Analyzing the molecular basis underlying anatomic and functional complexity of the mouse brain with MERSCOPE™

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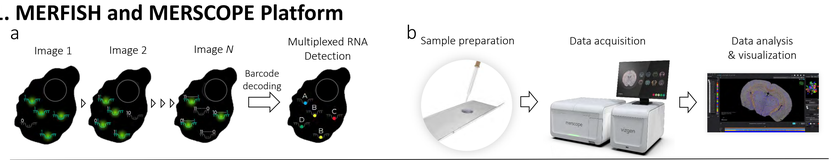
ABSTRACT

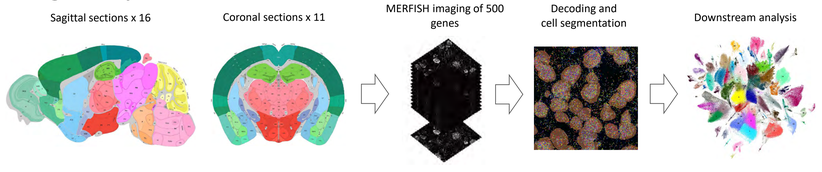
Single-cell sequencing has provided numerous novel insights into cell heterogeneity and cell-type-specific adaptation of the nervous system. However, most sequencing-based techniques require cell dissociation and consequently lose the spatial information of the analyzed cells, which prevents directly connecting the cell types to specific anatomic and functional features. The rapid development of spatially resolved genomic assays enables molecular analysis in the tissue context, with the potential of revealing how single-cell gene activity orchestrates the structure and of complex tissues like the nervous system. Here, we demonstrated the use of the MERSCOPE™ Platform to generate a transcriptionally defined and spatially resolved single-cell mouse brain atlas. By performing multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) assays with a 500-gene panel designed for cell typing, we obtained over two million cells with precise gene expression and spatial information across the mouse brain. Clustering analysis of the gene expression data resolved all major cell populations as well as detailed neuron and non-neuron subtypes across different brain regions. Importantly, all these molecularly determined cell types were precisely mapped to their original locations. By assessing the relationship between molecular and anatomic features of identified cell types, we found that both excitatory and inhibitory neuron subtypes exhibit significant variation in gene expression and spatial distribution along multiple axes of different brain structures. Furthermore, the high-resolution spatial transcriptomic data enabled us to assess the spatial relationship and cell-cell interactions across different cell types. Altogether, our work not only created a molecularly defined and spatially resolved mouse brain cell atlas, but also demonstrated the power of MERFISH measurements generated by the MERSCOPE™ Platform in analyzing the molecular basis underlying the anatomic and functional complexity of the nervous system.

1. MERFISH and MERSCOPE Platform

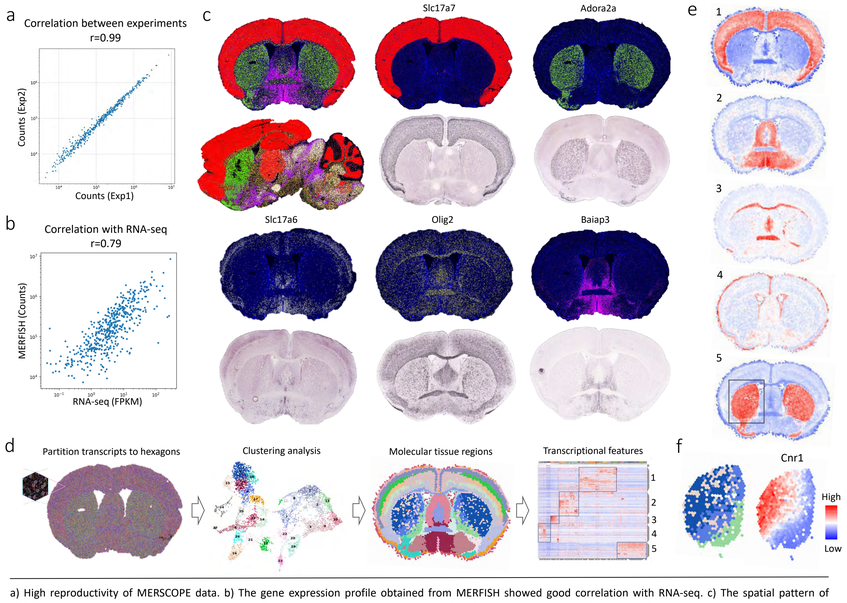


a) MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization) enables in situ transcriptomic profiling at single-cell resolution. b) MERSCOPE is an end-to-end solution of MERFISH technique.

