Technical Note

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Automated Dispensing and Assay of Organoids in Matrigel® Domes Using the BIO ONE and Incucyte®

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Abstract

Advances in biomaterials and stem cell engineering have enabled the development of three dimensional (3D) organoids that more accurately recapitulate human tissue architecture than traditional 2D cultures or animal models. These systems have rapidly become essential tools for studying human development, disease mechanisms, and personalized therapeutic responses. Despite considerable progress, many current approaches for generating organoid models in multi-well plates remain constrained by limited scalability, technical complexity, and poor reproducibility. In this technical note, we describe a workflow addressing the challenges with the set-up and analysis of organoids in multi-well plates. We show how automated dispensing combined with automated continuous non-invasive live-cell imaging and analysis of organoids, supports increased throughput and generation of robust data suitable for compound profiling and mode of action studies.

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Introduction

Developed from pluripotent stem cells or adult stem cells, organoids are three-dimensional (3D), *in vitro* cell models that recapitulate and retain many structural and functional aspects of their *in vivo* counterparts. Consequently, they have gained keen interest as systems to model and study human tissue development, disease progression and provide relevant systems for drug discovery, offering personalized and regenerative medicine opportunties[1].

While organoids represent promising *in vitro* platforms for drug discovery, technical challenges can hinder their integration into discovery pipelines. Additionally, non-standardized methods across different research institutions make it challenging to reproduce and compare data across studies. For organoid assays to yield robust and reproducible data, it is essential to minimize or control as many sources of variance as possible. In organoid assay workflows, the handling and dispensing of viscous, temperature-sensitive, and costly matrices are key sources of variability. A common technique for organoid-based drug screening involves embedding organoids in an extracellular matrix (ECM), such as Matrigel®, and seeding into multi-well plates, with organoid culture medium added on top[2]. One such technique involves dispensing a single droplet of organoid-Matrigel® suspension into multi-well plates, followed by polymerization of Matrigel® at 37 °C to create a 'dome' like structure in the well. While this approach helps minimize the volume and therefore cost per well of ECM used, droplet dispense into each well is often performed using a manual pipette, making this an arduous, labor-intensive method, with limited throughput and prone to human error. During the workflow, the operator needs to be aware of and maintain specific temperatures of the materials and consumables used. To avoid premature polymerization of Matrigel®, the organoid-Matrigel® suspension being dispensed needs to be kept cold. To facilitate Matrigel® polymerization, ensuring stable positioning and attachment of the droplet in the well, the assay plate needs to be kept warm. With all these factors considered, the success of this time-consuming, manual approach is often dependent on operator experience.

By combining precision low-volume dispensing with controlled handling of temperature-sensitive materials, like Matrigel®, the CELLINK BIO ONE platform offers a targeted solution to several of the sources of variability in organoid assay preparation. Its small footprint enables sterile operation within biosaftey cabinets, while its open design provides unobstructed access to consumables and samples in confined workspaces.

3D organoid cultures present various challenges for live-cell imaging. Their complex architecture and density can hinder penetration of light and their spatial positioning in different focal planes within an extracellular matrix can demand complex image acquisition and analysis pipelines. Easy to use, automated advanced image software tools which support the handling and analysis of extensive volumes of complex data are necessary.

The Incucyte® Live-Cell Analysis System enables kinetic monitoring and quantification of cell cultures using HD Phase-contrast and brightfield images, which are automatically quantified via integrated software. Purpose built Incucyte® Organoid Analysis Software enables automated monitoring and quantification of organoid growth and proliferation within an incubator without perturbing these sensitive cultures.

In this technical note, we describe the combined use of the CELLINK BIO ONE automated bio-dispenser and Sartorius Incucyte® Live-Cell Analysis System in a workflow for the 3D *in vitro* model creation and assay of hepatic organoids in 96-well microwell plates. This approach is suited and amenable to organoid compound profiling and efficacy testing for drug discovery.

Materials and Methods

An illustration of the protocol steps involved in using the BIO ONE bio dispenser and Incucyte® Live-Cell Analysis System in the assay of hepatic organoids is shown in Figure 1.

