



BIOINFO 2023



Introduction to Spatially Resolved Biology *(and its practical application)*

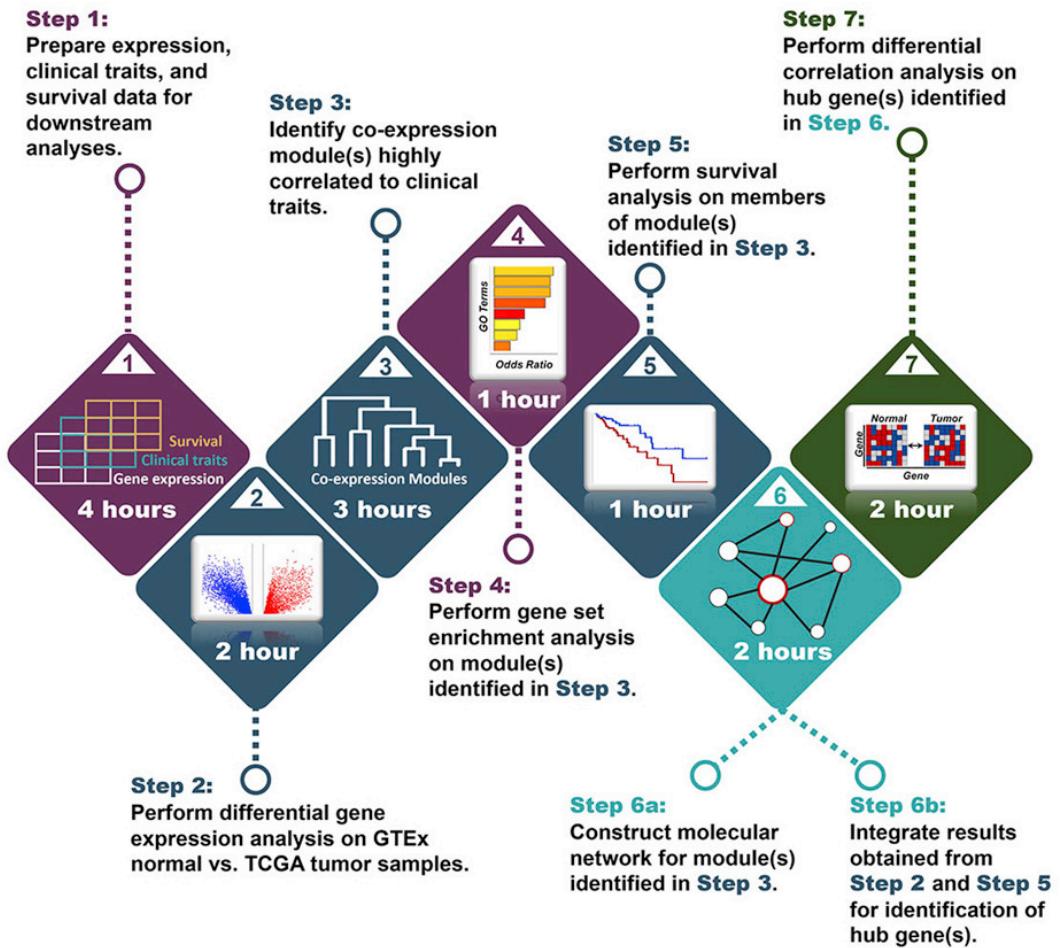
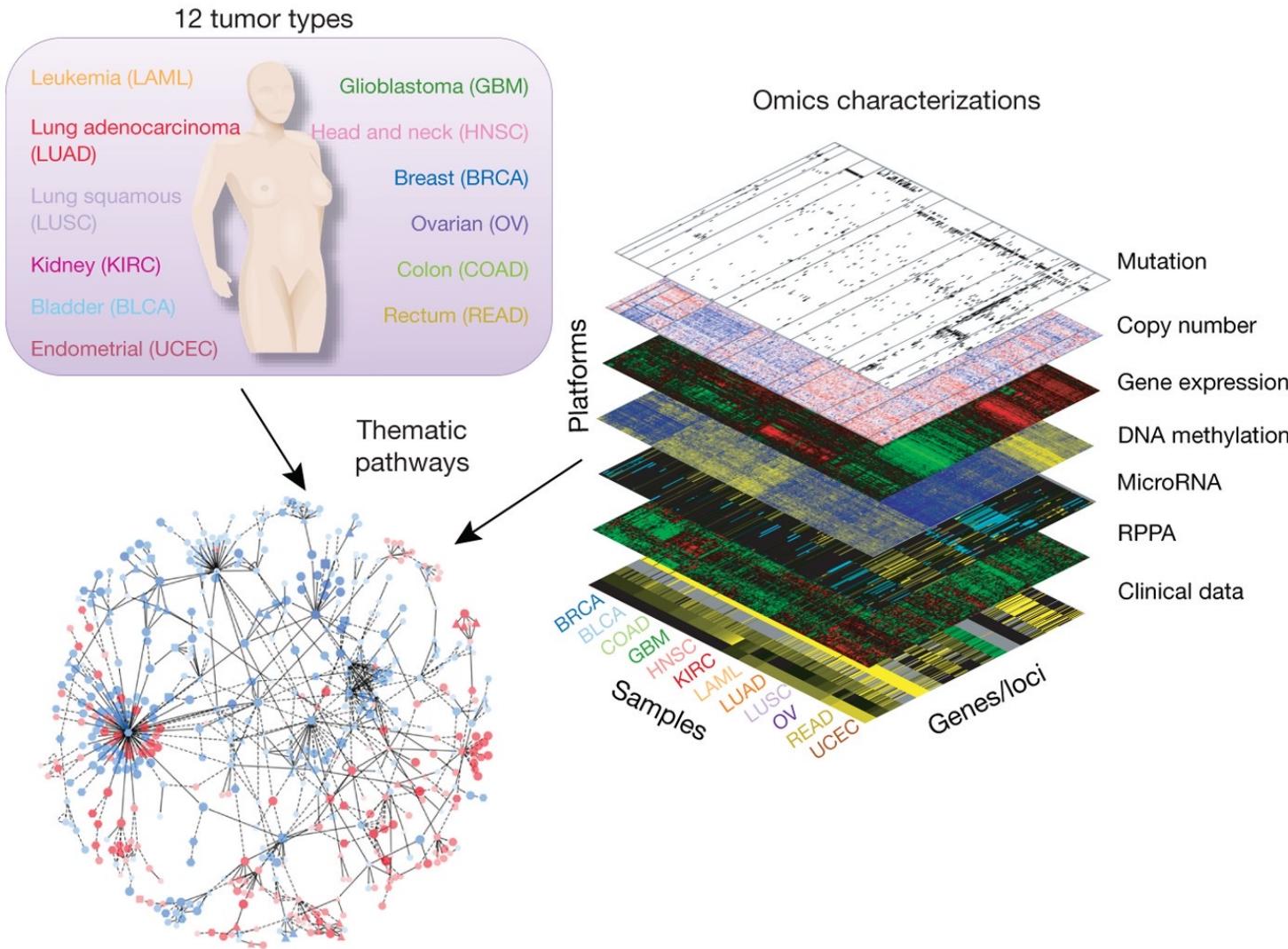
Jungmin Choi, Ph.D.

Department of Biomedical Sciences
Korea University, College of Medicine

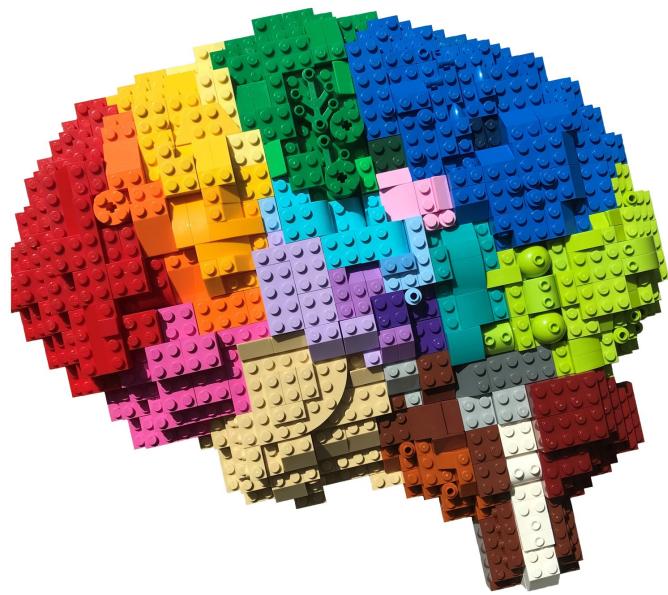
Topics Explored in Today's Lecture

- **Introduction**
 - A broad overview of single-cell data and spatially resolved experimental techniques
 - **Computational methodology and frameworks**
 - Hands-on exercise for beginners
- A 5-min break**

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-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)

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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 Organized by James Eberwine in 2009 (Alongside Junhyong Kim)



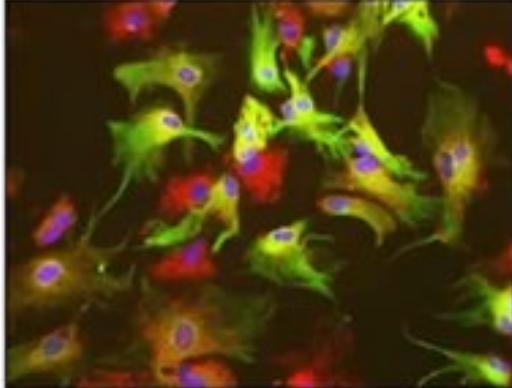
<https://pbs.twimg.com/media/EJRjv6sUwAARHO8?format=jpg&name=4096x4096>

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

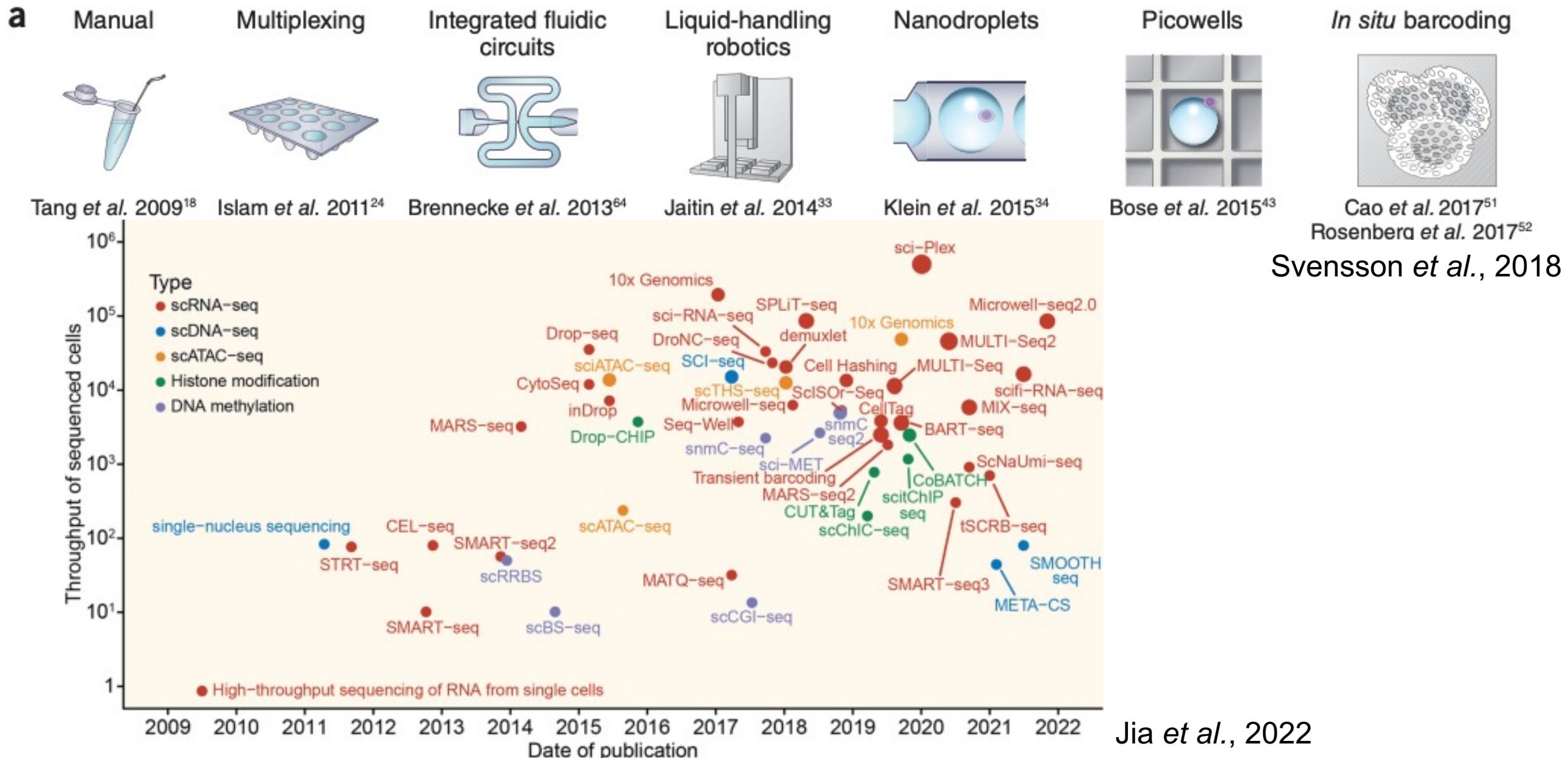
Ben Simons, Cambridge University, UK

Jonathan Sweedler, University of Illinois

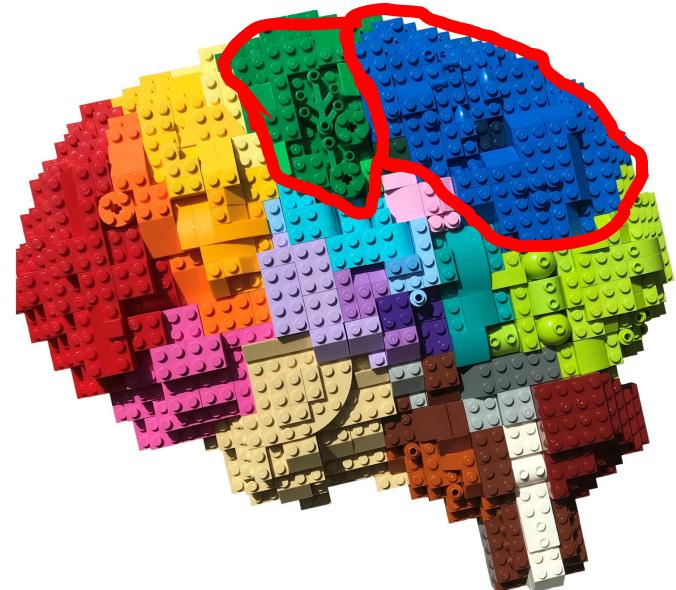
Daojing Wang, Lawrence Berkeley National Laboratory

