Dissecting neurodegeneration mouse models with an automated high-plex spatial platform

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ABSTRACT

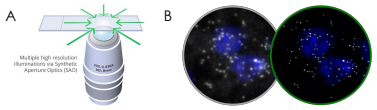
Single-cell RNA sequencing (scRNA-seq) has fundamentally expanded our understanding of tissue composition and heterogeneity. While this methodology enables unbiased and comprehensive identification of cell types based on their transcriptomic profiles, it does not provide positional information in the tissue architecture context.

Rebus Biosystems has developed a highly automated platform that combines Synthetic Aperture Optics (SAO), fluidics engineering and data processing to enable multiplex analysis of gene targets across large tissue sections with minimal user intervention. Here, we used the Rebus Biosystems platform to quantify expression levels of 31 target genes across three experimental conditions.

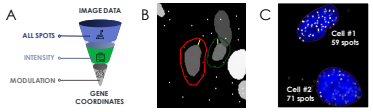
First, we obtained fresh frozen mouse brain sections from two transgenic lines modeling a neurodegenerative disease and a third wild-type line serving as a control. We mounted one section from each of the three conditions (genotypes) on the same glass coverslip. Sections mounted together corresponded to the same antero-posterior levels of the hippocampus and adjacent somatosensory cortex. We ran nine of these three-section arrays to acquire single mRNA molecule quantitative data for 31 genes using on-system cyclic single-molecule RNA fluorescence in situ hybridization (smFISH) chemistry. Detected mRNA spots were automatically assigned to individual DAPI-stained nuclei to render a single-cell gene-expression matrix. Like with scRNA-seq approaches, cell-type associated genes were used for unbiased classification of single cells into several distinct neuronal and glial cell types. Then, the spatial information was used to further dissect each cell type into distinct anatomical compartments and explore spatial relationships between cells.

The expression levels of disease-associated genes were compared across experimental conditions, for each cell type and anatomical structure individually. The combination of single cell gene expression and spatial data allows for fine dissection of tissue heterogenicity, while the balanced tissue-array design minimizes technical variability and enables powerful statistical comparisons

SYNTHETIC APERTURE OPTICS

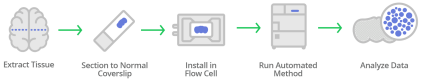


The SAO Concept. The resolution of a 20X air objective is dramatically improved by using SAO technology. **A**, The sample is illuminated by a series of structured light pairs (green arrows) that are created by the interference of excitation laser beams. **B**, The series of low-resolution images are then reconstructed using proprietary software to generate a single image that has equivalent resolution of a 100X oil immersion objective. This combination of illumination and reconstruction breaks the long-standing resolution limits of long working distance air objectives and enables large fields-of-view with high resolution.



Spot Detection Software. Processes large scale, whole tissue image data sets and quantifies gene counts and locations. **A**, The software's algorithm analyzes both the raw and reconstructed images to detect mRNA spots, using multiple filtering steps to remove false positives and generate high fidelity quantitative data. **B,C**, Individual cells are segmented using nuclear staining images and transcripts are assigned using distance thresholds to generate a spatially annotated single cell gene expression matrix.

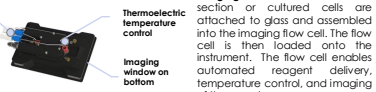
WORKFLOW



Spatial Genomics Workflow. Once genes of interest are selected, primary probes are designed for each gene and loaded to the imager. Fresh-frozen tissue is sectioned and fixed onto functionalized glass and assembled into the imaging flow cell using included gaskets. The flow cell is installed to the imaging platform by connecting the cooling and reagent lines. The instrument runs an automated method that includes optional tissue pre-treatment steps, followed by hybridization of all primary probes in one step. Subsequent cycles of reagent delivery, imaging, and neutralization of 3 genes per cycle are performed until all genes are imaged. Raw images are further processed for individual cell segmentation and transcripts quantification, using Rebus' proprietary software.

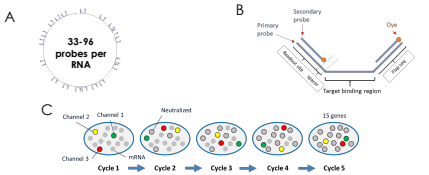
HARDWARE

Rebus Biosystems' Spatial Omics Imaging Platform is a fully-integrated platform for multiplex analyte detection. The platform is equipped with a DAPI LED and laser lines for multiplexing 3+ analytes per cycle, with the SAO-enabled optics to rapidly image large areas at high resolution. Coupled with automated fluidics, the platform enables a multitude of assays for macromolecule detection.



