

BRUKER SPATIAL BIOLOGY

Industry-leading spatial solutions for high-plex multiomic tissue exploration and discovery

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Senior Field Application Scientist

Calgary Spatial Omics Seminar November 7th, 2024



Applied Spatial Omics Centre

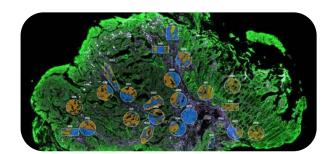


Platforms optimized to deliver industry-leading performance for your application objective

Platforms excel with FFPE tissues and diverse sample or disease types

Bulk Regional

2019 GeoMx® DSP



• High-plex, whole transcriptome

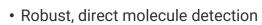
Fast and flexible platform

· High sensitivity with user-

defined tissue sampling

• ex situ sequencing readout

RNA, 570+ protein (multiomics)



nCounter®

- · Rapid turnaround, minimal handson time
- Flexible assays (up to 800-Plex)
- Simplified data analytics

Spatial biology

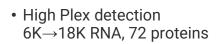
Single Cell and Sub-Cellular



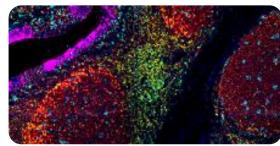




CellScapeTM



- Accurate cell segmentation
- Comprehensive data analysis with AtoMx™ SIP
- In situ imager



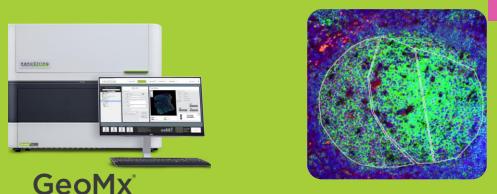
Best in class resolution

- Highest dynamic range
- Flexible assays, high-plex ~100 proteins
- Cyclic, highly multiplexed IF

2008

Spatial Biology Demands Multiple Levels of Plex, Resolution, and Throughput





Whole Transcriptome (WTA) (18,000+ plex) > 570-plex Proteins (IPA)

Gain insights into complex tissue microenvironments

Simultaneously co-detect RNA and protein

Discover and develop biomarkers

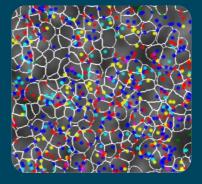
Run multi-omic cohort studies

How do multicellular areas of interest (AOIs) compare?

Both platforms available through ASOC

CELLULAR EXPLORATION





Up to 6375-Plex RNA; 72-plex Protein Human WTx in 2025

Map tissue cellular composition

Discover cell-cell interactions (niches)

Identify mechanisms of cell behavior and response

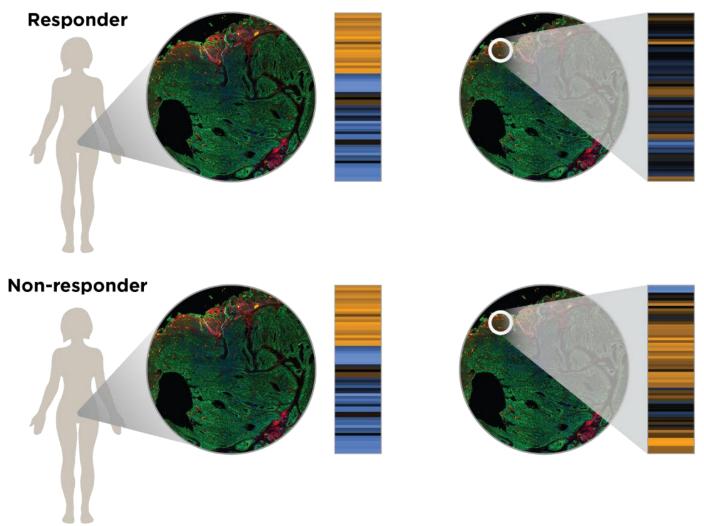
Discover biomarkers

How do **single cells** compare?



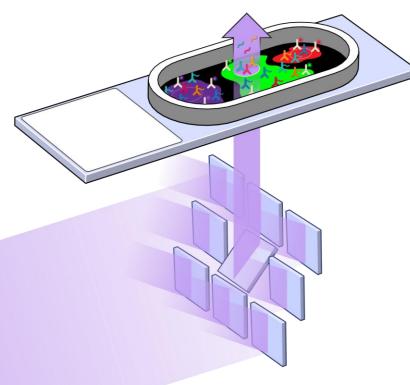
The Challenge of Heterogeneous Tissue

Spatial profiling has the potential to reveal actionable differences in biology

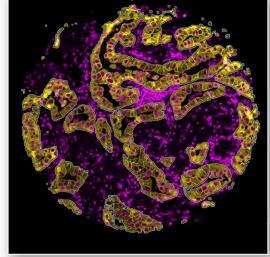




GeoMx DSP Adaptive Optics



Stain

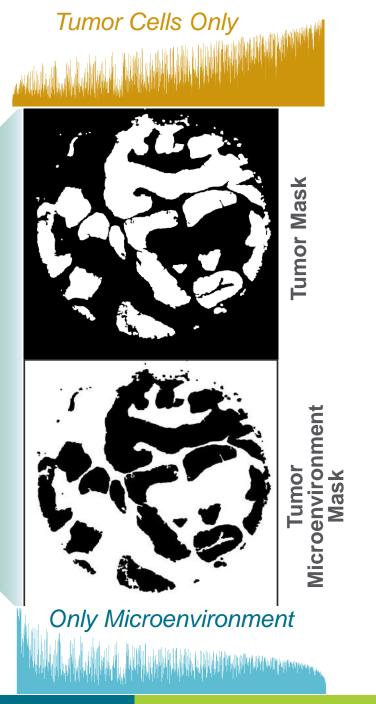


Whole Transcriptome

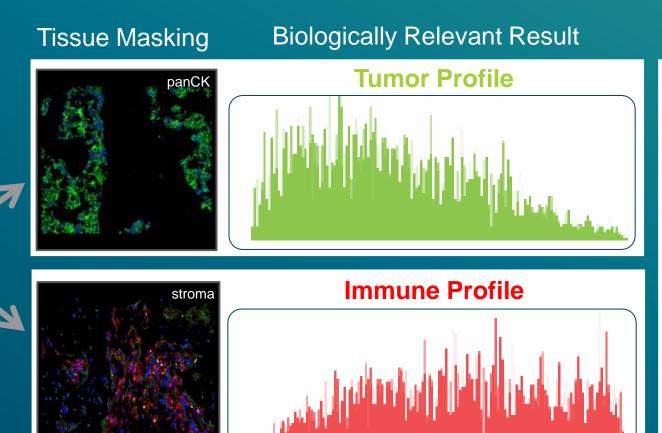
Expression Level



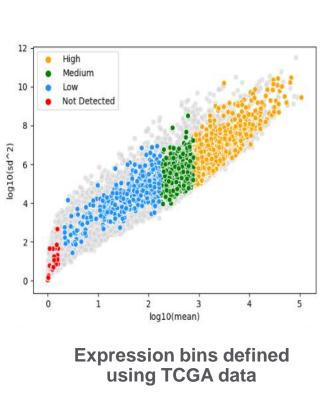
Digital micromirror chip (DMD) ~ 1 million 1um² mirrors



GeoMx Delivers High Sensitivity to Detect Low, Medium, and High Expressors with 5+ Logs of Dynamic Range

