10x GENOMICS

Discovering Spatial Biology at “10x” Resolution

Northwestern University – February 15th, 2024

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Three complementary platforms

A comprehensive toolkit to address the complexity of biology

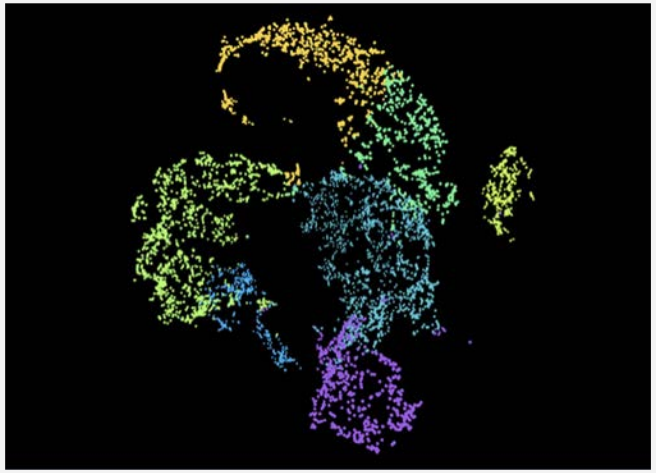
Hypothesis Generating

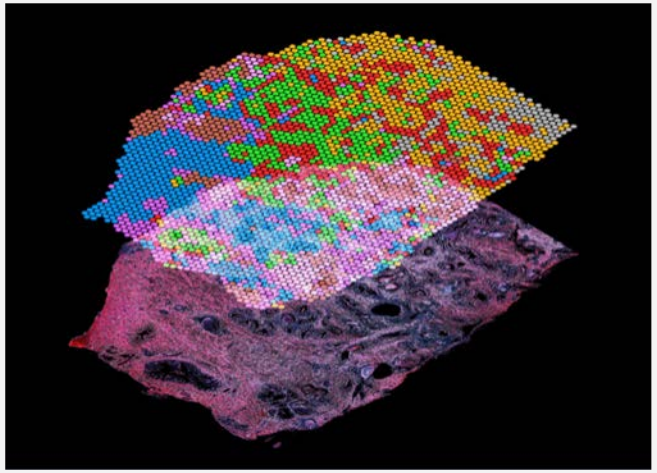
Hypothesis Testing

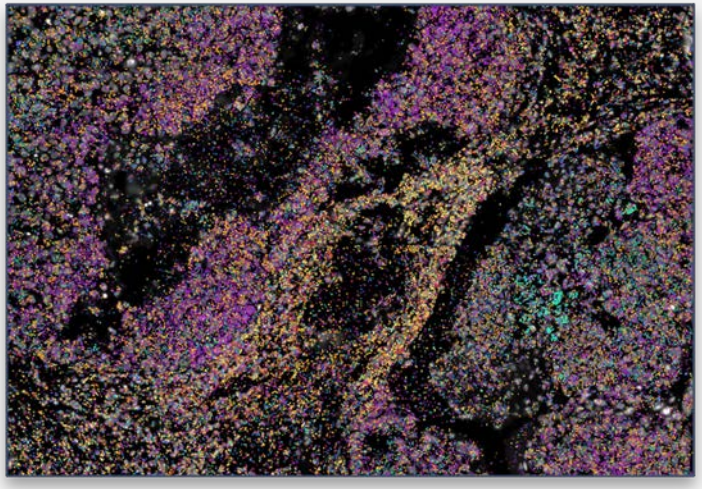
Chromium Single Cell

Visium Spatial

Xenium In Situ







NGS

Integrated Microscopy

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10x Products are Empowering Impactful Science

7,100+

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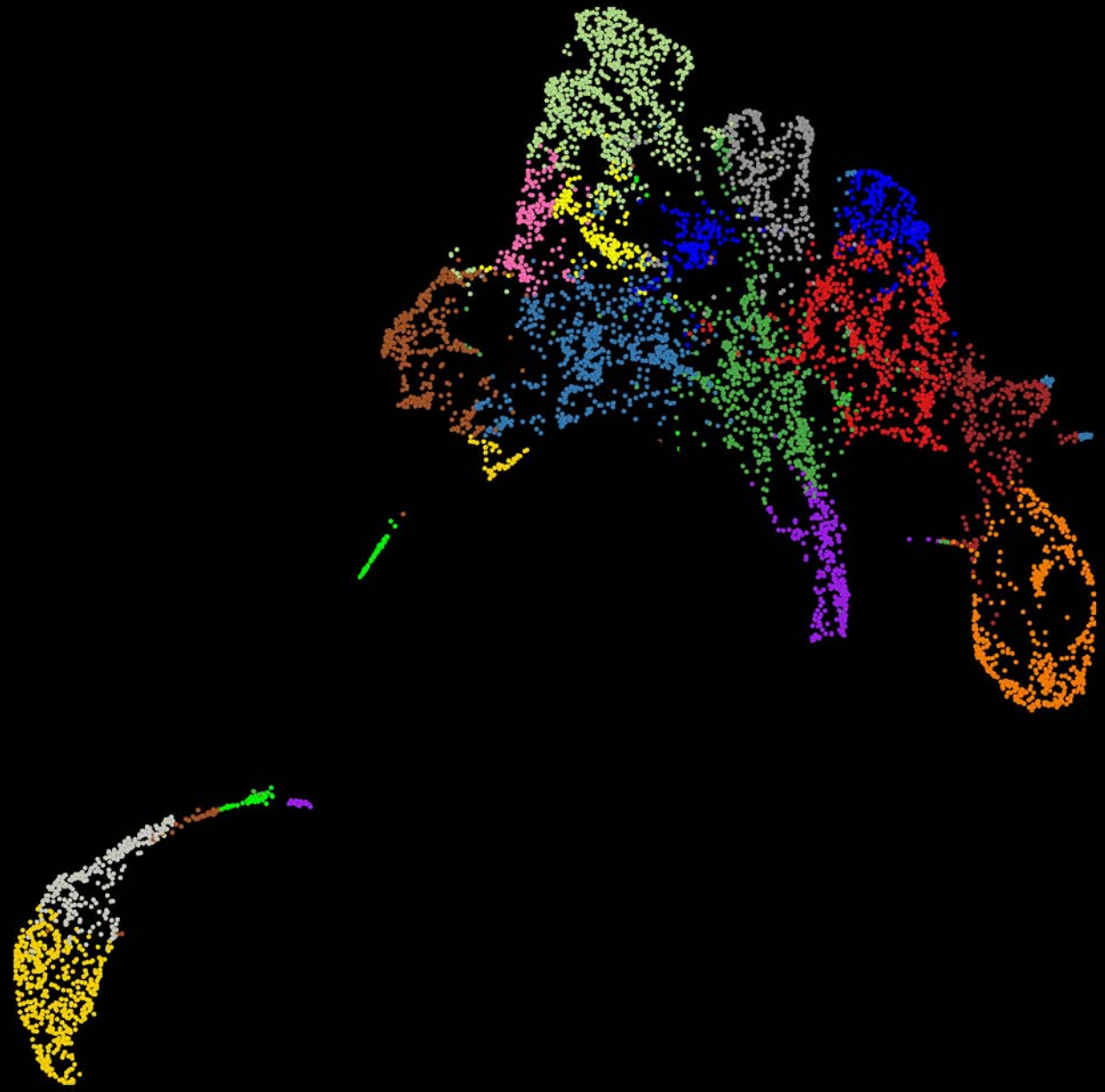
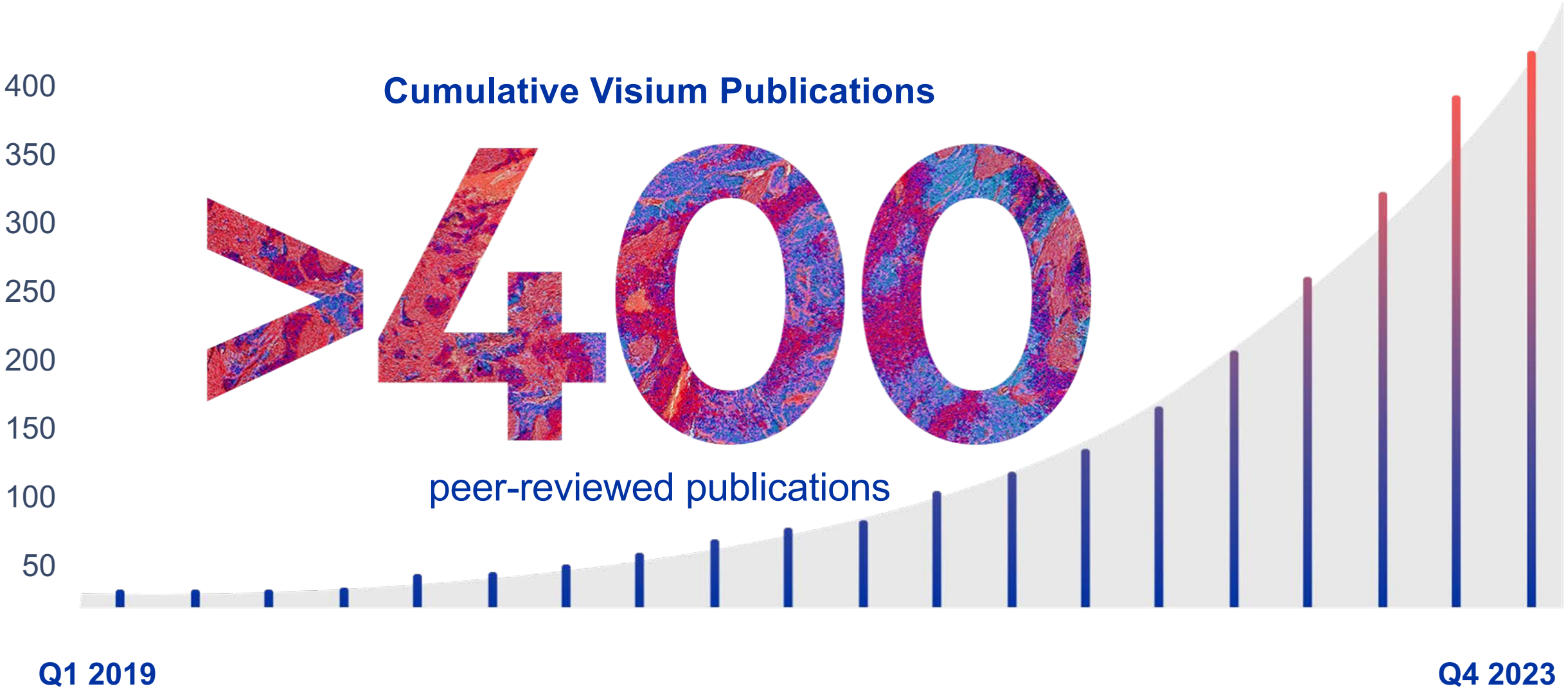
Visium Spatial



created with https://gganimate.com/

Visium Has Powered Impactful Research



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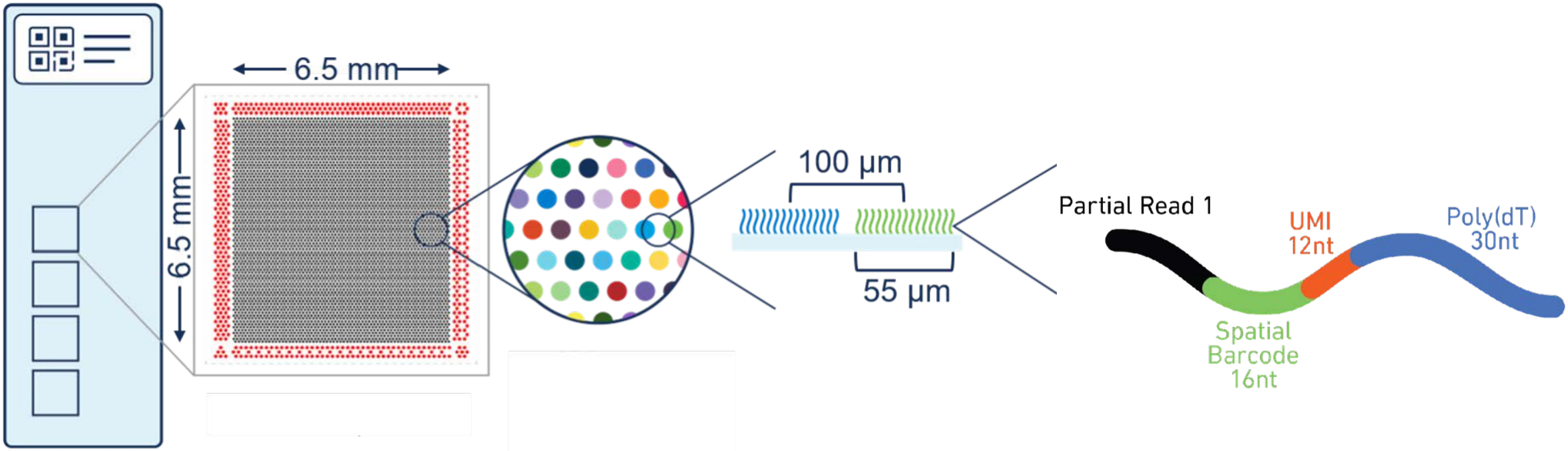
Spatial capture technology for whole transcriptome analysis

Visium Spatial Gene Expression Slide

Capture Area with ~5000 Barcoded Spots

Visium Gene Expression Barcoded Spots



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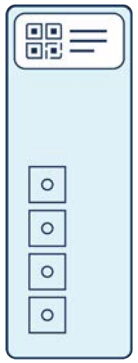
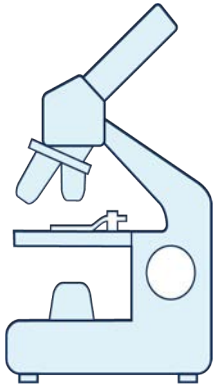
Visium Spatial Gene Expression

Tissue Prep

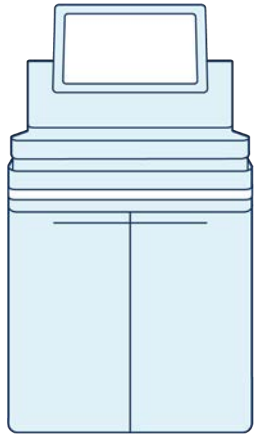


Imaging

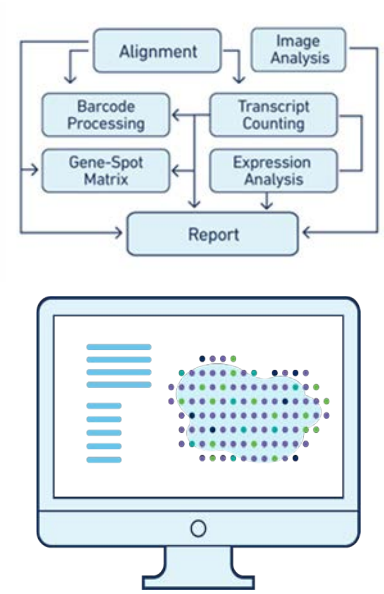


Barcoding & Library Construction

Sequencing



Data Analysis & Visualization



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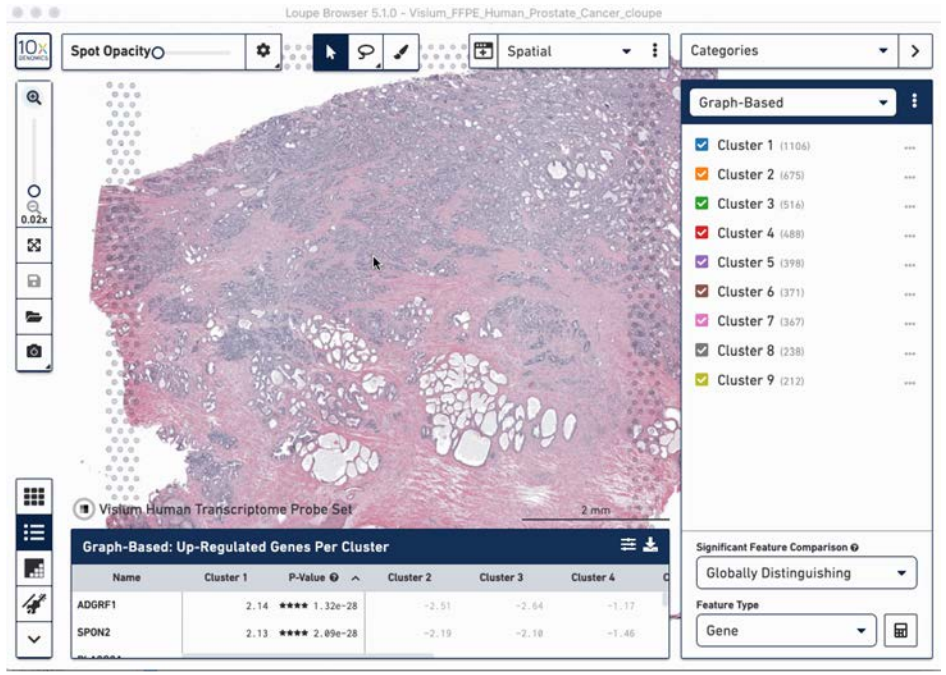
Visium Spatial Gene Expression

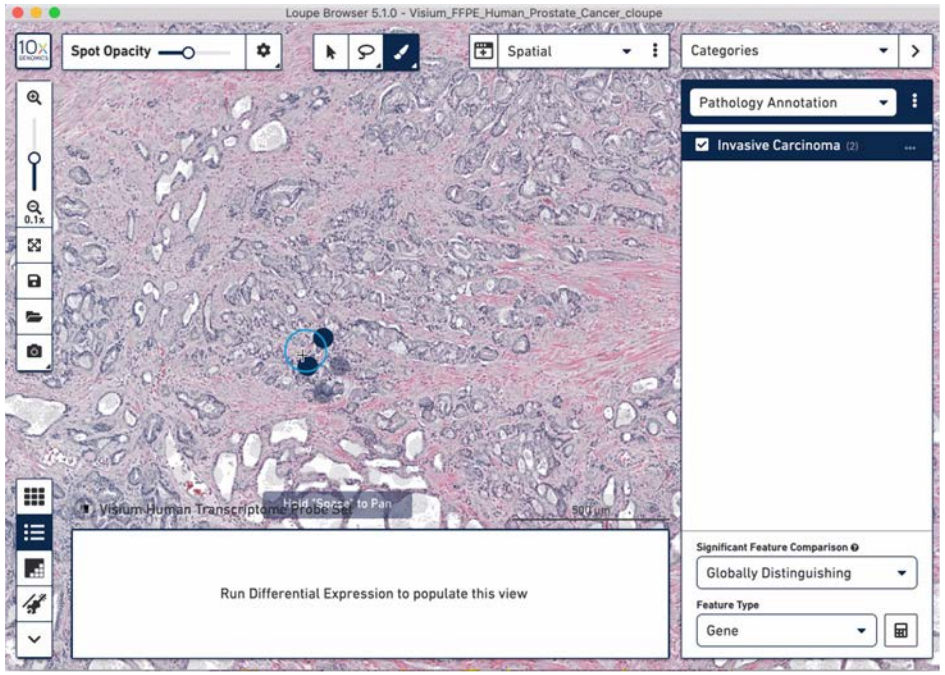
Streamlined Data Analysis

Easy-to-use analysis software that combines histological and gene expression data

Data Exploration in Loupe Browser



Flexible Spot Selection for Region Annotation



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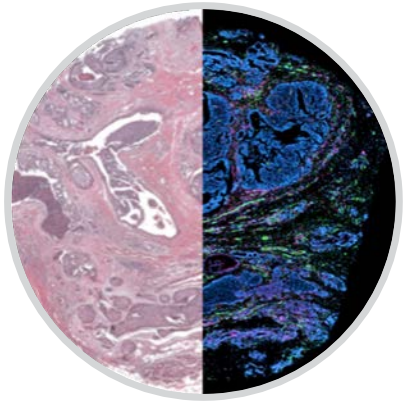
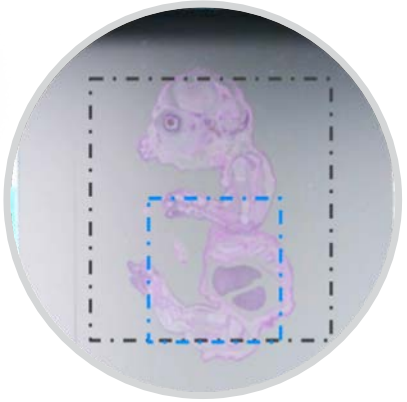
Get MORE from your samples

Expanded sample formats

H&E or IF with gene expression



Choice of best tissue section



Flexible tissue size

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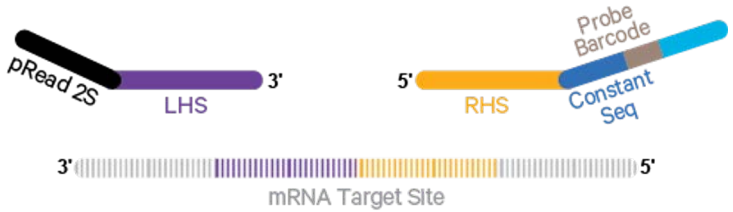
10x Probe-Based Chemistries Provide Highly Specific and Customizable Workflows

Chromium Gene Expression Flex



Visium for FFPE

Xenium

Technical Note

CG000621 | Rev A

Custom Probe Design for Visium Spatial Gene Expression and Chromium Single Cell Gene Expression Flex

