#504

A novel spatial multi-omic approach for biological discoveries in colonic diseased tissues using a comprehensive Immuno-Oncology protein panel

Rudy Van Eijsden[1], Alyssa Rosenbloom[1], Shilah Bonnett[1], Christine Kang[1], Mark Conner[1], Megan Vandenberg[1], Erin Piazza[1], Brian Filanoski[1], Rhonda Meredith[1], Hye Son Yi[1], Lori Hamanishi[1], Eduardo Ignacio[1], Nadine Nelson[2], Michael Prater[2], Vik Devgan[1], Melanie Moon[1], Lesley Isgur[1], Terence Theisen[1], Margaret Hoang[1], Gary Geiss[1], and Joseph M. Beechem[1*]

[1]NanoString® Technologies, Seattle, WA, USA , [2]abcam plc, Discovery Drive, Cambridge, UK

NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

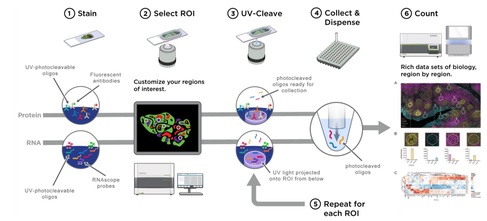
Abstract

The advancement of spatially resolved, multiplex proteomic and transcriptomic technologies has revolutionized and redefined the approaches to complex biological questions pertaining to tissue heterogeneity, tumor microenvironments, cellular interactions, cellular diversity, and therapeutic response. While spatial transcriptomics has traditionally led the way in plex, multiple studies have demonstrated a poor correlation between RNA expression and protein abundance, owing to transcriptional and translational regulation, target turnover, and most critically, post-translational protein modifications. Therefore, a more holistic, ultra-high-plex, and high-throughput proteomic atlas approach becomes critical for the next phase of discovery biology. Here, we present a barrier-breaking, spatial proteomics panel that was designed to accelerate scientific discoveries.

A Digital Spatial Profiler platform is uniquely suited to support high-plex proteomics, allowing for the simultaneous analyses of proteins from discrete regions of interest (ROIs) in FFPE tissue sections while preserving spatial context. The assay relies upon abcam antibodies coupled to photocleavable DNA barcodes readout with NGS sequencing, allowing for theoretically unlimited plex. Here we introduce the Human Immuno-Oncology Proteome Atlas (IPA), a 570+ antibody-based proteomic discovery panel, compatible with immunohistochemistry on FFPE tissues with NGS readout. IPA is the highest-plex, most comprehensive, antibody-based multi-omic panel to date focusing on key areas of immuno-oncology, oncology, immunology, epigenetics, metabolism, cell death, and signaling pathway regulation.

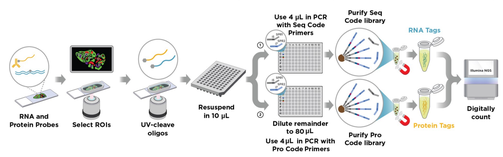
Here we demonstrate the performance of IPA on various cell lines and tissue. Additionally, we show the power of IPA, using the spatial multi-omic assay along with the GeoMx® Whole Transcriptome Atlas (> 18,000 transcripts), a 30-plex custom antibody panel and microbiome-curated RNA custom spike-in (~42 transcripts) to evaluate 70 different colon disease samples across 4 pathologies including adenocarcinoma, hyperplasia, and chronic inflammation. This is the highest-plex multi-omic (~610-plex proteins and >18,042 genes) study ever implemented for spatial biology. When we compared the diseased tissue to normal tissue, we observed an upregulation of specific pathways associated with tumorigenesis and inflammation. Furthermore, we observed distinct differences in proteomic and transcriptomic landscape between pathologies. The cutting-edge, data-driven, expert-curated IPA panel is at the forefront of spatial proteomics, empowering the researcher for the acceleration of biological discoveries.

