Novel Methods for Culture and Development of Stem Cell-Derived 2D and 3D Models

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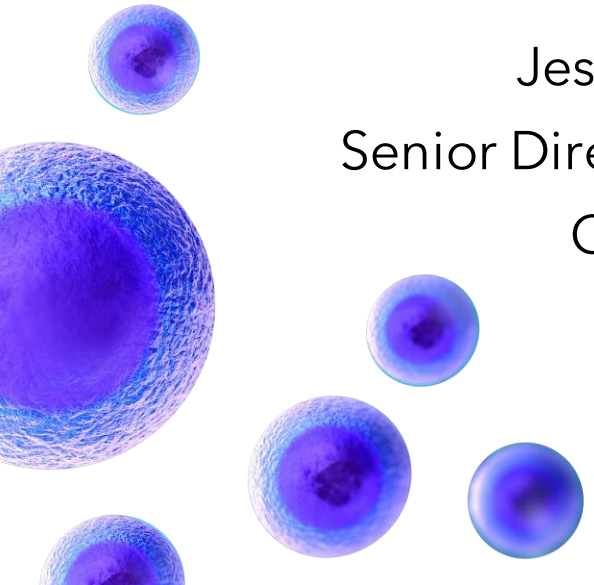
STEMCELL Technologies



Key Learning Objectives

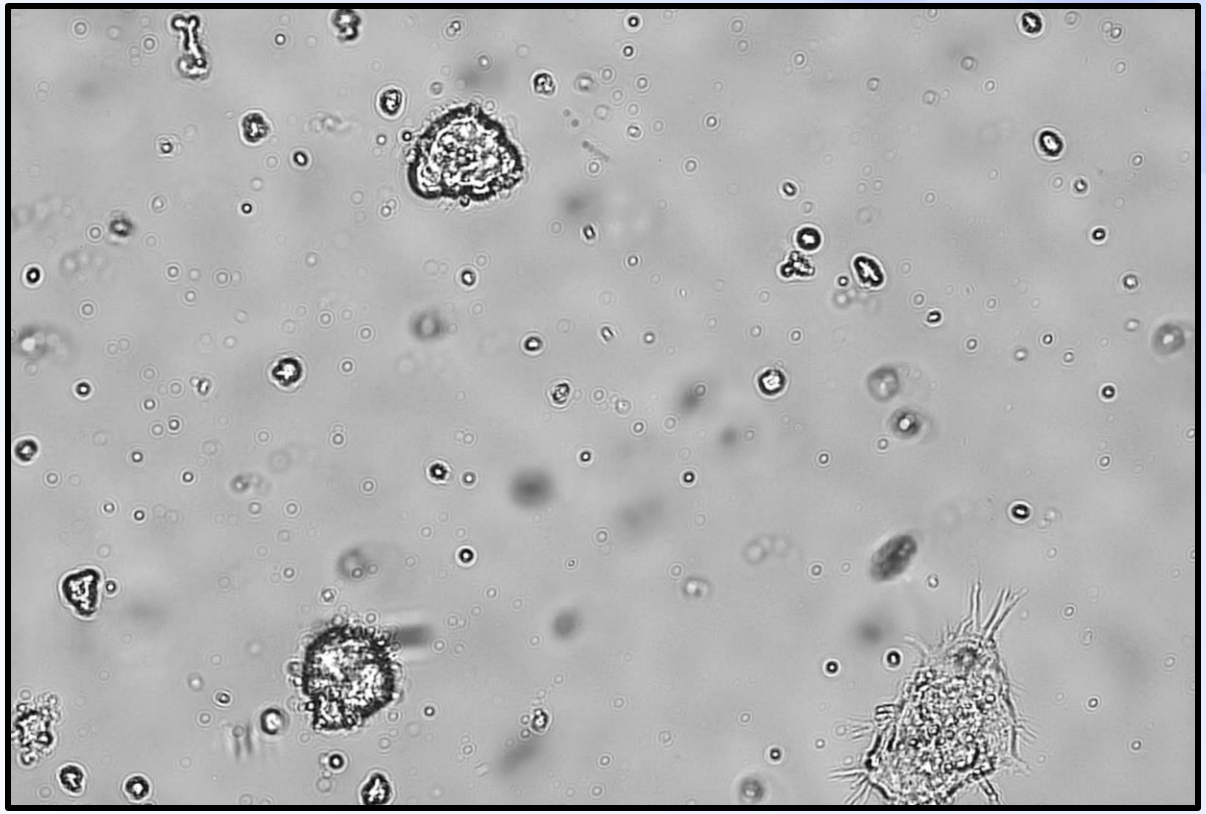
1. Demonstrate a protocol that helps to get to the desired clone in the shortest time possible with a demonstrated savings of valuable reagents, media, and plastic.

2. The use of hPSC-derived models to model human health, development, and disease

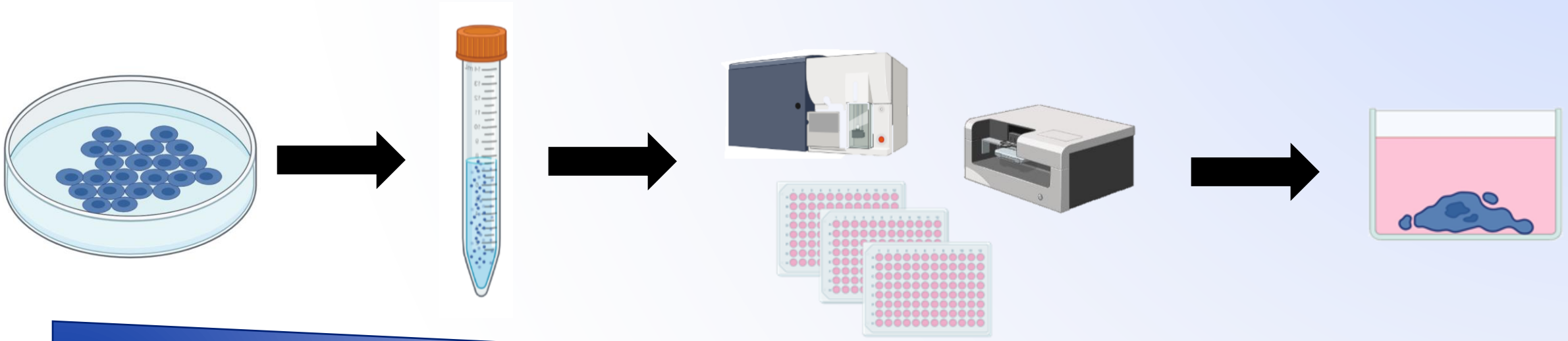
3. Differentiation and characterization of hPSC-derived choroid plexus organoids

The Challenge of iPS Cell Line Development

- Poor Viability
  - Inefficient
  - Difficulty proving monoclonality
  - Manual Isolation
  - Loss of pluripotency

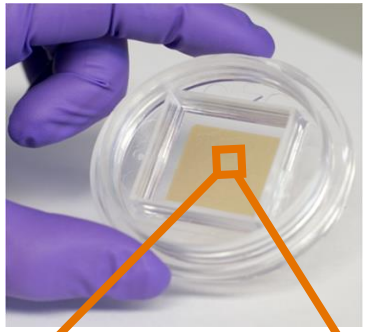


The Challenge of iPS Cell Line Development



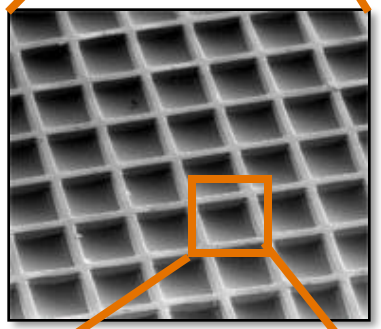
Viability

Flask-like Culture + Single-Cell Spatial Segregation



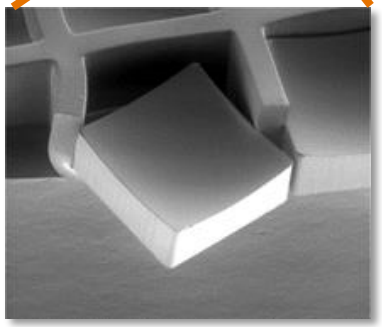
CellRaft® Array

- Multiple formats
- Easy to use
- Contiguous media



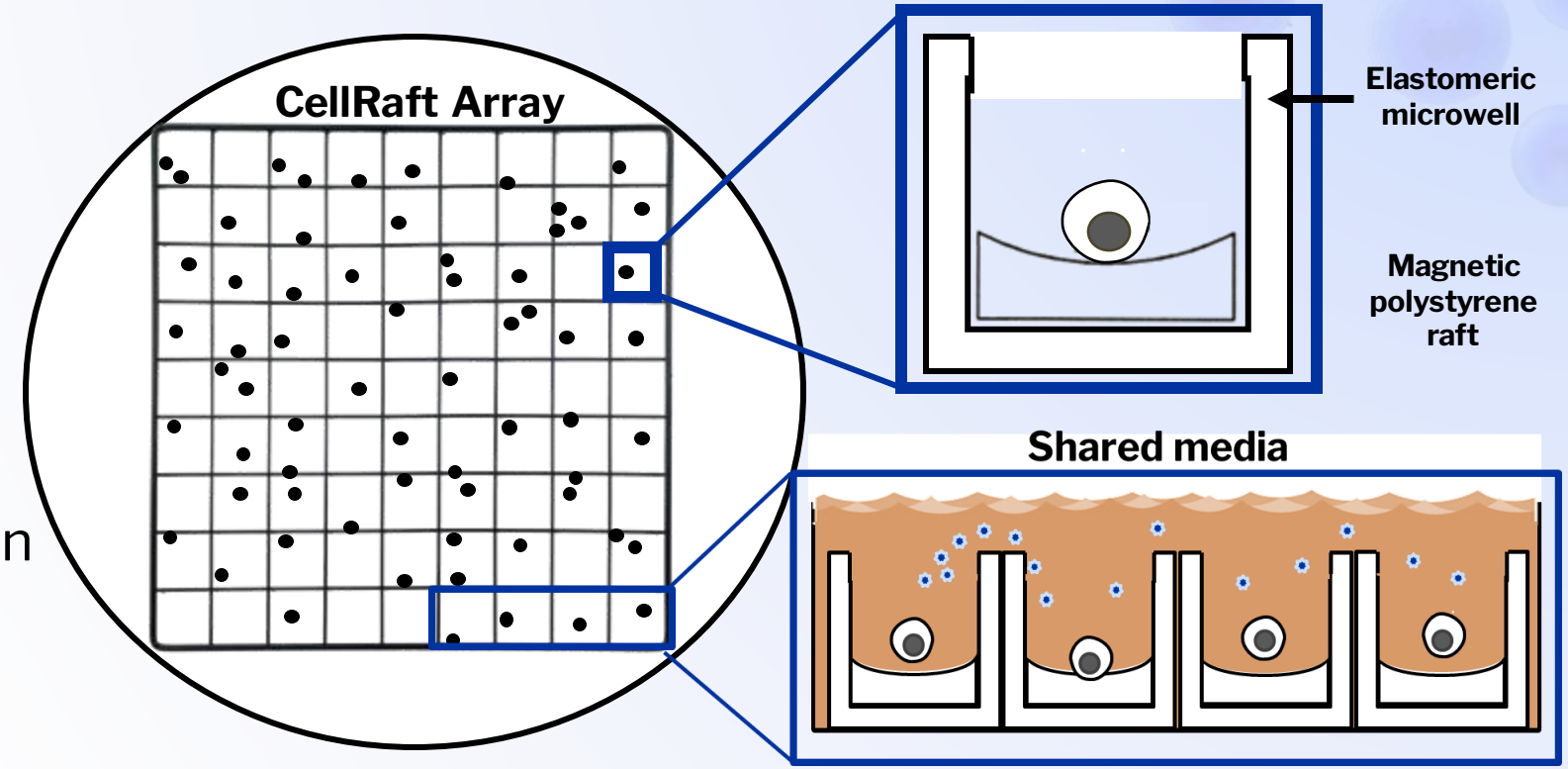
Microwells

- Elastomeric
- Spatial Segregation



CellRaft®

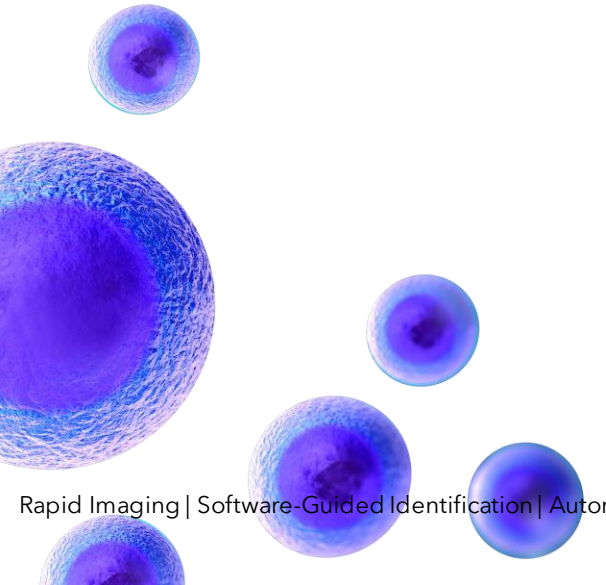
- Magnetic polystyrene



Poll Question #1

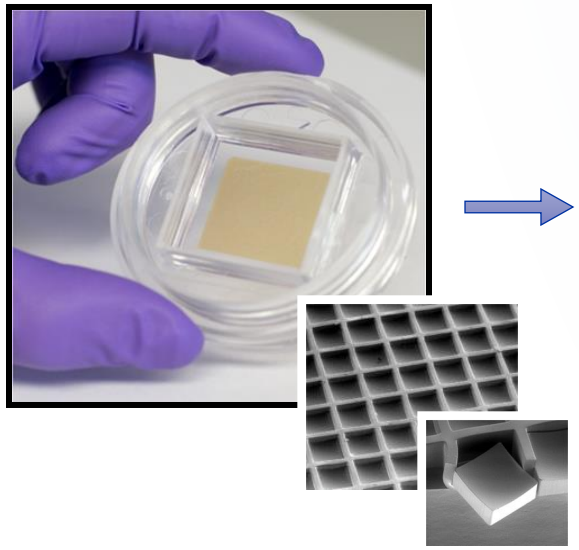
CellRaft Technology

How Does it Work?



