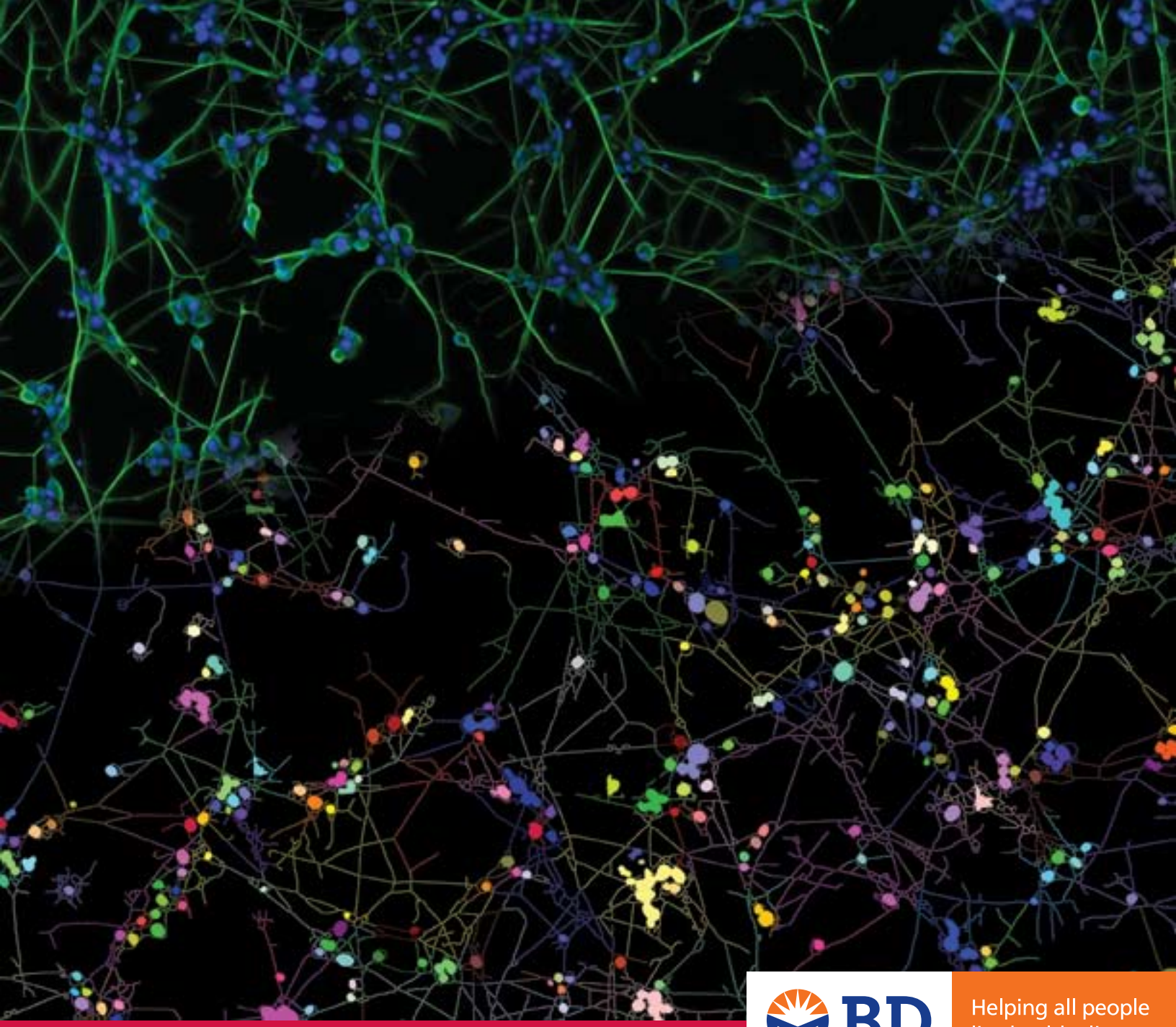

BD

Helping all people live healthy lives

High Content Cellular Analysis

Hardware | Software | Reagents | Labware

Table of Contents

Introduction

Flow cytometry and imaging provide comprehensive cell analysis

Automated high-content imaging technologies are becoming increasingly popular tools within the cell analysis laboratory.

Flow cytometry technology is a tool of choice for the life scientist seeking quantitative analysis of cell populations. Flow cytometry yields high cell throughput and multiplexible fluorescence intensity measurements, advantages unmatched by other analytical technologies. With added cell sorting (FACS) capability, researchers can collect subpopulations of interest for expansion and further analysis, enhancing "flow's" value as a cell biology research tool. Common applications of flow cytometry include population analysis using biomarkers, phosphorylation profiling of signaling pathways, and multiplexed bead arrays.

Imaging is an ideal companion technology to flow cytometry. In addition to multiplexed fluorescence intensity measurements of cell populations, imaging provides the ability to measure cellular features such as size, shape, and movement. Since cells can be visualized without removing them from their culture environment, they can be analyzed over time to provide real-time information about cellular responses to stimuli. Coupled with 3-dimensional confocal techniques, live-cell imaging can provide a level of biological detail not possible using conventional analytical tools.

In combination, flow cytometry technology and high-content imaging provide a comprehensive cell analysis solution.

Imaging complements flow cytometry and FACS

Flow Cytometry	Imaging
Provides statistical analysis of cell populations via fluorescence intensity	Measures fluorescence intensity, localization, cell motility, and morphology
Provides single-cell resolution using PMT detection technologies	Provides subcellular resolution using CCD camera technologies
Rapid analysis of thousands to hundreds of thousands of cells	Rapid analysis of one to thousands of cells
Cells must be prepared in suspension. Also compatible with multiplexed bead arrays	Is ideally suited for adherent cells and tissues
Multiplexible (12+ colors)	Multiplexible (4+ colors)
Cells are normally discarded but can be collected following sorting	Cells can be revisited and monitored over time
Key strengths: • Cell preparation (sorting) • Statistical population analysis • Low abundance events	**Key strengths:** • Morphological measurements • Spatial analysis • Images can be reanalyzed • Visual data

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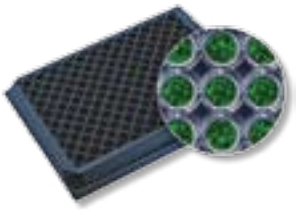
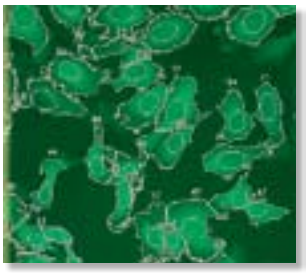
What is high-content cell analysis?

Cells are plated into multiwell plates, culture slides, or onto other imaging compatible substrates.

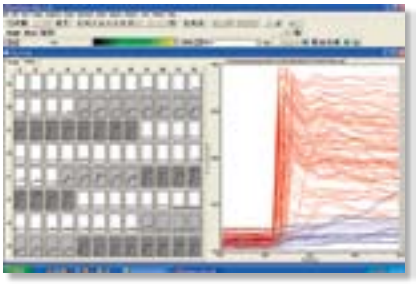


With the BD Pathway™ high-content cell analyzers, images are captured in either confocal or widefield modes providing the best possible images for analysis. Proprietary software algorithms segment the image.

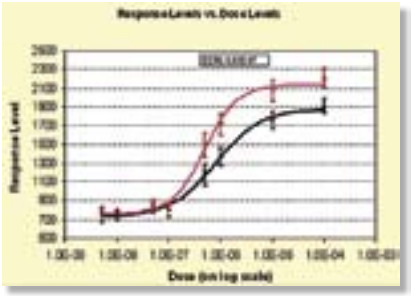


The segmented image may be further divided into regions of interest (ROI) where fluorescence measurements are made. These measurements can include fluorescence intensity ratios, granularity, morphological features, fluorescence localization, and more.

Individual cells may then be classified into different categories based on end-point or kinetic response profiles at the cell, well, or plate level.



Data is analyzed and presented within the provided software, or it can be exported for analysis within third party software.



BD Pathway™ Hardware

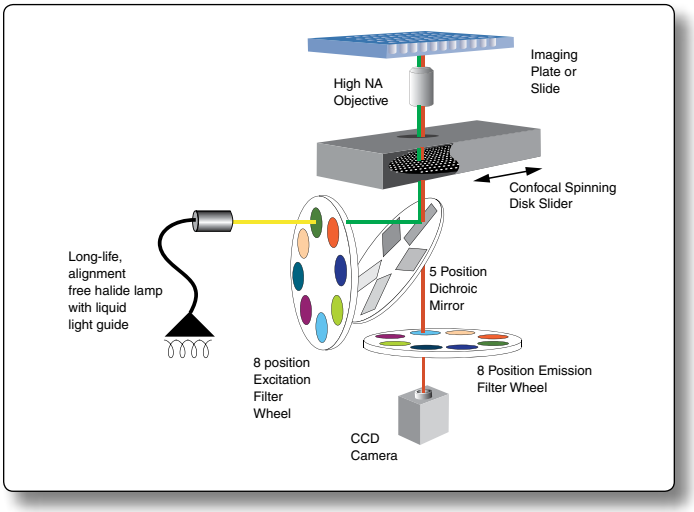
BD Pathway 435 High-Content Cell Analyzer

The BD Pathway 435 system is a compact benchtop for high-content cellular imaging and is ideally suited for endpoint (fixed cell) biological assays. Light from a mercury metal halide lamp, introduced through a liquid light guide, provides full-spectrum illumination. The lamp is engineered for long life and never needs alignment. The laser auto focus, fast filter changers, spinning disk confocal optics, and a high-resolution CCD camera enable the BD Pathway 435 system to record high-quality fluorescence images from multiwell plates and slides. A transmitted light canopy provides the ability to capture bright-field images that can be overlaid onto fluorescence images. The system comes with powerful, flexible imaging processing and analysis software.

