Unlocking Insights in Alzheimer's Disease from Archived Brain Tissue Samples with High Sensitivity Spatial Transcriptomics

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Introduction

The advent of tools enabling researchers to easily perform spatial transcriptomics with single-cell resolution has enabled a revolution in the understanding of the nervous system. However, tissue samples with degraded RNA or extensive crosslinking present challenges for *in situ* gene expression measurements. The majority of human brain samples are archived using fresh frozen methodologies, but the RNA quality is often too low for useful transcriptome analysis due to variable collection conditions. Accurately mapping gene expression in frozen brain tissue *in situ* requires a spatial transcriptomics technique with high detection efficiency. Multiplexed Error Robust in situ Hybridization (MERFISH) 2.0™ directly profiles the transcriptome of intact tissues with high sensitivity and accuracy. The 3 cm^2 imageable area allows measurement from multiple samples on the same slide, removing batch effects and other artifacts when studying paired conditions.

Here we demonstrate spatial transcriptomics from healthy and Alzheimer's Diseased (AD) human cortical tissue simultaneously on the MERSCOPE Ultra™ Platform. In addition, we performed protein co-staining for Aβ plaques in the same experiment. We demonstrate how combining high sensitivity spatial transcriptomics with readout methods increases the scope and scale of spatial transcriptomics experiments Both tissues were run with a 960-gene panel customized for neuronal cell typing and neurodegeneration. We used this data to construct a spatially resolved single-cell atlas across healthy and diseased human brain tissue, mapped and cataloged different neuronal cell types, and analyzed gene expression by proximity to Aβ plaques. These types of measurements are only possible through the combination of spatial imaging, gene expression quantification, and protein detection.

