Celigo™ image cytometer

Applications guide

Table of contents

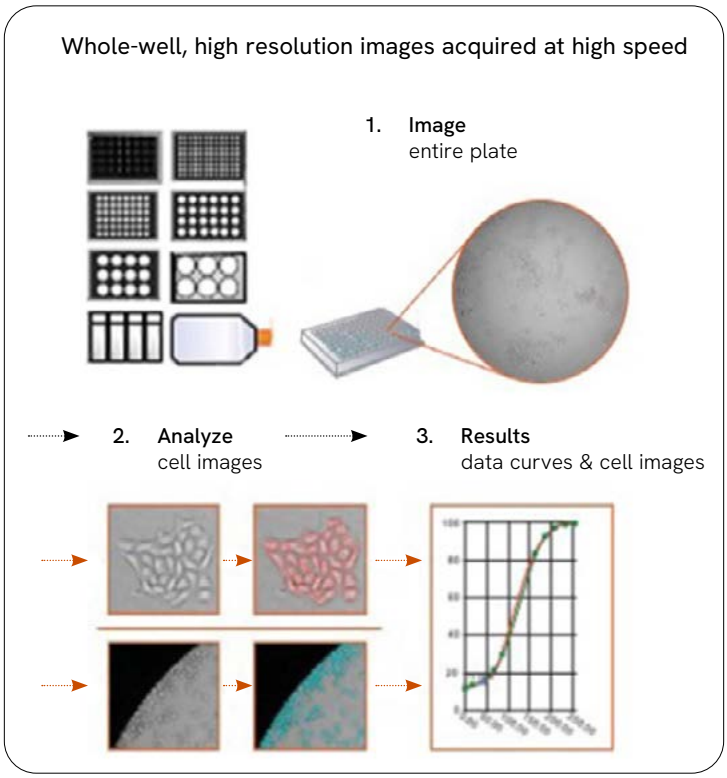
Table of contents

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.



Celigo image cytometer applications

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

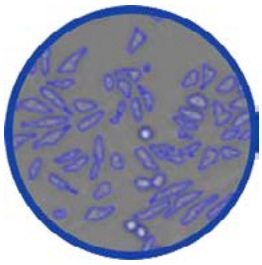
Direct cell counting, visualization and documentation of all cells, using gating interface, ADCC, direct NK cell killing, CAR-T, CDC

1 Growth tracking / proliferation

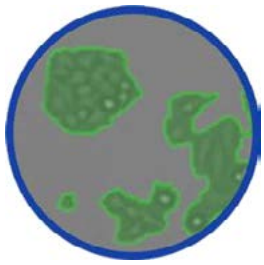
Proliferation (any well format) ~6 min to results per 96-well plate.

Determination of the growth characteristics of primary cells and cell lines can be analyzed in only a few minutes using the **Celigo™ image cytometer**. Cell density can be accomplished either through direct cell counting or using texture analysis (cell confluence).

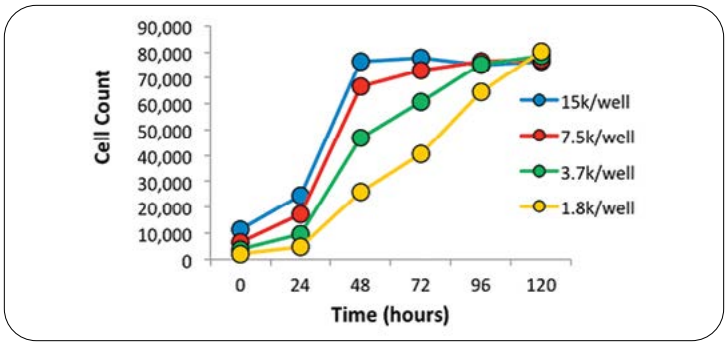
Direct cell count



Cell confluence

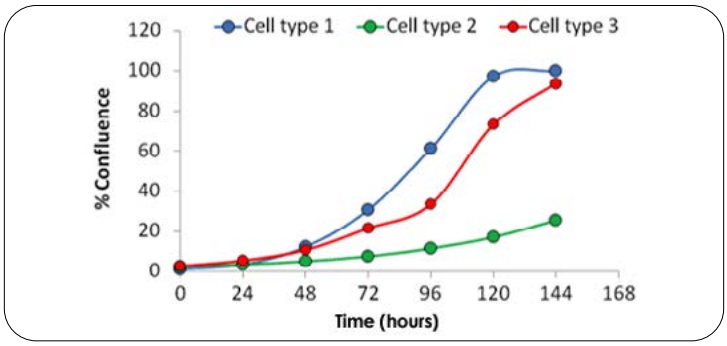


Cell growth tracking using the Celigo



Growth curves for CHO (DUXB11) cells grown in 96-well plates over 120 hours. Cells were imaged and counted using the Celigo Cell Counting application.

Streamlined cell culture maintenance using the Celigo



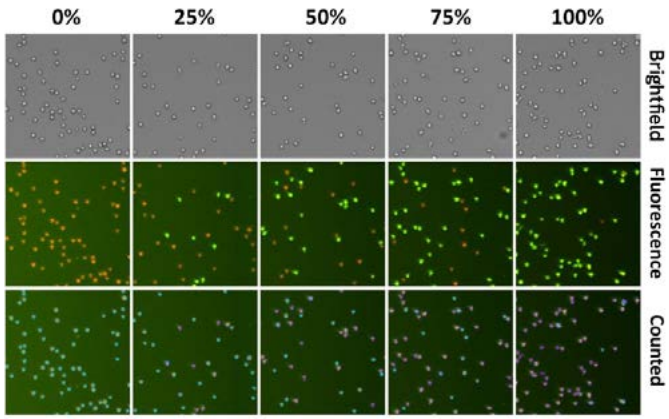
Example of output for monitoring adherent cell cultures in 96-well plates. Cells can be imaged and counted in culture vessels without staining or harvesting. The Celigo updates and returns growth curves automatically.

For research use only. Not for use in diagnostic procedures.

High-throughput cell counting and viability with AOPI (96-well plate) ~9 min to results.

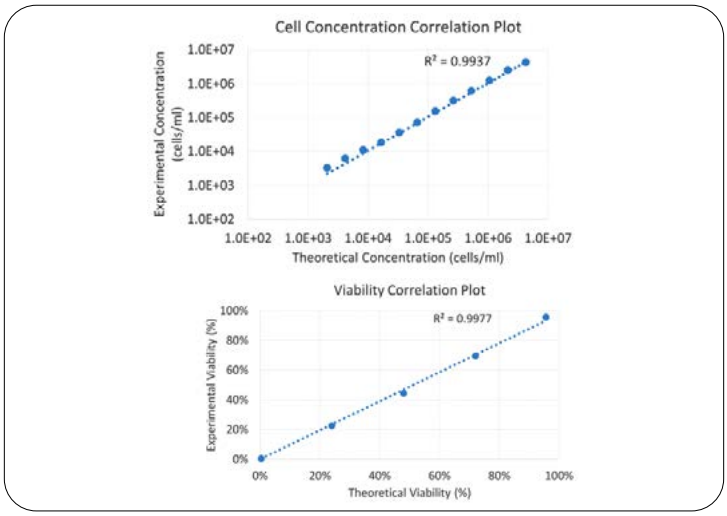
High-throughput cell counting and viability determination of 96 samples can be accomplished in 9 minutes on the Celigo™. A mixture of Acridine Orange and Propidium Iodide (AOPI) was used to highlight and determine viability of nucleated cells. Debris and non-nucleated cells were ignored. This method is beneficial for counting samples from multiple different sources as cell samples are stable in the AOPI reagent for 45 minutes both in terms of viability and cell count. This allows the scientist to spend time gathering samples from multiple sources in a single 96-well plate prior to the 9-minute count on the Celigo.

Counting and viability with AOPI



Brightfield, Merged AO and PI images, and counted AOPI images. Here, heat killed Jurkat cells were mixed with live Jurkat cells in specific ratios to achieve viabilities from 0 to 100%.

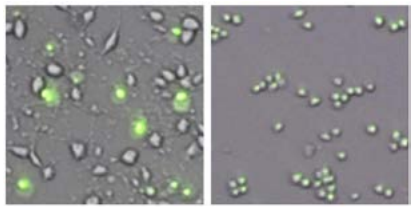
High-throughput cell counting on the Celigo with AOPI has a high dynamic range



Apoptosis (any well format) ~7 min to results per 96-well plate.

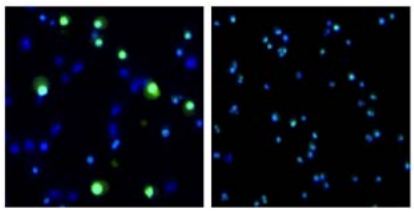
Apoptosis can be quantified in real time (kinetic) in parallel to brightfield proliferation assays, or as an endpoint measurement. In both assays, apoptotic cells are highlighted by the green fluorescent Caspase 3/7 reagent. This reagent can be included with the added treatments and maintained in the culture medium for the duration of the assay.

Brightfield and Caspase 3/7 positive cell count



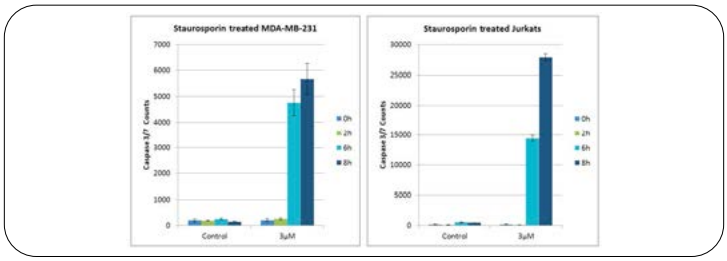
Brightfield and green fluorescent images of MDA-MB-231 (left) and Jurkat (right) cells

Caspase 3/7 positive and total cell count (Hoechst)



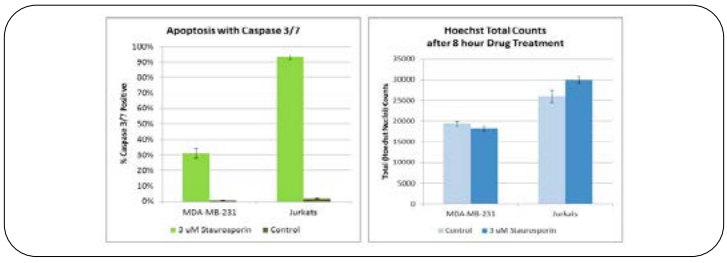
Blue (Hoechst 33342) and green (Caspase 3/7) fluorescent images of MDA-MB-231 (left) and Jurkat (right) cells

Kinetic monitoring of apoptosis



Staurosporin induced apoptosis over 8 hours in both adherent and suspension cell lines.

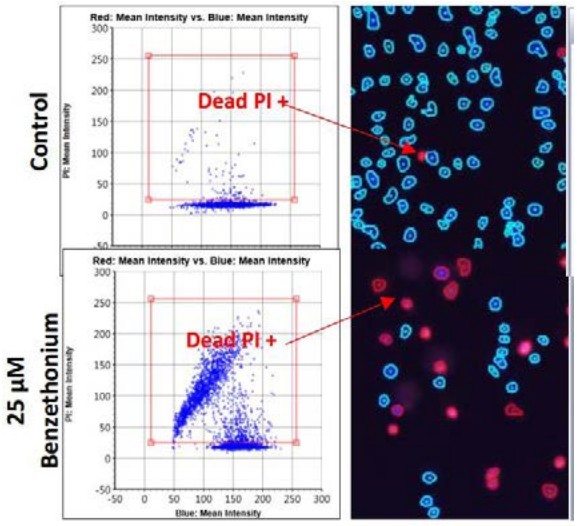
End point “mix and ready” assay for % Caspase 3/7

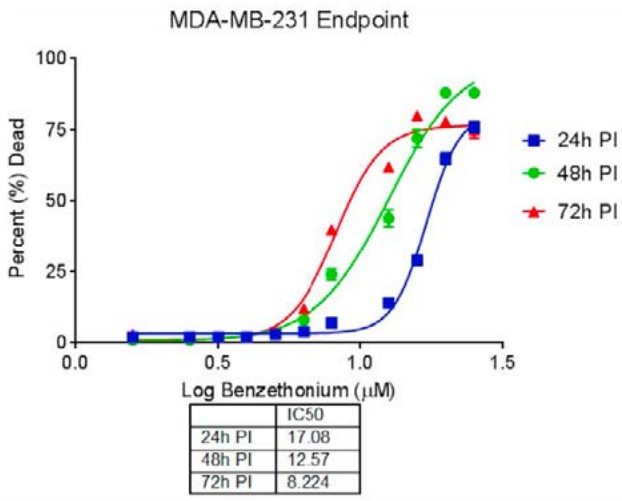


Left: Staurosporin induced more apoptosis in Jurkat cells than MDA-BM-231 cells.

