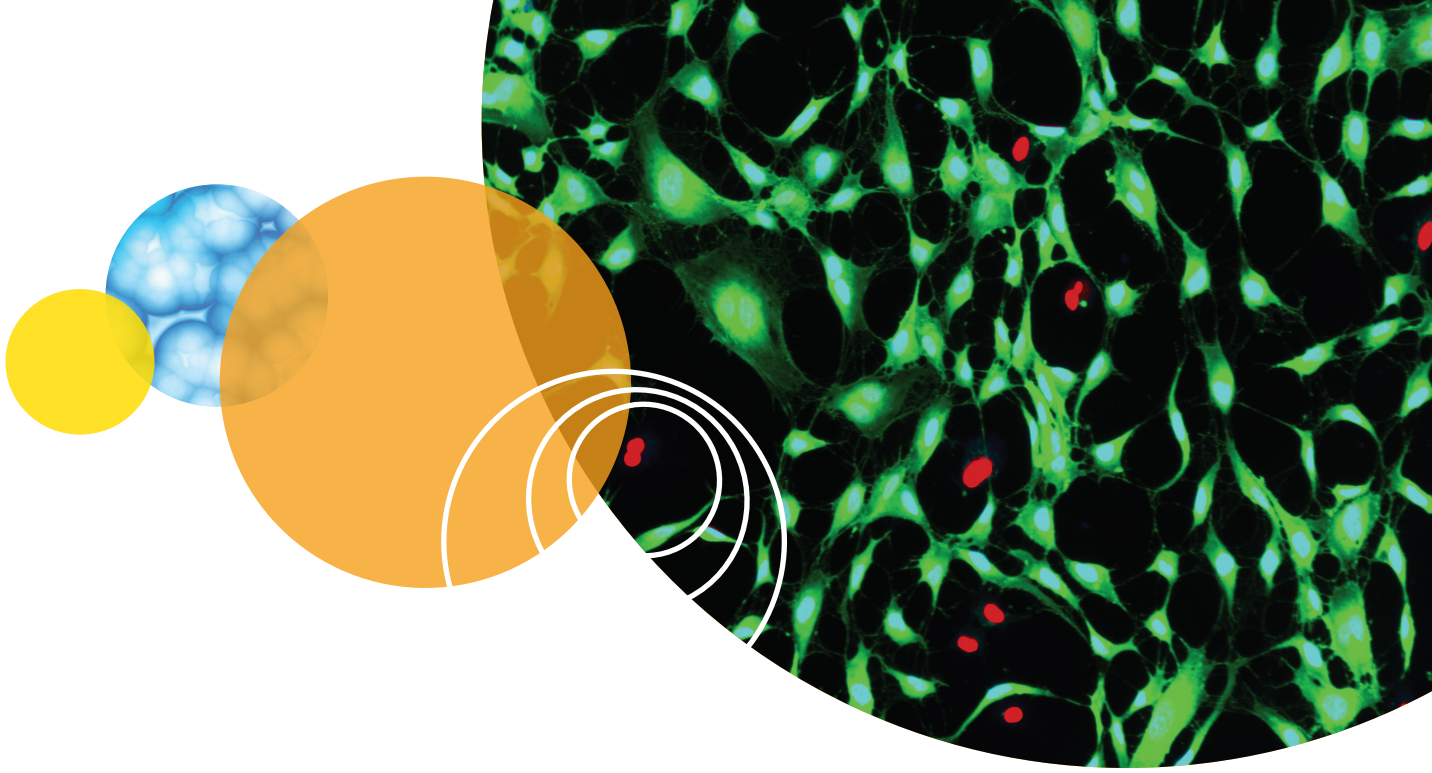


Count Cells Like a Pro

Cell counting and phenotypic characterization are important subsets of cytometry used in various applications within life science research. Our SpectraMax® MiniMax™ 300 Imaging Cytometer, with the SpectraMax® i3x Multi-Mode Microplate Reader, adds cellular imaging assays in a small footprint and eliminates cell staining for cell counting and confluence measurements with StainFree™ Cell Detection Technology. This field-upgradeable option with one-of-a-kind brightfield cell segmentation, green and red fluorescent channel detection, and a simple workflow provides you with cellular analysis capability without the need for a complex imaging system. Here, we describe the analysis of a variety of commonly used cell types.

eBook contents

For more information, visit
moleculardevices.com

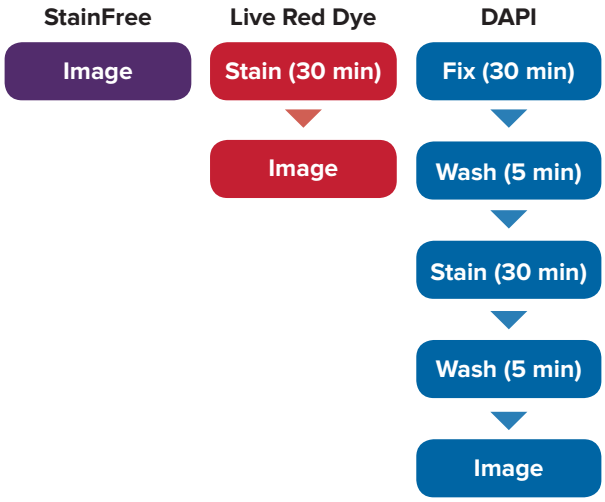
MOLECULAR DEVICES

About StainFree Cell Detection Technology

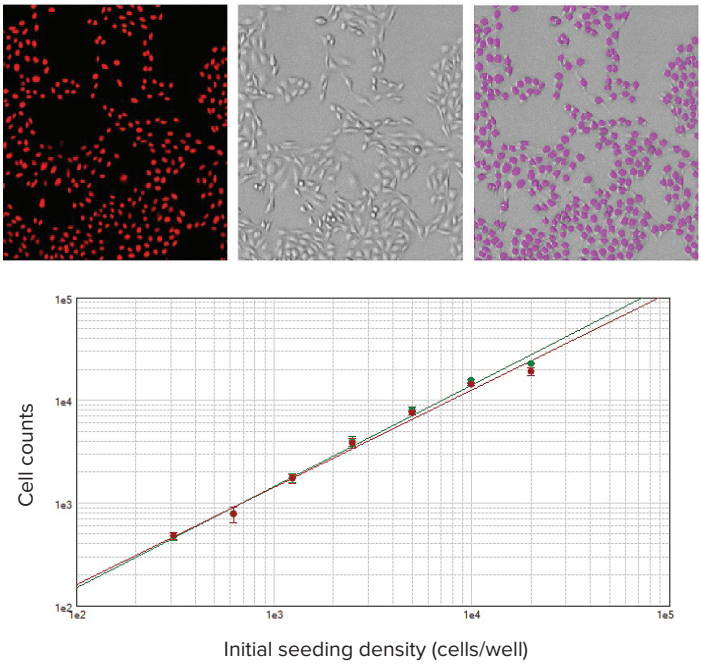
Imaging cell-based assays typically requires the use of fluorescent probes that can be toxic to living cells or may only function in fixed cells. A label-free method for analyzing cell counts and cell confluence enables you to quantitatively monitor cell proliferation and health without time-consuming workflows that may disrupt cell viability.

The SpectraMax i3 Multi-Mode Microplate Reader with the SpectraMax MiniMax 300 Imaging Cytometer uses unique, patent-pending, StainFree Cell Detection Technology that allows you to perform cell proliferation, cytotoxicity, and other assays without nuclear stains like DAPI, which intercalates with DNA, or live cell dyes that are actually toxic to cells in the long term.

Download Application Note



Workflow for cell analysis with StainFree technology vs. Live Red Dye vs. DAPI. The StainFree workflow saves about 70 minutes compared to fixation and staining with DAPI. Moreover, cells analyzed using StainFree technology remain fully viable and can be used in additional assays.

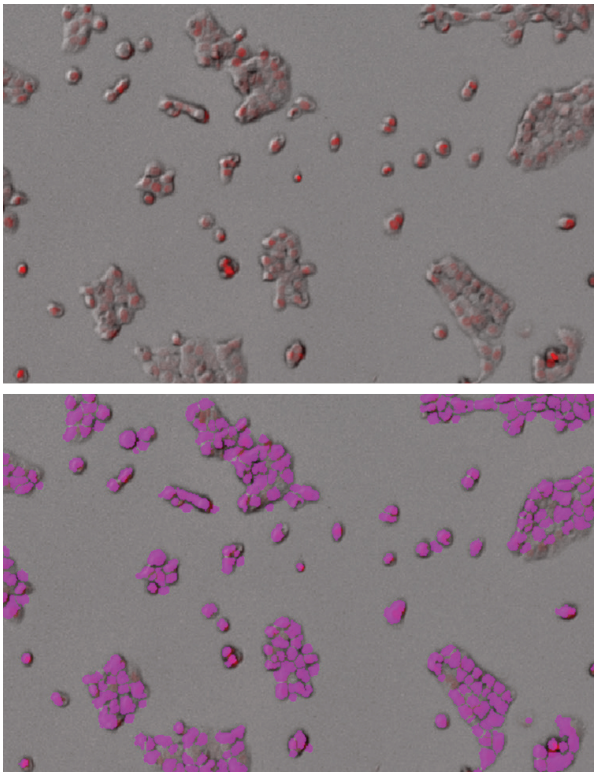


Cells counted with the MiniMax cytometer. Cells stained with Live Red Dye were imaged in the red fluorescent **(top left)** or transmitted light **(top middle)** channel. StainFree cell counts **(top right, purple masks)** correlate very closely to cell counts based on red nuclear staining, as shown in the graph (green circles: StainFree counts; red circles: red nuclear counts). The r^2 values for both curves were 0.99.

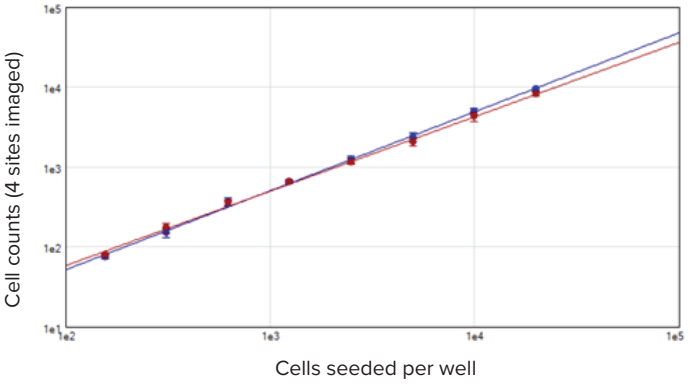
A431 Cells

The epidermoid carcinoma cell line A431 expresses abnormally high levels of epidermal growth factor receptor (EGFR) and is a useful model for studying EGFR-mediated signaling, which regulates growth, survival, proliferation, and differentiation in mammalian cells. Derived from the epidermis of an 85-year old female patient and established by D. J. Giard et al., A431 cells have proven to be a valuable model system for examining the cell cycle, apoptosis, and cancer.

