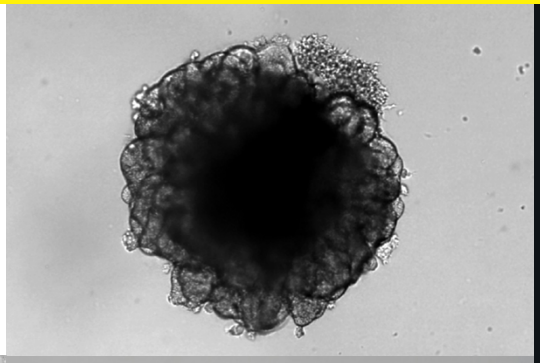
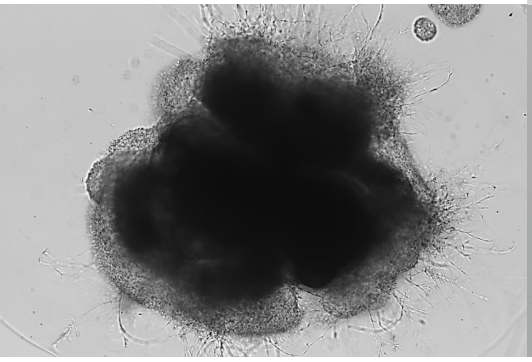
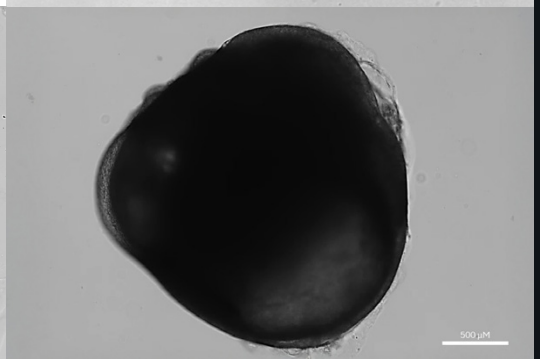
Monitoring and Automating Brain Organoid Differentiation using the CellCelector

Practical Tips for Automated Organoid Transfer, Live-cell Imaging, and Analysis







Technical Note

This technical note provides an overview of methods used in differentiation and monitoring of 3D brain organoids using the CellCelector Flex Platform. It outlines protocols and practical tips for automated organoid transfer, and discrete live-cell imaging of cerebral organoids in suspension.

Introduction

Brain organoids, generated from human pluripotent stem cells (hPSC), are three-dimensional (3D) *in vitro* models which exhibit cellular composition and organization that mimic the developing brain *in vivo* and are increasingly utilized to study human brain development and disease. However, brain organoid differentiation is technically difficult due to long protocols, sensitivity to perturbations, and heterogeneity. Since brain organoids are typically cultured in suspension and grow to dense objects of considerable size (3 – 5 mm in diameter), they are challenging to image and quantify, with methods predominantly being qualitative in nature or limited to end-point analyses. We have previously described a comprehensive workflow utilizing live-cell analysis and HTS by Cytometry for monitoring the unguided differentiation of iPSC-derived cerebral organoids over 40 days. In this technical note, we focus on how the CellCelector Flex can be used for gentle, automated organoid transfer and live-cell analysis of organoid growth and morphology at discrete timepoints throughout differentiation.

Required Materials

- Human iPSCs of choice
- Materials required for brain organoid differentiation (e.g., STEMdiffTM Cerebral Organoid Kit, STEMCELL Technologies, Cat. No. 08570)
- 96-well ULA round-bottom plate (e.g., Corning®, Cat. No. 3363)
- 6-well ULA flat-bottom plate (e.g., Corning®, Cat. No. 3471)
- 200 µL wide-bore pipette tips (e.g., Axygen®, Fisher Scientific, Cat. No. 14-222-730)
- CellCelector Flex and Incubator Flow Box

Experimental Workflow

Human iPSCs were differentiated into cerebral organoids over 40 days using a commercially available kit and four stage unguided protocol and differentiation.[1] As discussed previously[2], a comprehensive workflow was used to temporally monitor and characterize brain organoids using multiple Sartorius platforms (Figure 1). The methods below describe the use of the CellCelector Flex as part of this approach for automated organoid transfer and discrete live-cell analysis of cerebral organoids.

