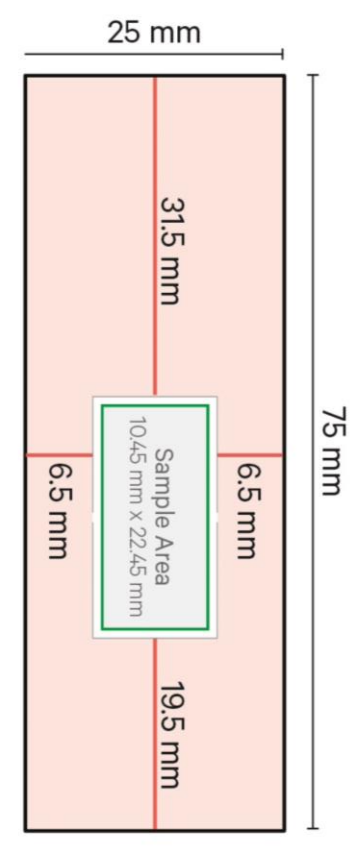


From Method Development to Discovery:
Innovating Spatial Transcriptomics with Xenium

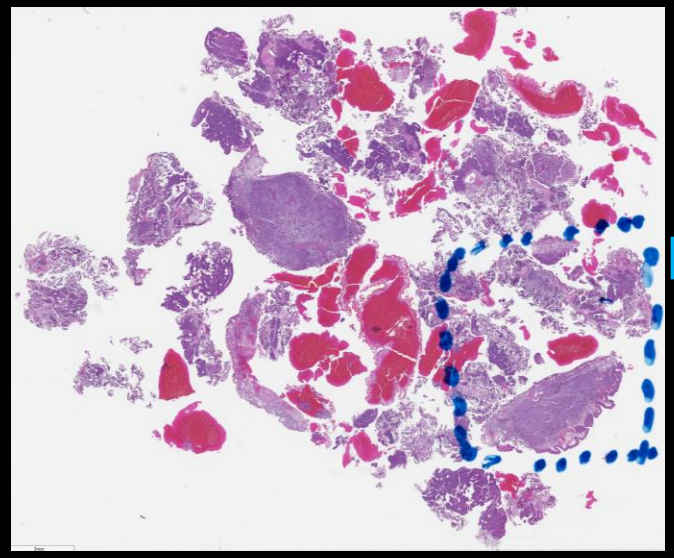
Gabriela De la Cruz
Facility Director
Pathology Services Core
Lineberger Comprehensive Cancer Center
UNC-CH School of Medicine

Xenium Specialized Tissue Placement

Xenium Slide Sample Area

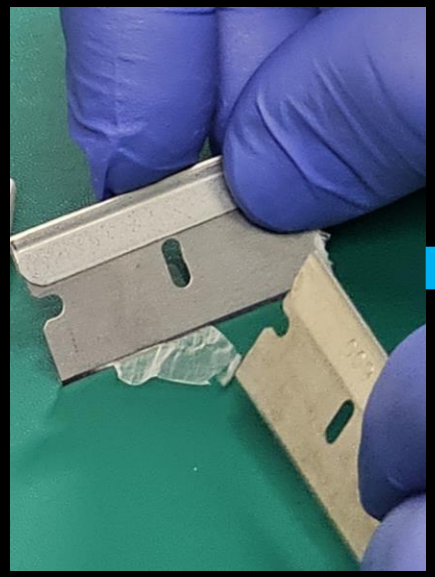


Annotated H&E



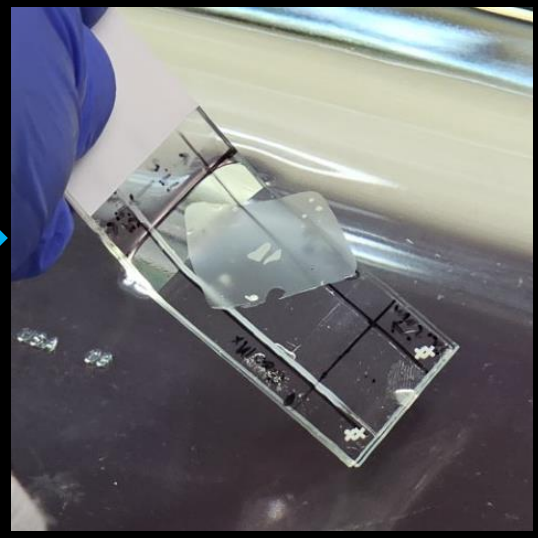
Annotated H&E or other stained slides guide are of interest identification

