

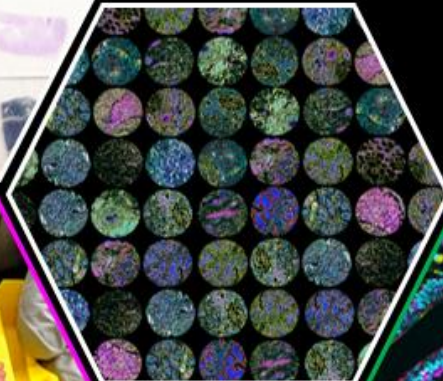
HISTOLOGY



- Process/embed and sectioning
 - Formalin-fixed
 - Frozen

- Tissue decalcify
- RNase-free technique
- H&E, other routine stains
- Special stains

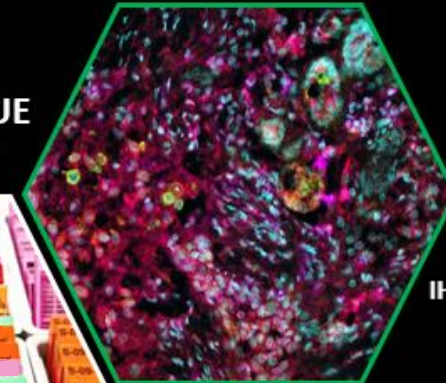
TMA CREATION



CONTROL TISSUE REPOSITORY



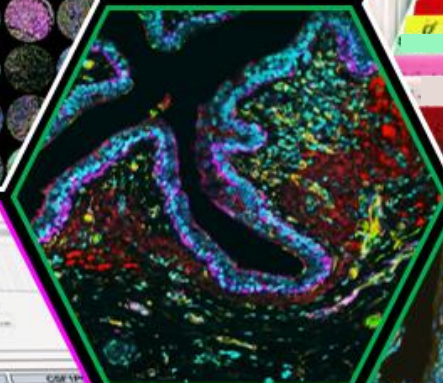
IHC/ISH



IHC & ISH:

- Chromogenic (BF)
- Immunofluorescent (IF)
- Automated or manual
- Single or multiplex
- Validation & optimization of antibodies

IMAGING



- Imaging Systems:
 - Vectra
 - Nuance
 - APERIO

MOLECULAR SERVICES



- DNA/RNA extraction:
 - FFPE
 - Fresh tissue

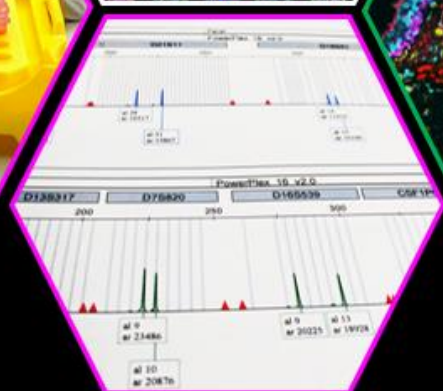
- Tissue collection:
 - FFPE sections/curles
 - Tissue block punch
 - Macrodissections

- Molecular Profiling
 - nCounter MAX

Short tandem repeat (STR) analysis:

- Cultured human cells
- Extracted DNA

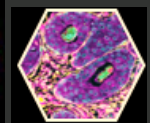
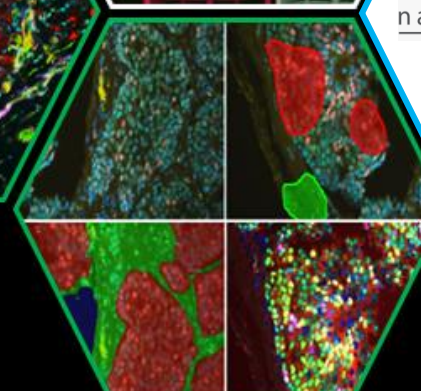
HUMAN CELL LINE AUTHENTICATION



SPATIAL -OMICS

- GeoMx: RNA and protein
- Visium w/ CytAssist
- Visium HD for higher resolution
- Xenium and MERSCOPE support

MORPHOMETRIC ANALYSIS



- Board-certified pathologist
- Certified histotechnologist
- Experienced staff

Functional Spatial Multiomics with GeoMx DSP: Integrating Biological Layers to Advance Mechanistic Biomarker Discovery

Grant M. Barthel
sRAM – Bruker Spatial Biology
3/25/2026

Best-in-class solutions for every layer of biology

Spatial Biology

GeoMx®



Unmatched discovery multiomics to profile from cells to tissue structures for spatial breakthroughs at scale

CosMx®



High sensitivity, subcellular imaging of the whole transcriptome for complete pathway-level spatial insights

CellScape™



Flexible subcellular proteomics for rapid and quantitative spatial phenotyping

Cellular Biology

nCounter®



Highly robust single or multiomics bulk analysis for rapid biomarker discovery and development

Beacon®



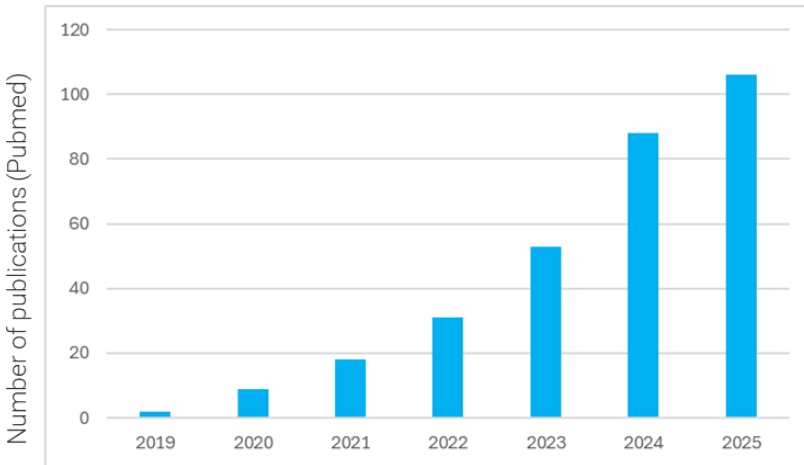
Functional, multi-parameter profiling of live single cells

GeoMx DSP uniquely enables comprehensive tissue biology analysis and streamlined discovery-to-translation workflow



- Spatial resolution
- Scalable throughput
- FFPE compatibility
- Whole transcriptome
- High-plex pathway-centric protein panel
- High PTM content
- Functional state measurement
- Multi-layer integration
- Translational readiness

GeoMx DSP spatial transcriptomics, proteomics and multiomics increasingly contributes to high-impact discovery



Science

Subventricular zone radial glial cells maintain inhibitory neuron production in the human brain

nature

Cancer-induced nerve injury promotes resistance to anti-PD-1 therapy

npj | precision oncology

Published in partnership with The Horrell Institute, University of Minnesota

Article



<https://doi.org/10.1038/s41698-025-00963-0>

The development of a high-plex spatial proteomic methodology for the characterisation of the head and neck tumour microenvironment

Cancer Cell

Multi-omic landscape of human gliomas from diagnosis to treatment and recurrence

nature immunology



Resource

<https://doi.org/10.1038/s41590-025-02161-3>

A single-cell spatial chart of the airway wall reveals proinflammatory cellular ecosystems and their interactions in health and asthma



Integrated spatial multi-omics profiling of *Fusobacterium nucleatum* in breast cancer unveils its role in tumour microenvironment modulation and cancer progression



Multi-scaled transcriptomics of chronically inflamed nasal epithelium reveals immune-epithelial dynamics and tissue remodeling in nasal polyp formation

nature medicine



Article

<https://doi.org/10.1038/s41591-025-03957-4>

Microbial signals in primary and metastatic brain tumors

nature medicine



Article

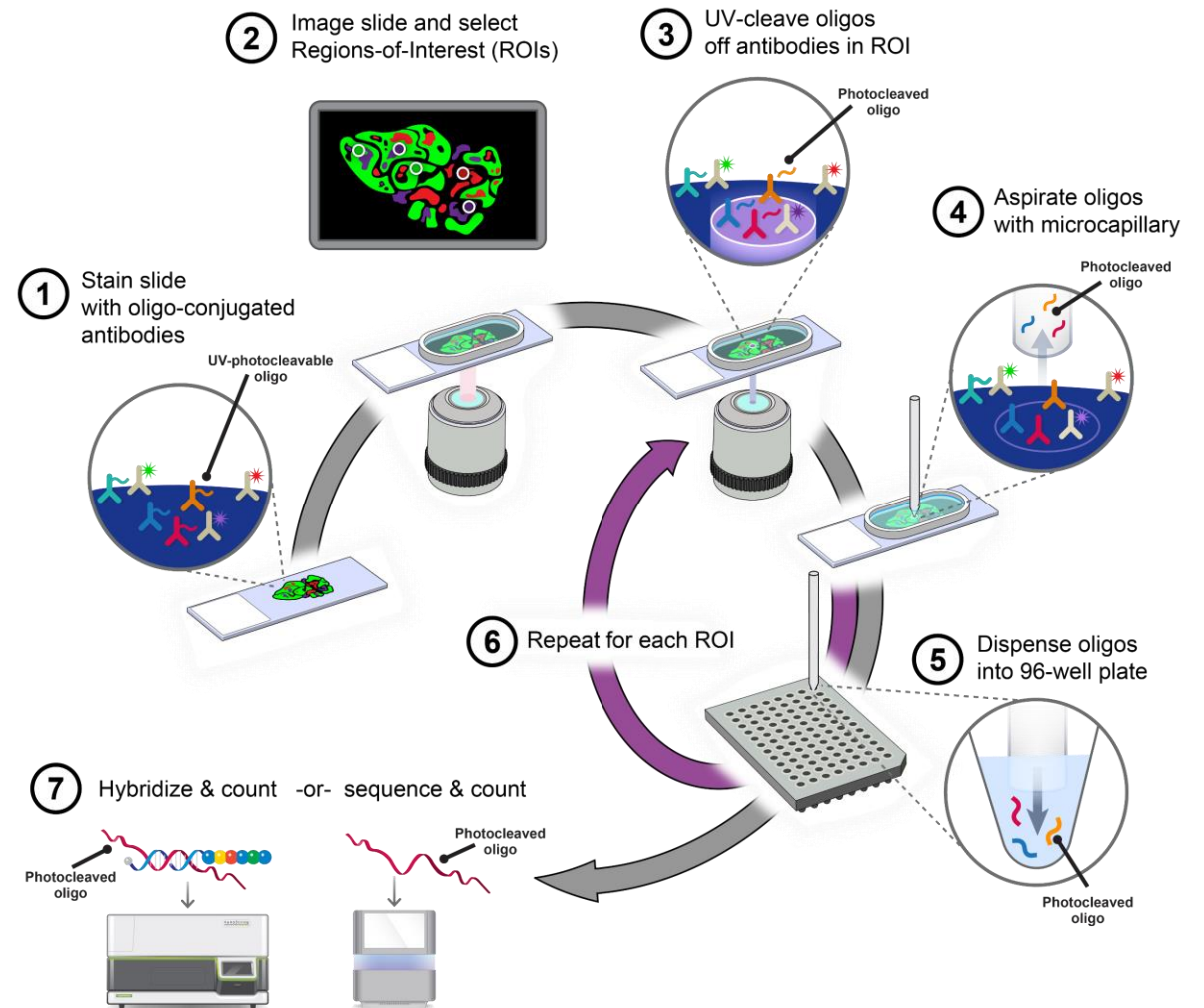
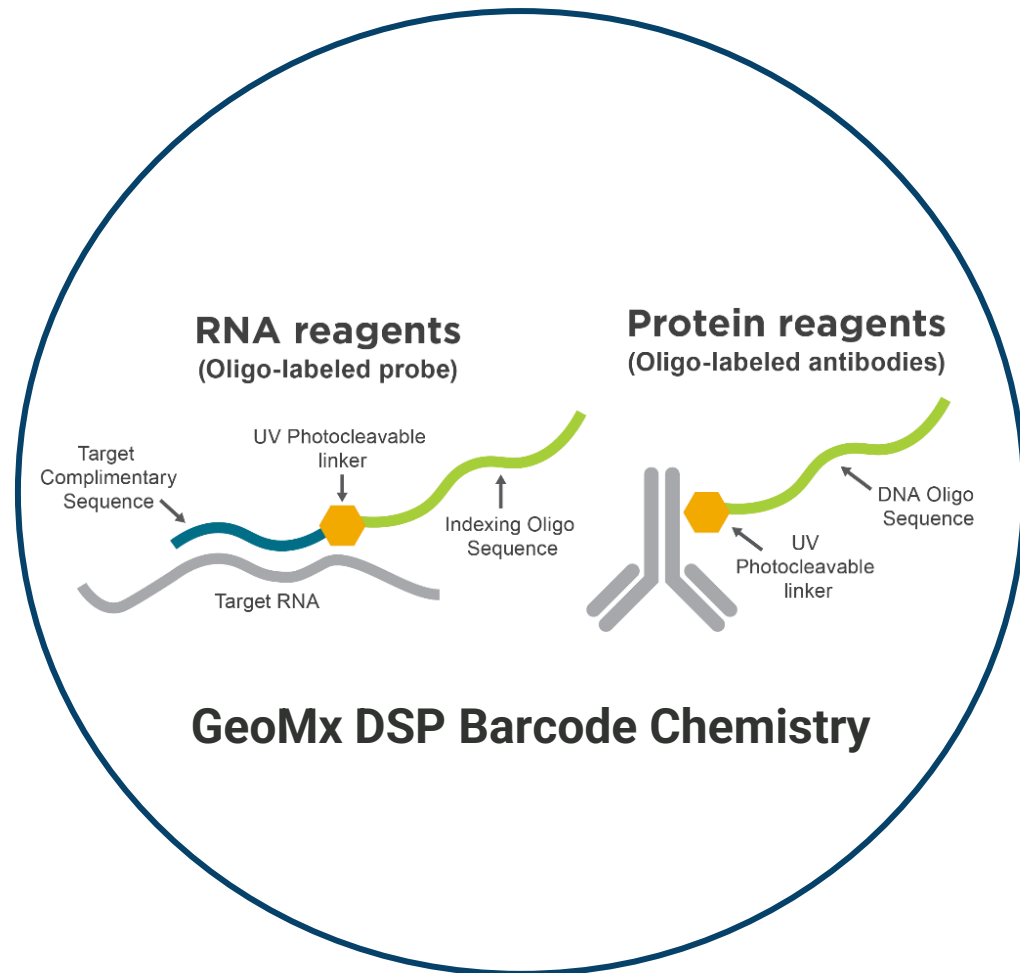
<https://doi.org/10.1038/s41591-025-03625-7>

Broad rim lesions are a new pathological and imaging biomarker for rapid disease progression in multiple sclerosis

Molecular Cancer

The landscape of dynamic tumor immunophenotyping on neoadjuvant chemotherapy combined with trastuzumab for the treatment of HER2-positive breast cancer

GeoMx DSP's core barcoding chemistry and sequencing readout enables theoretically unlimited plex



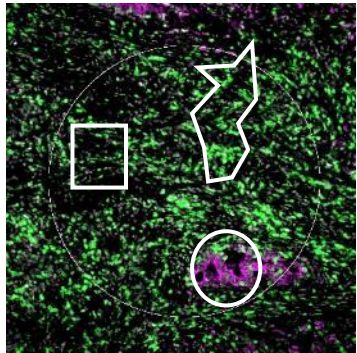
Unique Profiling Modalities to Interrogate Tissue Samples



Geometric

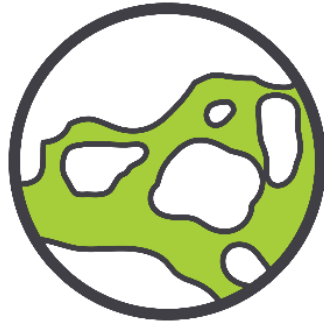


CD3 PanCK DNA

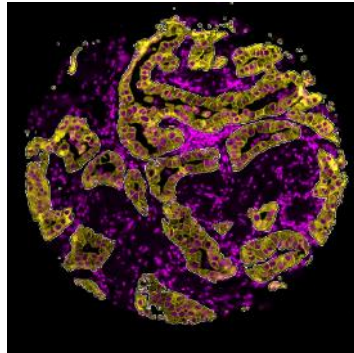


What is the heterogeneity of expression in different regions of my tissue?

Segmentation

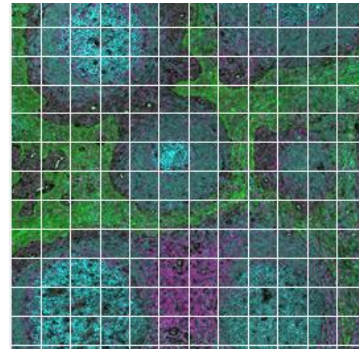
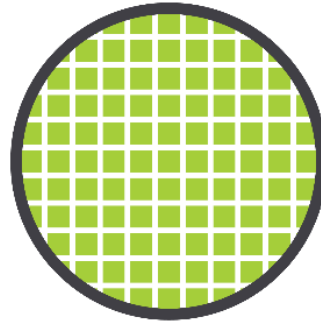


PanCK DNA



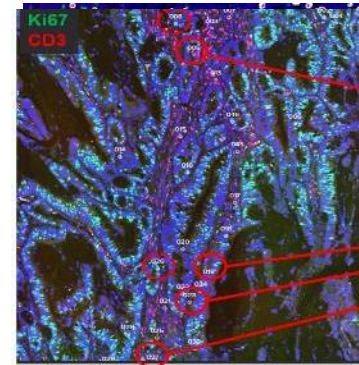
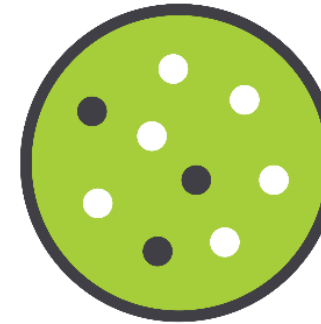
What is the expression profile of distinct biological compartments (e.g., Tumor-TME)?

Gridded



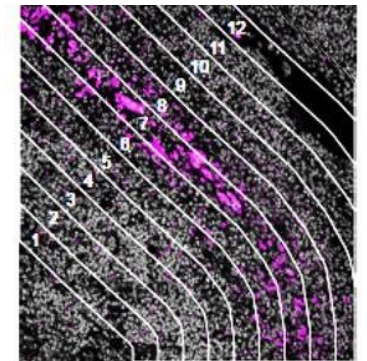
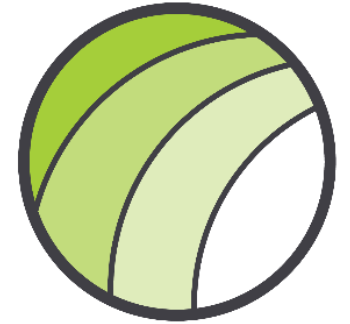
What novel targets are uncovered with deep mapping of a specific tissue region?

Cell Type Specific



What is the expression profile of a specific cell population in my tissue?

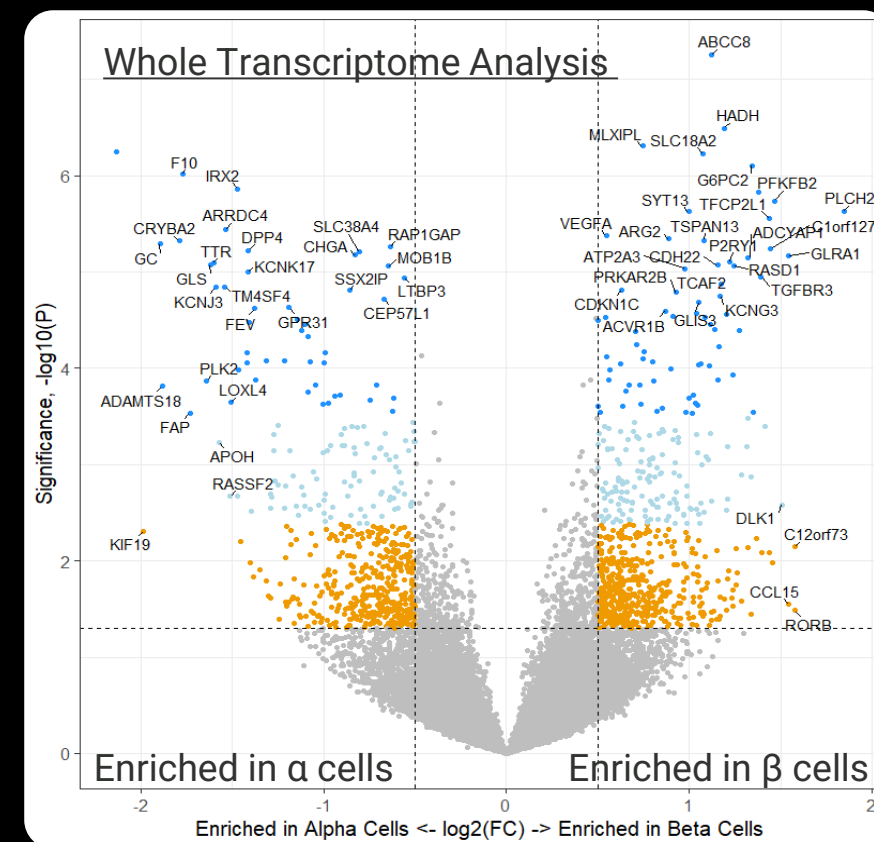
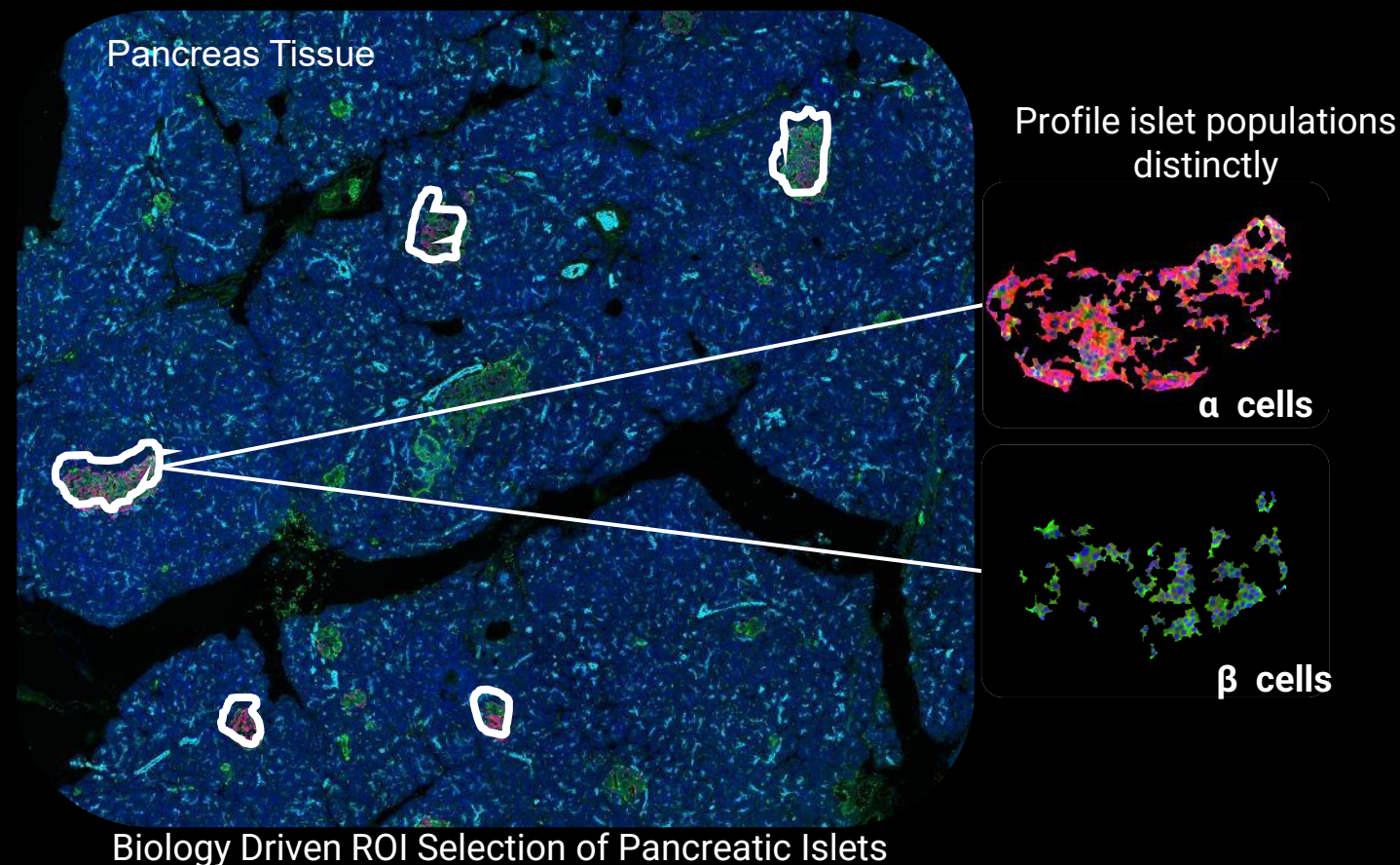
Contour



How does the immune environment change on either side of an infiltrate boundary?

