BD Pathway Bioimaging Systems

BD

Helping all people live healthy lives

Cellular Imaging for High-Content Analysis

Delivering industry-leading bioimaging, BD Pathway™ high-content cell analyzers combine superior image quality, flexible image capture, and live-cell analysis to address a wide range of applications. BD Pathway systems provide fluorescence intensity measurements, kinetic imaging, and morphological analysis, including subcellular imaging.

Both the benchtop BD Pathway 435 and the stand-alone BD Pathway 855 provide high performance and ease of use to improve workflow and productivity. Cellular images can be captured in either confocal or widefield mode to deliver high-resolution images for subsequent analysis. A unique optical spinning disk allows operators to switch between confocal or widefield modes, minimizing background fluorescence and maximizing image quality. An innovative motionless stage with movable optics enhances image stability for loosely adherent and live cells.

Powerful acquisition software allows administrators to easily develop templates for predefined routine or specialized applications. Researchers can visualize both endpoint and kinetic image data in a wide range of formats.

To support a wide range of applications, BD Biosciences also provides BD™ Bioimaging Certified Reagents ideally suited for optimal image acquisition and analysis.

As part of BD Biosciences ongoing commitment to bring innovative tools to life scientists for use in emerging areas of cell research, BD Pathway systems are backed by world-class technical and application support.

High-Content Analysis

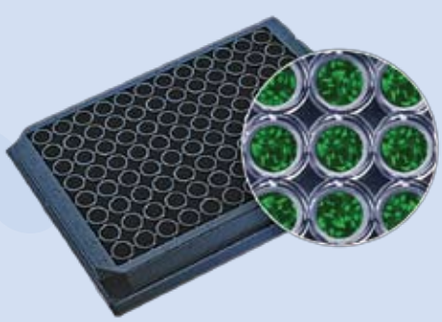
BD Pathway Systems

The benchtop BD Pathway 435 and stand-alone BD Pathway 855 feature precision optics with flexible and easy-to-use controls and comprehensive acquisition and analysis software. Images are captured using a unique confocal spinning disk slider. The confocal mode enables a BD Pathway instrument to deliver high-resolution images without the background fluorescence often associated with widefield imaging systems. The laser-based autofocus capability of the BD Pathway systems enables rapid acquisition of high-quality images. The systems can also use camera-based autofocus or combined autofocus modes when more control over image acquisition is required.

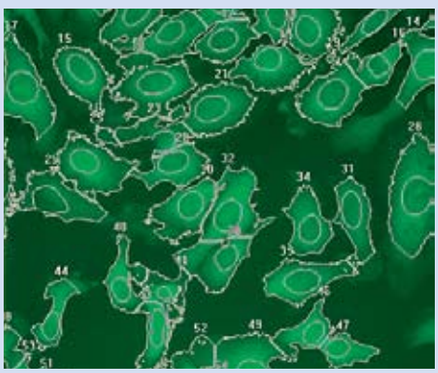
BD Pathway 435 Benchtop System

The BD Pathway 435 system is a compact benchtop platform ideal for endpoint biological assays. Light from a mercury metal halide lamp introduced through a liquid light guide provides illumination from 360 nm to 700 nm. A transmitted light canopy provides the ability to capture bright-field images that can be overlaid onto fluorescent images. The lamp requires no light alignment and is rated to last 2000 hours.

High-Content Cell Analysis Workflow



Cell samples are prepared on multiwell plates, culture slides, or other imaging-compatible substrates.

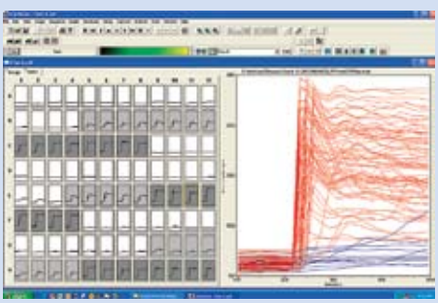


Images are captured in either confocal or widefield modes, delivering the best possible analysis methods.

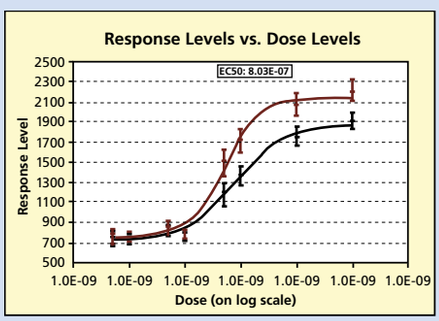
The segmented image can be further divided into regions of interest where measurements, such as fluorescence intensity ratios, granularity, morphological features, and fluorescence distribution, are made.

