Abstract #6104

Scan to learn more

New developments in spatial transcriptomics unlock insights into the tumor microenvironment with MERFISH2.0 and MERSCOPE® Ultra™

Manisha Ray, Renchao Chen, Justin He, Bin Wang, Bing Yang, Timothy Wiggin, Lizi Maziashvili, Jichuan Zhang, Alex Genshaft, Brittany Auclair, Shawn Wang, Hao Wang, Sudhir Tattikota, Jiang He, George Emanuel

Vizgen, Inc. 61 Moulton St, Cambridge, MA, 02138

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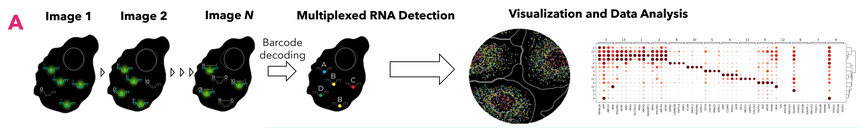
Introduction

The emergence of tools enabling researchers to perform high-plex spatial transcriptomics with single-cell resolution has revolutionized our understanding of tumor development. However, tissue samples with degraded RNA or extensive crosslinking present challenges for gene expression measurements. For decades, cancer tissue samples have been collected and preserved using formalin-fixation and embedding in paraffin (FFPE), which is not optimal for preserving RNA integrity. This has limited the depth of insights researchers can obtain from archival samples.

The Multiplexed Error-Robust Fluorescence in situ Hybridization (MERFISH) technology facilitates direct RNA profiling *in situ* with high sensitivity and resolution. The high accuracy and high plexity are enabled by tiling probes along the length of a transcript. This tiling presents unique challenges in samples with fragmented RNA, such as in FFPE. The MERFISH 2.0 chemistry and sample preparation workflow were developed specifically to improve transcript detection efficiency for up to 1000 genes in low quality tissues. These improvements can be applied to tissues of up to 3cm^2 using the MERSCOPE® Ultra™ Platform. Here we applied the improved capabilities of MERFISH 2.0 to profile human and mouse samples over a range of sample qualities.

