



KSBi-BIML 2024

Introduction to Single-cell Biology

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Korea University, College of Medicine



Topics Explored in Today's Lecture

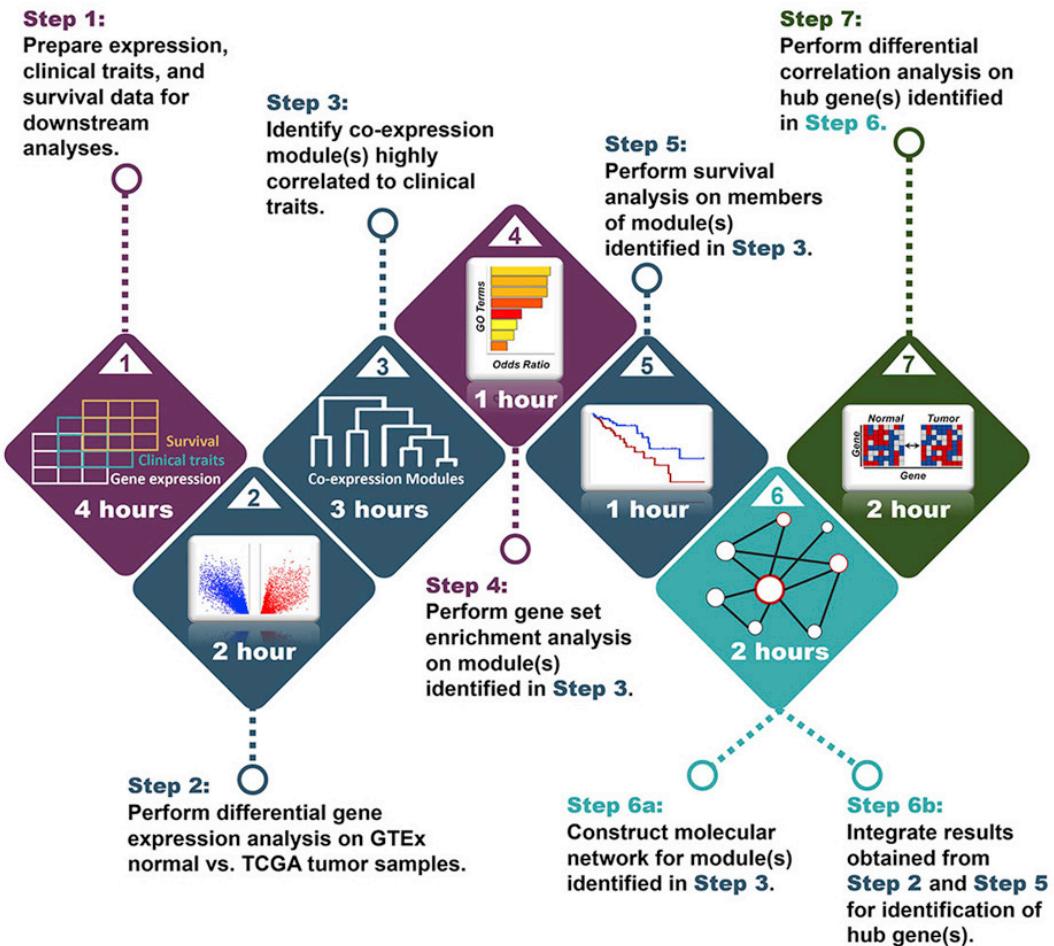
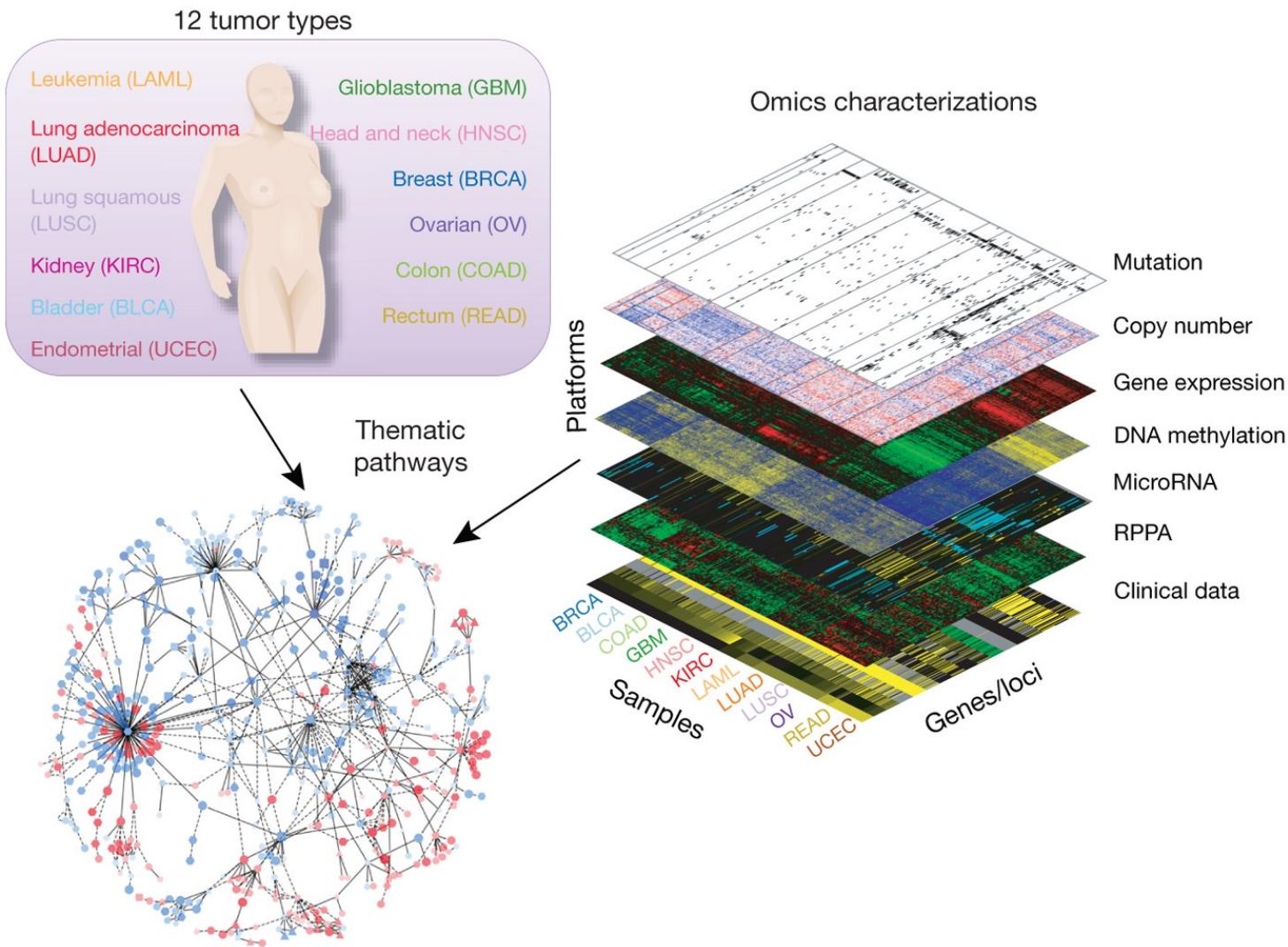
- **Introduction**

- A broad overview of single-cell data and spatially resolved experimental techniques

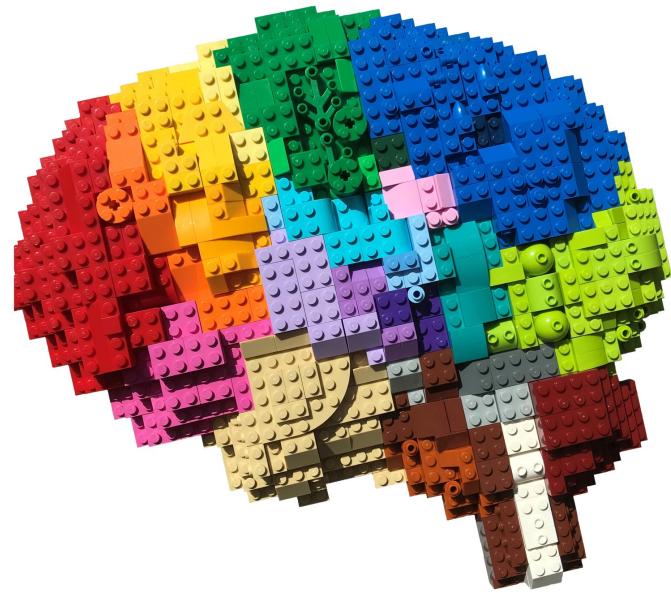
- **Computational methodology and frameworks**

- Different flavors of currently available methods for spatially resolved data analysis

Major Successes Large-scale Consortia such as TCGA and PCAWG through Bulk Genomics



Single-cell Data Provides Valuable Insights into the Complexity of Biological Systems



Brain tissue



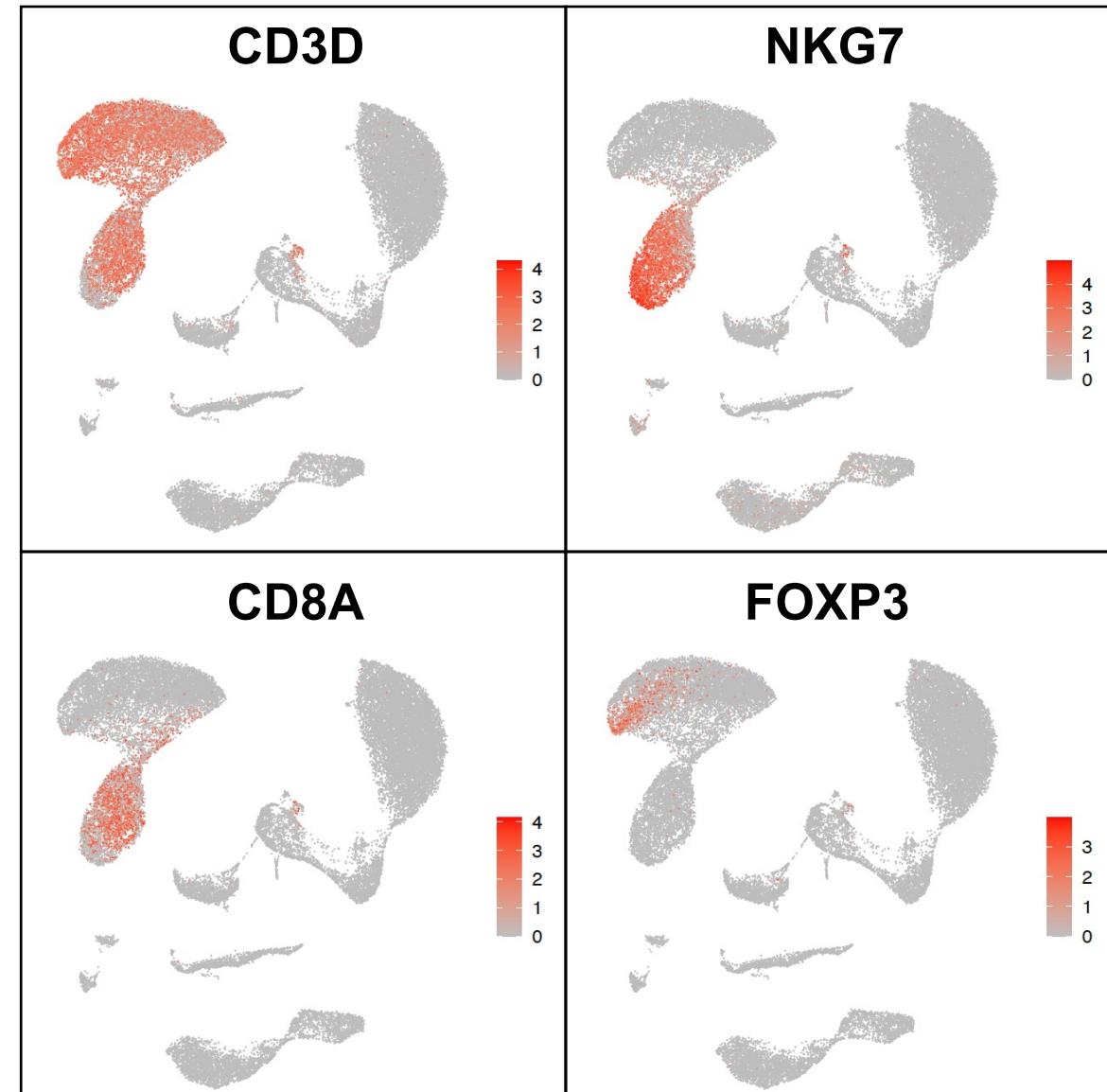
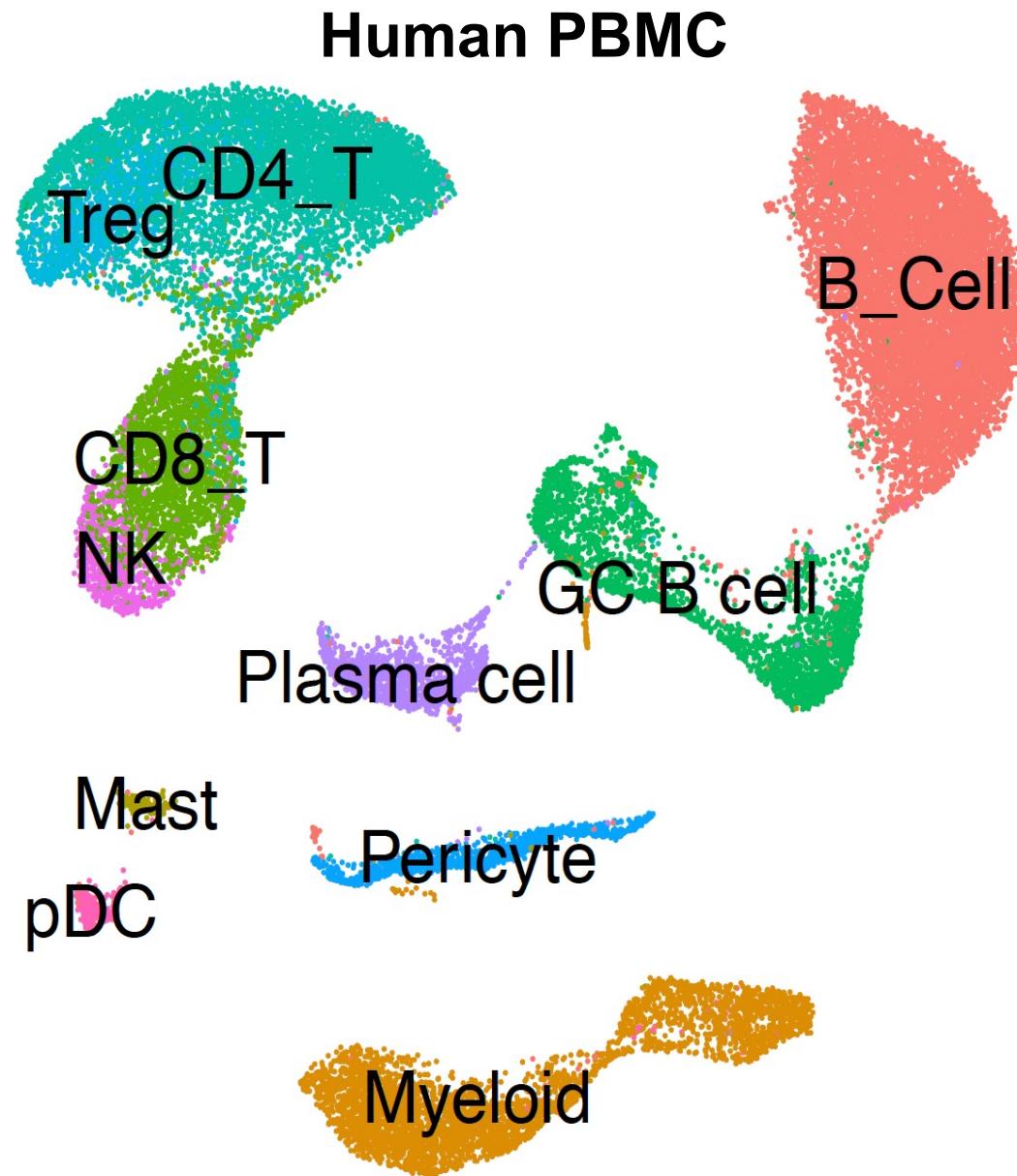
Bulk genomics