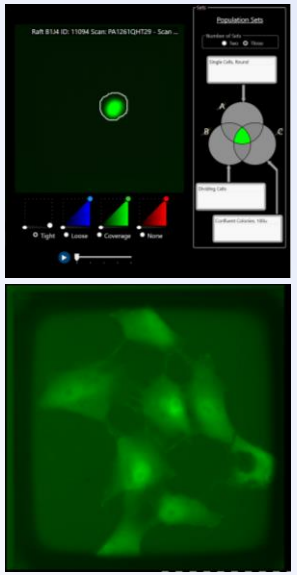
CellRaft Technology Overview

CellRaft® Array

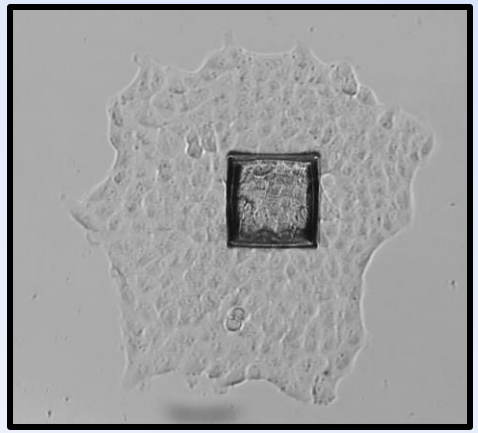


CellRaft AIR® System

CellRaft Cytometry™



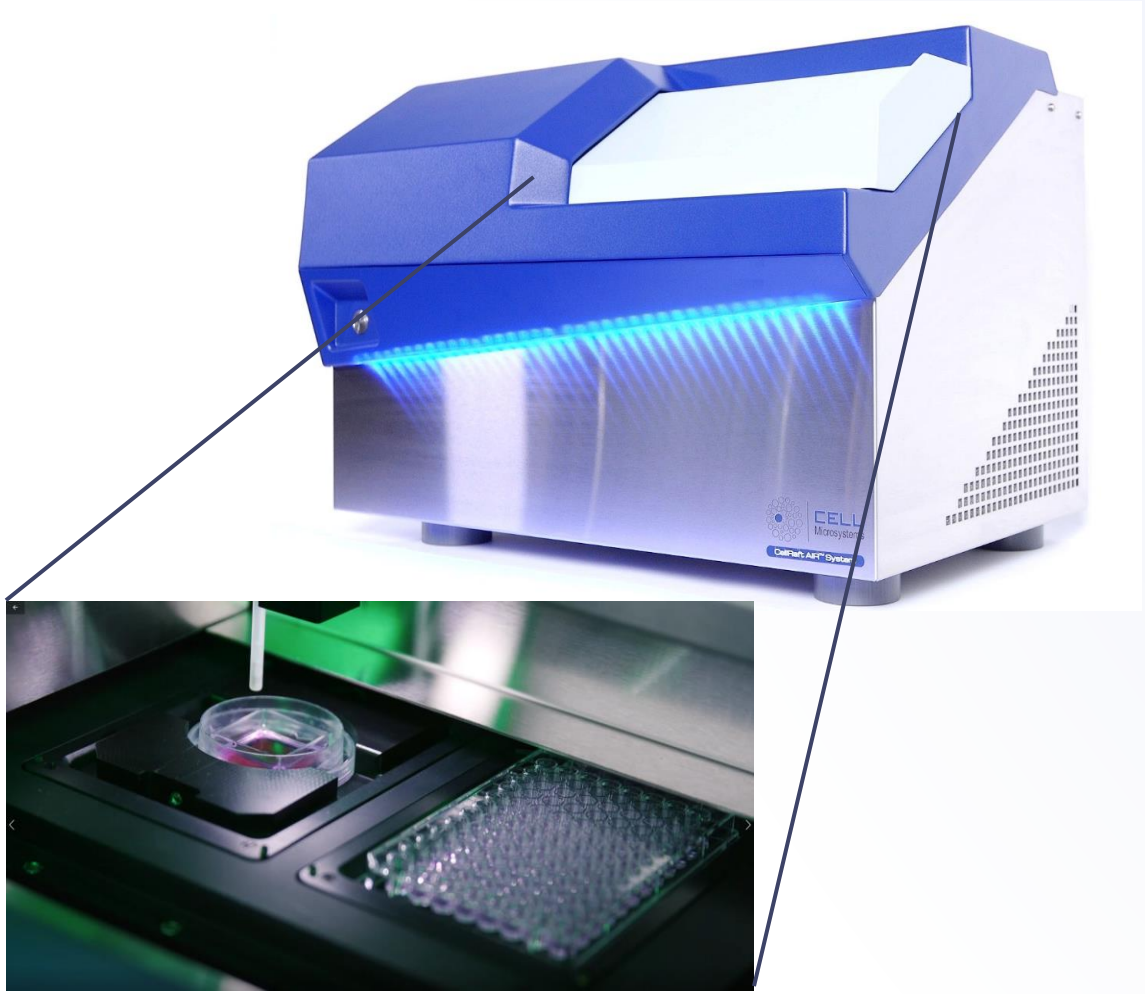
96-well plate



Flask-like Culture Conditions ✚ Single-cell Separation ✚ Image-based, Software-guided Selection

= Automated Retrieval of High Viability Cells, Colonies, or Organoids

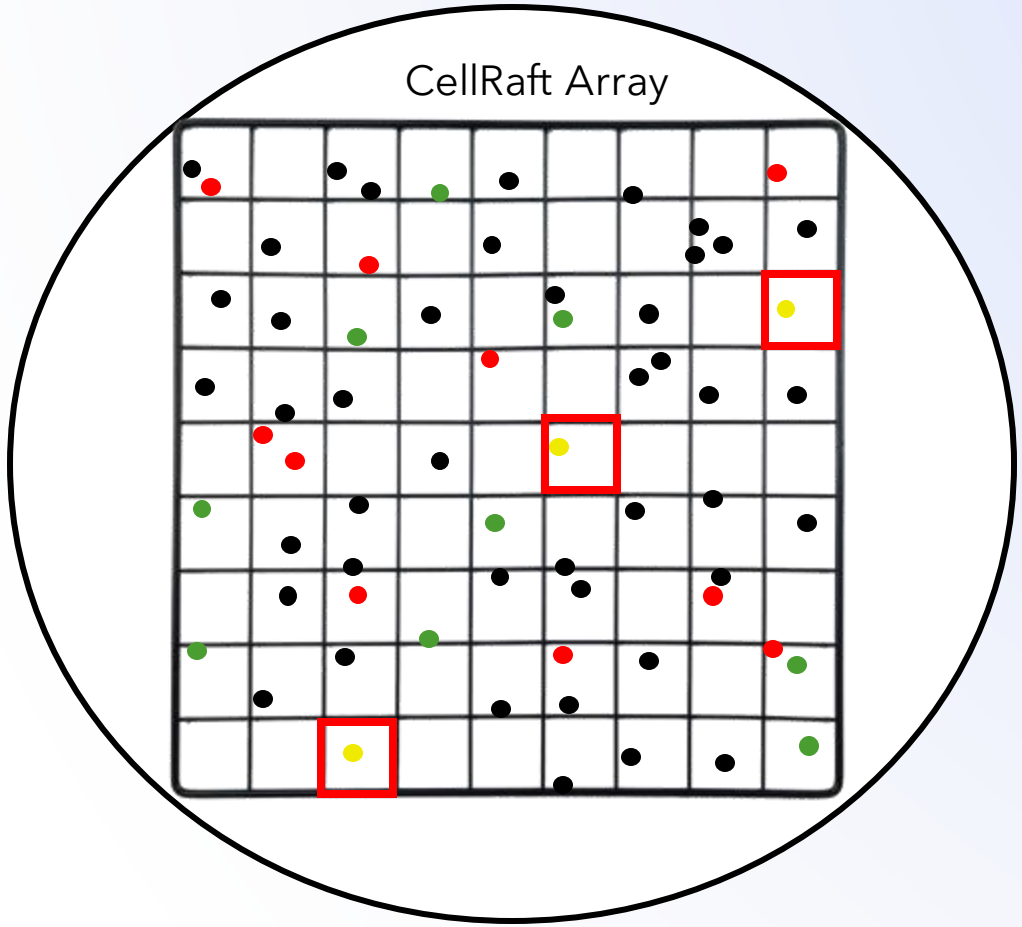
CellRaft AIR Instrument



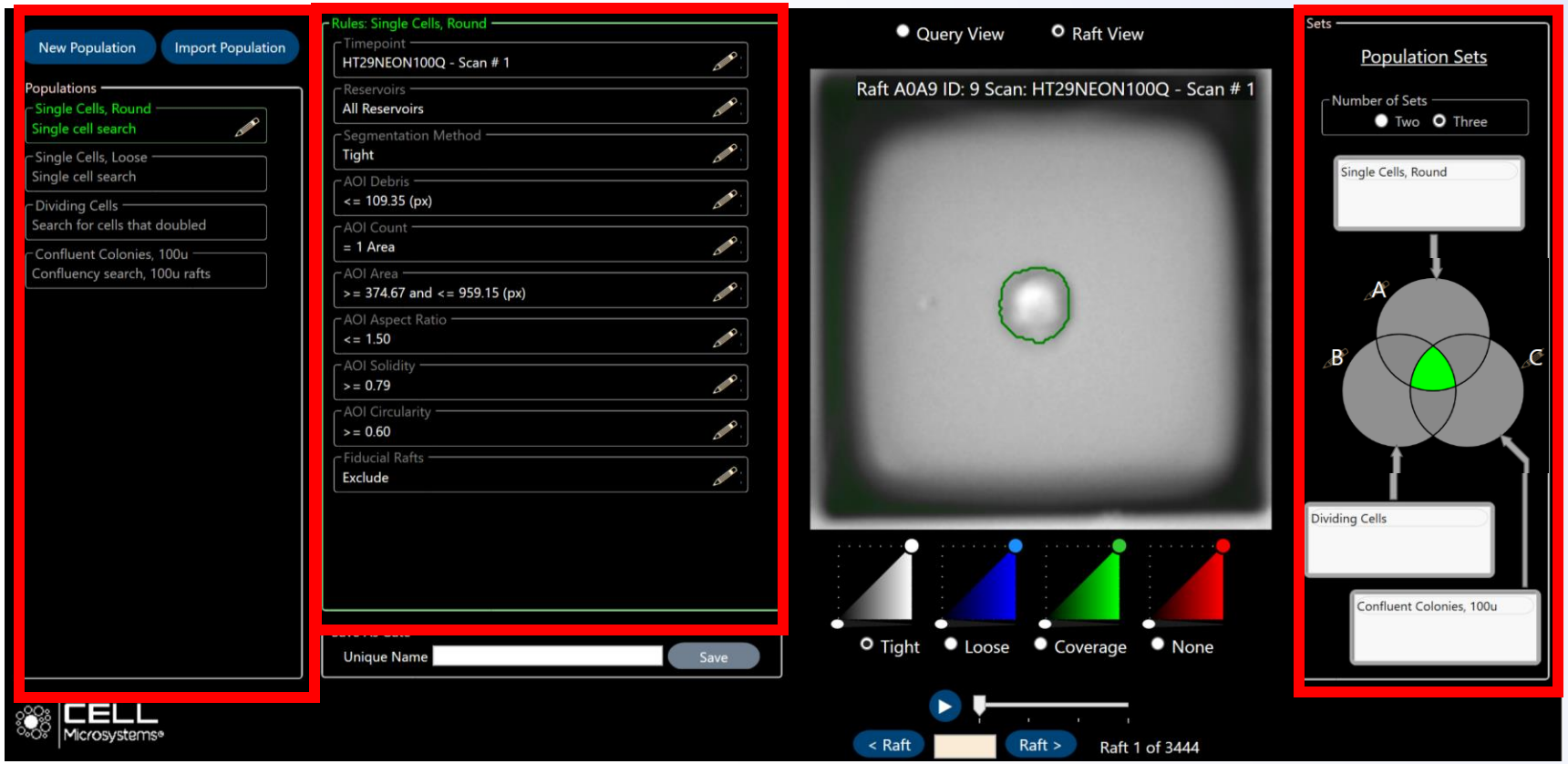
Automated:

- **Imaging**
  - **Brightfield** and **3-color fluorescent** inverted microscope
- **Identification**
  - **10X** Magnification
  - In-depth software analysis of images

CellRaft Cytometry: Software Guided CellRaft Selection

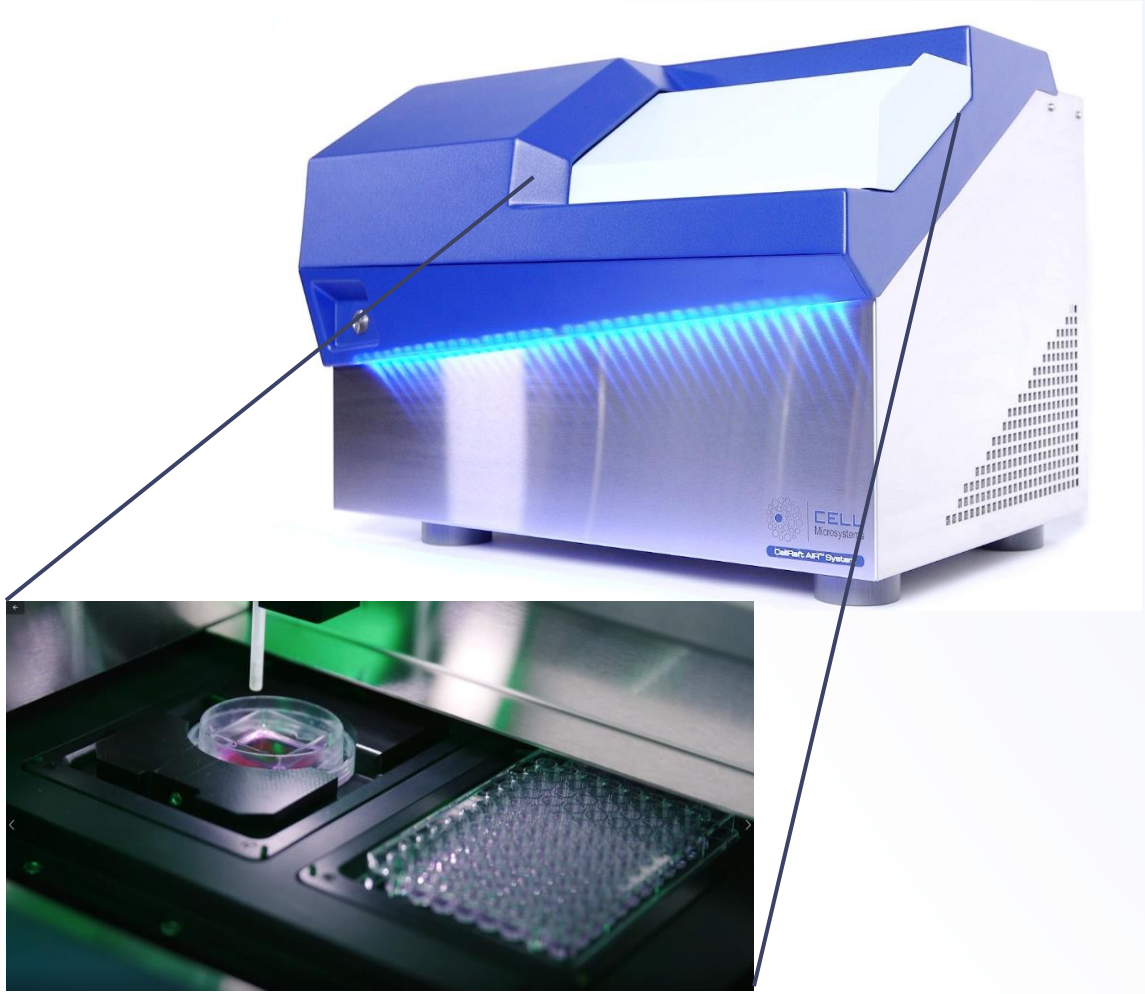


CellRaft Cytometry: Software Guided CellRaft Selection



- Advanced brightfield analysis
- Offline analysis frees up instrument
- Define unique subpopulations
- Create sub-sets
  - Easily identify desired CellRafts
- Rapidly map desired CellRafts for isolation

