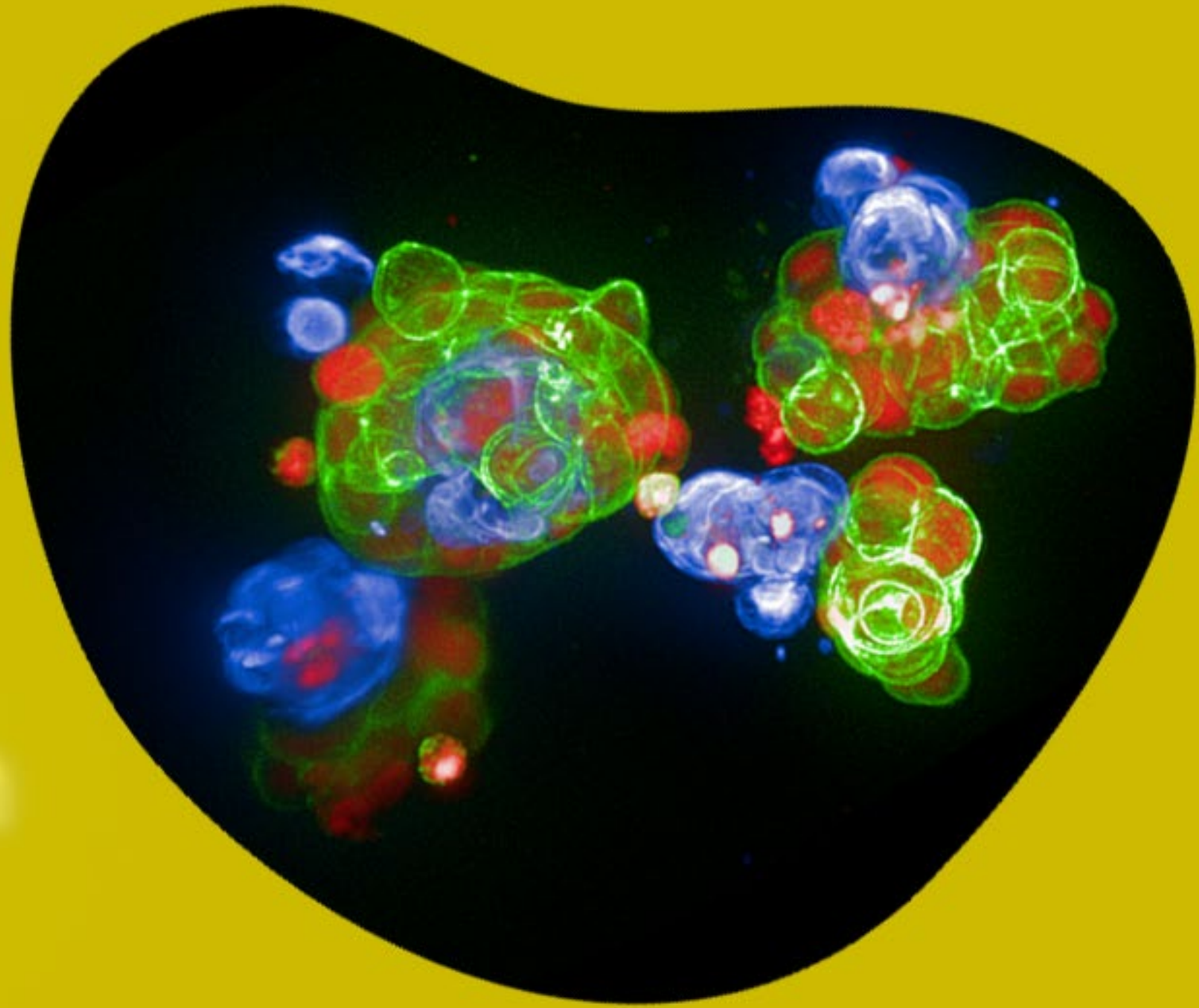
# Harness the power of 3D.



