# Using the power of AI in automated cell culture

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## Introduction

The drug discovery industry relies heavily on automation technologies to enhance efficiency, reduce errors, and ensure reproducibility of assays. Despite these benefits, in-house implementation of end-to-end automation is challenging due to the need for specialized expertise, integration of hardware and software, and the optimization of biological assays for automated workflows.

To address these challenges, the CellXpress.ai™ Automated Cell Culture System was developed. Designed for automated cell culture, it integrates a liquid handler, incubator, imager, and AI-enabled image analysis solution, all controlled by a single software platform. This system features a modular workflow design, breaking down common lab workflows into phases that allow flexibility in protocol design. The AI-assisted automated decision-making function can inform users or trigger subsequent steps in the cell culture process.

Here, we describe the automation of three common biological workflows (iPSC, colorectal cancer (CRC) organoids, spheroids) that we have developed on the CellXpress.ai system. For iPSC culture, the system automates processes such as seeding, feeding, imaging, and analysis, with AI monitoring cell morphology and confluence. For CRC organoids, it automates medium exchange, passaging, and imaging, with real-time AI-driven decision-making ensuring optimal growth conditions. For spheroid culture, it automates formation, culture, and analysis, assessing spheroid size, viability, and morphology through integrated imaging and AI tools. Overall, the CellXpress.ai system addresses the key challenges of automation in drug discovery, enabling researchers to focus on scientific inquiries while achieving consistent and reliable results.

## Materials and methods

### CellXpress.ai Automated Cell Culture System

The CellXpress.ai Automated Cell Culture System provides a workstation for automated cell and organoid culture. It includes an automated imager, a liquid handler, and an incubator, controlled by an easy-to-use software. The system can be expanded or further integrated with external components via 2 external ports.


### Cell culture

**Automated 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 coated 6-well plates (cat. #354277, Corning). Once the cells are established in culture, the plates were transferred to the CellXpress.ai for maintenance. The “Feeding” phase in the software was setup to do daily media change. mTeSR media was added to the media container and stored on deck (at 4°C) for the duration of the experiment.

**Automated colorectal cancer organoid culture:** Colorectal cancer (CRC) organoids (Line ISO68, Cellesce) were handled according manufacturer’s instructions. Briefly, organoids were thawed quickly at 37°C, gently resuspended and washed in media. Pellet containing organoids were resuspended in 80% Matrigel and manually dispensed into a deep well plate. The CellXpress.ai was used to pipette 40µl of organoid suspension into a 24well plate. The plate kept at 37°C for 15 mins to allow the Matrigel to set, before the addition of complete media.

**Monitoring and maintenance:** Monitoring of iPSC was carried with the CellXpress.ai built-in imager. The “monitoring” phase was set up in the protocol to image the wells every 12 hours. To quantify confluency, an image analysis protocol that utilizes a pre-trained, deep learning model was used to segment iPSC colonies. Decision making component was also added to the protocol such that when iPSC confluency reaches > 80%, the user is informed via email. Passaging may also automatically be triggered in this way.

The protocol used to monitor of CRC organoids is similar to that for iPSC. Daily imaging of the plate was scheduled. Media exchange was carried out every three days. Passaging occurred every 7 days.

## Results

### Automated 2D and 3D cell cultures

Recent advances in cell biology have spurred the development of increasingly complex models of human disease. These advances include new cellular models, such as patient-derived induced pluripotent stem cells (iPSC), new culture methods, such as three-dimensional organoids. Many of the workflows involving these recent cellular models can be carried out by skilled technicians, however, this manual approach becomes increasingly impractical when translating these workflows to high-throughput drug discovery environments.

To overcome this challenge, we developed an automated cell culture platform capable of performing typical cell culture tasks such as seeding of cells, changing media, monitoring and passaging (Figure 1).

