

In Situ Single-cell Transcriptomic Imaging in Formalin-fixed Paraffin-embedded Tissues with the MERSCOPE™ Platform

vizgen

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Introduction

Formalin-fixed paraffin-embedded (FFPE) tissues are widely used clinical sample types for histology and molecular diagnosis. However, due to RNA degradation and protein cross-linking, FFPE samples are often challenging for single-cell transcriptomic analysis. Vizgen's *in situ* spatial genomics solution, the MERSCOPE™ Platform, built on multiplexed error robust *in situ* hybridization (MERFISH) technology, directly profiles intact tissue's transcriptome with sub-cellular spatial resolution.

Here, we present a workflow to perform *in situ* single-cell transcriptomic imaging in FFPE tissue sections and demonstrate MERFISH imaging in more than 10 FFPE tissue types from mouse and human donors, including archival clinical samples. Hundreds of thousands of cells for each tissue type were captured, generating hundreds of millions of counts for profiled genes. Notably, we found that our FFPE workflow maintains the high accuracy and detection efficiency of our fresh frozen (FF) protocol in matched samples. Furthermore, we demonstrate that the MERSCOPE FFPE workflow is compatible with **protein imaging** through simultaneous cell boundary staining and Vizgen's newly released protein co-detection product, enabling true cell atlasing in complex tissue like cancer. **Combining these technologies to fit your experimental design, the MERSCOPE Platform makes spatial genomics inquiries accessible with diverse sample preservation methods.**

Materials & Methods

MERFISH is a massively multiplexed single molecule imaging technology to simultaneously measure the quantity and distribution of RNA species across a whole tissue slice (FIGURE 1).¹

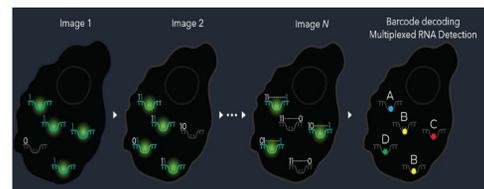


FIGURE 1. Illustration of MERFISH process.

To perform MERFISH, target RNA species are labeled with a tile of oligo probes containing different barcodes. Each barcode is then fluorescently detected in sequential round of imaging, and the optical barcode generated from these multiple rounds of imaging with '1' indicating fluorescence signal and '0' indicating no fluorescence, will help to resolve different RNA species.

We used various MERSCOPE Gene Panels in combination with the MERSCOPE Cell Boundary Staining Kit and MERSCOPE Protein Stain co-detection reagents to profile FFPE tissues. Samples were run on our commercially available MERSCOPE Platform (FIGURE 2). Results were visually inspected with the MERSCOPE™ Visualizer.



FIGURE 2. Vizgen's commercially available MERSCOPE Platform (left). MERSCOPE allows for 1cm² of imageable area and is compatible with 500 gene panels combined with 9 auxiliary stains. MERFISH data generated by MERSCOPE is available in mouse brain, mouse liver and 8 FFPE human tumors for visualization or to download at www.vizgen.com (right).

Results

MERSCOPE FFPE workflow enables highly accurate and sensitive *in situ*, single-cell transcriptomic imaging in FFPE samples

To demonstrate MERFISH in FFPE samples, we used a 483-plex mouse brain receptor gene panel to image FFPE mouse brain. MERFISH counts were compared with bulk RNA-sequencing (RNA-seq), and the spatial distribution of select genes were compared against Allen Institute's mouse brain map.² Furthermore, to evaluate the sensitivity and accuracy of the MERSCOPE FFPE workflow, we compared the MERFISH results obtained from fresh frozen samples against the data from FFPE samples obtained from matching brain regions (FIGURE 3).