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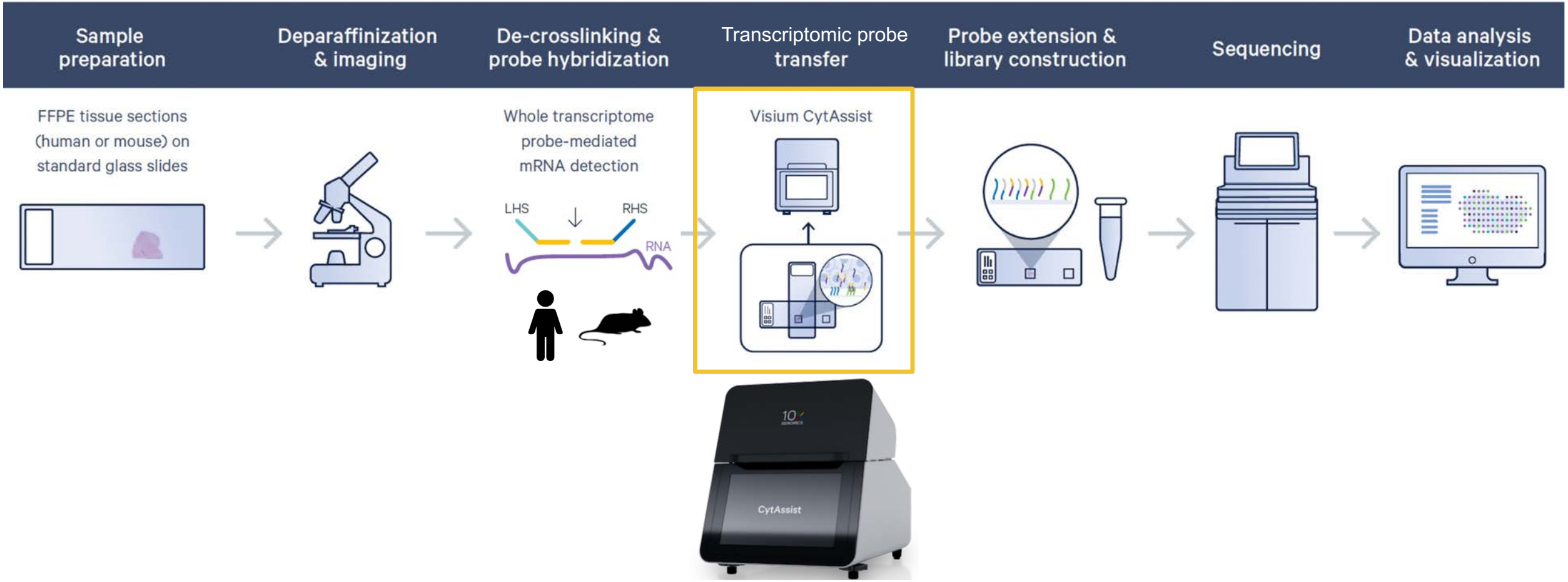
Simplifying Your Visium Workflow with CytAssist

Seamlessly integrate with standard histology sample preparation



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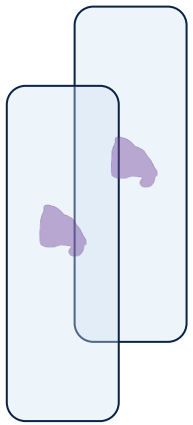
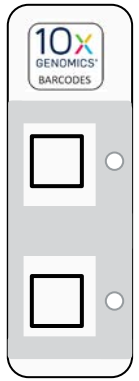
Simplifying Your Visium Workflow with CytAssist

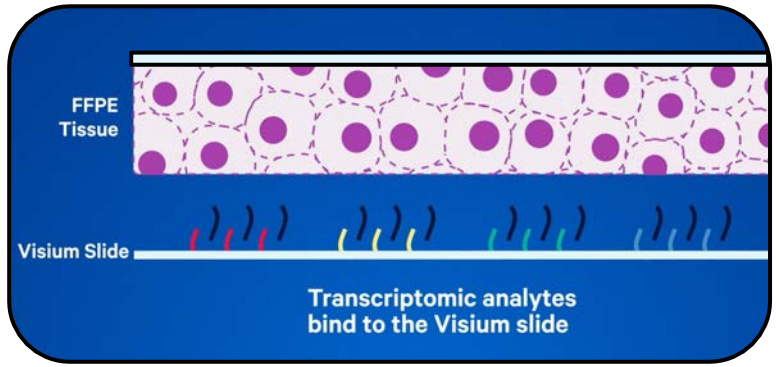
Seamlessly integrate with standard histology sample preparation

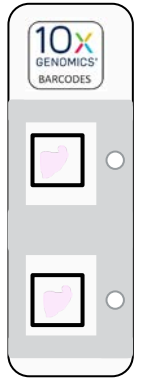
Two tissue slides
Stained sections on standard glass slides





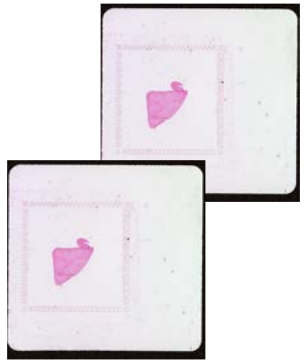
Visium slide
Two Capture Areas





Visium slide
Target molecules now captured on slide

Two tissue images
Spatial orientation for sequencing data



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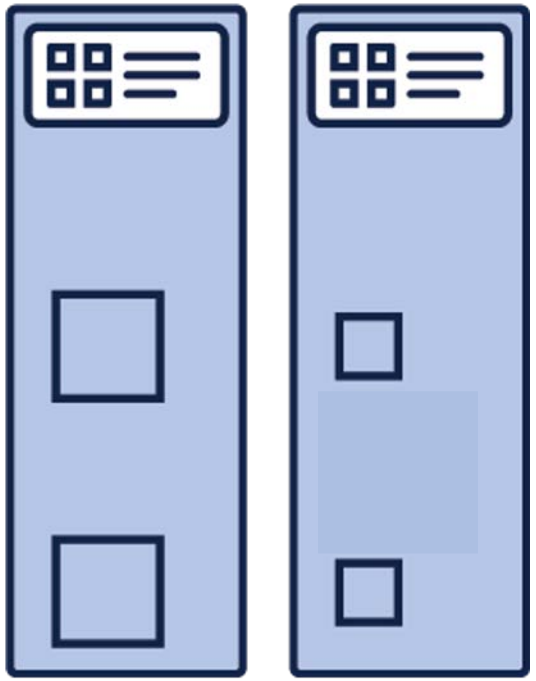
Profiling tissues of different sizes

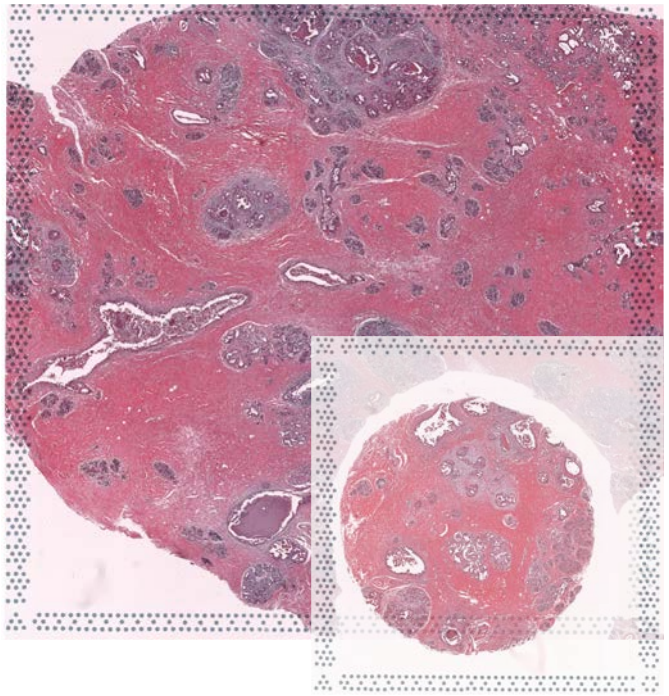
FFPE Human Breast Cancer



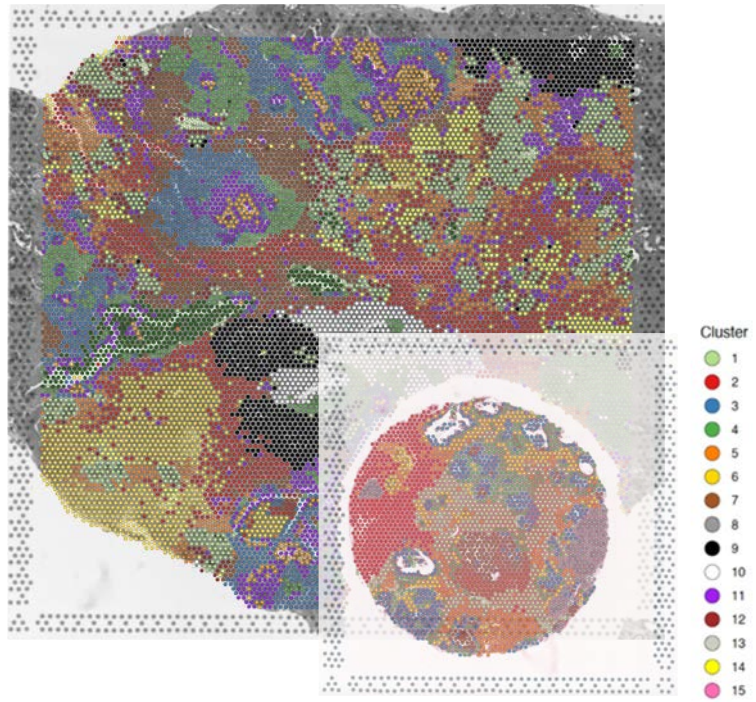
Visium slide, 11 mm

Visium slide, 6.5 mm

H&E



Gene clustering



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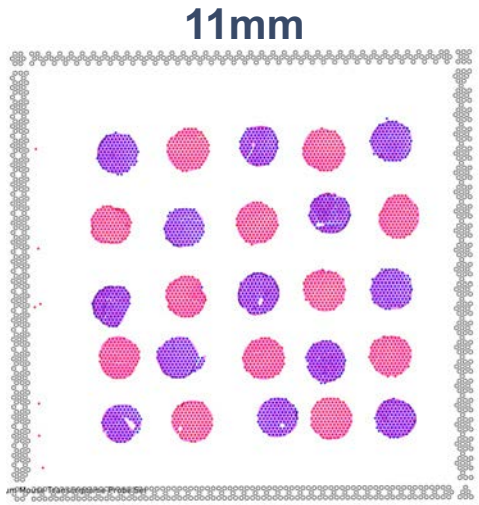
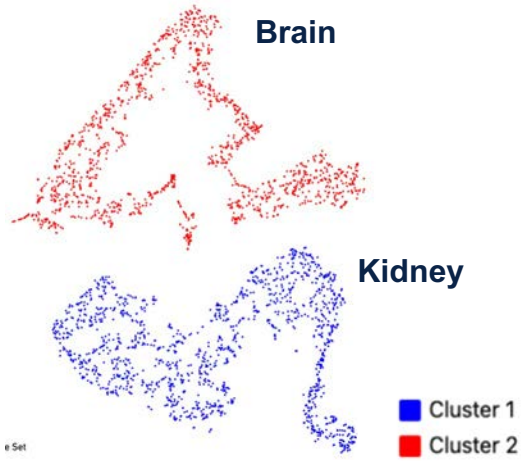
Visium CytAssist is Compatible with Tissue Microarrays

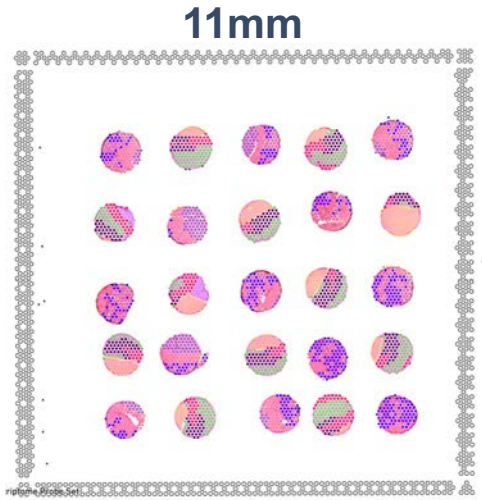


Kidney and Brain in alternate order

Sample punches

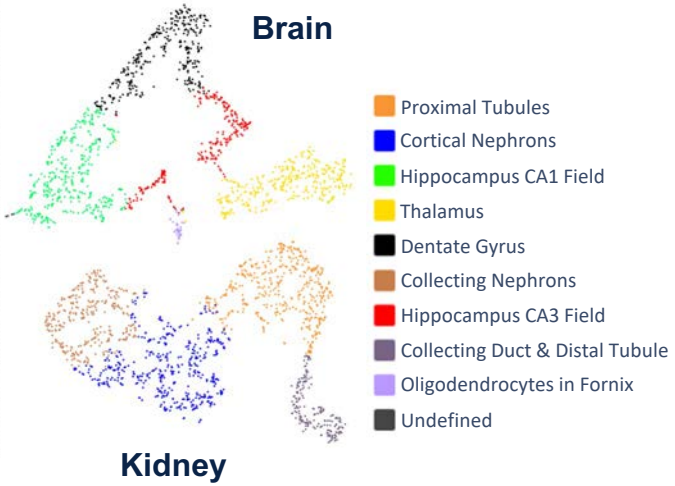


t-SNE plot



Kidney and Brain in alternate order

Sample punches



t-SNE plot

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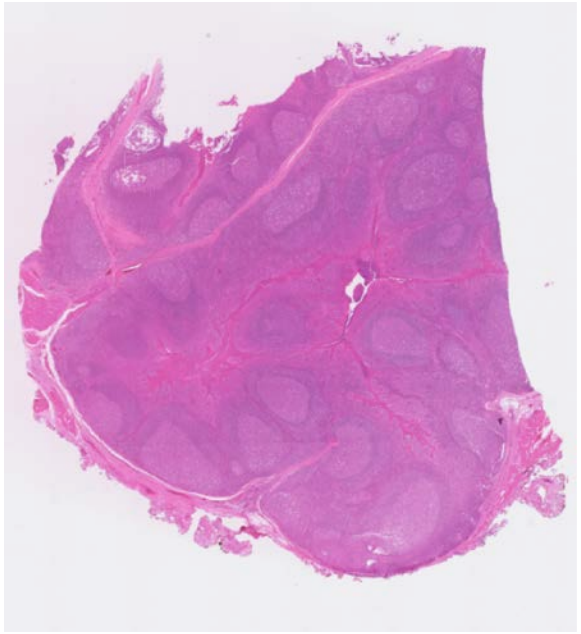
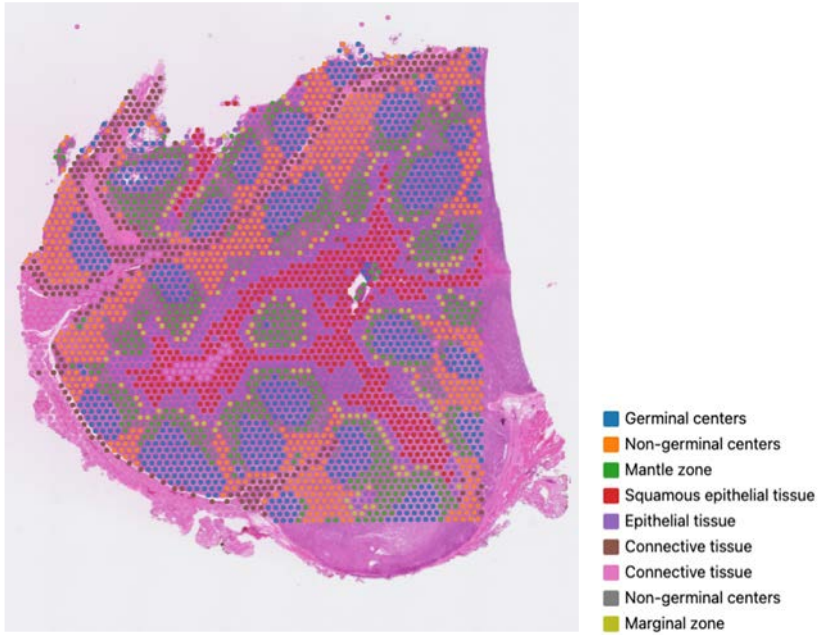
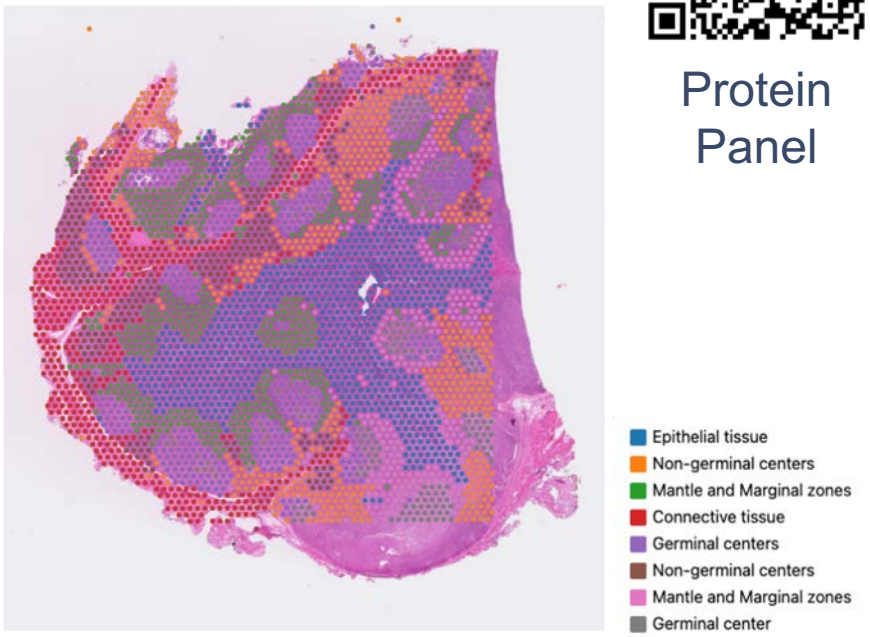
Visium CytAssist Gene and Protein Expression

Protein Panel

Tonsil - FFPE







Morphology

H&E-stained image

RNA

Gene expression clustering
(Whole transcriptome)

Protein

Protein clustering
(Immune cell profiling panel)

- 31 human immune cell-focused markers + 4 isotype controls
- Includes cell surface proteins and intracellular proteins

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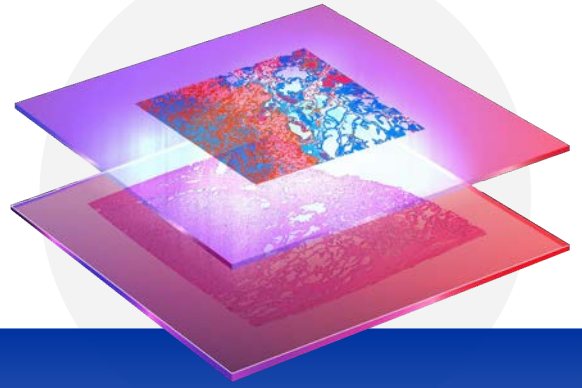
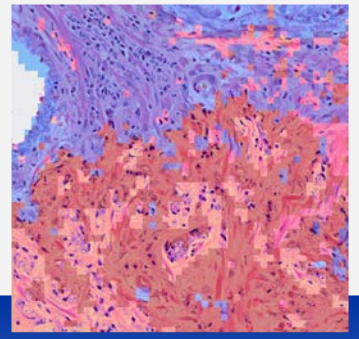
Introducing Visium HD

The spatial discovery power you want with the resolution and data quality you need



Unparalleled Spatial Discovery

Whole transcriptome gene expression analysis



Resolved at Single Cell Scale

Capture Area with continuous lawn of 2 x 2 µm barcoded squares

Data Quality You Can Trust

Accurate transcript localization enabled by Visium CytAssist

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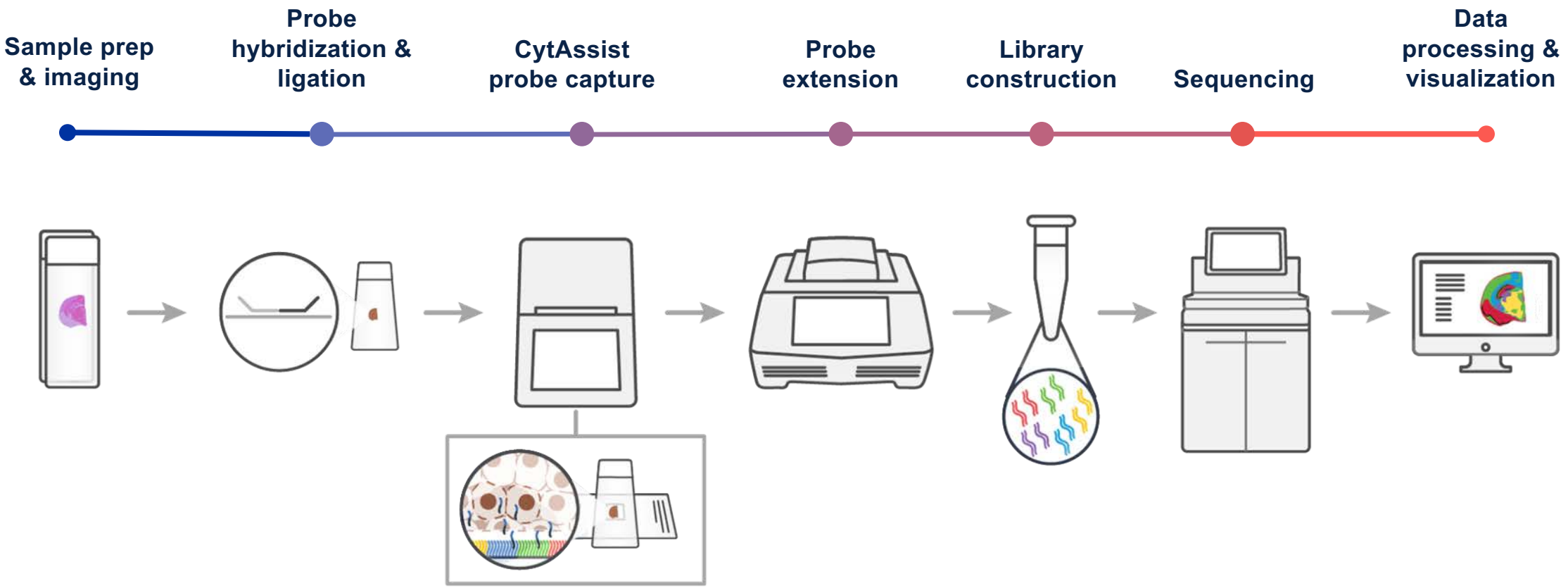
Visium HD Workflow Powered by CytAssist

