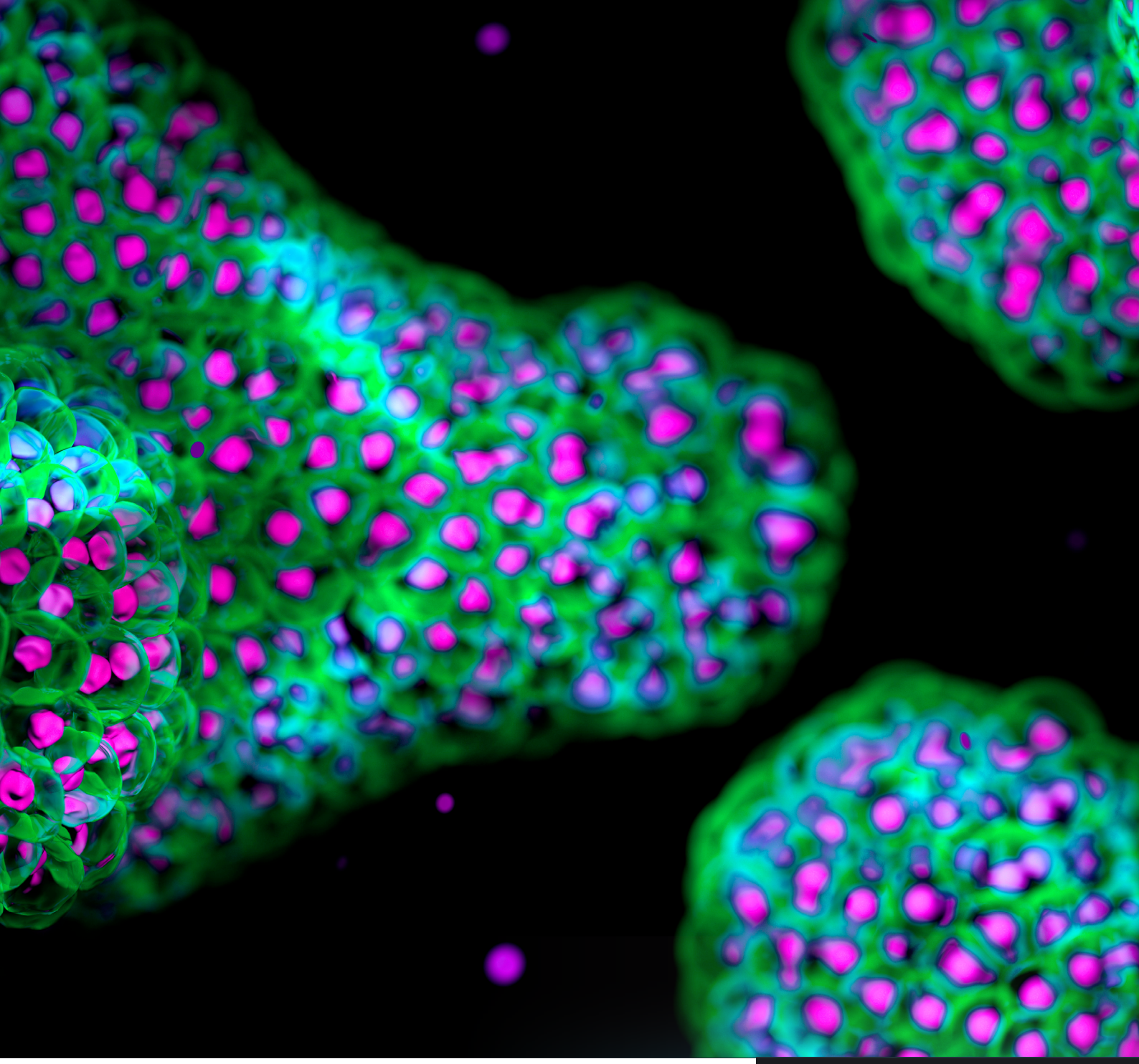

The Future of 3D Cell Analysis: From Isolation to Insight

Simplifying Progress

Get Started

SARTORIUS

Introduction

Three-dimensional (3D) cell models, such as organoids and spheroids, are transforming medicine by providing crucial insights for understanding diseases and developing targeted treatments. However, working with 3D cell models presents unique challenges. These structures are more complex than 2D cultures and require careful handling to maintain their integrity and viability. They also need frequent monitoring as they grow and mature to ensure the expected function. Additionally, high-throughput processing is critical for efficiently generating and analyzing large volumes of data, as traditional methods may not suffice for the scale required in modern research.

Life science instrument technology companies are meeting these demands with high-throughput solutions that support different stages of the workflow. For monitoring cell health, morphology, maturation, and function, live-cell imaging and analysis platforms offer unique benefits tailored to 3D culture. First, they provide non-disturbing continuous monitoring while the cells grow, helping to preserve cellular biology. Second, real-time kinetic tracking offers valuable insights into gene expression and cell behavior in response to varied conditions. The ability to isolate single cells, spheroids, and organoids with minimal damage is also crucial for maintaining high viability rates and ensuring the integrity of the research. Automated systems that can accurately detect, isolate, and transfer 3D structures are invaluable to high-throughput workflows.

This eBook explores the challenges and solutions in working with complex 3D models, emphasizing the importance of standardized workflows for high-throughput cell manipulation and analysis. It features application notes on culturing, maintaining, and characterizing induced pluripotent stem cells (iPSCs), as well as label-free, real-time live cell assays for 3D organoids embedded in Matrigel®. The featured technologies collectively enhance the reproducibility, efficiency, and physiological relevance of 3D cell models, accelerating breakthroughs from the bench to the bedside.

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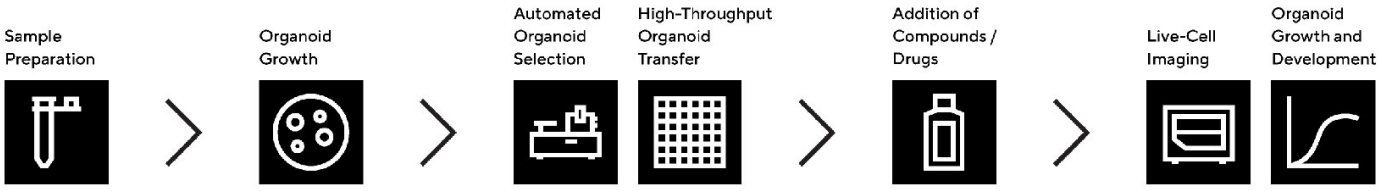
Workflow

Live-Cell Imaging as the End-Point Assay



Molecular Analysis as the End-Point Assay

Explore the Workflow, Here:

Application Note: Solutions for the Culturing, Maintaining and Characterization of Induced Pluripotent Stem Cells

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Kirsty McBain — Scientist - Sartorius
Nicola Bevan — Manager - Sartorius

Introduction

From drug discovery to organoid modeling of disease, stem cells are increasingly being used in research as a vital tool for scientific investigation. The current trend away from animal models and the push to more relevant systems for simulating the human body require flexible and specific tools to achieve this goal. Induced Pluripotent Stem Cells (iPSCs) are produced from normal tissue, through the forced expression of key transcription factors[1], providing a limitless supply of these precious cells for research and development. Due to the very specialized nature of these cells, their maintenance and culture is more intensive than most cell lines. For this reason, it is important that solutions for the culture and maintenance of these cell types are readily and widely available. Characterization of stem cells can be difficult and unreliable, depending on the methodology used, which is why it is important to develop robust techniques for monitoring stem cells throughout culture and experimental testing. If conditions are not optimal during the maintenance of iPSCs, their pluripotency can be lost.

Reproducibility is highly prized in research and automated solutions can provide high levels of consistency in method and data generation. The CellCelector Flex is an automated platform for targeted cell identification and picking that is not only highly accurate, but also very gentle on cells, providing an ideal solution when working with delicate iPSCs. The Incucyte® Live-Cell Analysis platform automates the imaging processes of iPSC workflows, allowing cells to be monitored over time to analyze changes in morphology and colony formation from within the incubator. This limits the disturbance to precious iPSC culture plates, but also enables real-time tracking of cell growth and health metrics.

Further characterization of iPSCs can be performed on the iQue® High-Throughput Screening (HTS) Cytometer, investigating changes in expression of pluripotency markers integral to maintaining stemness, providing an overview of the status of iPSCs.

This application note discusses the novel solutions provided by Sartorius platforms for the culture, maintenance, and characterization of iPSCs, during research and development.

Many traditional methods for culturing, monitoring and characterizing iPSCs can:

1. Be inconsistent and unreliable, resulting in seeded populations with high levels of heterogeneity, cell death and differentiation
2. Require regular disturbance of culture plates to monitor growth and confluency, with no integrated options for analysis
3. Demand large volumes of precious sample for analysis, resulting in less material for downstream applications
4. Necessitate the use of a variety of techniques to measure multiple characteristics

Methods

The following methods outline a flexible, in-depth workflow for growth and characterization of iPSCs using multiple Sartorius platforms.

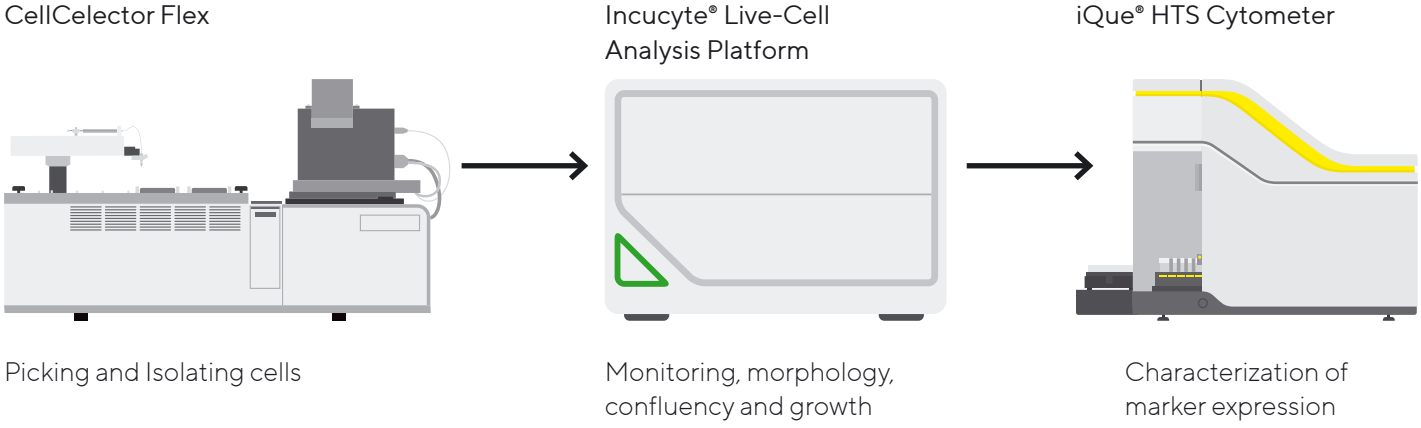


Figure 1. Schematic showcasing the use of Sartorius platforms in iPSC culture.
Using the three Sartorius instruments, CellCelector Flex, Incucyte® and iQue®, iPSCs can be picked and seeded, pluripotency tested, and growth and confluency monitored.

