Single Cell Core at Harvard Medical School

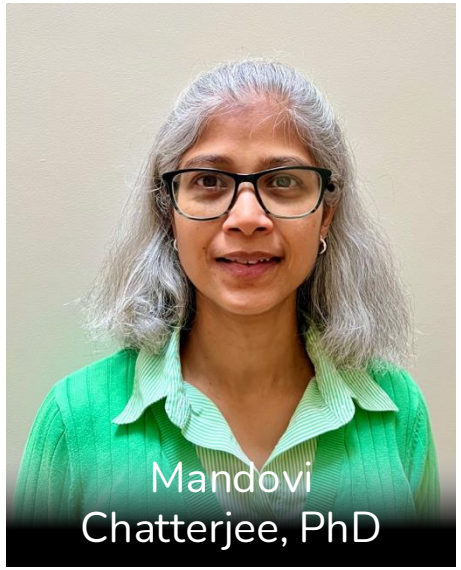
Pratyusha Bala, PhD

Associate Director, Spatial Transcriptomics

200 Longwood Ave
Armenise Room 517
Boston, MA 02115

Single Cell Core: Team

Core Director



Associate Director

Faculty Advisors:
Allon M. Klein, PhD
Jeffrey Moffitt, PhD
Christophe Benoist, MD, PhD

Funding:
HMS Foundry Award Program

https://singlecellcore.hms.harvard.edu

TECHNOLOGIES SUPPORTED BY SINGLE CELL CORE

10X GENOMICS

Parse BIOSCIENCES

Fluent BioSciences

PacBio

Oxford NANOPORE Technologies

vizgen

Complete GENOMICS

TaKaRa

https://singlecellcore.hms.harvard.edu

Single modality:

- Single cell and single nuclei RNA-seq
  - 3’- & 5’-gene expression
  - Fixed RNA profiling
- Single cell ATAC-seq

Multiple modalities (Multiome)

- CITE-seq- cell surface receptor w/ scRNA-seq
- Combined scATAC-seq and scRNA-seq

Long-read sequencing

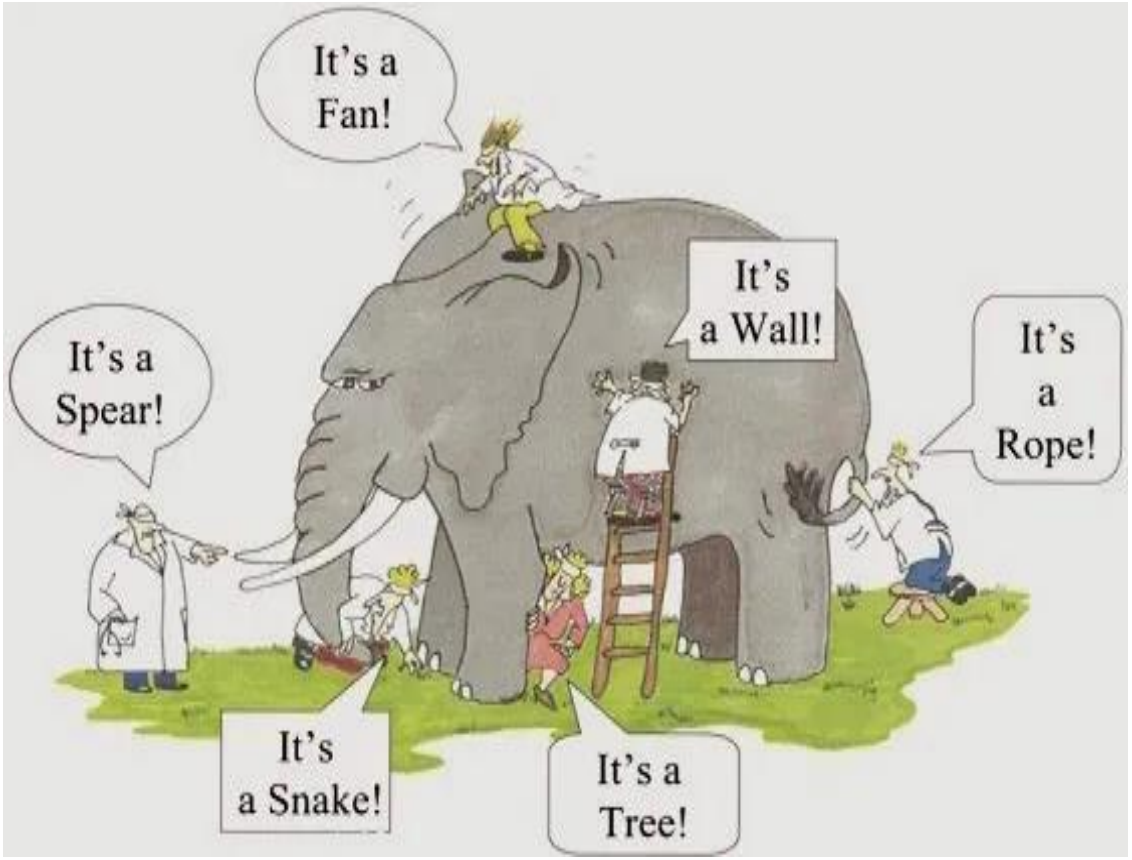
- PacBio (Kinnex) & Oxford Nanopore Technologies

Spatial transcriptomics

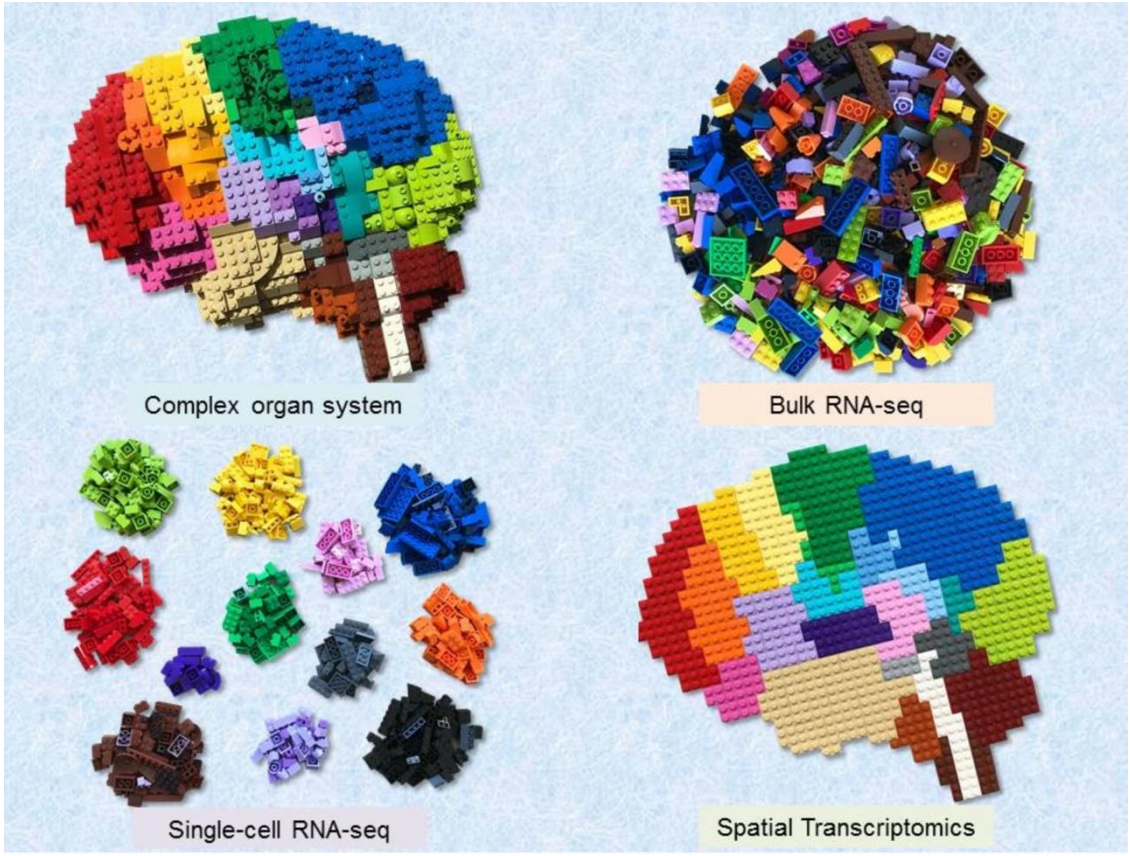
- Image-based
  - MERSCOPE (From Vizgen)
- NGS-based
  - VISIUM/VISIUM HD (From 10X Genomics)
  - STOmics (From Complete Genomics)
  - Slide-seq/ Slide-Tag (From Takara)

Why do we need spatial context?