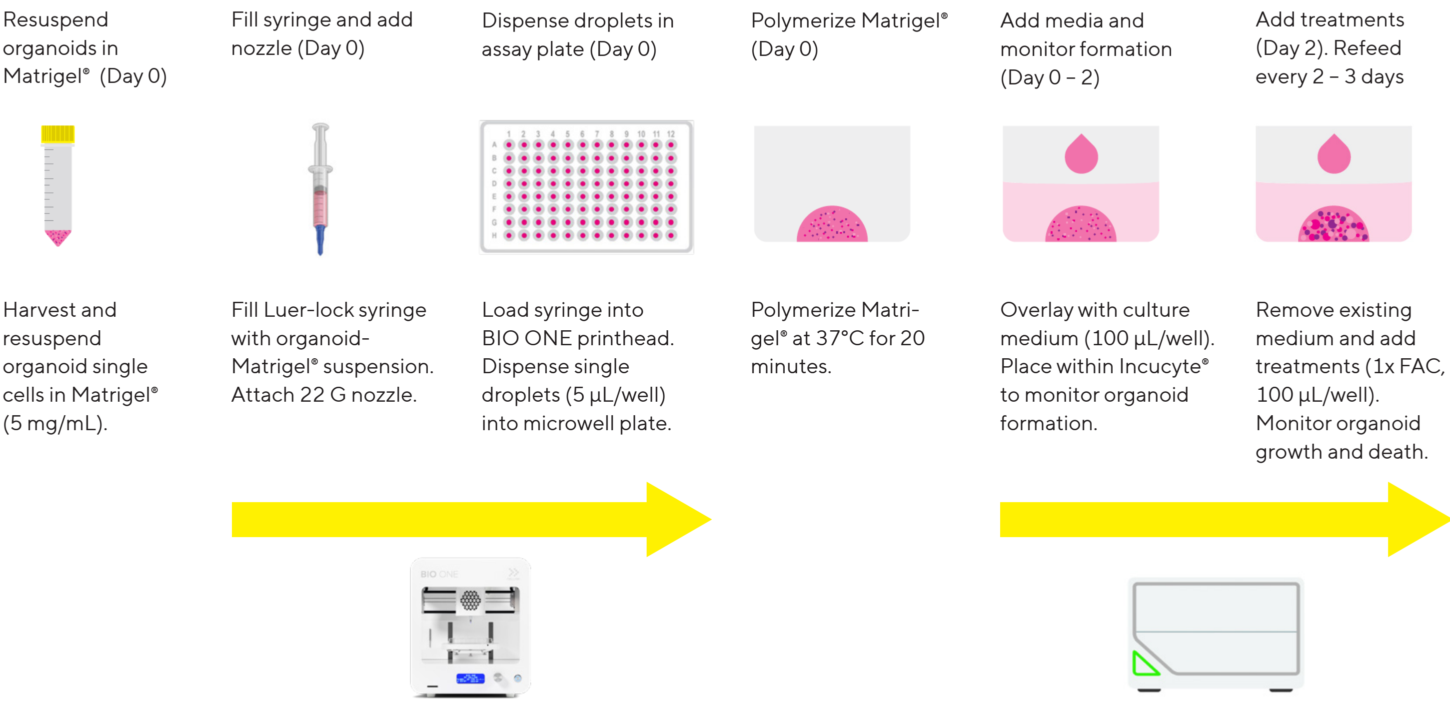


Figure 1. Protocol quick guide. Using the CELLINK BIO ONE bio dispenser and Sartorius Incucyte® Live-Cell Analysis System, a schematic showing steps involved in the 3D *in vitro* model creation and assay of hepatic organoids.

Organoid culture

Mouse hepatic organoids were cultured in accordance with the Incucyte® Organoid culture QC protocol (**Incucyte® Organoid Culture QC**). Briefly, organoids were embedded in Matrigel® (100%) domes (30 µL/well) in 24-well flat bottom TC-treated microplates and cultured in complete hepatic organoid growth medium (750 µL/well, HepatiCult™ growth medium supplemented with Penicillin-Streptomycin). The Incucyte® Live-Cell Analysis System was used to monitor organoid formation and growth for up to 7 days, with brightfield (BF) images (4x magnification) acquired at 6-hour intervals. Media refreshes were performed every 2 – 3 days. Materials used are outlined below (Table 1). At maturity, organoids were either passaged or used for assays.

