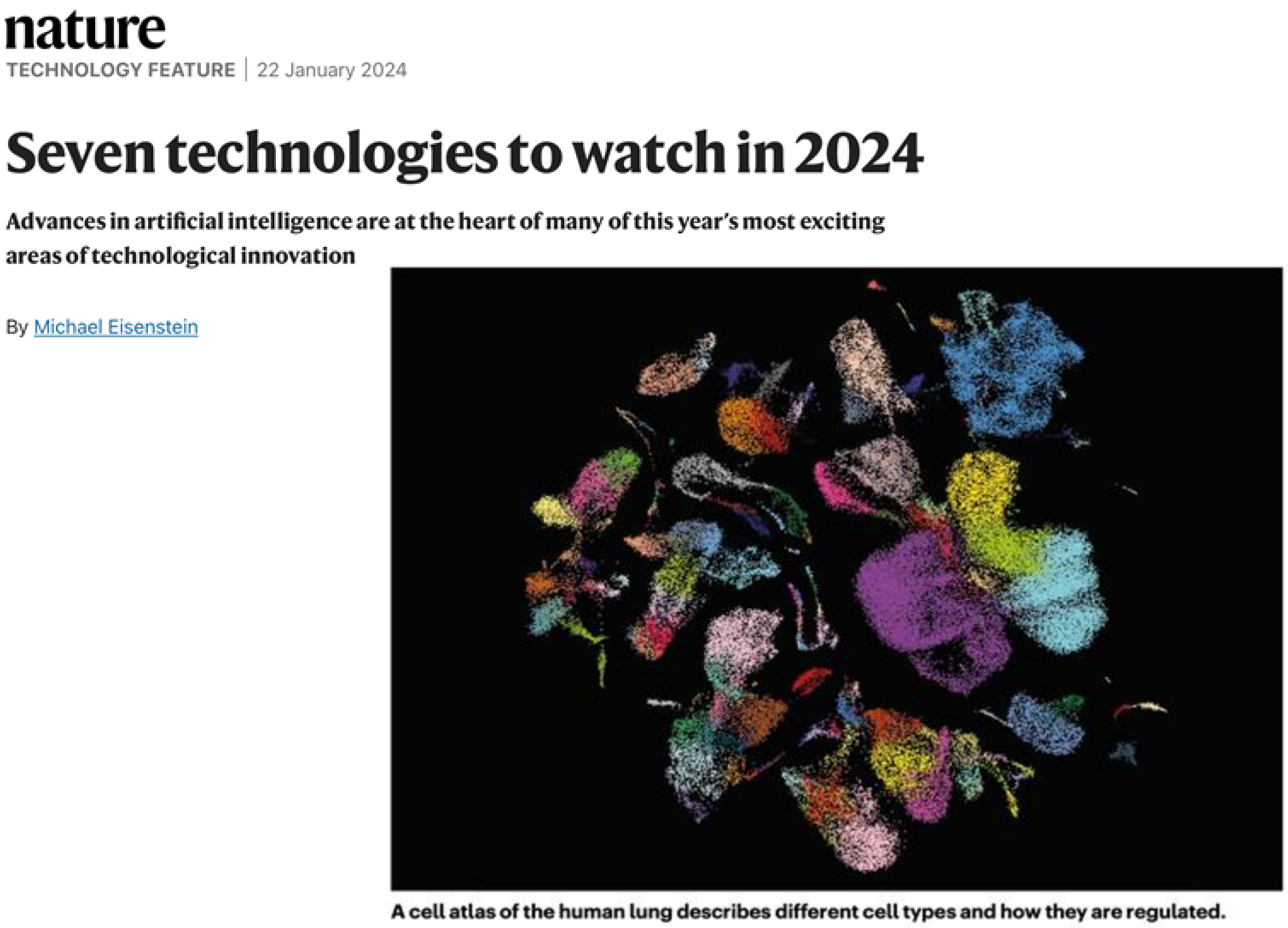
Exploring Omics with CAT: A Gateway to Genomic and Spatial Insight

Decoding the Spatial Dimension of Gene Expression: A Comprehensive Introduction to Spatial Transcriptomics Platforms, Bioinformatics Analysis, and Their Applications in Biological Research.

Bo Young Ahn and **Heewon Seo**
Applied Spatial Omics Centre
Cumming School of Medicine
University of Calgary

June 17, 2025

Envision New Dimension: Spatial Omics



nature

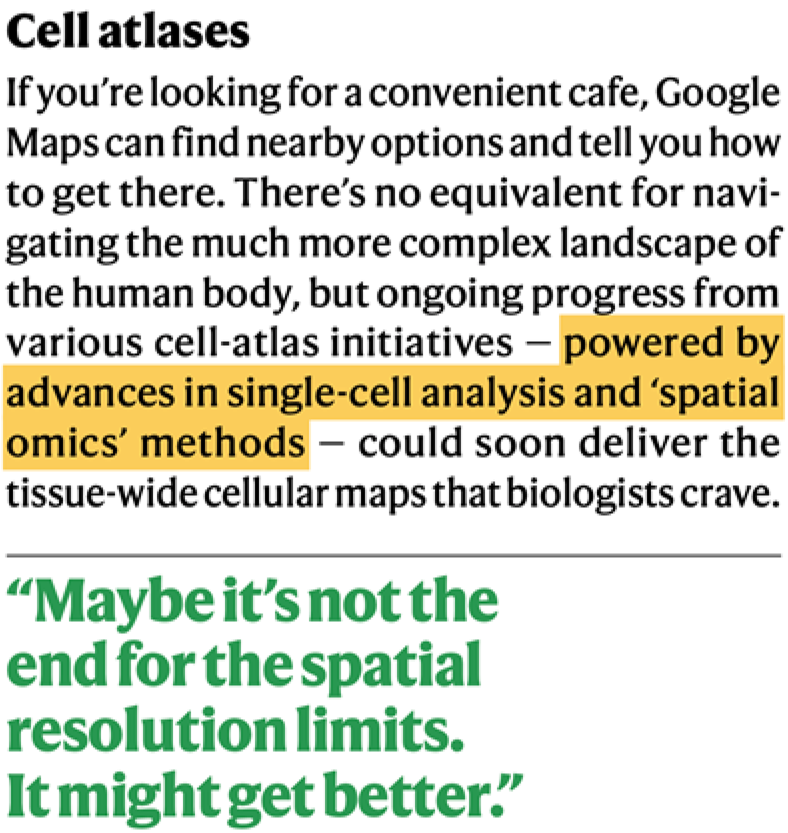
TECHNOLOGY FEATURE | 22 January 2024

Seven technologies to watch in 2024

Advances in artificial intelligence are at the heart of many of this year's most exciting areas of technological innovation

By Michael Eisenstein

A cell atlas of the human lung describes different cell types and how they are regulated.



Cell atlases

If you're looking for a convenient cafe, Google Maps can find nearby options and tell you how to get there. There's no equivalent for navigating the much more complex landscape of the human body, but ongoing progress from various cell-atlas initiatives – powered by advances in single-cell analysis and 'spatial omics' methods – could soon deliver the tissue-wide cellular maps that biologists crave.

> **"Maybe it's not the end for the spatial resolution limits. It might get better."**

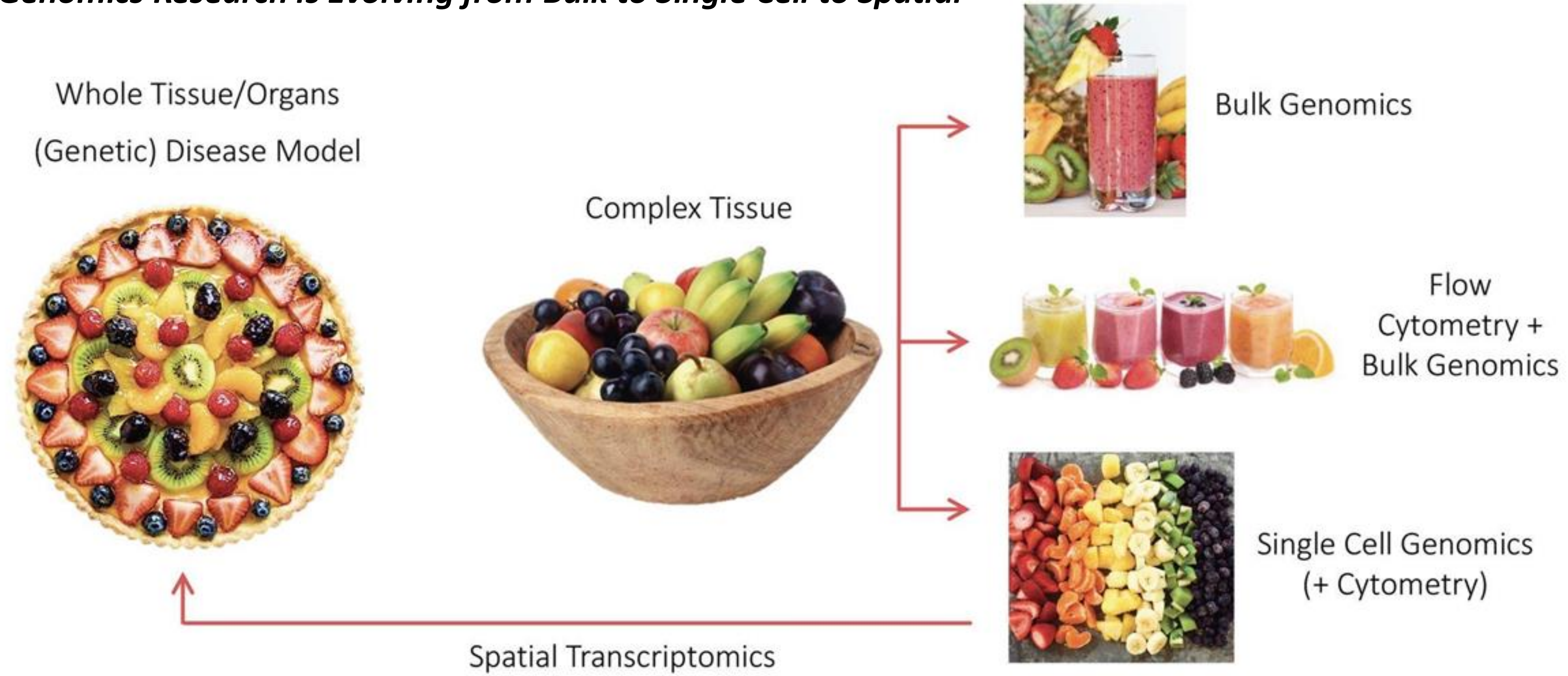
What is Spatial Omics?

- Spatial Omics includes:
  - Spatial Transcriptomics
  - Spatial Proteomics

- Spatial Omics is an overarching term for all methods that assign **transcriptomics/proteomics data to the original location within the tissue**.

Spatial Transcriptomics in Layman's Term I

Genomics Research is Evolving from Bulk to Single Cell to Spatial



Whole Tissue/Organs

(Genetic) Disease Model

Complex Tissue

Bulk Genomics

Flow
Cytometry +
Bulk Genomics

Single Cell Genomics
(+ Cytometry)

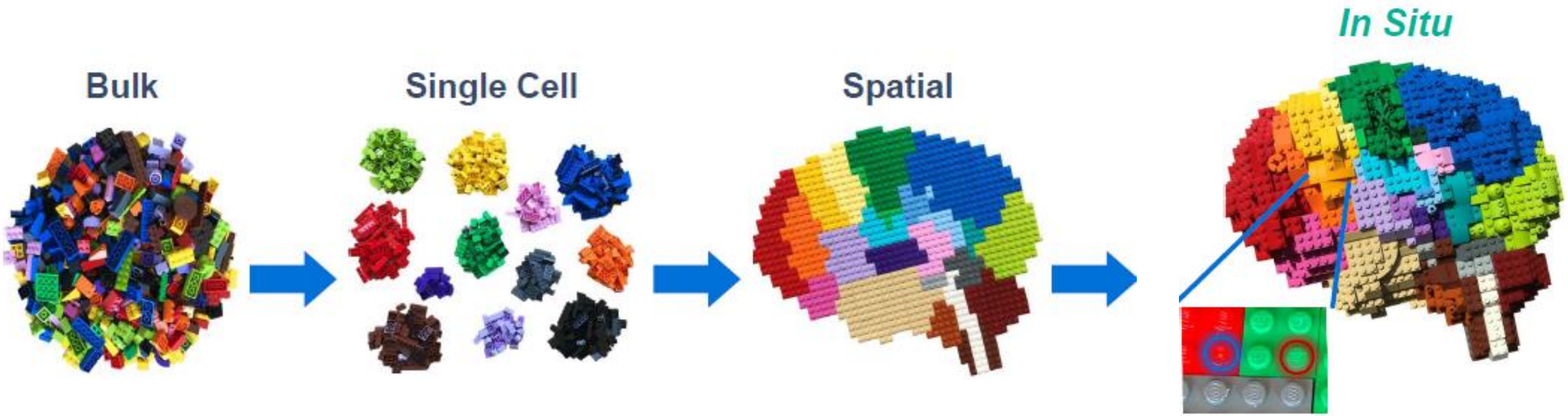
Spatial Transcriptomics

https://twitter.com/LGMartelotto/status/1186745128615985152

Spatial Transcriptomics in Layman's Term *II*

Getting the Best View of Biology and Disease

Overview of legacy and current "omic" methods



https://twitter.com/bioxia7/status/1261464021322137600?s=12

Spatial Transcriptomics in Canadian's Term

Bulk-Player Information

Single-Player

Spatial-Players

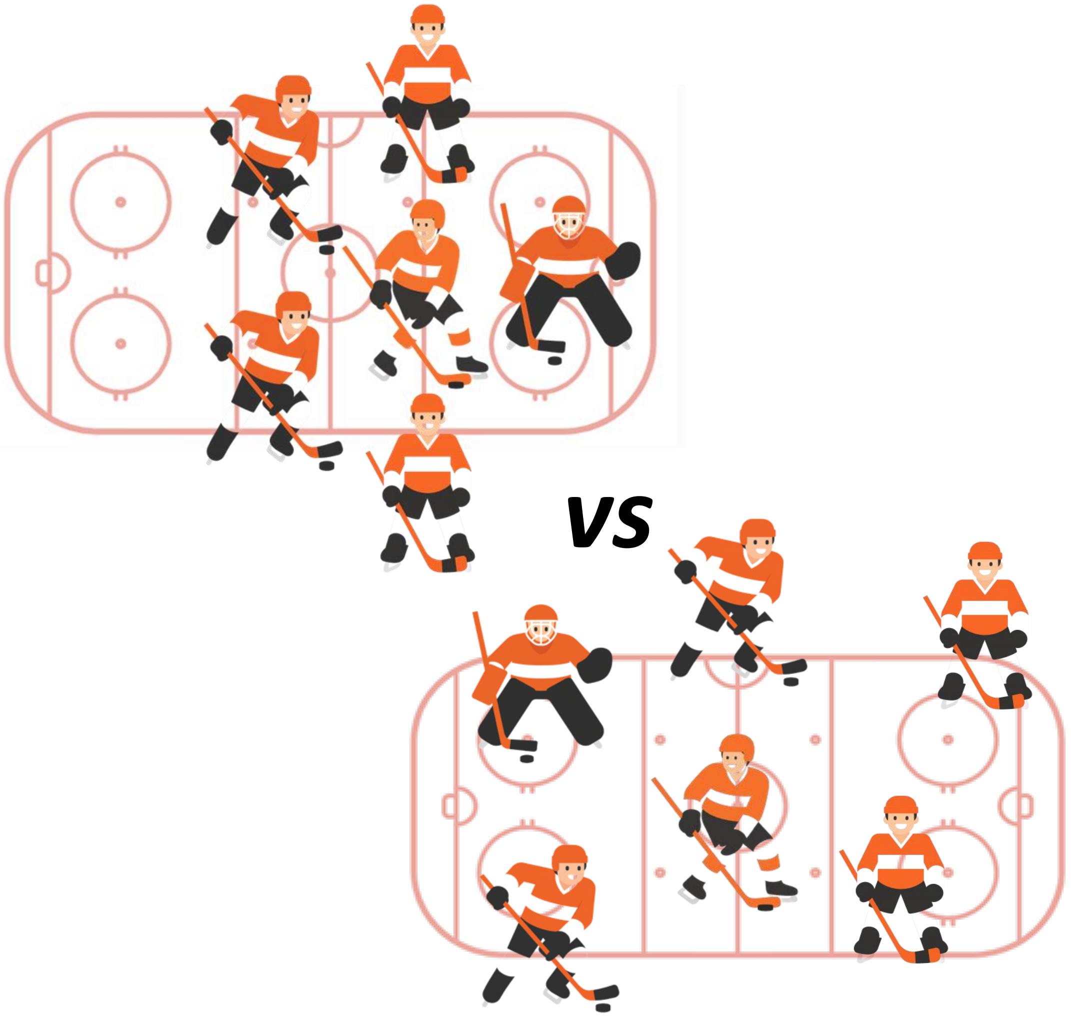
Spatial Transcriptomics in Canadian’s Term

