Seamless science for 3D cell models.

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3D cell culture workflow solutions

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Discovery with a fresh perspective

More than ever, researchers look to 3D cell cultures, multicellular spheroids, 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 2D cell cultures. 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 solutions, you can culture, treat, and analyze 3D cell cultures - and begin generating more physiologically relevant data to power better-informed decisions.

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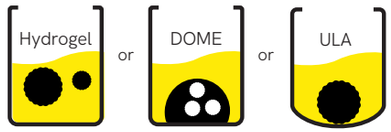
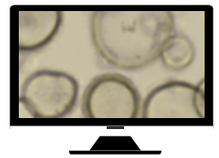
A 3D portfolio that's stronger together





Culture	Treatment	Model analysis	Data analysis
▪ Hydrogels ▪ Microplates ▪ Automated liquid handlers ▪ Cell counters and image cytometers ▪ CRISPR technologies	▪ Automated liquid handlers ▪ CRISPRi/siRNA reagents	▪ **Cellular imaging** - Image cytometers - Reagents & consumables - High-content imaging ▪ **Cell health and marker detection** - Luminescence assays - Alpha/HTRF immunoassays - Plate readers ▪ **Omics** - Oligo-conjugated antibodies	▪ Harmony™ high-content imaging & analysis ▪ Signals Image Artist™ high-content image analysis & management ▪ Signals Research Suite™ data processing and data-driven analytics.

Provide the right genomic context

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.

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

We offer 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

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

Read more

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.

Read more

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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 brightfield, fluorescent, and plate-based automated cell counters that can generate highly reliable results. They come with predifined 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™ Ascend™ automated 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.

Learn more

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.

Learn more

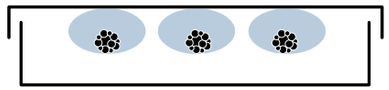
Seeding methods for growing cells in 3D

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.

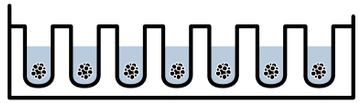
Alternative platforms simulate the microphysiological conditions with more sophistication. For example, microfluidic devices enable you to add nutrient or chemical gradients, and bioprinters allow you to create more physiological shapes.

Non-Scaffold Method

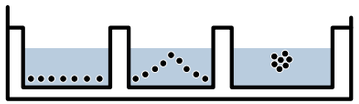
Hanging drop



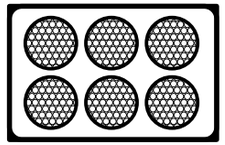
Ultra low attachment plate



Magnetic levitation

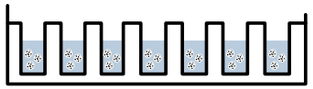


Micropatterned plates



Scaffold Method

Biological hydrogels



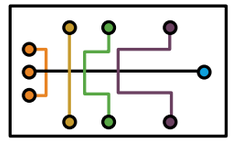
- Collagen
- Basement membrane extract (BME)
- Alginate
- Fibrin
- Hyaluronic acid (HA)
- Cellulose

Synthetic hydrogels

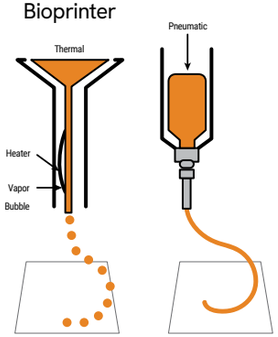
- Polyethylene glycol (PEG)
- Polylactic acid (PLA)
- Polyglycolic acid (PGA)
- Synthetic peptides

Specialized Platforms

Microfluidic devices



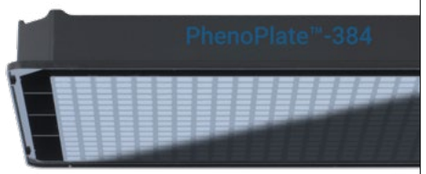
Bioprinter



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
- Excellent 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.

Learn more

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

Learn more

Support cell growth with animal-free hydrogels

Our **GrowDex® hydrogels** allow 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

Guide

See how to reduce cost and time to set up reliable HTS/HCS using 3D cell culture models.

