MERSCOPE Reveals the Transcriptional Organization of the Mouse Brain

George Emanuel, Jiang He, Justin He—Vizgen, Inc

Abstract

We mapped 483 genes with MERSCOPE™ across full mouse brain coronal slices. Enabled by MERFISH technology, MERSCOPE measures the position of individual molecules with single molecule resolution and high detection efficiency. For this demonstration, we constructed a panel of canonical cell type markers and nonsensory G-Protein coupled receptors (GPCRs) to spatially profile nonsensory GPCR expression across the brain with cellular context. Nonsensory GPCRs in the brain mediate signaling and may play vital roles behind brain ageing and neurodegenerative disorders. Our map demonstrates that MERSCOPE is a leading tool for molecular atlassing, enabling greater insight into healthy versus diseased tissue.

Introduction

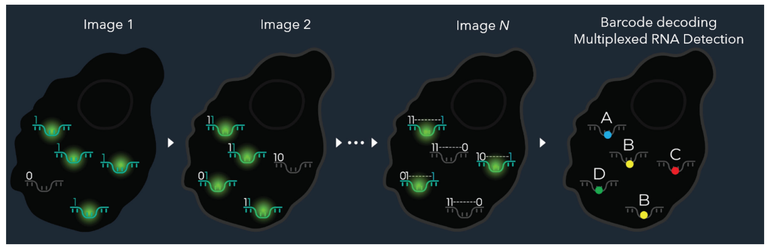

FIGURE 1. MERFISH encoding and readout scheme. For a MERFISH measurement, each gene is assigned a unique binary barcode and the barcodes are optically detected using sequential rounds of single molecule FISH. For each imaging round, the genes assigned barcodes containing a 1 bit for the corresponding bit position appear as single molecule FISH spots while genes assigned barcodes containing a 0 bit remain dark. The pattern of single molecule FISH spots across the imaging rounds allows 100's to 1000's of transcripts to be spatially resolved.

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, Harvard University.[1] 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 (Figure 1). MERFISH technology provides high detection efficiency with nanometer-scale resolution enabling the 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. Data output for each measurement includes the list of all detected transcripts and their spatial locations in three dimensions, the gene counts per cell matrix, additional spatial cell metadata, cell boundary polygons, and high resolution DAPI and Poly T mosaic images. The Vizgen MERSCOPE platform includes Data Visualization and Analysis software and the outputs are compatible with tools developed by the academic community.

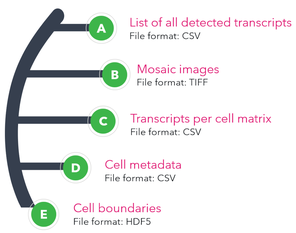

FIGURE 2. MERFISH Measurement Data Output

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7. Vizgen MERFISH Mouse Brain Receptor Map: https://info.vizgen.com/mouse-brain-data

vizgen

WEB: vizgen.com
INFO: info@vizgen.com | MEDIA CONTACT: brittany.auclair@vizgen.com
37 Spinelli Place | West Cambridge Science Park | Cambridge, MA 02138

MERSCOPE Study Design

FIGURE 3. Vizgen MERSCOPE Platform. The MERSCOPE platform contains a high resolution fluorescence imaging system to perform the single molecule imaging required for MERFISH and a fluidic system for extinguishing the signal and restaining between each imaging round to automatically perform a full MERFISH measurement.

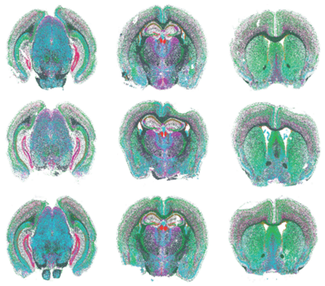

FIGURE 4. MERFISH measurements across 9 full mouse brain coronal slices. Each panel depicts the expression pattern for 8 of the genes from the 483 gene measurement for each of the 3 biological replicates at 3 positions across the mouse brain.

To develop our MERFISH Mouse Brain Receptor Map we constructed a panel that consisted of 483 total genes including canonical brain cell type markers, nonsensory GPCRs, and receptor tyrosine kinases to spatially profile nonsensory GPCR expression across the brain with cellular context. MERFISH measurements were conducted for three full coronal slices across three positions in the brain with three biological replicates for each position to determine the exact location of the targeted transcripts.

