The CellXpress.ai Automated Cell Culture System: For automated, robust brain organoid generation

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Introduction

Protocols for brain organoid generation have significantly advanced in recent years, establishing these models as essential tools in drug discovery, disease modeling, and personalized medicine. Despite this progress, brain organoid systems continue to face challenges such as poor reproducibility and complex, labor-intensive protocols that hinder scalability. Although differentiation protocols from induced pluripotent stem cells (iPSCs) into brain organoids vary across systems, they typically require media exchange over many months together with continuous monitoring. This lengthy cultivation process contributes to high variability between wells and plates, which remains a major limitation.

Here, we demonstrate the reliable generation of brain organoid models using the CellXpress.ai® Automated Cell Culture System. Reproducibility was achieved by rigorous quality control of iPSC cultivation to ensure high-quality input material. We then reduced variability by optimizing liquid handling steps to minimize organoid loss or damage during media aspiration and dispensing. Extended hands-off time was achieved by using the system's "Smart Media Module," which enables on-deck reagent storage for multiple days. To enable cultivation of free-floating organoids, we integrated an in-incubator rocking device into the CellXpress.ai system, to provide continuous media agitation during the entire maturation process.

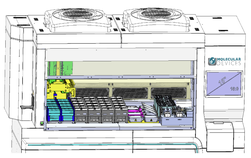
Monitoring was achieved by the built-in automated imager in combination with the IN Carta® Image Analysis Software image segmentation and classification package. Organoid tracking over many passages can be achieved via the "Cell Journey" feature and by using the 2x objective the system can acquire an entire 6-well plate of free-floating organoids with full coverage.

We also showed the seamless integration of external devices such as the ImageXpress® Confocal HT.ai High-Content Imaging System for either endpoint assay, or advanced monitoring by utilizing the CellXpress.ai system's back port.

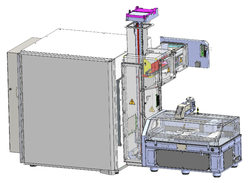
CellXpress.ai Automated Cell Culture System

The CellXpress.ai system

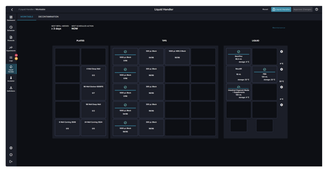
Liquid handling



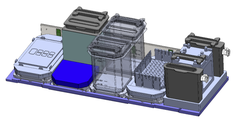
Automated microscopy, incubation, and robotics



Unified software environment



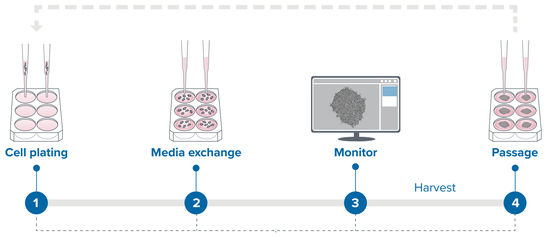
Smart media module



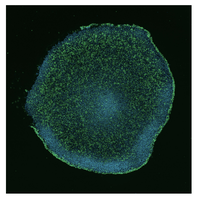
The CellXpress.ai Automated Cell Culture System integrates hardware and software to seed, feed, and monitor both 2D and 3D cell culture and can utilize machine learning-assisted image analysis to ensure consistent treatment of the cell culture.

Organoid generation workflows

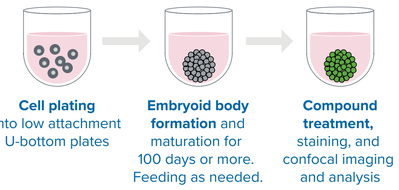
iPSC cultivation



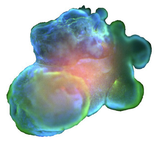
iPSC colony stained for pluripotency marker