Tissue Scoring



Sections scored to remove excess tissue

Tissue Placement



Mock slides with the capture area guide sectioning

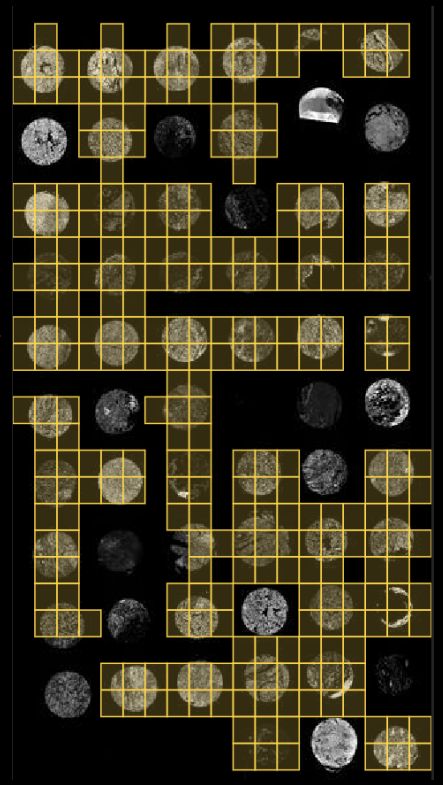
TMA Tissue Map & Core Groups

Xenium Slide #1 **Xenium Slide #2**

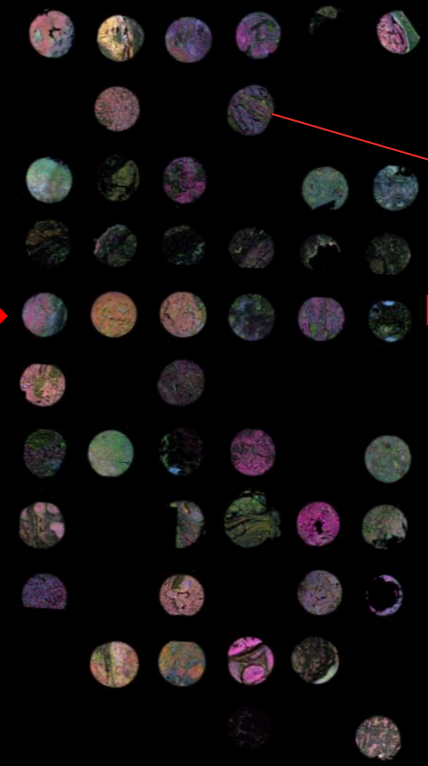
5	6	7	8	9	10	11	12	13	14	15	16	
Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	1
Lymph Nodes	Tumor	Lymph Nodes	Tumor	Tumor	Lymph Nodes	Lymph Nodes	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	2
Tumor	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	Tumor	Lymph Nodes	3
Controls	Controls	Controls	Controls	Controls	Controls	Controls	Controls	Controls	Controls	Controls	Tumor	4
Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	5
Tumor	Lymph Nodes	Tumor	Tumor	Lymph Nodes	Lymph Nodes	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	Lymph Nodes	6
Tumor	Tumor	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	Tumor	7
Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	8
Tumor	Lymph Nodes	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Lymph Nodes	Tumor	9
Lymph Nodes	Tumor	Tumor	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Lymph Nodes	Tumor	Tumor	Tumor	10
Empty	Empty	Empty	Controls	Lymph Nodes	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	Tumor	11

