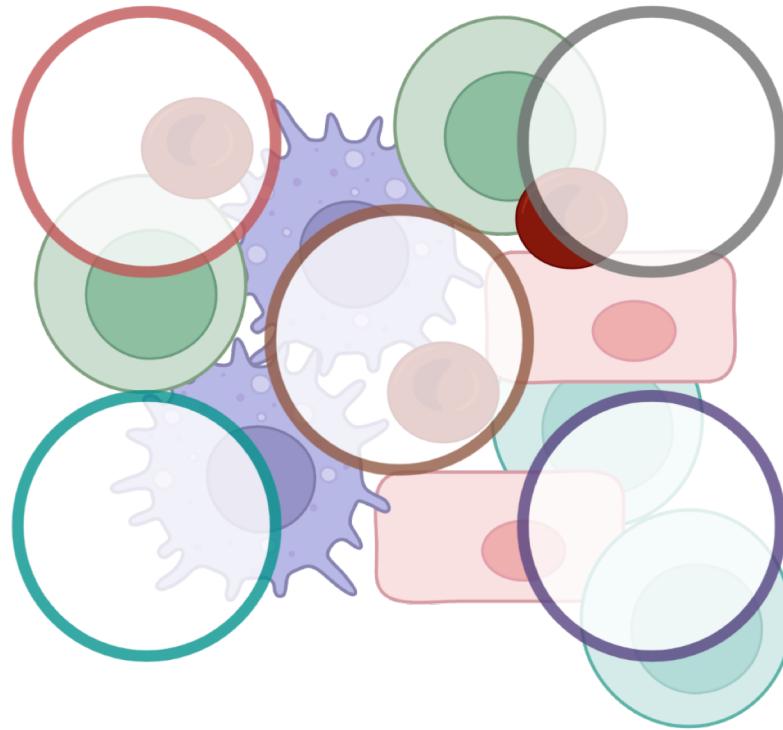
- Pre-processing
- Clustering
- Cell segmentation (imaging-based)
- Deconvolution (sequencing-based)
- DGE
- Pathway analysis
- Cell-cell communication (neighbors)

What is usually done?

- Pre-processing
- Clustering
- Cell segmentation (imaging-based)
- *Deconvolution (sequencing-based)*
- DGE
- Pathway analysis
- *Cell-cell communication (neighbors)*

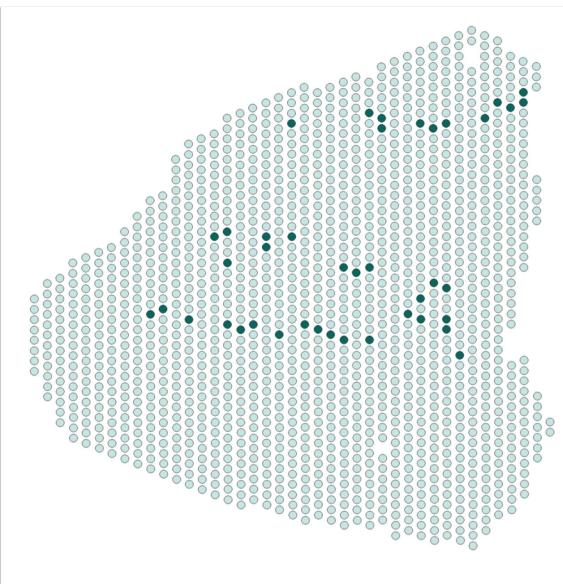
Automated pipelines:
STtools
Squidpy
Tangram
Spacemake
Sparrow

Spot deconvolution



Spot deconvolution

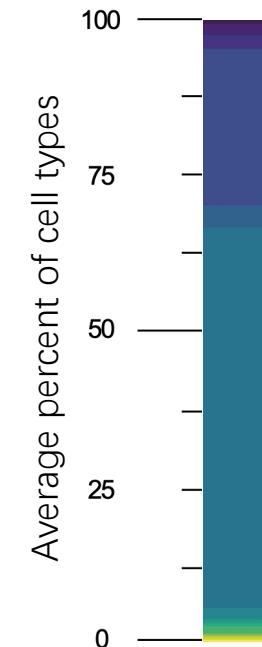
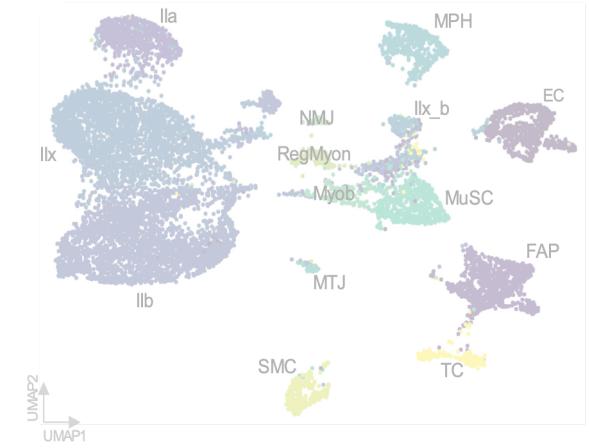
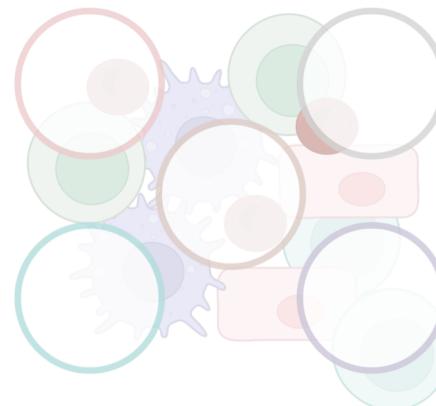
Neuromuscular junction