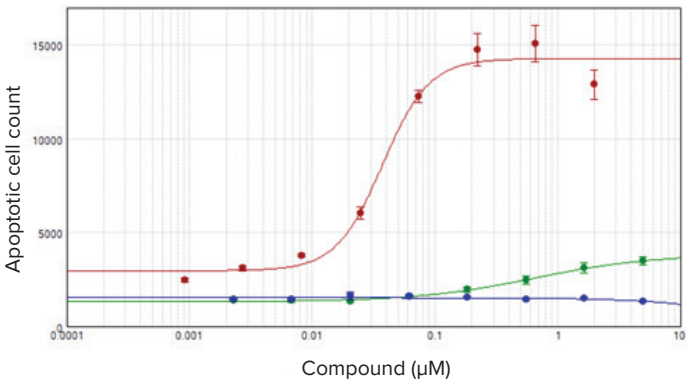
Click here for tips and instrument settings.



A431 cells were stained with EarlyTox™ Live Red Dye and then imaged using the SpectraMax MiniMax cytometer. Cells were identified with a user-defined custom setting. **Top:** overlaid transmitted light and red fluorescent images; **Bottom:** cells identified using StainFree analysis (purple masks show cells identified by the software).



StainFree cell counts vs. fluorescent cell counts. A431 cells were seeded at densities ranging from 156 to 20,000 cells per well, and nuclei were stained using EarlyTox Live Red Dye. Images were acquired from 4 sites per well, and a region of interest in the middle of the wells was selected for analysis. Cells were then counted by using either the StainFree method (blue plot) or by counting red-fluorescent nuclei (red plot).

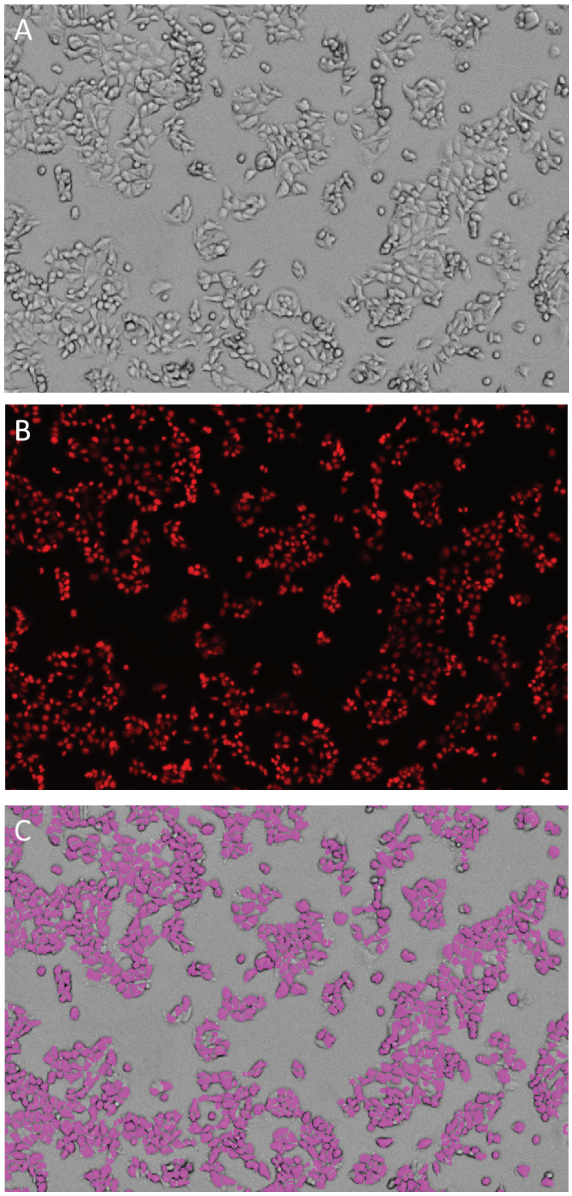


EarlyTox™ Caspase-3/7 NucView 488 Assay. A431 cells were treated with serial dilutions of staurosporine (red plot), camptothecin (green plot), and etoposide (blue plot) for 24 hours and then assayed for apoptosis using the EarlyTox Caspase-3/7 NucView™ 488 Assay Kit. Cells expressing caspase-3/7 were stained green fluorescent and were imaged and counted using the SpectraMax MiniMax cytometer. Results were plotted as apoptotic cell count vs. compound concentration using SoftMax Pro Software.

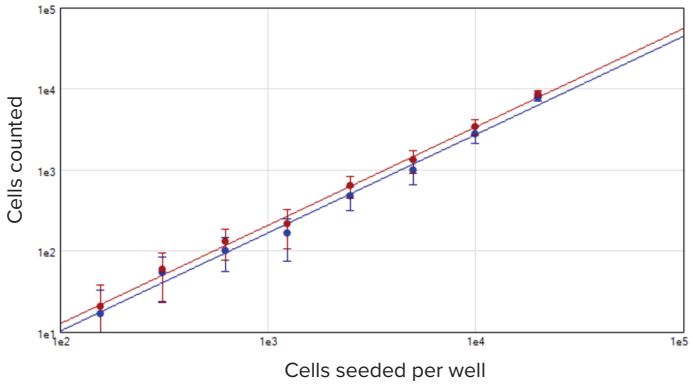
MCF-7 Cells

Derived in 1970 from a nun named Sister Catherine Frances (Helen Marion) Mallon, this cell line was named after the Michigan Cancer Foundation, where it was generated. MCF-7 is one of the few breast cancer cell lines to express substantial levels of estrogen receptor (ER) alpha, thus it offers researchers a valuable model system for the study of ER-positive breast cancers. Type 'MCF-7 cells' into PubMed and you will find over 20,000 references to this remarkable cell line.

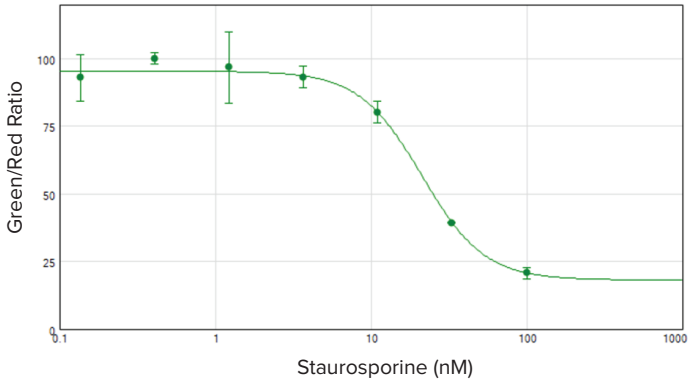
Click here for tips and instrument settings.



MCF-7 cells were stained with EarlyTox Live Red Dye and then imaged using the SpectraMax MiniMax cytometer. Cells were identified with a user-defined custom setting. **(A)** transmitted light image; **(B)** nuclei stained with EarlyTox Live Red Dye; **(C)** cells identified using StainFree analysis (purple masks show cells identified by the software).



StainFree cell counts vs. fluorescent cell counts. MCF-7 cells were seeded at densities ranging from 156 to 20,000 cells per well, and nuclei were stained using EarlyTox Live Red Dye. Images were acquired from 4 sites per well, and a region of interest in the middle of the wells was selected for analysis. Cells were then counted by using either the StainFree method (blue plot) or by counting red-fluorescent nuclei (red plot).

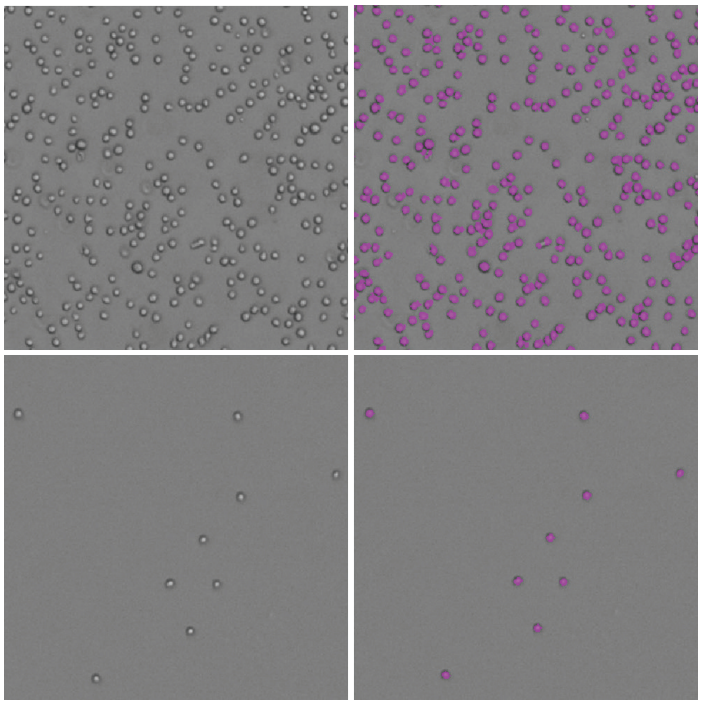


EarlyTox Live/Dead Assay. MCF-7 cells were treated with serial dilutions of staurosporine for 24 hours and then assayed using Molecular Devices EarlyTox™ Live/Dead Assay Kit. Live cells were stained with Calcein AM (green dye), and dead cells were stained with an ethidium homodimer (red dye). The plate was read on the SpectraMax i3x Multi-Mode Microplate Reader. Results were plotted as ratio of green/red vs. compound concentration, and an IC_{50} value of 21.2 nM was calculated using SoftMax Pro Software.

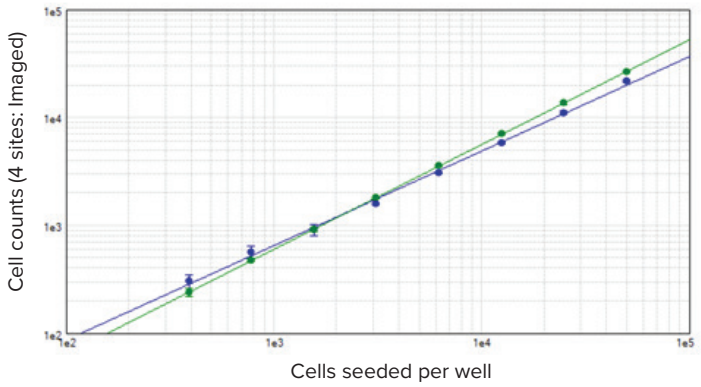
Jurkat Cells

Established in the late 1970s from the peripheral blood of a young boy with T-cell leukemia, Jurkat cells are an immortalized T-lymphocyte cell line. They have been used to study T-cell leukemia, T-cell signaling, and cancer cell sensitivity to drug treatments. These small, round cells grow readily in suspension and have also been used as a model system for various cell viability and apoptosis assays as demonstrated here.