GeoMx® Digital Spatial Profiler Workflow

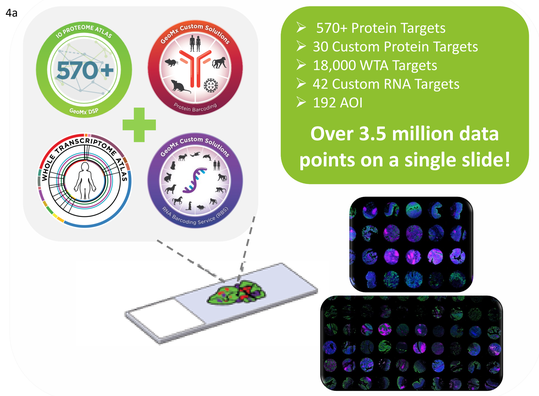


GeoMx Immuno Oncology Proteome Atlas is fully compatible with existing and well validated GeoMx Digital Spatial Profiler workflows

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

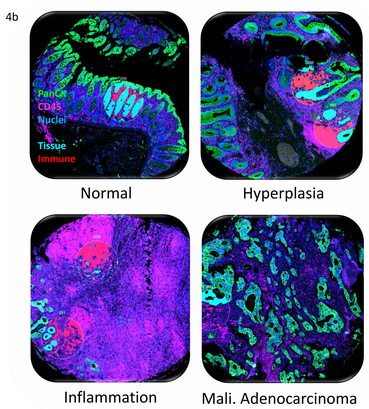


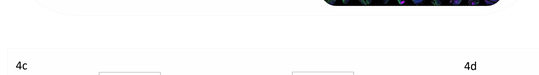
Spatial Proteogenomics reveals reduced phosphorylation of Cytokeratin 8 in Malignant Adenocarcinoma compared to chronically Inflamed colon

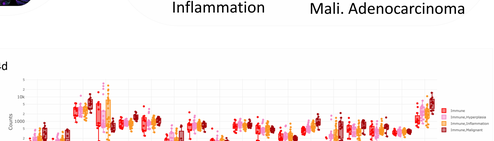


- 570+ Protein Targets
- 30 Custom Protein Targets
- 18,000 WTA Targets
- 42 Custom RNA Targets
- 192 AOI

Over 3.5 million data points on a single slide!







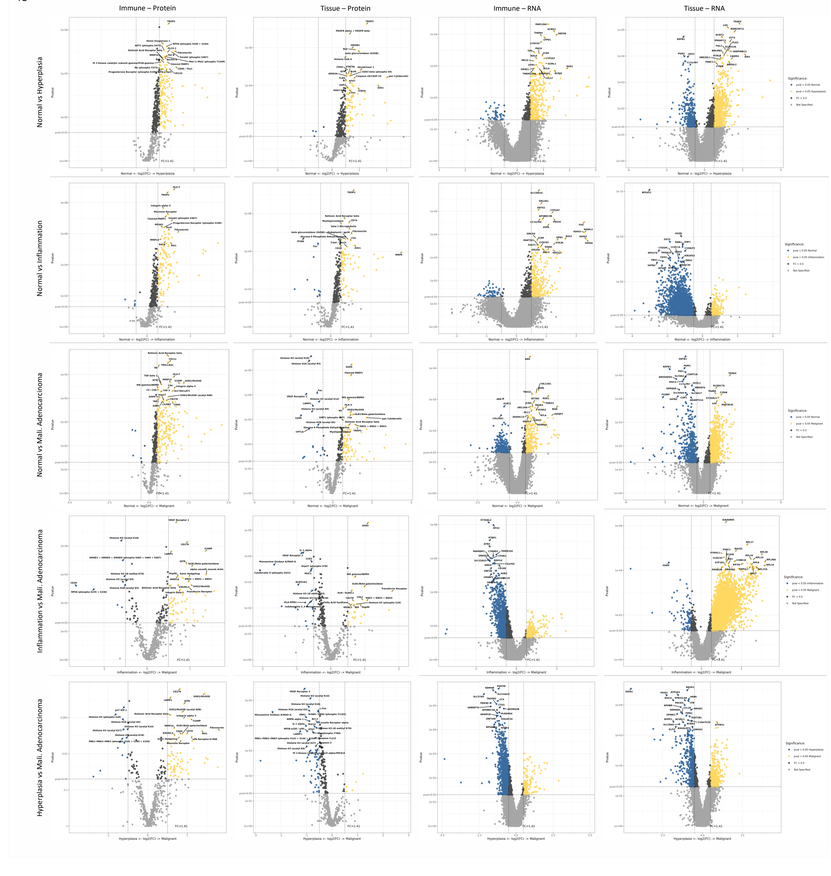
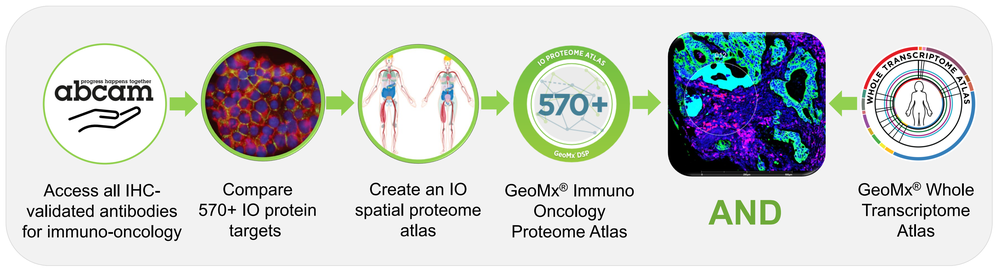
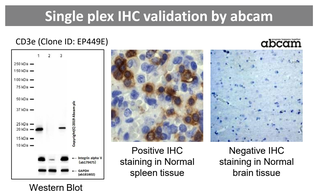


