

Morphology Driven High-Plex Spatial Analysis of Tissue Microenvironments

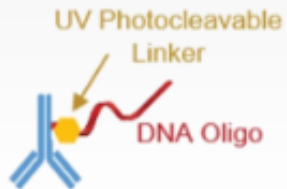
For SITC Spatial Biology Award

September 2019

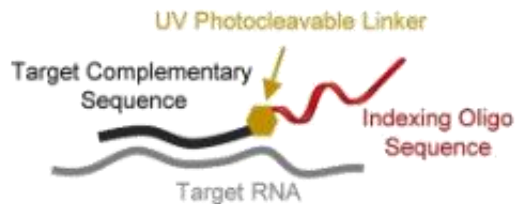
GeoMx™ DSP Enables Spatial, High-Plex Protein & RNA Profiling

High-Plex Mixtures of Proprietary Reagents

Protein reagents
Oligo-labeled antibodies



RNA reagents
Oligo-labeled probe



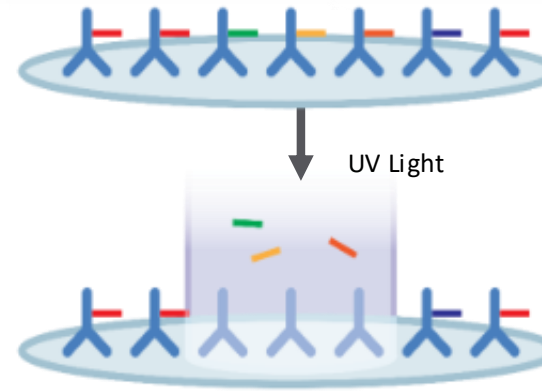
Profile Regions of Interest on FFPE slide

GeoMx™ Digital
Spatial Profiler

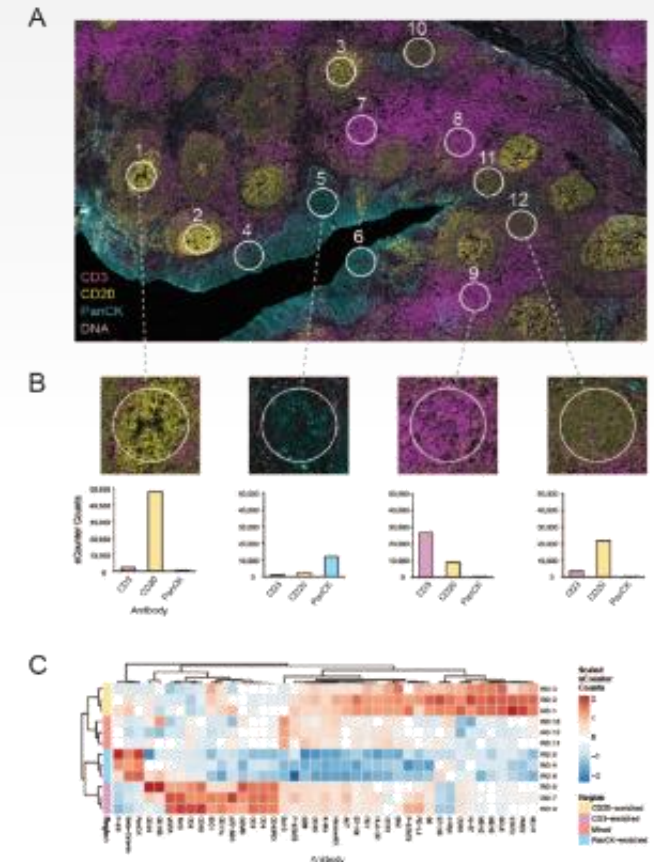


Label FFPE Slide
with Probe Mix

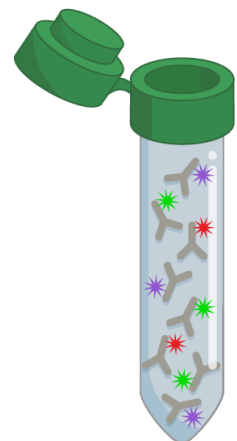
Illuminate
Region of Interest,
as Small as a
Single Cell



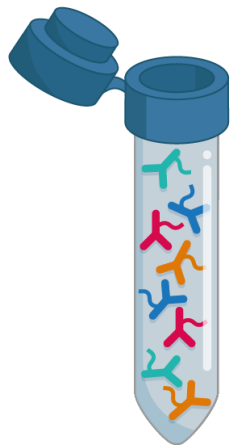
Rich Data Sets of Biology, Region by Region



