Simplifying Progress

Automating 3D culture model systems: Delivering robust, reproducible solutions to complex tissue model culture

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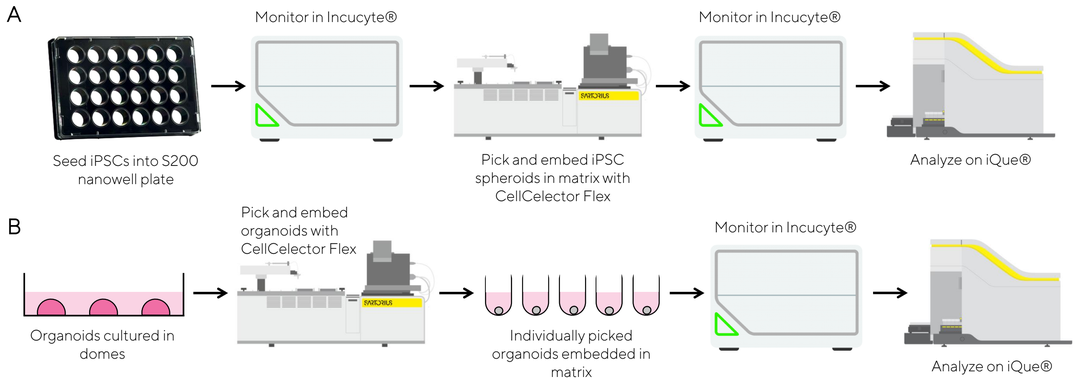
Introduction

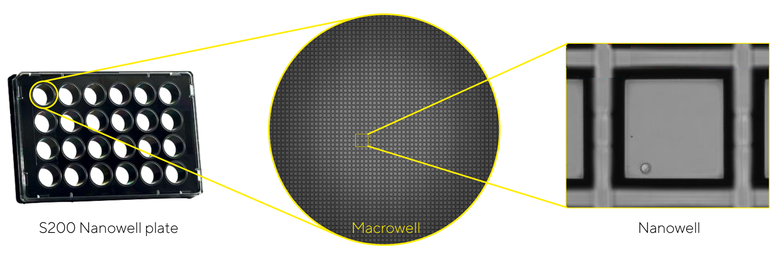
- 3D models such as spheroids and organoids are becoming increasingly relevant for the study of disease and development.
- Generation of these models can prove to be highly complex, expensive and resource intensive, necessitating efficient and reliable methods for their development, monitoring and characterization.
- Further complexity is inherent in the production of disease models that contain populations of cells with specific markers or genetic mutations associated with disease.
- Development of such models requires accurate manipulation of cells to successfully create a physiologically relevant system.
- In this study, spheroids were cultured from individual iPSCs to produce clonal iPSC spheroids derived from a single cell, while human iPSC derived hepatic organoids were selected from Matrigel® domes based on their attributes and transferred for further culture and analysis.
- Here, we outline a simple, standardized, and robust workflow using CellCelector Flex to identify and pick 3D cell models for further development based upon key growth and morphological attributes.
- During the workflow, the Incucyte® Live-Cell Analysis System and the iQue® HTS Cytometer were used to monitor growth and morphological phenotype over time and phenotypic characterization through marker expression analysis.
- This approach simplifies the culture and generation of organoids and monoclonal spheroids from iPSCs for drug discovery, development, and toxicity studies.

3D model workflows

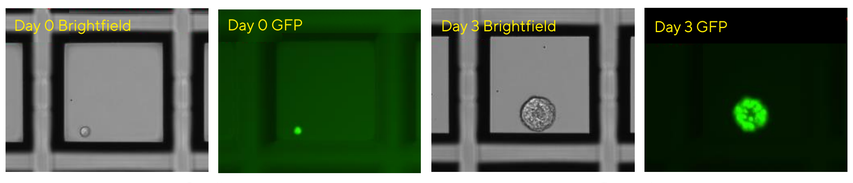


- The 3D model workflows outline the production of (A) iPSC spheroids from single cells and (B) the identification and isolation of individual organoids from Matrigel® dome cultures.
- Over a period of 10 days, monoclonal iPSC spheroids were cultured from individual GFP-expressing cells.
- Isolation of organoids based on specific characteristics provides researchers with a powerful tool for 3D cell model development and research.

