Single-cell Multi-modal Profiling of *In Vitro* Neuronal Networks: Linking Morphology, Function, and Transcriptomics

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INTRODUCTION

Single-cell technologies have revolutionized our understanding of cellular heterogeneity, yet current high-throughput methods face critical limitations. Conventional approaches rely on enzymatic dissociation and harsh microfluidic processing that disrupt functional networks and artificially alter transcriptomic states. These constraints are especially problematic for brain cells relevant to neuro-immunological and neurodegenerative diseases, which are large, fragile, and dependent on intact functional units. We developed a high-throughput, multi-modal platform enabling longitudinal studies (days to weeks) of individual cells, co-cultures, and neuronal networks, followed by endpoint transcriptomics without cell dissociation. The platform captures thousands of cells in CellCage™ Enclosures (CCE), generating linked imaging, functional, and transcriptome data. Flexible CCE designs support diverse strategies—from isolating single cells to capturing somas within intact neuronal networks or configurations supporting axonal outgrowth. Deep-learning morphology quantification, cell tracking, functional assays, and mRNA sequencing are linked across thousands of individual CCE per experiment.

Using this platform, we establish assays combining image-based phenotypic analysis with transcriptomics, including microglia phagocytosis and fluorescent imaging in neurons and astrocytes. Neurons remain functionally intact within networks until lysis, preserving cell-cell interactions lost during dissociation. We also establish glial-neuronal co-cultures where neurons form functional networks first, then microglial cells are introduced to the network to permit glial-neuron interactions such as phagocytic activity and surveillance behaviors while capturing transcriptomic changes in neurons. By enabling transcriptomic study of clinically relevant functions while preserving cell integrity, this platform provides a powerful tool for pathway analysis and target discovery in neuro-immunological research.

A NOVEL MICRO-3D PRINTING TECHNOLOGY ENABLES HIGH-THROUGHPUT EVALUATION OF CELL PHENOTYPES AND TRANSCRIPTOMES

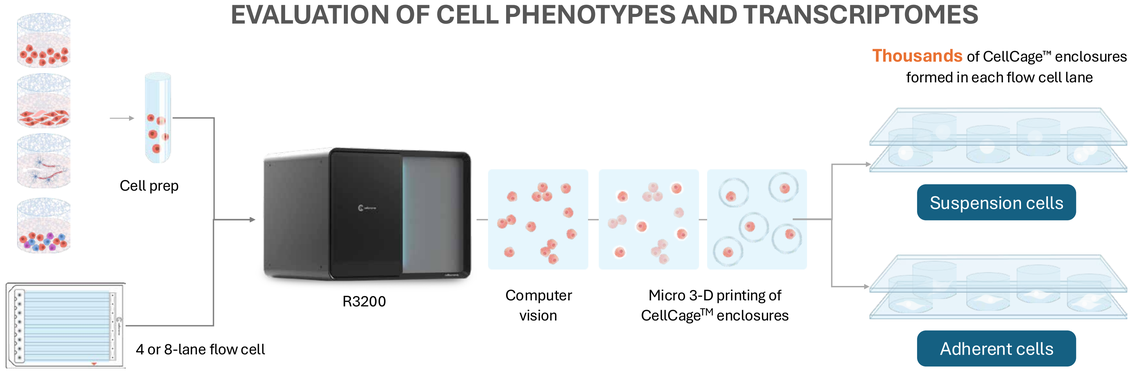
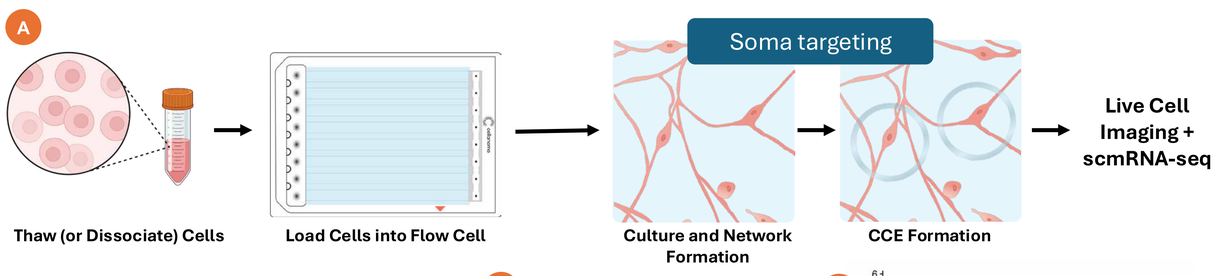
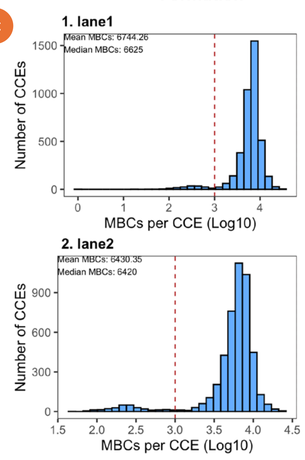
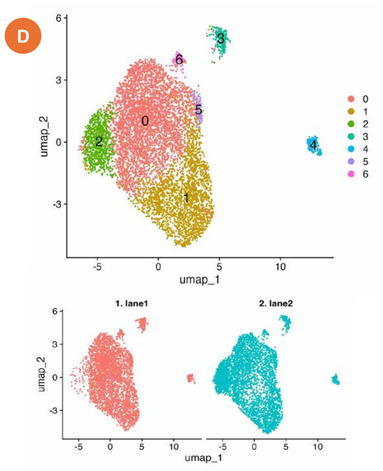


