Morphology Driven High-Plex Spatial Analysis of Tissue Microenvironments

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nanoString

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

Smart Panels: Biomarkers, Pathways and Signatures.

miRNA
(Human, Mouse, Rat)

Gene Fusion
(lung, leukemia)
and CNV

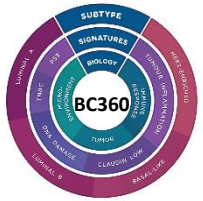
Vantage 3D
(Protein and RNA)

Gene Expression

Oncology

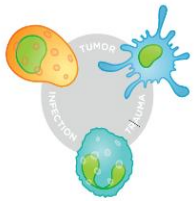
PanCancer
- Pathways
- Immune Profiling
- Progression

360 Panels

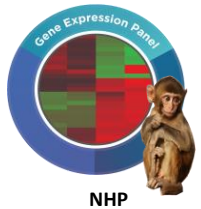


Immunology

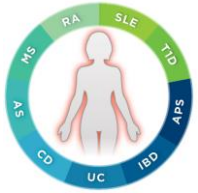
Immunology V2



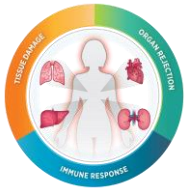
Myeloid Innate Immunity



NHP Immunology

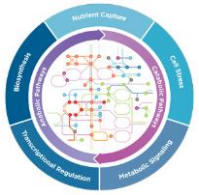


Autoimmune Profiling & Discovery



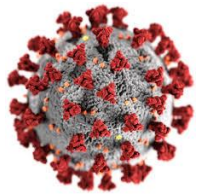
Human Organ Transplant

CAR-T



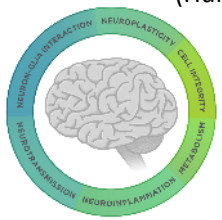
Metabolic Pathways

Fibrosis

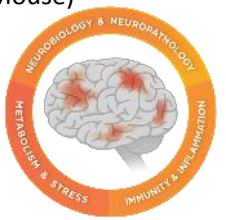


Neuroscience

(Human, Mouse)



Neuropathology



Neuroinflammation

Alzheimer's Disease

Glial Profiling

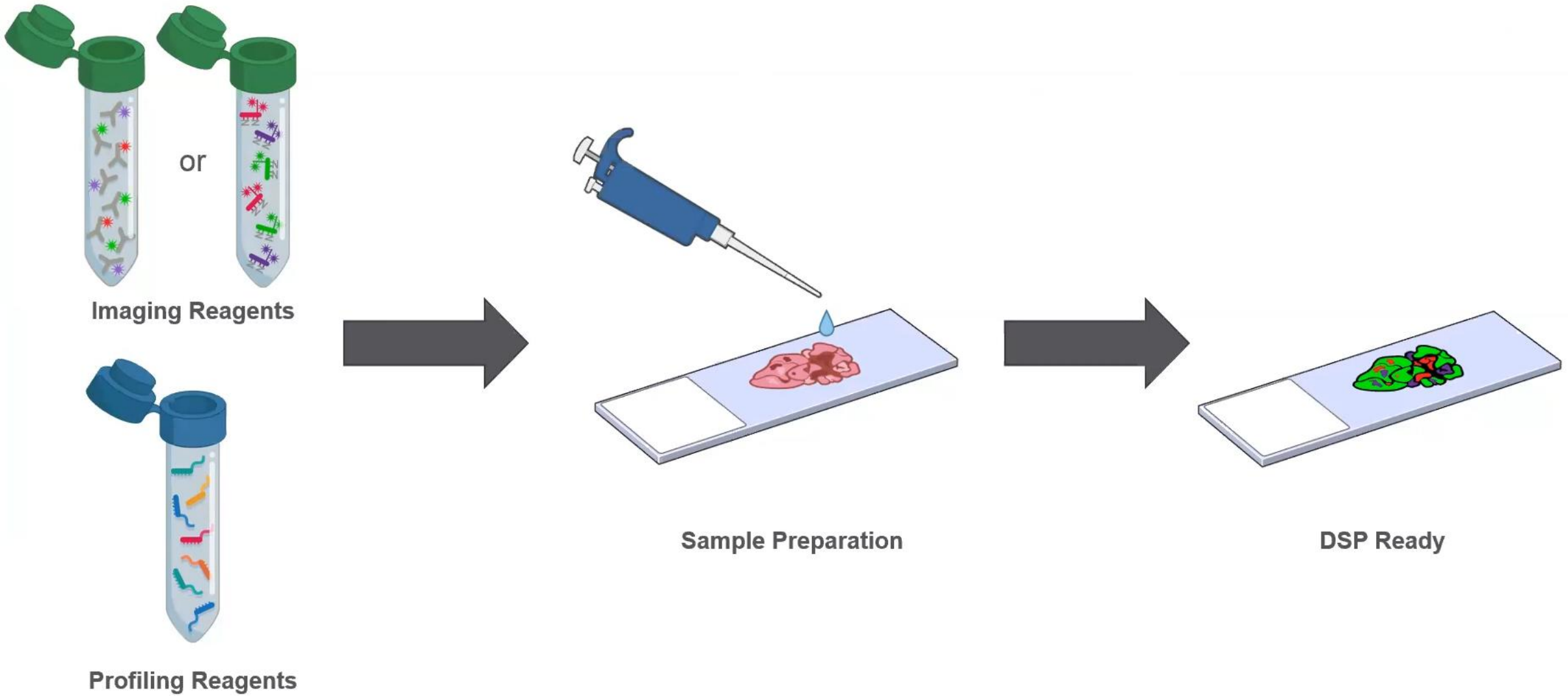
Panel Plus

Spike in up to 30 additional targets

About the GeoMx Digital Spatial Profiler (DSP)

nanoString®

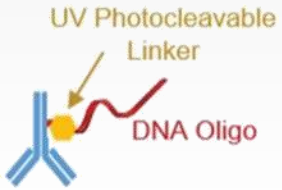
Imaging and Profiling in One Assay



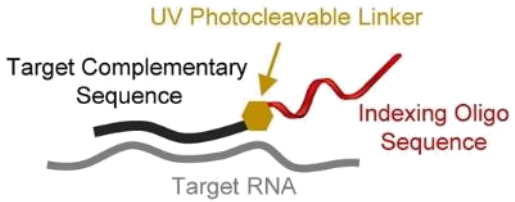
GeoMx™ DSP Enables Spatial, High-Plex Protein & RNA Profiling