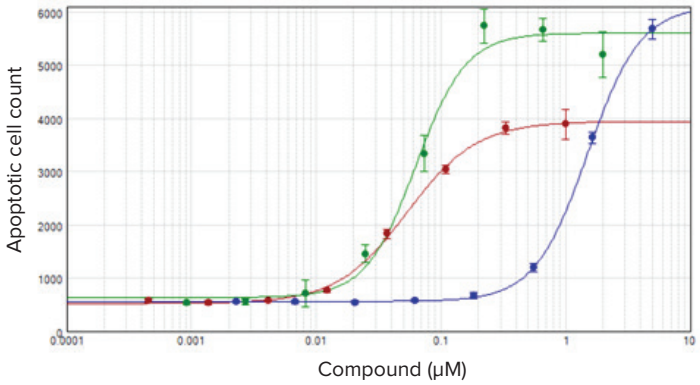
Click here for tips and instrument settings.



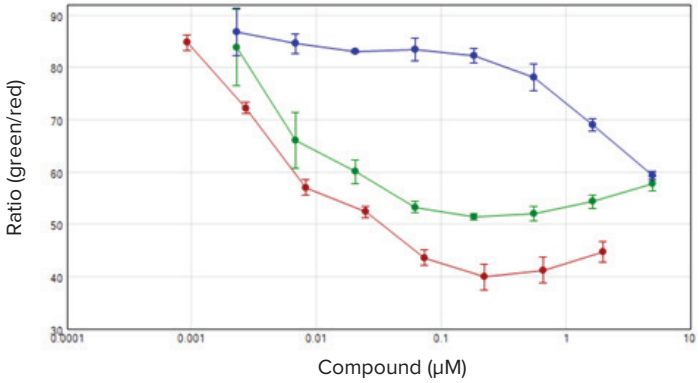
StainFree cell counts. Jurkat cells were imaged using the SpectraMax MiniMax 300 Imaging Cytometer, and cells were identified using the predefined 'CellsC' setting. Shown on the left are the original transmitted light images and on the right are the same images with purple masks indicating cells identified by the software. Two cell densities are shown: 50,000 and 1562 cells seeded per well of a 96-well plate.



StainFree cell counts vs. fluorescent cell counts. Jurkat cells seeded at densities ranging from 390 to 50,000 cells per well were counted using StainFree technology (blue dots), or they were stained with EarlyTox™ Live Cell Assay dye and green fluorescent cells were counted (green dots). Cell counts obtained with both methods agreed closely across the entire range of cell densities. Cell counts were measured for four sites imaged per well.



EarlyTox Caspase-3/7 NucView 488 Assay. Jurkat cells were treated with staurosporine (red plot), camptothecin (green plot), or etoposide (blue plot) for 28 hours and then assayed for apoptosis using the EarlyTox™ Caspase-3/7 NucView 488 Assay Kit. Cells expressing caspase-3/7 were stained green fluorescent and were imaged and counted using the SpectraMax MiniMax 300 Imaging Cytometer.

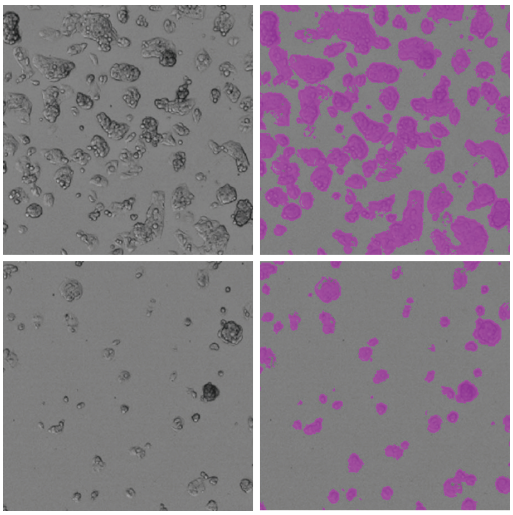


EarlyTox Live/Dead Assay. Jurkat cells were treated with serial dilutions of staurosporine (red plot), camptothecin (green plot), and etoposide (blue plot) for 24 hours and then assayed using Molecular Devices EarlyTox Live/Dead Assay Kit. Live cells were stained with calcein AM (green dye), and dead cells were stained with ethidium homodimer (red dye) and read on a SpectraMax i3x Multi-Mode Microplate Reader using the WellScan feature, which performs multiple reads spaced regularly across the well. Results were plotted as ratio of green/red vs. compound concentration. All compounds used in this experiment caused a dramatic reduction in cell viability within 24 hours.

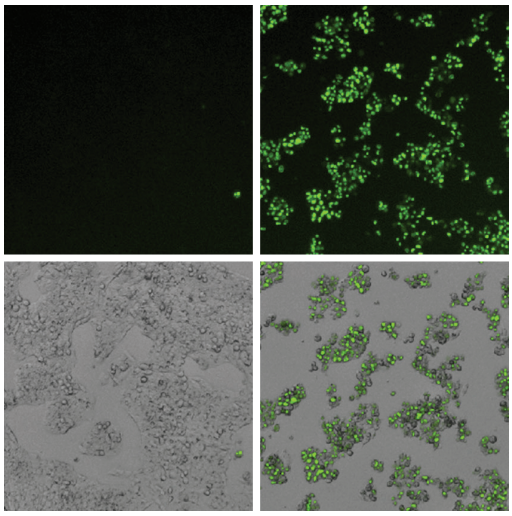
HepG2 Cells

HepG2 is a cell line derived from the liver tissue of a patient with hepatocellular carcinoma (HCC). It is often used as a model system for HCC as well as for studies of drug metabolism and toxicity. Cultures are adherent, with epithelial morphology, and tend to grow in small aggregates that make the counting of individual cells difficult. Under the right culture conditions, they can form distinct apical and basal surfaces amenable to studies of liver disease.

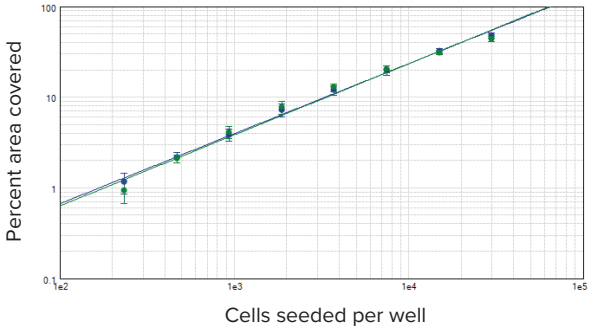
Click here for tips and instrument settings.



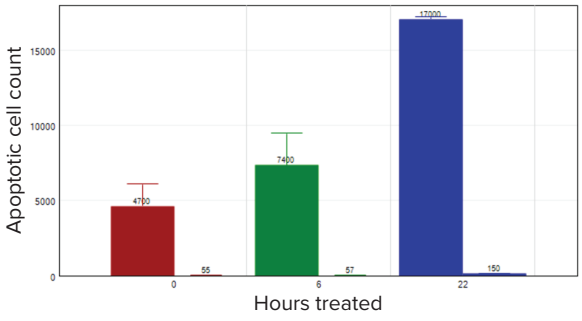
StainFree determination of % covered area. HepG2 cells were imaged using the SpectraMax MiniMax 300 Imaging Cytometer and cells were identified using a custom, user-defined analysis setting. Shown on the left are the original transmitted light images, and on the right is the same image with purple masks indicating cells identified by the software. Two cell densities are shown for comparison.



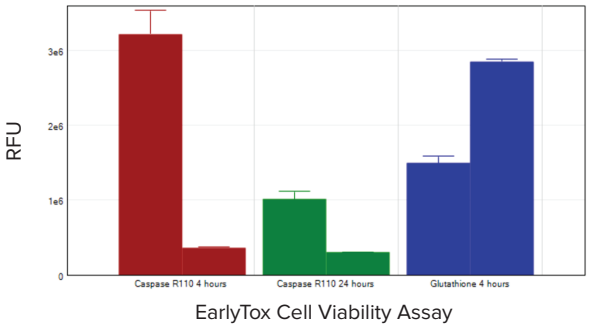
EarlyTox Caspase-3/7 NucView 488 Assay. HepG2 treated with media control **(left panels)** or 200 µM capsaisin **(right panels)** were assayed with the EarlyTox Caspase-3/7 NucView 488 Assay Kit and imaged using the SpectraMax MiniMax 300 Imaging Cytometer with green fluorescent and transmitted light (TL) channels. **Top row:** fluorescence image showing apoptotic cells labeled with green fluorescence. **Bottom row:** overlaid TL and fluorescence images showing minimal apoptosis in control cells and nearly 100% apoptosis in treated cells.



StainFree vs. fluorescent analysis of % covered area. Confluence of HepG2 cells seeded at densities ranging from 234 to 30,000 cells per well was analyzed using StainFree technology (blue dots), or they were stained with EarlyTox Live Cell Assay dye and fluorescent area was analyzed (green dots). The percent area covered for a region of interest of the image for both methods agreed closely across the entire range of cell densities.



EarlyTox Caspase-3/7 NucView 488 Assay (time course). HepG2 treated with 200 µM capsaicin (left bar), or media control (right bar), for 4.5 hours (red bars), 6 hours (green bars), or 22 hours (blue bars). Apoptosis was assessed using the EarlyTox Caspase-3/7 NucView 488 Assay Kit. Cells were incubated with 5 µM of NucView 488 substrate and imaged at the time points indicated.

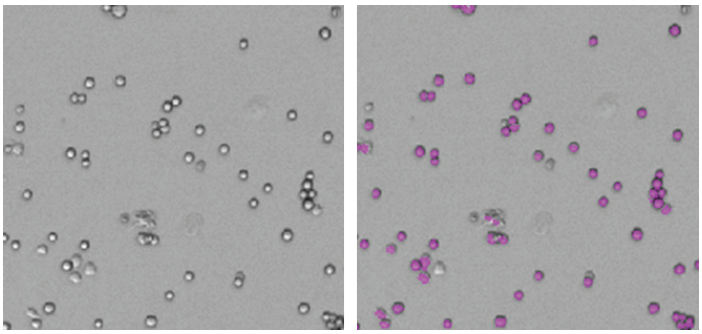


EarlyTox Caspase-3/7 R110 Assay Kit and EarlyTox Glutathione Assay Kit. HepG2 treated with 200 µM capsaicin (left bar of each pair) or media control (right bar of each pair) were assayed using the EarlyTox™ Caspase-3/7 R110 Assay Kit (red and green bars), which measures caspase activity, or EarlyTox™ Glutathione Assay Kit (blue bars), which measures the decrease in glutathione, an early indicator or apoptosis. Both assays were detected on the SpectraMax i3x Multi-Mode Microplate Reader using preconfigured protocols in SoftMax Pro Software.

