



2023 제17회 KOGO 통계유전체 워크샵

Introduction to Spatially Resolved Biology

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Korea University, College of Medicine

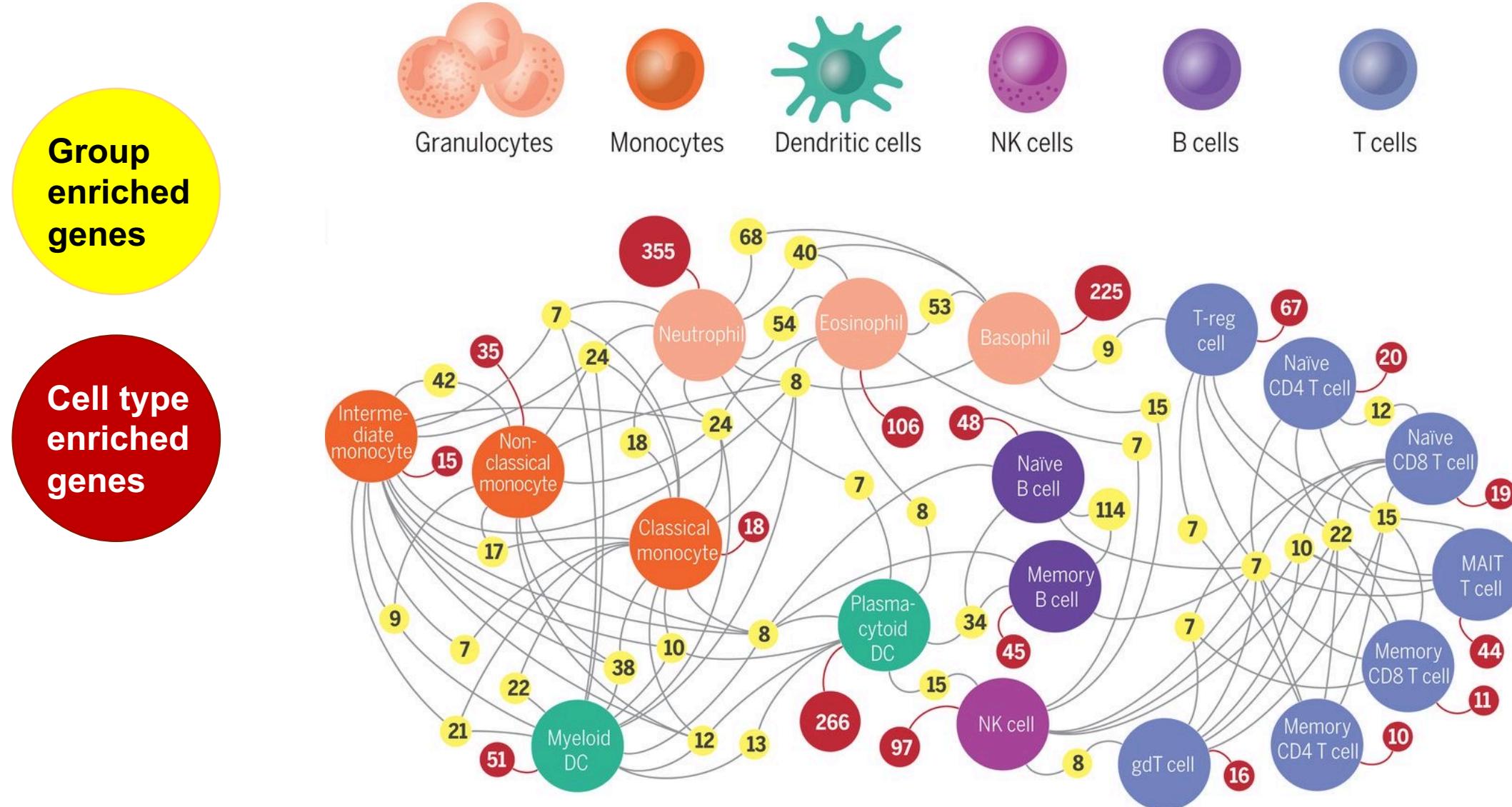
Topics Explored in Today's Lecture

- **Introduction**
 - A broad overview of single-cell data and spatially resolved experimental techniques
- **Computational methodology and frameworks**
 - Different flavors of currently available methods for spatially resolved data analysis

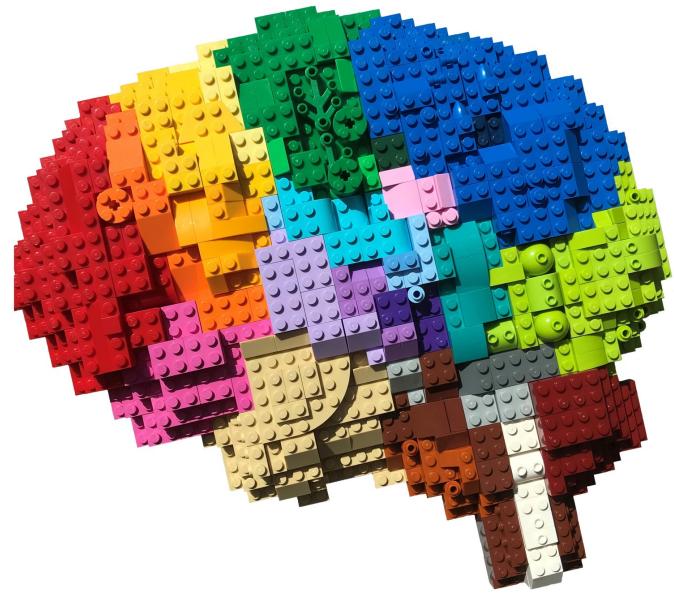
**Human Body
is Made of
37 Trillions
of Cells**



Each cell expresses a set of different genes that enable them to carry out specialized functions



Single-cell data provides valuable insights into the complexity of biological systems



Brain tissue



Bulk genomics

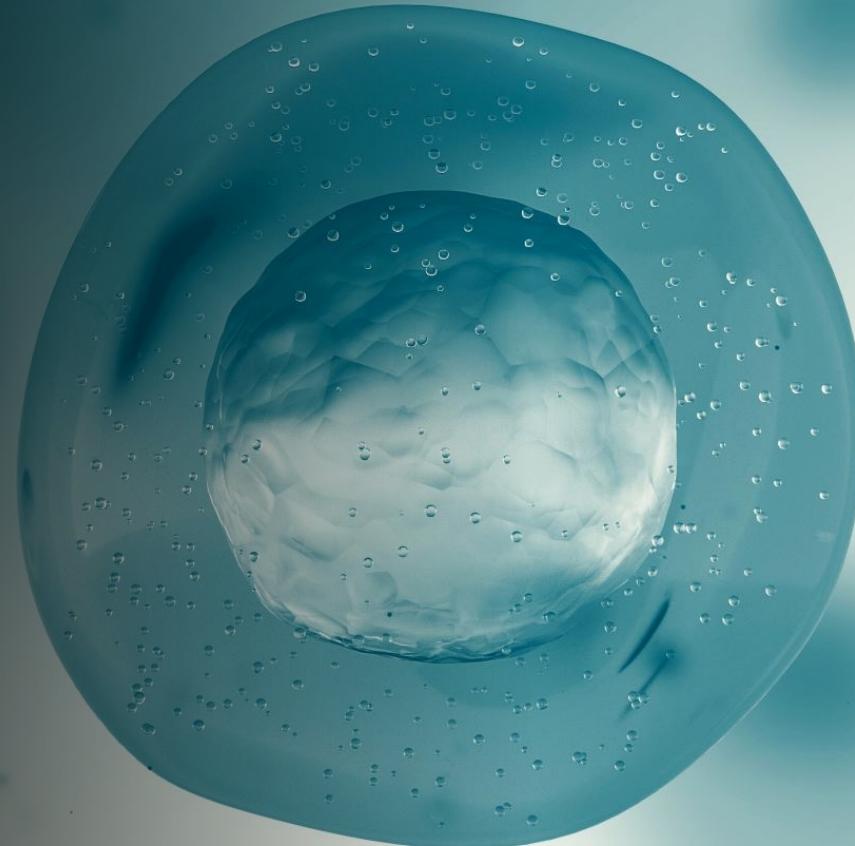


Single-cell genomics

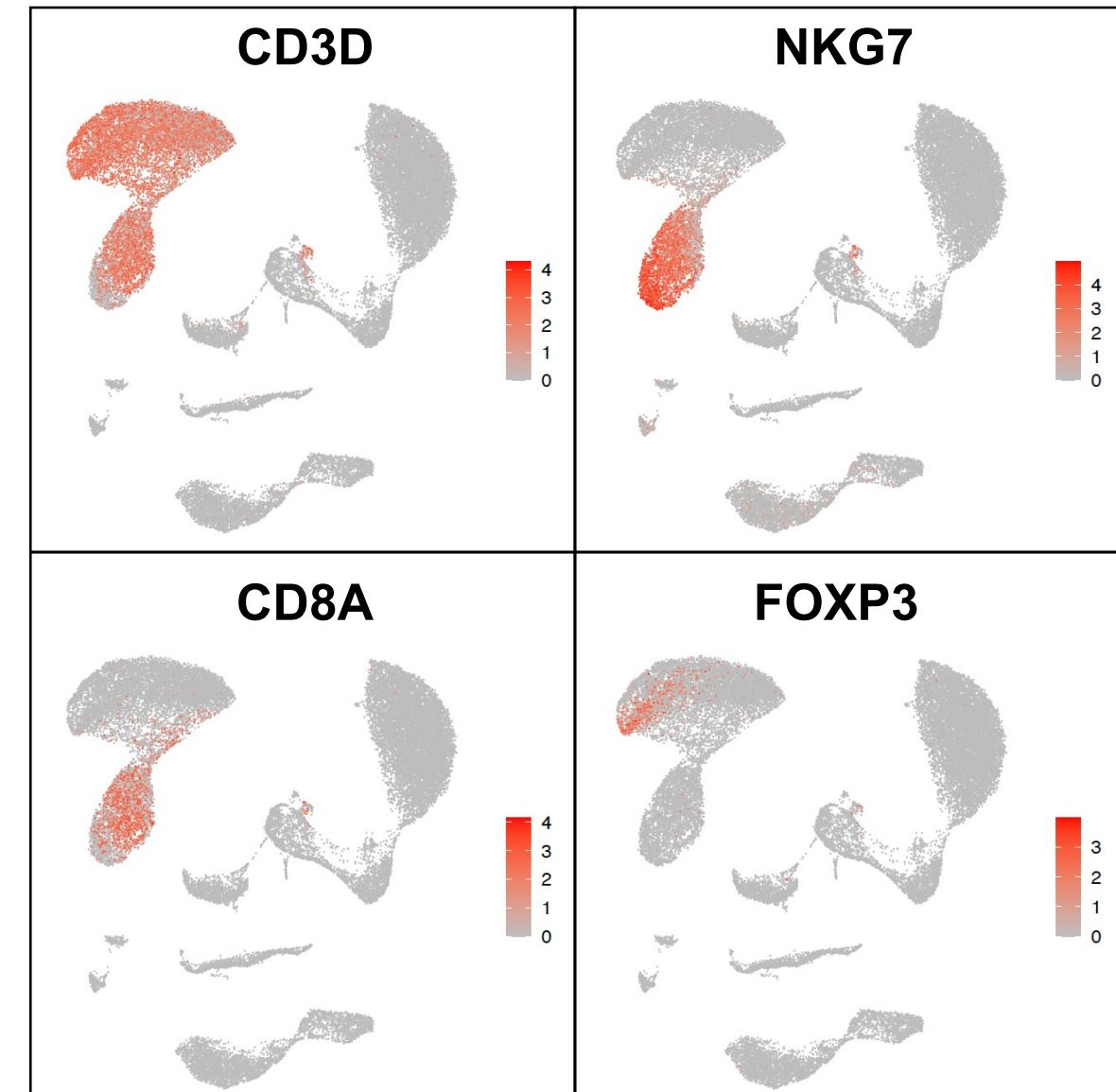
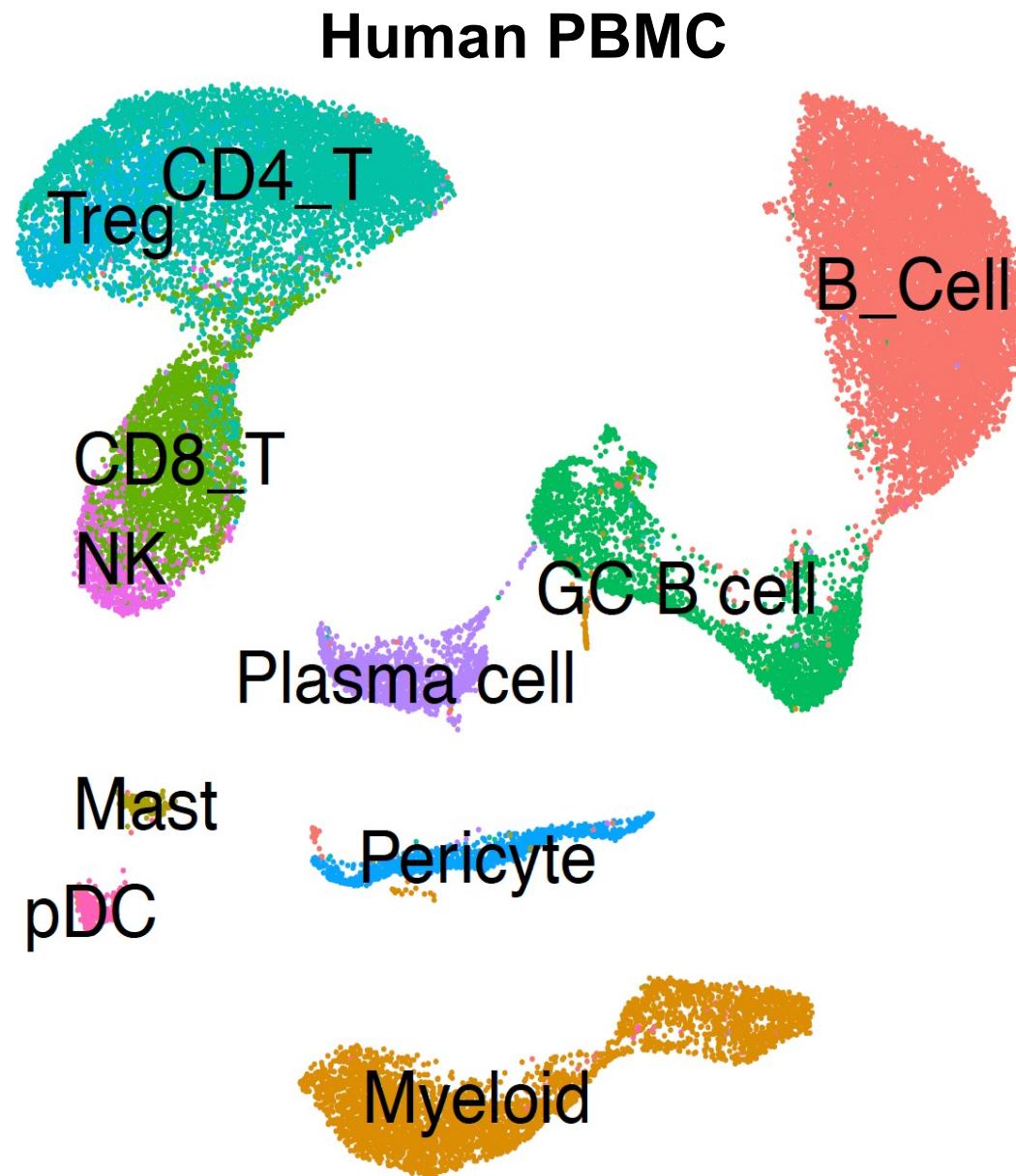
The average behavior measured in millions of cells (bulk genomics)
does not necessarily reflect the behavior in individual cells



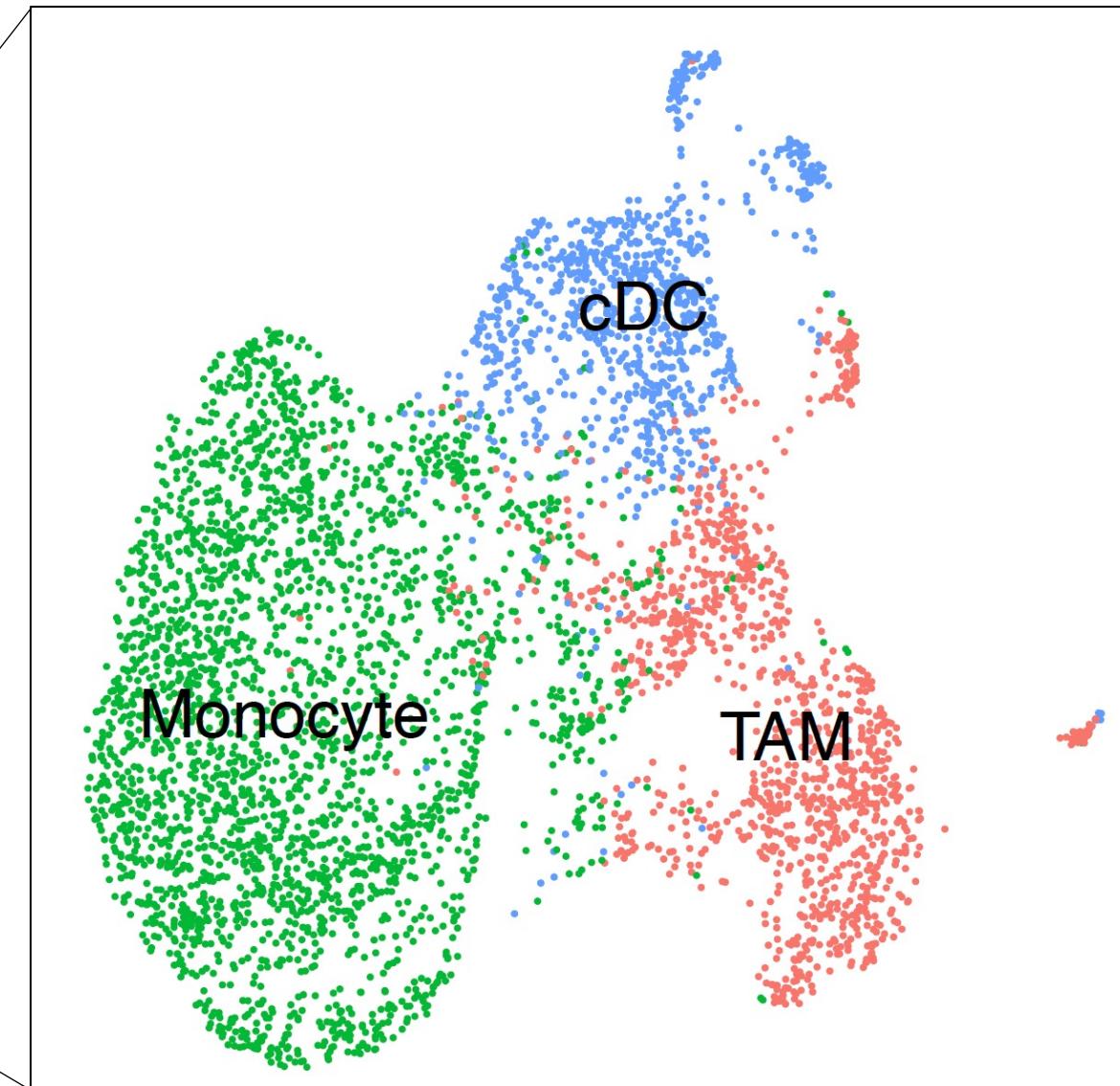
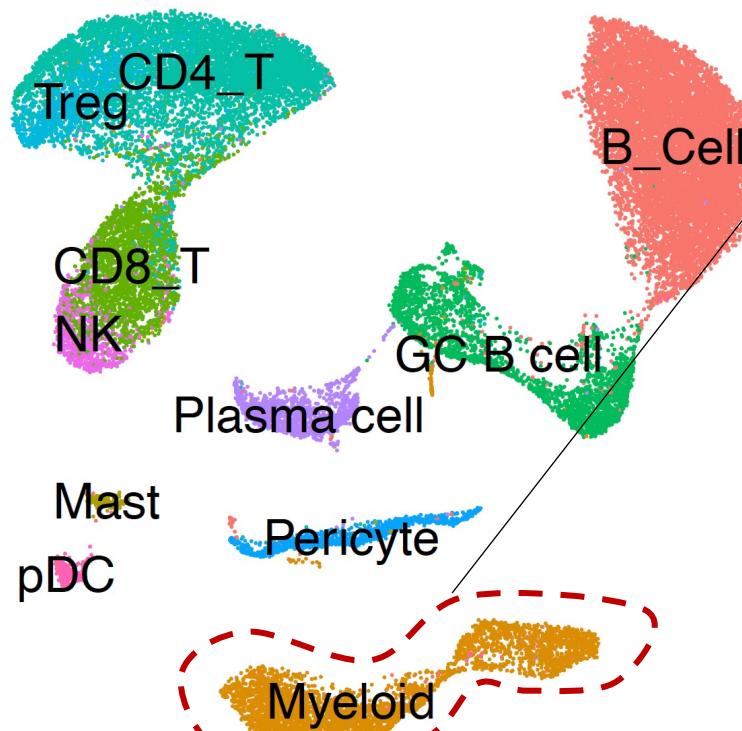
Why care single cells?



