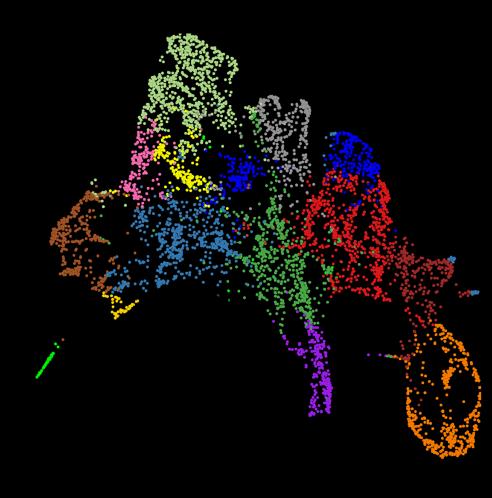
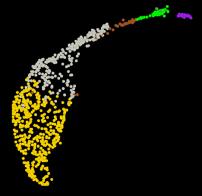
# Visium Spatial





# Your Local 10x Genomics Team



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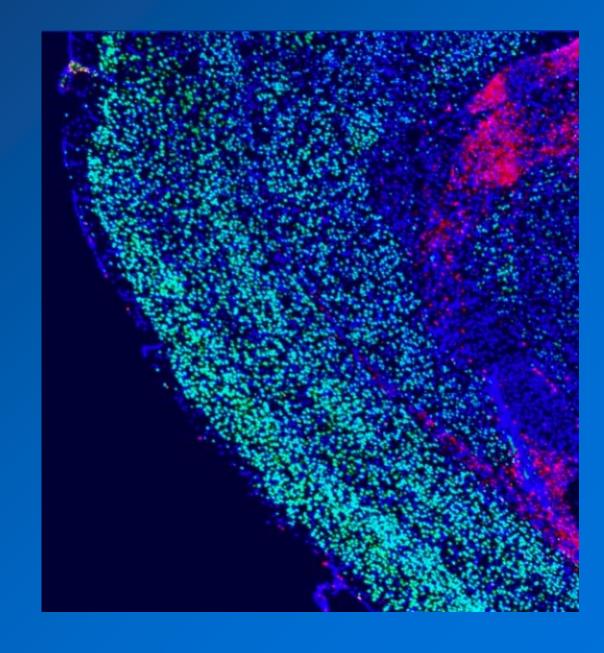


# Master Biology with the 10x Toolkit Visium Spatial

Josh Talboom, Ph.D. Science & Technology Advisor (STA) San Diego & OC, CA

GRT Hub Workshop - Spatial Transcriptomic Data Analysis 9/28/2023

The complexity of biology limits our understanding of disease and our abilities to improve human health.



# Fundamental Challenges Remain in Treating Disease

Many diseases still pose major threats to public health

ALZHEIMER'S →

CANCER -

AUTOIMMUNE DISORDERS

98%

failure in Alzheimer's drug development

Kim, C. Kwon et al. Alzheimer's Disease: Key Insights from Two Decades of Clinical Trial Failures. *Journal of Alzheimer's Disease* 1: 83–100 (2022).

**20-30**%

response rates for immune checkpoint blockade

Kumar, N, Papillon-Cavanagh, S, Tang, H, et al. A multi-omic single cell sequencing approach to develop a CD8 T cell specific gene signature for anti-PD1 response in solid tumors. *Int J Cancer*. Vol. 151 Issue 11, 2043–2054 (2022).

4+ yrs.

for diagnosis of autoimmune disorders

Diagnosing Autoimmune Diseases, *Benaroya Research Institute*, Oct. 2017, www.benaroyaresearch.org/blog/post/diagnosing-autoimmune-diseases.



# 10x Solutions Are Transforming the Understanding of Disease

10x provides the tools researchers use to solve the tough questions that remain in biology & disease

#### Cancer

#### Cell

Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies

#### nature medicine

A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer

#### **Autoimmune**

#### Science

Single-cell eQTL mapping identifies cell type-specific genetic control of autoimmune disease

#### nature

Interpreting type 1 diabetes risk with genetics and single-cell epigenomics

#### Neuroscience

#### nature genetics

Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease

#### nature immunology

Microglia use TAM receptors to detect and engulf amyloid β plaques

#### Other fields

#### Science

Pathogenic variants damage cell composition and single cell transcription in cardiomyopathies

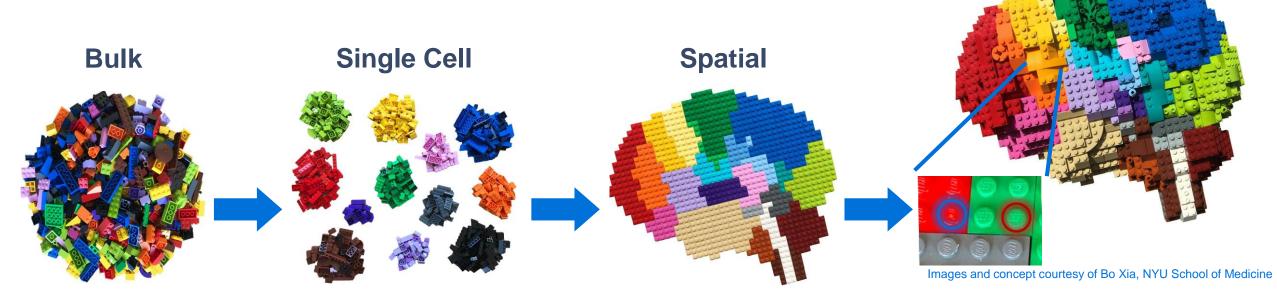
#### **Cell Genomics**

Retinal ganglion cell-specific regulation in primary open-angle glaucoma



# **Getting the Best View of Biology and Disease**

Overview of legacy and current "omic" methods



- General snapshot of the whole target "ome"
- Lacks cellular or subcellular resolution and spatial context
- Cell-by-cell view of the whole target "ome"
- Method unable to retain spatial context
- 2D view of the whole target "ome" across several cells
- Valuable for initial discovery at the system, organ, or tissue level
- Current solutions do not offer single cell or subcellular resolution

• 3D view of 500-1000 "ome" targets in *situ* at subcellular resolution

In Situ

- Suitable for focused deeper dives into cell-by-cell biological mechanisms
- Not practical for discovery at the system or organ level

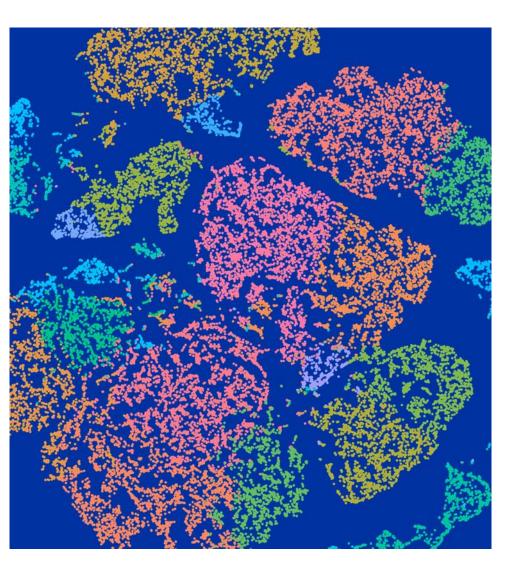






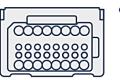
# Chromium

RESOLVE TUMOR HETEROGENEITY, CELL BY CELL

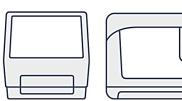


- Partitions and barcodes cells in minutes
- Analyzes 100s to millions of cells
- Leverages scale of NGS while preserving cellular identity
- FFPE compatible (Gene Expression Flex)
- Perform Single Cell:
  - Gene expression
  - Protein expression
  - Functional genomicsby CRISPR
- Immune repertoire and corresponding antigens
- Epigenetics
- Multiomic analyses

**REAGENTS & CHIPS** 



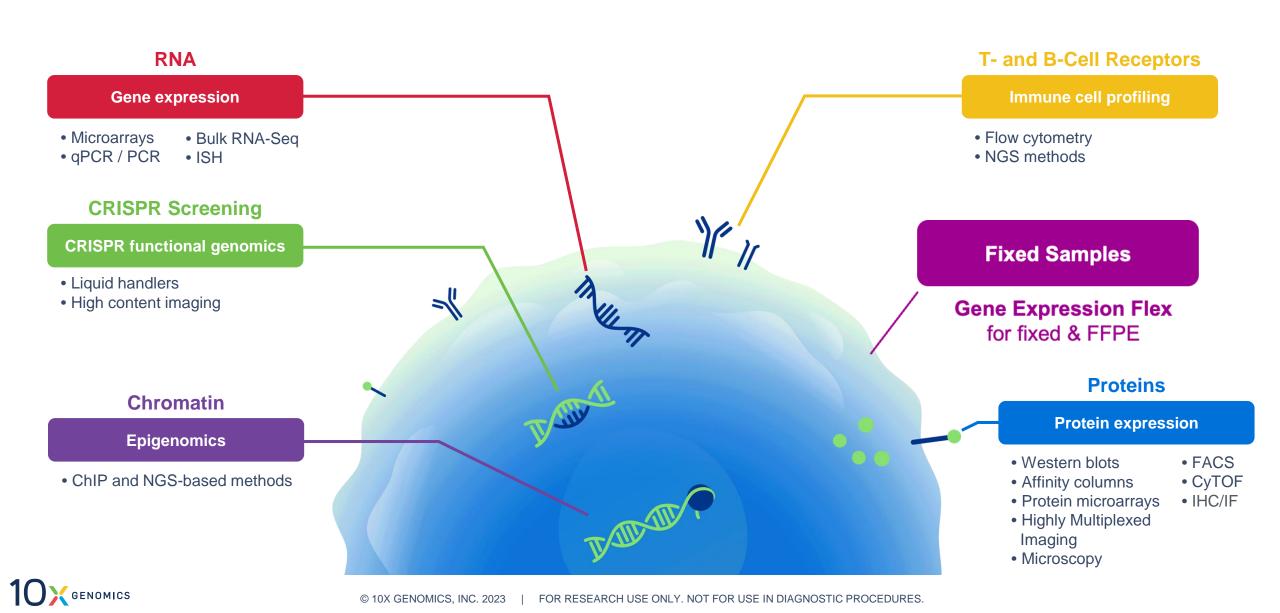
INSTRUMENTS



SOFTWARE



# **Chromium – Replacing the Legacy Toolkit Across Biology**



## **Chromium – Instrument**



## **Chromium X**

Next generation single cell system that runs all standard 80K cells/run assays and HT & Fixed assays with up to 1M cells/run



