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Monitoring Brain Organoid Differentiation and Development Leveraging Live-Cell Imaging and Cytometry

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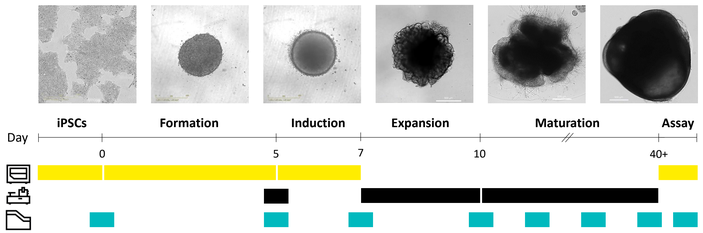
Introduction

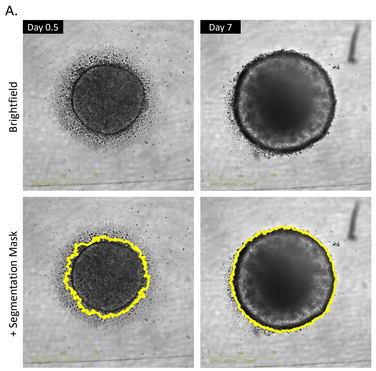
- hPSC-derived brain organoids are physiologically relevant 3D *in vitro* models that mimic human brain development and cellular organization.
- They are increasingly employed to study neurodevelopment, neuropathology, and drug screening but face challenges due to long protocols, sensitivity, large size, heterogeneity, and limited temporal methods for objective monitoring.
- This workflow incorporates live-cell analysis and high-throughput screening (HTS) by cytometry to monitor cerebral organoid differentiation.
- The data exemplifies how this approach enables objective assessment of cerebral organoid differentiation by temporally monitoring growth, morphology, and marker expression, to support data-driven QC decisions and neurodevelopmental or toxicity studies.

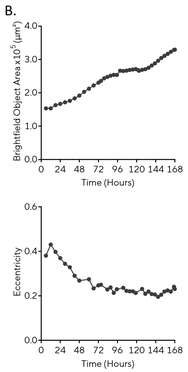
iPSC-derived Cerebral Organoid Differentiation Workflow



- Schematic shown for cerebral organoid generation from human iPSCs using a commercial kit (STEMdiff™ Cerebral Organoid Kit). The four-stage unguided protocol begins with an intermediate aggregation formation stage, followed by neuroepithelia expansion 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® Live-Cell Analysis System, yellow boxes) or discrete live-cell analysis (CellCelector Flex, black boxes) and HTS by cytometry to assess relevant markers (iQue® HTS Platform, teal boxes).

Continuous Live-Cell Analysis of Organoid Formation and Induction



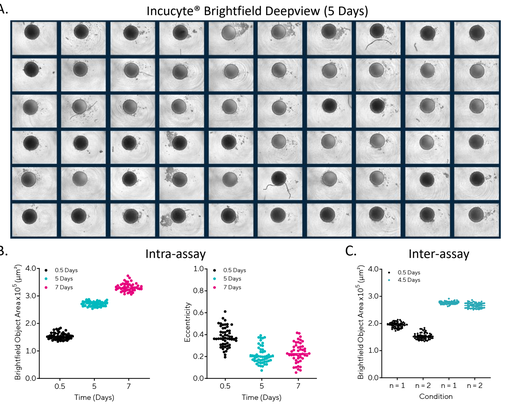


- Organoid formation and induction were performed in 96-well round-bottom ULA plates and images were acquired using the Incucyte® spheroid scan type every 6 hours over 7 days.

A. Representative timelapse brightfield images show rapid aggregation of iPSCs into compact organoids with the expected morphology. Organoids were symmetrical and exhibited smooth round edges with rosette-like patterning by day 7. Integrated analysis software accurately segmented the organoids (yellow outline).

B. Quantification revealed a temporal increase in organoid area (top graph) and an initial decrease in eccentricity (bottom graph) with aggregation that then remained relatively stable.

High Assay Robustness for Organoid Formation and Induction



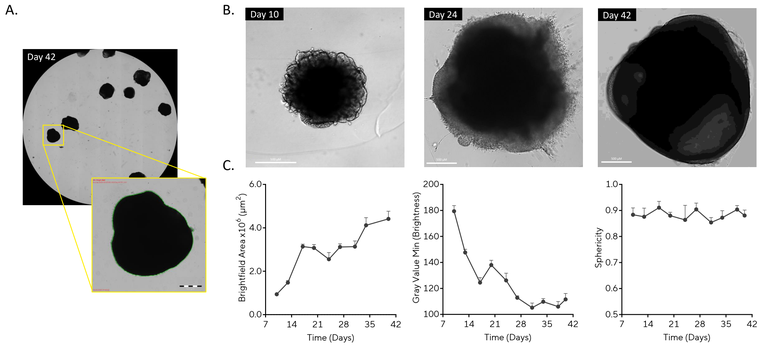
- As homogeneity is crucial in initial stages to maximize differentiation efficiency, we examined the reproducibility of organoids throughout formation and induction.

