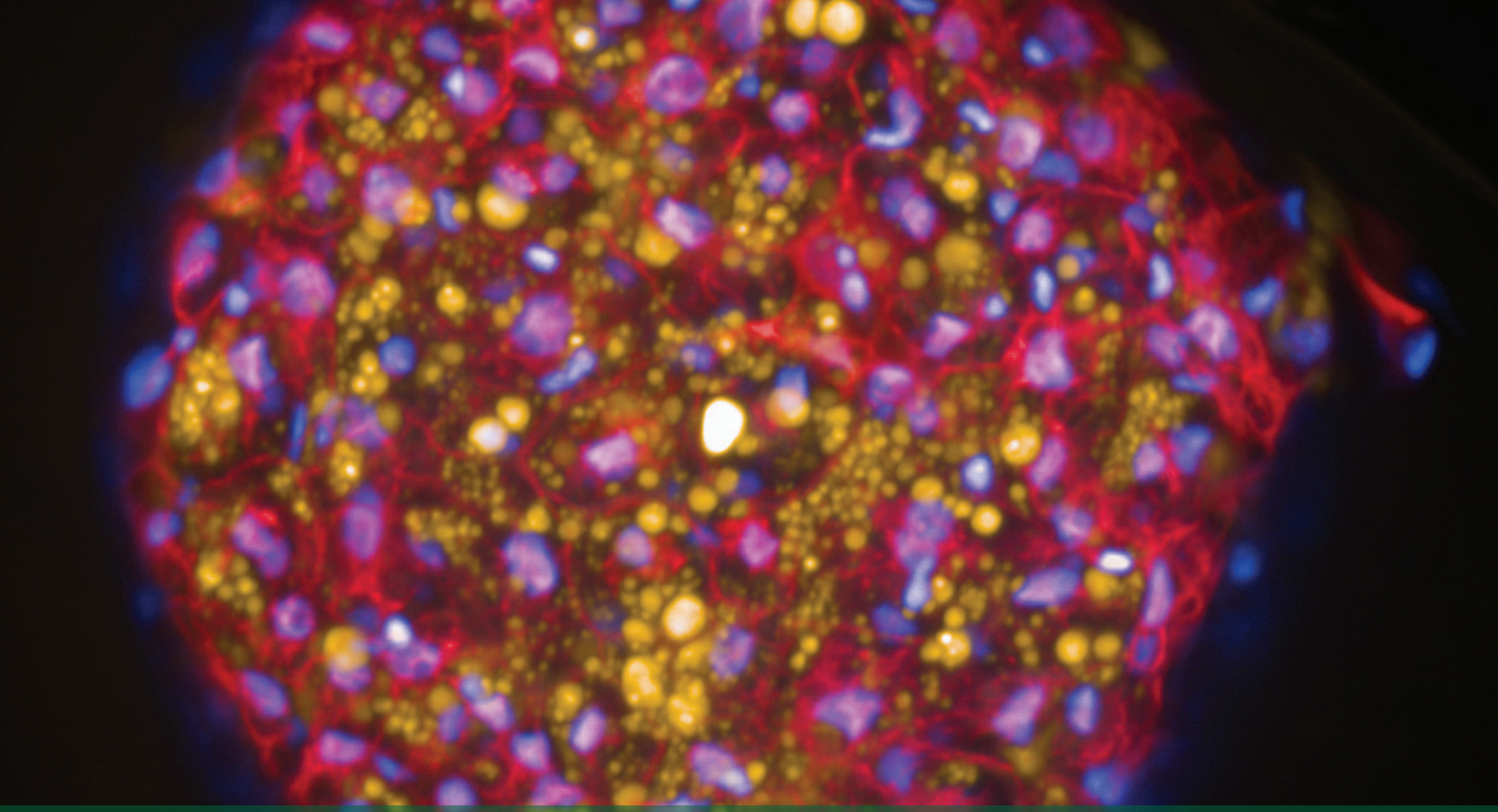
SOLUTIONS FOR 3D CELL CULTURES

- Microplates and Reagents
- High-Content Imaging Systems
- High-Content Software and Informatics

Solutions for 3D Cell Cultures

For research use only. Not for use in diagnostic procedures.