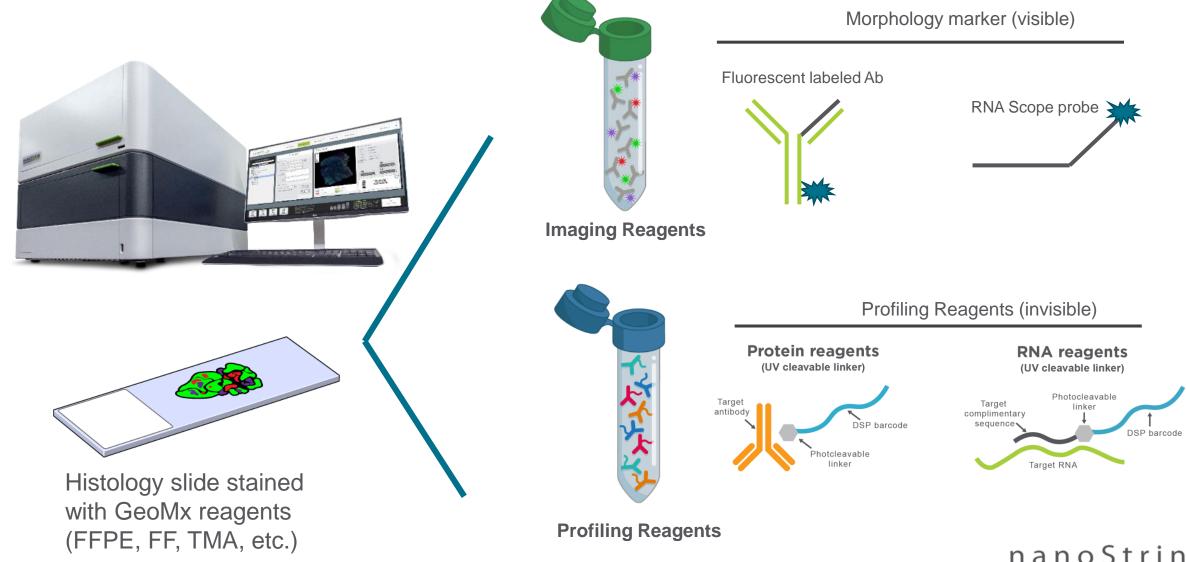


Dynamic Expression Range



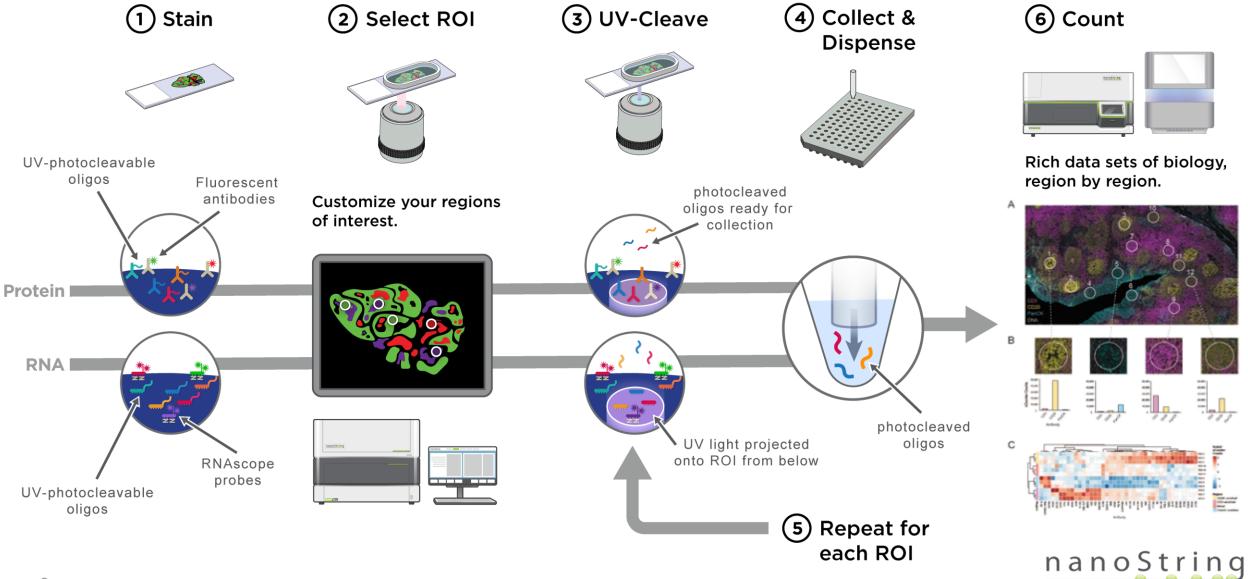


GeoMx Digital Spatial Profiler – How it works

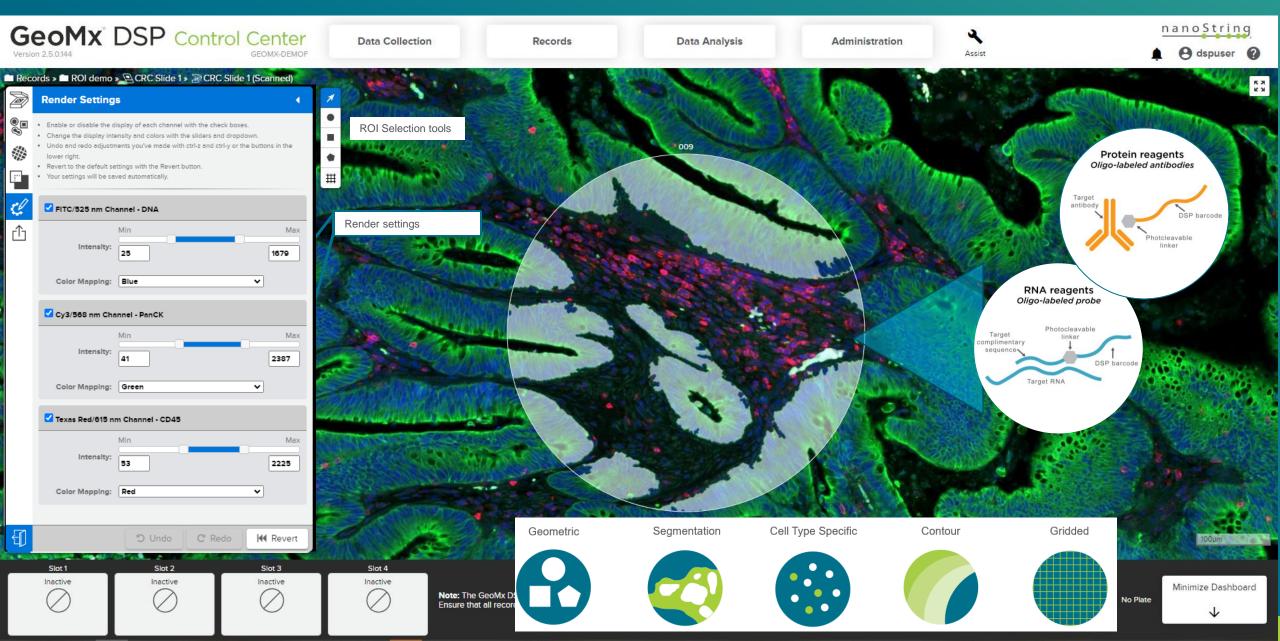


GeoMx Workflow





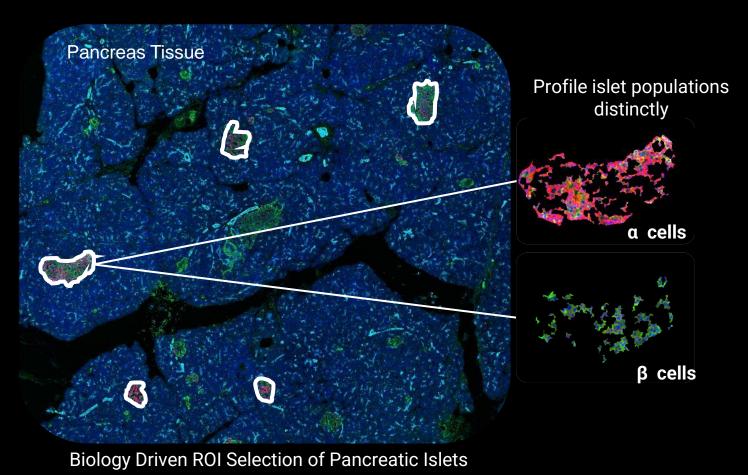
GeoMx Region of Interest (ROI) Selection and Segmentation

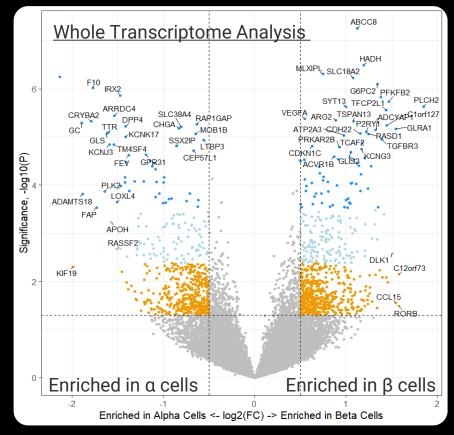




Focus on the biology of interest and maximize discovery with whole transcriptome exploration (WTA Assay)

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





>8000 genes detected



GeoMx® Validated in 400+ Peer-Reviewed Publications

GeoMx Covers







JCI insight



Immuno-oncology



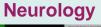


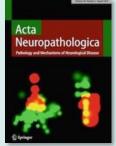


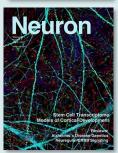
























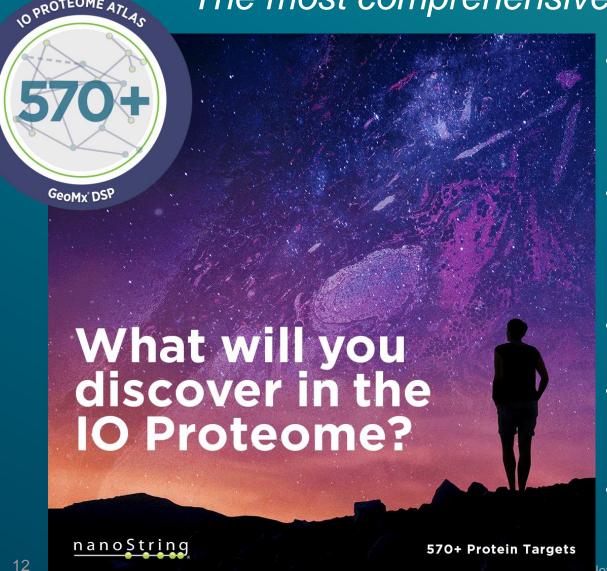






Introducing the GeoMx® IO Proteome Atlas (IPA)

The most comprehensive spatial proteomics panel



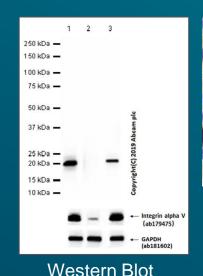
- Covers virtually every IO protein target in the world's largest IHC-compatible antibody catalog <u>Abcam</u>
 clone list available
- 570+ protein targets across cancer, metabolism, infectious disease, organ transplant, epigenetics, autoimmunity, and post-translational modifications
- Curated by IO subject matter experts
- Comprehensive coverage of the Hallmarks of Cancer, across 77 functional pathways
- Ability to add up to 40 of your own protein targets

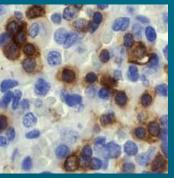
<u>nano String</u>

GeoMx® IPA delivers results you can trust Independent functional validation of reagents using orthogonal methods

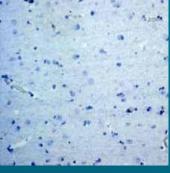
Single-plex IHC validation by Abcam

CD3e (Clone ID: EP449E)





Positive IHC staining in Normal spleen tissue



Negative IHC staining in Normal brain tissue

Multiplex validation by NSTG

Measured on 90 pellet CPA and an FDA-approved screening array (TMA) with tumor and normal tissues.

Assessed:

- Clear positive signal in positive control cells & tissues
- Clear negative signal in negative control cells & tissues
- Phospho-specific antibodies also validated using established models, including phosphatase inhibitor treatment prior to fixation









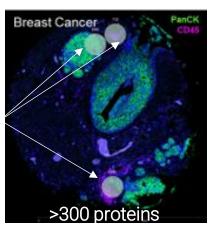


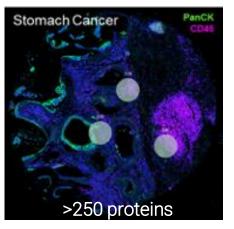


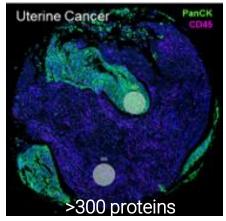
GeoMx DSP has the highest plex spatial protein assay in the world to discover previously unexplored biology across any tissue and disease

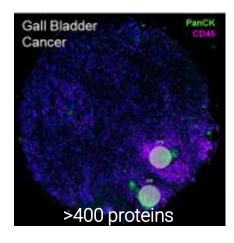
Profile 570+ protein targets from each tissue section: *Discover the next PD-L1 biomarker*

Regions of interest



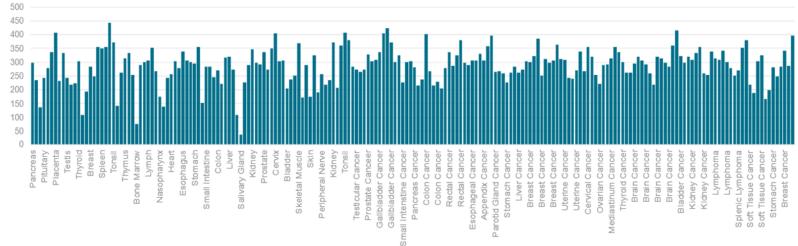








Proteins detectedper AOI per Tissue Type





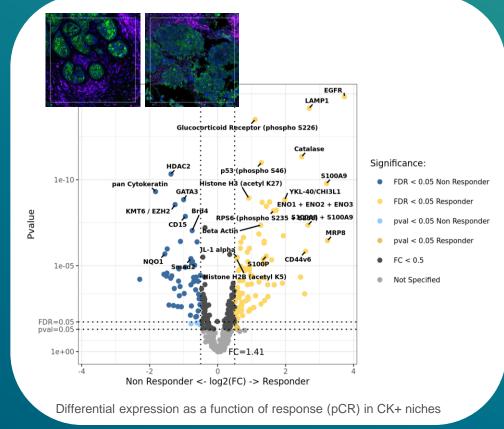
GeoMx IPA Reveals Novel Protein Markers with Implications for Patient Stratification

Clinical early stage HER2+ breast cancer tumor biopsies for responders and non-responders to neoadjuvant (TCHP)-treatment were analyzed using the GeoMx IPA.

