

Enhanced Spatial Transcriptomics Enables High-Resolution Profiling of Neuronal Transcription Factors in the Developing Nervous System



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Introduction

The advent of imaging-based high-plex spatial transcriptomics tools with single-cell resolution has transformed our understanding of the structure, function, and development of the nervous system. These technologies provide the ability to visualize gene expression in its native spatial context, enabling deeper insights into cellular identity, organization, and communication. However, detecting genes expressed at low levels—especially in samples with compromised RNA quality—remains a challenge for downstream biological analysis. Transcription factors, in particular, are difficult to profile due to their typically low abundance, transient expression, and tight regulation across developmental stages. To address this, the Multiplexed Error-Robust Fluorescence In Situ Hybridization 2.0 (MERFISH 2.0) chemistry and sample preparation workflow were developed to enhance transcript detection efficiency for up to 1,000 genes. This technology enables highly sensitive, spatially resolved RNA profiling directly in tissue, while preserving cellular architecture and gene expression patterns. In this study, we applied MERFISH 2.0 to comprehensively profile transcription factors during mouse development. Two panels, each containing 815 genes, were designed to cover the full repertoire of known mouse transcription factors. Multiple developmental stages of mouse embryos and postnatal brains were sectioned onto MERSCOPE Ultra® slides and analyzed using both panels. The resulting data enabled detailed spatial and single-cell analyses, demonstrating that MERFISH 2.0 provides robust detection of transcription factors throughout embryonic and early brain development. High-resolution, spatially resolved profiling of transcription factors at the single-cell level opens new avenues for studying neuronal development, function, and disease. This approach will facilitate exploration of transcriptional dynamics that drive lineage commitment, neurogenesis, and brain regionalization, offering a powerful tool for investigating gene regulation in complex tissues.

Graphic Overview

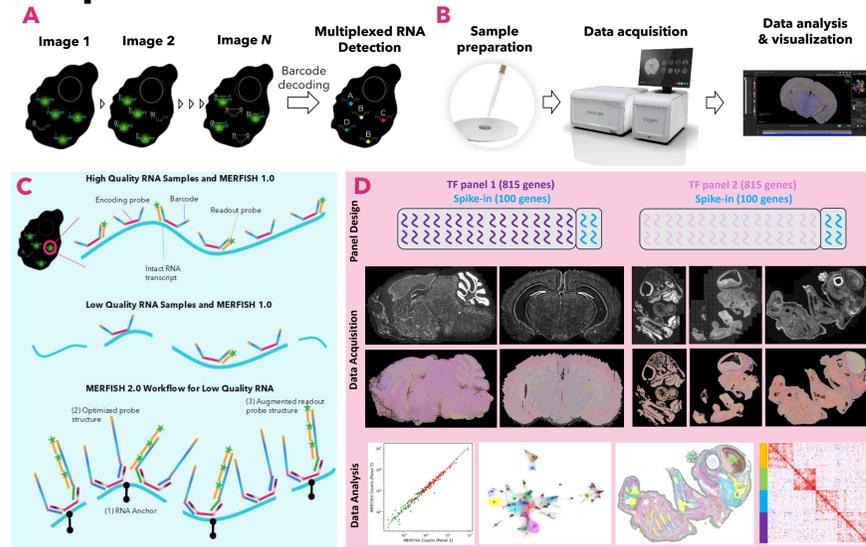


FIGURE 1. The MERSCOPE Platform and experimental design. **A**) MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization) uses binary barcodes to encode individual mRNA species, enabling in situ profiling of hundreds of genes at single-molecule resolution. **B**) The MERSCOPE Platform provides a complete solution for MERFISH—from sample preparation through imaging, analysis, and visualization. **C**) MERFISH 2.0 chemistry and workflow were developed to enhance RNA capture and detection in samples with lower RNA quality, such as archival brain tissue. Improvements include: (1) optimized anchoring chemistry to better capture RNA fragments; (2) an optimized probe structure enabling more efficient target binding; and (3) enhanced readout probes that substantially improve signal-to-noise during imaging. **D**) Two 815-gene panels were designed to target mouse transcription factors (TFs). Fresh-frozen mouse brain and FFPE mouse embryo samples were profiled using both TF panels, each supplemented with 100 spike-in genes. These MERFISH datasets enabled identification of diverse cell populations and characterization of transcriptional programs underlying tissue architecture and development.