3D high-content screening

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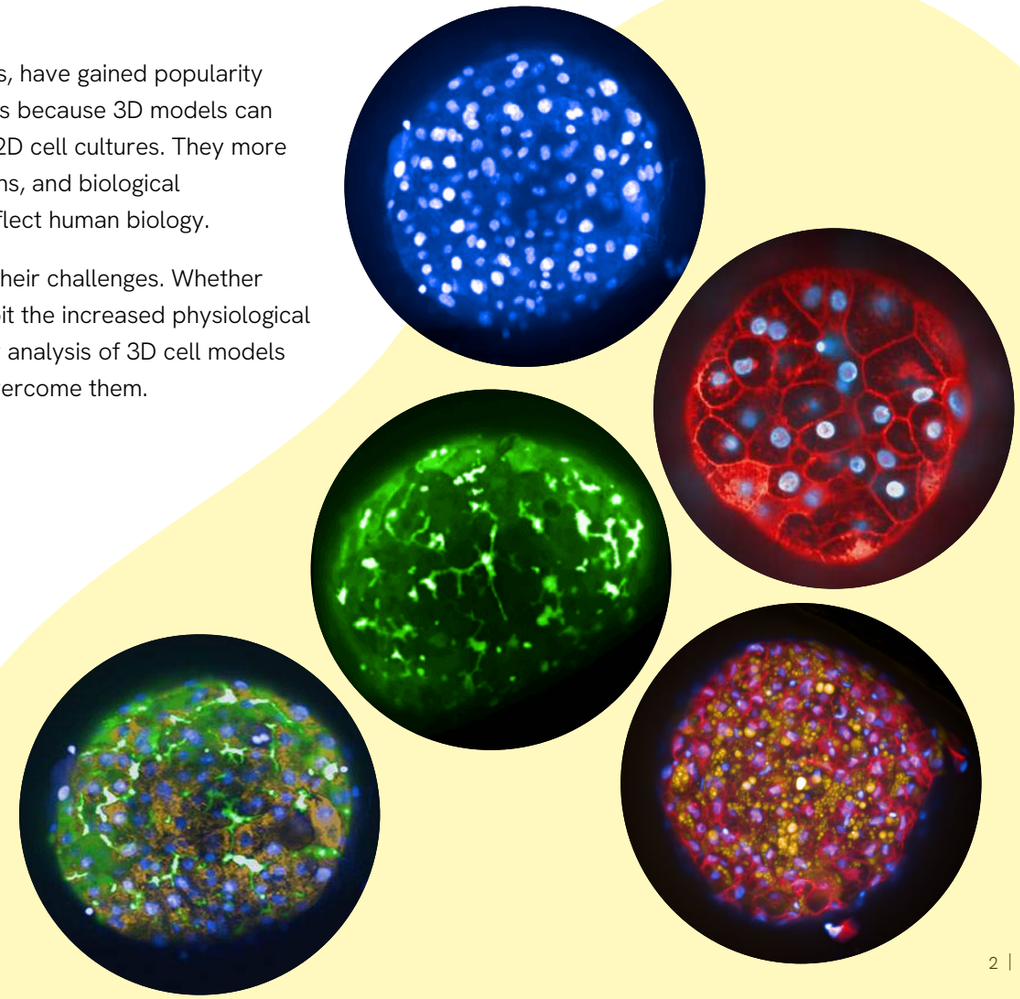
# Master 3D cell model imaging

3D cell cultures, such as spheroids, organoids, and microtissues, have gained popularity as tools for research, drug discovery and toxicity studies. That's because 3D models can be more physiologically relevant than biochemical assays and 2D cell cultures. They more closely represent the microenvironments, cell-to-cell interactions, and biological processes that occur *in vivo*, providing data that may better reflect human biology.

3D cell model imaging and analysis workflows are not without their challenges. Whether you're familiar with high-content screening and looking to exploit the increased physiological relevance of complex 3D cell models, or you want to take your analysis of 3D cell models to the next level, you'll need the right tools and strategies to overcome them.

**Explore this guide to learn more about:**

- Rapidly and confidently generating high-quality images from large, thick cell samples
- Efficiently reducing imaging times and handling large volumes of data
- Streamlining image analysis without the need for transfer between software



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# Your moment of clarity

High-quality images are crucial for successful high-content analysis (HCA). But capturing them isn't always easy for large, thick 3D structures such as spheroids. Revvity's imaging solutions can help address these challenges. Read on for tips about getting the best images for your analysis.

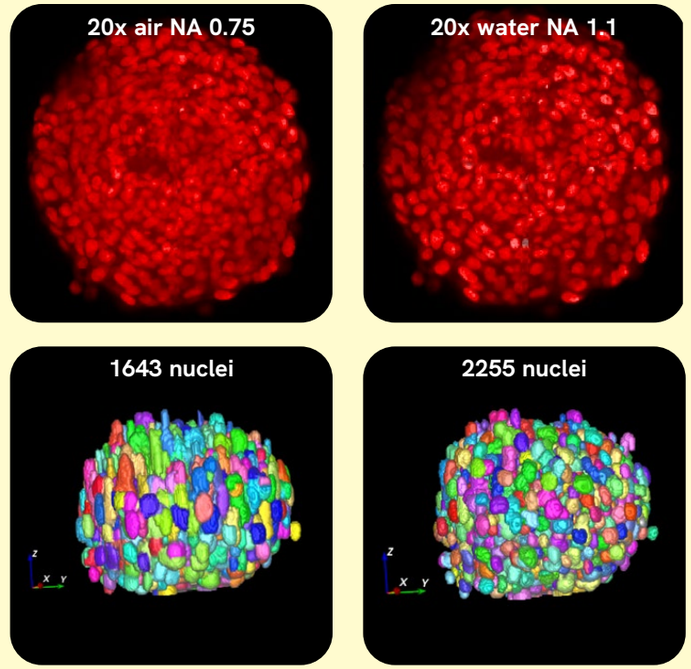
## Strategies for high-quality images

**Confocal imaging** removes the out-of-focus light, enabling optical sectioning of samples, with a better signal-to-noise ratio, and higher XYZ resolution than widefield imaging, while maintaining fast Z scans for high-throughput requirements.

**Water immersion objectives** capture up to four times more light than air objectives and provide higher XYZ resolution, to help you can resolve intracellular details even from deep within 3D structures.

**Spheroid sizes** up to approximately 500 µm diameter are advisable in order to image and analyze intracellular details.

**Optical clearing** of 3D cell models helps increase the amount of light getting inside the spheroids to excite fluorochromes as well as remove biomaterial that blocks the fluorescent signal reaching the cameras.

