

OPTIMIZING
**Cellular Imaging
and Analysis**

FEATURED ARTICLES:

**Automated, Robust Brain
Organoid Generation**

**Imaging and Analysis
of 3D Cell Models**

**High-Throughput, Kinetic
Cytotoxicity Profiling**

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Advances in Cellular Imaging and Analysis

Cell behavior can now be studied in physiologically relevant environments.

Cellular imaging and analysis have evolved significantly over the past decade, driven by the growing need to study cell behavior in physiologically relevant environments. While traditional two-dimensional (2D) culture systems remain an important foundation for biomedical research, their limitations are increasingly clear. Cells in monolayer cultures fail to capture the complexity of tissue architecture, signaling gradients, and microenvironmental heterogeneity found in living organisms. In response, researchers have turned to three-dimensional (3D) models such as organoids and spheroids, which better reproduce *in vivo* conditions and enable deeper insights into disease, development, and therapeutic responses.

Advancing from 2D to 3D models

The shift from 2D to 3D cell culture reflects a re-evaluation of how experimental systems model biological reality. While 2D cultures offer simplicity and low cost, they lack the cellular organization and gradient conditions seen in tissues. In contrast, 3D structures—composed of multiple cell types arranged in physiologically relevant

architectures—allow for nutrient and oxygen gradients that influence cellular metabolism, gene expression, and response to stimuli.

Imaging for organoid and spheroid analysis

As the complexity of cell models increases, so does the need for precise imaging and analysis. High-content imaging systems allow researchers to assess viability, morphology, and growth patterns without disrupting the culture. In spheroid assays, imaging can reveal differences in proliferation and death rates within core versus peripheral regions. For organoids, imaging enables longitudinal monitoring to capture changes as they develop and mature.

Sophisticated software algorithms are increasingly integrated into imaging workflows, enabling automated segmentation, quantification of viability markers, and assessment of structural integrity. The result is a richer dataset that captures spatial heterogeneity within the 3D structures. Such imaging approaches provide a way to correlate cellular

responses with microenvironmental conditions, improving the interpretability and reproducibility of experimental results.

Automation and quality control in 3D culture

Automation in cellular imaging and culture addresses two critical needs: scalability and reproducibility. Automated culture systems can track individual organoids from initial seeding to maturity, ensuring consistent feeding, environmental control, and mechanical agitation. By incorporating image-based assessments at key points in the growth cycle, these platforms facilitate early intervention when growth or morphology deviates from expected patterns.

Standardized automated workflows, such as the Molecular Devices solutions discussed in [Chapter 2](#), typically include steps such as embryoid body formation in optimized plate formats, transfer to larger wells for maturation, and continuous media exchange. Integration with external imaging systems expands analytical capacity, allowing for high-content confocal microscopy, volumetric analysis, and assessment of neuronal activity in brain organoids. Combining imaging data with metadata from culture systems generates comprehensive records that support quality assurance.

Imaging for dynamic analysis

While endpoint imaging provides valuable structural information, live-cell imaging introduces the ability to observe dynamic processes in real time. This is particularly relevant for drug discovery, where treatment effects may not be adequately captured by static snapshots. Apoptosis, cytotoxicity, and proliferation can all occur over varying

timescales, influenced by dose, cell type, and microenvironment. By continuously tracking cells in both 2D monolayers and 3D spheroids, live-cell imaging preserves the temporal dimension of cellular responses.

Time-lapse microscopy combined with advanced image analysis allows for detailed trajectory tracking of individual cells or cell clusters. In spheroids, live-cell imaging can reveal how apoptosis propagates from nutrient-depleted cores outward, or how treatment-induced changes alter spheroid integrity over time. In organoids, dynamic imaging can highlight the emergence of specialized structures, synapse formation in neuronal models, or shifts in metabolic activity under experimental conditions.

Applications

Integration of advanced imaging with quantitative analysis enhances the suitability of both 2D and 3D systems for drug screening. Measuring viability, growth rates, and morphological changes over time provides a multi-parameter dataset that can improve hit validation, identify off-target effects, and support mechanistic investigations. In cancer research, spheroid models can be exposed to gradients of drug concentration, enabling spatially resolved efficacy measurements. In neuroscience, brain organoids can be analyzed for changes in neuronal activity patterns following candidate compound treatment.

Mechanistic studies benefit from combining imaging data with other molecular assays. Imaging can identify which regions within an organoid respond to treatment, guiding subsequent targeted sampling for transcriptomics, proteomics, or metabolomics. Such integration of multi-modal data helps build a more complete picture of cell behavior and drug action.



Chapter 3 describes how organoid viability assays and spheroid growth assays can be imaged and analyzed using the CELENA® X High Content Imaging system from Logos Biosystems, while **Chapter 4** covers several applications of the Celloger® Pro in apoptosis and cytotoxicity assays.

Ensuring rigor and reproducibility

The expansion of 3D cell culture and advanced imaging creates new demands for rigor in experimental design. Variability in culture conditions, imaging parameters, or data processing can undermine reproducibility. Automated systems help mitigate these issues by controlling

environmental factors, standardizing workflows, and applying consistent image acquisition settings. Complemented by well-defined protocols and validated analysis algorithms, these approaches strengthen the reliability of conclusions drawn from complex cellular models.

The future of cellular imaging is likely to involve further integration of automation, artificial intelligence-driven image interpretation, and multi-modal data acquisition. As 3D models continue to diversify—from vascularized organoids to microphysiological chips—the ability to visualize and quantify events in real time will remain a critical capability for understanding biology and advancing therapeutic development.

The CellXpress.ai Automated Cell Culture System for Automated, Robust Brain Organoid Generation

New workflow helps overcome reproducibility and scalability challenges.

Felix Spira, Ph.D., and Sandra Grund-Gröschke, Ph.D.

Introduction

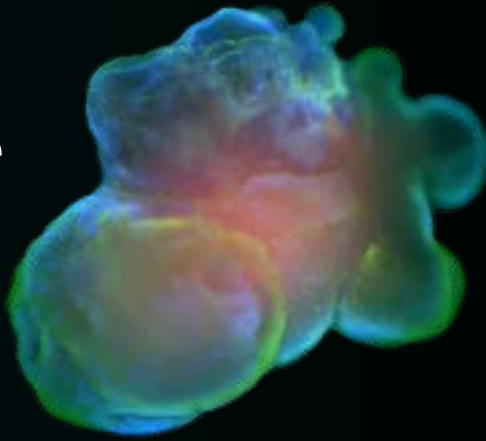
Neurological diseases such as Alzheimer's and Parkinson's remain a major global health challenge. Despite significant investment in drug discovery and development pipelines, progress has been hindered by several bottlenecks. One fundamental challenge was the lack of reliable *in vitro* and *in vivo* models to guide compound screening and lead optimization accurately. Traditional workflows with cell lines and animal tests often do not recapitulate the complexity of the human brain and its disorders. This changed with the development of the first brain organoid model in 2014 by Lancaster et al.¹ Brain organoids are complex 3D miniaturized models of parts of the human brain that are derived

Benefits

- **Enhanced consistency.** Feeding organoids automatically means they're cared for on a set schedule, even on weekends and holidays. That consistency not only reduces human error but also leads to more reliable downstream assays.
- **Decreased cross-contamination risk.** No mix-up of media or mishandling of plates. Automated handling keeps every step standardized and secure.
- **Decreased contamination risk.** Built-in H₂O₂ decontamination system for the incubator and Hepafiltered positive pressure inside the liquid handling cabinet to prevent contamination of samples.
- **Effective time management.** Increased hands-off time enables scientists to focus on more critical tasks while accelerating their research and development timelines.
- **Imaging becomes reliable and repeatable.** Automated imaging systems take care of the routine image acquisition, and deep learning-based analysis enables rigorous quality control and monitoring.
- **Integration with external instruments.** Compatibility with image acquisition and analysis tools engenders end-to-end iPSC culture automation to advance neurodegenerative disease modelling.

Rethinking Neuroscience Research

The new reality
in brain organoid
automation



The CellXpress.ai® Automated Cell Culture System is redefining what's possible in neuroscience labs. Purpose-built for complex models like iPSC-derived neurons and cerebral organoids, the system automates every step—from seeding and feeding to monitoring and passaging—to **reduce hands-on time by up to 90%**.

Its integrated rocking incubator delivers continuous, gentle media agitation to maintain consistent nutrient distribution and support optimal organoid health. Meanwhile, AI-powered image analysis enables real-time decision-making to keep your cultures on track—even when you're away from the bench.

The result? Cell models you can count on, insights you can act on, and time to focus on discoveries that move neuroscience forward.



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from human induced pluripotent stem cells (iPSCs). Unlike traditional 2D cultures, brain organoids contain multiple cell types that interact with each other and recapitulate the architecture and structure of real tissue. This complexity makes them superior models to study brain development and diseases.

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.²

Despite this progress, the translation of brain organoids into tools for drug development continues to face challenges because of poor reproducibility and complex, labor-intensive protocols that hinder scalability. Although differentiation protocols from induced pluripotent stem cells (iPSCs)

into brain organoids vary across model systems, they typically require media exchange over several months, together with continuous monitoring. This lengthy cultivation process contributes to high variability between wells and plates, which remains a major limitation.

The CellXpress.ai[®] Automated Cell Culture System is designed to overcome these challenges. The system combines a liquid handler, imager, and incubator into a unified platform, allowing for seeding, feeding, passage, and monitoring both 2D and 3D cell cultures (Figures 1 and 2).