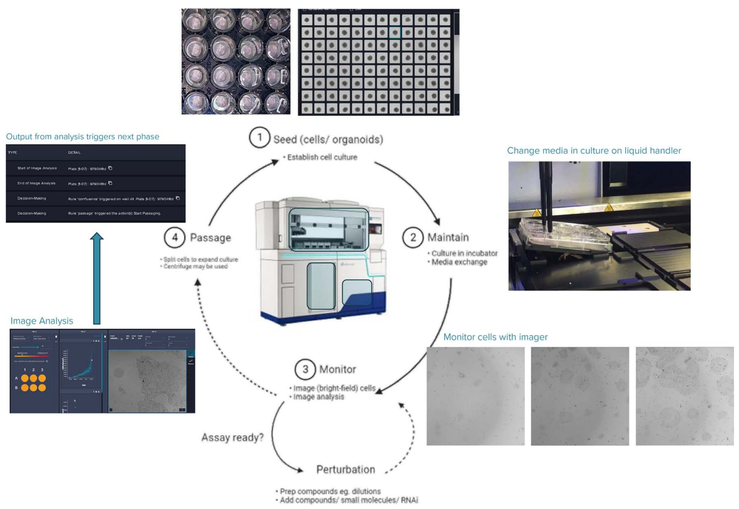



**Figure 1.** Basic processes required in cell culture is shown here. 1) Cell seeding can be automated on a liquid handler. This may involve seeding of cell suspension for 2D cultures or handling of organoids mixed in Matrigel. 2) Cells are maintained in an incubator, and media changes can be scheduled and carried out on the liquid handling deck. 3) Cells are monitored with an imager. This is followed by on-the-fly, AI-enabled image analysis which can be configured to trigger downstream steps based on the results. 4) Passaging is often carried out when iPSC are confluent. In this case, a decision-making protocol can be set up to trigger the passaging step.

### AI-based image analysis

In addition to maintaining cells in culture with regular media changes, it is also important to carry out routine monitoring of cells to ensure that the cells are growing as expected and detect anomalies (such as contamination). In most small-scale settings, monitoring usually involves visual inspection of cells. This is rarely feasible in high throughput environment. Thus, we have automated cell culture monitoring using an image-base, quantitative approach.

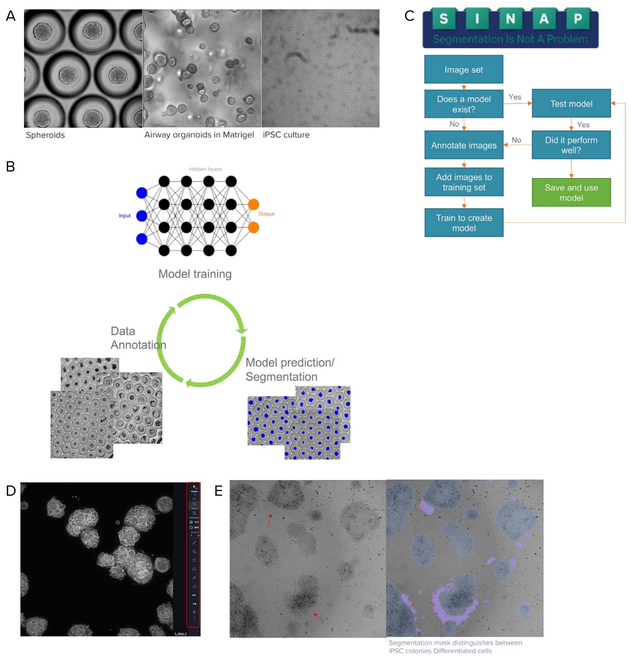
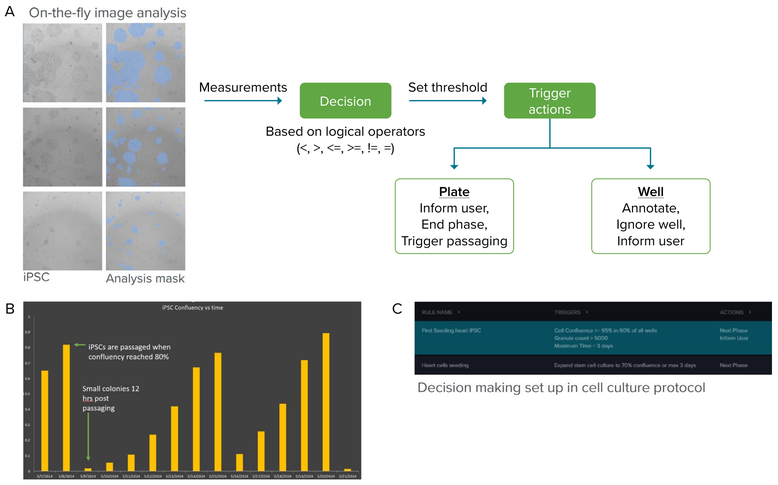



**Figure 2.** Bright field transmitted light imaging is commonly used to image cells in culture. However, segmentation of transmitted light images is challenging due to the meniscus effects and shading or edge artifacts. A) Example of artifacts (Well edge effects, heterogenous background, and debris in media) observed in transmitted light images. B, C) Deep-learning can be used for segmentation of challenging images. The workflow starts with data annotation followed by model training and validation. This process continues iteratively until the model is satisfactory. D) The image analysis software, IN Carta, includes a deep-learning based segmentation tool (SINAP). Shown here is the SINAP interface for image annotation to create training data. Images are loaded in the main panel while the annotation tools (red box) are on the right. E) Models to segment differentiated and undifferentiated cells may be trained using SINAP. Arrows point to areas of differentiation. Right: Overlay of segmentation mask shown.

