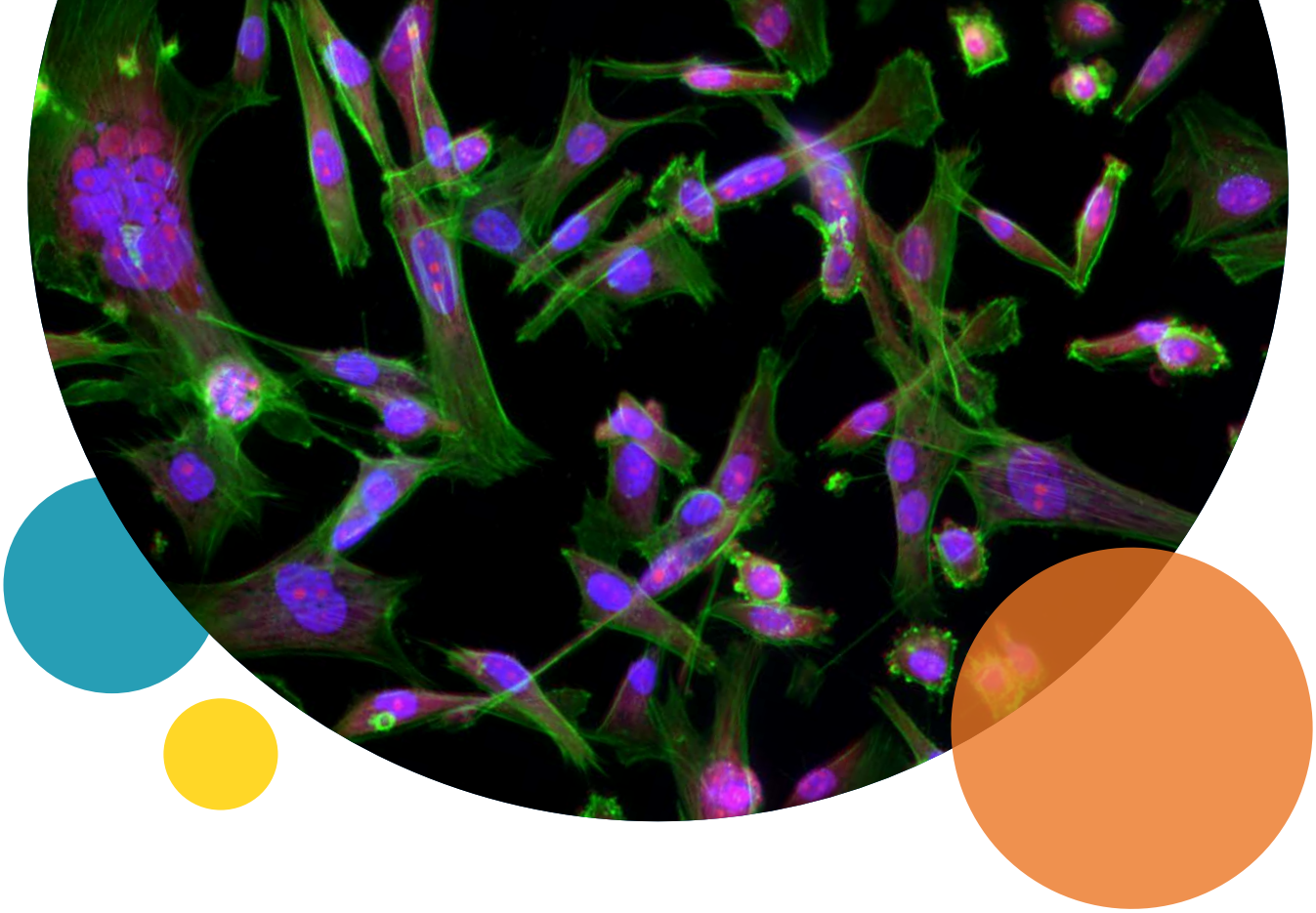

Cellular Imaging Insights

Elucidating complex cellular pathways within 2D and 3D cellular structures in response to chemical stimulation or stress is the goal of many researchers. The capacity to which scientists have access to and can adopt emerging cellular imaging and analysis technology fuels their level of understanding. With cost efficient, high-throughput instruments, such as the ImageXpress® imaging portfolio, researchers can gain insight and expedite their studies while better understanding the interaction of cells under different conditions. This eBook provides new tools to better understand complex imaging models.

eBook contents

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Counting cells with transmitted light

Label-free cellular assays are required for a multitude of biological applications that monitor the cell number, proliferation, health, confluency, and cytotoxicity. These applications necessitate efficient and robust transmitted light (TL) imaging and analysis capabilities providing precise segmentation for quantitation of cells and assessment in variety of cell responses and morphologies. Here, we demonstrate how to accurately segment and quantify unlabeled cells using transmitted light.