Figure 4: a) Samples were treated with the GeoMx Spatial Proteogenomic workflow. Samples were interrogated for RNA transcripts with the GeoMx Whole Transcriptome Atlas and a custom set of RNA probes. Samples were interrogated for Protein targets with the GeoMx Immuno Oncology Proteome Atlas and a custom set of Protein probes. **b)** Two FFPE colon tissue arrays, covering multiple human colon pathologies, were interrogated. Sample cohorts cover diverse colon tissue pathologies: normal tissue, hyperplasic tissue, chronically inflamed tissue, and malignant adenocarcinoma of colon tissue. Tissue was fluorescently stained for PanCK (green) and CD45 (magenta) proteins as well as nuclei (Syto13, blue). Tissue regions of interest were segmented into immune (red) and tissue (cyan). **c)** Expression of Cytokeratin 8, Cytokeratin 8 (phospho S431), and Cytokeratin 17 protein targets and expression of corresponding RNA genes: KRT18, KRT17, and KRT8 across colon pathology cohorts. **d)** Expression of immune cluster of differentiation (CD) protein targets and corresponding genes (RNA) across colon pathology cohorts. **e)** Differential expression of RNA or Protein analytes in segmented tissue or immune populations between pathology cohorts.

What if You Could…



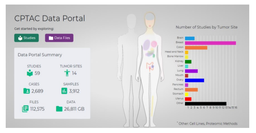
Single plex IHC validation by abcam

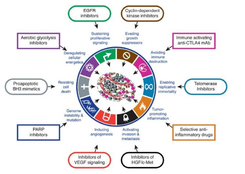


Example single plex antibody validation by abcam: Each antibody was evaluated for target specificity and compatibility with IHC workflow by abcam internal pathology and immunohistochemistry experts.