Bulk-Player Information

Single-Player

Spatial-Players

Spatial Transcriptomics in Canadian's Term



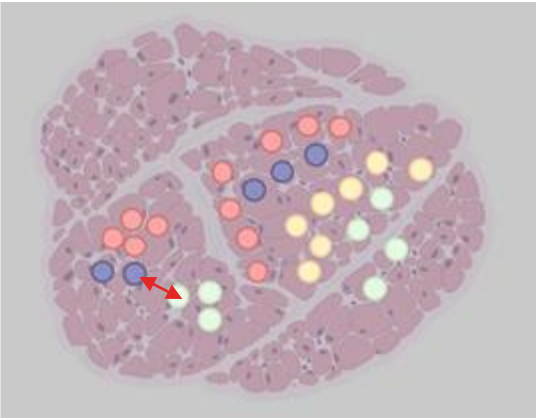
Bulk-Player Information
Bulk Genomics

Single-Player
Single Cell Genomics

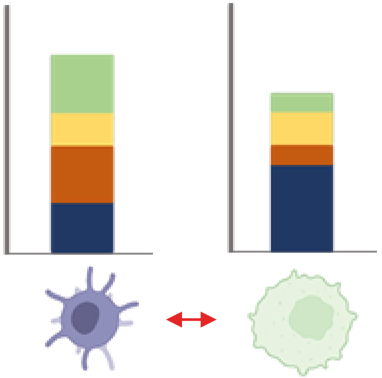
Spatial-Players
Spatial Genomics

Advances in Spatial Transcriptomics

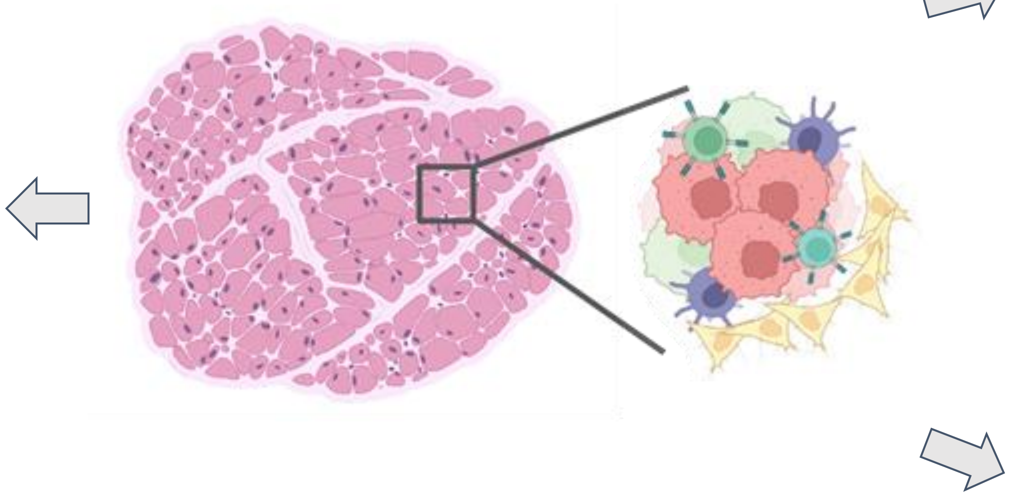
Spatial Transcriptomics



Map the locations of cell types across a tissue

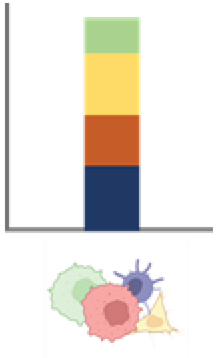


Cell-cell interaction



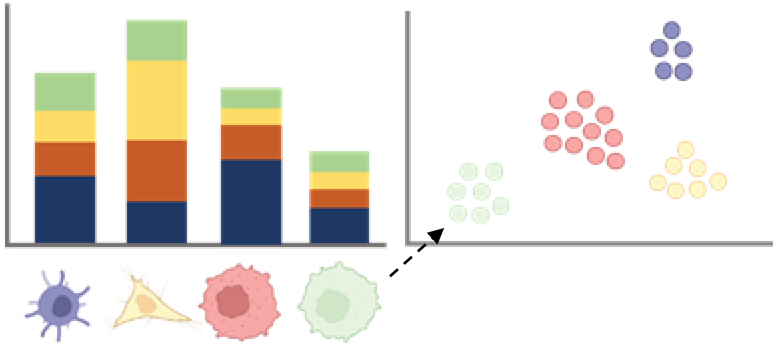
Property	Bulk	Single-cell	Spatial
Cell-type	X	O	O
Location	X	X	O

Bulk Genomics



Average gene expression

Single-cell Genomics

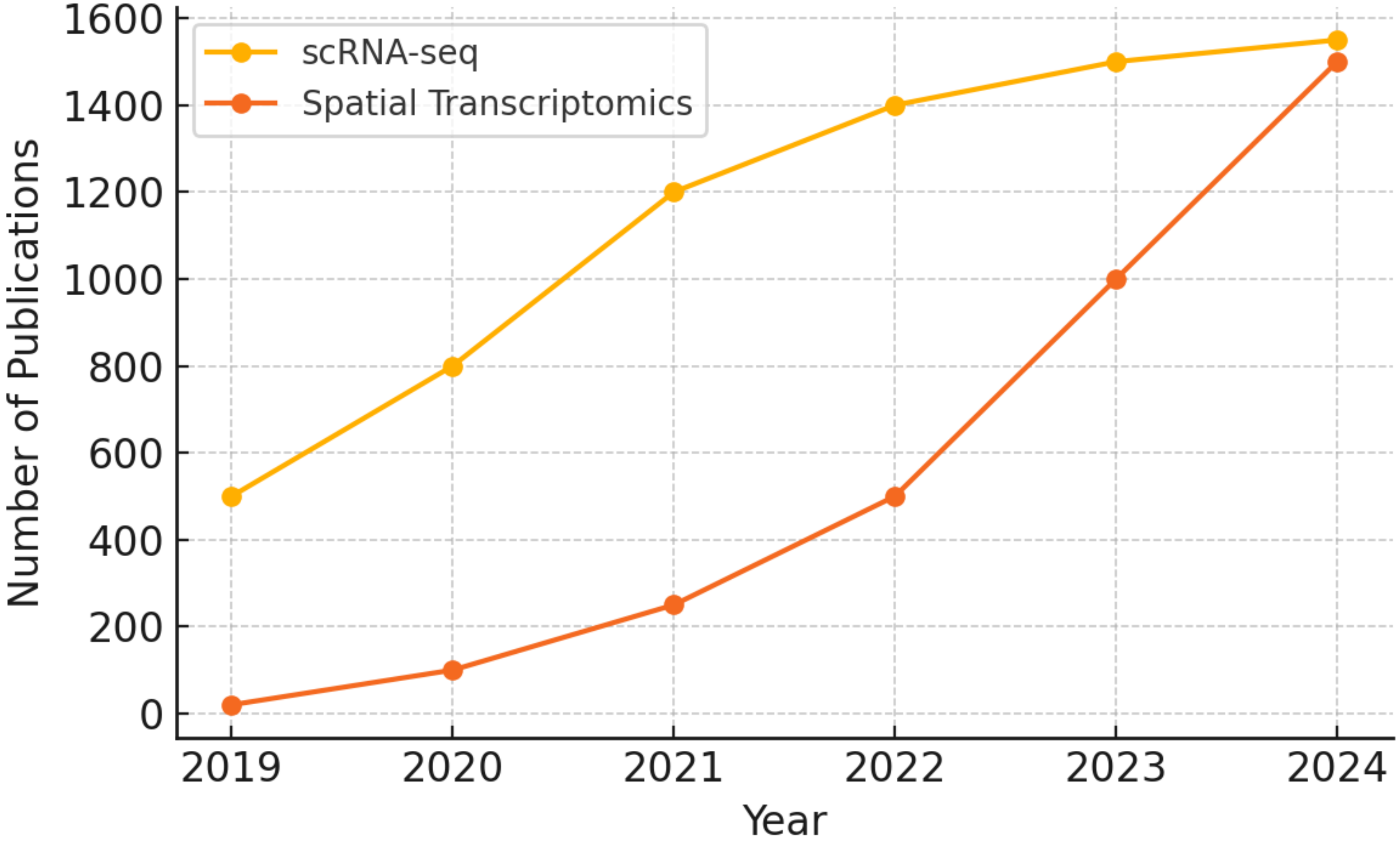


Cell-type gene expression

Cell-type clusters

Why it's important

Publications Trends (2019-2024)



PubMed search trends (2019–2024)

Why it's important

ASOC Applied Spatial Omics Centre
Centre for Advanced Technologies
UNIVERSITY OF CALGARY

History of Spatial Transcriptomics

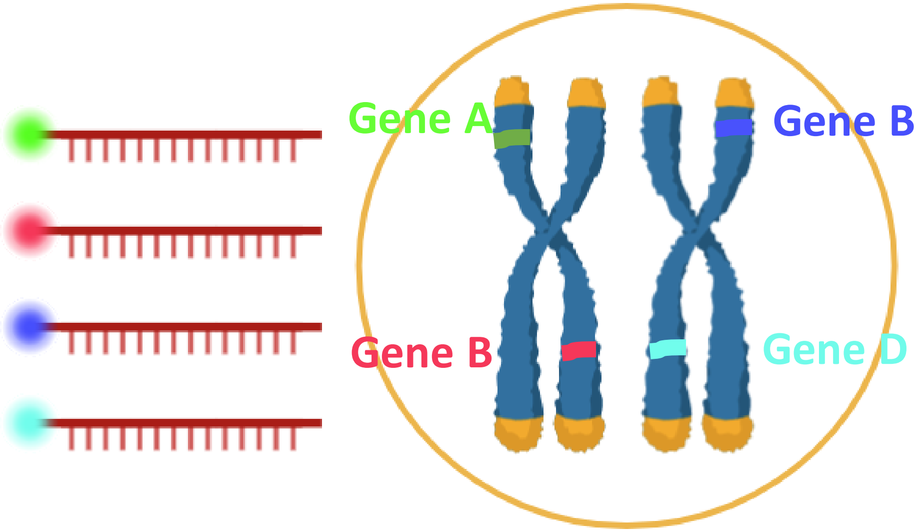
- ***Spatial transcriptomics isn't brand new***;

It began in the 1970s but could only handle one gene at a time.

Dr. Gall lab reported a DNA-DNA hybridization method (*in situ* hybridization) using radioactive labeling for detecting the cellular localization of DNA sequences (*PNAS* 1969).

- The recent rise in spatial transcriptomics comes from the ability to measure hundreds, thousands, or even whole transcriptome at once!

Fluorescence in-situ Hybridization (FISH)



Four Main Key Spatial Transcriptomics Technologies

Sequencing-based method:

- *in situ* capture

  ex, Visium
- Region of interest (ROI)-based

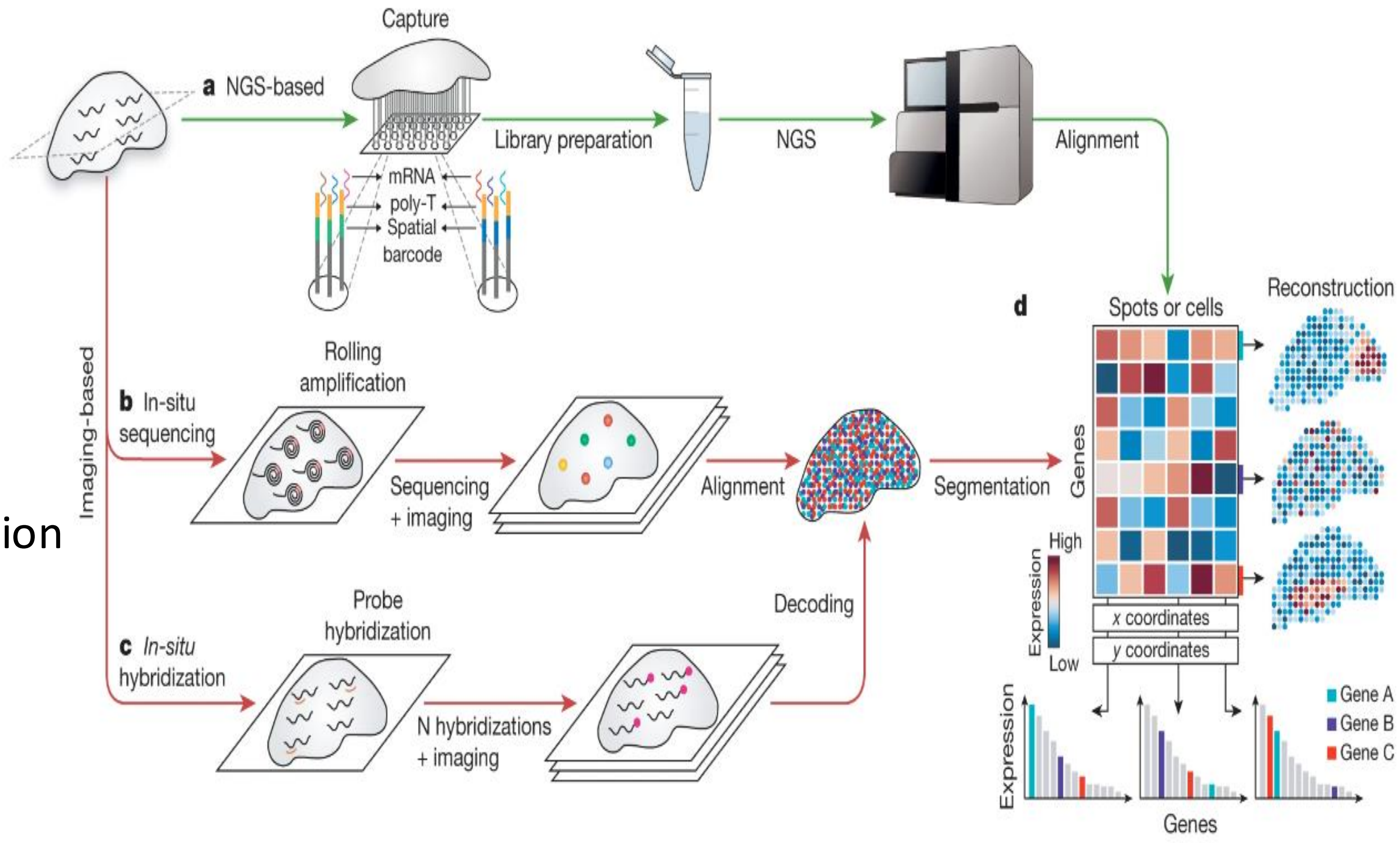
  ex, GeoMx

Imaging-based method:

- Fluorescence in situ hybridization (fISH)

  ex, MERFISH, CosMx
- *in situ* sequencing (ISS)

  ex, Xenium



Rao et al., Nature (2021)

Four Main Key Spatial Transcriptomics Technologies

Sequencing-based method:

- ***Need Sequencing***

  ex, Visium

- Region of interest (ROI)-based

  ex, GeoMx

Imaging-based method:

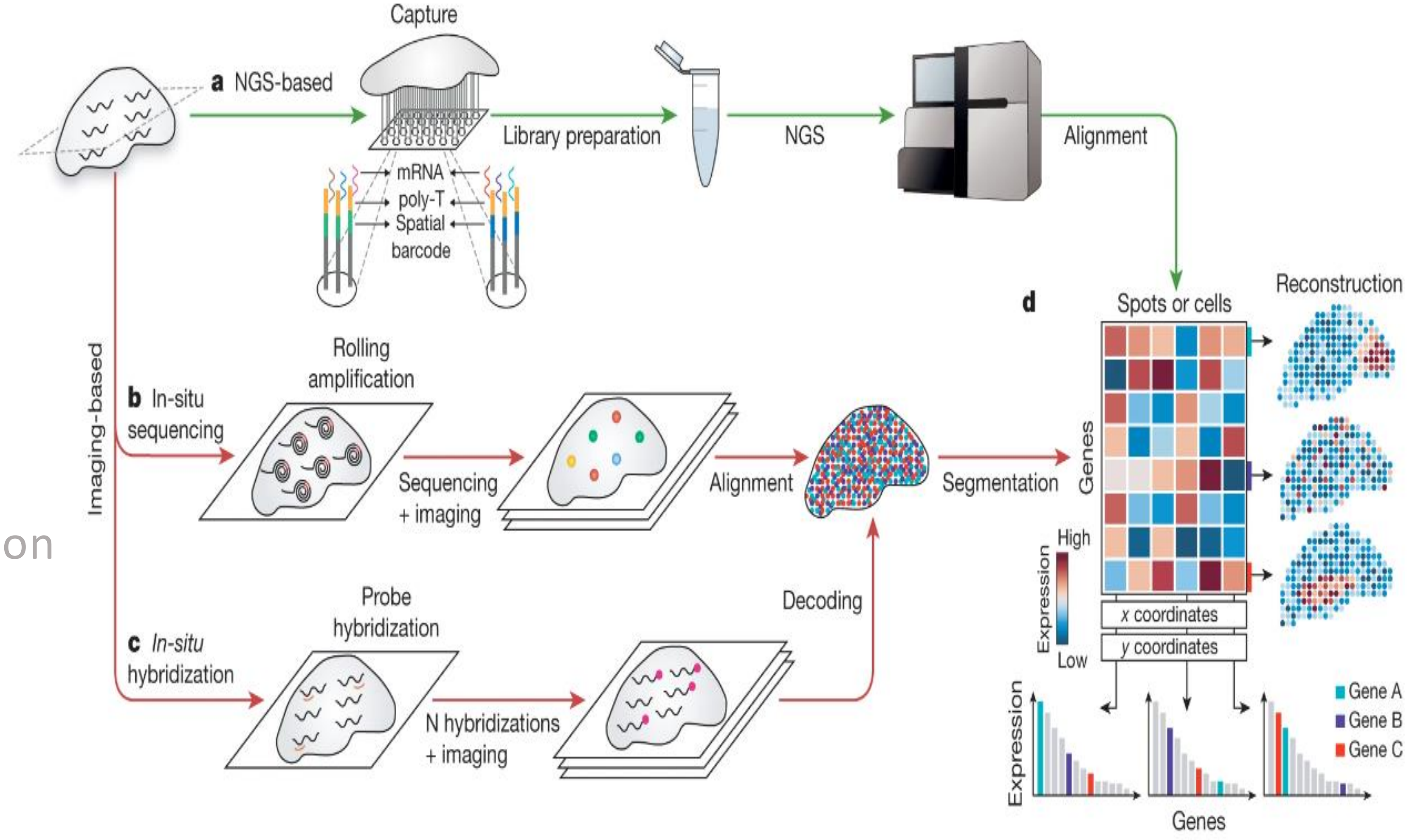
- Fluorescence in situ hybridization

  No Need Sequencing

  Longer Scanning Time

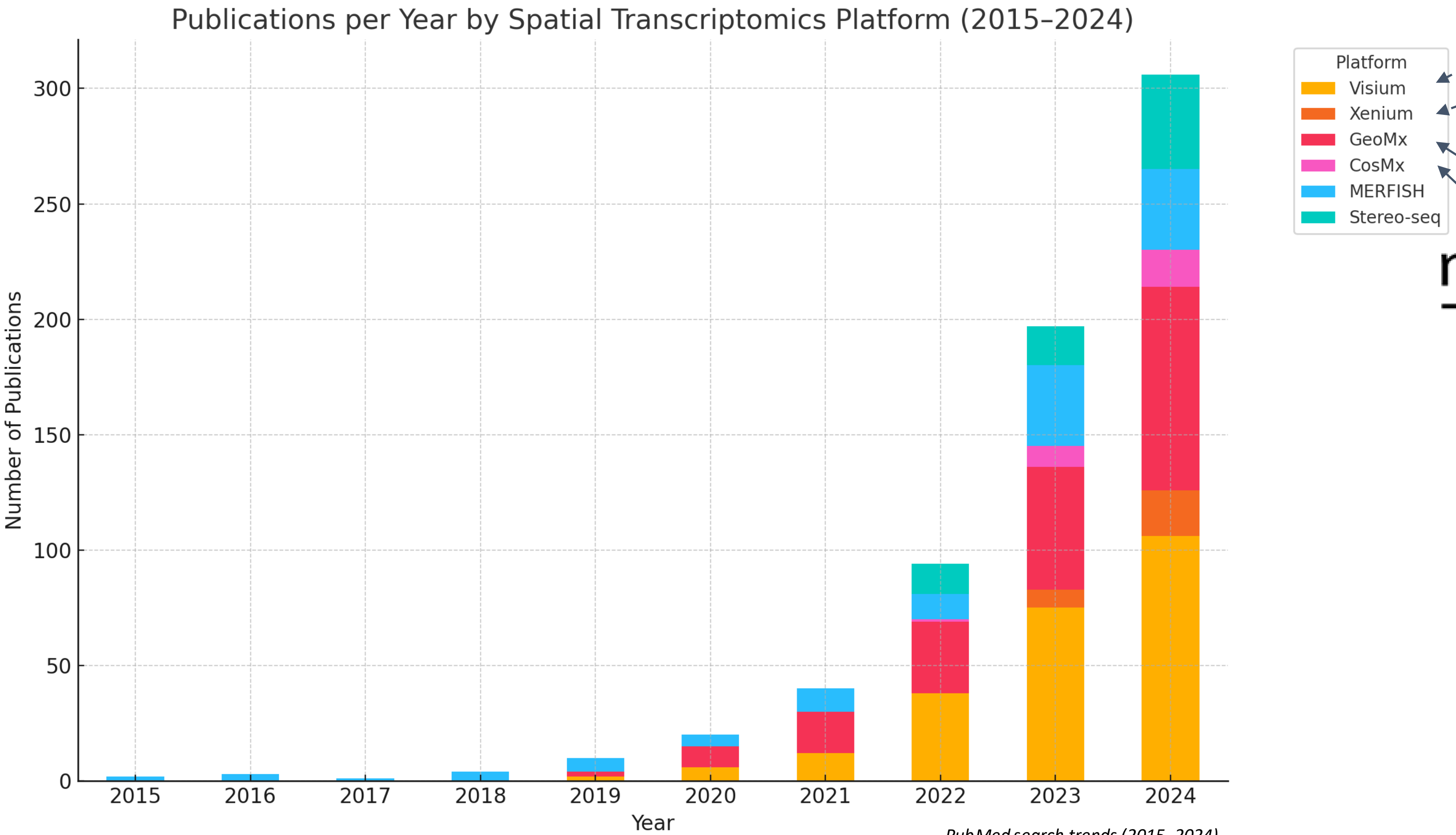
- *in situ* sequencing (ISS)

  ex, Xenium



Rao et al., Nature (2021)

Publications in Spatial Omics (2015-2024)



PubMed search trends (2015–2024)

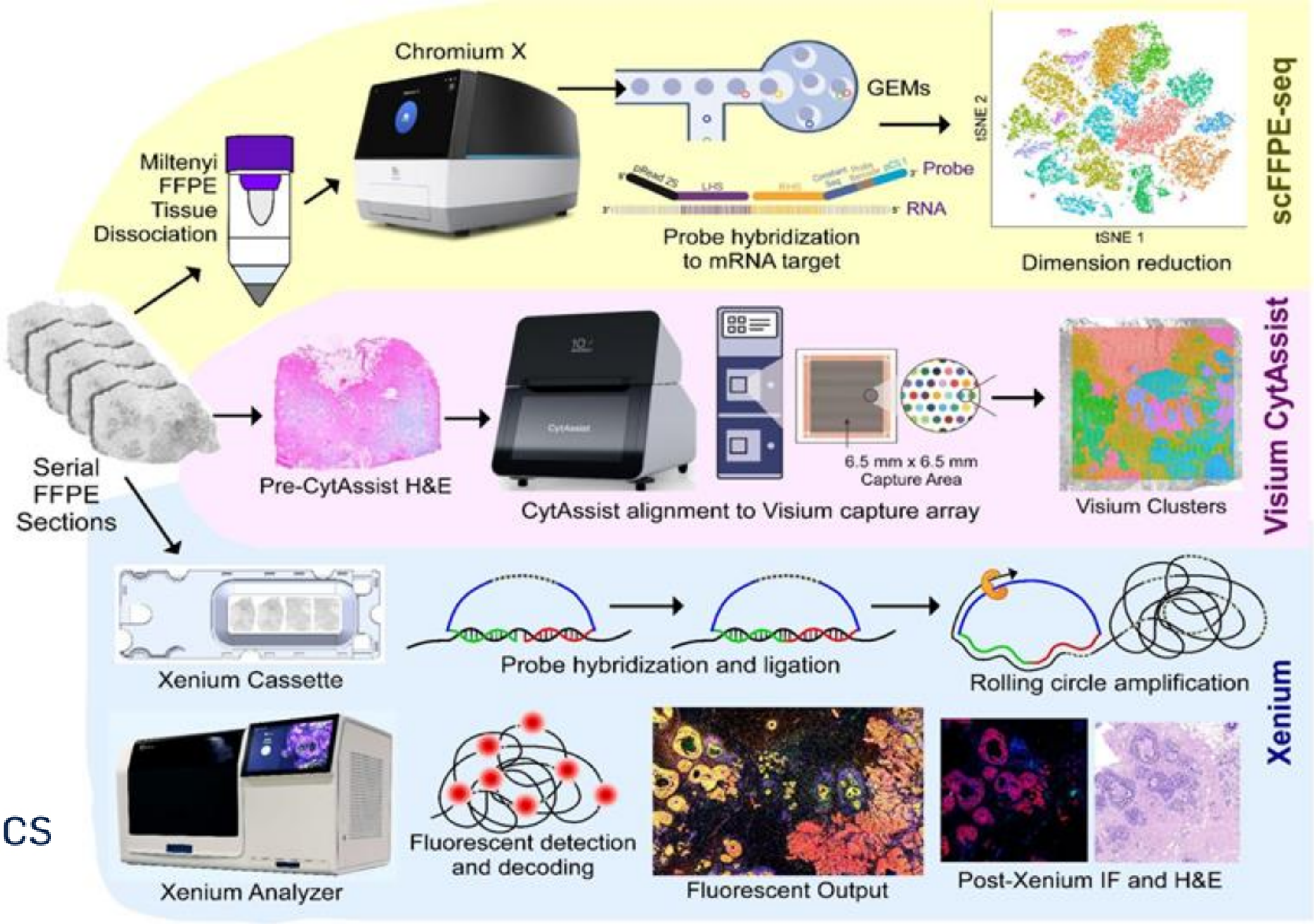
10X GENOMICS

nanoString

Spatial Omics Platforms: *10X Genomics*

Three Pillars of 10X Genomics

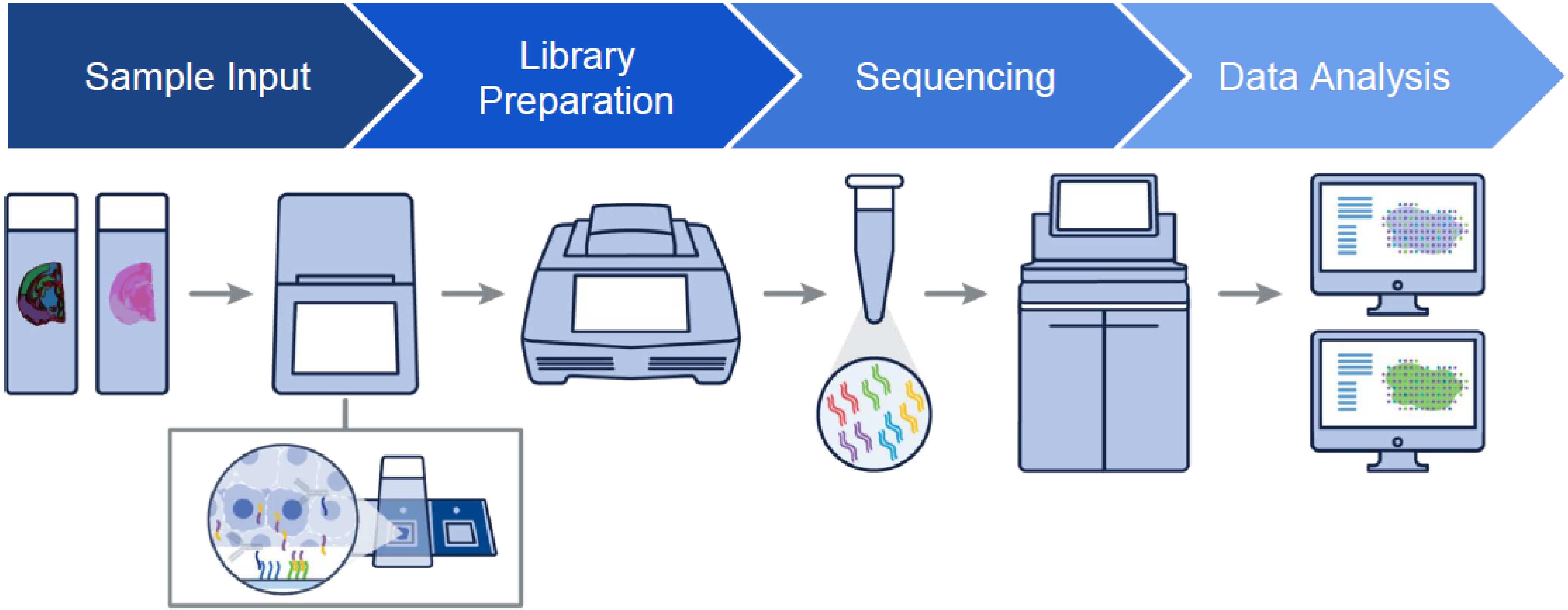
- Outlook for single-cell
- Spatial and In Situ Platforms



https://www.biorxiv.org/content/10.1101/2022.10.06.510405v2

ST analysis workflow

Visium Spatial Gene Expression



Sample Preparation
User-supplied human samples
Freshly Placed FFPE Sections
Archived H&E Slides

Library Preparation
Visium CytAssist
10x Genomics Kits
User-supplied third-party reagents

Sequencing
10x Genomics library-compatible sequencer (e.g., Illumina sequencer)

Analysis
Space Ranger analysis pipeline
Loupe Browser visualization tool

Source: 10X Genomics

ST analysis platform – 10X Genomics: Visium

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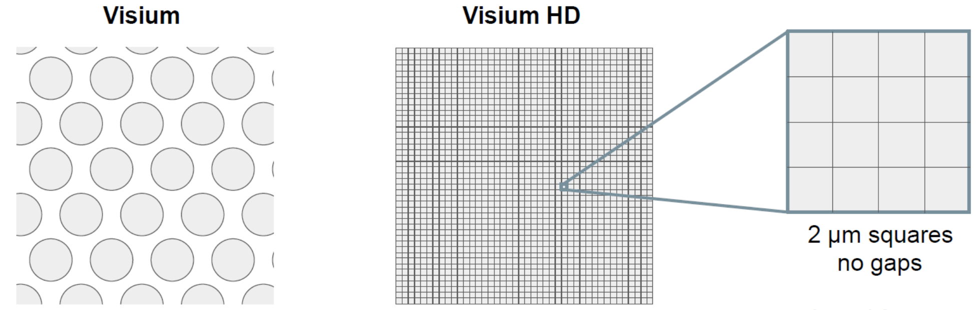
Visium Spatial Gene Expression

First-Generation assays (55 um barcoded spot)

- **v1 3’ Gene Expression**: 3’ ploy(A) capture-based chemistry for fresh frozen tissues from diverse species
  Resolution of 1–10 cells per 55 µm barcoded spot
- **v2 WT Panel Gene Expression**: Probe-based chemistry for human and mouse FFPE, fresh frozen, and fixed frozen tissues
  Resolution of 1–10 cells per 55 µm barcoded spot

High-Definition assay (2 um barcoded spot) – *Single-Cell Resolution*

- **HD 3’ Gene Expression**: 3’ ploy(A) capture-based chemistry for fresh frozen tissues from diverse species
  Resolution of single cell per 2 µm barcoded spot
- **HD WT Panel Gene Expression**: Probe-based chemistry for human and mouse FFPE, fresh frozen, and fixed frozen tissues
  Resolution of single cell per 2 µm barcoded spot



55 µm spots, hexagonally arranged

8 µm squares, no gaps

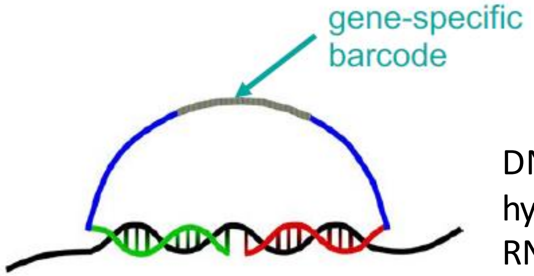
2 µm squares no gaps

Actual feature size

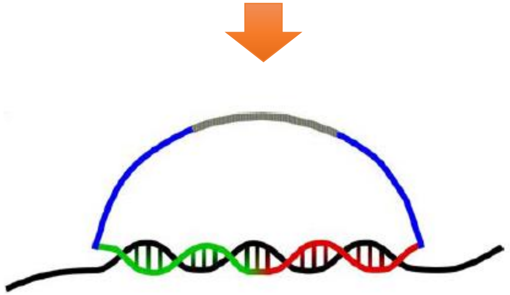
Source: 10X Genomics

Xenium *in situ*

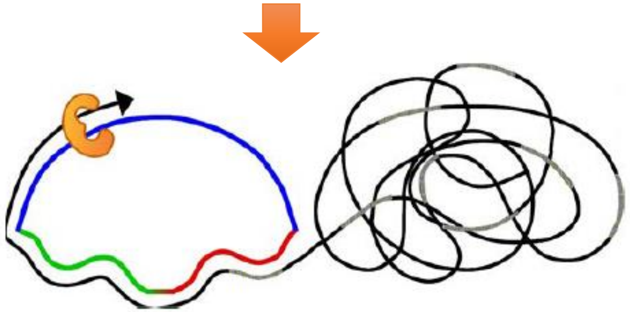
Section tissue onto 12 mm x 24 mm area



DNA probes hybridized to target RNA

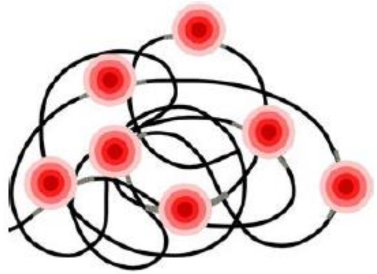


Highly specific ligation

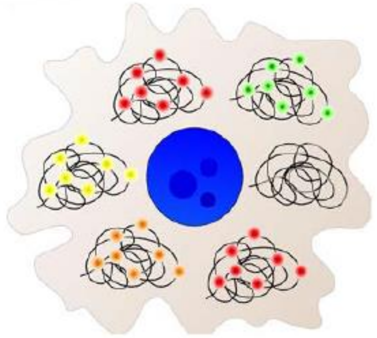


Multiple copies of barcode generated

Chemistry and Imaging



Fluorescent oligos hybridize to amplified probes



Multiple cycles of probe binding and stripping

Onboard analysis decodes fluorescent signal across cycles to transcripts

10X Genomics

ST analysis platform – 10X Genomics: Xenium

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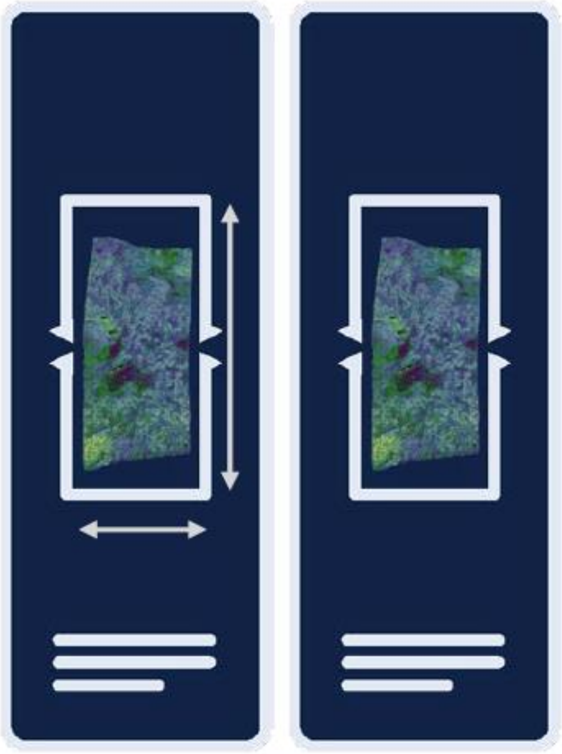
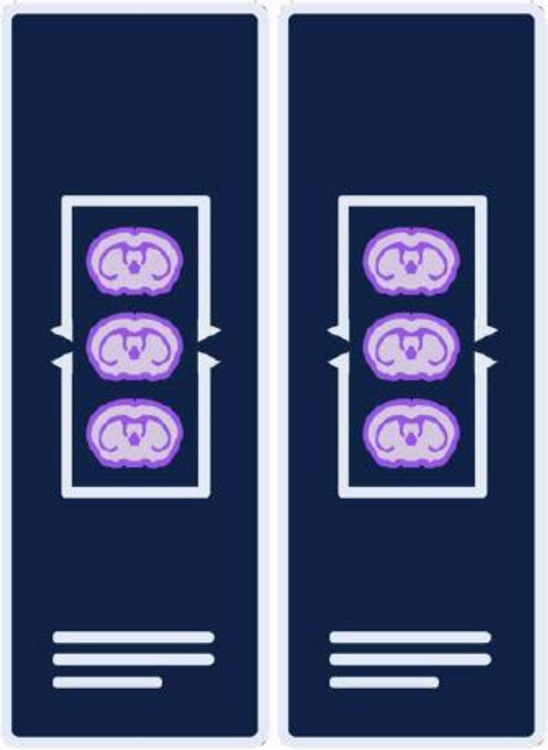
Xenium *in situ*