# Chromium Single Cell Gene Expression Flex

Remove the constraints of standard single cell workflows by profiling fixed RNA

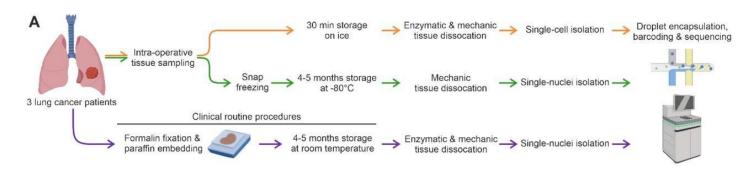


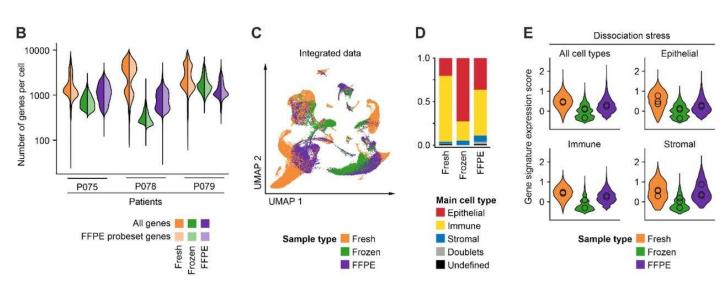
# Flex – Profiled Fresh & FFPE Lung Cancer Tissue

Trinks et al. bioRxiv, April 2023

## **Key Takeaways:**

- Fresh and FFPE samples generated high-quality single cell data
- Highly consistent behavior between samples
- Flex made single-cell profiling of fresh tissue easier
- Flex enabled single-cell profiling of FFPE tissues & reduced cost:
  - Retrospective analysis annotated with long-term clinical follow-up data
  - Easy integration of single cell and spatial data
  - Facilitates routine insights utilizing histological
     & molecular features





https://doi.org/10.1101/2023.04.25.538273



# Flex – Flexibility

Multiple sample types, scale, & options

#### **Broad sample compatibility**

#### In-line multiplexing

# Flexible sample and cell input number



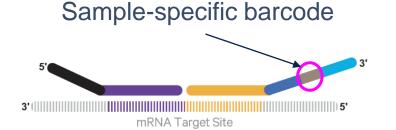
Fresh tissue Frozen tissue



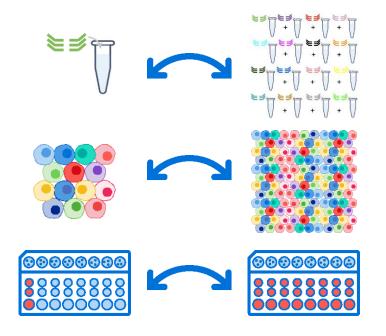
FFPE tissue



Cell suspensions
Nuclei suspensions



5-fold increase in scale over other multiplexing techniques



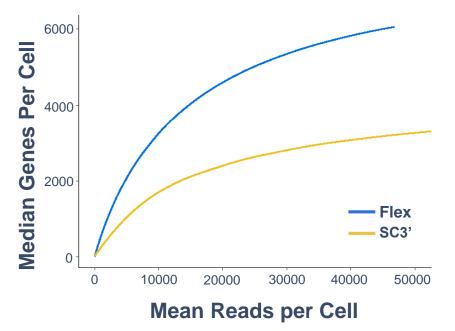
- Up to 128 samples per chip
  - i.e.,16 samples per lane with the 4 x 16 Multiplex Kit
  - Up to 1M cells per chip
    - i.e., 128K cells per lane with the 4 x 16 Multiplex Kit



# Flex - Industry Leading Single-Cell Sensitivity

High sensitivity at low read depth

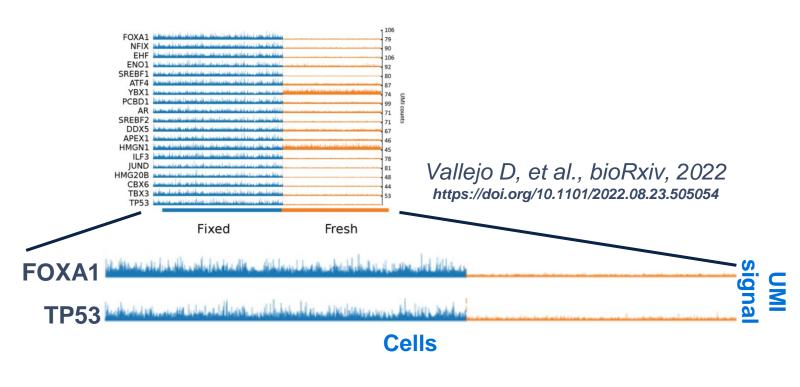
#### Ovarian cancer nuclei



Substantial reduction in sequencing cost

#### **Transcription factor detection**

#### Prostate cancer cell line (LNCaP)

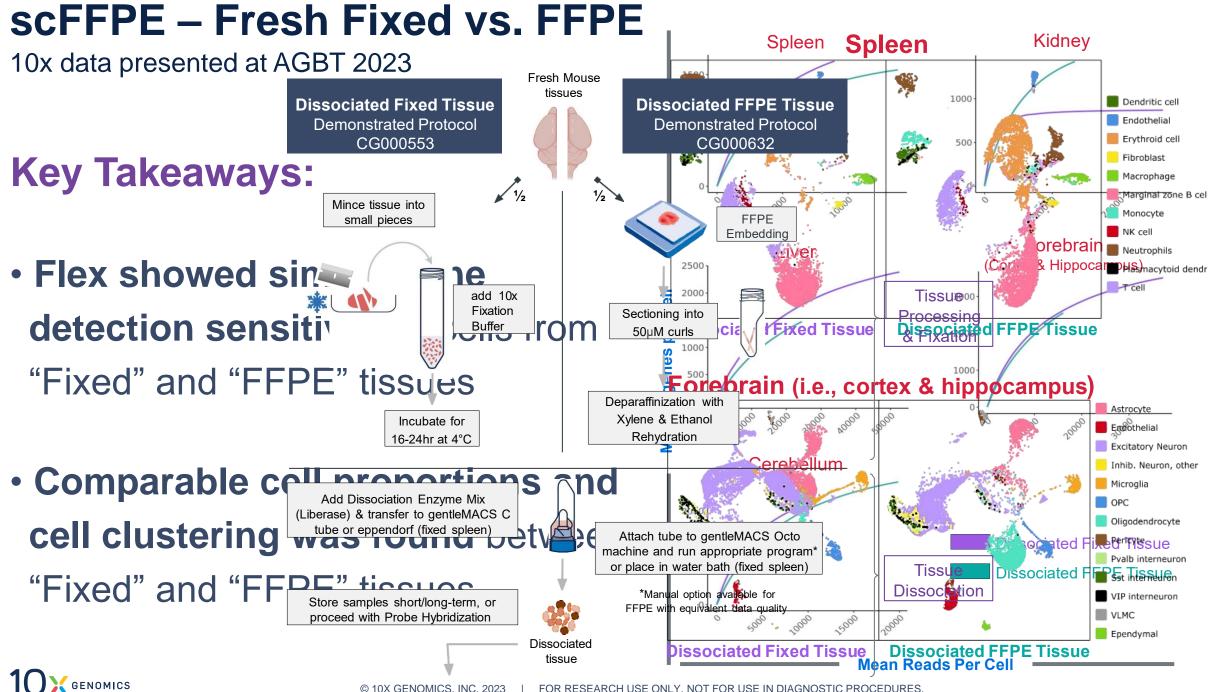




# Chromium Single Cell Gene Expression Flex Single Cell FFPE (scFFPE)

Utilizing the Flex assay to profile cells & nuclei from FFPE tissue

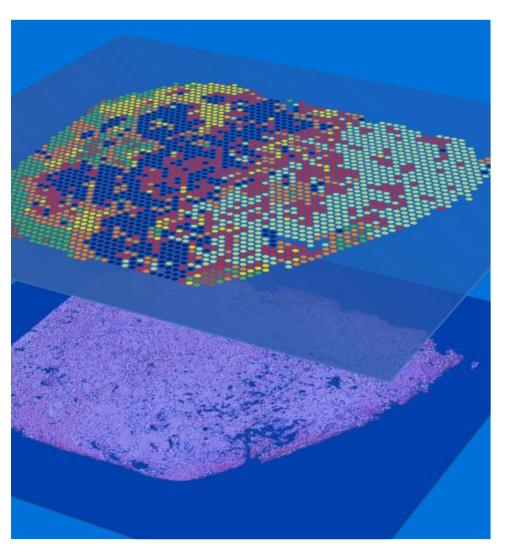




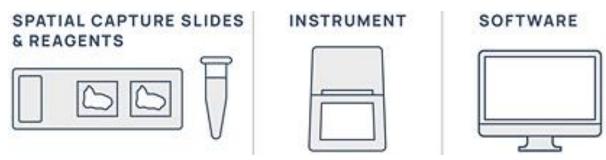


# Visium

#### **EXPLORE THE SPATIAL ARCHITECTURE OF GENE & PROTEIN EXPRESSION**



- Histological & whole transcriptomic information from an entire tissue section
- Slide contains arrayed barcoded oligos representing spatial locations
- Leverages NGS while preserving spatial location
- Unbiased gene & protein expression in tissue context
- Compatible with Fresh Frozen, Fixed Frozen, 4% PFA Fixed, & FFPE