High-Plex Mixtures of Proprietary Reagents

Protein reagents
Oligo-labeled antibodies



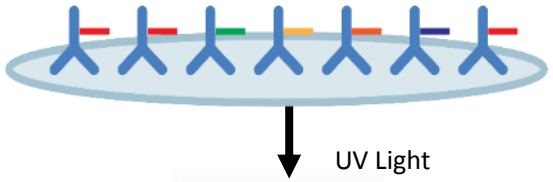
RNA reagents
Oligo-labeled probe



Profile Regions of Interest on FFPE slide

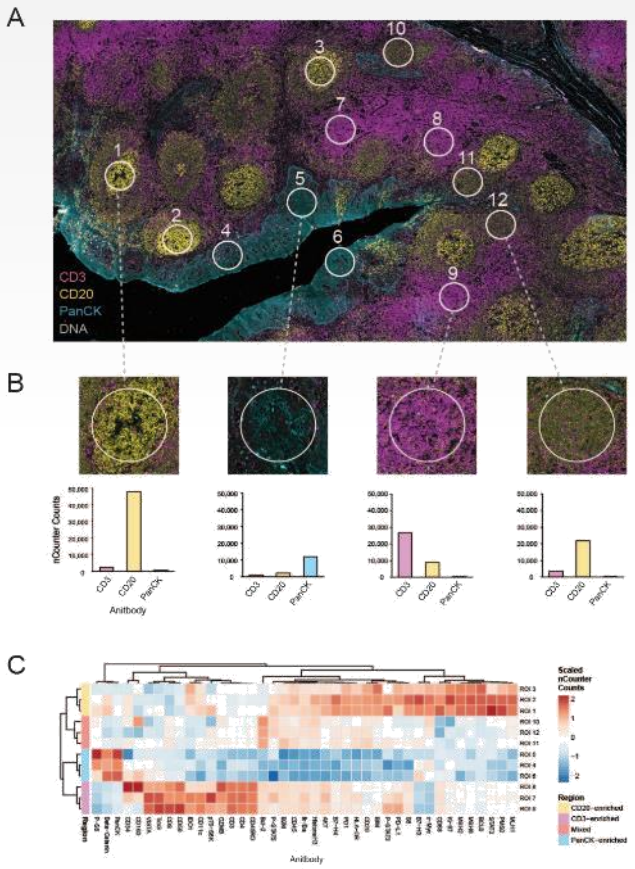
GeoMx™ Digital Spatial Profiler

Label FFPE Slide with Probe Mix

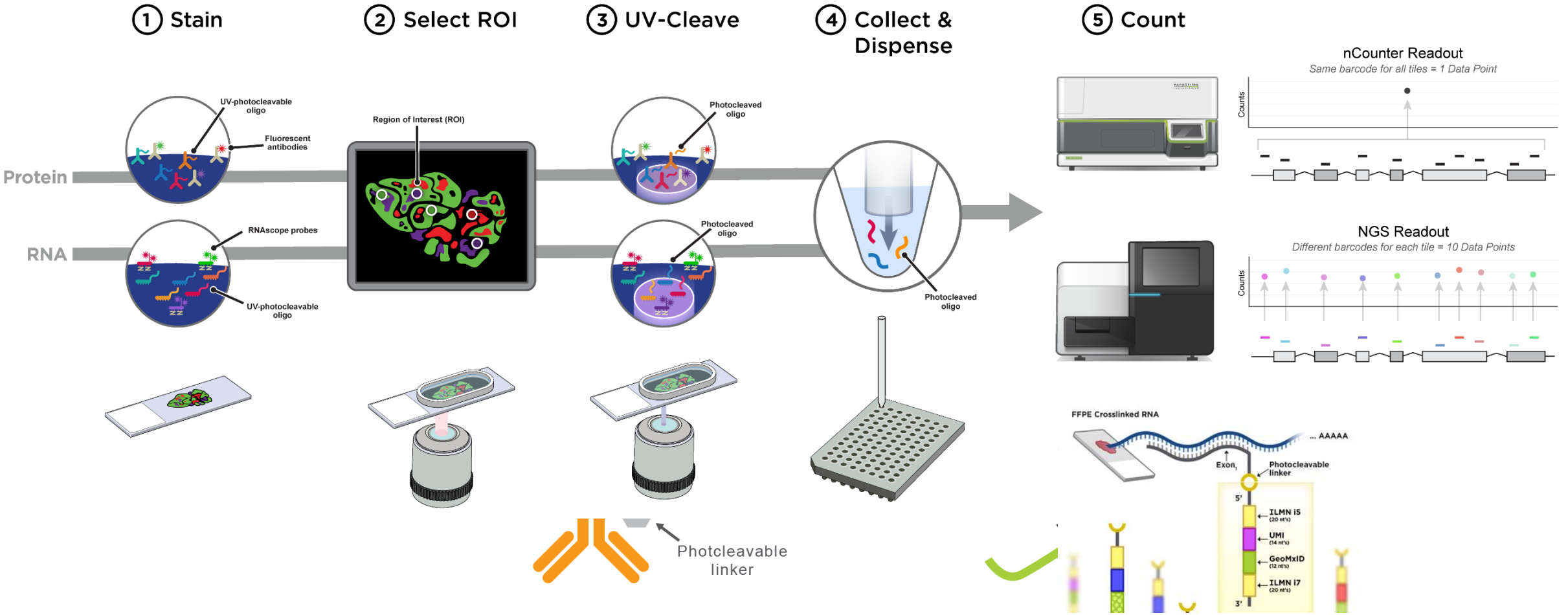


Illuminate Region of Interest, as Small as a Single Cell

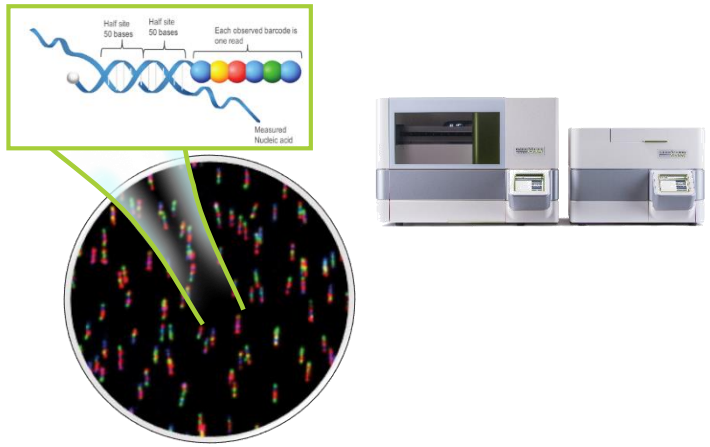
Rich Data Sets of Biology, Region by Region