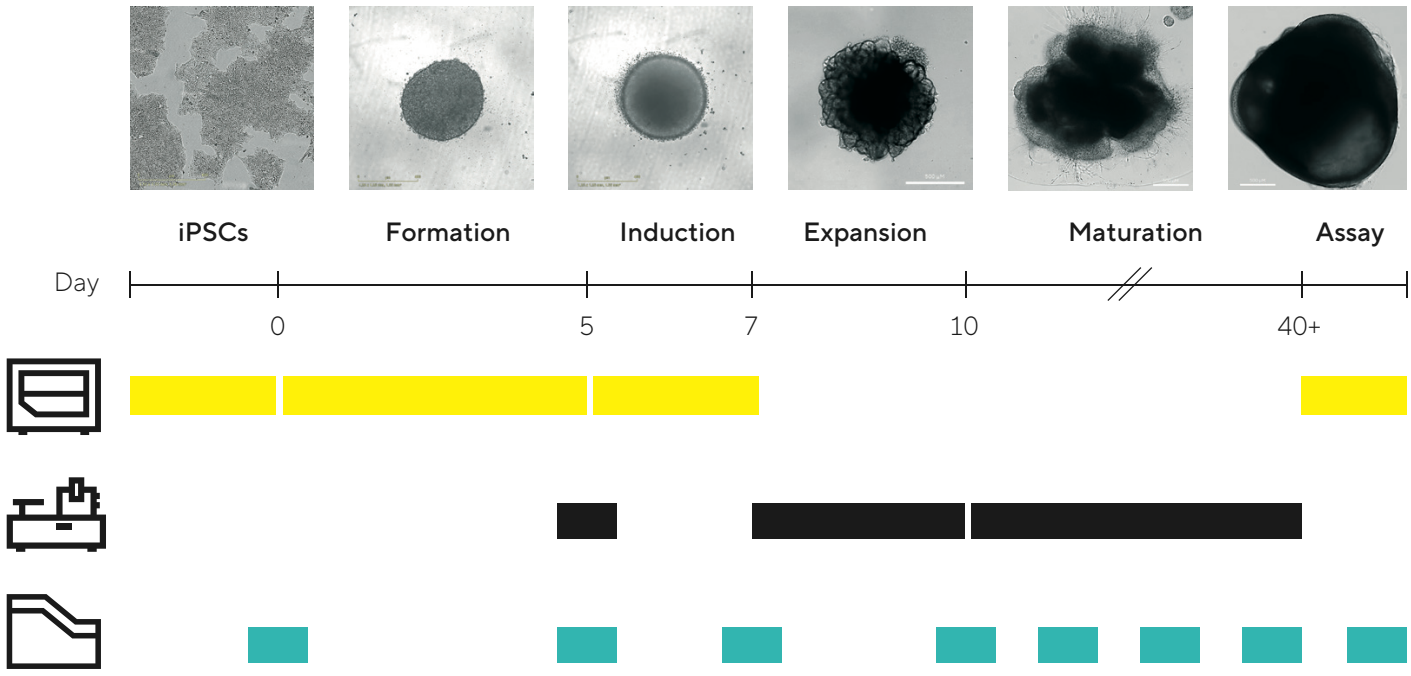


Figure 1. Workflow for the generation, monitoring, and characterization of iPSC-derived cerebral organoids. Schematic shown for cerebral organoid generation from iPSCs which begins with an intermediate aggregation formation stage, followed by neuroepithelia expansion in Matrigel™ and a period of maturation until day 40 when organoids are considered early mature and exhibit cortical-like regions. The workflow incorporates multiple Sartorius instruments for the monitoring and characterization of differentiation through continuous (Incucyte®, yellow boxes) or discrete live-cell analysis (CellCelector Flex, black boxes) and high-throughput screening by cytometry to assess relevant markers (iQue®, teal boxes).

Organoid Transfer

Following formation on Day 5, 60 organoids were individually transferred from inner wells of a 96-well ULA round-bottom plate to a second 96-well ULA round-bottom plate containing 200 µL/well of induction medium. PBS was added to edge wells of 96-well plates, which were avoided to minimize risk of edge effects to organoid growth. Organoid transfer was automated using the CellCelector Flex and parameters used are shown below (Table 1; Figure 2). An Incubator Flow Box (37 °C, 50% humidity, gassing off) was used to maintain a physiological environment. Parameters may need to be optimized dependent on the iPSC line being used and organoid size on Day 5, if need be, a larger capillary can be used (1200 µm diameter).

