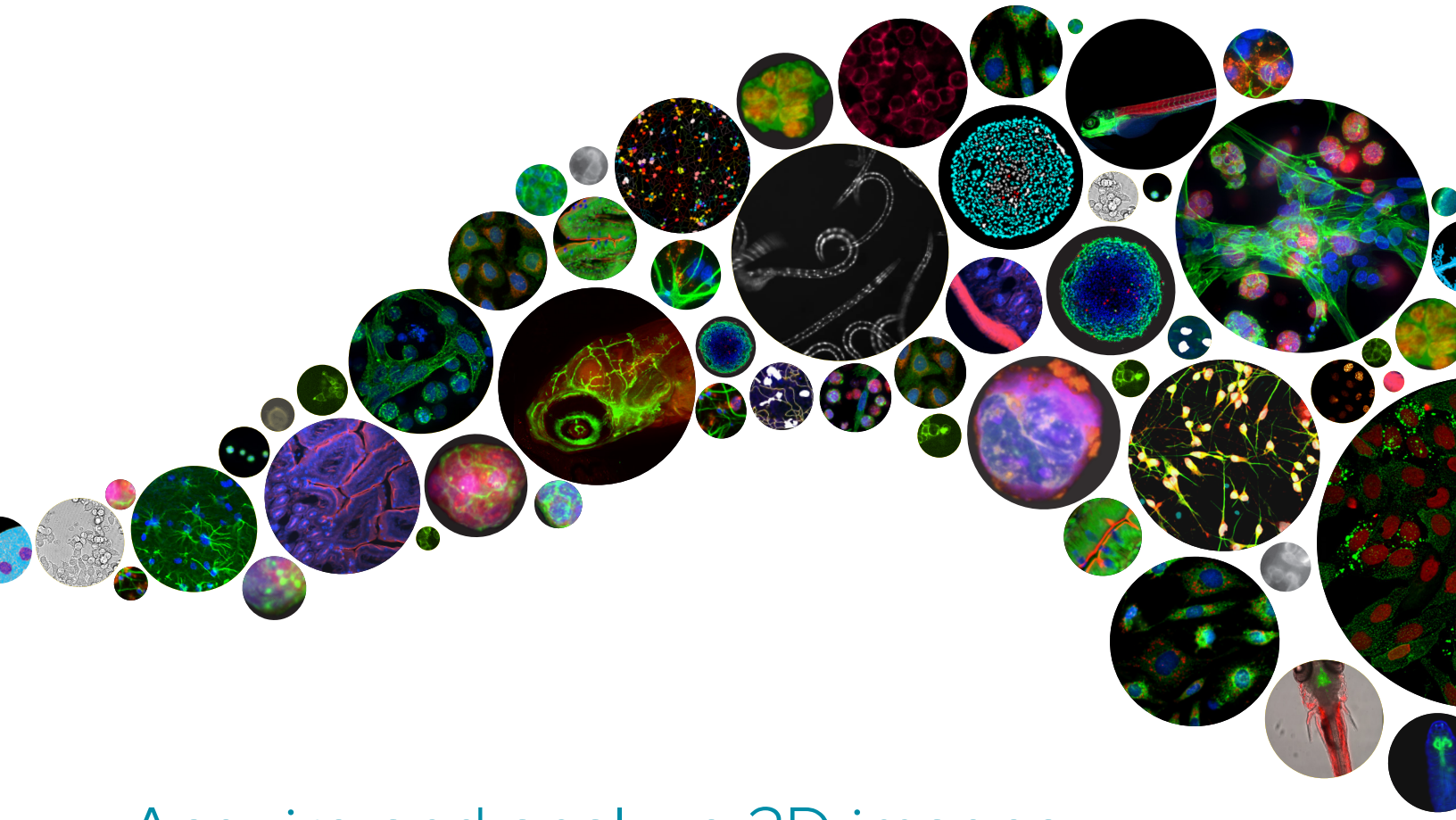

Acquire and analyze 3D images like a pro

High complexity cellular models such as three-dimensional (3D) spheroids better mimic *in vivo* environments compared to simpler two-dimensional (2D) models. While the development of quantitative assays using 3D cultures has emerged as an attractive investigative tool to understand complex biology, challenging 3D image acquisition and analysis workflows have hindered wider adoption by the screening and automated imaging communities.

Next-generation high-content, high-throughput tools for microscopy offer innovative and automated techniques for evaluating this complex biology. With new technology like the ImageXpress® Micro Confocal High-Content Imaging System and the MetaXpress® 3D Analysis Module with 3D Viewer, screening these models within a single, integrated interface will dramatically reduce the time to discovery.

