Validating scRNAseq data from human fetal brain using automated, highly-multiplexed *in situ* mRNA detection with single-cell and single-transcript resolution

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Abstract

Background: Single-cell RNA sequencing (scRNAseq) has fundamentally expanded our understanding of tissue composition and heterogeneity. While this methodology enables unbiased and comprehensive identification of distinct cell types based on their transcriptomic profiles, it does not provide positional context in the tissue architecture, which is critical for understanding the interactions between cells and their native tissue microenvironment. Spatial validation of scRNAseq data using in situ techniques enables investigation of tissue-specific expression patterns. Emerging technologies that provide genome-wide views of gene expression can aid in screening but cannot provide data on native gene expression from full tissue areas at single cell resolution. Rebus Biosystems has developed an automated platform that combines Synthetic Aperture Optics (SAO), fluidics engineering, and single-molecule RNA Fluorescence In Situ Hybridization (smRNA FISH) chemistry to probe 30+ gene targets across large tissue sections with the ability to resolve and count individual transcripts in single cells. Here, we describe a demonstration of the technology platform to visualize and quantify spatial gene expression of 15 genes in the developing human fetal brain.

Methods: A panel of genes identified by single-cell transcriptomics was selected according to their specificity and expression level in order to map all major cell types and most cell subtypes. Assays consisting of multiple target probes and corresponding fluorescent readout probes were designed for each gene. A fresh frozen human fetal brain was sectioned to a glass coverslip, fixed, and assembled into the imaging flow cell. Target probes for all genes were hybridized in one step. Readout probes for 3 genes were then hybridized, imaged, and removed during automated readout cycling. SAO enabled imaging of entire tissue sections with comparable lateral resolution to a 100x oil immersion lens while achieving more than an order or magnitude improvement in throughput.

Results: A full experiment, from flow cell assembly to the probing of 15 genes in five cycles was completed within one day for a whole human fetal brain section. Single RNA molecules were consistently detected with good signal strength, sensitivity, and specificity. Target RNA molecules were counted and assigned to nuclei with customized algorithms for downstream clustering and mapping of cell types to XY space.

Conclusions: The results obtained with this robust, automated platform enabled validation of functionally distinct cell populations identified by scRNAseq and further elucidate the spatial distribution of developmental gene expression programs at the single cell level. This integrated platform provides a powerful new tool for expansion into long non-coding RNAs, introns, and proteins.

Integrated Fluidics and Optics

Figure 1. Rebus Biosystems' Spatial Omics Imaging Platform is a fully-integrated platform for multiplex analyte detection. **a**. The platform is equipped with a DAPI LED and laser lines for multiplexing 3+ analytes per cycle with automated fluidics. **b**. The tissue or cell sample is loaded into the flow cell and connected to integrated fluidics. **c**. SAO-enabled optics rapidly image large areas at high resolution. Together this platform enables a multitude of assays for macromolecule detection.

Automated smFISH Chemistry

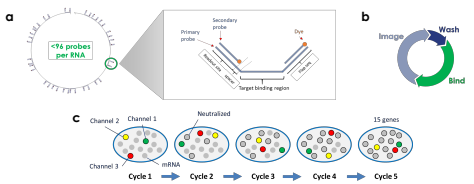

Figure 2. Optimized Cyclic smFISH Chemistry to Support Quantification of Highly Multiplexed in situ Data. a. Unique primary probes are synthesized to each mRNA target with <96 primary probes hybridizing to each target transcript, depending on the length of the mRNA exon. The probe architecture allows two secondary probes to bind each primary probe. **b**. A set of three fluorophore-labeled secondary readout probes are used to reveal the gene locations and are subsequently neutralized after imaging. **c**. Each RNA is read once with a set of 3 readout probes until all genes are imaged. The cyclic workflow to reveal 15 gene targets is shown.