Read now

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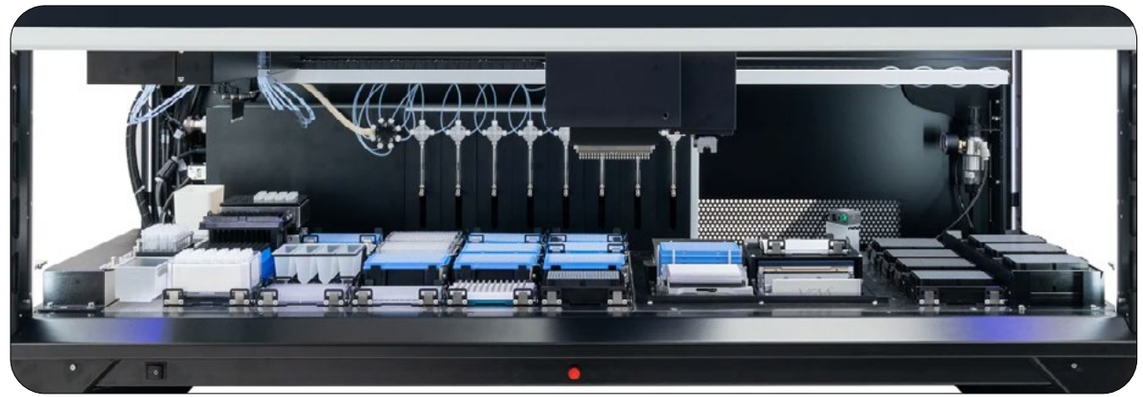
Automated cell seeding for thermal hydrogels

Animal-derived hydrogels like the mouse tumor basement membrane extract (BME) 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 **Fontus™ 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.

Read now

Monitor 3D cell cultures

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.

Our **Celigo™ imaging cytometer** automates imaging analysis of 3D cell models, significantly reducing the effort it takes to quantify spheroid number, growth, shape, and cytotoxic effects. The Celigo allows 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.

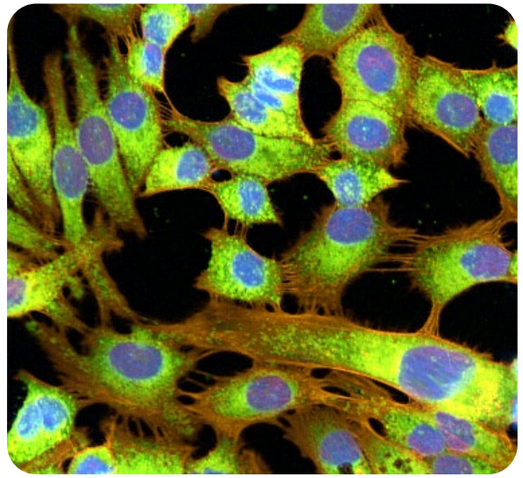
Learn more

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.

Learn more

On the web

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.

Learn more

Image your samples in 3D

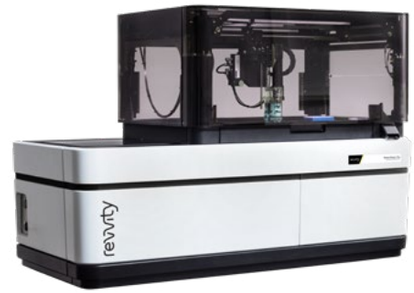
Designed with 3D cell models in mind, our high-content analysis systems let you quickly and easily generate content-rich, physiologically relevant data from 3D samples.

Spinning-disk confocality allows you to acquire image stacks with improved signal-to-noise ratios and high X, Y, and Z resolution. 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.

Operetta CLS™
High-Content Analysis System



Opera Phenix™ Plus
High-Content Screening System

Guide

Learn hints and tops for successful 3D cell model imaging.