Variability can be reduced by optimizing the liquid handling steps of CellXpress.ai, thereby reducing contamination risk and minimizing organoid loss or damage during media aspiration and dispensing.

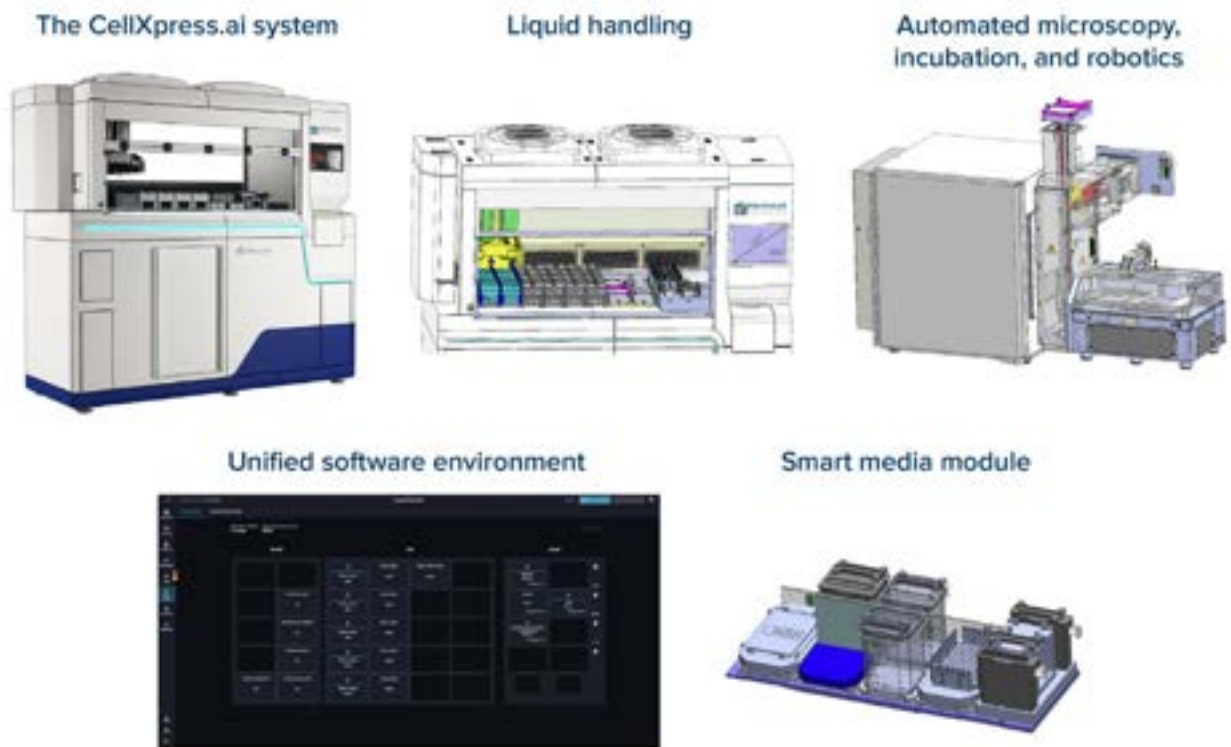


Figure 1. The CellXpress.ai system's built-in hardware and software.

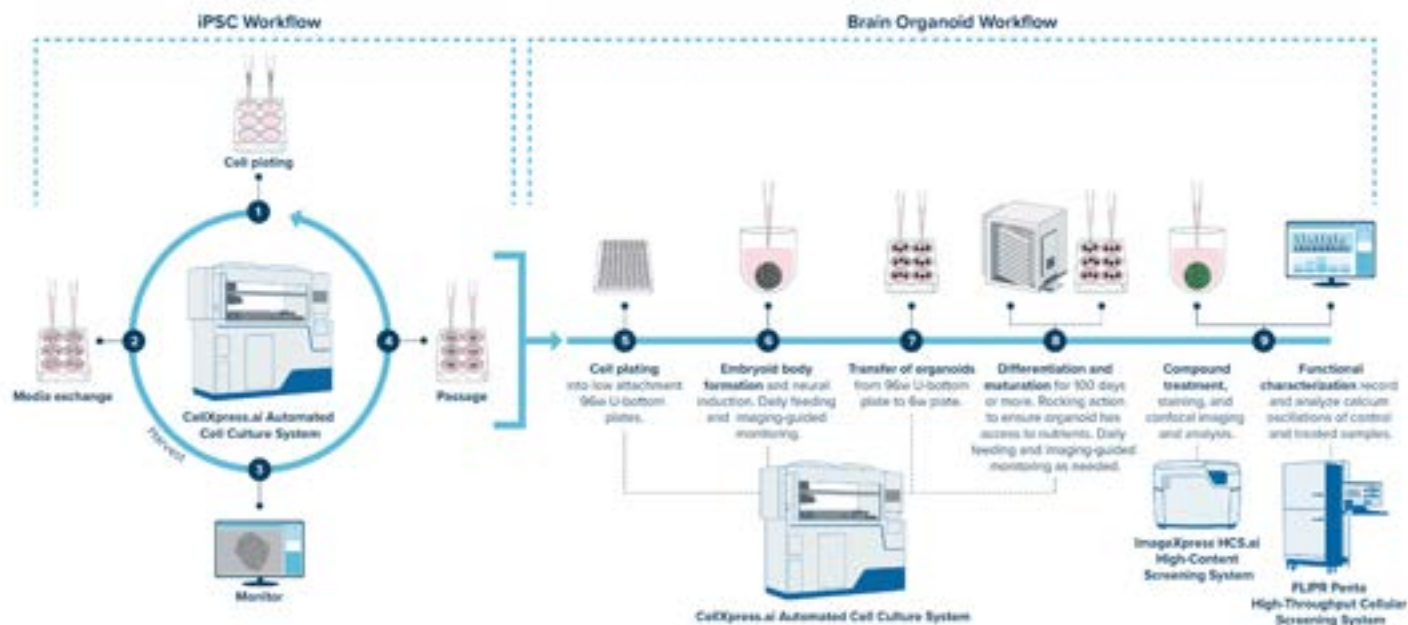


Figure 2. The CellXpress.ai system supports cultivation of both human iPSCs and brain organoids.

The “Smart Media Module” of the system significantly enhances hands-off time by allowing on-deck reagent storage for multiple days. In addition, those media modules will pre-heat the media in advance to ensure the media is at the allocated temperature once feeding starts and is kept at this temperature until all plates are processed. Media is automatically cooled down after the feeding event for on-deck media storage (Figure 1). In addition to media containers, reservoir plates can also be stored on deck.

The CellXpress.ai Automated Cell Culture System can integrate a rocking incubator to enable the cultivation of free-floating organoids, such as brain organoids, by providing continuous media agitation during the entire maturation process. This rocking incubator can hold up to six racks with a mix-and-match configuration that allows both static and rocking conditions. Thereby, stem cells can be cultivated in the same incubator as the

brain organoids. This feature is key to providing an end-to-end workflow for the entire process of brain organoid generation. Furthermore, the CellXpress.ai system allows constant monitoring of the culture via its built-in imager and utilizes machine learning-assisted image analysis via the incorporation of the IN Carta® Image Analysis Software.

In this application note, we describe the automated brain organoid generation workflow using the CellXpress.ai system, starting from iPSCs up to differentiation and maturation of complex brain organoids (Figure 2). For stem cell cultivation, single-cell or fragment passaging methods were established. For differentiation of stem cells into brain organoids, the starting material is critical. Stem cells should exhibit healthy, non-differentiated colonies. To control the stem cell quality, we deployed the IN Carta image analysis software, which utilizes advanced artificial intelligence and enables colony segmentation to distinguish between healthy and

differentiated colonies. We implement a workflow to generate brain organoids using a rocker instead of the classically used orbital shaker. Organoid quality was assessed via functional assays and whole-mount staining. We also demonstrated the seamless integration of external devices, such as the ImageXpress® Confocal HT.ai High Content Imaging System, for advanced monitoring by utilizing the CellXpress.ai system's back port.

Methods

iPSCs cultivation

The human induced pluripotent stem cell (iPSC) line WTC-11 (Gm25256, Coriell Institute for Medical Research) was used to generate brain organoids. Fragment passaging was performed according to the protocol described in the application note [Automation of iPSC culture, passaging, and expansion with the CellXpress.ai Automated Cell Culture System](#). For the generation of brain organoids, iPSCs were single-passaged using TrypLE and seeded into 96-well U-bottom plates.

Cerebral organoids

For the generation of cerebral organoids, the StemCell Technologies kit (Catalog #08570) was used as described in the protocol. For maturation, plates were either placed on an orbital shaker or a rocker for comparison.

Forebrain organoids

Forebrain organoids were generated in a 96-well U-bottom plate (Greiner, Kremsmünster, DE). On day 12, organoids were either transferred into 6w ultra-low attachment plates (Greiner, Kremsmünster, DE) or further cultivated in 96-well U-bottom plates. For maturation, plates were either placed on an orbital shaker or a rocker for comparison.

Midbrain organoids

Midbrain generation was performed according to Renner et al. (2021).³

Image analysis

The IN Carta image analysis software, which utilized a machine learning-based protocol, was used to segment undifferentiated and differentiated iPSC colonies. Based on image analysis, decision-making rules were set in the protocol to enable both automated iPSC culture passaging and to exclude wells where differentiation was present.

Cell characterization

For quality control of iPSC colonies, those were fixed with 4% paraformaldehyde and stained for pluripotency markers.

Functional activity of brain organoids was measured by assessing the calcium flux using the FLIPR® Calcium 6 Assay Kit (Molecular Devices) according to the manufacturer's protocol.

