High-Content Analysis Solution

Accelerating Organoid Research: High-Throughput Imaging and Analysis of Human Intestinal Organoids (HIOs)

CellVoyager CV8000 High-Content Analysis System

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Researchers at Baylor College of Medicine developed a fast, scalable workflow that pairs 2D Human Intestinal Organoid (HIO) monolayers with high-throughput imaging on the Yokogawa CellVoyager CV8000 and automated analysis tools, enabling unbiased phenotyping and dramatically improving throughput and reproducibility in organoid studies.

Introduction

Pharmaceutical and biotechnology organizations face increasing pressure to accelerate drug discovery while meeting sustainability and ethical mandates. As the industry decreases reliance on animal models, in vitro systems such as Human Intestinal Organoids (HIOs) have become central to disease modeling, microbiome studies, and therapeutic screening.

Despite their value, 3-dimensional HIOs present substantial imaging and analysis challenges—particularly for high-volume workflows. Conventional confocal microscopy is slow, labor-intensive, and biased toward manually selected fields of view, while flow cytometry sacrifices 3D structure and spatial context. These constraints limit throughput, reproducibility, and scalability.

To address these challenges, researchers at Baylor College of Medicine developed a rapid, high-throughput pipeline for 2-dimensional imaging, maintaining spatial context while allowing easy quantitative analysis of HIO phenotypes. The workflow combines automated acquisition with scalable image analysis to enable unbiased, reproducible characterization across large HIO cohorts. Imaging was performed on the CellVoyager CV8000 High-Content Screening (HCS) System, a dual microlensed spinning-disk confocal platform that enables high-speed volumetric imaging while maintaining sensitivity and minimizing photobleaching—critical for 2D heterotypic cellular models such as HIOs. Images were automatically reconstructed and analyzed using the Yokogawa CellPathfinder and open-source CellProfiler analysis software.

Fig. 1 CellVoyager CV8000 High Content Imaging system

Instrument and Application Overview

Two-dimensional HIOs effectively mimic the multicellular architecture and single layer of cells comprising the intestinal epithelium, enabling physiologically relevant evaluation of epithelial dynamics, host–microbe interactions, and drug responses. However, imaging and analyzing samples using traditional methods presents challenges:

Manual confocal microscopy is very time consuming, subjective and can miss subtle cell morphologies when selecting a single field of view compared to full well imaging.

Flow cytometry dissociates tissue, eliminating spatial context, limiting longitudinal studies and is not conducive to analysis of multiple samples for high-throughput analyses.

Automated 3D quantification with traditional microscopy methods is challenging due to variable morphology, heterogeneous 3D structure, and very large datasets of multi-plane image stacks.

To meet screening-scale demands, researchers require a platform capable of objective acquisition, high-speed volumetric imaging, whole-well or representative coverage, and reproducible quantification.

Instrument	Application	Key Benefit
Yokogawa CellVoyager CV8000	High-content screening of 2D HIO monolayers	Rapid, high-resolution confocal imaging with automated sample indexing, focusing, and environmental control option
Yokogawa CellPathfinder	Automated image correction, stitching and projection	Seamless integration with CV8000 acquisition
CellProfiler	Quantification and normalization	Powerful, open-source analysis software

Experimental Workflow

The Baylor researchers developed a standardized pipeline to enable rapid, unbiased quantification of HIO monolayers in a 96-well format.

1. Sample Preparation

- Donor-derived HIO monolayers are seeded on collagen-coated 96-well plates.
- Treatments include microbial supernatants, compounds, or biologics.
- EdU incorporation and antibody staining (e.g., chromogranin A) provide proliferation and cell-identity readouts.

2. Automated High-Content Imaging (CV8000)

- Whole-well datasets acquired using spinning-disk confocal optics at low magnification for complete coverage.
- Multi-laser excitation (e.g., 405/488 nm) and simultaneous camera acquisition support rapid dual-channel imaging.
- A typical well (nine fields, short z-stack, two fluorophores) is completed in **~1.5 minutes**.
- Built-in corrections include dark-frame subtraction, flat-field correction, geometric correction, spectral unmixing, and channel alignment.