Cell Culture and Maintenance

Picking and Seeding iPSCs
Individual cells and colonies were picked using the CellCelector Flex with the Adherent Colony Picking Module and seeded into tissue culture plates for further expansion and downstream processing. Images were taken prior to and post picking to monitor and record the effects of colony manipulation using the CellCelector Flex. Propidium Iodide (PI) staining was undertaken on iPSC colonies after seeding by adding PI at a concentration of 500 nM and incubating for 3 minutes, rinsing twice with PBS and resuspending in growth medium (mTESR Plus) for imaging.

Thawing and Culturing iPSCs

Cells (ATCC-DYS0100 cells derived from human foreskin fibroblasts) were thawed and plated onto Vitronectin XF™ (1:25 dilution in CellAdhere™ Dilution Buffer) precoated 6-well plates at a seeding density of 1x10⁶ cells/well in 1 mL growth medium (mTESR™ Plus) supplemented with Y-27632 (ROCK inhibitor, 10 µM) and incubated at 37°C. iPSCs were monitored using the Incucyte® system to assess confluency, colony formation, and general cell morphology and health. The confluence of colonies was analyzed using the integrated Incucyte® AI confluence software algorithm. Passages were performed every 3-4 days at approximately 60-70% confluence using Gentle Cell Dissociation Reagent and replated at 1x10⁵ cells/well. Medium changes were performed daily during the week, while double volume medium changes were performed on Friday to account for no medium changes over the weekend. For the non-optimized iPSC culture, cells were grown as above except using RPMI 1640 medium supplemented with 10% FBS, L-glutamine 2 mM, Penicillin/Streptomycin 100 µg/mL.

Characterization and Monitoring of Pluripotency

Pluripotency Characterization: iQue® Platform

iPSCs were dissociated to single cells during passage and at specified timepoints using Gentle Cell Dissociation Reagent. Single cell suspensions were stained with cell surface marker antibodies (in PBS + 2% FBS) for one non-pluripotent marker, SSEA-1, and two pluripotency markers, SSEA-4 and TRA-1-60, in addition to the iQue® Membrane Integrity (B/Red) Dye, for viability analysis. Cells were seeded at 2x10⁴ cells/well in a V-bottom 96-well plate and stained with the cocktail of antibodies described (RT in the dark for 30 minutes). To wash plates, PBS + 2% FBS (100 µl) was added, prior to centrifugation (300 x g, 5 minutes), then aspirated. Plates were shaken (3000 rpm, 60 seconds) and the samples resuspended in PBS + 2% FBS (20 µL), prior to being analyzed on the iQue® HTS Cytometer. Analysis of data was performed using the iQue Forecyt® software after compensation had been optimized for each of the antibodies.

Monitoring Pluripotency and Cell Health: Incucyte® System

During the experiments, iPSCs were monitored for changes in morphology and confluency using the Incucyte® Live-Cell Analysis platform. Cultured iPSCs lines were monitored by high definition (HD) phase contrast at 4-hour intervals using a repeating scan schedule at 10X. Nuclear to cytoplasmic ratios were calculated by staining iPSC nuclei using the Incucyte® Nuclight Rapid Red Dye (1:1000) and measuring the cytoplasmic area (confluence mask) and the nuclear area (fluorescence mask) using basic masking to quantify pluripotency/normal iPSC morphology.

Intracellular and Surface Marker Studies

iPSC and control THP-1 cells were seeded at 2x10⁴ cells/well in a V-bottom 96-well plate and fixed, permeabilized and stained according to the protocol found in the following tech note: Intracellular Staining Assay for iQue® HTS Platform. Pluripotency markers, SSEA-4, TRA-1-60, Oct 3/4 and Sox-2 were analyzed, while SSEA-1 expression was used as a marker for non-pluripotency. Analysis was performed on the iQue Forecyt® software after compensation had been optimized for each of the antibodies.

Results

Developing workflows for the culture and characterization of stem cells such as iPSCs is vital in producing consistent, reproducible and robust data. Using the Sartorius platforms showcased here (Figure 1), we can highlight the benefits of the approaches described for culturing iPSCs that are healthy and pluripotent while monitoring and characterizing these stem cells for key markers of health and stemness.

Picking iPSCs Using the CellCelector Flex Is Fast, Gentle and Reliable

It is important when working with any cell system, but notably stem cells such as iPSCs, to maintain good cell health. The data here highlights the delicate, gentle picking and seeding capability of the CellCelector Flex. When stained with Propidium Iodide (PI), a stain that indicates cell death, manual manipulation of iPSCs produces an increased

number of PI positive cells when compared to the CellCelector Flex, indicative of fewer healthy cells (Figure 2A). The CellCelector Flex colony also has less debris and more tightly defined borders (Figure 2B).
The flexibility and power of the CellCelector Flex is exemplified by its capabilities, it is able to pick single iPSCs or whole iPSC colonies from a tissue culture plate.

This provides the opportunity to select ideal colonies from cultures on a standard plate for further propagation. Additionally, portions of colonies can be selected for further culture. This is useful if a portion of the colony spontaneously differentiates. Differentiated sections can be removed or pluripotent sections can be picked for passaging or analysis (Figure 2C-F).

