Spatially Resolved Transcriptomics with the GeoMx Digital Spatial Profiler

Tony Zucca MSc
GeoMx DSP Technical Sales Specialist

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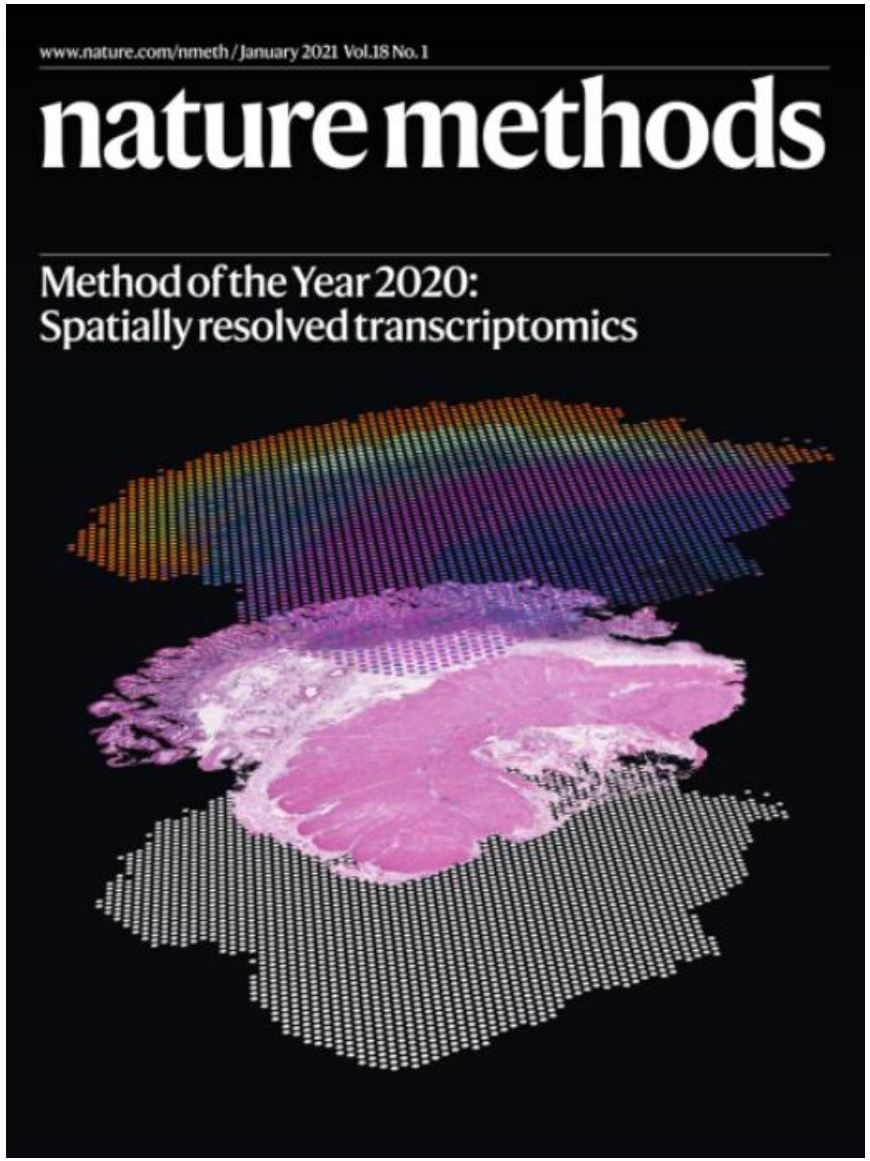

Editorial | Published: 06 January 2021

Method of the Year 2020: spatially resolved transcriptomics

Nature Methods **18**, 1(2021) | Cite this article

16k Accesses | **220** Altmetric | Metrics

Spatially resolved transcriptomics methods are changing the way we understand complex tissues.

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Tools for Biomarker Discovery and Translational Research

Founded: 2003
Headquartered: Seattle, WA

3,200 Publications To Date

GeoMx™ Digital Spatial Profiler
Launched 2019

nCounter® Analysis System
Launched: 2008

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Continued Innovation With New Platform: Spatial Molecular Imager (SMI) H2 2022 launch

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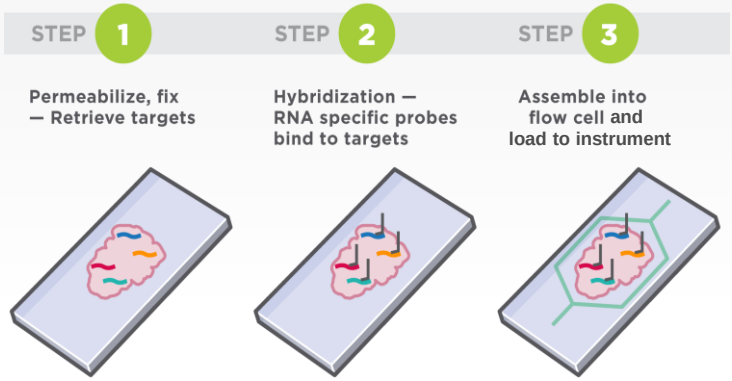
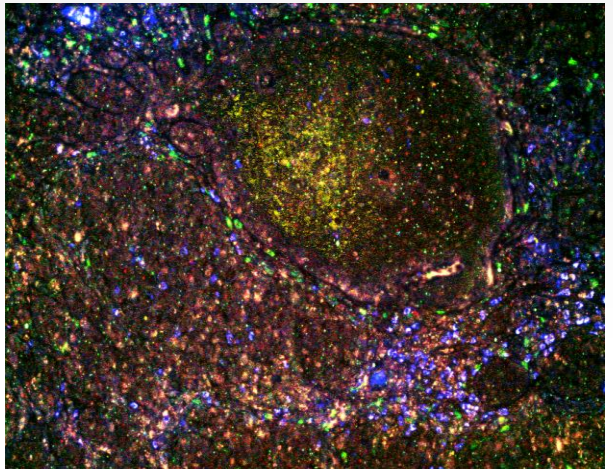
New Platform: Spatial Molecular Imager (SMI) 2022 launch

Easy Sample Preparation



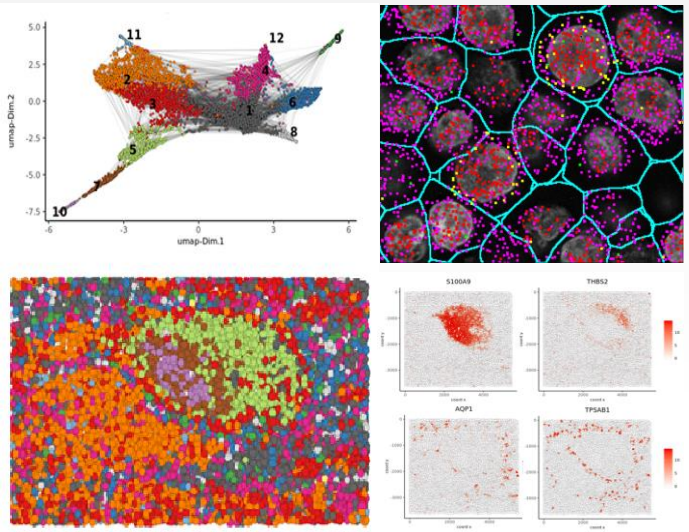
Standard ISH processing steps with ~1 hour hands on time. Works with FFPE, Fresh Frozen, Organoid

Integrated Readout



Fully automated cyclic imaging chemistry with integrated readout (*No other Instrument Required)*

Interactive Data Analysis



Big data from thousands of single cells, biological interpretation and interactive visualization

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New Platform: Spatial Molecular Imager (SMI) 2022 launch

- **HIGH RESOLUTION** – subcellular with 3-Dimensional resolution
- **LARGE PANEL** – demonstrated up to 1,000-plex and increasing
- **HIGH SENSITIVITY** – accurately detect low copy number genes
- **ANALYZE RNA and PROTEIN** with any sample type (FFPE, Fresh frozen, Organoid)

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New Platform: Spatial Molecular Imager (SMI) 2022 launch

- Evan Newell, Fred Hutchinson Cancer Research Center **"In-situ visualization and measurement of tumor infiltrating TCR clones on intact FFPE renal cell carcinoma (RCC) tissue using spatial molecular imager“**
- Matthew Freedman, Dana-Farber Cancer Institute **"Highly sensitive transcriptomic-based pooled CRISPR screening enabled by spatial molecular imager“**
- Erin Piazza, NanoString Technologies, Inc. **"Mapping cell type, cell state, and cell-cell interactions with 1000-plex single cell gene expression assay using spatial molecular imaging“**
- Patrick Danaher, NanoString Technologies, Inc. **"Unsupervised discovery of tissue substructures using spatial molecular imaging of gene expression"**

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Why High-plex Digital Spatial Profiling

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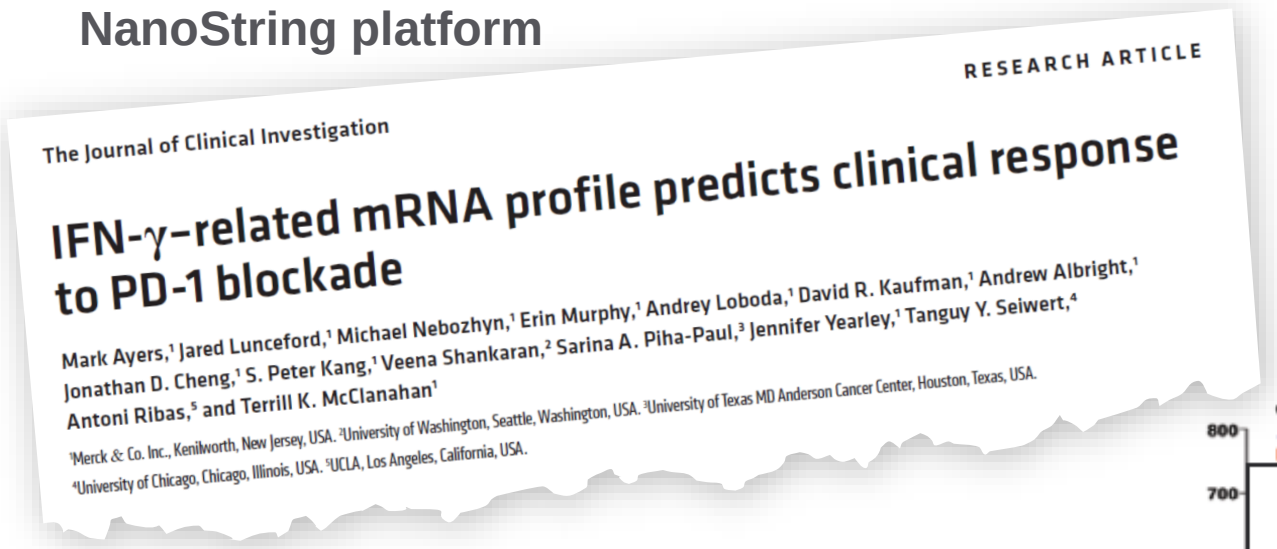
Gene Expression Signatures Improve Patient Selection

NanoString + Merck collaboration: discovered & developed using NanoString platform



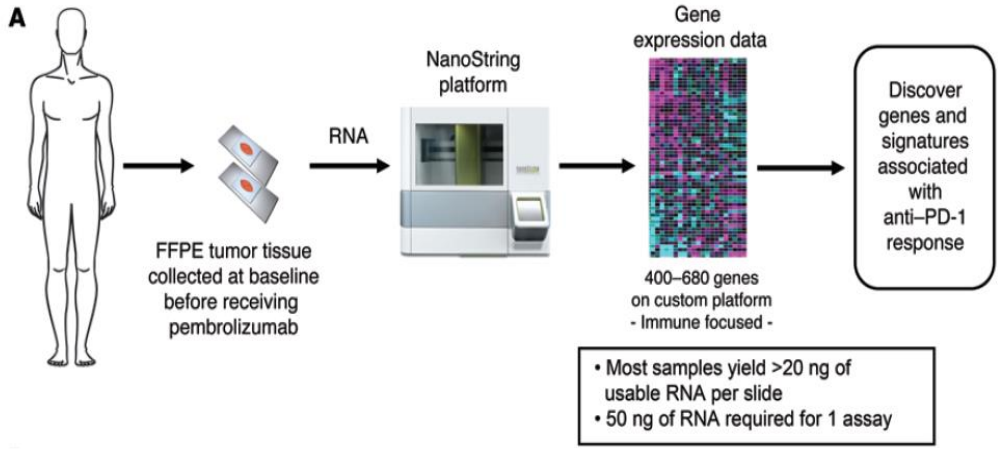
RESEARCH ARTICLE

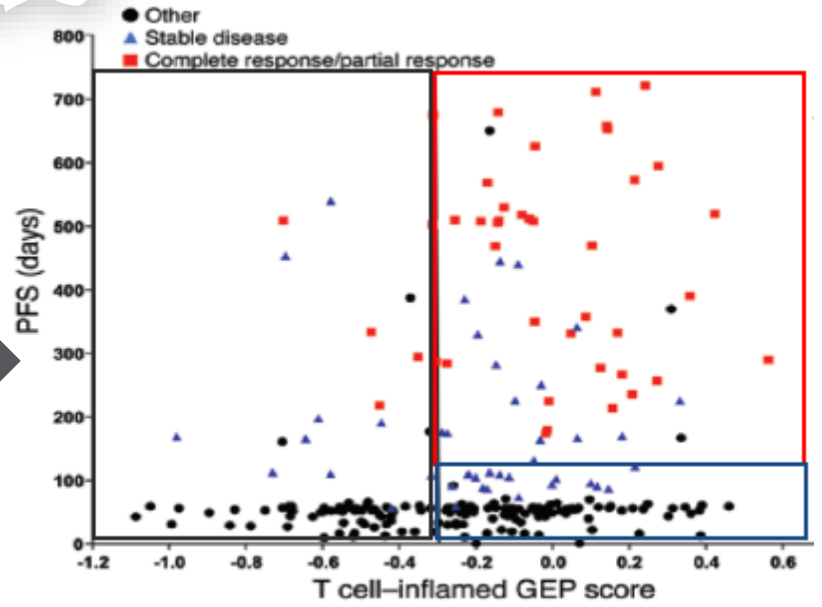
The Journal of Clinical Investigation

IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade

Mark Ayers,[1] Jared Lunceford,[1] Michael Nebozhyn,[1] Erin Murphy,[1] Andrey Loboda,[1] David R. Kaufman,[1] Andrew Albright,[1] Jonathan D. Cheng,[1] S. Peter Kang,[1] Veena Shankaran,[2] Sarina A. Piha-Paul,[3] Jennifer Yearley,[1] Tanguy Y. Seiwert,[4] Antoni Ribas,[5] and Terrill K. McClanahan[1]

[1]Merck & Co. Inc., Kenilworth, New Jersey, USA. [2]University of Washington, Seattle, Washington, USA. [3]University of Texas MD Anderson Cancer Center, Houston, Texas, USA. [4]University of Chicago, Chicago, Illinois, USA. [5]UCLA, Los Angeles, California, USA.