For quality control of organoids, whole-mount staining was performed according to the protocol published by Renner et al.⁴ In short, organoids were first cleared by using benzyl alcohol and benzyl benzoate. After successful clearing, the organoids were stained. High-content imaging was performed using the ImageXpress HT.ai system.

Results

Automated stem cell cultivation

For the generation of brain organoids, the quality of the starting material is critical, meaning that high-quality stem cells are required to generate high-quality brain organoids. By using the CellXpress.ai system, we were able to maintain

human iPSC cultures for several passages. Here, consistent growth of stem cell colonies could be observed by continuous increase in cell numbers and confluency (Figure 3A). For cultivation of stem cells on the CellXpress.ai system, single-cell and fragment passing protocols were established (Figure 3B). Here, passaging could be automatically triggered by decision-making when confluence reaches a certain threshold.

The compatibility of the CellXpress.ai system with the IN Carta image analysis software facilitated the implementation of deep learning for colony segmentation to discriminate between healthy (Figure 3C) and differentiated cells (Figure 3D). Using those

established protocols, we were able to show constant growth of human iPSCs, which we verified over several plates (Figure 3E).

iPSCs differentiation into brain organoids

The CellXpress.ai system software possesses several protocol phases, which can be combined as needed to set up a protocol. These phases include feeding, imaging, seeding, and passaging. By using the various features of the CellXpress.ai system, we were able to establish a brain organoid generation workflow. This protocol includes seeding of iPSC into 96-well U-bottom plates and regular feeding and image acquisition cycles of 96-well and 6-well plates (Figure 4A). Thus, the CellXpress.

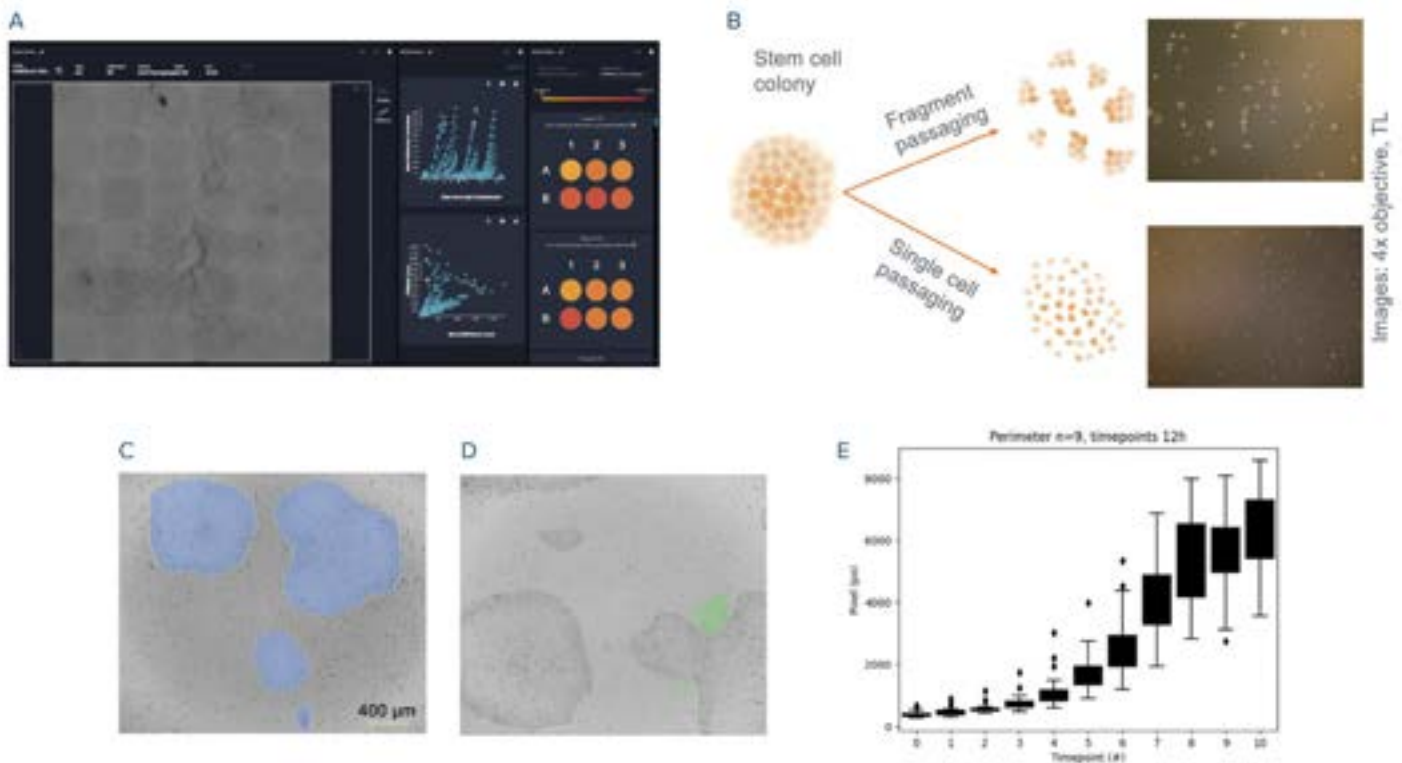


Figure 3. Automated stem cell culture. (A) The CellXpress.ai system user software showing stem cell cultivation workflow. (B) Different passaging methods built into the CellXpress.ai system. (C, D) Deep learning for colony segmentation to discriminate between healthy colonies (C) and differentiated cells (D). (E) Quantification of colony growth monitored over 6 days. Boxplots show the average of 9 plates.

ai system increases walkaway time by performing error-prone and repetitive tasks during organoid development with unparalleled precision. The built-in CellXpress.ai system imager allows fast and full-well acquisition of 96-well and 6-well plates. Thereby, every organoid can be monitored in high resolution, providing important details such as budding of organoids (Figure 4B–C).

For the successful maturation of brain organoids, it is critical to keep the organoids in motion, because these organoids are incredibly hungry and need a constant flow of nutrients and oxygen. Although standard protocols involve an orbital shaker, their automation remains a challenge. However, there

are automated solutions that involve a rocker. Here, we aimed to test whether organoids grown on a rocker are comparable to those grown on a shaker (Figure 4D). We were able to successfully grow cerebral organoids on a rocker and those showed characteristic features such as budding of the organoid surface around day 10 (Figure 4E). We compared the area of organoids grown on a shaker and rocker after 48 days of culture and observed no difference (Figure 4E).

High-resolution image acquisition of brain organoids

Combining the automation capabilities of the CellXpress.ai system with the imaging prowess of

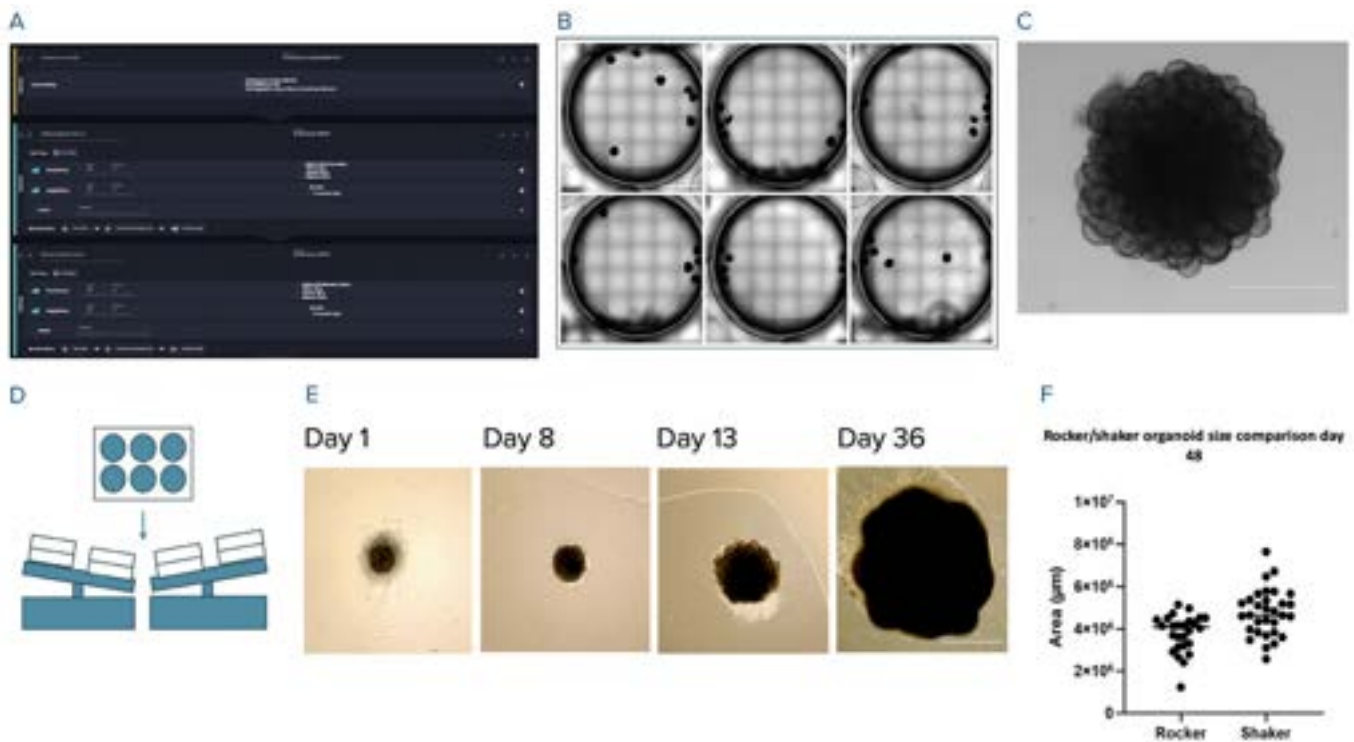


Figure 4. Differentiation of human iPSC into brain organoids. (A) Image showing protocol setup in the CellXpress.ai system user software (B) Representative images of stitched full-well acquisition of the 6-well plate. (C) Enlarged organoid of C. (D) Cartoon of the cultivation of brain organoids on a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