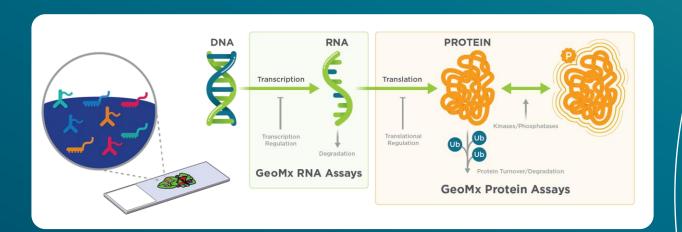
- GeoMx IPA data revealed:
 - New and "<u>provocative</u>" protein biomarkers with potential implications for patient stratification.
 - Clear differences in the phospho-protein profiles of responders vs non-responders.
 - Enrichment of proteins associated with the ERBB signaling pathway in responders.
- GeoMx® IPA data showed concordance with CosMx 1K-plex RNA data.



E. Aubrey Thompson, PhD Professor Mayo Clinic

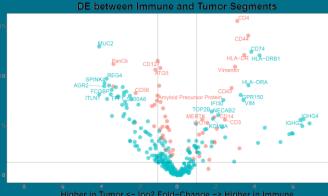


GeoMx Spatial Proteogenomics: Assay RNA and Protein on same slide!

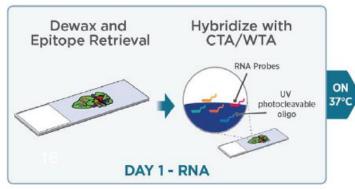


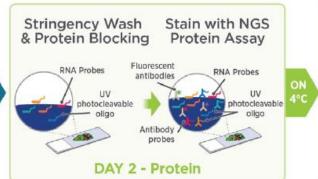


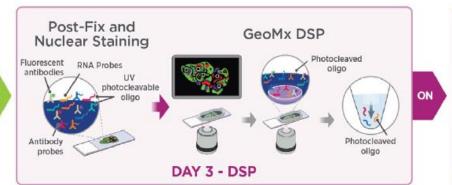
White Paper Showcases Highest Ever Protein and RNA Detection From a Single FFPE Section (WTA + IPA)



Higher in Tumor <- log2 Fold-Change -> Higher in Immune



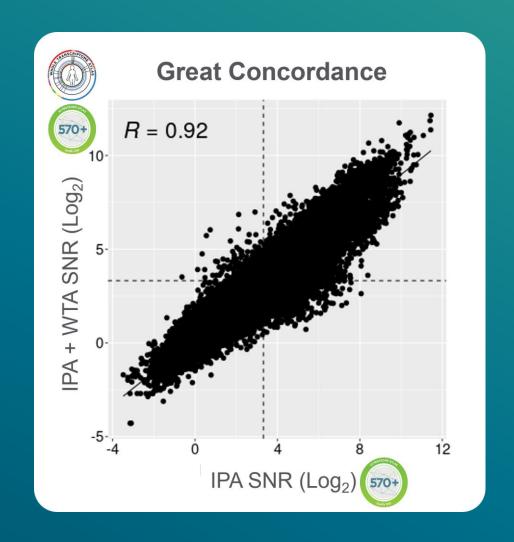






IPA assay performance remains consistent for IPA alone and IPA + WTA



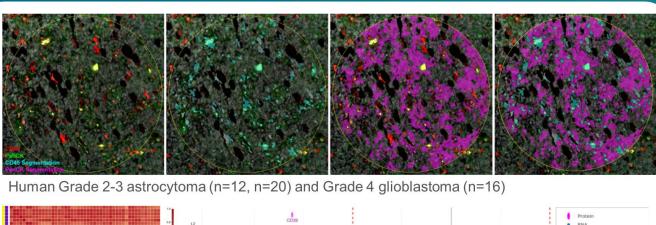


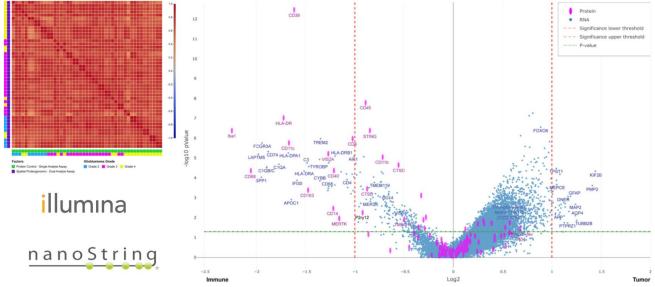






Spatial Proteogenomics: Co-detection of whole transcriptome and 570 proteins



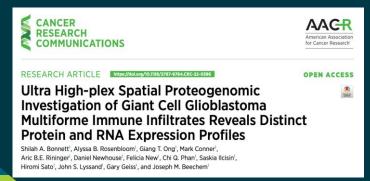


Highest Ever Protein and RNA Detection From a Single FFPE Section

 Detection of Cell Composition and Function

Spatial Proteogenomics Drives Research Further

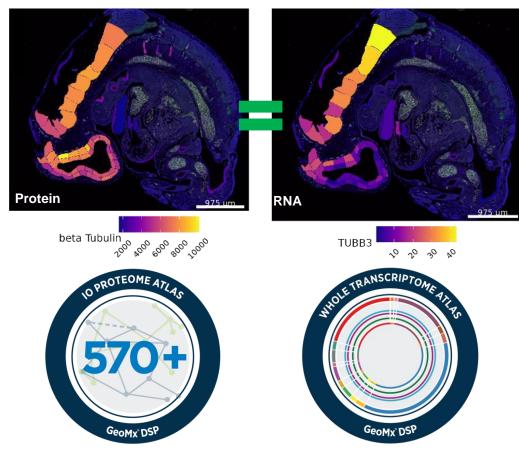
Novel workflow saves
 precious samples and
 enables researchers to
 study the relationship
 between RNA and Protein





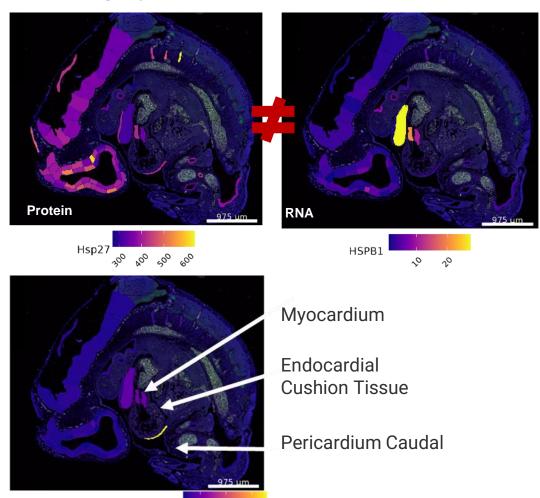
The only spatial platform to interrogate the whole transcriptome and high-plex protein discovery from the same slide simultaneously

Interrogate correlative and post-translational dynamics all from a single precious section



8-week human embryo data from a single tissue section

In collaboration with: BS de Bakker, MJB van den Hoff et al. Dutch Fetal Biobank, Amsterdam UMC



Hsp27 (phospho S78)

FFPE Tissue from the Influenza Epidemic of 1918 (>100 years old!)







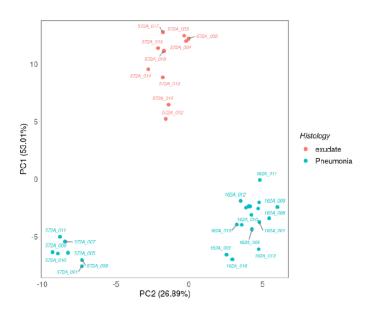
Uncoupling pathways involved in immunotherapy resistance: insights from deep tissue profiling

Arutha Kulasinghe, Ph.D.
Queensland University

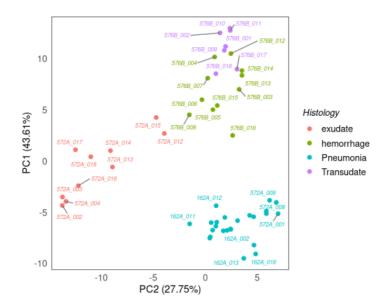


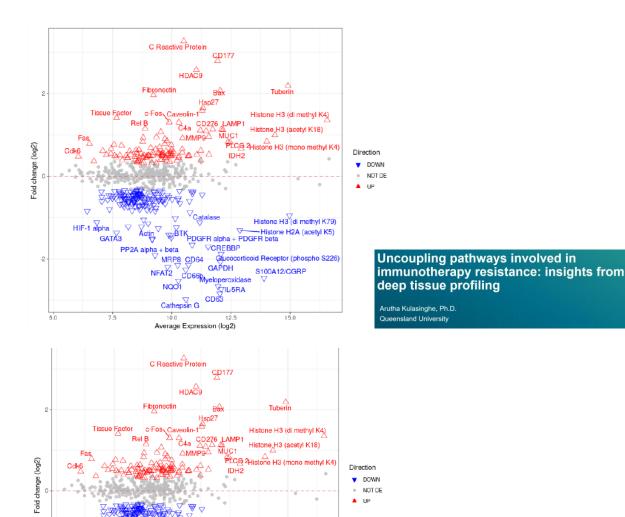
IPA (570-plex protein), WTA (18,000-plex RNA)