Non-inflamed tumors **rarely respond**

Almost all responses have T-cell inflamed tumors

Not all inflamed tumors respond

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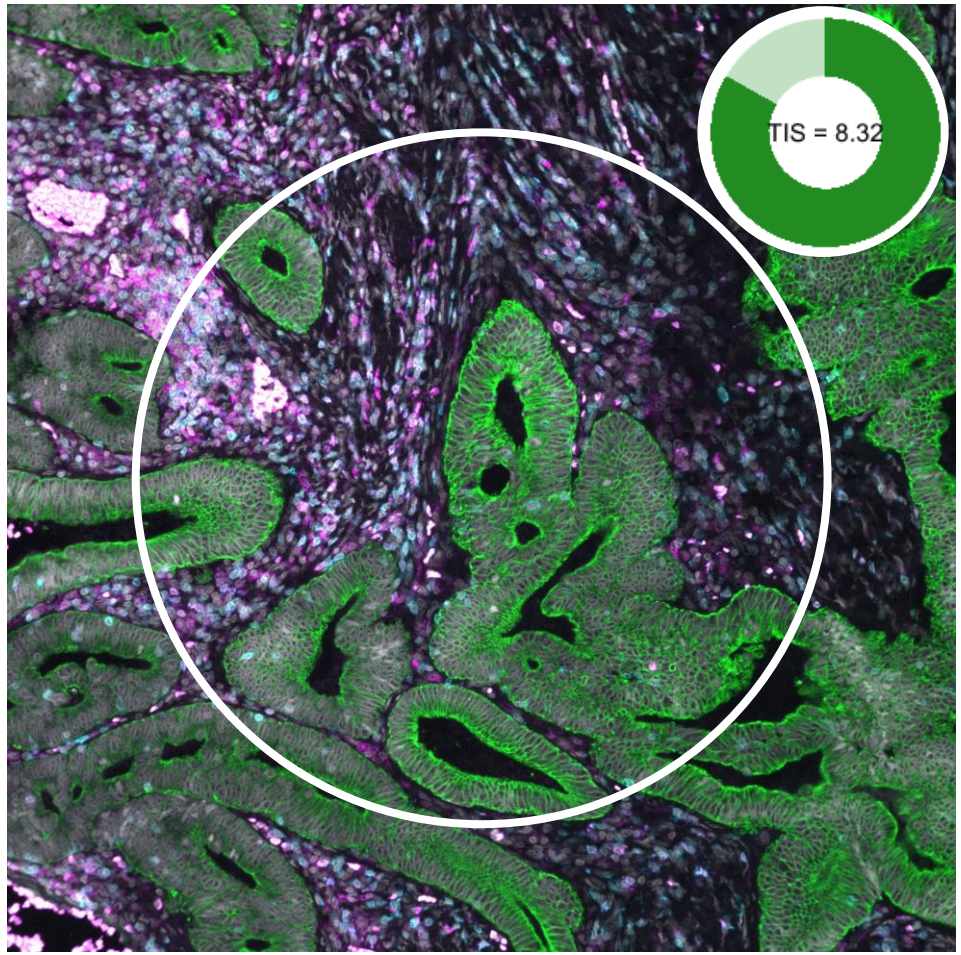
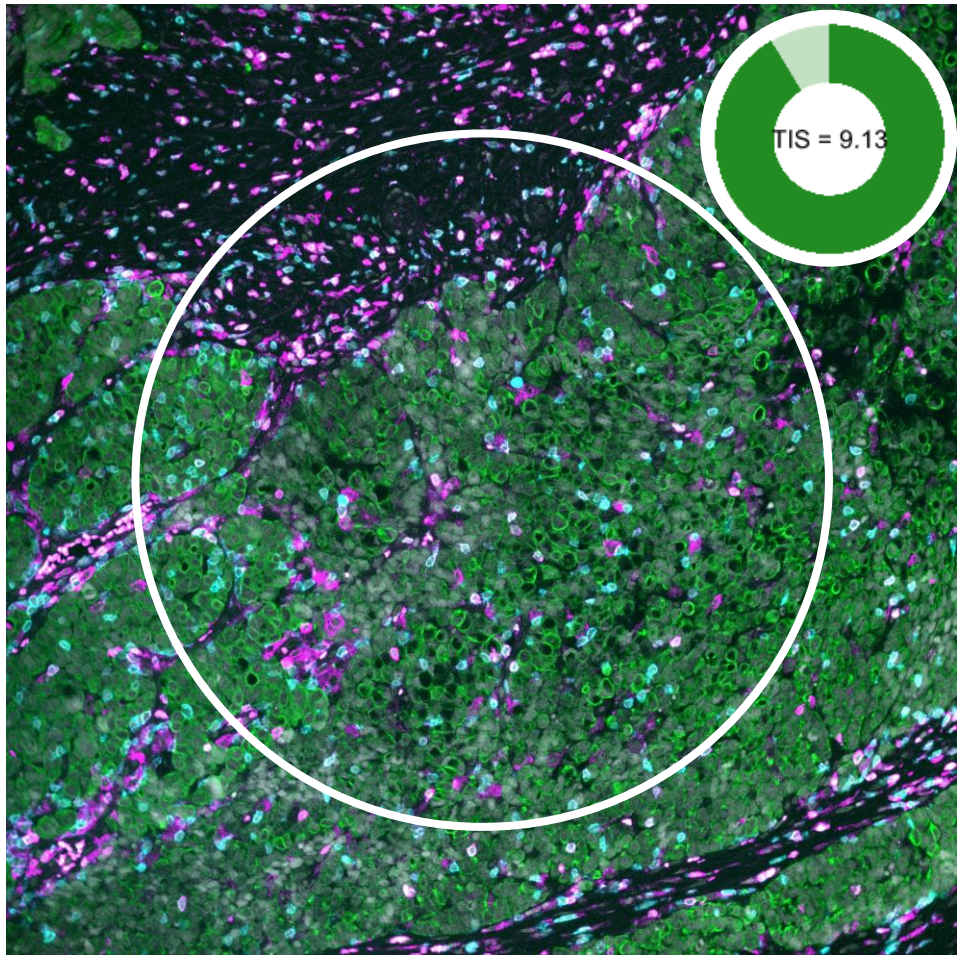
Biology that is Spatial Cannot be Resolved by Bulk or Single Cell Analysis

Hot but Excluded Tumor

Highly Infiltrated Tumor

Pan-CK
CD45
CD3
Syto13





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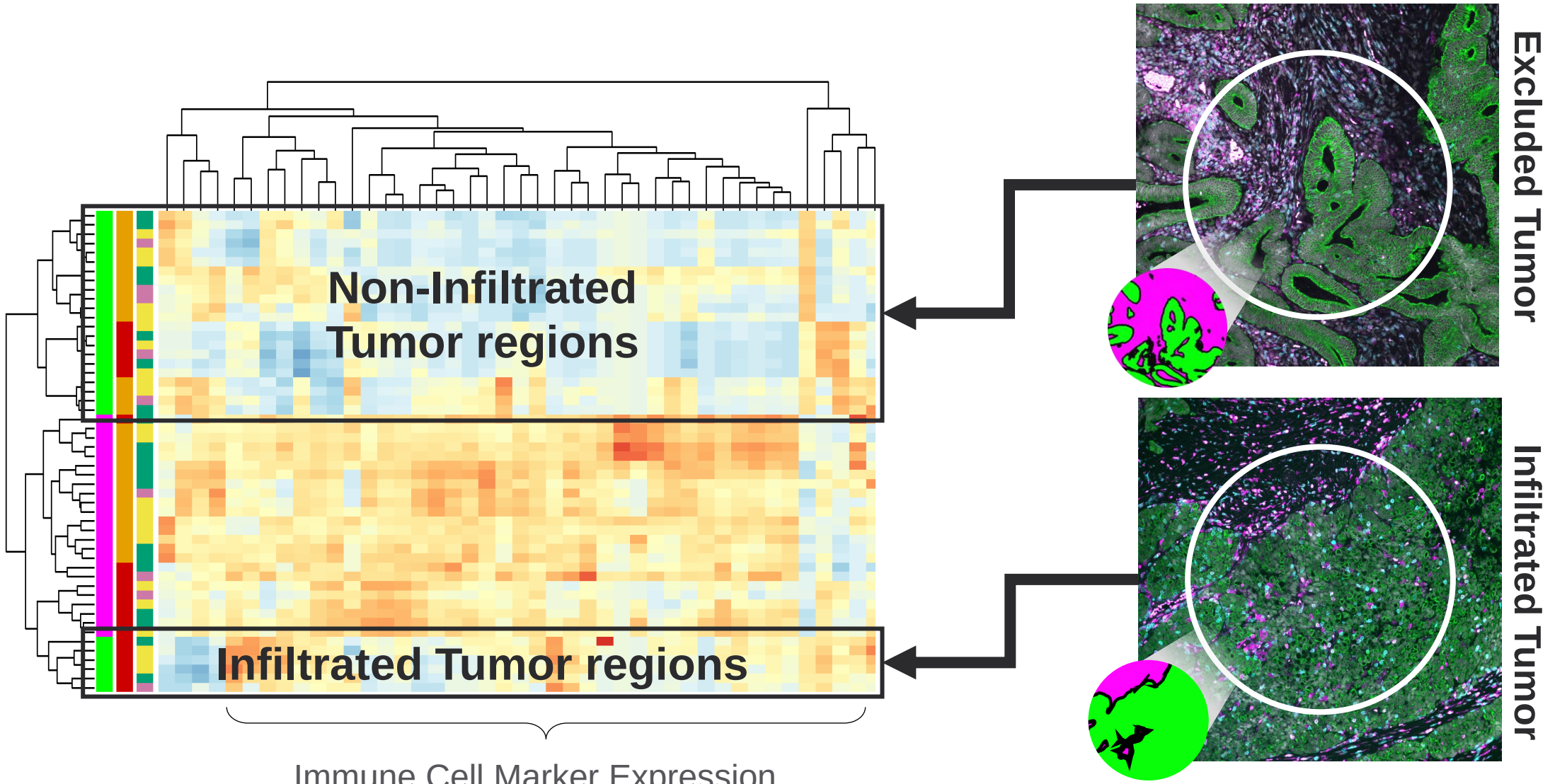
GeoMx DSP Profiling Identifies and Dissects Cells in Infiltrated Tumors



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About the GeoMx Digital Spatial Profiler (DSP)

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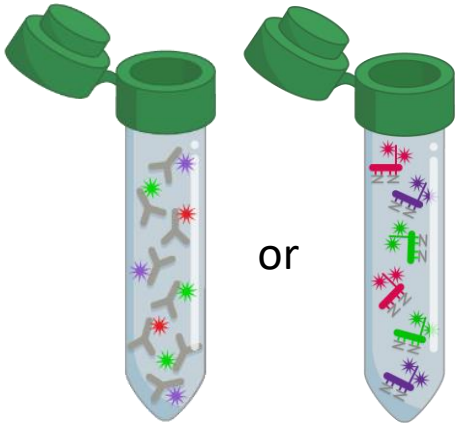
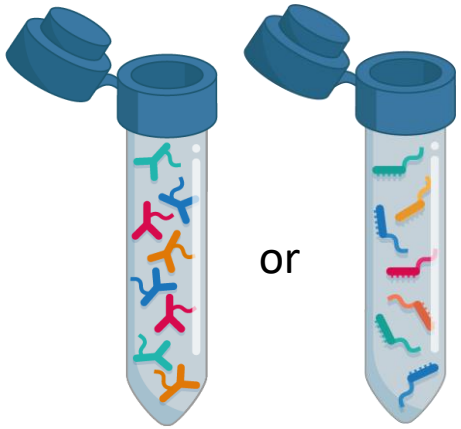
Imaging and Profiling in One Assay



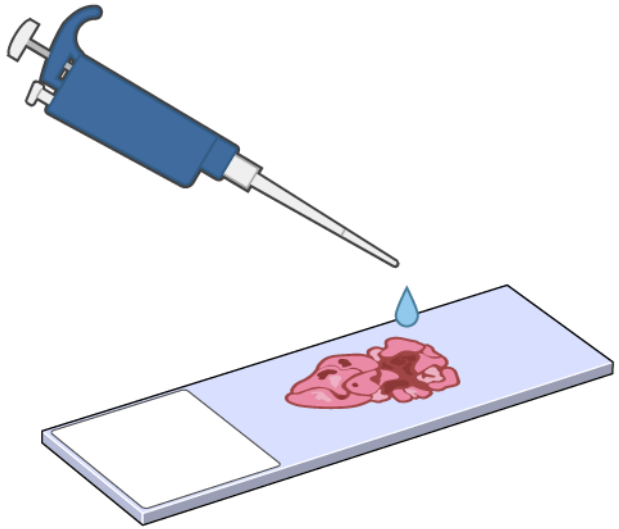
or

Imaging Reagents (Morphology Markers)
(Up to 4 colors)

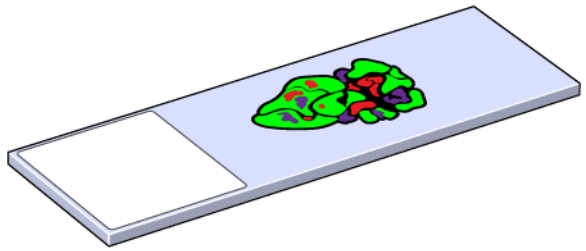


or

Profiling Reagents
(High Plex)



Sample Preparation*



DSP Ready

*Can be automated on Leica Bond Systems

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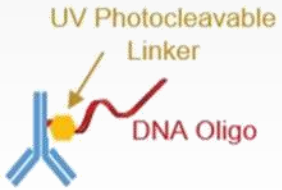
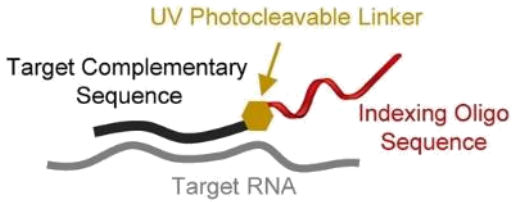
GeoMx™ DSP Enables Spatial, High-Plex Protein & RNA Profiling