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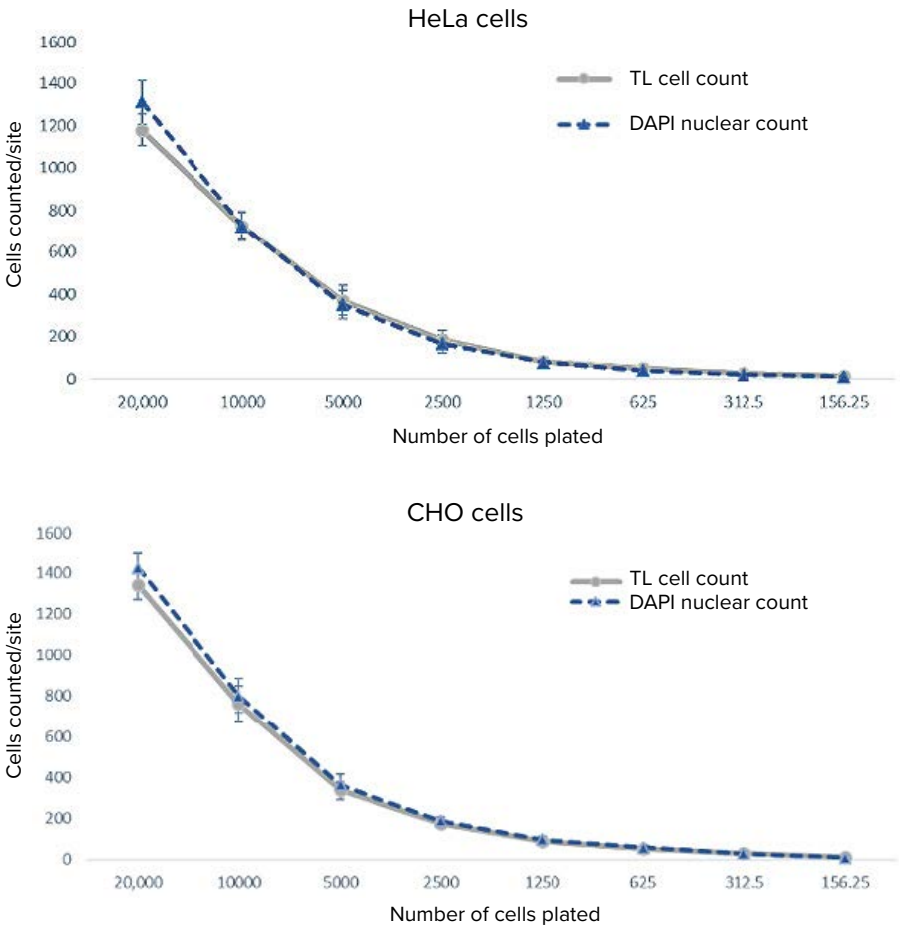
BENEFITS

- Generate data similar to fluorescent cell counting without adding potentially harmful stains
- Optimize analysis of different types of cells using preconfigured algorithms
- Run precise segmentation for quantitation of cells and assessment for a variety of cell responses and morphologies



Comparison of total cell counts for HeLa and CHO cells based on the TL Cell Segmentation and Count Nuclei Objects module. The cells were diluted 1:2, starting from a concentration of 20,000 cells per well.

Cytotoxicity assessment using automated cell imaging and live/dead assays

Live/dead assays are utilized in a wide variety of research applications including investigation of cytotoxic effects of various compounds, treatments, or changes in gene expression. Automated cellular imaging and analysis provide an optimal method to assess cell viability and cell death. Here, we describe the use of the ImageXpress® Pico Automated Cell Imaging System and CellReporterXpress Automated Image Acquisition and Analysis Software to image cells treated with EarlyTox™ Live/Dead Assay Kit.