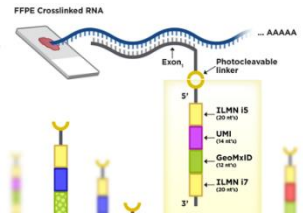


GeoMx® Digital Spatial Profiler: How it works

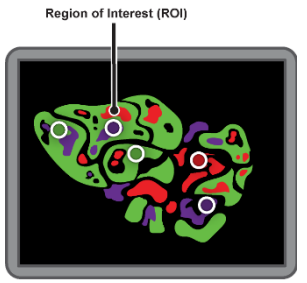


Digital Count Data with spatial information



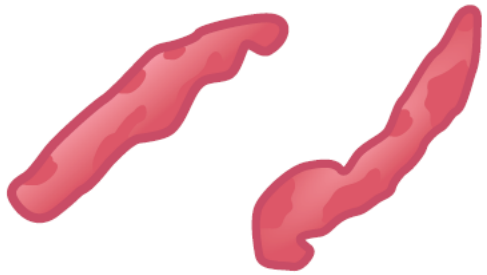


Target	Sample 1	Sample 1	Sample …	Sample …	Sample 12
Protein or RNA	Segment 1	Segment 2	Segment …	Segment …	Segment 24
SPP1	8,002	201	####	####	948
GAPDH	7,452	1,621	####	####	1,370
PLA2G2A	6,884	449	####	####	948
HSP90AB1	2,751	915	####	####	632
TGFBI	2,096	816	####	####	1,054
TIMP1	2,034	473	####	####	948
PGK1	1,427	1,420	####	####	632
MCL1	1,320	1,374	####	####	421
FAT1	1,303	208	####	####	948
STAT3	1,270	1,554	####	####	1,054
PLG	1,129	7,935	####	####	527
XRCC5	1,113	1,854	####	####	1,791
COL1A1	1,080	272	####	####	1,054
ERBB2	1,028	106	####	####	421

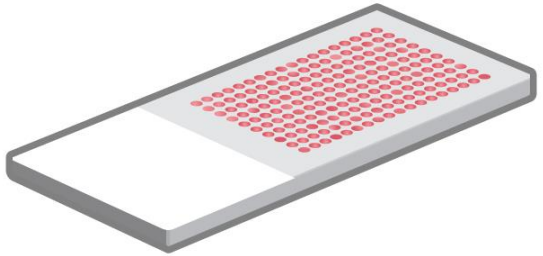


Analyze Any Sample Types with Selection Based on Experimental Design, Not Technology

