# Dive deeper into disease pathology with single cell and spatial multiomics

Nordic Pathology Meeting 2025

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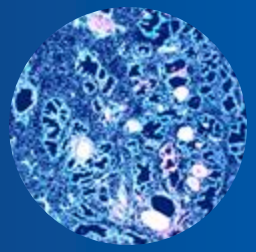
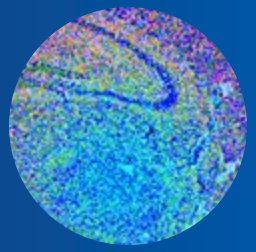
# Biology is immensely complex

## And needs to be measured at large scale and high resolution



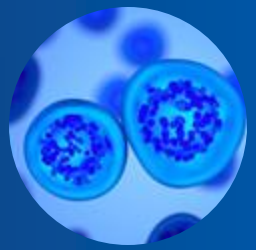
### Enormous complexity within each cell

Interactions of millions of different molecules and molecular machines



### 40 trillion cells in the human body

Each cell contains its own set of expressed genes



### Cells form tissues, which form organs

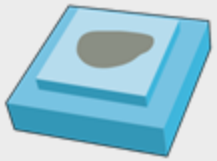
Each tissue comprises a large diversity of cells and cell types

10X GENOMICS

# New technological innovations are unlocking insights from clinical research samples at **scale**

**Table Stakes**

### FFPE Compatible



*Unlock single cell and spatial insights in archival tissue*

### High-Throughput

*Sufficiently power insights across heterogeneous samples*

### Cost Efficient

*Multiplexing, automation, and throughput reduce cost/insight*

**10x Enabled**

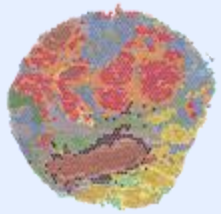
### Cellular Resolution

*Discover cell-type-specific biomarkers*

### Multiple Features Per Cell

*Simultaneously measure multiple features from a sample*

### Spatial Context

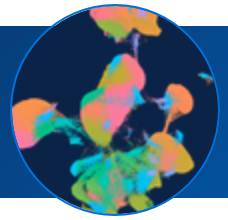


*Resolve spatial distribution of biomarkers in tissue*

# From discovery to development with complementary workflows

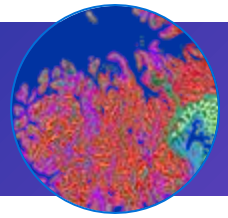
Discovery → Preclinical → Clinical trials

**Chromium** Single Cell



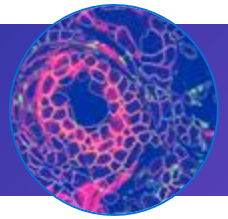
Unbiased whole transcriptome results for dissociated samples, up to millions of cells

**Visium** Spatial



Unbiased whole transcriptome results for tissue sections with spatial context and single cell–scale resolution

**Xenium** Spatial



Targeted results for up to 5,000 genes with subcellular spatial context

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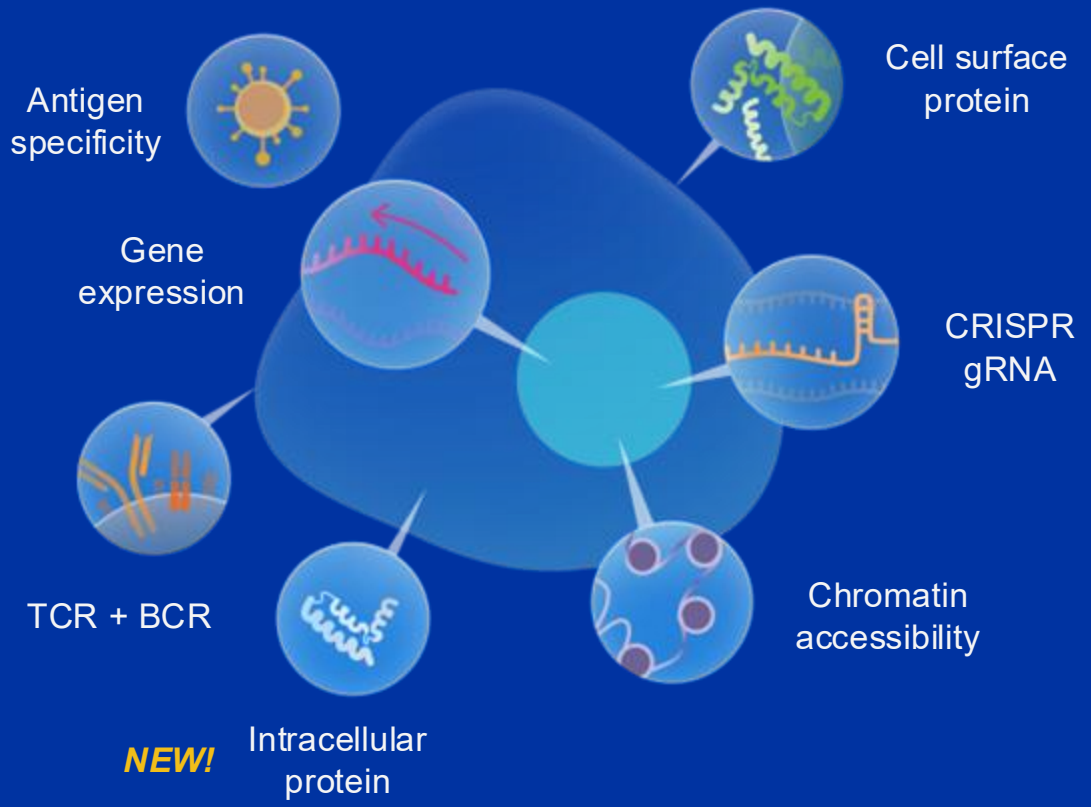
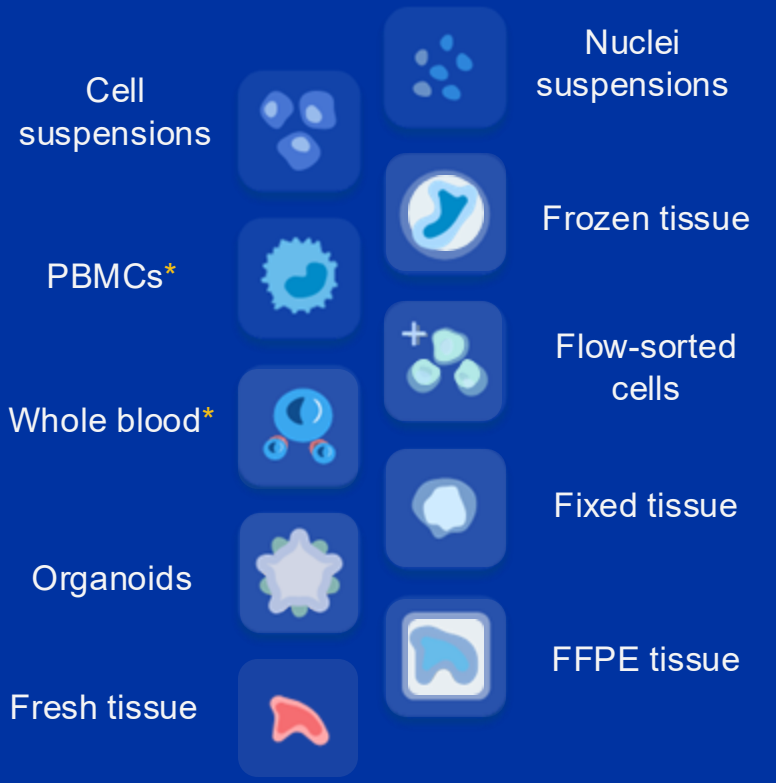
# Comprehensive multiomics and sample access

## Multiomics




Species agnostic

## Sample types




* ***NEW!*** Fixation protocols

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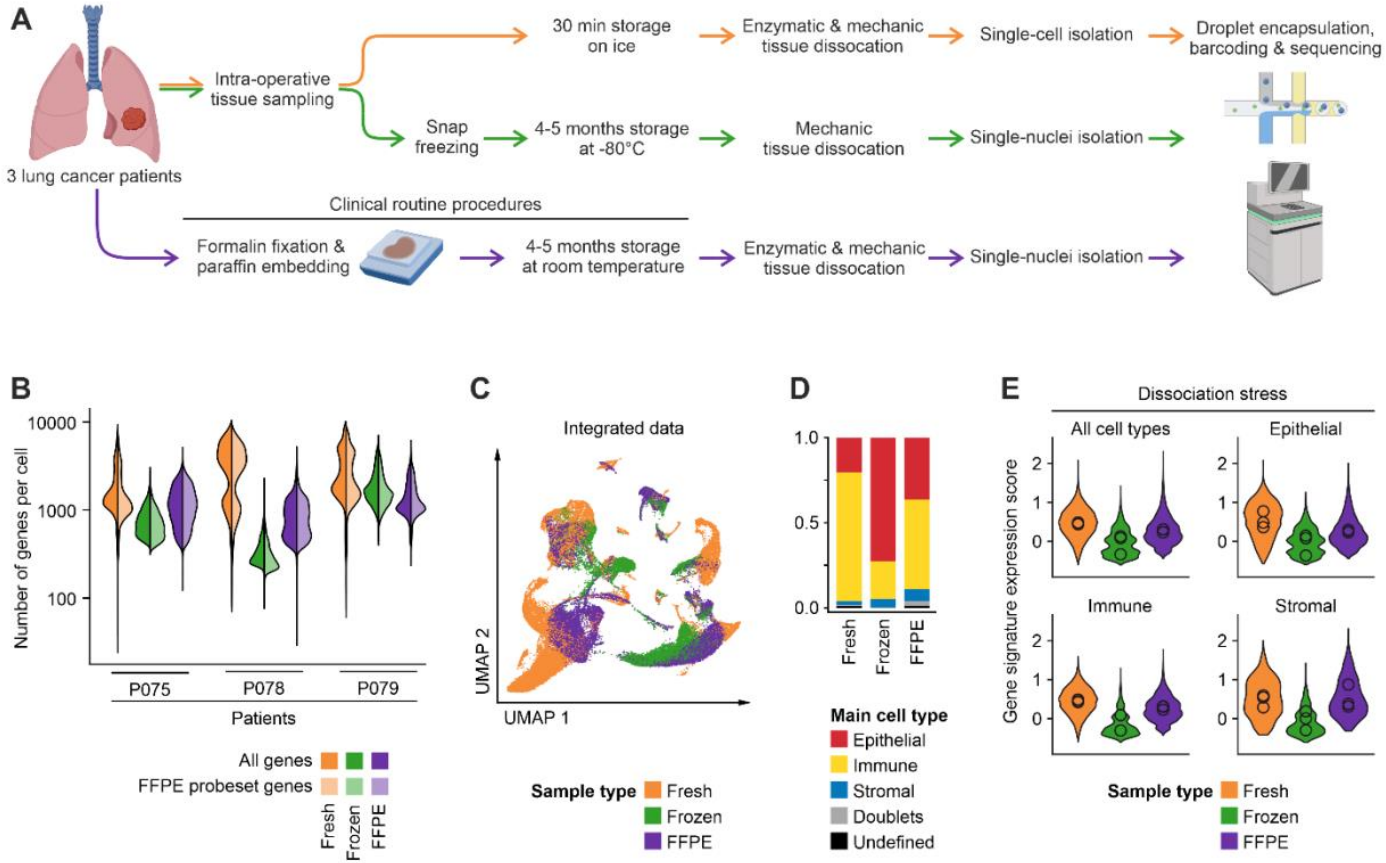
# Robust detection of clinically relevant features in single-cell RNA profiles of patient-matched fresh and FFPE lung cancer tissue

Researchers benchmarked single-cell gene expression profiles from patient-matched fresh, cryopreserved and FFPE cancer tissue.

**FFPE tissue robustly preserved clinically relevant information on cell types and patient characteristics comparable to fresh tissue and can be employed for the robust detection of clinically relevant traits on the single-cell level.**

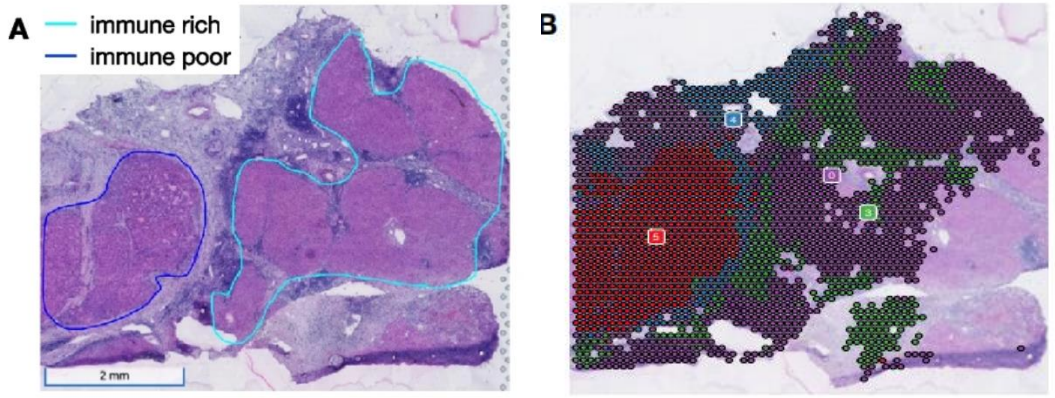
doi: https://doi.org/10.1101/2023.04.25.538273;

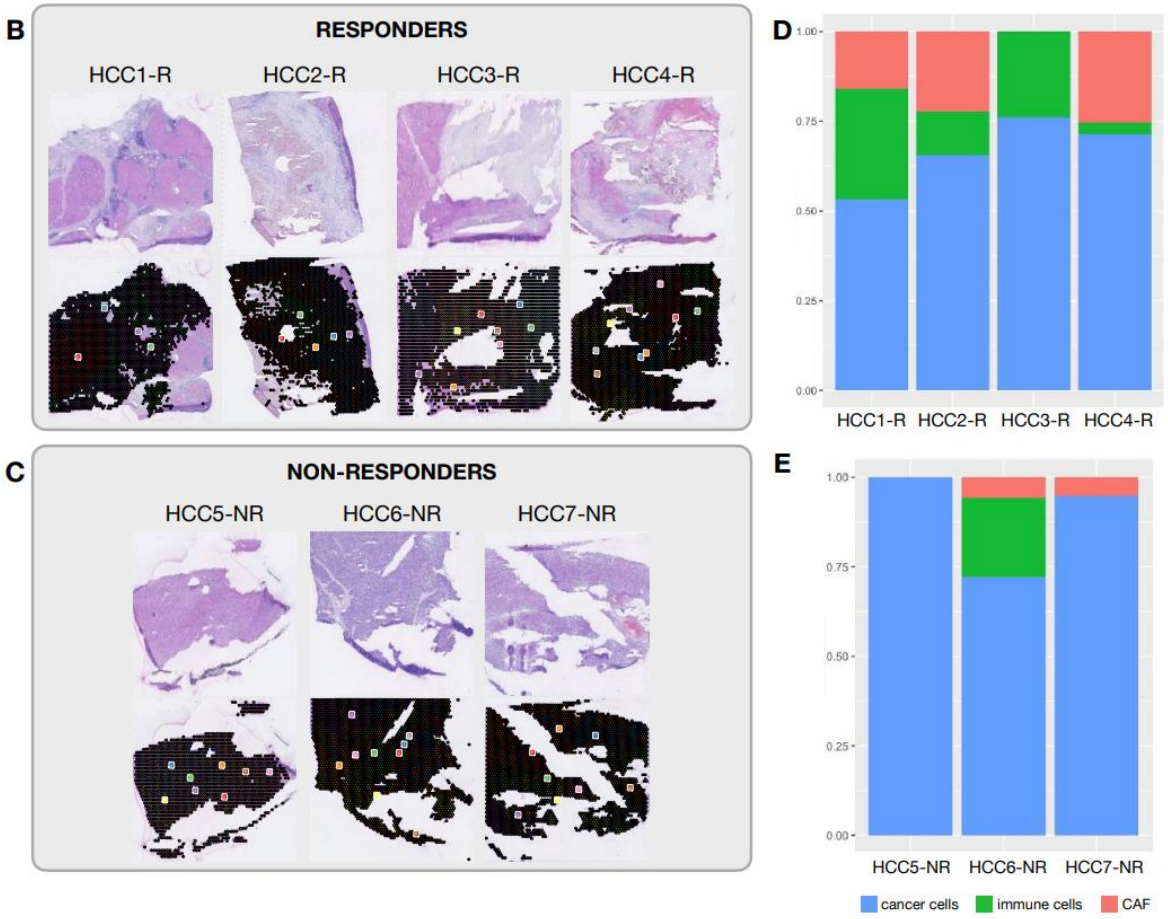
Disease characterization



# Spatial transcriptomics analysis of neoadjuvant cabozantinib and nivolumab in advanced hepatocellular carcinoma identifies independent mechanisms of resistance and recurrence

Data show that responses to modern systemic therapy in HCC are associated with distinctive molecular and cellular landscapes and provide new targets to enhance and prolong responses to systemic therapy in HCC.