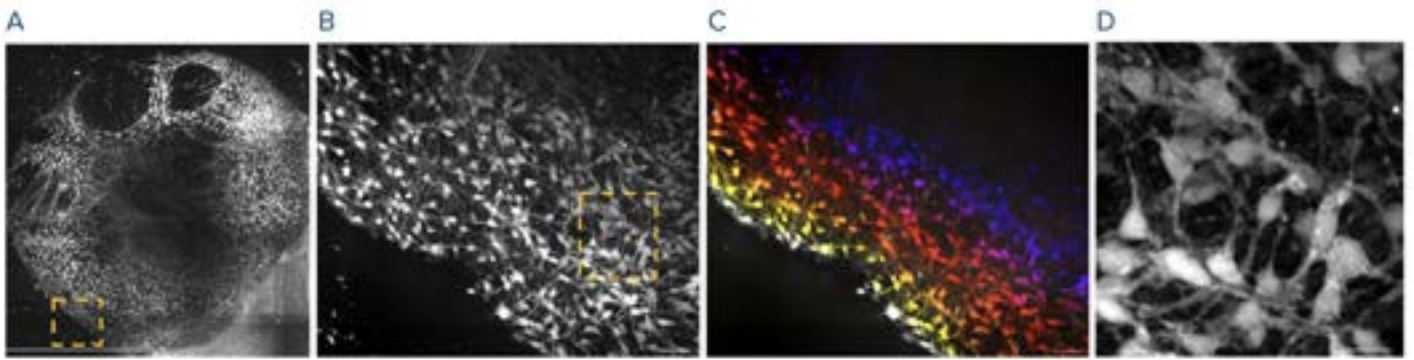


Figure 5. High-resolution image acquisition. Exemplary images of brain organoids. (A) Stitched max projection of high-resolution image, 6x6 tiling, 200 z-planes – 2 μ m distance between planes. (B) Zoom into the region indicated in A. (C) Spatial colored projection of region indicated in A to show cortical layers. (D) Zoom into the region indicated in B. Scale bar: A: 1mm, B–D: 20 μ m; A–D: Objective 20x water immersion on the ImageXpress HT.ai system.

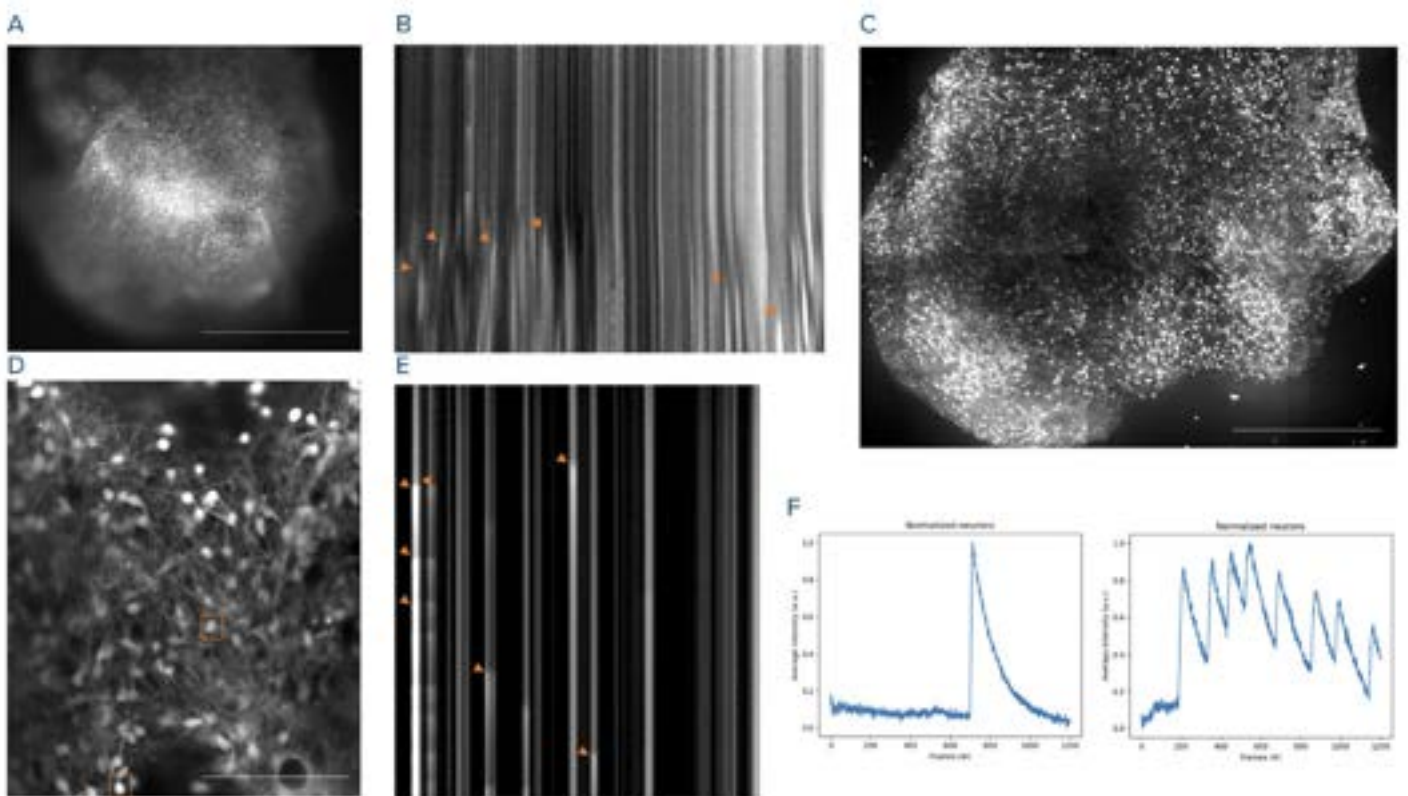


Figure 6. Image acquisition and analysis. (A) Stream acquisition of a calcium-stained brain organoid. (B) Kymograph of A indicating calcium activity. (C) Max projection of high-resolution Z-stack of organoid A. (D) High-resolution stream acquisition of neuronal network of organoid in C. (E) Kymograph of neuronal network of D. (F) Single neuronal traces of D, neurons are indicated by orange boxes. A, B: 4x objective build in the CellXpress.ai system's automated microscope, 70 ms framerate. C–E: The ImageXpress HT.ai system 20x water immersion. D–F: Step-size 2 μ m, frame rate 20 ms. Scale bar A, C = 1 mm, D = 10 μ m

ImageXpress HT.ai system facilitated the acquisition of high-resolution images of brain organoids. The ImageXpress HT.ai system maintains high resolution and image quality in different levels of magnification, enabling both a holistic view of the organoid and a detailed view of regions, with cortical layers clearly visible (Figure 5A-D). The multiple modes of imaging collectively substantiate the viability and functionality of the neurons in the brain organoids.

Analysis of cerebral organoids

Functional activity of the cerebral organoids was determined using the Calcium 6 Assay kit to track changes in intracellular calcium concentration over

time, which reflects neuronal activity. To measure neuronal activity, the stream acquisition function of the ImageXpress HT.ai system was used, which revealed the generation of functionally active brain organoids. (Figures 6A–F)

Midbrain organoid generation on the CellXpress.ai system

Using the CellXpress.ai system, we established an automated midbrain generation workflow (Figure 7A). Growth of midbrains was tracked over time displays using the system's integrated cell journey feature (Figure 7B).