2. Design of experiments

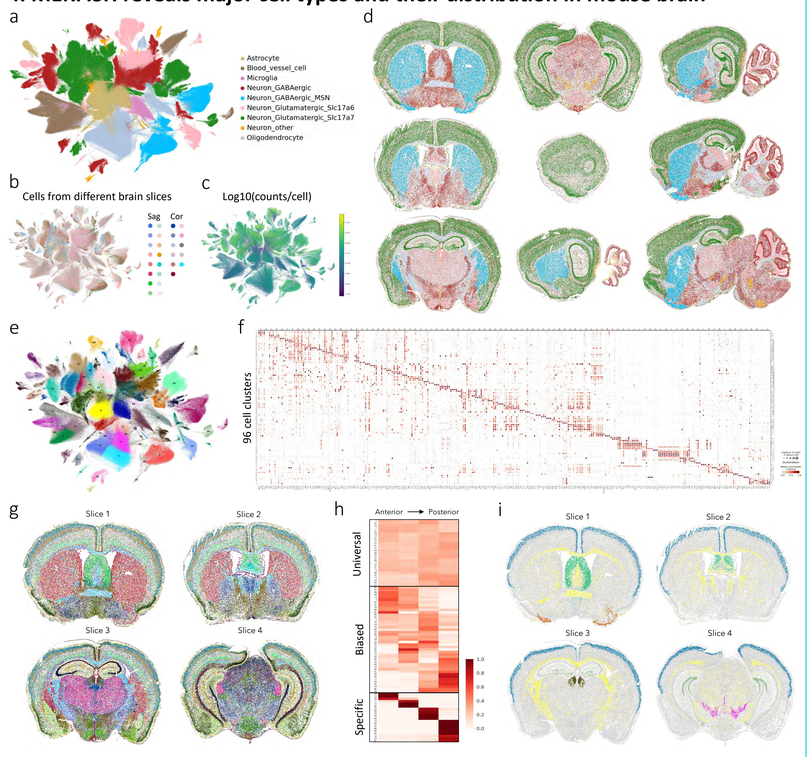


3. Spatial transcriptomic profiling of mouse brain with MERFISH



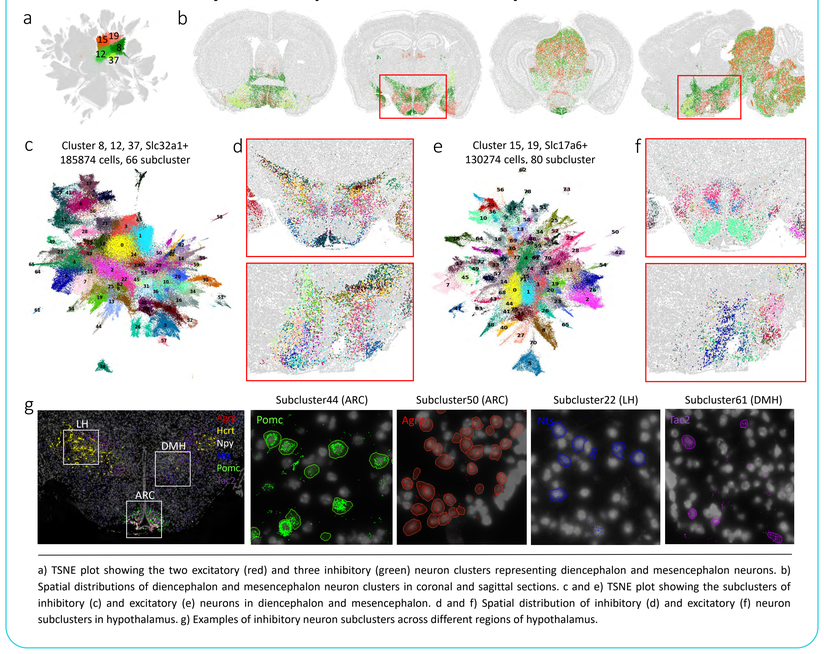
a) High reproductivity of MERSCOPE data. b) The gene expression profile obtained from MERFISH showed good correlation with RNA-seq. c) The spatial pattern of different genes as revealed by MERFISH and ISH. d) Molecular tissue regions could be identified by analyzing the information of transcripts. e) Groups of genes are enriched in different molecular tissue regions. f) Gene expression heterogeneity in striatum subregions.

