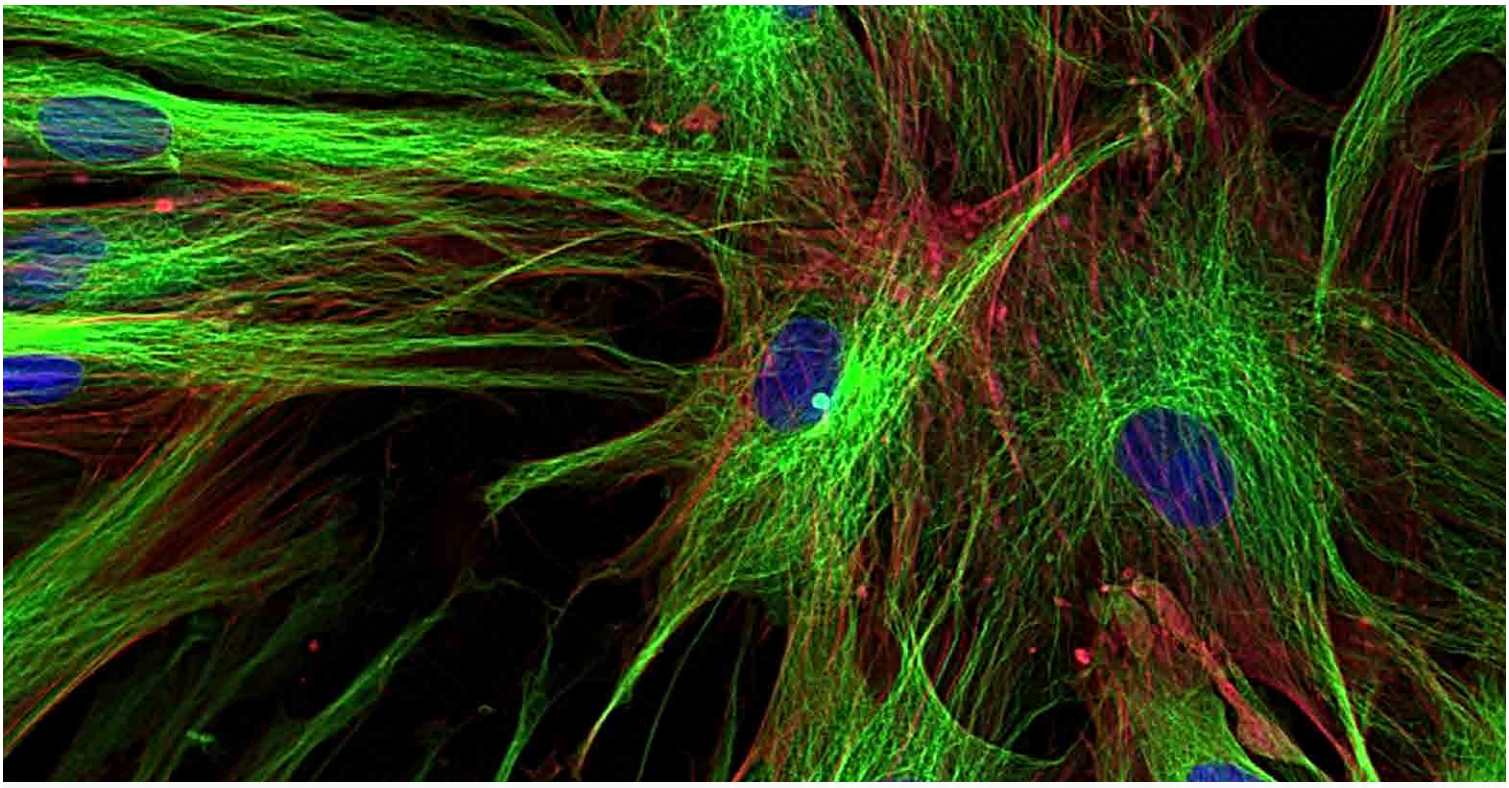
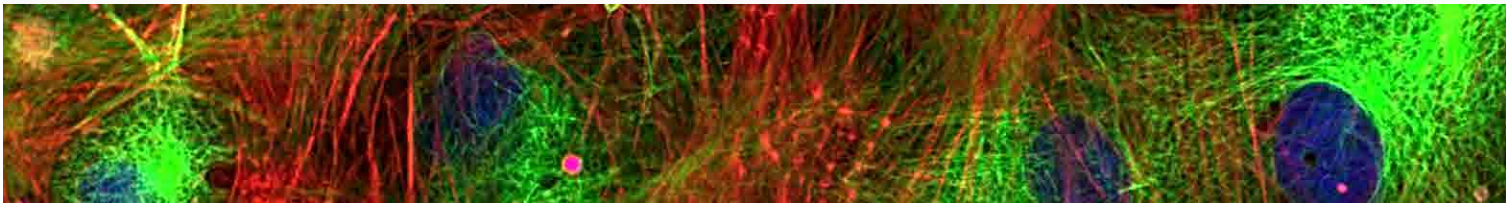


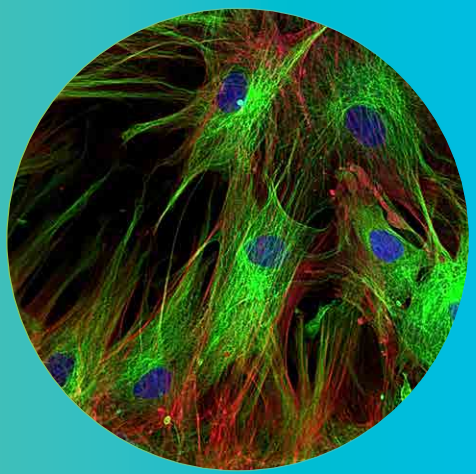


Focus: Cellular Analysis

Cellular Analysis refers to a broad range of technologies that are used to investigate the basic cell biology as it relates to cell health, proliferation, function, and death.