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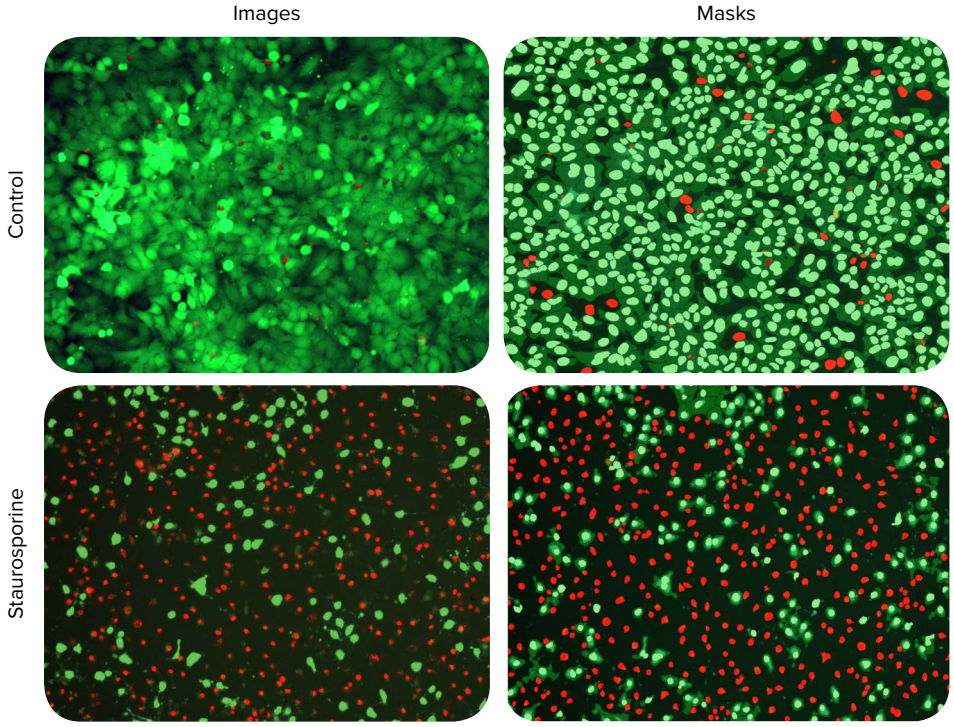
BENEFITS

- Efficiently measure cell viability using a no-wash homogenous assay protocol
- Accurately quantify live or dead cells
- Quickly generate statistically relevant results using preconfigured analysis modules



Representative images of negative control cells and cells treated with 0.1 µM of staurosporine. **Left:** 10X images of Hoechst nuclei stained (blue), Calcein AM-stained (green) and EthD-III-stained (red) HeLa cells. **Right:** Analysis masks show nuclei of live cells in green and nuclei of dead cells in red.

Detection of autophagy using automated imaging

Autophagy is a highly regulated process of degrading and recycling damaged proteins and organelles in response to cellular stress, such as nutrient deprivation, viral infection and genotoxic stress. Since dysregulation of autophagy has been established to play a role in various neurodegenerative diseases and cancers, the discovery of novel therapeutic agents targeting various stages along this process has emerged as a promising new approach for drug therapies. Here, we demonstrate the reliability and efficiency of automated imaging to screen assays exploring compound effects on autophagy.

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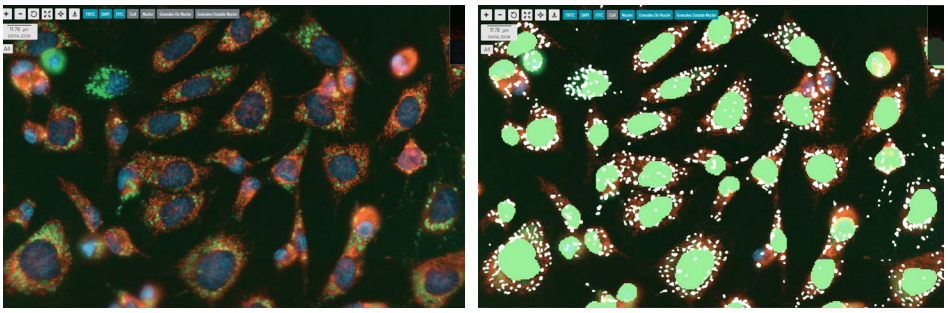
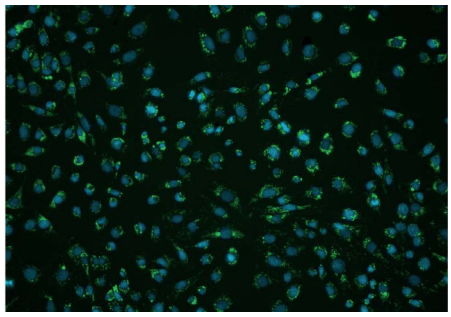
BENEFITS

