Monitoring growth and characterization of compound effects in 3D intestinal organoids by high content imaging

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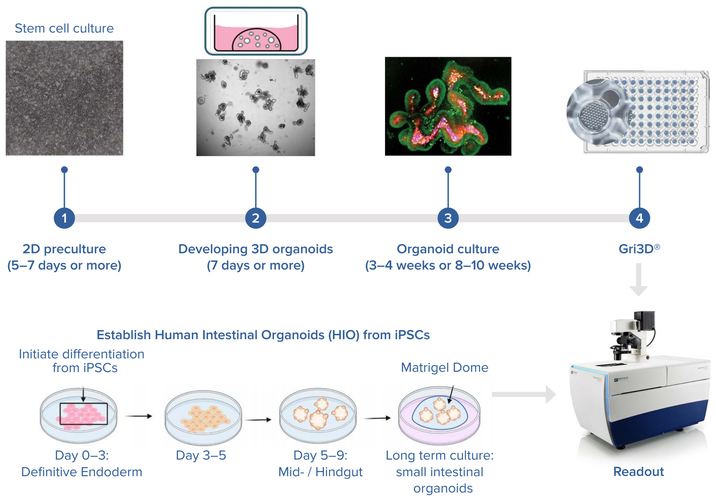
Summary

3D cell models representing various tissues were successfully used for studying complex biological effects and tissue architecture, however, the complexity of 3D models remains a hurdle for the wider adoption in research and drug screening. We describe the automated integrated cell culture and a high-content imaging system that allows automated monitoring, maintenance, characterization of organoids, and testing of the effects of various compounds. The integrated system included a confocal imaging system, automated incubator, liquid handler, and collaborative robot. We developed methods for the automation of the seeding, and media exchange, as well as monitoring the development of mouse intestinal organoids. In addition, this method allows automation of compound testing and evaluation of toxicity effects.

3D intestinal organoids were developed from human iPSC-derived cells cultured in Matrigel. Using an automated liquid handling system allowed automated seeding cells in Matrigel droplets followed by automated media addition and media exchanges. Organoids were monitored using imaging in transmitted light. Then machine-learning-based image analysis allowed detection of organoids and characterization of their size and density. For endpoint measurements, organoids were stained with fluorescently labeled antibodies or viability dyes and imaged using the automated confocal imaging system. Advanced image analysis allowed by 3D reconstitution and complex phenotypic evaluation of organoid structures, including characterization of organoid size and complexity, cell morphology and viability, and presence of differentiation markers. We demonstrated the concentration-dependent toxicity effects of several anti-cancer drugs.

We further increased the complexity by generating human intestinal organoids in Gri3D micropatterned U-bottom-shaped microwells in the hydrogel. We followed the development and self-organization of healthy human intestinal organoids over time which allowed the assessment of phenotypic features at a single-organoid level in an automatable high throughput workflow. We demonstrate the tools for increasing throughput and automation of organoid assays and compound screening and also propose analysis approaches and descriptors that allow gaining more information about these complex models.

Methods



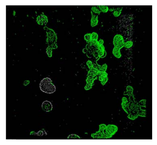
Cell cultures

3D intestinal organoids: 3D intestinal organoids were derived from 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 7–10 days. Intestinal organoids were then passaged- dissociated and re-plated into fresh Matrigel domes. Human rectal organoids developed were cultured on Gri3D® and labeled with SPY555-FastActTM and imaged over 7 days.

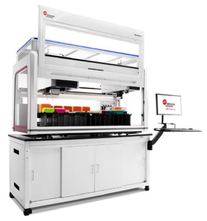
Cell monitoring and imaging

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. iPSC colony images were acquired in TL with approximately 100 µm offset to increase contrast at the edge of the colonies. For lung and intestinal organoids, Z-stack images were acquired with the 4X or 10X objectives using confocal mode. MetaXpress or IN Carta™ Image Analysis Software were used for all analysis.

