Vizgen MERSCOPE™ Platform Grant Application Resource

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The MERSCOPE™ Platform Overview

The Vizgen MERSCOPE™ Platform (Figure 1) is the first and only commercial solution for MERFISH technology, enabling massively multiplexed, error-robust, single-cell *in situ* transcriptomic imaging and protein co-detection. The MERSCOPE Platform was commercialized in 2022 and has since been described in impactful papers demonstrating the breadth of its applications, including oncology[1], immunology[2], neuroscience[3], and developmental biology[4]. The MERSCOPE Platform solution includes reagents, the MERSCOPE™ Instrument, and software to streamline the full process from sample to high-quality MERFISH data.
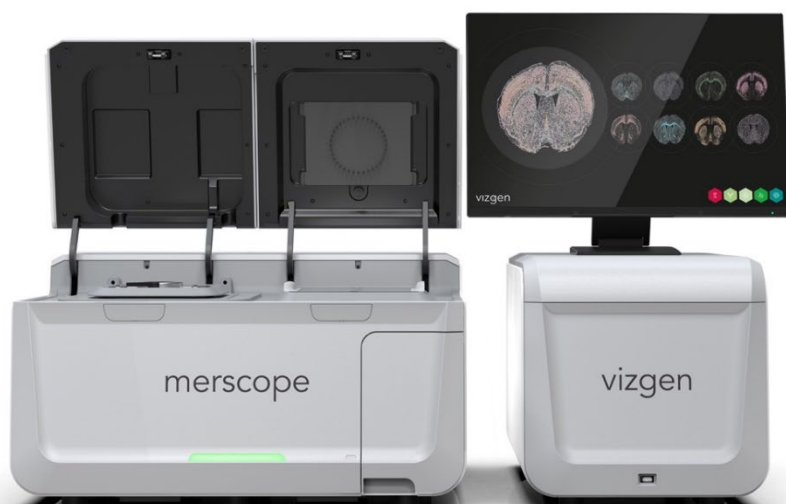

Figure 1. The Vizgen MERSCOPE Platform. The platform consists of the MERSCOPE™ Instrument (left) and the MERSCOPE™ Analysis Computer (right).

MERFISH (Multiplexed error-robust fluorescence *in situ* hybridization) is a spatially resolved single-cell transcriptome profiling technology developed in the lab of Dr. Xiaowei Zhuang at Harvard University[5]. MERFISH combines the power of single-cell transcriptomics with spatial biology by directly visualizing and counting RNA transcripts from 100's to > 10,000 genes in cells or tissue slices. This is achieved by massively multiplexing single molecule fluorescence *in situ* hybridization (smFISH) through error robust barcoding, combinatorial labeling, and sequential imaging. The recent release of the MERSCOPE™ Protein Stain Reagent Kit enables the simultaneous detection of RNA and proteins in a single run. By building upon the gold standard of RNA quantification (smFISH) and protein immunofluorescence staining, MERFISH leads the nascent spatial genomics field with unmatched sensitivity and detection efficiency. This enables multiomic mapping of the molecular, cellular, and functional composition of biological systems with preserved spatial context, providing insight into the biologically relevant organization of tissues in health and disease. The MERSCOPE Platform is compatible with an ever-expanding list of sample types, including adherent or suspended cells, fresh or fixed frozen tissues, and formalin-fixed paraffin-embedded (FFPE) samples enabled by our forthcoming MERSCOPE™ FFPE Sample Preparation Kit (to be released in Q4 2022).

The MERSCOPE Instrument integrates high-resolution imaging, fluidics, and image processing into automated hardware to deliver precise measurements. Additionally, Vizgen provides a suite of software tools to support the MERSCOPE Platform. MERSCOPE is validated with human and mouse tissues but is compatible with virtually any sample with a well-annotated transcriptome. Prior to initiating the MERFISH run on MERSCOPE, users build their custom gene panel through the Vizgen Gene Panel Design Portal. Custom gene panels are currently available in three sizes: up to 140 genes, up to 300 genes, and up to 500 genes. In addition to custom panels, Vizgen also provides a growing number of enabled options to be released as pre-designed gene panels. These panels are specially curated by our highly skilled, in-house scientists in collaboration with expert

researchers in their respective fields. These targeted, curated gene panels allow researchers to focus on data generation and analysis, not on labor-intensive design and target selection steps.

