Lung organoids for disease modeling and toxicity assessment by 3D high-content imaging and analysis

Oksana Sirenko, Mary Kasinos, Angeline Lim, Matthew Hammer, Felix Spira, Celeste Glazer
Molecular Devices, LLC, San Jose, CA

Summary

3D cell models representing different tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. 3D organoids have the ability to organize the way that it more closely recapitulates key aspects of human tissue. However, complexity of 3D assays remains a hurdle for the wider adoption of organoid models in research and drug screening. New advanced tools for imaging and analysis, as well as assay automation are critical for increased quality of information, throughput, and precision of complex biological models.

Developing organoids comprised of spherical objects with complex morphology including cavities and vesical structures. Increase in size (from <30 to 1000 µm) and complexity was monitored during eight weeks of development. Advanced image analysis allowed 3D reconstitution and complex analysis of organoid structures, characterization of cell morphology and viability, as well as determining expression level of differentiation markers. Organoids were monitored using transmitted light, then stained and imaged through Matrigel using automated confocal imaging. Image analysis included conventional and AI-based tools.

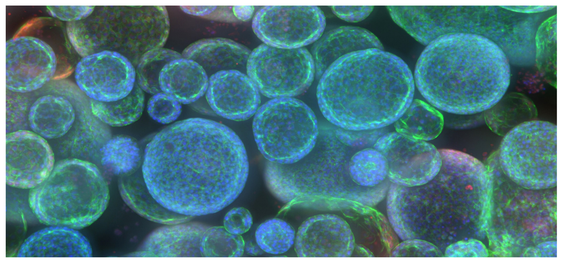
In this study, we describe the automated culture and a high-content imaging method that allowed monitoring and characterization of growth and differentiation of lung organoids. Primary human lung epithelial cells were cultured in Matrigel supplemented with a mixture of growth factors that promote the formation of 3D structures recapitulating the morphological and functional characteristics of the airway. Organoids then self-organized into complex multi-lineage structures.

Methods

Cells: 3D lung organoids

3D lung organoids were derived from primary human lung epithelial cells (ScienCells, Co.). Cells were expanded for two weeks in 2D according to the protocol from ScienCells. Then the cells were seeded in 90% Matrigel (Corning) into Matrigel domes using reagents according to the protocol from Stem Cell Technologies (PneumaCult™ Airway Organoid Kit). Cells formed 3D organoids and were fed every second day for two weeks using PneumaCult Airway Organoid seeding media, then differentiated for another six weeks using PneumaCult Airway Organoid differentiation media. Organoids were grown in different plate formats (see Results section), and then treated with compounds or stained with various markers for morphology and viability assessment. The growth and development of organoids were monitored in transmitted light every week using 4X or 10X magnification. Images of organoids stained with different markers (shown below).

A



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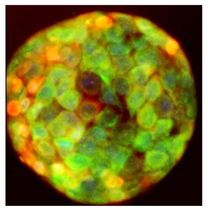


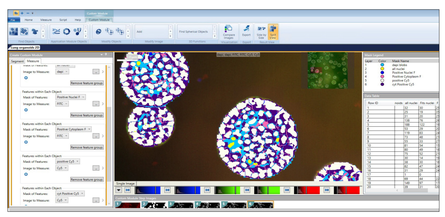
Figure 1. Monitoring complexity of organoid structure using confocal imaging. A. Live organoids were stained with Hoechst nuclear dye (blue), MitoTracker, (red) and Calcein AM (green). Confocal images were taken 5–10 µm apart, 10X objective. B. Fixed organoids were stained with Ac Tubulin (green) and ZO-1 (red) were taken with 20X objective.

Organoid imaging and 3D analysis

3D lung organoids were stained by adding staining reagents in staining buffer to half of the media typically for two hours. Then staining reagents were removed, washed with PBS for 30 min and fresh media was added. Organoids samples were imaged live or were fixed with 4% formaldehyde and stored at RT for later imaging. Organoid samples were imaged using ImageXpress® Confocal HT.ai High-Content Imaging System with water immersion option. Images were taken using 4X and 10X magnifications with confocal option, 20X or 40X images were taken with confocal and water immersion, and Z-stack of images was taken 3–10 µm apart, depending on magnification covering range 150–250 microns deep. Image analysis was done in 3D using MetaXpress® High-Content Imaging Acquisition and Analysis Software.