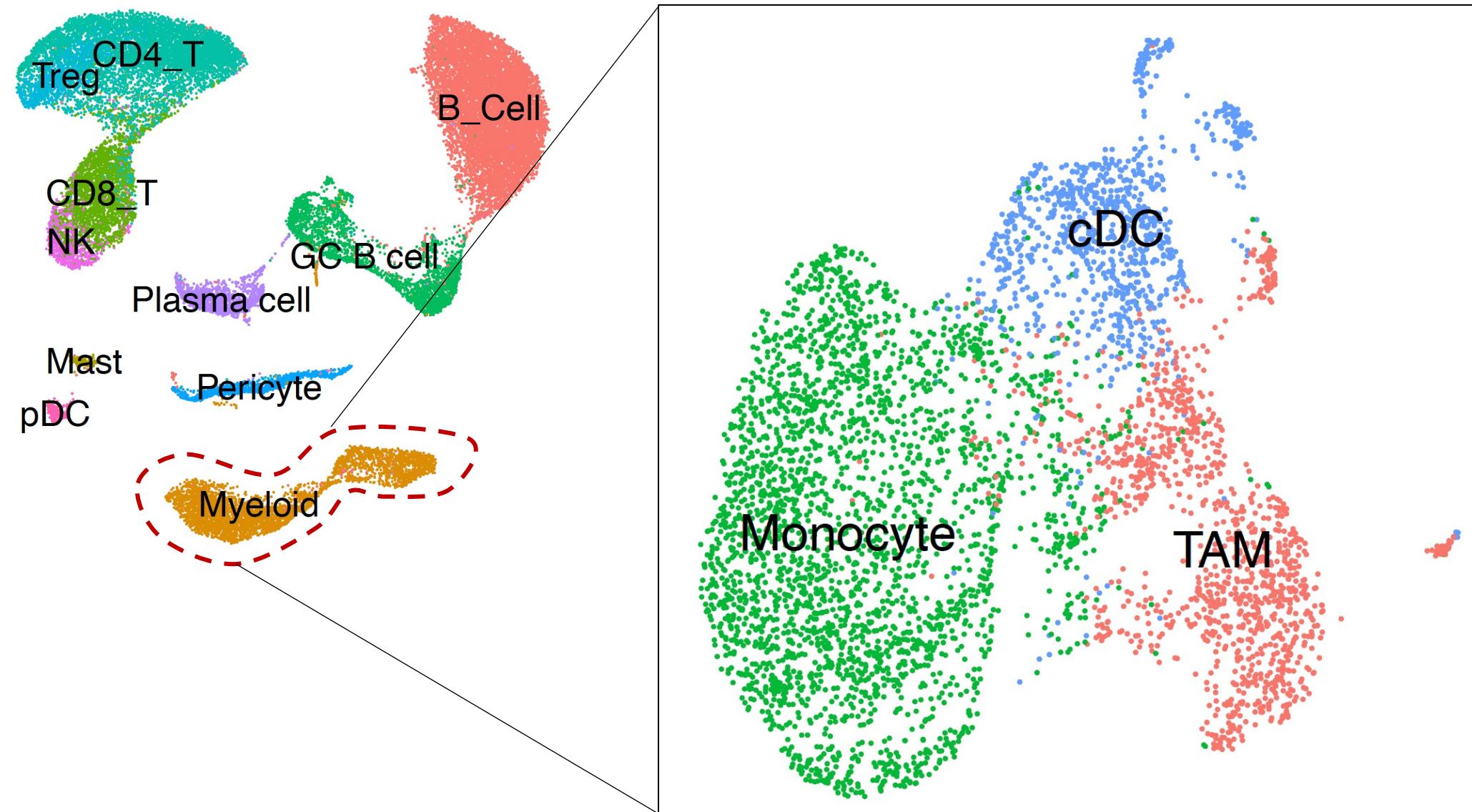
Single-cell genomics

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

Single Cells Can Identify And Characterize Cell Types

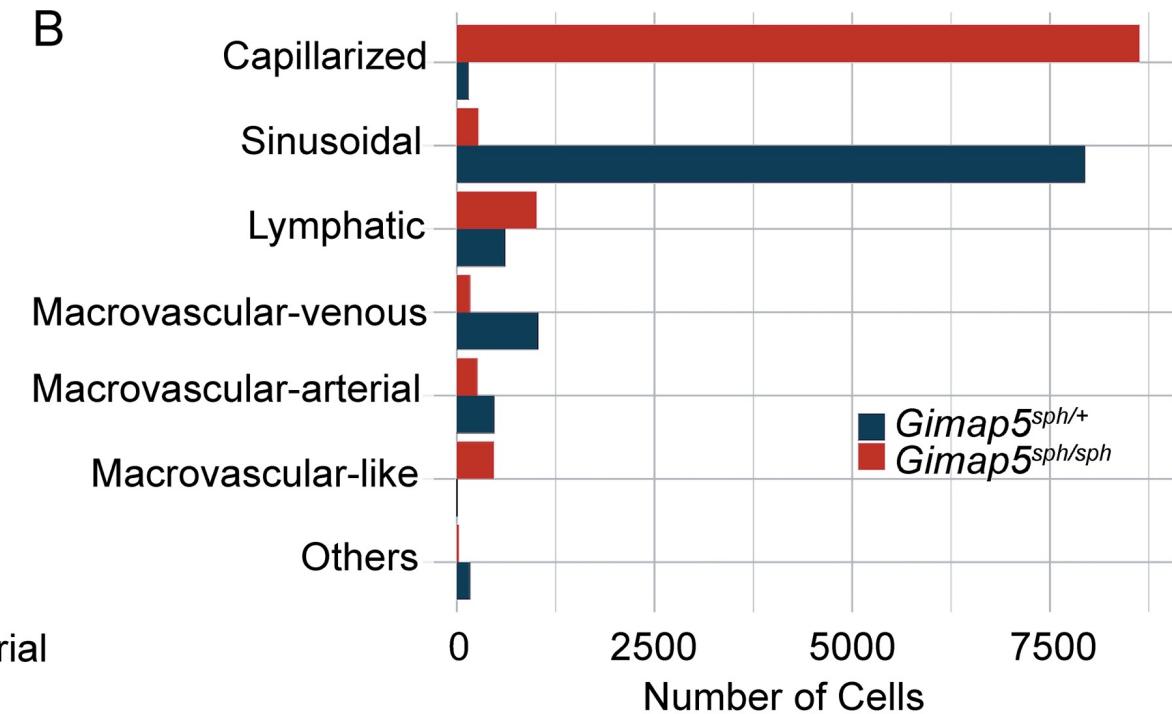
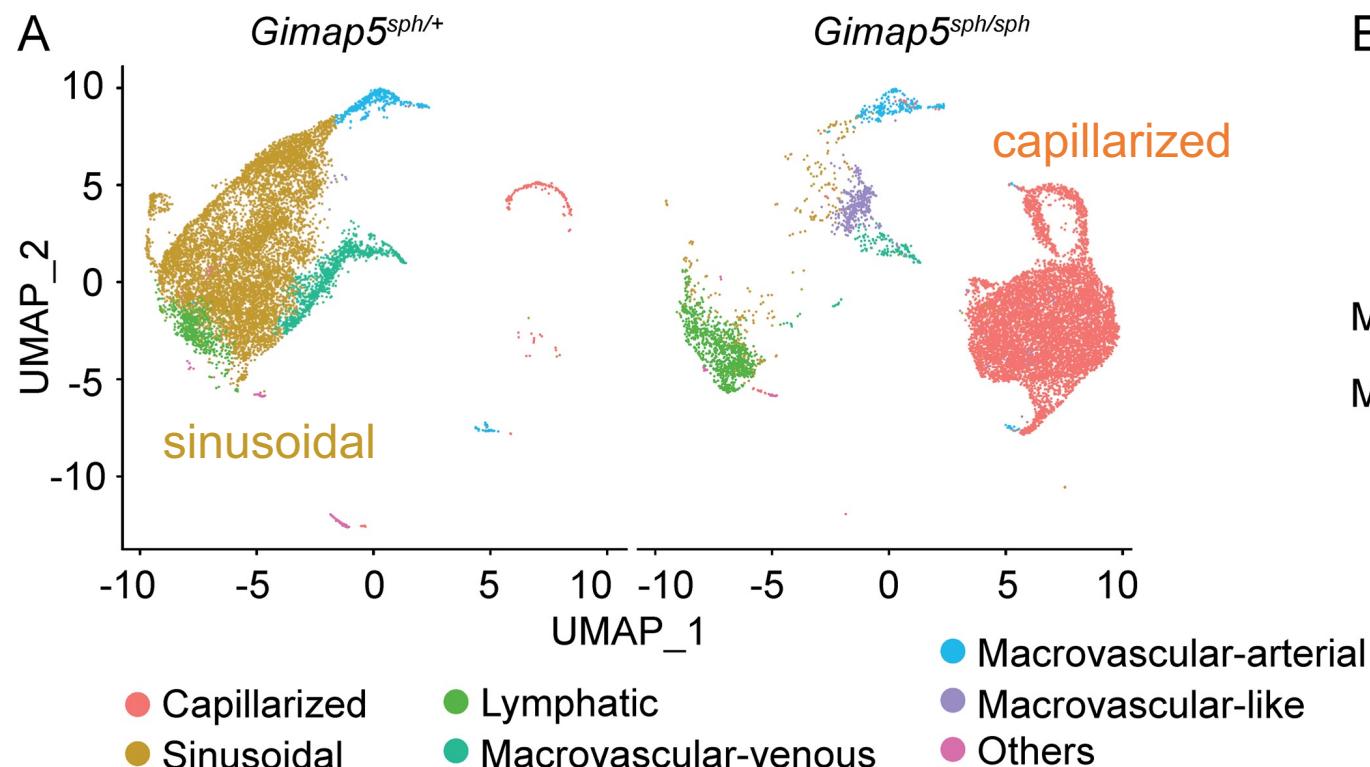


Cellular Heterogeneity Can Be Even Further Explored



Single-cell Data Can Elucidate The Cellular Composition And Dynamics Of Cell State Changes

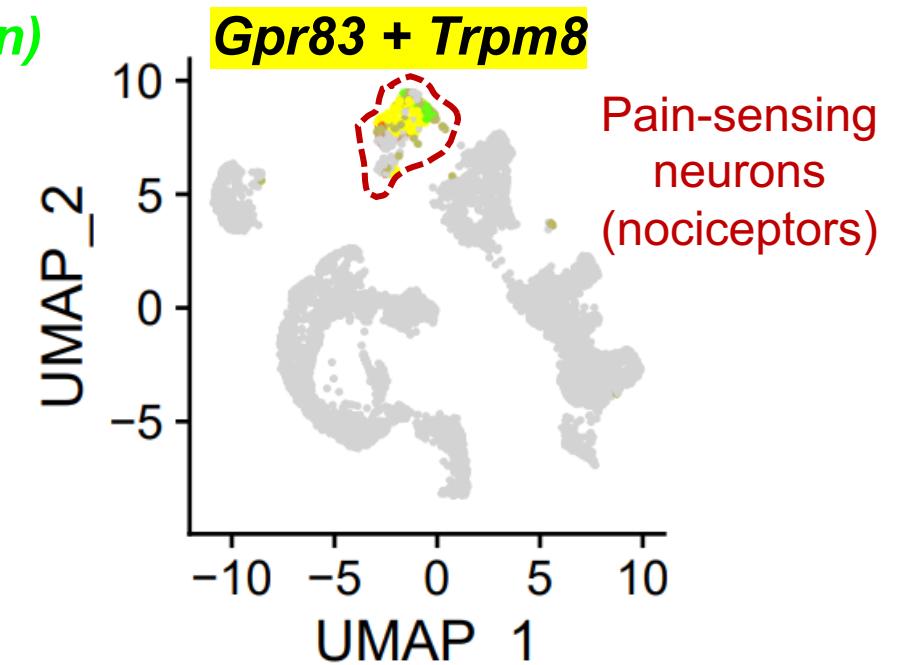
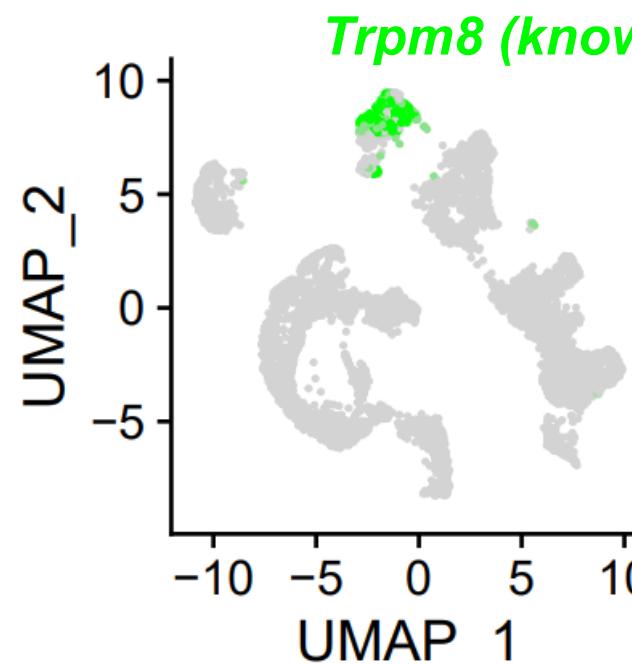
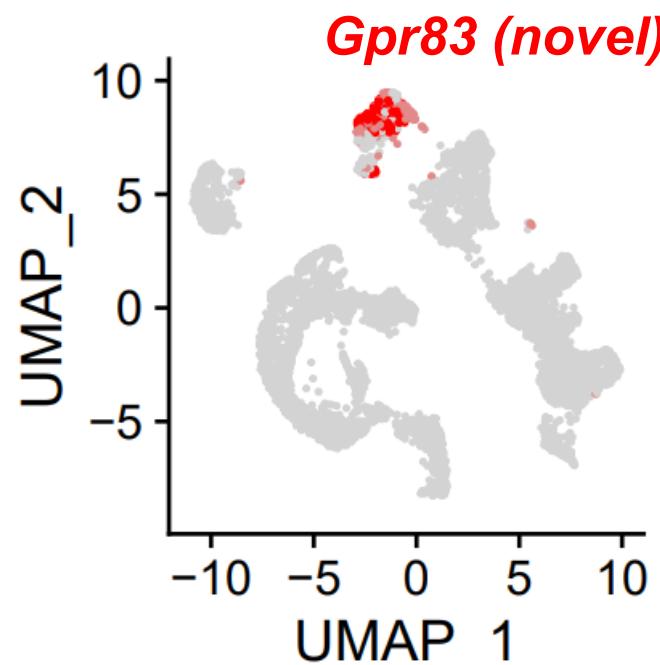
Mouse endothelial cells



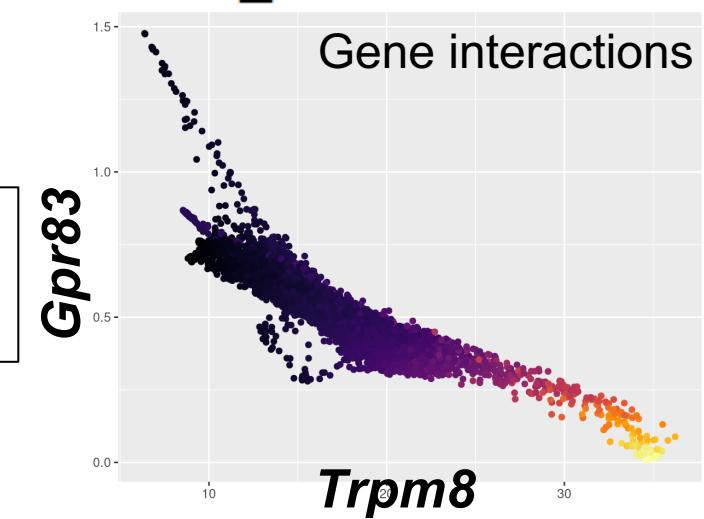
The homozygous deletion of *Gimap5* leads to the accumulation of a specific type of endothelial cells, resulting in portal hypertension

Single Cells Can Identify Strongly Associated Genes

Mouse Dorsal Root Ganglia (DRG)

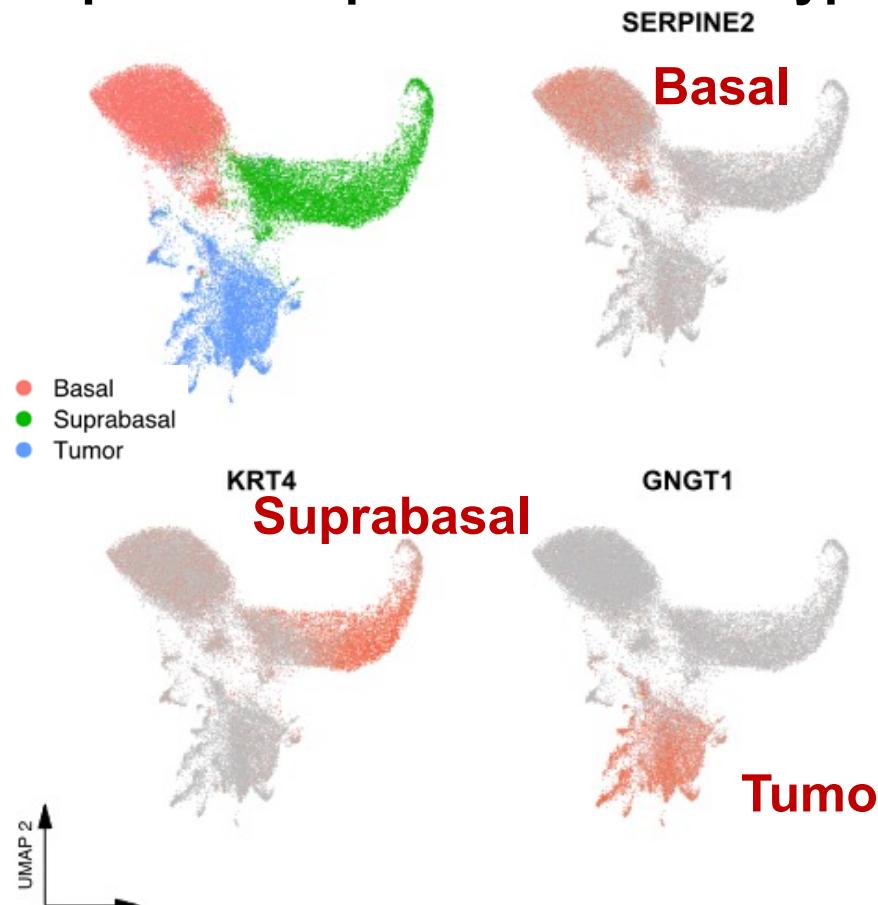


The exclusive interaction of *Gpr83* and *Trpm8* in pain-sensing neurons implicates their crucial roles

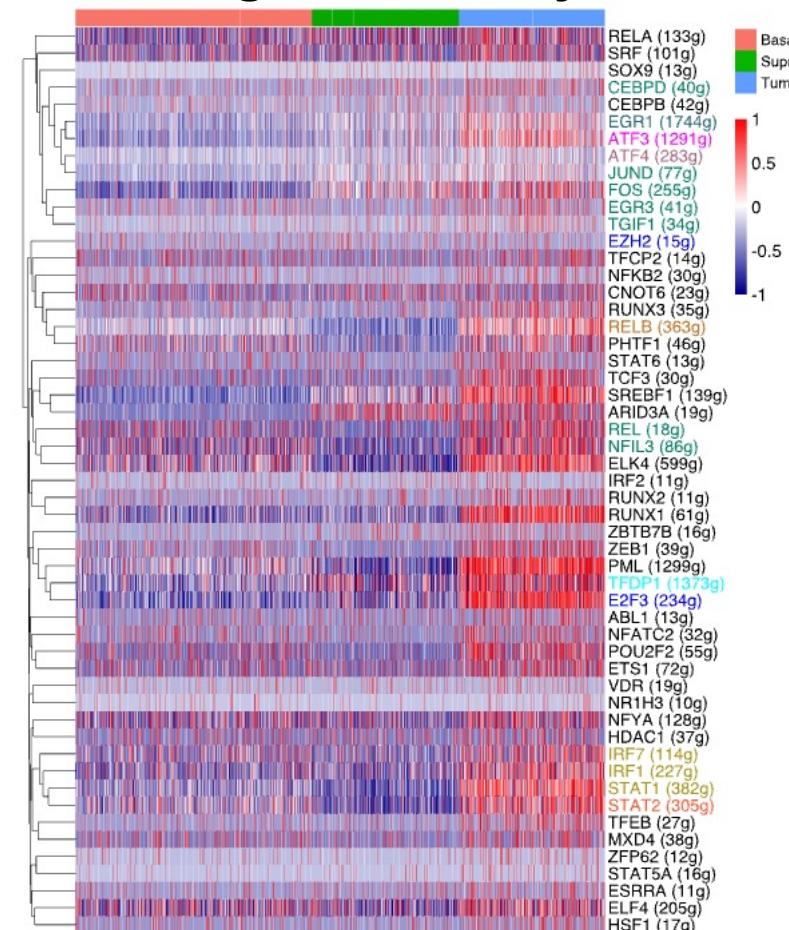


Single Cells Provide Insights Into Gene Regulatory Networks And The Effects Of Specific Genes On Cellular Function

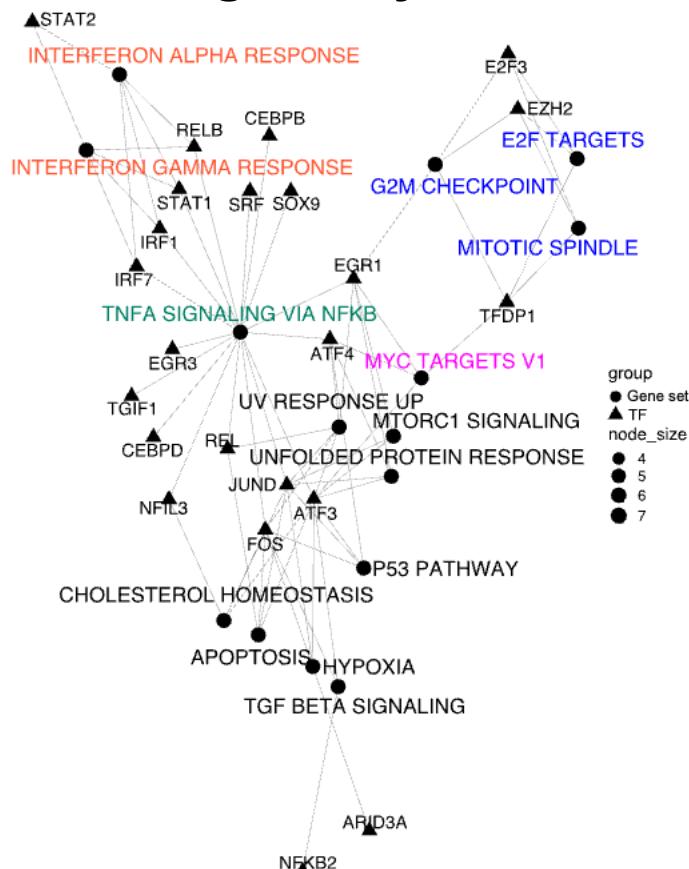
Squamous epithelial sub-cell types



Regulon Activity



Gene Regulatory Network

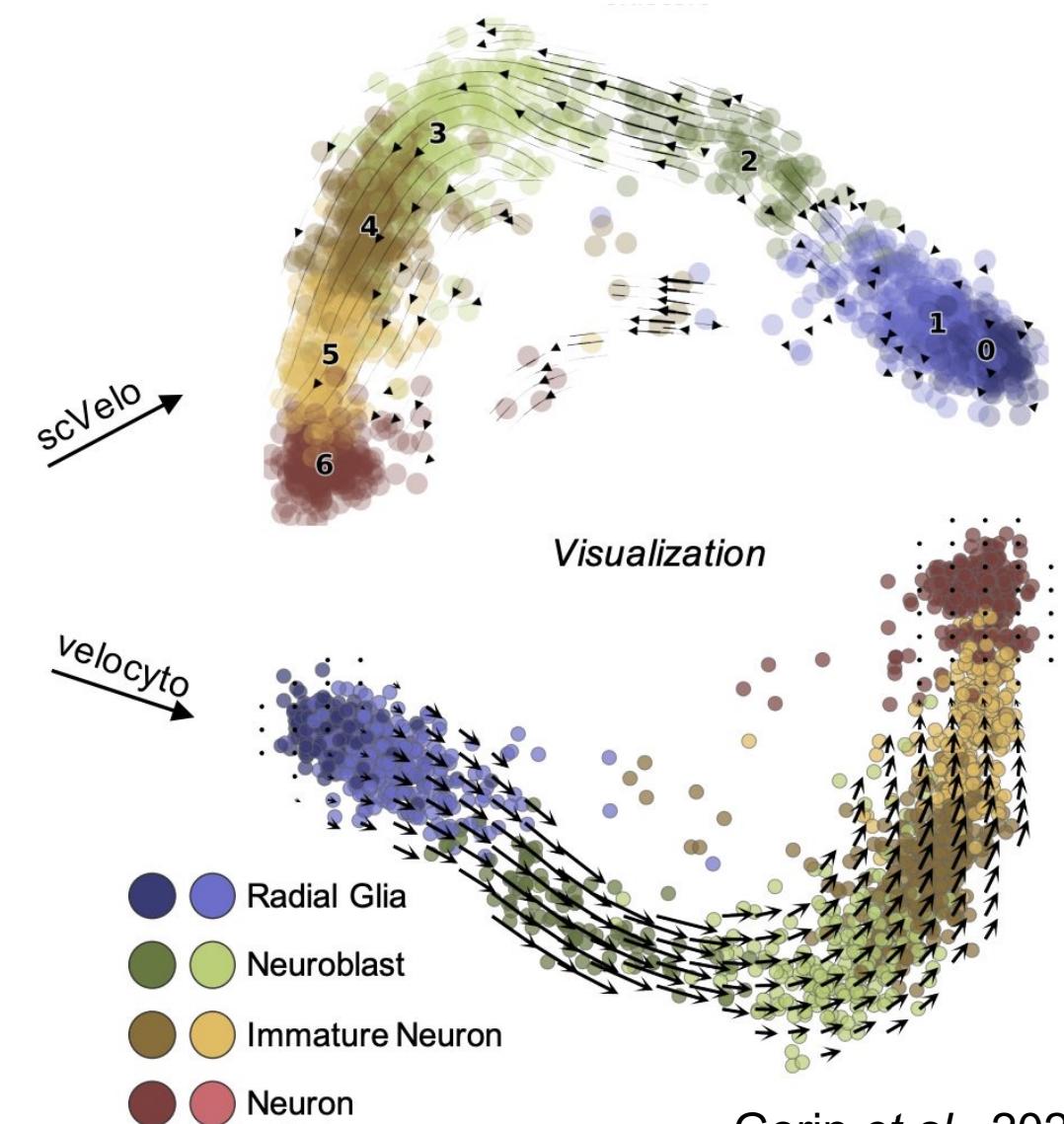
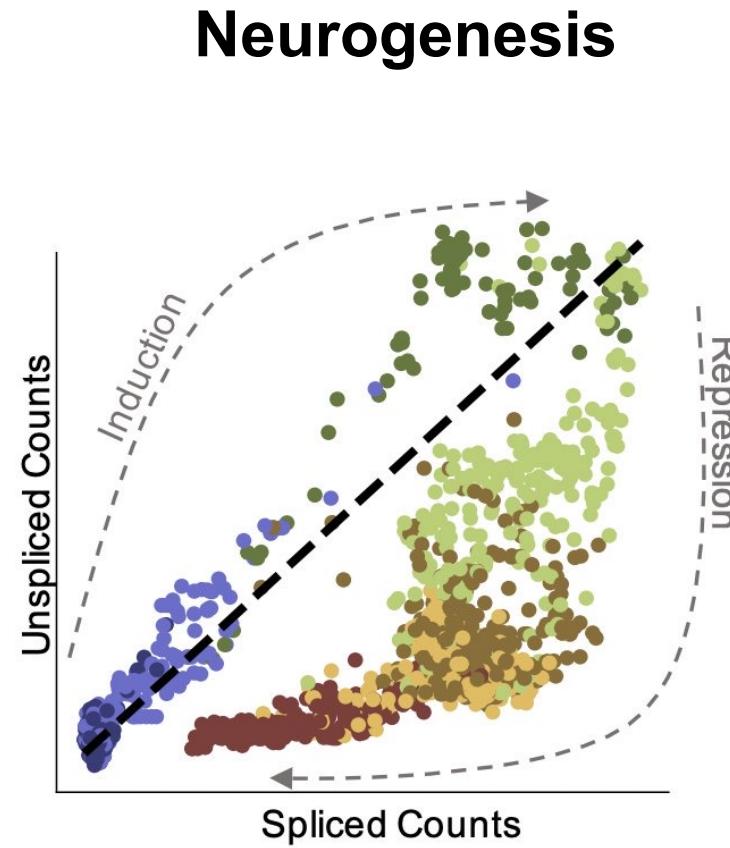