Transforming standard histological sections into high-resolution, whole transcriptome gene expression data



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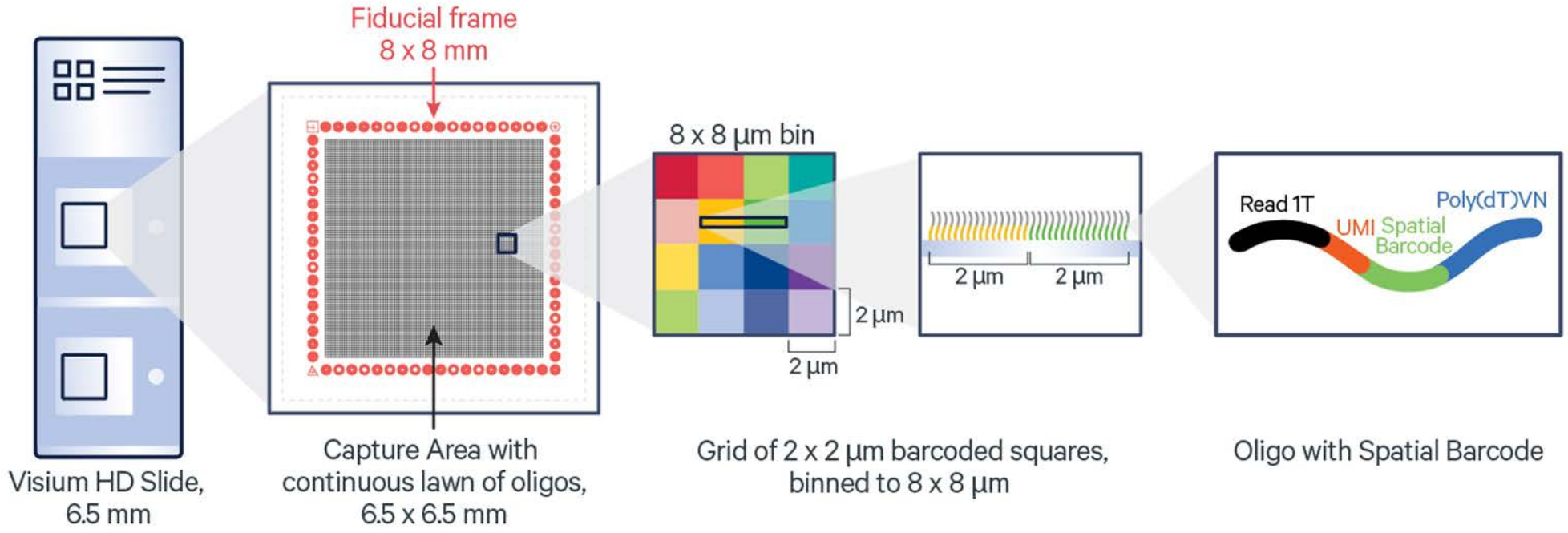
Resolved at Single Cell Scale

Sample prep & imaging | Probe hybridization & ligation | CytAssist probe capture | **Probe extension** | **Library construction** | Sequencing | Data processing & visualization



Data is output at 2 µm and multiple bin sizes. The 8 µm bin is a recommended starting point for visualization and analysis.

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Streamlined, Easy-to-Integrate Data Analysis

Sample prep & imaging

Probe hybridization & ligation

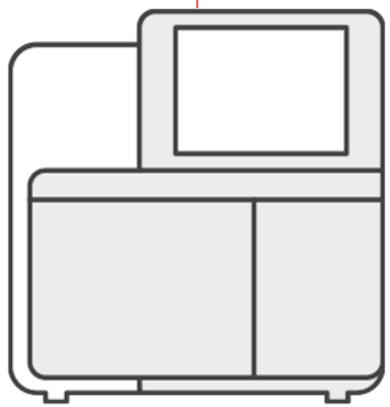
CytAssist probe capture

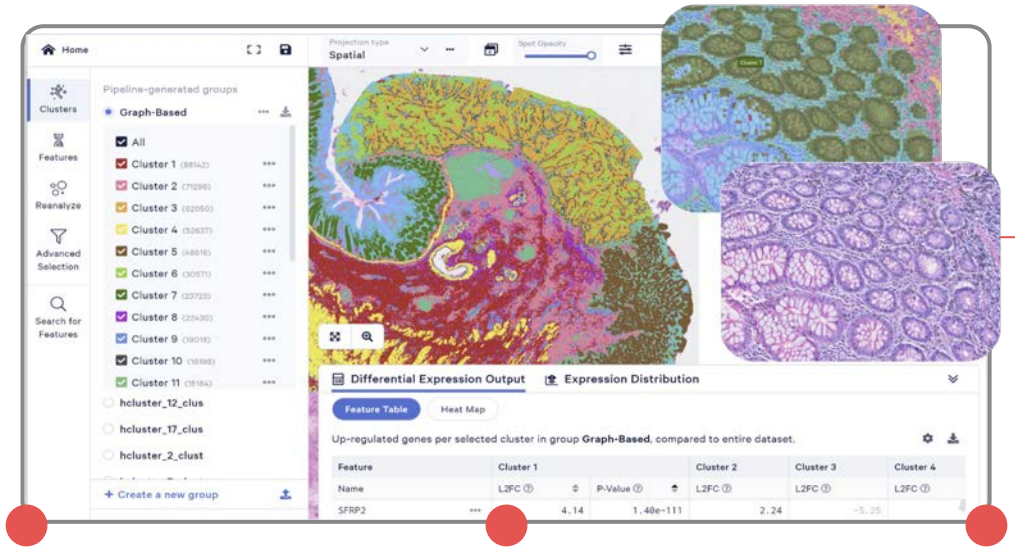
Probe extension

Library construction

Sequencing

Data processing & visualization





Sequencing Rec: 275M+ reads for a fully covered 6.5 mm Capture Area

Explore data in 10x-developed Loupe Browser

Data output at multiple bin levels: 2 µm, 8 µm (Loupe file provided), 16 µm

Flexible binning in increments of 2 µm

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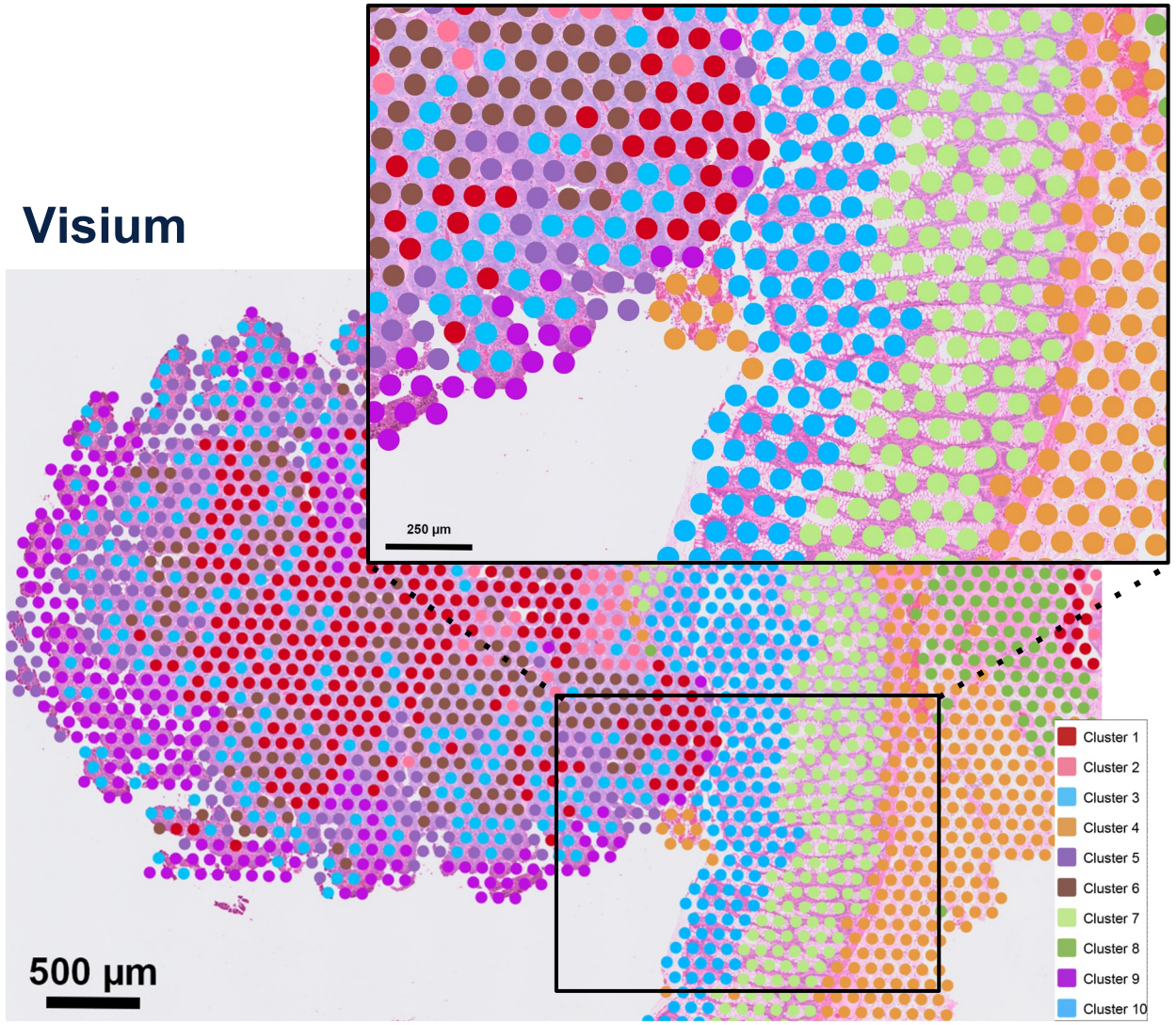
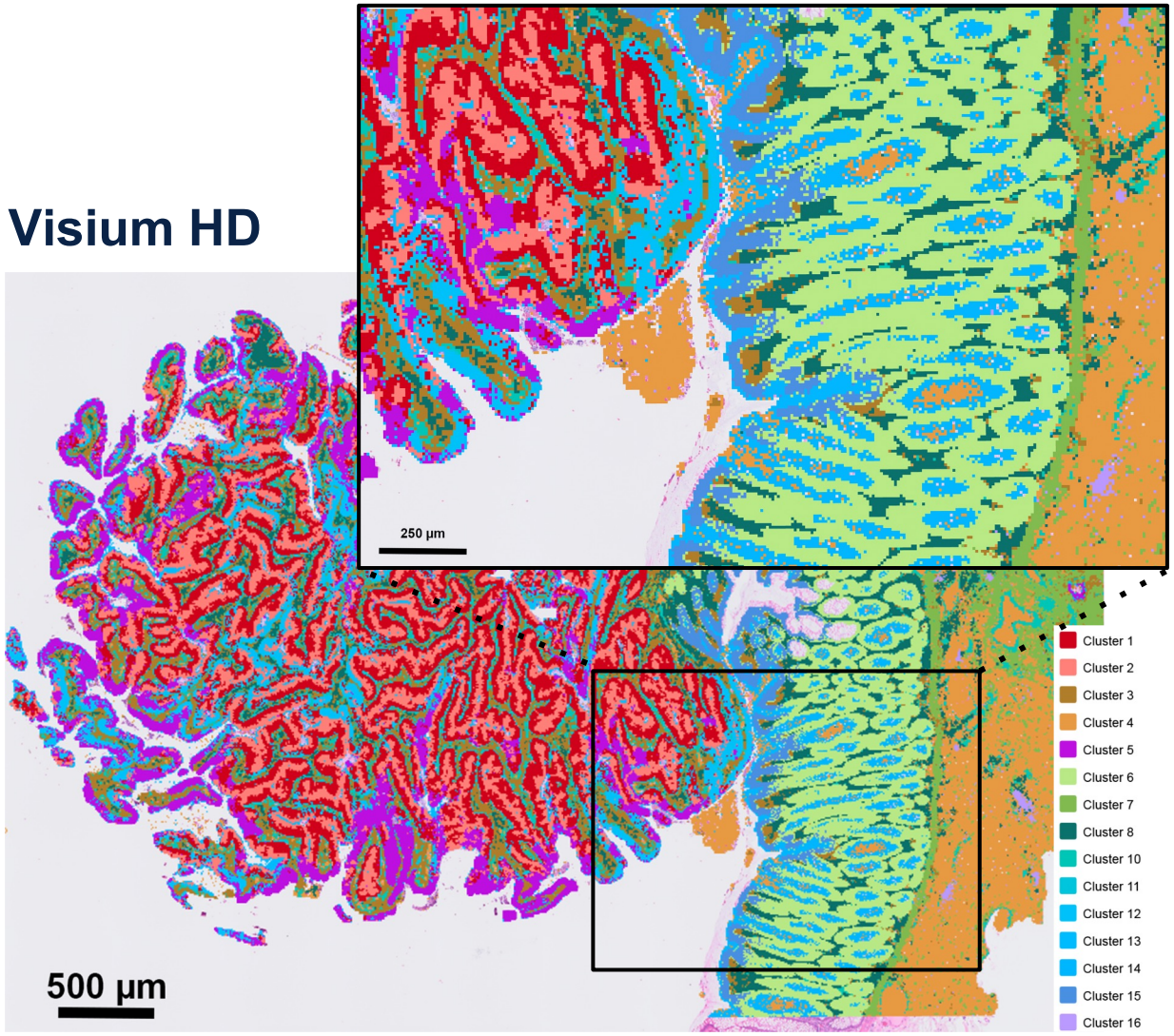
The power of Visium HD

Visium



Visium HD



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FFPE human colorectal cancer

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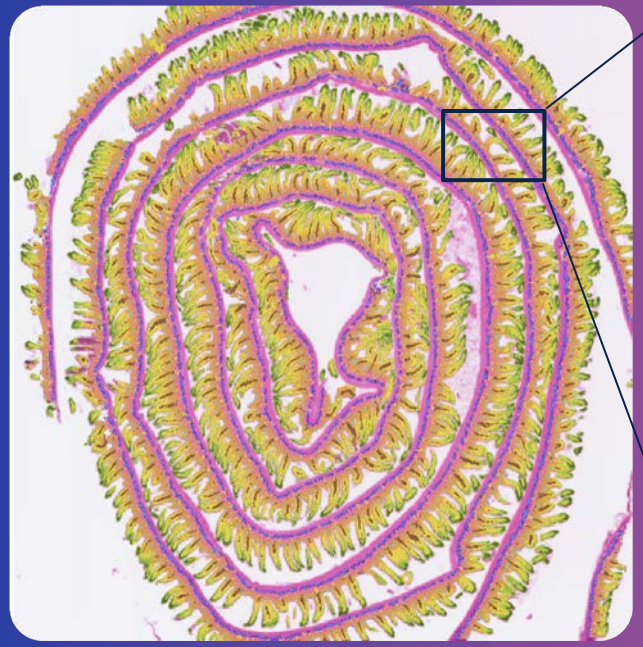
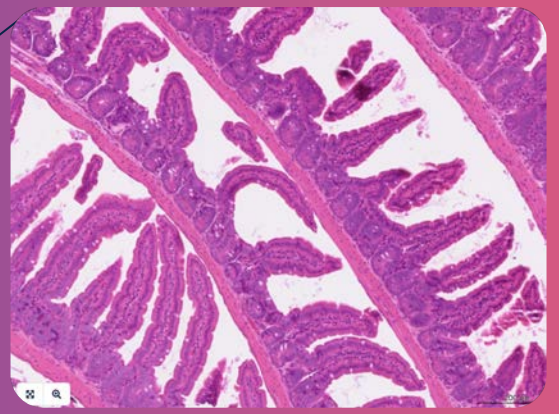
Generate Accurate and Highly Specific Spatial Maps

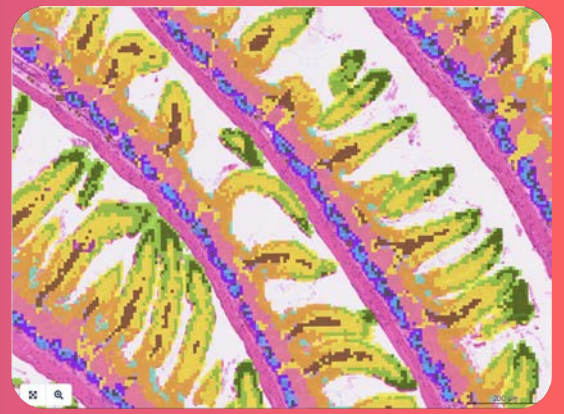
Visium HD precisely replicates known biology



Mouse Intestine
Gene Expression
Clustering



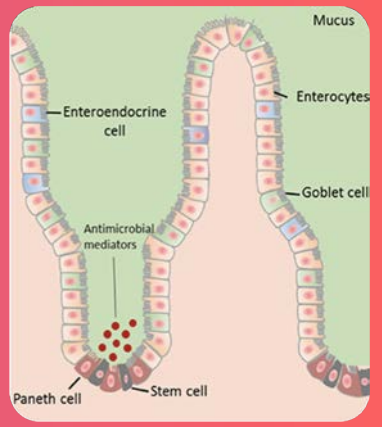
H&E image



HD: Unbiased Clustering

Lyz1
(paneth cell marker)

Muc2
(goblet cell marker)



Small Intestine

Herath *et al. Front Cell Infect Microbiol* (2020).

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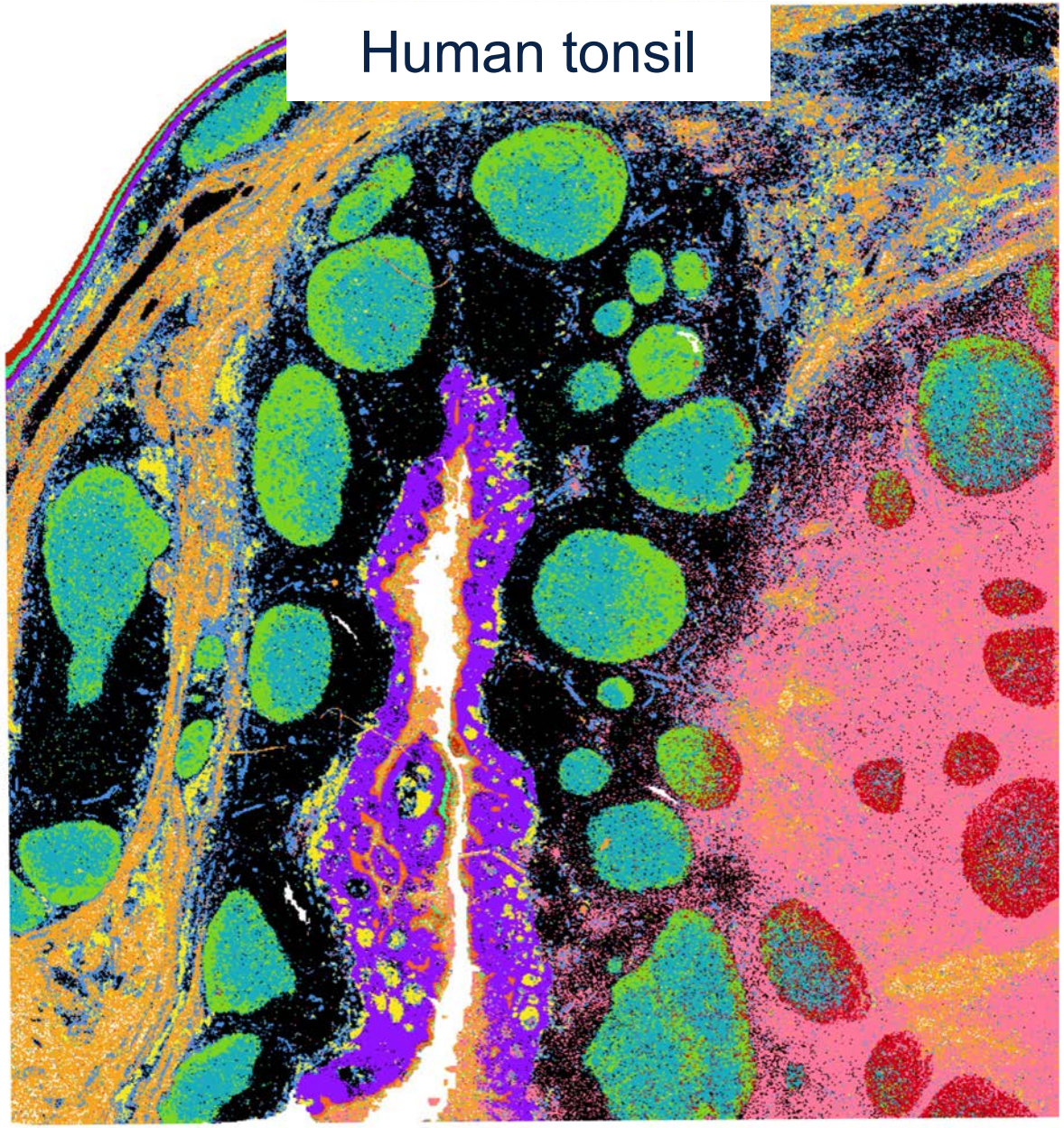
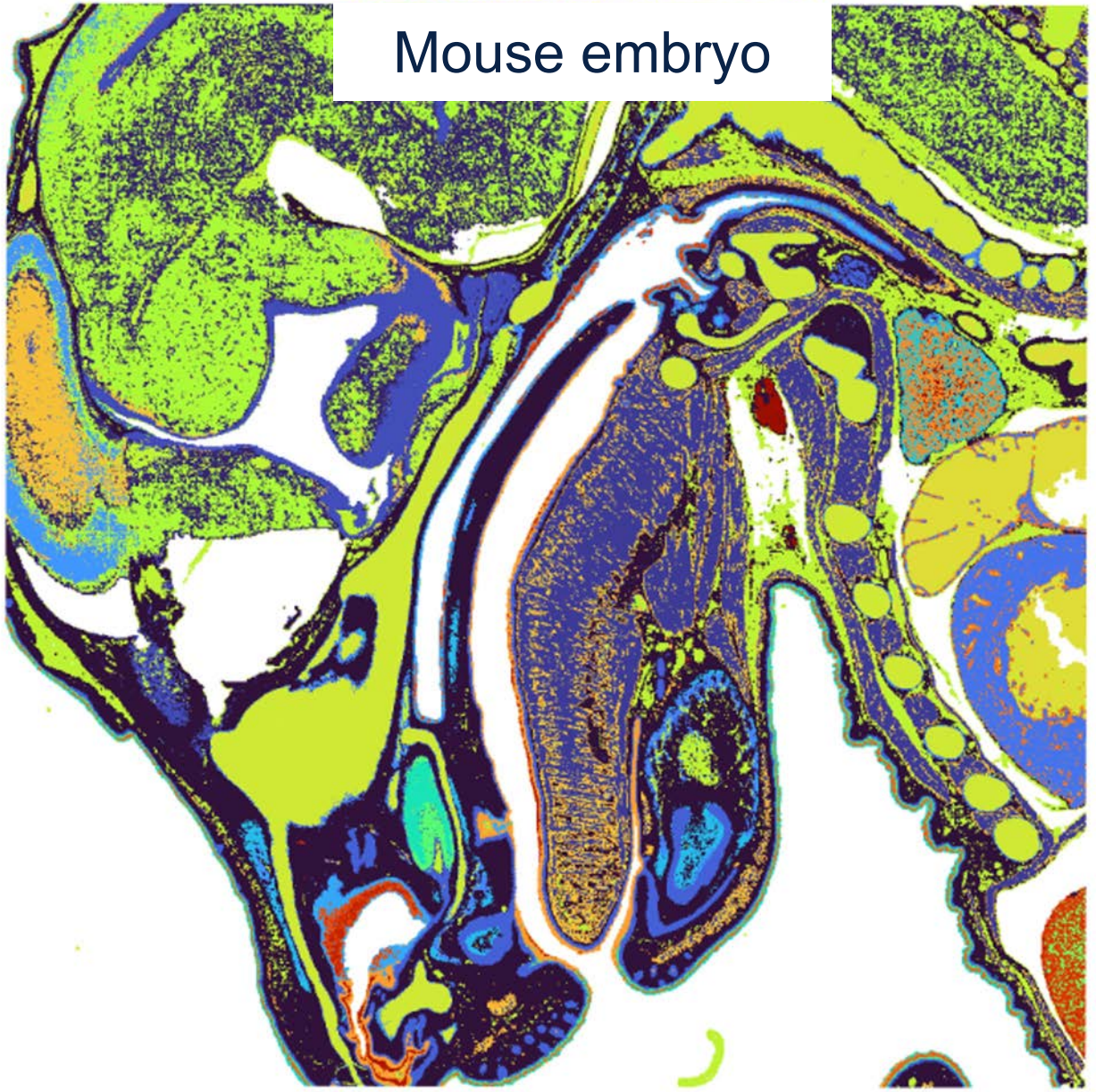
Human tonsil



