Abstract 6674

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Combining Vizgen's MERSCOPE® spatial transcriptomics and InSituPlex® spatial proteomics profiling to unravel the complexities of the tumor-immune microenvironment

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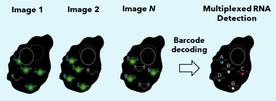
Introduction

Despite remarkable results for many patients, cancer therapies have still a poor response rate. Biomarkers used to select patients for treatment are few and only single-biomarker assays are clinically approved. Here we aim to enable a comprehensive understanding of the molecular, morphological and functional landscape of tumors using a multi-omics approach **combining spatial transcriptomics and proteomics** assays. Vizgen's MERSCOPE® Ultra™ platform allows for high-resolution, spatially resolved analysis of up to 1000 genes, enabling the study of molecular and cellular signatures associated with the Tumor-immune MicroEnvironment (TiME), while Ultivue's InSituPlex® (ISP) spatial proteomics assay allows for pathology-grade high-throughput detection of tumor and immune cell interactions, functions and spatial distribution. We demonstrate the ability to orthogonally validate findings and expand on the biomarker-driven protein data with pathway-level data from the transcriptomic data. The integration of gene expression profiling enabling screening of a broad range of targets, and multiple immunofluorescence (mIF) assays for target validation, allows for a holistic understanding of spatial and molecular insights of the TiME. These complementary technologies can be used in parallel for biomarker discovery and immuno-oncology pathways interrogation providing synergistic insights into the complex and dynamic TiME.

Materials and Methods

Background

MERFISH 2.0

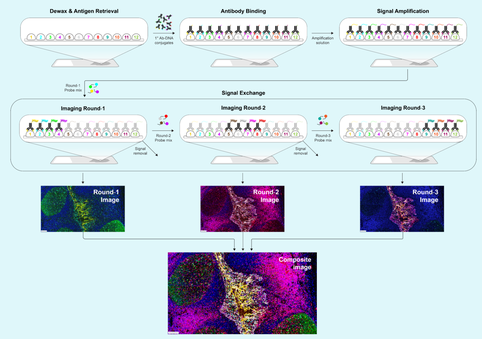


MERFISH 2.0 uses binary barcodes to encode different mRNA species, which enables *in situ* profiling of hundreds to thousands of genes at single-molecule resolution.

MERSCOPE Ultra

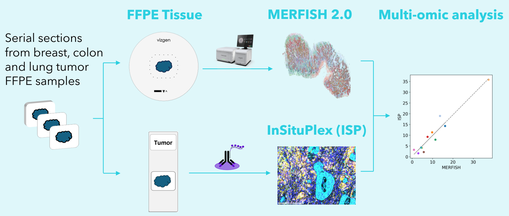
The MERSCOPE Ultra Platform provides an end-to-end solution for the MERFISH 2.0 technique, from sample preparation to data analysis and visualization.

InSituPlex (ISP)



The ISP assay allows multiplexed immunofluorescence proteomic staining of up to 12 biomarkers in a single kit. Barcode tags on each antibody are amplified simultaneously, and markers are visualized in sets of 4 at a time. Images are co-registered and analyzed using the STARVUE™ image data science platform.

Methods



Formalin-fixed paraffin-embedded (FFPE) specimens from multiple tumors were cut into 5µm sections for both technologies. Serial sections were profiled with Vizgen's MERSCOPE Ultra Platform and 815-plex Breast cancer and ImmunoOncology (IO) panels with MERFISH 2.0 chemistry, or with a 12-plex OmniVUE mIF panel including markers for antibody-drug conjugate (ADC) targets. The resulting spatial transcriptomic data was segmented using Cellpose2 and processed for single cell analysis downstream using both UMAP and spatial projections for visualization. Immunofluorescence images were co-registered, then exported for analysis using Ultivue's STARVUE™ platform, which quantifies the expression of each marker across the region imaged.

Results

MERFISH 2.0 generates high quality spatial transcriptomics data across tumor types

Table 1: Transcript counts from human FFPE cancer samples

Sample	Gene panel (815-plex) description	Total cells	Total counts	Counts/100 µm²	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