Focus on the biology of interest and maximize discovery with whole transcriptome exploration

What genes are differentially expressed in alpha and beta cell populations in the Islets of Langerhans?

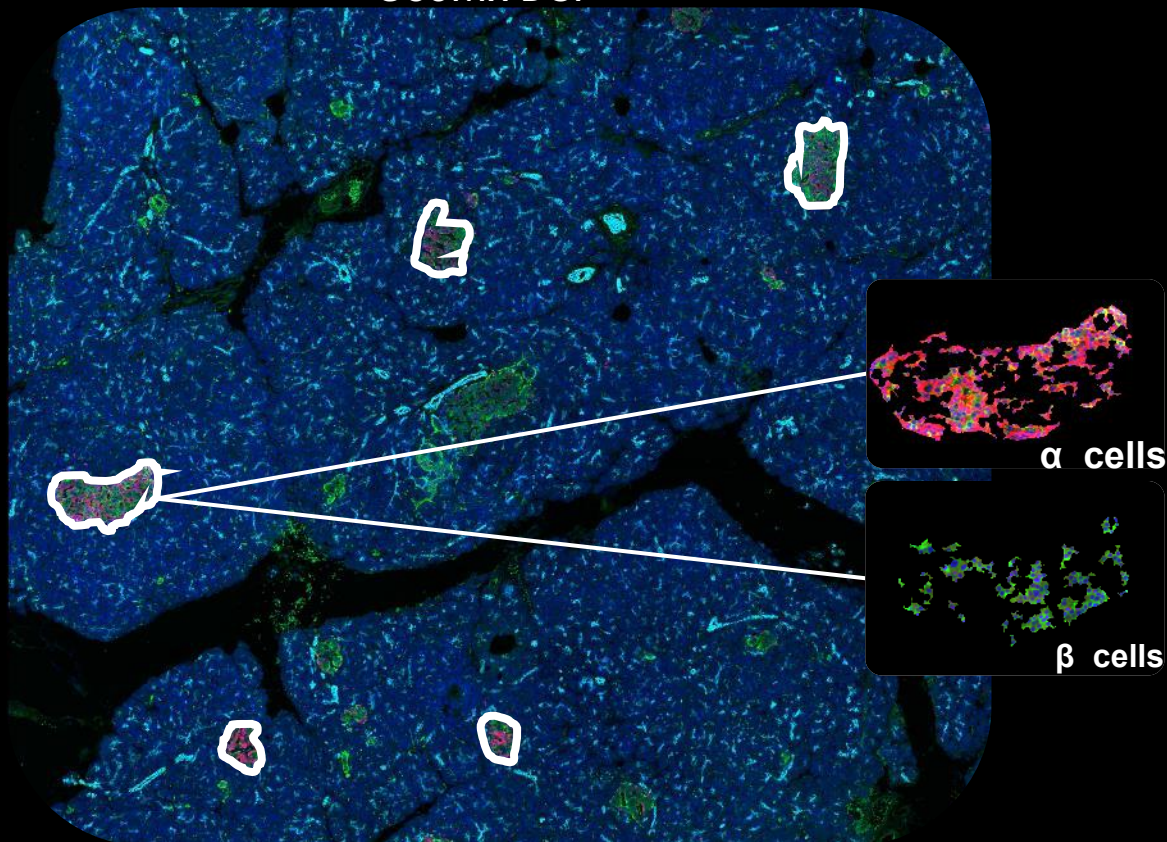


>8000 genes detected

GeoMx DSP captures critical biology and tissue organization lost with array-based methods

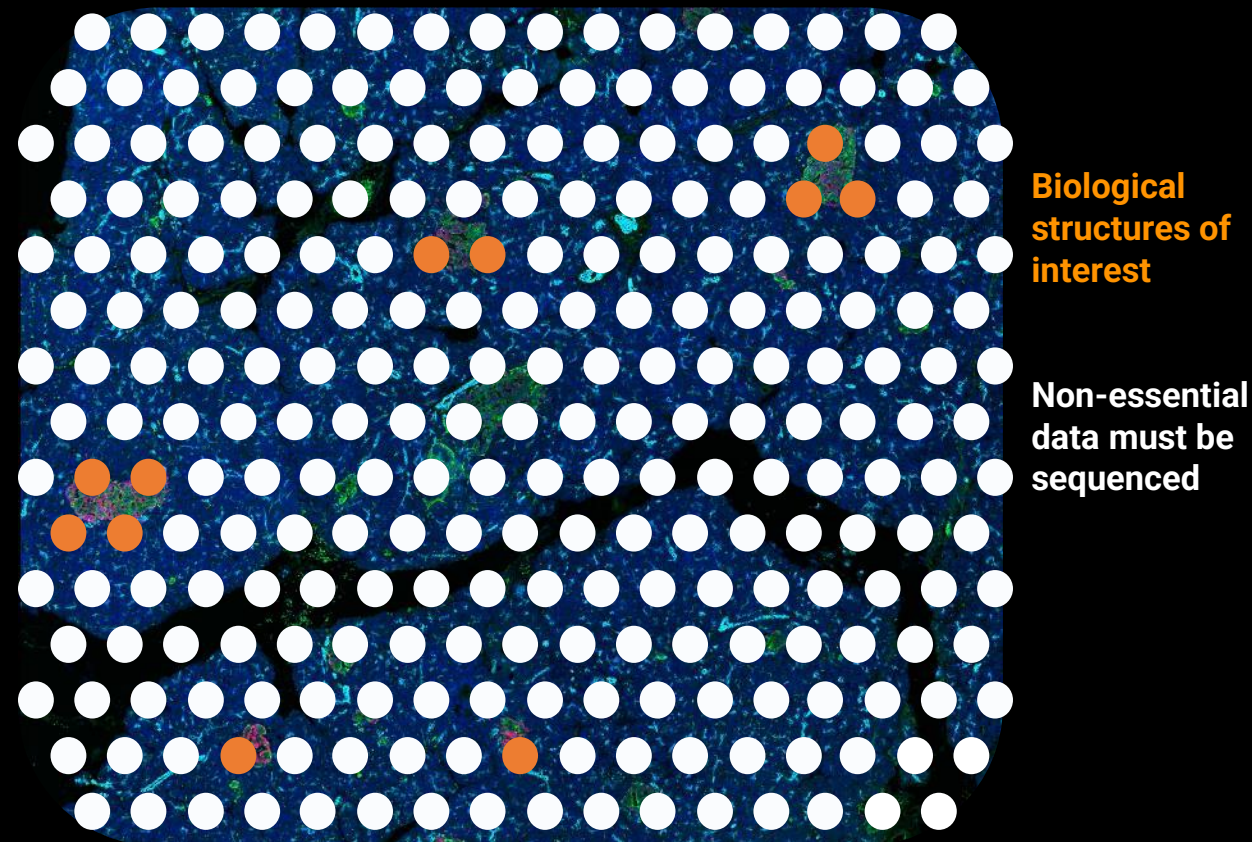
Explore tissue without non-essential sequencing costs

GeoMx DSP



Relevant biological structures with high sensitivity

Array-based method



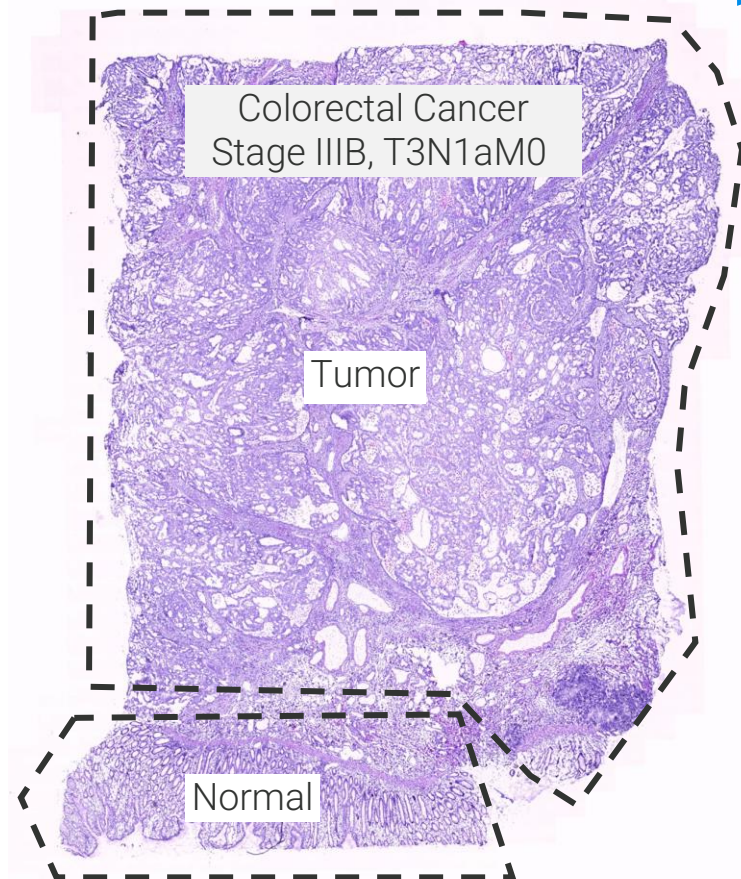
Mixed cells in array spots with low sensitivity

High-throughput and cost-effective spatial multiomic analysis of defined tissue regions of interest



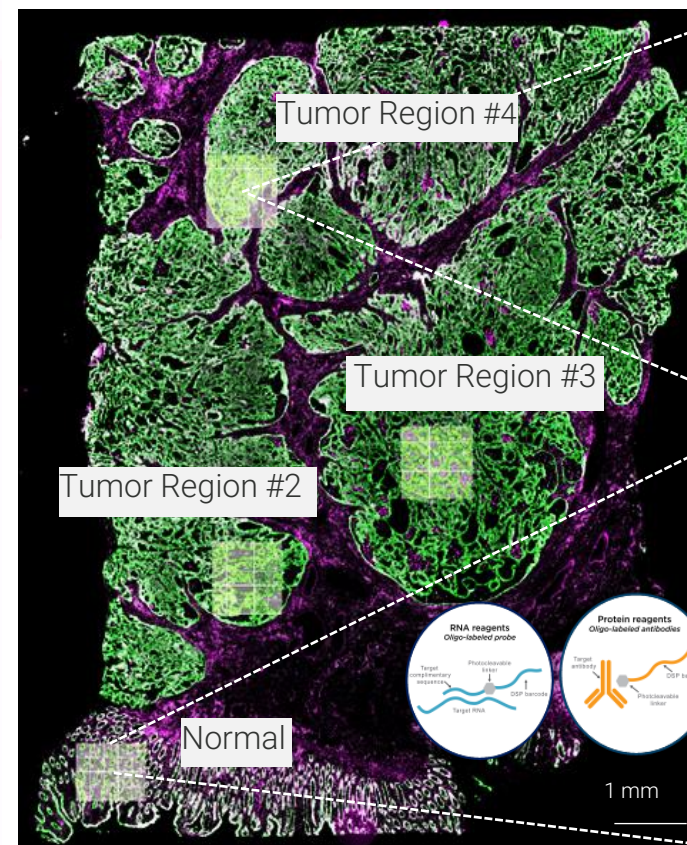
Visualize **Tissue Morphology**

H&E Stain

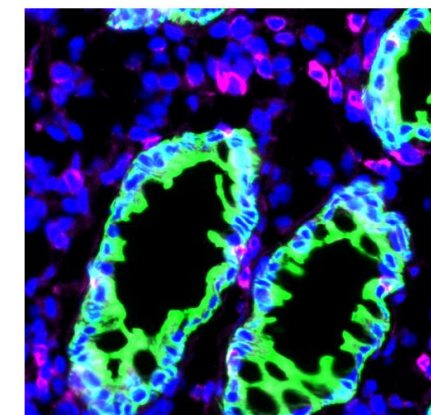
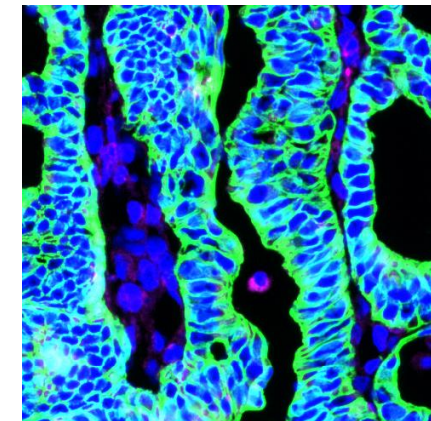


Focus on **Regions-of-Interests (ROIs)**

Immunofluorescence + Oligo-tagged RNA probes and antibodies



PanCK **CD45**

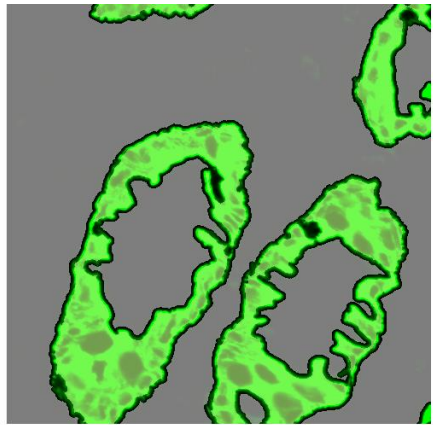
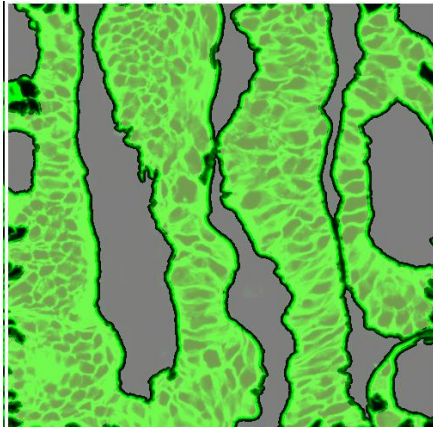


Precise collection of RNA and protein information from enriched cell populations for downstream analysis

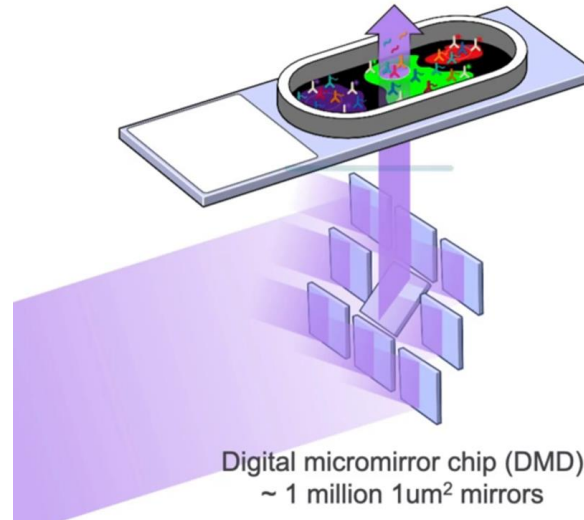
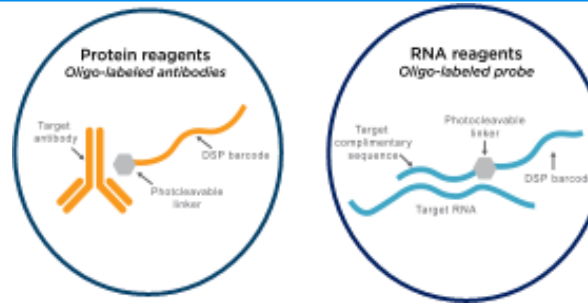


Area of Illumination

PanCK+ CD45- cells

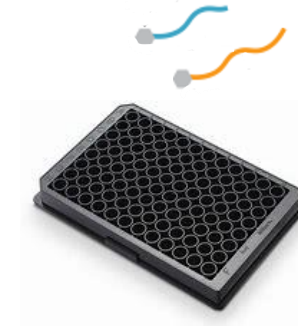


UV-Directed Oligo Cleavage



Digital micromirror chip (DMD)
~ 1 million 1 μ m² mirrors

Oligo Collection

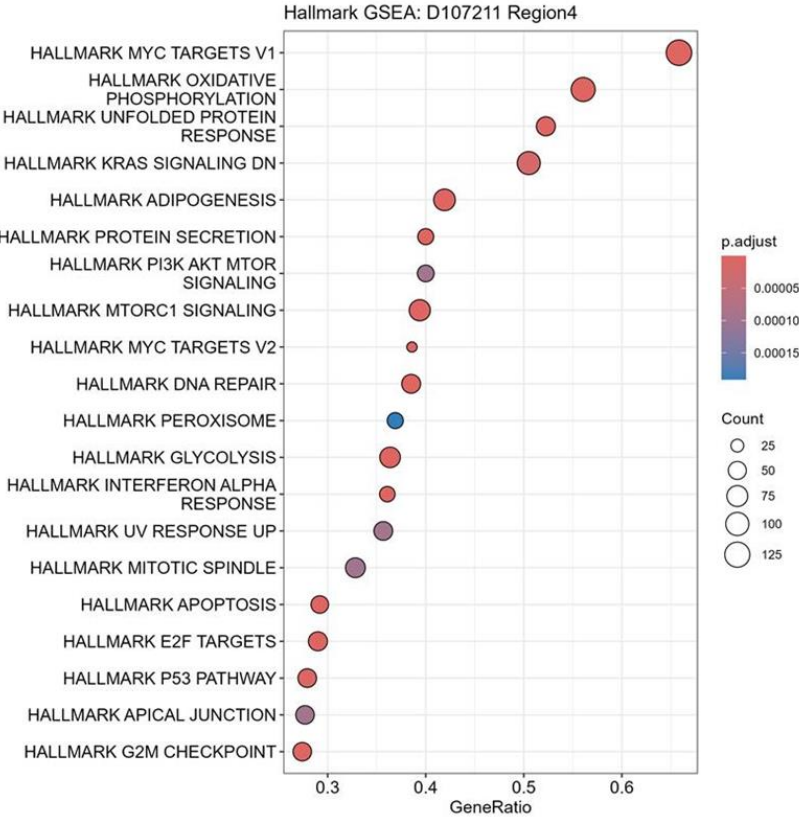
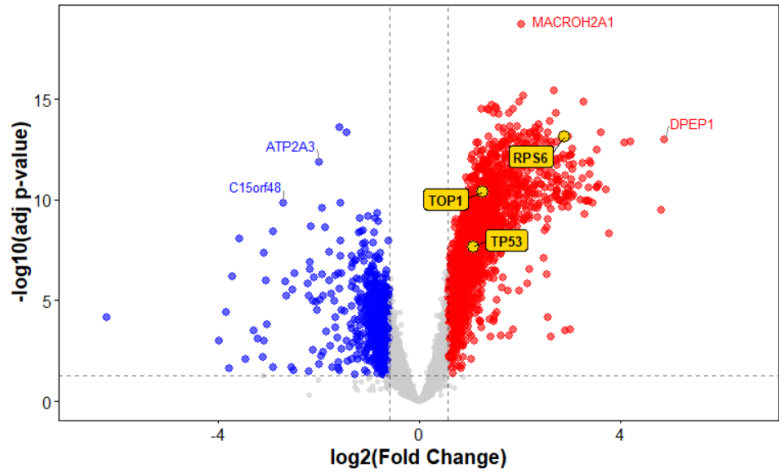


Count



Spatial whole transcriptome analysis identifies available pathways potentially driving tumor growth

Normal Region vs Tumor region



Data interpretation based on RNA analysis

Biological pathways	Enrichment of
Proliferation and cell cycle	MYC, E2F targets, G2M checkpoints
DNA damage and stress repair	DNA repair, UV response, p53 signaling
Oncogenic pathways	KRAS, PIK3-AKT-mTor, p53 signaling
Apoptosis	CASP8, CASP4, IGF2R, BCL2L1

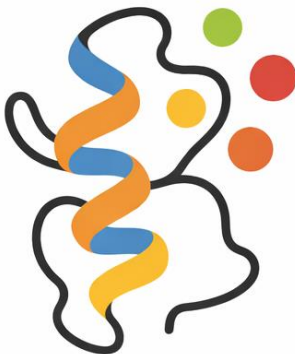
- A highly proliferative and stressed tumor driven by oncogenic pathways – Are the pathways active?
- Actively engaging DNA repair and p53 pathways – Which DNA damage response pathways are active?
- Simultaneously primed for apoptosis and survival – Which program is really being executed?