PerkinElmer®
For the Better



3D CELLULAR MODELS ARE THE SHAPE OF THINGS TO COME

Researchers are increasingly looking to 3D cell cultures, microtissues, and organoids as a way to bridge the gap between 2D cell cultures and *in vivo* animal models. That's because 3D models provide more physiologically relevant conditions than biochemical assays and 2D cell cultures, as they more closely mimic the microenvironments, cell-to-cell interactions, and biological processes that occur *in vivo*. Plus, they show a higher degree of morphological and functional differentiation – again, similar to *in vivo* cell characteristics.

But there are challenges to this technology, and you'll need the right tools to overcome them: Growing consistent, reproducible 3D cultures can be problematic, and imaging large, thick cell samples can be extremely difficult – while handling the huge volumes of data these 3D cell experiments produce could be the most pressing challenge of all. [1]

With our best-in-class solutions, you can grow, detect, and analyze 3D cell cultures – and begin generating more physiologically relevant data that can power better-informed decisions.

GROW

Carefully designed microplate technologies for producing consistent 3D cultures – time after time

DETECT

Sensitive detection and high-content imaging solutions for generating high-quality data

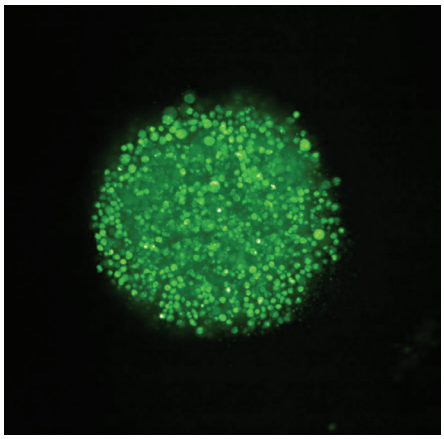
ANALYZE

Powerful, intuitive informatics solutions for handling high volumes of data and uncovering insights

CONSISTENT SPHEROIDS FOR REPRODUCIBLE RESULTS

Specialized cell models require highly specialized methods and tools. And for consistent, reproducible results, you need to grow uniform 3D cultures to minimize batch-to-batch variability.

That's why we offer a variety of microplates and advanced surface coatings to meet the needs of unique cell types and applications.



Spheroid tissue formed over three days in the 96-well CellCarrier ULA plate imaged on the Opera Phenix system.

MICROPLATES

CellCarrier™ Spheroid ULA Microplates

These ultra low-attachment microplates are ideal if you need an economical method to grow uniform spheroids and have no coculturing or media-exchange requirements. The synthetic plate coating ensures minimal cell-plate adhesion, which promotes uniform, single-spheroid formation.
(Product No. 6055330, 10 pack; 6055334, 40 pack)

CellCarrier Spheroid ULA

InSphero GravityTRAP™ ULA Microplates

Ideal for long-term cultivation, these plates use a SureXchange™ tapered ledge that facilitates easy medium exchange and prevents microtissue loss. The flat-bottom observation chamber enables brightfield and fluorescent microscopy for measuring size and function. *(Product No. ISP-09-001)*

InSphero GravityPLUS™ Microplates

For outstanding size consistency, this hanging-drop plate features SureDrop™ microtechnology, with variation in diameter of 5% or less across an entire 96-well plate. Hanging-drop spheroid formation is recommended for cocultures and primary cells. *(Product No. ISP-03-001, 10 pack)*

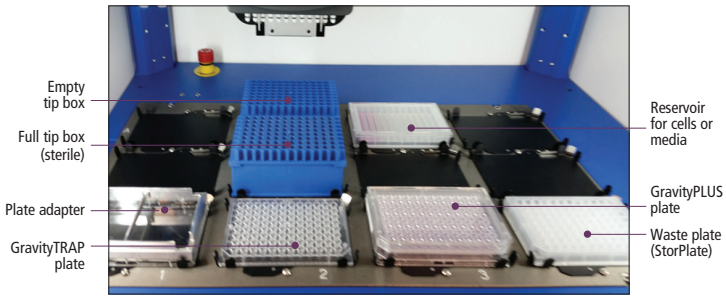
InSphero GravityPLUS Hanging-Drop System