Picking height	40 µm
Illumination	Brightfield; Objective 4x
Source Vessel	96-well ULA round-bottom plate
Destination Vessel	96-well ULA round-bottom plate
Air gap	5 µl
Picking volume	8 µl
Capillary diameter	500 µm (disposable tips)
Picking Module	Semi-solid media picking tool

Table 1: Parameters for brain organoid automated transfer on Day 5 using the CellCelector.

Automated picking using the CellCelector is accurate and gentle and offers several advantages over manual transfer. This includes minimizing human error and improving reducibility, transfer with low injection volumes, and full documentation of transferred organoids including before and after picking images and a record of each organoid's characteristics (e.g., size, sphericity and eccentricity).

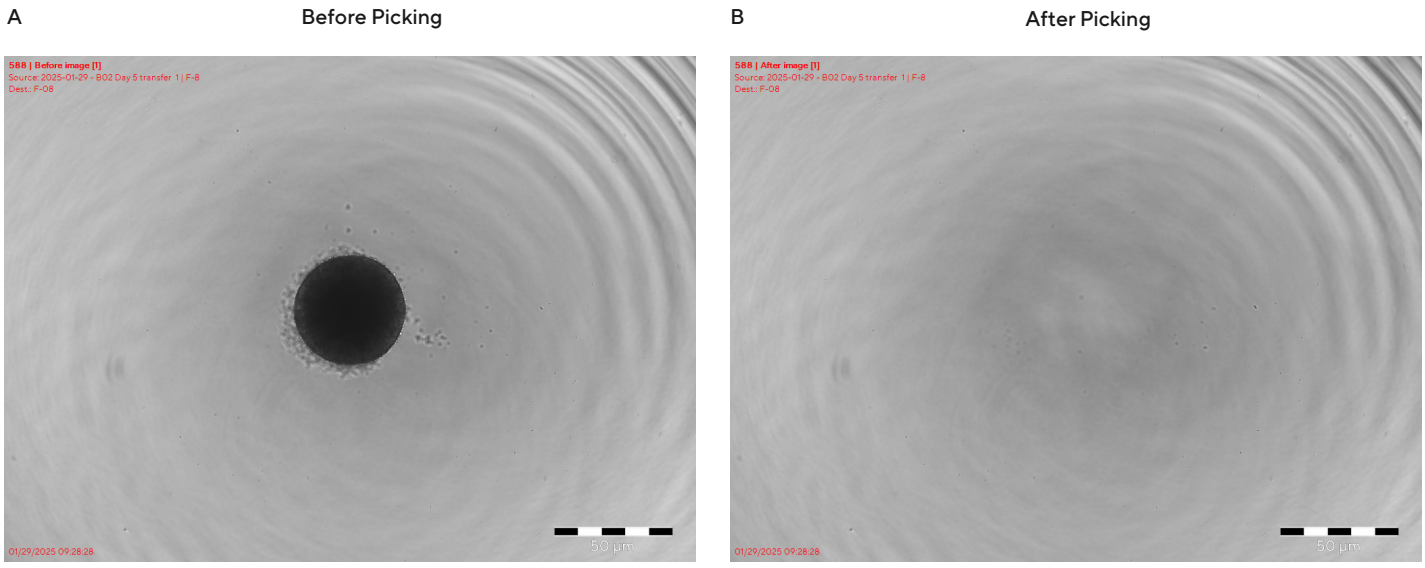


Figure 2. Automated brain organoid transfer. (A) CellCelector Brightfield images of source well before and after (B) organoid picking.

Organoid Imaging and Analysis

From Day 10 onwards organoids embedded in Matrigel™ droplets were matured in 6-well ULA plates on an orbital shaker (74 RPM, at 37 °C). Due to condensation forming on plate lids with shaking, it is recommended to keep a spare sterile lid for imaging. Every 3 – 4 days, full refeeds of maturation medium were performed and brightfield images (4x magnification, 75% of well imaged on slowest stage speed) were acquired using the CellCelector Flex with an Incubator Flow Box (37 °C, 50% humidity, gassing off) to monitor organoid growth and development up to 50 days. Images were analyzed using integrated CellCelector software, which enables export of metrics for each organoid (ROI) in the field of view (Figure 3).