Integrating RNA and protein layers: From descriptive biology to mechanistic insights

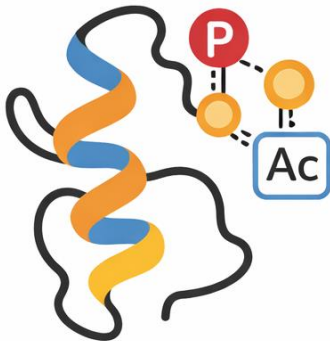
RNA



Protein



Protein Translational Modification (PTM)



What it answers	What biological programs are primed?	Is the machinery present?	What is actually active right now?
What it defines	Potential	Capacity	Activity

RNA pathway enrichment ≠ pathway activation

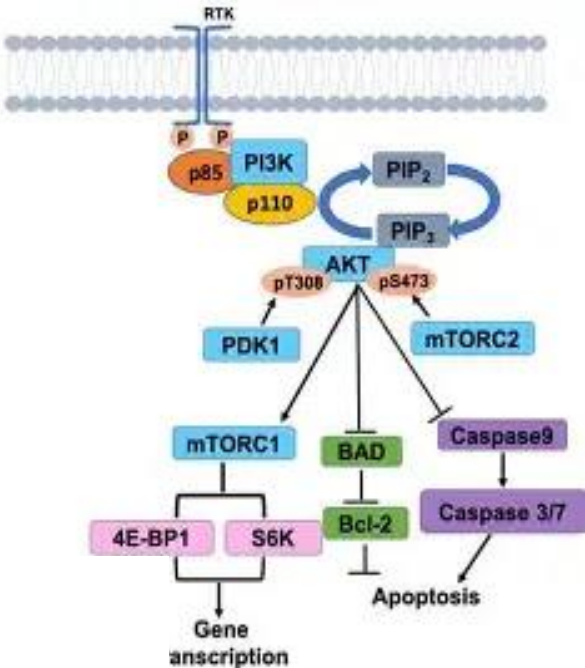
GeoMx Discovery Protein Atlas (DPA): A spatial functional signaling panel enriched in PTMs for mechanistic discovery

Curated selection of **1200+** extensively validated antibodies targeting **core biological processes** that govern cellular behavior across tissues and disease contexts.

130+ PTMs covering major signaling pathways for **direct measurement of functional states**

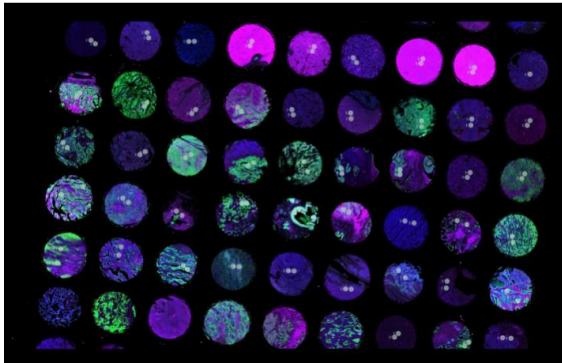


Signaling pathways	
RTK Signaling	Translation Control
PI3K–AKT–mTOR	Metabolic Rewiring
MAPK Cascade	AMPK / Energy Sensing
JAK–STAT Signaling	Redox Stress Response
NFκB Signaling	Cytoskeleton / Adhesion
TGFβ / SMAD Signaling	Vesicle Trafficking
WNT / β-Catenin	Proteostasis
DNA Damage Response	Autophagy
p53 Axis	Apoptosis
Cell Cycle Regulation	Epigenetic Regulation





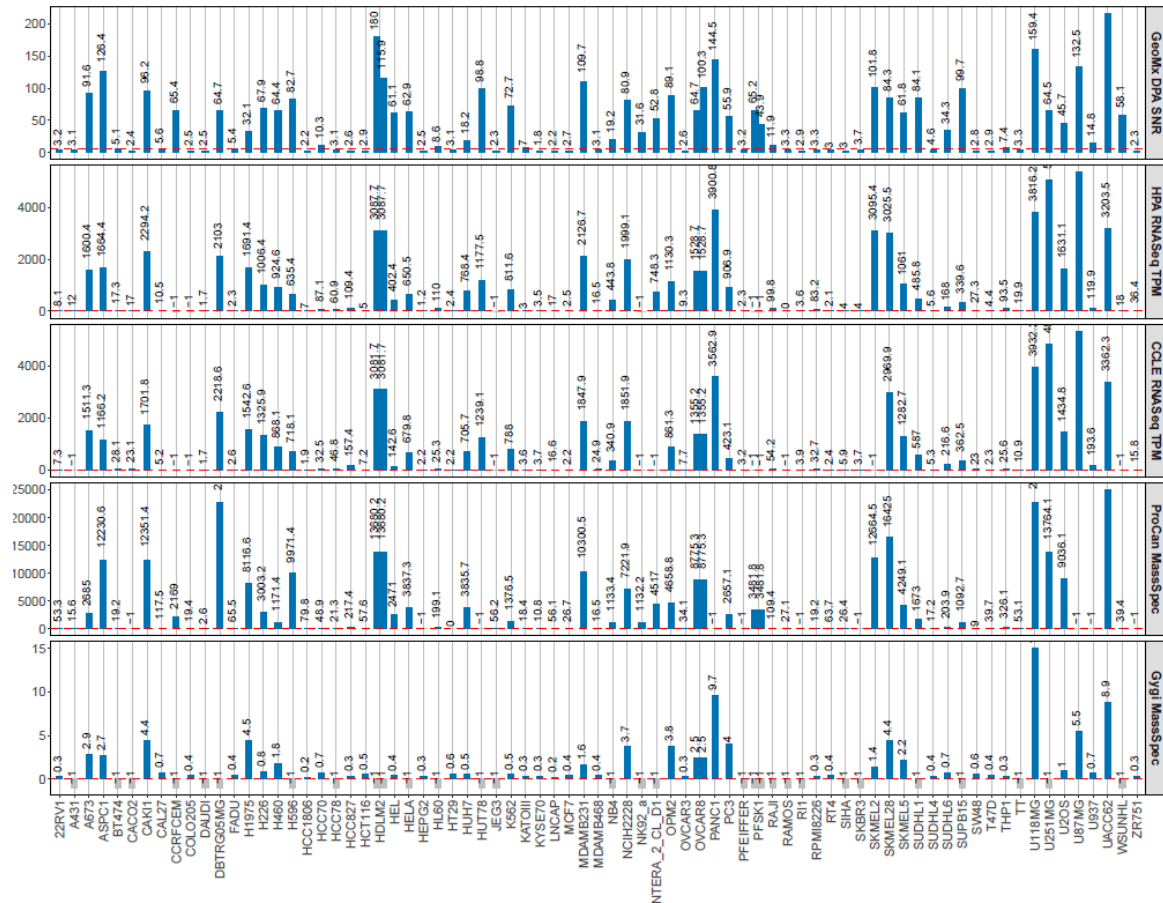
Antibodies are further validated at multi-plex level on multiple tissues



	MERGED	#b02547
Wild-type HeLa cells		
VIM knockdown HeLa cells		

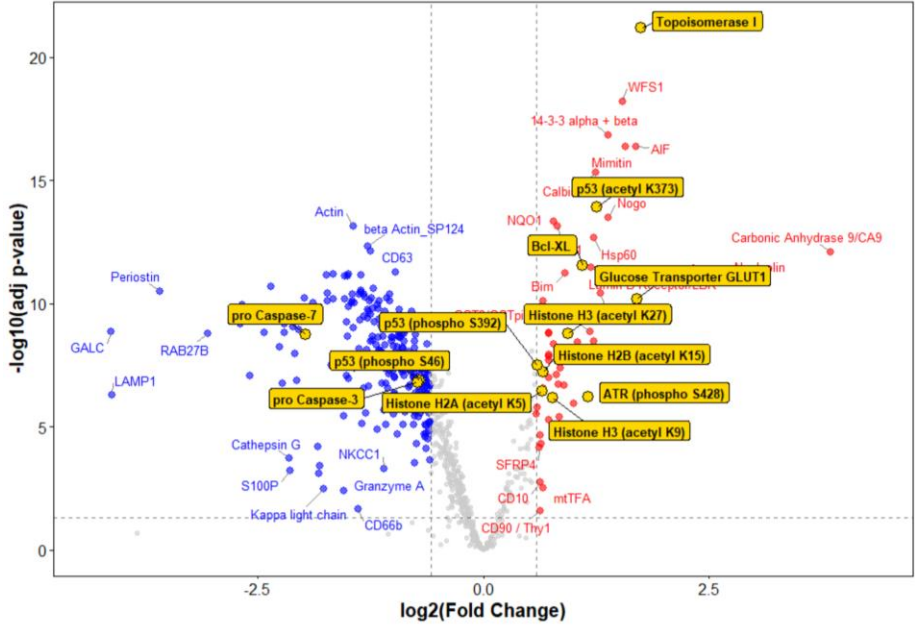
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Vimentin



Spatial functional protein data complements RNA data and directly measures pathway activity through extensive PTM analysis

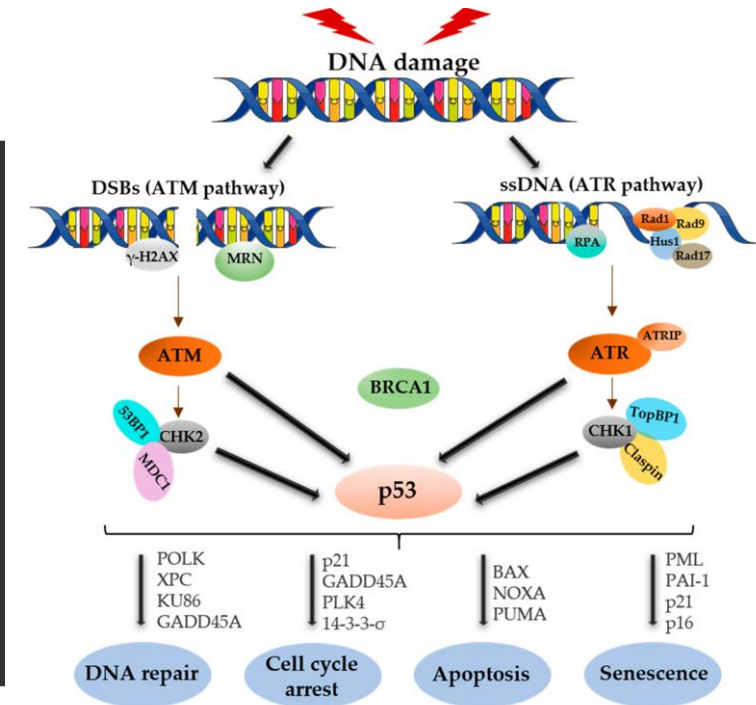
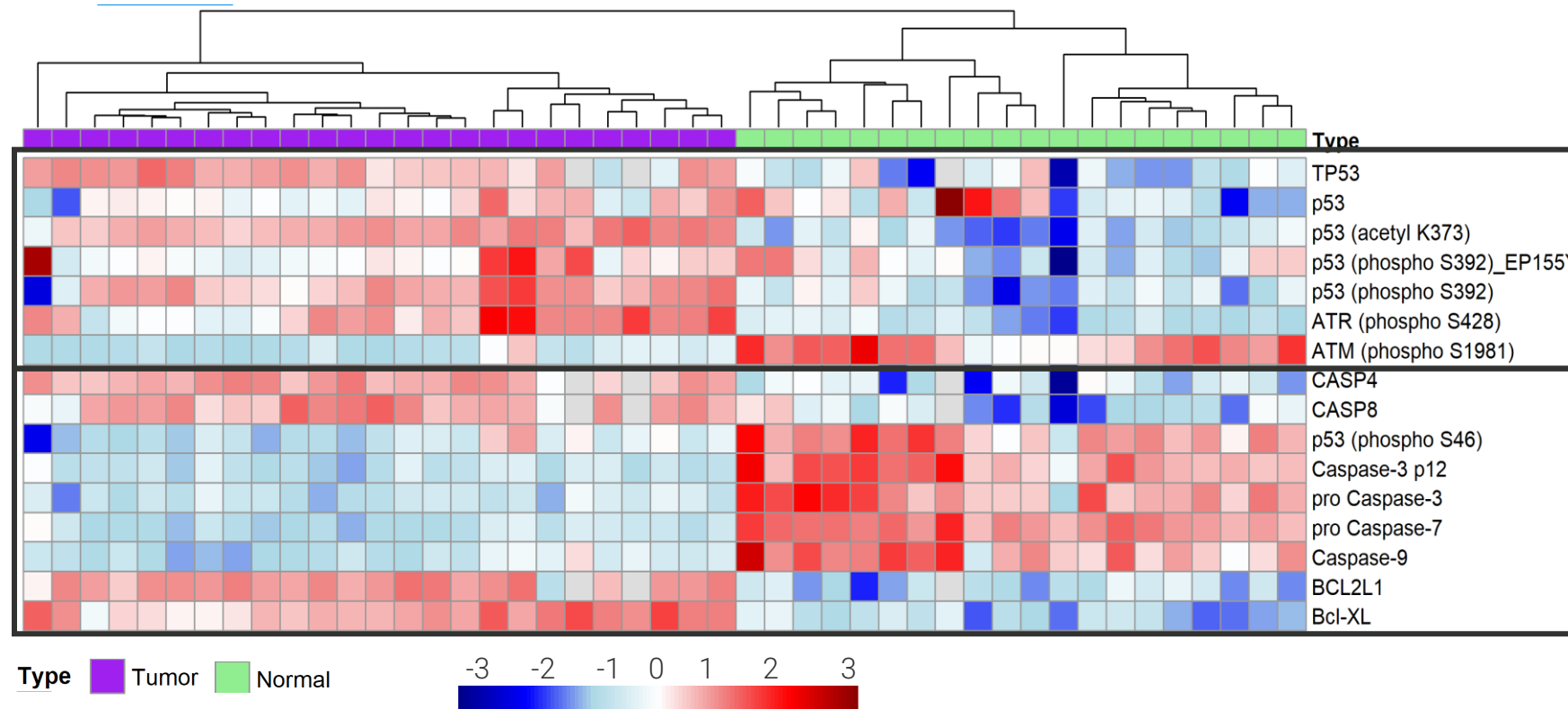
Normal Region vs Tumor region



332 differentially expressed proteins
68 differentially expressed PTMs

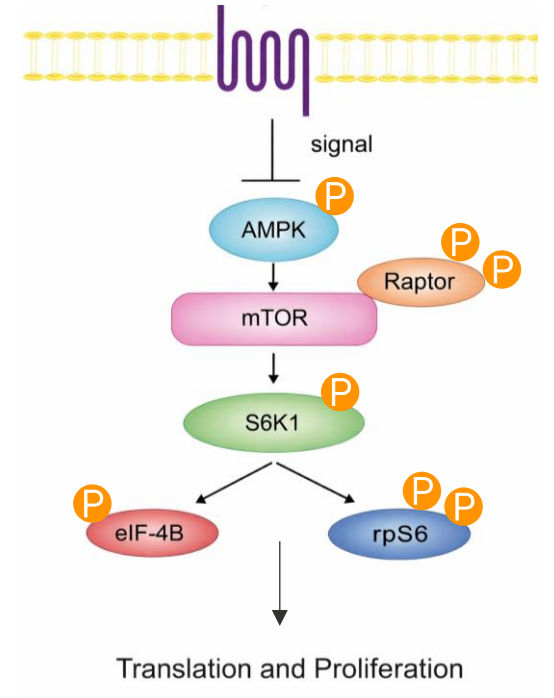
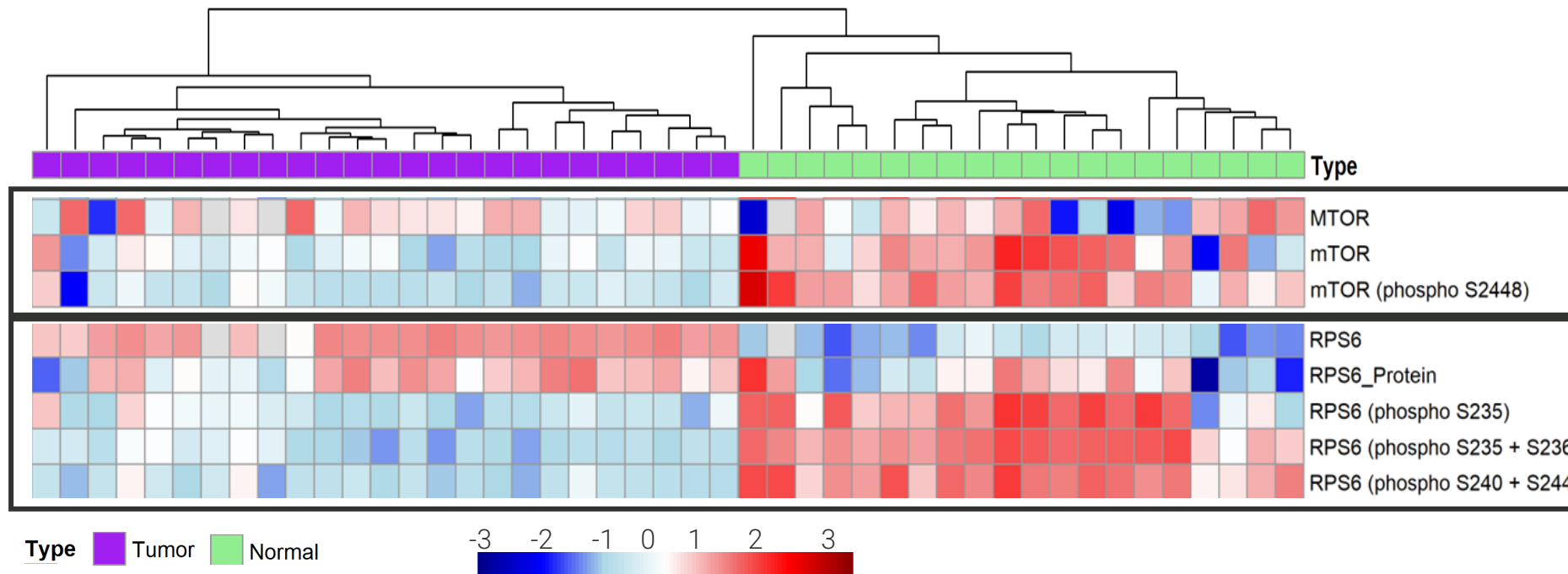
Biological Pathway	Key Protein / PTM Changes	Functional State
DNA Damage Response	ATR (pS428) ↑, ATM (pS1981) ↓, Topoisomerase I ↑	Replication stress–driven DDR
p53 Signaling	p53 (acetyl K373) ↑, p53 (pS392) ↑, p53 (pS46) ↓, TP53INP1 ↑	Active p53 stress response
PI3K–AKT–mTOR	AKT (pS473) ↓, mTOR (pS2448) ↓, RPS6 (pS235/236, pS240/244) ↓, eIF4EBP1 ↑	Attenuated pathway activity
Cell Cycle / Proliferation	E2F4 ↑, Topoisomerase I ↑, mitotic regulators mixed	Sustained proliferative drive
Epigenetic Regulation	Histone H3 (K27ac, K9ac) ↑, H2A/H2B acetylation ↑	Active chromatin state
Apoptosis	Cleaved Caspase-3 ↓, Caspase-9 ↓, Bcl-xL ↑, Bim ↑	Survival favored over apoptosis execution
Metabolic Reprogramming	GLUT1 ↑, SLC7A5 ↑, Glutaminase ↑, IDH2 ↓, PDHA1 ↑	Metabolic adaptation
Oxidative Stress / Redox	SOD2 ↑, NQO1 ↑, GSTpi ↑	Enhanced antioxidant defense,
Cytoskeleton / Adhesion	Integrins mixed, E-cadherin ↓, cytokeratins ↓	Structural remodeling

Spatial multiomics confirms and refines the role of DNA damage response and p53 in driving tumor survival



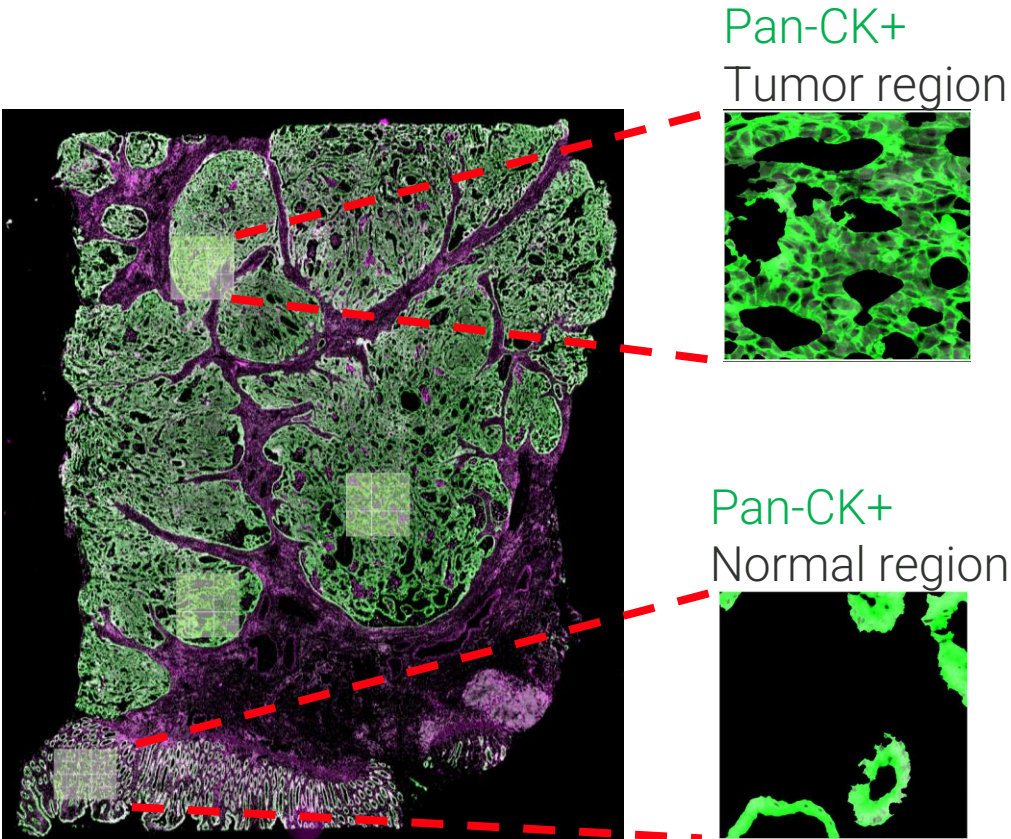
- RNA, total protein and PTMs strongly correlate and confirm activation of p53 pathway
- DNA damage response is driven by ssDNA repair pathway and phosphorylated ATR
- Both apoptosis and survival are primed at RNA level, but protein data indicate apoptosis suppression and survival program execution