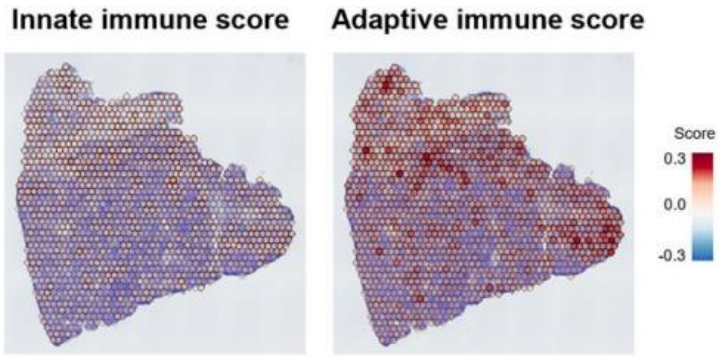



https://doi.org/10.1186/s13073-023-01218-y

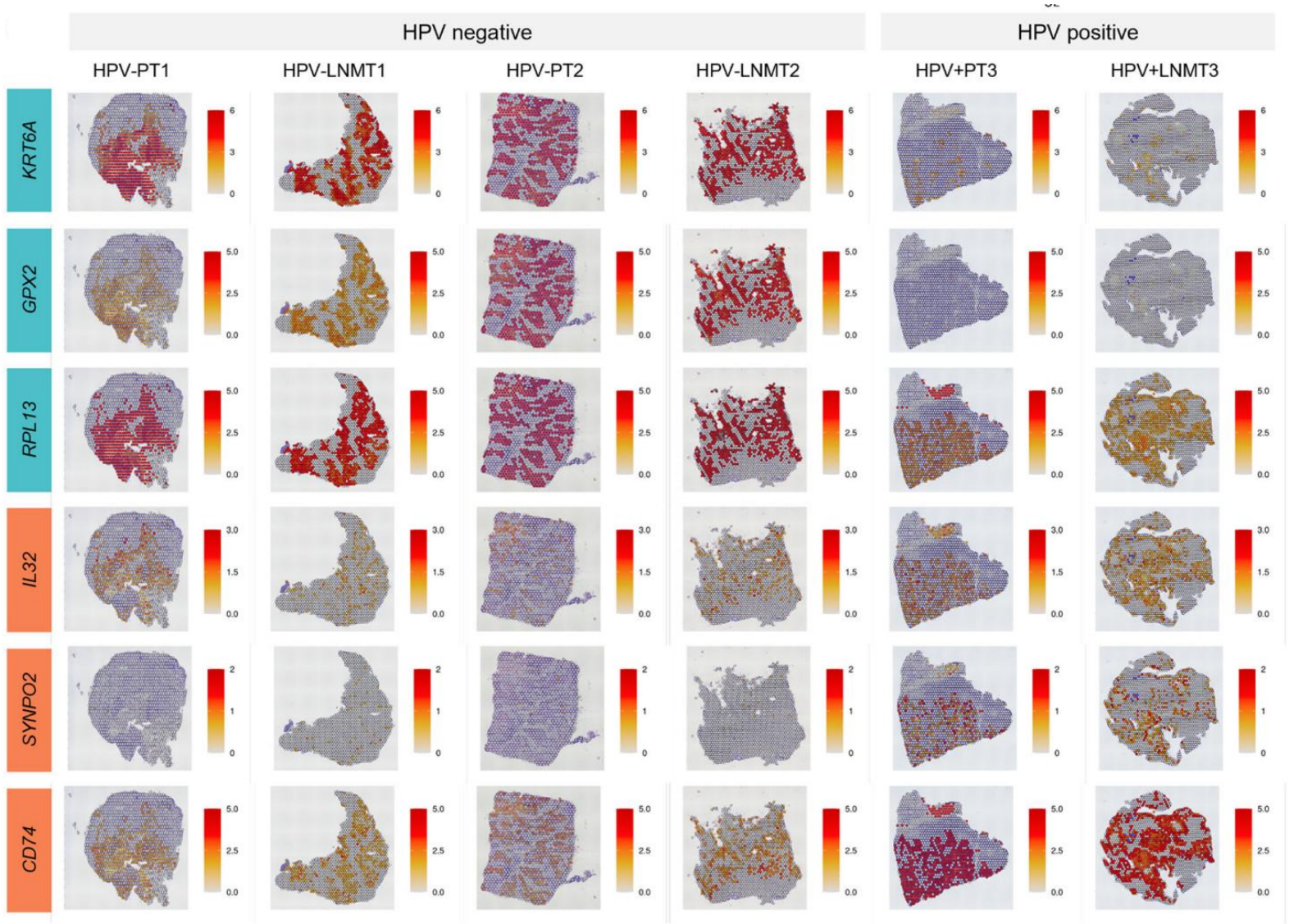
# Deciphering head and neck cancer microenvironment: Single-cell and spatial transcriptomics reveals human papillomavirus-associated differences

HPV +




Disease characterisation

DOI: 10.1002/jmv.29386

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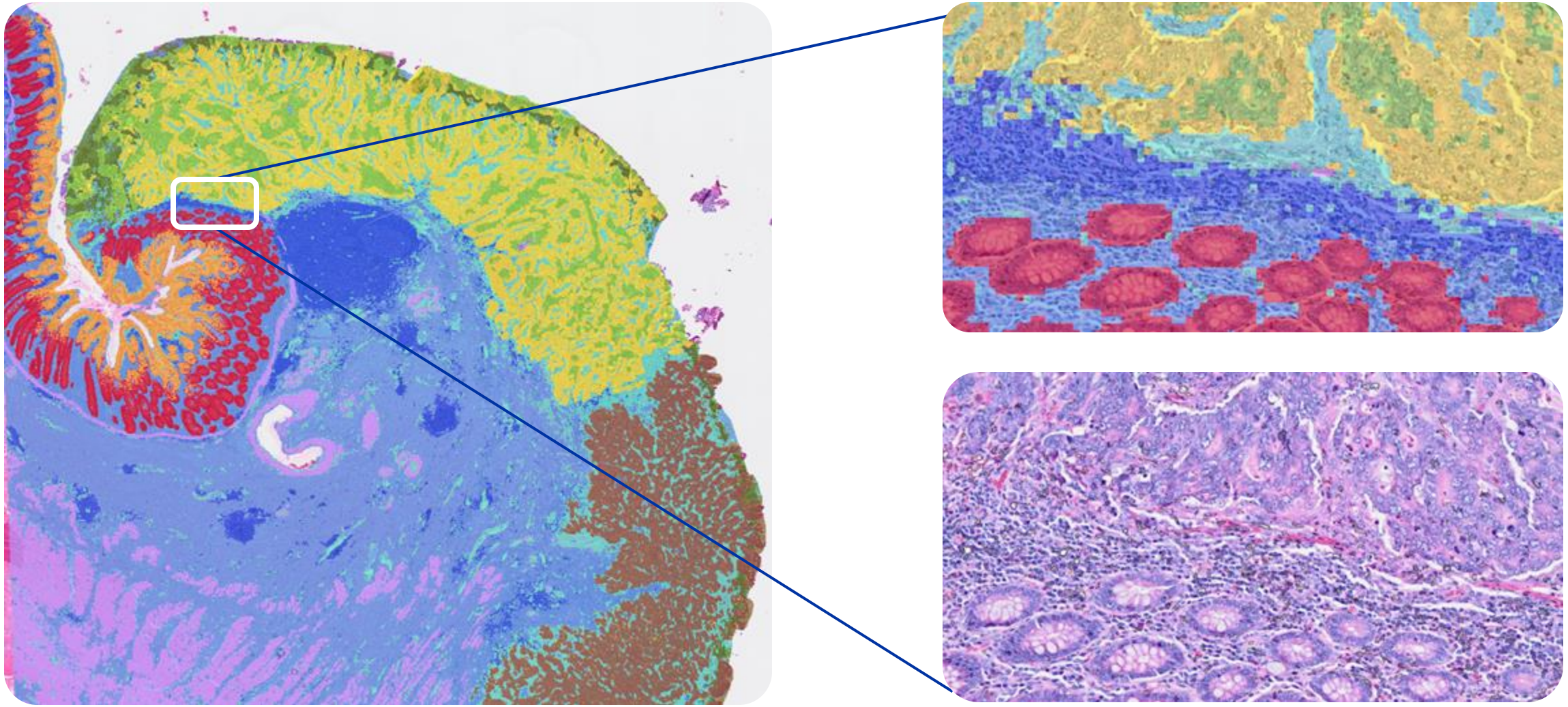
# Visium HD: Enhancing Histology with the Whole Transcriptome

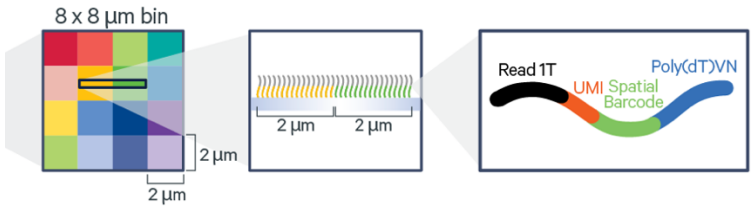
Colon Cancer FFPE: **18,058** genes detected

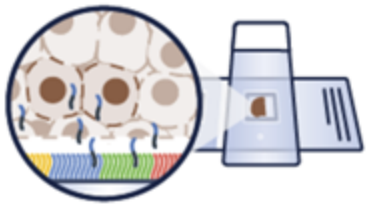


# Utilizing the Power and Ease of CytAssist

Two tissue slides

Visium HD slide

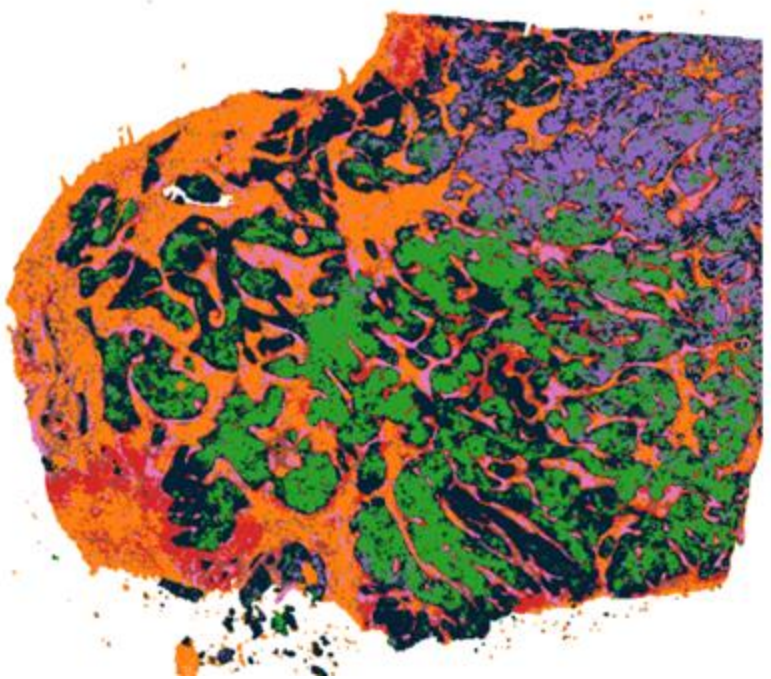


**Visium HD slide**
*Target molecules now captured on slide*

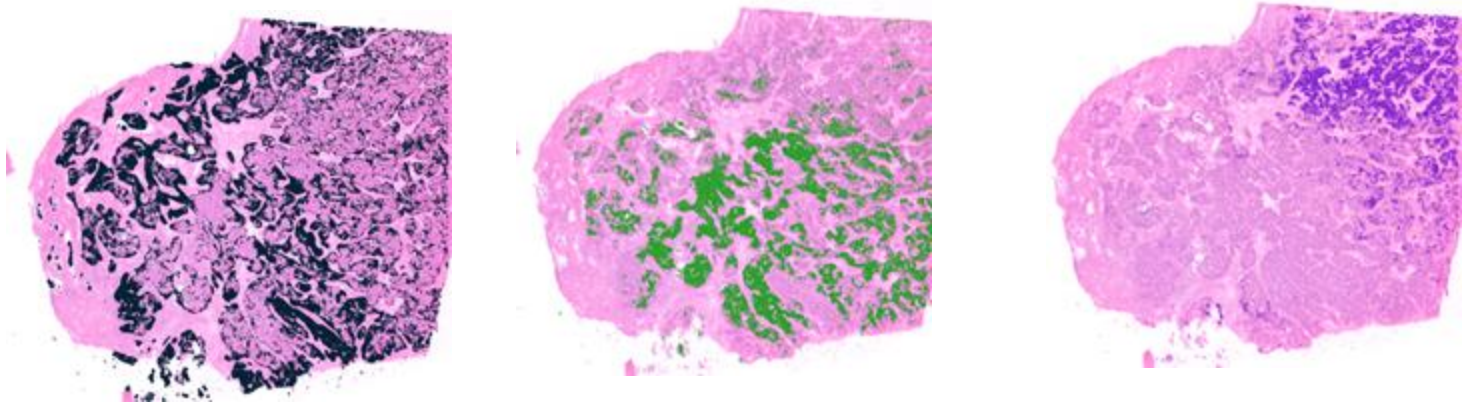
# Explore the Tumor Microenvironment with High Sensitivity

## Fresh frozen human breast cancer



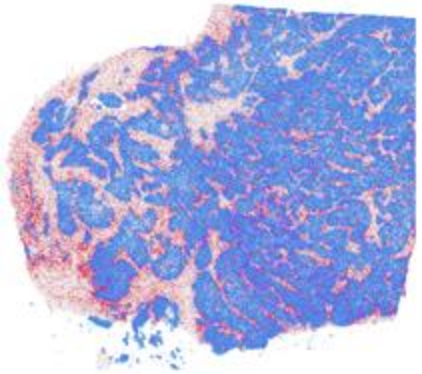
Unsupervised clustering (8 µm x 8 µm bins)

## Identify distinct cancer epithelial cell clusters and overlay H&E image



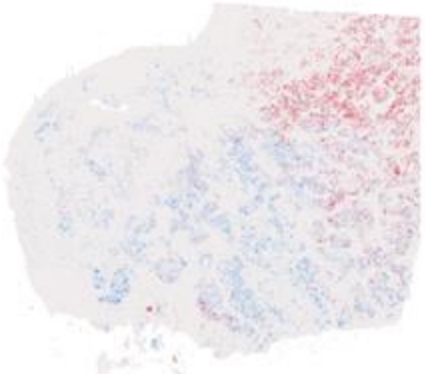
## Reliably differentiate known cancer marker expression within the TME