The BD Pathway 435 system is designed for:

- Labs needing a dedicated high-content image acquisition and analysis workstation
- Researchers that primarily work on endpoint applications with fixed cells or tissues
- Labs that routinely conduct fluorescence image documentation and analysis

Schematic representation of the BD Pathway 435 light path



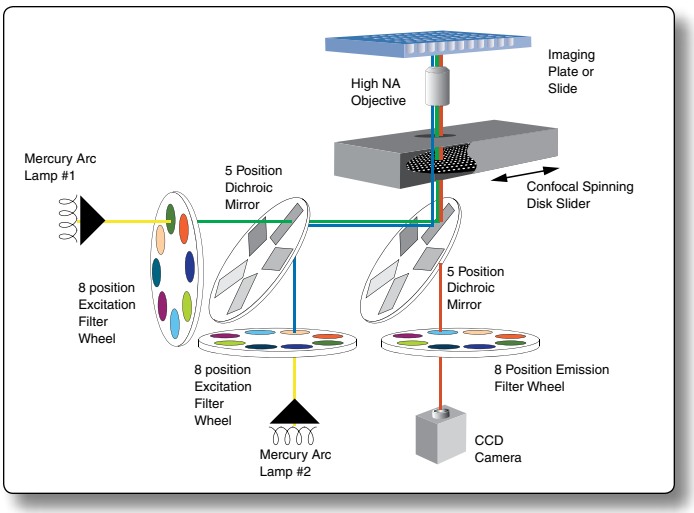
BD Pathway 855 High-Content Cell Analyzer

The BD Pathway 855 system offers the ultimate in flexibility for high-content imaging of live and fixed cells. Equipped with environmental control and liquid handling, the system has full-spectrum (340–700 nm) illumination, laser auto focus, and fast filter changers. These powerful features, along with spinning disk confocal optics and a cooled CCD camera, enable the BD Pathway 855 system to rapidly record high-resolution fluorescence images from multiwell plates and slides. The system comes with powerful imaging software to perform a broad range of fluorescence-based kinetic and endpoint biological assays.

The BD Pathway 855 system is designed for:

- Core facilities or labs needing a versatile solution for live cell and endpoint fluorescence imaging
- Researchers conducting live cell assay development for which viability of the cells is important
- Investigators requiring a variety of dynamic imaging modes on live cells with image-as-you-add capability.

Schematic representation of the BD Pathway 855 light path.



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Hardware Features and Benefits

Feature	Benefits	BD Pathway™ 435	BD Pathway™ 855
High-performance laser-based autofocus	High image acquisition speed can be obtained (under 5 minutes per 96-well plate). The system also can be switched to camera-based autofocus or combined autofocus modes when more control over image acquisition is needed.	✓	✓
Real-time, true-optical confocal spinning disk	Permits automated switching between widefield and confocal imaging with movable disk. Delivers high-resolution 3-D images without background fluorescence often associated with widefield imaging systems.	✓	✓
High-precision x,y,z linear-motor positioning	Allows high-speed, precise image montage (tiling) without the need for software processing. Increased cell counts are possible by capturing multiple adjacent images per sample.	✓	✓
Proprietary motionless stage with movable optics	Ensures sample stability during image acquisition. Also allows loosely adherent and suspension cells to be imaged.	✓	✓
Flexible computer-controlled independent excitation, emission, and dichroic filters	Provides high-speed, fully automated imaging of fluorescently labeled samples.	✓	✓
Long-life, alignment-free illumination	Permits full-spectrum (360-700 nm) imaging. No light alignment required. Long lamp life (1500 hours) and low maintenance cost.	✓	
Dual-mercury arc-lamp illumination	Permits full-spectrum (340-700 nm). Imaging allows the illumination of the sample through one of up to 16 different excitation filters.		✓
Robot compatibility	Allows high-throughput, unattended instrument operation.	✓	✓
On-stage liquid handling	Enables a variety of dynamic imaging modes on live cells with image-as-you-add capabilities.		✓
Environmental control	Keeps cells alive during extended imaging periods.		✓
Transmitted light	Permits superimposition of transmitted light images onto fluorescence images to assist with localization of cellular events.	✓	✓
Binocular eyepiece	Allows direct viewing of cells in both fluorescence and transmitted light modes.		✓
Interchangeable objectives	Enables a wide range of imaging options at multiple resolutions.	✓	✓

BD Pathway™ Software

Powerful, flexible, yet easy to use

The BD Pathway software provides a suite of integrated tools to accomplish the tasks of high-content cellular analysis—from image acquisition and analysis to data analysis and visualization. The software is designed to accommodate the needs of many users, from users needing push-button control of pre-defined applications to advanced assay developers. The software utilizes several easily accessible interfaces.

Routine Assay User	Occasional/Custom User	Assay Developer
Predefined applications are activated through a single push-button launch window.	A number of step-through Application Wizards are provided to allow optimization of commonly run applications.	Advanced users can build custom instruction sets through an intuitive macro builder that controls all components of the system.

Routine Assay User

Easy as 1-2-3

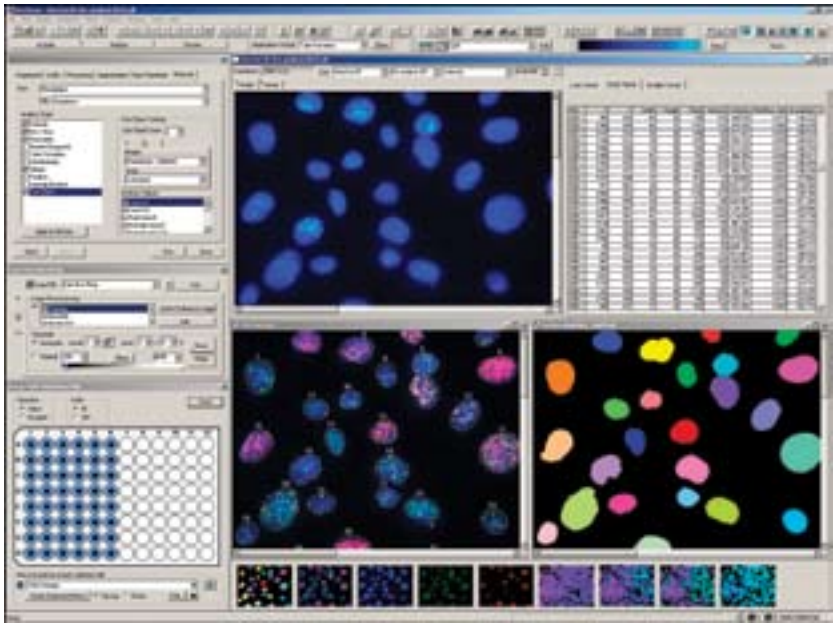
Users wanting to run preconfigured applications can easily do so with the **Assay Launch** dialog. Whether provided by BD or developed by the laboratory, new applications can be added to the launch dialog for easy access at any time.

The Assay Launch dialog provides the ability to:

1. Select a preconfigured application
2. Select the wells of the plate to be analyzed
3. Run the assay

There is also a convenient tool for making any necessary edits to the application.

Assay launch dialog for simple control of routine applications.



Once selected, the application launches an interface allowing the user to view progress in realtime.