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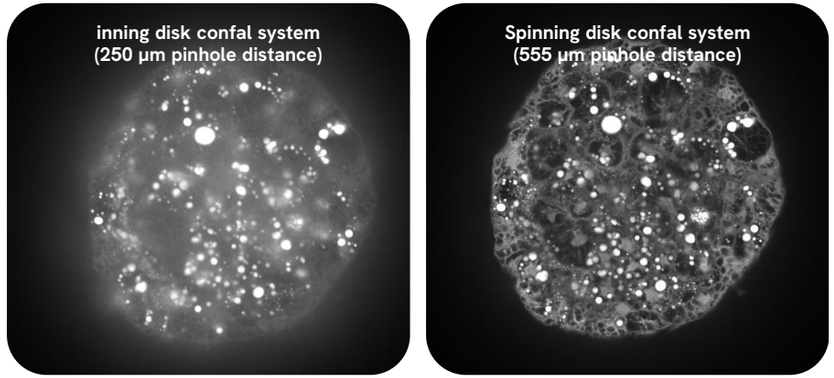
# Improved 3D imaging with confocal HCS systems

The advantage of using confocal spinning disk imaging is that it provides effective optical sectioning of 3D samples by reducing out-of-focus light. Early spinning disk confocal instruments like the Opera QEHS had pinhole-to-pinhole distances of 250 µm. However, thick 3D samples benefit from a wider pinhole distance up to 555 µm, as this approach allows light penetration while reducing the haze stemming from pinhole crosstalk.

See the difference in the images below. Steatosis microtissues were stained with CellMask™ DeepRed and Nile Red stains on two systems. The image on the right was achieved using the Opera Phenix spinning disk confocal system (555 µm pinhole distance), while the image on the left was produced using a 250 µm pinhole distance. Both images were taken using 40x water immersion objectives.




PRODUCT SPOTLIGHT

## Opera Phenix OptIQ™ system

Featuring capabilities that support 3D applications, this high-content screening system contains:

- Confocal scanning with 555 µm pinhole-to-pinhole distance for reduced crosstalk in thick specimens
- Proprietary Synchrony™ Optics, enabling effective z-sectioning and simultaneous acquisition of up to four channels with reduced crosstalk, supporting high-quality 3D imaging at high speed

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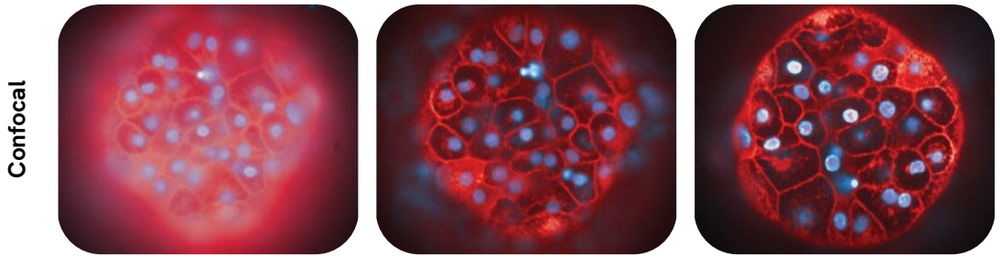
# The benefits of water immersion objectives for 3D HCS

Choosing an objective lens is critical to achieving high-quality images, and it's especially important for 3D cell models.

Water immersion objectives capture up to four times more light than air objectives, provide higher XYZ resolution, and match the refractive index of water in cells. The images can hlep you to resolve intracellular details even from deep within 3D structures.

The images below compare results using air and water immersion objectives. Human liver microtissue (single plane) was imaged on the Operetta™ CLS™ system in confocal mode with air and water immersion objectives. Observing the three images below, the 40x water immersion objective (40 x W) generated the sharpest image.

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## Water immersion objectives

Our water immersion objectives:

- Improve light collection
- Enhance 3D imaging
- Reduce bleaching

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# Clearing strategies for 3D spheroids

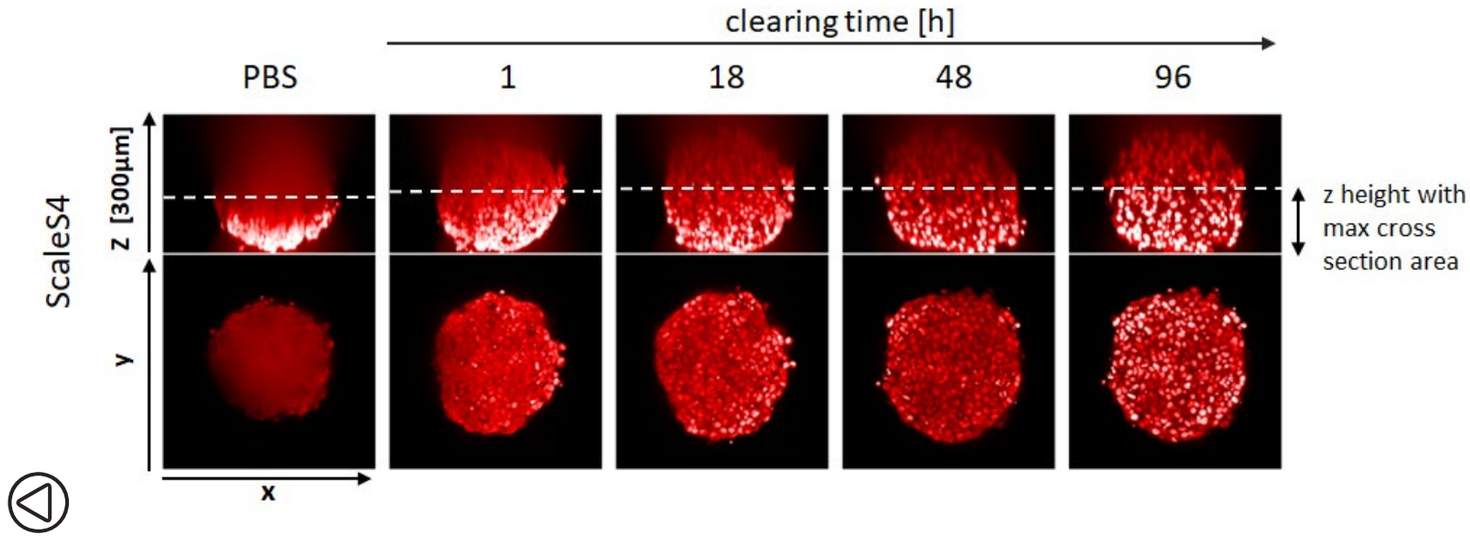
Reduced light penetration from increased light absorption and scattering properties can make it challenging to acquire 3D high-content images of solid spheroids. Optical clearing techniques are one solution to enhance imaging depth.

