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Simplifying Progress

Label-free, real-time live cell assays for 3D Organoids embedded in Matrigel®.

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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 compound mechanisms of action and assessment of CFTR function *in vitro*.
- These data exemplify the amenability of this approach for real-time compound profiling across a range of disease areas.

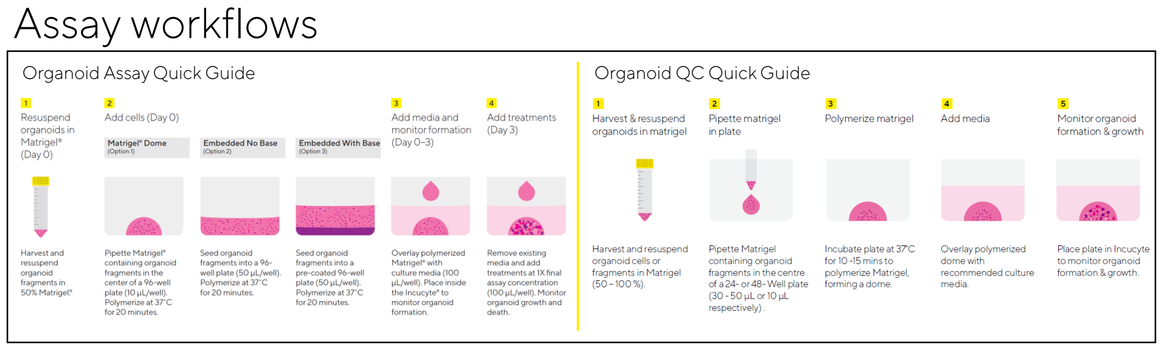
Incucyte® System for live-cell analysis: Methodology

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.

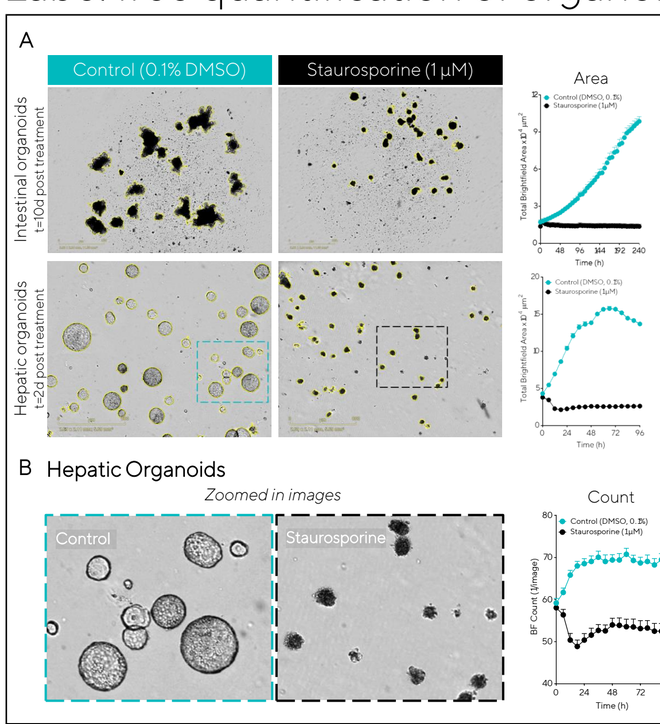
Incucyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