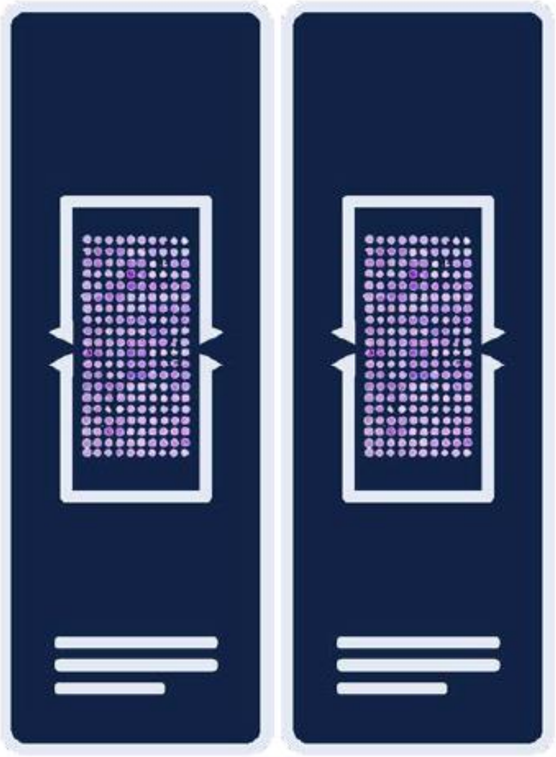
Two 10.5 x 22.5mm sections

~2M cells



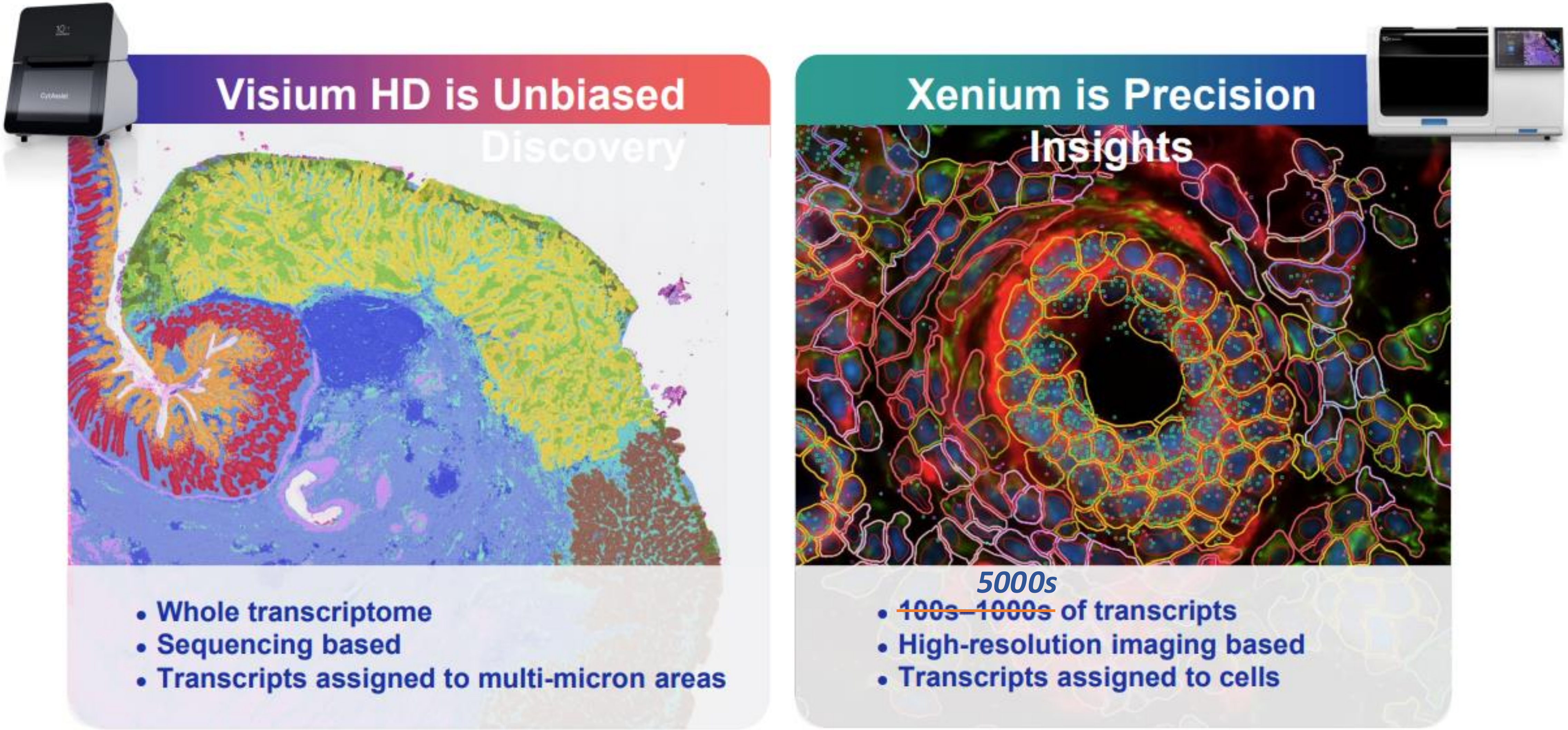
Six 6 x 10mm mouse brain sections

~1M cells



~Four hundred 1 x 0.6mm biopsies in TMAs

~1M cells



Visium HD is Unbiased Discovery

- Whole transcriptome
- Sequencing based
- Transcripts assigned to multi-micron areas

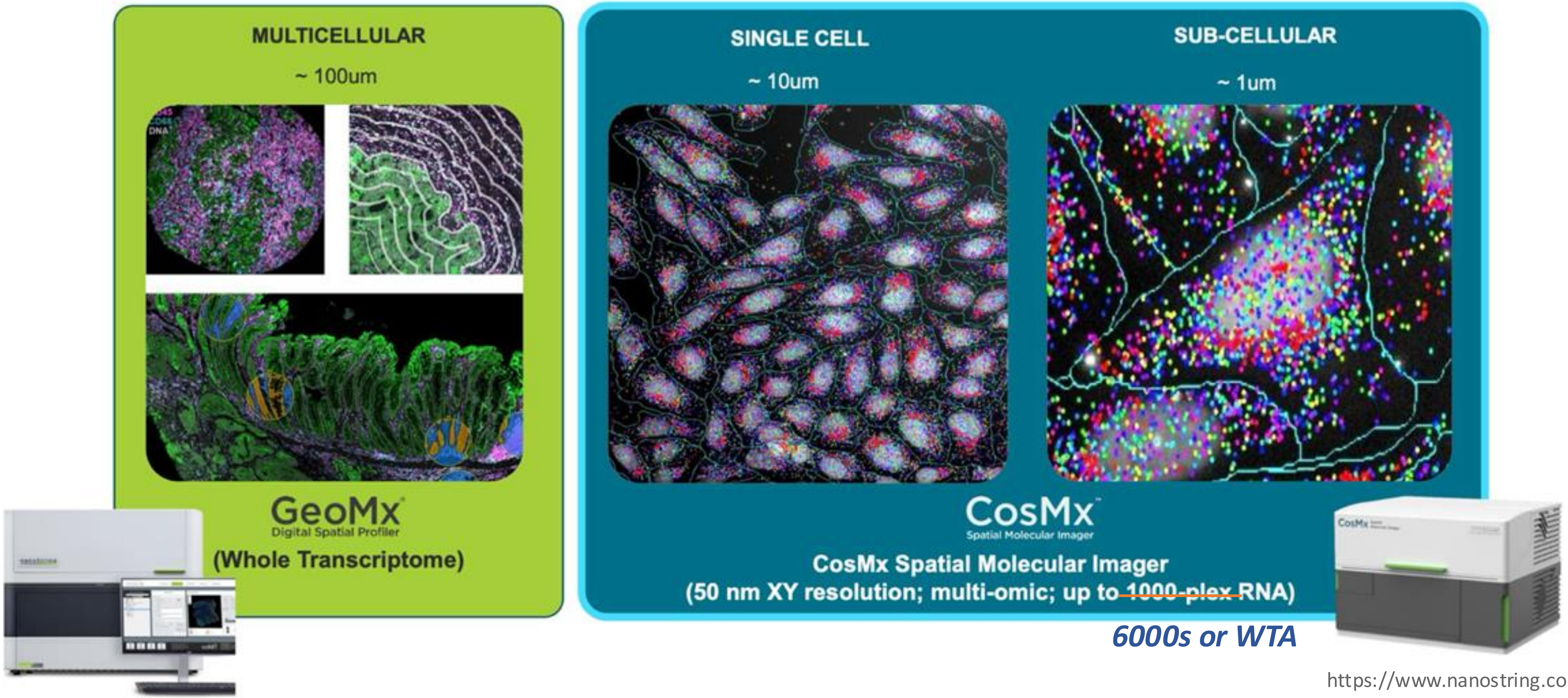
Xenium is Precision Insights

- ~~100s–1000s~~ 5000s of transcripts
- High-resolution imaging based
- Transcripts assigned to cells

10X Genomics

ST analysis platform – 10X Genomics: Visium HD vs Xenium

Spatial Omics Platforms: *Nanostring*



MULTICELLULAR

~ 100um

GeoMx Digital Spatial Profiler

(Whole Transcriptome)

SINGLE CELL

~ 10um

SUB-CELLULAR

~ 1um

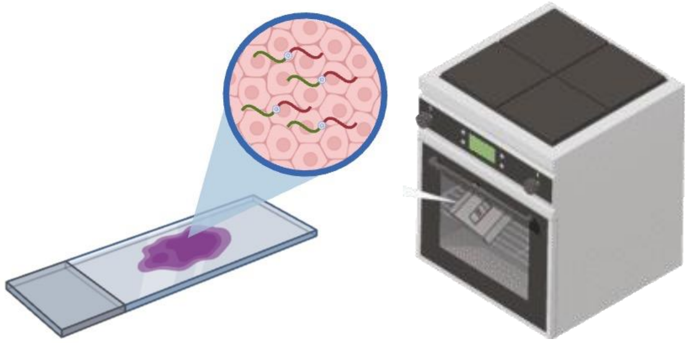
CosMx Spatial Molecular Imager

CosMx Spatial Molecular Imager
(50 nm XY resolution; multi-omic; up to ~~1000-plex~~ RNA)

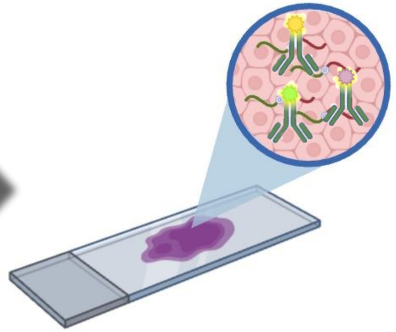
6000s or WTA

https://www.nanostring.com/

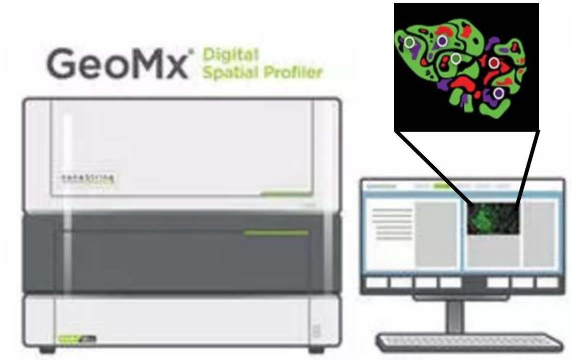
GeoMx Digital Spatial Profiler (DSP)



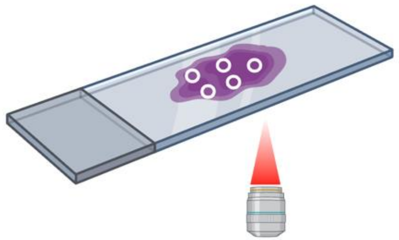
Hybridization with barcoded RNA probes



Stain with morphology markers

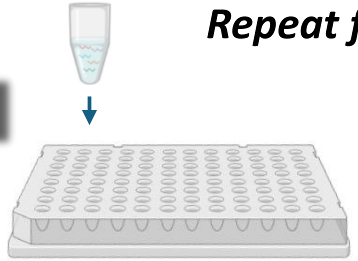


Scan and Define Region of Interest (ROI)



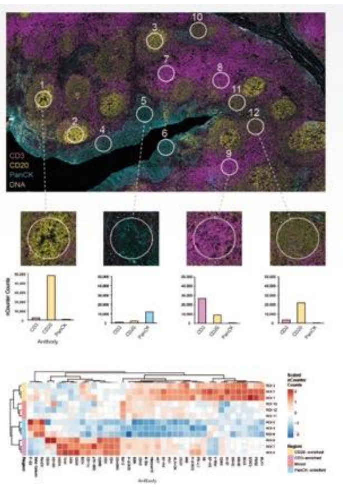
Probes release from each area-of illumination (AOI) with UV light

Repeat for each ROI

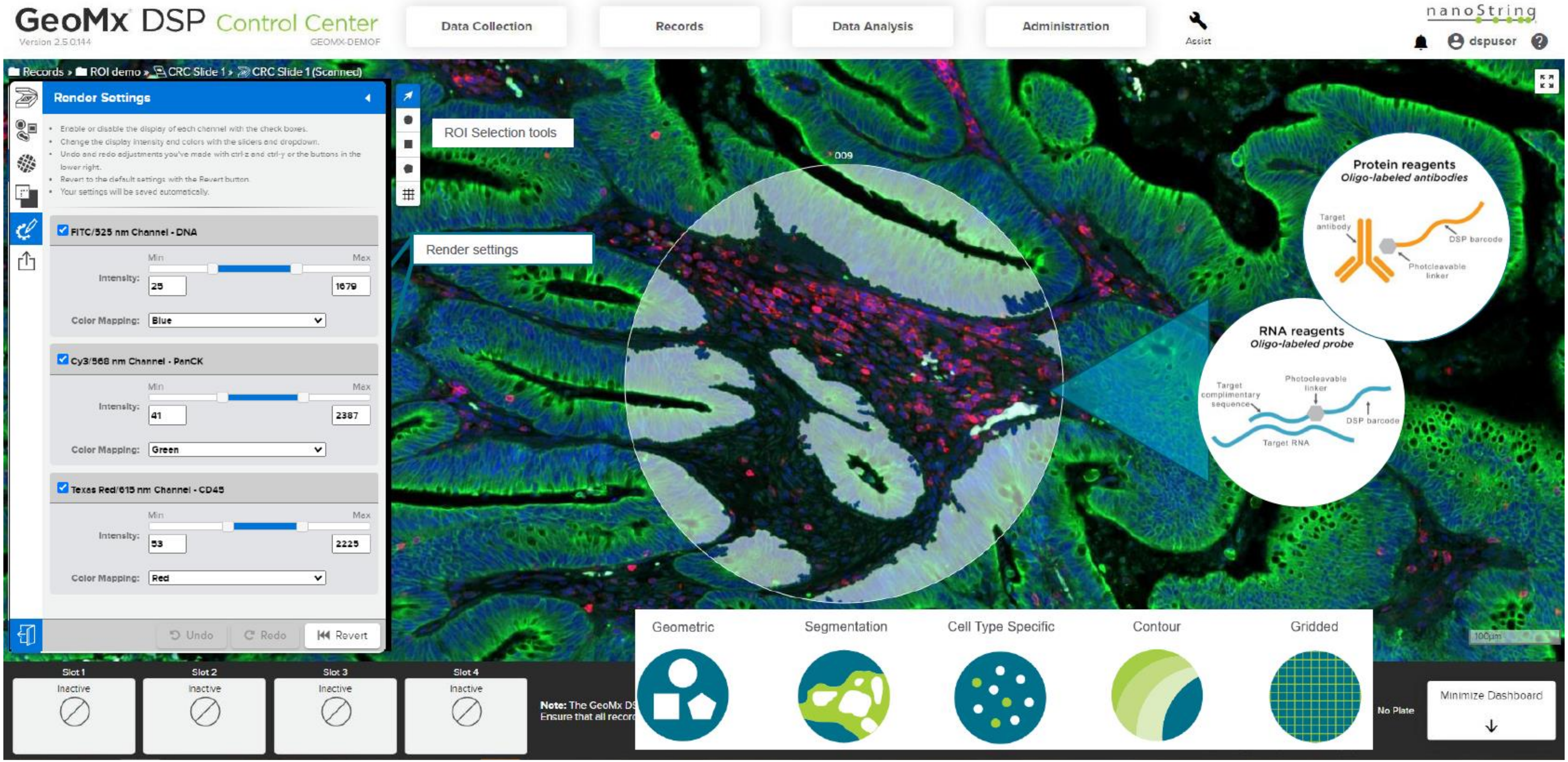


Dispense the UV-cleaved oligos into a 96-well plate

Each AOI is uniquely indexed during library preparation for NGS readout



GeoMx Region of Interest (ROI) Selection and Segmentation



Render Settings

- Enable or disable the display of each channel with the check boxes.
- Change the display intensity and colors with the sliders and dropdown.
- Undo and redo adjustments you've made with ctrl-z and ctrl-y or the buttons in the lower right.
- Revert to the default settings with the Revert button.
- Your settings will be saved automatically.