1. Single cells can identify and characterize cell types

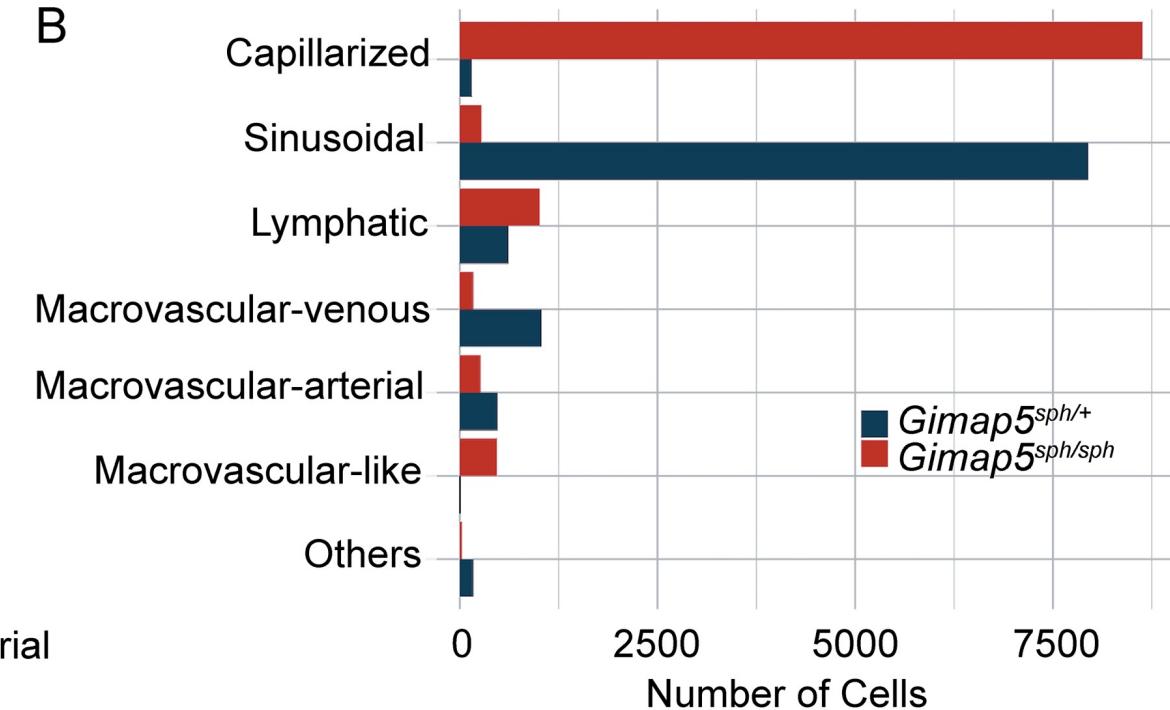
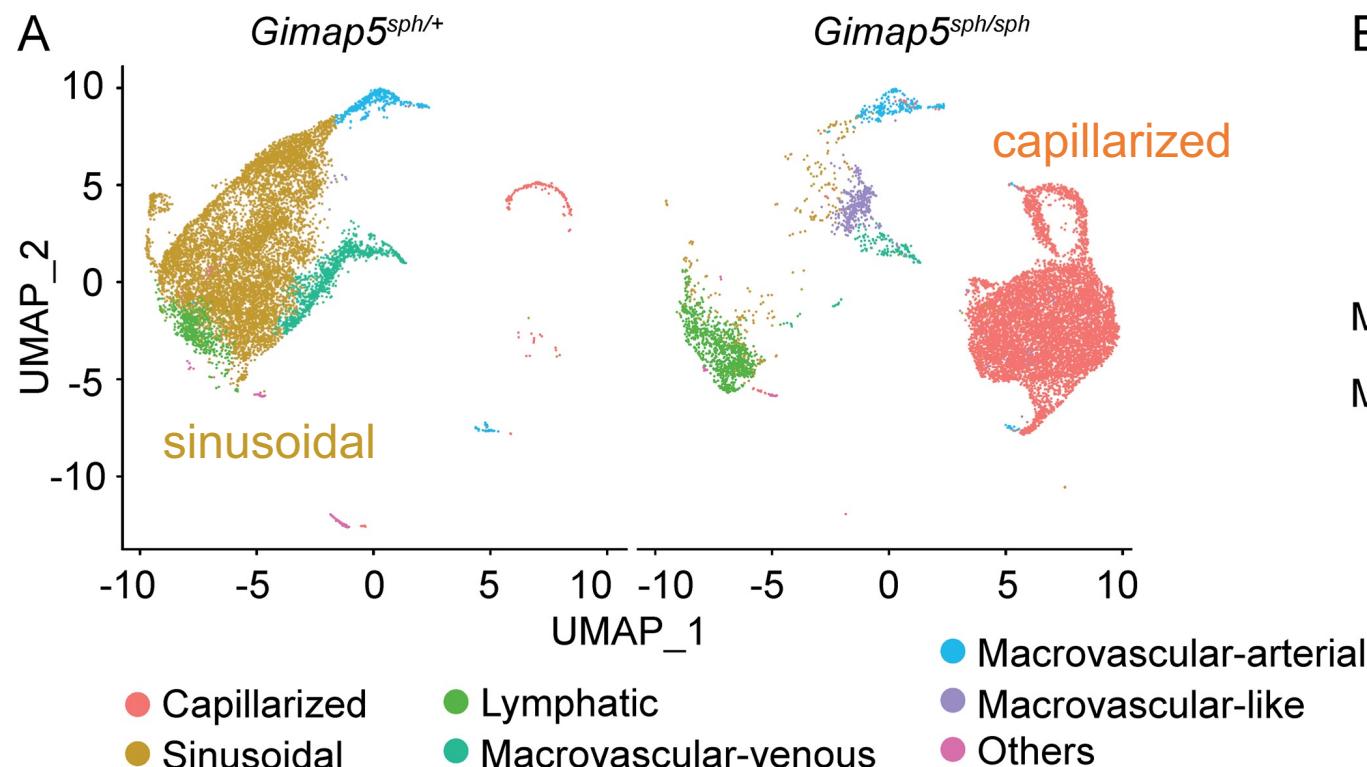


1. Cellular heterogeneity can be even further explored



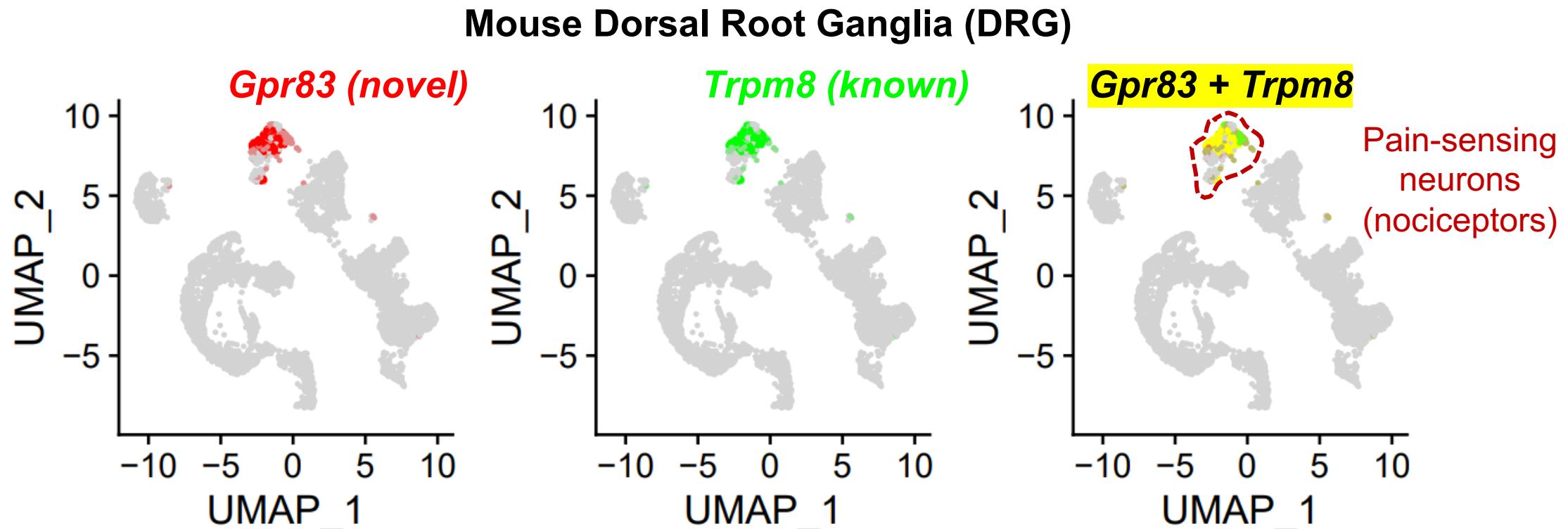
2. Single-cell data can elucidate the cellular composition and dynamics of cell state changes

Mouse endothelial cells

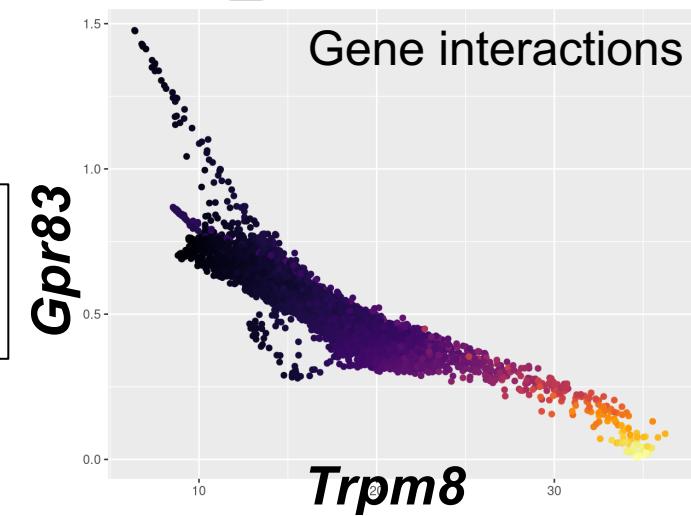


The homozygous deletion of *Gimap5* leads to the accumulation of a specific type of endothelial cells, resulting in portal hypertension

3. Single cells can identify strongly associated genes

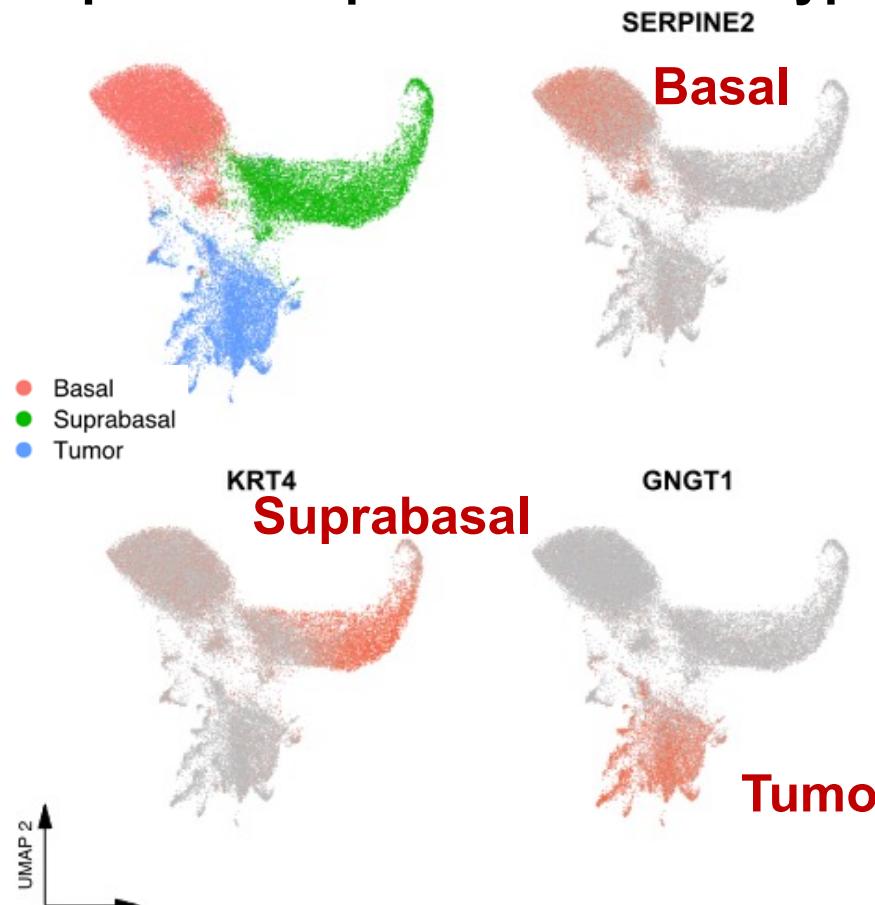


The exclusive interaction of *Grp83* and *Trpm8* in pain-sensing neurons implicates their crucial roles

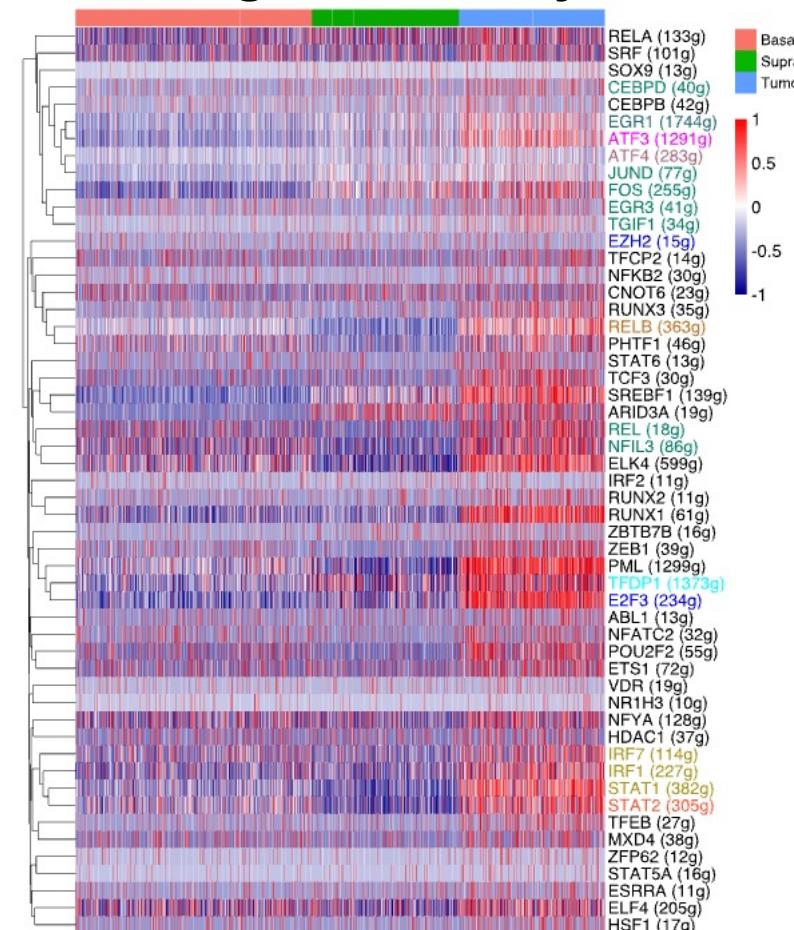


4. Single cells provide insights into gene regulatory networks and the effects of specific genes on cellular function

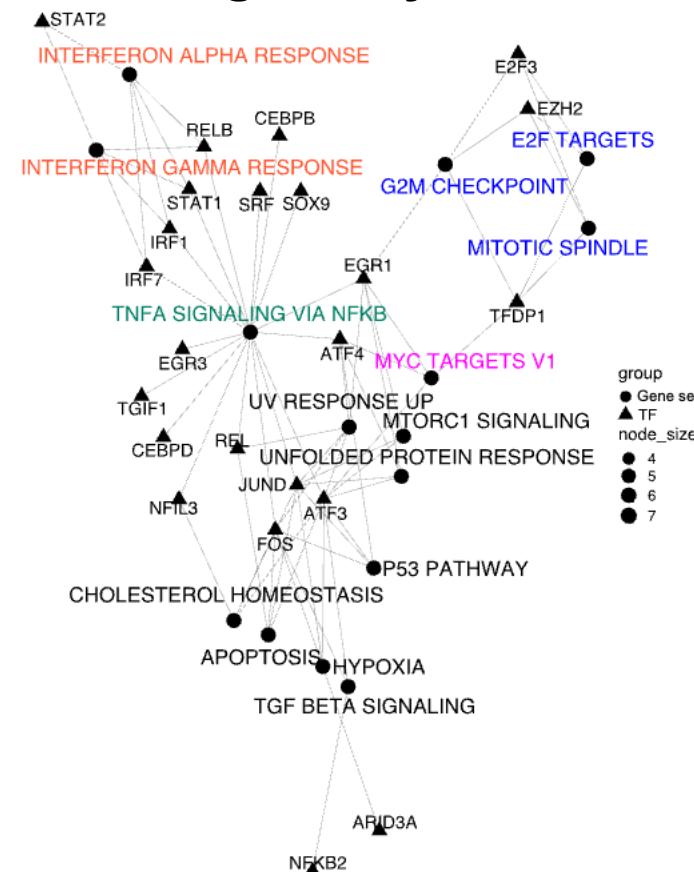
Squamous epithelial sub-cell types



Regulon Activity



Gene Regulatory Network

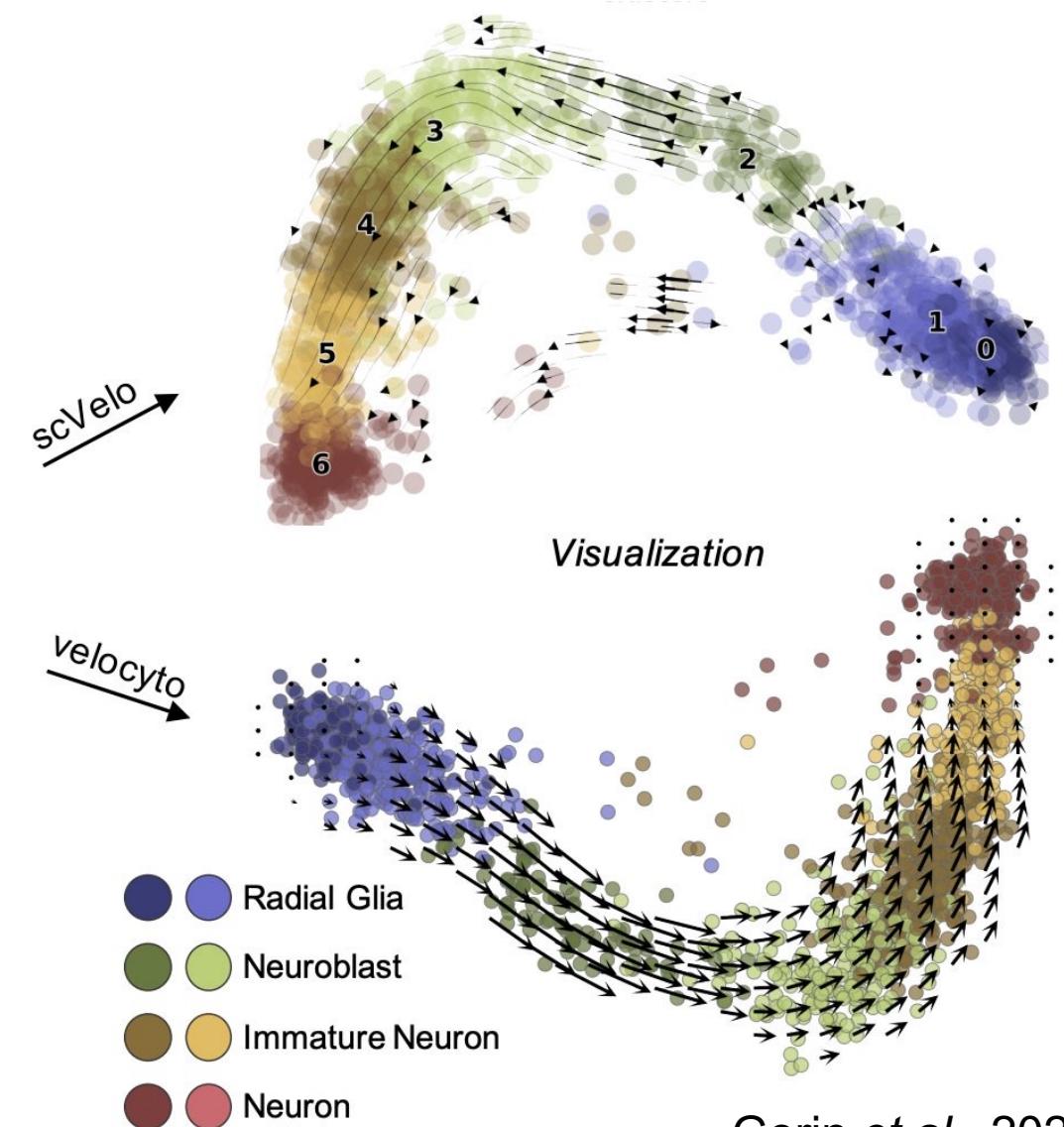
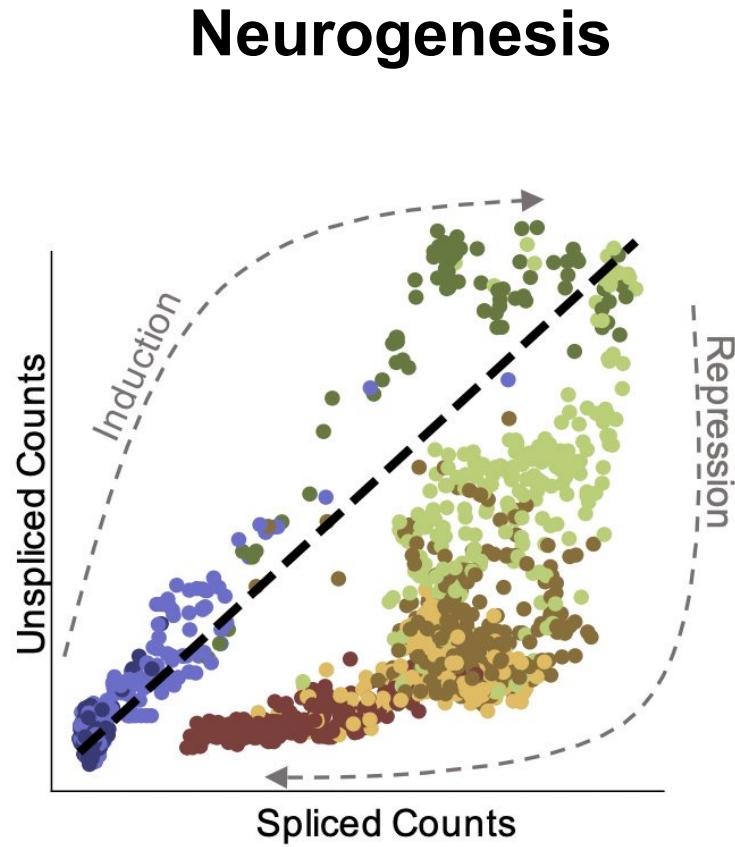


unpublished

Tumors exhibit activated TNF α , INF γ , and Cell Cycle gene sets compared to Basal and Suprabasal Cells

5. Single-cell data can predict a continuum from static snapshots of cell state

A sequential trajectory
of cliff diving



Single cell genomics was pioneered by James Eberwine (Upenn) in the early 1990

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1663–1667, March 1990
Biochemistry

Amplified RNA synthesized from limited quantities of heterogeneous cDNA

(cerebellum/guanine nucleotide-binding protein/T7 RNA polymerase/Purkinje cell)

RUSSELL N. VAN GELDER*,†, MARK E. VON ZASTROW‡, ANDREA YOOL§, WILLIAM C. DEMENT*,
JACK D. BARCHAS‡, AND JAMES H. EBERWINE‡¶

*Nancy Pritzker Laboratory of Behavioral Neurochemistry, *Sleep Research Laboratory, Department of Psychiatry, and §Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305

Communicated by Seymour Benzer, November 13, 1989

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 3010–3014, April 1992
Neurobiology

Analysis of gene expression in single live neurons

(amplified, antisense RNA/expression profile/mRNA complexity/pyramidal cell)

JAMES EBERWINE*†‡, HERMES YEH§, KEVIN MIYASHIRO*, YANXIANG CAO*, SURESH NAIR*,
RICHARD FINNELL*¶, MARTHA ZETTEL§, AND PAUL COLEMAN§

Departments of *Pharmacology and †Psychiatry, University of Pennsylvania Medical School, Philadelphia, PA 19104; and Department of §Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642

Communicated by George Koelle, December 2, 1991



The very first CSHL Single Cell Workshop in 2009 organized by James Eberwine

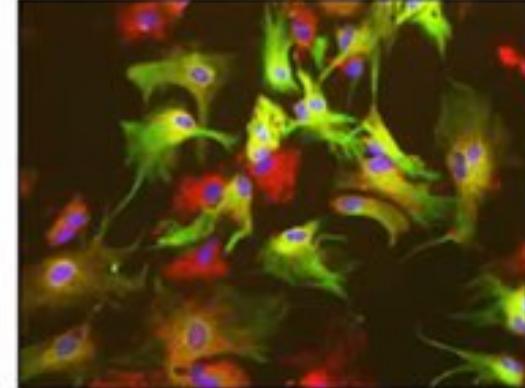


WELCOME

WORKSHOP

ON SINGLE CELL TECHNIQUES

JULY 16-19, 2009



PLIMPTON CONFERENCE ROOM
BECKMAN BUILDING
COLD SPRING HARBOR LABORATORY

A three-day workshop with leaders in the field, junior faculty, postdoctoral fellows and graduate students to discuss new, cutting-edge developments and techniques in single cell analysis in bacterial, yeast, plant and animal systems.