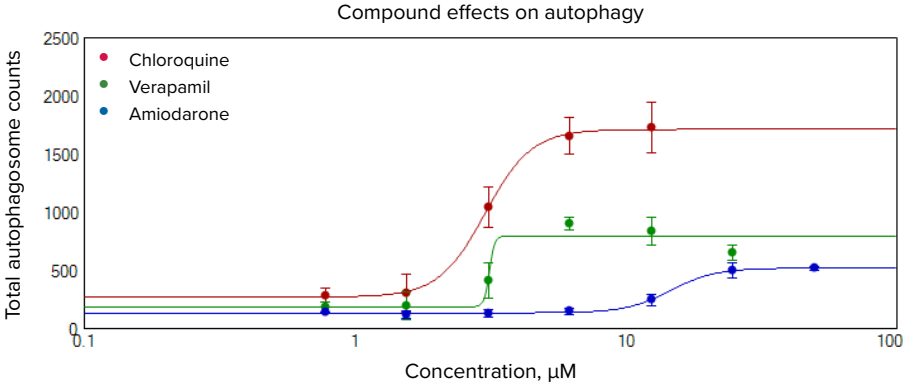
- Simplify detection and analysis of compound effects on autophagy
- Identify new therapeutic agents that target autophagy earlier in drug development



Autophagy image analysis. Left: Image of PC12 neuroblastoma cells treated with chloroquine for 24h and stained with Cyto-ID reagent, MitoTracker Orange, and Hoechst dyes. Images were taken by the ImageXpress® Nano Automated Imaging System, 40X magnification. Autophagy particles (green), mitochondria (red), and nuclei (blue). **Right:** Analysis masks shown for autophagy particles and nuclei after using Granularity application module in CellReporterXpress software. Nuclei (green) and autophagosomes (white).



Autophagy detection assay. Image of PC12 neuroblastoma cells treated with chloroquine for 24h and stained with Cyto-ID autophagy detection kit. Images were taken by the ImageXpress Nano system, 20X magnification. Autophagy particles are indicated in green, and nuclei shown in blue.



Compound effects on autophagy. Concentration-dependent responses for autophagy indicated for three compounds using "total granules" (total autophagosome count) as a readout. Chloroquine (red), verapamil (green), and amiodarone (blue).

Evaluation of mitochondrial integrity and mitochondria membrane potential using automated cell imaging and analysis

Mitochondrial function, a key indicator of cell health, can be assessed by monitoring changes in mitochondrial membrane potential. Cationic fluorescent dyes are commonly used to assess more in-depth information about mitochondrial health. Here, we evaluated mitochondrial damage using the ImageXpress® Pico Automated Imaging System and CellReporterXpress software.

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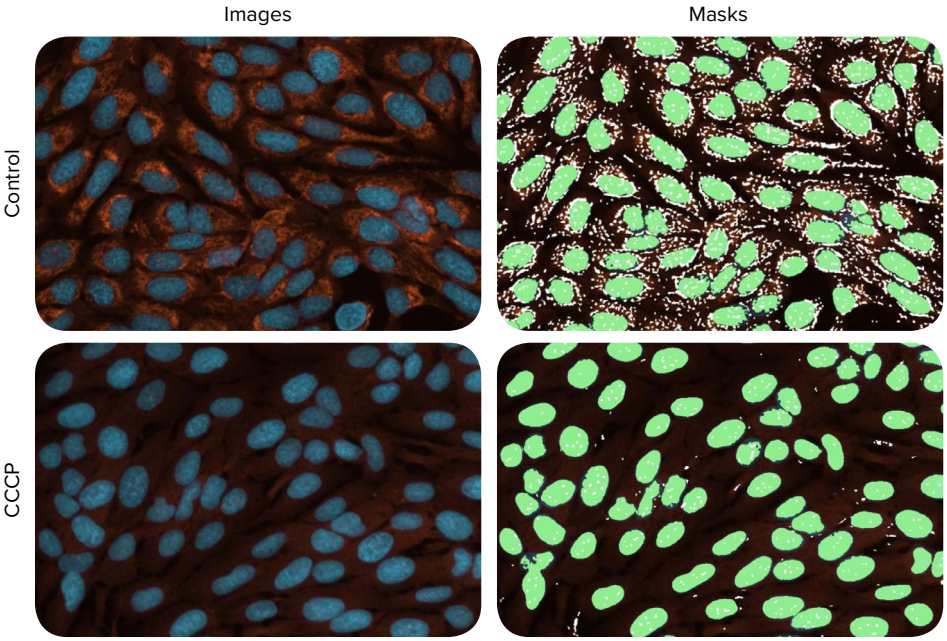
BENEFITS

