Automation for organoid assays – An integrated system with high-content imaging

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Summary

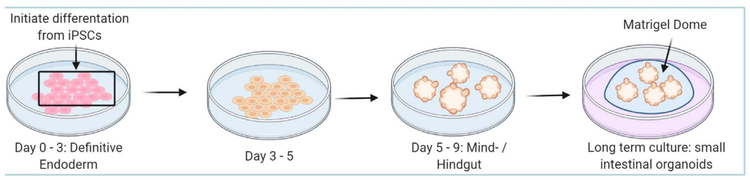
Three-dimensional (3D) cell models that represent various tissues are being successfully used in drug discovery and disease modeling to study complex biological effects and tissue architecture. However, the complexity of 3D models remains a hurdle for their wider adoption in research and drug screening. It is a long and labor-intensive process, yet, by implementing high-throughput screening and automated processing, there can be a significant reduction in the time and effort involved. Here, we describe 3D organoid culture automation that includes monitoring, maintenance, and characterization of organoids. In addition, we evaluated the feasibility of automation in a 3D culture workflow using the BioAssemblyBot® by Advanced Solutions (BAB)—a multitool robot with liquid handling, plate transferring, and bioprinting capabilities. We developed methods for automated seeding and media exchange as well as monitoring the development of intestinal organoids, and colorectal and rectal organoids.

This method allows high-throughput compound testing and evaluation of toxicity effects. 3D organoids were developed and cultured in Matrigel®. The liquid handling tool on the BAB allowed for automated seeding of cells in Matrigel droplets followed by media addition and media exchanges. Organoids were monitored using imaging in transmitted light. Later, deep learning-based image segmentation was used to detect organoids and characterize them based on multiple parameters. For endpoint measurements, organoids were stained with fluorescently labeled antibodies/viability dyes and imaged using the ImageXpress® Confocal HT.ai High-Content Imaging System. Advanced image analysis and 3D reconstruction allowed for complex phenotypic evaluation of organoid structures, including organoid size and complexity, cell morphology and viability, and the presence of differentiation markers. Also, the concentration-dependent toxicity effects of organoids in response to several anti-cancer compounds were also evaluated.

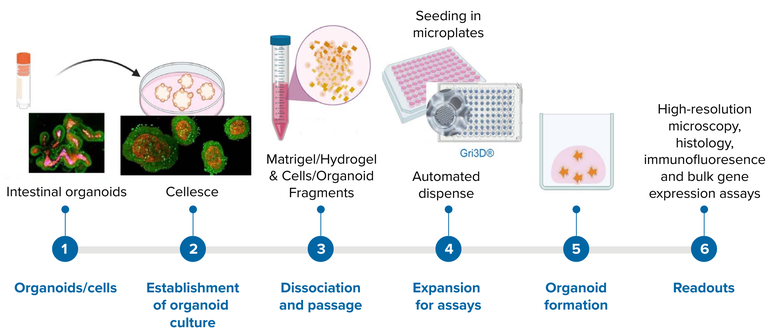
We demonstrate here the tools for increasing throughput and automation of organoid assays and compound screening and propose analysis approaches and descriptors that allow gaining more information about these complex models.

Methods

Establish Human Intestinal Organoids (HIO) from iPSCs



Automated dispense, expansion and assays (OIC, BAB)

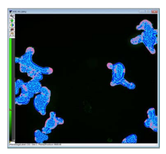


Cell culture, monitoring, and imaging

3D Organoid models: Organoids were derived from mouse and human iPSCs (STEMCELL Technologies). Cells were cultured and differentiated according to the STEMCELL Technologies protocol. Cells were seeded in 50% growth-factor reduced Matrigel (Corning) domes in a 24-well plate format and were fed every second day with fresh media for 1-2 weeks. Organoids were then passaged, dissociated, and re-plated into fresh Matrigel domes. Colorectal organoids (CRC) were obtained from Cellesce and maintained as per protocol. Human rectal organoids developed were cultured on Gri3D® and labeled with SPY555-FastAct™ and imaged over 7 days.

Imaging: Transmitted light (TL) of fluorescent images were acquired on the ImageXpress Confocal HT.ai High-Content Imaging System (Molecular Devices) using MetaXpress® High-Content Image Acquisition & Analysis Software. Z-stack images for the organoids were acquired with the 4X or 10X objectives using confocal mode. MetaXpress or IN Carta™ Image Analysis Software was used for all analysis.

ImageXpress Confocal HT.ai High-Content Imaging System



MetaXpress High-Content Image Acquisition & Analysis Software

BioAssemblyBot (BAB)

Organoid Innovation Center (OIC – Hamilton Star)

Results

Culturing/imaging 3D organoid models

The organoid culture was started from primary mouse intestinal cells/ human iPSC cells, and then organoids were grown in Matrigel domes using reagents and protocol from STEMCELL Technologies. Colorectal organoids were obtained from Cellesce and maintained as recommended. Briefly, organoids were first expanded, then mixed with GF-reduced Matrigel and seeded into Matrigel domes in 24-well or 96-well plate formats.

