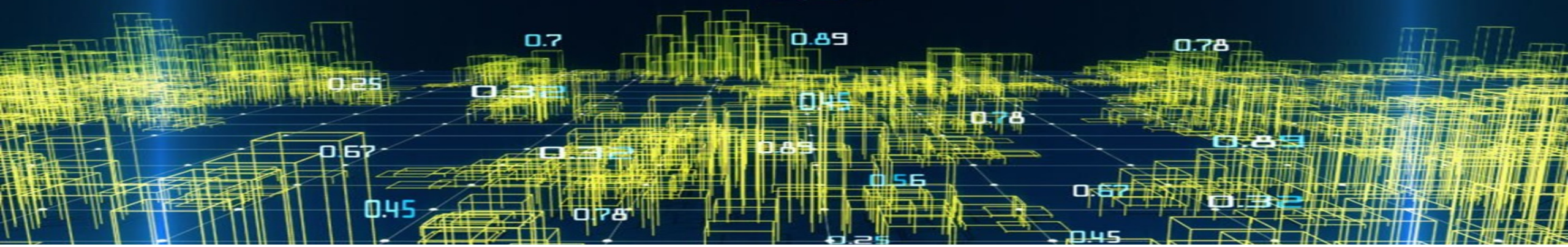
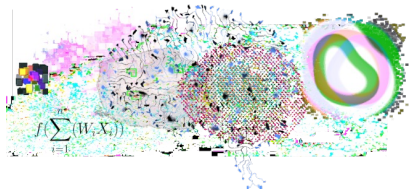


Advances in Spatial Transcriptomics and Computational Approaches

Guillaume MARCY

AI / ML for the analysis of single-cell spatial transcriptomics (UCBL Lyon) – 15th October 2025



Université Claude Bernard Lyon 1

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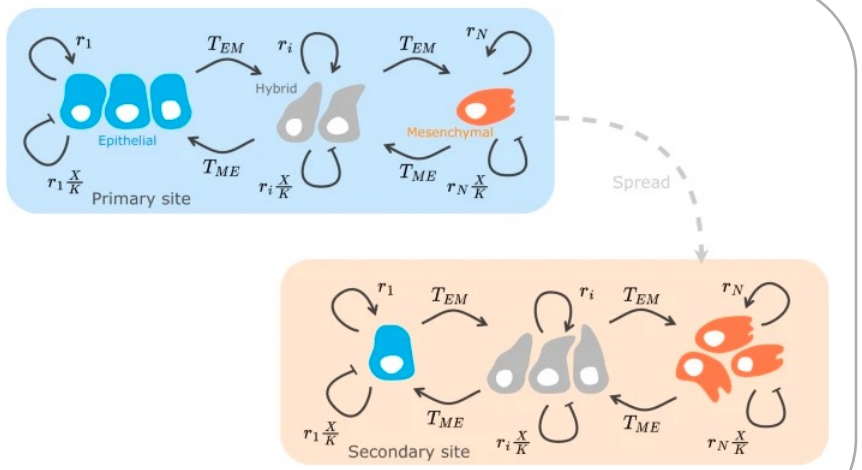
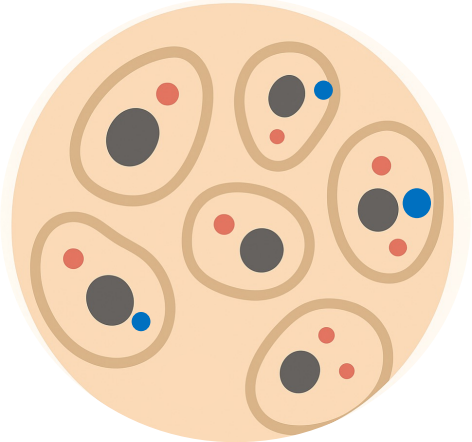
UNIVERSITÉ DE LYON

Why is Spatial Transcriptomics (ST) Important ?



Saumil Shah et al. 2023. npj

- Position of a cell relative to its cellular or non-cellular environment influences its exposition to signals
  - → Cellular phenotype
  - → Function of the tissue

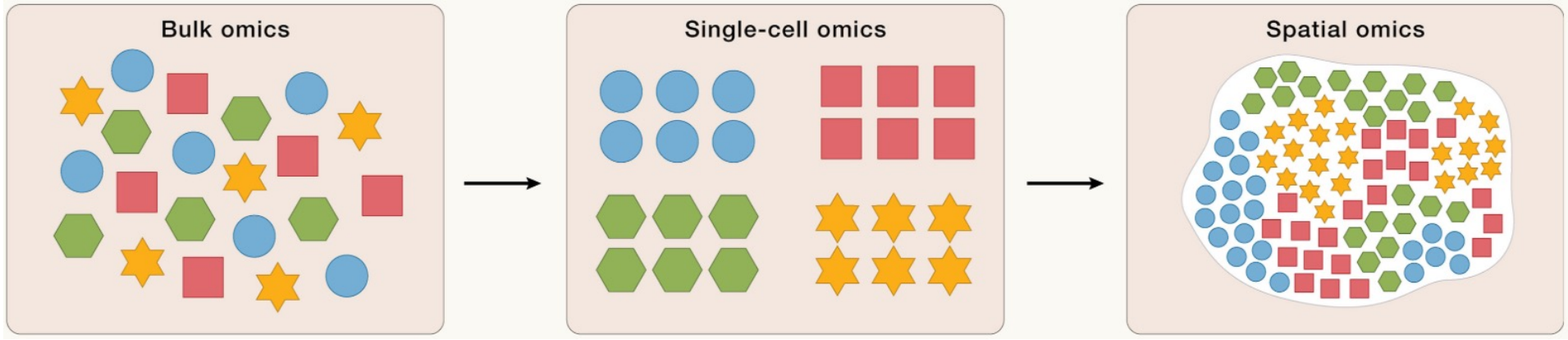


- Sub-cellular localization of mRNAs can vary depending on the function of the gene
  - → Target proteins to a specific location

ST aims to count the number of transcripts of each gene at distinct spatial locations in a tissue

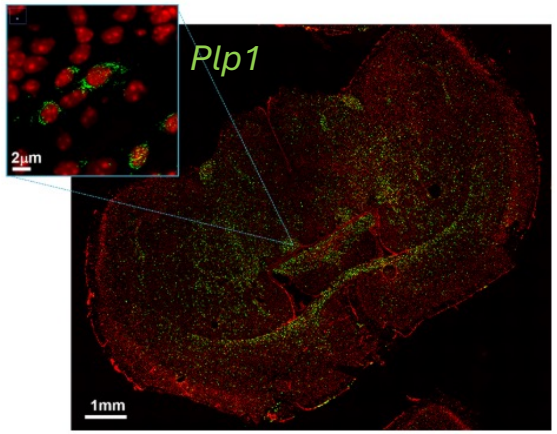
How Does it Works ?