After users receive the Gene Panel, they should measure the RIN and DV200 values of the tissue samples and use the MERSCOPE Sample Verification Kit to assess the RNA quality of each sample; this will ensure that only high-quality samples are imaged. In samples where autofluorescence is prevalent, tissues are placed under the MERSCOPE™ Photobleacher to remove excess background signal. Tissue samples can be stained using the MERSCOPE™ Cell Boundary Staining Kit to mark cell boundaries and enable individual cell analysis. The proteome is also an important target for many researchers, and Vizgen offers the MERSCOPE Protein Stain Reagent Kits to allow researchers to detect up to 6 proteins simultaneously with hundreds of RNA species. The MERSCOPE Instrument control software enables automated image processing to extract the relevant information from the raw images. Finally, the MERSCOPE™ Vizualizer interactive software supports the visualization and analysis of the data generated from a MERFISH experiment. The MERSCOPE Platform allows users to get from sample to high-quality spatial genomics data without the need or cost of sequencing.

The Vizgen team offers input and technical support at each step of the process, from ordering gene panels through data analysis. Additionally, the Vizgen Lab Services program allows researchers to leverage our in-house sample processing and data analysis expertise to accelerate their research and yield fundamental insights faster.

Key Performance Features

The MERSCOPE platform provides many key advantages, enabling researchers to gain unprecedented insight into spatial transcriptomics (Figure 2).
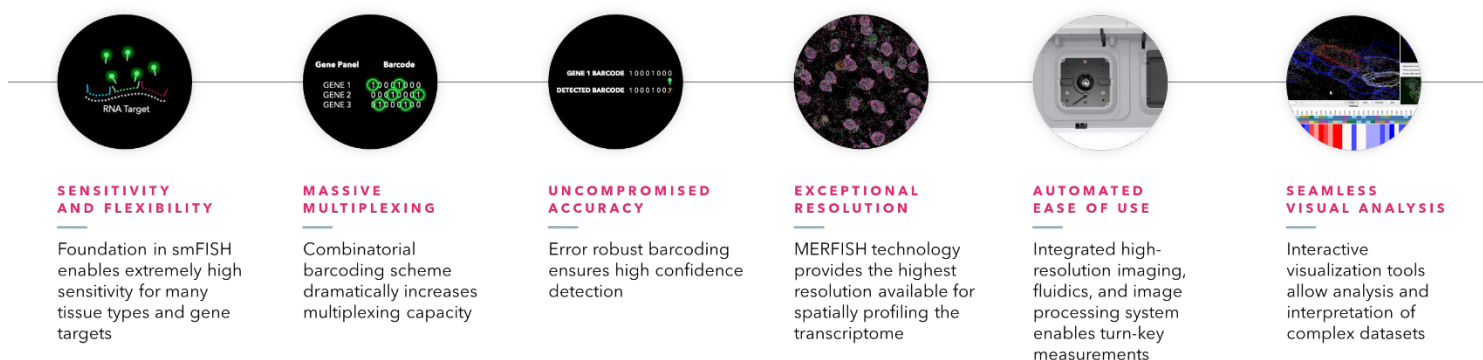

SENSITIVITY AND FLEXIBILITY

Foundation in smFISH enables extremely high sensitivity for many tissue types and gene targets

MASSIVE MULTIPLEXING

Combinatorial barcoding scheme dramatically increases multiplexing capacity

UNCOMPROMISED ACCURACY

Error robust barcoding ensures high confidence detection

EXCEPTIONAL RESOLUTION

MERFISH technology provides the highest resolution available for spatially profiling the transcriptome

AUTOMATED EASE OF USE

Integrated high-resolution imaging, fluidics, and image processing system enables turn-key measurements

SEAMLESS VISUAL ANALYSIS

Interactive visualization tools allow analysis and interpretation of complex datasets

Figure 2. A subset of key performance features of MERSCOPE. Vizgen's MERSCOPE Platform provides users with unparalleled insight into multiomic spatial structure on a biologically relevant scale.

- **Sample Input Flexibility**: MERSCOPE is commercially validated in human and mouse species and is applicable to any sample containing a well-annotated transcriptome. The workflow is compatible with cell culture, fresh and fixed frozen, and FFPE sample preparation.
- **High Throughput Processing**: Profile up to several hundred thousand cells in a single sample at low cost per cell and single molecule resolution across the whole tissue slice; approximately one day to image 1 cm^2 area for a panel of ~500 genes.
- **Customizable Gene Panel Design**: Intuitive software to build panels that enables the Vizgen team to provide automatic and immediate feedback to the end user about the suitability of the genes selected for a MERFISH measurement.
- **Massive Multiplexing Capability**: Current commercial kits for MERSCOPE support custom multiplexing of up to 500 genes. MERSCOPE provides the highest level of multiplexing with single-cell resolution as compared to other commercially available instruments.
- **Protein Co-detection**: Simultaneously detect up to 6 proteins with primary antibodies hosted from

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mouse, rabbit, goat, rat, human and chicken.