Instrument and software

ImageXpress Confocal HT.ai system with water immersion option



MetaXpress software with Custom Module Editor

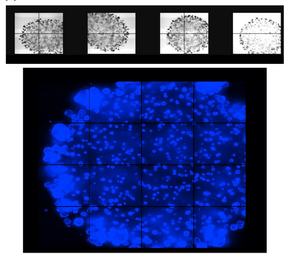
Results

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 Stem Cell Technologies. Briefly, cells were expanded in 2D, then mixed with GF-reduced Matrigel and seeded into Matrigel domes in 24-well plates or other plate formats.

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

A



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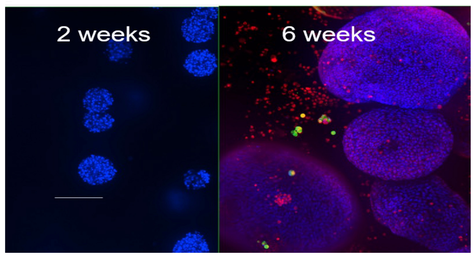
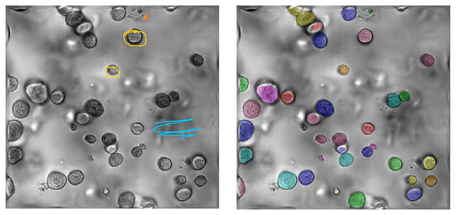


Figure 2. A. Organoids in Matrigel dome four weeks in culture, TL images (4X) or stained with Hoechst dye (10X). Mean diameters of organoids increased over time. B. Comparison of organoid size, two weeks stained with Hoechst (blue), six week's samples stained with Hoechst and MitoTracker (red). Organoids were imaged using the confocal option, Z-stack of 23 images 10 µm apart. Maximum projection images.

Monitoring organoid growth and 3D image analysis

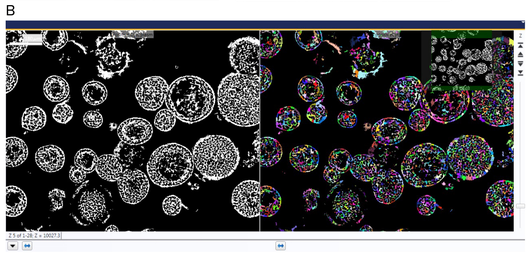
Organoid growth and development were monitored using transmitted light imaging or using Hoechst stain. Automated imaging of live organoids in Matrigel was done using confocal option, 4X or 10X magnification. Image analysis of a single Z-plane or 3D projection was done to determine the approximate number and size of organoids. Time-dependent increase in size was observed over 6–8 weeks of culture.

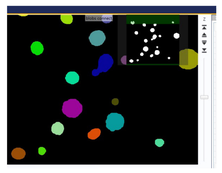
A



AI-based analysis in transmitted light. "Find blobs" analysis detects organoids in CME or SINAP (IN Carta™), then objects defined in 3D space.

B





"Cell scoring" analysis detects nuclei, positive-negative cells, and cytoplasm, then combines in 3D space, defining numbers, volumes, and distances.

Figure 3. Image analysis recapture complexity of organoid structures using 3D image analysis. Organoids (A), cells or sub-cellular structures (B) can be detected, counted, and characterized using the Custom Module Editor. Analysis performed in each Z-plane, and then software combined the information to provide measurements in 3D space.