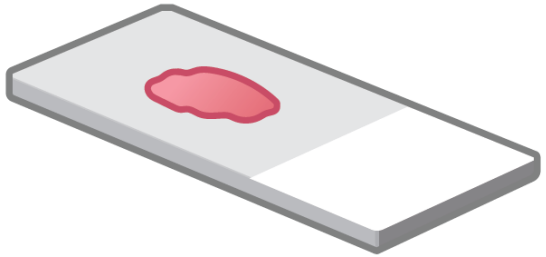
Core Needle Biopsy



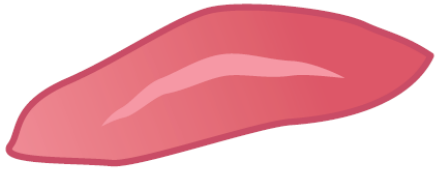
FFPE Tissue Block



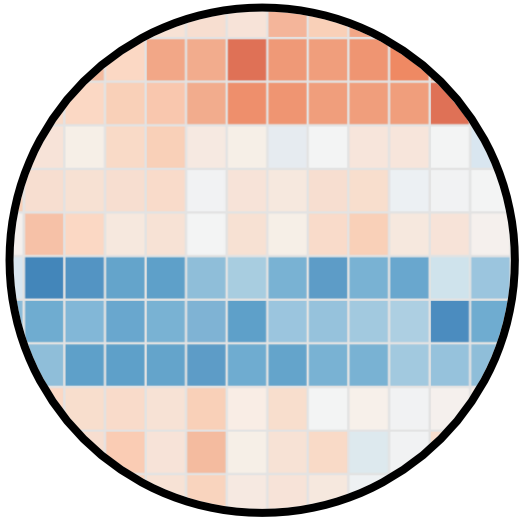
Tissue Microarray



Slide Mounted Tissue



Fresh Frozen Tissue



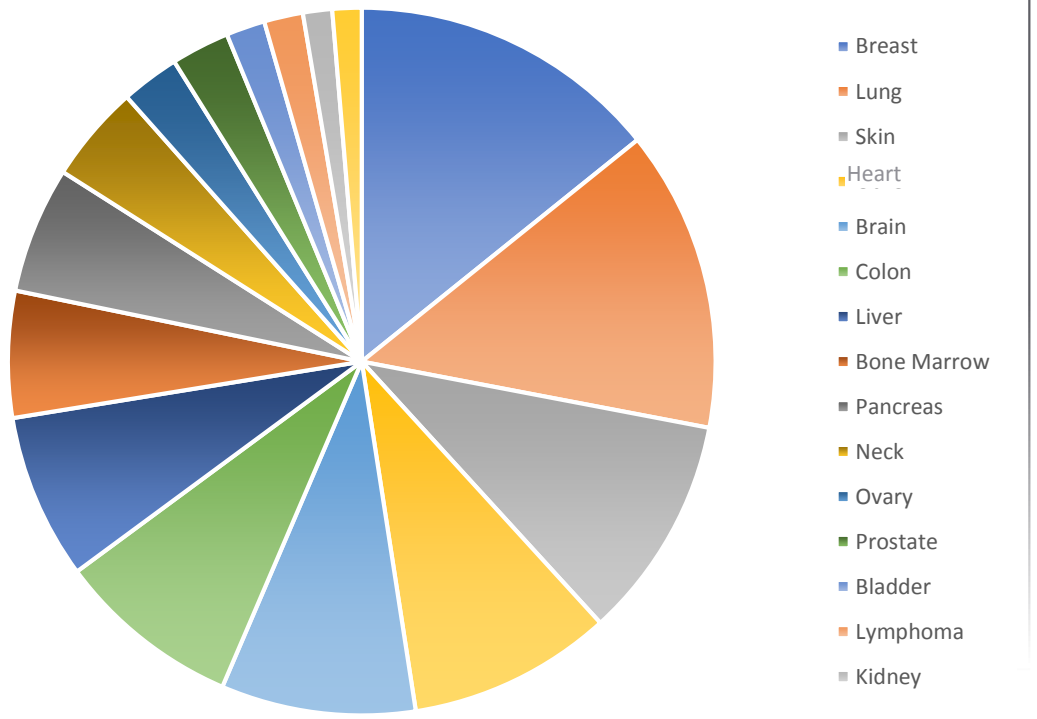
Trust ALL Data

Robust Chemistry Validated by Multiple Labs Drives Rapid Publications

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

25+ publications since November 2018

> 30 Tissue Types Studied



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

Protein Assays for nCounter®

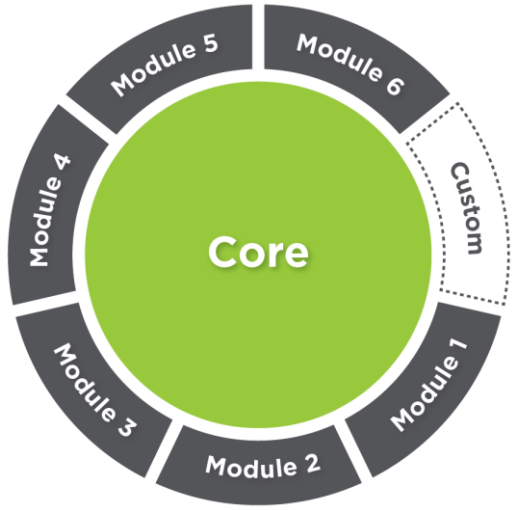
>140 targets for cancer and immunology

- 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



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

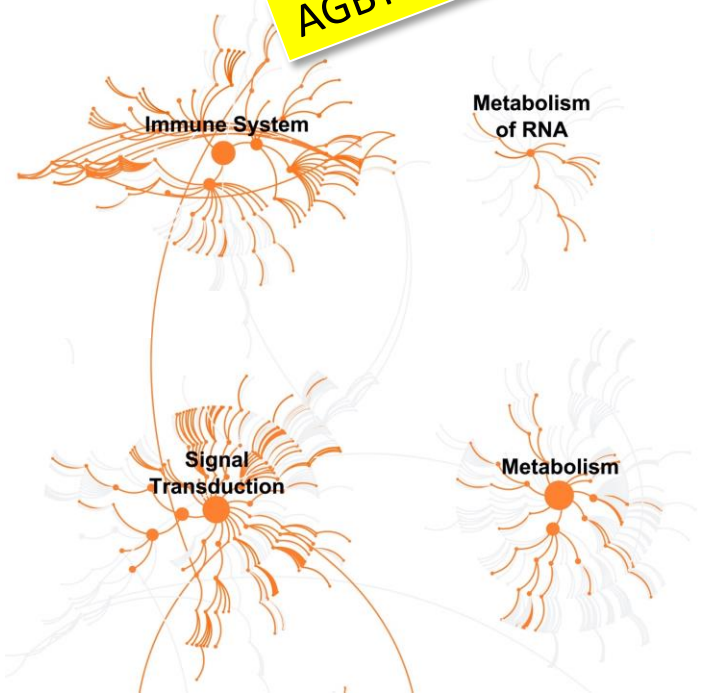
Reactome Coverage
(expanded limited region schematically depicted)