- **Full Tissue Analysis**: MERSCOPE provides information across the whole tissue section rather than targeted regions or a percentage of the tissue as with other commercial platforms.
- **Subcellular Resolution**: Scaling from whole tissue section to single-cell and subcellular imaging down to 100 nm. No need to select areas of interest.
- **Unparalleled Sensitivity**: Provides the highest detection efficiency for identifying RNA for many tissue types and gene targets. Detect nearly all the transcripts expressed from the targeted genes.
- **Detection Accuracy**: Error-correcting barcoding schemes and combinatorial labeling and imaging to ensure the highest detection accuracy.
- **Detection Efficiency**: Greater than 70-fold advantage in detection efficiency over the latest competitive data available[6].
- **No Additional Sequencing**: MERSCOPE directly quantifies RNA and does not require downstream sequencing. The platform outputs the complete data that is most relevant for reaching biological conclusions without the need to wait for sequencing samples to be processed and does not require any additional instrumentation upstream or downstream.
- **Visualization & Analysis Software**: MERSCOPE Vizualizer allows you to combine spatial context at the single-cell level seamlessly. No need to overlay H&E and/or IHC images to get spatially resolved single cell data.

The MERSCOPE Platform Workflow

The MERSCOPE workflow (Figure 3) begins with selecting a gene panel. For custom panels, the intuitive MERSCOPE gene panel design software enables automatic and immediate feedback to the end user about the suitability of the genes selected for a MERFISH measurement. The software ensures users target genes with individual abundance thresholds below 800 FPKM (Fragments Per Kilobase of transcript per Million mapped reads) and a total abundance of ~9000 FPKM. For highly abundant genes not suitable for standard MERFISH measurements, sequential FISH imaging of select gene targets can be conducted using the auxiliary channels. Once the gene panel is designed, the samples are hybridized with the designed probes and loaded to a flow chamber for imaging. A reagent pack containing readout probes is used to readout the barcodes on the MERSCOPE platform. By decoding the signal from raw images, the location and quantity of different genes will be resolved and then the data can be explored through Vizgen's visualization software. Before initiating a full MERSCOPE run, the MERSCOPE™ Sample Verification Kit can be used to verify that the RNA quality after sample preparation will result in adequate MERFISH imaging quality.
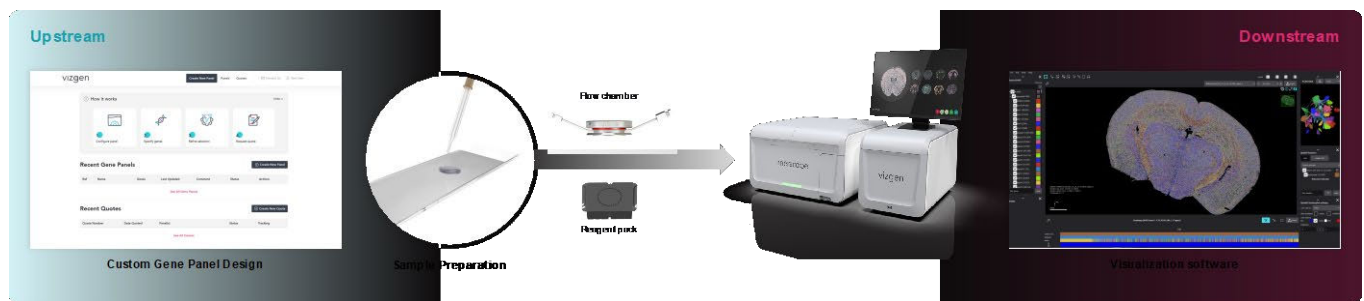

Figure 3. The MERSCOPE Platform Workflow. First, gene panel parameters are selected using the MERSCOPE gene panel design software. Next, the user prepares the sample and loads the MERSCOPE slide into the flow chamber, after which the fully automated instrument run proceeds. The MERSCOPE Instrument Software automatically processes the raw images to output spatial genomics measurements in a format ready for immediate downstream analysis.