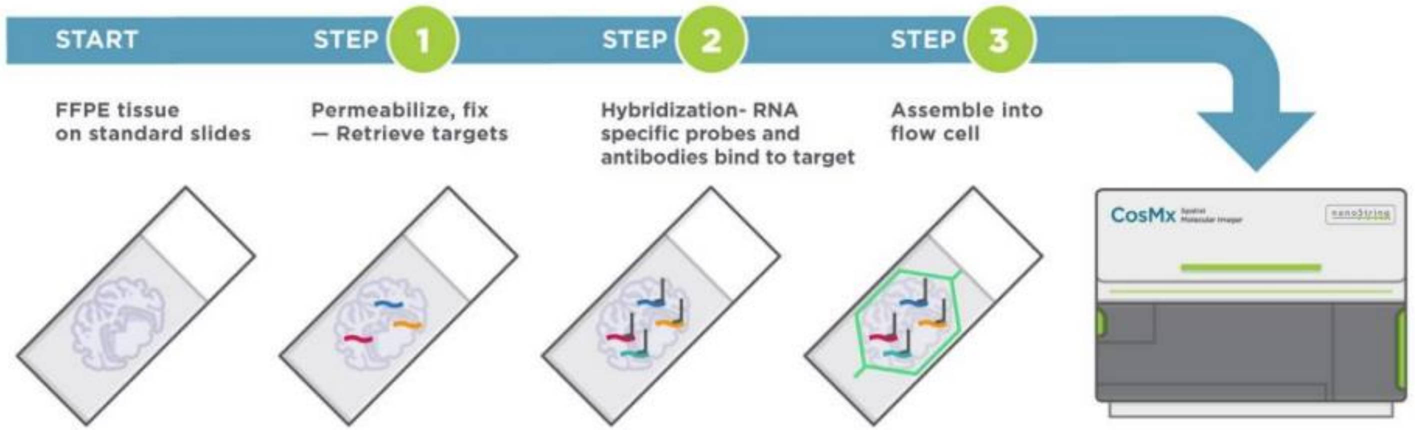
☑ **FITC/525 nm Channel - DNA**
Intensity: Min 25 Max 1679
Color Mapping: Blue

☑ **Cy3/568 nm Channel - PanCK**
Intensity: Min 41 Max 2387
Color Mapping: Green

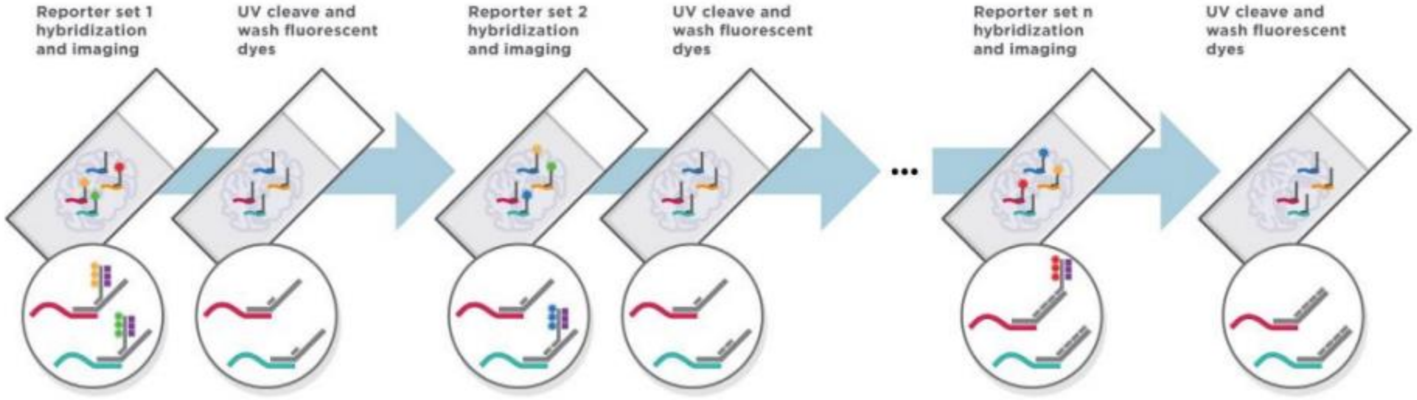
☑ **Texas Red/615 nm Channel - CD45**
Intensity: Min 53 Max 2225
Color Mapping: Red

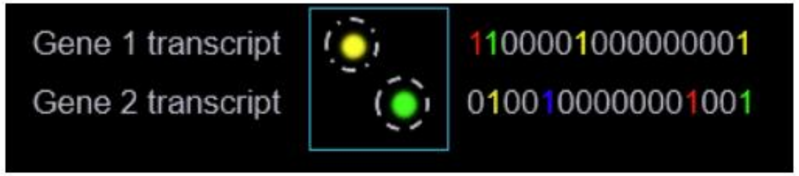
Note: The GeoMx D
Ensure that all record

CosMx Spatial Molecular Imager



Simple and streamlined sample prep workflow.

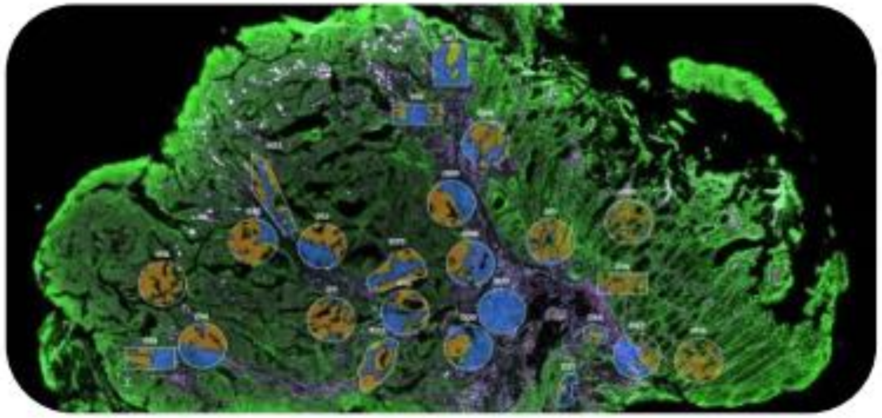




Nanostring

2019

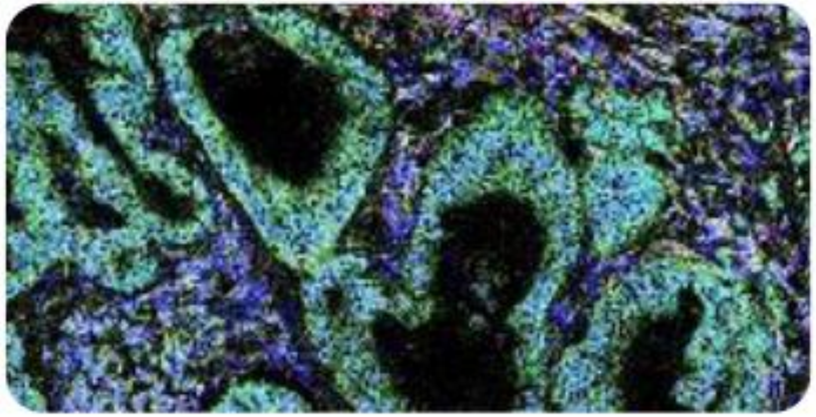
GeoMx® DSP



- High-plex, whole transcriptome RNA, 570+ protein (multiomics)
- Fast and flexible platform
- High sensitivity with user-defined tissue sampling
- *ex situ* sequencing readout

2022

CosMx™ SMI



- High Plex detection 6K→18K RNA, 72 proteins
- Accurate cell segmentation
- Comprehensive data analysis with AtoMx™ SIP
- *In situ* imager

Nanostring

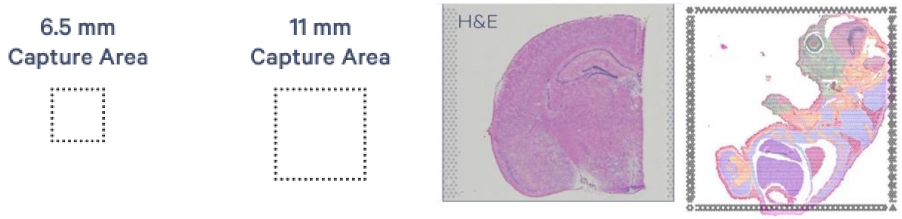
ST analysis platform – Nanostring: GeoMx vs CosMx

How Many

- **Visium: 6.5 mm or 11 mm**

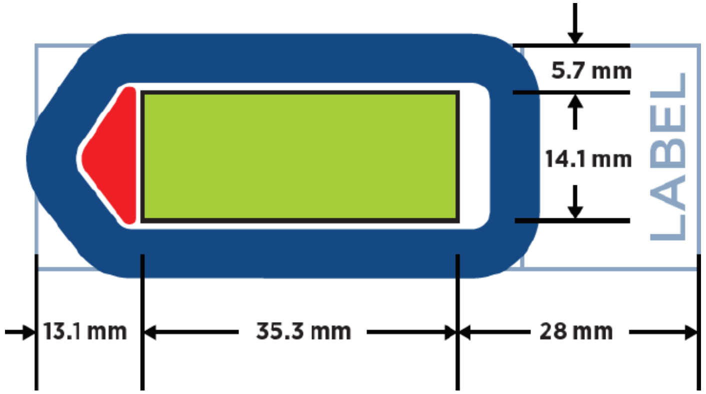
2/3 whole mouse brains or mouse embryo per capture area

Tissue microarray (TMA) is available for Visium CytAssist



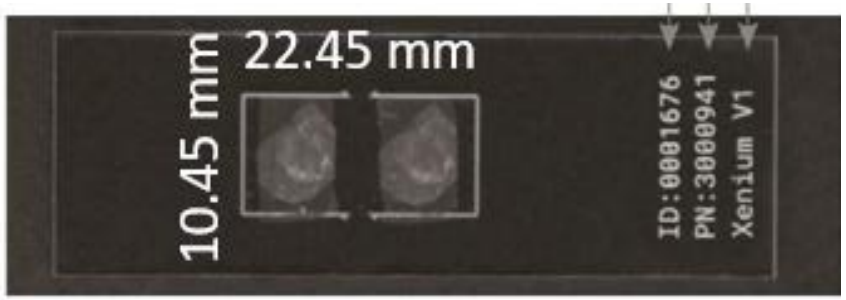
- **GeoMx: 35.3 mm X 14.1 mm**

4 whole mouse brains



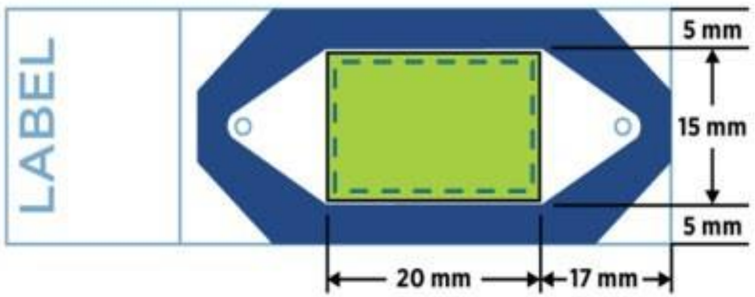
- **Xenium: 10.45 mm X 22.45 mm**

3 whole mouse brains



- **CosMx: 20 mm X 15 mm**

3 whole mouse brains



Adapted from 10X Genomics

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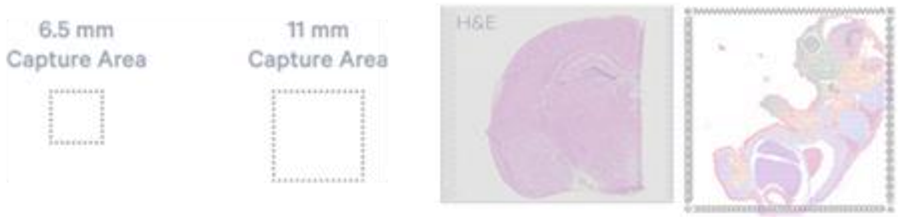

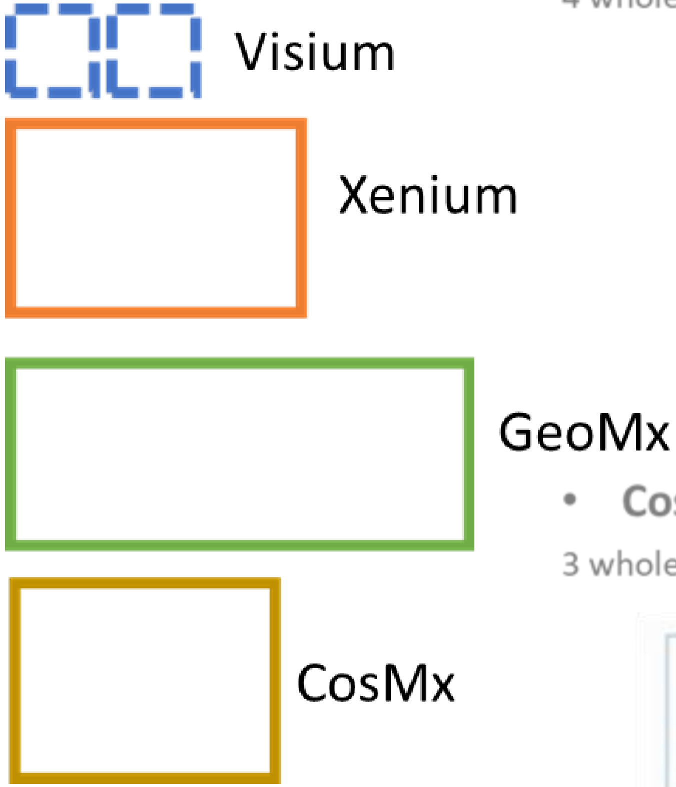

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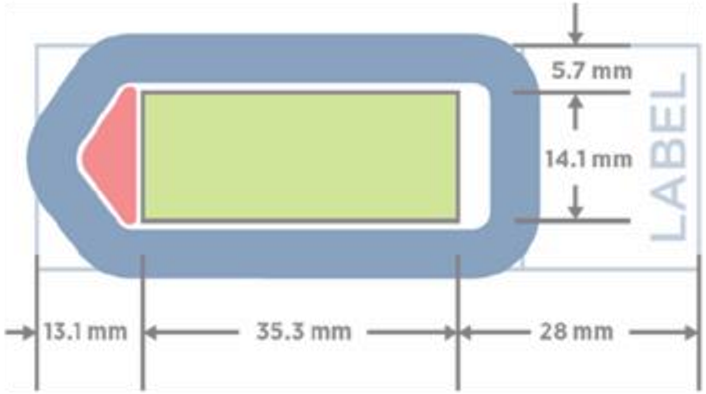
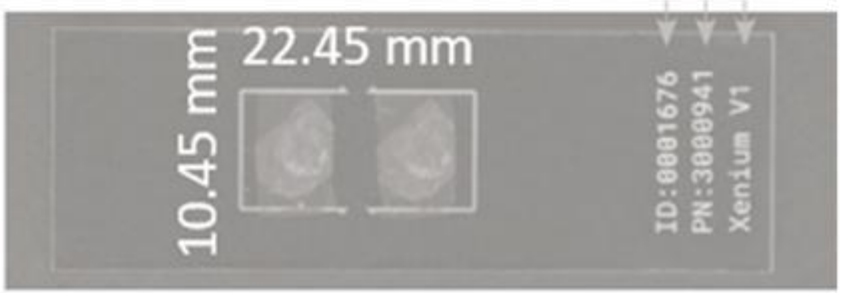
4 whole mouse brains



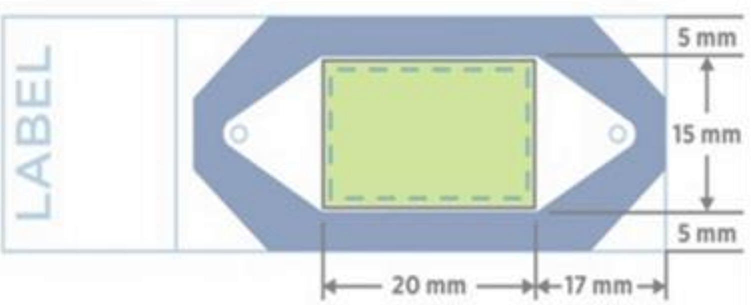
- **Xenium: 10.45 mm X 22.45 mm**

3 whole mouse brains



- **CosMx: 20 mm X 15 mm**

3 whole mouse brains



Adapted from 10X Genomics

Let me introduce
Applied Spatial Omics Centre (ASOC)

ASOC, *Applied Spatial Omics Centre*

ASOC provides comprehensive services in **histology, spatial transcriptomics, and bioinformatic analysis**, empowering researchers with the tools and expertise needed to drive innovations in biomedical research

Our Team member

Bo Young Ahn, PhD
Senior Spatial Biology Specialist

Shiying Liu, MSc
Cell & Molecular Biology Specialist
With CGE

Kim Goring, MSc
Histology Specialist

Heewon Seo, PhD
Lead Bioinformatician

End-to-end Spatial Omics Service

Project Initiation & Experimental Design

Quality Control Test & Histology Service

- Project Review and Platform & Service Overview
- Comprehensive RNA & DNA Purification for Genomic Research
- Core Histology Services for Spatial Transcriptomics
- Hands-On Histology Training for Trainees – *Launching Soon!*

Spatial analysis platform Run & Read out

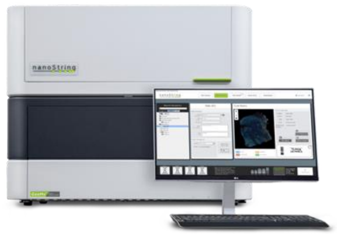
Visium CytAssist

Xenium

Chromium w/ CHGI

10X Genomics

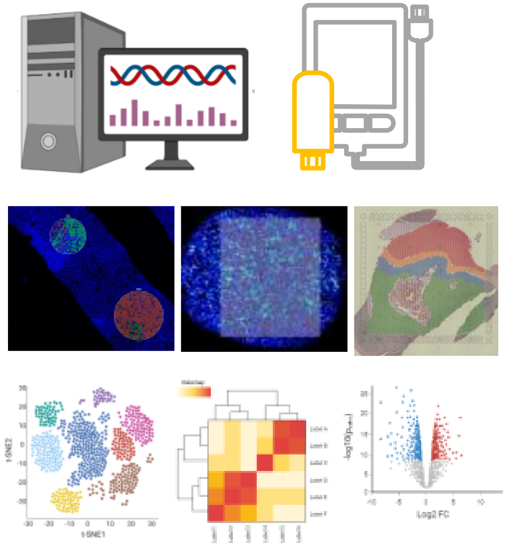
CosMx



GeoMx

Nanostring

DATA Processing & Analysis



- Custom Bioinformatics Analysis Services
- One-on-One Workshops in Spatial Data Analysis

How We Help

ASOC Applied Spatial Omics Centre

Centre for Advanced Technologies

UNIVERSITY OF CALGARY

Obstacles of Spatial Omics Analysis for individual lab

- **High cost, expertise, and/or need for specialized equipment**

- **A wide range of technologies to choose from**

  in situ hybridization, in situ sequencing, and in situ capturing technologies *etc*

- **Tissue of interest may require additional optimization**

Why Work with Us

Affordable

- Services at cost, non-profit

- Centralization ensures top-tier equipment access

Expertise

- Specialized team dedicated to research support

- Seasoned staff with continuous, open communication channels

- End-to-end service

Adaptable

- Customizable options and rates

- Diverse services for all tech needs

- Open to faculty and external clients

Free consultation!!

