More than ever, researchers look to 3D cell cultures, microtissues, and organoids 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. And handling the huge volumes of data these 3D cell experiments produce could be the most pressing challenge of all.

With our best-in-class solutions, you can seed, process, detect, and analyze 3D cell cultures – and begin generating more physiologically relevant data to power better-informed decisions.
Seamless Science for 3D Cell Models

A 3D Portfolio That’s Stronger Together

**SEEDING**
1. Horizon cell lines, primary cells, iPSCs and edited cell lines

**SAMPLE PROCESSING**
2. Nexcelom Cellaca™ cell counters
3. JANUS® G3 liquid handling workstation for automated cell seeding
4a. PhenoPlate™ microplates, and GrowDex® hydrogels
4b. CellCarrier™ ULA-coated round-bottom wells (round spheroid formation)
5. Nexcelom Celigo live-cell imaging for assay development
6. Treatment
7a. PhenoVue™ fluorescent probes, antibodies, and cell painting kits
7b. Drug discovery reagents, including HTTR®, Alpha, and luminescence assays
7c. HIVE™ scRNAseq solution (sample storage and single-cell profiling)
8a. High-content imaging platform including Opereetta® CLS® and Opera Phenix® Plus
8b. Multimode plate readers including VICTOR® Nivo™, EnSight® and EnVision®
8c. LabChip® GX Touch™ nucleic acid analyzer

**READOUT**
9. NGS platform
10. Signals Image Artist™ next-generation high-content image analysis and management

**ANALYSIS**
Signals VitroVivo™ data management and analysis

**INTRODUCTION**
- INTRODUCTION

**WORKFLOW**
- WORKFLOW

**SEED**
- SEED

**SAMPLE PROCESSING**
- SAMPLE PROCESSING

**READOUT**
- READOUT

**ANALYZE**
- ANALYZE

**CONTACT US**
- CONTACT US
Seeding Methods for Growing Cells in 3D

The first critical step in your 3D cell culture workflow is seeding, using specific methods and growth conditions. Methods are selected depending on the biological question, complexity of model, and readout technology. Ultralow-attachment (ULA) plates are coated with hydrophilic polymer to prevent cells from sticking to the surface and allowing them to aggregate. Cells can also be cultured in hydrogels, which mimic the extracellular matrix and support cell growth in three dimensions.

Alternative platforms simulate the microphysiological conditions with more sophistication. For example, microfluidic devices enable you to add nutrient or chemical gradients, and bioprinters allow you to create more physiological shapes.

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PerkinElmer
For the Better
Targeting the Most Relevant Cell Types

Selecting the right cell model for your application is critical to research success – especially when working with 3D models, in which experimental setup is more tedious and time consuming than with 2D models.

Spheroids typically consist of one or more cell types, either primary cells or cell lines, that proliferate and aggregate to form a three-dimensional structure. Organoids are derived from organ-specific stem cells (iPSCs, ESCs, or adult stem cells) and undergo differentiation and self-organization, mimicking at least one function of tissue or an organ.

Complex structures such as spheroids and organoids are better at mimicking the in vivo microenvironment than 2D models. Gene-editing tools such as the CRISPR-Cas9 system or siRNA allow researchers to control gene expression and introduce disease-specific mutations. And the combination of gene-editing and 3D cell models can improve our understanding of molecular mechanisms. These modifications are mostly introduced before building the three-dimensional structure, though there are also ways to deliver genes into organoids.

We offer a catalogue of more than 3,000 Horizon off-the-shelf edited cell lines and a variety of excellent gene-modulation reagents. In particular, the Edit-R™ all-in-one system and Accell siRNA reagents work well with 3D cell models.

Edit-R All-in-One CRISPR System

Horizon’s Edit-R™ All-in-one system combines CRISPR single-guide RNA and Cas9 nuclease expression into a single lentiviral packaged vector.

Accell siRNA Reagents

A novel siRNA platform for difficult-to-transfect cells, Accell siRNA reagents are modified to require no transfection reagent or viral vector for delivery and are available as individual reagents and in SMARTpool format.
Concentrate on Cell Counting

Before seeding cells, you need to determine cell concentration. Manual cell counting is tedious work, so automated cell counters are today’s method of choice. These are available in slide or microwell plate-based versions, depending on the throughput needs and readout type.