- Assess more in-depth information about mitochondrial integrity and membrane potential in response to compound treatment
- Rapidly produce multiple readouts
- Generate assay results more efficiently with preconfigured protocols



Evaluation of mitochondrial integrity and membrane potential by automated imaging. 20X images of control U2OS cells and cells treated for 60 min with CCCP were imaged with the ImageXpress Pico system and analyzed using the CellReporterXpress software. **Left:** Images of control cells and cells treated with 1 µM of CCCP, stained with MitoTracker Orange (orange) and Hoechst nuclear dye (blue). **Right:** Image analysis masks showing the nuclei (green) and mitochondria particles (white). Treatment with inhibitor of oxidative phosphorylation CCCP resulted in loss of membrane potential.

Phenotypic characterization of anti-cancer drug effects

Discovery and evaluation of anti-cancer therapies is an active area of research that includes development of cell-based models, screening for novel drugs, comparison of drug efficacy, and understanding the mechanisms of action. In contrast to target-based drug discovery, phenotypic drug discovery has been shown to produce a greater number of first-in-class drugs with novel mechanisms of action. Here, we provide an efficient method for monitoring and analyzing the cellular effects of anti-cancer compounds using automated cellular imaging.

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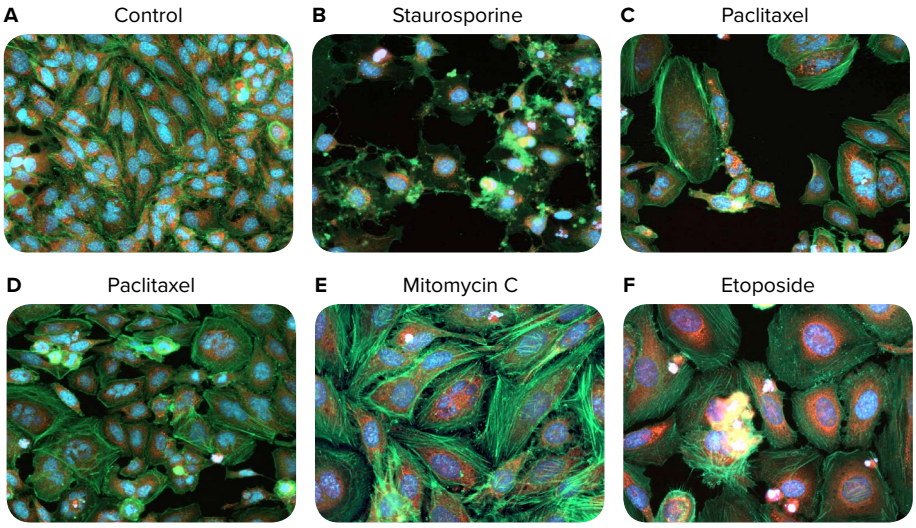
BENEFITS

- Enable multiple readouts to characterize phenotypc effects of tested compounds
- Monitor simultaneous cytotoxic effects of live and fixed cell staining protocols
- Improve drug discovery success and productivity



40X images of HeLa cells stained with AlexaFluor488 phalloidin. Representative images show phenotypic changes of HeLa cells in response to compound treatments. Images were acquired with the ImageXpress Nano system and CellReporterXpress software using a 40X Plan Fluor objective. Note the following changes in phenotypes: apoptotic nuclei, cell detachment, changes in cell area and intensities of phalloidin stain, changes in nuclear number and shape. Drug concentrations for each compound are as follows: **B)** staurosporine (0.12 µM), **C)** paclitaxel (5.1 x 10^{-4} µM), **D)** paclitaxel (1.7 x 10^{-4} µM), **E)** mitomycin C (0.41 µM), and **F)** etoposide (3.7 µM).