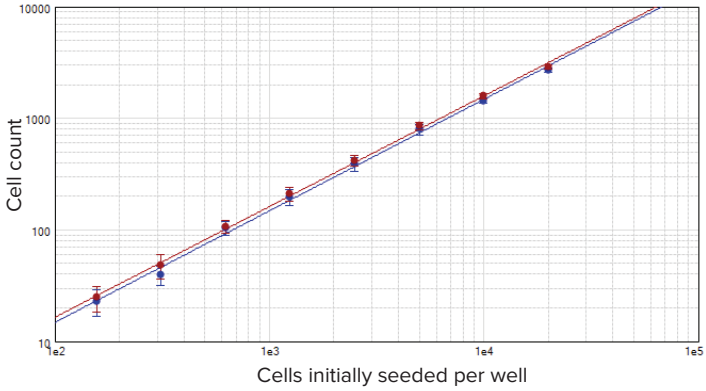
RBL Cells

The establishment of rat basophilic leukemia (RBL) cell lines in the 1970s offered researchers a convenient, if imperfect, model system for the study of difficult-to-obtain mast cells. RBL-2H3 cells subcloned from one of the original RBL cell lines have been used to study the biochemical pathways for secretion in mast cells. Some RBL cell lines are grown as suspension cultures and others are adherent. Because the morphology of the cells under different growth conditions, or even within the same culture, can vary, the ability to create a customized StainFree analysis setting is extremely useful in ensuring accurate cell counts.

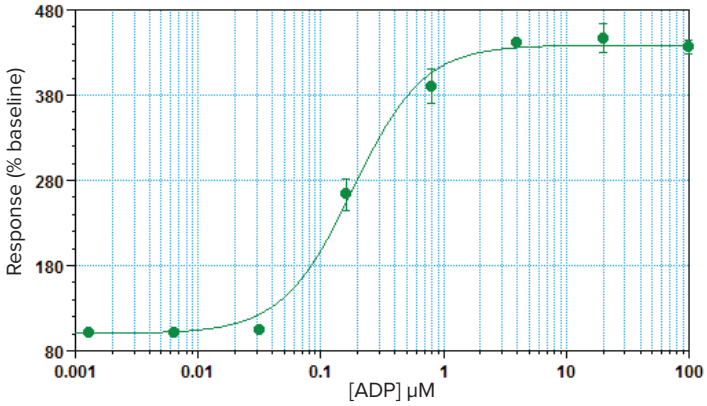
Click here for tips and instrument settings.



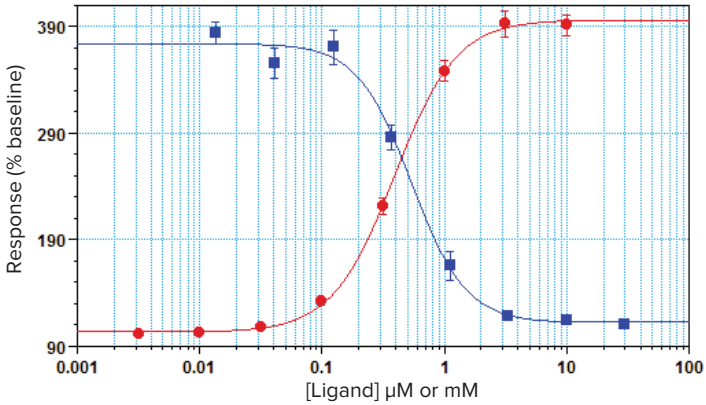
StainFree cell counts. RBL-1 cells were imaged using the SpectraMax i3 MiniMax 300 Imaging Cytometer, and cells were identified using a custom, user-defined analysis setting. Shown on the left is the original transmitted light image, and on the right is the same image with purple masks indicating cells identified by the software.



StainFree cell counts vs. fluorescent nuclear counts. RBL-1 cells seeded at densities ranging from 156 to 20,000 cells per well were counted using StainFree technology (blue dots), or they were stained with EarlyTox Live Red Dye and red fluorescent nuclei were counted (red dots). Cell counts obtained with both methods agreed closely across the entire range of cell densities. Cell counts were measured within a user-defined region of interest (ROI).



FLIPR Calcium 6 Assay on FlexStation 3 Multi-Mode Microplate Reader. RBL-2H3 cells were seeded at 30,000 cells per well in a 96-well plate and incubated overnight. The next day, growth medium was removed, FLIPR® Calcium 6 Assay dye plus probenecid were added to the wells, and cells were incubated for 2 hours. A kinetic fluorescence assay was then performed on a FlexStation® 3 Multi-Mode Microplate Reader. The onboard pipettor delivered the agonist ADP to assay wells, while fluorescent signal indicating calcium mobilization was simultaneously measured from the bottom of the wells by the plate reader.

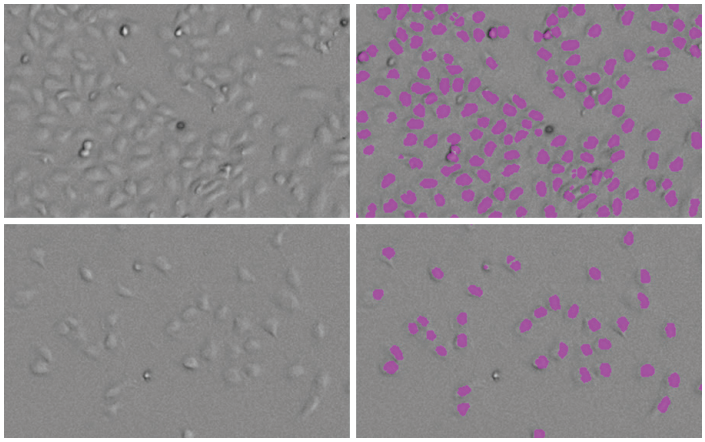


Fura-2 QBT Calcium assay on FlexStation 3 Multi-Mode Microplate Reader. RBL-2H3 cells were seeded at 30,000 cells per well in a 96-well plate and incubated overnight. The next day they were loaded with Fura-2 QBT™ dye for 60 minutes after removal of growth medium. For IC_{50} studies, inhibitor YM-58483 was added cells for 45 minutes prior to assay. A kinetic fluorescence assay was then performed on a FlexStation 3 Multi-Mode Microplate Reader. The onboard pipettor delivered $CaCl_2$ to wells at the EC_{80} concentration (for inhibitor assay, blue dots), or in a titration series (red dots). Fluorescent signal indicating calcium mobilization was simultaneously measured from the bottom of the wells by the plate reader.

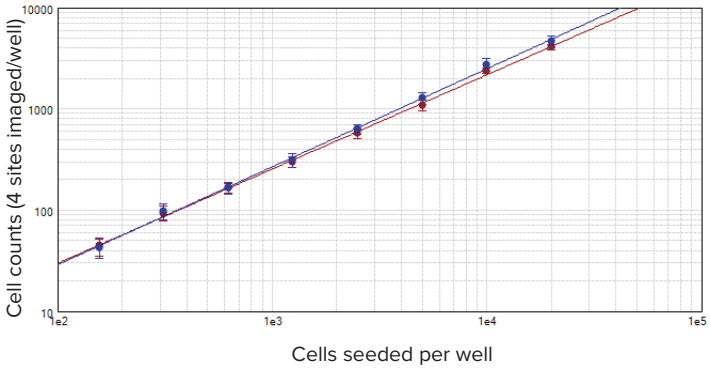
HUVEC Cells

Human umbilical vein endothelial cells (HUVEC) are primary cells isolated from the vein of the umbilical cord. They are a model system for studying endothelial cell function, with applications including hypoxia, inflammation, oxidative stress, response to infection, and both normal and tumor-associated angiogenesis. Endothelial cell activation, an inflammatory response, can be induced by cytokines such as TNF-α and IFN-γ and results in upregulation of cell adhesion molecules like VCAM-1/CD106, whose upregulation can be quantified using fluorescently labeled antibodies and cellular imaging.

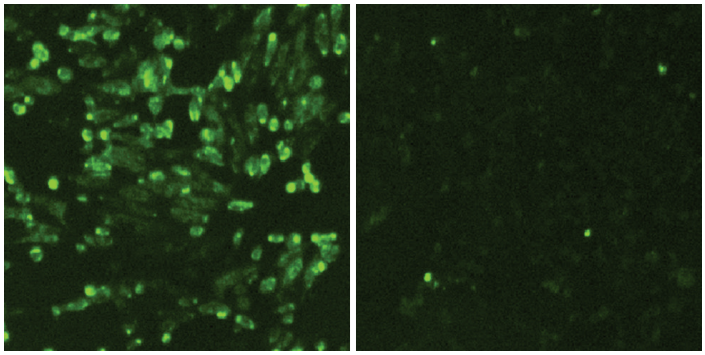
Click here for tips and instrument settings.



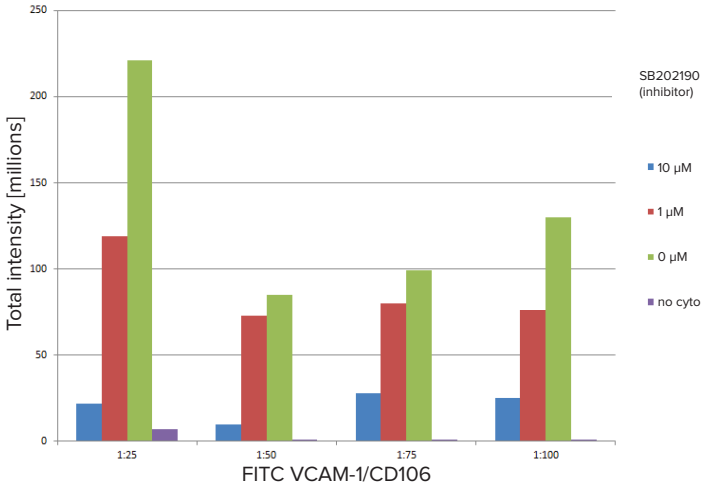
StainFree cell counts. HUVEC were imaged using the SpectraMax i3 MiniMax 300 Imaging Cytometer, and cells were identified from transmitted light images using the predefined 'CellsB' analysis setting. Shown on the left are the original transmitted light images, and on the right are the same images with purple masks indicating cells identified by the software. Cells seeded at high **(top row)** and low **(bottom row)** densities are shown.



StainFree cell counts vs. fluorescent nuclear counts. HUVEC seeded at densities ranging from 156 to 20,000 cells per well were counted using StainFree technology (blue circles), or they were stained with EarlyTox™ Live Red Dye and red-fluorescent nuclei were counted (red circles). Cell counts obtained with both methods agreed closely across the entire range of cell densities.



Cytokine-induced VCAM-1/CD106 expression in HUVEC. HUVEC treated with cytokines TNF-α and IFN-γ (left) or untreated (right) were stained with FITC-labeled antibody against VCAM-1/CD106 and imaged with the SpectraMax MiniMax 300 Imaging Cytometer..

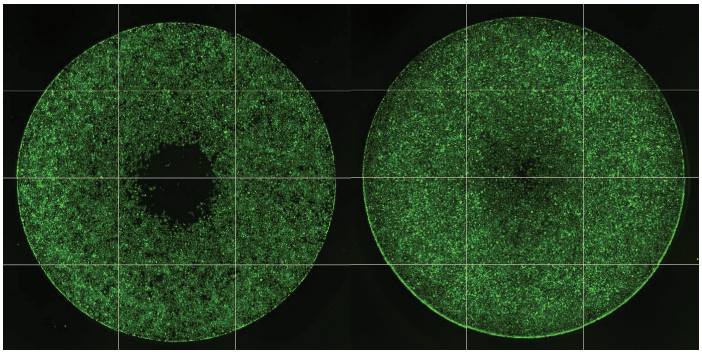


Inhibition of cytokine-induced VCAM-1/CD106 expression in HUVEC. HUVEC were seeded at 10,000 cells per well in a 96-well plate and allowed to attach and grow overnight. They were treated with the cytokines TNF-α and IFN-γ, as well as different concentrations of the p38 MAP kinase inhibitor SB202190, for 24 hours and then stained with a FITC-labeled antibody to VCAM-1/CD106. Controls without inhibitor or cytokines were included.