InSphero GravityPLUS Hanging-Drop System

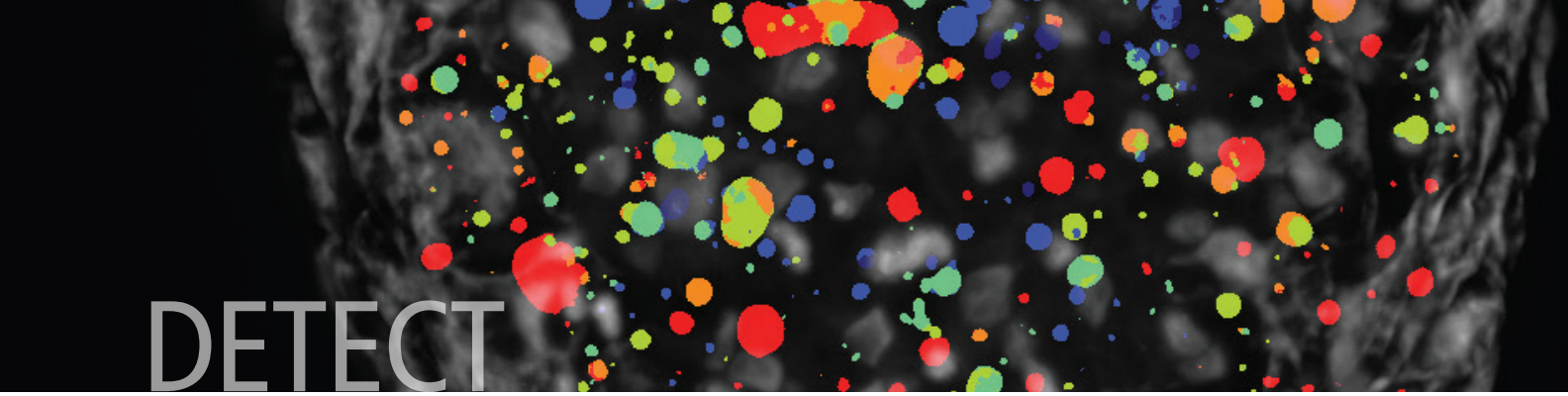
This system includes the GravityPLUS plate for the formation of spheroids in a hanging-drop environment, combined with the GravityTRAP ULA plate for spheroid capture and analysis. *(Product No. ISP-06-010, 3 pack; ISP-06-001,10 pack)*

AUTOMATION

Our liquid-handling automation workstations enable you to seed and transfer 3D cell cultures into plates for cellular imaging and long-term culture. With the Zephyr™ workstation, the deck can be set up to automate pipetting steps: initial cell seeding into the GravityPLUS hanging-drop plate, media exchange, prepping the GravityTRAP culture plate, harvesting from the hanging-drop plate, and transferring into the culture and assay plate.[2]



Zephyr G3 Automated Workstation



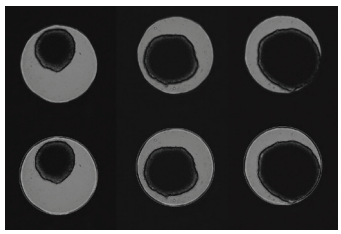
DETECTION SOLUTIONS THAT SHED MORE LIGHT ON YOUR CELLS

There are many ways to measure the biological response of 3D models to treatments, and the choice of method depends on the question asked and the specifics of the model system used. Parameters such as microtissue size can be repeatedly monitored using brightfield imaging or determined via robust endpoint assays. More complex functions can be measured by sensitive detection of secreted proteins or detailed analysis of spheroid structure and organization. Each of these readouts poses its unique challenges that can be overcome with the right tools. Our line of sensitive, yet powerful microplate readers and high-content analysis systems, plus innovative reagents, is designed for 3D cell culture detection, and together with our specially designed microplates, can shed more light on to your sample. The result? Better results – every time.