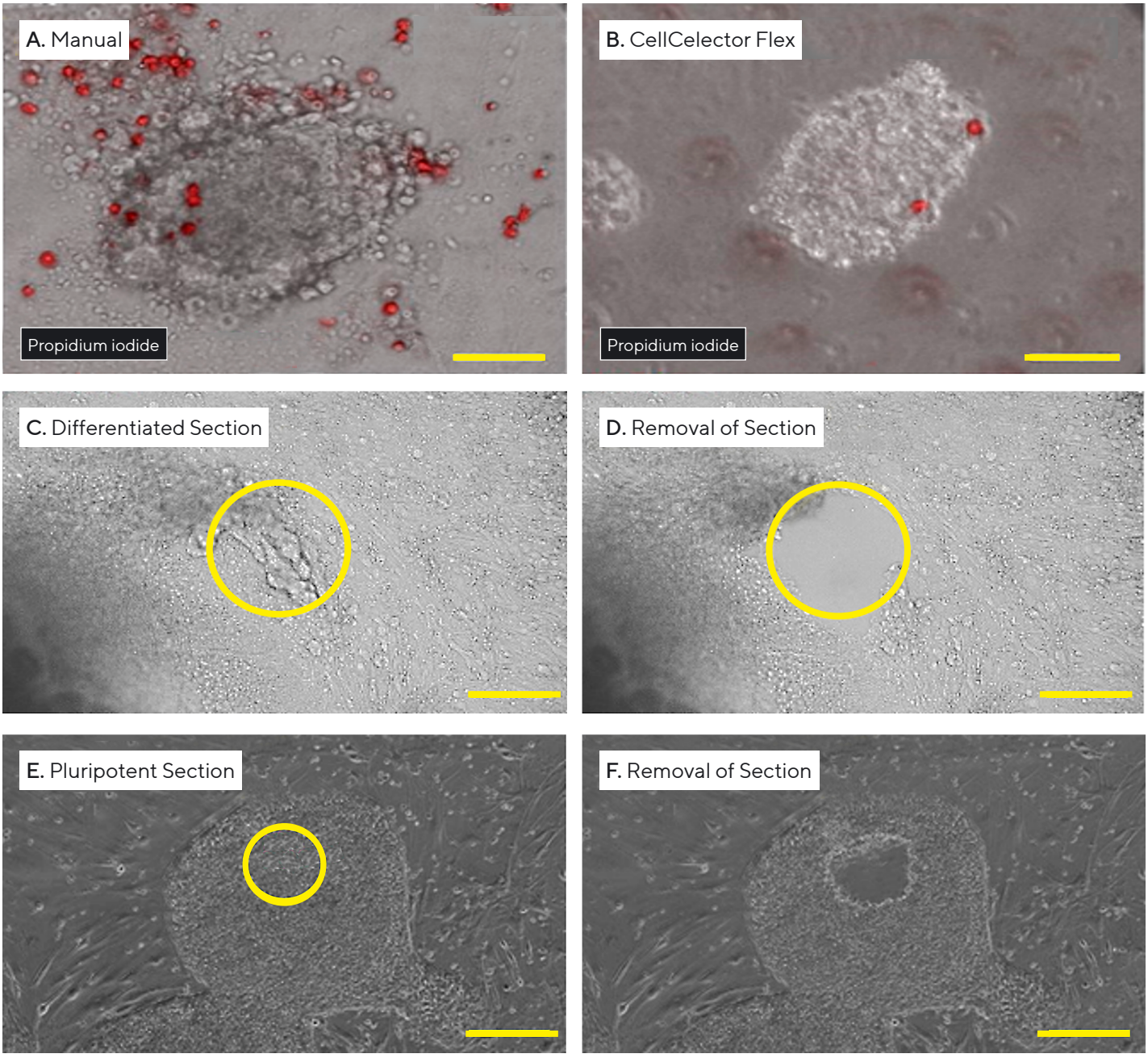


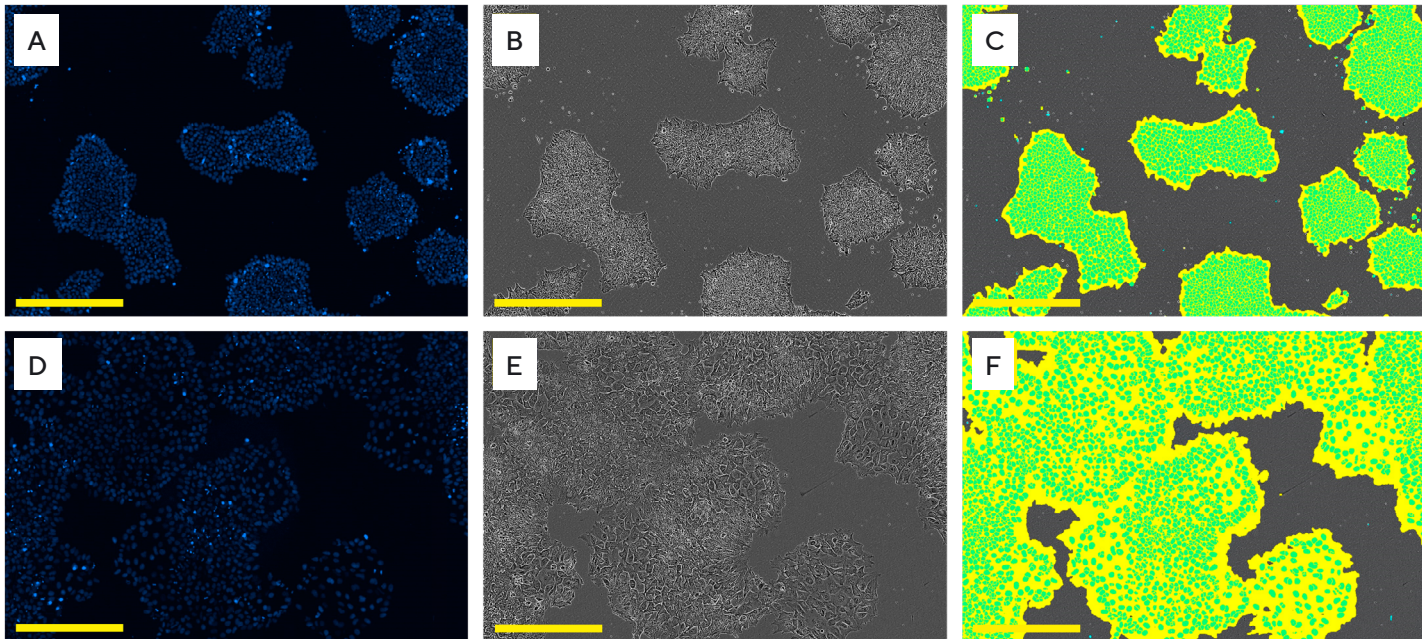
Figure 2. Picking iPSCs using the CellCelector Flex is accurate, fast, gentle and reliable.
Micrographs taken using the CellCelector platform highlighting iPSC colonies selected by the system. (A) Manually and (B) CellCelector picked and seeded iPSC colony stained with propidium iodide (PI) to identify cell death. (C) Micrograph depicting an area of differentiation in a stem cell colony prior to picking with the CellCelector. (D) The same area of the culture plate shown in (C) after removal. (E) Micrograph of a large iPSC colony grown on a feeder layer, prior to picking a section of pluripotent cells. The bottom right of the colony has indications of spontaneous differentiation. (F) The colony in (E) after picking using the CellCelector Flex, the area of pluripotent cells targeted by the machine has been collected for further culture. Scale bar equals 500 µm.

Monitoring Morphology and Pluripotent Potential During iPSC Culture

The CellCelector Flex can be used within the same workflow as another Sartorius platform, the Incucyte® Live-Cell Analysis platform. This system provides tools for monitoring cells during culture within the incubator, so changes in morphology can be recorded and analyzed without requiring removal of culture plates. In the following case, losses in morphological indicators of pluripotency can be observed, recorded, and subsequent analysis can be performed to quantify these changes.

Incucyte® images of iPSCs after 2 days in culture, show a marked difference in morphology between the optimized and non-optimized culture conditions. iPSCs grown in optimized conditions form tightly packed colonies with clearly defined edges, that 'glow' under phase images (Figure 3B), by contrast, non-optimized iPSCs are much more spread out and no longer form tightly packed colonies, they are beginning to resemble fibroblast cells (Figure 3E). Nuclear staining using Incucyte® Nuclight Rapid Red Dye also highlights the separation of the cells when grown in non-optimized conditions (Figure 3D), nuclei are much more spread out and lose the tight distribution found in optimized conditions (Figure 3A). Quantification of these morphological differences was performed using the Incucyte® Adherent Cell-by-Cell scan at 10X magnification and nuclear and cytoplasm area measurements were made using the Basic Analyzer and AI Confluence analysis (micrographs in Figure 3C, F) using the following equation to provide a nuclear/cytoplasm ratio, a standard measurement used when studying iPSCs. The graph in Figure 3G illustrates the reduction in this ratio in the non-optimized conditions, from 0.6 to 0.4. The more iPSC like, and thus pluripotent, a cell is, the higher the nuclear/cytoplasm ratio.

$$\frac{\text{total nuclei area}}{\text{total cytoplasmic area}} = \text{nuclear/cytoplasm ratio}$$



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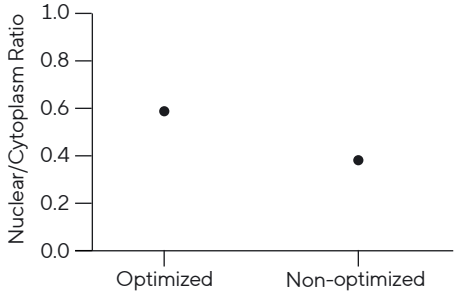


Figure 3. Monitoring morphology and pluripotent potential during iPSC culture. Incucyte® images of iPSCs grown under optimized (mTESR Plus) and non-optimized (RPMI) conditions. (A, D) Fluorescent images of iPSCs stained with Nuclight Rapid Red Dye comparing nuclear density between conditions. (B, E) Phase contrast images of the same iPSCs showing morphological differences between the two variables. (C, F) Analysis masking on the Incucyte® depicting confluency and nuclear masking that can be used to determine the nuclear/cytoplasm ratio illustrated in (G). Scale bar equals 400 µm.

Changes in iPSC Marker Expression Analyzed with the iQue® HTS Platform

To investigate further the losses in pluripotency in iPSCs when cultured in non-optimal conditions, surface marker expression of specific pluripotency markers can be analyzed with the iQue® HTS Platform, requiring as little as 10 µL per sample.

iPSCs grown in non-optimized conditions show rapid loss of pluripotency marker expression compared to optimized conditions (Figure 4). This indicates a loss in pluripotency correlating with the data collected on the Incucyte® platform (Figure 3). After 2 days in culture (Figure 4A), analysis of non-optimized conditions shows a decrease in expression of pluripotency markers SSEA-4 (97.3 ± 0.8%), TRA-1-60 (89.8 ± 0.9%), and the pluripotent population (34.6 ± 0.3%), with a further decrease after 4 days of treatment (SSEA-4 63.4 ± 2.9%, TRA-1-60 58.9 ± 2.9%, pluripotent population 19.3 ± 3.0%) when compared with optimized conditions (Figure 4B). In contrast, for the optimized iPSCs, no marked differences in expression profile over the time course of these studies was observed (95 ± 0.4% for pluripotent markers and less than 1.8 ± 0.5% for SSEA-1). (Figure 4A, B). In addition, the increase in non-pluripotent marker SSEA-1 expression (57.5 ± 0.7%) is clear as early as 2 days post treatment (Figure 4A) and remains high throughout culture.

In Figure 4C, (dot plots taken directly from iQue Forecyt® software) there is a clear shift in SSEA-1 expression between the optimized (1.63% SSEA-1 positive) and non-optimized conditions (57.5 % SSEA-1 positive) (upper two dot plots). The lower plots further illustrate the shift away from pluripotent marker expression in the non-optimized conditions, where the optimized iPSCs present a compact population in the upper right quadrant of the plot (SSEA-4+, TRA-1-60+) while the non-optimized iPSCs present a much more spread population shifting into the TRA-1-60 negative portion of the plot.