For more information on products that fit each step in this workflow, visit **vwr.com/cellular-analysis.**

2020



Phenotypic cell-based assays with a new automated imaging system and cell analysis software

By Oksana Sirenko, PhD; Felix Spira, Matthew Hammer, Jayne Hesley, and Kayla Hill,
Molecular Devices LLC, San Jose, California, USA

AUTOMATED CELL-BASED ASSAYS

There is a great need to automate complex cell-based assays with multi-parametric readouts while maintaining high data quality and precision. Highly predictive assays with biologically relevant and complex cell models necessitate the use of robust image acquisition and analysis platforms for the phenotypic characterization of cellular and subcellular responses. This study uses a compact, automated imaging system to develop several multi-parametric assays utilizing iPSC-derived cardiomyocytes, neurons, and hepatocytes. The ability to analyze multiple readouts enabled the monitoring of multiple cellular phenotypes and biological processes including cell viability, apoptosis, mitochondria membrane potential, autophagy for defining mechanisms of toxicity in live or fixed cells, and examples of label–free cell analysis for evaluation of cytotoxicity and cell proliferation. Here, we present several assay models that will be useful for both academic and biopharma environments. We also demonstrate the utility of a new, automated imaging system for the expansion of biological research into both standard and complex assays for the generation of more relevant data in addition to scientific breakthroughs.

INSTRUMENTATION: AUTOMATED IMAGER AND IMAGE ANALYSIS SOFTWARE

Cell-based assays were performed using the ImageXpress® Pico Automated Cell Imaging System in combination with the CellReporterXpress® Automated Imaging Acquisition and Analysis Software. The imager provides four fluorescence channels plus transmitted light and colorimetric assays with a variety of magnifications, environmental control (EC), injectors, and time-lapse capacity to enable automatic monitoring of cell proliferation, differentiation, compound toxicity, and a variety of other cell-based assays. The analysis software uses novel algorithms for object recognition that simplify the workflow and analysis and provide multi-parametric readouts.

METHODS

Cell Culture: Human iPSC-derived neurons, cardiomyocytes, or hepatocytes and the appropriate media were purchased from Cellular Dynamics International, Fujifilm Co (CDI). Cells were plated into 384-well black clear bottom plates (Greiner) at a

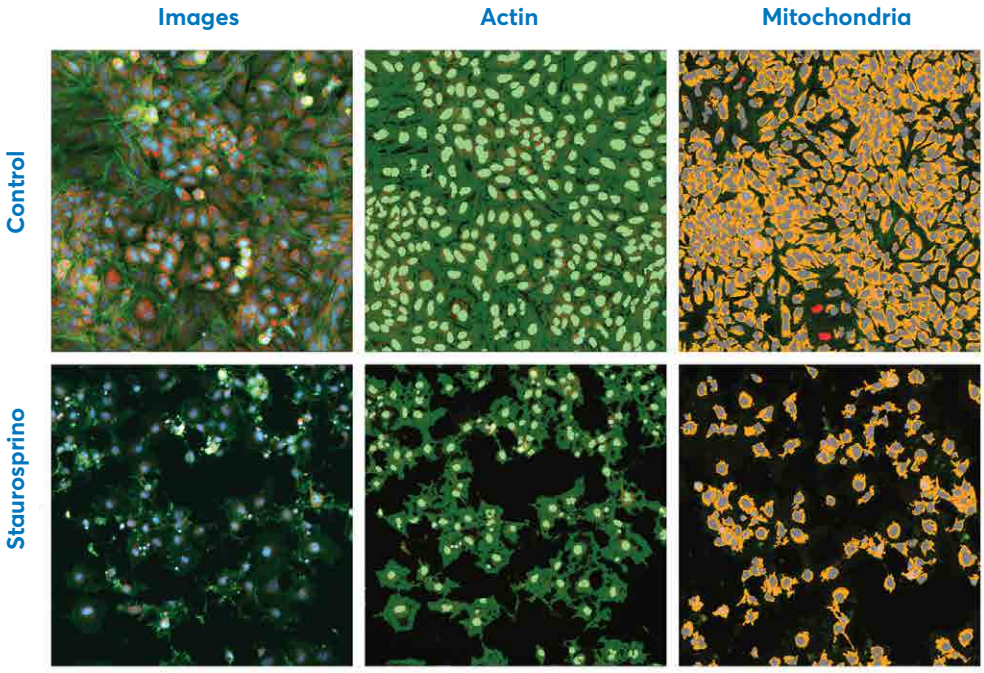


Figure 1. Images and the analysis masks for multi-parametric analysis. HeLa cells were treated for 72 hours, then stained with a nuclear stain (Hoechst 33342), actin cytoskeleton stain (AlexaFluor® 488 (AF488) labeled phalloidin), and MitoTracker Orange CMTMRos. Images and analysis masks compared for control cells and cells treated with 0.1µM staurosporine. Cells were imaged with the DAPI, FITC, and TRITC using a 10x Plan Fluor objective. The images (left) show nuclei (blue), actin cytoskeleton (1:100, green), mitochondria (orange). Images were analyzed using the Cell Scoring analysis modules optimized for the quantitation of phalloidin positive cells (middle) and MitoTracker Orange positive cells (right). The analysis masks: light green - positive nuclei, red - negative nuclei, green - actin cytoskeleton, orange - intact mitochondria.