Viability with Hoechst and PI (any well format) ~15 min to results per 96-well plate.

Cell viability can be quantified as a single, endpoint measurement in live cells using Hoechst 33342 and Propidium Iodide (PI). This assay was performed on live cells without fixation.



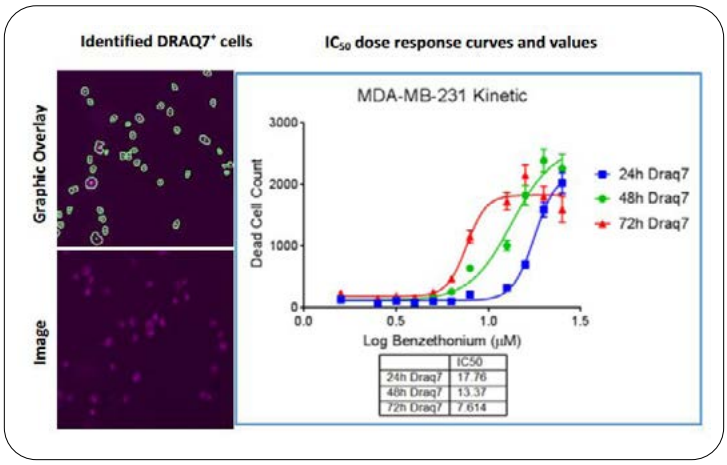


	IC50
24h PI	17.08
48h PI	12.57
72h PI	8.224

Total cell count was determined by enumerating Hoechst 33342 stained nuclei. Dead cells were highlighted with cell impermeant dyes such as PI or DRAQ7™. The Celigo™'s gateing interface allows for direct visual confirmation of correct gate placement: cells that fall into the red gate were highlighted with a red ROI in the cell image.

Kinetic Viability with DRAQ7 (any well format) ~7 min to results per 96-well plate.

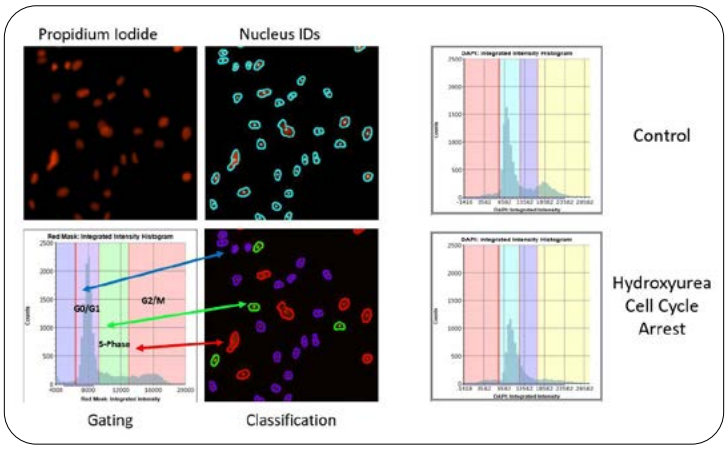
Cell viability can be quantified in real time (kinetic) over the length of the assay. In this assay, PI or DRAQ7™ was added to the culture medium. Only dead cells were highlighted by the nucleic acid stain. Repeated imaging of each sample provided time course cell death data.



Dead cell count was determined by detecting DRAQ7™ stained nuclei. % Cytotoxicity can also be calculated by counting total cell number in brightfield images (not shown).

Cell cycle analysis (any well format) ~7 min to results per 96-well plate.

Determination of population cell cycle stage can be performed as a standalone assay or following any monitoring with brightfield (such as a proliferation assay). Fixed cells were stained with PI and analyzed within the Celigo™ gating tab. Additionally, results could have been exported to flow cytometry software for automated analysis.

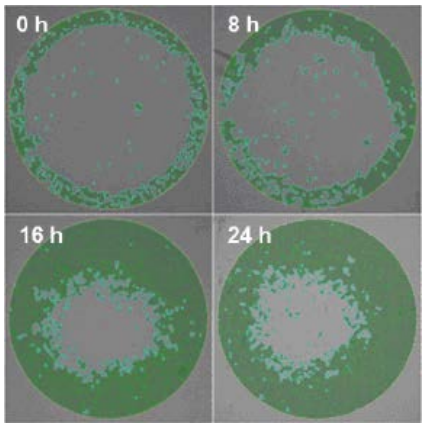


Wound healing (96- and 384-well format) ~6 min to results per 96-well plate.

The Celigo™ image cytometer has been used to provide automated, rapid assessment of wound healing using the Oris™ Platypus plate technology. Automated segmentation of cells or confluency in both brightfield and fluorescence provided a quantitative output of wound healing / migration.

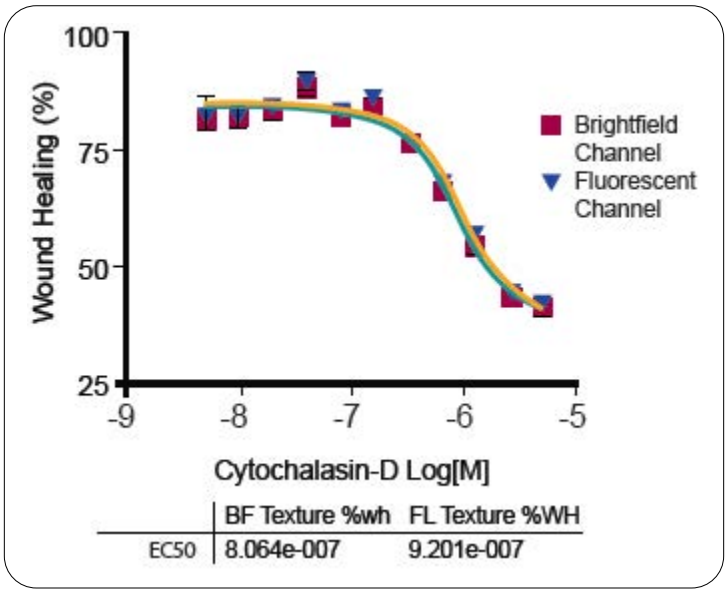
Dose-response of % wound healing measured with the Celigo cytometer using brightfield and fluorescence.

Wound area fill view



Wound area fill view at 0, 8, 16 and 24 hours. Confluence detection of the area covered within the wound was followed over time and a pseudo color green fill mask is added to aid visualization.

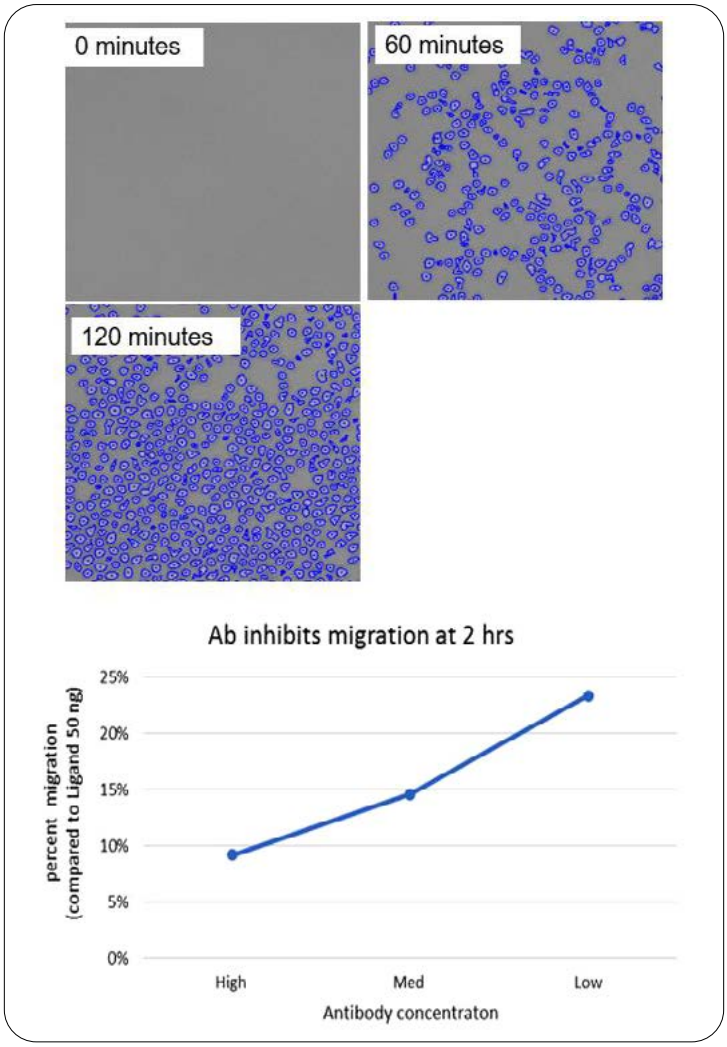
Dose-Response Curve



HT1080 cells were plated in a 96-well Oris™ plate and allowed to grow to confluency. Upon removal of the plug the cells were treated with a dose response of Cytochalsin D and the confluence readout was read in brightfield or in fluorescence after staining with CellTracker™ Green.

Migration/ chemotaxis ~14 min (24-well plate) or 6 min (96-well plate) to results per plate

Cell migration assays can be read on the Celigo™. Assays can be performed in Transwell systems in either 24- or 96-well format. For suspension cells such as T cells or monocytes, the cells that migrate through the membrane and fall to the bottom of the culture plate are counted in brightfield images. For adherent cells, cells can be labeled with a live fluorescent marker, and in combination with fluorescently opaque membranes (corning), those that migrate through the membrane and adhere to the underside can be counted.



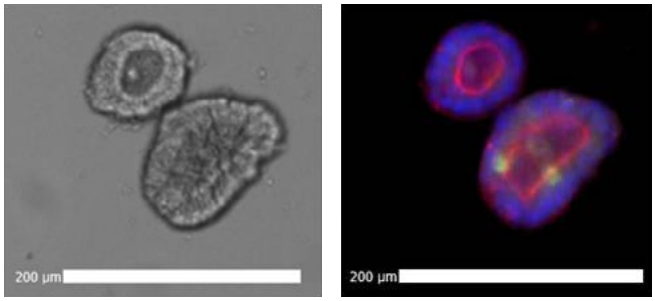
Monocytes were plated at 100K cells per transwell (96-well format) and allowed to migrate for up to 3 hours. At hourly intervals, the plates were imaged on the Celigo (6 min scan and analysis), and the number of cells on the surface of the culture dish were counted. In this assay, only 10% of the monocytes migrated in the presence of high antibody concentration, compared to no antibody control.

2 Organoids, 3D co-cultures

3D tumor organoid cultures (96- to 384-well format) ~2 to 25 min plate.

The Celigo™ image cytometer has been used for the evaluation of 3D tumor organoid cultures in both brightfiled and multiple fluorescent channels. In the example below, tumor organoids were grown in the presence of docetaxel. Total organoid area per docetaxel dose was evaluated in brightfiled images. Organoids were then stained with DAPI, Phalloidin, and phospho-Histone H3.

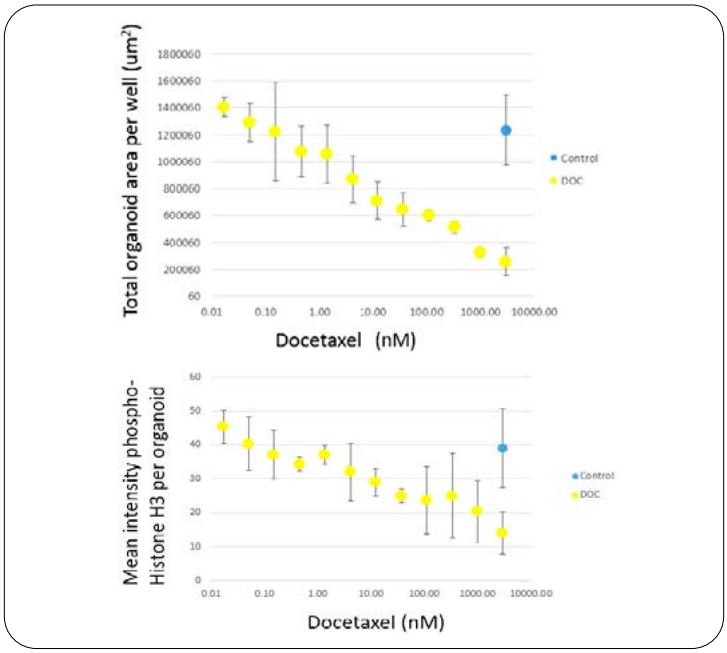
3D Tumor Organoid culture



Left: Brightfield image of 3D tumor organoids.