Graphical Overview

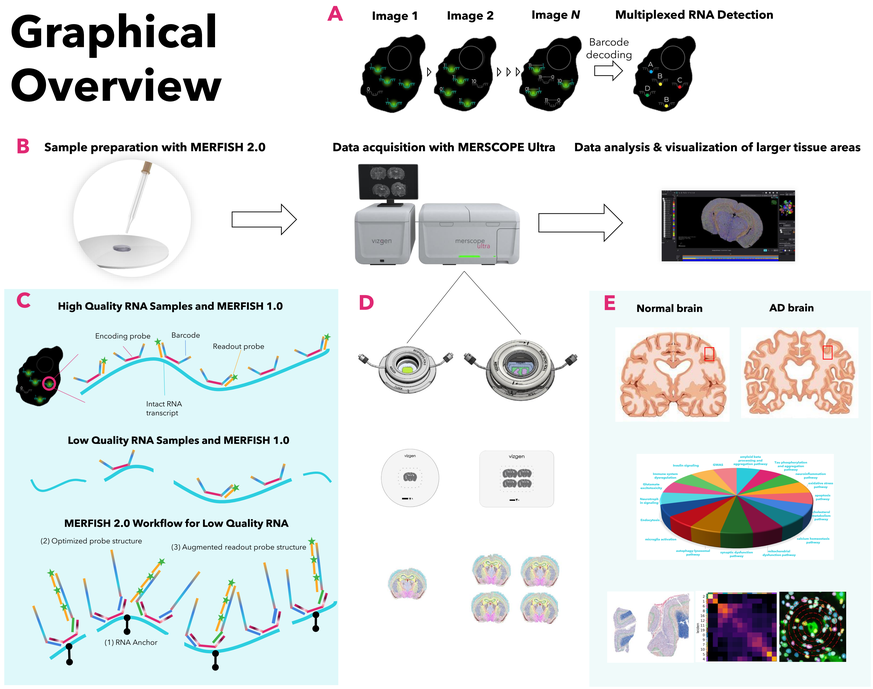

FIGURE 1: Methods and experimental design. A) MERFISH uses binary barcodes to encode different mRNA species, which enables *in situ* profiling of hundreds to thousands of genes at single-molecular resolution. **B)** The MERSCOPE Platform is an end-to-end solution for MERFISH, from sample preparation to data analysis and visualization. **C)** We developed the MERFISH 2.0 chemistry and workflow to improve capture and detection of RNA from lower RNA quality samples, such as archival brain tissue. The MERFISH 2.0 workflow introduces 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. **D)** The MERSCOPE Ultra Platform enables acquisition of spatial data of up to 3cm^2 without sacrificing speed or quality. Using two flow cells, the MERSCOPE Ultra can support both Standard and Large slides. **E)** Frozen archival human brain blocks from healthy and AD brain were sectioned onto the same MERSCOPE Large slide. The MERSCOPE sample preparation workflow was run on Large slides using a MERFISH 2.0 960-plex gene panel containing markers for both cell typing and neurodegeneration pathways. In addition, the sections were stained for co-detection of Aβ protein. Analysis of both brains in the same experiment enabled identification of cell-type-specific molecular and cellular adaptations in the disease state.

MERFISH 2.0 substantially improved RNA detection efficiency in archival human brain tissue

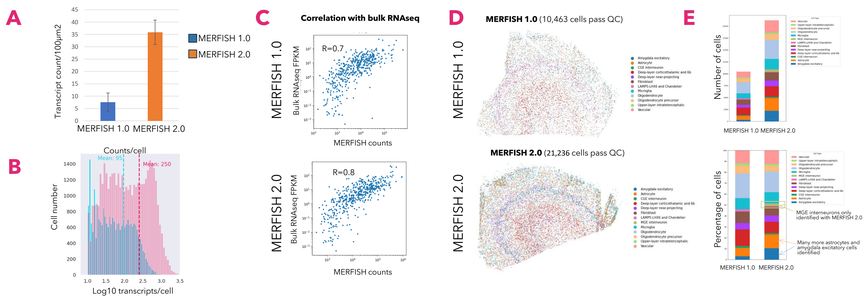

Figure 2: MERFISH 2.0 substantially improved RNA detection efficiency in human brain. To evaluate the performance of MERFISH 2.0 chemistry, samples from human cortex were run with matched 815-plex MERFISH 1.0 or 2.0 gene panels. RNA transcript counts per 100μm^2 **(A)** and counts per cell (**B**) were used to evaluate sensitivity. **C)** Correlation of MERFISH counts with bulk RNAseq show high correlation with biological references. Higher sensitivity resulted in more cells passing QC **(D)** and more cell types **(E)** identified with MERFISH 2.0.

Simultaneous profiling of molecular and spatial heterogeneity in normal and Alzheimer's Disease tissue

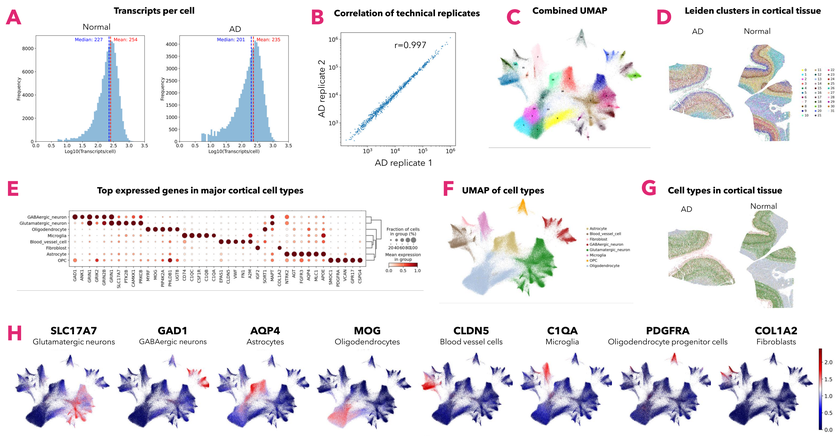

FIGURE 3: Simultaneous profiling of molecular and spatial heterogeneity of normal and AD human cortex. A) Histogram showing the distribution of transcript counts per cell from normal and AD brain samples in a single MERSCOPE Ultra experiment. **B)** Correlation between technical replicates of MERFISH 2.0 showed high reproducibility. Leiden clustering of MERSCOPE data from human normal and AD brain cortical sections shown in UMAP **(C)** and spatial **(D)** projections. **E)** The top genes per Leiden cluster were used to assign cell types, with the highest expressed genes per cell type shown by dot plot. UMAP **(F)** and spatial distribution **(G)** of major cell clusters in normal and AD human cortex. **H)** UMAPs colored by the marker genes of the major cell types present in human cortex.