Materials	Supplier	Cat. No.	Final Concentration
Mouse Hepatic Organoids	STEMCELL Technologies	70932	-
HepatiCult™ Organoid Growth Medium (mouse)	STEMCELL Technologies	06030	-
Penicillin-Streptomycin (10,000 U/mL)	Gibco	15140	1%
Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning®	356321	100% for routine culture
DMEM/F-12 with 15 mM HEPES	STEMCELL Technologies	36254	-
Costar® 24-well flat-bottom TC plate	Corning®	2536	-

Table 1. Materials used for routine hepatic organoid culture.

Organoid dispensing

To minimize culture contamination, the BIO ONE was placed and operated within a cell culture laminar flow hood. Prior to organoid harvest, the BIO ONE printhead and printbed temperatures were set to 2 °C and 30 °C, respectively. 96-well non-TC assay plates were pre-warmed in a 37 °C incubator for at least 30 minutes prior to use and 3 mL Luer-lock syringes were kept at 4 °C until needed for filling.

For preparation of assay plates, mature hepatic organoids were harvested from routine 24-well culture plates, enzymatically dissociated into single cells, and resuspended in complete organoid growth medium containing Matrigel® (5 mg/mL)[2]. In brief, organoids were collected in cold DMEM/F-12 by mechanical disruption of the Matrigel® domes, transferred to a conical tube (15 mL) and washed twice by centrifugation (200 g, 4 °C, 5 minutes) in cold DMEM/F-12. The supernatant was aspirated, the cell pellet resuspended in TrypLE and the tube gently agitated in a water bath (5 minutes, 37 °C). TrypLE was diluted by filling the tube to maximum volume with DMEM/F-12 and gently passing the suspension through a 40 µm strainer followed by centrifugation (200 g, 4 °C, 5 minutes). The pellet was resuspended in a low volume (0.1 – 0.5 mL) of complete organoid assay medium (HepatiCult™ growth medium supplemented with Penicillin-Streptomycin and ROCK inhibitor), cells counted and diluted to the required density in complete organoid assay medium containing Matrigel® (5 mg/mL).

Matrigel® printing was performed according to the **BIO ONE protocol**. To minimize bubbles, a pre-chilled Luer-lock tip syringe was filled slowly with the organoid-Matrigel® suspension. Once filled, a 22 G nozzle was attached and the syringe loaded into the pre-cooled BIO ONE printhead. The automatic calibration function was performed ahead of dispensing a single droplet (5 µL) into the center of each well of the 96-well assay plate. BIO ONE settings are outlined in Table 3. After dispensing, plates were moved to a 37 °C incubator for 20 minutes to polymerize the Matrigel®. Polymerized Matrigel® domes were overlaid with complete hepatic organoid medium (100 µL) containing ROCK inhibitor.

Compound treatment

Two days post seeding (day 0 assay), once organoids had formed, organoid growth medium was aspirated and organoids treated with 1x final assay concentration (FAC) of complete organoid growth medium (minus ROCK inhibitor) containing vehicle control (0.1% DMSO) or compounds (100 µL/well, triplicate wells). 100% re-treatments were performed every 2 – 3 days. Brightfield images (4x magnification) were acquired using the Incucyte® Live-Cell Analysis System and Organoid Scan Type every 6 hours for 5 days. The Incucyte® Organoid Analysis Software was used to segment and quantify brightfield (BF) organoid images. Materials used are outlined below (Table 2).

Materials	Supplier	Cat. No.	Final Concentration
HepatiCult™ Organoid Growth Medium (mouse)	STEMCELL Technologies	06030	-
Penicillin-Streptomycin (10,000 U/mL)	Gibco	15140	1%
DMEM/F-12 with 15 mM HEPES	STEMCELL Technologies	36254	-
TrypLE Express	Gibco	12604013	-
Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning®	356321	5 mg/mL for assay
Cell strainer (40 µm)	Corning®	431750	-
Y-27632 dihydrochloride (ROCK inhibitor)	Tocris	1254	10 µM
BD Plastipak 3 mL Syringe Luer-Lock Tip	BD Plastipak	309658	-
22 G nozzle	CELLINK	NZ4220005001	-
VWR® 96 flat bottom, non-TC plate	VWR	374-2781	-
Staurosporine	Sigma	19-123	1 µM
Camptothecin	Tocris	1100	1 µM
5-Fluorouracil	Tocris	3257	100 µM

Table 2. Materials used for hepatic organoid assay.