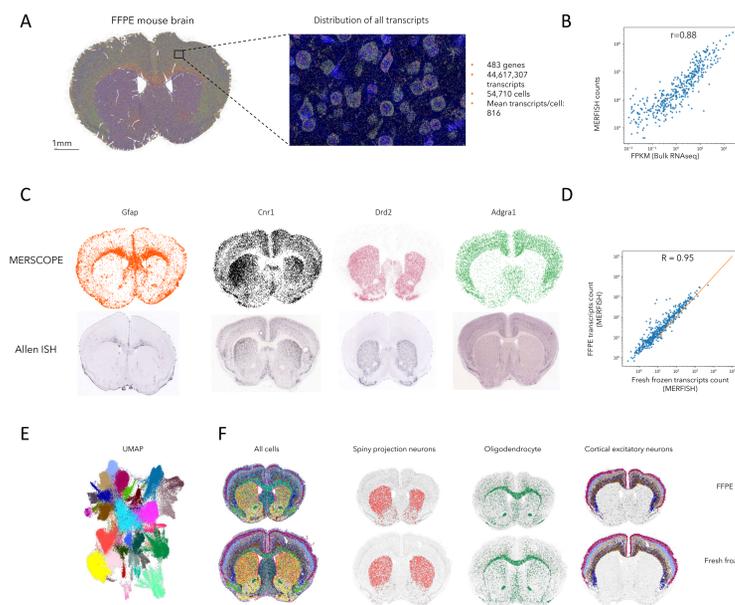


FIGURE 3. Demonstration of MERFISH workflow in an FFPE mouse brain A) sample was imaged by MERSCOPE using a 483-plex mouse brain receptor panel. The spatial distribution of all transcripts are shown across the coronal section of the brain (left) and a zoomed-in cortical region with DAPI overlay (right). Scale bars represent 1 mm. B) Correlation of MERSCOPE counts with bulk RNA-sequencing data, indicating the measurement is highly sensitive and accurate. C) Spatial distribution of 4 select genes from the MERSCOPE measurement (top), and *in situ* hybridization (ISH) data from Allen Institute's mouse brain map (bottom). D) Correlation between MERFISH mouse brain from FF sample and FFPE sample. E) UMAP visualization of cell types identified in mouse brain. F) Spatial distribution of cell types identified in FFPE (top) and fresh frozen (bottom) mouse brain.

MERSCOPE FFPE workflow is applicable to different FFPE samples

To demonstrate that Vizgen's FFPE workflow generates reliable data and is robust across tissue types, we performed MERFISH on 12 different human samples from both healthy and diseased patients using a 244-plex gene panel. Then we compared the MERFISH results to bulk RNA-seq data from the same tissue type (FIGURE 4).

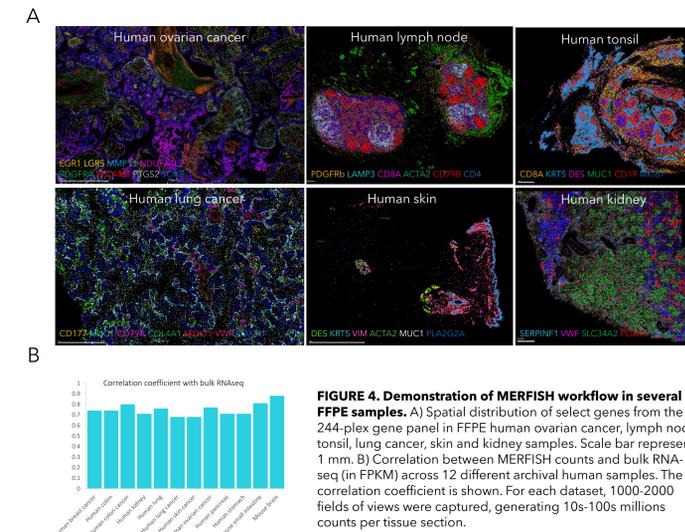


FIGURE 4. Demonstration of MERFISH workflow in several FFPE samples. A) Spatial distribution of select genes from the 244-plex gene panel in FFPE human ovarian cancer, lymph node, tonsil, lung cancer, skin and kidney samples. Scale bar represents 1 mm. B) Correlation between MERFISH counts and bulk RNA-seq (in FPKM) across 12 different archival human samples. The correlation coefficient is shown. For each dataset, 1000-2000 fields of views were captured, generating 10s-100s millions counts per tissue section.

