From Microcavities to Morphological Insights: High-Throughput 3D Organoid Imaging and Analysis with Gri3D® and CQ1

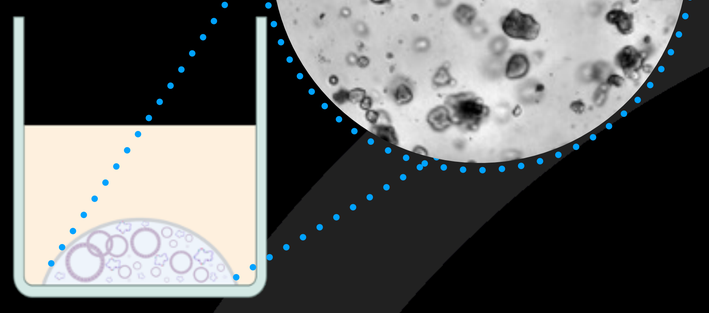
Ballabio, M.[1], Wieland, F. A.[2], Clapés Cabrer, M.[1], Meyer, M.[3], Hoehnel-Ka, S.[1]

[1] SUN bioscience SA, Lausanne, Switzerland - [2] Yokogawa Life Science Europe, Basel, Switzerland - [3] Doppl SA, Lausanne, Switzerland

INTRODUCTION

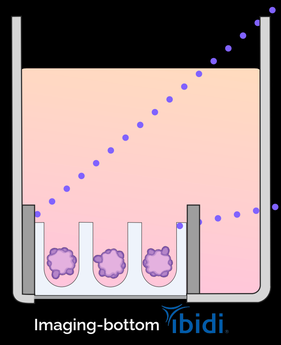
Organoids bridge traditional cell culture and animal models, offering promise in various fields, and are traditionally cultured in solidified extracellular matrix (ECM). This method presents multiple challenges, including **high-content imaging in 3D** due to sample heterogeneity and variations in focal planes [1].

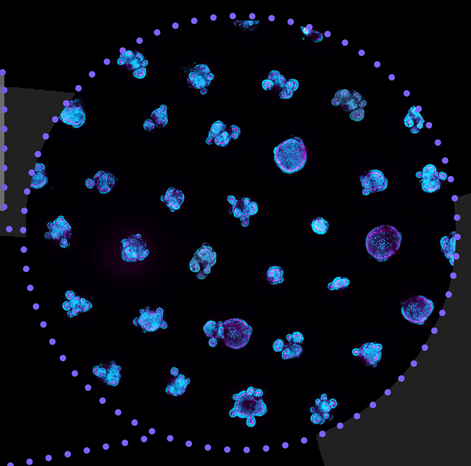
ECM-dome 3D culture



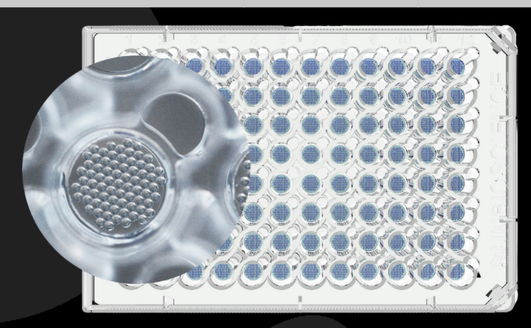
Microwell array in Gri3D®

- U-bottom microcavities
- Cell-repellent PEG hydrogel
- Pipetting port
- Imaging-bottom (180 µm)



