Developing a novel technology converging single-cell transcriptomics with high-resolution, live cellular behaviors at high throughput

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INTRODUCTION

Understanding cellular functions requires longitudinal evaluation of single cells receiving cues from the environment or other cells. However, studies around these phenomena are bottlenecked – current single-cell and spatial technologies capture static snapshots, and are not integrated with direct analysis of live, single-cell behaviors. Here, we report a novel technology with preliminary results demonstrating longitudinal assessment of live cellular behaviors, including cell-cell interactions, and generation of single-cell RNA-sequencing data integrated with cytokine secretion at high throughput.

LEVERAGING CELLANOME TECHNOLOGY FOR MULTI-MODAL PROFILING OF CELLS

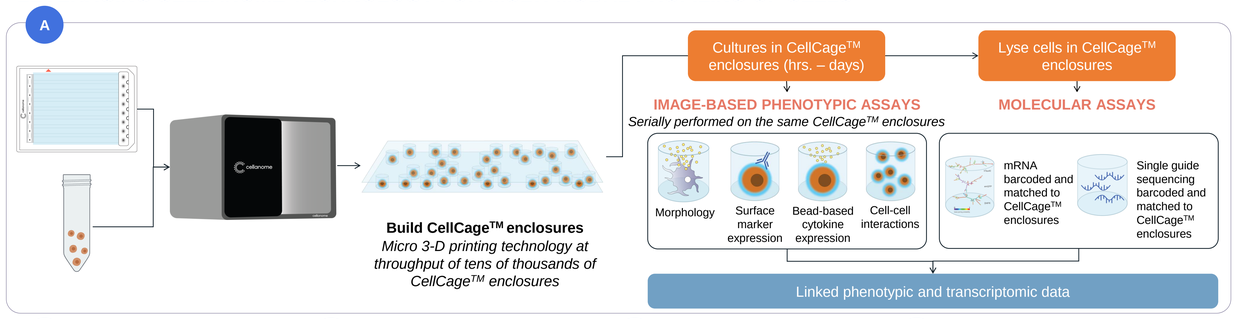


Fig 1. Cellanome's Technology enables the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures
(A) Tens of thousands of cells are mixed with hydrogel precursor and loaded on an 8-lane flow cell. Cell positions are identified and CellCage™ enclosures (CCEs) automatically generated around cells with light-guided polymerization. Bio-compatible CCEs can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine beads). CCEs are permeable to reagents enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells (e.g., small molecules, immunofluorescent antibodies). Cells are lysed within CCEs to generate positionally barcoded cDNA for downstream library prep and sequencing off the instrument. **(B)** Fluorescent imaging of the same NK-92 cell in CCEs following staining with α-CD7-FITC, α-CD56-PE, α-CD45-CY5 and DAPI. **(C)** Brightfield imaging of activated human T cells and NK-92 cells on days 0, and 2 of culture in CCEs. **(D)** Cellanome technology is compatible with diverse cell types, including adherent cells. Images represent a examples of adherent cell types tested to be viable on Cellanome's flow cells. **(E)** Flow cells for sequencing experiments are designed to be compatible with suspension and adherent cells by barcoding either the bottom (suspension) or top (adherent) surfaces. **(F-i)** CCEs are formed across entire flow cell lanes at the scale of ten of thousands. **(F-ii)** In a single representative field of view (FOV), overview of how imaging data is linked to mRNA. Encapsulated cells are serially imaged across brightfield and fluorescence channels. In these representative images, 3 fibroblast markers are detected on the same cells. After imaging data is collected, Cellanome's computer vision capabilities segment cells, identify barcode positions on the flow cell surface, and match them to constructed CCEs. Cells are then lysed within CCEs and mRNA captured to the barcodes on the flow cell's surface prior to cDNA synthesis.