The Blind men and the Elephant
- John Godfrey Saxe

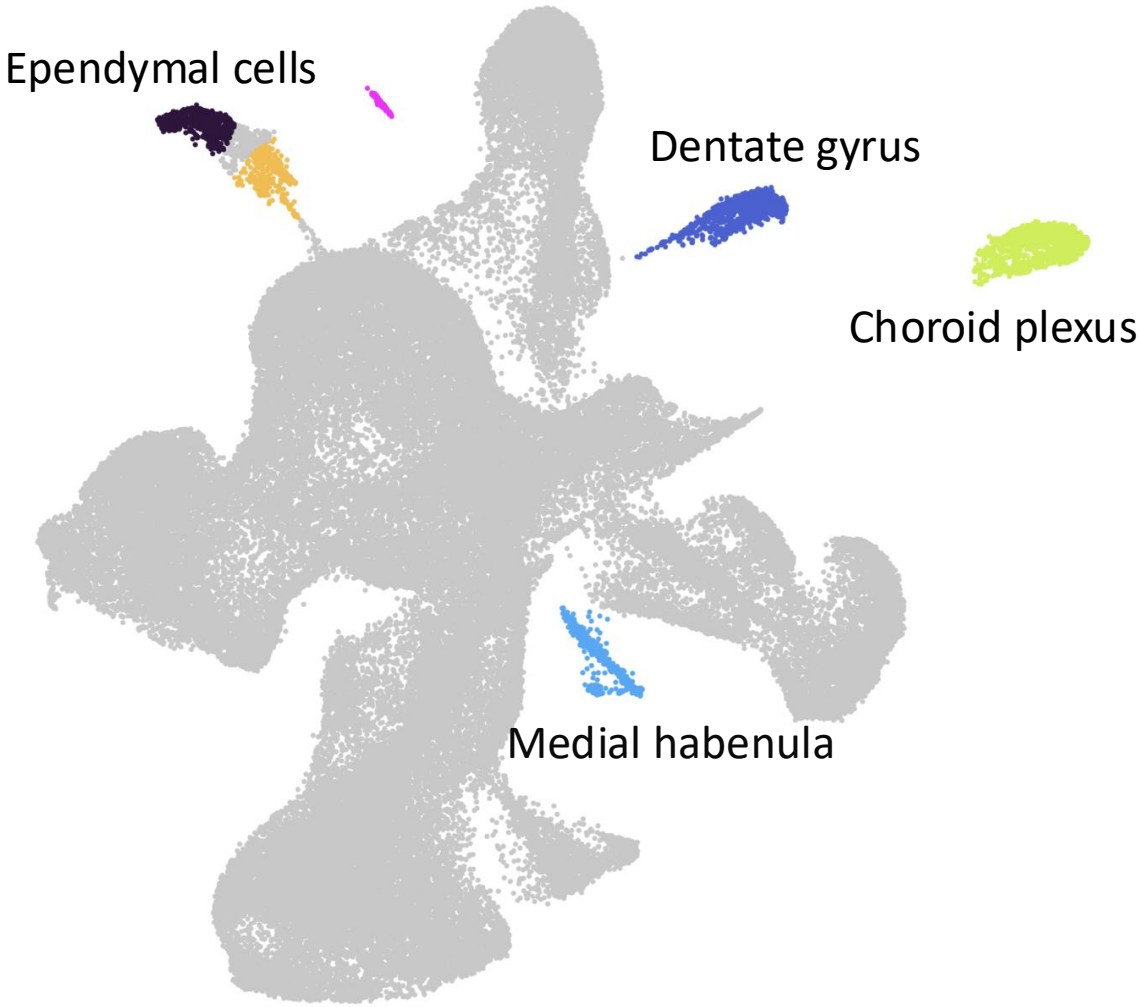


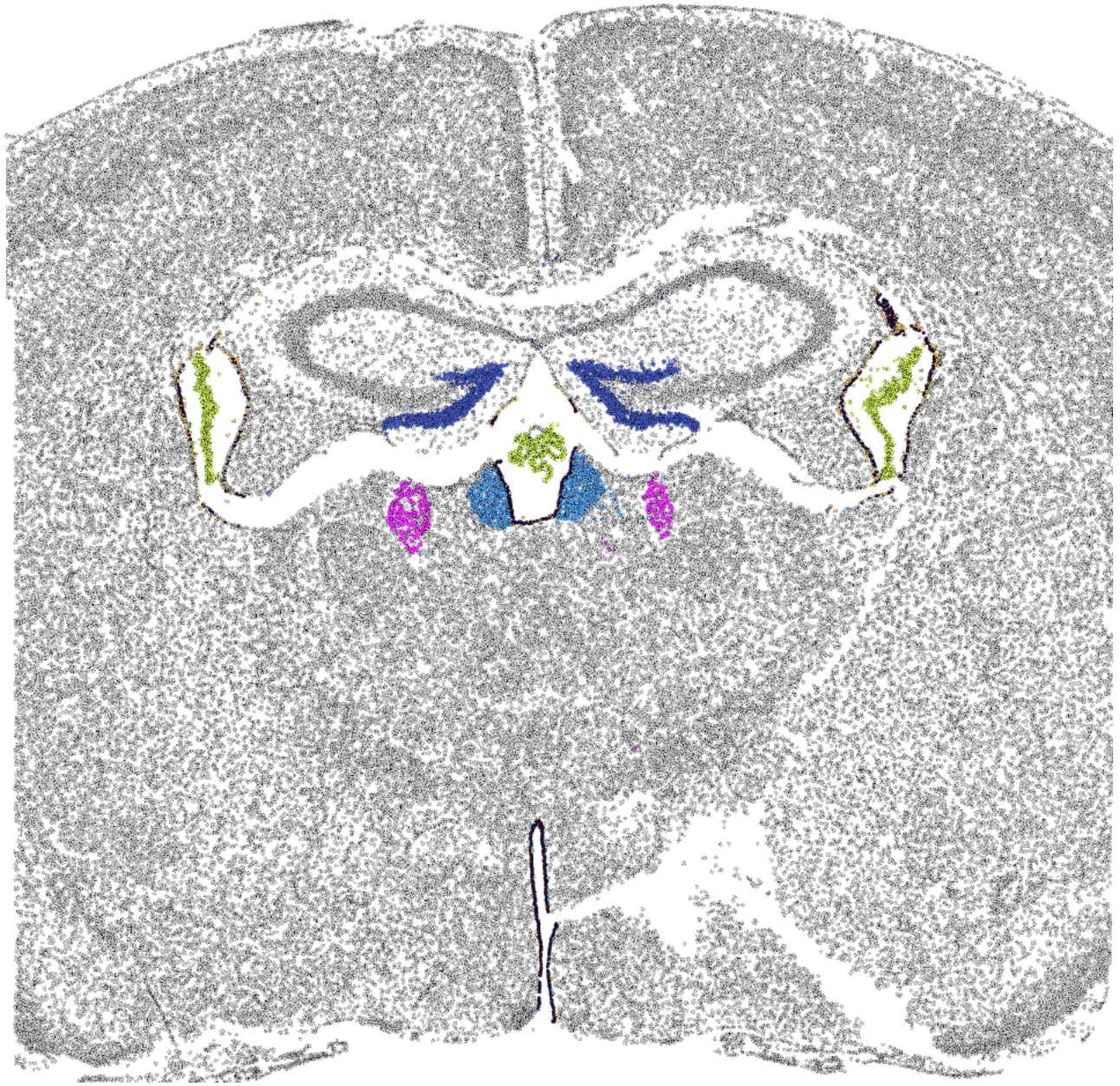
https://boxia2018.wixsite.com



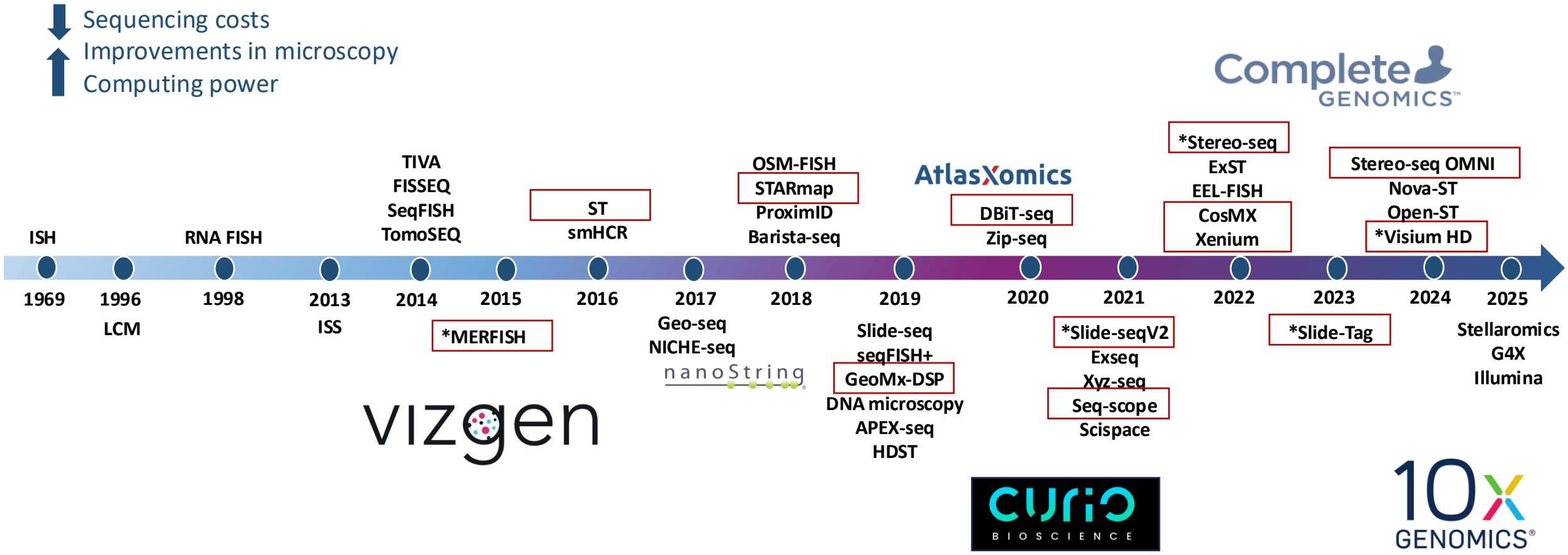
Moral: Limited perspectives can lead to misunderstanding or conflict when we fail to see the full picture

MsBrain dataset
cells=75515





The technology evolution



Adapted from Du et.al, 2023 and Yue et.al,2023

* Technologies SCC currently offer

Sample considerations

- Species and Tissue type
  - Sample preparation

Fresh Frozen

- Highest detection sensitivity when well preserved

PFA Fixed Frozen

- Easier to preserve RNA for certain tissue types

Formalin fixed paraffin embedded

- Well-preserved tissue morphology
- Only option for archival tissues

Archived slides

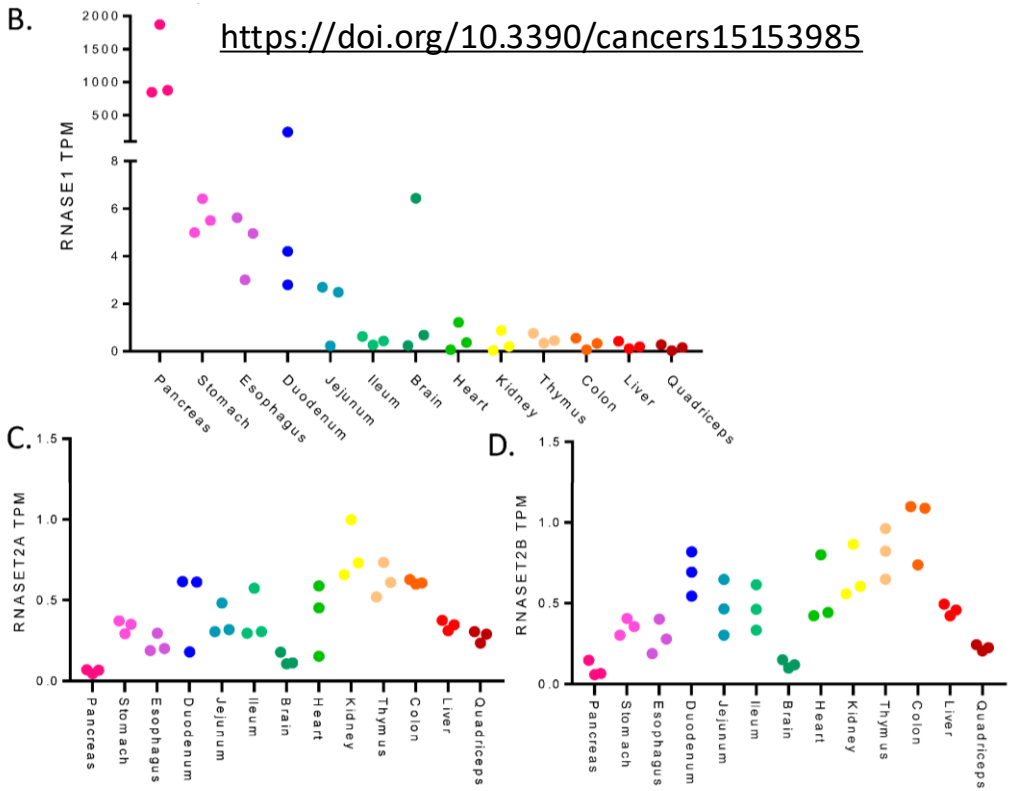
- ❖ Morphology and RNA integrity
- ❖ Find the best sample preparation method for your tissue type
- ❖ Can incorporate IF or boundary stains

RNA integrity

Tissue Extrinsic

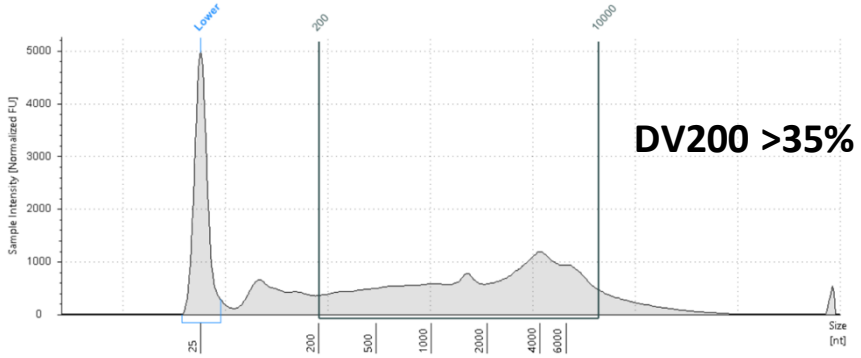
- Environment and reagents
- Harvesting methods

Tissue Intrinsic



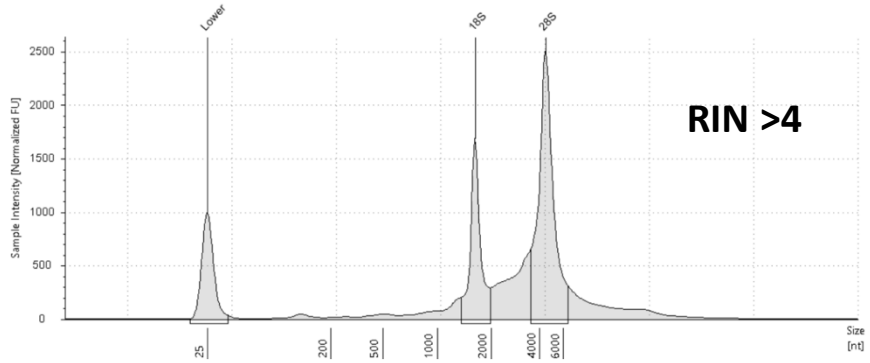
https://doi.org/10.3390/cancers15153985

RNA Quality control



Region Table

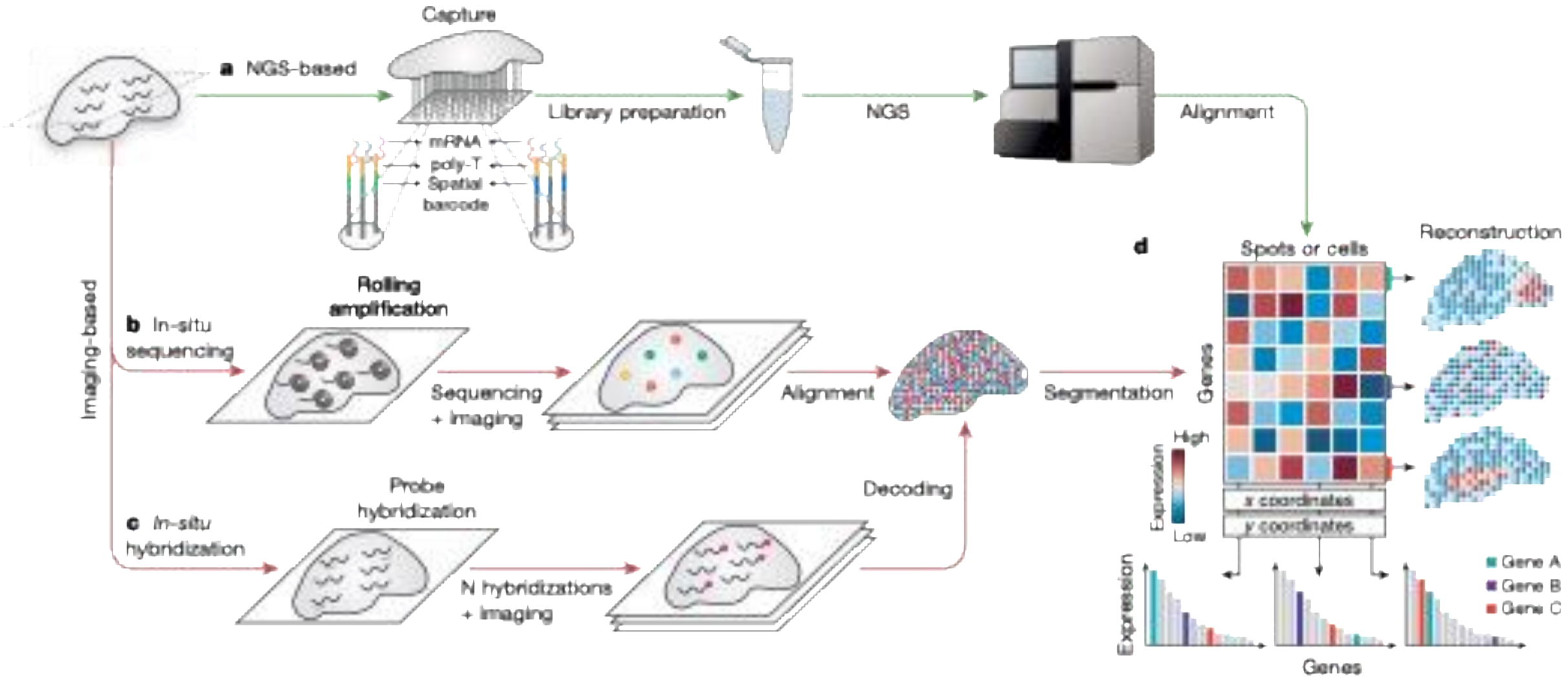
From [nt]	To [nt]	Average Size [nt]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
200	10000	5126	51.5	29.6	74.90		



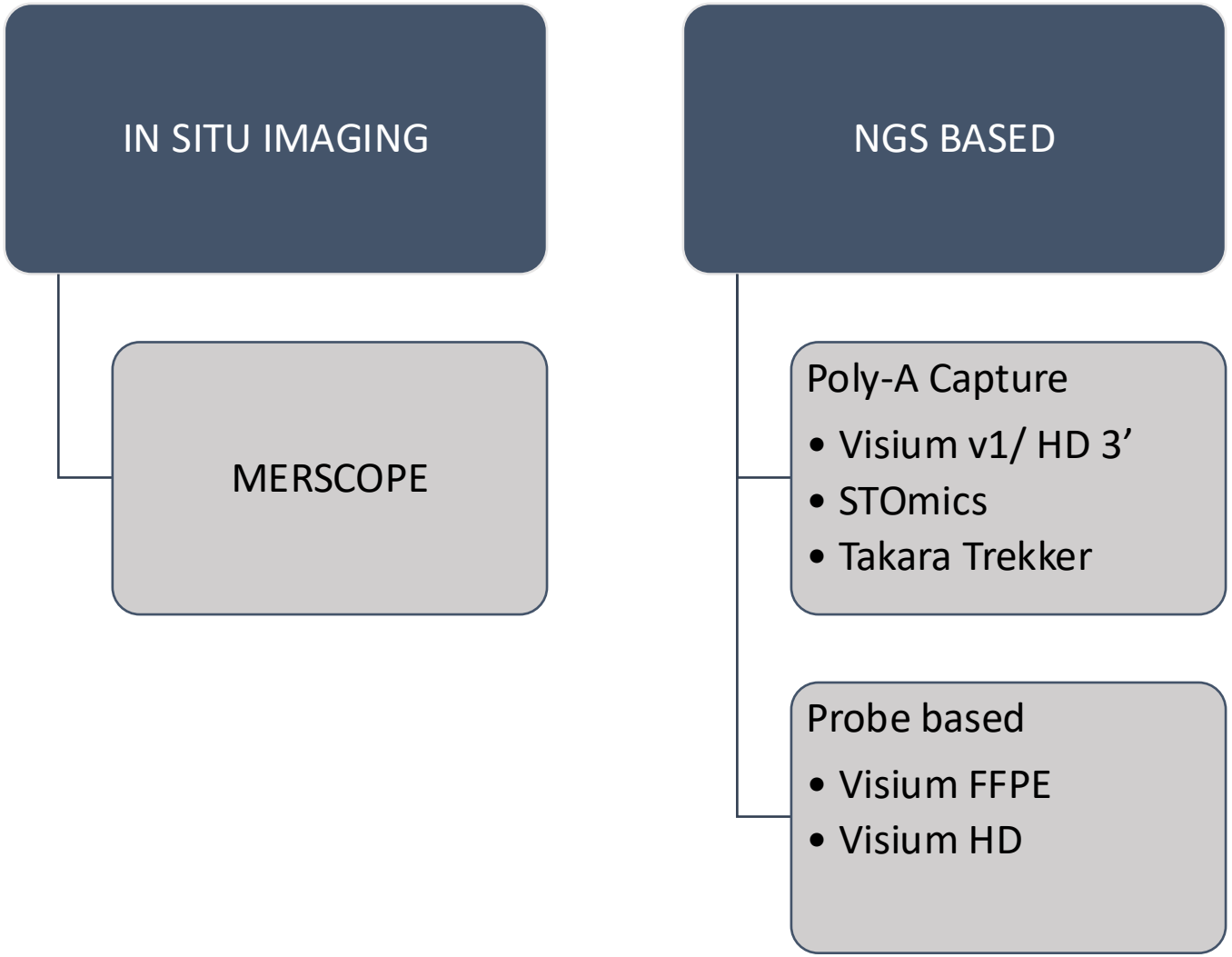
Sample Table

Well	RINe	28S/18S (Area)	Conc. [pg/µl]	Sample Description	Alert	Observations
B1	9.4	2.2	2530			