EC_{50} (µM) ± Standard Deviation					
Analysis Readouts	**Staurosporine**	**Mitomycin C**	**Paclitaxel**	**Etoposide**	**Doxorubicin**
Number of actin positive cells	0.012 ± 0.001	0.078 ± 0.140	$5.46 \times 10^{-4} \pm 3.48 \times 10^{-5}$	0.820 ± 0.340	0.020 ± 0.012
Actin positive cell total area	0.067 ± 0.009	4.952 ± 0.354	$8.56 \times 10^{-4} \pm 5.69 \times 10^{-5}$	13.54 ± 1.229	0.649 ± 0.054
Actin positive cell total integrated intensity	0.038 ± 0.005	3.614 ± 0.378	$6.33 \times 10^{-4} \pm 4.21 \times 10^{-5}$	10.10 ± 1.301	0.501 ± 0.078
Number of cells with intact mitochondria	0.013 ± 0.001	0.110 ± 0.045	$4.50 \times 10^{-4} \pm 2.64 \times 10^{-5}$	0.543 ± 0.190	0.014 ± 0.006
Mitochondria positive cell total integrated intensity	0.017 ± 0.002	1.691 ± 0.222	$4.18 \times 10^{-4} \pm 2.98 \times 10^{-5}$	6.336 ± 1.086	0.197 ± 0.053

density of 10,000 cells per well and cultured as recommended by protocols from CDI. HeLa or PC12 cell lines (ATCC) were cultured according to manufacturer's recommendations. Treatment with compounds was performed 24 hours post plating; cultures were exposed to treatment for three days, or as indicated in the figures.

Cell Staining: To assess phenotypic changes, cells were stained live using a mixture of three dyes: the viability dye Calcein AM (1mM), the mitochondria potential dye MitoTracker Orange (0.2mM), and the Nuclear dye Hoechst (2 mM). For visualizing the actin cytoskeleton, cells were fixed with 4% formaldehyde and stained with AlexaFluor® 488 (AF488) labeled phalloidin stain. Neurons were stained with anti-TuJ-1 antibodies (BD Biosciences).

ASSESSMENT OF CELL MORPHOLOGY AND VIABILITY

Imaging and analysis methods provide efficient tools for characterization of multiple readouts including cell viability, characterization of cell shape, cell adhesion and spreading, cytoskeleton integrity (morphology), and mitochondria potential.

RESULTS

Compound-specific effects on neurite outgrowth

Phenotypic readouts for neurite outgrowth (iCell Gaba Neurons, CDI) included quantitative characterization of the extent and complexity of neural networks by multiplexed measurements. We have characterized multiple measurements and tested several known neurotoxic compounds. Neurite outgrowth was characterized by the extent of the outgrowth (length of total outgrowth or mean outgrowth per cell), the number of neurite processes (total number of processes), and the extent of branching (total number of branches and mean number of branches per cell) (See Fig. 2, top right).

Phenotypic changes in hepatocytes

iPSC-derived human hepatocytes present a valuable cellular model that can closely resemble the phenotypes and functionality of primary hepatocytes, while minimizing variability and other limitations of primary cells. Automated