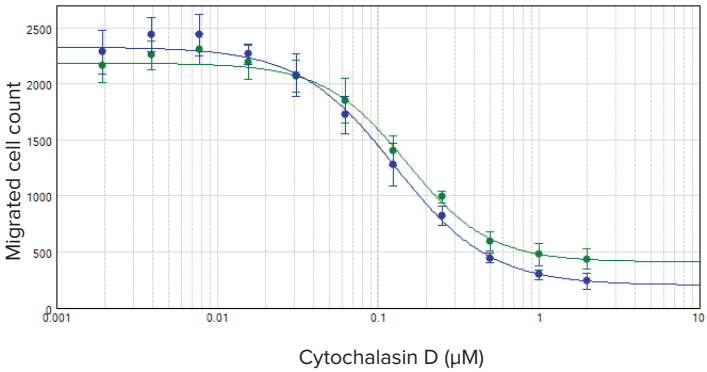
HT1080 Cells

The fibrosarcoma cell line HT1080 was generated in 1972 from tumor biopsy tissue. Interestingly, the patient had not been treated with radiation or chemotherapy at the time of biopsy, so the cells lack the sort of unwanted mutations that are commonly caused by these treatments. Because HT1080 cells are highly invasive, they are often used as models for cell invasion and migration. Researchers have been able to identify a number of potential therapeutic agents for cancer on the basis of their ability to inhibit the invasive behavior of HT1080 cells.

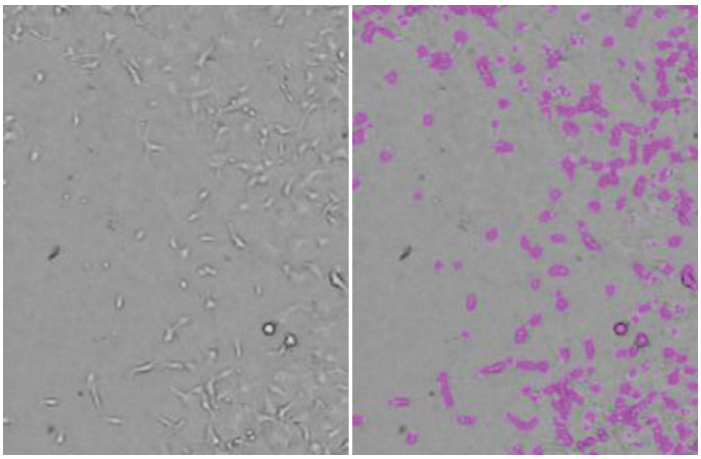
Click here for tips and instrument settings.



Imaging cell migration. The Oris™ Pro Cell Migration Assay from Platypus Technologies was used to measure migration of HT1080 cells treated with cytochalasin D **(left)** or DMSO control **(right)**. This assay uses a non-toxic biocompatible gel (BCG) to form a cell-free zone on culture surfaces. After seeding cells into the 96-well plate, the BCG dissolves permitting cells to migrate into the well centers (area indicated by yellow circles). The results were imaged on the SpectraMax MiniMax 300 Imaging Cytometer, and cells that had migrated in the well centers were counted using the region of interest (ROI) selection feature in SoftMax Pro Software.



Quantifying cell migration. Cells were treated with cytochalasin D, and cells that subsequently migrated into well centers were counted using StainFree Technology (blue circles) or fluorescent cell analysis (green circles). EC_{50} values for cytochalasin D were 0.13 µM and 0.15 µM, respectively, confirming that cells need not be stained in order to accurately assess cell migration with the Oris Pro Assay.

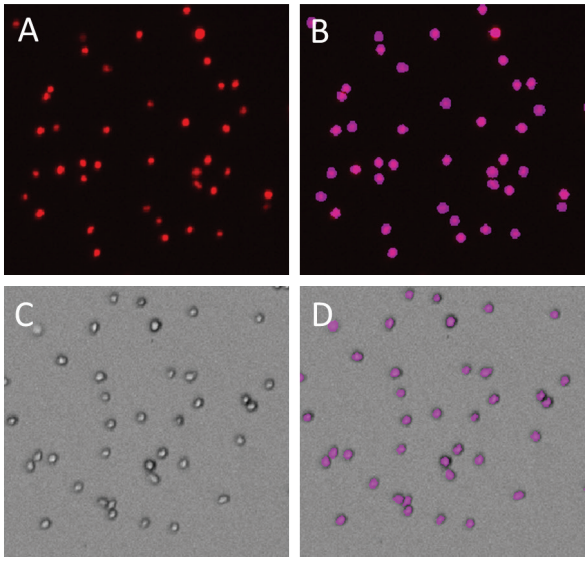


StainFree cell counts. HT1080 cells were imaged using the SpectraMax MiniMax 300 Imaging Cytometer, and cells were identified using the predefined 'CellsD' analysis setting. Shown on the left is the original transmitted light image, and on the right is the same image with purple masks indicating cells identified by the software.

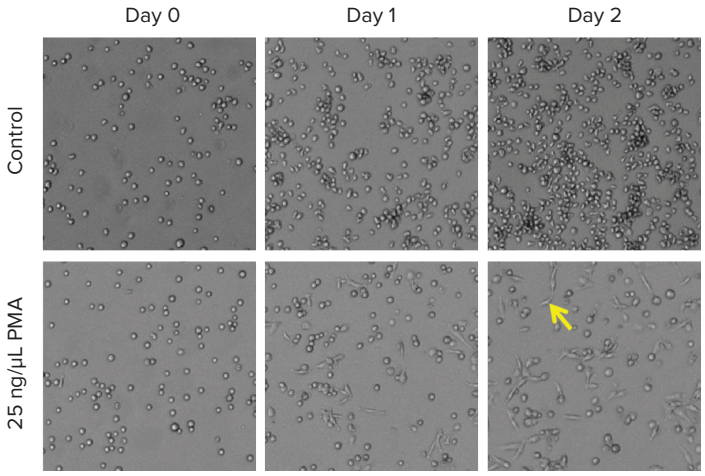
THP-1 Cells

Derived from the blood of an acute monocytic leukemia patient over 30 years ago, the human monocytic cell line THP-1 is widely used to study the function of monocytes and macrophages, as well as in leukemia research. Normally THP-1 cells grow in suspension, but they can be induced to differentiate into adherent macrophage-like cells, which express IL-8 and other markers, and can thus serve as a model system for differentiation.

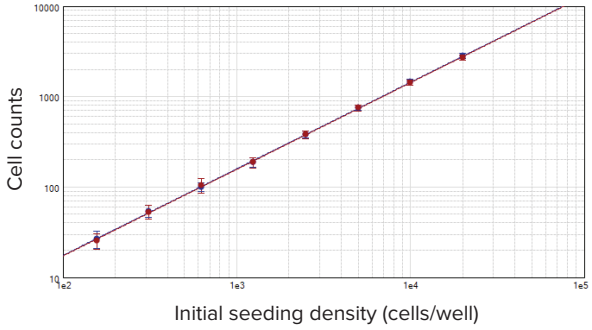
Click here for tips and instrument settings.



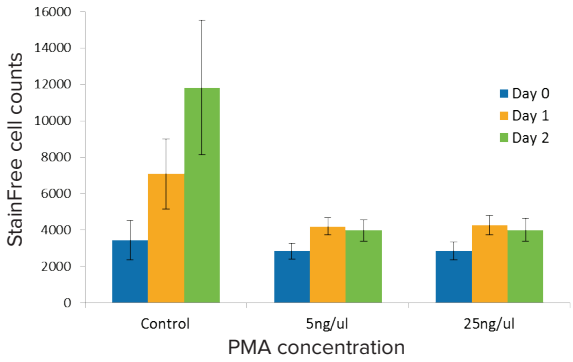
Undifferentiated THP-1 cells imaged on the MiniMax cytometer. (A) THP-1 cells stained with EarlyTox Live Red Dye. **(B)** Purple object masks showing cells identified by SoftMax Pro Software in the red fluorescent imaging channel. **(C)** Cells imaged in the TL channel. **(D)** Cells identified by StainFree analysis in the TL channel.



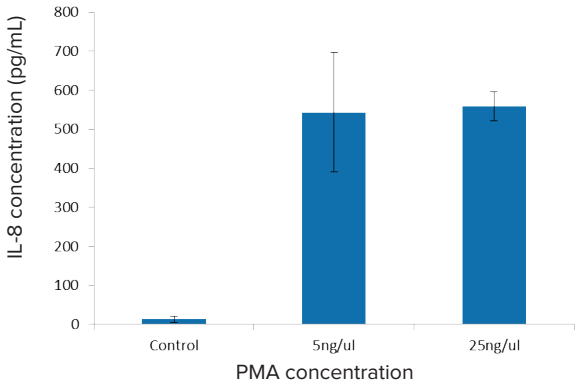
THP-1 differentiation time course. THP-1 cells were seeded at 20,000 cells per well in a 96-well black-wall, clear-bottom plate. They were treated with 25 ng/µL PMA or with DMSO control. Cells were imaged on Day 0, Day 1 post-addition, and Day 2 post-addition. The yellow arrow indicates a differentiated cell with adherent, flattened morphology.



StainFree and fluorescent nuclear counts of undifferentiated THP-1 cells. THP-1 cells seeded at densities ranging from 156 to 20,000 cells per well were counted the day after seeding using StainFree technology (blue circles), or their nuclei were counted after staining with EarlyTox Live Red Dye (red circles). Cell counts obtained with both methods differed by 5% or less at all cell densities.



THP-1 cell proliferation. Cell proliferation was measured in PMA-treated or DMSO-treated control cells using StainFree technology. PMA treatment halts cell proliferation, but control cells continue to divide.

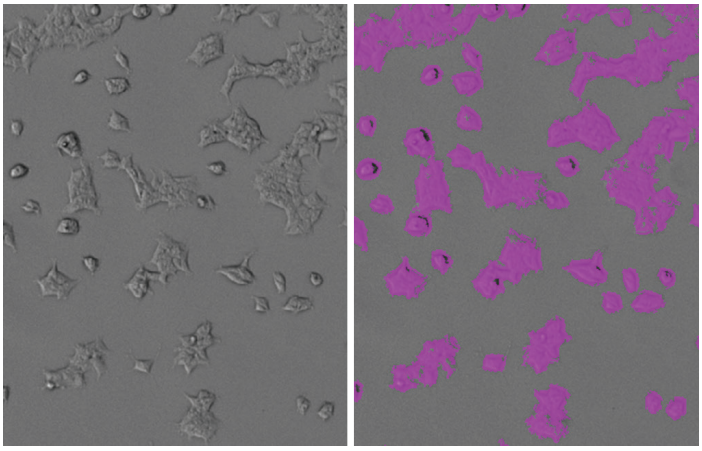


IL-8 ELISA. IL-8 concentration measured via ELISA in PMA- and DMSO-treated THP-1 cells. Cells were seeded at 24,000 cells per well, and supernatants were collected for assay 48 hours after treatment was initiated. The absorbance-based IL-8 ELISA was detected using a SpectraMax i3 microplate reader.

