Molecular Atlassing with MERSCOPE™

A New Spatial Transcriptomics Technique Accurately Reveals the Organization of the Mouse Brain Transcriptome

ABSTRACT

Building molecular atlases to fully understand the structure and function of each cell within the brain is now a key goal of neuroscience research. Atlas initiatives using single-cell RNA sequencing can characterize cell types based on their RNA expression profiles. However, tissue organization is lost when cells are dissociated for single-cell sequencing, making it difficult to study how cellular heterogeneity contributes to tissue function. Furthermore, accurately characterizing each cell within the brain is challenging due to the low expression of many functionally important genes such as nonsensory G-Protein coupled receptors (GPCRs) which are not well captured by other technologies. A true spatial transcriptomics technology with high detection efficiency and single-molecule resolution is required to build accurate and complete molecular atlases. Vizgen's MERSCOPE™ Platform for *in situ* spatial genomics enables the direct profiling of the spatial organization of intact tissue with subcellular resolution. MERSCOPE is built on multiplexed error robust *in situ* hybridization (MERFISH) technology that uses combinatorial labeling, sequential imaging, and error-robust barcoding to provide the highest detection efficiency and resolution available for spatial genomics.[1] In a single experiment, MERSCOPE can spatially profile hundreds of thousands of cells with high accuracy and reproducibility. To demonstrate the power of MERSCOPE, we constructed a custom MERFISH gene panel of 483 genes to spatially profile both canonical cell type markers and nonsensory GPCR expression across the brain t. Nonsensory GPCRs in the brain mediate signaling and may play vital roles behind brain aging and neurodegenerative disorders.[2] However, these genes are difficult to analyze.[3] Our experiment successfully detected multiple lowly expressed GPCRs including the oxytocin receptor (Oxtr)[3], thyroid stimulating hormone receptor (Tshr)[4], and insulin receptor (Insr)[5]. The mouse brain receptor map demonstrates MERSCOPE as a leading tool for molecular atlassing, enabling scientists to find greater insights into healthy versus diseased tissue.

INTRODUCTION

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 sections. This is achieved by massively multiplexed 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 (FIGURE 2). The Vizgen MERSCOPE Platform includes the MERSCOPE™ Vizualizer data visualization and analysis software, and the data outputs are compatible with tools developed by the academic community.

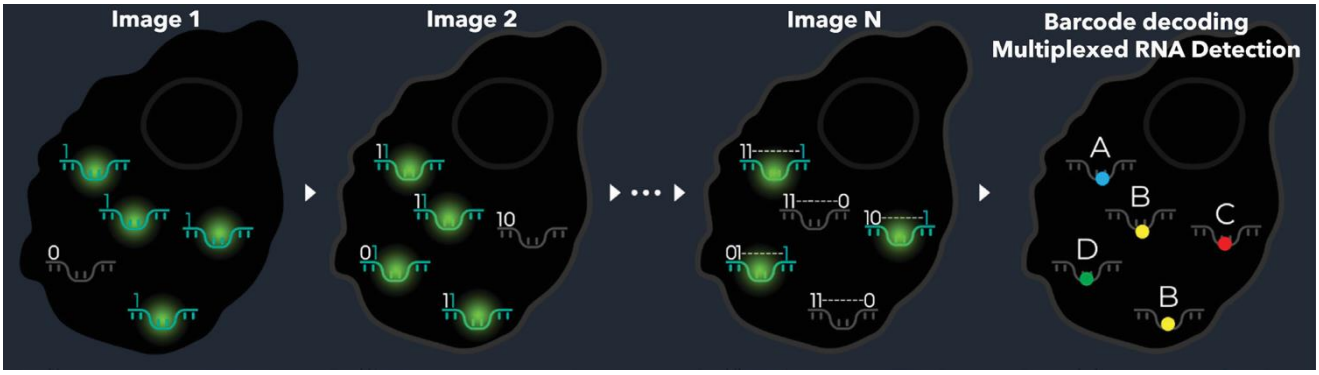

FIGURE 1: MERFISH encoding and readout scheme. For a MERFISH measurement, each gene is assigned a unique binary barcode. The barcodes are optically detected using sequential rounds of single-molecule FISH. For each imaging round, the genes assigned barcodes containing a 1 bit for the corresponding bit 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.

