GeoMx DIGITAL SPACE PROFILER: APPLICATIONS EXAMPLES

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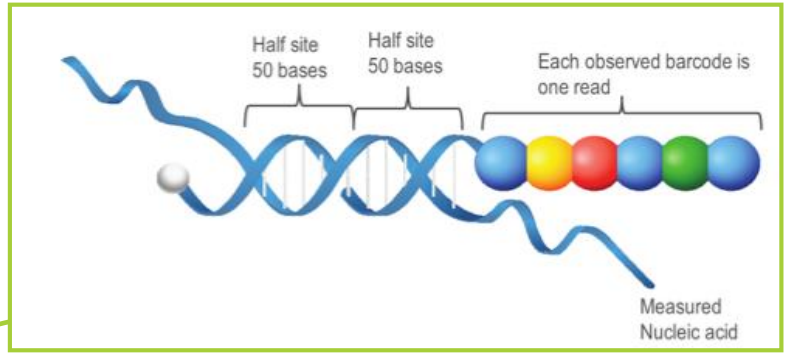
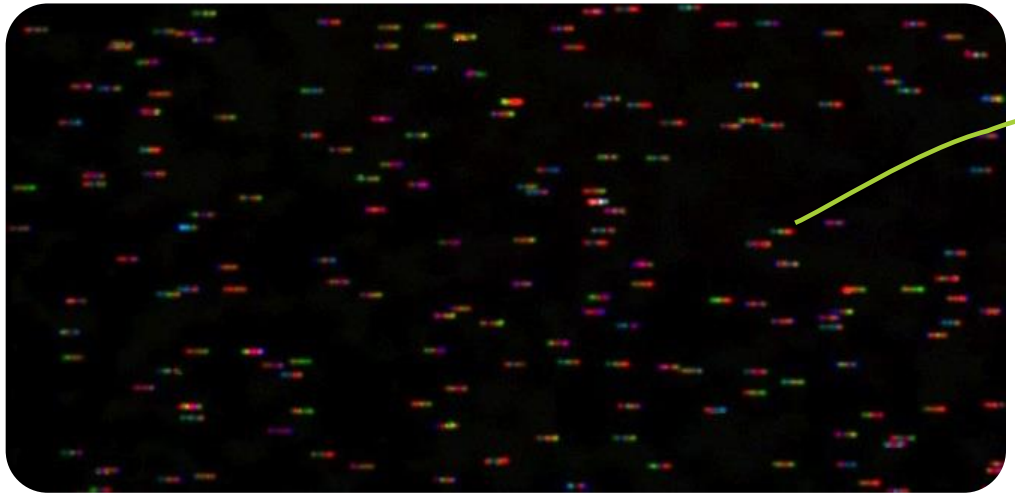
Digital Pathology & AI
London, 07/12/2018

1 Digital counting of single molecules

- Probes up to 800 genes simultaneously
- **Digital gene expression** applied to biological pathways



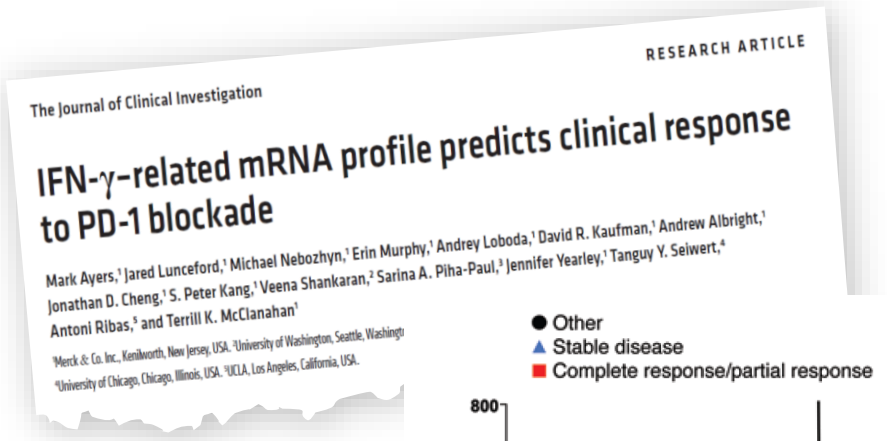


nanoString
TECHNOLOGIES

Single molecule fluorescent barcodes
each attached to an individual nucleic acid molecule

1 Molecular imaging: for what?

BULK ANALYSIS


RESEARCH ARTICLE

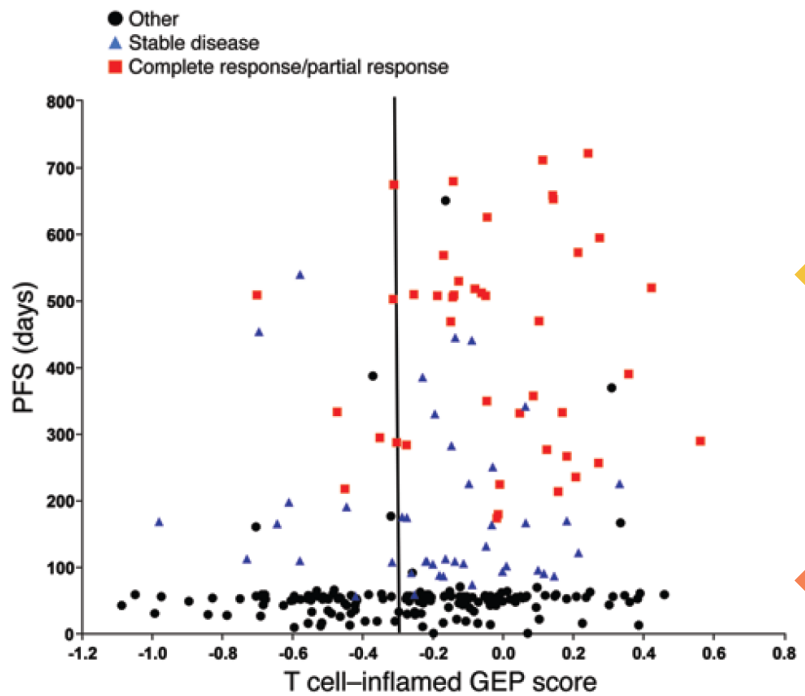
The Journal of Clinical Investigation

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, Washingt
[4]University of Chicago, Chicago, Illinois, USA. [5]UCLA, Los Angeles, California, USA.

18 genes



Non-inflamed tumors **rarely respond**

Almost all responses have T-cell inflamed tumors

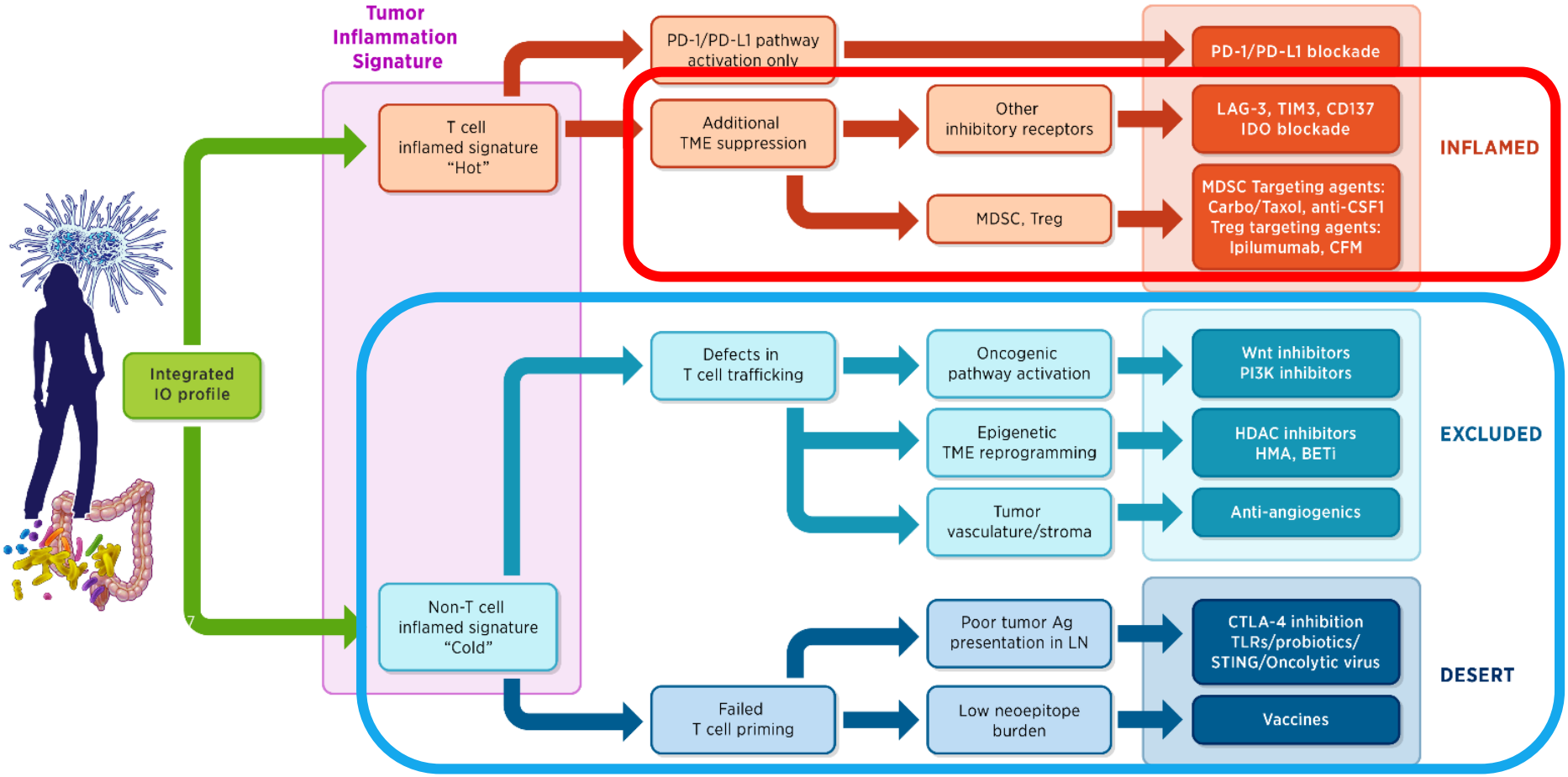
Not all inflamed tumors respond

Ayers et al. Journal of Clinical Investigation. 2017. https://doi.org/10.1172/JCI91190

1 Molecular imaging: for what?

770 genes

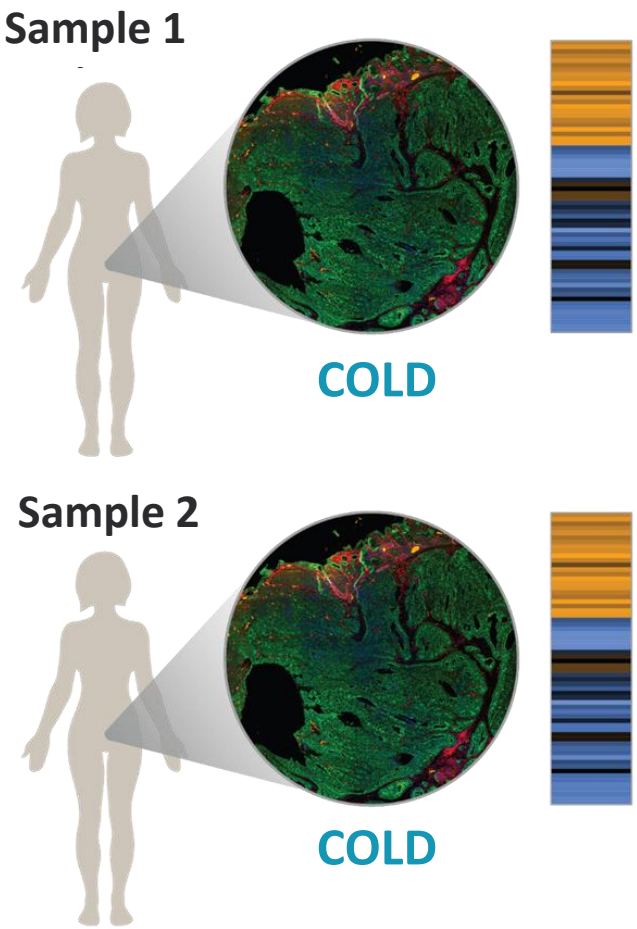
BULK ANALYSIS



Ayers et al. Journal of Clinical Investigation. 2017. https://doi.org/10.1172/JCI91190

