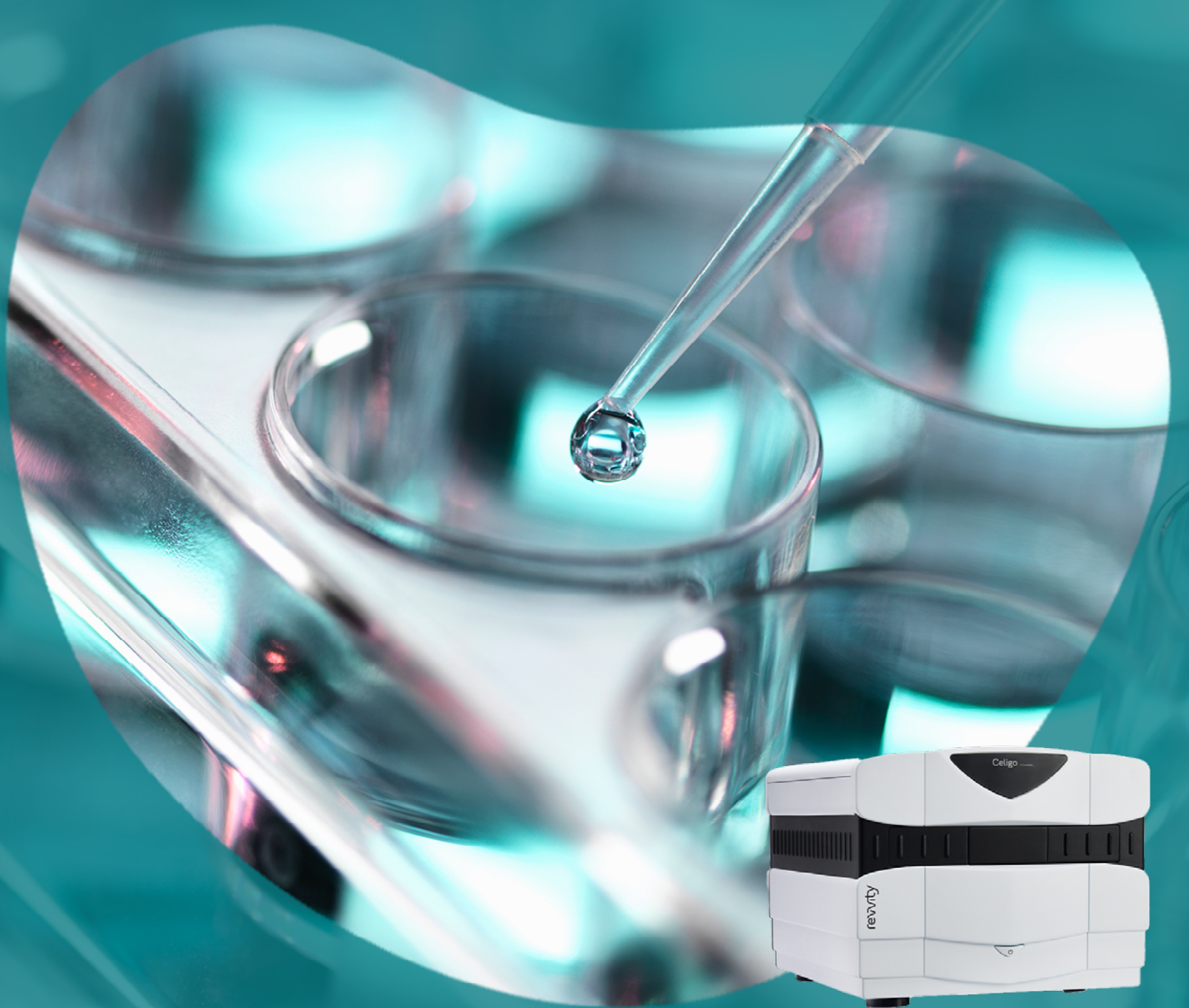


Whole-well
imaging.



revvity



Celigo™ image cytometer



Celigo image cytometer overview

The benchtop Celigo image cytometry system provides high-throughput, whole-well imaging and quantitative data through image analysis in brightfield 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 microplates from 6 to 1536-well and T-flask formats.

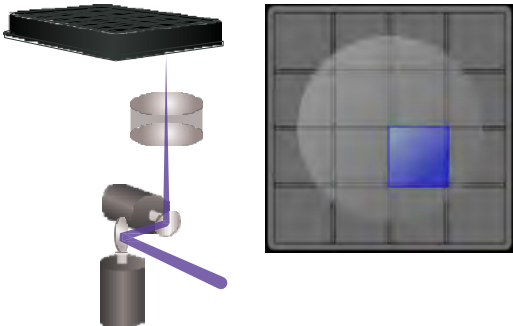
The workflow based intuitive software provides concurrent imaging and analysis; kinetic analysis such as time-lapse growth tracking, flow cytometry-like gating analysis and reporting of cell populations. Cell images of specific populations may be displayed with color overlays.

The Celigo system 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.

Core technology

Proprietary optics

Whole-well images are captured moving the plate once per well.



Illumination

Flat illumination and excellent edge-to-edge contrast images in every well.

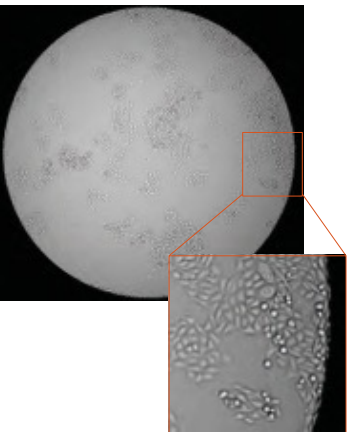
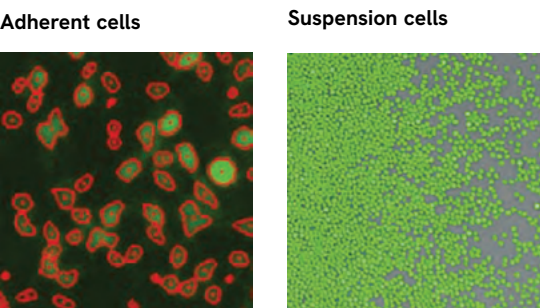


Image segmentation

Brightfield and fluorescence algorithms for segmentation of adherent and suspension cells.

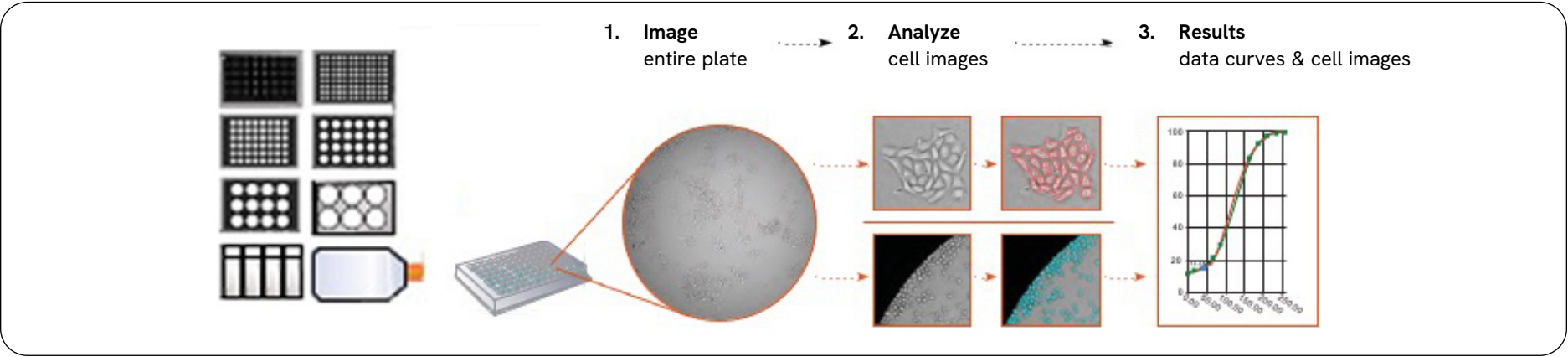


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Whole-well, high resolution images acquired at high speed



Image

- 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 brightfield and 4 fluorescent channels

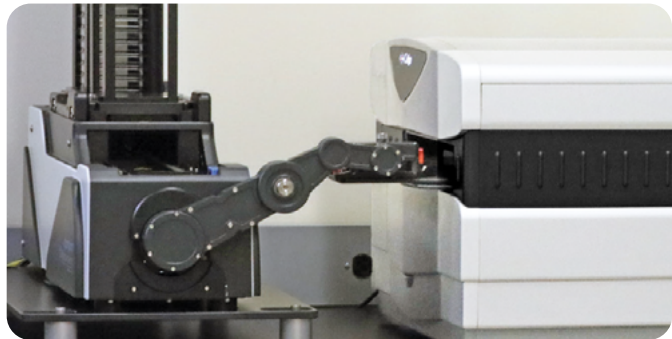
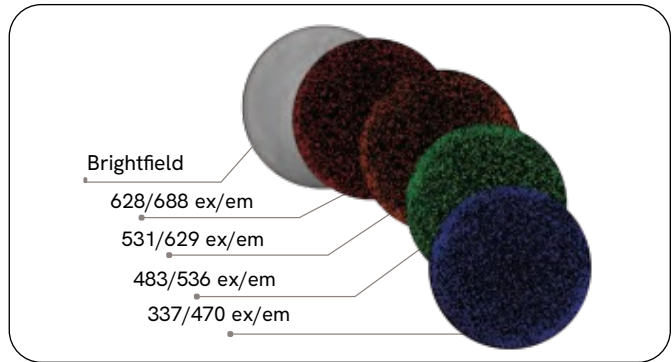
Analyze

- Fast scanning for image acquisition and analysis with minimal plate movement ensuring minimal sample disruption
- Quantify cells and colonies with a non-invasive method
- Measure adherent cells without trypsinization

Results

- Save experiment settings – quickly run the same assay on many plates without additional set up
- System automatically stitches multiple fields of view into a single full resolution image

Imaging channels



Celigo easily integrates with robotic arms, plate stackers, automated incubators and liquid handlers.