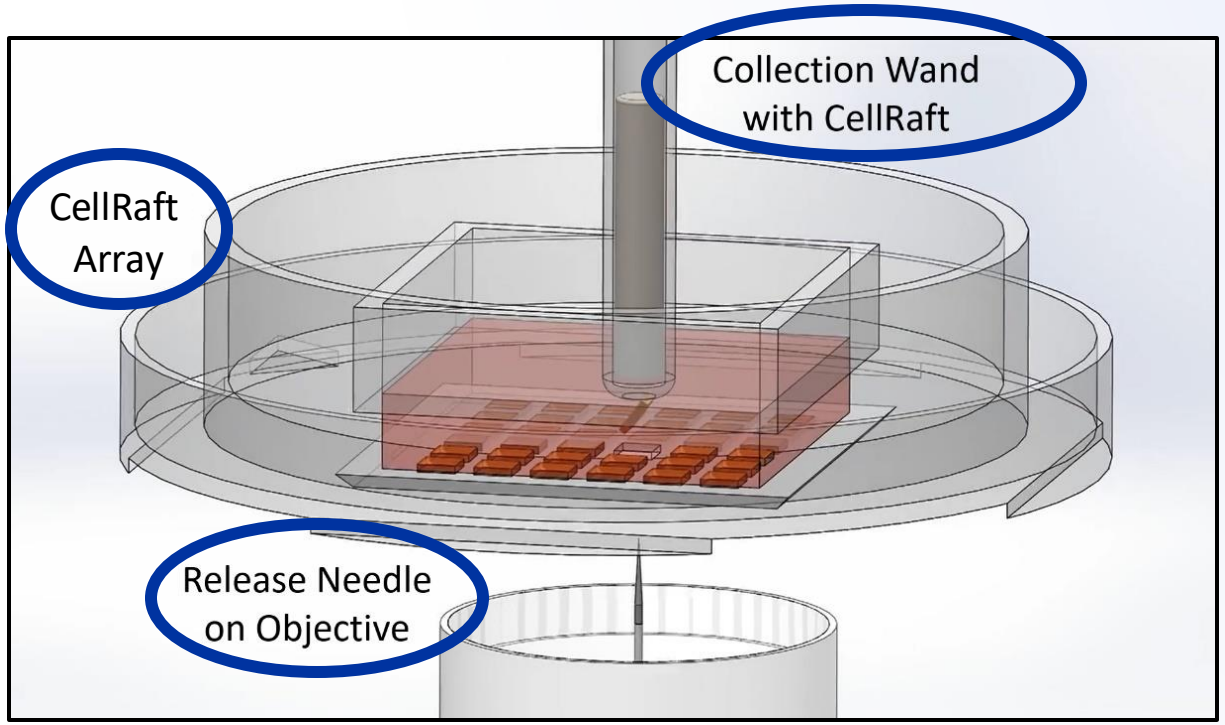
CellRaft AIR Instrument



Automated:

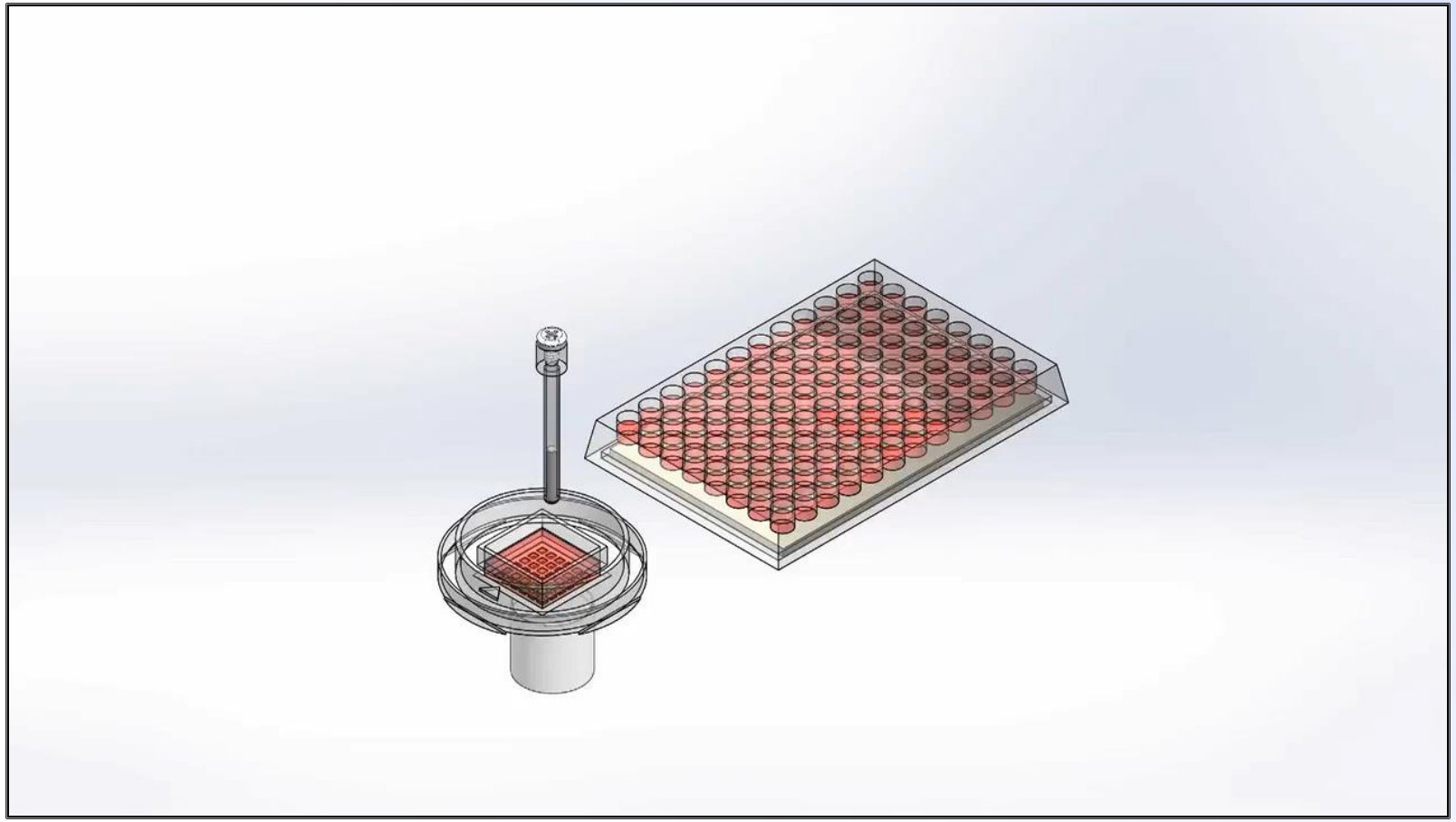
- **Imaging**
  - **Brightfield** and **3-color fluorescent** inverted microscope
- **Identification**
  - **10X** Magnification
  - In-depth software analysis of images
- **Isolation**
  - **CellRaft release and transfer** to 96-well collection plate

CellRaft AIR: Automated Clone Retrieval



- Cells remain on Raft during isolation
  - No need to trypsinize or remove cells
- CellRafts are releasable and retrievable
- Magnetic Rafts collected with magnetic wand

CellRaft AIR: Automated Clone Retrieval



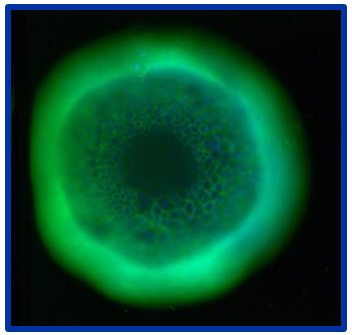
Broad Cell Line Compatibility

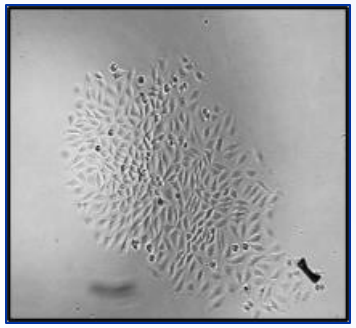
Over 80 cell lines characterized on array

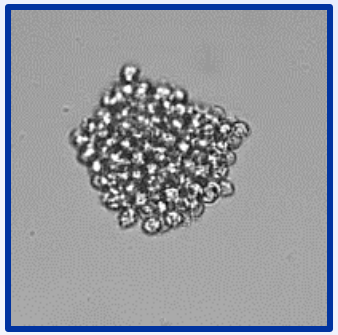
Primary Cells
- iPSC
- ESC
- Organoids

Adherent Cells
- HEK 293T
- CHO
- HeLa
- LLC

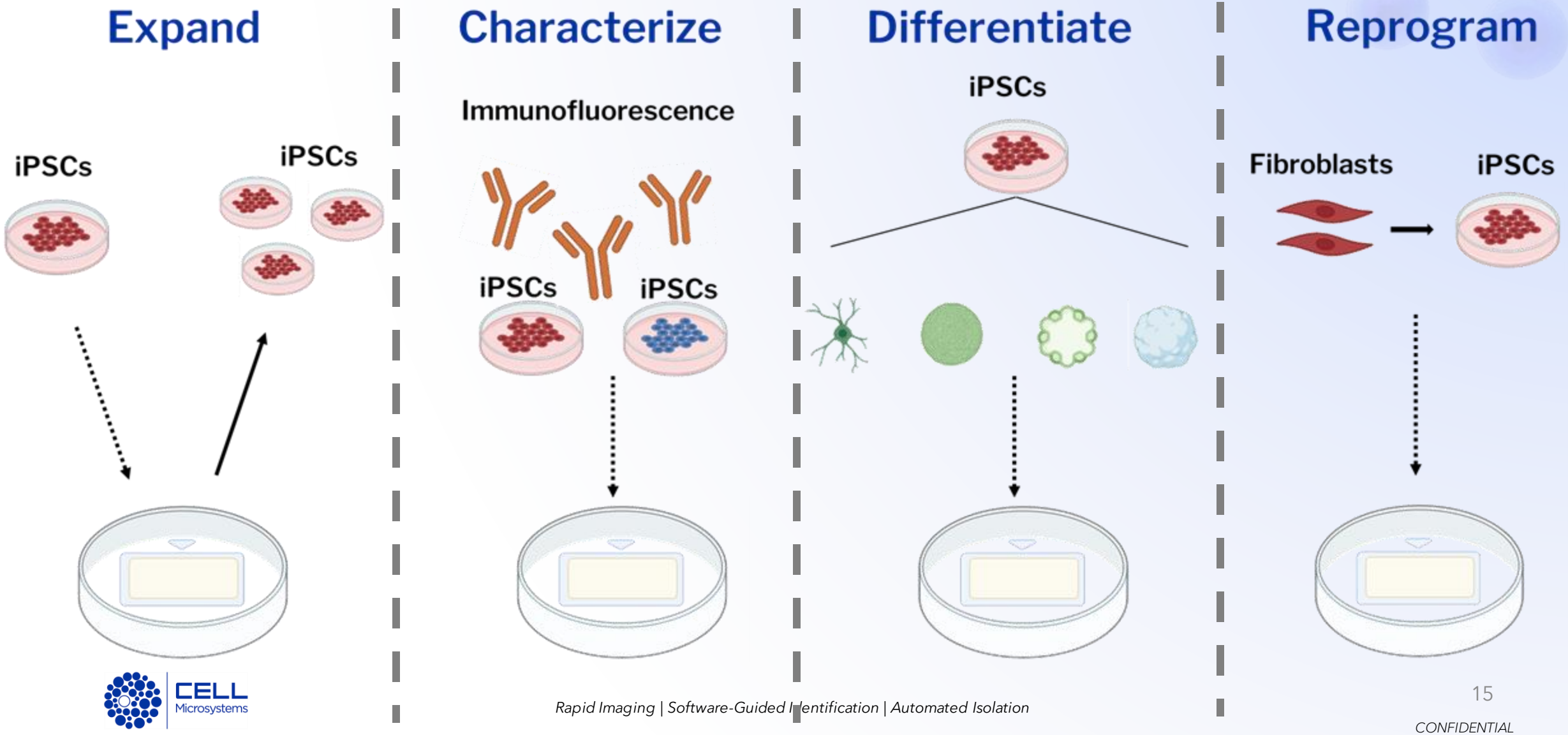
Suspension Cells
- Raji
- Jurkat
- THP1
- TS543