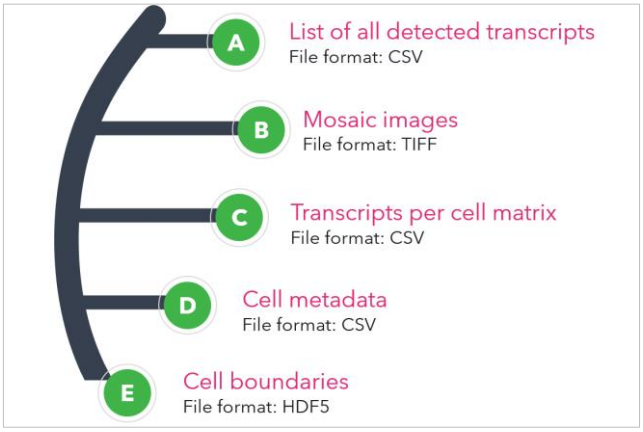

FIGURE 2: MERFISH Measurement Data Output.

MERSCOPE STUDY DESIGN

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 the brain with cellular context. MERFISH measurements were conducted for full coronal sections across three different positions in the mouse brain with three biological replicates per position to determine the exact location of the targeted transcripts (FIGURE 3).

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

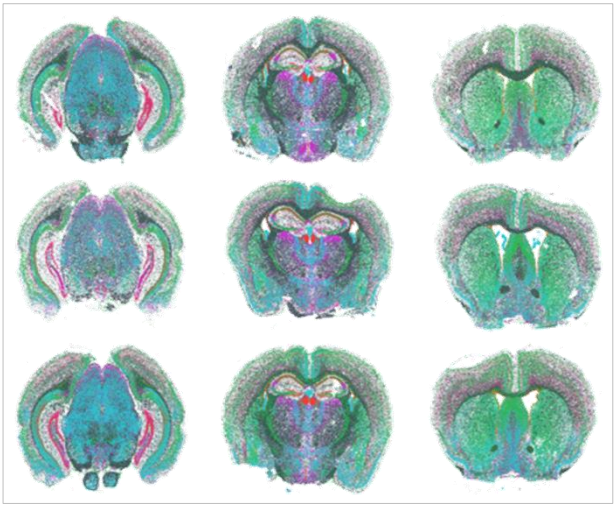

FIGURE 3: MERFISH measurements across 9 full mouse brain coronal sections. Each panel depicts the expression pattern for 8 of the genes from the 483 gene measurement for each of the 3 biological replicates at 3 positions across the mouse brain.

RESULTS

We ran the MERFISH measurements using the 483 gene panel across the three coronal mouse brain sections each with three replicates on the MERSCOPE Platform to generate a Mouse Brain Atlas (FIGURE 3). From these measurements, MERSCOPE achieved quantitative, single-molecule resolution spatial transcriptome profiling across the full coronal sections. At any position in the coronal section, we can zoom in to explore the cellular and molecular composition ofthe sample (FIGURE 4). We compare the number of transcripts detected to bulk sequencing and found a strong correlation (r=0.88) between biological replicates (FIGURE 5). From MERSCOPE's cell segmentation, we were able to perform

single-cell spatial analyses. We clustered cells by gene expression to identify different cell types and map the spatial organization as well as the variation in gene expression of these cells (FIGUREs 6 and 7).

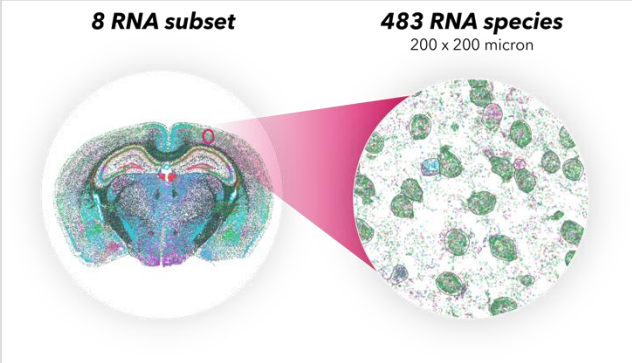

FIGURE 4: 8 RNA subset from MERFISH measurement of 483 transcripts.

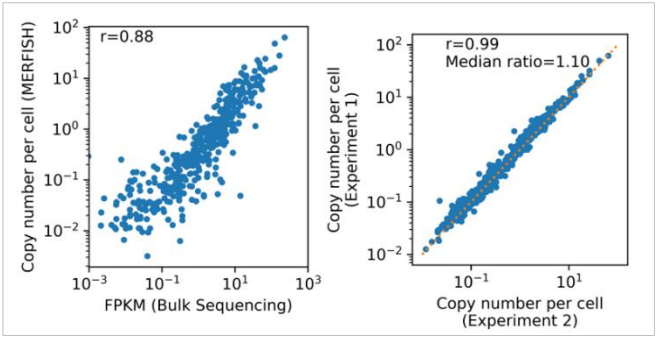

FIGURE 5: Correlation plot comparing bulk RNA seq data to MERFISH measurement.

