Automation of 3D organoid culture workflow with deep-learning based image analysis

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Introduction

3D cell culture as a model system is increasingly popular because it recapitulates the *in vivo* microenvironment better than 2D cell cultures. Organoids have the capacity for stable differentiation and rapid growth and, as such, the organoid model system offers huge potential in disease modeling, drug screening and precision therapy.[1,2]

The technology available for using organoids as a model system is still in its infancy compared to the more established 2D culture or animal models. More development is needed to address reproducibility of organoids between batches and to standardized the process of organoid culture. Current protocols for the generation and maintenance of organoids are complex, time consuming and requires extensive manual handling.

To overcome some of the complexities involved in the culture process, we sought to develop an end-to-end workflow that uses automation and deep-learning analysis tools for the growth, maintenance, and monitoring of organoids in culture. Automated cell culture has the potential to increase quality and quantity of experiments and allows for long-term cell culture maintenance with less manual intervention. To automate the process for growing organoids, we set up a workcell that consists of an incubator, liquid handler, high-content imager, plate reader, and centrifuge. All the instruments in the workcell are accessible by a software-controlled robotic arm to transfer cell culture plates between instruments. Using lung organoids as the 3D model, we developed protocols to seed and plate cells in Matrigel® domes using the workcell's liquid handler. Culture plates containing cells can be transferred from the incubator to the liquid handler for scheduled media changes. Because organoids are typically cultured longer than 2D cell cultures, it is important to monitor growth of organoids as a form of quality control. The scheduler in the software can be programmed to move the plates between the incubator and the high-content imager. Brightfield images of the organoids are acquired and then analyzed using AI-based analysis tools which enables measurement of organoids (e.g. size, shape, texture) over time. Overall, we show the feasibility of using an automated workcell for the culture and monitoring of 3D pulmonary (lung) organoids. These results provide the foundational workflow which can be adapted for other 3D cell models such as intestinal, brain or patient-derived tissues and enable scaling-up of organoid production for other downstream applications.

Methods

Cell cultures

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. Differentiation was carried out for another six weeks using the PneumaCult Airway Organoid differentiation media.

Image acquisition and analysis

All images were acquired on the ImageXpress® Confocal HT.ai High-Content Imaging System (Molecular Devices) using the MetaXpress® High-Content Image Acquisition and Analysis Software. The growth and development of organoids were monitored with bright field imaging every week using 4X or 10X objectives. Z-stack imaging was carried out with "best focus" projection image selected.

IN Carta® Image Analysis Software was used for analysis in the monitoring phase. The "Export to IN Carta" function in MetaXpress software was used to import images into IN Carta software. SINAP was used to carry out segmentation of all images. Each model was trained and verified before being used in the analysis protocol.

Workcell construction and design

The workcell consists of the following primary components: Hotel for storage of microtiter plates, LiCONiC STX44 automated incubator with wave function, Aquamax Microplate Washer (Molecular Devices), HiGTM 4 automated centrifuge (BioNex Solutions Inc), SpectraMax iD5 Multi-Mode Microplate reader, ImageXpress Confocal HT.ai system, ImageXpress® Pico Automated Cell Imaging System (Molecular Devices), and Biomek i7 Automated Workstation (Beckman Coulter Life Sciences).

The plate handling robot is a PreciseFlex400 robot on a 2 meter rail to access all plate nests.

We use Green Button Go® scheduling software which enables one unified location for control of all devices as well as setup of scheduling the overall workflow.

Results

The process of culturing cells in 3D is more complex than in 2D (Figure 1B) thus, limiting its use in high-throughput applications such as drug discovery. To overcome this, we set up a robotics-driven automation workcell (Figure 1A) and sought to develop an end-to-end workflow for organoid culture and maintenance.

Automation-enabled workcell for 3D organoid culture

Figure 1. The instruments in the workcell that support automation of the cell culture process is illustrated, and picture of the workcell is shown (A). The PreciseFlex400 serves as the robotic arm to move plates between each of the instruments within the workcell. B) Workflow showing the main steps for 3D cell culture is represented. Cells (IPSCs or adult stem cells) are pre-cultured and expanded in 2D before they are used to create organoids. Most organoid culture requires an extracellular matrix (ECM) usually in the form of hydrogel-based matrix or the commercially available Matrigel. Differentiation of stem cells into specific cellular fate is controlled by the addition of factors in the culture media at specific timepoints. Growth and maturation of organoids can be monitored with high-throughput imaging using brightfield microscopy until they are assay-ready.

