Enhancing Drug Discovery: Integrating High-Content Imaging with Traditional Plate-Based Screening for Comprehensive Analysis and Mechanistic Insights

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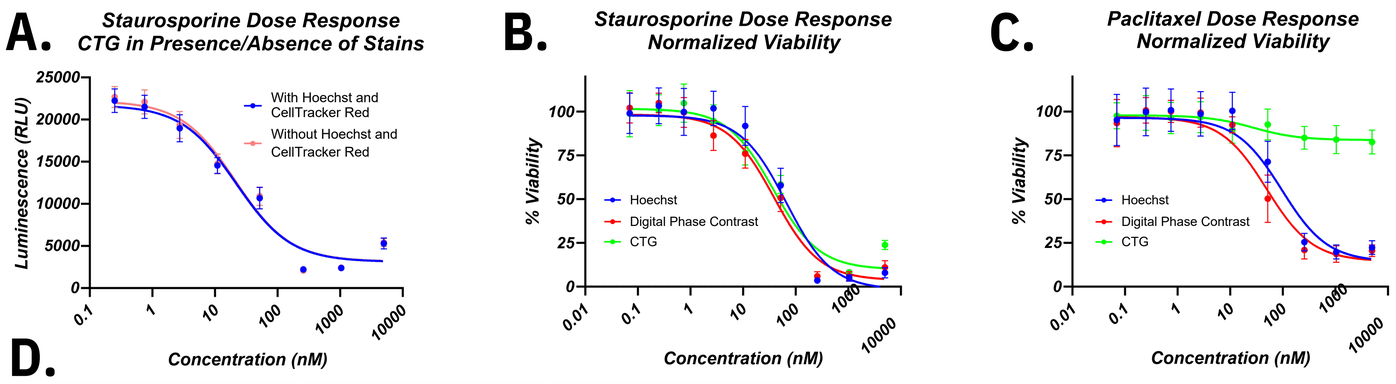
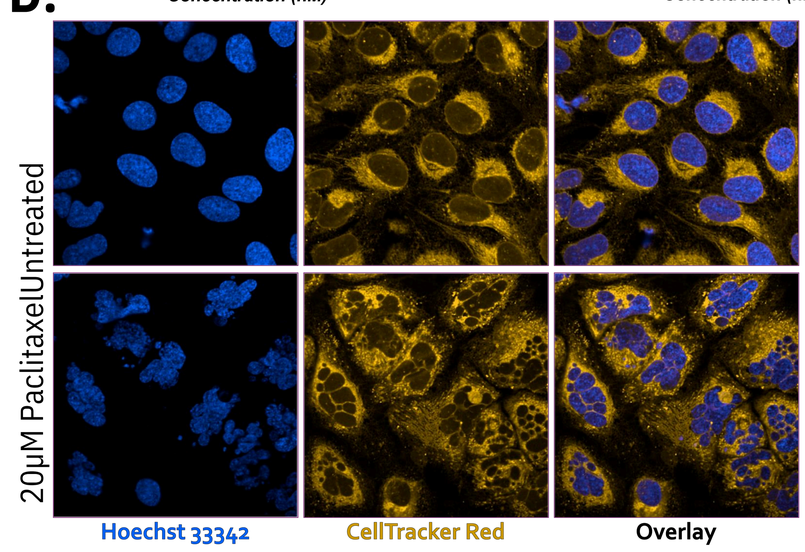
Introduction

The integration of high-content imaging into traditional plate-based small molecule screening methodologies represents a transformative advancement in drug discovery, offering a more comprehensive and detailed analysis of potential therapeutics. Conventional plate-based assays, while efficient and high-throughput, primarily provide quantitative data limited to specific biochemical interactions or cellular responses. In contrast, high-content imaging enables the visualization and quantification of multiple cellular parameters simultaneously, offering a multidimensional perspective on the effects of both large and small molecules. One of the primary benefits of incorporating high-content imaging approaches into traditional plate-based assays is the ability to obtain a more holistic view of compound activity. While plate-based assays might indicate a change in enzyme activity or receptor binding, high-content imaging can reveal how these changes translate into broader cellular phenotypes. These capabilities are crucial in understanding the nuanced effects of small molecules that might be overlooked by traditional assays. This multifaceted approach ensures that compounds not only hit their intended targets but also produce the desired therapeutic outcomes without off-target effects, enhancing the robustness and reproducibility of data without adding significant time or cost. By employing a bit of creativity to program design, high-content imaging can be performed concurrently with traditional plate-based assay formats in the same microplate by imaging live cells prior to lysis steps or by immunofluorescence after supernatants are collected. This integration enhances the understanding of the mechanisms of action (MoA) of potential therapeutics, allowing for the identification of promising drug candidates with greater precision and confidence to ensure that compounds are not only effective but also safe and well-characterized at the cellular level. This poster outlines the application of high-content imaging techniques in supplementing conventional plate-based cytotoxicity assays, enhancing the depth and breadth of data, and elucidating phenotypic and mechanistic profiles of potential therapeutics in early stage drug discovery and development.