We offer a broad range of Nexcelom™ brightfield, fluorescent, and plate-based automated cell counters that can generate highly accurate results. They come with predefined settings for frequently used assays and cell types to ensure consistent results from sample to sample. Or you can easily build custom assays and cell types to fit your experimental needs – and modules for 21 CFR Part 11 compatibility are also available.

**Cellometer™ K2 Fluorescent Cell Counter**

This cell counter enables viewing, analyzing, and reporting on complex or messy samples with as little as 10 µl of cell sample, generating counts, concentration, viability, and size in less than 60 seconds.

**Cellaca™ MX High-Throughput Cell Counter**

This system automatically counts as many as 24 samples in minutes while requiring as little as 25 µl of cell sample. It provides multiple fluorescent filter options with autofocus function and can perform cell-based assays, including viability, vitality, and apoptosis.
The Right Plate Makes All the Difference

Before starting your experiment, you need to choose a culture format. U-bottom ultralow-attachment (ULA) plates are easy to use and need no special equipment, giving a single spheroid per well. Or you can use an imaging plate in combination with a hydrogel, which mimics the in vivo microenvironment. Multiple spheroids/organoids are formed in one well, supported by the extracellular matrix.

PhenoPlate™ Microplates

PhenoPlate microplates deliver both performance and superior images for high-content applications, with:

- Optimal clarity and fast autofocusing from excellent flatness of the plate bottom
- Superior image quality from high optical quality of cyclic olefin imaging surface
- Better well access when using water immersion and high-NA objectives with ultralow plate bottom

They’re also available with different coatings to suit your application.

CellCarrier™ Spheroid ULA Microplates

A unique ULA-coated surface in round-well plates enables the formation of consistently round spheroids from numerous cellular models. The microplate coating helps eliminate satellite spheroid growth for easier data acquisition and analysis. Additional features include:

- Unique design made specifically for 3D spheroids
- Automation compatibility for quick, hassle-free analysis
- Compatible with high-content imaging systems
Support Cell Growth with Animal-Free Hydrogels

Our GrowDex® hydrogels mimic the extracellular matrix and support cell growth and differentiation, enabling convenient, scalable animal-free culture of 3D spheroids and organoids. These hydrogels are composed of natural cellulose fibers extracted from wood sourced from sustainable and responsibly managed forests — a proven solution for automated cell culture and high-content screening.

Manufactured to the highest standards with strict quality-control criteria to ensure lot-to-lot reproducibility, these hydrogels provide a host of other benefits:

- No animal biomolecules interfere with readouts
- Can be used and stored at room temperature — perfect for liquid-handling systems
- Supplied in prepacked syringes
- Simple protocol — mix GrowDex hydrogels with media and cells
- Matrix composition can be optimized by supplementing cell culture media with specific biomolecules
- Transparent, no autofluorescence, and tried and tested on our high-content imaging systems
Automated Cell Seeding for Thermal Hydrogels

Animal-derived hydrogels like the mouse tumor basement membrane extract (BME) matrigel is one of the most frequently used hydrogels for 3D cell cultures. Though it’s well established, it’s not convenient, as it gelates at room temperature and pipetting must be performed at ~4 °C. Our JANUS® liquid handler, equipped with a cold block, can accommodate thermal hydrogels, providing a more convenient way to automate your cell seeding.

WHITE PAPER
Researchers have developed an automated, high-throughput assay that enables the growth, treatment, and analysis of organoids grown from prostate cancer patient-derived xenografts (PDXs). The approach can be used to quantify changes in the growth of heterogeneous 3D cultures to candidate drugs or compound libraries and across whole wells or specific subpopulations of organoids.

WEBINAR
Learn how a group at Mount Sinai School of Medicine developed a pipeline to evaluate patient-derived organoid models for precision medicine.
**Bioprint Your Cell Models**

Bioprinters allow you to form hydrogels with properties matching various tissue types and physiologically relevant matrix environments. You can form these 3D cell cultures with high precision at quantities and consistencies suitable for HTS by depositing cells and matrix components into standard microplates. Bioprinted tissue can be handled and treated similarly to established protocols and processes and allows for the use of 3D cell cultures in drug discovery campaigns using common screening techniques and equipment.