Designing an Immuno Oncology Proteome Atlas

Curated and validated IO Content
- Use Clinical Proteomic Tumor Analysis Consortium (CPTAC) data
- Focus on post-translational modifications
- Use high quality abcam antibodies
- Employ Immuno Oncology subject matter experts
- Human Specific
- 570+ Proteins, Mapped to 556 Unique Genes
- 77 Functional Annotations
- All Hallmarks of Cancer





AMPK Signaling	Epigenetic Modifications	Lipid Metabolism	Proteotoxic Stress	Complement System	HIF1 Signaling	NO Signaling	TH1 Differentiation
Androgen Signaling	ERBB2 Signaling	MET Signaling	Purinergic Signaling	Cytotoxicity	Hippo Signaling	Notch Signaling	TH17 Differentiation
Apoptosis	Estrogen Signaling	MHC I/II	RAGE Signaling	Differentiation	IDH1/2	NRF2 Signaling	TH2 Differentiation
Autophagy	FGFR Signaling	Mitochondrial Metabolism	Senescence	DNA Damage Repair	IL Signaling	Oxidative Stress	TLR Signaling
Cancer	FoxO Signaling	mTor Signaling	T Cell Exhaustion	Drug Resistance	Immortality & Stemness	P53 Signaling	TNF Signaling
Cell Adhesion	Glutamine Metabolism	Myc	T-Cell Checkpoints	EGFR Signaling	Insulin Signaling	P13-Akt Signaling	Treg Differentiation
Cell Cycle	Glycolysis-Glucose Transport	Myeloid Inflammation	TCR Signaling	EMT	Interferon Response	PDGF Signaling	Type 1 & 2 Interferon Signalling
Chemokine Signaling	Hedgehog Signaling	NF-kB Signaling	TGF-Beta Signaling	Endocytosis	JAK-STAT Signaling	Prostoglandin Inflammation	VEGF Signaling
							Wnt Signaling

GeoMx Immuno Oncology Proteome Atlas Functional Pathways

GeoMx Immuno Oncology Proteome Atlas Public Dataset
Visit
GeoMx® IO Proteome Atlas | NanoString

Multi-plex Validation: Sensitivity and Specificity

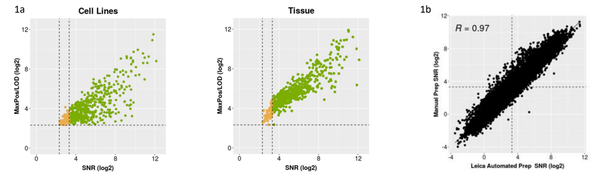
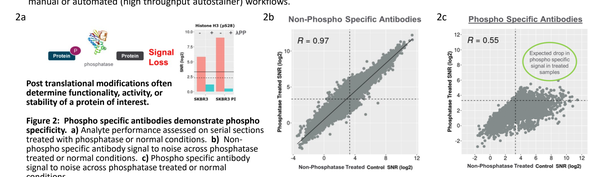


Figure 1: GeoMx Immuno Oncology Proteome Atlas (IPA) analytes demonstrate robust specificity and sensitivity and performs equivalently with either manual or automated sample prep. a) Target sensitivity and specificity performance measured across homogenous cell lines and cancer and normal tissues. **b)** GeoMx IPA analyte performance assessed on serial sections prepared with manual or automated (high throughput autostainer) workflows.



Post translational modifications often determine functionality, activity, or stability of a protein of interest.

Figure 2: Phospho specific antibodies demonstrate phospho specificity. a) Analyte performance assessed on serial sections treated with phosphatase or normal conditions. **b)** Non-phospho specific antibody signal to noise across phosphatase treated or normal conditions. **c)** Phospho specific antibody signal to noise across phosphatase treated or normal conditions.

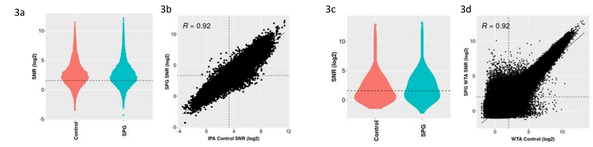
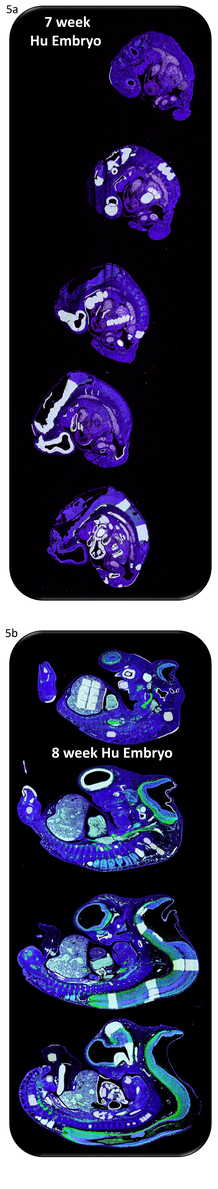