Spatially multiomics correct potentially misleading information provided by RNA alone



- RNA analysis indicated enrichment of oncogenic pathway PI3K-AKT-mTOR
- Protein analysis demonstrates that although primed, this pathway is not executed
- RPS6 mRNA and total protein are upregulated in tumor regions, but PTMs are shut down

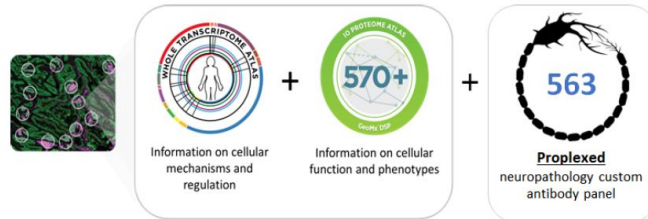
A More Complete View of Cancer Biology Driven By the Integration of Spatial Transcriptomics and Proteomics



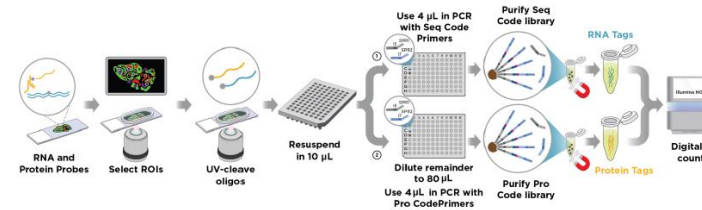
RNA/Protein	Hallmark pathway (RNA)	Representative markers (Protein / PTM)	Interpretation
Concordant	MYC Targets / E2F / G2M Checkpoint	E2F4 ↑, Topoisomerase I ↑, Histone H3 acetylation ↑	Proliferative transcriptional programs are functionally executed via chromatin opening and replication machinery
Concordant	DNA Repair	ATR pS428 ↑, p53 acetyl-K373 ↑, p53 pS392 ↑	RNA "DNA repair" reflects ATR-biased replication stress , not balanced repair
Concordant	Oxidative Phosphorylation	AIF ↑, IDH2 ↑, PDHA1 ↑, mtTFA ↑	Mitochondrial engagement with stress-adapted remodeling, not simple ETC upregulation
Concordant	Apoptosis (stress-associated)	BCL-XL ↑, TP53INP1 ↑, Bim ↑	Apoptotic program transcriptionally engaged but shifted toward survival
Discordant	PI3K-AKT-mTOR / mTORC1	AKT pS473 ↓, mTOR pS2448 ↓, RPS6 pS235/236 ↓	Pathway transcriptionally present but canonically inactive at signaling level (feedback or rewiring)
Discordant	Hypoxia / HIF signaling	HIF-1α ↓, CA9 ↑	Microenvironmental hypoxia signatures persist despite reduced HIF-1α protein
Discordant	Immune / Interferon response	HLA-A ↓, HLA-C ↓, CD3 ↓, CD8 ↓	RNA immune signatures do not translate to functional immune presence (immune exclusion)
Discordant	Glycolysis	GLUT1 ↑, Hexokinase I/II ↓	Metabolic rewiring favors nutrient uptake and redox control, not full glycolytic flux

WTA combined with >1.1K proteins to Reveal Biomarkers Associated with Alzheimer Disease

Highest Plex Spatial Multiomics



Spatial Proteogenomic Workflow #2: Seq Code RNA Assay and Pro Code Protein Assay



"Digital Spatial Profiler platform is uniquely suited to support high-plex proteomics...Here, we use a barrier-breaking, highest plex spatial multiomics solution to explore the connection between tau neuropathology and senescence in human Alzheimer's disease (AD)"

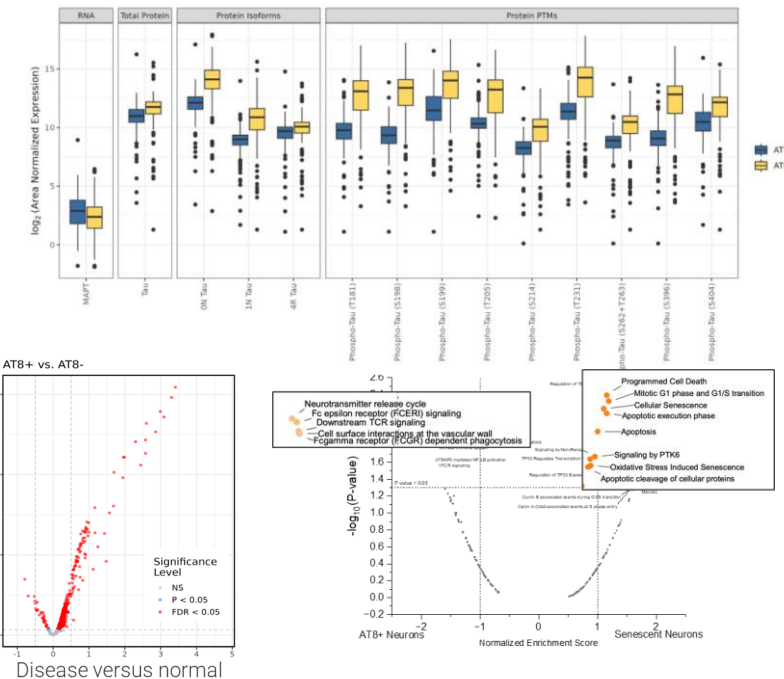
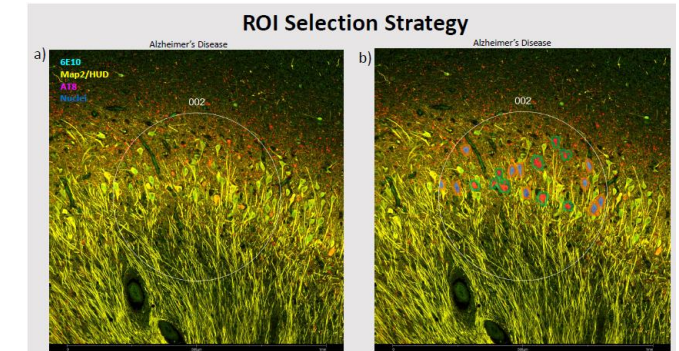


Miranda Orr, PhD
Miranda Orr, PhD

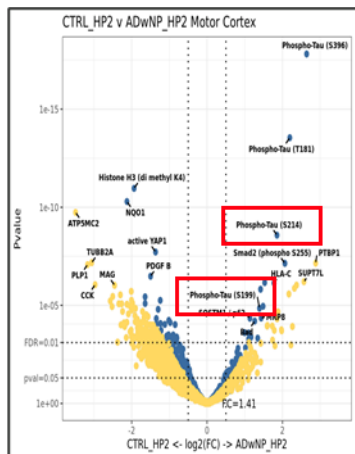
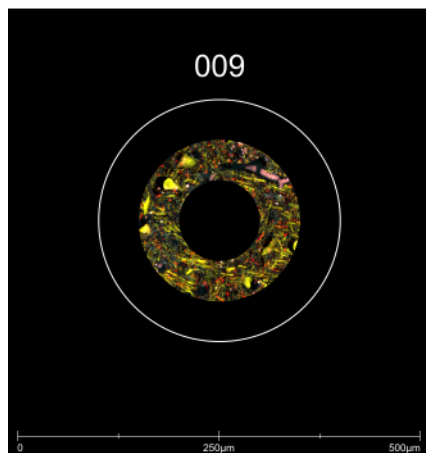


Findings

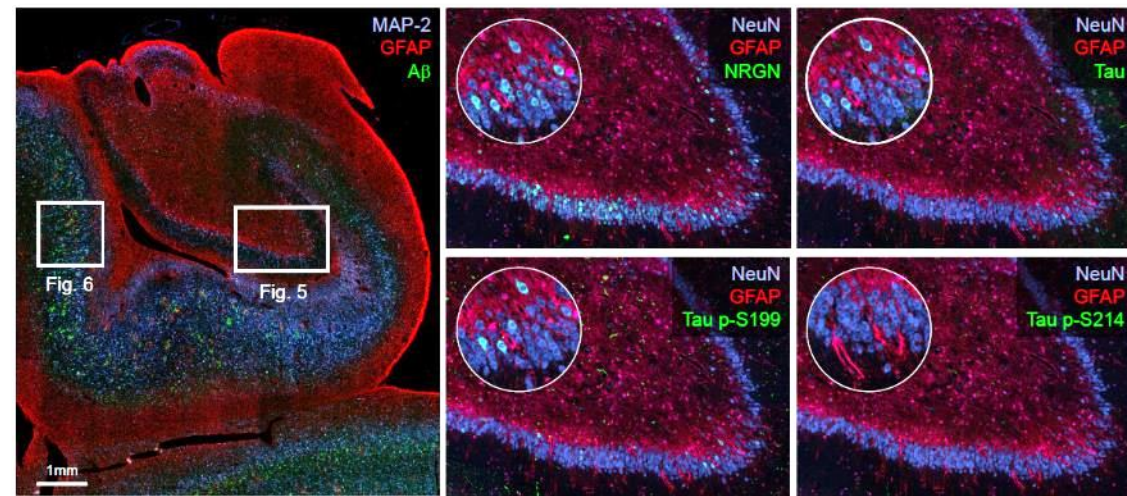
- In human Alzheimer's brain, measured over 15X more dysregulated proteins than had ever been.
- No differences in expression levels of the *MAPT* gene coding for Tau protein was observed between normal and disease neurons
- Several phosphorylated forms of Tau protein were on the other hand identified as potential disease biomarkers
- Integrating gene expression, protein abundance, isoform diversity, and post-translational modifications is essential to fully understand tau biology.



Unlocking a spatial protein biomarker discovery-to-validation workflow not possible to date: the only path available to screen >1200 antibodies



abcam



- Screened >1K proteins
- Identified biomarkers implicated in Tauopathy
- Each clone in the panel is readily available as single-vial reagent
- Requirement for *in-situ* single cell validation at scale



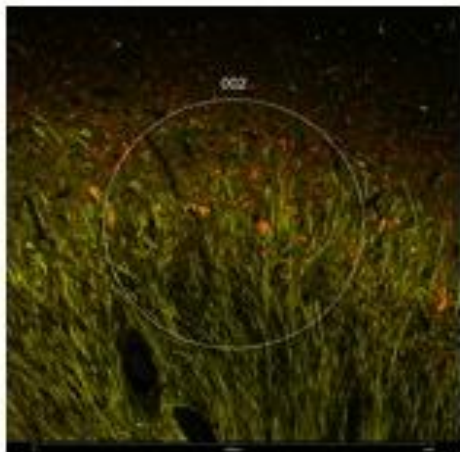
Miranda Orr, PhD
Washington University in St. Louis



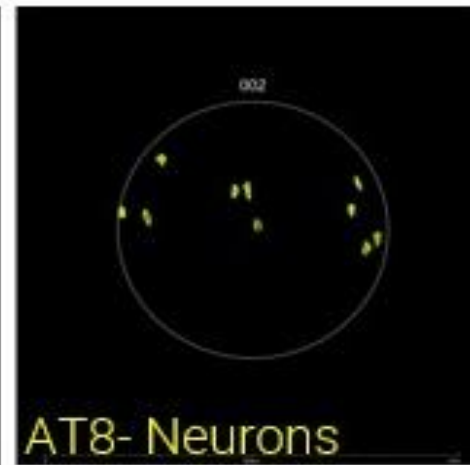
- Designed custom 30-plex panel
- 20 unknown clones (incl. 8 phosphospecific ABs)
- Ideation to result: <1 month
- AB success rate ~85%

Single-cell spatial resolution for protein interrogation from niches to rare and individual cells

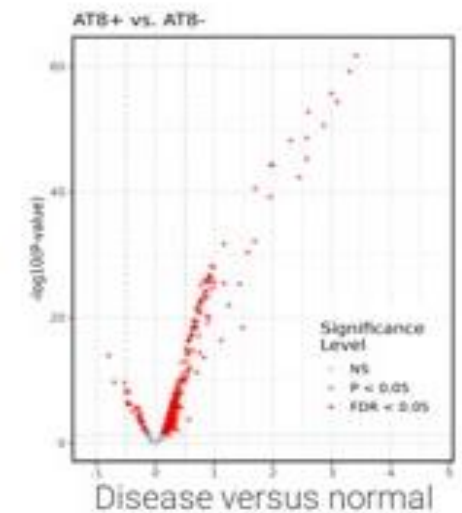
Select ROI



Segment and enrich rare cells



Perform differential analysis



Miranda Orr PhD



Single-cell segmentation to investigate the expression of 1200+ proteins on enriched rare AT8+ and AT8- cells. *Data generated in collaboration with Dr. Miranda Orr*

Integration of proteomics, transcriptomics and glycomics for a comprehensive characterization of different glioma types and grades



Cancer Cell

Multi-omic landscape of human gliomas from diagnosis to treatment and recurrence

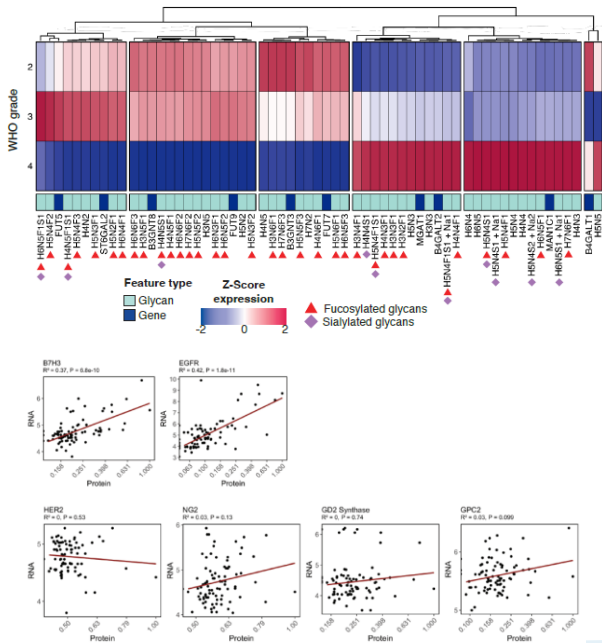
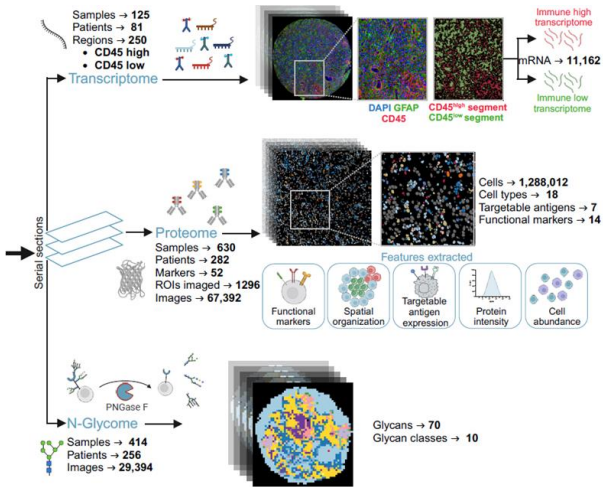
“Combining these modalities (proteomics, transcriptomics and glycomics), we established direct correlations between cell composition, protein expression, gene expression, and glycan profiles within the same spatial context across our diverse glioma cohort.....Multi-modal data analysis across these lesions provided new insights into how the glioma TME is shaped by tumor classification and treatment, revealing potential explanations for the limited efficacy of TA-targeted immunotherapies...”



Michael Angelo
Associate Professor of Pathology



Article

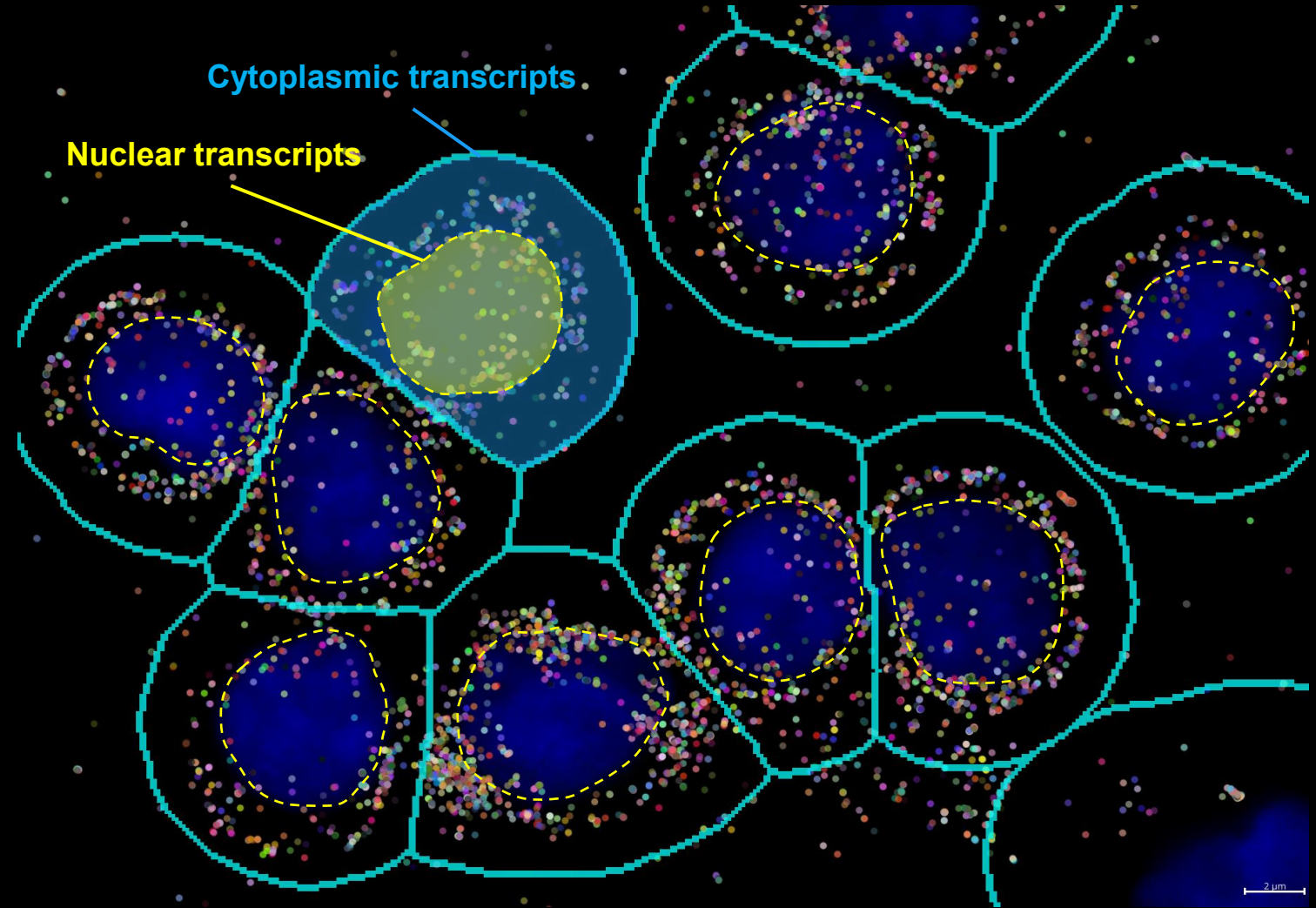


- Spatial proteomics (MIBI), transcriptomics (GeoMx) and glycomics (MALDI-MSI) were performed on serial sections from patients with different type and grade of glioma
- MIBI protein panel identified changes in cell composition correlating with glioma type and grade as well as tumor antigens frequently expressed across all glioma types and grades
- GeoMx WTA + identified 11K genes differentially expressed in immune-rich and -poor regions
- MALDI-MSI glycomics revealed expression patterns of 70 N-Glycans associated glioma grade and type
- Integration of the data from the 3 single-omics revealed how transcriptomics could only partially predict tumor antigen or N-Glycan expression thus highlighting the need to integrate different modalities for a more precise and comprehensive biology and disease understanding.