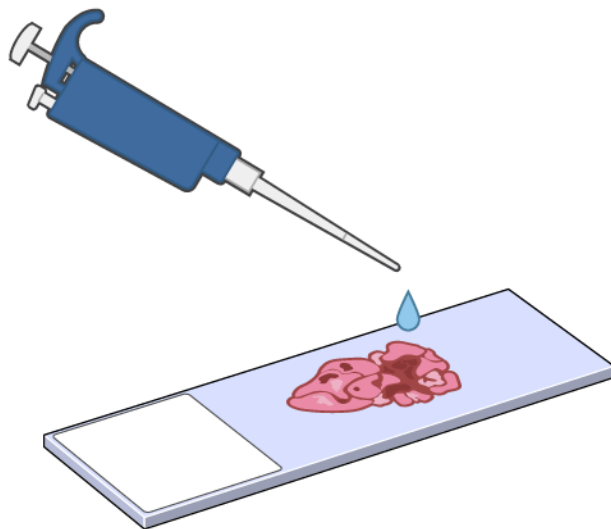
Imaging and Profiling in One Assay



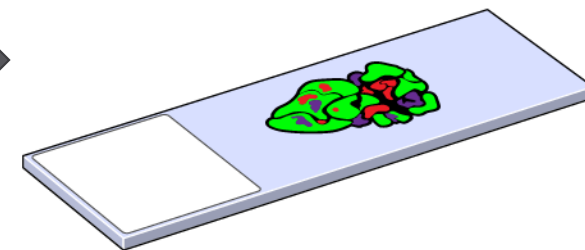
Imaging Reagents



Profiling Reagents



Sample Preparation

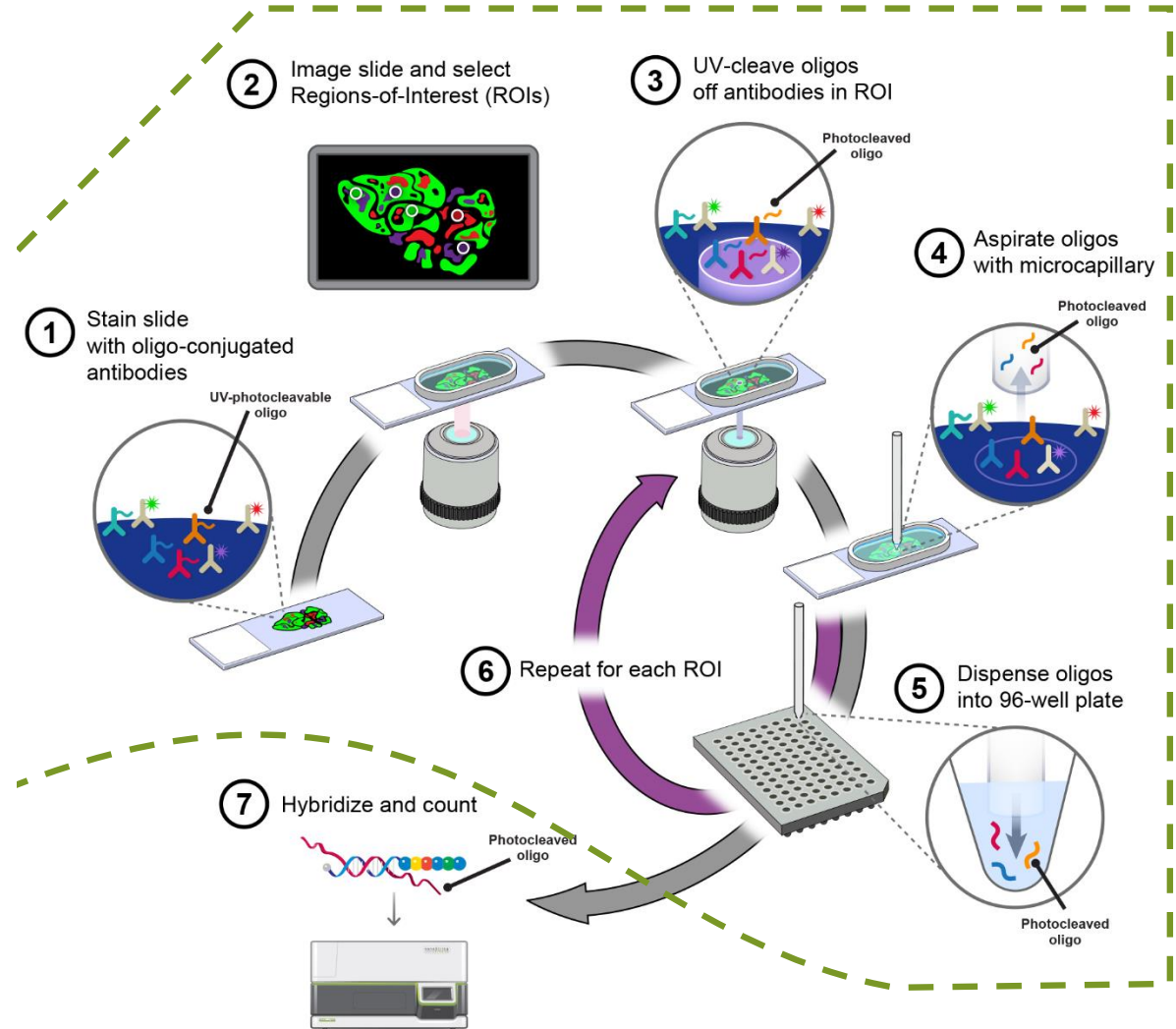


DSP Ready

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



GeoMx Digital Spatial Profiler
Your GPS for Immuno-Oncology



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DSP GeoMx has Numerous Advantages