Spatial Transcriptomics Technologies



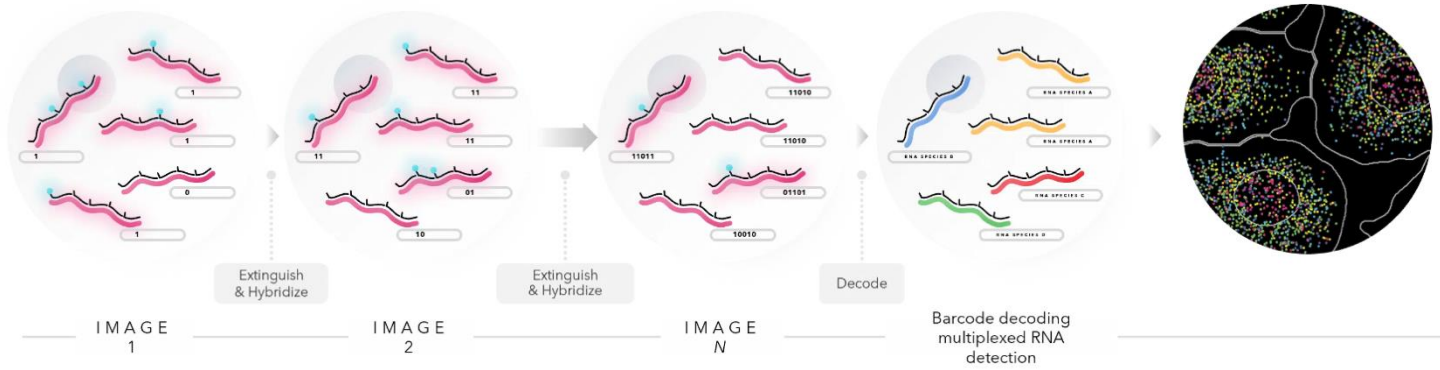
SCC: Spatial Transcriptomics technology repertoire



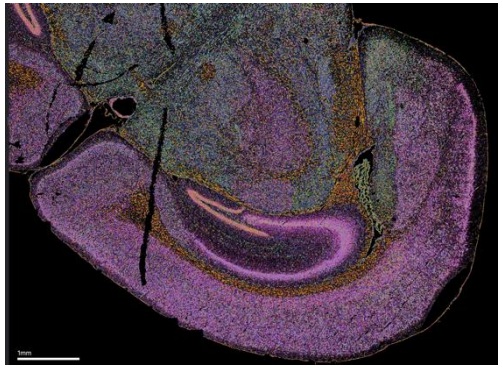
vizgen

MERSCOPE

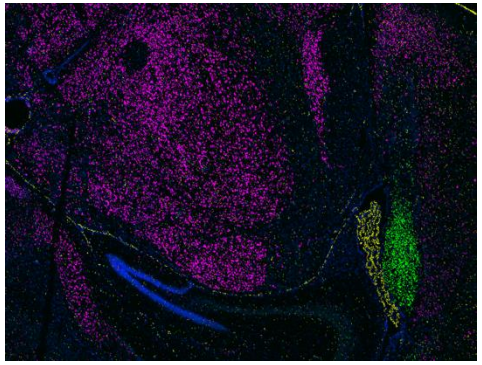
Multiplexed Error-Robust Fluorescence in situ Hybridization (MERFISH)



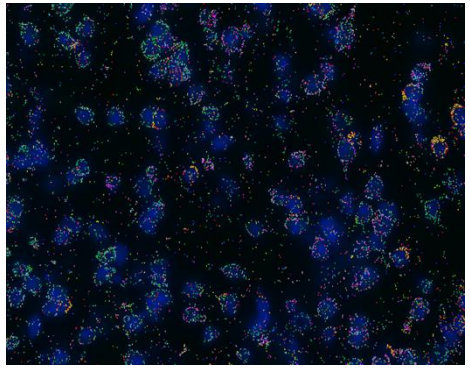
https://vizgen.com/technology/
Chen et.al, Science 2015



Mouse brain



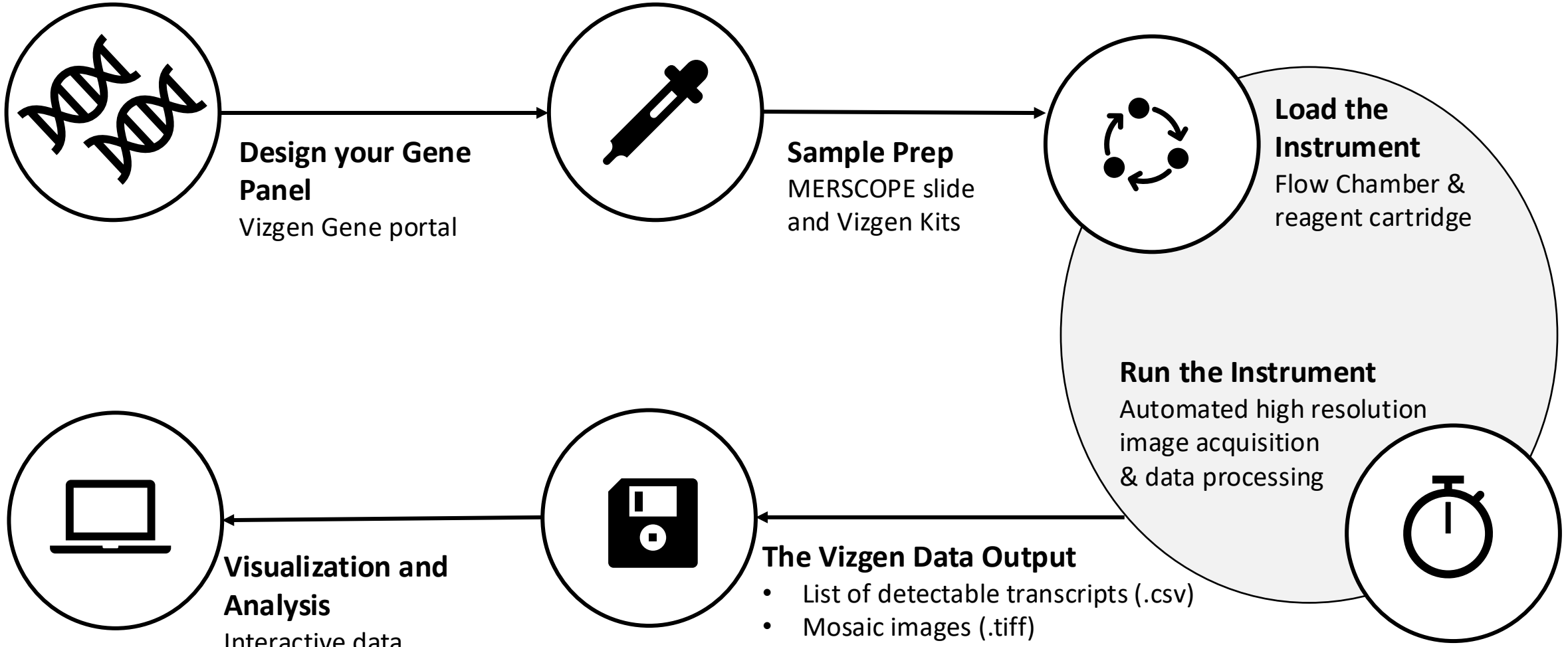
High sensitivity



Sub-cellular resolution

- 100-1000 genes, **High sensitivity**
- Fresh frozen and FFPE compatible
- **100 nm resolution**

MERSCOPE WORKFLOW



Design your Gene Panel
Vizgen Gene portal

Sample Prep
MERSCOPE slide and Vizgen Kits

Load the Instrument
Flow Chamber & reagent cartridge

Run the Instrument
Automated high resolution image acquisition & data processing

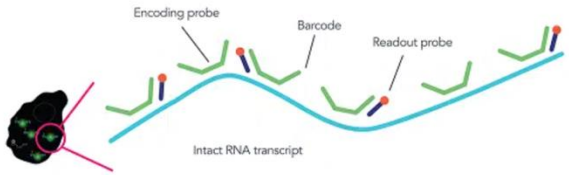
The Vizgen Data Output

- List of detectable transcripts (.csv)
- Mosaic images (.tiff)
- Transcripts per cell matrix (.csv)
- Cell metadata (.csv)
- Cell boundaries (.hdf5)

Visualization and Analysis
Interactive data visualization & analysis software

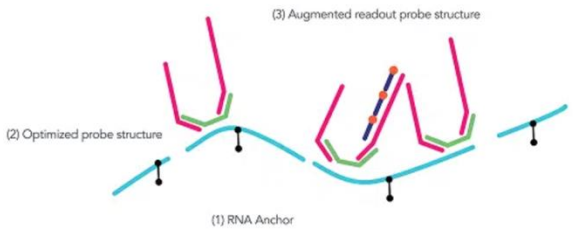
MERSCOPE V2.0 CHEMISTRY

High Quality RNA Samples and traditional MERFISH

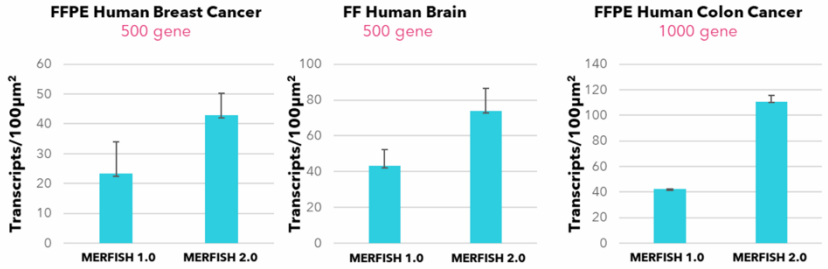


Low Quality RNA Samples and traditional MERFISH

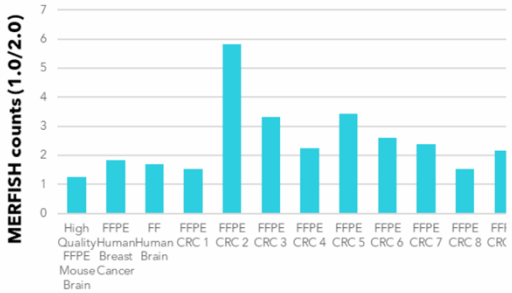
MERFISH 2.0 Workflow for Low Quality RNA



Improved sensitivity in a wide range of samples with MERFISH 2.0



Fold of improvement with MERFISH 2.0



MERSCOPE PREDESIGNED PROBE PANELS

Oncology and Tumor Microenvironment

Characterize tumor heterogeneity, immune infiltration, and signaling pathways with single-cell spatial precision.

Neuroscience and Brain Mapping

Profile neuronal and glial cell populations, connectivity, and gene expression across defined brain regions.

Immunology and Inflammation

Visualize immune cell localization and cytokine signaling to reveal tissue-specific immune responses.

Developmental and Regenerative Biology

Trace spatial gene expression through differentiation or regeneration to uncover lineage relationships.

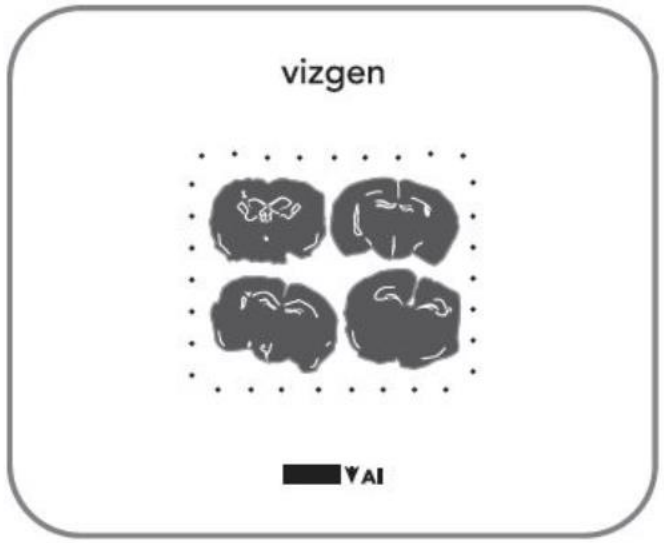
Organ and System-Level Profiling

Map cell types and molecular interactions in key organs, such as lung, liver, and kidney, with subcellular resolution.

Method of Choice

- Resolution: Sub-cellular
- Plexity: upto 1000 genes
- Sensitivity
- Scale

ULTRA slide



Up to 4 coronal mouse brain sections on a single slide

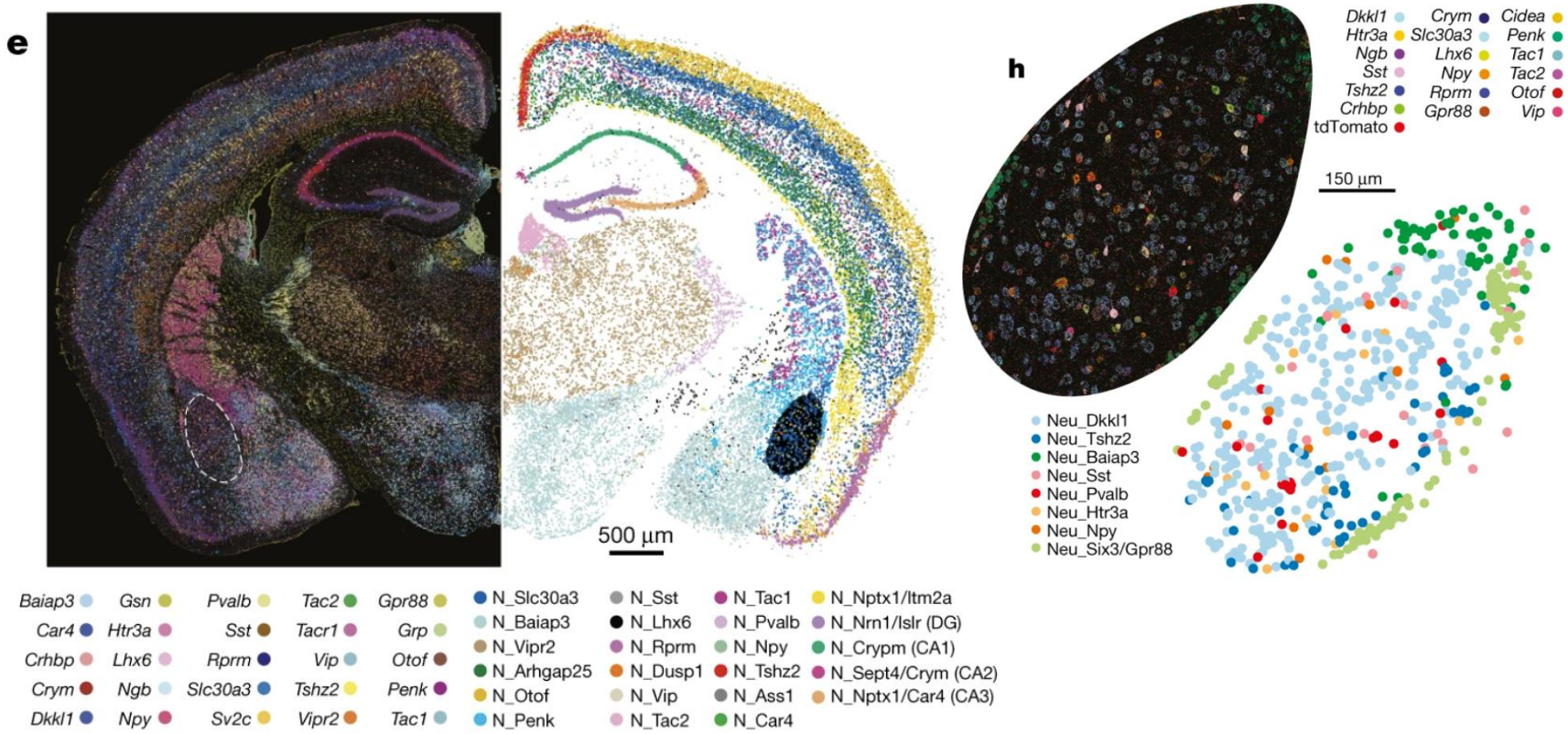
Article

Spatial transcriptomics reveal neuron–astrocyte synergy in long-term memory

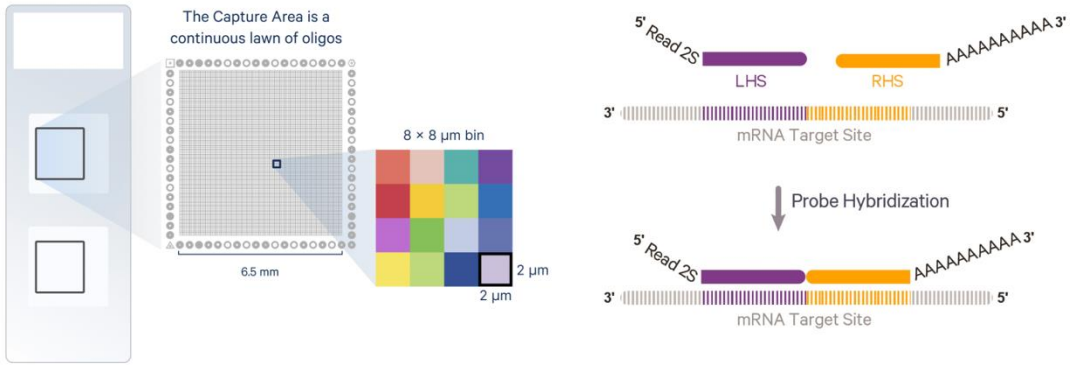
https://doi.org/10.1038/s41586-023-07011-6

