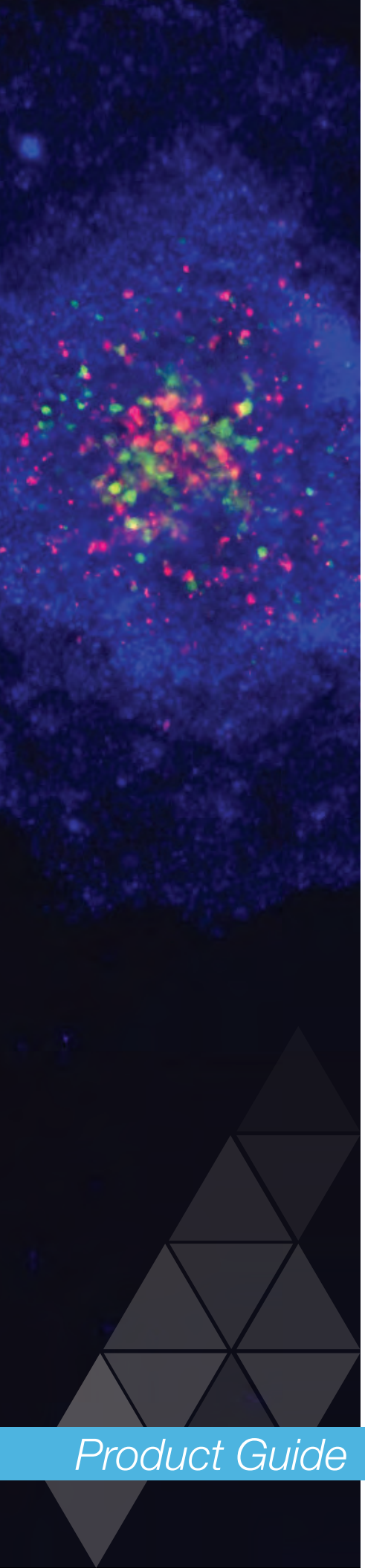
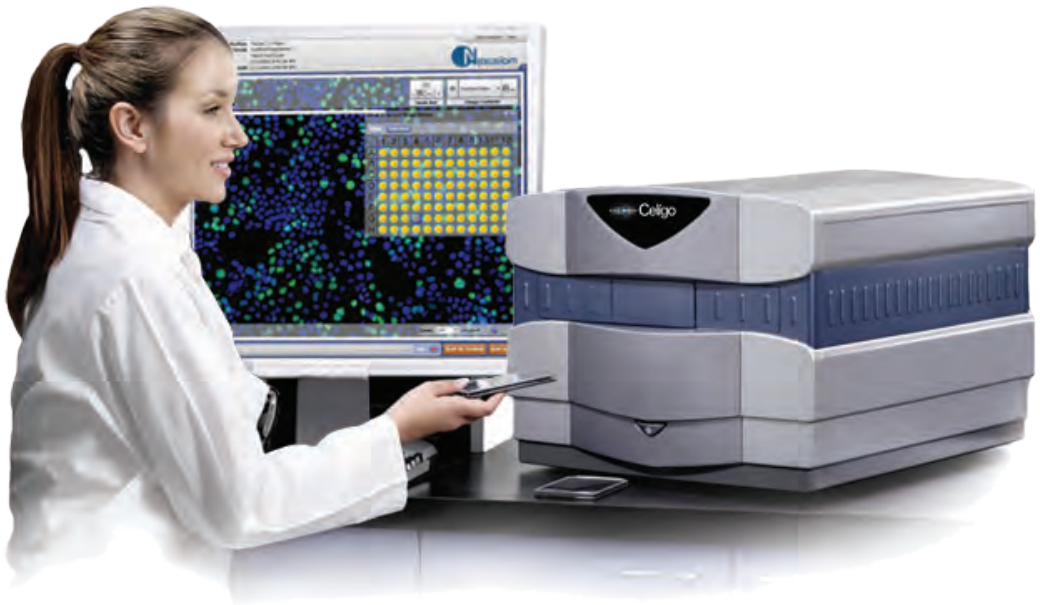


Celígo®

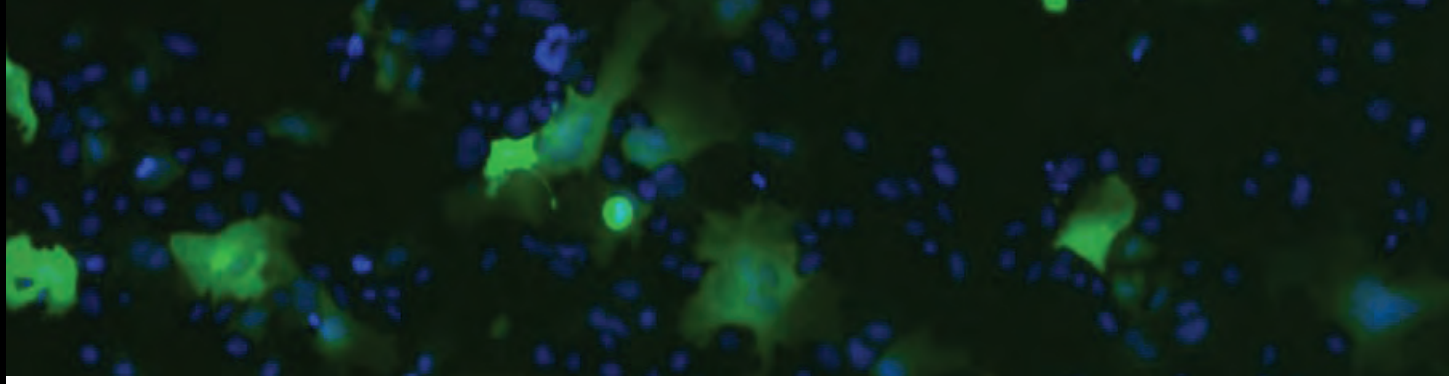
Image Cytometry



Every Cell. Every Well.

Product Guide

Nexcelom Bioscience

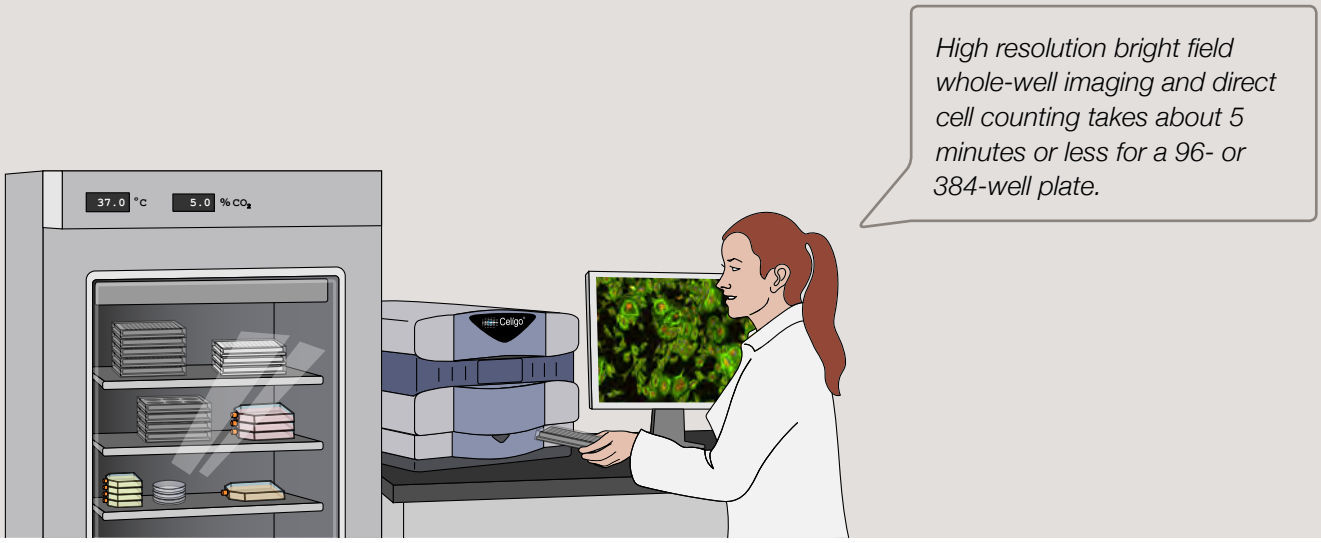


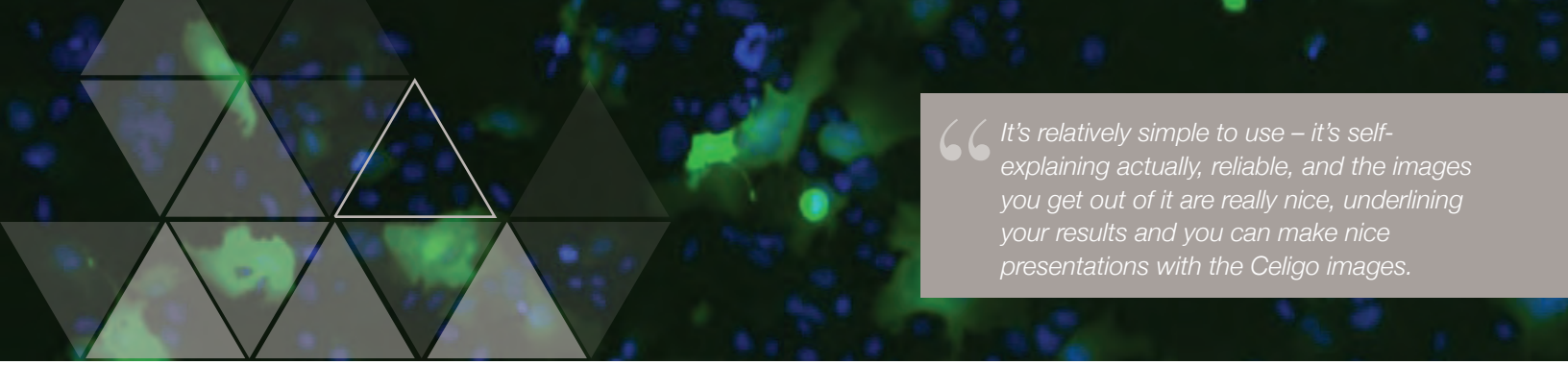
Celigo Image Cytometer

The bench-top Celigo image cytometry system provides high-throughput whole-well imaging and quantitative data through image analysis in bright field and up to four fluorescent channels, for a wide variety of cell-based assays. It is routinely used to investigate adherent and suspension cells, 3D tumor spheroids and colonies of iPSC and cancer stem cells. It is compatible with microwell plates from 6 to 1536-well and T-flask formats.