This eBook highlights a few 3D cell model applications as well as our solutions for acquiring and visualizing quantitative data.

eBook contents

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High-content imaging and 3D models

In recent years, there have been significant advances in automated microscopy and imaging for delivering more predictive, physiologically-relevant measurements for drug discovery and environmental toxicity applications. Implementation of more complex assays and 3D models requires high resolution to capture publication-quality images and data. Enhanced assay sensitivity can be achieved by taking advantage of the optical properties of confocal imaging, capturing images with a high signal-to-noise ratio while reducing out-of-focus light for crisper images and accurate cellular detail.

Utilization of 3D cellular assays adds value to research and screening campaigns, spanning the translational gap between 2D cell cultures and whole-animal models. By reproducing important parameters of the *in vivo* environment, 3D models can provide unique insight into the behavior of stem cells and developing tissues *in vitro*.

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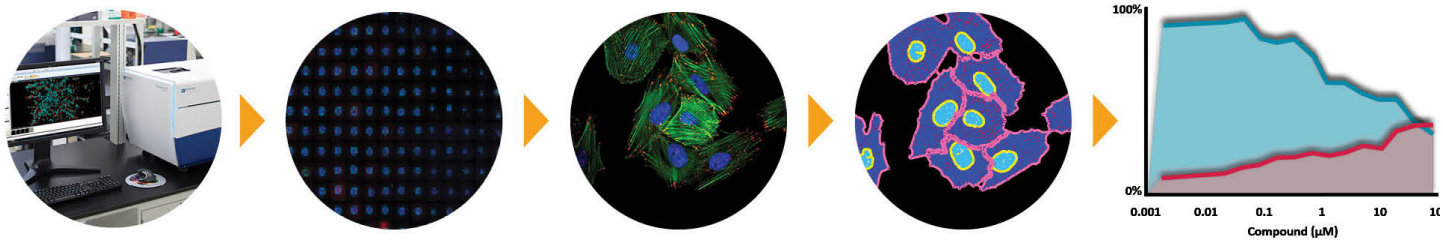
- Improve predictivity and relevance of cell based screening assays
- Utilize models that closely mimic *in vivo* responses to drug treatment, particularly drug resistance
- Reliably image spheroids following a step-by-step workflow from plating to analysis



3D cancer spheroids

Compared with assays utilizing adherent cells grown in 2D monolayers, 3D cancer cell cultures are believed to more closely mimic tumor response to drug treatment *in vivo*. Still, due to the labor-intensive and inconsistent nature of 3D models, the development of assays employing cancer spheroids has proven challenging. Here, we describe a method for cancer spheroid analysis compatible with high-throughput screening. This approach was used to measure and quantify compound effects on cell growth in a semi-solid matrix using high-content imaging and 3D analysis.

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- Develop high-throughput, high-content image acquisition and analysis methods for complex 3D assays
- Characterize dose-dependent phenotypic effect on human cancer spheroids in response to anti-cancer compound treatment
- Streamline analysis workflow using an integrated analysis solution
- Accelerate delivery of accurate 3D measurements

