### SARTORIUS

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

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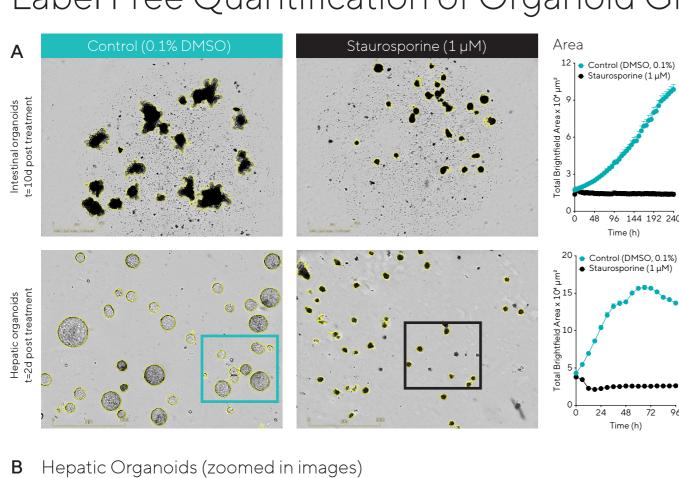
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#### Summary and Impact

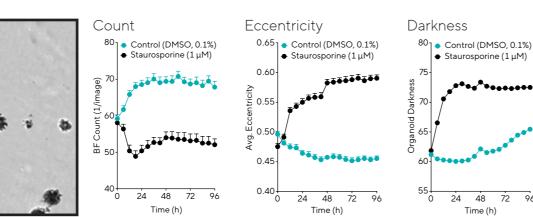
As organoids exhibit structural, morphogenetic, and functional properties that recapitulate *in vivo* pathophysiology, they are increasingly being used *in vitro*. To successfully use these models across a variety of research disciplines and applications, technology pipelines to image & quantify these complex structures are key. Here, we demonstrate simple, robust workflows for monitoring and quantifying organoid growth, death and morphology. Incucyte®'s Organoid Analysis Software Module enables the ability to kinetically visualize and quantify distinct organoid morphologies embedded in Matrigel®. These validation data demonstrate the ability to characterize the differentiation and maturation of organoid cultures in 24-well plates and assess treatment effects on organoid growth in 96-well microplates. Integrated, label-free size and morphology metrics enabled real-time elucidation of intracellular pathway modulation. These data exemplify the amenability of this approach for real-time compound profiling and mechanism of action studies.

#### Label Free Quantification of Organoid Growth and Death



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**Figure 1:** 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). Changes in organoid size and shape were kinetically tracked over time (4-10 d). 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 induced loss of distinctive rounded hepatic organoid phenotype (increased eccentricity) and increased darkness over time (B).



#### Probing Mechanisms of Action Using Morphology Metrics

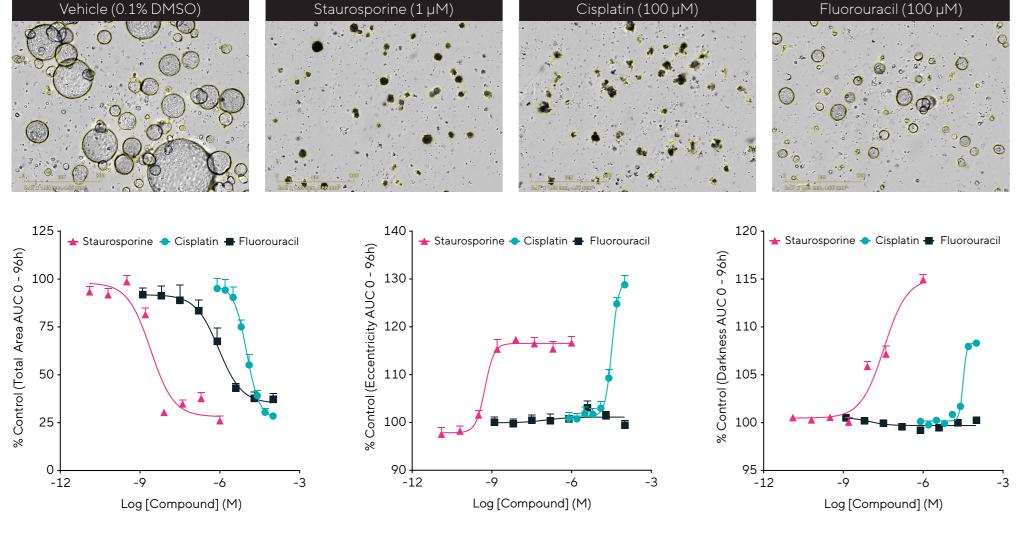
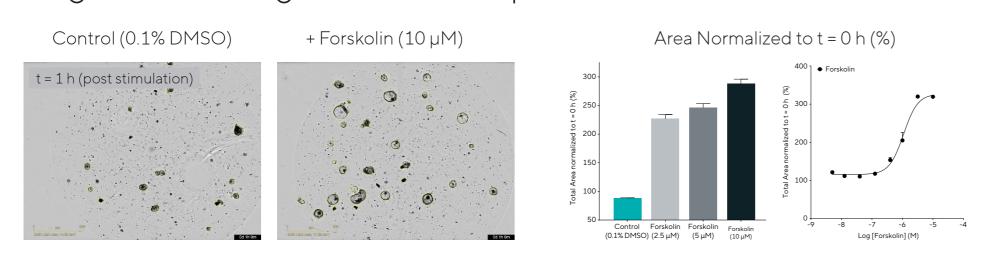


