# Machine learning-assisted automation of complex 2D and 3D cell culture and assay models

Oksana Sirenko, Angelina Chopoff, Krishna Macha, Marco Lindner, Felix Spira | Molecular Devices, LLC

## Introduction

The complexity of cell models has increased with model types such as iPSC-derived cells, patient-derived models, and 3D organoids. The latter of which shows great promise to increase translational efficacy of research and facilitate the development of new drugs since tissue structure and disease phenotypes from these complex models demonstrate better predictive responses. However, challenges associated with the practical adoption of complex assays, including reproducibility and the ability to scale up, have limited their widespread adoption as a primary screening method in drug discovery.

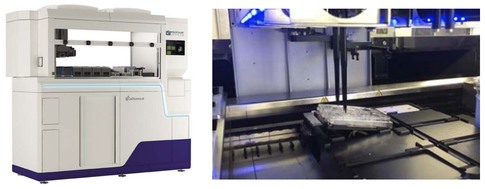
To alleviate the limitations that come with labor-intensive cell culture protocols, we developed the CellXpress.ai™ Automated Cell Culture System. This new system automates the entire cell culture process to improve workflows and make assays more reliable and reproducible. The system contains four essential components for cell culture automation: liquid handling, incubation, imaging, and AI-powered software that automates complex protocols, scheduling, and image-based analysis in one unified environment. Critically important is the software's efficient decision-making capabilities to enable fully automated cell culture processes.

We demonstrated the full automation of several popular cell culture processes. First, we show automated maintenance and expansion of regular and iPSC cell lines. The maintenance and expansion of cell cultures were controlled by imaging-based automated decision making. Cells were cultured with automated periodic media exchange and maintained using imaging and image analysis. Then, based on the selected cell area threshold, cell passaging was automatically triggered using automated decision-making instead of user interaction. The system also provided images, graphs of the selected measurements, and sent notification to the user alerting them to process milestones.

In the second example, iPSC cells were induced for cardiac differentiation by using appropriate media. Cell were then harvested and seeded into u-bottom 96-well plates where they formed spheroids and underwent daily media exchanges with monitoring by imaging. The third example demonstrates automated seeding, media exchange, and passaging of intestinal organoids in Matrigel domes. Organoid size, area, and density were measured by image analysis using a trained machine learning-based protocol, then several triggers for passaging were applied, including organoid size, complexity, and density criteria.

The methods described above fully automates cell culture for standard or complex protocols, allowing scientists to increase productivity, reproducibility, and scale for their research or compound screening assays and automates complex, labor-intensive tasks.

## Methods



CellXpress.ai Automated Cell Culture System and automated media exchange in 6-well plates

**Conventional Cell Lines:** The materials used include HCT116 cells, media with McCoy, 10% FBS, and 1% Pen Strep. Corning 500 mL McCoy's 5A (product number 10-050-CV), ThermoFisher Penicillin-Streptomycin (catalog number 15070-063), FBS (VWR 97068-085), Corning 6-well clear plate (product number 3516), 1000 µL tips (CO-RE Hamilton, sterile and non-filtered), Corning 0.25% Trypsin (product number 25-053-CI), and the CellXpress.ai system and software. All the required materials should be present in the liquid handler, which is essentially a fume hood with features such as laminar flow to sustain sterility. An existing plate with HCT116 cells must also be placed in the incubator for the instrument to begin its maintenance.

In the CellXpress.ai system software, we created the protocol using a phase "Feeding and Passaging" for our fully or partially automated experiments. This protocol includes a feeding step every 48 hours using the McCoy media. When feeding activates, 1400 uL of liquid is aspirated from the experiment's active plate and replaced with 1400 µL of McCoy. The imaging step occurred every 8 hours. The imaging protocol used 4X imaging in transmitted light. Typically, we used 2x2 sites in the center of each well of the 6-well plate. Several sites per well can be increased for a more accurate cell density assessment. Image analysis used protocols from the IN Carta® Image Analysis Software called "4x TL HCT116 confluence". This image analysis protocol determined the area covered by cells from transmitted light images. The total cell area (cell area covered by cells) was plotted over time making graphs reflecting cell area over time. Importantly, we included the imaging analysis rules that would inform the user when it was time to trigger cell passaging or to proceed with the automated passaging step without user participation.

**IPSCs** (ATCC 201B7) were cultured in Vitronectin (STEMCELL Technologies) in 6-well plates and passaged according to the STEMCELL Technologies protocols for cluster or single-cell passaging. The cluster passaging method was as follows. Transmitted light images were acquired on the CellXpress.ai system using 4x or 2x magnifications. The CellXpress.ai system software was used for all analysis. Image analysis was done using pre-trained machine-learning protocols with IN Carta image analysis software. ReLeSR™ reagent, and mTeSR™ media were from STEMCELL Technologies.