unpublished

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

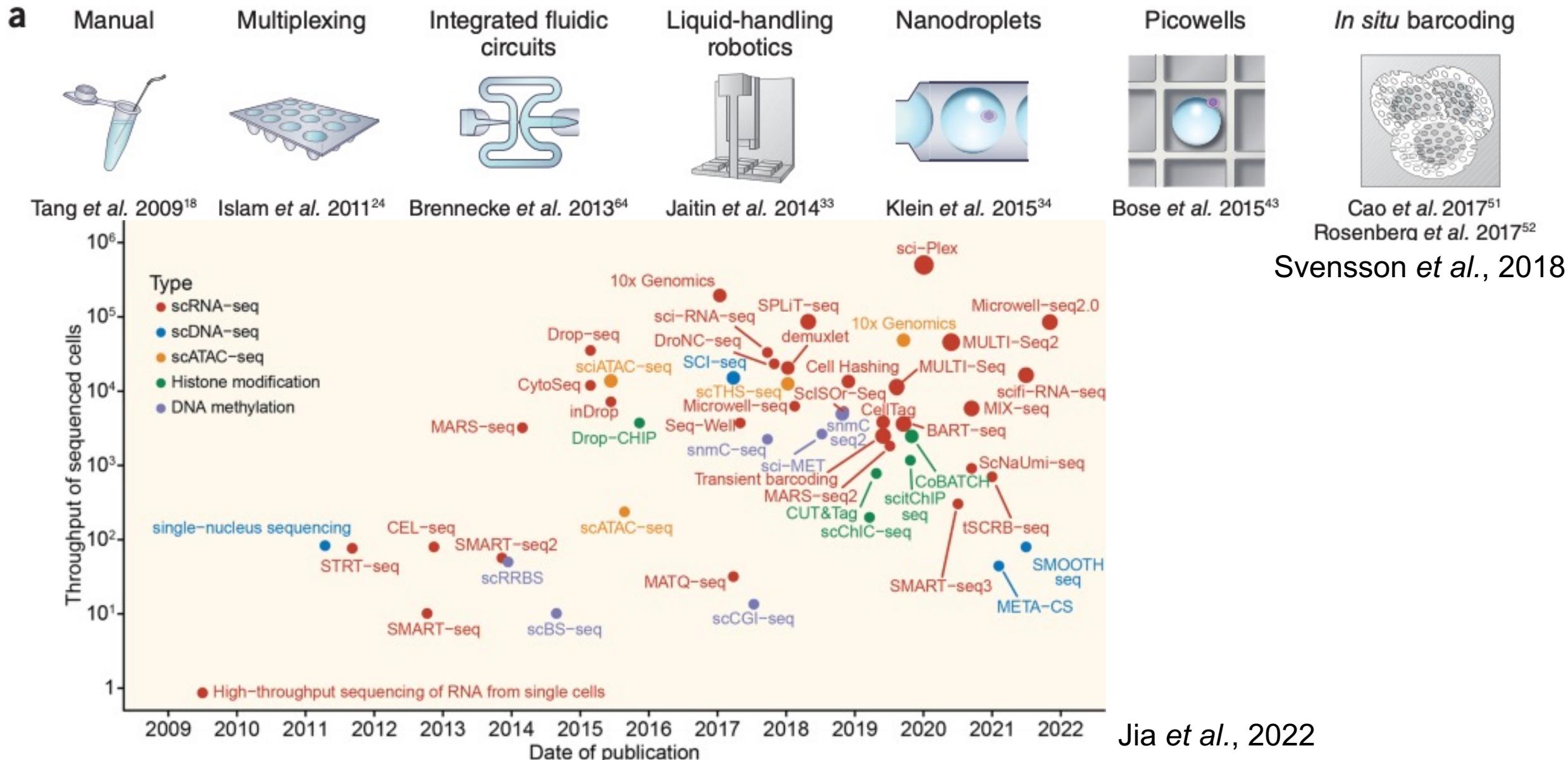
Single-cell Data Can Predict A Continuum From Static Snapshots Of Cell State

A sequential trajectory of cliff diving

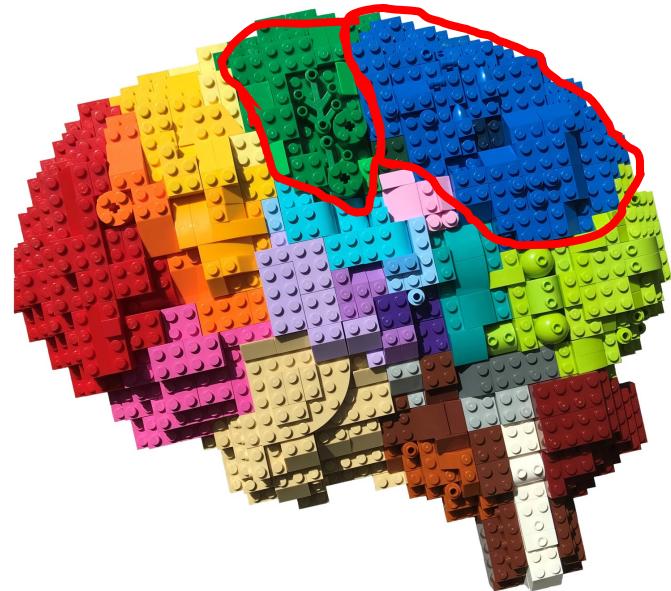


Gorin et al., 2022

The Field Of Single-cell Sequencing Technology Has Experienced Exponential Growth



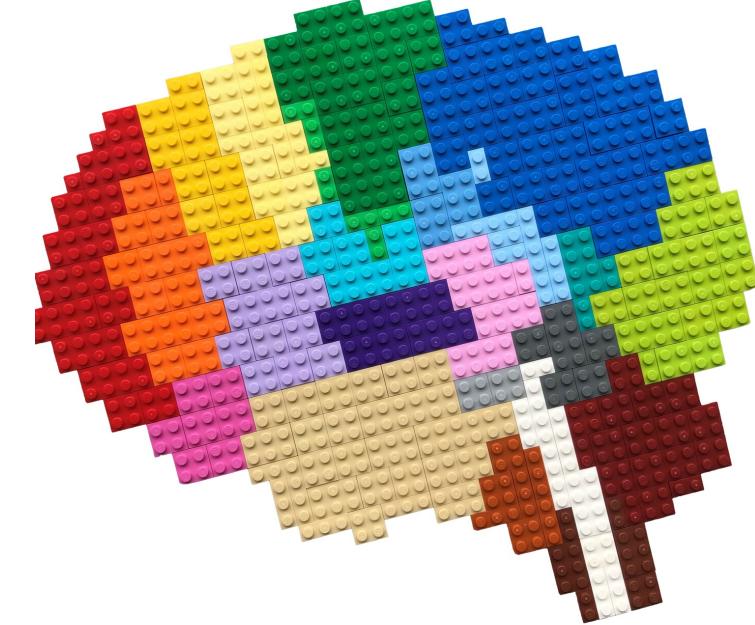
Unfortunately, Single-cell Transcriptomics Lacks Spatial Information Due To Cell Dissociation



The original brain



single-cell RNA Sequencing

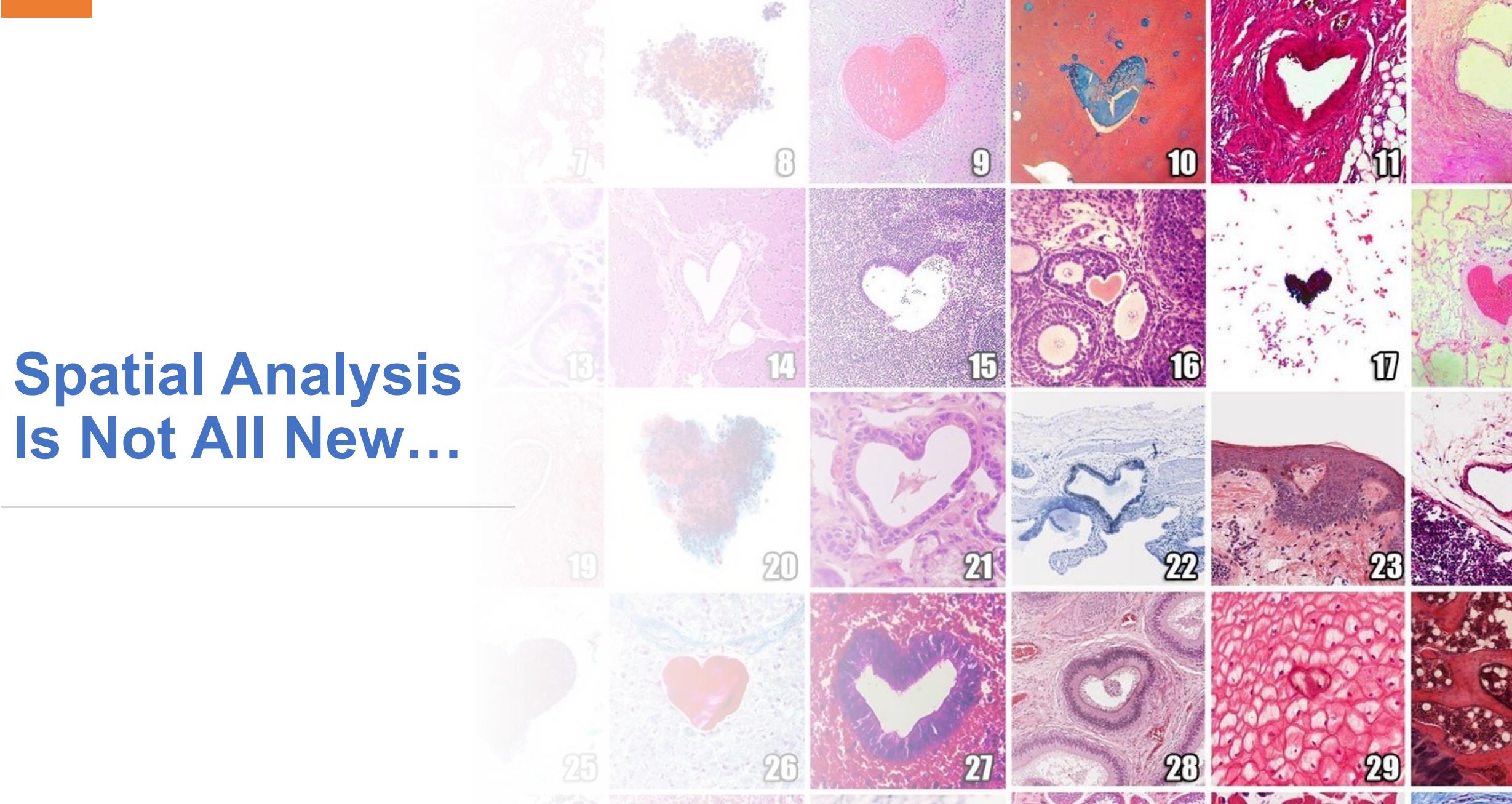


Spatially resolved transcriptomics

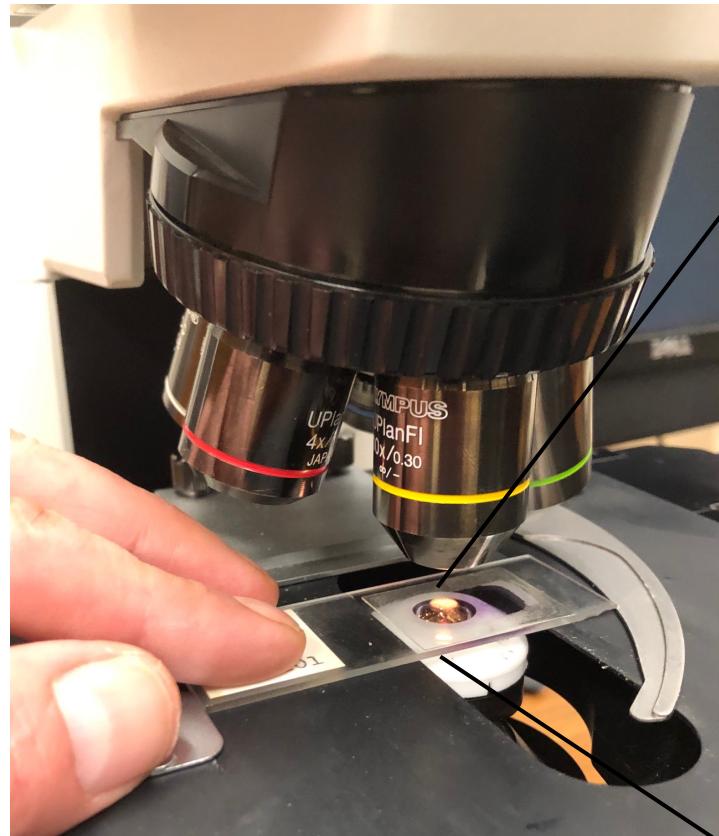
The first law of geography

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

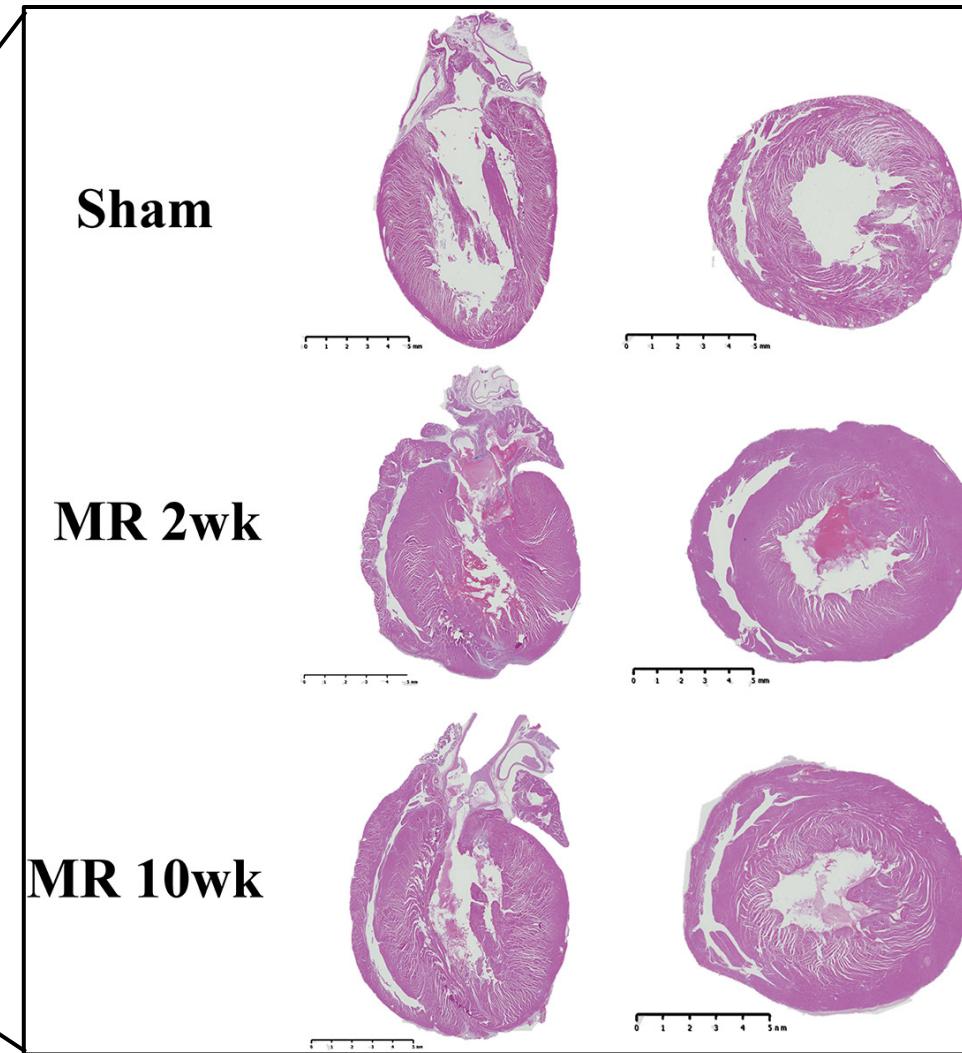
Spatial Analysis Is Not All New...



The Drawbacks Of Conventional Histology Techniques



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

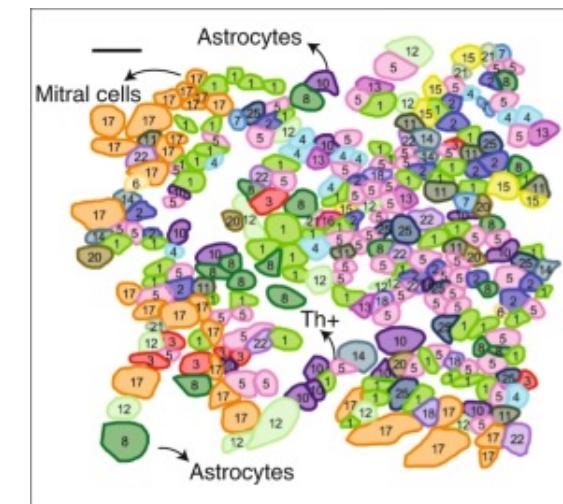
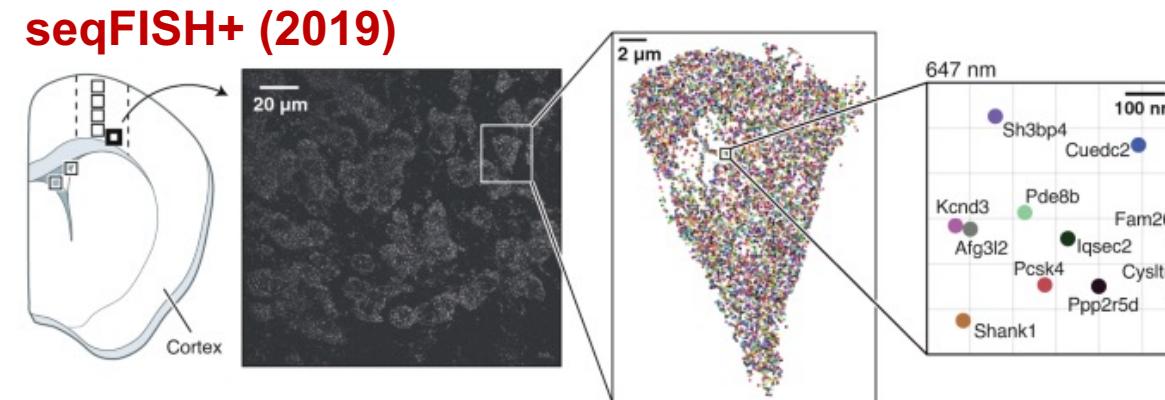
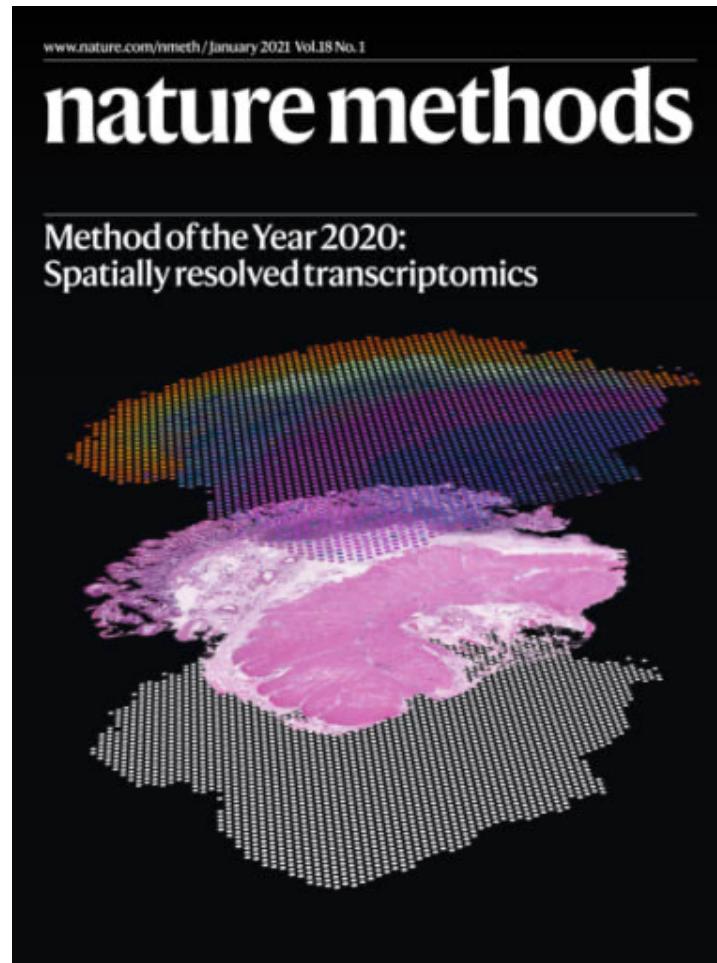