CELLCAGE ENCLOSURE™ (CCE) FORMATION STRATEGIES AND CONFIGURATIONS

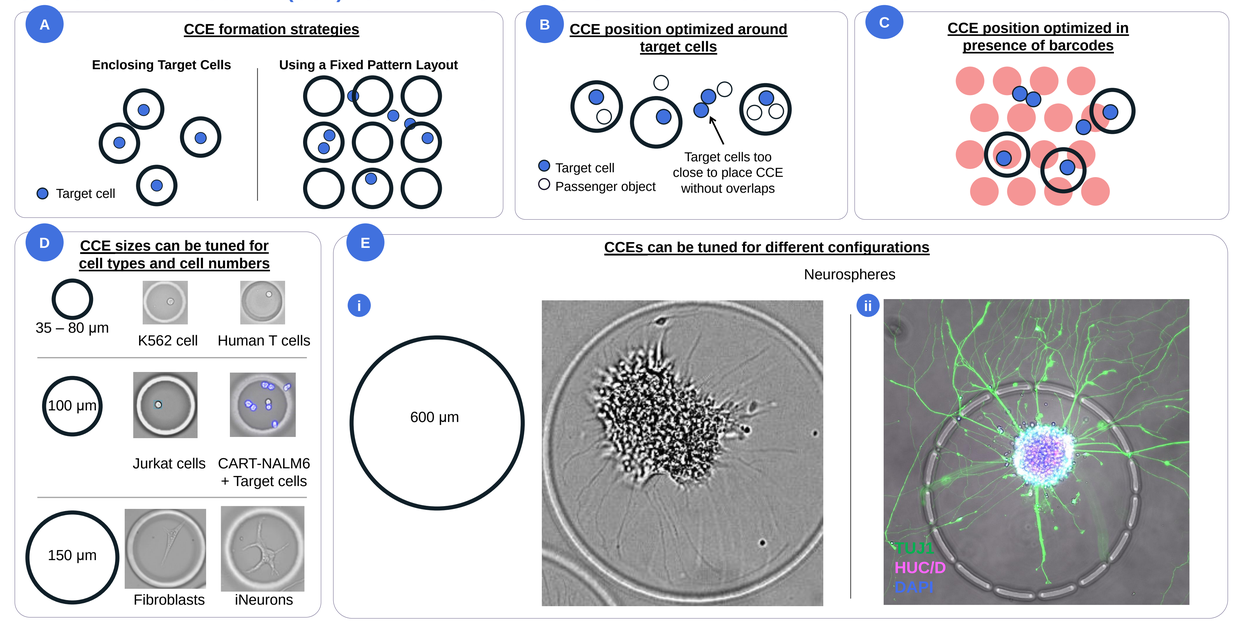
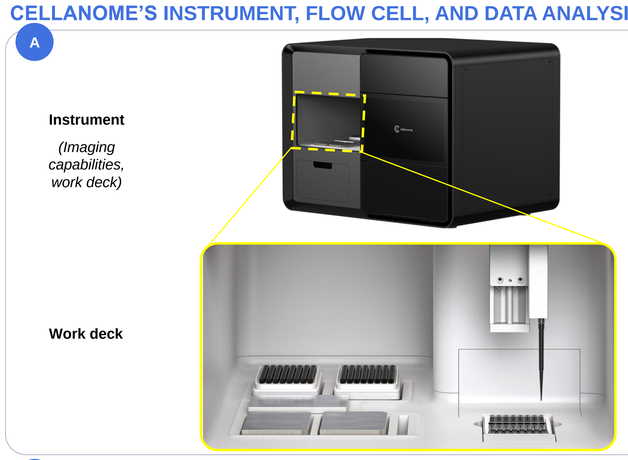


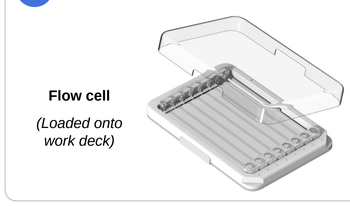
Fig 2. Dynamic CCE formation strategies and configurations are being developed to enable a variety of cell biology assays
(A) CCEs can be formed around target cells, or in a fixed grid pattern. **(B)** The optimal placement of the CCEs is determined by an optimization algorithm according to a set of rules using as input the position of the cells, **(C)** position of barcodes, and overlap between CCE and barcodes, as detected by computer vision. **(D** and **E)** CCE formation is dynamic, enabling cell size to be tuned based on needs of cell types, numbers in each individual CCE, and desired throughput across the entire flow cell. Smaller CCEs (35 – 80 µm) can be formed around smaller cells, which results in higher throughput across the flow cell, and larger CCEs (100 - 600 µm) can be generated to accommodate larger cells, multiple cells per CCE, or **(E-i)** multi-cellular aggregates like neurospheres. **(E-ii)** CCEs can be further configured to craft different shapes. Windowed CCEs are a representative example of CCEs that can be configured to have gaps in the enclosure. When leveraged for neurospheres, this configuration can enable studies around axon pathfinding or synapse formation.