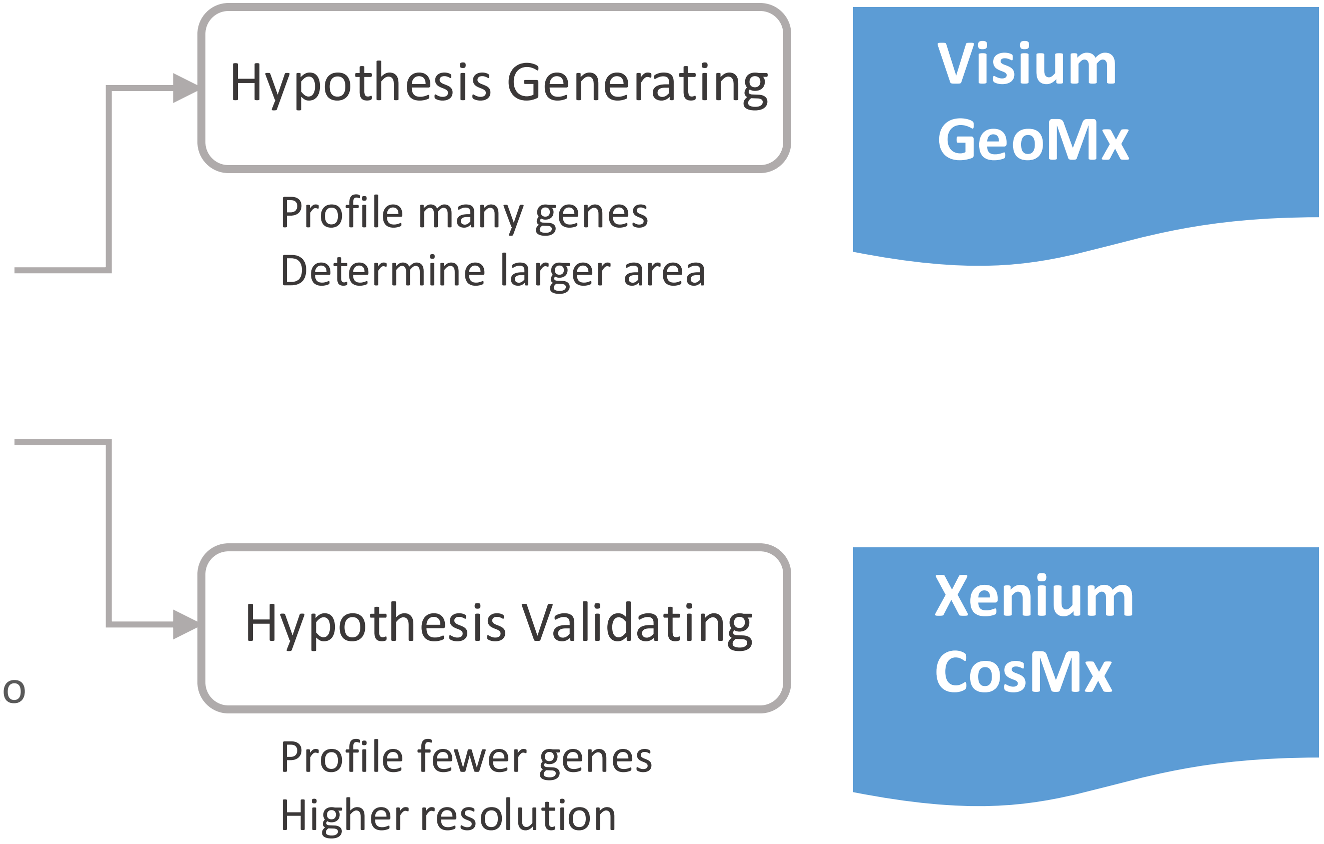
https://asoc.ucalgary.ca/

ASOC@ucalgry.ca

How to Choose the Right Spatial Transcriptomics Platform

What is the goal of the spatial experiment?

1. What species are the samples from?
2. How are the tissue samples prepared?
3. What is the stage of the project?
4. For the experiment, how many control and test samples will be included?
5. How many sections of each sample type can fit onto a slide with the chosen platform



Hypothesis Generating

Profile many genes
Determine larger area

Visium
GeoMx

Hypothesis Validating

Profile fewer genes
Higher resolution

Xenium
CosMx

How Much

Contact us!

We'll collaborate with you to tailor the project and LOWER the costs

Why Bioinformatics Matters in Spatial Omics

- **High-dimensional complexity**
  - Spatial omics datasets are massive, thousands of genes across thousands of spatial locations
  - Requires sophisticated computational tools to preprocess, normalize, and reduce noise
- **Integration of modalities**
  - Spatial omics often combines transcriptomic, proteomic, and imaging data
  - Enables the integration and comparison of these layers for a systems-level view
- **Interpretation at scale**
  - Manually analyzing these data is infeasible
  - Scales insights through automated workflows, machine learning, and statistical modeling

Bioinformatics Applications in Spatial Omics

1. **Data preprocessing and quality control**
   - Spot/barcode filtering, image registration, spatial resolution adjustment
2. **Spatial clustering and cell type/state annotation**
   - Identifying regions with distinct molecular signatures.
3. **Spatially variable gene detection**
   - Identifying genes with expression patterns that correlate with tissue structure
4. **Cell-cell communication and neighborhood analysis**
   - Inferring interactions between nearby cells based on ligand-receptor expression

What Bioinformatics Can Deliver to Scientists

- **Hypothesis generation**
  - Reveal unexpected spatial patterns that can lead to novel biological questions
- **Data-driven validation**
  - Support or refine experimental findings with quantitative spatial evidence
- **Interdisciplinary insights**
  - Enable collaborations between biologists and clinicians by transforming complex spatial data into interpretable results
- **Scalable and reproducible research**
  - Bioinformatics pipelines enable reproducible, transparent, and sharable analyses across teams and projects

What to Ask When Visiting a Core Facility

- Focus on *experience*, not just *information*
  - Don’t settle for answers that can be easily found in protocols or online resources
- Ask about practical expertise
  - Inquire about how they’ve handled similar data, experiments, or projects
- Evaluate problem-solving skills
  - Look for evidence of how the team responds to unexpected challenges and improves outcomes over time

- **Assess the team’s real-world experience!**

Why Work with ASOC?

- Committed team focused on enhancing data quality

  1. Have you assessed your data quality, and can you demonstrate improvement over time?

- Hands-on experts who actively analyze data

  2. Do you actively engage with your data to generate insights?

- Research-driven collaborators who align with your goals and contribute to publications

  3. Do you have the expertise to understand the current landscape and align with the goals of the field?

- **Experience matters when adapting protocols to specific needs!**

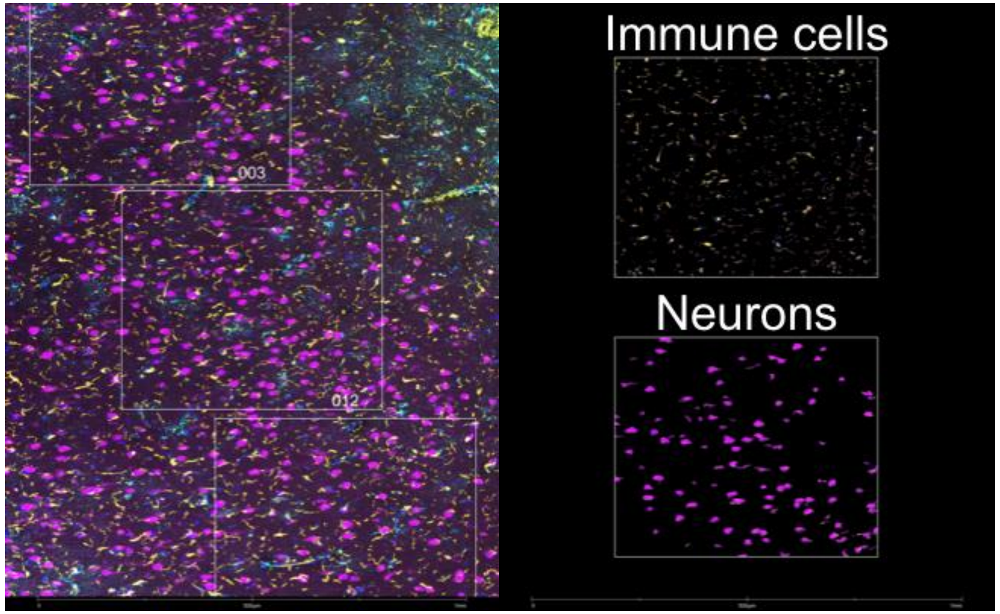
1. Quality Control and Data Integrity

Have you assessed your data quality, and can you demonstrate improvement over time?

- Spatial omics platforms produce a variety of metrics due to the complexity of the processes involved
  - Collaboration between wet- and dry-lab is crucial
- Establishing robust quality control workflows
  1. **Collect quality metrics** at both the pre-analysis (*run-level*) and post-analysis (*interpretation-level*) stages
  2. **Identify trends** that impact analytical outcomes, e.g., the number of genes retained in downstream analysis
  3. **Adjust operational strategies** based on QC insights to optimize the performance of spatial omics platforms
  4. **Review and compare QC metrics across runs** to ensure consistency and drive continuous improvement

Region of Interest (ROI) Strategy & Optimization

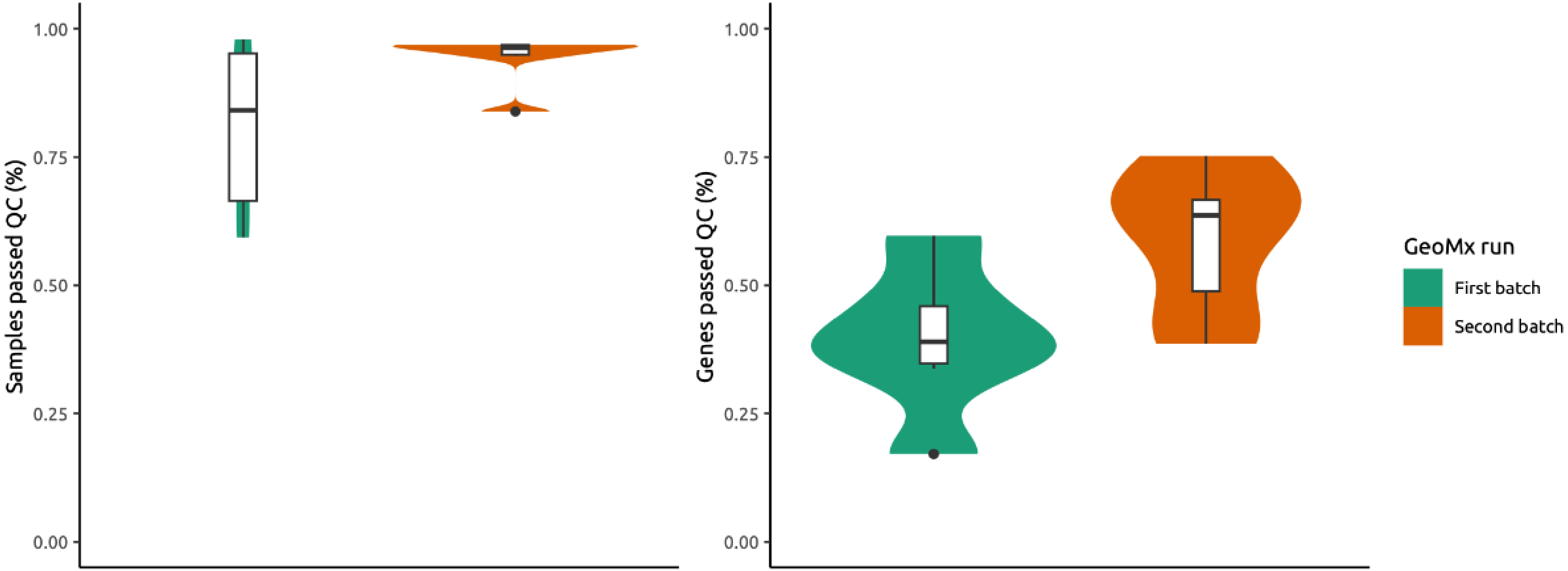
- In case of *GeoMx*:
  - Users often aim to include **as large an area as possible** to capture more data for analysis
  - Unaware of the *trade-offs* associated with larger ROI
  - Larger ROIs tend to accumulate higher background signal
    - Non-specific negative control probes that do not target mRNA
  - True biological signals must compete with elevated background noise, impacting downstream interpretation
- **Revealed a clear trend**:
  - Larger ROIs are associated with a higher number of genes being excluded during downstream processing



One of the brain ROIs from Nanostring.com

Improving ROI Selection and Data Quality in GeoMx

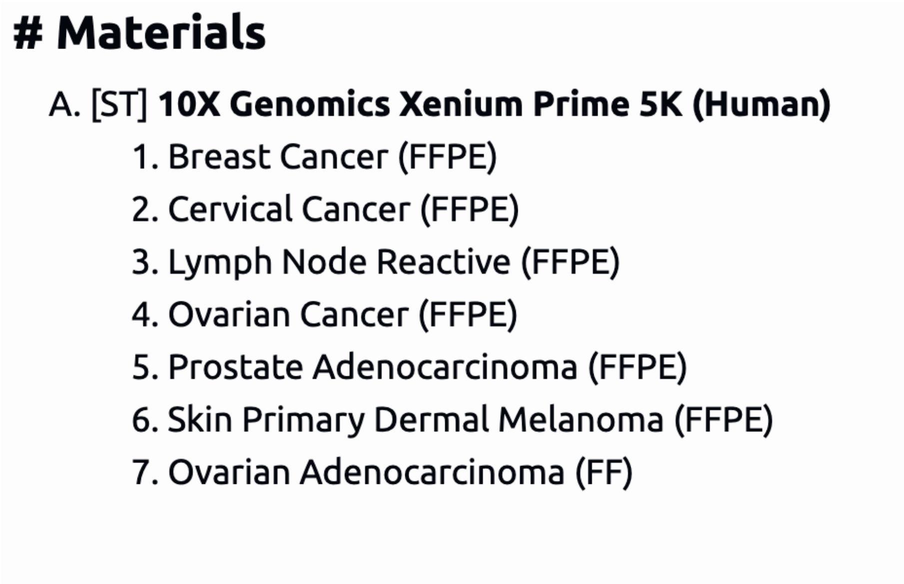
- **Gained expertise in strategic ROI selection** to help users obtain the *highest-quality* data
  - Provided guidance to customers on maximizing signal quality while minimizing background interference
  - Violin plots: first batch (*before* Summer 2024) and second batch (*after* Summer 2024)



2. Encountered Technical Biases

Do you actively engage with your data to generate insights?

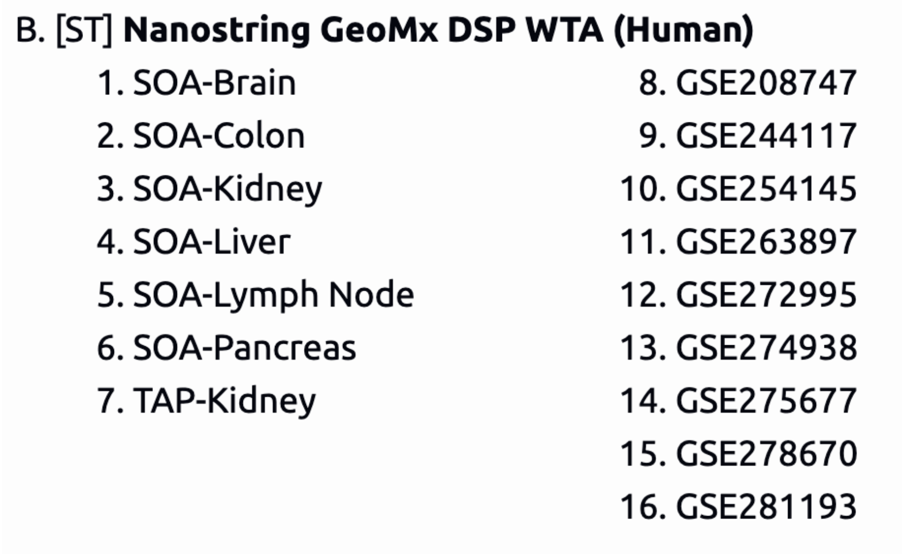
- Spatial transcriptomics technologies still face limitations in
  - **Sensitivity and specificity**
  - **Technical variability**
- Recurrent observation: **consistent *high* or *low* gene coverage regardless of tissue type**
  - Evaluated pre-designed panels using public datasets
    - Seven Xenium In Situ datasets with the Xenium Prime 5k panel
    - 16 GeoMx DSP datasets with the Human Whole Transcriptome Atlas (WTA)



Materials

A. [ST] **10X Genomics Xenium Prime 5K (Human)**

1. Breast Cancer (FFPE)
2. Cervical Cancer (FFPE)
3. Lymph Node Reactive (FFPE)
4. Ovarian Cancer (FFPE)
5. Prostate Adenocarcinoma (FFPE)
6. Skin Primary Dermal Melanoma (FFPE)
7. Ovarian Adenocarcinoma (FF)