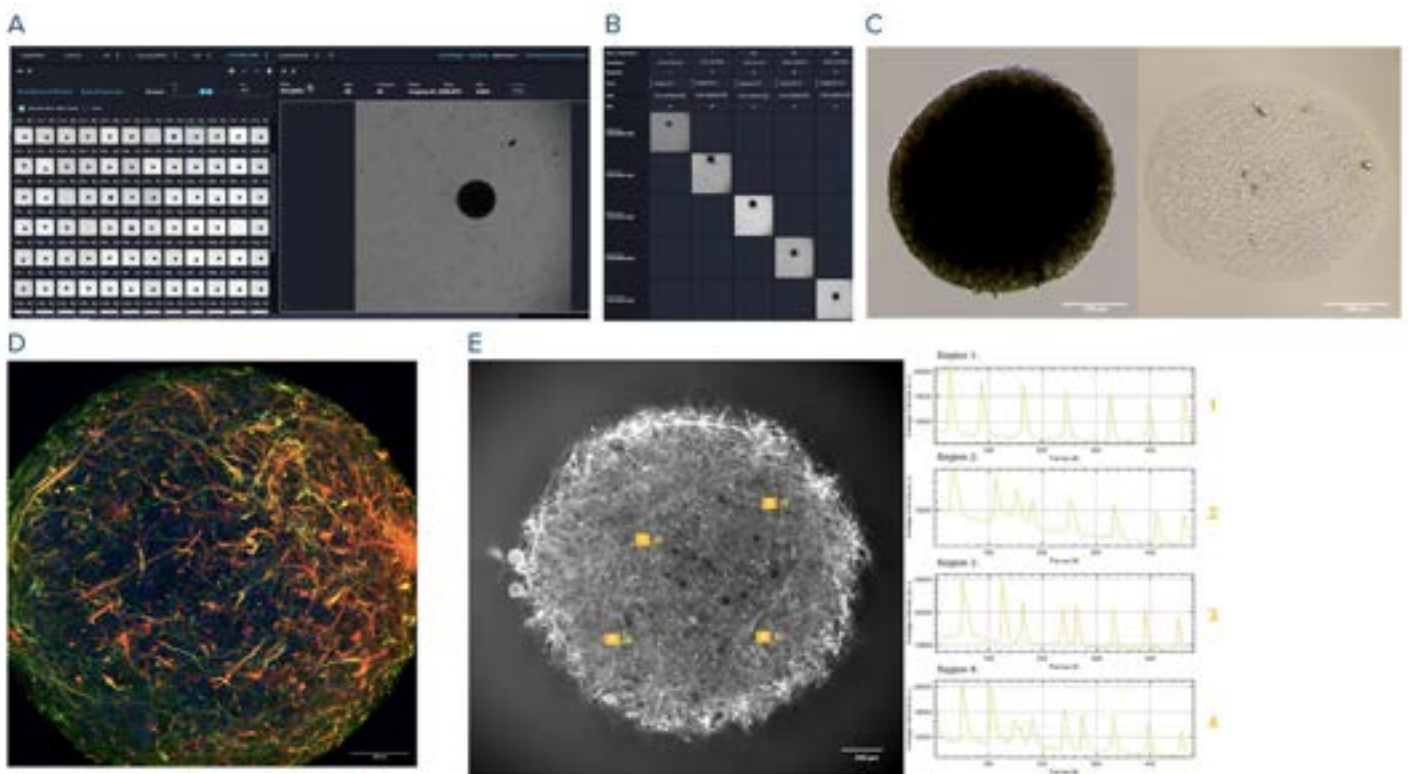


Figure 7. Midbrain generation on the CellXpress.ai system. (A) User software showing midbrain organoids cultivated on the CellXpress.ai system. (B) Cell journey to follow individual organoids over time. (C) Pre and post-clearing images. (D) Maximum projection of a whole-mount stain of a cleared midbrain organoid. (E) Stream acquisition of a calcium-stained midbrain, including single neuron traces for four regions (1–4). A, B: The CellXpress.ai system's built-in microscope, 4x air objective. C: Evos XL 10x air objective, D, E: The ImageXpress HT.ai system 20x water immersion objective.

For quality control of midbrain organoids, we performed whole-mount staining and calcium imaging. For whole-mount staining of midbrain organoids, we performed optical clearing for enhanced light penetration (Figure 7C). After successful clearing, the organoids were stained with fluorescence antibodies, and imaging was performed using the high-content ImageXpress HT.ai system. Whole-mount staining with immunofluorescence markers yields high-quality images of midbrain organoids, clearly depicting morphological and phenotypical features (Figure 7D). In addition, calcium-stained midbrain organoids were monitored, and single neurons selected from four different regions of the organoid were traced for neuronal activity, demonstrating functionality throughout the midbrain organoid (Figure 7E).

Phenotypic classification of brain organoids

Understanding organoid phenotype is critical to differentiate between “good” and “bad” organoids.

Hereby, excluding those organoids that show unwanted phenotypes from further processing, such as feeding and imaging, will in turn reduce waste of expensive media and time. The phenoglyphs tool of the IN Carta software allows phenotypic classification of brain organoids (Figure 8A).

While classification can be performed manually, the IN Carta software deep learning algorithm can be implemented to accelerate the process. The confusion matrix in Figure 8B reveals a consistent match between manually determined and automatically annotated phenotypic labels, highlighting the utility of the IN Carta software in automated brain organoid expansion workflows.

Conclusion

This application note demonstrates that brain organoids generated using the CellXpress.ai Automated Cell Culture System are comparable in size and neuronal activity to those produced manually. The system successfully supported the

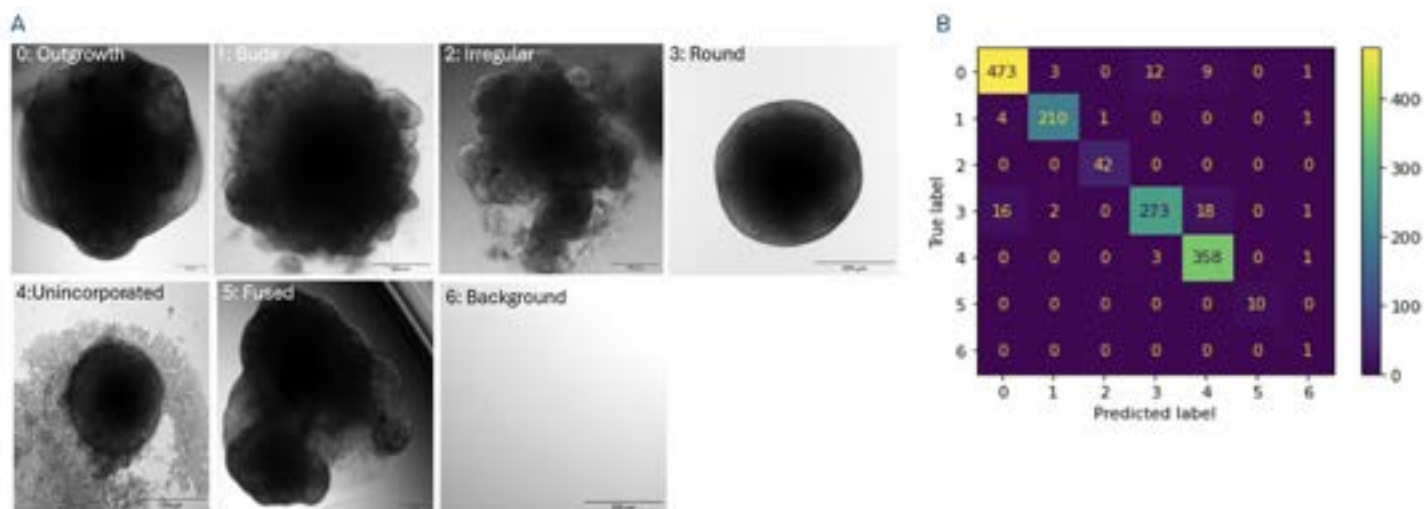


Figure 8. (A) Representative images of classes used for organoid classification. (B) Confusion matrix between automatically-annotated dataset and the ground truth dataset. Objective 4x air; Scale bars: 200 μ m

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 production, from healthy stem cells to mature, functional brain organoids, within a single automated platform.

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Additional Resource

CellXpress.ai Automated Cell Culture System

Imaging and Analysis of 3D Cell Models Using the CELENA[®] X High Content Imaging System

Study demonstrates that platform provides a reproducible and quantitative method to study 3D cell models.

Key features

- High quality imaging and analysis of 3D cell models using the CELENA[®] X High Content Imaging System
- Optimized pipelines for analyzing organoid and spheroid samples

Introduction

Two-dimensional (2D) cell culture systems have been the standard for a wide variety of biological research because they are relatively inexpensive and easy to study. However, a growing body of evidence suggests that three-dimensional (3D) cell models can provide a more accurate representation of real cell environments than 2D cell culture systems.

Organoids, for example, are organ-specific 3D cell models derived from human stem cells. They are designed to mimic the functionality and structure of human organs revealing the complex nature of

that types of tissue. Moreover, 3D spheroids can represent a gradient of nutrients and oxygen between cells located in both outer and inner layers which is more relevant to physiological environments.

Thanks to these advantages, 3D models are notably useful for studying various types of cancers. Here, we describe how organoid viability assays and spheroid growth assays can be imaged and analyzed using the CELENA[®] X High Content Imaging System and the CELENA[®] X Cell Analyzer software.

Materials and Methods

Patient-derived colorectal cancer (CRC) organoid samples were obtained from Seoul Asan Medical Center. Organoid samples were treated with DMSO and different concentrations of Cisplatin (10, 20, and 40 μM). They were stained with Calcein AM (Life Technologies, C3099) and Ethidium Homodimer-1 (EthD-1; Life Technologies, E1169). Spheroid samples were prepared by plating HeLa cells on a 96-well, round bottom plate with densities of 0.5, 1, 5 $\times 10^3$ cells/well. Prepared samples were imaged over 14 days (Day 1, 4, 6, 7, 8, 11, 13, and 14).

Acquired images were analyzed using CELENA® X Cell Analyzer software.

Both organoid and spheroid samples were imaged using a 4X LWD high NA objective. An EGFP filter cube (Ex470/30, Em530/50), and a RFP filter cube (EX530/40, Em605/55) were used for multi-channel imaging.

Organoid viability assay

The organoid viability assay is a useful tool for anticancer drug screening, but requires a different strategy of imaging and analysis compared to 2D cell culture. For example, organoids have multiple focal planes making it difficult to acquire in-focused images for multiple organoids. For live/dead cell viability of the single organoid, a different analysis strategy is required since individual cells in an organoid do not exist as a single live/dead status. To address this issue, we took an advantage focus merge software module of the CELENA® X High

Content Imaging System after acquiring Z-stack images from multi-channel fluorescence.

Cisplatin treated CRC organoid samples were imaged on 5 different focal planes. Images were then processed using the 'MergeFocus' module to merge 5 different focal planes (Figure 1).