The protocol for 3D intestinal organoids* that used primary mouse intestinal cells was developed based on 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.

## Results

### Automated monitoring and media exchanges and passaging of 2D culture (HCT116 cell line) with the CellXpress.ai system

The current study aimed to develop protocols for the fully automated cell culture. We used the Feeding with Passaging phase and set a protocol that would conduct media exchanges every 48 hours, take images of plates in transmitted light with 4X magnification every 6 hours, and perform cell passaging when triggered automatically or manually (see a screenshot below). In the passaging step, we set 1 to 10 as the cell splitting ratio.


**Figure 1.** Settings for Feeding with Passaging protocol used for automated cell culture. System aspirates 1400 µL and dispenses 1400 µL of McCoy media in an active plate, with tilting.

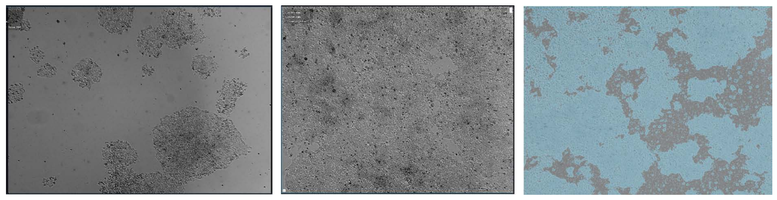


**Figure 2.** HCT116 cells with low, medium, and high confluency (cell area sum 1.6E7, or 3.1E7 µm$^2$ respectively, per imaged area). The image analysis mask (in blue) defines area covered with cells.

While the feeding and imaging tasks are performed periodically (depending on the timing set in the protocol) the passaging step is active, and can be triggered either manually by a scientist, or automatically by the instrument. In order to trigger the passage step automatically, the trigger value must be selected based on the imaging results. Image analysis is done automatically after each imaging step. The Passaging step was triggered when the cell covered area (Area Sum) reached the number that corresponded to cell confluency at which we would typically perform passaging. This number was empirically chosen as 30,000,000 square microns (Figure 2).

In the first variant of the protocol, we actioned the software to send a notification to the user when the plate needed to be passaged, meaning that cells reached a threshold confluency. When users received this message, they triggered passaging by activating the Passage button, which can be done remotely. In the fully automated protocol, the instrument proceeded with passaging when the cell density reached threshold, without the participation of a scientist. Passaging can be done for an expansion scenario (increased number of plates), or in maintenance mode, when the number of plates is not increased.

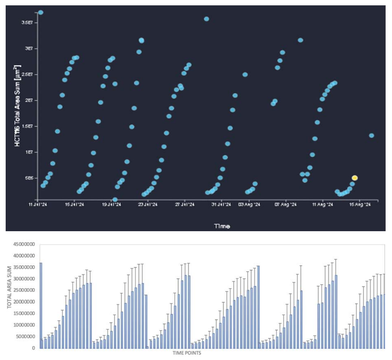



**Figure 3.** The graphs show monitoring cell area over the time and a fraction of the plate was imaged with 4 sites at 4X magnifications. Cell areas were averaged per plate. Missed points appeared during pausing the instrument for trainings. Bar graph below shows averaged values and standard deviations between wells in 6-well plate during cell culture.

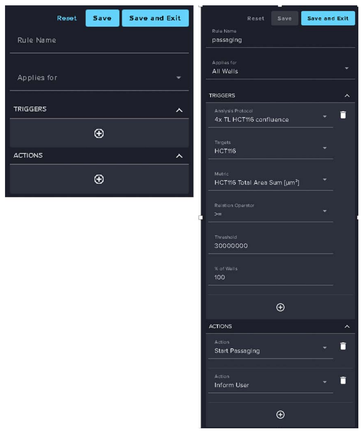



**Figure 4.** Rule in CellXpress.ai software used in both remotely automated and fully automated experiments.

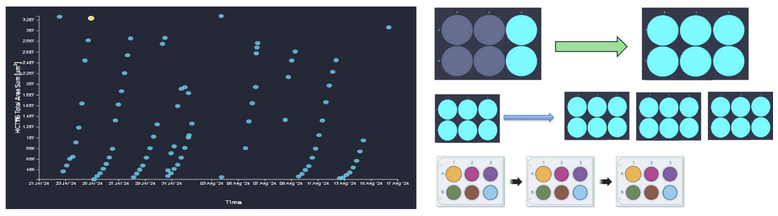



**Figure 5.** Growth patterns of HCT116 cells for the fully automated experiment on the CellXpress.ai system. Total Area Sum in square microns vs. time where error bars are the standard deviation of the total area sum µm$^2$ of each well of the remotely automated experiment. Note: missing time points in graphs were due to pausing protocol when the instrument was used for training/demos, the protocol proceeded as expected after un-pausing.