Figure 3: GeoMx Immuno Oncology Proteome Atlas performs equivalently in standalone and spatial proteogenomic workflows and has no effect on GeoMx Whole Transcriptome Atlas performance. a) Violin plots of $\log_2$ signal to noise ratio of IPA analytes (protein) across cell line array serial sections in a standalone or spatial proteogenomic (with WTA (RNA)) assay configuration. **b)** Pearson correlation of $\log_2$ signal to noise ratio of IPA analytes (protein) across cell line array serial sections in a standalone or spatial proteogenomic (with WTA (RNA)) assay configuration. **c)** Violin plots of $\log_2$ signal to noise ratio of WTA analytes (RNA) across cell line array serial sections in a standalone or spatial proteogenomic (with IPA (protein)) assay configuration. **d)** Pearson correlation of $\log_2$ signal to noise ratio of WTA analytes (RNA) across cell line array serial sections in a standalone or spatial proteogenomic (with IPA (protein)) assay configuration.

Spatial Proteogenomics on 7 and 8 week Human Embryo shows distinct temporal organ differentiation



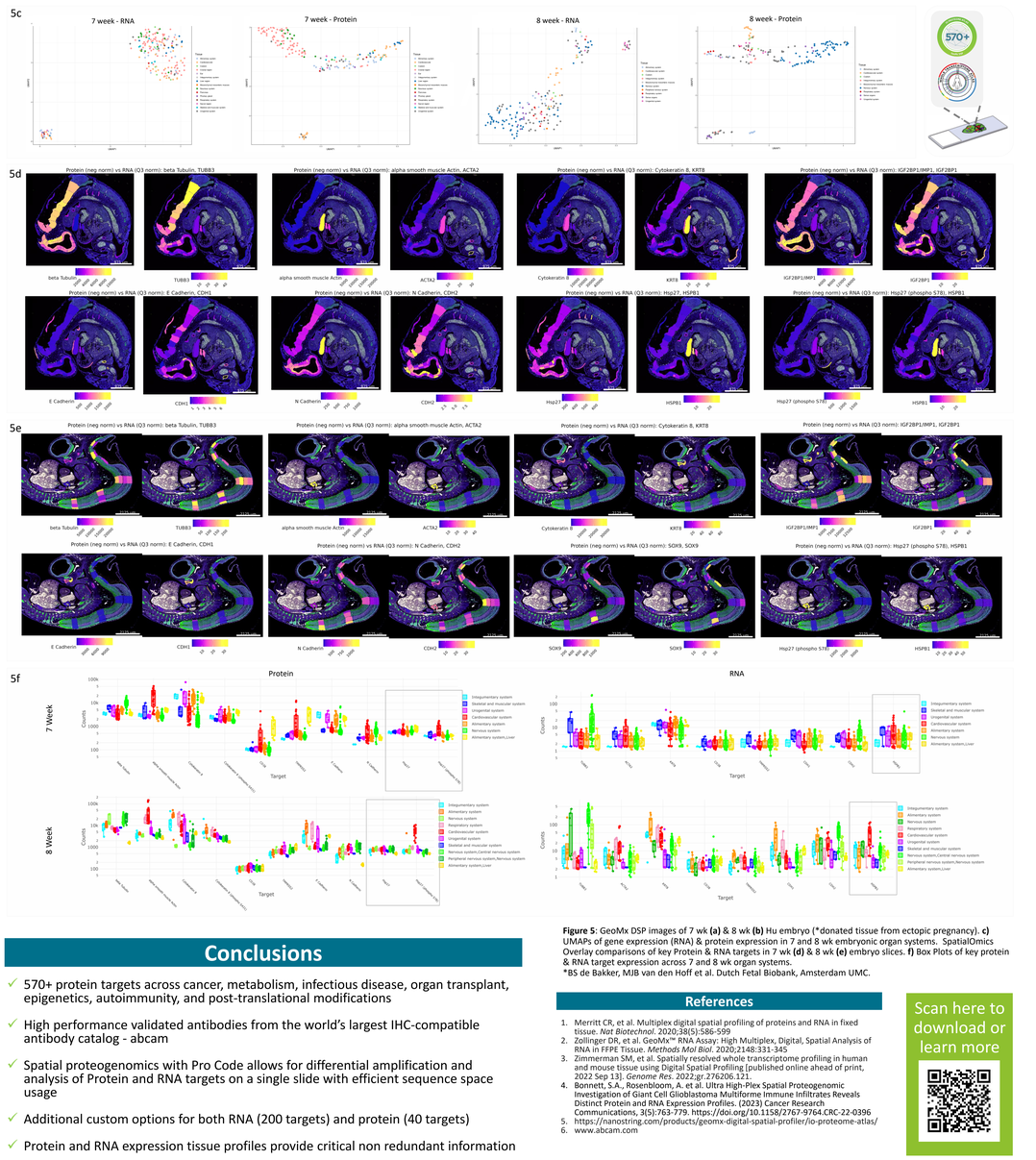


Figure 5: GeoMx DSP images of 7 wk **(a)** & 8 wk **(b)** Hu embryo (*donated tissue from ectopic pregnancy). **c)** UMAPs of gene expression (RNA) & protein expression in 7 and 8 wk embryonic organ systems. SpatialOmics Overlay comparisons of key Protein & RNA targets in 7 wk **(d)** & 8 wk **(e)** embryo slices. **f)** Box Plots of key protein & RNA target expression across 7 and 8 wk organ systems.
*BS de Bakker, MJB van den Hoff et al. Dutch Fetal Biobank, Amsterdam UMC.

Conclusions