## Results

### Image-based AI for automated decision making

Confluency, is a common metric used to determine whether cells are ready for passaging. In non-automated workflows, confluency is usually a subjective measure based on the user’s cell culture experience. Similarly, in the culture of adult stem cell derived organoids (such as intestinal), factors such as size and morphology is used to determined when passaging should be carried out. Here, we have integrated an AI-based image analysis approach in the CellXpress.ai system to make “decisions” in cell culture.




Decision making set up in cell culture protocol

**Figure 3.** A) Monitoring of cell cultures such as iPSC culture starts with image acquisition followed by analysis using IN Carta’s SINAP. Measurement output is then used to trigger future actions such as “inform user” via email or to start passaging. B) iPSC cultures are imaged daily with Confluency used in the decision-making setup. Graph shows iPSC confluency over time. C) Example of cell culture protocol with decision making rules set up.

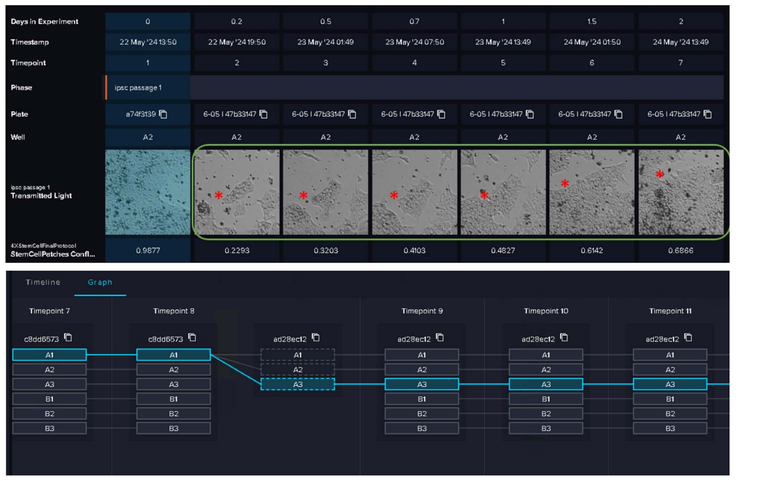



**Figure 4.** For quality control, the CellXpress.ai software includes a“widget” setup for custom dashboard view over time (A). Samples from each well is tracked in the software. For example, at time point8, well A1 was passaged and seeded into well A3 of the new plate.

### Automated 3D cell culture (organoids and spheroids )

Patient-derived colorectal cancer organoids (CRC) were cultured in the CellXpress system. CRCs were manually suspended in Matrigel and transferred to the CellXpress for seeding into 24well plates as domes (Figure 5). Once domes were polymerized (15min in incubator), culture media was added off-center to prevent disruption of organoid containing domes. For spheroids, HCT116 cells suspension was loaded on the CellXpress.ai for seeding into 96 well ultra low attachment coated U bottom plate.

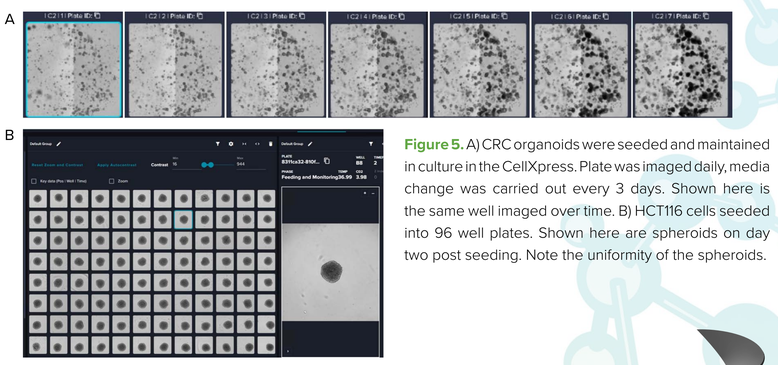



**Figure 5.** A) CRC organoids were seeded and maintained in culture in the CellXpress. Plate was imaged daily, media change was carried out every 3 days. Shown here is the same well imaged over time. B) HCT116 cells seeded into 96 well plates. Shown here are spheroids on day two post seeding. Note the uniformity of the spheroids.

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