Fig 1. Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. Tens of thousands of cells in suspension are mixed with hydrogel precursor and loaded on a 4 or 8-lane flow cell. Positions of cells are identified and CellCage™ enclosures are generated around cells with light-guided polymerization in an automated fashion. CellCage™ enclosures are permeable to reagents (e.g., small molecules, antibodies) enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells. Cells can be lysed within CellCage™ enclosures to generate barcoded cDNA for downstream library prep and sequencing off the instrument.

CAPTURING INTACT NEURON SOMA IN CELLCAGE™ ENCLOSURES







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CCE-level Summary Statistics

Dataset	CCE Formed	CCE Retained	Percent Recovery	Median MBCs	Median Reads	Median Genes	Mito (%)	Ribo (%)
Lane 1	4,032	3,781	93.8%	6,813	20,545	2,940	8.39	4.79
Lane 2	4,692	4,391	93.6%	6,622	18,168	2,947	6.82	4.87

Fig 2. Using CellCage™ Enclosures to study connected neurons. (A) bit.bio ioGlutamatergic neurons were thawed and loaded into the flow cell. Cells were cultured for 7 days to allow for network formation. On the instrument, cells were automatically detected and CellCage™ Enclosures (CCE) created around individual neuron soma. After they were enclosed, neurons were imaged and transcriptome collected without cell dissociation. (B) Representative image showing 5 neuron soma captured inside of CCE. (C) Quality control of transcriptome data showing high number of molecular barcodes (MBC) collected for the majority of CCE. (D) Merged data from two lanes of neuron data. Top plot shows clusters identified by transcriptomic differences. Bottom plot shows the data points split for each lane. (E) Summary statistics of two lanes of neuron data showing the number of CCE, molecular barcodes, and genes recovered.