Merged images were then analyzed to evaluate viability of organoids. The strategy was to compare Calcein AM and EthD-1 signals from organoids. Calcein AM produces intense green fluorescent signal by esterase activities in live cells, while EthD-1 is a dead cell-specific red fluorescent dye. The viability of organoids was measured by comparing green and red fluorescent signals. Images obtained from GFP and RFP channels were overlaid using the 'ImageMathOverlay' module. Overlaid images were used to identify all objects expressing fluorescent signals with the 'IdentifyPrimaryObjects'. Intensity and size of objects were quantified using the 'MeasureObjectIntensity' and 'MeasureObjectSizeShape' modules (Table 1).

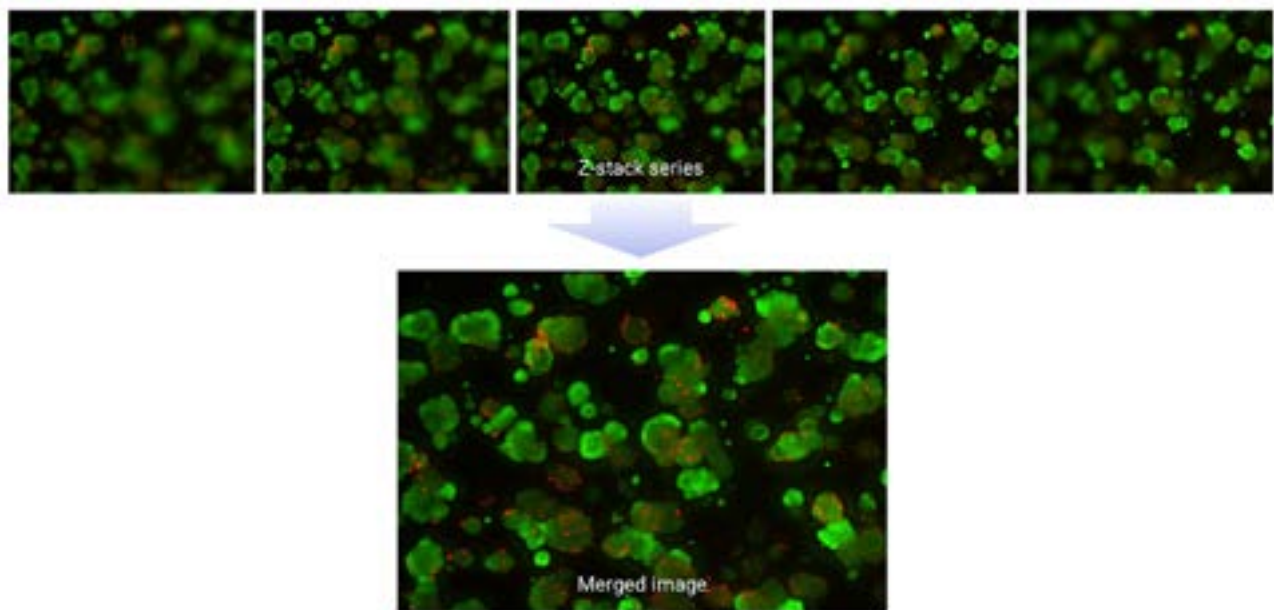


Figure 1. Images taken on 5 different focal planes were processed to create a merged image.



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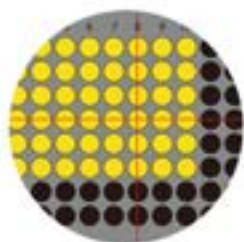


Image acquisition

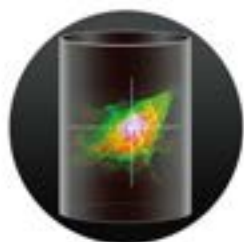
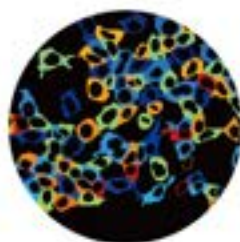


Image analysis



Data analysis

Table 1. Description of the pipeline used to assess cell cycle with CELENA® X Cell Analyzer		
Order	Module	Use
1	ImageMathOverlay	Overlays images obtained from GFP and RFP channels
2	IdentifyPrimaryObjects	Identifies objects on the overlaid image
3	MeasureObjectIntensity	Measures intensity of identified objects
4	MeasureImageAreaOccupied	Measures area of identified objects
5	GrayToColor	Converts gray images to color images

Color composite images showing both green and red signals were made with the 'GrayToColor' module (Figure 2B). The analyzed data showed Calcein AM signal decreases while EthD-1 signal increases with the higher Cisplatin concentration (Figure 2, A and B).

Spheroid growth assay

Spheroid growth according to the seeding density was assessed by measuring the area, major axis

Table 2. Description of the pipeline used to assess spheroid growth with CELENA® X Cell Analyzer		
Order	Module	Use
1	EnhanceOrSuppressFeatures	Suppresses the intensity of certain pixels
2	Invert	Inverts bright and dark areas
3	IdentifyPrimaryObjects	Identifies spheroid samples
4	MeasureObjectSizeShape	Measures size of identified objects
5	OverlayOutlines	Places outlines around identified objects

length, and perimeter of spheroid samples. The 'EnhanceOrSuppressFeatures' module was used to distinguish spheroid samples from the background. Bright and dark areas were inverted using the 'Invert' module. Spheroids were then identified using the 'IdentifyPrimaryObjects' module. The 'MeasureObjectSizeShape' module measured the size of identified objects selecting options including Area, MajorAxisLength, and Perimeter. Brightfield Images presenting the outlines around spheroids were produced using the 'OverlayOutlines' module (Figure 3A).

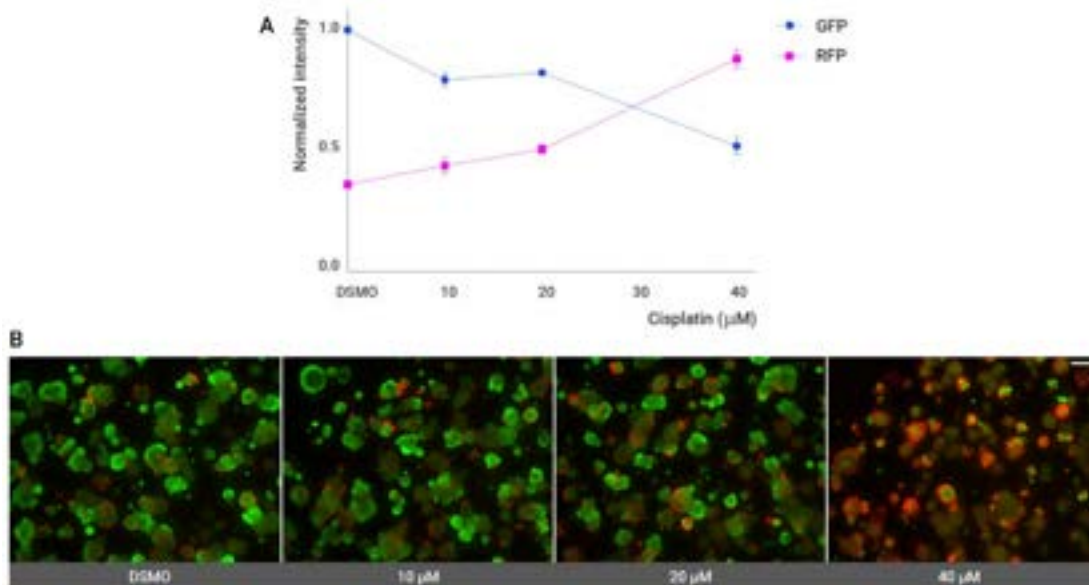


Figure 2. (A) The graph showing normalized intensity of GFP and RFP. (B) Images acquired using CELENA® X system showing change in green and red fluorescence (scale bar: 200 µm).

The growth and morphology of HeLa cells were imaged for 14 days using the CELENA® X High Content Imaging System (Figure 3A). These images were used to measure the Area, MajorAxisLength, and Perimeter of spheroids. The growth curves showed that sizes vary according to different seeding densities of spheroids (Figure 3B).

Conclusion

In this study, we demonstrated how to analyze 3D cell models using CELENA® X High Content

Imaging System. High content imaging combined with analysis for organoid viability and spheroid growth provided a reproducible and quantitative method to study 3D cell models. Furthermore, the 'MergeFocus' module was found to be a highly effective tool for analyzing Z-stack images of 3D cell models. In conclusion, the CELENA® X High Content Imaging System combined with the CELENA® X Cell Analyzer Software would be advantageous for 3D model imaging and analysis.