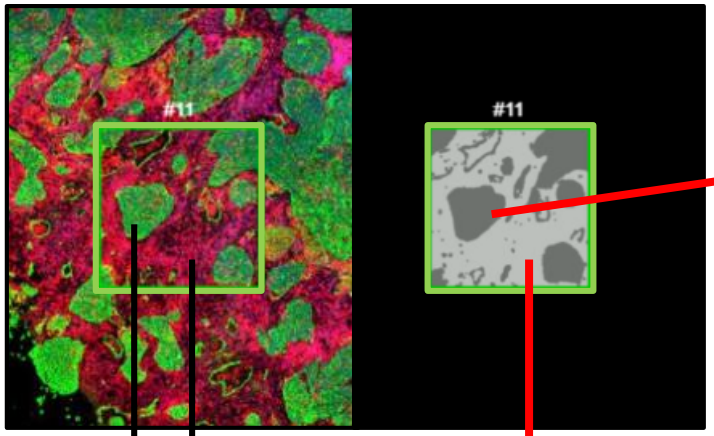
1 Molecular imaging: for what?

SPATIALLY RESOLVED ANALYSIS



TRUE PERSONALIZATION of IMMUNOTHERAPY

1 Molecular imaging: for what?



High levels of STAT3 expression in tumor only indicates highly proliferative tumor associated with poor prognosis

Treatment options may include anti-STAT3 agents like JAK1/2 inhibitors to block STAT3

TME

Tumor

High levels of STAT3 expression in MDSCs have immunosuppressive effects and associated with poor prognosis

Treatment options may include removal of MDSCs using CSF-1R

Jim Allison (MDAnderson)
Nobel Prize Medicine (2018)

Tumor vs Tumor microenvironment

Myeloid-derived suppressor cells (MDSCs)

1 Molecular imaging: challenges

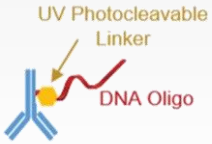
- Keeping the **tissue architecture to integrate spatial information** (and avoid microdissection)
- **Resolution** (down to single cell)
- High **multiplexing** (more is better)
- **Sensitivity & dynamic range** (for RNA+++)
- **Multiple analytes** (RNA + proteins)
- **Customization of panels / No panel** (discovery)
- **Cost-effectiveness**

2 GeoMx Digital Space Profiler

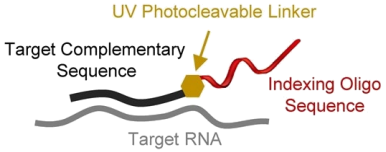
FONDATION
Hospices Civils de Lyon

High Plex Mixtures of Proprietary Reagents

Protein reagents
Oligo-labeled antibodies



RNA reagents
Oligo-labeled probe



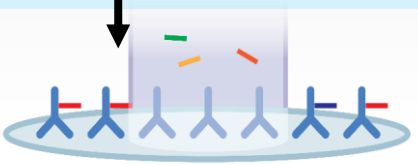
Profile Regions of Interest on FFPE slide

How it Works

Label FFPE Slide with Probe Mix

UV Light

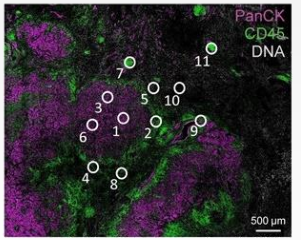
Illuminate Region of Interest, as Small as a Single Cell

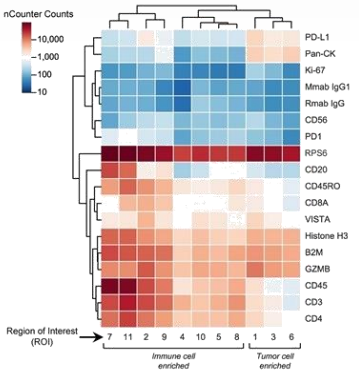


Analyze Barcodes on nCounter

Rich Data Sets of Biology, Region by Region

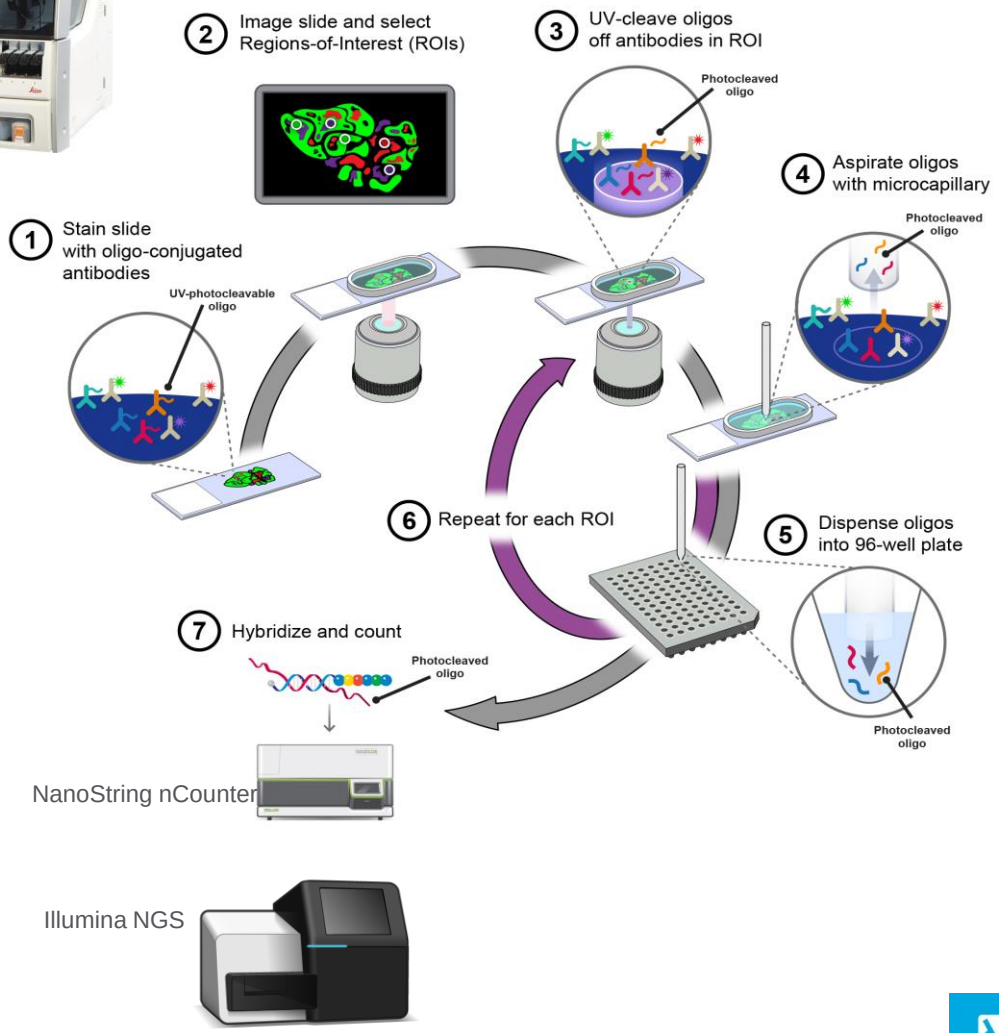
Lung Cancer (NSCLC)





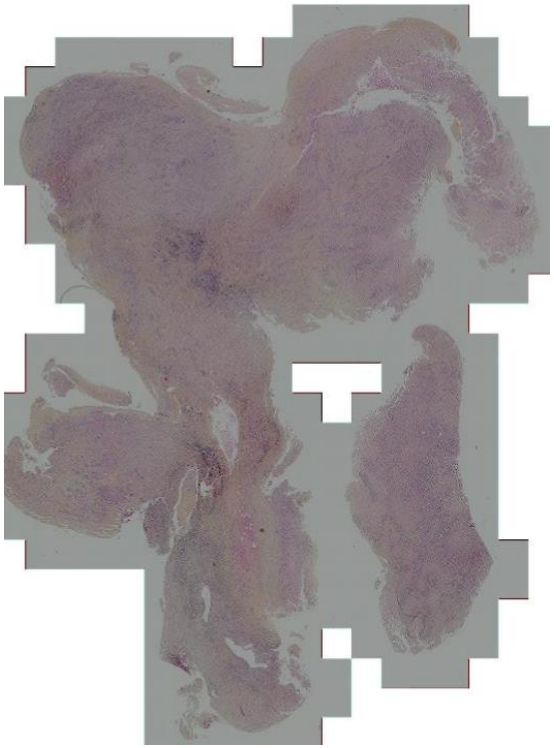
❷ GeoMx Digital Space Profiler

GeoMx™ Digital Spatial Profiler
Your GPS for Spatially-Resolved Biology



❷ GeoMx DSP: Imaging

16SD07504



H & E

fluorescence

20X overlay

Visualization markers:

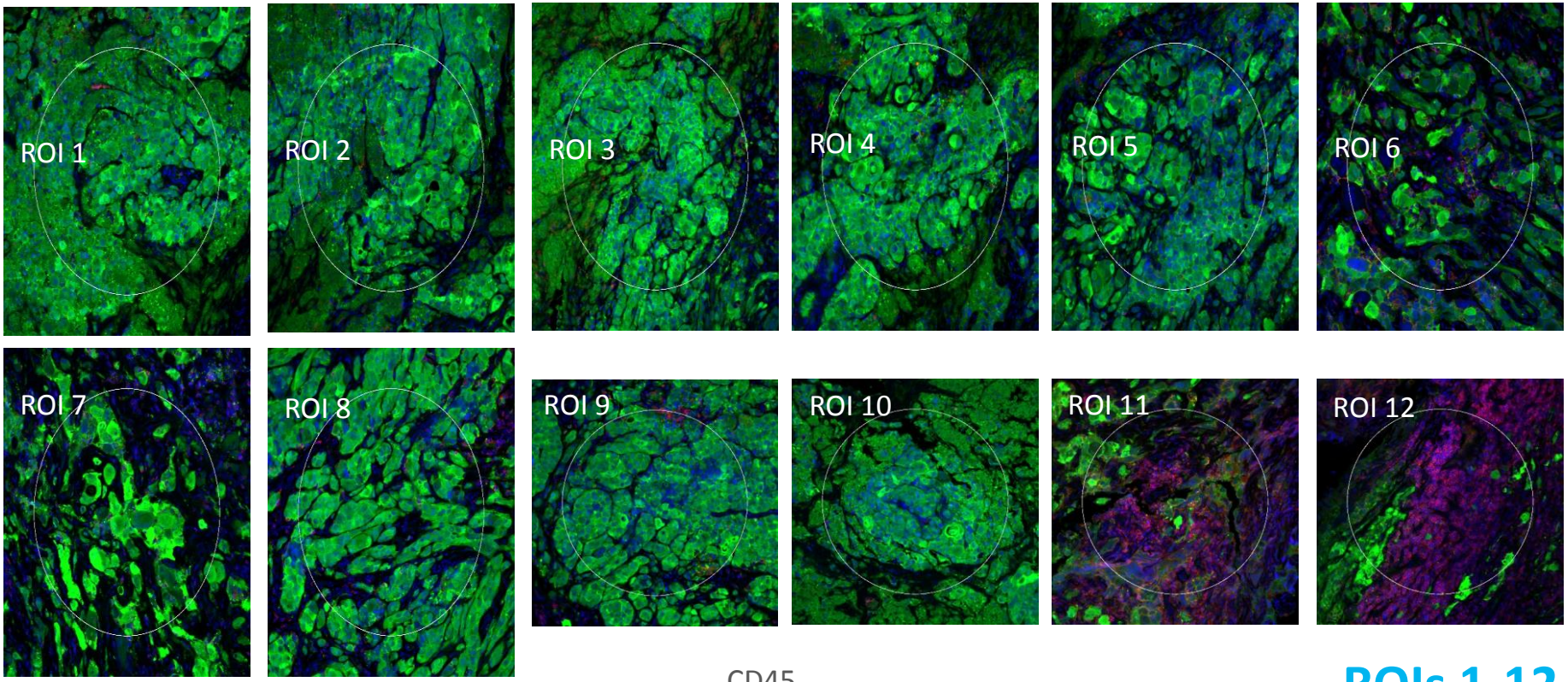
CD45 (red),

S-100B (green),

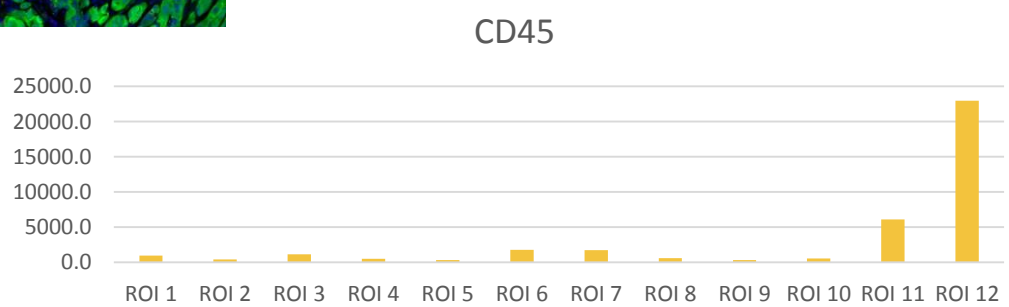
DNA (blue)