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Fluorescent assays

Cell cycle, viability/cell health, internalization & phagocytosis, co-culture, surface proteins & antibodies, cell secretion, transfection/transduction, apoptosis, migration

Brightfield

Adherent cell growth tracking, suspension cell counting, embryoid 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

Virology

Viral titer, viral infection, antibody neutralization, transduction efficiency, cytopathic effect, CAR T cell-mediated cytotoxicity

iPSC reprogramming

Fibroblast doubling, iPSC colony counting, embryoid body formation, immunostaining for differentiation

Migration | Invasion

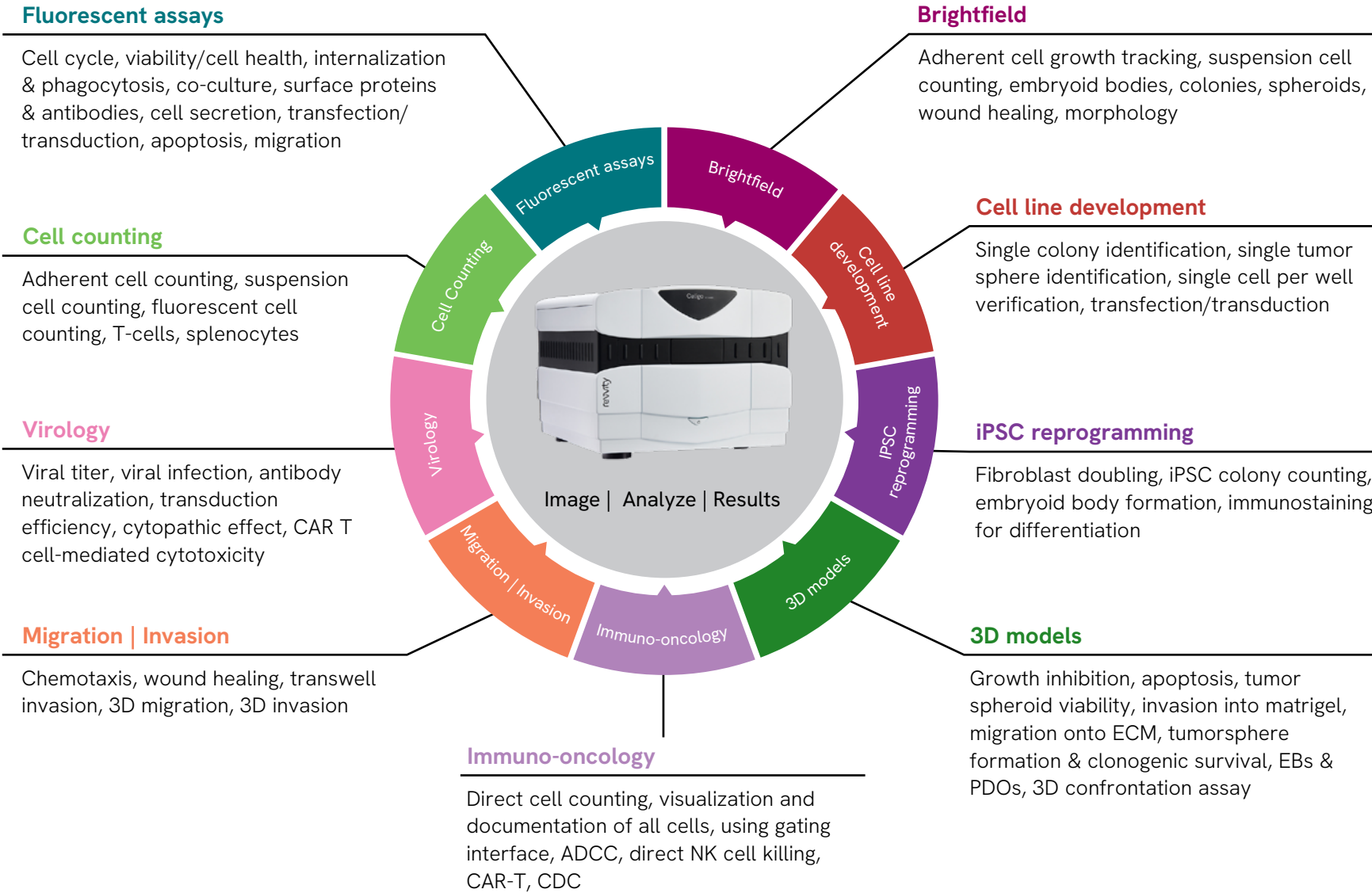
Chemotaxis, wound healing, transwell invasion, 3D migration, 3D invasion

3D models

Growth inhibition, apoptosis, tumor spheroid viability, invasion into matrigel, migration onto ECM, tumorsphere formation & clonogenic survival, EBs & PDOs, 3D confrontation assay

Immuno-oncology

Direct cell counting, visualization and documentation of all cells, using gating interface, ADCC, direct NK cell killing, CAR-T, CDC





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Integrated cell imaging and analysis in the same software

Oncology

- Apoptosis
- Cell cycle
- Cell viability
- Cytotoxicity
- Cell migration
- Cell morphology
- Immunophenotyping
- Metabolism
- Wound healing
- Chemotaxis

Immuno-oncology

- Direct cell-mediated cytotoxicity
- Antibody-dependent cell-mediated cytotoxicity (ADCC)
- Complement-dependent cytotoxicity
- Antibody-mediated cytotoxicity

3D tumor spheroid modeling

- Growth tracking
- Growth inhibition
- Apoptosis | Viability
- Invasion | Migration
- Patient derived organoids (PDOs)

Stem cell research

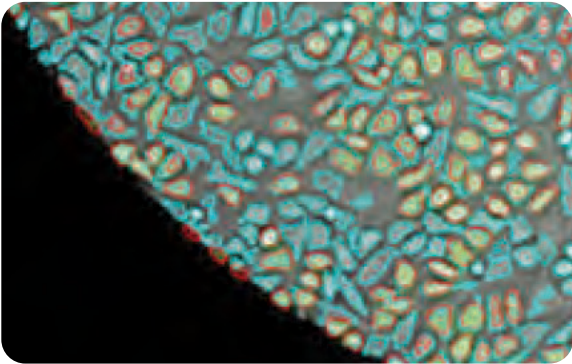
- iPS cell line generation
- Embryoid body morphology
- Stem cell marker analysis

Virology assays

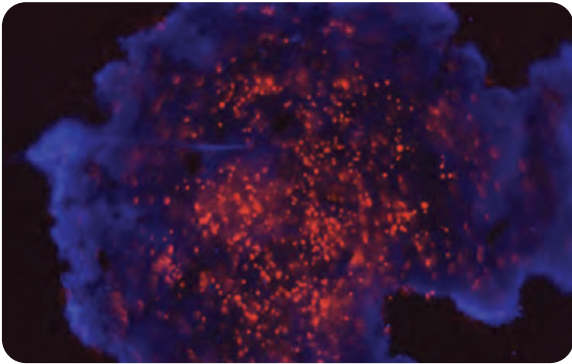
- Foci and plaque counting
- Antibody neutralization
- Hemagglutination assay

Cell line development and biologics production

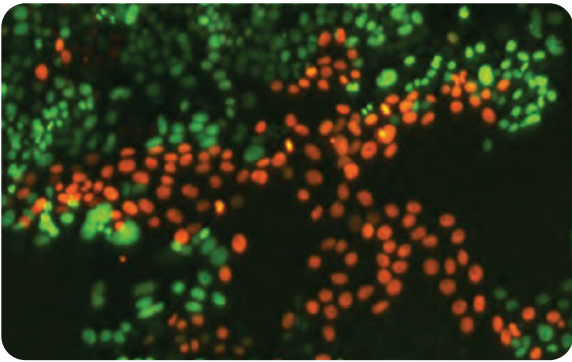
- Single cell detection
- Transfection | Transduction efficiency
- CRISPR gene editing monitoring
- Single cell to single colony monitoring
- Growth tracking
- Hybridoma screening
- Routine quality control



Cells counted to the well edge



Propidium iodide and Hoechst stained 3D spheroid



Surface marker stained iPS cells

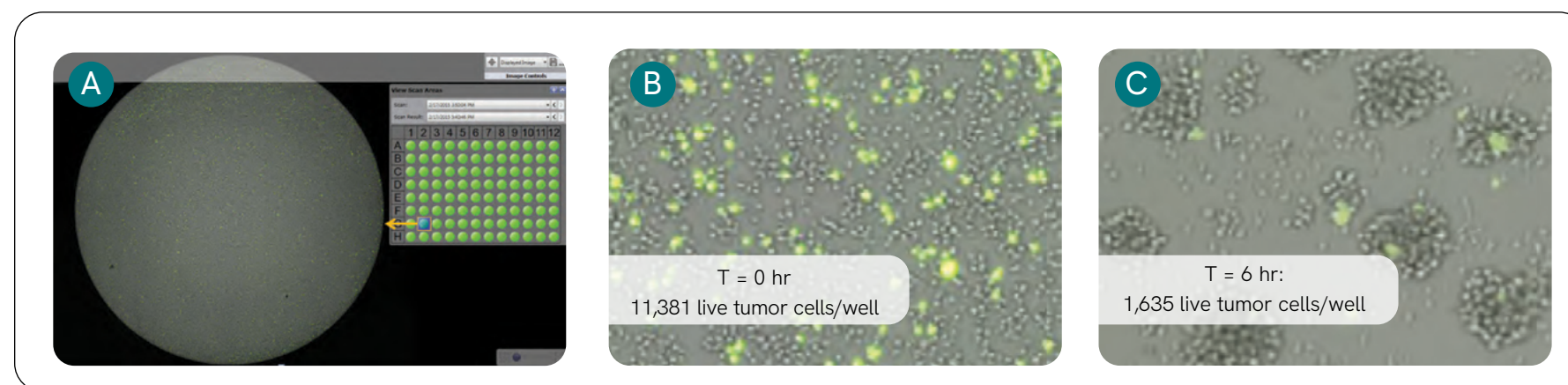
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Immuno-oncology

Accurate & consistent cell-mediated cytotoxicity assay for immuno-oncology

The Celigo system images and counts cells directly within the experimental environment, while other methods often require the removal of cells from the experimental plate to perform end-point assays. Improve your data by performing consistent and reliable direct cell counts. Immuno-oncology assays additionally benefit from Celigo's increased sensitivity since 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 highest number of data points. Typically, only a few minutes are required for imaging and analyzing a 96- or 384-well plate.*

Quantify killing & visualize formation of immune complexes: Direct cell counting assay in a 96-well plate



Whole-well cell counting

Acquire brightfield and fluorescent whole-well, high resolution images (A).

Auto-save hi-resolution images

Celigo images are used to verify results and are automatically saved for all wells (A).

Kinetic measurement of tumor cell killing

Images from the same 96-well plate (B, C) were acquired at 0 hours and 6 hours during a non-destructive, kinetic assay using Calcein AM. Celigo images verify the decrease of Calcein positive live tumor cells from 11,381 to 1,635 within the same well.



