Comprehensive Profiling of a Mouse Brain Integrating Spatial Transcriptomics, Single-cell RNA Sequencing & Single-cell DNA Methylation Profiling

Manisha Ray, Melanie Masuda, Maggie Nakamoto, Austin Holmes, Brent Biddy, Dominic Skinner, Sanika Khare, Renchao Chen, Hao Wang, Shichen Wang, Felix Schlesinger, Andrew Adey

Background

Introduction

Spatial transcriptomics, single-cell RNA sequencing (scRNA-Seq), and single-cell DNA methylation sequencing offer complementary views of cellular biology. By integrating these data types, we aim to achieve an unprecedented understanding of a biological system's complexities. This study demonstrates how combining these approaches yields spatially resolved information about cellular epigenetic states and transcriptomic patterns within an efficient and cost-effective workflow.

Methods

Mouse brain slices of varying thickness (10 µm and 300 µm) were collected from three distinct brain regions. The 10 µm samples were subjected to spatial transcriptomics profiling using Vizgen's 500-gene pan-neuron panel on the MERSCOPE platform. The 300 µm slices were used for transcriptome-wide RNA profiling with ScaleBio's scRNA-Seq kit and genome-wide DNA methylation sequencing using their scMET kit.

Seurat was employed for processing and analyzing both the ScaleBio scRNA-Seq and Vizgen spatial data. Each dataset underwent normalization with SCTransform and clustering via the default Seurat workflow. Subsequently, the datasets were integrated using Seurat's anchor-based canonical correlation analysis (CCA) approach. In contrast, scMET data was processed through ScaleBio's Seq Suite pipeline and visualized using the Amethyst tool.

Results

All three platforms yielded high-quality data, exhibiting robust cell, gene, and transcript recovery. Vizgen data displayed anticipated spatial patterns of cell-type-specific genes, offering a reliable spatial reference for imputation. Unbiased clusters identified in the ScaleBio scRNA data showed clear spatial resolution upon integration with Vizgen data and projection onto brain slices. The observed and imputed spatial patterns demonstrated high concordance with the Allen Brain Atlas when compared at similar brain regions, further validating the quality of the spatial and single-cell data and their integration.

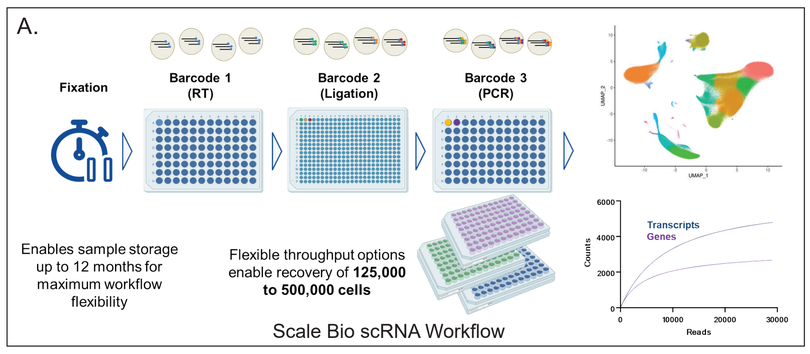
Moreover, the imputed spatial patterns of differentially expressed genes absent from the Vizgen panel but identified as hallmarks of multiple spatially resolved clusters aligned with Allen Brain Atlas data. This confirmed that accurate spatial patterns can be inferred even for genes exclusively present in the single-cell data.

Leveraging single-cell DNA methylation patterns, we successfully identified diverse cell types that corroborated with both the spatial and transcriptomics datasets. Furthermore, clustering based on CH methylation levels helped distinguish between neuronal and non-neuronal cell types.

Conclusions

This study highlights the power of integrating spatial transcriptomics, single-cell RNA sequencing, and single-cell DNA methylation sequencing. By simultaneously examining epigenetic states and transcriptomic patterns at single-cell resolution within a spatial context, we gain a more comprehensive understanding of the intricate cellular landscape of the mouse brain. This integrated approach holds immense promise for unraveling the complex interplay between gene expression, epigenetic regulation, and spatial organization in various biological systems.