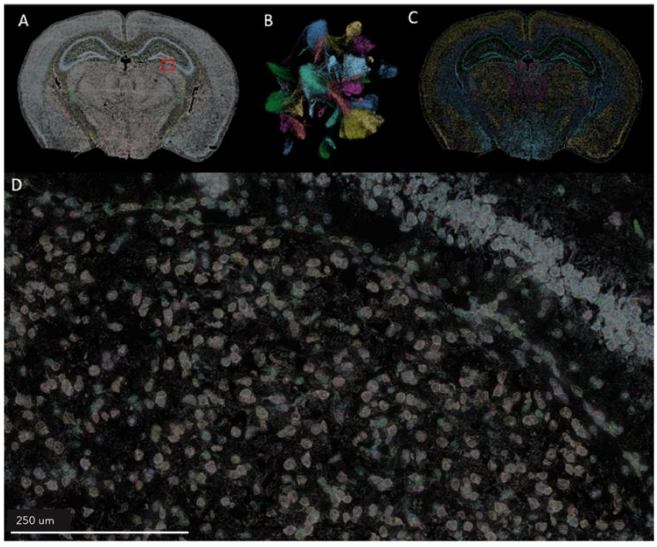

FIGURE 6: MERFISH measurement of 483 genes across one of the 9 coronal sections output highlighting (A) all detected transcripts, (B) a UMAP constructed from the cell by gene data and colored by cell type clustering results, and (C) the spatial organization of the detected cells colored by their cell type classification. Across the full mouse brain coronal section, MERSCOPE maps the transcriptome with single-molecule and single-cell resolution (D). Each transcript is depicted as a point and the segmented cell boundaries are drawn as lines colored by the assigned cell type.

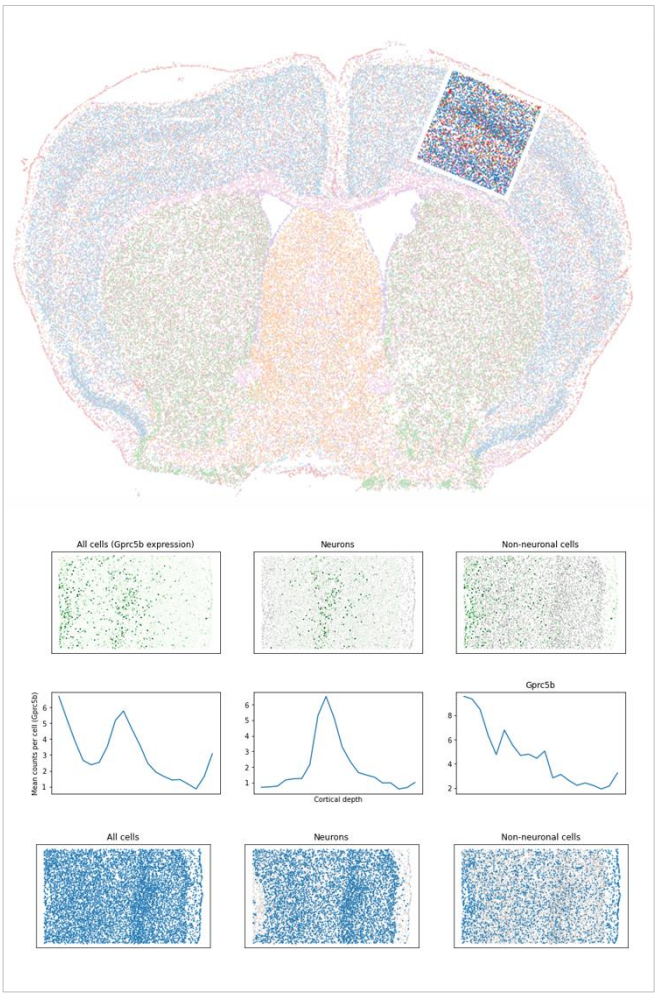

FIGURE 7: Cell-type dependent spatial distribution of Gprc5b expression. From cell clustering results, cells were categorized as either neuronal or nonneuronal. The Gprc5b expression across all cells indicates two peaks of high expression, one deep in the cortex and one in the mid cortex. By grouping clustered cells into neuronal and non-neuronal populations, the cell-type-dependent spatial Gprc5b expression can be quantified. 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 accurately spatially profile a large panel of genes across whole tissue sections 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 MERFISH gene panel measured across three whole mouse coronal sections on the MERSCOPE instrument. Each coronal section had three replicates measured for a total of nine samples. We showcased the ability of MERSCOPE to detect lowly expressed GPCRs which mediate signaling and may play vital roles behind brain aging and neurodegenerative disorders but are difficult to detect with other technologies.[2] Since these gene are During our experiment, MERSCOPE measured GPCRs including Oxtr[3], Tshr[4], and Insr[5]. 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[6] for researchers to access and explore.

REFERENCES

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