I.   Sample Preparation

The MERSCOPE workflow is compatible with cell cultures and fresh, fixed frozen or FFPE tissue

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sections. To prepare a sample with the MERSCOPE workflow, the tissue of interest is sectioned and adhered to a MERSCOPE Slide (a 40mm round coverslip). The tissue section is then fixed with a fixation buffer and made permeable to the hybridization probes by overnight incubation. These encoding probes contain different barcodes that hybridize with the targets of interest. Then the sample is embedded in a gel matrix and cleared to remove any unnecessary component of tissue that may interfere with the measurement. Sample tissues with autofluorescence can be placed under the MERSCOPE Photobleacher to remove the excess background signal. Cell boundary stain be used to mark cell boundaries and enable individual cell analysis, and/or stained with protein stain for the co-detection of proteins and RNA in a single MERFISH experiment.

II.   Imaging on the MERSCOPE Instrument

Once the sample preparation is complete, the MERSCOPE slide is secured into the flow chamber, and then loaded into the instrument along with the imaging cartridge (Figure 5). Users can select up to 1cm^2 area of the MERSCOPE slide to image down to 100 nm resolution, providing imaging of whole tissue sections at single-cell, and even subcellular, resolution. Such unparalleled sensitivity and resolution allow the capture of hundreds of thousands of cells, permitting the identification of rare cell types and functional states on a biologically meaningful scale. During the fully automated instrument run, readout probes are introduced throughout multiple rounds of imaging and an optical barcode is generated. Imaging time varies from about 10 hours for small areas to 30 hours for 500 gene imaging on 1cm^2 of tissue. The MERSCOPE Platform automatically processes the raw images to output the spatial genomics measurements in a format that is ready for immediate downstream analysis.

III.   Visualization and Analysis

The MERSCOPE Instrument Software (in combination with the MERSCOPE Analysis Computer) automatically processes the raw images to output spatial genomics measurements in a format ready for immediate downstream analysis. The output includes the list of all detected transcripts and their spatial locations in three dimensions (CSV files), the mosaic images (TIFF), output from the cell segmentation analysis: the transcripts per cell matrix (CSV), the cell metadata (CSV), the cell boundaries (HDF5), various logs and tasks, a summary .png file, and a binary for use with the MERSCOPE Vizualizer software to visualize and analyze the data. The MERSCOPE Platform includes the MERSCOPE Vizualizer Data Visualization and Analysis software. Once analysis is complete, the output files can be transferred from the Analysis PC for downstream visualization and analysis using the Vizualizer software. The data outputs are also compatible with tools developed by the academic community.

Biological Applications

Vizgen is developing the next generation of genomics tools to expand on the capabilities of spatially resolved transcriptomics in a variety of sample types (Figure 4). This technology will aid the acceleration of biological research and discovery to advance the understanding of human health and disease. MERFISH is a game-changing technology in single-cell genomics with broad applications in both fundamental biology and medicine, ranging from basic science to drug discovery, to clinical pathology.
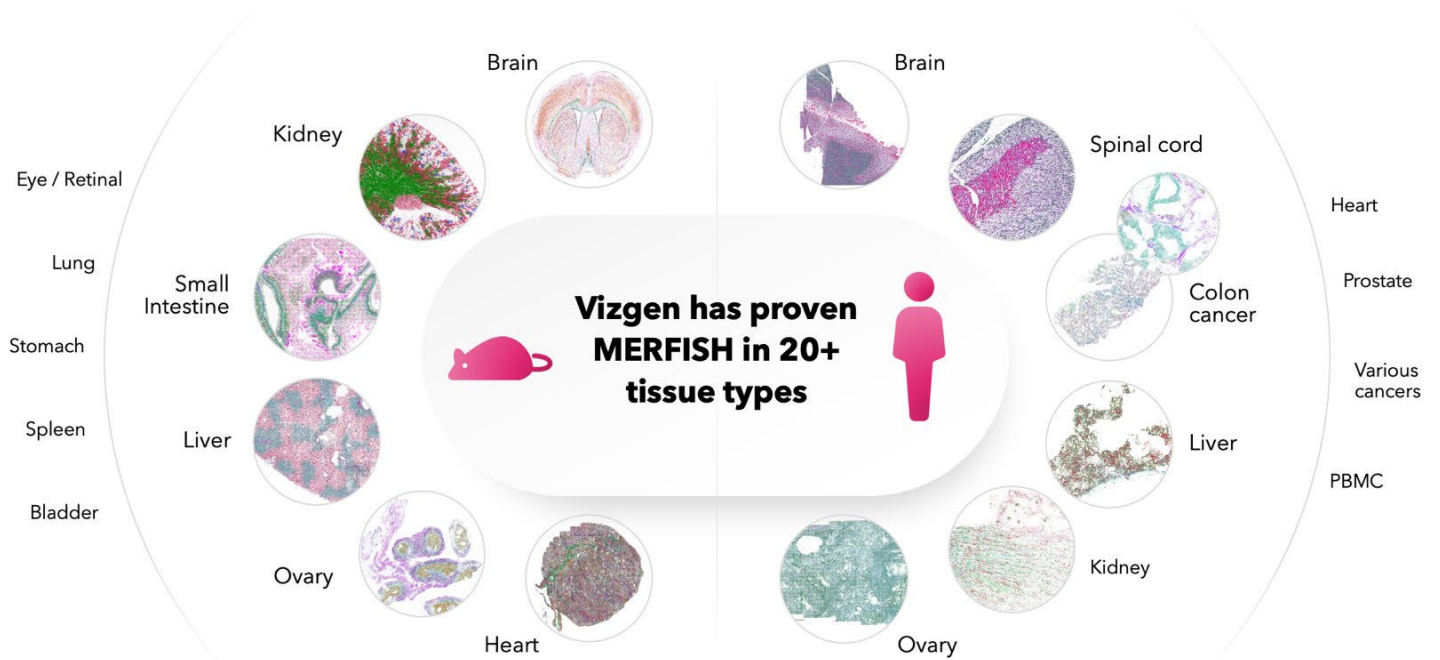
Figure 4. Sample types compatible with MERFISH. MERFISH technology has been demonstrated on many different sample types as depicted above.