Discrete live-cell imaging allows the monitoring and quantification of key stage-specific morphological features that develop temporally during cerebral organoid expansion and maturation (Figure 4), whilst accounting for an expected degree of heterogeneity. Notably, the approach also enables go/no go decisions to be made for individual organoids throughout differentiation, for example if they don't exhibit the expected budding morphology, lose border integrity, or develop large cysts suggestive of off-target differentiation.

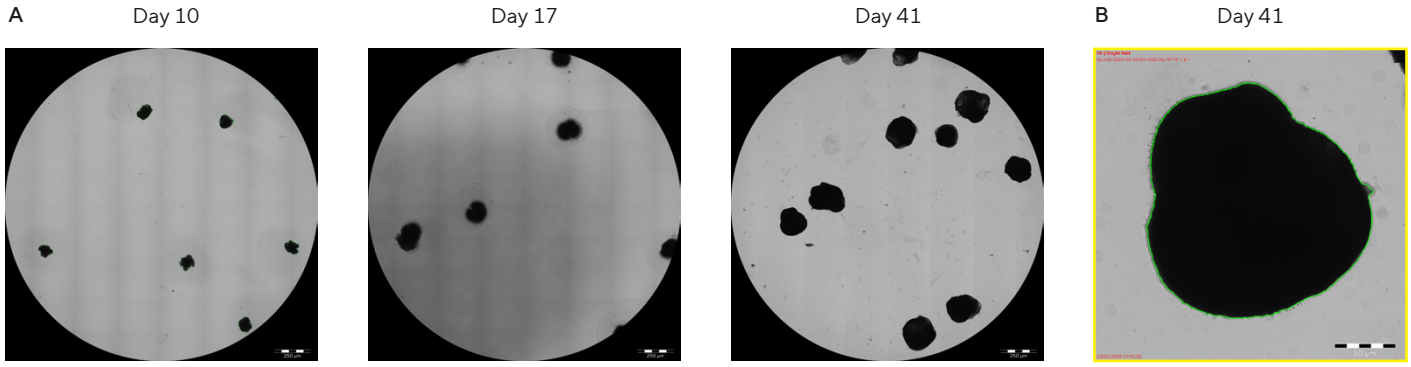
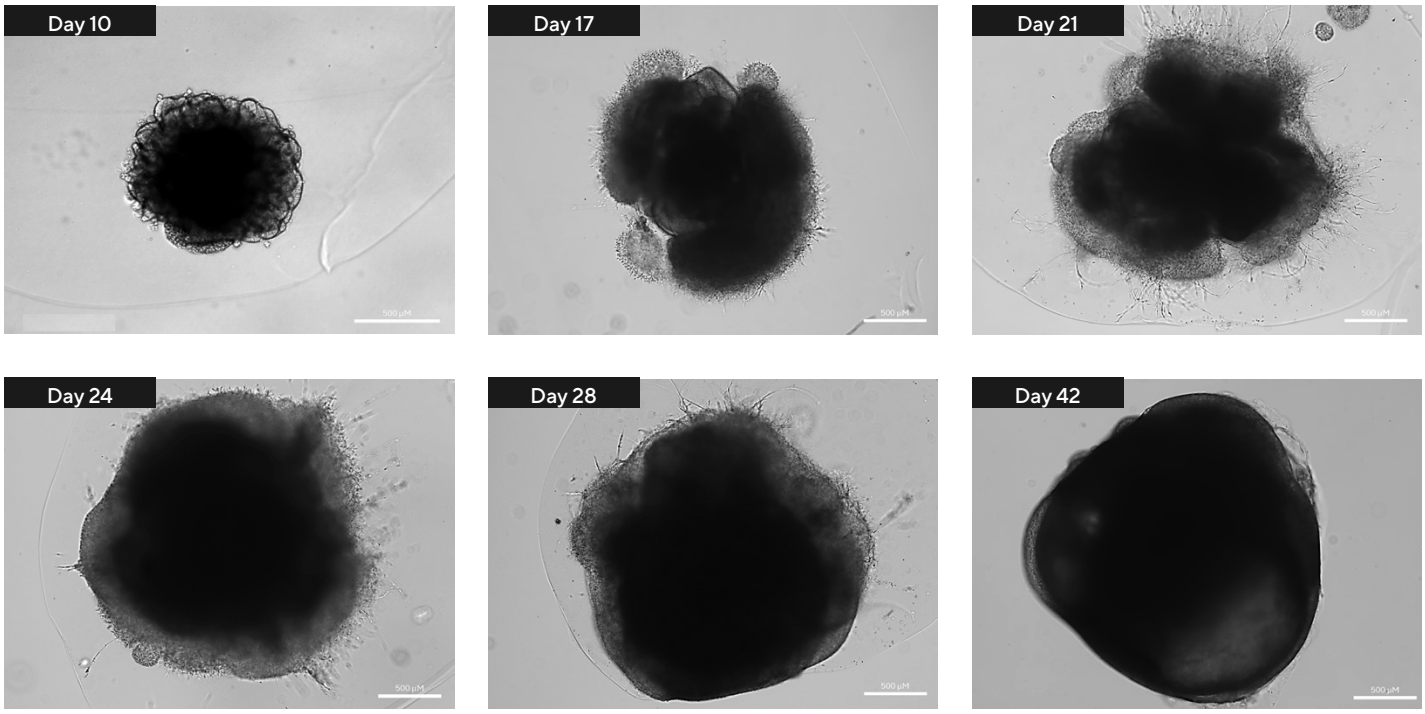


