Transform the complexities of 3D cell culture into a reliable and translatable science: Automation of 3D organoid culture and organoid analysis

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Introduction

Attrition in the therapeutic pipeline can often be attributed to a lack of translational efficacy from the pre-clinical phase to the clinic. Organoids show great promise as a game-changer in disease modeling and drug screening since they better resemble tissue structure and functionality and show more predictive responses to drugs. However, challenges associated with the practical adoption of organoids, such as assay complexity, reproducibility, and the ability to scale up have limited their widespread adoption as a primary screening method in drug discovery.

To alleviate the bottlenecks that come with labor-intensive manual protocols, we developed the CellXpress.ai Automated Cell Culture System. This revolutionary solution automates the entire organoid culture process for prolonged, complex workflows. The CellXpress.ai system uses machine learning to autonomously manage media exchange, plating, passaging, organoid monitoring, endpoint assays, and complex image analysis. Here we present results from the automation of several commonly used organoid protocols, including the culture of 3D organoids in matrix domes or in low attachment plates. Healthy intestinal organoids were cultured, passaged, and expanded in Matrigel domes (24-well). Organoids were cultured with automated media exchange and monitored by imaging every 24h. After 5–6 days, organoids were automatically collected, purified from Matrigel and dispersed, then mixed with fresh Matrigel and re-pated. Organoids self-organized and developed complex crypt structures. Organoids were monitored in transmitted light, and machine learning-based image analysis evaluated organoid number, size (by area), and optical density. For the endpoint assay (96-well), organoids were stained for viability markers and monitored for concentration and time-dependent effects of compounds on healthy intestinal organoids (toxicity evaluation), or patient-derived colorectal cancer organoids (drug screening).

Automation of cell culture processes powered by machine learning decision-making has incredible potential to bring 3D biology to another level, allowing for increased throughput and reproducibility.

Methods

Automation of organoid workflow

The powerful new CellXpress.ai Automated Cell Culture System automates the entire cell culture process with an integrated incubator, liquid handler, and image-based decision-making. This hands-off automated solution manages demanding feeding and passaging schedules by monitoring the development of cell cultures with periodic imaging and analysis, and leverages machine learning to initiate passaging, endpoint assay, or troubleshooting steps.

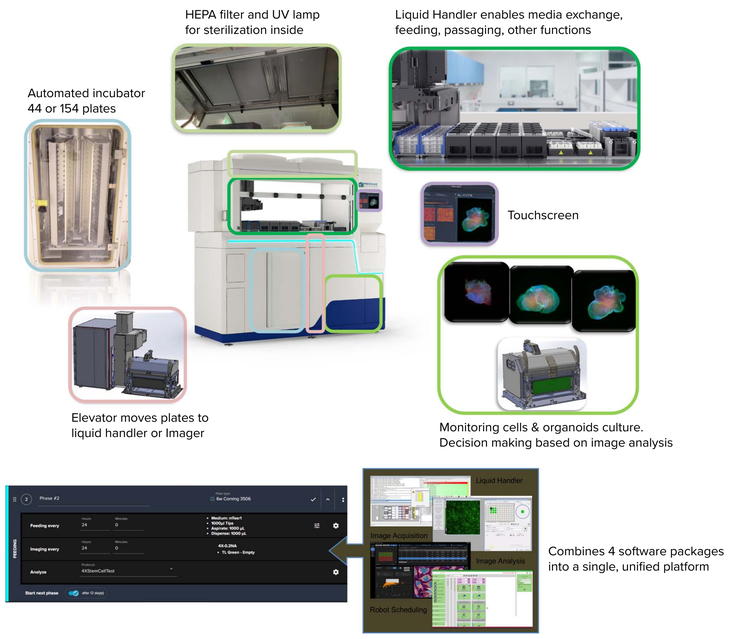


Figure 1. CellXpress.ai system components and functionality

Cell culture protocols

3D intestinal organoids* were derived from primary mouse intestinal cells using established methods (STEMCELL Technologies). Cells were cultured and differentiated 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 or Cultrex (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.