Supporting Data

MERSCOPE workflows on all supported sample types are highly accurate and reproducible when compared to bulk RNA sequencing data, as illustrated in Figure 5.
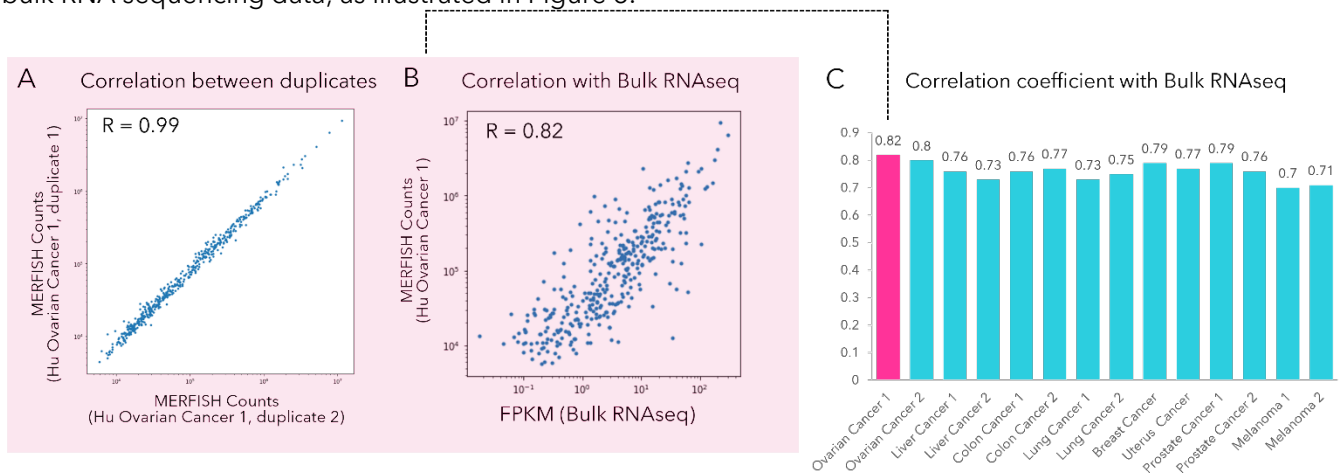

Figure 5. MERSCOPE FFPE workflow is highly sensitive, accurate and reproducible. A) Correlation of MERSCOPE data between two human ovarian cancer slices from the same patient. Correlation coefficient is 0.99, indicating the measurement is highly reproducible. B) Human ovarian cancer sample 1 was analyzed by MERSCOPE using a 500 gene panel, and adjacent sections were analyzed by bulk RNA sequencing. Correlation analysis between MERFISH counts and FPKM values from bulk RNA sequencing is shown. The correlation coefficient is 0.82, indicating the measurement is highly accurate. C) Correlation analysis between MERSCOPE data and bulk RNA sequence was performed across 14 cancer samples, and correlation coefficients show high accuracy across multiple cancer types and replicates.