High-Plex Mixtures of Proprietary Reagents

Protein reagents
Oligo-labeled antibodies



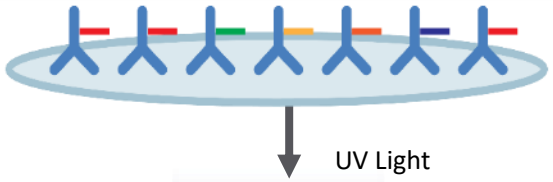
RNA reagents
Oligo-labeled probe



Profile Regions of Interest on FFPE slide

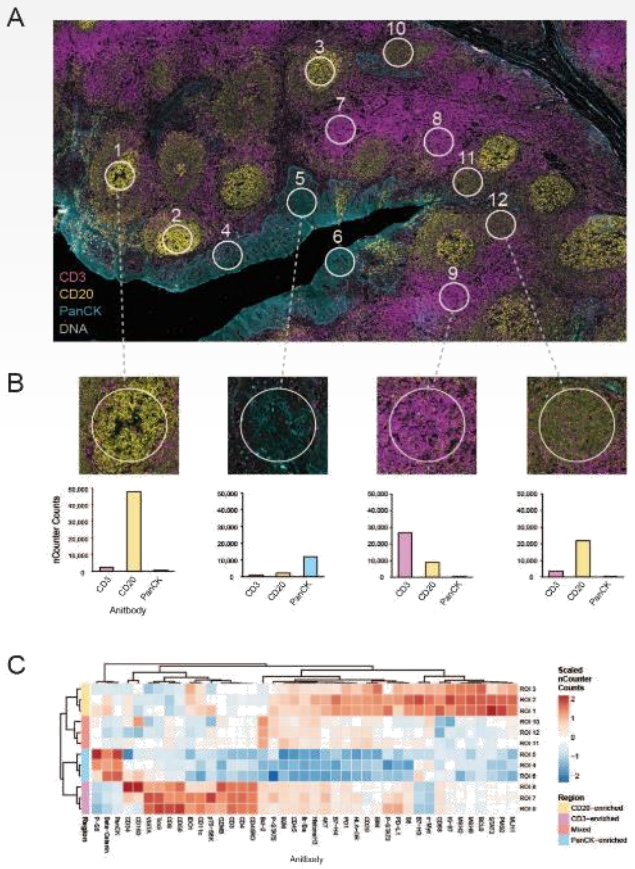
GeoMx™ Digital Spatial Profiler

Label FFPE Slide with Probe Mix



Illuminate Region of Interest, as Small as a Single Cell

Rich Data Sets of Biology, Region by Region



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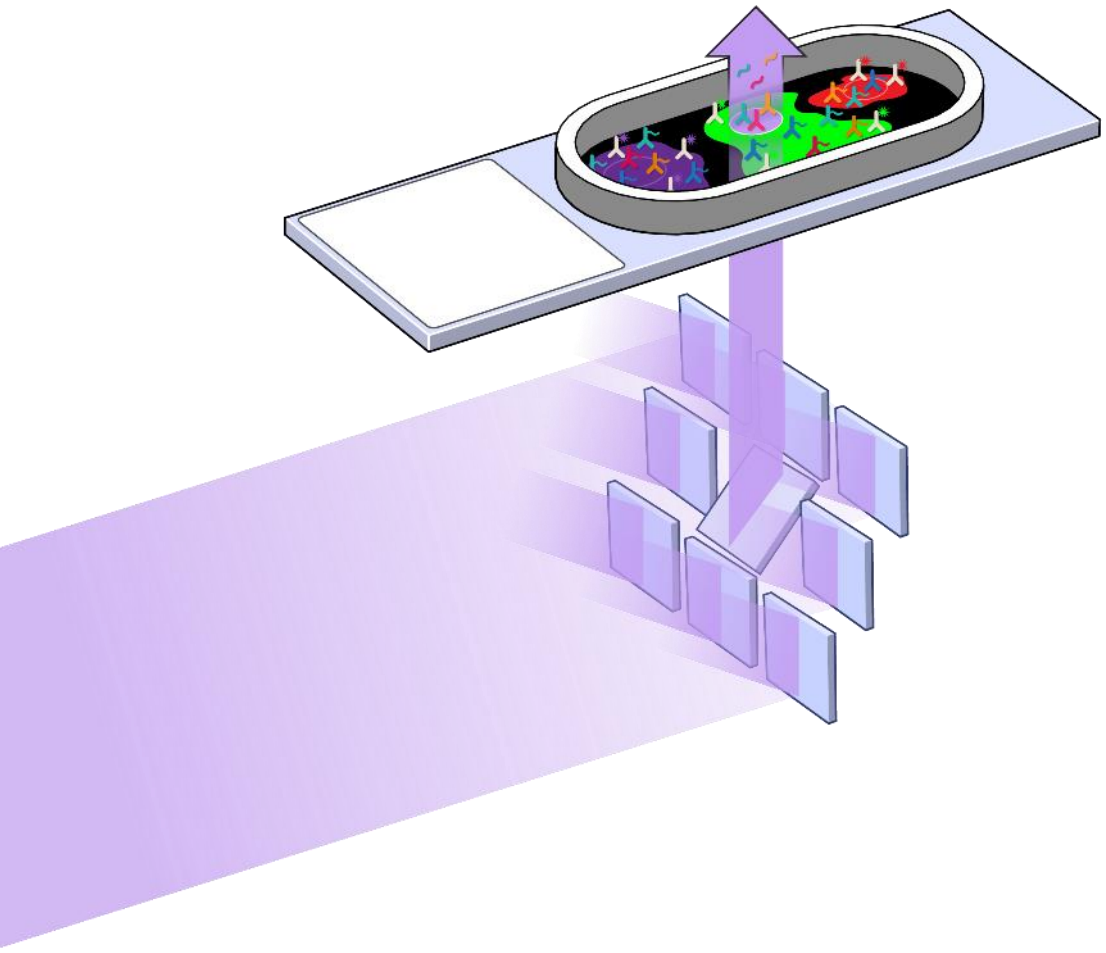
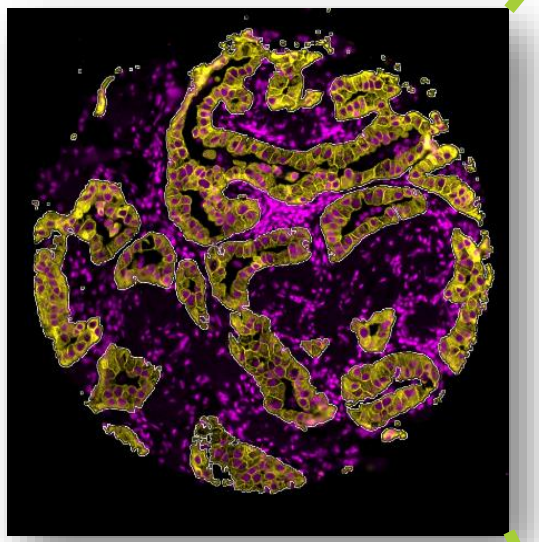
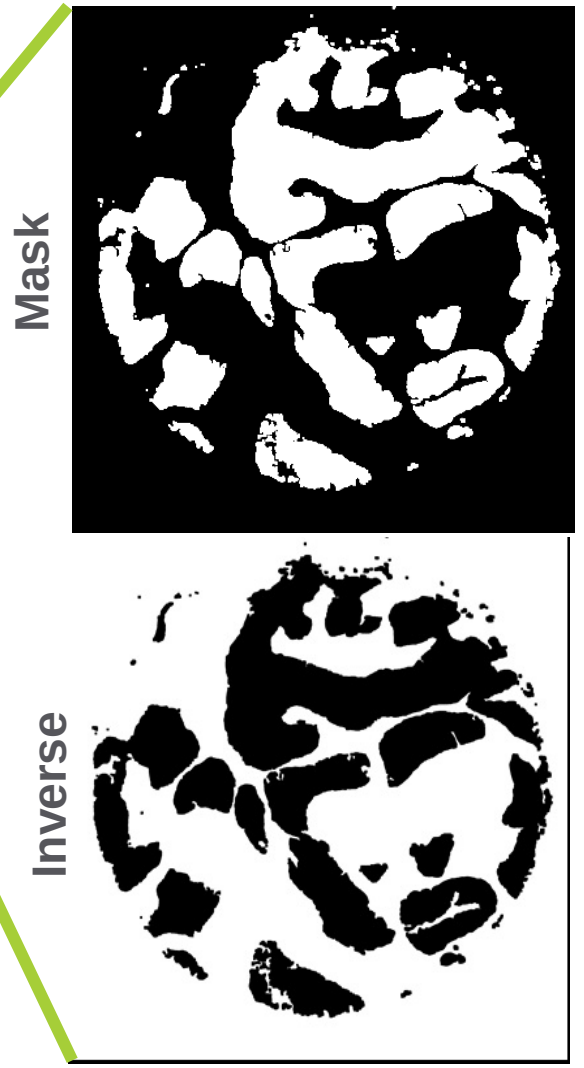
Optical Technology Enables Biologically Precise ROI Selection







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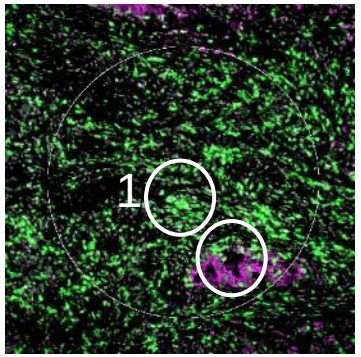
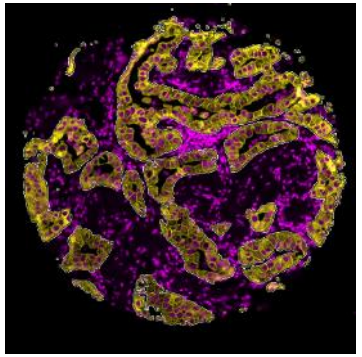
Five Unique Profiling Modalities Designed to Interrogate Tissue Samples

Geometric



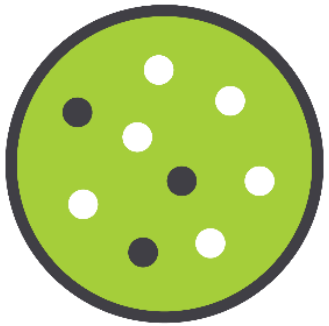
What is the heterogeneity of expression in different regions of my tissue?

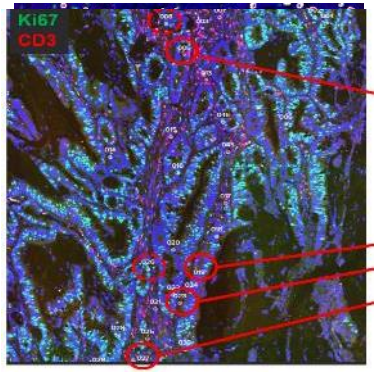
Segmentation



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

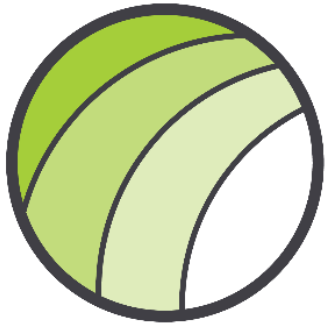
Cell-type Specific

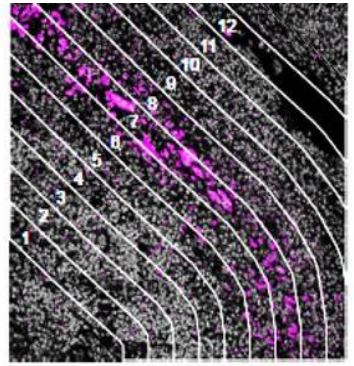




What is the expression profile of a specific cell population in my tissue?

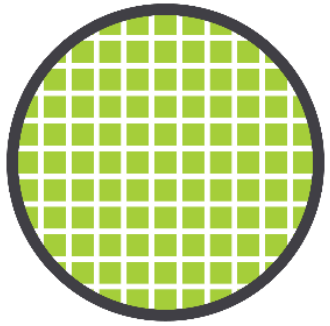
Contour

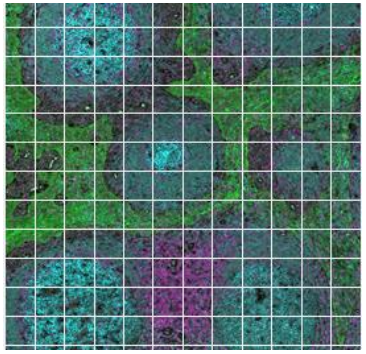




How does the immune environment change on either side of an infiltrate boundary?

Gridded





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

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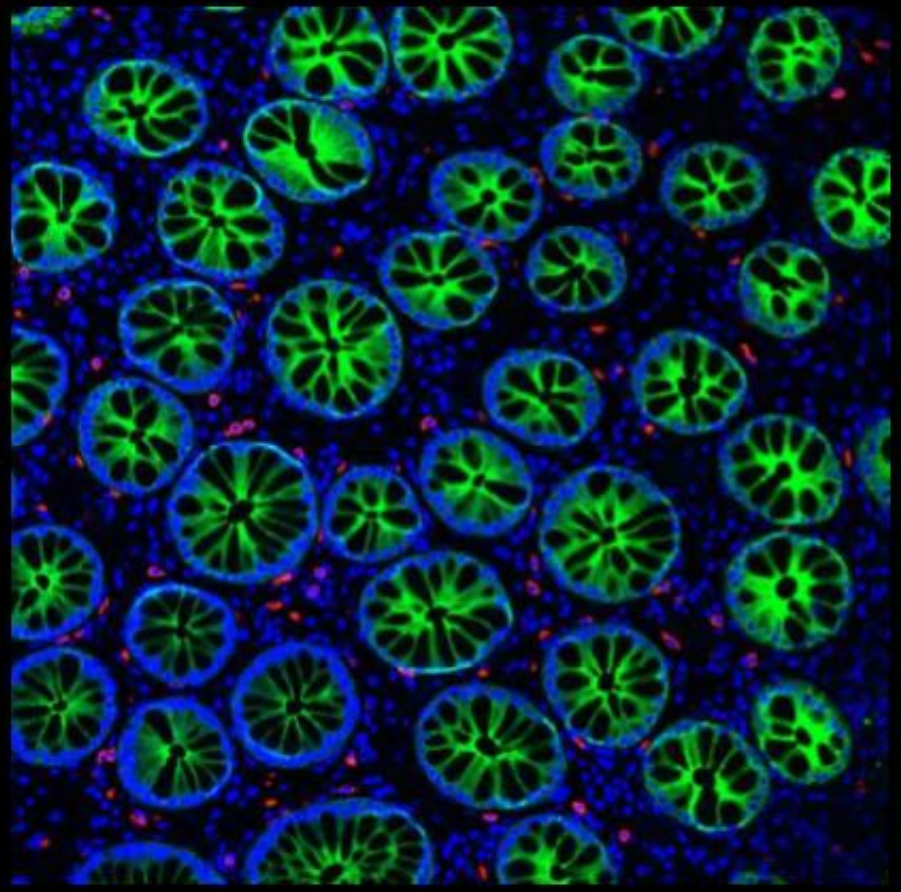
Rare-Cell Profiling: Tissue Image (Left), Automated Digital-Mirror Light-Profile (right)



Inflammatory Bowel Disease: Blue = nuclear stain, Green = PanCytokeratin, Red = Tryptase, Mast-Cell Marker

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GeoMx Digital Spatial Profiling enables high-parameter Protein and RNA spatial profiling of FFPE and FF

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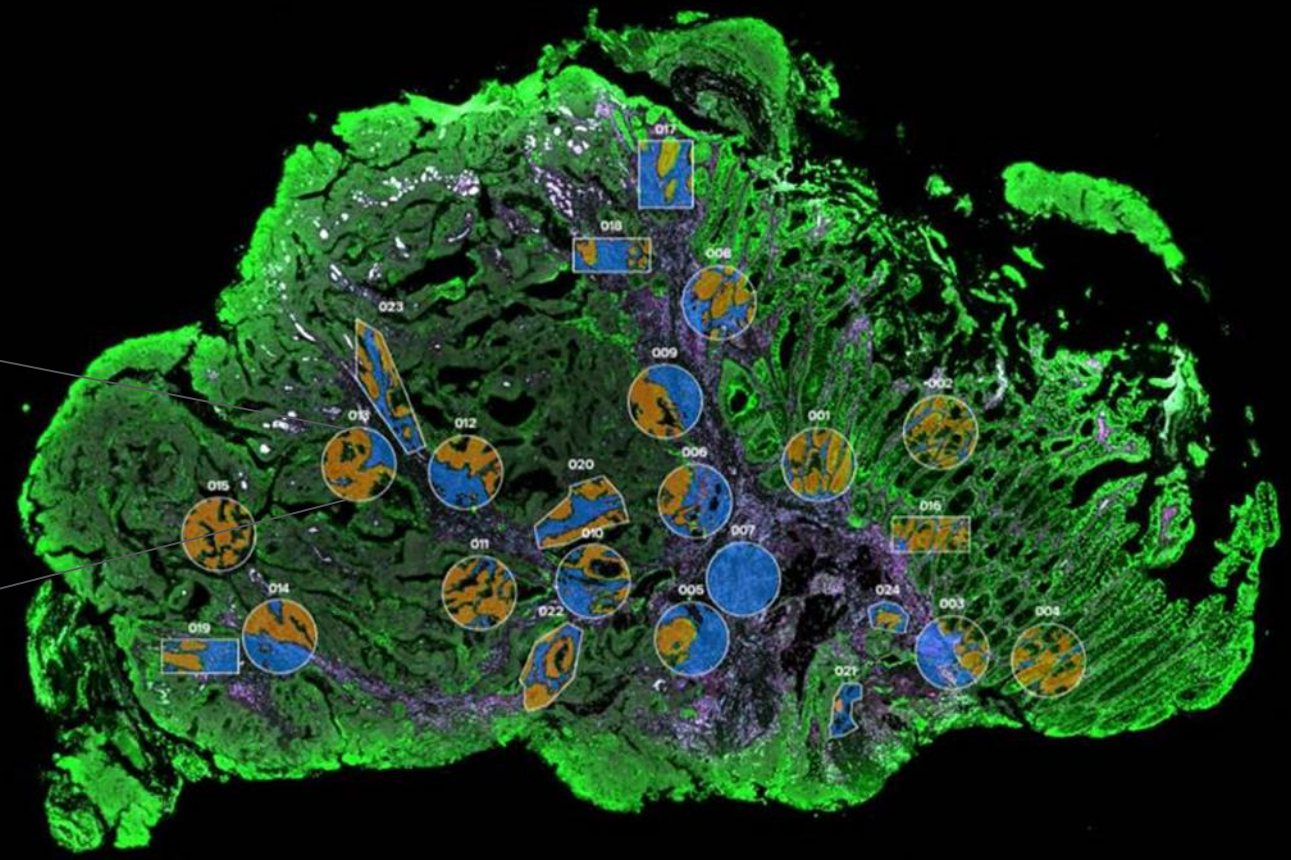
GeoMx Digital Spatial Profiling enables high-parameter Protein and RNA spatial profiling of FFPE and FF