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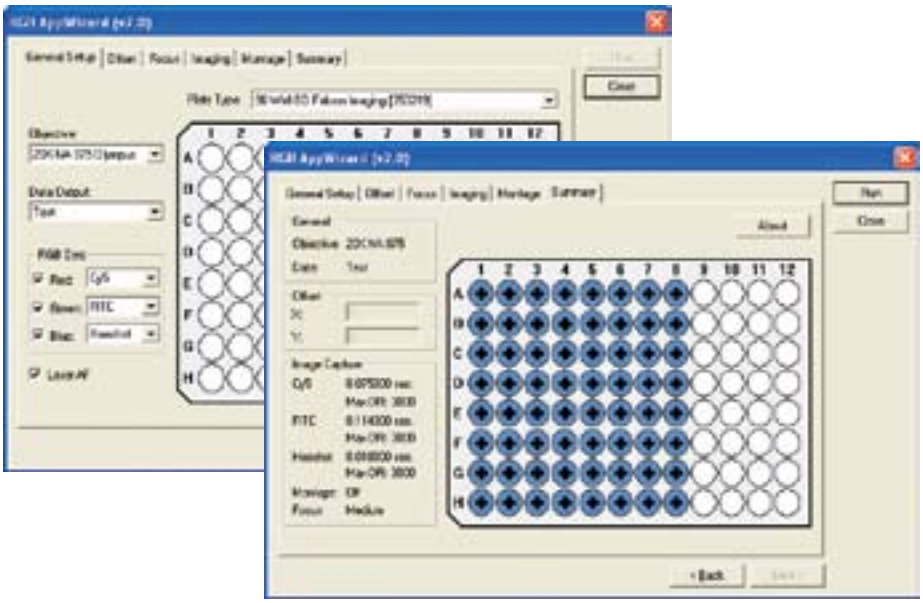
BD Pathway™ Software *(Cont'd)*

Occasional/Custom

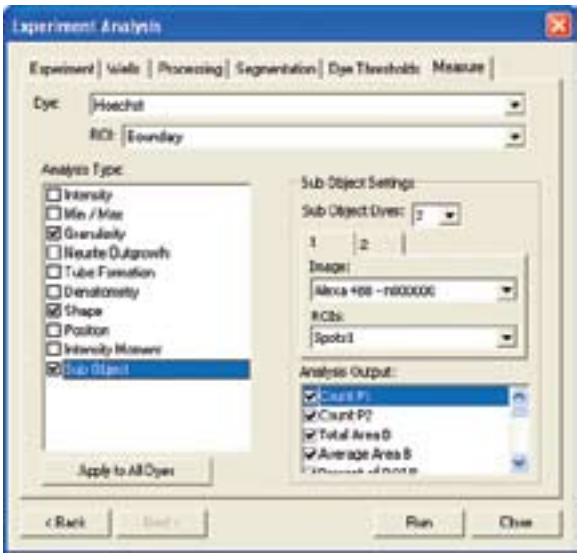
User

Step-by-step set up of applications

Users requiring easy optimization of their routine high-content experiments can utilize BD Pathway's unique Application Wizards. These preconfigured wizards provide guided access to a number of key settings of the instrument. The user simply steps through the dialog boxes, making any necessary adjustments, reviews the summary tab, and launches the application. The Wizards require only basic knowledge of imaging, allowing the cell biologist to configure the system using clear, easy to understand language.



Application Wizards provide guided, step-through instructions in easy to understand language.



You can apply the settings used in the running of an Application Wizard to multiple plates during reanalysis.

For more advanced analysis, additional tools are available to step the user through customized analysis algorithms. The user simply selects the experiment data, the analysis type, and adjusts some basic settings. Then, the user can experiment with a variety of settings before selecting those to apply. Once defined, these settings can be saved for consistent application across any number of plates.

BD Pathway™ Software *(cont'd)*

Assay Developer

Full instrument control

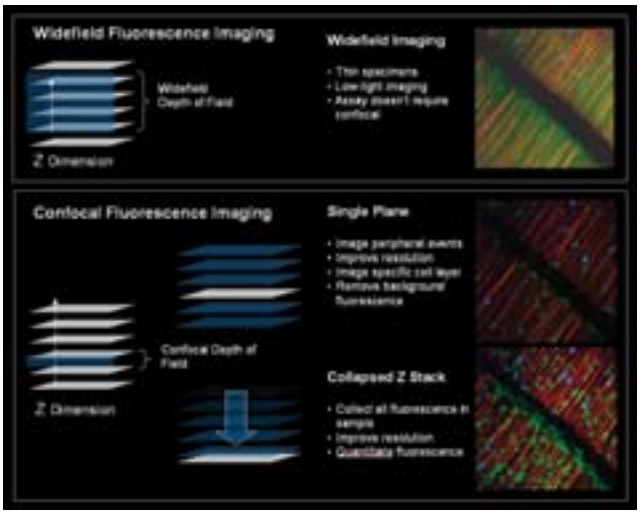
The power and flexibility of the BD Pathway software allows advanced users to develop novel applications unique to their experimental needs. An intuitive, step-wise macro builder allow the user to interact with every setting of the instrument and make precise adjustments to the system. The user can select from hundreds of defined "actions" to assemble into a macro (instruction set) for the instrument. The step-wise macro builder provides unprecedented flexibility in image acquisition

Advanced Features

The BD Pathway software includes a comprehensive set of advanced features for image acquisition, image visualization, and live cell dynamic imaging (BD Pathway 855 instrument) to meet your ongoing research needs.

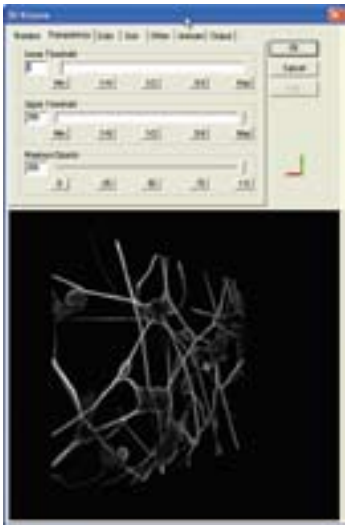
Confocal Z Stack acquisition

The BD Pathway system provides a selectable spinning disk confocal, which allows users to take advantage of both widefield and confocal imaging methods. Based on experimental conditions and imaging requirements, the confocal capability provides the flexibility to remove out-of-focus haze for improved image quality and analysis. A plate-wide collapsed Z Stack provides the highest quality image while retaining all the fluorescence information throughout the depth of the specimen.



3-D visualization

The 3-D volume rendering tool is built in and allows image Z Stacks to be viewed from any angle. The image views can also be rendered to develop a 3-D rotational projection and video sequence.



3D volume rendering of an image Z stack for image visualization.

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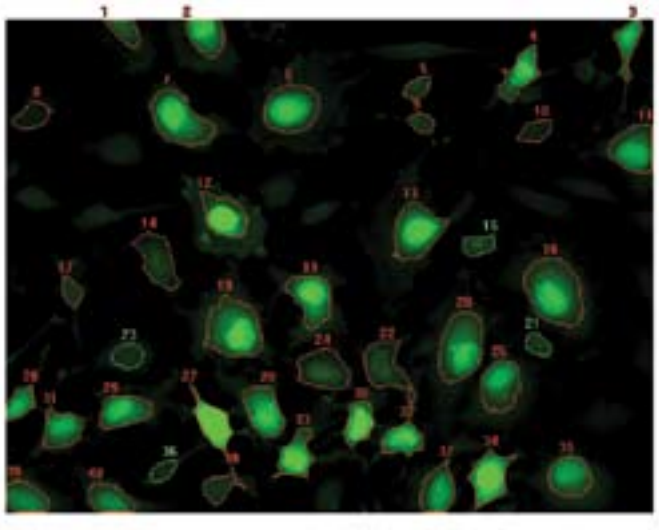
BD Pathway™ Software *(cont'd)*

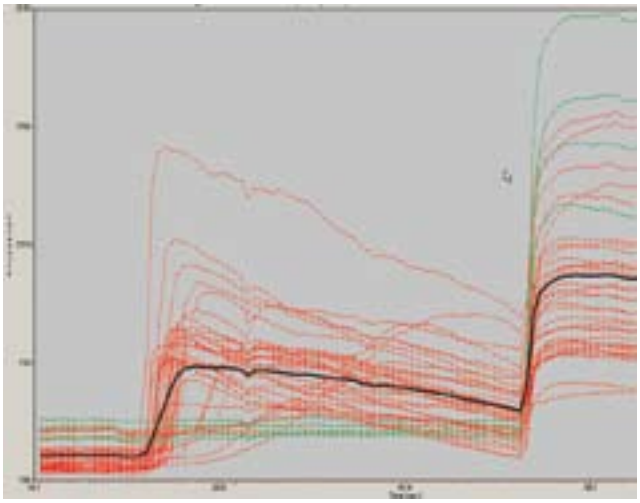
Live cell dynamics

The BD Pathway™ 855 system supports a variety of live cell imaging applications such as calcium kinetics using single or dual wavelength dyes.

With the included liquid handling capability, compound additions can be coordinated with imaging to ensure analysis of events in real-time.