High content Imaging

Fluorescent (FL) images were acquired on the ImageXpress® Confocal HT.ai High-Content Imaging System (Molecular Devices) using MetaXpress® High-Content Image Acquisition and Analysis Software. For intestinal organoids, Z-stack images were acquired with the 4X or 10X objectives using confocal mode. MetaXpress or IN Carta® Image Analysis Software was used for all analysis

Results

Automation of 3D organoids cell culture

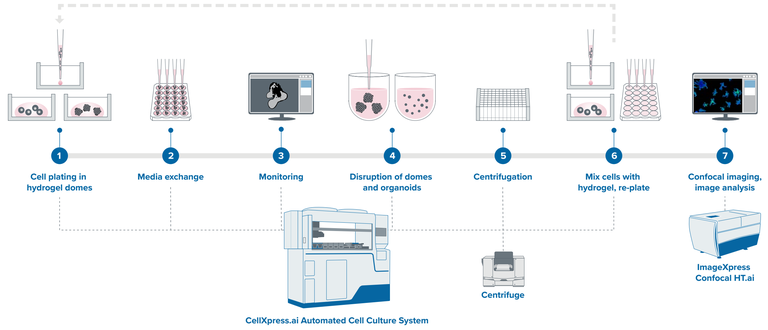
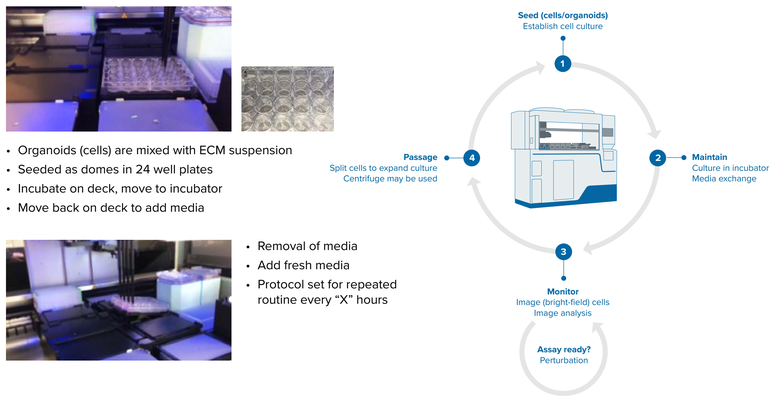
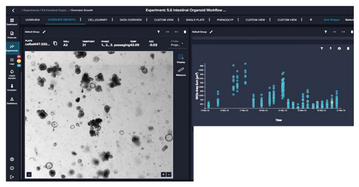


Figure 2. Schematic diagram of automated organoid culture and passaging protocol

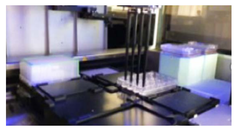


- Organoids (cells) are mixed with ECM suspension
- Seeded as domes in 24 well plates
- Incubate on deck, move to incubator
- Move back on deck to add media

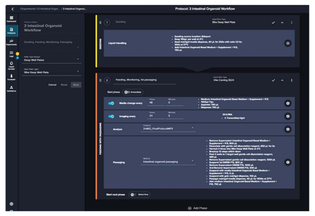
- Removal of media
- Add fresh media
- Protocol set for repeated routine every “X” hours



- Routine imaging of plate (defined by user)
- On-the-fly image analysis using pre-trained deep-learning based model (or customized model)
- Data analytics tools to view growth of organoids over time
- Results can be used to trigger other processes/protocols



- Removal of media
- Release of organoids from ECM, centrifugation (external)
- Fragment organoids
- Add fresh ECM/Matrigel
- Seed into new plate



- Full workflow protocol set in “phases” defined by user:
- Feeding/monitoring, seeding, passaging
- Each step defines main attributes (source and destination plates, pipetting protocols, volumes, media, imaging settings, analysis protocols)
- Main steps can be modified by fine-tuning parameters, that allow further optimization
- Decisions for the process can be made automatically based on imaging analysis

Figure 3. Steps of the organoid culture and passaging protocol

Organoid culture in Matrigel domes was performed according to the basic STEMCELL Technologies recommended protocol for mouse intestinal organoids. Organoids were grown and passaged in 24 well plate format with single dome 40–50µl, 50-60% Matrigel or Cultrex.