Technology Overview



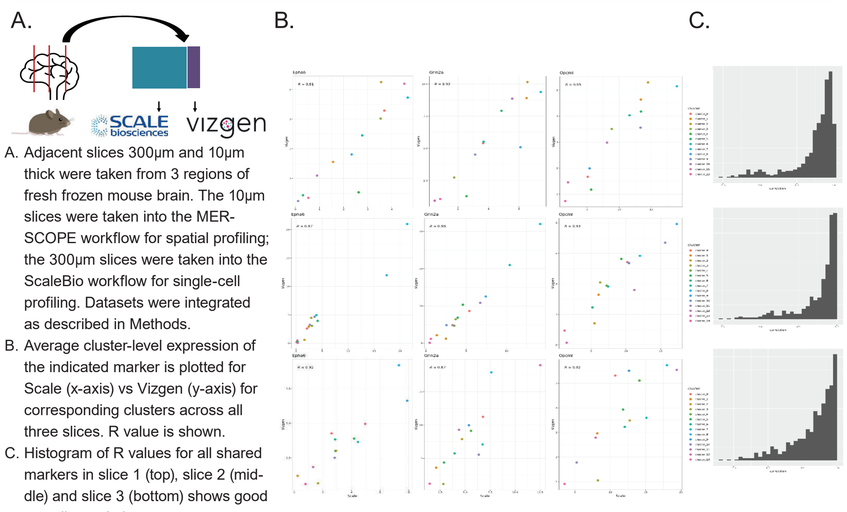
A. ScaleBio™ scRNA-Seq workflow: Isolated nuclei are fixed and undergo sequential barcoding to generate libraries Right: UMAP projection and sensitivity plots are shown from 684,859 cryopreserved PBMCs that were profiled using two ScaleBio scRNA kits.

B. ScaleBio™ scMET workflow: Nuclei isolation and fixation, nucleosome disruption and indexed tagmentation, sorting and bisulfite conversion, followed by library generation and sequencing, and bioinformatic analyses.

C. Vizgen MERFISH spatial imaging with MERSCOPE: End-to-end workflow including custom panel design, sample preparation, Vizgen's commercially available MERSCOPE Platform, and data visualization by the MERSCOPE Vizualizer® software.

D. MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization) uses binary barcodes to encode different mRNA species, which enables in situ profiling of hundreds of genes at single-molecular resolution.

Experimental Design and Data QC for Integration

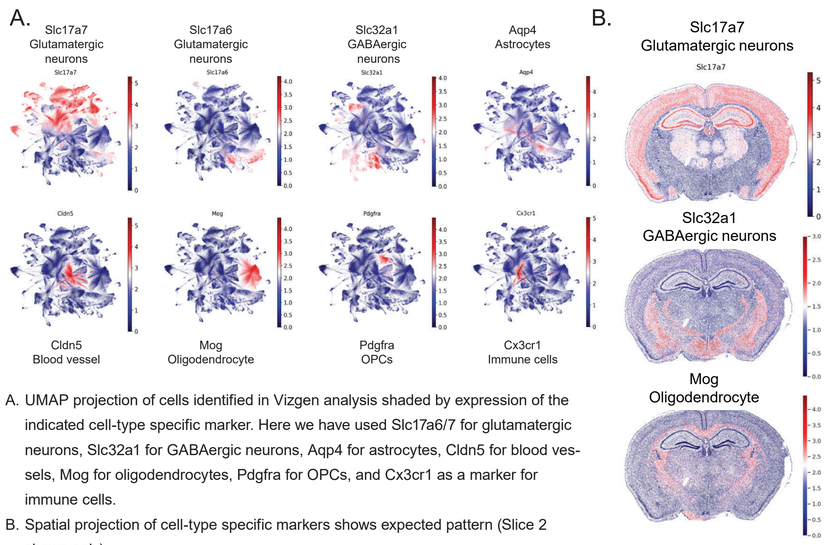


A. Adjacent slices 300µm and 10µm thick were taken from 3 regions of fresh frozen mouse brain. The 10µm slices were taken into the MERSCOPE workflow for spatial profiling; the 300µm slices were taken into the ScaleBio workflow for single-cell profiling. Datasets were integrated as described in Methods.

B. Average cluster-level expression of the indicated marker is plotted for Scale (x-axis) vs Vizgen (y-axis) for corresponding clusters across all three slices. R value is shown.

C. Histogram of R values for all shared markers in slice 1 (top), slice 2 (middle) and slice 3 (bottom) shows good overall correlation.