**BLOG**

See how 3D cell cultures can be used in a small-molecule screening workflow by combining the RASTRUM bioprinter with AlphaLISA® technology.

**POSTER**

Check out this workflow for cell painting–based phenotypic screening of 3D cell cultures using a RASTRUM bioprinter with high-content confocal imaging and machine learning.
Monitor 3D Cell Cultures Live

Three-dimensional cell models usually need to grow for days or weeks, and that’s why it’s important to have a gentle, noninvasive method of following growth and proliferation over time. As fluorescent dyes can negatively influence cell health, brightfield imaging is an ideal solution.

Both the Celigo™ imaging cytometer and the MuviCyte™ live-cell imaging system automate imaging analysis of 3D cell models over time, significantly reducing the effort it takes to quantify cell size, number, growth, shape, and cytotoxic effects. Both instruments allow for fluorescence imaging to complement brightfield results for end-point viability analysis and other applications.

ON THE WEB
Learn more about our Celigo imaging cytometer, developed to fully automate imaging and analysis of 3D cell models.

ON THE WEB
See how our MuviCyte live-cell imaging system provides optimal cell culture conditions even for long-term assays.
Stain for Imaging Readouts

Cellular imaging techniques such as high-content analysis rely on the ability to detect and distinguish between cellular compartments and organelles. High-quality data depends on high-quality images made possible by bright fluorescent dyes.

Building on our extensive expertise in imaging instrumentation, fluorescent dye chemistry, and assay development, our PhenoVue™ reagents are designed to help you get the best from your cellular imaging applications. Our portfolio includes:

- Organelle and cell compartment probes
- Fluorescent secondary antibodies
- Cell painting kits

ON THE WEB

Learn how our PhenoVue suite of cellular imaging reagents complements our proven high-content screening instruments and image analysis software.

TECHNICAL NOTE

Optical clearing can increase limited penetration depth in 3D imaging. Learn about innovative clearing strategies for 3D spheroids using the Opera Phenix® high-content screening system.
Our advanced detection technologies provide a wider dynamic range, expanded signal stability, increased sensitivity, and the option for no-wash assays. These reagents are perfect for GPCRs, kinases, epigenetics, PPIs, and the quantification of a wide range of biomarkers, including cytokines – and for uncovering new insights from your 3D cell models.

**TRF and Alpha Technologies**

Neither TRF nor Alpha technologies are disadvantaged by the background effects usually visible in fluorescent assays.

TRF assays use lanthanide chelates or kryptates with long fluorescence lifetimes, and the detected signal consists of only the excited lanthanides, as in DELFIA® assays, or of fluorophores excited via TR-FRET, as in HTRF® or LANCE® assays.

**Alpha** is a no-wash, bead-based technology in which a donor bead is excited. When an acceptor bead is in close vicinity through binding to the target, a cascade of chemical reactions is set in motion, creating an amplified signal.

**APPLICATION NOTE**

Read about using AlphaLISA® biomarker kits on 2D and 3D breast cancer cell culture models.

**POSTER**

Learn more about validation of HTRF technology on 3D cell models.

**APPLICATION NOTE**

See how researchers are utilizing HTRF phospho-/total protein assays to analyze cell signaling pathways in 3D.
Keep an Eye on Cell Health

Luminescence Reagents

Our ATPLite™ 3D and ATPLite 1step 3D reagents 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, making the assay automation-friendly and compatible with any of our multimode plate readers.

Fluorescent Reagents

Our portfolio of fluorescent reagents and kits enables you to measure cell-health parameters such as viability, vitality, or apoptosis. They’re optimized to work with our Cellometer or Celigo systems and with 3D cell models.

3D Tumor Spheroids Treated at Different Drug Concentrations and Stained with Propidium Iodide

Measuring Cell Health

Viability: Measured using propidium iodide, a DNA-binding dye that enters cells with compromised membranes

Vitality: Identification of the number of enzymatically active cells using Calcein-AM, which converts from nonfluorescent to a brightly fluorescing dye in healthy cells

Apoptosis: Detection of an activated executioner protein Caspase 3/7 is a strong indicator for poor cell health
Determine Single-cell Transcriptomic Information

Three-dimensional cell models are complex structures derived from primary tissue cells, adult stem cells, or pluripotent stem cells. They're often highly heterogeneous, containing many different cell types. So it's important to determine single-cell transcriptomic information to understand the complexity of the model. Single-cell RNA sequencing (scRNAseq) provides transcriptional profiling of individual cells, enabling you to understand which genes are expressed and in what quantities and how cells differ within a biological sample.