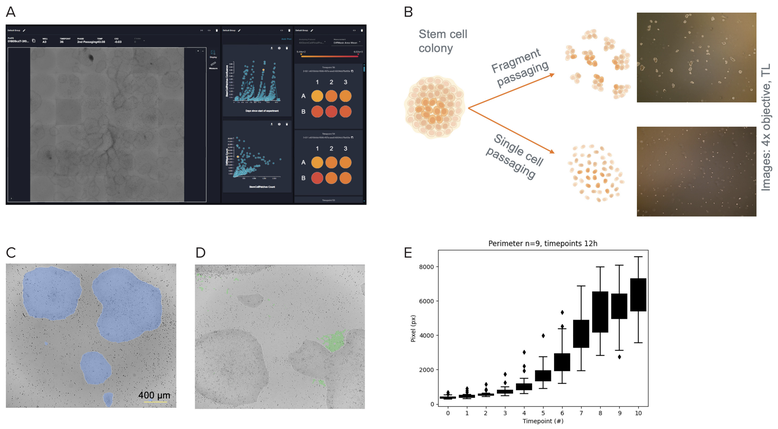
Organoid generation



Whole mount stain of a brain organoid

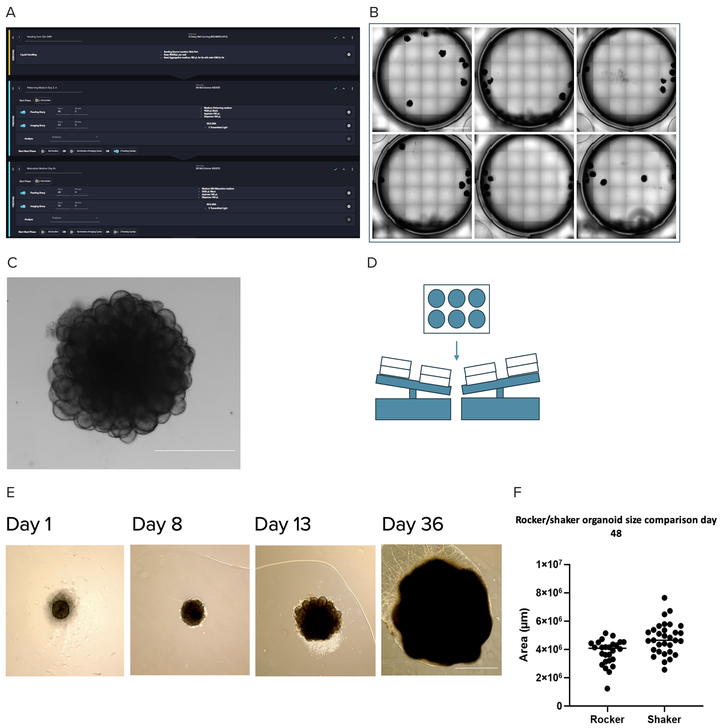


Automated stem cell cultivation



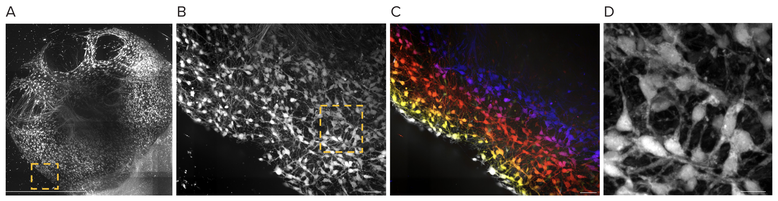
(A) The CellXpress.ai system software showing stem cell cultivation workflow. (B) Different passaging methods built into the CellXpress.ai system. (C, D) Deep learning for colony segmentation to discriminate between healthy colonies (C) and differentiated cells (D). (E) Quantification of colony growth monitored over 6 days. Boxplots shows average of 9 plates.

iPSCs differentiation into brain organoids



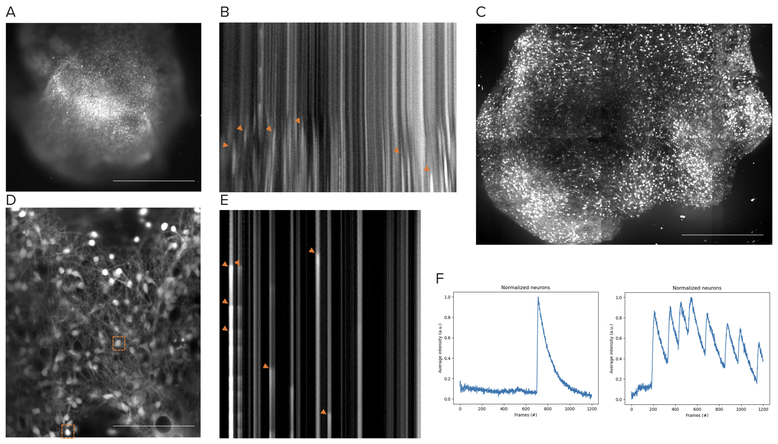
(A) Image showing protocol setup in the CellXpress.ai system software (B) Representative images of stitched full-well acquisition of 6-well plate. (C) Enlarged organoid of C. (D) Cartoon of cultivation of brain organoids on rocker. (E) Representative single images of organoids over 36 days. (F) Quantification of organoid sizes of the organoids cultivated on a rocker and shaker. Scale Bars B: 10 mm, D: 20 µm, C and F: 600 µm; Objective 2x air using the CellXpress.ai system built in microscope (B,C), 4x Evos (E)

