# Whole-brain spatial validation of single-cell gene expression with sub-micron resolution

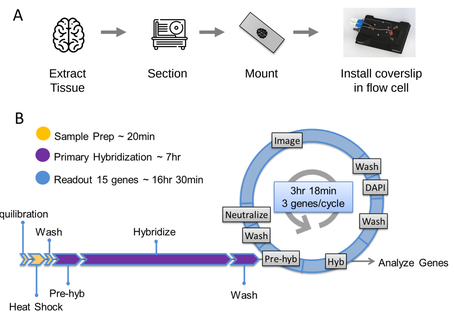
**Irene Oh[1],** Josh Ryu[1], Raymund Yin[1], Hojin Lee[1], Zenjoe Green[1], Aparna Bhaduri[2,3], Matthew Keefe[4,5], Tomasz Nowakowski[4,5,6], Mangyin Matthew Mo[1], Brett Cook[1], Noel Jee[1]

1 Optical Biosystems, Inc. 2 Department of Neurology, University of California, San Francisco, San Francisco, CA, USA, 3 Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA, USA, 4 Department of Anatomy, University of California, San Francisco, San Francisco, CA, USA, 5 Department of Psychiatry, University of California, San Francisco, San Francisco, CA, USA, 6 Chan Zuckerberg Biohub, San Francisco, CA, USA

## ABSTRACT

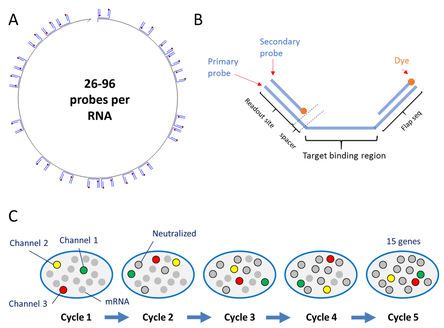
Single-cell RNA sequencing (scRNA-seq) has fundamentally expanded our understanding of tissue composition and heterogeneity. Various scRNA-seq approaches enable comprehensive screening of complete tissues but require tissue dissociation and the removal of individual cells from their biological context. Validation of differential gene expression *in situ* is, therefore, of high interest. Emerging spatial technologies focus on genome-wide expression data *in situ* but lack the depth, resolution, scale, or speed necessary to interrogate spatial gene expression with true single-cell resolution. Furthermore, researchers generally focus on a handful of relevant genes determined from scRNA-seq data or broad spatial screening platforms. Optical Biosystems (OBI) has developed an automated platform that combines Synthetic Aperture Optics, fluidics engineering, and single-molecule RNA fluorescence in-situ hybridization (smRNA FISH) chemistry to probe up to 30 gene targets across a large tissue sections with sub-micron resolution. Here we demonstrate the capabilities of the platform in a human brain tissue section and a mouse brain tissue section. smRNA FISH probes were designed to detect each gene of interest and multiplexed sequentially on each tissue section. Imaging data was collected, processed, and analyzed to quantify individual RNA molecules across 100,000+ single cells in 0.3 – 0.5 $cm^2$ tissue sections. Our imaging data accurately reproduced cell type abundance as determined from the scRNA-seq data and validated the expected spatial location of different cell types within the whole tissue. These data exhibit a powerful and robust automated platform that can translate biological insights from transcriptomic screening experiments into a spatial context and provide a foundation for expansion into long non-coding RNAs, mitochondrial RNAs, introns, splice variants, chromatin structure, and proteins.

## WORKFLOW




**Spatial genomics workflow.** Once genes of interest are selected, primary probes are designed for each gene and loaded to the imager. In contrast to other in situ hybridization workflows, there is no additional sample preparation before loading the sample into the flow cell, and all chemistry is performed inside the instrument. **A,** Fresh-frozen tissue is sectioned and fixed onto functionalized glass and assembled into the imaging flow cell using included gaskets. **B,** After the sample is loaded, the flow cell is loaded onto the imaging platform by connecting the cooling and reagent lines. The instrument runs an automated method that includes a heat-shock step and optional tissue clearing step, followed by hybridization of all primary probes in one step. Automated cycles of reagent delivery and subsequent imaging read out 3 genes per cycle until all genes are imaged. Raw images are further processed for downstream analysis.