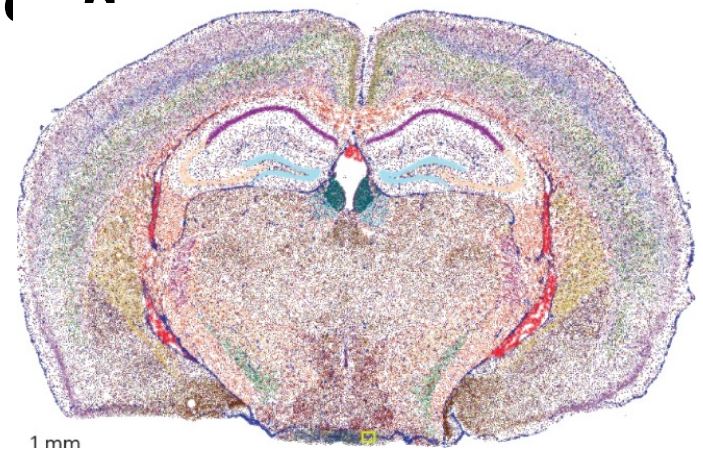
1 Sequencing-based ST technologies – From high throughput sequencing world



Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

2 Imaging-based ST technologies – From imaging w[illegible]



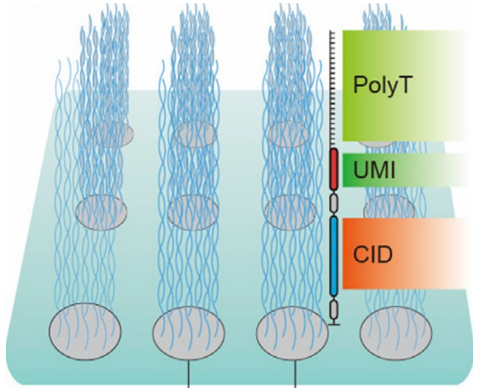


Salas et al. 2025. ***Xenium***

How Does it Works ?

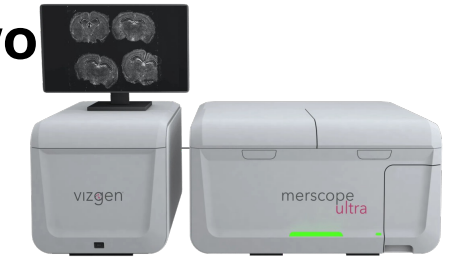
1 Sequencing-based ST technologies – From high throughput sequencing world

- **Extract** mRNAs from tissue while keeping the spatial information
- mRNAs are profiled using **sequencing**



2 Imaging-based ST technologies – From imaging wo

- **Imaging** mRNAs directly within tissue via microscopy
  - Hybridization of mRNAs to fluorescently labelled probes
  - Direct sequencing of barcodes associated to amplified mRNAs



Merscope by Vizgen

Xenium by 10X Genomics

Different Approaches for Different Answers

? How many genes ?

? What gene capture efficiency ?

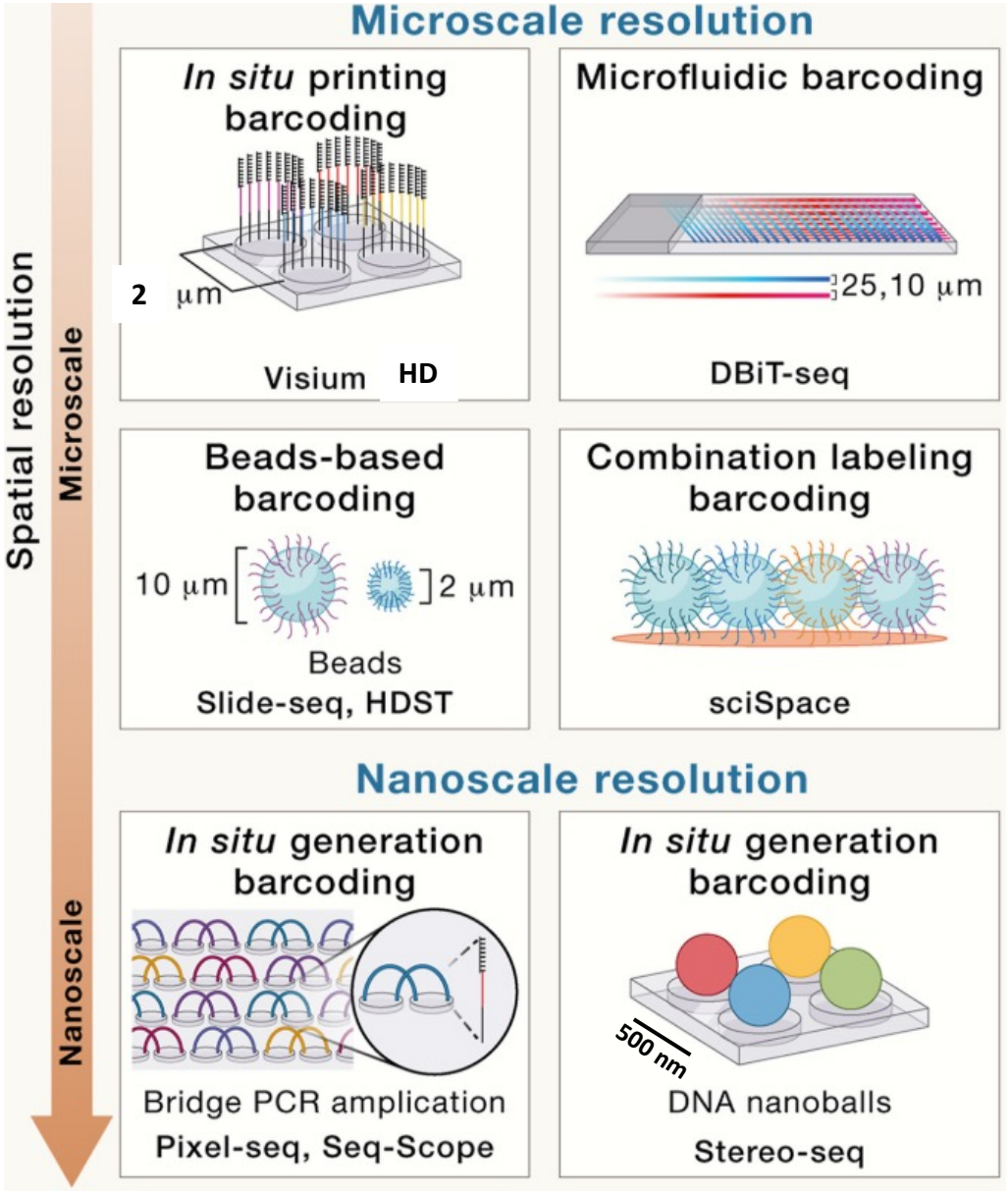
? Which kind of genes ? → PolyA mRNAs, microRNAs, lncRNAs …

? What spatial resolution ? → Single cells versus anatomical regions/domains

? What is the size of the tissue ? → Full embryo versus dissected regions

? Which kind of samples ? → Fresh Frozen (FF), Bioarchives/biopsies (FFPE)

Sequencing-based Spatial Transcriptomics



Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

Common steps

- concept of scRNA-seq library + **spatial barcode**
- spatially barcoded mRNAs are **sequenced**
- reads contain spatial barcode + gene sequence