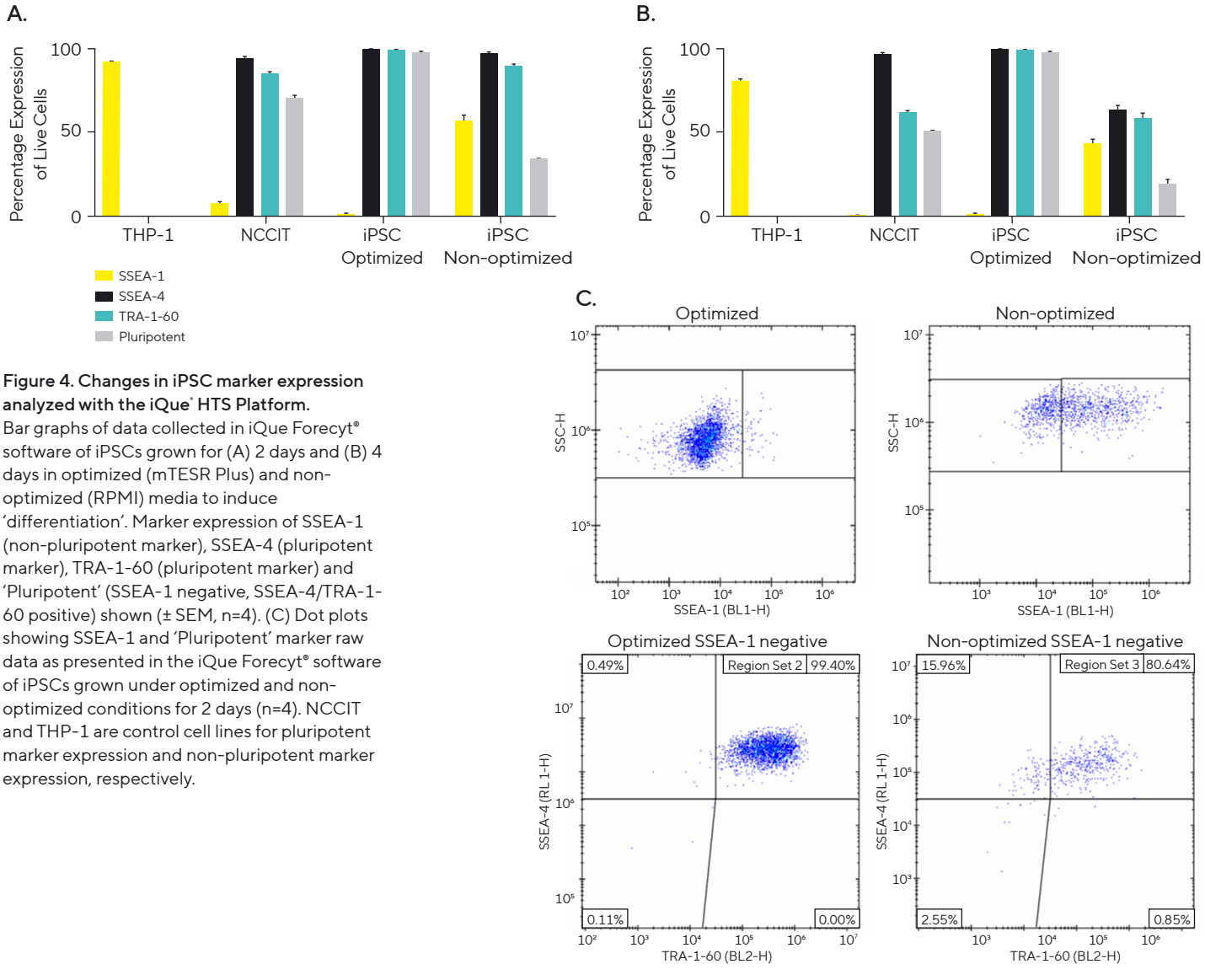


Figure 4. Changes in iPSC marker expression analyzed with the iQue® HTS Platform.
Bar graphs of data collected in iQue Forecyt® software of iPSCs grown for (A) 2 days and (B) 4 days in optimized (mTESR Plus) and non-optimized (RPMI) media to induce 'differentiation'. Marker expression of SSEA-1 (non-pluripotent marker), SSEA-4 (pluripotent marker), TRA-1-60 (pluripotent marker) and 'Pluripotent' (SSEA-1 negative, SSEA-4/TRA-1-60 positive) shown (± SEM, n=4). (C) Dot plots showing SSEA-1 and 'Pluripotent' marker raw data as presented in the iQue Forecyt® software of iPSCs grown under optimized and non-optimized conditions for 2 days (n=4). NCCIT and THP-1 are control cell lines for pluripotent marker expression and non-pluripotent marker expression, respectively.

Surface and Intracellular Marker Staining Provides Solutions for High-Throughput Cellular Characterization

Using the iQue® HTS Platform to monitor intracellular markers in addition to surface markers further characterizes the pluripotency of cells.

Using THP-1 cells as a non-pluripotent control, iPSCs were fixed, permeabilized and stained for the surface markers SSEA-1, SSEA-4 and TRA-1-60, in addition to the intracellular markers Oct 3/4 and Sox 2 (Figure 5). Dot plot data taken directly from iQue Forecyt® software, clearly show the expression of pluripotency markers SSEA-4, TRA-1-60, Oct 3/4 and Sox 2 in iPSC cells (black) and the non-pluripotent marker, SSEA-1, only expressed in the THP-1 control cell line (yellow) (Figure 5A). The heatmap in Figure 5B illustrates this expression pattern in a plate view configuration, where black is high expression and yellow is low expression, exemplifying the flexibility of data presentation in the iQue Forecyt® software. Analysis of this data as a bar graph in Figure 5C further highlights the contrasting expression profiles of the two cell types. The ability to characterize a range of marker expression in cell lines, including iPSCs, via a flexible multiplexed workflow, exemplifies the power and utility of Sartorius platforms such as the iQue® HTS Cytometer.

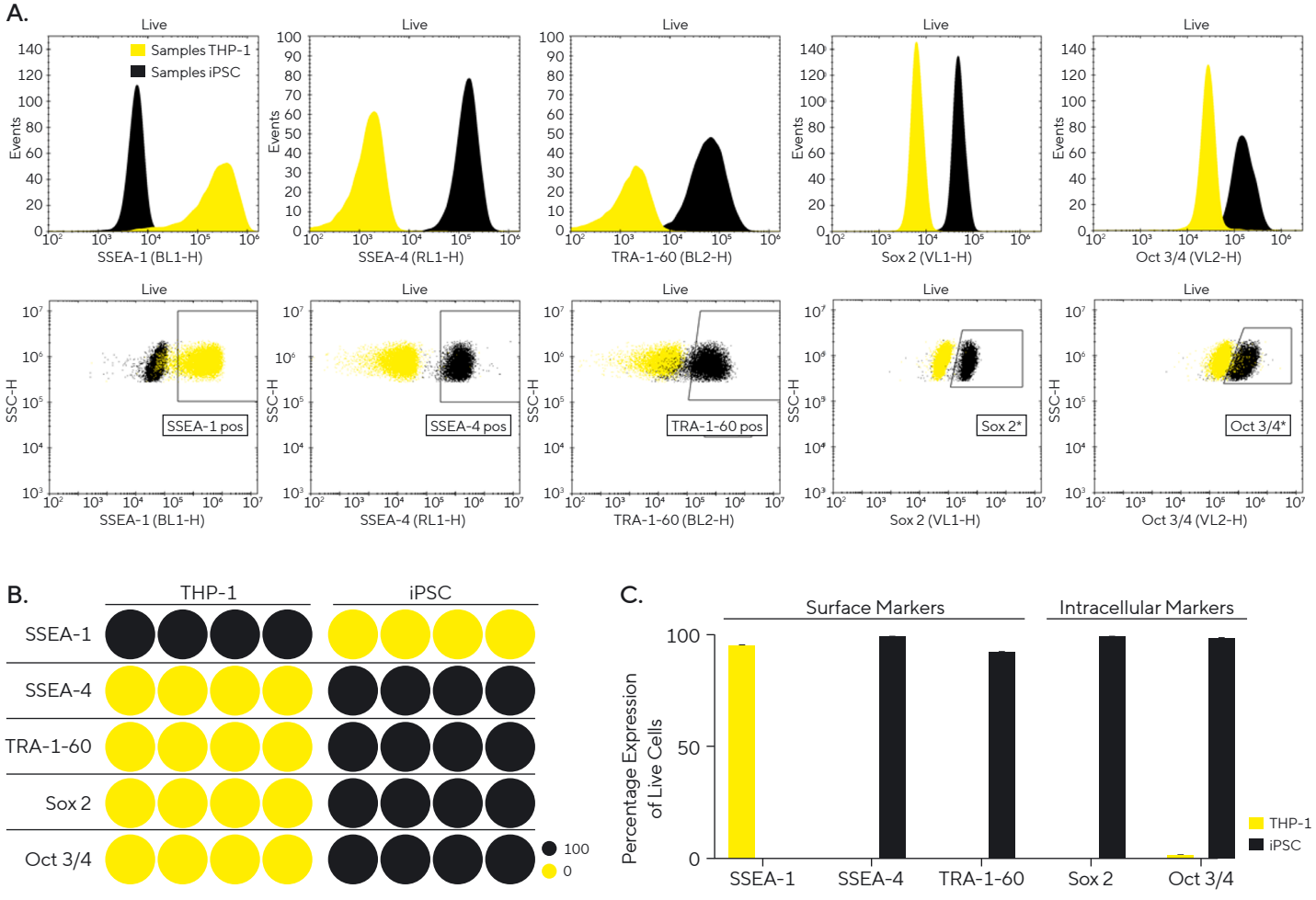


Figure 5. Surface and intracellular marker staining provides solutions for high throughput cellular characterization.
SSEA-1 was used as a marker of normal, non-pluripotent cells, while SSEA-4, TRA-1-60, Sox 2, and Oct 3/4 were all used to characterize pluripotent cells. (A) Histograms and dot plots created in the Forecyt software system for iQue®, showing the expression of various surface and intracellular markers in iPSC and control cells (n=4). (B) Heatmap from iQue Forecyt® illustrating the expression of the same markers, representing the plate map and expression profile per well. (C) Bar graph showing marker expression data in 3rd party software (± SEM, n=4).

Cell Selection and Retrieval: Tissue-Derived Organoids

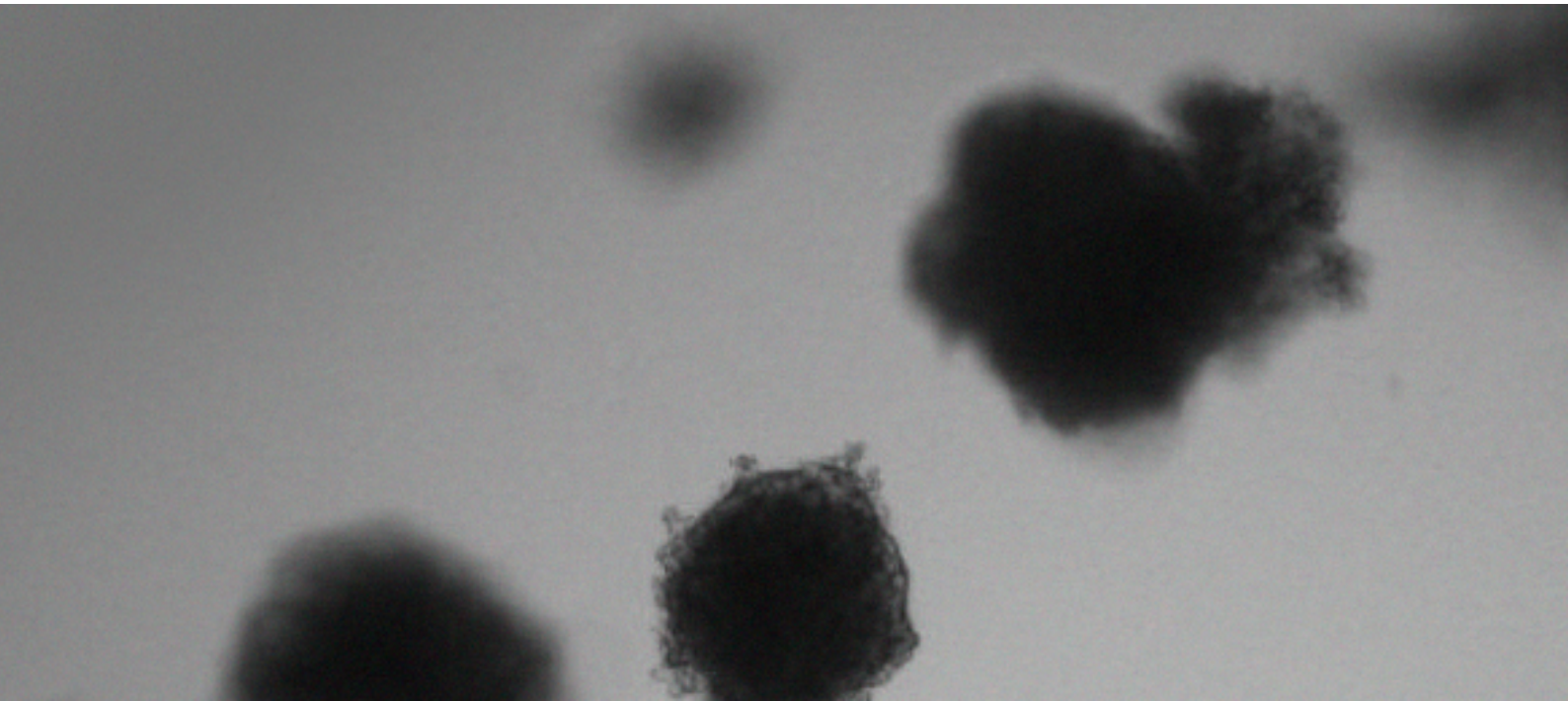
Automated Workflows for the High-Throughput Selection and Picking of Complex 3D Structures