Antibody-dependent cell-mediated cytotoxicity (ADCC) assays

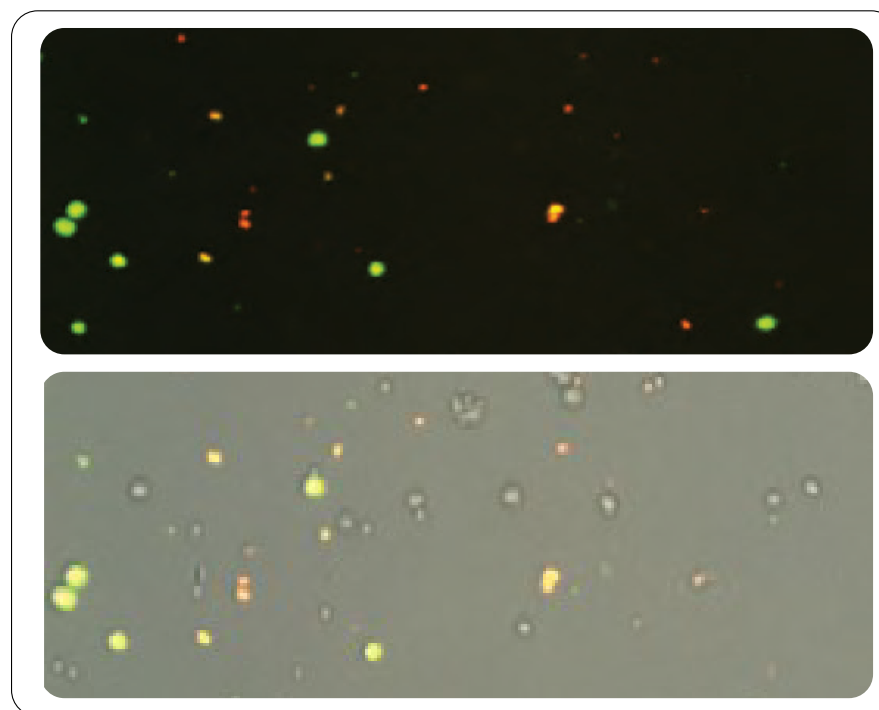
NK cell mediated cytotoxicity effects on targeted cells

Live Calcein AM-positive cells were counted to determine the effect of a bimodal fusion protein (M7824) on NK cell-mediated cytotoxicity in co-cultures.

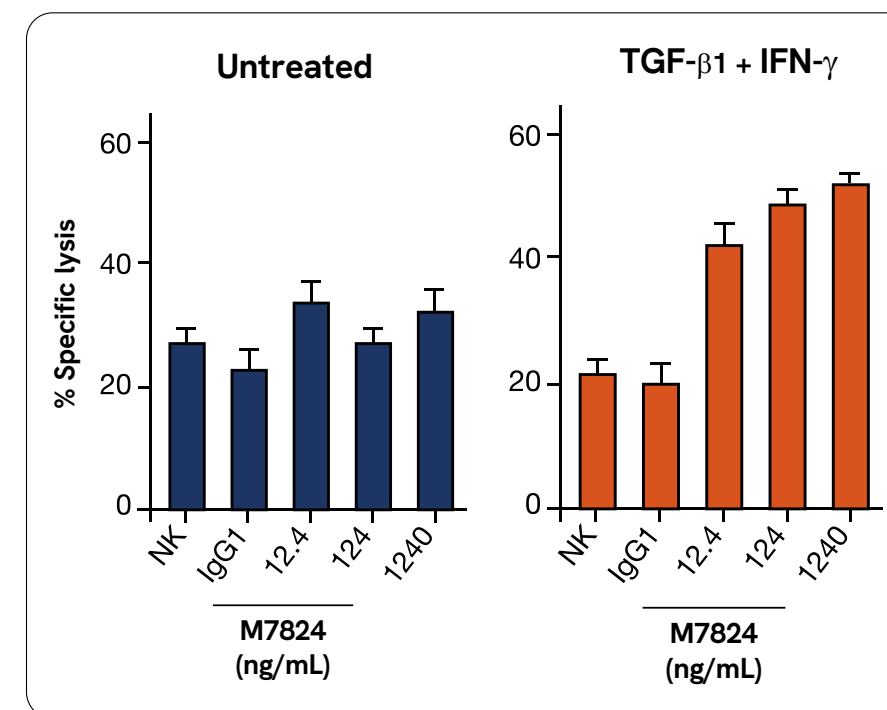
Target cells (A549) were pretreated with TGF- β 1 and IFN- γ and stained with Calcein AM (CAM). The NK cells were isolated from PBMCs and co-cultured with the A549 cells.

- Cytotoxicity imaged in co-cultures
- Percent specific lysis determined

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Fluorescent and brightfield images of A549 treated with M7824 proteins. After 16 hours of co-culture with NK cells, propidium iodide (PI) was added, and live cells (CAM+/PI-) target cells were counted.



Specific Lysis (%): Pretreatment with TGF- β 1 and IFN- γ sensitized A549 target cells to ADCC by M7824.



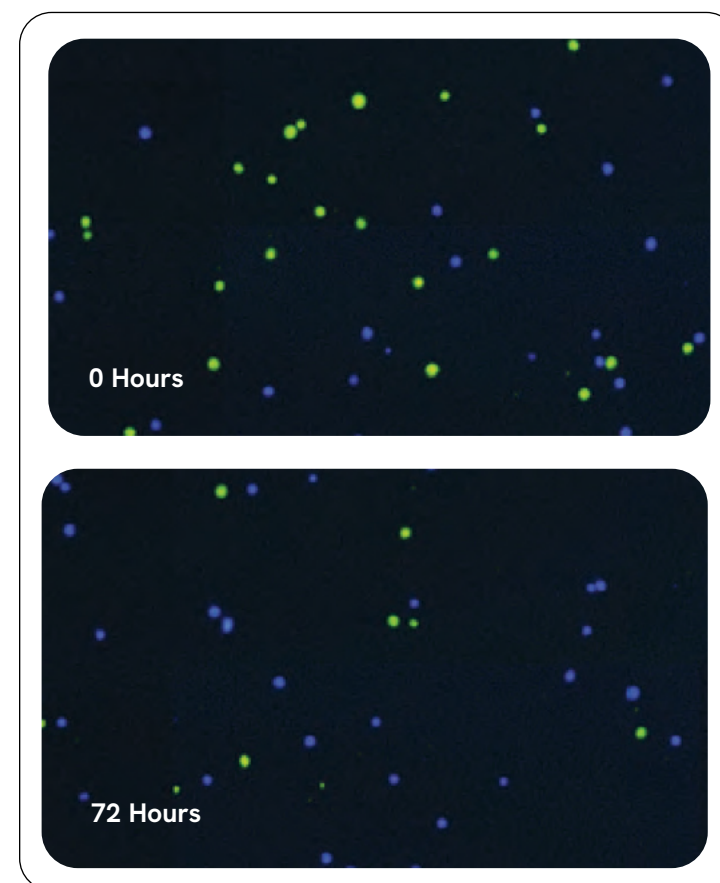
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Tracking bispecific antibody induced cytotoxicity in complex co-culture

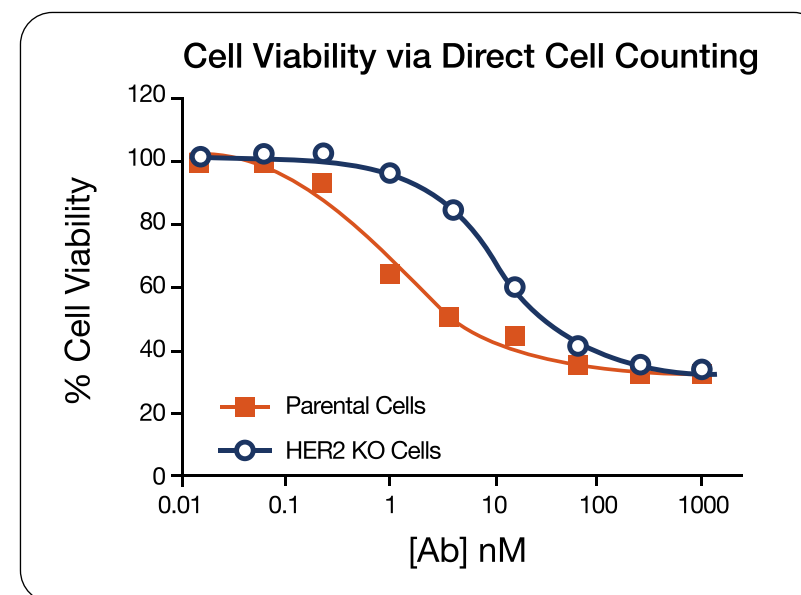
Multi-valent antibody analysis (DuetMab Assay)

The Celigo was used to directly count co-culture of prenatal cells and HER2 KO cells stained with CellTracker™ Green and Violet, respectively.

The assay investigated the selective cytotoxicity effect of the monovalent bispecific IgG (DuetMab) on a co-culture of prenatal cells and HER2 KO cells.



Fluorescent images of time 0 and 72 hours of prenatal and HER2 KO cells incubated with DuetMabs



Cell viabilities were calculated by direct cell counting, which showed differences in DuetMabs dose response between the prenatal and HER2 KO cell types (IC50: 1.08 nM and 10.71 nM, respectively).



Drug screening

Discover combination therapies, investigate mechanisms of drug resistance and signaling pathways

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.

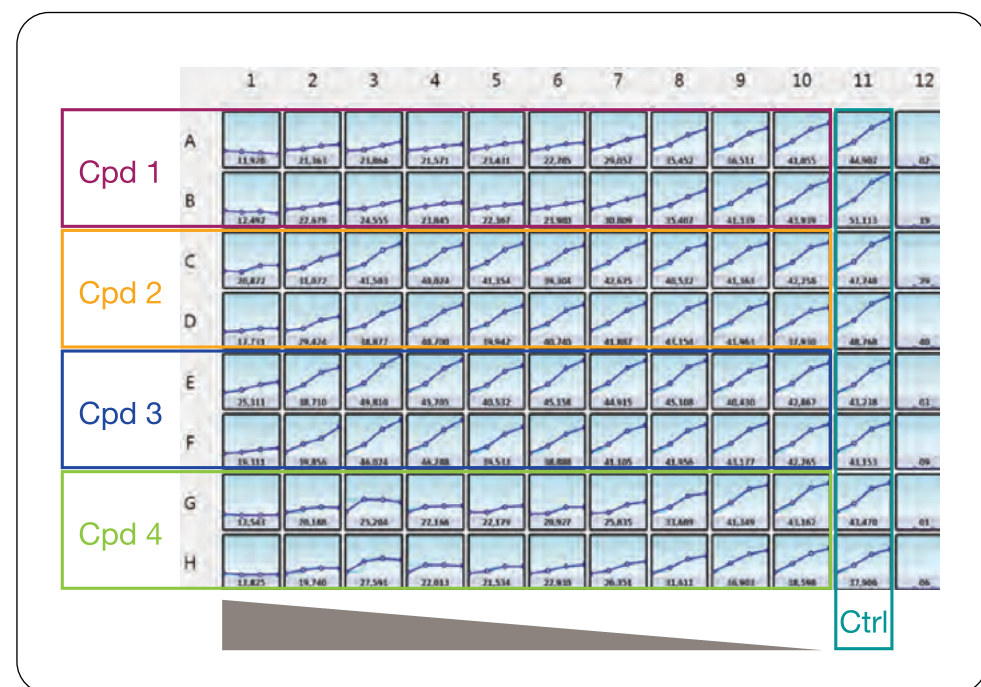
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- Count and analyze cells directly in experimental plates
- Up to 5 imaging channels (e.g. BF, far red, red, green, blue)
- Whole-well imaging for more accurate analysis
- Direct high-throughput counting of suspension and adherent cells