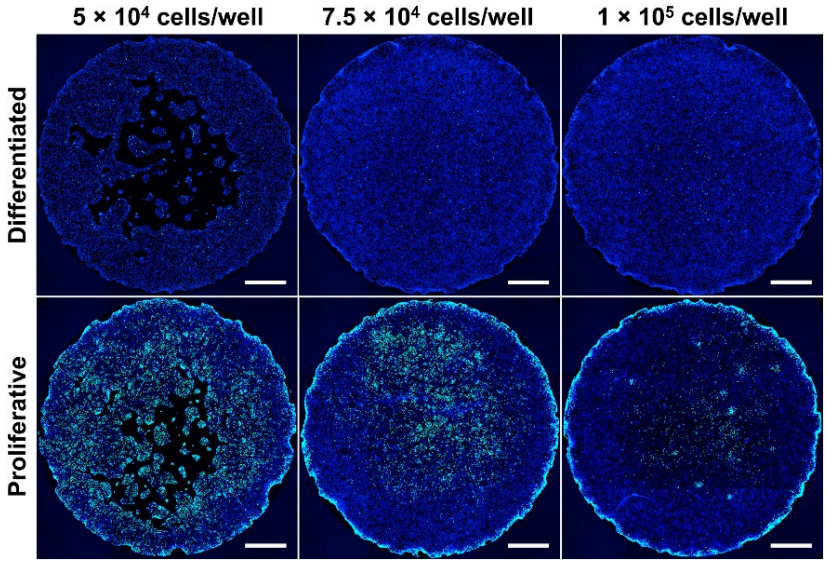

Fig. 2 Effects of monolayer plating density on cell proliferation. Reconstructed whole well images of monolayers from a jejunal HIO line plated with varying cell densities and cultured for two days in differentiation medium or proliferation medium (L-WRN conditioned medium). HIOs were then pulsed with EdU for 24 hours and EdU-stained to label proliferating cells (green), then stained with DAPI (blue) to label remaining nuclei. Images were then taken at 4× magnification (scale bar = 100 µm)

3. Image Reconstruction and AI-Ready Formatting (CellPathfinder and CellProfiler)

- Nine tiles are stitched into a seamless whole-well image and a maximum-intensity projection was created using CellPathfinder.
- Central crop masks are applied to maximize area containing cells for analysis in CellProfiler.
- 16-bit TIFFs and metadata are exported for downstream analysis or open-source tools.

4. Quantification & Normalization (CellProfiler)

- Adaptive, pixel- and object-based algorithms quantify nuclear EdU or cytoplasmic marker signal.
- Fluorescence is normalized to DAPI for consistent cell density correction.

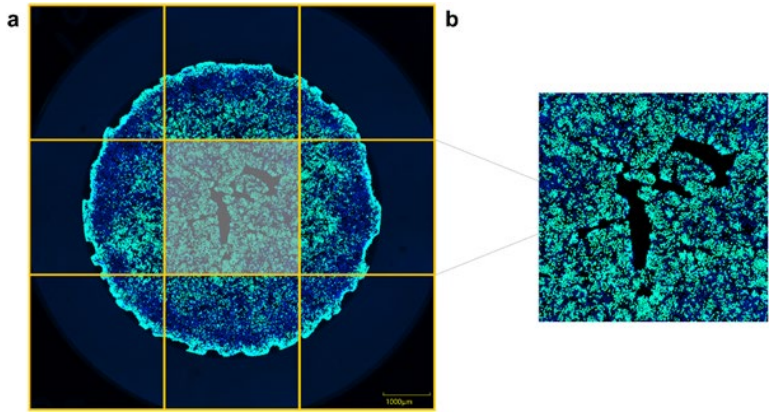

Fig. 3 CV8000 image reconstruction and postprocessing. a) The whole well of an HIO monolayer was imaged on a CV8000 confocal by obtaining nine images of the well at 4 × magnification. Images were postprocessed and partial-well images were stitched together in CellPathfinder to reconstruct the whole-well image. b) To avoid edge effects, a central crop area was obtained from each reconstructed whole-well image by using the CellProfiler crop function before quantification.