Received: 16 March 2023

Wenfei Sun[1,2,6], Zhihui Liu[2,3,6], Xian Jiang[2], Michelle B. Chen[1], Hua Dong[4], Jonathan Liu[5], Thomas C. Südhof[2,3]✉ & Stephen R. Quake[1,5]✉



Visium HD spatial gene expression



https://www.10xgenomics.com/platforms/visium/

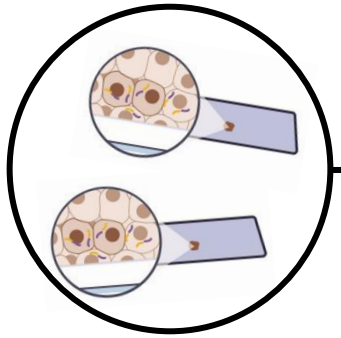
- High dimension, genome scale investigation
- Human and mouse compatible**, Probe based**
- All species, HD 3', **poly-A capture**
- **2 µm minimum resolution**
- Fresh frozen, Fixed frozen, FFPE and archived slides

Bin Metrics Overview

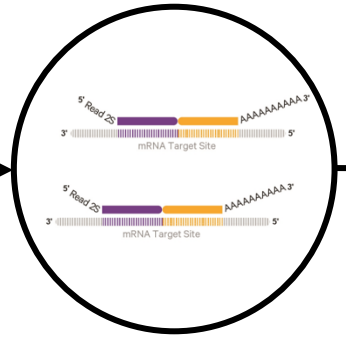
Bin Size (µm)	8 µm	16 µm
Number of Bins Under Tissue	639,062	160,296
Mean UMI Counts per Bin	657.0	2620.7
Mean Genes per Bin	523.0	1589.1

MsBrain_Fresh frozen

VISIUM HD WORKFLOW



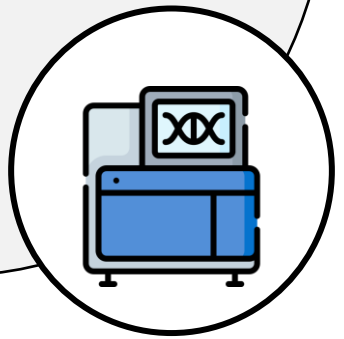
Staining
H&E or IF supported



Probe Hybridization
Mouse and Human

CytAssist
Probe capture

Library preparation and Sequencing



Spaceranger Data Output
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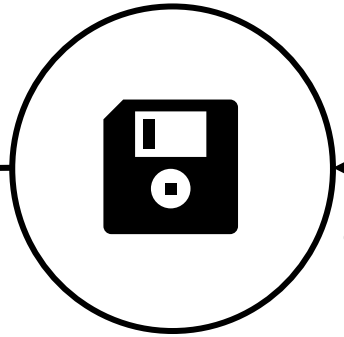
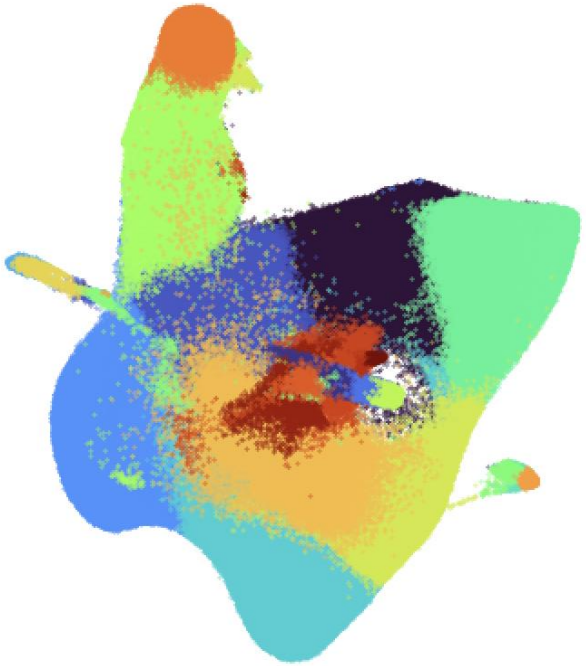
- raw_feature_bc_matrix/
- filtered_feature_bc_matrix.h5
- web_summary.html
- metrics_summary.csv

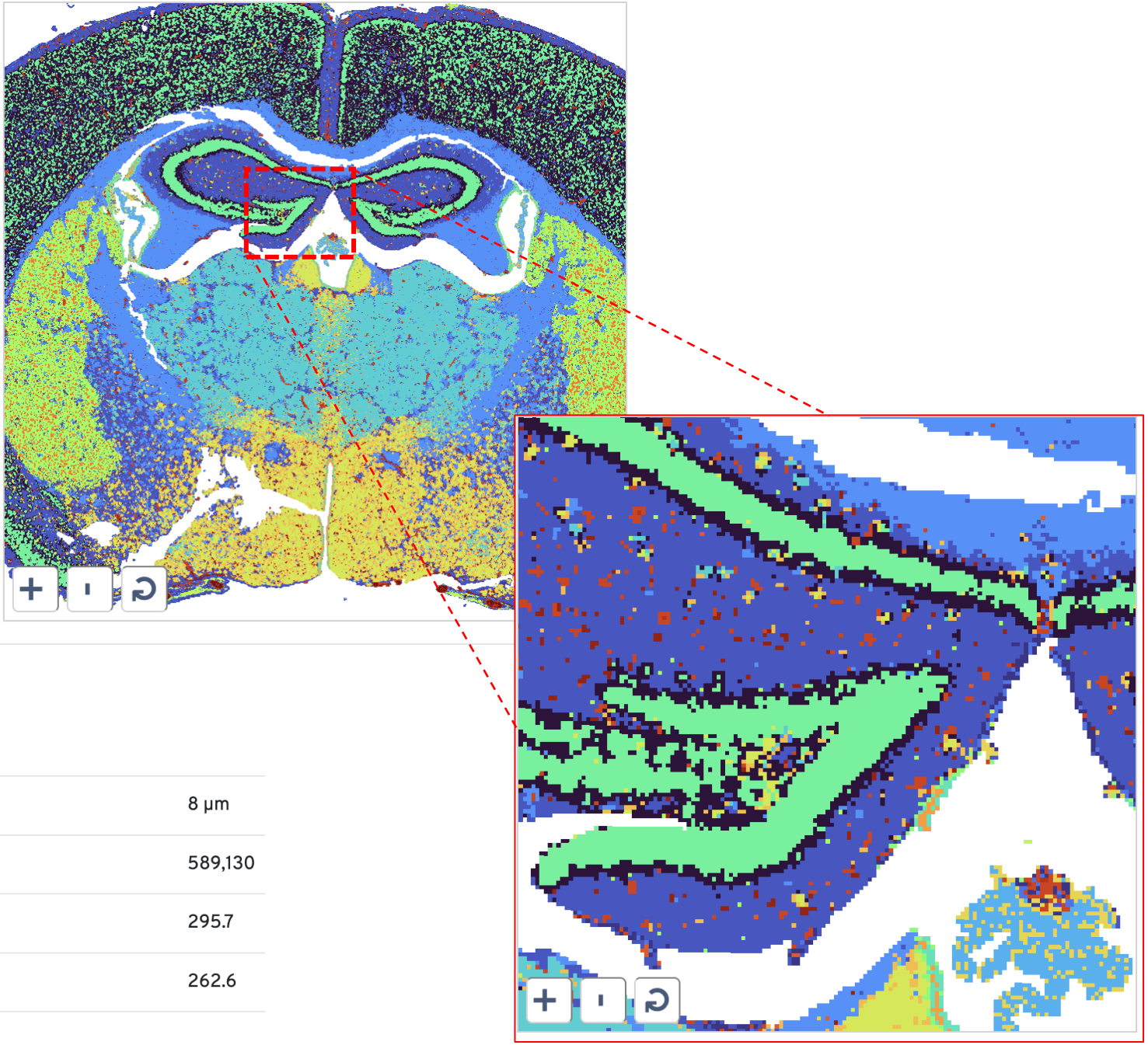


Visualization and Analysis
Interactive data visualization & analysis software

UMAP Projection of 8µm bins colored by Graph-based clustering



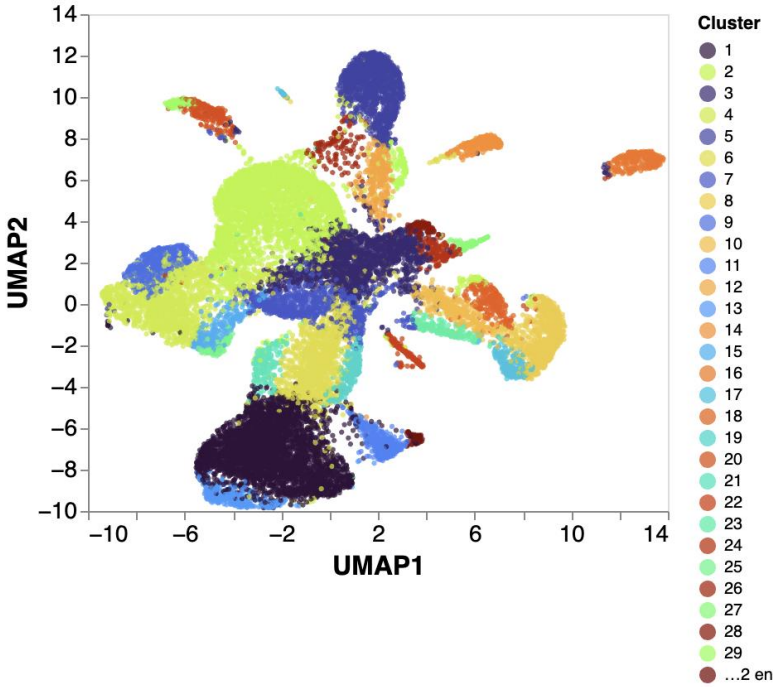
Tissue plot with 8µm bins colored by Graph-based clustering

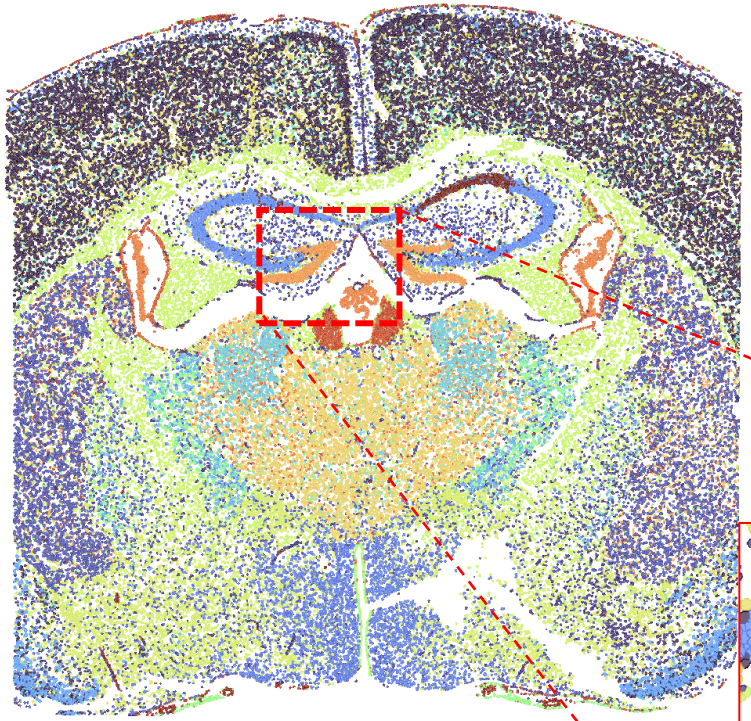


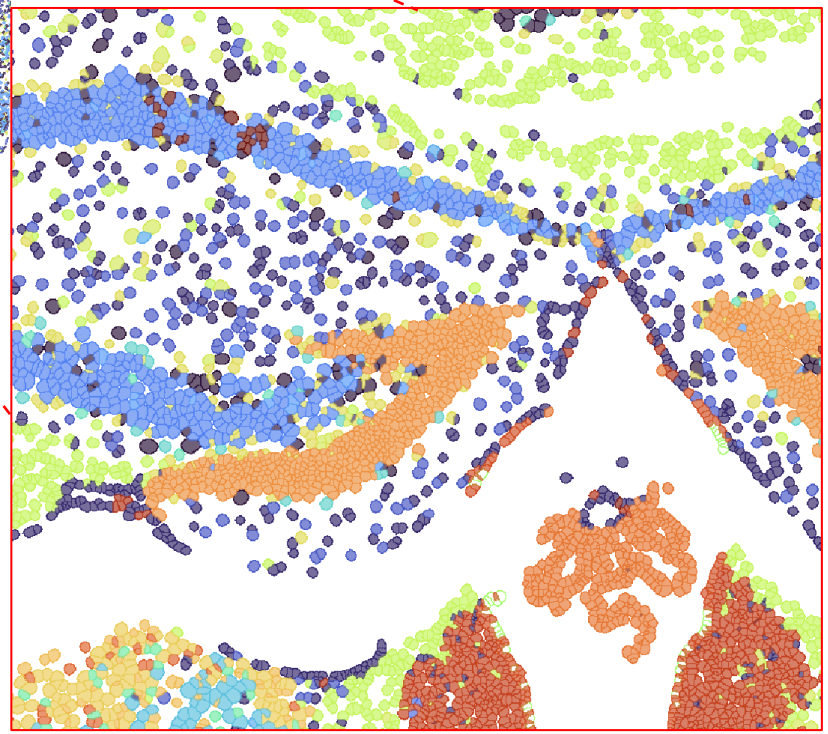
Bin Metrics Overview

Bin Size (µm)	8 µm
Number of Bins Under Tissue	589,130
Mean UMI Counts per Bin	295.7
Mean Genes per Bin	262.6

