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Application Note

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Real-time Live-Cell Analysis of 3D Organoid growth in Matrigel® domes

Introduction

Recent advances in organoid technology have opened up new horizons for translational human disease research, disease modeling, regenerative medicine and predictive precision therapies[1].

Organoids are differentiated primary micro tissues formed from a variety of stem cells (SCs) that can be established within 3D extracellular matrices to mimic *in-vivo* architecture and genetic diversity[1]. As self-organizing and self-renewing structures, organoids have a distinct advantage over traditional monolayer culture techniques and hold unprecedented potential for various applications[1,2]. In order to effectively use these models in basic research, disease modeling and drug screening, specific and reliable *in-vitro* culture and analysis methods are required.

Currently, characterization and optimization of organoid cultures are limited in their ability to objectively monitor these 3D structures as they form and grow over time. The Incucyte® Organoid Analysis Software Module provides a solution to standardize and automate organoid culture workflows, simplifying culture characterization and optimization.

Assay Principle

This application note describes the use of the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software Module to study the formation and growth of organoid cultures. A proprietary Brightfield (BF) image acquisition approach enables real-time kinetic imaging of 3D organoids embedded within Matrigel® domes. Organoid size, count and morphology measurements are automatically plotted over time to provide insight on organoid differentiation and maturation characteristics.

Here we describe validation methods and data demonstrating the ability to kinetically visualize and quantify organoid formation and growth in Matrigel® domes.

Find out more: www.essenbioscience.com/en/applications/cell-monitoring-workflows/organoid-qc/