NEURON-MICROGLIA CO-CULTURES WITH SINGLE NEURON TRANSCRIPTOME

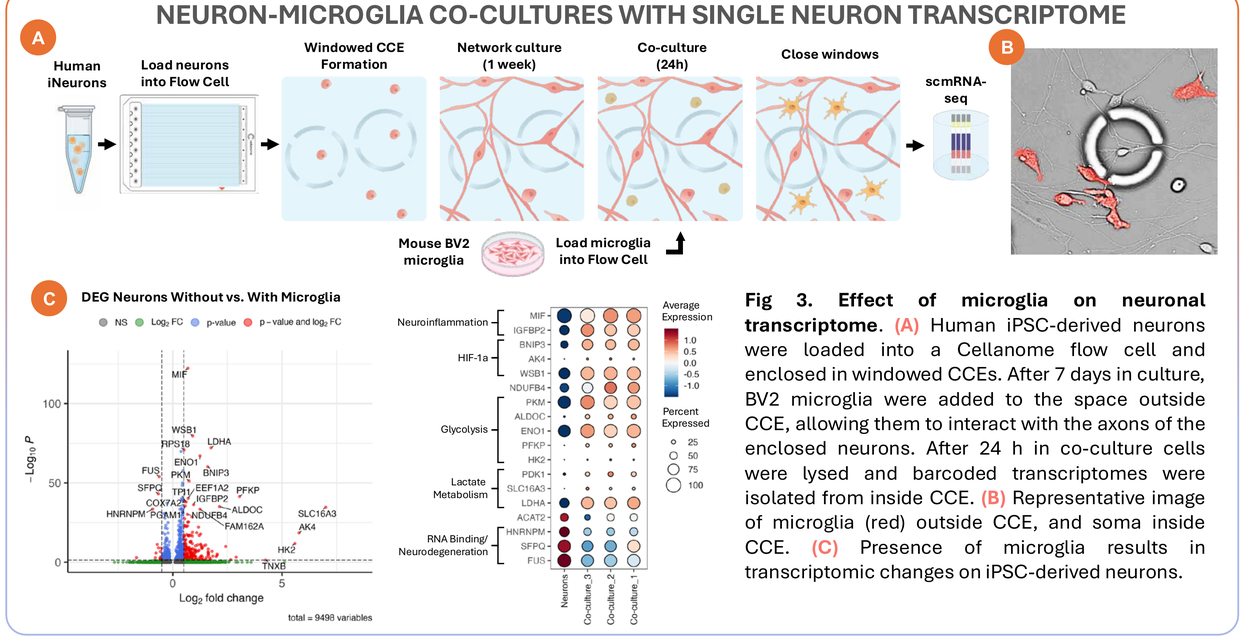

Fig 3. Effect of microglia on neuronal transcriptome. (A) Human iPSC-derived neurons were loaded into a Cellanome flow cell and enclosed in windowed CCEs. After 7 days in culture, BV2 microglia were added to the space outside CCE, allowing them to interact with the axons of the enclosed neurons. After 24 h in co-culture cells were lysed and barcoded transcriptomes were isolated from inside CCE. (B) Representative image of microglia (red) outside CCE, and soma inside CCE. (C) Presence of microglia results in transcriptomic changes on iPSC-derived neurons.