# **Visium – How it Works**



# Visium – Use Case in Drug Discovery

Lyubetskaya et al., 2023, Cell Reports Methods

## **Key Takeaways:**

- Over 80K Visium FF & FFPE spatial spots were profiled across several human & mouse tissues
- Transcriptional data with spatial context identified unique features across several tumors
- Cell-type & depth features are distinctly resolved across different tumors
- Tumor-specific biomarkers were identified in patient pancreatic cancer FFPE tissue samples

System and modality	Goal	Conclusion
Rat colon	Determine resolution, fidelity, and reproducibility of ST using a normal, well structured tissue with our spatial validation framework	ST can reliably and with high resolution capture spatial information consistent with pathology annotation and known cell types
Syngeneic tumors	Assess the ability of ST to capture tumor and microenvironment heterogeneity in a reductionist model	ST reflects inter and intra- tumoral heterogeneity, including immune infiltration and depth-specific biology, in two mouse syngeneic oncology models
Single donor	Use a single donor to compare frozen and FFPE ST modalities to support viability of ST for FFPE clinical cohorts	The FFPE probes-based ST protocol was consistent with polyA-based frozen and FFPE protocols, with few notable exceptions due to non-specific probes
Clinical cohort  *	Determine the utility of ST for putative target discovery in a clinical cohort	Analysis of a small clinical cohort with our spatial validation framework showed consistency between ST and digital pathology and allowed for the identification of putative tumor biomarkers

https://doi.org/10.1016/j.crmeth.2023.100340



# **Visium Spatial**

# **Direct Placement Assays**

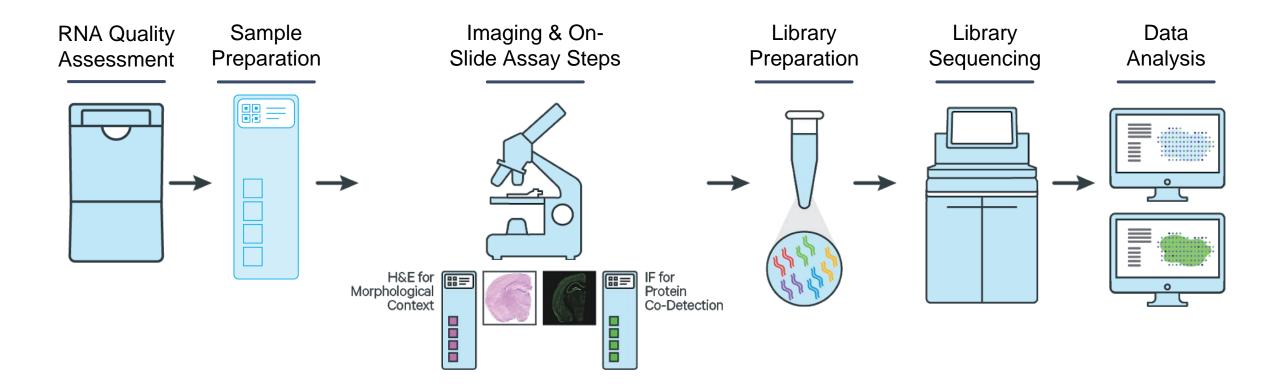
Keep the spatial context of gene expression without the need of an instrument

Fresh Frozen (RT-based) & FFPE (probe-based) v1



### Visium – Direct Placement Workflow

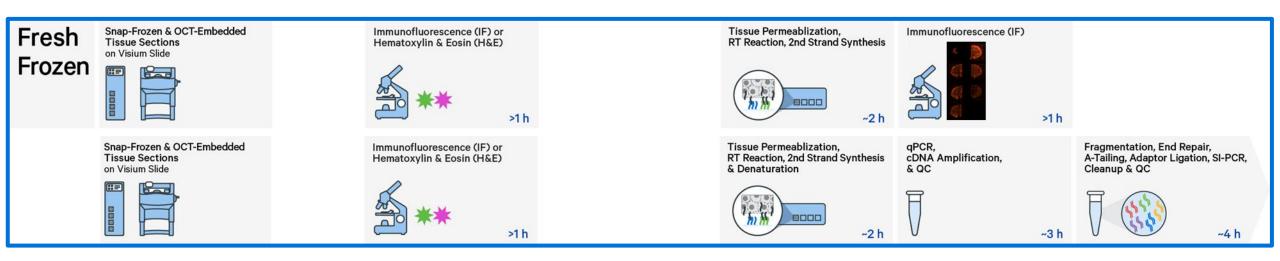
No instrument required - Fresh Frozen & FFPE v1 assays

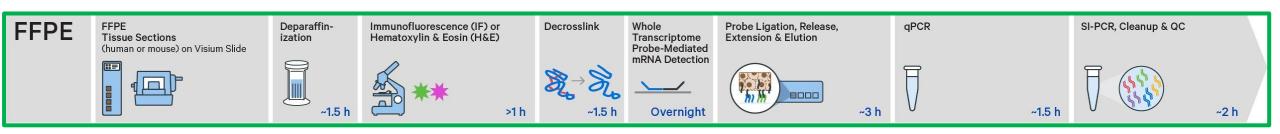




### **Visium – Direct Placement Workflows**

No instrument required - Fresh Frozen & FFPE v1 assays

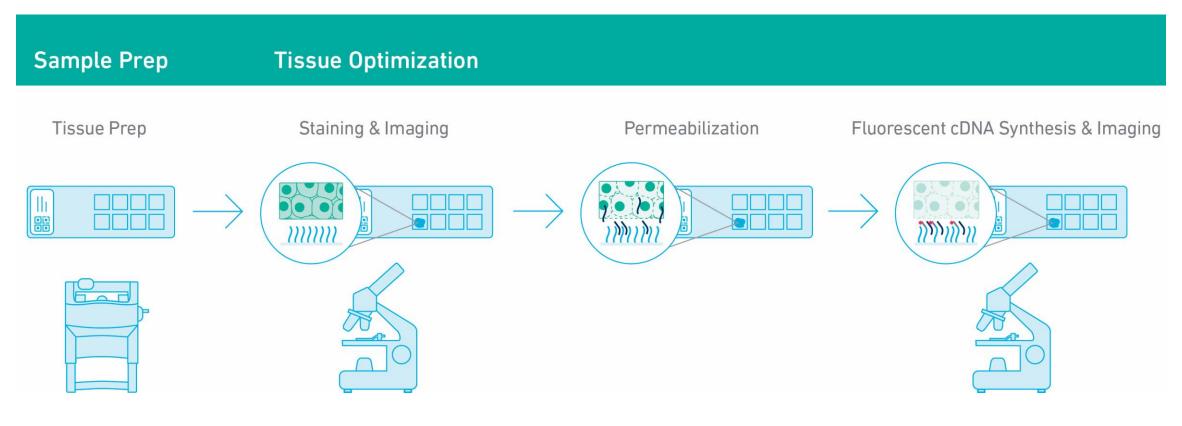






# Visium – Fresh Frozen - Tissue Optimization Workflow

Direct placement v1 assay



Section Tissue on to Visium Slide

- 1. Fix Tissue
- 1. Non-Barcoded mRNA Capture
- 1. Remove Tissue

- 2. H&E Stain
- 3. BF Image

2. Fluorescent Image



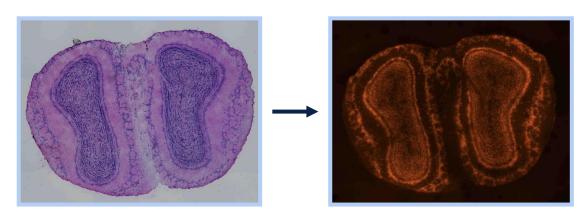
# Visium – Fresh Frozen - Tissue Optimization Workflow

Direct placement v1 assay

Use optimal permeabilization time for Gene Expression Experiment

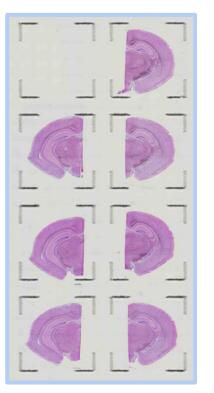
#### **Purpose**

 To determine the optimal permeabilization time, ensure tissue compatibility, and become familiar with the workflow