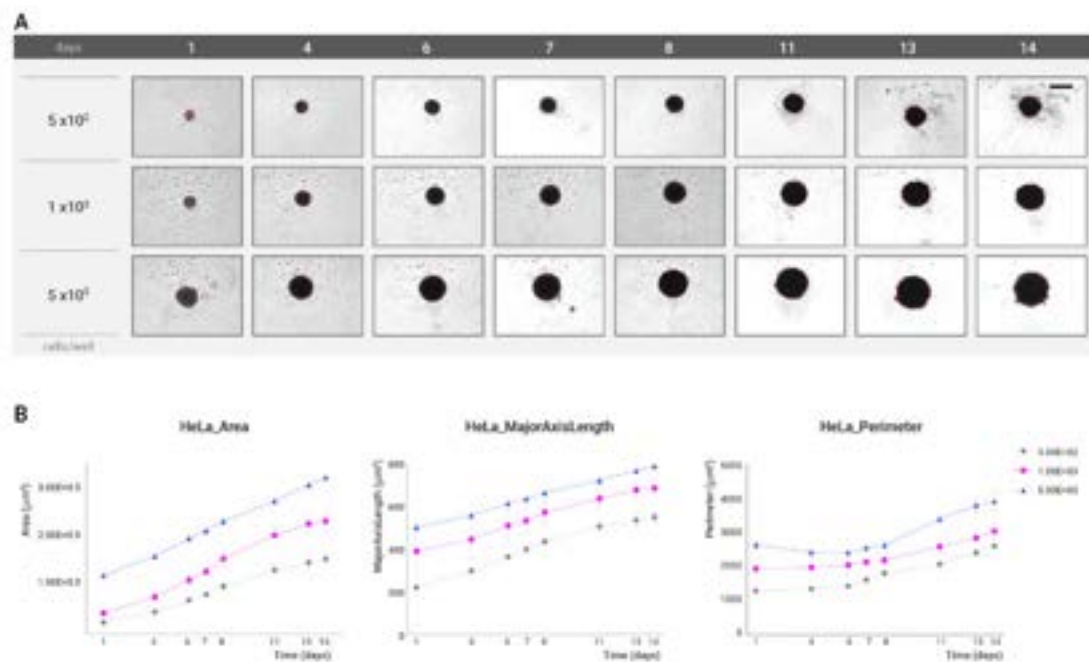


Figure 3. (A) The montage showing the growth of spheroids over time with different seeding densities (Scale bar: 500 μm). (B) The graphs showing the Area, MajorAxisLength, and Perimeter of spheroids. Spheroids have bigger sizes according to the seeding density.

Additional Resource

CELENA® X High Content Imaging System

High-Throughput, Kinetic Cytotoxicity Profiling with Celloger[®] Pro

Live-cell imaging for drug screening and quantification in 2D and 3D models

Introduction

Cell-based assays have become an essential approach in modern drug discovery and biomedical research. Among various techniques, live-cell imaging offers a unique advantage: it enables monitoring of cellular processes over time, in contrast to conventional static endpoint measurements that may overlook critical temporal changes in cell fate.^{1,2} This capability provides researchers with richer information and more comprehensive data interpretation, particularly when assessing drug-induced apoptosis and cellular viability.^{3,4,5}

The Celloger[®] Pro, a live-cell imaging system developed for both 2D monolayer cultures and 3D spheroid models, integrates automated time-lapse microscopy with advanced image analysis. This allows for continuous tracking of individual cells or complex tissue-like structures under controlled incubation conditions. In this chapter, several applications of the Celloger[®] Pro in apoptosis and cytotoxicity assays are introduced. Each section illustrates how the system can reveal cellular

dynamics that are otherwise difficult to capture with traditional assays, while also highlighting the platform's versatility for drug screening and mechanistic studies.

Section 1: Quantitative Viability Analysis and IC50 Determination

Continuous imaging of the same sample across multiple time points eliminates the need for parallel endpoint assays, reducing reagent consumption and labor costs. We used staurosporine, a protein kinase inhibitor,⁶ to assess the drug-induced cytotoxicity in H2B:GFP-labeled U-2OS osteosarcoma cells. Cells were exposed to a gradient of staurosporine concentrations, and the loss of membrane integrity was monitored using ethidium homodimer-1 (EthD-1, 4 μ M) staining. This approach enabled visualization of morphological degradation and membrane permeabilization in a dose- and time-dependent manner (Figure 1).

Using the system's auto-counting capability, cell viability was quantified as the proportion of EthD-1-negative cells relative to the initial (0 h)

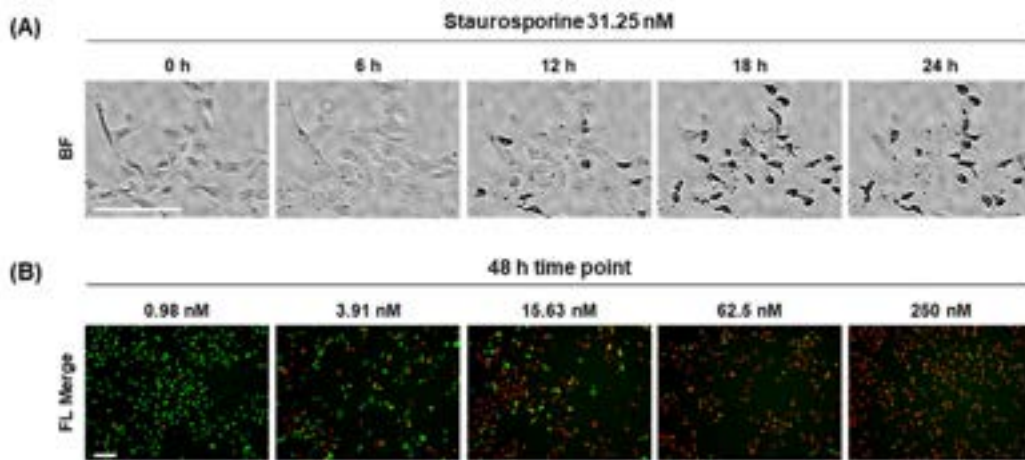


Figure 1. Representative imaging of staurosporine-induced cytotoxicity in U-2OS cells using 4X magnification lens. Scale bar, 200 μm . (A) Bright-field cropped images of cells treated with 31.25 nM staurosporine and imaged at 0~24 h on the Celloger[®] Pro, showing morphological degradation. (B) Two-color fluorescence images at 48 h after exposure to increasing concentrations (0.98~250 nM).

GFP-positive cell count. The heatmap (Figure 2A) illustrates a progressive decline in viability over time with increasing drug concentration. Dose-response curves at multiple time points (Figure 2B) reveal that the inhibitory effect became stronger with prolonged exposure, as indicated by the leftward shift of the curves and the lower half-maximal inhibitory concentration (IC₅₀) values⁷ at later time points. Time-resolved regression analysis (Figure 2C) confirmed this trend, showing that IC₅₀ declined and converged to 2.945 nM by 48 h, consistent with an accumulative drug effect.

Together, the live-cell imaging approach enhances experimental efficiency and reproducibility, while providing reliable kinetic cytotoxicity profiles that strengthen the accuracy of IC₅₀ determination for large-scale screening.

Section 2: Time-Resolved Imaging of Apoptosis with Multiplexed Dyes

Apoptosis, or programmed cell death, is a central mechanism exploited in cancer therapy, as many

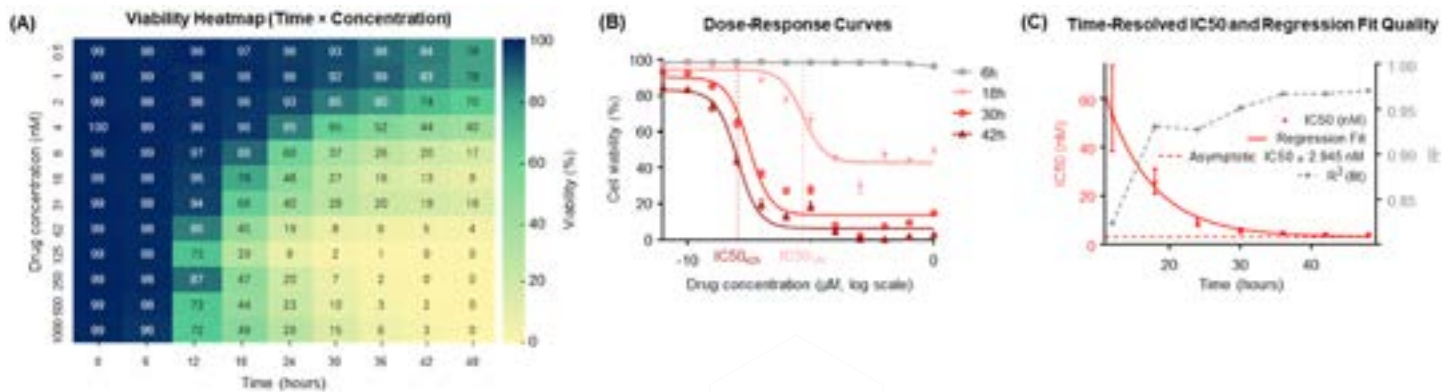


Figure 2. Quantitative viability and IC₅₀ kinetics of staurosporine-treated U-2OS cells. (A) Viability heatmap across drug concentration (y-axis) and imaging time (x-axis). Each tile shows viability (%) quantified by automated analysis on the Celloger[®] Pro. Color scale, 0~100% viability. (B) Nonlinear regression curves and IC₅₀ calculated at each indicated timepoint. Two spotted lines compare IC₅₀ values at 18, 42 h. (C) Time-resolved IC₅₀ values estimated at 12~48 h from nonlinear regression of concentration-response curves. Red markers show IC₅₀ with 95% confidence intervals (left y-axis). Gray markers/line show corresponding regression R² (right y-axis).