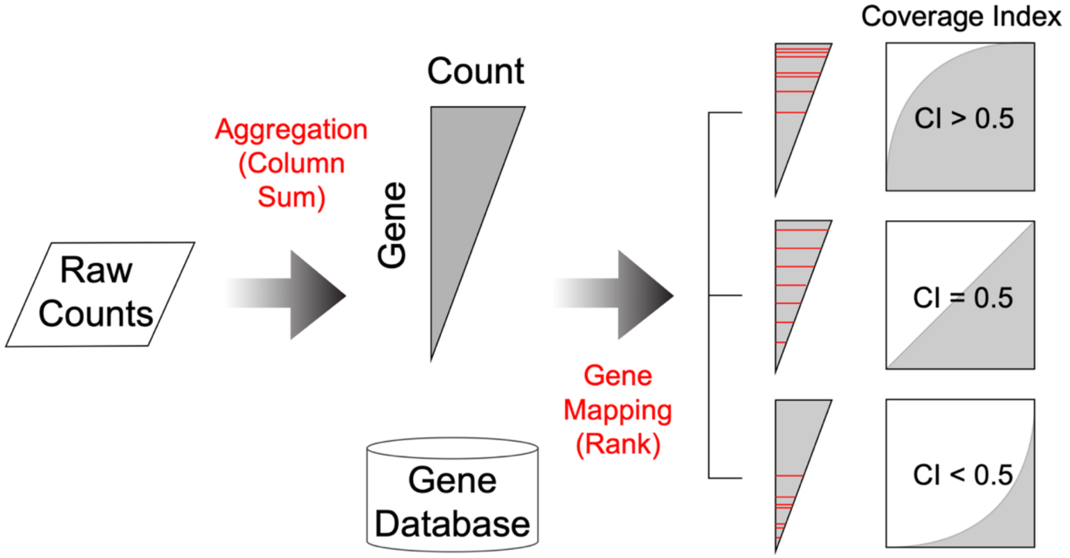
B. [ST] **Nanostring GeoMx DSP WTA (Human)**

1. SOA-Brain
2. SOA-Colon
3. SOA-Kidney
4. SOA-Liver
5. SOA-Lymph Node
6. SOA-Pancreas
7. TAP-Kidney
8. GSE208747
9. GSE244117
10. GSE254145
11. GSE263897
12. GSE272995
13. GSE274938
14. GSE275677
15. GSE278670
16. GSE281193

SOA, spatial organ atlas
TAP, technology access program

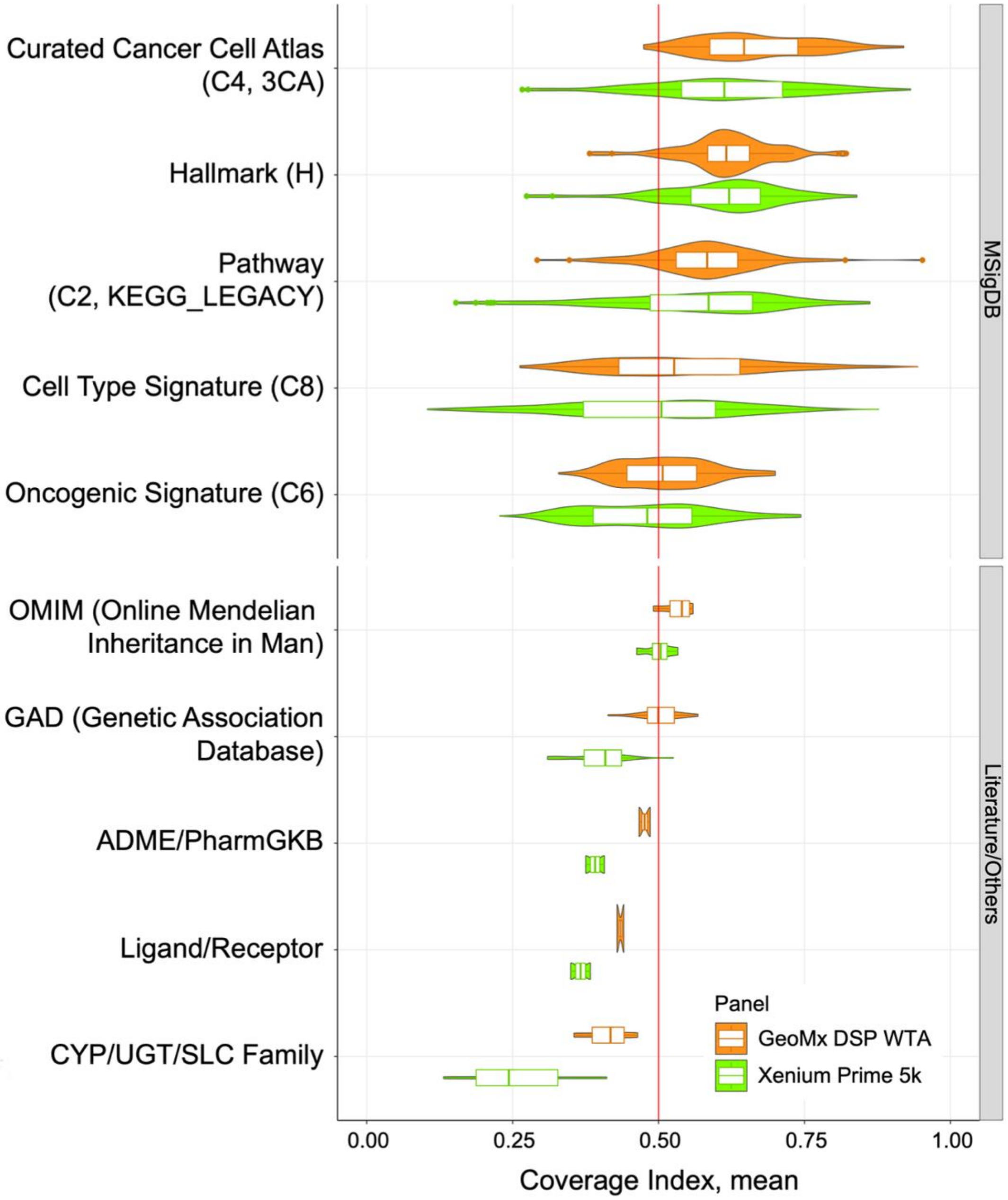
Evaluating Technical Variability in Spatial Transcriptomics

- **Coverage Index (CI)**
  - Quantitative metric to assess the representation of established gene signatures
  - **Higher CI values denote greater coverage** among gene members within a representative gene list, while lower CI values indicate reduced coverage



Evaluating Technical Variability in Spatial Transcriptomics

- **Cancer-related gene lists exhibit the highest CI values**
  - Suggesting that pre-designed panels are optimized for oncology research
- **Ligand/receptor genes show lower representation**
  - Indicating potential gaps in panel design
- The findings highlight the need for customized panel designs to ensure adequate gene coverage in diverse biological and clinical applications



3. Translating Data into Biological Understanding

Do you have the expertise to understand the current landscape and align with the goals of the field?

- Linked spatial expression patterns to clinical entities or anatomical contexts
- Supported scientists in **hypothesis generation** by:
  - Generating genome-wide profiles
  - Identifying spatially variable genes
  - Mapping cell-cell communication and microenvironment structures
- **Data-driven validation** by:
  - Executing a targeted panel assay
  - Collaborating in downstream integrative analyses
  - Combining spatial data with omics/pathology data

Hypothesis Generation

- **Conducting genome-wide exploratory data analysis** to uncover underlying patterns and trends
- **Identifying biologically relevant features** through statistical and computational approaches
- **Mapping extracted features** to established biological pathways and disease mechanisms
- **Performing clustering analyses** to reveal sample subgroups or functional modules
- **Integrating prior knowledge** using biomedical ontologies and curated databases
- **Formulating biologically plausible and testable hypotheses** based on data-driven insights
- **Ensuring analytical reproducibility** and comprehensive documentation to support future validation efforts

Data-driven Validation

- **Comparing findings with established benchmarks or gold-standard datasets** to confirm biological relevance, e.g., cell-type markers
- **Designing robust computational workflows to evaluate generated hypotheses** using independent datasets
- **Incorporating technical and biological replicates** to assess consistency, reproducibility, and biological variability in validation outcomes
- **Visualizing validation results** through interpretable plots and metrics
- **Documenting validation strategies and outcomes** to ensure transparency and reproducibility of analytical conclusions

Harvesting Insights: Validated Findings and Scholarly Contributions

- Multiscale spatial transcriptomics for *hypothesis generation* and *validation*
    - GeoMx enabled **a comprehensive regional overview** by identifying broad spatial transcriptomic patterns across tissue sections
    - Xenium provided **high-resolution insights at single-cell and subcellular levels**, allowing for precise mapping of gene expression within individual cells
- The integration of GeoMx and Xenium **bridged the gap between tissue-wide transcriptomic profiling and single-cell resolution data**

> Sci Adv. 2025 May 2;11(18):eadt8976. doi: 10.1126/sciadv.adt8976. Epub 2025 Apr 30.

Impaired parvalbumin interneurons in the retrosplenial cortex as the cause of sex-dependent vulnerability in Alzheimer's disease

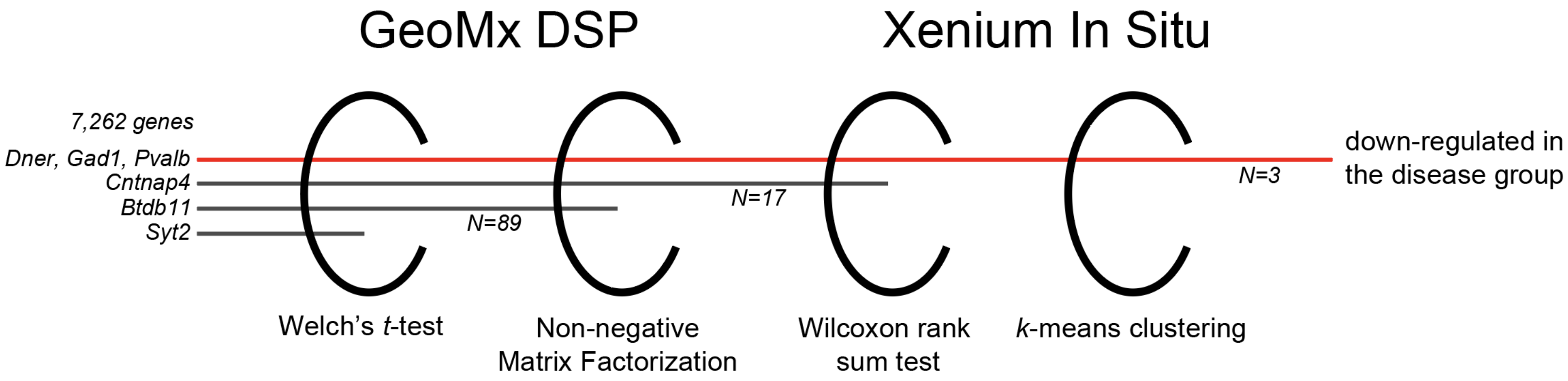
Dylan J Terstege[1,2], Yi Ren[1,2], Bo Young Ahn[3], Heewon Seo[3], Kabirat Adigun[1,2]; Alzheimer’s Disease Neuroimaging Initiative; Liisa A M Galea[4,5,6,7], Derya Sargin[2,8,9], Jonathan R Epp[1,2]

Affiliations + expand

PMID: 40305608 PMCID: PMC12042879 DOI: 10.1126/sciadv.adt8976

Companion Paper Under Review

- Developed **robust computational workflows** to evaluate data-driven hypotheses using multiple spatial transcriptomics datasets
  - **Integrated two complementary spatial transcriptomics platforms** to comprehensively characterize cells of interest
  - Leveraged bulk RNA-seq data from the GeoMx platform (*broader coverage*) to classify 10x Xenium-derived cell types (*higher resolution*), addressing the limitation of canonical cell type markers in disease contexts



Advancing Spatial Omics Through Bioinformatics at ASOC

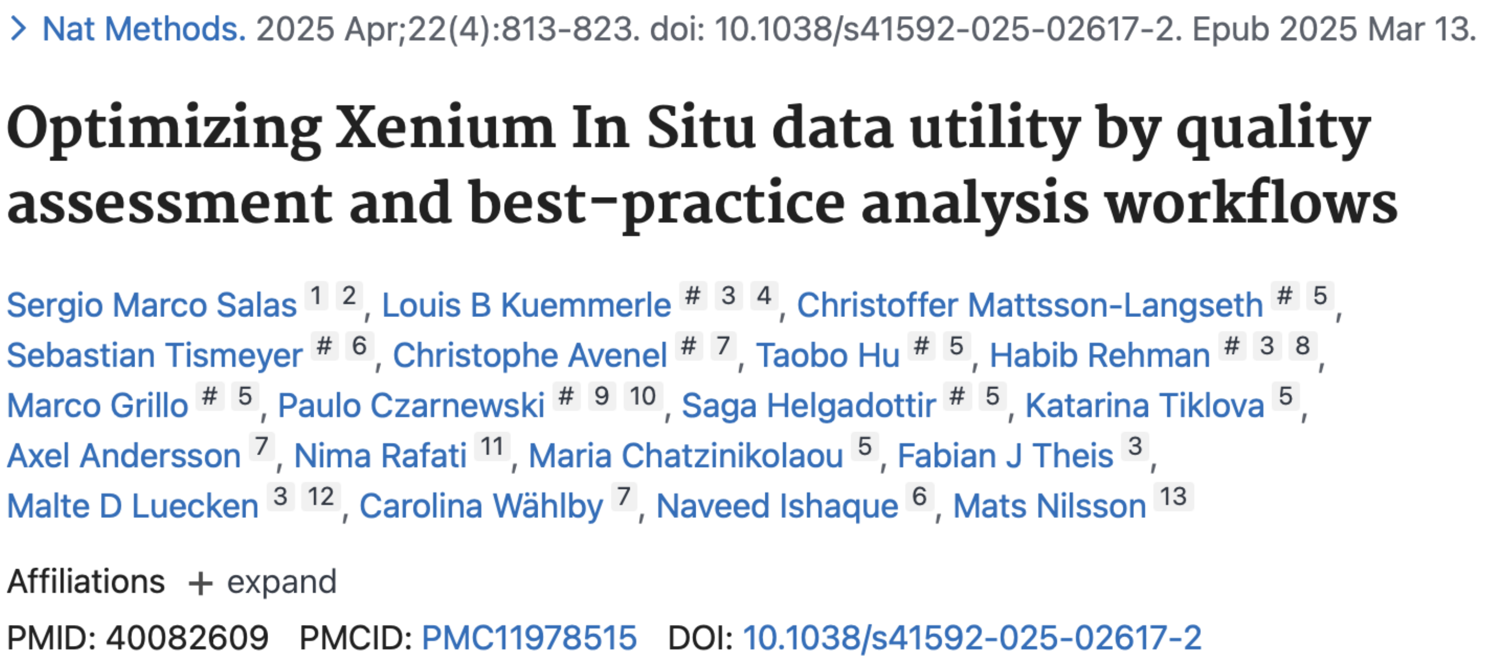
1. **Quality control and data integrity**
2. **Encountered technical biases**
3. **Translating data into biological understanding (publications)**

- ASOC Bioinformatics team aims to accelerate spatial omics research and its applications in clinical practice and provide customized and case-by-case analysis services for the life science research community

4. **Pipeline development and workflow standardization**
5. **Scalability, reproducibility and data sharing**

4. Pipeline Development and Workflow Standardization

- Spatial omics is still emerging — standardized workflows are lacking
  - **Vendor tools and third-party software cover only a small portion of the full analysis workflow**
    - *Visium Spatial/HD* and *Xenium In Situ* from 10X Genomics
    - *GeoMx DSP* and *CosMx SMI* from Nanostring
- **Workflow standardization and best practices**
  - Developed and maintained analysis pipelines for all four major spatial omics platforms
  - Created modular and scalable pipelines adaptable to diverse project needs
  - Ensured timely deployment of pipelines to match rising demand
  - Hosted training workshops and created documentation to support researchers



> Nat Methods. 2025 Apr;22(4):813-823. doi: 10.1038/s41592-025-02617-2. Epub 2025 Mar 13.

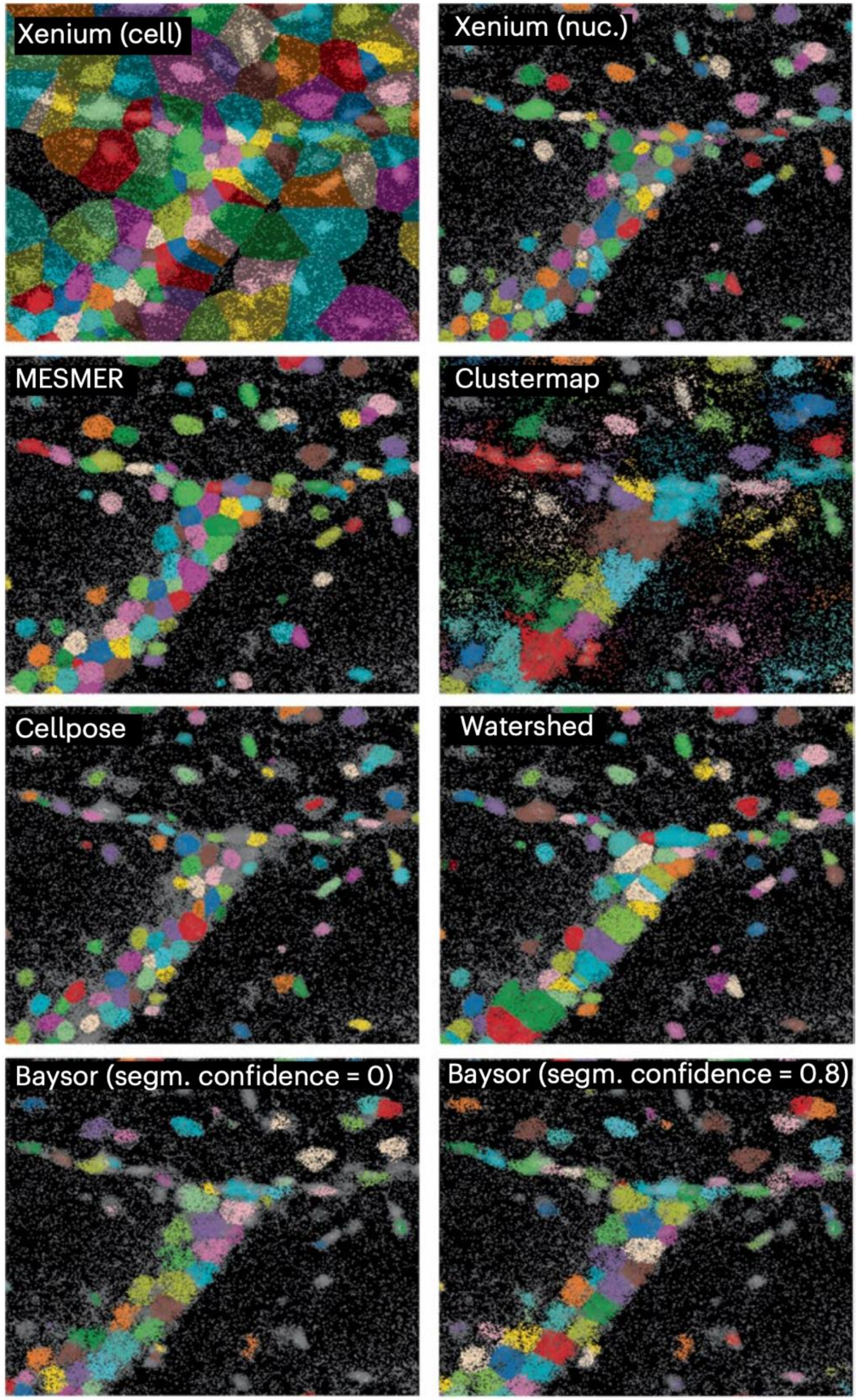
Optimizing Xenium In Situ data utility by quality assessment and best-practice analysis workflows