Dispensing Parameters	Setting
Well plate	96-well plate
Printbed temperature	30 °C
Printhead temperature	2 °C
Extrusion rate	20 µL/s
Extrusion volume	10 µL
Retraction volume	5 µL
Droplet volume	5 µL
Z-offset	0.7 mm
Retract rate	20 µL/s
Postflow stop time	0.5 s
Z-lift between wells	30.0 mm

Table 3. BIO ONE Droplet Print function parameters. Settings used for dispensing single droplets (5 µL per well) of organoids (0.5K cells per well) in Matrigel® (5 mg/mL) through a 22 G nozzle.

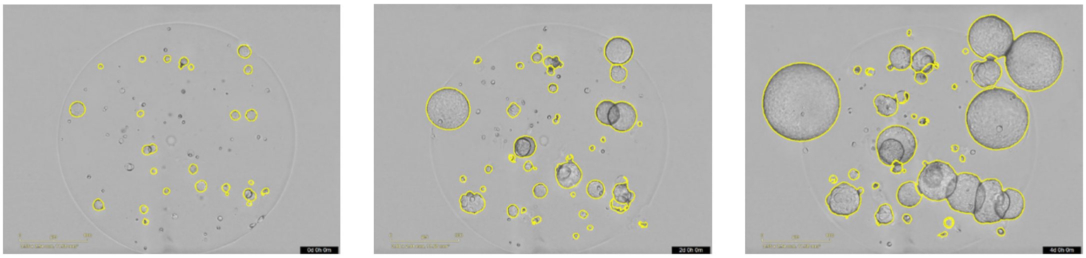
Results

Assay quality

Using the BIO ONE, single droplets of Matrigel®-embedded organoids were dispensed into each well of 96-well assay plates, only requiring approximately 6 minutes per plate. Uniform droplet shape and size was attained across the micro-well plate, with all organoids within the dome automatically located and imaged using the Incucyte® organoid software (Figure 2A). To illustrate reproducibility of dispense, organoid morphology and growth was assessed across 6 plates prepared on two separate days (3 plates per test occasion).

Incucyte® real-time, integrated, organoid image capture and brightfield image segmentation was used to quantify organoid size. Cystic morphology typical of hepatic organoids and comparable organoid size (area) across assay days 0, 2, 4 was attained for all six plates, showing that an extrusion-based method and BIO ONE parameters selected for droplet dispense were not detrimental to organoid formation and growth (Figure 2B).

A



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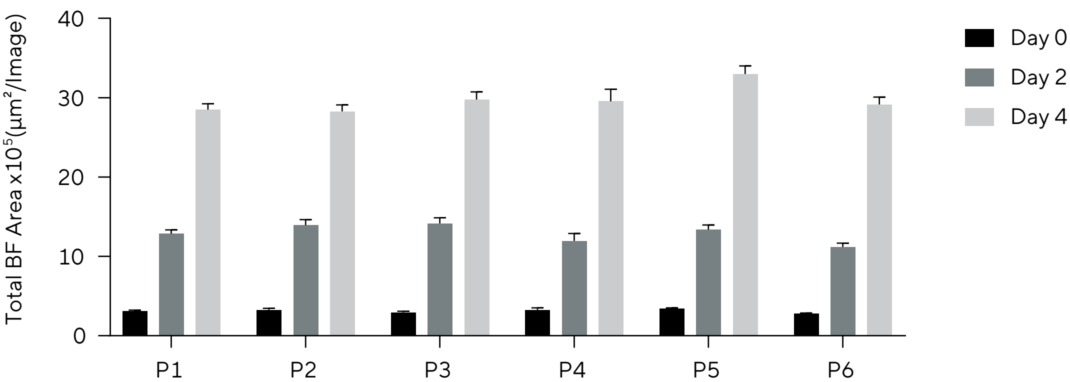

Figure 2. Acquisition and quantification of hepatic organoids. Using the BIO ONE, droplets of organoids embedded in Matrigel® were dispensed into 96-well plates and monitored using live-cell analysis. Organoids were allowed to form for 2 days prior to start of assay. (A) Representative Incucyte® brightfield images with outline segmentation mask (yellow) are shown on assay days 0, 2 and 4. (B) Bar graph showing total organoid area at assay day 0, 2, 4 for 6 plates (P1 – P6). Data shown as mean ± SEM, n= 15 replicates per plate.