Right: Merged blue (DAPI), red (Phalloidin) and green (phospho-Histone H3) fluorescent images.

Organoid growth in response to Docetaxel

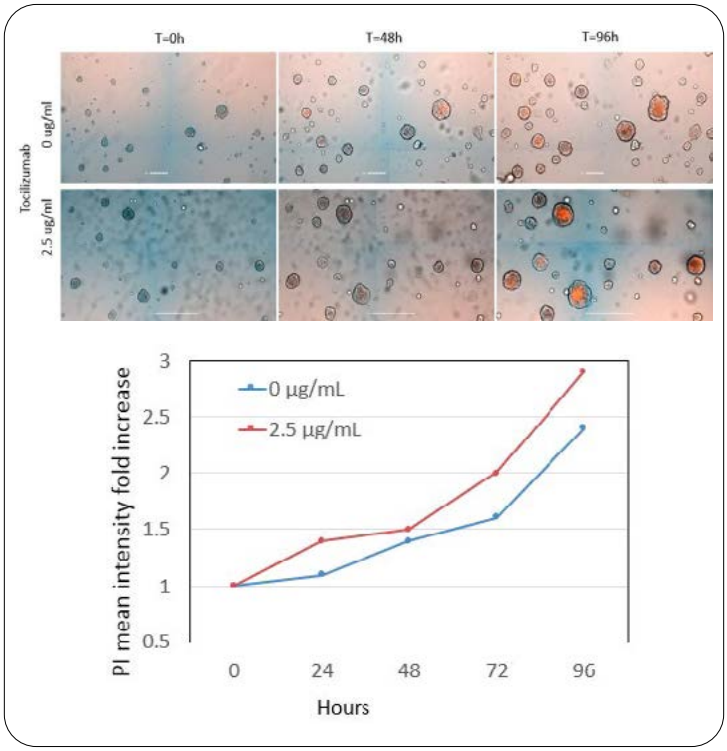


Top: Total Organoid area per well reflects both organoid size and number of organoids generated in each condition.

Bottom: The mean intensity of phosphor-Histone H3 per organoid reflects the number of proliferating cells per organoid.

Kinetic viability in tumor PDO with PI (96-well format) ~10 min per plate.

The Celigo™ image cytometer has been used for monitoring viability of 3D tumor organoid cultures with Propidium Iodide (PI). In the example below, esophageal tumor organoids were grown in the presence of Tocilizumab, an anti Il-6 antibody. Each organoid was detected in the Brightfield image and mean PI intensity per organoid was evaluated. An increase in PI mean fluorescence intensity indicates more dead cells per organoid.



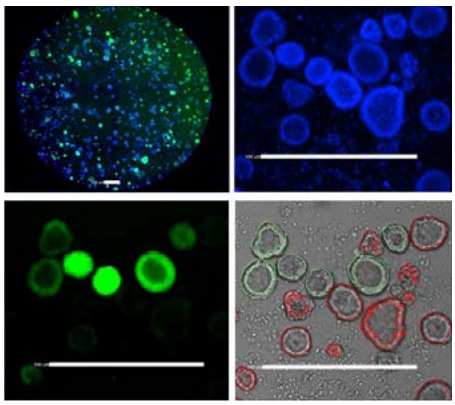
Top: Merged brightfield and red fluorescent images of esophageal adenocarcinoma organoids cultured in the presence of Tocilizumab (anti Il-6) over 96 hours. PI was added to the culture medium at T=0h to highlight cells with a compromised membrane.

Bottom: quantification of fold increase in mean PI fluorescence intensity per organoid over 96 hours for cultures with and without Tocilizumab.

Quantitative fluorescence analysis of PDO (6- to 96-well format) ~20-40 min per plate.

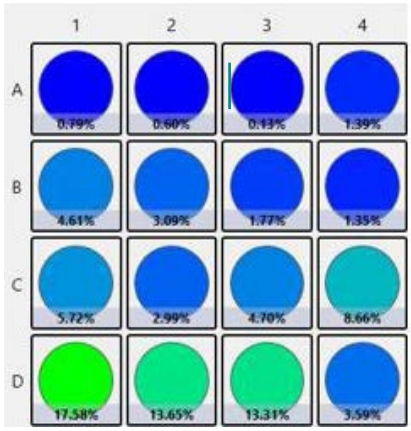
The Celigo™ image cytometer has been used for the quantitative evaluation of fluorescence expression in 3D tumor organoid cultures in both brightfiled and multiple fluorescent channels. Fluorescence can be the result of antibody based staining for specific biomarkers or the expression of a reporter gene. In the example below, all cells were stained with Hoechst 33342, including non PDO associated cells. Then, using gating all small (single) cells were excluded from analysis so that green fluorescence could be evaluated in the PDO population alone.

Brightfield and fluorescent images



Whole well image of merged Hoechst and green fluorescent images. Individual PDO can be seen in detailed Blue (Hoechst 33342), Green and Brightfield images. Red lines in Brightfield image indicates PDO that are green negative, while green lines indicate those classified as green positive.

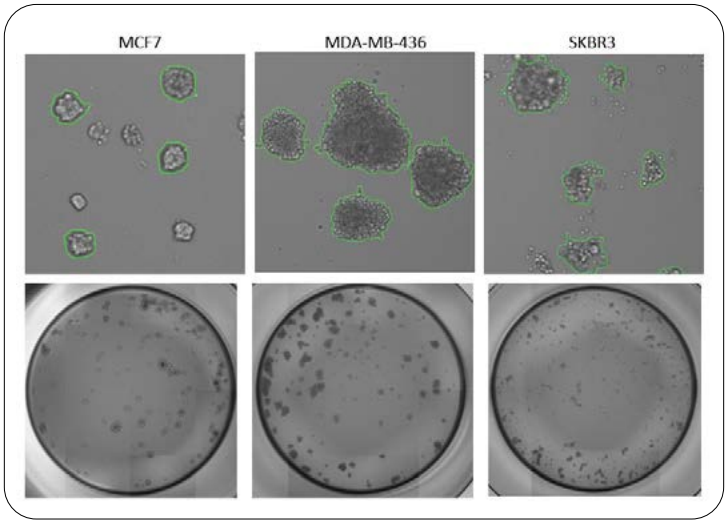
% of green positive organoids



The percentage of total organoids that are green positive in each well (subset of a 96-well plate).

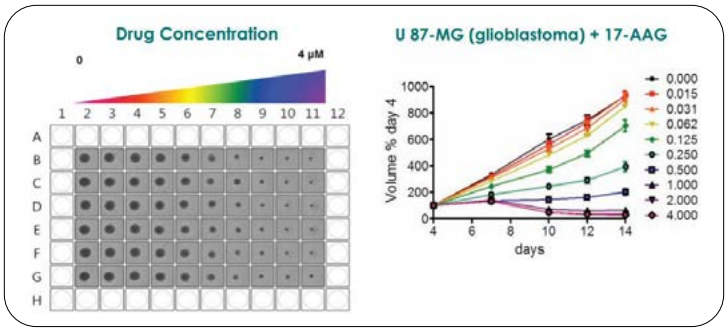
3D spheroid formation assay (6- to 96-well format) ~5 min per plate.

The Celigo™ image cytometer can be used to detect and measure a wide variety of 3D spheroid types. Multicellular tumor spheroid morphology is cell line dependent. The Celigo detection parameters can incorporate characteristics such as size, roundness and boarder smoothness.



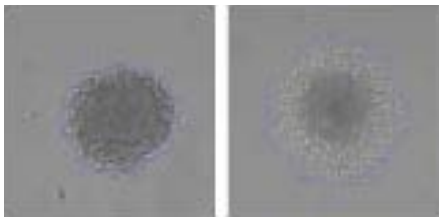
3D tumor spheroid screening (96- to 384-well format) ~2 min per plate.

The Celigo™ image cytometer has been developed to fully automate imaging and analysis of tumor spheres. Brightfield imaging is suitable for time course screening assays such as in the example below where the impact of 17-AAG on tumor growth was assessed. As a model of metastatic virulence, migration of tumor cells can be assessed by embedding spheroids in extracellular matrix and quantifying the extent of migration in response to drug treatment.



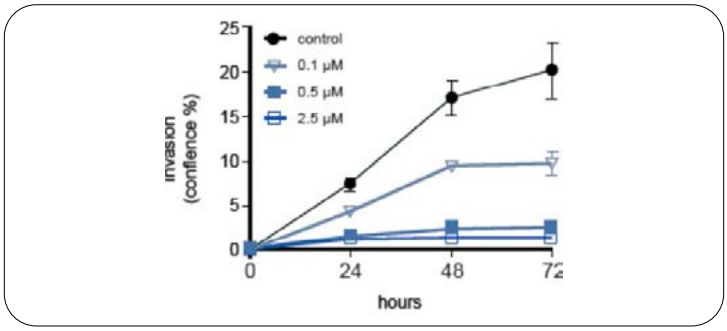
Spheroid growth inhibition screen. Vince et al. BMC Biology 2012, 10:29, March 2012.

Outward migration of tumor spheroid cells into matrix.



Brightfield images of non-migrating (left) and migrating (right) cells from tumor spheroids imbedded in extracellular matrix gel. The total area of migrating cells can be detected and quantified. In the line plot: 17-AAD treatment inhibits cell migration in a dose dependent manner.

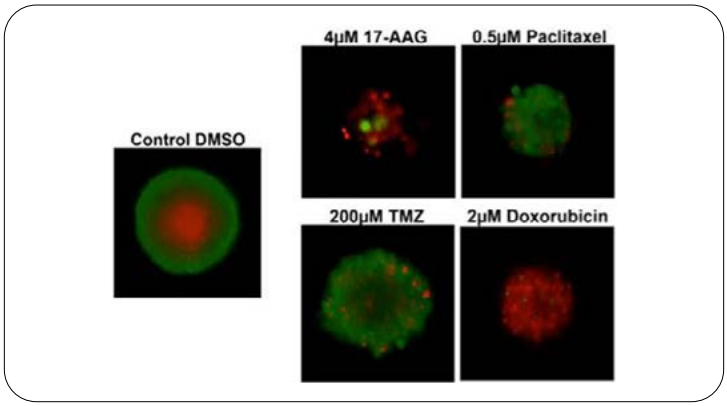
17-AAG inhibits cell migration away from tumor spheroid as a model of metastasis.



3D tumor spheroid functional analysis with viability (96- to 384-well format) ~3 min per plate.

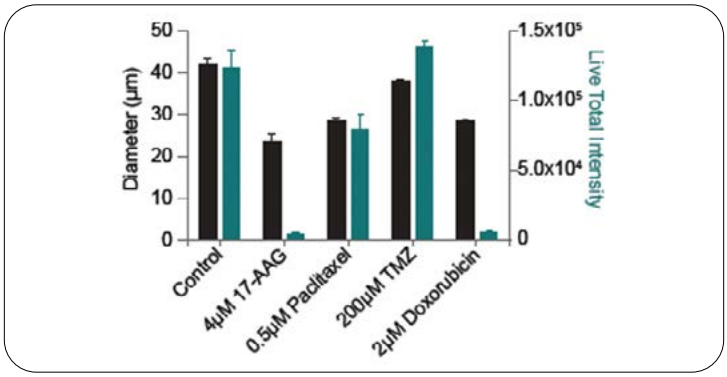
The Celigo™ image cytometer has been developed to fully automate imaging and analysis of tumor spheres. In addition to the assessment of spheroid growth in brightfield images, fluorescence enables the determination of spheroid viability. In the example below Calcein AM (green) highlights live metabolically active cells, while propidium iodide highlights dead cells. Here, 17-AAG and Paclitaxel both led to spheres smaller than control, albeit through a different mechanism (17-AAG treated spheroids were mostly dead, while Paclitaxel treated spheres were mostly live. Additional fluorescent stains for caspase 3/7 activity and hypoxia are available for similar readouts.

Live/Dead tumor spheroid staining



Tumor spheroids were incubated with Calcein AM (green) to highlight metabolically active cells and propidium iodide (red) to highlight dead cells.