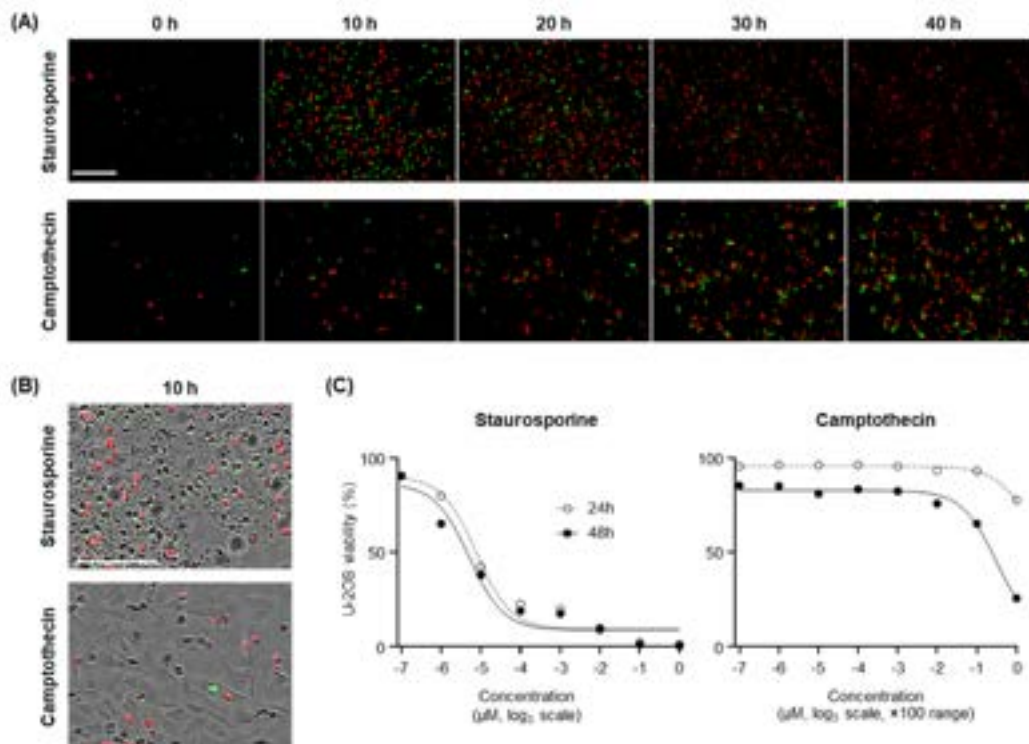
anti-cancer drugs are designed to trigger apoptosis in tumor cells.⁸ Traditionally, detection relies on endpoint assays such as western blotting or flow cytometry, which cannot capture the onset, progression, or heterogeneity of apoptotic responses. To resolve these temporal dynamics, we employed a dual-dye strategy combining a caspase activation probe (CellEvent™ Caspase-3/7 Green, 2 μM) with a cell death marker (EthD-1). U-2OS cells were treated with either staurosporine (1.37 nM ~ 1 μM) or camptothecin, a topoisomerase I inhibitor that induces DNA damage (137 nM ~ 100 μM).

The apoptotic profiles of the two drugs were clearly distinct. Staurosporine triggered a rapid increase in caspase activity, with maximal green fluorescence observed between 10 h and 20 h.

By contrast, camptothecin induced a gradual and sustained increase up to 40 h, consistent with its DNA damage–dependent and cell cycle–specific mode of action (Figure 3). Brightfield/fluorescence overlays at 10 h further revealed pronounced shrinkage and morphological changes in staurosporine-treated cells.

Quantitative viability analysis across drug concentrations highlighted this divergence: staurosporine caused a sharp viability loss even at low concentrations, whereas camptothecin maintained relatively high viability up to 33 μM, with significant reductions appearing only after 48 hours. A modest downward shift in regression curves was also evident at 48 h for both drugs, likely reflecting cumulative toxicity.

Figure 3. Comparative apoptosis dynamics induced by staurosporine and camptothecin in U-2OS cells. (A) Time-lapse two-color fluorescence images of cells treated with staurosporine (top panel, 0.11 μM) or camptothecin (bottom panel, 11.1 μM), acquired at 0–40 h. CellEvent™ Caspase-3/7 (2 μM) is shown in green; EthD-1 (4 μM) in red. Scale bars, 200 μm. (B) Representative bright-field/fluorescence overlays at 10 h for staurosporine (top) and camptothecin (bottom). (C) Dose–response curves of cell viability (%) measured at 24 h (open symbols) and 48 h (filled symbols). Left: staurosporine; right: camptothecin. X-axis: log₃-transformed drug concentration. Data points represent means, and curves show nonlinear regression fits.



Section 3: Drug Response Assessment in 3D Spheroids

While two-dimensional (2D) cultures provide useful insights, they do not fully replicate the physiological complexity of tissues. Three-dimensional (3D) spheroid cultures more closely mimic *in vivo* tumors by establishing gradients of oxygen, nutrients, and drug penetration, which strongly influence therapeutic responses. As such, 3D models represent a valuable platform for translational research.

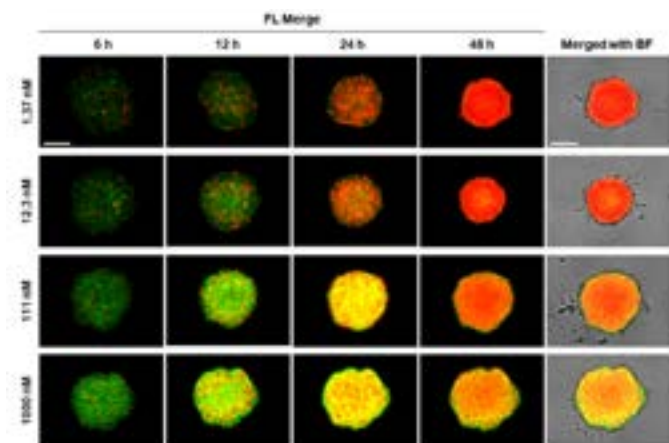
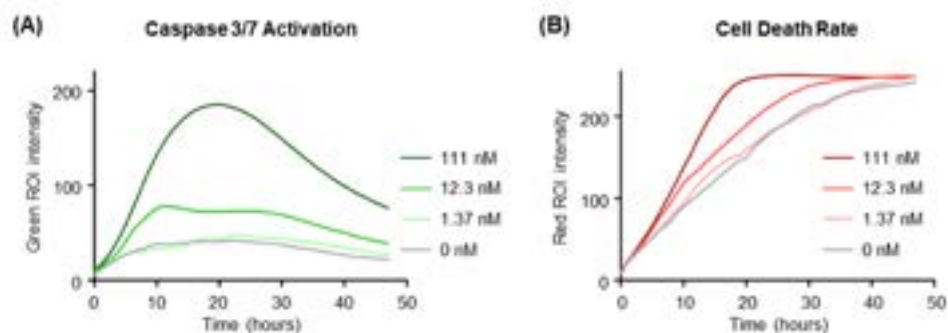


Figure 4. Apoptosis induction in U-2OS spheroids. Fluorescence images of spheroids treated with 1.37~1000 nM staurosporine and acquired at 6~48 h using the Celloger® Pro. Merged images show CellEvent™ Caspase-3/7 (green) and EthD-1 (red). Scale bar, 200 μ m.

Figure 5. Quantitative analysis of fluorescence intensity in U-2OS spheroids. Spheroids were treated with staurosporine at each dose. A circular ROI was defined around each spheroid at 0 h and applied across all time points using Celloger® Pro Analysis software. (A) Mean ROI intensity in the green channel (Caspase-3/7). (B) Mean ROI intensity in the red channel (EthD-1).



To investigate apoptosis dynamics in this context, U-2OS spheroids were generated and treated with varying concentrations of staurosporine. Unlike monolayer cultures, spheroid analysis required a region-based intensity approach, as single-cell resolution was not feasible. Circular regions of interest (ROIs) were applied to each spheroid to quantify mean fluorescence intensity for apoptotic (green) and dead (red) cell markers.

Apoptotic onset occurred at a similar time point across concentrations (~6 h), with higher doses accelerating caspase activation (Figure 4). At the highest concentration (1000 nM), cell death began at 12 h, producing strong signals in both green and red channels that appeared yellow when merged. At lower concentrations, green fluorescence was much weaker, indicating limited or transient caspase activation.

Regardless of dose, green signal intensity reached its maximum around 20 hours and then declined (Figure 5), consistent with staurosporine-induced transient caspase activation also observed in 2D cultures. In contrast, red fluorescence steadily increased and plateaued by 30 h, with a faster rise observed under high-dose treatment. It reflects progressive membrane disruption even when apoptotic signaling was less evident.

These results highlight the value of time-resolved monitoring in 3D models. Continuous live-cell

imaging reveals where and when it occurs within a complex structure—an insight critical for preclinical evaluation of compounds targeting solid tumors.

Conclusion

Celloger® Pro not only serves as a powerful imaging tool but also as an integrated analytical platform. Its ability to capture and quantify dynamic cell death processes provides a more comprehensive understanding of drug action, surpassing the limitations of conventional endpoint assays.

Compounds that appear similarly effective in endpoint assays may, in fact, differ substantially in the kinetics, extent, and consistency of their cytotoxic effects. By continuously tracking cell fate, the Celloger® Pro reveals kinetic features such as the timing of caspase activation and the spatial progression of apoptosis within 3D spheroids for high-throughput screening.

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Additional Resource

Celloger® Pro

Celloger[®] Pro

Seamless Imaging, Limitless Insights



Key Applications of Celloger[®]

Cell function



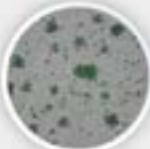
Sublocalization



Adipogenesis



Transfection efficiency



NK cell killing assay



Actin dynamics



Mitochondrial membrane potential



Phagocytosis

Cell movement & morphology



Cell monitoring



Wound healing



Spheroid formation



Neurite outgrowth



Zebrafish observation

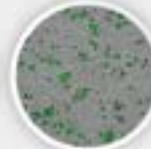
Cell viability



Spheroid cytotoxicity



Apoptosis



Cytotoxicity

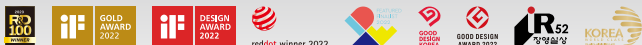
Real-time cell monitoring
inside incubator

Multi-point imaging with fully motorized camera

Equipped with **dual fluorescence** (green & red) and bright field

User **interchangeable** objective lens

List of Awards (Celloger[®] Series)



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