The high-resolution data generated on MERSCOPE instruments enables imaging of transcripts down to a subcellular level, enabling cell type identification and atlassing. Figure 6 shows the spatial mapping of different cell types identified in human breast cancer using MERFISH data.
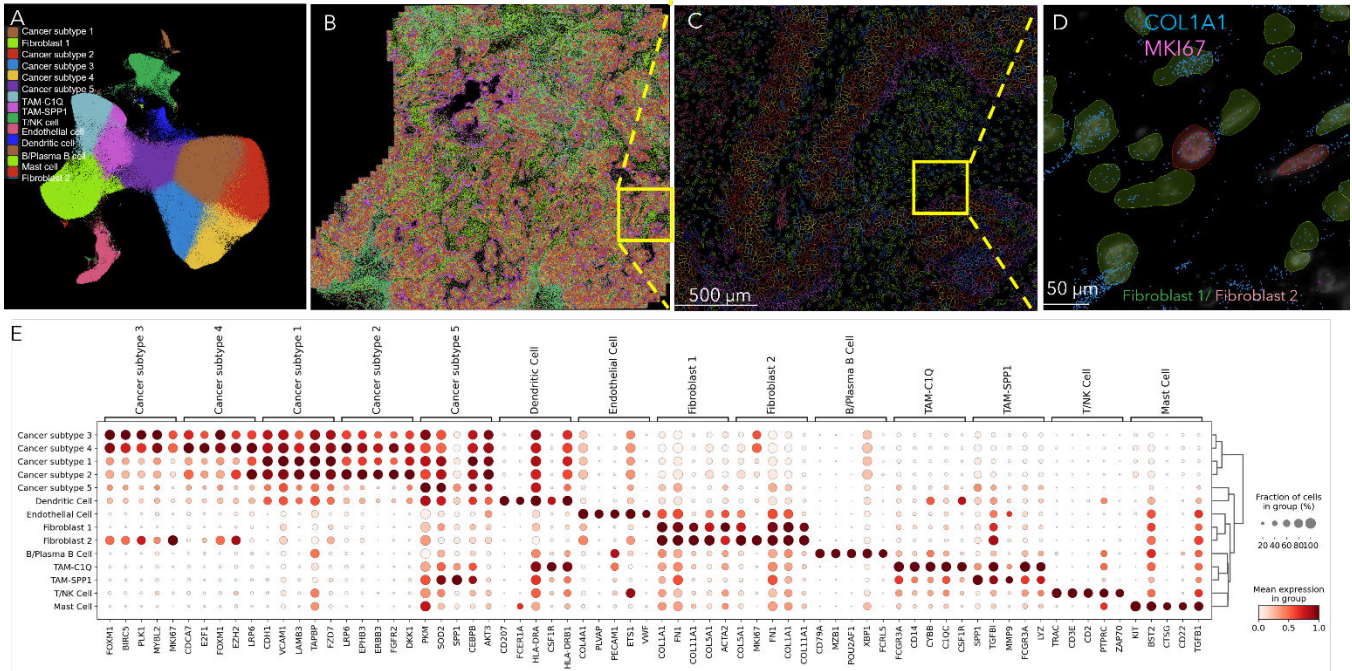

Figure 6. MERSCOPE cell type identification and mapping in human breast cancer. A) UMAP visualization of different cell types identified in human breast cancer generated from MERFISH transcript data. B) Spatial distribution of 14 identified cell types across the tissue. C) Spatial distribution of identified 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.