## Results

### Automated cell culture of iPSC and organoids with automated decision making

We also automated iPSC culture with automated decision-making and performed according to the basic STEMCELL Technologies recommended protocol for human iPSC cells. The protocol was tested with two commercially available iPSC cell lines and automated using basic iPSC protocols recommended by STEMCELL Technologies. The protocol was set up for media exchange of mTeSR every 24 hours on the liquid handler and monitored by imaging every 12h. Deep learning-based image segmentation was used to detect iPSC colonies, determine the cell area and confluency, and detect cell phenotype changes or differentiation. Confluency was used for decision making. Passaging events are marked by arrows in Figure 6. Images acquired during monitoring were analyzed and measurements were used for automated decision making. Passaging steps were triggered automatically or by user decision after receiving notification. In the presented example, 70% of cell confluency for 100% of wells was used as the decision-making point for cell passaging.

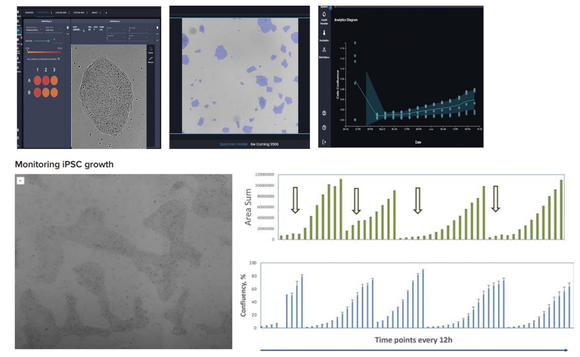



**Figure 6.** iPSC cell imaging followed by On-the-fly image analysis using pre-trained deep-learning based model for recognition stem cell colonies. Representative image of stem cell colonies and graph showing the total Area Sum or % confluency for iPSC culture in 6-well plate for two independent experiments. Passaging steps indicated with arrows. Error bars indicate SDEV between the wells in 6 well plates.

Automating the 3D culture was the most challenging task. Organoid passaging was done using a combination of pipetting steps and external centrifugation steps to optimize for the mouse organoids workflow. Modification of the flow rates, pipetting steps and repeats, centrifugation speed, etc. were optimized 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 external centrifugation, after which organoids were dispersed with rigorous pipetting, remixed with fresh Matrigel, and re-plated. We performed continuous culture of intestinal organoids for 3–5 passages for more than a month. Automated seeding of organoid domes allowed consistent size and accurate centering of domes in 24-well plates. Organoids grew as expected during automated culture, consistent with typical intestinal organoids morphology. Images were taken daily using 2X objective and image analysis was done using a pre-trained machine-learning model. The image analysis found organoids and determine their number, mean and total area, density, roundness, granularity, and other morphological criteria. The software also reviewed organoid domes during the culture and provided on-the-fly analysis and timed course plots representing various measurements. Decision for passaging included combination of organoid area and density measurements and was triggered upon receiving e-mail notification.

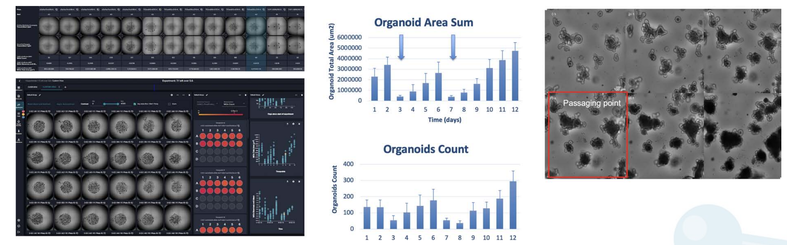



**Figure 7.** Imaging of 3D organoids with 2X-4X magnification followed by on-the-fly image analysis using pre-trained deep-learning based model for recognition organoids. Representative images of organoids in 24-wells shown; graphs showing the total Area Sum or organoid count in 24-well plate. Passaging steps indicated with arrows. Error bars indicate SDEV between the wells in 24-well plates.

## Summary

- We demonstrated a fully automated protocol for HCT116 cell culture, iPSC, and 3D organoids including media exchanges, imaging, and passaging using the CellXpress.ai system.
- Machine learning–based image analysis was utilized to obtain measurements for triggering automated passaging when cells reached a required cell density threshold, with both manual and automatic passaging options.
- The process described here overcomes the challenges of manual handling of cell culture, including complex cultures of iPSC cells and organoids, enabling automated cell maintenance, expansion, and assay set-up. Cell culture automation offers a great potential to reduce labor costs and increase productivity, throughput, and reproducibility.
- Automating key processes greatly reduced manual labor and effort spent by scientist for cell culture, showing promise for more reproducible and scalable cell assay experiments.

HUB Organoid Technology used herein was used under license from HUB Organoids. To use HUB Organoid Technology for commercial purposes, please contact bd@huborganoids.nl for a commercial use license.

MOLECULAR DEVICES