*LGALS1* *ERBB2*



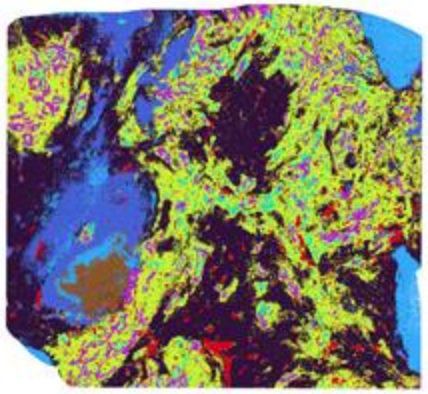
*TOP2A* *SLPI*

*HMGCS2* *CYP1B1*

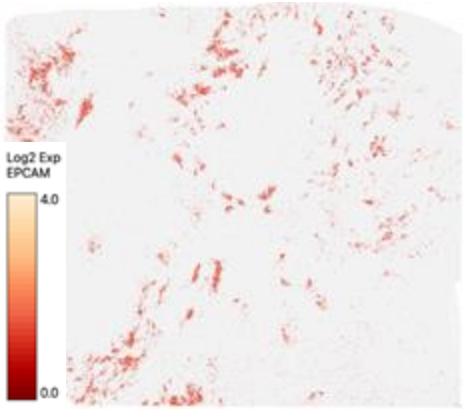


# Precisely Reveal Tumor Heterogeneity in Fixed Frozen Tissues

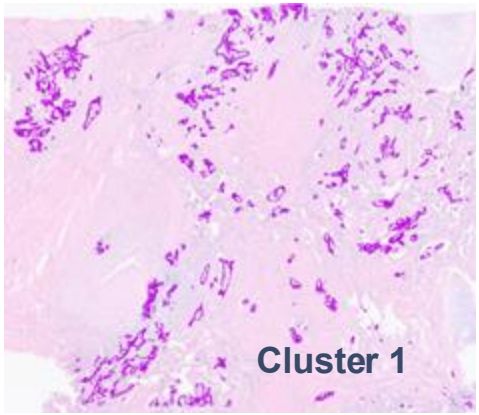
## Human lung cancer

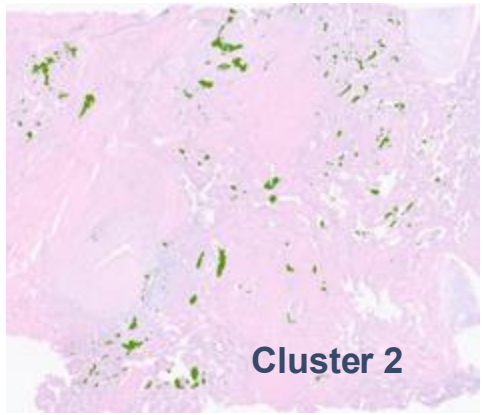


## Identify epithelial cells

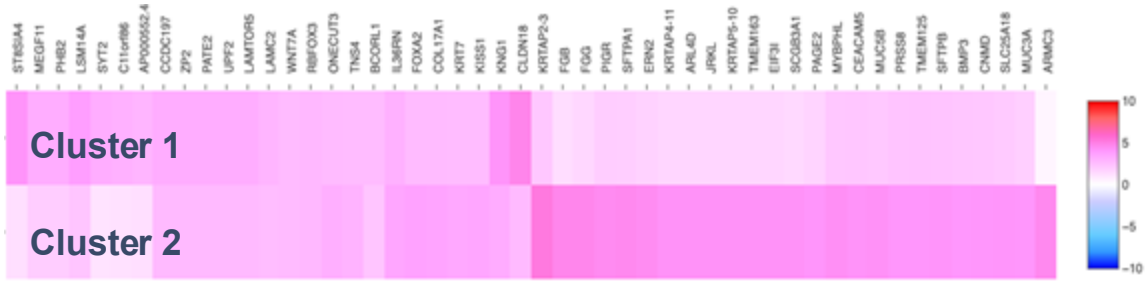



## Differentiate between different epithelial cell populations

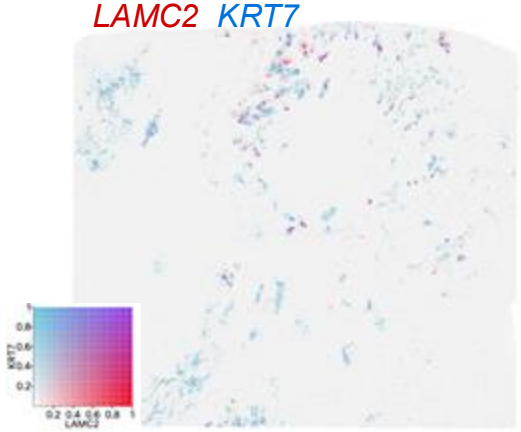


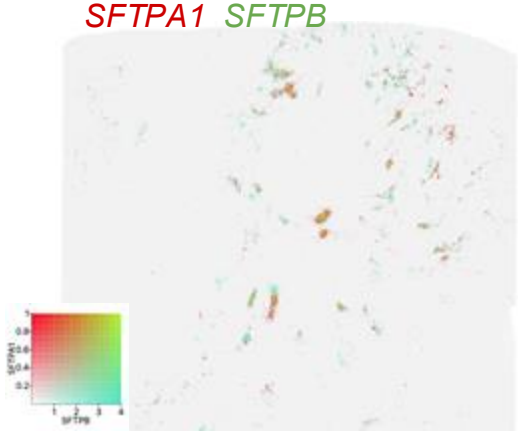

## Reveal differential gene expression & gene localization




*LAMC2* *KRT7*



*SFTPA1* *SFTPB*



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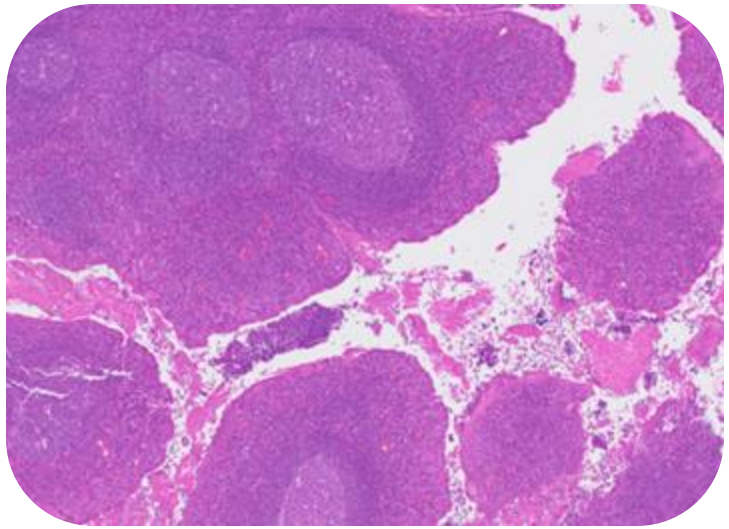
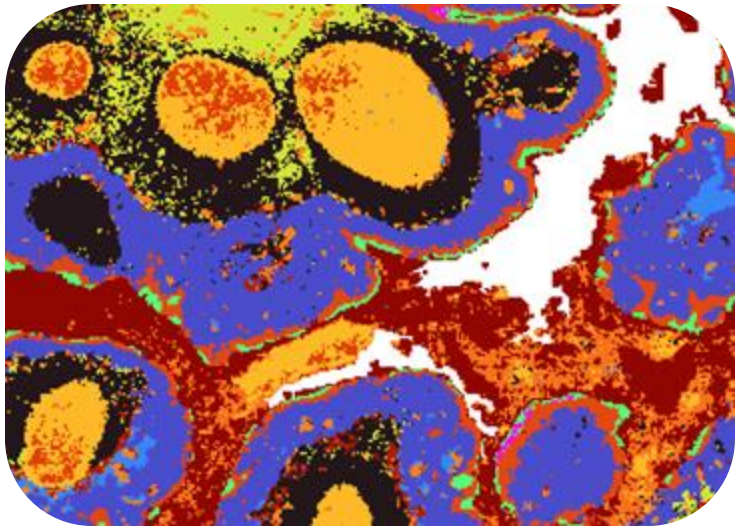
# Further resolve biology with cell segmentation

Mapping the tissue heterogeneity of human tonsil sample with cell segmentation

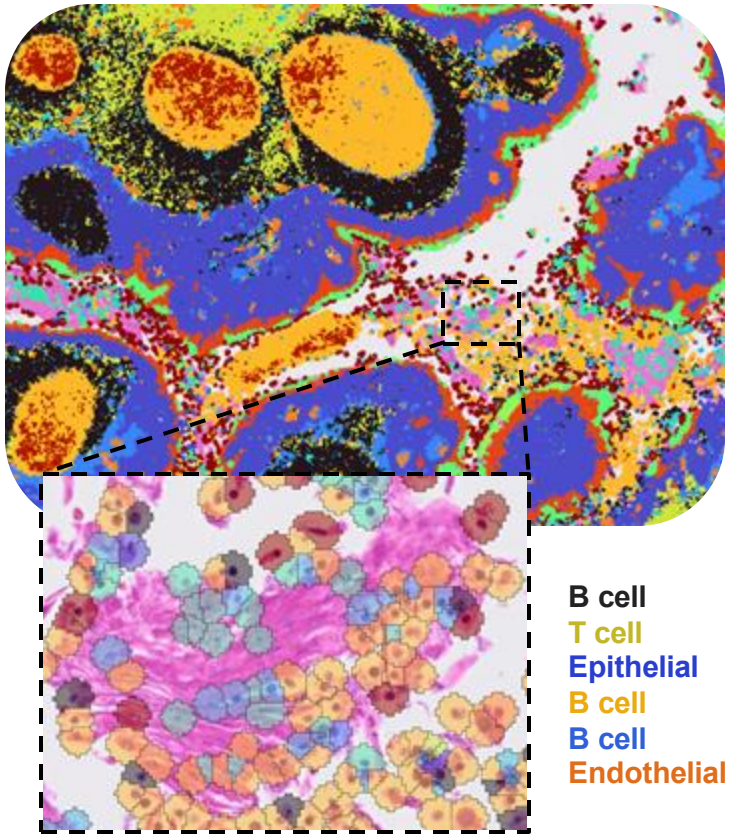
## H&E image



## 8 µm bin clustering



## Segmentation-based clustering

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# Xenium is the industry-leading single cell spatial imaging platform

## Superior performance

- Highly sensitive and specific
- Strong correlation with scRNA-seq
- Deep cellular phenotyping and annotation

## Comprehensive and flexible

- Customizable panels up to 5,000 genes or full custom
- Same section IF, H&E, and Visium HD
- Compatible across species and tissue types

## Fast and easy to use

- Industry-leading speed and throughput
- Simple, tissue-agnostic universal protocols
- Fast and intuitive data analysis

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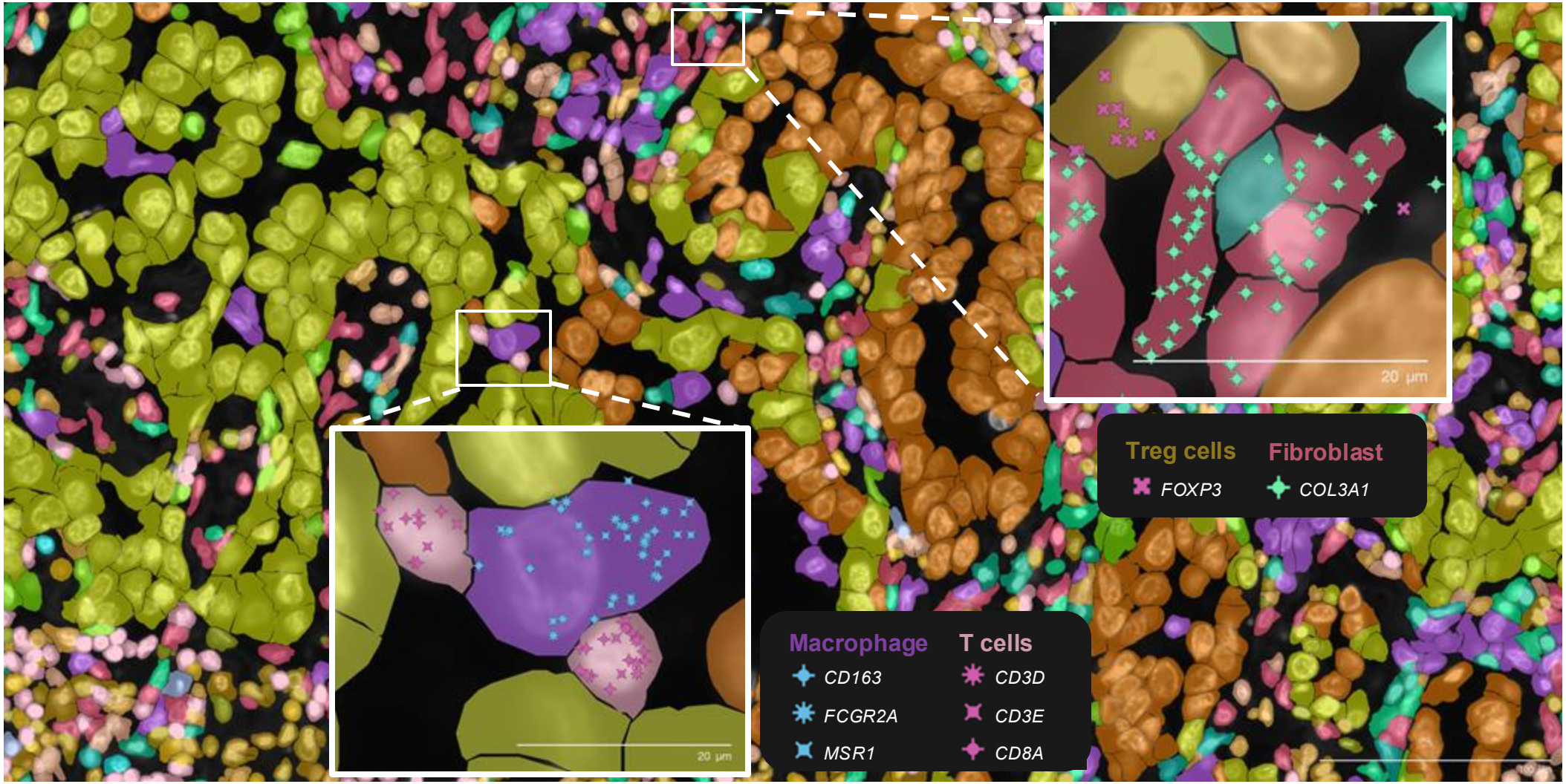
# Unveiling cell identity with **enhanced morphology** and **transcript mapping**



# Broad panel menu uniquely suited to address multiple applications

## Tissue/application-specific panels (~250-380-plex)

Multi-tissue, Immuno-Oncology, Brain, Breast, Lung, Colon, Skin

Multi-tissue, Brain

**Add up to 100 custom targets**

## Exploratory pan tissue and pathway panels (~5000-plex)

## Fully custom panels (up to 480-plex)

...

## Advanced customization

Isoform/translocations

Xenografts

Exogenous sequences

TCR/BCR profiling

Expressed SNVs

## Sample types

FFPE tissue

Fresh tissue

Frozen tissue

Fixed tissue

PBMCs/ cell suspensions

Adherent cells

Organoids

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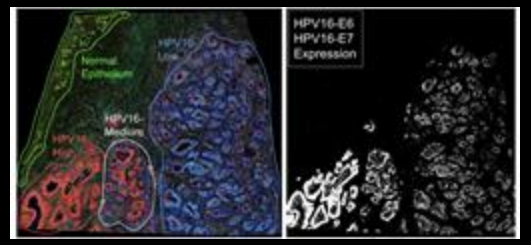
# Xenium advanced customization enables unique applications

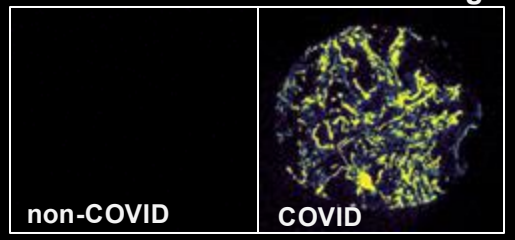
## Exogenous sequences

### HPV in human FFPE cervical cancer




FFPE Human Cervical Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes - Public dataset

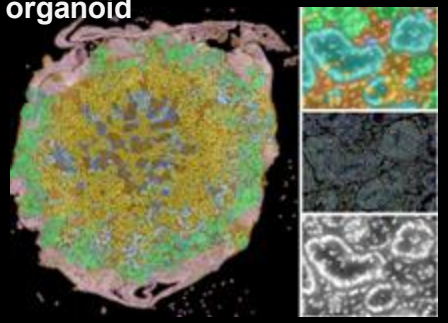
### COVID RNA in FFPE human lung




Emanuel Wyler, PhD, MDC Berlin

## Unique model systems

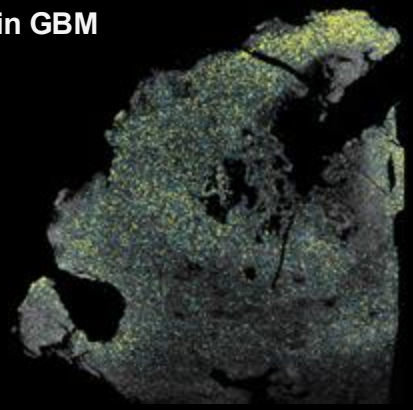
### Kidney organoid



Alex Combes, PhD, Monash University

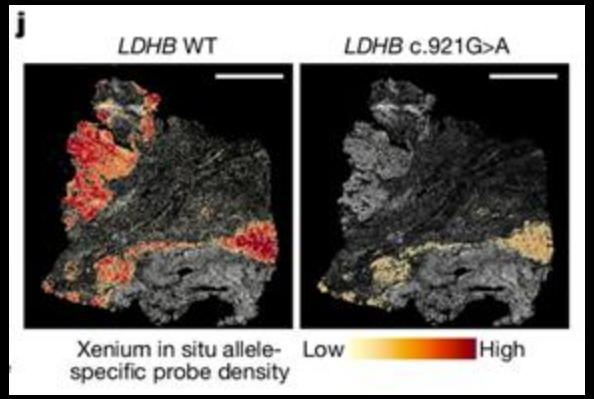
## Splicing isoform, SNV

### EGFRvIII splice variant in FFPE human brain GBM



Simon Gregory, PhD, Duke University

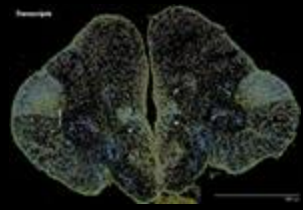
### *LDHB* SNV in human FFPP CRC Metastasis




Ding et al. Tumour evolution and microenvironment interactions in 2D and 3D space. *Nature* (2024).