Sergio Marco Salas[1,2], Louis B Kuemmerle[#,3,4], Christoffer Mattsson-Langseth[#,5], Sebastian Tismeyer[#,6], Christophe Avenel[#,7], Taobo Hu[#,5], Habib Rehman[#,3,8], Marco Grillo[#,5], Paulo Czarnewski[#,9,10], Saga Helgadottir[#,5], Katarina Tiklova[5], Axel Andersson[7], Nima Rafati[11], Maria Chatzinikolaou[5], Fabian J Theis[3], Malte D Luecken[3,12], Carolina Wählby[7], Naveed Ishaque[6], Mats Nilsson[13]

Affiliations + expand

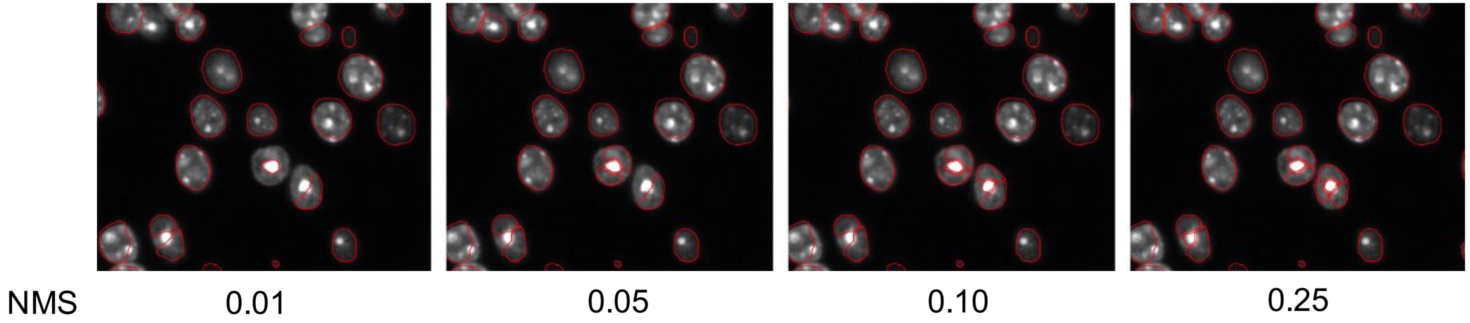
PMID: 40082609 PMCID: PMC11978515 DOI: 10.1038/s41592-025-02617-2

- Current consensus on data analysis for Xenium In Situ.
  - Comparison of cell identification using various segmentation algorithms (see right)
  - Utilized a default parameter set to ensure a fair comparison

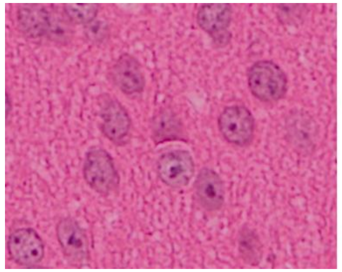


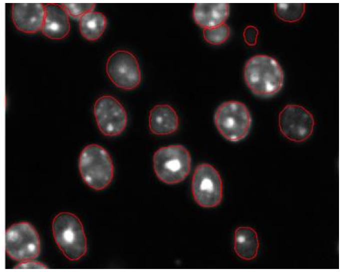
Xenium In Situ Workflows at ASOC

- Employ a selection of consensus methods, i.e., **StarDist**
- Parallelize computational processes with <u>a diverse array of parameters</u>
  - e.g., healthy mouse brain (100 µm)

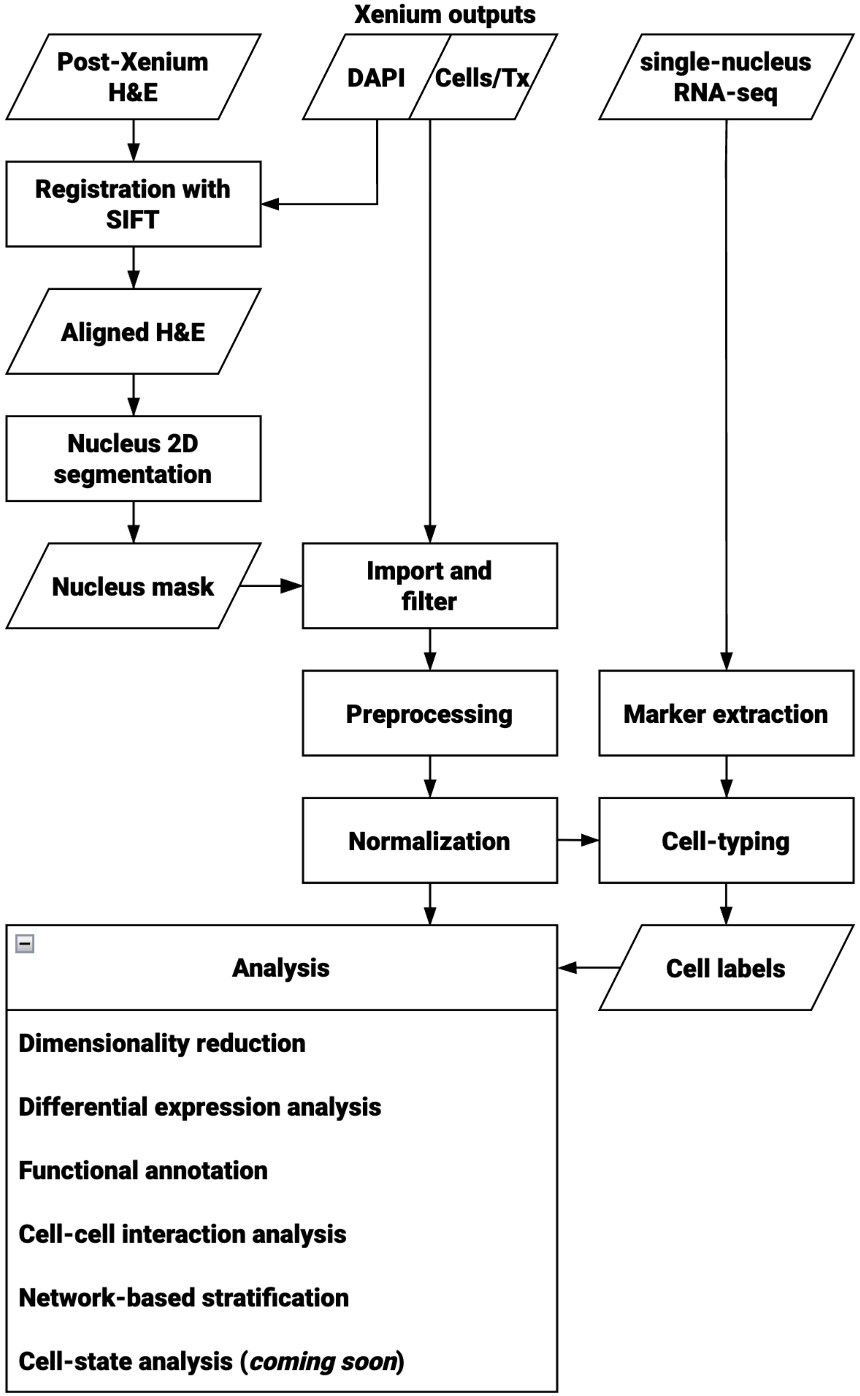


- **Optimize output** to address the biological questions


H&E


Best result

NMS, non-maximum suppression


Xenium outputs

Post-Xenium H&E

DAPI

Cells/Tx

single-nucleus RNA-seq

Registration with SIFT

Aligned H&E

Nucleus 2D segmentation

Nucleus mask

Import and filter

Preprocessing

Marker extraction

Normalization

Cell-typing

Cell labels

Analysis

- Dimensionality reduction
- Differential expression analysis
- Functional annotation
- Cell-cell interaction analysis
- Network-based stratification
- Cell-state analysis (*coming soon*)

5. Scalability, Reproducibility and Data Sharing

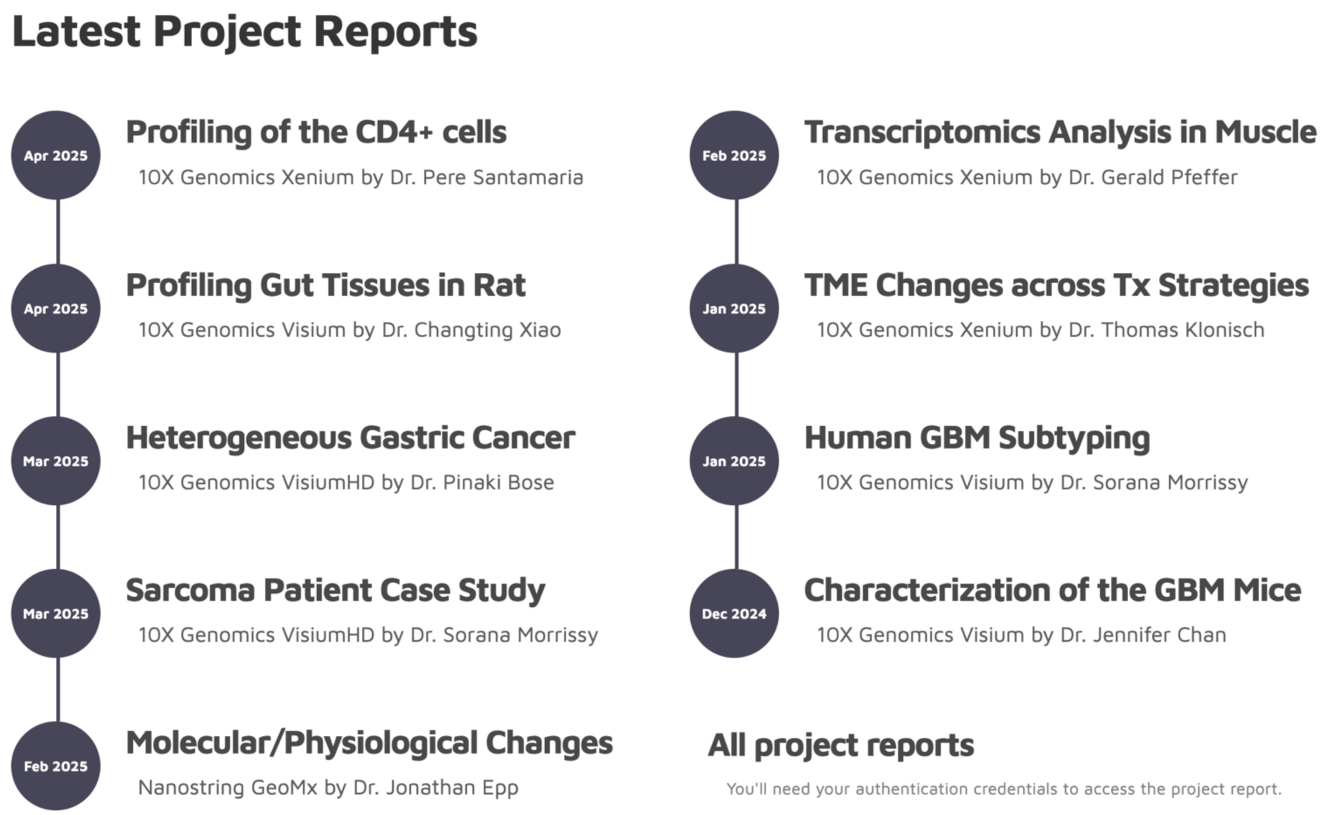
- **Built reproducible analysis environments** with version-controlled pipelines
- **Ensured compatibility** with downstream analysis platforms
- **Supported collaborative data sharing** through structured results, interactive visualizations, and documentation

Scalable and Reproducible Analysis Environments

- **Designed the workflows to be scalable** across different computing infrastructures, from local machines to high-performance clusters
- **Packaged all computational workflows into a Docker application** to ensure reproducibility across environments
- **Provided full source code and documentation publicly** via GitHub for transparency and community use
- **Ensured that analyses can be reliably reproduced and audited** by peers or reviewers
- **Facilitated easy deployment of the analysis environment** without manual setup or dependency issues

Project Reports and Data Sharing

- ASOC portal (ASOC.ucalgary.ca) provides *one-click* access to project reports—no downloads or client-side backups required



Latest Project Reports

Apr 2025
Profiling of the CD4+ cells
10X Genomics Xenium by Dr. Pere Santamaria

Apr 2025
Profiling Gut Tissues in Rat
10X Genomics Visium by Dr. Changting Xiao

Mar 2025
Heterogeneous Gastric Cancer
10X Genomics VisiumHD by Dr. Pinaki Bose

Mar 2025
Sarcoma Patient Case Study
10X Genomics VisiumHD by Dr. Sorana Morrissy

Feb 2025
Molecular/Physiological Changes
Nanostring GeoMx by Dr. Jonathan Epp

Feb 2025
Transcriptomics Analysis in Muscle
10X Genomics Xenium by Dr. Gerald Pfeffer

Jan 2025
TME Changes across Tx Strategies
10X Genomics Xenium by Dr. Thomas Klonisch

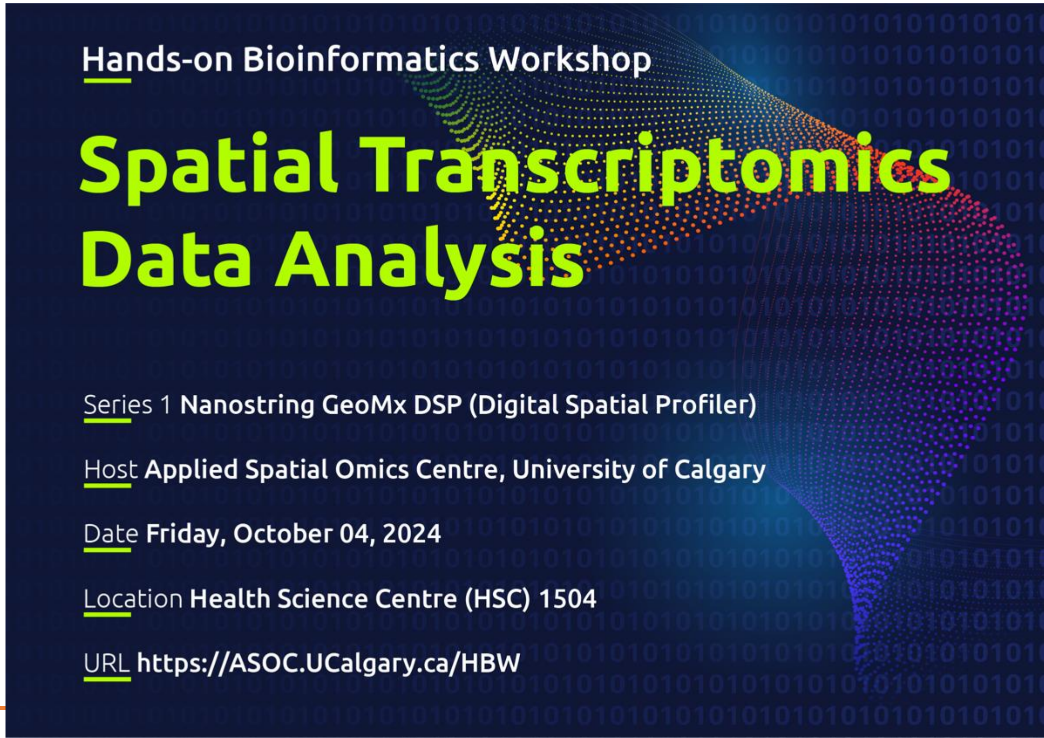
Jan 2025
Human GBM Subtyping
10X Genomics Visium by Dr. Sorana Morrissy

Dec 2024
Characterization of the GBM Mice
10X Genomics Visium by Dr. Jennifer Chan

All project reports
You'll need your authentication credentials to access the project report.

Educational Activities and Beyond

- **Hosted a hands-on bioinformatics workshop** focused on best practices for GeoMx data analysis
- **Provided step-by-step guidance on running Docker-based workflows** in participants' own working environments, e.g., laptops
- **Offered comprehensive training materials and reusable code examples** to support independent learning and application
- **Presented real-world case studies to demonstrate end-to-end reproducibility** and **equip participants to analyze their own data effectively**



Hands-on Bioinformatics Workshop

Spatial Transcriptomics Data Analysis

Series 1 **Nanostring GeoMx DSP (Digital Spatial Profiler)**

Host **Applied Spatial Omics Centre, University of Calgary**

Date **Friday, October 04, 2024**

Location **Health Science Centre (HSC) 1504**

URL **https://ASOC.UCalgary.ca/HBW**

Acknowledgements

- Cumming School of Medicine
- Centre for Advanced Technologies (CAT)
  - Roman Krawetz, Simon Hirota, and Angela Krawetz

- CGE
  - Derrick Rancourt
  - Cameron Fielding
  - Ken Ito
  - Yaping Yu
  - Shiying Liu
  - Robyn Flynn
- CHGI
  - Paul Gordon
  - Shelly Wegener
  - Jene Weatherhead
  - Juli Kriel
  - Rebecca Walchyshyn

Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases

Alberta Children's Hospital Research Institute

Alberta Health Services

Alberta Cancer Foundation

POET Precision Oncology Experimental Therapeutics

Hotchkiss Brain Institute

INNOVATION.CA Canada Foundation for Innovation | Fondation canadienne pour l'innovation

Arnie Charbonneau Cancer Institute

McCaig Institute for Bone and Joint Health

ASOC Applied Spatial Omics Centre

Centre for Advanced Technologies

University of Calgary