High-speed phenotypic profiling using a next generation high-content imaging platform with AI-enabled image analysis

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Introduction

High-content imaging is an important cornerstone in the drug discovery process. Unlike assays that measure a single parameter, high-content imaging captures multiple cellular features and thus provides a richer understanding of the cellular response to perturbations. Current imaging platforms may be limited in image acquisition speed, low signal to background, and lack of data management. To address these limitations, we developed a next generation high-content imager, the ImageXpress® HCS.ai High-Content Screening System (Molecular Devices), with fully redesigned hardware and software. Improvements to x, y, and z stage movement and autofocus versatility result in higher throughput while the new state-of-the-art optical light path, including optimized light sources and a higher quantum efficiency camera means reduced exposure times, low background, and enhanced signal-to-noise ratio.

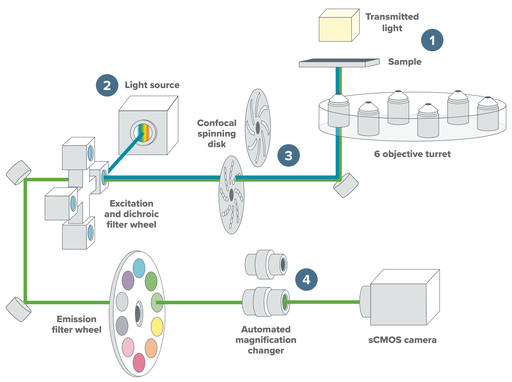
Here, we will present three example assays and their results to illustrate how the new imager with its modern software interface follows a logical workflow from set-up to analysis. For each assay, Z-prime score was more than 0.5:

1. G-protein coupled receptor assay
2. Cell painting
3. Spheroid viability

On average, we show a 40% improvement in image acquisition speed when compared to current systems. The signal:noise ratio also showed significant improvement over other current laser-based systems (over 2X improvement). The enhancement in speed, coupled with the system's high image quality, makes the HCS.ai system well-suited for typical 2D and 3D high-throughput imaging applications.

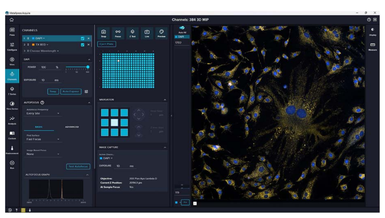
Methods

Image acquisition

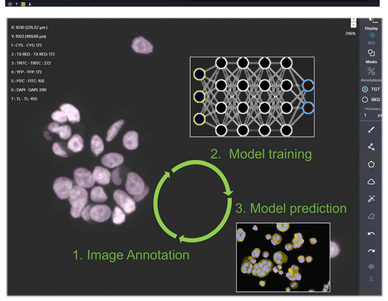


The ImageXpress HCS.ai system is redesigned with improved optics and hardware to support fast image acquisition without compromising image quality. 1) It supports standard SBS format labware and slides, 2) offers either laser or LED light source, 3) has four spinning disk geometry options available, 4) automated magnification changer offers up to 12 effective magnifications in one system (including four water immersion options).

Image acquisition software + AI-enabled image analysis



Redesigned image acquisition software (MetaXpress® Acquire) offers an intuitive, user-friendly environment that is quick for novices to master while remaining flexible and customizable for advanced users. There are also several new features, including easily controlled transmitted light acquisitions and identification and the centering "rare" objects in a well for high magnification acquisition.



IN Carta® Image Analysis Software includes two AI-enabled tools for image analysis.

- SINAP (Segmentation Is Not A Problem) is a deep learning-based tool for image segmentation. Pre-trained models (such as for nuclei, cells) can be customized by users to fit their analysis goals. Tools such as SAM (Segment Anything Model) allow users to more easily annotate images.
- Phenoglyphs is a machine-learning, classification-based tool which uses measurements of segmented features post-image analysis.

Results

Up to 50% shorter image acquisition times

To evaluate instrument speed performance, cells were imaged using various settings and the image acquisition times were compared to similar high-content screening imagers. Acquisition was on average 50% faster for images acquired in widefield, 38% faster for images acquired in confocal (Figure 1.) For assays such as cell painting, acquisition was completed under 96mins (confocal, water immersion, 4FL channels + TL) compared to other similar systems which took ~162 mins.

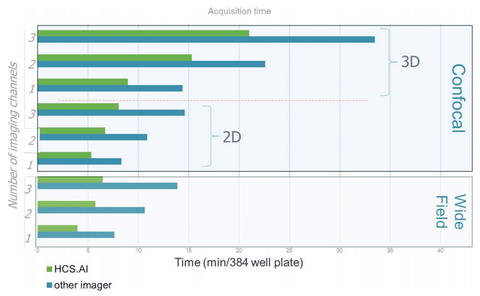


Figure 1. Average acquisition speeds of three runs for each image acquisition set up shown (four instruments, 20X). For 3D imaging, 5 Z-planes were acquired while for 2D imaging, 1 focused plane was acquired. For widefield speed tests, only 2D imaging was performed.