The work-flow based intuitive software provides concurrent imaging and analysis; kinetic analysis such as time-lapse growth tracking and flow cytometry-like gating analysis and reporting of cell populations. Cell images of specific populations may be displayed with color overlays through a gating selection.

The Celigo product allows users to perform high-speed, fully automated imaging and quantification of a wide range of cell types across complex sample types. It enables an extensive menu of applications including label-free cell counting, confluence-based cell growth tracking, killing assays, apoptosis, cell cycle analysis, migration and invasion assays, as well as cellular assays for receptor internalization, protein expression and detection, phosphorylation and phagocytosis.

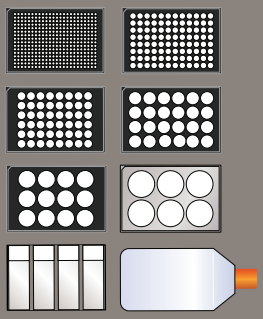


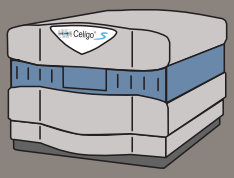


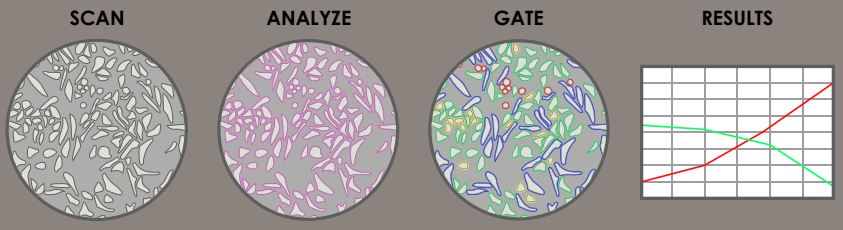
> *It's relatively simple to use – it's self-explaining actually, reliable, and the images you get out of it are really nice, underlining your results and you can make nice presentations with the Celigo images.*

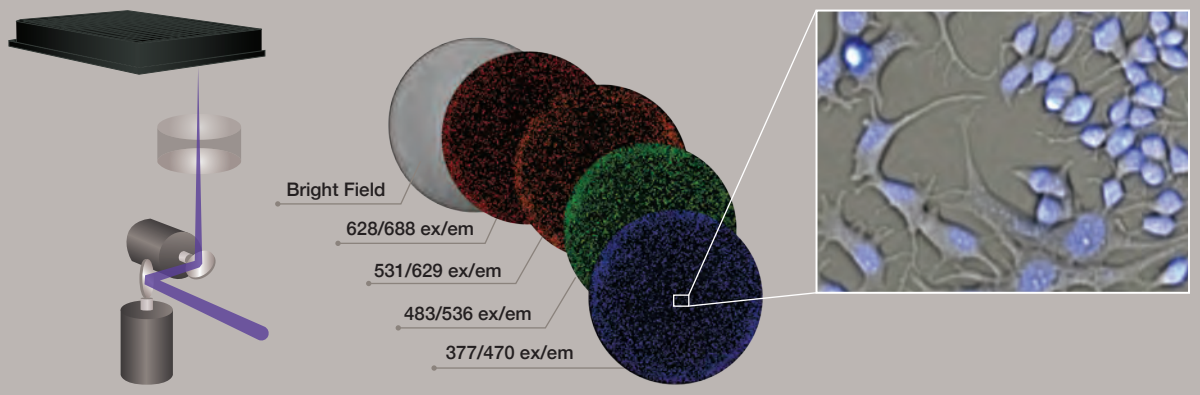
Whole-Well, High Resolution Images Acquired at High Speed

- Proprietary optical design enables uniform illumination and consistent edge contrast
- Image and count every cell in each well: 0 - 100,000 cells/96-well
- 5 imaging channels with bright field and 4 fluorescent colors
- Fast scanning for image acquisition and analysis with minimal plate movement ensuring minimal sample disruption
- Accurately quantify cells and colonies with a non-invasive method
- Ability to save experiment settings – quickly run the same assay on many plates without additional set up
- Measure adherent cells without trypsinization
- Powerful, easy-to-use image analysis software – accessible for everyone in the lab
- System stitches multiple fields of view into a full resolution image
- Easily integrates with robotic arms, plate stackers, automated incubators and liquid handlers

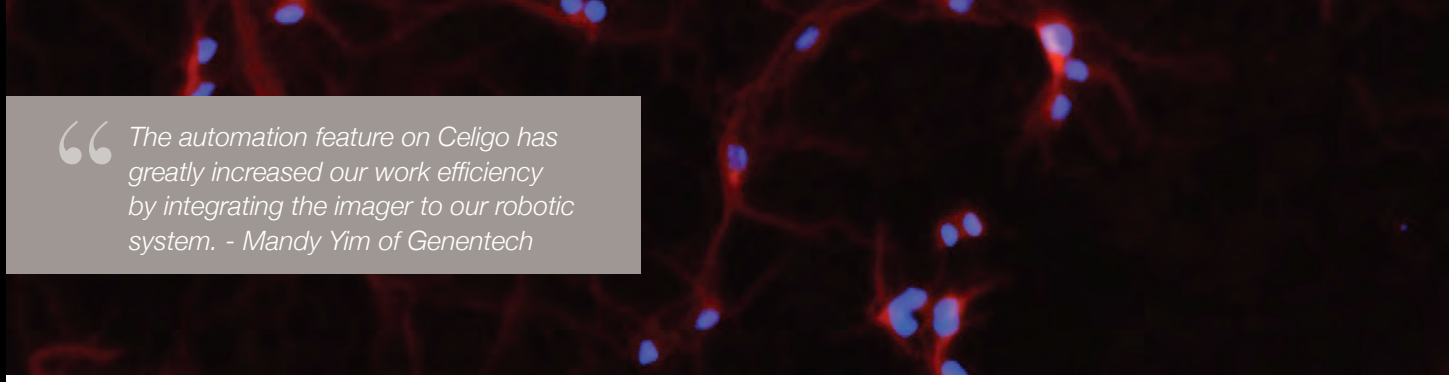








Celigo Applications



> *"The automation feature on Celigo has greatly increased our work efficiency by integrating the imager to our robotic system. - Mandy Yim of Genentech*

Fluorescent Assays

Cell Cycle, Viability/Cell Health, Internalization & Phagocytosis, Co-Culture, Surface Proteins & Antibodies, Cell Secretion, Transfection/Transduction, Apoptosis, Migration

Bright Field

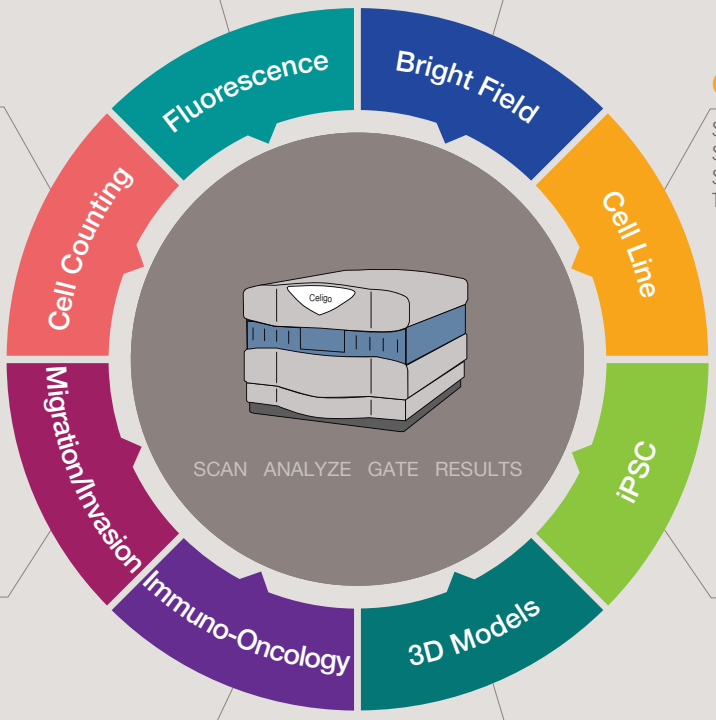
Adherent Cell Growth Tracking, Suspension Cell Counting, Embroyid Bodies, Colonies, Spheroids, Wound Healing, Morphology