Technology Access Program: Melanoma treated with anti-PD1+ IDO1 inhibitor

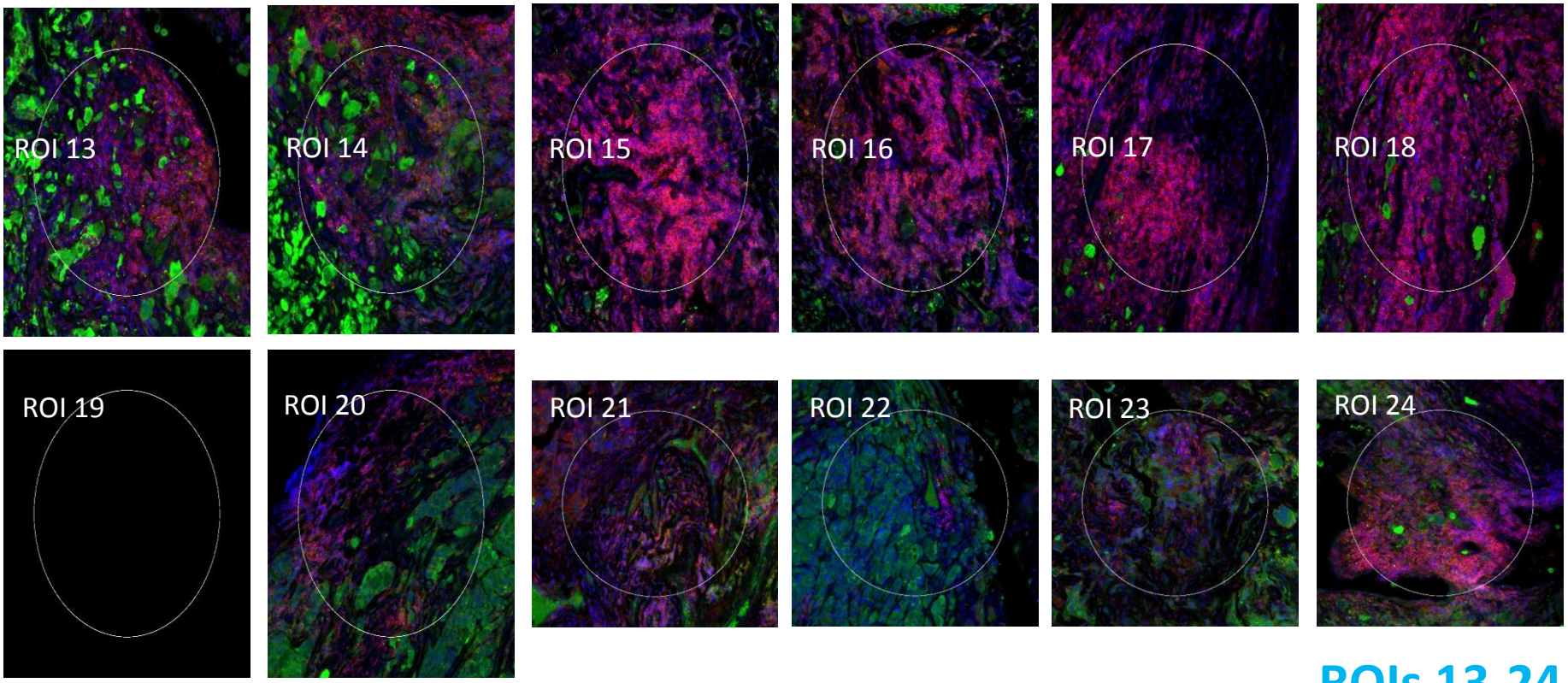
2 GeoMx DSP: Imaging



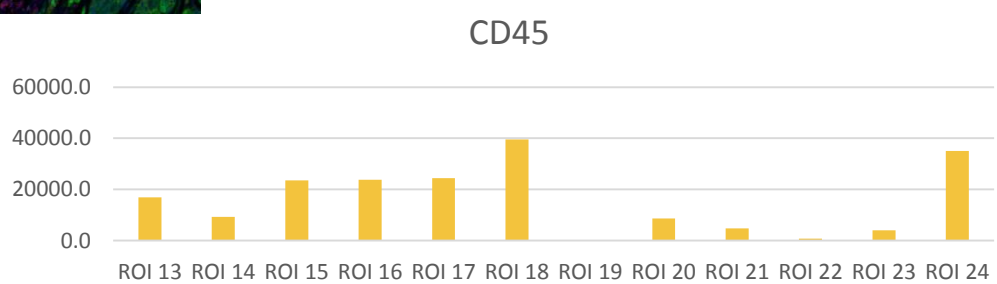
ROIs 1-12



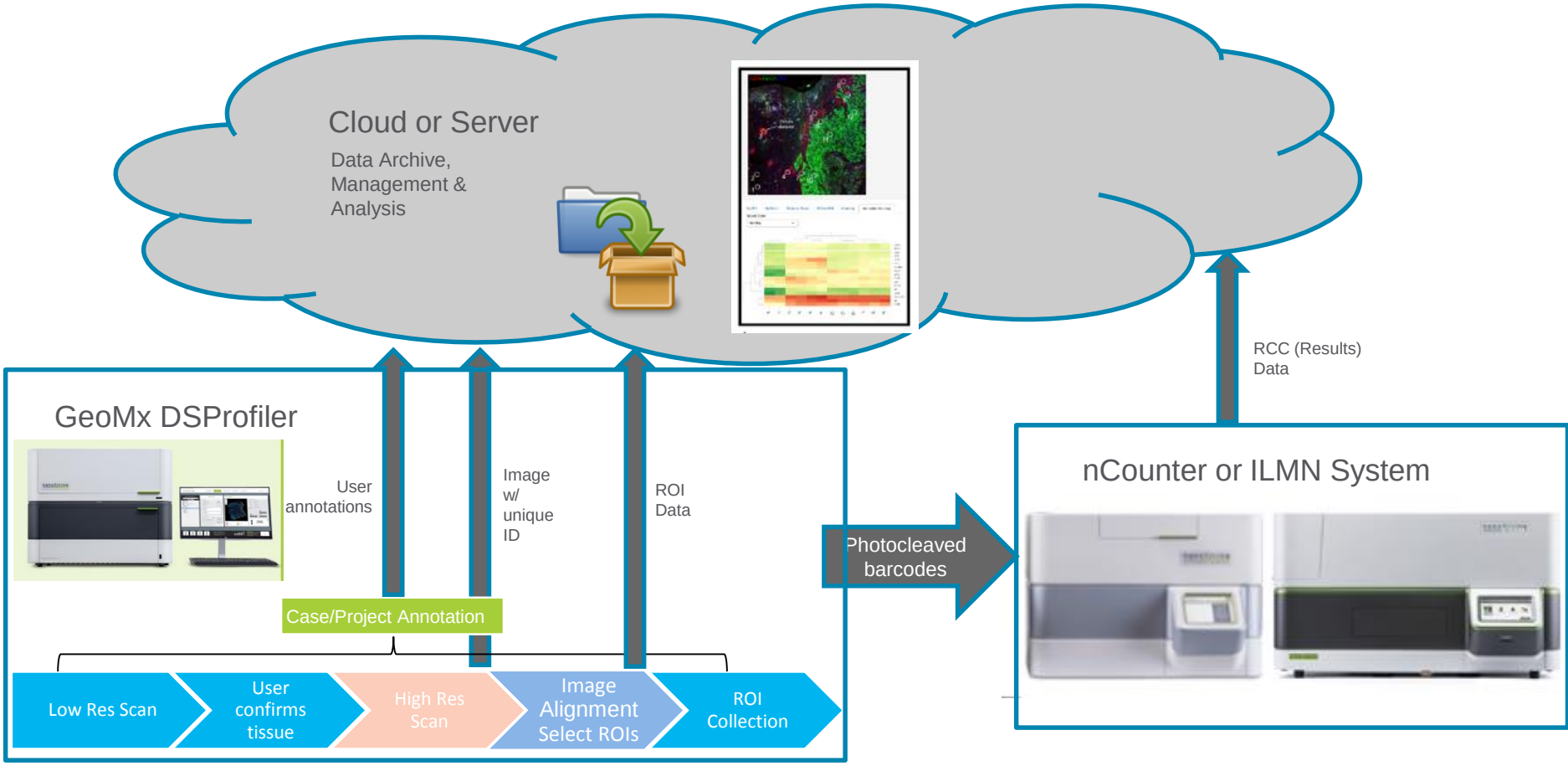
2 GeoMx DSP: Imaging



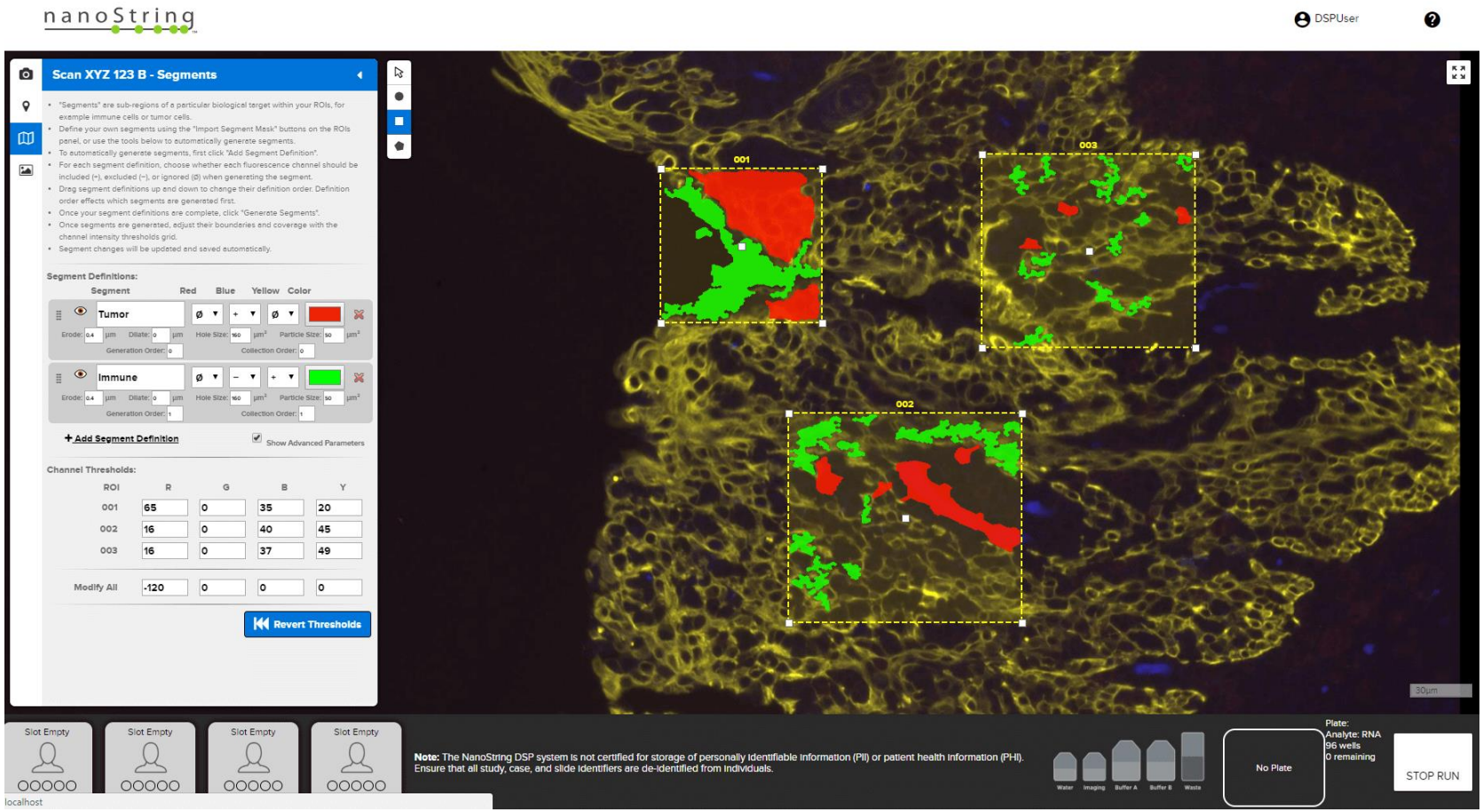
ROIs 13-24



2 GeoMx DSP: Software

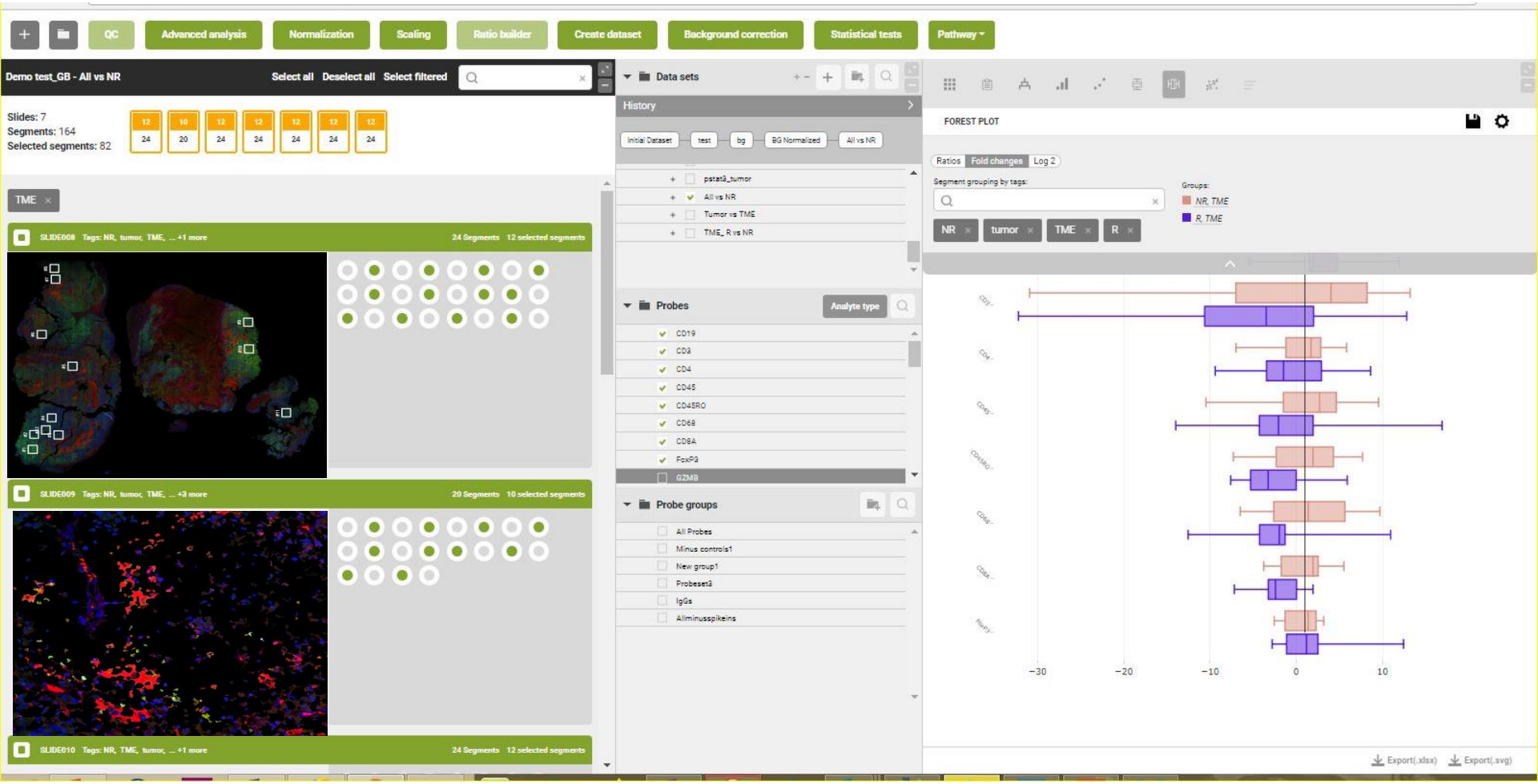


2 GeoMx DSP: Software



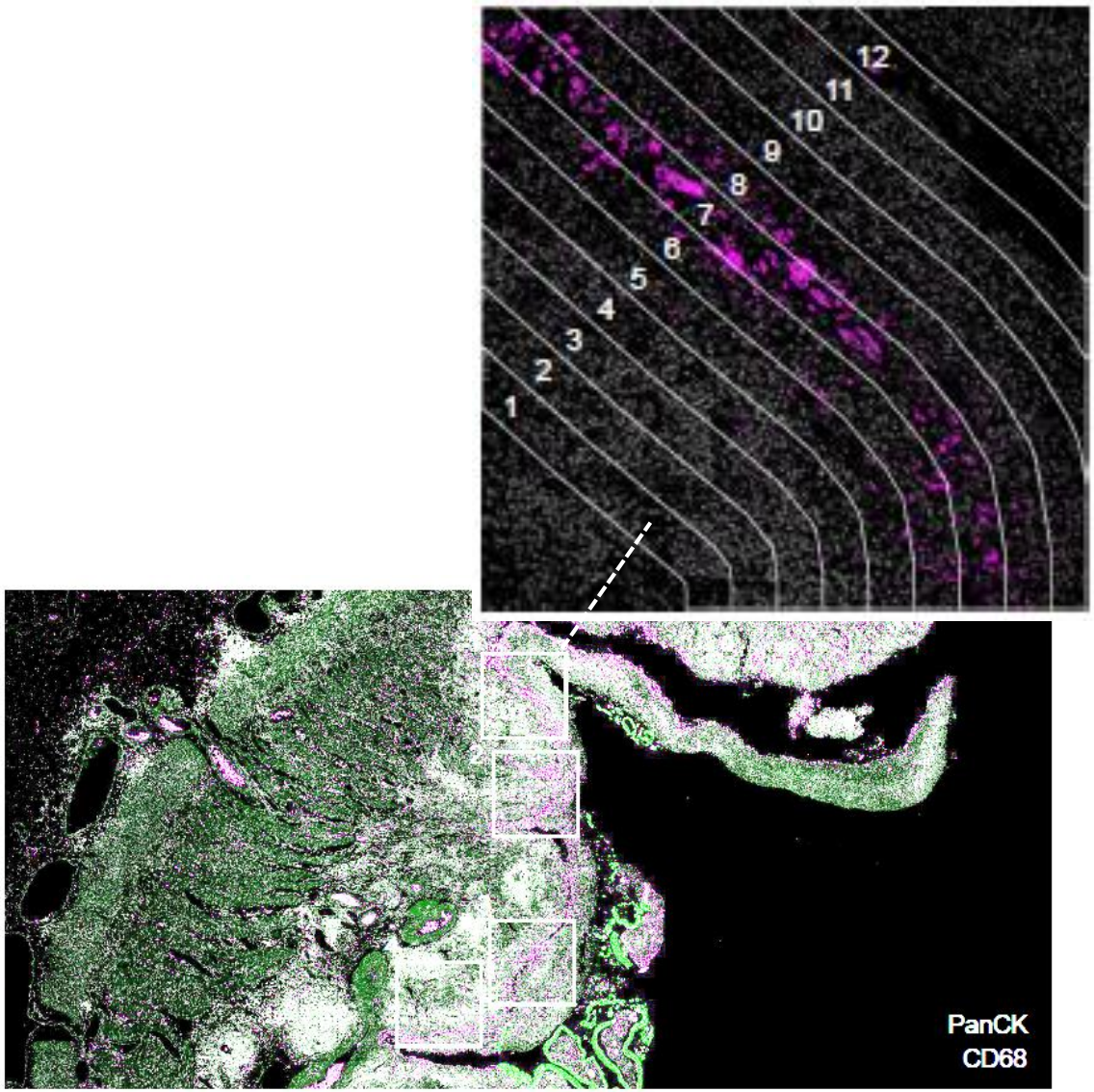
Automatic segmentation tumor / microenvironment