Figure 3. Imaging and analysis of cerebral organoid maturation using the CellCelector A) Representative overview brightfield images shown for one well at 4x (70% well imaged) over time. B) Using integrated analysis, at a single time-point each organoid is assigned a display number (ROI) which can be matched to object-by-object data and facilitates the exclusion of organoids from analysis (e.g., due to blurring or not in field of view). Example organoid segmentation is also shown (green outline).

A



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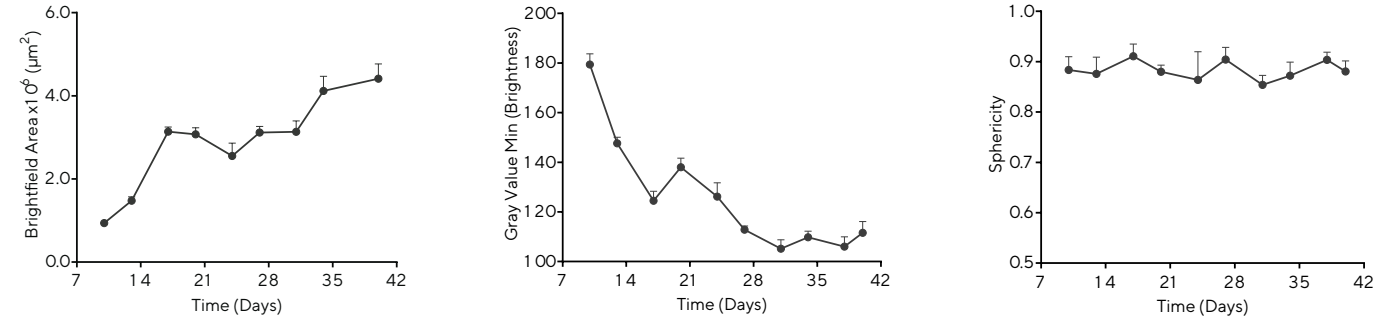

Figure 4. Temporal quantification of cerebral organoid maturation. Organoids were matured from Day 10 until Day 40 and were monitored using discrete live-cell analysis. A) Representative brightfield images of organoids over time showing distinct morphological features that occur temporally. B) Quantification of organoids showed an increase in brightfield area over time, and a decrease in grey value min (brightness) as a dense core forms whilst sphericity remains relatively stable. Data presented as mean ± SEM, n = 6 replicates

References

1. STEMCELL Technologies. STEMdiff™ Cerebral Organoid Kit. Product Information Sheet. 2024. Accessed June 12, 2025. https://cdn.stemcell.com/media/files/pis/10000003530-PIS_05.pdf
2. Trigg J. et al. A comprehensive workflow for quantifying cerebral organoid differentiation. Accessed July, 2025. https://www.sartorius.com/en/applications/life-science-research/advanced-cell-systems/advanced-cell-systems-resources/comprehensive-workflow-for-quantifying-cerebral-organoid-differentiation#id-1734612

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