Enable All iPSC Workflows



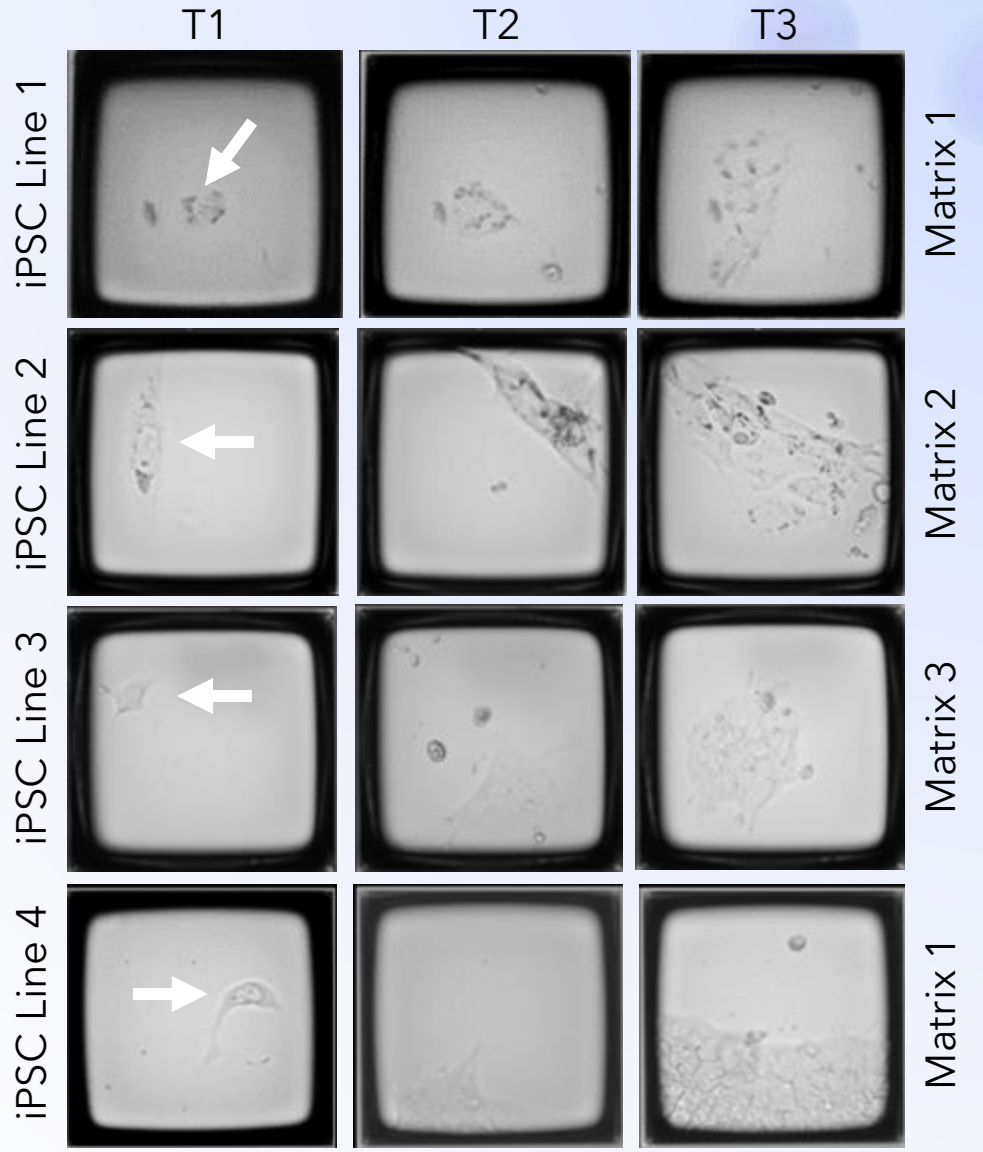
Rapid Imaging | Software-Guided Identification | Automated Isolation

Expand: Improve Efficiency and Efficacy of iPSC Cloning

- Track-and-trace from single cell to clone
- Screen more cells
  - **500X** more cells/consumable
- Compatible with any iPSC coating
  - **1000X** less coating/cell screened
- Compatible with any iPSC media
  - **2000X** less media/cell screened

	iPSCs screened/plate	uL Coating/cell	uL Media/cell
CellRaft Array	60,000	0.05	0.05
96 well plate	96	52.1	104.2

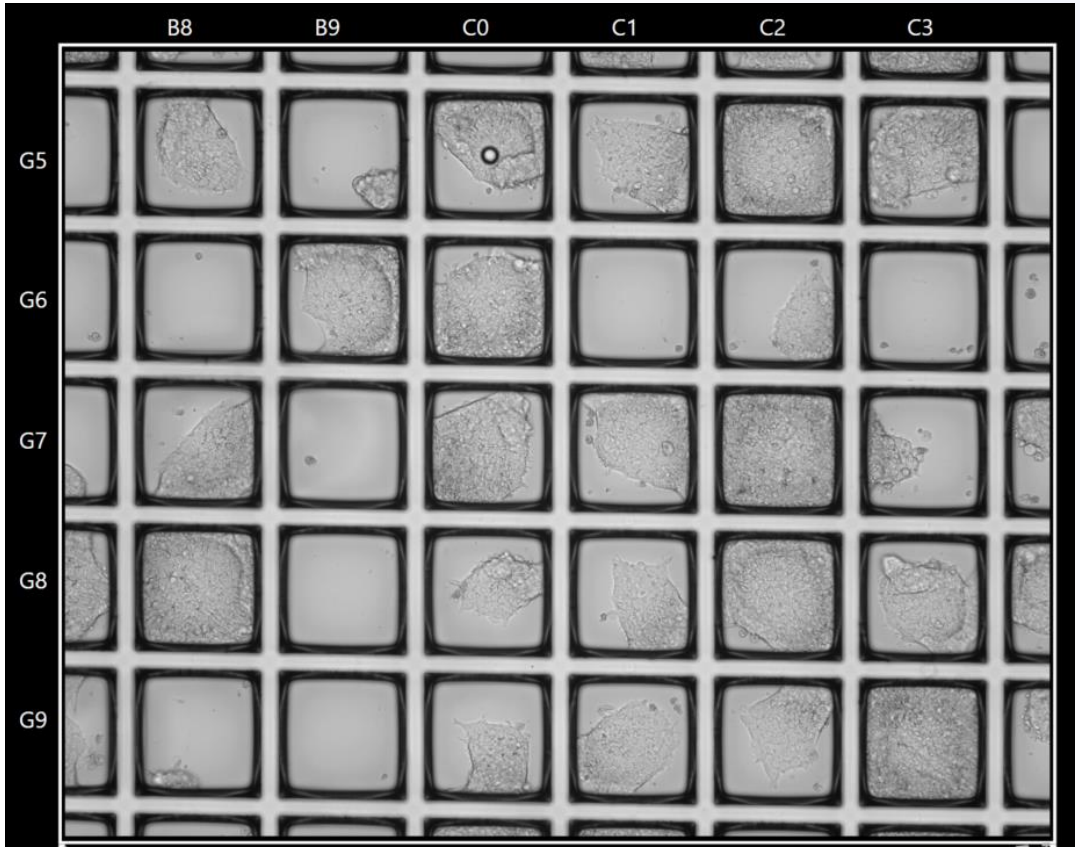
CELL Microsystems *Compared to 96wp



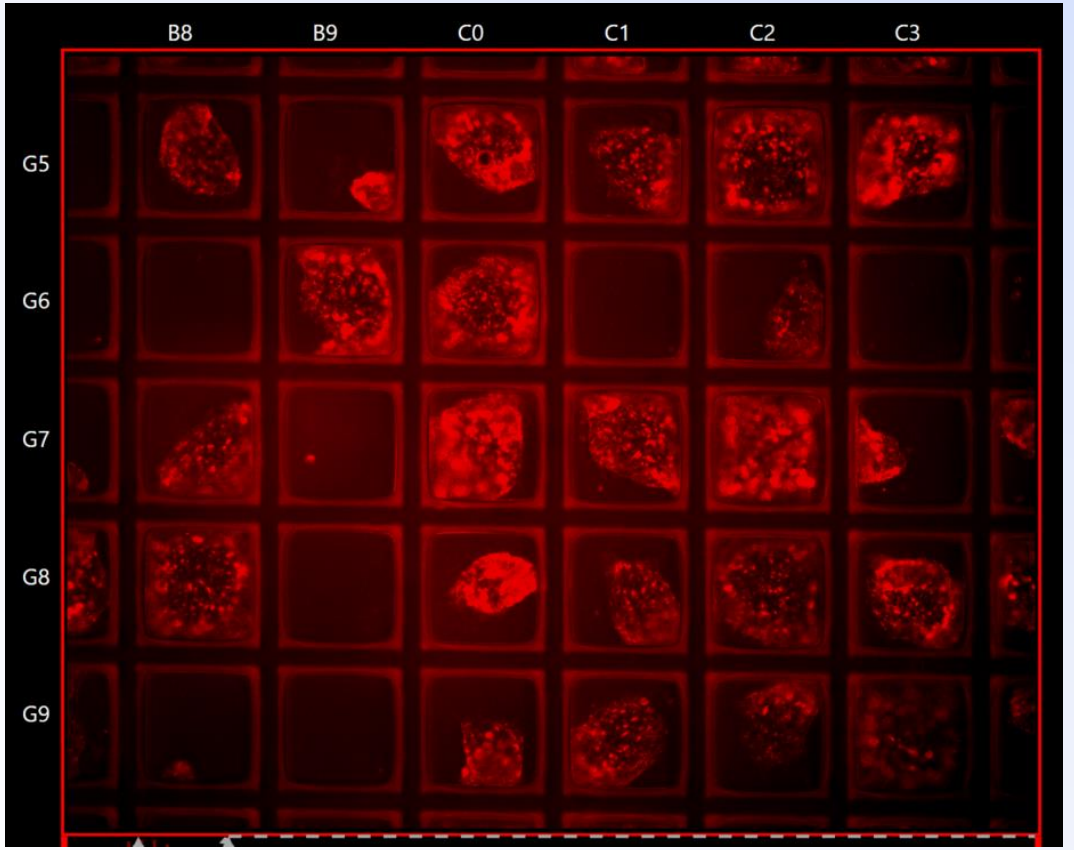
mTeSR™ Plus and CellAdhere™ Laminin-521

Characterize: Confirm Pluripotency with Live Staining

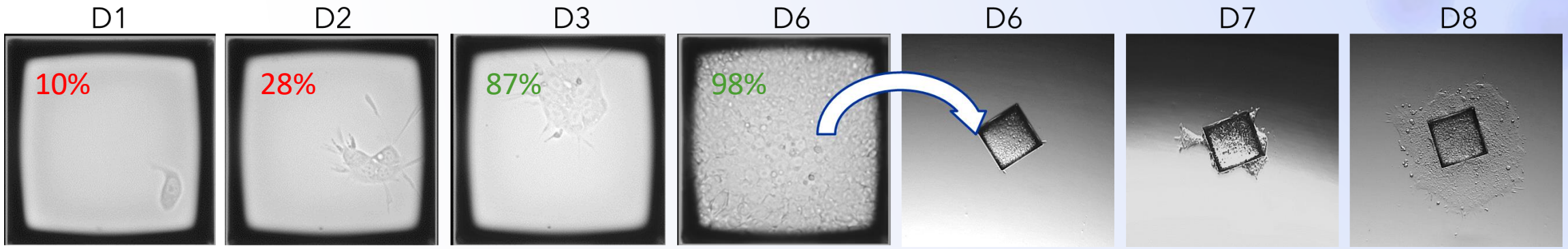
Brightfield

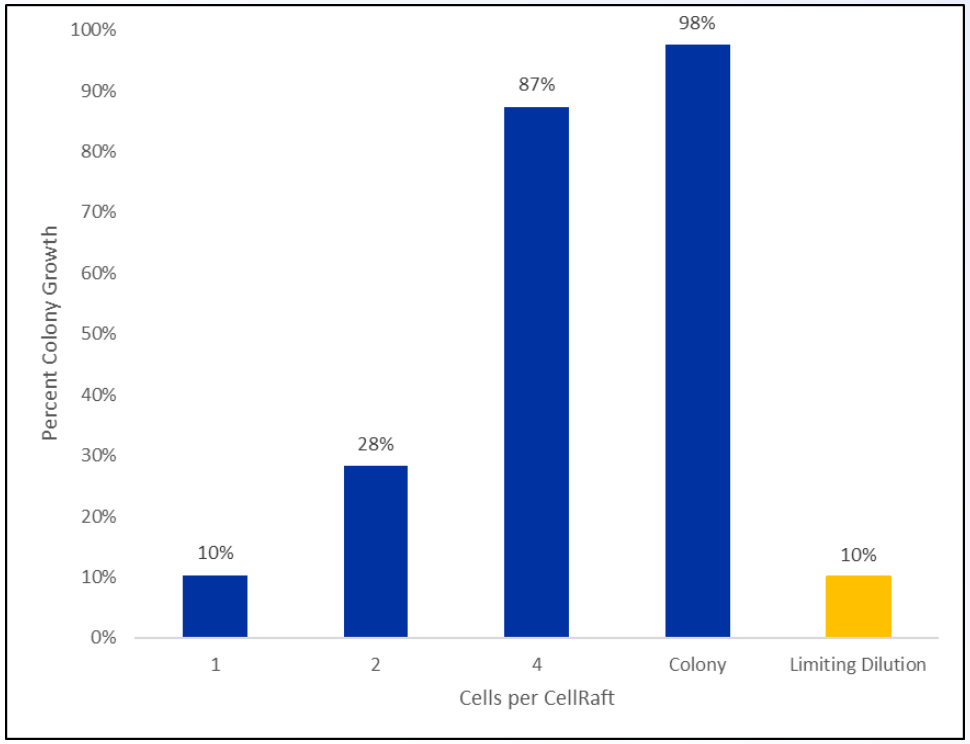


TRA-1 60



High-Efficiency Clonal Outgrowth from Single iPSCs





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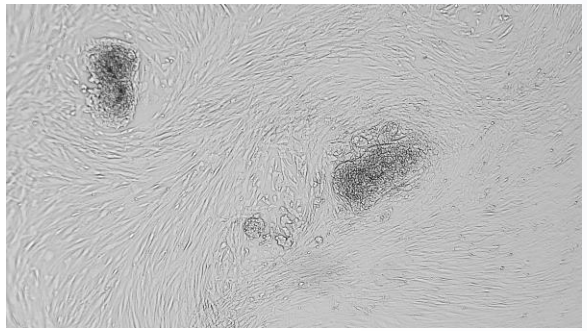
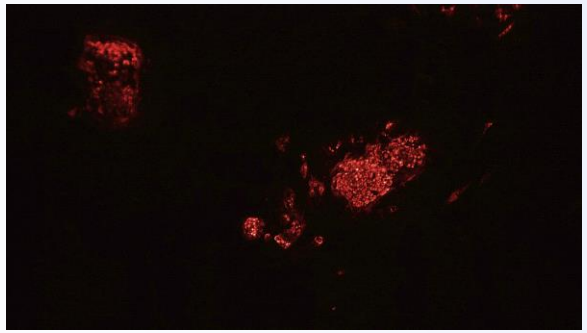
Reprogram: Direct-to-Array iPSC Reprogramming