To assess assay quality, controls on each plate were used to calculate a Z′ value, where values greater than 0.4 indicate robust results. Two days post seeding, organoids were treated with vehicle (0.1% DMSO) or staurosporine (1 µM), for 5 days.

Comparing the size (area) of organoids exposed to vehicle and staurosporine, Z′ values of ≥ 0.4 (range 0.40 – 0.57) were returned from 3 independent experiments (Figure 3).

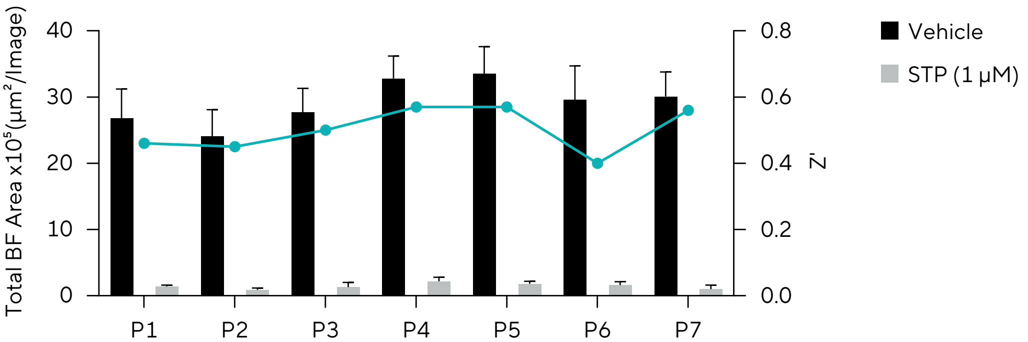

Figure 3. Assay quality. Using the BIO ONE, droplets of organoids embedded in Matrigel® were dispensed into 96-well plates and monitored using live-cell analysis. Organoids were allowed to form for 2 days prior to treatment with vehicle (0.1% DMSO) or staurosporine (STP, 1 µM) for 5 days. Bar graph showing total organoid area 5 days post treatment and Z′ values for 7 plates generated across 3 separate test occasions. Data shown as mean ± SD n= 15 (vehicle) or 6 (STP) replicates per plate.

Compound pharmacology

To assess compound specific effects on organoid growth and morphology, organoids were allowed to form for 2 days prior to treatment with staurosporine (STP, protein kinase inhibitor), camptothecin (CMP, DNA topoisomerase inhibitor) or 5-fluorouracil (5-FU, thymidylate synthetase inhibitor).

Brightfield images show compound-specific effects on organoid size and morphology, with STP causing a marked inhibition of organoid area and darkness (Figure 4A). Incucyte® microplate view showing organoid area times-course plots for each well in the plate demonstrate comparable responses between replicate wells (Figure 4B). At the highest concentration evaluated an attenuation of organoid size was observed across all compounds. STP treated organoids shrank to a size smaller than when first exposed to the cytotoxic agent. This effect was less pronounced and delayed to ~ 72h for CMP treated organoids, while 5-FU appeared cytostatic, causing little or no change to organoid size (Figure 4C).

In contrast, marked temporal differences in organoid darkness were noted for each compound. STP caused a rapid and sustained increase in organoid darkness while a more delayed response coinciding with the area time-course data was observed for CMP. 5-FU caused little to no effect on organoid darkness (Figure 4D). Together, these metrics offer an insight into the cytotoxic and cytostatic mechanisms of compounds.

Concentration response curves (CRCs) representing the area under the curve (AUC) analysis of total area time-course data (0 – 120 hours, Figure 5) were constructed. All compounds caused a concentration dependent inhibition of organoid growth, returning pIC_{50} values of 8.9 ± 0.0 for STP, 7.8 ± 0.2 for CMP and 5.6 ± 0.1 for 5-FU (area CRCs, Figure 5D).