Xiaowei Zhuang, Harvard University

The Field of Single-Cell Sequencing Technology has Experienced Exponential Growth



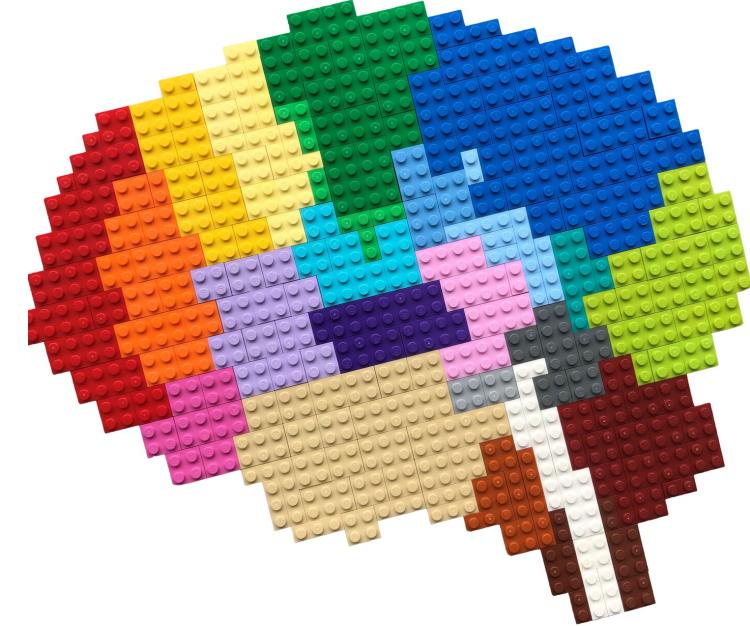
Unfortunately, Single-cell Transcriptomics Lacks Spatial Information due to Cell Dissociation



The original brain



single-cell RNA Sequencing



Spatially Resolved Transcriptomics

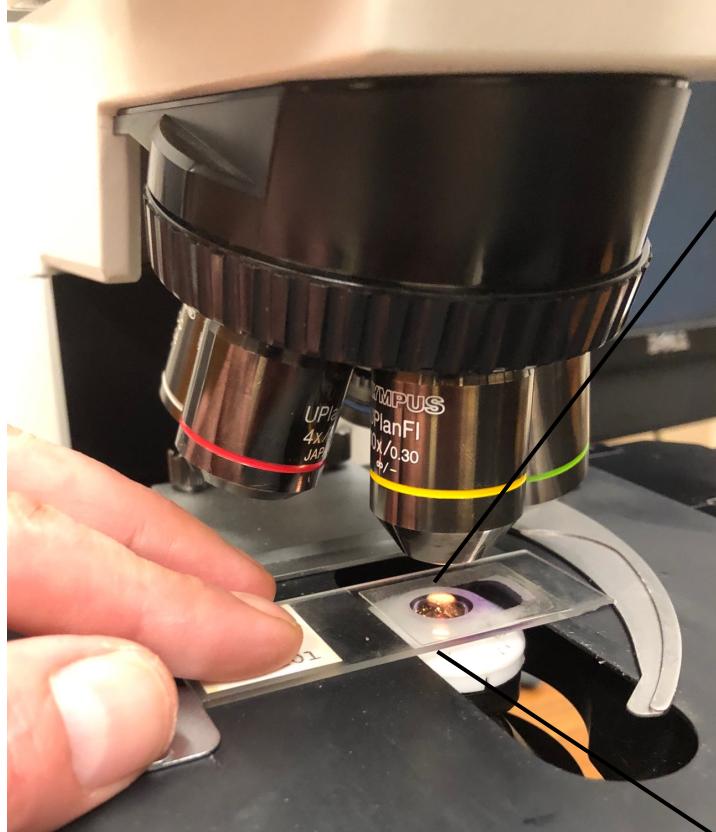
The first law of geography

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

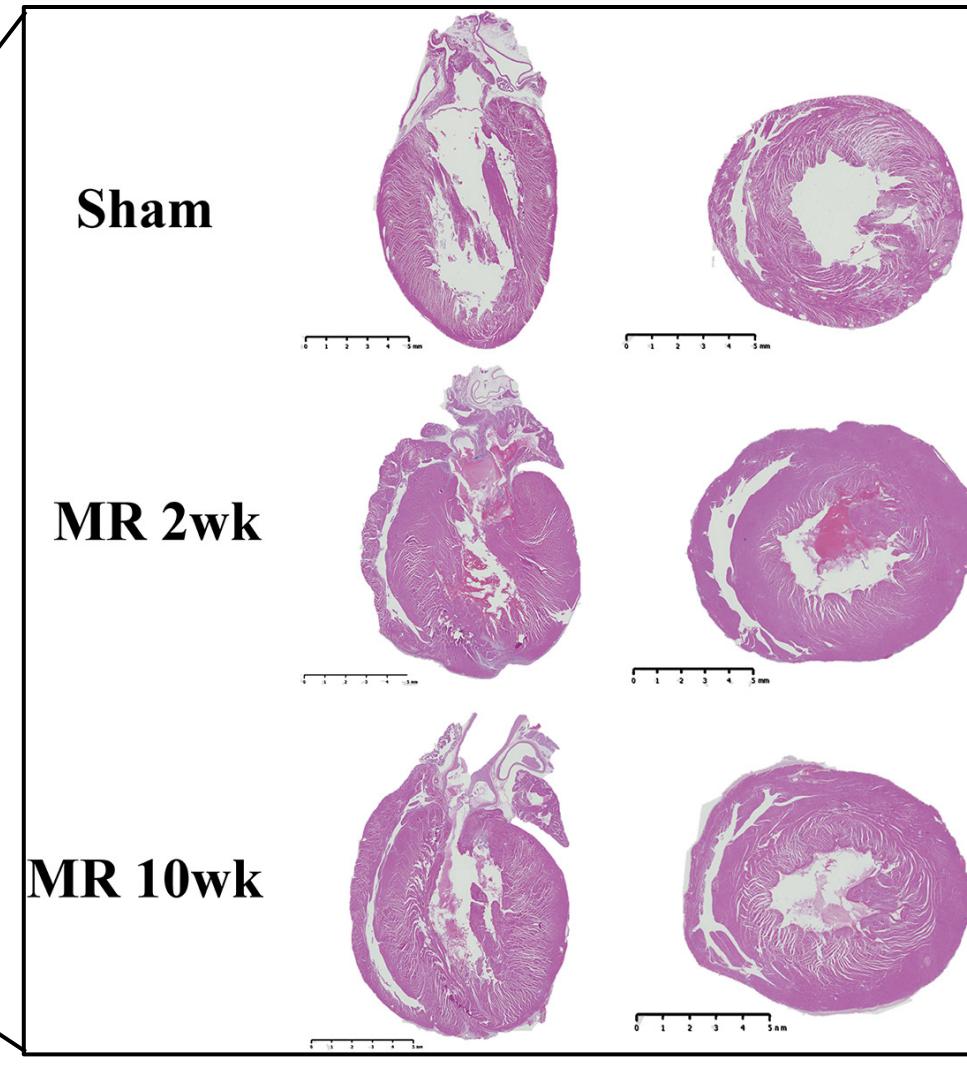
Spatial Analysis is Not New...



The Drawbacks of Conventional Histology Techniques



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

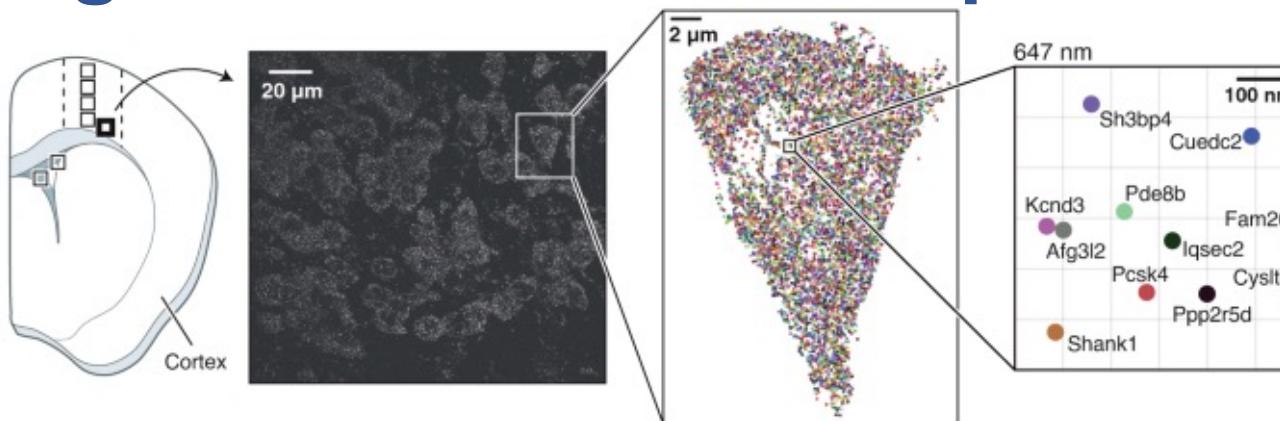


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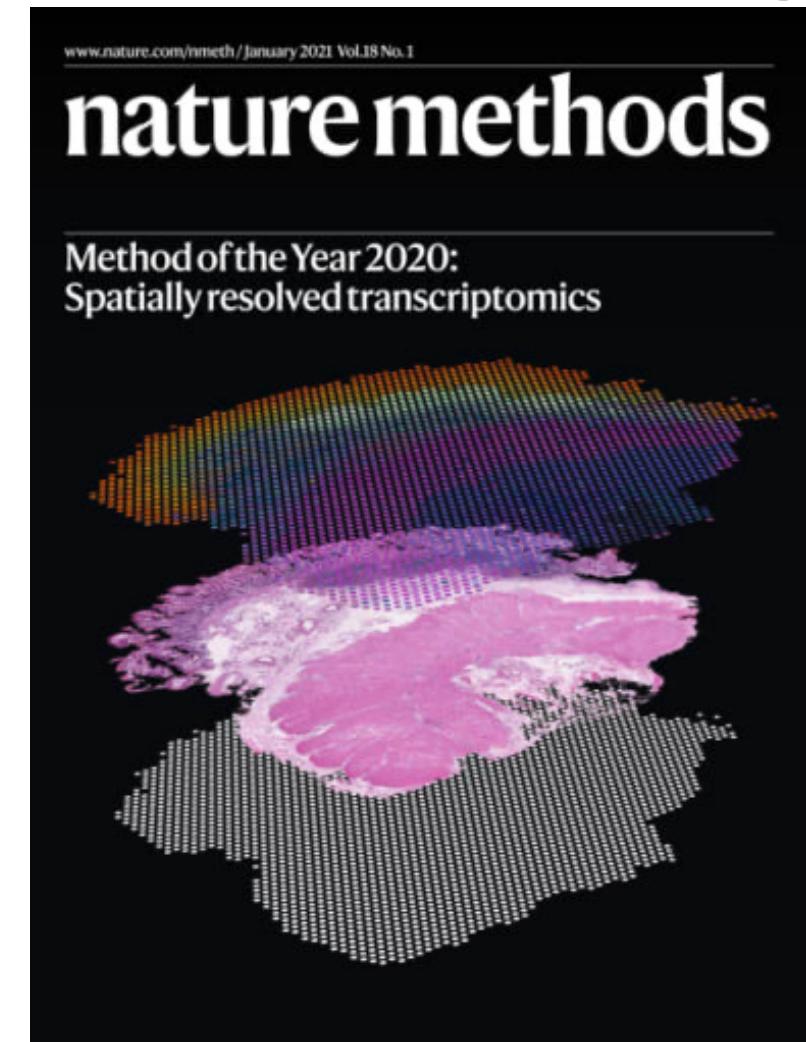
- *Low plex*
- *Targeted*
- *Signal*
- *Precision*
- *Qualitative*