See the difference in the images below. They demonstrate that an optical clearing solution, in this case ScaleS4, can increase the imaging depth of spheroids over time. The same spheroid was imaged at four different time points after the addition of ScaleS4.

## Optical clearing solutions

These help enhance the imaging and analysis of your samples by:

- Removing lipid and protein molecules, which contribute to light scattering effects
- Modifying the refractive indices within the sample
- Increasing imaging depth of spheroids

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# Reduced imaging time, reduced data volume

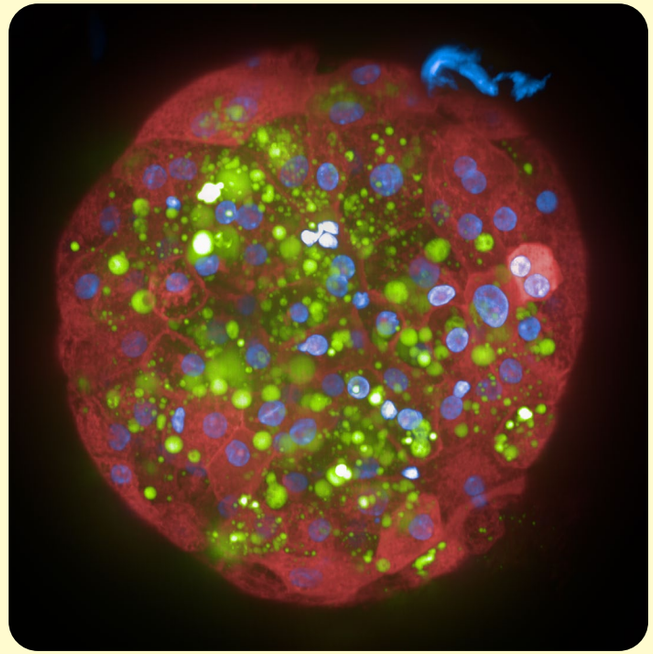
Some of the biggest challenges of 3D cell model imaging are long run times and large volumes of data. Often, only part of the total well area — specifically where the 3D model is situated — is of interest.

Learn more about saving time during imaging and acquiring high-resolution data from your region of interest:

**Reduce imaging time** with 3D cell models that require multiple planes and fluorescent channels by using a **spinning disk** confocal imaging system that combines laser-based excitation with two or four cameras and acquires images at high frame rates.

**Capture up to four times more light** with **water immersion objectives** than air objectives, to help reduce imaging times while still obtaining high-quality images.

**Reduce acquisition and analysis time and maintain data storage space** using intelligent technology that lets you **pre-scan** at low magnification to locate your 3D cell model, then automatically **rescans** at higher magnification with the object centered in the image.



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# Fast and intelligent image acquisition

For efficient high-content imaging and analysis, harness the power of pre-scanning and rescanning technology with PreciScan intelligent acquisition software.

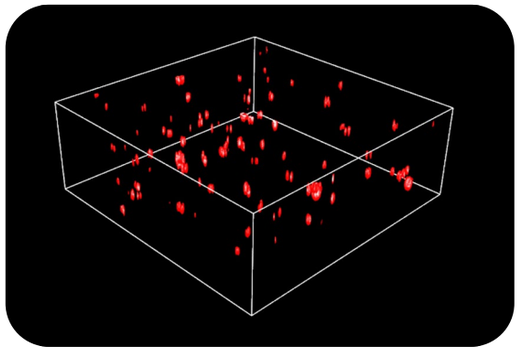
The PreciScan plug-in for Harmony™ HCA software uses intelligent image acquisition to pre-scan at low magnification to locate your object of interest, for example a spheroid. Then it automatically rescans it at a higher magnification with the object in the center of the image.

PRODUCT SPOTLIGHT

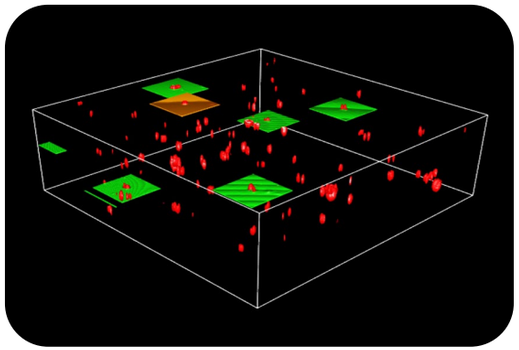
## PreciScan software

In 3D microtissue and rare event studies, this optional plug-in helps reduce acquisition and analysis times with:

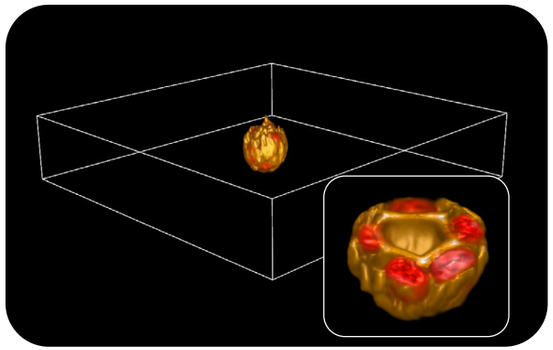
- A fully automated workflow of low-magnification pre-scan, image analysis, and higher-magnification rescan
- Targeted scanning of your object of interest



**1. Pre-scan organoids with low-magnification z-stack**



**2. Identify xy and z positions of organoids**



**3. Rescan individual organoids with high-magnification z-stack**

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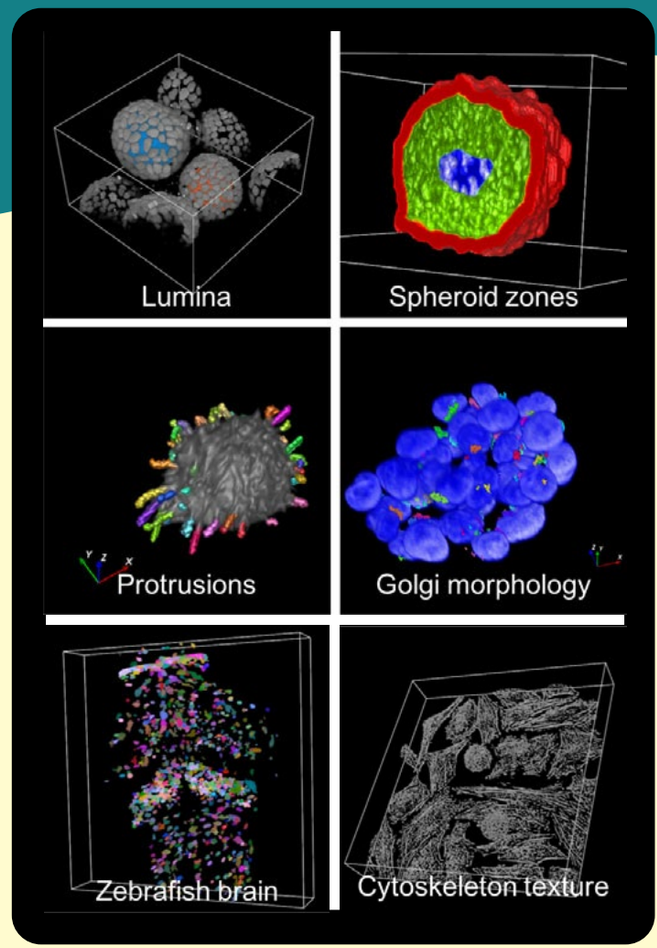
# The insights are in the details

If you're analyzing 3D cell models in 2D or 2.5D for simplicity and throughput, you could be missing valuable phenotypic information.




With our Harmony high-content imaging and analysis software, you can automatically analyze your 3D cell models in 3D, helping you get more from the time you've invested in developing your cell models. You can segment and quantitate volume, morphology, intensity, texture, positions, and distances in 3D, to better visualize and understand your 3D cell models and accelerate 3D image analysis.

**Examples of use include:**

- Evaluate cell killing and infiltration of immune cells on solid tumor models in immunotherapy studies
- Quantify the number of lumina inside 3D objects
- Analyze heterogeneity of your model system in situ
- Calculate the number of oligodendrocytes in a zebrafish brain
- Examine single cell morphologies, including protrusions and Golgi volumes, and visualize intricate 3D cytoskeleton textures

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# 3D volumetric analysis of luminal spaces inside cysts and organoids

Harmony high-content imaging and analysis software building blocks help provide a simple strategy for 3D analysis of spherical cysts and their hollow spaces.

The image below shows an example analysis strategy using these building blocks to calculate volumetric and positional properties for MDCK cysts, an extensively studied model system.

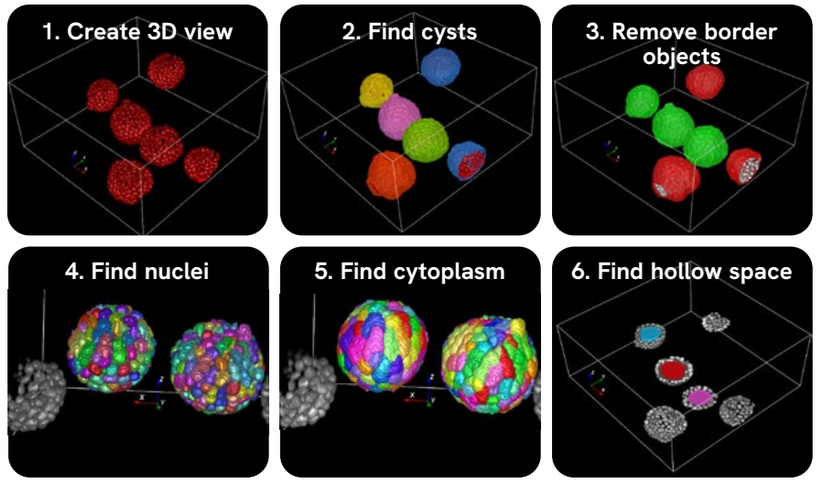
A similar approach can be applied for more complex cell models with luminal spaces, such as intestinal organoids.