Morphology driven profiling



Seamless integration with
current pathology workflow

Multiplex

Many analytes on one tissue slice in a single pass

Multi-Analyte

High plex spatial analysis of both protein and RNA

Quantitative

Simple counts delivered to you in an elegant
integrated software

High resolution

Limit of detection down to single cell

Non-destructive

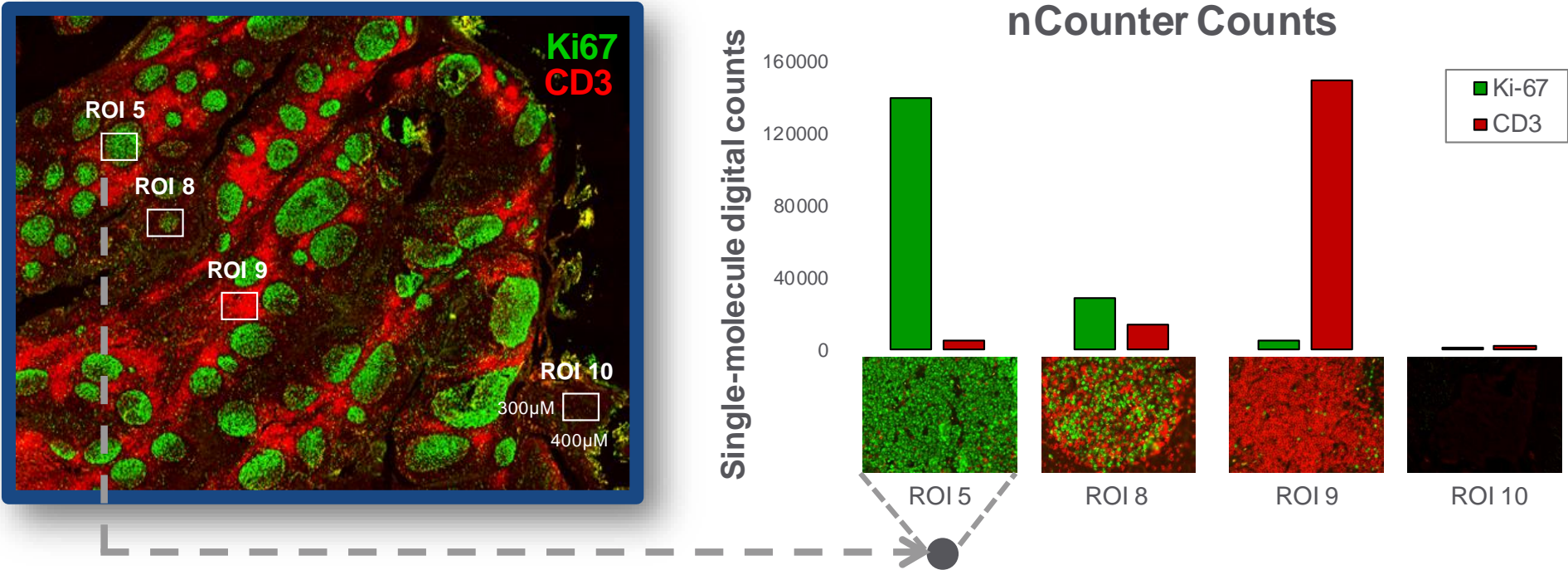
Sample is only touched by light

Throughput

Up to 12 sections per day

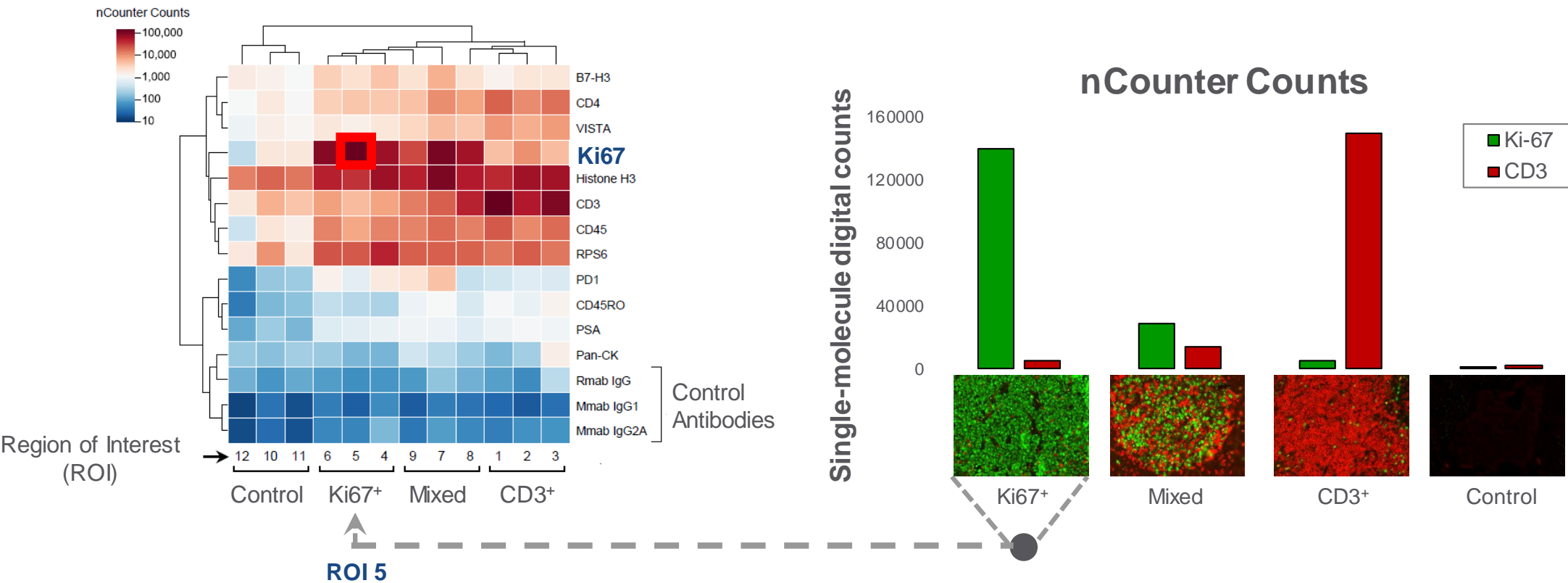
Single-molecule Counting of Photocleaved Oligos