<https://images.app.goo.gl/sPBzLdEAm5uA9qjh6>

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

Integrating molecular methodologies can further increase the power of spatial analysis

Spatially Resolved Transcriptomics Unravels The Spatial Organization Of Gene Expression Within Tissues And Organ

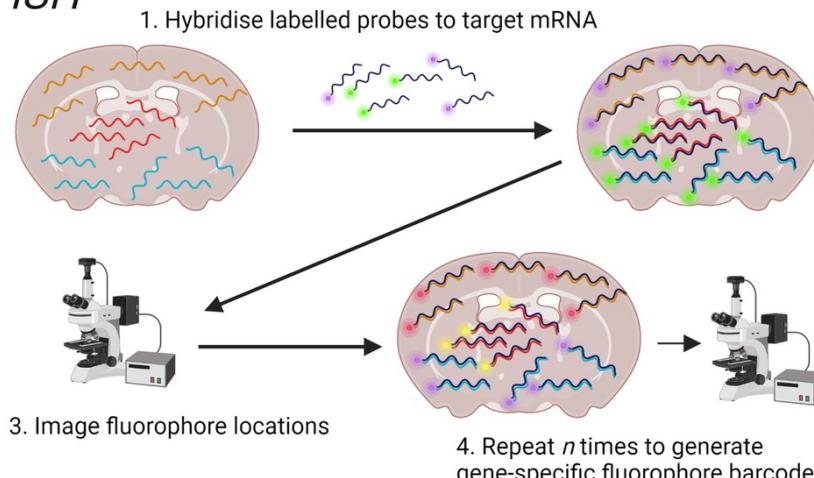


SRT provides valuable insights into the biological processes that underlie development, disease, and homeostasis

Spatially Transcriptomics Methods Can Be Broadly Classified Into Two Categories

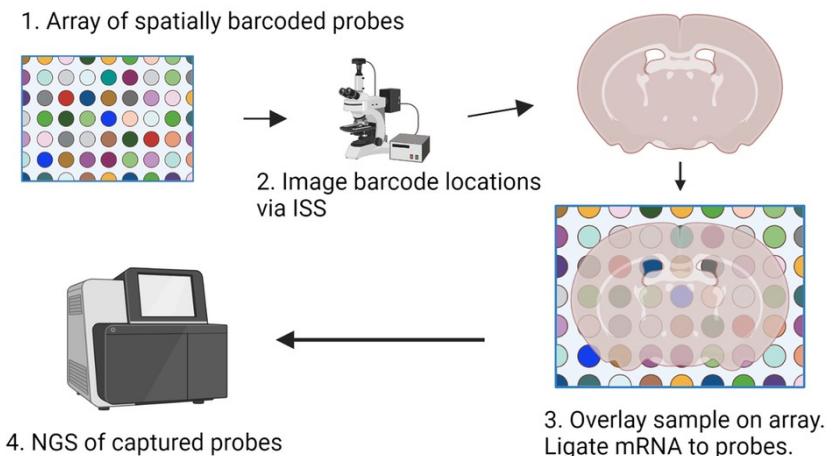
1. Imaging methods

ISH

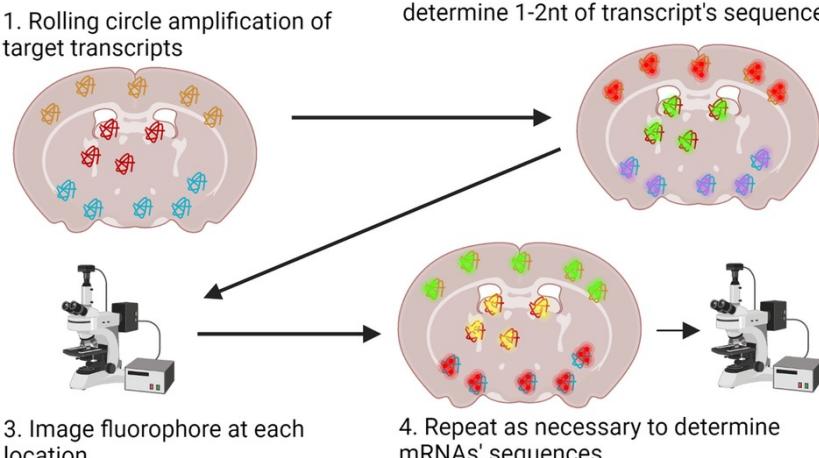


2. Sequencing methods

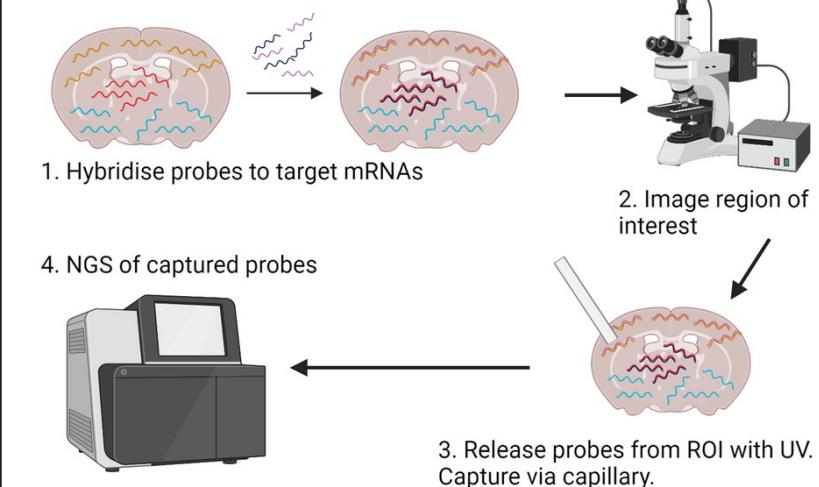
Arrays



ISS

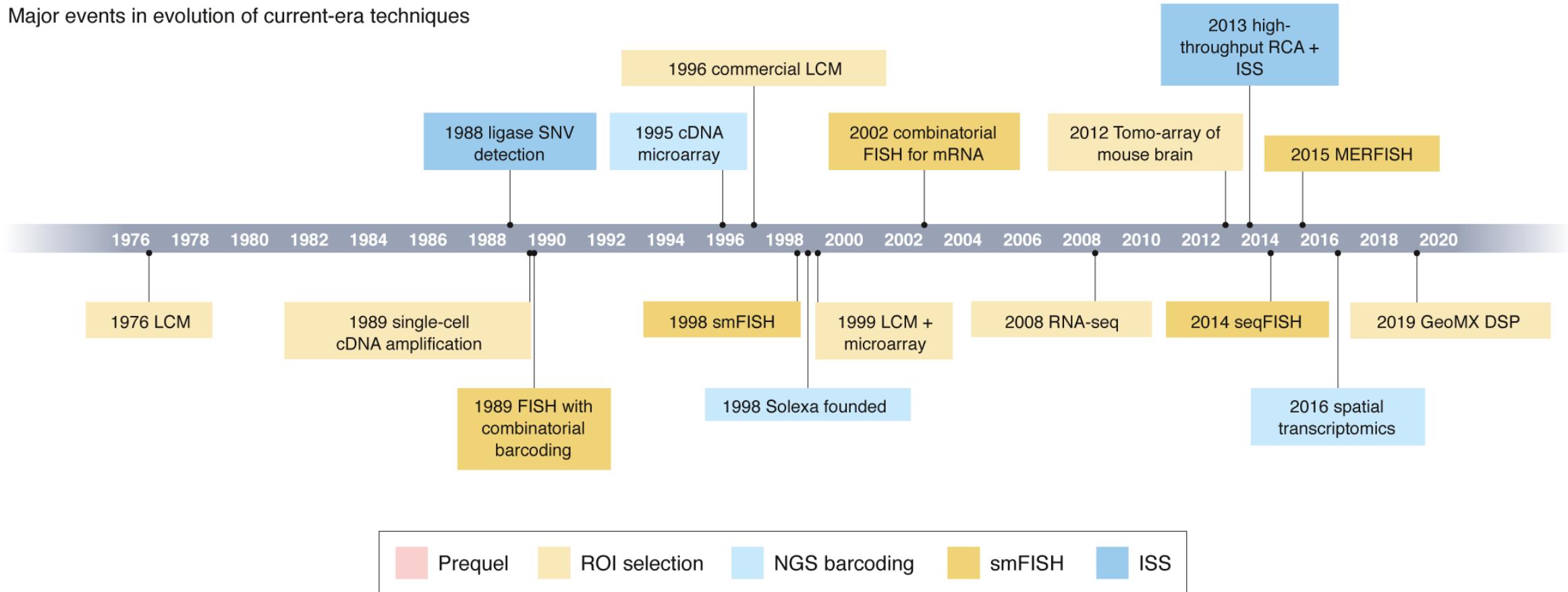


Microdissection



Timelines Of Major Events

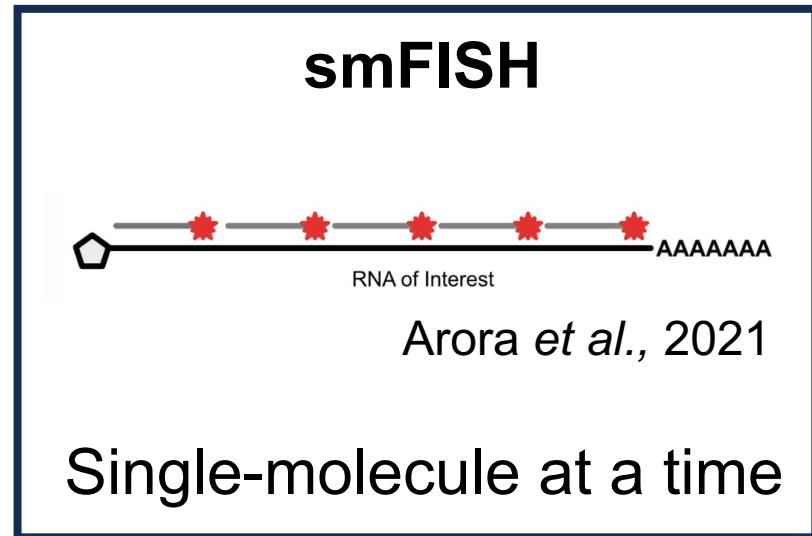
c Major events in evolution of current-era techniques



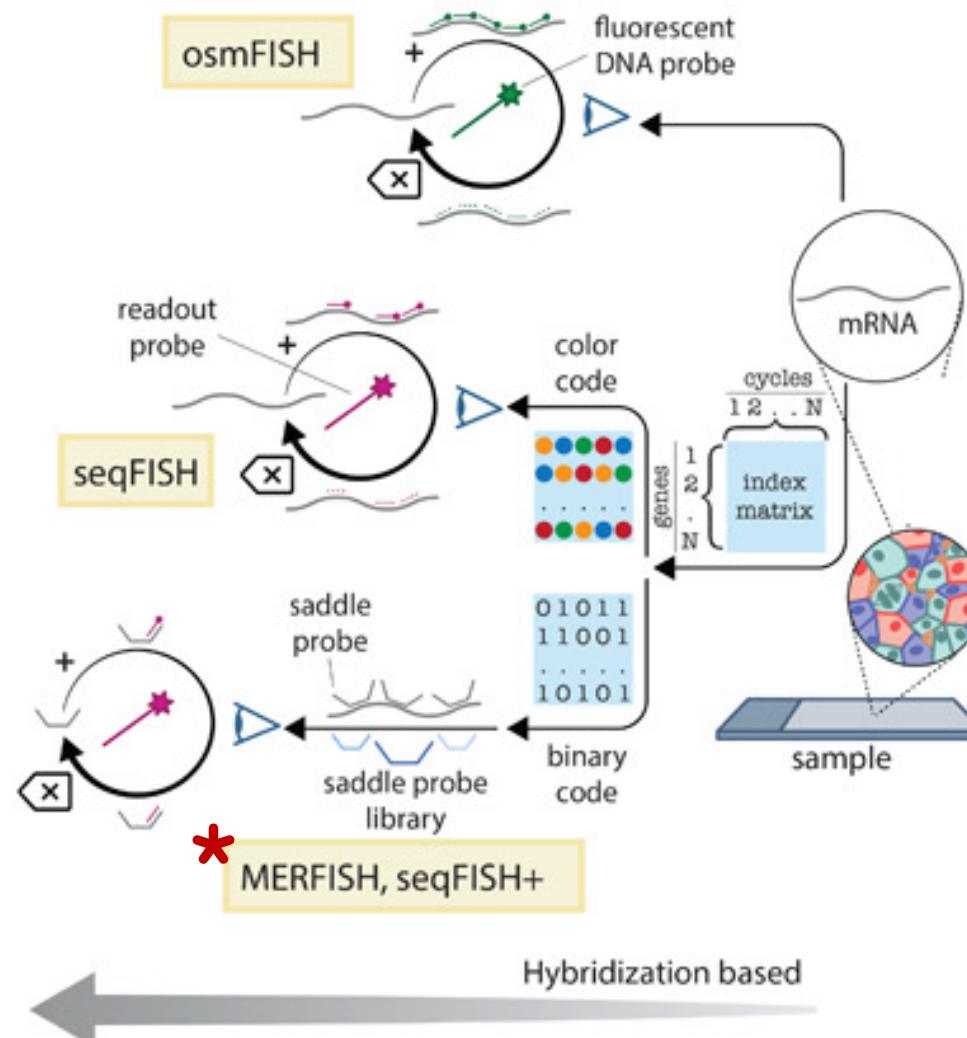
Moses et al., 2022

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

Fluorescence *In Situ* Hybridization (FISH)

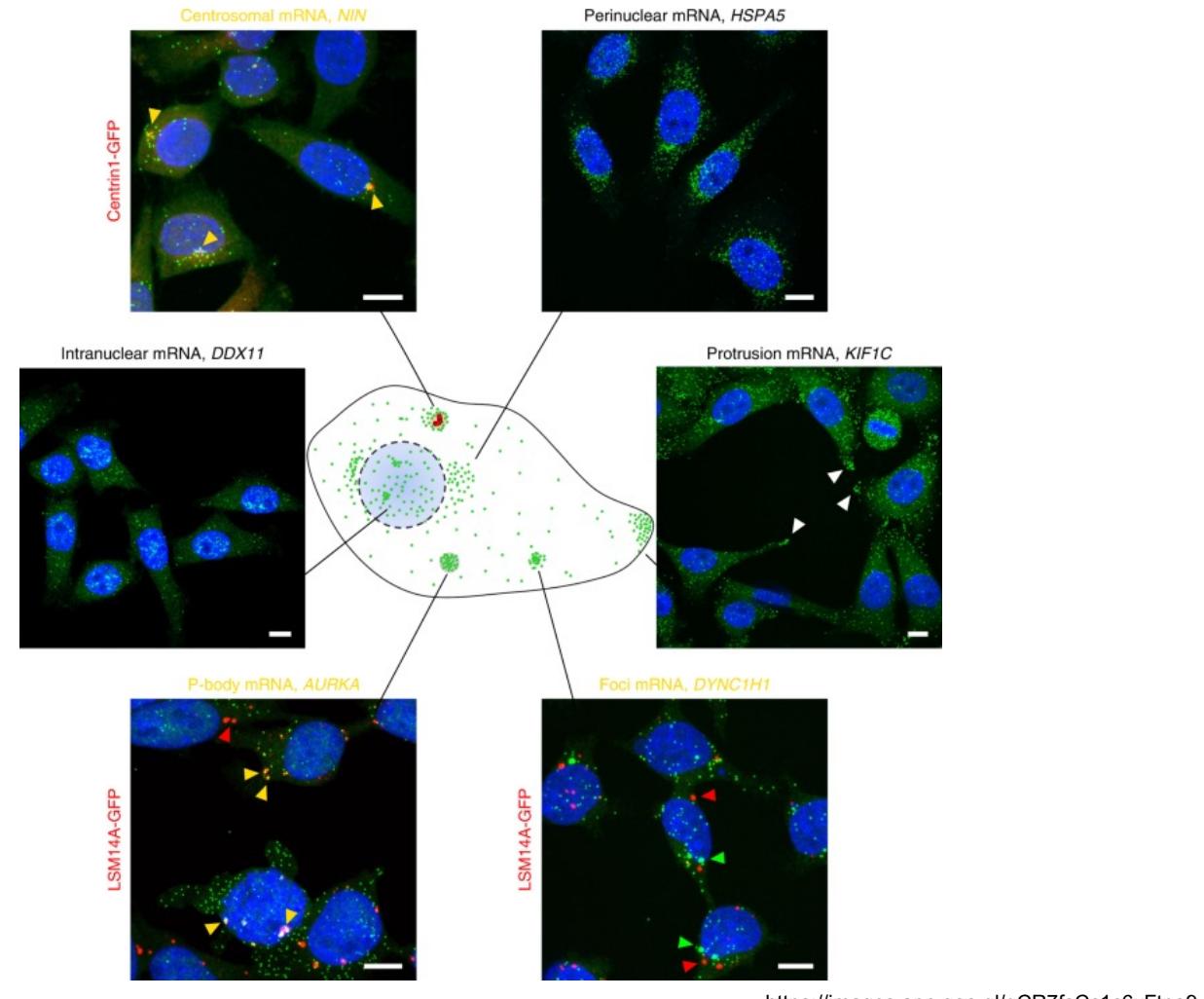
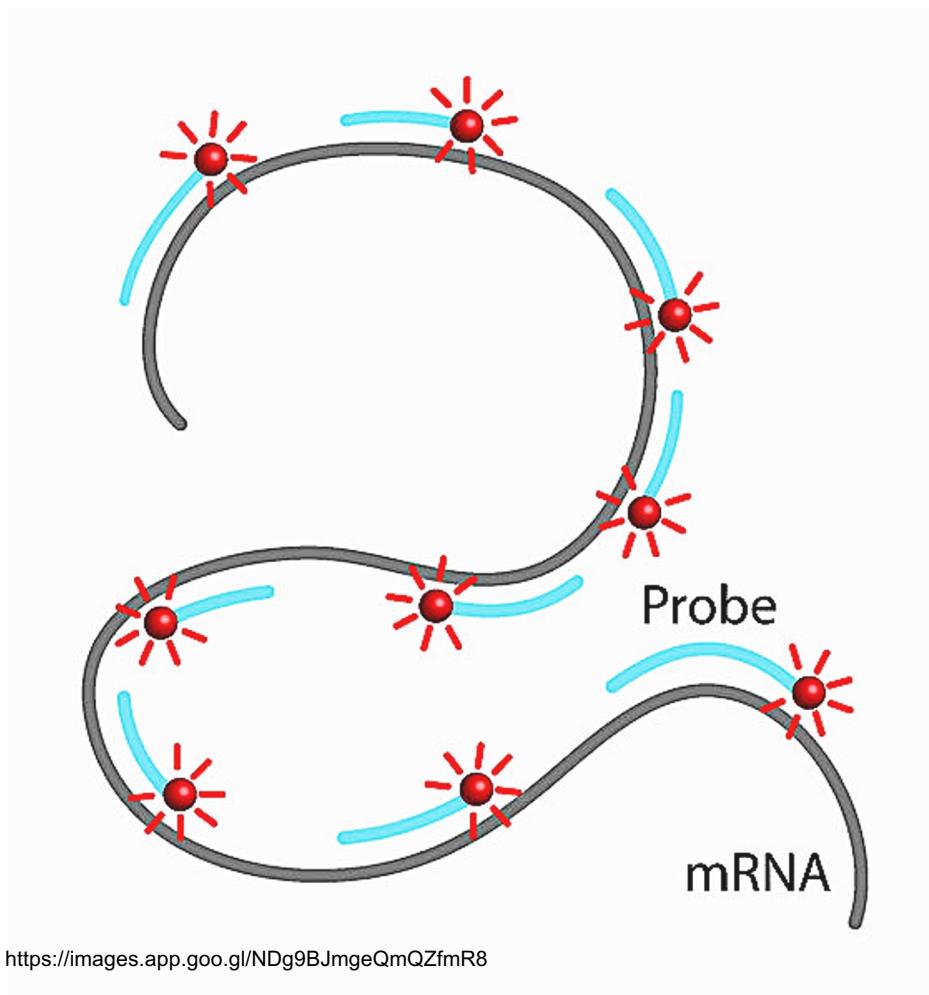


- Label probes for specific targets, hybridize in place
- Require “*a priori*” defined targets (100~1,000)
- Offer subcellular resolution



