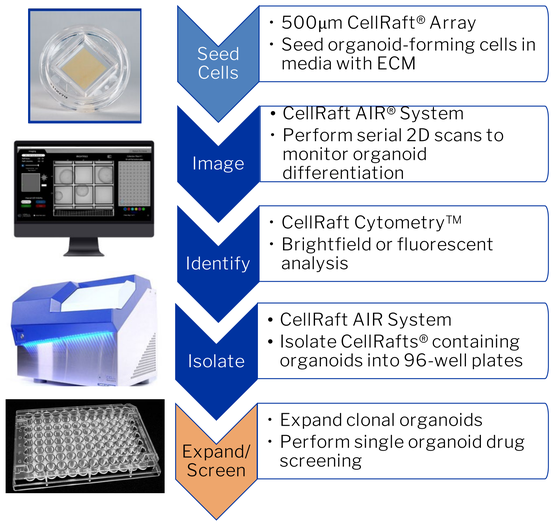
# ACCELERATING THE USE OF iPSC-DERIVED ORGANOIDS IN DRUG DISCOVERY AND PERSONALIZED MEDICINE

**Allysa Stern**, Lexi Land, Cole Peeler, Keith Williams, Rob McClellan, Brandon Thompson, Steven Gebhart, Jessica Hartman
Cell Microsystems, Inc. Durham, NC USA

## BACKGROUND

- Workflows for generating iPSC-derived organoids are often inefficient, labor-intensive, and suffer from limitations of heterogeneity and throughput.
- Our key objective is to demonstrate the flexibility and key features of the **CellRaft® Technology** that provide **solutions** to many of the **pain points** of organoid workflows.
- Using the CellRaft Technology, we have demonstrated **streamlined, reproducible** organoid workflows that offer reliable imaging, software-guided selection, and automated isolation of single organoids for downstream applications.

## METHOD




## RESULTS

- Using the CellRaft Array, thousands of single human iPSCs can be monitored for **monoclonality** and **organoid differentiation**.
- iPSC-derived organoids were **differentiated** on-array into neural organoids, including cerebral and choroid plexus.
- Clonal iPSC organoid formation was achieved with a high degree of **efficiency (>17%)** and CellRafts with organoids were **isolated** with the AIR System **(>90% efficiency)**.
- Day 28 cerebral organoids, isolated from the CellRaft Array and matured in 96-well plates, show significant **neural outgrowth** and are positively stained for live neurons.
- **Single, mature** cerebral organoids **screened** for acute alcohol-mediated apoptosis demonstrate a **dose-dependent** activation of Caspase 3/7.

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- **Monitor differentiation of iPSC-derived organoids**
- **Reliable, efficient clonal organoid generation**
- **Fully automate isolation of viable, intact organoids**
- **Generate custom organoid assay plates for propagation, drug screening, or other analyses**

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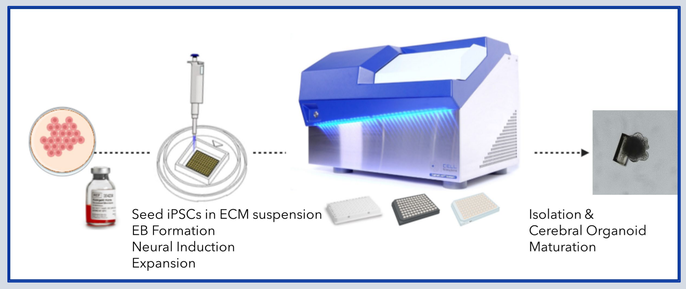
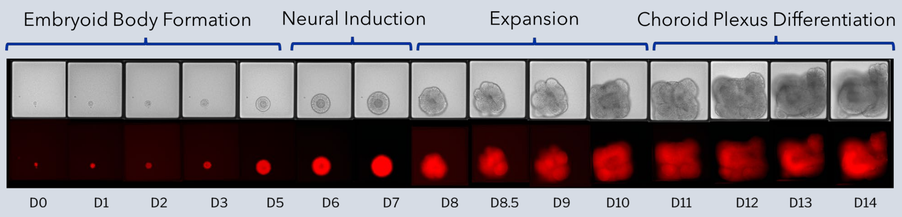
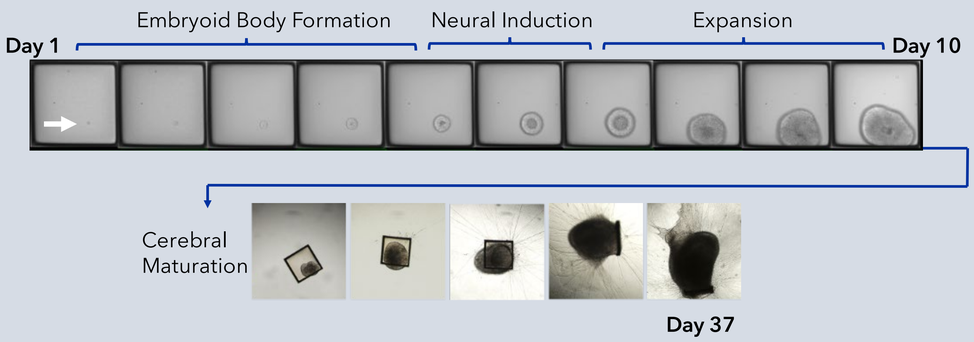
### iPSC-Differentiated Neural Organoids