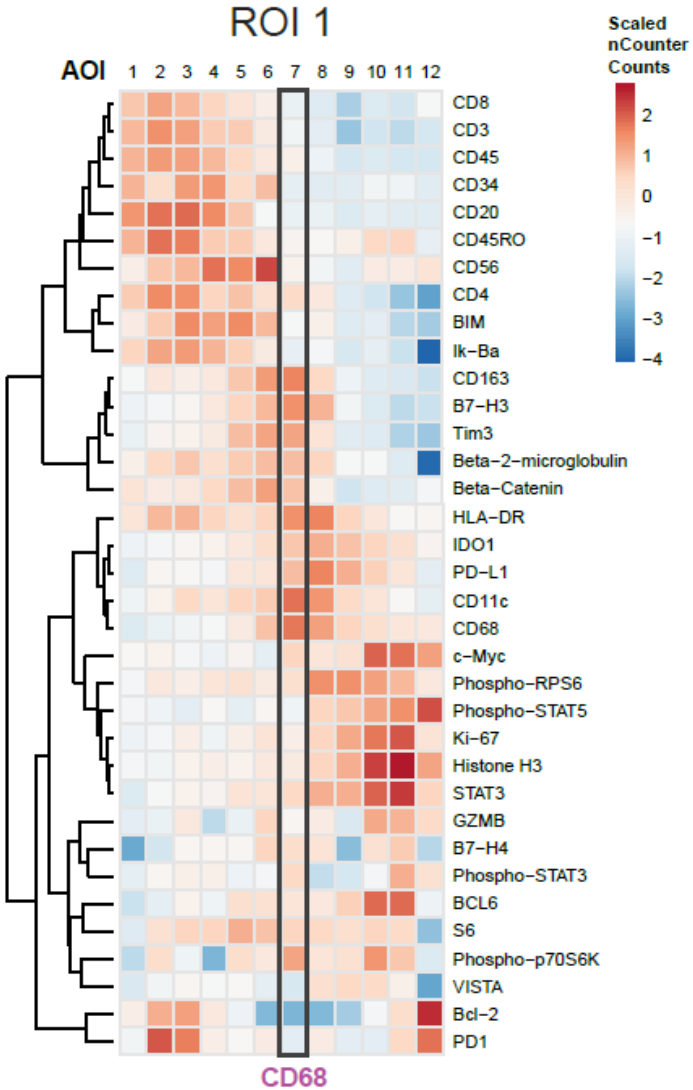
2 GeoMx DSP: Software



Comparison between ROIs

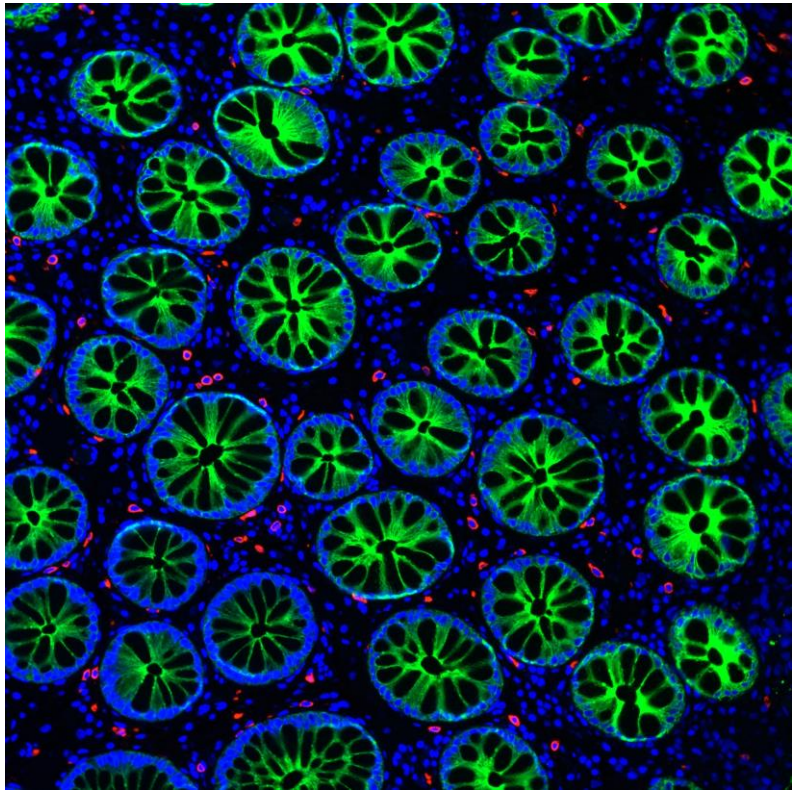
❷ GeoMx DSP: tailored ROIs selection





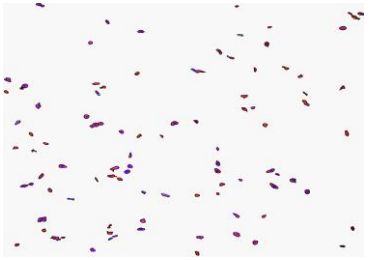
❷ GeoMx DSP: tailored ROIs selection

Profiling in the "shape" of Inflammatory Bowel Disease

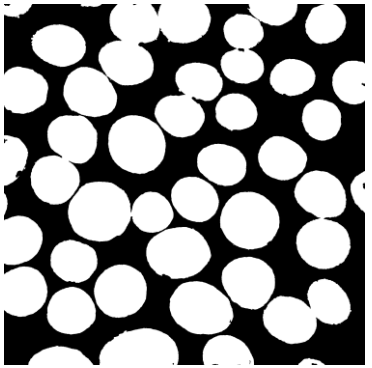


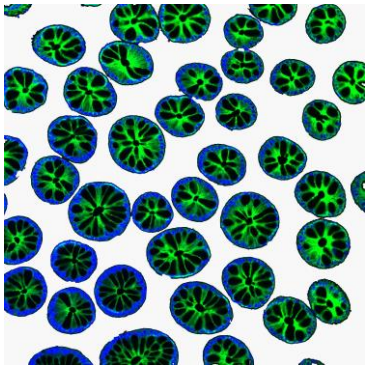
Blue = nuclear stain
Green = PanCytokeratin
Red = Tryptase, Mast-Cell Marker