TMA does not fit in Xenium Sample Area, map helps split into 2 Xenium slides

Tumor + Control ROIs

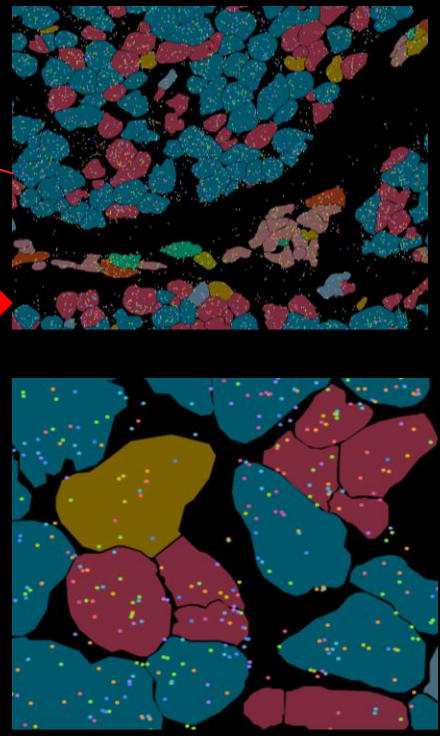


Tumor + Control Genes



Map guides region of interest selection for most effective data grouping and analysis: Lymph Nodes vs Tumor + Controls

Closeup of Tumor Genes



Cell clusters & Transcripts of tumor core

H&E Protocol & Image Files

Post Xenium Assay, H&E Modification

Test H&E with available reagents

- Reagents recommended by company on backorder
- Use duplicate slide of tissue from Xenium QC Run

↓

Stain Too Dark

- Modify protocol to decrease incubation times
- Use other duplicate slide of tissue from Xenium QC Run

↓

Used New Protocol on Xenium QC Slide

- Reproduce results on Xenium experimental slides
- Save updated protocol

Post Xenium Assay, Image File Conversion

Image file is SVS format

- Not compatible with Xenium Explorer
- Need H&E image to correlate data to transcripts

↓

Use QuPath to convert to OME-TIFF

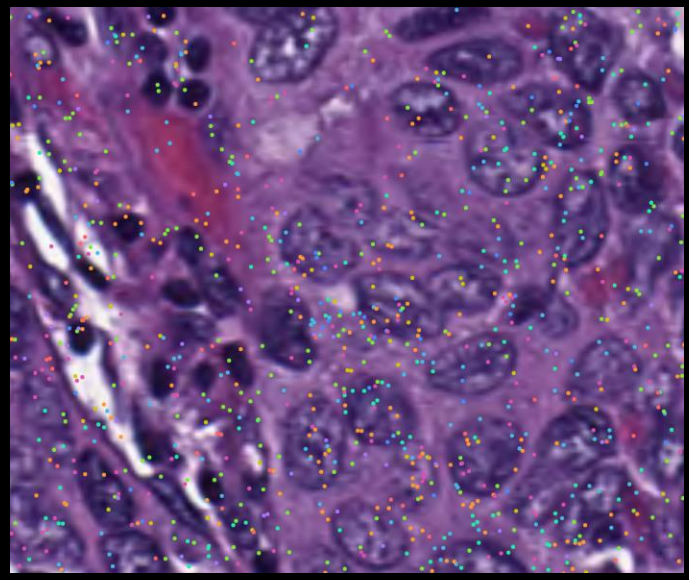
- Compatible with Xenium Explorer
- Can convert H&E's or IF images