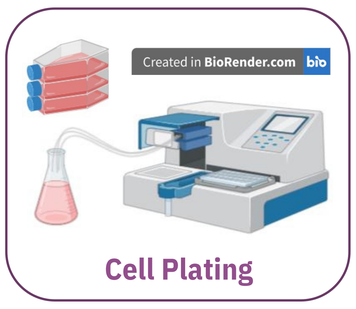
Comparison of Plate Reader and HCI Outputs





Assessing Cell Viability and Morphological Changes through High-Content Imaging: Cell viability was assessed by both CellTiter-Glo® Luminescent Cell Viability Assay Reagent (CTG) and high-content imaging. Staurosporine or Paclitaxel treated U2OS cells were incubated for 24 hours prior to assessment of cell viability. **(A)** To initially compare effects of cellular stains, wells labeled with fluorescent HCI stains were processed with CTG and compared to those without staining. **(B)** Morphological changes were assessed using Hoechst 33342 and CellTracker™ Red CMPTX Dye (blue line), stain-free Digital Phase Contrast (DPC) (red line), and CTG (green line). Cells treated with Staurosporine, a pro-apoptotic compound that can arrest the cell cycle at the G2/M phase, exhibit nearly identical dose response curves using the three different methods of quantification (top left graph). **(C)** Cells treated with Paclitaxel, a microtubule stabilizer which blocks cell division and proliferation, show minimal change in cell viability as measured by CTG reagent. **(D)** However, Paclitaxel treated cells analyzed by high-content imaging reveal dramatic morphological changes indicative of mitotic catastrophe.

Assay Design and Workflow



Cell Plating

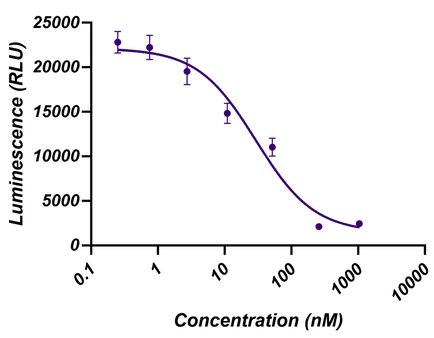
Thermo Scientific® Multidrop® Combi Dispenser

Compound Addition

EchoTM555Acoustic Liquid Dispenser

Fluorescence Readout

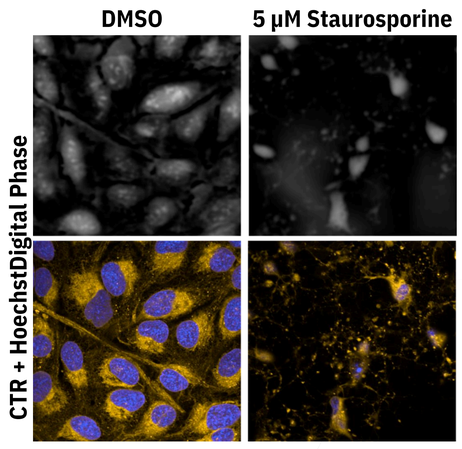
Revvity EnVision® Multimode Plate Reader



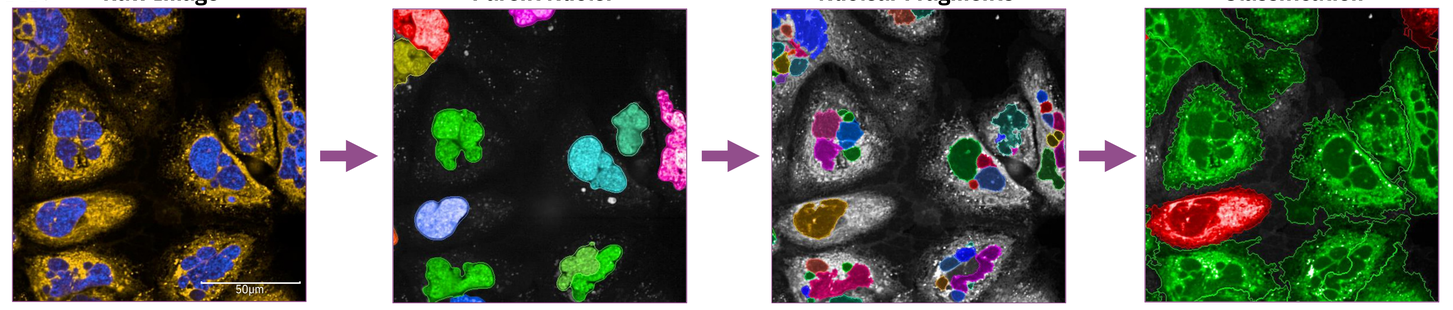
Fully-Automated Assay Workflow: Cell viability assays using traditional luminescence-based viability dyes such as the CellTiter-Glo® assay employ standard instrumentation required for cell seeding, compound addition, and fluorescence quantification. The time and cost impact of an additional high-content imaging step is minimal, requiring cost-effective cell stains (i.e. Hoechst nuclear stain, CellTracker™ Red CMTPX Dye), a short wash step, and a streamlined imaging step that can be simply incorporated into an already fully-automated viability assay using the PerkinElmer® Cell::Explorer™ platform. Adherent U2OS osteosarcoma cells were seeded in 384-well plate format and treated with cytotoxic reference compounds using the EchoTM 555 Acoustic Liquid Dispenser. Compound treated cells were then incubated at 37˚C before they were processed though the traditional cell viability workflow or with the addition of a high-content imaging step. The traditional plate-based assay workflow was performed both with and without the additional high-content imaging step to directly compare percent viability of compound treated cells normalized to a vehicle only (i.e. DMSO) treated control.