A



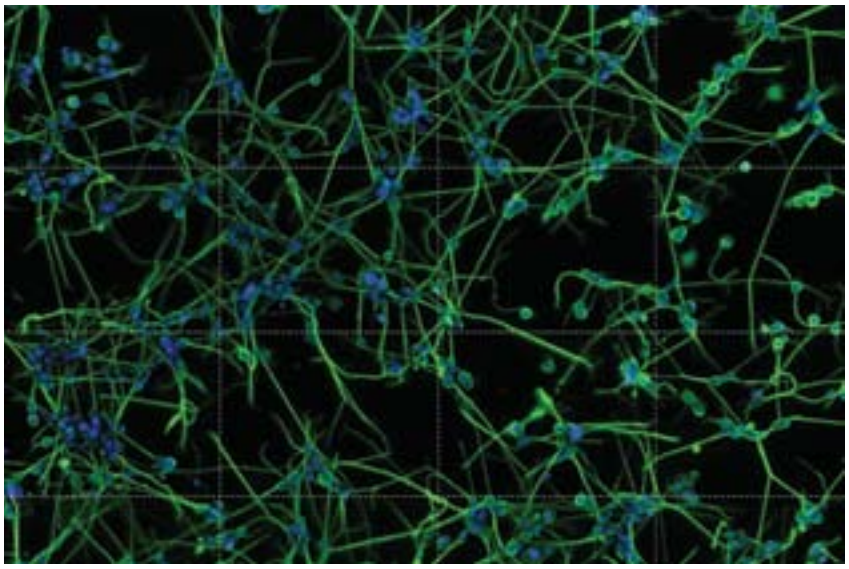


B

Calcium response in Fluo-4® labeled HeLa cells after stimulation with an agonist. Each line in Panel B represents intensity over time for single-cell response to the specific agonist (first drug addition) and positive control (second drug addition). Cells have been classified using the BD Pathway software into positive responders (red) and negative responders (green) for the specific agonist. Black line is the average response of all cells. Panel A is a segmented image from a single time point after drug addition showing positive (red) and negative (green) cells with cell ROI (region of interest) numbers.

Image montage (tiling without software stitching)

The BD Pathway™ system offers advanced hardware and software capabilities for building the image montage (tiles.) Experiments that require multiple locations, a larger cell population, or a single expanded field of view image can easily be set up through a montage capture dialog. For example, Neuron length may be measured over a number of fields of view with a high degree of precision and accuracy.

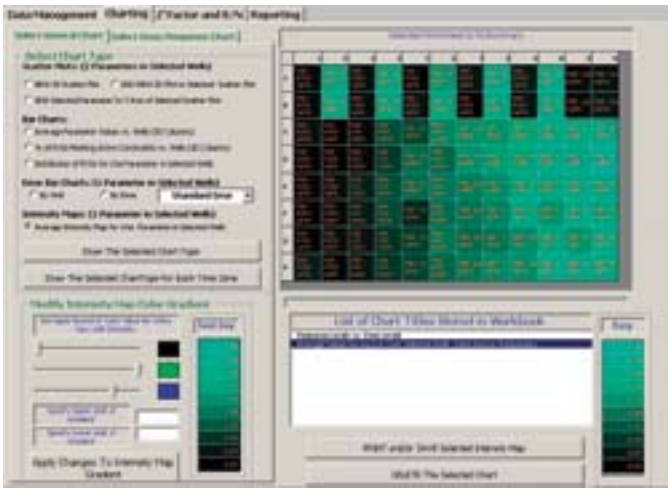


BD Pathway™ Software *(cont'd)*

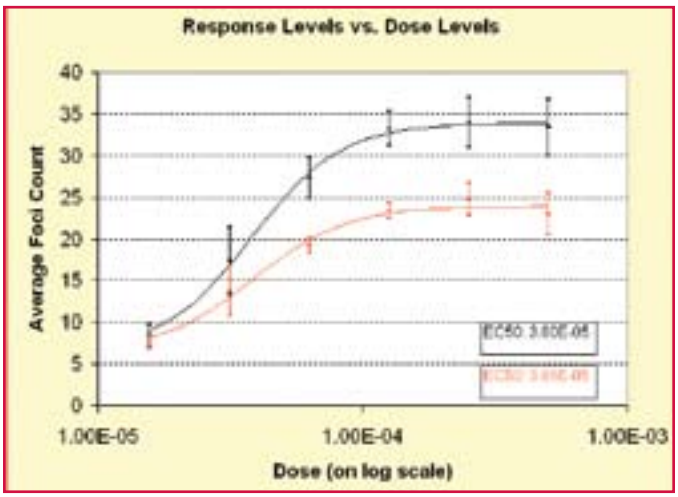
Data Analysis and Visualization

The BD Pathway system includes a wide range of data analysis and visualization tools for high-content image data. Endpoint and kinetic data can be analyzed using the BD Pathway system. Examples of data analysis and visualization tools include:

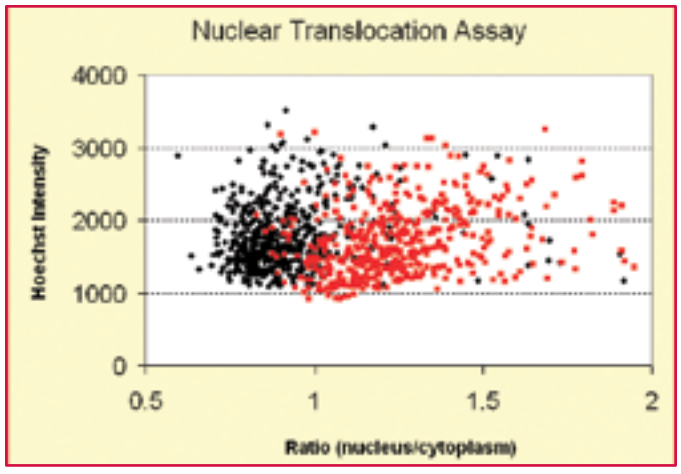
- Bar charts
- Scatter plots
- Dose-response curves
- Heatmaps
- Cell-by-cell or well-by-well analysis
- Cell scoring (percentage of cells responding)
- Z-scores



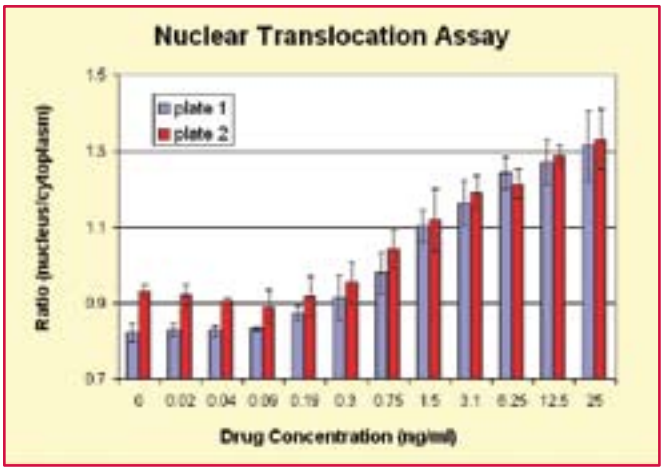
Heatmap display showing average value of a measurement parameter on a well-per-well basis. The heatmap provides an excellent overview of a drug's dose response activity.



Multiple dose-response curves can be plotted on the same chart allowing, comparison between different experimental conditions.



Scatter plot example showing the comparison between area of the cell and rate of rise parameters.



Bar chart comparing the response levels of two data sets.

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Software Features and Benefits

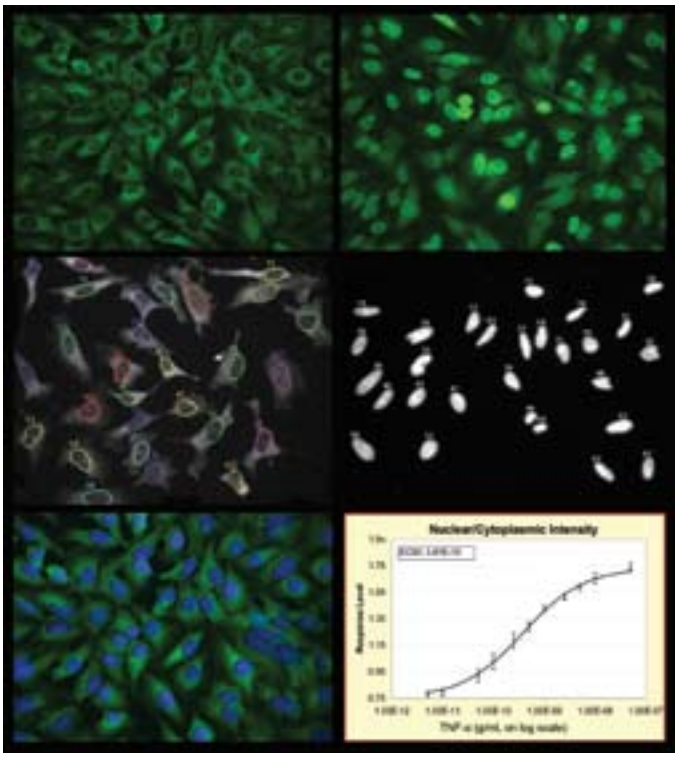
Feature	Benefits	BD Pathway™ 435	BD Pathway™ 855
High-content analysis software included	Most high-content applications can be run without the need to purchase applications-specific software.	✓	✓
Powerful analysis capabilities	Flexible software provides a variety of analysis tools, enabling a broader range of applications.	✓	✓
Comprehensive software suite	From image acquisition and analysis to data analysis and visualization, BD Pathway™ software provides a complete solution.	✓	✓
Flexible export of images and data	Support for unencrypted 16-bit TIFF, JPEG, and BMP file formats, plus export of delimited text files, allows you to migrate your data to third party software provider solutions.	✓	✓
Integrated 3-D volume rendering	Easy capture and visualization of 3-D images and movies.	✓	✓
Advanced kinetic control with scheduling	Run a number of dynamic live-cell applications including calcium flux imaging and time-lapse microscopy.		✓

Applications

The power and flexibility of the BD Pathway™ system enables researchers to develop and perform a wide array of high-content biological applications. A number of preset applications together with application wizards for assay customization are available, making it possible to correlate basic biological processes, with quantifiable measurements. The BD Pathway high-content cellular analyzer provides a balance between ready-to-run applications and complete assay design, as well as novel advanced applications.