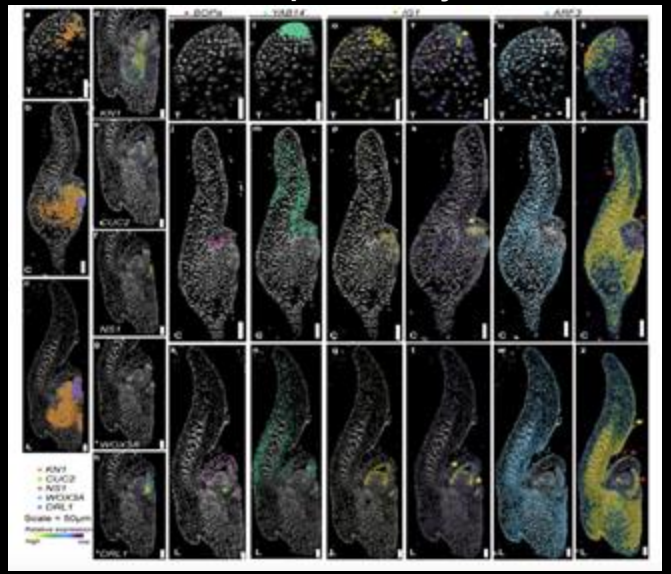
## Non-human, non-mouse species

### Tilapia brain



https://x.com/10xGenomics/status/1767939225843015683

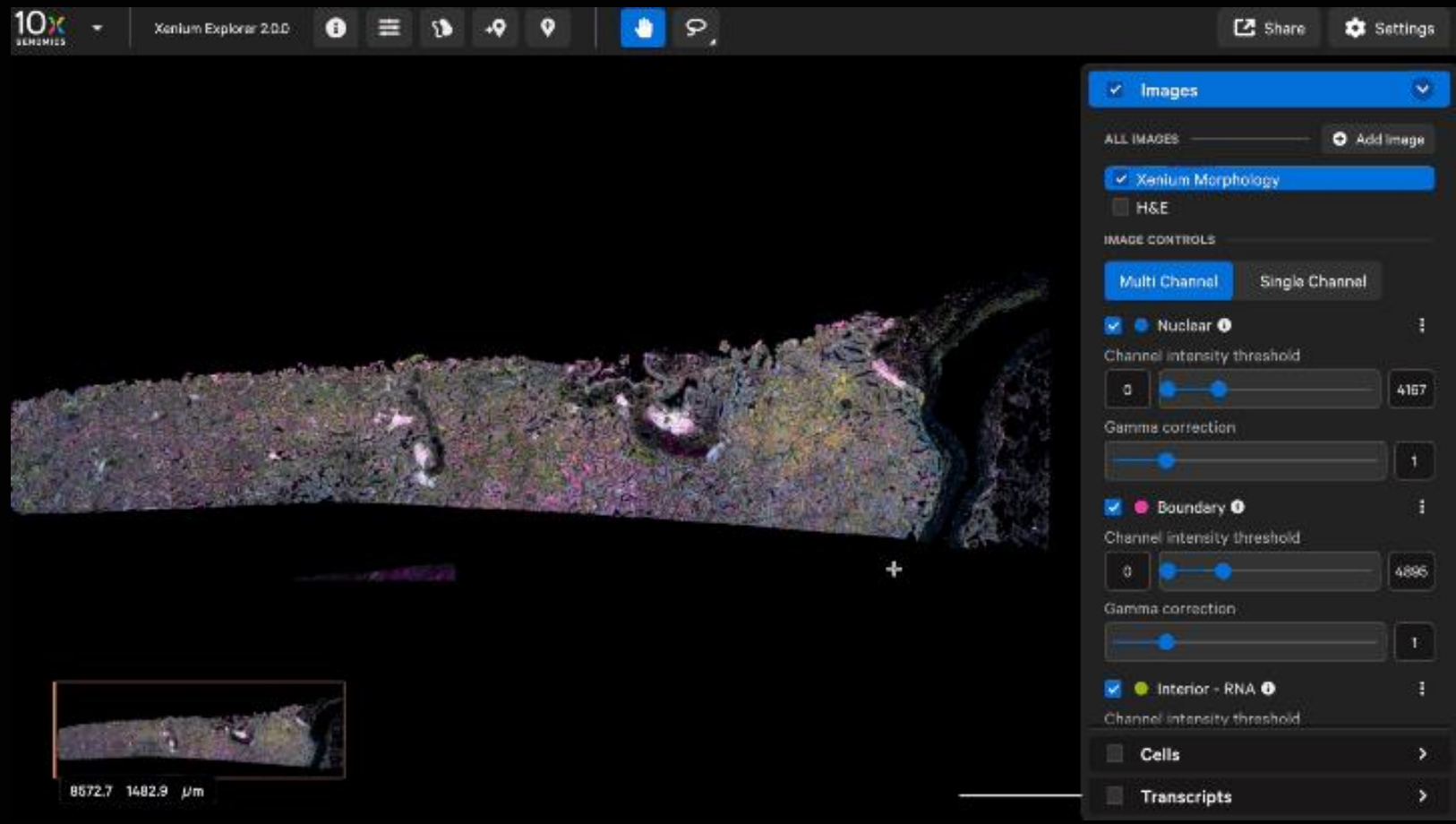
### Maize plant embryo



Scanlon et al. Multiplexed transcriptomic analyses of the plant embryonic hourglass. *BioRxiv* (2024).

# Xenium Explorer: Intuitive visualization & analysis

Begin exploration immediately without the need for off-instrument processing




**View cell morphology, evaluate cell segmentation, interrogate transcript subcellular localization, analyze regions of interest**

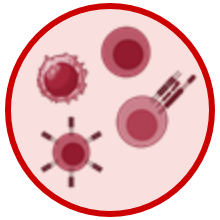
# Single-slide RNA and protein profiling for precision insights

## Human FFPE tissue

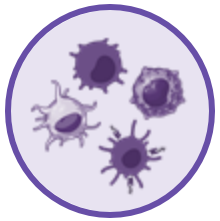


**Compatible with tissue/application RNA panels including RNA customization (~250-480 plex)**

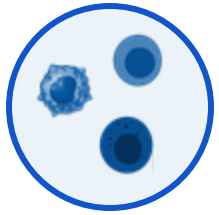
Multi-tissue, immuno-oncology, brain, breast, lung, colon, skin



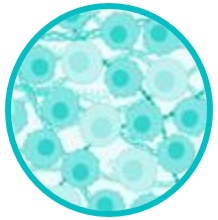
**Immune Cell Typing A**
CD3E, CD4, CD8A, CD20



**Immune Cell Typing B**
HLA-DR, CD68, CD11c, CD138



**Immune Cell Typing C**
GranzymeB, CD163, CD16, CD56



**Cellular Characterization**
E-Cadherin, Vimentin, αSMA, CD45

**Proliferation & Differentiation**
CD45RA, CD45RO, PCNA, Ki-67

**Immune Checkpoint**
PD-1, PD-L1, VISTA, LAG-3

**Tumor Environment**
PanCK, CD31, β-catenin, PTEN

**Protein customization on the roadmap**

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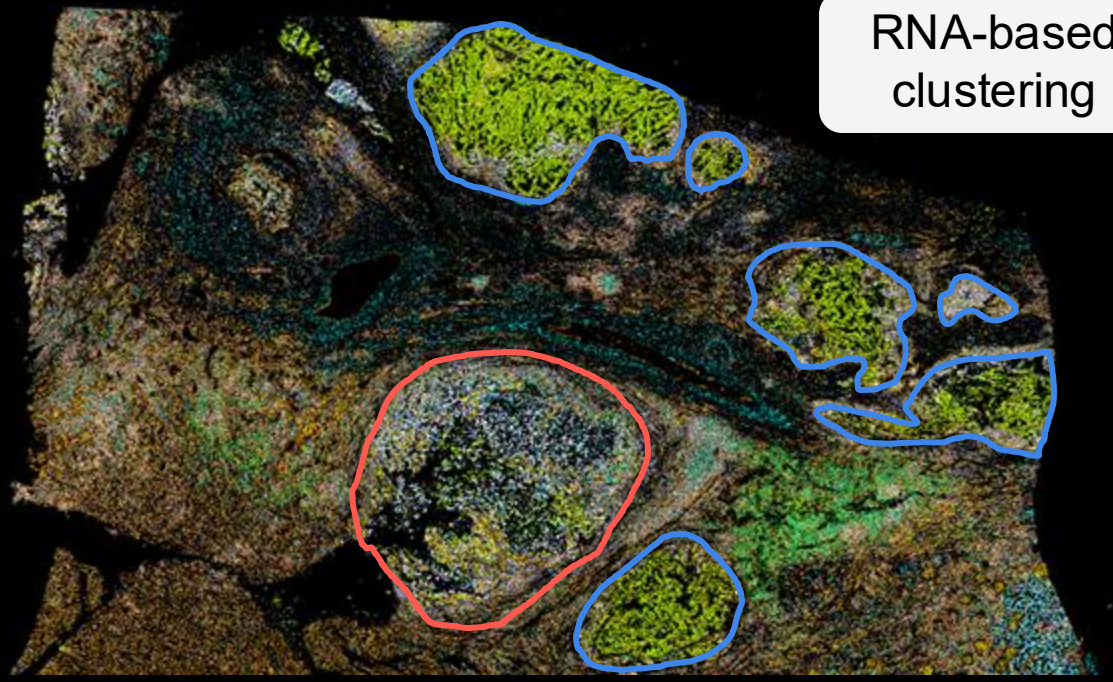
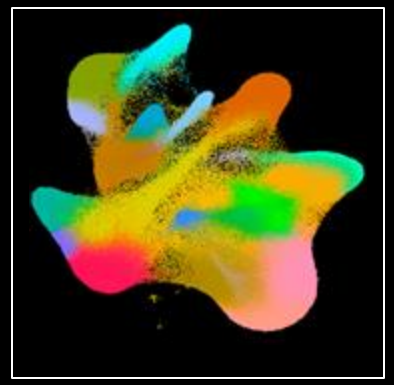
# Proteogenomic-specific insights from Xenium

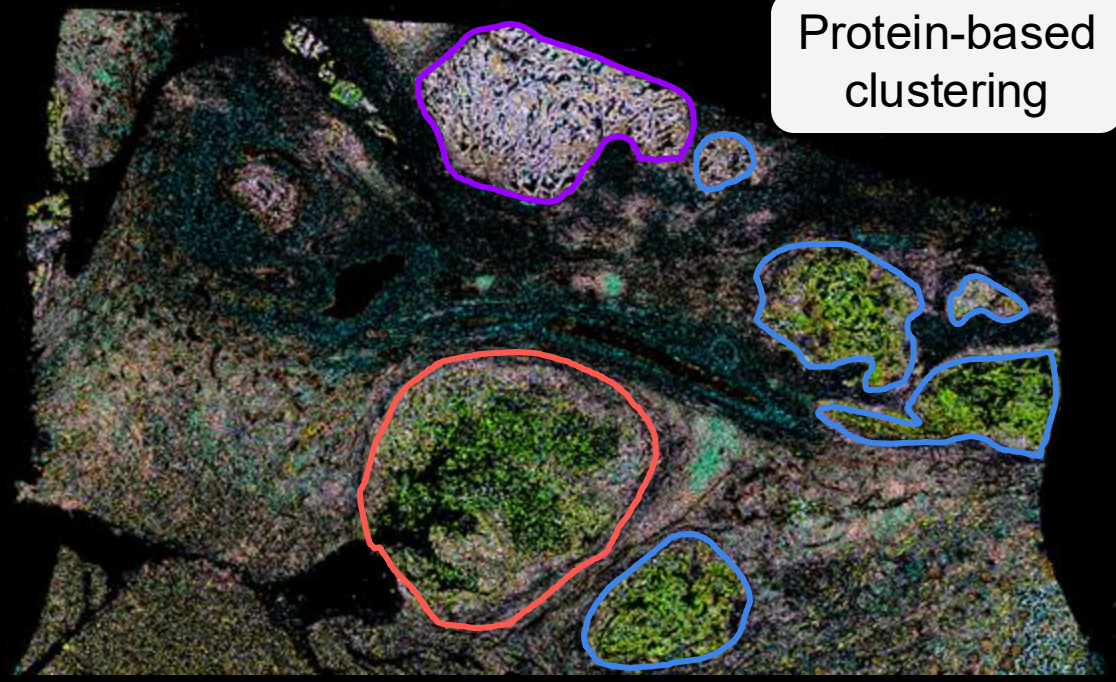
RNA-based clustering

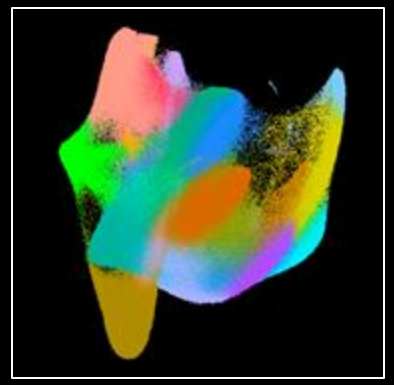




- T cells (CD38$^+$)
- Cancer associated fibroblasts (HIF1A$^+$)
- T cells (CXCL9$^+$)
- Endothelial cells
- Macrophages
- Mesangial/tubular epithelial cells
- B cells (IL7R$^+$)
- T cells (CXCL9$^+$ CXCL12$^+$)
- Podocytes
- Tumor cells (LRP2$^+$)
- Memory B cells
- B cells (MS4A6A$^+$)
- Activated macrophages
- Smooth muscle cells
- Proximal tubular cells
- Distal convoluted tubule cells
- Collecting duct cells
- Plasma cells
- Tumor cells (SYNPO$^+$)
- Cancer associated fibroblasts (MYH11$^+$)
- T cells (CD38$^+$ CXCL10$^+$)
- Tubular epithelial cells

Protein-based clustering





- T cells (CD11c$^+$)
- Exhausted cytotoxic T cells
- Exhausted T cells
- Endothelial cells
- Macrophages
- Epithelial cells
- NK cells
- T helper cells
- Tubular epithelial cells type I
- Tumor cells (CD138$^+$)
- B cells
- Dendritic cells (HLA-DR$^+$)
- Dendritic cells (HLA-DR$^-$)
- Smooth muscle cells
- Proximal tubular cells
- Distal convoluted tubule cells
- Collecting duct cells
- Dendritic cells (CD31$^+$ HLA-DR$^+$)
- Tumor cells (CD138$^-$)
- Endothelial cells (VISTA$^+$)
- Naive T cells
- Tubular epithelial cells type II

10x GENOMICS

# Leveraging the 10x Tool Kit to Understand Cancer

## High resolution mapping of the tumor microenvironment using integrated single-cell, spatial and in situ analysis

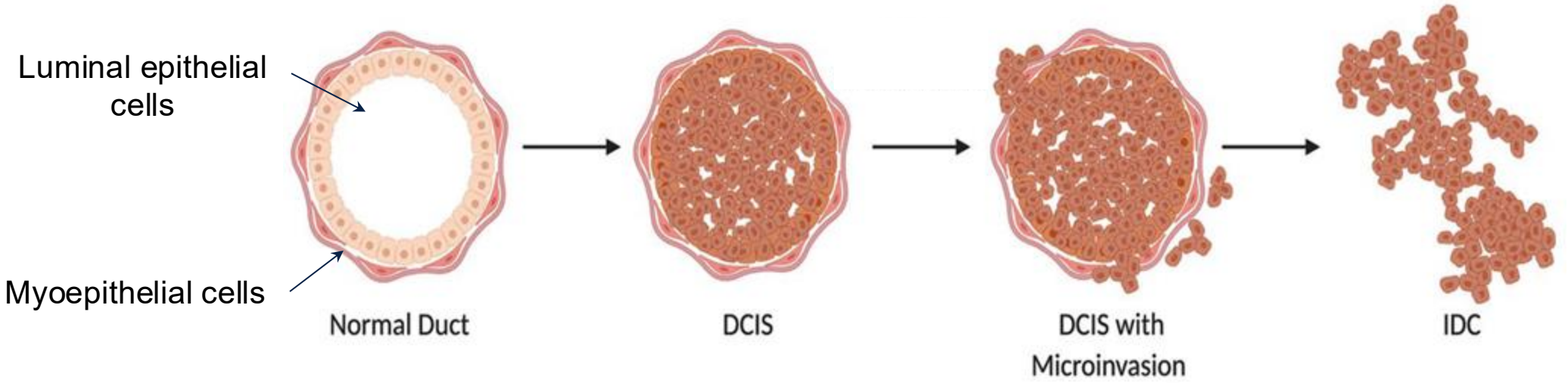
Amanda Janesick, Robert Shelansky, Andrew D. Gottscho, Florian Wagner, Stephen R. Williams, Morgane Rouault, Ghezal Beliakoff, Carolyn A. Morrison, Michelli F. Oliveira, Jordan T. Sicherman, Andrew Kohlway, Jawad Abousoud, Tingsheng Yu Drennon, Seayar H. Mohabbat, 10x Development Teams & Sarah E. B. Taylor

nature communications

- Cancer is highly heterogeneous: **what's** going on, **where**?
- The most relevant samples tend to be FFPE
- This preprint shows how comprehensive single cell and spatial technologies can transform our understanding of tumorigenesis and the cancer microenvironment.