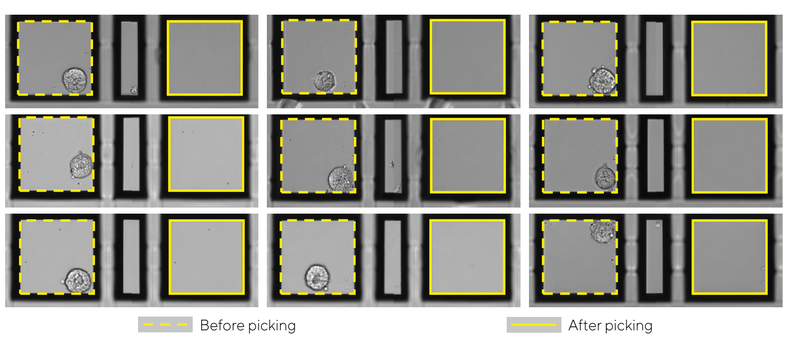
Monoclonal iPSC spheroid generation



- S200 nanowell plates provide thousands of nanowells in one macrowell of a standard tissue culture plate.
- Nuclight Green GFP expressing iPSCs were seeded into the nanowell plate at a density to encourage a single cell to deposit into each nanowell.
- After 3 days in culture, healthy GFP expressing iPSC spheroids had formed across multiple wells.



Picking and embedding iPSC spheroids



- iPSC spheroids were identified and picked using the CellCelector Flex.
- Individual wells were scanned and analyzed to identify those spheroids expressing GFP that were derived from a single cell.
- Only those spheroids were targeted for picking.
- The CellCelector picked each spheroid from its individual nanowell and resuspended it in hydrogel.
- The hydrogel embedded spheroid was seeded into a well of medium in a 96-well ULA plate.
- The plates were then transferred to the Incucyte® for monitoring.

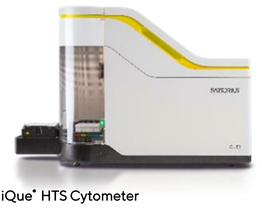
CellCelector Flex, Incucyte® & iQue® Systems

CellCelector Flex Cell Picking Platform

An automated cell selection and picking platform providing solutions to complex experimental methods such as clone development and 3D spheroid and organoid culture.

Incucyte® Live-Cell Analysis System

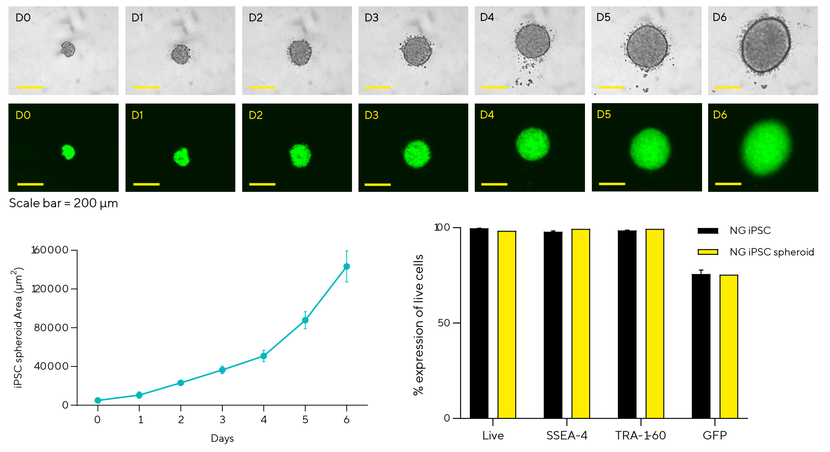
A fully automated phase contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.