Lymphoid Tissue

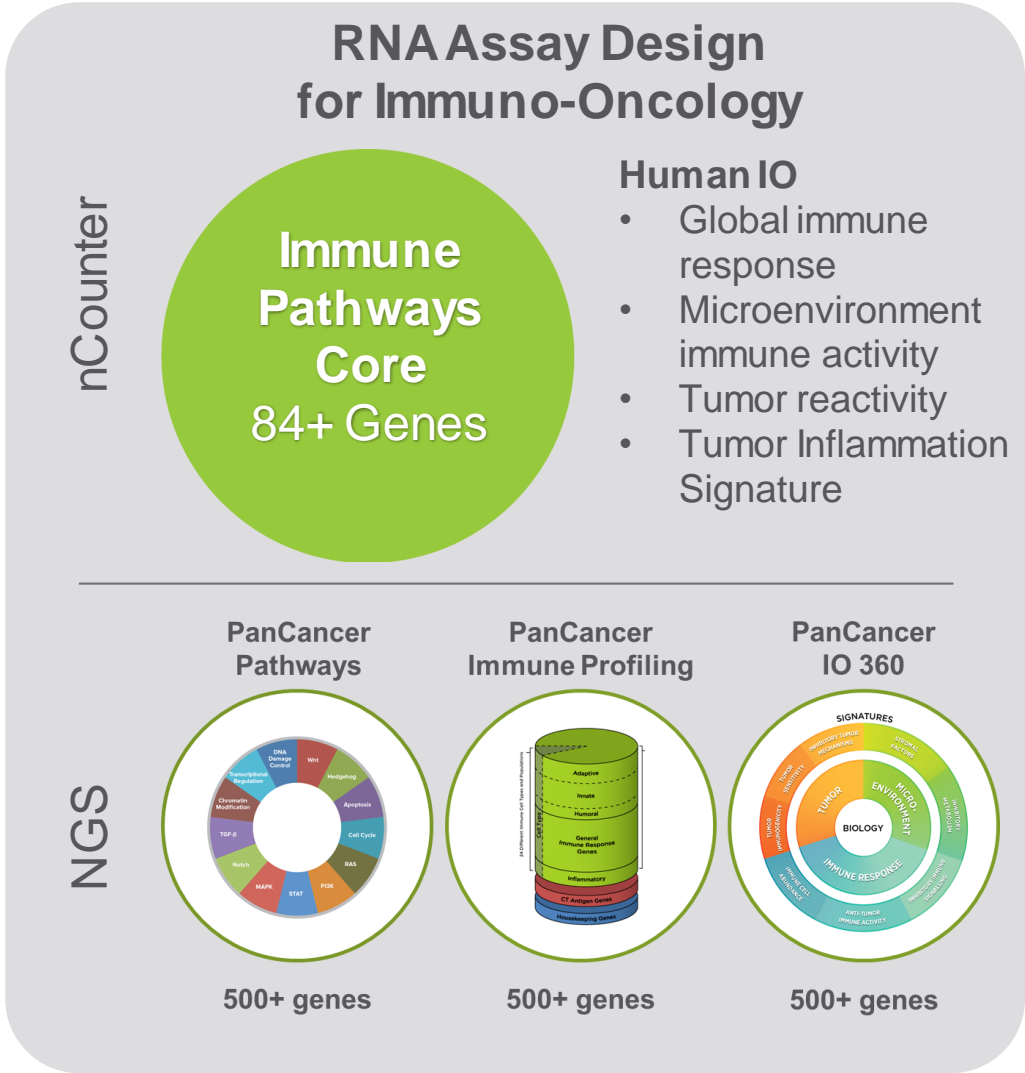
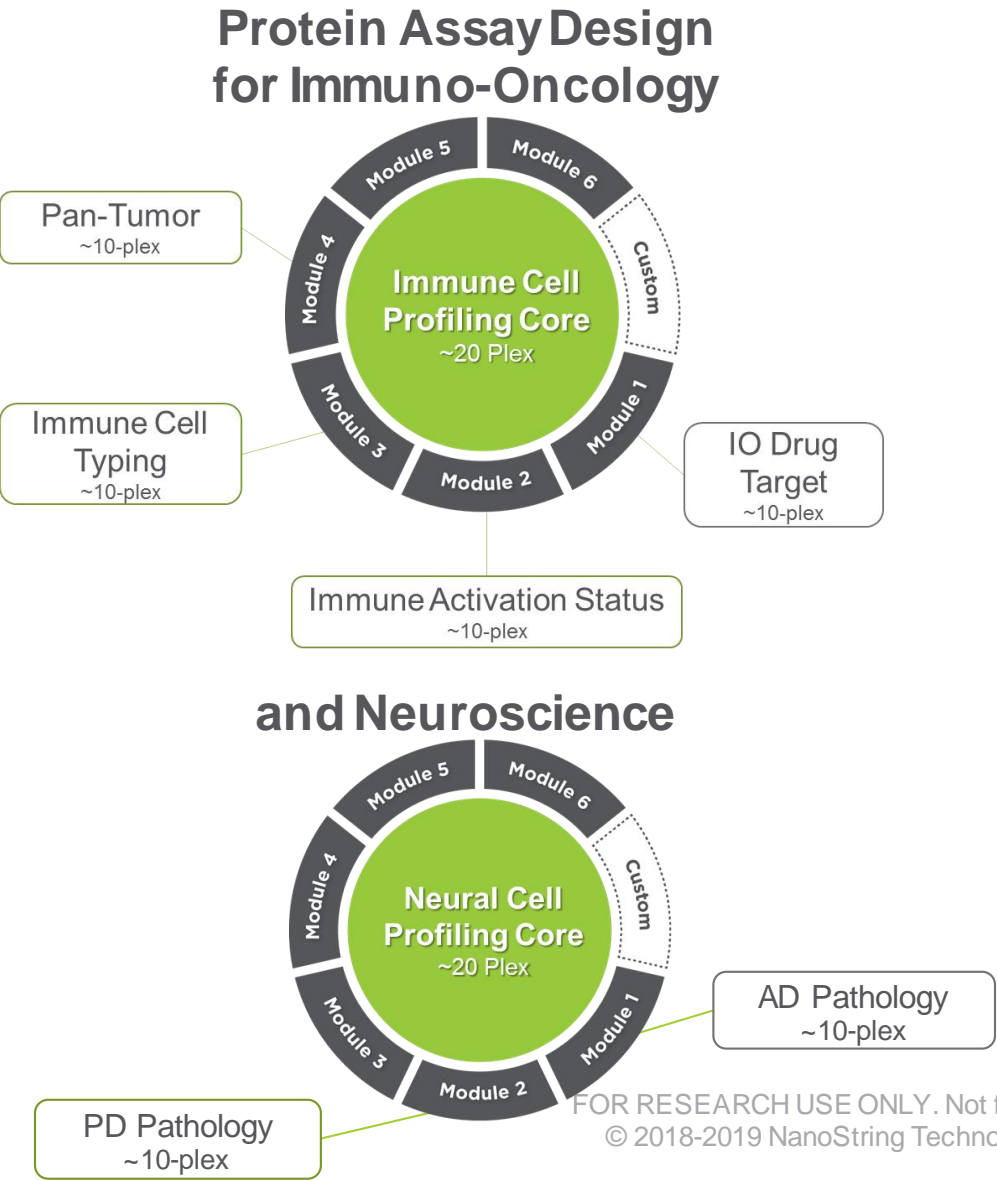


Single-molecule Counting of Photocleaved Oligos

Lymphoid Tissue



Flexible and validated content design to fit a range of applications and plex needs



Currently Available IO Protein Content

GeoMx™ Immune Cell Profiling Panel Human Protein Core for nCounter®
PD-1
CD68
HLA-DR
Ki-67
Beta-2-microglobulin
CD11c
CD20
CD3
CD4
CD45
CD56
CD8
CTLA4
GZMB
PD-L1
PanCk
SMA
Fibronectin
Controls
Rb IgG
Ms IgG1
Ms IgG2a
Histone H3
S6
GAPDH

GeoMx™ IO Drug Target Panel Human Protein Module for nCounter®
4-1BB
LAG3
OX40L
Tim-3
VISTA
ARG1
B7-H3
IDO1
STING
GITR

GeoMx™ Immune Cell Typing Panel Human Protein Module for nCounter®
CD45RO
FOXP3
CD34
CD66b
FAP-alpha
CD14
CD163

GeoMx™ Immune Activation Status Panel Human Protein Module for nCounter®
CD127
CD25
CD80
ICOS
PD-L2
CD40
CD44
CD27

GeoMx™ Pan-Tumor Panel Human Protein Module for nCounter®
MART1
NY-ESO-1
S100B
Bcl-2
EpCAM
Her2
PTEN
ER-alpha
PR

GeoMx™ Immune Cell Profiling Panel Mouse Protein Core for nCounter®
PD-1
MHC II
CD11b
Ki-67
F4/80
CD11c
CD19
CD3e
CD4
CD45
CD8a
CTLA4
GZMB
PD-L1
PanCk
SMA
Fibronectin
Controls
Rb IgG
Rt IgG2a
Rt IgG2b
Histone H3
S6
GAPDH

GeoMx™ IO Drug Target Panel Mouse Protein Module for nCounter®
LAG3
OX40L
Tim-3
VISTA
B7-H3
GITR