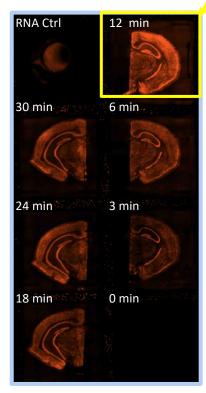


 Recommend testing serial sections of a single tissue type and section thickness per Tissue Optimization slide before proceeding to the Gene Expression experiment

#### **Brightfield image**



#### Fluorescent image



Mouse brain

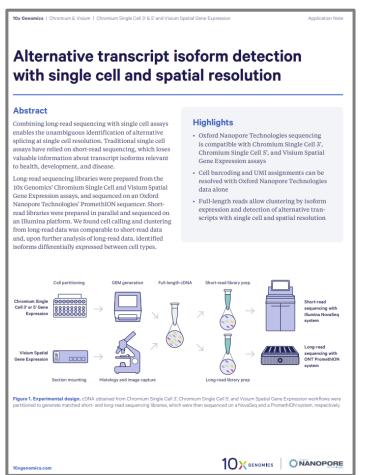


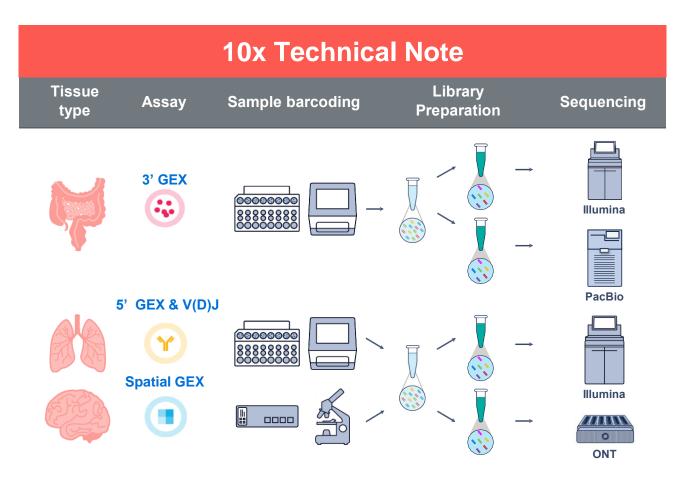
### Visium – Fresh Frozen - Isoform Detection

Enabling new insights via isoform-level alternative splicing for the 10x RT-based 3', 5', & Visium FF assays







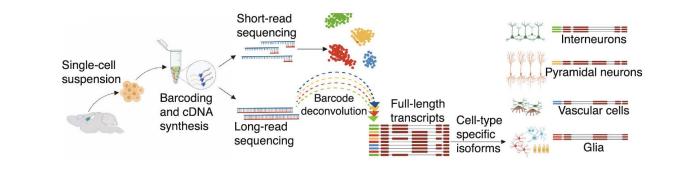


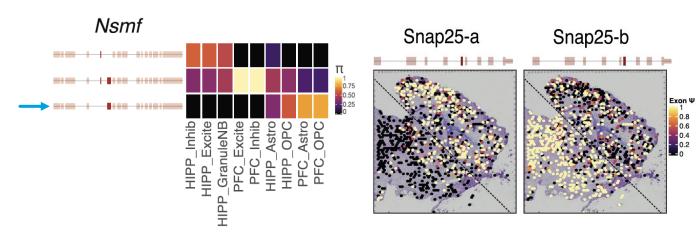
### Visium – Fresh Frozen - Isoform Detection

Joglekar et al., Nat Comm, 2021

## **Key Takeaways:**

- 10x Genomics Single Cell and Visium
   Spatial Gene Expression solutions were combined with long-read sequencing to investigate the differential isoform expression (DIE) between brain regions
- Adding spatial analysis anchored singlecell DIE observations and localized mRNA splicing
- Spatial transcriptomics combined with longread sequencing yields a spatially resolved splicing map, bringing closer a full isoform map of the brain





doi.org/10.1038/s41467-020-20343-5



# **Visium Spatial**

# **CytAssist Enabled Assays**

Simplifies Visium prep/workflow & broadens sample access

FFPE/probe-based v2



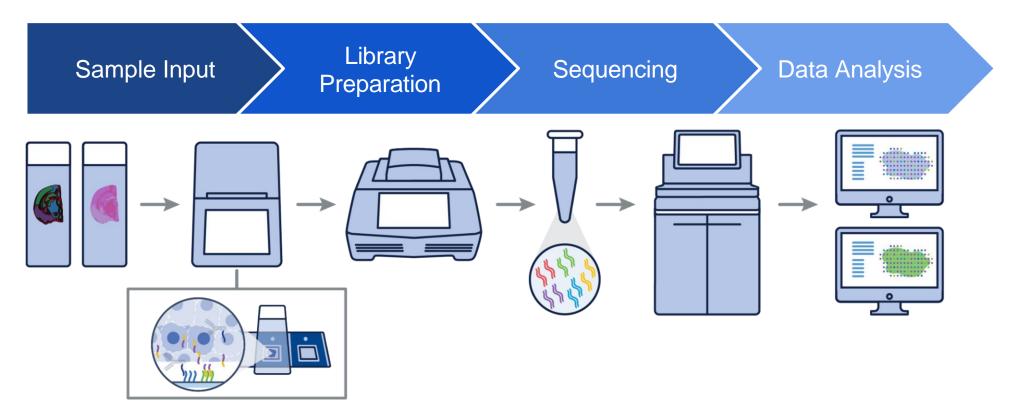
# Visium – CytAssist Simplifies Prep & Broadens Access

Precision analyte capture and spatial barcoding





# **Visium CytAssist – Workflow**



#### **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



# **Visium CytAssist – Workflow**

Seamlessly integrate with standard histology FFPE & FF sample preparation

### Two tissue slides Two tissue images Stained sections on Spatial orientation for standard glass slides sequencing data BARCODES Visium Slide **Visium Slide** Two capture areas Target probes now captured on slide Visium Slide Transcriptomic analytes



bind to the Visium slide

# Visium CytAssist – Rescues Challenging Sample Type

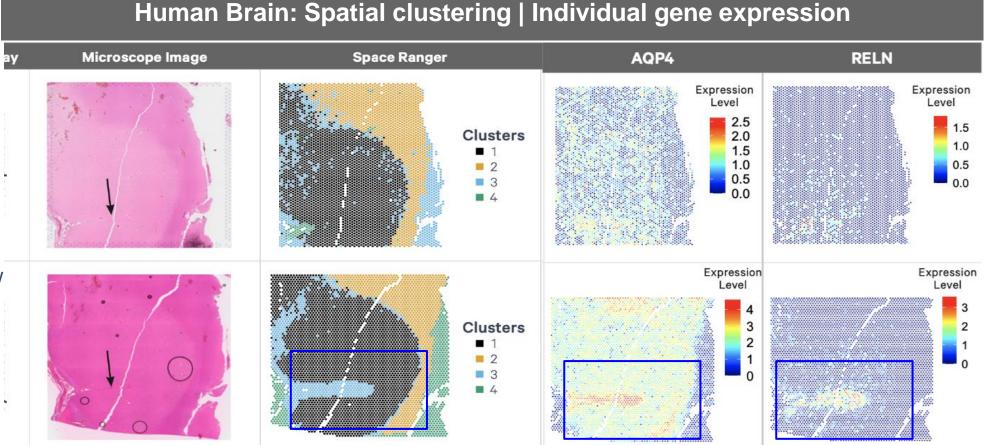
Human Brain | Spatial Clustering | Individual Gene Expression

#### **Spatial Clustering**

 Boxed region highlights layers of gene clusters forming around a capillary

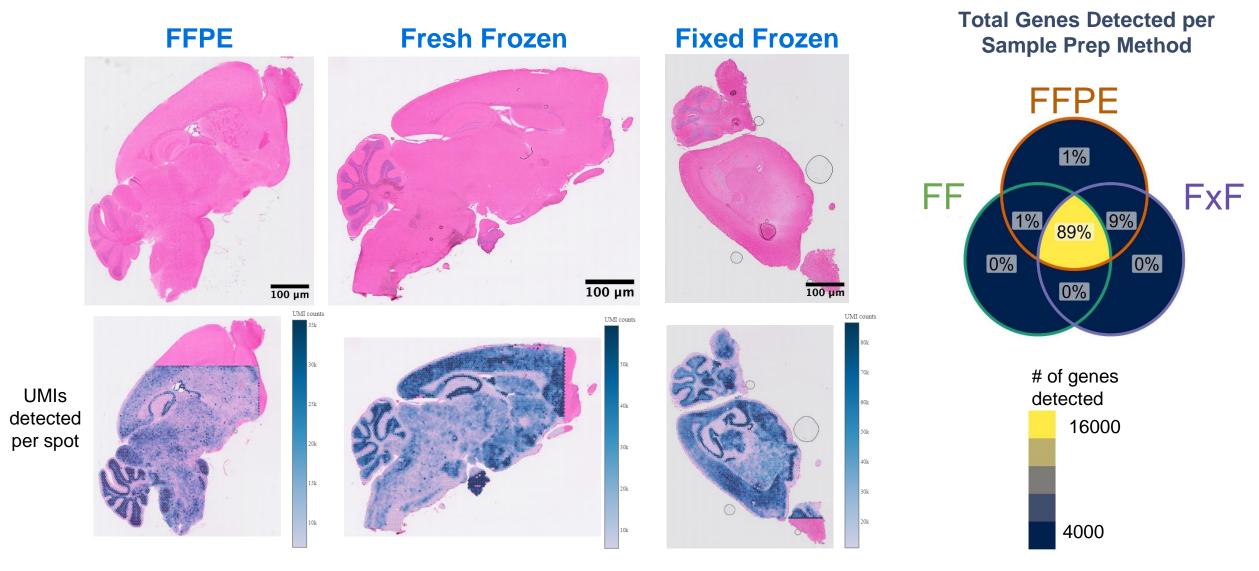
# Individual Gene Expression

- Gene maps from DP show less distinction between signal and background
- AQP4 and RELN regulates the blood- brain barrier, seen in the cluster near the capillary