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

Assay workflows

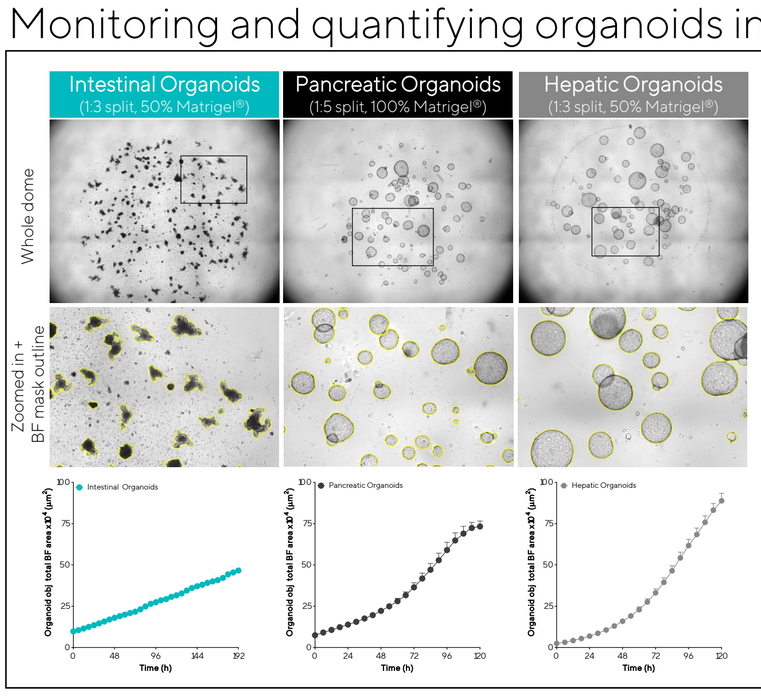


Label free quantification of organoid growth and death



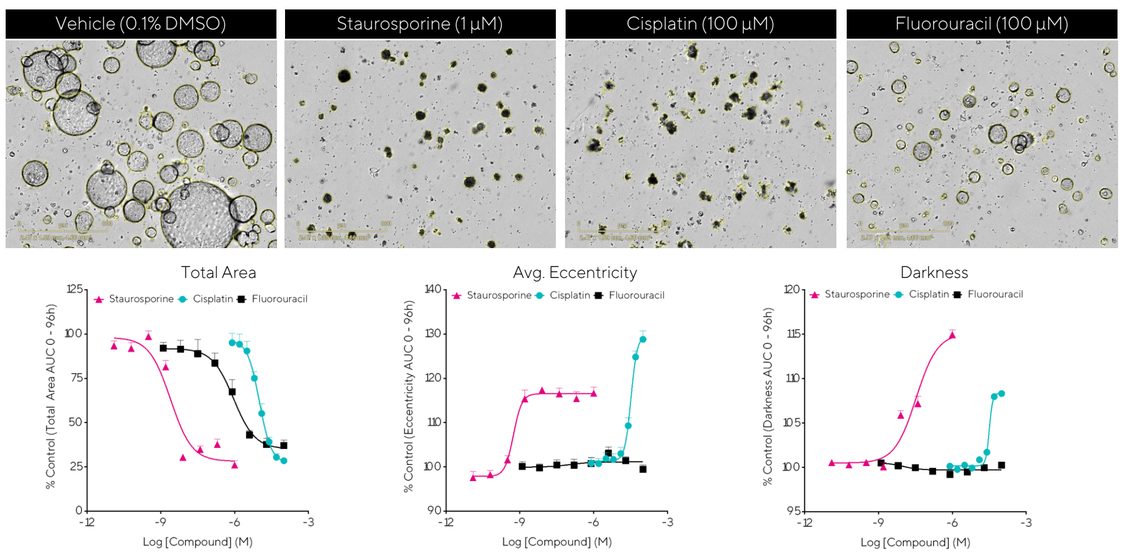
- Mouse intestinal (1:6 split) and hepatic fragments (1K cells/well) were embedded in Matrigel® (50%) in 96-well plates and allowed to form organoids for 3 days prior to treatment (vehicle or staurosporine; STP).
- Changes in organoid size and shape were kinetically tracked over time (4 – 10 d).
- Brightfield (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).

Monitoring and quantifying organoids in Matrigel® domes



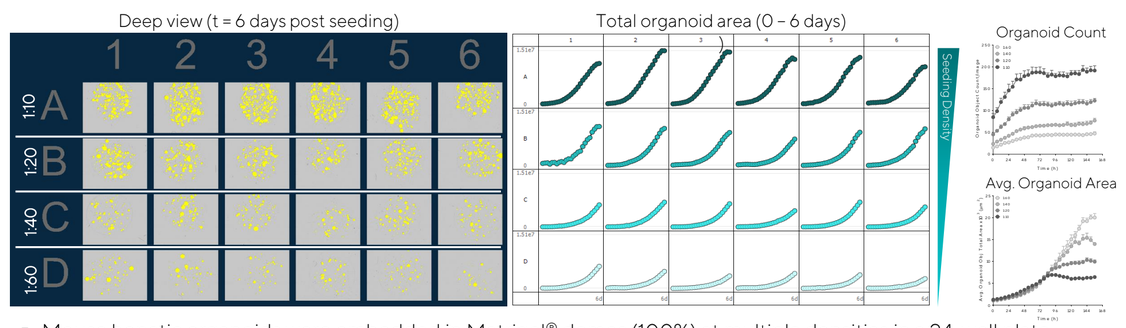
- Mouse intestinal, pancreatic and hepatic organoids were embedded in Matrigel® domes in 24-well plates and imaged every 6 hours.
- Organoid growth, differentiation and maturation was measured using Incucyte®'s automated Organoid Analysis Software Module which tracks changes in organoid size (area) over 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 time-courses 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.