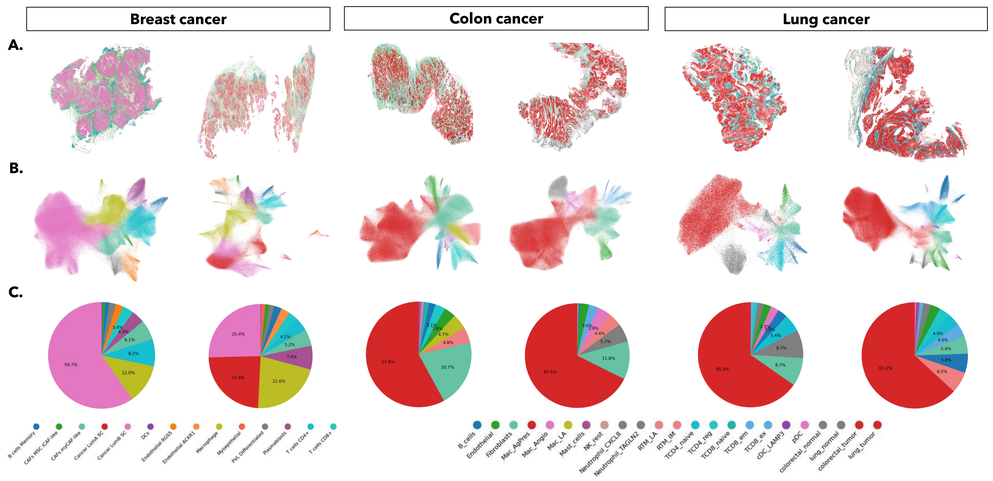


Figure 1: MERFISH 2.0 was performed on FFPE tissues from a range of human cancers (**Table 1**). Plots showing the spatial distribution of cells using MERSCOPE Ultra (**A**), the uniform manifold approximation & projection (UMAP) plots of cell types (**B**), and pie charts representing the cell type composition of all the tumor types (**C**). Legends underneath show cell types identified from the 815-plex human Breast cancer (left) or ImmunoOncology (right) gene panels.

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

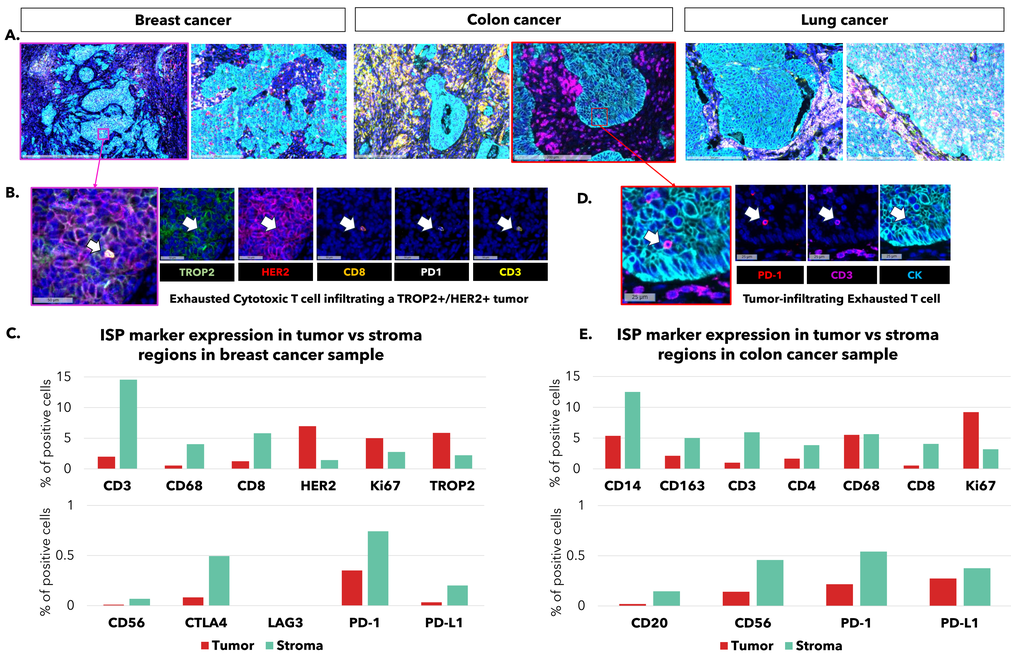


Figure 2: ISP assays allow for detailed phenotype evaluation in tumors, and quantification of marker or cell prevalence in different areas of tissue. (**A**) Selected ROIs of FFPE tissue blocks analyzed in Figure 1, after staining with 12-plex OmniVUE panels. (**B**) Breast cancer sample stained using a 12-plex ADC OmniVUE panel shows HER2 and TROP2 expression and cytotoxic T cell infiltration. (**C**) Comparative expression of immune markers on cells in tumor versus nontumor regions of the breast cancer sample in **A**. Tumor region is identified via CK expression. (**D**) Colon cancer sample stained with a 12-plex OmniVUE panel that highlights an infiltrating exhausted T cell phenotype. (**E**) Comparative expression of immune markers on cells in tumor versus nontumor regions of the colon cancer sample in **D**.