Our applications take advantage of our live-cell kinetic and confocal capabilities, and can be run in multi-plate high-throughput and/or low-throughput modes, depending on the need. The systems support a large variety of plate and slide types, with flexibility to modify existing applications or to develop your own.

Imaging applications can be divided into the following four assay modes involving the measurement of fluorescence intensity, fluorescence distribution, morphological changes in fluorescently labeled structures, and migration of fluorescently labeled cells.



Assay Modes

	Fluorescence intensity	Fluorescence distribution and colocalization	Morphology	Motility and migration
When to use	• Biological response appears as a change in fluorescence intensity but other fluorescence detection methods are ineffective • Few cells are available (e.g. primary cells, stem cells) • Cell population is heterogeneous	• Total fluorescence does not change within a cell following activation • Identify where within a cell an event occurs • Colocalize cellular events	• Biological response measured by changes in cell size or shape	• Biological response is measured as cellular movement
Example of BD Pathway application	• Calcium kinetics • Mitotic index • Live/dead • Cell viability • Cytotoxicity • Cell cycle • Apoptosis • Mitochondrial health (JC-1,TMRE, MitoTracker) • Steatosis • IkB degradation • Reactive oxygen species • Whole organism imaging (***c. elegans***, arabidopsis, zebrafish) • Tissue arrays	• Cytoplasmic/ nucleus translocation assays • DNA damage (and repair) • Mitotic defects (monopolar spindles) • Apoptosis • Synaptic junction localization • FISH • 3-D cell imaging (stem cells, pancreatic islets) • Transfluor® GPCR (receptor internalization)	• Tube formation (angiogenesis) • Neurite outgrowth • Micronucleus assay • Morphometric analysis (tubulin) • Apoptosis (nuclear size, granularity)	• Cell migration • Chemotaxis • Cell invasion

Modes can be multiplexed to develop unique applications

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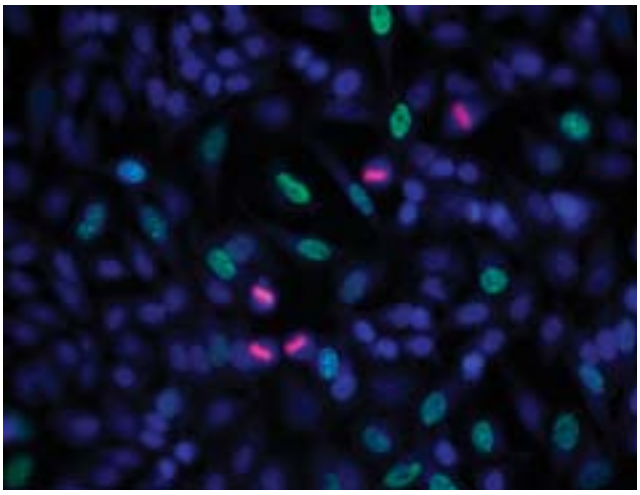
Applications *(cont'd)*

Fluorescence Intensity Applications

Quantitative Cell Cycle Analysis

Many drugs impact cell cycle in some way. Whether carrying out drug screening experiments or analyzing cell cycle directly in an academic lab, determining the percentage of cells in a particular phase of the cell cycle is very important. Two of the most commonly studied phases are S phase (DNA synthesis) and M phase (Mitosis). BD Biosciences antibody reagents enable the study of both using a simple one-step staining procedure.

A



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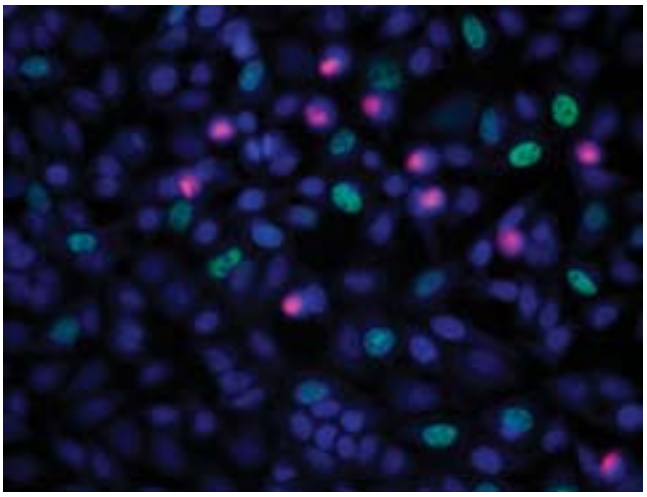

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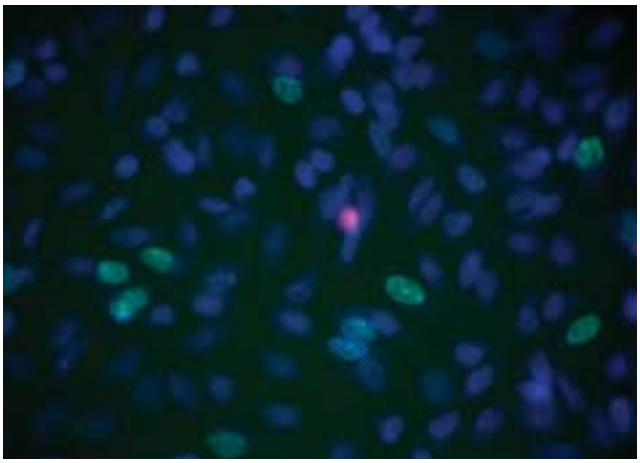

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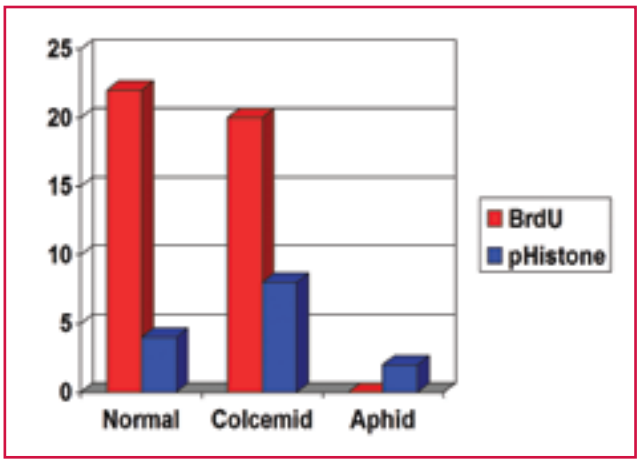


Effects of colcemid and aphidicolin on the cell cycle. HeLa cells were seeded into a BD Falcon™ Imaging 96-well plate cultured overnight, then treated for 2 h with either 500 ng/mL colecimid (Panel B), 500 ng/mL aphidicolin (Panel C) or vehicle control (Panel A). M and S phase cells were detected in a single staining step using the BD Bioimaging Cell Cycle Kit which uses a directly conjugated anti-phospho-Histone H3 antibody to detect M phase cells and a directly conjugated anti-BrdU antibody to detect cells in S phase. Merged pseudocolored 20X images are shown in Panels A–C. The BrdU signal is pseudocolored green, the phosho-Histone H3 signal is pseudocolored red and Hoechst stained cells are pseudocolored blue. Data was analyzed using BD Pathway™ system software and shows that colcemid caused an increase in the percentage of cells in M phase over controls and that aphidicolin produced a dramatic decrease in the number of cells in S phase.

