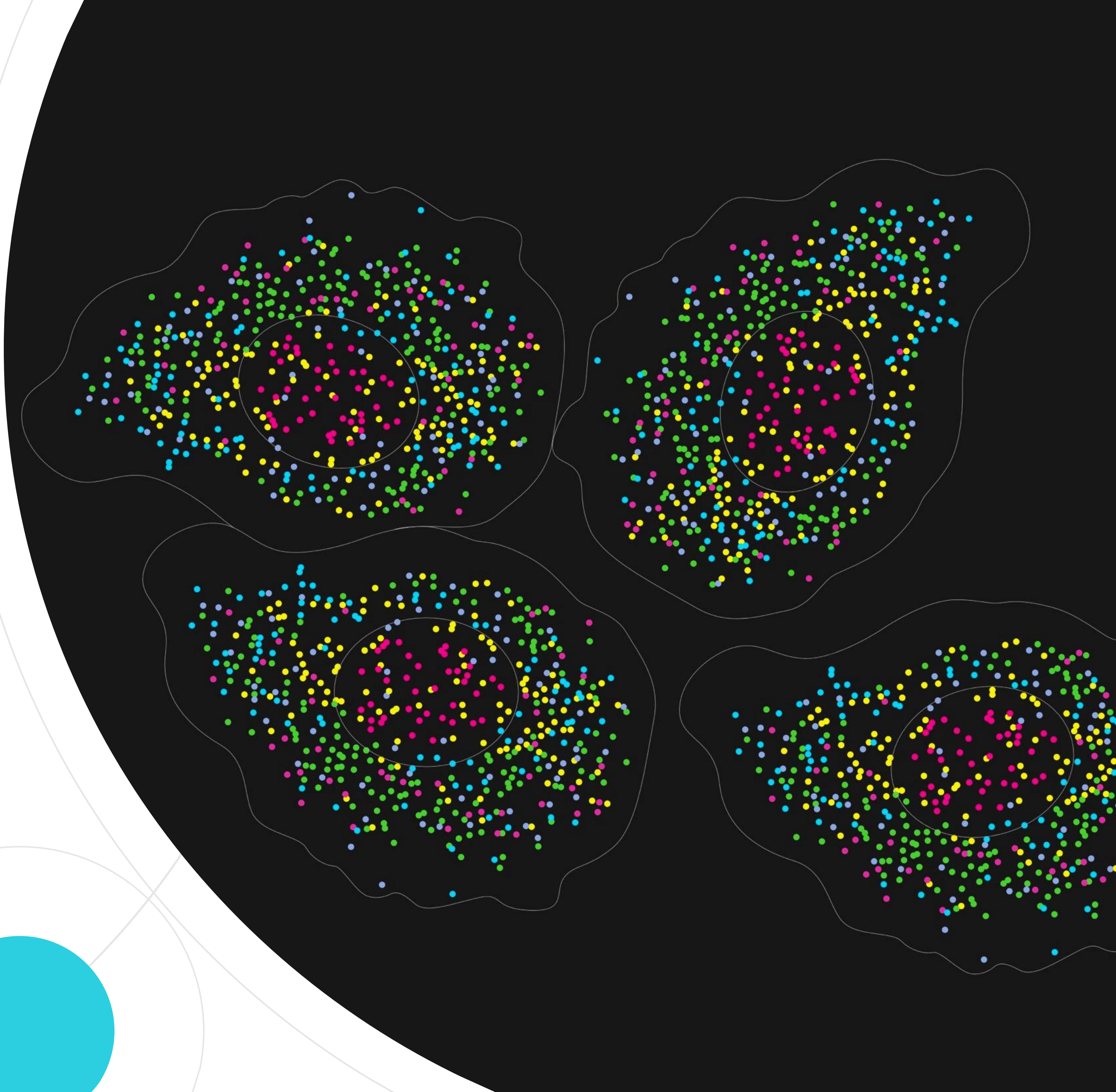
Multi-omic profiling of the tumor-immune microenvironment with MERSCOPE® spatial transcriptomics and InSituPlex® spatial proteomics

Manisha Ray, PhD

Direction of Applications Development



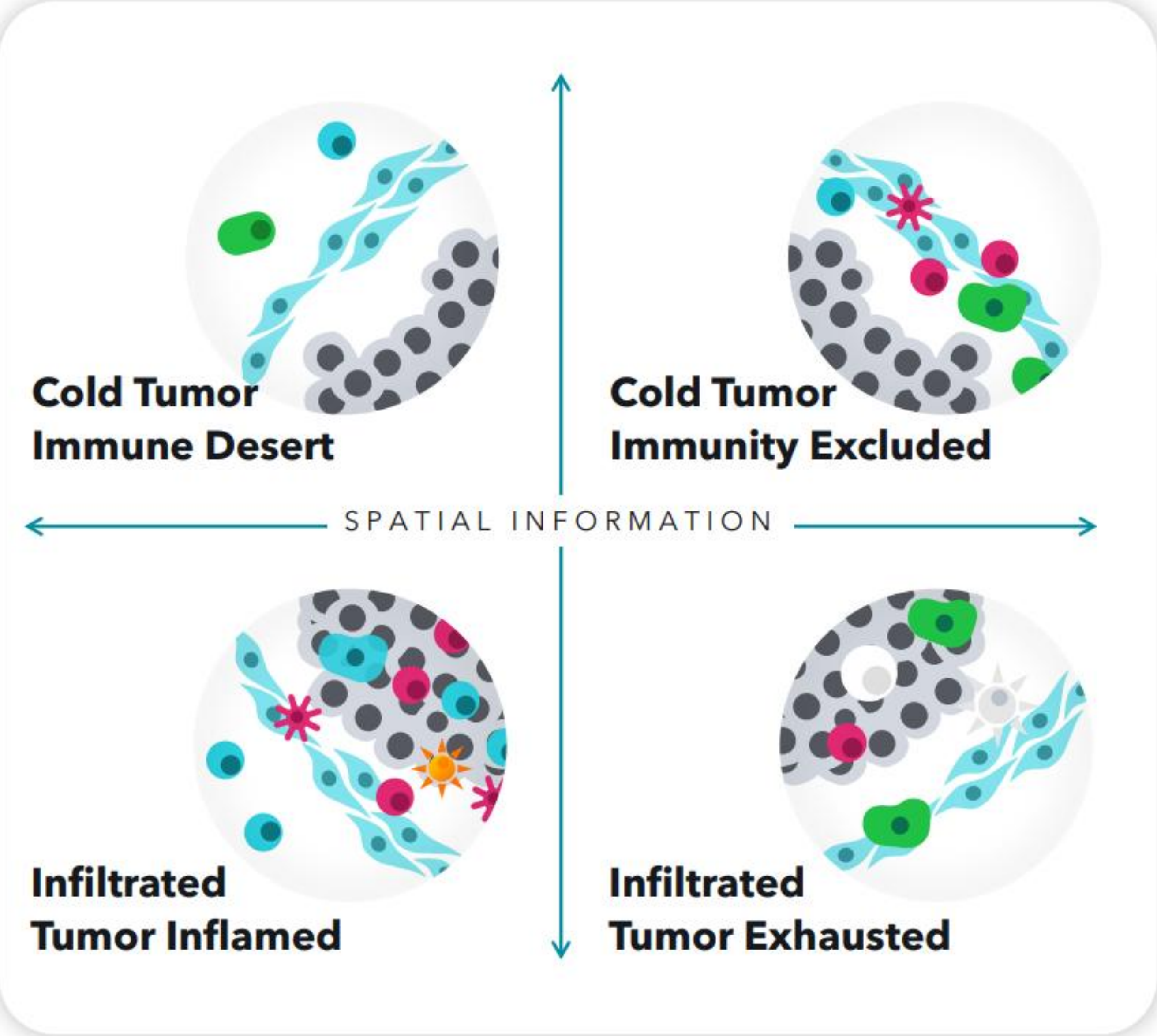
> "In all honesty, you can hardly find a problem in biology and medicine that is not a spatial problem."

PROF PHYSIOLOGY AND SPATIAL CORE DIRECTOR, MAYO CLINIC

Spatial Insights from In Situ 'Omics Are Essential

EXAMPLE: ONCOLOGY

- Locating specific immune cell types relative to the tumor identify different types of cancer activity
- These profiles directly inform how patients may respond to different therapies

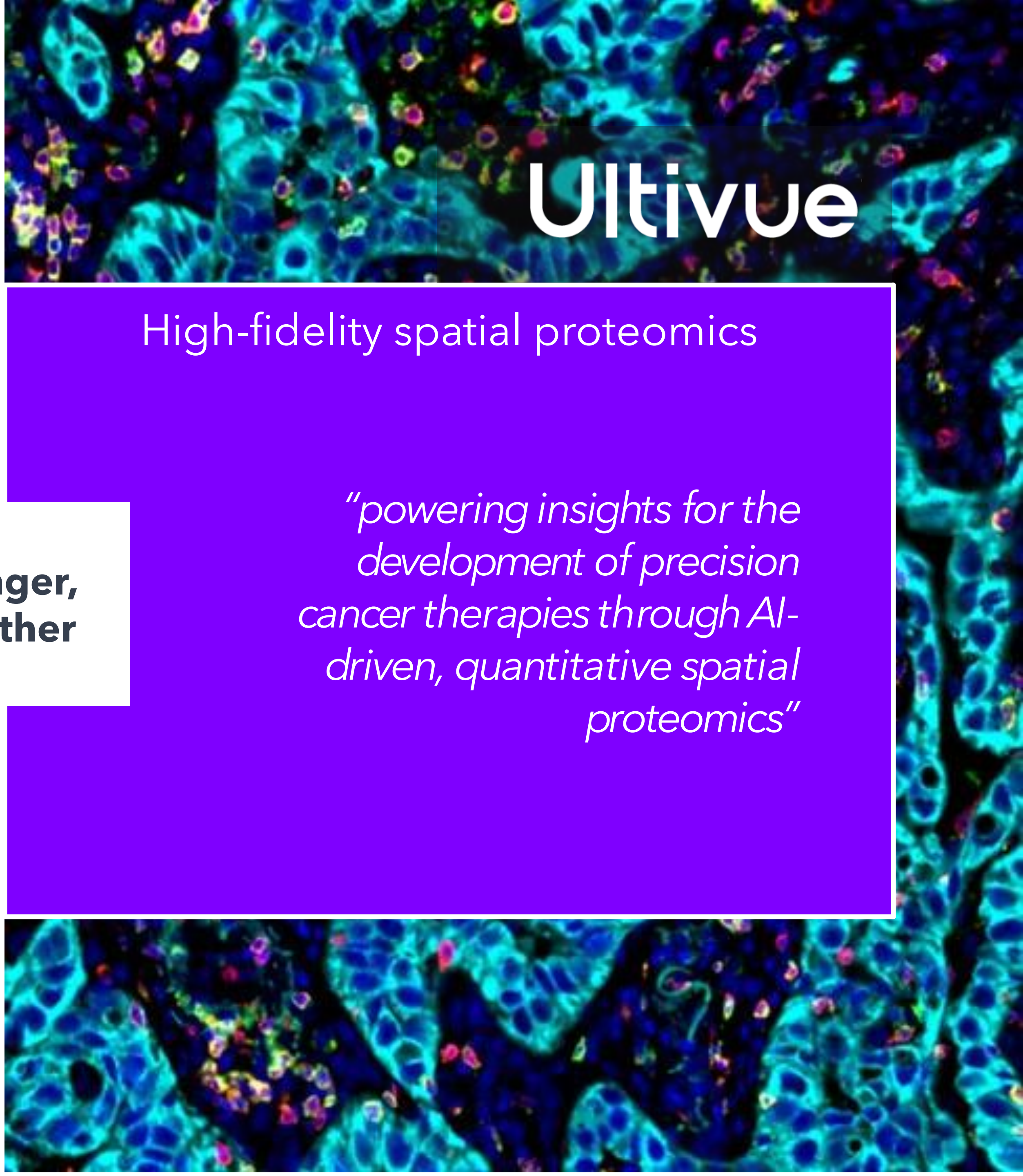


vizgen

Single-cell spatial transcriptomics

"dedicated to pioneering the next generation of genomics, providing tools that demonstrate the possibilities of in situ single-cell spatial genomics"