Integrating molecular methodologies can further increase the power of spatial analysis

Spatially Resolved Transcriptomics (SRT) Unravels Spatial Organization of Gene Expression Within Tissues and Organ

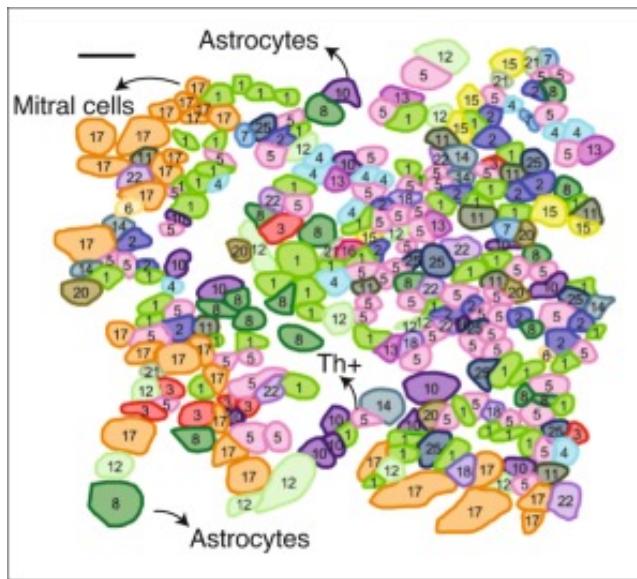


<https://images.app.goo.gl/wQGSbQo6U6oJVFVR8>



<https://images.app.goo.gl/kzcF5TVb54Nb44By7>

seqFISH+ (2019)