Multi-parametric assessment of cell phenotypes

The ability to investigate a variety of cellular responses provides important information about mechanism of action. Automated cellular imaging is an efficient method for characterizing multiple readouts including the assessment of cell viability, characterization of cell shape, cell adhesion and spreading, cytoskeleton integrity, and mitochondria potential. Here, we streamline the workflow by performing simultaneous image acquisition and analysis using the ImageXpress Pico Automated Cell Imaging System and preconfigured analysis protocols.

BENEFITS

- Streamline the capture of various cell phenotypes
- Obtain numeric data quickly using preconfigured analysis protocols
- Assess a variety of morphological changes in response to compound treatment

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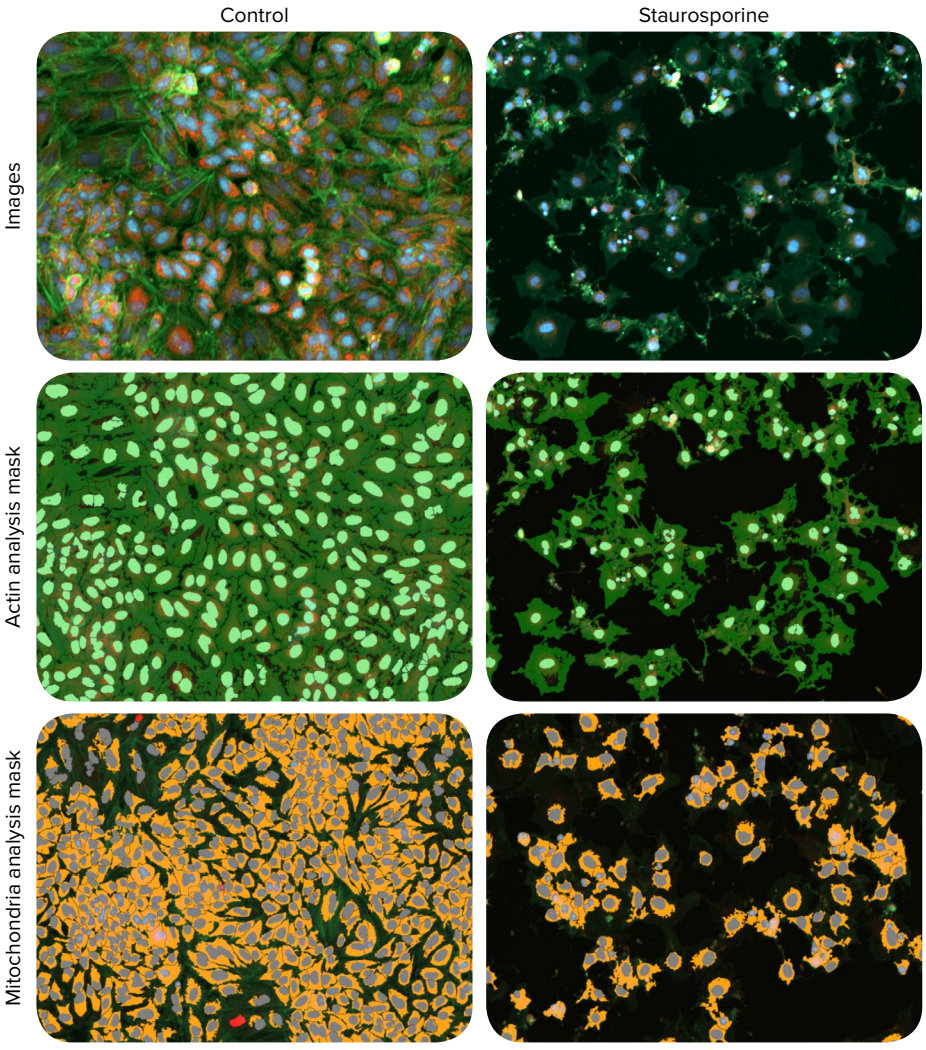

Image and analysis masks for multi-parametric analysis of cell viability and morphology. HeLa cells were treated with several compounds for 72 hours and then stained with a nuclear stain (Hoechst 33342) actin cytoskeleton stain (AlexaFluor 488 (AF488) labeled phalloidin) and a mitochondrial stain (MitoTracker Orange CMTMRos). Representative images and analysis masks show a comparison of control cells and cells treated with 0.1 µM staurosporine. The cells were imaged with DAPI, FITC, and TRITC channels using the ImageXpress Pico system with a 10X Plan Fluor objective.