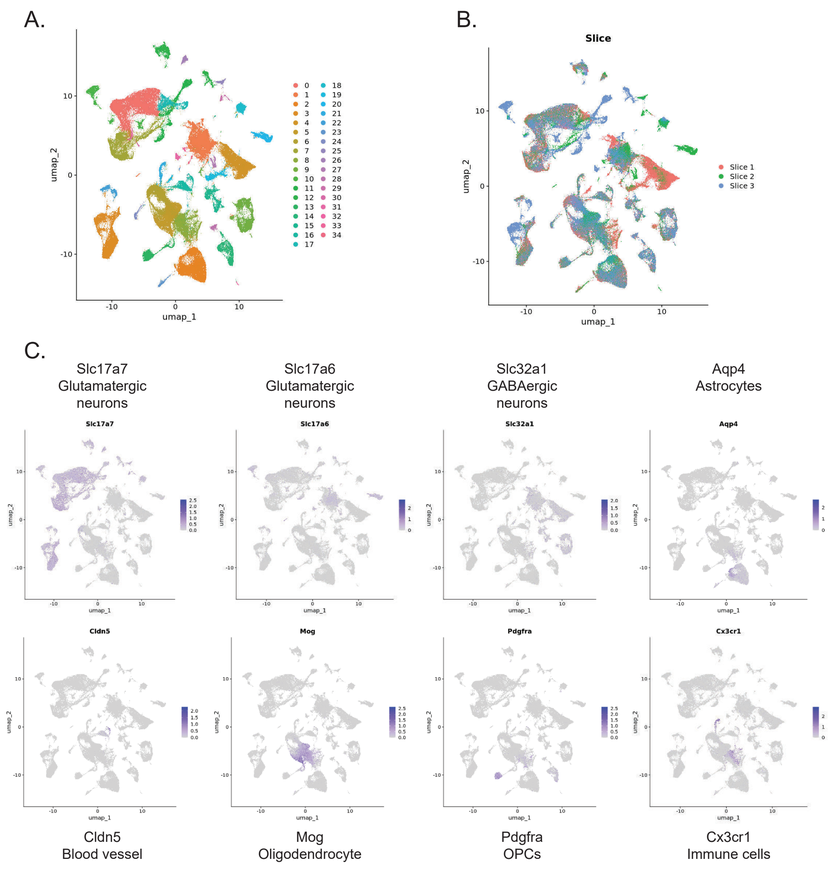
MERSCOPE Shows Expected Spatial Pattern of Known Genes



A. UMAP projection of cells identified in Vizgen analysis shaded by expression of the indicated cell-type specific marker. Here we have used Slc17a6/7 for glutamatergic neurons, Slc32a1 for GABAergic neurons, Aqp4 for astrocytes, Cldn5 for blood vessels, Mog for oligodendrocytes, Pdgfra for OPCs, and Cx3cr1 as a marker for immune cells.

B. Spatial projection of cell-type specific markers shows expected pattern (Slice 2 shown only).

Scale scRNA -seq Identifies Distinct & Relevant Cell Types



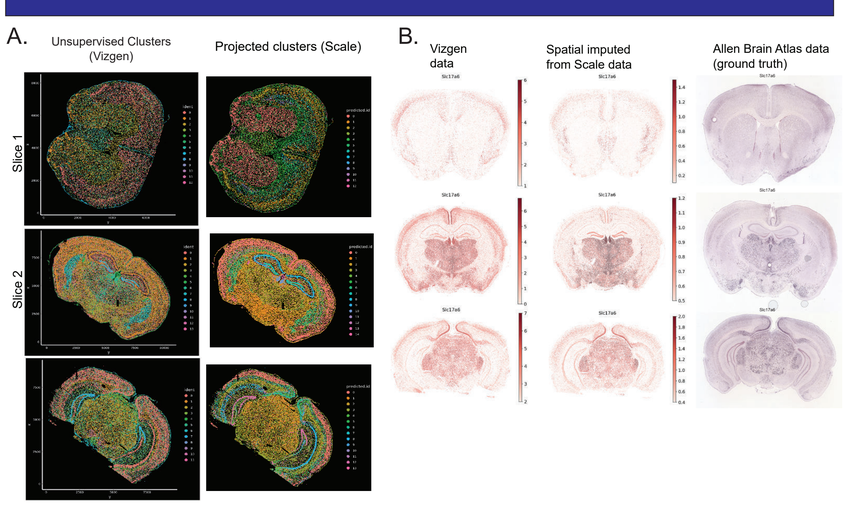
A. UMAP showing all 206,921 recovered cells profiled in the ScaleBio single-cell RNA-seq workflow colored by clusters

B. UMAP projection from A marking cells recovered across the 3 slices

C. UMAP projection of cells identified by Scale scRNA-seq analysis shaded by expression of the indicated cell-type specific marker. Here we have used Slc17a6/7 for glutamatergic neurons, Slc32a1 for GABAergic neurons, Aqp4 for astrocytes, Cldn5 for blood vessels, Mog for oligodendrocytes, Pdgfra for OPCs, and Cx3cr1 as a marker for immune cells. We see good concordance between cell types identified and their respective proportions in the examined population between spatial, RNA, methylation datasets.

D. Dot blot denoting percentage of cells expressing specified marker genes in each cluster along with the average expression levels.