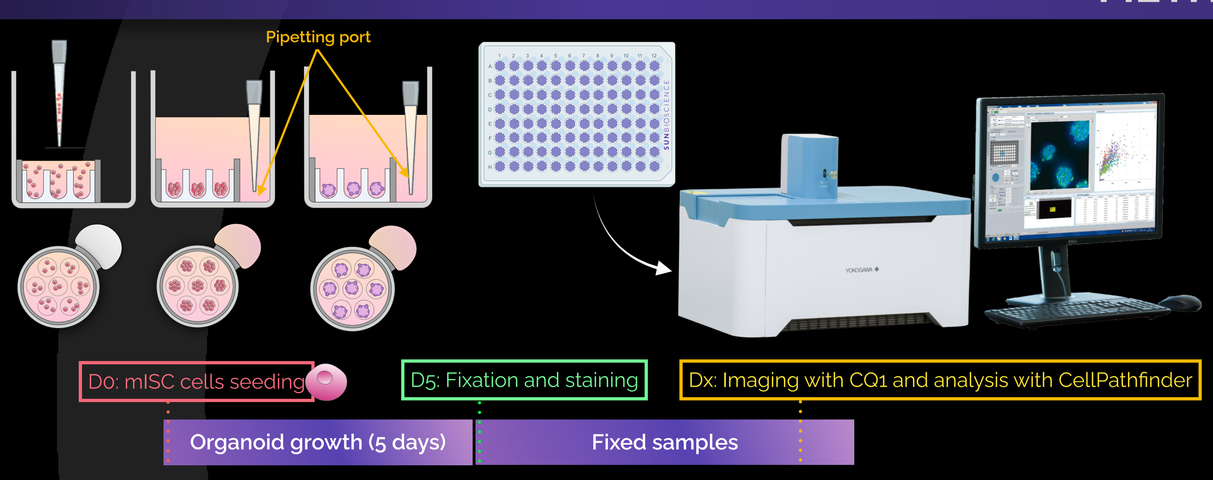


Feature	ECM-dome	Gri3D®
HOMOGENEITY	■	■■■
LOCATION (x,y,z)	■	■■■
AUTOMATION	■	■■■
CELL-CELL INTERACTIONS	■■	■■■
PHYSIOLOGICAL RELEVANCE	■■	■■■
THROUGHPUT	■	■■■



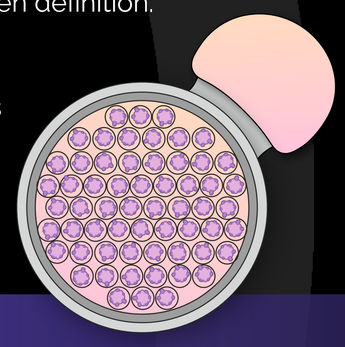
To tackle these hurdles, we introduce Gri3D®: an innovative **hydrogel microwell 96WP** system designed for uniform cell seeding, efficient aggregation, and the generation of individual microtissues in **suspension-like conditions** [2]. Importantly, the resulting microtissues are tesselated all within the **same focal plane**, enabling simultaneous high-resolution imaging. Here, we combined the plate with Yokogawa's CQ1, a **dual-microlens spinning disk imaging confocal microscope**, which has proven to be successful in high-content imaging of 3D spheroid models [3]. In this study, we demonstrate high-content imaging of mouse intestinal organoids cultured in Gri3D® plates using Yokogawa's CQ1 high-content imaging system and CellPathfinder software, facilitating precise segmentation and analysis.

METHODS



Mouse intestinal organoids are cultured in Gri3D® plates for five days. Subsequently, the microtissues are fixed with paraformaldehyde, stained, and imaged directly on an imaging-bottom plate, with Yokogawa's CQ1. The transparent hydrogel enables high-resolution imaging of organoids in microcavities, with Z-stack imaging revealing intricate 3D structures. CellPathfinder software facilitates segmentation and analysis, yielding insights into organoid characteristics such as nuclei count, epithelial thickness, and lumen definition.

Model: Mouse Intestinal organoids
Gri3D® Format: 400 µm, 121 µwells - imaging bottom - black walls
Seeding density: ~200 cells per microwell (24.2k cells per well)
Cell types: Mouse intestinal stem cells (mISC)
Culture time: 5 days



