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New developments in spatial transcriptomics using MERFISH 2.0™ unlock insights into the tumor microenvironment

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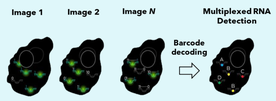
Introduction

Despite remarkable results for many patients, the response rate for immunotherapy varies from one patient to the next. While technological advances have made it possible to study single cells in their native context, samples with lower RNA quality remain challenging for capturing the full complexity and dynamic nature of the tumor-immune microenvironment. Vizgen's MERSCOPE Ultra™ platform allows for high-plex, high-resolution, spatially resolved spatial transcriptomic analysis of up to 1000 genes, enabling the study of whole transcriptomic signatures associated with tumor status and the immune system. The Multiplexed Error-Robust Fluorescence in Situ Hybridization (MERFISH) technology facilitates direct RNA profiling in situ with high sensitivity and sub-cellular resolution. With MERFISH 2.0, an enhanced MERFISH workflow that substantially improves the sensitivity of RNA detection, we profiled multiple tumor samples from patients and elucidated how the local immune environment influences gene expression and protein activity in the tumors.

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 technology, from sample preparation to data analysis and visualization.

Experimental design

Formalin-fixed paraffin-embedded (FFPE) specimens from breast, colon, and lung tumor indications were cut into 5µm sections for spatial transcriptomic measurement. Two tumor samples were measured for each indication. An 815-plex Human Immune-Oncology (IO) Panel was used for lung and colon cancer, and an 815-plex Human Breast Cancer Panel was used for breast cancer samples using the MERFISH 2.0 workflow. The resulting data was used for single cell and spatial analyses.

Analysis

Spatial Transcriptomics

MERFISH counts were calculated per gene across the tissue (Figure 1A)

Cell-by-Gene Matrix

The resulting counts were assembled into a single-cell-by-gene matrix, which was used to perform uniform manifold approximation & projection (UMAP) analysis and Leiden clustering

Cell Types

The top genes per Leiden cluster were used to assign cell types at the single-cell level (Figure 1 B&C)

Spatial Neighbors

The cell type data was used to perform the spatial nearest neighbor analysis of the tumor and its microenvironment

Neighborhood Composition Matrix

Spatial analysis reveals spatial domains or niches of cell types

Niches

The gene and spatial data were used to assess the top differential genes between spatial niche and between different tumor samples

Results

MERFISH 2.0 generates high quality spatial transcriptomics data across tumor types

Table 1: Transcript counts from human FFPE cancer samples

Tissue type	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

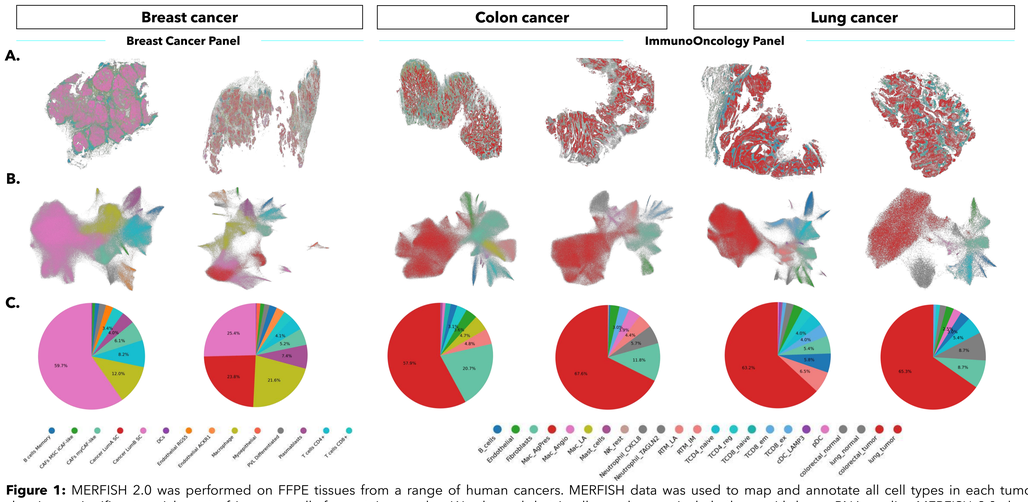


Figure 1: MERFISH 2.0 was performed on FFPE tissues from a range of human cancers. MERFISH data was used to map and annotate all cell types in each tumor, showing a significant enrichment of immune cells for certain samples. We showed that in all samples, particularly those with lower RNA quality, MERFISH 2.0 shows excellent detection efficiency for RNA transcripts and subsequent quality of downstream data analysis. (**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.

