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Application Note

TOWARDS A SINGLE-CELL ATLAS OF THE MOUSE BRAIN USING AN AUTOMATED SPATIAL OMICS PLATFORM

The REBUS ESPER™ spatial omics platform is a fully-integrated, automated instrument that delivers high-throughput, quantitative, single-molecule, single-cell data with spatial context and allows analysis of biological molecules directly in and across large tissue sections. The first assay developed for this platform, the ESPER™ High Fidelity assay, is based on gold-standard single-molecule fluorescent in situ hybridization (smFISH) chemistry. This assay's high sensitivity and specificity allow for identification of cell types with only a few genes. Here, we demonstrate the ability of the Rebus Esper platform combined with the Esper High Fidelity assay to identify all major cell types of the mouse brain and faithfully reconstruct the known tissue architecture using spatial data from only 17 cell type-specific genes and 7 reference genes.

Introduction

High-resolution spatial transcriptomics, a complementary approach to single-cell sequencing

Single-cell RNA sequencing (scRNA-seq) is allowing for the unbiased identification of distinct cell types based on their transcriptomic profiles. However, scRNA-seq protocols require mechanical or enzymatic tissue dissociation to isolate individual cells, thereby compromising the ability to correlate gene expression data with anatomical and morphological information.

A variety of techniques have emerged in recent years to characterize single cells in tissues, specifically through analysis of gene expression. However, these techniques force users into one or more compromises. Ex situ sequencing techniques increase throughput but at the cost of resolution. Single-molecule FISH techniques offer high resolution but low throughput. Still other techniques that use barcoding schemes for FISH or in situ sequencing have relatively high resolution and throughput, but at the cost of dynamic range, sensitivity and specificity.

The Esper High Fidelity assay is ideal for researchers who have done early, discovery-focused experiments and now need to validate their results and further refine their hypotheses. The high specificity and sensitivity of the assay, combined with the resolution and throughput of the Rebus Esper platform, uniquely position it for detection of critical biomarkers and gene signatures, especially in relatively rare cells.

Here we demonstrate the capabilities of the Rebus Esper platform and Esper High Fidelity assay in a whole mouse brain tissue section, showing that the major cell types were accurately identified using the high-quality single-cell data from 24 genes, of which only 17 were cell type-specific. The CellxFeature matrix automatically generated by the system allowed mapping of the annotated cell types back to their original locations in the tissue, revealing the tissue architecture and spatial relationships between cells. Our results are highly consistent with previously published brain atlas data of the main cell type proportions, including small and hard to resolve cells like microglia.

Methods

A fully integrated, automated workflow for spatial transcriptomics

Sample Preparation

Fresh frozen brain tissue from a 10-week-old mouse was sectioned at 10µm onto a pretreated glass coverslip and fixed with 4% PFA. No additional pre-run sample pre-treatment was necessary. The coverslip was then assembled into the imaging flow cell and loaded onto the Rebus Esper instrument.

Multiplexed smFISH Chemistry

The integrated fluidics system of the Rebus Esper instrument allowed automated use of the Esper High Fidelity assay. Unique primary probes synthesized to each of 24 mRNA targets were hybridized to all target transcripts in one step. The primary probe architecture allows two readout probes to bind each primary probe. A set of three fluorophore-labeled readout probes were used to reveal the gene locations of three genes per cycle and were subsequently neutralized after imaging (1). The cyclic workflow to reveal 15 gene targets is shown in Figure 1, though in the experiment described here eight cycles were used.

Synthetic Aperture Optics Imaging

The Synthetic Aperture Optics (SAO) technology at the heart of the Rebus Esper platform dramatically improves the resolution of a 20X air objective, allowing for high-throughput, high-resolution imaging (2). For each readout cycle, the sample was illuminated by a series of high-resolution light patterns that were created by the interference of excitation laser beams. A series of low-resolution images were then reconstructed using the Esper Process software to generate a single image with resolution equivalent to that of a 100X oil immersion objective. All imaging and processing steps were run automatically, without user action.