Label-free kinetic cytotoxicity assays

Multi-drug growth inhibition

- 96-well plate set-up with 4 compounds at 10 concentrations and a control
- Scans the plates at multiple time points over hours or days
- Automatic growth curve generation for each well



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Automatically quantify 2D migration | invasion assays

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

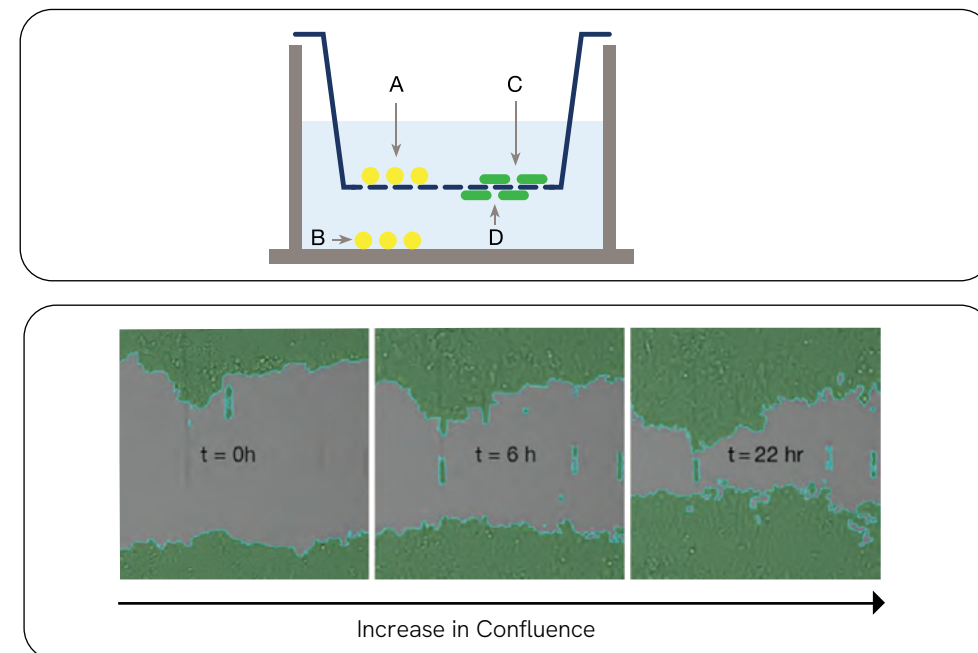
Suspension cells located inside the insert (A) migrate through the porous membrane toward the chemoattractant in the bottom of the plate.

Adherent cells located on the top of an ECM coated insert (C) invade and migrate to the bottom side of the membrane insert.

Celigo automatically images and counts the migrated suspension and adherent cells (B, D).

Image and quantify wound healing assays

The entire well is imaged at multiple time points. Software automatically, and concurrently with imaging, calculates the cell confluence in each well.

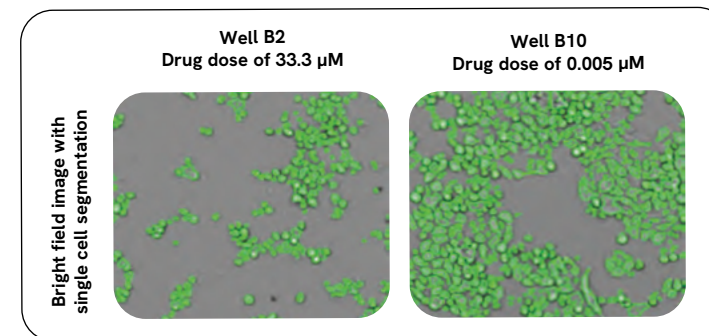
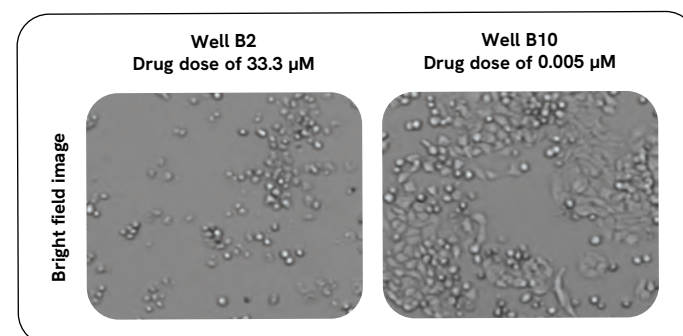


Monitor growth inhibition using direct cell counting

Dose-dependent growth inhibition

Generated images are from two wells, B2 and B10, post drug treatment at doses of 33.3 μM and 0.005 μM respectively.

- Visual determination of growth inhibition (greater in B2 than B10).
- Identification and segmentation of individual cells (green) for each drug treatment.



3D tumor models

Image cytometry enabling high-throughput 3D tumor spheroid screening

3D tumor spheroids are highly complex models increasingly used for cancer drug screening assays due to their better physiological relevance. Unlike 2D models, where cells can be enumerated and analyzed individually, the 3D model, which consists of clustered cells, requires a more advanced analysis method to characterize and quantify the effect of drugs on the entire spheroid. Therefore, to create a multi-parametric analysis platform, various orthogonal assays are required to screen the drug compounds. Celigo's 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.

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Growth inhibition studies in microplates

1. Form and treat 3D tumor spheroids

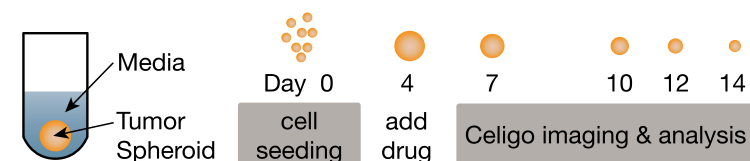
Four days after seeding, spheroids were treated with compounds for 72 hours in u-bottom ultra low attachment 96-well plates.

2. Analyze 3D tumor spheroids

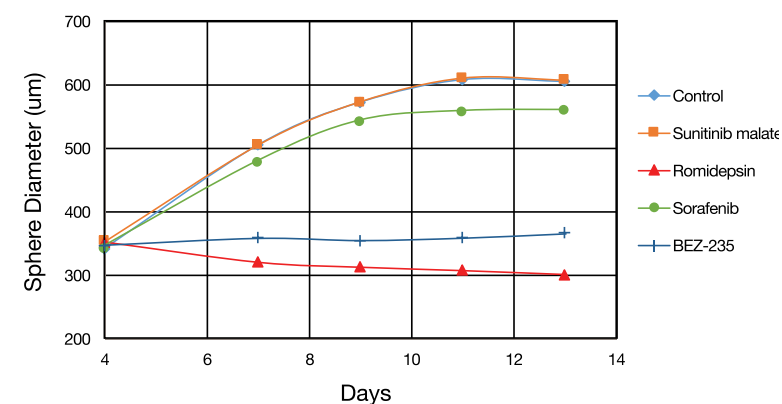
Whole-well images are acquired and analyzed at multiple days after adding drug compounds.

Tumor sphere diameter under different drug treatments is exported into Excel for further data analysis.

1. Experimental Setup



2. Growth Inhibition of U-87 MG Tumor Spheroids by BEZ and Romidespin



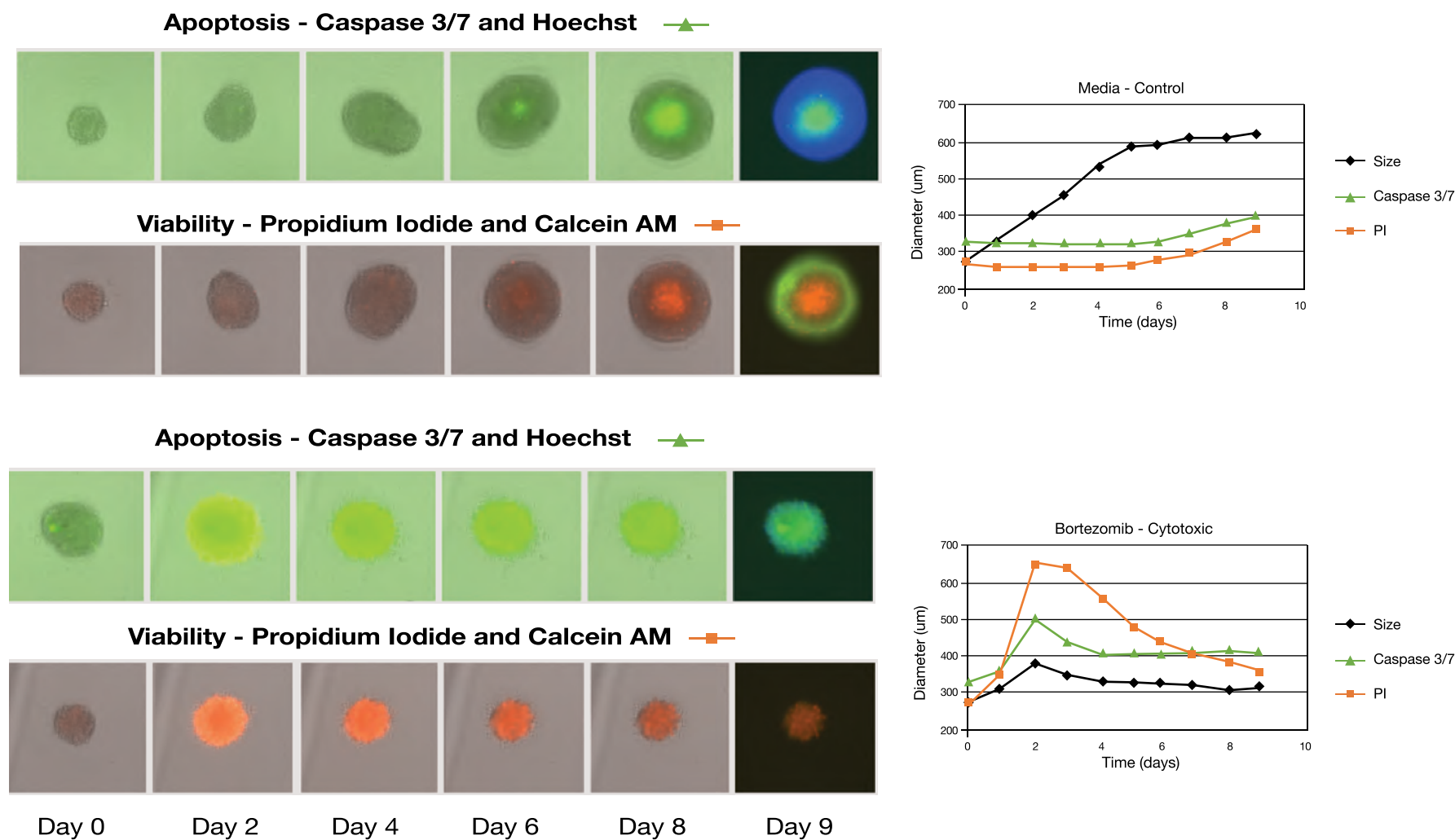


Real-time kinetic apoptosis and viability screening

Measure the effects on apoptosis and viability with fluorescent image analysis.