Stronger, Together



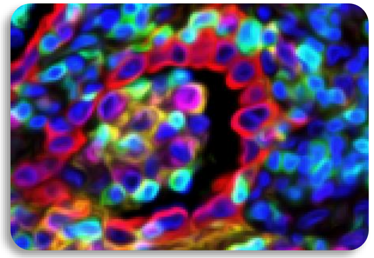
Ultivue

High-fidelity spatial proteomics

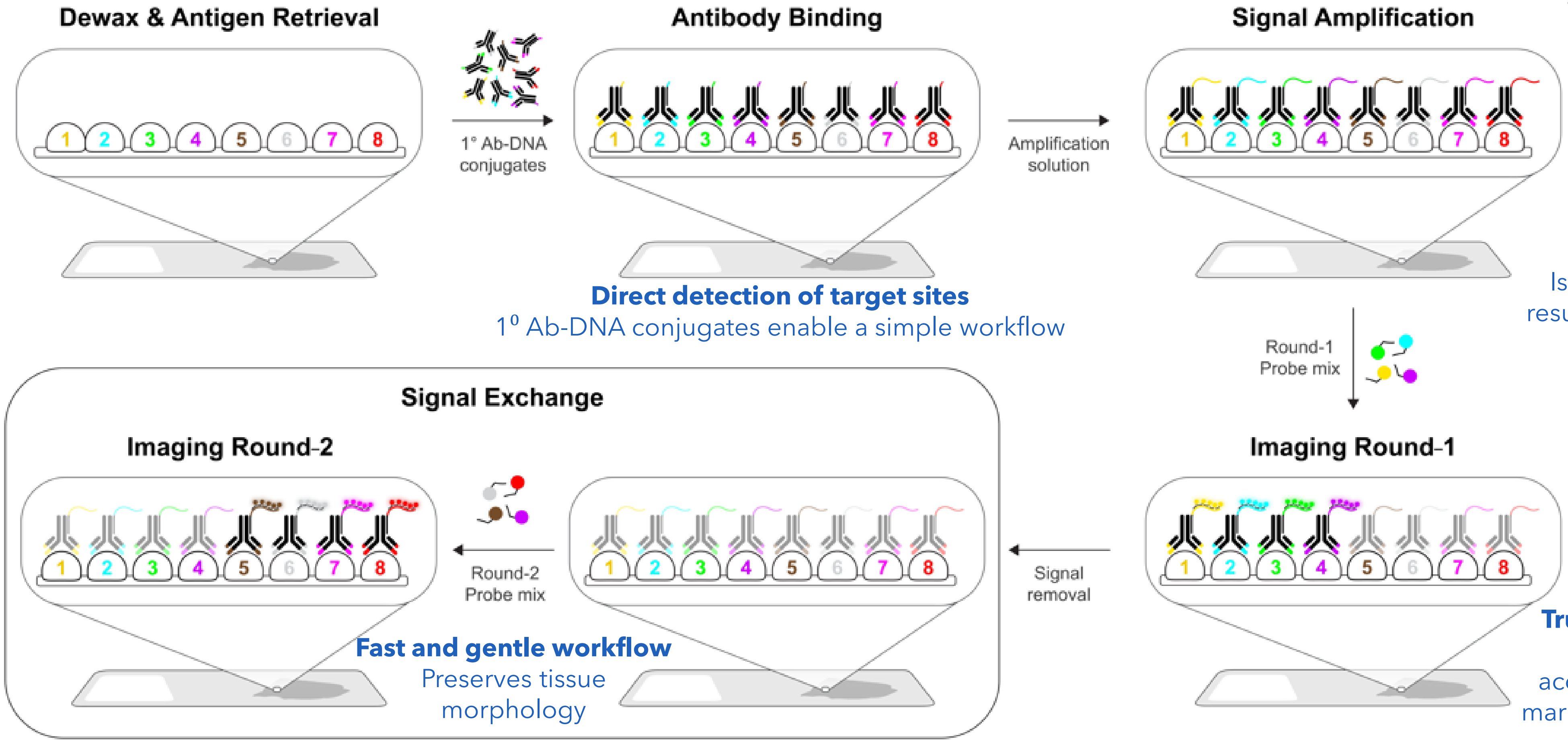
"powering insights for the development of precision cancer therapies through AI-driven, quantitative spatial proteomics"

InSituPlex® (ISP) Assay for Spatial Proteomics

The TME is Complex: Your Assay Shouldn't Be!



Minimal imaging time with low false negatives



Direct detection of target sites
1^0 Ab-DNA conjugates enable a simple workflow

Direct Signal amplification
Isometric amplification results in numerous copies of barcodes per Ab

True signal visualization
No post-processing: accurate co-localization of markers via direct labeling of probes

Fast and gentle workflow
Preserves tissue morphology

Next Generation Integrated "Samples-to-Insights" with STARVUE

mIF Image

- Configurable panels of <12 targets
- Simple high-throughput workflow
- High specificity & dynamic range

Image Stacking

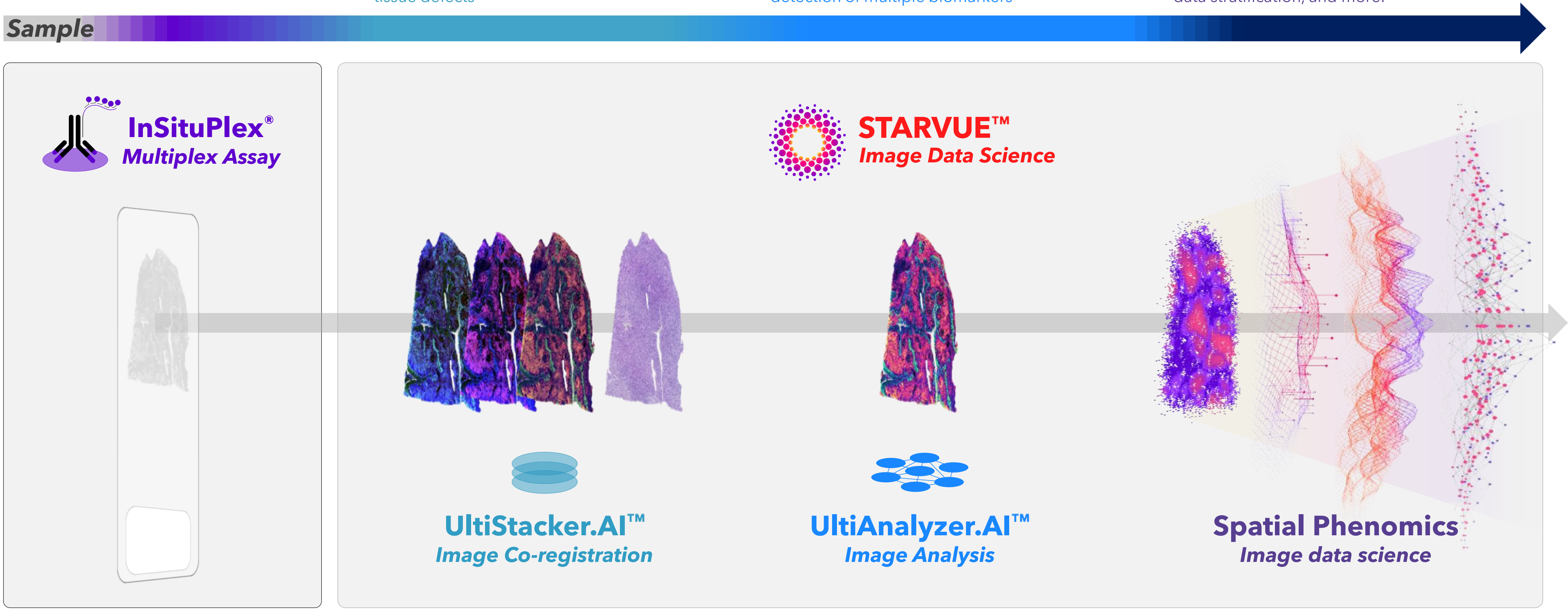
- Robust co-registration, micron-level accuracy
- Extremely high throughput (100's of samples/hr)
- Nearly perfect co-registration even with large tissue defects

Image Analysis

- Deep-learning enabled whole-slide analysis
- Highly scalable cloud-based infrastructure
- Custom phenotyping through robust co-detection of multiple biomarkers

Biological Insights

- Intercellular dynamics: ROIs vs. whole section
- Advanced spatial phenomics: Clinical data integration, Endpoint analysis, Cohort-level data stratification, and more.



Sample

InSituPlex®
Multiplex Assay

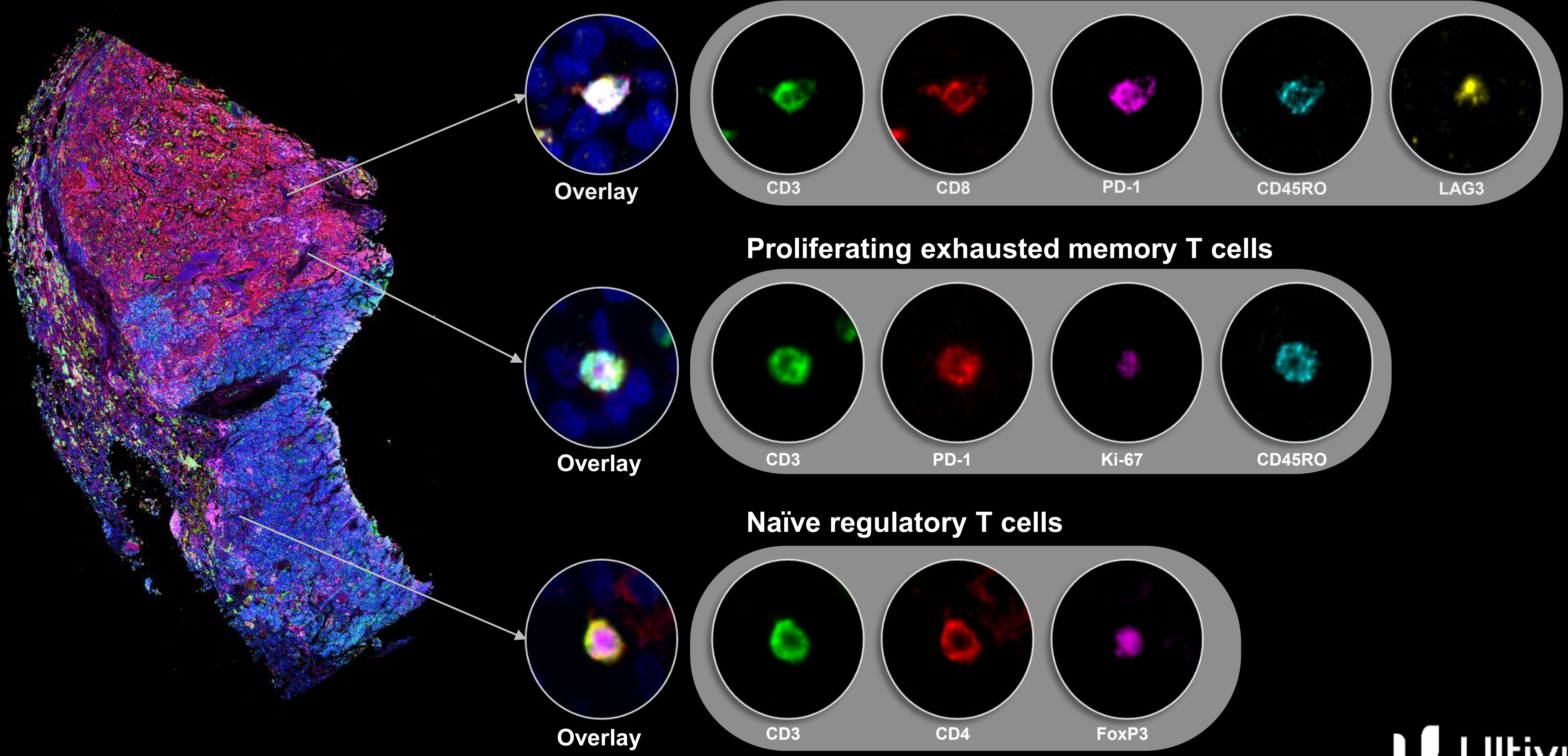
STARVUE™
Image Data Science

UltiStacker.AI™
Image Co-registration

UltiAnalyzer.AI™
Image Analysis

Spatial Phenomics
Image data science

ISP facilitates high resolution cellular phenotyping through co-detection of up to 12 biomarkers in a single cell