Plating organoids was started from organoids suspension in Matrigel which was placed into a pre-chilled 96-well deep well block. The suspension was pipetted by 4 pipette tips and dispensed into 24-well plate, by 4 tips at a time. Plating into a 96-well format was also tested.

Feeding organoids was done by media removal/addition of fresh media, 4 wells at a time.

Imaging/monitoring organoids was done in transmitted light using CellXpress.ai automated cell culture system with 2x or 4x magnification. Image analysis was performed using a machine learning-based protocol. The analysis evaluated organoid number, mean and total area, density, and a number of other measurements. Passaging organoids was set for every 4 days, or as user-directed, or depending on automatic decision-making based on one or more selected measurements (e.g. total area or organoid density).

Passaging organoids. For passaging organoids, the combination of pipetting steps and external centrifugation steps was optimized for the mouse organoids workflow. Modification of the flow rates, pipetting steps and repeats, centrifugation speed, etc. can be customized by changing appropriate “fine-tuning” steps. For the passaging process, media was removed and Matrigel domes were incubated with Gentle cell dissociation reagent, then rigorous pipetting was done to break Matrigel domes. The mix was harvested into a 96 deep-well block. Then optional pooling of two wells into one was performed, followed by centrifugation on an external centrifuge with a speed of 400g for 5 min. The block was then placed back, the media was removed, and organoids were washed once. After a second centrifugation, most of supernatant was aspirated, and then organoids were broken with rigorous pipetting using smaller tips. Then fresh Matrigel was added to the appropriate volume, mixed, and seeded into a new plate.

Staining/Imaging of organoids for endpoint assay was done using FL imaging with the ImageXpress Confocal HT.ai instrument.

Results

Organoid culture and monitoring

Figure 4. A. Representative images (4X) of organoid culture taken at different time point of the continuous culture. B. Graphical presentation of organoid analysis: organoid count and skewness over the time. Skewness includes combination of optical density, granularity, other optical parameters and the value is useful in defining time for organoid passaging. C. Imaging of a single well over the time showing the “cell journey”. D. Tiled 4X images of organoid domes from 24-well plate. E. Presentation of organoid count and organoid area sum over time. Averages and STDEV calculated from 24 wells of the plate.

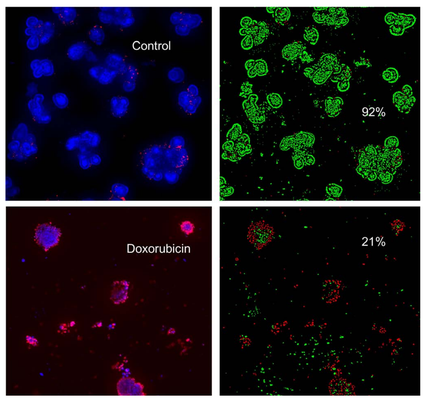


Figure 5. Confocal images of intestinal organoids taken after treatment with compounds and staining with Calcein AM, MitoTracker and Hoechst nuclear stain. Cells were imaged using IXM HT.ai imaging system with the DAPI, FITC and Texas Red channels, confocal, 10X objective. Red: MitoTracker; Green: Calcein AM; Blue: DAPI

Summary

- Organoid technologies are considered a game-changer in disease modeling and drug screening, since they better resemble tissue structure and functionality, and show more predictive responses to drugs.
- Challenges associated with the practical adoption of organoids, such as assay complexity, lack of reproducibility, and the ability to scale up screening have limited their widespread adoption as a primary screening method in drug discovery.
- We demonstrate here how researchers can alleviate the bottlenecks that come with labor-intensive, complex protocols to increase productivity for drug screening.
- A powerful new solution, CellXpress.ai Automated Cell Culture System, allows labs to automate the entire cell culture process—from assay set-up to screening and data analysis—with machine learning-powered workflows to make assays more reliable and reproducible.
- Automated cell culture processes powered by imaging and machine learning-controlled decision-making has a great potential to bring 3D biology into another level, increasing throughput and reproducibility, and enabling a variety of high-throughput drug discovery and precision medicine applications.

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