4-1BB, CD11c, CD14, CD163, CD19, CD27, CD3, CD56, CD66b, CD68, CD8A, CD20, CD3, CD4, CD45, CD45RO, GZMB, HLA-DR, Histone H3, ICOS, IDO1, Ki-67, LAG3, OX40L, PD1, PD-L1, PTEN, STAT3, STAT3-P, VISTA, B7-H3 (CD276), B7-H4, Bcl-2, Beta-2-microglobulin, Beta-Catenin, CD44, Pan-Cytokeratin, GZMB, Ki67, S100B, S6, STING



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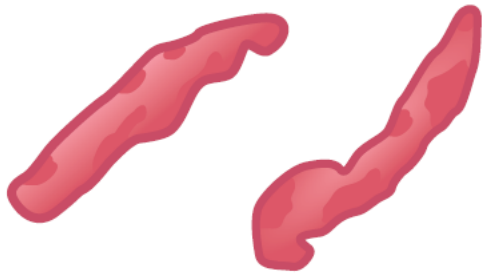
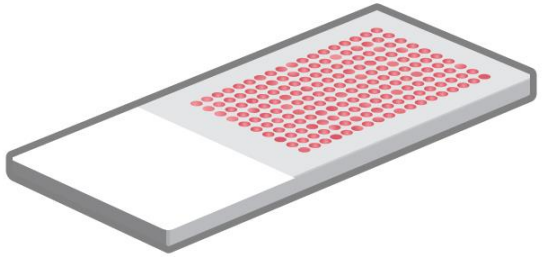
Analyze Any Sample Types with Selection Based on Experimental Design, Not Technology

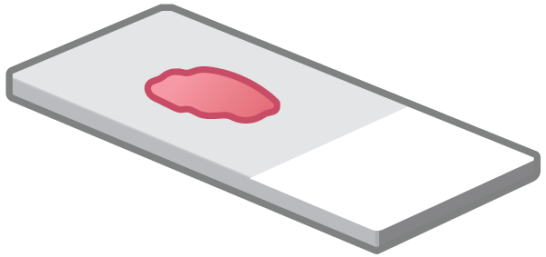
Core Needle Biopsy



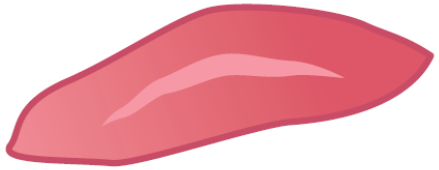
FFPE Tissue Block



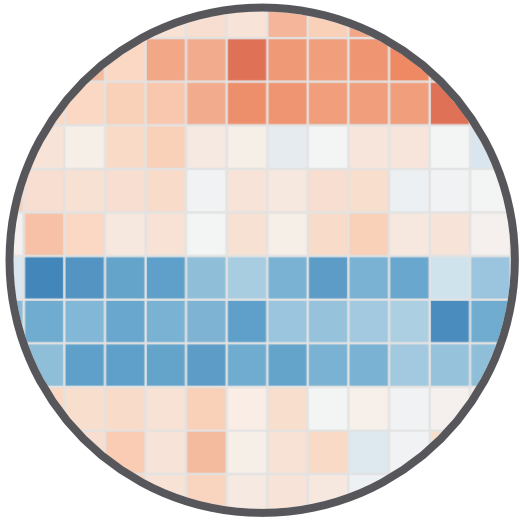
Tissue Microarray



Slide Mounted Tissue



Fresh Frozen Tissue



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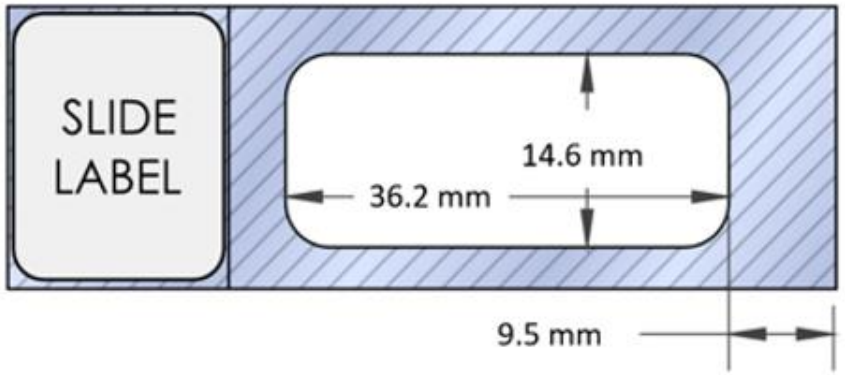
Large Area & Standard Slide for Ease of Use

Sample Guidelines

- 4 µm-6 µm unstained sections mounted on adhesive/positively charged slides are required, e.g., Superfrost Plus; Leica X-tra-adhesive (Cat#: 3800050). For TMA, bone marrow tissue and mRNA DSP samples, Leica Bond plus slides (Cat# S21.2113.A) are recommended.
- For mRNA DSP samples, blocks under 3 years are preferred, and fresh-cut sections stored at 4°C in a desiccator for short term storage (2 weeks) is recommended.
- Ideally, tissue sections should be placed in the center of the slide and be no larger than 36.2 mm wide by 14.6 mm high. If sections are larger than this size or placed off center, it is possible that the tissue located in the blue area cannot be measured.



- Complete the Customer Information and Project Information sections below.
- Populate the sample manifest (see FFPE Information tab below) or provide an alternate sample manifest with your order.
- Ship the contents of the order at room temperature.

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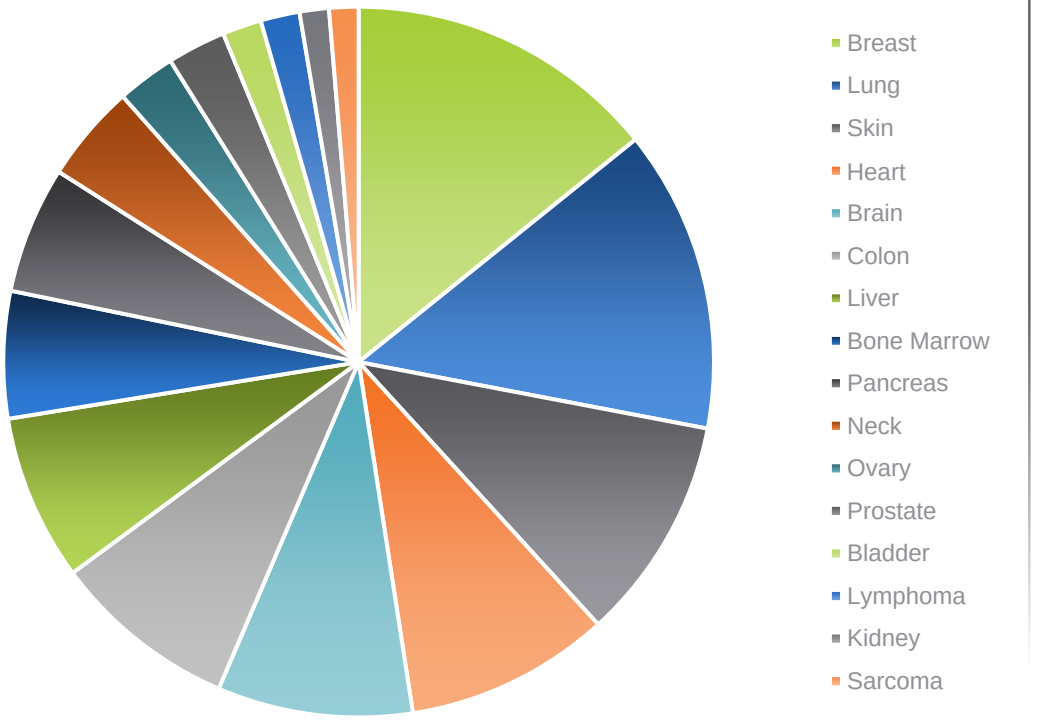
Trust ALL Data

Robust Chemistry Validated by Multiple Labs Drives Rapid Publications

>2,000+ DSP Technology Access Program samples run since November 2016

40+ publications since November 2018

> 30 Tissue Types Studied



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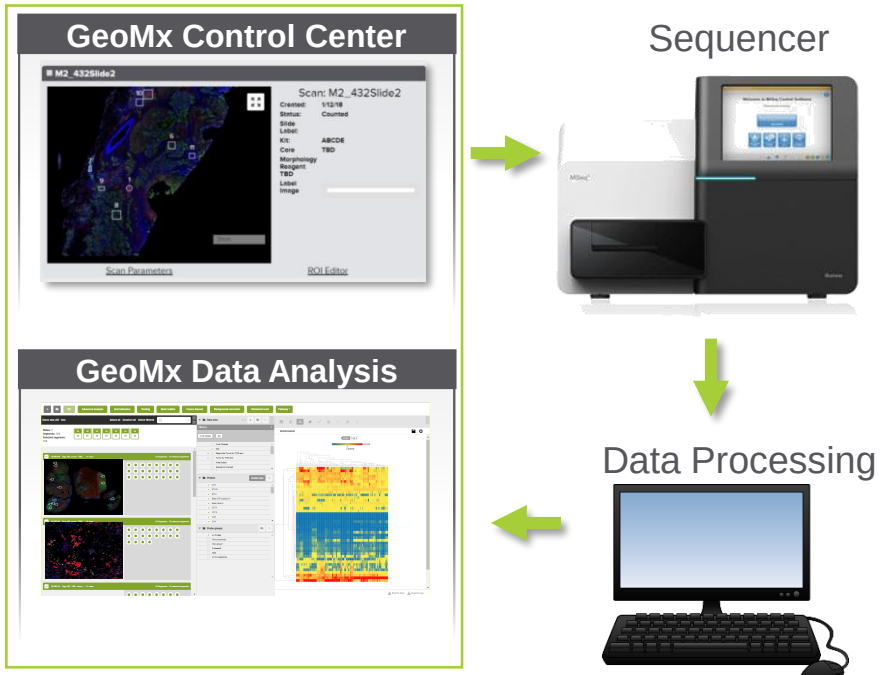
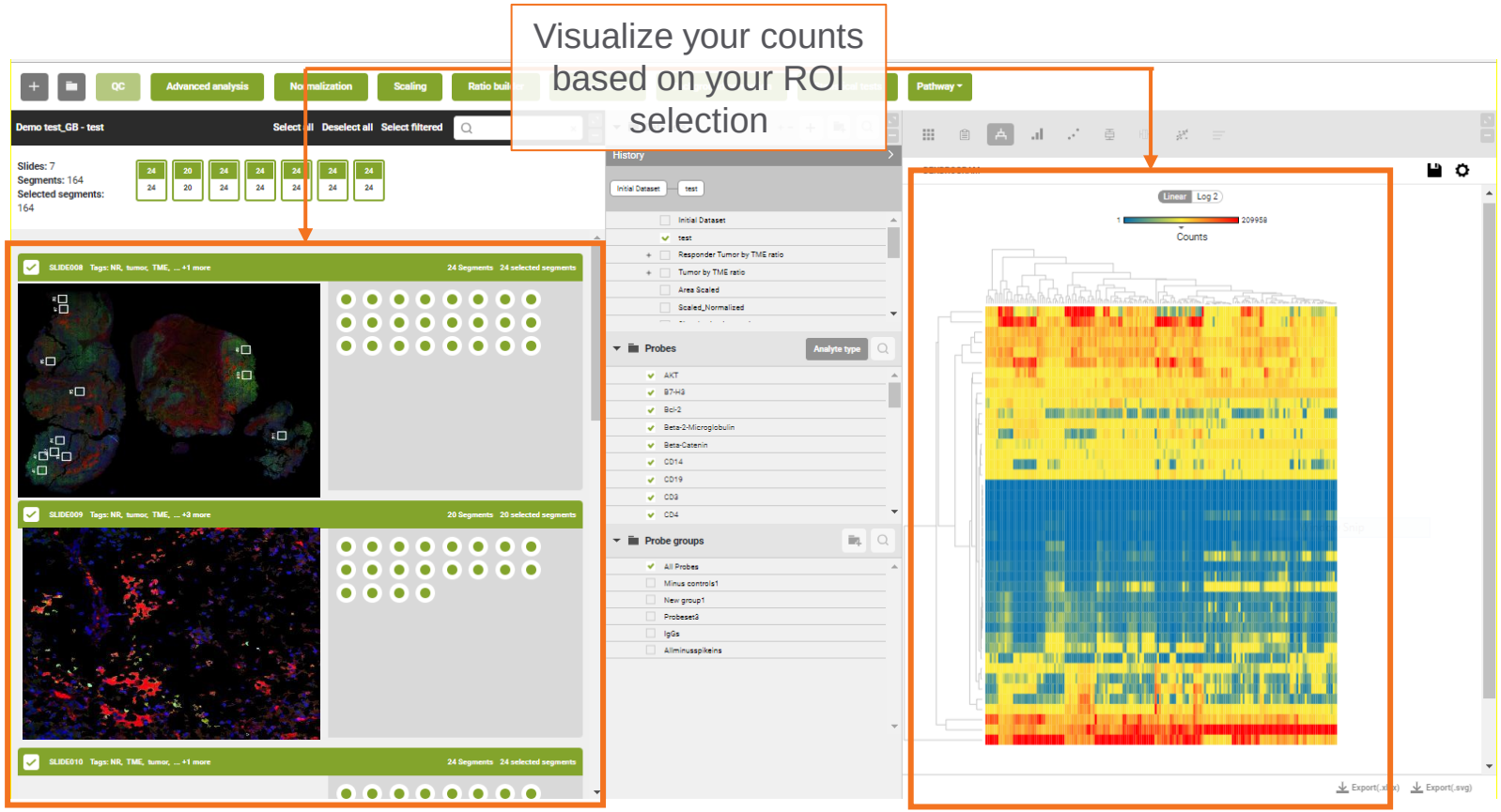
Plug-and-play Data Analysis Suite

Integrative data processing tools with existing NGS workflow



Interactive data analysis suite that connects quantitative data to spatial context



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Protein and RNA Panels

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Flexible & Validated Content Designed to Fit a Range of Applications & Plex Needs

Protein Assays for nCounter®

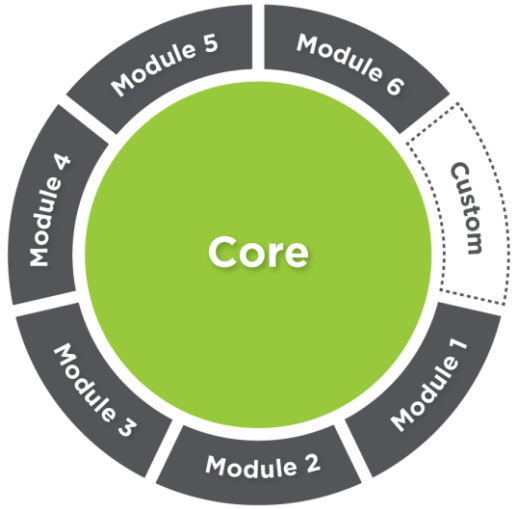
>140 targets for cancer and immunology

- Immune Cell Profiling Panel
- IO Drug Target Panel
- Immune Activation Status Panel
- Immune Cell Typing Panel
- Pan-Tumor Panel
- MAPK Signaling Panel
- PI3K/AKT Signaling Panel
- Cell Death Panel