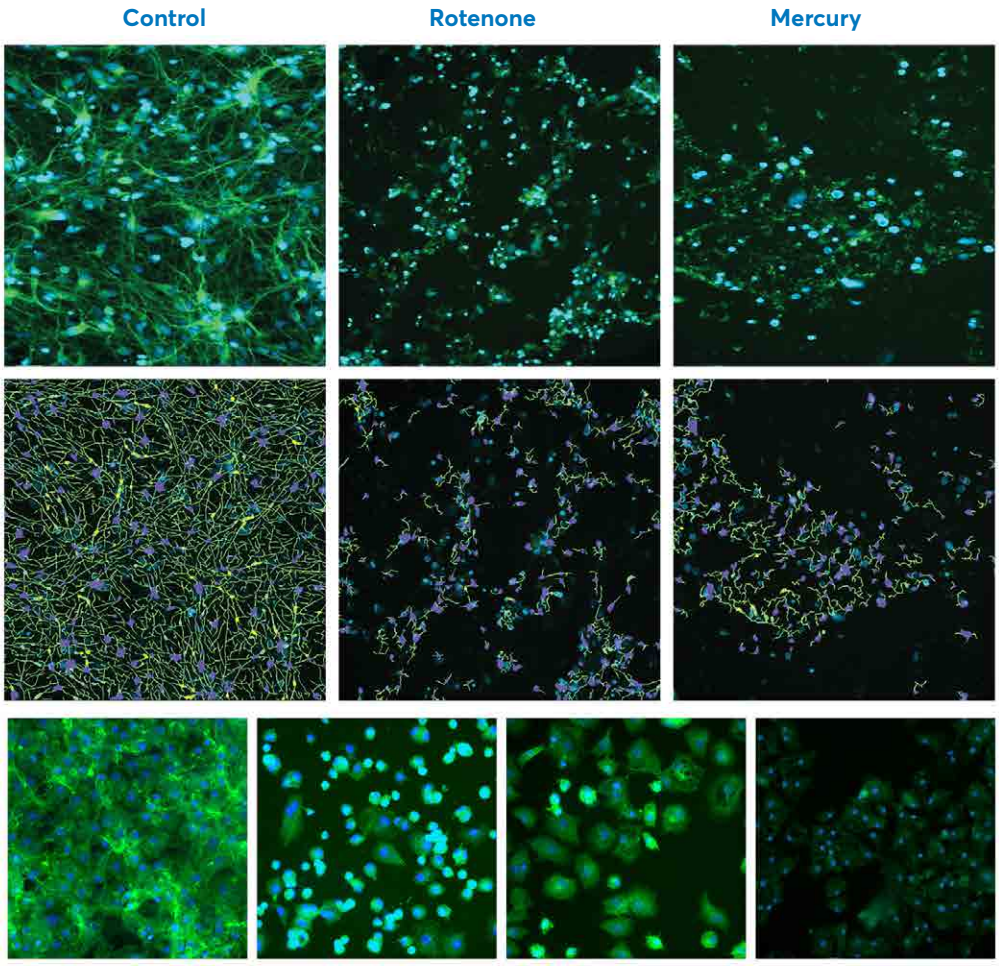
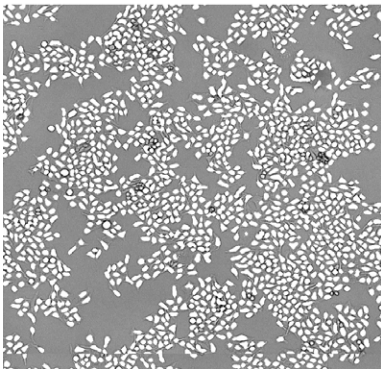


Figure 2. Images of β-tubulin (green) stain and the software analysis traces shown for the control and compound treated cells. iCell Neurons were treated with compounds for 3 days, then were fixed and stained with AF488-conjugated anti-β-tubulin (TUJ-1) antibodies (1:100, BD Biosciences). Images were taken by the ImageXpress Pico system, 10x Plan Fluor objective and FITC and DAPI channels. Images were processed using the Neurite Tracing analysis algorithm. Analysis masks on the right show the outgrowth (green), as well as cell bodies (blue), and branching points (pink). Disruption of neurite networks was measured for neurons treated with 1μM of indicated compounds. EC50s defined by decrease in total outgrowth were 6.1μM for rotenone and 0.07μM for methyl mercury; EC50s defined by decreased numbers of branches were 2.8μM for rotenone and 0.071μM for methyl mercury.

Figure 3. Assessment of changes in hepatocyte morphology after treatment with hepatotoxic compounds. Hepatocytes were plated and treated with compounds for 72h, stained with a combination of Hoechst (2μM), MitoTracker Orange (0.2μM), and then fixed and stained with AF488- conjugated Phalloidin (1:100). Images were taken by ImageXpress Pico system, using a 10x Plan Fluor objective, and DAPI, TRITC, and FITC channels. Images were processed using the Cell Scoring analysis algorithm. Composite images of actin, nuclei, and are shown for the control and compound treated cells. Disruption of the cytoskeleton, variations in cell spreading, and cell death were observed for hepatocytes treated with 30μM of indicated compounds.



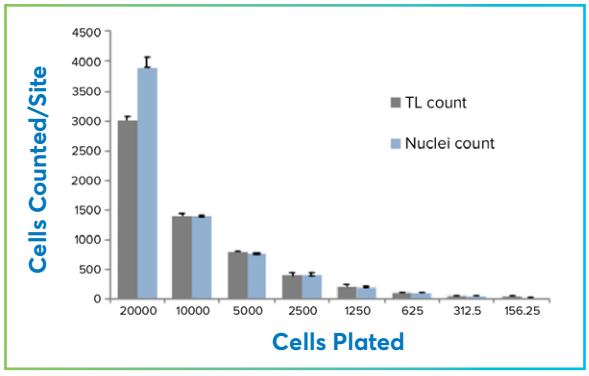


Figure 4. HeLa cell images in transmitted light (10x objective, 2ms exposure) with white analysis masks (left), and a comparison of cell quantitation using transmitted light segmentation vs. fluorescent nuclear count (right). CellReporterXpress software contains a preconfigured application module for quantifying cell count in transmitted light.

imaging and analysis can be used for evaluation of hepatotoxic effects of compounds (See Fig. 3).

Counting cells using tansmitted light and apoptosis assay

The analysis software allows image segmentation and cell counting without using dyes. This application is especially useful for monitoring cell proliferation and cell death over time. In addition, we describe an assay for apoptosis detection using a fluorescent marker (See Fig. 4).