An estimated 90% of the ~370 nonsensory GPCRs in the brain mediate signaling and may play vital roles behind brain aging and neurodegenerative disorders[2] but are difficult to analyze because of their structural properties, low abundance, and lack of highly specific antibodies.[3] In this study we aimed to offer spatial information about these functionally relavent but low expressing genes, while also generating an atlas of the mouse brain

Results

1. MERSCOPE quantitatively mapped the mouse brain with single molecule resolution

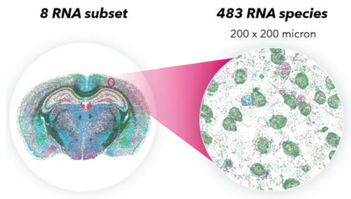

FIGURE 5. 8 RNA subset from MERFISH measurement of 483 transcripts.

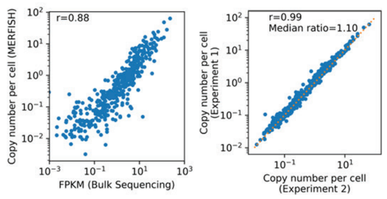

FIGURE 6. Correlation plot comparing bulk RNA seq data to MERFISH measurement.

We ran the MERFISH measurements using the 483 gene panel across the three coronal mouse brain slices each with three replicates on the MERSCOPE platform to generate a Mouse Brain Atlas. From these measurements, we achieved single molecule resolution spatial transcriptome profiling across the full coronal slices. At any position in the coronal slice we are able to zoom in to explore the cellular and molecular composition of the sample. We compare the number of transcripts detected to bulk sequencing and found strong correlation (r=0.88) and strong correlation between biological replicates. From MERSCOPE's cell segmentation, we were able to cluster cells by gene expression to identify different cell types and map the spatial organization of these cells.

Results (continued)

2. MERSCOPE enabled single cell spatial analysis

FIGURE 7. MERFISH measurement output highlighting (A) all detected transcripts, (B) cell by gene data, and (C) the spatial organization of the detected cells.

3. Single-cell resolution enabled mapping the spatial, cell-type-dependent variation in gene expression

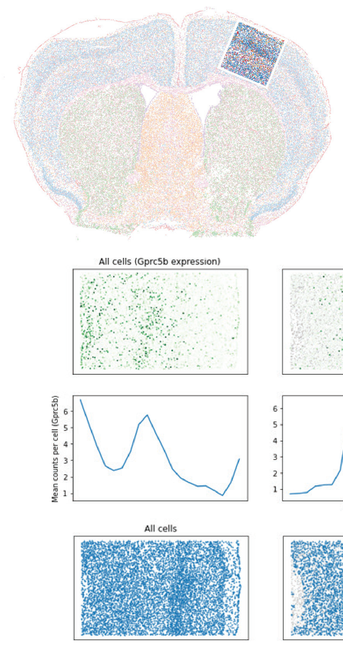

FIGURE 8. Cell-type dependent spatial distribution of Gprc5b expression. From cell clustering results, cells were categorized as either neuronal or non-neuronal. The Gprc5b expression across all cells indicates two peaks of high expression, one deep in the cortex and one in the mid cortex. By deconvolving the signals from neuronal and non-neuronal cells, the peak in the mid cortex is dominated by expression within neuronal cells while the peak of high Gprc5 expression deep in the cortex is dominated by expression within non-neuronal cells.

Conclusion

Vizgen's MERSCOPE platform harnesses MERFISH technology to provide the resolution and the detection efficiency needed to accuracy spatially profile a large panel of genes across whole tissue slices down to the subcellular level. The Vizgen MERSCOPE is the first and only commercial platform solution for MERFISH technology and includes reagents, the MERSCOPE instrument, and software to streamline the full process from sample to high quality MERFISH data. To demonstrate the power of MERSCOPE we developed the MERFISH Mouse Brain Receptor Map. Our map contains the exact position of transcripts from a custom 483 gene panel across three full mouse coronal slices across three replicates on the MERSCOPE instrument. We showcased the ability of MERSCOPE to detect GPCRs, which mediate signaling and may play vital roles behind brain ageing and neurodegenerative disorders, but are lowly expressed and difficult to capture with other technologies. The GPCRs measured by MERSCOPE during our experiment include Oxtr (oxytocin receptor)[4], Tshr (Thyroid stimulating hormone receptor)[5], and Insr (insulin receptor)[6]. The ability to detect lowly expressed genes such as GCPRs and completely spatially profile expression across the brain with cellular context can assist scientists with gaining a deeper understanding of brain tissue structure and function. Our data set is publicly available[7] for researchers to access and explore.