Spot Detection and Nuclei Segmentation

For each image, Esper Process spot detection software analyzed both raw and reconstructed data for potential gene transcripts using multiple filtering steps to remove false positives and generate high fidelity data. Single-cell expression data was generated using nuclei segmentation and attribution of detected transcripts to each nucleus using a maximum distance threshold. The tissue-wide CellxFeature matrix is the final output of Esper Process. This single file containing the number of transcript copies for each gene for each cell was used for subsequent clustering analysis.

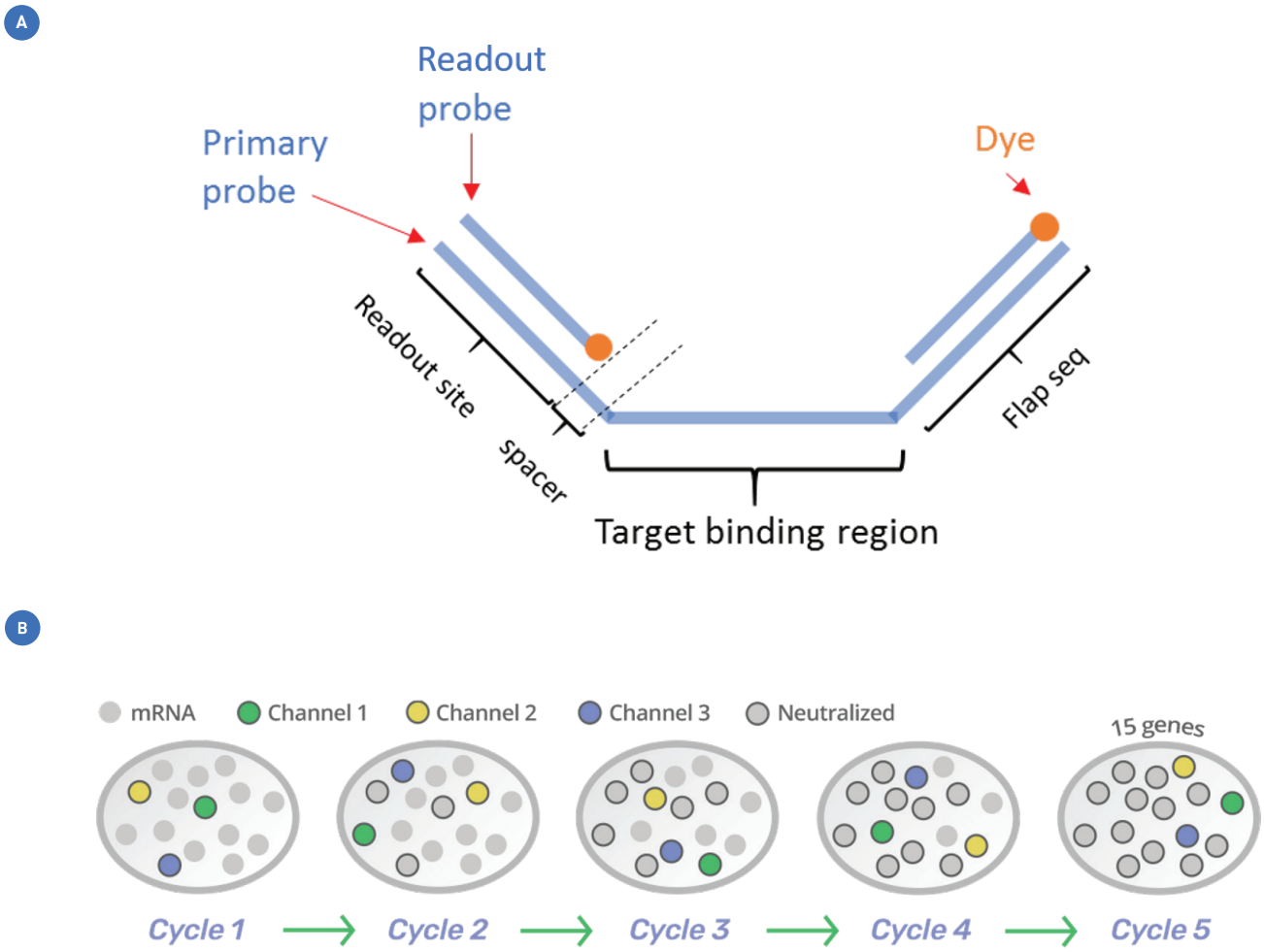

Figure 1. Esper High Fidelity: Optimized smFISH chemistry for cyclic detection of RNA targets.