Exhausted memory cytotoxic T cells

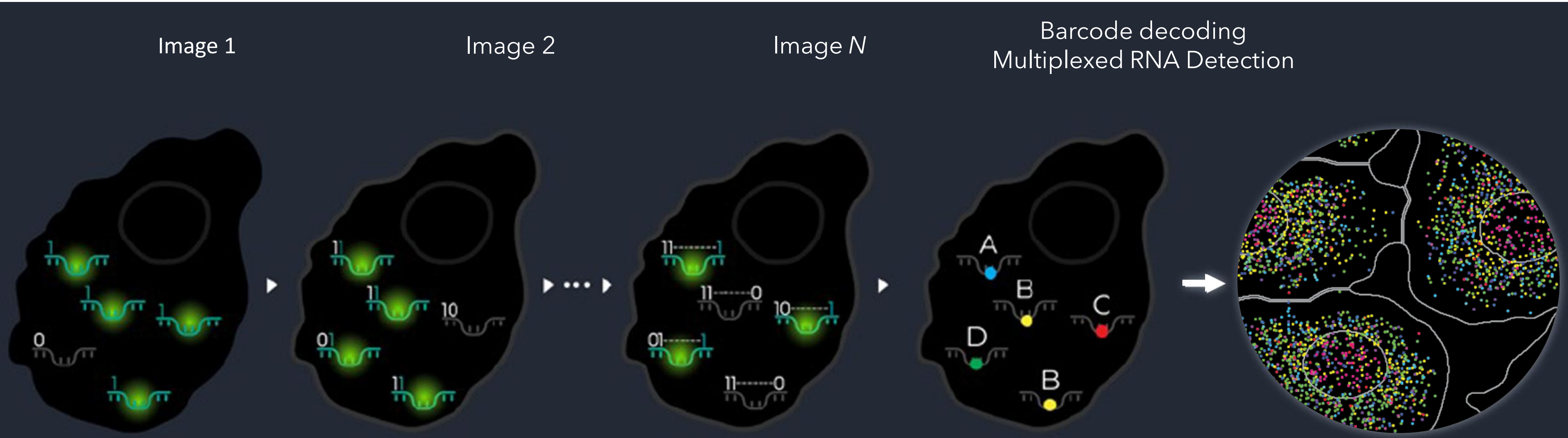
Proliferating exhausted memory T cells

Naïve regulatory T cells

MERFISH: Highly multiplexed, Spatially Resolved, Single-cell Gene Expression Profiling by RNA Imaging

Platform technology from Harvard: Multiplexed Error-robust Fluorescence in situ Hybridization (MERFISH)

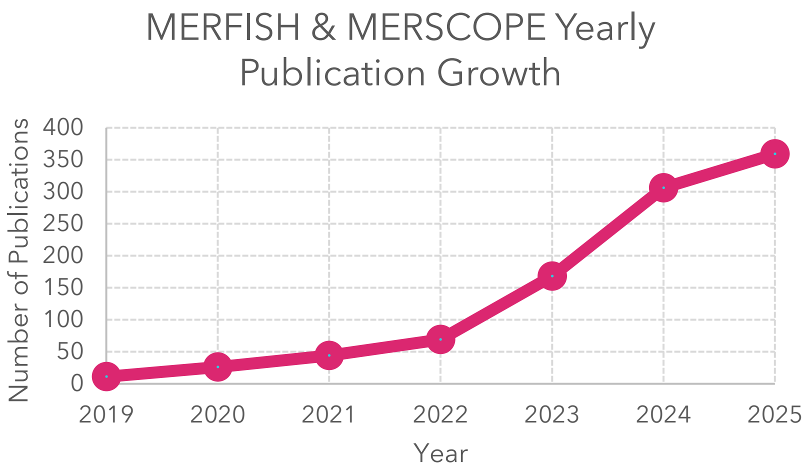
COMBINATORIAL LABELING | SEQUENTIAL IMAGING | ERROR ROBUST BARCODING



"Method of the Year 2020: spatially resolved transcriptomics" ---- **Nature Methods**

Chen et al, Science, 2015; Moffitt et al, PNAS, 2016; Moffitt et al, PNAS, 2016; Emanuel et al, Nature Methods, 2017; Wang et al, Sci. Rep, 2018;
Moffitt et al, Science, 2018; Wang et al, PNAS, 2019; Xia et al, Sci. Rep, 2019; Xia et al, PNAS, 2019;

MERFISH is the Leading Single-cell Resolution *In-Situ* High-Plex Spatial Transcriptomics Technology with over 300 Published Papers & Preprints



Science AAAS
Cell
nature
nature methods
nature biotechnology
PNAS Proceedings of the National Academy of Sciences of the United States of America
nature COMMUNICATIONS
SCIENTIFIC REPORTS natureresearch
Cancer Cell

100s of peer-reviewed publications and preprints across many therapeutic areas such as neuro, oncology, cardio, and infectious disease; in high-impact journals

CANCER RESEARCH UK
THE UNIVERSITY OF BRITISH COLUMBIA
BROAD INSTITUTE
Stanford University
ALLEN INSTITUTE
HARVARD MEDICAL SCHOOL
HARVARD UNIVERSITY
Yale University
The Rockefeller University
salk
Beth Israel Lahey Health Beth Israel Deaconess Medical Center
UCI University of California, Irvine
Alnylam PHARMACEUTICALS

100s of researchers in academia, pharma, and service organizations are using Vizgen technologies to further their research and drug development

MERSCOPE

smFISH × **Combinatorial Barcoding System** = **MERFISH**

Direct in situ RNA quantification

Custom gene panels of 100's of targets

Highly multiplexed RNA detection enabling single cell analysis

High accuracy and consistency due to error robustness

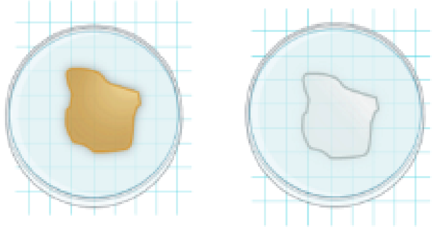
\+

An easy-to-use, automated platform

MERSCOPE™ Ultra Designed From the Ground Up for Optimal Performance

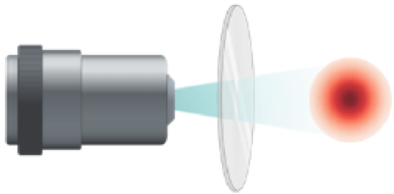
Sample Prep

Tissue
background clearing



Microscopy

Superior
optical resolution



+

Chemistry

Multi-site probes
for target detection

Code design

Error robustness

DETECTED
BARCODE

010101001

CORRECTED
BARCODE

010101000

Each design feature plays an important role in increasing
sensitivity and **specificity**

=

Unparalleled Data Quality

Assay and Sample Flexibility

Multiple Flow Cells Provides Flexible Imaging Options

FCX-S

Up to 1.25cm^2 imaging area

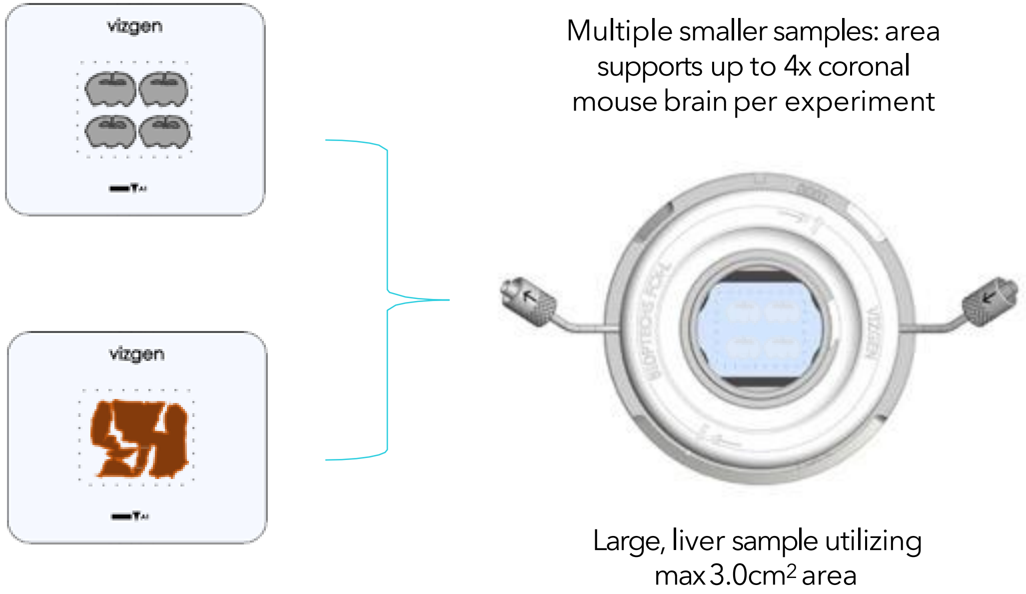
FCX-S ideal for small samples or initiating optimization of new tissues

FCX-L

Up to 3.0cm^2 imaging area

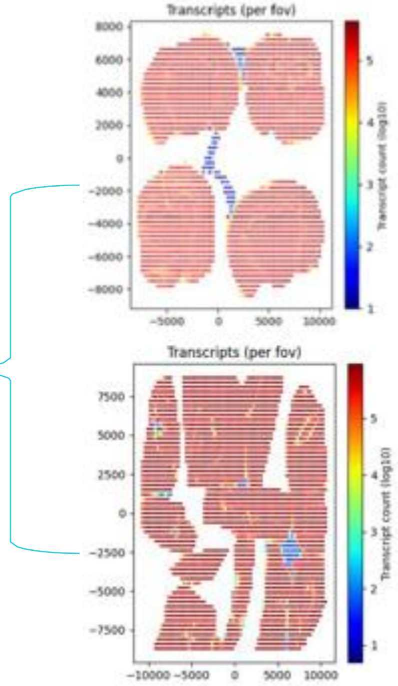
FCX-L supports multiple smaller samples or larger sample types e.g. whole brain, archival FFPE tissues, TMAs

Maximizes per sample economics



Multiple smaller samples: area supports up to 4x coronal mouse brain per experiment

Large, liver sample utilizing max 3.0cm^2 area



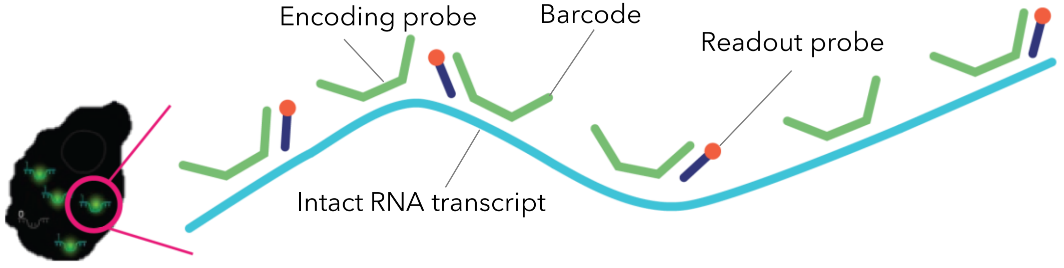
Example Data*
- 2,245 fovs
- 198,567 transcripts/fov
- 412,314,031 transcripts total
- 481,398 cells
- 541 transcripts/cell

Example Data*
- 3,196 fovs
- 565,009 transcripts/fov
- 1,763,967,579 total transcripts
- 1,285,017 cells
- 817 transcripts/cell

*Data provided based on development testing. Validated performance specifications are not available until launch.

MERFISH 2.0: Improved Sensitivity for Low Quality RNA

High Quality RNA Samples and traditional MERFISH

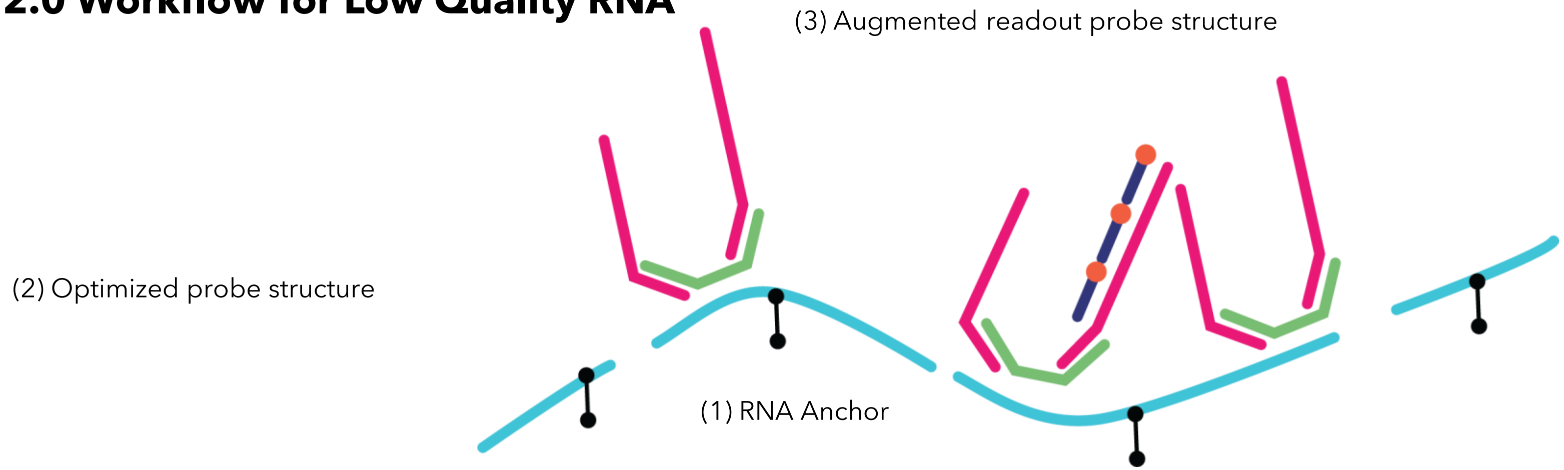


In intact RNA samples, multiple encoding probes bind along the transcript, generating strong fluorescent signals at the '1' barcode positions.