Apoptosis: Cells treated with staurosporine stained with Caspase-3/7 NucView dye

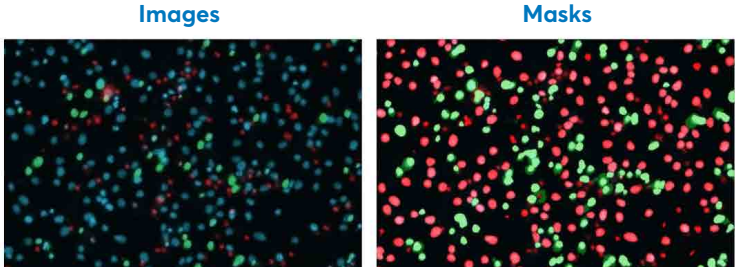


Figure 5. Images of HeLa cells treated with 0.3µM of staurosporine. Cells were stained for 30 min with EarlyTox Caspase-3/7 NucView 488 dye (Molecular Devices), Ethidium Homodimer III, and Hoechst 33342 nuclear dye. Live cells were imaged using the 10x objective. The Hoechst nuclear stain is shown in blue, apoptotic nuclei indicated by green, and nuclei of dead cells labeled in red. Apoptosis protocol was performed for the analysis. Masks of apoptotic cells indicated in green, live cells in red.

SUMMARY

In this article, we presented several examples of multi-parametric assays utilizing different cell types, including neurons, hepatocytes, and HeLa cells. We established that multiple readouts allowed better discrimination between different cell phenotypes and compound effects. This study demonstrates the breadth of imaging assays possible using the ImageXpress Pico system and CellReporterXpress software for evaluation of various biological effects. Furthermore, we demonstrated that the ability to generate multi-parametric readouts from the pre-configured analysis modules in CellReporterXpress provides deeper insight into the critical pathways being studied. The ImageXpress Pico offers a solution to enhance assay throughput, while providing a multitude of measurements to elucidate more relevant information from assays.

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White Cap	3–8 mL	12 mL	Sterile	50/Box	**89048-934**
White Cap	3–8 mL	12 mL	Sterile	50/Box; Case of 10 Boxes	**89048-932**
White Cap, Prefilled	3–8 mL	12 mL	Sterile	50/Box; Case of 10 Boxes	**89218-662**
50 mL Leucosep™ Tubes					
Blue Cap	15–30 mL	50 mL	Sterile	25/Box	**89048-938**
Blue Cap	15–30 mL	50 mL	Sterile	25/Box; Case of 12 Boxes	**89048-936**
Blue Cap, Prefilled	15–30 mL	50 mL	Sterile	25/Box; Case of 12 Boxes	**89136-192**

Pioneering imaging with innovation:

New Leica Microsystems THUNDER Imagers

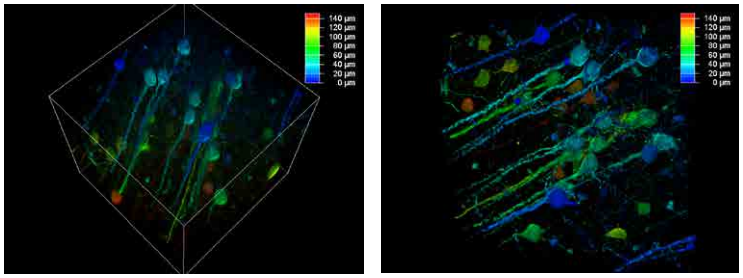
By Jan Schumacher, Louise Bertrand – Leica Microsystems

Life science research is seeing a massive shift away from traditional 2D cell culture to modern 3D cell culture models (e.g., spheroids or organoids) because it is more physiologically relevant. Similarly, with other types of specimens such as tissue sections or model organisms, there's a continuing trend for more extensive 3D imaging, as seeing biology in its context often is the key to breakthrough insights.

The challenge for researchers imaging these thicker samples is the image background (BG), mainly originating from out-of-focus regions of the observed sample, significantly reduces the contrast, the effective dynamic range, and the maximal possible signal-to-noise ratio of the imaging system. The recorded images show a typical haze and, in many cases, do not provide the level of detail required for further downstream analysis. Therefore, those working with thick 3D samples either use alternative microscopy methods or can try to reduce the haze by post-processing a series of images. The gold standard in removing out-of-focus BG are pinhole-based scanning systems, where the pinhole of a confocal system excludes light from out-of-focus layers, so only light from the in-focus layer reaches the detector.

THUNDER Imagers mark a new class of instruments for high-speed, high-quality imaging of single cells, tissues, whole organisms, or other thick, 3-dimensional specimens. THUNDER Imagers take advantage of Leica's proprietary opto-digital solution, Computational Clearing, as their core technology to generate high resolution and high contrast images using traditional widefield microscopes which efficiently differentiates and eliminates the BG from the signal. Computational Clearing removes the typical out-of-focus blur associated with widefield imaging in real-time, clearly revealing focal planes deep within the sample enabling the meaningful use of 3D specimens. The data generated from Leica's THUNDER Imager systems is quantifiable, allowing imaging deep within a sample resulting in improvements in image resolution.