3D tumor spheroid growth and viability

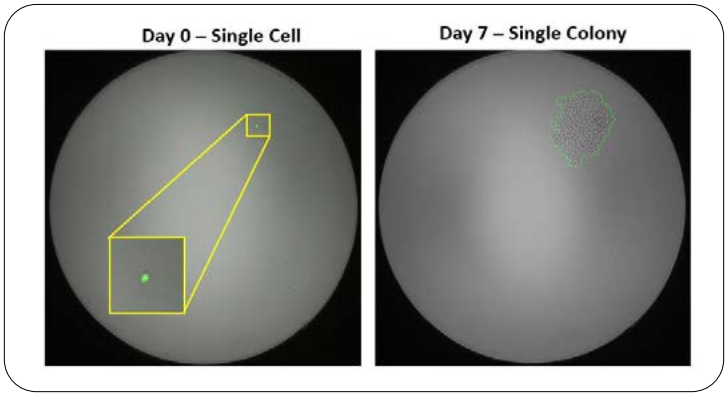


Control spheroid diameter reflected tumor growth while live intensity reflected cell viability and metabolism. Both 4 µM 17-AAG and 0.5 µM Paclitaxel (top row) led to smaller spheroids but led to dramatically different viabilities.

3 CLD and stem cells

Confirmation of cell clonality (any well format) ~6 min to results per 96-well plate.

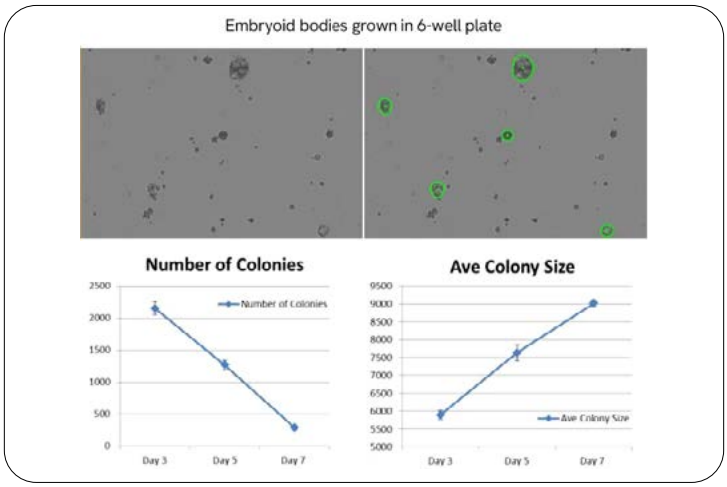
In the cell line development workflow, it is often necessary to be able to document the clonal origin of a custom cell type. With the Celigo™, initial single cell deposition by FACS or manual dilution can be confirmed by imaging on Day 0. In the example below, pre staining the sorted population with Calcein AM served as a secondary differentiation between cell and debris. The fluorescent Calcein diffused out of the cells within hours. Imaging and analysis on Day 7 highlighted wells with a single colony of sufficient size. Only wells with one colony and a single cell on Day 0 will moved forward in the development process.



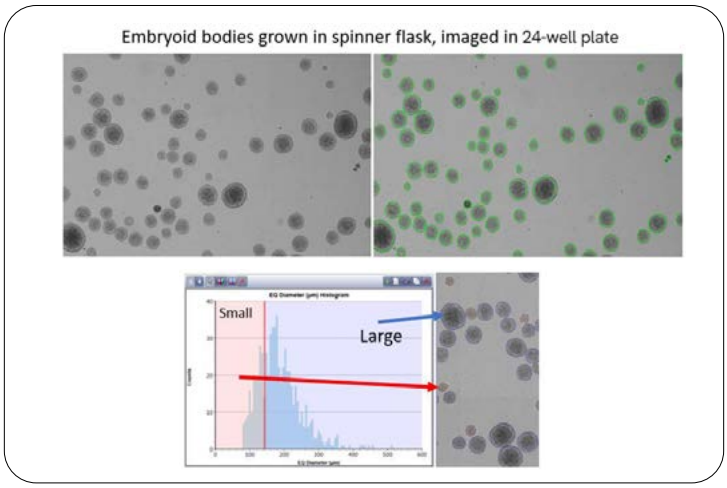
Whole well (96-well) images on Day 0 and Day 7 of cells FACS seeded as single cells and allowed to grow to colonies. Cells were stained with Calcein AM prior to sorting as a secondary differentiator between cells and debris. The fluorescent Calcein diffused out of the cells after 24 hours.

Embryoid body formation assay (any well format) ~5 min to results per plate.

The Celigo™ image cytometer can be used to detect and measure a wide variety of 3D spheroid types. Embryoid bodies can be generated in multi well plates and monitored by repeated imaging, or samples can be removed from spinner flasks at defined time points for determination of EB size and diameter.



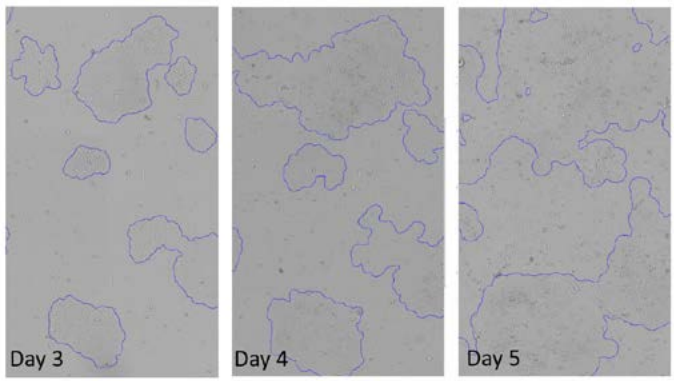
Representative raw and counted image of EB formation on Day 5 of culture in 6-well plates. Repeated imaging of the same cultures allowed for non-invasive monitoring of EB formation. In this image, single cells and small clusters of stem cells were considered too small to count and were therefor not included in the count (not circled).



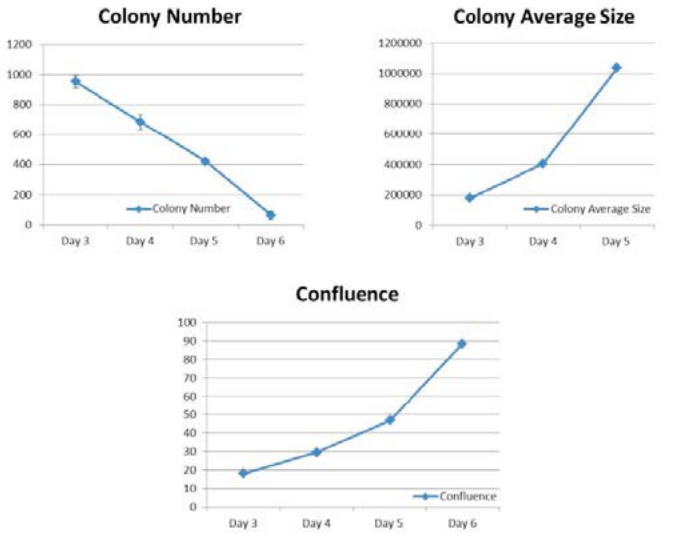
Representative raw and counted image of EB formation from cells grown in spinner flasks. Here, 1 mL was sampled from the spinner flask and placed in a 24-well plate. All EB were detected and could be further segmented based on EB measured diameter. Classification of EB as either small or large could be visually confirmed based on the color of the outline. Small EB are red, Large are blue.

iPSC colony growth (6-well format) ~4 min to results per 6-well plate.

In the iPSC generation workflow cells are seeded at limited dilution to form clonal colonies. The Celigo™ can be used to identify iPSC colonies and measure their number and size over multiple days.



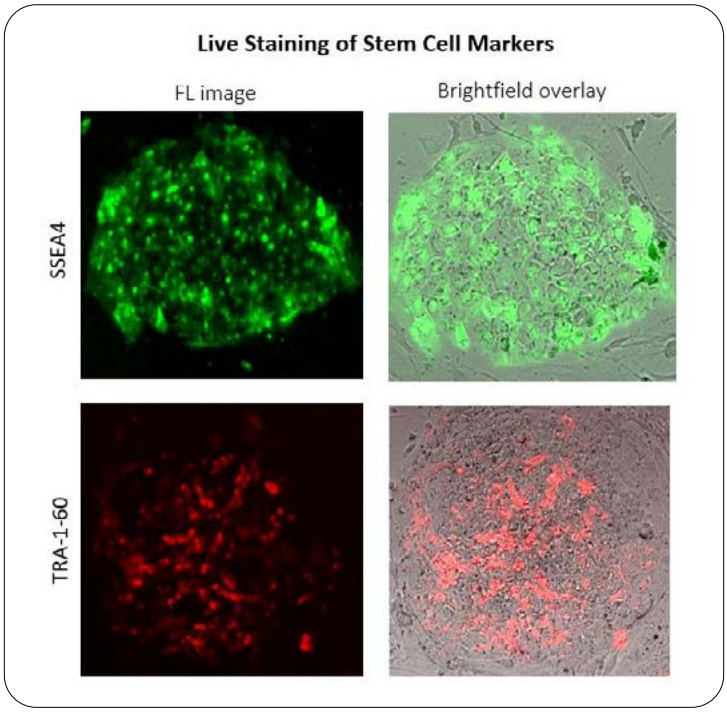
Representative images of iPSC colony growth in a 6-well plate.



Seeded as single cells, iPSC grew into multiple small colonies which then merged to form larger colonies over time. This is reflected in the above images and graphs.

Identification of iPSC colonies (6- and 12-well format) ~15 min to results per 6-well plate.

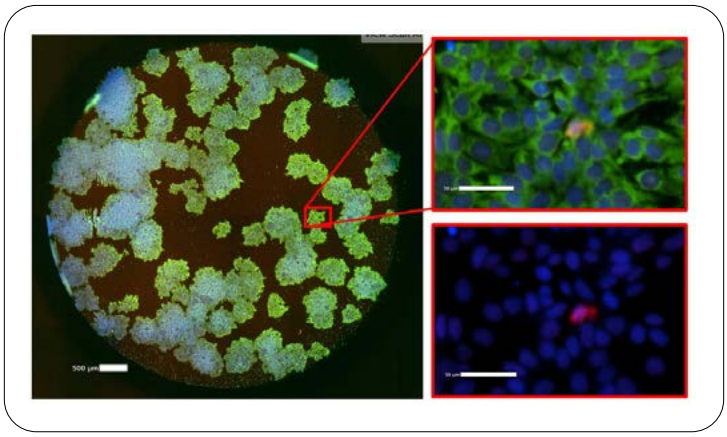
In the iPSC generation workflow cells were seeded at limited dilution to form clonal colonies. The Celigo™ was used to identify putative iPSC colonies on the basis of live staining for stem cell markers such as SSEA4, TRA-1-60 and others. Positive colonies can then be picked by hand or robot for further subculturing.



iPSC colonies were identified based on live staining for stem cell markers.

Immunostaining for rare cells (any well format) ~40 min to results per 96-well plate.

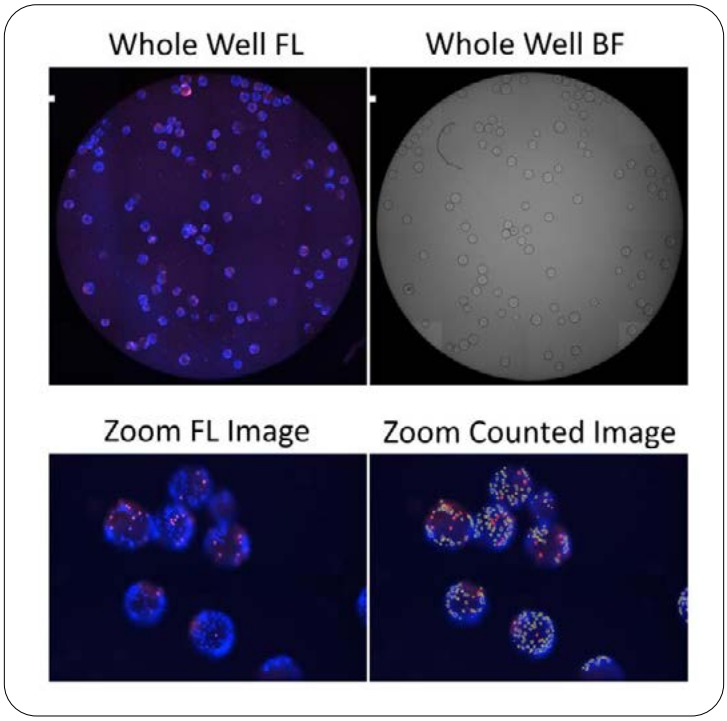
The Celigo™ can be used to detect rare cells in a population based on differential immunostaining. In the following example the number of remaining stem cells post differentiation was evaluated on the basis of OCT 4 expression. Cells were fixed and stained with Hoechst, a proprietary differentiation marker (green) and Oct4 (red). Four OCT4 positive cells were identified by the Celigo software in a background of ~30,000 OCT4 negative cells.