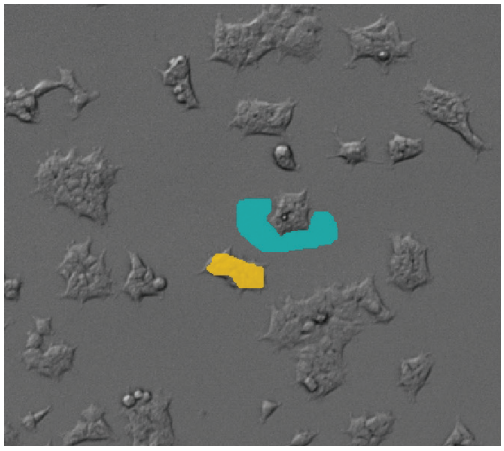
HEK293-GFP Cells

Human Embryonic Kidney 293 cells, also known as HEK293, 293 cells, or even as HEK cells, are a cell line derived from human embryonic kidney. In the early 1970's, Alex van der Eb isolated these cells in his laboratory at the University of Leiden, Holland, and another member of his lab transformed them by adenovirus, giving rise to the cell line used today. HEK293 cells are easy to grow and transfect, so they have been used extensively in transfection-based experiments, protein expression, and vaccine production.

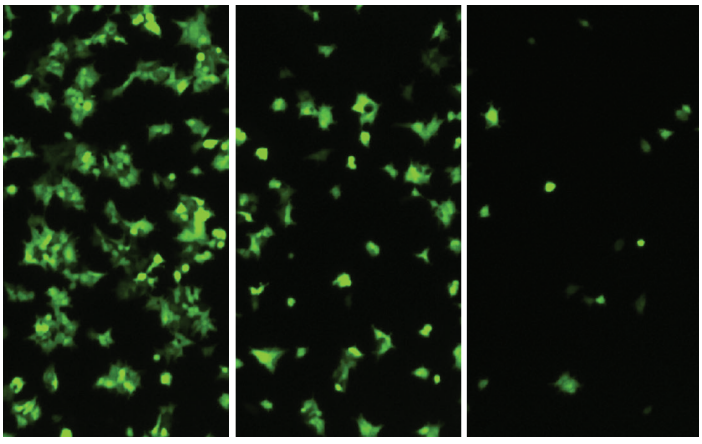
Click here for tips and instrument settings.



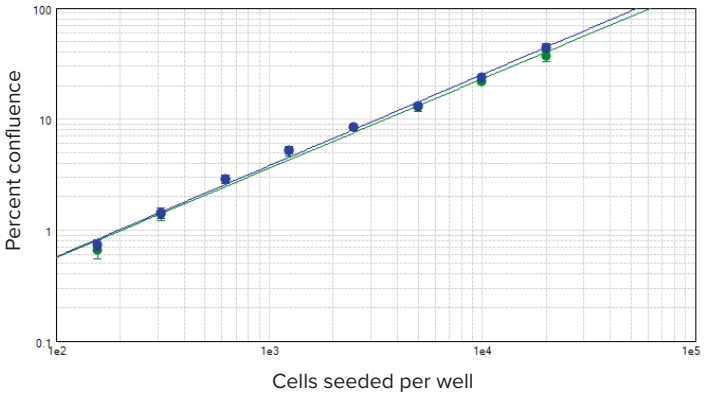
StainFree identification of HEK293-GFP cells. HEK293-GFP cells were imaged using the SpectraMax i3 MiniMax 300 Imaging Cytometer, and cells were identified using a custom field analysis setting. Shown on the left is the original transmitted light image, and on the right is the same image with purple masks indicating confluent areas identified by the software.



Defining cellular and background areas. Use of drawing tools in SoftMax Pro Software to define cellular (yellow) and background (blue) areas of the images.



HEK293-GFP cells imaged on the MiniMax cytometer. HEK293-GFP cells seeded at 20000, 5000, and 1250 cells per well and imaged in the green fluorescence channel of the MiniMax cytometer.

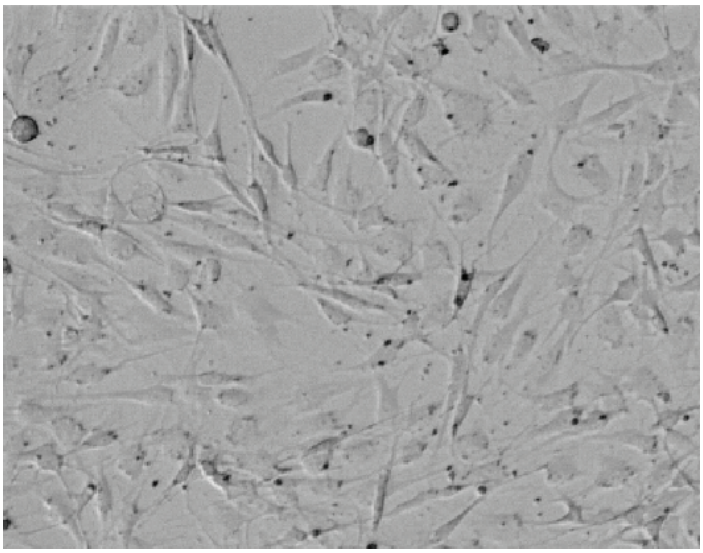


StainFree vs. fluorescence analysis. Comparison of confluent areas measured with StainFree analysis (blue circles) and fluorescence (green circles) analysis. StainFree percent confluence agrees almost perfectly with fluorescence-analyzed confluence.

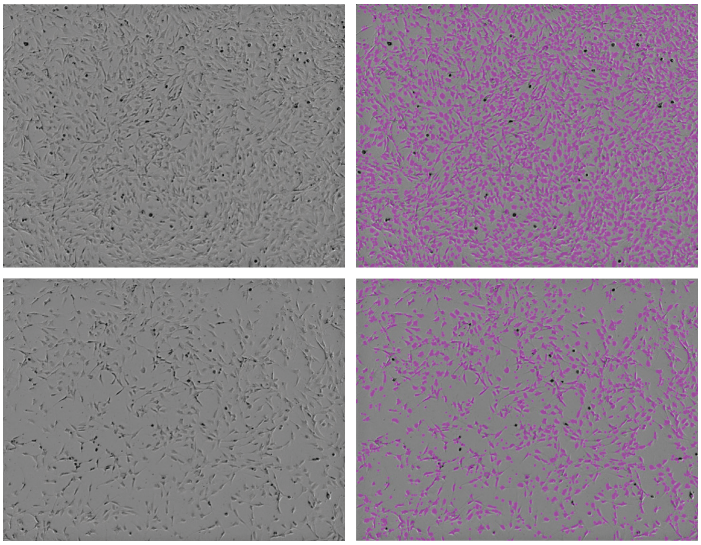
Rat Aortic Endothelial Cells

Rat aortic endothelial cells have been used in a variety of scientific studies including cell-cell adhesion, migration, angiogenesis, and the investigation of cellular signaling pathways involved in endothelial function under normal and disease conditions. Since they are primary cells, their use in drug screening can be tricky. Live cell imaging and measurement of cell confluence prior to running an assay can be critical to success.

Click here for tips and instrument settings.



Transmitted light image of rat aortic endothelial cells. Image shows the range of morphologies and cellular processes within a single primary culture.



StainFree counting of rat aortic endothelial cells. Rat aortic endothelial cells were imaged using the SpectraMax i3 MiniMax 300 Imaging Cytometer, and cells were identified using a custom analysis setting. Shown on the left are the original transmitted light images (top, higher density culture; bottom, lower density culture), and on the right are the same images with purple masks indicating cells identified by the software.

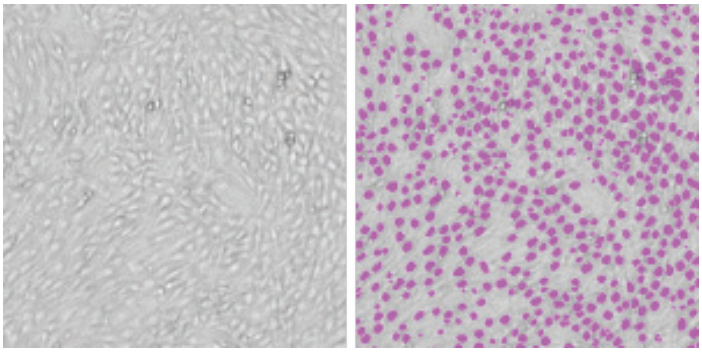
Images were generously provided by Dr. Virginia Huxley at the University of Missouri, Columbia, MO, with assistance from our field application scientist, Randy Milano.

NIH3T3 Cells

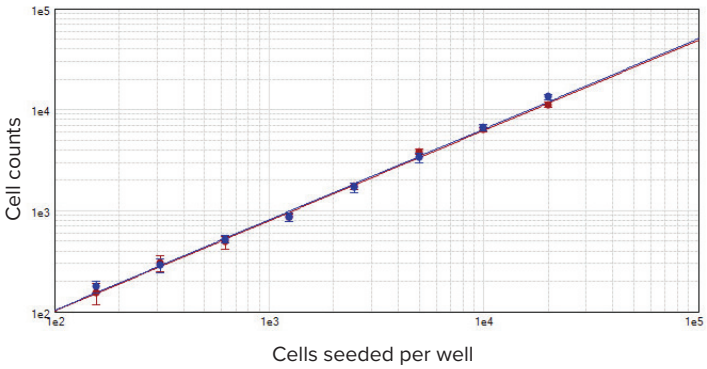
Established in 1963 by scientists George Todardo and Howard Green, the NIH3T3 cell line was derived from mouse embryonic fibroblasts. The cells spontaneously immortalized and were named after their culturing protocol, “3-day transfer, inoculum 3x105 cells”. Now the standard fibroblast cell line, they are known as excellent transfection hosts and as models for induced stem cell pluripotency experiments. They are also useful as feeder cells in human embryonic stem cell research and are often used for culturing keratinocytes, as the growth factors from NIH3T3 are favorable to keratinocyte growth.

The elongated structure and variable cell size of NIH3T3 can pose difficulties for image analysis. However, using StainFree technology, we are able to count these irregular-shaped cells with great accuracy.

