Intestinal organoids for automated screening assays. High content imaging and analysis of organoid morphology

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Summary

3D cell models representing various tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. However, complexity of 3D models remains a hurdle for the wider adoption in research and drug screening.

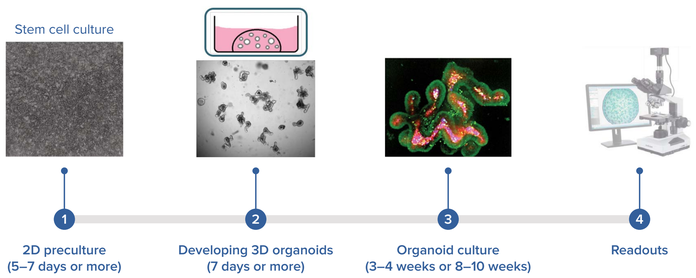
Here we describe a workflow for automation of organoid culture. The automated method utilizes an integrated work-cell, consisting of several instruments providing automated cell culture, monitoring, and a high-content imaging. The integrated system included the ImageXpress® Confocal HT.ai High-Content Imaging System, automated CO_2 incubator, automated liquid handler (Biomek i7), and collaborative robot. We developed methods for automation of the seeding, media exchange, as well as monitoring development of intestinal organoids. In addition, this method allows automation of compound testing and evaluation of phenotypic changes.

3D intestinal organoids were developed from primary mouse intestinal cells cultured in Matrigel. Cells were cultured in a media that promote the formation of 3D structures recapitulating the morphological and functional characteristics of intestine. Organoids self-organized and developed into complex structures resembling intestinal crypt formation. Developing organoids comprised objects with protrusions, cavities and vesical structures. Using automated liquid handling system allowed automated seeding cell in Matrigel droplets into 24 well plates, 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, diameter, and density. For endpoint measurements, organoids were then stained with fluorescently labeled antibodies or viability dyes and imaged using an 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, as well as determining presence and expression levels for differentiation markers. We characterized multiple quantitative descriptors that could be used for studying disease phenotypes and compound effects. 3D image analysis provided quantitation of the organoids number, size distribution, complexity, cell content, viability, volumes, as well as quantitation of cell proliferation and expression of specific markers. We demonstrated concentration-dependent effects of several compounds that have been known to cause toxicity (doxorubicin, cisplatin, Mitomycin C, Taxol).

Described methods demonstrate the tools for increase of throughput and automation in organoid assays and compound screening, and also propose analysis approaches and descriptors that allow gaining more information about the complex systems, disease phenotypes and compound effects.

Methods



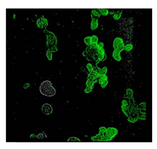
Cell cultures

3D intestinal organoids: 3D intestinal organoids were derived from primary mouse intestinal cells (STEMCELL Technologies). Cells were cultured in Matrigel domes according to the STEMCELL Technologies protocol. IntestiCult™ Organoid Growth Medium (STEMCELL Technologies) was utilized for cell culture. 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.

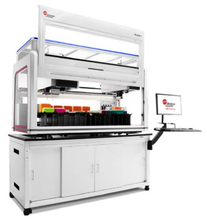
Cell monitoring and imaging

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 and 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 intestinal organoids

Organoid culture was started from primary mouse intestinal cells and then organoids were 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

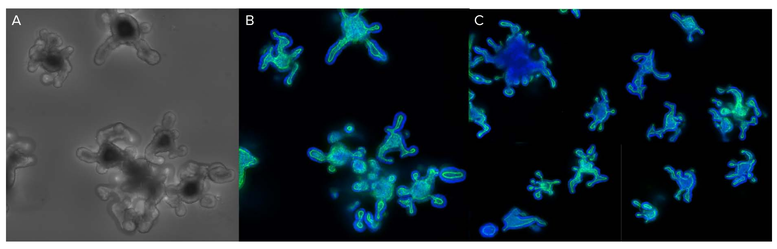


Figure 1. A. Organoids in Matrigel dome after four weeks in culture, TL image (10X). B. Organoids 10 days in culture stained with Hoechst dye (blue) and Calcein, AM (green), 10X. C. Organoids imaged using confocal option, Z-stack of 28 images taken 13 µm apart. Maximum projection images presented.

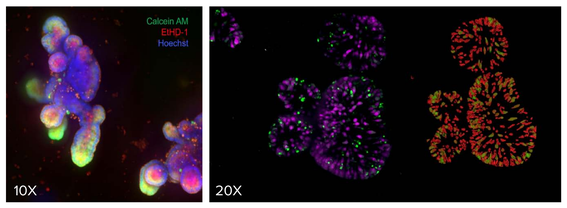
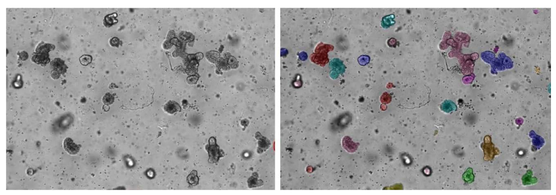


Figure 2. After six weeks of development, organoids were treated and stained to detect dead cells and imaged using ImageXpress Confocal HT.ai system. Numbers of EtHD-1 positive (dead) and negative (live) cells were counted using 3D analysis and used to determine EC_{50}. Hoechst nuclear dye (blue) and EtHD-1 (red) and Calcein, AM (green).