0%
100%

2019

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

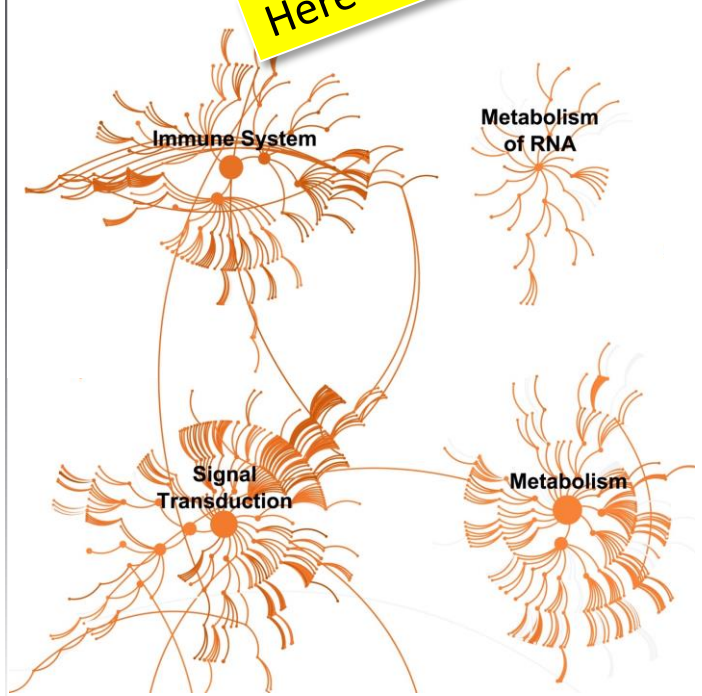
AGBT 2019



2020

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

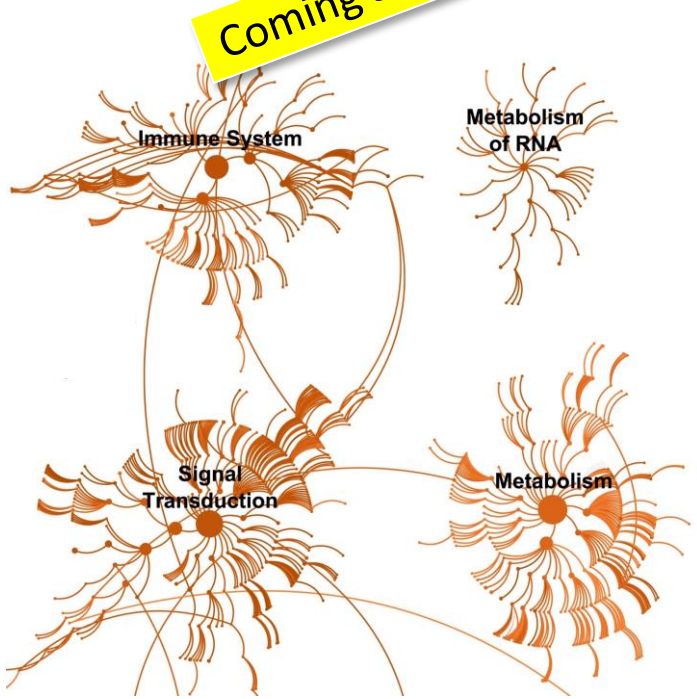
Here NOW



2021

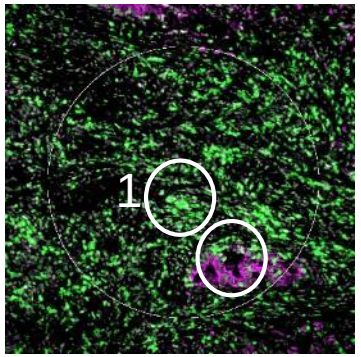
Whole Transcriptome Atlas
- NGS read-out
- 18,000+ genes

Coming soon



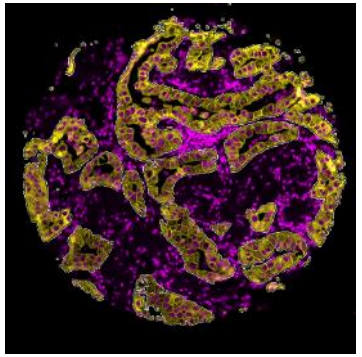
Five Unique Profiling Modalities Designed to Interrogate Tissue Samples

Geometric



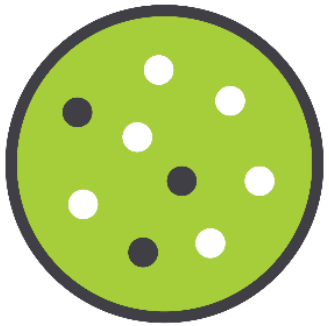
What is the heterogeneity of expression in different regions of my tissue?

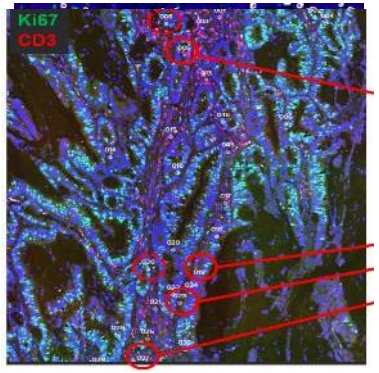
Segmentation



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

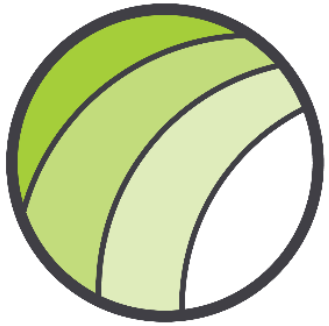
Cell-type Specific

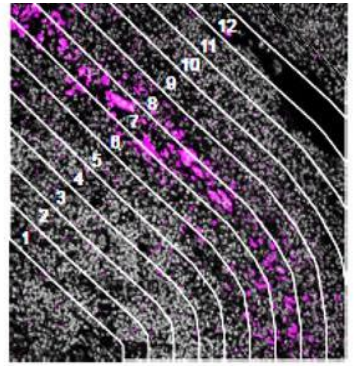




What is the expression profile of a specific cell population in my tissue?

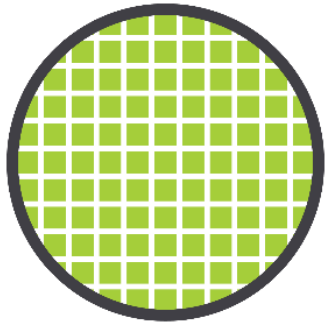
Contour

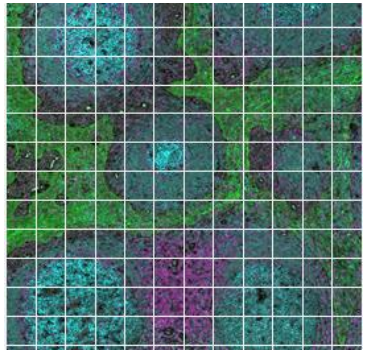




How does the immune environment change on either side of an infiltrate boundary?