Automated scanning, detection and gating of complex 3D structures based on a range of morphological parameters:

- Organoid sizes from 300 µm to 3.5 mm
- Successful embedding of spheroids and organoids in 100% Matrigel® into plates with or without cell culture membranes
- Low (1 µL) media injection volumes
- No aspiration of neighboring clones

Tissue-Derived Organoid Research

Organoids are self-organizing, 3-dimensional systems which retain many physiological characteristics of the native tissue from which they are derived. Accordingly, these miniaturized models have significant advantages over the use of traditional immortalized cell lines in providing accurate information on human disease modelling and can be used in the fields of drug screening, rare disease research, personalized medicine, and many others.



Key Advantages of the CellCelector in Organoid Research

- Automated scanning, detection and gating of complex 3D structures based on morphological and fluorescence parameters
- Gentle picking of a wide range of organoid sizes, ranging from 300 µm to 3.5 mm
- No changes in 3D structure or morphology following picking and transfer
- Organoid transfer with exceptionally low (1 µL) injection volumes of surrounding media into either 100% hydrogel, liquid media or any other medium
- Successful embedding of spheroids and organoids in 100% Matrigel® into plates with or without cell culture membranes
- Full documentation of transferred organoids – from source vessel to destination plate

Organoid Research: CellCelector Advantages

The CellCelector Flex has a number of inherent hardware features which are crucial for generating successful results within all organoid structures:

Cooled Destination Plates
The use of the optional cooled deck tray can maintain hydrogel temperature at ~0 °C, thus preventing any polymerization before the organoid structure is deposited (Fig.1). Increasing the temperature of deck tray up to 40 °C facilitates optimal polymerization.

Automatic Morphology Measurements and Gating
Automatically identify desirable organoids based on a range of morphological parameters, including area, diameter, sphericity, and the presence and structure of neighbouring organoids (Fig. 2).

Automated Picking Correction for Organoids in Suspension
Non-adherent organoids may move between scanning and picking. By using the automated correction pick-up functionality, organoids which might have moved can be easily picked within a pre-defined detection area (Fig. 3).

Morphology Preservation Following Transfer
Comparison of organoid images before (from the source plates) and after (from the destination plate) transfer shows that the organoids retain their morphology and structure due to the very gentle transfer. Additional downstream analysis confirmed internal structure preservation (Fig. 4).

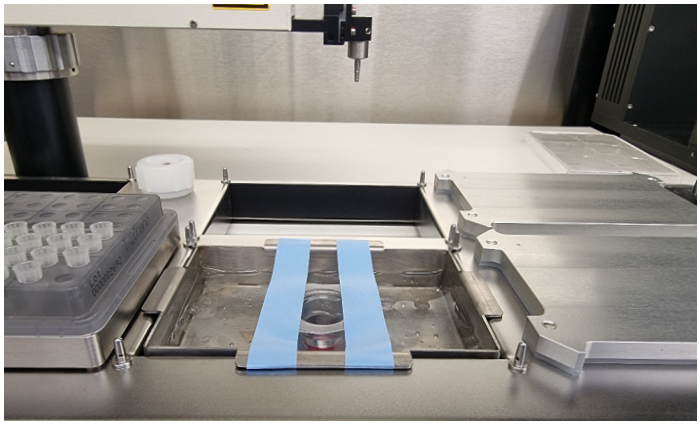


Figure 1: Large volume hydrogel bath placed on the cooled deck tray and kept at 0°C

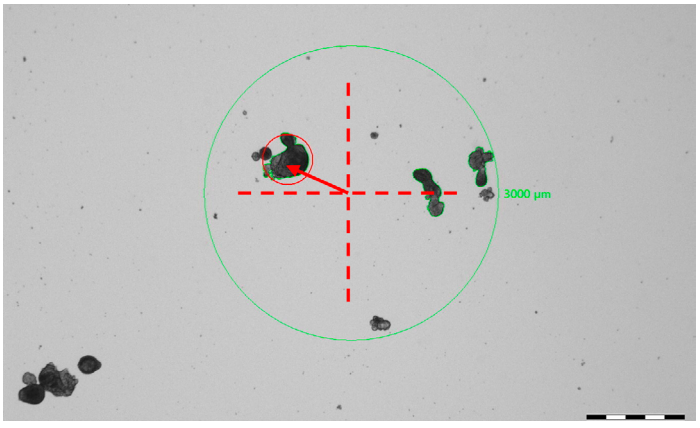


Figure 3: Automated picking correction for lung organoids in suspension

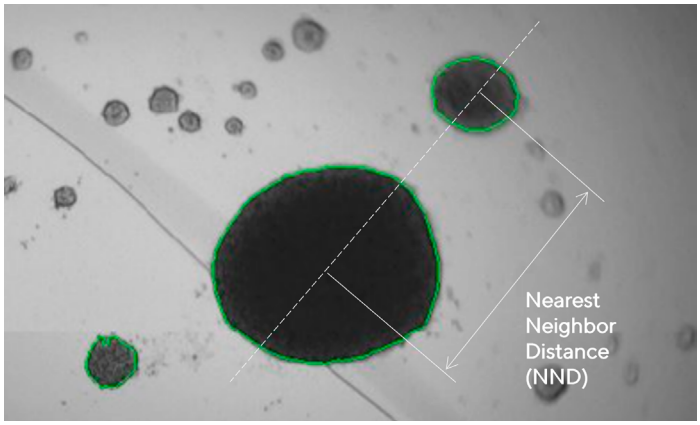


Figure 2: Nearest Neighbour Distance between a large heart organoid and its satellite organoid

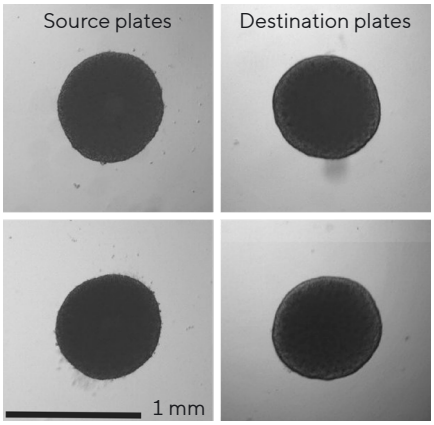


Figure 4: 700 µm heart organoids maintained their structure following gentle transfer

Picking From and Deposition Into 100% Hydrogel

Picking From Different Hydrogels
Organoids can be easily picked from a variety of hydrogels or liquid media without disturbing surrounding structures. In this example, organoids were efficiently picked from Matrigel® despite high organoid density across different planes (Fig. 5).

Bubble-Free Deposition Into Hydrogel
Controlling aspiration speed, volume and destination temperature parameters allows 100% bubble-free organoid deposition into small volumes of hydrogel (<10 µL) or liquid media. Different approaches can be taken to achieve this.

Destination plates can be kept at a continuously low temperature by the cooled deck tray allowing small hydrogel volumes to be aspirated and deposited without any polymerization, before the organoid is deposited directly into the hydrogel (Fig. 6). Conversely, both the hydrogel and the organoid can be aspirated in a single movement, before bubble-free deposition into the destination plate of choice (Fig. 7).

A key feature of both approaches are the low media volumes (<1 µL) aspirated with the organoid before deposition, therefore ensuring the organoids are surrounded by the optimal environment required for further growth and development.