# Exploring the Molecular Mechanisms Driving Breast Cancer Progression to Invasive Phenotypes

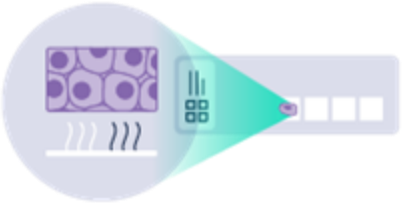


# Exploring Breast Cancer Biology with 10x Genomics

10k cells target

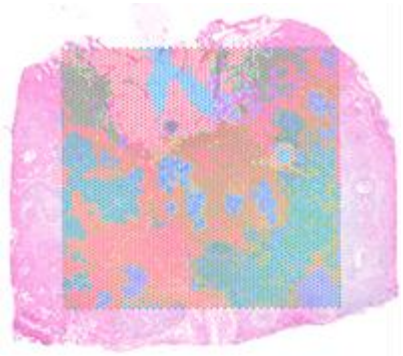
FFPE tissue dissociation

Serial FFPE sections

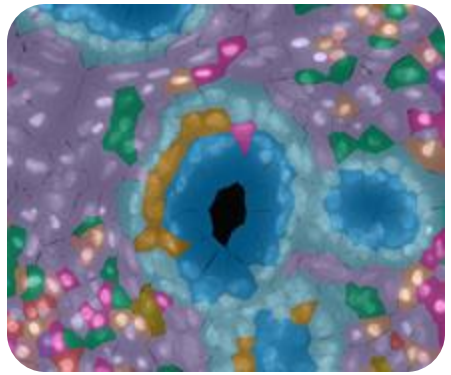


Chromium Single Cell Gene Expression Flex

Visium Spatial



Xenium In Situ

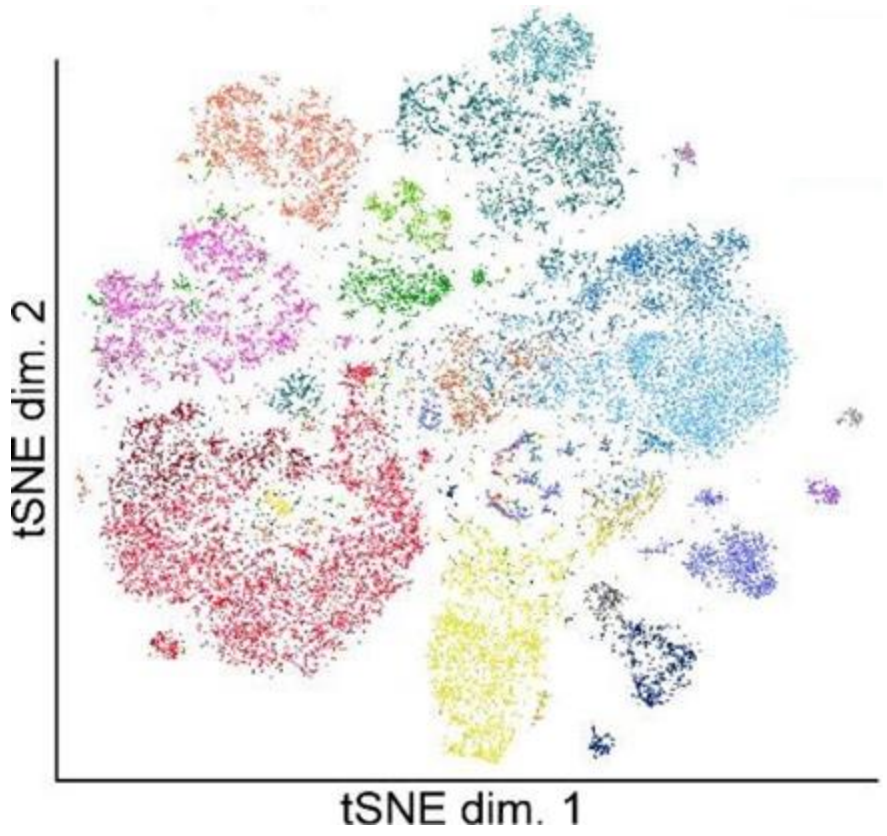


**Whole transcriptome**

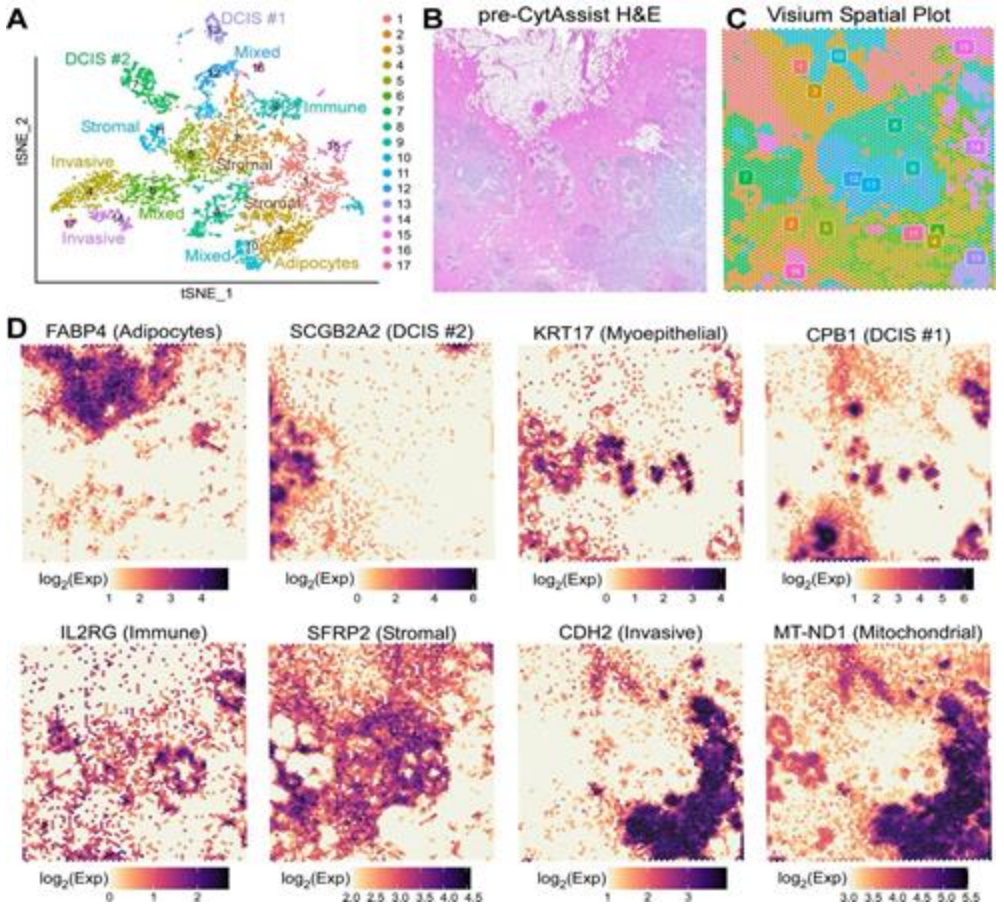
**In Situ gene expression**

# Chromium and Visium Resolve Main Cellular Components and Reveal Molecularly Distinct Cancer-Associated Cells

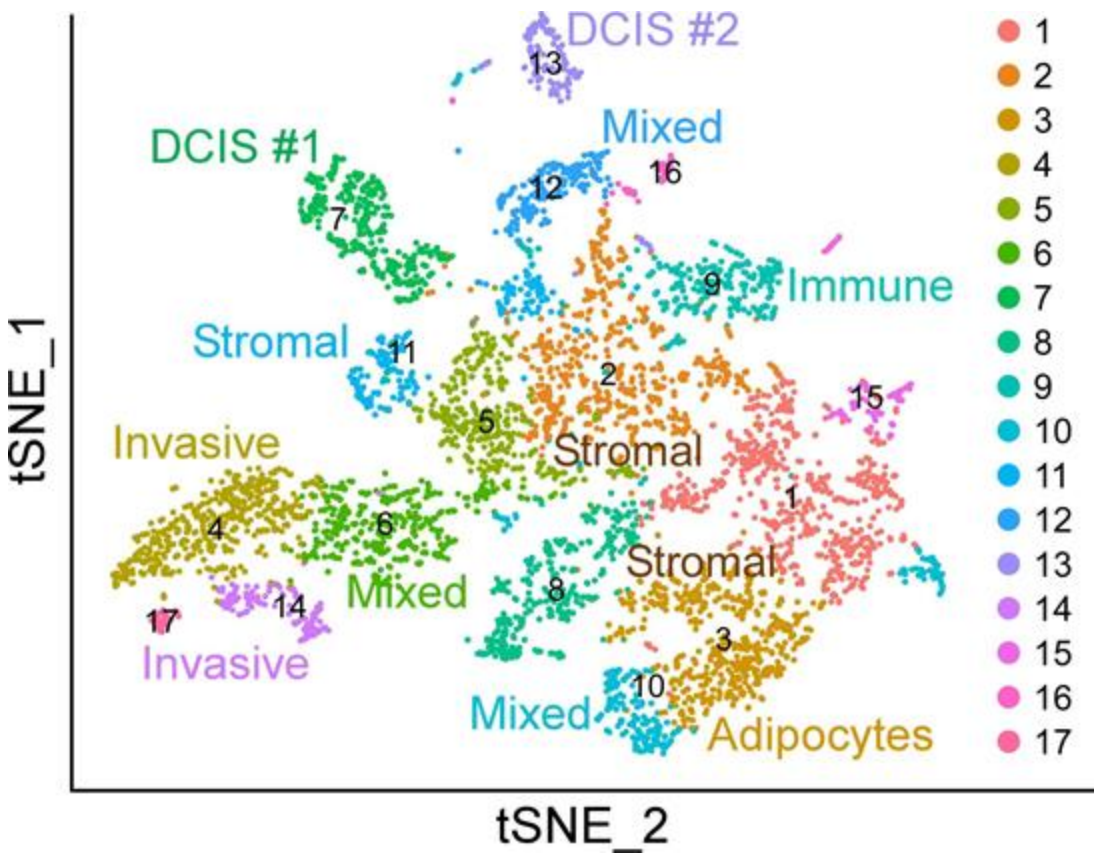
Single Cell Whole Transcriptome

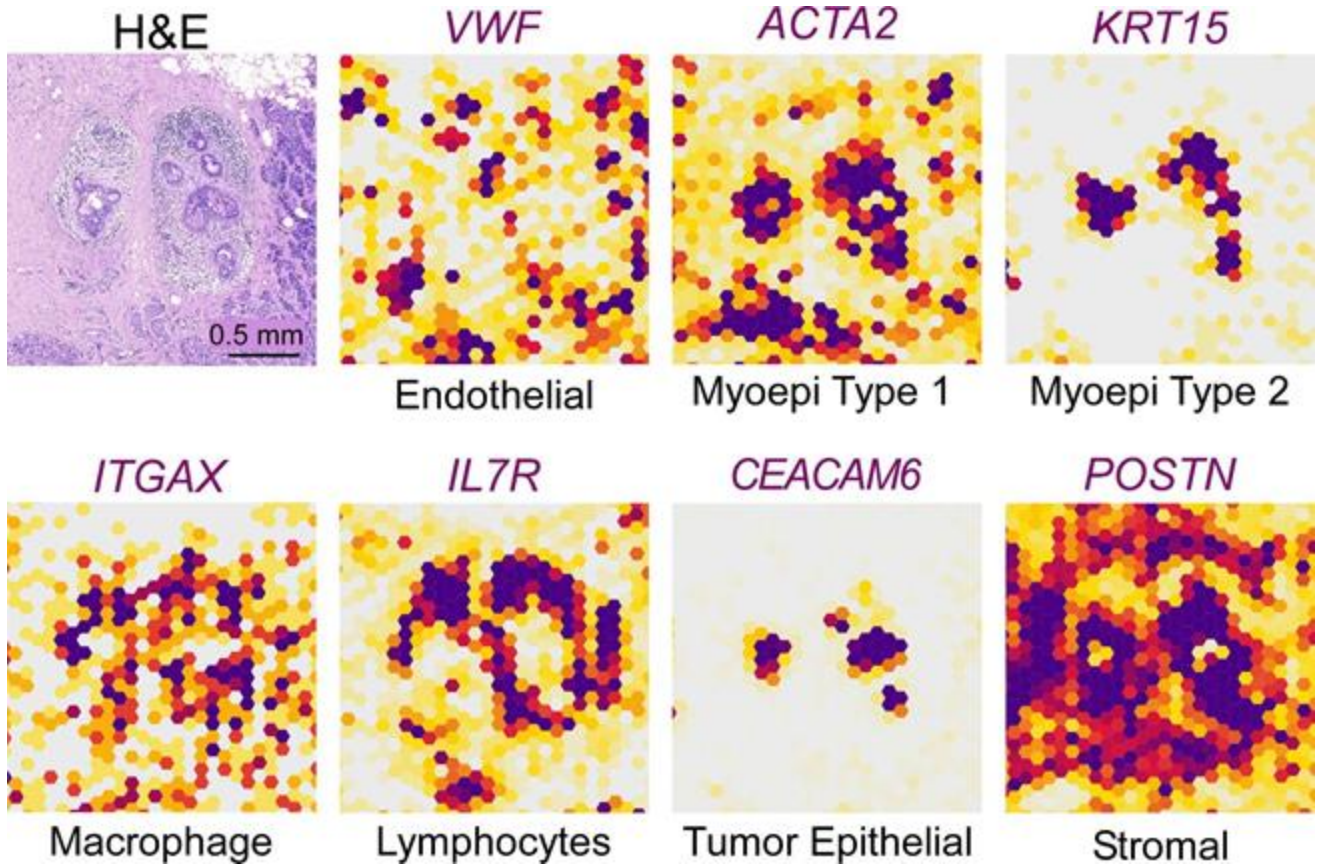



Spatial Whole Transcriptome

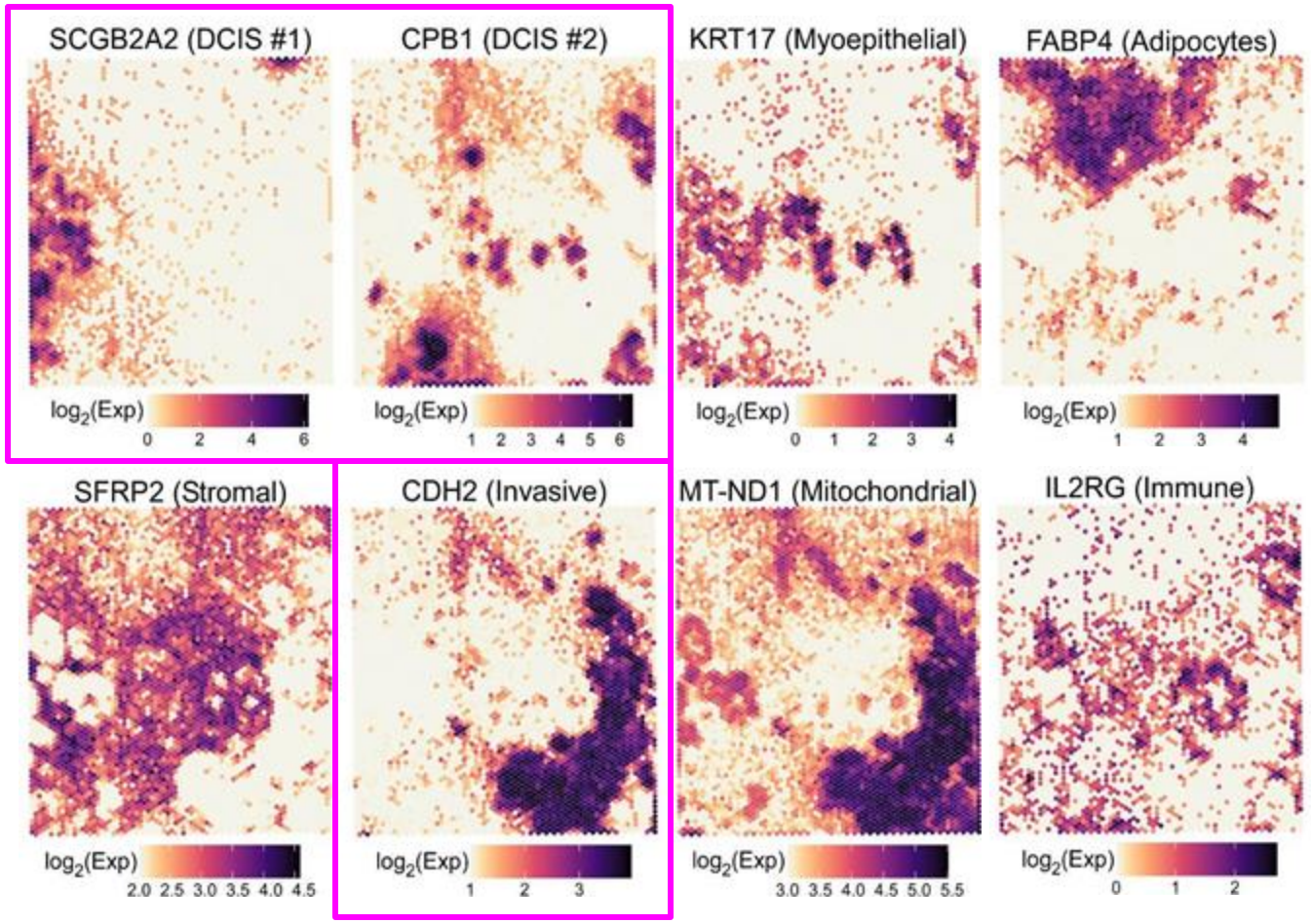


# 17 Unique Clusters Identified Using Visium

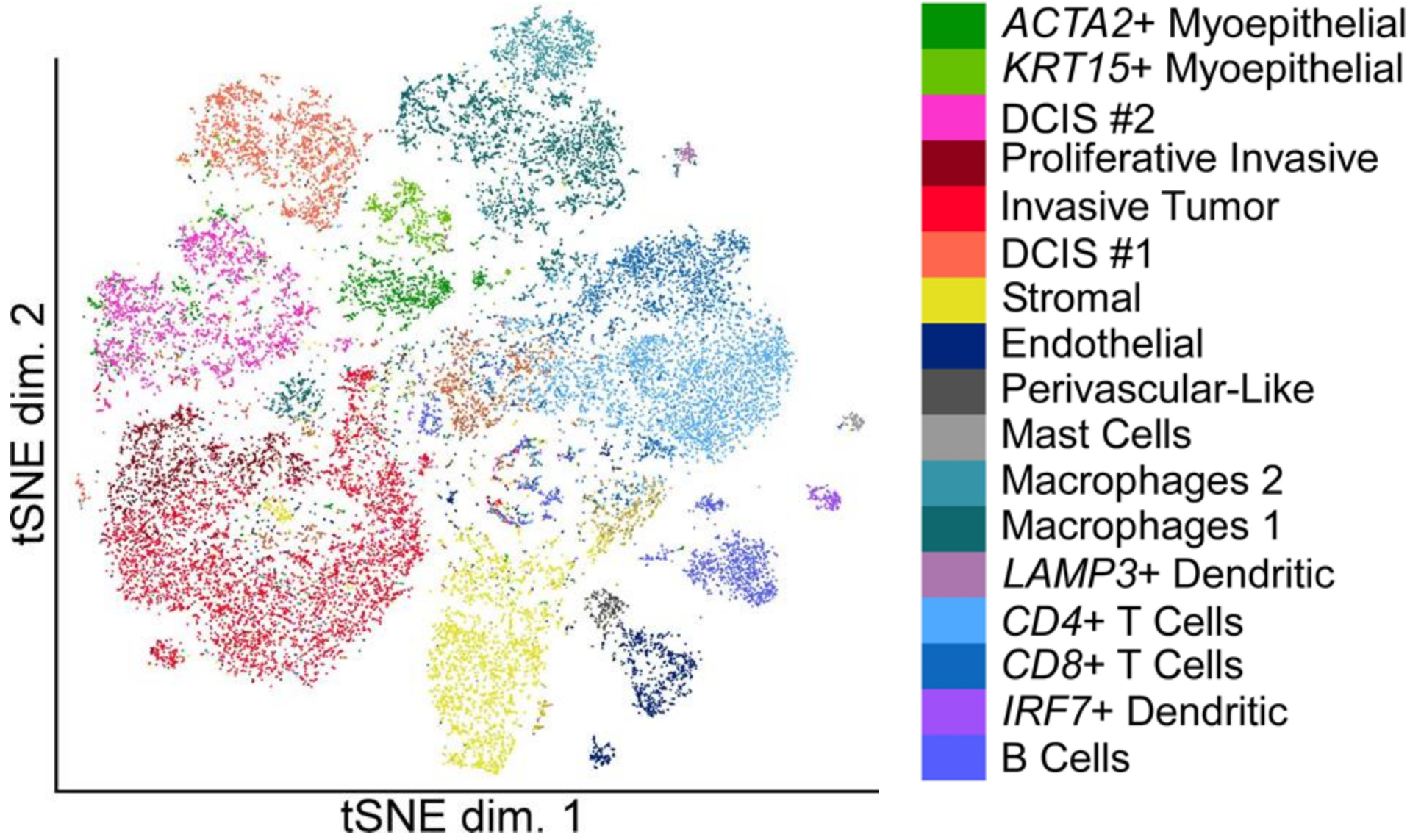


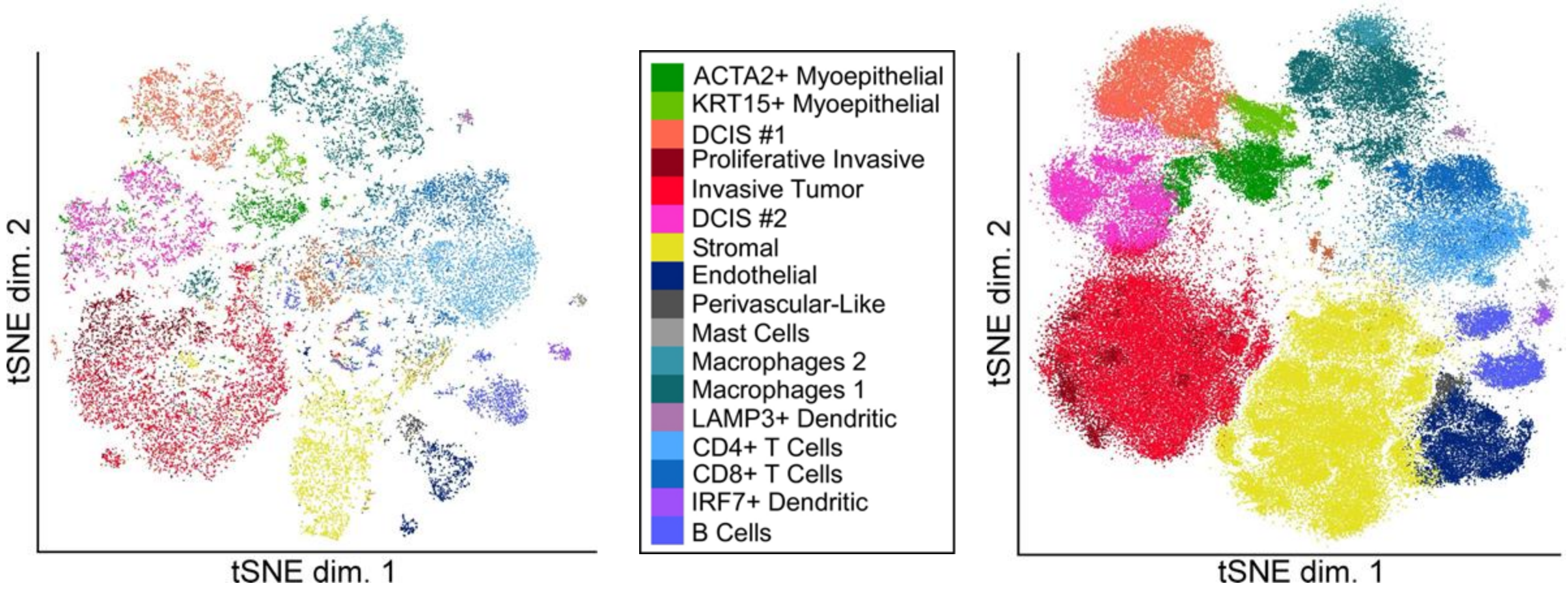
# Key Takeaways from the Integration of Chromium and Visium