MERSCOPE FFPE workflow is compatible with protein co-detection

We performed MERFISH with protein co-detection in human colon cancer to quantify 347 transcripts, 6 protein markers, and 3 cell boundary markers resulting in the detection of over 65 million transcripts (FIGURE 5).

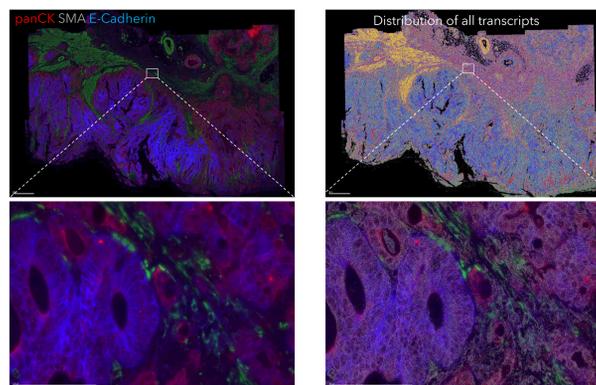


FIGURE 5. FFPE human colon cancer protein co-staining. FFPE human colon cancer section visualized with three proteins (left, panCK, SMA, and E-Cadherin) and all transcripts (right). The top row shows the whole sample, the bottom row shows an inset. Scale bars are 1 mm and 100 μ m on the top and bottom, respectively.

MERSCOPE cell segmentation workflow enables true cell atlasing in dense tissue

To enable single-cell analysis, we immunostained the plasma membrane and nucleus in dense tissue using Vizgen's MERSCOPE Cell Boundary Staining Kit. A deep learning-based cell segmentation algorithm was then applied to identify the cell boundary for each cell. After assigning each transcript to identified cells, the gene expression profile in each cell was used for cell clustering analysis. This workflow enables true cell atlasing in dense tissues such as human liver cancer (FIGURE 6).

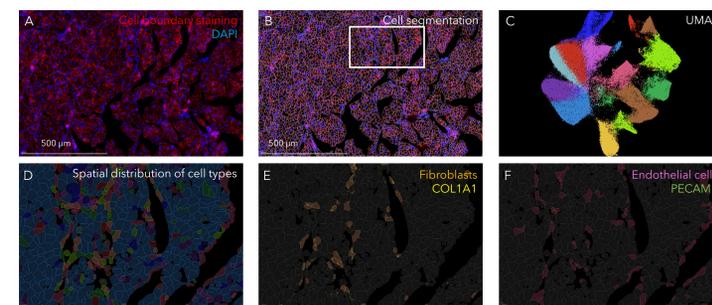


FIGURE 6. Single cell analysis in FFPE human liver cancer. A) FFPE human liver cancer was immunostained with Vizgen's MERSCOPE Cell Boundary Staining Kit, with plasma membrane staining shown in red and nucleus staining (DAPI) in blue. B) A deep learning-based cell segmentation algorithm was used to segment cells. The polygon masks for each identified cell are shown. C) UMAP visualization of different cell types identified in human liver cancer generated from MERFISH transcript data. D) Spatial distribution of 17 identified cell types across the tissue from boxed region in B). E) Spatial distribution of fibroblasts in boxed region from B. Fibroblast marker gene COL1A1 is shown in yellow. F) Spatial distribution of endothelial cells in boxed region from B. Endothelial marker gene PECAM1 is shown in green.