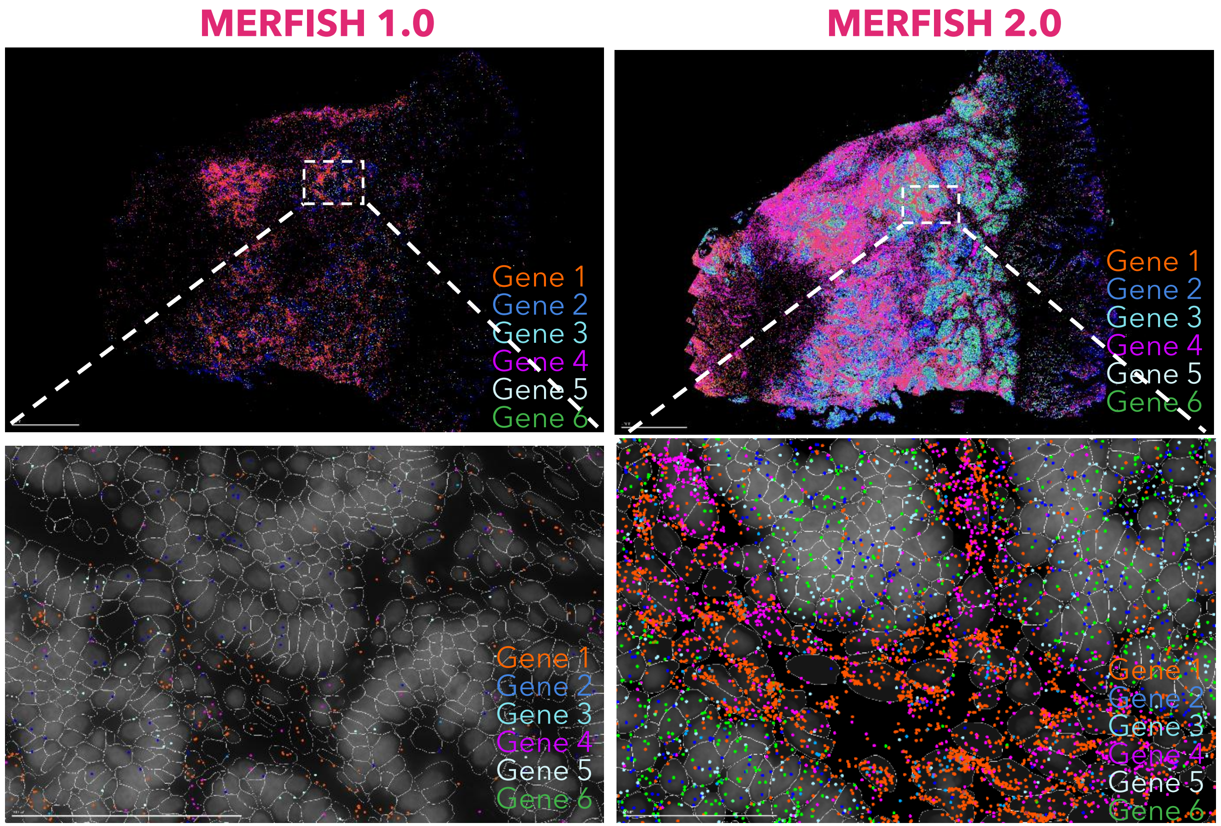
Low Quality RNA Samples and traditional MERFISH

In degraded samples, such as archival FFPE tissues, RNA fragmentation leads to fewer probe binding sites, reducing signal intensity. At lower RNA quality, background noise can obscure signals, making transcript quantification more challenging.

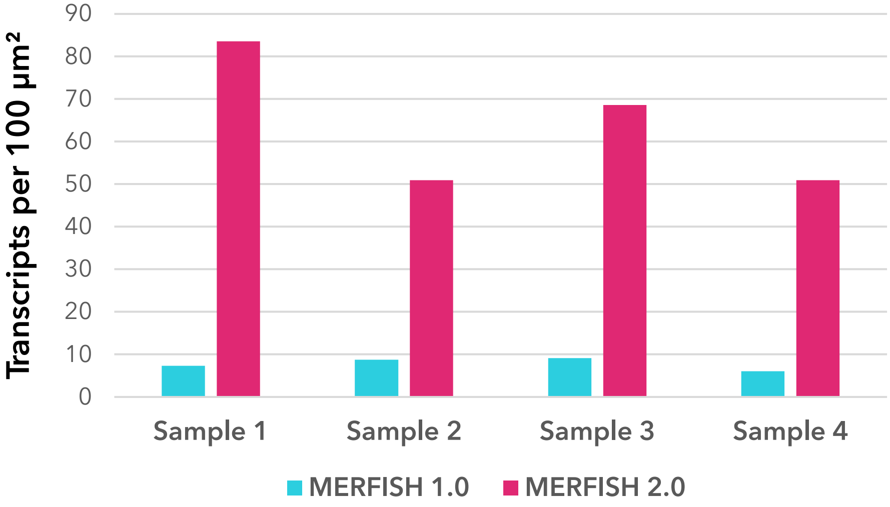
MERFISH 2.0 Workflow for Low Quality RNA



MERFISH 2.0 substantially improves transcript detection efficiency in FFPE human colorectal cancer samples



Comparison of detection efficiency



> "We decided on MERFISH due to the high sensitivity and the relatively low requirements for tissue samples. Our expectations were greatly surpassed by the astonishing (subcellular) resolution of MERFISH 2.0, especially when compared to MERFISH 1.0. This will allow us to mechanistically test the main hypothesis of the project using this data alone, Thank you very much!!!"
>
> **University Hospital Tübingen, Germany**

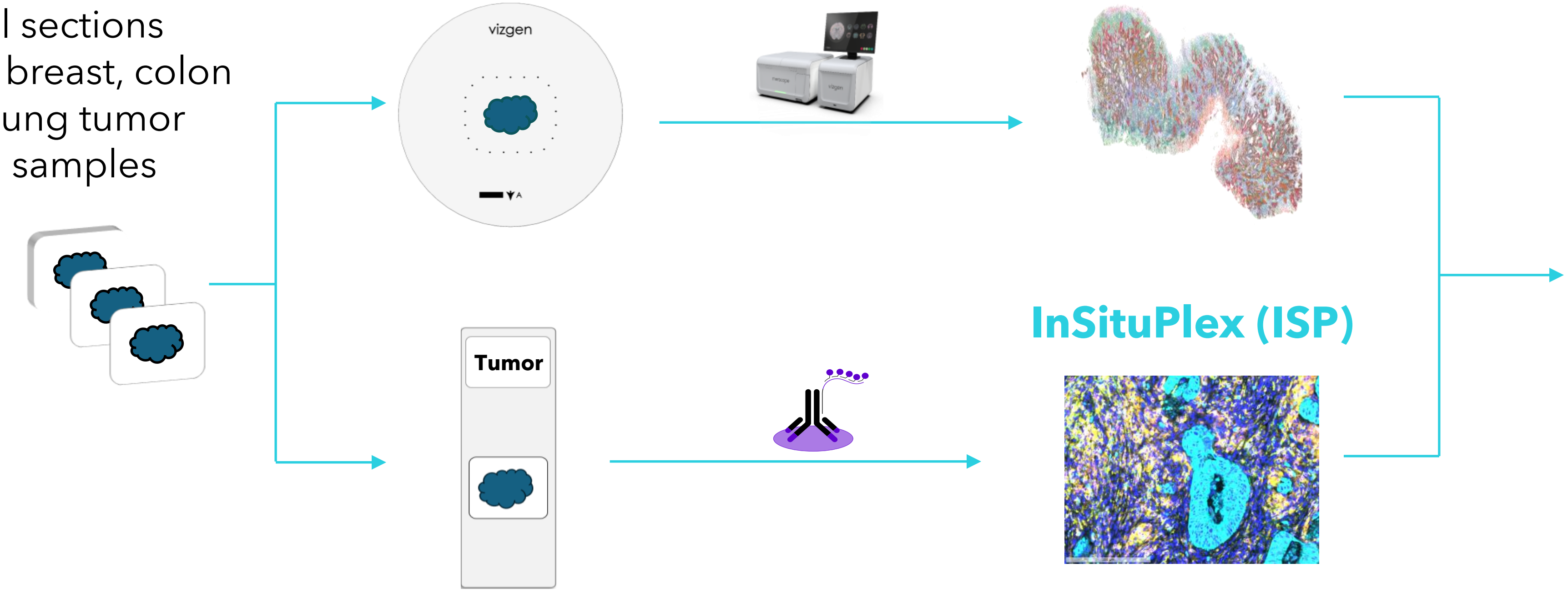
Generating matched multi-omic data with MERFISH 2.0 and ISP

Sectioning

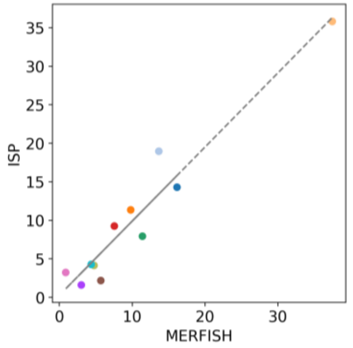
Data collection

Analysis of each dataset

Multi-omic analysis


Serial sections from breast, colon and lung tumor FFPE samples

InSituPlex (ISP)



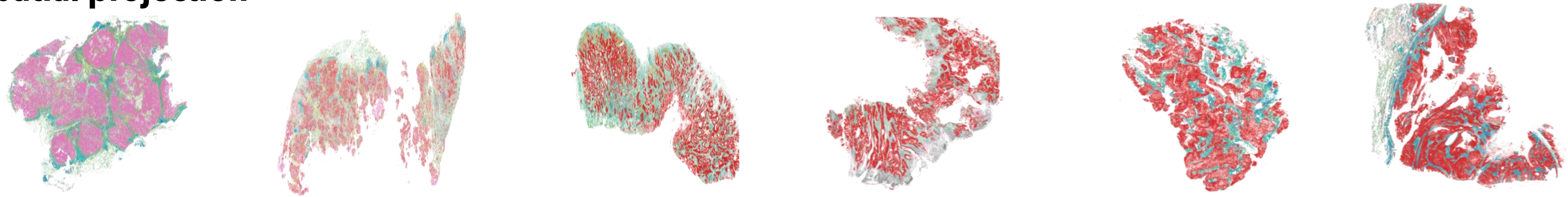
All tumor samples generated high MERFISH counts

Sample	Gene panel (815-plex) description	Total cells	Total counts	Counts/100 μm^2	Median transcripts/cell
Breast Infiltrating ductal carcinoma	Human Breast Cancer panel	767,974	382,677,293	366	324
Breast Tumor	Human Breast Cancer panel	270,489	56,728,287	130	143
Colon Adenocarcinoma, Invasive	Human ImmunoOncology (IO) panel	703,879	150,234,785	206	102
Colon Adenocarcinoma, Invasive	Human ImmunoOncology (IO) panel	561,747	151,001,514	165	143
Non-Small-Cell Lung Cancer (NSCLC)	Human ImmunoOncology (IO) panel	112,337	39,145,995	185	212
Non-Small-Cell Lung Cancer (NSCLC)	Human ImmunoOncology (IO) panel	629,612	209,290,557	214	178