Cell Counting

Adherent Cell Counting, Suspension Cell Counting, Fluorescent Cell Counting, T-cells, Splenocytes

Cell Line Development

Single Colony Identification, Single Tumor Sphere Identification, Single Cell Per Well Verification, Transfection/Transduction



Migration/Invasion

Chemotaxis, Wound Healing, Transwell Invasion, 3D Migration, 3D Invasion

iPSC Reprogramming

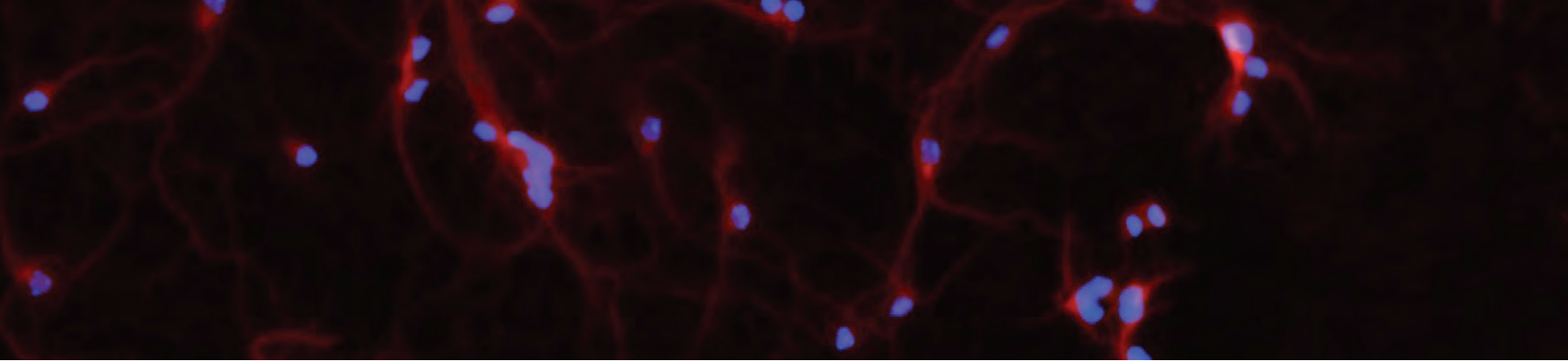
Fibroblast Doubling, iPSC Colony Counting, Embryoid Body Formation, Immunostaining for Differentiation

Immuno-Oncology

Direct Cell Counting, Visualize and Document All Cells, Using Gating Interface, ADCC, Direct NK Cell Killing, CAR-T, CDC

3D Models

Growth Inhibition, Measure Apoptosis, Tumor Spheroid Viability, Quantify Invasion into Matrigel, Measure Migration onto ECM, Tumorsphere Formation & Clonogenic Survival, EBs & PDOs, 3D Confrontation Assay



Integrated Cell Imaging and Analysis in the Same Software

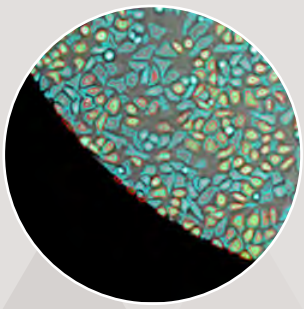
Oncology
- Apoptosis
- Cell cycle
- Cell health
- Cell viability
- Cytotoxicity
- DNA synthesis
- Cell migration
- Cell morphology
- Tumor sphere analysis
- Migration / invasion assays
- Wound healing
- Expression analysis

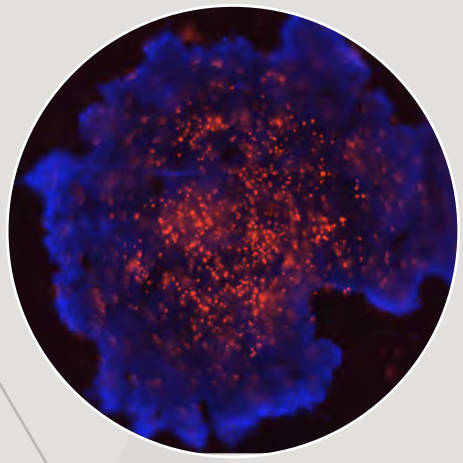
Cell Culture
- Cell counting
- Cell confluence
- Growth tracking
- Cell line generation
- Single cell detection
- Colony counting
- Transfection efficiency

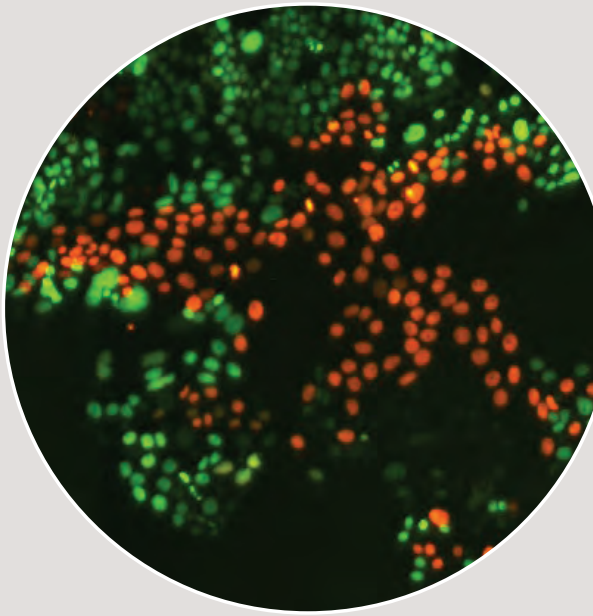
Stem Cell Research
- iPS cell line generation
- Embryoid body morphology
- Stem cell marker analysis

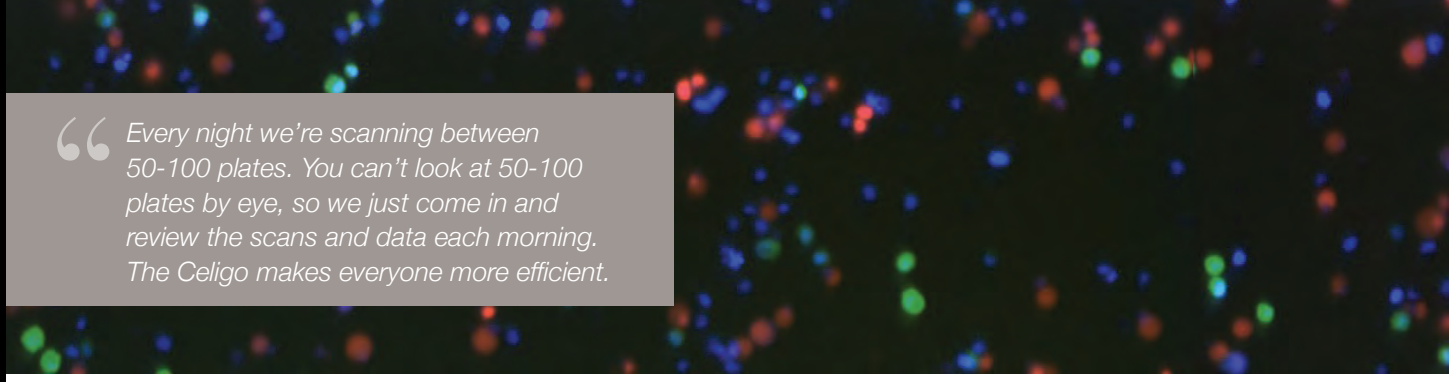
High Throughput
- Robotic integration
- Cell health
- Proliferation
- Fluorescent assays

Bio-Production
- Cell secretion
- Cell line monitoring
- Routine quality control









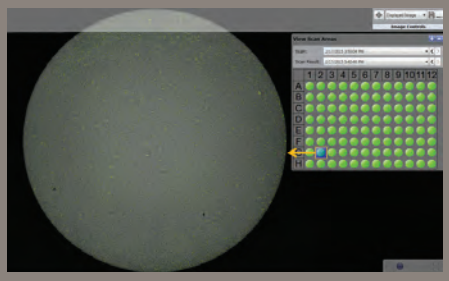
> *Every night we're scanning between 50-100 plates. You can't look at 50-100 plates by eye, so we just come in and review the scans and data each morning. The Celigo makes everyone more efficient.*

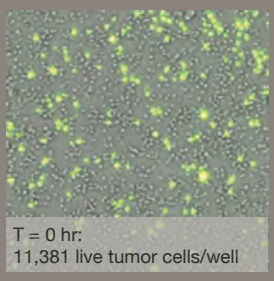
Accurate & Consistent Cell-Mediated Cytotoxicity Assay for Immuno-Oncology

The direct cell counting-based technology replaces traditional release assays for cytotoxicity measurements. Celigo is used to count every live target cell in every well at different time points. Data consistency and accuracy are improved due to direct counting of the same individual cells co-cultured with effector cells. This method is highly sensitive because cell death is measured at the individual cell level. Consequently, fewer target and effector cells are required, allowing for effective use of precious primary cell samples to generate the most number of data points. Typically, only a few minutes are required for imaging and analyzing a 96- or 384-well plate.

