Using AI-analysis tools and a next-generation high-content screening platform to improve 3D screening assay data capturing

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Introduction

Next-generation high-content imaging

Image-based high-content screening (HCS) is a potent drug discovery strategy that characterizes drug effects through the quantification of image-based features that describe cellular changes within or among cell populations. With the rising interest in 3D biological models, there is an increasing demand for an imaging platform that not only acquires high-throughput, high-quality images in 3D samples, but also enables complex image analysis. Here, we introduce the next generation in high-content imaging, the ImageXpress® HCS.ai High-Content Screening System (Molecular Devices). The system is equipped with flexible confocal spinning disk options, modular hardware, and a modern, intuitive software interface. The new imager was designed for greater precision and speed and leverages state-of-the-art optics with enhanced signal:noise ratio.

Here, we demonstrate the use of the HCS.ai system on two common 3D models. In the first application, we cultured patient-derived colorectal cancer (CRC) organoids embedded in an extracellular matrix (ECM) layer. These organoids were treated with a subset of compounds and then fluorescently labelled. A low magnification scan was done to identify the location of the organoids in the wells then we acquired only objects-of-interest with higher magnification. The images exhibit phenotypic differences between organoids treated with different type and concentration of compounds. The images were then analyzed using deep-learning segmentation to obtain multiparametric data to quantify differences and demonstrate a high Z-score. A second application shows how to obtain sharper images in the deep layer of spheroids and organoids by using the optional deep tissue spinning disk compared to standard spinning disk geometries. Overall, the HCS.ai system renders fast, high-throughput acquisition and high-quality images using an intuitive software interface. These combined improvements to acquisition speed, image quality, and machine learning-assisted analysis enable the use of more assays and models for both research and 3D drug screening.

Methods

Figure 1. ImageXpress HCS.ai High-Content Screening System

- High-speed hardware autofocus with multi-surface detection
- >5 mega pixel sCMOS camera with 95% peak quantum efficiency
- Linear encoded voice coil stages with better than 25 nm resolution
- 4-position excitation dichroic and 10-position emission filter wheel
- 6-position automated objective changer with 2X–90X magnification
- Standard spinning disk plus deep tissue spinning disk (50 µm pinhole)
- Water immersion option (20X–60X) and environmental control option

Results

Improved S:N ratio allows high-quality 3D imaging

To showcase the image quality of the HCS.ai system acquiring 3D samples, we imaged HCT116 spheroids and compare the S:N ratio across the entire Z-planes. Our results show an overall 2.6X improvement in S:N ratio and thus render more nuclei counts as compared to other imagers.

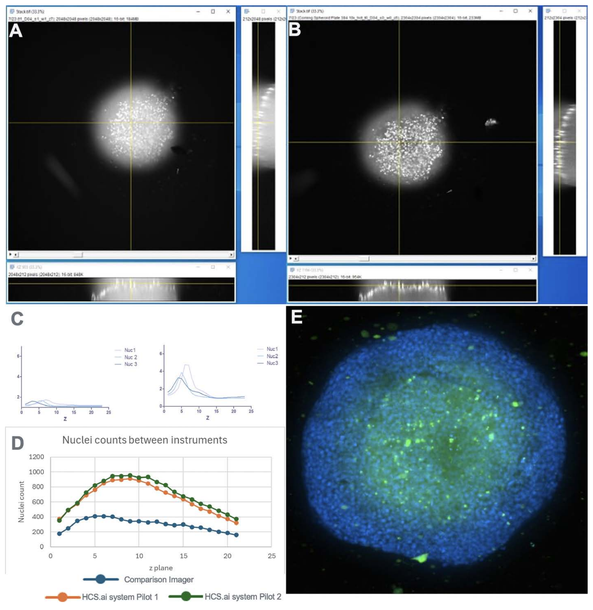
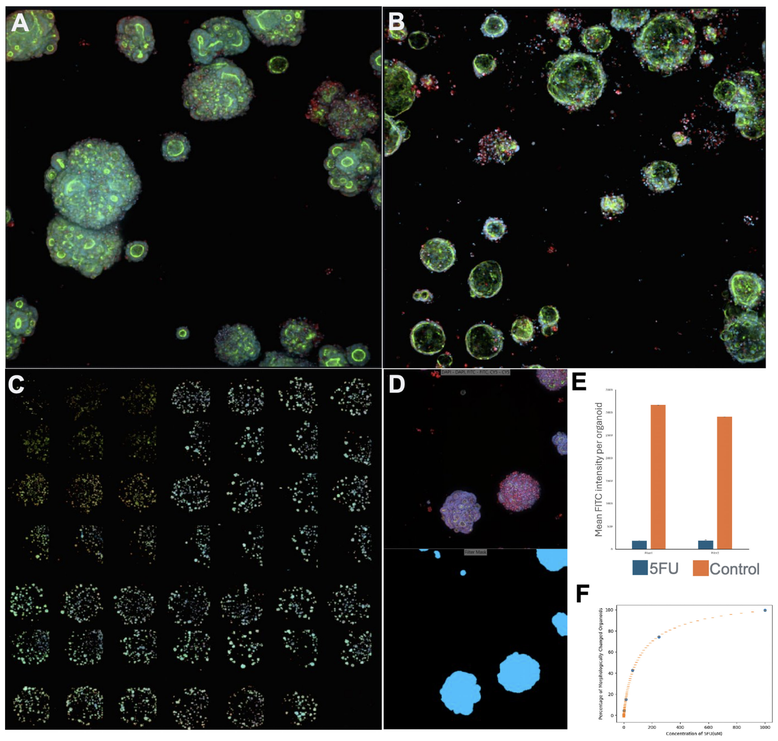