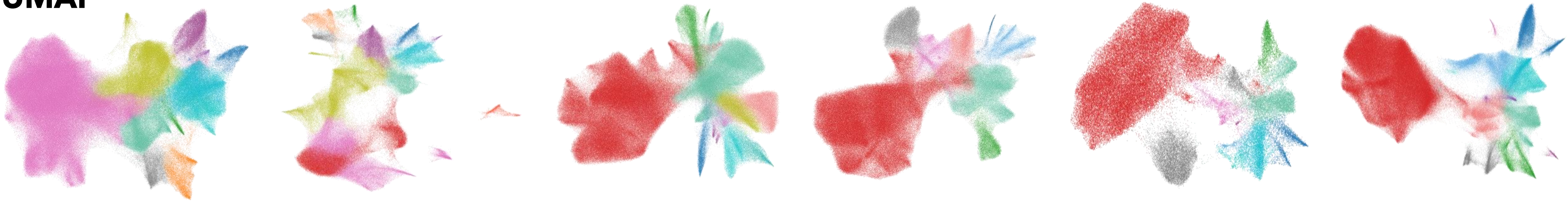
MERFISH 2.0 generates high quality spatial data across tumor types

Breast cancer | **Colon cancer** | **Lung cancer**

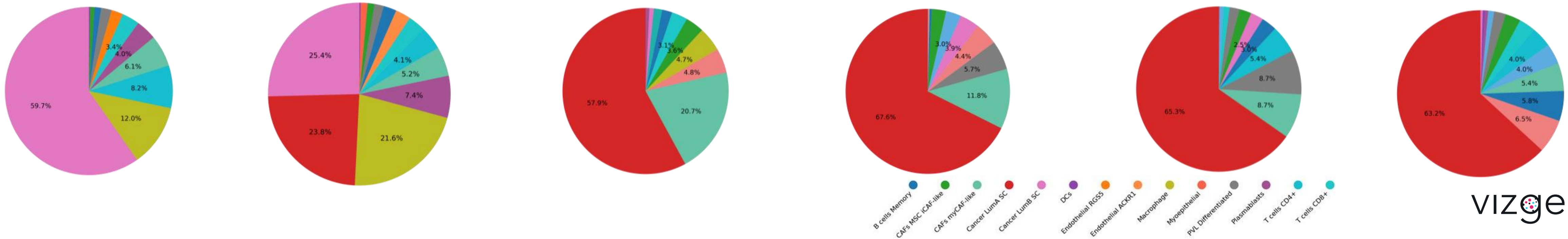
A. Spatial projection



B. UMAP

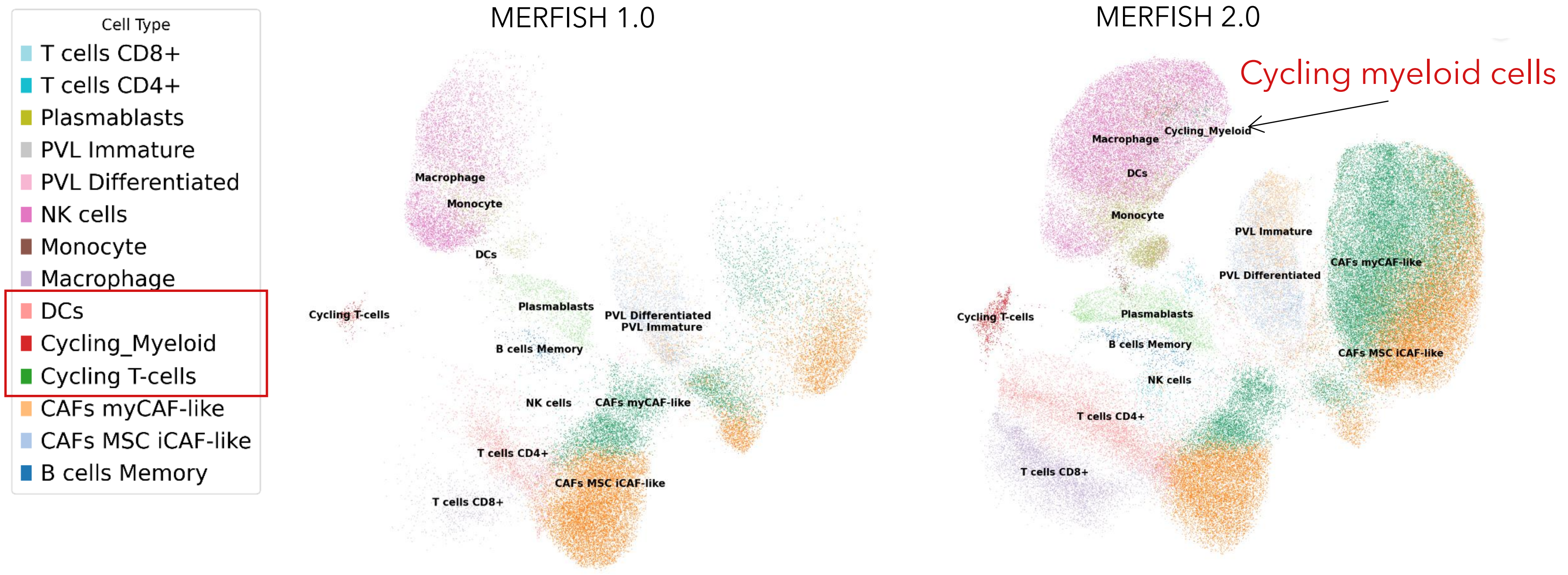


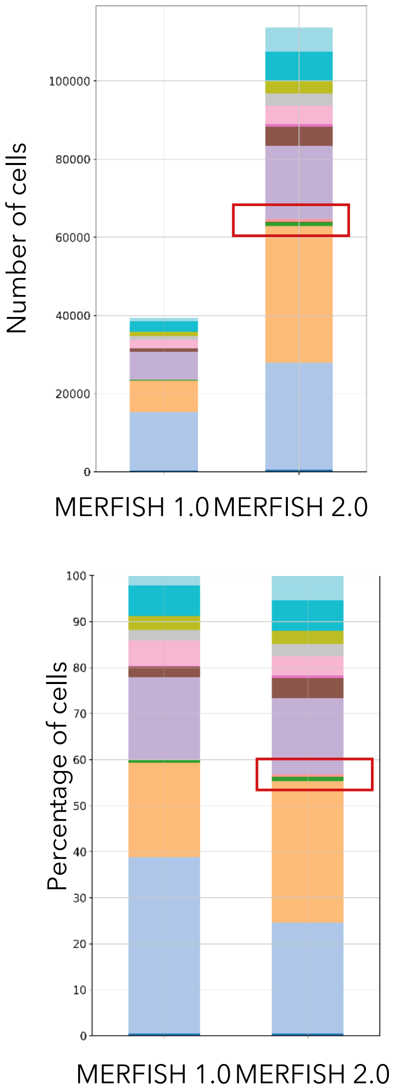
C. Cell type distribution



vizgen

Deep cell typing of immune cells with MERFISH 2.0





- Cycling myeloid cells are identified by MERFISH 2.0
- CD8 T cells, monocytes, and others are better captured by MERFISH 2.0, with fraction of cells increased

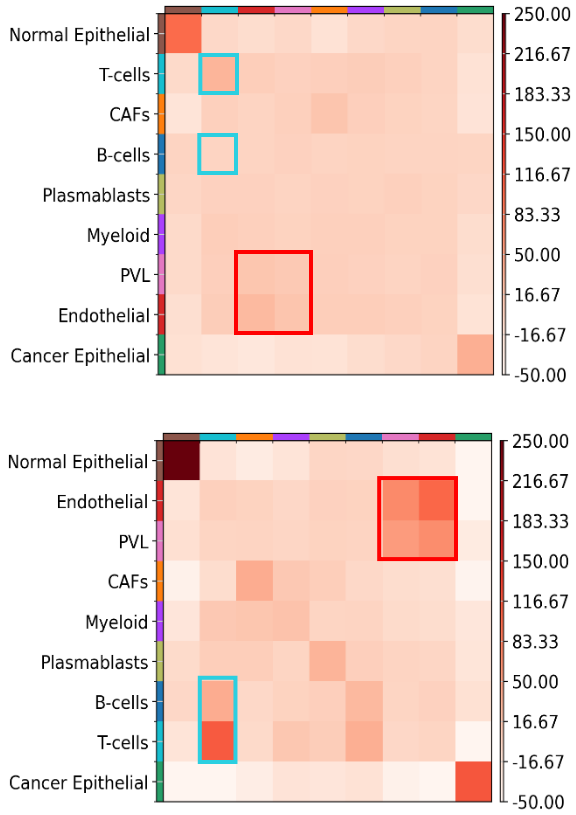
Re-clustered using known immune cell markers

MERFISH 2.0 Uncovers Cell-Cell Interactions

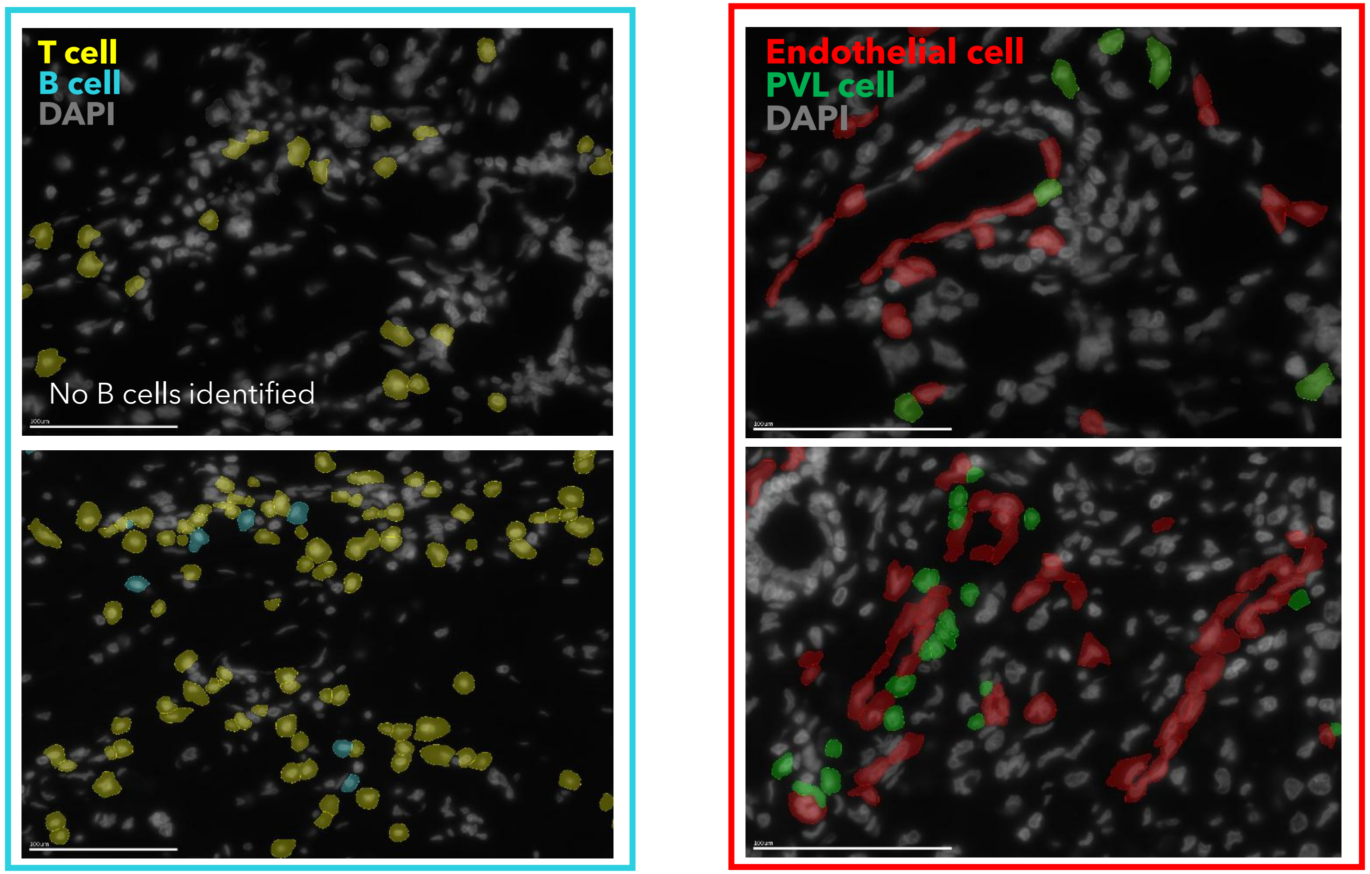
Spatial enrichment of major cell types

MERFISH 1.0

MERFISH 2.0



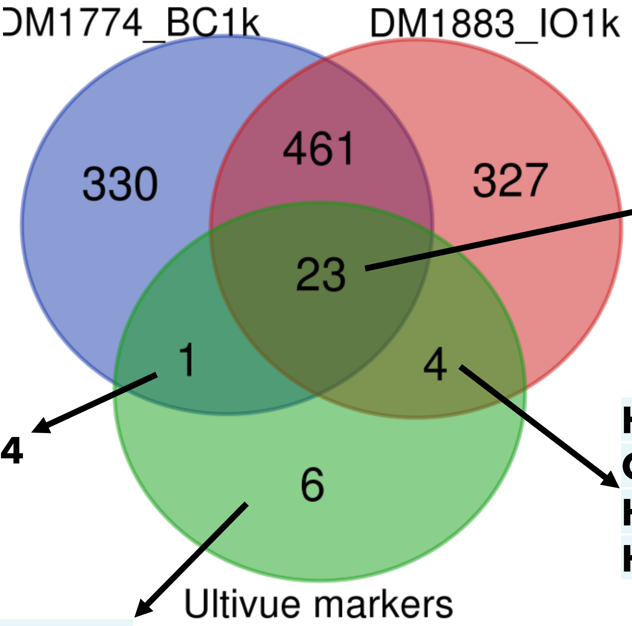
Improved cell-cell interaction analysis with MERFISH 2.0