Workshop on Single Cell Techniques

July 16 - 19, 2009

Abstract Deadline: April 17, 2009

Organizers:

James Eberwine, University of Pennsylvania Medical School

Xiaoliang Sunney Xie, Harvard University

Typical Program

We are pleased to announce a new summer **Workshop on Single Cell Techniques**, which will be held at Cold Spring Harbor Laboratory, New York. The meeting will begin at 7:30 p.m. on Thursday, July 16, and will conclude with lunch on Sunday, July 19, 2009. The proposed meeting will assemble the leaders in the field, together with junior faculty, postdoctoral fellows and graduate students, to discuss new, cutting-edge developments and techniques in single cell analysis in bacterial, yeast, plant and animal systems.

Tentative Topics:

Genomics

Proteomics

Metabolomics

Imaging Single Cells

Discussion Leaders:

Paul Blainey, Stanford University

Norman Dovichi, University of Washington

Scott Fraser, California Institute of Technology

Ralf-Peter Jansen, University of Munich, Germany

Junhyong Kim, University of Pennsylvania

Kaiqin Lao, Applied Biosystems

Carolyn Larabell, National Center for X-Ray Tomography

Charles Lieber, Harvard University

Jennifer Lippincott-Schwartz, National Institutes of Health

Zenobi Renato, Swiss Federal Institute of Technology, Switzerland

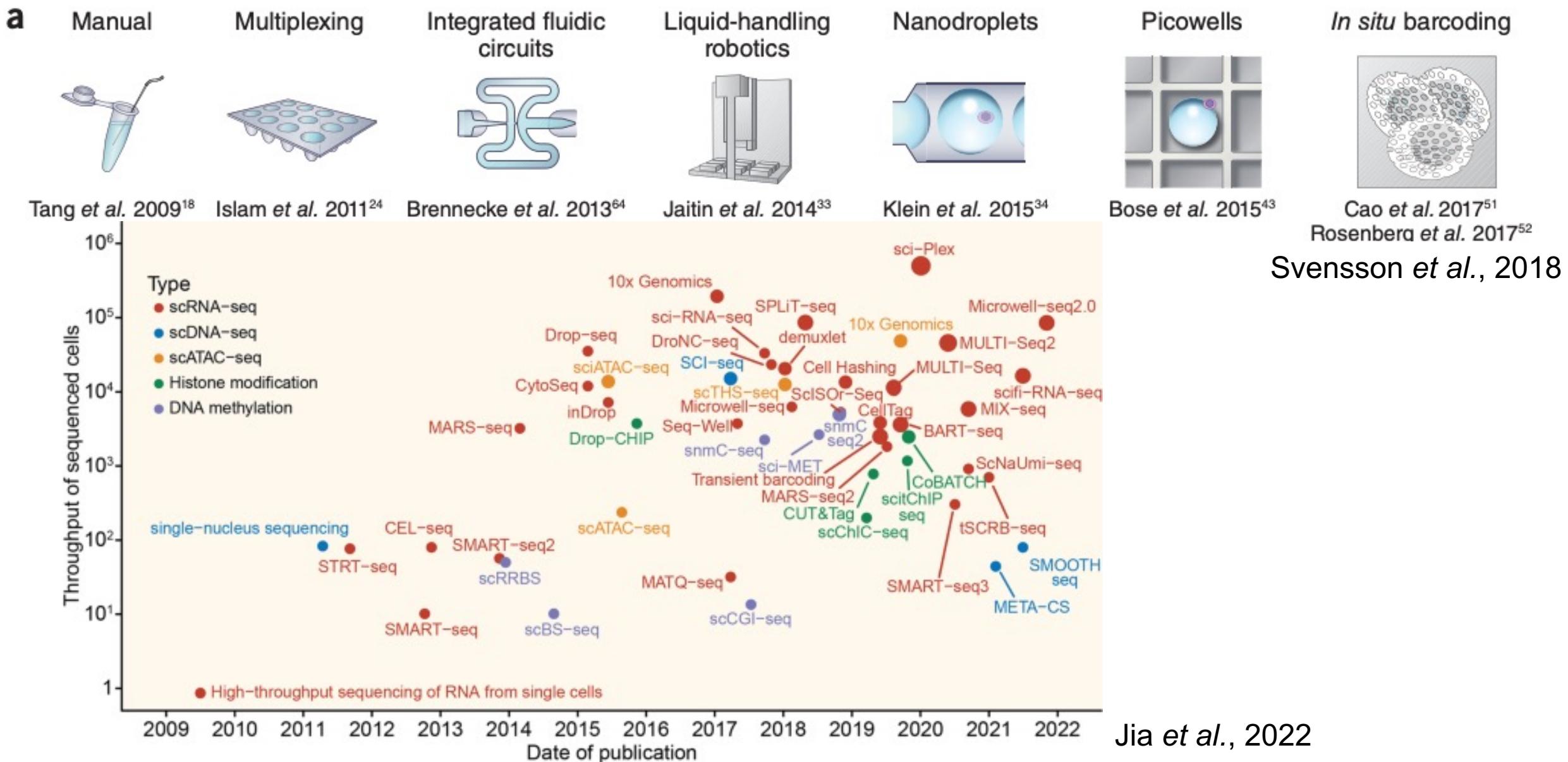
Ben Simons, Cambridge University, UK

Jonathan Sweedler, University of Illinois

Daojing Wang, Lawrence Berkeley National Laboratory

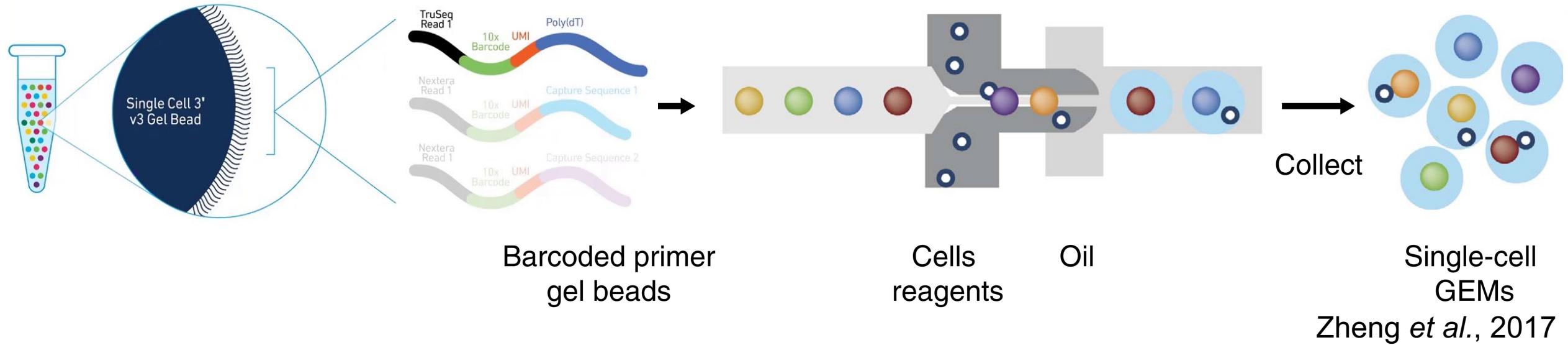
Xiaowei Zhuang, Harvard University

The field of single-cell sequencing technology has experienced exponential growth

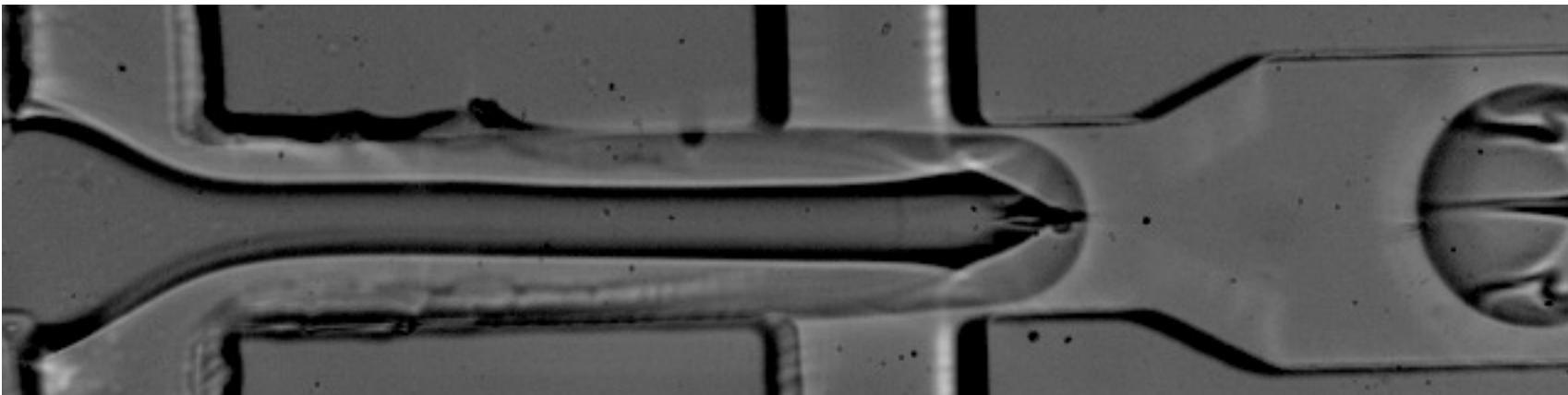


GAME-CHANGER

Microfluidic droplet-based cell isolation

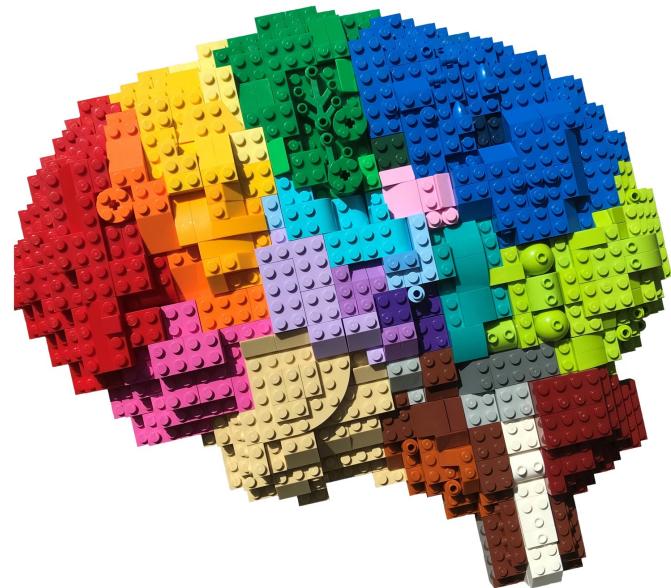


Zheng *et al.*, 2017



A gif image from dropseq.org

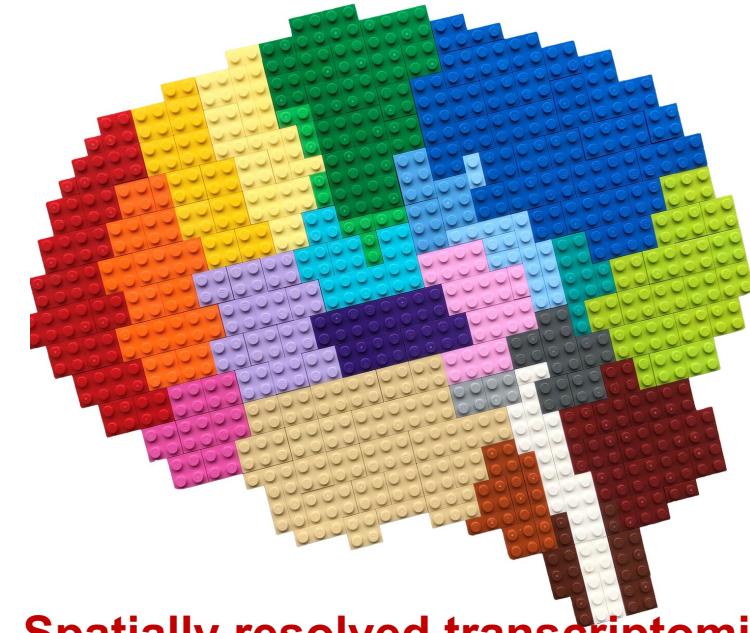
Unfortunately, single-cell transcriptomics lacks spatial information due to cell dissociation



The original brain



single-cell RNA Sequencing



**Spatially resolved transcriptomics
(SRT)**

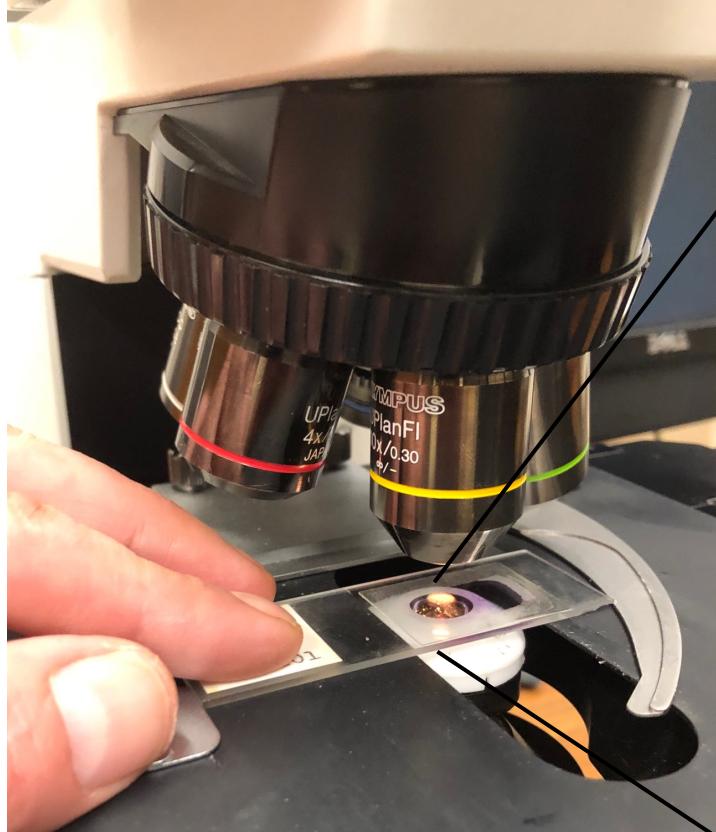
The first law of geography

*Everything is related to everything else,
but near things are more related than distant things*

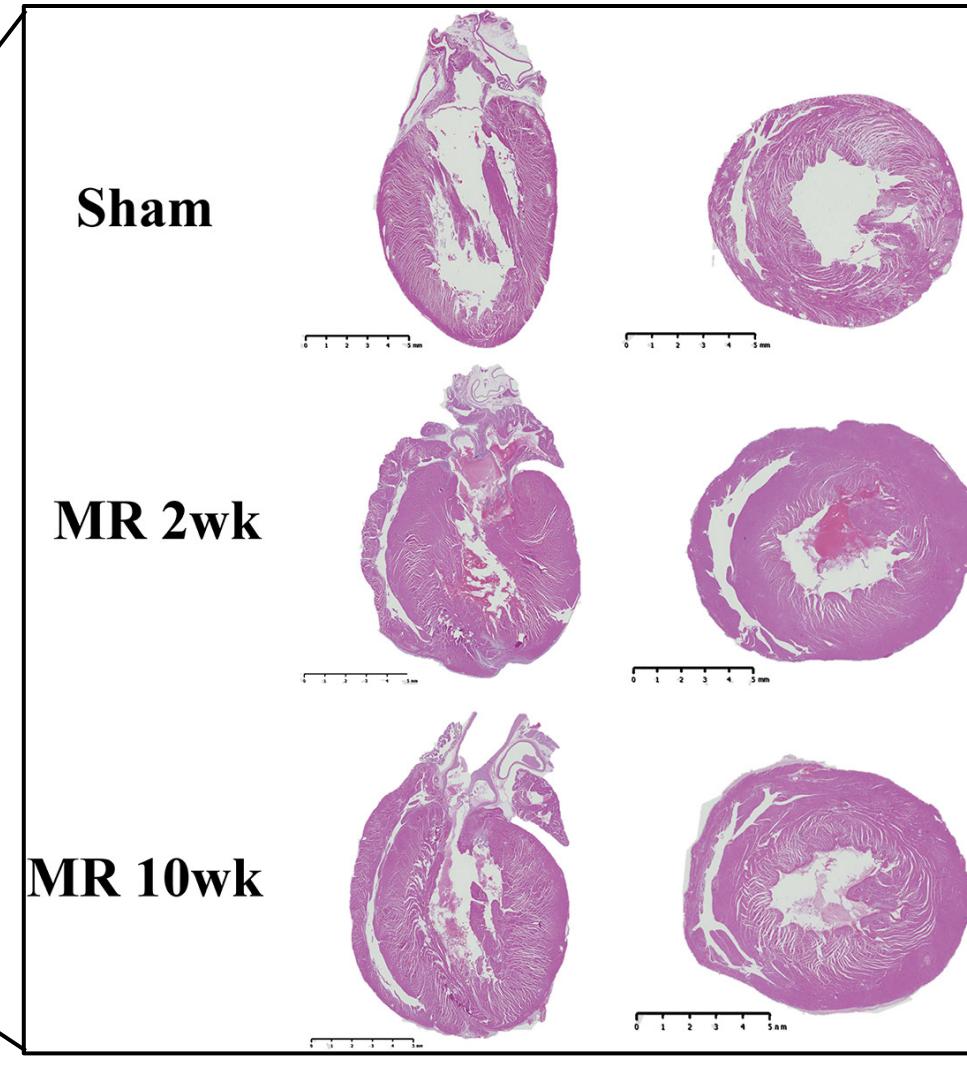
Spatial Analysis is Not New...