Mast-Cell ROI mask

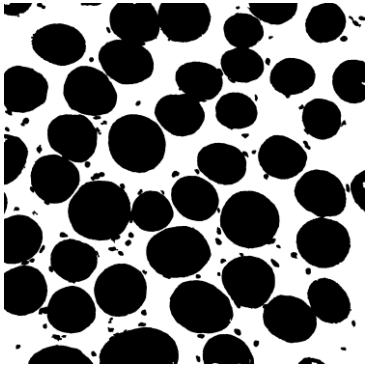


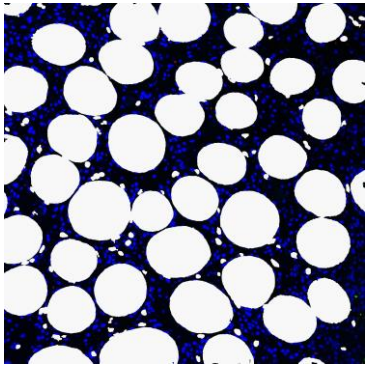
Intestinal Crypt ROI mask



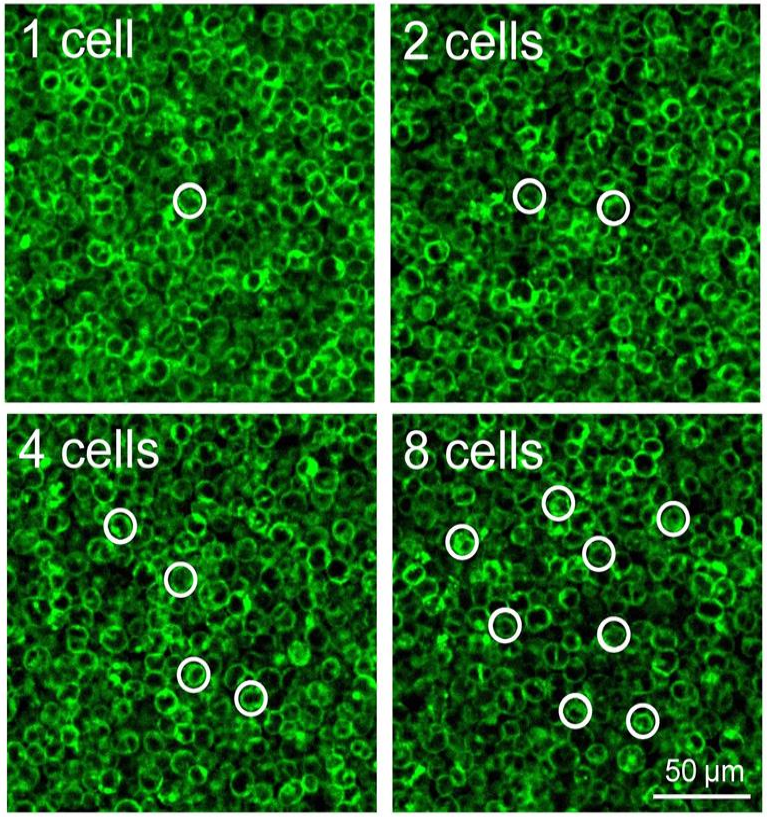


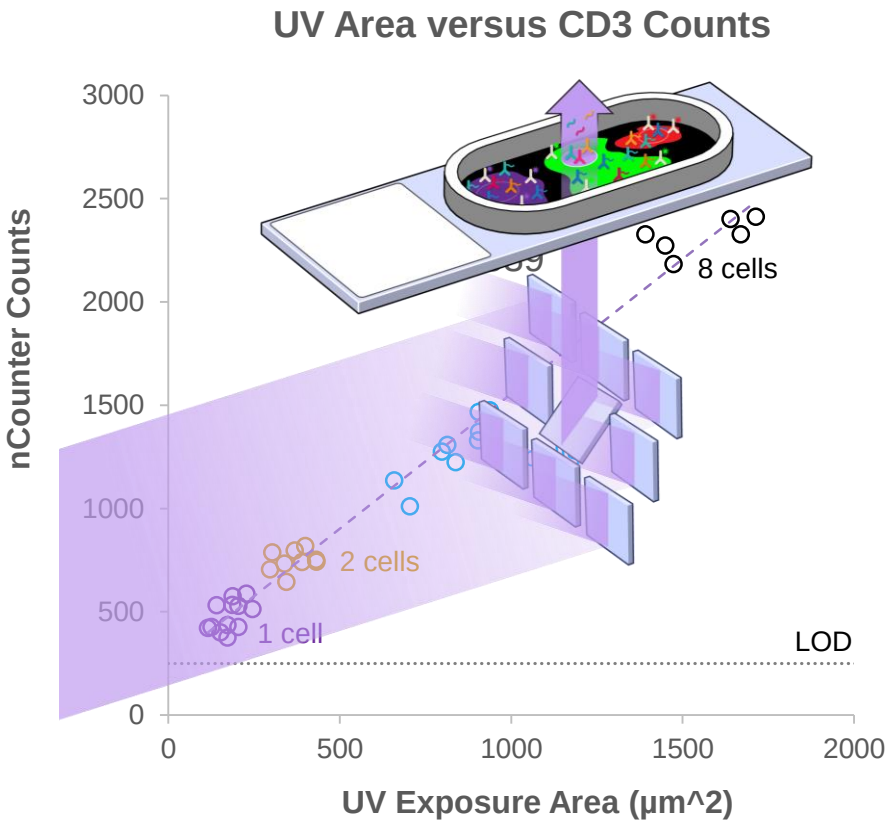
Connective tissue ROI mask



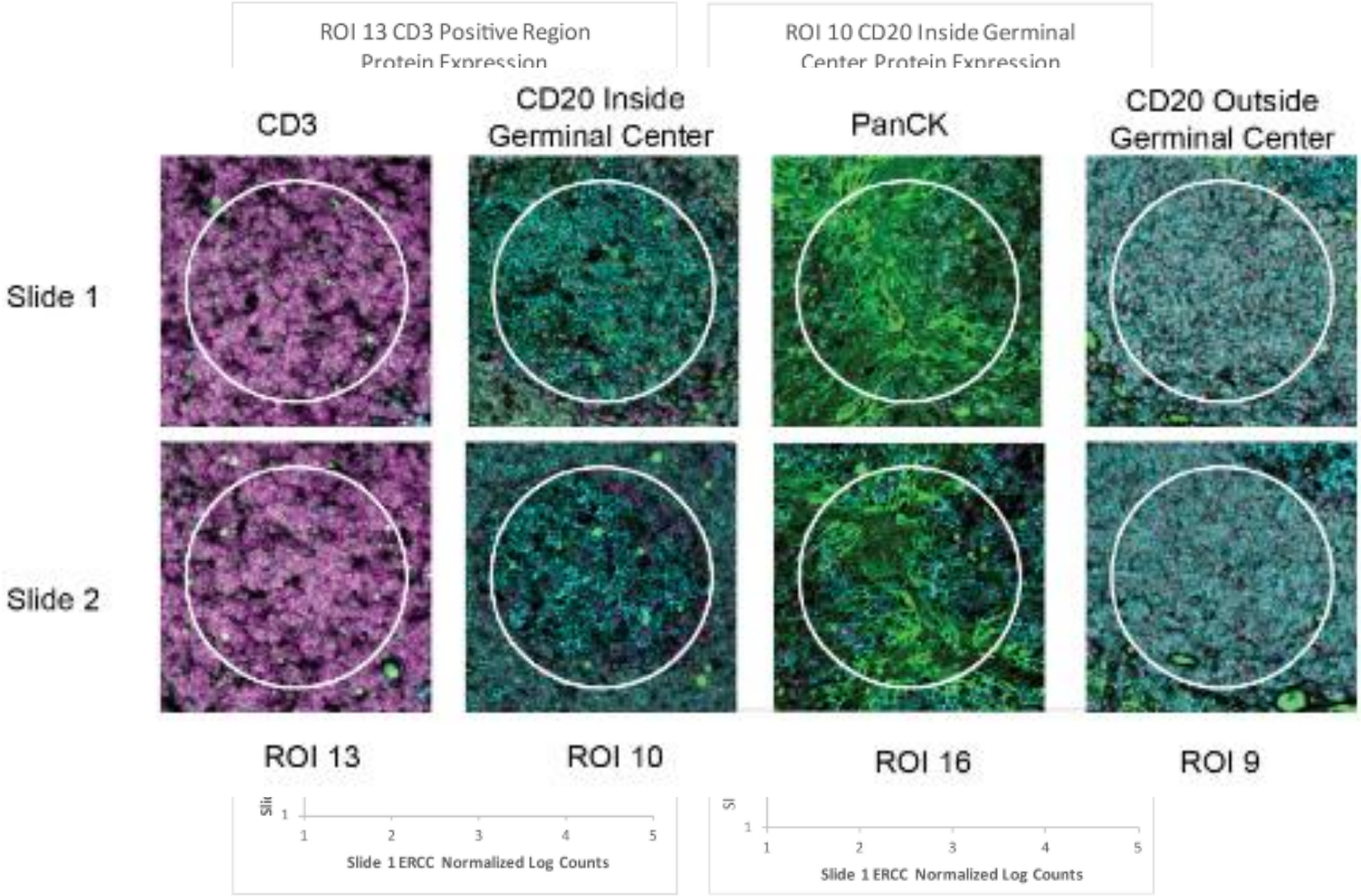


❷ GeoMx DSP: down to single cell



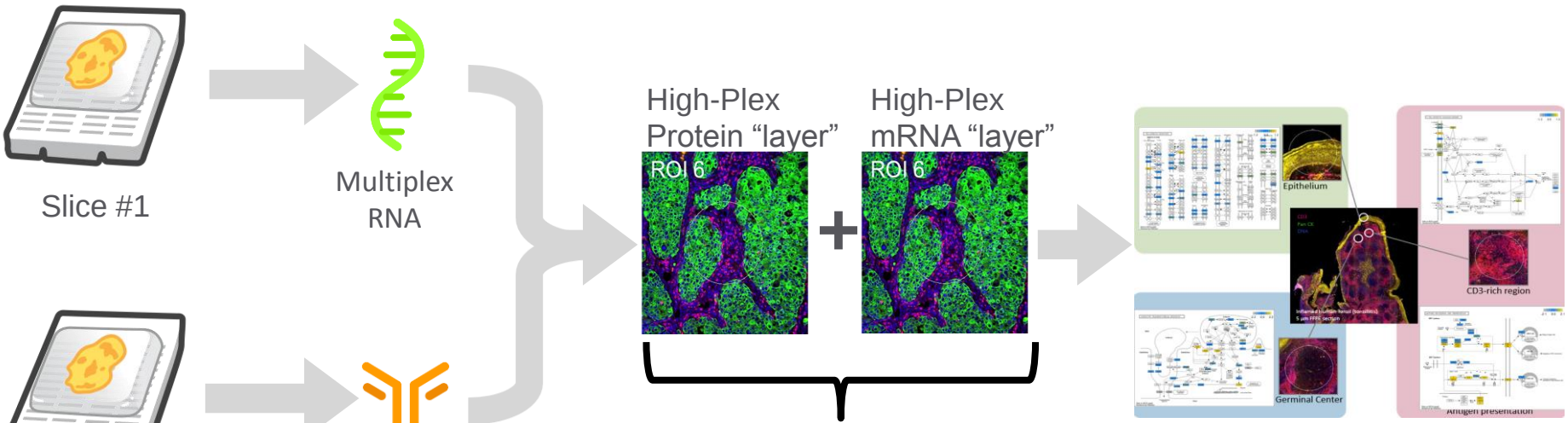


❷ GeoMx DSP: Reproducibility



50-plex Immuno-Oncology protein panel on 5um FFPE tissue sections

❷ GeoMx DSP: RNA & proteins



Co-Registered with Single-cell resolution

Complete spatially resolved biological pathways: **mRNAs + proteins + protein-PTMs**

3 Examples in Immuno-Oncology

Core Cell Profiling

Beta-2-microglobulin
CD11c
CD20
CD3
CD4
CD45
CD56
CD68
CD8
CTLA4
GZMB
Histone H3
Ki-67
PD1
PD-L1
Pan-Cytokeratin
S6

Drug Target Module

4-1BB
ARG1
B7-H4
B7-H3
LAG3
GITR
OX40L
IDO1
Tim-3
STING
VISTA

Activation/Inhibition Module

CD127
PD-L2
CD25
CD40
CD80
CD40L
CD86
HLA-DR
ICOS
CD27

Cell Typing Module

CD45RO
Gamma
FOXP3
Delta TCR
CD34
CD14
CD66b
FAPalpha

Tumor Module

MART1
EpCAM
NY-ESO-1
Her2/Erb
S100B
B2
Bcl-2

Trafficking Module

CXCR3
CXCL9
CD31
E selectin
CXCL10
L-selectin
CXCL11
P-selectin

Cytokine Module

Interferon gamma
IL-17
IL-6
TGF beta
TNFalpha

- Human IO Proteins panel

❸ Examples in Immuno-Oncology

- 96-gene-plex (~ 1000 mRNA probes) for RNA detection

CCL5	LAG3	CD3E	CXCL10	IFNGR1	Multi-CK	VSIR (VISTA)	OAZ1
CD27	NKG7	CD4	DKK2	IL12B	pan-Melanoma	Custom 1	POLR2A
CD274 (PDL1)	PSMB10	CD40	EPCAM	IL15	PDCD1	Custom 2	RAB7A
CD276 (B7-H3)	PDCD1LG2	CD40LG	FAS	IL6	PECAM1	Custom 3	SDHA
CD8A	STAT1	CD44	FOXP3	ITGAM (CD11B)	PTEN	Custom 4	Neg 1
CMKLR1	TIGIT	CD47	GZMB	ITGAV	PTPRC (CD45)	Custom 5	Neg 2
CXCL9	AKT1	CD68	HAVCR2 (TIM3)	ITGAX (CD11C)	STAT2	Custom 6	Neg 3
CXCR6	ARG1	CD74	HIF1A	ITGB2	STAT3	Custom 7	Neg 4
HLA-DQA1/2	B2M	CD86	ICAM1	ITGB8	TBX21	Custom 8	Neg 5
HLA-DRB	BATF3	CSF1R	ICOSLG	LY6E	TNF	Custom 9	Neg 6
HLA-E	BCL2	CTLA4	IFNAR1	MKI67	TNFRSF9 (41-BB)	Custom 10	Neg 7
IDO1	CCND1	CTNNB1	IFNG	MS4A1 (CD20)	VEGFA	UBB	Neg 8