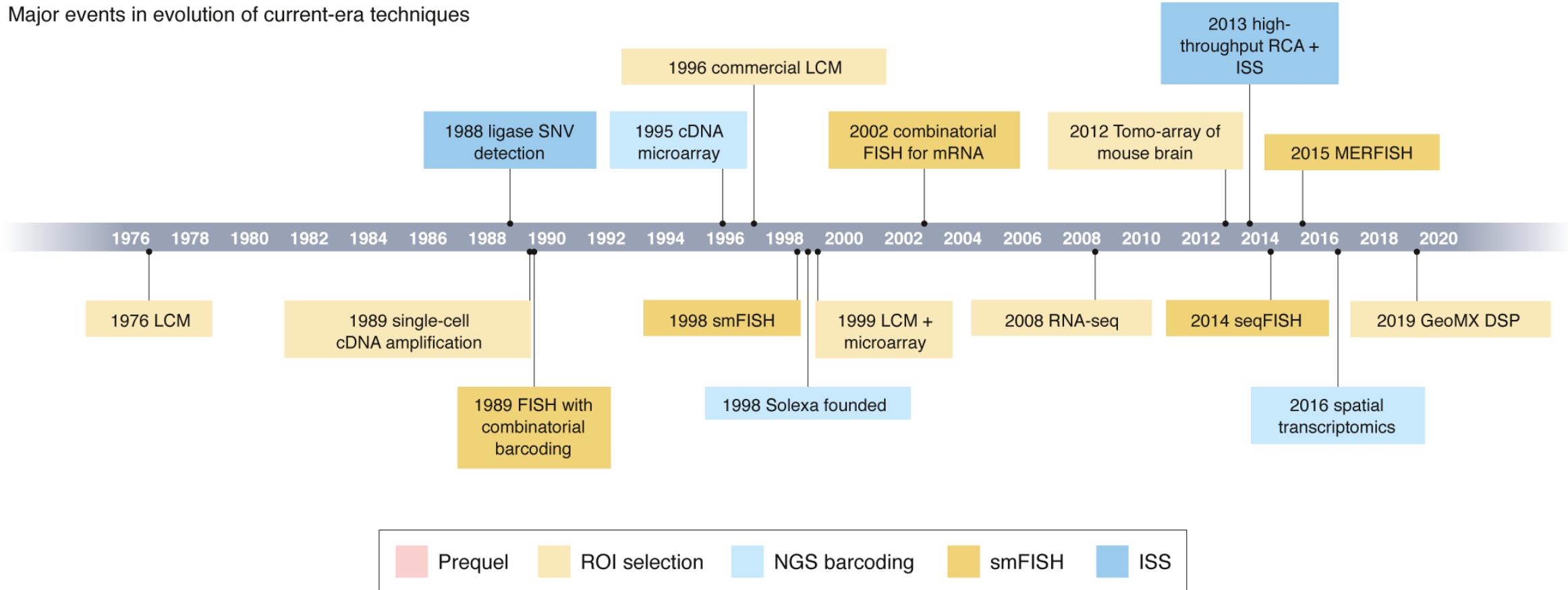


<https://images.app.goo.gl/RV7pES9ro3PXa3PA8>

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

Timelines of Major Events

c Major events in evolution of current-era techniques

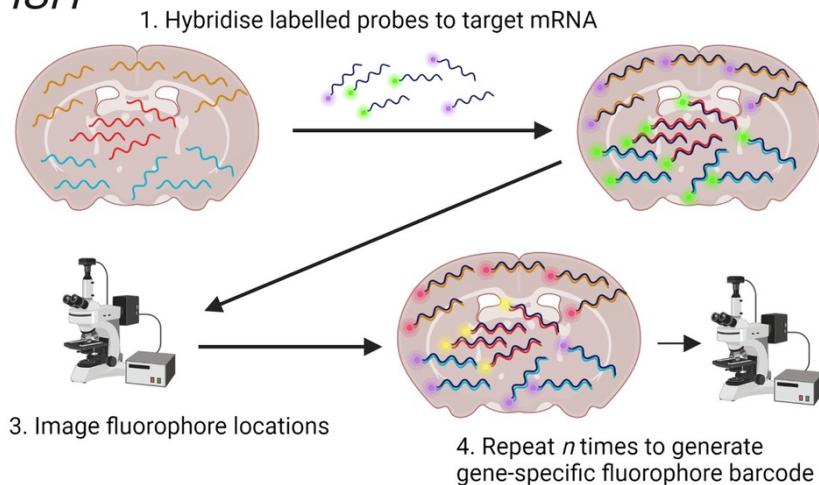


Moses et al., 2022

Spatially Transcriptomics Methods can be Broadly Classified into Two Categories

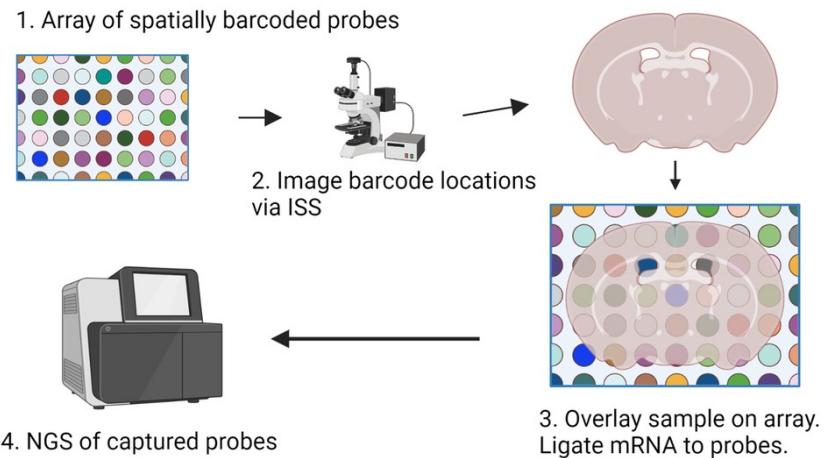
1. Imaging methods

ISH

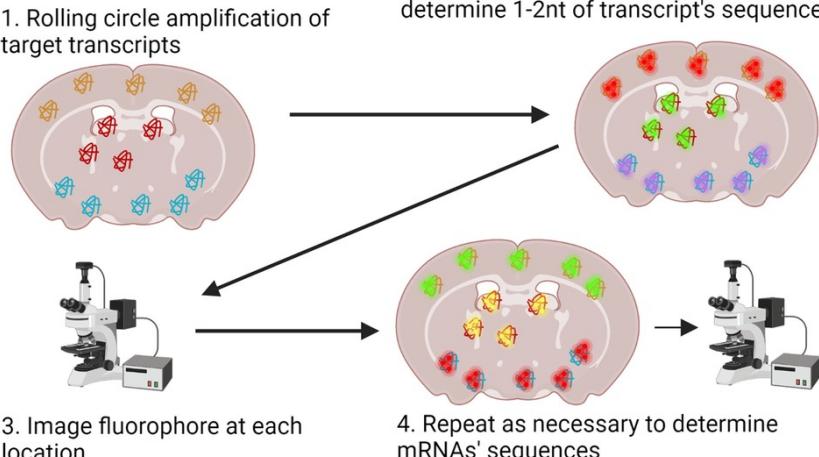


2. Sequencing methods

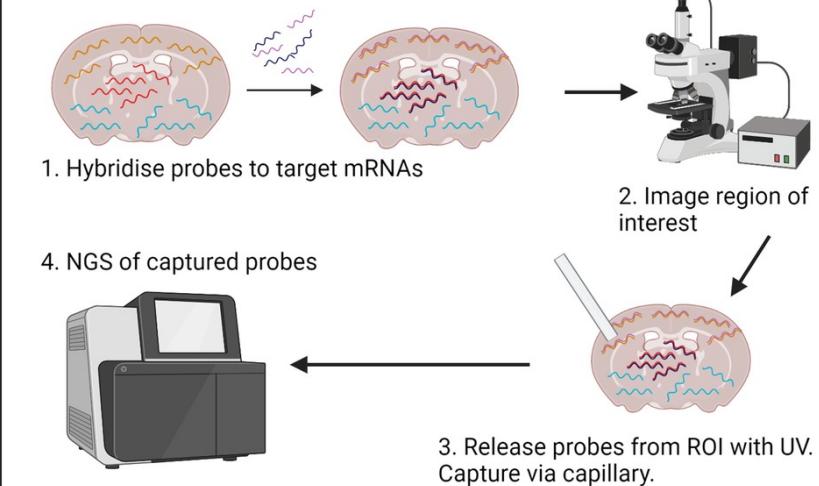
Arrays



ISS

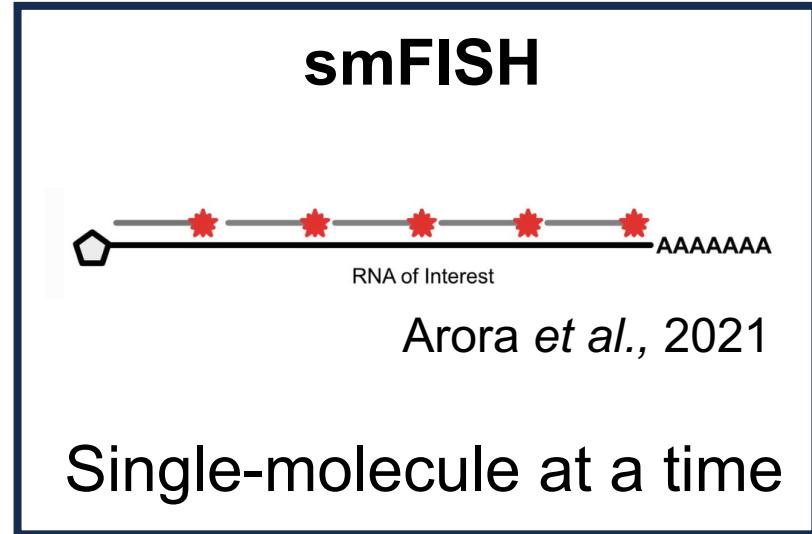


Microdissection

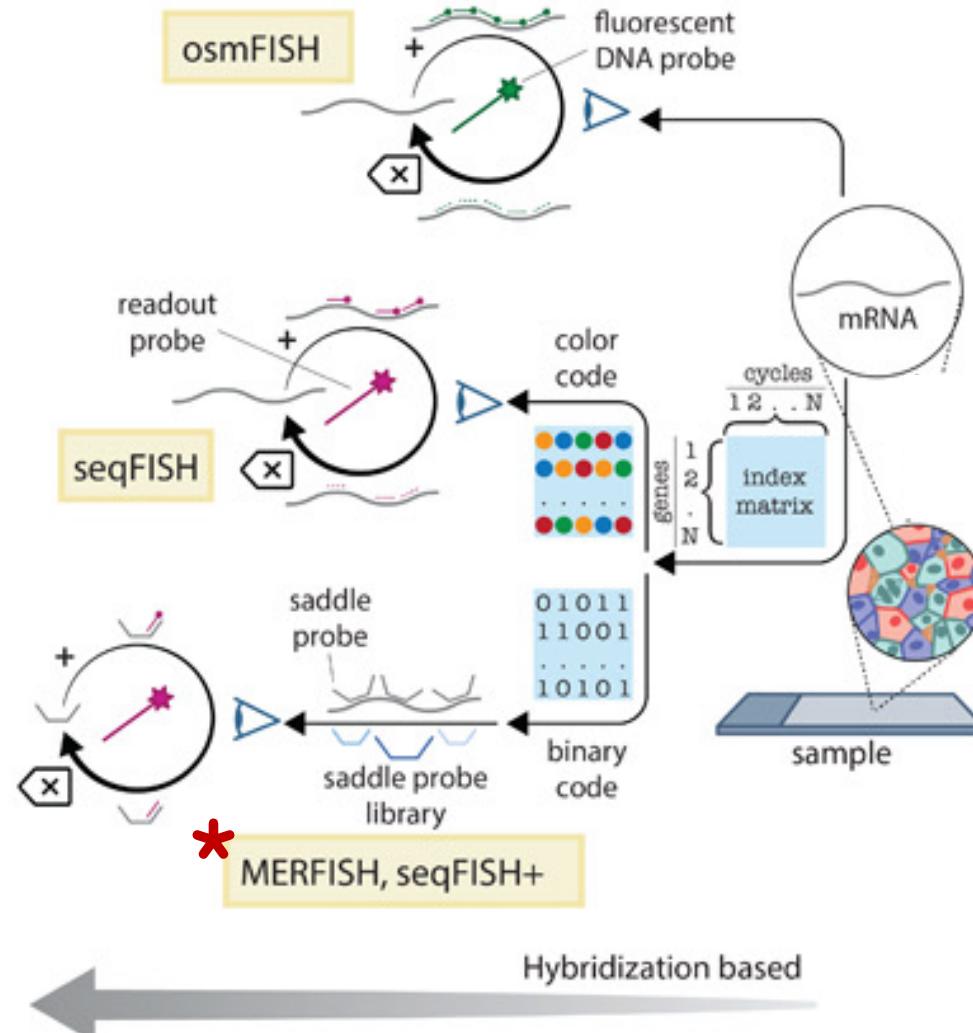


1. Image-based: *in situ* hybridization-based technology

Fluorescence *In Situ* Hybridization (FISH)

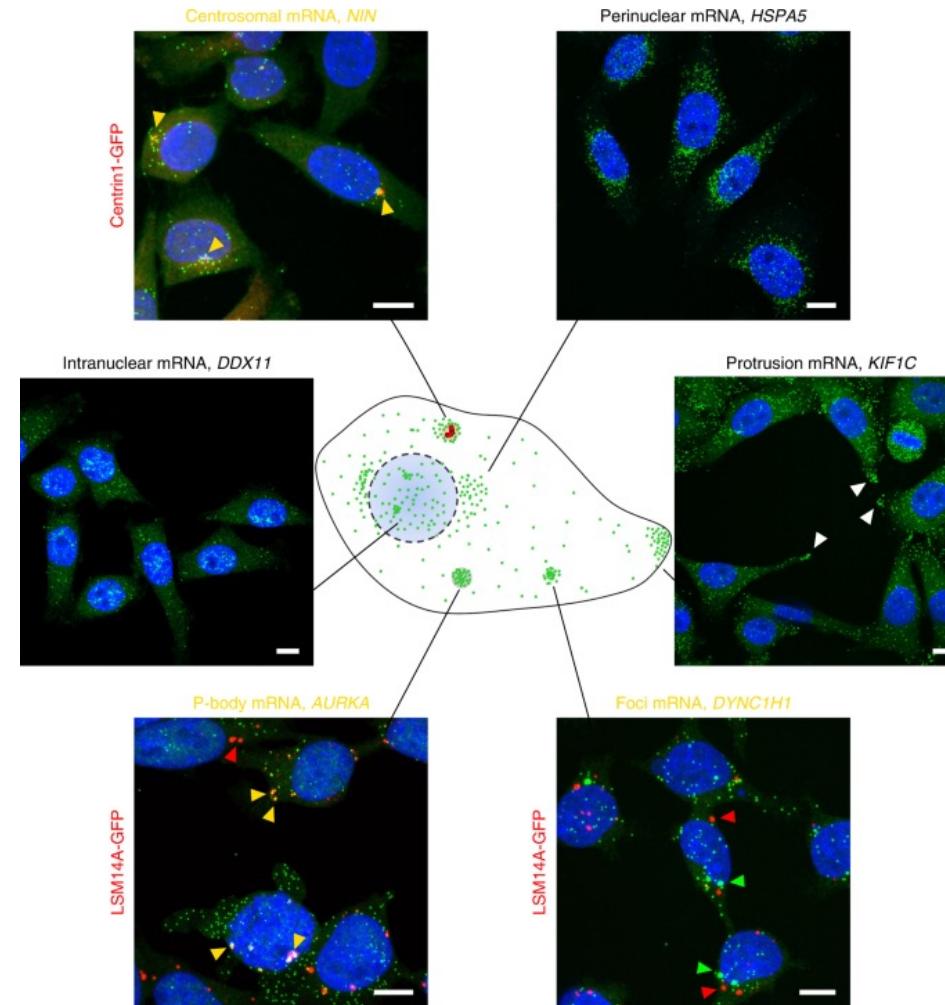
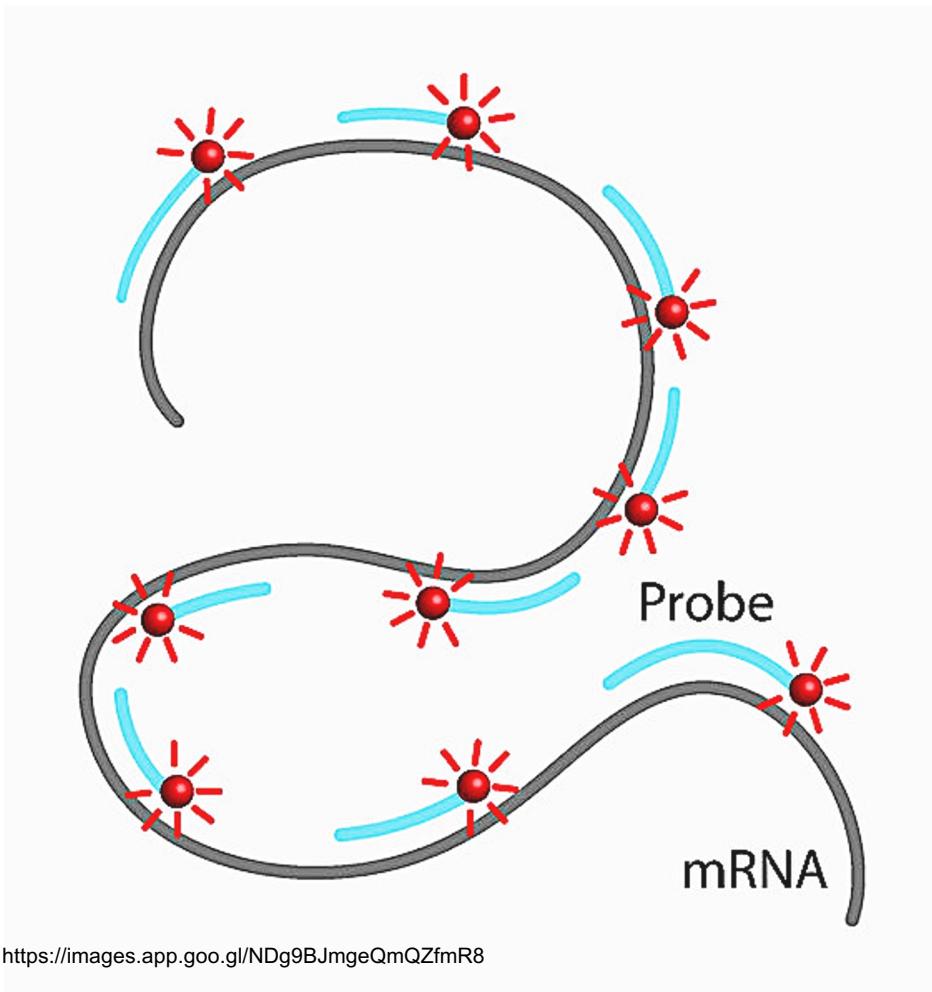


- Label probes for specific targets, hybridize in place
- Require “*a priori*” defined targets (100~1,000)
- Offer subcellular resolution



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.

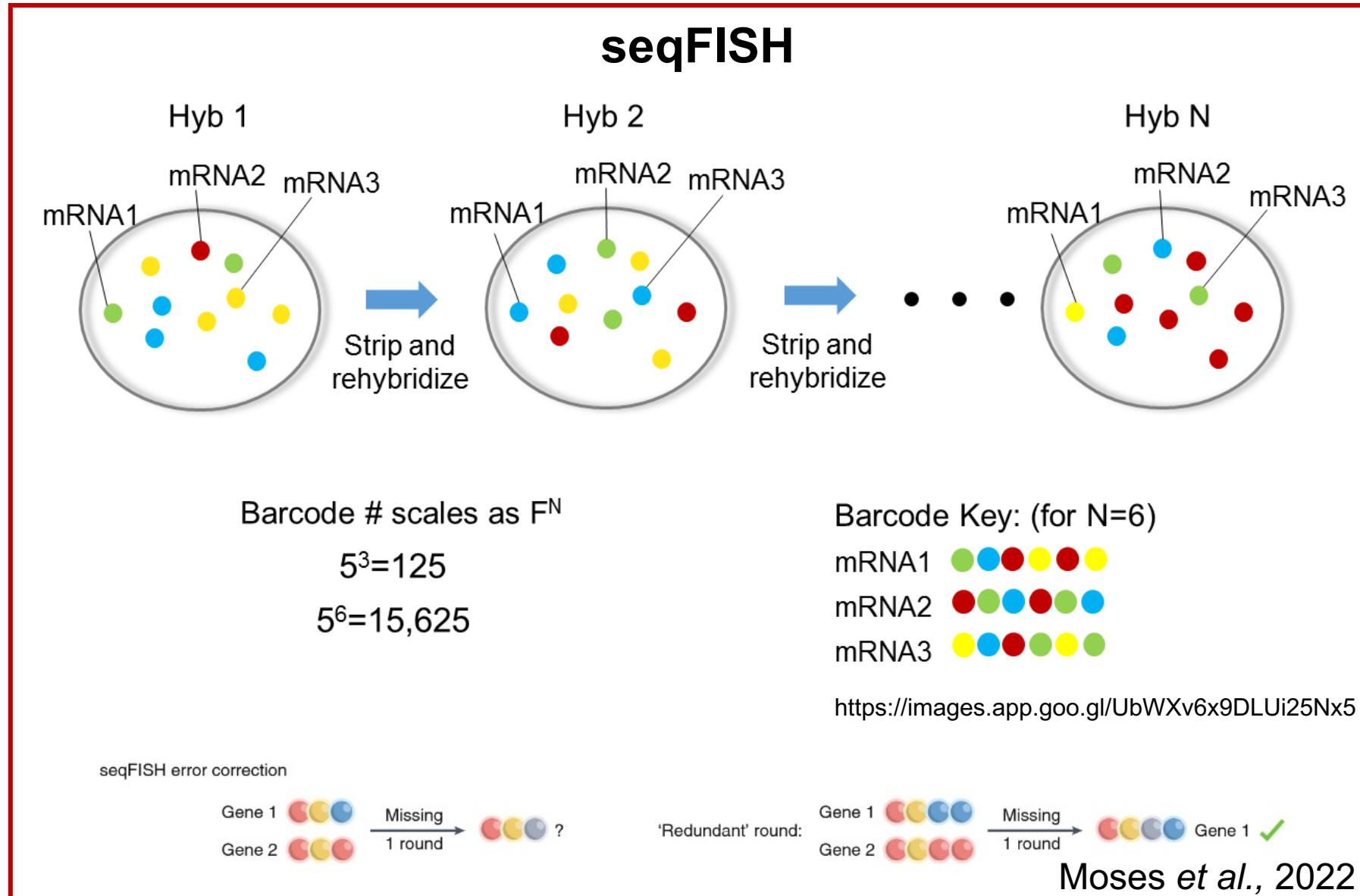
1. Image-based: *in situ* hybridization-based technology sequential Fluorescence In Situ Hybridization (seqFISH)