>75 targets for neuroscience

- Neural Cell Profiling Panel
- AD Pathology Panel
- PD Pathology Panel
- Alzheimer's Pathology Extended Panel
- Autophagy Panel Panel
- Glial Cell Subtyping Panel

Core + Module design for flexibility with up to 96-plex



Ordering Screen → Post-Conjugation Screen → Sensitivity / Specificity Screen → Interaction Screen → TMA Screen

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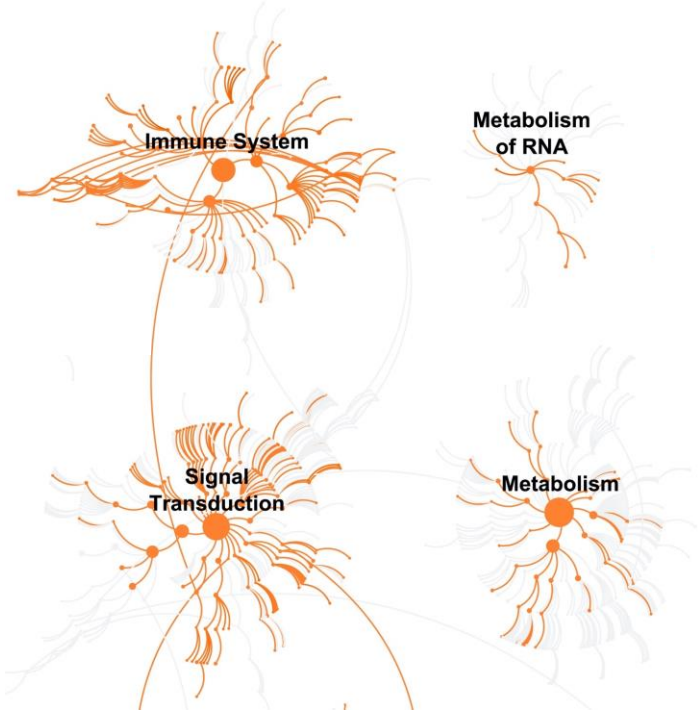
GeoMx DSP Products Advance from 84-plex to 18,000-plex

Reactome Coverage
(expanded limited region schematically depicted)

0%
100%

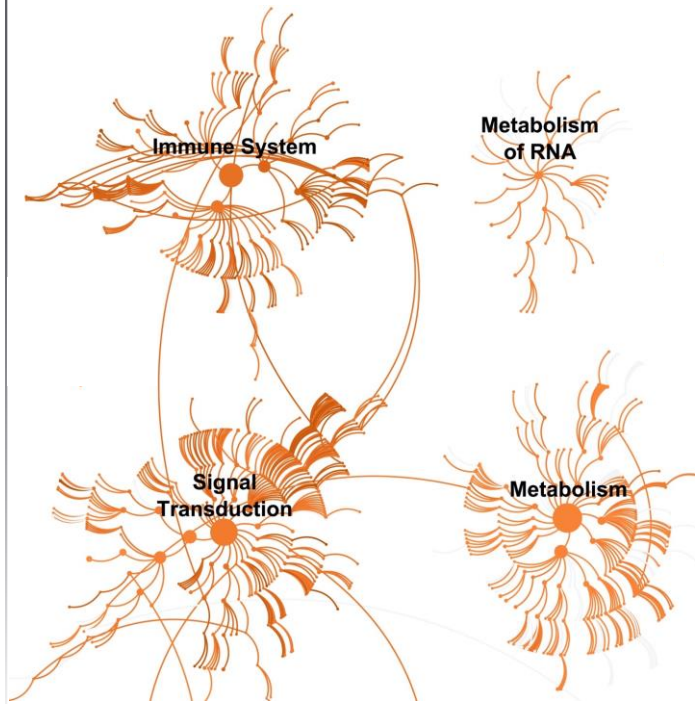
2019

Immune Pathways RNA Panel
- nCounter read-out
- 84 genes



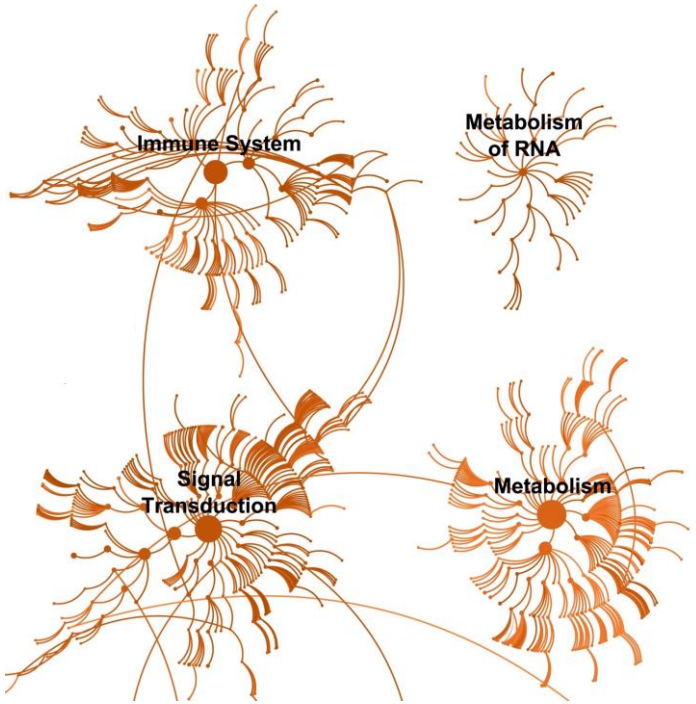
2020

Cancer Transcriptome Atlas
- NGS read-out
- 1,800+ genes



2021

Whole Transcriptome Atlas
- NGS read-out
- 18,000+ genes



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GeoMx Publications and Case Studies

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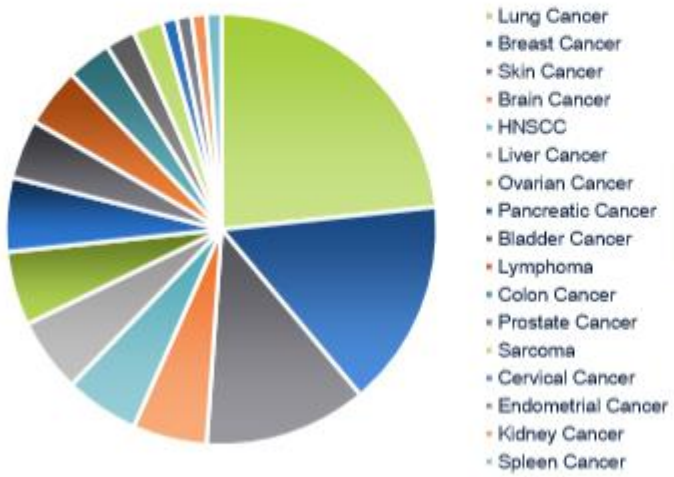
GeoMx®DSP high-throughput and extensive data analysis suite, really helps to shorten the time between experimentation, discovery and publication

Unexpected discovery of B-Cell involvement in therapeutic response using a 45-plex protein Immuno-Oncology panel.

Technology Access Program
Over 2,000 samples processed across 30 sample types



Less than 12 months

Submitted: 13-April-2018

Wargo J, et al, Nat Med 2018; 24(11) 1649-54

Blank CU, et al. Nat Med 2018; 24(11) 1655-61

9-months, 23 days

Submitted: 05-Feb-2019

Helmink B, Wargo J, et al, Nature 2020; 7:293

Carita R, Jonsson G, Nature 2020; 7:293

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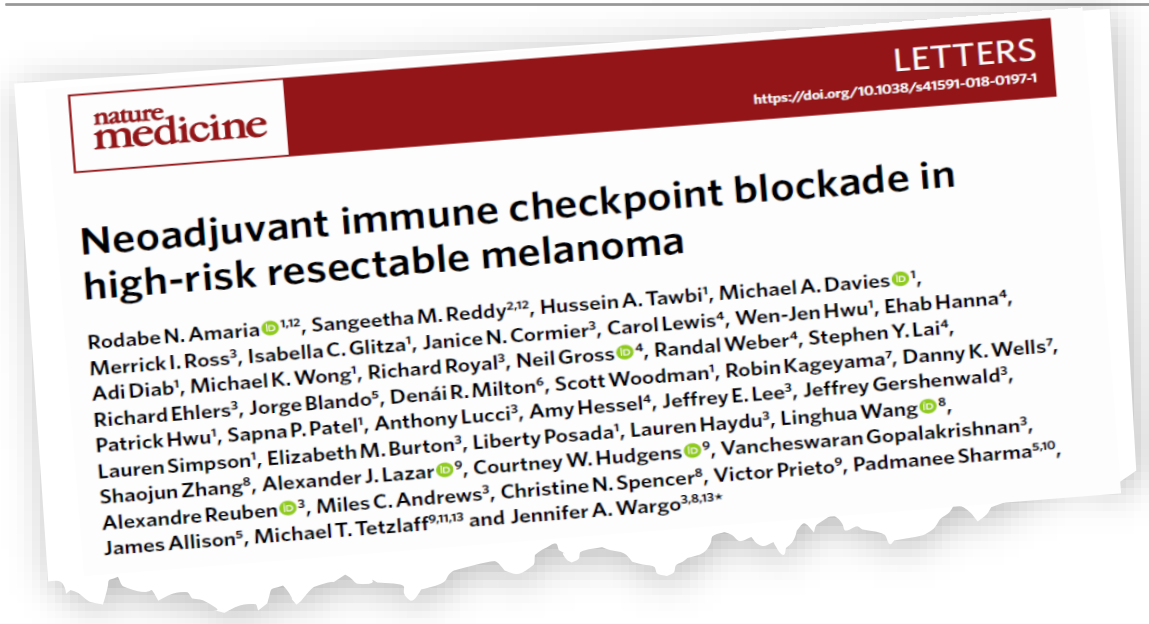
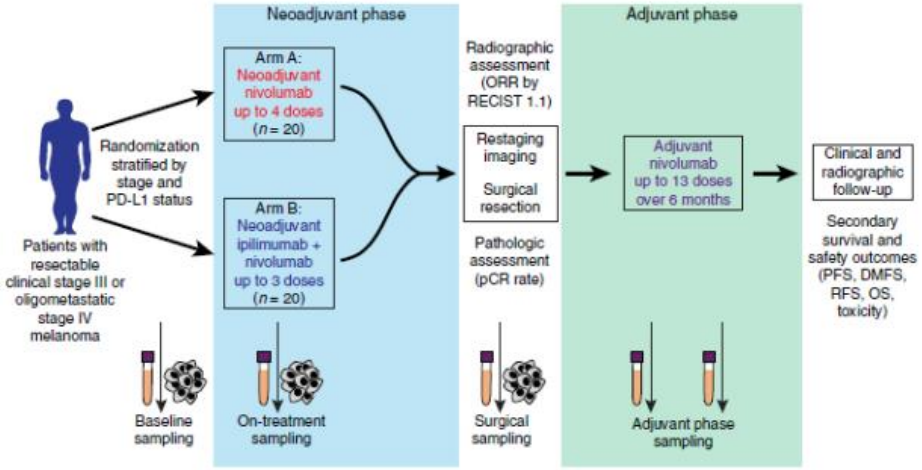
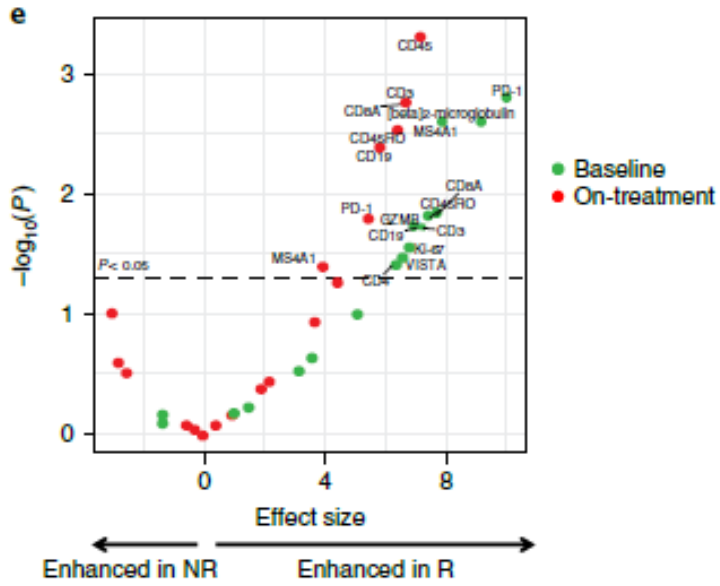
The Role of B cells in immune checkpoint blockade







THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Jennifer Wargo, MD

"With the help of the platform, researchers found multiple immune markers associated with response to immune checkpoint blockade, and some of these were B cell markers," *noted MD Anderson's Jennifer Wargo, who is also senior author of the Nature Medicine paper."*

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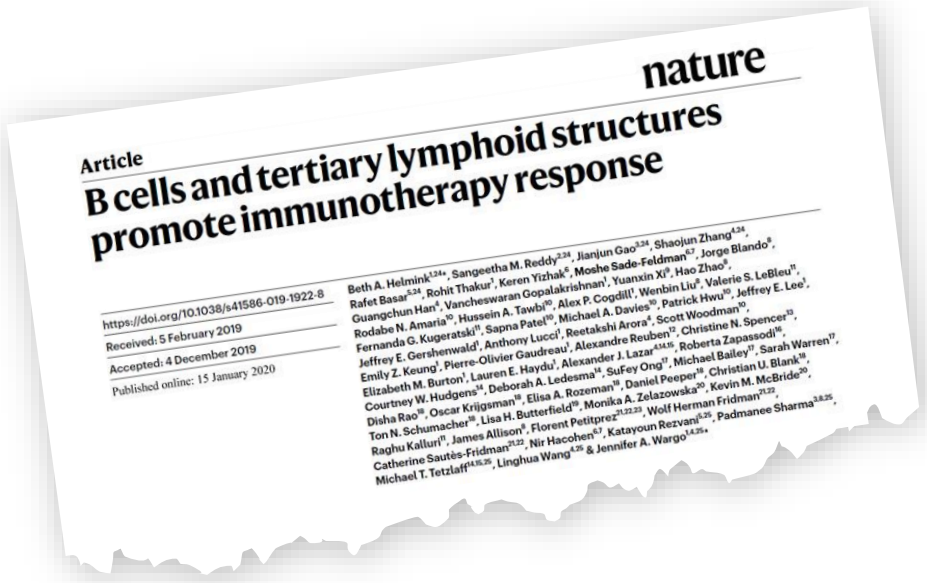
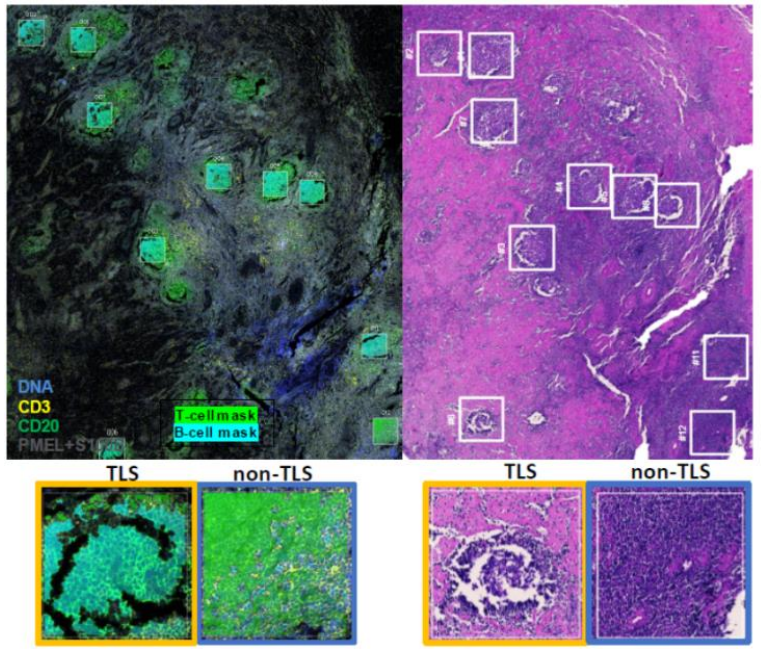
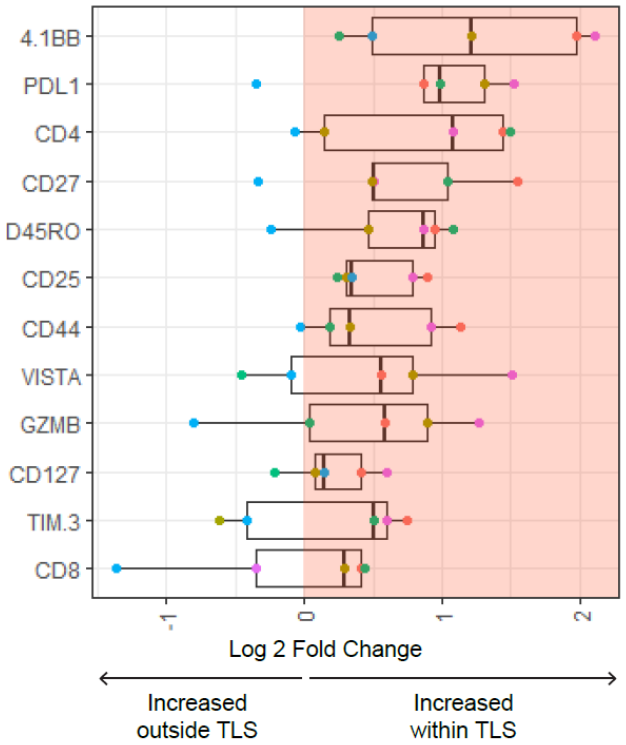
A deeper dive into the role of B cells and tertiary lymphoid structures