# Recall Our Comprehensively Annotated scFFPE-seq Data…

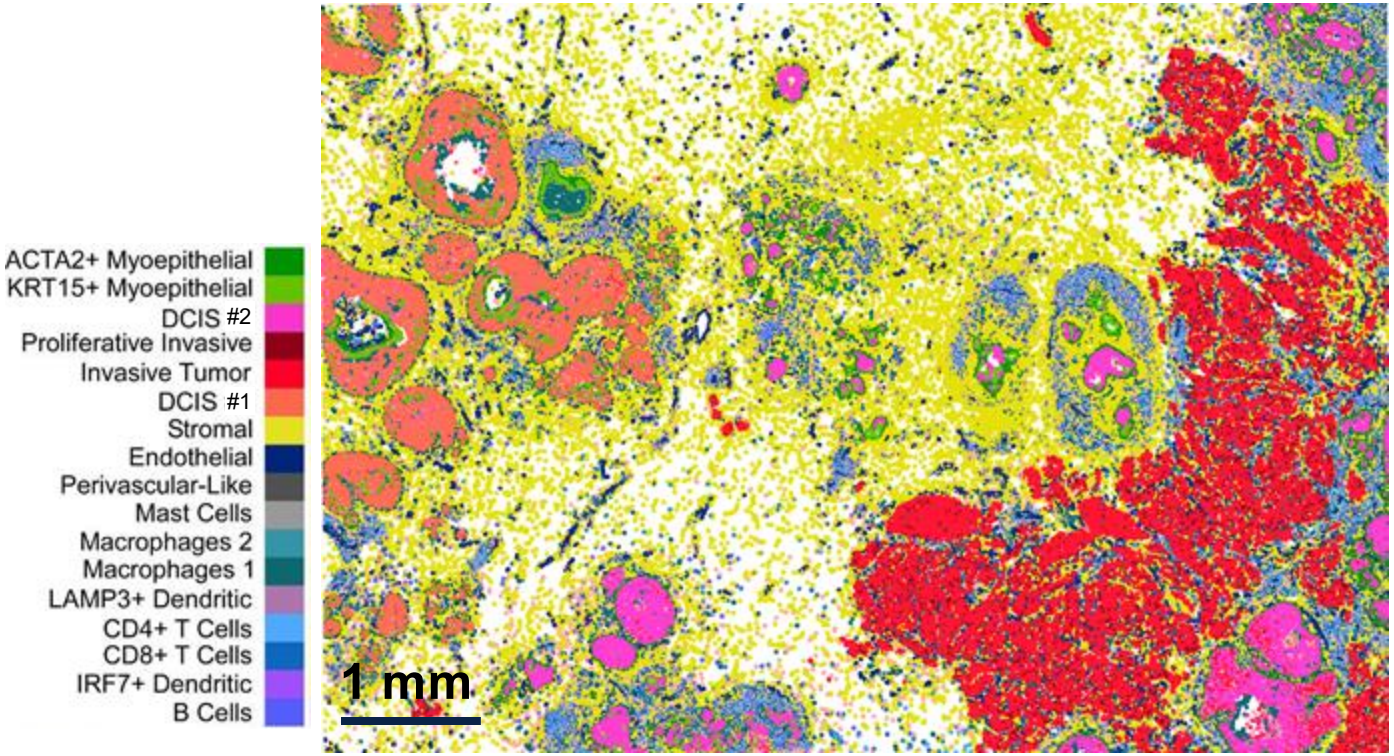


# Cell Types in Xenium Data Identified Using scFFPE-seq Annotations

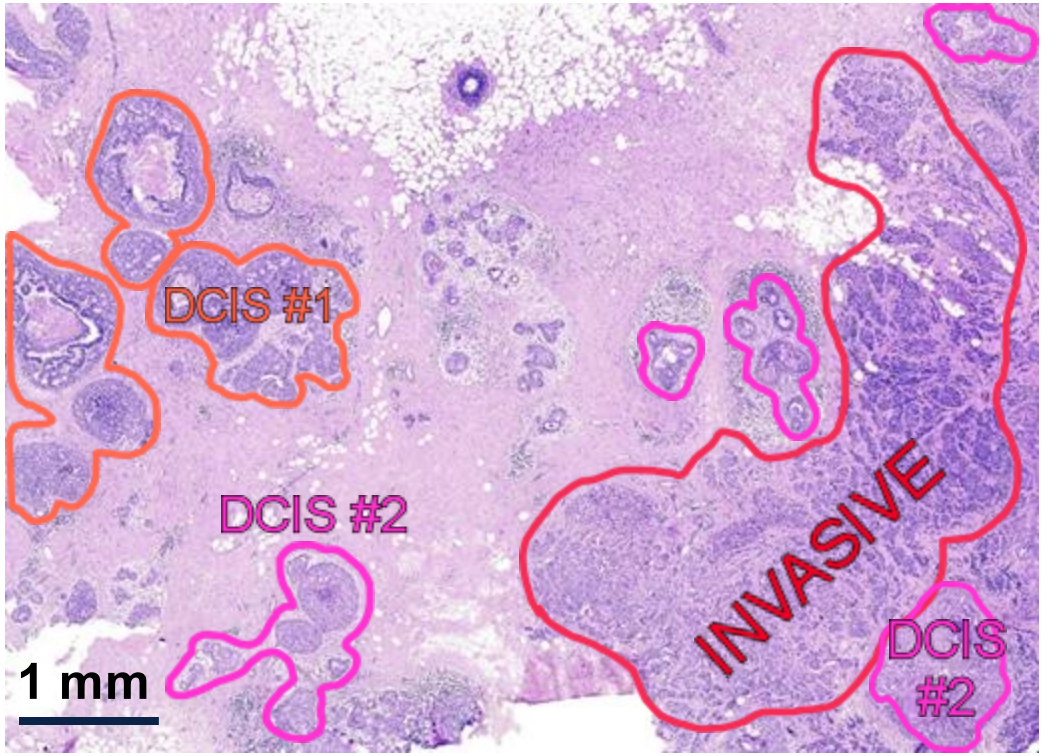


# Xenium Used to Identify the Cellular Composition and Molecular Markers of Tumor Subtypes

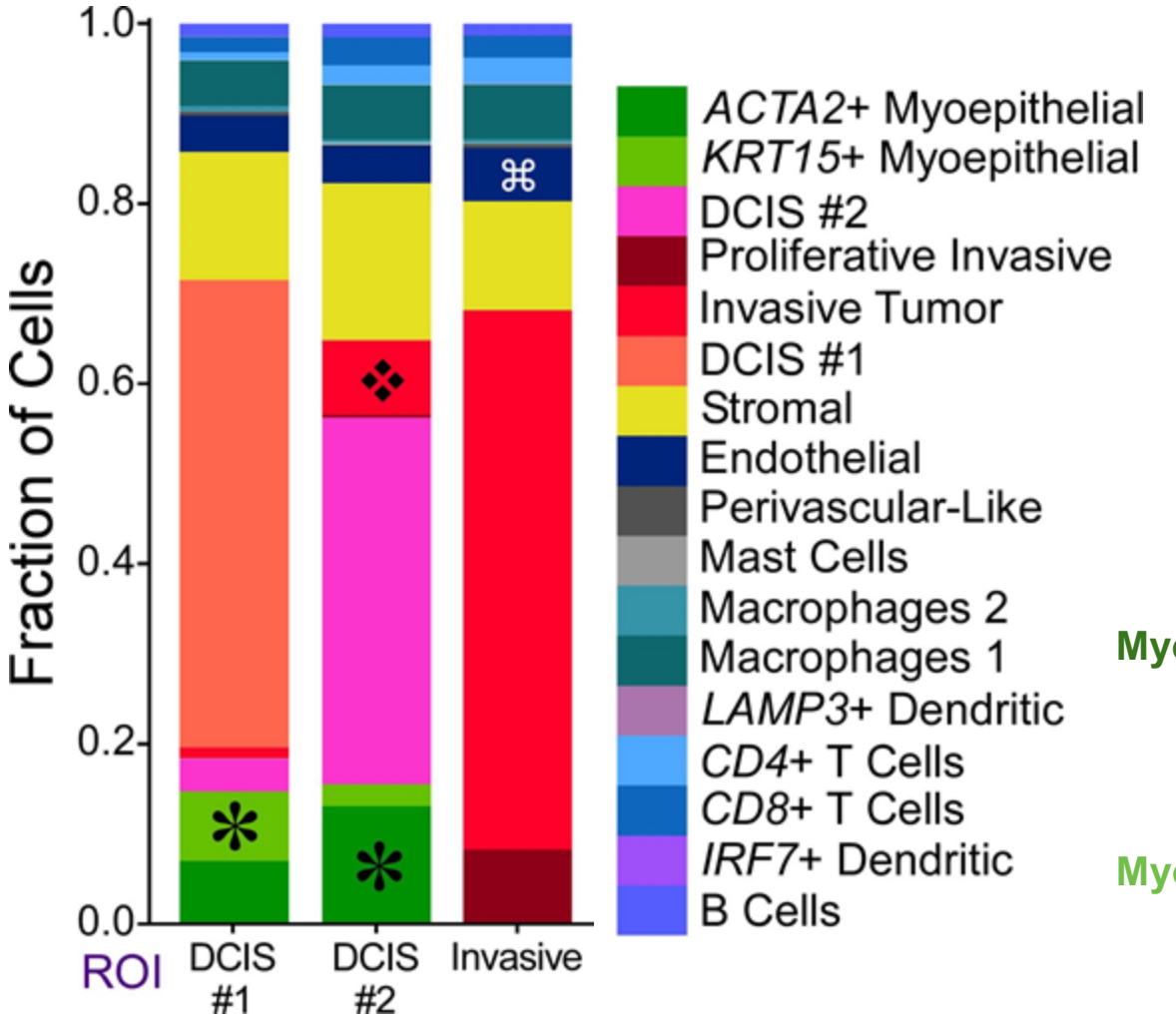
## Xenium Cell Annotations

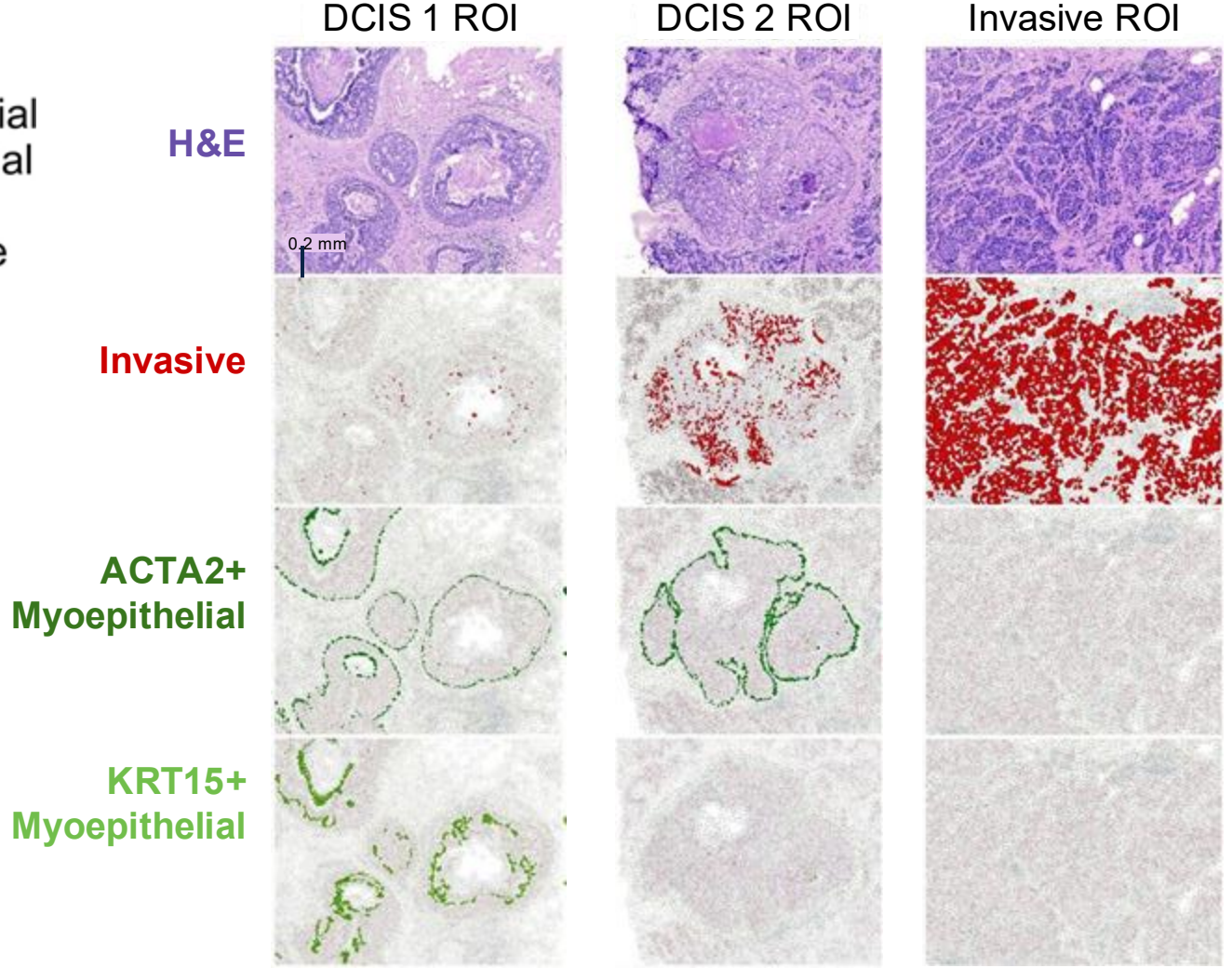