# **Visium CytAssist – Robust Performance Across Samples**





# Visium Spatial – CytAssist Enabled Gene and Protein Expression

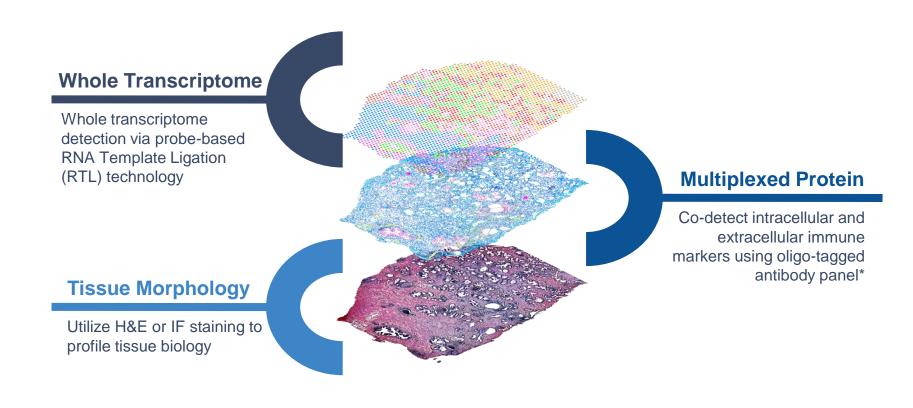
Broaden data collection from the same tissue section

FFPE/probe-based v2



### Visium CytAssist – Spatial Gene and Protein Overview

Probes and oligo-tagged antibodies enable RNA and protein co-detection



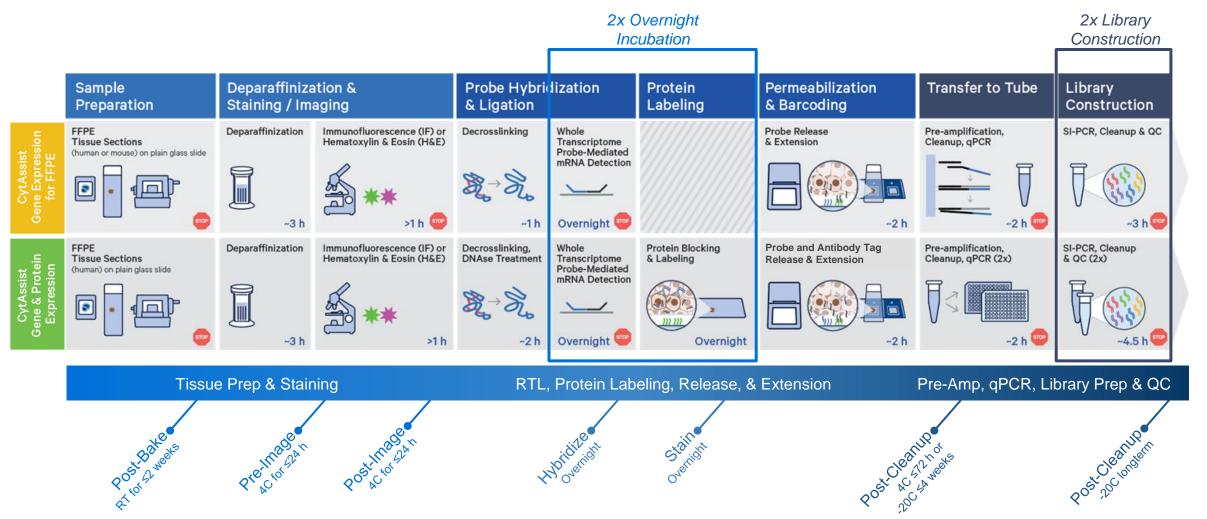
\*Additional targets may be detected by spiking in oligo-tagged antibodies



### **Visium CytAssist – Workflow**

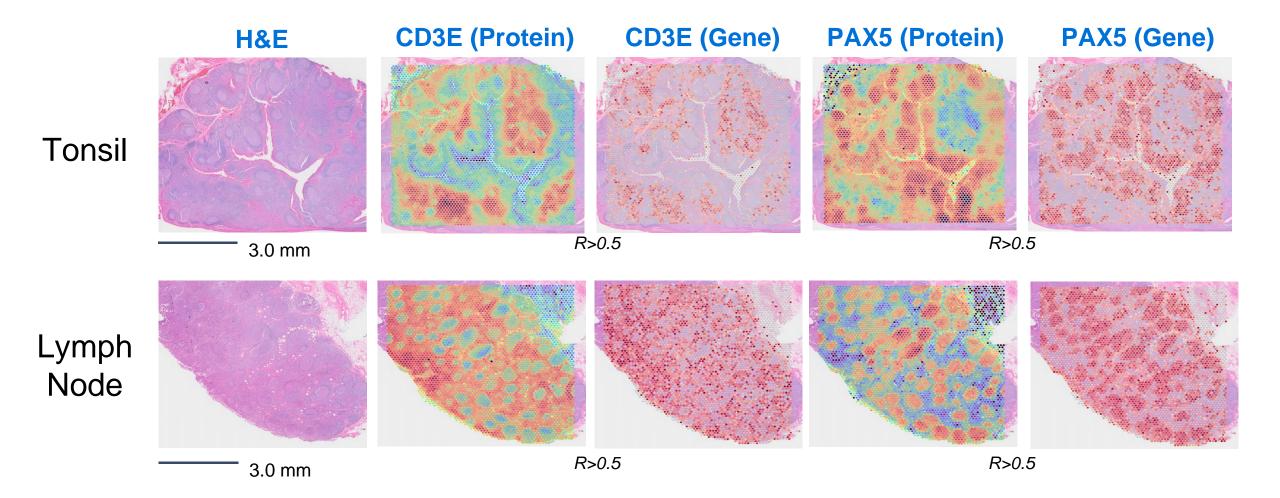
Spatial Gene and Protein Expression

### Addition of an overnight step and ~3 hours of workflow





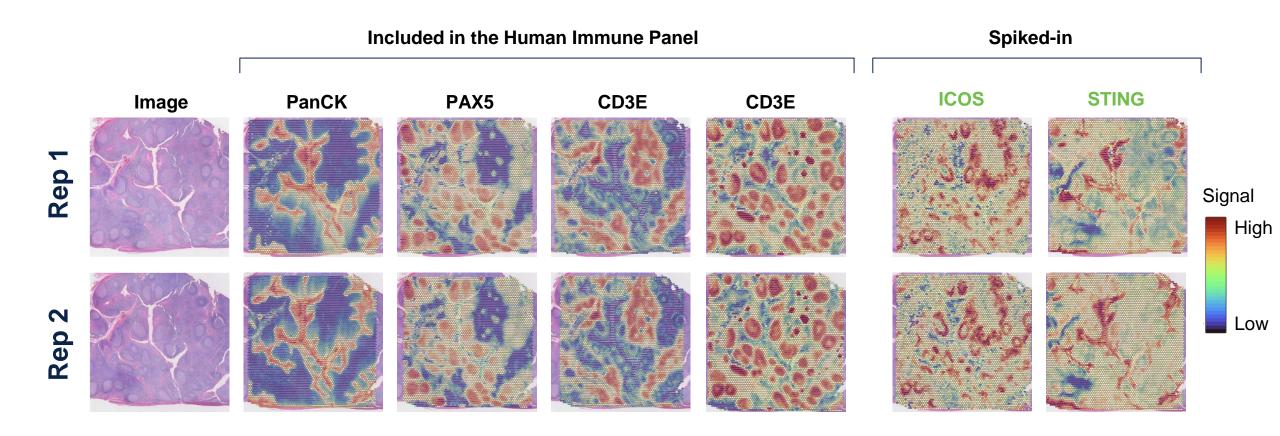
### Visium CytAssist – Gene & Protein on a Single Tissue Section