CELLANOME'S INSTRUMENT, FLOW CELL, AND DATA ANALYSIS FEATURES



Imaging capabilities	
Optics	• Brightfield • Fluorescence excitation: 405nm, 470nm, 560nm, 630nm • Emission filters: Brilliant violet, FITC, CY3, CY5, multi-band (4 channels)
Objectives	• 4x, 10x
Camera	• CMOS sensor
Spatial light patterning	• Digital micromirror device (DMD)

Work deck	
Computer	• On-board compute with GPU
Liquid handling and fluidics	• Fully integrated fluidics with pipetting based dispensing & syringe pump-based perfusion
Sterility	• HEPA air filtration, UV work-deck sterilization
Thermal Management	• On-deck reagent chiller • Flow cell temperature control: 15º C – 65º C



Flow cell specifications

All flow cells	
Lanes	8
Material	Glass
Height	75 microns

Flow cell configurations to enable diverse assays

Cell categories	Features
Adherent cells	• Different coating options to enable varied cell types (e.g., fibronectin, laminin) • Barcoded and non-barcoded options
Suspension cells	• Barcoded and non-barcoded options

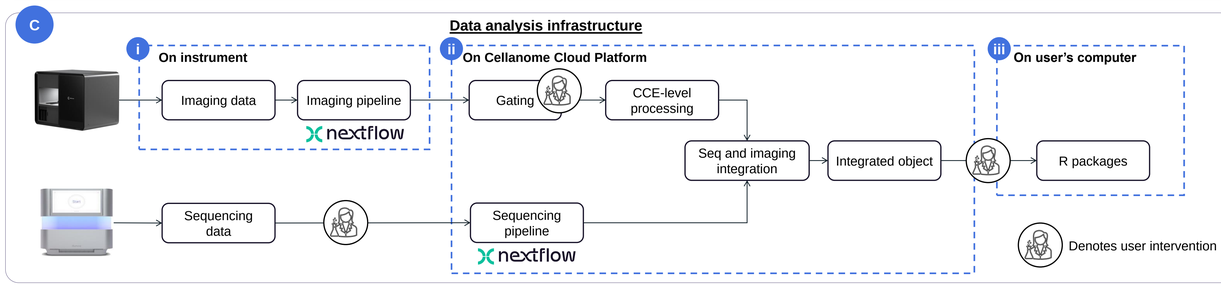
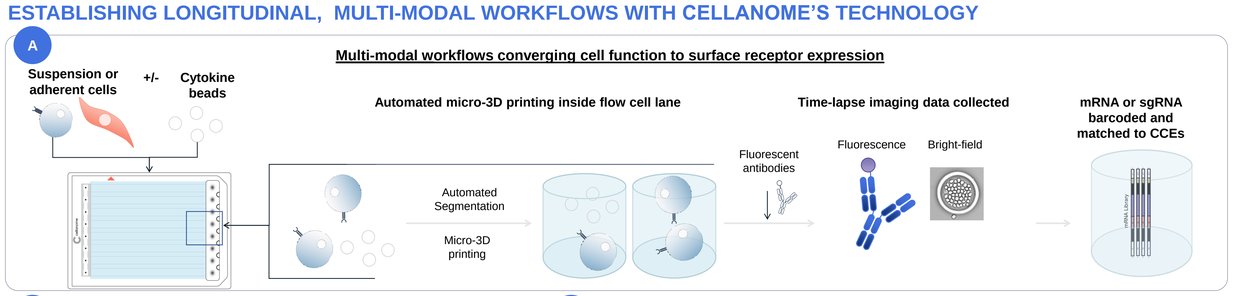
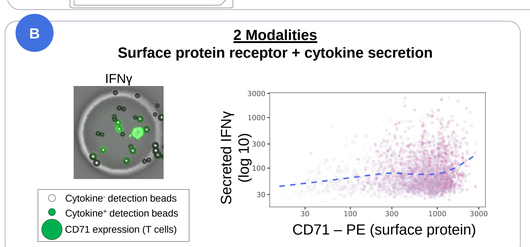
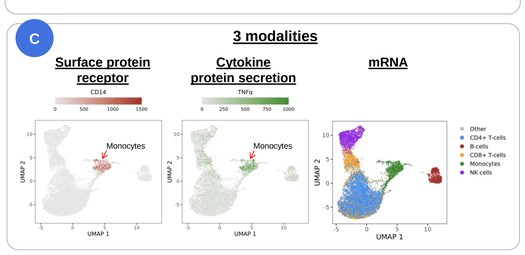


