Platform for Large-Scale Analysis of Brain Cell Types at Single-Cell Resolution Spanning Longitudinal Multi-Modal Measurements

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INTRODUCTION

Single-cell technologies have revolutionized our understanding of cellular functions and heterogeneity. However, current high-throughput methods face limitations for the study of 1) cells inadequately identified by protein markers, 2) large or fragile cells that lose viability under harsh microfluidic conditions, and 3) cells needing disruption from their functional units for transcriptional analysis. Many brain cell types fall into one or more of these categories. To address, we developed and leveraged a high-throughput, multi-modal platform facilitating the longitudinal (days to weeks) study of individual cells, co-cultures, or neuronal networks without enzymatic perturbation. Single cells or co-cultures can be encircled within CellCage™ enclosures, called CCEs, in which cells can be cultured and imaged for days to weeks to evaluate morphological changes over time due to differentiation, maturation, or proliferation. After, they are processed within CCEs for downstream sequencing (mRNA, CRISPR guides). Notably, this enables the capture of transcriptomics linked to longitudinal data around disease-relevant cellular functions, including phagocytosis, neuron-astrocyte interactions, and calcium signaling from cells that are functionally intact at the time of processing. This technology can become a key tool for unlocking physiologically relevant insights to advance the study of neuro-immunological and neurodegenerative diseases.

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

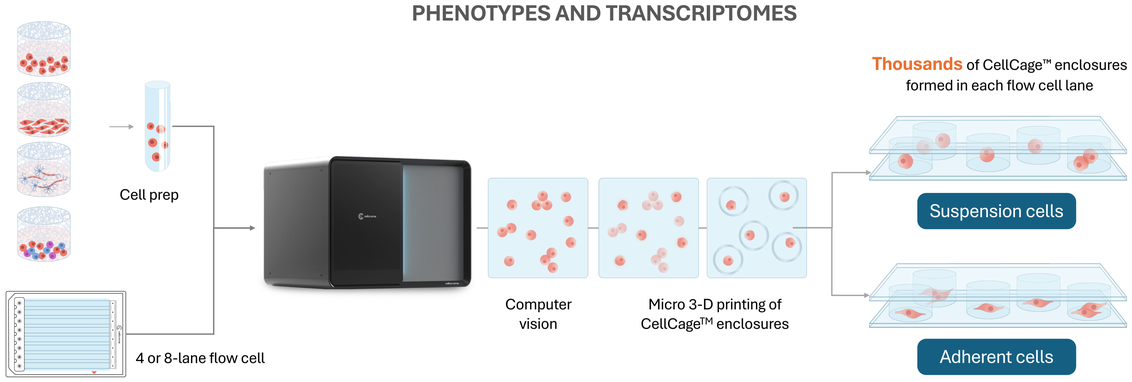


Fig 1. Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. Tens of thousands of cells in suspension are mixed with hydrogel precursor and loaded on a 4 or 8-lane flow cell. Positions of 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.