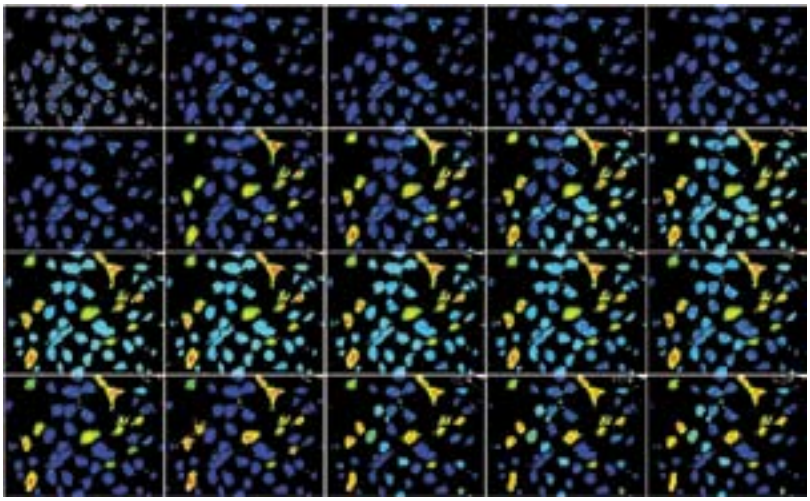
Applications *(cont'd)*

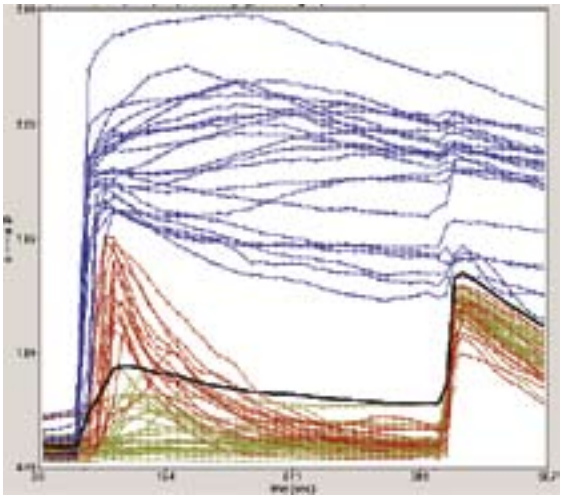
Calcium Kinetics

Simultaneous Measurement of Calcium Concentration and Mitochondrial Membrane Potential in Astrocytoma Cells Using Two Ratiometric Fluorescence Probes

Measurement of cytosolic free calcium concentration has been employed successfully to unravel intracellular signaling pathways, identify G-protein coupled receptors (GPCRs), and cAMP levels using specifically modified ion channels. Ratiometric dyes such as Fura-2 or JC-1 overcome the problem of uneven dye loading by exploiting a shift in the excitation (Fura-2) or emission (JC-1) wavelengths upon binding of calcium. This shift is independent of the dye concentration and can be extracted by taking two images in rapid succession and dividing them to generate a ratio measurement. The BD Pathway™ system enables the user to measure calcium changes reliably using ratiometric dyes.

These experiments demonstrate how the BD Pathway 855 high-content cell analyzer can be used to make simultaneous single-cell kinetic measurements of calcium and mitochondrial membrane potential changes.





Calcium kinetics. Stably transfected (VR1 receptor) NIH 3T3 cells were plated in a BD Falcon™ Imaging 96-well plate and cultured overnight. Cells were then labeled with Fura-2 and placed into the environmental chamber of a BD Pathway 855 system where the cells were maintained at 37°C and 5% CO_2. Specific and control agonists were added using on-board fluidics, and the cell responses were measured. The image mosaic on the left shows the time series of pseudocolored (for intensity) images from a single well. The first image in the series shows the single-cell ROIs imprinted on the image. The panel on the right shows the intensity over time for each cell. The kinetic data is further classified by the software into response types based on rate of rise and rate of fall parameters for the specific agonist. Cell line courtesy of Dr. Mike Iadarola, NIDCR, NIH, Bethesda, MD.

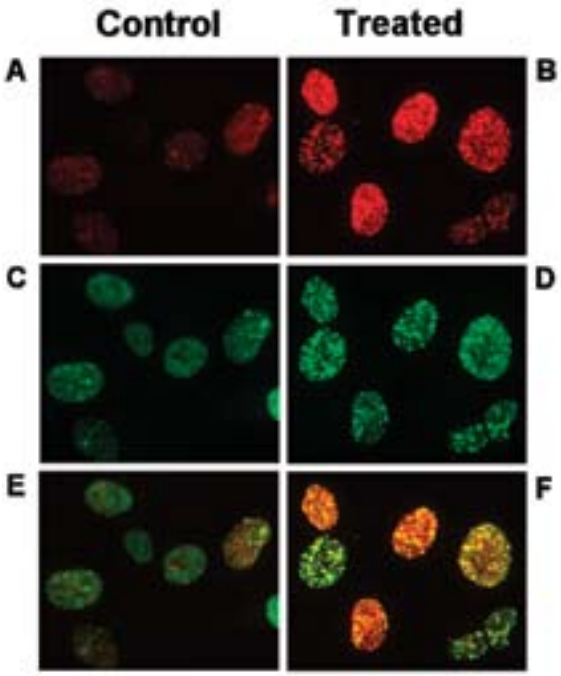
Applications *(cont'd)*

Fluorescence Distribution and Colocalization Application

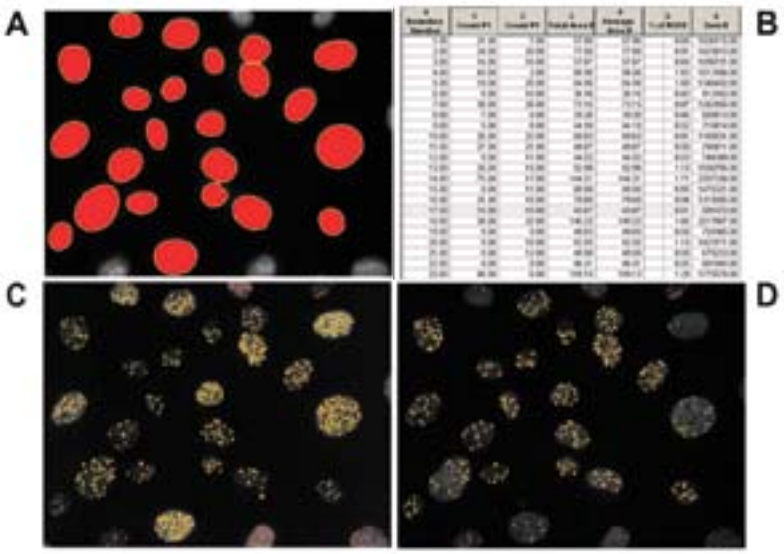
DNA Damage and Repair: Quantitation of Sub-Cellular Events Using Automated Confocal Imaging

Cancer prevention treatment and aging are of major relevance in understanding the mechanisms by which cells respond to, and repair, DNA damage. Additionally, by assessing the effect of compounds in drug screening campaigns on DNA integrity, an early indication of genotoxicity can be detected.

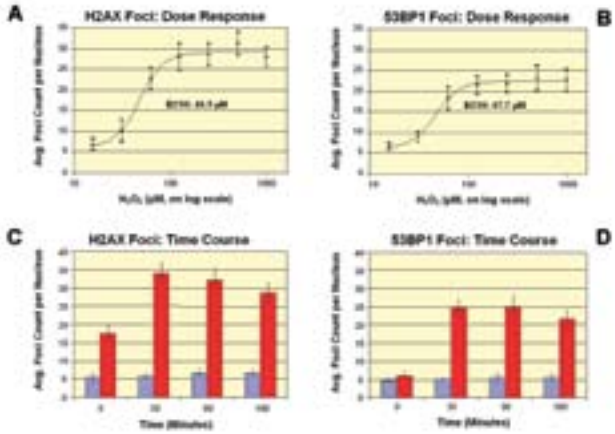
The DNA damage and repair application measures the presence of DNA damage and its repair using antibody probes. This application also detects proteins involved in the cellular response to DNA damage.



Induced DNA damage. HT1080 cells were plated in a BD Falcon™ Imaging 96-well plate and cultured overnight. Cells were then treated with a dilution series of H_2O_2 to induce DNA damage, or with vehicle control; cells were allowed to recover for 30 min. Cells were then fixed and permeabilized and stained with antibodies to detect DNA damage (phospho-H2AX, pseudocolored red, Panel A and B) and DNA repair (53BP1, pseudocolored green, Panel C and D). Cropped 40X confocal images from a control and a treated well are shown in panels A–F. Panel E and F are merged pseudocolored images and show the colocalization of the two probes as a yellow to orange color.



Data analysis. Using customizable and highly flexible software tools, automated image analysis was performed. Nuclear boundaries were generated using the Hoechst images (Panel A). The foci images from the two probes, phospho-H2AX (Panel C), and 53BP1 (Panel D), were segmented and analyzed within the nuclear boundaries. The BD Pathway™ software allows analysis of many different parameters such as foci count, intensity, size, area and colocalization (Panel B).



Dose-response curves. Dose-response curves and EC50 values generated by the BD Pathway software are shown for phospho-H2AX (Panel A) and 53BP1 (Panel B) probes. N=6 ± SEM.