Materials and Methods

Improving the MERSCOPE Workflow with MERFISH 2.0 and MERSCOPE Ultra



Unified protocol for all sample types

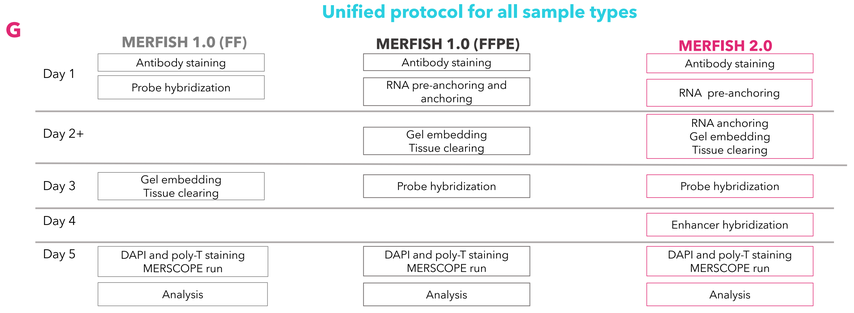


FIGURE 1. Improving the MERSCOPE Workflow with MERFISH 2.0 and MERSCOPE Ultra. A) MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization) uses binary barcodes to encode different mRNA species, which enables *in situ* profiling of hundreds to thousands of genes at single-molecular resolution and higher level spatial and genomic analyses. **B)** The MERSCOPE Ultra Platform provides an end-to-end solution for the MERFISH technique. Here we describe technical improvements to the entire method, including the MERFISH chemistry (**C-D**), instrument (**E**), and analysis (**F**). **C)** MERFISH 1.0 performs well in high quality samples with intact RNA, where many probes can bind along the length of the transcripts to yield sufficient readout signal. However, in lower quality samples, such as some archival FFPE tissues, RNA fragmentation can occur, leading to loss of RNA fragments and fewer available binding sites, which reduces signal intensity. Low intensity fluorescent signal may be lost to background, complicating signal differentiation and accurate transcript quantification. **D**) We developed the MERFISH 2.0 chemistry to enhance MERFISH detection in samples with low quality RNA. The enhanced detection results from 1) optimized anchoring to better capture of RNA fragments; 2) optimized probe structure to enable efficient binding with the targets; and 3) enhanced readout probes that significantly increase the signal-to-noise ratio during imaging. **E)** The MERSCOPE Ultra Platform improves upon the original MERSCOPE platform while maintaining high quality. Using two flow cells, the MERSCOPE Ultra is capable of using of both Standard 40 mm round slides and Large 47 x 57 mm slides. Combined with high resolution optics, the platform can image to 3cm^2 without sacrificing quality. **F)** Improvements to the computational and analytic pipelines, including dedicated graphics processing, compression, and more efficient decoding allow for faster imaging and analysis. **G**) Together, these improvements result in a unified workflow capable of running fresh frozen, fixed frozen, and FFPE samples.

Results

MERFISH 2.0 substantially improved RNA detection efficiency in a wide range of samples from human and mouse.

Sample	815-plex gene panel
Fresh frozen human brain	Human Brain Panel
FFPE human breast cancer	Human Breast Cancer Panel
FFPE human lung cancer TMA	Human ImmunoOncology Panel
FFPE human breast cancer TMA	Human Breast Cancer Panel
Fresh frozen mouse brain	Mouse Pan Neuro Panel
Fixed frozen TMA of mouse tissues	Pan Mouse Panel

Table 1: Panel information

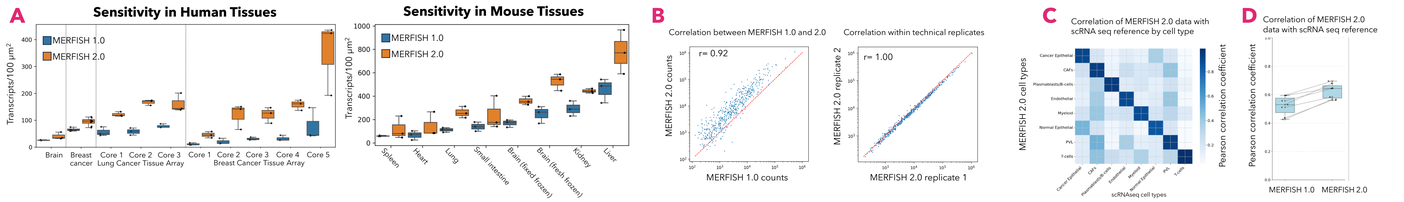
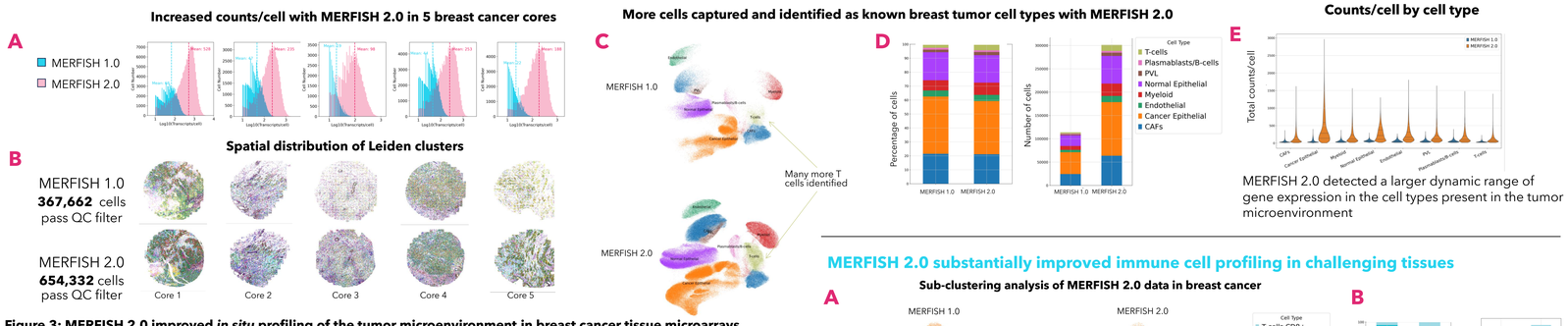