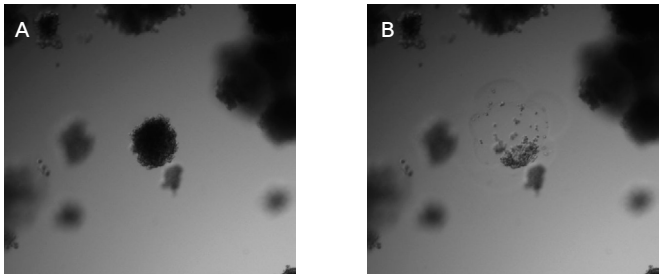


Figure 5: Accurate organoid selection and transfer from areas of high organoid density, (A) before and (B) after selection and transfer

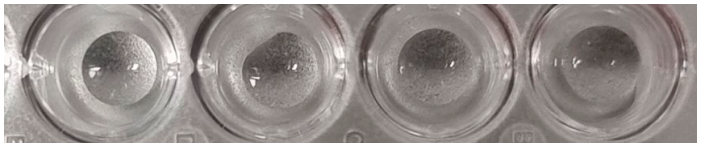


Figure 6: Photograph of bubble-free 10 µL and 20 µL Geltrex® droplets in U-bottom 96 well plates 90 mins after initial deposition

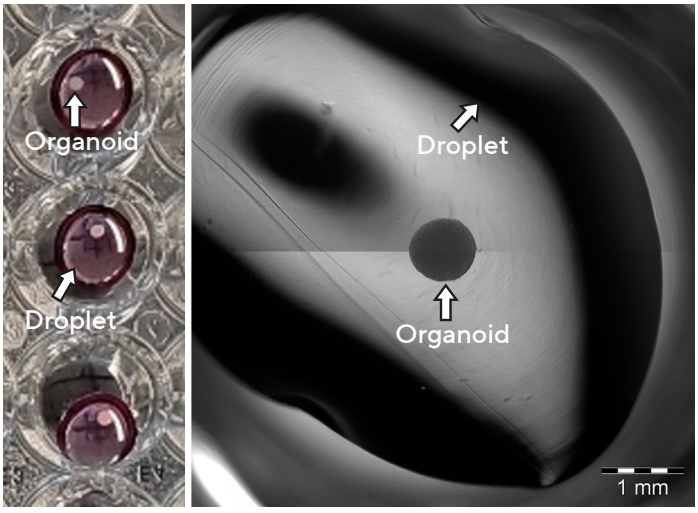
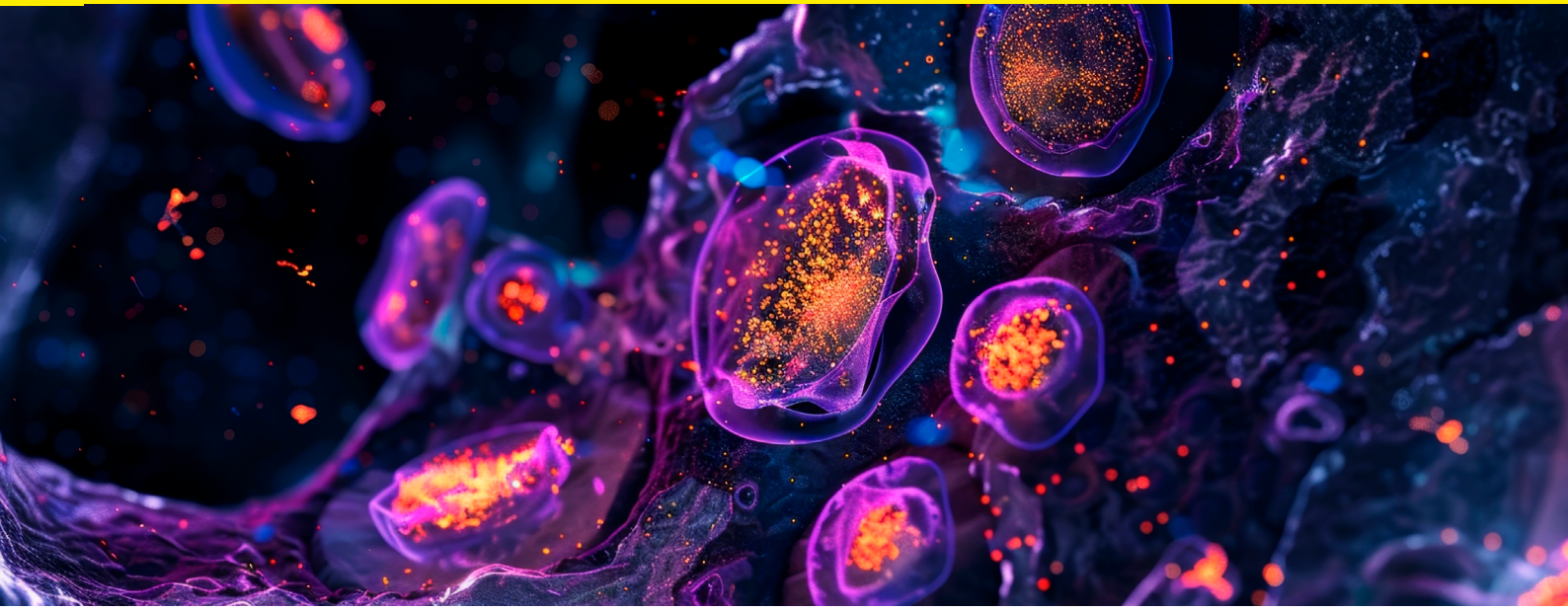


Figure 7: Photograph and scanning of the destination plate to verify deposits and the absence of air bubbles



Application Note: Label-Free, Real-Time Live Cell Assays for 3D Organoids Embedded in Matrigel®

Miniver Oliver[1], Tim Jackson[1], Sandra Leibel[2], Kalpana Barnes[1], Tim Dale[1]
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2. Rady Children's Hospital, University of California, San Diego

Introduction

Advances in preclinical *in-vitro* models are crucial for both basic research and drug development across a range of applications. Organoid technologies are increasingly being used as in-vitro models of human development and disease as they exhibit structural, morphogenetic and functional properties that recapitulate in-vivo pathophysiology.1 To successfully use these models across a variety of research disciplines and applications, approaches that reduce variability and technology pipelines to image and quantify these complex cell models are required.

Currently, techniques to robustly characterize and visualize these models may be limited by one or more of the following:

- Time-consuming, expensive or laborious acquisition processes.
- Use of third party analysis software. Random, end point assessments or indirect (e.g. ATP)
- readouts that may overlook key morphological changes over time.
- Requirement to label cells (fluorescence-based quantification), which may be challenging and not amenable to a range of cell types.

The Incucyte® Organoid Analysis Software Module provides a solution to standardize and automate organoid acquisition and analysis workflows, simplifying characterization of these complex cultures.

Assay Principle

This application note describes the use of the Incucyte® Live-Cell Analysis System along with the Incucyte® Organoid Analysis Software Module to study the growth or death of organoids, label-free. A proprietary brightfield image acquisition approach enables real-time kinetic imaging of 3D organoids embedded within a matrix (Matrigel®). Organoid size, count and morphology measurements are automatically plotted over time to gain in-depth organoid characterization following perturbation. Here we describe validation methods and data demonstrating the ability to kinetically image and quantify the growth, death and morphology of organoids embedded in Matrigel®.

Materials and Methods

Quick Guide

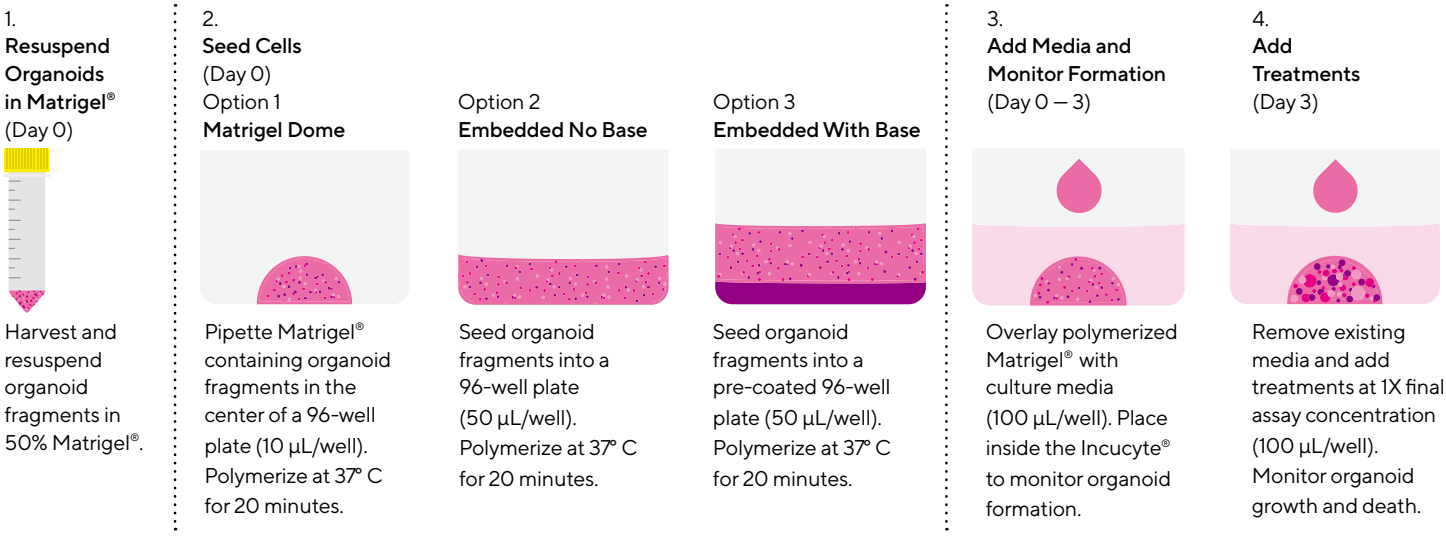

Figure 1: Incucyte® Organoid Assay Workflow

1. Organoids of interest are harvested according to model-specific guidelines and resuspended in 50% Matrigel®.
2. Matrigel® containing organoid fragments is pipetted into each well of a 96-well tissue culture treated plate utilizing any of following assay formats:
   - **Matrigel® Dome** (Pre-warmed plate; 10 µL/well)
   - **Embedded No Base** (Pre-chilled plate; 50 µL/well)
   - **Embedded With Base** (Pre-coated plate; 50 µL/well)
3. Plate is placed in a humidified incubator to polymerize Matrigel® (37° C, 20 minutes).
4. Cell type-specific growth media is added on top of polymerized Matrigel® (100 µL/well).
5. Organoid formation is monitored in an Incucyte® (Organoid Assay scan type, 4x, 6-hour repeat scanning, 0 – 3 d).
6. Post formation, treatments are added (100 µL at 1x final assay concentration (FAC) per well).
7. Organoid growth and death is monitored within an Incucyte® every 6 hours for up to 10 days. Organoid metrics (e.g. size, count, eccentricity) are reported in real-time based on brightfield image analysis.

Organoid culture reagents were obtained from StemCell Technologies unless otherwise noted. Mouse intestinal (#70931), hepatic (#70932), human brain (healthy or patient derived; prepared externally) and human lung organoids (cultured by University of California San Diego[2]) were embedded in Matrigel® (Corning #356231 or #354277 brain organoids) in 96-well flat bottom TC-treated microplates (Corning #3595).