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

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



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

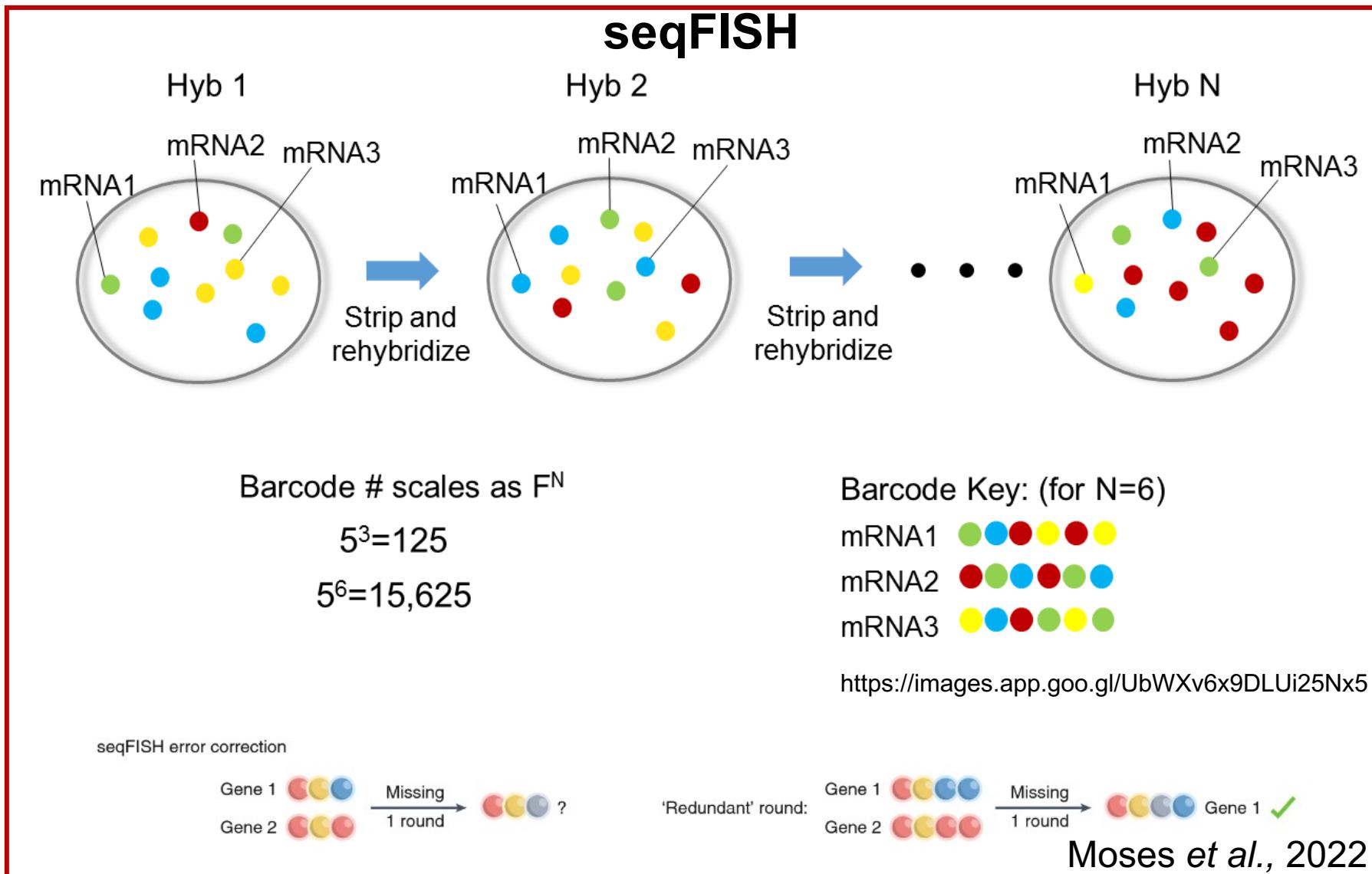
1. Image-based: *in situ* hybridization-based technology

sequential Fluorescence In Situ Hybridization (seqFISH) from Long Cai Lab (Caltech)

smFISH



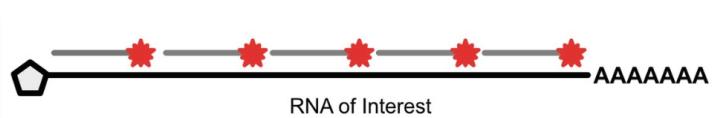
Single-molecule at a time



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

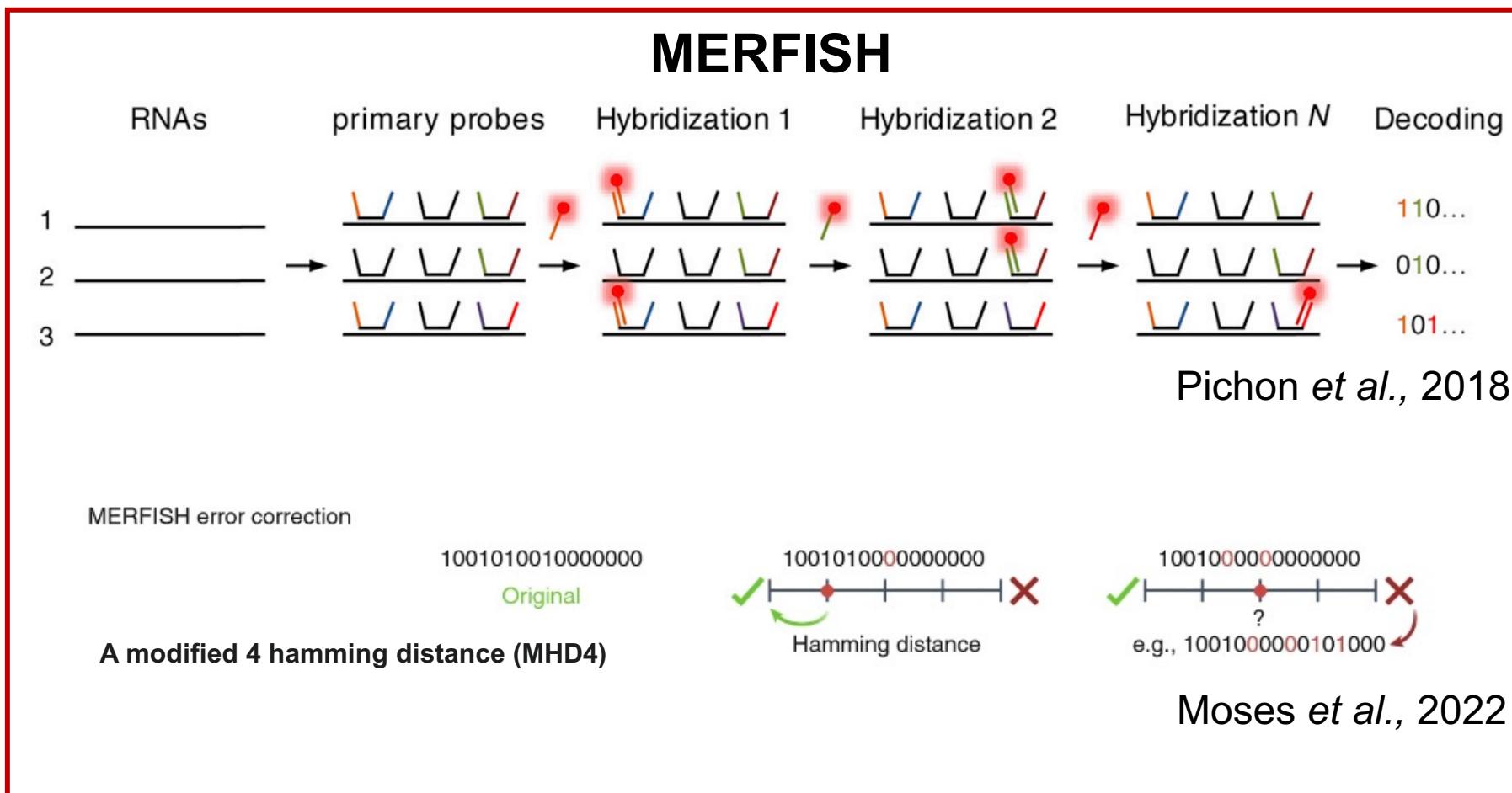
Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH) from Xiaowei Zhuan Lab (Harvard)

smFISH



Arora et al., 2021

Single-molecule at a time



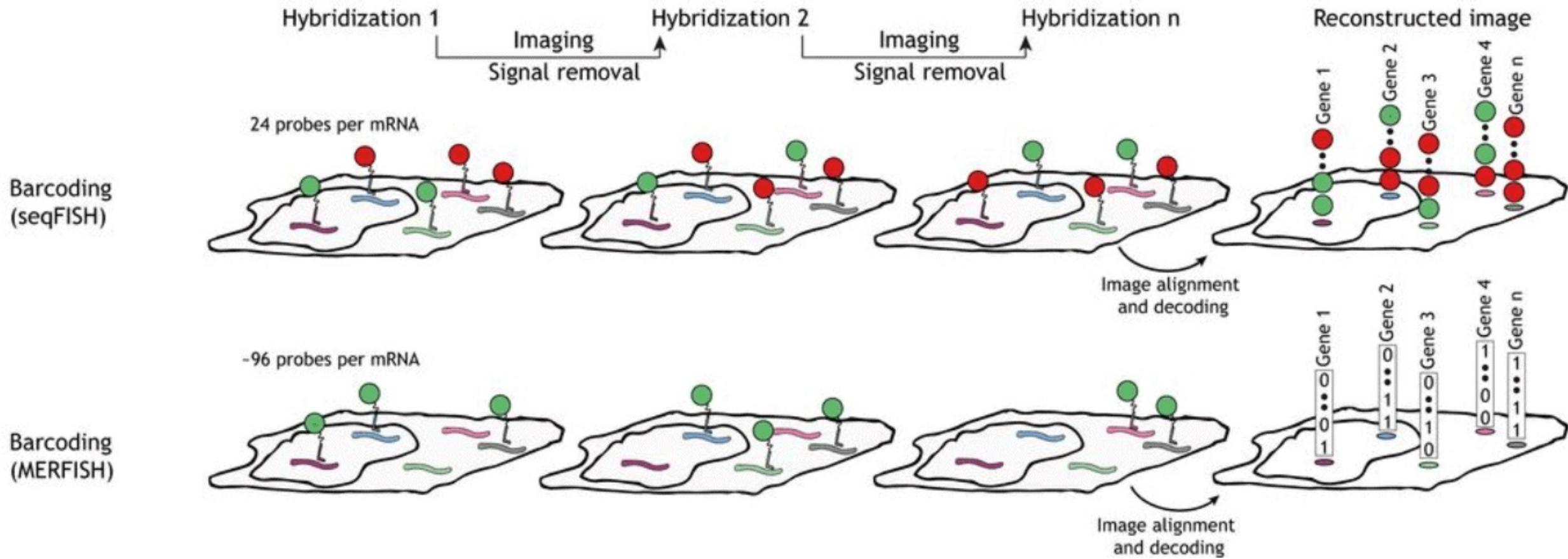
- COMBINATORIAL LABELING
- SEQUENTIAL IMAGING
- ERROR ROBUST BARCODING TO MINIMIZE THE HYBRIDIZATION FAILURE

<https://vizgen.com/wp-content/uploads/2022/10/MERFISH-Technology-Overview.png.webp>

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

seqFISH VS MERFISH

Multiplexing transcriptomes

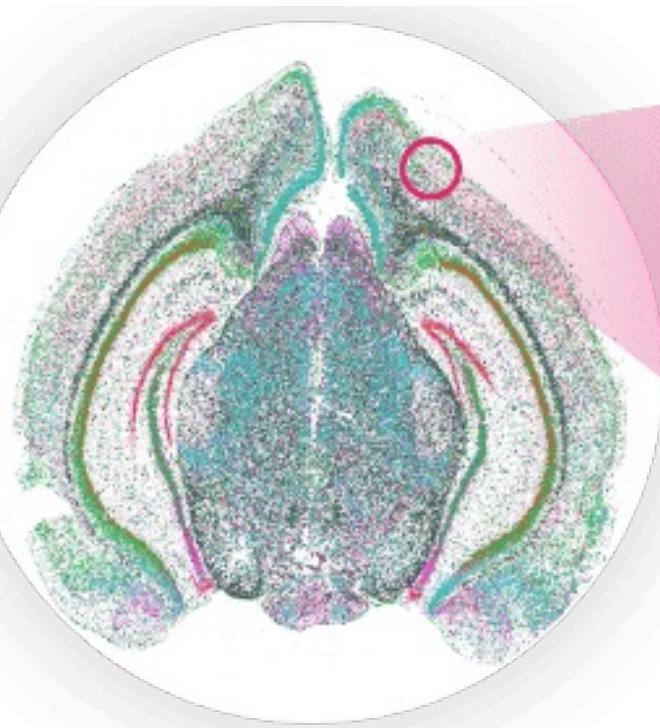


1. Image-based: *in situ* hybridization-based technology

The MERSCOPE platform, offering MERFISH technology, is commercially available through Vizgen

VIZgen

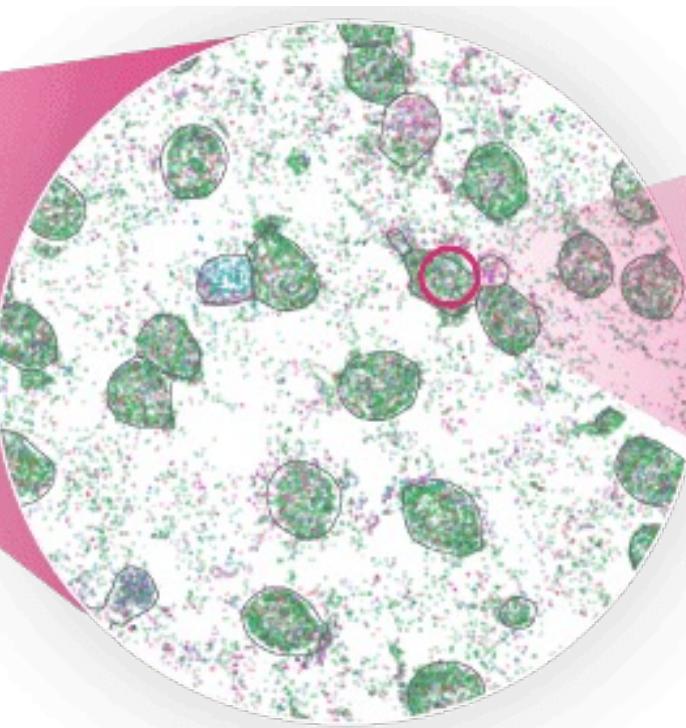
Imaging area:
1x1 cm



WHOLE SECTION

9 x 7 mm

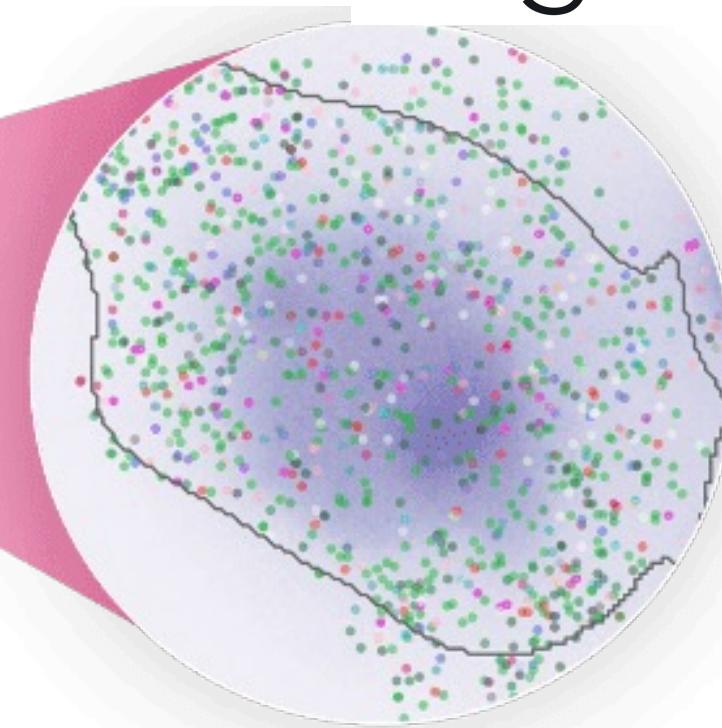
Organization of tissue



WIDE FIELD OF VIEW

200 x 200 micron

Cell interaction/function



SUB-CELLULAR

12 x 12 micron

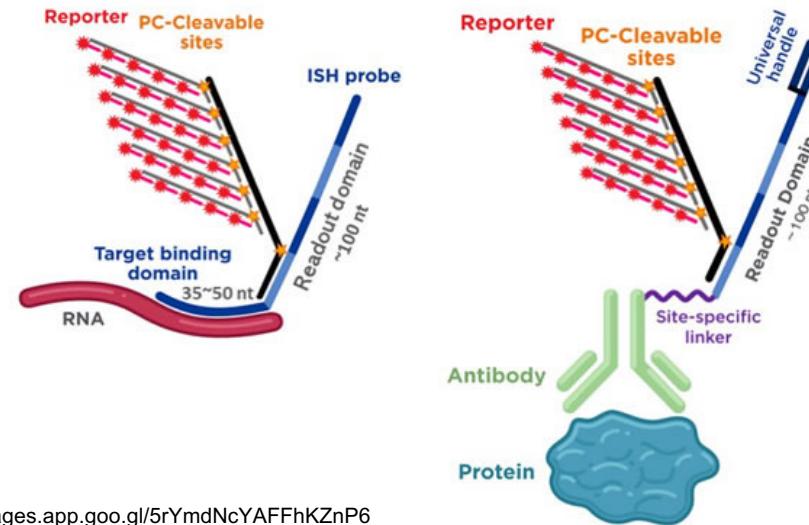
L2/3 IT Glutamatergic neuron

- Vizgen provides gene panel up to 500 genes

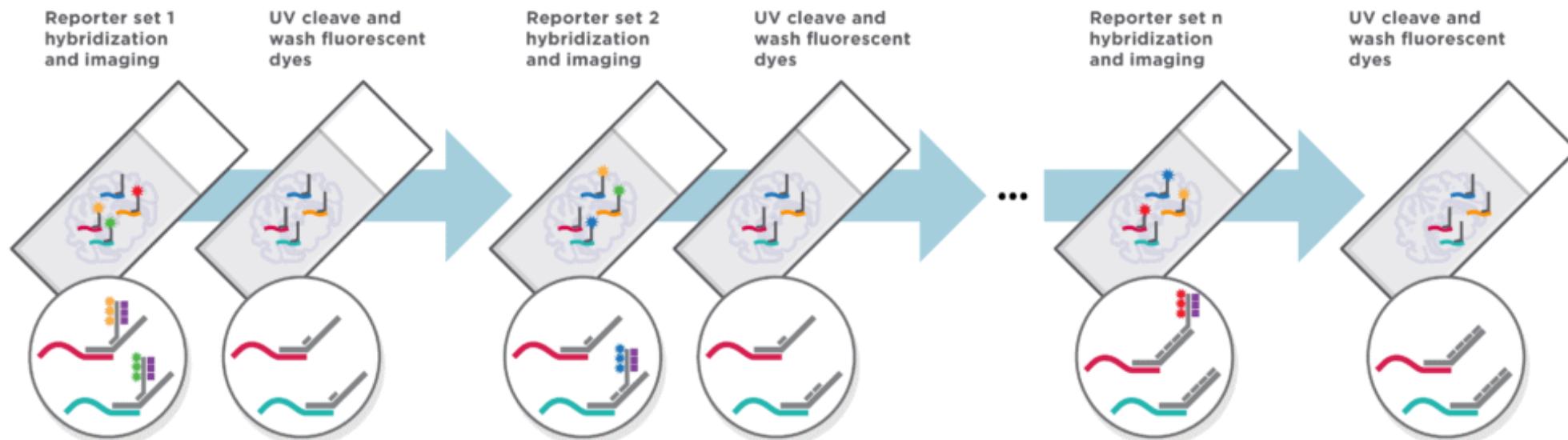
<https://images.app.goo.gl/8D3Y413MnsjBBrEdA>

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

CosMx Spatial Molecular Imager



<https://images.app.goo.gl/5rYmdNcYAFFhKZnP6>

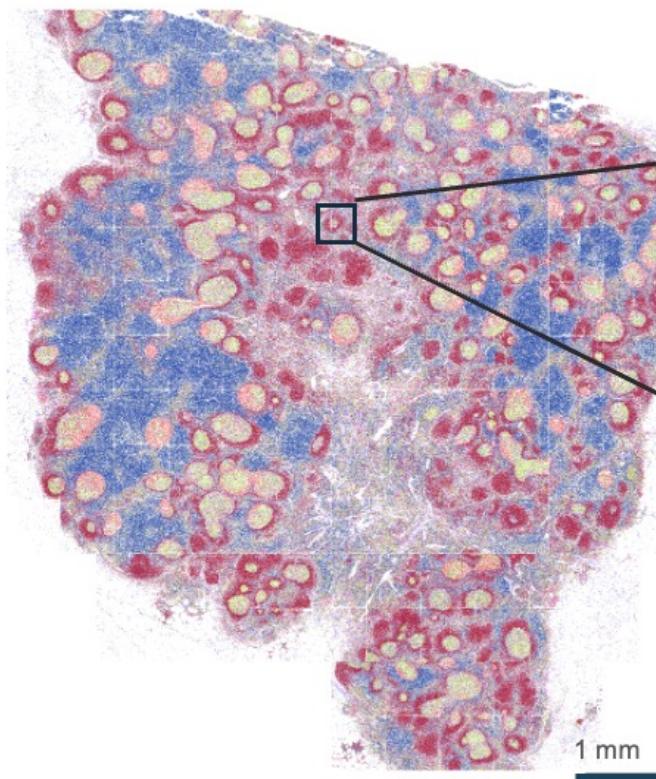


<https://images.app.goo.gl/9LWuD5tm8zEqghzn6>

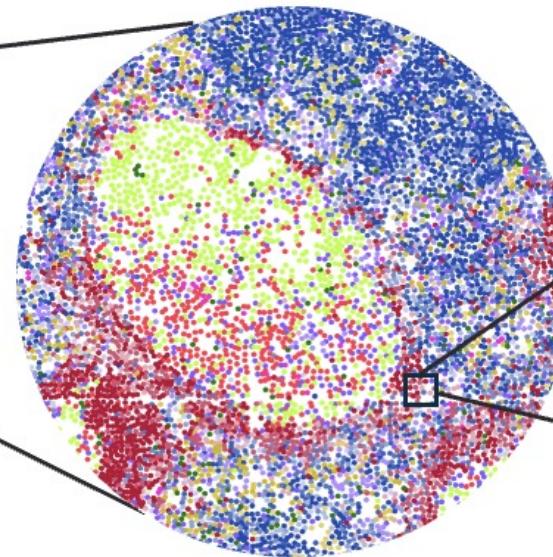
1. Image-based: *in situ* hybridization-based technology

NanoString's CosMx Spatial Molecular Imager (SMI)

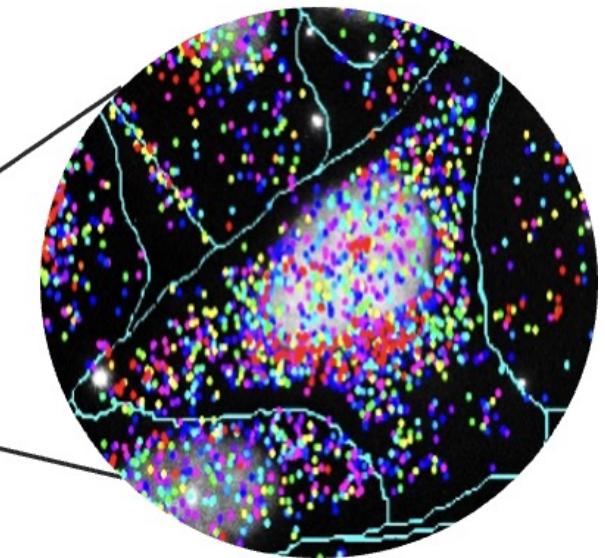
Whole Tissue Section



Single-cell



Subcellular



~50 nm in the XY plane

100um

10um

<https://images.app.goo.gl/vDTVoocyhcBRzTHR8>

CosMx SMI enables high-resolution imaging of more than 1,000 RNA and over 64 protein analytes within morphologically intact whole tissue sections.