Results

Benchmarking MERFISH 2.0 sensitivity and accuracy

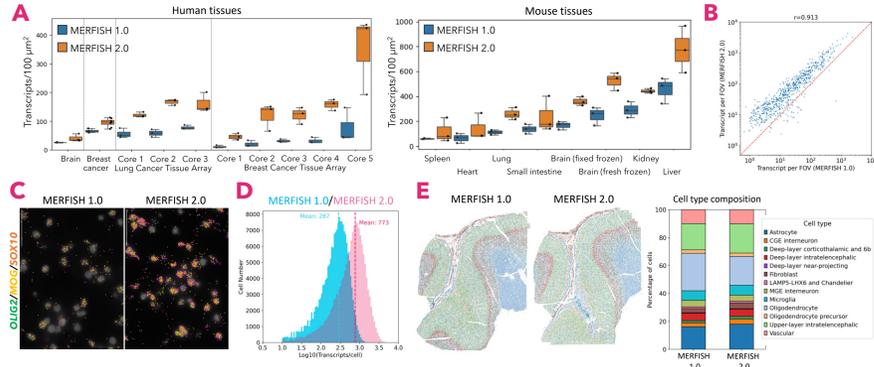


FIGURE 2. Benchmarking MERFISH 2.0 sensitivity and accuracy. **A**) Box plots showing increased transcript detection sensitivity with MERFISH 2.0 across multiple human (left) and mouse (right) tissues. **B**) Scatter plot illustrating transcript count correlation between matched MERFISH 1.0 and MERFISH 2.0 experiments. **C**) Representative transcripts from a human brain sample processed using MERFISH 1.0 or MERFISH 2.0. **D**) Histogram of transcript counts per cell from a human brain sample processed by MERFISH 1.0 or MERFISH 2.0. **E**) Spatial distribution (left) and proportional abundance (right) of major cell populations detected in human brain samples processed by MERFISH 1.0 and MERFISH 2.0.

