# Organoids for disease modeling and *in vitro* drug screening: Automated culture monitoring, imaging, and analysis of complex biological systems

Oksana Sirenko[1], Angeline Lim[1], Michael Hayes[2], Matthew Hammer[1], Mary Kassinos[1], Felix Spira[1], Dan DiSepio[1], Bhagya Wijayawardena[2], Constantin Radu[1], Dan O'Connor[1], Celeste Glazer[1] | [1]Molecular Devices, LLC, San Jose, CA, [2]Beckman Coulter Life Sciences, Indianapolis, IN

## Summary

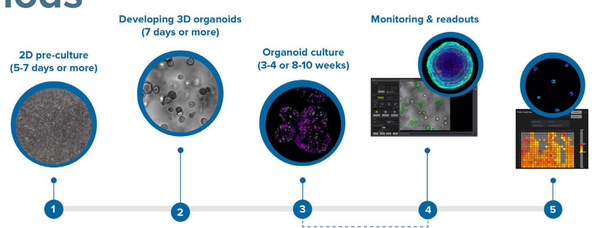
3D cell models representing various tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. However, the complexity of 3D models remains a hurdle for wider adoption in research and drug screening.

We describe 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. We demonstrated methods for automation of three complex workflows: iPSC culture, 3D lung organoids, and intestinal organoids. The automated, integrated system included an ImageXpress® Confocal HT.ai High-Content Imaging System, automated incubator, Biomek i7 hybrid workstation, and a robotic device.

iPSC were cultured, passaged, and monitored daily by automated imaging. Cell phenotypes, density, and colony sizes were automatically detected and characterized by AI-based image analysis. 3D lung organoids were developed from primary human lung epithelial cells cultured in Matrigel with a mixture of growth factors. Organoids were monitored using transmitted light, then stained and imaged through Matrigel using automated confocal imaging. Image analysis included conventional and AI-based tools. Developing organoids comprised spherical objects with complex morphology including cavities, protrusions, and vesicle structures. Increases in size and complexity were monitored during 6-8 weeks of development. Advanced image analysis allowed 3D reconstitution and complex analysis of organoids, cell morphology, viability, and differentiation markers. We characterized multiple quantitative descriptors of organoids that could be used for studying disease phenotypes and compound effects. We measured concentration-dependent effects of several drugs that have been known to cause lung toxicity. Intestinal organoids were developed from primary mouse intestinal cells and were studied for effects of inflammatory cytokines or anti-cancer drugs.

The workflow demonstrates the usefulness of automation and advanced high-content imaging for increased throughput and information in organoid assays, critical for compound screening.

## Methods




### Cell cultures

**iPSC culture:** Human iPSC cells adapted to feeder-free conditions (SC102A-1, System Biosciences) were thawed and cultured in Complete mTeSRTM Plus culture medium (STEMCELL Technologies) in Matrigel (cat. #354277, Corning) coated plates. Media was changed every day except once per week when a double volume of media was added to skip changing media on one day. Cells were passaged using the enzyme-free reagent ReLeSRTM (STEMCELL Technologies) at a 1:6–1:10 split ratio.

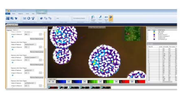
**3D lung organoids:** 3D lung organoids were derived from primary human lung epithelial cells (ScienCell). Cells were cultured and expanded in 2D according to ScienCell protocol. For 3D organoid culture, the PneumaCult™ Airway Organoid Kit (STEMCELL Technologies) was used according to manufacturer's protocol. Briefly, cells were seeded in 90% Matrigel (Corning) domes in 24-well plate format (1 dome per well) and were fed every second day for two weeks using the PneumaCult Airway Organoid seeding media. Then differentiation was carried out for another six weeks using the PneumaCult Airway Organoid differentiation media.

**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 according to the STEMCELL Technologies protocols.

### Cell monitoring and imaging

Transmitted light (TL) of fluorescent images were acquired on the ImageXpress Confocal HT.ai 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 and Analysis Software

Biomek i7 automated liquid handler

## Results

### Culturing and imaging 3D lung organoids

Organoid culture was started from primary lung epithelial cells (see Methods section), and then organoids were grown in Matrigel domes using reagents and protocol from STEMCELL Technologies. Briefly, cells were first expanded in 2D, then mixed with GF-reduced Matrigel and seeded into Matrigel domes in 24-well or 96-well plate formats.

2D expansion → Matrigel dome 3D → Differentiation → Experiment

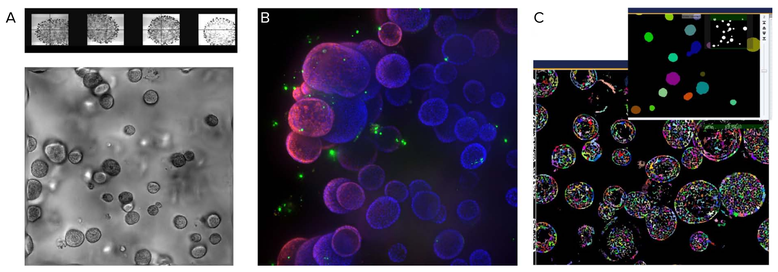



**Figure 1.** A. Organoids in Matrigel dome after four weeks in culture, TL images (4X, 10X). B. Organoids six weeks in culture stained with Hoechst dye (blue) and MitoTracker (red), 10X. Organoids imaged using confocal option, Z-stack of 23 images 10 µm apart. Maximum projection image presented. C. Image analysis using Custom Module Editor (MetaXpress software). Finding organoids, cells, and subcellular structures