Material & Methods

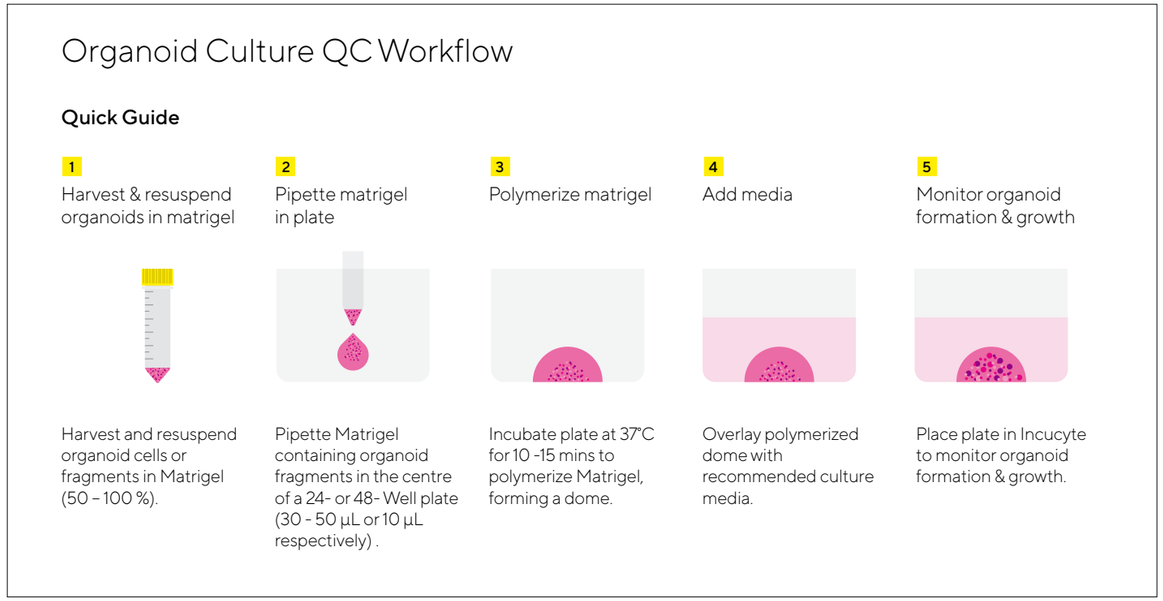

Figure 1. Assay Workflow

1. Organoids of interest are harvested according to model-specific guidelines and organoid pellet is re-suspended in Matrigel® (50 – 100%)
2. Matrigel® containing single cells or organoid fragments is pipetted into the center of a well in a 24-well or 48-well tissue culture treated plate to form a dome (10 µL in 48-well plate, 30 µL- 50 µL in 24-well plate).
3. Plate is placed in a humidified incubator to polymerize Matrigel® (37° C, 10 – 15 minutes).
4. Cell type-specific growth media is added on top of solidified dome (250 µL/well in 48-well plate or 750 µL/well in 24-well plate).
5. Organoid formation and growth is monitored in an Incucyte® (Organoid scan type, 4X, 6 hour repeat scanning, 5 – 10 days). Organoid size (maturation) is reported in real-time based on brightfield image analysis.

Organoid culture reagents were obtained from StemCell Technologies unless otherwise noted. Mouse Intestinal (#70931), Pancreatic (#70933) and Hepatic (#70932) organoids were embedded in Matrigel® domes (Corning #356231) in 24-well or 48-well flat bottom TC-treated microplates (Corning #3526, 3548 respectively) and cultured in organoid growth medium (IntestiCult™ OGM Cat. #06005; PancreaCult™ OGM #06040; HepatiCult™ OGM #06030) supplemented with 100 units/100 µg per mL Pen/Strep (Life technologies). Organoid formation and growth was monitored in an Incucyte® at 6 hour intervals for up to 10 days.

Monitoring and quantifying organoid growth in Matrigel® domes.

Mouse intestinal, pancreatic and hepatic organoids were embedded in Matrigel® domes (50% or 100%) in 24-well plates and imaged every 6 hours.

Organoid growth, differentiation and maturation was measured using Incucyte's automated Organoid Software Analysis Module which tracks changes in organoid size (area) over time.

Figure 2 illustrates the software's ability to visualize as a single in focus 2D image, individual organoids embedded throughout the Matrigel® dome (top). Zoomed in BF images (bottom) and time-courses revealed cell type-specific morphological features and growth profiles respectively. Note the comparable rapid growth (time-courses) and size (BF images) of mature hepatic and pancreatic organoids in contrast to intestinal organoids, which appear smaller and exhibit a distinct budding phenotype as they mature.

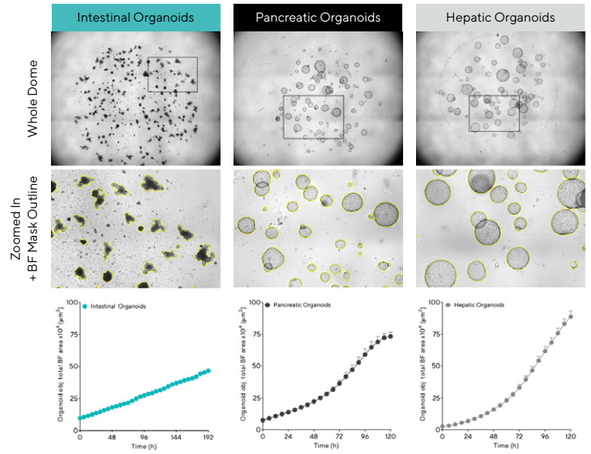

Figure 2. Acquisition and quantification of organoid growth in Matrigel® domes. Mouse intestinal (1:3 split, 50% Matrigel), pancreatic (1:5 split, 100% Matrigel®) and hepatic organoids (1:40 split, 100% Matrigel®) were embedded in Matrigel® domes in 24-well plates and imaged every 6 h in an Incucyte. 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). Time-course plots showing the individual well total BF area (µm²) over time (h) demonstrate cell type specific organoid growth. All images captured at 4X magnification. Each data point represents mean ± SEM, n = 4 wells.

Optimization and characterization of organoid cultures using real-time kinetic measurements.