Brightfield

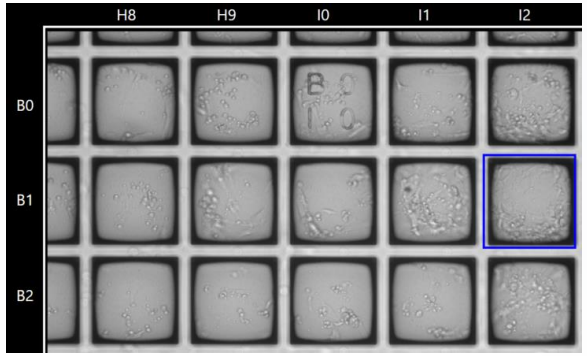
TRA-1 60

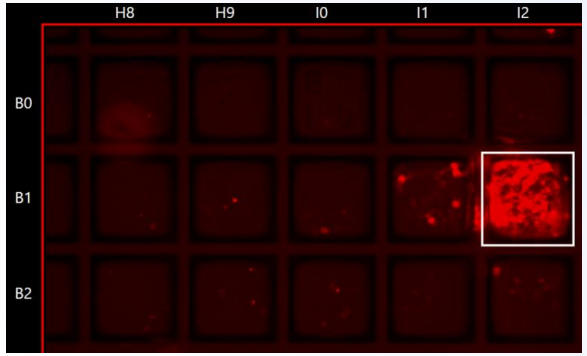
6-well plate

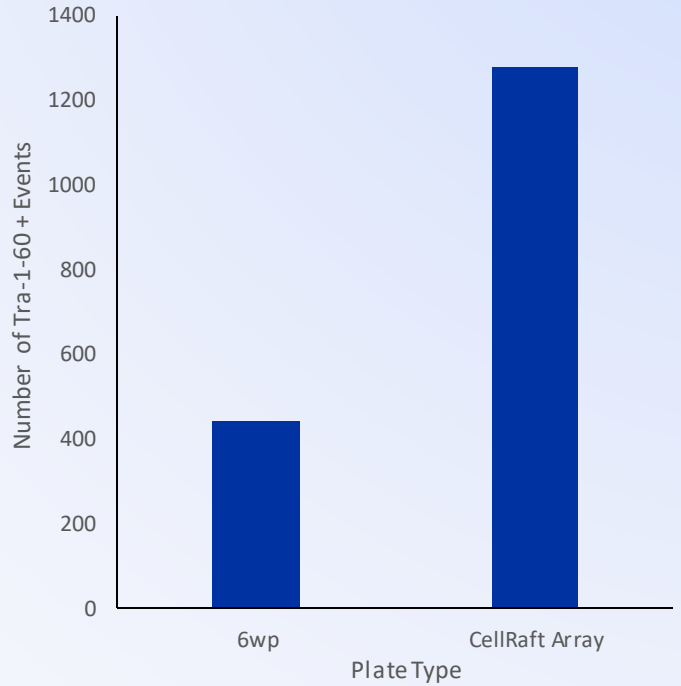




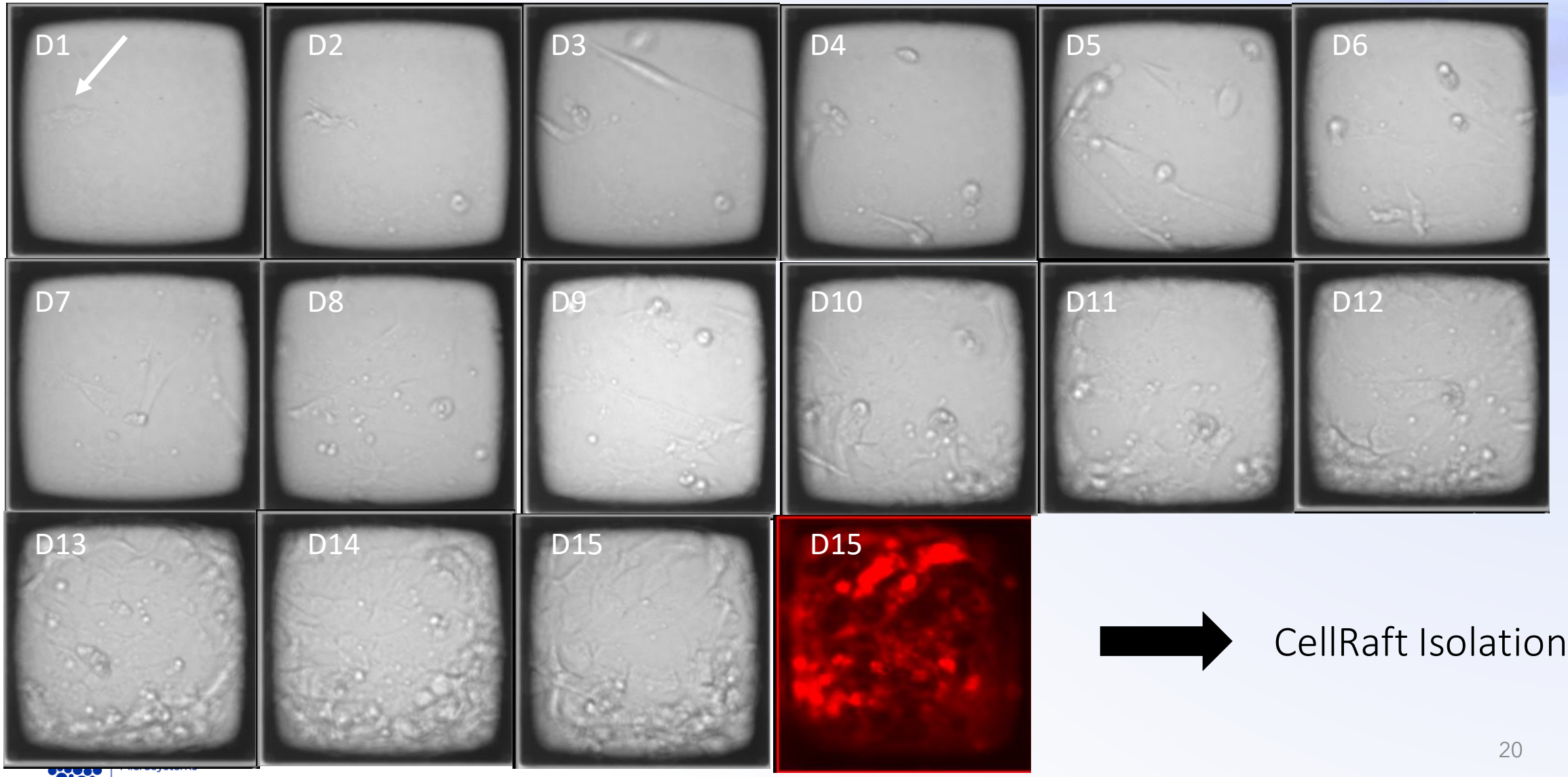
CellRaft Array





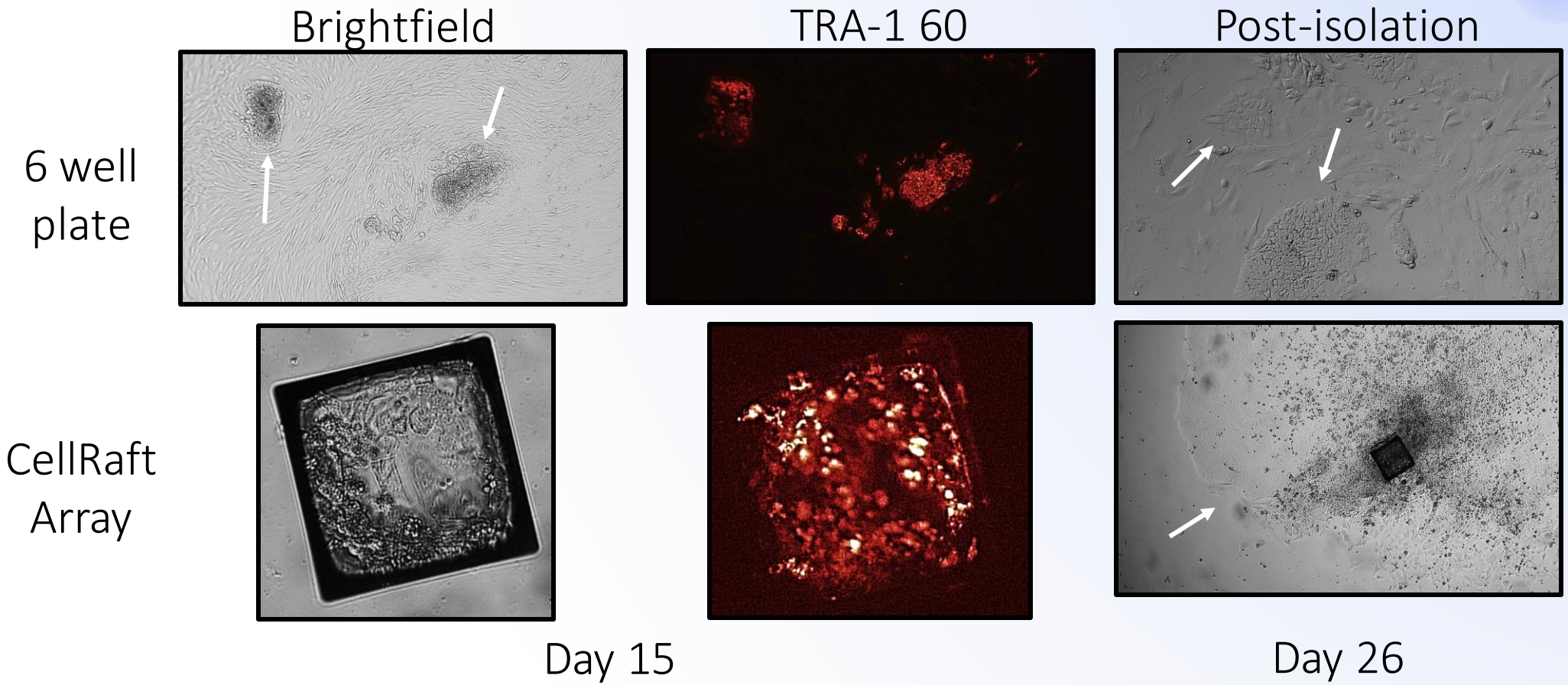


Reprogram: Direct-to-Array iPSC Reprogramming



CellRaft Isolation

Reprogram: Direct-to-Array iPSC Reprogramming

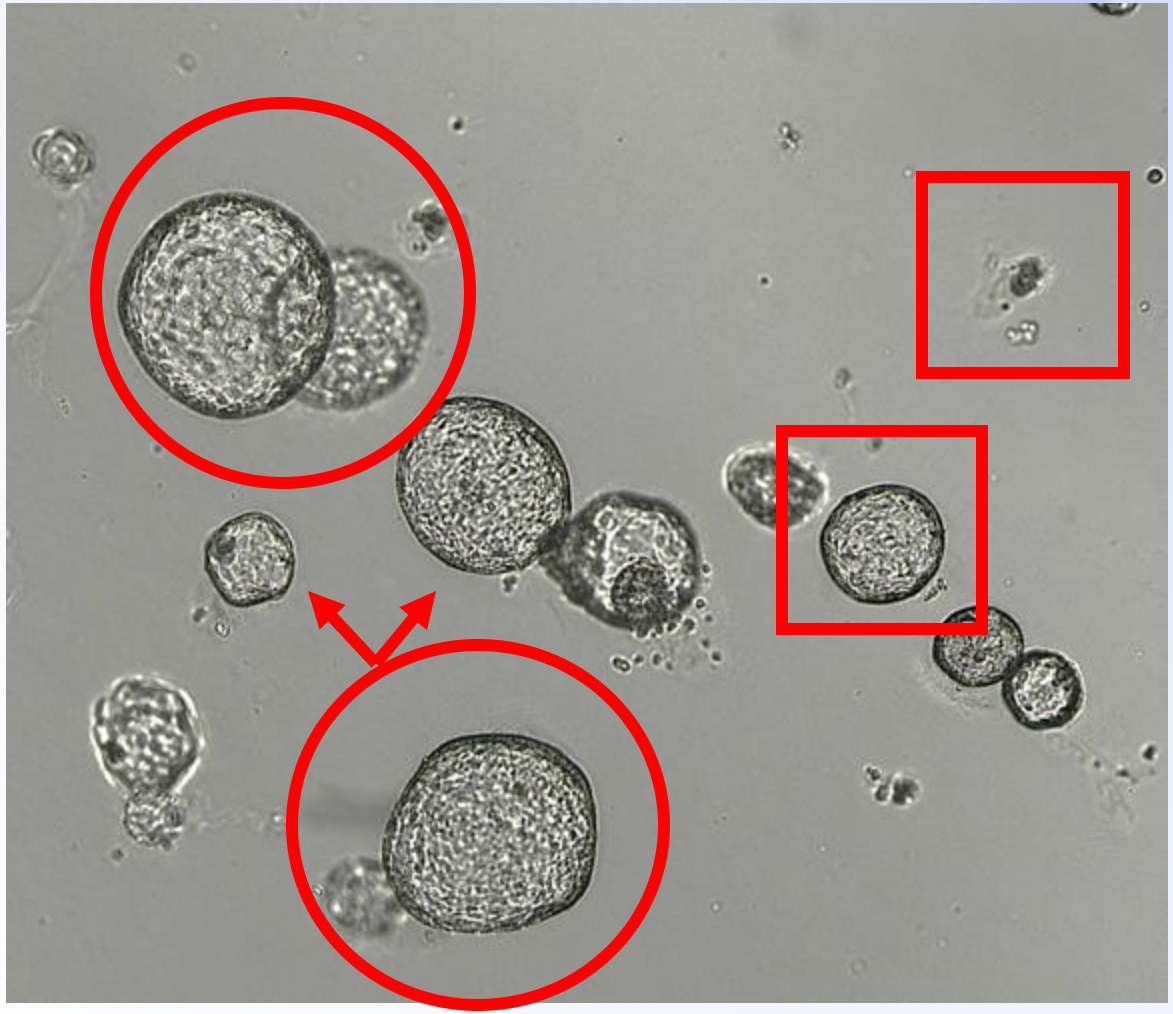


Improve the Efficiency of 2D iPSC Workflows

- **Track and trace** iPSCs from single cell to clone
  - Characterize
  - Edit
  - Reprogram
- Increase clonal iPSC generation by **25X** compared to limiting dilution
- Confirm pluripotency on-array prior to clonal expansion
- Reduce **time, consumables, materials, and labor** required for iPSC clonal development
- Enable **2D and 3D** applications including cell line development, reprogramming, and differentiation

Grow, Image, and Isolate 3D iPSCs

- Traditional dome culture challenges:
  - Multifocal imaging
  - Heterogeneity
  - Viability inconsistencies
  - Pooled readouts



