SARTORIUS

Compendium

Incucyte® Applications: 3D Cell Culture

Introduction

Our understanding of human diseases and the development of therapeutic strategies to combat them are being advanced with the use of ground-breaking *in vitro* 3D cell culture models, including single and multicellular tumor spheroids and organoids. These models can display structural, morphogenetic and functional properties that resemble *in vivo* pathophysiology, creating a more physiologically relevant setting for predictive and translational research.

Tumor spheroids have a microenvironment that more closely resembles that of tumors *in vivo* and as a result, offer significant advantages over monolayer cultures. In conventional 2D culture platforms, tumor cells are grown on non-biological rigid surfaces in excess culture medium, producing hyper-oxygenated and hyper-nourished cells with unrestricted and non-physiological proliferation characteristics. A more realistic setting involves recreating the physiologic heterogeneity inherent to a 3D tumor structure and providing a microenvironment similar to the *in vivo* situation, which includes key interactions between the tumor and the extracellular matrix (ECM). In these 3D spheroid cultures, the cell environment can be reproduced with higher accuracy including cell-cell and cell-matrix interactions.

Similarly, organoids are rapidly becoming a powerful tool for both basic research and drug discovery, spanning a wide range of applications including oncology, regenerative medicine, disease modeling, and drug screening. These 3D organotypical structures can be grown *in vitro* to produce miniaturized versions of the organs from which they were derived. As self-organizing and self-renewing 3D structures, organoids offer a distinct advantage over traditional monolayer culture techniques and offer a more physiologically relevant milieu in which to understand complex biology with greater clarity. When established with 3D extracellular matrices, the cultures can recapitulate the *in vivo* architecture, spatial organization, and genetic diversity of the cell populations found in the original organ with remarkable fidelity.

In this compendium of applications, we demonstrate the power and flexibility of the Incucyte® Live-Cell Analysis System for the study of spheroids and organoids in foundational research, disease modeling and drug screening.

Find out more: www.sartorius.com/incucyte

Label-free Spheroid and Organoid Models

Conventional techniques for generating and quantitatively analyzing spheroids and organoids are time consuming, laborious, costly, and/or lack reproducibility, prohibiting the effective utilization of these advanced models.

Incucyte® 3D Tumor Spheroid Assays
Effective analysis of 3D tumor spheroids can be challenging as these heterogeneous models are multi-faceted and dynamic. Traditional plate reader assays focus on a single end-point and lack image-based analysis which is needed to provide morphological information and the ability to confirm data within images. Conventional imaging systems are inherently difficult to adapt to kinetic analyses of *in vitro* culture models, suffering from:

- Incomplete data resulting from missed information between imaging intervals
- Multiple uncontrolled environmental fluctuations resulting from the movement of plates from the incubator to the imaging system and lengthy 3D image acquisition protocols outside the incubator
- Time-consuming development of optimal image acquisition parameters
- Complex image processing requiring expert operators to generate quantitative information

Incucyte® 3D Tumor Spheroid Assays offer an integrated turnkey solution to automatically monitor and quantify tumor spheroid formation, growth and health in real-time inside the tissue culture incubator.

Application Notes 1 and 2 describe how brightfield image analysis, in combination with phase contrast imaging performed on the Incucyte® Live-Cell Analysis System, allows for label-free study of 3D spheroid morphology, growth and shrinkage in 96- and 384-assay formats. Incucyte® HD phase contrast images facilitate comprehensive visualization of spheroid morphological features (shape, size) and intercellular compaction (loose aggregates vs. compact spheroids) characteristic for each cell type. Brightfield provides the means for objective spheroid kinetic quantification and cell dependent growth rate profile assessment for diverse types of spheroids.

The Incucyte® Live-Cell Analysis System can be used to provide kinetic, non-perturbing physiological characterization of multi-cellular spheroid growth and viability and is amenable to pharmacological discovery and development as detailed in Application Note 3.