MULTIMODE DETECTION WITH IMAGING

EnSight® Multimode Plate Reader

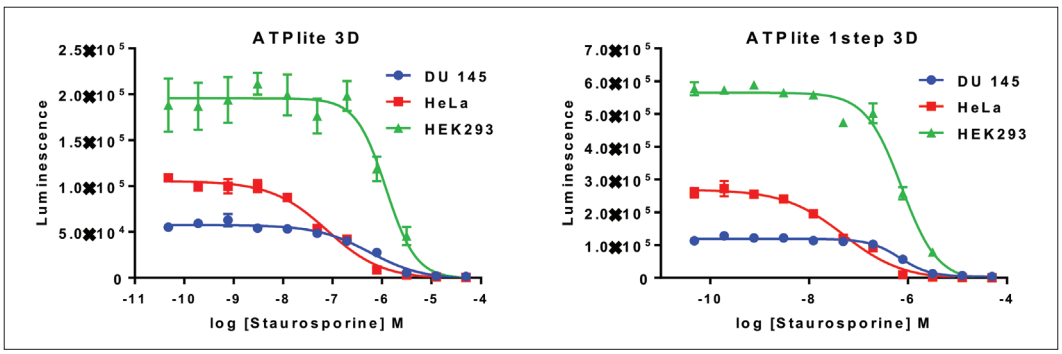
The EnSight multimode plate reader combines multiple fluorescence and luminescence detection technologies with image cytometry in a single benchtop platform. Imaging is fast – in fact, a 384-well plate can be imaged and analyzed in less than five minutes. With the EnSight reader, you can monitor the growth and health of your 3D cell cultures by measuring size, shape, and more. Plus, you can combine imaging with other technologies such as AlphaLISA® to detect secreted proteins.[3,4]



Spheroids imaged in GravityTRAP plate on the EnSight multimode plate reader and measured to determine their size.

Luminescence Reagents

Our ATPlite™ 3D and ATPlite 1step 3D reagent kits for cell viability and proliferation provide a simple, robust protocol for ATP-content endpoint measurements of 3D spheroids. The protocol ensures reliable spheroid lysis and works directly in the culture plate, avoiding issues caused by transferring samples to luminescence-compatible multiwell plates, making the assay automation-friendly. For best performance, use with the EnSight or any other system from our family of plate readers.

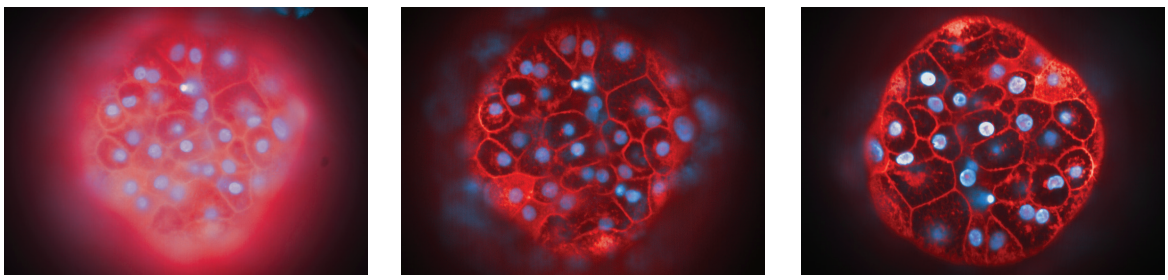


Cytotoxicity study of DU 145, HeLa, and HEK293 spheroids treated with staurosporine, using CellCarrier™ Spheroid ULA and InSphero GravityTRAP microplates, ATPlite 3D and ATPlite 1step 3D assays, and EnSight plate reader.[5]

HIGH-CONTENT ANALYSIS SYSTEMS

Designed with 3D cell models in mind, our high-content analysis systems let you quickly and easily generate content-rich, physiologically relevant data from 3D samples with enabling technologies:

- **Spinning-disk confocal microscopy** enables you to rapidly acquire image stacks with improved signal-to-noise ratios and high X, Y, and Z resolution. Pinholes in the confocal plane allow only light from the focal plane to pass while rejecting out-of-focus light. Images can be acquired at very high frame rates with minimal sample illumination, making spinning-disk confocal microscopy ideal for imaging 3D spheroids and live samples at high speed with minimal photobleaching.
- **Water immersion objectives** allow for higher numerical apertures than air objectives, so they capture up to four times more light and provide a higher resolution in X,Y, and Z. That means you get more detail faster and can image deeper into 3D structures, and delicate live-cell samples can be imaged with less photodamage.