Optimized Image Processing

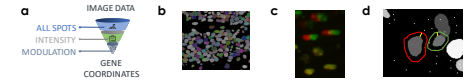

Figure 3. Fully automated image processing generates Cell x Feature Matrix for downstream clustering analysis and precise spatial mapping. Processes large scale, whole tissue image data sets and quantifies gene counts and locations. **a**. The software's algorithm analyzes both the raw and reconstructed data for gene transcripts using multiple filtering steps to remove false positives and generate high fidelity data that result in the detection of individual transcripts. **b**. Accurate nuclei segmentation in high cell density areas is enabled by deep learning. **c**. Images from each cycle are registered within 1µm. **d**. Single cell expression data is generated by attributing detected RNA FISH spots to each nuclei using an adjustable perimeter threshold.

Targeted Spatial Transcriptome of the Human Fetal Brain

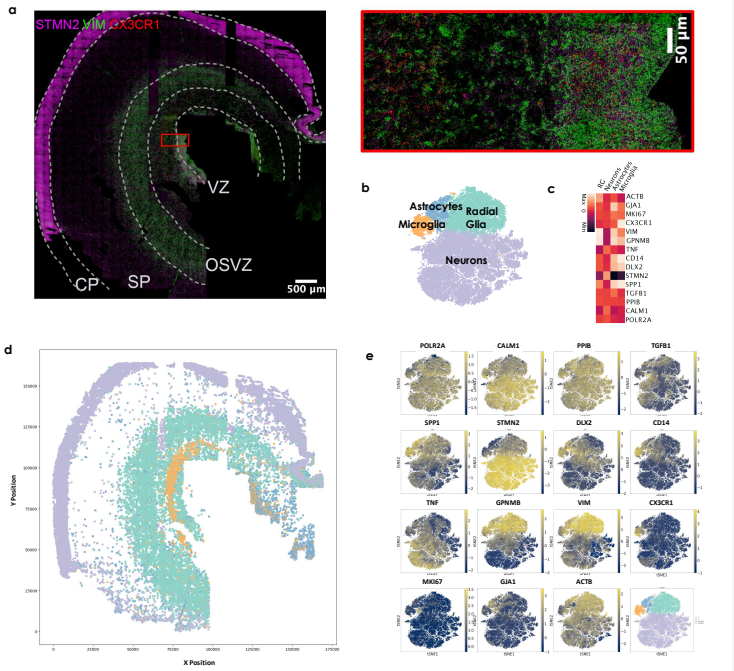

Figure 4. Single cell spatial transcriptome of the developing human cortex.
a. Composite image of a human gestational week 20 (GW20) somatosensory cortex showing spatial gene expression of STMN2 (purple), VIM (green) and CX3CR1 (red). Dotted lines show borders of different cortical regions. CP, cortical plate; SP, subplate; OSVZ, outer subventricular zone; VZ, ventricular zone. The red bounding box shows a zoomed-in section rotated 90° on the right. **b**. tSNE visualization of cell clusters based on distinct expression profiles of different marker genes. In the first round, all cells were segmented based on DAPI, identified based on gene expression level and then clustered to reveal major cortical cell types. **c**. Heatmap visualization of four main cell types with distinct expression profiles. **d**. clusters mapped backed to XY space. **e**. relative expression maps for each gene shown on the cluster plot.

Conclusions

Rebus Biosystems has demonstrated a robust and automated method for detecting 30 gene targets via smRNA FISH with sub-cellular resolution across large tissue sections. Unlike previous methods, these technology improvements ensure no compromise is made in choose between speed or resolution when conducting experiments in spatial transcriptomics.

In this study, this robust, automated platform enabled validation of functionally distinct cell populations identified by scRNAseq and further elucidated the spatial distribution of developmental gene expression programs at the single cell level.

Using the spatially annotated Cell X Feature Matrix, we were able to dissect the main cell types present in the human fetal brain, map their spatial distribution, and resolve the expression gradients of key genes involved in development.

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Disclosures

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