PCA of genes in regions of 1918 influenza virusinfected lungs



PCA of proteins in regions of 1918 influenza virusinfected lungs





Cathepsin G

Average Expression (log2)



GeoMx® DSP for robust tissue exploration

UNBIASED PLEX MULTIOMICS

Measure whole transcriptome and 570+ proteins on the same slide

BIOLOGY-DRIVEN

Profile meaningful regions to minimize noise and simplify analysis

HIGH SENSITIVITY

Detect low, medium, and high expressors with 5 logs of dynamic range

SCALABLE THROUGHPUT

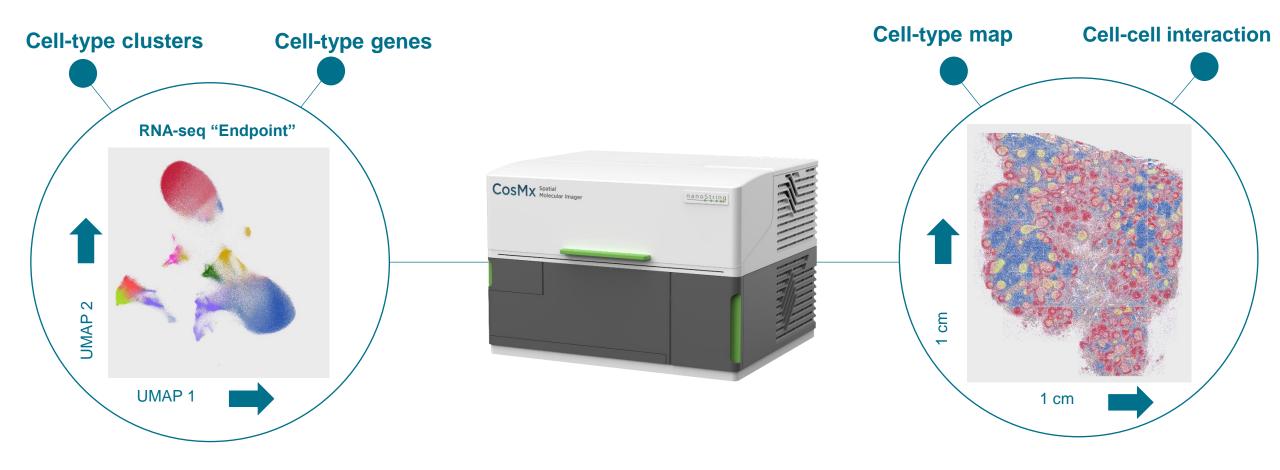
Process up to 4 slides at a time and scale with automated workflows

ANY SAMPLE

Analyze FFPE, FF, Whole Mount, TMA, organoid



Advancing from scRNA-Seq to Spatially-Resolved Single-Cell (CosMx™ SMI)

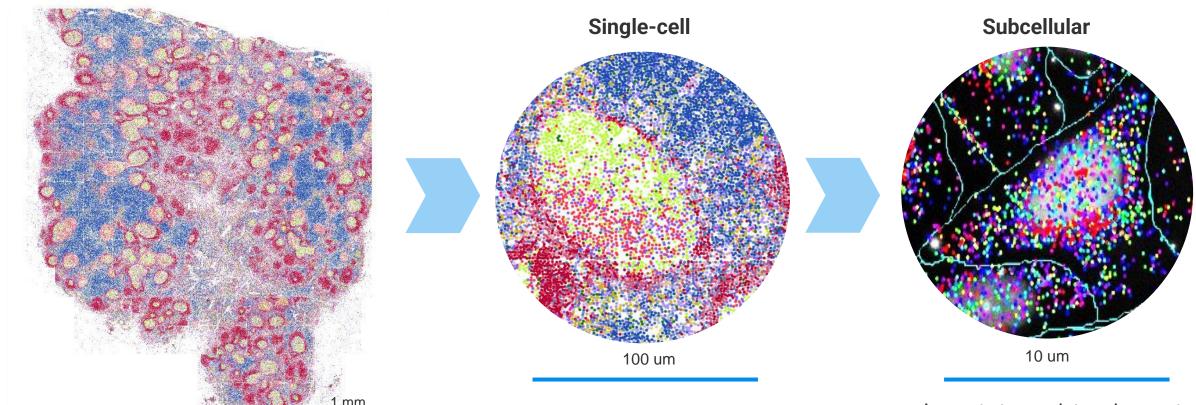






CosMx SMI: the highest-plex (6000+ plex) single-cell spatial platform Capture the story of every cell-to-cell interaction from an entire tissue section

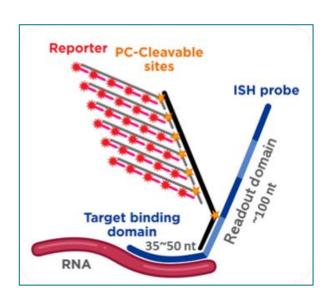
Resolve millions of cells and billions of transcripts at single-cell and subcellular resolution



1.4 million cells analyzed across entire **CosMx** lymph node FFPE tissue section (1K RNA)

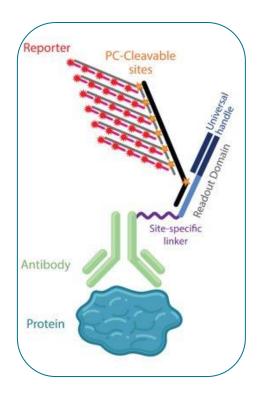
Accurate transcript assignment enabled by multi-modal, ML segmentation algorithm

High-Plex CosMx™ RNA and Protein Panels



RNA Panels Consist of Pooled ISH Probes

- Five ISH probes per target
- Panels include ERCC Negative Probe controls, non-target-binding
- Currently up to a commercial 6175-Plex + 200 custom targets (whole transcriptome in 2025)

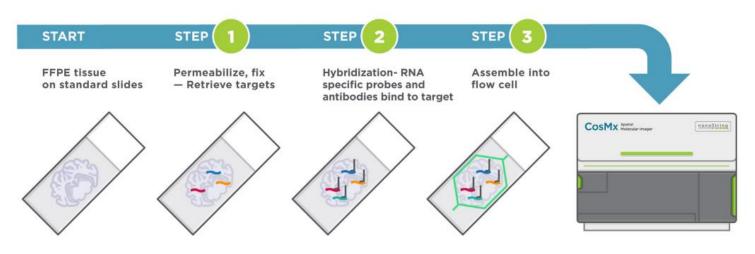


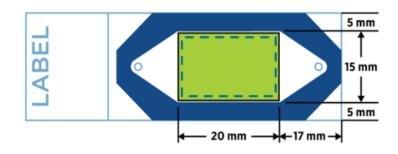
Protein Panels Consist of Pooled Antibodies

- Monoclonal antibodies w/ site-specific conjugation
- IgG controls, non-target-binding
- Currently up to 64-Plex + 8 custom targets

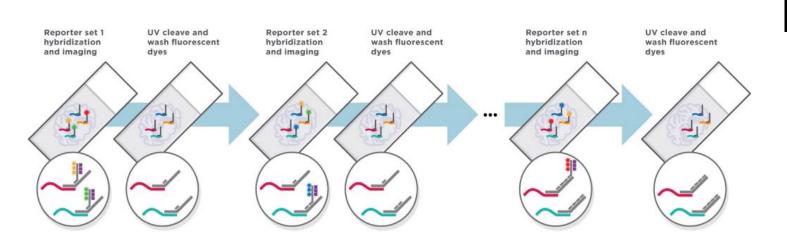


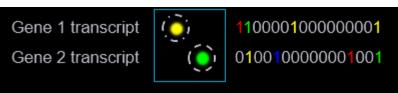
CosMx™ Uses Single Molecule In Situ Hybridization Chemistry with Cyclic Imaging and Optical Barcodes





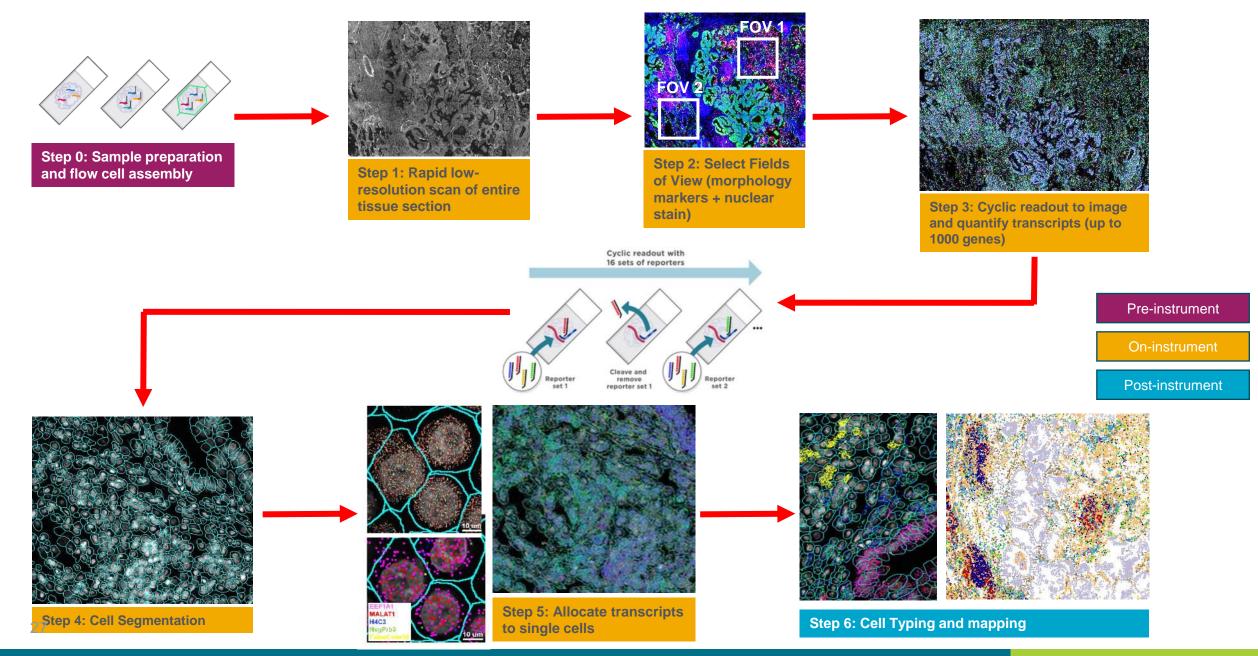
Simple and streamlined sample prep workflow.