The Drawbacks of Conventional Histology Techniques



<https://images.app.goo.gl/1xRUgDgMHkhxQVeI7>

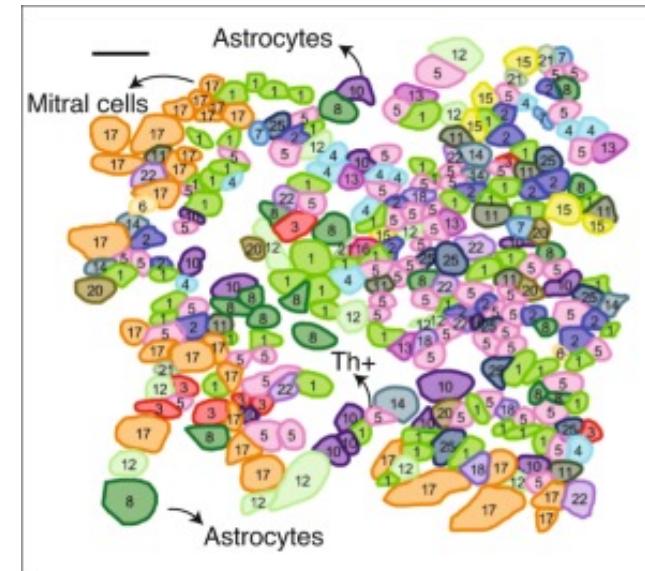
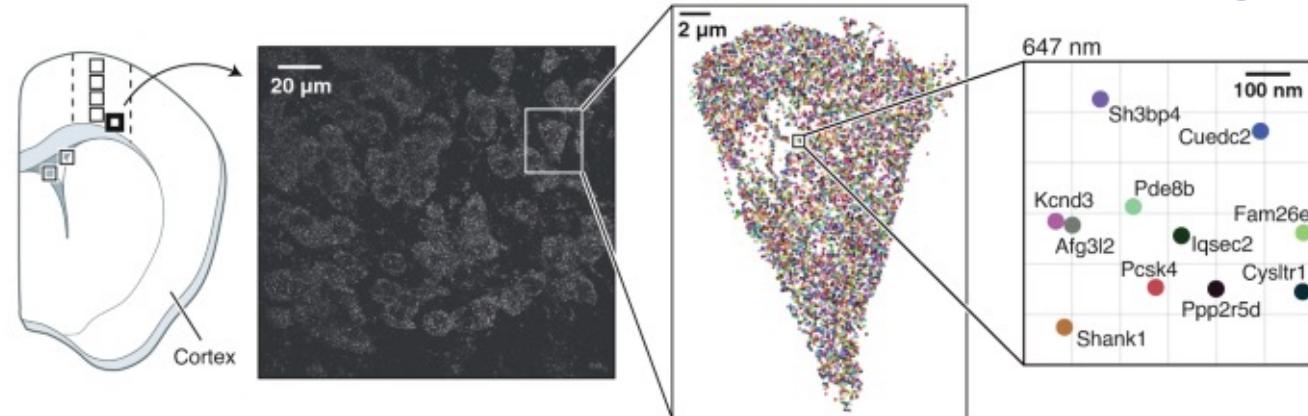
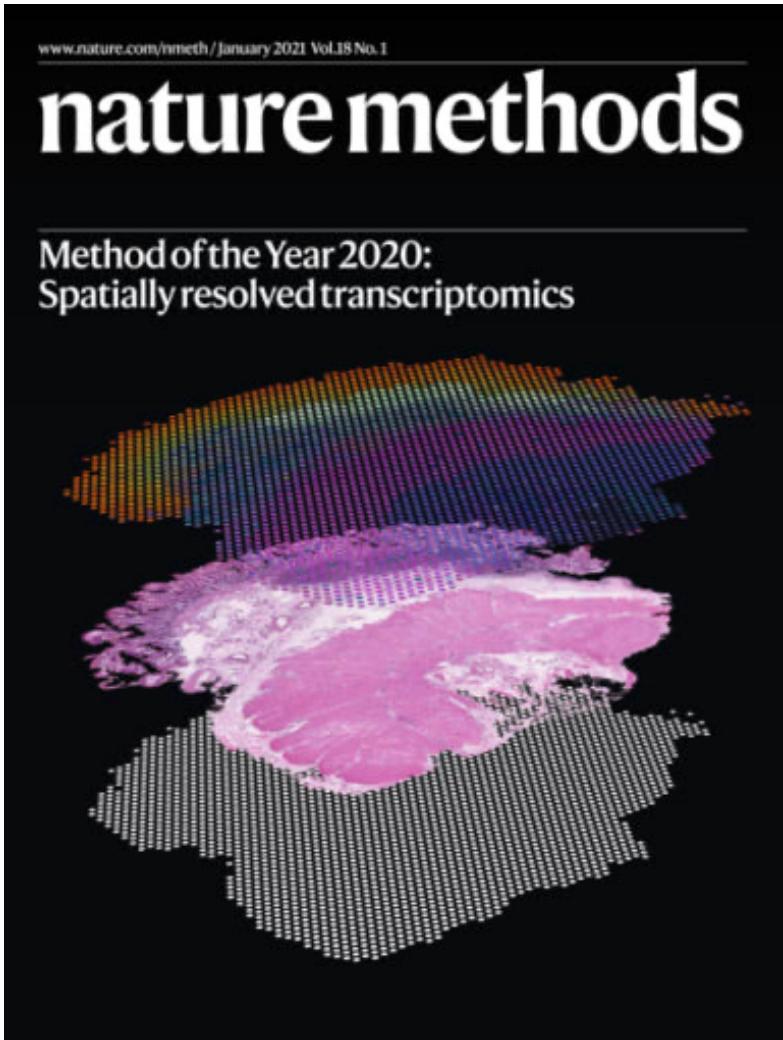


<https://images.app.goo.gl/sPBzLdEAm5uA9qjh6>

- *Low plex*
- *Targeted*
- *Signal*
- *Precision*
- *Qualitative*

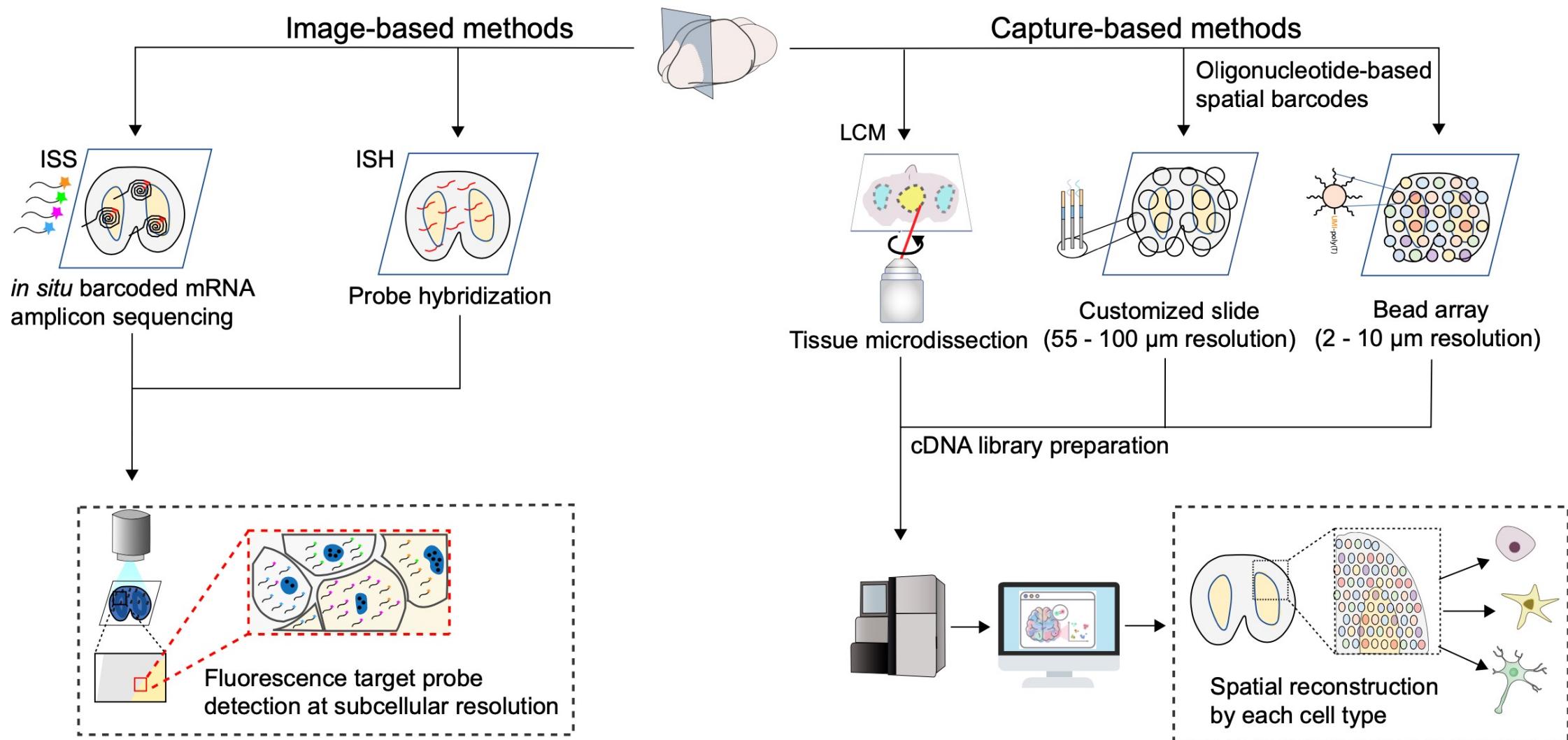
Integrating molecular methodologies can further increase the power of spatial analysis

Spatially Resolved Transcriptomics (SRT) unravels the spatial organization of gene expression within tissues and organ

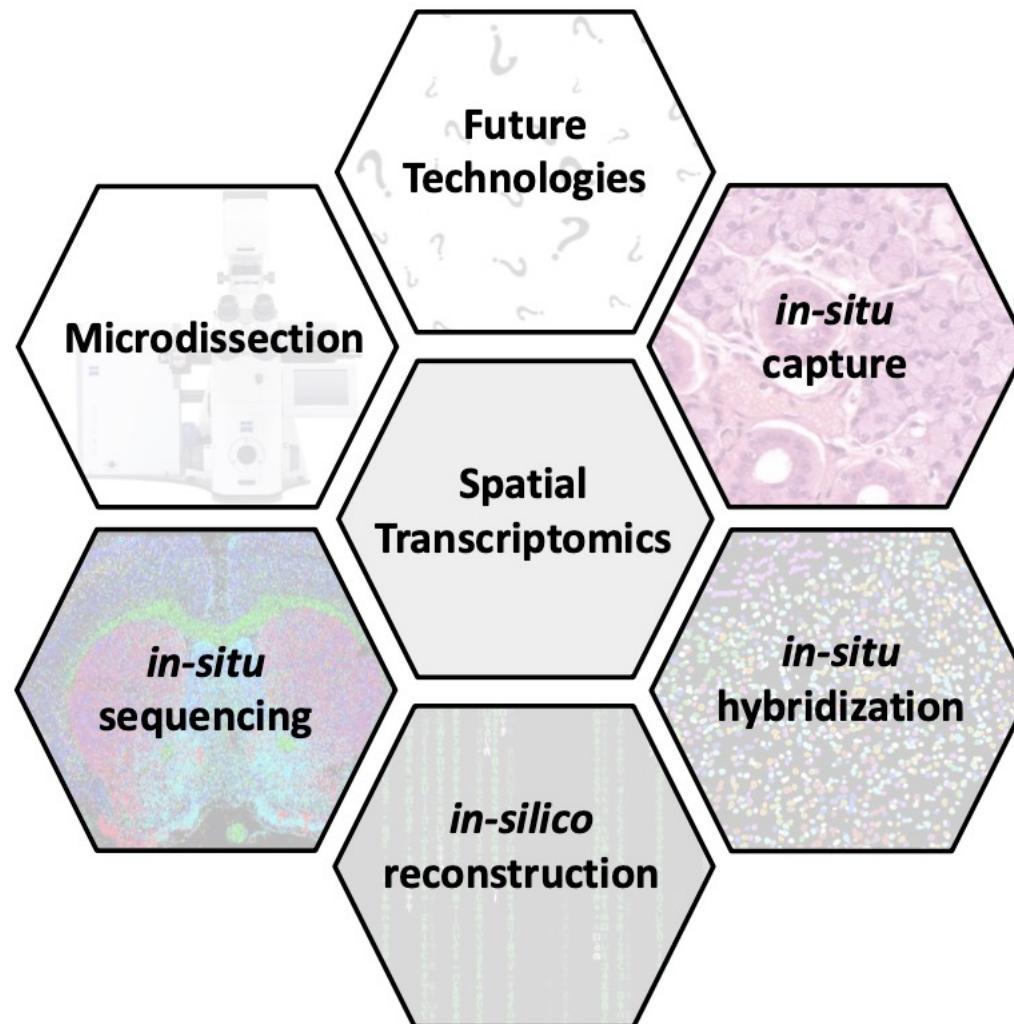


SRT provides valuable insights into the biological processes that underlie development, disease, and homeostasis

Spatially Resolved Transcriptomics (SRT) methods can be broadly classified into two categories



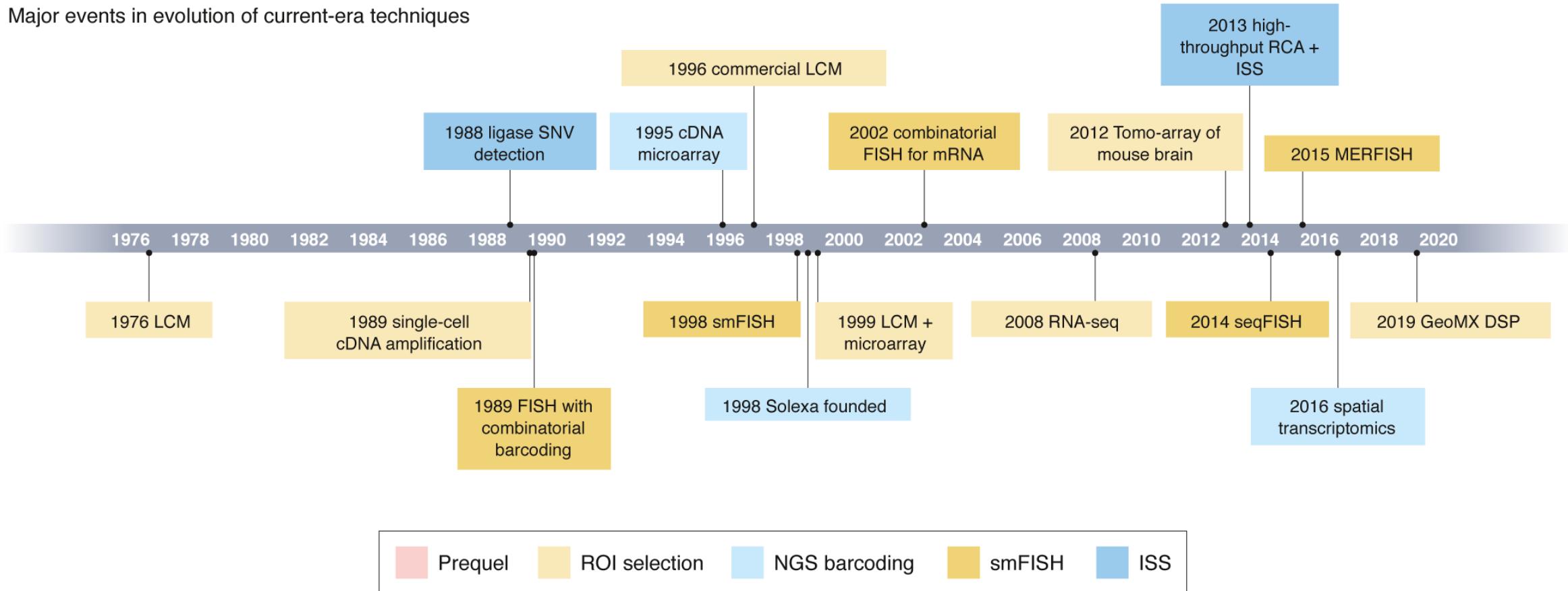
Various experimental spatially resolved techniques



Asp et al., 2020

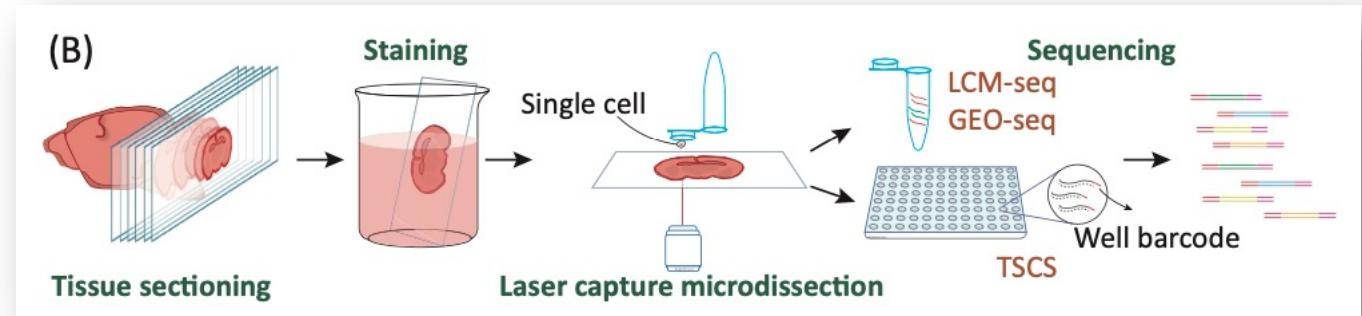
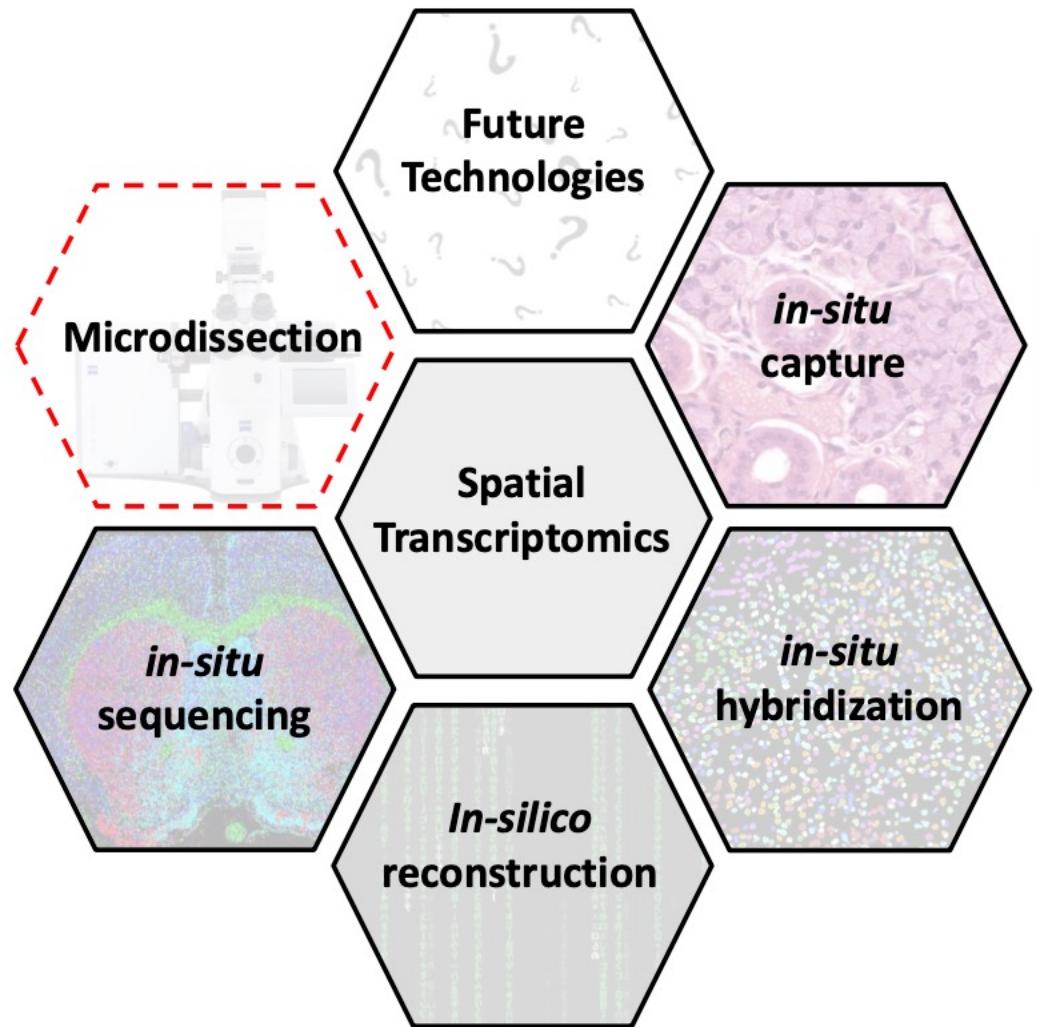
Timelines of major events

c Major events in evolution of current-era techniques



Moses et al., 2022

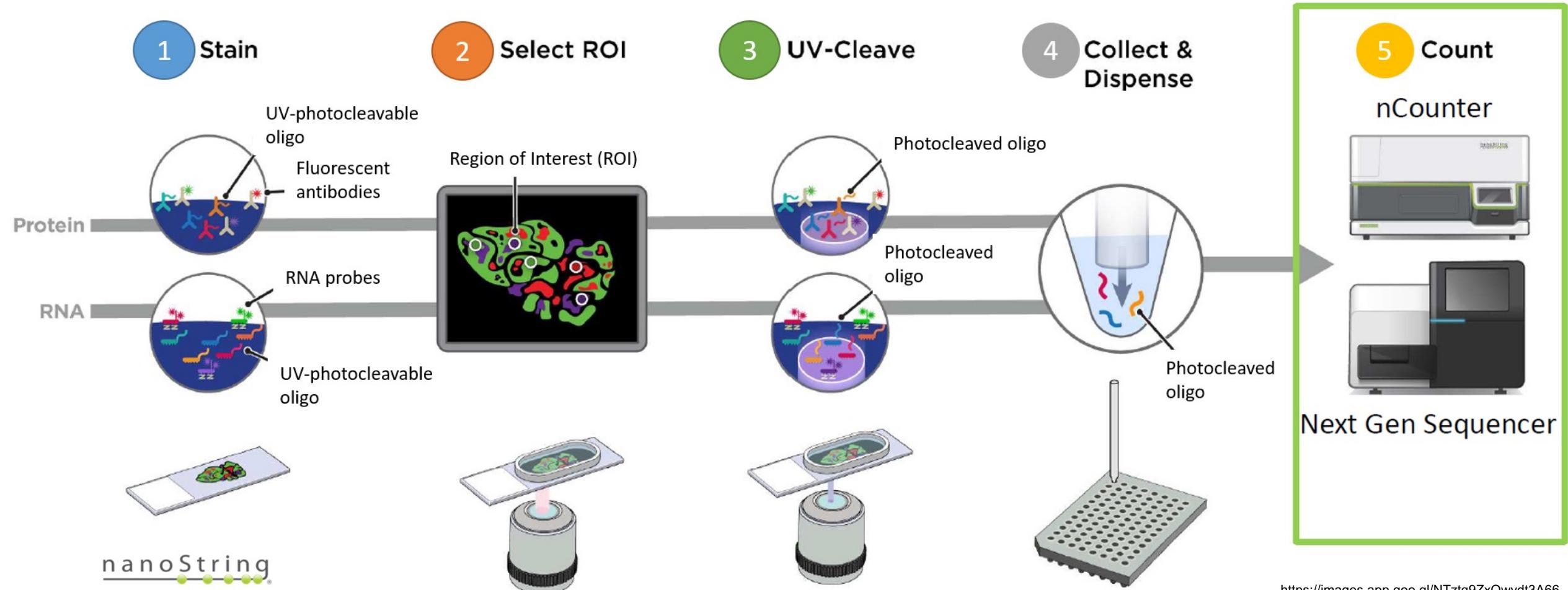
1. Microdissection-based technology



Liao et al., 2021

- Dissect a region of interest (ROI), place isolates in separate wells, and sequence (bulk or single cell)
- Examples:
 1. Physical dissection - Laser Capture Microdissection (LCM), Tomo-seq (bulk), GEO-seq (single cell) and STRP-seq
 2. Optical marking - NICHE-seq, ZipSeq and **GeoMx DSP**