A, Probe architecture allows two readout probes to bind each primary probe, **B**, During each cycle, a set of three of fluorophore-labeled secondary readout probes are used to reveal transcript locations and are subsequently neutralized after imaging. Representative example of cyclic chemistry demonstrating analysis of 15 genes (5 cycles X 3 genes each cycle).

Results

High-quality data that can be used for cell type identification and mapping

RNA Detection

A full experiment, from flow cell assembly to the probing of 24 genes in eight cycles, was completed within two days for a section whole mouse brain measuring approximately 50mm^2. In total, 1.4x10^7 transcripts were detected from 120,605 cells across 300 fields of view. Single RNA molecules were consistently detected with good signal strength, sensitivity, and specificity (Figure 2).

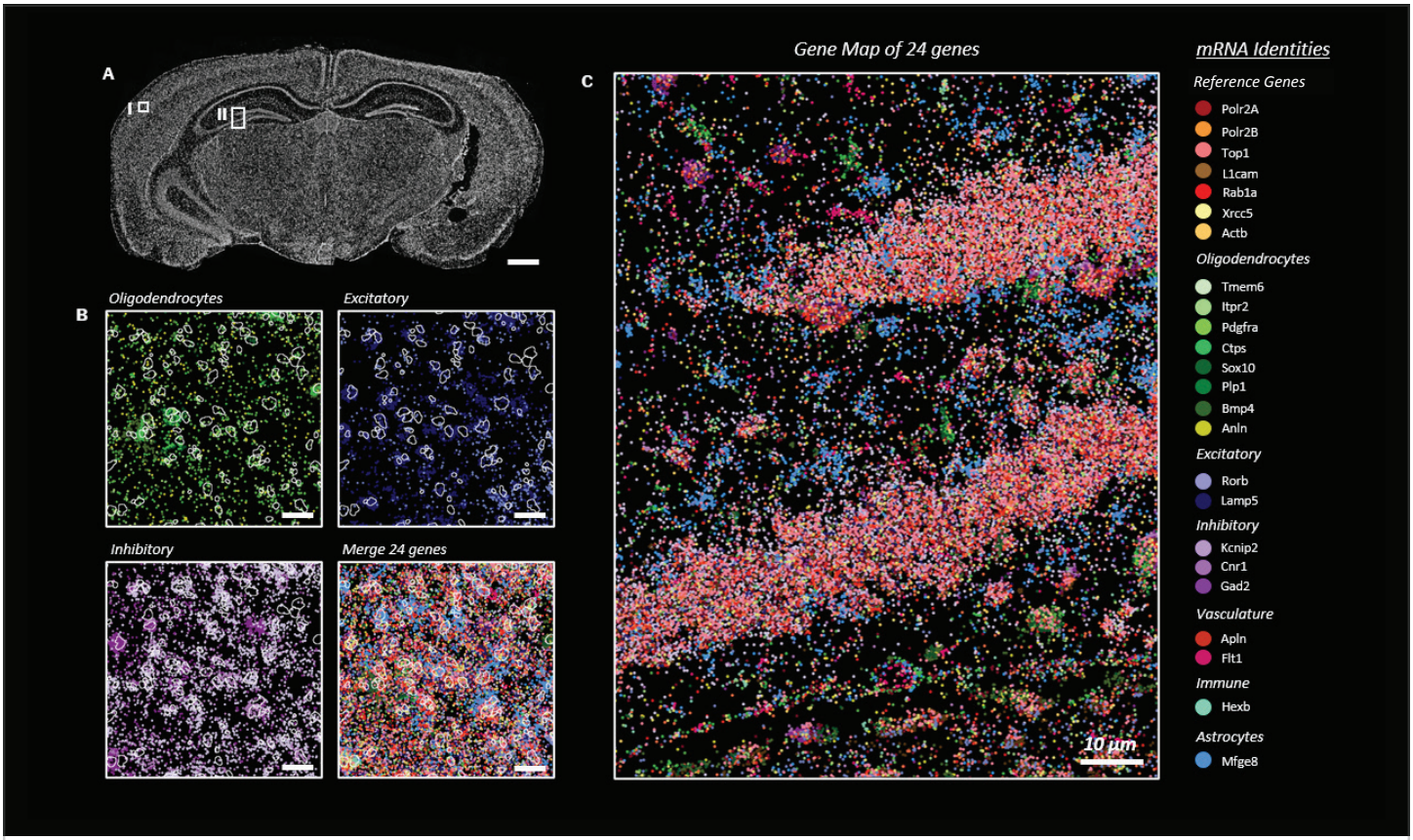

Figure 2. Full mouse brain section displaying mRNA positions of 24 genes.

A, DAPI-labeled nuclei of a coronal section (Bregma = -1.85) from a 10-week-old mouse brain. **B**, Close-up views of brain region I indicated by white rectangle in A. Nuclear boundaries are highlighted and mRNA transcript locations corresponding to gene groups or entire 24 gene panel, as indicated at right, are shown. Each colored dot represents a single transcript. **C**, Close-up view of region II indicated in A showing mRNA locations of all 24 genes.

Clustering and Cell Type Mapping

Dimensionality reduction and unbiased clustering was carried out using Leiden and UMAP algorithms with the Python package Scanpy. Cell type identities were annotated manually based on marker gene expression, resulting in 18 distinct types based on the expression levels of the 24 measured genes (Figure 3A-C).