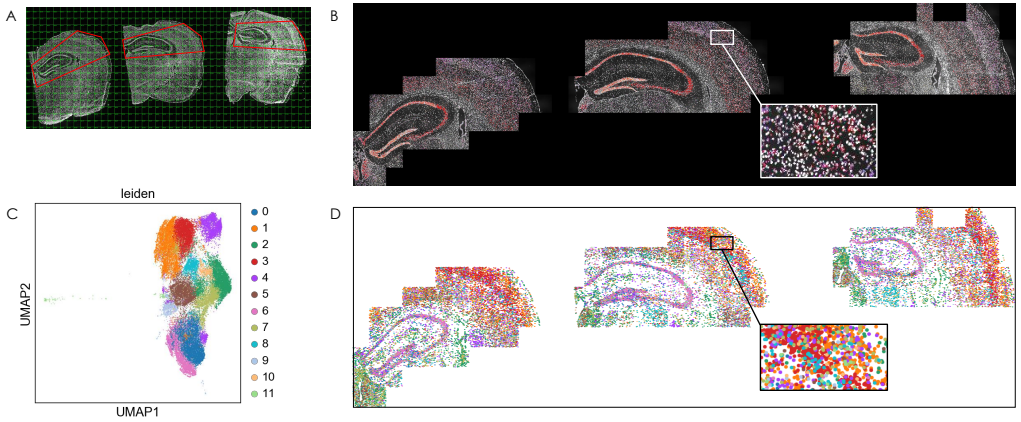
Imaging Flow Cell. A tissue section or cultured cells are attached to glass and assembled into the imaging flow cell. The flow cell is then loaded onto the instrument. The flow cell enables automated reagent delivery, temperature control, and imaging of the sample.

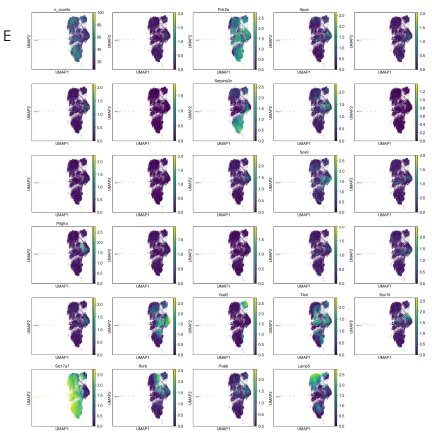
CHEMISTRY



Chemistry to Support Quantification of Highly Multiplexed in situ Data. Unique primary probes are synthesized to each mRNA target with between 33-96 primary probes hybridizing to each target transcript, depending on the length of the mRNA exon. **A**, The probe architecture allows two secondary probes to bind each primary probe. **B**, A set of three of fluorophore-labeled secondary readout probes are used to reveal the gene locations and are subsequently neutralized after imaging. **C**, The cyclic workflow to reveal 15 gene targets is shown. Each RNA is read once with a set of 3 readout probes until all genes are imaged.

COMPARING CELL TYPE COMPOSITION AND GENE EXPRESSION LEVELS BETWEEN THREE CONDITIONS





Single cell gene expression data allows unsupervised cell-type clustering

A, Image of DAPI staining from a single coverslip containing the three mouse sections (corresponding to three different genotypes). The image is a composite of individual fields of view, indicated by the green grid. The red outlines represent the selected regions of interest for subsequent subcellular cyclic smFISH. The complete automated method, including sample pretreatment, all hybridizations, imaging, and washing was 32 hours. A single z-plane of 20X depth of field (~3µm) was acquired for each field of view. **B**, Detected mRNA spots for 31 genes in the hippocampus and cortical regions. The RNA spots are color coded by gene and overlayed on the DAPI image. **C**, Unsupervised clustering of single cells. RNA spots were assigned to individual cells (identified using DAPI images) and the gene expression matrix was processed using Scanpy to generate a UMAP plot, annotated using Leiden algorithm. Each dot corresponds to one cell, color coded by cluster identity. **D**, Plotting the annotated cells back to the XY space reconstructs the tissue organization and displays the specific distribution of cell types. **E**, Expression levels for each gene visualized on UMAP plot show that most clusters correspond to specific cell types (some gene names are omitted due to disclosure agreements).

Conclusions

Rebus Biosystems has demonstrated a robust and automated method for detecting up to 30 gene targets via smRNA FISH with subcellular resolution across large tissue sections. Unlike previous methods, these technology improvements ensure no compromise is made in choosing between speed or resolution when conducting experiments in spatial transcriptomics. Here we show a practical application of the system.

In this study, we demonstrate the platform's ability to accurately identify the main cell types of the adult mouse brain using high quality single cell data from 31 genes.

The XY mapping of annotated clusters allow for further dissection of the individual cell types by anatomical structure (For example, cell type 1 in hippocampus vs cell type 1 in cortex).

Differences in gene expression between the three genotypes (the three sections) can be quantified for each cell type and anatomical structure. As the three sections were run in parallel, technical variation is minimized, increasing the statistical power and reducing bias.

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