Mouse embryo



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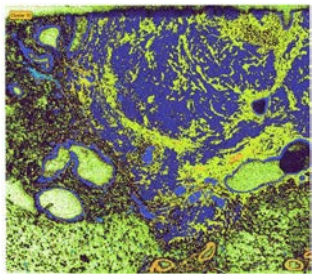
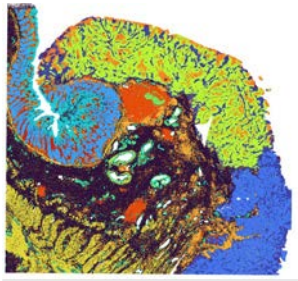
Visium HD: Built for FFPE

Human

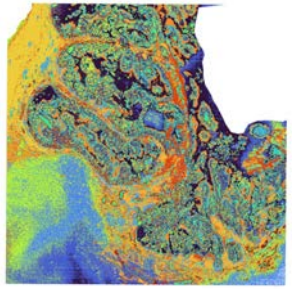
Breast cancer



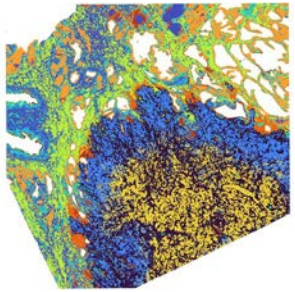
Colon cancer



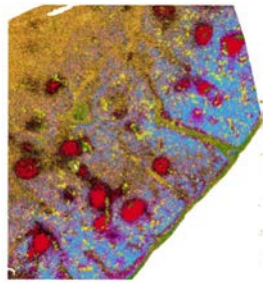
Ovarian cancer



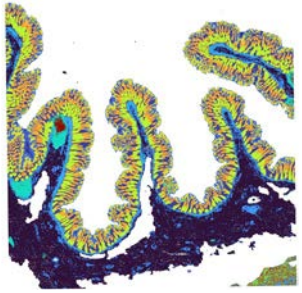
Prostate cancer



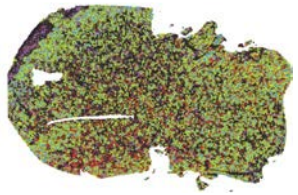
Spleen



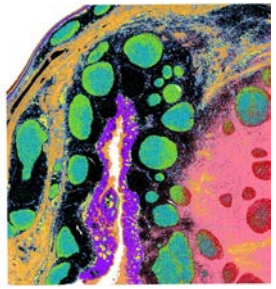
Colon



Brain

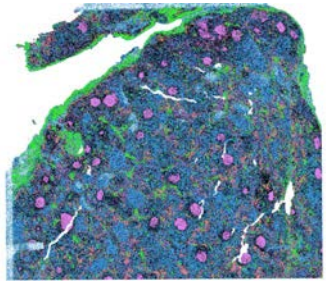


Tonsil

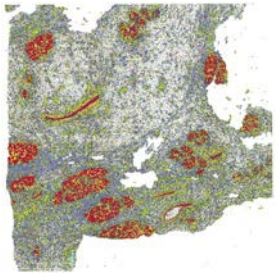


Pancreas

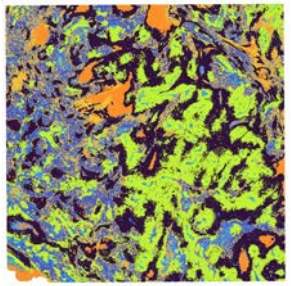
Lymph node



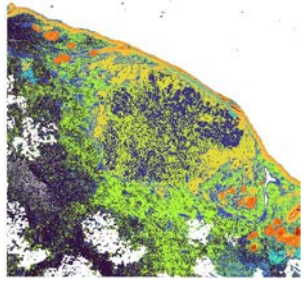
Breast



Lung cancer

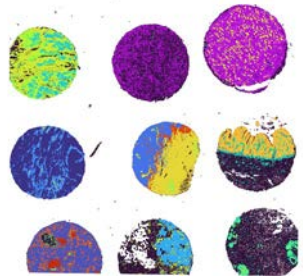


Skin melanoma



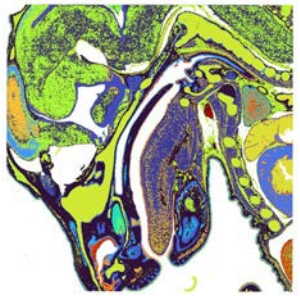
Liver cancer

TMA

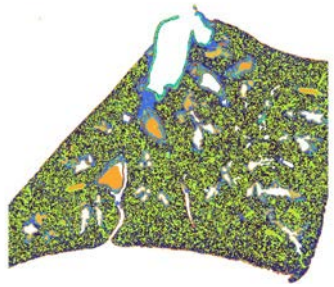


Mouse

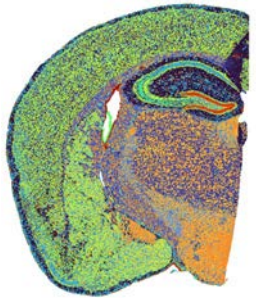
Embryo



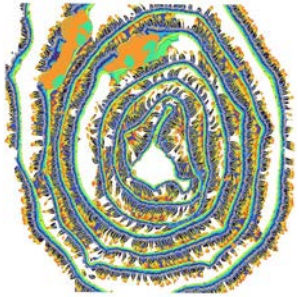
Lung



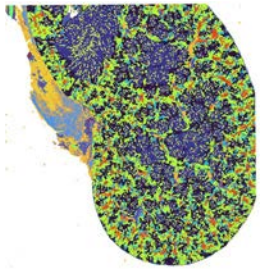
Brain



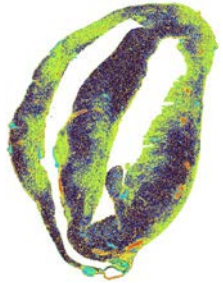
Small intestine



Kidney



Heart



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Unsupervised clustering (8 µm x 8 µm bins)

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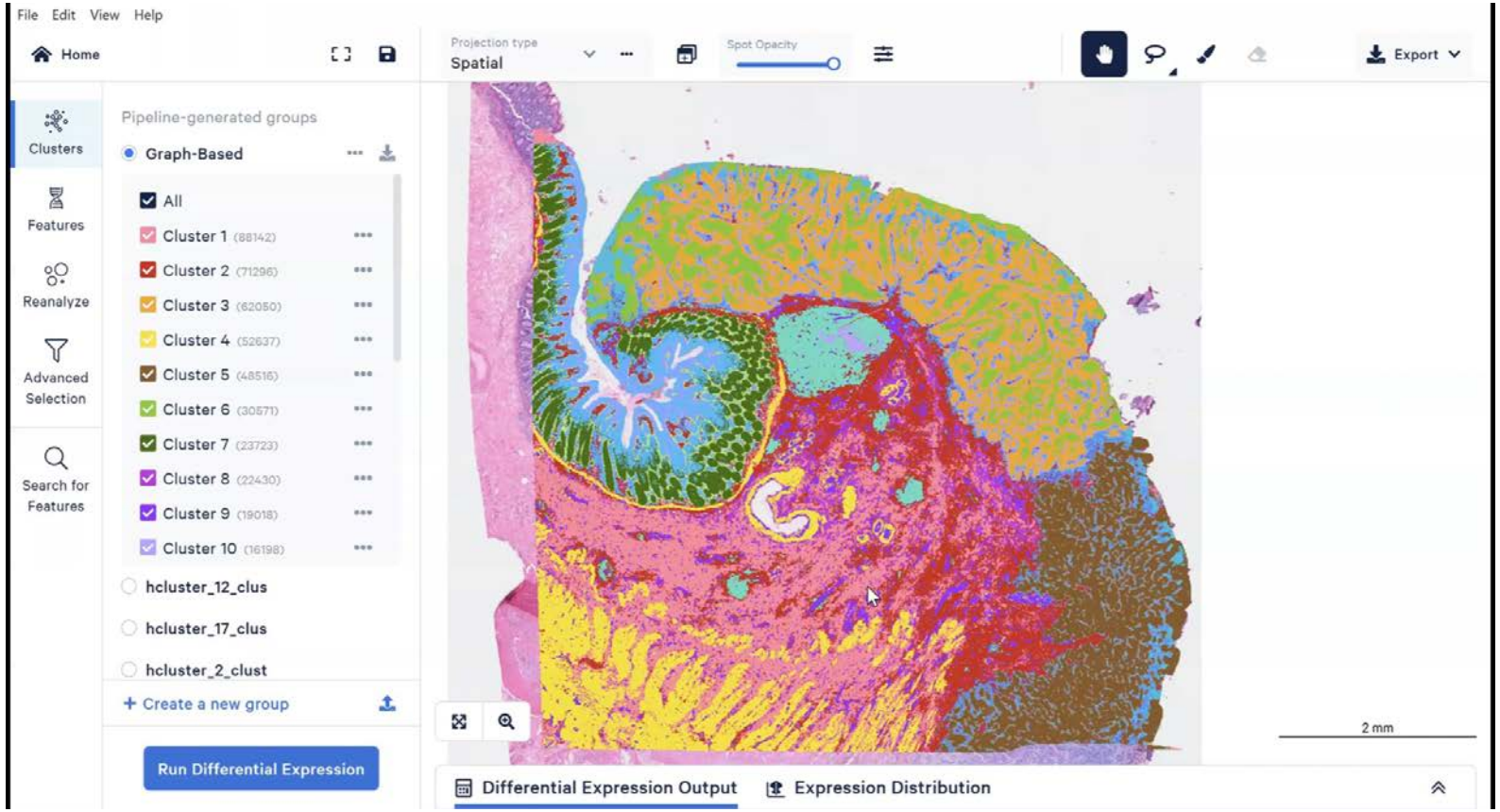
Easy-to-Use Analysis Accelerates Discovery



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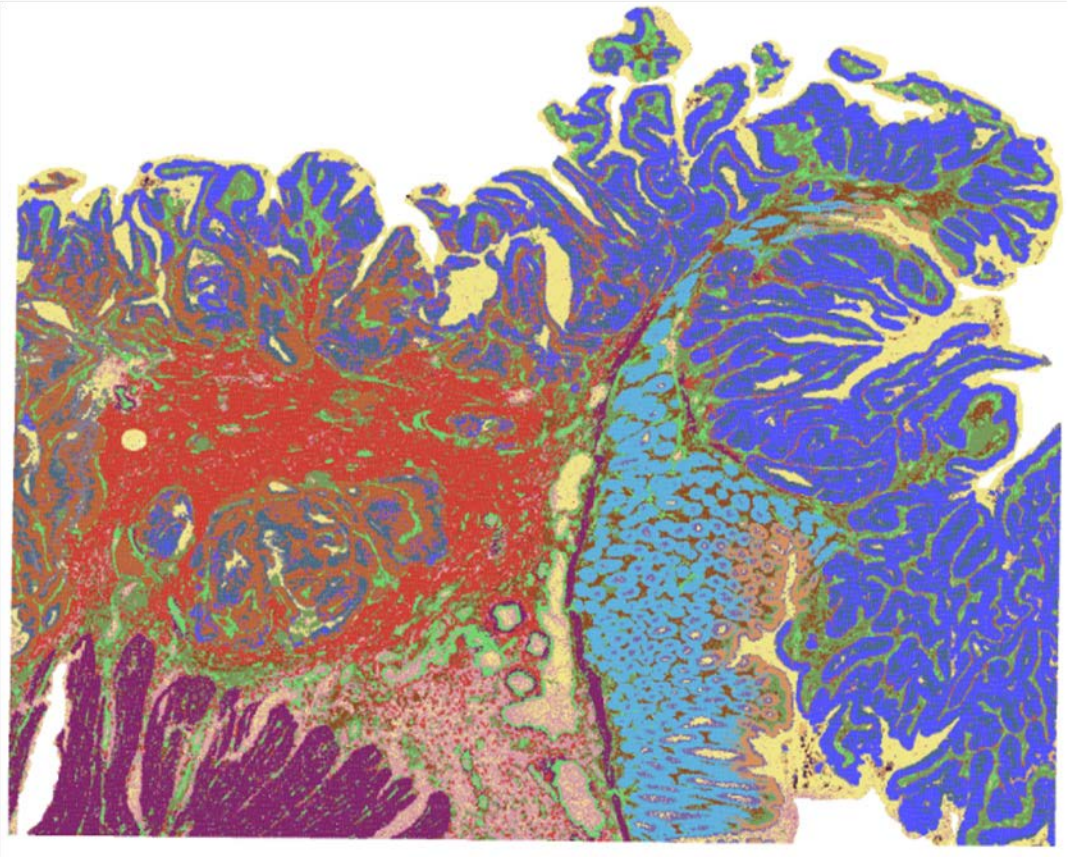
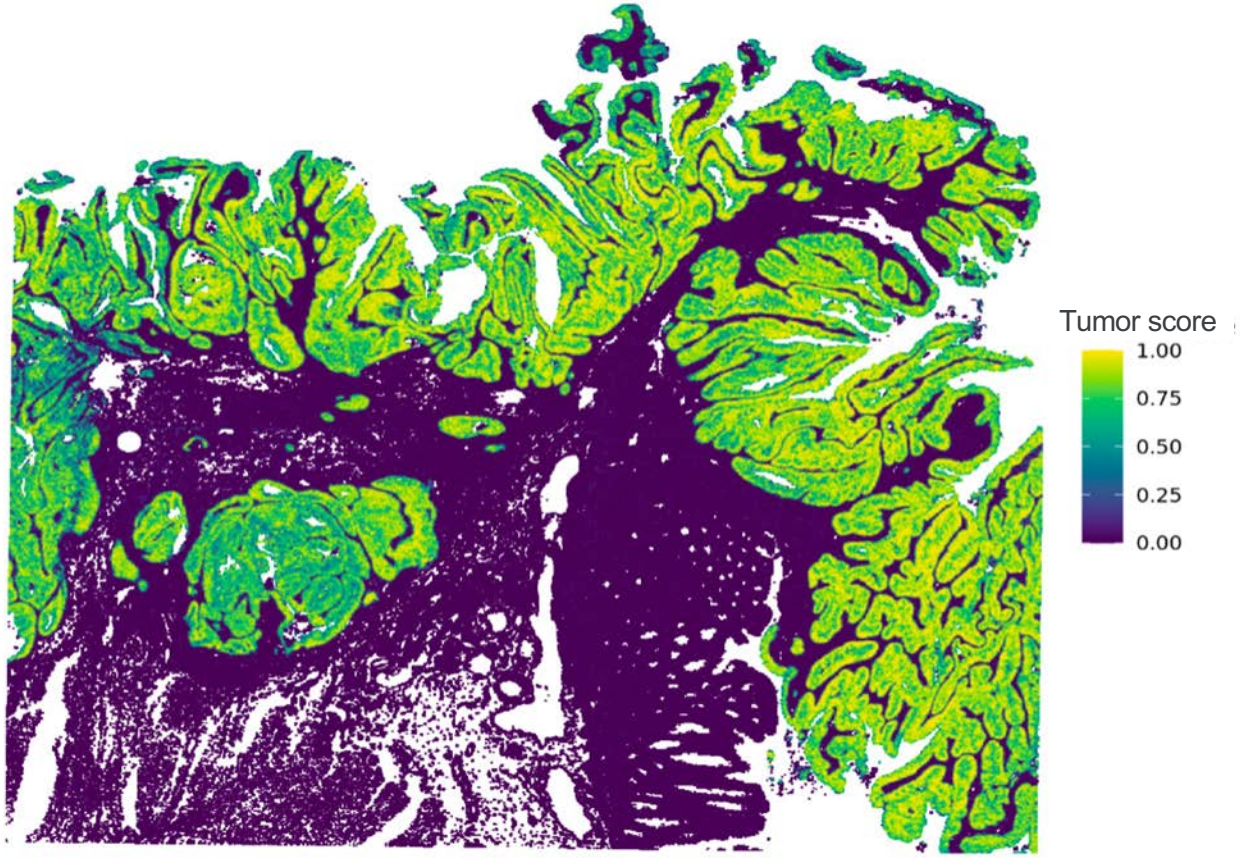
Visium HD identifies tumor regions with high resolution



Unsupervised clustering



Tumor deconvolution using scRNA-seq

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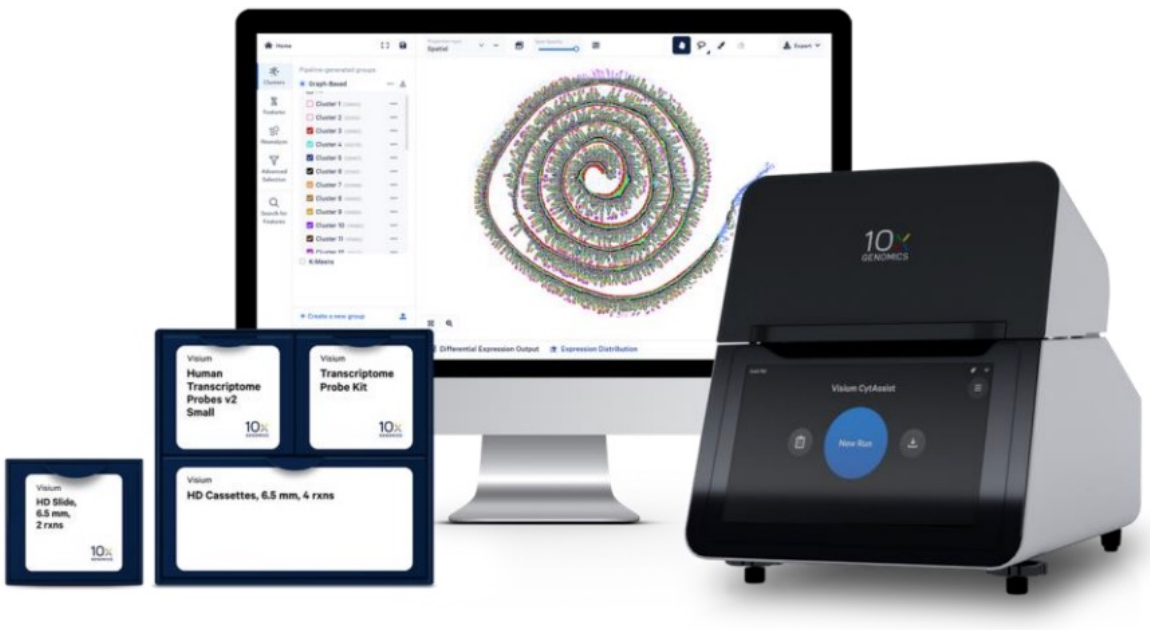
Order Today: Visium HD Shipping This Quarter