- ✓ 570+ protein targets across cancer, metabolism, infectious disease, organ transplant, epigenetics, autoimmunity, and post-translational modifications
- ✓ High performance validated antibodies from the world's largest IHC-compatible antibody catalog - abcam
- ✓ Spatial proteogenomics with Pro Code allows for differential amplification and analysis of Protein and RNA targets on a single slide with efficient sequence space usage
- ✓ Additional custom options for both RNA (200 targets) and protein (40 targets)
- ✓ Protein and RNA expression tissue profiles provide critical non redundant information

References

1. Merritt CR, et al. Multiplex digital spatial profiling of proteins and RNA in fixed tissue. *Nat Biotechnol*. 2020;38(5):586-599
2. Zollinger DR, et al. GeoMx™ RNA Assay: High Multiplex, Digital, Spatial Analysis of RNA in FFPE Tissue. *Methods Mol Biol*. 2020;2148:331-345
3. Zimmerman SM, et al. Spatially resolved whole transcriptome profiling in human and mouse tissue using Digital Spatial Profiling [published online ahead of print, 2022 Sep 13]. *Genome Res*. 2022;gr.276206.121.
4. Bonnett, S.A., Rosenbloom, A. et al. Ultra High-Plex Spatial Proteogenomic Investigation of Giant Cell Glioblastoma Multiforme Immune Infiltrates Reveals Distinct Protein and RNA Expression Profiles. (2023) Cancer Research Communications, 3(5):763-779. https://doi.org/10.1158/2767-9764.CRC-22-0396
5. https://nanostring.com/products/geomx-digital-spatial-profiler/io-proteome-atlas/
6. www.abcam.com

Scan here to download or learn more

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.
www.nanostring.com | info@nanostring.com
© 2024 NanoString Technologies, Inc.