GeoMx functional spatial multiomics transforms RNA-based hypotheses into mechanistically grounded insights

- Whole transcriptome combined with 1200+ proteins and PTMs on the same tissue regions enables **cell-type and region-specific** direct measurement of **pathway potential and activity**
- Spatial pathway analysis at the protein level is critical to:
 - **Confirm, refine and correct** information provided by RNA alone
 - Distinguish **active vs inactive pathways**
 - Select **mechanistically relevant biomarkers**
 - **Reduce false positives** from RNA-only signatures
 - Bridge discovery to **clinically actionable biomarkers**
 - Enable **more accurate prediction of therapeutic response**
- Integration of GeoMx DPA and CellScape XR supports a seamless and rapid transition from discovery to translation

Subcellular localization of RNA transcripts in PBMCs



Current needs and challenges in Spatial Biology

What are the most important features to you in a spatial biology platform? (1=highest, 9=lowest; n=128)

	1	2	3	4	5	6	7	8	9
1 Panel size	30	23	18	17	12	10	8	1	2
2 Spatial resolution	27	24	22	16	11	6	6	4	3
3 Multiomics	20	23	17	16	7	12	11	7	4
4 Cost per sample	14	7	19	19	22	15	9	7	4
5 FFPE compatibility	11	8	15	11	13	6	12	31	14
6 Ease of use	8	15	15	12	24	20	11	11	1
7 Up-front cost	4	2	1	10	9	13	13	17	53
8 Bioinformatics included	3	6	5	11	11	9	28	21	29
9 Cost per run	2	15	10	8	9	29	20	17	8

Created with [Datawrapper](#)



Spatial Omics Technology Survey Reveals 'Long Tail' of Interest Across Many Platforms

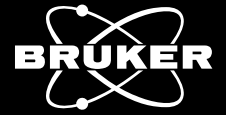
Aug 13, 2025 | [Andrew P. Han](#)

[Premium](#)

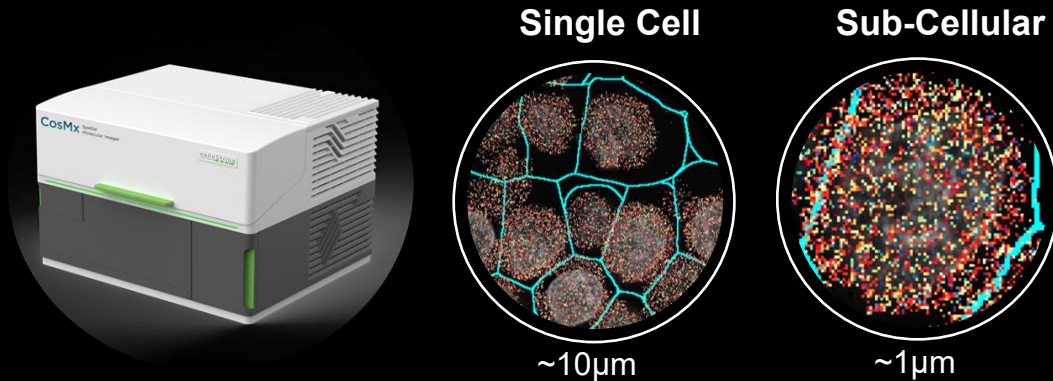
[Save for later](#)

- There is no single spatial instrument able to address all the critical features
- Use of different instruments in a single study is often optimal

Two complementary technologies for a complete, high-plex multiomic study of cell and tissue biology

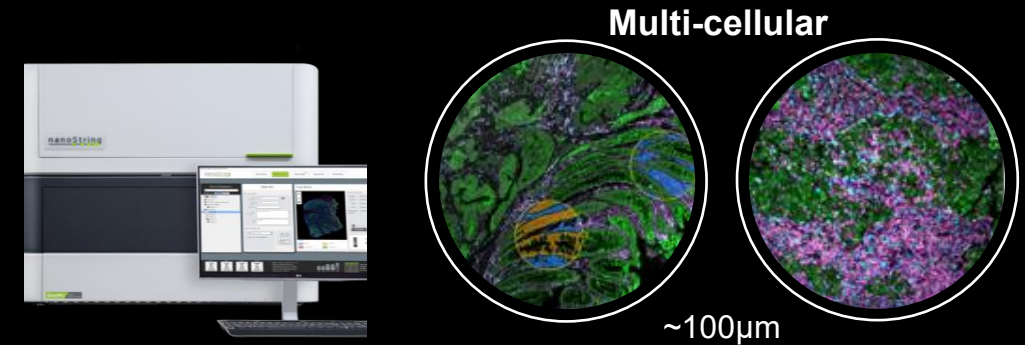


CosMx Spatial Molecular Imager



- **UNBIASED DISCOVERY** – Imaging-based whole-slide analysis
- **GENOMIC BREADTH** – The ONLY spatial platform enabling same slide whole transcriptome and protein analysis at single-cell and sub-cellular resolution
- **MULTI-OMIC CAPABILITY** – Whole transcriptome + up to 76 proteins

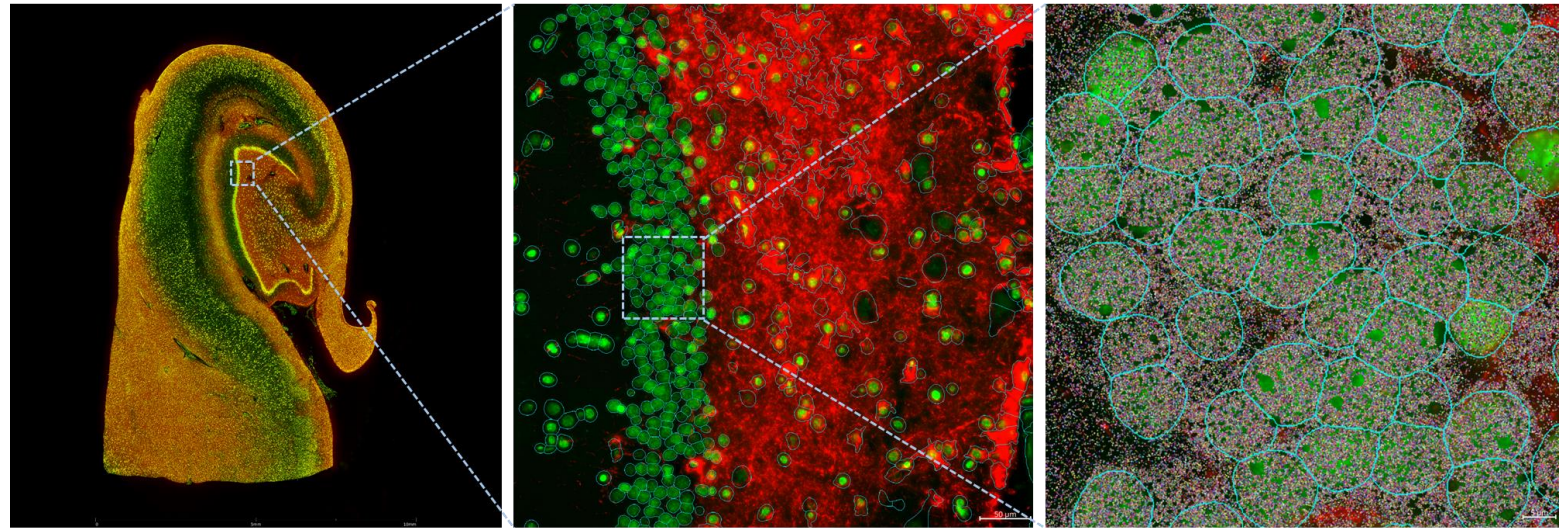
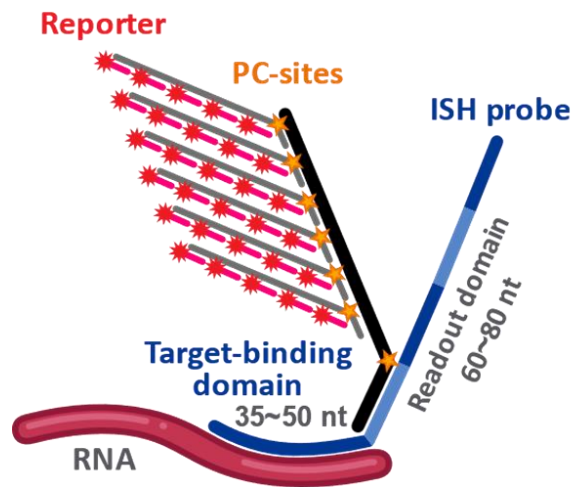
GeoMx Digital Spatial Profiler



- **HIGHEST MULTI-OMIC PLEX**– The ONLY spatial platform enabling same-slide whole transcriptomics and 1K+ protein multiomic analysis
- **HIGH-THROUGHPUT** – Up to 20-40 slides per week
- **REGIONAL, CELL POPULATION SPECIFIC DISCOVERY** – NGS-based ROI/AOI analysis

CosMx[®] WTX: **first** and **only** imaging platform to traverse subcellular spatial whole transcriptome biology from an FFPE tissue section

Why? Direct hybridization chemistry (no amplification) for maximum sensitivity and specificity



Robust and Scalable **FFPE** and FF chemistry

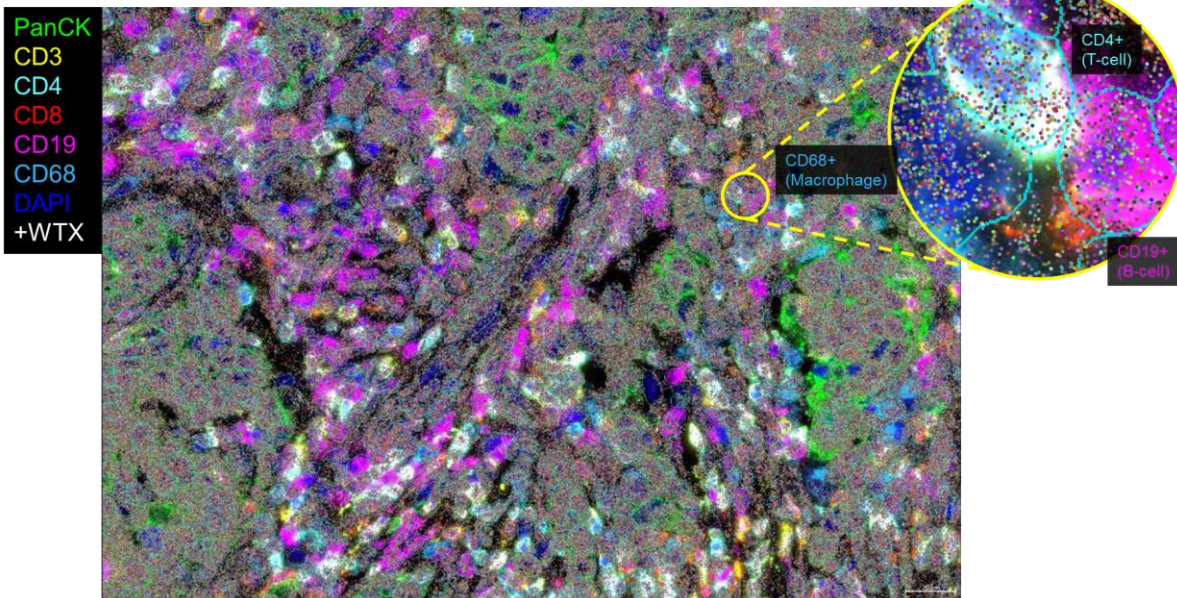
- No complex, uneven, or enzymatic amplification
- Control of optical crowding—**essential** for plex

High Fidelity and Complete Transcriptomics

- Designed and validated for **FFPE**
- >99% protein-coding transcriptome
- **Accurate** cell segmentation

CosMx[®] Same-Cell Multiomics with RNA and protein for deeper spatial exploration

Enhance your transcriptomic findings with protein-based context and phenotypic information

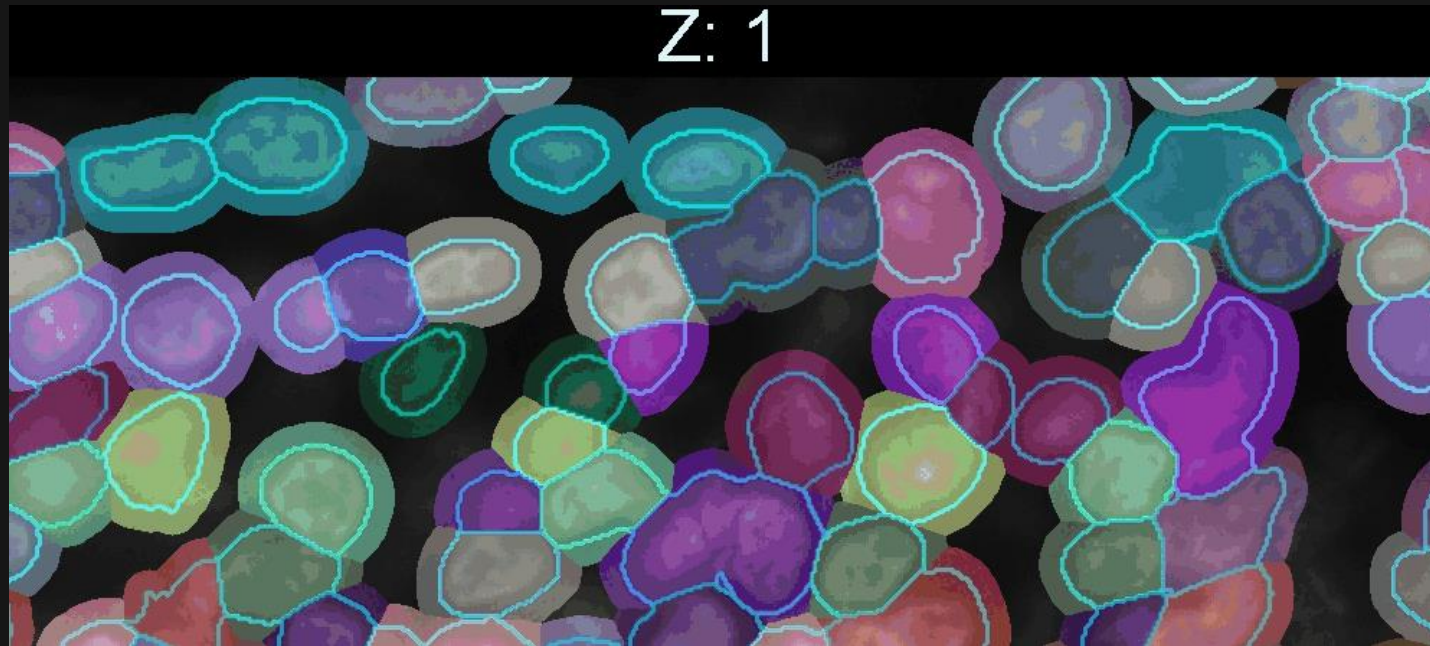


- Combine with any Human RNA assay (up to WTX)
- Add up to 8 of your own protein targets for custom investigation

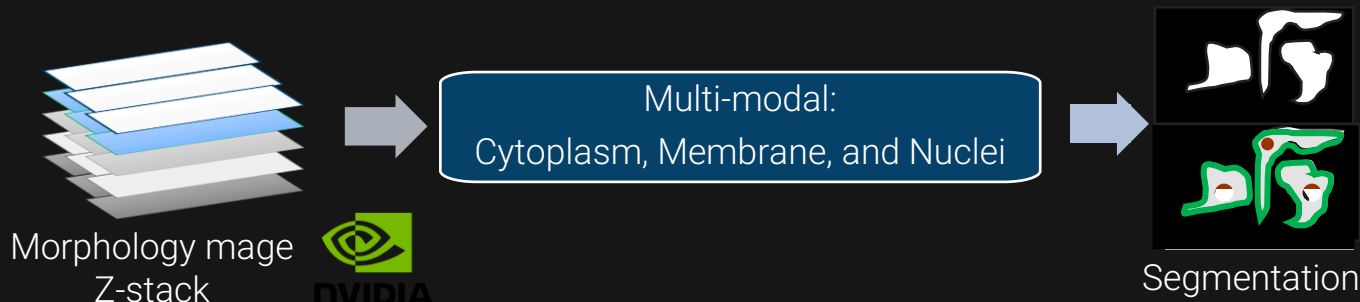
Use CosMx Same-Cell Multiomics to:

- Reveal RNA-Protein relationships at high plex
- Incorporate tissue architecture into your spatial analysis
- Refine and validate cell types using integrated multiomic methods

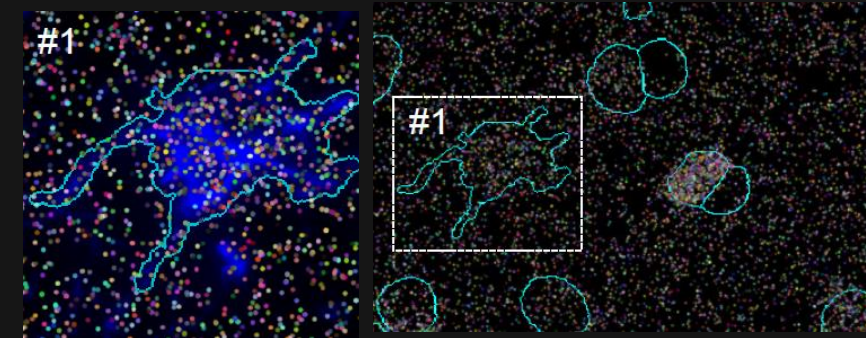
Taking our best-in-class segmentation to 3D: the most accurate AI models for transcript assignment to individual cells in space



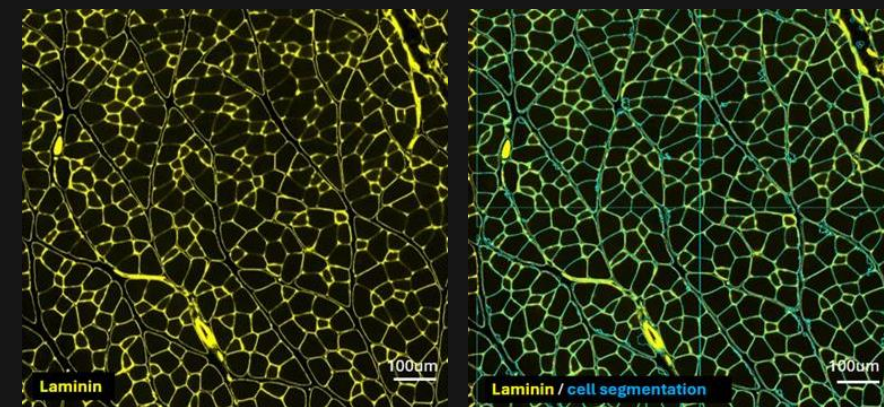
Pancreas tissue example above: **> 20% transcripts change assignments when advancing to 3D**



Neuro-specific AI model

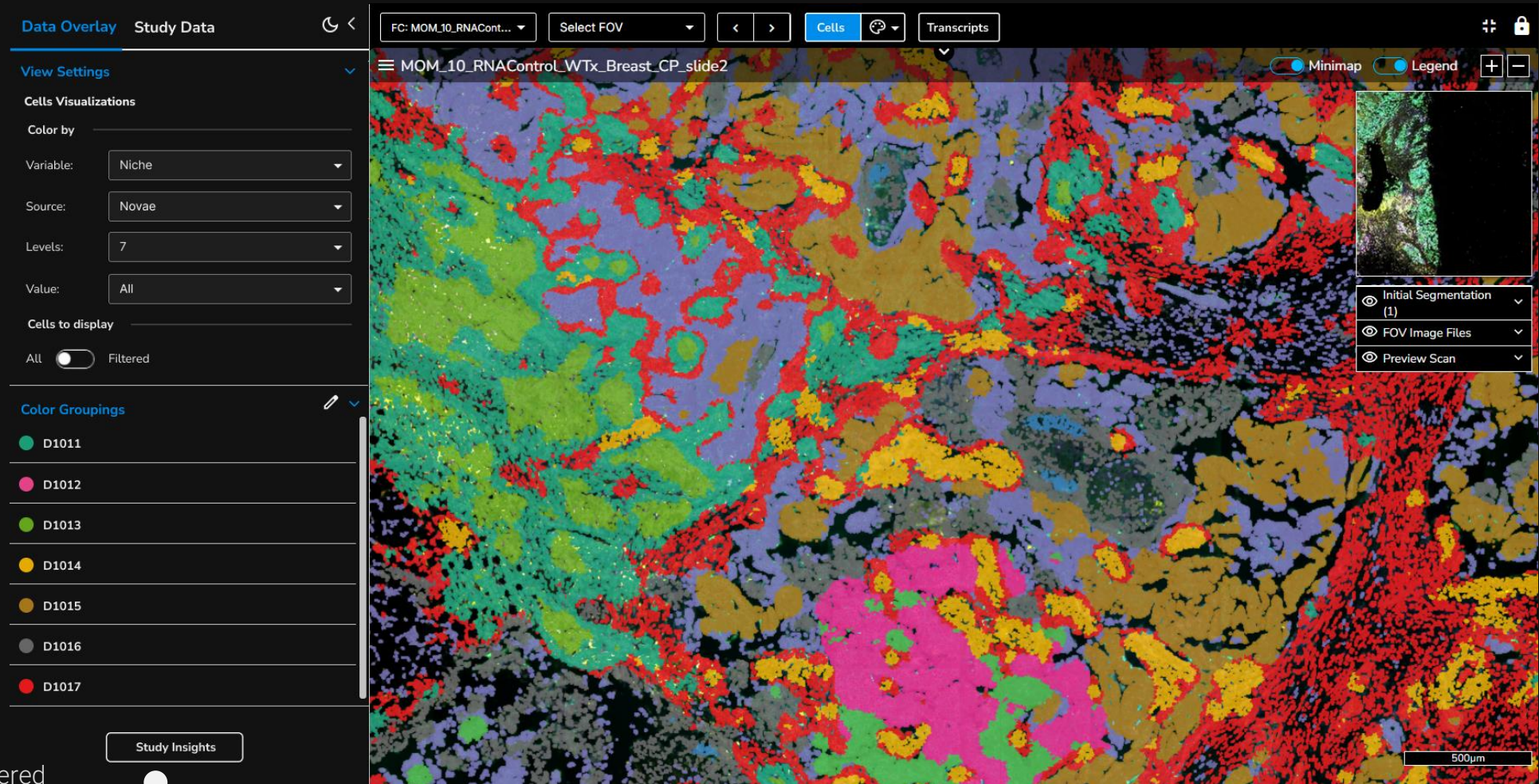


Muscle-specific AI model



Introducing AtoMx **Spatial Discovery**: “image-first” and conversational data analytics

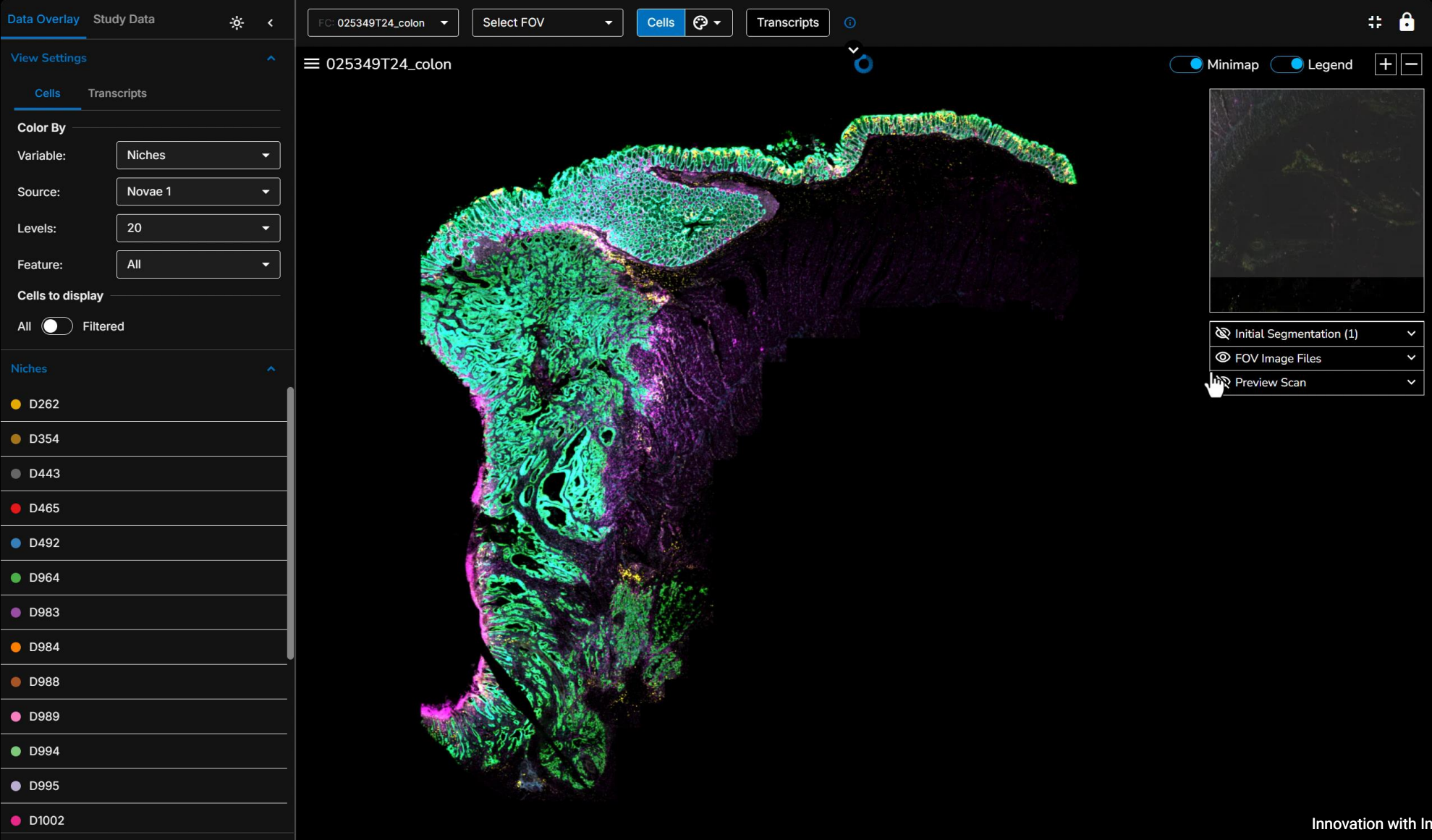
Empowering biologists to ask and answer their own questions real-time at the study-level