The MERSCOPE™ Cell Boundary Staining Kit enables users to mark cell boundaries to facilitate cell segmentation for single-cell analysis. The MERSCOPE Cell Boundary Staining Kit contains 3 cell boundary stains, each with different qualities for cell segmentation in different tissues. Figure 7 shows an example of how the cell segmentation workflow enables distinct identification of cell populations across dense tissues in FFPE human liver cancer.
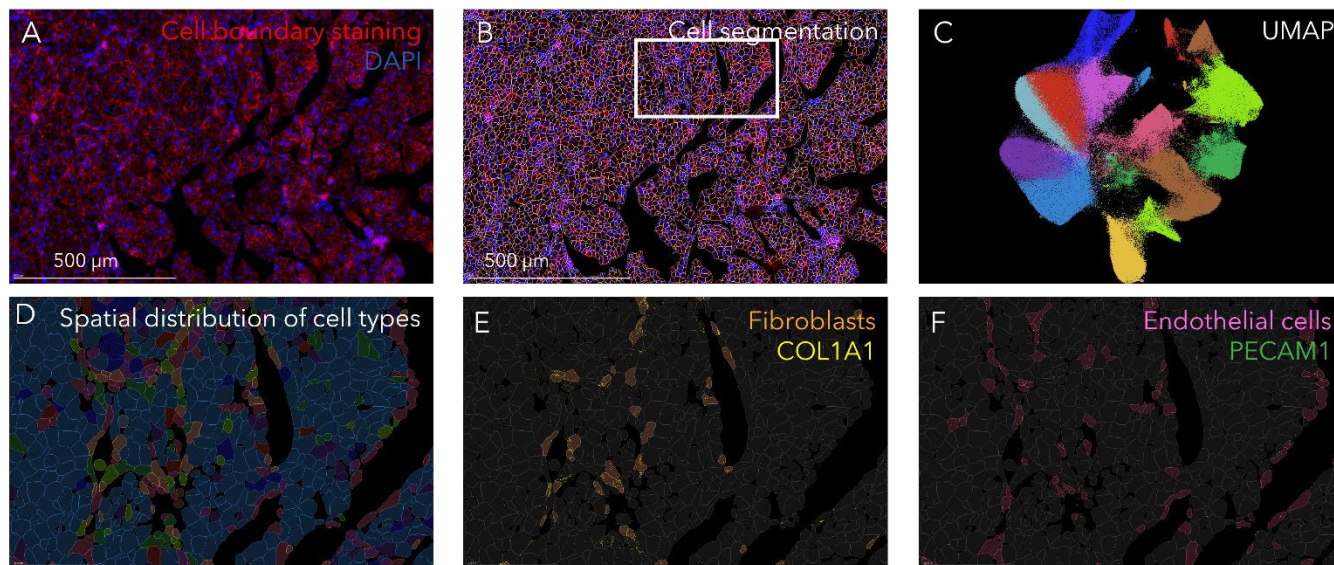
Figure 7. MERSCOPE cell segmentation workflow enables true cell atlassing in dense tissue. A) FFPE human liver cancer was immunostained with Vizgen's cell boundary staining kit; plasma membrane staining shown in red and nucleus staining in blue. B) Deep learning-based cell segmentation algorithm was used to segment cells. The polygon masks for each identified cell are shown. C) UMAP visualization of 17 different cell types identified in human liver cancer generate from MERFISH transcript data. D) Spatial distribution of identified cell types across the tissue in boxed region from B. E) Spatial distribution of fibroblasts in boxed region from B. Fibroblast marker gene COL1A1 shown in yellow. F) Spatial distribution of endothelial cells in boxed region from B. Endothelial marker gene PECAM1 shown in green.

Available Data Releases

- MERSCOPE FFPE Human Immuno-oncology: contains 16 total datasets from 8 FFPE tumor tissue types including breast, colon, lung, liver, skin, prostate, uterine and ovarian, each measuring 500 genes totaling over 4 billion transcripts and 9 million cells cumulatively.
- Vizgen MERFISH Mouse Liver Map: MERFISH measurement of a gene panel containing 347 total genes. The dataset includes the list of detected transcripts, gene counts per cell matrix, additional spatial cell metadata, cell boundary polygons, and DAPI images.
- Vizgen MERFISH Mouse Receptor Map: MERFISH measurement of a gene panel containing 483 total genes including canonical brain cell type markers, GPCRs, and RTKs measured on 3 full coronal slices across 3 biological replicates. The dataset includes the list of detected transcripts, gene counts per cell matrix, additional spatial cell metadata, cell boundary polygons, and DAPI images.

	Immuno-oncology	Liver	Brain Receptor
Species	Human	Mouse	Mouse
Tissue(s)	8 tumor types - colon - liver - melanoma - ovarian - prostate - lung cancer - breast cancer - uterine cancer	Liver	Brain
Gene Panel Size	500	347	483
Number of Samples	16	4	9
Total Transcripts Detected	4,129,432,299	1,205,513,113	~55,000,000
Total Cells	8,696,580	1,275,831	~700,000

The most up-to-date list of available data releases can be found at www.vizgen.com/data-release-program/.