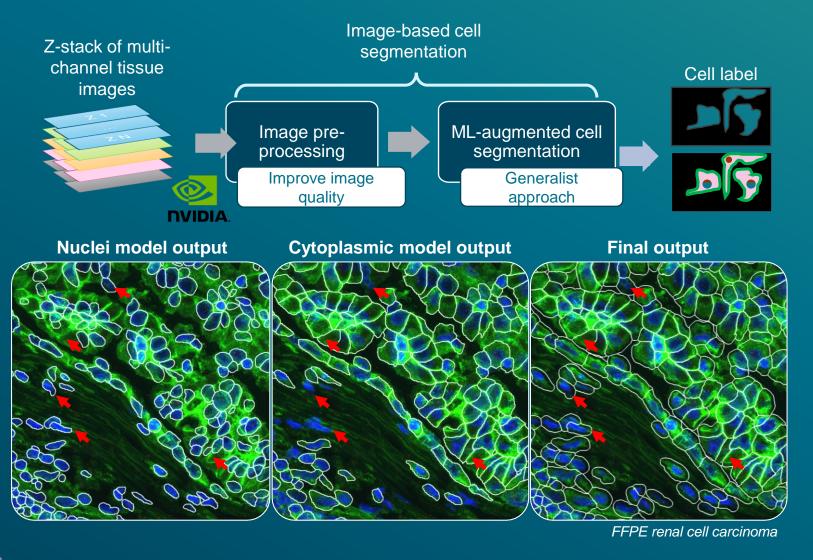


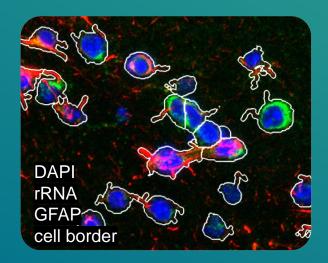


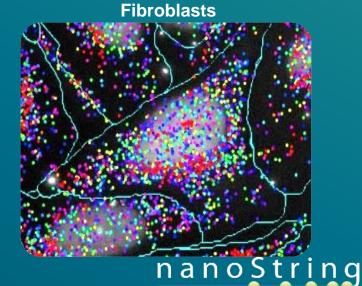
CosMx™ Spatially Resolves Molecules (in situ Imaging)



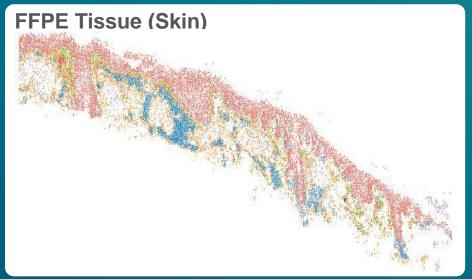
Robust segmentation of cell boundaries and compartments

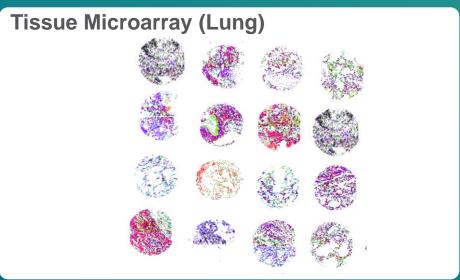


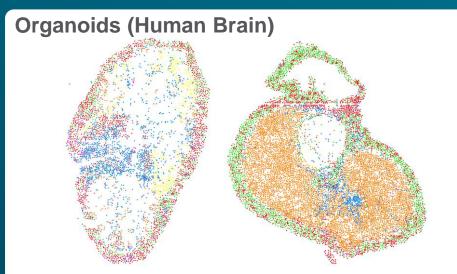


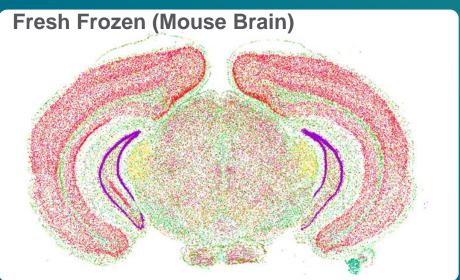


Generates Robust Data with All Samples: FFPE to Fresh Frozen



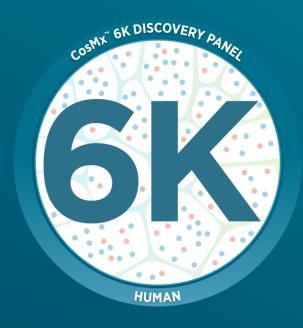








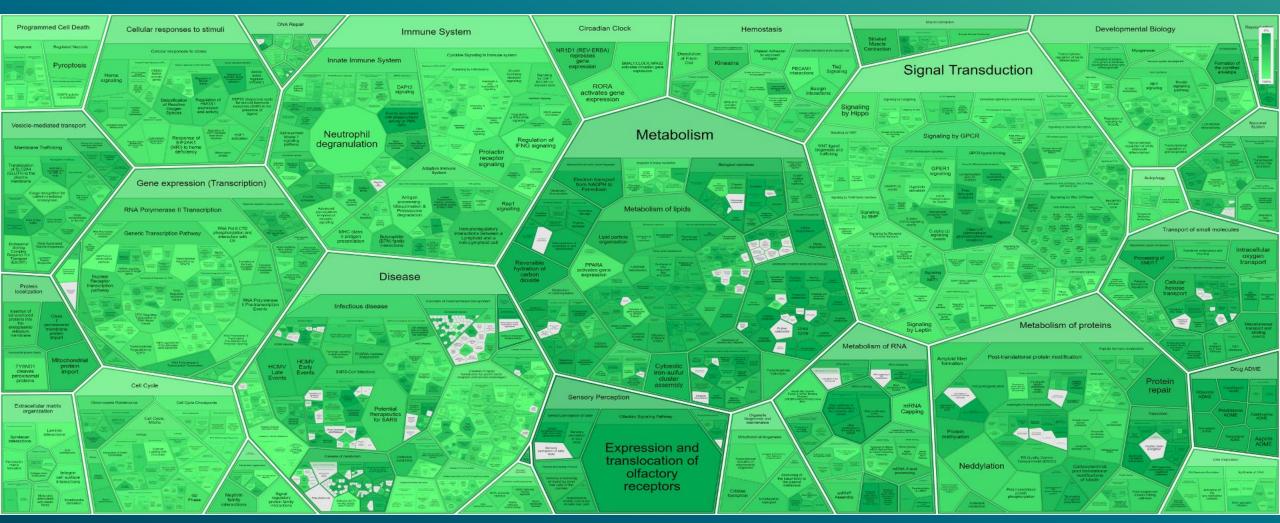
CosMx™ 6K Discovery RNA Panel



- 6175 curated RNA targets
- Customizable with up to 200 targets
- Accurate, multi-modal cell segmentation
- Compatible with wide range of tissues
- Profile nearly the entire human reactome
- Available for human tissue
- Human Neuro coverage



CosMx 6000-plex Panel: Virtually the Entire Reactome



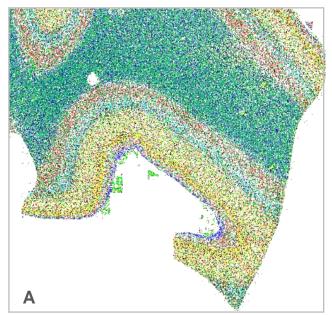


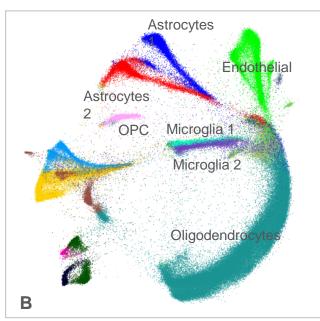


CosMx™ 6K Discovery Public Dataset

Tissue type	Human frontal cortex
Plex	6078 gene targets (Pre-commercial)
Scan area	102.7 mm ²
Total cells	194,065
% cells pass QC	97.2%
Total transcripts detected	331,132,591
% of Transcripts Assigned to a Cell	49%
Sensitivity (Mean Transcripts per cell)	839
Sensitivity (Mean transcripts/um3)	1.29
Genomic Breadth (Mean unique genes per cell)	544
Genomic Breadth (Unique genes detected above LOD)	3364
False Positives (Mean negatives/target/cell)	0.027

First Single-Cell Spatial Dataset with over 6000 Genes





Cell type map of human frontal cortex tissue. (A) Spatially resolved cell type map of entire tissue section. (B) UMAP projection of cell type clusters.



QR code to download 6K dataset



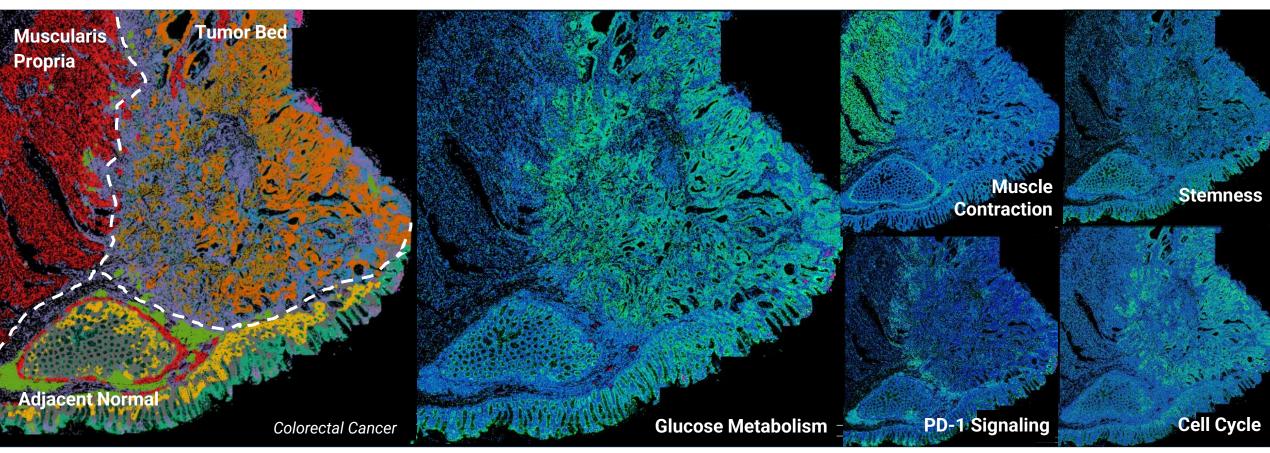


Move beyond individual gene analysis to understand functional pathways of biology at single-cell resolution