Benchmarking the spike-in gene panel

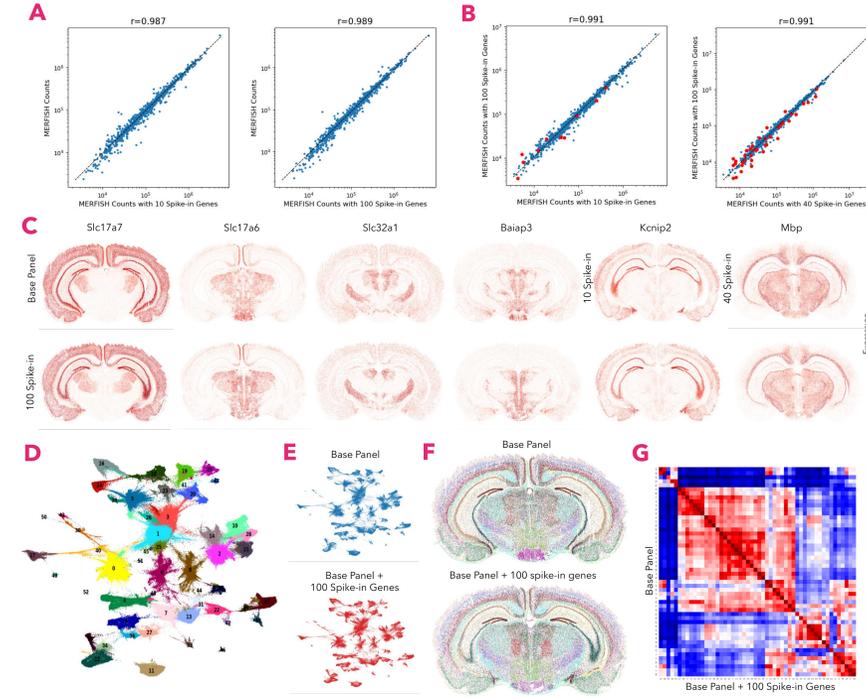


FIGURE 3. Benchmarking the spike-in gene panel. **A**) Scatter plots showing transcript count correlations between replicate MERSCOPE 2.0 runs using the base 815-gene panel (left) or the base panel supplemented with 100 spike-in genes (right). **B**) Scatter plots comparing transcript counts from experiments using the base panel plus 10, 40, or 100 spike-in genes. **C**) Expression patterns of selected genes detected in mouse brain using the base panel or the base panel supplemented with 10, 40, or 100 spike-in genes. **D**) UMAP visualization of cell clusters identified from datasets generated with the base panel or the base panel plus 100 spike-in genes. **E**) UMAP showing cells from the base-panel experiment (blue) or the base panel plus 100 spike-in genes (red). **F**) Spatial distribution of cell types detected in mouse brain samples profiled with the base panel or the base panel plus 100 spike-in genes. **G**) Heatmap showing gene expression correlations across cell clusters from mouse brain profiled with the base panel or with the base panel plus 100 spike-in genes.

Profiling mature mouse brain sample using two TF panels with spike-in genes

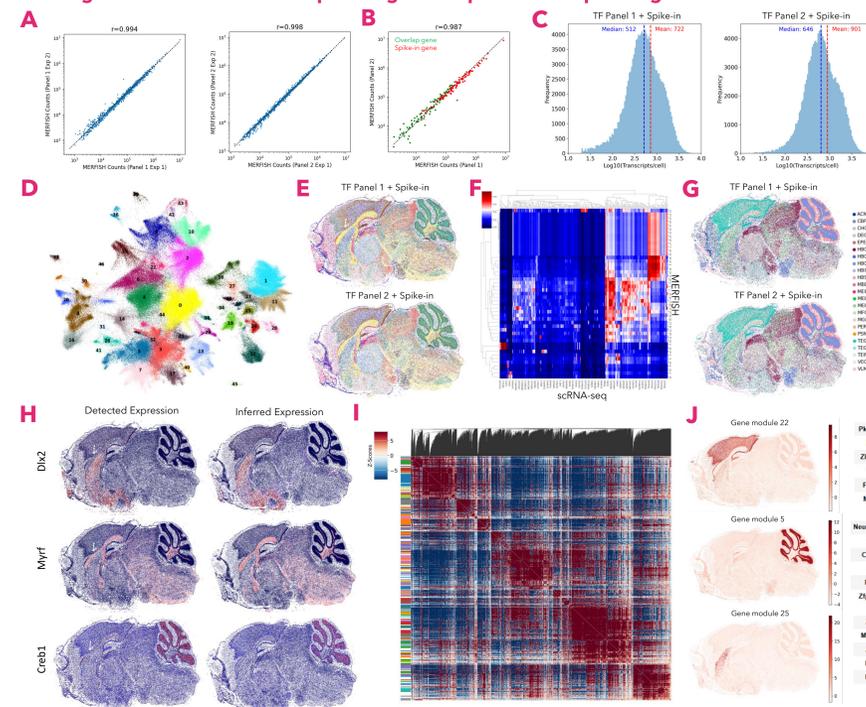
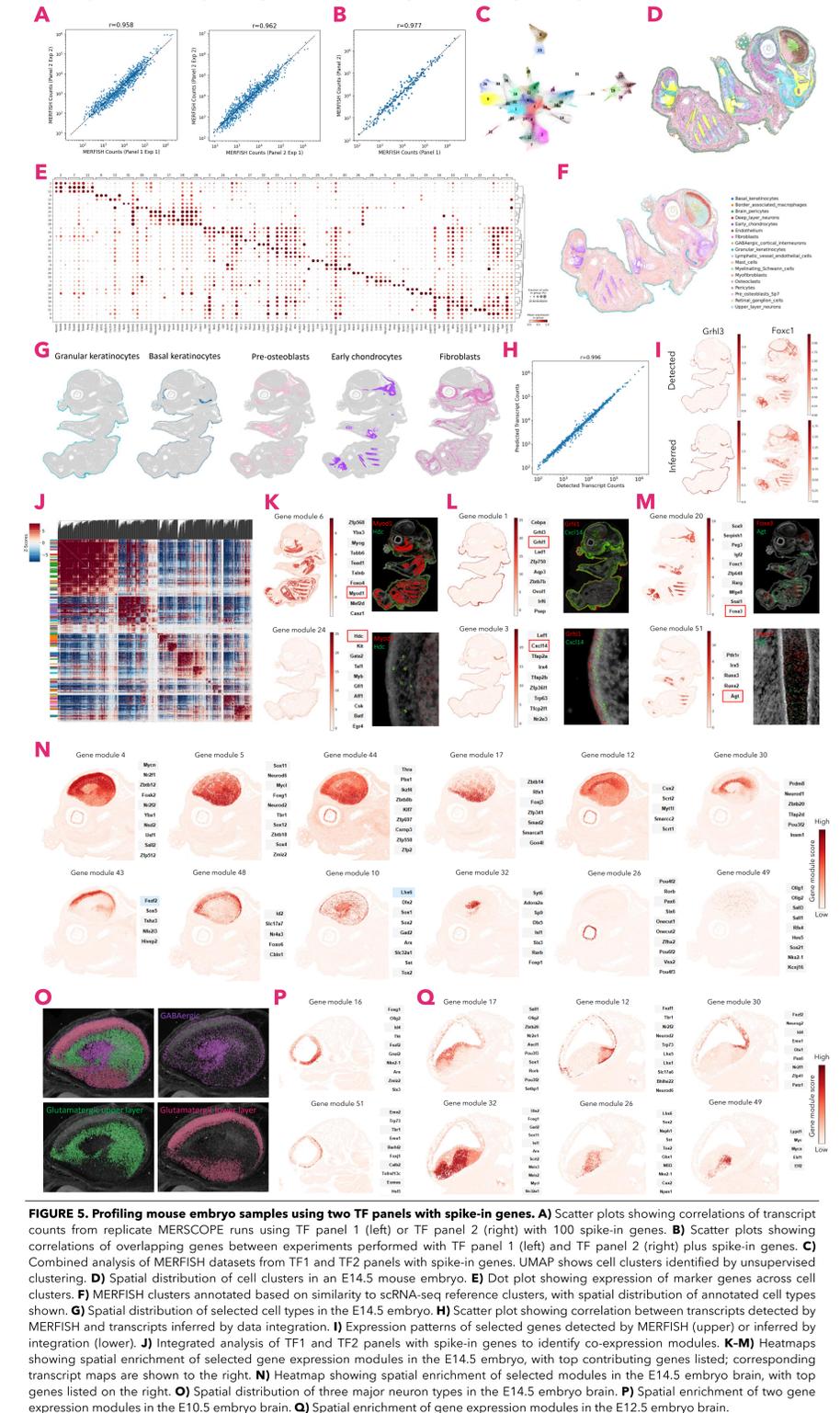