smFISH



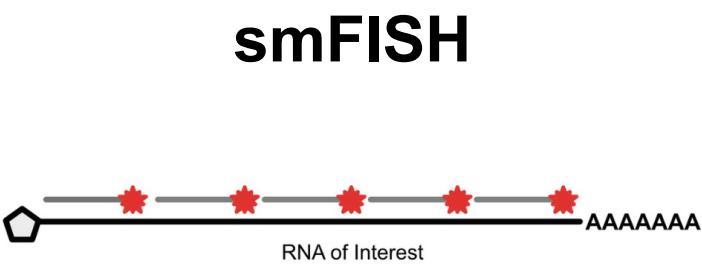
Arora et al., 2021

Single-molecule at a time



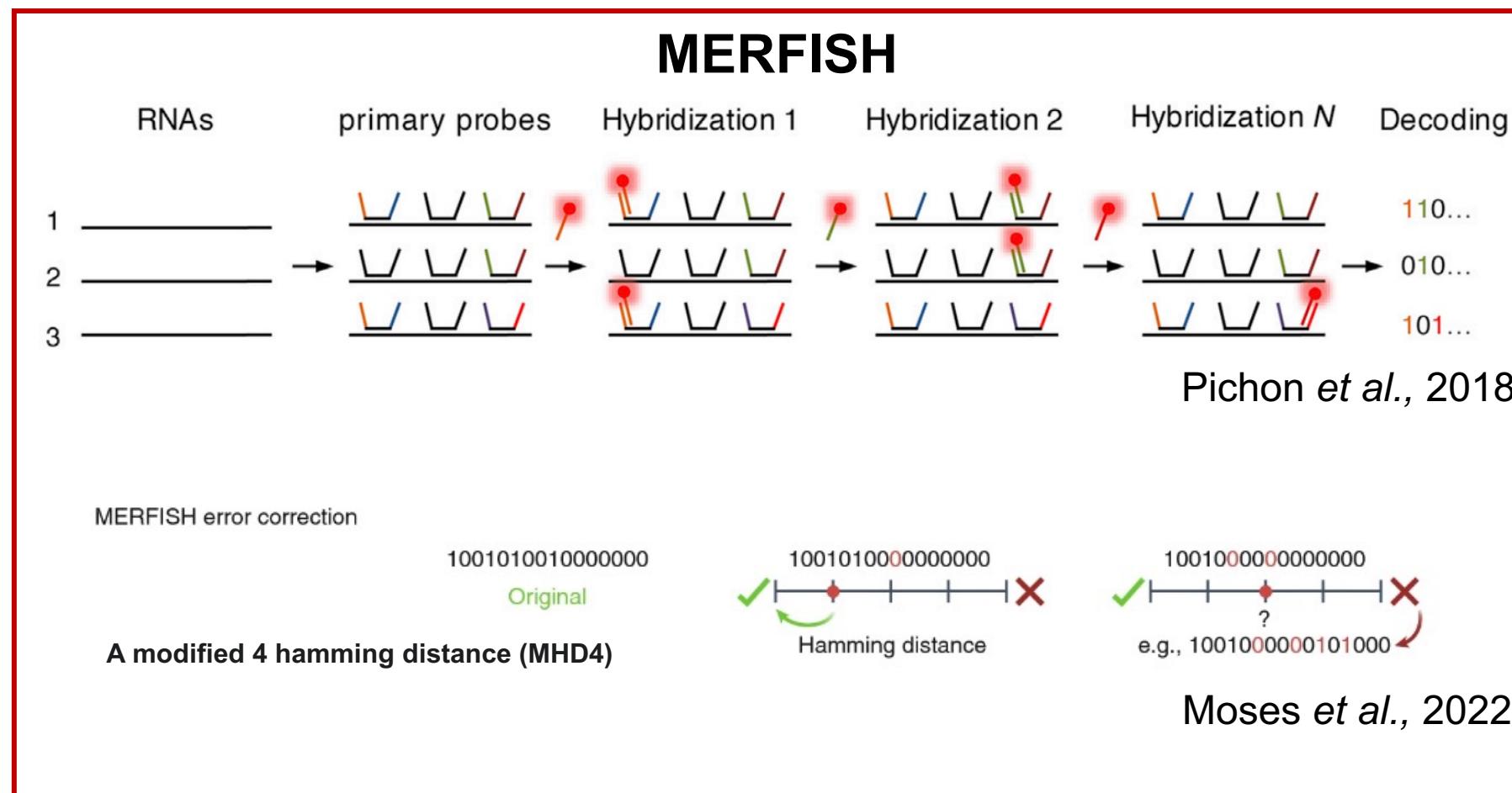
1. Image-based: *in situ* hybridization-based technology

Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH)



Arora et al., 2021

Single-molecule at a time



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

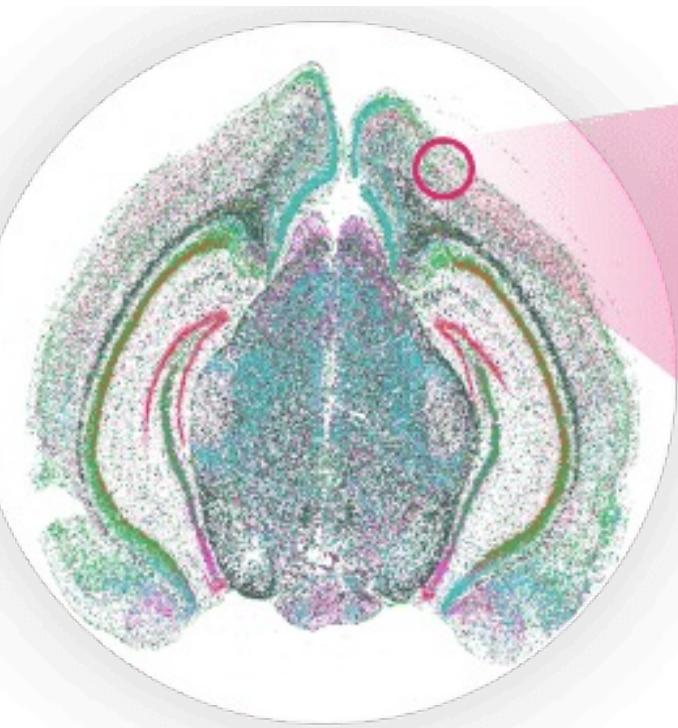
<https://vizgen.com/wp-content/uploads/2022/10/MERFISH-Technology-OverView.png.webp>

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

MERFISH is commercially available at Vizgen as MERSCOPE platform

VIZgen

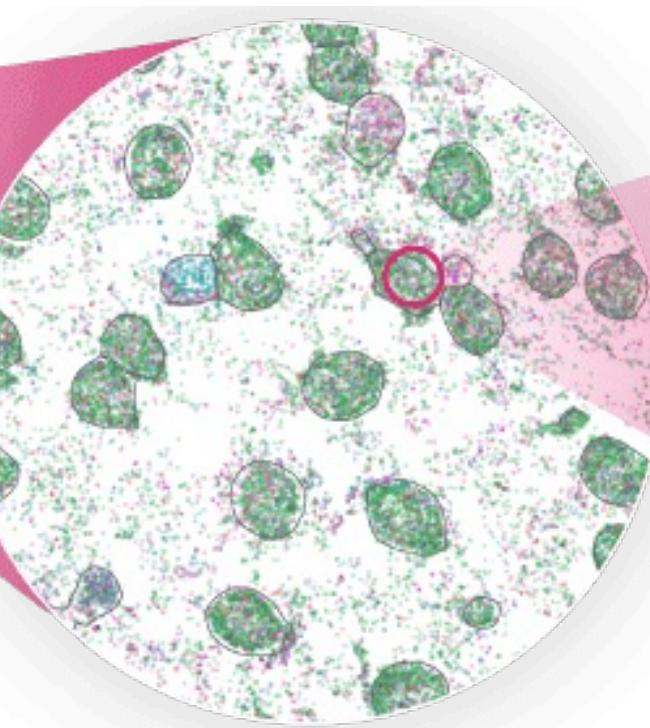
Imaging area:
1x1 cm



WHOLE SECTION

9 x 7 mm

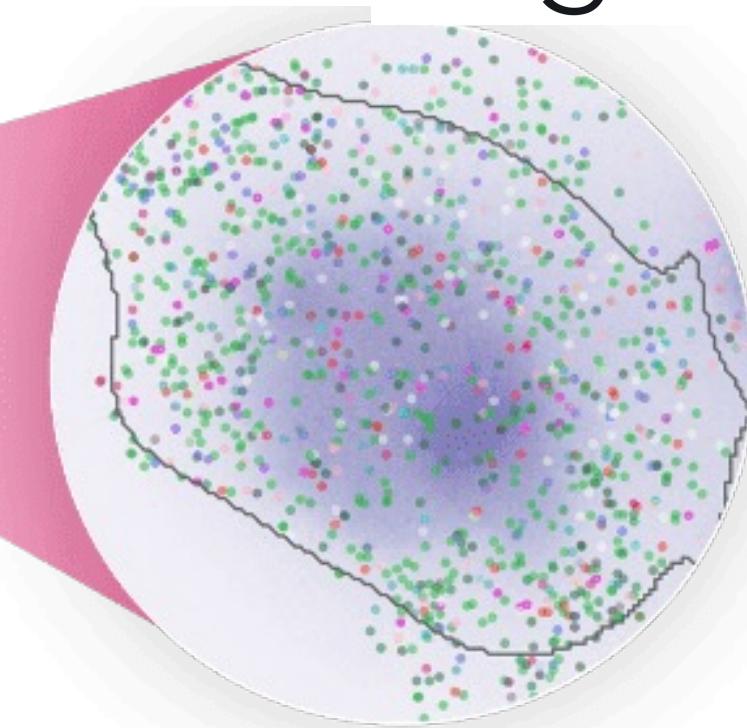
Organization of tissue



WIDE FIELD OF VIEW

200 x 200 micron

Cell interaction/function



SUB-CELLULAR

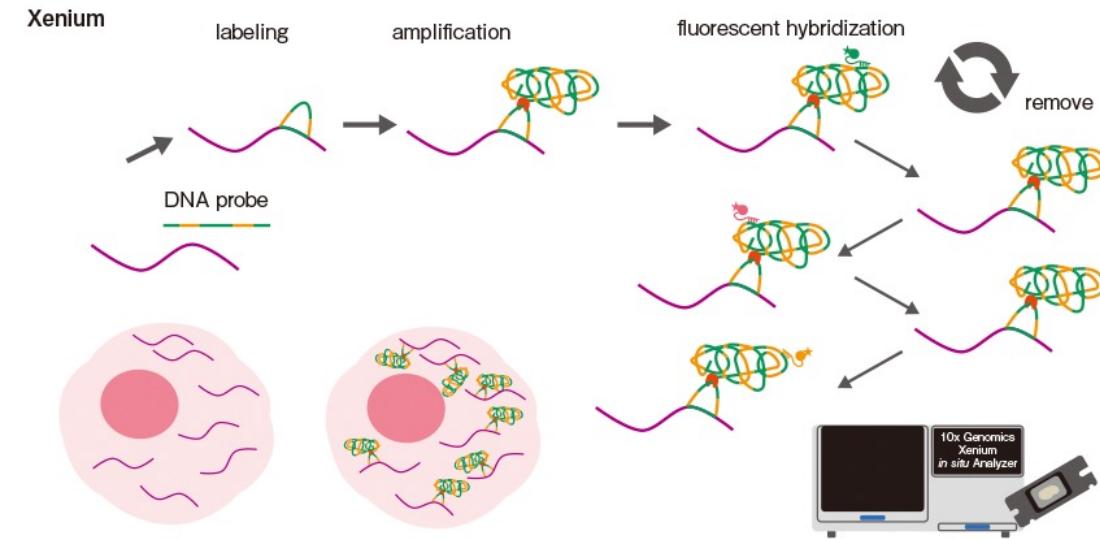
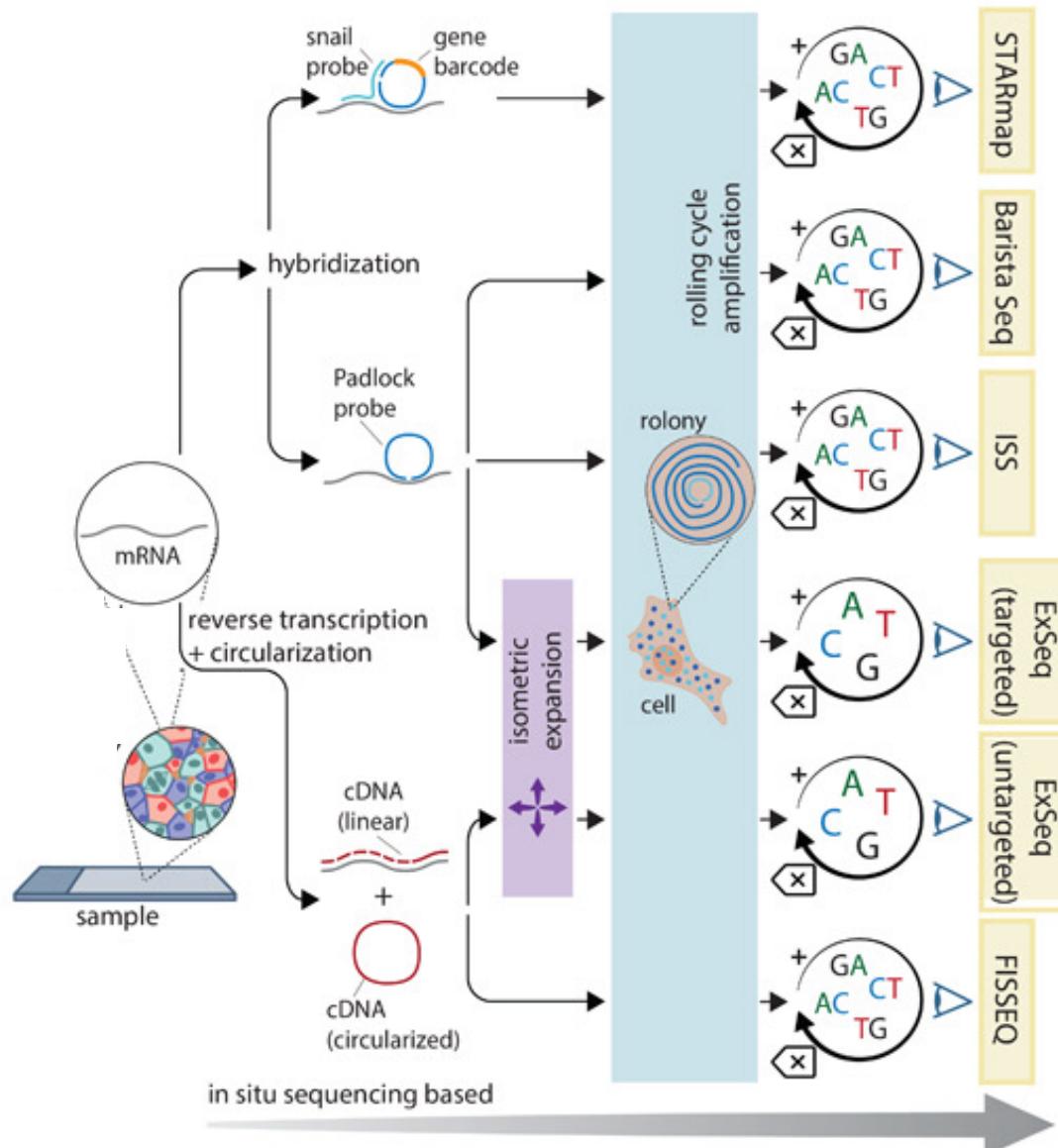
12 x 12 micron