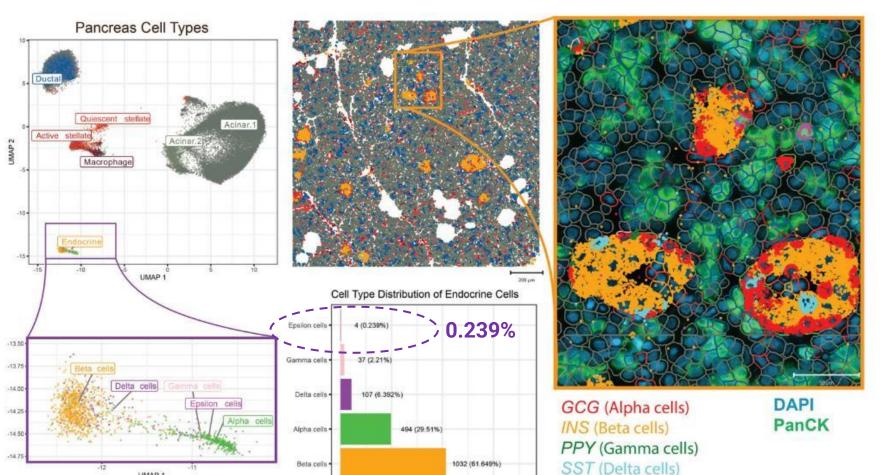
Project the Reactome directly onto tissue with CosMx 6K Discovery





The only single-cell spatial platform to demonstrate whole transcriptome biology: FFPE public dataset available

Commercially available for the CosMx SMI in 2025



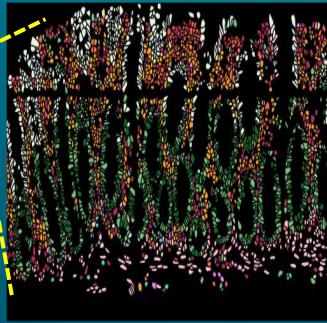


Resolve rare cell types with subcellular spatial context (healthy human pancreas)

GHRL (Epsilon cells)

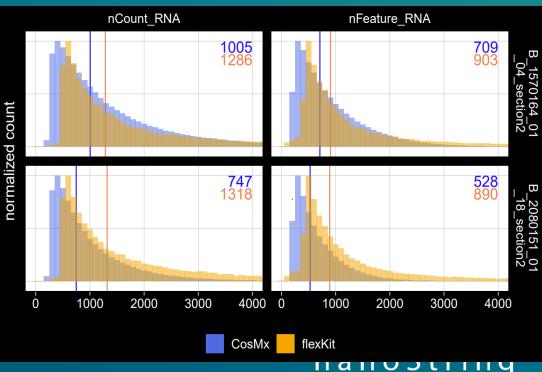
CosMx WTx Panel: True Single-Cell Spatially-resolved Whole Transcriptome with Dr. Holger Heyn (CNAG, Barcelona)





Tubulovillous adenomas (early CRC)

- Total single cells = 1.4 Million cells
- Transcripts = over 1.4 Billion
- Transcripts per cell > 1,000
- Close to snRNA-seq on all key metrics
- Less than 1-cent per transcriptome



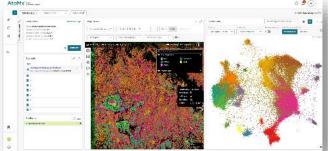


First comprehensive cloud platform for single-cell spatial informatics that scales to any plex and throughput

Cloud platform fully integrated with CosMx from sample to data for analysis

Demo of AtoMx this afternoon!

Multi-sample and Iterative **Analytics Suite**



Tunable Machine-learning Cell Segmentation Toolkit



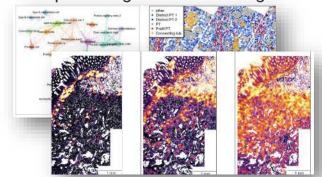
Opensource Portable and Flexible Data formats







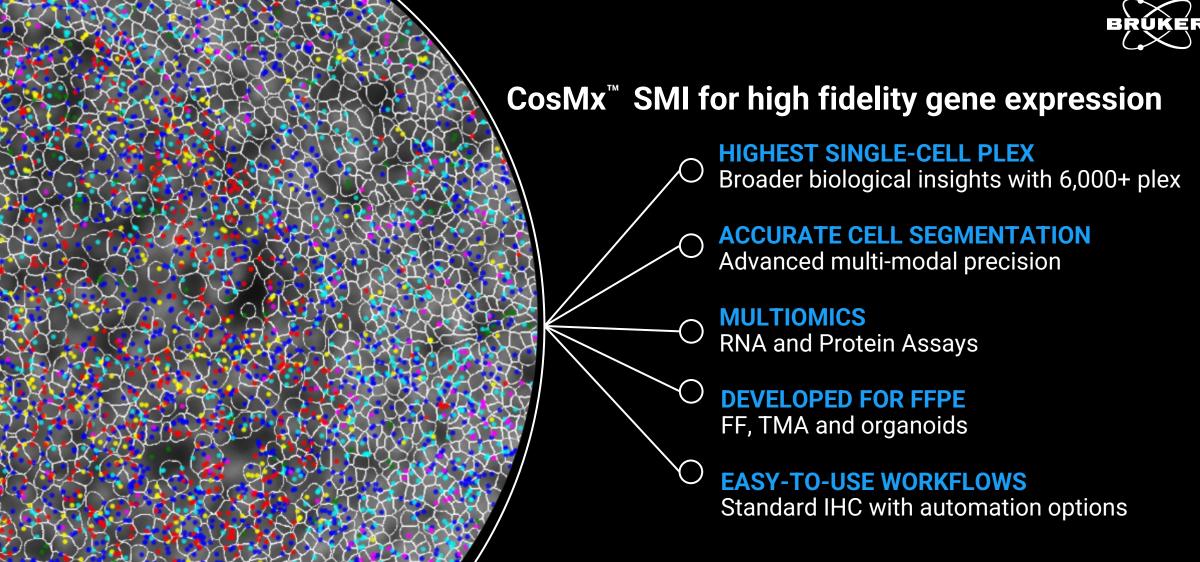
New Spatial Algorithms for Single-cell





Spatial Informatics Platform







CellScape™ Precise Spatial Proteomics



4-sample holder



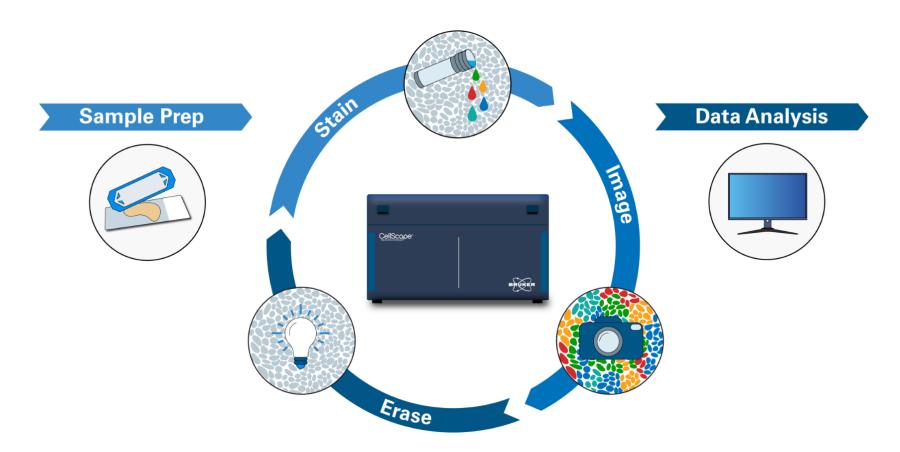
Integrated fluidics

Enclosed imaging system

Connected software



Stain. Image. Repeat.



Samples are prepared with CellScape™ Whole-Slide Imaging Chambers. Cycles of staining, imaging, and signal removal are automatically completed by the instrument. Images are digitally overlaid to achieve high-plex spatial proteomic datasets.



Straightforward, reliable technique with open-source antibodies

Rigorously validated panels and open-source antibodies get you productive sooner, at less cost

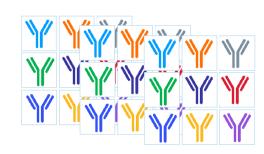
Use Our Antibodies



Reliable, modular, and robustly validated VistaPlex™ Assay kits for core applications:

- Immune profiling
- Tissue architecture
- Immune cell subtyping

Beyond our panels, leverage our database of over 350 CellScapecompatible antibodies



Use Your Antibodies

- Capture the advantage of biomarker flexibility and customization — Use the clones you know
- Primary monoclonal antibodies for high specificity
- Faster and more straightforward antibody validation - no custom conjugation needed
- Directly conjugated antibodies



The most comprehensive and customizable off-the-shelf modular antibody panels . . . Build and expand your high-plex assay at any time

Mix and match pre-validated panels can be applied today, tomorrow, or next month

Tissue Architecture

(Myo-) Epithelia: Beta catenin, CD138, E-cadherin, podoplanin, SMA

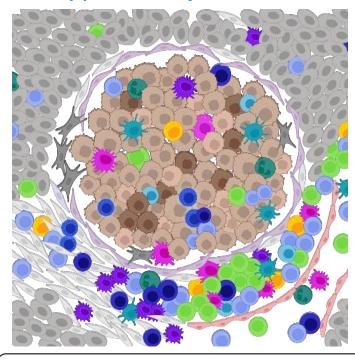
Endothelia: CD31, CD34, Podoplanin

Other: Vimentin, CollagenIV

Myeloid

Macrophages: CD11b, CD11c, CD14, CD163, iNOS, CD206

Dendritic Cells: HLA-DR, CD123, DC-SIGN, CD80, CD86



Immune Profiling

Lymphocytes. CD45, CD45RA, CD27 T-cells. CD3, CD4, CD8, FoxP3 B-cells, Macs. CD20, CD68, Cell States. GrnB, PD-1, PD-L1, Ki67

Tumor, PanCK

NK Cells

CD56, NKG2D, CD161, CD57, NCR1

Checkpoints

LAG-3, OX40, TIIM-3, CTLA-4, TIGIT

Activation / Proliferation

ICOS, CD40, CD40L, IL2RA, IDO1, VISTA, IFNG, PCNA

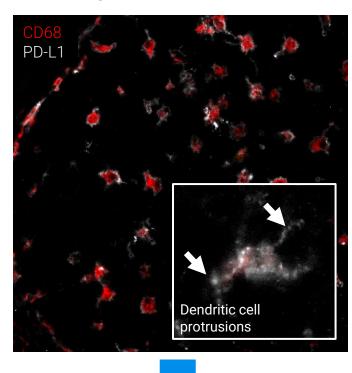




What is quantitative spatial phenotyping?