High sensitivity of MERFISH 2.0 enables sub-profiling of glutamatergic neurons within cortical layers

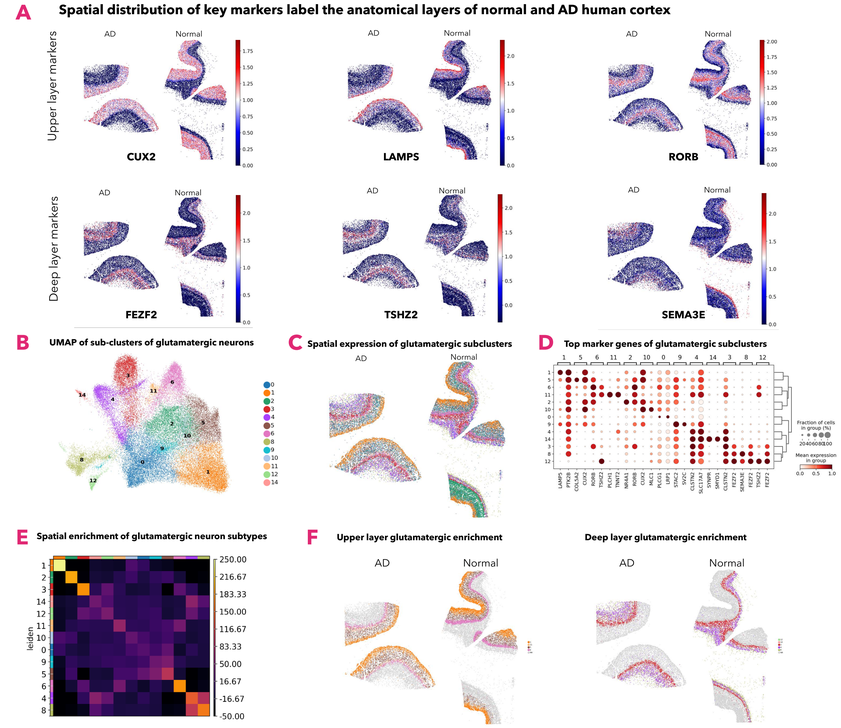

FIGURE 4: Sub-profiling of glutamatergic neurons showed layer-specific expression of neuronal subtypes. A) Spatial heatmap of the expression of markers for upper layer and deep layers of the cortex. Examination of individual markers for glutamtergic neurons further highlights the anatomical structures within the cortex. The data was re-clustered for sub-types of glutamatergic neuron, and the resulting Leiden clusters were plotted on **B)** UMAP, **C)** spatial distribution of glutamatergic neuron sub-clusters, along with top marker genes (**D**). **E)** Heatmap showing the neighborhood enrichment of different glutamatergic sub-clusters. **F)** Spatial distribution of different glutamatergic sub-clusters within the layers of human cortex.

Conclusions

- Genes with lower expression are much more likely to be detected with MERFISH 2.0, leading to a reduced cell-dropout rate in single cell analysis, and producing the accurate, robust data needed for confident spatial analyses.
- The MERSCOPE Ultra Platform with a 960-gene MERFISH gene panel enabled the accurate spatial profiling of hundreds of mRNA in intact tissue context, allowed for simultaneous study of health and AD human cortical tissue, removing any potential batch effects
- The 960-gene human brain panel allowed for deep profiling of neuronal subtypes and mechanistic investigation of neurodegenerative pathway changes associated with Alzheimer's Disease.
- Co-detection of Aβ protein showed changes in cellular composition in spatial proximity to plaques, and uncovered genomic changes within cell types in contact with plaques.
- Gene expression of known neuronal markers such as ATPB24 and SYT1 was altered by proximity to the plaque within glutamatergic and GABAergic neurons.
- **These types of mechanistic investigations into Alzheimer's Disease are only possible through the combination of spatial imaging, gene expression quantification, and protein detection.**

Spatial multiomic profiling revealed cellular and molecular changes associated with AD