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



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

FISSEQ was acquired by 10X Genomics and developed into Xenium

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

Xenium *in situ*

10X
GENOMICS

Imaging area:
12x24 mm

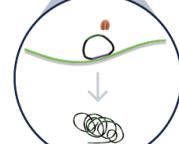
FFPE or FF Tissue



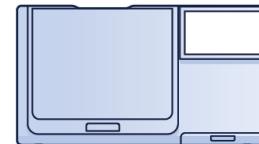
Permeabilization or
deparaffinization/decrosslinking
& probe hybridization



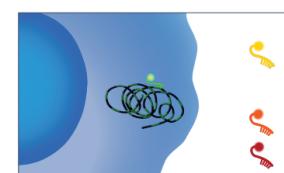
Probe ligation
& enzymatic amplification



Load slides onto the
Xenium Analyzer



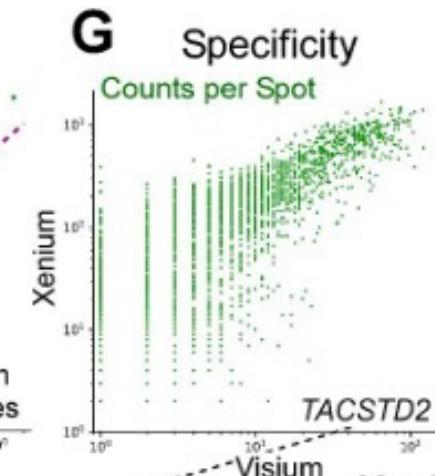
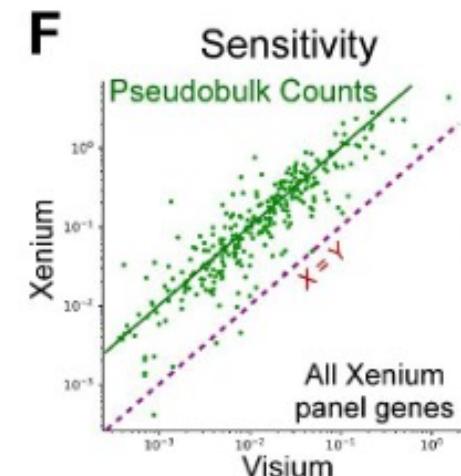
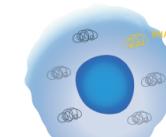
Successive rounds of fluorescent
probe hybridization, imaging &
probe removal



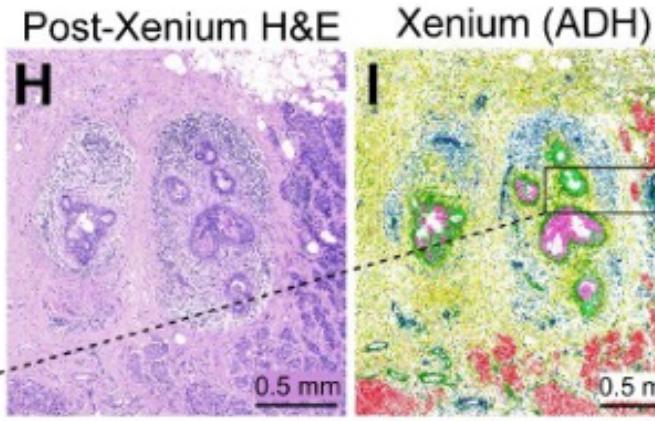
Optical signature generation



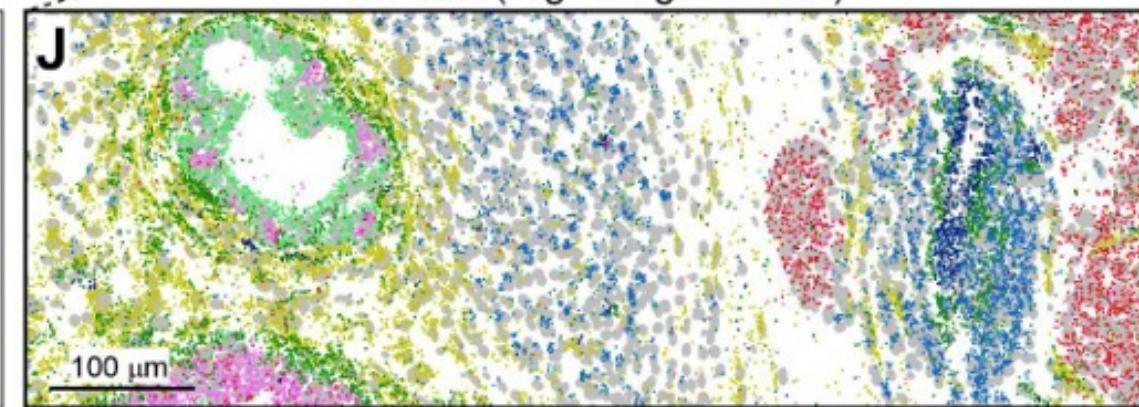
Target gene identification



Breast cancer



Xenium (High Magnification)

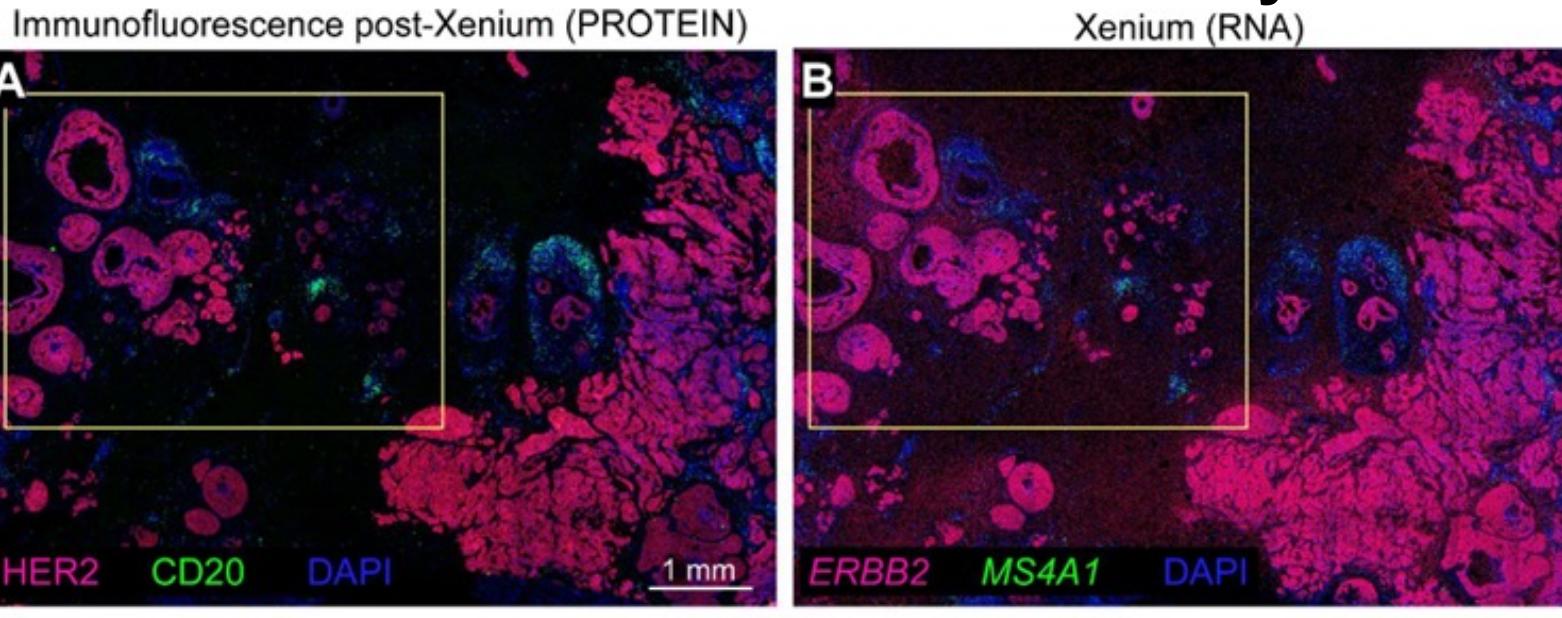


Janesick et al., 2022

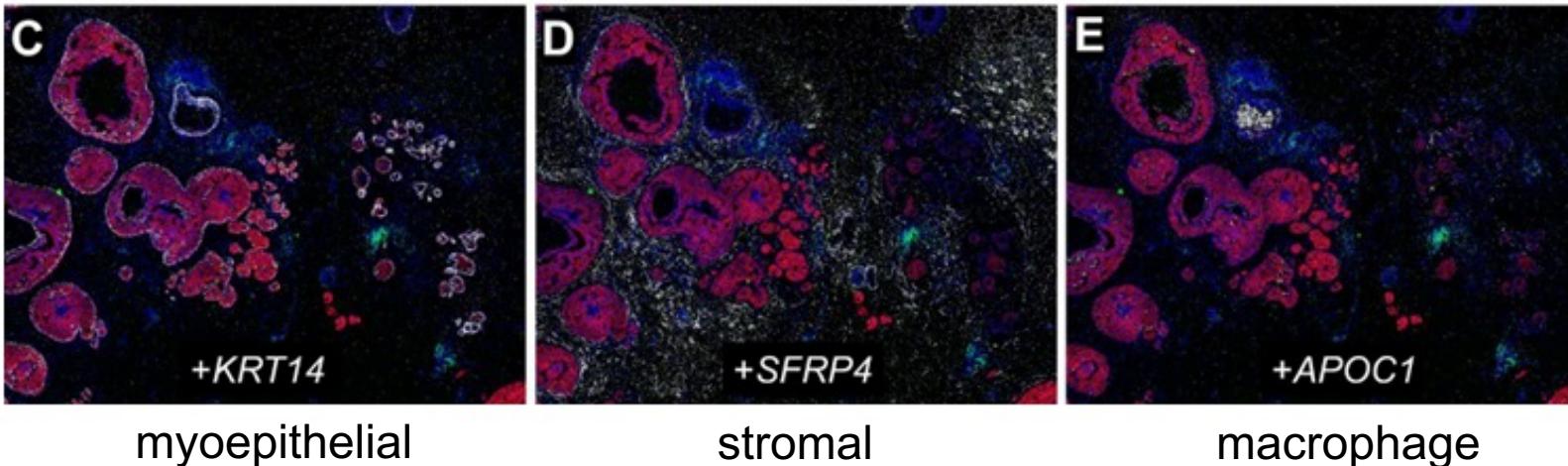
2. Image-based: *in situ* sequencing-based technology