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

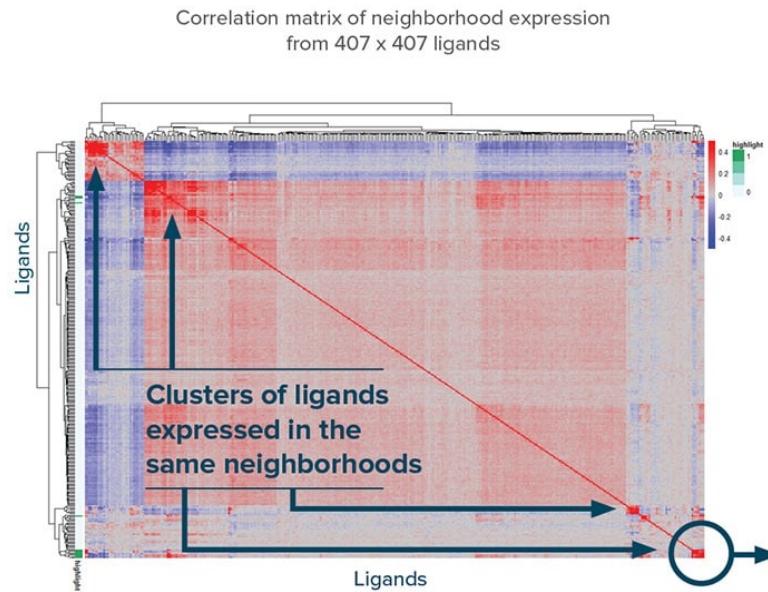
NanoString's CosMx Spatial Molecular Imager (SMI)

<https://images.app.goo.gl/vDTVoocyhcBRzTHR8>

CosMx SMI enables high-resolution imaging of more than 1,000 RNA and over 64 protein analytes within morphologically intact whole tissue sections.

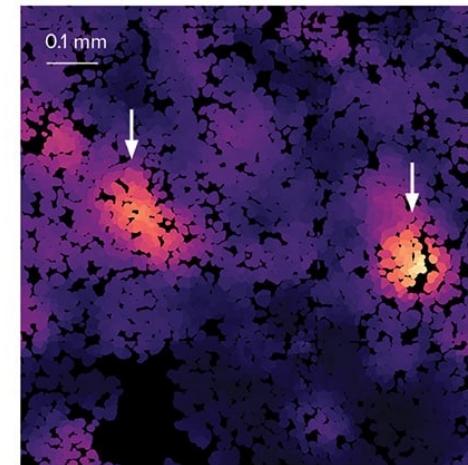
1. Image-based: *in situ* hybridization-based technology

NanoString's CosMx Spatial Molecular Imager (SMI)

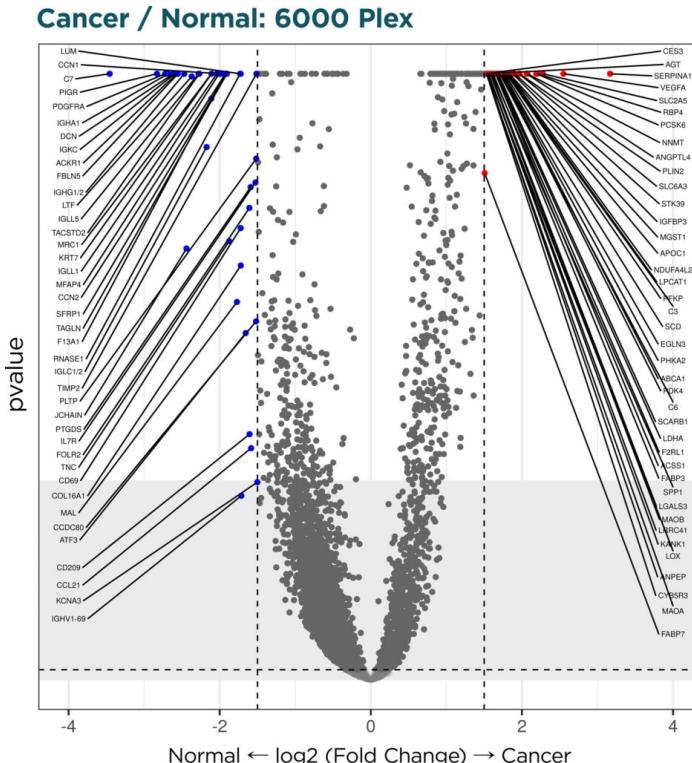


<https://images.app.goo.gl/iEp7L4hnvkU6REM4A>

One cluster of co-expressed ligands in sporadic hotspots

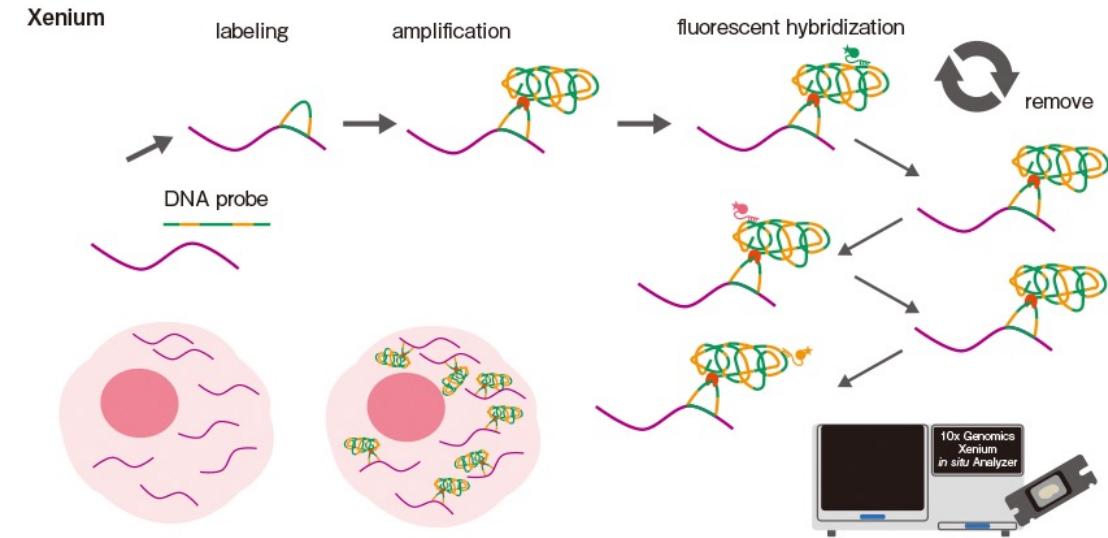
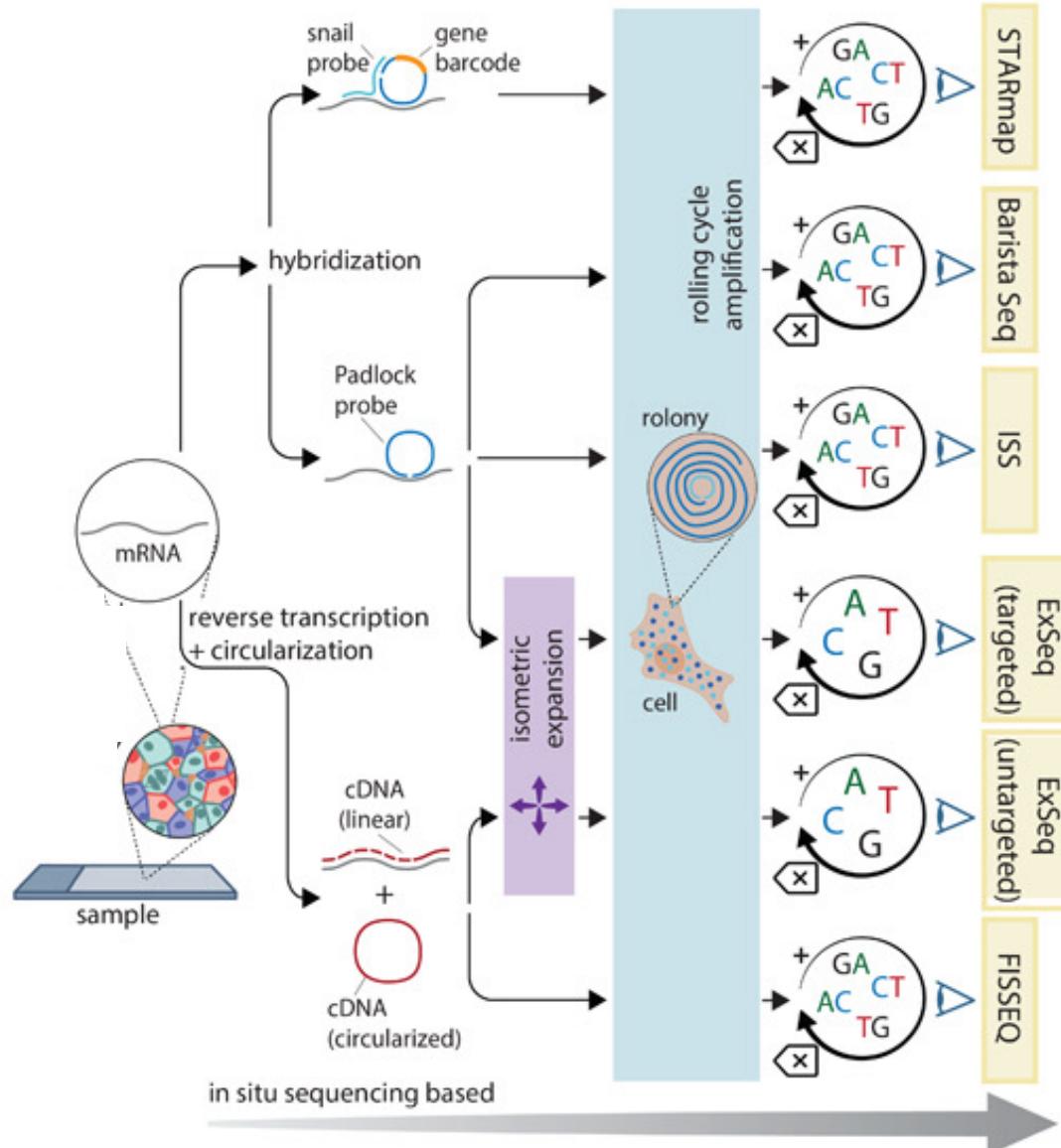


One cluster: CXCL9, PTPRC, HLA-E, HLA-DPA1, HLA-DPB2, HLA-DQA1, HLA-DMA, HLA-DMB, HLA-DQA2, HLA-DQB1, HLA-DRA



The CosMx™ 6K Discovery panel enables to spatially analyze virtually the entire transcriptome at the single cell level

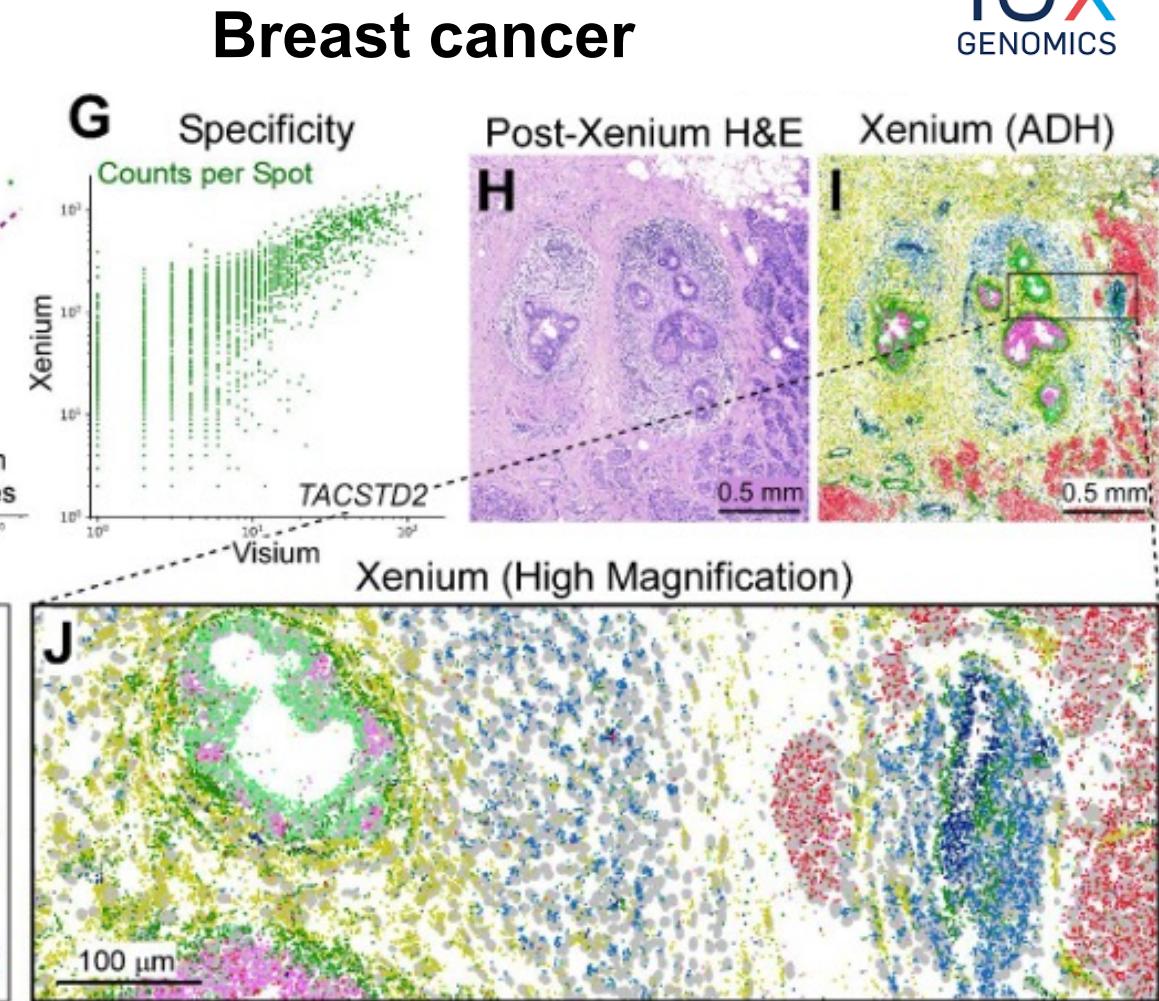
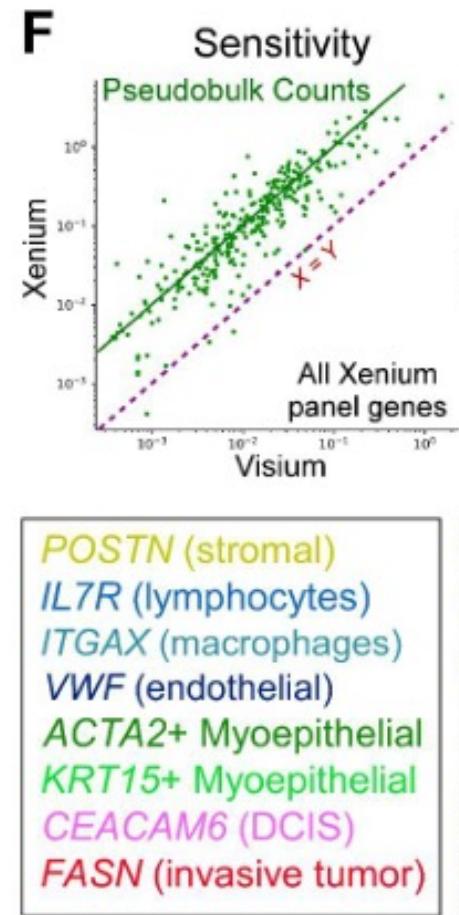
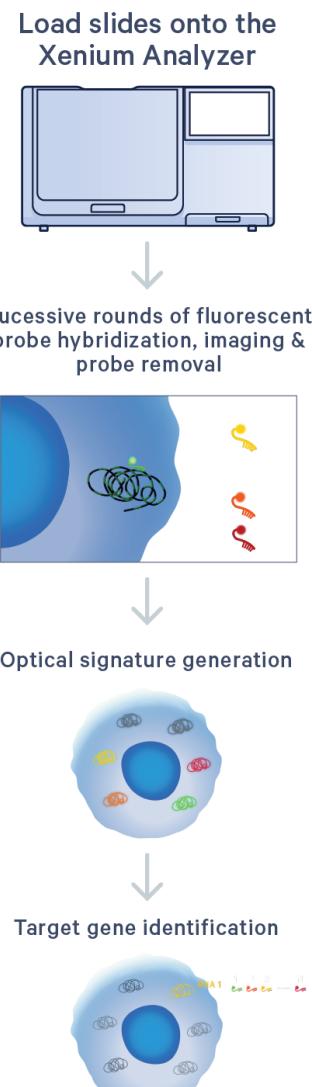
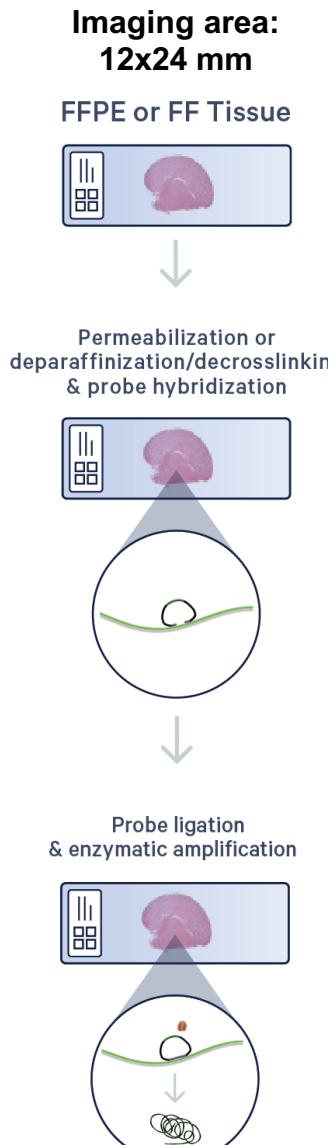
2. Image-based: *in situ* sequencing-based technology



- Sequence the transcripts in place
- Some rely on “*a priori*” defined targets
- Offer subcellular resolution