Spatially-distinct changes in immune cell activation





THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Jennifer Wargo, MD

Major Finding of Publication

B cells organize into tertiary lymphoid structures (TLS) in melanoma and are a biomarker of response to immunotherapy

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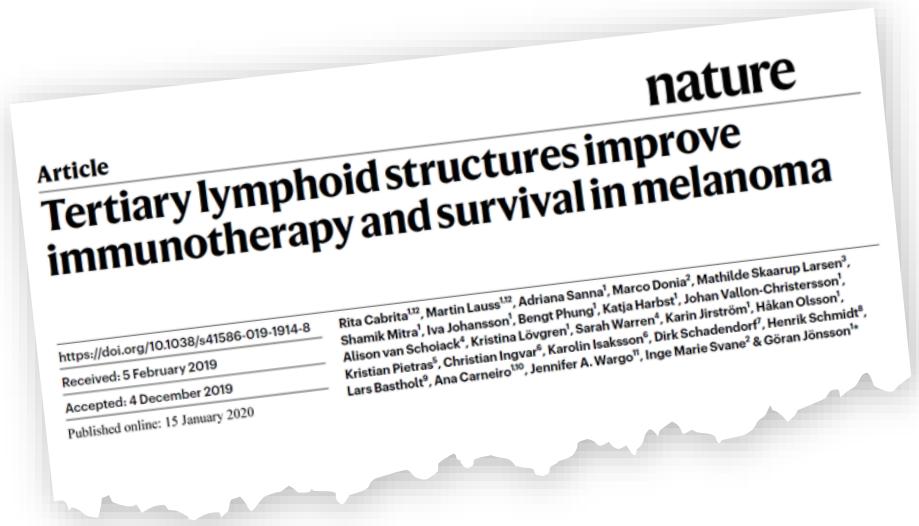
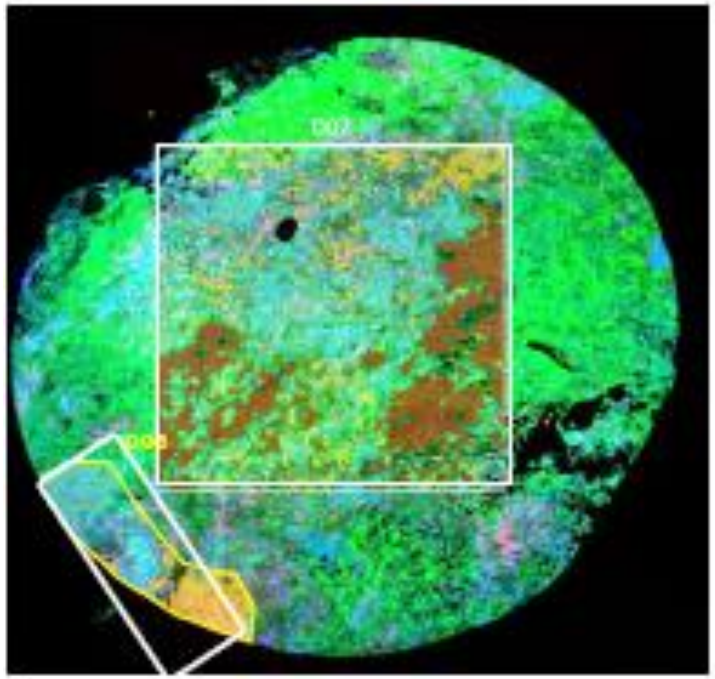
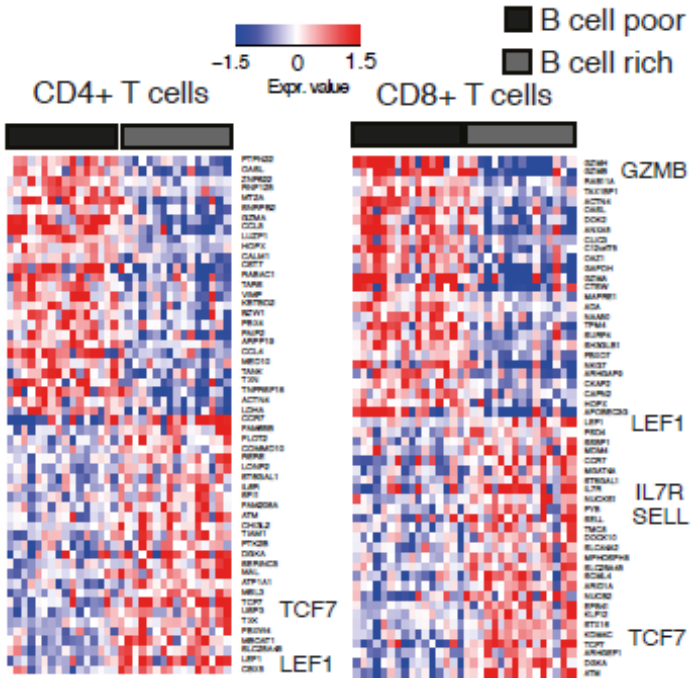
A deeper dive into the role of B cells and tertiary lymphoid structures



Spatially-distinct interplay of B and T cells





LUND UNIVERSITY

Göran Jönsson

Key finding of publication

B cells in TLS synergize with killer T cells that could ultimately target tumour cells.

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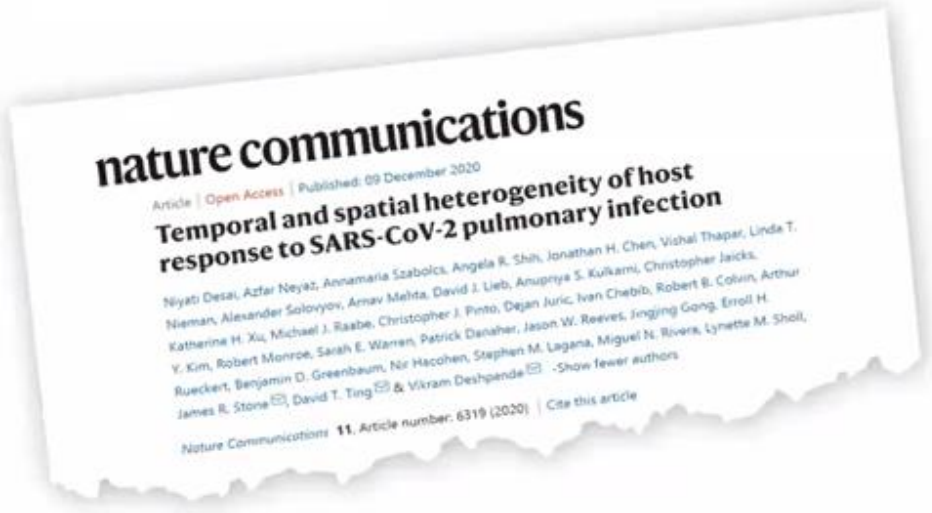
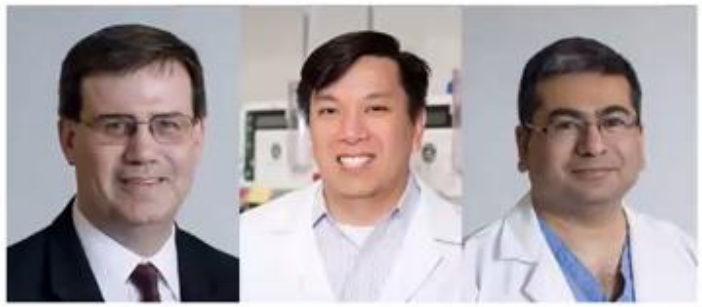
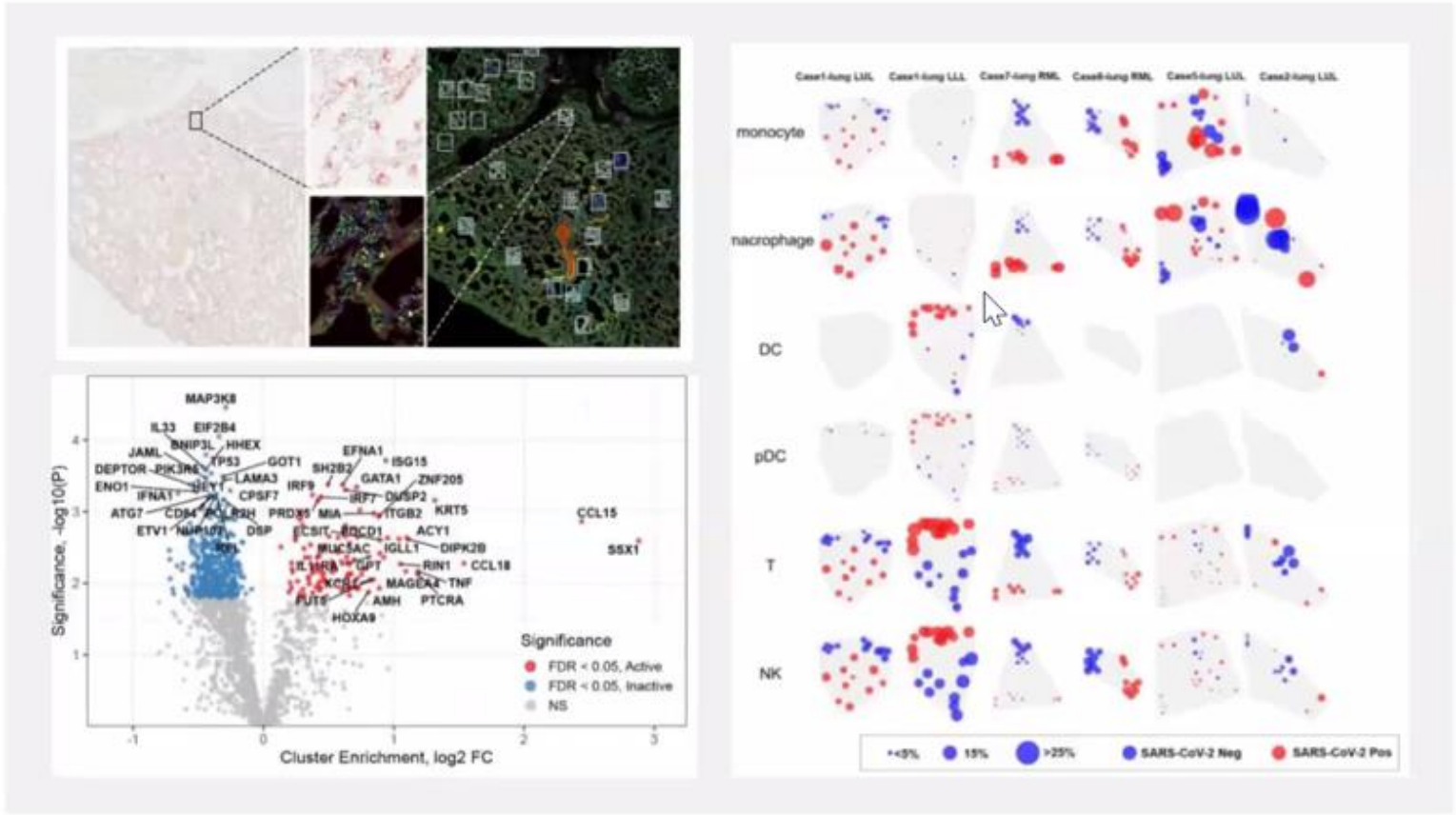
Isolating the Heterogeneity of SARS-CoV-2 Lung Infection





James Stone MD, PhD | David Ting MD | Vikram Deshpande MBBS

MASSACHUSETTS GENERAL HOSPITAL



"The use of the GeoMx digital spatial profiler has provided unprecedented spatial transcriptomic and proteomic analysis of the intra-pulmonary heterogeneity of SARS-CoV-2 infection."