Figure 2: MERFISH 2.0 substantially improved RNA detection efficiency in a wide range of samples from human and mouse. To evaluate the performance of MERFISH 2.0 chemistry, samples from human (fresh frozen brain, FFPE breast cancer, FFPE lung cancer tissue microarray (TMA), FFPE breast cancer TMA) and mouse (fixed frozen spleen, heart, lung, small intestine, brain, kidney, liver and fresh frozen brain) were run with the MERFISH 1.0 and 2.0 workflow. Matched 815-plex MERFISH 1.0 or 2.0 gene panels were used as listed in **Table 1**. **A)** RNA transcript counts per 100µm^2 were used as a metric to evaluate sensitivity for MERFISH 1.0 (blue) or MERFISH 2.0 (orange). Each condition represents at least 3 samples (N=3). **B**) The Pearson correlation in counts between MERFISH 1.0 and MERFISH 2.0 was calculated for each set of samples. The Pearson correlation coefficient for all samples was above 0.8, indicating both methods generated high quality data. The data for a representative set of lung cancer samples is shown (left). Correlation between technical replicates of MERFISH 2.0 approached 1 (right), indicating high reproducibility. Gene counts from both chemistries were compared to single-cell RNA sequencing (scRNAseq) data as a biological standard across major cell types. **D**) MERFISH 2.0 data showed an increase in correlation with scRNAseq data relative to MERFISH 1.0, and high correlations within cell types (**C**).

MERFISH 2.0 improved *in situ* profiling of the tumor microenvironment in breast cancer tissue microarrays



MERFISH 2.0 detected a larger dynamic range of gene expression in the cell types present in the tumor microenvironment

Figure 3: MERFISH 2.0 improved *in situ* profiling of the tumor microenvironment in breast cancer tissue microarrays

FFPE human breast cancer TMA samples (5 cores) were run with 815-plex Breast Cancer Panels designed with MERFISH 1.0 and 2.0 chemistry. The resulting data was used for spatial and single-cell analyses. **A**) Histograms showing the transcripts per cell with MERFISH 1.0 and 2.0 chemistry in each core of the TMA. The mean counts per cell are shown for each chemistry. **B**) Spatial distribution of Leiden clusters in each core with MERFISH 1.0 and 2.0 chemistry showed much greater cell capture rate across the tissue with MERFISH 2.0. **C**) UMAP displaying the different cell types identified in the breast cancer samples. **D**) Cell types were plotted by percent and abundance for each chemistry. **E**) Violin plot showing the dynamic range of RNA transcripts counts in select cell types in the human breast cancer cores.

MERFISH 2.0 enabled better spatial characterization of cell-cell interactions and gene co-expression patterns