Intelligent time-lapse imaging to enhance live cell assay development

The ability to monitor responses in living cells over a specific period of time offers cell biologists several key advantages for assay development. For routine cell-based screening, time-course results can determine the correct time to read end-point assays. High-content time-lapse imaging can also be used to characterize kinetics for multiple reactions at once, and to monitor cell proliferation or death. Here, we evaluated complex time lapse experiments using the ImageXpress Micro system.

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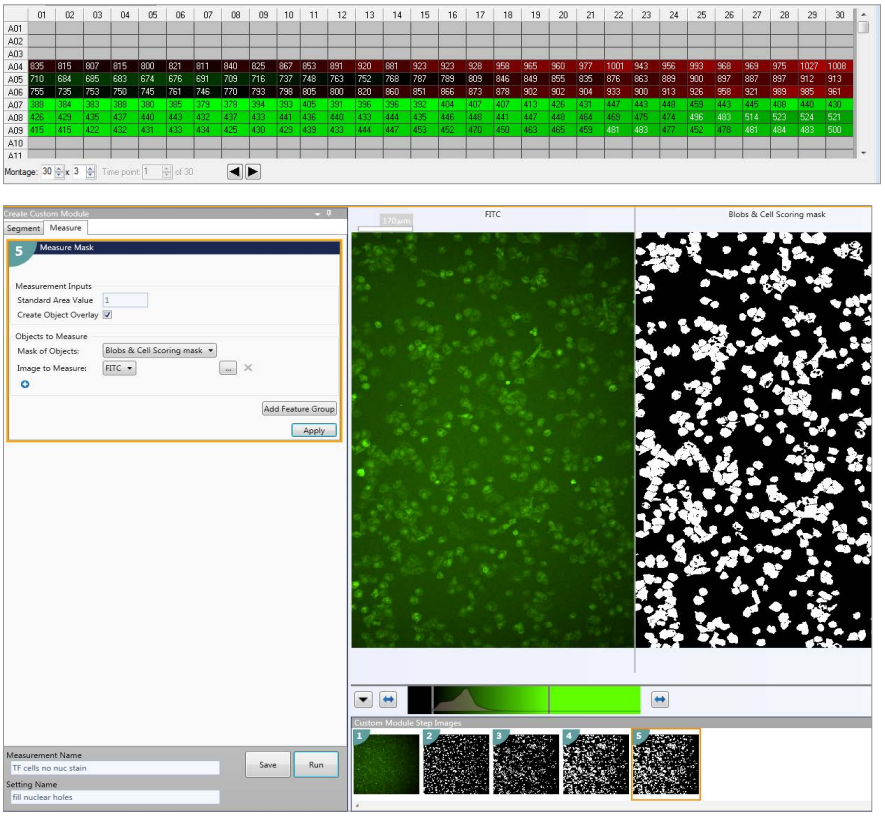
BENEFITS

- Characterize kinetics for multiple reactions at once
- Track effect of treatments on cell proliferation in real time
- Simplify complex time lapse experiments
- Decrease analysis times by up to 40X with parallel image processing



Determine cell proliferation rate in significant number of cells due to large field of view. Top: Time vs. well heat map data for GFP-expressing U2OS cells grown overnight. The clone in wells A04:A06 had higher cell counts than the clone in wells A07:A08. **Bottom:** A custom module accurately identified cells, even when flat-field (shading) correction was not used during image acquisition.

Automation of 3D spheroid cultures in ultra-low attachment plates

Three-dimensional (3D) cell cultures offer greater physiological relevance than monolayer cultures for cellular interaction studies and compound screens. Manual manipulations of these cultures can be laborious, and challenges are amplified as sample throughput increases. Automation provides the fine control necessary to plate and process 3D cell cultures at high volume. Here, we highlight the use of ultra-low attachment (ULA) plates to improve plating consistency and spheroid formation over traditional methods. The ImageXpress Micro Confocal system was used to measure and confirm spheroid size and volume consistency, and to further expedite the acquisition and analysis of cell-level data.