Automation workcell layout and software control

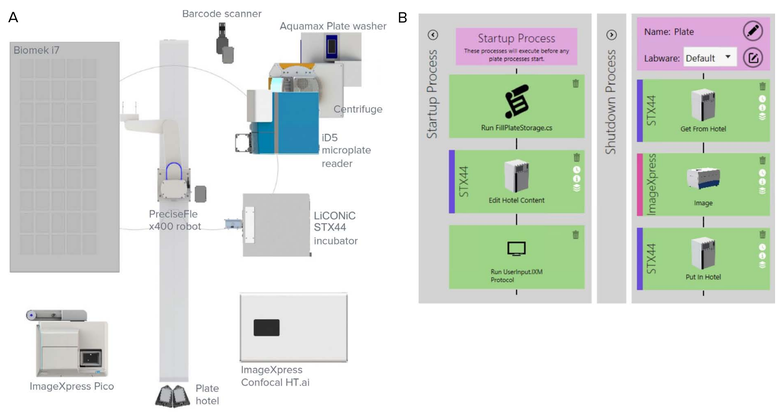

Figure 2. Layout of the individual instruments in the workcell is illustrated in (A). The instruments are controlled by a integrated software (Green Button Go) that allows for set up of processes. An example of the process to monitor cells in culture is shown in (B). Here, the plates are moved from the incubator to the ImageXpress Confocal HT.ai for imaging in brightfield and then back to the incubator. The process can also be scheduled, and plates that need to be imaged can be entered as a list to enable easier batch processing. More complex routines that includes the liquid handler for media exchanges (feeding) can also be implemented.

Results

Automated pulmonary (lung) organoid culture

3D organoids are powerful models with many biomedical applications. Because they can be derived from any patient, organoids hold huge potential in drug discovery and in personalized therapeutics. Here, we developed protocols on the liquid handler for cell seeding in Matrigel and for media changes.

To initiate the lung organoid culture, we used the Biomek i7 Automated workstation to dispense cells with Matrigel as a dome in each well (Figure 3). The liquid handler can be programmed to dispense at specific location within each well. The liquid property, dispense volume and speed can be also be controlled within the software to ensure accurate pipetting volumes.

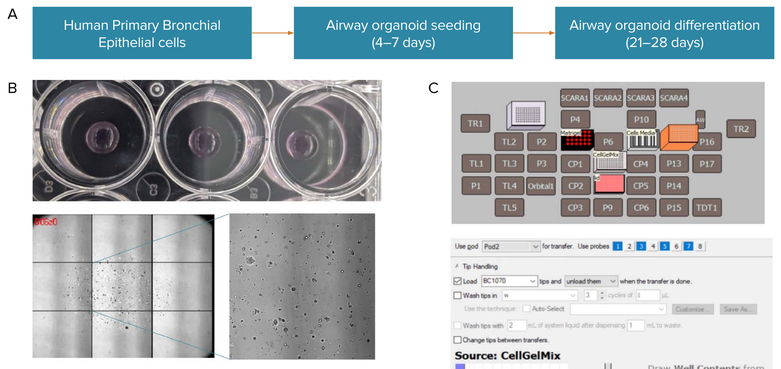

Figure 3. Lung organoid culture with liquid handling. Main steps in the culture of lung organoids is shown in (A). HUBECs are grown and expanded in 2D culture before they are dissociated and then mixed with Matrigel for 3D culture. This is then followed by differentiation and maintenance that can take up to 28 days in culture. B) The Biomek i7 Automation workstation is used to dispense the Matrigel and cell mixture as a dome in the center of the well (top). Nine sites from each well were imaged the following day. Images from one well are shown. C) Representation of the deck layout of the Biomek i7 (top). For seeding in multiple plates, the cold plates may be used to maintain the temperature of Matrigel to prevent polymerization. Example screenshots of the method used to transfer Matrigel into domes in a 24 well plate are shown.

Deep learning-based model for analysis of lung organoids

Automated image analysis is an integral part of an automation enabled platform. The ability to monitor cells and organoids in real time and extract meaningful information is dependent on robust image analysis of label-free transmitted light images. To monitor the quality of developing organoids, we used AI-based segmentation to analyze images acquired with brightfield imaging. Growth of lung organoids can be monitored by measuring their diameters over time.

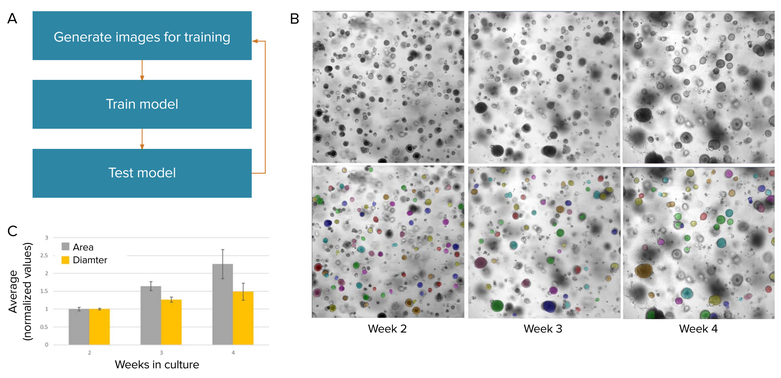

Figure 4. Applying AI-based method to analyze lung organoids. A) Overview of the SINAP workflow in IN Carta software to generate a model for analysis of lung organoids. B) Images of lung organoids grown in Matrigel dome. These images usually have high, non-homogenous background which prevents robust object segmentation. Using SINAP, a model was created to segment lung organoids (mask shown in colored overlay). B) Graph showing change in average lung diameter and area over 2, 3 and 4 weeks in culture (normalized, error bars represent standard deviation between replicate wells).

Conclusions

- We designed and applied an automated approach for the culture, maintenance and monitoring of 3D organoids.
- We show that an AI-based approach can be successfully used to generate robust segmentation for the analysis of label-free biological modes such as 3D organoids.

References

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