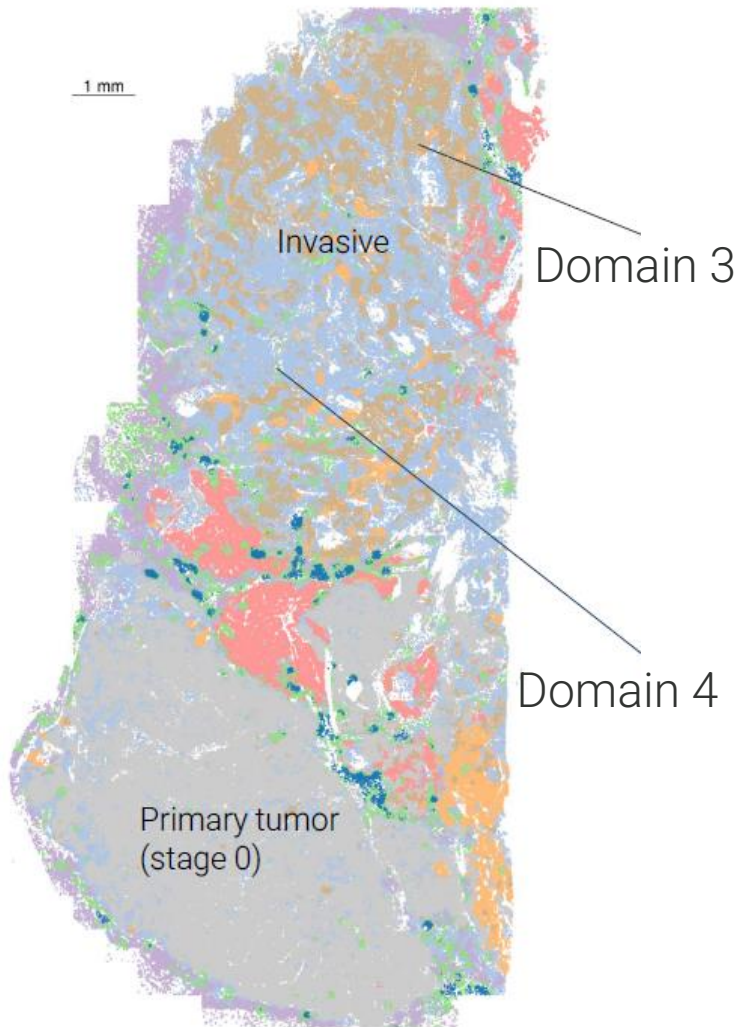
Export for AI-powered
analysis insights



Leverage complete biology to deliver more robust insights through LLM models: limited data penalizes your LLM outputs



AtoMx image-first will make an “AI/ML/LLM data package” that allows you to have a “conversational analysis” with your data...



Enabled: AtoMx data package exported directly to scientist who collected this data

Enabled: Scientist can utilize a Natural language query of their data on their favorite LLM (or AI/ML package) package.

For example: Using Gemini Pro the Scientist can simply ask: **What are the key biological differences of domain 3 versus domain 4?**

Gemini Pro “digestion” of CosMx AtoMx data-package and answer to specific query

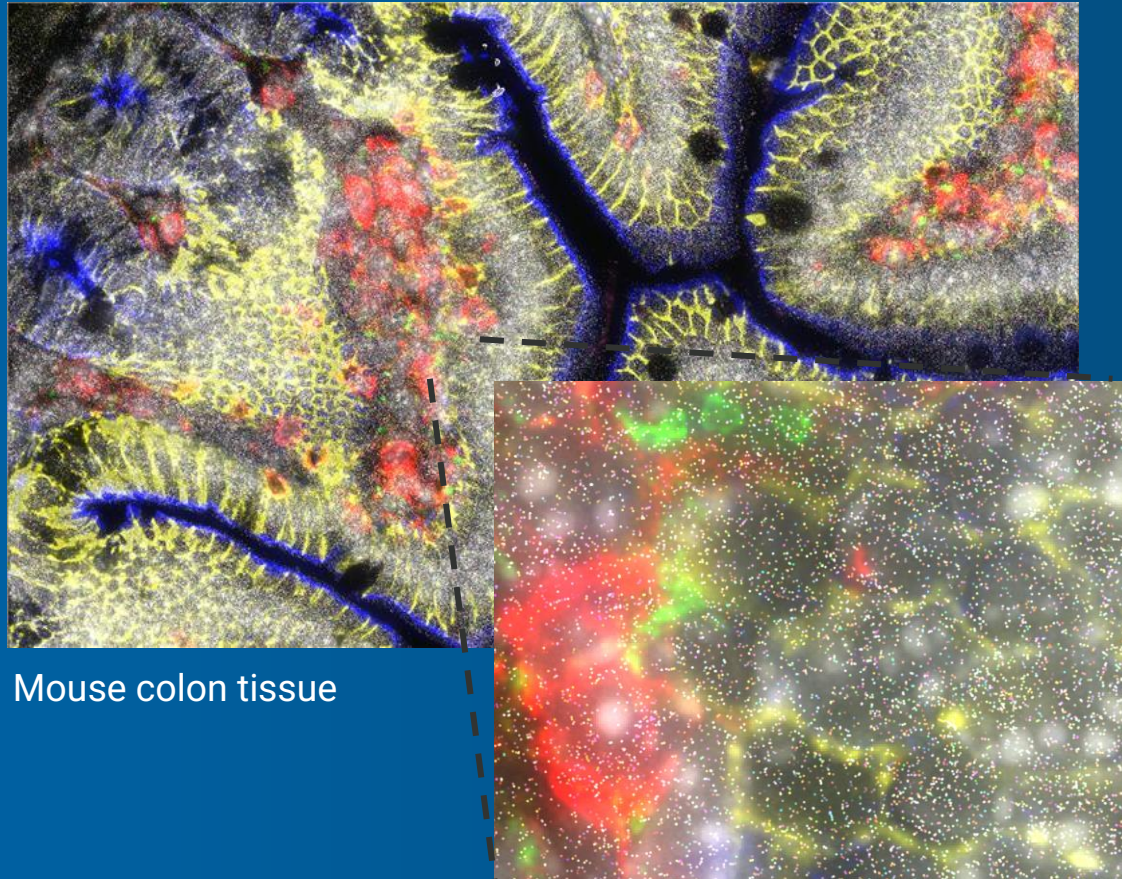
“Domain 3 pathways are characteristic of a highly proliferative tumor environment and metabolic activity, which aligns with the cell type composition of domain 3 (mostly tumor cells). Eukaryotic Translation Elongation: High in domain 3 (mean=0.36) and very low in domain 4 (mean=0.01). This indicates a strong signal for active protein synthesis and rapid growth in domain 3. Protein Translation is High in domain 3 (mean=0.17) and very low in domain 4 (mean=0.00). This pathway is a fundamental process for protein production, further supporting the proliferative nature of domain 3. Selenamino Acid Metabolism is High in domain 3 (mean=0.28) and very low in domain 4 (mean=0.00). This pathway is involved in cellular stress response and metabolism, indicating a distinct metabolic state in domain 3.”

(source: response from Gemini Pro after prompted with spatial discovery AtoMx input)

CosMx 2026 roadmap drives more applications to advance single-cell and subcellular spatial biology

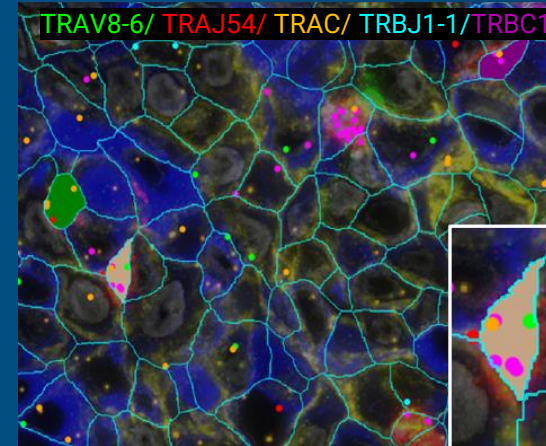


CosMx Mouse Whole Transcriptome (>21K targets)

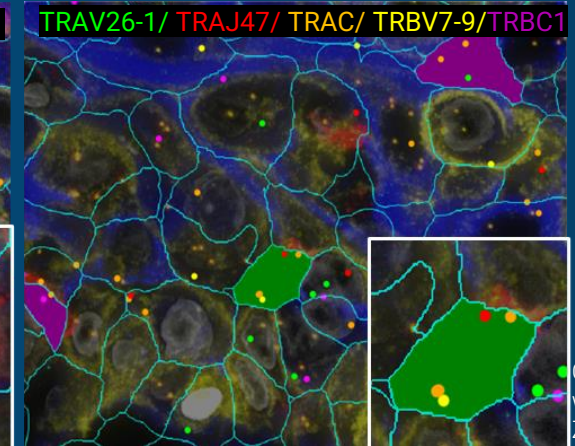


T cell Receptor assay (V-D-J focus)

Tumor reactive T cell

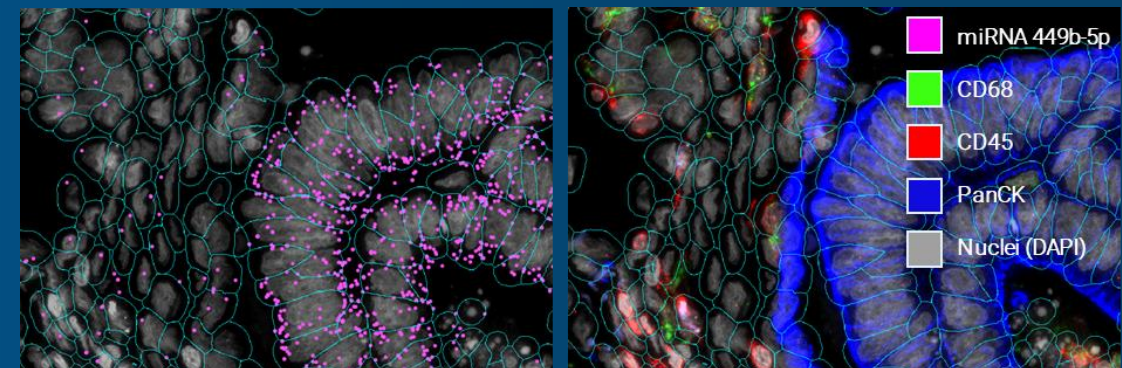


T cell bystander



Collaboration
with Dr. Joe
Zenga

miRNA assay in development



Bruker Spatial Biology: Best-in-class technologies for every layer of biology

What are the most important features to you in a spatial biology platform? (1=highest, 9=lowest; n=128)

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Created with [Datawrapper](#)

Spatial Biology

GeoMx®



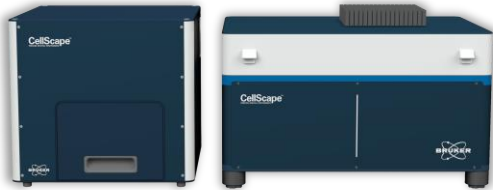
Unmatched discovery multiomics to profile from cells to tissue structures for spatial breakthroughs at scale

CosMx®



High sensitivity, subcellular imaging of the whole transcriptome for complete pathway-level spatial insights

CellScape™



Flexible subcellular proteomics for rapid and quantitative spatial phenotyping

Office Hours 2:00 - 4:00PM

Room HSLC: 1229

Ask us about special seminar promo!

Thank you

A fluorescence microscopy image showing a complex tissue structure. The image features a large, irregularly shaped mass in the upper left quadrant, primarily colored in bright green, which likely represents the tumor cells. This mass is surrounded by a dense network of smaller, more organized structures, possibly representing the tumor microenvironment, including stromal cells and blood vessels. The background is a mix of green, yellow, and blue, with some red lines indicating specific cellular or structural components. The overall texture is highly detailed, showing individual cells and their interactions.

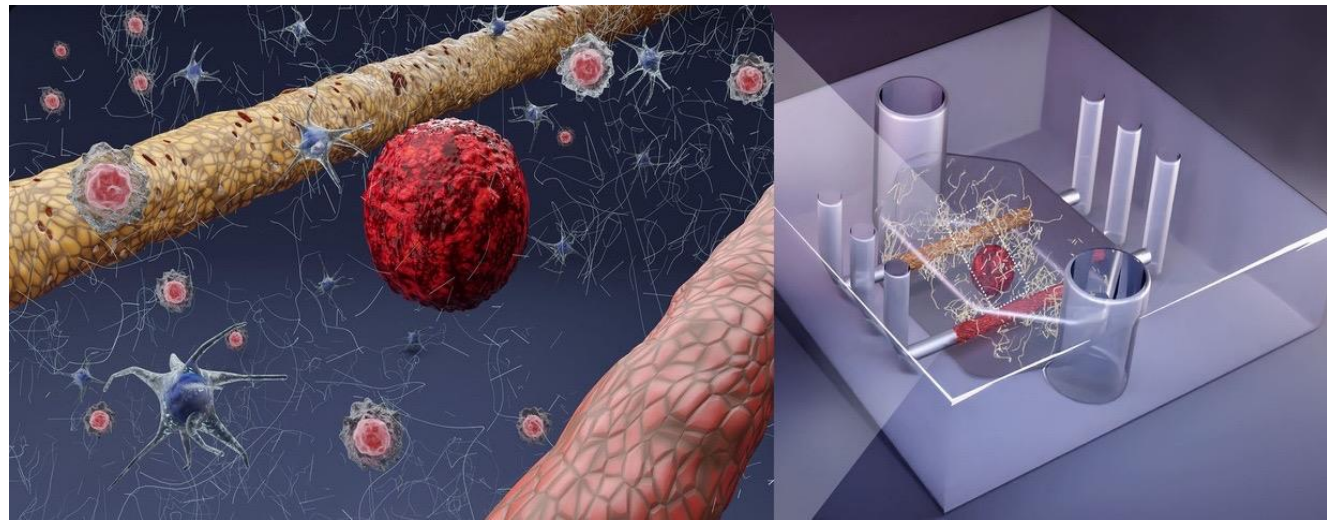
**Spatial profiling of the head and neck cancer
tumor microenvironment.**

**Sheena Kerr, PhD
Research Assistant Professor
UW Madison
3/25/2026**

Guiding complex microenvironment modeling



- Microphysiological systems aim to recapitulate organ form and function.
- How to improve fidelity of model to original tissue site?



Adeel Ahmed, PhD



Rene Welch Schwartz, PhD

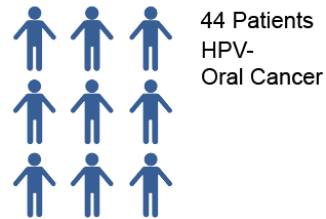


Irene Ong, PhD



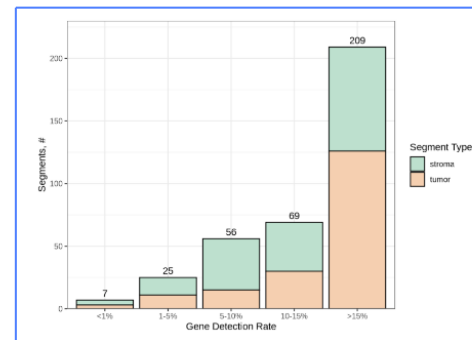
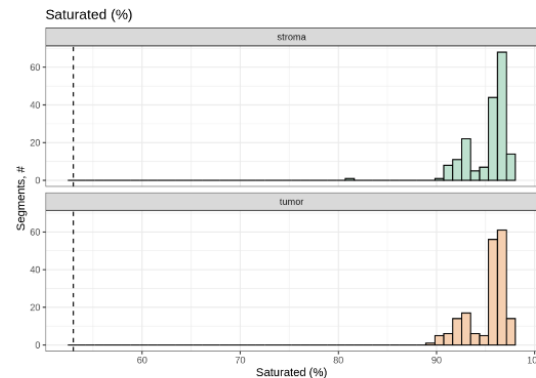
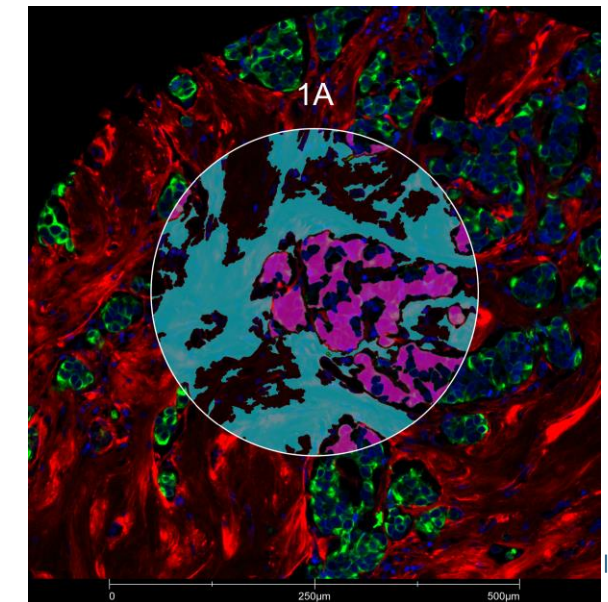
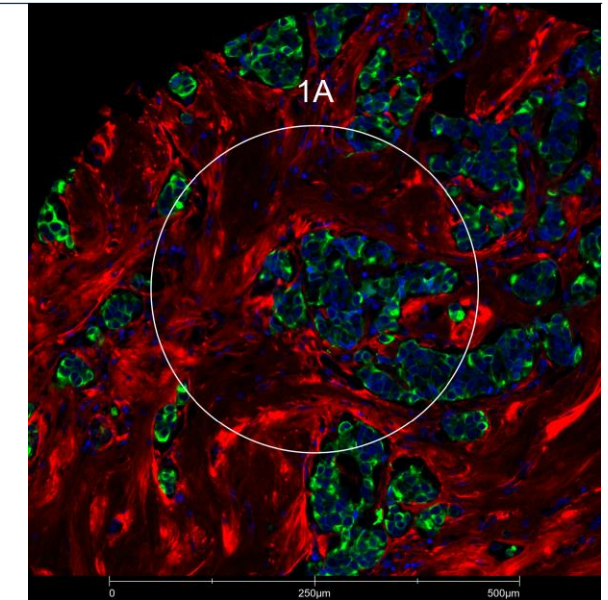
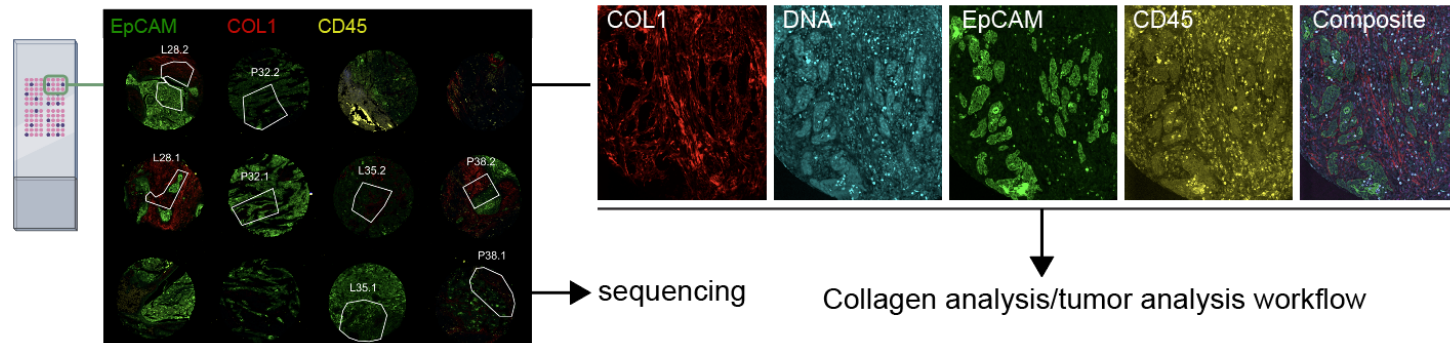
UW Madison HN SPORE

Profiling multiple tumor types on a TMA



44 Patients
HPV-
Oral Cancer

44 Patients
2 tissue sites (primary and lymph node)
176 tissue cores
2 ROI (tumor + Stroma)



Analyzing the data

QC Metrics

Read saturation **>90%** *for most segments*

Trimming rate **~95%** *for most segments*

Stitching rate **~95%** *for most segments*

Gene Filtering — LOQ

$\text{LOQ} = \text{geomean}(\text{negative controls}) \times \text{geomSD}(\text{negative controls})$

Genes and segments with signals consistently below LOQ were excluded. LOQ-based filtering balances sensitivity and specificity for low-abundance transcripts in spatial ROIs.