→ **Spatial location x gene matrix**

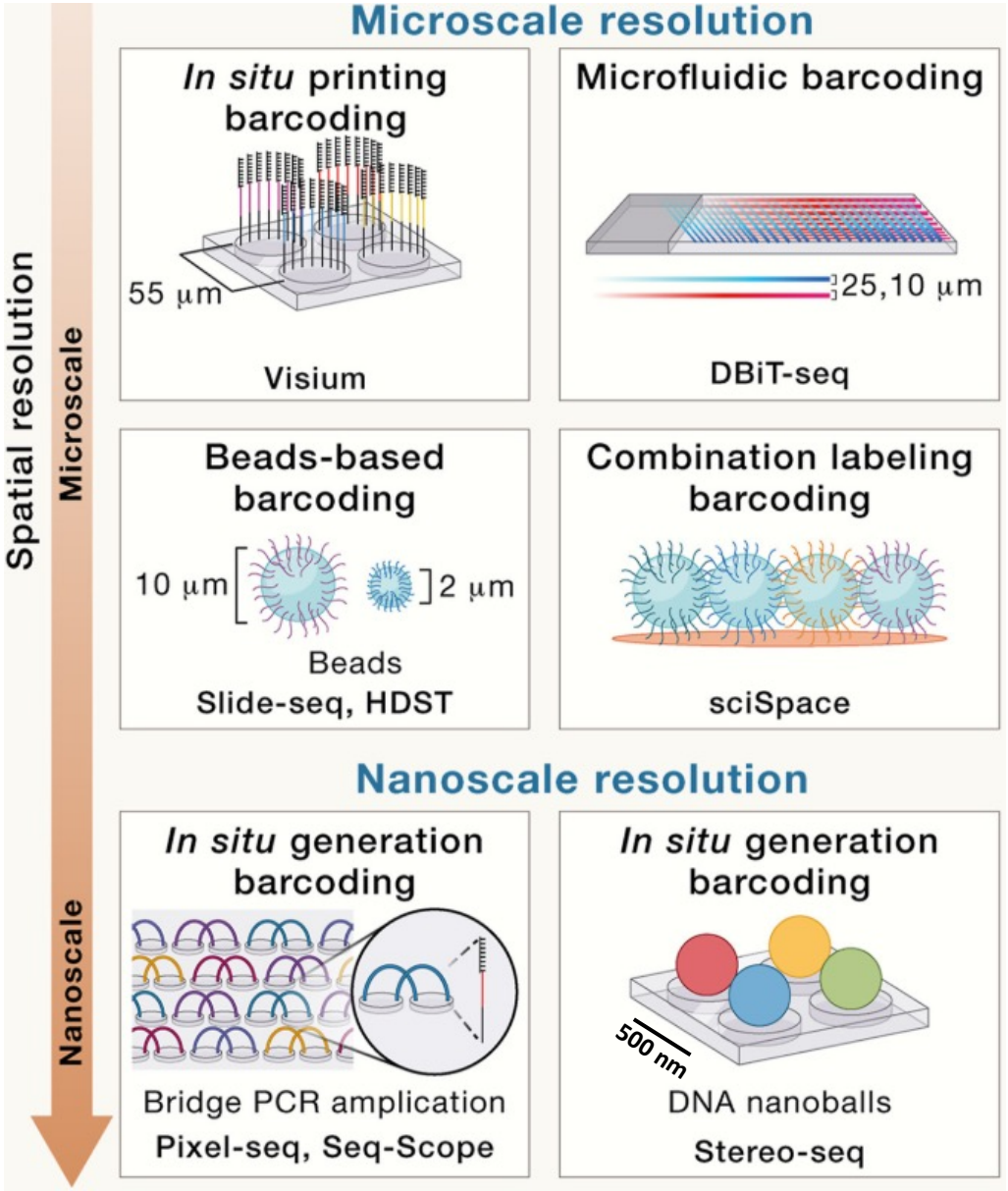
☺

- Full transcriptome

☹

- Low capture efficiency
- Single cell Resolution

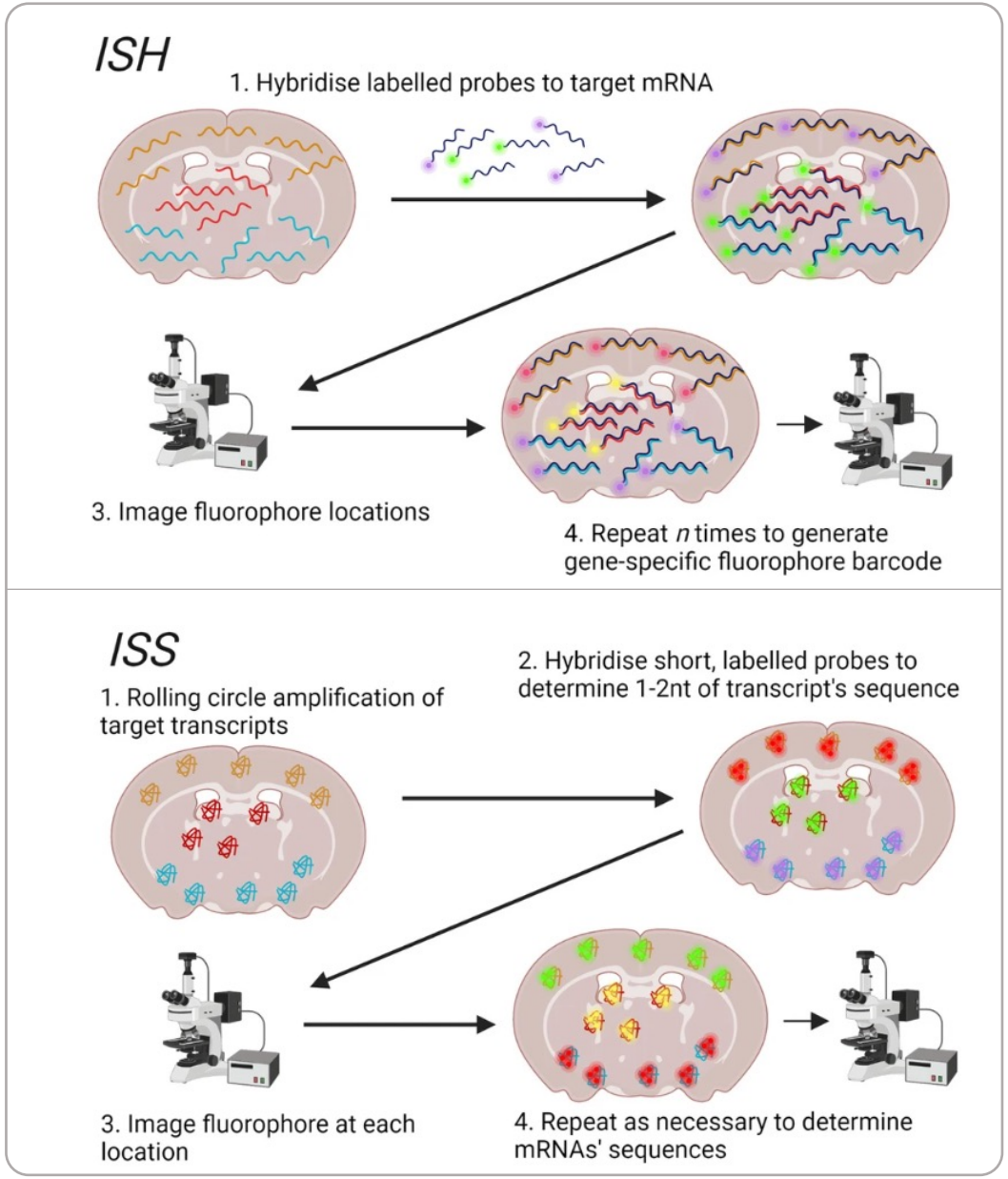
Sequencing-based Spatial Transcriptomics