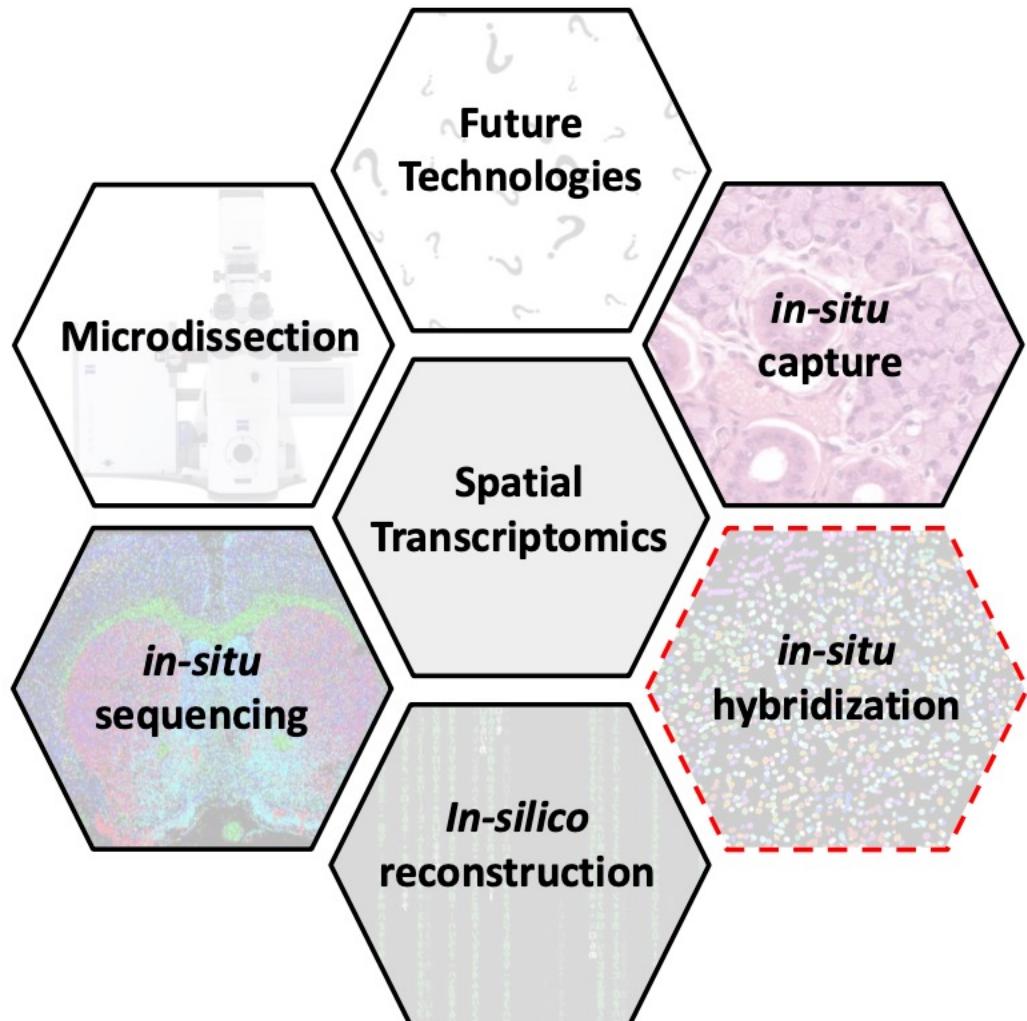
1. Modern microdissection-based technology Nanostring's GeoMx Digital Spatial Profiling (DSP)



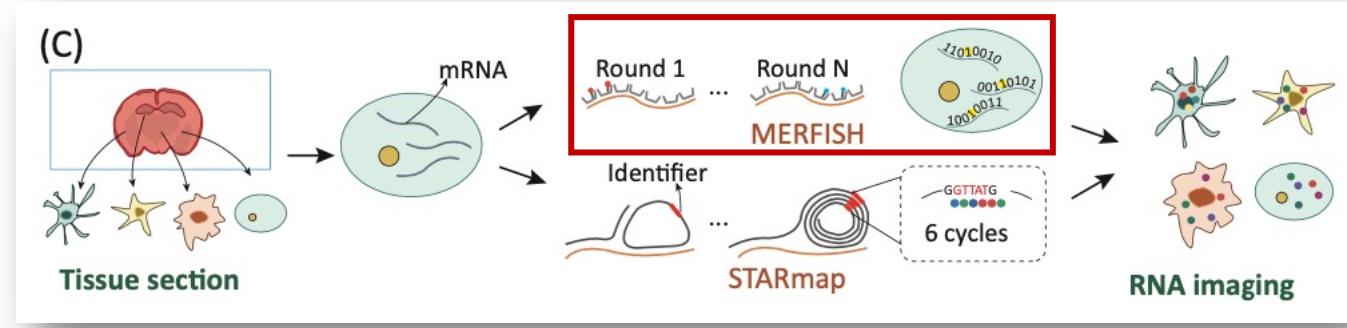
<https://images.app.goo.gl/NTztq9ZxQwydt3A66>

- Nanostring provides gene panel with 1,833 genes and human and mouse whole-transcriptome panels with over 18,000 genes.
- Nanostring also features 90-plex protein assays

2. Image-based: *in situ* hybridization-based technology



Asp et al., 2020



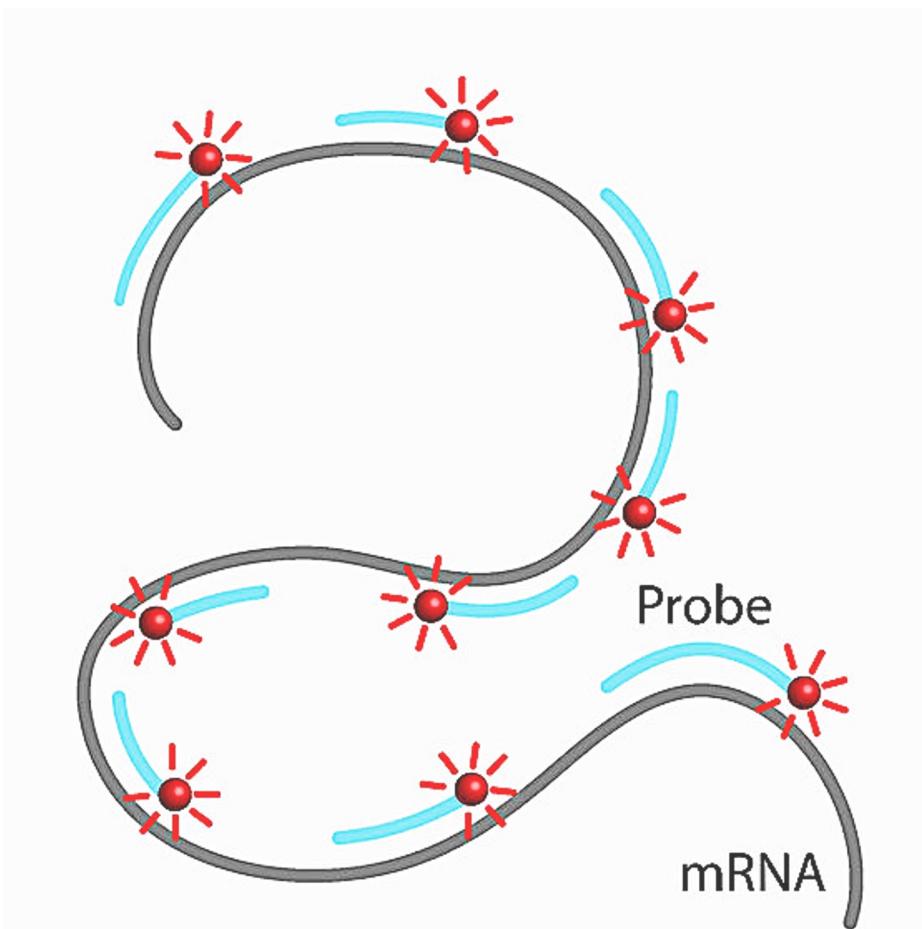
Liao et al., 2021

- Label probes for specific targets, hybridize in place
- Require “*a priori*” defined targets (100~1,000)
- Offer sub-cellular resolution.
- Examples) **smFISH**, **seqFISH**, **MERFISH**, CosMx, seqFISH+, EEL, and RNAscope etc.

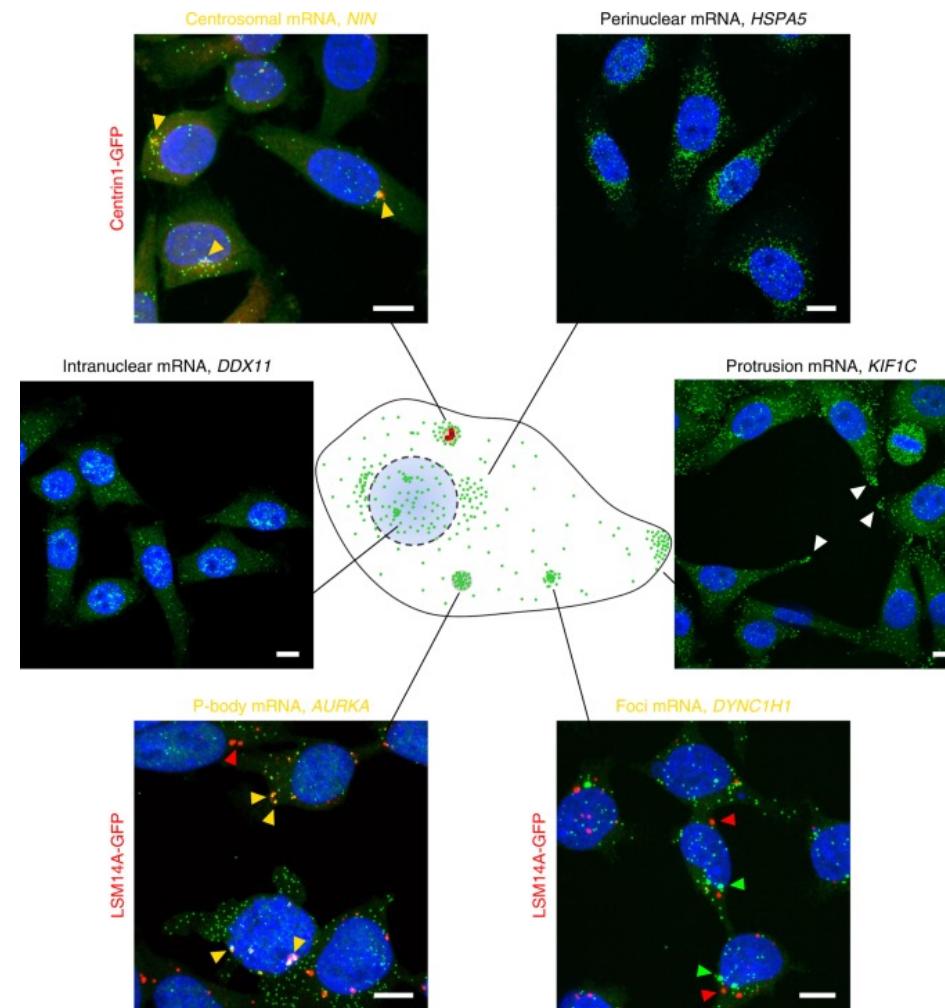
* FISH – Fluorescence *In Situ* Hybridization

2. Image-based: *in situ* hybridization-based technology

single-molecule Fluorescence *In Situ* Hybridization (smFISH)



<https://images.app.goo.gl/NDg9BJmgeQmQZfmR8>



<https://images.app.goo.gl/qCR7fsCc1c6xFtppe9>

smFISH detects low abundance RNAs, distinguishes different RNA isoforms and visualizes RNA localization and RNA dynamics by binding specifically to target.

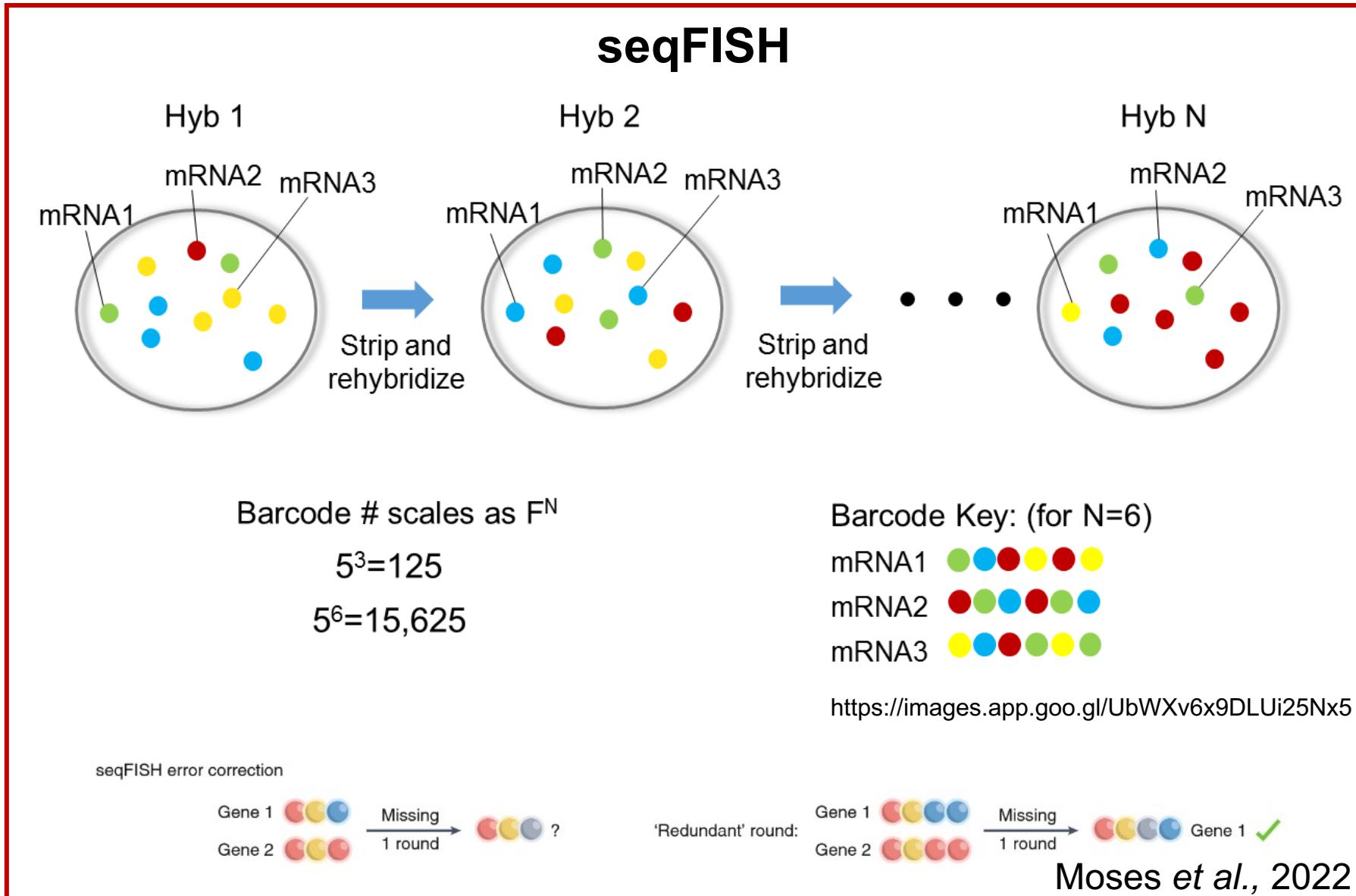
2. Image-based: *in situ* hybridization-based technology sequential Fluorescence In Situ Hybridization (seqFISH)

smFISH



Arora et al., 2021

Single-molecule at a time



2. Image-based: *in situ* hybridization-based technology

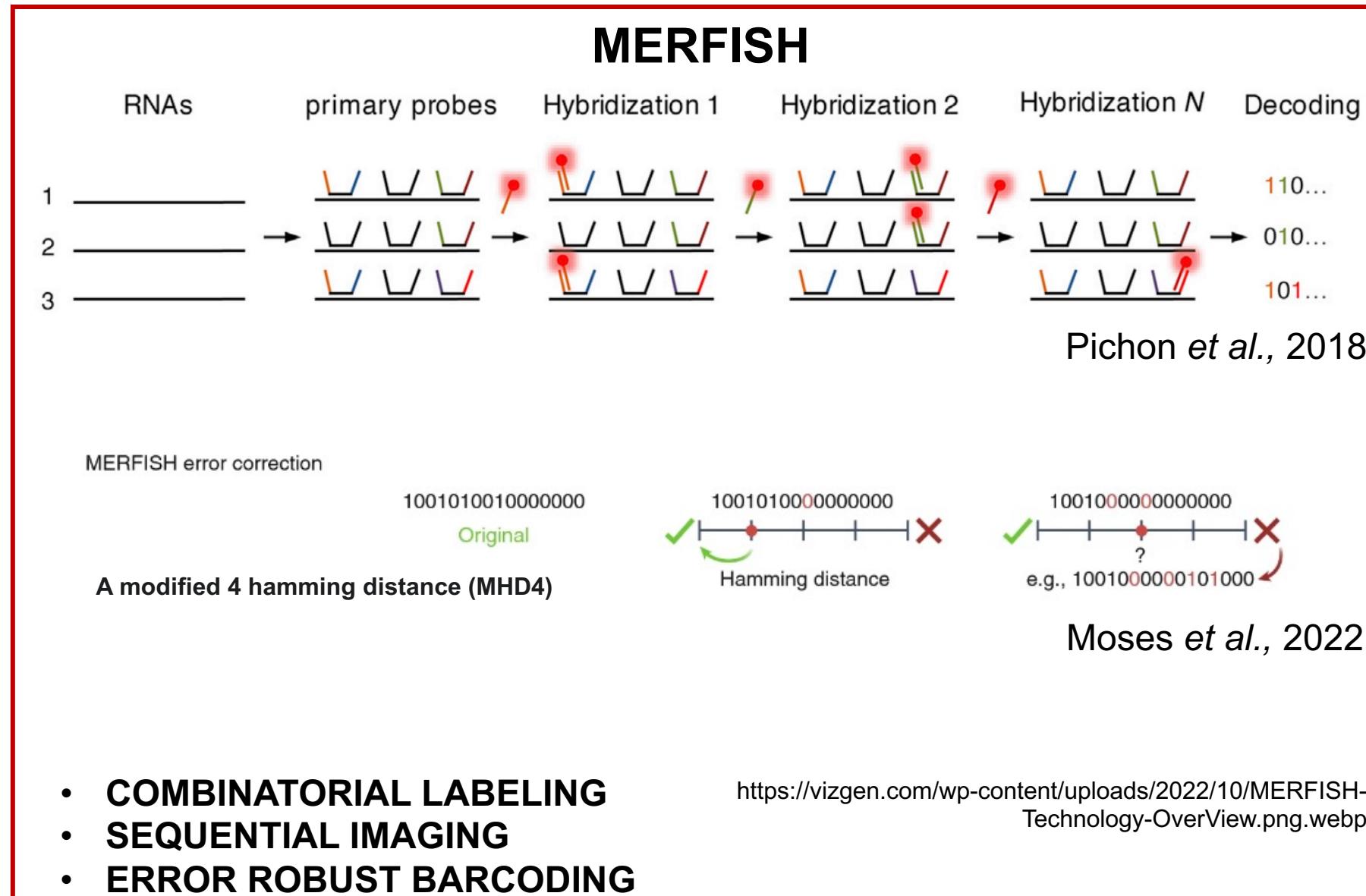
Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH)