Spatial enrichment analysis reveals T & B cell as well as PVL & Endothelial cell interaction

vizgen

Comparisons of MERFISH gene panels with ISP validated protein markers



FUT4

FOLR1
KRT8
TACSTD2
KRT18
KRT19
F3

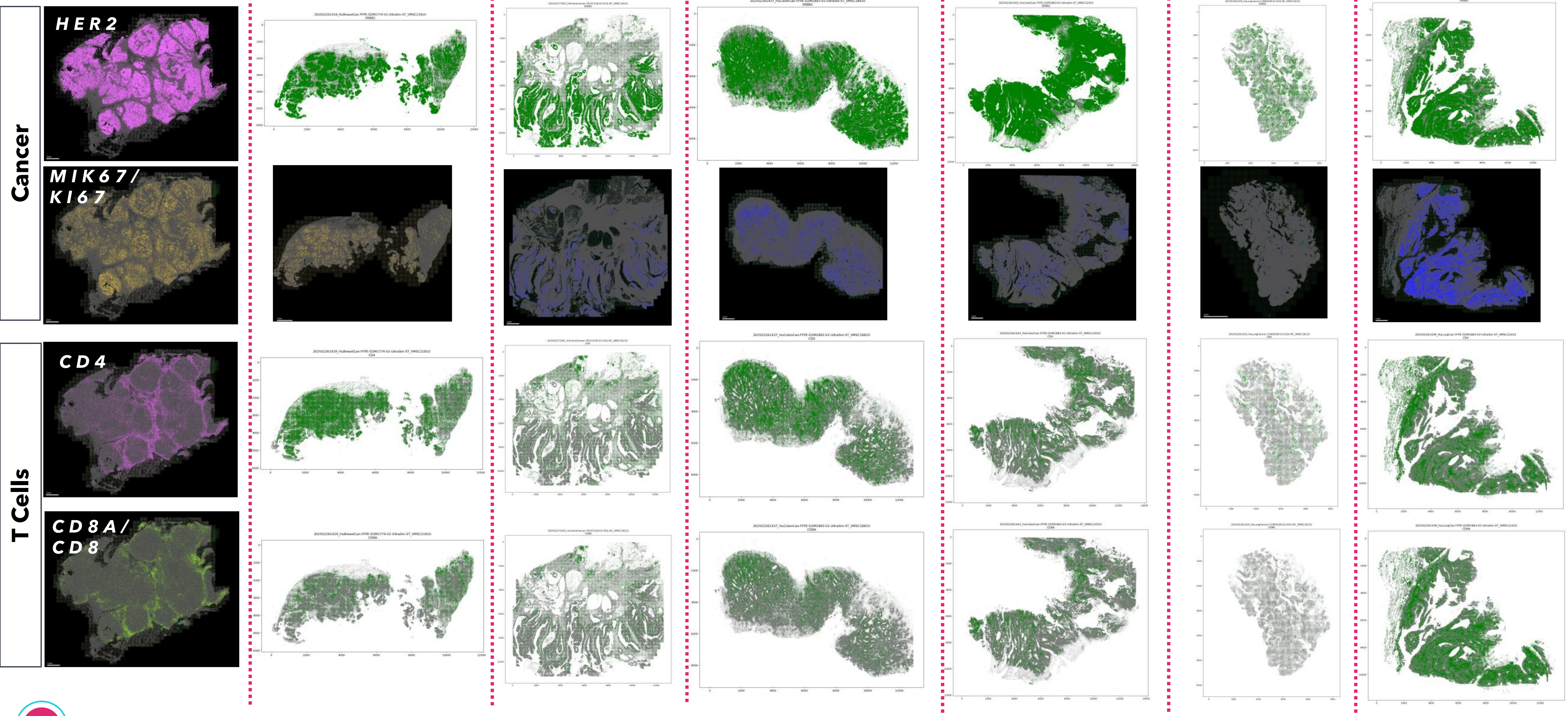
HLA-DRA
CTLA4
HLA-DPB1
HLA-DRB1

CD4
FOXP3
NCAM1
LAG3
SOX10
CD8A
CD3D
MRC1
CD68
PTPRC
CD14
ITGAM
CD163
MS4A1
ERBB2
GZMB
ITGAX
CD3G
PDCD1
CD8B
CD3E
CD274
MKI67

- ➢ Compared MERFISH 1000plex Breast Cancer and IO panels with validated ISP markers
- ➢ Assess RNA expression of overlapping genes using MERFISH data
- ➢ Developed 12-plex protein panel for each cancer indication based on the expression patterns of the select set of genes.

ULTIVUE MARKER COMPARISONS – **ENRICHMENT OF T CELL MARKERS**

Breast Cancer		**Colon Cancer**			**Lung Cancer**	
1291297B	**ILS45390PT2-0**	**761515PB**	**1319400B**	**1324256B**	**1298263B**	**1300266B**



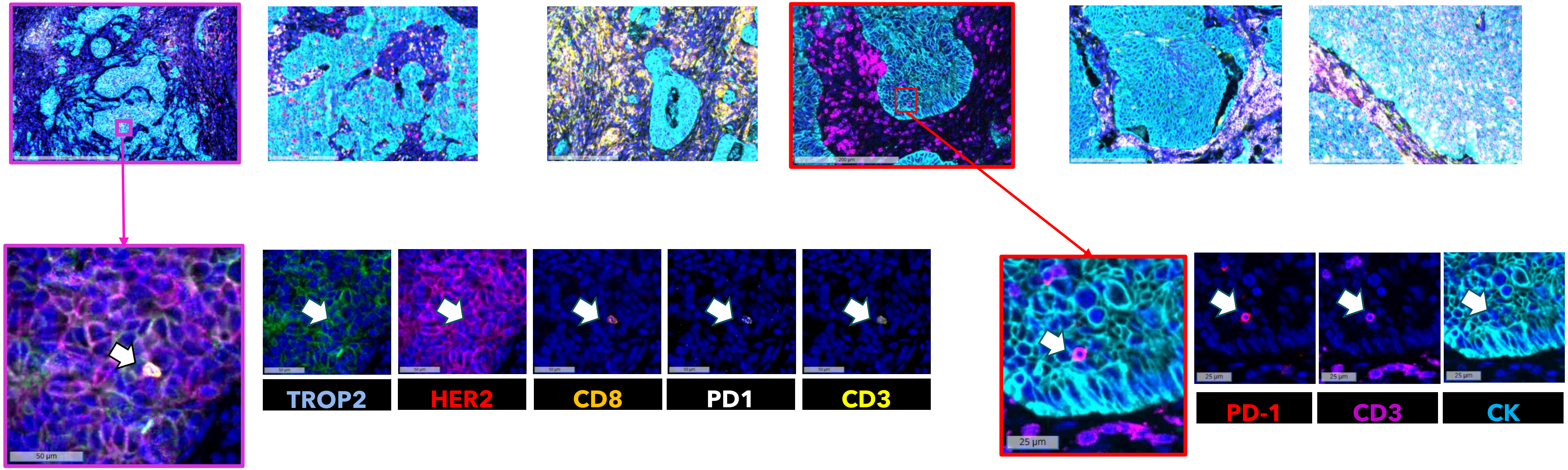
vizgen

InSituPlex assays with 12-plex OmniVUE panels generate high quality spatial proteomics data across tumor types