40xhNA NA 0.75 widefield | 40xhNA NA 0.75 confocal | 40xW NA 1.1 confocal

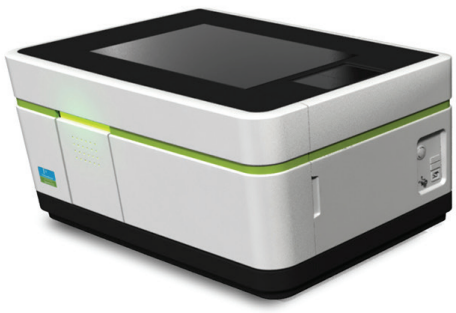
Confocal microscopy (middle and right) captures higher resolution images than nonconfocal (left), and water immersion (right) provides even greater detail, as shown by these images of 3D InSight™ Human Liver Microtissues from InSphero labeled with Hoechst (nuclei, blue) and CellMask™ Deep Red plasma membrane stain acquired on the Operetta CLS system.[6]

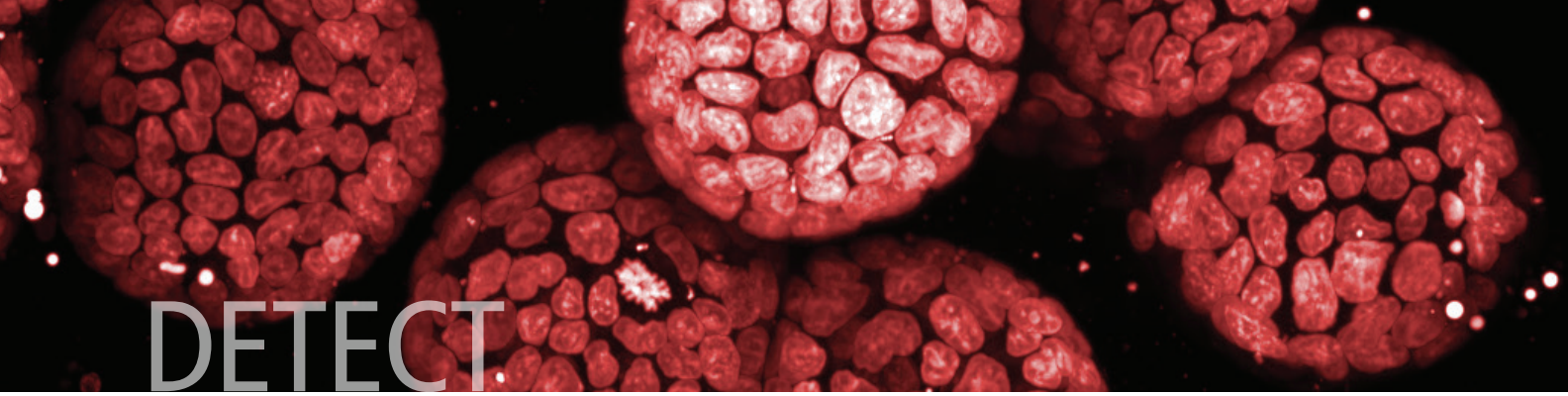
Operetta CLS™ High-Content Analysis System

This powerful imaging solution lets you uncover deep biological understanding with its unique combination of technologies – automated water-immersion objectives, eight high-power excitation LEDs, true confocal optics, and an ultrasensitive sCMOS camera – providing flexibility, sensitivity, and resolution for 3D imaging.

Opera Phenix™ High-Content Screening System

Here is the premier confocal solution for 3D high-content screening. Its Synchrony Optics™ enables simultaneous multicolor confocal image acquisition for speed and sensitivity – no compromise. It features a microlens-enhanced dual-disk design with a pinhole distance optimized for thick samples such as 3D cell models, and dual-field excitation that separates excitation of neighboring spectral channels in space and time. The result is fast multichannel 3D acquisition at high image quality.

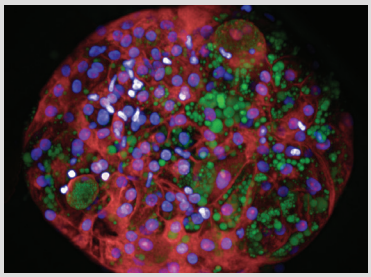




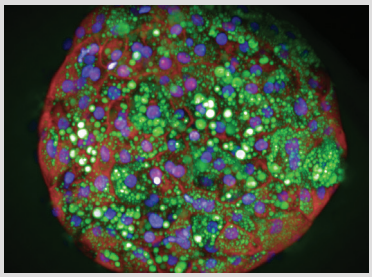
DETECT

PHENOTYPIC HIGH-CONTENT ASSAY TO STUDY STEATOSIS IN 3D LIVER SPHEROIDS

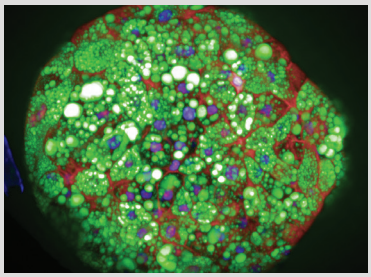
Several drugs are associated with the potential for drug-induced hepatic steatosis. In this example, lipid accumulation is analyzed in InSphero's 3D InSight™ Human Liver Microtissues labeled with Hoechst (blue, nucleus), CellMask Deep Red (red, membranes), and NileRed (green, lipid droplets). Stack imaging was performed on the Opera Phenix system using a 20xW objective.