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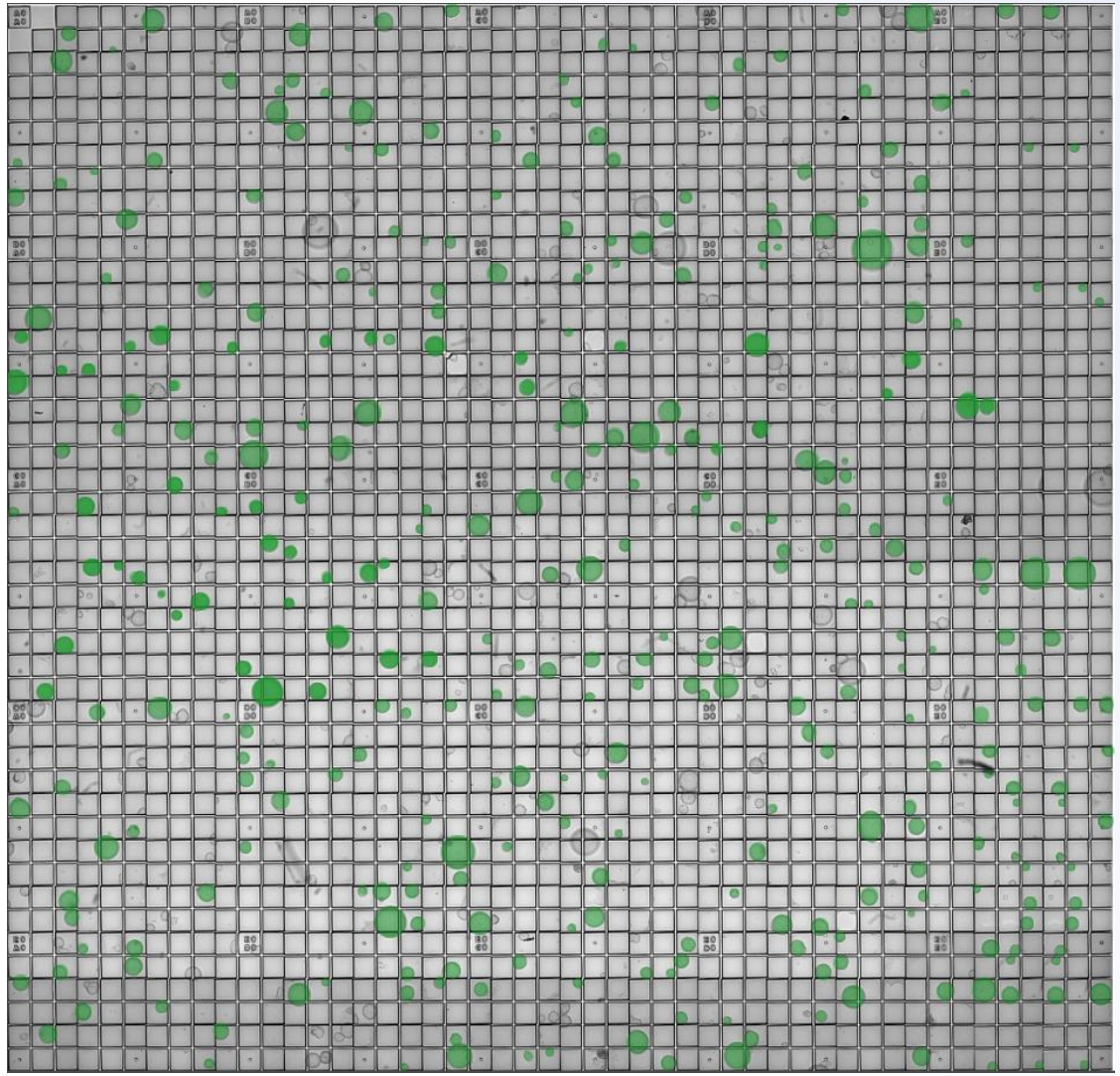
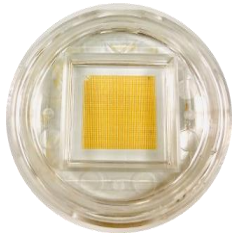
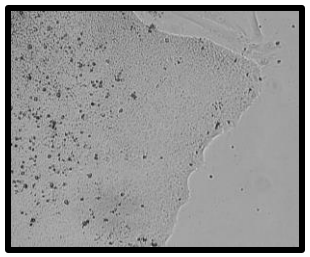
Outside the Dome: A Novel Culture Method for Organoids



- Grow and maintain hundreds of individual organoids
- Serially image the same organoid over time
- Phenotypically characterize to identify individual organoids of interest

Outside the Dome: A Novel Culture Method for Organoids





Prepare

- 3D CellRaft Array (500µm CellRafts)

Dissociate

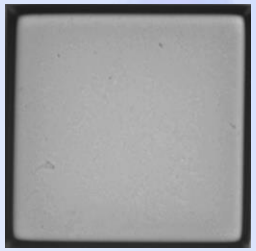
- Enzymatic dissociation of iPSCs into single-cell suspension

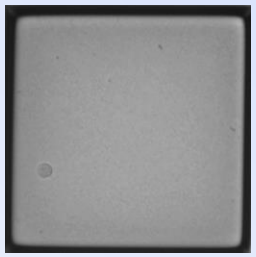
Culture

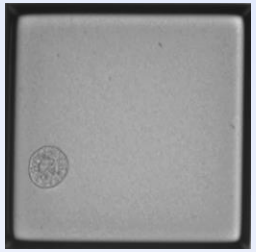
- Seed single-cell iPSCs in CellRaft Suspension culture in ECM

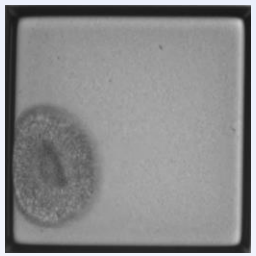
Scan

- CellRaft AIR System
- Perform serial 2D scans to monitor organoid development









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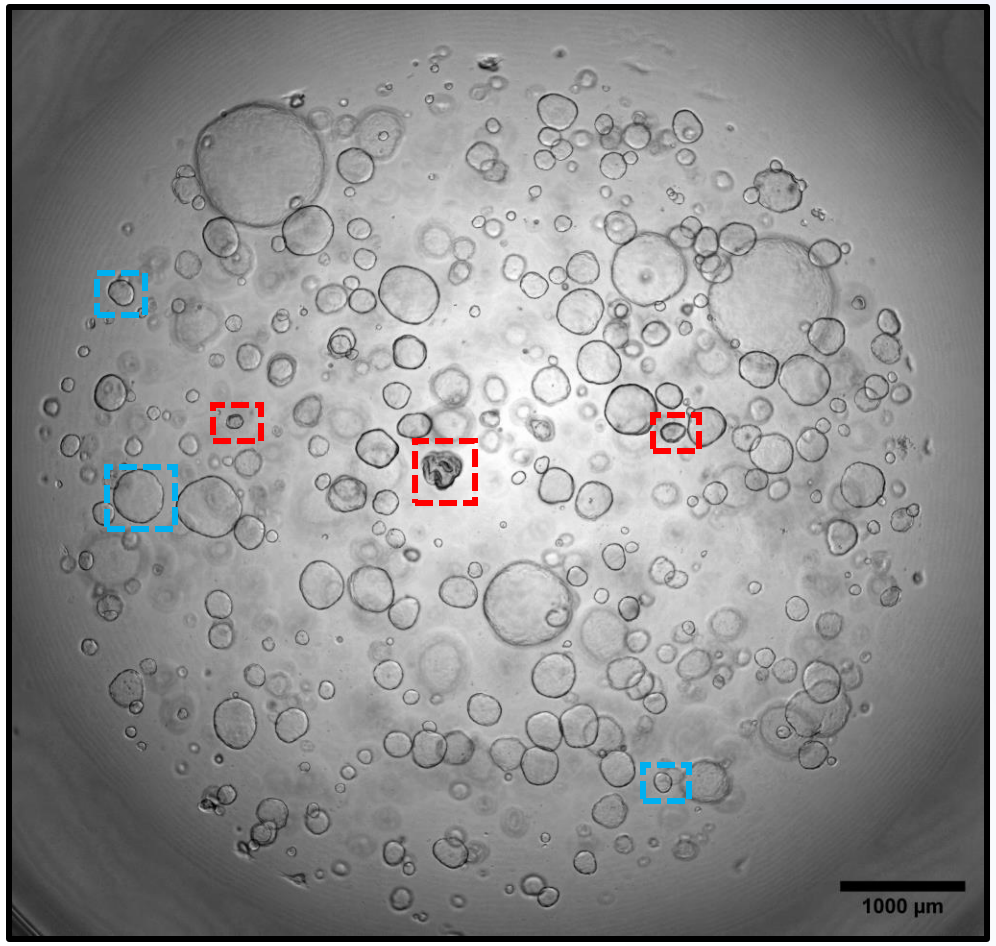
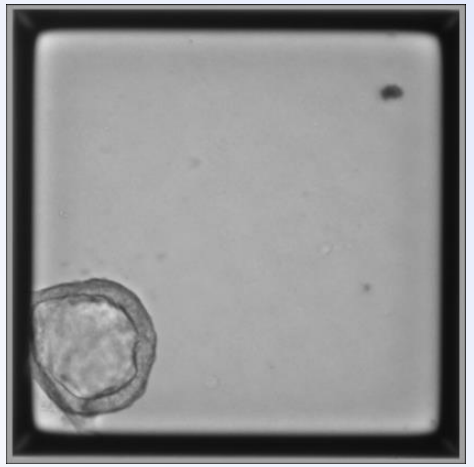
Comparing Dome vs CellRaft Array Suspension Culture

Dome Culture

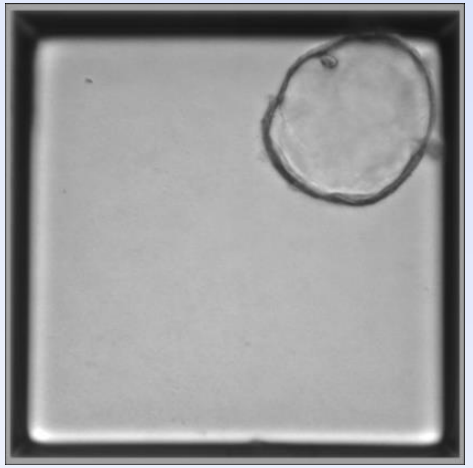


CellRaft Array Suspension Culture

Columnar organoid

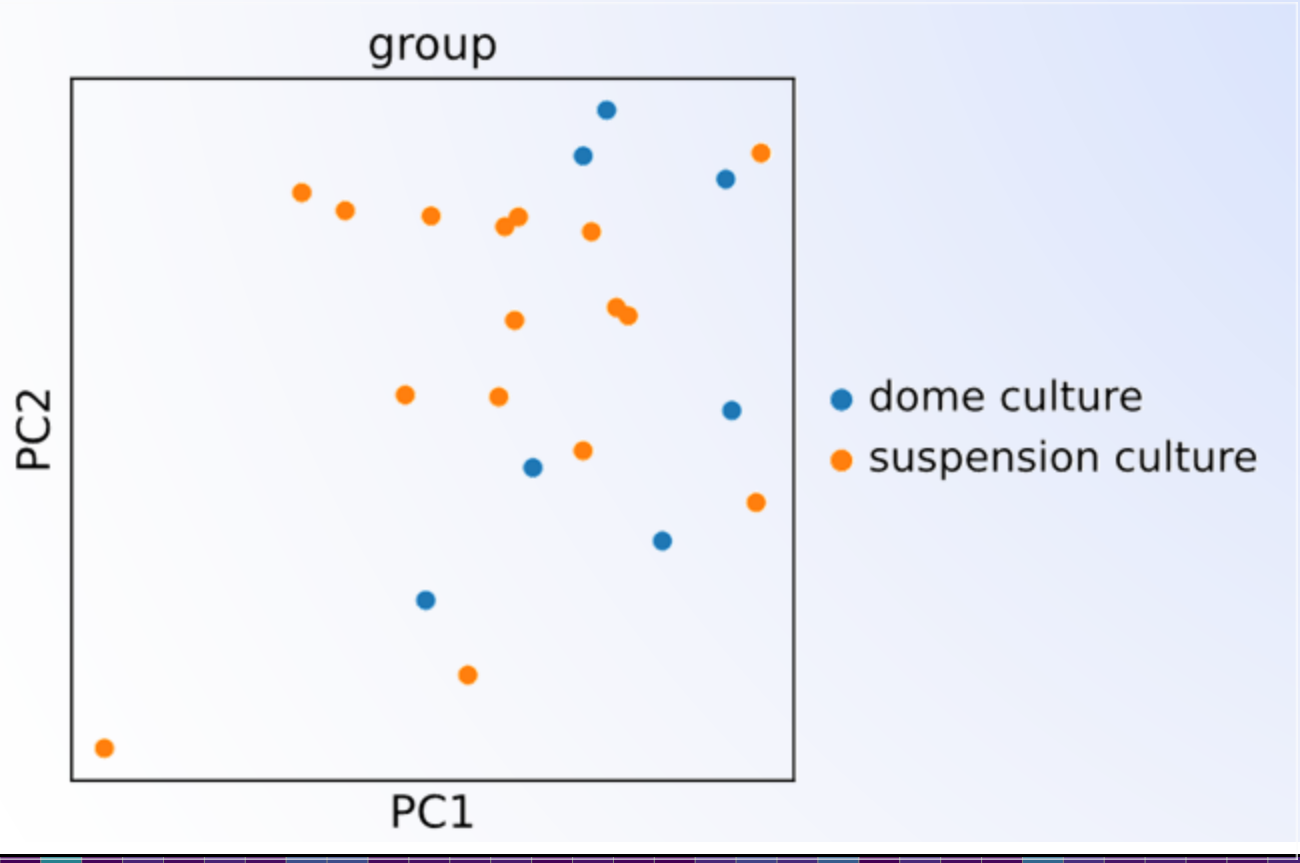


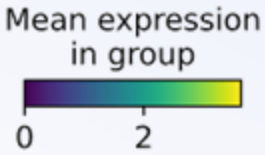
Cystic organoid



Primary mouse gastric stem cells
IntestiCult™ Organoid Growth Medium (Mouse)