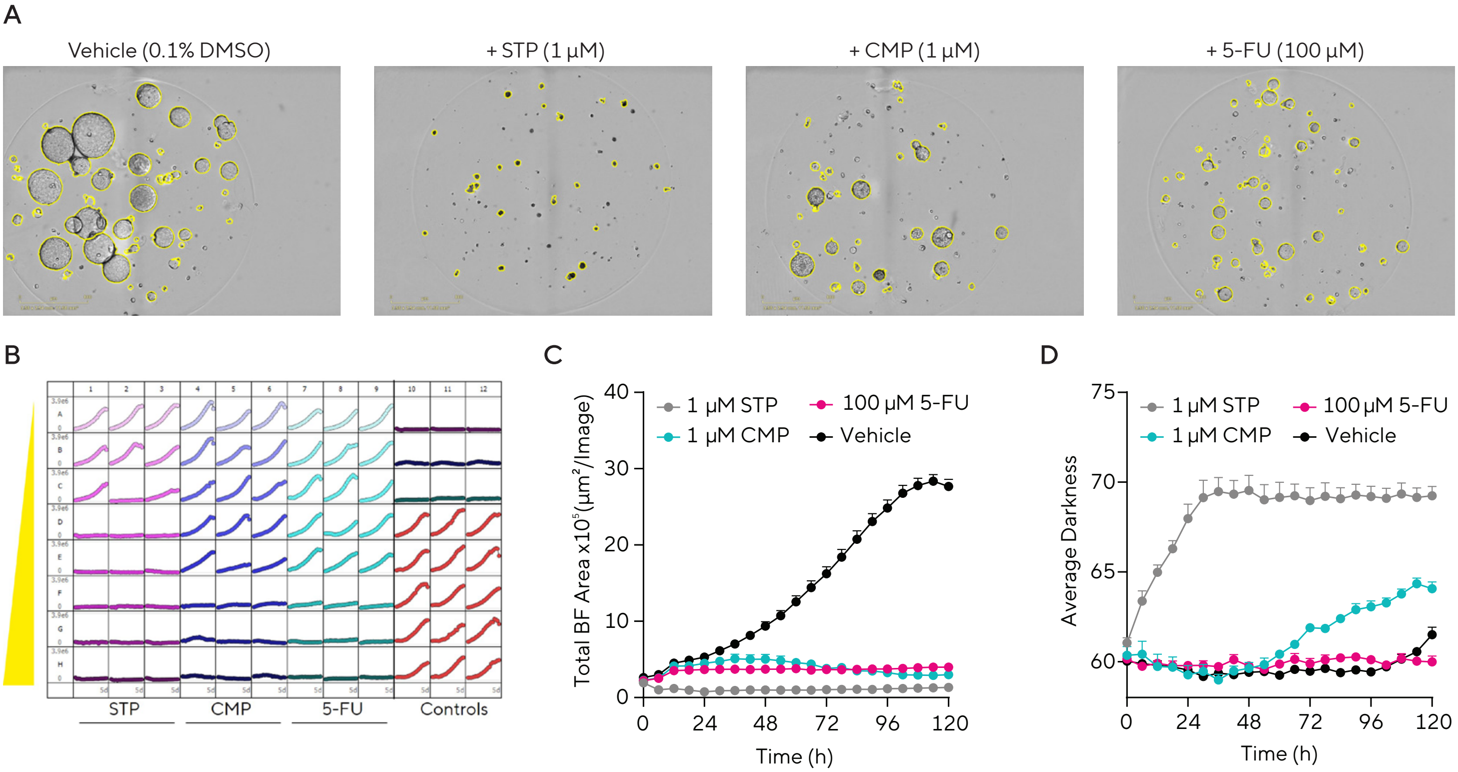

Figure 4. Compound induced cytotoxicity in hepatic organoids. Organoids were allowed to form for 2 days prior to treatment with concentration ranges of cytotoxic compounds STP, CMP and 5-FU. (A) Incucyte® brightfield images with outline segmentation mask (yellow) taken 3 days post treatment show compound specific effects on organoid size and morphology. (B) Microplate view of area vs time displays concentration dependent inhibition of proliferation. Time-courses of organoid area (C) and organoid darkness (D). Data shown as mean ± SEM, n= 3 replicates per plate (15 replicates for vehicle).