Control



Low concentration of Oleic Acid

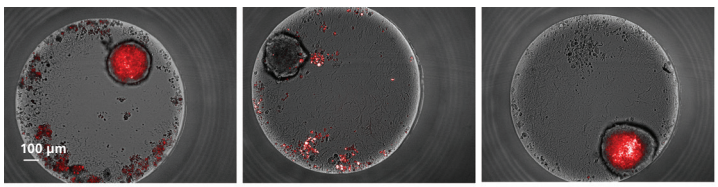


High concentration of Oleic Acid

Functional NIR Agents

Designed for *in vivo* preclinical imaging, our near-infrared (NIR) fluorescent agents such as ProSense 680 and HypoxiSense 680 can be used with our Opera Phenix and Operetta CLS high-content analysis platforms. These NIR solutions are invaluable in oncology, where the same tumor model can be investigated both *in vitro* and as xenograft *in vivo*. We offer both targeted and activatable NIR agents with excitation maxima below 700 nm, making them well suited for a variety of *in vitro* HCS applications.

ProSense 680 | MMPSense 680 | HypoxiSense 680



Overlay of brightfield and fluorescence images of 3D InSight™ Human Tumor Microtissues stained with ProSense 680, MMPSense 680, and HypoxiSense 680 results in characteristic staining patterns.[7]

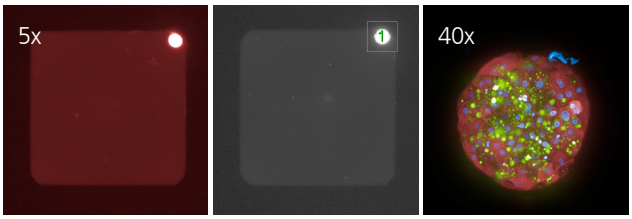
INFORMATICS SOLUTIONS THAT TAKE YOU FROM IMAGE TO INSIGHT

Harmony® Imaging and Analysis Software

Simple, yet powerful, Harmony software drives the Operetta CLS and Opera Phenix HCS systems. It provides a complete solution for 3D cell culture models with an intuitive, automated workflow-centric interface to set up and run automated 3D imaging assays, visualize images in 3D, export 3D movies, and store, retrieve and present results in meaningful ways.

Speed Up 3D Image Acquisition

With the PreciScan feature, you can prescan at low magnification to locate where spheroids have grown, then automatically rescan at higher magnification with the spheroid centered in the image. This technology saves significant acquisition and analysis time, as well as data storage space.