PRODUCT SPOTLIGHT

## Harmony software

This intuitive software offers building blocks to help simplify your 3D high-content assays. You can:

- Efficiently perform 3D segmentation and analysis
- Define zones and quantify spatial differences within spheroids
- Create an efficient strategy for 3D analysis of spherical cysts




**7. Calculate cyst specific properties**

| Cyst centroid X in image [µm] | Cyst centroid Y in image [µm] | Cyst centroid Z in image [µm] |
|---|---|---|
| -117 | 13.1 | 47 |
| 86.5 | -38.5 | 66.6 |
| -1.7 | -22.5 | 58 |
| **Cyst volume [µm³]** | **Cyst surface area [µm²]** | **Cyst sphericity** |
| 168879 | 17877 | 0.83 |
| 217712 | 21292 | 0.82 |
| 254947 | 23239 | 0.84 |
| **Lumen volume [µm³]** | **Number of nuclei per cyst** | **Cell volume [µm³] – mean per cyst** |
| 33415 | 139 | 813 |
| 63350 | 130 | 1080 |
| 59776 | 179 | 922 |

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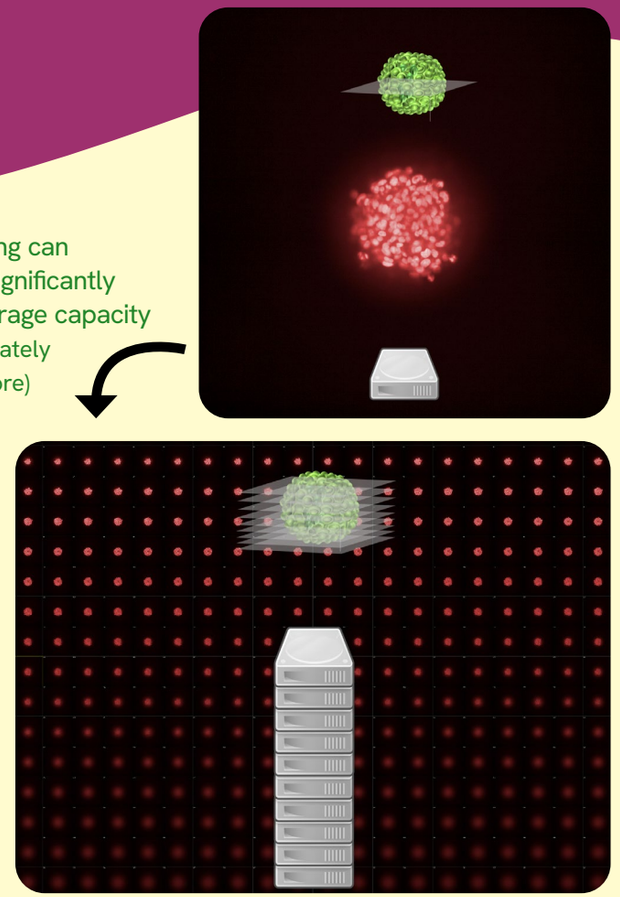
# Efficient data management and faster data insights

Working with large sample numbers in microplates means that 3D data sets are extremely large and processing the data can be laborious and time consuming. Automatic data transfer is important, so you can focus on what matters most — your research.

Cloud deployment options give you high-volume image data storage that's scalable and can support your lab. Using Revvity's Harmony imaging and analysis software and Image Artist platform, you can:

- Manage your data to help achieve faster insights
- Image, visualize, and analyze complex cellular models in 3D
- Acquire images at the same time data is being transferred
- Expand your cloud-based storage, as needed

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# 3D HCS in action

Explore the following links to see how Revvity HCS instruments are used to help advance the field of 3D high-content screening.

The following publications reference the use of Revvity instruments. Their inclusion does not imply endorsement by the authors.

**Comparison of Two Supporting Matrices for Patient-Derived Cancer Cells in 3D Drug Sensitivity and Resistance Testing Assay (3D-DSRT)**

Researchers describe the optimization and use of DSRT of patient-derived cancer cells in 3D growth-supporting matrices using HCS.

Read more

**A Microenvironment-Inspired Synthetic 3D Model for Pancreatic Ductal Adenocarcinoma Organoids**

Authors demonstrate the use of HCS in the development of a fully synthetic hydrogel extracellular matrix for studying pancreatic cancer cells *in vitro*.

Read more

**Advanced High-Content Screening Applications of Clonogenicity in Cancer**

Researchers showcase how they used HCS and PreciScan to quantify the number and size of clonogenic colonies in 2D and 3D culture models, highlighting how the methods shown have broad utility for HCS drug discovery.

Read more

**Machine Learning-Assisted High-Content Analysis of Pluripotent Stem Cell-Derived Embryos *In Vitro***

Researchers couple HCS with machine learning to analyze the ability of multiple cells lines to form ITS embryos and determine factors that could promote their generation.

Read more

**Pancreatic Cancer Organoids in the Field of Precision Medicine: A Review of Literature and Experience on Drug Sensitivity Testing with Multiple Readouts and Synergy Scoring**

Authors summarize their findings in using organoids and HCS to elicit important insights into designing the right personalized treatments for pancreatic cancer patients.

Read more

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# Imaging systems for 3D cell models

Our Operetta CLS and Opera Phenix OptIQ spinning disk confocal high-content imaging systems can help you quickly and easily generate content-rich, physiologically relevant data from diverse 3D samples.