↓

Import images to Xenium Explorer

- Compare morphology to transcripts
- Compare transcripts to protein data

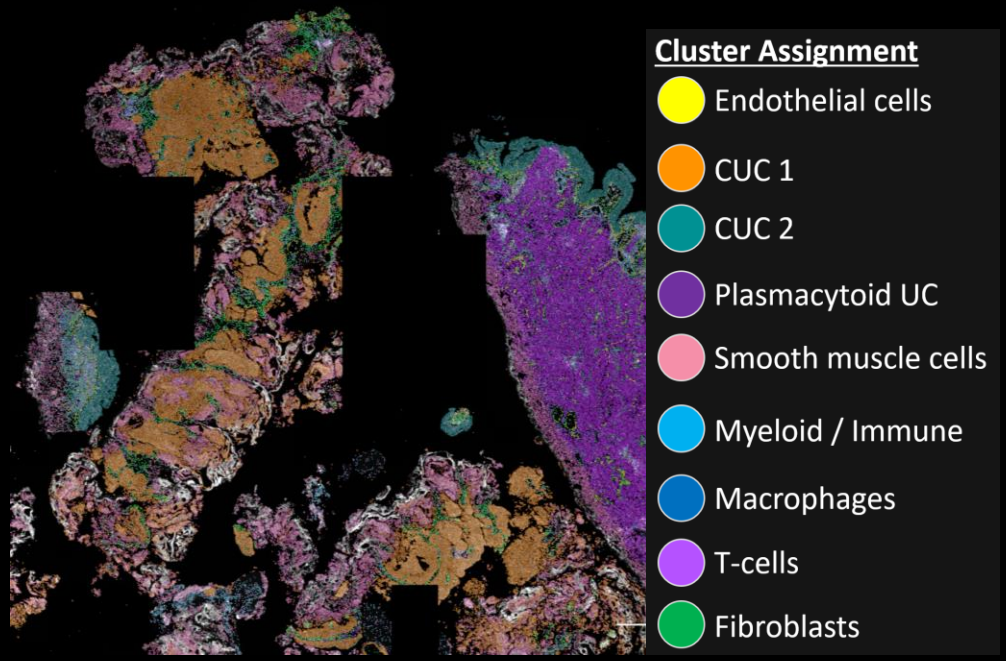
H&E + Transcripts



Spatially resolved transcripts in TMA core stained with H&E post Xenium assay after file conversion and import into Xenium Explorer

Results & Quality Metrics

Cell Type Map



Spatial mapping identified distinct tumor microenvironment components with unique profiles

Panels & Key Metrics

10X Multi-tissue + cancer panel:
377 genes
Custom bladder cancer panel:
100 genes

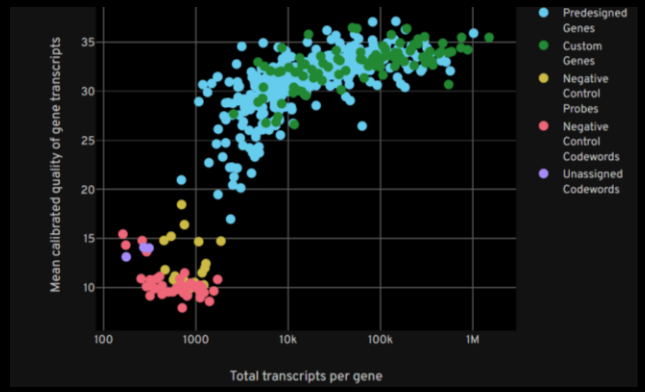
320,917
Number of cells detected

43
Median transcripts per cell

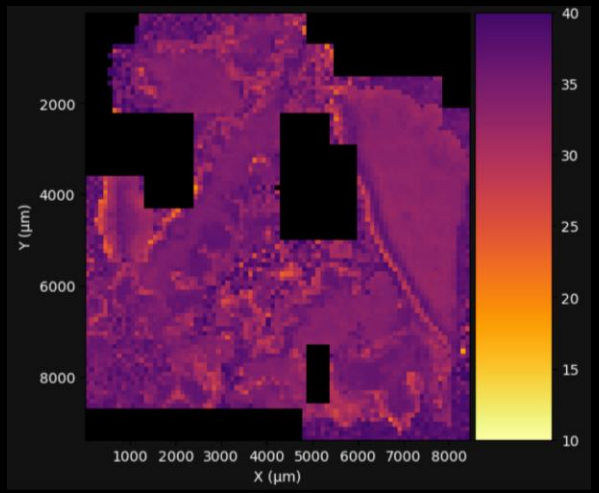
26,458,989
Total high quality decoded transcripts

RNA Quality Scores

Q-Scores > 20 are good!