### Visium CytAssist – Antibody Spike-In Data

**Human Tonsil** 





### Visium Spatial – CytAssist Enabled

Roadmap: Visium HD

Enabling NGS-based whole-transcriptome at 2 µm resolution

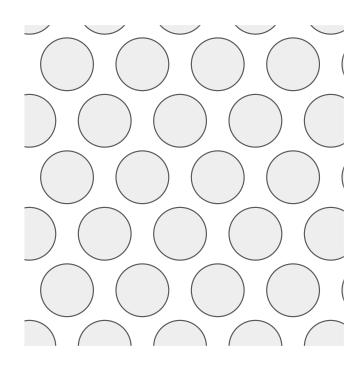
FFPE/probe-based v2



### **Visium CytAssist – Roadmap**

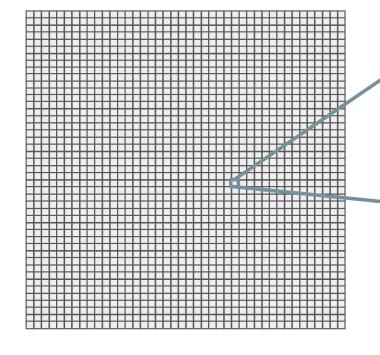
Visium HD

### **Visium**



55 μm spots, hexagonally arranged

### **Visium HD**



8 µm squares, no gaps

2 μm squares no gaps

Actual feature size

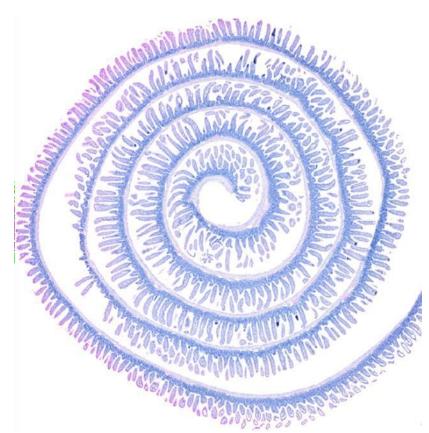


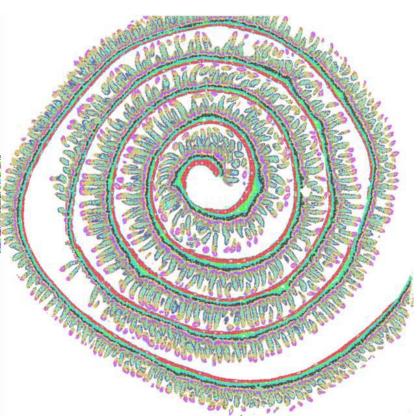
### **CytAssist – Higher Resolution: Visium HD**

FFPE mouse intestine

### **H&E** staining



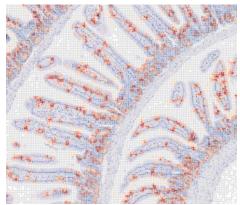




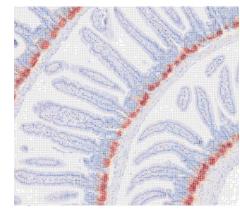
R&D consumables, reagents, workflows, software being used to generate and process the data

### Marker gene expression

Muc2 (goblet cell marker)

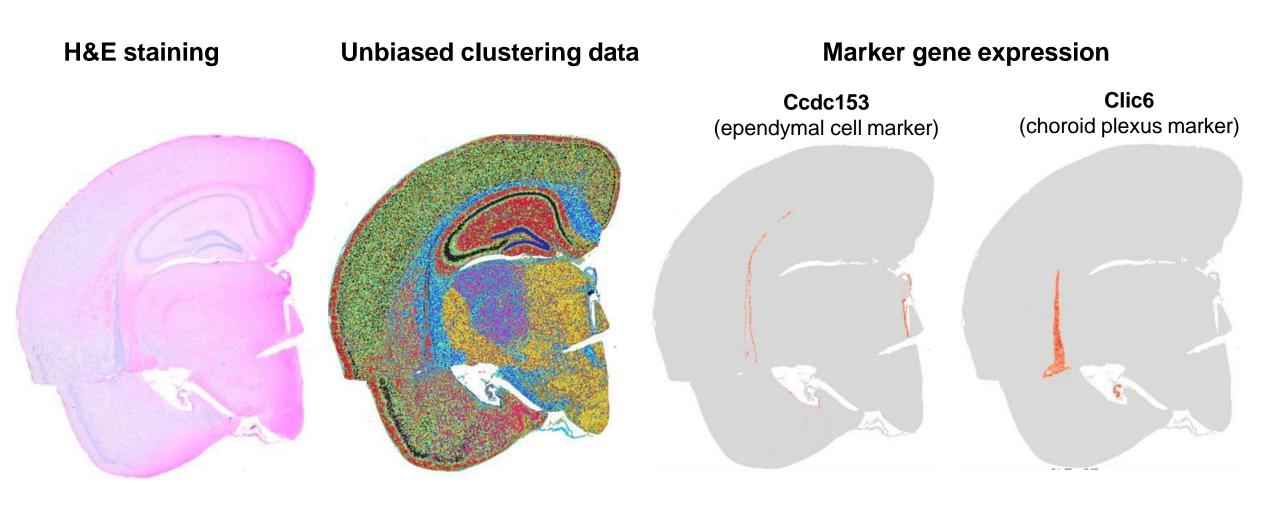


Lyz1 (paneth cell marker)



### **CytAssist – Higher Resolution: Visium HD**

FFPE mouse brain



R&D consumables, reagents, workflows, software being used to generate and process the data



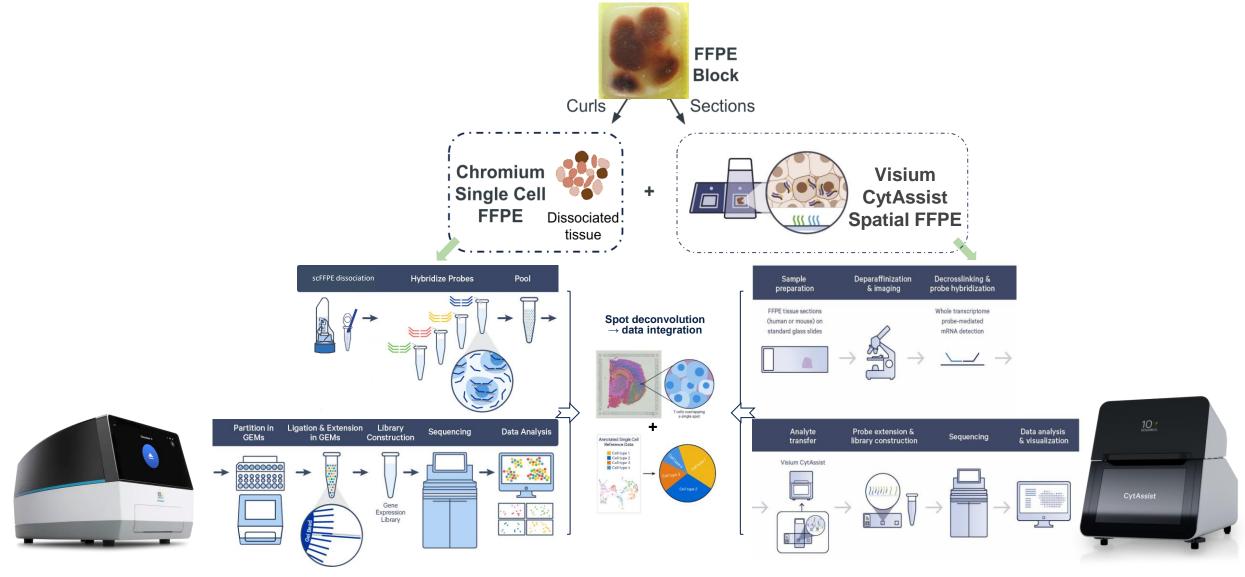
### **Chromium Single Cell & Visium Spatial**

## **Data Integration**

Uniting data from Chromium Flex's scFFPE with Visium's FFPE on CytAssist

### Data Integration – Flex and CytAssist from same FFPE Tissue

Example using FFPE human prostate cancer tissue





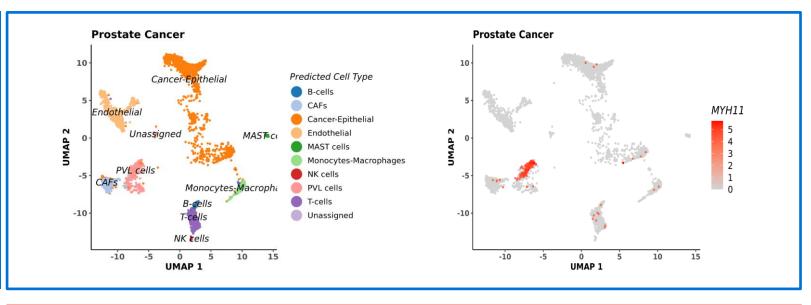
### Data Integration – Flex and CytAssist from same FFPE Tissue

Example using FFPE human prostate cancer tissue

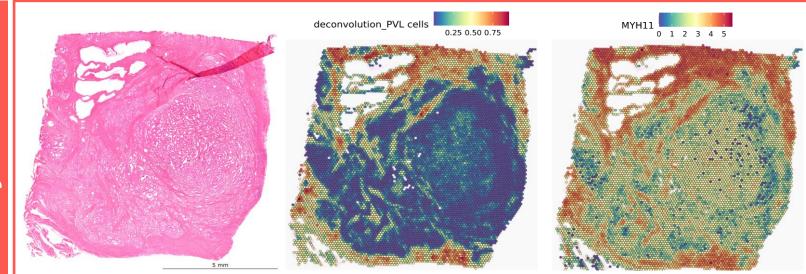
### **Key Takeaways:**

- Combine single-cell resolution with spatial context
- Uncover new biological insights
- Enabled by shared probe architecture