Tumor spheroids were treated with different drug compounds and screened to measure the effects on apoptosis and viability. The drug effects are characterized by measuring caspase 3/7 and propidium iodide (PI) fluorescent intensities over time.

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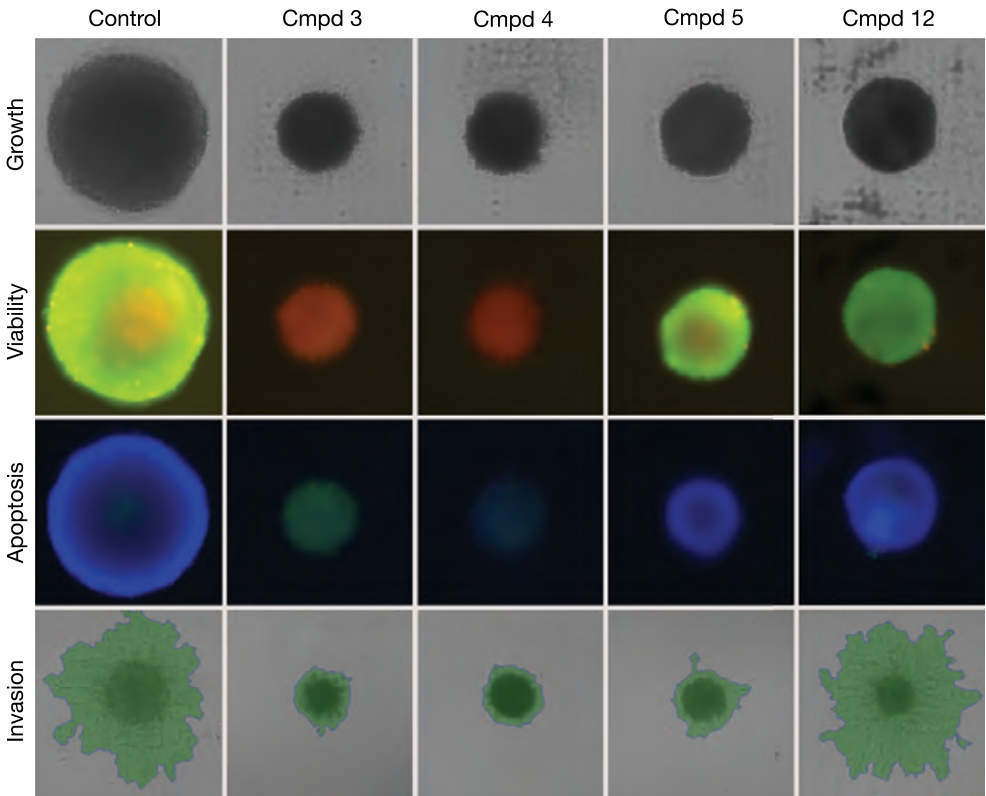
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Tumor spheroid drug screening

Visualize drug compound screening

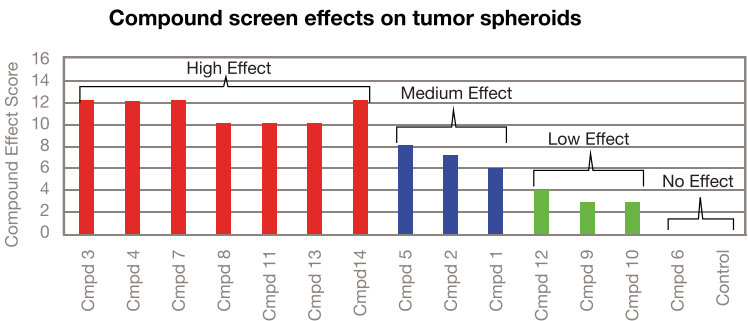
Spheroid growth, viability (calcein AM/PI), apoptosis (Caspase 3-7/Hoechst) and invasion are analyzed.

Brightfield and fluorescent images acquired by the Celigo visually confirm the state of the spheroid under different drug treatments.



Drug screening data analysis

Data generated from image analysis: Compound 3 and 4 were highly cytotoxic to the spheroids, while compound 5 and 12 showed cytostatic effects.



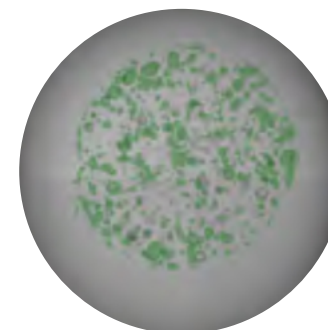
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Growth tracking and viability of organoids

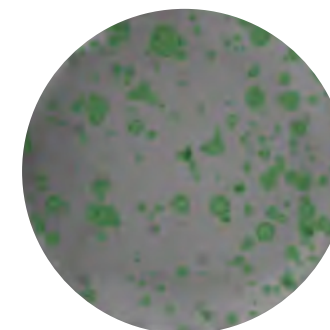
3D organoid analysis

Organoids are a 3D in vitro culture system derived from self-organizing stem cells. They can recapitulate the *in vivo* architecture, functionality, and genetic signature of original tissues and are thought to be a relevant model for evaluating drug effects.

In the examples shown here, green segmentation is added to bright field images for quick and easy identification of Celigo counted organoids.



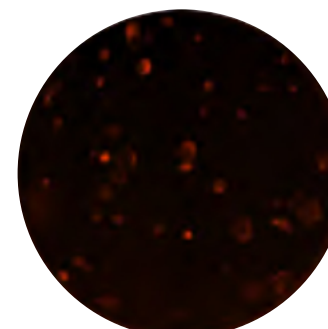
Dome Method



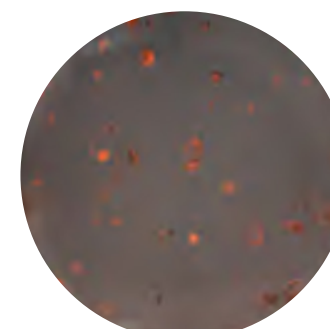
Spin Method

Viability determination

Addition of non-perturbing dyes like propidium iodide (PI) generates valuable viability data, as seen in these whole-well images.



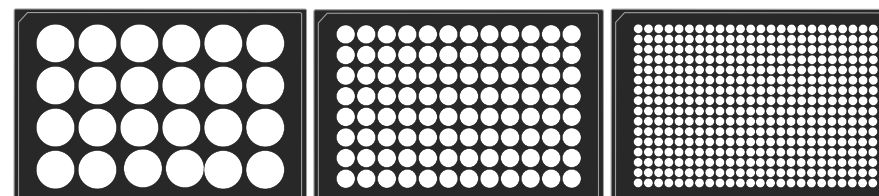
Propidium Iodide



Brightfield and PI Overlay

Perform organoid analysis in 24- to 384-well plates

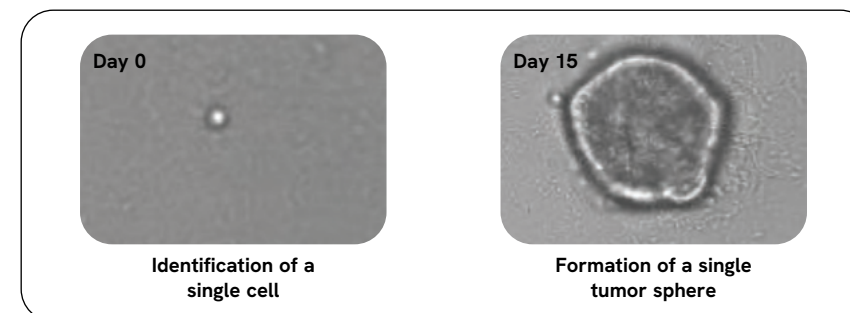
Whole-well imaging for organoid screening can be done in 24-well plates down to 384-well plates.



Monitor tumor sphere formation in microwell plates: single cell to a single sphere

Single cell to single tumor sphere

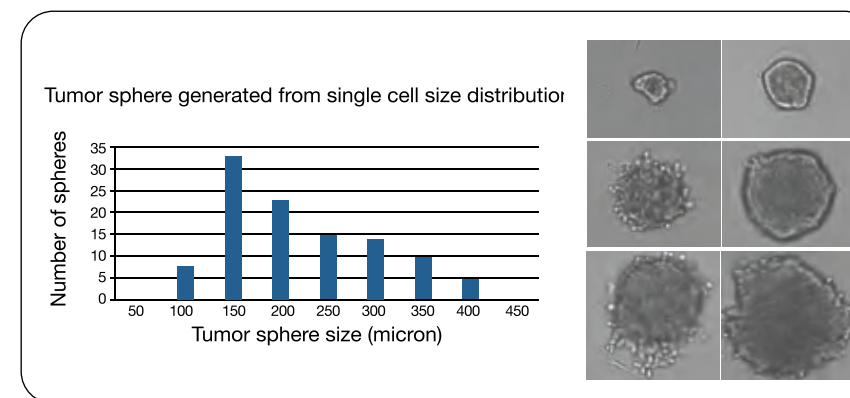
Use whole-well imaging to confirm a single cell per well (shown on Day 0) and verify its formation into a single tumor sphere (shown on Day 15).



Quantification of tumor sphere size

Each tumor sphere (shown at right) grew from a single cell clone seeded on day 1.

- Images show the variation in formed tumor sphere size and morphology.
- A size distribution graph was generated showing the variation of tumor sphere sizes.