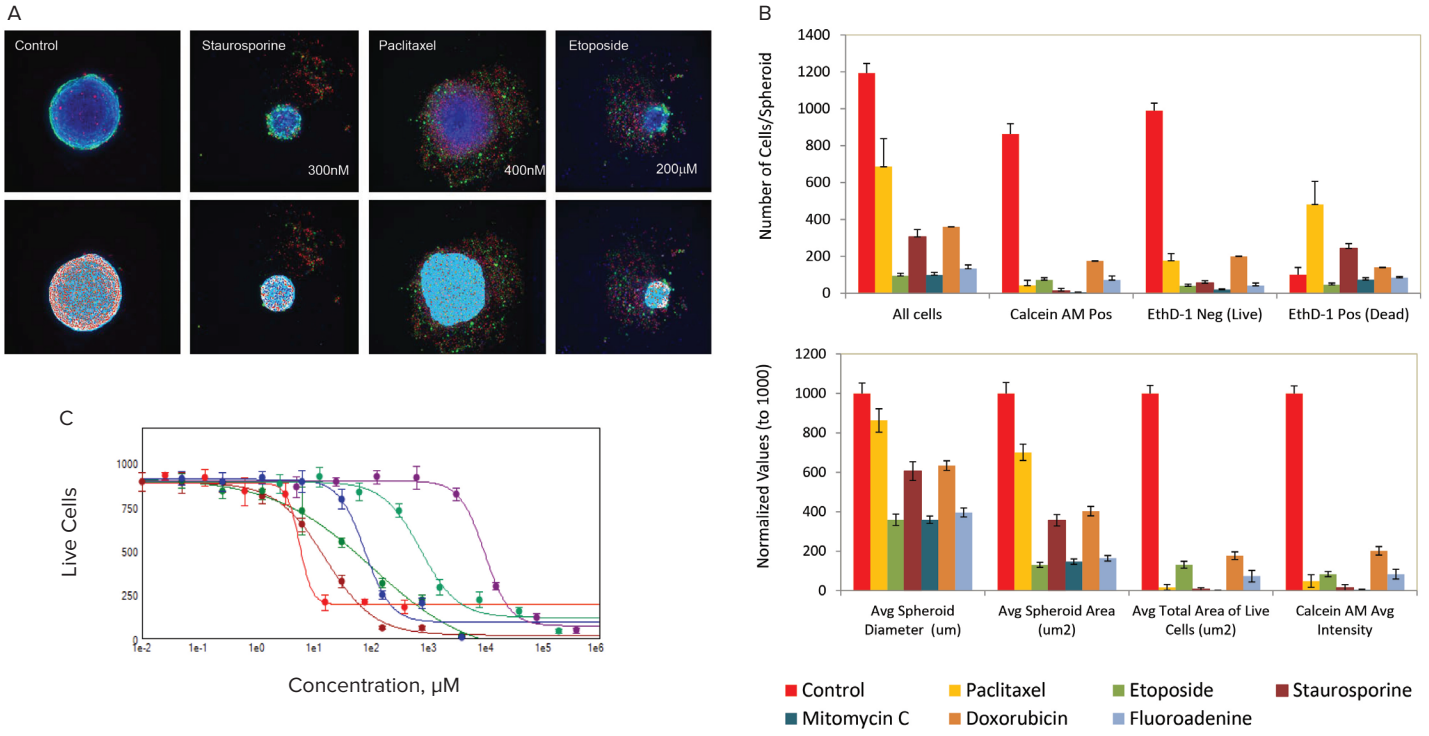

Figure 1. Maximum projection images of spheroids representing various phenotypes. (A) Image analysis read-outs derived as a result of Nuclei Count and Cell Scoring analysis. **(B)** Bar graphs: control (0.1% DMSO), paclitaxel 150 nM, etoposide 200 µM, staurosporine 300 nM, mitomycin C 1 µM, doxorubicin 1 µM, fluoroadenine 100 µM. Geometric or average intensity values were normalized to DMSO controls (set to 1000). **(C)** Concentration–dependent effects and 4-parameter curve fits of selected compounds. Red-paclitaxel, dark red-staurosporine, blue-doxorubicin, green-mitomycin C, teal-etoposide, purple-fluoroadenine.

Whole organisms

Whole organisms provide the highest relevance to human biology and are used to study entire, interactive systems. These models can be used to predict treatment effects in humans due to their inherent complexity. Compared with cultured cell-based assays, screens utilizing whole organisms allow for more comprehensive evaluation of biological responses by accounting for the influence of multiple cell types interacting within a complete system. For example, the zebrafish model has gained favor as an alternative to mammalian systems for drug profiling due to improved cost, throughput, and reduced ethical concerns. We used the ImageXpress Micro system and MetaXpress software to automate imaging screens of zebrafish, examining changes in angiogenesis, gene expression, ototoxicity, and neurotoxicity.

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View 3D Rotation of a Zebrafish

- Visualize and measure whole-body phenotypes in a single image
- Generate crisply focused 2D and 3D images of whole organisms with automated, high speed Z stacking
- Evaluate thousands of compounds in a few days using high-content imaging and 3D analysis software