High quality MERFISH 2.0 data reveals distinct spatial neighborhoods across sample types

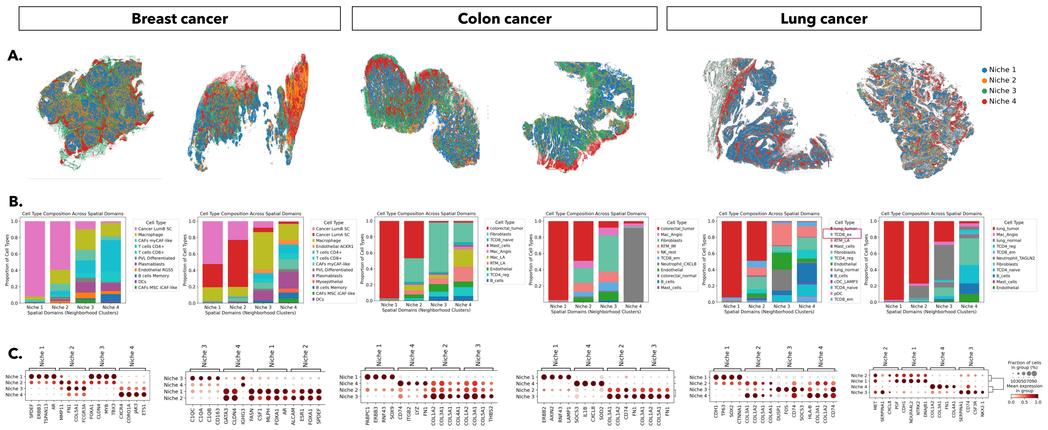


Figure 2: Spatial analysis reveals "spatial domains" or niches (see workflow in Methods) within multiple cancer indications. (**A**) Spatial projections overlaid with each spatially-defined domain after neighborhood analysis. (**B**) Bar graphs represent the cellular composition of each niche. Notably, we observed increased exhausted T cell (TCD8_ex) infiltration in highly infiltrated NSCLC (left) vs the minimally infiltrated NSCLC (right). (**C**) Dot plots show the marker gene enrichment for each niche.

Immune infiltration alters the cellular neighborhood and gene expression of NSCLC tumors