Figure 2: Hepatic organoids were treated with known chemotherapeutic agents & imaged every 6 h for 4 days. Brightfield images (2d post treatment) show compound-specific effects on organoid size and morphology. Concentration response curves (CRCs) represent the area under the curve (AUC) analysis of time-course data. All compounds exhibited concentration dependent inhibition of organoid growth (area), yielding IC50 values of 3 nM for staurosporine (STP), 9.7 μM for cisplatin (CIS) and 0.78 μM for fluorouracil (5-FU). Increases in eccentricity and darkness indicative of 3D structure disruption and cell death respectively were only observed in STP and CIS-treated organoids (cytotoxic MoA). Differences between the size and morphology readouts illustrated the cytostatic mechanism of 5-FU.

#### Organoid Enlargement in Response to Forskolin Stimulation



**Figure 3:** Intestinal organoids formed for 3d were treated with increasing concentrations of an adenylyl cyclase activator, forskolin, and imaged in an Incucyte® every 15 – 20 minutes for up to 7 hours. Brightfield (BF) images show effects of increased cAMP concentrations on organoid size and phenotype. Bar chart of BF area normalized to t = 0 h demonstrates that an increase in size is forskolin concentration dependent. Following stimulation, organoids increased in size, exhibited a more rounded shape and a clear lumen.

#### Incucyte® System for Live-Cell Analysis: Methodology



# 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.

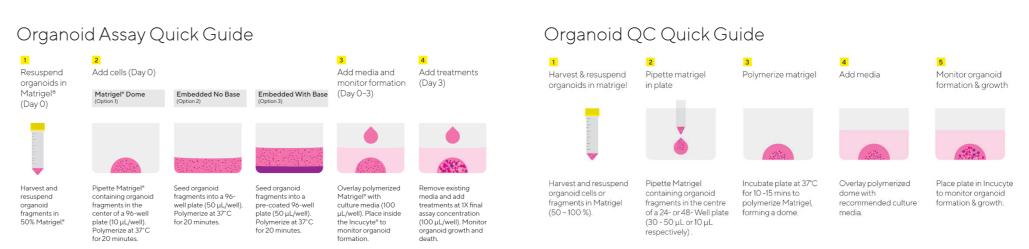


Incucyte® Software
Fast, flexible and powerful control
hub for continuous live-cell analysis
comprising image acquisition,
processing and data visualization.



Sartorius Reagents and Consumables A suite of reagents, kits and protocols for cell health and function screening.