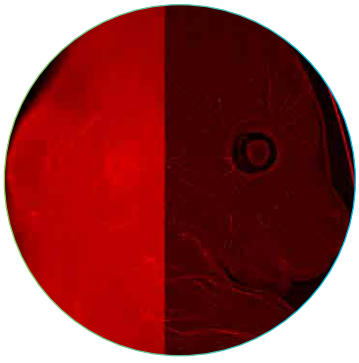
The maximum depth that can be imaged is highly sample dependent; density of fluorophores, absorption, or homogeneity of local refractive indices within the sample directly affect the amount of scattered light within the sample. Most high-magnification widefield imaging experiments utilize tissue sections that are a maximum depth of 50µm, as it is believed that no more information can be retrieved beyond this thickness. Computational Clearing enables deep imaging (up to 150µm) by removing the scattered light component in live specimens so imaging can be done under physiological conditions. THUNDER Imager experiments conducted with live samples can, of course, be used for fixed samples as well.



THUNDER Imagers improve upon widefield sensitivity and resolution, while providing easily understood image processing, speed, and automation for complex experiments to streamline your research workflow.

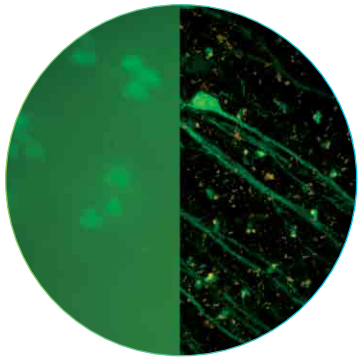
THUNDER Imagers are designed to meet tomorrow's demanding applications:

Model Organisms



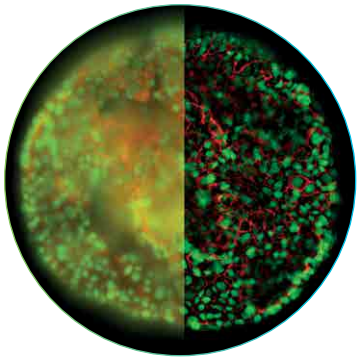
In this E12-14 mouse (wt sample), neurofilaments are stained in red to assess neuronal outgrowth. The mouse was cleared with the ScaleS reagent. Sample courtesy Yves Lutz, Centre d'imagerie, IGBMC (France).

Tissue Sections



YFP mouse brain slices stained with GFAP-A647. Imaged with a THUNDER Imager Tissue. Courtesy Dr. Hong Xu, University of Pennsylvania, Philadelphia (USA).

3D Cell Culture



HeLa cell spheroid stained with Alexa Fluor 568 Phalloidin (Actin) and YOYO 1 iodide (Nucleus).

DO YOU WANT TO LEARN MORE?

Are you looking for an easy-to-use 3D imaging solution that offers high speed, amazing image quality and stunning workflow efficiency? Take a deeper look at the THUNDER technology in the full-length technical note on vwr.com/leica and get in touch with your VWR Life Science Specialist to discuss how you can take your imaging to the next level with THUNDER.

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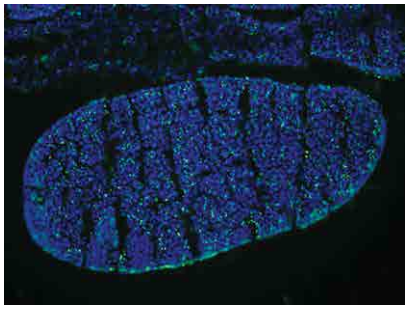

Description	Antigen	Size	Cat. No.
Rabbit Host			
Anti-Collagen Type I Antibody	COL1A1	100 µL	**RL600-401-103.01***
Anti-Osteopontin Antibody	SPP1	200 µL	**RL100-401-404**
Anti-MCL-1 Antibody	MCL1	100 µL	**RL600-401-394**
Anti-SMT3 Antibody	SMT3	500 µg	**RL200-401-428**
Anti-PPARA Antibody	PPARA	100 µL	**RL600-401-421**

Description	Antigen	Size	Cat. No.
Anti-ACTB Antibody	ACTB	200 µg	**RL600-401-886**
Anti-TUBA1B Antibody	TUBA1B	200 µg	**RL600-401-880**
Mouse Host			
Anti-6xHis Antibody [clone: 33D1.D2.G8]	6xHis	100 µg	**RL200-301-382**
Anti-BrdU Antibody [clone 29G6.E8]	BrdU	100 µg	**10802-262***

* These products are not currently available in Canada. Please contact your VWR Life Science Specialist to find a similar product available in your region.

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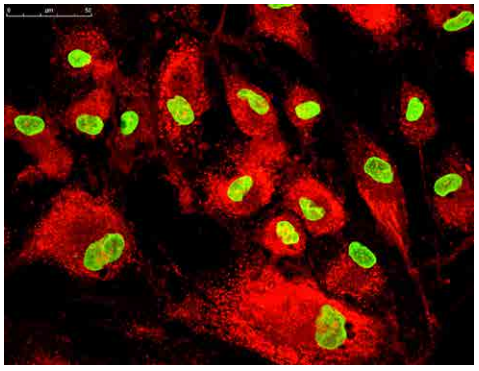

The Bacteria Live/Dead Staining Kit enables a two-color fluorescent staining on both live bacteria (green) and dead bacteria (red) using the two nucleic acid dyes DMAO and EthD-III. DMAO is a green-fluorescent dye which stains both live and dead bacteria with intact and damaged cell membranes while the red-fluorescing EthD-III only stains dead bacteria with damaged cell membranes. With an appropriate mixture of both dyes, viable bacteria with intact cell membranes are stained green, whereas bacteria with damaged cell membranes fluoresce red. The kit is applicable to most bacteria types for viability and cytotoxicity studies, and suitable for fluorescence microscopy and flow cytometry.