4. MERFISH reveals major cell types and their distribution in mouse brain



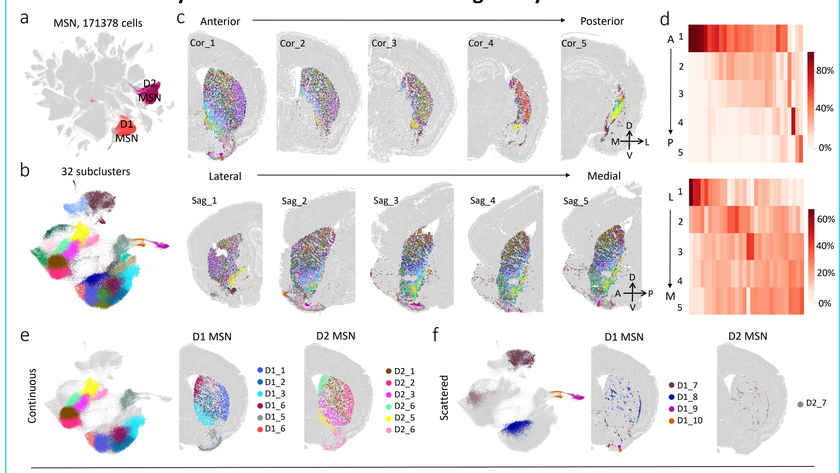
a) TSNE plot showing the major cell groups revealed by single-cell clustering. b) TSNE plot showing the cells from different tissue sections. c) TSNE plot showing the transcript counts across different cells. d) Spatial distribution of the cell groups in coronal and sagittal sections. e) TSNE plot showing the 96 cell clusters (coded with different colors) revealed from MERFISH data. f) Dot plot showing the expression patterns of marker genes across the 96 cell clusters. g) Spatial distribution of different cell clusters. h) Heatmap showing the proportion of different cell clusters along the anterior-posterior axis. i) Examples of cell types with universal and region-specific distribution.

5. Neuronal diversity in diencephalon and mesencephalon



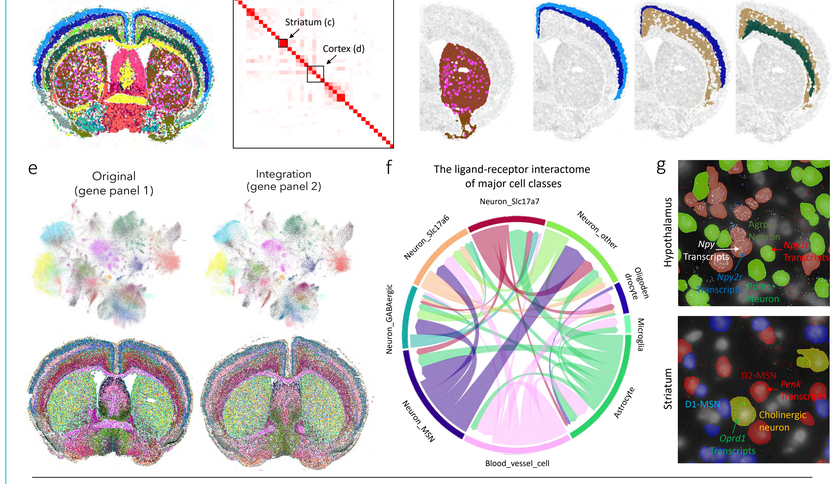
a) TSNE plot showing the two excitatory (red) and three inhibitory (green) neuron clusters representing diencephalon and mesencephalon neurons. b) Spatial distributions of diencephalon and mesencephalon neuron clusters in coronal and sagittal sections. c and e) TSNE plot showing the subclusters of inhibitory (c) and excitatory (e) neurons in diencephalon and mesencephalon. d and f) Spatial distribution of inhibitory (d) and excitatory (f) neuron subclusters in hypothalamus. g) Examples of inhibitory neuron subclusters across different regions of hypothalamus.

6. MSN diversity underlie the anatomic heterogeneity of striatum



a) TSNE plot showing the D1 and D2-MSN neuron clusters. b) TSNE plot showing different MSN subclusters. c) Spatial distribution of different MSN subclusters in coronal (upper) and sagittal (lower) sections. d) Heatmap showing the proportion of different MSN subclusters along anterior-posterior (upper) and medial-lateral (lower) axis. e and f) MSN subclusters showing continuous (e) or scattered (f) spatial patterns.

7. MERFISH data provides anatomic and functional insights into mouse brain



a) The alpha-shapes of different cell clusters in a coronal section. b) Heatmap showing the cell cluster neighborhood overlap across cell clusters. The box regions representing MSN and cortical neuron clusters are shown in c and d. c) D1 and D2-MSNs are well mixed in striatum. d) Examples of different cortical neuron clusters showing either little (left and middle) or significant (right) overlap with other cortical neuron clusters. e) Integration MERFISH data of different gene panels. f) Integration of MERFISH and scRNA-seq data enable transcriptome-wide analysis of ligand-receptor interaction across major cell groups. g) MERFISH data enable high resolution cell-cell interaction analysis in situ.

Conclusions

- High-throughput single-cell spatial transcriptomic profiling of mouse brain with MERSCOPE platform
- Identify major cell type and their spatial distribution across various brain regions with MERFISH data
- MERFISH data enables detailed cell typing in different brain regions
- MERFISH data provides insights into the anatomic heterogeneity and functional complexity of mouse brain

Future directions and potential applications

- Integrate single-cell RNA-seq/epigenetic data to achieve genome-wide/multi-omic spatial profiling
- Map neuron type-specific connection by combine MERFISH with neural tracing
- Link transcriptionally define neuron types to different functions with neuronal activity staining/imaging
- Analyze cell type-specific transcriptional changes in different disease models

Acknowledgements

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