CytAssist





# Chromium Flex & Visium CytAssist Probe Customization

Unlock limitless possibilities via cross-platform shared chemistry

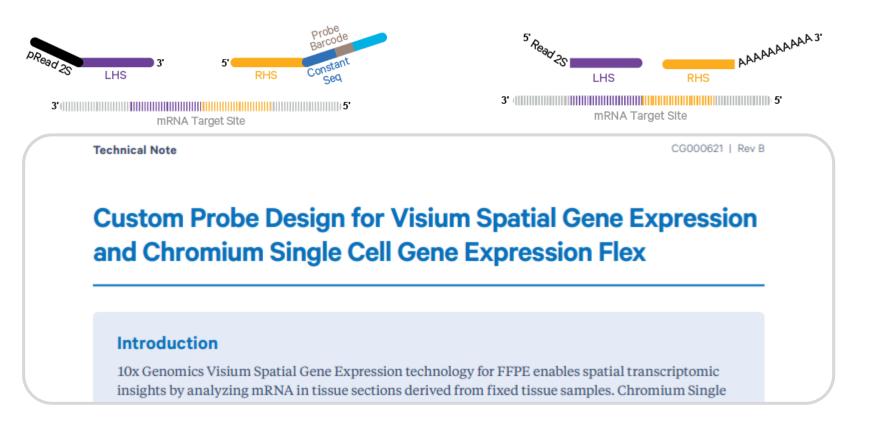
### 10x Probe-Based Chemistries

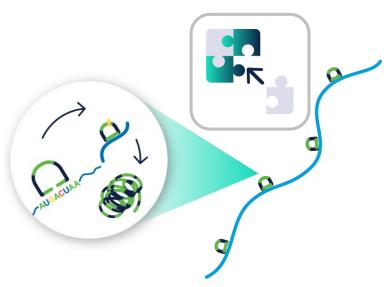
Provide highly specific & customizable workflows

# **Chromium Gene Expression Flex**

### **Visium for FFPE**

### Xenium

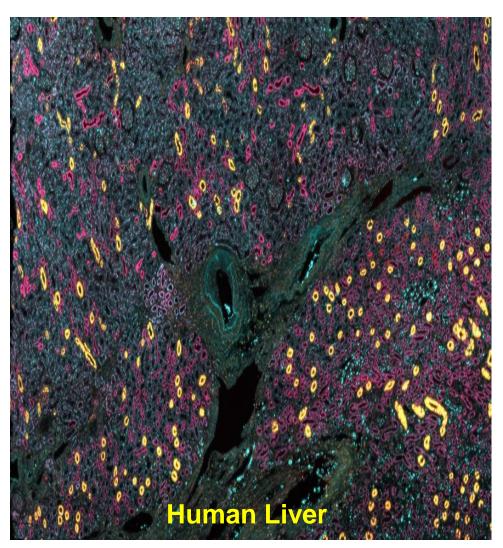




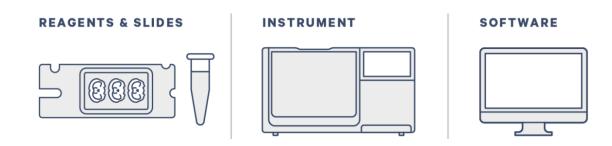


## Xenium

### UNCOVER CELL-CELL INTERACTIONS DRIVING FUNCTION

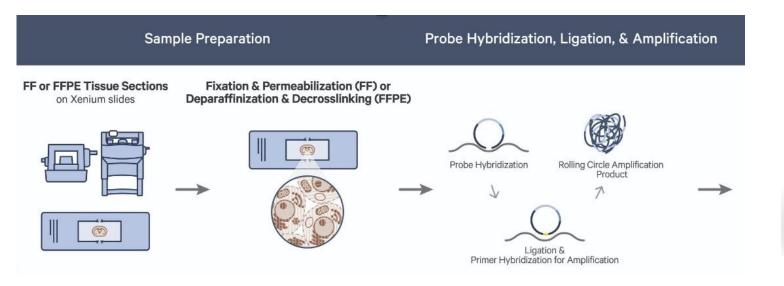


- Reveal the relationship between cellular structure and function with <u>single base-pair resolution</u>
- End-to-end in situ platform with imaging readout (no NGS required)
- Hundreds of RNA targets with subcellular resolution
- Highly specific and sensitive
- High throughput, fast time to results
- Compatible with Fresh Frozen and FFPE



### **Xenium – A Simple Workflow with 3-4hrs of Hands-On Time**

From sections to instrument start in 2-3 days



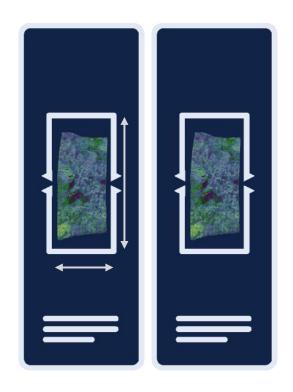


Simple and robust benchtop workflow with only ~4 hours hands on time

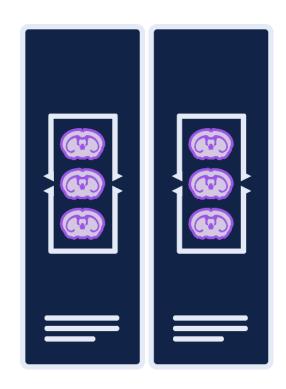
Fully automated decoding and end-to-end analysis



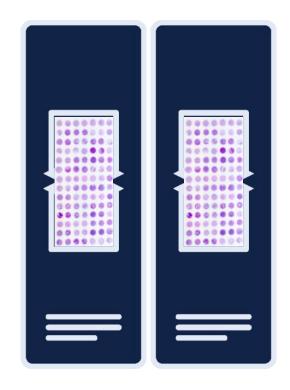
### Xenium – Leading Analyzable Area for Maximum Flexibility



**2 sections** 10.5 x 22.5mm



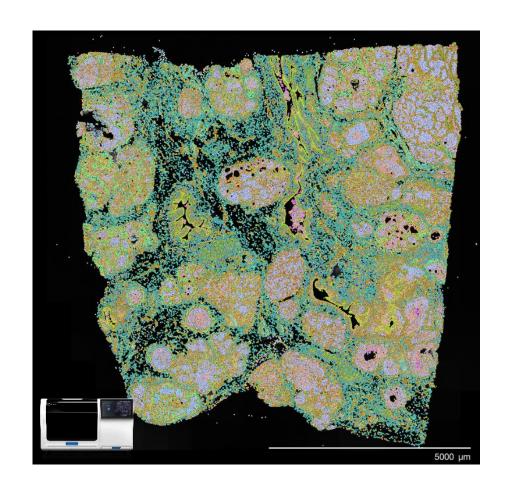
6 mouse brains



196 biopsies



### Xenium – Post Run: Whole-Transcriptome - Visium Cytassist



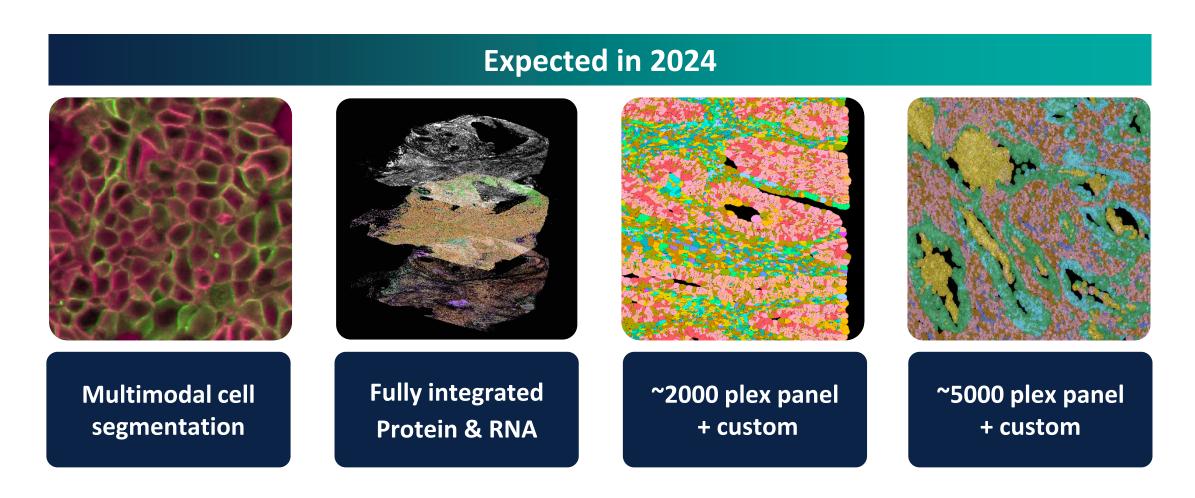


**Human FFPE breast infiltrative ductal carcinoma** 



### Xenium – Roadmap

Broader cell segmentation, protein multiomics, and increased RNA plex



<sup>\*</sup>Final product release timelines and configurations subject to change



### Xenium – Rapid Adoption in the World's Leading Labs

# 10x Genomics Surpasses 100 Xenium Analyzer Shipments

PR Newswire - Tue Aug 29, 3:30PM CDT Partnership Content

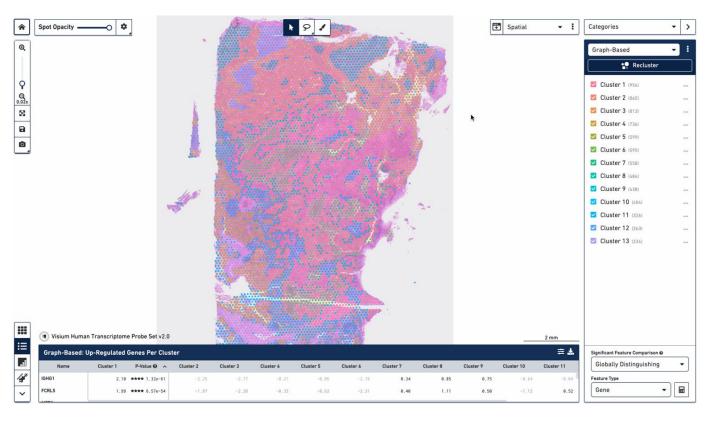
Rapid growth and adoption of Xenium Analyzer demonstrates strength of company's innovation engine, broad commercial reach and operational scale





## Conclusion

### **Explore Visium datasets with Loupe Browser 7.0 Today**



- Explore clustering, visualize genes of interest, annotate tissue regions, and calculate differential expression without any coding!
- Download Loupe Browser free and explore public datasets, including TMAs and variety of mouse and human tissues
- Redesigned Version 7.0 now available!