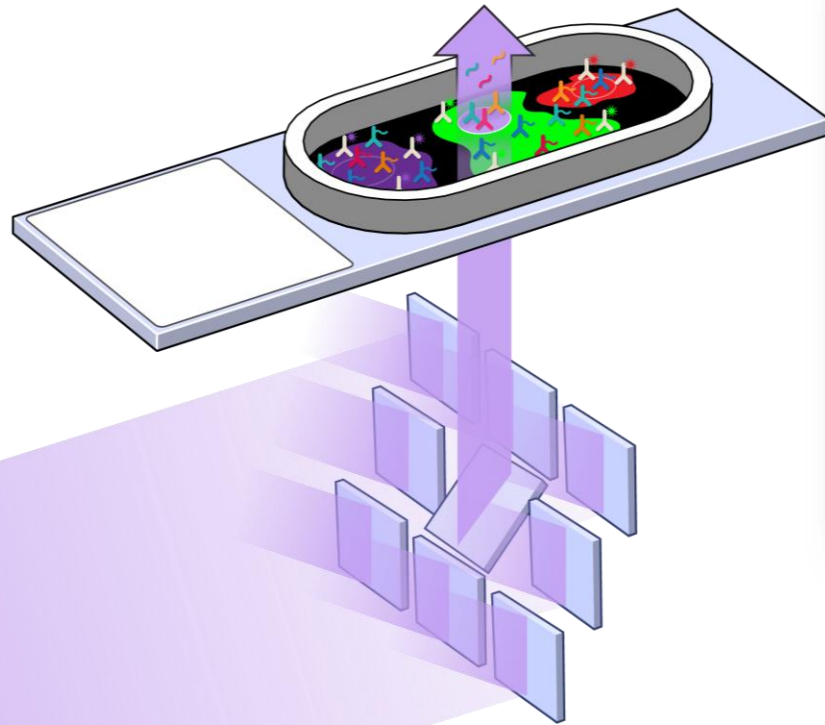
Currently Available IO RNA Content

GeoMx™ Immune Pathways Panel

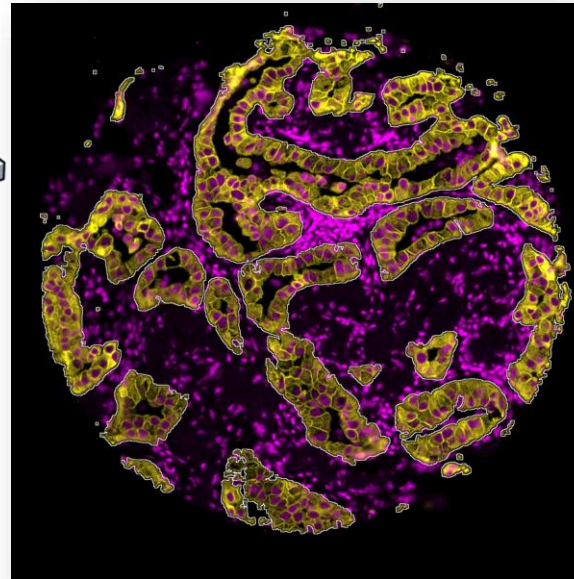
Human RNA Core for nCounter®

AKT1	CD3E	CMKLR1	FOXP3	IFNAR1	ITGB8	PECAM1	TNFRSF9
ARG1	CD4	CSF1R	GZMB	IFNG	KRT	PSMB10	VEGFA
B2M	CD40	CTLA4	HAVCR2	IFNGR1	LAG3	PTEN	VSIR
BATF3	CD40LG	CTNNB1	HIF1A	IL12B	LY6E	PTPRC	Internal Reference Genes
BCL2	CD44	CXCL10	HLA-DQ	IL15	MKI67	STAT1	
CCL5	CD47	CXCL9	HLA-DRB	IL6	MS4A1	STAT2	
CCND1	CD68	CXCR6	HLA-E	ITGAM	NKG7	STAT3	
CD27	CD74	DKK2	ICAM1	ITGAV	pan-melanocyte	TBX21	
CD274	CD86	EPCAM	ICOSLG	ITGAX	PDCD1	TIGIT	
CD276	CD8A	FAS	IDO1	ITGB2	PDCD1LG2	TNF	

Optical Technology Enables Flexible ROI Selection



Stain



Mask

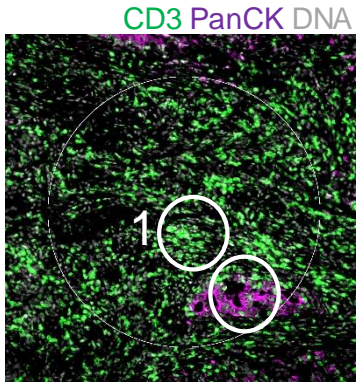


Inverse



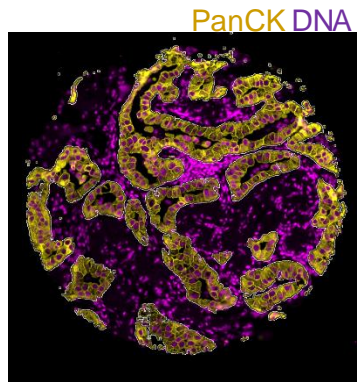
Five Unique Profiling Modalities Designed to Interrogate Tissue Samples

Geometric



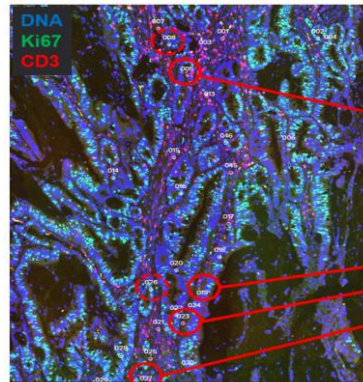
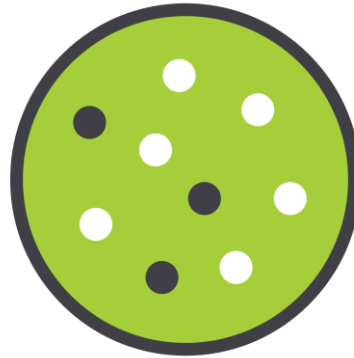
What is the heterogeneity of expression in different regions of my tissue?

Segmentation



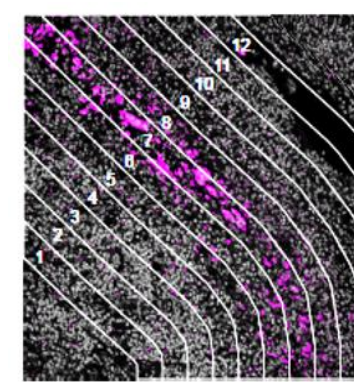
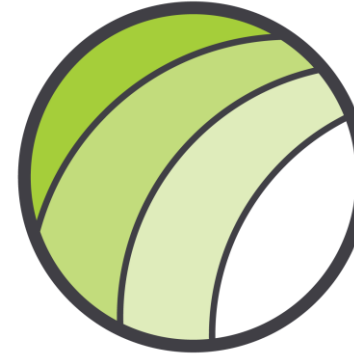
What is the expression profile of distinct biological compartments (e.g., Tumor-TME)?