- **Unparalleled spatial discovery:** Whole transcriptome gene expression analysis
- **Resolved at single cell scale:** Capture Area with grid-patterned 2 x 2 µm barcoded squares
- **High data quality:** Precise instrument-driven probe capture
- **Broad access to diverse tissues:** Archived or newly sectioned FFPE with no optimization required
- **Compatible with histology workflows:** H&E or IF on the same section

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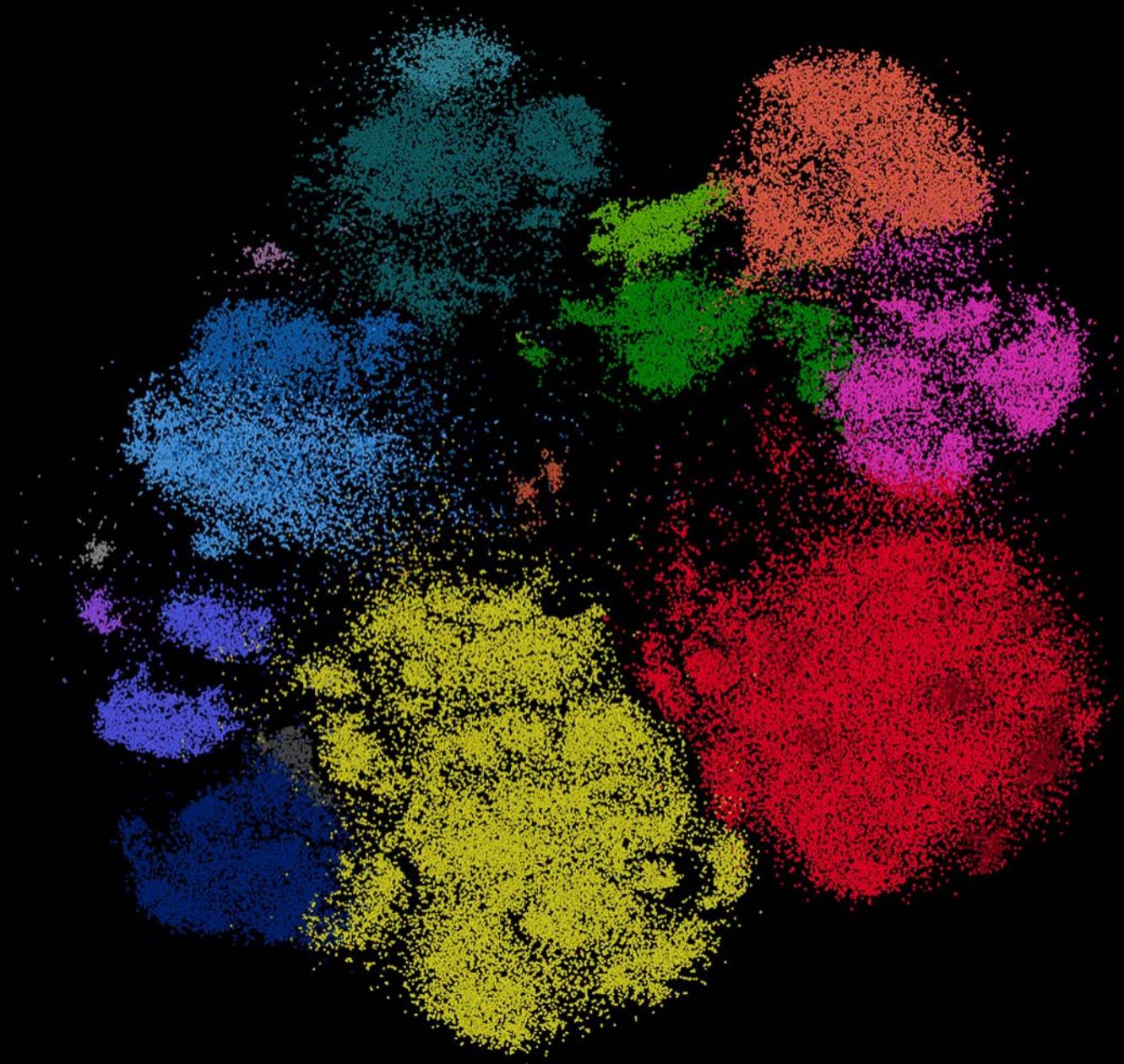
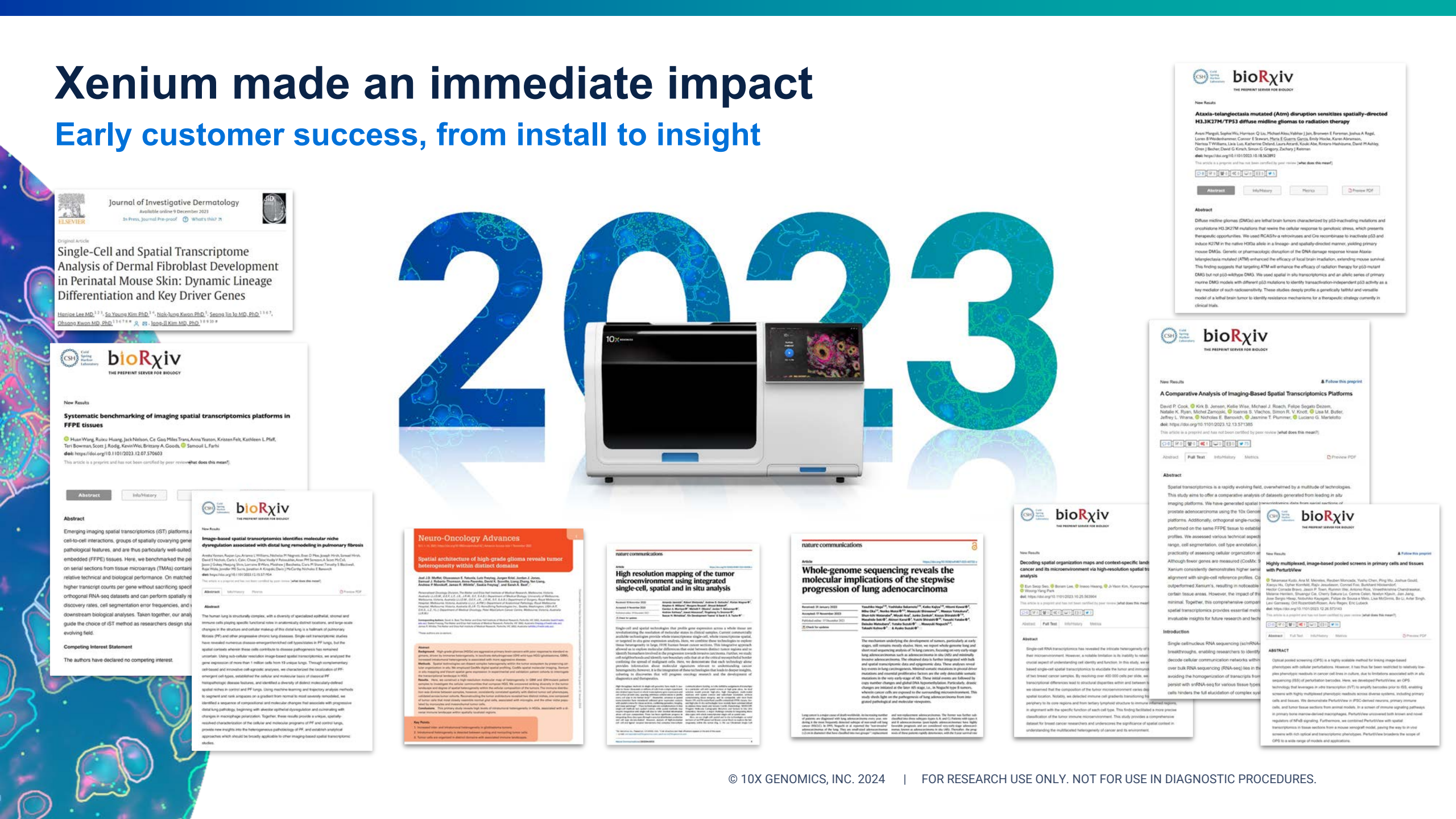
Xenium
in situ



Stephen Williams

Xenium made an immediate impact

Early customer success, from install to insight



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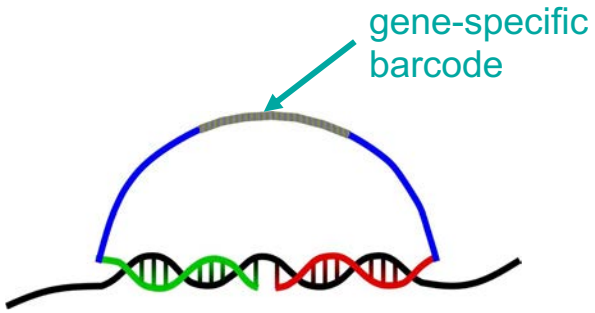
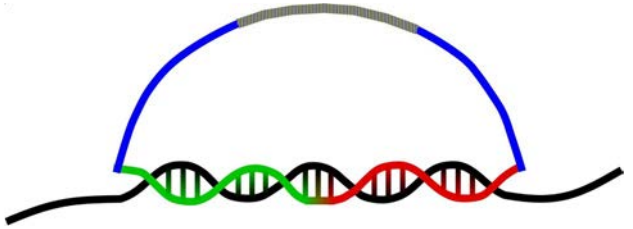
Before Xenium Workflow

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

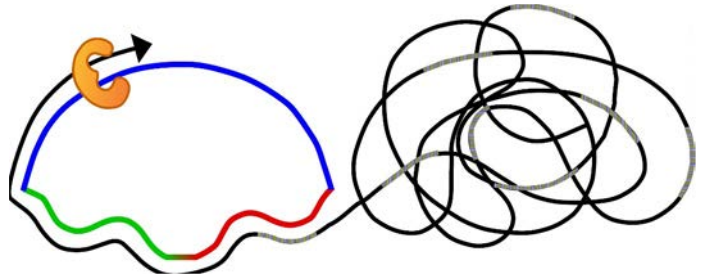
Section tissue onto 12 mm x 24 mm area



DNA probes hybridized to target RNA



Highly specific ligation



Multiple copies of barcode generated

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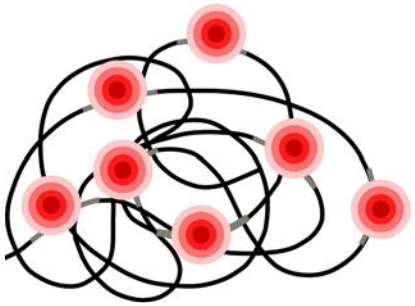
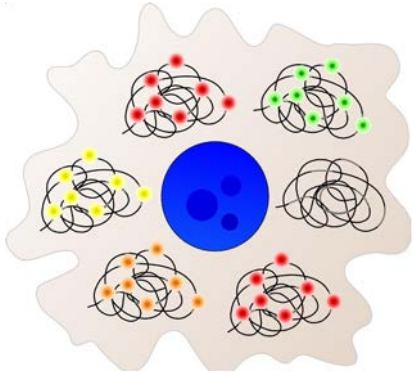
Onboard analysis includes in-line decoding

Achieves precise X,Y,Z localization of transcripts with reliable quality

Chemistry and Imaging

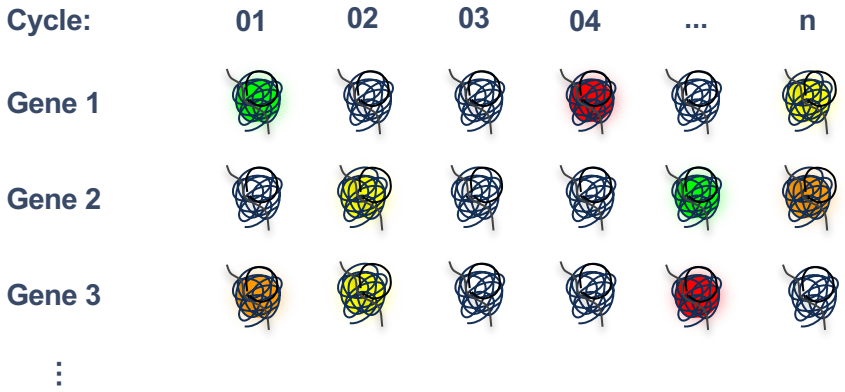


Fluorescent oligos hybridize to amplified probes



Multiple cycles of probe binding and stripping

In-line decoding



Onboard analysis decodes fluorescent signal across cycles to transcripts

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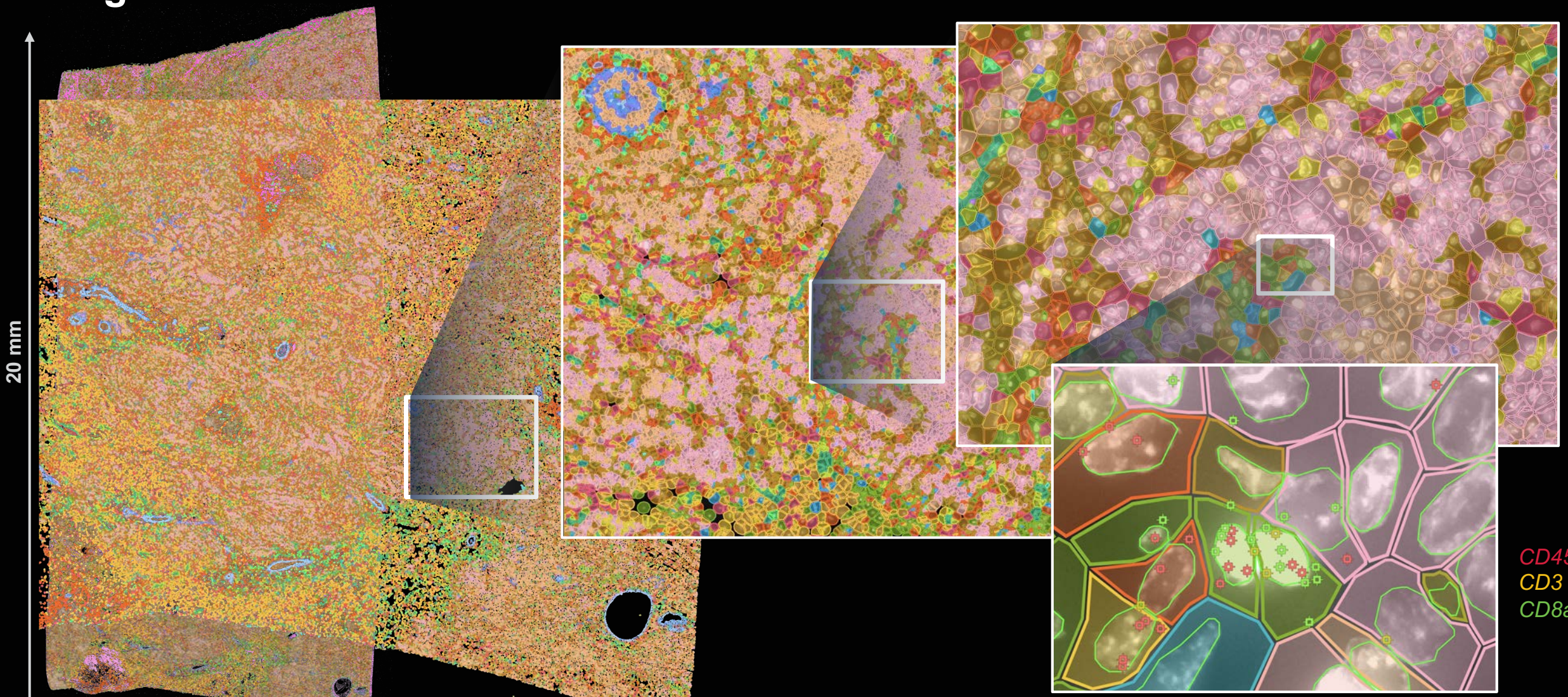
Large FFPE Human Breast Cancer Section With ~1M Cells



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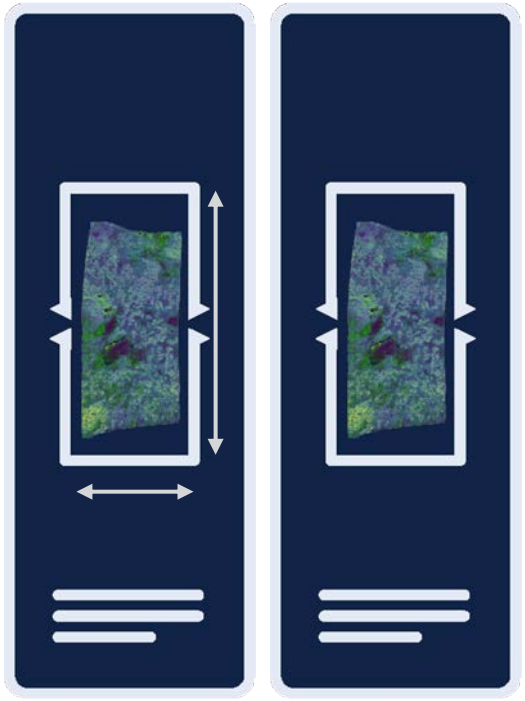
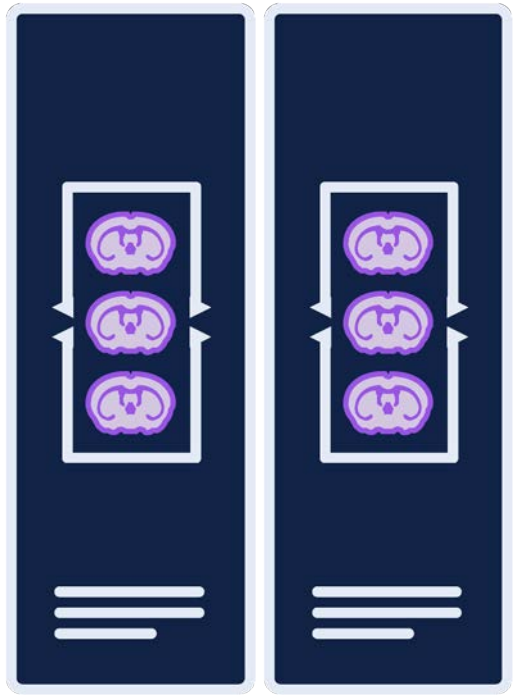
Leading analyzable area allows for maximum flexibility



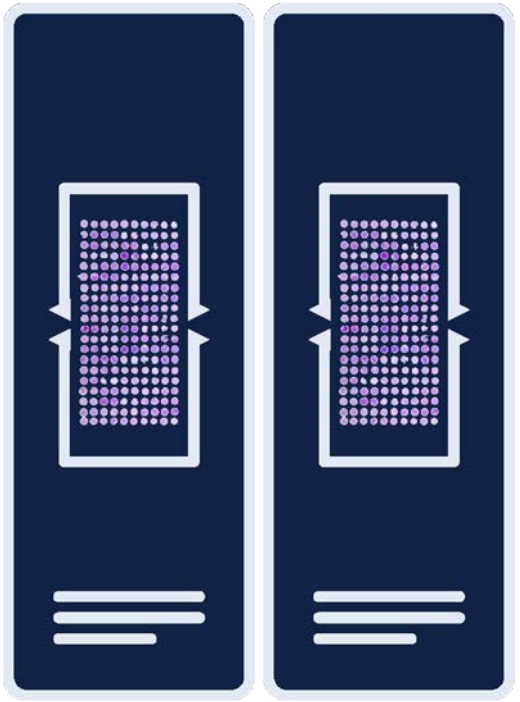
Two 10.5 x 22.5mm sections

~2M cells



Six 6 x 10mm mouse brain sections

~1M cells



~Four hundred 1 x 0.6mm biopsies in TMAs

~1M cells

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Xenium Panel and Custom Menu Offers Maximum Flexibility