Defining optimal culture conditions and regimes are critical for establishing healthy cultures for use in downstream studies. Objectively defining parameters such as seeding densities, passage frequency and ensuring cultures have differentiated and display appropriate morphology is key.

Measuring proliferation to optimize growth conditions and seeding densities

To optimize organoid seeding density, mouse hepatic organoids were embedded in Matrigel® domes (100%) in 48-well plates at multiple split ratios (1:10 – 1:40 split). BF images and quantification of organoid area demonstrated that organoid growth rate and size is proportional to the number of cells seeded (Figure 3). Organoids seeded at the highest density appeared larger (> 500 µm diameter) and exhibited rapid growth reaching maximal size (73.4×10^4 µm² ± 2.3 mean ± SEM, n = 14 wells) within 120 h (Figure 3, BF images and total area time-course respectively). Conversely, at lower densities while the organoid maturation phase was extended (28.6×10^4 µm² ± 2.9 mean ± SEM, n = 14 wells at 120 h, total area), the greatest growth potential (size) was observed (Figure 3, average area time-course).

Measuring morphological features to define optimal organoid maturation phase

To define optimal organoid passaging frequency, mouse hepatic organoids were embedded in Matrigel® domes (100%) and imaged in an Incucyte® for 8 days. Hepatic organoids are typically ready for passaging when

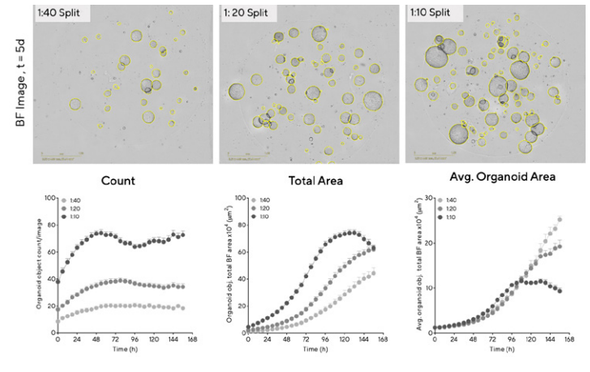

Figure 3. Determine optimal conditions for maximal organoid expansion. Mouse hepatic organoids were embedded in Matrigel® domes (100%) in 48-well plates at multiple seeding densities. BF Images (5 d post seeding) and time-courses of individual well area and count demonstrate density-dependent organoid growth. Data were collected over 168 h at 6 h intervals. All images captured at 4X magnification. Each data point represents mean ± SEM, n=14 wells.

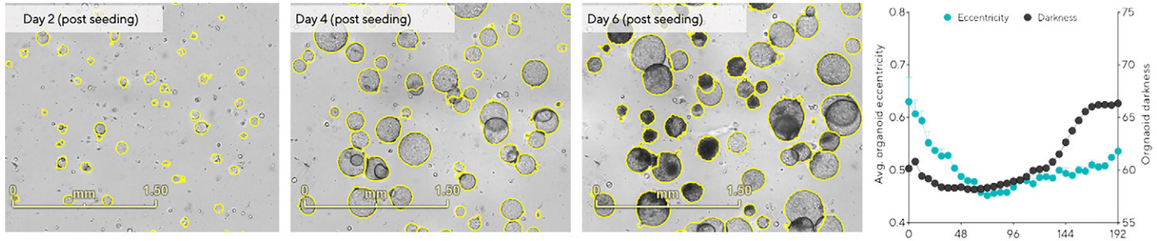

Figure 4. Define cell-type specific passage frequency using integrated morphology metrics. Hepatic organoids were embedded in 100% Matrigel® domes (1:10 split) in 24-well plates. Tracking changes in organoid eccentricity (object roundness) and darkness (object brightness) enabled rapid assessment of optimal culture passage periods. Images (day 6) and time-course data demonstrate that organoids that reached maximal size collapsed (increased eccentricity) and darkened (increased darkness). Data were collected over 192 h at 6 h intervals. All images captured at 4x magnification. Each data point represents mean ± SEM, n=6 wells.