Fig 3. Cellanome instrument, consumables, and data analysis infrastructure
(A) Images and key features of Cellanome instrument with integrated CCE formation, microscopy, and automated reagent delivery capabilities that enable a variety of cell biology assays. **(B)** Image and features of flow cells on which cells are enclosed in CCEs and reagents are delivered. Flow cell configurations are available to accommodate adherent or suspension cells, and experimental goals around cell phenotyping / function only, or cell phenotyping / function linked to sequencing data (e.g., mRNA, single-guide sequencing for CRISPR screens). **(C)** Data analysis is supported with an on-board image analysis and cloud-based visualization and analysis platform. **(i)** Imaging data is processed locally on the instrument by an analysis pipeline written in Nextflow. The pipeline segments all the objects in an image and calculates various features including the intensity of each object across all the fluorescent channels, and morphological properties such as size, eccentricity, etc. **(ii)** The results are then uploaded to the Cellanome Cloud Platform where they are further processed in an interactive workflow to define populations of interest (see below) and integrated with sequencing data results. **(iii)** The integrated data can be downloaded from the Cellanome Cloud Platform and further processed using R packages internally developed. The integrated data contains gene expression measurements and longitudinal imaging-based features matched at the level of individual CCEs.

ESTABLISHING LONGITUDINAL, MULTI-MODAL WORKFLOWS WITH CELLANOME'S TECHNOLOGY







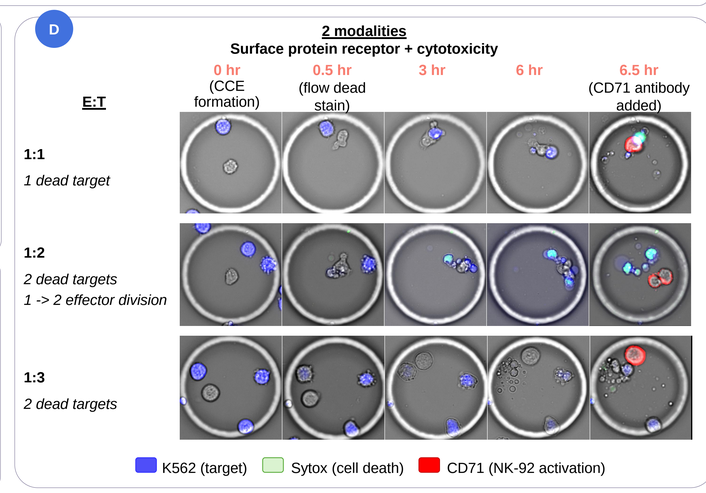


Fig 4. Longitudinal workflows converging imaging and sequencing data can be leveraged for multi-modal evaluation of cell behavior, including interactions
(A) Schematic of workflow for multi-modal assays on Cellanome's Technology. POC was established for assays converging cytokine secretion or cytotoxicity to surface markers. For the following assays, PBMCs activated with α-CD3 / α-CD28 in flask, or cell lines (e.g., NK-92, K562) are mixed with hydrogel precursor and loaded onto a Cellanome flow cell. If conducting a cytokine secretion assay, then cytokine beads are mixed with cells and hydrogel precursor. After loading, cells were detected and CCEs formed around cells alone or with proximal cytokine beads. Media containing reagents that label cells or beads, including α-CD71 (lymphocyte activation), α-CD14 (monocytes), α-IFNγ / α-TNFα (cytokine beads) were perfused onto the flow cell. Subsequently, time-lapse images of cells within CCEs were collected for multi-modal evaluation. **(B)** Signal intensity of cytokine beads and surface receptor expression can be quantified and linked to perform pairwise analysis across modalities, with each dot representing an individual CCE with a single T cell from PBMCs. **(C)** PBMCs from a healthy donor were rested overnight and stimulated with LPS for 75 minutes. Cell type annotation based on gene expression data was performed using SingleR and reveals an expected distribution of cell types. Monocytes (as identified by gene expression) express CD14 (surface receptor assay) and secrete TNFα (cytokine secretion assay) following LPS stimulation. As expected, expression of multiple pro-inflammatory cytokines is enriched specifically in the monocyte compartment. **(D)** Demonstrating longitudinal assessment of cell killing capabilities converged to activation markers by individual cells. Representative longitudinal images of individual Effector cells (NK-92) co-encapsulated with one more Target cells (K562). Representative images of brightfield and fluorescence imaging data to monitor cell activation (CD71 expression on NK-92), viability (both NK-92 and K562), and proliferation (both NK-92 and K562). This data is coupled with the evaluation of K562 cell death for direct evaluation of killing by individual NK-92 cells in a manner that enables evaluation of kinetics, validation of NK-92 viability, and NK-92 proliferation to ensure increased killing in a single CCE is due to killing, as opposed to increased numbers of NK-92 cells from proliferation.