Mpz

Chrne

Expression level
0 1 2 3 4 5



Muscle fibers

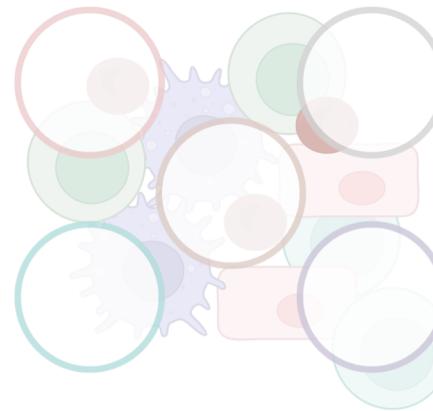


Neuromuscular junction

- EC
- FAP
- IIa
- IIb
- IIx
- IIx_b
- MPH
- MTJ
- MuSC
- Myob
- NMJ
- RegMyon
- SMC
- TC

- EC
- FAP
- IIa
- IIb
- IIx
- IIx.b
- MPH
- MTJ
- MuSC
- Myob
- NMJ
- RegMyon
- SMC
- TC

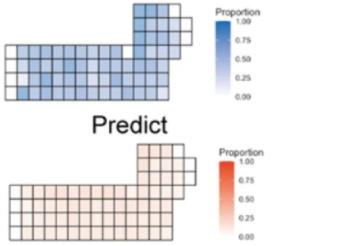
Spot deconvolution



Accuracy

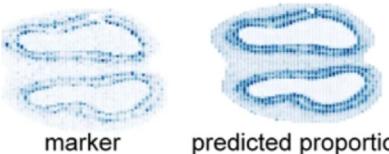
JSD & RMSE

Ground truth



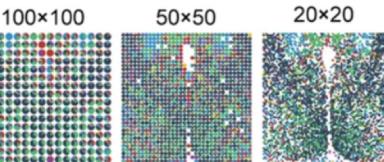
Predict

Correlation of cell type & marker genes



Robustness

Various number of cell types, genes, spots & datasets



Stability among replicate experiments, different normalization methods & hyperparameters



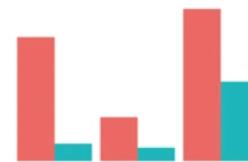
Usability

Quality of software & paper

code quality
installation procedure
document quality

...

Computational time



18 methods

With scRNA-seq reference:

Probabilistic-based:

stereoscope, RCTD, DestVI,
STRIDE, CARD, SpatialDecon,
Cell2location

NMF-based:

NMFReg, SpatialDWLS,
SPOTlight

Graph-based:

DSTG, SD²

Deep learning-based:

Tangram

Optimal transport-based:

SpaOTsc, NovoSpaRc

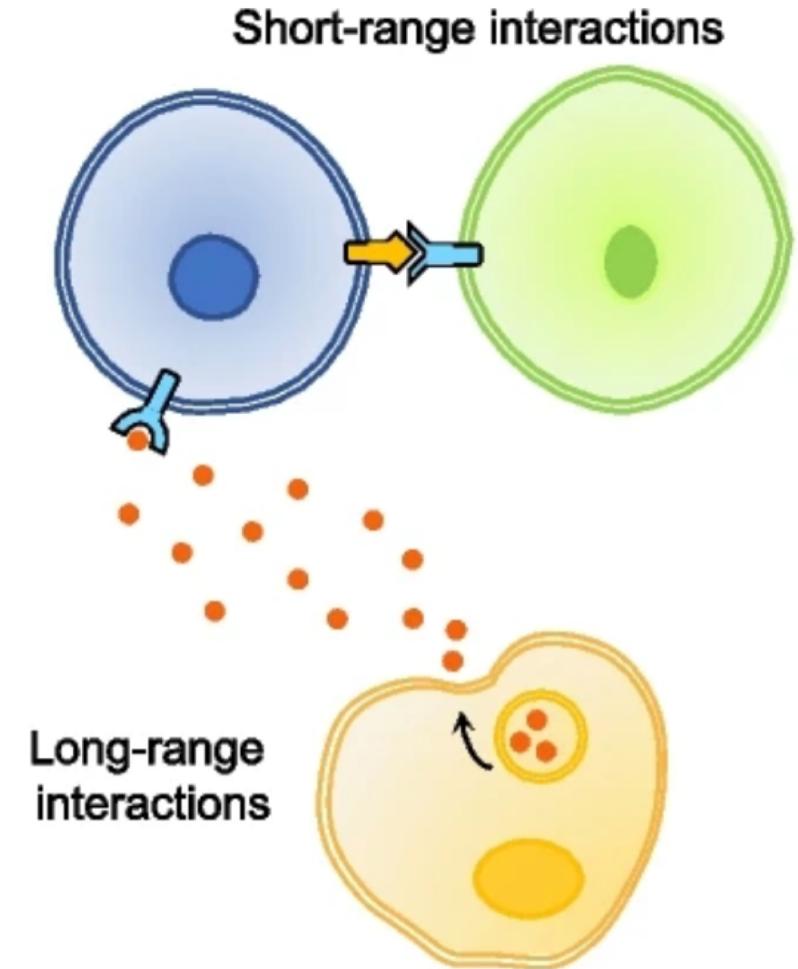
Without scRNA-seq reference:

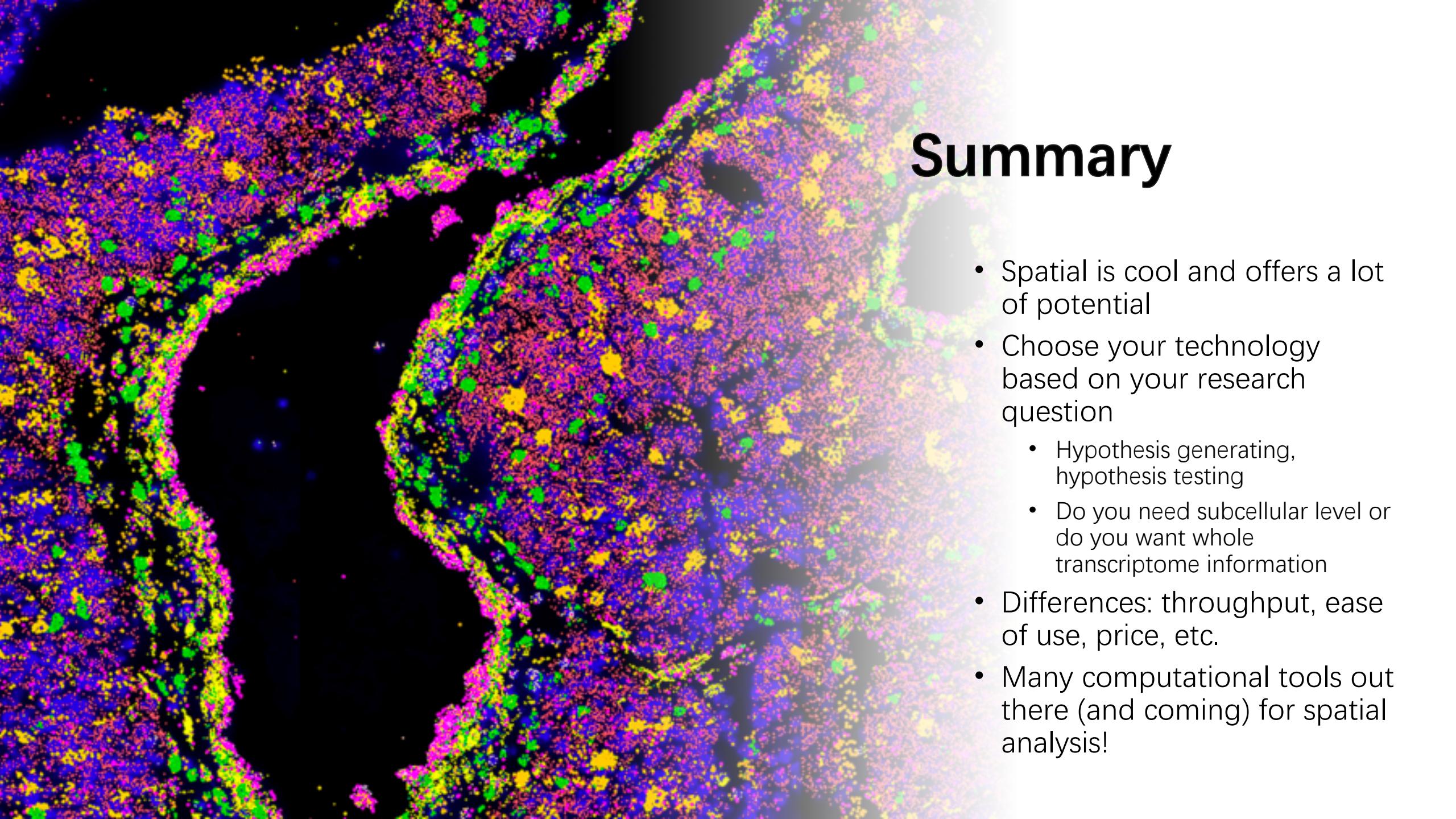
Berglund, E. et al.
STdeconvolve
SpiceMix

Cell-cell communication (CCC)

Tools:

- COMMOT
- CellChat
- CellPhoneDB
- NicheNet
- ICELLNET



A grayscale image of a tissue section, possibly a brain slice, showing various cellular structures. Colored dots (red, green, blue) are scattered across the image, representing spatial transcriptomic data. These dots are more concentrated in certain areas, indicating specific gene expression patterns.

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!

Thank you for your attention!