Improved image quality – high signal, low background

The optical light path in the HCS.ai system was redesigned to improve the signal-noise ratio (SNR). To assess the image quality of 3D structures on this new system, spheroids were formed in a U-bottom plate, fixed and stained with a nuclei marker (Hoechst), and imaged. SNR was calculated from 4 instruments and are shown in Figure 2. On average, we obtained a SNR of 4.4 – over 2-fold improvement compared to a similar imager. The high SNR improved sensitivity and we were able to detect at least 2X more nuclei at depth, compared to a similar imager.

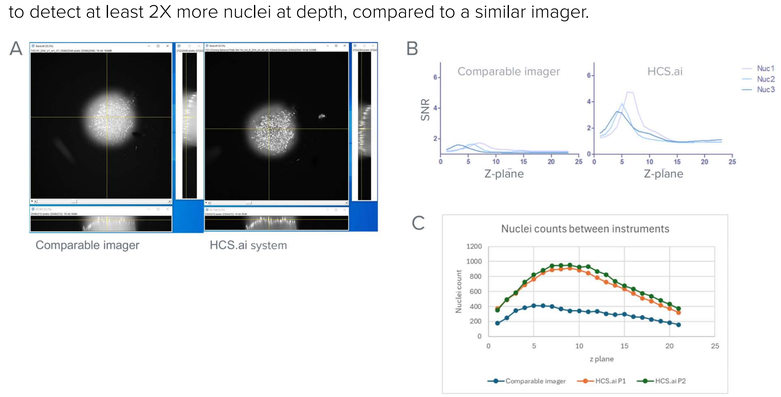


Figure 2. Evaluation of SNR in 3D samples. A) Representative spheroids (single plane, 10x) shown here with their corresponding orthogonal sections. The images are displayed with similar scaling settings. Note the improve contrast with images acquired on the HCS.ai system. B) Plot showing the SNR of 3 nuclei in Z. Step size of each Z-plane is 6µm. C) Spheroid images were analyzed and the number of nuclei in each Z-plane shown (data from two HCS.ai systems). Note that the number of detectable nuclei is at least twice that of the comparable imager.

Assay performance: GPCR Assay (Transfluor®)

G-protein coupled receptors (GPCR) are the largest class of pharmaceutical targets and used widely in cell-based screens. Upon GPCR activation, ß-arrestin in the cell cytoplasm translocate to pits and endocytic vesicles (Figure 3A). Here, U2OS cells expressing GFP tagged ß-arrestin were treated with isoproterenol (GPCR agonist) which allowes for imaging and analysis of internalized GPCR vesicles using high-content imaging. The number of GFP puncta was used to calculate the Z-prime score for the assay (0.75 and 0.67) (Figure 3C).

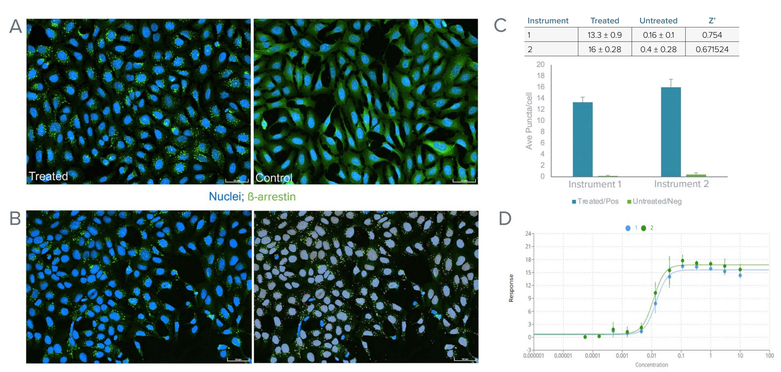


Figure 3. Evaluation of the GPCR assay. A) Cells with activated GPCR (treated with isoproterenol) shown on the left, negative control on the right. Note the presence of ß-arrestin punctae (green) in the treated samples. B) Images were analyzed using IN Carta image analysis software. Shown here is a pair of images, with the analysis mask for nuclei and punctae shown on the right. C) Average of 8 technical replicates from two HCS.ai systems shown. D) Dose response plot shown here from two HCS.ai systems. EC_{50} = 0.014 (1), 0.011 (2). (Dose response curve generated using Quest Graph™ Four Parameter Logistic (4PL) Curve Calculator. AAT Bioquest, Inc., 7 Jan. 2025)

Results

Cell painting

Cell painting is a highly multiplexed image-based assay that uses up to six fluorescent dyes to label eight cellular components. This has been shown to be a very powerful approach to characterize cellular profiles, providing proxy indicator of cell state, gene expression, and even mechanism of action for drugs. Here, MCF7 breast cancer cells were treated with a small set of compounds followed by the cell painting assay (Figure 4). Images were acquired on the HCS.ai system and analyzed using IN Carta image analysis software. Data analytics were carried out in StratoMineR™ software a cloud-based platform developed for the analysis of multiparametric data, such as in phenotypic profiling assays.