FIGURE 4. Profiling mature mouse brain using two TF panels with spike-in genes. **A**) Scatter plots showing correlations of transcript counts from replicate MERSCOPE runs using TF panel 1 (left) or TF panel 2 (right) with 100 spike-in genes. **B**) Scatter plots showing correlations of overlapping genes between experiments performed with TF panel 1 (left) and TF panel 2 (right) plus spike-in genes. **C**) Histograms showing transcript count per cell distributions from mouse brain samples profiled with TF panel 1 (left) or TF panel 2 (right) plus spike-in genes. **D**) Combined analysis of MERFISH datasets from TF1 and TF2 panels with spike-in genes. UMAP shows cell clusters identified by unsupervised clustering. **E**) Spatial distribution of cell clusters in mouse brain profiled with TF1 (upper) and TF2 (lower) panels plus spike-in genes. **F**) Heatmap showing pairwise correlations between MERFISH-derived cell clusters and scRNA-seq clusters. MERFISH clusters were annotated based on similarity to scRNA-seq reference clusters. **G**) Spatial distribution of annotated cell types in mouse brain profiled with TF1 (upper) and TF2 (lower) panels plus spike-in genes. **H**) Expression patterns of selected genes as detected directly by MERFISH (left) or inferred by data integration (right). **I**) Integrated analysis of TF1 and TF2 panels with spike-in genes to identify co-expression modules. **J**) Heatmap showing spatial enrichment of gene expression modules in mouse brain, with top contributing genes listed on the right.

Profiling mouse embryo samples using two TF panels with spike-in genes



Conclusions

- MERFISH 2.0 substantially increases RNA detection sensitivity while maintaining high accuracy.
- Spike-in genes provide additional flexibility in MERSCOPE panel design, enabling broader applications.
- Profiling mature mouse brain with TF panels and spike-in genes reveals transcriptional programs underlying neural cell diversity.
- Profiling mouse embryos across developmental stages with TF panels and spike-in genes identifies spatiotemporal enrichment of gene modules in the developing mouse brain.