UMAP of Segmented Cells





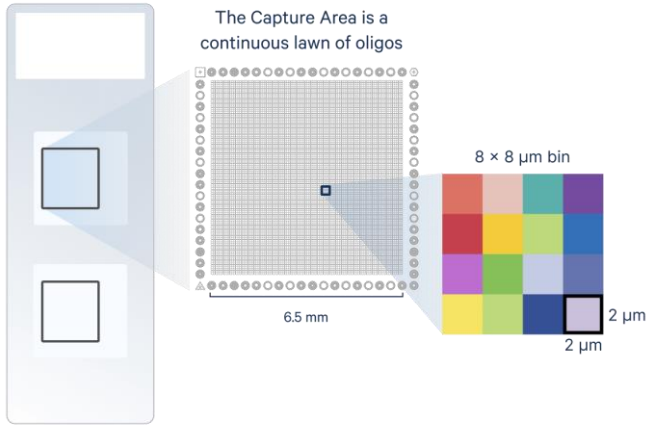


Cell Segmentation Metrics

Number of Cells	75,515
Reads in Cells	70.0%
UMIs in Cells	77.0%
Mean Reads per Cell	3,268.6
Median Genes per Cell	1030.0
Median UMIs per Cell	1306.0

Method of Choice

- Discovery: Genome scale
- Near cellular Resolution
- High- throughput
- Archived/stored samples



nature genetics

Article

https://doi.org/10.1038/s41588-025-02193-3

High-definition spatial transcriptomic profiling of immune cell populations in colorectal cancer

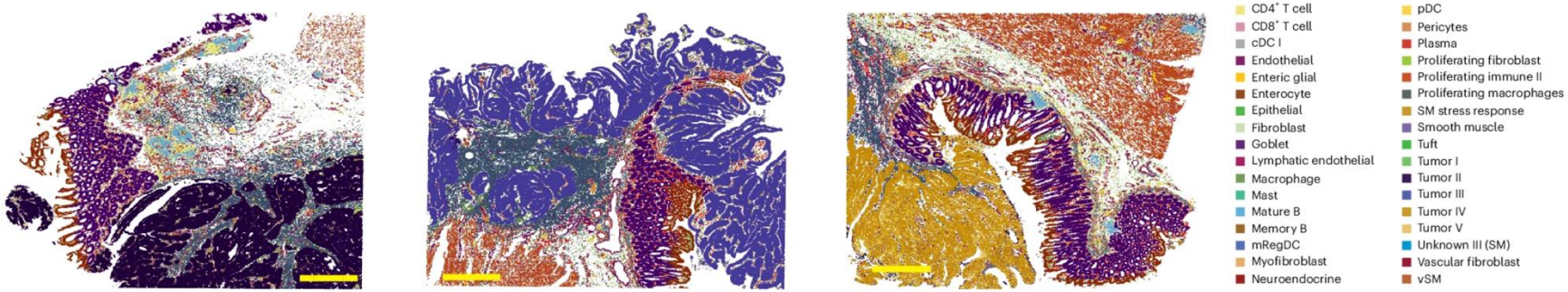
Received: 13 June 2024

Accepted: 14 April 2025

Published online: 5 June 2025

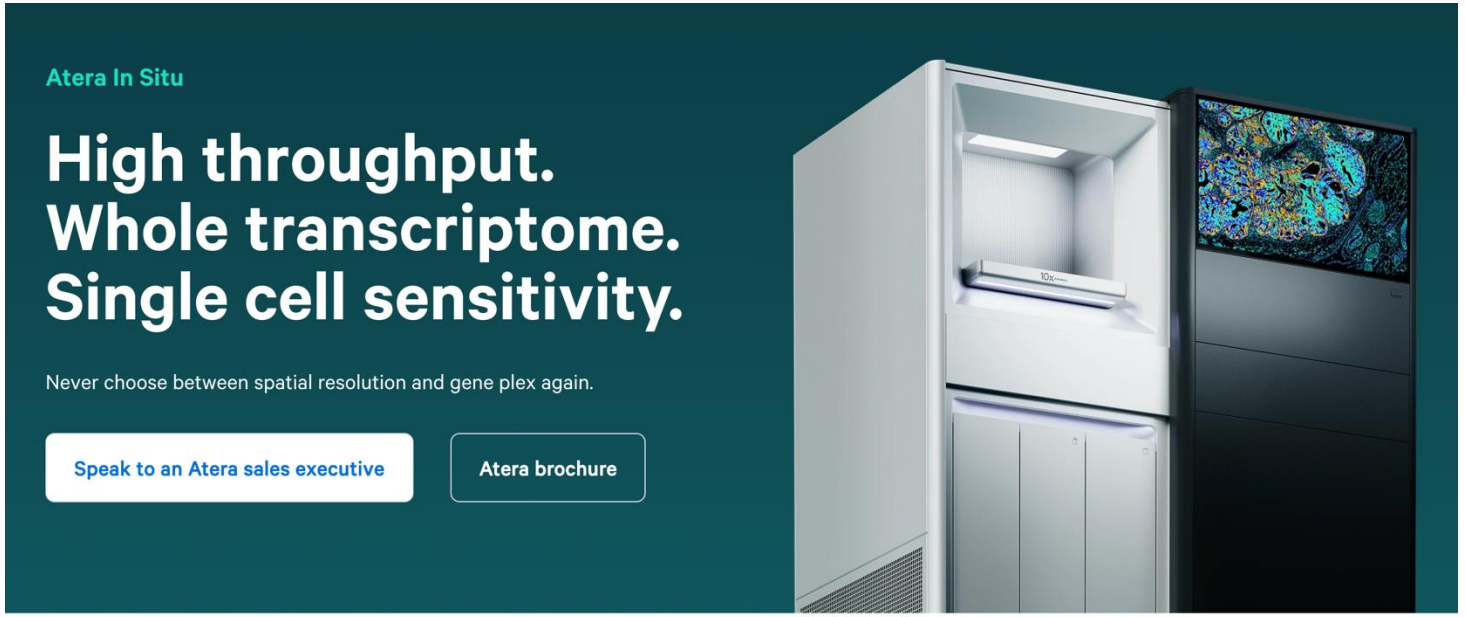
Check for updates

Michelli Faria de Oliveira[1,2], Juan Pablo Romero[1,2], Meii Chung[1,2], Stephen R. Williams[1], Andrew D. Gottscho[1], Anushka Gupta[1], Susan E. Pilipauskas[1], Seayar Mohabbat[1], Nandhini Raman[1], David J. Sukovich[1], David M. Patterson[1], Visium HD Development Team* & Sarah E. B. Taylor[1]



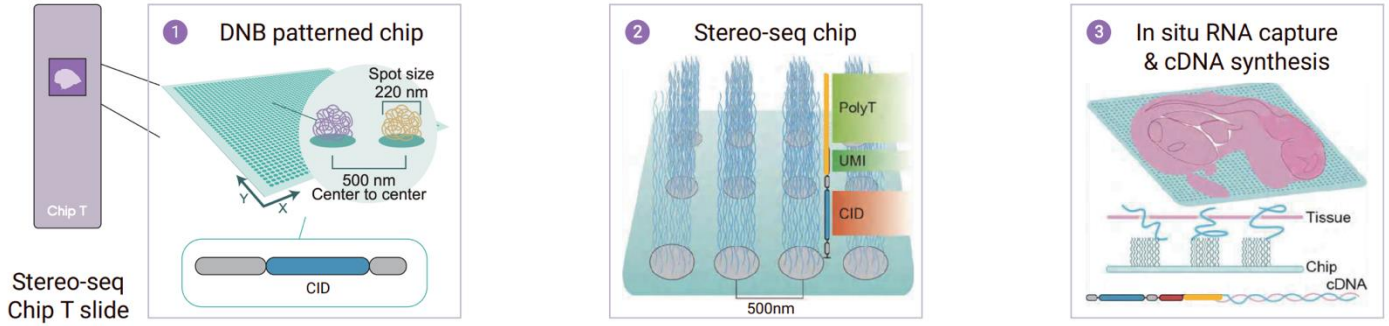
Updates

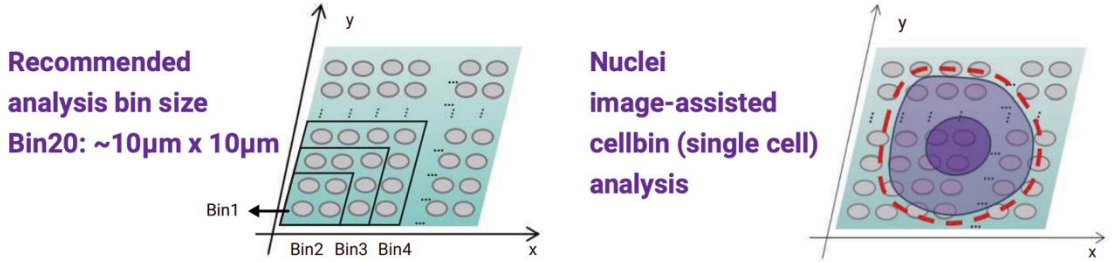
- HD 3’ - poly-A capture at HD resolution
- 11mm capture area available
- Atera insitu: 18000 plex Imaging based Spatial



STOmics

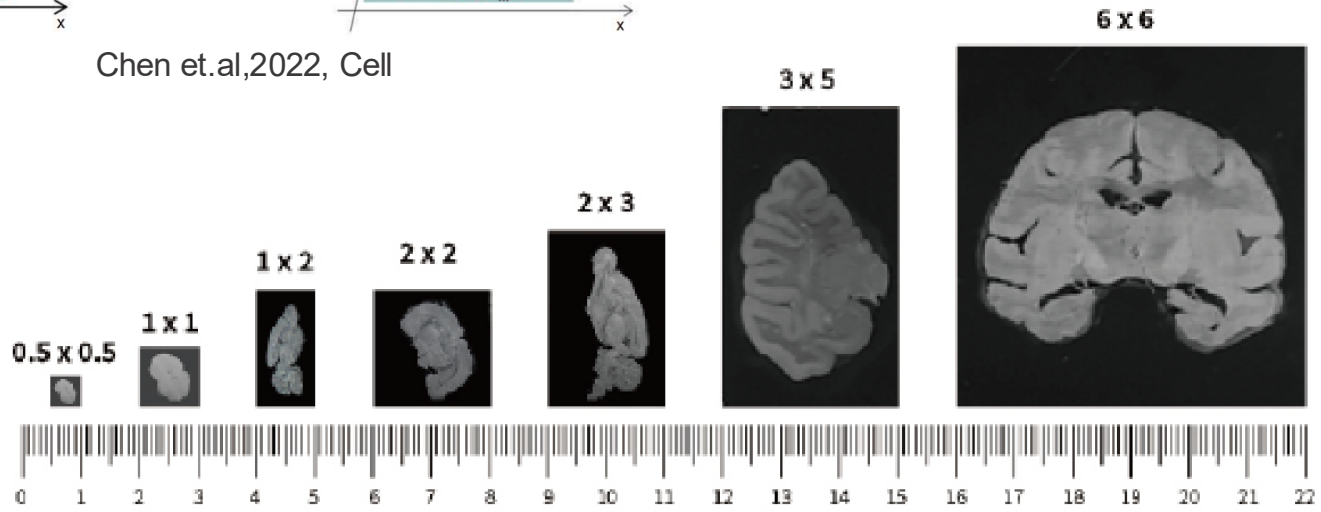
Stereo-seq spatial gene expression



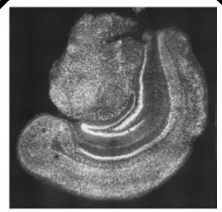


- Genome scale investigation
- Fresh frozen and FFPE compatible, **all eukaryotes**
- 500nm resolution, **5mm^2 ,1cm^2, 1*2cm, 2*3cm**