RESULTS

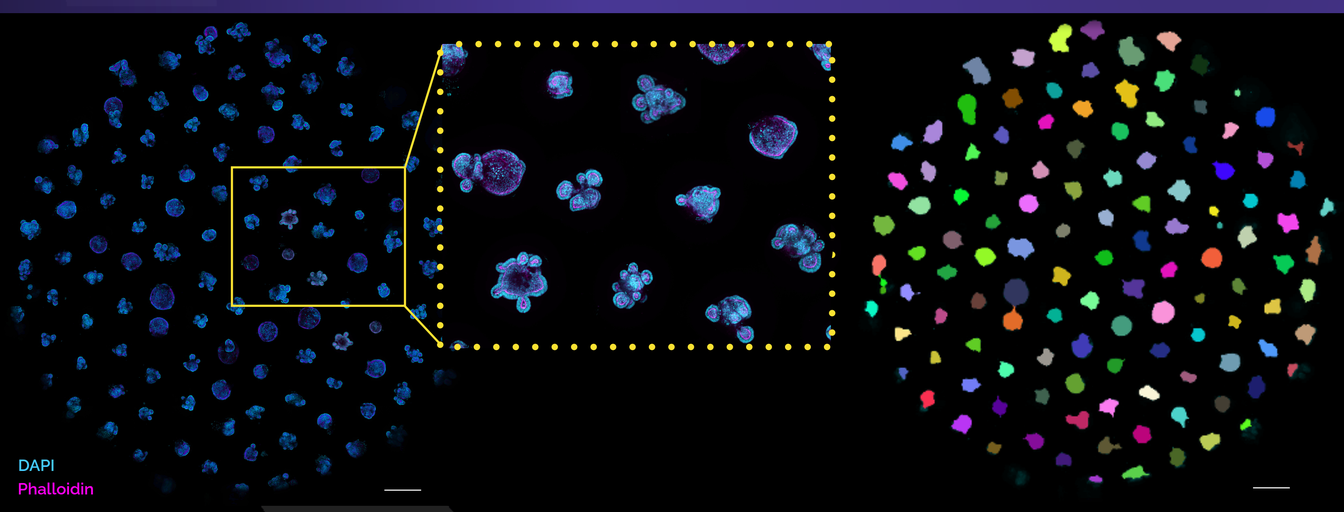


Figure 1) Organoid fluorescence imaging and segmentation of one single Gri3D® well, containing 121 3D microtissues. Left: 4x stitched images taken on a Yokogawa CQ1. Maximum intensity projection of stained mouse intestinal organoids. DAPI in Blue and Phalloidin in Magenta. Right: Segmentation, labeling, and counting of the organoids in Yokogawa's CellPathfinder software. Scale bars 400 µm.

Gri3D® 96WP 400 µm microwells are used to scale up the generation of intestinal organoids. 121 microtissues are formed within one well, all in the same focal plane. After five days, the organoids are incubated with the antibodies of interest, fixed, and immunostained on Gri3D® following an automatable protocol (Figure 1. Left). The images are analyzed with CellPathfinder, allowing to automatically count, label, and segment the 121 microtissues (Figure 1. Right).

Because the hydrogel is transparent (95% water), high-magnification (20x) Z-stack imaging can be performed directly on-plate using the CQ1, and further 3D analyses can be conducted with CellPathfinder to extract numerous features. In this instance, we successfully segment the cell nuclei, measure the epithelium thickness, and accurately define the hollow lumens inside the mouse intestinal organoids in 3D (Figure 2).

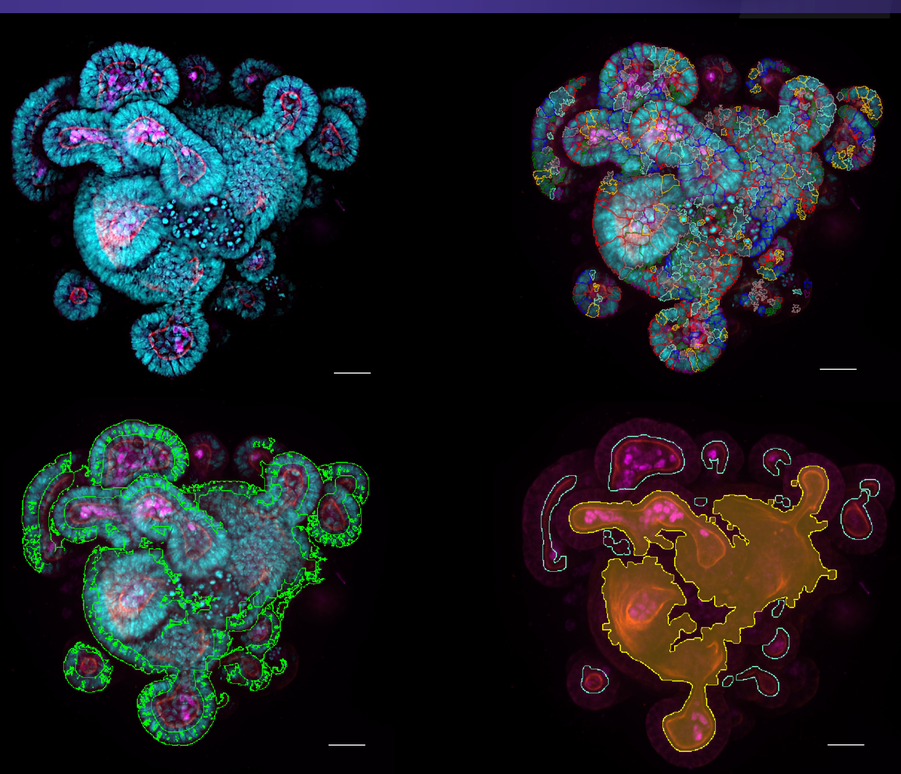


Figure 2) Organoid analysis in CellPathfinder. 20x maximum intensity projection and analysis of a mouse intestinal organoid in Gri3D® 400µm imaging-bottom black-walled plate. Top left: unmasked image before analysis. Top right: nuclei detection. Bottom left: epithelium thickness. Bottom right: lumen definition. Phalloidin in Red, MitoTracker in Magenta, DAPI in Cyan. Scale bars 20 µm.

The integration of Yokogawa's CQ1 high-content system with SUN bioscience's Gri3D® plates represents a significant advancement in **3D imaging for organoid research**. The combined use of Yokogawa's cutting-edge imaging technology and SUN bioscience's innovative Gri3D® plates addresses previously encountered challenges by enabling **faster image acquisition** and more **accurate data**. This synergistic approach opens new avenues for advancing organoid-based research, paving the way for deeper insights into complex biological systems and accelerating the development of novel therapeutics.

REFERENCES

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Contact info: enquiries@sunbioscience.ch

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