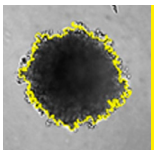
Whitepaper 1 describes the importance of live-cell monitoring for complex, recurrent workflows and, with the ability to perform multiparametric imaging techniques in a non-perturbing manner, its relevance and value to 3D cell culture models is demonstrated.

Incucyte® Organoid Culture QC
In order to exploit organoid models for meaningful basic research, disease modelling or drug screening, specific and reliable culture and analysis methods are required, including tightly controlled, optimized and documented steps throughout the organoid workflow beginning with culture quality control. Currently, characterization and optimization of organoid cultures are limited in their ability to reproducibly form and monitor these 3D cell models as they form and grow over time.

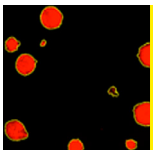
The Incucyte® Live-Cell Analysis System offers a turnkey solution consisting of a validated 3D cell culture protocol and software module for real-time visualization and label-free objective quantification to optimize organoid culture conditions. Our Organoid Analysis Guide (eBook 1) and Organoids in Drug Discovery (eBook 2) provide a comprehensive handbook that includes:

- Translational applications of organoids
- Criteria for successful organoid culture and expansion
- Imaging and objective analysis of organoids using leading-edge technology

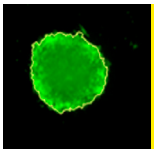
Resources Highlighted in This Section



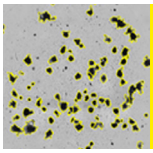
Application Note 1:
Label-Free, Real-Time Live-Cell Assays for Spheroids: Incucyte® Brightfield Analysis



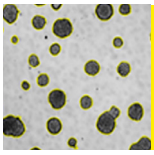
Whitepaper 1:
Live-Cell Monitoring – Optimizing Workflows for Advanced Cell Models



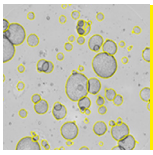
Application Note 2:
Validation and Pharmacological Utility of Real-Time, Live-Cell Assays for Single 3D Spheroids



eBook 1:
Organoid Analysis Guide



Application Note 3:
Validation of Real-Time, Live-Cell Assays for 3D Multi-Spheroids Formed on Bio-Matrices



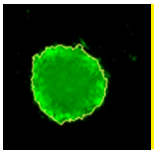
eBook 2:
Organoids in Drug Discovery

Spheroid Viability/ Health Assays

Incucyte® 3D Single and Multi-spheroid Assays are a turnkey, integrated solution to automatically track and quantify tumor spheroid formation, growth and health in real-time inside the tissue culture incubator. Incucyte® assays address the shortcomings of conventional imaging systems which are inherently difficult to adapt to kinetic analyses of *in vitro* culture models.

Detailed information about these assays can be found in Application Notes 2 and 3 and several of our website pages.

Resources Highlighted in This Section



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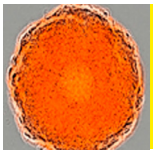
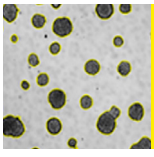
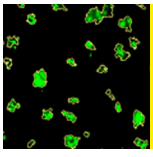
Validation and Pharmacological Utility of Real-Time, Live-Cell Assays for Single 3D Spheroids



Web Page:
Incucyte® Live-Cell Assays for Single-Tumor Spheroids



Application Note 3:
Validation of Real-Time, Live-Cell Assays for 3D Multi-Spheroids Formed on Bio-Matrices



Web Page:
Incucyte® Live-Cell Assays for Multi-Tumor Spheroids

Organoid Applications

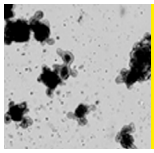
To successfully establish and accelerate organoid relevance to clinical outcomes, approaches are required that can reduce variability while also increasing insight. Innovations in culturing *ex vivo* human models has opened a world of scientific possibilities but are still limited by the technologies used to characterize these complex cell models. These limitations include:

- Introduction of variability due to lack of environmental control and inadequate 3D cell culture protocols that do not compromise organoid cell health
- Low-throughput and cannot be scaled for screening
- Subjective random assessment of organoid cell growth, death or morphology
- Require time-consuming, expensive and manual processes for acquisition of organoid images
- Require third party software for end-point analysis, or additional fluorescent markers — with limited quantitative information

In Application Note 4, we demonstrate use of the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software Module to facilitate kinetic assessment of organoid formation and growth. Results of the study demonstrated the ability to automatically locate and analyze 3D organoids embedded within Matrigel® domes; use of integrated, real-time label-free metrics to optimize and define culture conditions and regimes; optimize the timing for passaging or extending cultures based on integrated morphological parameters.

Incucyte® Organoid applications kinetically monitor and quantify organoid differentiation (Organoid Culture QC) or growth and death (Organoid Assay) undisturbed inside your incubator. We can capture and quantify distinct organoid morphologies and track cell death via changes in size (Total Brightfield Area) using Incucyte® Organoid Analysis Software Module.

Resources Highlighted in This Section



Application Note 4:
Real-Time Live-Cell Analysis of 3D Organoid Growth in Matrigel® Domes

3D Spheroid Co-Culture Models

Current methods for assessing the growth and shrinkage of tumor spheroids are often limited by time-consuming, expensive and/or laborious assay workflows. These may include fluorescent probes that perturb the biology, an end-point analysis that might miss insightful temporal information, or indirect biochemical readouts that overlook valuable morphological insight.

The Incucyte® Live-Cell Analysis System allows for direct, multiplexed measurements of immune cell-mediated killing of tumor cells via the combination of real-time, automated analysis along with non-perturbing, live-cell reagents—directly in the cell culture incubator.

Application Note 5 demonstrates how the Incucyte® Live-Cell Analysis System enabled analysis of 3D multi-spheroid co-cultures with either stromal or immune cells over time and is applicable to compound testing.

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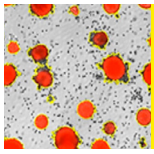


Application Note 5:
Real-Time Live-Cell Analysis of Multi-Spheroid, Co-Cultured, 3D Tumor Assays

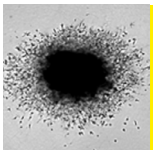
Single-Spheroid Invasion Assays

The ability to invade surrounding tissues and seed secondary sites is a defining feature of malignancy. Several Incucyte® assays can be used to dissect the sequential steps of metastasis, including 'scratch wound' migration and invasion assays, chemotaxis experiments, and growth and colonization studies. Combining these live-cell assays with 3D cell culture models has also provided valuable information which might otherwise be overlooked (Whitepaper 2).

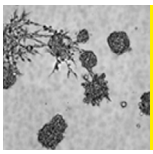
The Incucyte® Live-Cell Analysis System enables accurate assessment of the invasive potential of tumor spheroids over time, inside the incubator. Poster 1 describes how this label-free analysis delivers deeper insight into the invasion, progression and response to treatments of tumor spheroids.

Detailed information about invasion assays can also be found on our website.

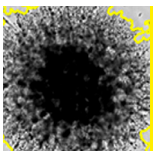
Resources Highlighted in This Section



Poster 1:
Real-Time, Live-Cell Analysis of 3D Tumor Spheroid Invasion



Whitepaper 2:
Exploring the Hallmarks of Cancer With Real-Time Live-Cell Analysis



Web Page:
Incucyte® Single Spheroid Invasion Assay

Conclusion

Tumor spheroids and human organoid systems continue to enable a greater understanding of complex disease mechanisms and are playing a larger role in discovery and development of novel therapeutics. The ability to objectively define parameters such as seeding density, passage frequency and morphology is essential to establishing healthy cultures and new applications continue to expand and unlock the full potential of these more physiologically relevant 3D cultures.

[i] Dutta, D., Heo, I., and Clevers, H. (2017). **Disease modeling in stem cell-derived 3D organoid systems.** *Trends in Molecular Medicine.* 23(5), 393-410.

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