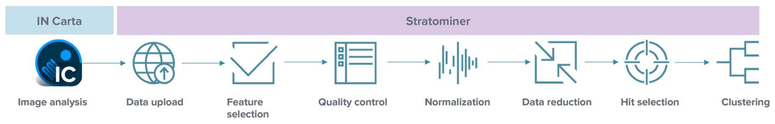


Image analysis to data analytics workflow using IN Carta software followed by StratoMineR.

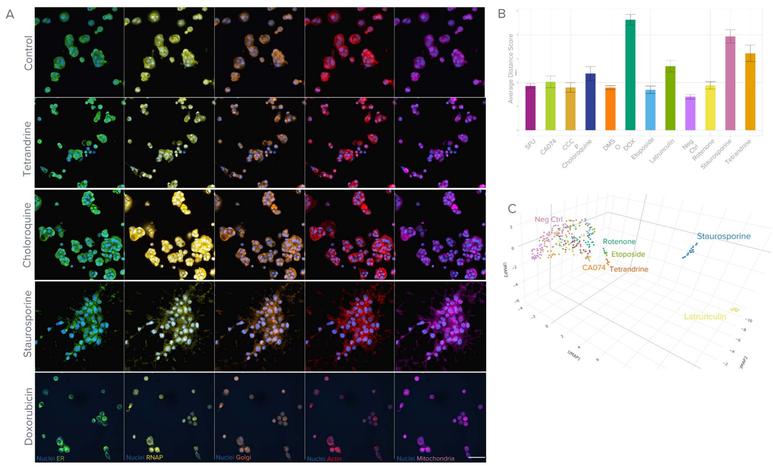


Figure 4. Cell painting assay on the HCS.ai system. A) Representative images of control and treated MCF7 cells. Scale=50µm. Images were analyzed using IN Carta image analysis software. SINAP was used for nuclei segmentation. 246 measurements per cell from the assay was uploaded to the StratoMineR software for further analysis. B) Graph showing the average physical distance score for compounds used. C) UMAP representation of the phenotypic profiles.

3D spheroid assay

Cell-cell interactions is important in many cellular processes such as metabolism, cell signaling, and gene expression. As such, there is an increasing use of 3D cellular models—such as spheroids—in assays. To evaluate the HCS.ai system on 3D samples, spheroids (HCT116 cells) were formed in U-shape, ultra low attachment plates. Spheroids were treated with compounds and their effects were assessed using Caspase 3 (apoptosis marker) staining (Figure 5). Analysis of the number of apoptotic nuclei/total nuclei in the untreated vs. positive control compound treated spheroid yielded a Z-prime average of 0.8 (n=2 instruments).

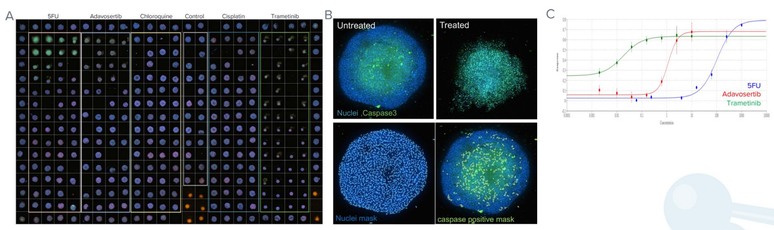


Figure 5. Spheroids imaged on the HCS.ai system. A) Overview image of whole plate from the IN Carta software. Spheroids were stained with Hoechst (Blue), caspase3 (green), ethidium homodimer (orange) and MitoTracker (magenta). B) Spheroid images were collected with Z-stacking in 10X. Max. projection images shown here. Analysis was carried out in the IN Carta custom module editor. C) Dose response curve (caspase positive cells/total number of cells). EC_{50}: 5FU (Fluorouracil) 103µM; Adavosertib 1.13µM; Trametinib 0.018µM.

Conclusions

- Newly designed high-content imager improved acquisition speeds by an average of 40%, enabling faster screening compared to similar imagers.
- Image quality increased more than 2-fold in SNR, enabling detection of more objects in 3D structures.
- The HCS.ai system is compatible with highly multiplexed assays which gave good clustering based on phenotypic profiles.
- Both 2D and 3D assays yielded high Z' scores (>0.5), demonstrating the suitability of the using the HCS.ai system for high-throughput screening.

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