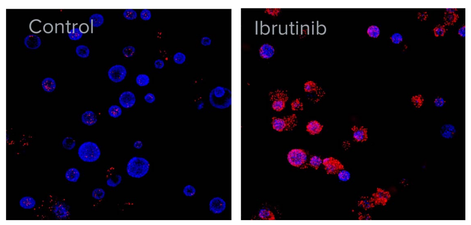
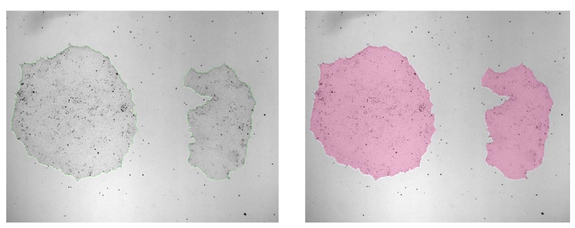



**Figure 2.** After six weeks of development, organoids were treated with 10 µM of Ibrutinib for 72 h. Organoids were then stained with Hoechst nuclear dye (blue) and EtHD-1 (red) to detect dead cells and imaged using ImageXpress Confocal HT.ai system. Numbers of EtHD-1 positive (dead) and negative (live) cells were counted using 3D analysis and used to determine $EC_{50}$.

### Maintenance and monitoring iPSC culture

iPSC were cultured and monitored daily by automated imaging. Cell phenotypes, density, and colony sizes were automatically detected and characterized by AI-based image analysis.

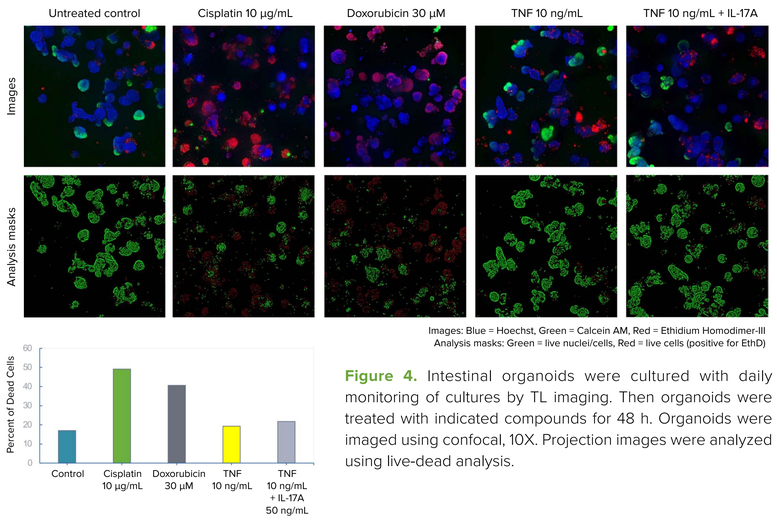


AI-based image analysis module of In Carta software (SINAP) used for segmentation of transmitted light images and detection of iPSC cells and colonies. Image in transmitted light and corresponding analysis masks shown.

**Figure 3.** iPSC cells were cultured using STEMCELL Technologies protocols with daily monitoring of culture in TL, 4X objective, with approximately 100 µm offset. Transmitted light images were analyzed using AI-based module of In Carta software SINAP. Then colonies 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 (doxorubicin or cisplatin) or with inflammatory cytokines for 48 hours. Next, organoids were stained and imaged using ImageXpress Confocal 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.

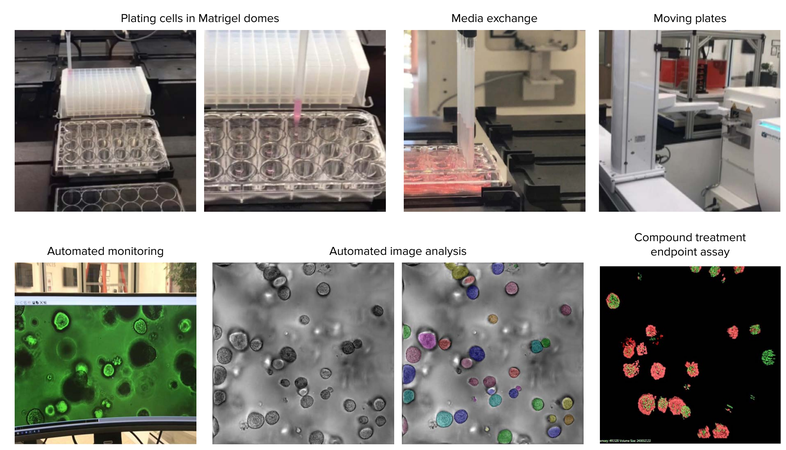
## Results

### 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 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




## 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 and iPSC colonies.
- Confocal imaging in combination with 3D analysis allows complex, quantitative analysis of cellular content of organoids as well as count and measurements of cells with different phenotypes (cell count, live-dead assessment, cell scoring for specific markers, etc.). The methods can be used for testing effects of different compounds, toxicity evaluation, and disease modeling.

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