	VisiumHD	Stereo-seq	Slide-seq (Curio seeker)
Company	10X Genomics	STOMICS	Takara/Curio
Sample type	FF & FFPE	FF & FFPE	FF
Species	Agnostic (FF) Hu & Mu (FFPE)	Agnostic	Agnostic
Area	6.5 x 6.5 mm	10 x 10 mm + Larger & custom	10 x 10 mm
Resolution	2 μm	0.5 μm	10 μm
Bin size	8 and 16 μm^2 & SC	Custom>SC	10 μm^2
Capture efficiency /10μm²	250 genes	300 genes (Up to 1500 genes/Neuron)	250 genes

Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

Imaging-based Spatial Transcriptomics



Common steps

- **design** oligonucleotide probes targeting predefined set of genes through hybridization
- quantification of transcripts via **microscopy imaging**

→ **Spatial location x gene matrix**

☺

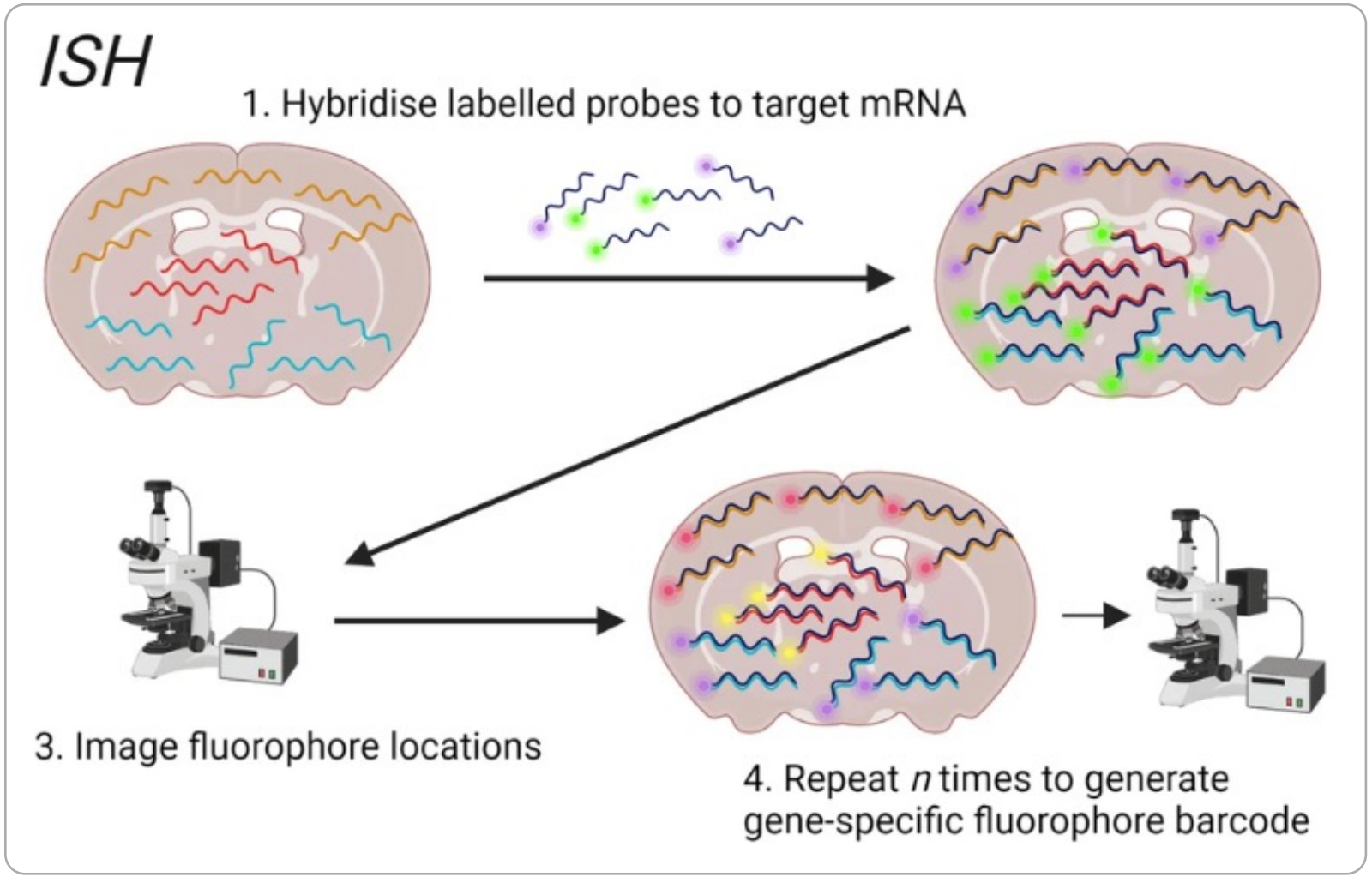
- All RNAs, not only mRNAs
- Subcellular resolution

☹

- Targeted panel of genes

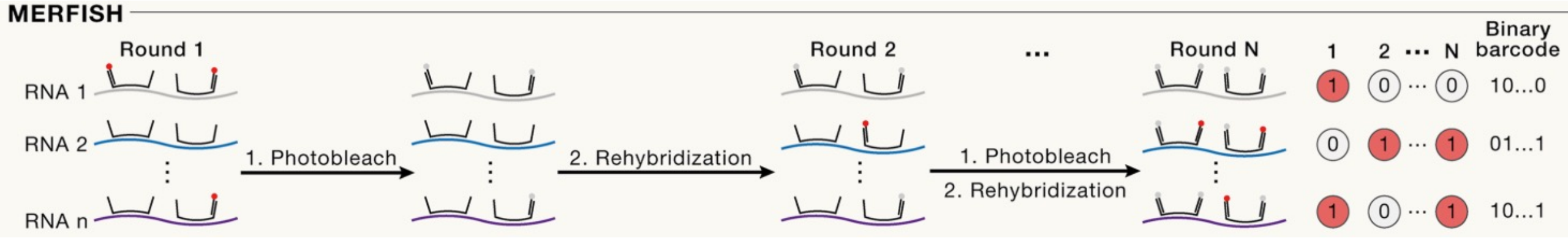
Williams et al. 2022. Genome medicine. An introduction to ST for biomedical research.