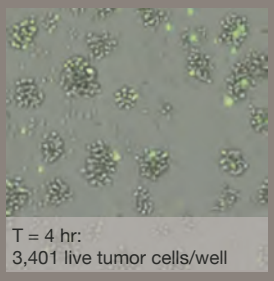
Citations:
Somanchi SS, et al. PLoS ONE. 2015 Oct 10(10)
Kummerow C, et al. Eur J Immunol. 2014 Jun 44(6):1870-2

Quantify killing & visualize formation of immune complexes, through a direct cell counting assay in a 96 well plate

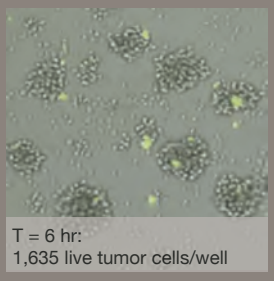




T = 0 hr:
11,381 live tumor cells/well



T = 4 hr:
3,401 live tumor cells/well

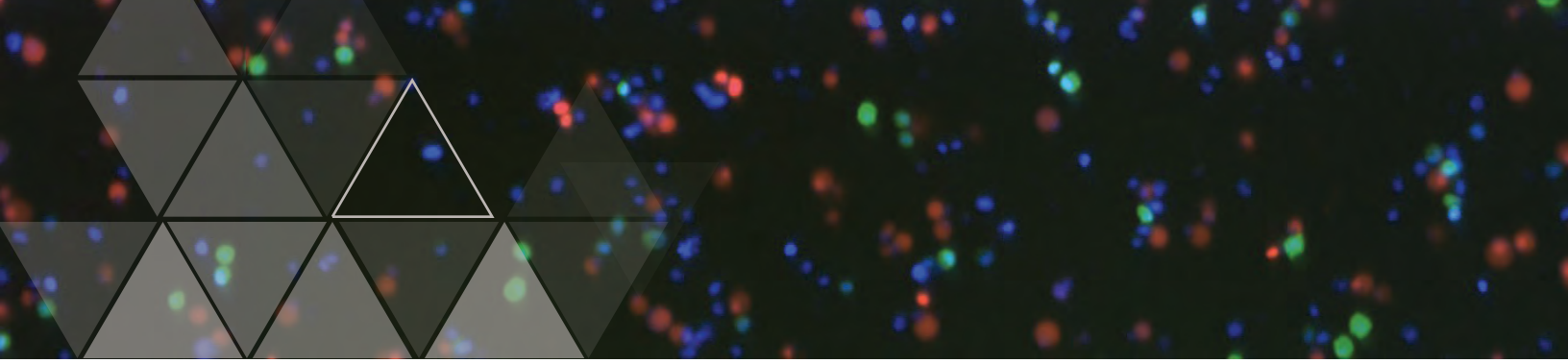


T = 6 hr:
1,635 live tumor cells/well

Count every cell in every well
By imaging whole wells at high resolution with bright field images showing all cells, and fluorescent images showing calcein AM-labeled target tumor cells, the Celigo simultaneously acquires and counts every cell in every well.

Kinetic measurement of tumor cell killing
The panel of images above show a non-destructive, kinetic assay using calcein AM. The same 96-well plate was imaged at 0 hr, 4 hr and 6 hr, showing a decrease of calcein positive live tumor cells from 11,381 to 1,635 cells within the same well.

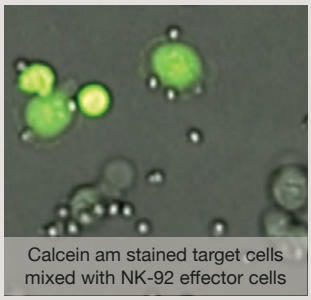
Auto-save hi-res images
The same images show a gradual increase in the formation of immune complexes, which is visualized and automatically documented for all wells.



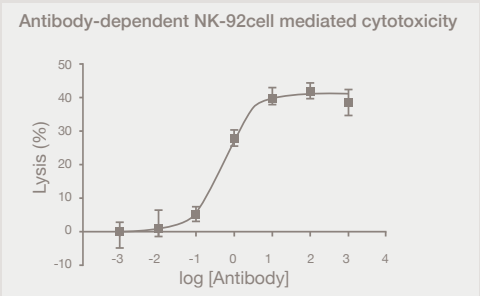
Typical stains and labels for target cells
- Fluorescent proteins: GFP, RFP, etc.
- Cell tracer dyes: calcein AM, CFSE, CMFDA, etc.
- Viability dyes: PI, DAPI, AO, Hoechst, etc.

Commonly imaged effector cells
- CIK cells
- NK cells
- Neutrophils
- CAR-T cells

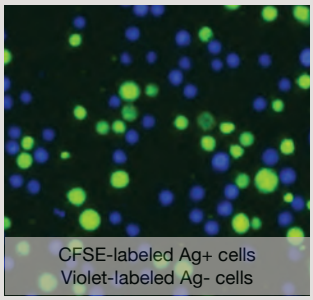
NK-92 Cell Mediated Killing



Calcein am stained target cells mixed with NK-92 effector cells



Mixed Tumor Target Cells

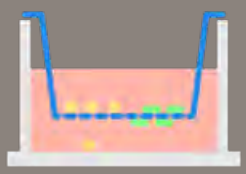


CFSE-labeled Ag+ cells
Violet-labeled Ag- cells

Automatically quantify 2D migration / invasion assays

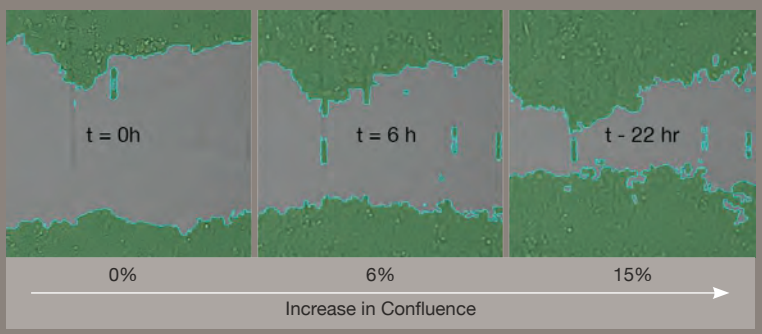
Image and quantify transwell-based chemotaxis, invasion and migration of suspension and adherent cells.

Suspension cells located inside the insert migrate through the porous membrane toward the chemoattractant from the bottom plate. Celigo is used to automatically count migrated cells in the bottom plate.

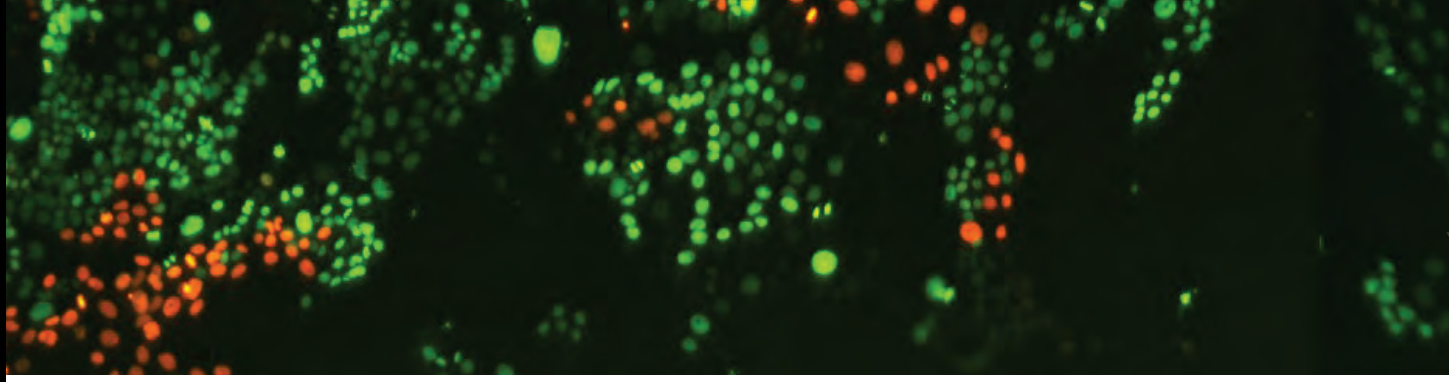


Adherent cells located on the top of the insert with an ECM coated membrane invade and migrate to the bottom side of the membrane. Celigo is used to automatically image and count cells on the bottom side of the insert membrane.

Image and quantify wound healing assays

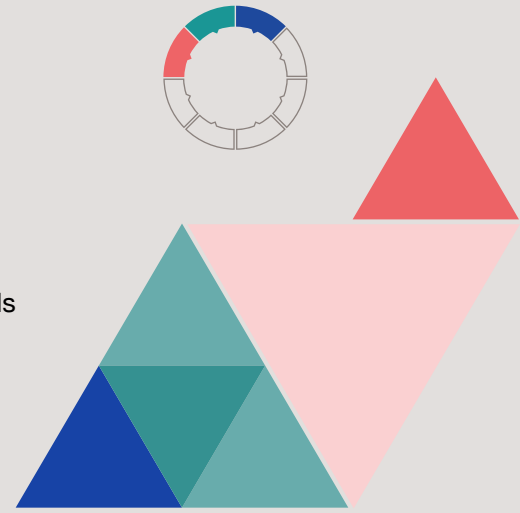


In a wound healing migration assay, a wound is produced on a fully confluent cell layer by a mechanical tool or generated by a device inhibiting cell growth in a defined area. Celigo is used to image the entire well with a wound at multiple time points. Confluence is automatically calculated for each well. The increase of confluence is obtained for conditions which enhance cell migration.