Organoids were cultured in cell type-specific organoid growth medium (e.g. IntestiCult™ OGM Cat. #06005; #06040; HepatiCult™ OGM #06030; STEMdiff™ Cerebral Organoid Kit Cat. #08570) supplemented with 100 units/100 µg per mL Pen/Strep (Life technologies). Organoid formation, growth and death was monitored in an Incucyte® at 6-hour intervals for up to 10 days.

Visualizing and Quantifying Differential Organoid Phenotypes in a 96-Well Assay Format

To evaluate the ability of the Incucyte® Organoid Analysis Software Module to accurately track organoid growth in 96-well plates, mouse intestinal, hepatic, or human whole lung organoids[2] were embedded in Matrigel® (50 %) and bright-field (BF) images were acquired every 6 hours (Figure 2A).

Organoids were automatically located and changes in size (area) were kinetically tracked using Incucyte' s Organoid Software Analysis Module. BF area segmentation shown in yellow (Figure 2) enabled label-free quantification of organoid growth and illustrates the software's ability to accurately segment individual objects embedded in Matrigel® across a range of cell types.

Acquired BF images (6 d post seeding) and time-courses (Figure 2A) revealed cell type-specific morphological features and temporal growth profiles respectively. Individual lung organoids appeared larger (400 µm – 1 mm diameter) than Intestinal (100 - 400 µm) or hepatic (50 - 500 µm) organoids and reached maximal size rapidly (90 h, 22.1 x 10^4 µm^2 ± 2.5 mean ± SEM, n = 3 wells).

To further demonstrate the software's ability to distinguish organoid morphological differences, human brain organoids derived from healthy- or epilepsy- iPSCs (induced pluripotent stem cells) were embedded in 50% Matrigel® and imaged over 8 days (Figure 2B). Figure 2B illustrates that these cultures exhibited comparable growth (avg. area bar chart) but displayed distinct phenotypes (BF images). Morphology-related parameters tracking changes in object roundness (eccentricity) or brightness (darkness) were utilized to exemplify differential organoid phenotypes. Mature healthy organoids appeared darker and rounded (decreased eccentricity), while an increase in eccentricity was observed in patient organoids as they formed loose, disorganized aggregates (Figure 2B).

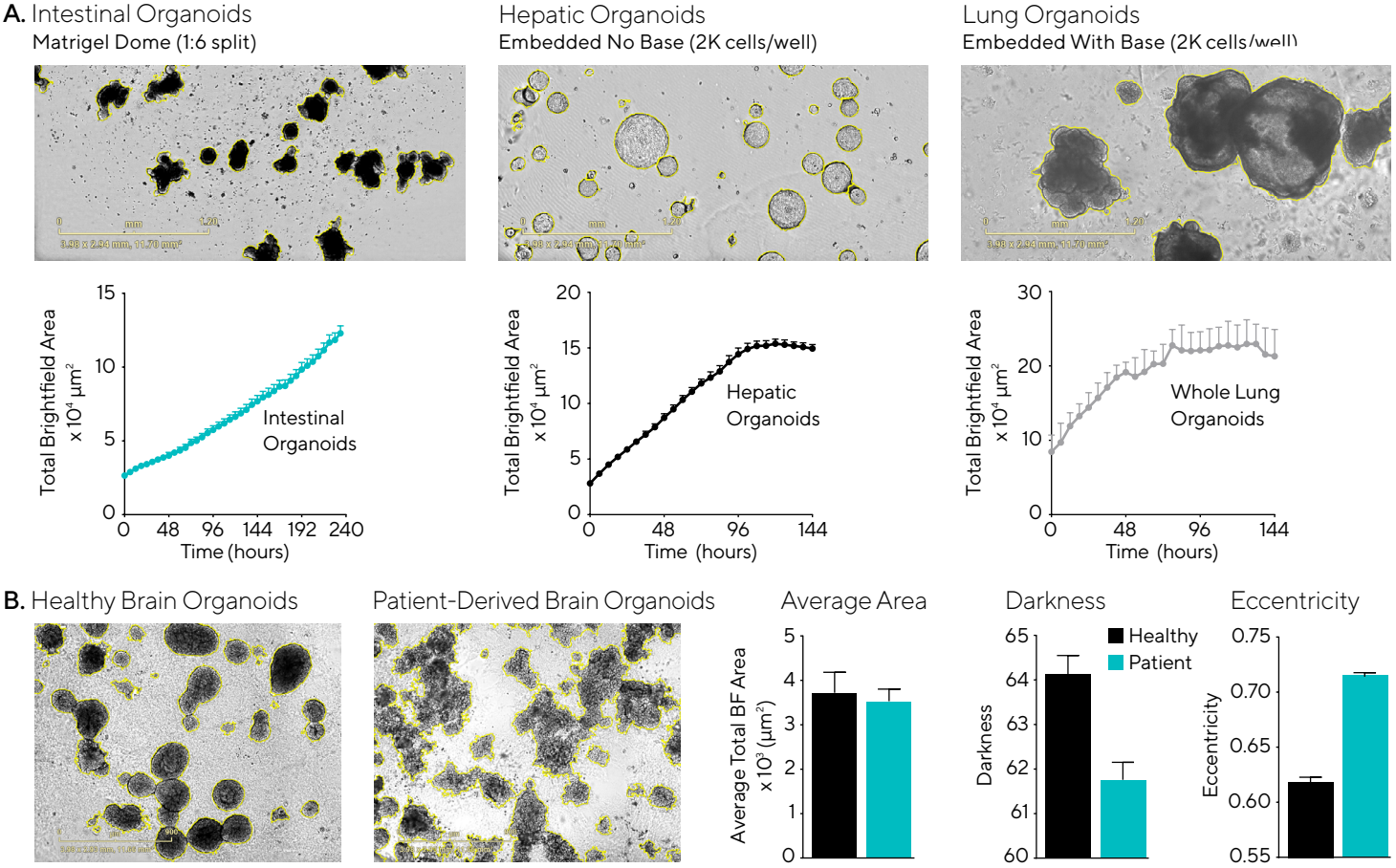

Figure 2: Acquisition and quantification of distinct organoid morphologies. Mouse intestinal (1:6 split, dome), hepatic (2K cells/well) and whole lung organoids (2K cells/well) were seeded (50% Matrigel®) into 96-well plates and imaged in an Incucyte®. Brightfield (BF) images (6 days post seeding) and time-course plots of the individual well total BF area (µm^2) over time (hours) show distinct organoid phenotypes and demonstrate cell type specific organoid growth, respectively (A). Healthy or diseased human brain organoids (2K cells/well) were embedded in 50% Matrigel® and imaged over 8 days. Images (6 days) and bar graphs demonstrate growth capabilities and differential phenotypes of healthy vs diseased organoids (B). All images captured at 4x magnification. Each data point represents mean ± SEM, n = 3 - 12 wells.

Quantifying Organoid Growth and Death Over Time

To assess the impact of treatments on organoid growth and morphology, intestinal and hepatic organoid fragments were embedded in Matrigel® (50%) and allowed to form organoids for 3 days prior to treatment with protein kinase inhibitor staurosporine (1 µM, STP). Changes in organoid size and shape were kinetically monitored and quantified over time (4 - 10 days).

Figure 3A demonstrates that vehicle treated intestinal or hepatic organoids increase in size (10-fold or 3-fold respectively) and number (Figure 3B) over time while a marked reduction is observed in the presence of STP.

Time-courses and zoomed in BF images shown in figure 3B illustrate the effect of STP on hepatic organoid morphology. A concomitant increase in darkness and eccentricity was observed as STP induced cell death and elicited loss of distinctive rounded phenotype over time.