Description	Size	Cat. No.
Bacteria Live/Dead Staining Kit	100-1000 assays	**10180-608**

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1X Solution Composition: 135 mM NaCl, 2.7 mM KCl, 11 mM phosphate buffer. Does not contain magnesium or calcium.

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1 L	97062-948

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Heat Shock Isolation

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10 g	Heat Shock Isolation	97063-138

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Grade	30% Solution
Protein Synonyms	BSA, Fraction V, Cohn Fraction V, Bovine Serum Albumin
Protein/Peptide Name	Albumin
Source	Serum
Species	Bovine

Size	Cat. No.
50 mL	97063-626
50 mL	97063-630
500 mL	97063-624

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Description	Sensitivity	Range	Cat. No.
Human IL-6	<0.3 pg/mL	4.69 pg/mL–300 pg/mL	76175-452
Human IL-10	<0.5 pg/mL	7.8 pg/mL–500 pg/mL	76175-454
Human TNF-α	<1 pg/mL	15.6 pg/mL–1,000 pg/mL	76175-416
Murine IL-6	<1 pg/mL	15.6 pg/mL–1,000 pg/mL	76175-426
Murine TNF-α	<1 pg/mL	15.6 pg/mL–1,000 pg/mL	76175-436

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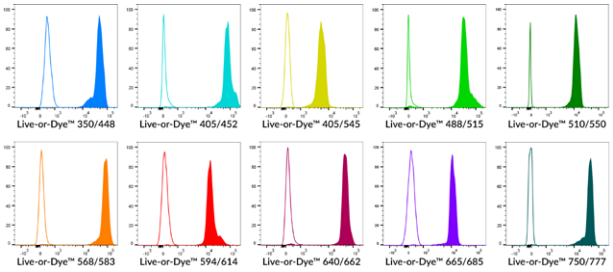

Description	Excite/Emit, nm	Size	Cat. No.
Live-or-Dye™ 350/448	350/448	200 Assays	**10018-168**
Live-or-Dye™ 405/505	405/505	200 Assays	**10018-182**
Live-or-Dye™ 405/452	405/452	200 Assays	**10018-170**
Live-or-Dye™ 488/515	488/515	200 Assays	**10018-172**

Description	Excite/Emit, nm	Size	Cat. No.
Live-or-Dye™ 568/583	568/583	200 Assays	**10018-174**
Live-or-Dye™ 594/614	594/614	200 Assays	**10018-176**
Live-or-Dye™ 640/662	40/662	200 Assays	**10018-178**
Live-or-Dye™ 750/777	750/777	200 Assays	**10018-180**

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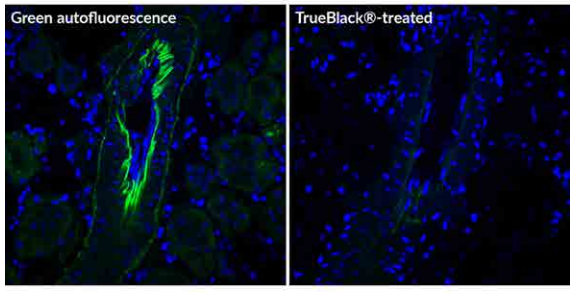
- Clears the way for fluorescence imaging of human and aged animal tissues



Autofluorescence interferes with immunofluorescence staining. TrueBlack developed by Biotium is a superior alternative to traditionally used Sudan Black B. It effectively eliminates lipofuscin autofluorescence from neuronal tissues like the brain and retina; it can also reduce non-lipofuscin autofluorescence from RBCs, extracellular matrix components etc. Treatment is simple, rapid, and has minimal effect on other fluorescent markers.

Description	Size	Cat. No.
TrueBlack Lipofuscin Autofluorescence Quencher, 20X in DMF	1 mL	**10119-144**

CORNING

Demanding research needs all the support it can get

TRANSWELL® INSERTS, CORNING®

VARIETY OF SURFACE TREATMENTS FOR A DIVERSE ARRAY OF APPLICATIONS

Transwell® inserts are suitable for a variety of applications such as angiogenesis, co-culture, epithelial cell polarity, migration, invasion, tissue engineering, toxicity testing, and transport and permeability studies. Inserts provide independent access to both apical and basolateral plasma membranes of the cell monolayer.

No. of Wells	Insert Size	Pore Size	Well Area	Packaging	Cat. No.
Polyester Tissue Culture-Treated Inserts					
6	24 mm	0.4 µm	4.67 cm^2	6/Plate	**29442-074**
6	24 mm	3.0 µm	4.67 cm^2	6/Plate	**29442-076**
12	12 mm	0.4 µm	1.12 cm^2	12/Plate	**29442-078**
12	12 mm	3 µm	1.12 cm^2	12/Plate	**29442-080**
24	6.5 mm*	0.4 µm	0.33 cm^2	12/Plate, 4 Plates	**29442-082**
24	6.5 mm	3 µm	0.33 cm^2	12/Plate, 4 Plates	**29442-084**
Polycarbonate Tissue Culture-Treated Inserts					
6	24 mm	0.4 µm	4.67 cm^2	6/Plate	**29442-104**
12	12 mm	0.4 µm	1.12 cm^2	12/Plate	**29442-086**
12	12 mm	3 µm	1.12 cm^2	12/Plate	**29442-088**
24	6.5 mm	8.0 µm	0.3 cm^2	12/Plate, 4 Plates	**29442-120**

*6.5 mm membrane diameter are packaged 12 inserts in a 24-well plate, 4 plates per case.