Read now

Technical note

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

Read now

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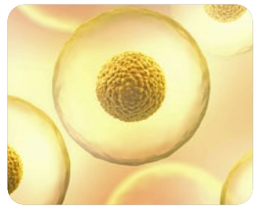
Analyze your bioprinted cell models

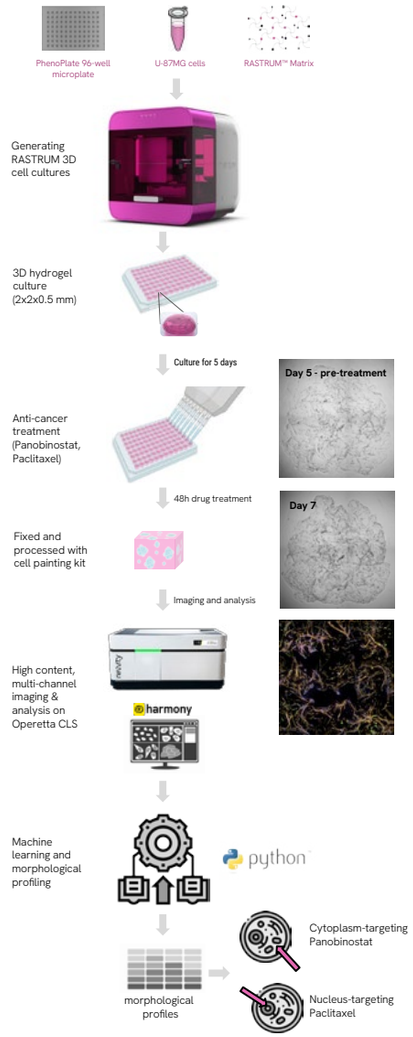
Bioprinters allow you to form hydrogels with properties matching various tissue types and physiologically relevant matrix environments. You can form these 3D cell cultures with high precision at quantities and consistencies suitable for HTS by depositing cells and matrix components into standard microplates. 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.



Technical note:

Explore our Technical Note unveiling a robust workflow for advanced cytotoxicity analysis in 3D cell cultures. This method addresses challenges in reproducibility, scalability, and imaging accuracy, enhancing drug testing accuracy and physiological relevance.

Read now



Workflow of high-throughput generation of 3D cell cultures using RASTRUM™ platform followed by drug treatment, cell painting and high content imaging and analysis using the Operetta CLS leading to morphological profiling of cells in 3D.

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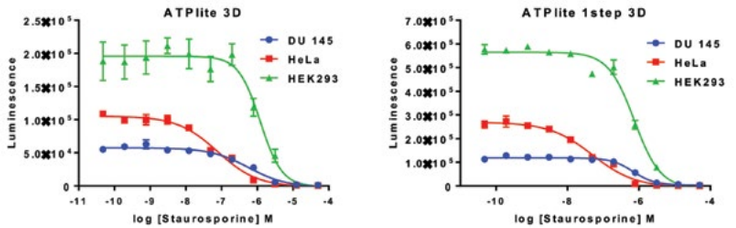
Keep an eye on cell health

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.



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.

Application note

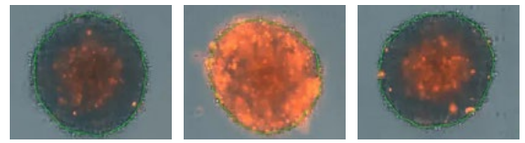
Learn about proliferation and cell death analyses of 3D cultures.

Read now

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



Control *High drug dose* *Medium drug dose*

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

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Shed more light on your sample

Our advanced immunoassays provide protein detection with wide 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

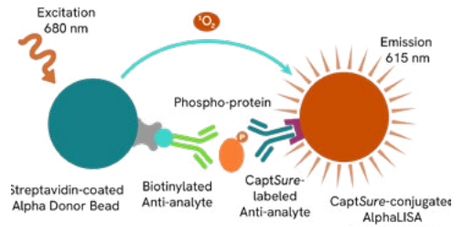
Read about using AlphaLISA™ biomarker kits on 2D and 3D breast cancer cell culture models.

Learn more

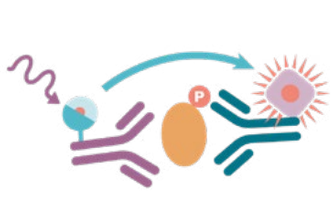
Application note

See how researchers are utilizing HTRF phospho-/total protein assays to analyze cell signaling pathways in 3D.