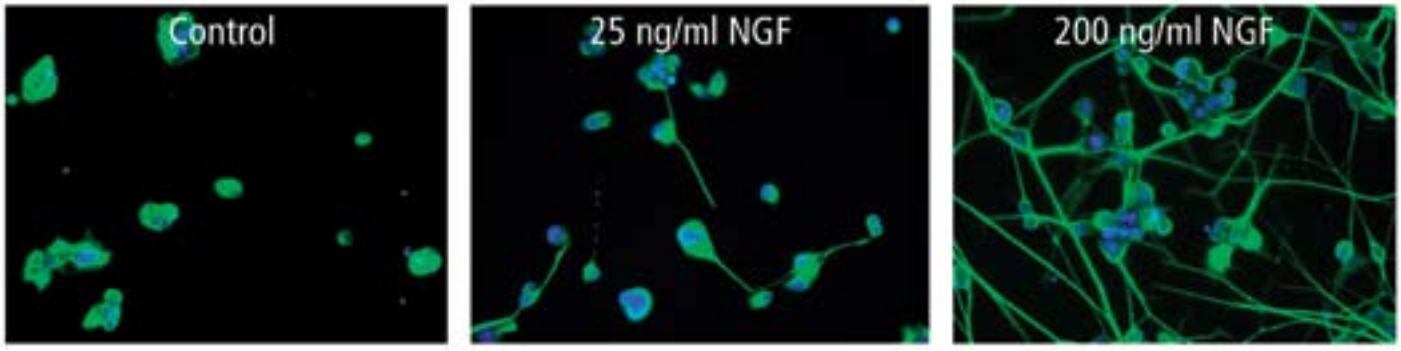
Applications *(cont'd)*

Morphology Application

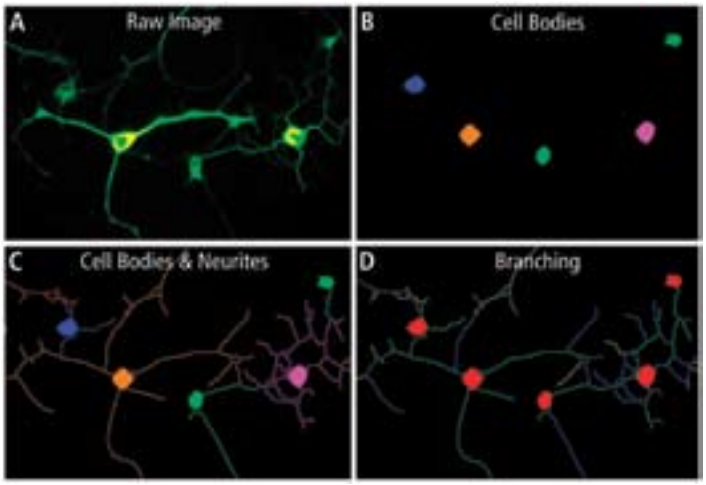
Quantitative High-Content Analysis of Neurite Outgrowth

Changes in the pattern of neurite outgrowth have been implicated in neurodegenerative disorders as well as traumatic injuries. Measurement of neurite outgrowth using an automated image-based assay can be beneficial in the research, screening, and validation phases of the drug discovery process.

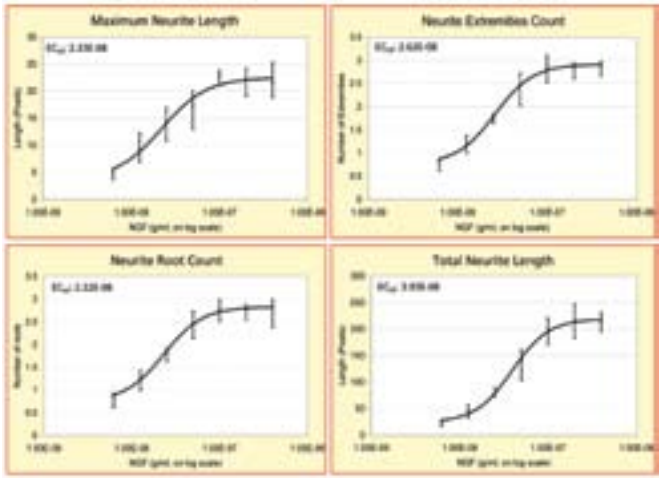
This is a multi-parameter assay in which the software segments the neuronal cell body and analyzes fluorescently labeled neurites with respect to their total length, maximum length, root count, extremities count, node points, and segment count.



NGF-induced neurite outgrowth. PC-12 cells were plated in a BD Falcon™ Imaging 96-well plate coated with rat tail collagen and cultured overnight. Cells were then subjected to a neurite differentiation protocol that included a dilution series of nerve growth factor (NGF) for 10 days. Cells were then fixed and permeabilized and stained with anti-tubulin antibody and Hoechst. Representative 20X pseudocolored images of control and treated wells are shown; neurites are pseudocolored green and nuclei are pseudocolored blue.



BD Pathway™ software-generated image and segmentation masks. Panel A displays a pseudocolored neurite image. Segmentation masks displaying cell bodies (Panel B, colors randomly assigned by the software), cell bodies and neurites (Panel C), and neurite branch levels (Panel D, primary, secondary, tertiary and quaternary branches are colored green, blue, yellow and cyan, respectively).



Dose-response curves. NGF dose-response curves and EC_{50} values generated by the BD Pathway software are shown for four measurement parameters, maximum neurite length, neurite extremity count, neurite root count, and total neurite length. N=10 ±SEM.

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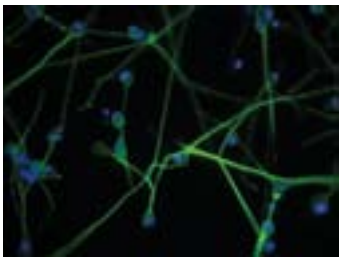
Reagents

BD™ Biosciences Bioimaging Certified Reagents are certified for optimal performance based on an internal qualification program. For BD to certify a reagent, an antibody must pass a number of tests that focus on high-content applications for which reproducibility is essential. These tests include:

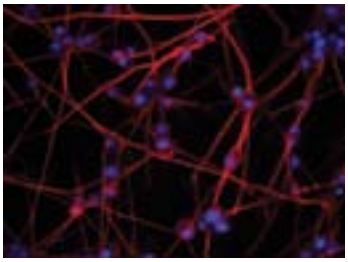
- Exceeding a signal intensity threshold over background in commonly used cell lines
- Working with widely used fixation and permeabilization methods
- Localizing to the appropriate region within a cell
- Showing an appropriate response following stimulati

* For a current list of Bioimaging Certified Reagents, please visit us at www.bdbiosciences.com/bcr

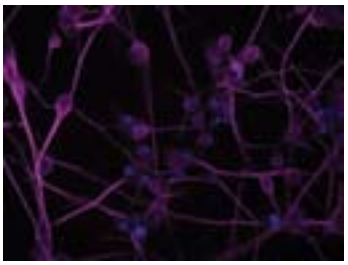
A



B



C



Representative images using BD Bioimaging Certified primary conjugated antibodies. Pseudo-colored merged images of nerve growth factor treated PC12 cells. Nuclei are psuedo-colored blue in all images.

Neurites were stained with:

Panel A: Alexa Fluor® 488 conjugated anti ß-tubulin antibody (green)

Panel B: Alexa Fluor® 555 conjugated anti ß-tubulin antibody (red)

Panel C: Alexa Fluor® 647 conjugated anti ß-tubulin antibody (magenta)

Images were acquired using a 20X objective (0.75 NA).

Cell Cultureware for Bioimaging

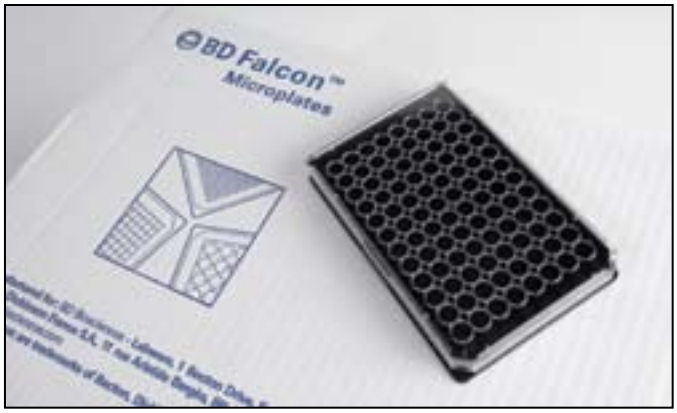
BD Falcon and BD BioCoat™ Microplates for Imaging Applications

BD Biosciences also offers microplates to support manual and automated high-throughput imaging settings. With black walls to minimize well-to-well crosstalk, these plates feature an especially thin, clear bottom that has been specifically developed for imaging applications. Our imaging plates are available in 96 or 384 well formats, either uncoated (tissue culture treated surface) or coated with Collagen I or Poly-Lysine.

Microplates for Manual and Automated High-Throughput Imaging Applications

DESCRIPTION	VOLUME	WELL SHAPE	QTY/PACK	QTY/CASE	CAT. NO.
BD BioCoat 384-well Microplate, Black/Clear, Collagen I coated*	90 µL	Flat-Bottom	5	50	356667
BD BioCoat 384-well Microplate, Black/Clear, Poly-D-Lysine-coated*	90 µL	Flat-Bottom	5	50	356663
BD Falcon 96-well Imaging Plate, Black/Clear, Tissue Culture treated	390 µL	Flat-Bottom	8	32	353219
BD Falcon 384-well Microplate, Black/Clear, Tissue Culture treated	120 µL	Flat-Bottom	5	50	353962

* Collagen I and Poly-Lysine coatings are available through our custom coating program. Please contact your local BD Biosciences representative for more information.