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Cell line development through gene editing

Quantify and monitor 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/transduction efficiencies
- Rapid high-throughput imaging
- Direct documentation and verification of single cell to a single colony

Monitor cell health and expansion during cell line development

Quantify transfection efficiencies*

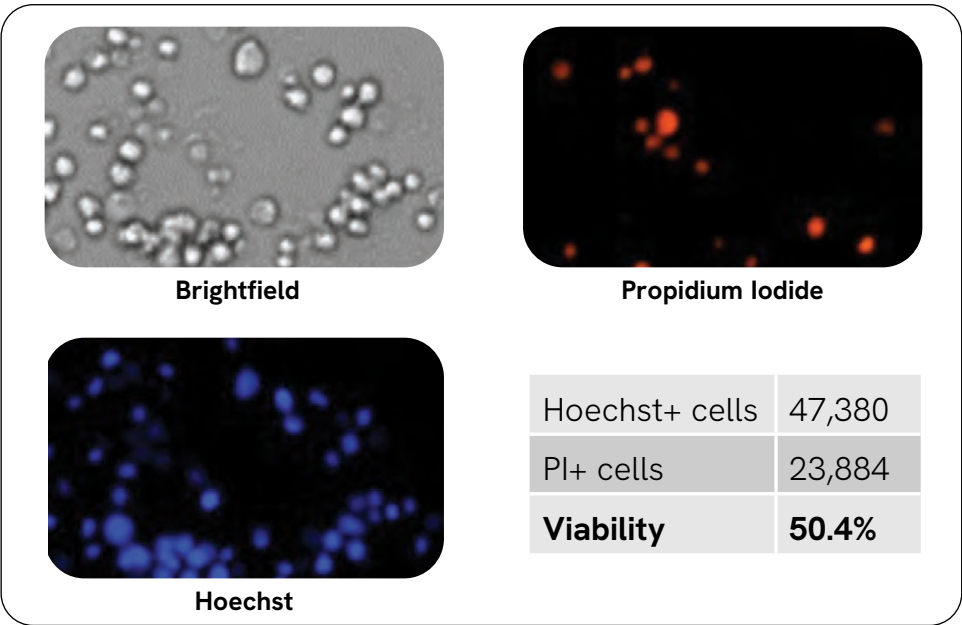
Whole-well imaging combined with the 2D gating interface allows for high-throughput testing of transfection constructs and as a quality control measure during expansion to verify cell line stability.

Monitor single cell to single colony formation†

Generated colonies are imaged and analyzed for colony number, size, shape and expression of a fluorescent marker indicative of a successful transfection.

Monitor cell expansion during batch culturing‡

During culture expansion, cell growth and viability were measured using a bright field and multi-channel fluorescent assay. (Images below BF = total cells, PI = dead cells, Hoechst = live cells)



*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

†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

‡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



Tracking Monoclonality

Within the Celigo software, the Single Colony Verification application creates and captures the scans for time course monitoring. The Celigo *Monoclonality Report* streamlines the detection and screening of single cell derived colonies over the course of multiple timepoints. Clones confirmed as monoclonal in origin, are selected for report export to produce a detailed image timeline. Colony formation data includes traceability to the Celigo scan acquisition date, time, and well location.

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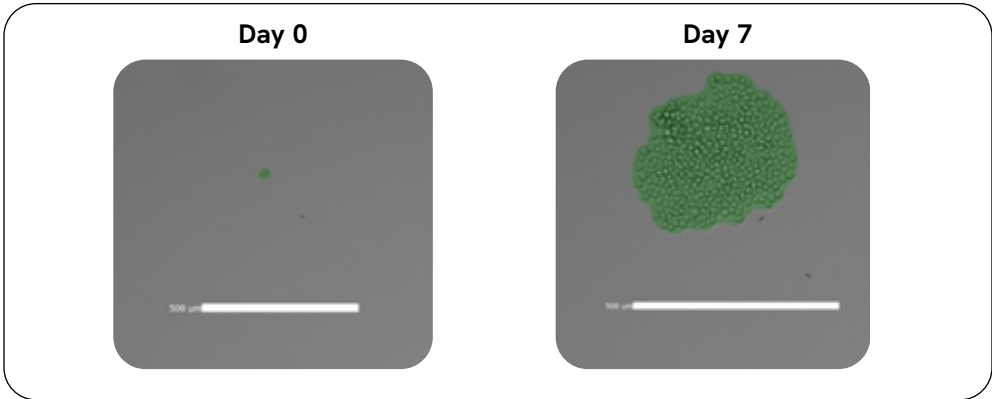


Figure 1: Cropped day 0 image displays a single counted cell within the whole well using brightfield imaging on Celigo. The cell was tracked over a 7-day period, as it formed into a colony. Identified single cell and colony are shown with green overlays.

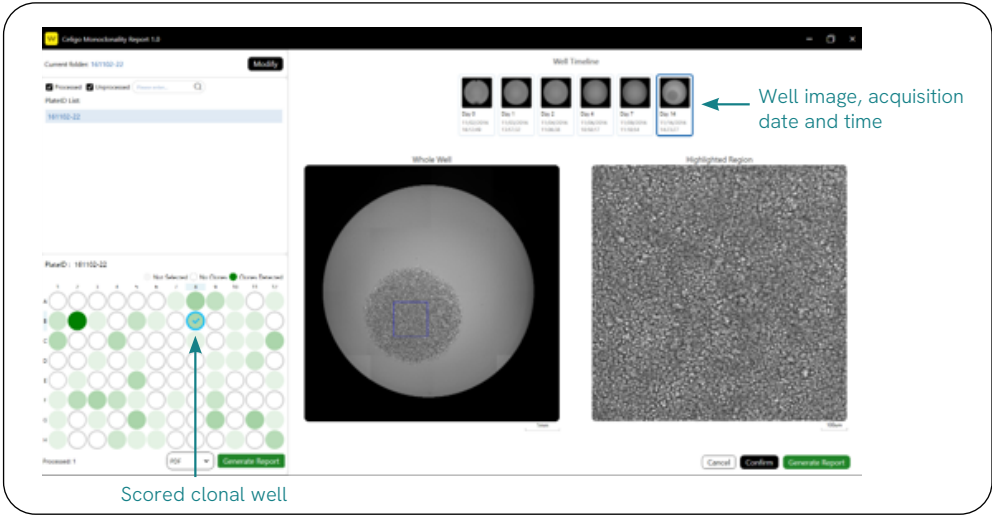


Figure 2: The Celigo monoclonality reporter categorizes wells for cellular growth using a heatmap, providing users with a visual cue for easier identification of clones of interest.

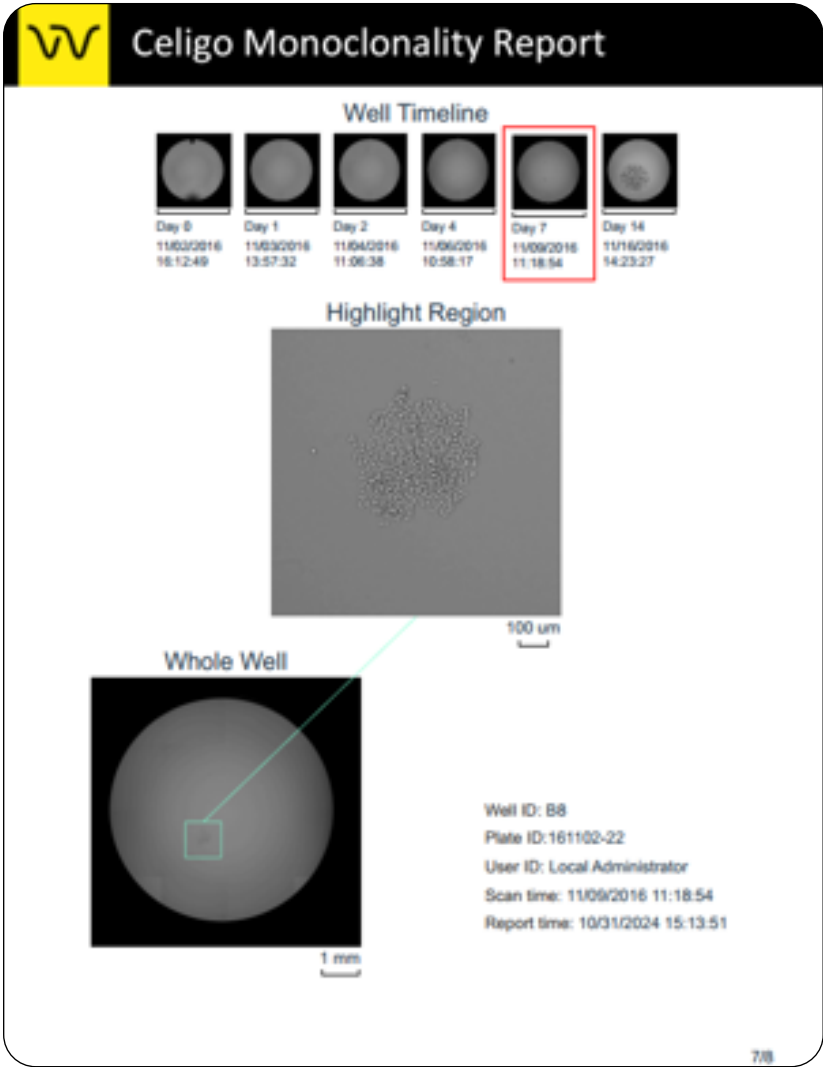


Figure 3: Clones of interest are selected for report export and provide users with well level documentation of colony formation.

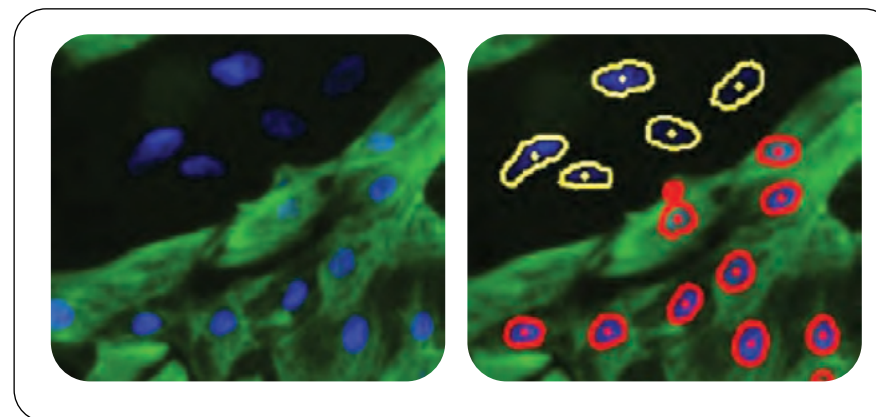


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Automated, high-throughput, characterization and differentiation of induced pluripotent stem cells

Celigo image cytometer is utilized during the multi-stage process of iPSC generation.

- Perform brightfield confluence and fluorescent viability assays.
- Track fibroblast growth, monitor cell expansion during pre and post freeze/thaw cycles.
- Image iPSC colonies using fluorescent fixed and live-cell markers for monitoring the re-programming process.
- Examine differentiation by imaging and quantifying embryoid bodies and cell-line specific markers



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.