## CHEMISTRY

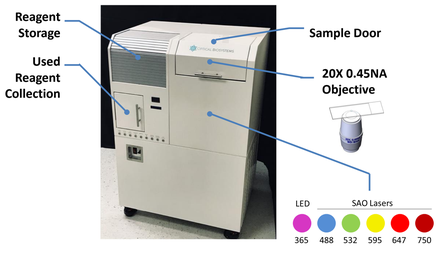



| | | | | | |
|---|---|---|---|---|---|
| Channel 1 | KCNE5 | EOMES | PDGFRA_CD | SATB2_CD | POLR2A |
| Channel 2 | FBLN2_CD | RELN | MKI67_CD | CD38 | PTPRZ1 |
| Channel 3 | LHX5 | NEUROD2 | SOX2 | TBR1 | TOP1 |

**Chemistry to Support Quantification of Highly Multiplexed in situ Data.** Unique primary probes are synthesized to each mRNA target with between 26-96 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.

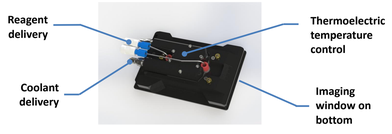
## HARDWARE

### Spatial Omics Imaging Platform



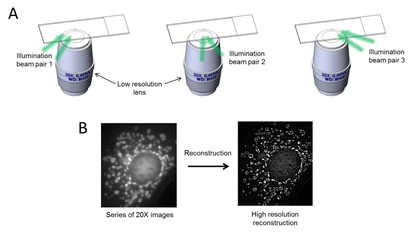

**Optical Biosystems' Spatial Omics Imaging Platform** is a fully-integrated platform for multiplex analyte detection. The platform is equipped with a DAPI LED and laser lines for multiplexing 3+ analytes per cycle, with the SAO-enabled optics to rapidly image large areas. Coupled with automated fluidics,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

### Imaging Flow Cell

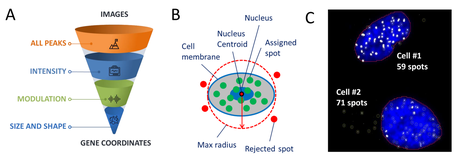



**Imaging Flow Cell.** A tissue section or cultured cells are attached to glass and assembled into the imaging flow cell. The flow cell is then loaded onto the instrument. The flow cell enables automated reagent delivery, temperature control, and imaging of the sample.

## 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 high-resolution 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 is equivalent to the 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 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,C,** Single cell expression data is generated using nuclei segmentation and attributing detected genes to each nucleus using a maximum distance threshold.