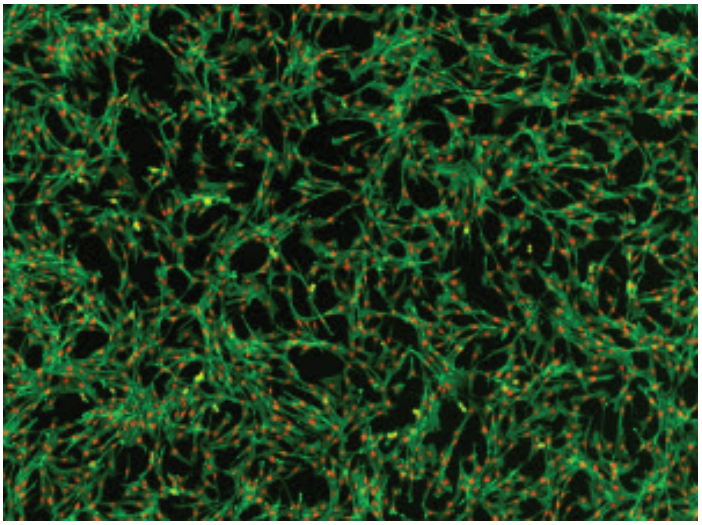
Click here for tips and instrument settings.



StainFree analysis of NIH3T3 cells. NIH3T3 cells were imaged using the SpectraMax MiniMax 300 Imaging Cytometer, and cells were identified using the predefined ‘CellsB’ analysis setting. Shown on the left is the original transmitted light image, and on the right is the same image with purple masks indicating cells identified by the software.



Cell counts of NIH3T3 cells. NIH3T3 cells were seeded in a 1:2 dilution series in a 96-well plate. The cells were fixed and stained with a nuclear dye. Following staining, the SpectraMax MiniMax 300 Imaging Cytometer was used to count cells using either the nuclear red staining (red curve) or StainFree Cell Detection Technology (blue curve) as the basis for cell identification. Both counting methods yield nearly identical results, and both curves have $r^2 > 0.99$.

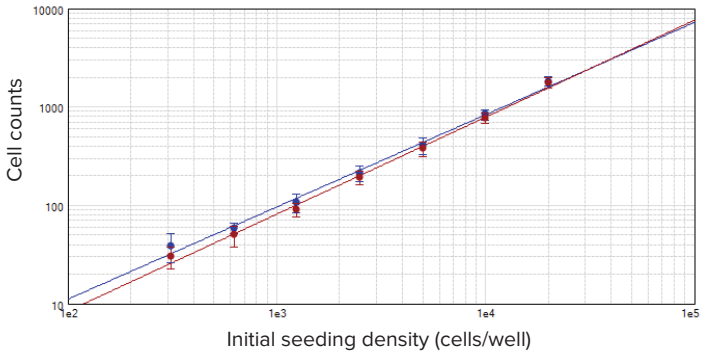


NIH3T3 cells stained. NIH3T3 cells stained with AlexaFluor 488-phalloidin and red nuclear dye. Cells were imaged on the SpectraMax MiniMax 300 Imaging Cytometer.

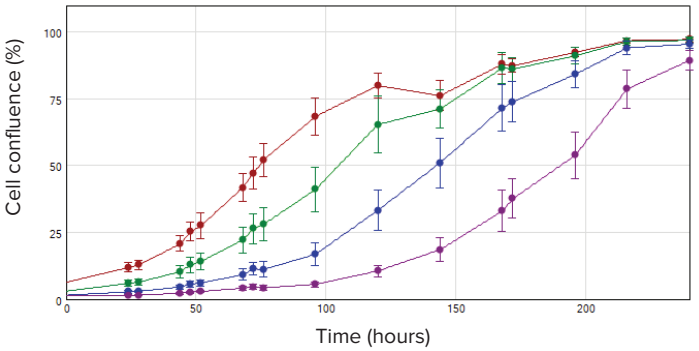
PC-12 Cells

First cultured by Greene and Tischler in 1976, PC-12 cells originated from a pheochromocytoma (neuroendocrine tumor) of the rat adrenal medulla. It was developed as a model cell line and an alternative to adrenal chromaffin primary cell cultures. PC-12 cells are able to differentiate into neuron-like cells in the presence of nerve growth factor or dexamethasone. Due to their differentiation ability and ease of culture, PC-12 cells are used in a variety of research areas ranging from drug efficacy to neurosecretion.

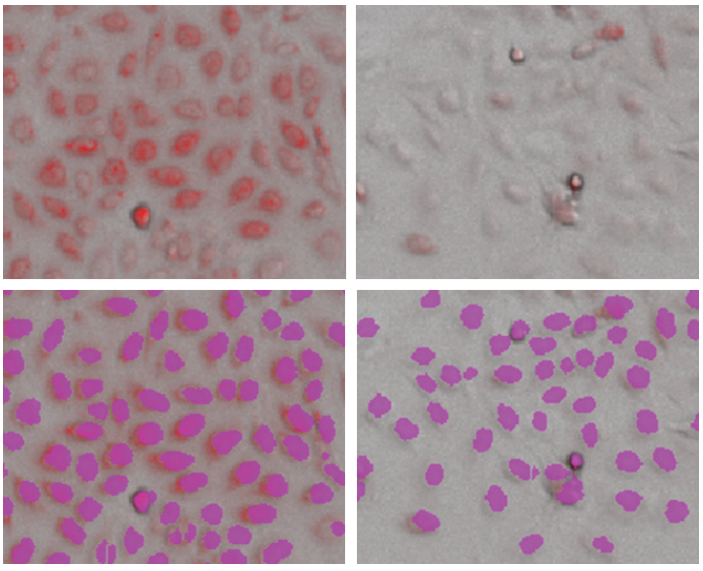
Click here for tips and instrument settings.



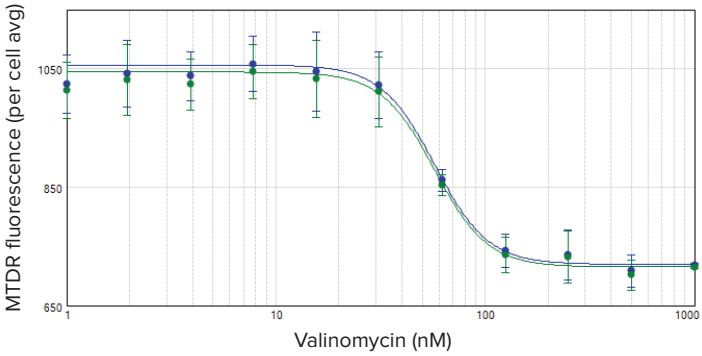
StainFree technology compared to fluorescence cell counting. PC-12 cells counted using StainFree Cell Detection Technology (blue circles) and red nuclear stain (red circles). Counts obtained from both methods agree closely, demonstrating that StainFree technology gives accurate cell counts while eliminating the need for fluorescent dyes ($r^2 > 0.99$ for each plot).



Growth curves obtained using StainFree covered area analysis. Growth curves of PC-12 cells over a ten-day period. Cells were originally seeded at 2000 cells (red), 1000 cells (green), 500 cells (blue), and 200 cells (purple). Cell confluence was measured every 24 hours using the Field Analysis feature in SoftMax Pro Software. The observed dip in confluence for the 2000-cell initial density population at 144 hours (*) is likely due to depletion of nutrients in the culture medium; medium was originally replaced every three days, but from 144 hours onward it was replaced daily.



StainFree analysis of PC-12 cells with mitochondrial staining. Top row shows transmitted light images overlaid with red fluorescent (mitochondrial) images. Bottom row shows StainFree analysis with individual cells (purple masks) identified by the software. Left, untreated cells; right, cells treated with 1 µM valinomycin. For each individual cell identified, the intensity of mitochondrial staining could be calculated.

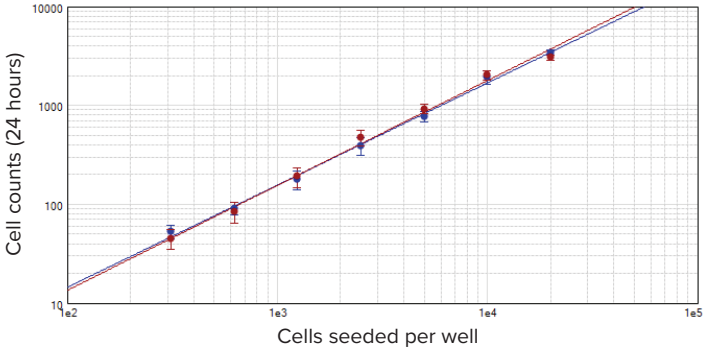


IC_{50} curves for PC-12 cells treated with valinomycin. PC-12 cells were treated with valinomycin and assayed for mitochondrial activity using MitoTracker™ Deep Red FM (MTDR) dye. Concentration-dependent response curves are shown for cells analyzed using StainFree Technology (blue) or fluorescent nuclear staining (green) to identify individual cells. Average mitochondrial fluorescence per cell is graphed vs. concentration of valinomycin. Nearly identical curves were obtained with both analysis methods, and in both cases the IC_{50} value was 57 nM.

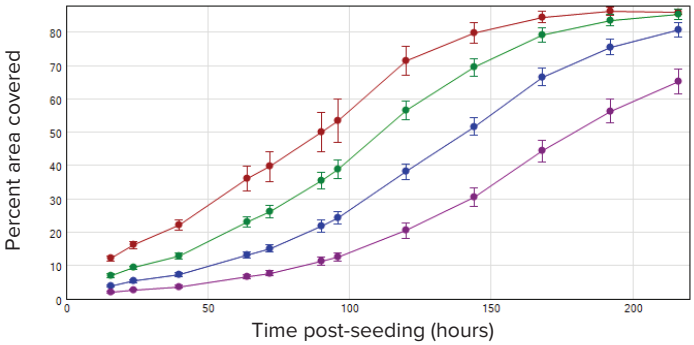
HeLa Cells

HeLa cells were the first cell lines to be grown in the lab and are still used in countless biomedical research projects today. They were derived from cervical cancer cells taken in 1951 from Henrietta Lacks, a patient who later died from the disease. Rebecca Skloot's bestselling book, "The Immortal Life of Henrietta Lacks", tells the fascinating story of her life and legacy.

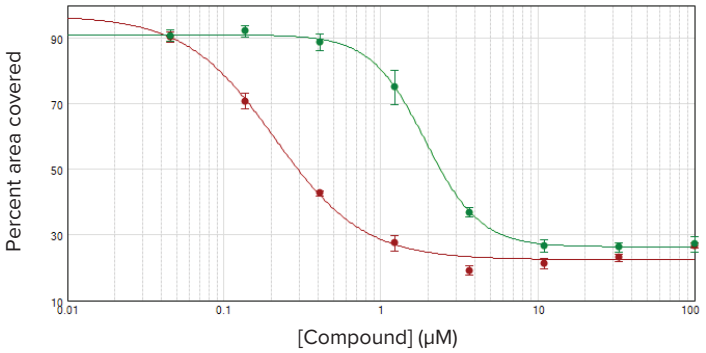
Click here for tips and instrument settings.