Figure 2. Spheroids were formed from HCT116 cells in U-shape ultra low attachment plate. Spheroids were stained with Caspase 3 (FITC), then fixed and stained with Hoechst (nuclei) and imaged on the HCS.ai system using the 10x objective and 60 µm pinhole spinning disk confocal. Z-stacking with 6 µm step size was acquired. A. Representative spheroids (single plane) shown with their corresponding orthogonal sections, acquired with comparison imager; B. acquired with the HCS.ai; C. S:N ratio across the entire Z-planes from 3 selected nuclei for the comparison imager and the HCS.ai; D. Nuclei counts comparison between the HCS.ai system and other imagers. E. Representative image showing DAPI staining; G. Representative image showing DAPI and Caspase 3 staining.

Better instrument, better 3D assay results

To evaluate instrument performance in 3D samples embedded in ECM such as Matrigel, we imaged the CRC organoids seeded in 96W iBidi plate. The average intensity of FITC channel for each organoid was then measured and used to calculate the z' score. An overall high z' score larger than 0.7 was achieved. Further classification analysis shows the increasing of 5FU concentration induces more morphologically changed organoids.



Results

Figure 3. CRC organoids were seeded in 96W iBidi polymer-bottom plate, treated with a small panels of compounds (5 days), and fixed and stained with Phalloidin (FITC), Hoechst (DAPI), and Ethidium homodimer (TRITC). A. Representative images of the untreated control organoids; B. Representative image of the organoids treated with 5FU; C. The overview of the plate; D. The segmentation masks of the organoids were generated with custom module editor (CME) embedded in IN Carta® Image Analysis Software and features associated with each organoid were extracted; E. Graph of the mean FITC intensity from 2 pilots; F. Further analysis shows the increasing trend of morphologically changed organoids through the increasing of 5FU concentration.

New QuickID workflow ensures targeted high-resolution images

The embedded QuickID Targeted Acquisition workflow has the ability for user to run workflow independently, image biological object at higher magnification objective following identification using low magnification objective with on-the-fly image analysis. The two special plates, i.e., the 384W IN Sphero plate and 96W iBidi plate with grown organoids were used to showcase the ability for targeted, high-resolution images. Note that objects from high-mag objective are in the center of the ROI.

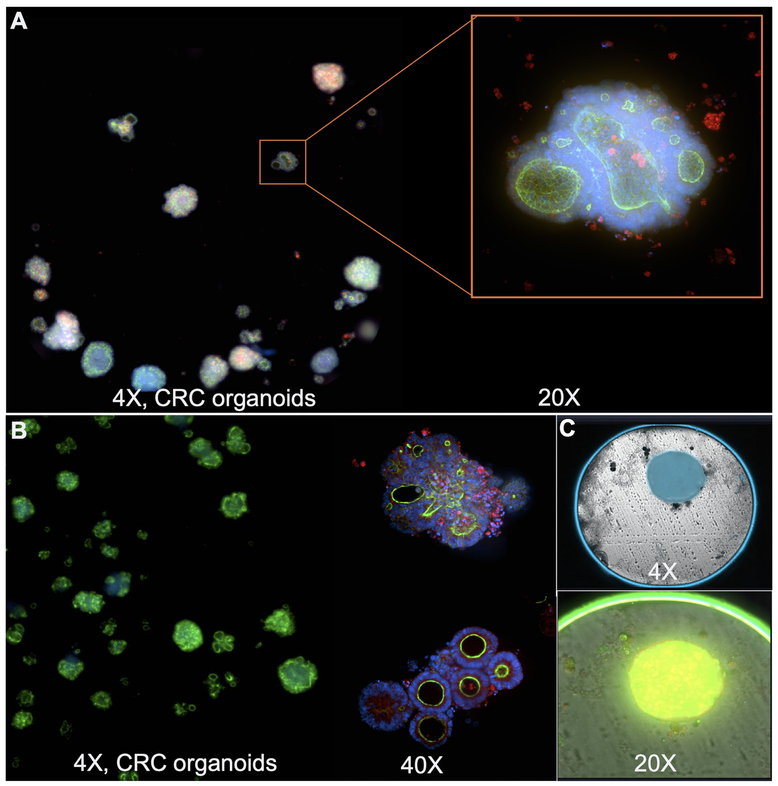


Figure 4. A. The same sample used in Figure 3 was imaged with QuickID with 4X objective, followed by IN Carta analysis to identify organoids using simple thresholding in the DAPI channel. Next, the 20X objective was used to acquire high-resolution images. B. The 40X objective was used to acquire high-resolution images using the same sample as in Figure 4A. C. Liver tissues were grown in 384W flat bottom IN Sphero plates with black walls and a small (1 mm) viewing window at the plate bottom. However, with the 20X objective, the FOV is insufficient to cover the all the organoids. QuickID was used to first identify liver tissues in 4X and then imaging was carried out with the 20X objective.

Conclusion

- The ImageXpress HCS.ai High-Content Screening System is a next-generation high-content imager with improved signal-to-noise ratio.
- The revamped QuickID targeted acquisition workflow ensures hassle-free and targeted high-resolution imaging.
- The improved image quality of the HCS.ai system generates better assay results from 3D models.