Pre-designed & validated panels

Human Breast
280 genes

Human Lung
289 genes

Human Brain
266 genes

Human Skin
260 genes

Add up to 100 custom targets

Human Multi-Tissue & Cancer
377 genes

Human Colon
322 genes

Mouse Brain
248 genes

Mouse Multi-Tissue
379 genes

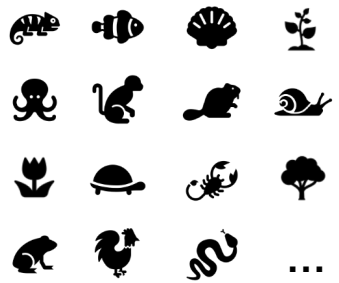
Standalone custom

480 custom genes

300 custom genes

100 custom genes

50 custom genes



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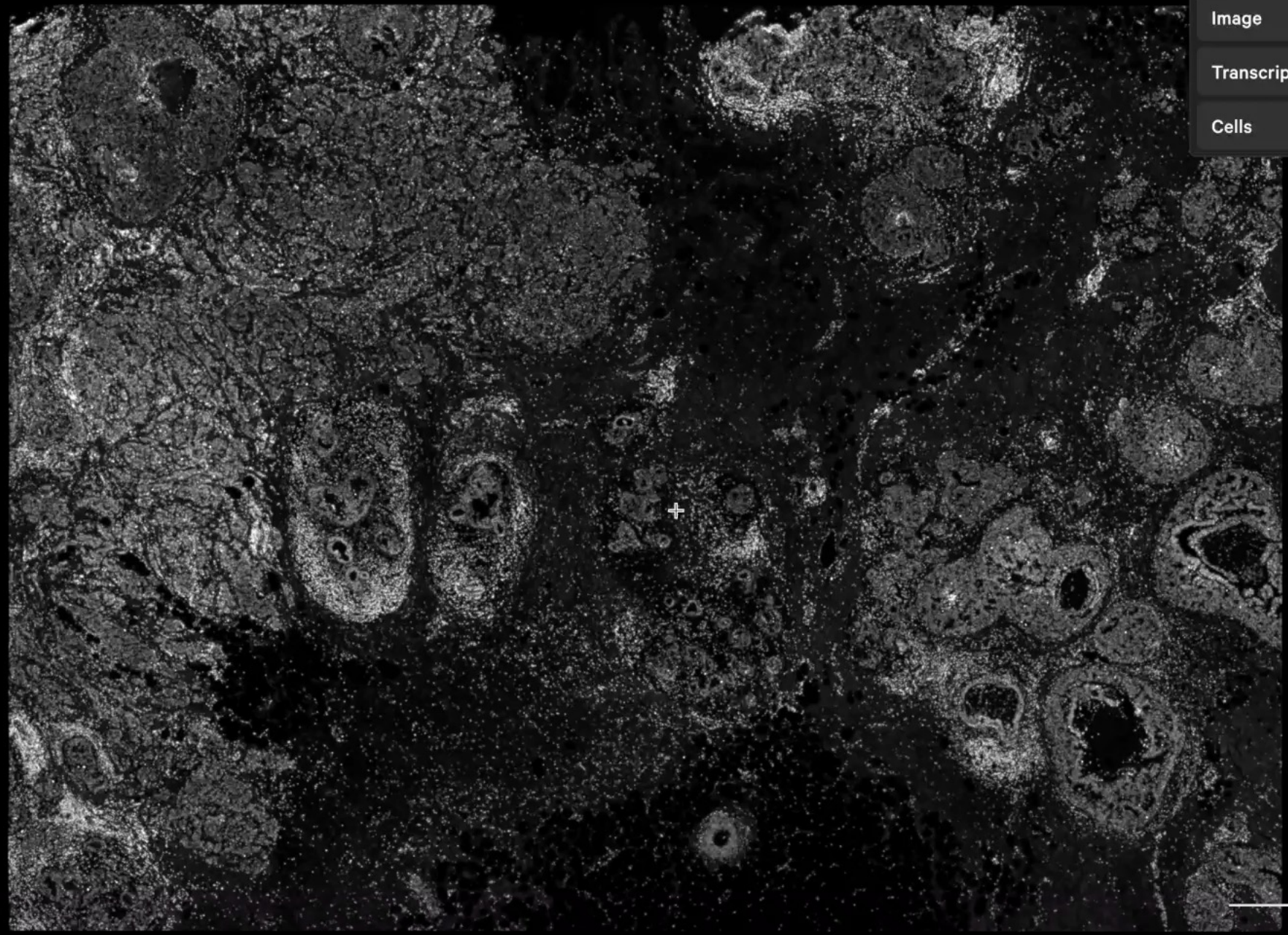
Explore the power of in situ with 10x Genomics Xenium Analysis technology >



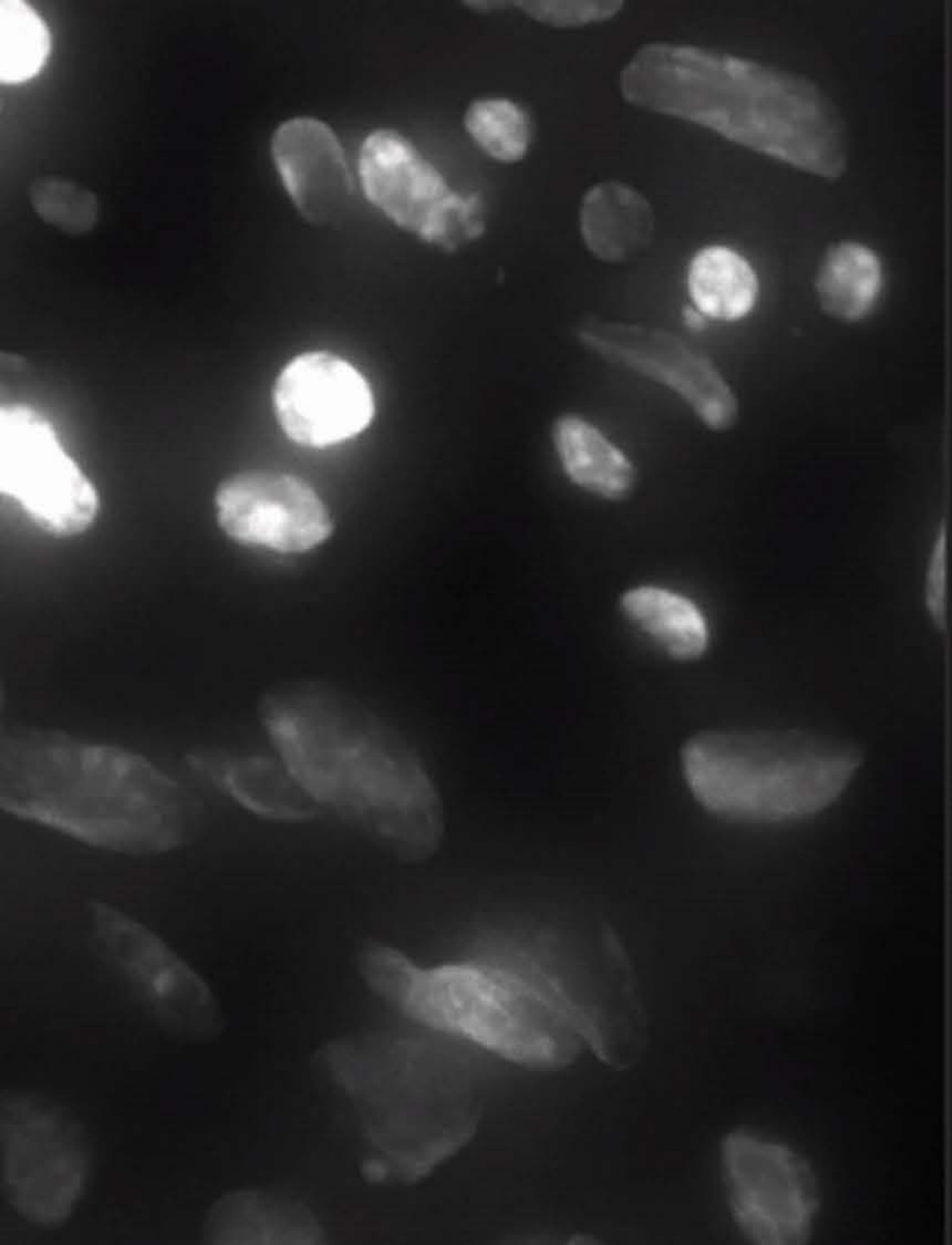
Image

Transcripts

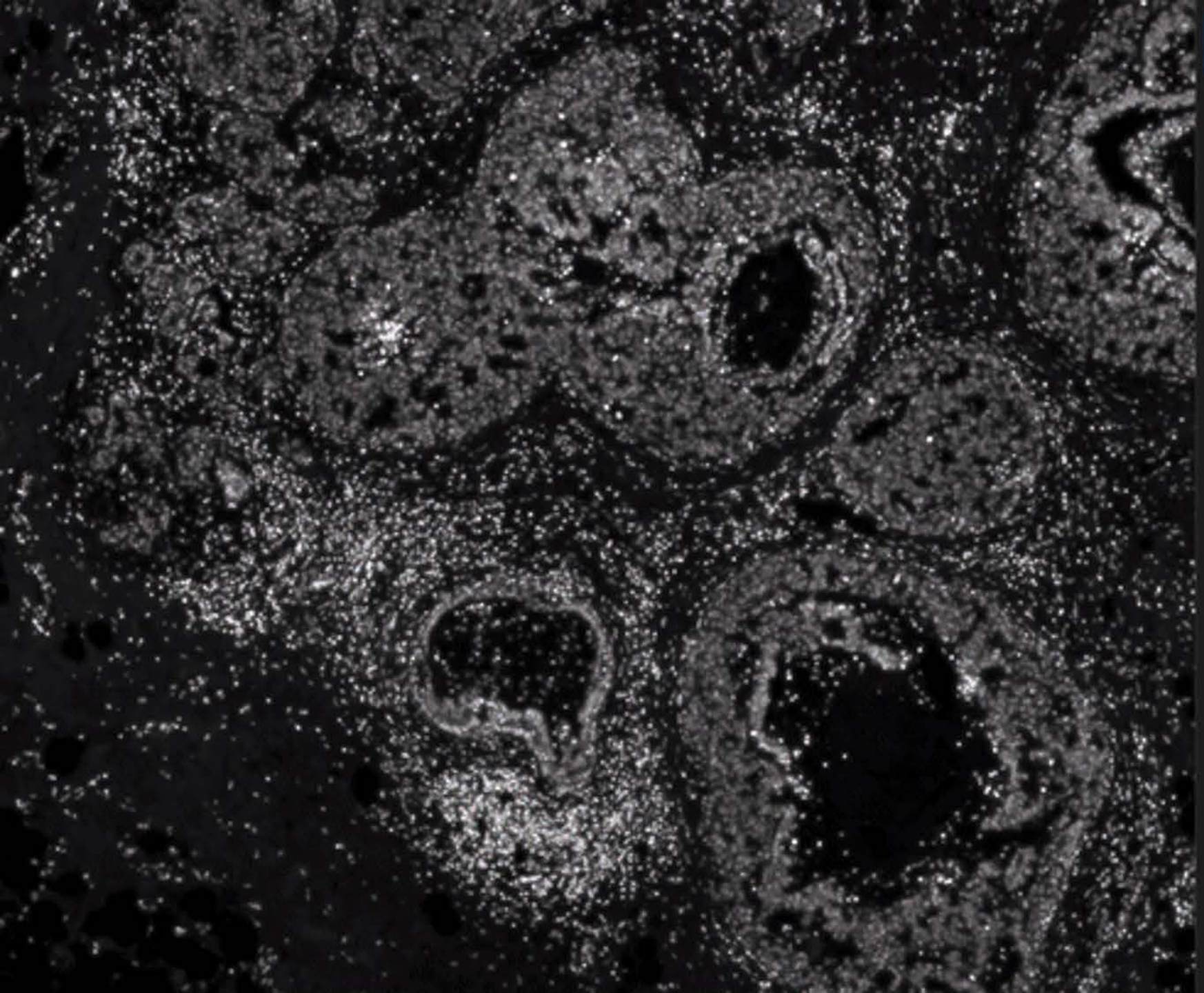
Cells

3948.4 2992.2 µm

2000 µm



- Genes (313)
  - Tumor General (9)
  - Tumor DCIS (4)
  - Tumor Invasive (1)
  - Proliferative (2)
  - Myoepithelial Subtype 1 (5)
    - ACTA2
    - DST
    - KRT14
    - KRT5
    - MYLK
  - Myoepithelial Subtype 2 (4)
  - Stroma (6)



Transcripts

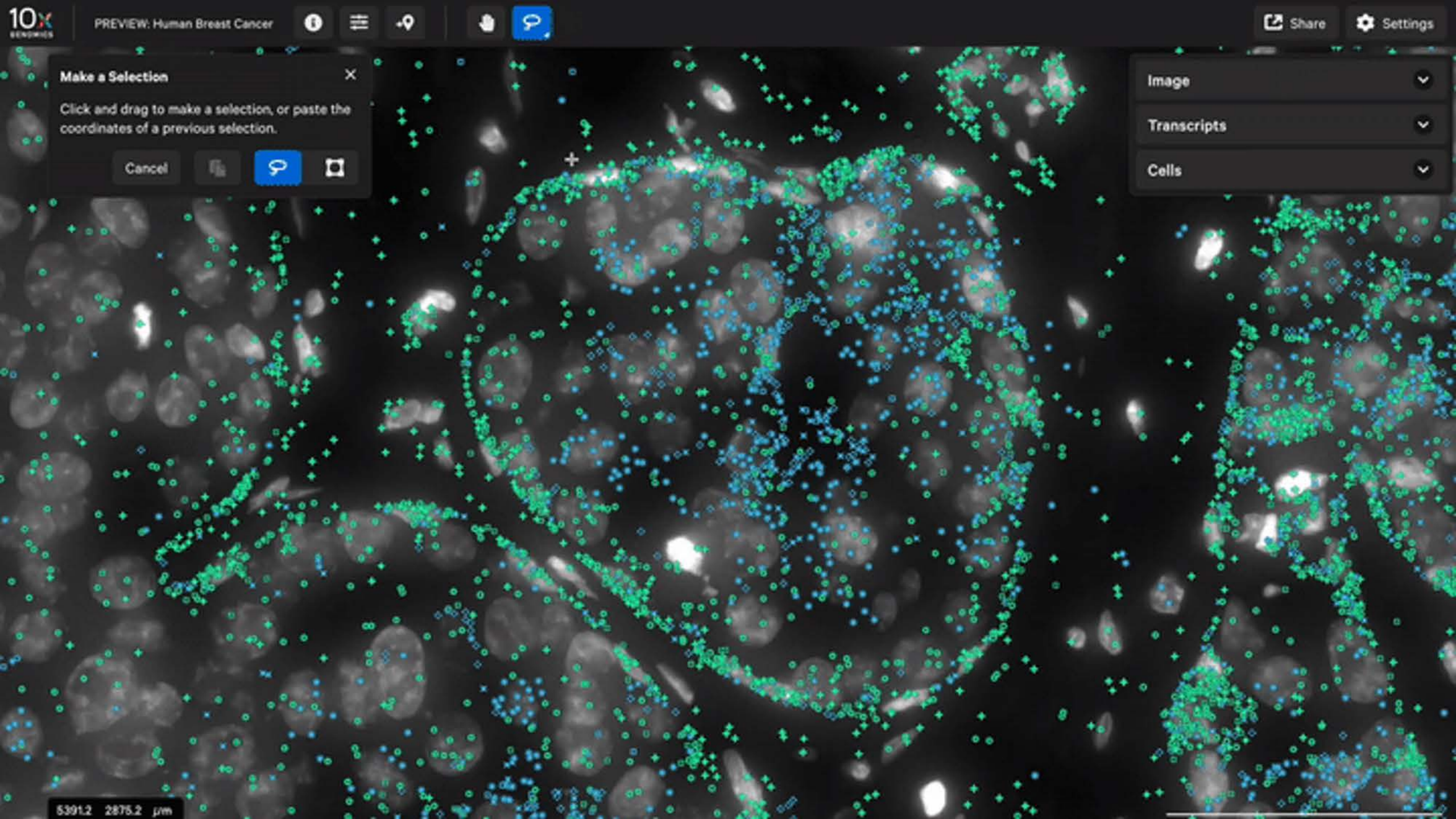
Filter transcripts

- Genes (313)
  - Tumor General (9)
  - Tumor DCIS (4)
  - Tumor Invasive (1)
  - Proliferative (2)
  - Myoepithelial Subtype 1 (5)
  - Myoepithelial Subtype 2 (4)
  - Stroma (6)
  - Endothelial Cells (4)
  - B Cells (3)
  - T Cells (8)
  - Macrophages (10)
  - Dendritic Cells (5)
  - Mast Cells (3)
  - Ungrouped (251)

0/313 genes selected

View transcripts as

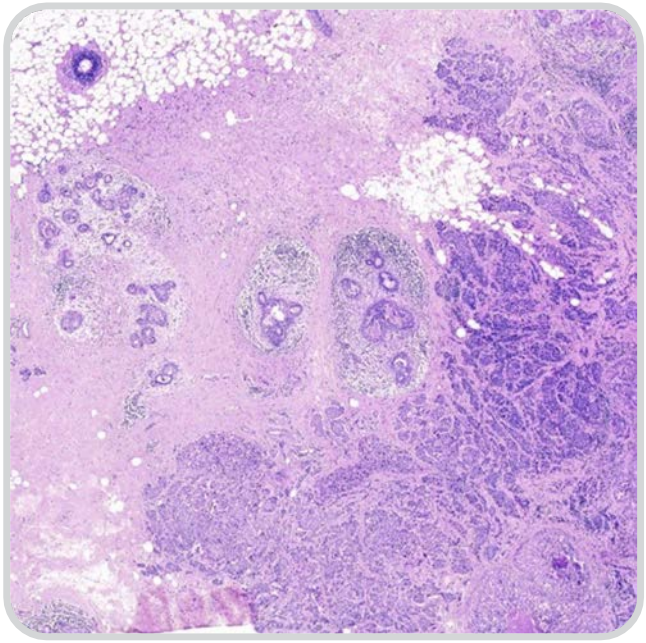
None | Density Map | Points


PREVIEW: Human Breast Cancer
Share
Settings
Make a Selection
Click and drag to make a selection, or paste the coordinates of a previous selection.
Cancel
Image
Transcripts
Cells
5391.2 2875.2 µm

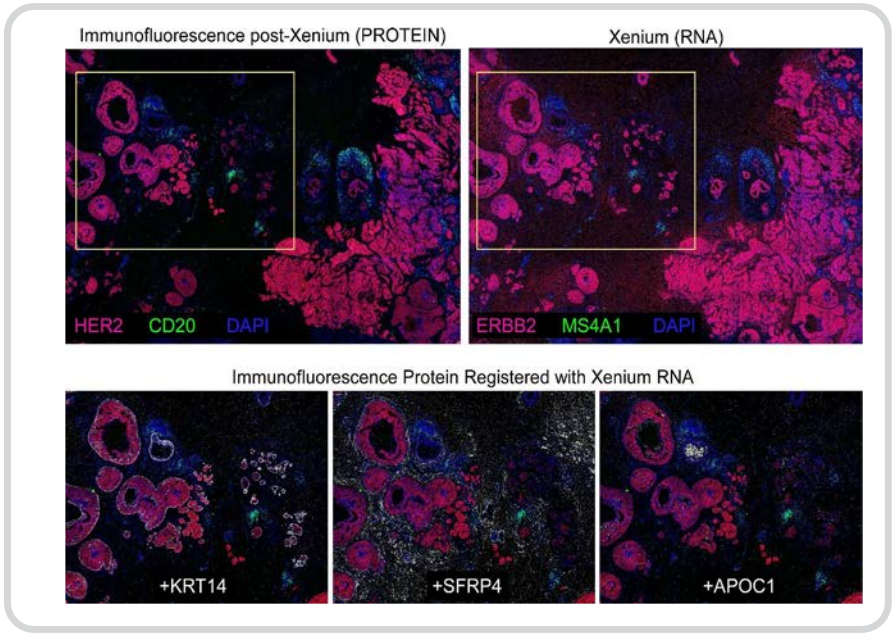
Xenium Leaves Tissue Morphology Intact

Gain additional insights from the same tissue section post run

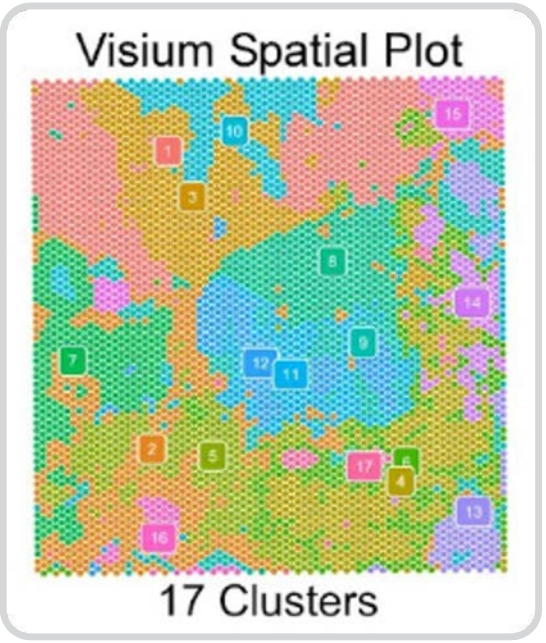
H&E staining



IF staining



Visium via CytAssist



These compatible workflows are done off Xenium Analyzer instrument. These use cases have worked well for the limited number of samples run at 10x and are not formally supported.