Matrigel dome 3D models (Manual/ Automation) ➔ Assay Development

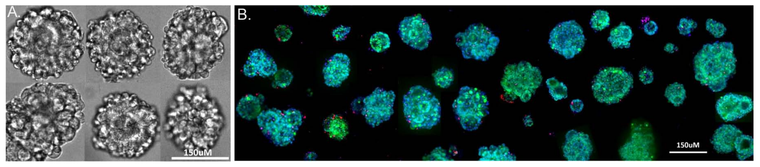


Figure 1. A. Cellesce Colorectal Organoids in Matrigel dome after one week in culture, TL image (10X). B. Organoids 10 days in culture stained with Hoechst dye (blue) and Cyto3D (Acridine orange and Propidium Iodide), 10X.

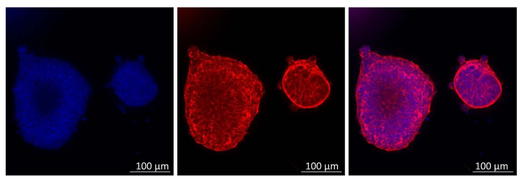
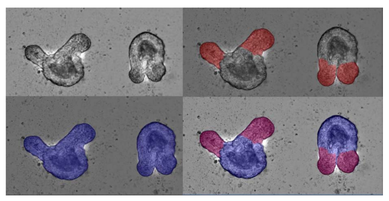


Figure 2. After differentiation of iPSCs, into small intestinal organoids (~ 2 weeks), organoids were stained and imaged using the ImageXpress HT.ai system at 20X. The number of cells was counted using 3D analysis and used to analyze the growth. Hoechst nuclear dye (blue) and Tx-Red phalloidin (red), Merged (Pink).

Maintenance and monitoring of organoids culture

Mouse Intestinal organoids (MIO) were cultured and monitored daily by automated imaging. Organoid growth, distribution, and size were automatically detected and characterized by AI-based image analysis.

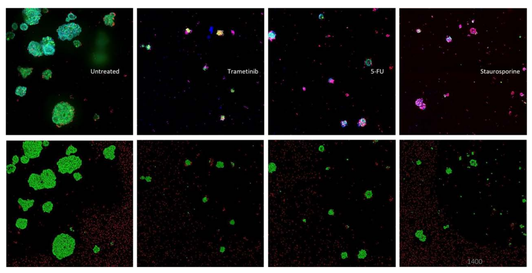


An AI-based image analysis module IN Carta® Image Analysis Software (SINAP) was used for segmentation of transmitted light images and detection of mouse intestinal organoids. Image in transmitted light and corresponding analysis masks are shown. Phenoglyphs, a machine learning-based classification tool, was used to classify all the images into immature, intermediate, and mature organoids.

Figure 3. Intestinal Organoids were cultured using STEMCELL Technologies protocols with daily monitoring of culture in TL, 10X objective. Transmitted light images were analyzed using an AI-based module of In Carta software SINAP. Then organoids were analyzed for their growth, size, number, and size of crypt domains (orange/pink).

Colorectal organoids – Drug screening

3D colorectal organoids procured from Cellesce were cultured in Matrigel domes for 2 days and then treated with anti-cancer compounds for 4 days. Next, organoids were stained and imaged using ImageXpress HT.ai. Cytotoxic effects were observed with anti-cancer compounds.

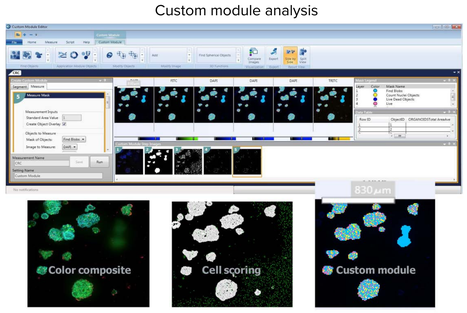


Images: Blue = Hoechst, Green = Acridine Orange, Red = Propidium Iodide
Analysis masks: Green = live nuclei and cytoplasm of cells, Red = live cells (positive for Dapi)

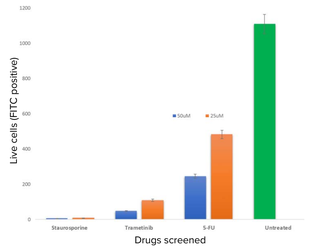
Drug Screening

Figure 4. Colorectal organoids were cultured with daily monitoring of cultures by TL imaging. Then organoids were treated with indicated compounds for 96h. Organoids were imaged using confocal, 10X. Projection images were analyzed using live-dead analysis. Image analysis using Custom Module Editor (MetaXpress software). Finding organoids, cells, and subcellular structures.