When cell identities were plotted in X,Y space, the expected cellular organization of the neocortex, archicortex (hippocampus & dentate gyrus), palocortex (amygdala & olfactory cortex), thalamus and hypothalamus were apparent. White matter (green oligodendrocytes) and structures like the ependymal layer and choroid plexuses were also evident (Figure 3D). Excitatory neuron subtypes revealed the layered structure of the cortex (Figure 3E), while inhibitory neurons were more evenly distributed (Figure 3F). Among non-neuronal cells, oligodendrocytes (green) concentrate in the white matter regions while astroglia (blue) are scattered throughout the gray matter. (Figure 3G).

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Comparison to Previously Published Data

The direct detection of transcripts enabled by the Esper High Fidelity assay achieves lower levels of drop-outs, fewer false positives, and a higher mRNA capture efficiency compared to typical droplet-based single-cell RNA sequencing methods. In this application note, we demonstrated the ability of this assay combined with the Rebus Esper platform to resolve the expected densities of the different cell types in the mouse brain using spatial transcriptomic information from only 17 cell type-specific genes. The data presented here are highly consistent with previously published atlas data (3) (Figure 3H). In addition to confirmation of gene expression levels and cell type composition, the multi-scale imaging enabled by the Rebus Esper instrument provides information about broad tissue architecture and the spatial relationships between cells.