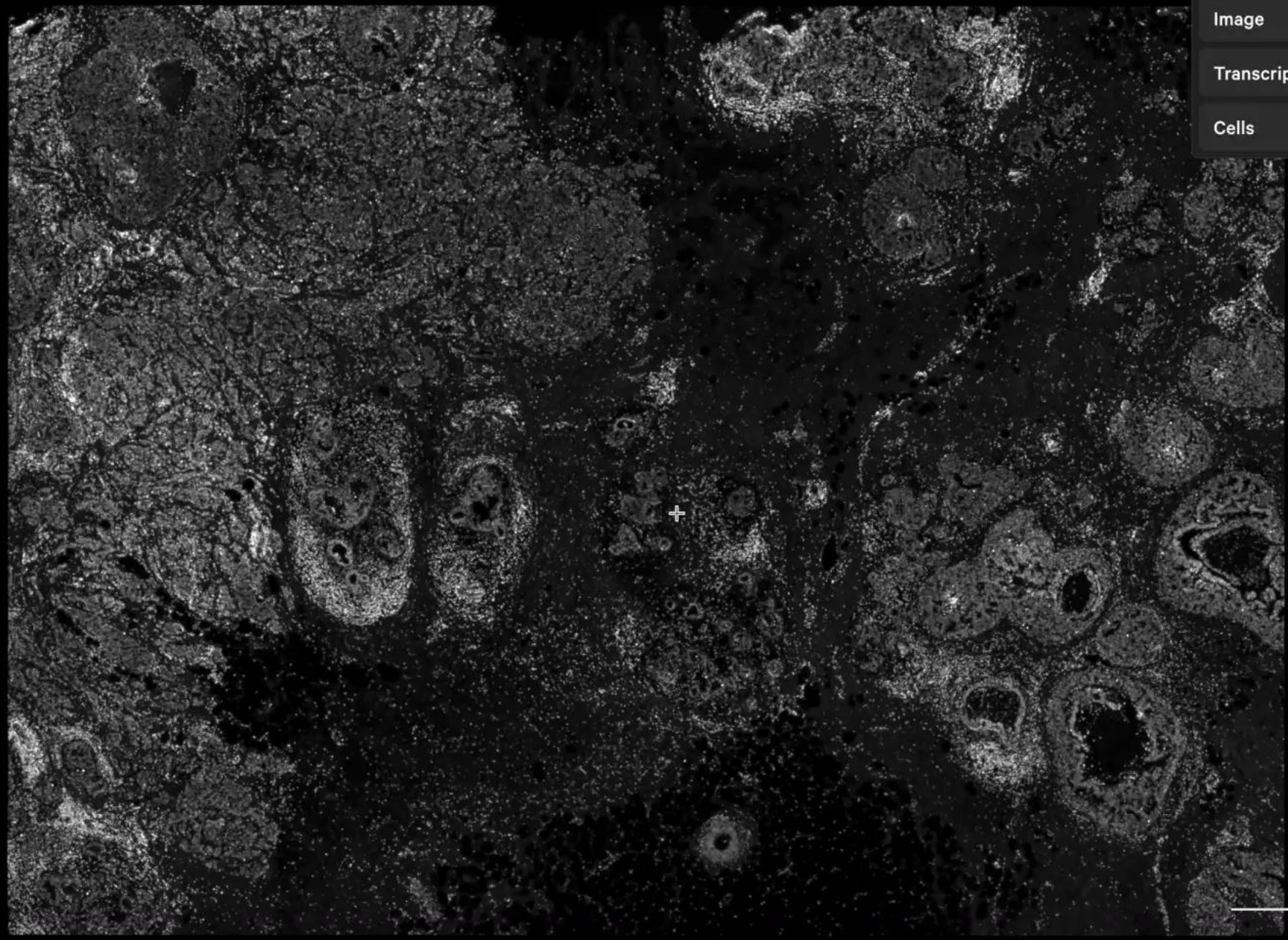

## Selection of Tumor ROIs



# Xenium Localizes Cancer-Associated Cell Subtypes on Breast Cancer FFPE Sample





Image

Transcripts

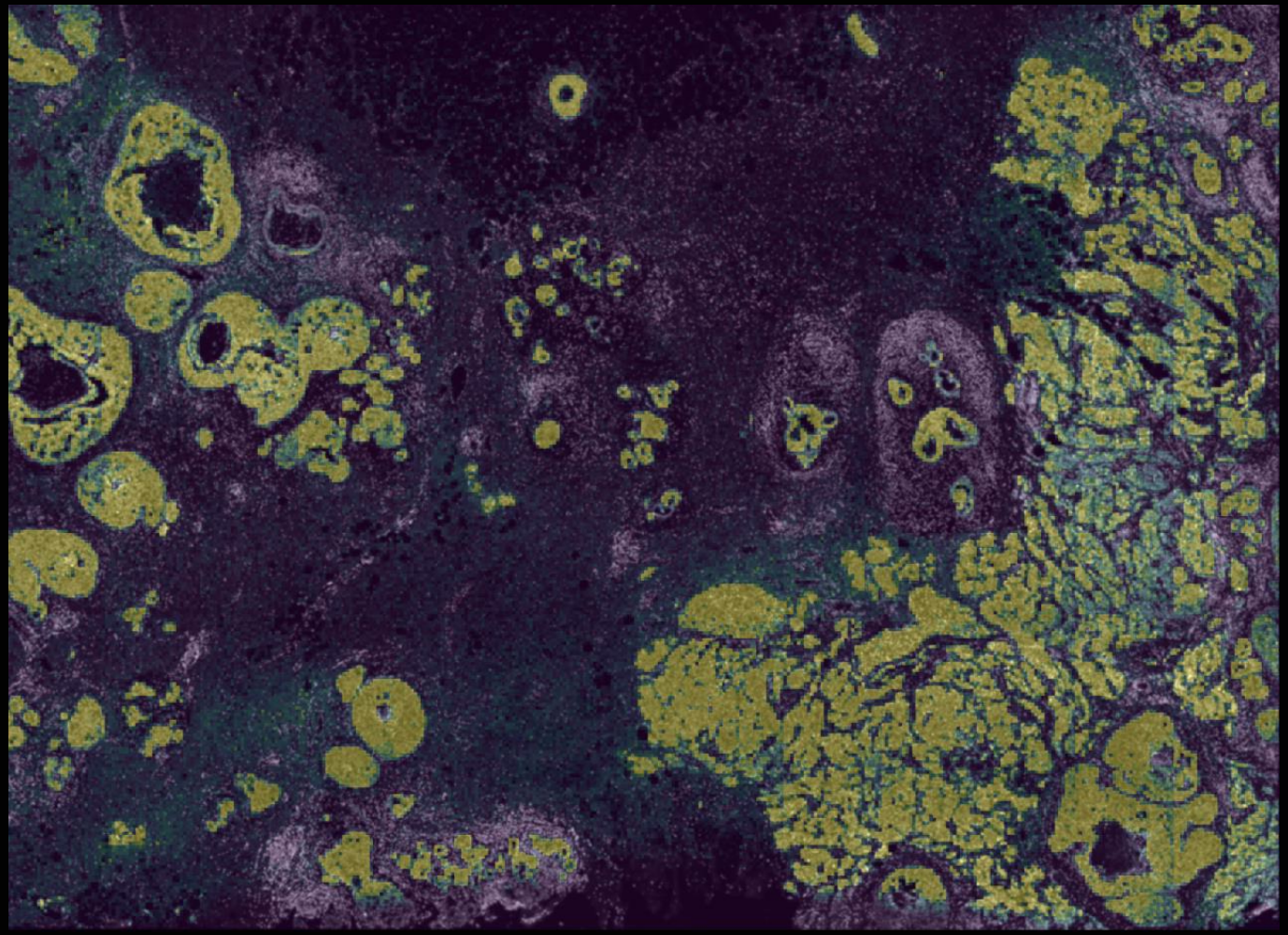
Cells

3948.4 2992.2 µm

2000 µm

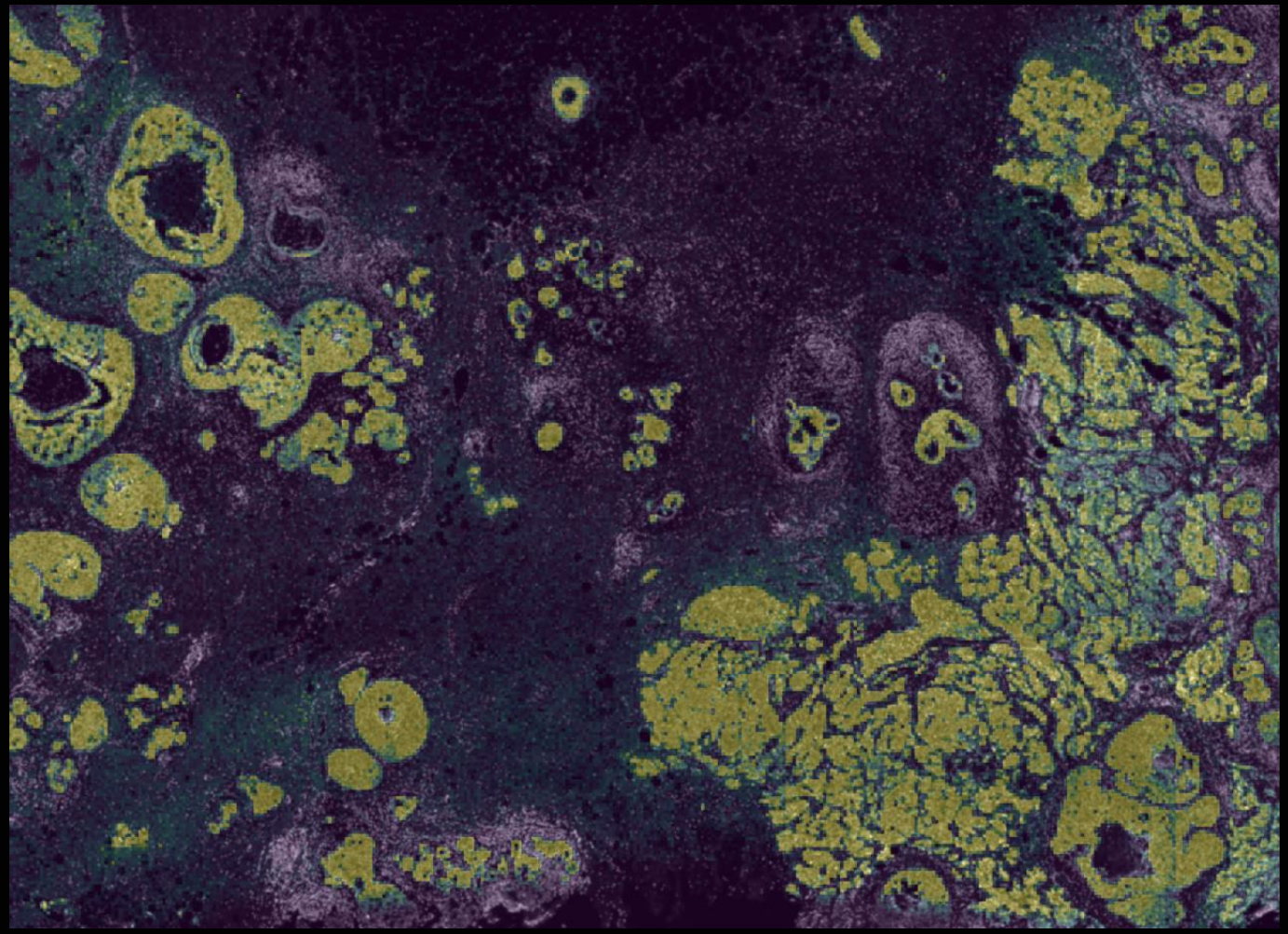
# Xenium Explorer: Gene Expression Visualization at Scale