Probing mechanisms of action using morphology metrics



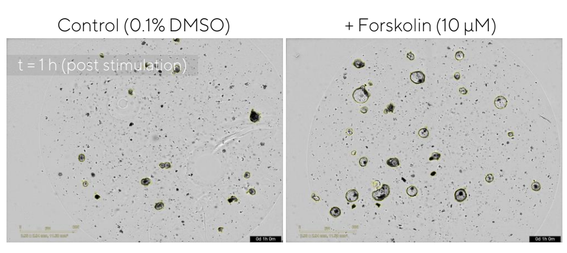
- Hepatic organoids were treated with known chemotherapeutic agents & imaged every 6 h for 4 days.
- Brightfield images (2d post treatment) show compound-specific effects on organoid size and morphology.
- Concentration response curves (CRCs) represent the area under the curve (AUC) analysis of time-course data.
- All compounds exhibited concentration dependent inhibition of organoid growth (area), yielding IC_{50} 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.

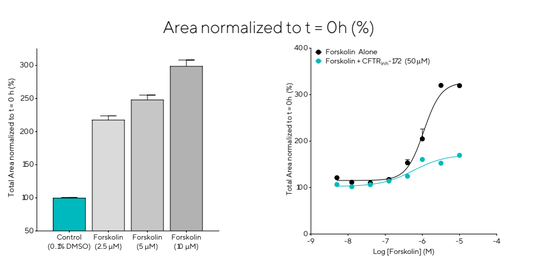
Real-time visualisation & quantification of culture conditions



- 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.

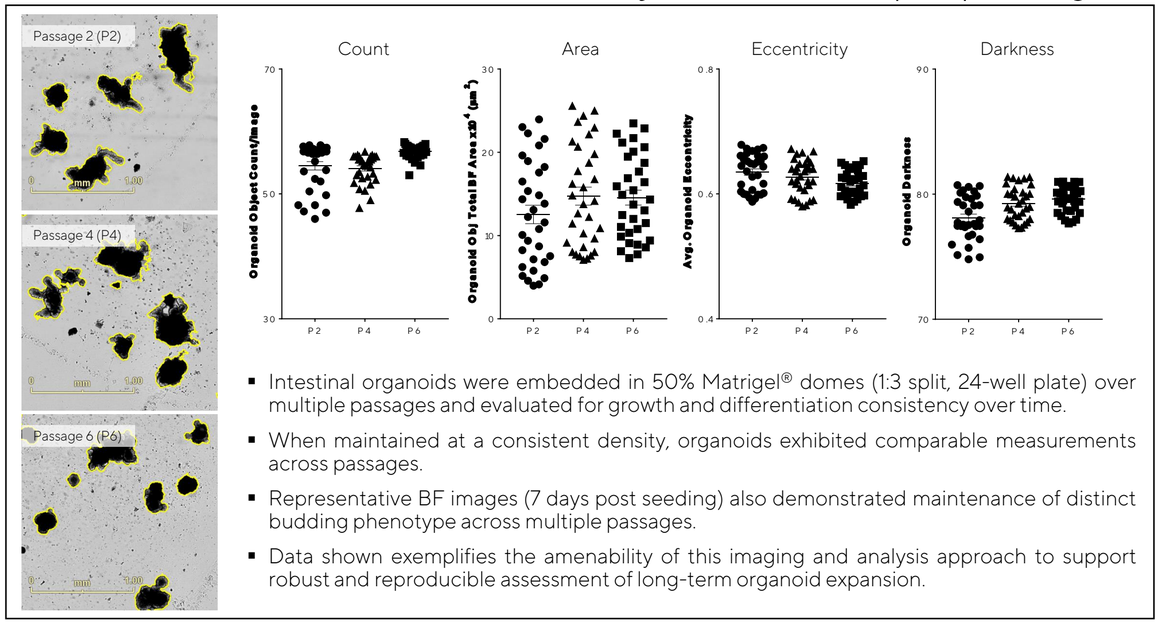
Organoid swelling in response to Forskolin stimulation





- Intestinal organoids formed for 3d were treated with increasing concentrations of forskolin and imaged in an Incucyte® every 15 – 20 mins for up to 7 hours.
- Brightfield (BF) images show effects of forskolin treatment on organoid size and phenotype.
- Following stimulation, organoids increased in size, exhibited a more rounded shape and a clear lumen.
- Bar chart of BF area normalized to t=0h demonstrates that swelling is forskolin concentration-dependent.
- In the presence of CFTR inhibitor $CFTR_{inh}$-172 the maximal response was reduced by > 50% (~150% at 10 µM) illustrating that swelling was cystic fibrosis transmembrane conductance regulator (CFTR)-dependent.

Growth & differentiation efficiency across multiple passages



- 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.