Whole-well blue, green and red fluorescent image. Detail area shows, rare OCT4 (+) (red) cell.

Cells on microcarriers ~10 min to results per 96-well plate.

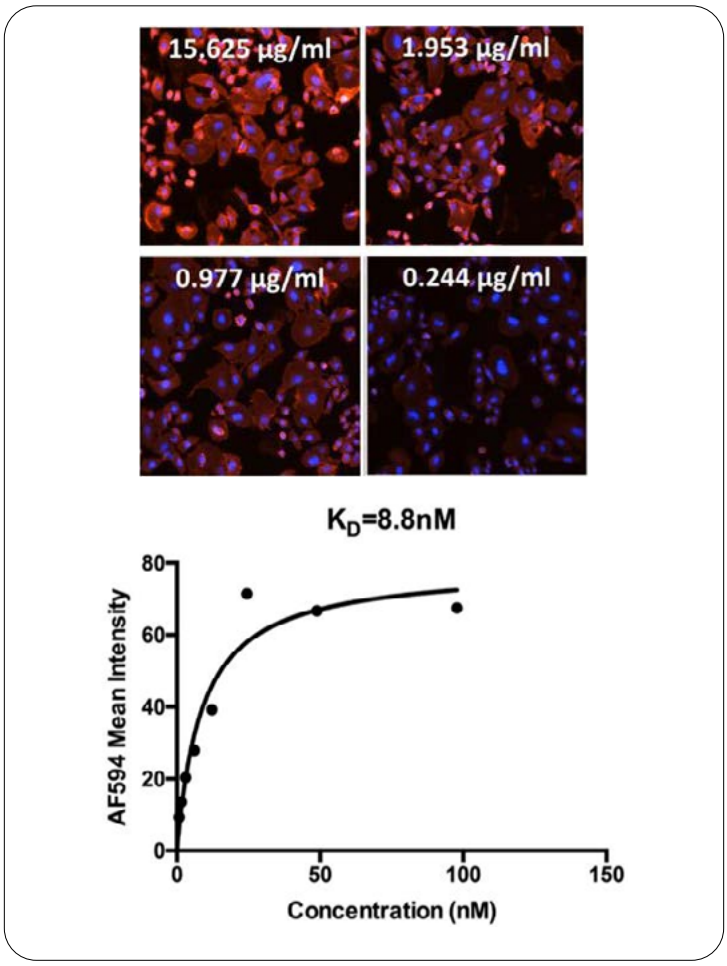
The Celigo™ can be used to estimate the average number of cells per microcarrier as well as overall viability, with the use of viability stains such as Hoechst 33342 and Propidium Iodide.



Cells grown on microcarriers were incubated with Hoechst 33342 and PI. These dyes will provided total and dead cell count respectively. Separately the number of microcarriers were counted in brightfield images allowing for the calculation of average cell number per microcarrier.

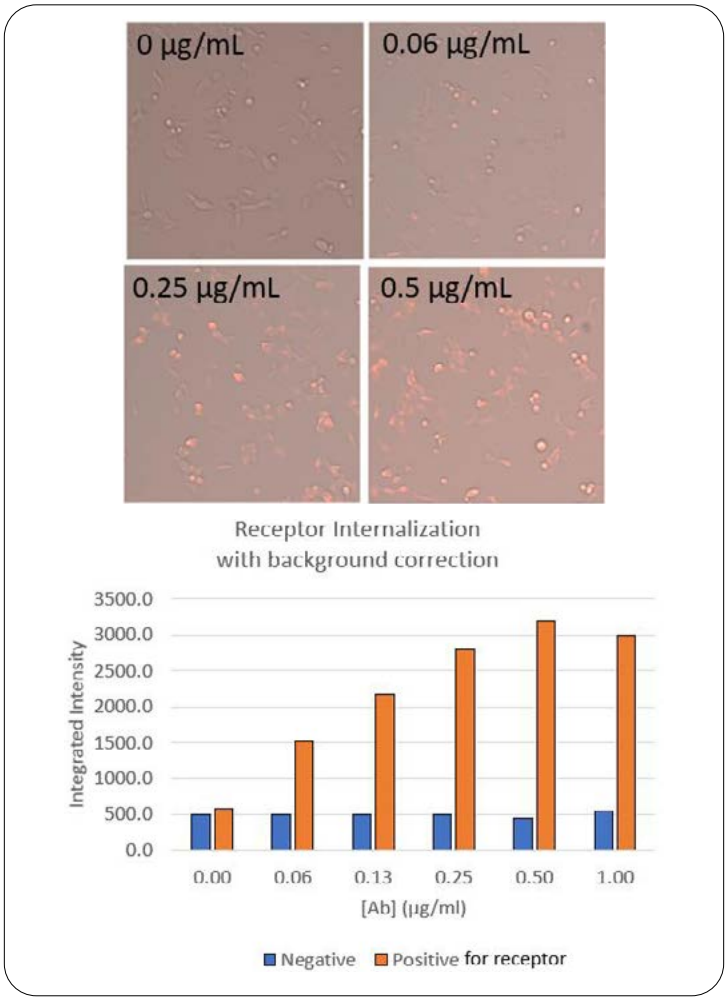
Antibody binding screening/K_D determination (96- to 1536-well format) ~20 min to results per 96-well plate.

Antibody binding can be rapidly assessed via fluorescence on Celigo™. In addition to High-throughput screening of multiple Ab solutions, (hybridoma supernatants for example), antibody binding kinetics can also be determined.



Antibody internalization (96- to 1536-well format) ~20 min to results per 96-well plate.

Antibody internalization can be determined on the Celigo™ by conjugating antibody with a pH sensitive dye such as pHrodo™ red. Upon internalization and localization in the lysosome, the pHrodo™ will fluoresce and can be quantified.

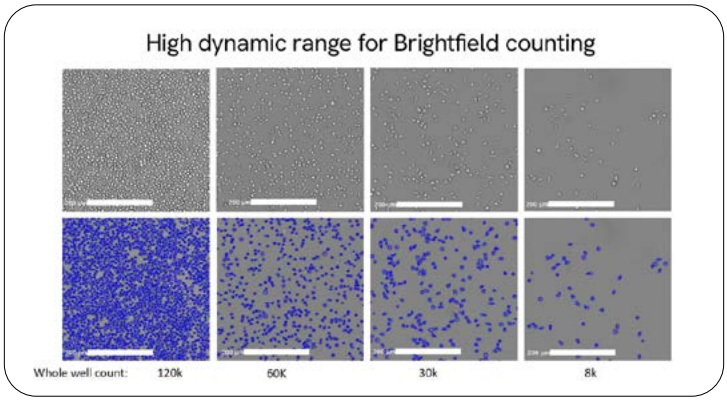


MDA MB 468 cells were incubated with varying concentrations of pHrodo™ labeled antibody. Only cells expressing the receptor of interest exhibited increases of red fluorescence, indicating internalization of the labeled antibody.

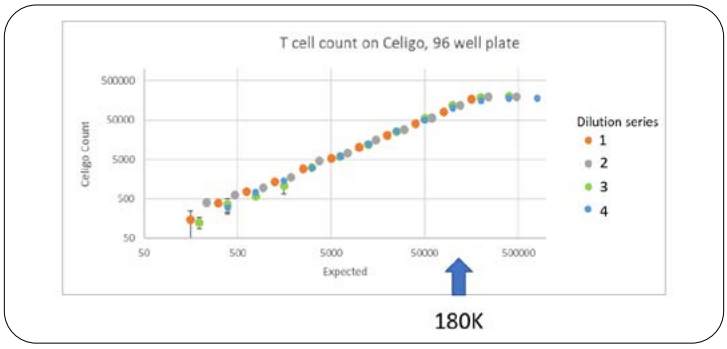
4 Immuno-oncology

T cell counting (all well formats) ~5 min to results per 96-well plate.

Counting of suspension cells like T cells can be performed in label free brightfield images in all well plate formats. Typically, this is accomplished in 96-well format to allow for technical replicates and for counting multiple samples. The Celigo™ has a high linear count range for cell densities up to 180,000 cells per 96-well. If sampling from large growth batches, keep in mind that a 20 µL sample from a stock of 30E6 cells/mL is 60,000 cells (well within the dynamic range for counting on the Celigo in a 96-well plate).



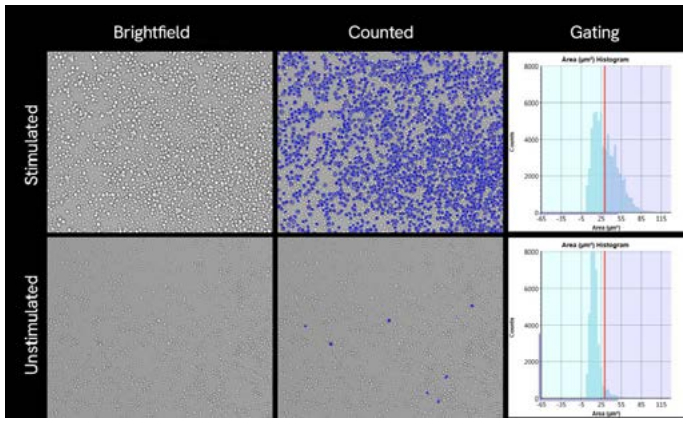
Representative brightfield and counted image for a range of T cell densities in a 96-well plate.



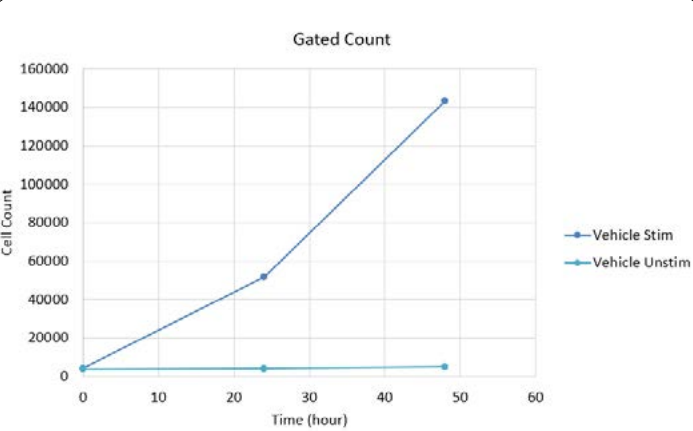
The Celigo counted T cells in a 96-well up to a density of 180,000 cells per well. In the above graph, each dot represents the mean of 3 replicates. Error bars are standard error (N=3).

T cell activation (96- and 384-well format) ~5 min to results per 96-well plate.

T cell activation can be assessed in label free brightfield images via changes in T cell morphology. Large, activated T cells are detected and counted over multiple timepoints. This assay was used to identify compounds that induce T cell activation prior to more quantitative assessment via expensive cytokine release assays.



Brightfield images of T cell cultures. Stimulated T cells had a larger diameter. These larger cells were selected for, and counted using the Celigo™ gating interface.

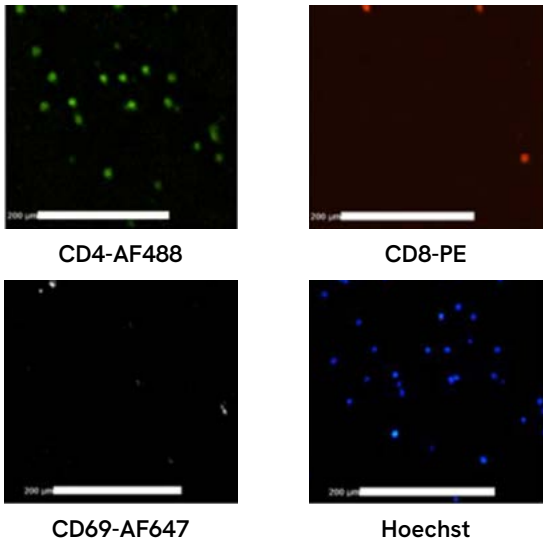


The time course increase in large T cells was quantified in brightfield images. Wells with the most stimulated cells were used for expensive cytokine release analysis.