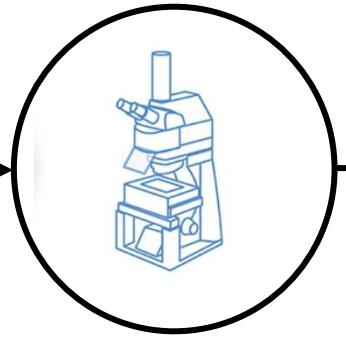
Chen et.al,2022, Cell



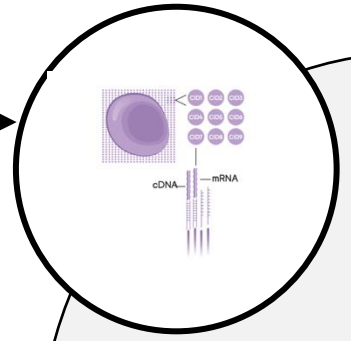
STOmics WORKFLOW



Permeabilization testing
Once for tissue and sample type

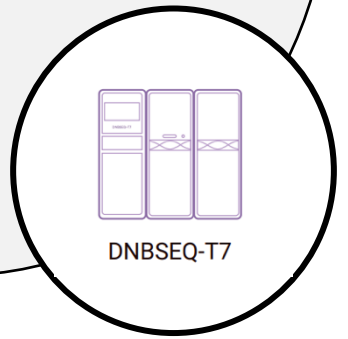


Staining & Imaging
H&E or IF supported



RNA Capture
cDNA Synthesis
PolyA or N'mer

Library preparation and Sequencing
DNB sequencer



STOmics Data Output
- Filtered_feature_bc_matrix/
- raw_feature_bc_matrix/
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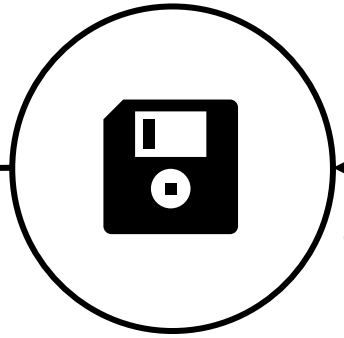
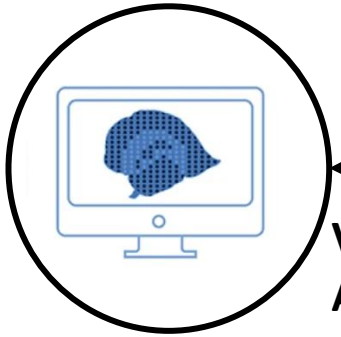
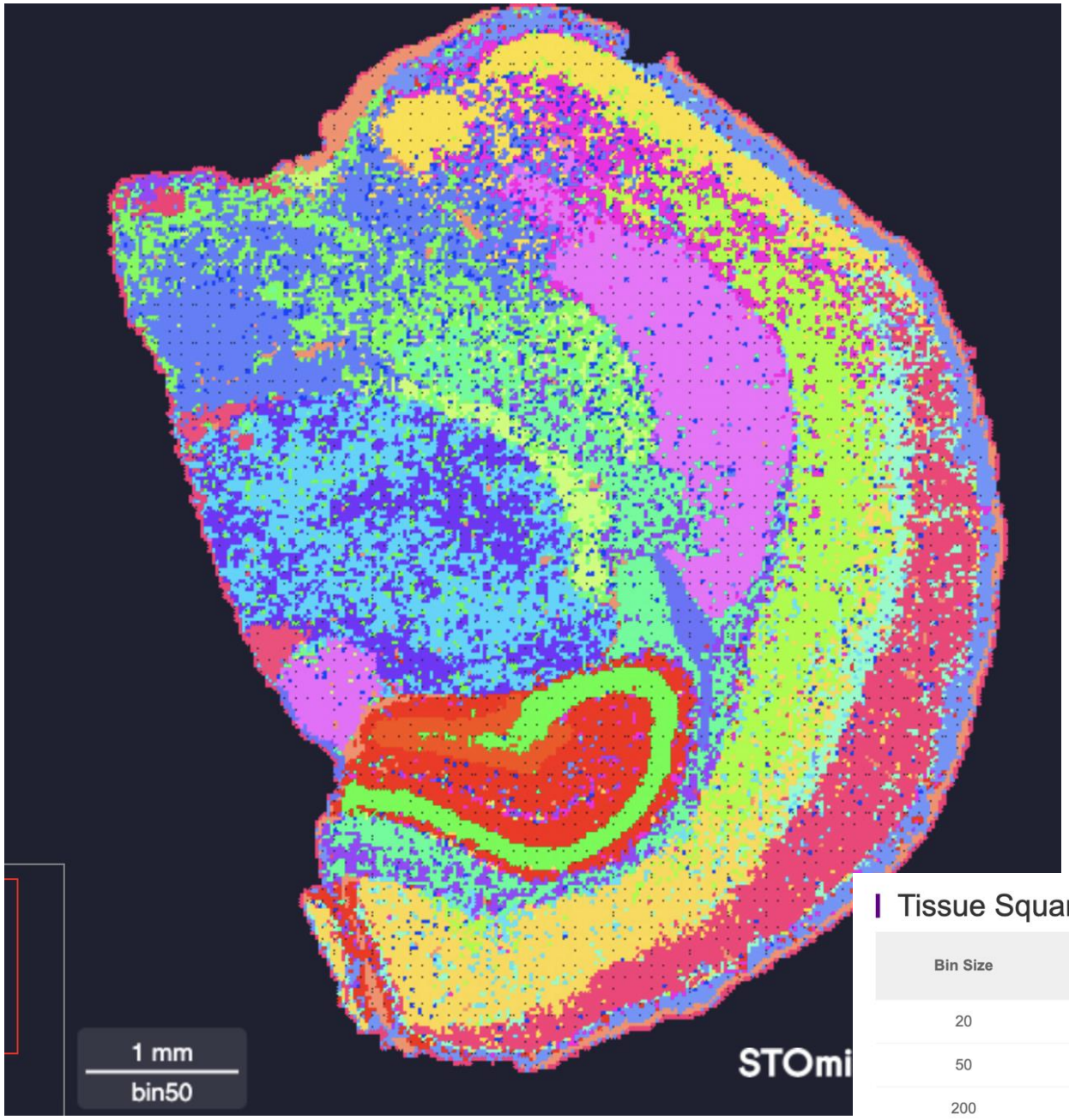
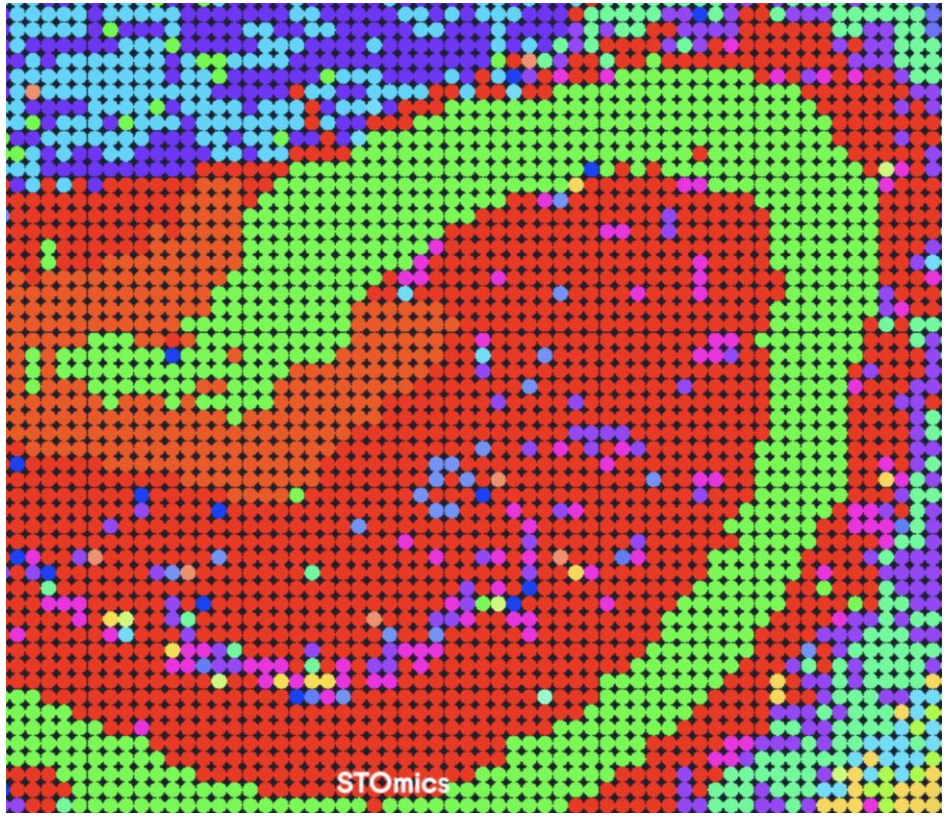


Visualization and Analysis
Interactive data visualization & analysis software

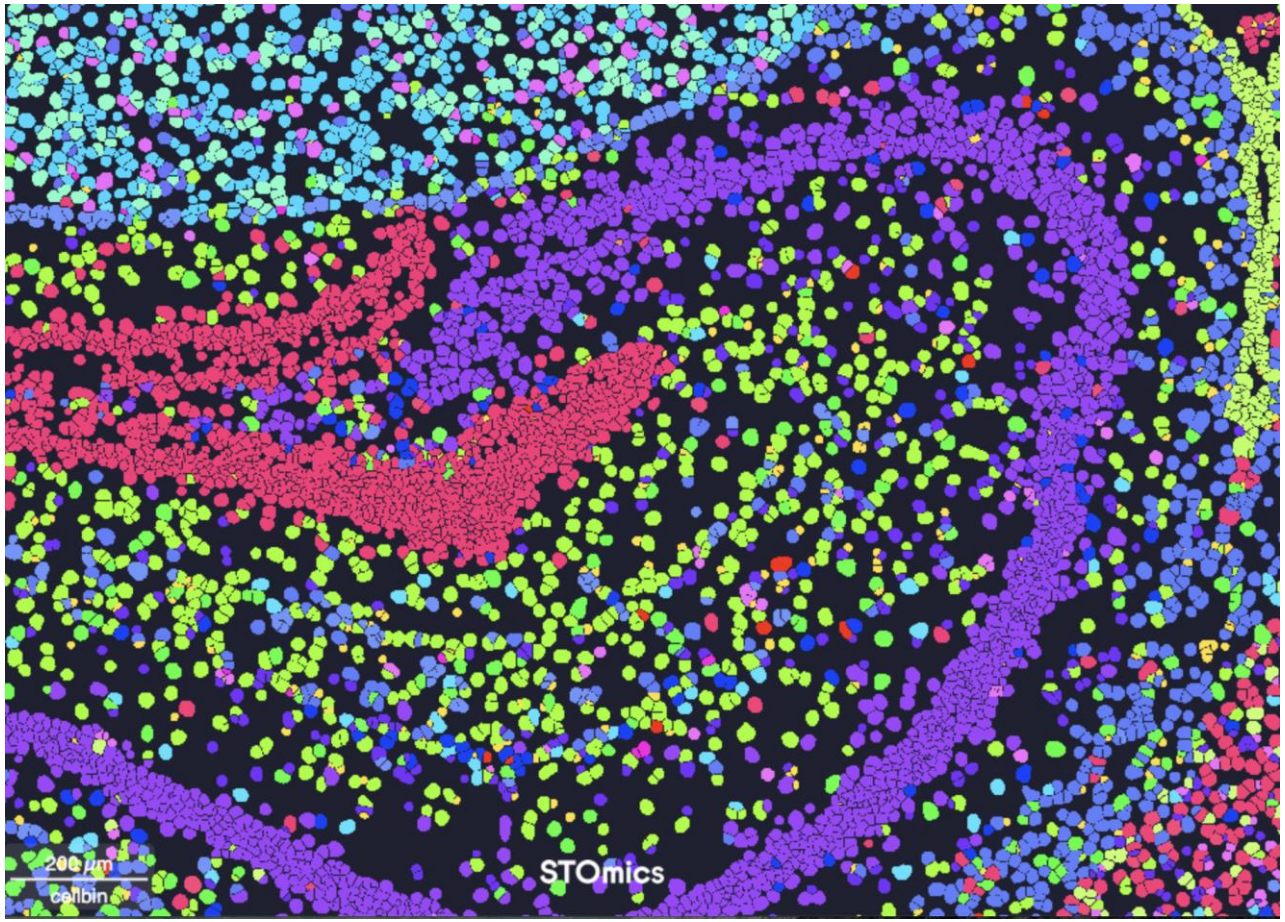






Tissue Square Bin Statistics

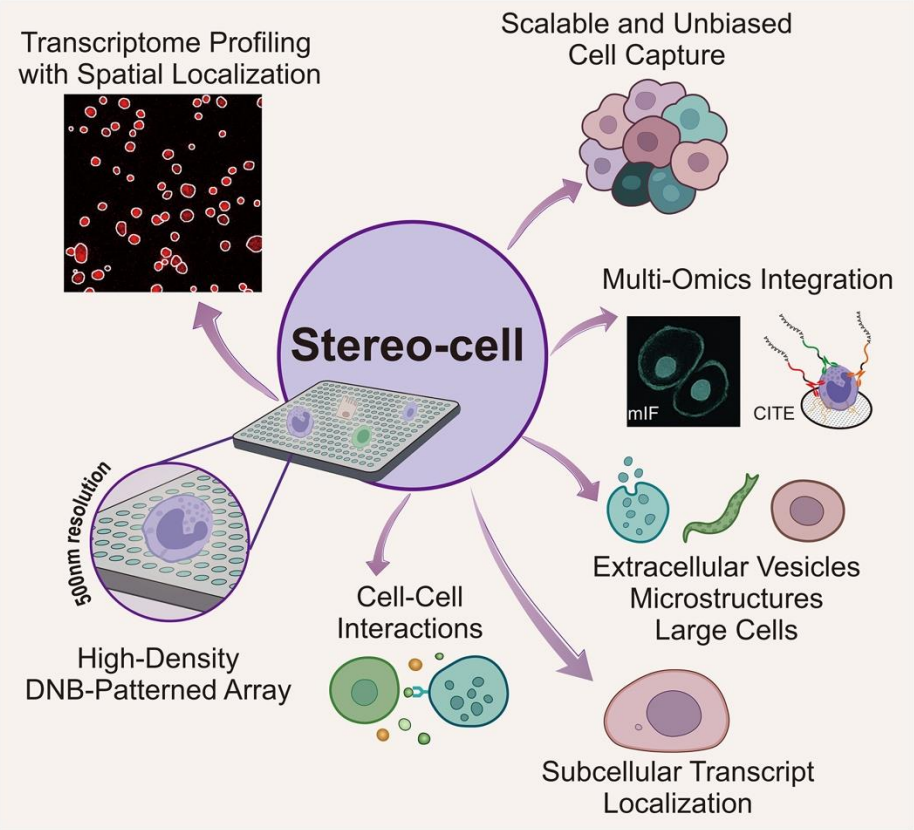
Bin Size	Mean Reads (per bin)	Median Reads (per bin)	Mean Gene Type (per bin)	Median Gene Type (per bin)	Mean MID (per bin)	Median MID (per bin)
20	3,814	3,071	324	282	462	377
50	23,655	21,294	1,534	1,479	2,868	2,593
200	365,533	366,481	8,061	8,482	44,318	44,309



Cell Bin Statistics

Cell Count	Mean Cell Area	Median Cell Area	Mean Gene Type	Median Gene Type	Mean MID	Median MID
70,035	912	877	794	732	1,314	1,133

Stereo-cell



Custom barcode capture with spatial transcriptomics

5'-CTGCTGACGTACTGAGAGGC-3'
the same sequence for Fw and Rv primers used in Stereo-seq

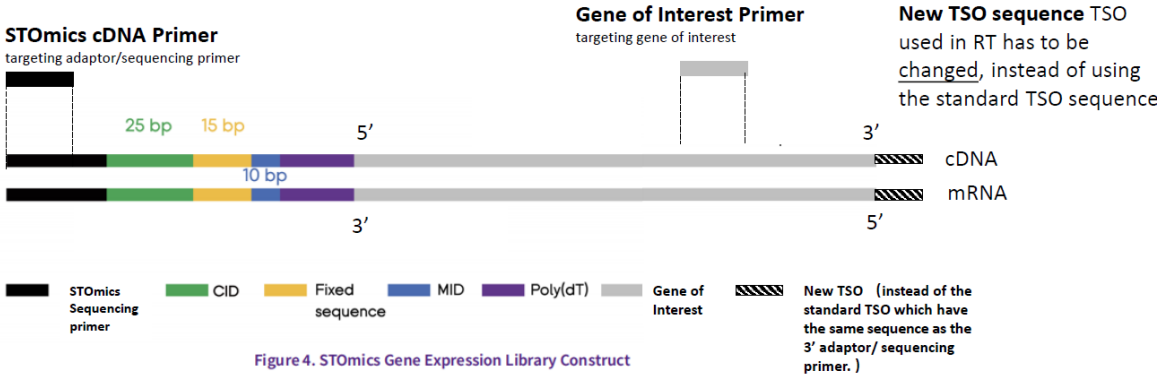
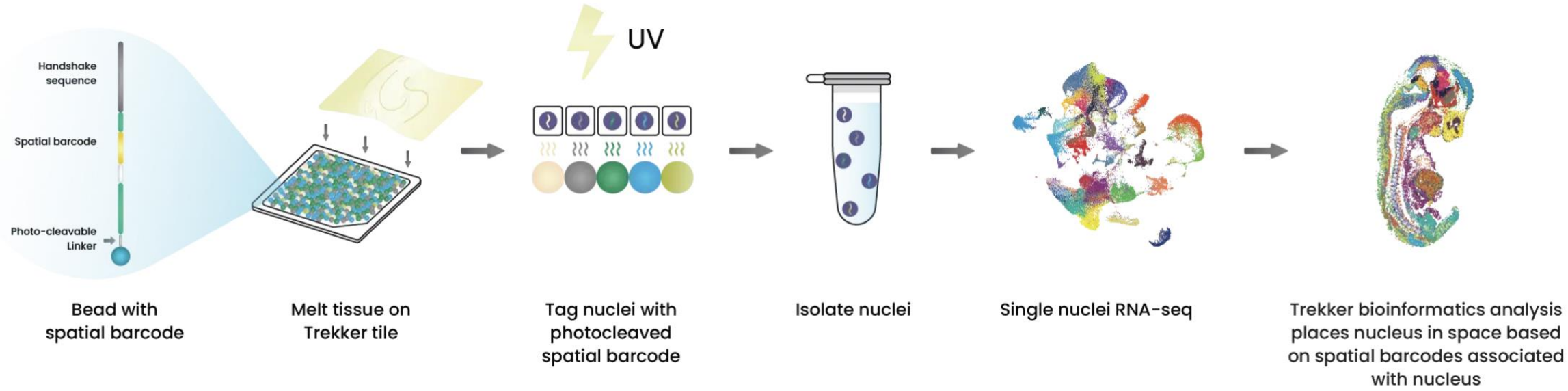