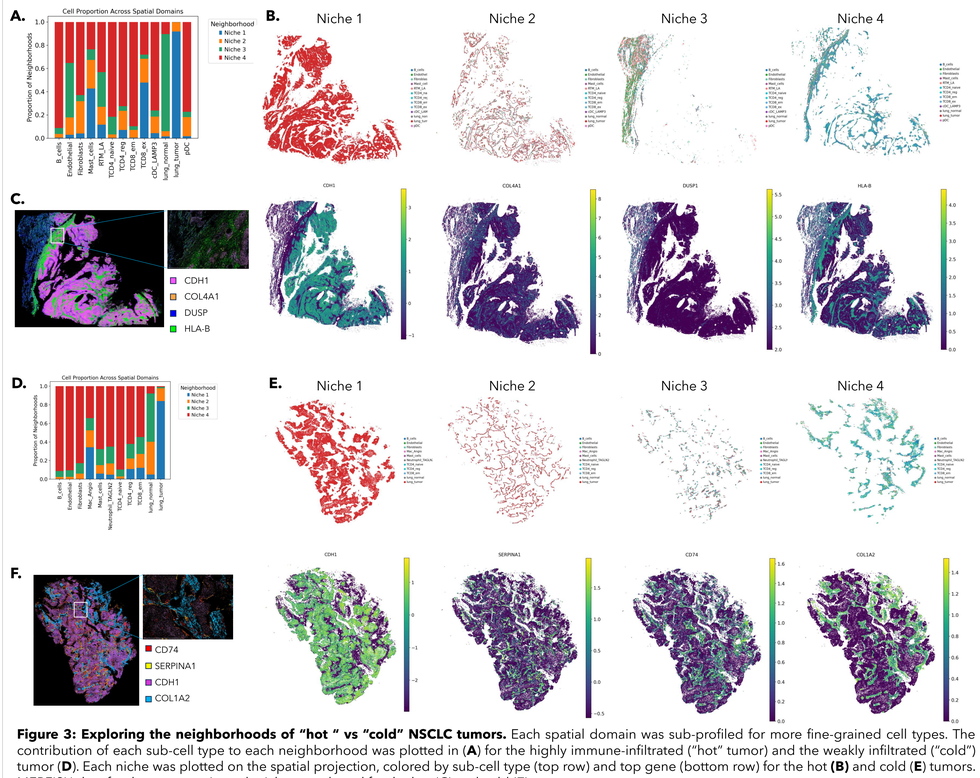


Figure 3: Exploring the neighborhoods of "hot " vs "cold" NSCLC tumors. Each spatial domain was sub-profiled for more fine-grained cell types. The contribution of each sub-cell type to each neighborhood was plotted in (**A**) for the highly immune-infiltrated ("hot" tumor) and the weakly infiltrated ("cold" tumor (**D**). Each niche was plotted on the spatial projection, colored by sub-cell type (top row) and top gene (bottom row) for the hot (**B**) and cold (**E**) tumors. MERFISH data for the top gene in each niche was plotted for the hot (**C**) and cold (**F**) tumors.

Tumor cells exhibit distinct expression patterns in proximity to immune cells

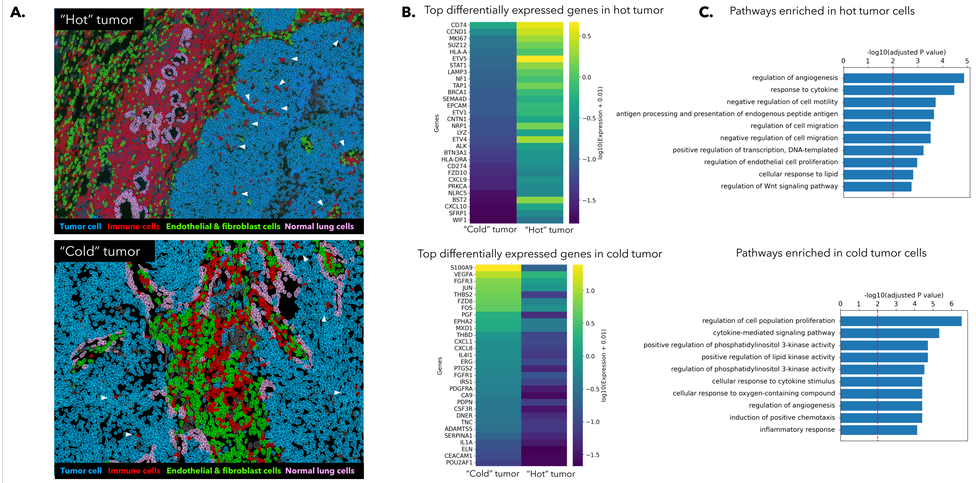


Figure 4: Immune cells altered the gene expression in tumor cells in a spatially-dependent manner. (**A**) Coloring tumor and immune cells within hot and cold tumors shows tumor-immune cell interactions at the tumor boundary only in the hot tumor (white arrows). (**B**) Differential expression analysis of the tumor cells between the two tumors revealed the top relative expressors in the hot and cold tumors. (**C**) GSEA analysis of the differentially expressed genes shows upregulation of immune response pathways in the hot tumor, contrasting with upregulation of cellular proliferation pathways in the cold tumor.

Conclusions

- **Spatially resolved transcriptomic profiling of tumor samples at the single-cell level offers significant opportunities for understanding how cancers develop and evolve in situ. These improvements enable detailed characterization of tumor heterogeneity and will unlock new insights and new avenues for therapeutic research. Spatial analysis using MERSCOPE Ultra revealed increased infiltration of cytotoxic T cells within most tumors.**
- **Spatial profiling data was used to identify and characterize spatially associated niches within each tumor sample.**
- **Different subsets and proportions of tumor and immune cells were found in each tumor, resulting in different neighborhood compositions.**
- **Analysis of the MERFISH 2.0 data revealed defined spatial domains enriched in immune-enriched NSCLC sample when compared with that of a minimally enriched counterpart, and different patterns of expression the tumor cells in proximity to immune cells.**
- **High-quality spatial transcriptomic data demonstrate how spatially-resolved interactions can lead to new mechanistic insights at the tumor-immune interface.**