Rare cell



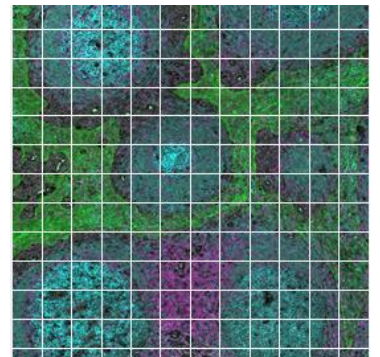
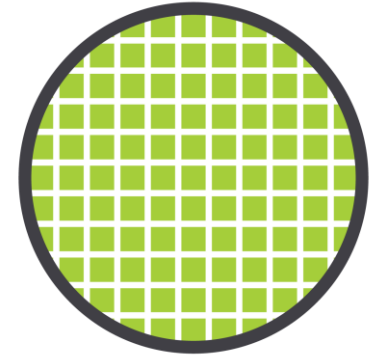
What is the expression profile of a specific cell population in my tissue?

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?

Integrating bulk and spatial profiling technologies for the discovery of RNA and protein biomarkers in muscle invasive bladder cancer



- **Background**

- ~80% of patients with muscle-invasive bladder cancer (MIBC) fail to respond to immunotherapies.
- This suggests existence of complementary immune evasion mechanisms.
- There is a need for comprehensive immune profiling of patient tumor samples.

- **Experimental Questions/Goals**

- Technical evaluation of bulk vs spatial genomics for assessing tumor microenvironment and defining MIBC molecular subtypes.
- Define mechanisms of immune evasion in PPAR γ ^{High} MIBC.
- Evaluate the role of TMB (tumor mutation burden) and tumor somatic mutations on immune evasion and PPAR γ expression.
- Establish a solution for comprehensive immune profiling in clinical samples where tissue is limiting.



Victoria Rimkunas, PhD

Associate Director

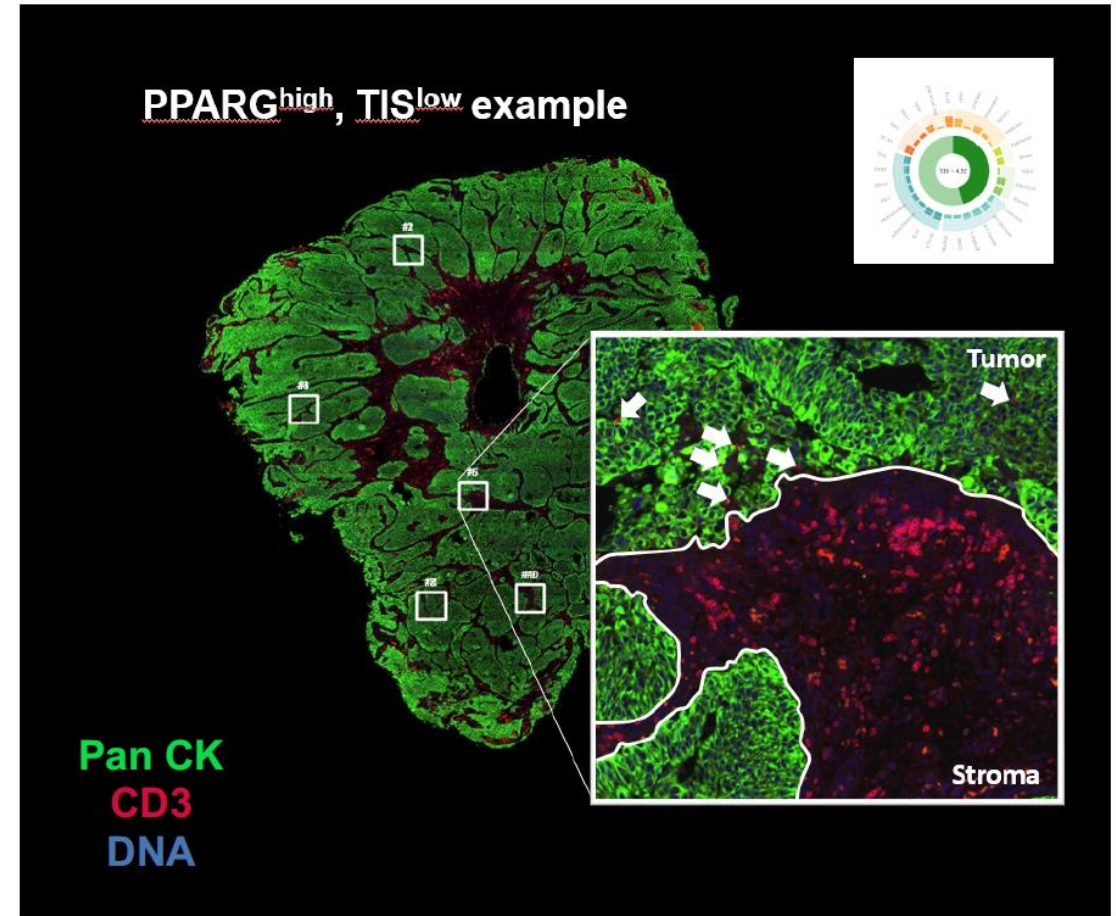
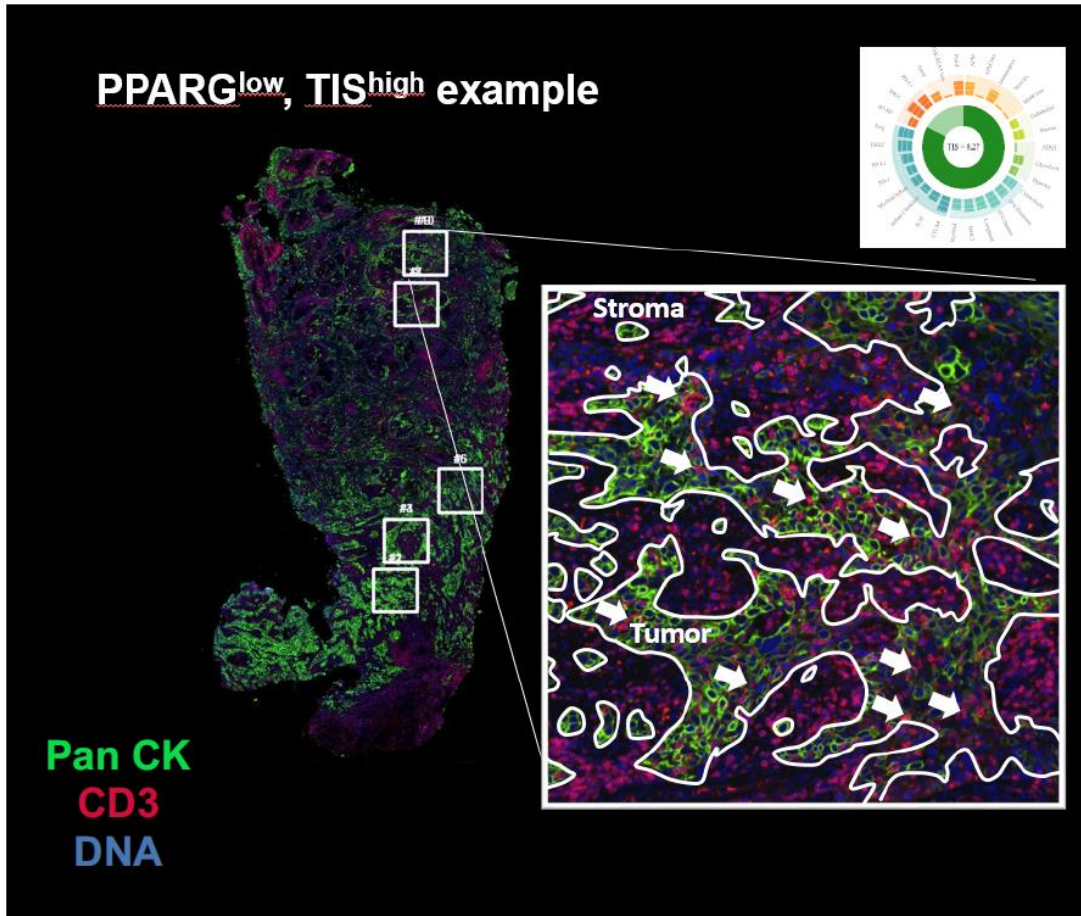
H3 Biomedicine