ImageXpress Confocal HT.ai
High-Content Imaging System



MetaXpress High-Content Image
Acquisition & Analysis Software



Biomek i7 automated
liquid handler

Results

Culturing and imaging 3D mouse and human intestinal organoids

Organoid culture was started from primary mouse intestinal cells/human iPSC cells, and then organoids were developed using grown in Matrigel domes using reagents and protocol from STEMCELL Technologies. 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 Expansion (Manual/Automation) ➞ Assay Development

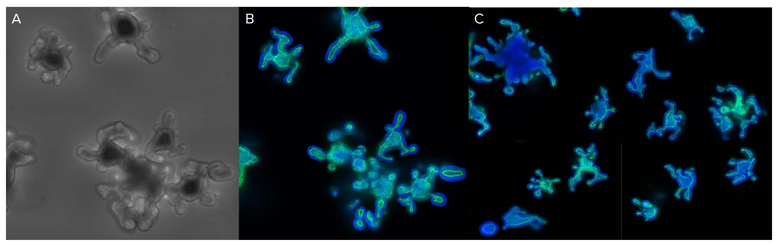


Figure 1. A. Organoids in Matrigel dome after four weeks in culture, TL image (10X). B. Organoids 10 days in culture stained with Hoechst dye (blue) and Calcein, AM (green), 10X. C. Organoids imaged using confocal option, Z-stack of 28 images 13 µm apart. Maximum projection image presented.

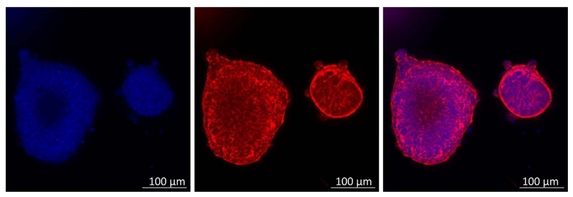
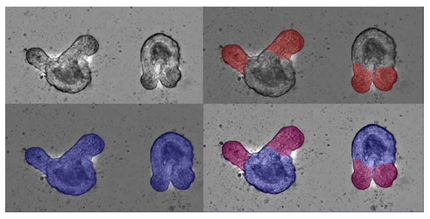


Figure 2. After differentiation of iPSCs, into small intestinal organoids (~ 2 weeks), organoids were stained and imaged using the ImageXpress Confocal system at 20X. The number of cells was counted using 3D analysis and used to analyze the growth. Hoechst nuclear dye (blue) and Tx-Red phalloidin (red),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

Maintenance and monitoring of organoids culture

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

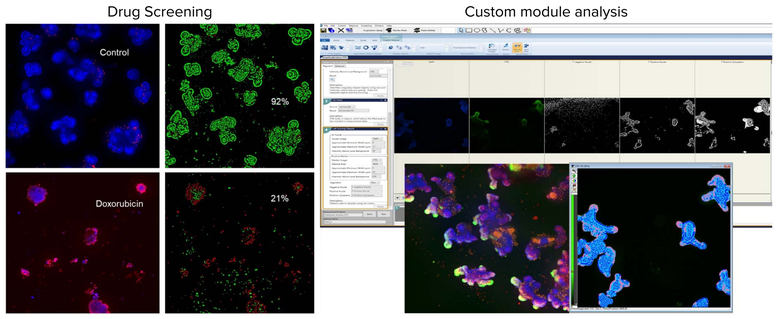


AI-based image analysis module of IN Carta software (SINAP) used for segmentation of transmitted light images and detection of mouse intestinal organoids. Image in transmitted light and corresponding analysis masks are shown. Phenoglyphs, a machine learning based classification tool, was used to classify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

Figure 3. MIOs 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).

Intestinal organoids – Drug screening

3D intestinal organoids derived from primary mouse intestinal cells were cultured in Matrigel domes for five days, and then treated with anti-cancer compounds for 48hrs. Next, organoids were stained and imaged using IXM-C. Cytotoxic effects were observed with anti-cancer compounds.



Images: Blue = Hoechst, Green = Calcein AM, Red = Ethidium Homodimer-III
Analysis masks: Green = live nuclei/cells, Red - live cells (positive for EthD)

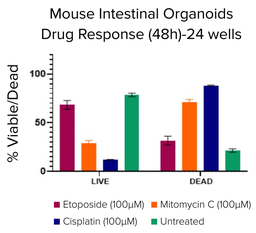


Figure 4. Intestinal organoids were cultured with daily monitoring of cultures by TL imaging. Then organoids were treated with indicated compounds for 48 h. Organoids were imaged using confocal, 10X. Projection images were analyzed using live-dead analysis. Image analysis using Custom Module Editor (MetaXpress software). Finding organoids, cells, and subcellular structures.