WORKFLOWS FOR STUDYING NEURONS IN CELLCAGE™ ENCLOSURES

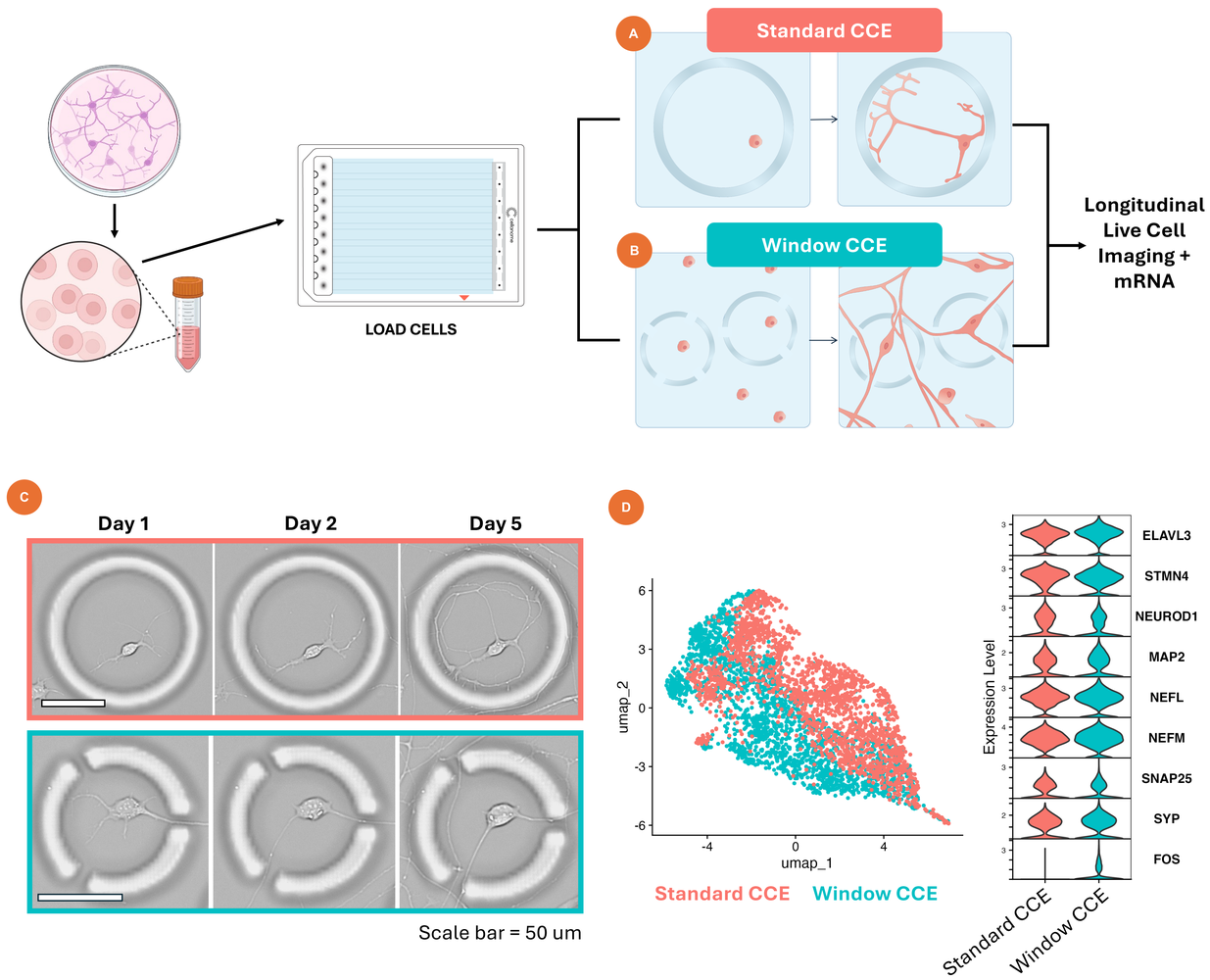


Fig 2. Using CellCage™ enclosures (CCE) to study isolated or connected neurons. (A) Dissociated neurons are loaded into the flow cell in a hydrogel precursor mix. On the instrument, cells are automatically detected and CellCage™ enclosures are created around individual neurons. Neurons are then incubated to allow for attachment and neurite extension within the CCE. (B) Same as (A), but the CellCage™ enclosures have openings that allow for neuronal outgrowth and network formation. (C) After they are enclosed, neurons can be imaged over time using brightfield and fluorescent imaging. (D) At the end of the experiment, the transcriptome of these neurons / somas can be isolated and linked to the imaging data. Single-neuron mRNA analysis shows separate clustering of neurons in standard CCEs (whole cell, isolated) vs window CCEs (soma, connected). Right panel demonstrated expression of neuronal genes in both conditions and upregulation of FOS in connected neurons.

MULTISPECIES NEURON-ASTROCYTE CO-CULTURES IN CELLCAGE ™ ENCLOSURES

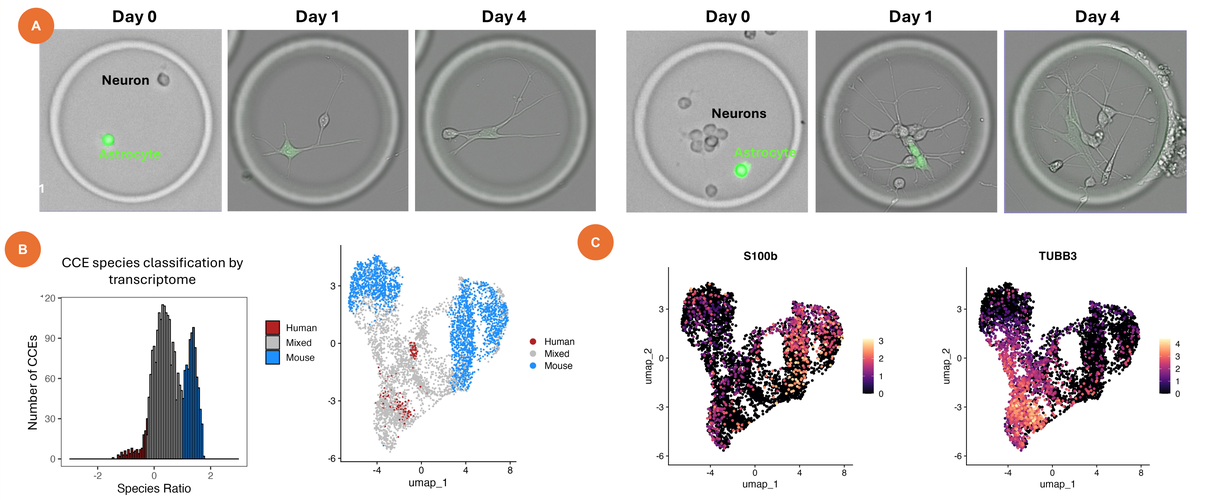


Fig 3. Linked image and transcriptome analysis astrocyte-neuron co-cultures. (A) Mouse astrocytes were labeled with CFSE dye (green) and mixed with human iPSC-derived neurons before loading them into a Cellanome flow cell. CCEs were formed using fluorescent targeting of astrocytes. Single astrocytes were encircled with a Poisson distribution of neurons, as "passenger cells," enabling co-cultures to span a distribution of astrocyte:neuron ratios from 1:1 to 1:8. (B) After 4 days in culture, cells were lysed and barcoded transcriptomes were isolated from each CCE. Transcriptome data can be matched to imaging data, resulting in a linked, multi-modal dataset. Gene expression analysis enables deconvolution of astrocyte and neuron data, using mouse and human genes. (C) Mouse transcripts express astrocyte markers (S100b, left), while human transcripts include neuronal makers (TUBB, right).