Fluorescent Staining and High-Content Imaging

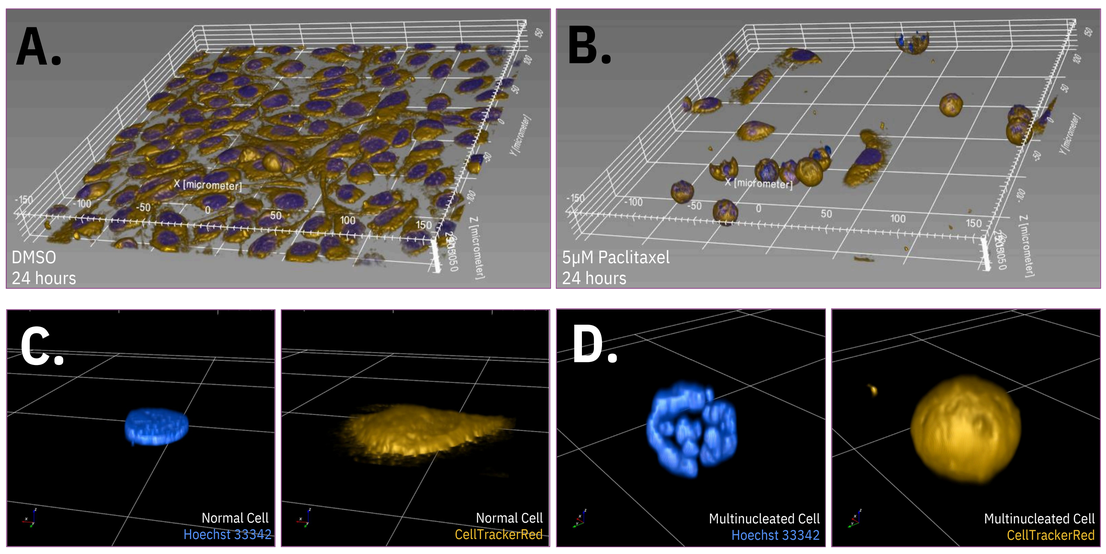
BioTek® EL406 Washer Dispenser
Revvity Opera Phenix® Microplate Reader



Uncovering and Classifying Phenotypes with HCI



Customizing Image Analysis to Reveal New Phenotypes: **(A)** Custom image analysis workflow to quantify and classify a multinucleation phenotype in Paclitaxel treated cells using commercially available cytoplasmic and nuclear stains. Quantifications relaying on nuclear morphology, cell texture, and fragmentation were analyzed using a linear classification algorithm to identify multinucleated cells in each test well population. **(B)** Phenotypic classification of Paclitaxel effects allowed for dose-response assessment of the onset of multinucleation and a better understanding of mitotic catastrophe at the cell population level. At higher concentrations of Paclitaxel (500 – 5,000nM), consistent shifts in multinucleation state were observed which are representative of the dynamic onset of cellular detachment, senescence, or cell death occurring in the cell population.



Increasing Cellular Dimensionality: Data from both cytotoxicity and imaging assays together suggest that the cell loss observed in the imaging assay was not representative of cell death after 24 hours of Paclitaxel treatment. To explore this, cells were imaged in a 22µm Z stack to identify cells that may have lost their adherent state but remained intact. **(A)** DMSO- treated control cells grew to high density with the expected flattened cell body morphology. **(B)** Cells treated with 5µM Paclitaxel demonstrated loss of cell density and adherence to the plate bottom. Clear differences can be observed in nuclear structure. **(C)** Normally functioning U2OS cells appeared largely flat and adhered to the plate bottom. In contrast, all lifted cells discovered in the Paclitaxel-treated wells presented a strong multinucleation phenotype.

Conclusions

- The introduction of a non-perturbing high-content imaging acquisition step into a standard assay workflow can add immense value to a discovery campaign.
- While some compounds have relatively straightforward anti-cancer properties, others produce more nuanced phenotypes that warrant more detailed investigation, though not necessarily at a greater time or materials cost.
- Utilization of both plate readers and confocal microscopes into a single automation platform allow for seamless integration of imaging into workflows with little to no effect on lead time or cost.

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