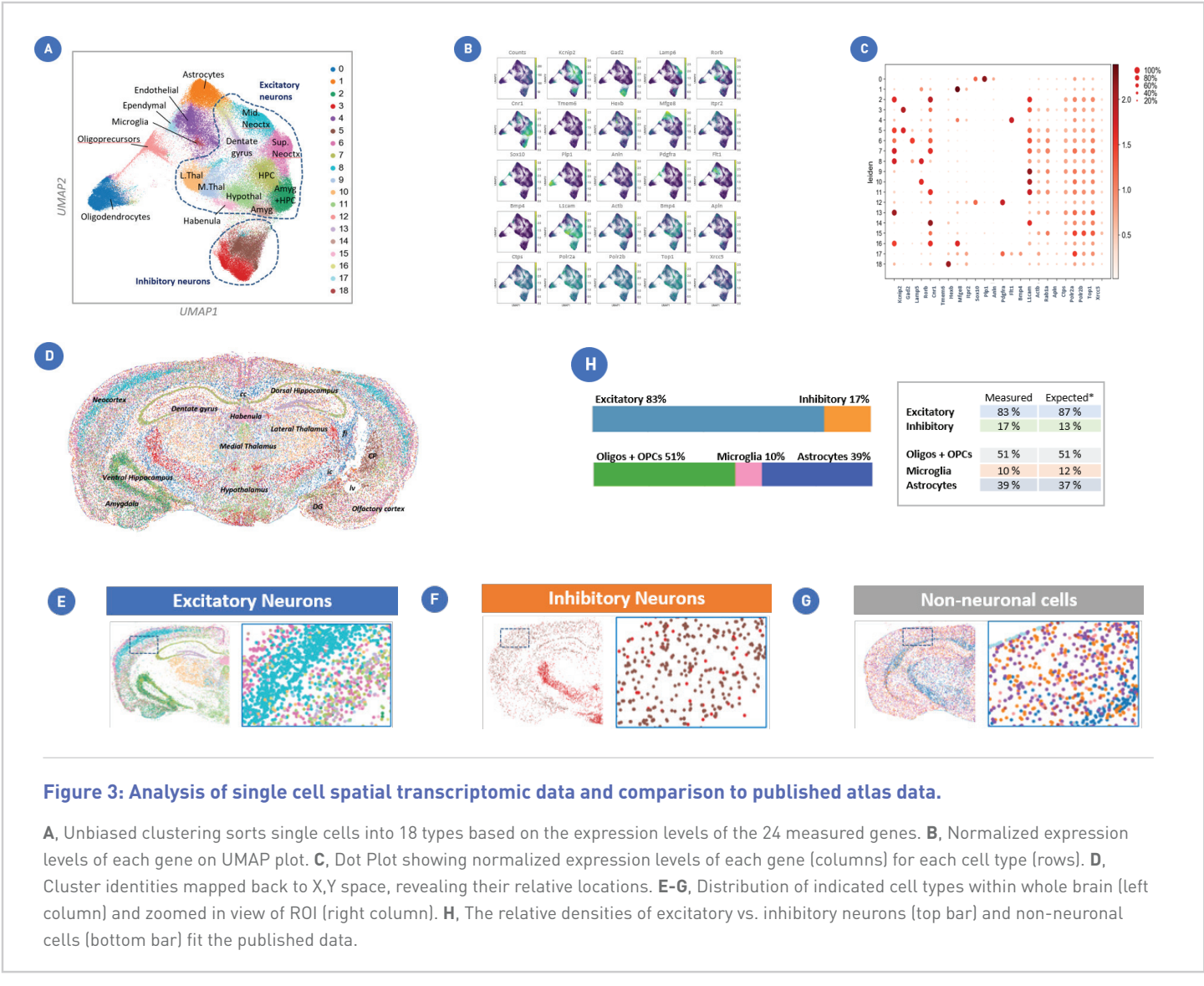

Figure 3: Analysis of single cell spatial transcriptomic data and comparison to published atlas data.

A, Unbiased clustering sorts single cells into 18 types based on the expression levels of the 24 measured genes. **B**, Normalized expression levels of each gene on UMAP plot. **C**, Dot Plot showing normalized expression levels of each gene (columns) for each cell type (rows). **D**, Cluster identities mapped back to X,Y space, revealing their relative locations. **E-G**, Distribution of indicated cell types within whole brain (left column) and zoomed in view of ROI (right column). **H**, The relative densities of excitatory vs. inhibitory neurons (top bar) and non-neuronal cells (bottom bar) fit the published data.

References

1. Codeluppi et al. Spatial organization of the somatosensory cortex revealed by cyclic smFISH. Nature Methods, 2018.
2. Ryu et al. Multibeam interferometric illumination as the primary source of resolution in optical microscopy. Applied Physics Letters, 2006.
3. C. Ero et.al. In An Atlas for the Mouse Brain. Front. Neuroinform., 2018.

2255 Martin Avenue, Ste. F
Santa Clara, CA 95050 USA

www.rebusbio.com
info@rebusbio.com