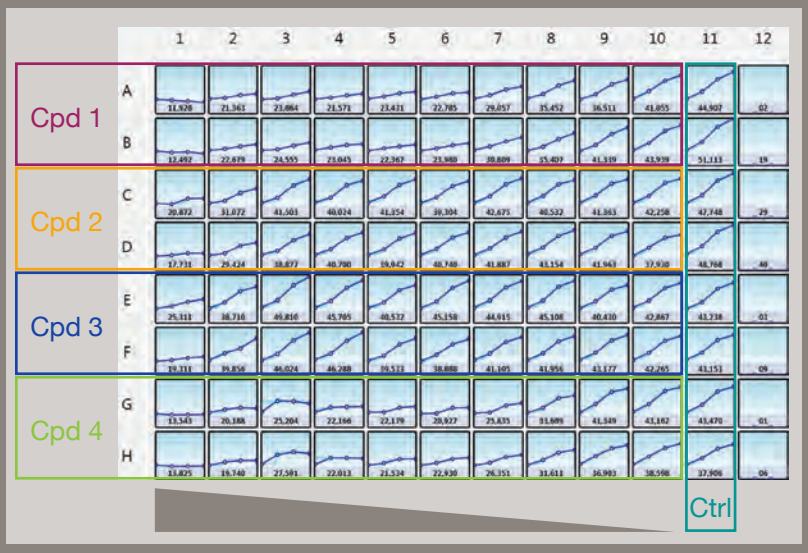
Investigate Mechanisms of Drug Resistance, Signaling Pathways; Discover Combination Therapies

Fast and high throughput cell counting assays are needed for drug discovery to satisfy the need to test an increasingly large number of cell models with large numbers of compounds and compound combinations. Gene editing technologies, such as CRISPR/Cas9, are rapidly expanding phenotypic *in vitro* cell models for oncology, and the success of immune and combination therapies has increased the number of compounds to be tested. Celigo image cytometer performs direct cell counting assays in micro-well plates, typically scanning a plate in a few minutes. With 5 imaging channels, multiple labeling options can be used for co-cultured tumor microenvironment models, such as cancer-associated fibroblasts (CAF) and immune cells, or even 3D tumor spheroids. In addition to adherent cells, imaging whole wells in bright field and fluorescent channels enables direct high-throughput counting of suspension cells in microwell plates, in which case cells are non-uniformly distributed in wells, so the ability to count every cell is essential for accurate data.



Citation:
Fu X, et al. Breast Cancer Res. 2014 Sep 11;16(5):430
Giuliano M, et al. Clin Cancer Res. 2015 Sep 1;21(17):3995-4003

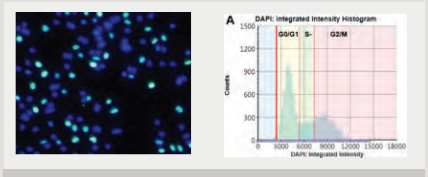
Label-free kinetic assays for cytotoxicity



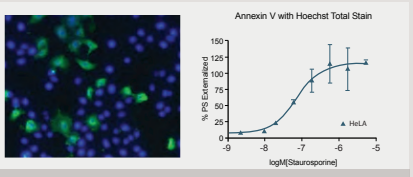
Multi-Drug Growth Inhibition
Growth inhibition: 4 compounds at 10 concentrations at 4 time points, with controls, in a 96-well plate. Compounds were added to the wells 24 hours after cell plating and the same plates were imaged at 4 time points: 0, 24, 48, 72 hours. Each whole-well plate was scanned within 5 minutes. Celigo software automatically generates the growth curves of total number of cells per well, showing dose-dependent growth inhibition for all compounds.

> *We really utilize the high-throughput aspect of the Celigo. You get really nice statistics. You get a lot of data points. Instead of only having 3 data points you get 96 data points. You can look at small changes and actually get some statistical significance out of it.*

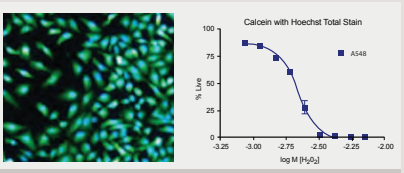
Commonly Used Functional Assays on Celigo



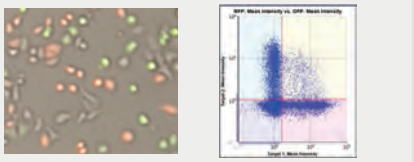
Cell Cycle by DAPI



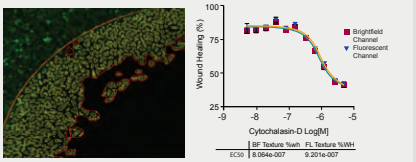
Apoptosis - PS Externalization



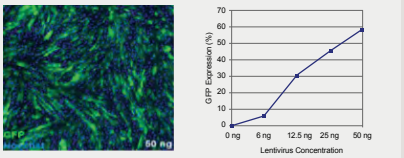
Cell Viability



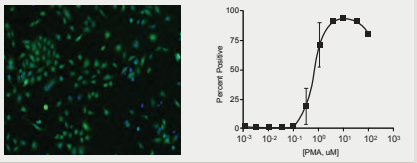
Transfection Efficiency



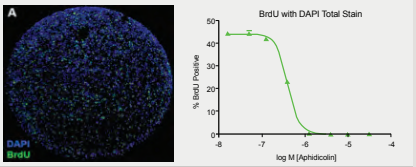
Migration



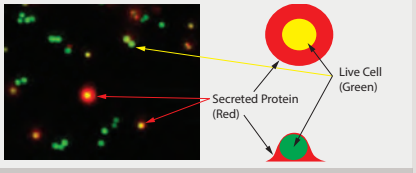
Viral Infection



ERK Phosphorylation

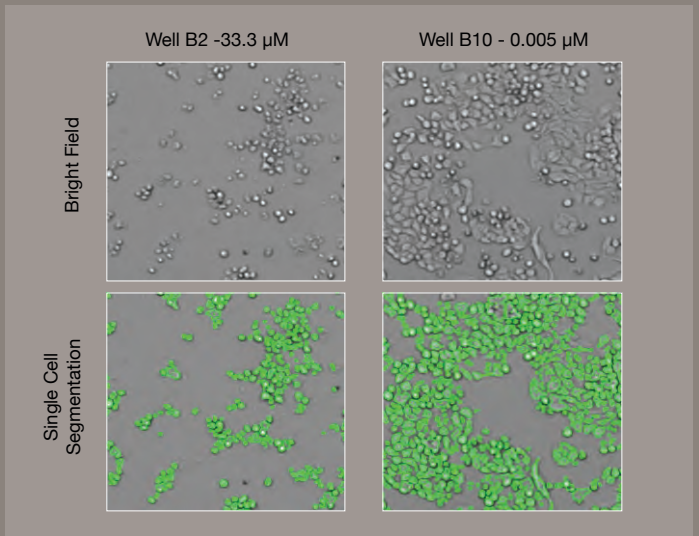


DNA Synthesis

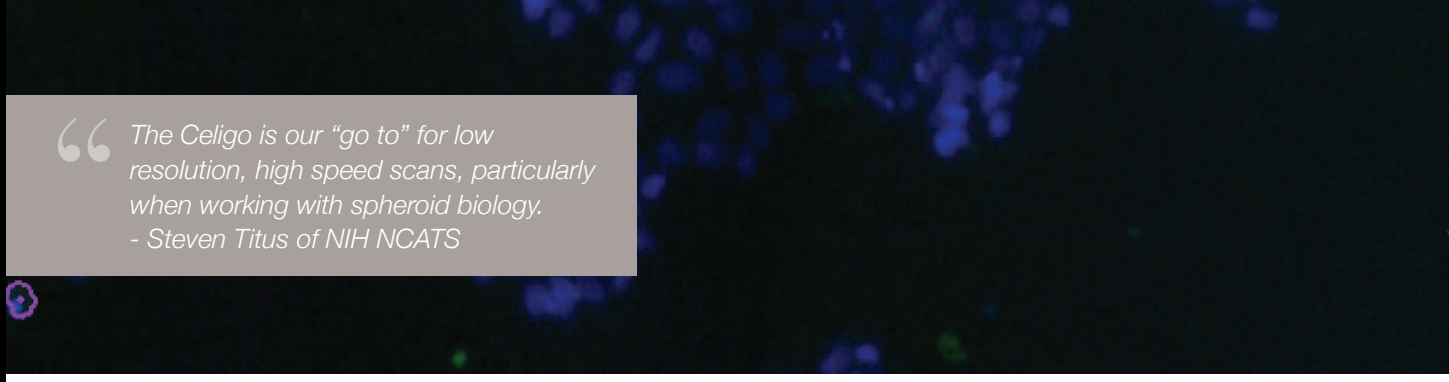


Secretion

Monitor growth inhibition using direct cell counting



Dose-Dependent Growth Inhibition
Acquired and analyzed bright field images were taken 72 hours post-treatment. HT-29 cell growth inhibition is seen in the higher drug dose well B2, compared to well B10. Individually identified and segmented HT-29 cells are outlined in green color for each drug treatment.