BENEFITS

- Automate plating, drug treatment, and staining of spheroids
- Reduce laborious workflows for analyzing spheroid consistency and drug cytotoxicity
- Generate more biologically-relevant levels of cell-cell interactions than monolayer cultures

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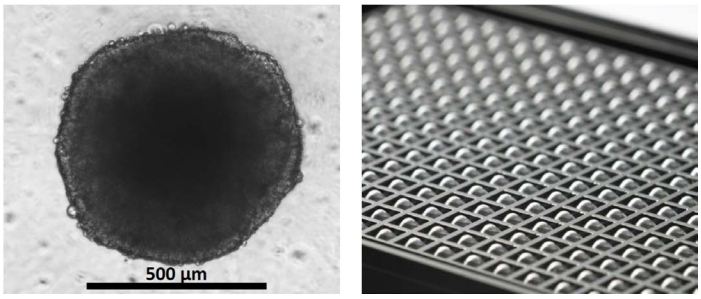
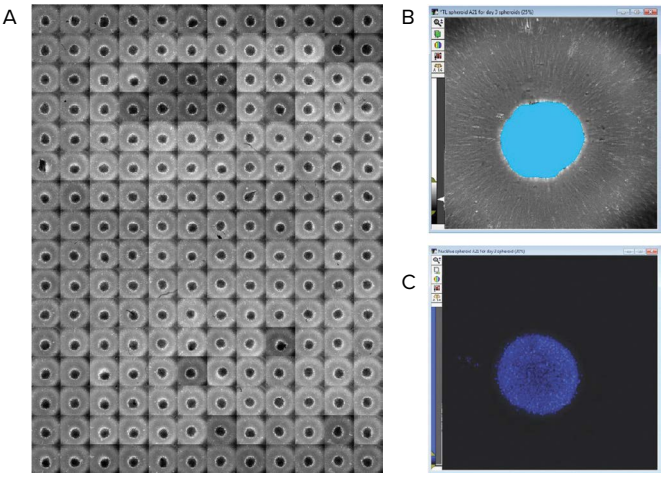
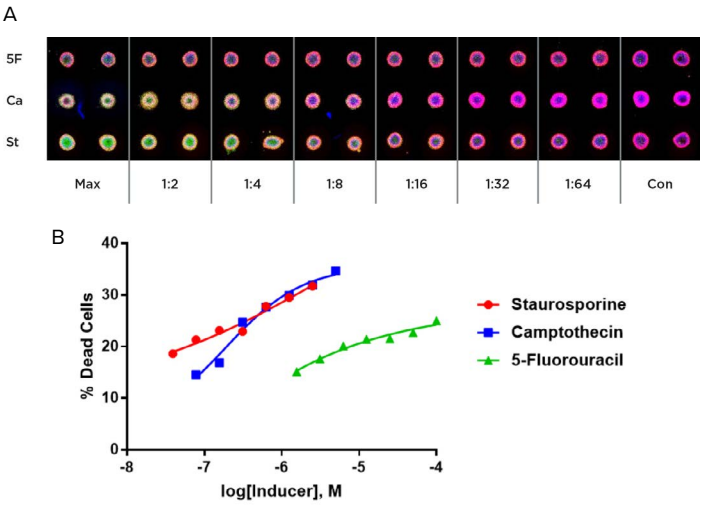


Image of a three-dimensional spheroid tumor model three days after plating 4000 HCT 116 cells in a Corning® ultra-low attachment plate (right).



Spheroid size and shape consistency. (A) Montage of 192 day 3 spheroids imaged at 10X using brightfield with the ImageXpress Micro Confocal system. **(B)** Spheroids were analyzed for circularity (shape factor, elliptical form factor) and size (perimeter and area) to ensure consistency across the well. **(C)** 32 wells were then stained for 24 hours with NucBlue so that cell nuclei could be counted and spheroid volume determined.



Apoptotic dose response. (A) 2D projections of Z-stacks acquired using the ImageXpress Micro Confocal system that identify live cells (red) and dead cells (green) for day 3 spheroids treated for 24 hours with dilution curves of 100 µM 5-fluorouracil (5F), 5 µM camptothecin (Ca), and 2.5 µM staurosporine (St). **(B)** A dose response curve showing the average percentage of dead cells in the duplicate spheroids for each condition.

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