Gene-Specific Transcript Quality



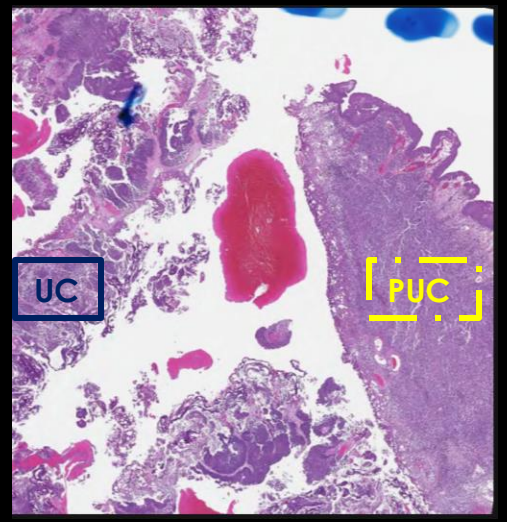
Mean Q-score Spatial Map

Unpublished – please do not copy/post

Results & Validation

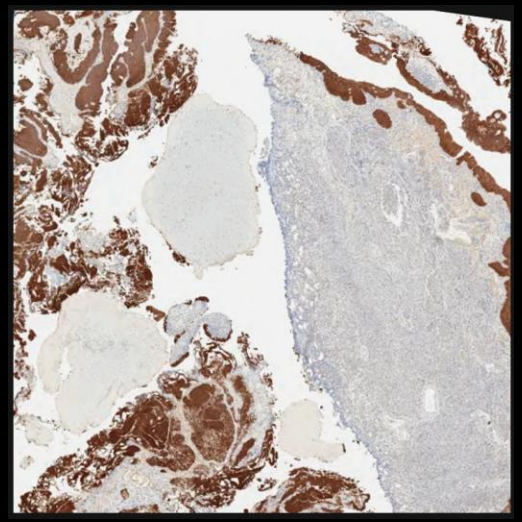
PUC shows loss of CDH1 (E-Cad) and increased SNAIL expression compared to Conventional UC

H&E



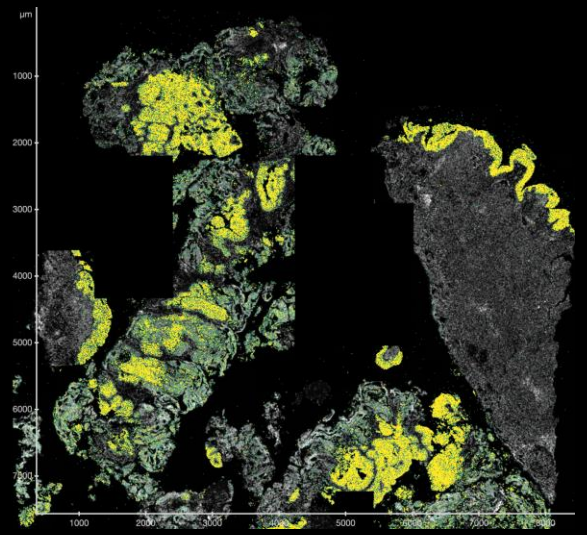
Evaluates tissue quality and tumor type/availability

E-cadherin IHC



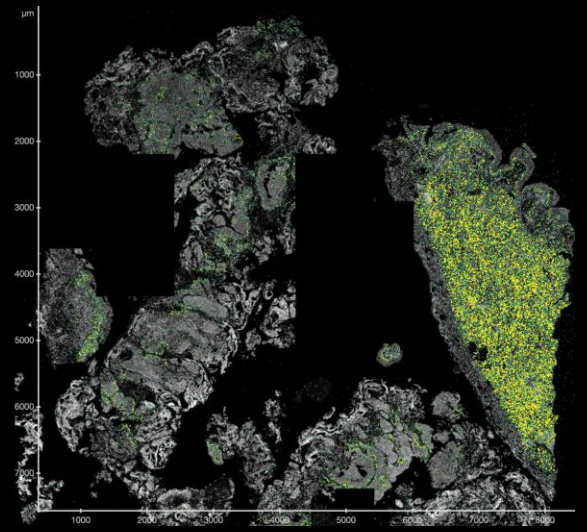
Correlates known histological features to genetic data

CDH1 Genes (E-cadherin)



Xenium CDH1 signal confirmed by IHC

SNAI2 (Snail)



Xenium SNAIL signal confirmed in PUC region according to H&E

Spatial transcriptomics is revolutionizing disease understanding—core facilities are at the forefront of this transformation.

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Secondary appointment in Genetics
UNC-CH

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Histologist
Pathology Services Core

→ For performing PUC Xenium assay and facilitating images

Nicholas Pankow
Histologist
Pathology Services Core

→ For performing PUC Xenium assay

UNC School of Medicine

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