References

- Spatially resolved, highly multiplexed RNA profiling in single cells (2015). <https://science.sciencemag.org/content/348/6233/aaa6090.full>
- Allen Institute for Brain Science (2004). Allen Mouse Brain Atlas [ISH Dataset]. Available from mouse.brain-map.org. Allen Institute for Brain Science (2011).
- MERSCOPE FFPE Human Immuno-Oncology Data Release: <https://info.vizgen.com/merscope-ffpe-solution>

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Single-cell, spatially resolved transcriptomic profiling in FFPE human tumors with MERSCOPE

We applied MERFISH to perform cell atlasing and immunoprofiling in 8 different FFPE human tumors by using a panel of 500 genes. Hundreds of thousands of cells were imaged in each sample, generating hundreds of millions of counts per sample. The MERSCOPE FFPE Human Immunology Data Release enabled us to map out all the major cell types in each tumor, including subtypes of immune cells, and characterize their gene expression profile at single-cell resolution (FIGURE 7).³

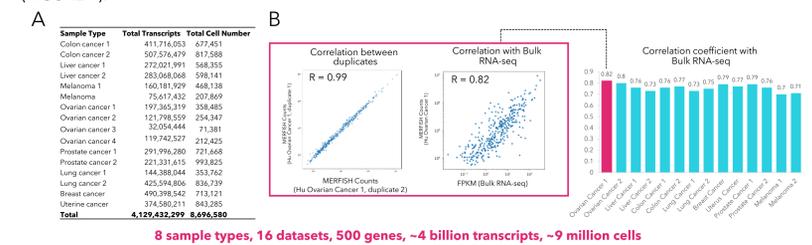


FIGURE 7. Summary of Vizgen's MERSCOPE FFPE Human Immuno-oncology Data Release. A) Table summarizing the FFPE Immuno-oncology Data Release, featuring 8 cancer types, ~4 billion transcripts and ~9 million cells. B) Correlation of MERSCOPE data between two human ovarian cancer slices from the same patient (Left), with bulk RNA-seq (middle), and the correlation coefficient with bulk RNA-seq for all measured samples (right), indicating the MERFISH measurement is highly accurate and sensitive.

MERSCOPE has superior data quality as compared to the CosMx™ Spatial Molecular Imager

We compared the human lung cancer dataset from the MERSCOPE FFPE Immuno-oncology Data Release and NanoString public dataset sample Lung_9_2 to evaluate the sensitivity and specificity of MERSCOPE (FIGURE 8). 5X more transcripts were detected for the overlapping genes with MERSCOPE.

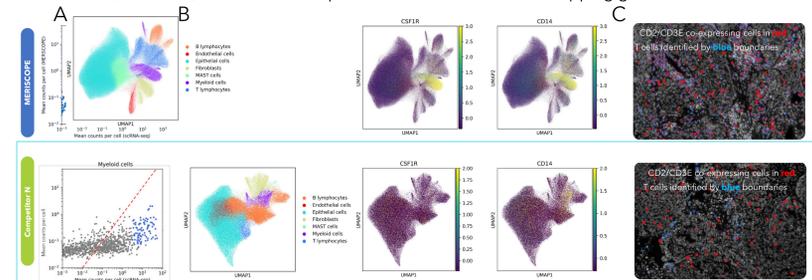


FIGURE 8. A) MERSCOPE has better specificity versus the CosMx™ Spatial Molecular Imager platform. The effective multiplexing for Myeloid cells was determined using a binary change point algorithm on mean counts for each gene detected by NanoString sorted by the corresponding abundance of the gene in scRNA-seq to identify the transition from low correlation with scRNA-seq to high correlation. B) MERSCOPE accurately recapitulates known biology in Myeloid cells. CSF1R and CD14 are key cell type markers in Myeloid cells. C) MERSCOPE confirms biological context for T cells markers.

Cell type identification and mapping in human breast cancer

We performed single-cell analysis on the human breast cancer data and annotated different cell types identified within the tumor. With high spatial resolution, MERSCOPE enables characterization of gene expression at single-cell level and helps to identify rare cell types (FIGURE 9).