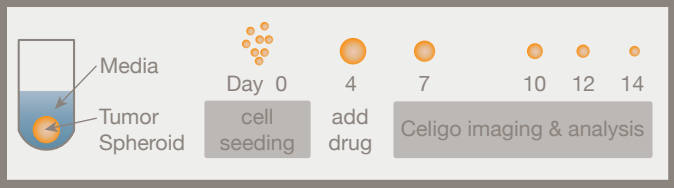
> *The Celigo is our "go to" for low resolution, high speed scans, particularly when working with spheroid biology.*
> *- Steven Titus of NIH NCATS*

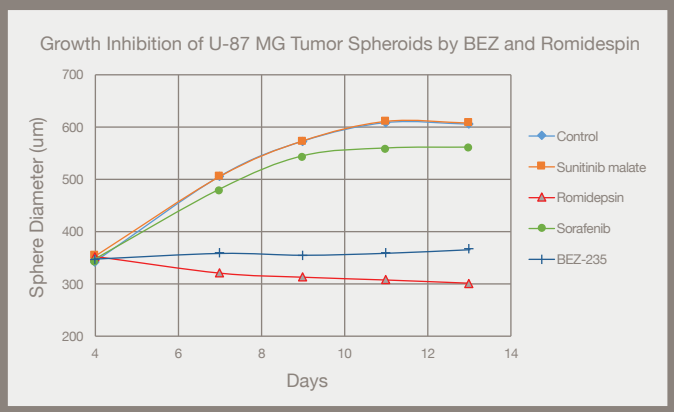
Image Cytometry Enabling High-Throughput 3D Tumor Spheroid Screening

3D tumor spheroid cancer models are used to screen for potential drug candidates due to its physiological relevance. Celigo is used to perform a variety of 3D tumor spheroid assays such as growth inhibition, viability and apoptosis by acquiring high-quality bright field and fluorescent images. The bright field images are used to define the area to quantify signals from up to 4 fluorescent channels. Tumor sphere formation assays are also easily performed to determine optimal cancer models for drug screening. High-throughput imaging and analysis is achieved by acquiring a single image from a single focal plane per well, which allows rapid screening of 96- and 384-well plates, typically in less than 2 minutes.

Citations:
Vinci M, et al. BMC Biol. 2012 Mar 22;10:29
Guo WM, et al. Mol Pharm. 2014 Jul 7;11(7):2182-9

3D tumor spheroid growth inhibition and viability in microwell plates

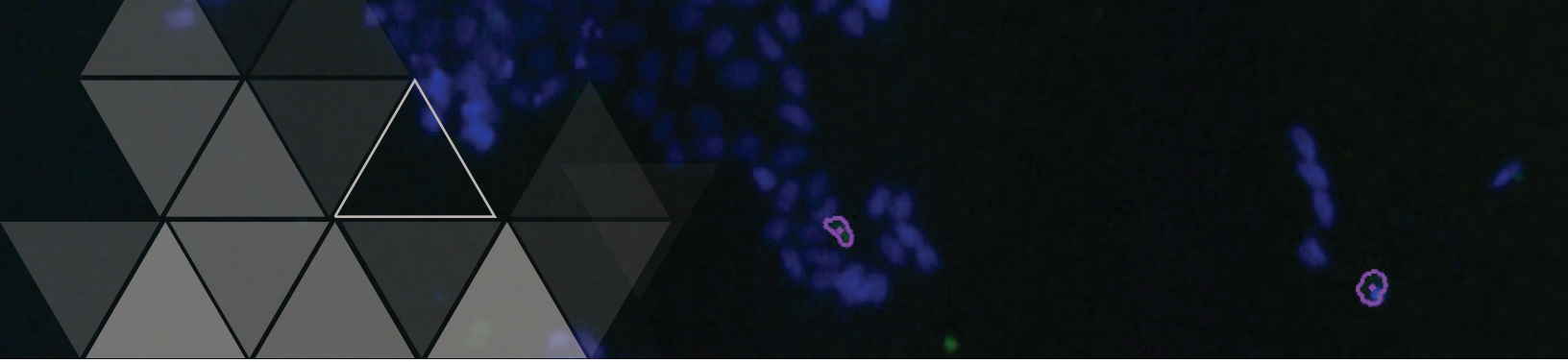




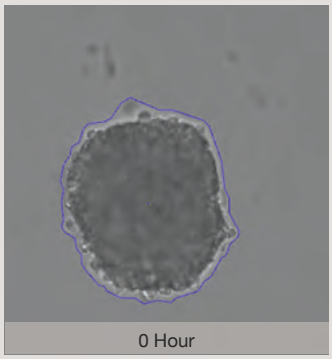
Form 3D tumor spheroids
Four days after seeding U-87 MG cells in u-bottom ultra low attachment (ULA) 96-well plates, drug treatments were added to the formed spheroids and images were acquired using bright field imaging.

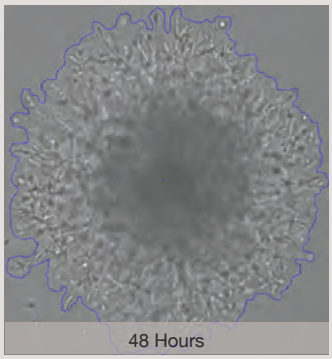
Measure spheroid size
The graph on the left shows drug-dependent spheroid diameters, over a 9 day treatment period, measured by the Celigo software. The images, shown at right, illustrate growth inhibition of U-87 MG tumor spheroids treated with BEZ and Romidepsin compared to the other drug treatments.

Monitor spheroid viability
On day 13, tumor spheroids were stained with live cell green dye calcein AM and dead cell red dye propidium iodide to examine viability. Using the Celigo software, the green and red fluorescent images, shown at right, were overlaid to show live and dead regions within the tumor spheroid.



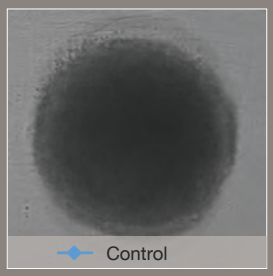
Invasion into Matrigel

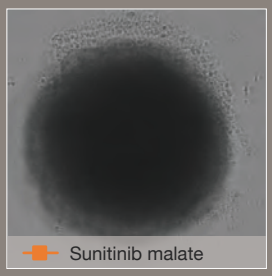


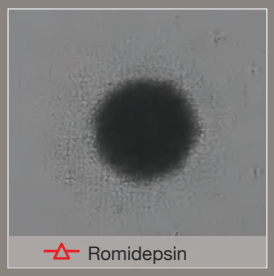


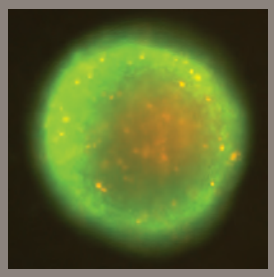
Other 3D tumor spheroid assays
- Invasion/Migration into Matrigel
- 3D tumor spheroid killing by T-cells
- Confrontation assay
- Embryoid Body

Typical stains and labels
- Calcein AM
- Propidium iodide
- Caspase 3/7
- Hoechst

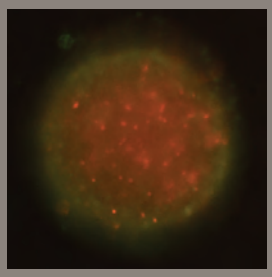




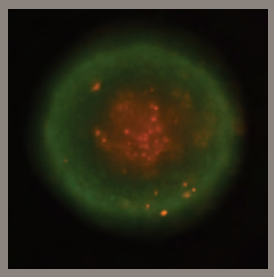




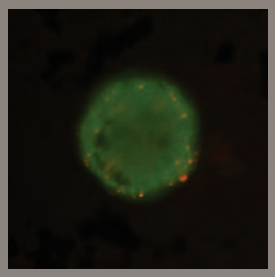
High viability, shown by bright calcein signal with minimal PI+ cell death



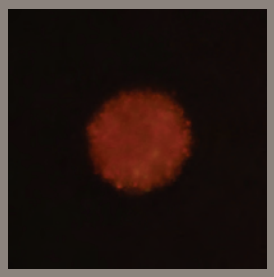
Low viability, shown by low calcein levels with high PI+ cell death