The HIVE™ scRNAseq solution is a combined sample-storage and single-cell profiling system that enables sample collection at different sites and times, together with sample processing at the collection location or at a centralized processing laboratory.

LabChip® GX Touch™ microfluidics technology is an unparalleled electrophoresis platform that offers high-sensitivity nucleic acid analysis, including gDNA and RNA integrity assessment, library preparation quality assurance, and PCR-fragment sizing and quantitation.

ON THE WEB
Read more about how the scRNAseq solution provides better resolution of individual cells and cellular subpopulations.

ON THE WEB
Learn more about how the LabChip® GX Touch™ nucleic acid analyzer's microfluidic technology generates reproducible, high-resolution data.
Seamless Science for 3D Cell Models

Image Your Samples in 3D

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.

Enabling Technologies for HCA

**Spinning-disk** enable you to 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.

**PreciScan** lets you prescan at low magnification to locate where spheroids have grown, then automatically rescan at higher magnification with the spheroid centered in the image. This saves acquisition and analysis time and data storage space.

### APPLICATION BRIEF

Learn five tips for a successful high-content screening assay with a 3D cell model system.

### TECHNICAL NOTE

Read more about how the Opera Phenix high-content screening system performance improves 3D imaging.

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Operetta® CLS™
High-Content Analysis System

Opera Phenix® Plus
High-Content Screening System
See More of What Your Cells Are Showing

High-throughput screening assays are still the method of choice to identify potential compounds in drug discovery, and many common assays can be transferred from 2D into 3D. You can investigate biological processes by quantifying protein concentrations and studying protein interactions, gene expression, or signaling pathways. Throughout the drug discovery workflow, it’s important to keep an eye on cell health by determining viability, proliferation, and toxicity.

Our **VICTOR® Nivo™** system packs all the latest major detection technologies in the industry’s smallest benchtop footprint – the perfect microplate reader for everyday biochemical and cell-based assays.

The **EnSight®** system offers both fast well-imaging and multimode detection capabilities for target-based and phenotypic screening, all in a single benchtop instrument.

The **EnVision®** reader provides ultrahigh throughput and maximum sensitivity across all detection technologies. With its exceptional speed and minimal downtime, it’s ideal for complex assays to drive your scientific breakthroughs.

**ON THE WEB**
Learn more about our complete line of multimode plate readers.

**READ NOW**
Analyze Data in Context

Our Harmony® software drives the Operetta CLS and Opera Phenix Plus systems and includes everything you need to analyze the most complex cellular models in 3D, reliably discriminate phenotypes, and turn your biological data into knowledge.

As datasets for 3D objects are often huge, Harmony software can be used together with Signals Image Artist™, our next-generation image analysis and management platform, enabling high-performance computing and storing and sharing of high-content screening and cell-imaging data.

Both products are based on easy-to-use assay building blocks with integrated artificial intelligence that make advanced image analysis straightforward. Data from Harmony software and all major high-content screening and cell imaging systems can seamlessly integrate with Signals Image Artist software.

This example shows MDGK epithelial cells which were cultured in Geltrex enriched medium, where they spontaneously form cysts. MDGK cysts are a well established model system to study epithelial tissues.
Manage Data to Uncover New Insights

**Signals VitroVivo™** is an intuitive, configurable screening workflow processor coupled with the unparalleled data visualization and analysis capabilities of the TIBCO® Spotfire® platform. Its flexibility makes it ideal for one-off assay development or more sophisticated applications, and it can support a long and growing list of techniques — even those that generate ultrahigh data volumes. You can now leverage a consistent, repeatable pattern for data acquisition as well as the data processing protocols themselves.

Other features include:

- Intuitive data capture
- Configurable calculation engine
- Ability to store and search all assay parameters
- Unique data handling for *in vivo/DMPK*
- Fully integrated Signals Image Artist software

1. Parse from Instrument
2. Join Metadata
3. Evaluate Data and Statistics
For more information visit www.perkinelmer.com/3DCellModels