### Visium CytAssist: Ready to Explore More

Simplifying spatial sample preparation and broadening sample access



### Your Sample, Your Spatial Story

- Spatial Gene Expression for FFPE
- Spatial Gene & Protein Expression for FFPE
- Spatial Gene Expression for Fresh Frozen
- Spatial Gene Expression for Fixed Frozen
- Spatial Gene Expression for Tissue Microarrays
- Spot Deconvolution
- Validation of Xylene Alternatives

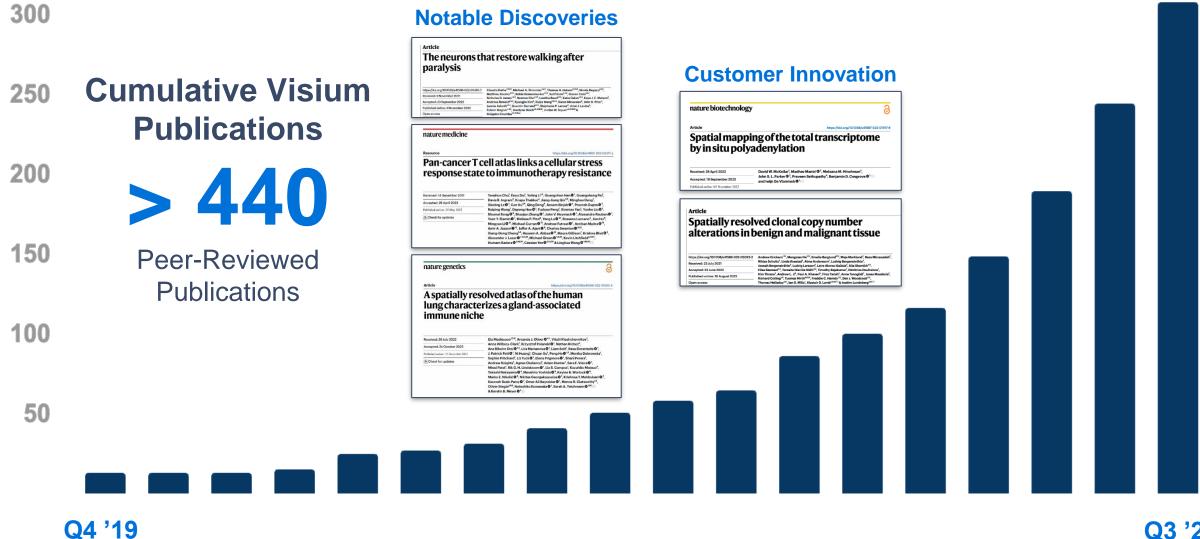
Sample Prep Recommendation for Skin & Bone

Visium HD Spatial Gene Expression



Soon

### Visium Is Powering Impactful Research





### **Biology's Most Comprehensive Toolkit**





# Thank you Want More Information?

Please Request a Follow-Up Meeting!



**Chromium Gene Expression Flex** 

Visium CytAssist

Xenium In Situ

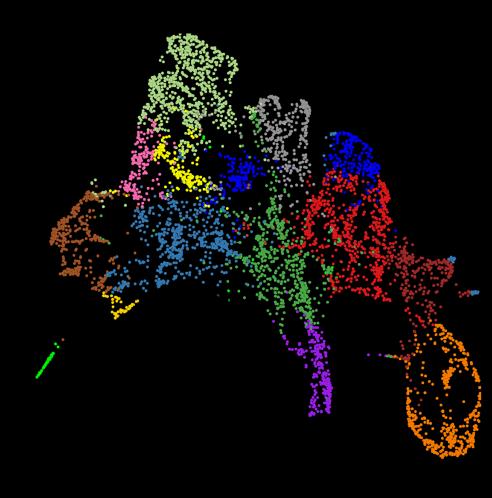


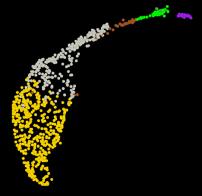
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# Visium Spatial





## **Appendix**

### scFFPE – Tested Tissues

Manual protocol - liberase + pestle dissociation

Tested Tissue	Age of Block	Amount of Tissue	Cells After Dissociation	Median UMIs per Cell (10k rps)	Median Genes per Cell (10k rps)
Human Healthy Lung	0 уо	50 um	~500k	~1500	~1000
Human Lung Cancer	0 уо	50 um	~1m	~1700	~1100
Human Breast Cancer	2 yo   10 yo	50 um	~2.3m   ~1.7m	~1000   ~1200	~700   ~1000
Human Alzheimer's Brain	3 уо	50 um	~350k	~1500	~1100
Human Glioblastoma	1 yo	50 um	~750k	~2000	~1400
Human Reactive Lymph Node	6 yo	50 um	~6.5m	~1100	~700
Human Healthy Lymph Node	4 yo	50 um	~2.3m	~1200 (5k rps)	~700 (5k rps)
Human Pancreas	3 уо	50um	~500k	~1500	~900
Human Healthy Liver	5 yo	50um	~350k	~1200 (5k rps)	~700 (5k rps)
Human Liver Cancer	4 уо	50um	~550k	~1500	~600
Human Ovarian Cancer	2 yo	50um	~1.5m	~1700	~1200
Human Skin Melanoma	5 уо	50um	~200k	~2500 (5k rps)	~1700 (5k rps)
Human Prostate Cancer	3 уо	50um	~700k	~1100	~750
Human Colorectal Cancer	2 yo	50um	~550k	~1500	~1000
Human Healthy Kidney	3 уо	50 um	~500k	~2000	~1300
Human Endocervical Cancer	3 уо	50 um	~1.1m	~1000	~700



### Visium CytAssist – Human Immune Cell Profiling Panel

Composition – 35 antibodies sourced from Abcam and BioLegend

Target	Cell Type or Marker	Clone ID	Vendor
alphaSMA	Structural marker	EPR5368	
CD3E	T cells	SP162	
CD4	T cells	EPR6855	
CD11b	Myeloid cells	EP1345Y	
CD11c	Dendritic cells	EP1347Y	
CD14	Myeloid cells	EPR3652	
CD16	Granulocytes/NK cells	EPR16784	
CD20	B cells	EP459Y	Abcam
CD27	Functional B cells	EPR8569	
CD31	Endothelial cells	EPR3094	
CD40	T and B lymphocytes	EPR20735	
CCR7/CD197	Functional B cells	EPR23192-57	
CXCR5	T cells	EPR23463-30	
EPCAM	Epithelial cells	EPR20532-222	
PDL1	Checkpoint marker	73-10	

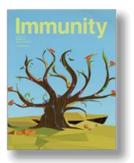
Target	Cell Type or Marker	Clone ID	Vendor	
BCL2	Apoptotic marker	100		
CD8A	T cells	C8/144B		
CD19	B cells	R109		
CD21	Dendritic and B cells	Bu32		
CD45RA	Functional T cells	HI100		
CD45RO	Functional T cells	UCHL1		
CD66b	Neutrophils	6/40c		
CD68	Macrophages	BL13756	Dialogond	
CD163	Macrophage marker	QA19A16	BioLegend	
CD138	Plasma cells	DL-101		
HLA-DR	Myeloid cells	LN3		
PanCK	Structural marker	AE-1/AE-3		
PAX5	B cells	1H9		
PCNA	Cell proliferation marker	PC10		
PD1	Checkpoint marker NAT1 ntin Mesenchymal cells 091D			
Vimentin				
IgG2a		MG2a-53		
lgG1 k	Isotype controls	MOPC-21	BioLegend	
IgG2b k	isotype controls	MPC-11	BioLegend	
lgG2a		RTK2758		

View Abcam or BioLegend website for IHC images and other details about antibody clones in Human Immune Cell Profiling Panel

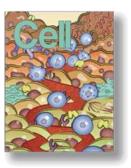


### 10x products are empowering impactful science

Cumulative count as of the 3<sup>rd</sup> quarter of 2023





















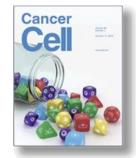










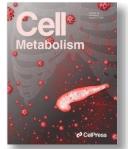












5,500+