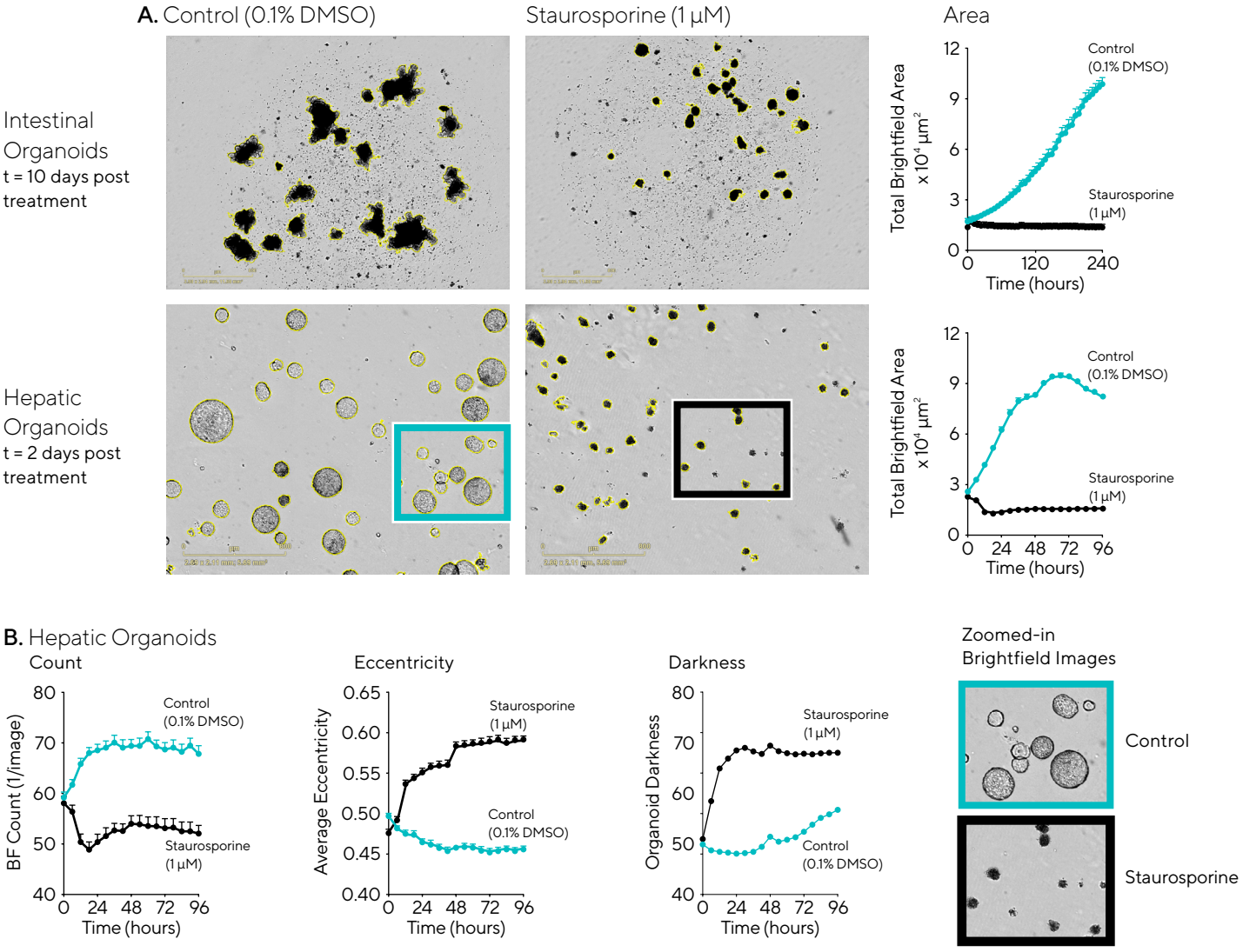

Figure 3: Perform automated label free quantification of organoids. Mouse intestinal (1:6 split) and hepatic fragments (1K cells/well) were embedded in Matrigel® (50%) in 96-well plates and allowed to form organoids for 3 days prior to treatment (vehicle or staurosporine; STP). Brightfield (BF) images (A) and corresponding time-courses of BF area (A) demonstrate the continued growth of vehicle treated organoids and the inhibitory effects of STP across both cell types. STP treated hepatic organoids lose distinctive rounded phenotype (increased eccentricity) and increase in darkness over time (B). Data were collected over a 96 - 240 hour period at 6 hour intervals. All images captured at 4x magnification. Each data point represents mean ± SEM, n=4 wells.

Probe Mechanisms of Action Using Real-Time Morphology Measurements

As patient-derived organoids (PDOs) retain the morphological and molecular characteristics of the tissue/tumour of origin, they are increasingly being used as *in-vitro* drug development models.[1] For these *in-vitro* drug studies, the ability to distinguish between cytotoxic and cytostatic cellular responses is crucial to establishing effective anticancer therapies.[3] Performing multi-parametric quantitative measurements is key to understanding these dynamic drug responses.

To exemplify drug-specific changes in organoids, hepatic organoids were formed for 3 days and subsequently treated with staurosporine (STP, protein kinase inhibitor), cisplatin (CIS, DNA synthesis inhibitor) or fluorouracil (5-FU, thymidylate synthetase inhibitor). Concentration response curves (CRCs) representing the area under the curve analysis of total area, eccentricity, or darkness time-course data (0 – 96 hours) were then constructed to discriminate between cytotoxic and cytostatic agents (Figure 4).

All compounds caused a concentration dependent inhibition of organoid growth, yielding IC_{50} values of 3 nM for STP, 0.78 µM for 5-FU and 9.7 µM for CIS (area CRC, Figure 4).

However, while attenuation of organoid size was observed across all compounds, increases in eccentricity and darkness indictive of 3D structure disruption and cell death respectively were only observed in CIS and STP-treated organoids.

STP induced notable changes in organoid eccentricity across a range of concentrations (1.6 nM – 1 µM, EC_{50} 0.5 nM) and evoked a concentration-dependent increase in organoid darkness (EC_{50} 53.3 nM), suggesting a strong cytotoxic mechanism of action (MoA). While concentration-dependent responses were also observed in CIS-treated organoids, substantially higher concentrations (50 -100 µM) were required to elicit comparable or greater effects on eccentricity (EC_{50} 32.5 µM) or darkness (EC_{50} 31.7 µM).

Conversely, 5-FU appeared to be more cytostatic, inhibiting organoid growth but not inducing cell death or disrupting distinct organoid phenotype. Differences between the size and morphology readouts support the cytostatic mechanism of 5-FU. Representative BF images confirm distinction between the cytotoxic MoA of STP and CIS and the cytostatic effects of 5-FU on hepatic organoids (Figure 4).

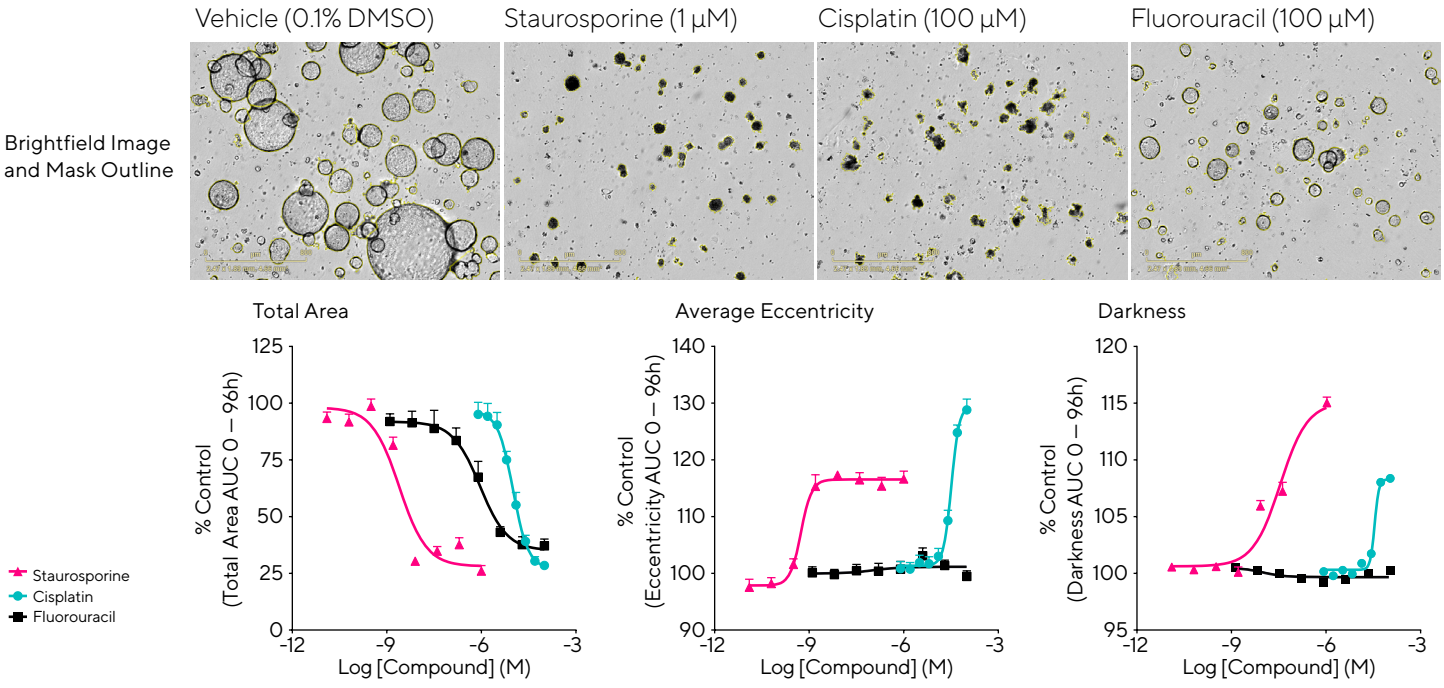

Figure 4: Distinguish between cytotoxic and cytostatic mechanisms of action using label-free measurements. Hepatic fragments were embedded (0.5K cells/well) in 50% Matrigel® and allowed to form organoids (3 days) prior to treatment. Brightfield images taken 2 day post treatment show compound-specific effects on organoid size and morphology. Concentration response curves (CRCs) of the area under the curve (AUC) analysis of area, eccentricity and darkness demonstrated differential profiles of cytotoxic (staurosporine and cisplatin) and cytostatic (fluorouracil) mechanisms of action. Data were collected over a 96-hour period at 6 hour intervals. Each data point represents mean ± SEM, n = 3 separate test occasions.

Conclusion

As the life sciences continue to shift toward more physiologically relevant models, three-dimensional (3D) cell systems like organoids and spheroids are becoming indispensable tools in both fundamental research and therapeutic development. This eBook has explored the technological innovations that are making 3D cell analysis more efficient, reproducible, and insightful than ever before.

By leveraging automated platforms such as the CellCelector Flex, Incucyte® Live-Cell Analysis System, and iQue® HTS Cytometer, researchers can now streamline workflows that previously required manual labor and carried high variability. These systems not only maintain the viability and integrity of delicate structures like iPSCs and organoids but also enable high-throughput, label-free, and kinetic analysis that accelerates the path from isolation to biological insight.

The detailed case studies and application notes presented—ranging from iPSC culture and characterization to real-time monitoring of organoid responses—underscore the critical need for integrated solutions that can keep pace with the complexity of 3D models. Whether identifying subtle changes in pluripotency markers or quantifying organoid swelling in response to pharmacological agents, the ability to automate and standardize these processes is central to advancing precision medicine and translational research. As these technologies mature and integrate further into laboratory ecosystems, the future of 3D cell analysis will be defined not just by what we can see under the microscope, but by the robust, data-rich insights we can generate—quickly, reliably, and at scale. Sartorius remains at the forefront of this transformation, enabling researchers to go beyond observation and toward actionable understanding in the dynamic world of advanced cell systems.

Further Reading

- Infographic: How to Characterize Organoids
- eBook: The Progress and Promise of iPSCs in Disease Modeling
- Blog: Unlocking the Potential of iPSCs: Expert Insights on Opportunities in Cell Therapy R&D
- Blog: Let's Talk About the Sticky Situation that is Transferring Organoids and Spheroids

Supporting Products and Solutions

Incucyte® Live-Cell Analysis System
The Incucyte® Live-Cell Analysis System enables real-time live-cell analysis directly inside your incubator. It provides quantitative, label-free in vitro approach for characterization and analysis of 2D and 3D cultures and cell models without ever having to remove cells from the incubator. The Incucyte® makes the process of acquiring, viewing, analyzing, and sharing images of living cells easier than ever before.
Learn More

CellCelector Automated Cell Selection and Retrieval Platform
The CellCelector Flex Platform is a fully automated cell imaging and picking system developed for screening, selection and isolation of single cells, clusters, spheroids, and organoids as well as single-cell clones and adherent colonies.
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