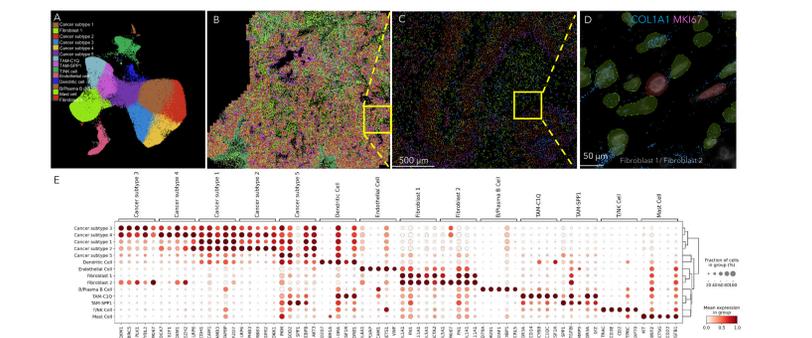


FIGURE 9. Breast cancer cell type mapping. A) UMAP visualization of different cell types identified in human breast cancer generated from MERFISH transcript data. B) Spatial distribution of 14 identified cell types across the tissue. C) Spatial distribution of identified cell types in boxed region in B. D) Spatial distribution of two types of fibroblasts (fibroblast 1 in green and fibroblast 2 in red) in boxed region in C. Both types of fibroblasts express COL1A1 gene, while fibroblast 2 expresses proliferation marker MKI67. E) Dot plot showing the marker genes for each cell type.

Characterization of immune cell types in breast cancer microenvironment

To further characterize the different immune cell types within the tumor microenvironment, we subclustered the T/NK cell population from the human breast cancer. We observed that Tregs are infiltrating into the tumor more than the regular CD4+ T cells (FIGURE 10).

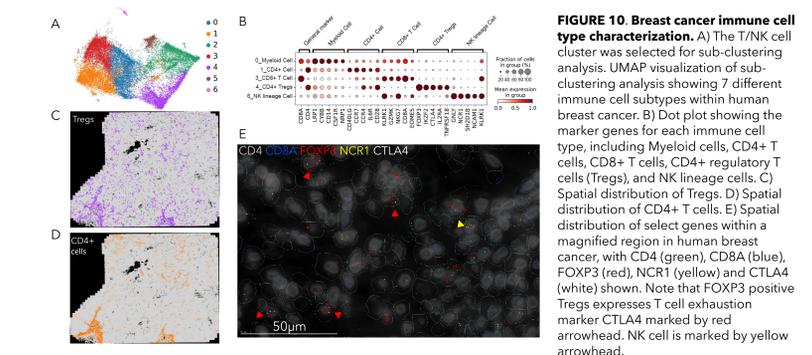


FIGURE 10. Breast cancer immune cell type characterization. A) The T/NK cell cluster was selected for sub-clustering analysis. UMAP visualization of sub-clustering analysis showing 7 different immune cell subtypes within human breast cancer. B) Dot plot showing the marker genes for each immune cell type, including Myeloid cells, CD4+ T cells, CD8+ T cells, CD4+ regulatory T cells (Tregs), and NK lineage cells. C) Spatial distribution of Tregs. D) Spatial distribution of CD4+ T cells. E) Spatial distribution of select genes within a magnified region in human breast cancer, with CD4 (green), CD8A (blue), FOXP3 (red), NCR1 (yellow) and CTLA4 (white) shown. Note that FOXP3 positive Tregs expresses T cell exhaustion marker CTLA4 marked by red arrowhead. NK cell is marked by yellow arrowhead.

Conclusion

- We demonstrated MERFISH in 10+ FFPE tissue types from mouse and human.
- MERSCOPE™ Platform FFPE workflow maintains high accuracy and sensitivity as compared to fresh frozen protocol, and has superior data quality as compared to the CosMx™ Spatial Molecular Imager platform.
- MERSCOPE FFPE workflow is compatible with antibody staining and offers true single-cell resolution for mapping and cataloging cell types in complex tissue.