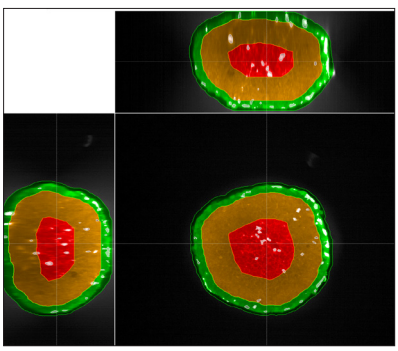
Step 1. Pre-scan at low magnification

Step 2. Identify object of interest

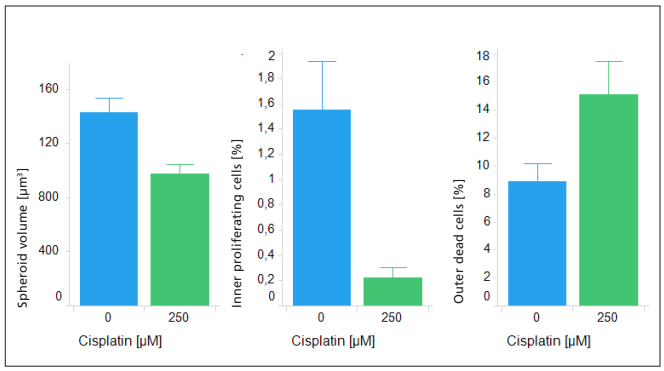
Step 3. Re-scan at high magnification

Easily Quantify Cellular Phenotypes – In Complex 3D Models

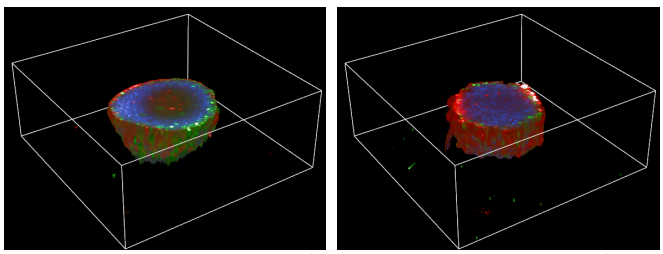
You can explore cell models by visualizing them in a 3D viewer and an XYZ viewer. Plus, you can measure morphology, volume, and texture in 3D; count nuclei within spheroids; and calculate XYZ positional properties. You can also quickly analyze your z-stack as maximum intensity projection, with 3D information preserved, using PlaneMap technology.



XYZ view showing positional analysis of dead cells using ring regions.
Green: Outer region
Orange: Inner Region
Red: Core Region



3D analysis of Cisplatin treated cancer spheroids shows that Cisplatin reduces the volume of 3D spheroids, reduces the percent of proliferating cells in the inner region, and increases the percent of dead cells in the outer and core regions (data not shown for core region).



3D view of cancer spheroids (cut-open) labeled with Hoechst (all cells: blue), LIVE/DEAD™ fixable cell stain (dead cells: red), and Ki-67 (proliferating cells: green), cleared with Visikol® HISTO-M™. Left: untreated; Right: Cisplatin treated.

Make Better Decisions Sooner

Export your results automatically into the **Columbus®** image data storage and analysis system, so you can access, reanalyze, and share image data from Opera Phenix and other HCS systems across your organization. You can also use **High-Content Profiler™**, powered by TIBCO Spotfire® software, to aggregate data, perform data QC and normalization, and perform multiparametric phenotypic analysis.

Image acknowledgments:

1. Inside cover / page 2 - 3D InSight™ Human Liver Microtissue, InSphero AG, labeled with Hoechst, CellMaskDeepRed and NileRed to analyze steatosis.
2. Page 4 - 3D InSight™ Human Liver Microtissue, Insphero AG, labeled with NileRed; lipid droplets pseudocolored in Harmony software according to z-height.

References:

1. Kriston-Vizi, J; Flotow, H (2017): *Getting the Whole Picture: High Content Screening Using Three-Dimensional Cellular Model Systems and Whole Animal Assays.* Cytometry A. Feb;91(2):152-159.
2. Greve, F; Fluri, D; Hinterneder, J.M; Garbow, N; Gerwe, B (2016): Application Note – *Automation of 3D Spheroid Production, Cell Culture and Analysis.*
3. Amin, J; Ramachandran, K; Williams, S.J; Lee, A; Novikova, L; Stehno-Bittel, L. (2016): *A simple, reliable method for high-throughput screening for diabetes drugs using 3D β-cell spheroids*, J. Pharmacol. Toxicol. Methods. 82:83-89.
4. Liu, FF; Peng, C; Escher, BI; Fantino, E; Giles, C; Were, S; Duffy, L; Ng, JC. (2013): *Hanging drop: An in vitro air toxic exposure model using human lung cells in 2D and 3D structures*, J. Hazard Mater. 261:701-710.
5. Hinterneder, J.N; Hurt, S; Hellmer, R; Dupriez V. (2017): Poster - *Rapid, reliable measurement of cellular proliferation and toxic compound effects on 3D spheroid cultures grown from multiple cancer cell lines with ATPliteTM 3D and ATPlite 1step 3D*. SLAS 2017.
6. Böttcher, K; Schreiner, A. (2017): Technical Note – *The Benefits of Automated Water Immersion Lenses for High-Content Screening.*
7. Waschow, M; Fassler, M; Böttcher K; Kelm, J. (2012): Application Note – *Quantitative Analysis of 3D Microtissue Growth and Biomarker Intensity.*

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013219B_02 PKI