PHAGOCYTOSIS AND MOPHOLOGY-BASED CLUSTERING ON MICROGLIAL CELLS

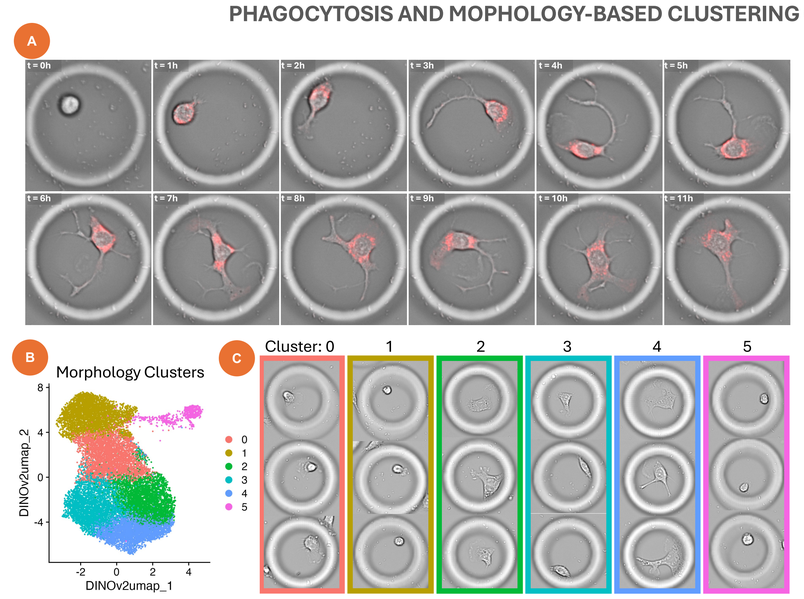
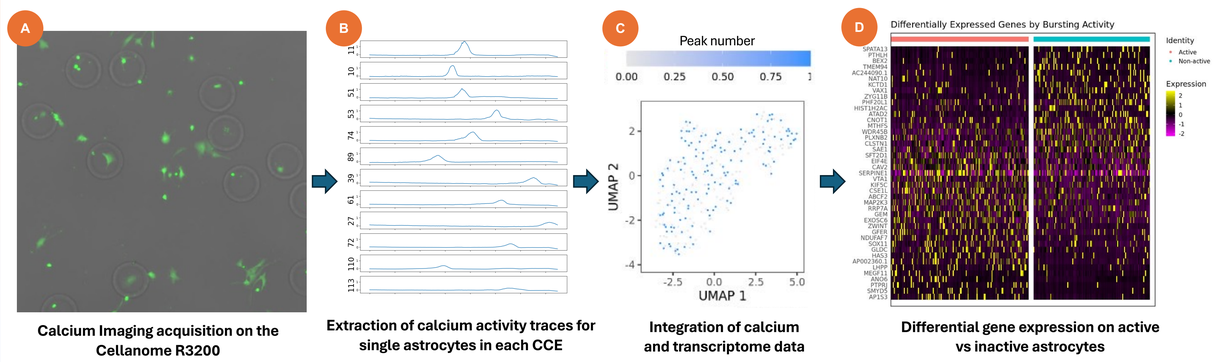


Fig 4. Integration of phagocytosis activity, morphology and transcriptome on BV2 microglial cells. (A) Time-lapse of a single BV2 microglial cell phagocytosing pHrodo™ BioParticles™. Images were acquired every hour over a 12h period. Increase of red signal shows phagocytosis of the particles. (B) Morphology-based 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.

INTEGRATED CALCIUM IMAGING AND TRANSCRIPTOME DATA FROM SINGLE ASTROCYTES IN CCEs



Calcium Imaging acquisition on the Cellanome R3200

Extraction of calcium activity traces for single astrocytes in each CCE

Integration of calcium and transcriptome data

Differential gene expression on active vs inactive astrocytes

Fig 5. Acquisition of calcium imaging and transcriptome data from single astrocytes in CCEs. (A) iPSC-derived astrocytes were enclosed in CCEs and imaged using Fluo4-AM calcium dye. Each FOV was imaged at 1.3 Hz for 5 minutes. (B) Calcium imaging data was processed to extract activity traces for single astrocytes in each CCE. (C) After calcium activity was measured, scRNA-seq data was collected from the same single cells, enabling linked scRNAseq and calcium activity data to be collected from the same cell. (D) Differential gene expression analysis was performed comparing active vs inactive astrocytes.