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, et al. EMBO Mol Med. 2014 Apr;6(4):504-18



Virology

Analysis of viral-based assays for vaccine, therapy and disease research: automation, high-throughput and multi-parameters

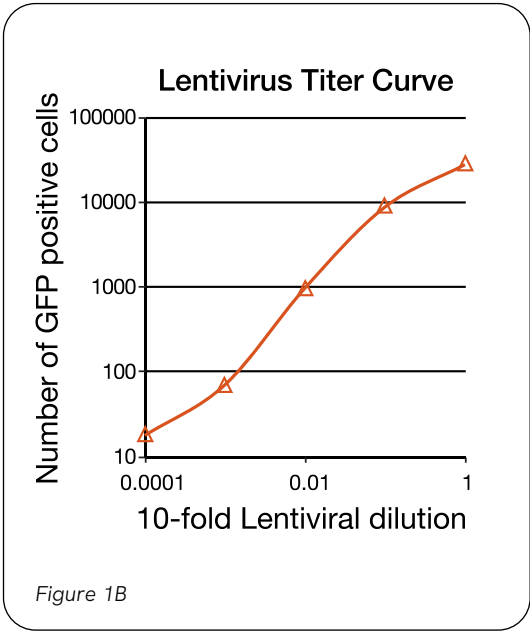
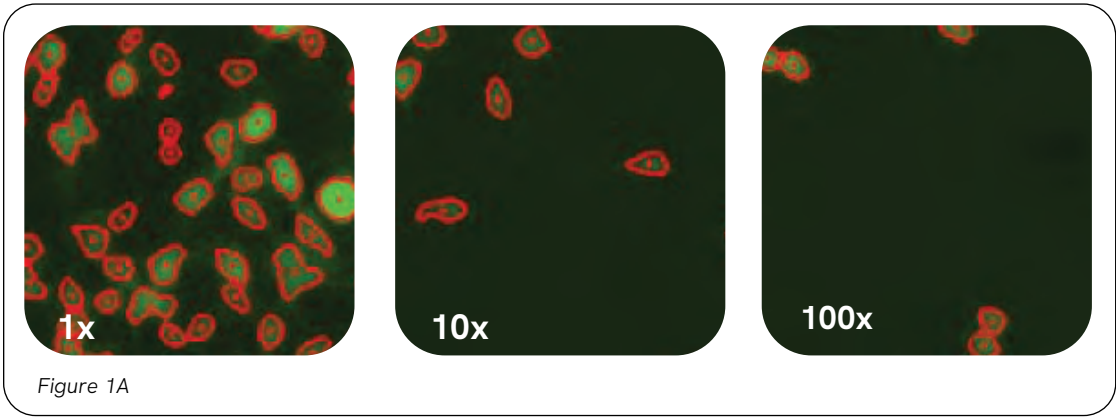
The Celigo imaging cytometer is an essential technology for the standardization and optimization of viral based assays. With rapid image acquisition and data analysis, viral plaque, antibody neutralization, and multiplexed high-throughput assays are achieved in minutes. Also, enhanced sensitivity, for measuring viral titers of low-level infections, is achieved through direct cell counts of whole-well images. The versatility of the Celigo instrument provides fast, efficient and reliable results for your virology research.

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 - Drug screening
 - 3D tumor models
 - Cell line development
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Large dynamic range for viral titers via direct cell counting

Count every infected cell to determine the viral titer

HEK293T cell seeded at 80% confluence and infected with a 10-fold serial dilution of GFP-labeled lentivirus. Post infection, cells were imaged on the Celigo (outlined in red; Figure 1A), and the fluorescent cell count for each well was used to generate a viral titer curve (see Figure 1B)



Yang ML, et al. Sci Rep. 2017 Mar 6;7:43829
Randhawa P, et al. Antiviral Res. 2014 Dec 112:113-9



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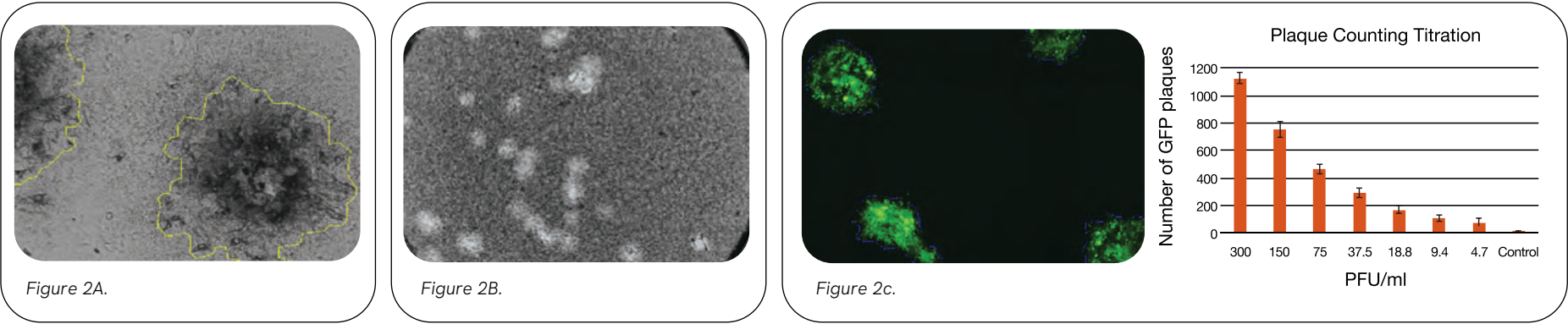
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Not for use in diagnostic procedures.

Increase throughput by automating plaque imaging and analysis

Count brightfield, fluorescent and stained viral plaques

Image and analyze plaques from cytolytic and non-cytopathic viruses: areas of infection in confluent cell culture indicated by horseradish peroxidase (HRP) for non-cytopathic viruses (Figure 2A) and zones of clearing for cytolytic viruses (Figure 2B).

Early viral infections can be seen by detecting zones of viral infection (plaques) by detecting the GFP signal within the infected cells (Figure 2C).

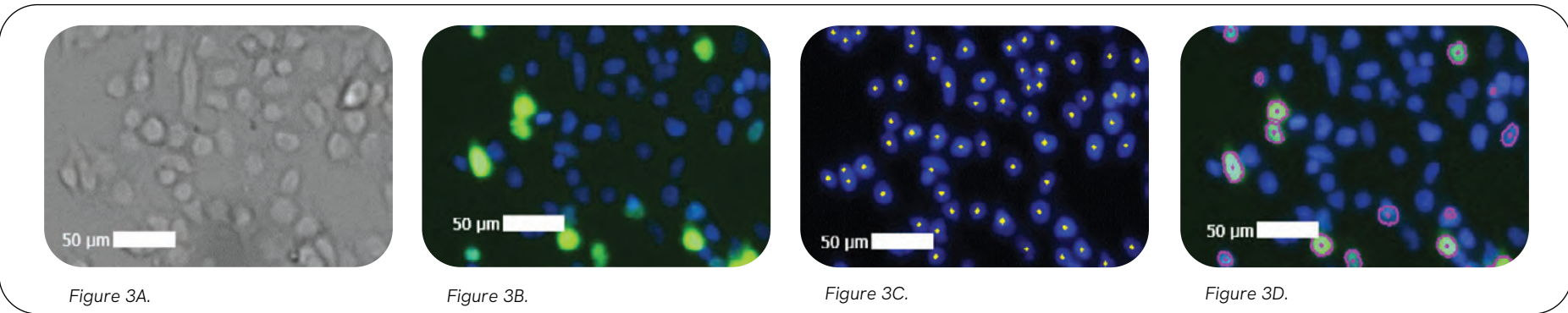


Perform real-time and end point monitoring of viral infections

Monitor the influenza virus infection

Influenza infected cells were fixed, stained and imaged (Figure 3A and 3B). The software identifies and enumerates the number of infected cells (figure 3D), as well as total number of cells (Figure 3C).

- Whole-well imaging allows for the identification of infected cells even at low viral titers.





Celigo specifications

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Software	Proprietary image acquisition and processing software Powerful analysis software/computer workstation Microsoft Windows				
Illumination/optics	1 LED-based enhanced brightfield 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 µm/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 (49.5 cm x 40 cm x 61 cm) 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				

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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

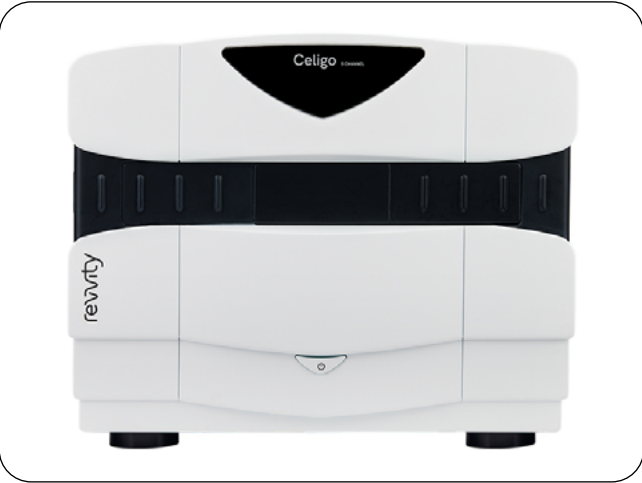
Supported plate types

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 Revvity Visiplate™ 1450606 plate	Revvity	Black
96-Well Corning™ 3603 plate	Corning	Black, white
96-Well Greiner™ 655090 plate	Greiner	Black, white
96-Well Greiner 675090 plate	Greiner	White, half-area
384-Well Corning™ 3542 plate	Corning	Low volume, black
384-Well Corning™ 3680 plate	Corning	Clear
384-Well Corning™ 3764 plate	Corning	Black, white
1536-Well Corning™ 3838 plate	Corning	Black, white



Celigo platform

The Celigo platform’s modular design allows you to customize for your laboratory’s needs. With automated microplate handling for either kinetic end-point analysis or time-point analysis, as well as complementary reagent kits, Celigo can improve your lab’s work-flow and increase its capabilities.



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Hardware

- Celigo BF Only
- Celigo 5 Channel

Software 5.X with Project Mode.

1. **Select project file**
2. **Load prepared plate & click run**

Acquisition, analysis, and data export steps are done automatically
3. **Get Results**
 - Report Templates: present data in customer defined layouts
 - Multiple FCS Express™ file export options
 - FCS and ICE file formats
 - Single file per plate or individual files per well
 - With images or without images in ICE file
 - Compression option
 - Auto open in templates
 - Security and Logging add-ons in FCS Express™

Stacker Automation

- Up to 50 plate capacity
- 15 sec transfer rate between plates
- Set up experiment and run
- Data exports automatically for each plate
- Handles plates with or without lids
- 6-well up to 1536-well plate formats
- Fits on standard lab bench
- Ideal for endpoint assays
- Easy to add on to existing Celigo instruments

Celigo Specific Reagents

- Formulations designed for Celigo
- Project files associated for each reagent kit



Automation solutions

Increase throughput and flexibility of your Celigo image cytometer with our custom automation solutions.