smFISH



Arora et al., 2021

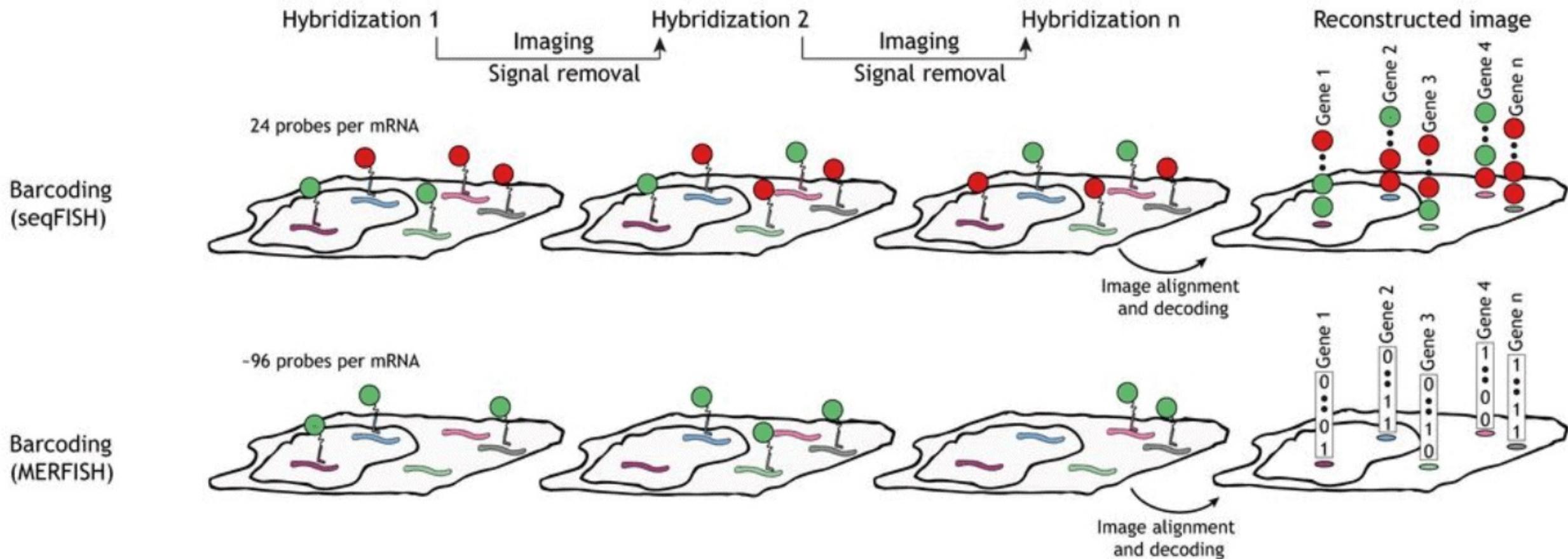
Single-molecule at a time



2. Image-based: *in situ* hybridization-based technology

seqFISH VS MERFISH

Multiplexing transcriptomes

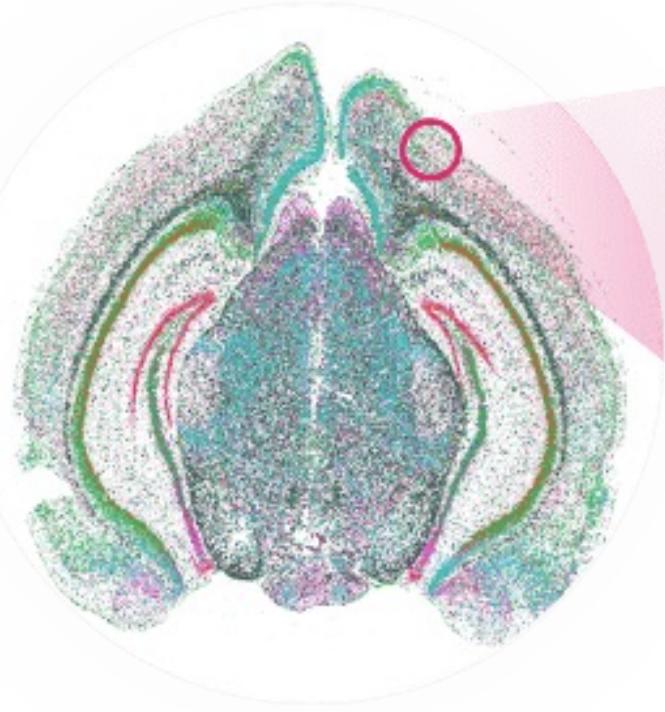


2. Image-based: *in situ* hybridization-based technology

MERFISH is commercially available at Vizgen as MERSCOPE platform

VIZgen

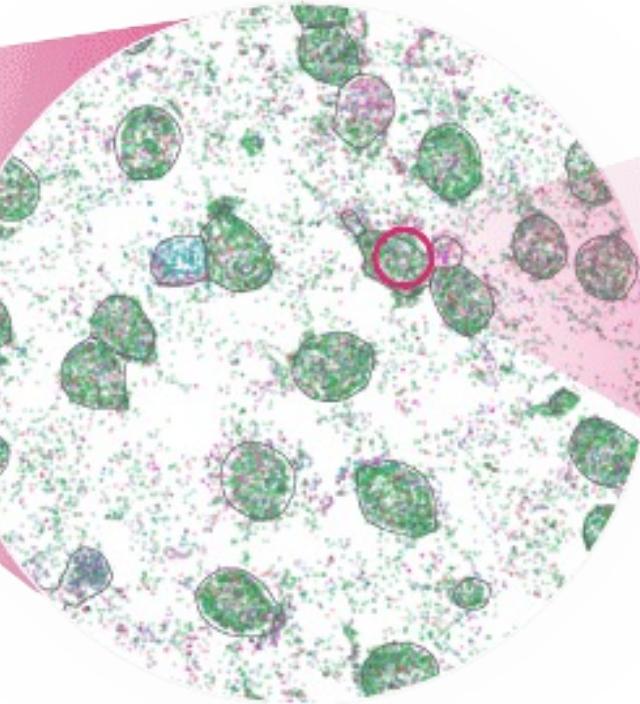
Imaging area:
1x1 cm



WHOLE SECTION

9 x 7 mm

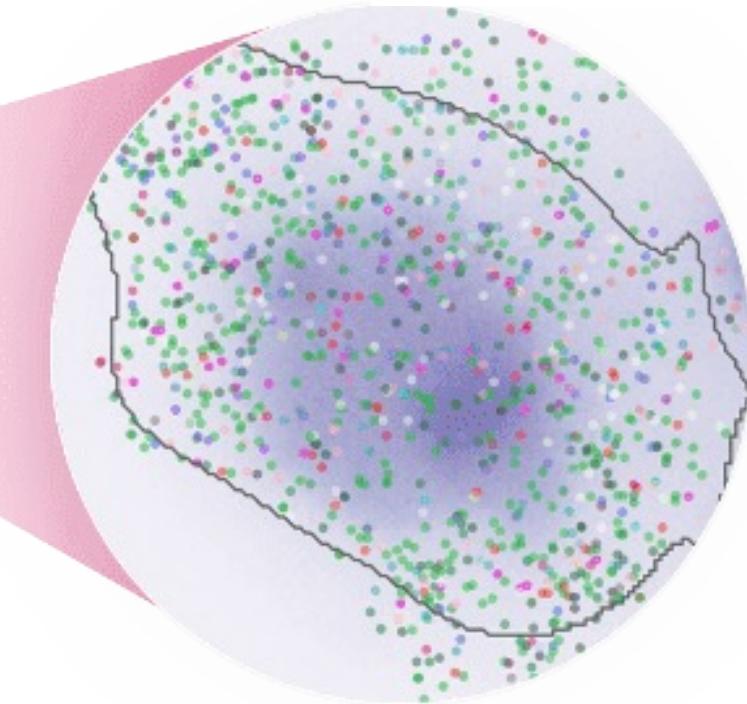
Organization of tissue



WIDE FIELD OF VIEW

200 x 200 micron

Cell interaction/function



SUB-CELLULAR

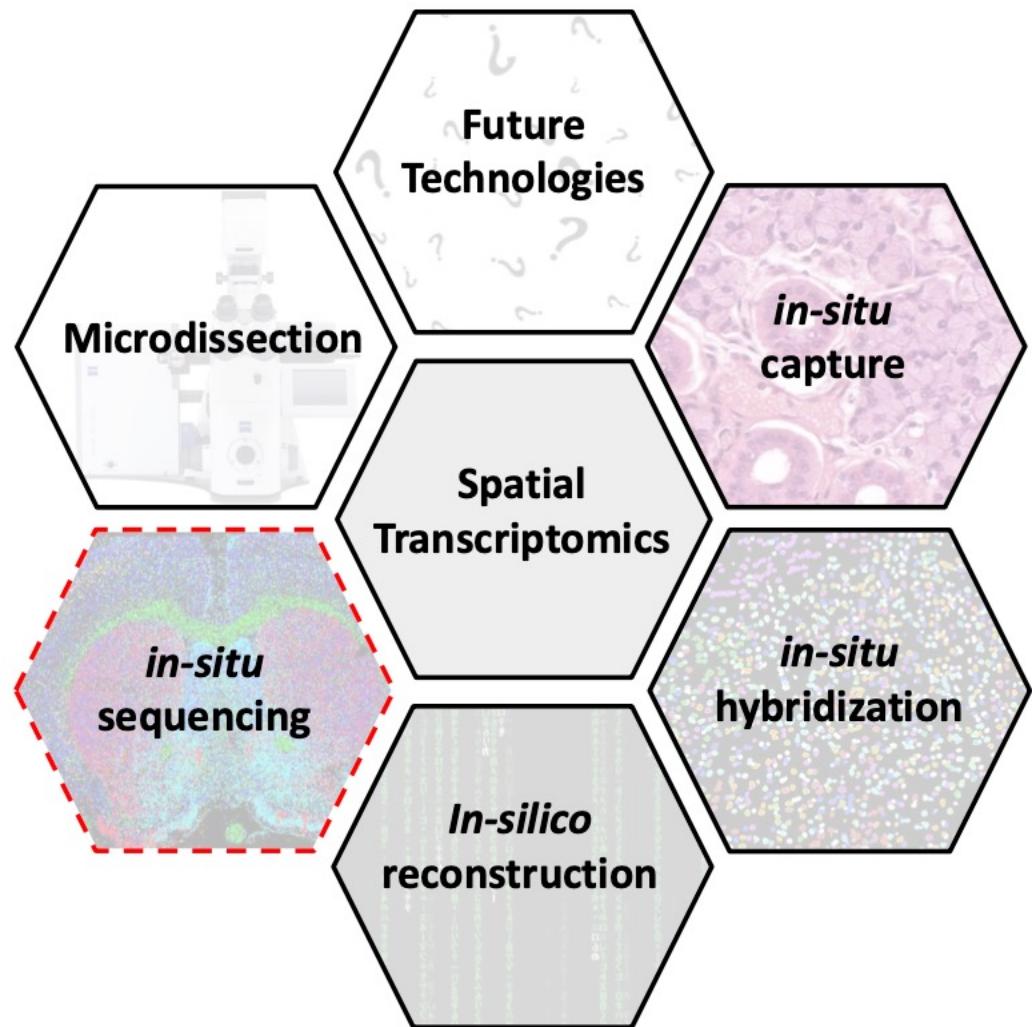
12 x 12 micron

L2/3 IT Glutamatergic neuron

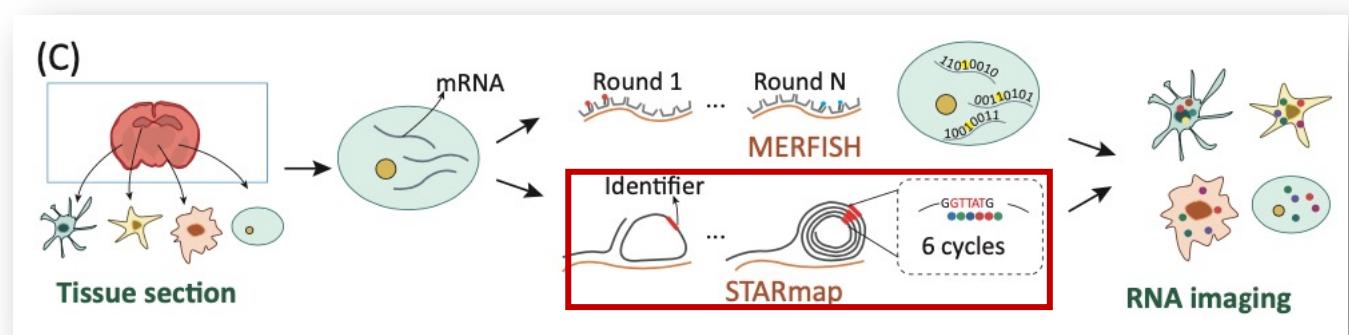
- Vizgen provides gene panel up to 500 genes

<https://images.app.goo.gl/8D3Y413MnsjBBrEdA>

2. Image-based: *in situ* sequencing-based technology



Asp et al., 2020

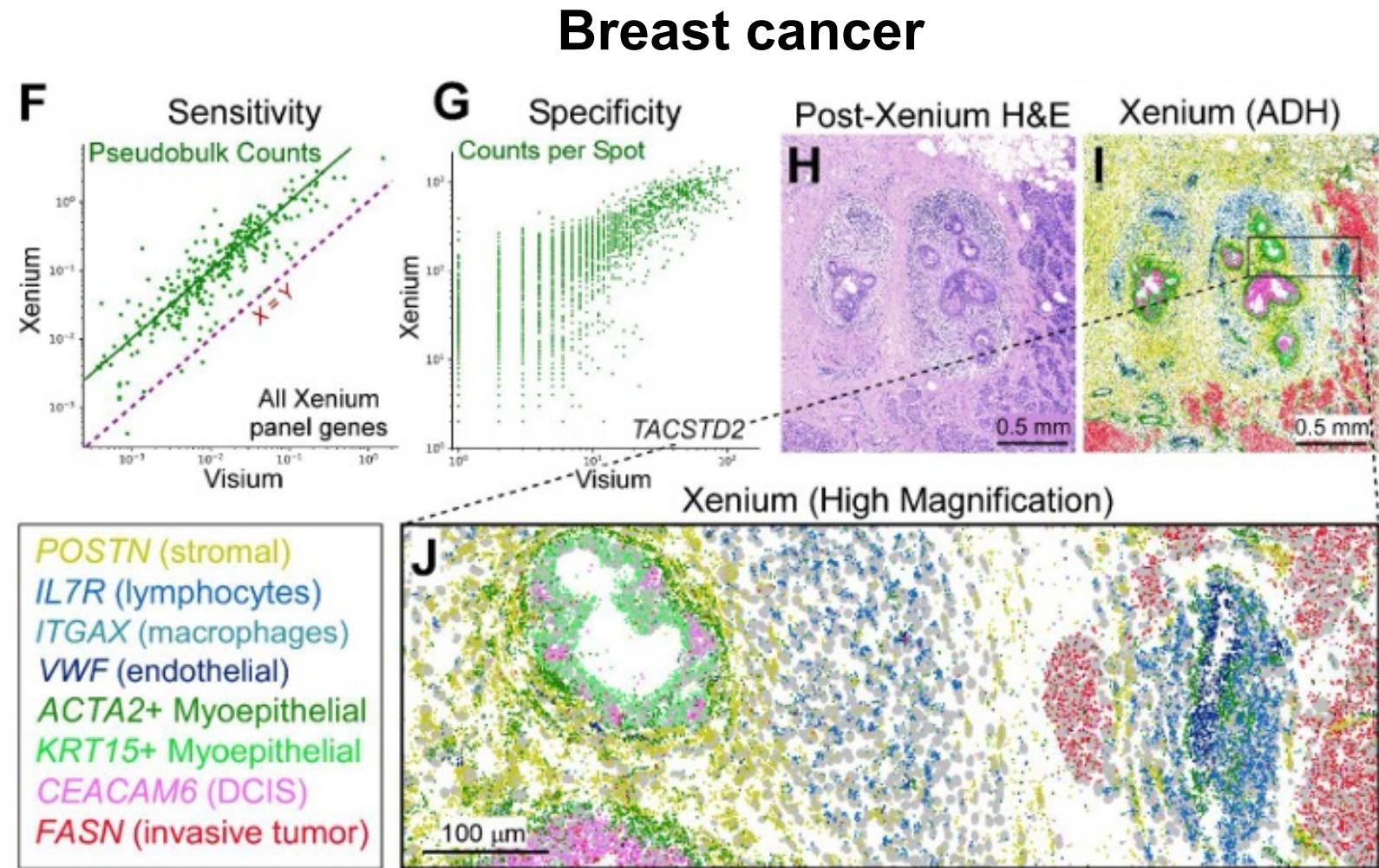
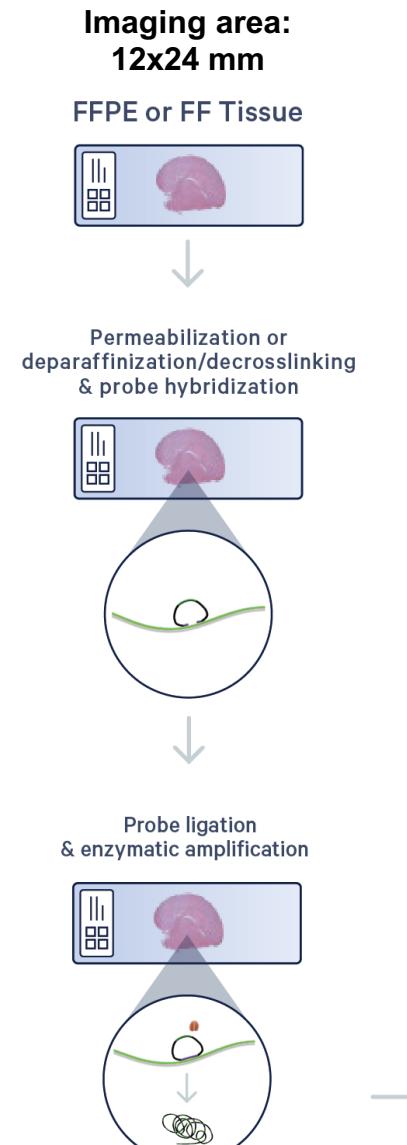


Liao et al., 2021

- Sequence the transcripts in place
- Offer sub-cellular resolution. Some rely on “*a priori*” defined targets
- Examples: ISS/Cartana (**Xenium**), FISSEQ, BaristaSeq, STARmap, and SCRINSHOT

2. Image-based: *in situ* sequencing-based technology

Xenium *in situ*

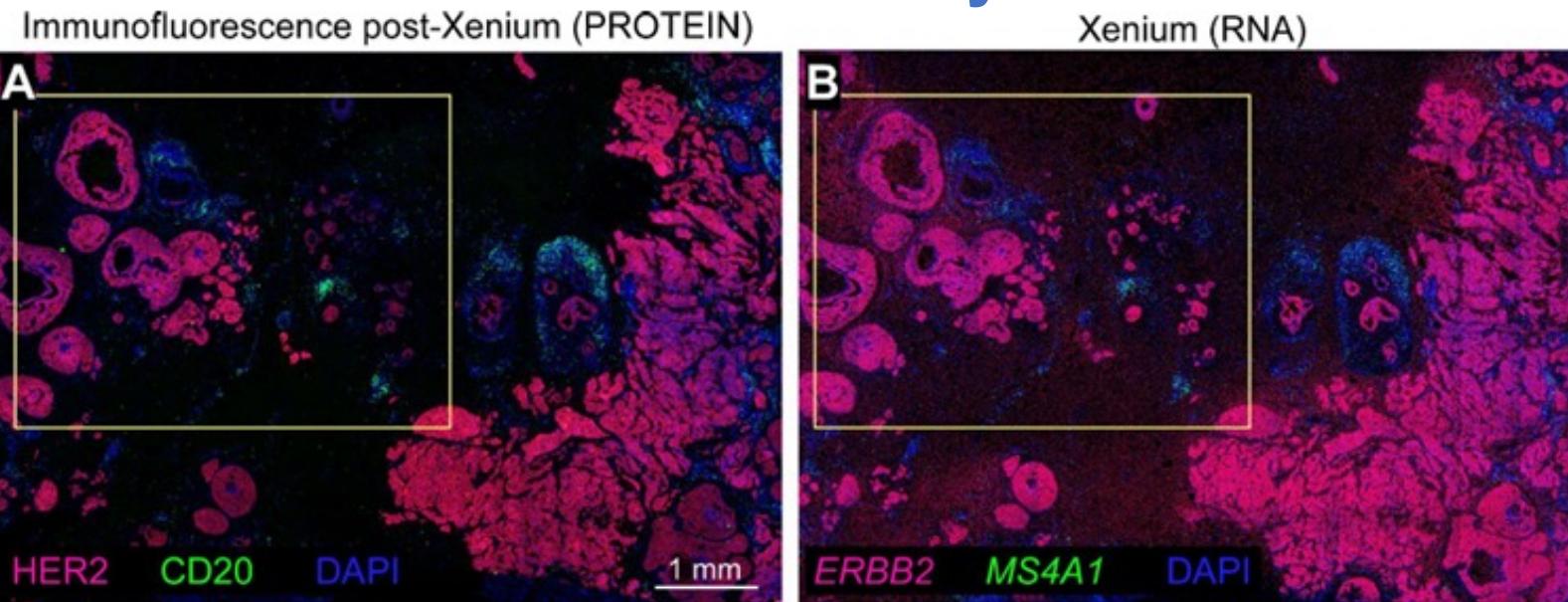


Janesick et al., 2022

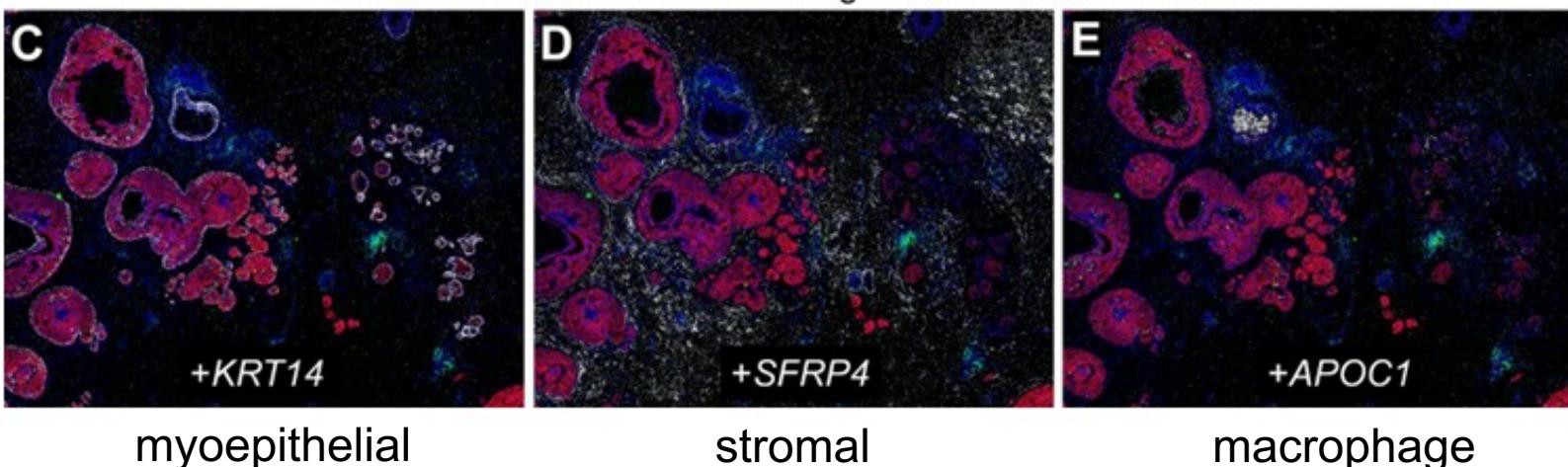
2. Image-based: *in situ* sequencing-based technology