MERFISH 2.0 and ISP show similar spatial distribution of RNA and protein biomarkers along with highly correlated expression in NSCLC with high and low levels of immune infiltration

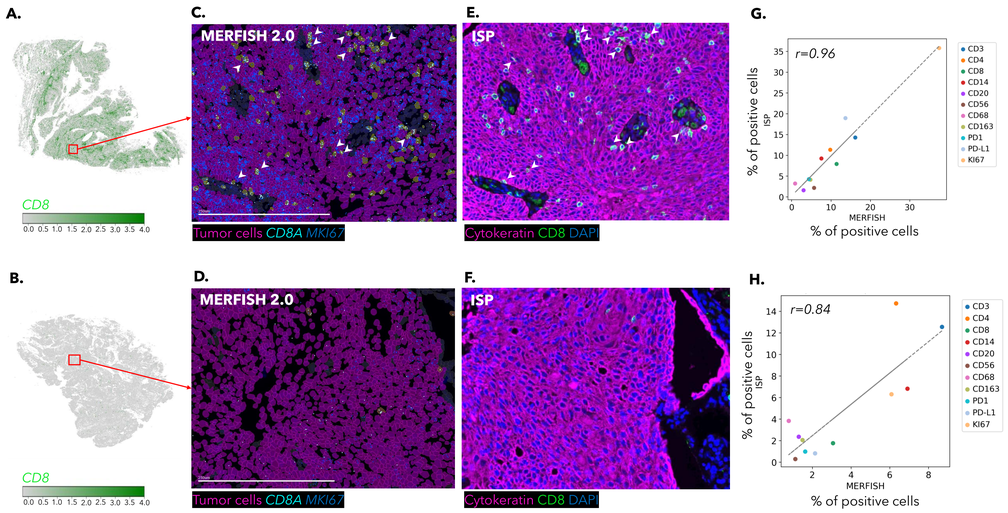


Figure 3: MERFISH 2.0 and ISP reveal good RNA–protein correlations in NSCLC samples displaying variations in immune cell infiltration. Spatial distribution of *CD8* mRNA expression in highly (**A**) and minimally infiltrated (**B**) samples shows increased enrichment in the highly infiltrated NSCLC sample. MERSCOPE images demonstrate increased infiltration of CD8+ exhausted T cells in the highly infiltrated sample (**C**) compared with that of minimally infiltrated sample (**D**). Dots represent *CD8A* in cyan and *MKI67* transcripts in blue, markers for CD8+ T cells and proliferating cancer cells, respectively. Spatial 12-plex proteomics by ISP reveal increased infiltration of CD8+ T cells in highly (**E**) compared with that of minimally infiltrated NSCLC (**F**), as well as similar patterns of expression as in MERFISH data (see arrowheads in **C**,**E**). mIF images show the expression of Cytokeratin (CK) in magenta, CD8 in green, and DAPI in blue, which mark cancer cells, T cells and nuclei, respectively. Correlation plots demonstrate percent of cells expressing RNA (from MERFISH 2.0 data) or protein (from the 12-plex ISP data), which correlate well in both highly (**G**) and minimally infiltrated (**H**) NSCLC samples.

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

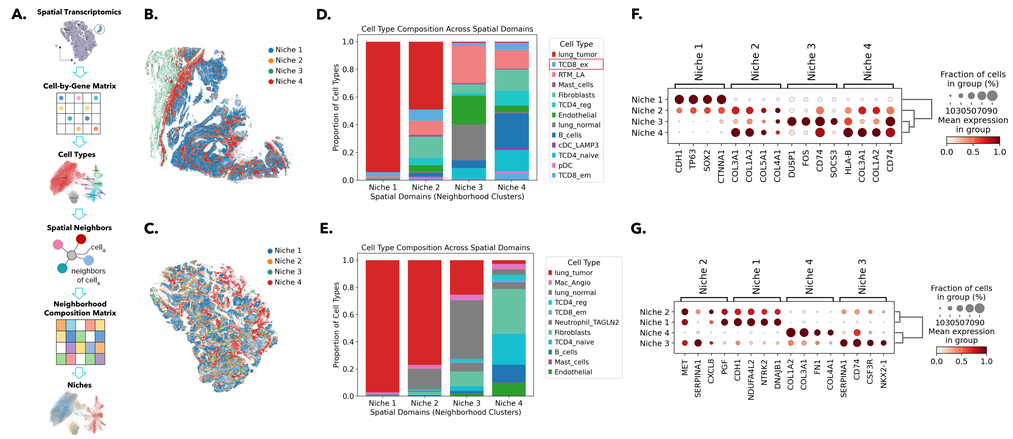


Figure 4: Spatial analysis reveals "spatial" domains (see workflow in **A**) in highly immune infiltrated (**B**) vs minimally infiltrated (**C**) NSCLC samples. Bar graphs represent cell type composition in each domain, showing increased exhausted T cell (TCD8_ex) infiltration in domains 1 & 2 in highly infiltrated NSCLC (**D**) vs the minimally infiltrated NSCLC (**E**). Dot plots show the marker gene enrichment in each domain in both the highly and minimally infiltrated NSCLC samples (**F, G**).

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.**