Figure 4. STOmics Gene Expression Library Construct

SCIENCE
21 Aug 2025, Vol 389, Issue 6762
DOI: 10.1126/science.adr0475

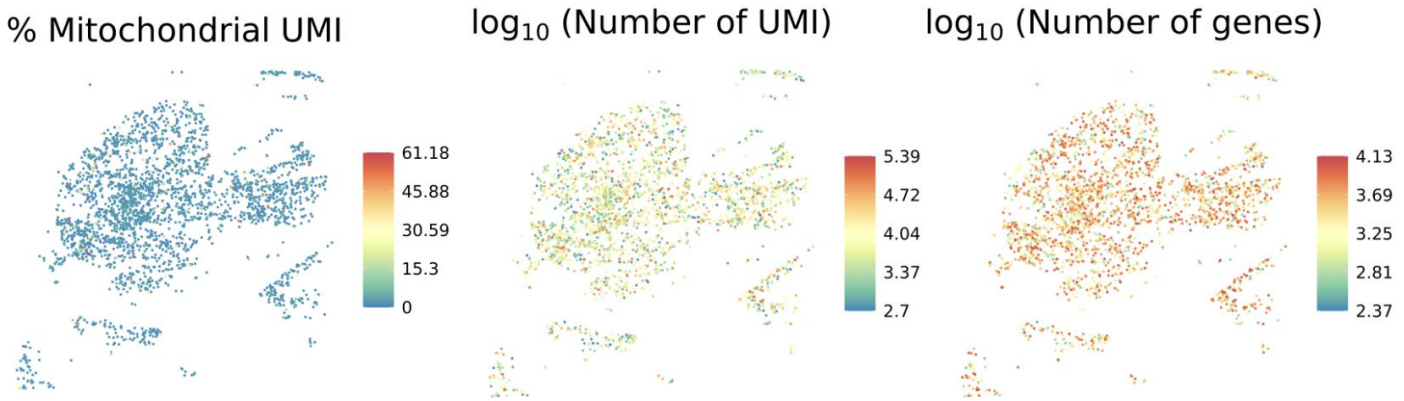
Slide Tag: Curio_Trekker or Takara Trekker



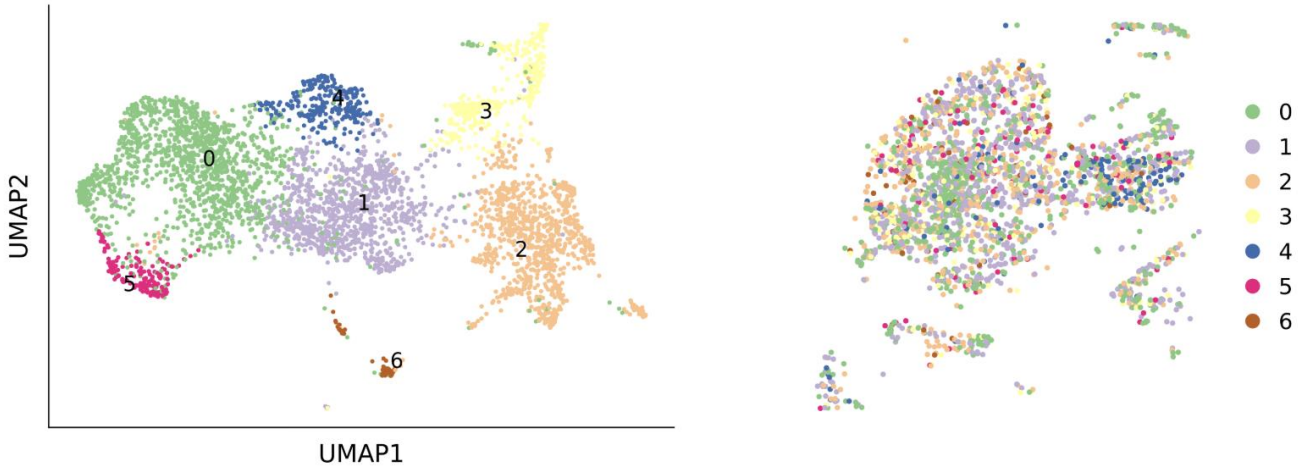
Single-cell workflow compatibility	
Supported protocols • 10x Chromium™ 3' RNA v3.1, v4 • BD Rhapsody™ WTA	User Demonstrated • Fluent PIP-Seq™ V • 10x Chromium™ Multiome ATAC + Gene Expression • ScaleBio™ Single-Cell RNA Kit

➢ Single cells with spatial tags

Ms Colonic xenografts: 5000 nuclei barcoded



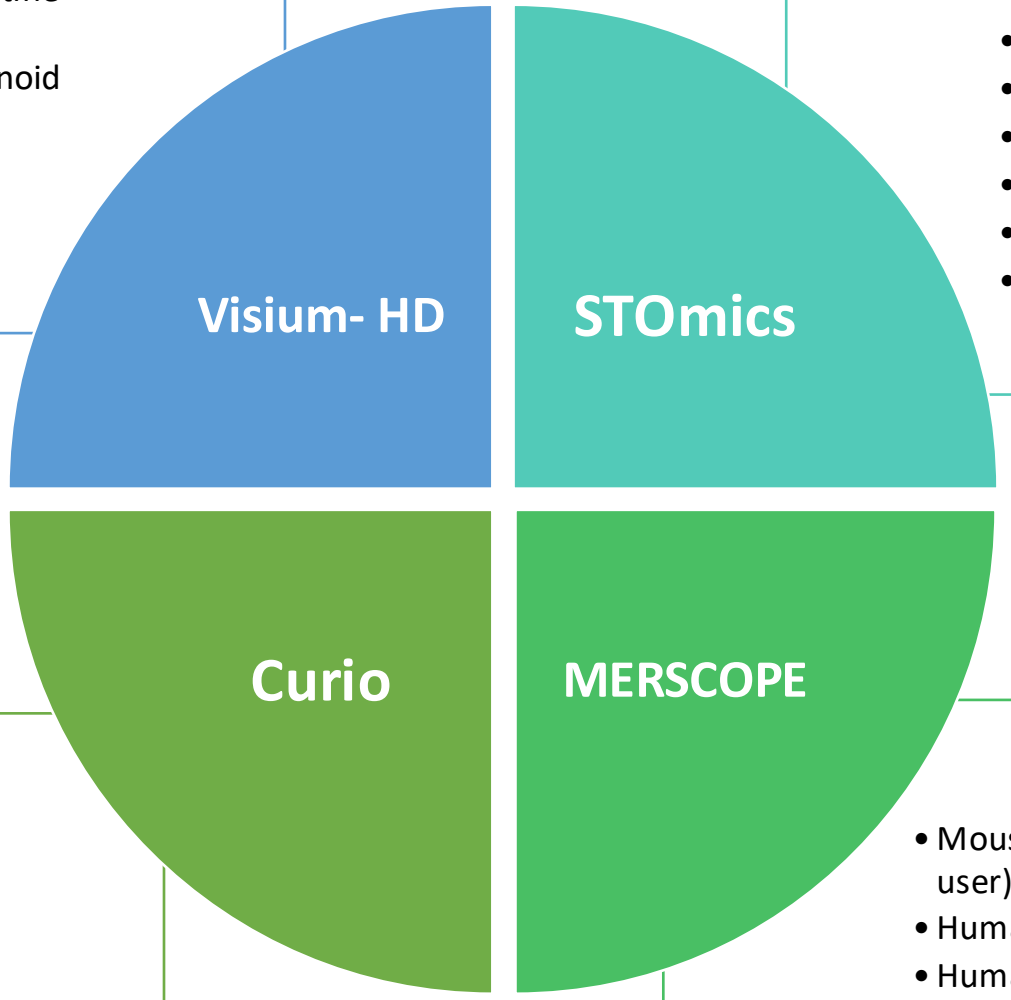
▶ Spatial Map of Cell Types



Updates

- FFPE Compatibility
- Highest depth in un-biased technologies
- One of the two commercially available spatial epigenomic technology

Our experiments so far



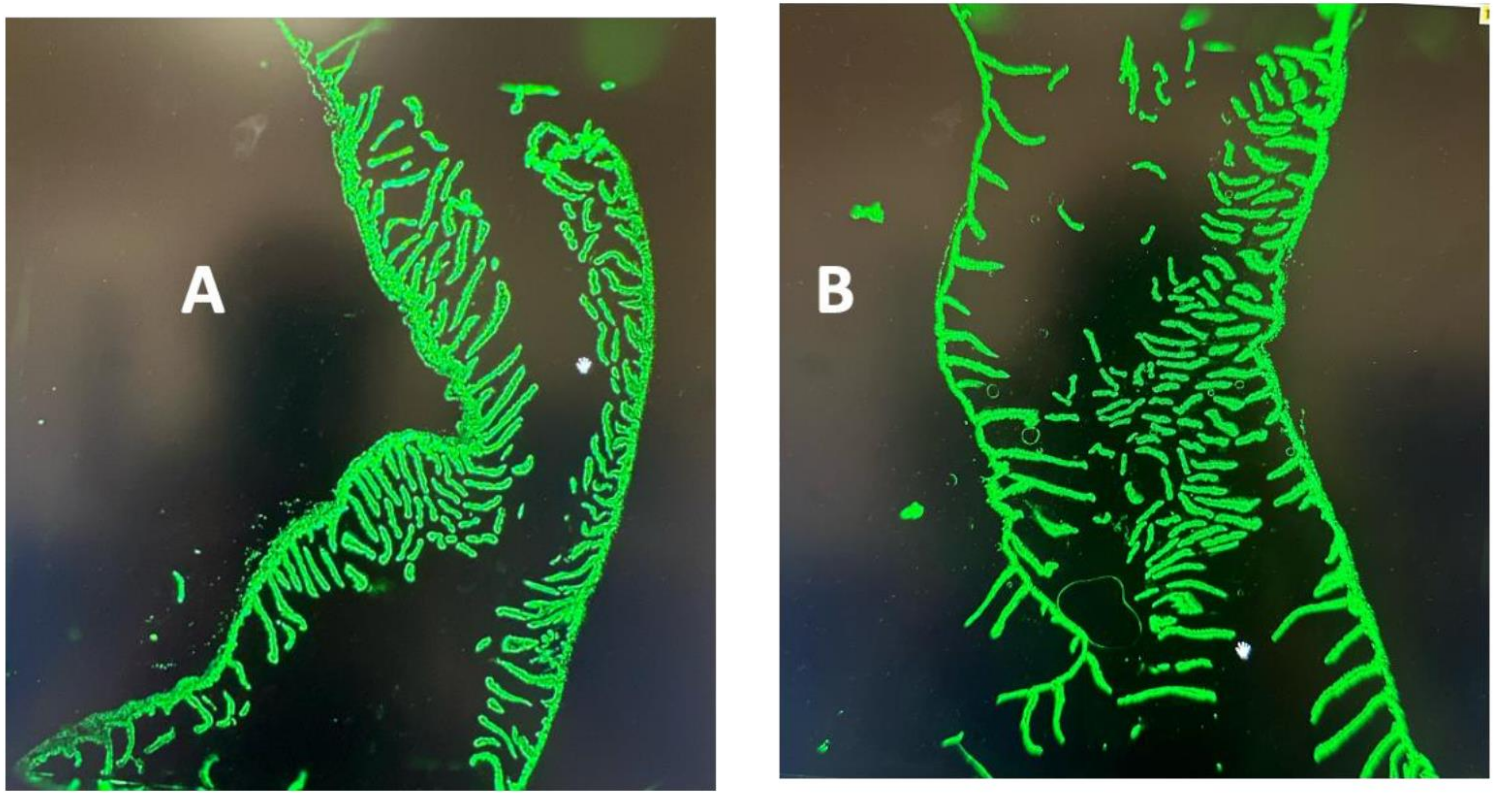
- Mouse brain and intestine (in-house)
- Human inner ear organoid
- Adipose organ on chip
- Mouse Spinal cord
- Mouse Muscle
- Human heart
- Colon xenografts

- Mouse brain and intestine (in-house)
- Mouse skull
- Mouse retina
- Chick retina
- Mouse full embryo
- Mouse bone-marrow
- Sea slug

- Mouse brain and intestine (in-house)
- Colon xenograft

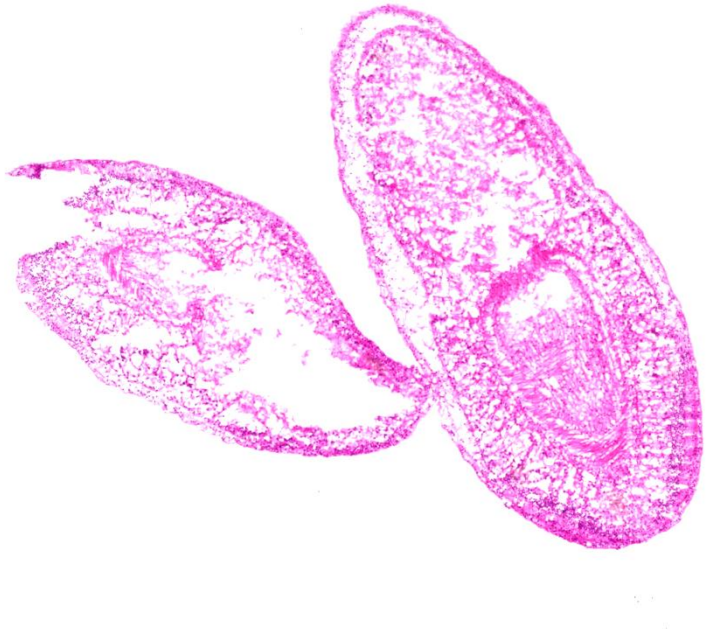
- Mouse brain (in-house and user)
- Human brain
- Human spinal cord
- Zebrafish heart