Integration Shows Distinct Spatial Expression of Single-cell Data

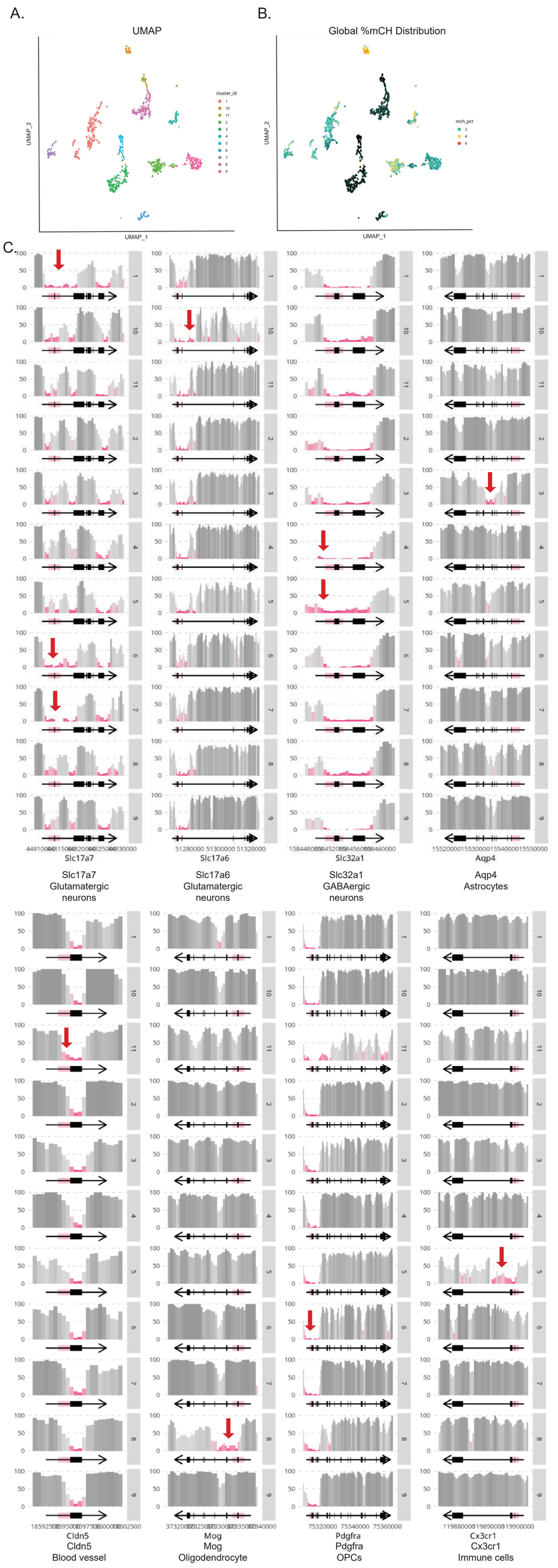


A. Left: clusters identified in Vizgen analysis projected onto brain maps. Right: Predicted scRNA-seq cluster identity for Vizgen spatial data after integration of spatial and scRNA-seq data. Clusters show distinct spatial expression with high spatial resolution.

B. Localization of Slc17a6 in the 3 slices in Vizgen, spatially imputed Scale, and Allen Brain Atlas data.

C. Localization of 3 genes not found in the Vizgen panel that were identified as differentially expressed in single-cell data in imputed and reference data.

Scale scMET Discerns Single-cell Methylomes & Gene Patterns



A. UMAP projection of 4295 single nuclei processed using the ScaleBio scDNA methylation kit clustered using their CG and CH methylation levels.

B. UMAP from A colored by %mCH; three distinct populations are observed - neurons (highest), oligodendrocytes (low), and glia (none).

C. Using Amethyst, we visualized mCG% at specific marker gene loci across the 12 distinct clusters from A. Lower %mCG indicates open and potentially transcriptionally active loci. Here we have used Slc17a6/7 for glutamatergic neurons, Slc32a1 for GABAergic neurons, Aqp4 for astrocytes, Cldn5 for blood vessels, Mog for oligodendrocytes, Pdgfra for OPCs, and Cx3cr1 as a marker for immune cells. We see good concordance between cell types identified and their respective proportions in the examined population between spatial, RNA, methylation datasets.

Conclusions

- Here we demonstrate the data from adjacent brain tissue slices that were run simultaneously through Vizgen MERSCOPE, Scale Bio scRNA-seq and scDNA methylation profiling yielding high quality spatial data, high throughput transcriptome-wide single-cell RNA data, and genome-wide single-cell DNA methylation data.
- We recovered and identified identical cell types across the three datasets demonstrating good concordance between the three platforms.
- We demonstrate the utility of Amethyst in visualizing complex genome wide single-cell DNA methylation data in identifying and curating cell types in a complex sample type.
- Integration of these three datasets provides comprehensive spatial transcriptomic and epigenomic analyses and offers valuable insights into gene expression organization within complex tissues.

References

- Rylaarsdam, Lauren E., et al. "Single-cell DNA methylation analysis tool Amethyst reveals distinct noncanonical methylation patterns in human glial cells." bioRxiv (2024): 2024-08.