Double Positive Breast Cancer (ERBB2+ & ESR1+ cells)



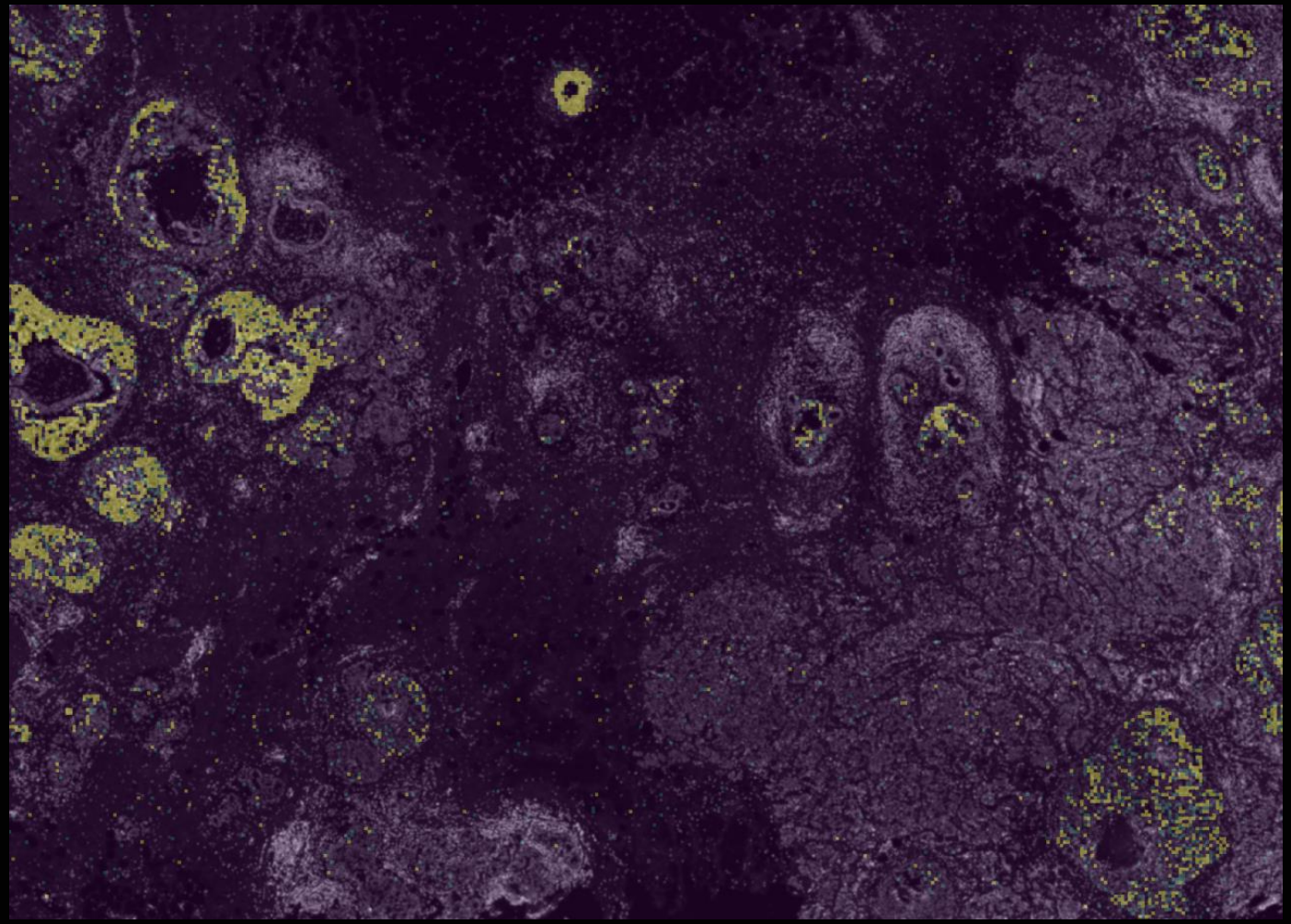
# Per-Gene Localization in Xenium Explorer

ERBB2+ cells



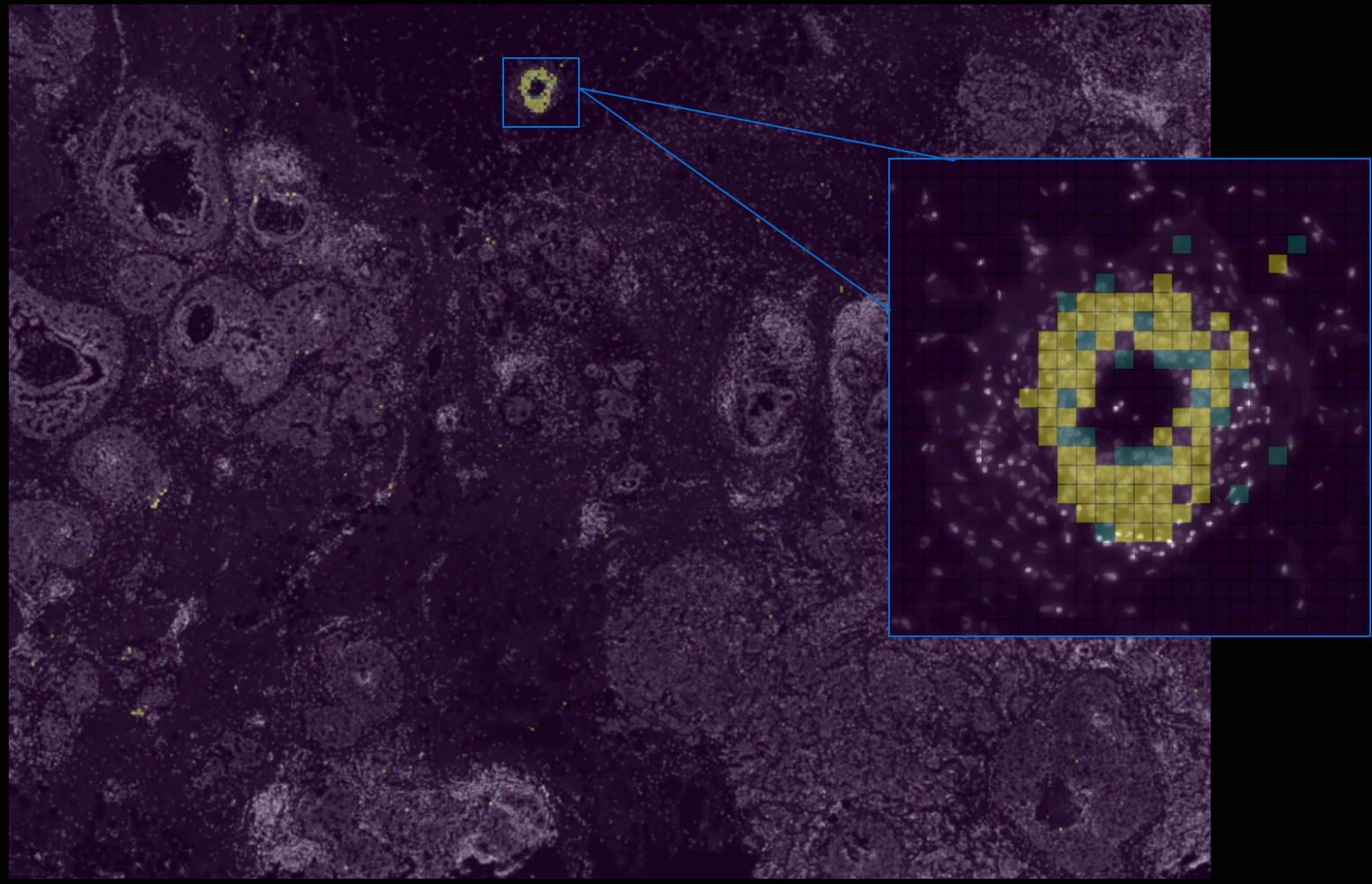
# Per-Gene Localization in Xenium Explorer

ESR1+ cells

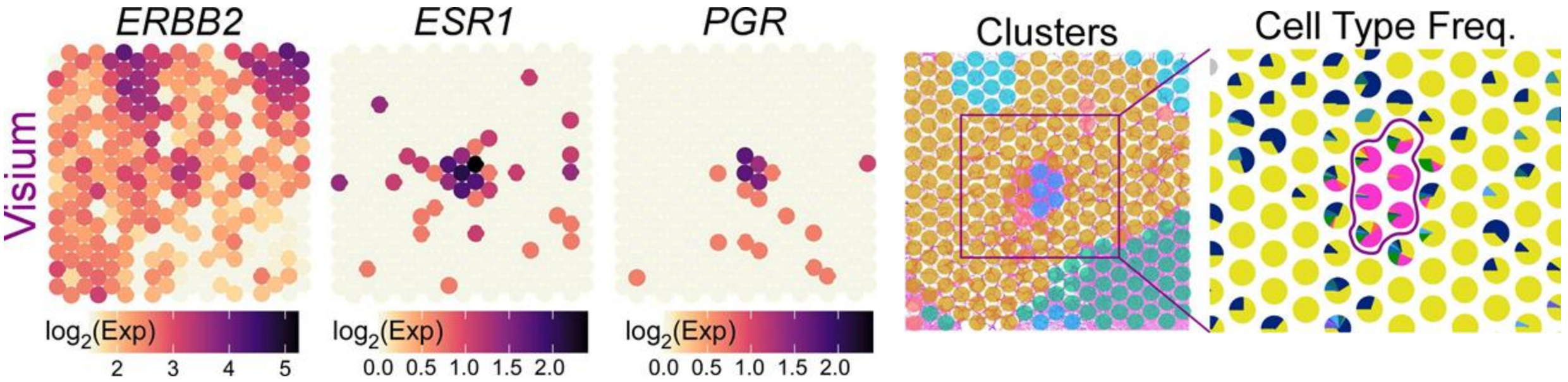


# Per-Gene Localization in Xenium Explorer

PGR+ cells – Unique Tumor Region that is Triple Positive!



# Visium and Xenium Integration Derives Differentially Expressed Genes in a Triple-positive Receptor ROI



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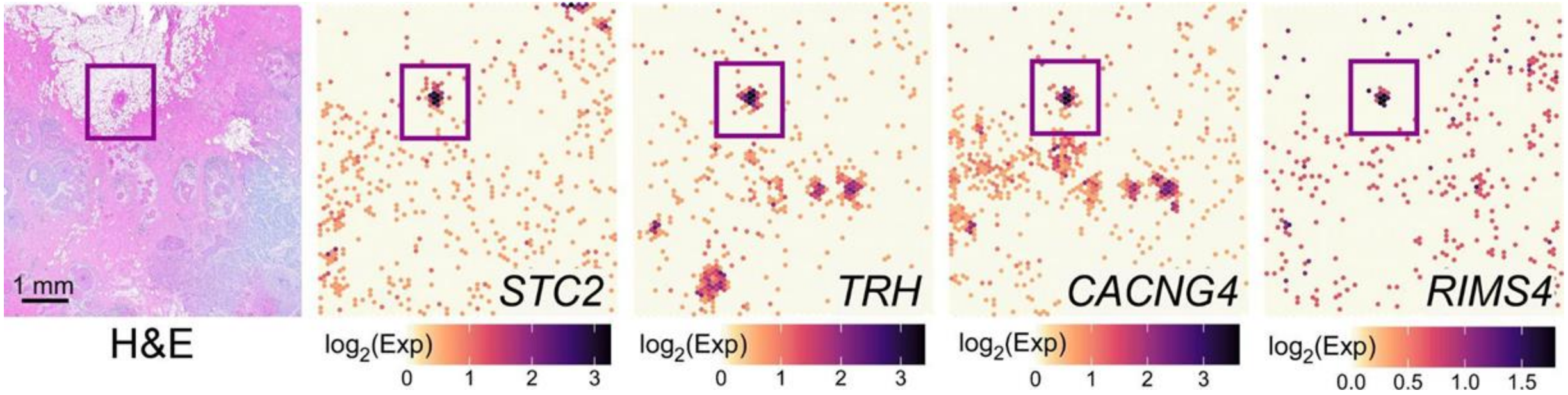

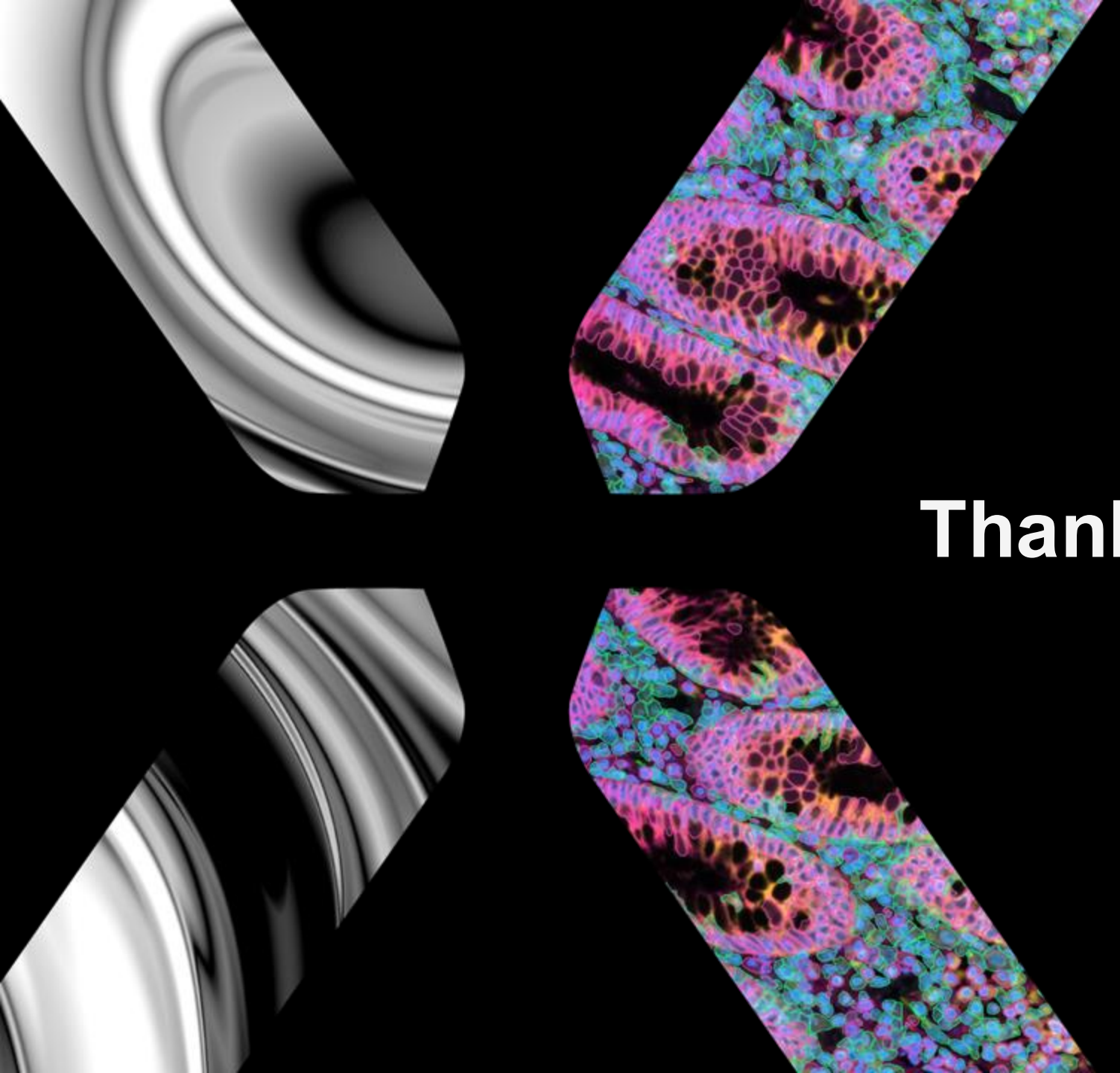

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# Thank you!

**Contact us:**

johanna.stergiadou@10xgenomics.com