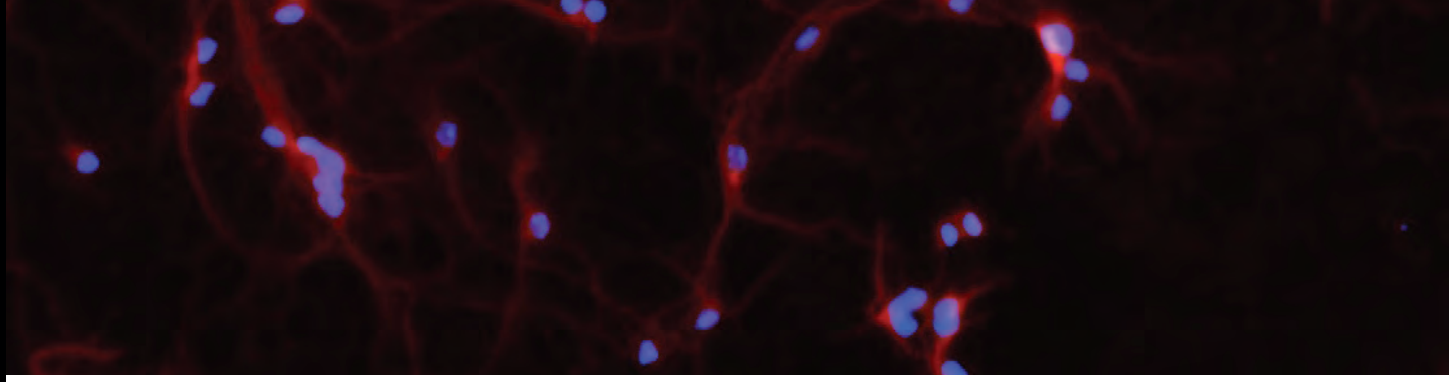
Lower viability, compared to control with hypoxic core shown by PI+ cells



Moderate viability with very low cell death

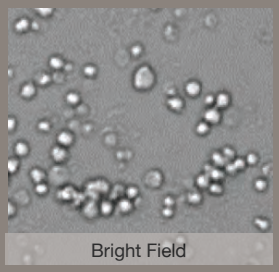


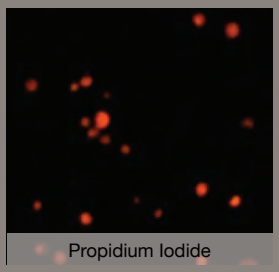
Very high cell death, shown by strong PI staining throughout the tumor spheroid

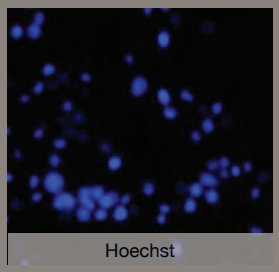


Quantify and Monitor CHO-Cell Line Development Mediated by CRISPR/Cas9 System

Chinese hamster ovary (CHO) cells are widely used as cell factories for the production of biologics. Gene editing technologies are used to alter function and to enhance production. During gene editing optimization and monitoring of stable clones, Celigo whole-well imaging allows for direct cell counting and quantification of transfection efficiencies. Celigo serves as a rapid high-throughput imaging platform for CHO cell line development, through direct documentation and verification of colony formation from a single cell.


Bright Field


Propidium Iodide


Hoechst

Hoechst+ cells	47,380
PI+ cells	23,884
Viability	50.4%

Quantify transfection efficiencies

Grav LM, Lee JS, Gerling S, et al. (2015) One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment. Biotechnol. J. 10, 1446-1456

CHO-S cell viability and growth was monitored every 3 days in preparation for gene editing, as well as on day 1 post-transfection. During transfection optimization, whole-well imaging allows for direct cell counting and measurement of transfection efficiencies during CHO-S cell line construction. While mCherry positive cell population represents precise CRISPR targeting, the presence of GFP signal signifies off-target effects. Using Celigo imaging combined with the 2D gating interface, allows high-throughput testing of transfection constructs and as a quality control measure during expansion to verify cell line stability.

Monitor cell expansion during batch culturing

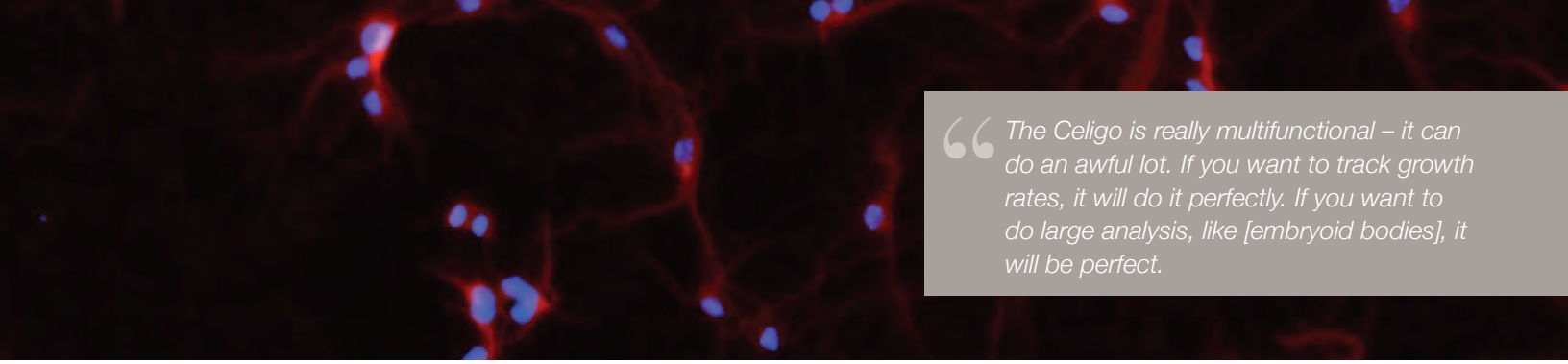
Hansen HG, Nilsson CN, Lund AM, et al. (2015) Versatile microscale screening platform for improving recombinant protein productivity in Chinese hamster ovary cells. Sci Rep. Dec, 11:5

During culture expansion, cell growth and viability were measured using a bright field and multi-channel fluorescent assay. In this example (images top left) Hoechst and PI are used for viability.

Monitor single cell to single colony formation

Lee JS, Kallehauge TB, Pedersen LE, Kildegaard HF (2015) Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway. Sci Rep. Feb 25;5

During clonal selection, a single cell was seeded per well and colony growth was monitored over the next 14 days. Generated colonies were imaged and analyzed for colony number, size, shape and expression of a fluorescent marker indicative of a successful transfection.

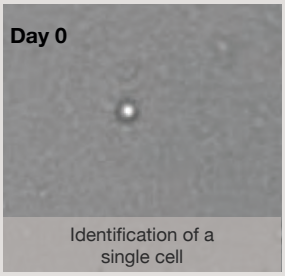


> *The Celigo is really multifunctional – it can do an awful lot. If you want to track growth rates, it will do it perfectly. If you want to do large analysis, like [embryoid bodies], it will be perfect.*

Monitor the tumor sphere formation from a single cell to a single sphere in microwell plates

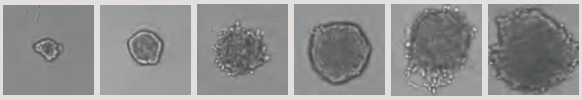
Single cell to single tumor sphere
A single cell is seeded per well and the formation of a tumor sphere is monitored over the next 15 days using the Celigo and the tumor sphere application. Celigo captures whole-well images on day 0 to document the presence of 1 cell per well. This data is used to verify that the formation of the sphere originated from a single cell.

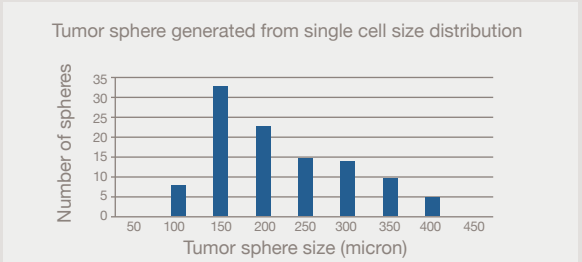
Quantification of tumor sphere size
On day 15 the entire 96-well plate is imaged and the tumor sphere size is automatically analyzed. The size distribution graph on the right shows a variation of tumor sphere sizes, (100 – 300 microns) that arose from single cell clones seeded on day one. The Celigo-captured images (top right) show the great variation in formed tumor sphere size and morphology.



Identification of a single cell

Formation of a single tumor sphere





Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells

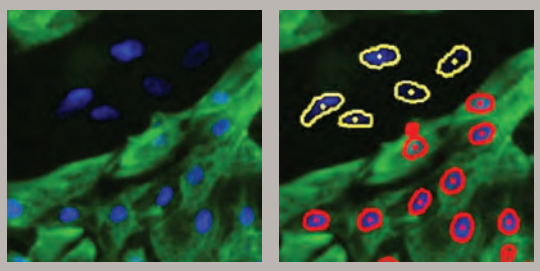
Celigo image cytometer is utilized during the multi-stage process of iPSC generation. Bright field confluence and fluorescent viability assays are used during fibroblast growth tracking, expansion and freeze/thaw cycles. Formation and imaging of iPSC colonies using fluorescent fixed and live-cell markers such as Tra-1-60, Oct4 and SSEA4 are used to monitor the re-programming process. Finally, the Celigo is used to examine differentiation by imaging and quantifying embryoid bodies and cell-line specific markers.

iPSC related markers:

Cellular markers: Nanog, Sox2, KLF4, MYC, Oct4

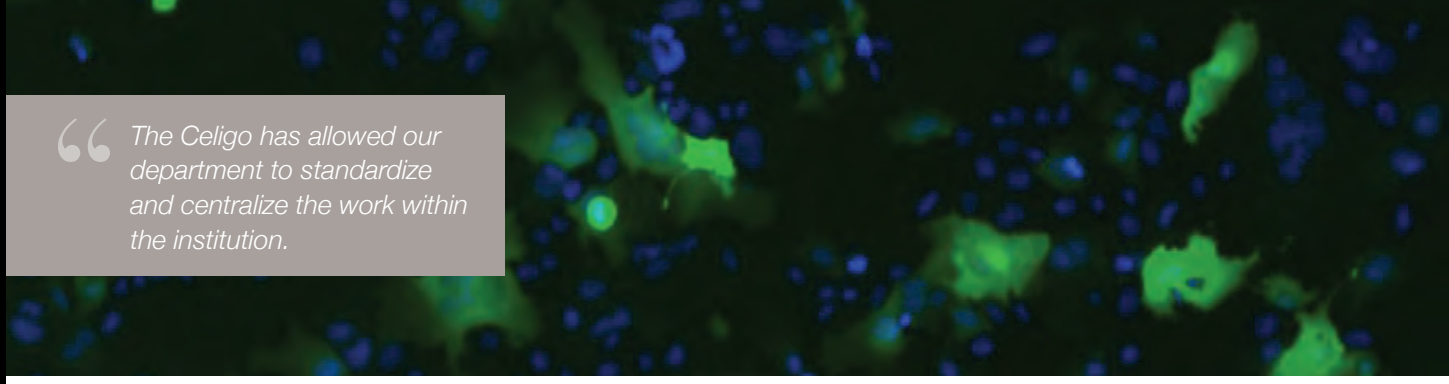
Cell surface markers: SSEA-3, TRA-1-60, TRA-1-81

Differentiation markers: Troponin-T, alpha-actinin



iPSC-derived cardiomyocytes stained with troponin-T and Hoechst. On the right, the same population of cells is analyzed using Celigo gating interface. Cells outlined in yellow represent troponin-T negative cells and those outlined in red are troponin-T positive differentiated cardiomyocytes.

Citations:
Paull D, et al. Nat Methods. 2015 Sep;12(9):885-92
Zhou H, et al. Stem Cell Rev. 2015 Aug;11(4):652-65
Proschel C, at al. EMBO Mol Med. 2014 Apr;6(4):504-18



> *The Celigo has allowed our department to standardize and centralize the work within the institution.*

Celigo Specifications

Software	Proprietary image acquisition and processing software Powerful analysis software/Computer workstation Microsoft Windows				
Illumination/Optics	1 LED-based enhanced bright field imaging channel with uniform well illumination 4 LED-based fluorescent channels Proprietary F-theta lens with superior well edge-to-edge contrast Galvanometric mirrors for fast imaging of large areas Large chip CCD camera (2024x2024 pixels) 1, 2, 4 or 8 um/pixel resolution				
Fluorescent Channels	Channel	Excitation	Dichroic	Emission	Typical Dyes
	Blue	377/50	409	470/22	Hoechst, DAPI
	Green	483/32	509	536/40	FITC, Calcein, GFP, AlexaFluor® 488
	Red	531/40	593	629/53	R-PE, PI, Texas Red, AlexaFluor® 568
	Far-Red	628/40	660	688/31	DRAQ5®, AlexaFluor® 647
Plate Compatibility	6, 12, 24, 48, 96, 384, 1536 well plates (black, white and clear wall plates) T-25 and T-75 flasks Slides and cell array plate profiles available upon request				
High-Speed Imaging	Less than 2 minutes per 384-well plate				
Weight and Dimensions	Dimensions: 19.5” W x 16”H x 24”D (49.5cm x40cm x 61cm) Weight: 117 lbs. (53 kg)				
Power Requirements	110-220 VAC 50-60 Hz				
Regulatory Compliance	CE marking				
Focusing modes	Hardware-Based Auto Focus, Image-Based Auto Focus, Manual Focus				

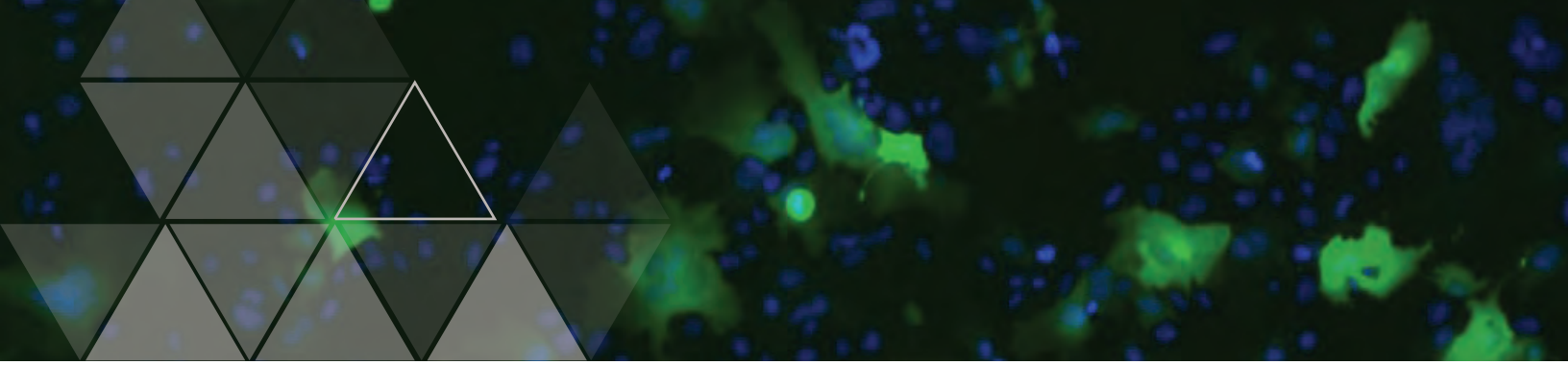


Plate Scan Times

Plate Type	Images / Well	Resolution (µm / pixels)	Typical Time
24-well Plate	1	2	< 7 minutes
96-well Plate	16	2	< 3.5 minutes
96-well Plate	16	1	< 5 minutes
384-well Plate	1	2	< 2 minutes
384-well Plate	4	2	< 7 minutes

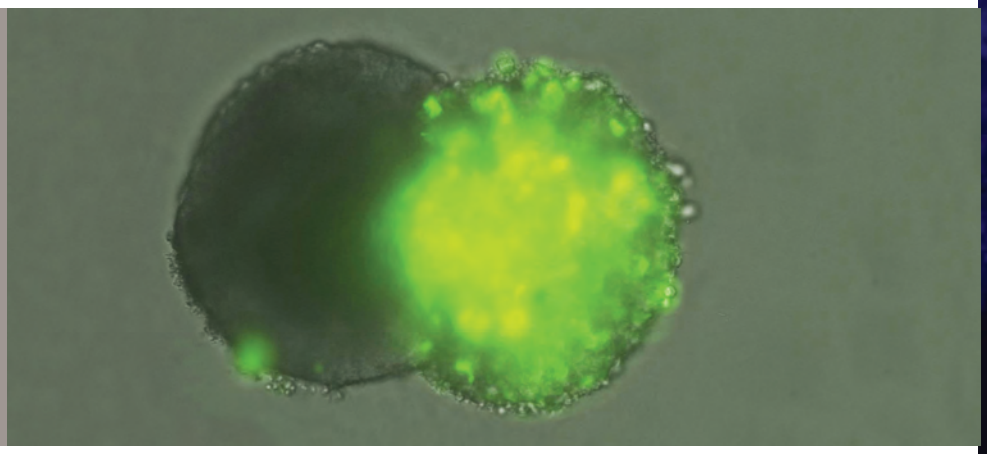
Recommended Plate Types

Plate Name	Manufacturer	Well Type
6-Well BD Falcon™ 353046 Plate	Corning	Clear
6-Well Corning™ 3516 Plate	Corning	Clear
12-Well Corning™ 3513 Plate	Corning	Clear
24-Well Corning™ 3524 Plate	Corning	Clear
24-Well PE Visiplate™ 1450606 Plate	Perkin Elmer	Black
Nexcelom^{3D} 96-well Ultra-low attachment treated round bottom multi-well plates	Nexcelom	Clear
96-Well Corning™ 3603 Plate	Corning	Black, White
96-Well Greiner™ 655090 Plate	Greiner	Black, White
Nexcelom^{3D} 384-well Ultra-low attachment treated round bottom multi-well plates	Nexcelom	Clear
384-Well Corning™ 3542 Plate	Corning	Low vol, Black
384-Well Corning™ 3680 Plate	Corning	Clear
384-Well Corning™ 3712 Plate	Corning	Black, White
1536-Well Corning™ 3838 Plate	Corning	Black, White

See our website for a complete listing of all recommended, supported and unsupported vessels

Nexcelom Bioscience
360 Merrimack Street, Building 9
Lawrence, MA 01843, USA

"We have one Celigo, completely integrated, and we have 5 people using it consistently. But more and more people outside our team are approaching us to use the Celigo. We've made it popular within the company. People are starting to realize the advantages of the robust system, and of what they can get out of it.



Innovation and Expertise in the Science of Cell Counting

Technical Seminars are an excellent way to introduce the Celigo image cytometer to a lab group or collaborators in different laboratories within an organization. A trained biologist will discuss and demonstrate the capabilities and advantages of the Celigo image cytometer for cell viability and cell-based assays.

On-Site Demonstrations are a convenient way to evaluate the Celigo system. We have regional demonstration laboratories where you can have a hands-on session to test your cells. Another option is to have an experienced Applications Specialist visit your lab with the Celigo system for a hands-on session to test your application.

Schedule a FREE on-line demonstration, on-site demonstration or technical seminar with a Nexcelom Applications Specialist today.

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