Normalization Strategy

75th Percentile

GeoMX default; scales to uniform 75th percentile

Median Normalization

Per-sample median scaling or DESeq median-ratio

Quantile Normalization

Enforces identical marginal distributions across all samples

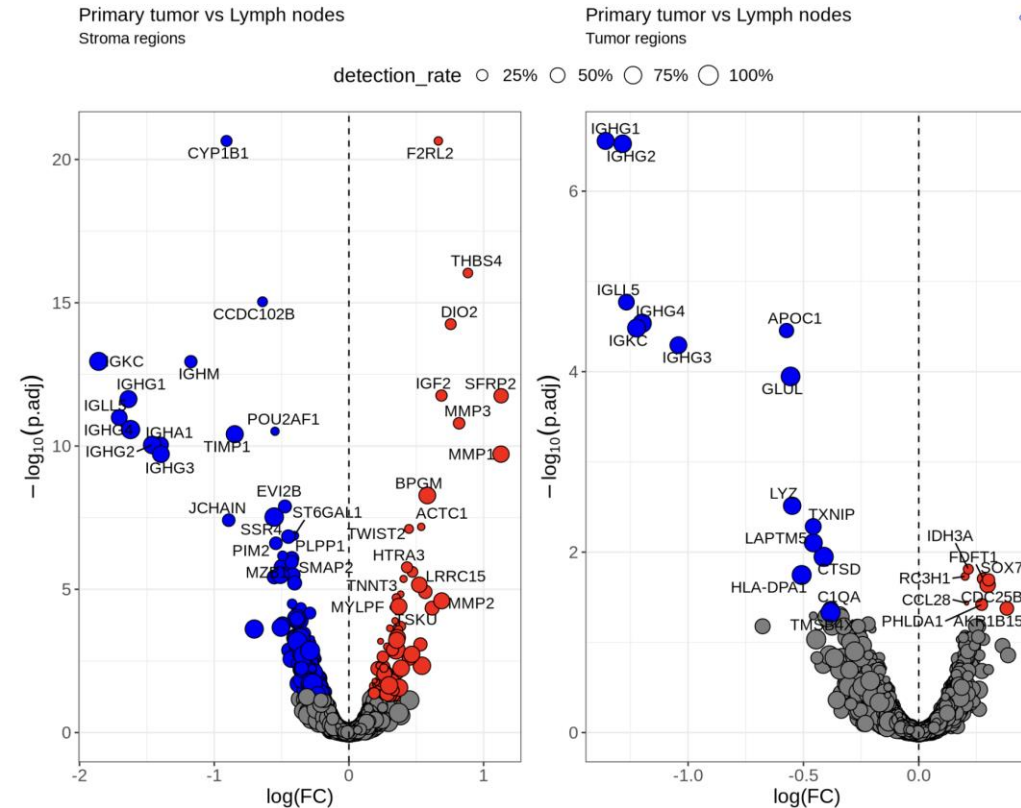
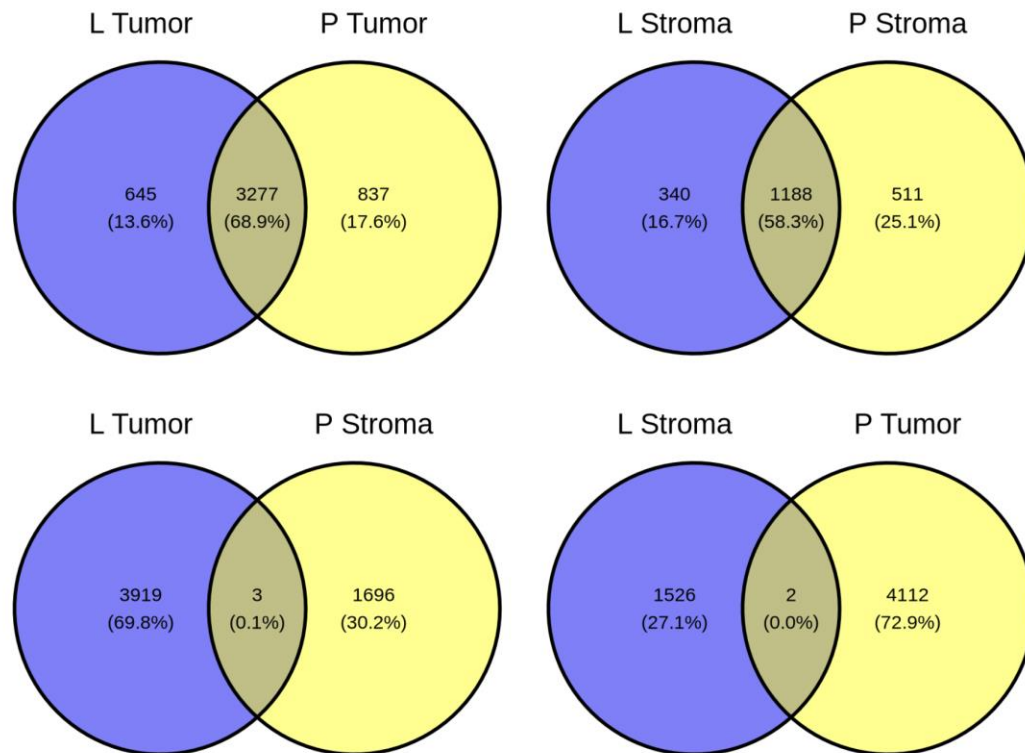
Smooth Quantile

Interpolates between within-group and global quantiles (Hicks et al. 2018) — **SELECTED**

Post-normalization UMAP: all four methods produce comparable clustering patterns with no apparent normalization artifacts.

Linear mixed effects model to account for interactions between tissue segment (ROI), tissue (primary, LN), random effects and residual error.

Stromal compartment shows greater variability



Stromal signatures between compartment

Tumor ROI

Upregulated in Lymph Node

- IGHG2 — IgG heavy chain (B cell infiltrate)
- CYP1B1 — cytochrome P450, drug metabolism
- CCDC102B — coiled-coil domain protein

Upregulated in Primary

- MMP3 — stromal matrix metalloprotease
- ACTA1 — skeletal actin isoform
- IGF2 — insulin-like growth factor

Relatively few DEGs — tumor cell transcriptome largely conserved between primary and metastatic sites

Stroma ROI

Upregulated in Lymph Node

- IGHG2 and immune-associated genes
- Complement and antibody effector genes

Upregulated in Primary

- MMP family members (ECM remodeling)
- F2RL2, SFRP2 — Wnt/ECM signaling
- IGF2, THBS4 — matrix-bound growth factors
- DIO2 — thyroid hormone metabolism

Stroma shows greater transcriptional divergence: immunostimulatory in LN, ECM-remodeling dominant in primary

Tumor vs Stroma- A pan stromal ECM signature



Key Finding: A robust ECM/stromal signature is upregulated in stromal ROIs irrespective of tissue site (primary vs. lymph node)

Upregulated in Stroma

COL1A1 / COL3A1 / COL5A1 Fibrillar collagens — structural ECM scaffold

POSTN Periostin — ECM glycoprotein, CAF activation marker

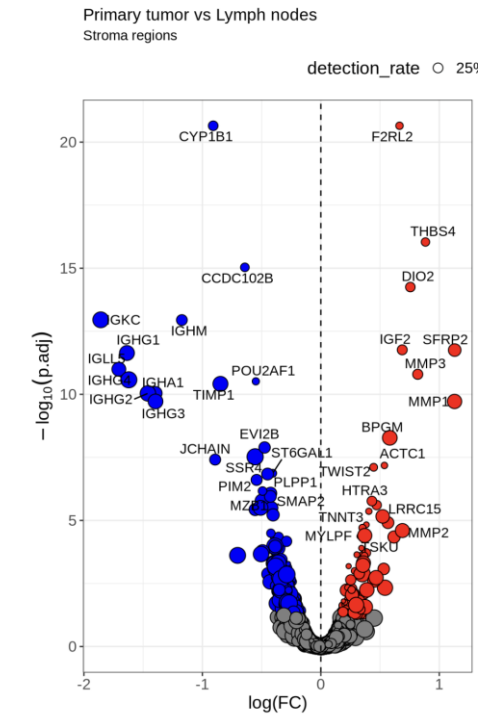
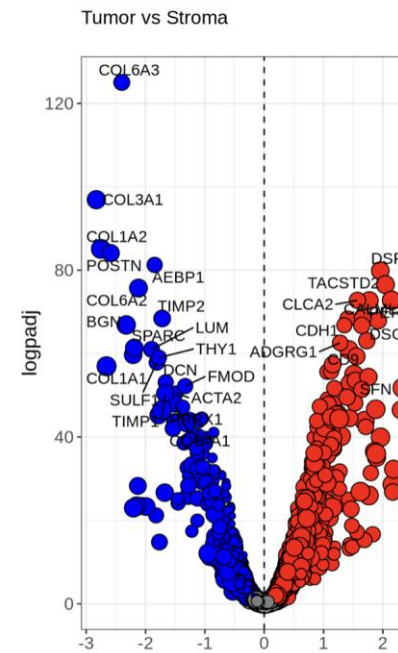
TIMP1 Tissue inhibitor of MMPs — ECM remodeling regulator

FMOD Fibromodulin — collagen fibril organization

ACTA2 Alpha-SMA — canonical myofibroblast / CAF marker

SPARC Matricellular protein — mediates collagen deposition

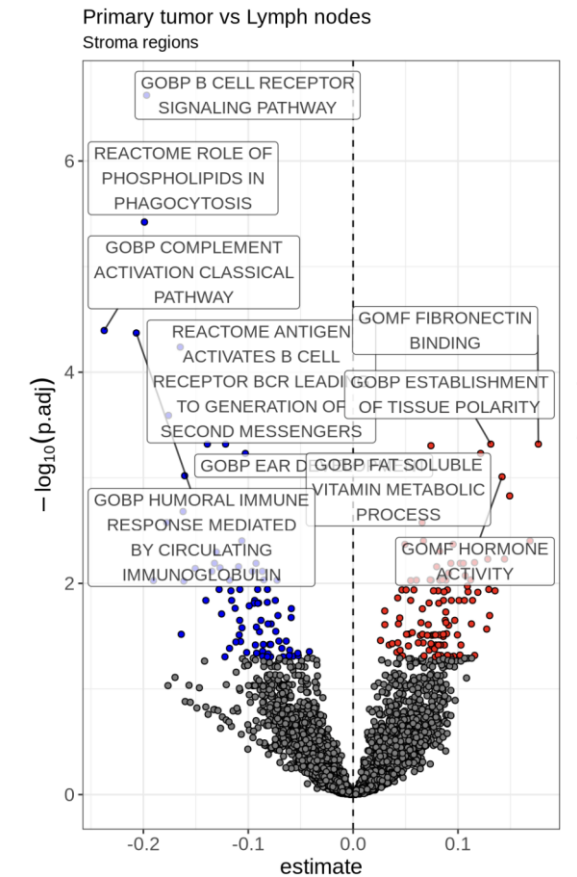
AEBP1 Transcription factor in fibroblast activation programs



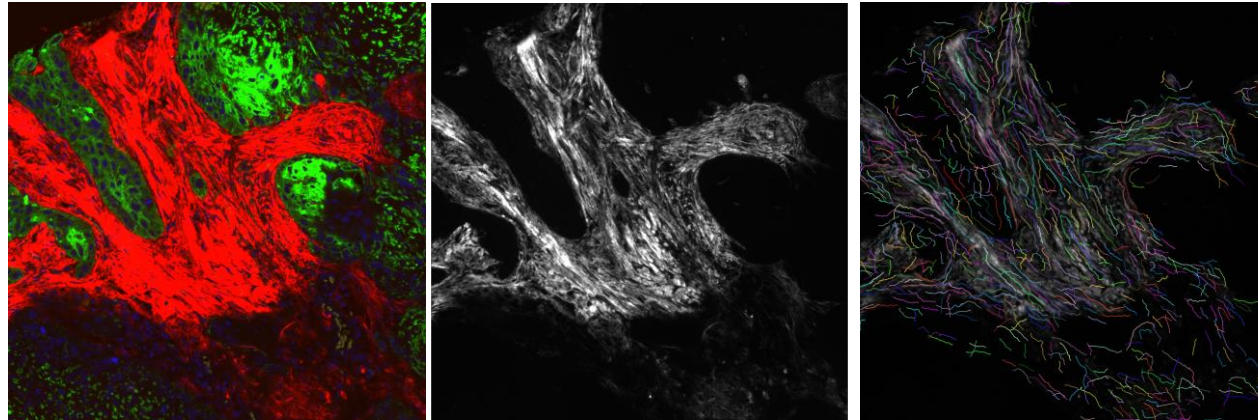
Gene set enrichment analysis (GSEA)

Databases queried:

Category	Representative Gene Sets	Enriched In
ECM / Collagen	EXTRACELLULAR_MATRIX_ORGANIZATION, COLLAGEN_FIBRIL_ORGANIZATION, COLLAGEN_BINDING	Stroma — Primary
Fibroblast / CAF	FIBROBLAST_ACTIVATION, ACTIN_BINDING, SMOOTH_MUSCLE_CONTRACTION	Stroma — Both sites
Immune Signaling	CYTOKINE_SIGNALING, INTERLEUKIN_SIGNALING, CHEMOKINE_SIGNALING	Stroma — LN
Integrin / Cell-Matrix	INTEGRIN_BINDING, CELL_SURFACE_RECEPTOR_SIGNALING, ECM_RECEPTOR_INTERACTION	Stroma — Primary
Angiogenesis	VEGF_SIGNALING, ANGIOPOIETIN_SIGNALING	Mixed



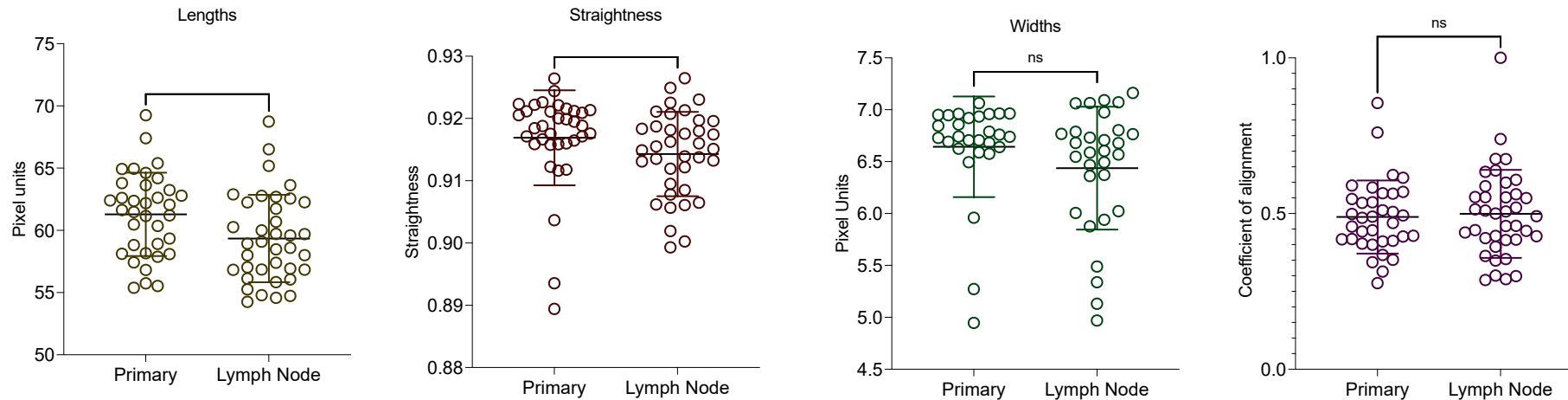
Comparing to physical collagen structures



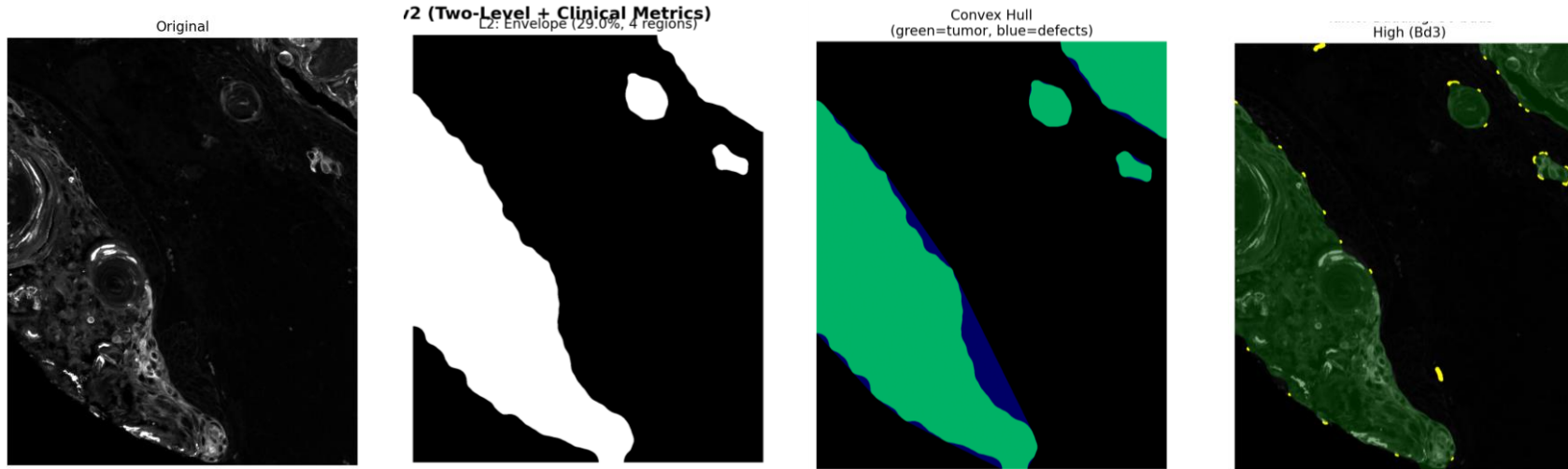
Composite

Collagen Channel

CT-FIRE Fiber ID



Leveraging imaging data



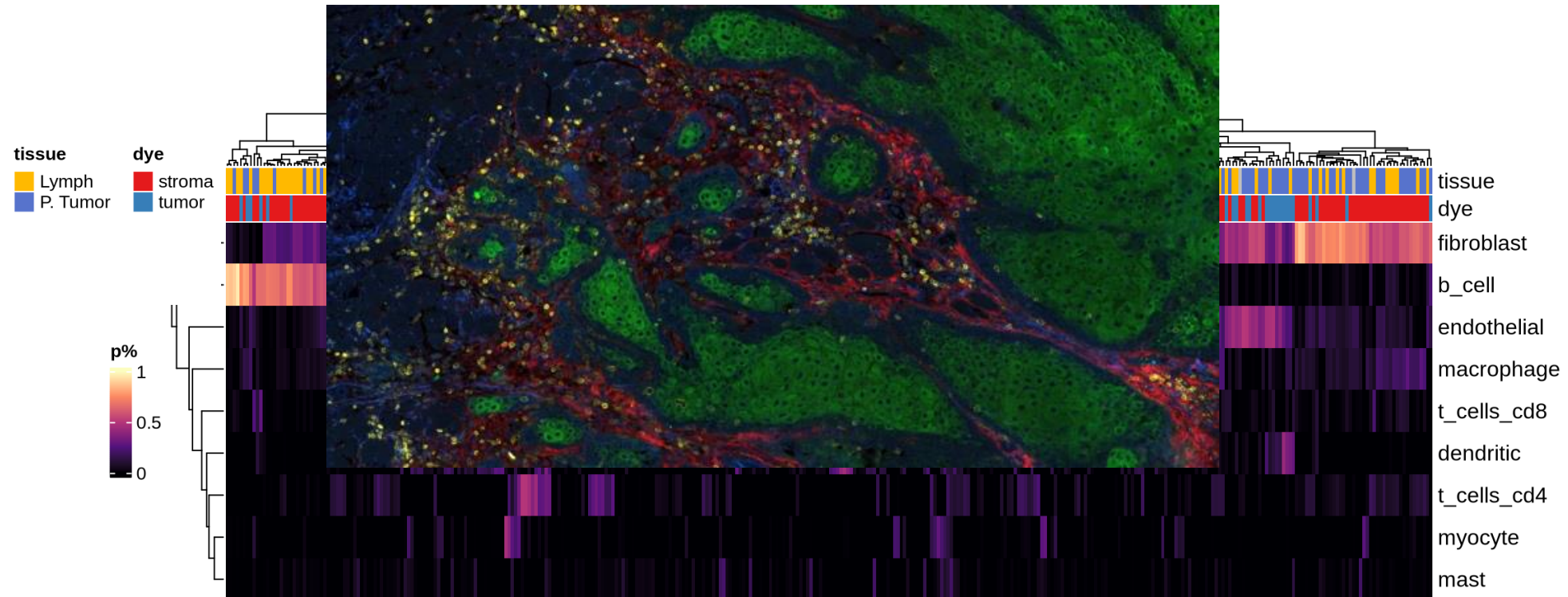
== INVASION ASSESSMENT: MODERATE ==
 Composite: 0.302 (0=smooth → 1=invasive)

— Tier 1: Clinical Metrics —
 TSR: 28.9% tumor / 71.0% stroma
 [Stroma-rich (HIGH RISK)]
 Budding: 30 buds → High (Bd3)

— Tier 2: WPOI + Shape —
 WPOI: IV-V (Scattered/infiltrative)
 Islands: 34 Satellites: 33
 Fragmentation: 0.562
 Main mass frac: 0.438
 Mean NN dist: 103 px

Metrics:
 Solidity
 Worst Pattern of Invasion
 Budding
 Tumor to Stroma Ratio

Understanding immune exclusion



Summary

Conclusions

- Stromal compartments carry the dominant transcriptional differences between primary HNC and lymph node metastases
- ECM remodeling genes define a shared pan-stromal program; immune genes distinguish LN from primary stroma
- Tumor cell transcriptomes are conserved across sites — the stroma, not the malignant cell, is the key stage-specific variable
- GeoMX spatial profiling provides compartment-level resolution unavailable in bulk RNA-seq

Next Steps

- Integrate DE and GSVA findings with patient clinical outcome data (survival, recurrence, treatment response)
- Correlate spatial transcriptomics with collagen fiber morphometry (alignment, density) from fluorescence imaging
- Develop HNC-specific ECM hydrogels for patient-specific microphysiological systems (MPS)
- Validate key fibroblast–immune co-regulatory genes in an independent validation cohort

Acknowledgements



MMB Lab

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Aaron Wieland, MD
Rong Hu, MD
Justine Bruce, MD

Ong Lab

Irene Ong, PhD
Rene Welch Schwartz, PhD

UWCCC Biobank
TRIP lab- Becky Baus
UW Biotechnology center





Spatial Biology Resources

Brightfield

Whole slide scanner

- High throughput (400 slides)
- 20x and 40x magnification
- Z-stacking capability
- ImageScope software

Aperio AT2



Vectra



Automated scanner

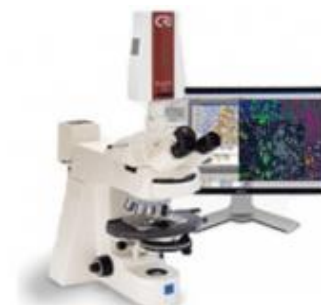
- Integrated machine learning software
- Brightfield and fluorescent
- 4x to 40x magnification
- Fields of view and TMA
- InForm software for analysis

Multispectral

Microscope

- Brightfield and fluorescent
- 4x to 40x magnification
- Nuance/ InForm software

Nuance



Spatial -Omics

GeoMx DSP



Visium with CytAssist



High-plex transcriptomics and proteomics

- Whole transcriptome analysis
- 1400+ proteome mapping
- 20x magnification
- Visium HD for higher resolution
- Xenium and MERSCOPE support
- Partnership with UW Biotech Center for NGS workflows