Custom module analysis



Human Colorectal Organoids
Drug Response (96h) – 96 wells



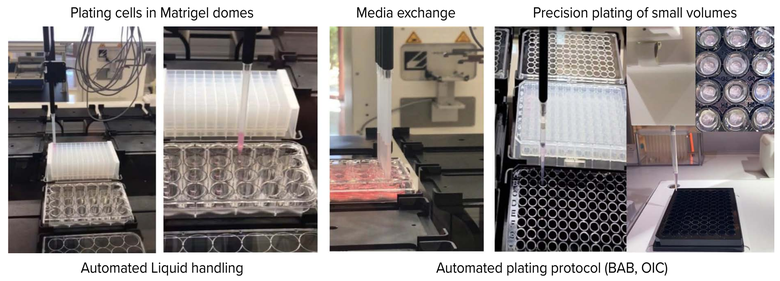
Results

Automation of cell culture and imaging protocols

Organoids provide a very useful tool for disease modeling and assessment of compound effects. Automated imaging and analysis of organoids are important for quantitative assessment of phenotypic changes in organoids and for increasing the throughput of experiments and tests.

We integrated the BAB, a dispenser, and a 3D printer with the ImageXpress HT.ai system for an automated dispensing and imaging workflow. We built an automated, integrated system that would allow automated monitoring, maintenance, and characterization of the growth and differentiation of organoids and stem cells, as well as testing the effects of various compounds. This solution also included IN Carta analysis software, automated CO_2 incubator, Hamilton Star liquid handler, collaborative robot, and rail, and additional, optional instruments (automated centrifuge, ImageXpress® Pico Automated Cell Imaging System, and plate reader).

Components of the automated organoid workflow



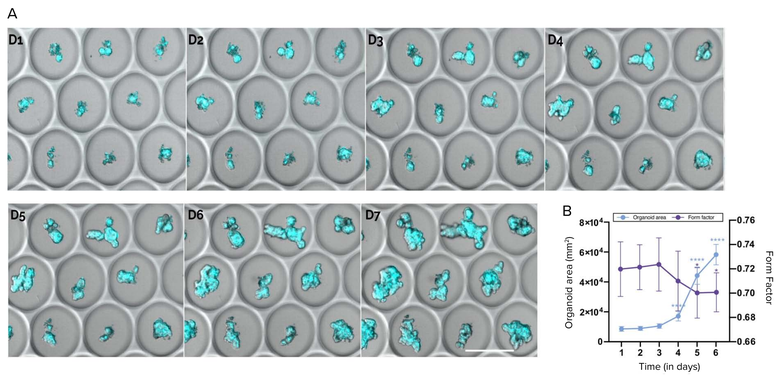


Figure 5. Growth of human rectal organoids on Gri3D® over 7 days. A. Overlays of TL (single plane) and SPY555-FastAct™ (maximum projection) of organoids over 7 days of culture. B. Quantification of organoid area and form factor over time. One-way ANOVA Dunnett's multiple comparisons, *P < 0.05, P**** < 0.0001, ns: non-significant. n=20. D: Day. Scale bar: 500 µm.

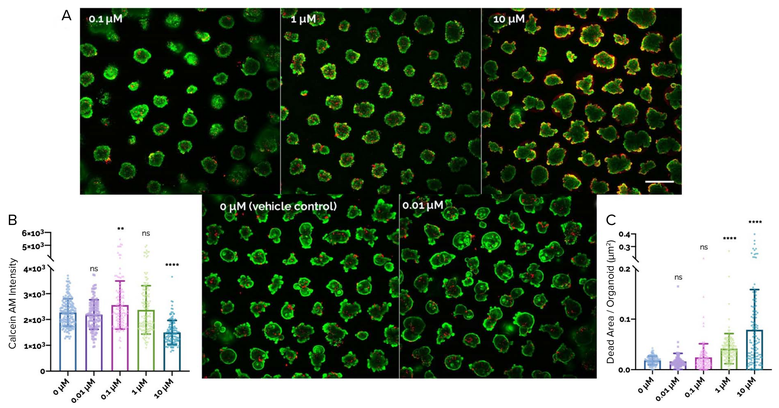


Figure 6. Response of human rectal organoids exposed to staurosporine. Live/Dead assay is performed on 7-day old organoids grown on Gri3D® after 24 hours of exposure to staurosporine. A. Maximum projection images of organoids after Live/Dead assay. Green: Calcein AM, live; red: EthD-1, dead. One-way ANOVA Dunnett's multiple comparisons, **P < 0.01, P**** < 0.0001, ns: non-significant. Scale bar: 500 µm, B. Calcein AM intensity per segmented organoid. C. Dead area per organoid. Error bars show standard deviation. Each dot represents an organoid.

Conclusions

- The process for organoid development can be automated by integrating several instruments, providing automated cell culture, maintenance, and differentiation of 3D cellular models that can be used for compound screening a variety of assays.
- Cell cultures can be monitored in transmitted light with the AI-based image analysis that allows the detection and characterization of organoids.
- Confocal imaging in combination with 3D analysis allows complex, quantitative analysis of cellular content of organoids. The methods can be used for testing the effects of different compounds, toxicity evaluation, and disease modeling.
- The combination of Gri3D® technology and a high-content imaging system with machine-learning algorithms allows the characterization of single organoids in one plane. Readily available patient-derived Cellesce organoids can be used postthaw for high-throughput assays.

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