Additionally, the Opera Phenix OptIQ system features a microlens-enhanced dual-disk design with a wide pinhole distance for thick samples such as 3D cell models.

*Operetta™ CLS™ high-content analysis system*

*Opera Phenix OptIQ™ high-content screening system*

### Automated water-immersion objectives

Capture up to four times more light than air objectives and provides higher resolution in X,Y, and Z, so you can image deeper into 3D structures and quickly gain more detail.

### Confocal spinning-disk technology

Well-suited for imaging 3D spheroids, this technology allows you to acquire image stacks with improved signal-to-noise ratios, high X, Y, and Z resolution, high frame rates, and reduced sample illumination.

### Intelligent image acquisition

Pre-scan at low magnification to locate spheroids, then automatically rescan at higher magnification with the spheroid centered in the image.

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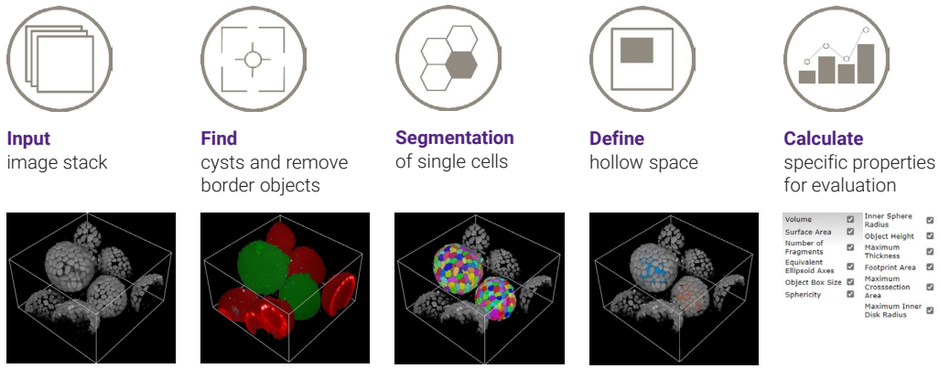
# Software made simple

Help simplify 3D image analysis with Harmony imaging and analysis software. Available for both Opera Phenix OptIQ and Operetta CLS system, this software can help you to:

- Speed up 3D image acquisition by targeted imaging of microtissues
- Better understand your cell models by exploring them in the 3D viewer and XYZ viewer
- Create Z stack overviews (or gallery)
- Produce 3D renderings and movies
- Analyze morphologies and volumes in 3D
- Calculate maximum intensity projections containing height profiles

As most datasets for 3D cell models are large, data from Harmony software and all major high-content screening and cell imaging systems can integrate with Image Artist, our next-generation image analysis and management platform.




MDCK epithelial cells cultured in a basement membrane matrix, where they spontaneously form cysts.

PRODUCT SPOTLIGHT

## Harmony high-content imaging and analysis software

Includes tools to assist in analyzing complex cellular models in 3D, reliably discriminate phenotypes, and turn your biological data into knowledge.

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## Image Artist high-content image analysis and management platform

Supports quick processing, analyzing, sharing, and storing large volumes of data generated by HCS and 3D cellular imaging, helping you move from data to insights.

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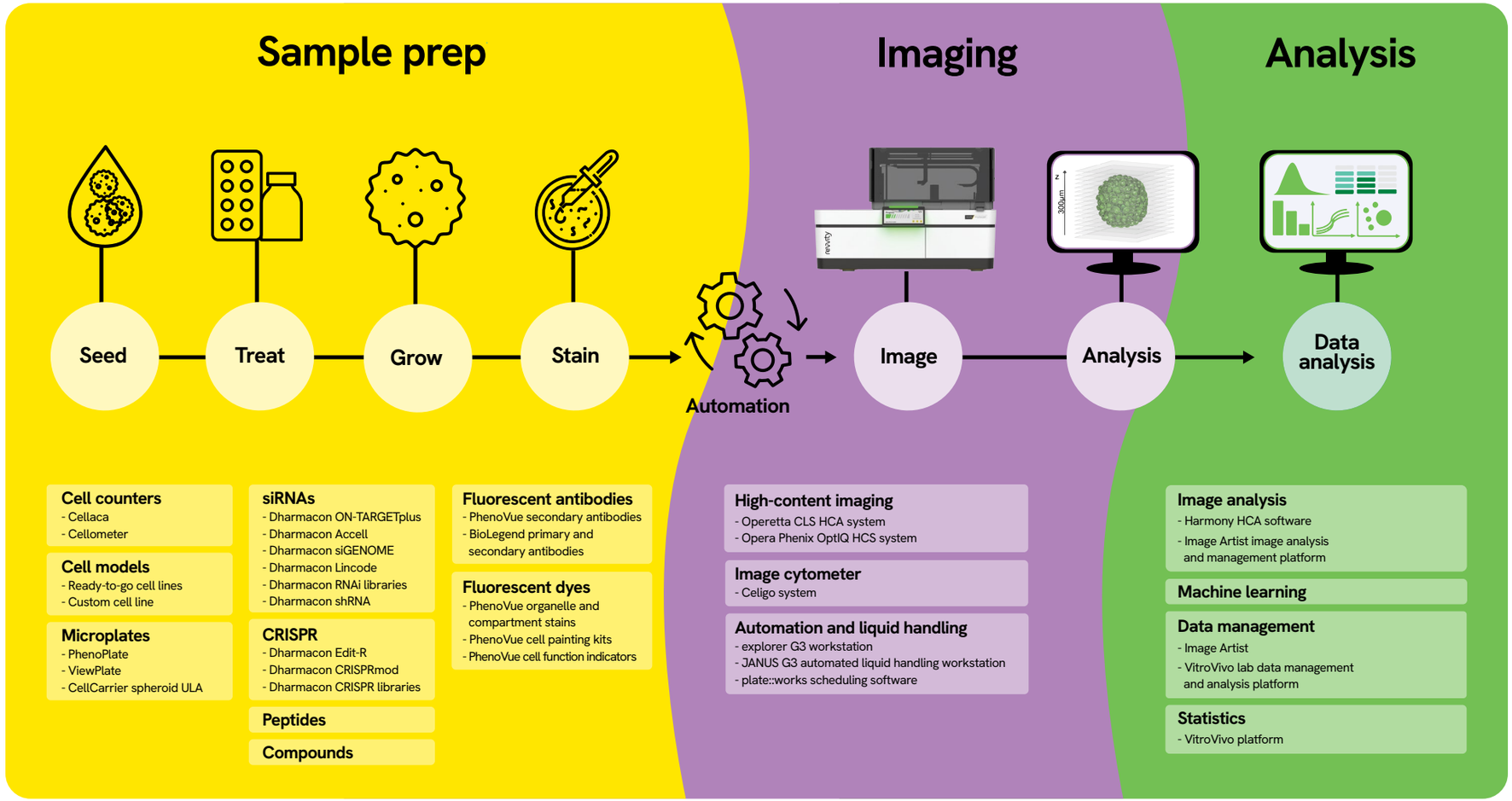
# Comprehensive HCS workflow solutions