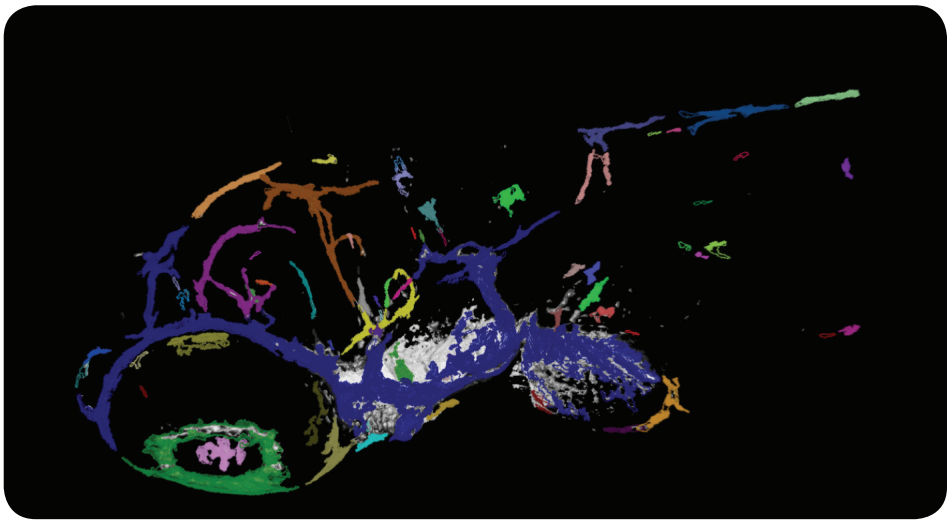

Figure 2. 3D segmentation tools can be used to connect touching fluorescent objects within the volume of the zebrafish sample and characterize those objects by intensity, shape, and size.

Stem cells

Stem cells provide an accessible source of primary-like cells that can be used in long-term studies to measure targeted responses in specific cell types and 3D tissues. Complex assays, such as 3D induced pluripotent stem cell (iPSC)-derived models, better represent tissue biology and cell interactions, improving the relevance of many toxicity and drug screening assays.

In one experiment, we used confocal imaging and 3D image analysis to characterize cellular information from a 3D matrix, yeilding a multi-parametric comparison of different spheroid phenotypes. We calculated IC_{50} values of compounds tested generated using 2D and 3D models to identify differences in the assay sensitivity to compound-induced effects. Analysis of compound toxicities included spheroid size (volume) and shape, cell number and spatial distribution, nuclear condensation, and intensities of cellular markers for viability, apoptosis and mitochondrial potential.

- Optimize image acquisition and analysis methods for multi-parametric characterization of spheroid phenotypes and determination of IC_{50} values
- Accelerate discovery by enabling rapid compound prioritization for *in vivo* testing and defining mechanisms of action
- Enhance development of relevant cell-based assays for efficient assessment of the hepatotoxicity of chemicals and drug candidates in high-throughput quantitative screening

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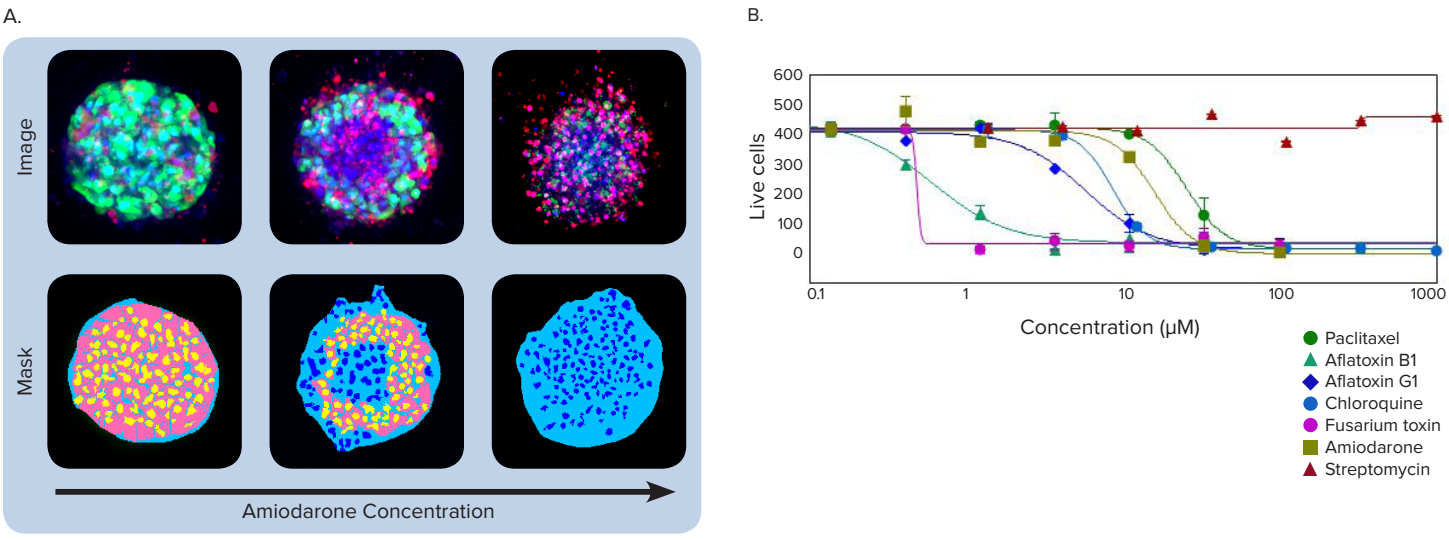