Sample specific problems: Tissue attachment

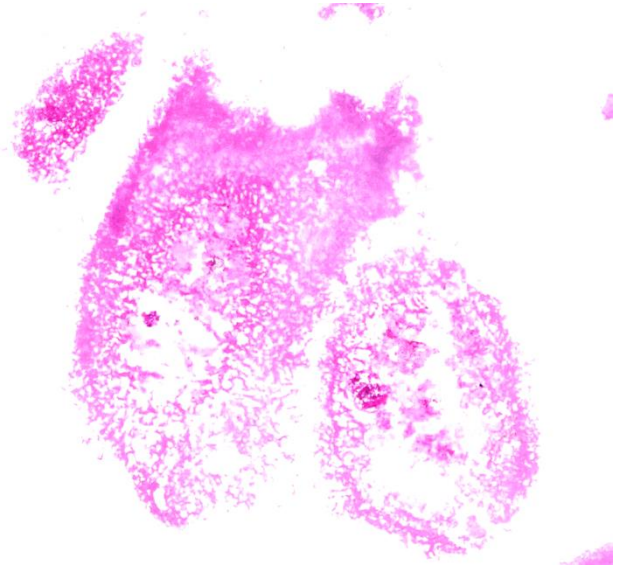


STOmics_Mouse Duodenum: Fixed Frozen tissue

Sample specific problems: Lysis/loss of morphology

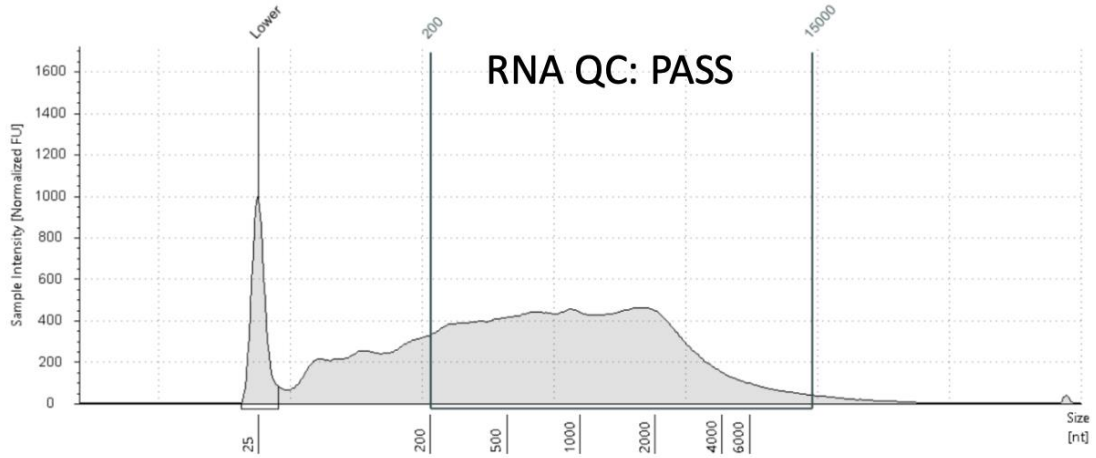


Marine_worms: Fixed_Frozen



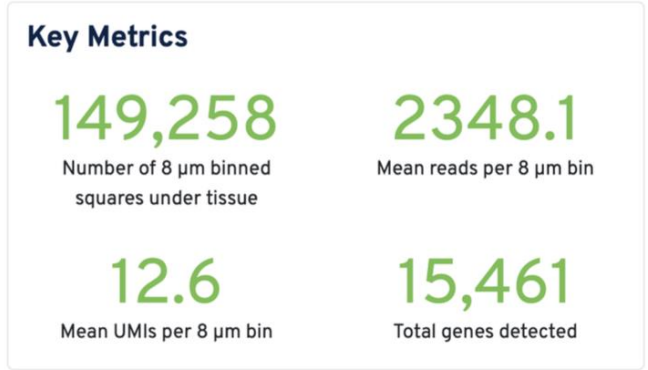
Marine_worms: Fresh_Frozen

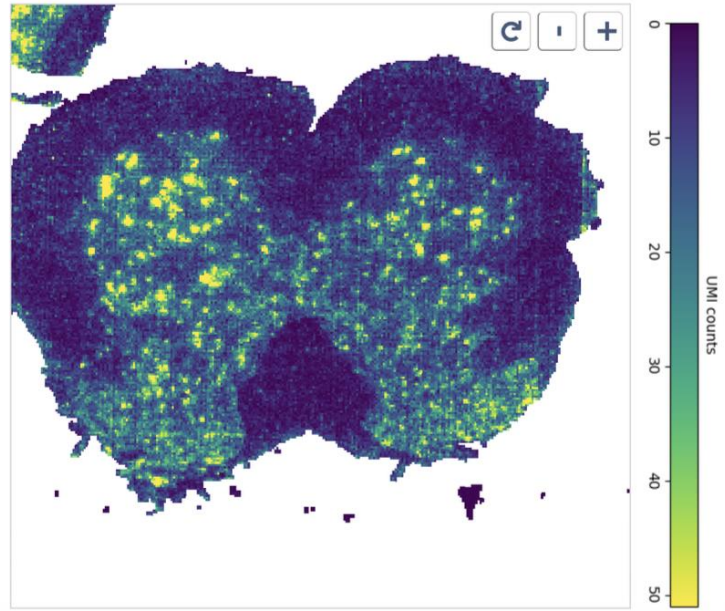
Sample specific problems: Probe binding failure?



Region Table

From [nt]	To [nt]	Average Size [nt]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
200	15000	4260	2820	1950	77.42		



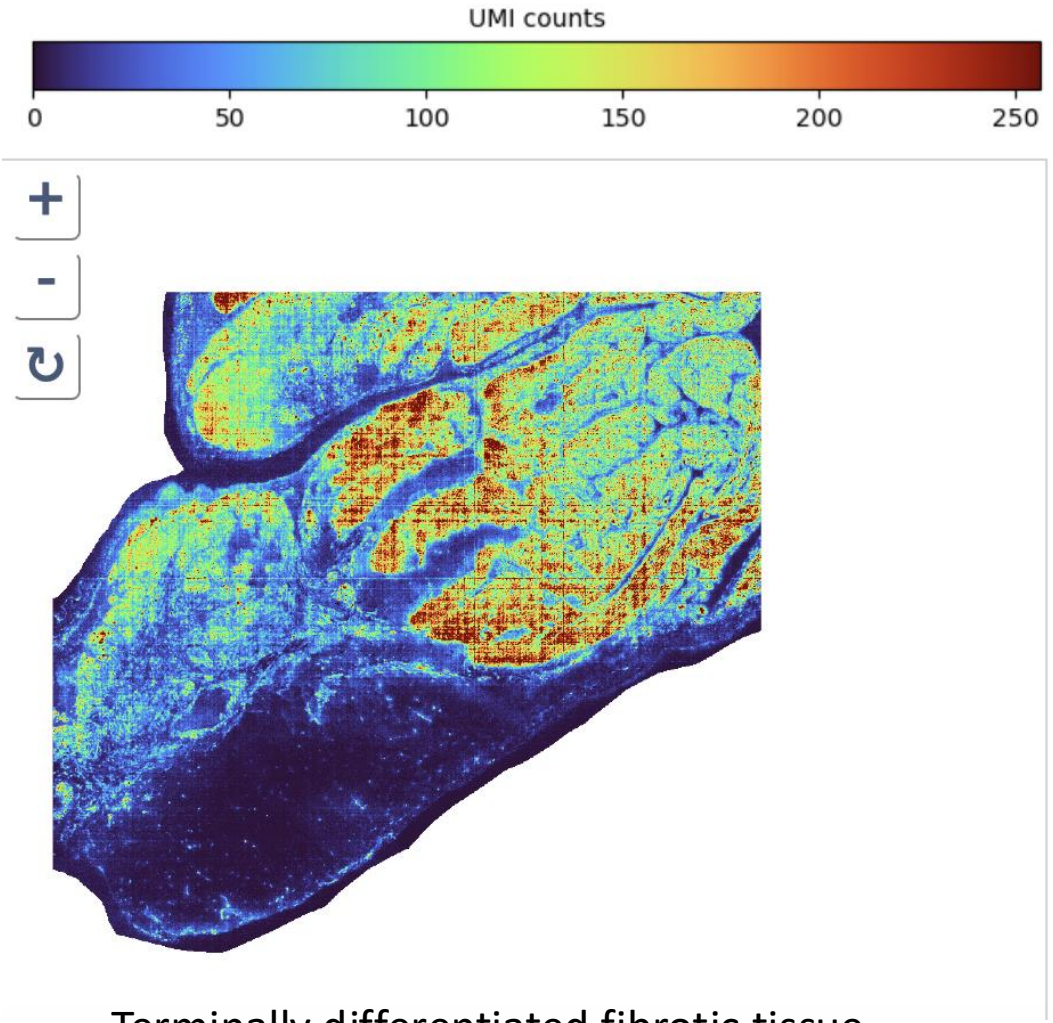


Visium HD_Mouse Spinal cord: FFPE

Low UMIs ≠ Bad Data always

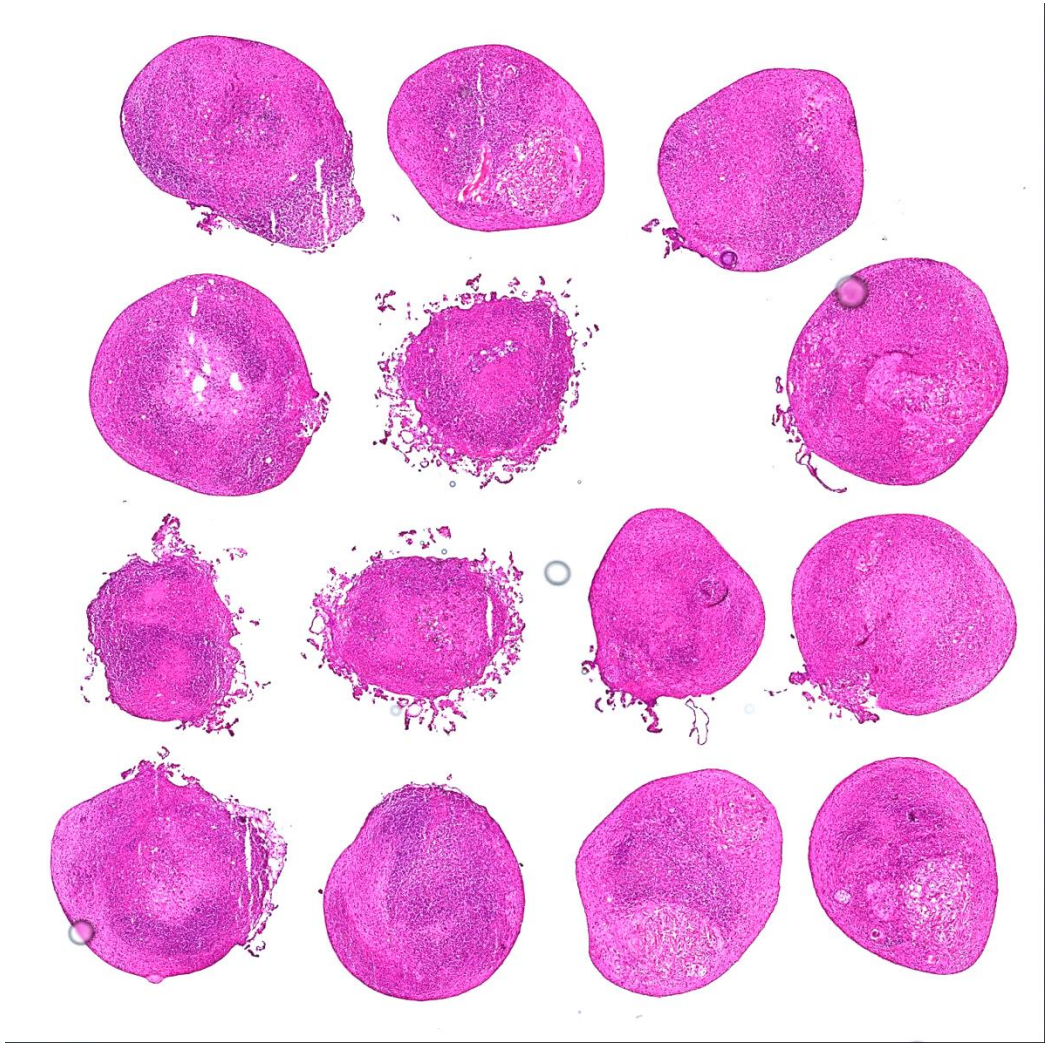
Key Metrics

486,254	1467.1
Number of 8 µm binned squares under tissue	Mean reads per 8 µm bin
71.1	17,328
Mean UMIs per 8 µm bin	Total genes detected



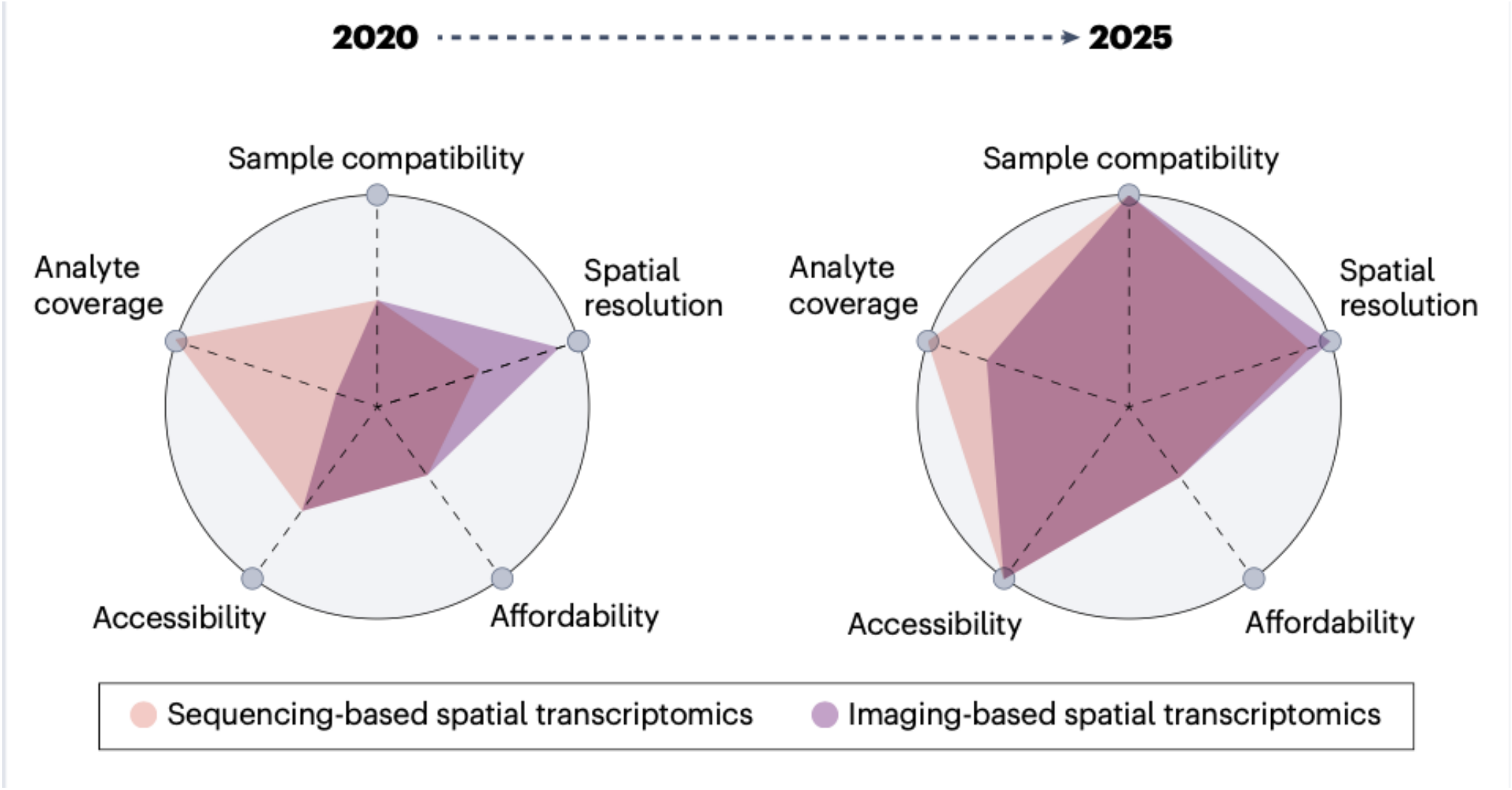
Terminally differentiated fibrotic tissue

Maximize data per experiment



6.5x 6.5mm
Visium HD area

Technology Evolution



Lázár, Enikő et al, *Nature Reviews Genetics*, 2026

Single cell core @ HMS: Spatial Transcriptomics

To enquire about our spatial transcriptomic services visit our website and schedule a free consultation.
https://singlecellcore.hms.harvard.edu/ spatial-transcriptomics-0

Technologies we offer

10X GENOMICS®

Visium HD
Visium HD 3’

vizgen

MERSCOPE

Complete GENOMICS

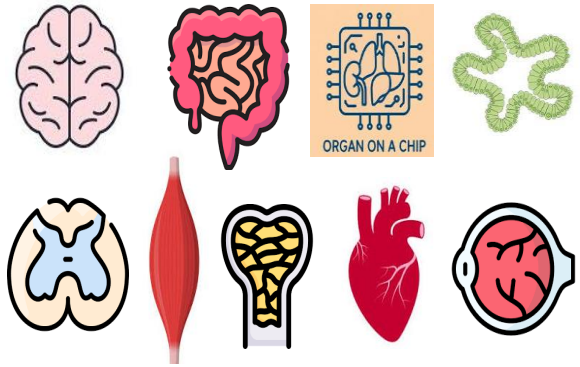
STOMics

TaKaRa

CURIO_Trekker
CURIO_Seeker

Tested so far

- FFPE Tissue
- Fresh Frozen Tissue
- Fixed Frozen Tissue



Our strengths

Block to Data service

Close collaboration with cores

- **Histopathology**
- **Imaging**
- **Sequencing**
- **Analysis**

Open to custom projects

Multiple technologies

Diverse sample expertise