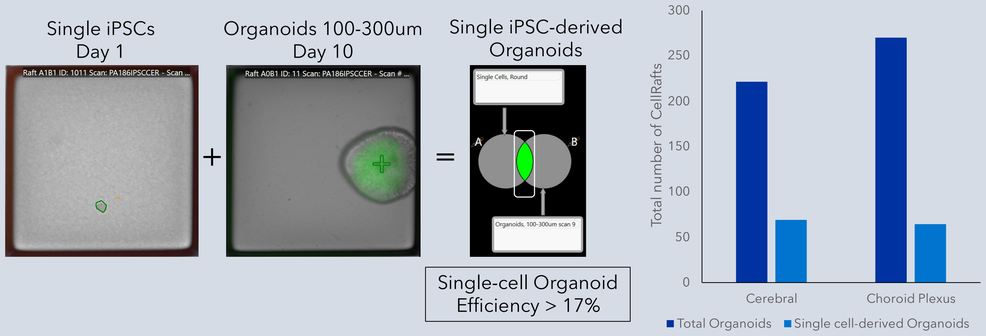



A mixed populations of edited (GFP or RFP) and unedited iPCS were seeded on the 500µm CellRaft Array in a suspension of Matrigel. The first several stages of organoid differentiation, embryoid body formation (days 1-5), neural induction (days 5-7), and expansion days (7-10), were performed on-array by performing media changes. The array was scanned every 24 hours to monitor organoid differentiation. At day 10, single organoids were isolated into 96-well plates into maturation media. Cerebral organoids were maintained in maturation media to day 42.

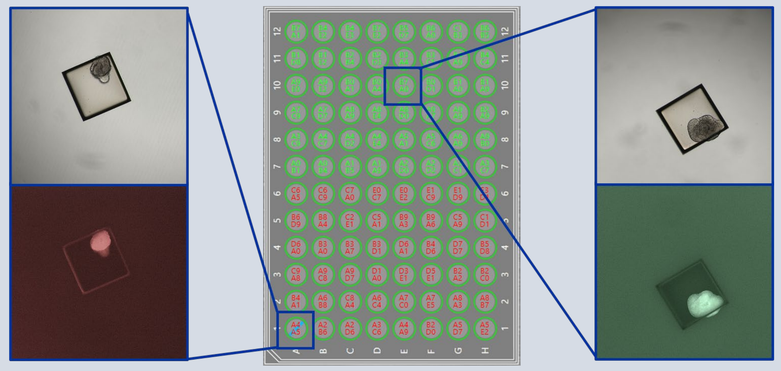
### Clonal Organoid Generation



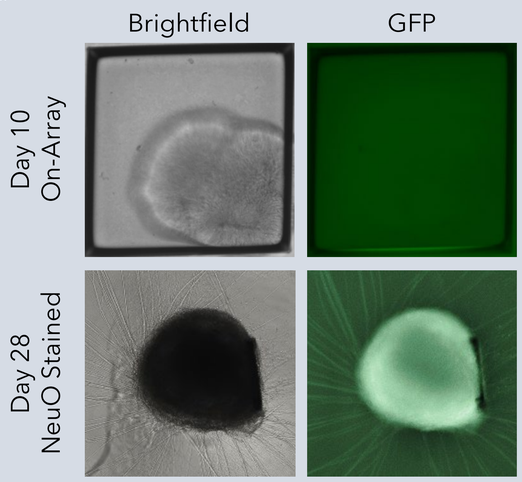

n = 3 arrays per organoid type

Using CellRaft Cytometry, user-defined populations can be built to identify CellRafts for isolation. For clonal workflows, populations identifying CellRafts with a single cell at day 1 and organoids at later timepoints can be overlayed to identify clonal organoids of interest, including fluorescence and morphological parameters.

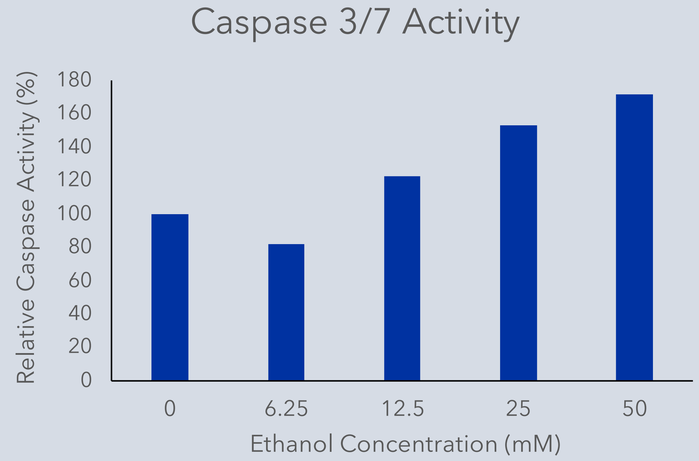
### Customized Organoid Assays for Drug Screening



The CellRaft AIR System performs automated isolation of CellRafts containing organoids of interest. Together, the CellRaft technology allows users to create custom assay plates of single organoids for downstream use, including propagation of edited, clonal organoids or edited organoids arrayed for downstream drug screening assays or further analysis.




On day 28, unedited organoids were stained using NeuroFluor NeuO, a membrane permeable fluorescent probe for detecting live neurons, as a visual confirmation of neural differentiation.




n = 6 organoids per dose

On day 43, mature cerebral organoids were exposed to a 5-point dose curve of ethanol (6.25-50mM, n = 6 wells per dose) to simulate acute alcohol exposure. After 6 hours of treatment, organoids were evaluated for alcohol-induced apoptosis using the Caspase-Glo 3/7 Assay (Promega). We observed a dose-dependent increase in caspase activity, supporting activation of apoptosis at the highest doses of ethanol exposure.