Figure 3. Phenotypic changes in the spheroids after compound treatment. (A) iPSC-derived hepatocyte spheroids treated with increasing concentrations of hepatotoxic compound (amiodarone) and stained with viability markers calcein AM (green), Hoechst (blue), and EthD-1 (red). Image analysis masks are shown below the composite images. **(B)** Dose-response curves for selected compounds.

Subcellular assays

Confocal imaging is commonly utilized for accurate identification of individual puncta, particularly in assays where co-localization of nuclear markers is measured. Assessing the co-localization of distinct markers can provide important insights into the biological pathways involved in a compound's mechanism of nuclear toxicity. Utilizing confocal imaging in combination with these assays provides rejection of out-of-focus light and results in improved signal-to-background ratios, thereby increasing assay robustness.

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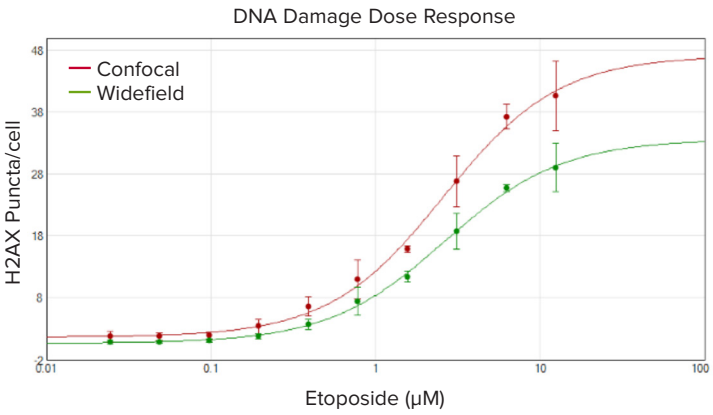
- Quantify genotoxicity using automated imaging and pre-configured software analysis modules
- Increase sensitivity to acquire high signal-to-background, clear images
- Measure co-localization of cellular markers to determine mechanisms of DNA damage
- Count individual puncta accurately using confocal imaging

A



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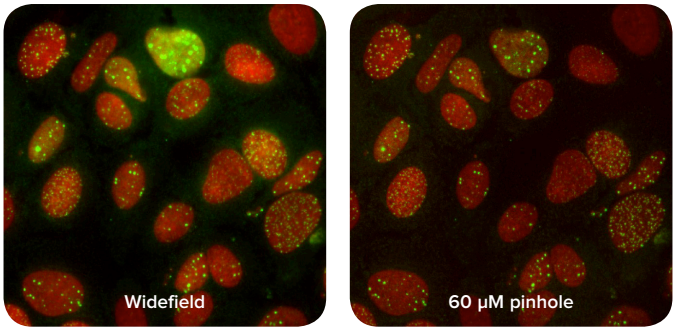

C

Parameter measured	Signal/Background		Z' Factor	
	Widefield	Confocal	Widefield	Confocal
Puncta count (H2AX)	2.6	8.3	0.17	0.57
Puncta area/ cell (H2AX)	10.2	19.6	0.42	0.54

Figure 4. A. Graph comparing DNA damage responses to etoposide treatment using confocal (red) vs. widefield (green) imaging. The number of H2AX-stained puncta per cell were plotted. **B.** Images acquired with a 40X PA (0.95NA) objective in confocal mode (right) illustrate improved nuclear puncta visibility and reduced background fluorescence compared to widefield images (left). **C.** The confocal images yielded a more robust assay, as reflected in the higher signal/background ratios and higher z' factors presented in the table.

3D imaging and analysis solutions

We offer a complete solution for quantitative 3D assays with reliable, high-throughput imaging instrumentation, sophisticated image analysis tools, and comprehensive data analysis software designed for handling multi-plate screening data. For further information, select the images or text below.

ImageXpress Micro Confocal High-Content Imaging System

MetaXpress High-Content Image Acquisition and Analysis Software

AcuityXpress High-Content Informatics Software

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