Imaging-based Spatial Transcriptomics – ISH technique



Hybridize RNAs to fluorescently labelled gene-specific probes

Imaging-based Spatial Transcriptomics – ISH Technique



Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

1. Each RNA is assigned a unique binary barcode e.g. Gene A : 0110100101011
2. Each RNA is targeted by several designed probes that contains 2 regions :
   - complementary RNA sequence
   - readout barcode sequences
3. Barcodes are read using sequential FISH rounds

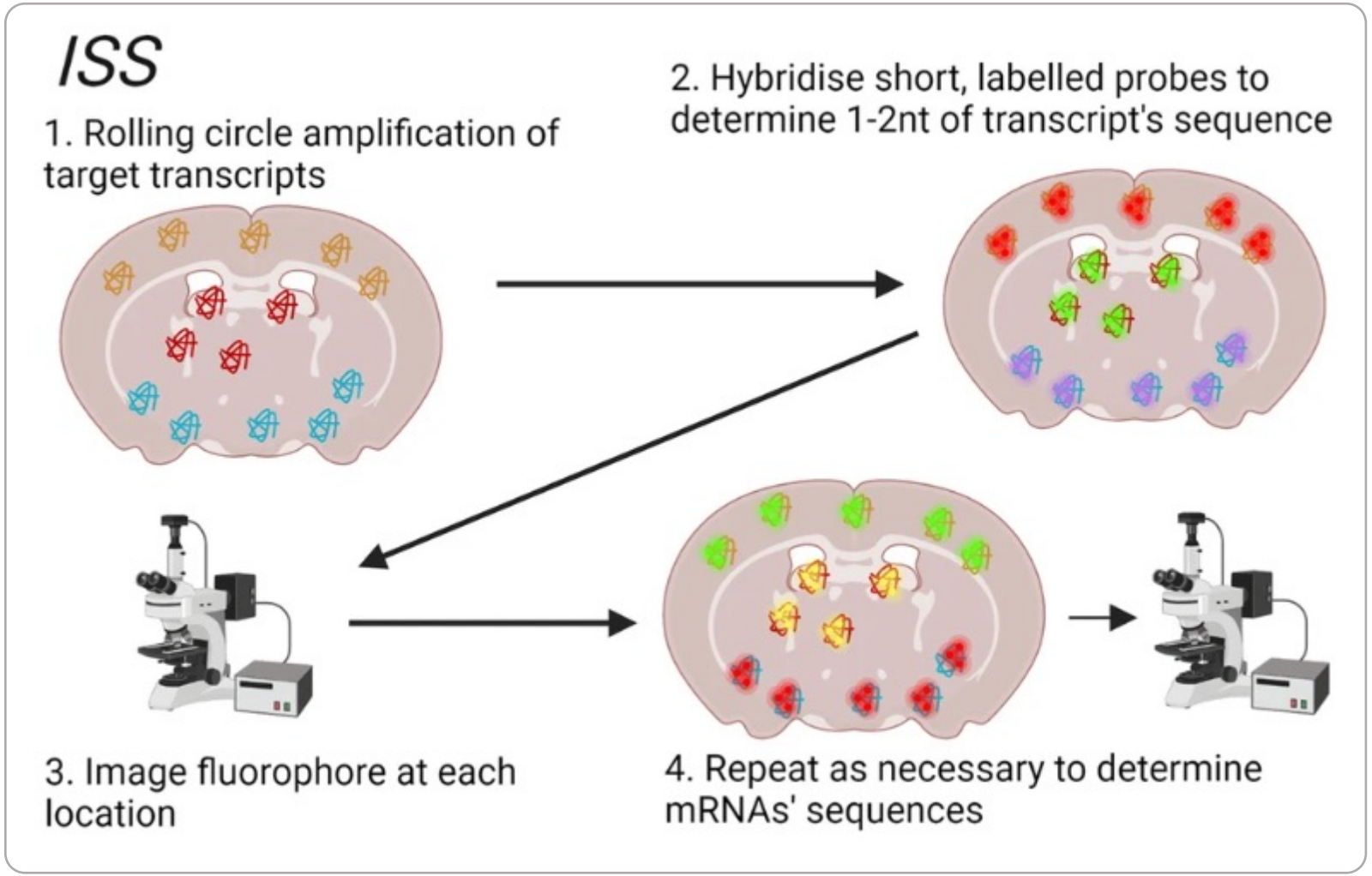
☺

- All RNAs, not only mRNAs
- Subcellular resolution

☹

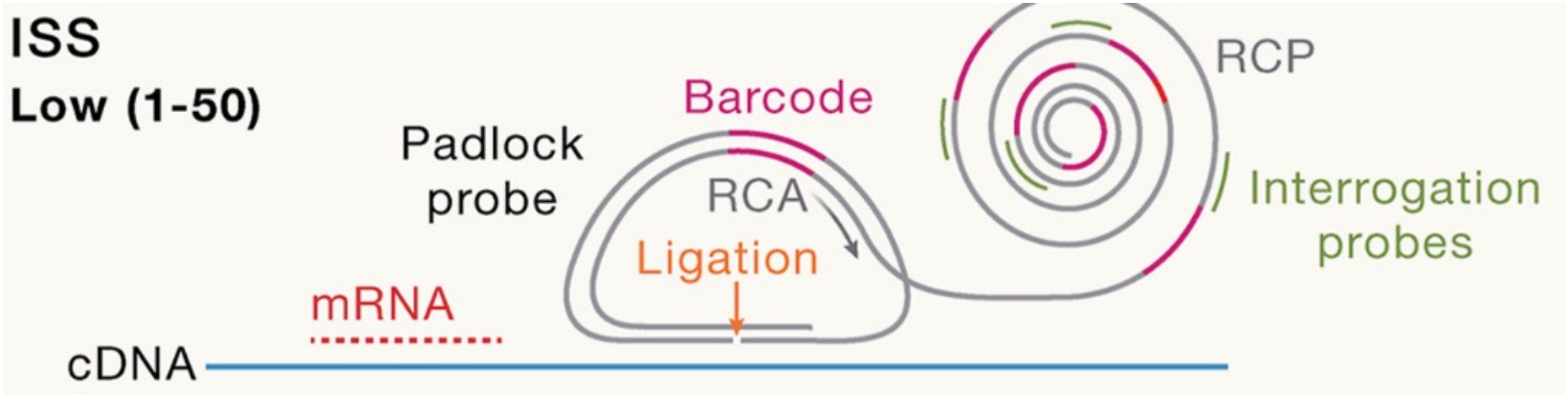
- Targeted, panel of genes

Imaging-based Spatial Transcriptomics – ISS Technique



Directly sequence amplified mRNAs inside the tissue section by sequencing by ligation technique

Imaging-based Spatial Transcriptomics – ISS Technique



Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

1. reverse transcription of the RNA
2. hybridization of padlock probes to the cDNA, containing a unique corresponding barcode
3. Rolling circle amplification (RCA) to produce many copies of the barcode
4. Read the barcode trough imaging the sequence of the barcode with fluorescently labelled probes targeting nucleotides

☺

- All RNAs, not only mRNAs
- Subcellular resolution

☹

- Targeted, panel of genes

Beyond Spatial Transcriptomics

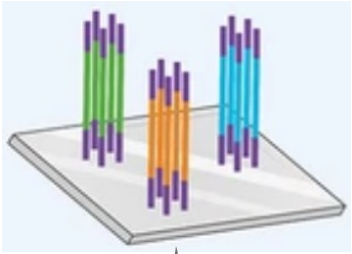
- Spatial ATAC-Seq (*Deng et al. 2022*) → Chromatin accessibility
- Spatial Cut&Tag (*Deng et al. 2022*) → Epigenetic landscape
- Spatial Proteomics → Macsima (Miltenyi), PhenoCycler (Akoya)…
- Spatial Metabolomics (*Ganesh et al. 2021*) → Metabolites quantification (mass spectrometry)