Gridded





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

Biomarkers Associated with Beneficial PD-1 Checkpoint Blockade in Non-Small-Cell Lung Cancer (NSCLC)

Background

- The majority of patients with advanced NSCLC do not respond to PD-1 axis blockade, particularly as monotherapies
- Since PD-1 checkpoint blockade is a standard of care for advanced stage disease, more robust predictive biomarkers are needed to optimally deliver these treatmetns

Experimental Question and Design

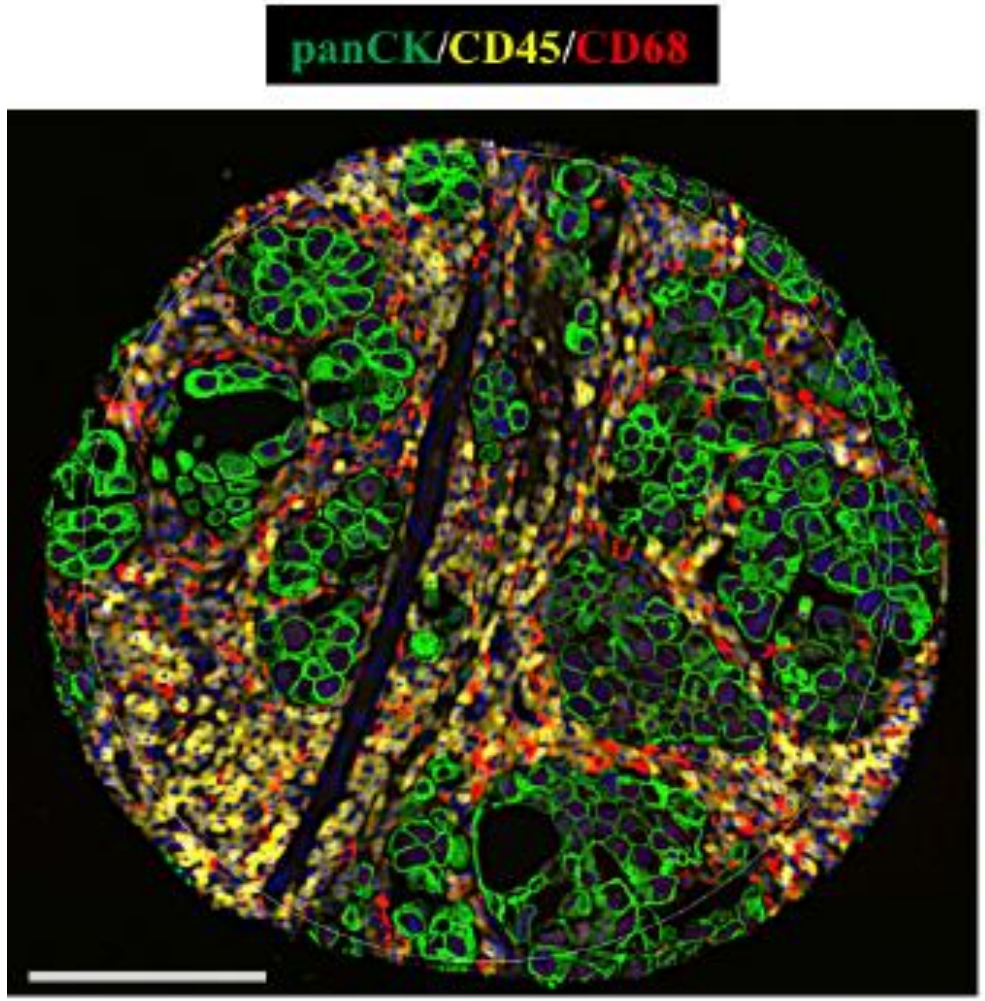
- Tissue microarrays were used to assay pretreatment biopsies from 53 advanced NSCLC patients who received single agent PD-1 checkpoint blockade
- The expression of 44 proteins was measured in 4 tissue compartments: tumor, macrophage, total immune, and non-immune stroma
- Expression of all markers across the 4 compartments was assessed for the ability to predict therapeutic response

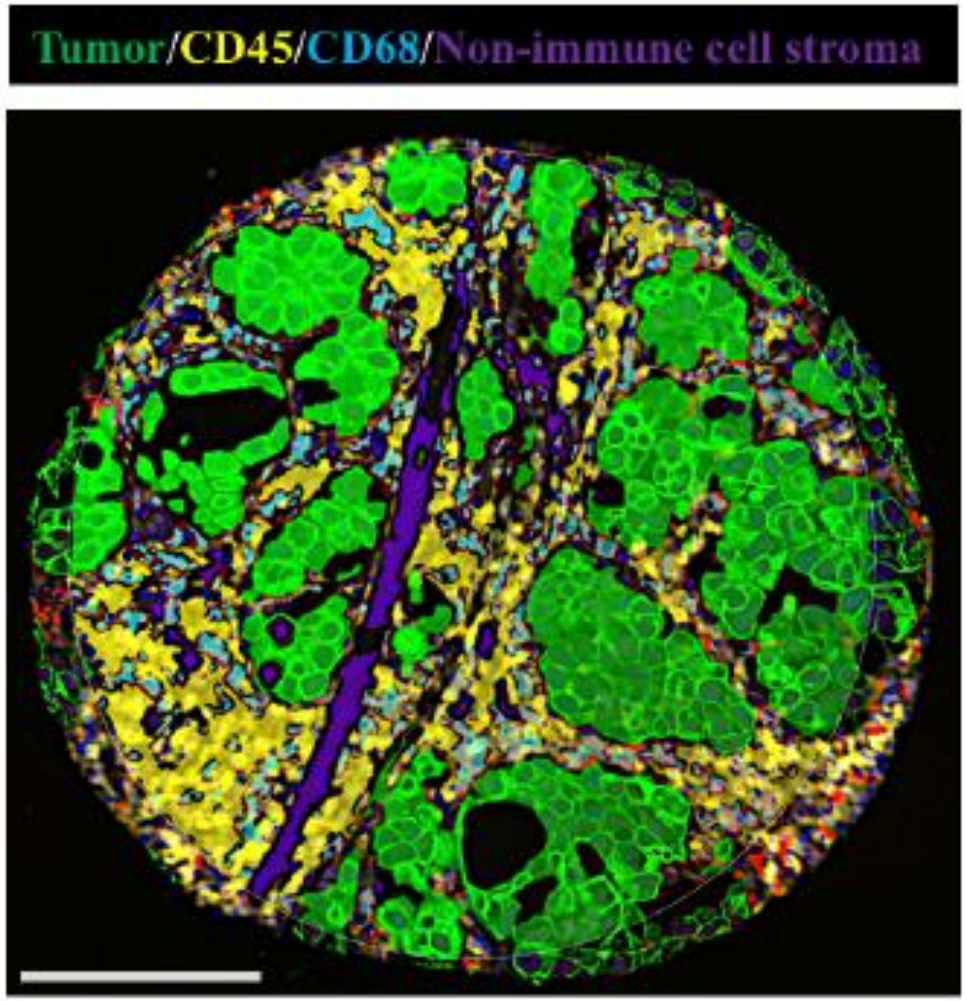
Results

- High expression of CD56 and CD4 in the total immune (CD45+) compartment was associated with progression free survival (PFS) and overall survival (OS)
- High levels of VISTA and CD127 in the tumor compartment were associated with immunotherapy resistance