Technical Specifications

Optics	BD Pathway™ 435	BD Pathway™ 855
Proprietary, fully integrated optical design	•	•
Selectable laser-based and/ or camera-based autofocus	•	•
Motionless stage handles 96 or 384 well plates, microscope slides, and chamber slides	•	•
X, Y resolution: 100 nm (mechanical)	•	•
Z resolution: 50 nm (mechanical)	•	•
5 images per second capture rate	•	•
Works with Olympus 4X, 10X, 20X, 40X objectives	•	•
Montage capability for large field imaging	•	•
Automation ready	•	• (requires robotics integration package)
Integrated temperature (ambient to 40°C) and CO_2 (ambient to 5%) control	n/a	•
Imaging modes	Confocal fluorescence, widefield fluorescence, brightfield	Confocal fluorescence, widefield fluorescence, brightfield
Illumination Sources	Single, EXFO X-cite, metal halide with liquid light guide; multi-position LED grid	Dual, HBO 100 W mercury arc; single automated LED
Confocal Unit		
Nipkow spinning disk	•	•
Disk sampling rate	1,000 fps	1,000 fps
Pinhole size	70 µM	70 µM
Filter Configurations		
Excitation	8	16
Dichroic	5	5
Emission	8	8
Automated control	•	•
Independent operation	•	•
Detection and Observation		
High resolution cooled CCD camera	12 Bit, high QE, effective pixels 1392 x 1040, pixel size 6.45 x 6.45 pixels	12 Bit, high QE, effective pixels 1392 x 1040, pixel size 6.45 x 6.45 pixels
Binocular eyepiece for direct sample viewing in confocal or widefield mode	n/a	•
Liquid Handling and Environmental Control		
On-stage pipet head with single-position syringe (2-100 µL)	n/a	•
Mixing capability (tituration)	n/a	•
Disposable pipette tips	n/a	•
Environmental Control (ambient to 38°C) and CO_2 (ambient 0-5% +/-.5%)	n/a	•

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Technical Specifications *(cont'd)*

Software	BD Pathway™ 435	BD Pathway™ 855
Advanced imaging software with hardware auto-detection capabilities	•	•
Automated focus and image acquisition	•	•
Automated segmentation and region of interest (ROI) identification	•	•
Interactive data and image navigation	•	•
Concurrent multiple ratiometric dye kinetics (US Patent 5,332,905)	n/a	•
Z sectioning and 3-D rendering	•	•
Hierarchical data classification algorithm	•	•
Data analysis module for Microsoft® Excel®	•	•
Preconfigured and user-configurable applications	•	•
Support for endpoint applications	•	•
Support for kinetic applications	•	•
BMP, TIFF, and TXT formats for data export	•	•
Computer (minimum specification)		
Pentium IV running Microsoft® Windows® XP Professional	•	•
2 GB RAM	•	•
256 MB video card	•	•
250 GB SATA hard drive	•	•
24" wide aspect ratio LCD flat panel display	Optional	•
DVD burner	•	•
Robotics Integration Package		
Access door with safety light curtain	Not required	Optional
Software interface to multiple scheduling systems	Optional	Optional
Physical Dimensions		
Width	33.5 inches (85 cm)	43 inches (109 cm)
Depth	19 inches (48 cm)	41 inches (104 cm)
Height	13.5 inches (34 cm)	66 inches (168 cm)

Technical and Training Support

Successful implementation of high-content analysis requires excellent technical support and application specific training. BD associates have years of experience in cell biology, high-content cell analysis, microscopy, and imaging. BD is a global business offering exceptional regional support to our customers worldwide. Here is a summary of some of our support and training capabilities.

- **BD Bioimaging Education Center.**
  This 2700 square-foot, state-of-the-art training center houses two classrooms, a wet lab, a microscopy suite, a conference center, and common area with a refreshment center. The center offers demonstration instruments, basic and advanced training classes, "getting started" courses, and technical presentations for a variety of BD technologies. This center complements our existing training facilities in San Diego, San Jose, Boston, Basel, Tokyo, and Seoul.

- **Technical Application Scientists.**
  Our field-based technical specialists support customers' high-content applications worldwide. These Masters or PhD level scientists with many years of relevant cell analysis expertise, ensuring your success in implementing high-content cellular applications.

- **Custom BD Pathway™ software services.**
  BD offers expert software scripting and optimization to support custom application development. If you are looking for custom high-content analysis research, our team of software and technical specialists are available to help you reach your goal. Our custom software support includes a feasibility study, product development, training, and support. Please contact your local sales representative for further details.

- **Customer support.**
  BD provides exceptional customer support through a combination of phone, e-mail, web, and field support services. We also can monitor and view our customers' experiments from our site, allowing for rapid diagnosis and resolution of the situation at hand. Our support team has knowledge of all of our product lines and is readily available to provide assistance.

- **Warranty and Extended Service Contract options.**
  The BD Pathway instrument includes a one-year service contract providing repair and preventive maintenance. We offer multiple tiers of extended service contracts based on customer needs.

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Ordering Information

Instrumentation and Accessories

Bioimaging Systems Products

DESCRIPTION	QTY	CAT.NO.
BD Pathway™ 855 Includes: Imager with binoculars, on-board liquid handling, confocal, standard filter set, Pentium IV computer, 24" LCD monitor, BD Pathway software (1 license), Olympus 20X/340 objective, Slide holder, installation and training (2 days). **NOTE: gantry glass panels/doors must be specified for each BD Pathway 855 quotation**	each	341036
BD Pathway™ 435 Includes: Bioimager, confocal, alignment-free long-life light source, standard filter set, Pentium IV computer, 20" wide-aspect ratio LCD monitor, BD Pathway software (1 license), Olympus 20X/340 objective	each	641250

BD™ Pathway Optional Software

DESCRIPTION	QTY	CAT.NO.
BD Pathway Software Online - Included with BD Pathway system	1 seat	341037
BD Pathway Software Offline	3 seats	341038
BD Image Data Explorer	4 seats	341039
Neurite Outgrowth with Tube Formation Algorithm	4 seats	346203
BD IPLab™ for BD Pathway system	4 seats	340989

BD Pathway Hardware Options

DESCRIPTION	QTY	CAT.NO.
Gantry glass panel set (black)	each	341047
Microscope slide holder	each	341045
Replacement BD Pathway emission filter wheel	each	341041
Replacement BD Pathway dichroic filter wheel	each	341042
Replacement BD Pathway excitation A filter wheel	each	341043
Replacement BD Pathway excitation B filter wheel	each	341044
Power conditioner/convertor - (non-U.S.) must specify country and voltage requirements	each	346547
HB0 103 Mercury arc lamp replacement bulbs	each	341032
Robotics Integration Package Includes automated door, nested tip holder, Automation Software Integration Kit** **must indicate for which robotics option: Thermo CRS *Polara* requires additional purchase of Polara adaptor from Thermo CRS	each	341046
Robotics tip tray	each	341040
Additional BD Pathway software license	each	341037
Additional on-site training	1 day	341062
Custom filters and configuration	inquire	
Extended Service Contract	year	341061

BD Pathway Additional Objectives

DESCRIPTION	QTY	CAT.NO.
Olympus PLAPO2X objective (Plan Apochromat; NA 0.08; 6.20 mm WD)	each	341052
Olympus UPLAPO4X objective (Plan Apochromat; NA 0.16; 13.0 mm WD)	each	341053
Olympus UPLFL4X objective (Universal Plan Fluorite; NA 0.13; 17.0 mm WD; 2.9 x 1.6 mm xyFOV)	each	341054
Olympus UPLFL10X objective (Universal Plan Fluorite; NA 0.3; 10.0 mm WD; 640 x 840 µm xyFOV)	each	341055
Olympus UPLAP10X objective (Universal Plan Apochromat; NA 0.4; 3.1 mm WD; 640 x 840 µm xyFOV)	each	341056
Olympus UAPO20X/340 objective (Universal Apochromat; NA 0.75; 0.55 mm WD; 420 x 320 µm xyFOV) NOTE: delivered with BD Pathway	each	341034
Olympus LCPLFL20X objective (Semi-Plan Apochromat; NA 0.4; 6.9 mm WD) Note: Long working distance lens	each	341067
Olympus UAPO40X/340 objective (Universal Apochromat; NA 0.9; 0.2 mm WD; 210 x 155 µm xyFOV)	each	341033
Olympus PLAPO40X objective (Plan Apochromat; NA 0.95; 0.13 mm WD)	each	341057
Olympus PLAPO60X objective (Plan Apochromat; NA 1.4; 0.15 mm WD)	each	341066

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