Multi-modalities

- Spatial ATAC-Seq + RNA expression (*Zhang et al. 2023*)
- Spatial Cut&Tag + RNA expression (*Zhang et al. 2023*)
- Spatial CITE-seq : Proteomics + RNA expression (*Liu et al. 2023*)

ST Data Analysis

❶ Sequencing-based ST technologies



Alignment and Mapping

❷ Imaging-based ST technologies

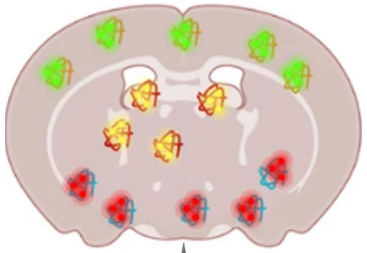


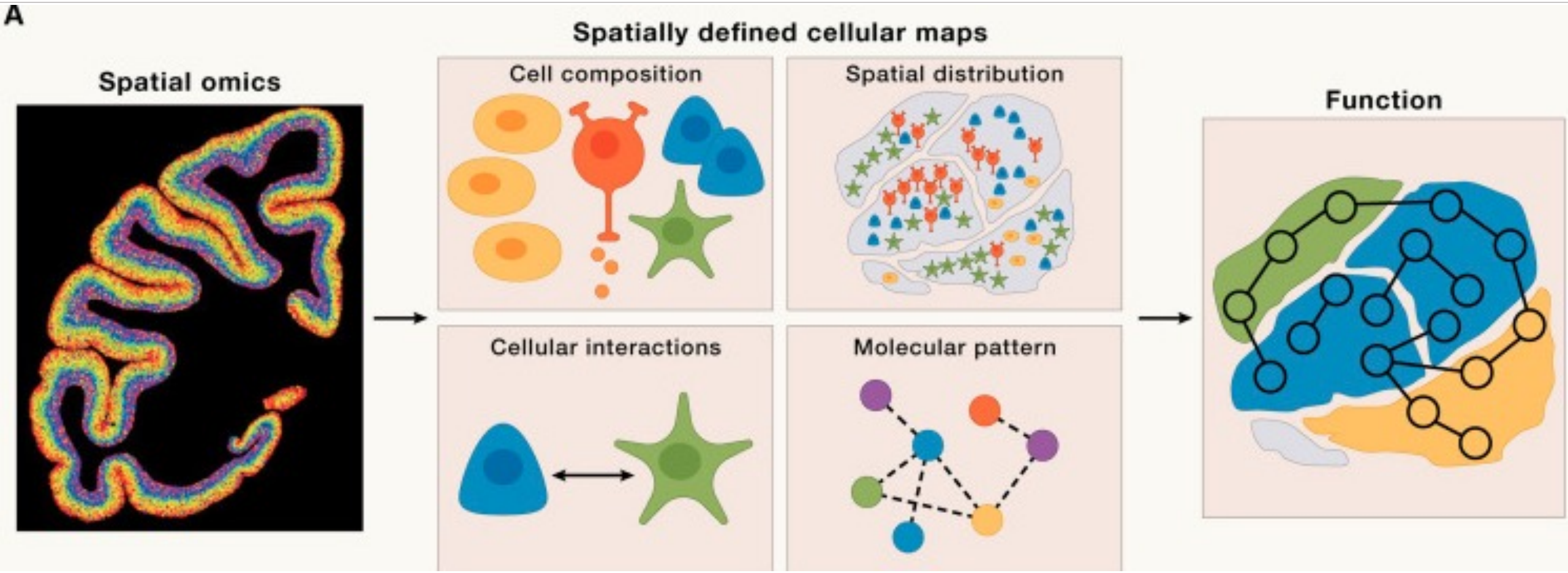
Image processing and RNA identification

Spatial location x Gene matrix (.h5 file)

	spot1	spot2		spotn
Gene1	3	2	.	13
Gene2	2	3	.	1
Gene3	1	14	.	18
…	.	.	.	.
…	.	.	.	.
…	.	.	.	.
GeneM	25	0	.	0

1. QC filtering
2. Normalization (log1p, scTransform, SpaNorm…)

What Do you Do with ST Data Analysis ?



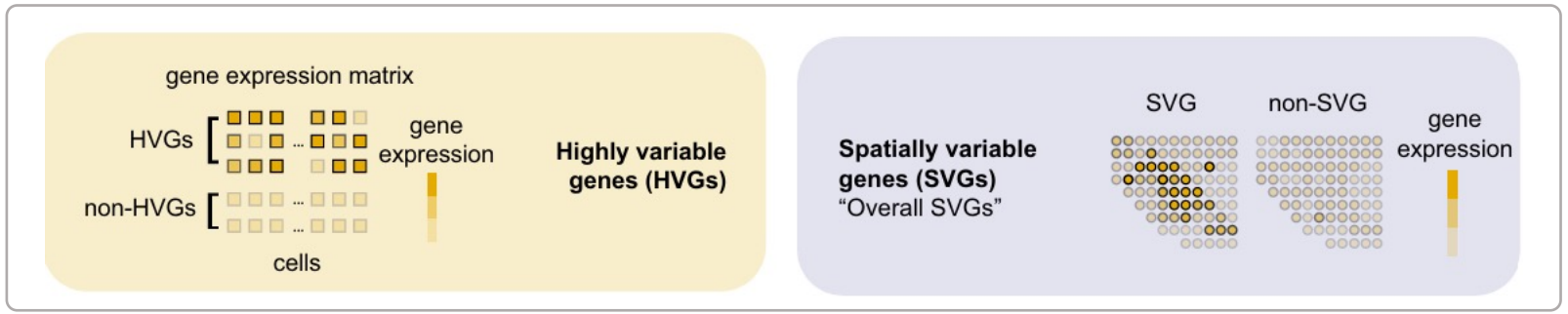
Liu et al. 2024. Cell Review. Spatiotemporal omics for biology and medicine.