Comparison to Related Instruments/ Technologies on the Market

- MERSCOPE offers significant improvements in resolution from whole tissue section to single-cell and subcellular imaging, down to 100 nm.
- MERSCOPE offers greater than 70-fold advantage in detection efficiency over array-based technologies[6]. The higher detection efficiency of MERSCOPE allows accurately quantifying lowly expressed genes.
- The MERSCOPE Platform enables the highest level of multiplexing with single-cell resolution as compared to other commercially available instruments.
- The MERSCOPE Platform offers entirely customizable gene panel designs through a gene panel design software. This allows the Vizgen team to provide automatic and immediate feedback to the end user about the suitability of the genes selected for a MERFISH measurement.
- MERSCOPE provides information across the whole tissue section rather than targeted regions or percentages of the whole tissue.
- MERSCOPE is validated with human and mouse tissues but compatible with virtually any sample with a well-annotated transcriptome.
- MERSCOPE directly quantifies RNA and does not require downstream sequencing.
- MERSCOPE is high throughput and can profile up to 1 cm^2 of tissue per day with single-molecule resolution across the whole tissue slice.
- The MERSCOPE Platform includes a proprietary Visualization and Analysis software allowing you to combine spatial context at the single-cell level seamlessly. There is no need to overlay H&E and/or IHC images to get spatially resolved single cell data.

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Ordering information

PRDOUCT NAME	DESCRIPTION	PART NUMBER
MERSCOPE Platform	The MERSCOPE Platform is an end-to-end solution for generating MERFISH measurements. Includes analysis computer, hardware and accessories.	10000001
MERSCOPE Photobleacher	Autofluorescent instrument accessory for clearing autofluorescence background from the tissue sample.	10100003
Training and Installation	Training and Installation MERSCOPE Platform.	40100001
MERSCOPE Slide Box	Sample slide for MERSCOPE Instrument. Functionalized for tissue samples to enable Vizgen sample preparation protocols. Pack of 20.	10500001
MERSCOPE Non-Beaded Slide Box	Sample slide for MERSCOPE Instrument. Specifically for cell culture samples only. Pack of 20.	10500002
MERSCOPE 140 Gene Panel	A custom gene panel comprised of encoding probes targeting 140 or fewer genes. 20 samples.	10400001
MERSCOPE 300 Gene Panel	A custom gene panel comprised of encoding probes targeting 140 to 300 genes. 20 samples.	10400002
MERSCOPE 500 Gene Panel	A custom gene panel comprised of encoding probes targeting 300 to or 500 genes. 20 Samples.	10400003
MERSCOPE 140 Gene Imaging Kit	Imaging reagent kit containing the reagents and cartridge for running a measurement on the MERSCOPE Platform for up to 140 genes.	10400004
MERSCOPE 300 Gene Imaging Kit	Imaging reagent kit containing the reagents and cartridge for running a measurement on the MERSCOPE Platform for up to 300 genes.	10400005
MERSCOPE 500 Gene Imaging Kit	Imaging reagent kit containing the reagents and cartridge for running a measurement on the MERSCOPE Platform for up to 500 genes.	10400006
MERSCOPE Cell Boundary Staining Kit	Kit containing a cell boundary staining reagent for staining the cell boundary in samples to enable cell segmentation in dense tissues. Human, Mouse and Rat. 20 Samples	10400009
MERSCOPE Sample Prep Kit	Kit containing sample preparation reagents for 20 samples. Compatible with fixed, fresh frozen tissue and cultured cells* For cultured cell samples, use MERSCOPE Slide (Non-beaded). 20 Samples.	10400012

MERSCOPE Sample Verification Kit (Human)	Kit containing smFISH probes and imaging reagents to do a single imaging run. 5 samples.	10400007
MERSCOPE Sample Verification Kit (Mouse)	Kit containing smFISH probes and imaging reagents to do a single imaging run. 5 samples.	10400008

References

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2. Hara T., Chanoch-Myers R., Mathewson D,. et al. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell. 2021 Jun 14;39(6):779-792.e11. doi: 10.1016/j.ccell.2021.05.002
3. Stogsdill JA, Kim K, Binan L, Farhi SL, Levin JZ, Arlotta P. Pyramidal neuron subtype diversity governs microglia states in the neocortex. Nature. 2022 Aug;608(7924):750-756. doi: 10.1038/s41586-022-05056-7. Epub 2022 Aug 10. PMID: 35948630.
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5. Wang, G., Moffitt, J.R. & Zhuang, X. Multiplexed imaging of high-density libraries of RNAs with MERFISH and expansion microscopy. Sci Rep 8, 4847 (2018). https://doi.org/10.1038/s41598-018-22297-7
6. Emanuel, G., & He, J. (2021). Using MERSCOPE to Generate a Cell Atlas of the Mouse Brain that Includes Lowly Expressed Genes. Microscopy Today, 29(6), 16-19. doi:10.1017/S1551929521001346

Key Publications

The efficacy of the MERSCOPE Platform is demonstrated in numerous peer-reviewed publications, many in top journals. For the most current list of MERSCOPE publications, visit https://vizgen.com/technology/#publications.

Resources

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