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Ultra-Low Attachment (ULA) surface enables 3D spheroid formation

CORNING® ELPLASIA® MICROCAVITY PLATES, CORNING®

SIMPLE 'PLUG AND PLAY' PROTOCOL FOR SCAFFOLD-FREE, SELF-ASSEMBLY SPHEROID FORMATION AT LARGE VOLUMES

- Spheroids can be formed and cultured for 21 or more days in the same plate
- Can produce between 79 to 15000+ spheroids per well, depending on plate format, under one culture condition
- Black opaque sidewalls to reduce well-to-well 'cross-talk'
- Well suited for fluorescent/luminescent assays
- Available in multiple formats, two well geometries and surface coating options

US ONLY

Description	Coating	Well Diameter	Cat. No.
Corning® Elplasia® 6-Well Round Bottom Micro-Cavity Plate	ULA	500 µm	**76337-210**
Corning® Elplasia® 24-Well Round Bottom Micro-Cavity Plate	ULA	500 µm	**76337-212**
Corning® Elplasia® 96-Well Round Bottom Micro-Cavity Plate	ULA	500 µm	**76337-214**
Corning® Elplasia® 6-Well Square Bottom Micro-Cavity Plate	Plasma Treated	200 µm	**76337-216**
Corning® Elplasia® 24-Well Square Bottom Micro-Cavity Plate	Plasma Treated	200 µm	**76337-218**
Corning® Elplasia® 96-Well Square Bottom Micro-Cavity Plate	Plasma Treated	200 µm	**76337-220**

*These products are not yet available in Canada. Please contact your VWR Life Science Specialist for information about when these will be available or for similar products currently available in your region.

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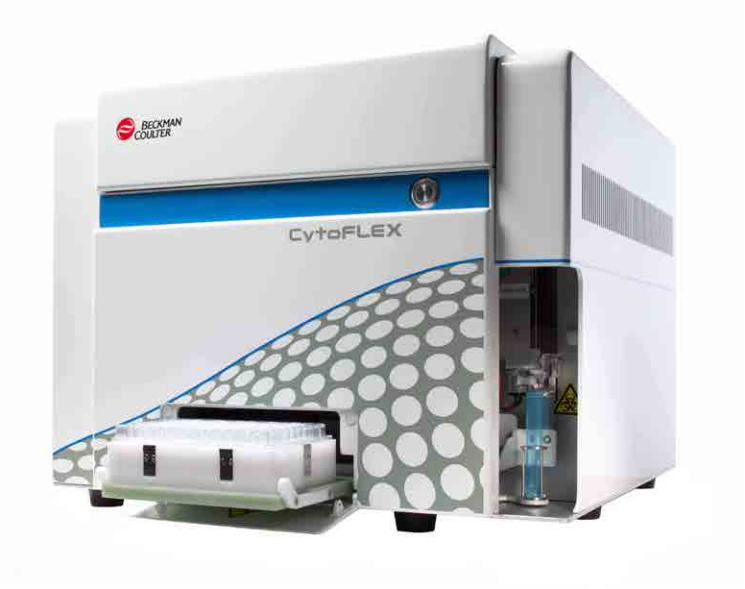

CYTOFLEX FLOW CYTOMETER, BECKMAN COULTER

DESIGNED TO DELIVER SUPERIOR PERFORMANCE WITH EASE OF INSTALLATION AND OPERATION FOR RESEARCH APPLICATIONS

Simplified system setup, data acquisition, analysis, and export of experimental results are integrated into a complete workflow solution with CytExpert software.

The CytoFLEX includes 13 band pass filters which can be repositioned as needed, and it is available with different configurations to provide the ultimate in application flexibility, including optional 96-well Plate Loader. Activate the number of channels needed initially and add channels later as research needs grow.

Dimensions	16.7 W x 13.4 D x 14" H
Operating System Compatibility	Windows® 10 Professional 64-bit
Operating Temperature	59 – 80.6 °F, non-condensing
Power	150 – 250W
Sensitivity	FITC <30 MESF; PE <10 MESF
Voltage	100 – 240V
Weight	51.6 lbs. (without Plate Loader), 61.7 lbs. (with Plate Loader)

Description	Cat. No.
CytoFLEX System B4-RO-VO	**76330-530**
CytoFLEX System B3-R1-VO	**76330-090**
CytoFLEX System B2-RO-V2	**76330-092**
CytoFLEX System B2-R2-VO	**76330-094**

Description	Cat. No.
Accessories	
CytoFLEX* Sheath Fluid	**76183-428**
Sheath Filter	**76183-334**
CytoFLEX Sheath Sensor	**76183-340**
Sheath Bottle Only	**76183-436**

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