Revvity offers solutions across the HCS workflow, from sample preparation to imaging and analysis.

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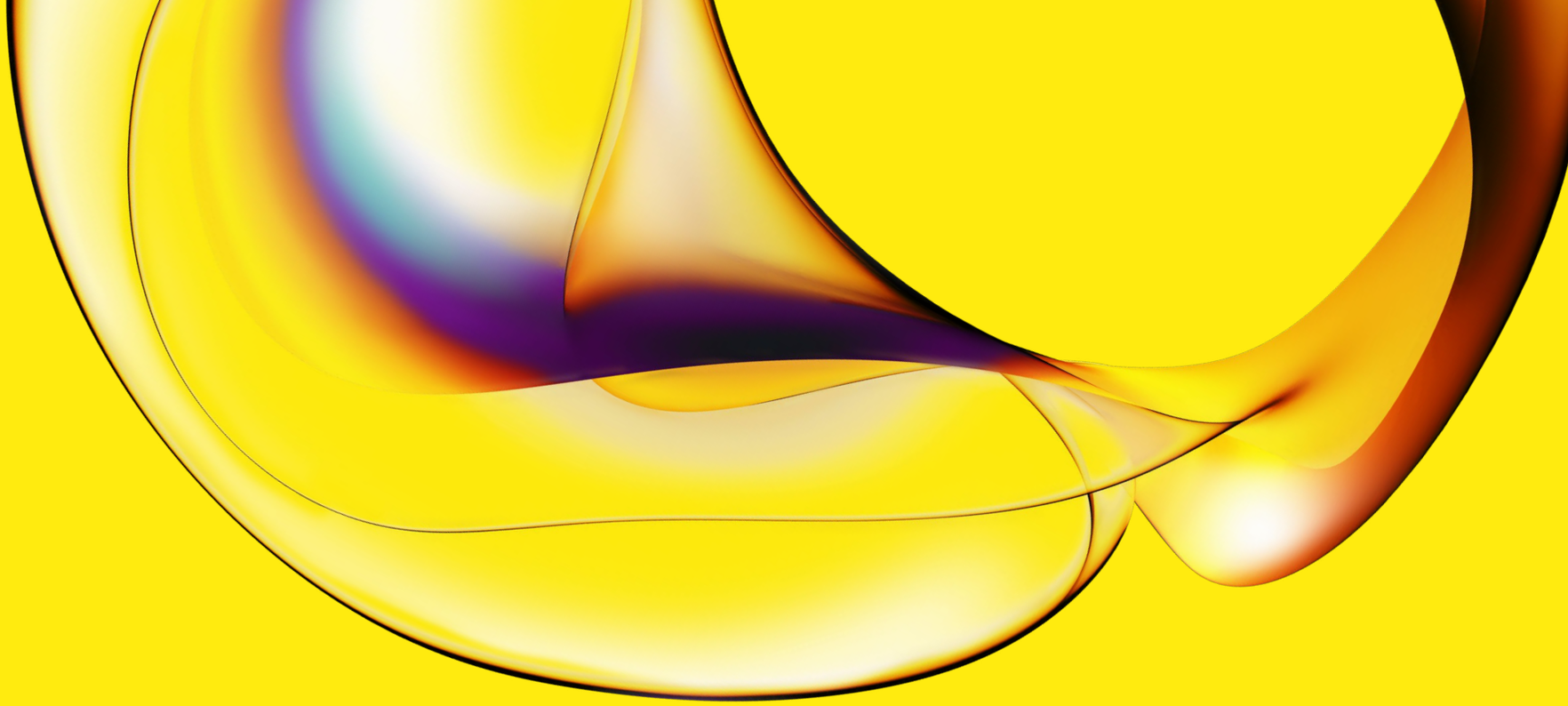

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