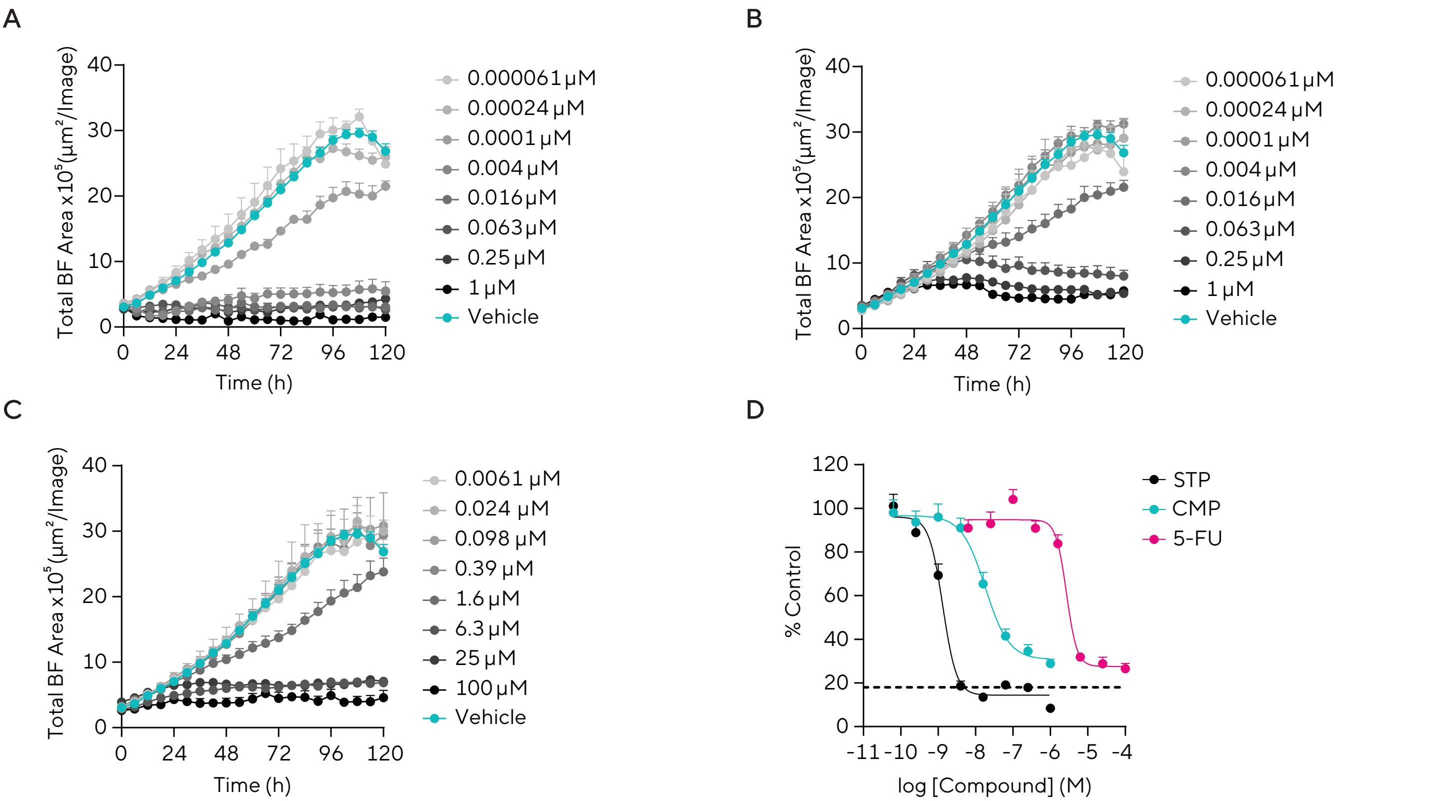

Figure 5. Compound pharmacology. Time-course of organoid area for organoids treated for 5 days with concentration ranges of STP (A), CMP (B) and 5-FU (C). Data shown as mean ± SEM, n= 3 replicates per plate. Inhibition concentration response curve for each compound with the dotted line representing vehicle control organoid size at time of compound addition, t=0h (D). Data shown as mean ± SEM, n= 3 separate test occasions.

Summary

In this study, we have demonstrated the effectiveness of using an automated approach for the successful assay of organoids. By leveraging BIO ONE's temperature-controlled, syringe-based extrusion and accommodation within a biosaftey cabinet, we dispensed low-volume, cell-laden Matrigel® domes with high consistency across 96-well plates. This supported rapid, reproducible, and scalable plate preparation while mitigating the variability and handling limitations inherent to manual approaches. Organoid dynamics was monitored using the Incucyte® Live-Cell Analysis System and Organoid Analysis Software. The integrated real-time quantitative label free metrics were used to assess compound-induced effects on organoid morphology and growth. The combined use of both instruments enables streamlined organoid assay plate production and assessment of organoids parameters appropriate for quantitative pharmacology.

References

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