Measurements of phenotypic changes and compound effects

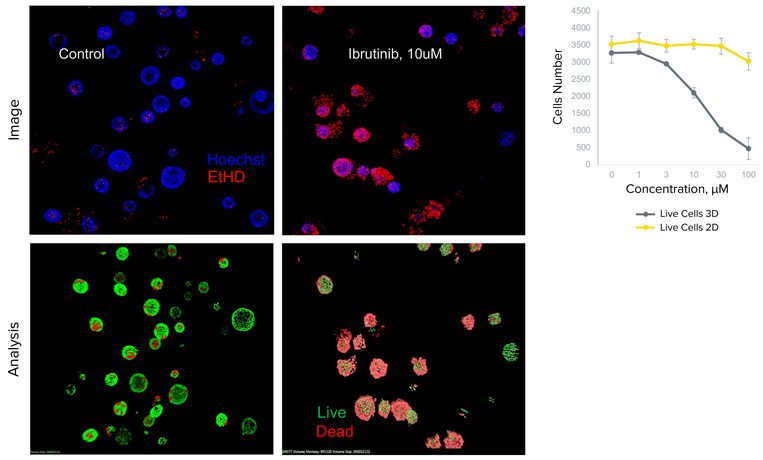


Figure 4. After six weeks of development, organoids were treated with different concentrations of Ibrutinib for 72h. Organoids were then stained with EtHD-1 to detect dead cells and imaged using ImageXpress Confocal HT.ai system. Numbers of EtHD-1 positive (dead) and negative (live) cells were counted using 3D analysis and used to determine EC_{50}.

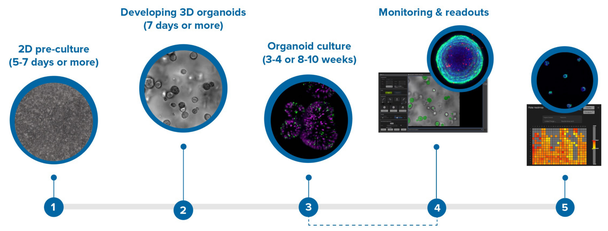
Results

Automation of culture and experimental 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 increases in throughput for experiments and tests.

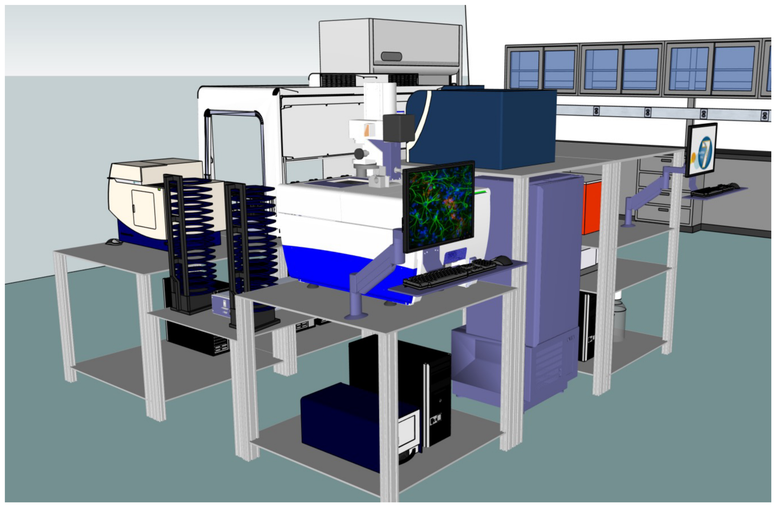
Confocal Imaging and 3D image analysis are especially useful for capturing complexity of 3D biological assays. Some anti-cancer drugs, such as Ibrutinib, that are used to treat leukemia have been shown to cause lung toxicity effects. We have tested toxicity of the anti-cancer drug Ibrutinib using 3D lung organoids.

We built an automated integrated system that allowed 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 included ImageXpress Confocal HT.ai system and analysis software, automated incubator, Biomek liquid handler, and a robotic device.



Automation of organoid workflow includes:

- Cell culture
- Monitoring of organoid development
- Media exchange
- Compound addition
- End-point assays
- Image analysis
- Data processing
- Scheduling



Individual components:

1. ImageXpress Confocal HT.ai system
2. Image analysis software
3. Multi-mode microplate reader
4. ImageXpress® Pico system
5. Automated liquid handler (Biomek i7)
6. Automated CO_2 incubator
7. Collaborative robot and rail
8. Barcode reader
9. Plate washer
10. Automated centrifuge

Conclusion

- 3D lung organoids were successfully formed and developed using established protocols. Development and growth of organoids were monitored in Matrigel by automated imaging that allowed evaluation of organoids size, volume, and complexity.
- Confocal imaging in combination with 3D analysis allowed quantitative characterization of cellular content as well as count and measurement cells with different phenotypes within organoids (cell count, live-dead assessment, cell scoring for specific markers, others). The model can be used for toxicity evaluation of pharmaceutical drugs and other compounds.
- The process for organoid development can be automated using integration of several instruments providing automated maintenance, expansion and differentiation of 3D cellular models for compound screening, toxicity assessment, and studying diseases.

MOLECULAR DEVICES