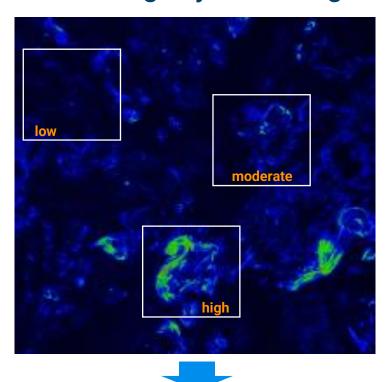
See the whole spatial neighborhood...

... At high resolution



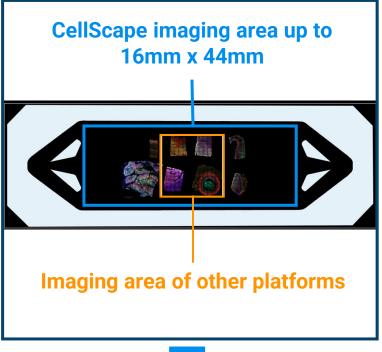
True single cell and subcellular detail (182 nm/pixel)

... Over a high dynamic range



Quantify relative abundance and identify low expressors (automated high dynamic range imaging)

... Across the whole slide

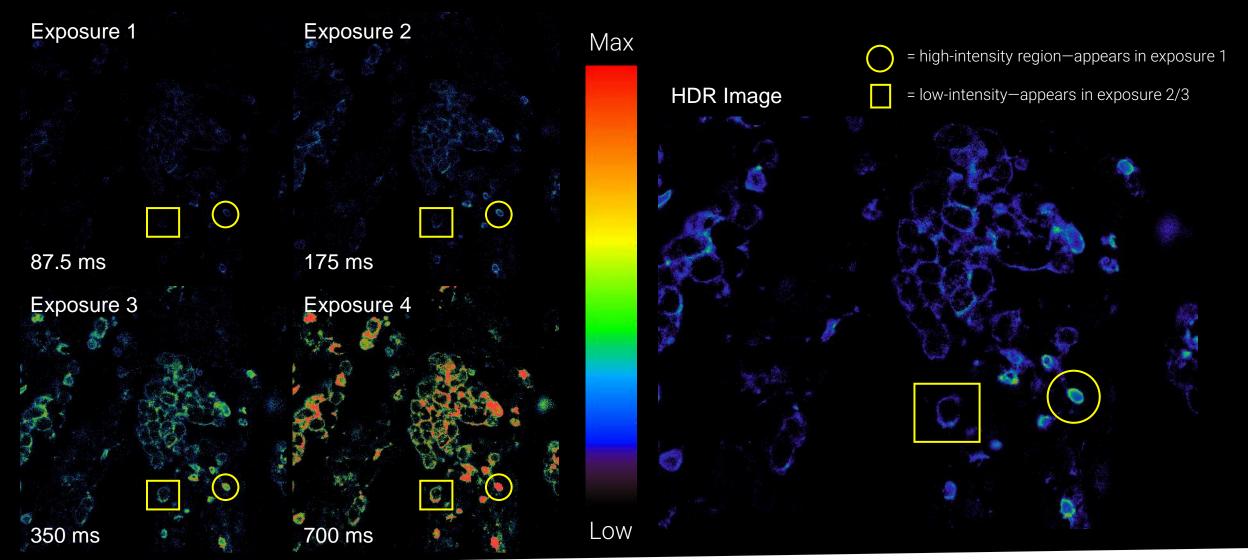




Increase throughput and reproducibility

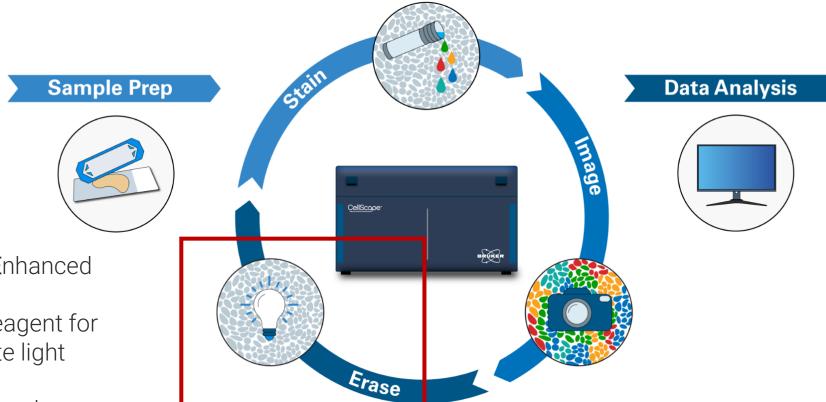


Our High Dynamic Range technology deploys multiple exposures to enable quantification of the full range of protein expression present in biology





Stain. Image. Repeat.



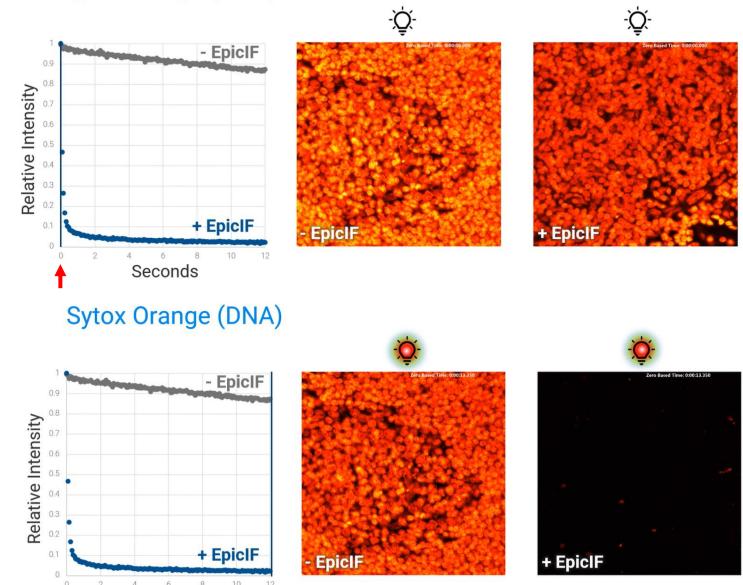
Newly launched EpicIF (Enhanced Photobleaching In Cyclic ImmunoFluorescence) reagent for photobleaching with white light

- Announced Oct 2024
- Signal removal from nearly any photostable fluorophore
- Non-destructive to tissue or epitopes
- https://www.brukerspatialbiology.com /EpicIF

Sytox Orange (DNA)

Seconds



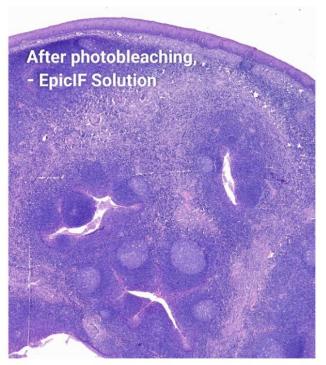


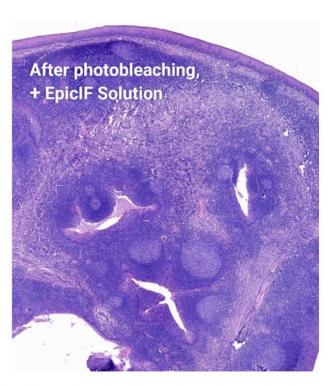


Modular sample re-examination is enabled by non-destructive and robust cyclic multiplexing

Photobleaching Preserves Epitope Stability and Tissue Integrity



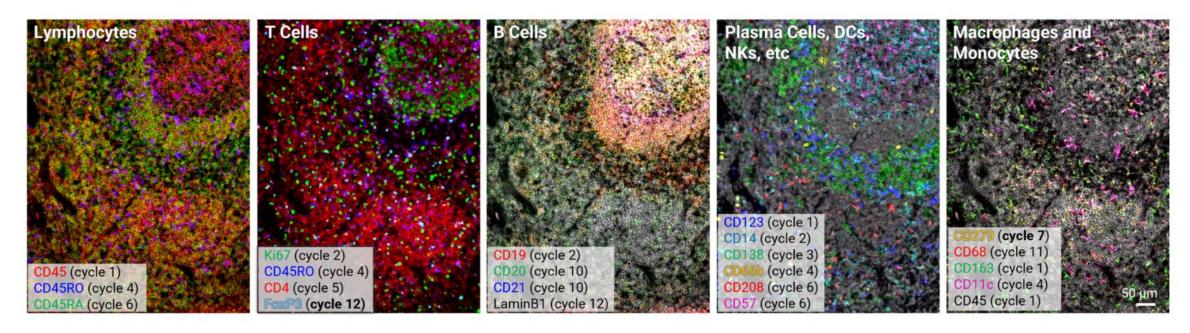




H&E staining of serial tonsil sections treated with (left to right) no photobleaching, 10 rounds of photobleaching without EpicIF solution, and 10 rounds of photobleaching with EpicIF solution. A pathologist concluded that the sections are the same with respect to integrity and preservation of cellular architecture.



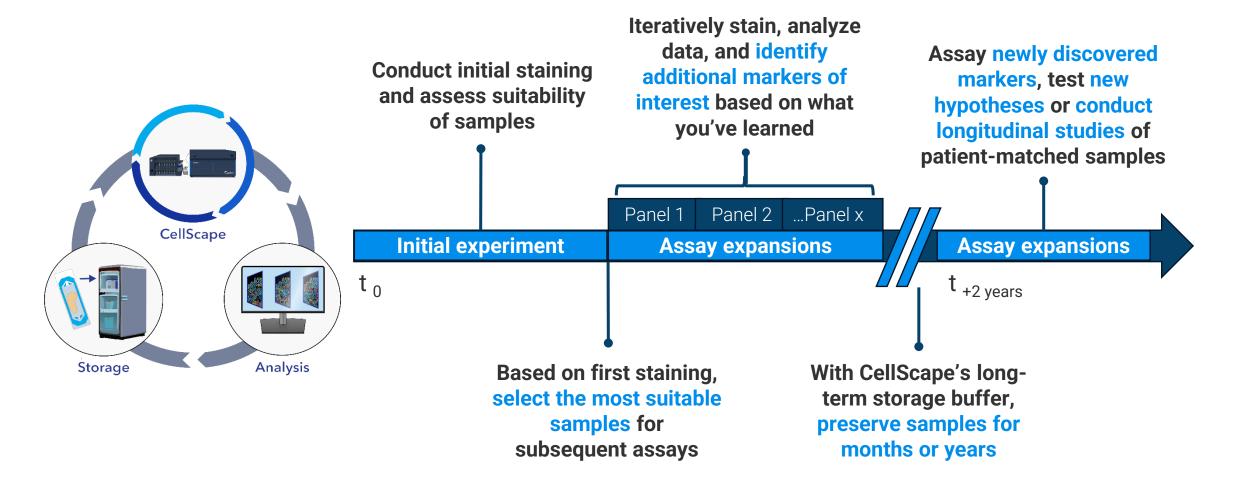
Stain. Image. Repeat.