Enabling Technologies

1. High-Speed, Gentle 2D Imaging

The CV8000 system delivers fast, low-phototoxicity volumetric imaging using the CSU-W1 dual Nipkow spinning-disk module and up to four sCMOS cameras for simultaneous multi-channel capture. Optical enhancements—such as optional 25 µm pinholes, high-efficiency microlens arrays, and water-immersion objectives—support deep, high-quality imaging of heterogeneously sized cellular specimens. Full environmental control enables stable, multi-day live-cell and HIO assays.

2. Advanced AI-Driven Analysis

Yokogawa’s imaging solutions deliver high-quality datasets that integrate effortlessly with open-source tools like CellProfiler. After acquisition, Yokogawa's CellPathfinder software enables automated stitching and correction ensuring clean whole-well images ready for downstream analysis. This compatibility allows researchers to leverage CellProfiler’s powerful segmentation, object detection and quantification within flexible workflows that support batch processing, multi-well comparisons, and report generation while maintaining scalability for enterprise data lakes and AI/ML pipelines. The techniques described in this application and in the referenced paper demonstrate that the resulting image-derived quantification shows strong correlation with flow cytometry data, reinforcing the robustness and reliability of the imaging-based approach.

3. Integrated Advantages for Modern Screening

The combined CV8000 + CellProfiler solution enables high-throughput, whole-well acquisition that removes selection bias and captures subtle phenotypes. It improves operational efficiency through reproducible, automated QC workflows and reduces reagent and energy use through 96-well formats and rapid acquisition. The platform supports diverse applications from toxicity and barrier-integrity assays to long-term live-cell and HIO imaging and remains future-ready through automation and AI-accelerated analytics.

Summary & Conclusion

The Yokogawa CellVoyager CV8000 HCS System, combined with CellProfiler analytics, provides an automated and scalable workflow for high-throughput phenotyping of 2D Human Intestinal Organoids. Fast and gentle, the dual microlens spinning-disk technology, water-immersion optics, whole-well reconstruction, and deep-learning-enabled analysis collectively overcome the traditional challenges of 3D organoid screening.

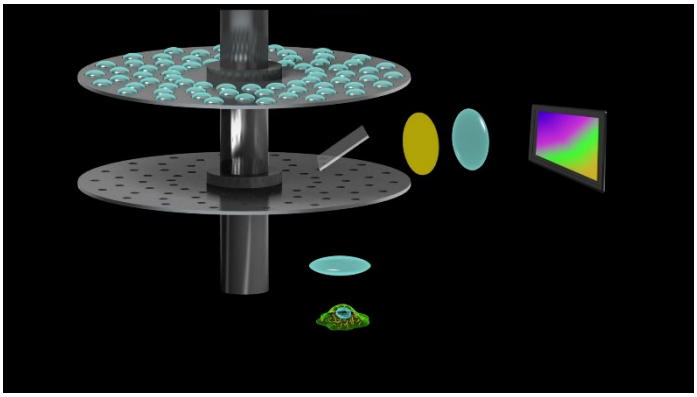

Fig. 4 Yokogawa's Dual spinning-disk with microlens confocal technology enables high-speed imaging while reducing phototoxicity and fluorescence photobleaching.

This application note reflects the strong synergy between organoid research and advanced imaging technology, made possible through the long and productive partnership between Baylor College of Medicine and Yokogawa. Yokogawa's support has been instrumental in establishing Baylor's robust high-throughput microscopy and high-content analysis resource over the past several years, laying the foundation for this introductory study and many future stories at the intersection of organoids and imaging.

Acknowledgements and references:
Research was done at Baylor College of Medicine by Faith M. Sawyer, in the laboratory of Professor Sarah E. Blutt.

For the original publication, please see:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0332418

For additional information about Yokogawa's High Content solutions, please visit:
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