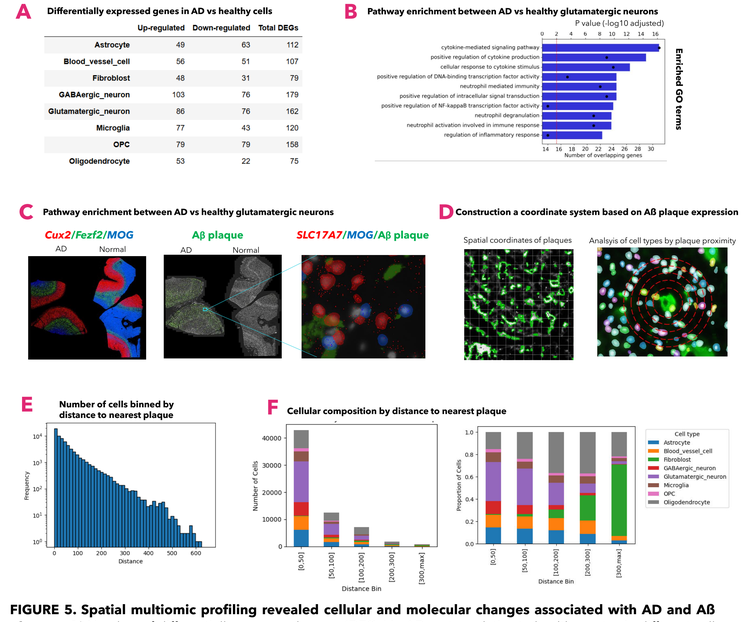

FIGURE 5. Spatial multiomic profiling revealed cellular and molecular changes associated with AD and Aß plaques. A) Number of differentially expressed genes (DEGs) in AD cortex relative to healthy cortex in different cell populations. **B)** Bar graph showing the GO terms enriched in the DEGs of glutamatergic neurons. **C)** Representative pictures of co-detection of Aβ plaques with MERFISH in human cortical samples. **D)** Antibody staining was used to identify the coordinates of Aβ plaques, which was used to calculate the distance of cells to Aβ plaques was determined. **E)** Histogram showing the distance of cells to the nearest Aβ plaques in the AD sample. **F)** The proportion of different cell populations grouped by distance to the nearest Aβ plaques.

Spatial multi-omic profiling revealed cellular and molecular changes associated with AD

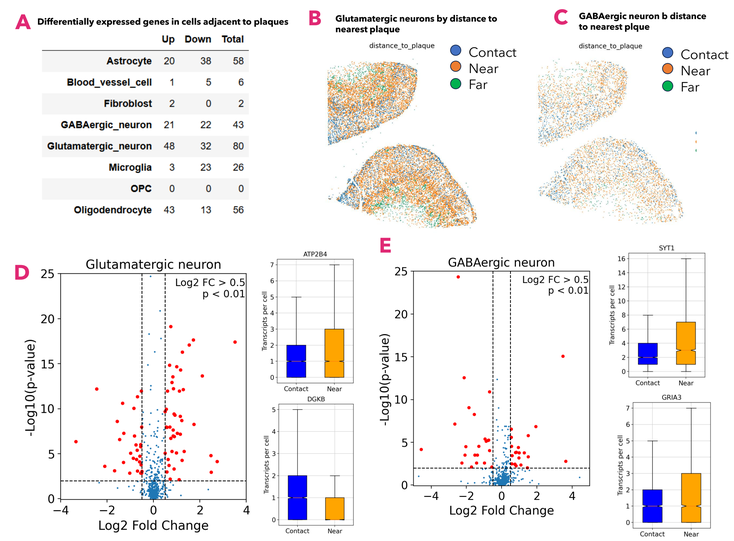

Figure 6: Neuronal gene expression altered by proximity to AB plaques. A) Number of differentially expressed genes in cells in contact with plaques vs further from plaques. Spatial relationships of glutamatergic neuron (**B**) and GABAergic neurons (**C**) to Aβ plaques. Volcano plots showing the DEGs between glutamatergic neuron (**D**) and GABAergic neurons (**E**) in and out of Aβ plaques. Box plots showing the examples of DEGs identified in glutamatergic neurons and GABAergic neurons, which are supported by literature.