the majority of organoids have reached their maximum growth and have not collapsed[3]. Representative BF Images (Figure 4) show that 2 days post seeding, cultures are not yet ready for passaging as the majority of organoids are less than 100 µm in diameter and exhibit clear lumens. A decline in eccentricity was also observed within 48 h as organoids formed and became more rounded (Figure 4, time-course). The optimal period for passaging this culture occurred between days 4 and 5, when most organoids within the dome had reached maximal size, exhibited a rounded morphology and had not collapsed (Figure 4). The time-course shows a marked increase in organoid darkness as collapsed organoids darkened over time (Figure 4, time-course, >96 h).

Tracking organoid differentiation and growth efficiency through passages

Under routine culture conditions, organoid morphology and growth capabilities are expected to remain consistent across multiple passages.

To assess intestinal organoid expansion and growth efficiency across passages a number of parameters were kinetically quantified (Figure 5). When maintained at a consistent density, intestinal organoids exhibited comparable count, area, eccentricity and darkness measurements across passages (Figure 5). Representative BF images (7 d) also demonstrate maintenance of distinct budding phenotype across multiple passages. Figure 5 exemplifies the amenability of this imaging and analysis approach to support robust and reproducible assessment of long-term organoid expansion.

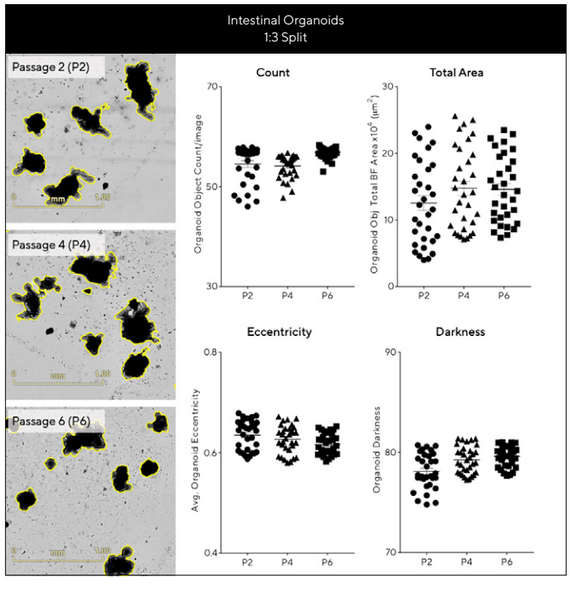

Figure 5. Assess growth and differentiation efficiency of organoids across multiple passages. Intestinal organoids were embedded in 50% Matrigel® domes (1:3 split, 24-well plate) over multiple passages and evaluated for growth and differentiation consistency over time. BF images (7 days post seeding) and corresponding plots show comparable morphology and growth across passages. Data were collected over 192 h at 6 h intervals. All images captured at 4X magnification. Each data point represents mean ± SEM, n=6 wells.

Conclusions

In this application note, we demonstrate the use of the Incucyte® Live-Cell Analysis system, in combination with the Incucyte® Organoid Analysis Software Module, to facilitate kinetic assessment of organoid formation and growth. We have demonstrated:

- The ability to automatically locate and analyze 3D organoids embedded within Matrigel® domes in both 24-and 48-well plates.
- The use of integrated, real-time label-free metrics to optimize and define culture conditions and regimes.
- Optimal periods for passaging or extension of organoid cultures based on integrated morphological parameters.
- Use of this approach to assess culture quality during extended passaging.

References

1 D. e. al., “Disease modeling in stem cell-derived 3D organoid systems,” Cell Press, vol. 23, no. 5, pp. 393-410, 2017.
2 G. e. al., “Long-term expansion, genomic stability and in vivo safety of adult human pancreas organoids.,” BMC Dev Biol, vol. 4, 2020.
3 “StemCell Technologies,” [Online]. Available: https://www.stemcell.com/mouse-hepatic-progenitor-organoid-culture-supplementary-protocols.html.

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