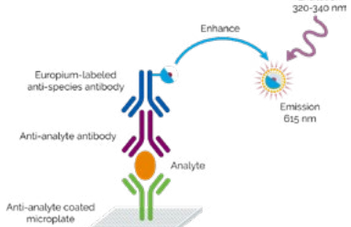
Learn more



Alpha



HTRF



DELFIA

Detect 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 **EnVision™ XCite** system offers a combination of filter based and monochromator based detection. It provides the flexibility required for busy multiuser labs - increasing productivity and elevating confidence in your scientific results.

The **EnVision Nexus™** reader provides remarkable speed and superior sensitivity across major detection technologies. It is the new standard in high-throughput screening on a brand-new innovative platform for your most demanding applications.

On the web

Learn more about our complete line of multimode plate readers.

Read now

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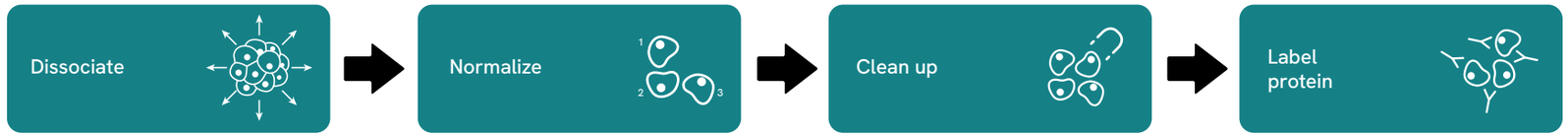
Assess relevancy of your models

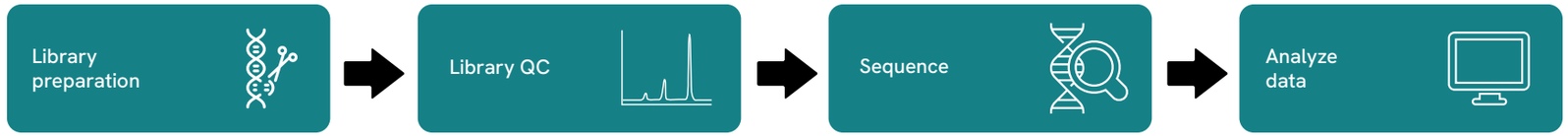
Three-dimensional cell models are complex structures derived from primary tissue cells, adult stem cells, or pluripotent stem cells. They're often highly heterogeneous, containing many different cells types. Single-cell RNA sequencing (scRNAseq) provides transcriptional information from individual cells, enabling you to understand which cell types are present in your model and how cells differ between samples.

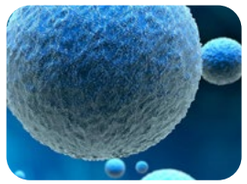
Upstream sample preparation



Partition and barcode

Downstream fragment analysis





On the web

Learn more about solutions for key steps of your single-cell sequencing journey -- working alongside your existing processing steps, making implementation simple.

Learn more

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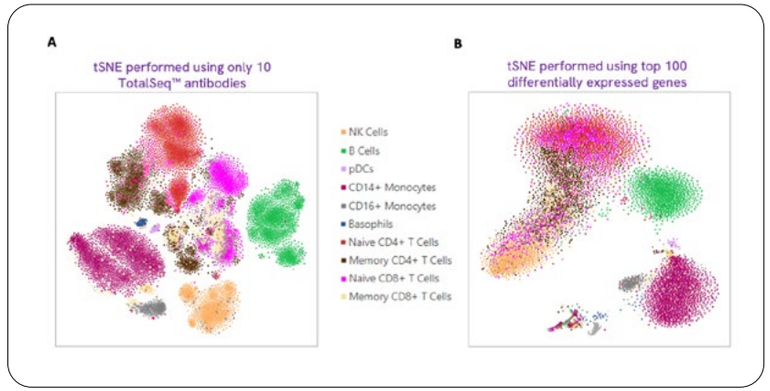
TotalSeq oligo-conjugated antibodies