– Desai et al., 2020 (pre-print)

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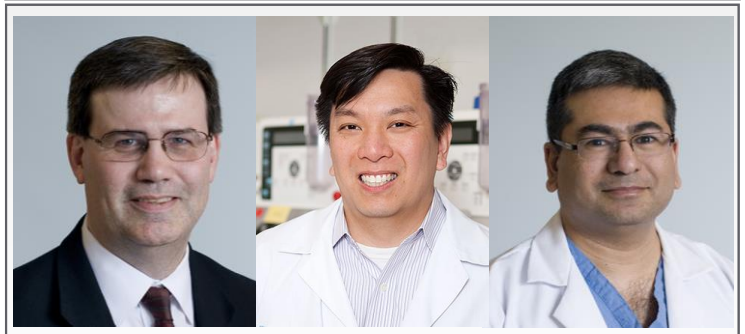
Spatial Heterogeneity of Host Response to SARS-CoV-2 Pulmonary Infection

Background

- **Relationship between SARS-CoV-2 infection and pulmonary disease severity is not fully understood.**
- **Immune responses amongst patients are varied and differences in these responses are likely to drive clinical outcomes.**

Experimental Question and Design

- **Can the GeoMx uncover hidden relationships between SARS-CoV-2 and patient immune responses in virus positive/negative airspaces of the lung?**
- **FFPE tissue sections from 5 infected patients were profiled with the GeoMx Cancer Transcriptome Atlas (CTA) and Protein assays.**
- **Viral positive/negative ROIs were selected with serial IHC sections with SARS-CoV-2 RNA-ISH staining**



James Stone
MD, PhD

David Ting
MD

Vikram Deshpande
MBBS

MASSACHUSETTS GENERAL HOSPITAL

Collaborative Effort
The Broad Institute
Memorial Sloan Kettering Cancer Center
Columbia University Irving Medical Center
Brigham and Woman's Hospital

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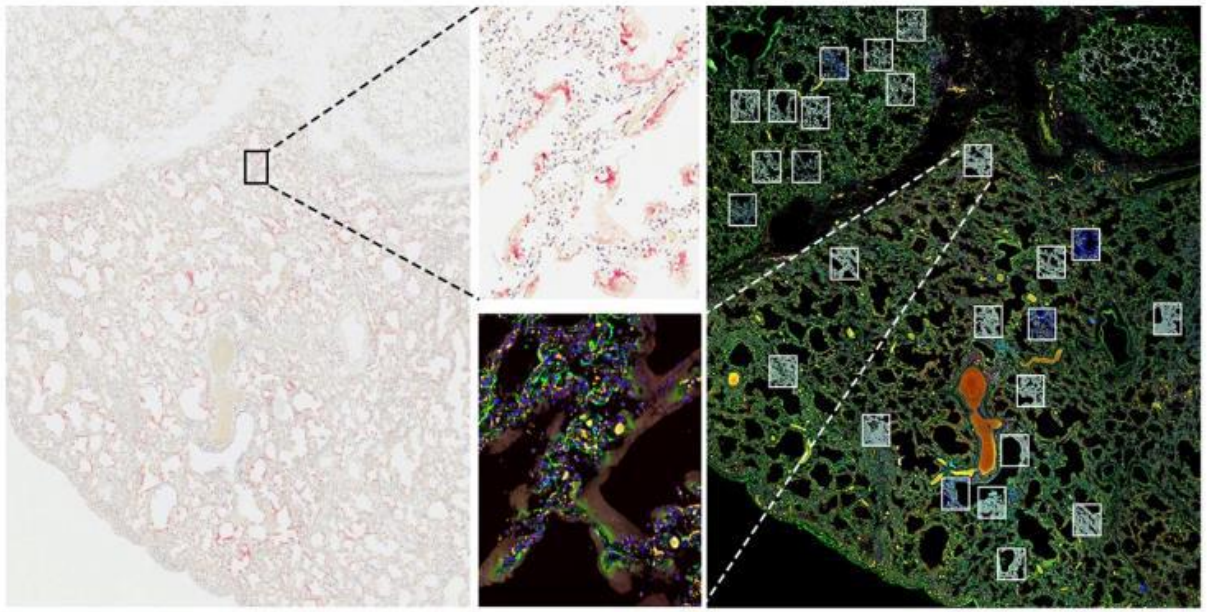
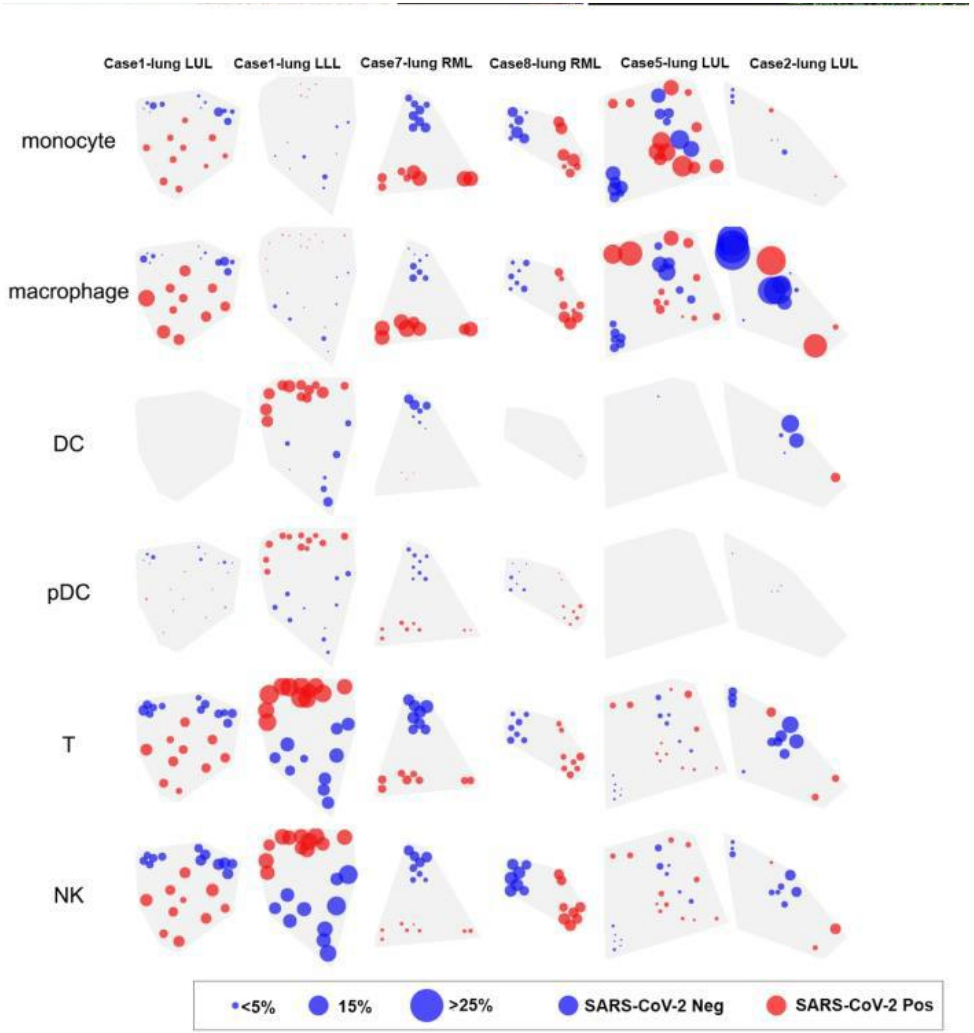
Immune cell deconvolution reveals remarkable heterogeneity in the localization of innate and adaptive immune cells





- Viral positive/negative ROIs were determined based on staining with the ACD RNA Scope SARS-CoV-2 probe
- ROIs were selected on lung tissue sections stained for DNA (Syto 13), Epithelium (PanCK), Immune Cells (CD45), and Macrophages (CD68)
- Immune cell deconvolution algorithm was run utilizing 1800+ plex CTA data
- High intra- and inter- patient heterogeneity was observed in the abundance and localization of macrophages, monocytes, dendritic cells, T cells, and NK cells

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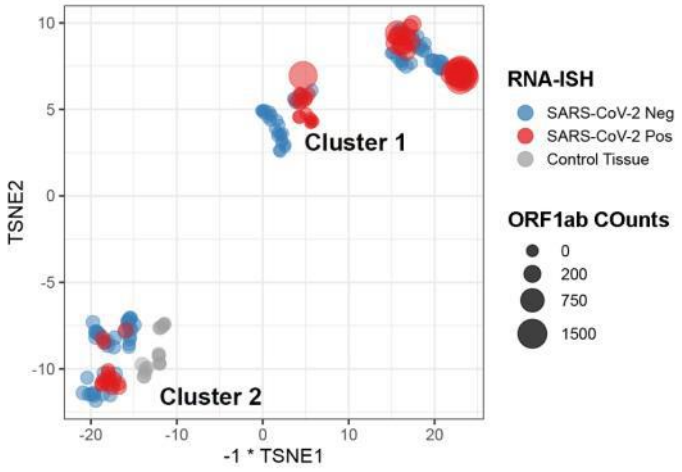
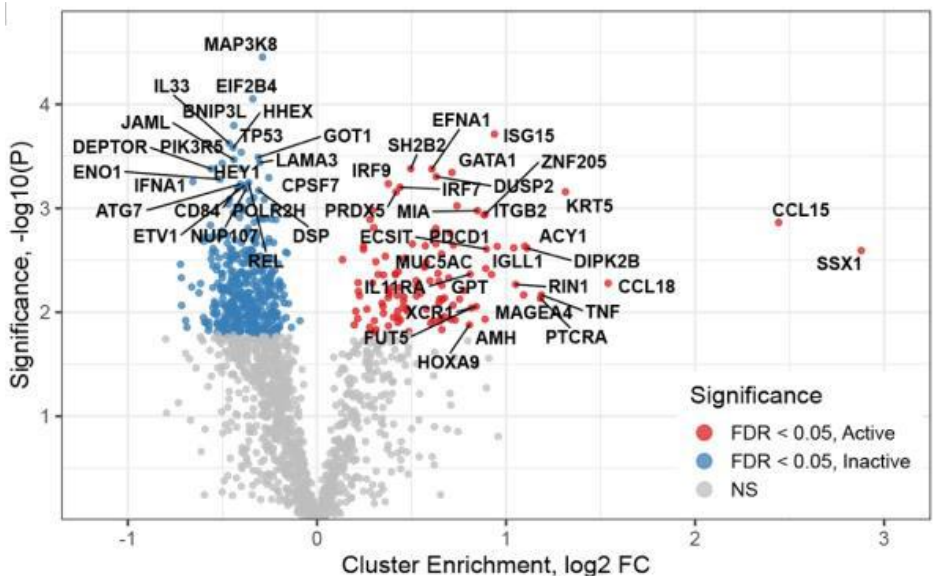
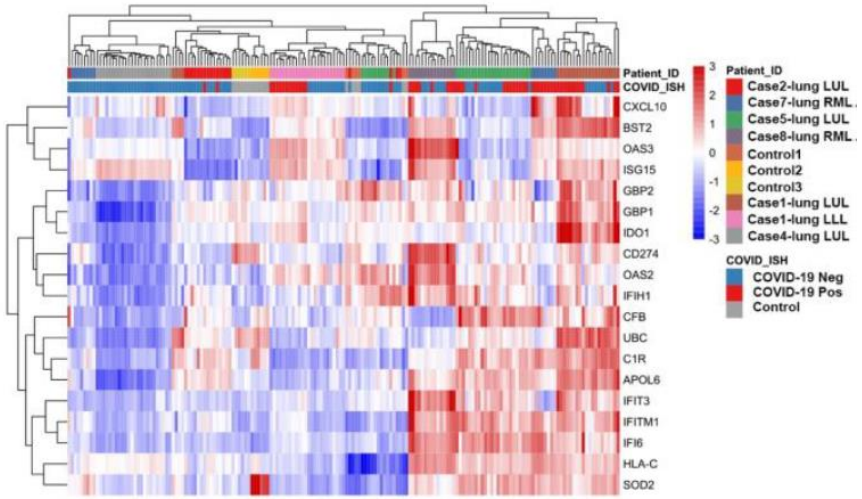
Significant interferon response gene expression and notable macrophage activity in virus positive ROIs







- **Clustering (tSNE) of the ROIs reveals two primary patient clusters, irrespective of the SARS-CoV-2 RNA-ISH positive/negative status of the ROI or patient viral load.**
- **Two outlier genes in particular SSX1 and CCL15 were highly elevated in virus high ROIs.**
- **CCL15 is a chemokine that is highly expressed in M1, as opposed to M2, macrophages; M1 macrophages are associated with SARS-CoV-2 high cases, indicating that CCL15 is important in the immune response to virus.**
- **Notably higher interferon response in virus high ROIs when compared to virus low ROIs.**

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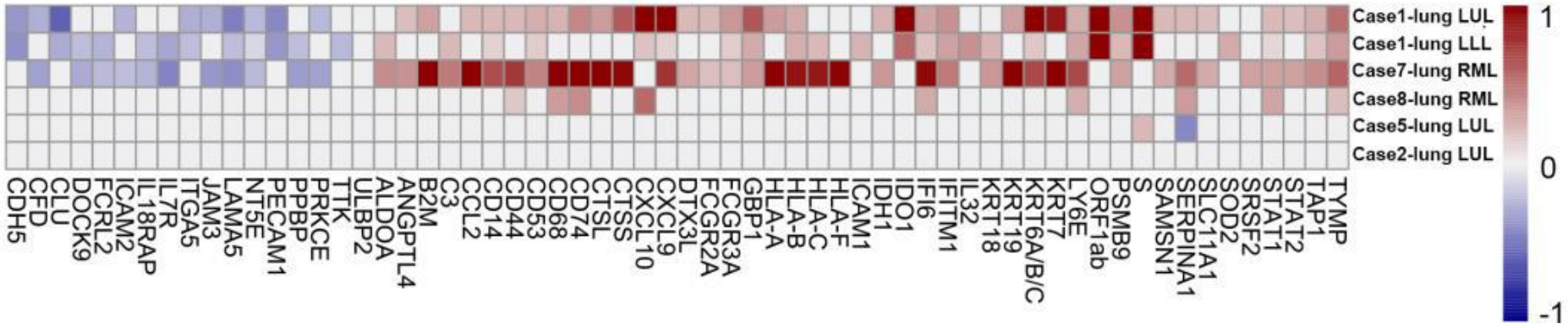
Spatial protein analyses across patients reveal enrichment of multiple immune checkpoint targets in virus-enriched ROIs



- **Immune checkpoint proteins CTLA4, PD-L1, and IDO1 are upregulated in virus positive ROIs, suggesting an immune microenvironment that is inhibitory to T-cell activation**

Summary

- **“The use of the GeoMx digital spatial profiler has provided unprecedented spatial transcriptomic and proteomic analysis of the intra-pulmonary heterogeneity of SARS-CoV-2 infection.” – Desai et al., 2020 (pre-print)**
- **Remarkable heterogeneity discovered in immune cell abundance, interferon response, and checkpoint markers as a function of virus localization.**

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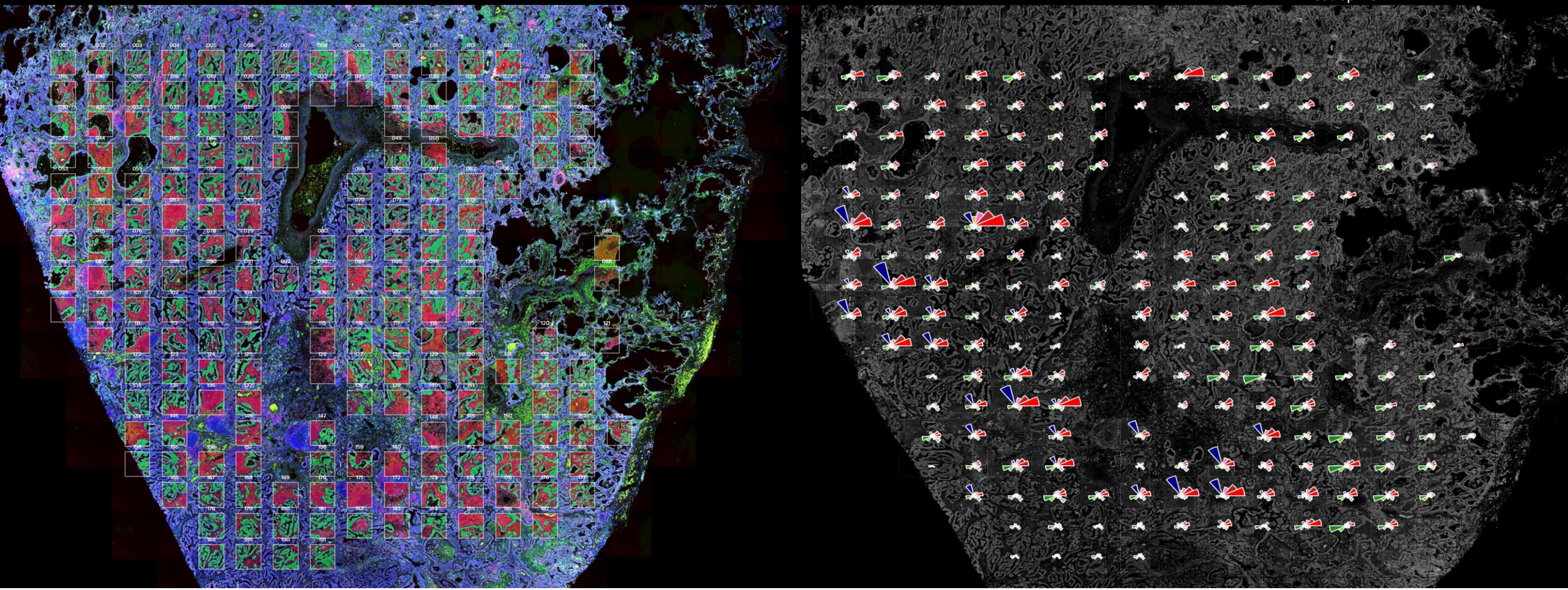
Spatially resolved, cell-subtype determination using RNA deconvolution