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

Xenium *in situ*

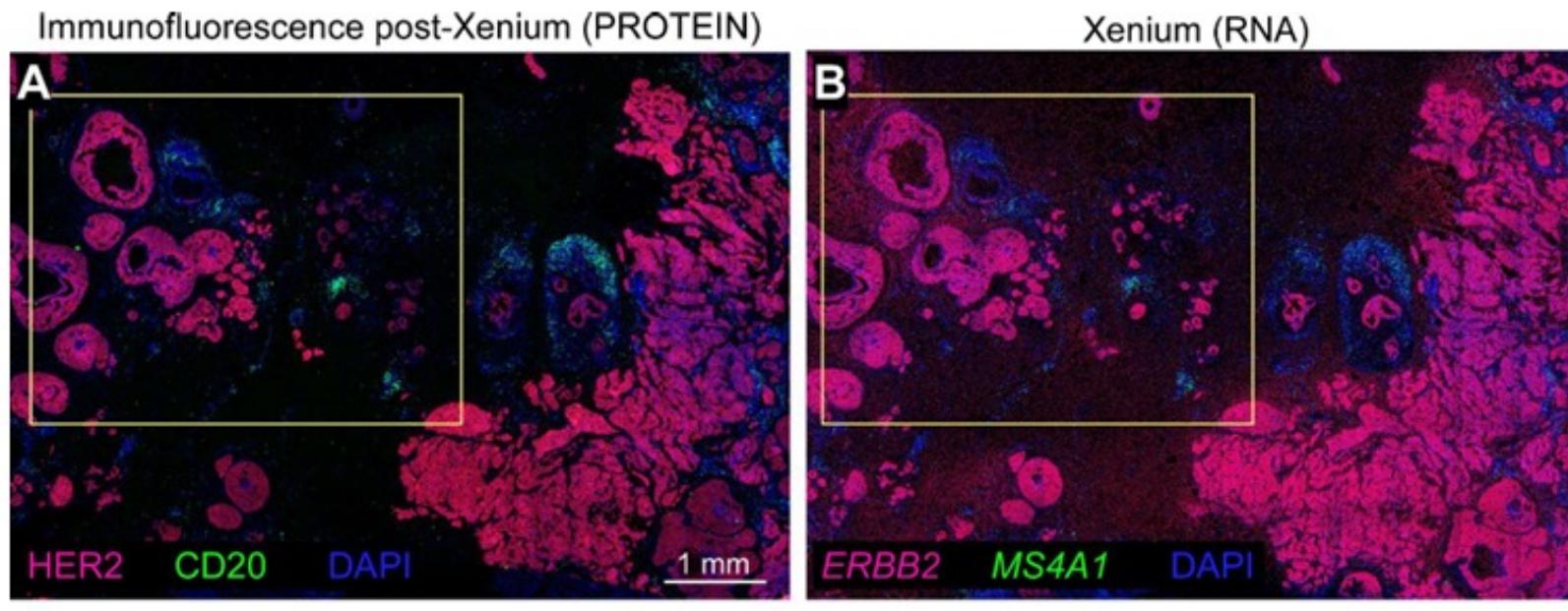


10X
GENOMICS

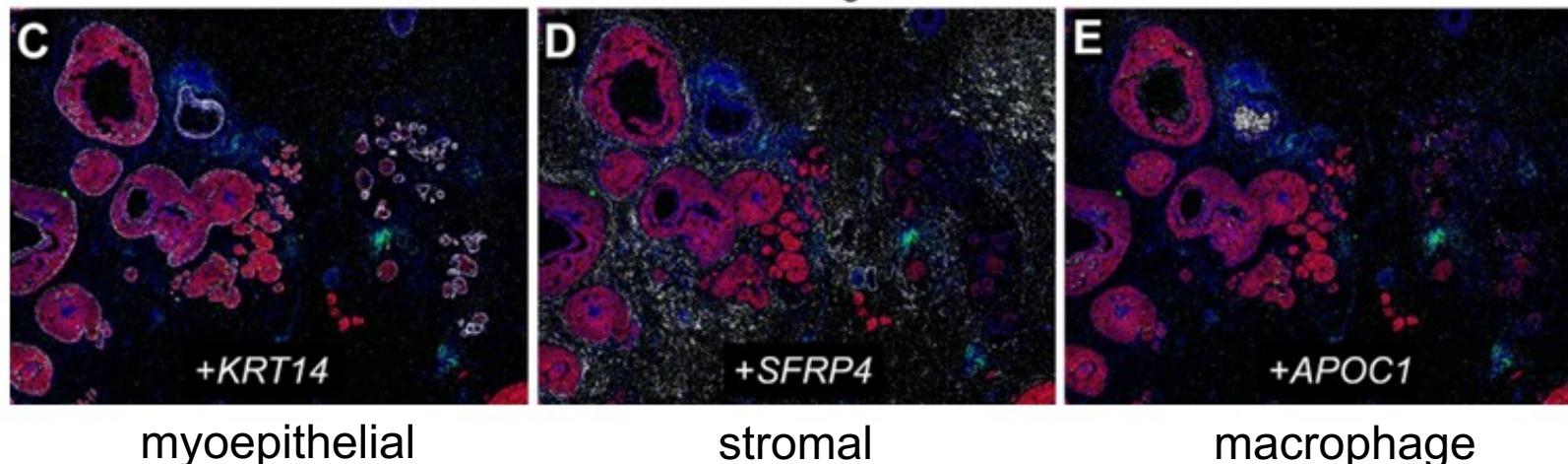
2. Image-based: *in situ* sequencing-based technology

RNA from Xenium and protein immunofluorescence can be visualized simultaneously

Breast cancer



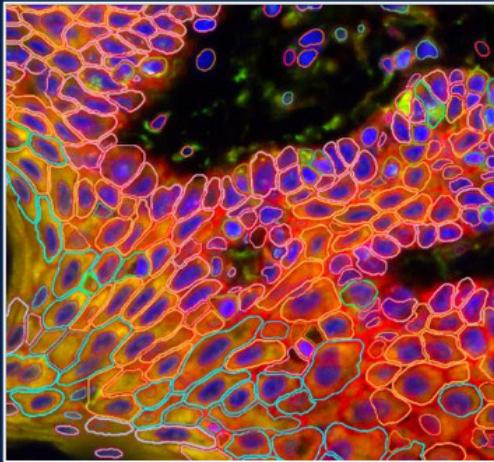
Immunofluorescence Protein Registered with Xenium RNA



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

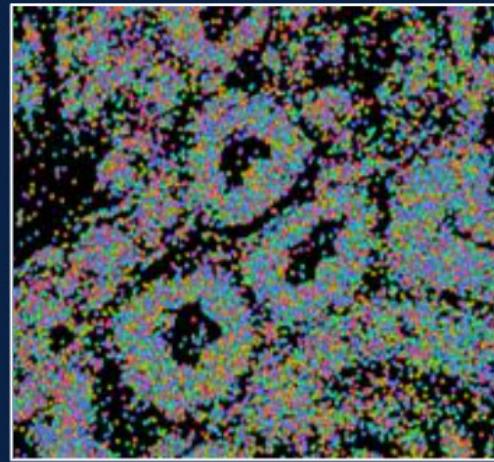
Upcoming Xenium Product Roadmap

Multimodal Segmentation
Broad compatibility



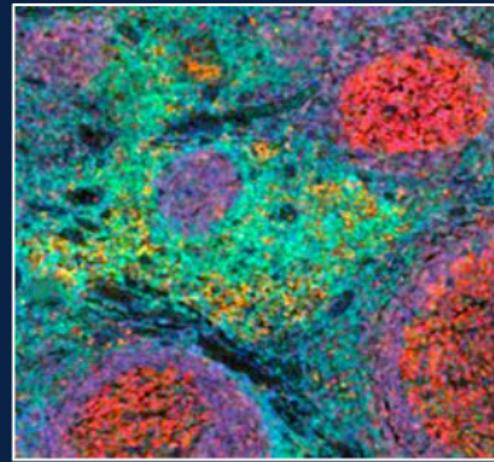
Shipping Q1

~5,000 Plex Panels
~5-day run
High quality data



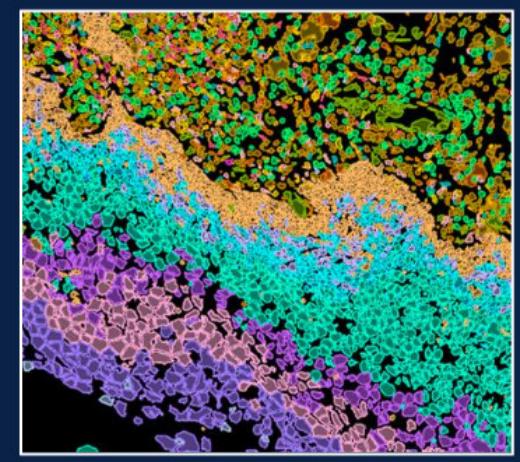
Q2 2024

In-line Multiplex Protein
Same slide as RNA



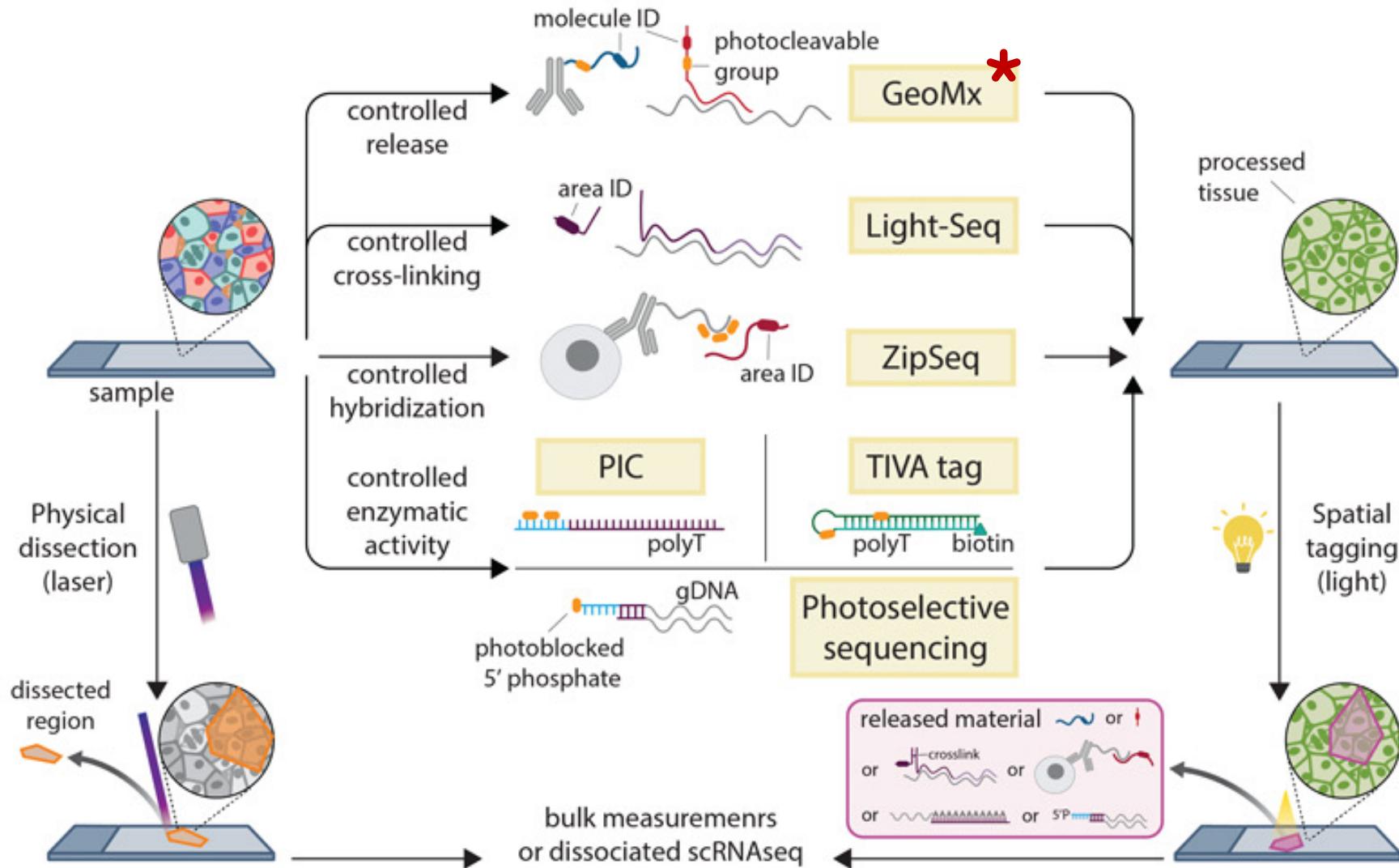
H2 2024

1,000–2,000 Plex Panels
Offers more flexibility



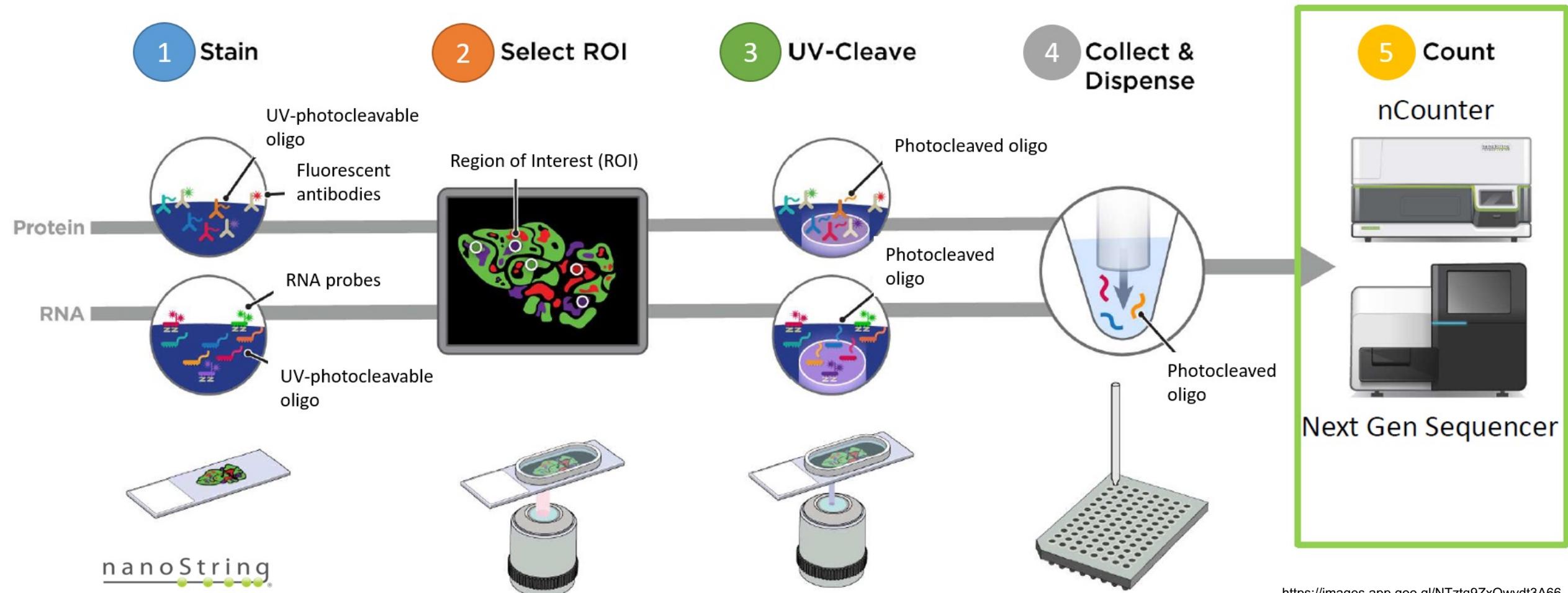
2024+

3. Sequencing-based: Microdissection-based technology



3. Sequencing-based: Microdissection-based technology

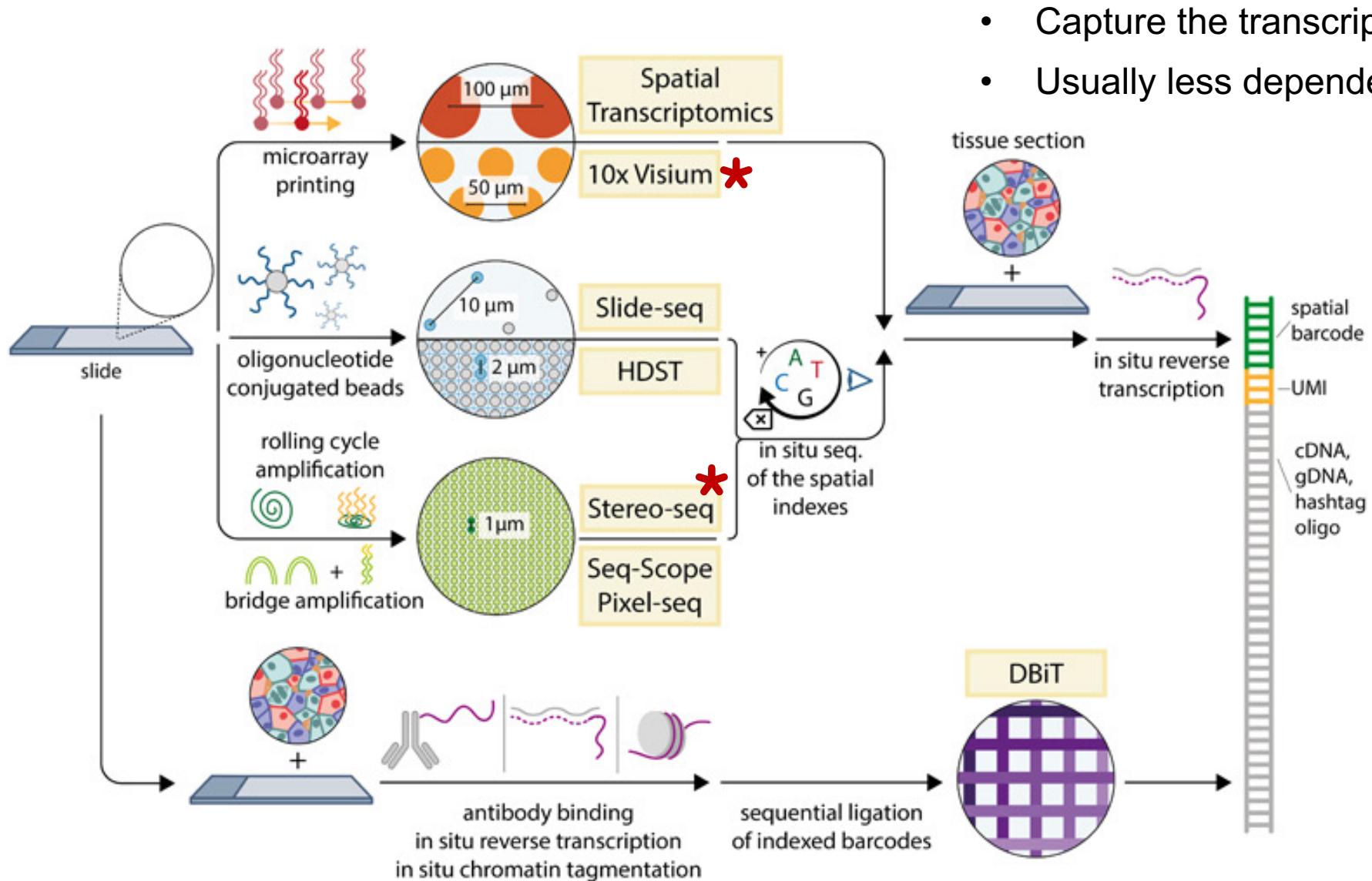
NanoString's GeoMx Digital Spatial Profiling (DSP)



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

- NanoString provides a 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

4. Sequencing-based: spatial barcoding technologies

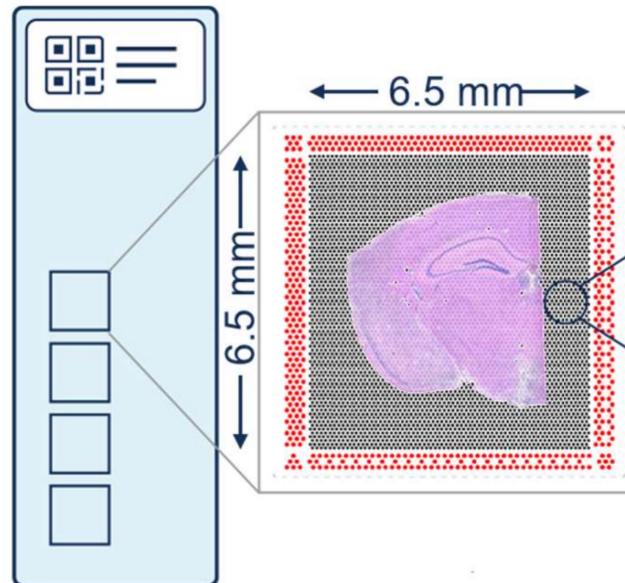


- Capture the transcripts *in situ* but sequence *ex situ*
- Usually less dependent on prior selection of targets