David Rimm, MD, PhD
Professor of Pathology and Medicine
Director of Pathology Tissue Services
Director of Translational Pathology
Yale University

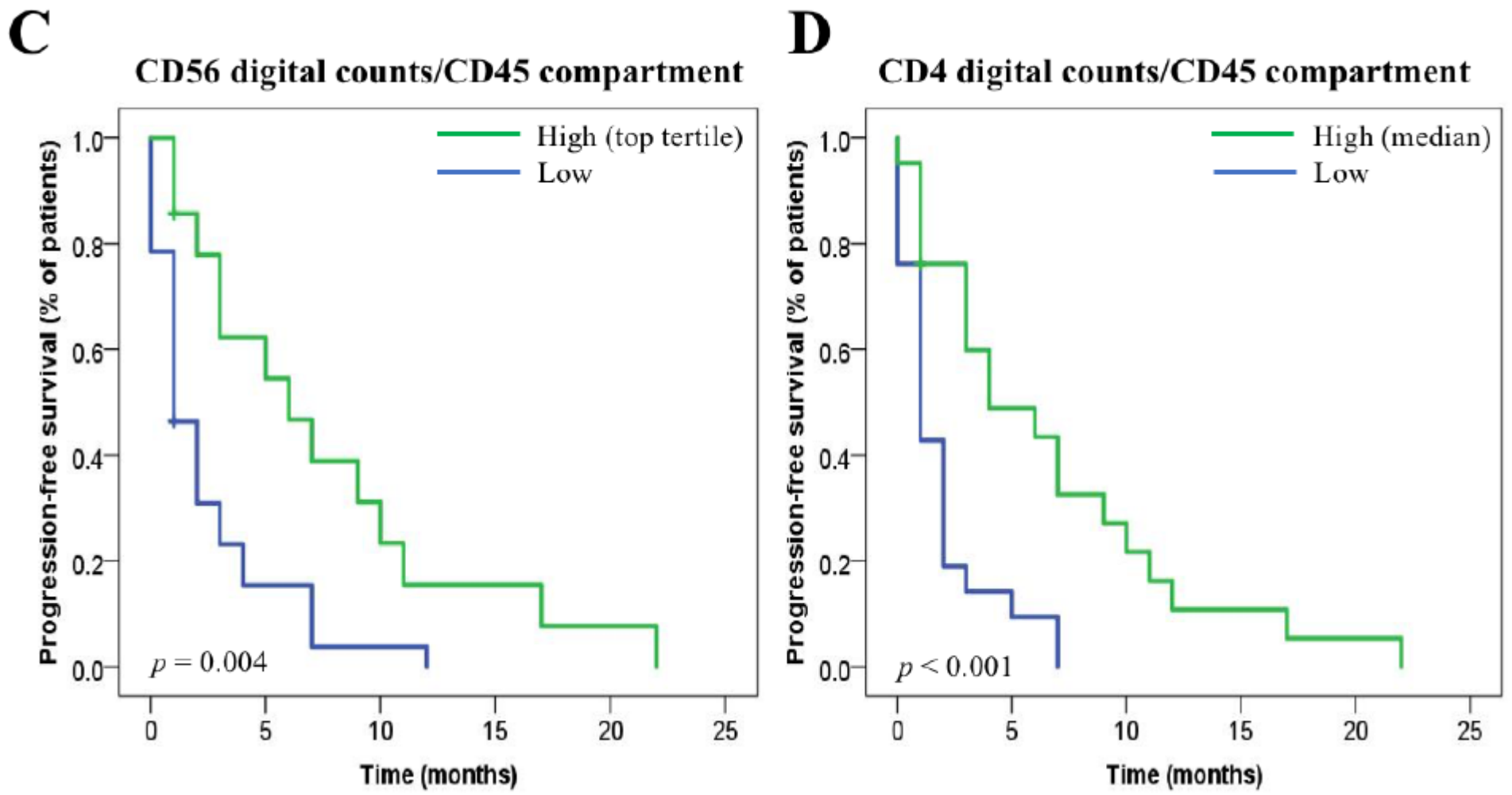
ROI Selection Strategy





Zugazagoitia et al., *Clin Can Res* 2020

Patient Outcome Associated with Compartment Specific Expression Profile



Zugazagoitia et al., *Clin Can Res* 2020

Identification of Predictive Biomarkers in Melanoma

Background

- Either single-agent or combinations of Immune checkpoint inhibitors are now regularly used in the clinic for the treatment of melanoma.
- Given the significant number of patients that are non-responsive to (ICI) Immune Checkpoint Inhibitors, the inflammatory toxicities and the lack of ability to satisfactorily predict either, there is a necessity to discover more predictive biomarkers

Experimental Question and Design

- Using tissue microarrays from pre-treatment biopsies, can DSP reveal novel predictive biomarkers either singularly or in combination across multiple biological compartments of the tumor and its immune microenvironment?
- The expression of 44 proteins was measured in the macrophage, T-cell and melanocyte compartments in the melanoma biopsies of 59 patients that underwent ICI therapy.