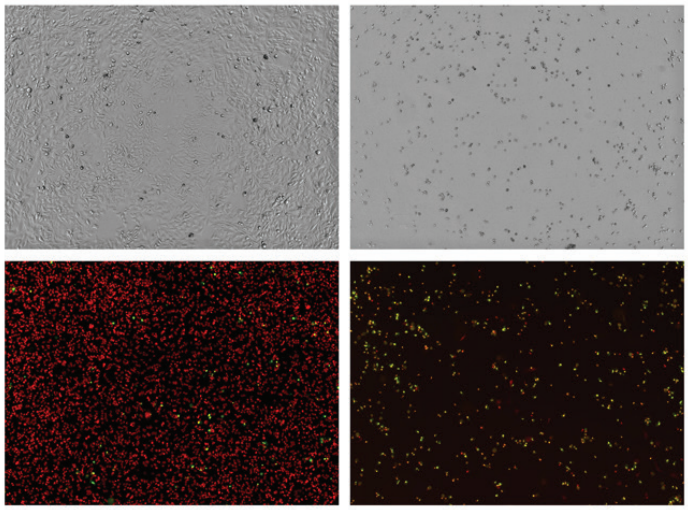
StainFree analysis compared to fluorescence cell counting. HeLa cells counted using StainFree Cell Detection Technology (blue circles) and red nuclear stain (red circles). Counts obtained from both methods agree closely, demonstrating that StainFree technology gives accurate cell counts while eliminating the need for fluorescent dyes (r > 0.99 for each plot).



Growth curves obtained using StainFree covered area analysis. HeLa cells were seeded at 4 initial densities in a 96-well plate: 250 (purple), 500 (blue), 1000 (green), and 2000 (red) cells per well. Cells were imaged with the transmitted light (TL) channel for 9 days. Percent area covered by cells at each time point was determined with StainFree analysis.



Cytotoxicity measured using StainFree technology. HeLa cells were seeded at 1000 cells per well and allowed to grow overnight. Then they were treated with anisomycin (red circles) or trichostatin (green circles) for 72 hours. Cytotoxicity was measured by calculating the percent area covered using StainFree analysis. IC_{50} curves were plotted with SoftMax Pro Software.

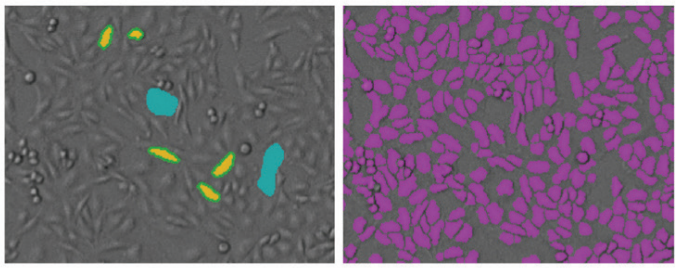


Images of HeLa cells treated with toxic compounds. Top row: HeLa cells were treated with low **(left)** or high **(right)** anisomycin and images with the transmitted light (TL) channel. **Bottom row:** HeLa cells were treated with low **(left)** or high **(right)** concentrations of staurosporine and assayed for cytotoxicity with the EarlyTox™ Cell Integrity Kit. Live cells are labeled red and dead cells are labeled both red and green.

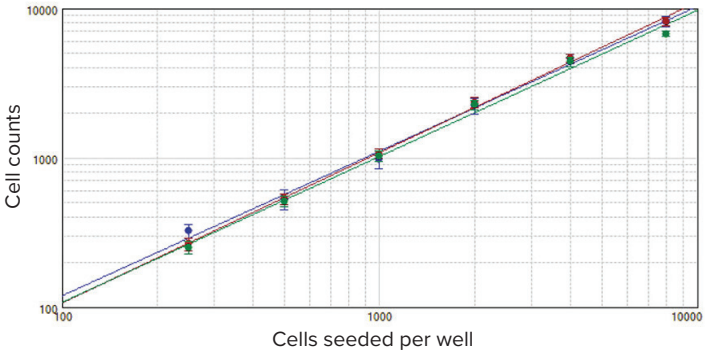
CHO Cells

Chinese Hamster Ovary (CHO) cells are an epithelial-like cell line commonly used in biological and medical research. Developed over 50 years ago, they have been used in innumerable studies ranging from genetics to cytotoxicity. They are highly amenable to transfection and have become the most popular cell line for manufacturing recombinant proteins, including therapeutics.

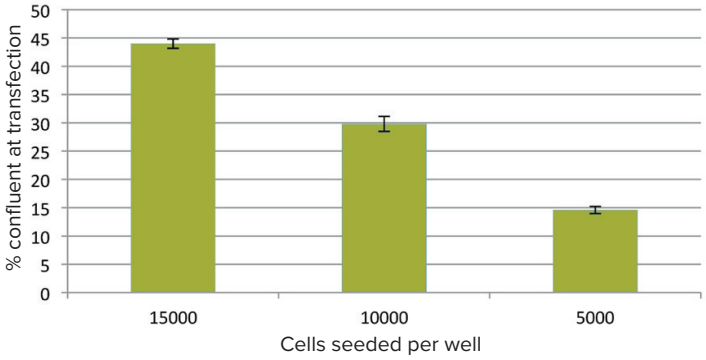
Click here for tips and instrument settings.



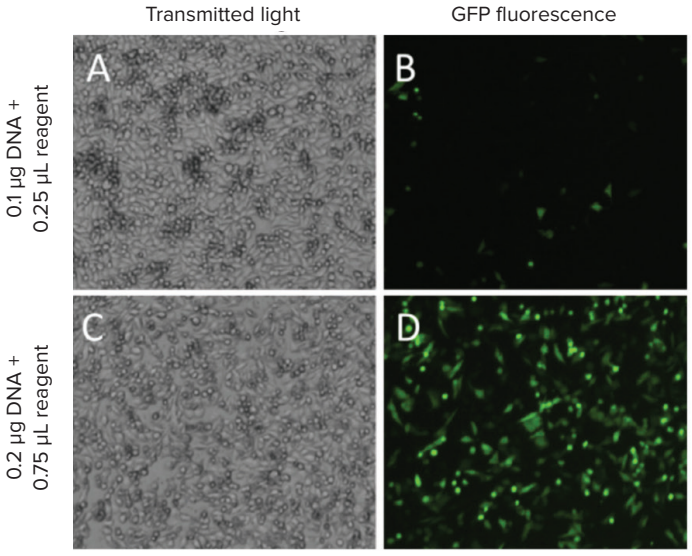
StainFree analysis of CHO cells using SoftMax Pro Software. CHO cells in these images were plated at 4000 cells per well in a 384-well microplate. Left: To create a new StainFree analysis setting, the mouse is used to 'draw' on the image, indicating individual cells (yellow) or non-cellular areas (blue). Right: Purple masks show the objects identified in the image by the software. Alternatively, a predefined image analysis setting can be used for one-click analysis of CHO cell counts.



StainFree technology compared to fluorescence cell counting. Cells counted using StainFree Cell Detection Technology (blue), red nuclear stain (red), and green whole-cell stain (green). Counts obtained from all three methods agree closely, demonstrating that StainFree technology gives accurate cell counts while eliminating the need for fluorescent dyes ($r > 0.99$ for each plot).



Cell confluence. Percent confluence of cells just prior to transfection was determined using StainFree technology. For initial seeding densities of 15000, 10000, and 5000 cells per well, the measured percent confluence after overnight growth was 44, 30, and 15, respectively.



Imaging of GFP-transfected cells. Transfected cells were imaged using the TL **(A, C)** and green fluorescent **(B, D)** channels of the SpectraMax MiniMax 300 Imaging Cytometer. Cells shown were seeded at 15000 cells per well in a 96-well plate. Two transfection conditions are shown, representing low **(B**) and high **(D)** transfection efficiencies. StainFree technology was used to identify individual cells, which could then be classified as positive or negative for GFP expression.

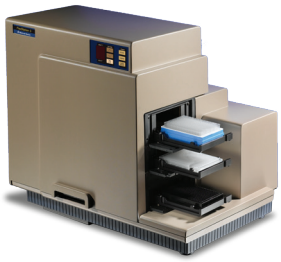
Cell Analysis Platforms & Reagents

For detailed information, select the images or text.

SpectraMax i3x Multi-Mode Microplate Reader with SpectraMax MiniMax 300 Imaging Cytometer

The SpectraMax i3x reader with the imaging cytometer enables quick imaging and analysis of cells and gives you a front-row view of phenotypic changes that accompany cytotoxicity, cell proliferation, and protein expression.

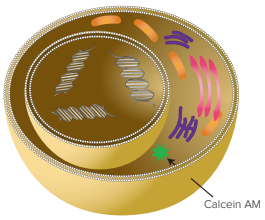
View Video | **Request Quote**



FlexStation 3 Multi-Mode Microplate Reader

The FlexStation 3 reader measures absorbance, fluorescence intensity, fluorescence polarization, luminescence, and time-resolved fluorescence. The reader integrates multi-mode microplate optics with precision, programmable liquid handling for perfectly timed, automated biochemical and cell-based assays.

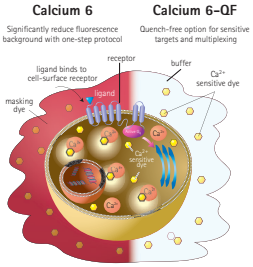
View Video | **Request Quote**



EarlyTox Cell Viability Assay Kits

The EarlyTox™ cell viability assay kits are a family of fluorescence-based reagents for the assessment of cell viability, cell proliferation, and various apoptosis events using mammalian cells. Optimized for use with microplate readers, these assay kits employ a no-wash, homogeneous assay protocol that enables characterization of a full concentration-response profile of test compounds.

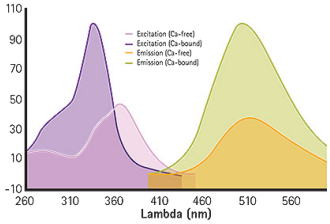
Download Data Sheet



FLIPR Calcium 6 Assay Kit

The FLIPR Calcium 6 Assay Kit measures changes in intracellular calcium during drug discovery and research. They deliver pre-optimized, homogeneous, fluorescence-based formulations to expedite assay development and screening of GPCR and ion channel targets.

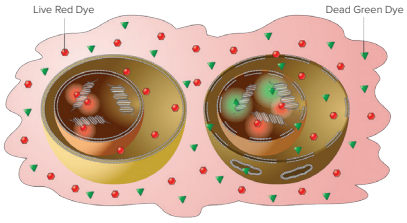
Download Data Sheet



Fura-2 QBT Calcium Kit

The Fura-2 QBT Calcium Kit is a simple, mix-and-read format that employs our proprietary masking technology with the industry-standard Fura-2 ratiometric calcium indicator. Streamline your current Fura-2 assay or utilize this no-wash reagent for calcium concentration determination or use with GFP-tagged proteins.

Download Data Sheet



EarlyTox Cell Integrity Kit

The EarlyTox Cell Integrity Kit offers a convenient way to determine live and dead cell populations by fluorescence imaging. Cytotoxicity mediated through a variety of cellular mechanisms can easily be monitored and quantified.

Download Application Note

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