RNA from Xenium and protein immunofluorescence
can be visualized simultaneously

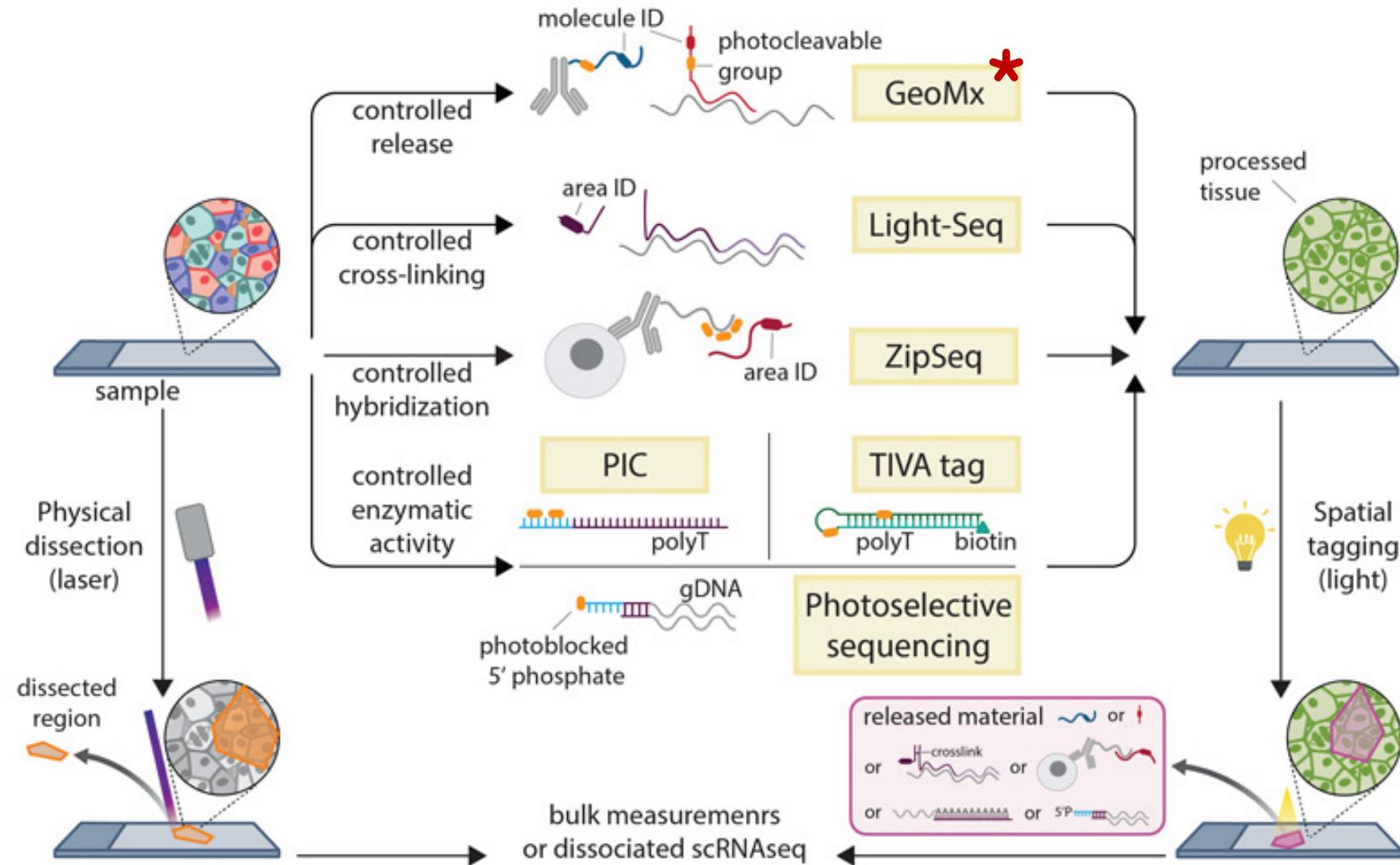
Breast cancer



Immunofluorescence Protein Registered with Xenium RNA

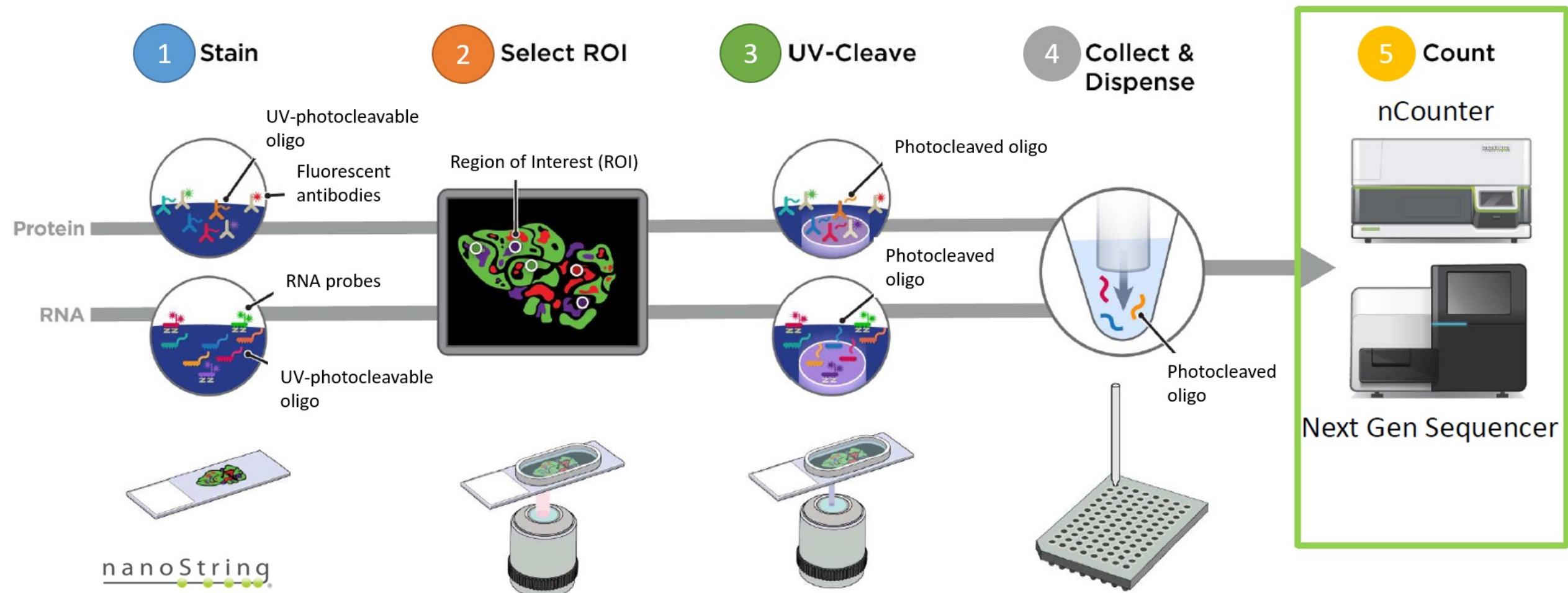


3. Sequencing-based: Microdissection-based technology



3. Sequencing-based: Microdissection-based technology

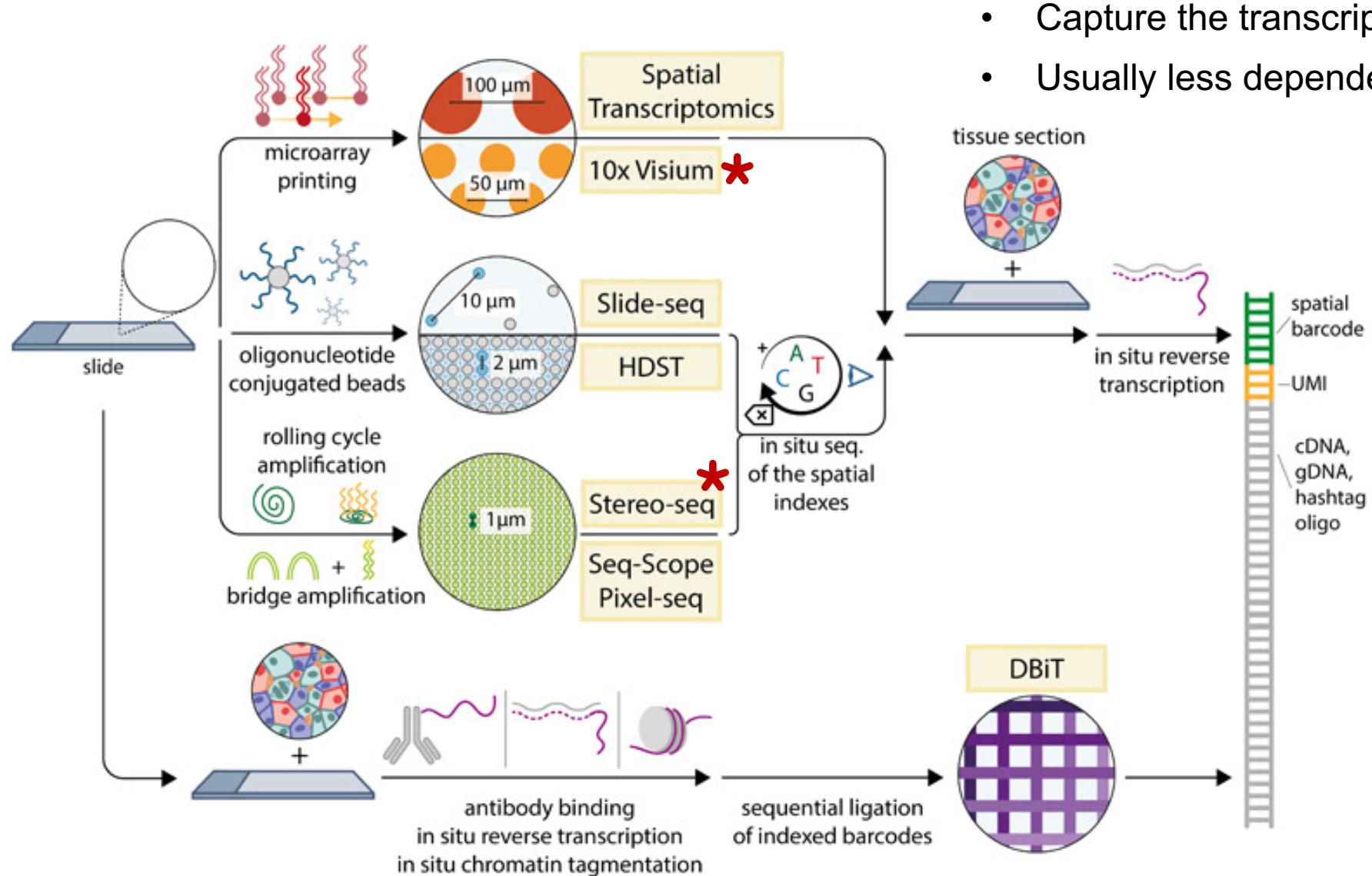
NanoString's GeoMx Digital Spatial Profiling (DSP)



<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

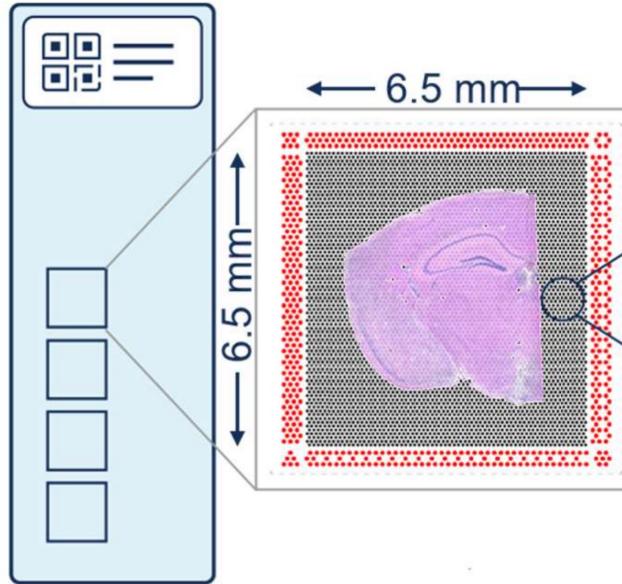


- Capture the transcripts *in situ* but sequence *ex situ*
- Usually less dependent on prior selection of targets

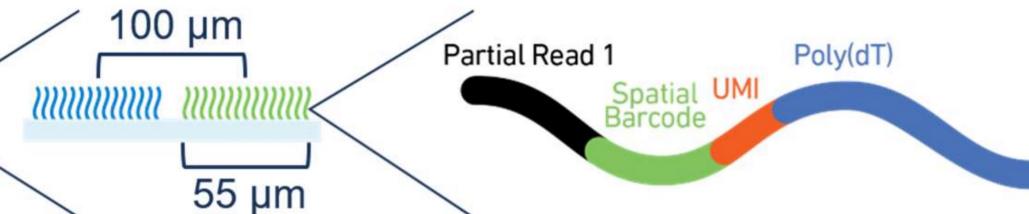
4. Sequencing-based: Spatial barcoding technologies