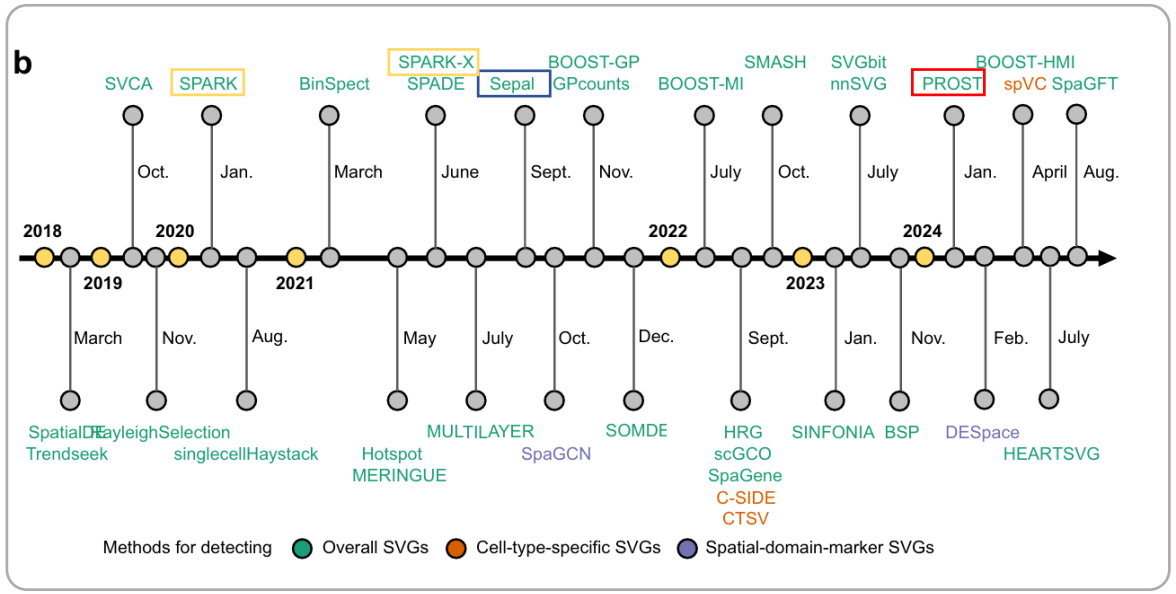
Tissue/Region Level

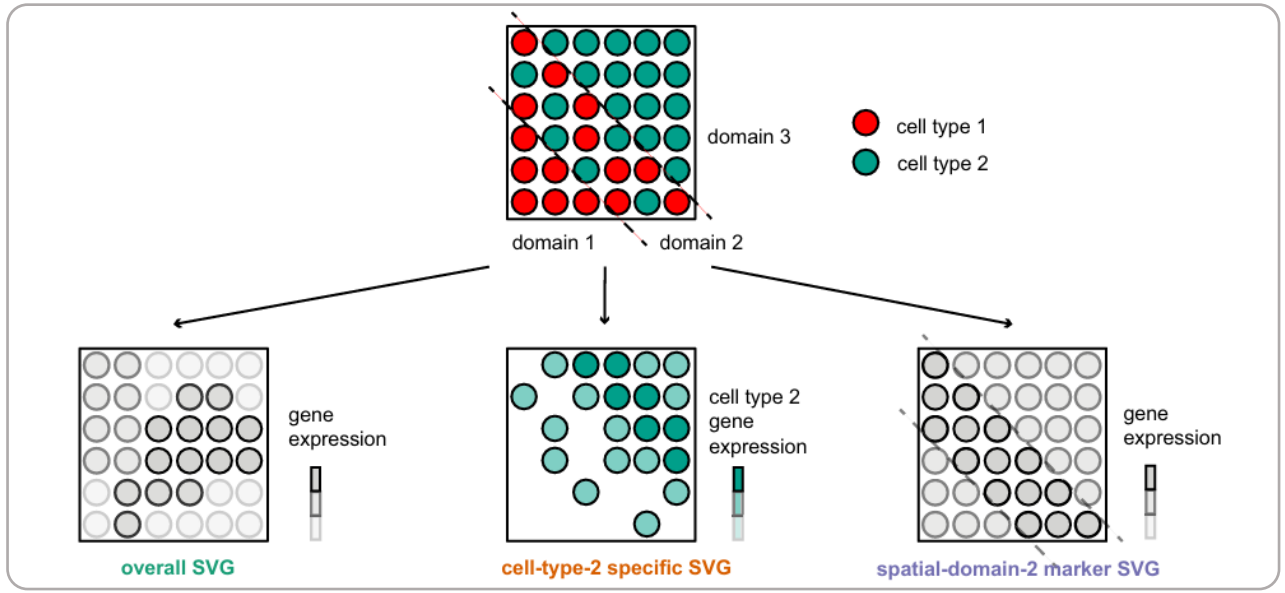
Cellular Level

Molecular Level

Spatial Variable Genes Detection







Guanao Yan et al. 2025. Review Nature Communication

Imaging-based technologies

Imputation of Missing Genes

Imaging-based ST technologies achieve a limited number of genes from the entire transcriptome. This restricted gene coverage limits the comprehensive understanding of the molecular landscape of the tissue.

- mapping single-cells onto the ST data → **Integration**
- imputing spatial gene expression from scRNA-seq data → **Prediction**

- **Mapping tools :** Seurat, LIGER, Harmony, Tangram, ENGEP , Tissue, ENGEP etc.
  - ☹ lack specific optimizations for spatial gene expression
- **Imputing tools :** SpaGE, stPlus, novoSpaRc, stDiff, SpatialScope, iss_patcher, SPRITE, **SpaIM, TransImpLR, stAI, TransImpute, SpotDiff, spRefine**
  - ☹ Actively developing area

Cellular Segmentation

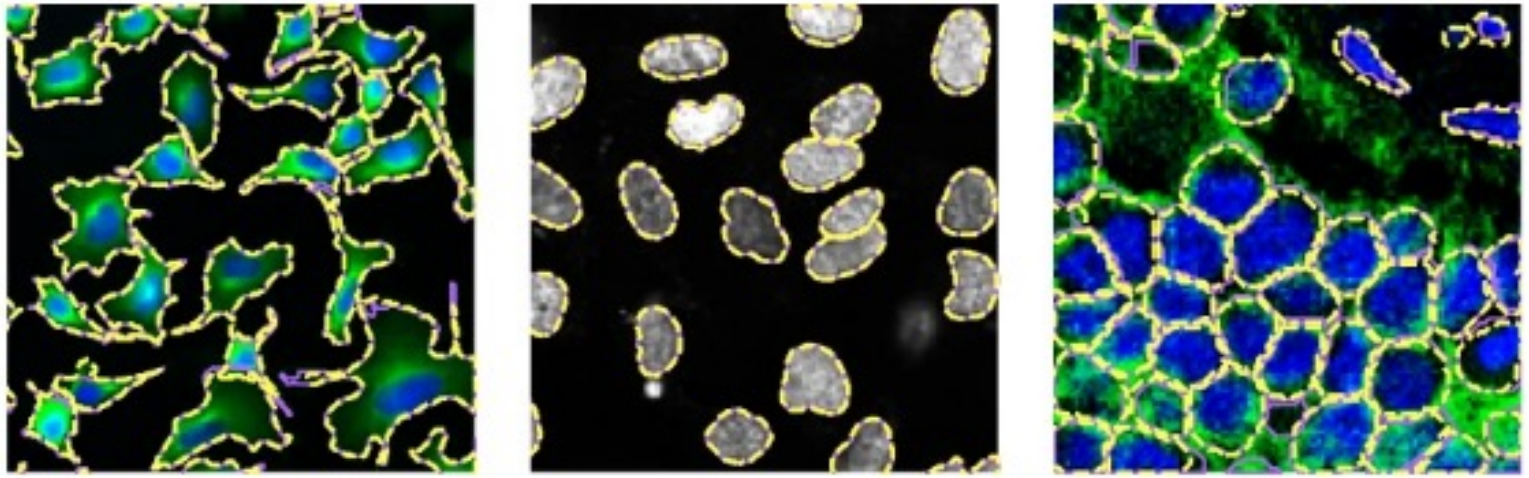
- Detected mRNAs (by sequencing or imaging) need to be attributed to individual cells

→ **Cellular segmentation on staining image**

- some commercially available ST platforms implemented cell segmentation in their pipeline (VisiumHD, Stereo-seq, Merscope, Xenium) through H&E, DAPI or other stainings

- segmentation tools : **CellPose**, **StarDist** → **Staining and Deep Learning**

Baysor, SAINSC → **Staining free**



Tringer et al. Cellpose3. 2025.