## ACKNOWLEDGEMENTS

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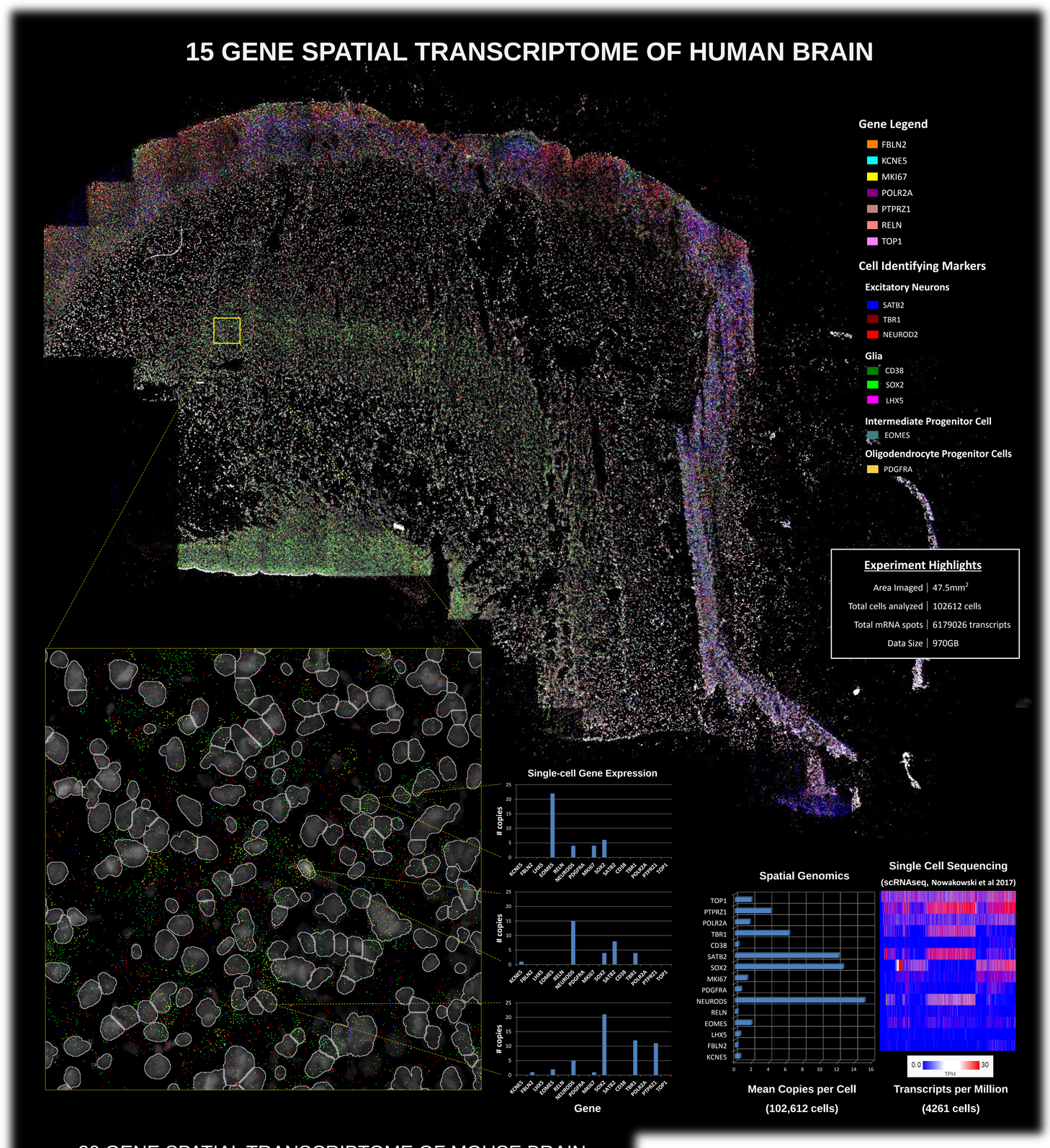
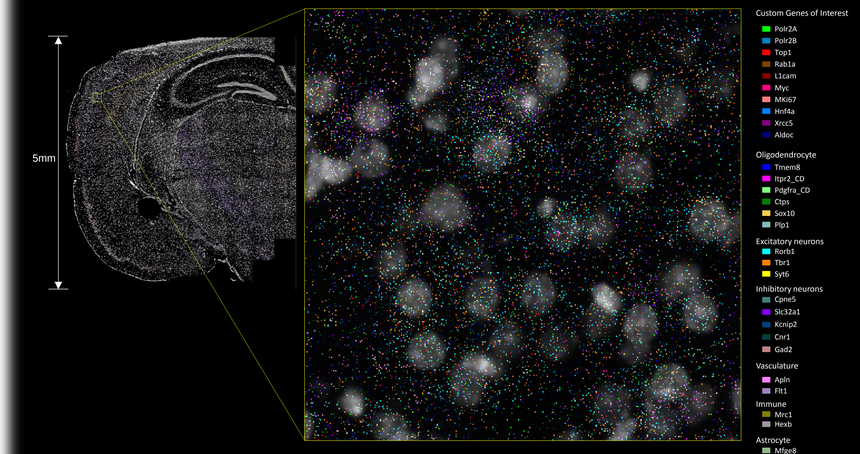
## 15 GENE SPATIAL TRANSCRIPTOME OF HUMAN BRAIN




## 30 GENE SPATIAL TRANSCRIPTOME OF MOUSE BRAIN




A single mouse brain slice was iteratively probed for 30 genes. A subset of the genes were cell-identifying markers.

## FUTURE DEVELOPMENT

Optical 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 that researchers do not have to choose between speed or resolution when conducting experiments in spatial genomics. As the methods undergo further rigorous validation, Optical Biosystems is developing additional features for the near future. Some of these are highlighted below.

**Sample compatibility**
- Formalin Fixed Paraffin Embedded (FFPE) tissues
- High-background tissues
- Multiple organisms

**Target repertoire expansion**
- Proteins
- Additional RNAs, including non-coding RNAs, mitochondrial RNAs, introns, splice variants
- DNA, including chromatin structure

**Assay versatility**
- Higher plex of up to 100 genes
- Broader coverage of the whole transcriptome
- Smaller gene targets

**Analysis tools**
- Software for large-scale visualization and annotation
- Automated spot detection and quantification
- Automated nuclei segmentation