Comparing Dome vs CellRaft Array Suspension Culture

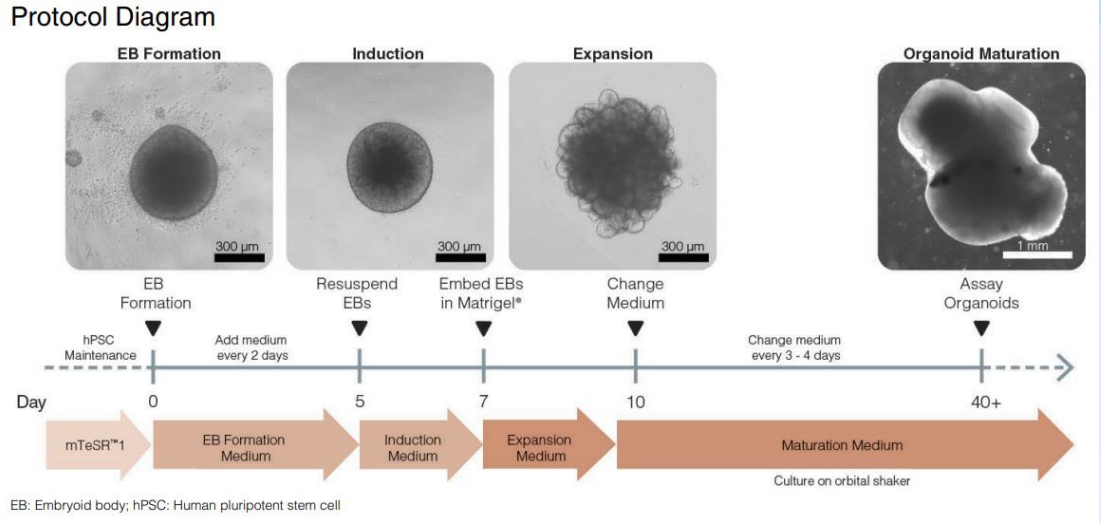




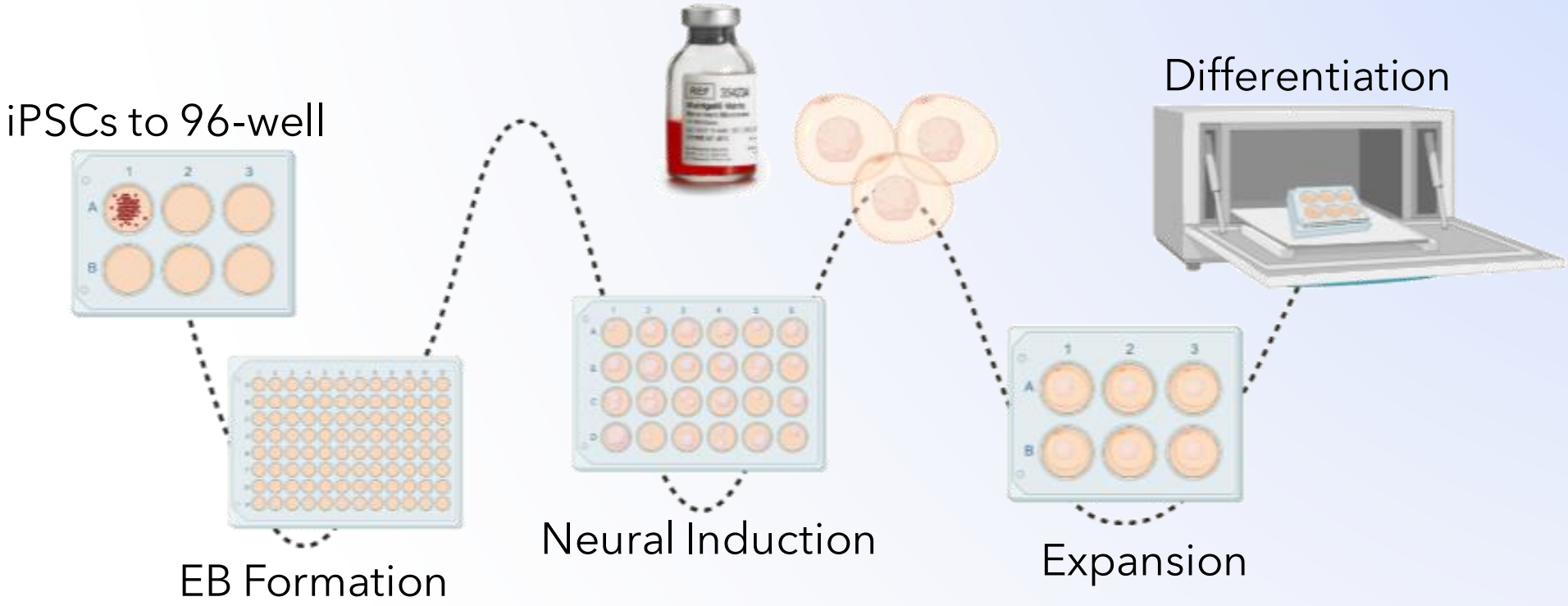
Research with iPSC-derived Organoids

- Developmental Biology
- Cancer
- Custom Disease Modeling

- Neuronal Organoids:
  - Brain development and evolution
  - Cancer
  - Alzheimer's Disease
  - Stroke

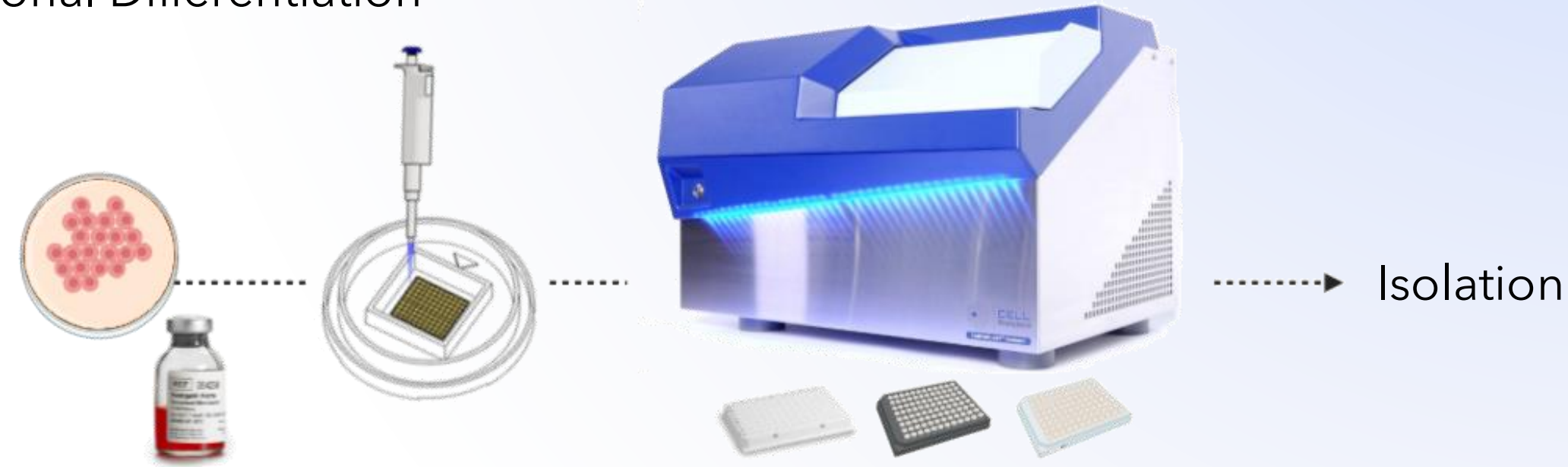


Neuronal Organoid Differentiation from iPS Cells

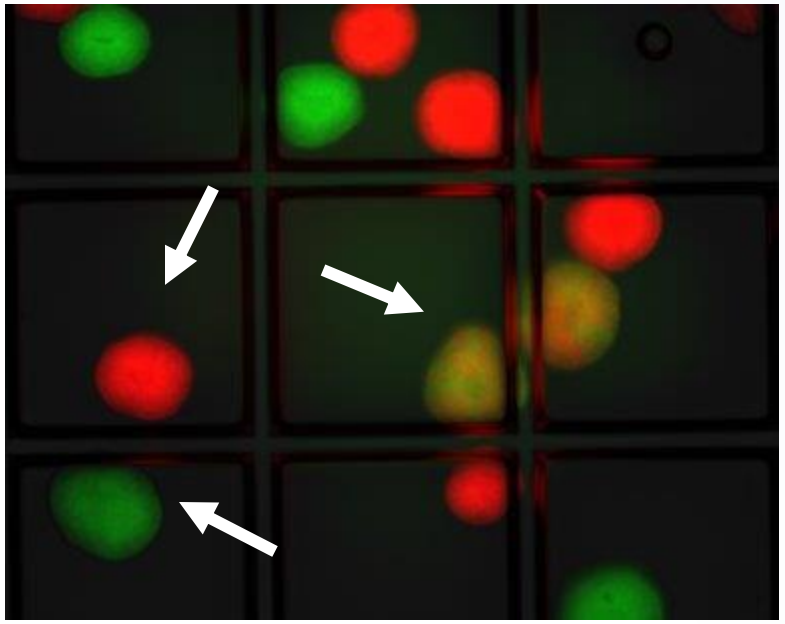


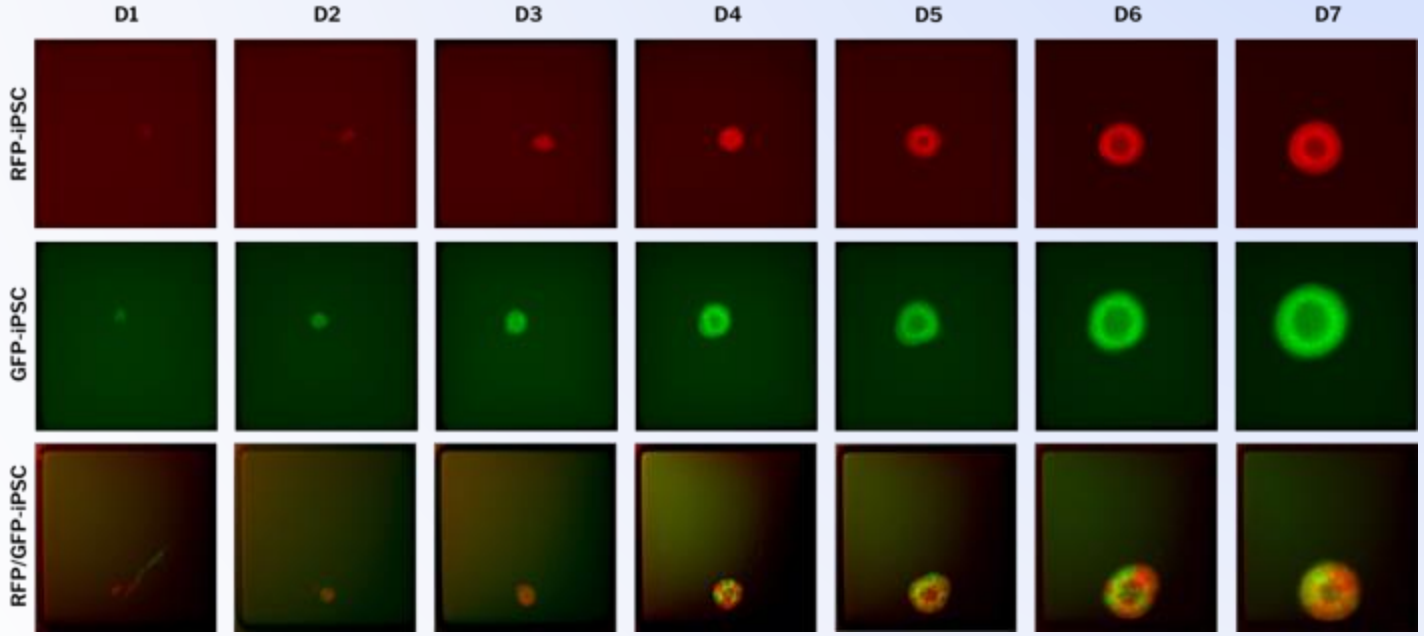
CellRaft Array Neuronal Organoid Workflow

- Seed iPSCs in ECM Suspension
- EB Formation
- Neural Induction
- Expansion
- Neuronal Differentiation