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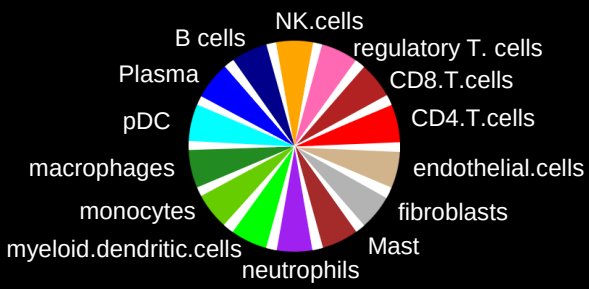
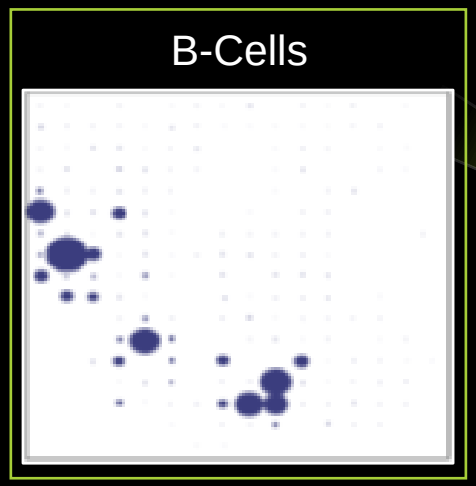
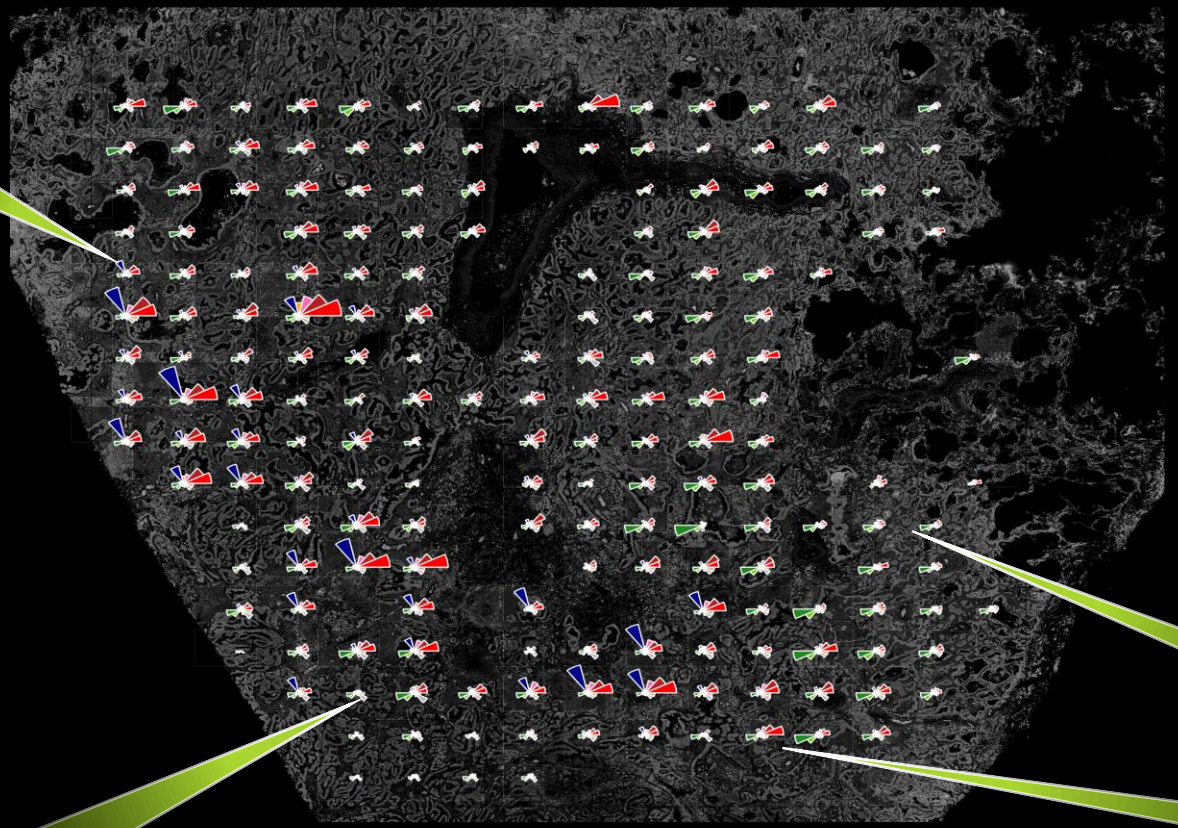
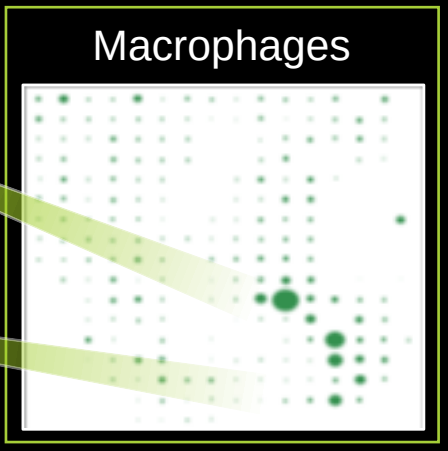
Cell subtypes vary considerably when spatially resolved









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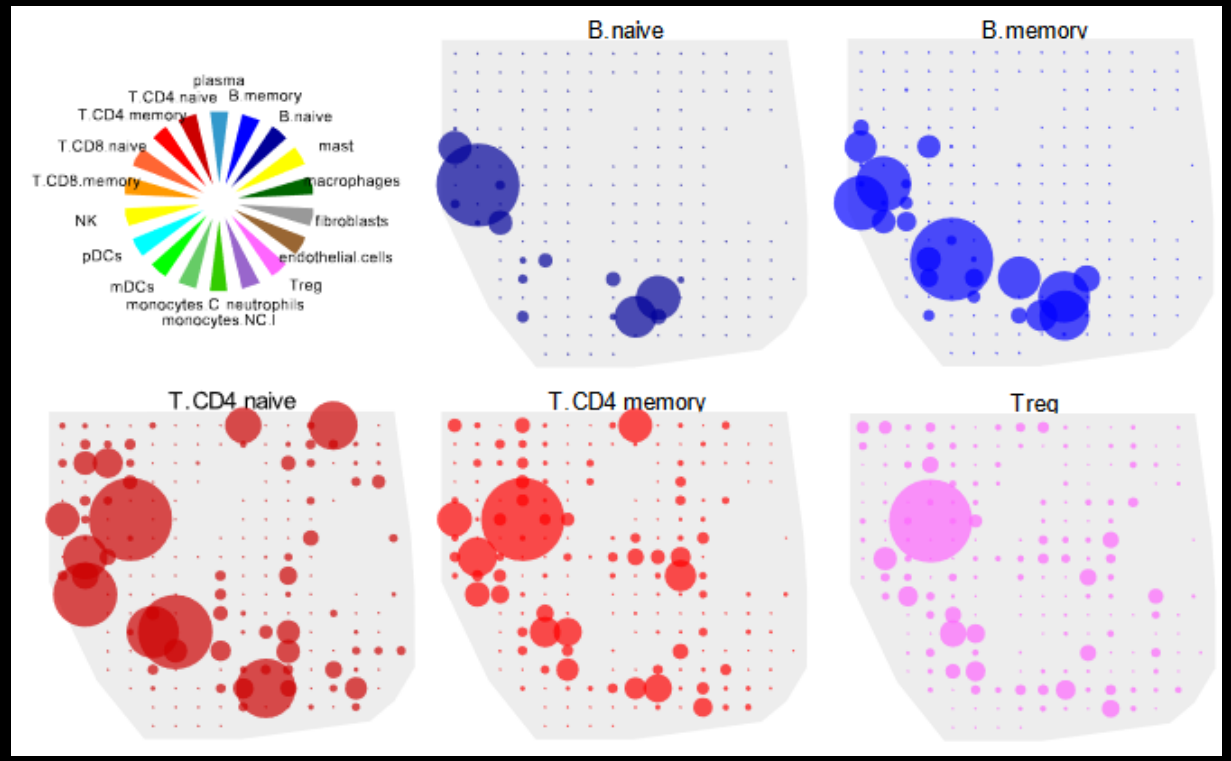
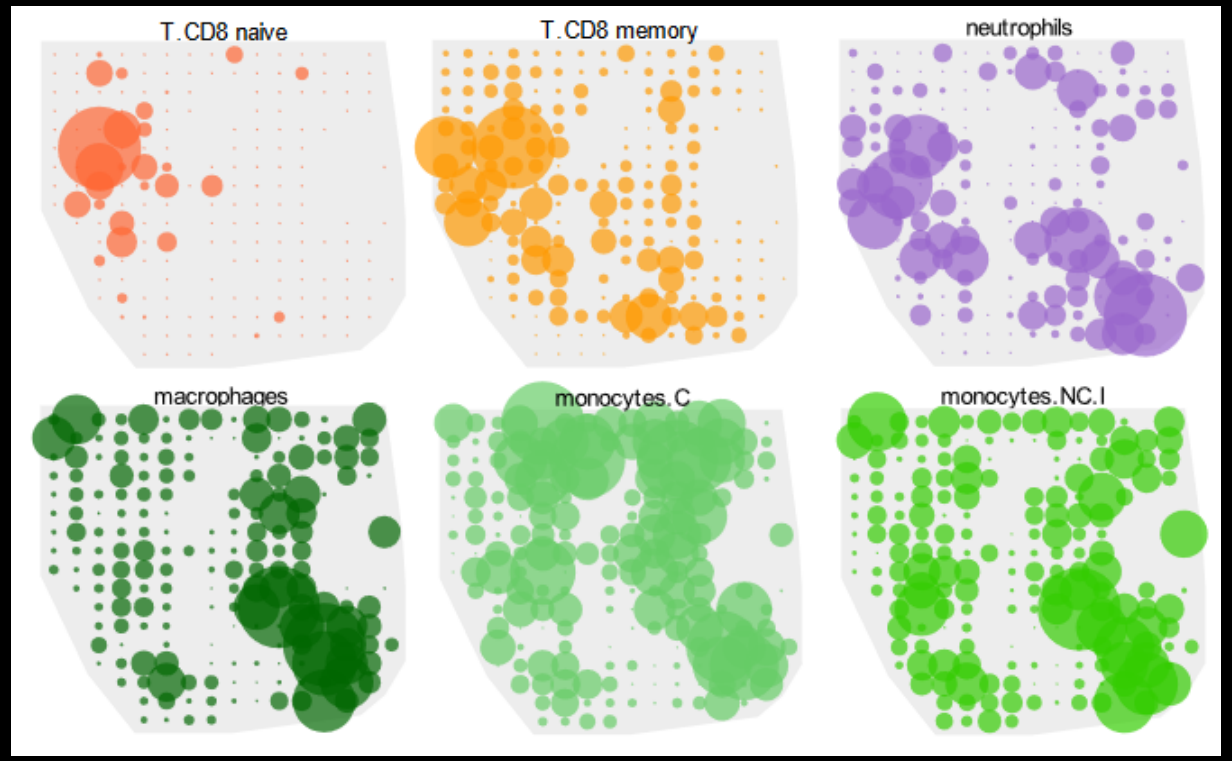
Cell subtypes vary considerably when spatially resolved





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Cell Deconvolution

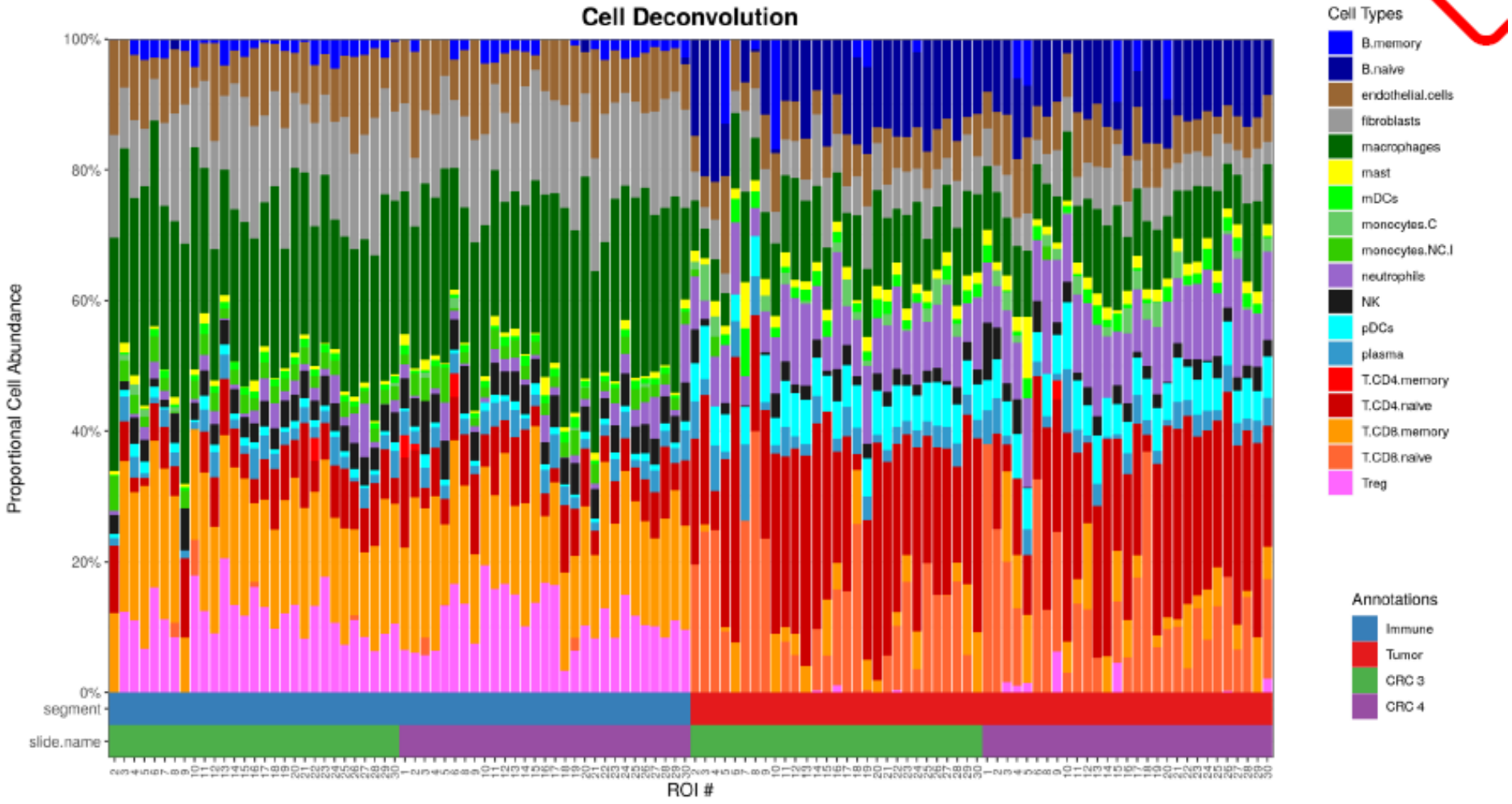
Example

Description

Gene expression patterns are deconvolved based on a training matrix of single-cell sequencing data. Estimated proportions of different cell types are inferred that explain overall expression patterns of each sample.

Results

- Immune and Tumor segments exhibit very different cell profiles, with Immune regions showing endothelial cells largely absent in the tumor, while myeloid dendritic cells are seen exclusively in the tumor.



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Ideally a gold standard spatial platform would have:

FF & FFPE Validated Workflows	Provides access to large sample set
RNA & Protein Compatibility	Ability to profile both enables comprehensive phenotypic profiling of expression, function, and activation state
Tunable Spatial Selection	ROI selection based on morphology markers
High-plex Capability	Up to 80+ plex protein & 18,000 plex Whole Transcriptome
High Throughput	Maximizes productivity in the lab with ~10 slides/day

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GeoMx™ Digital Spatial Profiler

GeoMx@nanostring.com