#### Assay Workflows



#### Monitoring and Quantifying Organoids in Matrigel® Domes

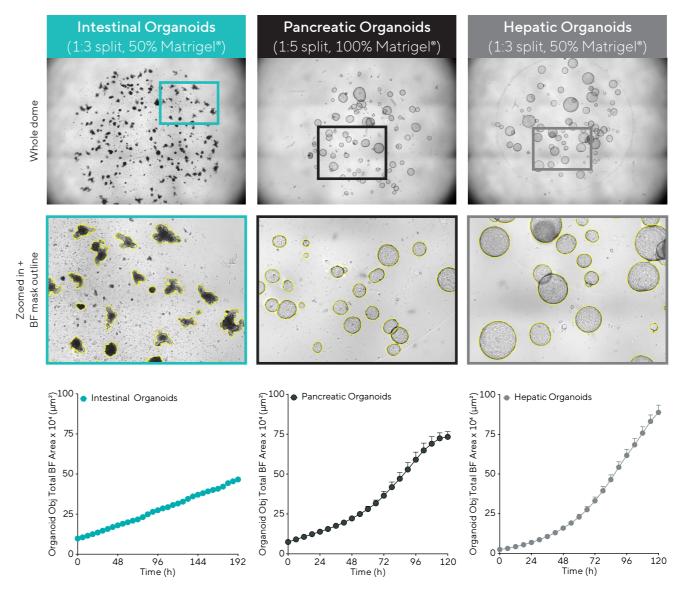
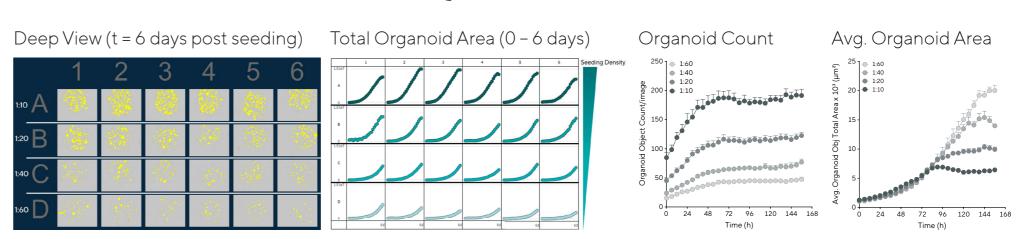


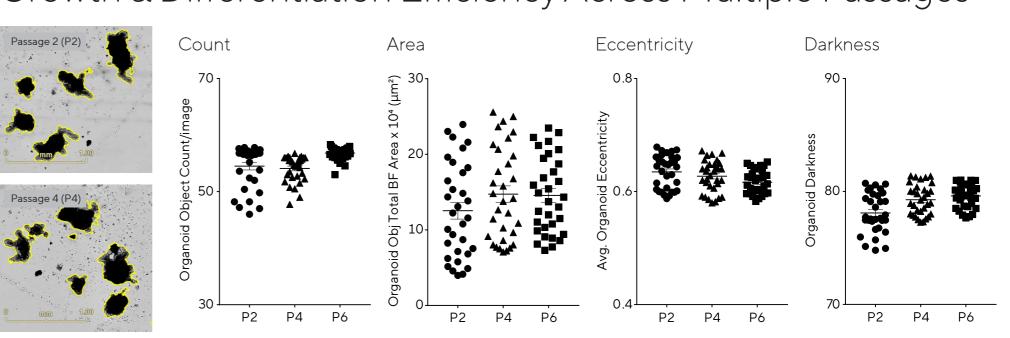
Figure 4: Mouse intestinal, pancreatic and hepatic organoids were embedded in Matrigel® domes in 24-well plates and imaged every 6 hours. Organoid growth, differentiation and maturation was measured using Incucyte®'s automated Organoid Analysis Software Module which tracks changes in organoid size (area) over time. Brightfield (BF) images of the entire Matrigel® dome (top) show organoid maturation 6 days post seeding. Note accurate segmentation (yellow outline mask) and distinct phenotypes of mature organoids (bottom). BF area timecourses demonstrate cell type specific organoid growth. Comparable hepatic and pancreatic organoid growth was observed, while intestinal organoids appeared smaller and exhibited a distinct budding phenotype.

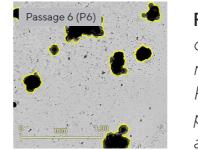
#### Real-Time Visualisation & Quantification of Culture Conditions



**Figure 5:** Mouse hepatic organoids were embedded in Matrigel® domes (100%) at multiple densities in a 24-well plate. Deep view images show brightfield images and segmentation mask overlay 6 days post seeding. Incucyte®'s real-time automated microplate vessel view and time-course plots demonstrate that organoid growth rate and size is proportional to cell number.

#### Growth & Differentiation Efficiency Across Multiple Passages





**Figure 6:** Intestinal organoids were embedded in 50% Matrigel® domes (1:3 split, 24-well plate) over multiple passages and evaluated for growth and differentiation consistency over time. When maintained at a consistent density, organoids exhibited comparable measurements across passages. Representative BF images (7 days post seeding) also demonstrated maintenance of distinct budding phenotype across multiple passages. Data shown exemplifies the amenability of this imaging and analysis approach to support robust and reproducible assessment of long-term organoid expansion.

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