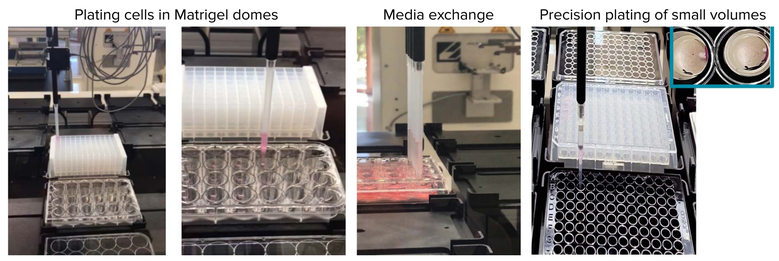
Results

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 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. The automated, integrated system includes ImageXpress Confocal HT.ai system and analysis software, automated CO_2 incubator, Biomek i7 liquid handler, collaborative robot, and rail, as well as additional optional instruments (automated centrifuge, ImageXpress® Pico system, plate reader).

Components of the automated organoid workflow



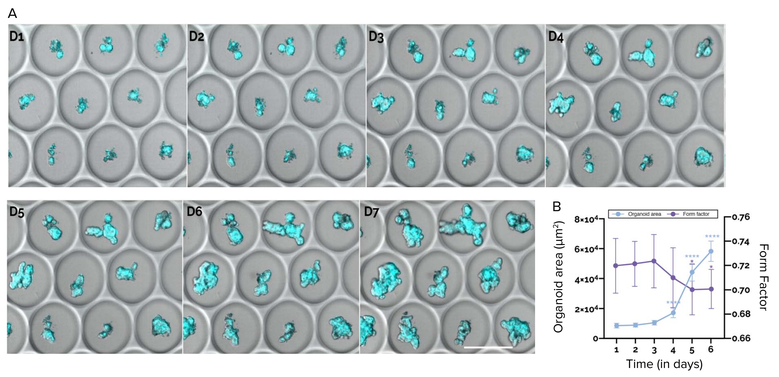


Figure 5. Growth of human rectal organoids on Gri3D® over 7 days. A. Overlays of TL (single plane) and SPY555-FastActTM (maximum projection) of organoids over 7 days of culture. B. Quantification of organoid area and form factor over time. One-way ANOVA Dunnett's multiple comparisons, *P < 0.05, P****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

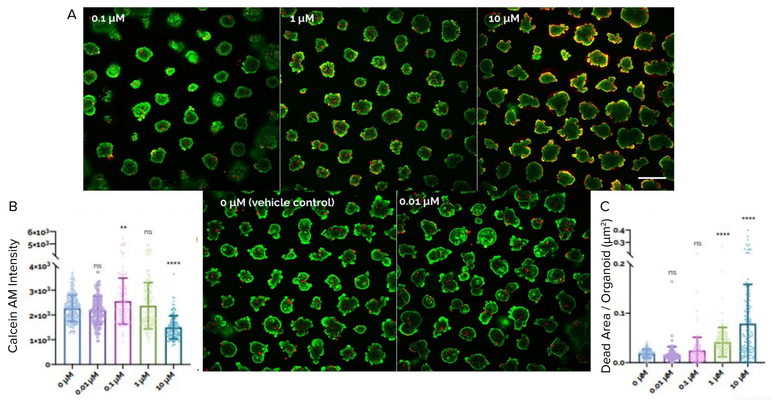


Figure 6. Response of human rectal organoids exposed to staurosporine. Live/Dead assay is performed on 7-day old organoids grown on Gri3D® after 24 hours of exposure to staurosporine. A. Maximum projection images of organoids after Live/Dead assay. Green: Calcein AM, live; red: EthD-1, dead. B. Calcein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

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 together with machine-learning algorithms allows the characterization of single organoids in one plane.

MOLECULAR DEVICES