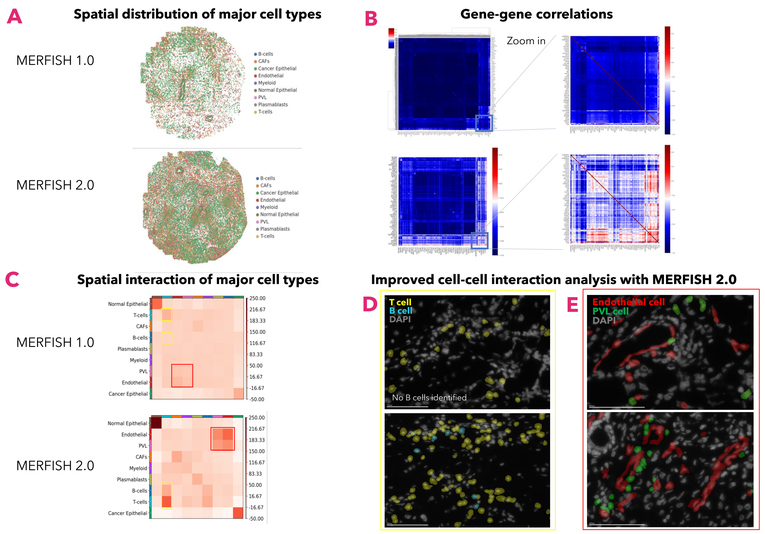


FIGURE 4. MERFISH 2.0 enabled better spatial characterization of cell-cell interactions and gene co-expression patterns. **A**) Spatial distribution of major cell types of the tumor microenvironment in a human breast cancer core**.** **B**) Gene-gene correlation analysis of the MERFISH data showed more co-regulated genes were identified with MERFISH 2.0 **C**) Spatial enrichment analysis of the major cell types showed greater spatial interactions of specific cell types with MERFISH 2.0. **D**) In particular, the T and B cell interactions were *only* observed with MERFISH 2.0, as B-cells did not appear in the MERFISH 1.0 region (yellow boxes). **E**) Similarly, the interaction between endothelial cells and PVL cells was only interpretable with MERFISH 2.0 (red boxes).

MERFISH 2.0 substantially improved immune cell profiling in challenging tissues

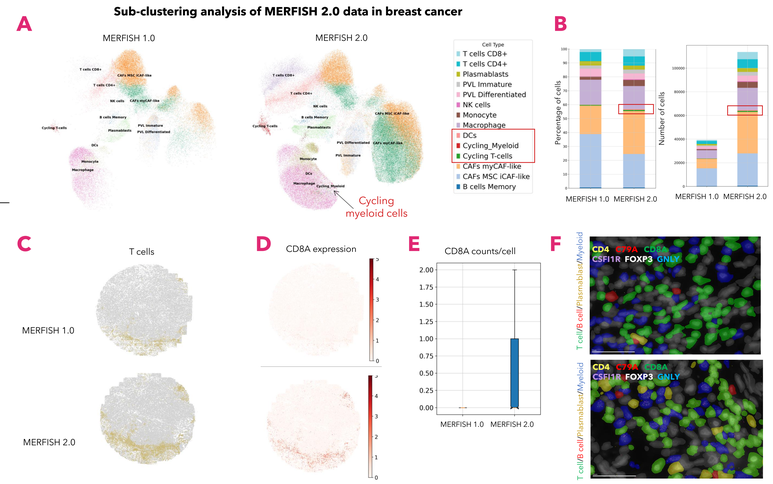


FIGURE 5. MERFISH 2.0 substantially improved immune cell profiling in challenging tissues. The increased sensitivity of MERFISH 2.0 enabled deeper investigation into the subtypes of immune cells present in the breast cancer sample. **A**) Sub-clustering analysis was performed to identify subtypes of cells in breast cancer, and annotated cell subtypes were shown in the UMAP. **B**) Identified cell types were plotted by percent and abundance for each chemistry. Cycling myeloid cells were only identified with the MERFISH 2.0 **C**) Spatial distribution of T cells and **D**) CD8A transcripts in a select core. **E**) Quantification of CD8A counts/cell for each chemistry. **F**) Spatial distribution of select immune cell types. MERFISH 2.0 was able to identify more immune cells and more transcripts within each cell for the representative field of view.

Conclusions

- MERFISH 2.0 substantially increased the sensitivity of the MERFISH technology, with up to **8X** improvement observed across multiple tissue types from mouse and multiple human cancers
- The improvement in sensitivity increased the number of cells passing QC filters, enabling the identification of more cell types and detection of more spatial interactions of specific cell types in human breast cancer samples.
- Profiling of the tumor microenvironment with MERFISH 2.0 uncovered cell types such as cycling myeloid cells that were previously undetectable by MERFISH 1.0.
- MERFISH 2.0 also enabled better characterization of gene co-expression patterns *in situ*.
- Spatially resolved transcriptomic profiling of archival tumor samples at the single-cell level offers significant opportunities for understanding how cancers develop *in situ*. These improvements will enable new genomic inquiries into how tumors interact with their environment, which in turn will open new areas of therapeutic research.