CHANGES IN ASTROCYTE TRANSCRIPTOME AND MORPHOLOGY WITH ACTIVATION

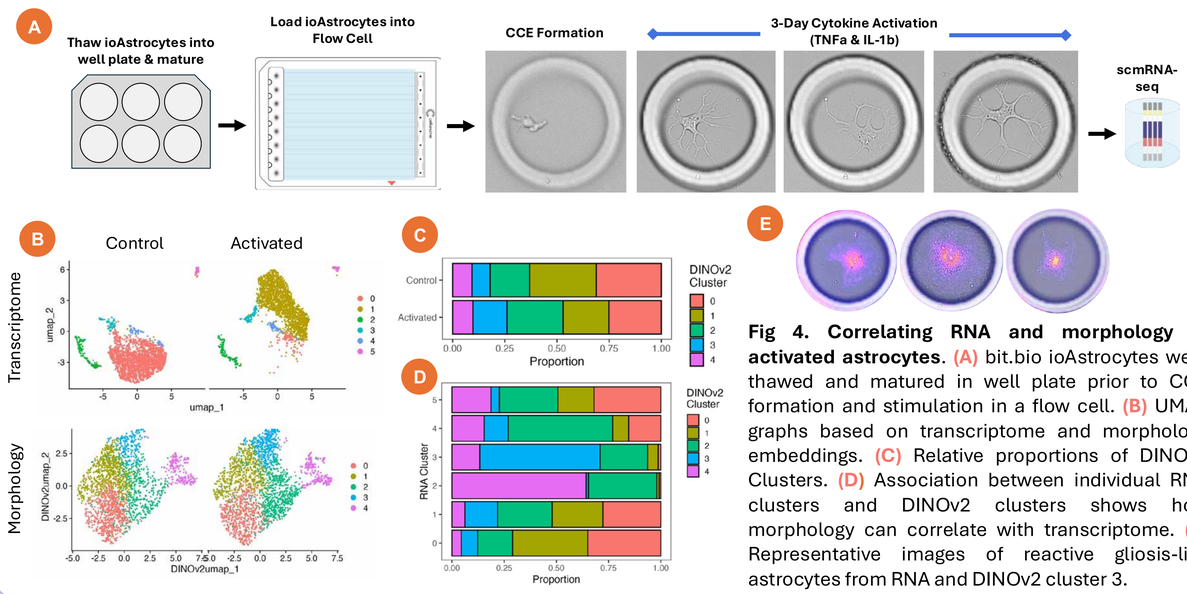

Fig 4. Correlating RNA and morphology in activated astrocytes. (A) bit.bio ioAstrocytes were thawed and matured in well plate prior to CCE formation and stimulation in a flow cell. (B) UMAP graphs based on transcriptome and morphology embeddings. (C) Relative proportions of DINOv2 Clusters. (D) Association between individual RNA clusters and DINOv2 clusters shows how morphology can correlate with transcriptome. (E) Representative images of reactive gliosis-like astrocytes from RNA and DINOv2 cluster 3.

MORPHOLOGY PREDICTS PHAGOCYTOSIS IN MICROGLIA

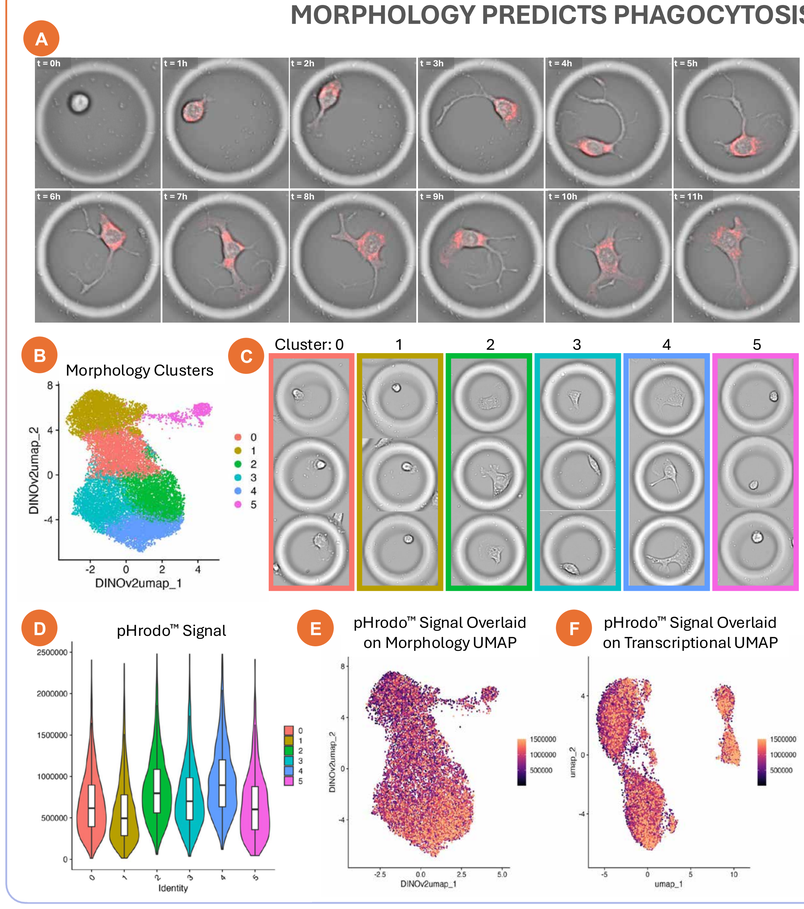

Fig 5. Integration of phagocytosis activity, morphology and transcriptome on BV2 microglial cells. (A) Time-lapse of a single BV2 microglial cell phagocytosing pHrodo™ BioParticles™. Images were acquired every hour over a 12h period. Increase of red signal shows phagocytosis of the particles. (B) Morphology-based clustering of single microglia. DINOv2 feature embeddings were calculated for each CCE and clustered in UMAP space. (C) Representative cells from each cluster in the morphology-based UMAP highlighting cluster differences. (D) Correlation of function and morphology. Violin plot shows the total signal from phagocytosed pHrodo™ BioParticles™ in each morphology-based cell cluster. Cluster 4 demonstrates the highest signal. (E) Visualization of functional phagocytosis readout on a morphology-based UMAP. Same data as in (D) overlaid on the morphology UMAP, showing increased signal in cluster 4 and 2. (F) Visualization of functional phagocytosis readout on a transcription-based UMAP. The same cells as in (E) but clustered based on mRNA readouts. Phagocytosis signal is broadly distributed across all clusters.