BD Pathway 855 Stand-Alone System
The BD Pathway 855 system offers the ultimate in flexibility for high-content imaging of live and fixed cells. The dual-mercury metal halide lamp's long life and alignment-free illumination deliver imaging across the full spectrum from 340 nm to 750 nm. Researchers can select among 16 different excitation filters suited for a broad range of applications.

Equipped with environmental control and liquid handling, the system can perform a wide range of fluorescence-based kinetic and endpoint biological assays. A binocular eyepiece allows for direct viewing of cells in both fluorescence and transmitted light modes.



Individual cells can then be classified into different categories based on end-point or kinetic response profiles at the cellular level.



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

Superior Image Capture and Analysis

Advanced, Versatile Optical Design

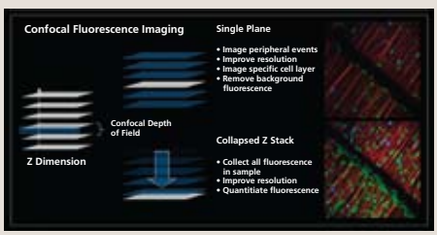
With BD Pathway high-content bioimagers, cellular images are captured in either confocal or widefield modes, providing the best possible images for analysis. To ensure optimal imaging and results, the BD Pathway system uses advanced optical features including autofocus, movable optics, and a confocal spinning disk.

Advanced optical systems in BD Pathway instruments provide even illumination at high intensity across the full spectrum, enabling a wide range of imaging options at multiple resolutions. Laser autofocus allows rapid image acquisition—under five minutes per 96-well plate. Different options for autofocus allow the BD Pathway system to switch from laser-based to camera-based or combined autofocus modes for applications requiring greater control over image acquisition. The unique real-time, true-optical spinning disk provides automated switching between widefield and confocal imaging. This capability delivers high-resolution images without background fluorescence often associated with widefield imaging systems.

High-precision x,y,z linear-motor positioning allows fast, precise image montage, also known as tiling, without the need for software processing. This capability helps achieve increased cell counts by acquiring multiple adjacent image fields per sample.

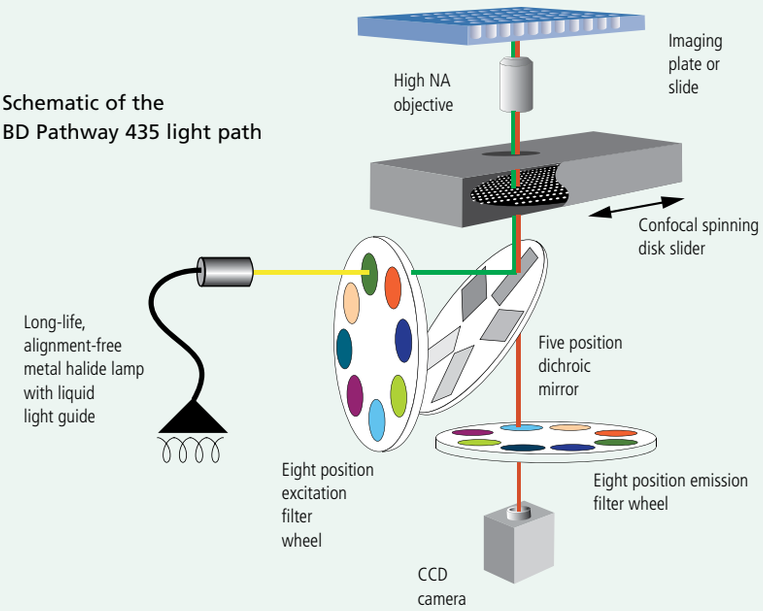
Confocal Z Stack Acquisition

A selectable spinning disk—the heart of the BD Pathway system—allows researchers to take advantage of both widefield and confocal imaging. The ability to select different objectives delivers better image quality and analysis. The 3-D capabilities provide quality imaging with collapsed Z stack for each field, while retaining the fluorescence information throughout the depth of the specimen, as shown here.