(Now: Director at Repair Therapeutics)

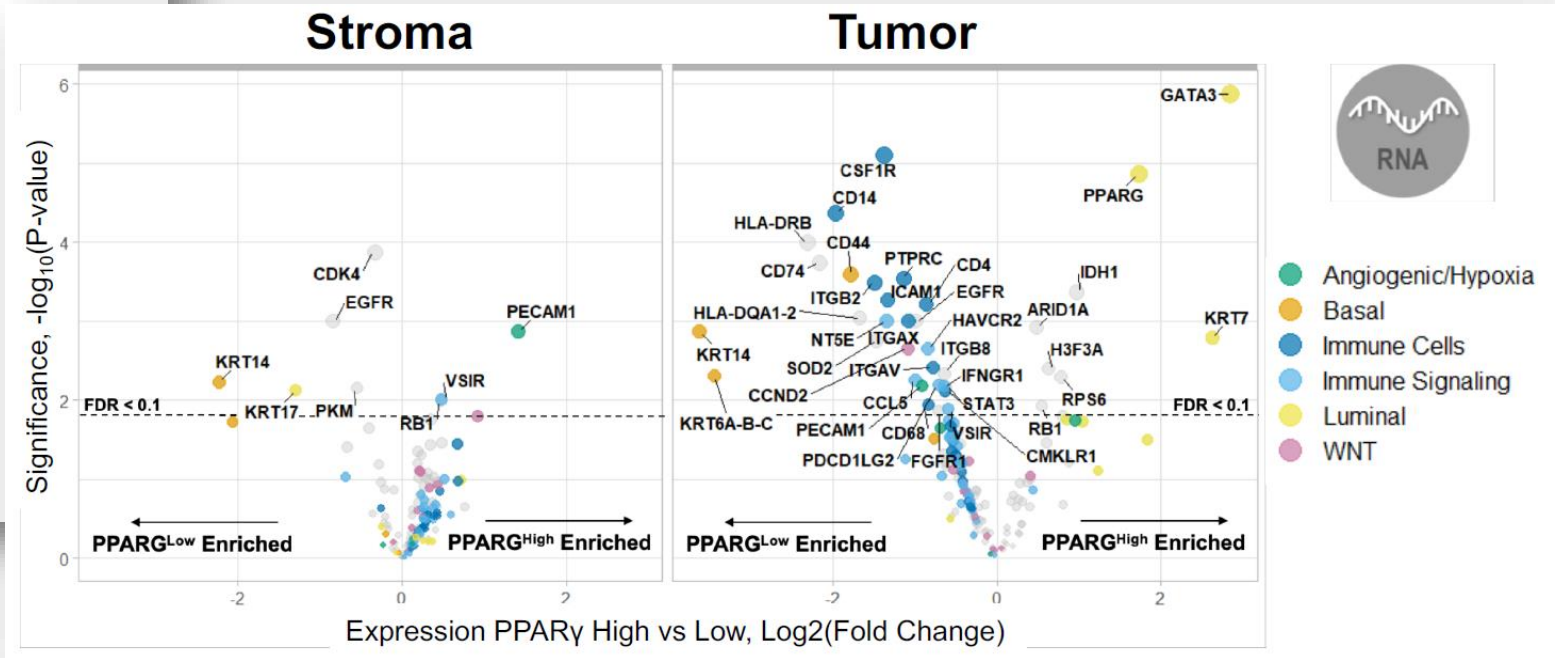
ROI Selection and Segmentation



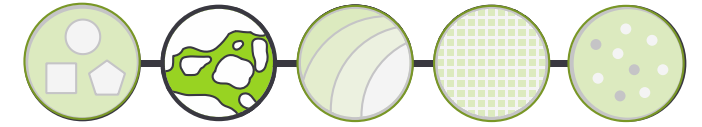
- Auto-segmentation for tumor (Pan-CK+) and Stroma (Pan-CK-)