Differentiation: Track Differentiation of Edited iPSCs



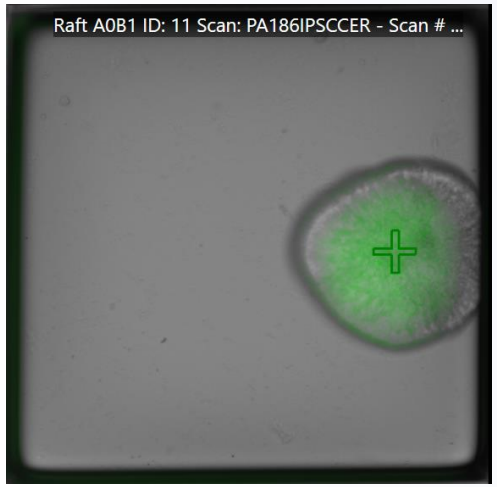


iPSC-derived Organoid Forming Efficiency

Single iPSCs
Day 1

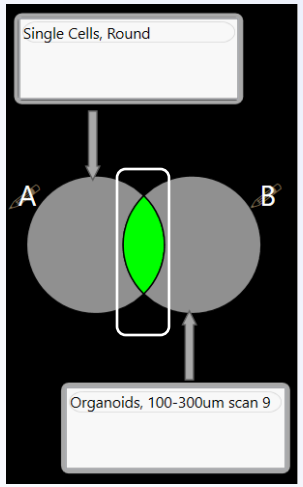
+

Organoids 100-300um
Day 10

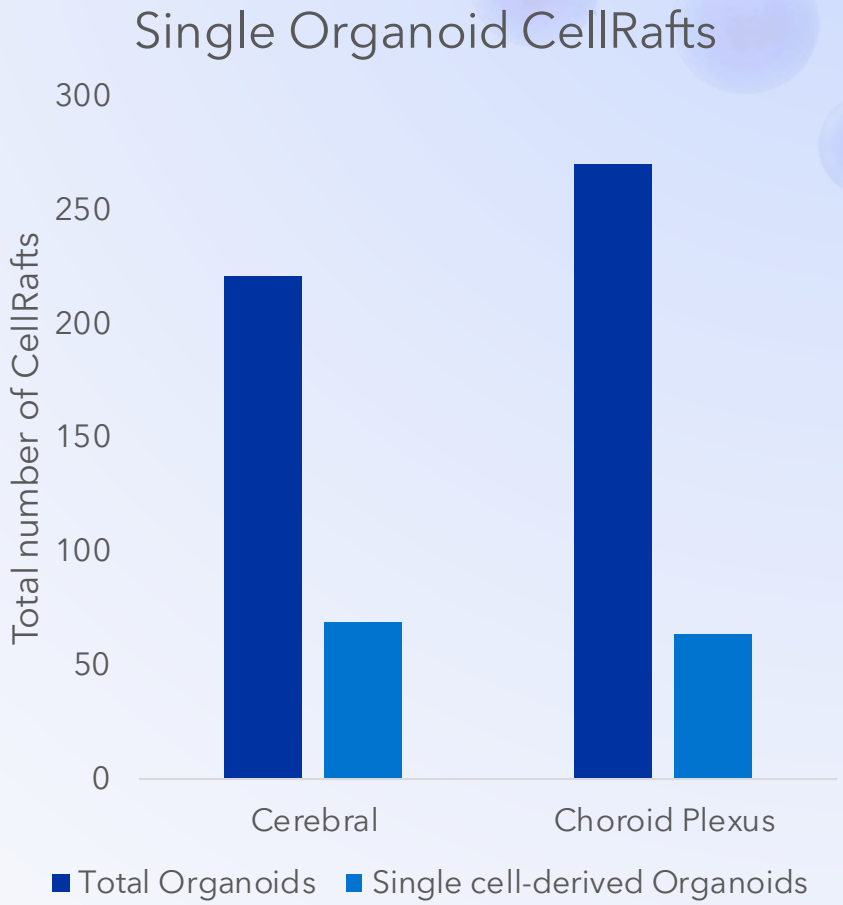


=

Single iPSC-derived
Organoids

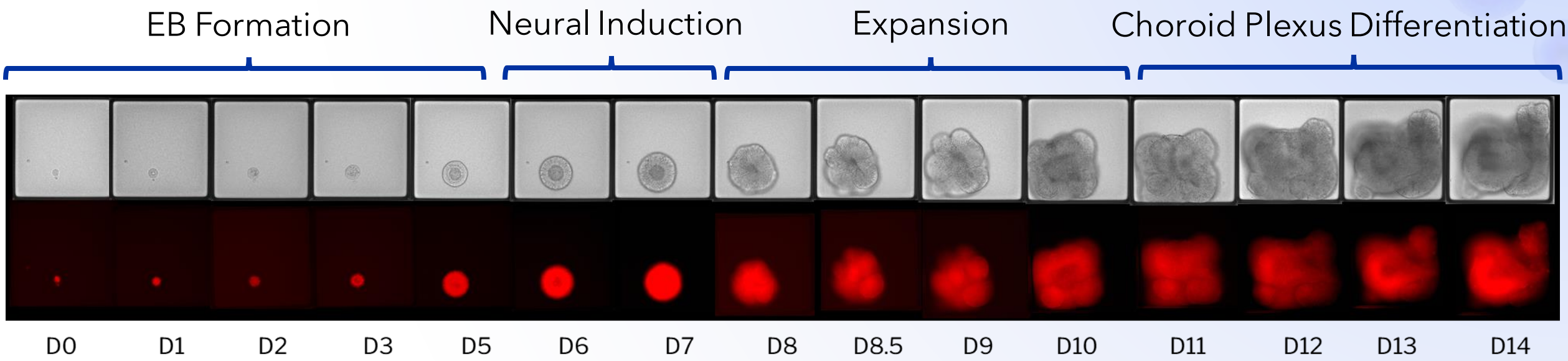


Single-cell Organoid Efficiency > 17%

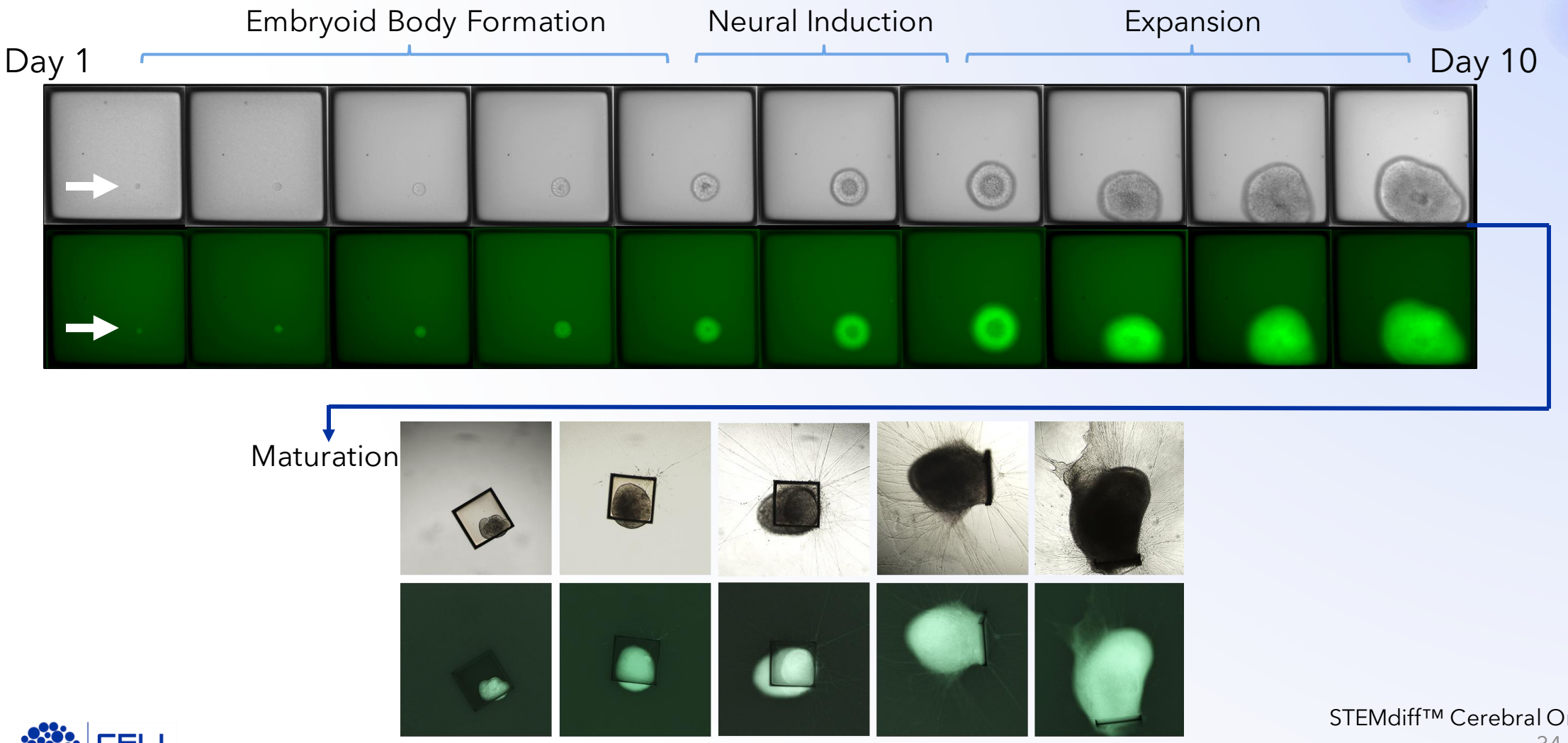


n = 3 arrays per organoid type

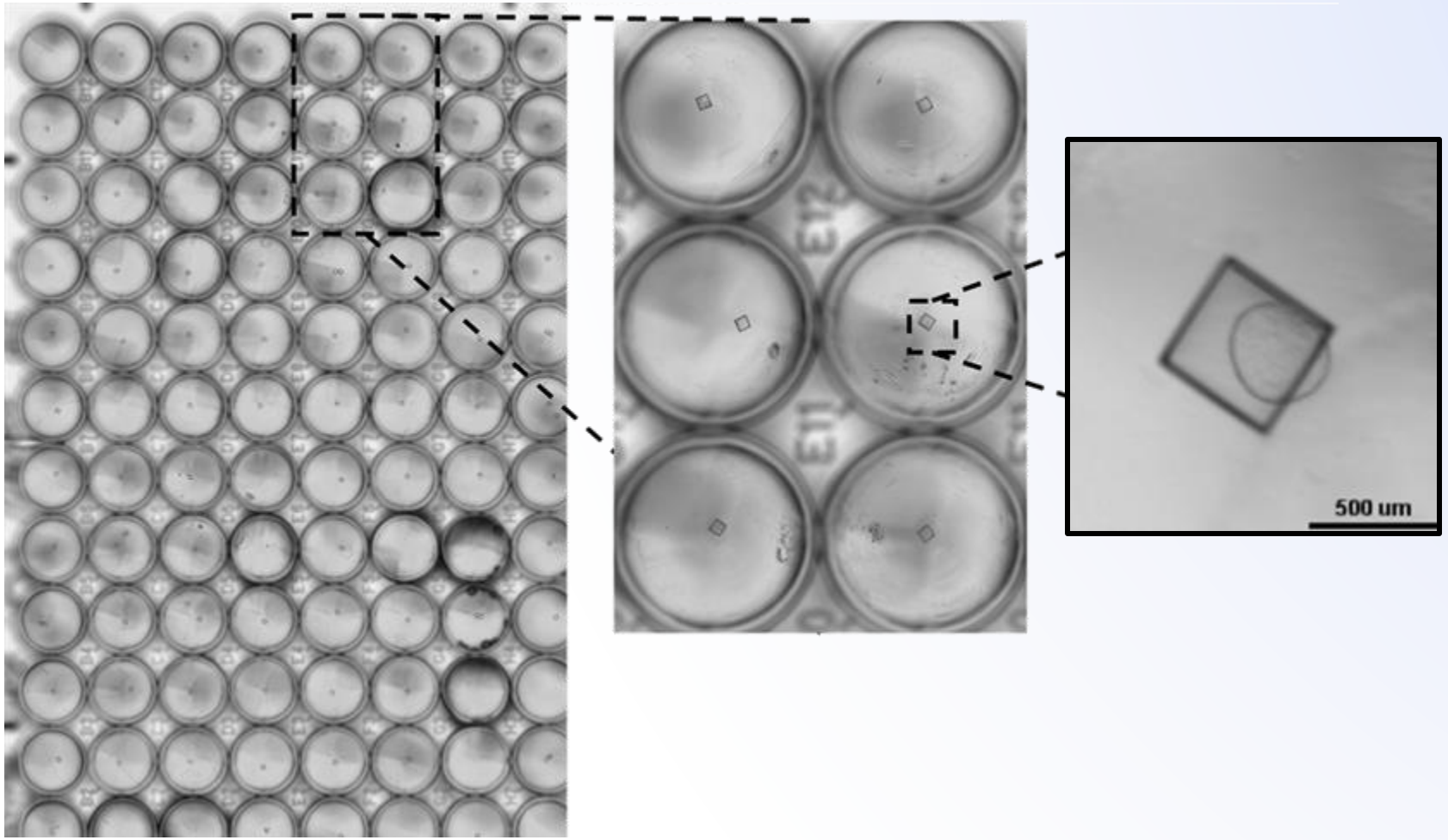
Differentiate: Choroid Plexus Organoid Differentiation



Differentiate: Cerebral Organoid Differentiation

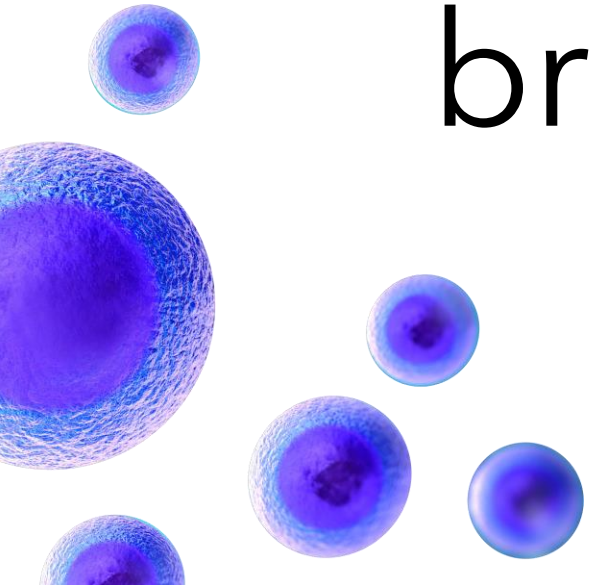


Create Custom iPSC-Derived Organoid Plates for Cell-based Assays



- Transcriptomics
- Drug Screening
- Toxicology
- Patient Derived

How would a full plate of characterized single organoids enable YOUR breakthrough research?



Poll Question #2

Acknowledgements

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University of North Carolina

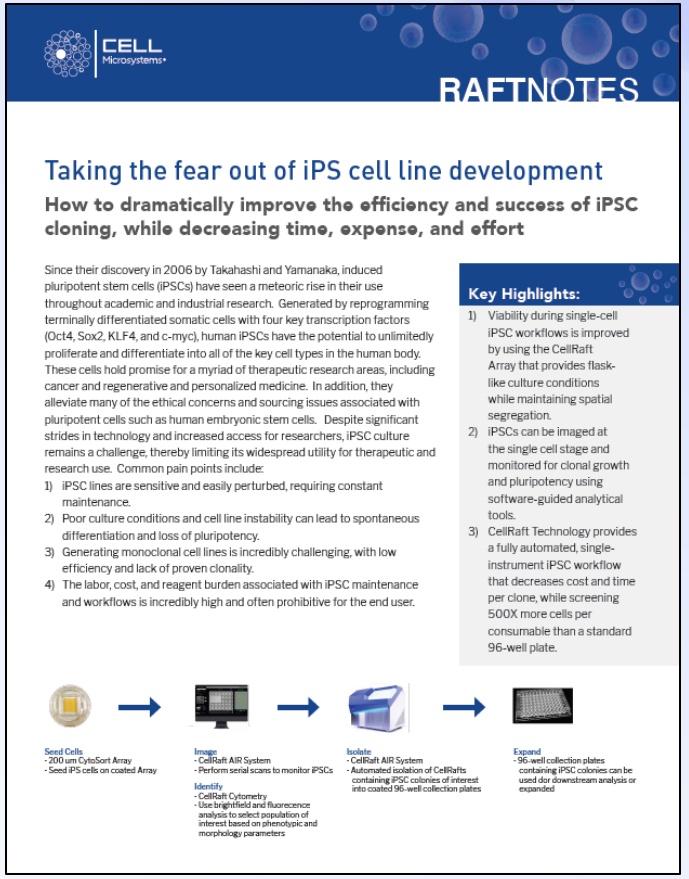
Jarrett Bliton

Scott Magness

Thank You!

Contact:
Jessica Hartman
jhartman@cellmicrosystems.com

Access the iPSC Application Note in the Resources Section of the Webinar



CELL Microsystems

RAFTNOTES

Taking the fear out of iPS cell line development

How to dramatically improve the efficiency and success of iPSC cloning, while decreasing time, expense, and effort

Since their discovery in 2006 by Takahashi and Yamanaka, induced pluripotent stem cells (iPSCs) have seen a meteoric rise in their use throughout academic and industrial research. Generated by reprogramming terminally differentiated somatic cells with four key transcription factors (Oct4, Sox2, KLF4, and c-myc), human iPSCs have the potential to unlimitedly proliferate and differentiate into all of the key cell types in the human body. These cells hold promise for a myriad of therapeutic research areas, including cancer and regenerative and personalized medicine. In addition, they alleviate many of the ethical concerns and sourcing issues associated with pluripotent cells such as human embryonic stem cells. Despite significant strides in technology and increased access for researchers, iPSC culture remains a challenge, thereby limiting its widespread utility for therapeutic and research use. Common pain points include:

1) iPSC lines are sensitive and easily perturbed, requiring constant maintenance.
2) Poor culture conditions and cell line instability can lead to spontaneous differentiation and loss of pluripotency.
3) Generating monoclonal cell lines is incredibly challenging, with low efficiency and lack of proven clonality.
4) The labor, cost, and reagent burden associated with iPSC maintenance and workflows is incredibly high and often prohibitive for the end user.

Key Highlights:

1) Viability during single-cell iPSC workflows is improved by using the CellRaft Array that provides flask-like culture conditions while maintaining spatial segregation.
2) iPSCs can be imaged at the single cell stage and monitored for clonal growth and pluripotency using software-guided analytical tools.
3) CellRaft Technology provides a fully automated, single-instrument iPSC workflow that decreases cost and time per clone, while screening 500X more cells per consumable than a standard 96-well plate.

Seed Cells
- 200 um CytoSort Array
- Seed iPS cells on coated Array

Image
- CellRaft AIR System
- Perform serial scans to monitor iPSCs

Identify
- CellRaft Cytometry
- Use brightfield and fluorecence analysis to select population of interest based on phenotypic and morphology parameters

Isolate
- CellRaft AIR System
- Automated isolation of CellRafts containing iPSC colonies of interest into coated 96-well collection plates

Expand
- 96-well collection plates containing iPSC colonies can be used dor downstream analysis or expanded

More Information:
cellmicrosystems.com