T cell activation via CD69 staining (multiple plate formats) ~20 min to results per 96-well plate.

The Celigo™ can be used to quantify T cell activation through the expression of CD69 in multiple well formats. In this example, fresh human PBMCs were incubated with dilutions of novel test antibodies as well as IgG and PMA controls for 24 hours. Following incubation, the samples were stained with fluorescent antibodies against CD4, CD8, CD69 and Hoechst 33342 then placed in a 96-well plate and imaged on the Celigo.

Staining of T cells with CD markers

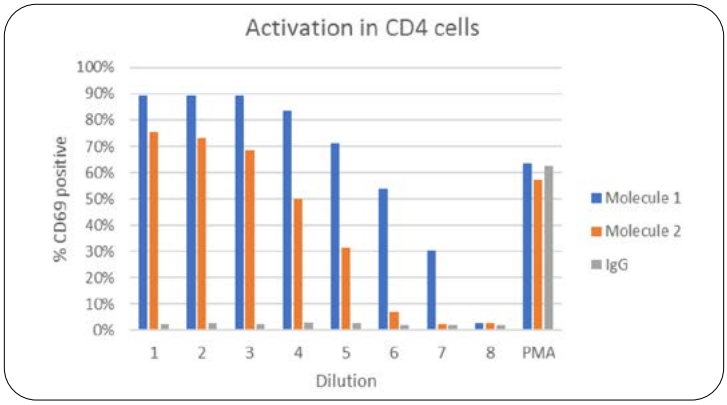


CD4-AF488 **CD8-PE**

CD69-AF647 **Hoechst**

PBMCs were incubated with a cocktail containing CD4-AF488, CD8-PE, CD69-AF647 and Hoechst 33342. Following incubation, cells were washed once in PBS and placed in a 96-well plate for imaging on the Celigo.

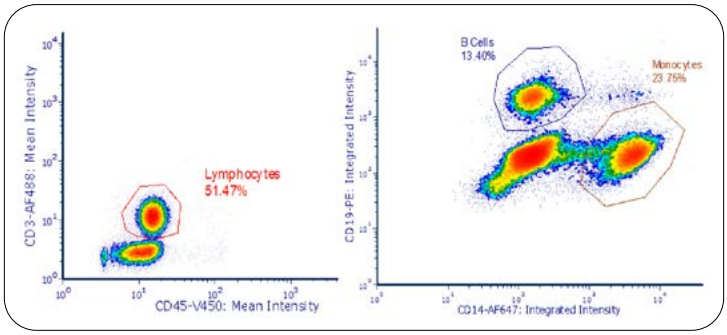
CD4 + T cell activation dose response (by CD69)



Test molecules 1 and 2 activated CD4+ T cells (% CD69) in a dose dependent manner.

Immunophenotyping of patient product (96-well plate) ~30 min to results

The Celigo™ can be used to immunophenotype primary cell samples based on the expression of specific surface markers. In this example, fresh human PBMCs were incubated with fluorescent antibodies against CD45, CD3, CD19 and CD14. Following incubation, the samples were washed once with PBS and placed in a 96-well plate and imaged on the Celigo. Replicate samples were also run on a Flow cytometer with comparable results (see table).



PBMCs were incubated with a cocktail containing CD3-AF488, CD45-V450, CD14-AF647 and CD19-PE. Following incubation, cells were washed once in PBS and placed in a 96-well plate for imaging on the Celigo. While scatter plot analysis can be done in the Celigo software, FCS files were exported for plotting in FCS Express™ from Denovo Software.

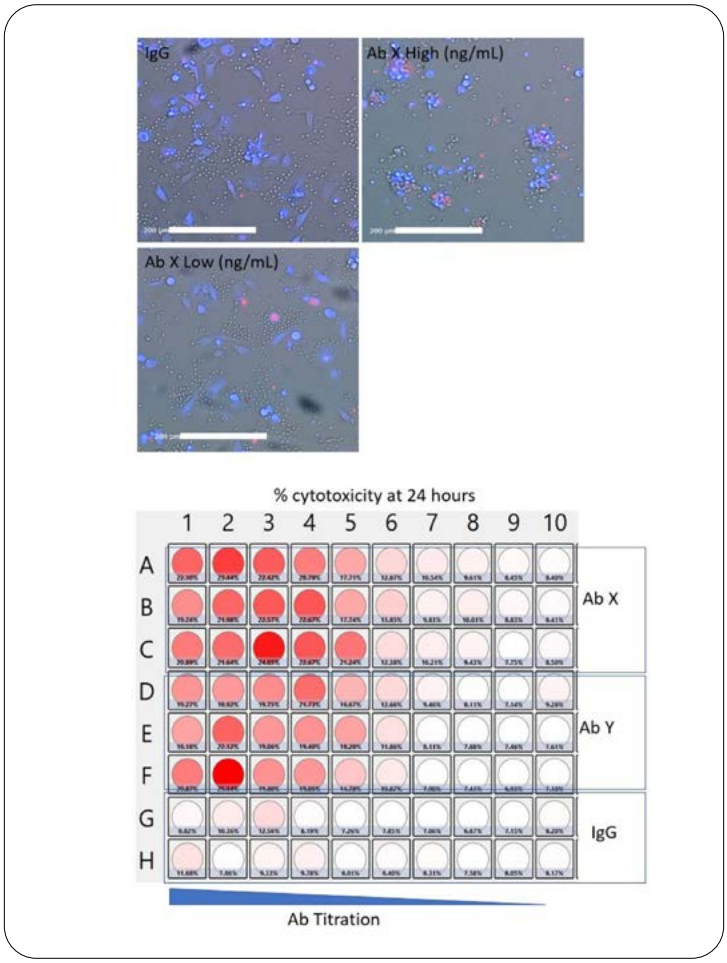
Subsets	% of Total Celigo	% of Total LSRII	# of Events	[cell/mL] x 10^6
Total Leukocytes (CD45+)	N/A 88.62%*	93.3%	68,482	102.72
T Cells (CD3+CD45+)	51.47%	45.8%	35,250	52.87
Monocytes (CD14+CD45+)	23.75%	23.8%	16,263	24.39
B Lymphocytes (CD19+CD45+)	13.40%	19.2%	9171	13.76

*Percent based on sum of subsets -100%

The Calculated Lymphocyte, B cell and Monocyte populations were comparable to analysis on the LSR II FACS instrument.

T cell mediated cytotoxicity (96-well format) ~18 min to results per 96-well plate.

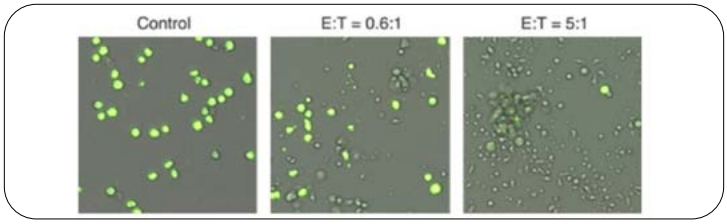
Immuno-oncology assays such as ADCC, CDC, NK cell, and T cell mediated killing can be read on the Celigo™. Assays can be performed in round bottom dishes as usual with final reading performed by transferring cells to flat dishes for analysis on the Celigo. Alternatively, killing assays can be performed entirely in flat bottom imaging dishes and monitored at multiple timepoints with the Celigo. The latter method is advantageous for assays with adherent target cells.



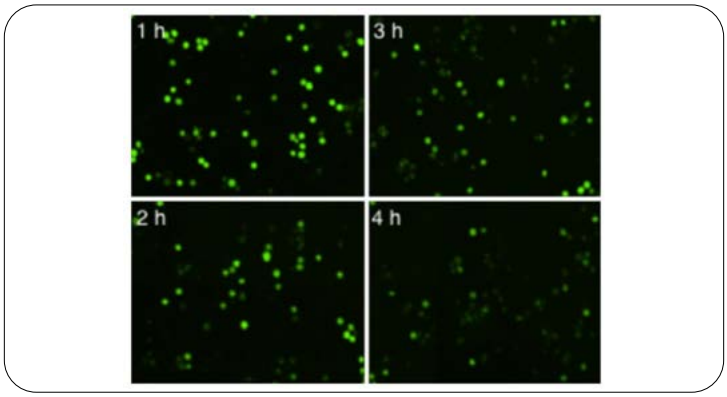
Adherent target cells were labeled with CellTrace™ Violet prior to incubation with PBMCs and antibodies of interest. Immune cell clustering were observed in images of wells with high antibody X concentrations, while low Ab X and IgG containing wells showed no or minimal clustering. Red indicates the level of cytotoxicity. At 24 hours there was a dose dependent cytotoxic effect for both Ab X and Ab Y, but not IgG.

NK cell mediated cytotoxicity (96-well format) ~10 min to results per 96-well plate.

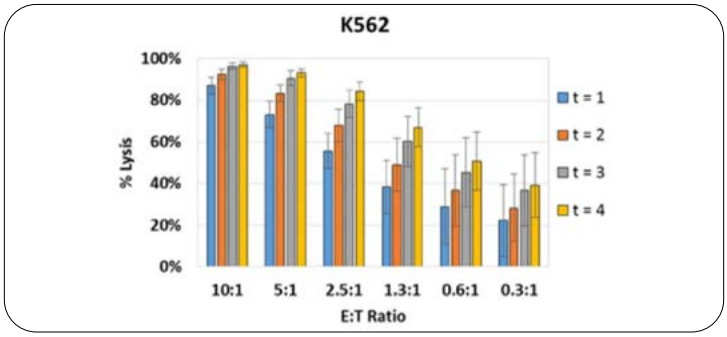
Immuno-oncology assays such as ADCC, CDC, NK cell, and T cell mediated killing can be read on the Celigo™. In these assays, target cells were labeled with bright green Calcein AM prior to incubation with effector cells. Once target cells were killed, they spilled their fluorescent contents rendering them non-fluorescent. NK- cell killing assays typically evolved over ~ 4 hours and were performed entirely in flat bottom imaging dishes and monitored at multiple timepoints with the Celigo.



Brightfield and Calcein AM overlay images showing E:T ratio dependent cell killing.



Green fluorescent images of Calcein loaded target cells. Fewer and fewer brightcells were observed over time indicating NK cell mediated killing of the target cells.

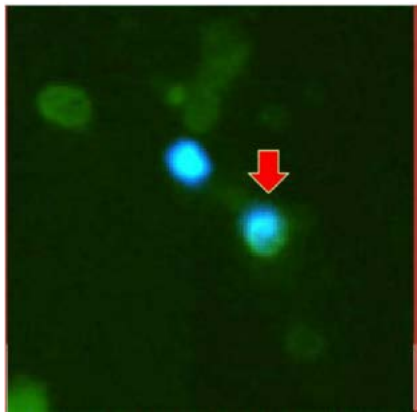


Quantification of NK cell mediated lysis of K562 target cells over 4 hours from a single 96-well plate. Target cell lysis increased both with increased effector cell concentration and with increased incubation time.

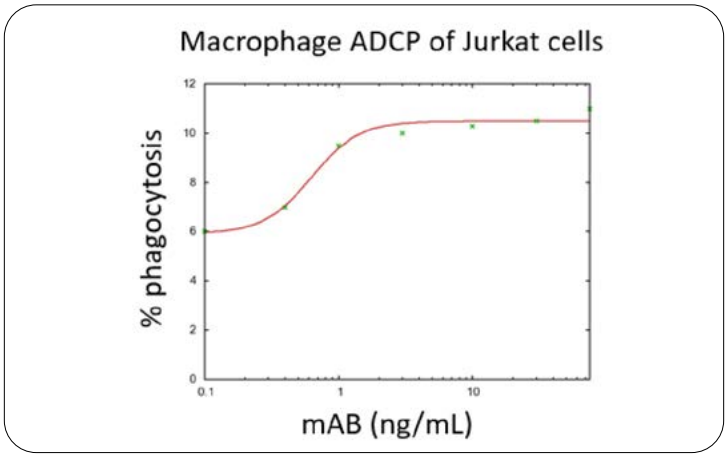
Phagocytosis/ efferocytosis/ADCP (multiple plate formats) ~15 min to results.

The Celigo™ has been used to quantitate the cellular internalization of media particles, antibodies and other cells through various mechanisms. With this class of assay, the item of interest to be internalized or phagocytosed is labeled with a fluorophore (CellTrace™, pHrodo™ etc). It is also possible to label the effector cell with a separate CellTrace™ dye. In the example below, Jurkat cells were stained with CellTrace™ violet and incubated with macrophages labeled with CD-14-FITC (green). When cultured together in the presence of a test antibody, an increase in macrophage phagocytosis of Jurkat cells was observed.