Breast cancer

Colon cancer

Lung cancer

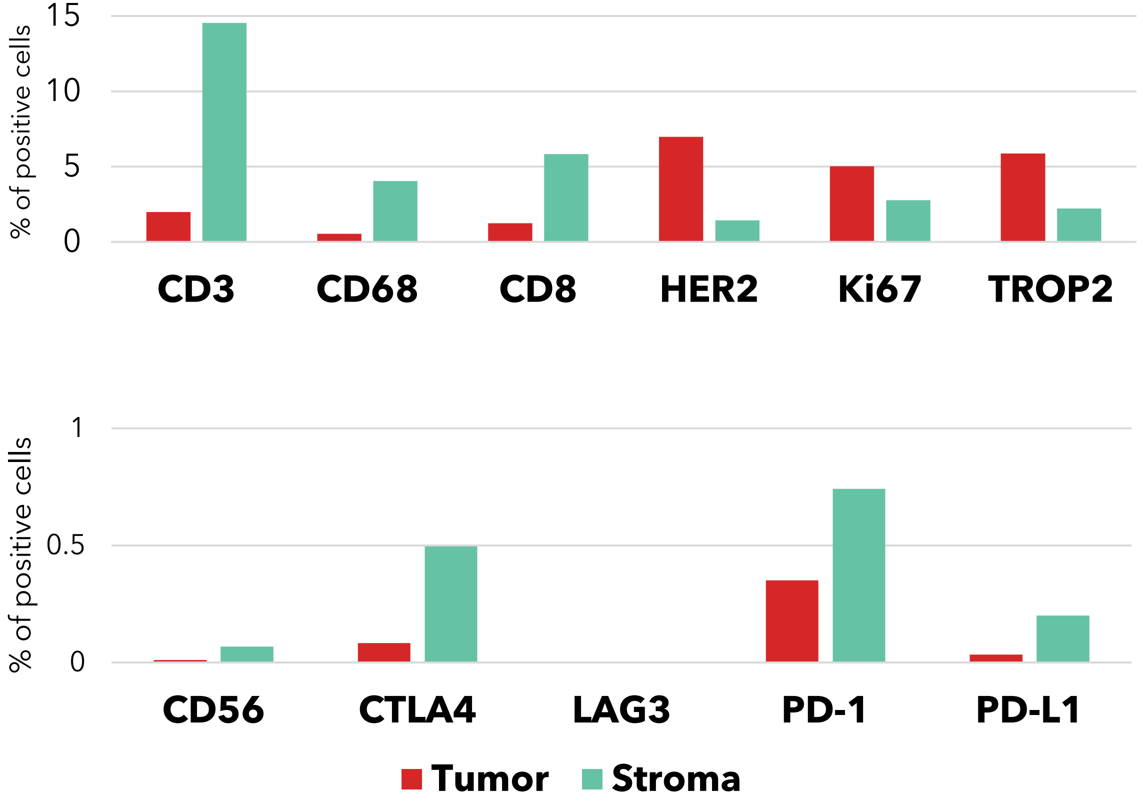


Exhausted Cytotoxic T cell infiltrating a TROP2+/HER2+ tumor

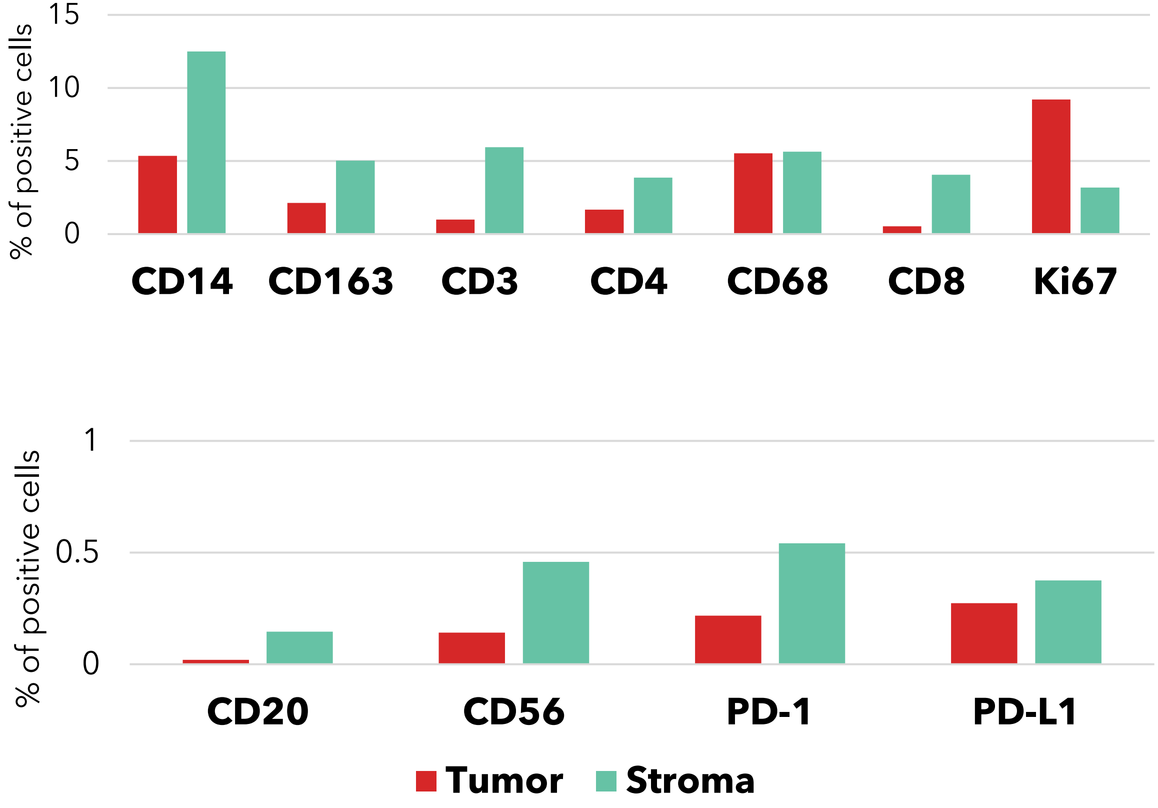
Tumor-infiltrating Exhausted T cell

Multiplexed protein markers characterize the tumor microenvironment

ISP marker expression in tumor vs stroma regions in breast cancer sample

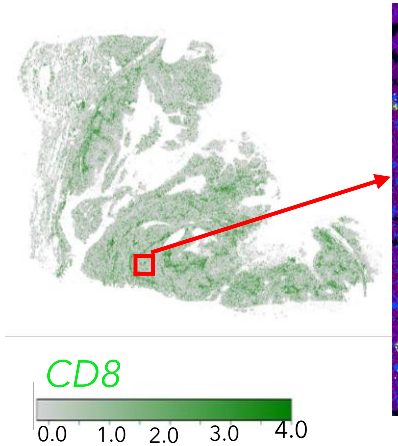


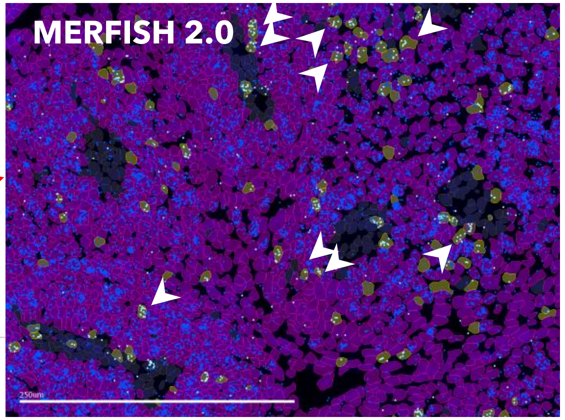
ISP marker expression in tumor vs stroma regions in colon cancer sample



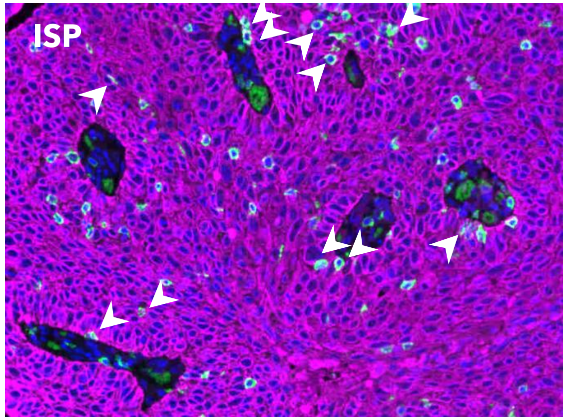
MERFISH 2.0 and ISP show similar spatial distribution of RNA and protein biomarkers along with highly correlated expression in NSCLC

Highly immune infiltrated NSCLC

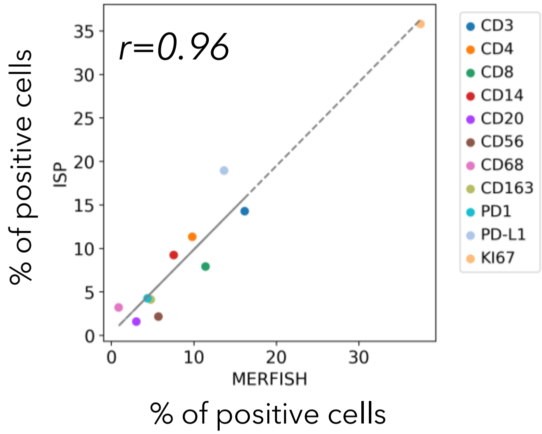




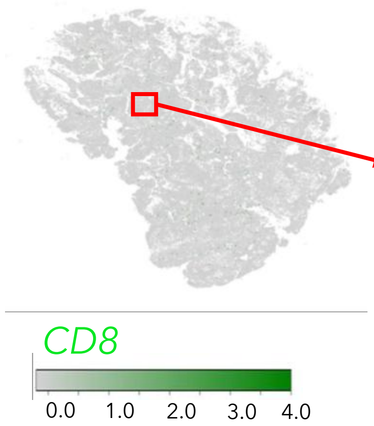
Tumor cells CD8A MKI67

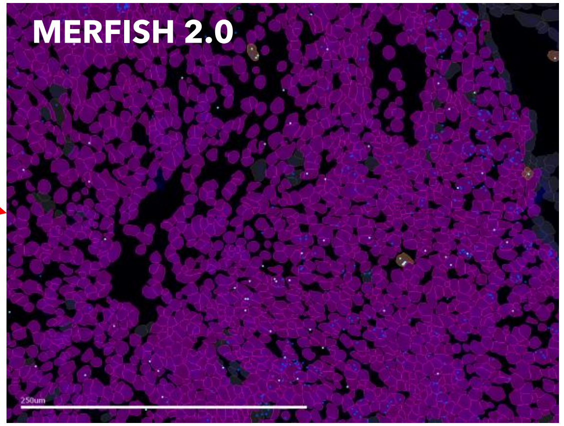


Cytokeratin CD8 DAPI

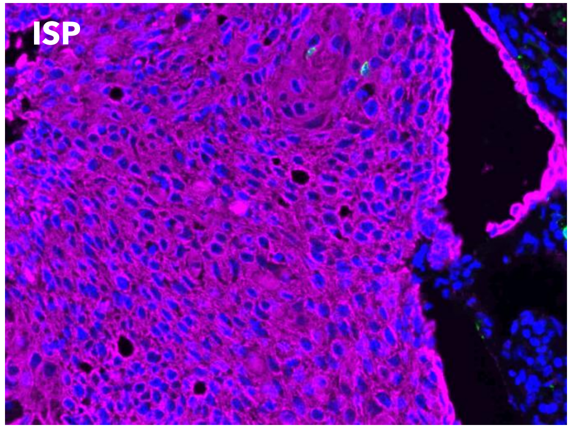


Minimally immune infiltrated NSCLC

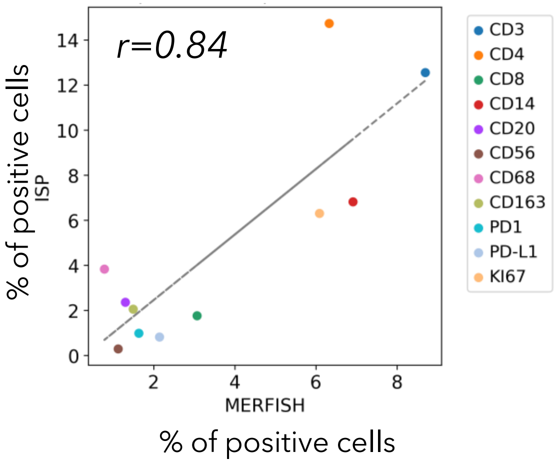




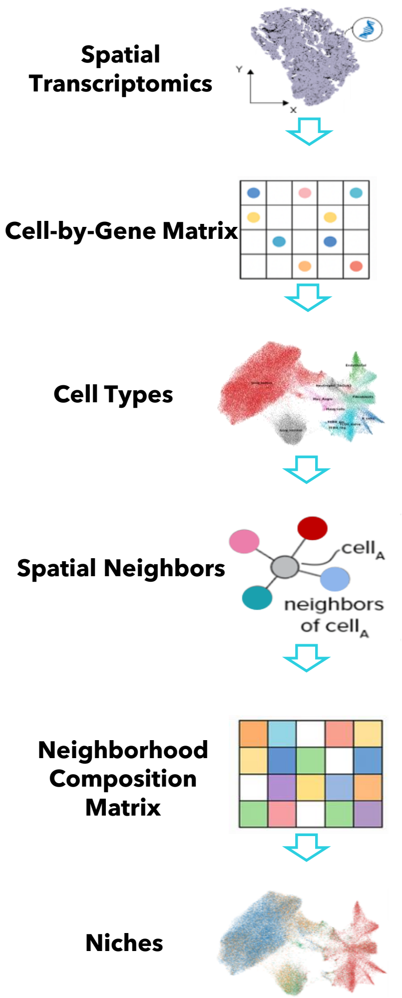
Tumor cells CD8A MKI67



Cytokeratin CD8 DAPI



Spatial phenotyping using MERFISH identifies spatial domains in highly and minimally infiltrated NSCLC samples