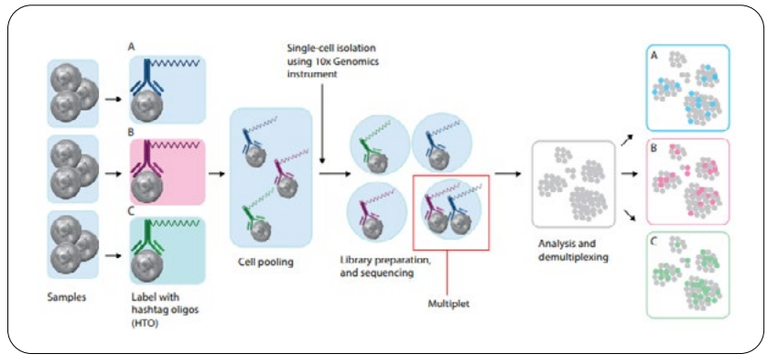
TotalSeq™ oligo-conjugated antibodies enable measurement of proteins at a single-cell level in applications that integrate simultaneous nucleic acid and protein detection, such as CITE-Seq or REAP-Seq, as well as those workflows available from 10x Genomics.

Barcoded antibodies can provide higher parameter phenotypic characterization when compared to CyTOF and other traditional cell analysis tools, by adding many more antibodies to characterize cellular proteins, and also convert single-cell RNA sequencing (scRNA-seq) into a true multiomic approach. This takes single-cell biology to unprecedented new levels by allowing:

- Combined insights, to increase sample clustering, reduce dropouts, and increase dimensionality (A)
- Pool samples, to reduce batch effects, for increased throughput by super-loading or pooling smaller sample sizes, often resulting in reduced experimental cost (B)



tSNE plots showing how the incorporation of results from TotalSeq antibody binding dramatically enhanced the resolution of the populations.



Multiplexing workflow using TotalSeq hashtags.

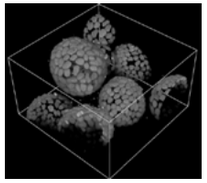
Analyze data in context

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.

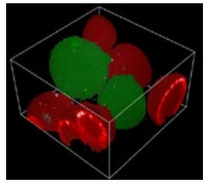
As datasets for 3D objects are often huge, Harmony software can be used together with **Signals Image Artist™,** our next-generation image analysis and management platform, enabling high-performance computing and storing and sharing of high-content screening and cell-imaging data.

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.

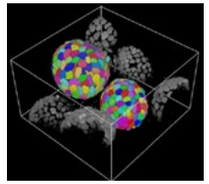
Input
image stack



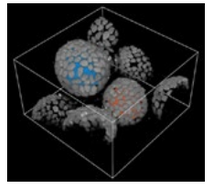
Find
cysts and remove border objects



Segmentation
of single cells

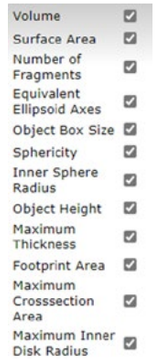


Define
hollow space



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

Calculate
specific properties for evaluation



Technical note

Learn more about 3D Volumetric and Zonal Analysis of Solid Spheroids.

Read now

Technical note

Learn more about 3D Volumetric Analysis of Luminal Spaces Inside Cysts or Organoids.

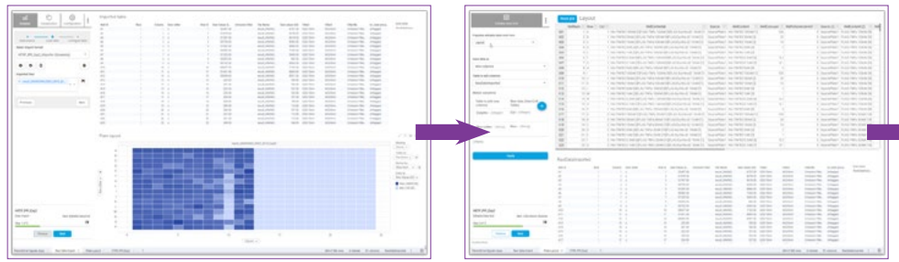
Read now

Analyze data to uncover new insights

Signals Research Suite™ 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.