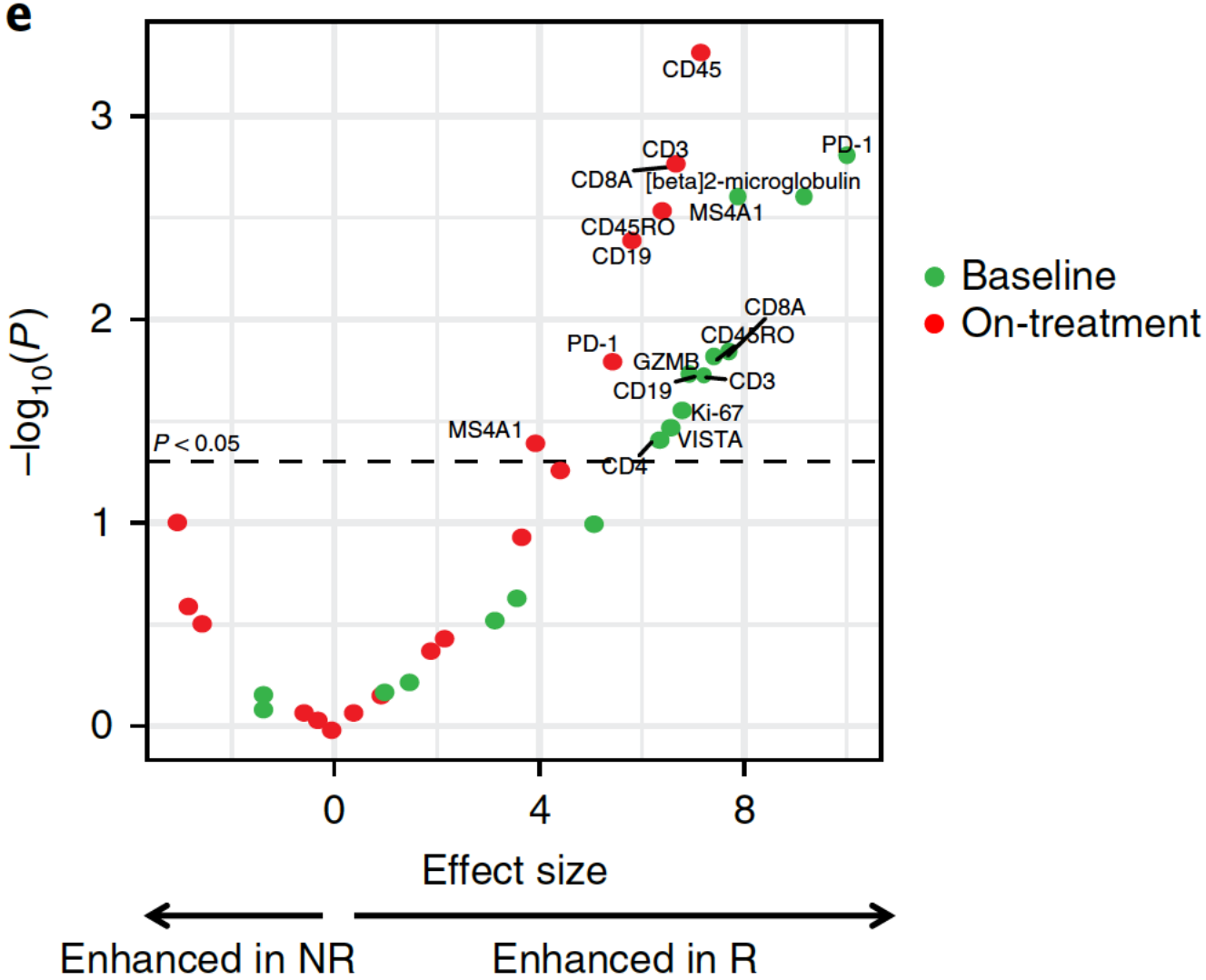
❸ Examples in Immuno-Oncology

nature medicine

e

Neoadjuv
nivoluma

Christian U. Blank[1,
Pia Kvistborg[2], Osc
Johannes V. van Thi
Lindsay G. Grijpink
Harm van Tinteren[3
and Ton N. Schuma



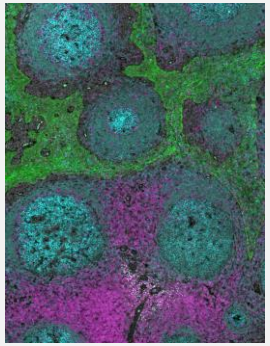
TERS
018-0197-1

ıa[4],
Wells[7],
d[3],
ıan[3],
rma[5,10],

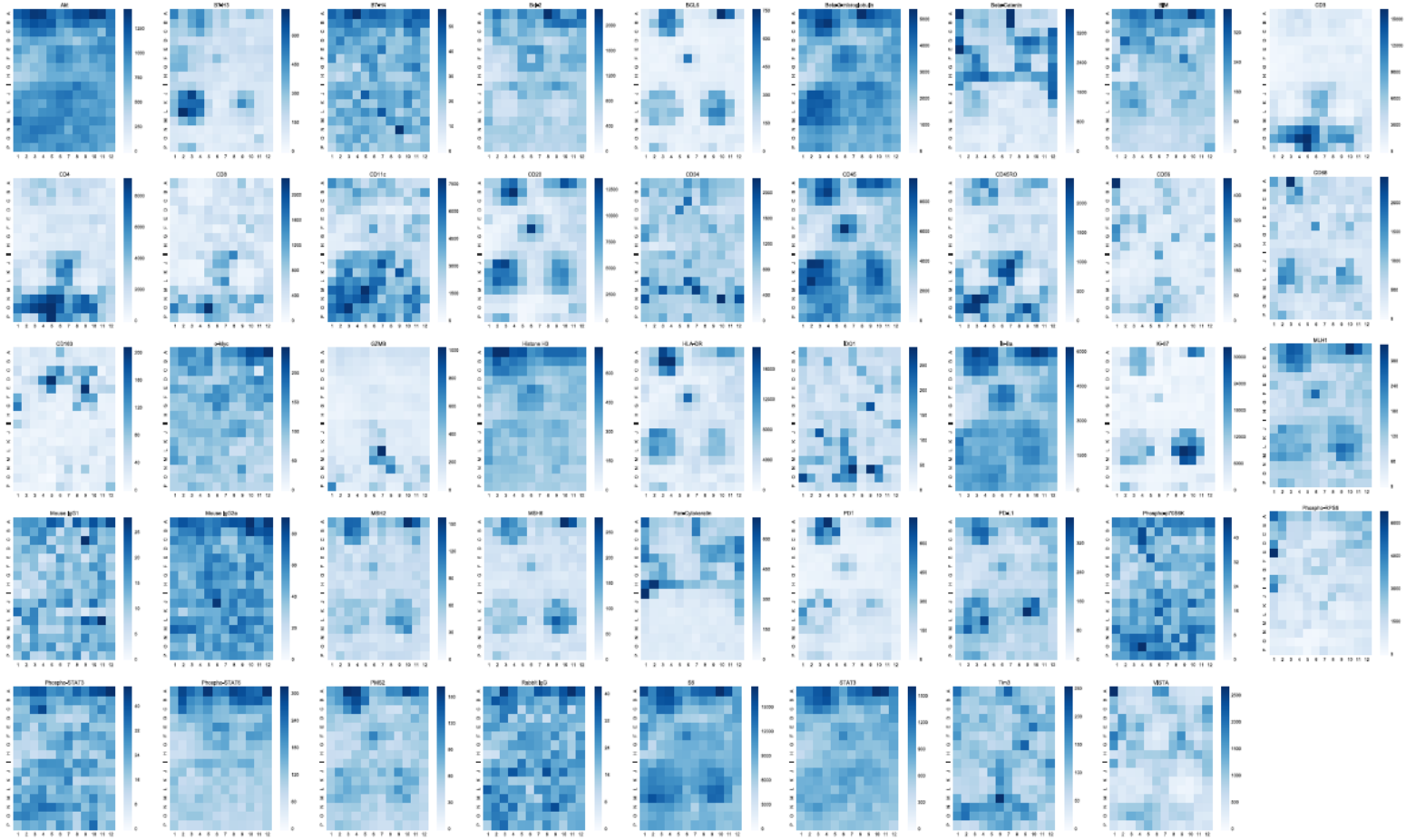
James Allison[5], Michael T. Tetzlaff[9,11,13] and Jennifer A. Wargo[3,8,13*]

3 Examples in Immuno-Oncology

Rastered Region



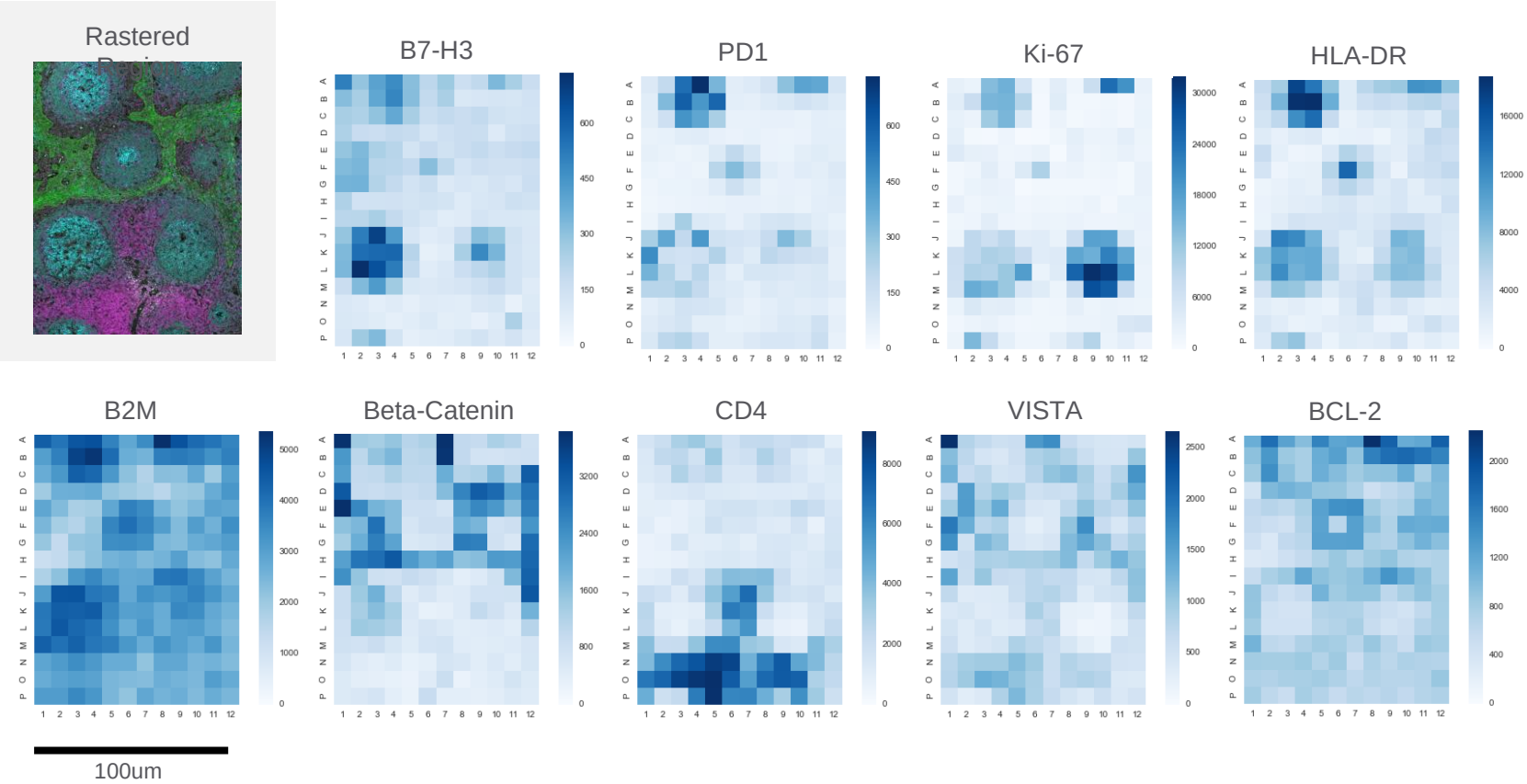
5 um FFPE
Lymphoid Tissue
PanCK = green
CD20 = blue
CD3 = purple