iQue® HTS Cytometer

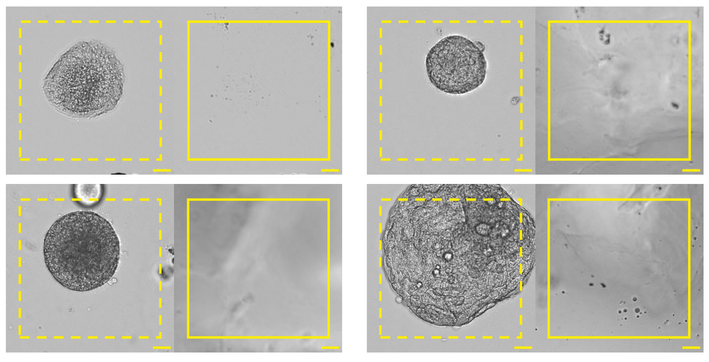
A high throughput screening cytometry platform with a patented sampling method allowing for rapid sample acquisition to deliver fast actionable results. Capable of handling 96 and 384 well plates.

Monitoring and characterizing iPSC spheroids



- Hydrogel embedded Nuclight Green (NG) iPSC spheroids were monitored in the Incucyte® for 6 days post picking to assess growth and morphology.
- Incucyte® micrographs highlight the steady growth rate of the spheroids while maintaining GFP expression.
- Analysis of Incucyte® captured images illustrate robust spheroid growth through brightfield area measurements.
- Marker expression analysis on iQue® shows high viability, pluripotent marker expression and GFP expression in iPSC spheroids when compared to source iPSCs.
- Spheroids isolated and seeded using CellCelector are healthy and maintain expression profiles.

Identification and isolation of organoids



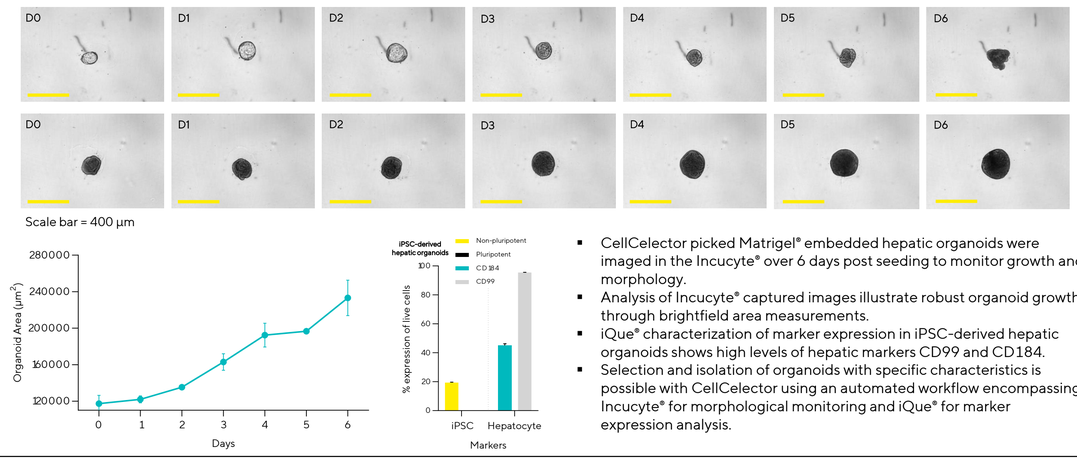
- iPSC derived hepatic organoids cultured in Matrigel® domes were identified and picked using the CellCelector Flex.
- Organoids were selected based on size and morphology, with different sizes selected to evaluate picking accuracy and success rate.
- Post picking, the organoids were embedded in a Matrigel® droplet and seeded into 96-well ULA plates.
- The plates were transferred to the Incucyte® for monitoring of growth and changes in morphology.

Before picking

After picking

Scale bar = 250 µm

Monitoring and characterizing organoid development



- CellCelector picked Matrigel® embedded hepatic organoids were imaged in the Incucyte® over 6 days post seeding to monitor growth and morphology.
- Analysis of Incucyte® captured images illustrate robust organoid growth through brightfield area measurements.
- iQue® characterization of marker expression in iPSC-derived hepatic organoids shows high levels of hepatic markers CD99 and CD184.
- Selection and isolation of organoids with specific characteristics is possible with CellCelector using an automated workflow encompassing Incucyte® for morphological monitoring and iQue® for marker expression analysis.