David Rimm, MD, PhD
Professor of Pathology and Medicine
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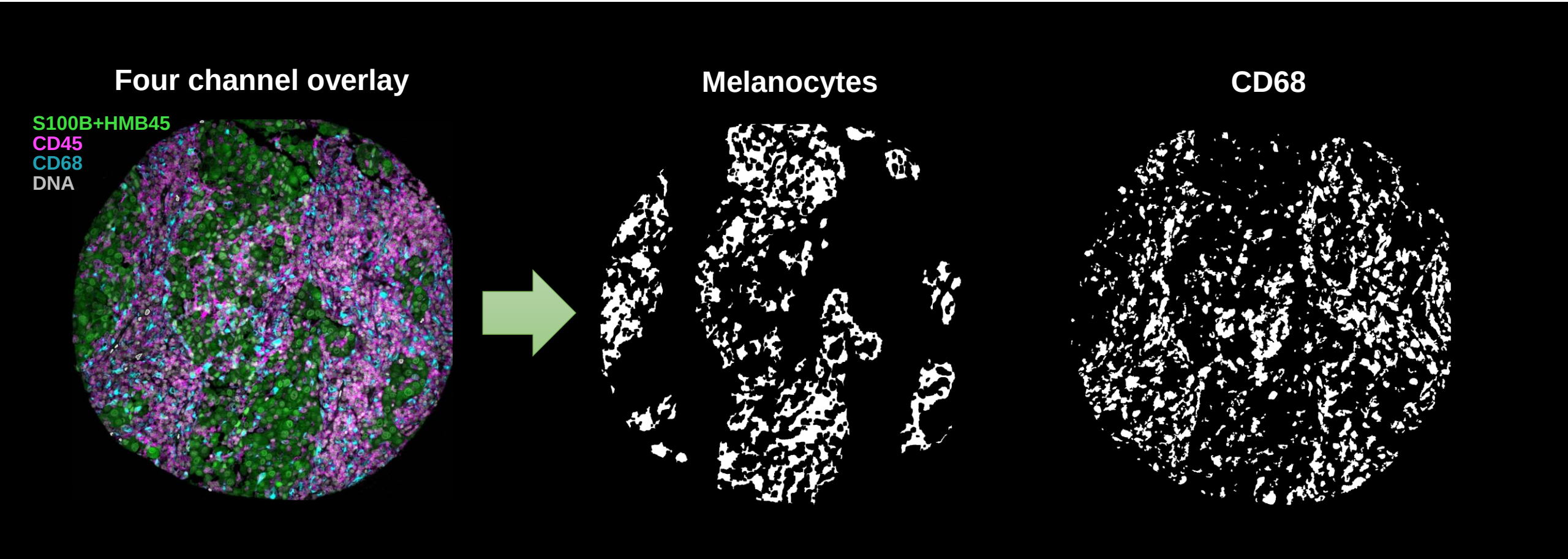
Director of Translational Pathology
Yale University

ROI Selection Strategy

- **Serial masks for each TMA core:**
  - 1. CD68 (macrophages)
  - 2. CD45 (all lymphocytes except macrophages)
  - 3. S100B+PMEL17 (melanocytes)
  - 4. DNA (non-tumor/non-lymphocyte cells) – remaining material from patient



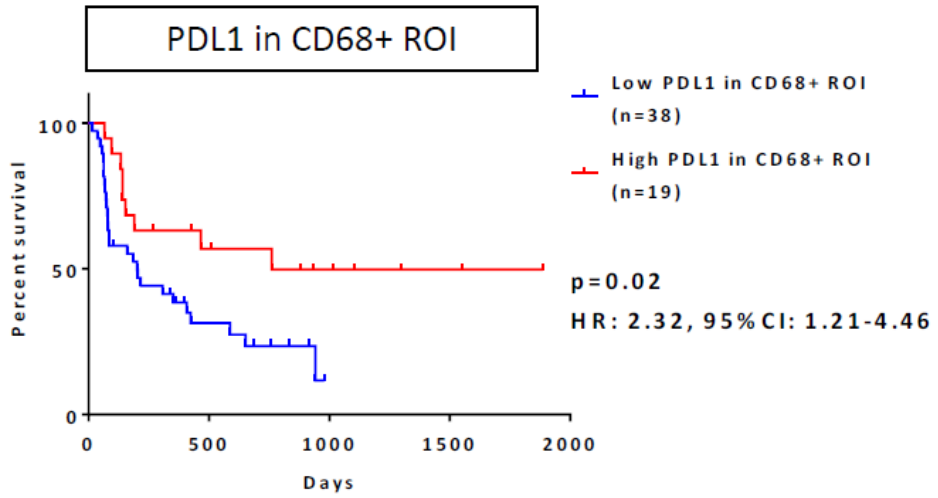
Patient Outcome Associated with Compartment Specific Expression Profile

- **Several probes, primarily in the stromal compartments, are associated with patient outcome**
  - PDL1 showed strongest association with OS in the CD68 compartment
  - B2M in CD45 compartment was associated with both PFS and OS as well
  - Results are independent of prior treatment

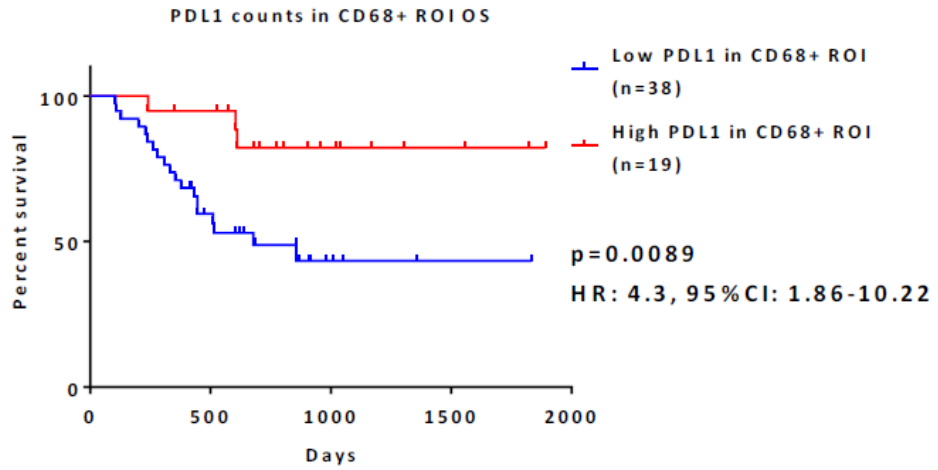
Multivariate Analysis

Probe	Mask	Outcome	P-value
B2M	CD45	PFS	**0.013**
PD1	CD45	PFS	*0.08*
IDO1	Tumor	PFS	0.1
PDL1	CD68	OS	**0.032**
B2M	CD45	OS	*0.055*
CD20	Tumor	OS	0.11

PFS



OS



GeoMx™ Digital Spatial Profiler

GeoMx@nanostring.com