RNA from Xenium and protein immunofluorescence can be visualized simultaneously

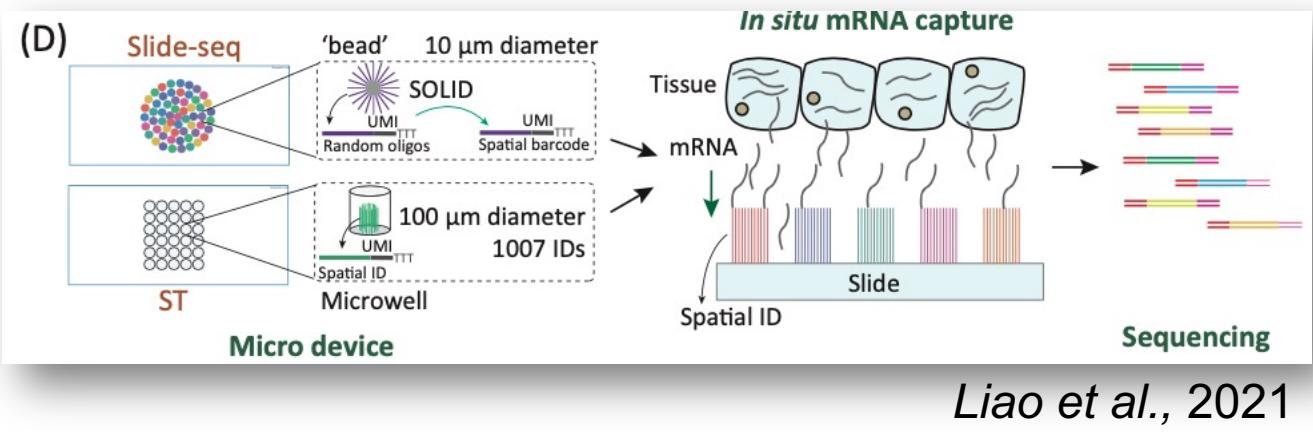
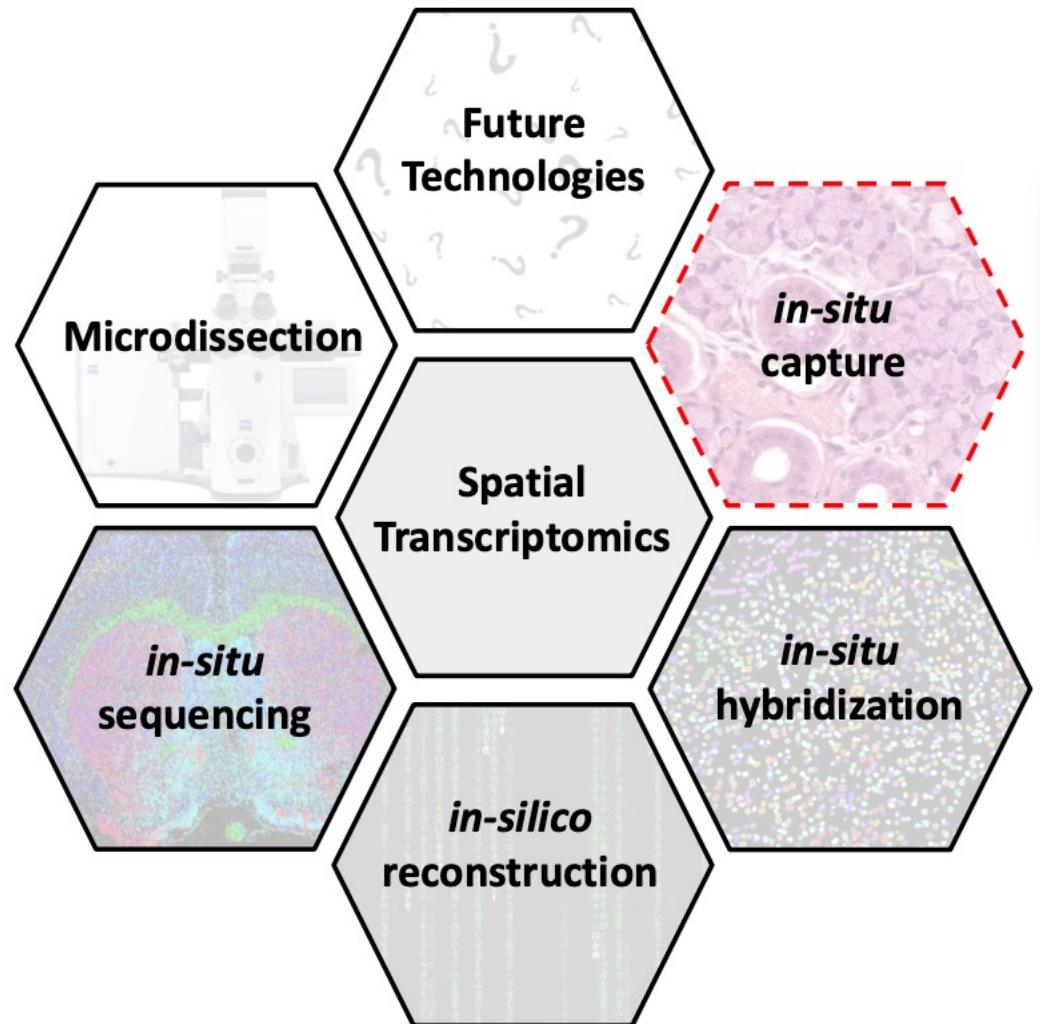
Breast cancer



Immunofluorescence Protein Registered with Xenium RNA



3. Capture-based: *in situ* capture-based technologies

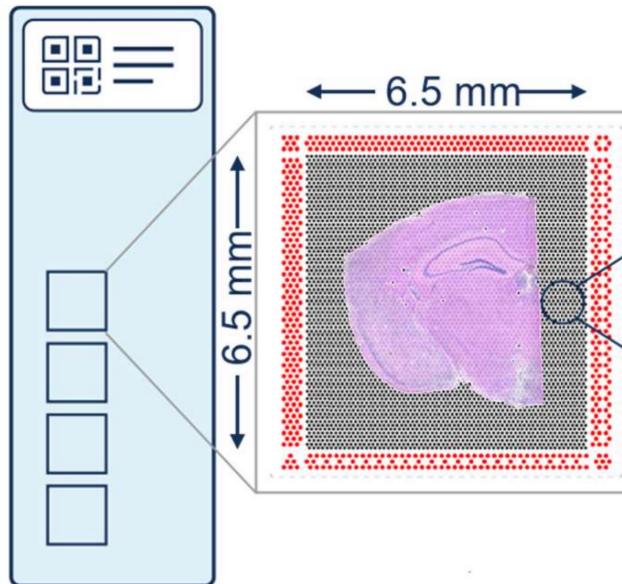


- Capture the transcripts *in situ* but sequence *ex situ*
- Usually less dependent on prior selection of targets
- Examples) Visium, Spatial Transcriptomics (ST), Slide-seq v2, HDST, APEX-Seq, and Stereo-seq

3. Capture-based: *in situ* capture-based technologies ::

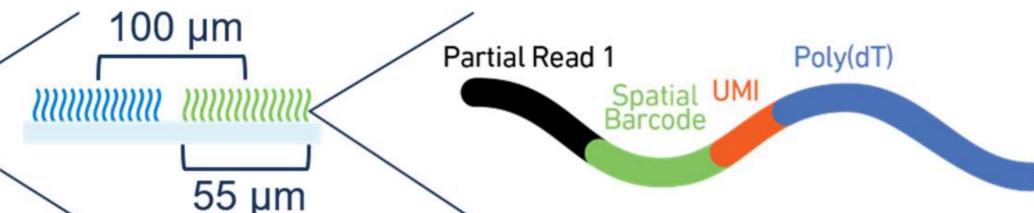
Visium

Visium Spatial
Gene Expression
Slide



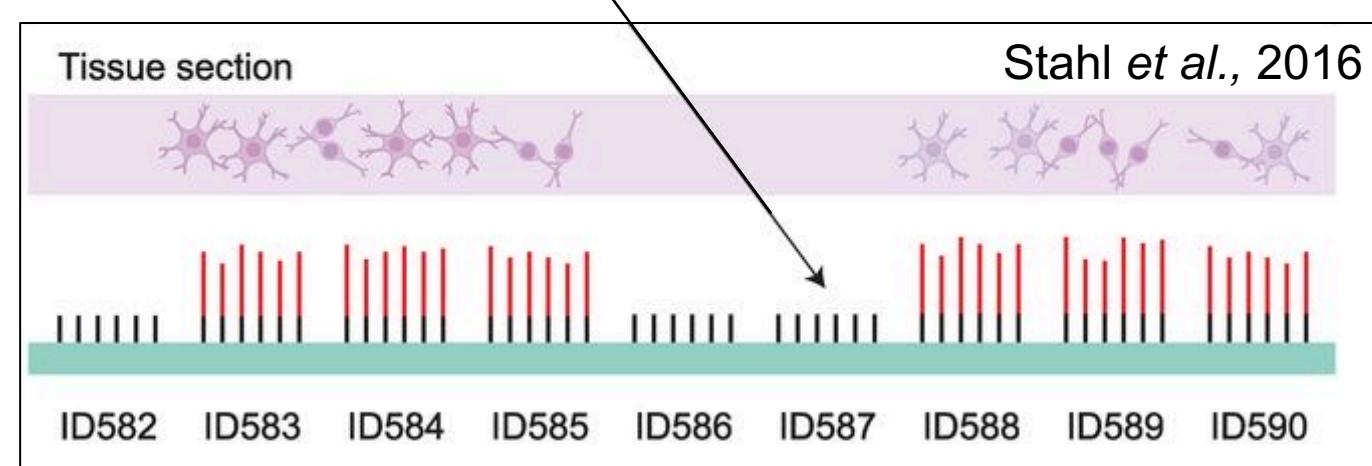
Capture Area with
~5000 Barcoded
Spots

Visium Gene
Expression Barcoded
Spots

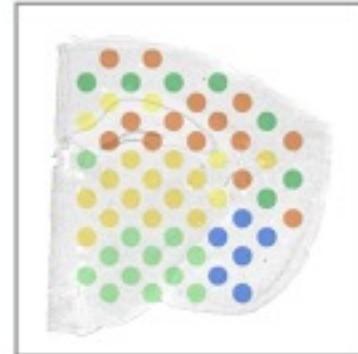


<https://images.app.goo.gl/2k36WDczUaUcBFTYA>

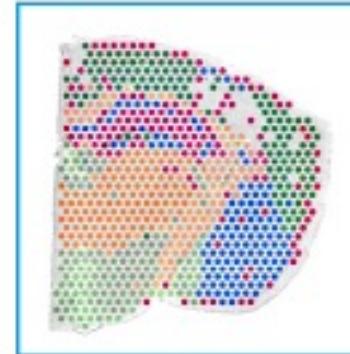
1. Array-based technique (4 arrays per slide)
2. 6.5 mm x 6.5 mm area to put the sample on
3. 4,992 spots arranged in a hexagonal grid
4. Array specs:
 - Spot diameter: 55 μ m
 - Center-to-center distance: 100 μ m



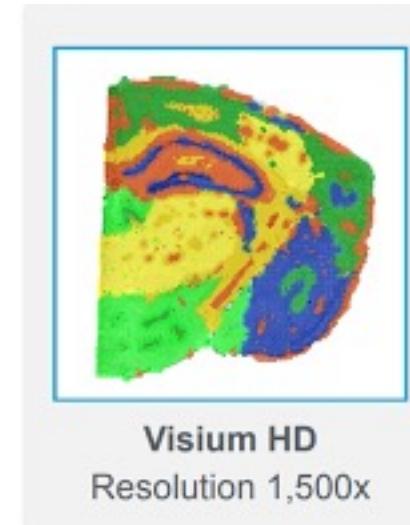
We are witnessing an evolution of Visium last decade



First Generation
ST



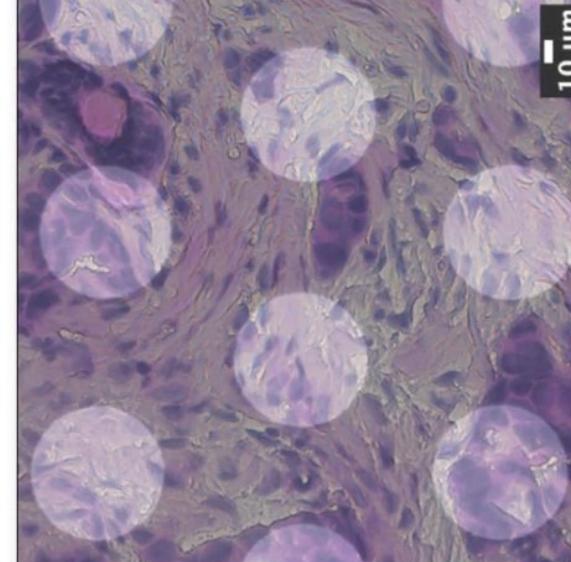
Visium
Resolution 4x



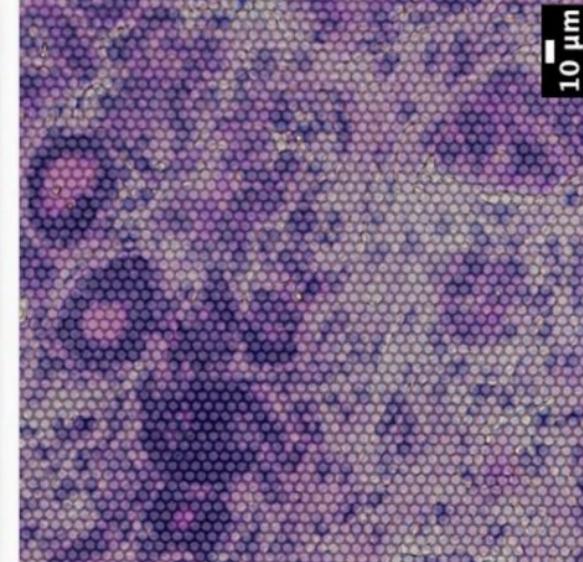
Visium HD
Resolution 1,500x

<https://images.app.goo.gl/5jH4HrQoE5VBPksZ9>

- Successor to Spatial Transcriptomics (ST)
- Approx. 1-10 cells contribute to each spot
Not a single-cell resolution!
- Data represented as [spot] x [gene] matrix
- You also get HE images of the same tissue



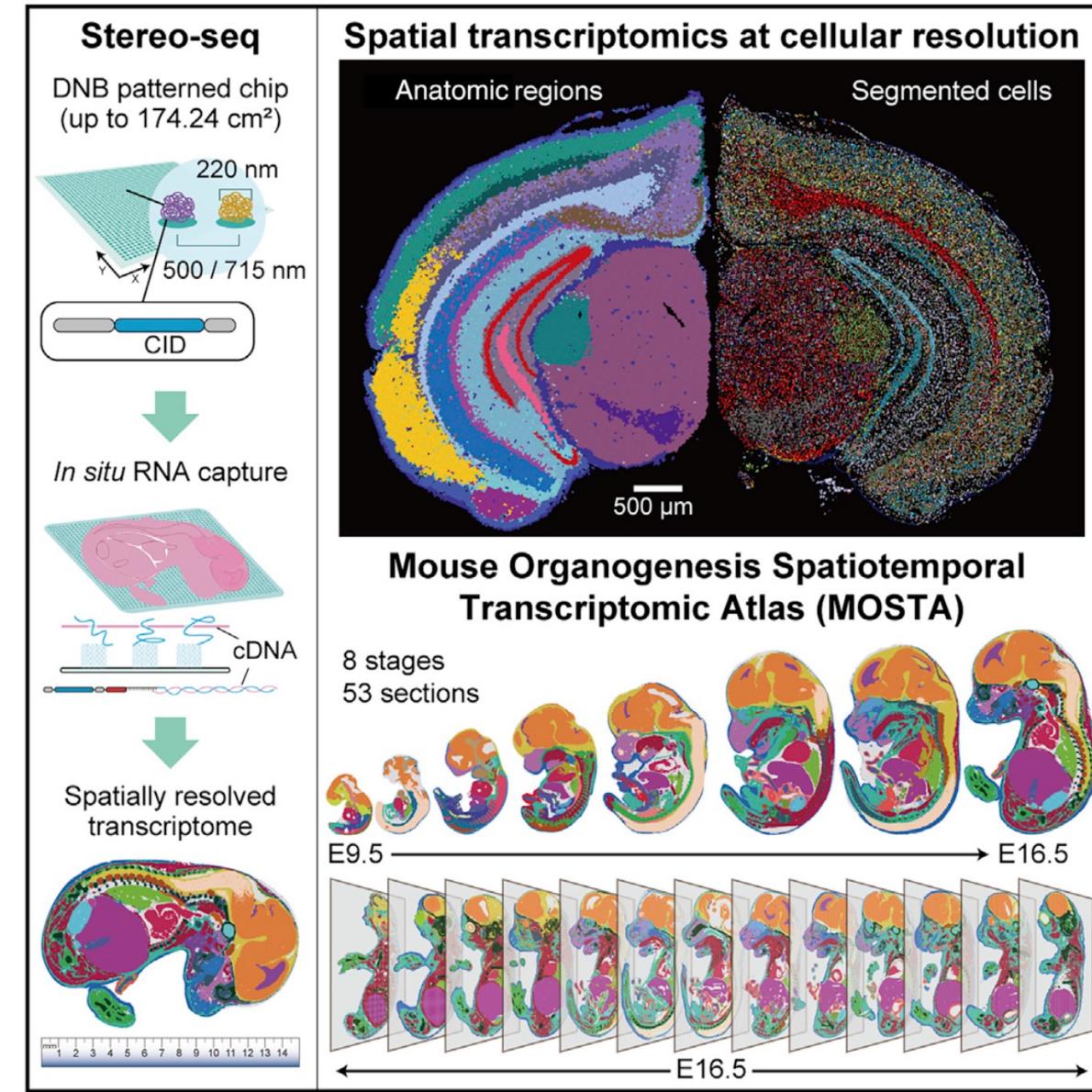
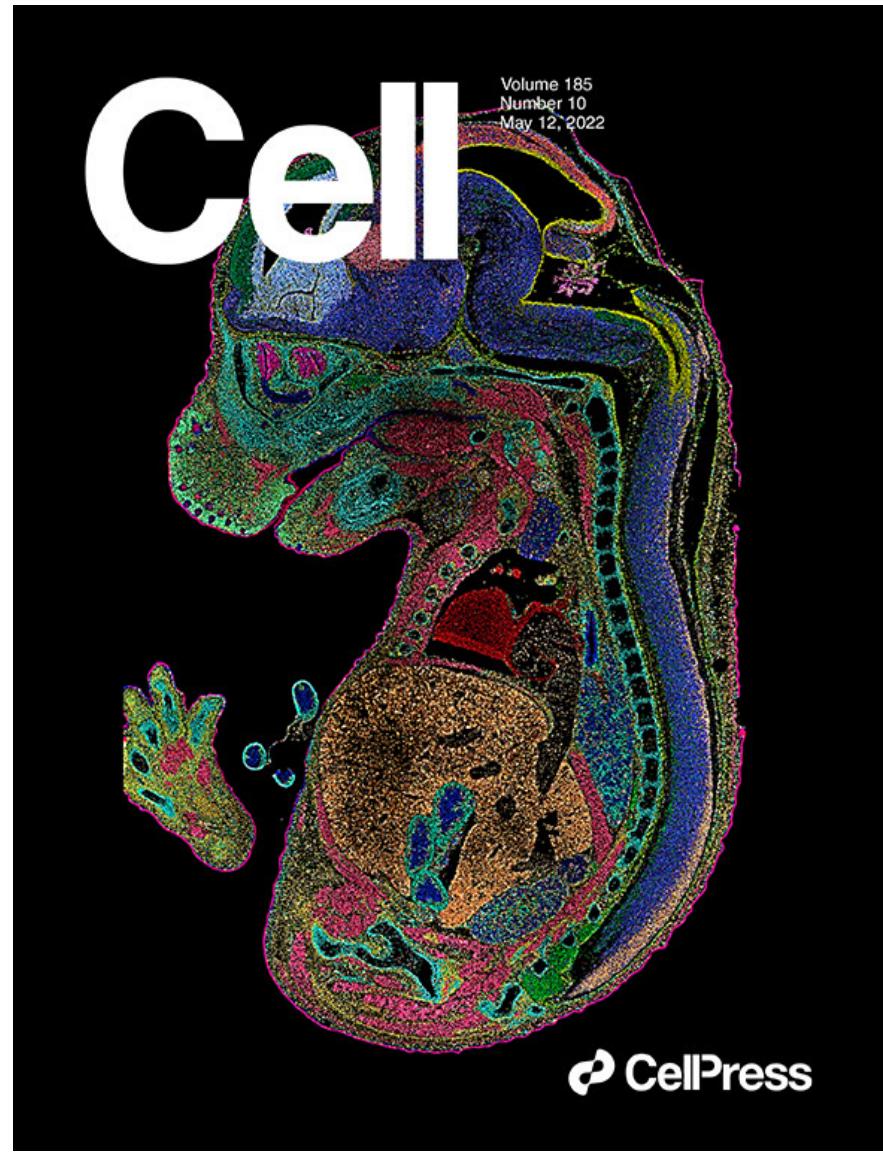
Visium