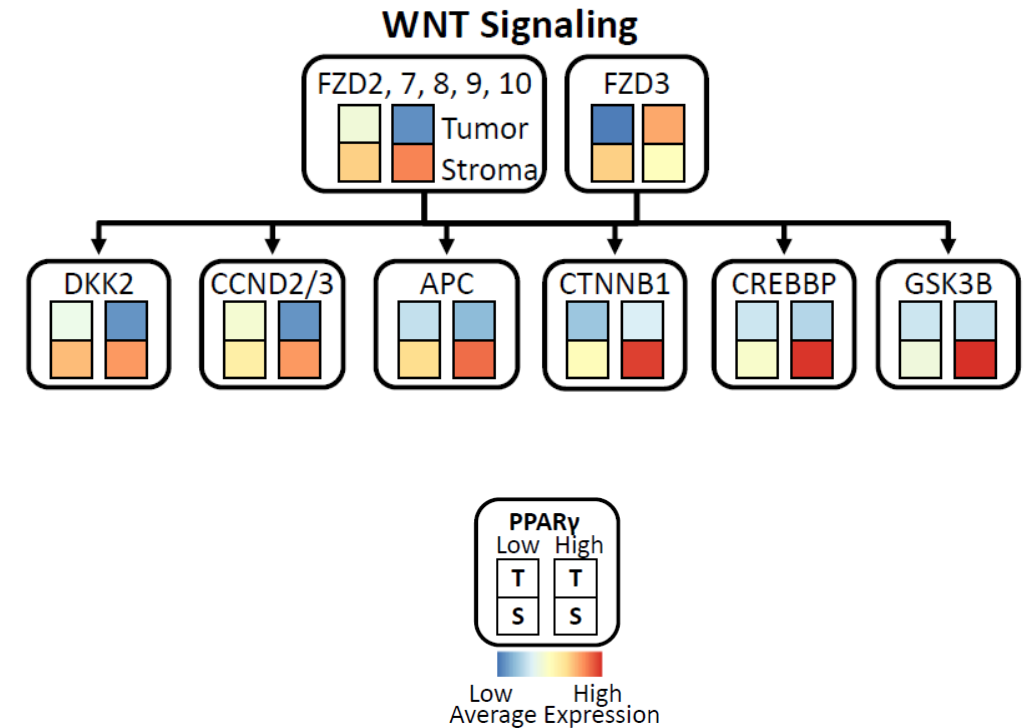
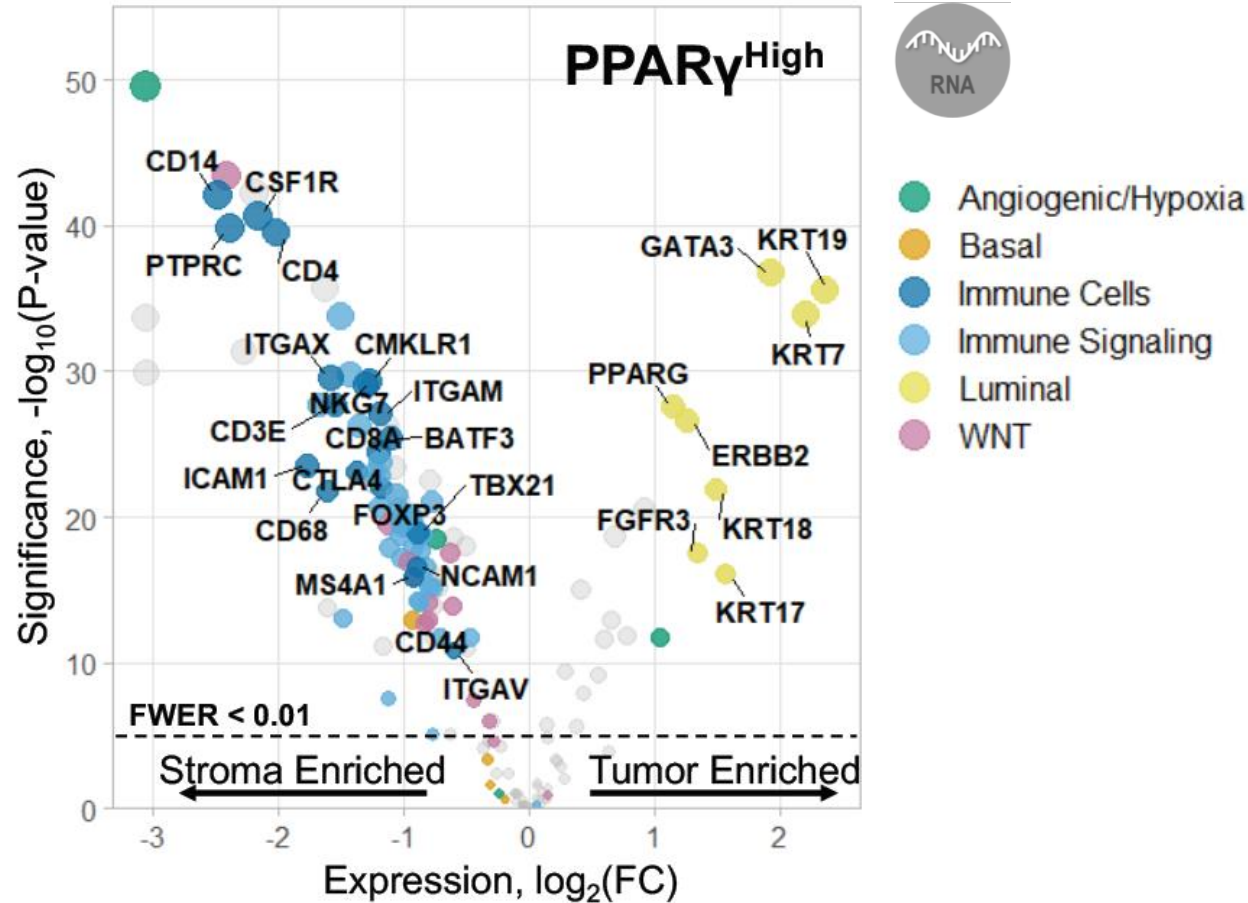
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Pathway analysis



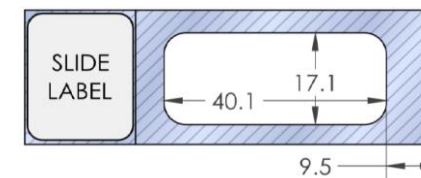
- Wnt signaling is enriched in stromal compartment of $\text{PPAR}\gamma^{\text{High}}$ tumors



Tip for Success

- **General Tips**

- Larger ROI give more robust counts
- 2 samples can be analyzed on 1 slide if they are both mounted within viewing area (white below)



- **Protein Profiling**

- Robust data achieved with at least 50 cells per ROI
- Visualization Markers Used Successfully: CD45, CD3E, CD8, CD68, CD11c, S100B+HMB45 (pan-melanoma), panCK, pan-cadherin, and more (just ask)

- **RNA Profiling**

- Robust profiling achieved with 200 cells per ROI
- Visualization Markers Used Successfully: CD8A, CD3E, CD68, PanCK AE1+AE3, DNA dye (e.g. Syto13)
- Others may also work but have not been tested

DSP GeoMx has Numerous Advantages

Morphology driven profiling



Seamless integration with
current pathology workflow

Multiplex

Many analytes on one tissue slice in a single pass

Multi-Analyte

High plex spatial analysis of both protein and RNA

Quantitative

Simple counts delivered to you in an elegant
integrated software

High resolution

Limit of detection down to single cell

Non-destructive

Sample is only touched by light

Throughput

Up to 12 sections per day

GeoMxTM Digital Spatial Profiler

GeoMx@nanosttring.com