High-resolution image acquisition of brain organoids



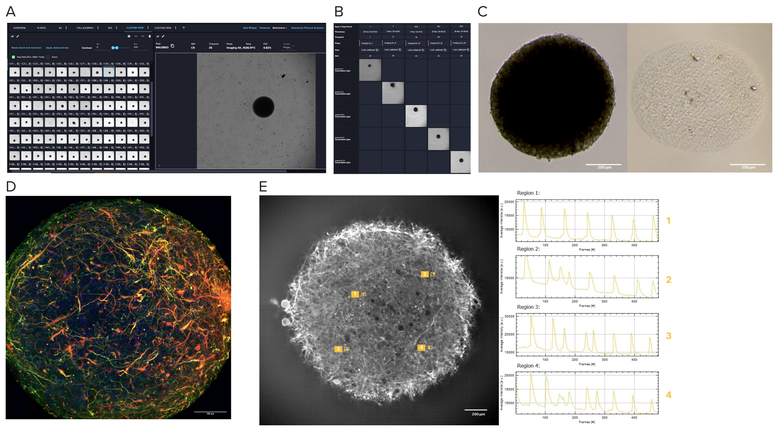
Exemplary images of brain organoids. (A) Stitched max projection of high-resolution image. (B) Zoom into region indicated in A. (C) Spatial colored projection of region indicated in A to show cortical layers. (D) Zoom into region indicated in B. Scale bar: A: 1mm, B–D: 20 µm; A–D: Objective 20x water immersion on the ImageXpress® Confocal HT.ai High-Content Imaging System

Analysis of cerebral organoids



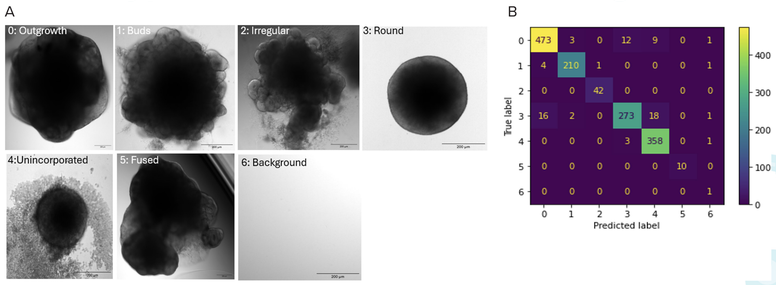
(A) Stream acquisition of a calcium-stained brain organoid. (B) Kymograph of A indicating calcium activity. (C) Max projection of high-resolution Z-stack of organoid A. (D) High-resolution stream acquisition of neuronal network of organoid in C. (E) Kymograph of neuronal network of D. (F) Single neuronal traces of D, neurons are indicated by orange boxes. A, B: 4x objective using the CellXpress.ai system's built-in automated microscope at 70 ms framerate. C–E: ImageXpress HT.ai system 20x water immersion. D–F: Step-size 2 µm, frame rate 20 ms. Scale bar A, C = 1 mm, D = 10 µm

Midbrain organoid generation on the CellXpress.ai system



(A) CellXpress.ai software showing midbrain organoids cultivated on the CellXpress.ai system. (B) Cell journey to follow individual organoids over time. (C) Organoid pre- and post-optical clearing. (D) Maximum projection of a whole-mount-stain of a cleared midbrain organoid. (E) Stream acquisition of a calcium-stained midbrain including single neuronal traces for four regions (1-4). A,B: the CellXpress.ai system built in microscope, 4x air objective. C: Evos XL 10x air objective, D, E: ImageXpress HT.ai system 20x water immersion objective.

Phenotypic classification of brain organoids



(A) Representative images of organoid classification. (B) Confusion matrix between automatically annotated dataset and ground-truth dataset. Objective 4x air; Scale bars: 200 µm

Conclusion

We demonstrated that brain organoids generated using the CellXpress.ai Automated Cell Culture System were comparable in size and neuronal activity to those produced manually. The system successfully supported the simultaneous cultivation of iPSC lines, free-floating brain organoids in 6-well plates, and single organoids in 96-well plates. The built-in imaging system enabled automated image acquisition across different plate formats and model systems, followed by reliable, label-free image segmentation and classification tailored to each model.

This fully integrated, end-to-end solution highlights the feasibility of scalable, robust, and high-throughput brain organoid generation—from healthy stem cells to mature, functional brain organoids—within a single automated platform.

MOLECULAR DEVICES

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