A. Brightfield deepview is shown for a 96-well plate (60 organoids in inner wells) at 5 Days and allows for the rapid assessment of morphology with organoids at this stage exhibiting a robust and uniform phenotype.

B. Comparison of individual organoids over time showed an increase in area with high intra-plate robustness. For eccentricity, we see a decrease over time with high intra-plate robustness, albeit with a slightly greater spread due to the segmentation of some debris around the organoid edges.

C. Comparison of brightfield area across experiments showed good levels of inter-assay robustness at 0.5 days with consistency increasing by 4.5 days (14.9 and 2.6 % CV, respectively).

Quantification of Organoid Maturation using Discrete Live-Cell Analysis



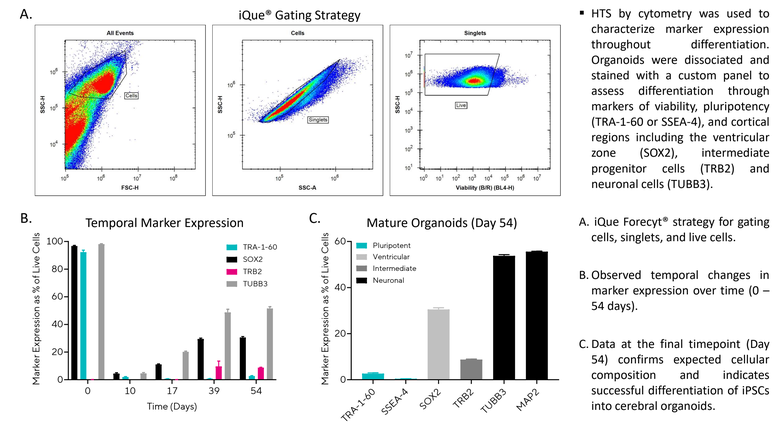
- In the next two stages, organoid neuroepithelia were expanded in Matrigel® droplets in 6-well plates (8 – 12 per well) for 3 days and then matured over an additional 30 days in suspension on a shaker. Organoids were imaged at 4x every 3 – 4 days on the CellCelector Flex.

A. Brightfield images were analyzed using integrated software, which enables export of metrics for each organoid (ROI) in the field of view (FOV) and allows for exclusion of organoids from analysis (e.g., not in FOV or object blurring).

B. Representative images show stage-specific morphological features that develop temporally. On Day 10 embedded organoids show a budding morphology evenly around the surface, which indicates successful expansion into neuroepithelia. Accompanying maturation, the buds merge and then contain neural rosettes with some neural crest outgrowth (Day 21 – 24) and eventually form organoids with a dense core, cortical layering and a smooth surface (Day 40).

C. Quantification of organoids showed an increase in area over time and a decrease in brightness as a dense core forms, whilst sphericity remains constant.

Characterization of Dynamic Changes in Marker Expression



- HTS by cytometry was used to characterize marker expression throughout differentiation. Organoids were dissociated and stained with a custom panel to assess differentiation through markers of viability, pluripotency (TRA-1-60 or SSEA-4), and cortical regions including the ventricular zone (SOX2), intermediate progenitor cells (TRB2) and neuronal cells (TUBB3).

A. iQue Forecyt® strategy for gating cells, singlets, and live cells.

B. Observed temporal changes in marker expression over time (0 – 54 days).

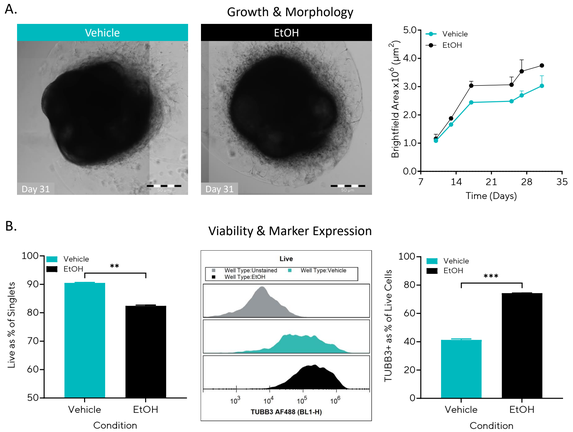
C. Data at the final timepoint (Day 54) confirms expected cellular composition and indicates successful differentiation of iPSCs into cerebral organoids.

Assessment of Ethanol Exposure During Brain Organoid Development

- This workflow was used to model prenatal alcohol exposure (PAE), which is linked to neurodevelopmental issues, including decreased cell viability, impaired neural differentiation and modified brain architecture.
- From Day 10, organoids were exposed to low levels of ethanol (EtOH, 50 mM) and monitored every 3 – 4 days using live-cell analysis. Viability and marker expression were assessed using HTS by cytometry on Day 31 (21 days post-exposure).

A. Quantification of brightfield images revealed similar temporal profiles for growth and morphology, with EtOH organoids being slightly larger.

B. Single-cell data revealed a significant decrease in cell viability ($p < 0.005$) and reduction in the neuronal marker TUBB3 ($D; p < 0.001$) for EtOH exposure compared to vehicle.



Data presented as mean + SEM, 3 – 60 replicates. Customers are solely responsible for obtaining all necessary licenses and approvals required in connection with the use or application of any products or services.