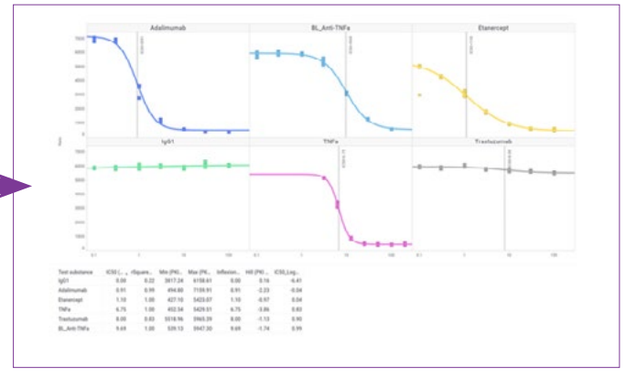
Other features include:

- 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

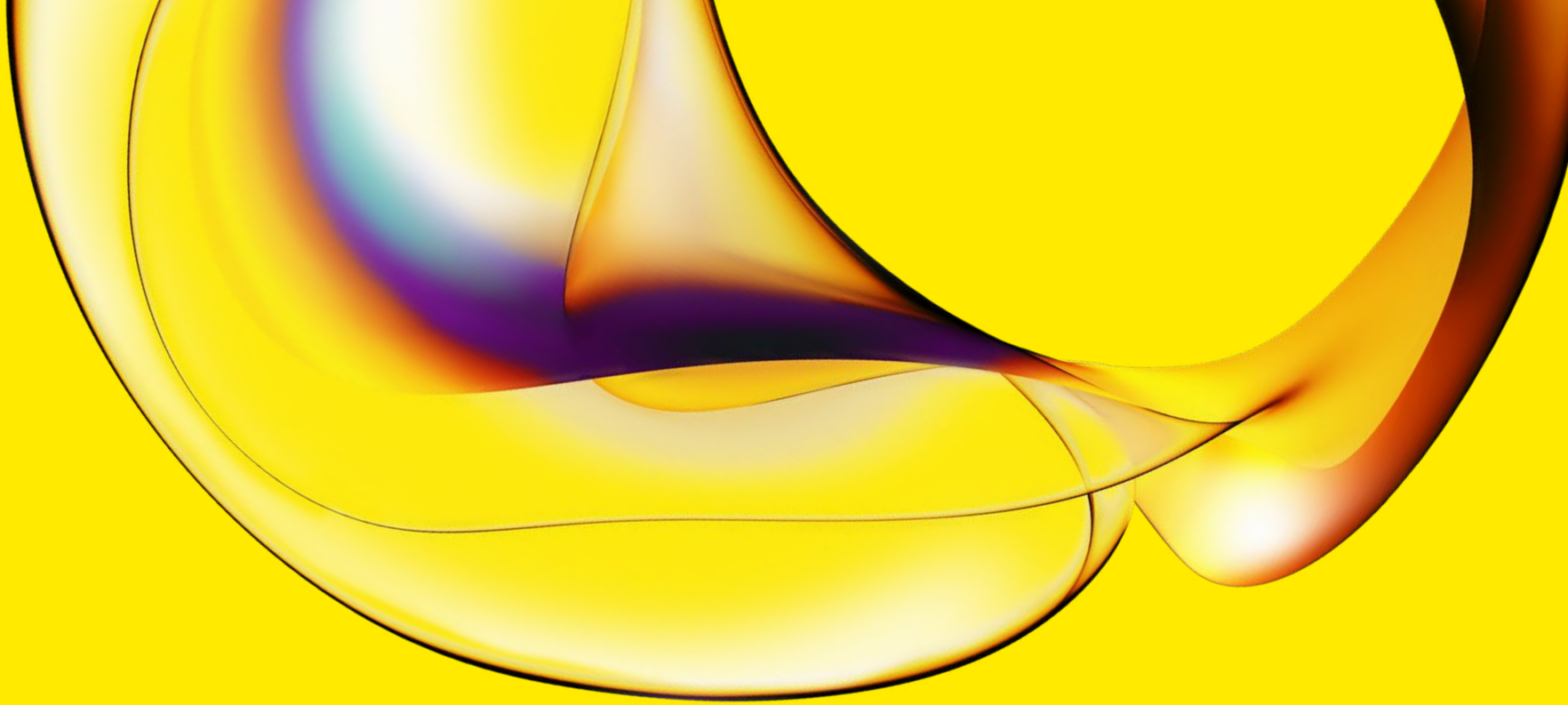


1. Parse from Instrument

2. Join Metadata



3. Evaluate Data and Statistics



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