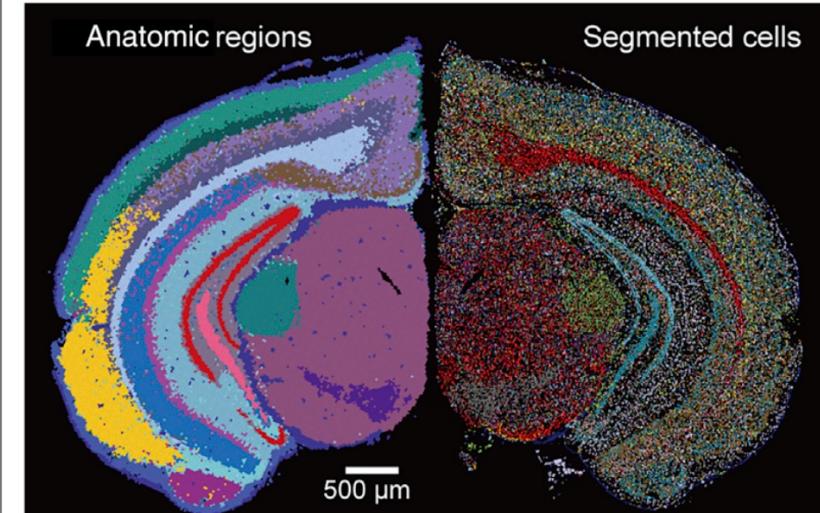
Visium HD (available soon)

<https://twitter.com/AlbertVilella/status/1367028429300916225/photo/1>

Stereo-seq is a transformational breakthrough in spatial transcriptomics technology

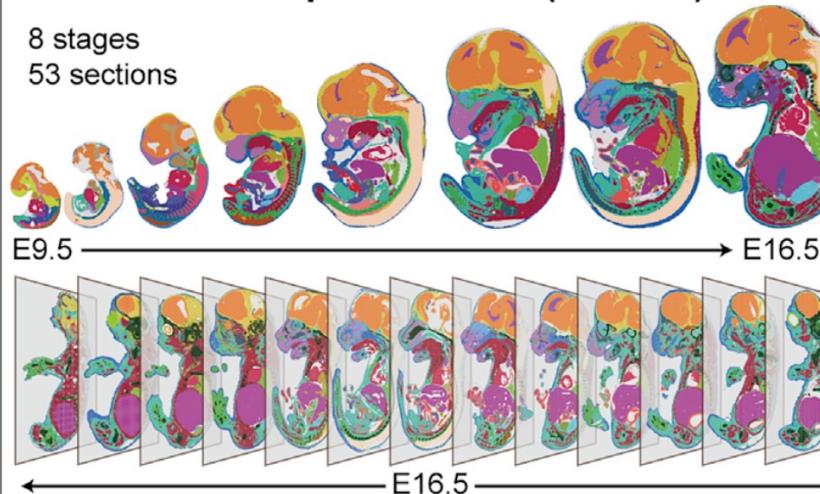


Spatial transcriptomics at cellular resolution



Mouse Organogenesis Spatiotemporal Transcriptomic Atlas (MOSTA)

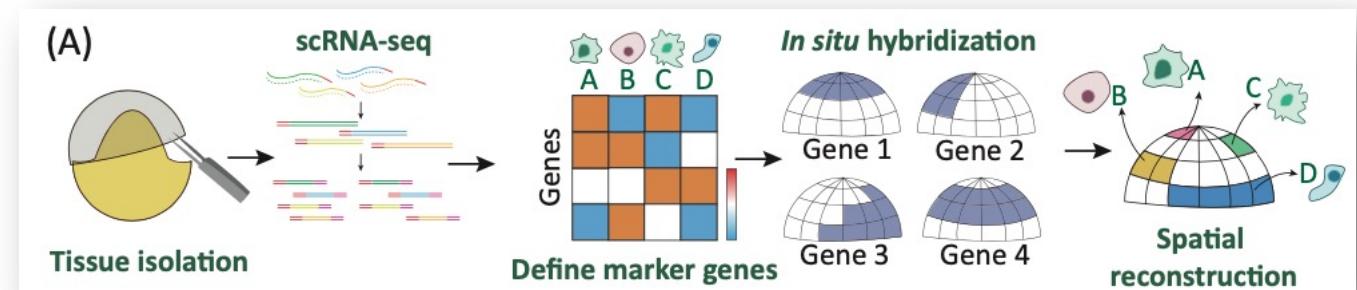
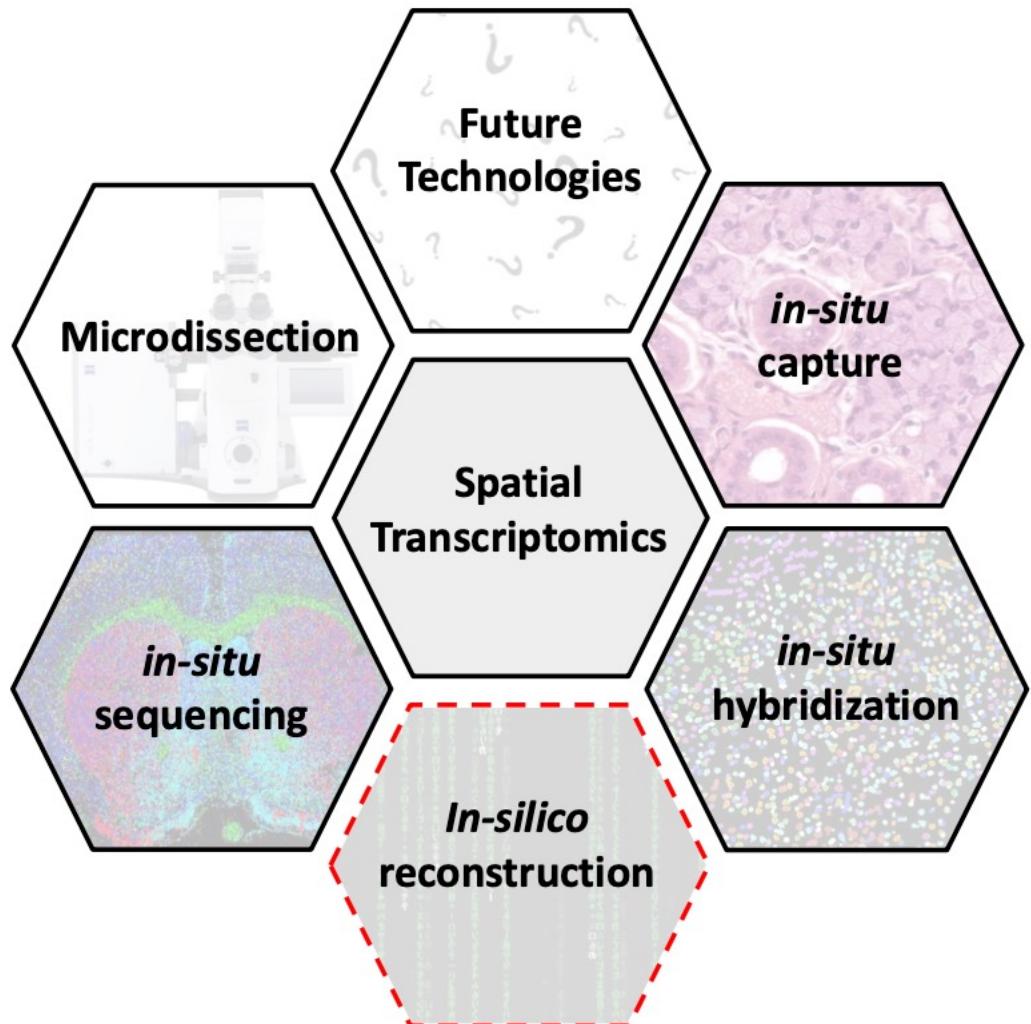
8 stages
53 sections



- Scalable
- High res
- Deep cov
- Not commercially available yet

Chen et al., 2022

4. *in silico* reconstruction technology



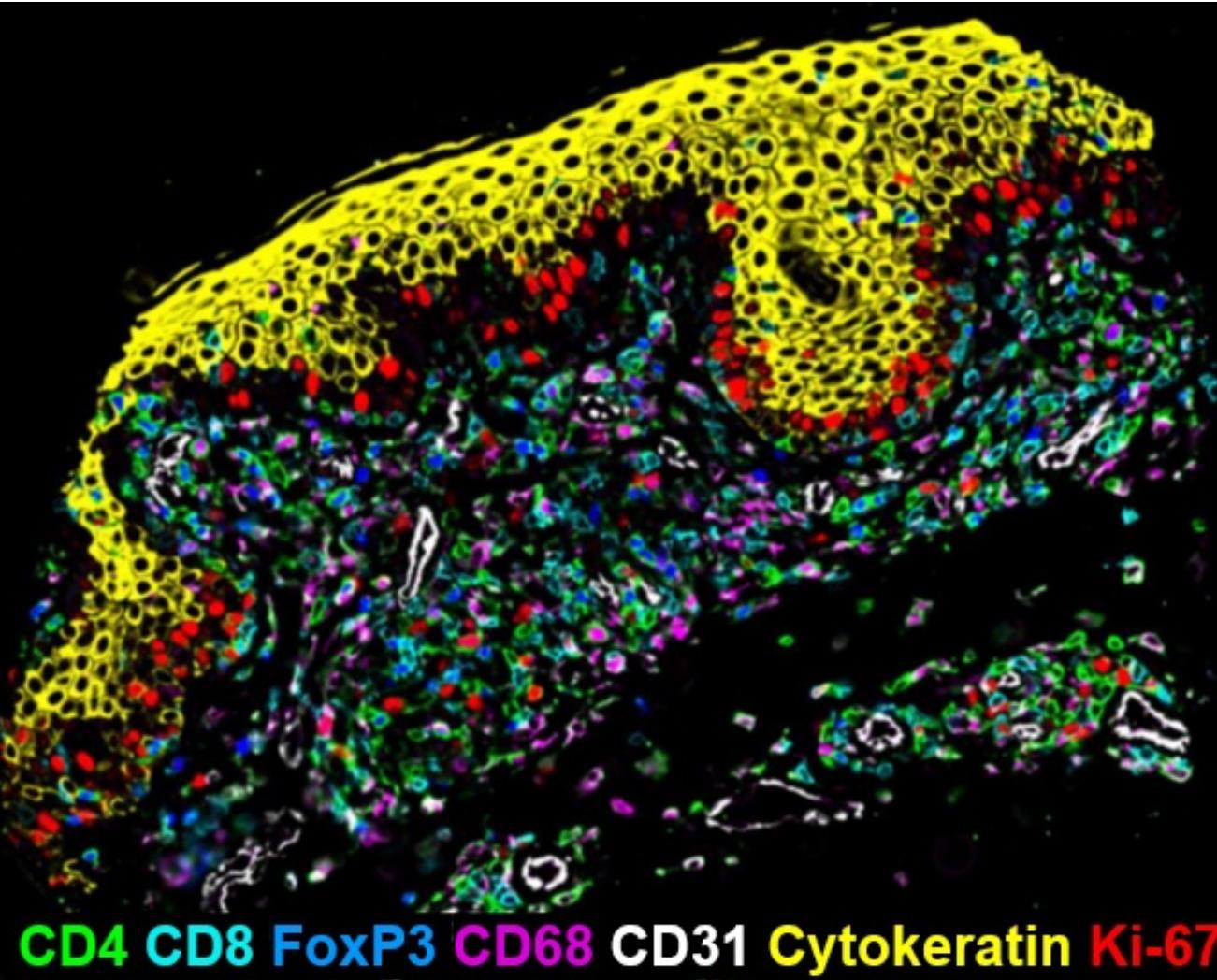
Liao et al., 2021

- Infer and reconstruct spatial structure from non-spatial data like single-cell transcriptomes
- At the core of this framework lies a structural correspondence hypothesis that cells in physical proximity share similar gene expression profiles
- Examples: novoSpaRc, CSOmap, and Seurat v3

Asp et al., 2020

5. Spatial proteomics offers direct measurements of protein localization and abundance in space

CODEX from Akoya



Several antibody-dependent technologies

1. Multiplexed IHC
 - t-CyCLF and CODEX
2. Imaging/spectrometry
 - Multiplexed ion beam imaging by time of flight (MIBI-TOF)
 - Imaging mass cytometry (IMC)
 - Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)

Recommended review literature on SRT

- Rao A, Barkley D, França GS, Yanai I. **Exploring tissue architecture using spatial transcriptomics.** Nature. 2021 Aug;596(7871):211-220. doi: 10.1038/s41586-021-03634-9. Epub 2021 Aug 11. PMID: 34381231; PMCID: PMC8475179.
- Longo SK, Guo MG, Ji AL, Khavari PA. **Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics.** Nat Rev Genet. 2021 Oct;22(10):627-644. doi: 10.1038/s41576-021-00370-8. Epub 2021 Jun 18. PMID: 34145435.
- Williams CG, Lee HJ, Asatsuma T, Vento-Tormo R, Haque A. **An introduction to spatial transcriptomics for biomedical research.** Genome Med. 2022 Jun 27;14(1):68. doi: 10.1186/s13073-022-01075-1. PMID: 35761361; PMCID: PMC9238181.
- Moses L, Pachter L. **Museum of spatial transcriptomics.** Nat Methods. 2022 May;19(5):534-546. doi: 10.1038/s41592-022-01409-2. Epub 2022 Mar 10. Erratum in: Nat Methods. 2022 Apr 19;; PMID: 35273392.
- Lee J, Yoo M, Choi J. **Recent advances in spatially resolved transcriptomics: challenges and opportunities.** BMB Rep. 2022 Mar;55(3):113-124. doi: 10.5483/BMBRep.2022.55.3.014. PMID: 35168703; PMCID: PMC8972138.

Can I try spatial transcriptomics?

- Which type of tissue is available:
Fresh-Frozen or Formalin-Fixed, Paraffin-Embedded?
- Is the RNA present in my sample of high quality?
- Can I successfully detect the genes I am interested?
- Are there sufficient samples for conducting biological replicates?
- Is the budget adequate for my research design?

Carefully evaluate these factors and consult with experts for valuable guidance

What do I need to use?

Depending on the experimental aim

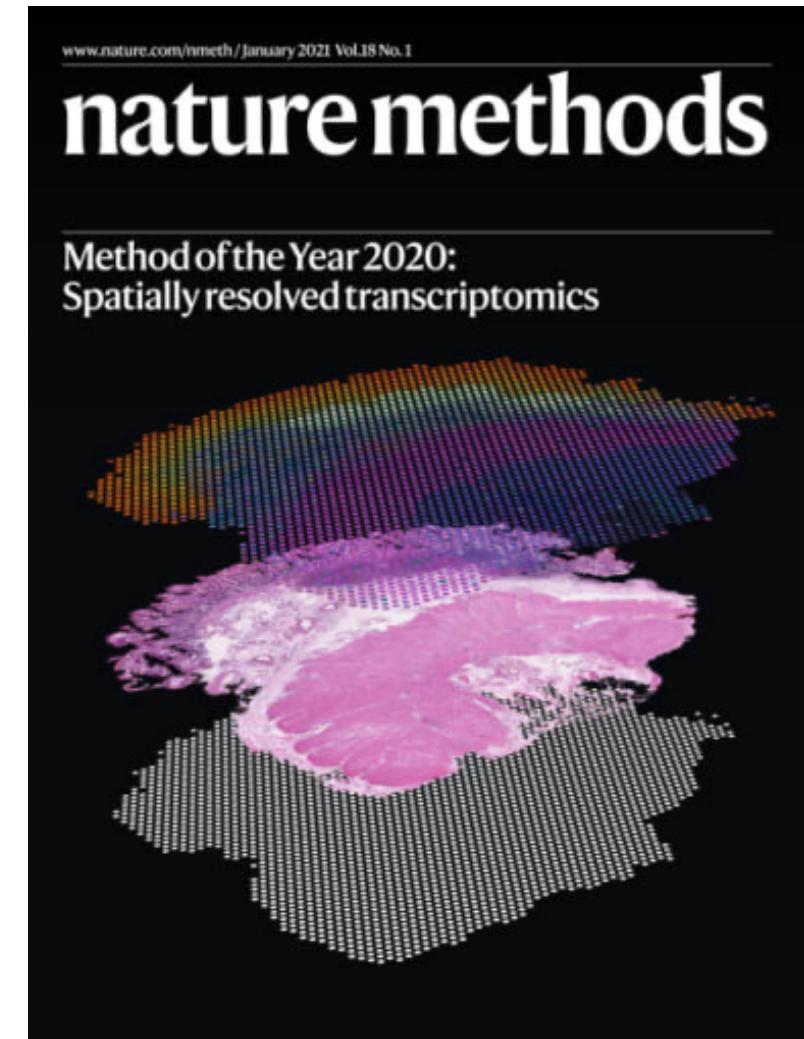
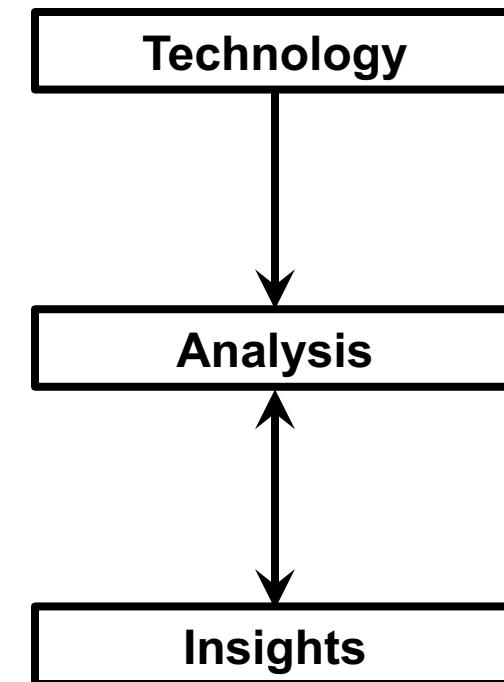
1. Hypothesis generating

- Sequence-based methods profiling gene systematically: Visium (10X), GeoMx, CosMx (Nanostring)

2. Hypothesis testing

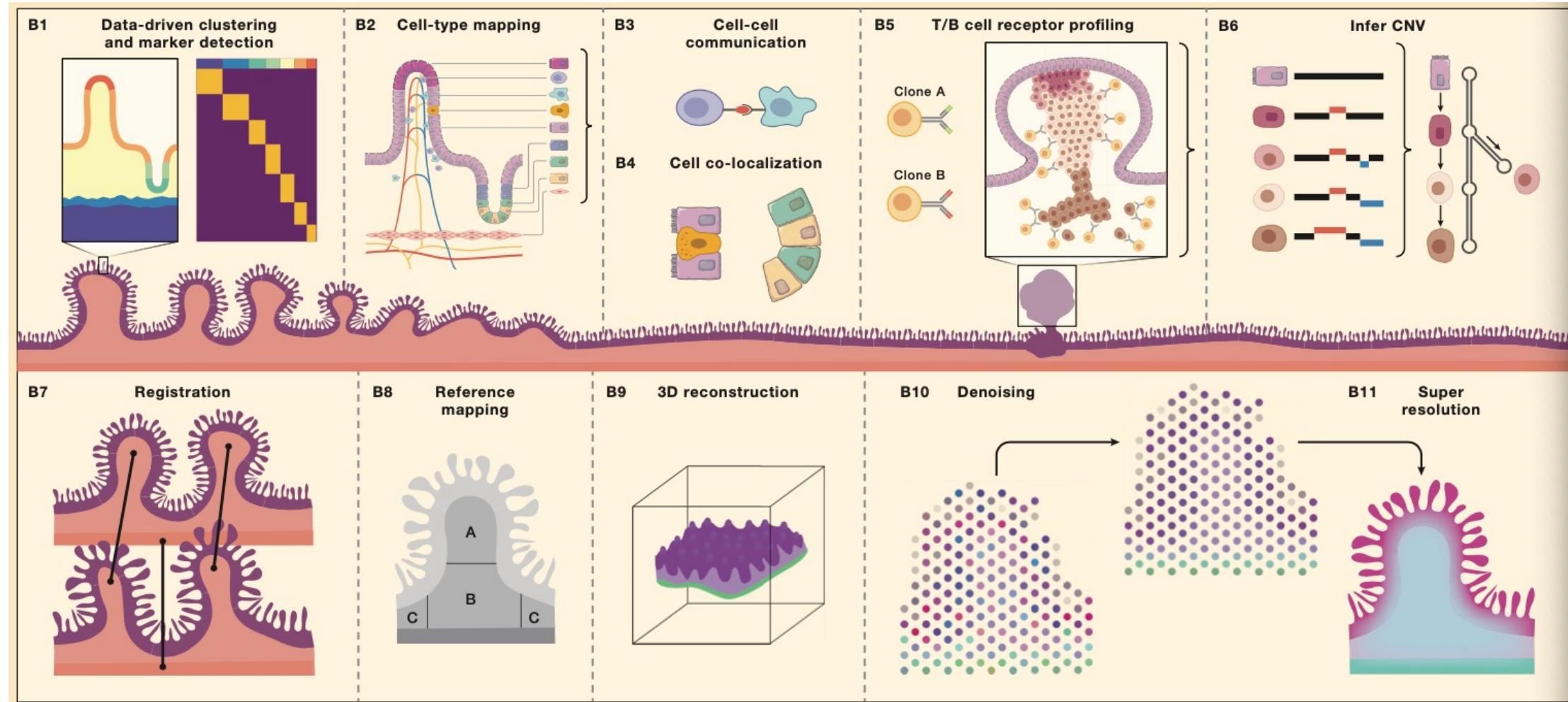
- Image-based methods profiling a few genes at high resolution and sensitivity: MERFISH (Vizgen) and Xenium (10X)

Where is the field moving?



Spatial omics technology and analysis algorithms coming to light

Snapshots of spatial transcriptomics applications



Larsson et al., 2023

Take home messages

- ✓ There are tons of spatial techniques out there
- ✓ In addition, an ever-increasing repertoire of computational methods!
- ✓ Spatial-omics data is already improving our understanding of human health and disease in research, diagnostic, and therapeutic setting

Questions?

Have fun with the hands-on SRT exercise



Jihyun Kim

Juhyeon Hong

Kwangmin Yoo