4. Sequencing-based: spatial barcoding technologies

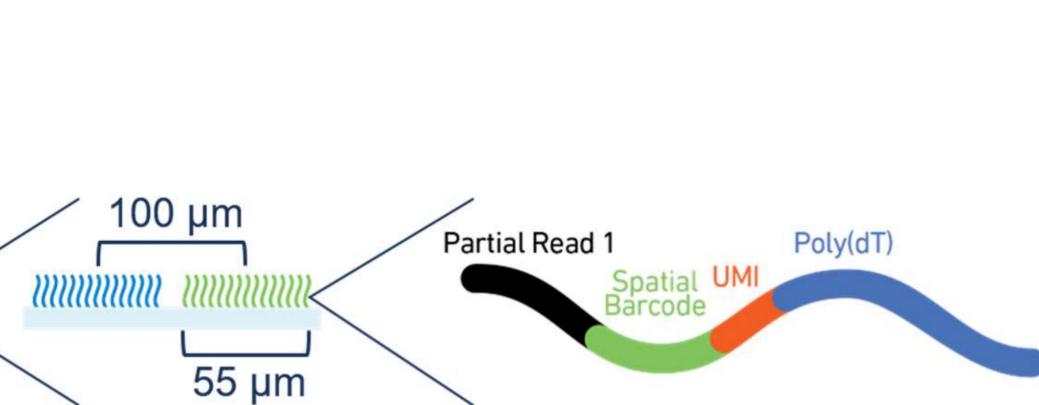
Visium by 10X Genomics

Visium Spatial
Gene Expression
Slide



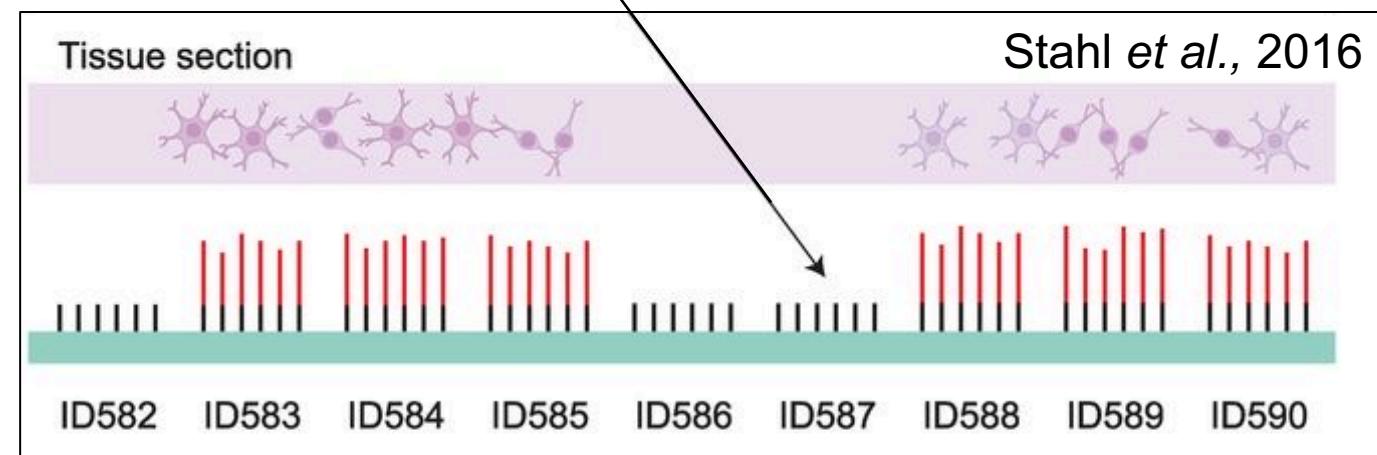
Capture Area with
~5000 Barcoded
Spots

Visium Gene
Expression Barcoded
Spots



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

10X
GENOMICS



Stahl et al., 2016

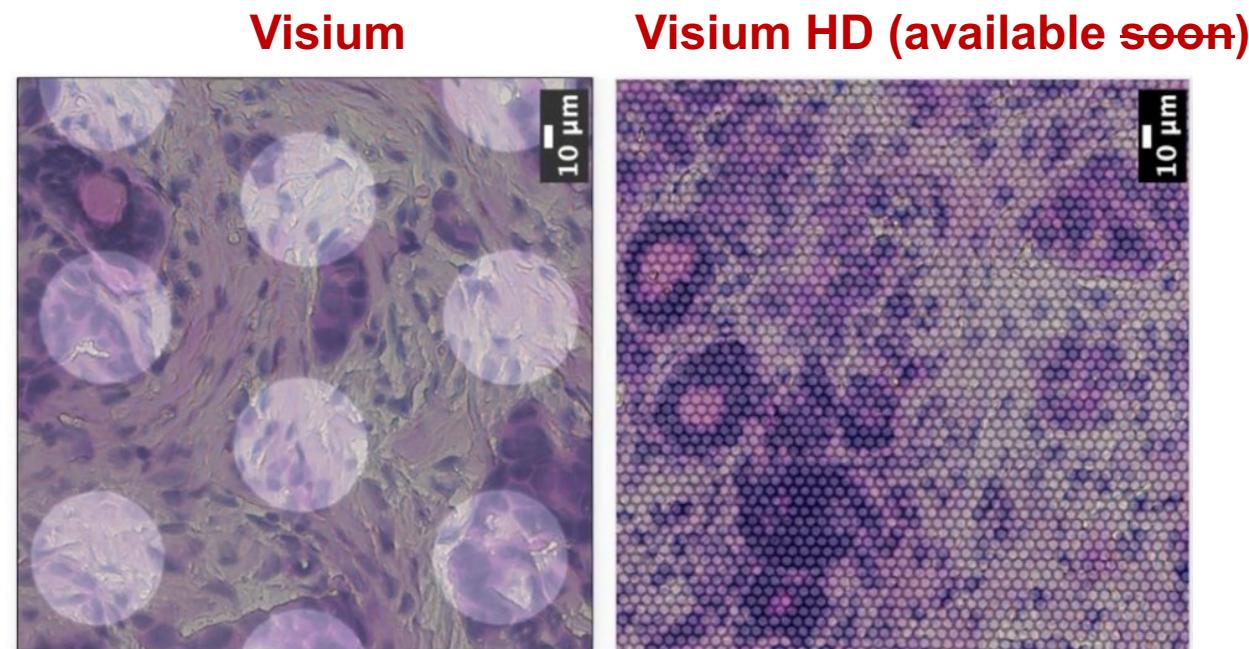
1. Array-based technique (4 arrays per slide)
2. 6.5 mm x 6.5 mm area to put the sample on
3. 4,992 spots arranged in a hexagonal grid
4. Array specs:
 - Spot diameter: 55 μm
 - Center-to-center distance: 100 μm

Over The Last Decade, We Have Observed The Evolution Of Visium



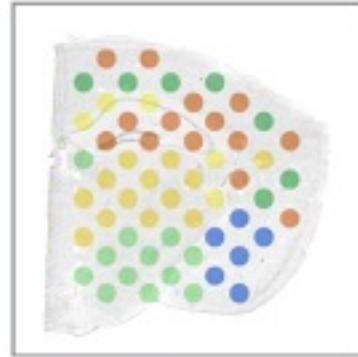
<https://images.app.goo.gl/5jH4HrQoE5VBPksZ9>

- 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



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

Visium HD Enables Spatially Resolved, Single Cell Scale Resolution Mapping Of Human Tissue

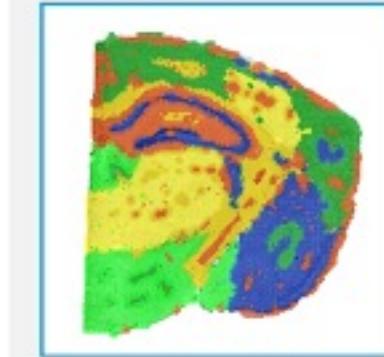


First Generation
ST

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



Visium
Resolution 4x

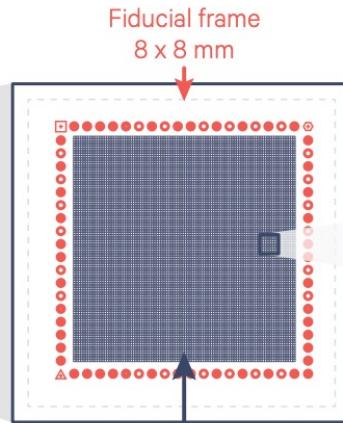


Visium HD
Resolution 1,500x

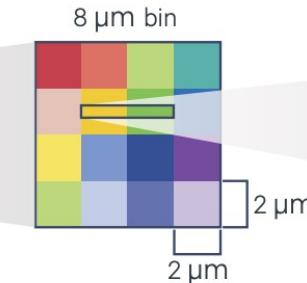
Visium HD Spatial Gene Expression



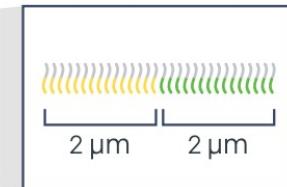
Visium HD Slide,
6.5 mm



Capture Area with
continuous lawn of oligos,
6.5 x 6.5 mm

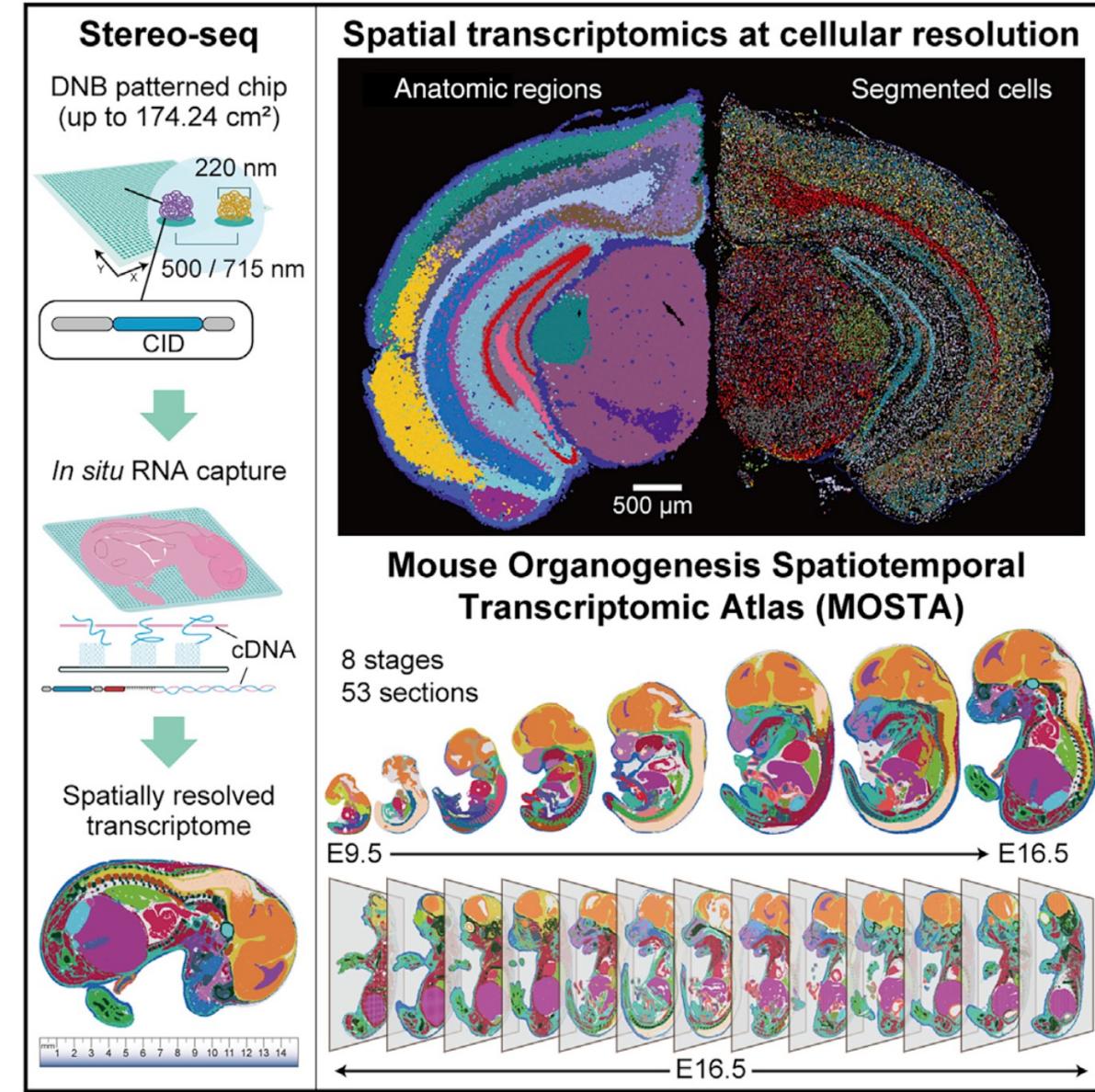
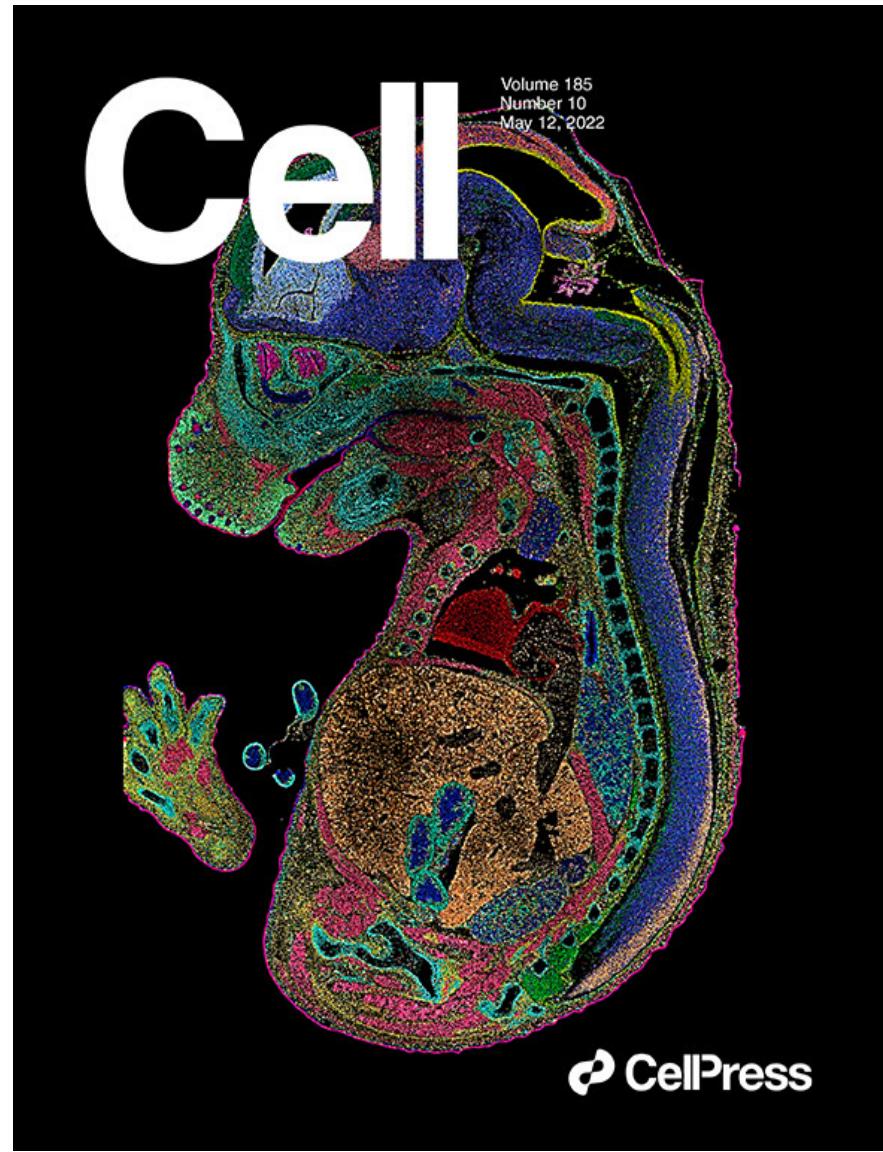


Grid of 2 x 2 μ m barcoded squares,
binned to 8 x 8 μ m



4. Sequencing-based: spatial barcoding technologies

Stereo-seq by BGI is a transformational breakthrough

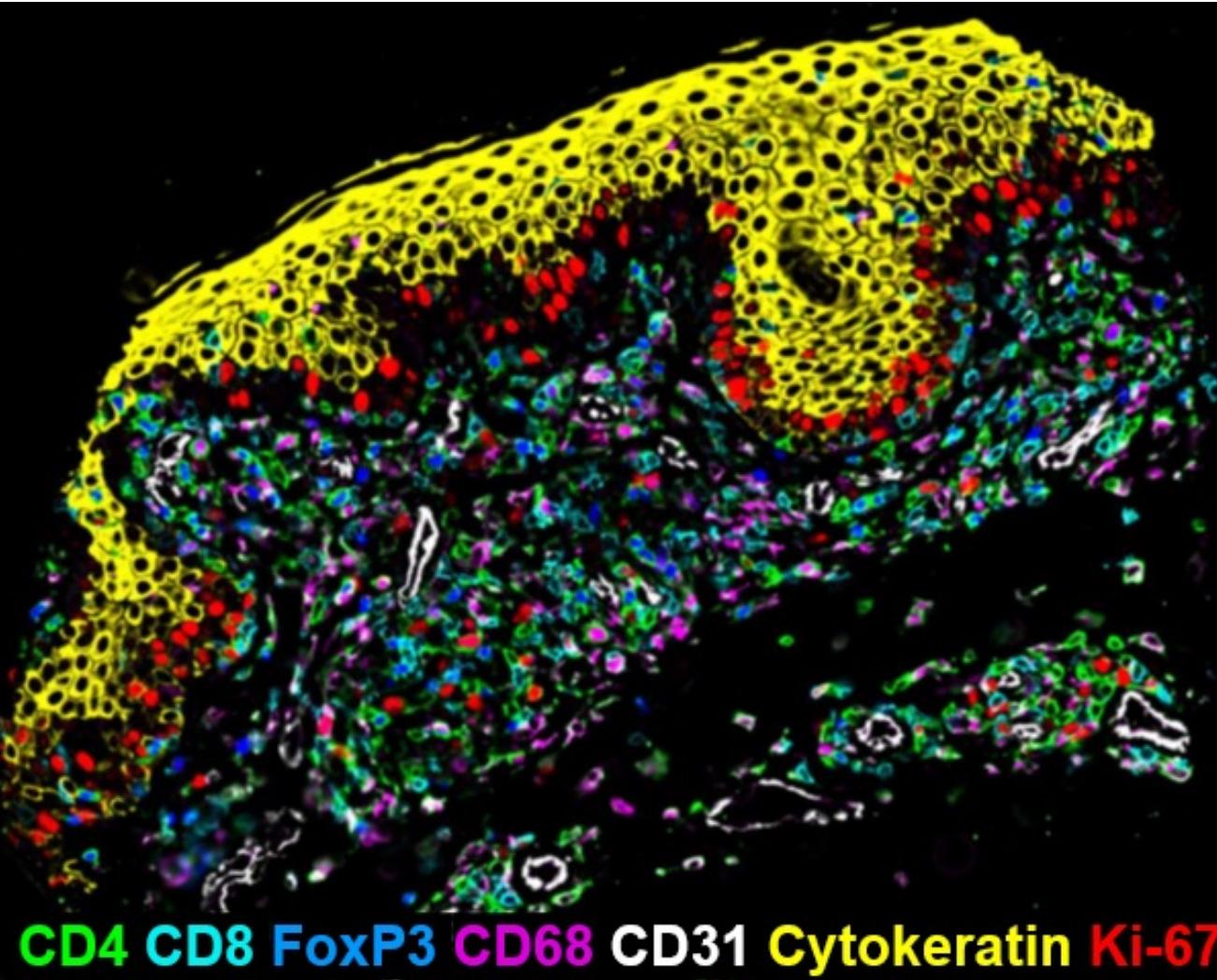


- Scalable
- High res
- Deep cov
- Not commercially available in Korea

Chen et al., 2022

5. Spatial Proteomics Offers Direct Measurements Of Protein Localization And Abundance In Space

CODEX (PhenoCycler) from Akoya



Several antibody-dependent technologies

1. Multiplexed IHC

- t-CyCLF and CODEX (PhenoCycler)
- PhenoCycler-Fusion 2.0 (101-plex)

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/high-plex image-based methods profiling gene systematically: Visium HD, Xenium-5K (10X), GeoMx, and CosMx-6K (Nanostring)

2. Hypothesis testing

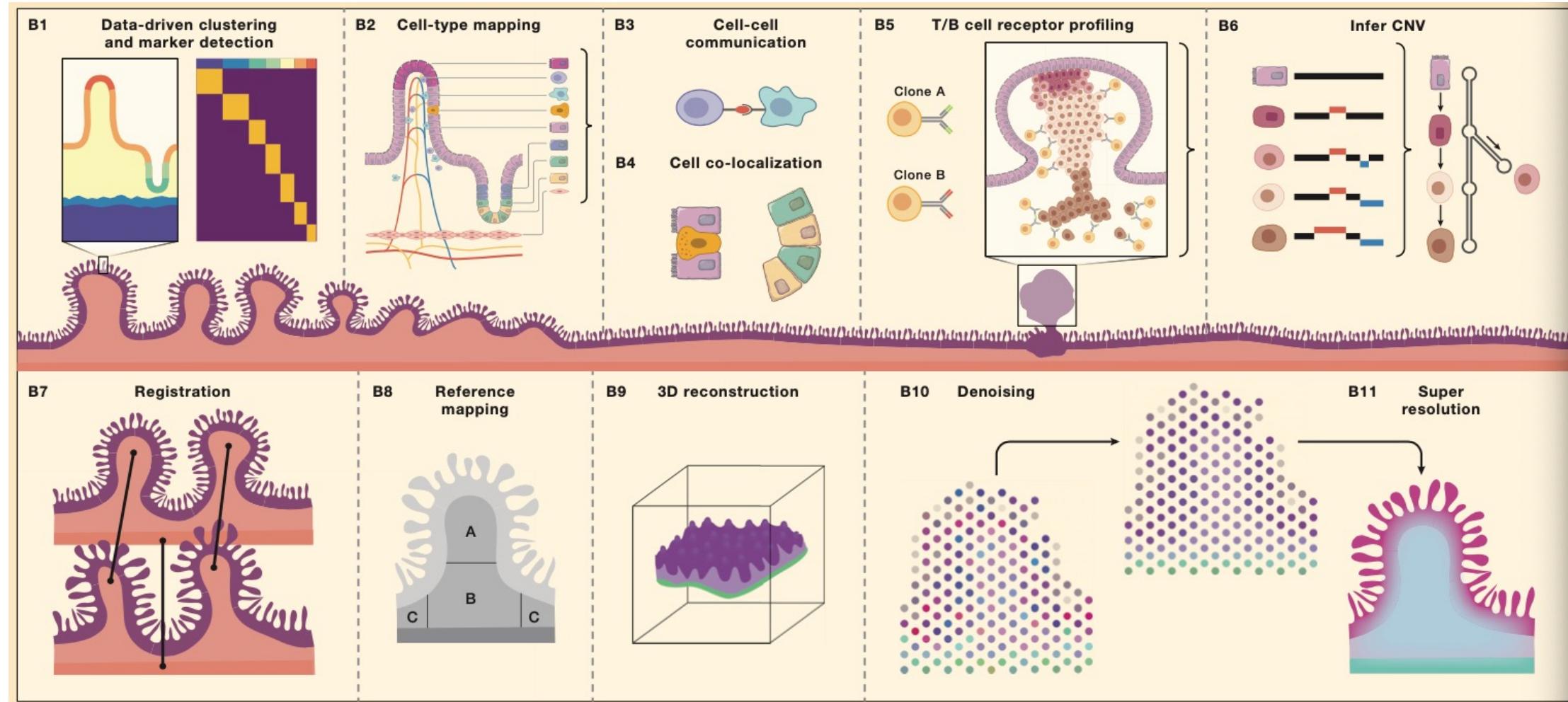
- Image-based methods profiling a few genes at high resolution and sensitivity: MERFISH (Vizgen), CosMx-1K (Nanostring) and Xenium-300 (10X)

Where Is The Field Moving To?

- **Spatial multi-omics technology and analysis algorithms are becoming available**
 - The simultaneous measurement of genomics, epigenomics, and genome organization in addition to transcriptomics and proteomics
- **3D profiling will be developed in the coming years**
 - Most spatial omics operate on thin tissue slices, essentially 2D
- **The technology will become more accessible and affordable for use**
 - As these technologies continue to mature and become more reliable, we can expect a decrease in costs.

This will allow for a more comprehensive understanding of complex biological systems like cancer

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



Dajun Lee

Juhyeon Hong

Kwangmin Yoo

Jihyun Kim

Hojin Lee

Harim Chun

Moonyoung Lee