Jurkat cells phagocytosed by macrophage



CellTrace™ violet labeled Jurkat cells (blue) were cultured with CD14-FITC labeled macrophages (green) and a test mAB. Red arrow indicates a macrophage that engulphed a Jurkat cell.

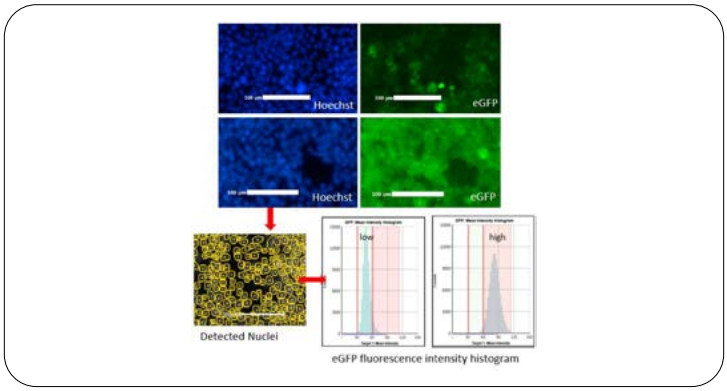


Macrophage phagocytosis of Jurkat cells was induced by the test antibody in a dose dependent manner.

Loss of fluorescence/ fusion protein degradation (multiple plate formats) ~11 min to results per 96-well plate.

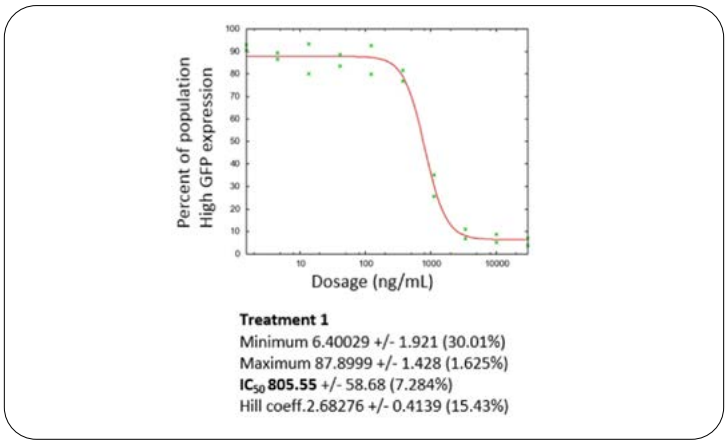
The Celigo™ can be used to quantify the per cell expression of a fluorescent protein such as eGFP. In this example, eGFP was fused to a cellular protein of interest. The cells were treated with a dilution of compound known to induce degradation of this protein. To quantify the per cell expression of eGFP, first live cells were labeled with Hoechst, then the mean intensity of eGFP fluorescence was calculated on a per cell basis. Using the Celigo gating interface the number of cells with high eGFP expression was calculated.

Count individual cells by nuclear stain, determine level of eGFP expression



Cells expressing an eGFP fusion protein were imaged in the Blue (Hoechst) and Green (eGFP) fluorescent channels. All cells were counted based on the nuclear label, the eGFP fluorescence was evaluated for each cell and plotted on a histogram. Cells were segmented into eGFP (high/positive) or eGFP (low/negative) populations.

Quantify impact of drug on loss of eGFP

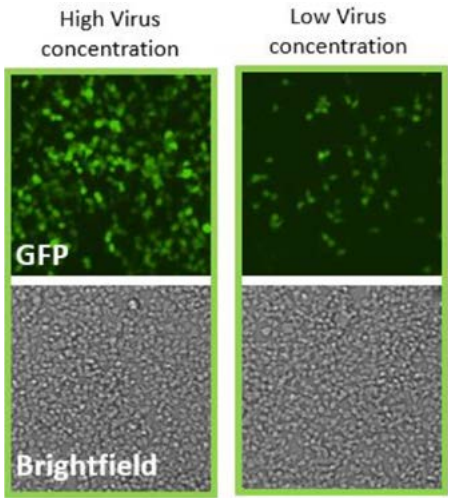


The Celigo counting results were easily exported as standard CSV files for graphing and analysis in programs such as Graphpad Prism.

Fluorescent protein transfection efficiency (multiple plate formats) ~10 min to results

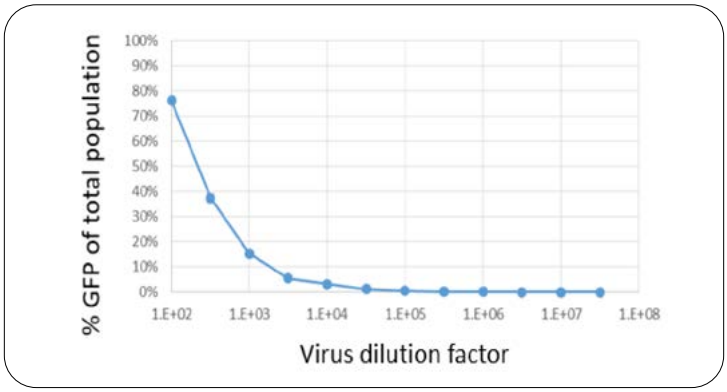
The Celigo™ can be used to determine the percentage of GFP expressing cells in a variety of multi well formats. In this example dilutions of lentivirus delivering GFP were introduced into confluent wells of a 96-well plate. After 24 hours, the percentage of GFP expressing cells was determined by counting cells both in green fluorescent images and brightfield images.

Whole well green fluorescent and brightfield imaging.



Confluent monolayers of cells were incubated with dilutions of lentivirus delivering GFP. Following 24 hours of incubation, cells were imaged and cells counted in both green fluorescent (GFP +) and brightfield (total cells) images.

Lentivirus transfection efficiency

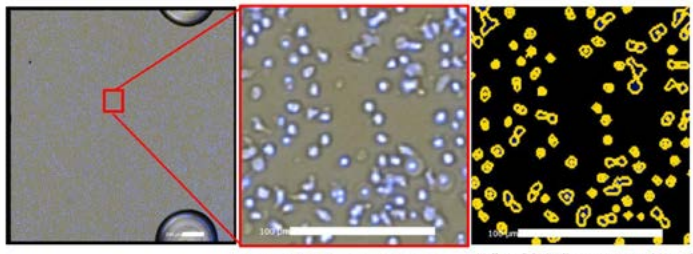


The percentage of cells expressing GFP were graphed in external programs such as MS excel. Cell counts were exported in CSV format for easy processing in data analysis software.

Cell count on Seahorse Bioanalyzer plates (96- and 24-well) ~2 min to results per 96-well plate.

Proper data analysis of Seahorse bioanalyzer data requires normalization to cell number. This was rapidly accomplished on the Celigo™ in 2 minutes per 96-well plate by counting the number of Hoechst 33342 stained nuclei. The Hoechst delivered according to the Agilent SOP by the Seahorse machine itself. Cells were also be counted in brightfield images.

Counting Hoechst 33342 stained cells

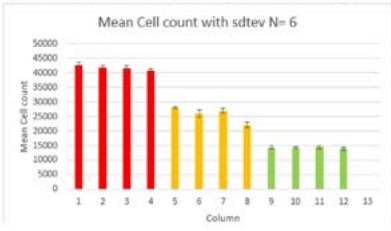


Confluent monolayers of cells were incubated with dilutions of lentivirus delivering GFP. Following 24 hours of incubation, cells were imaged and cells counted in both green fluorescent (GFP +) and brightfield (total cells) images.

The Celigo cell counts on Seahorse plates are accurate and consistent

Total Cell number

	1	2	3	4	5	6	7	8	9	10	11	12
A	4	46106	45269	44061	28183	27471	27981	21950	15756	14141	14732	5
B	43665	41764	41404	41214	28210	26879	27404	23176	14223	14385	15183	14665
C	42362	42955	43322	41402	28421	27149	27106	22981	14777	14514	13801	14232
D	43296	42360	42109	40739	28143	27110	27570	22703	14588	14705	15041	13954
E	43808	42050	41294	39399	28087	25558	26999	21681	13987	14150	13958	14307
F	42343	41174	41165	41599	27872	24960	27052	20841	15077	14837	14548	13281
G	41435	41715	40265	39986	27846	24171	25445	21631	13642	14017	14238	13380
H	2	43499	43442	35218	27989	25117	26732	22081	14522	13893	13636	4
Mean	42818	42003	41593	40723	28097	25971	26929	22169	14382	14435	14462	13970
CV	0.02	1%	2%	2%	1%	5%	3%	4%	4%	2%	4%	4%
stedev	924	610	1032	868	216	1260	760	922	531	316	566	546

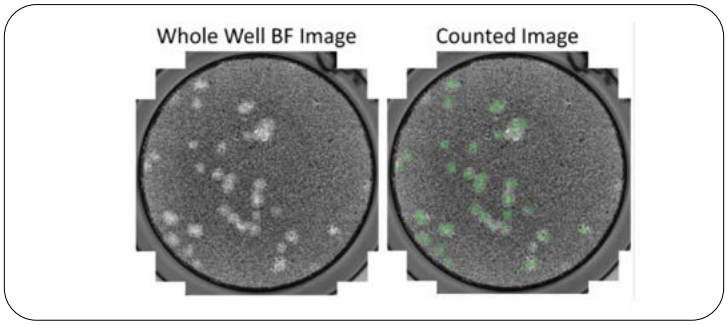


5 Virology

Open (Lytic) viral plaques (multiple plate formats) ~8 min to results.

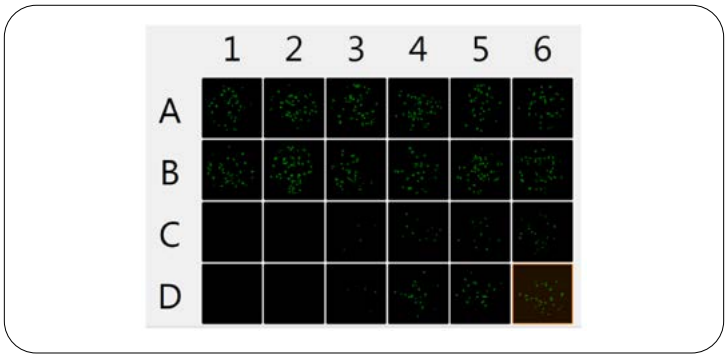
The Celigo™ can be used to count the number of open or lytic plaques in a confluent monolayer of crystal violet stained cells.

Whole well brightfield image and plaque count

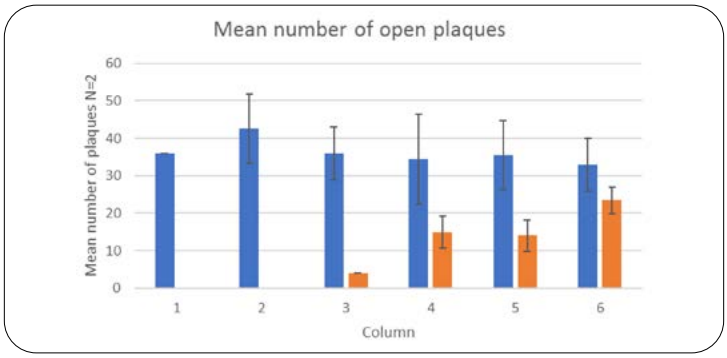


MDCK cells were plated to confluency in a 24-well plate. Following addition of virus and treatment an agar overlay was added. After 7 days of incubation overlay was removed and cells were stained with crystal violet.

Whole plate results view



Results view of whole plate, plaques were highlighted with a green texture to show plaque density.

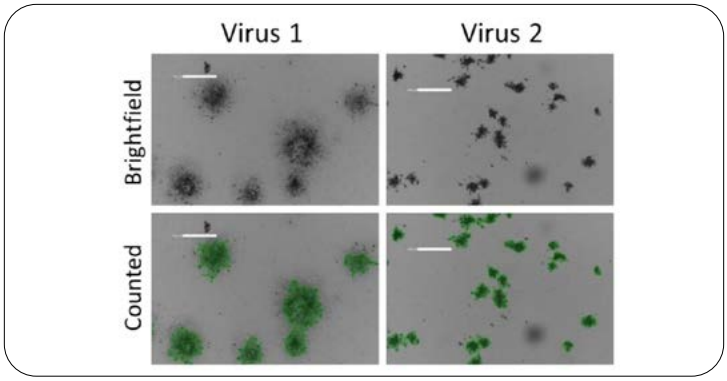


Plaque counts were reported by the Celigo software and can be exported in standard CSV file format for further analysis in programs like MS Excel.