Benefits:

- Higher throughput, increased flexibility, walk-away processing capabilities
- Error rate reduction, decrease in personnel time
- Increased utilization of Celigo enabling plate to be read without user attentions and/or overnight, enabling long term studies like clonal tracking
- Modular, scalable automation solution which grow with your needs

Scope of solutions:

Plate loading



Workcells



Workstations



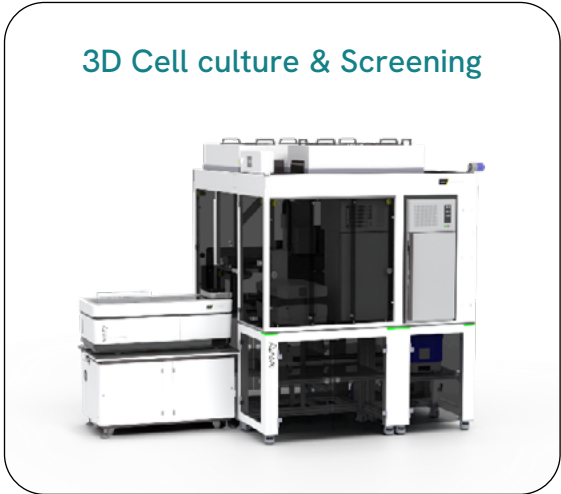
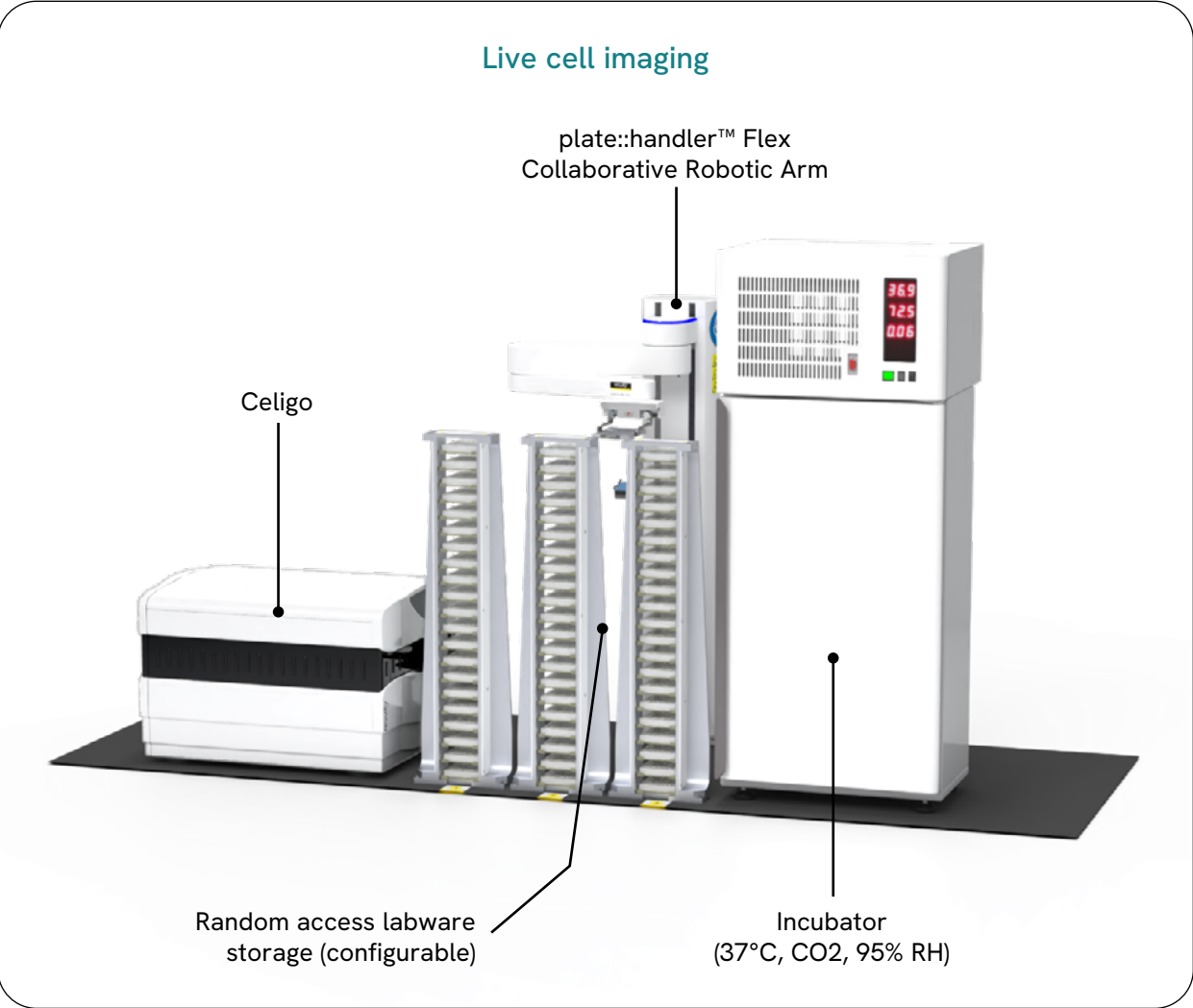
Platforms





Example configurations

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Reagents

ViaStain™ viability reagents

- Perform in-plate staining and analysis of suspension and adherent cells
- Measure percent viability and number live/dead cells

Name	Catalog #
AO / PI staining solution	CS2-0106
Calcein AM	CS1-0119
Calcein-AM / PI cell viability kit	CSK-0118
Calcein AM / PI / Hoechst viability kit	CSK-V0006-1
Hoechst/PI viability kit	CSK-V0005-1

ViaStain™ apoptosis reagents

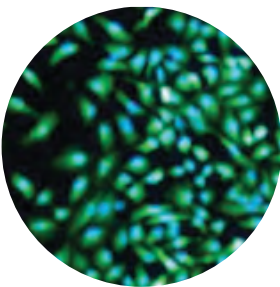
- Measure programmed cell death using various apoptosis detection reagents: Annexin V, Caspase 3/7-Live cell, Caspase 3 and Caspase 8
- Use Caspase 3/7 reagents for 2D and 3D cultures

Name	Catalog #
Live Caspase 3/7 Detection for 2D/3D Culture	CS1-V0002-1
Live Caspase 3/7 Detection for 2D/3D Culture with Hoechst	CSK-V0003-1
Annexin V-FITC	CS1-0114

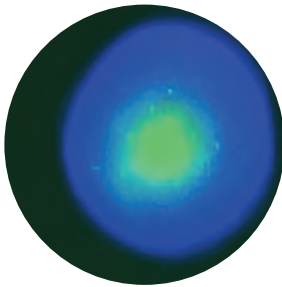
ViaStain™ proliferation/tracer reagents

- Fluorescent proliferation and cell-labeling dyes for cell identification, cell tracking and co-culture experiments

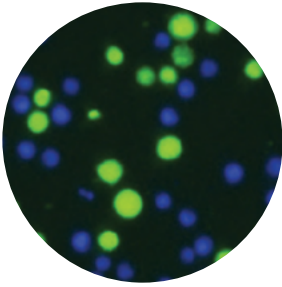
Name	Catalog #
CMFDA	CS1-P0001-1
CFSE	CS1-P0002-1
Tracer Blue	CS1-P0003-1
Calcein AM	CS1-0119



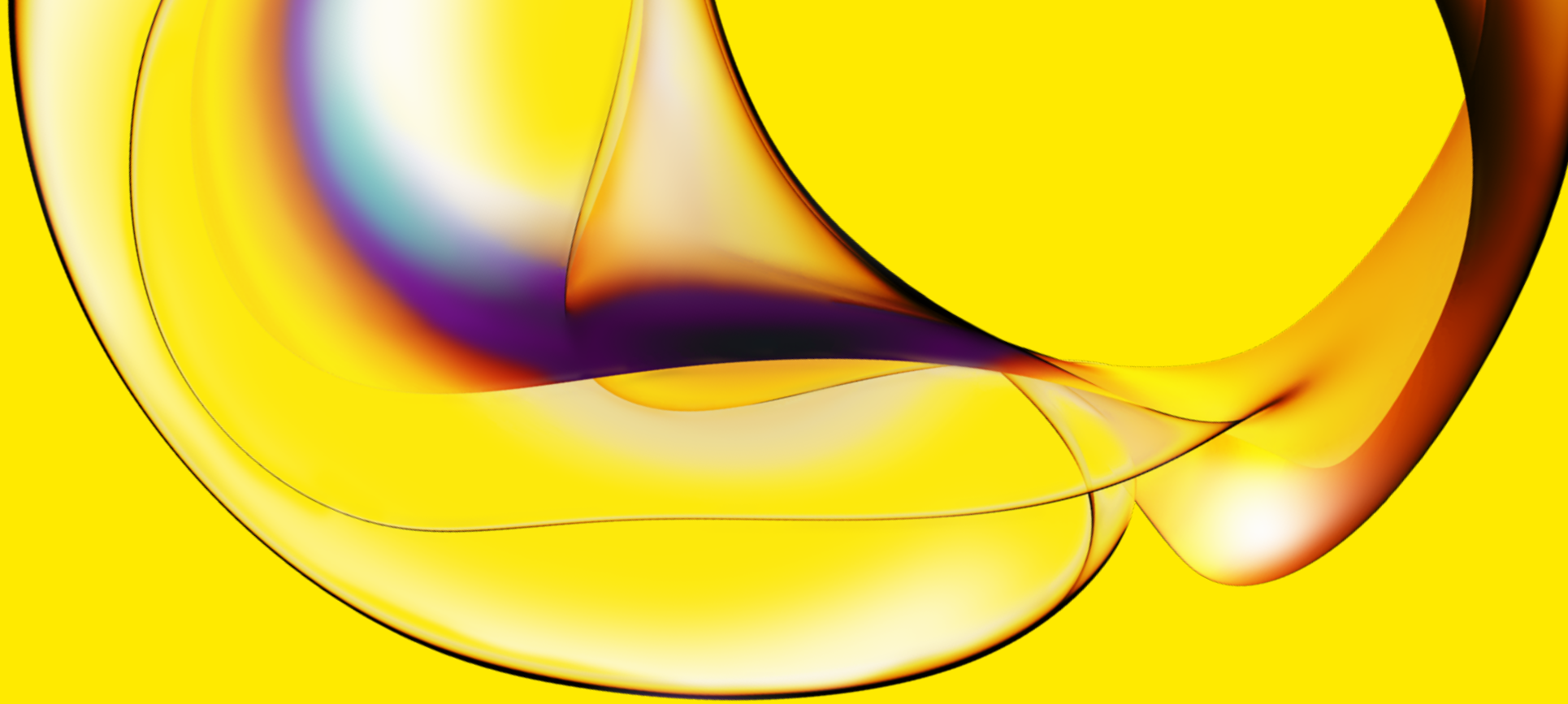
ViaStain™ viability



ViaStain™ apoptosis



ViaStain™ proliferation/tracer



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