48-plex image acquisition of an FFPE tonsil sample completed in 12 EpicIF cycles using fluorophores AF488, AF532, AF594, and AF647, with key markers indicated in legends.



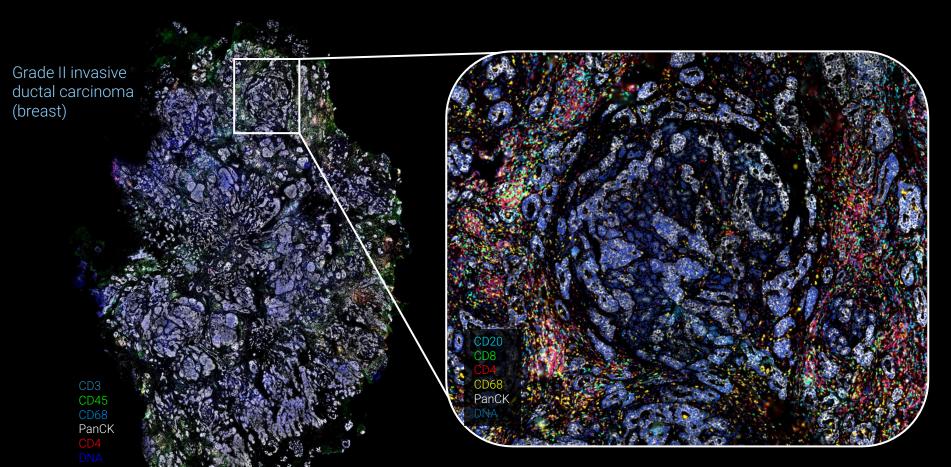
What hypothesis-driven spatial biology looks like





Initial Assay: Immune Profiling

Initial experiment



Observation:

Exceptionally strong immune infiltrate is accompanied by regional immune exclusion.

Follow-Up Question:

What is the mechanism of immune exclusion?

> **Assay Expansion** with CellScape

Innovation with Integrity | November 7, 2024 | 50 © 2024 Bruker

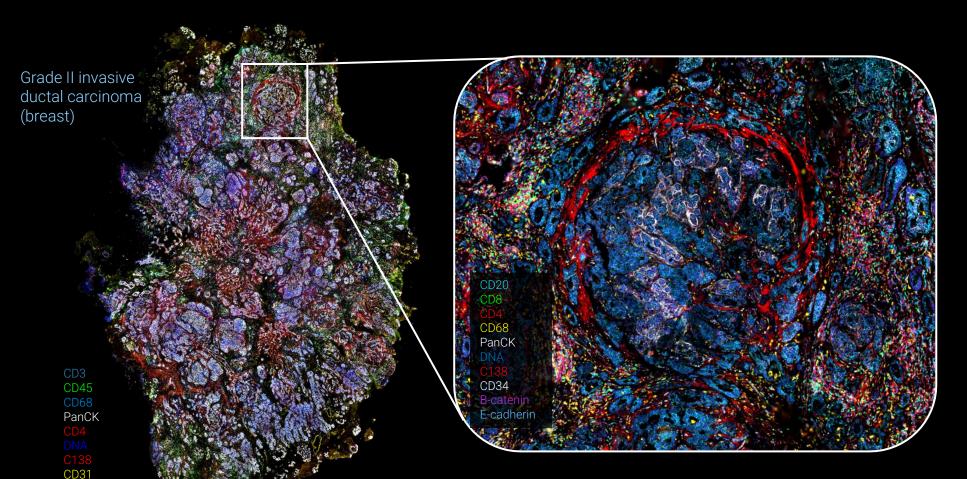
E-cadherin MUC1



Assay 2: Tissue Architecture Profiling

Initial experiment

Assay expansions



Observation:

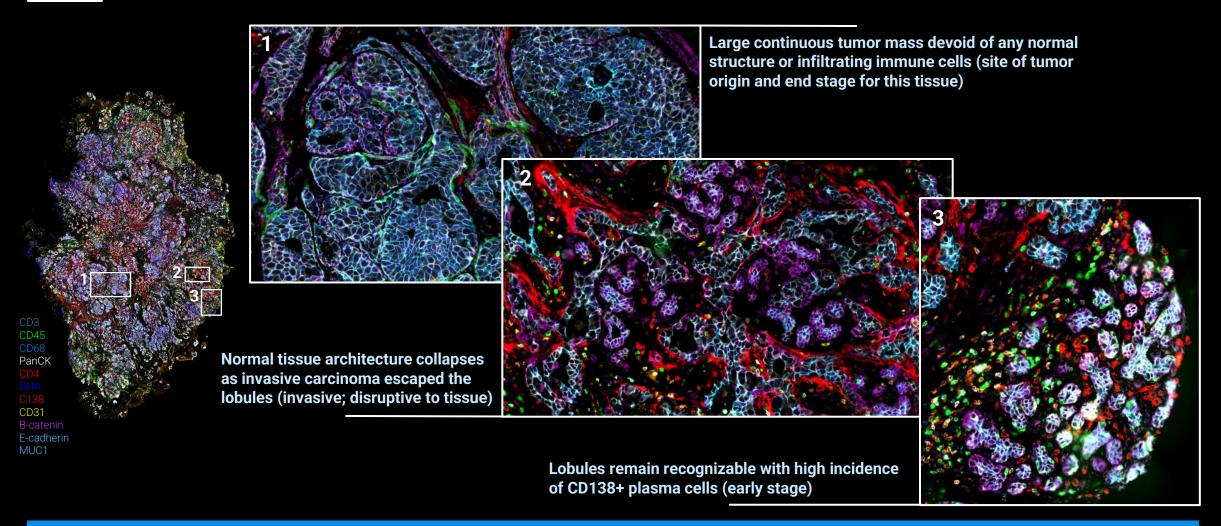
Immune infiltrate is compartmentalized by CD138+ plasma cells / epithelia, which may act as an immune barrier.

Conclusion:

This spatial arrangement within the tumor may determine the course of development for treatment.



Additional Assays: Immune-Architectural Profiling Illustrates Tumor Progression



Get the whole story for every sample



CellScape™ Precise Spatial Proteomics

QUANTITATIVE PERFORMANCEHigh resolution & high dynamic range (HDR)

STRAIGHTFORWARD & RELIABLE TECHNIQUE

Robust Assay Performance

STREAMLINED ASSAY DEVELOPMENT

Flexible marker selection and plex at scalable cost & throughput

EXPANDABLE ASSAYS

Re-examination enabled through gentle chemistry

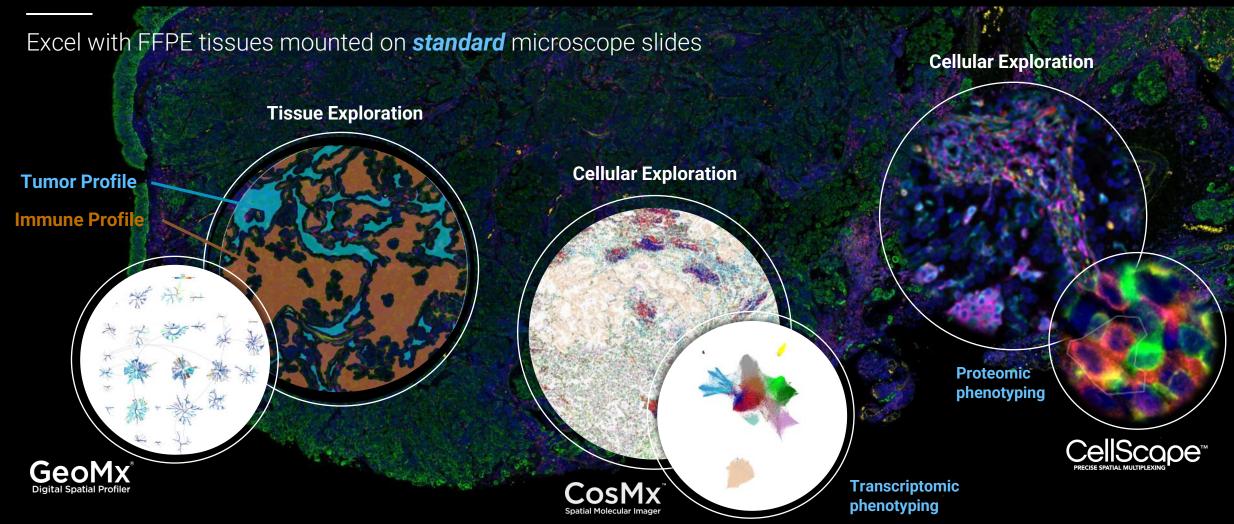
LARGEST IMAGING AREA

Whole tissues on standard slides





Comprehensive platforms to solve any spatial problem



Non-Small Cell Lung Cancer

AtoMx SIP: Streamlined, Scalable, Flexible

Instruments





GeoMx[®]







Standardized Data Formats

Seurat
OME-TIFF

3rd Party & Open-Source Tools

Open-Source Analytics

Digital Pathology Analysis

ROI Selection AI Algorithms

Benefits:

- Application can scale to needs of end user
- Ease of use (Chrome on client computer)
- More streamlined data sharing and AWS security
- Automated file transfer from CosMx to AtoMx



CosMx + AtoMx Integrated Workflow

CosMx Workflow



- Data is automatically transferred from CosMx to AtoMx
- Computationally-intensive processing in AtoMx (XYZ positions of fluor. spots decoded to transcripts)
- Data can be analyzed immediately after instrument completes



BRUKER SPATIAL BIOLOGY

Please see NanoString University and our website for more information. Thank you!



NanoString University https://university.nanostring.com/

Please reach out to us for project discussions and pricing options.

Wes Heydeck Amir Farooqui

Sr. FAS

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