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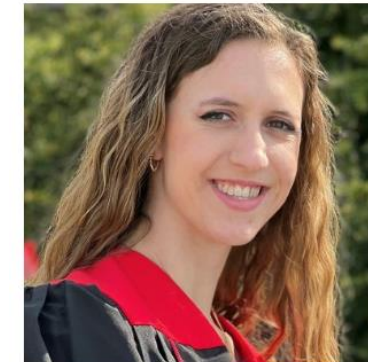
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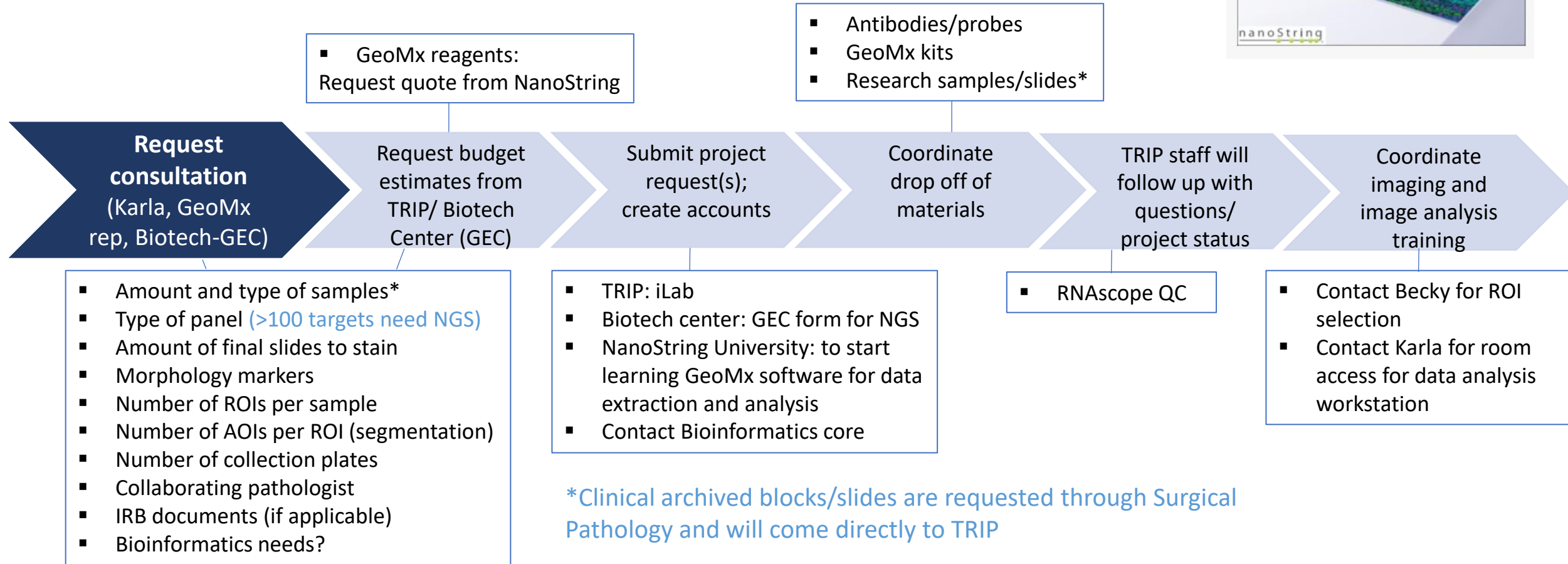
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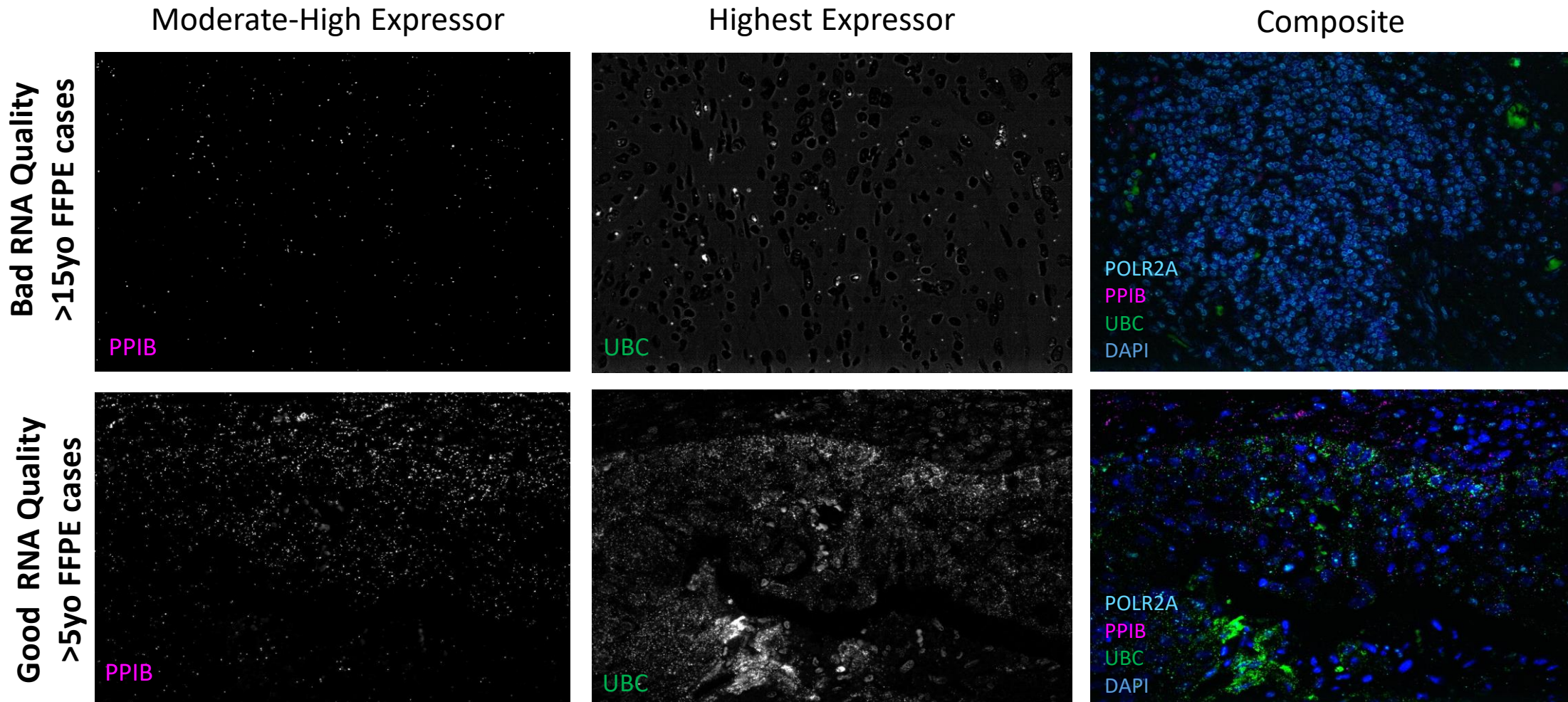
Workflow for project submission

Spatial Profiling: GeoMx



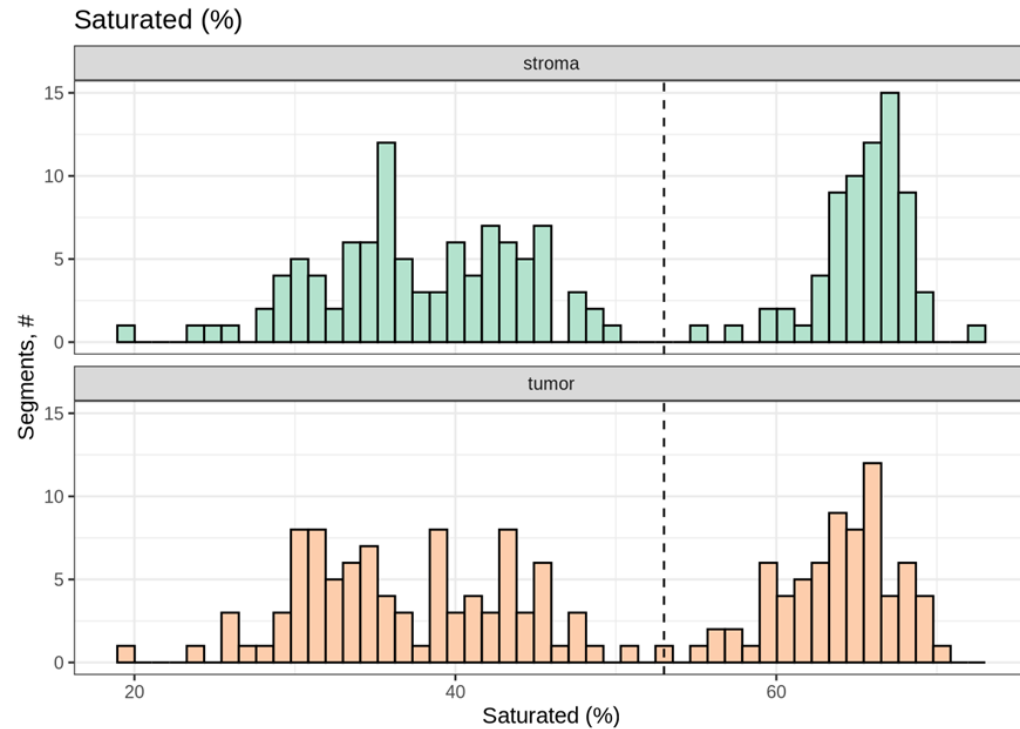
GeoMx Sample QC with RNAscope

RNAscope 3-plex Positive Control Probe



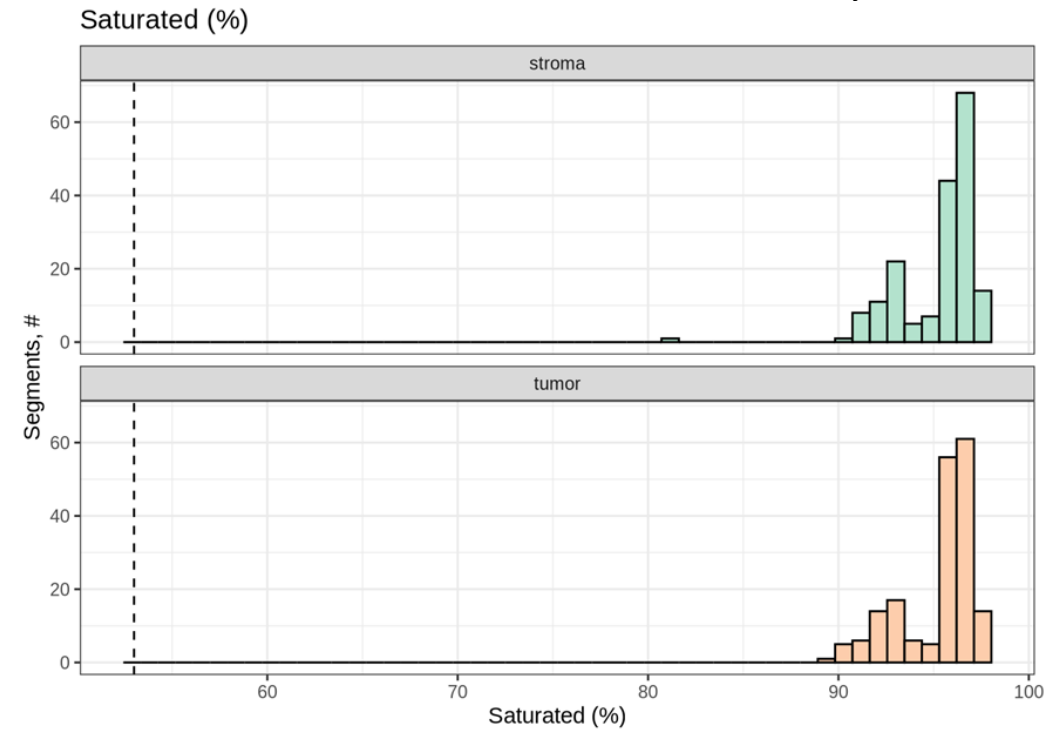
Data Quality: Sequencing saturation

TMA1: FFPE blocks are over 15yo



TMA1 -poor saturation across the samples

TMA2: FFPE blocks are under 5yo



TMA2 -good saturation across the samples

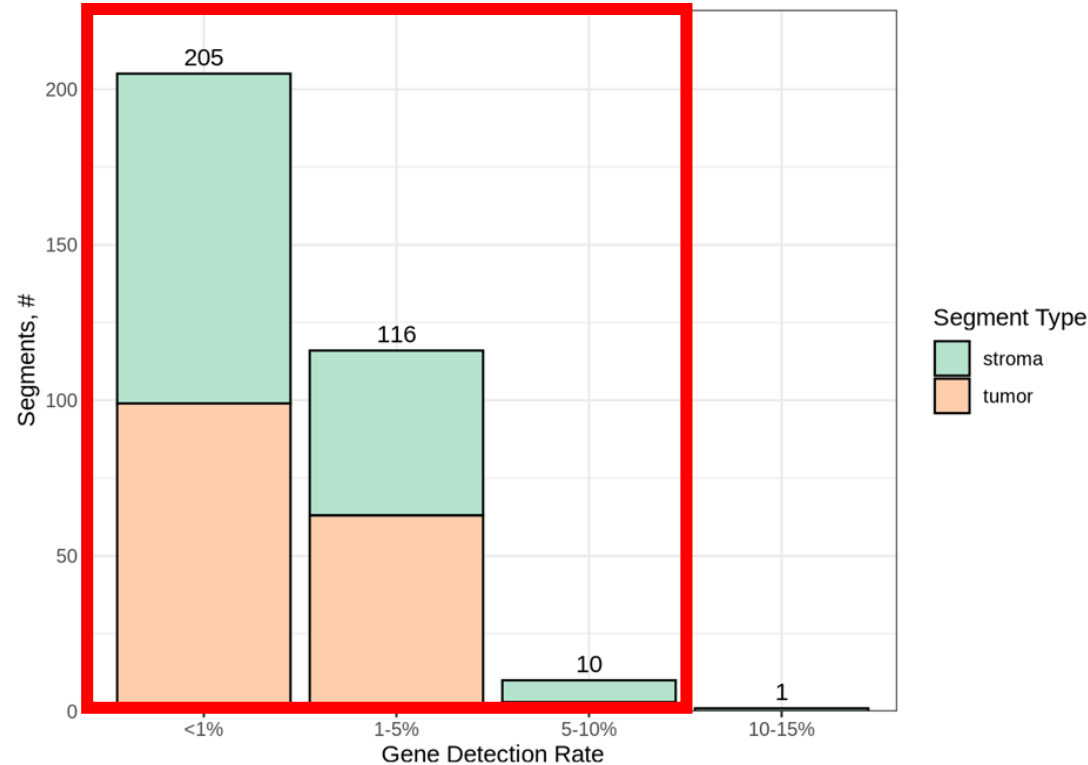
50% saturation = Resequencing necessary

TMA1: Half the reads fall below 50% saturation, 189 showed warning flag vs 143 that passed QC

TMA2: 100% passed QC

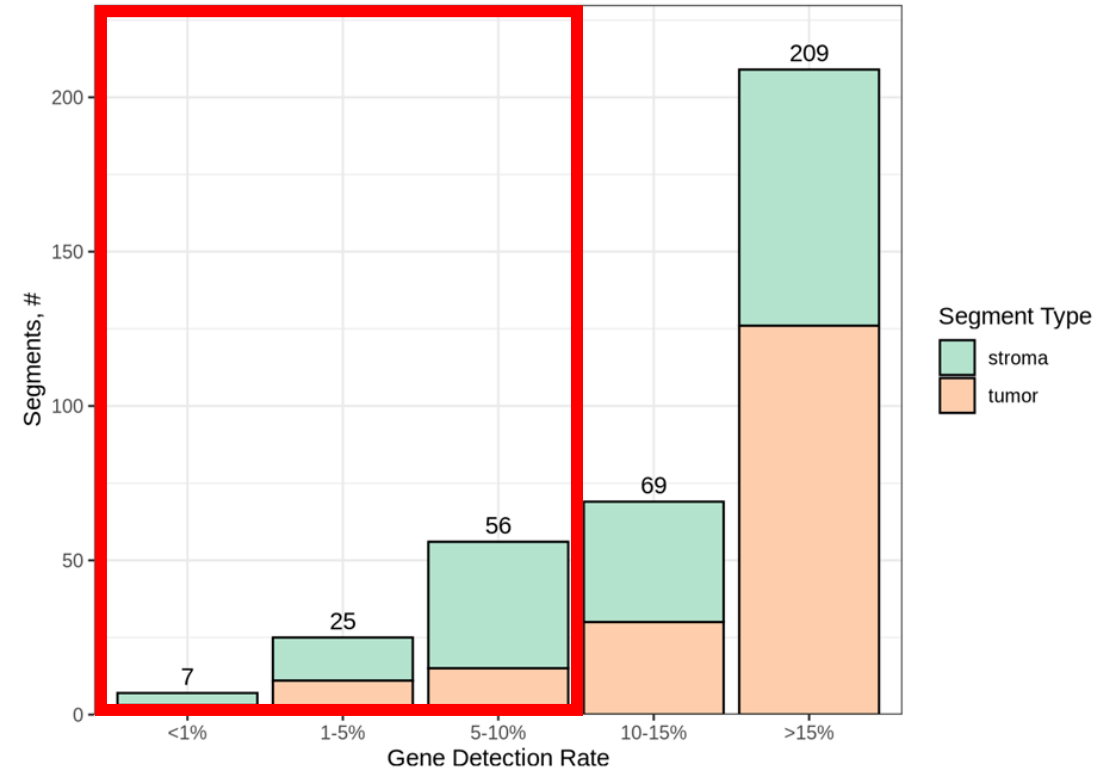
Data Quality: Gene detection rates

TMA1: FFPE blocks are over 15yo



TMA1: Poor gene detection, little usable data

TMA2: FFPE blocks are under 5yo



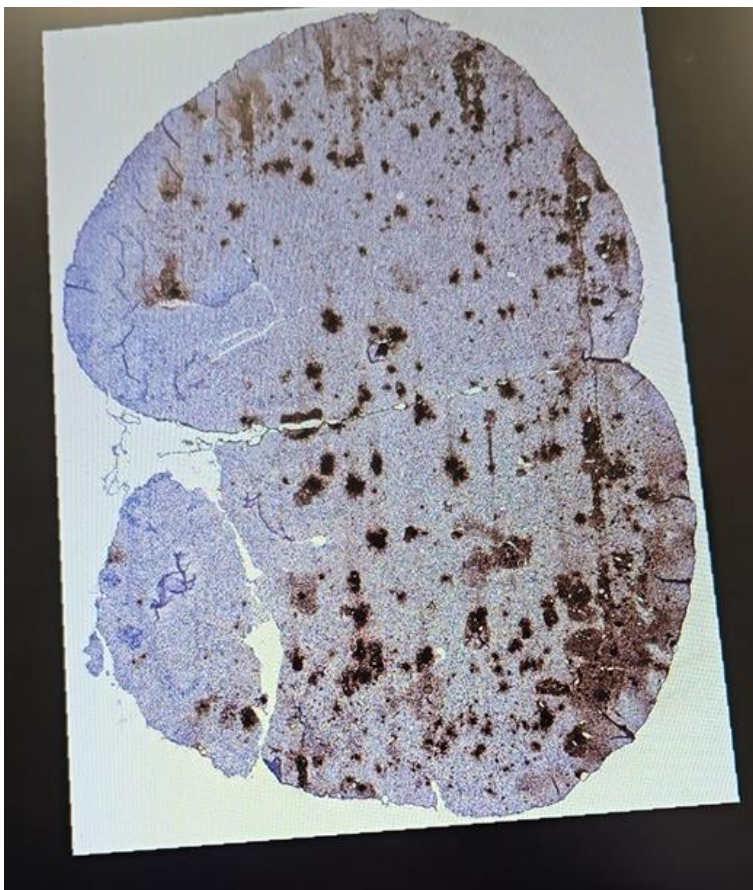
TMA2: Higher amount of genes fall into the desired categories

*Percentage of genes detected.

- Low gene detection rates suggest low numbers of reads available for that gene.
- Bruker-NanoString suggests removing reads below 10%



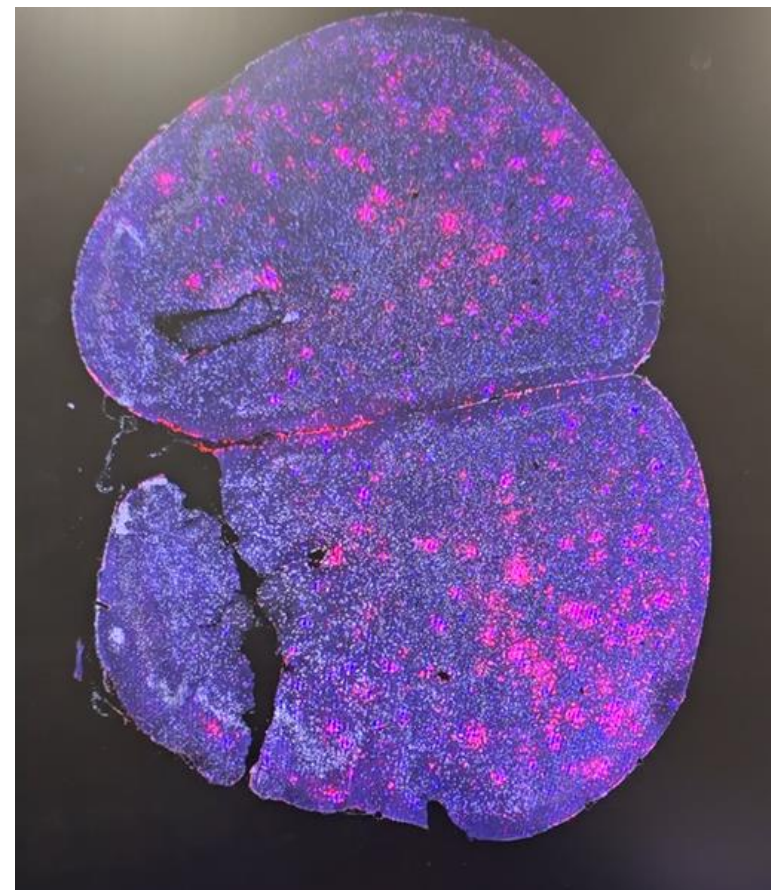
IHC-DAB Overlay



Upload Aperio scanned image of stained tissue



*Does not require morphology marker optimization = saves time & money!
-optimization ~\$1000/run



This is the view on the GeoMx screen of tissue



IHC Overlay Videos

Use Overlays for:

- *Non-segmenting projects
- *Antibodies that are not available fluorescently conjugated
- *Antibodies that are difficult to work with
- *Saving time and money
- *This can be used on segmenting projects with fluorescently labeled markers