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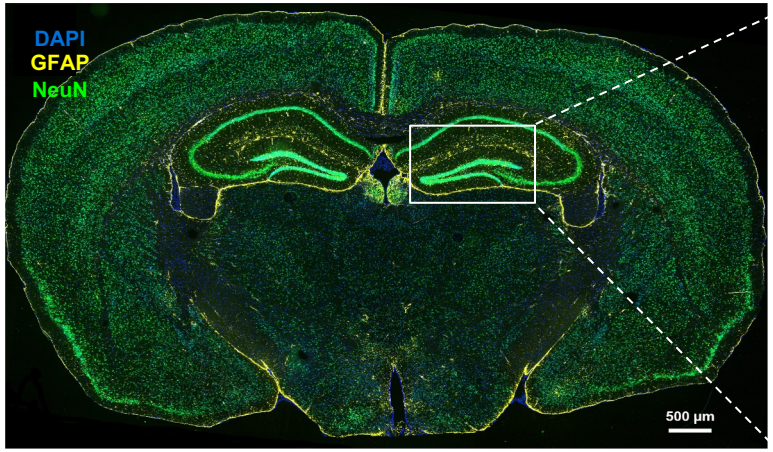
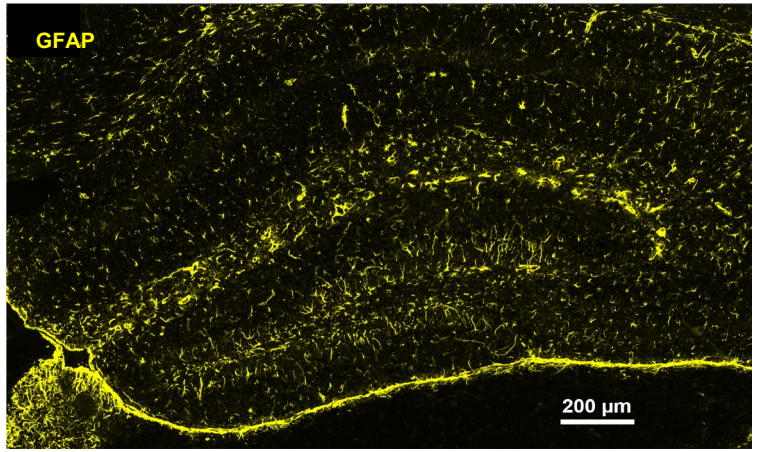
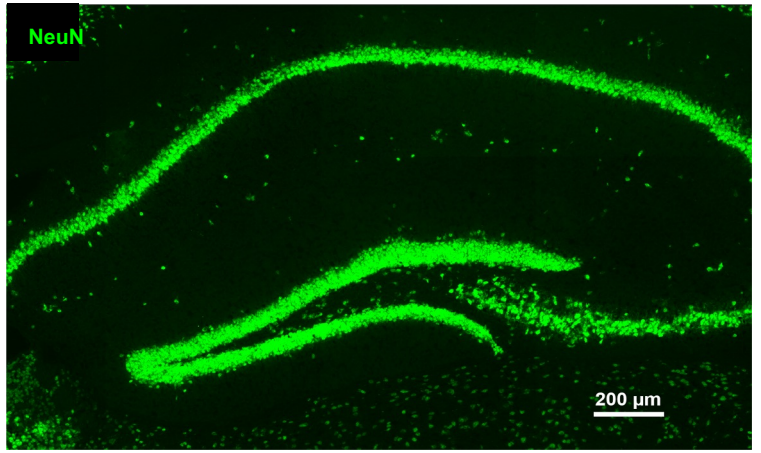
IF staining Post-Xenium is feasible on FF and FFPE samples

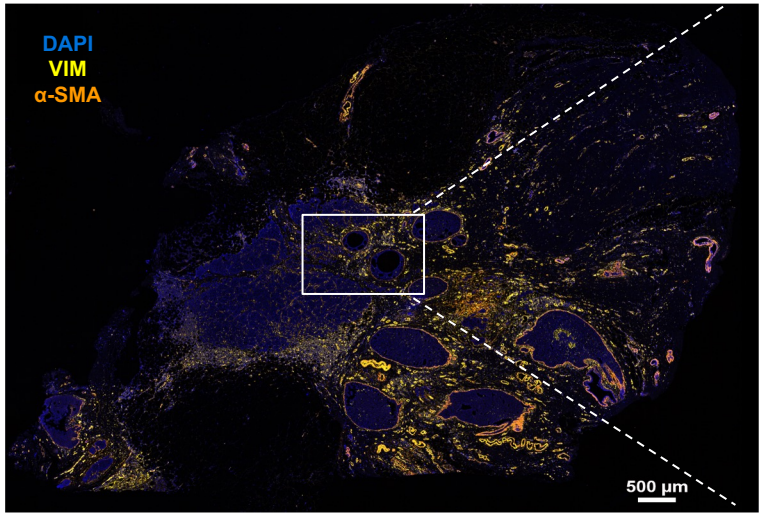
FF mBrain

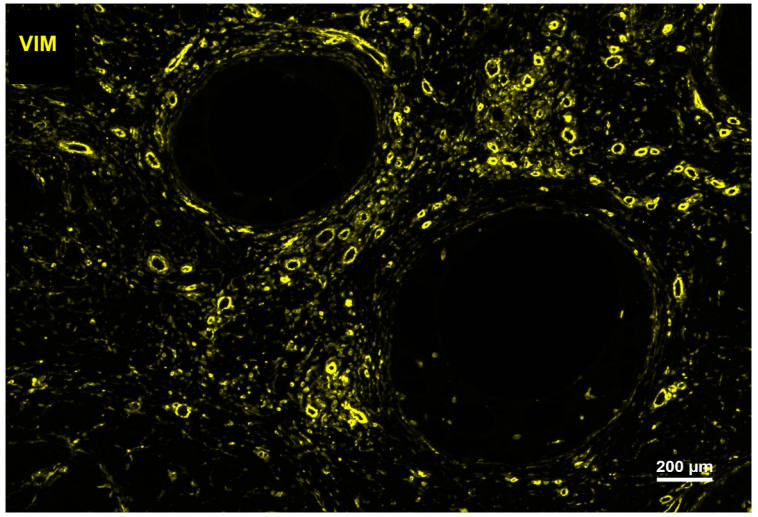


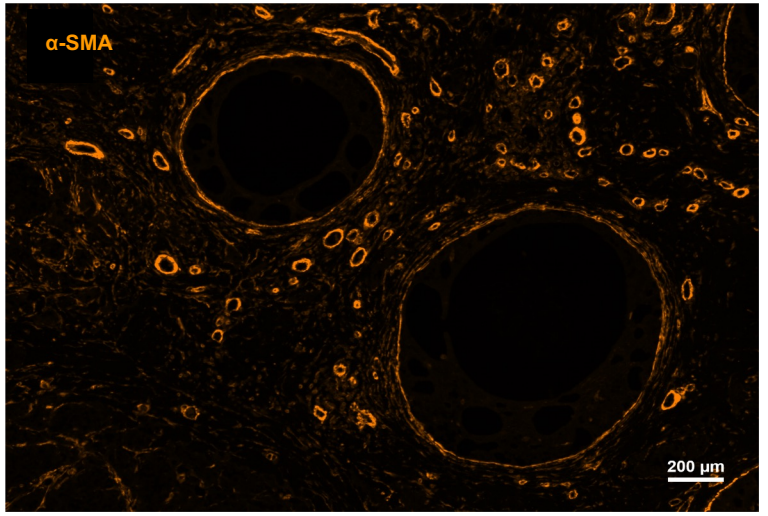




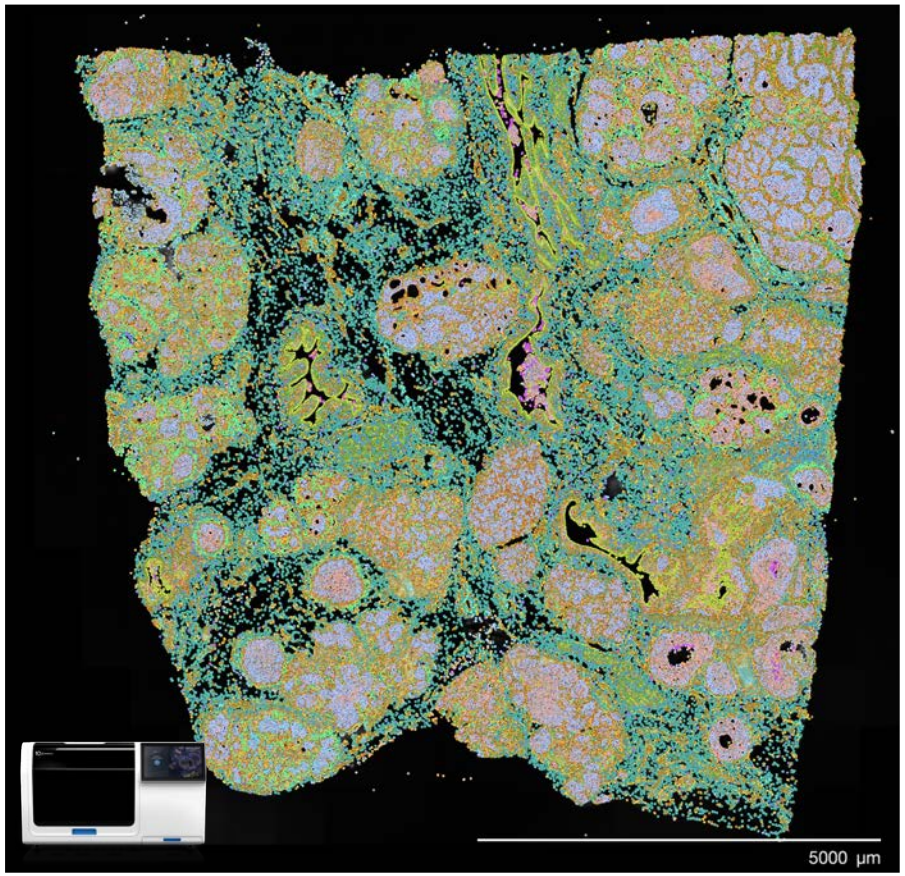
FFPE hBreast

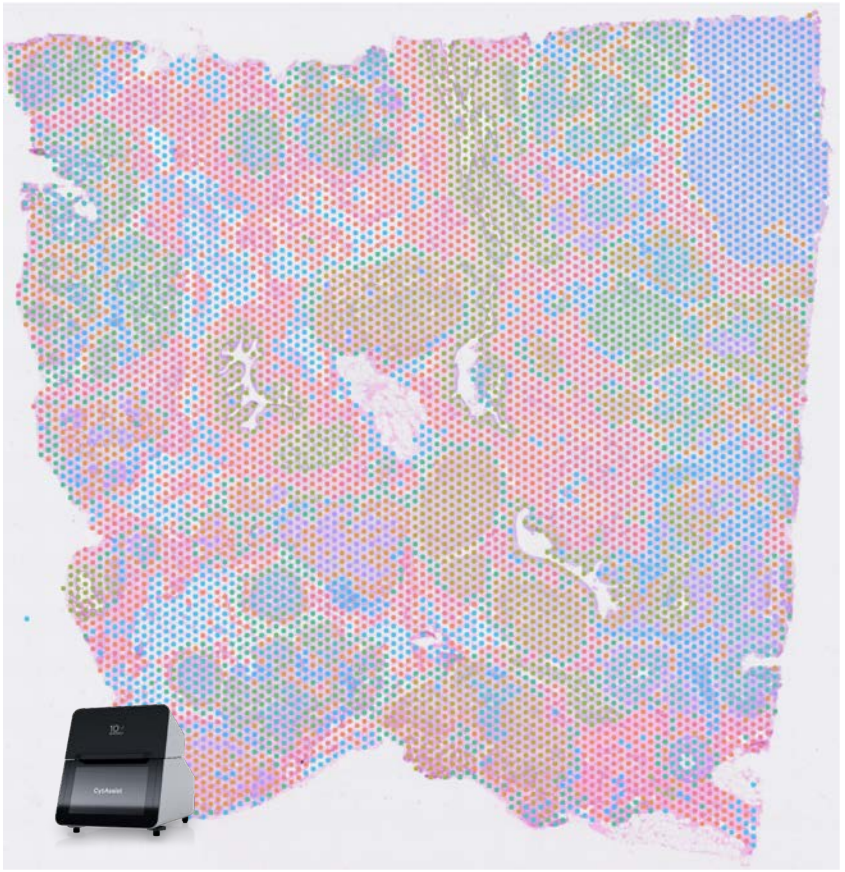






Running Visium Cytassist on the Same Xenium Assay Slide





Human FFPE breast infiltrative ductal carcinoma

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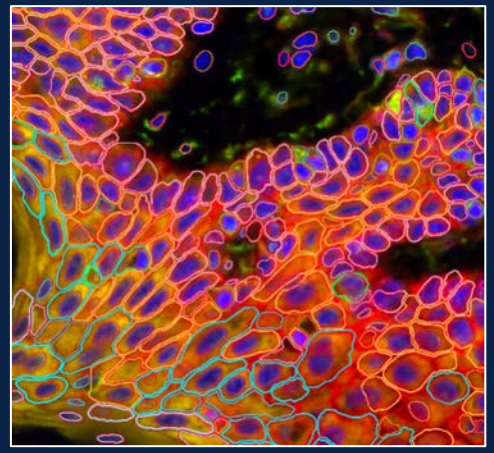
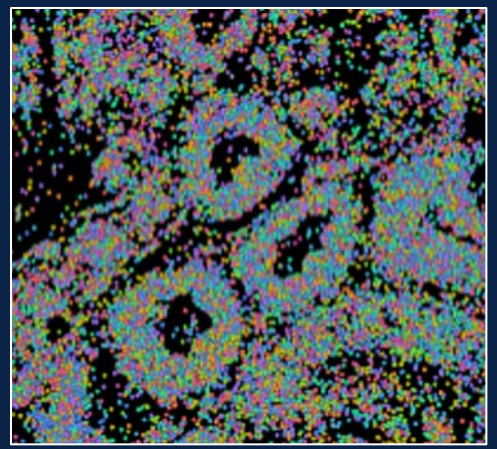
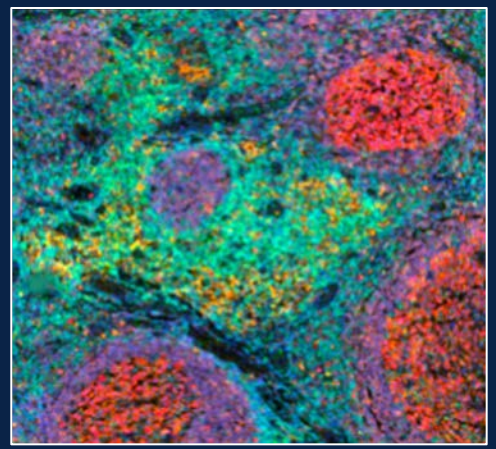
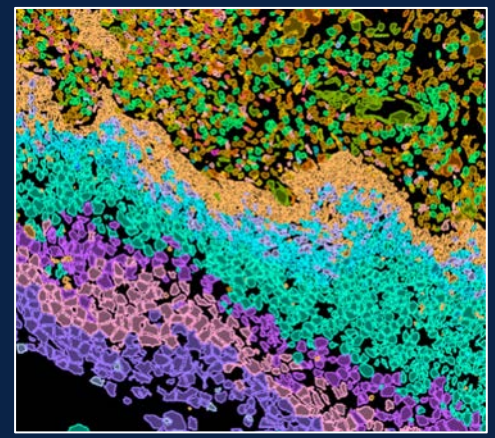
Increased Plexity and Integrated Protein Coming to Xenium

Multimodal Segmentation Broad compatibility	~5000 Plex Panels ~6 day run High quality data	In-line Multiplex Protein Same slide as RNA	1000-2000 Plex Panels Offers more flexibility
			
Shipping Q1	**Q2 2024**	**H2 2024**	**2024 +**

Final product release timelines and configurations subject to change

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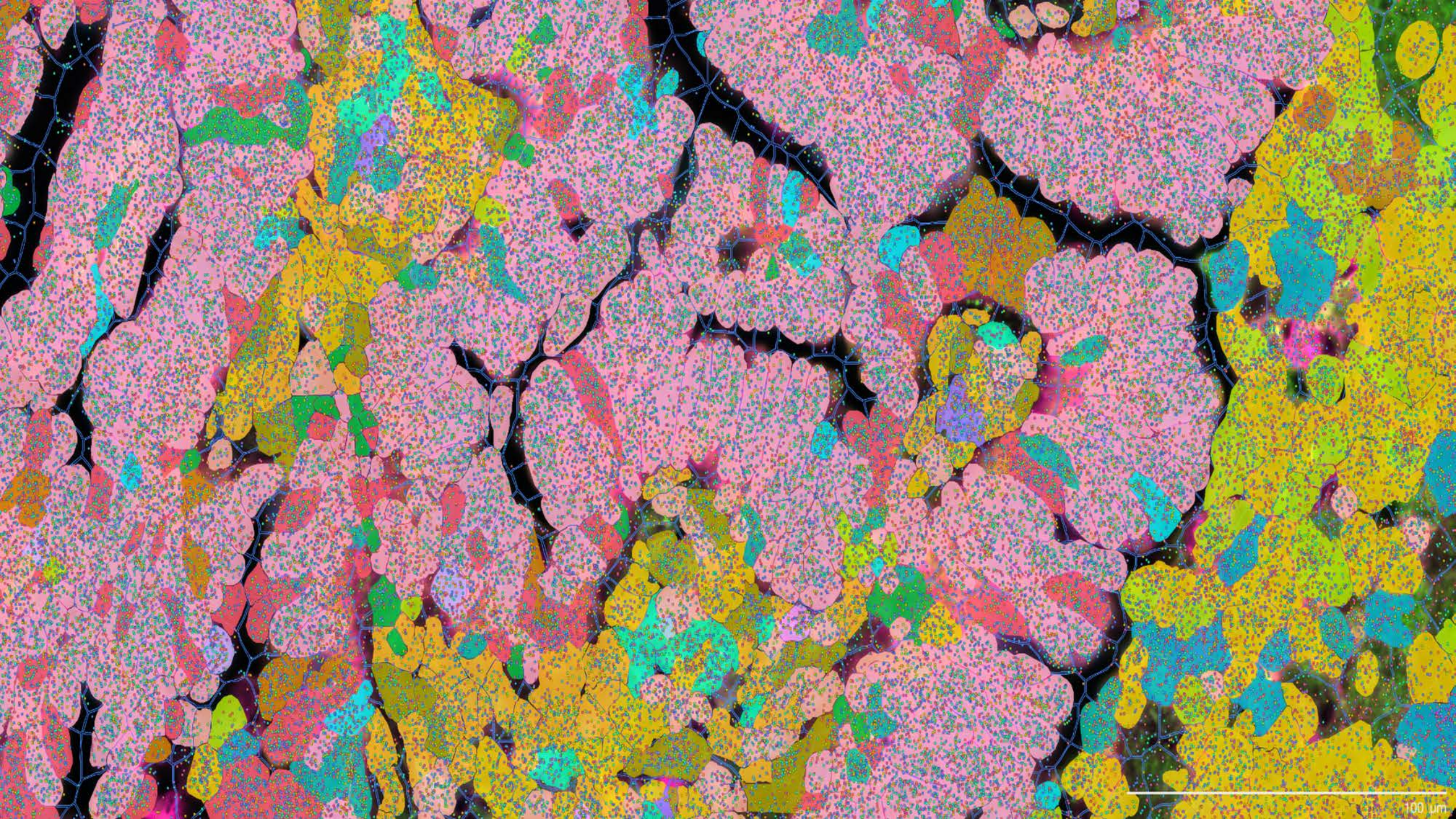
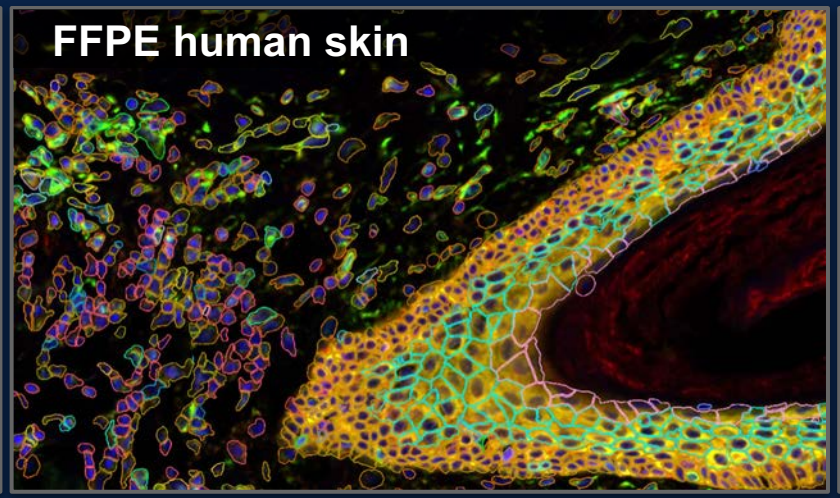
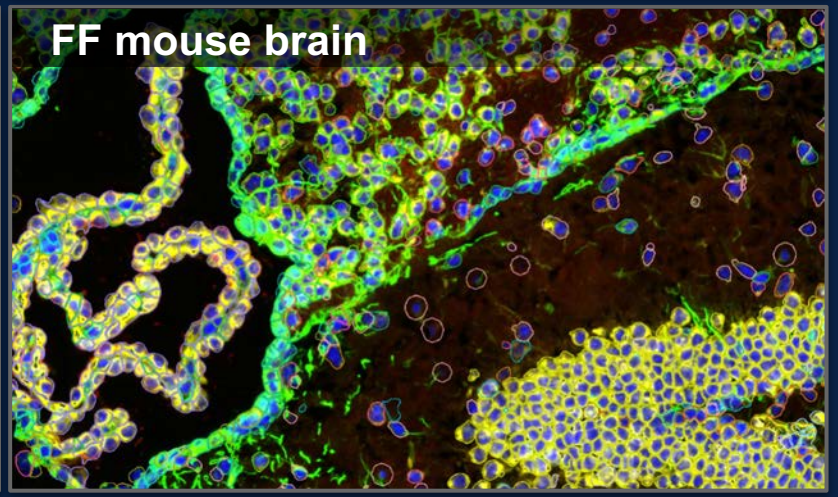

100 µm

Xenium multi-tissue stain-based cell segmentation improves cell delineation across different tissue types





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Xenium 5,000-plex will provide unmatched performance

Broad technology improvements enabling massive codebook expansion

5,000-plex

- Improved chemistry to allow for higher density and large codebook
- Maintains high per-gene sensitivity
- Dilution of high-expressing genes
- Practical to run in 1 working week

4 cm² in 6 days

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Note: Final product release timelines, configurations and estimated run times subject to change.