Visium by 10X Genomics

Visium Spatial
Gene Expression
Slide

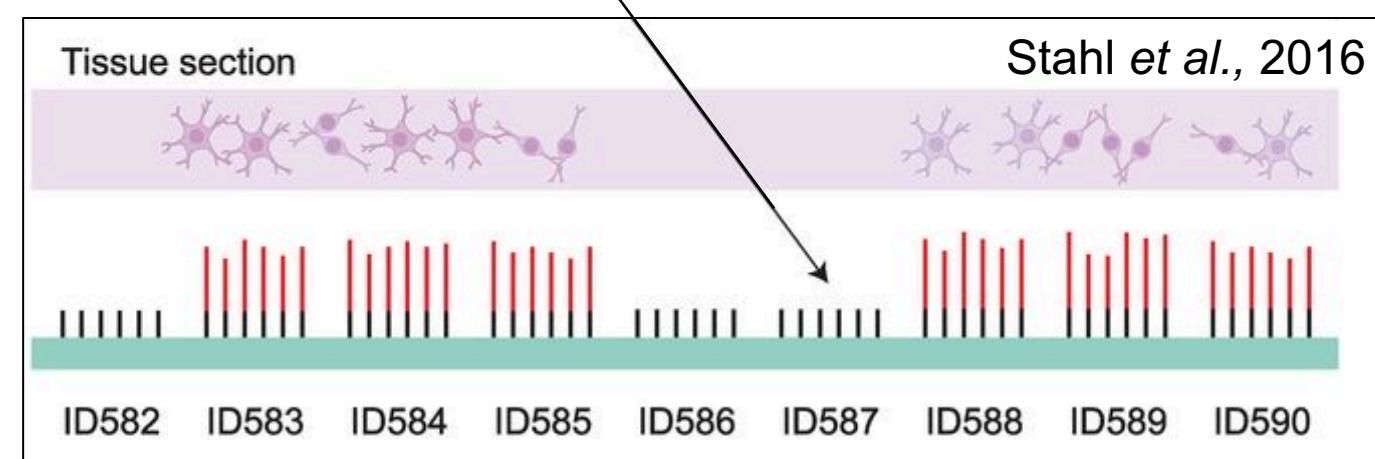


Visium Gene
Expression Barcoded
Spots



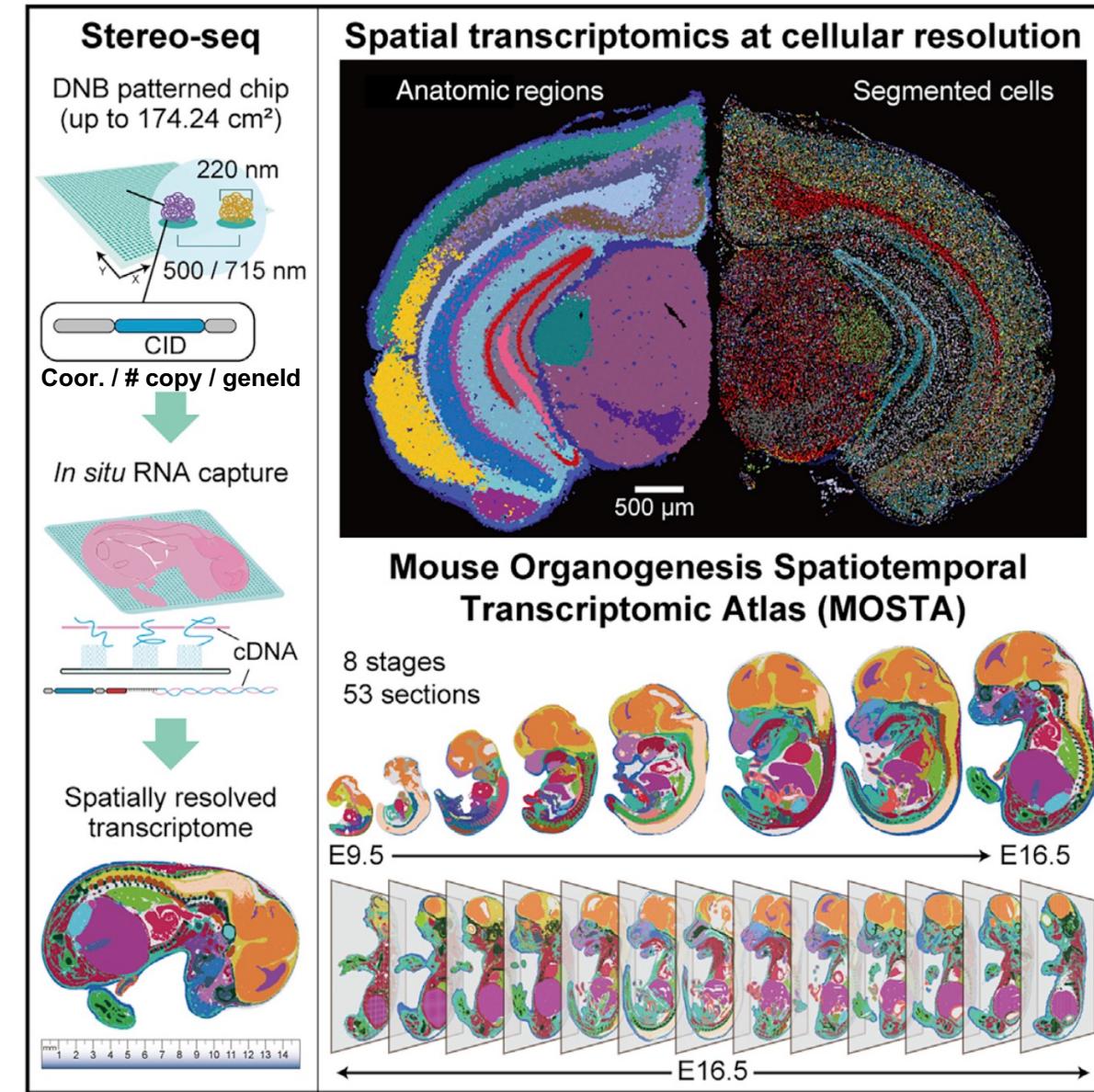
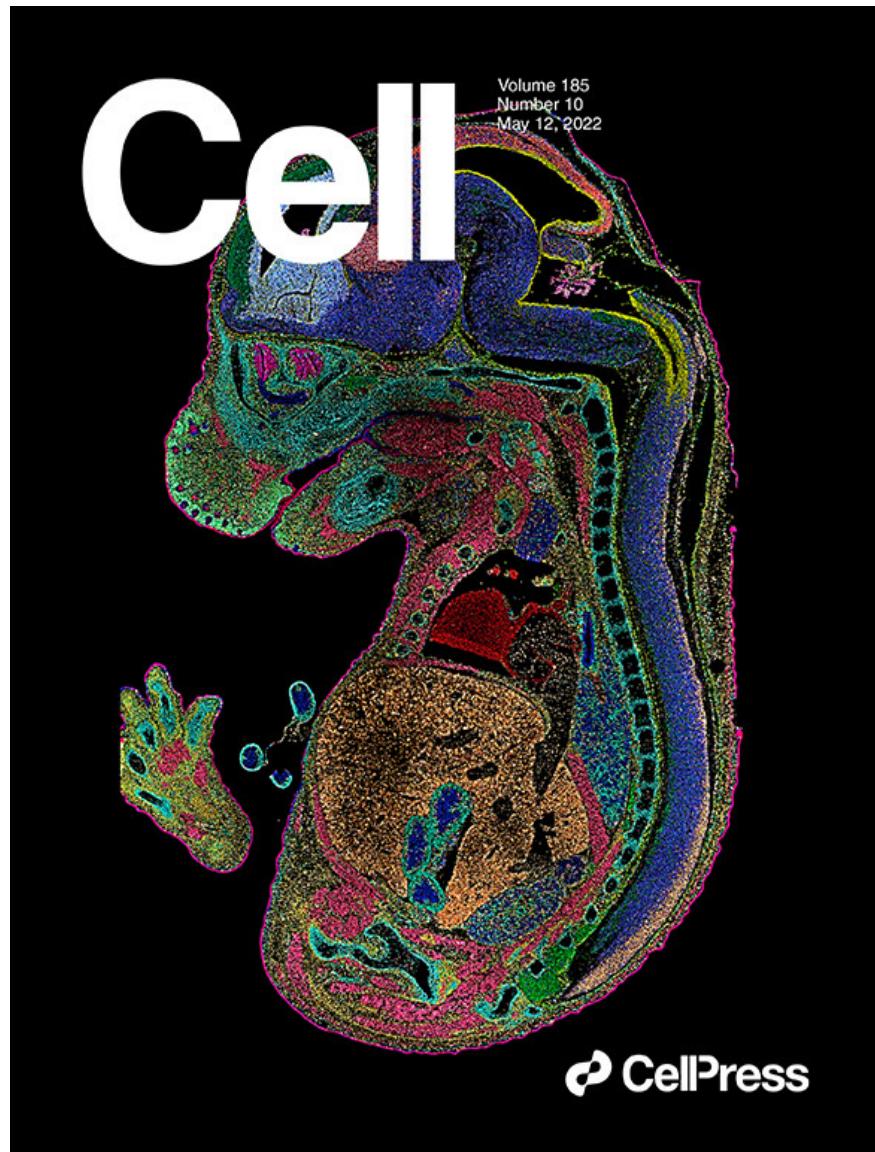
<https://images.app.goo.gl/2k36WDczUaUcBFTYA>

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 μm
 - Center-to-center distance: 100 μm



4. Sequencing-based: Spatial barcoding technologies

Stereo-seq by BGI is a transformational breakthrough

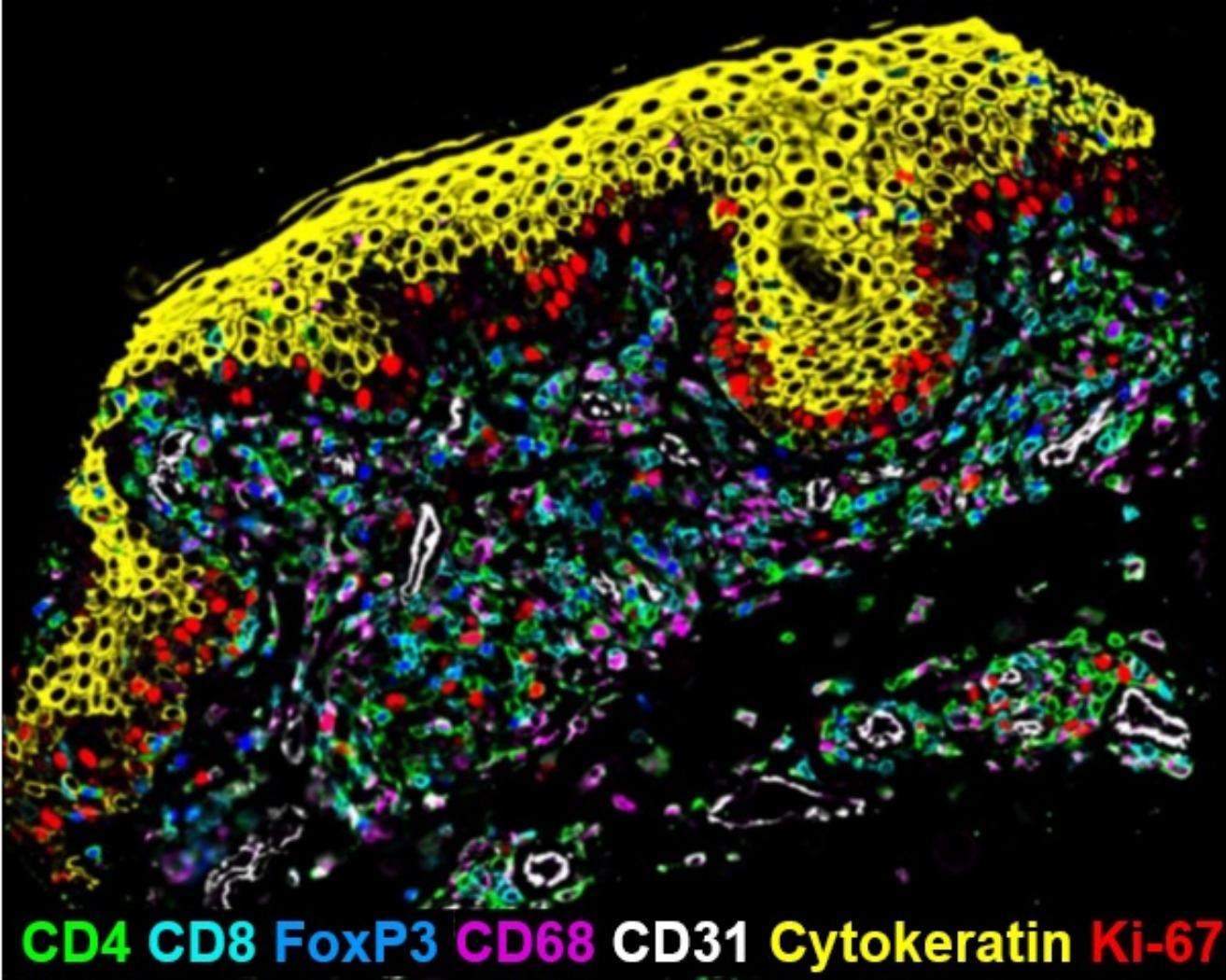


- **Scalable**
13cmx13cm
- **High res**
220nm
- **Deep cov**
#gene > 10,000
#UMI > 100,000
- **Not commercially available in Korea yet**

Chen et al., 2022

Spatial Transcriptomics Holds Great Promise for Revealing New Insights in Cancer Research

CODEX from Akoya



- Uncover tumor heterogeneity
- Identify the tumor microenvironment
- Analyze cell-cell interactions
- Examine tumor invasion interfaces
- Discover new biomarkers
- Provide prognostic information
- Validate previous findings

CD4 CD8 FoxP3 CD68 CD31 Cytokeratin Ki-67

Where is the Field Moving?

- **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

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 Technology Do I Need to Use?

Depending on the experimental aim

1. Hypothesis generating

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

2. Hypothesis testing

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

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

Recommended Review Literatures 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.

Questions?

Have fun with the hands-on SRT exercise



Jihyun Kim

Juhyeon Hong

Kwangmin Yoo