Viral Plaque assay (multiple plate formats) ~7 min to results per 96-well plate.

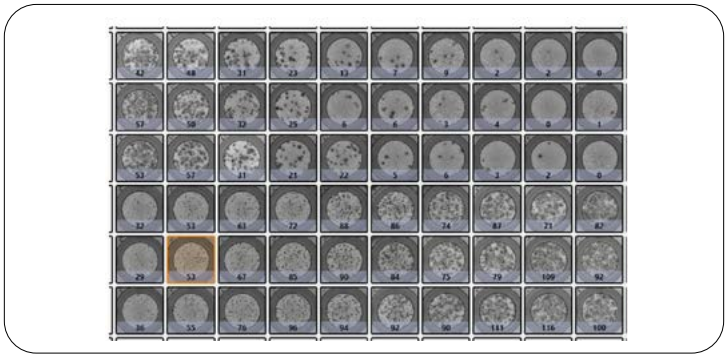
The Celigo™ can be used to count the number of HRP stained plaques in a variety of multi well formats. In this example two different viruses were diluted across a 96-well plate and allowed to incubate overnight under a semi solid medium. Infected cells were detected the following day by Immunohistochemical staining for virus coat proteins.

Brightfield detection of virus plaques



Different viruses can produce plaques with different morphologies. The counted plaques are highlighted with a green texture by the Celigo software.

Whole plate results view



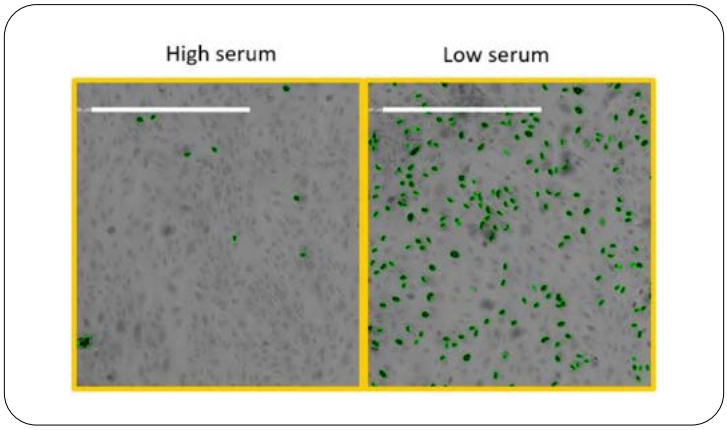
Whole plate results view. The number of counted plaques in each well is indicated.

Total Count	1	2	3	4	5	6	7	8	9	10	11	12	
A													
B		42	48	31	23	13	7	9	2	2	0		Virus 1
C		57	50	32	25	6	6	3	4	0	1		
D		53	57	31	21	22	5	6	3	2	0		
E		32	53	63	72	88	86	74	87	71	82		Virus 2
F		29	53	67	85	90	84	75	79	109	92		
G		36	55	76	96	94	92	90	111	116	100		
H													

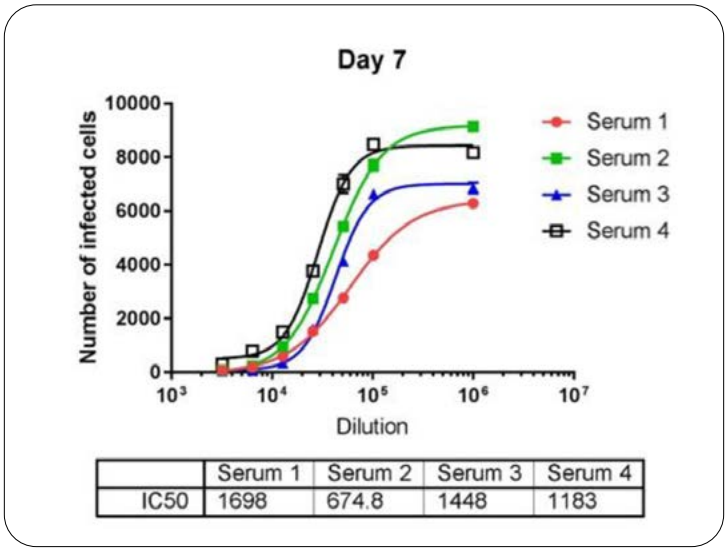
Viral infectivity/ microneutralization assay (multiple plate formats) ~7 min to results.

The Celigo™ can be used to count the number of virally infected cells in a variety of multi well formats. In this example individual infected cells were counted based on IHC staining for viral coat proteins.

Count individual infected cells



Confluent monolayers of ARPE-19 were incubated for 7 days with HCMV virus and dilutions of human serum. Individual infected cells were dark following IHC and were counted by the the Celigo (Green outlines).



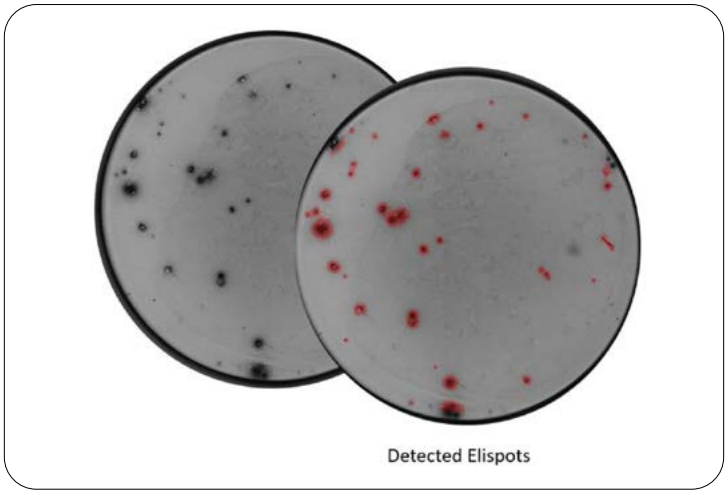
	Serum 1	Serum 2	Serum 3	Serum 4
IC50	1698	674.8	1448	1183

The Celigo counting results are easily exported as standard CSV files for graphing and analysis in programs such as Graphpad Prism.

ELIspot (96-well plate) ~7 min to results.

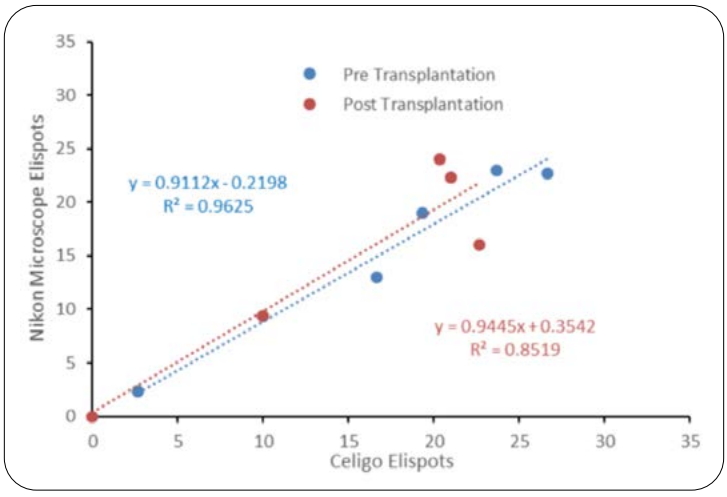
The Celigo™ can be used to count the number of ELIspots on PVDF membranes using brightfield images. Dark spots indicate cells that are secreting a molecule of interest (PBMC and IL-17 in this example). A comparison of the Celigo spot counting with image capture by standard microscope followed by spot counting with commercial ELIspot software showed good agreement between the two methods.

Count Dark Spots on PVDF Membrane



Brightfield image of 96-well ELIspot plate, PVDF bottom. Human PBMCs were seeded and assayed for IL-17 expression.

Celigo vs ELIspot commercial software

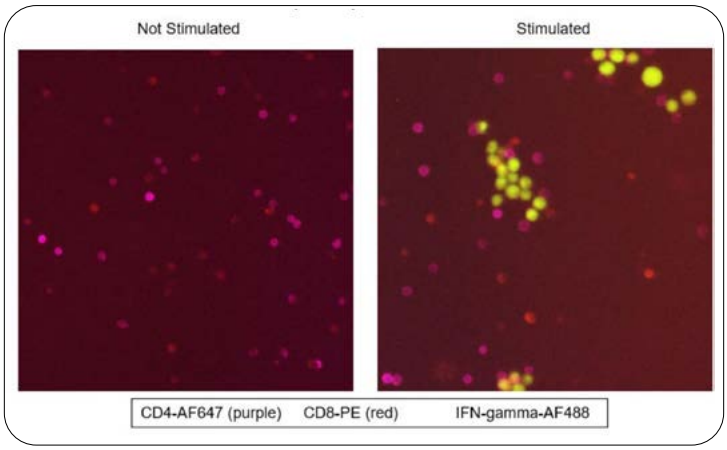


A comparison of spot counts by the Celigo and Microscope + commercial software found good agreement between methods.

ELIspot Fluorescence (96-well plate and others) ~15 min to results.

The Celigo™ can be used to perform fluorescent ELIspot assays on clear plastic multi-well plates. In the example below stimulated and unstimulated PBMCs were plated into wells that were coated with anti IFN-Gamma antibodies. After incubation, the plates were developed with a master mix of fluorescently tagged antibodies against CD4, CD8 and the IFN-Gamma capture antibodies. AF-488 highlighted cells secreting IFN-Gamma while the phenotype of the was be determined from the CD4 or CD8 label.

Fluorescence based ELIspot assay of PBMC INF-Gamma Secretion

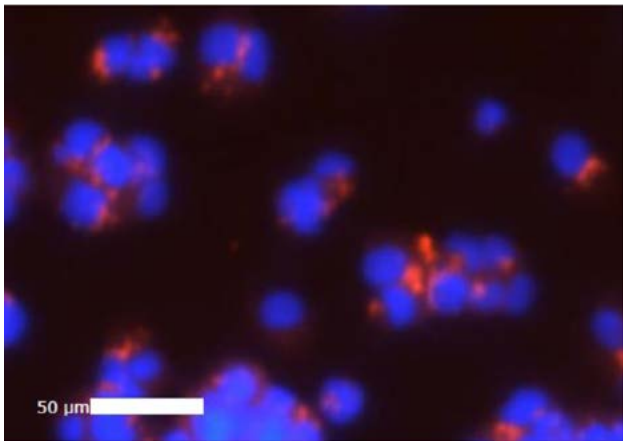


Merged, Far-Red (purple), Red (red) and Green (green) fluorescent images from the Celigo.

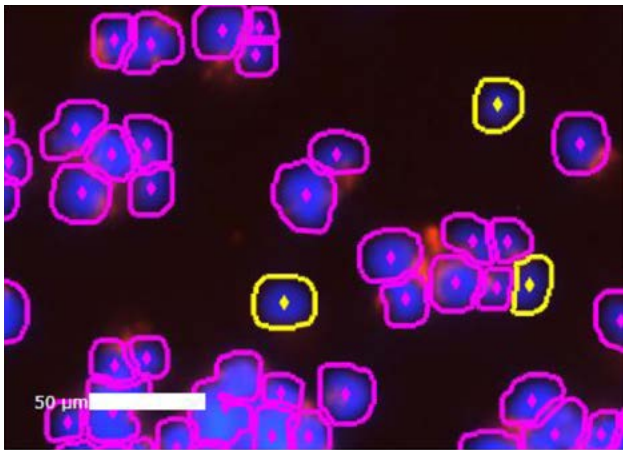
Celigo Applications 43 Quantification of intracellular parasites (96-well plate) ~10 min to results.

The Celigo™ can be used to detect and quantify intracellular parasites within host cells via immunostaining. In this example, perinuclear *Rickettsia* were detected by immunostaining following fixation. Individual host cells were detected by Hoechst 33342 nuclear stain. Hoechst stained nuclei were used as a mask to quantify *Rickettsia* signal on a per-cell basis

Immunostaining for intracellular rickettsia



Quantification of intracellular parasite



Merged Hoechst 33342 (blue) and Rickettsia immunostaining (red). Infected cells are highlighted in pink, uninfected cells in yellow (right image).

www.revvity.com

2033653

For research use only.
Not for use in diagnostic procedures.