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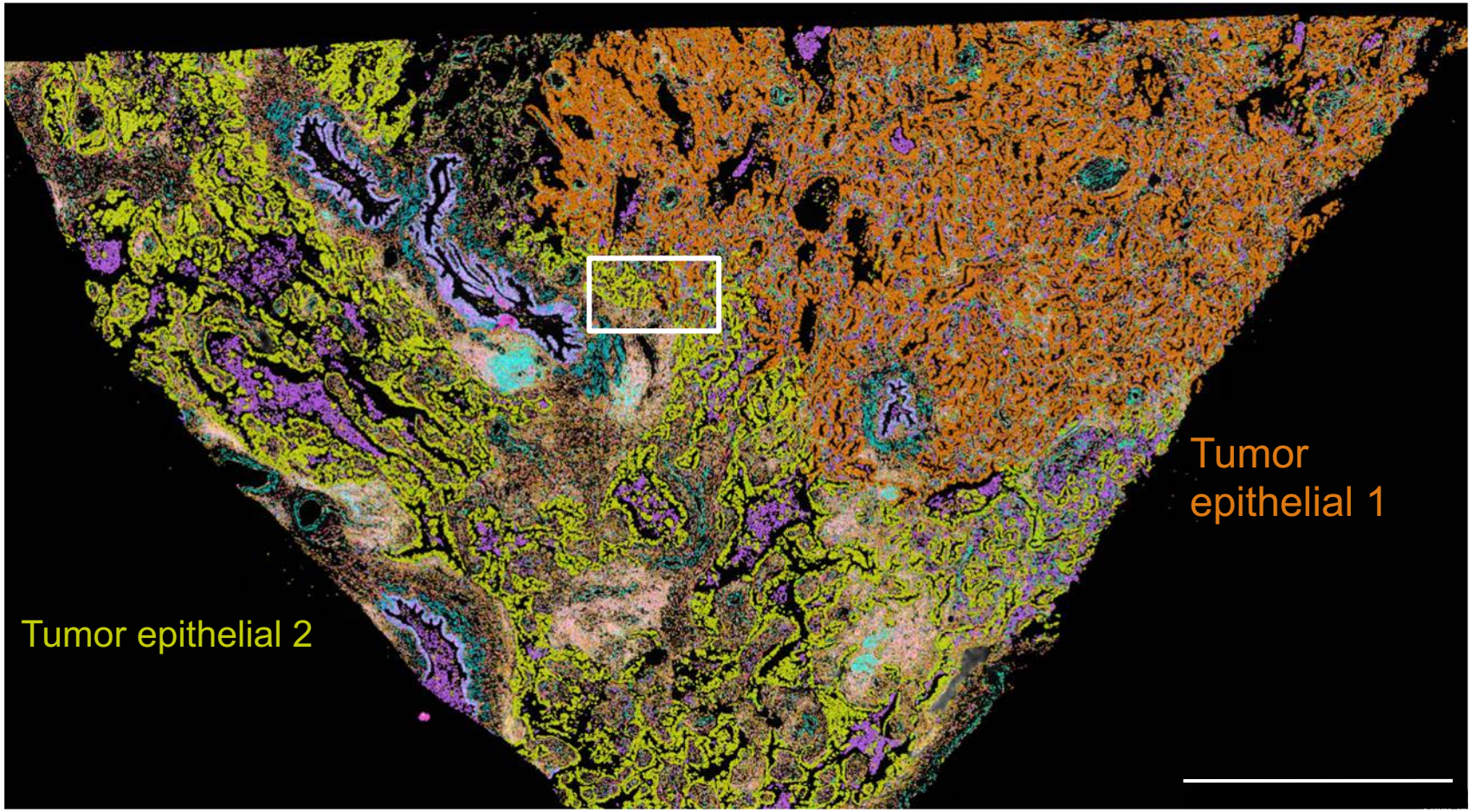
Xenium 5,000-plex assay detects a wide range of cell types in lung adenocarcinoma



Cells detected	289K
Median transcripts/cell	393
Transcripts per 100 μm²	877
False discovery rate	0.6%
Total high-quality transcripts	231M
DV200	39

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High-plexy RNA and protein profiling in the same Xenium run

ONE instrument run

ONE sample

TWO complementary readouts

- Menu of oligo-conjugated antibodies for seamless multiplexing on instrument
- Unified workflow for integrated RNA + protein profiling
- Validated, pre-titrated protein panels for ease of use

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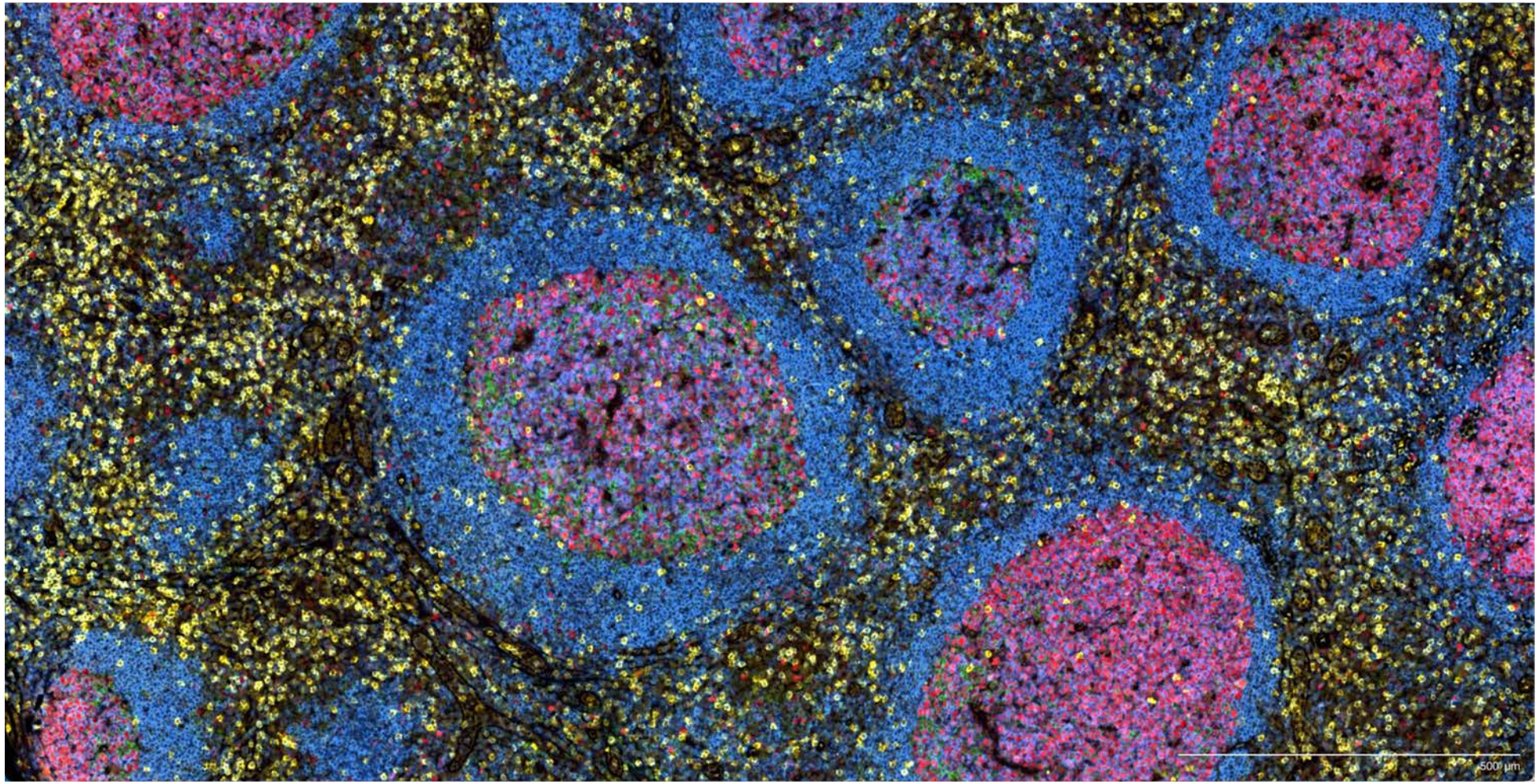
Simultaneous 20-plex protein + 1,000-plex RNA



CD45RA (Naive B & T cells) + PD-1 (T cells) + CD8A (Cytotoxic T cells) + PCNA (Proliferation)

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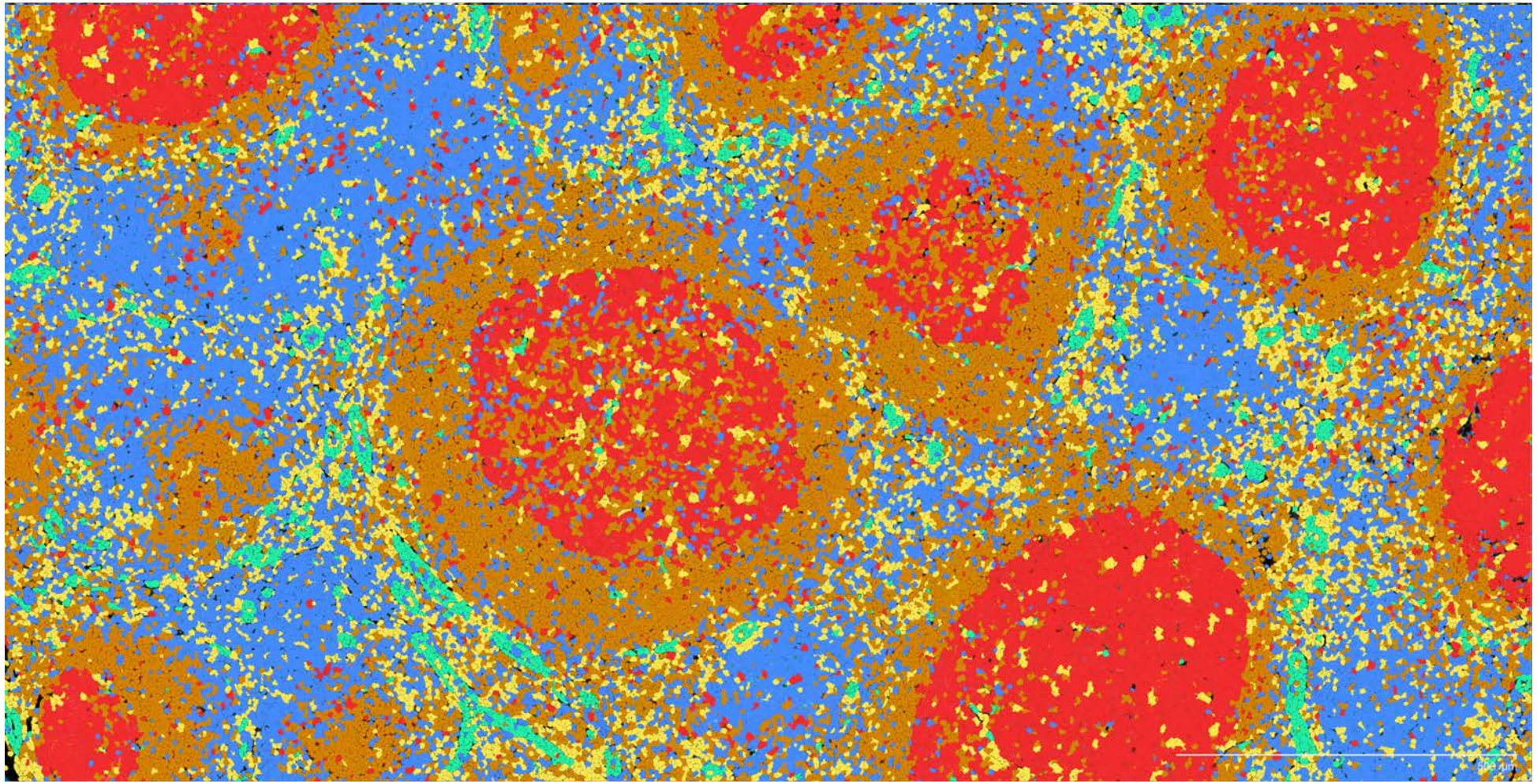
Simultaneous 20-plex protein + 1,000-plex RNA



RNA-assigned cluster labels

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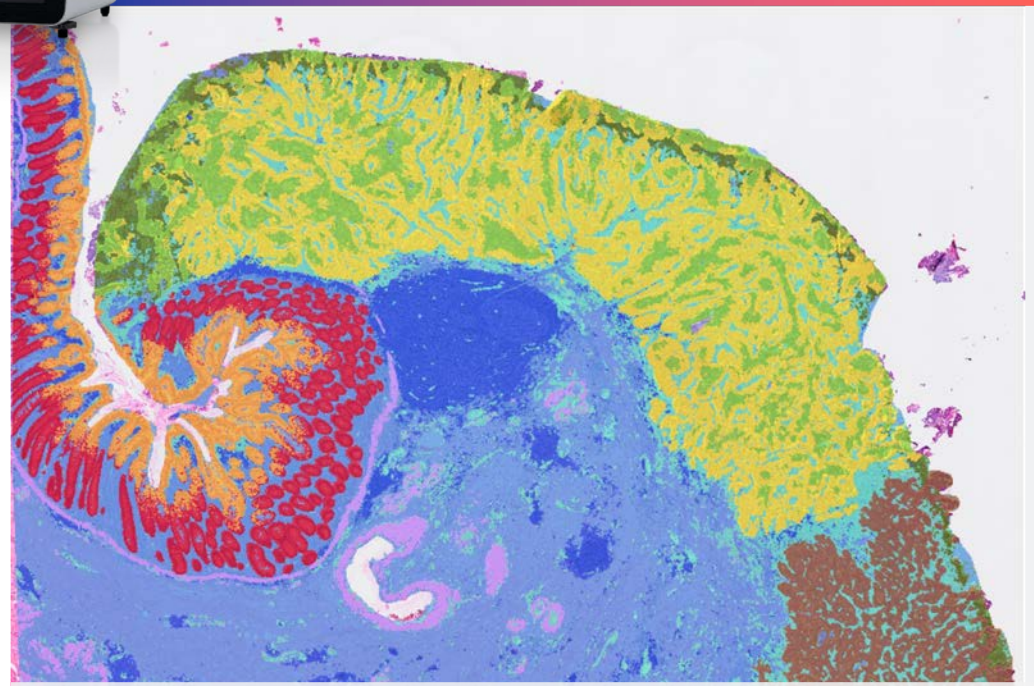
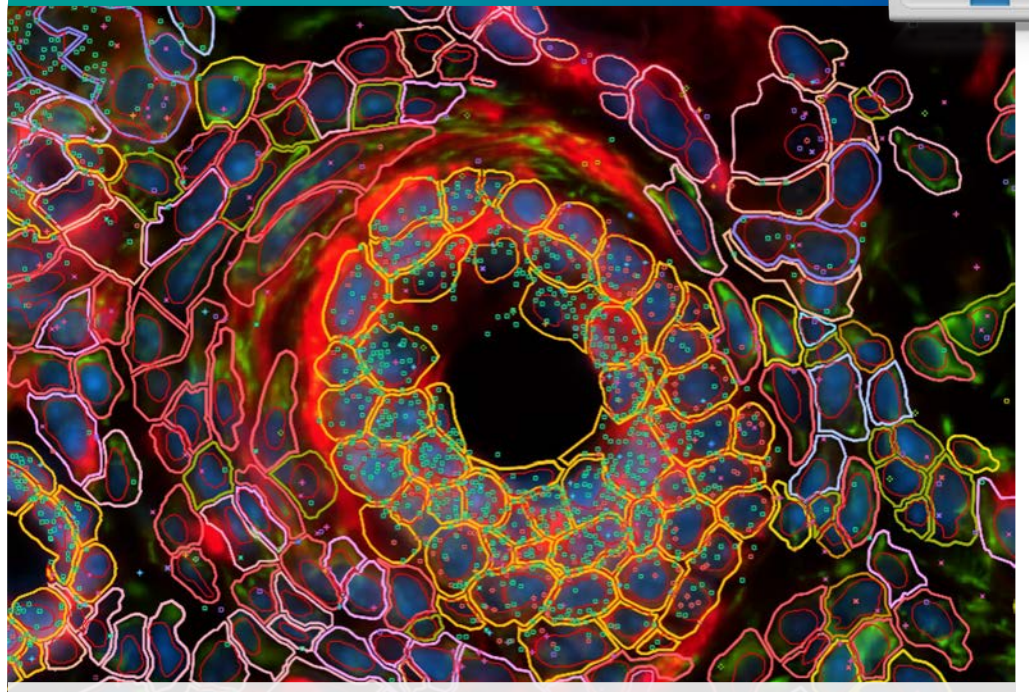
Unlock the Full Spectrum of Spatial Biology

Visium HD is Unbiased Discovery



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

Xenium is Precision Insights



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

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Chromium

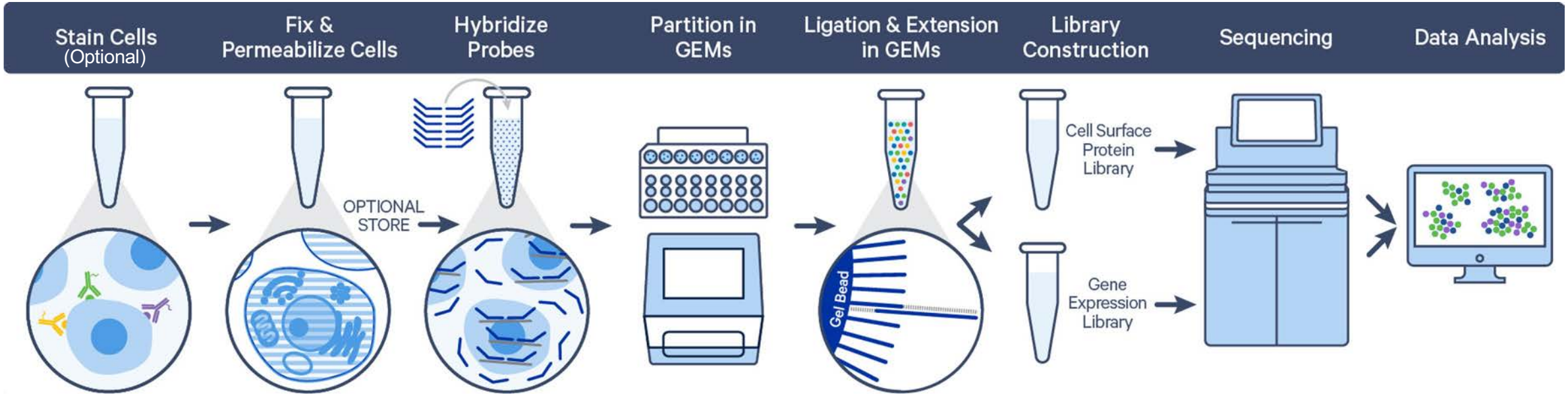
10x GENOMICS

Toolkit

Visium

Xenium

Single Cell Gene Expression Flex – A Streamlined Workflow



- Sensitive, probe-based **whole transcriptome assay**
- Assay compatible with Feature Barcode technology for **profiling cell surface proteins** (with TotalSeq™-C antibodies)
- Does not depend on polyA capture; covers more than 18,000 human or mouse genes

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Gene Expression Flex supports FFPE tissues

Two robust methods for FFPE dissociation

FFPE Block

FFPE Curls

Dissociated tissue

- We are providing **two methods** for FFPE tissue dissociation, as described in Demonstrated Protocol (CG000632)
  - Instrument-based workflow with the gentleMACS™ Octo Dissociator
  - Manual workflow with a pestle
- Appendix provides additional guidance regarding:
  - Cell counting recommendations
  - Cell yields derived from >20 samples with section thickness and cross-section size
- Each method has been extensively tested with the tissues listed below

HUMAN

- Brain (healthy, Alzheimer's, glioblastoma)
- Breast cancer
- Colorectal cancer
- Heart
- Kidney
- Lung (cancer, healthy)
- Ovarian cancer
- Lymph Node (reactive, healthy)
- Liver (cancer, healthy)
- Pancreas
- Prostate cancer
- Skin melanoma
- Testis
- Thymus
- Tonsil
- Spleen

MOUSE

- Brain (cerebellum, cortex, hippocampus)
- Heart
- Kidney
- Liver
- Spleen
- Thymus

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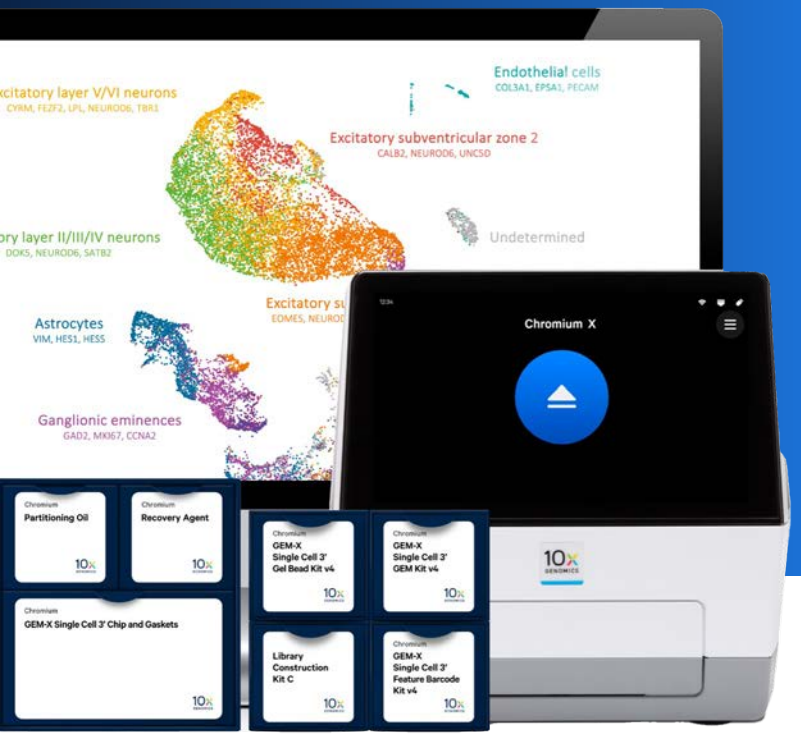
Introducing the Next Generation Single Cell Solutions

New versions of 3’ and 5’



- **Efficient partitioning**: tens of thousands of cells in 6 minutes
- **Built to scale**: 500–20K cells per channel, for up to 160K cells per run, run up to 8 samples in parallel
- **Cell size flexibility**: no lower limits
- **High cell capture rates**: up to 80% cell recovery
- **Low doublet rates**: 0.4% per 1,000 cells
- **Instrument compatibility**: Chromium X series

- Powered by **GEM-X Technology**

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Thank you! Questions?

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