Spatial Transcriptomics

Cell-by-Gene Matrix

Cell Types

Spatial Neighbors

Neighborhood Composition Matrix

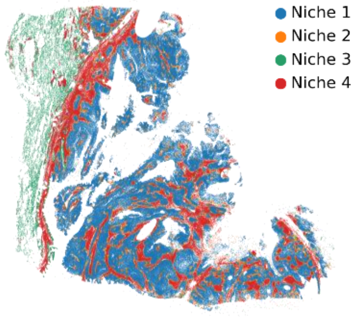
Niches

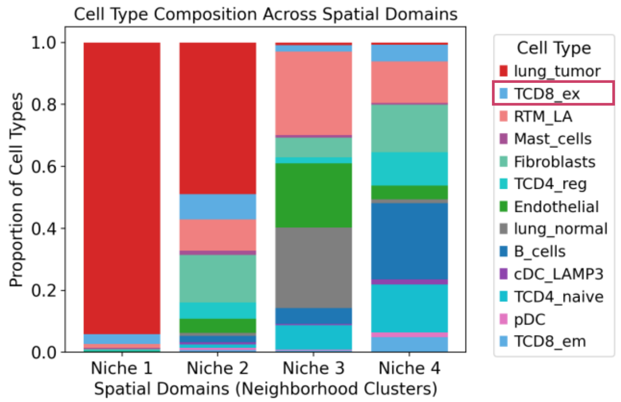
Spatial projection of niches

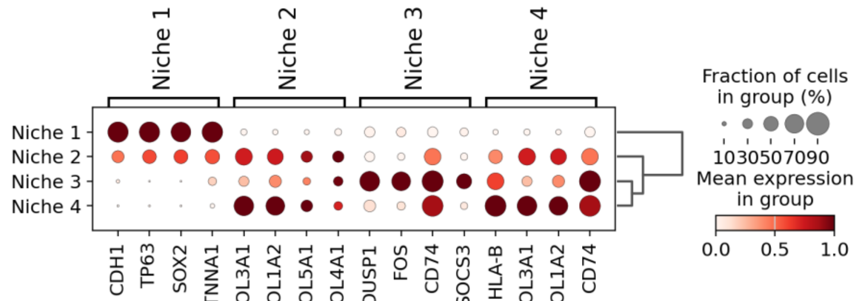
Cellular composition of each niche

Top genes expressed in each niche

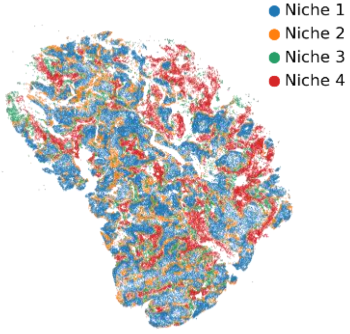
“Hot” tumor

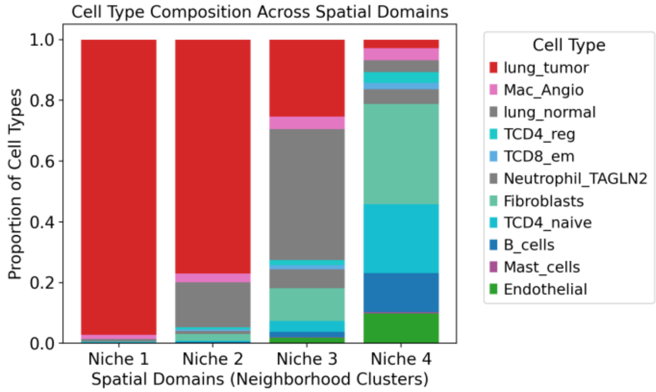


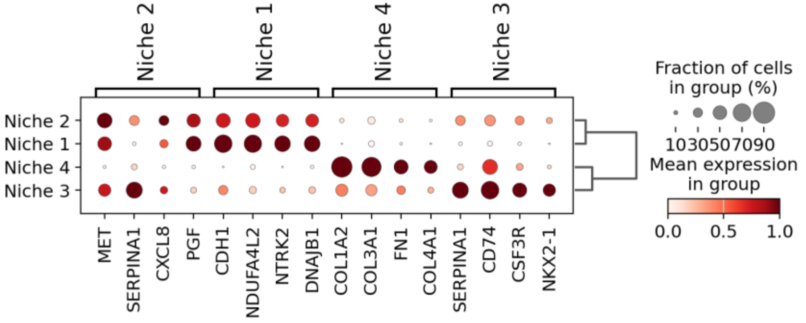




“Cold” tumor







Summary and conclusions

- Vizgen's MERFISH 2.0 and InSituPlex (ISP) spatial transcriptomics and proteomics platforms generate high quality data using human FFPE cancer samples.
- Spatial analysis using MERSCOPE Ultra revealed increased infiltration of cytotoxic T cells within most tumors, which was further confirmed using 12-plex OmniVUE panels.
- Importantly, spatial analyses of the MERFISH 2.0 and ISP data revealed a high degree of RNA–protein correlation in the lung cancer datasets.
- Correlation of RNA and protein data was very high (r=0.96) in a highly immune infiltrated NSCLC sample, though lower in a less-infiltrated sample. This could reflect biological differences in RNA or protein regulation in different cancer types.
- Analysis of the MERFISH 2.0 data also revealed defined spatial domains enriched in immune–enriched NSCLC sample when compared with that of a minimally enriched counterpart.
- The data generated by MERSCOPE Ultra and ISP clearly demonstrate that the two technologies have tremendous promise in characterizing RNA and protein at single cell and spatial resolution, using human FFPE cancer samples.

Next Steps

Multi-omic imaging

Image Stacking from multiple platforms

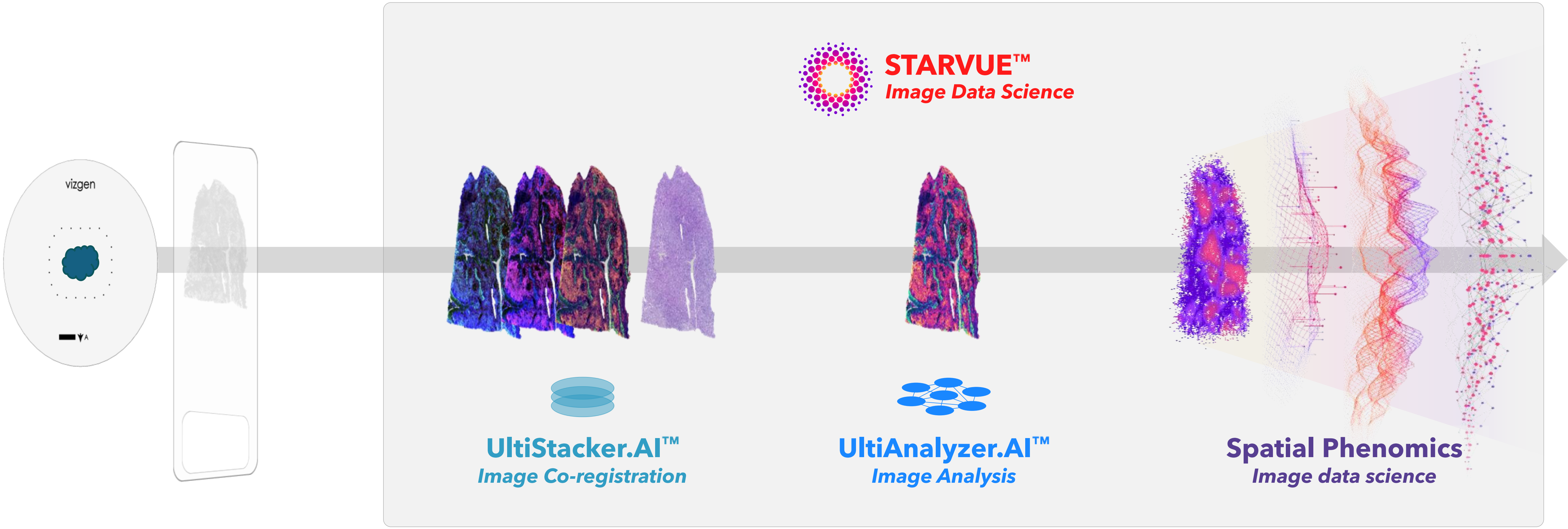
Image Analysis

- Deep-learning enabled whole-slide analysis
- Highly scalable cloud-based infrastructure
- Custom phenotyping through robust co-detection of multiple biomarkers

Biological Insights

- Intercellular dynamics: ROIs vs. whole section
- Advanced spatial phenomics: Clinical data integration, Endpoint analysis, Cohort-level data stratification, and more.

Sample



STARVUE™
Image Data Science

UltiStacker.AI™
Image Co-registration

UltiAnalyzer.AI™
Image Analysis

Spatial Phenomics
Image data science

Acknowledgements

Jiang He, VP of R&D and Co-Founder of Vizgen

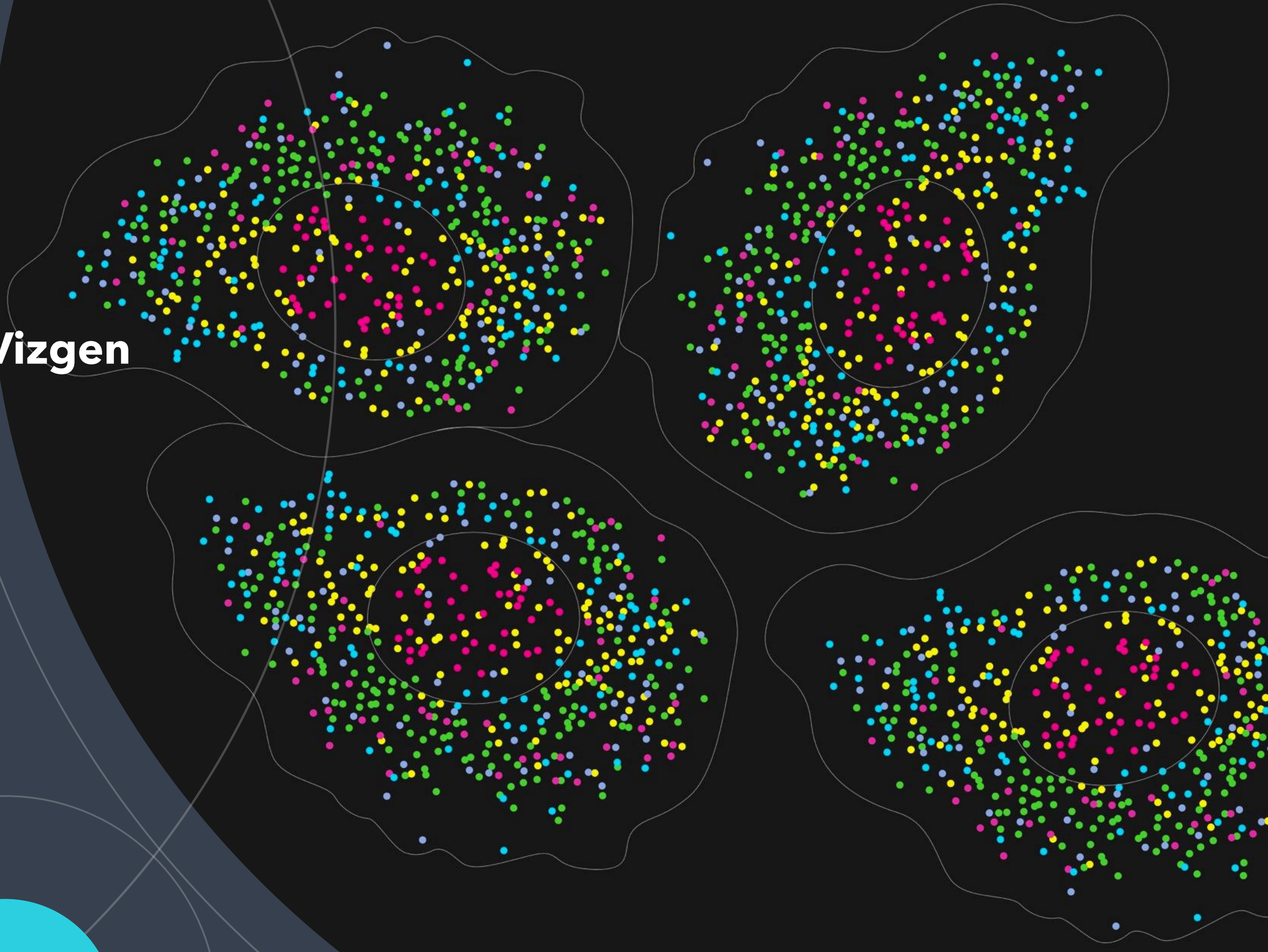
Proteomics

Kevin Hwang
Cassie Kysilovsky
Angela Vasaturo
Lorenz Rognoni

Transcriptomics

Renchao Chen
Sudhir Tattikota

All of the patients



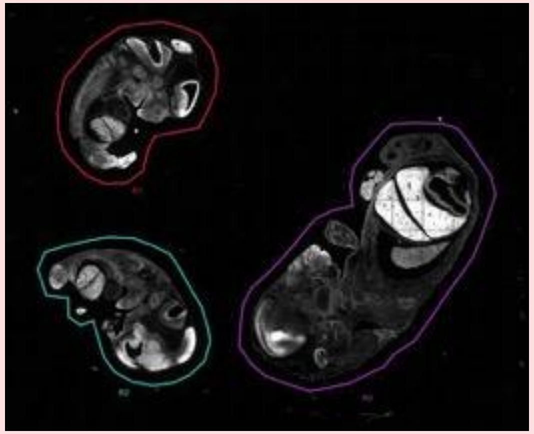
Flexibility to Combine Multiple Experiments on a Single Slide or Compare Conditions while Minimizing Batch Effects

Normal brain

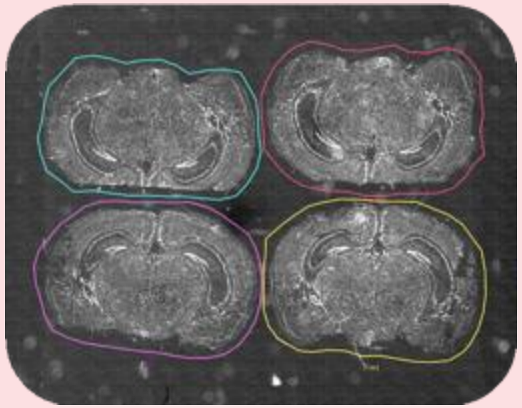
AD brain

Vizgen, Inc. internally generated data

Multi-stage mouse embryo



Vizgen, Inc. internally generated data



4x coronal adult mouse brain sections

Vizgen, Inc. internally generated data

150um of mouse retina serial sections

Image courtesy of the Sanderson Center for Optical Experimentation (SCOPE) at the UMass Chan Medical School