Cell numbers per
172 cells on aver
(113 min, 259 ma

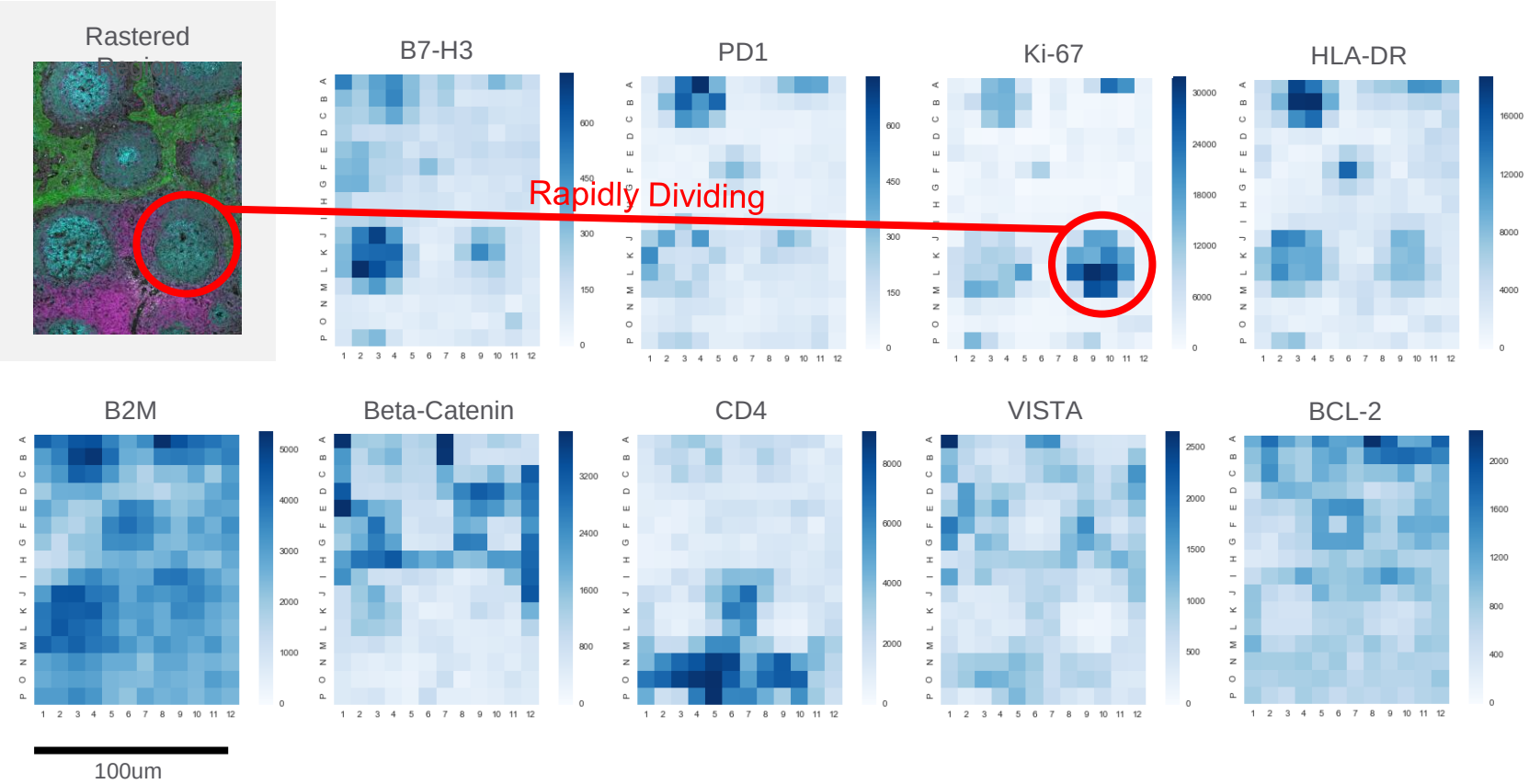
Spatial & functional organization of a lymphoid tissue

3 Examples in Immuno-Oncology



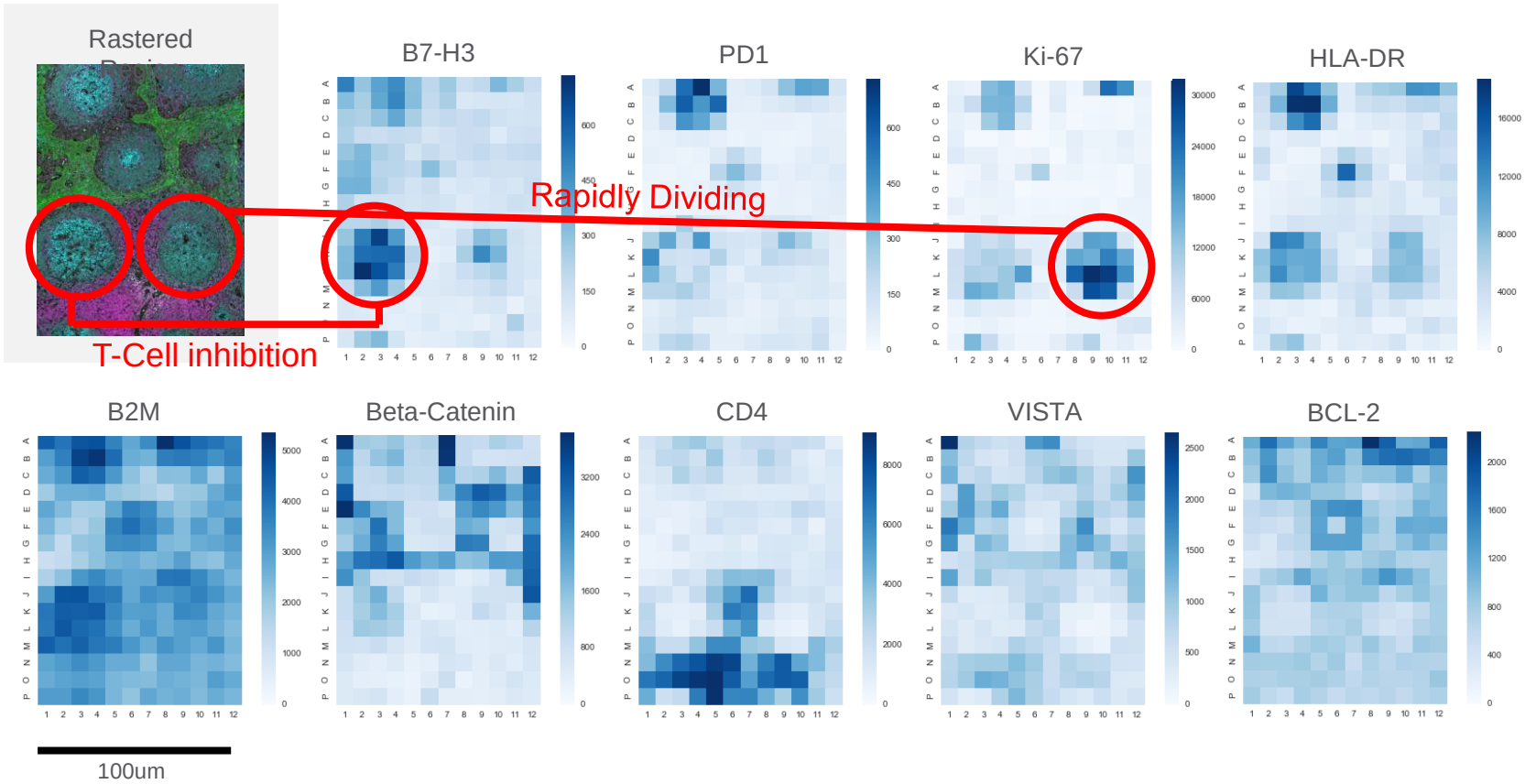
Spatial & functional organization of a lymphoid tissue

3 Examples in Immuno-Oncology



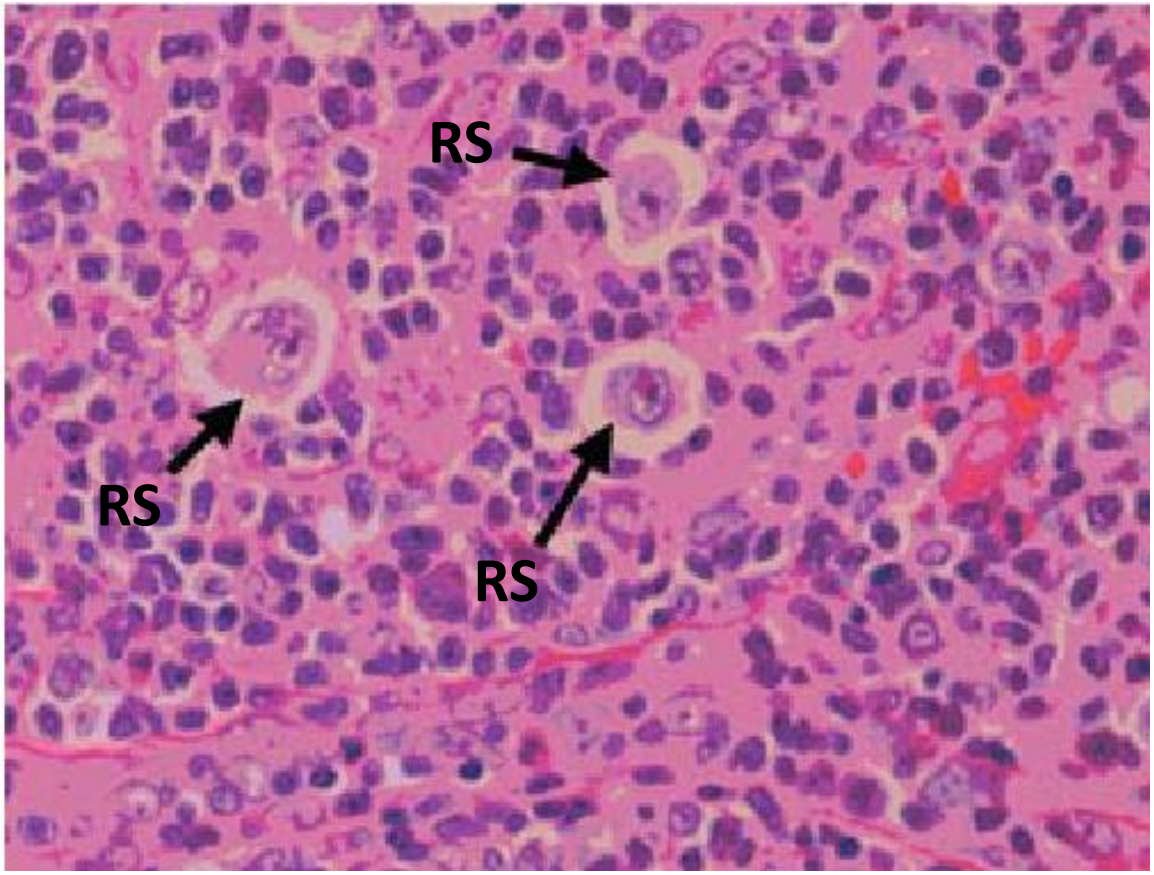
Spatial & functional organization of a lymphoid tissue

3 Examples in Immuno-Oncology



Spatial & functional organization of a lymphoid tissue

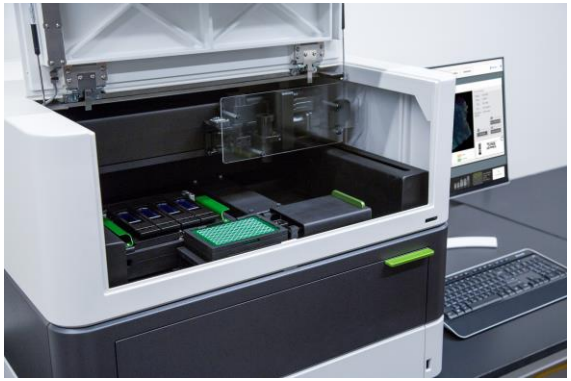
❸ Examples in Immuno-Oncology



Hodgkin lymphoma > immunological gradient around CD30+ cells

Antibodies to select ROI :
CD30 (Reed Sternberg cell), CD3 & CD68

4 GeoMx DSP outline



Multiplex	Many analytes on one tissue slice in a single pass
Multi-Analyte	High plex spatial analysis of both protein and RNA
Quantitation	Based on linear single-molecule counting: up to 6 logs
Single-cell	Limit of detection
Non-destructive	Sample is only touched by light
Throughput	Up to 20 sections per day

This is working, this is easy to implement

BUT how do we integrate this **level of complexity** into a clinical report that **benefit to the patient**?

Hospices Civils de Lyon

votre santé,
notre engagement

jonathan.lopez@chu-lyon.fr