Maintenance and monitoring iPSC culture

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

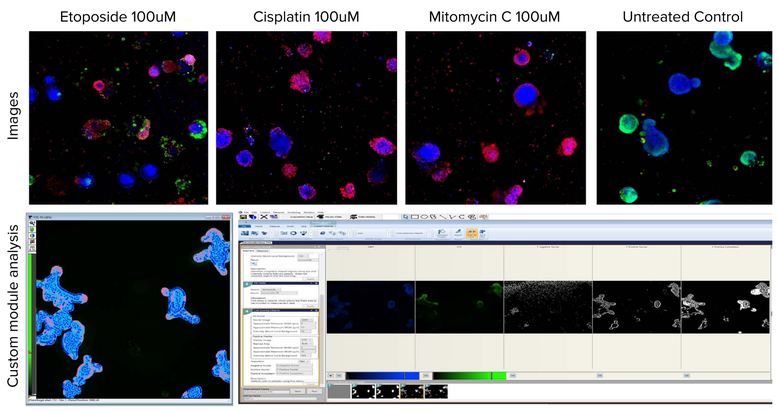


Machine learning-based image analysis module of IN Carta software (SINAP) used for segmentation of transmitted light images and detection of mouse intestinal organoids. Image in transmitted light and corresponding analysis masks shown.

Figure 3. MIOs were cultured using STEMCELL Technologies protocols with daily monitoring of culture in TL, 4X objective. Transmitted light images were analyzed using AI-based module of IN Carta software SINAP. Then organoids were analyzed for area, diameter, and other features.

Intestinal organoids

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 the ImageXpress HT.ai system. 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)

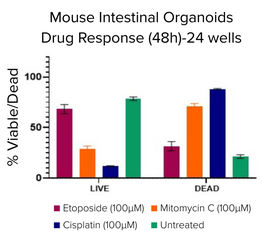


Figure 4. Intestinal organoids were cultured with daily monitoring of cultures by TL imaging. Then organoids were treated with indicated compounds for 48 h. Organoids were imaged using confocal, 10X. Projection images were analyzed using live-dead analysis. Image analysis was done using Custom Module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

Results

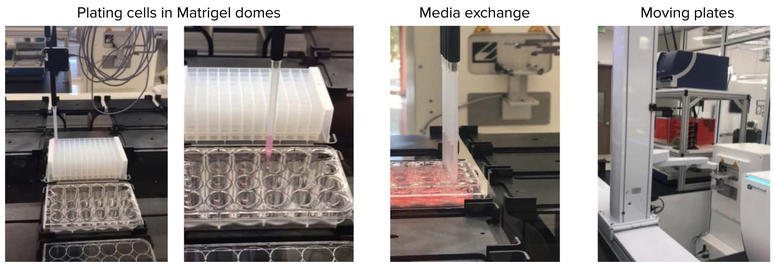
Automation of cell culture and imaging protocols

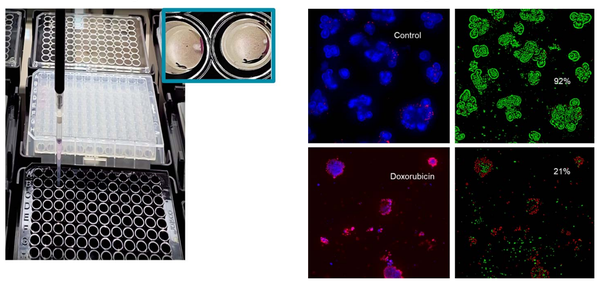
Organoids provide a very useful tool for disease modeling and assessment of compound effects. Automated imaging and analysis of organoids are important for quantitative assessment of phenotypic changes in organoids, and for increasing the throughput for experiments and tests.

We built an automated, integrated system that would allow automated monitoring, maintenance, and characterization of growth and differentiation of organoids and stem cells, as well as testing the effects of various compounds. The automated, integrated system includes the ImageXpress Confocal HT.ai system, analysis software, automated CO_2 incubator, Biomek i7 liquid handler, collaborative robot and rail, and additional optional instruments (automated centrifuge, ImageXpress® Pico Automated Cell Imaging System, plate reader).

Components of the automated organoid workflow

Time dependence of angiogenesis was modeled over five days. A time-dependent increase in the number and volume of sprouts was observed, as well as an increased number of cells or nuclei. Images were analyzed in 3D and the growth of angiogenic sprouts were characterized by multiple readouts including, total numbers of sprouts and nuclei, their intensities, volumes, distances between objects. The number (or average) of nuclei per individual sprout can also be determined (Figure 5). Secondary analysis was completed using Microsoft Excel.





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 effects of different compounds, toxicity evaluation, and disease modeling.

MOLECULAR DEVICES