Deconvolution

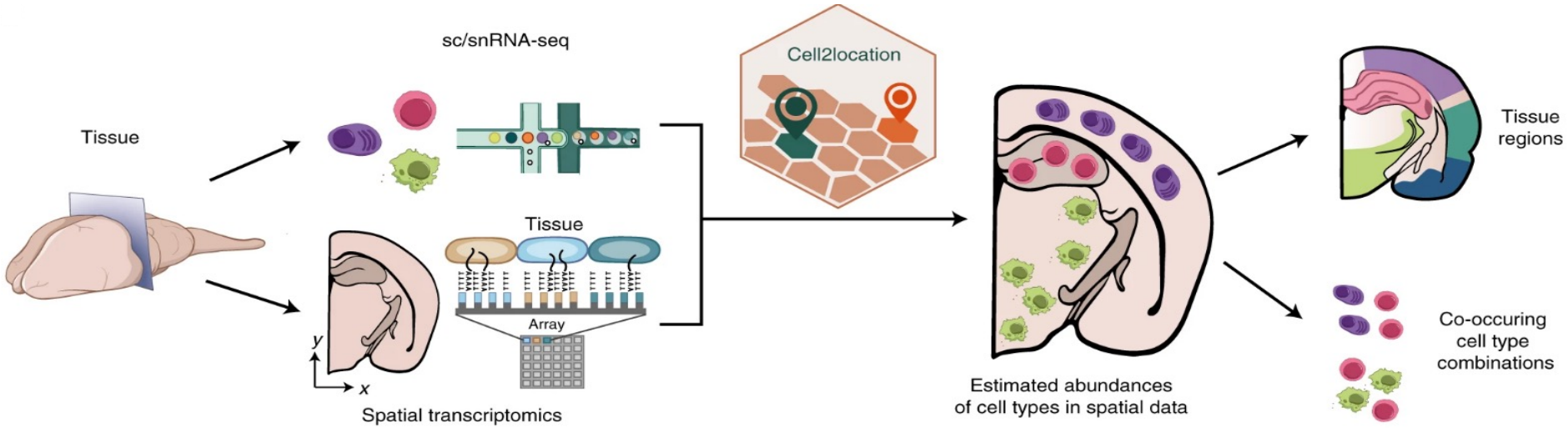
Microscale ST signals need to decompose cell types mixture for each spot

Methods depend on associated single scRNA-seq

Cell2location

1. Estimates cell types reference signatures from scRNA-seq datasets
2. Decomposes the mixture of counts from the reference cell types signature , estimating the cell type abundance at each spatial location

☺ Nanoscale platform ☹ Tissue Thickness



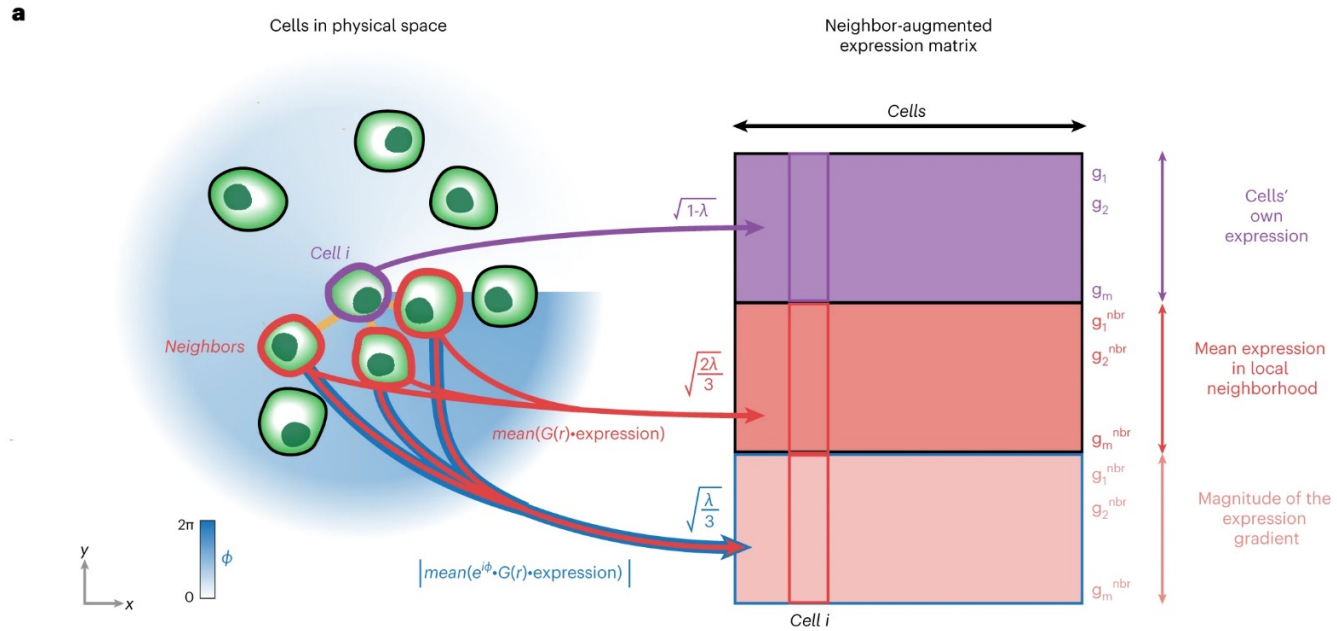
Kleshchevnikov et al. Cell2location. 2022. Nature Biotechnology

Spatial Domains

- Collective cell behaviors give rise to spatial domains which represent spatially organized and functionally distinct anatomical structures

→ **algorithms that look for neighborhoods in both gene expression and physical spaces**

- Tools : Giotto, BayesSpace, **SpaGCN**, SpiceMix, **GraphST**, BANKSY and **STAGATE**



Singhal et al. Banksy. 2024. Nature Genetics

Cell-Cell Communication

- Unravelling complex cell-cell communications in spatial microenvironments

→ **A lot of tools are available !!**

- tools employ a range of distinct strategies (e.g. statistics-based, expression-based, co-expression-based, correlation-based, network-based, and ML-based)
- popular tools are **NicheNet**, CellPhoneDB, CellChat
- CCC-Catalog (Cesaro et al. 2025) can be found at : https://sysbiobig.gitlab.io/ccc-catalog/

Conclusions

Biological Question → **Spatial Platform** → **Computational Approach**

- Sequencing-based ST
- Imaging-based ST

- Molecular Level
- Cellular Level
- Regional Level

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Inserm
La science pour la santé
From science to health

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Sergio Sarnataro (Spatial-Cell-ID)

Inria

Spatial-Cell-ID