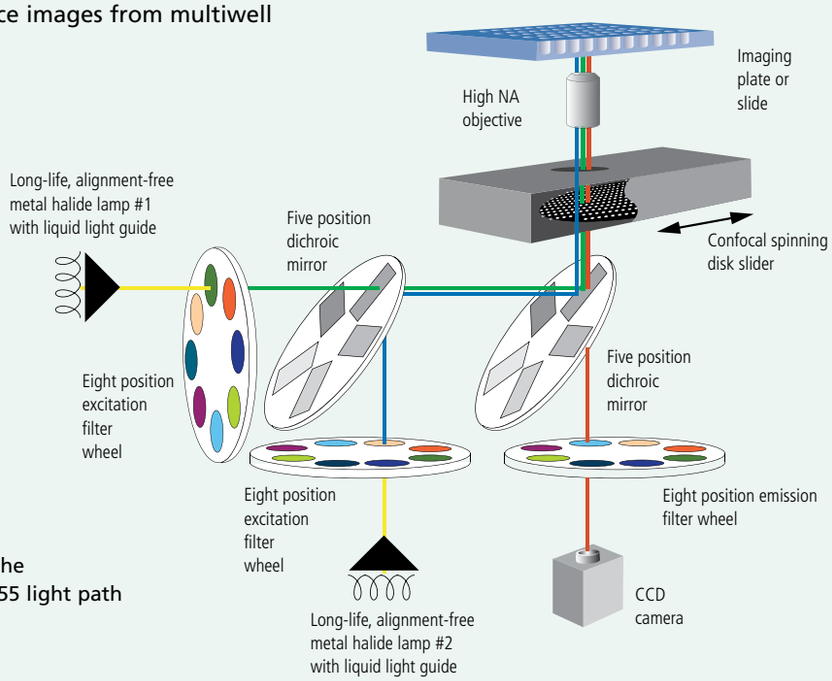


The system's unique motionless stage with movable optics ensures sample stability during image acquisition for loosely adherent and suspension cells. Transmitted light provides the ability to capture bright-field images that can be overlaid onto fluorescence images for precise localization of cellular events. Independent excitation, emission, and dichroic filters are flexible and computer controlled to provide high-speed, fully automated imaging of fluorescently labeled samples. This optical capability allows the system to record high-resolution fluorescence images from multiwell plates and slides.

Schematic of the
BD Pathway 435 light path

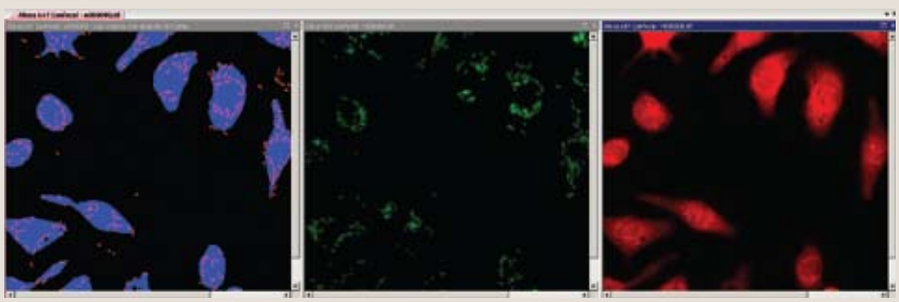


Schematic of the
BD Pathway 855 light path



Analysis of Macrophages

Using the BD Pathway Object Counting Tools feature, researchers can count and measure objects within objects to analyze subcellular regions via segmentation. This figure shows images of cells stained with a whole cell dye infected with *Listeria monocytogenes*. The *L. monocytogenes* are stained with BD Difco Listeria O Antiserum, followed by Alexa Fluor® 488 labeled rabbit secondary antibody.



From Setup to Results

Software that Simplifies High-Content Analysis

The BD Pathway software suite simplifies high-content cellular analysis and accommodates a full range of bioimaging applications.

Easy Setup and Acquisition

For routine analysis, predefined applications are set up by researchers and stored in the system for easy access with a single click. The Assay Launch dialog allows users to quickly select a routine application, enter the wells of the plate to be analyzed, and run the assay.

Intuitive Wizards for Applications

Applications can be added to the Assay Launch dialog for easy access at any time. Researchers can choose applications provided by BD. BD Pathway Wizards require only basic knowledge of imaging, allowing researchers to configure the system using clear, easy to understand directions. The user is guided through the dialog, reviews the summary tab, and launches the application.

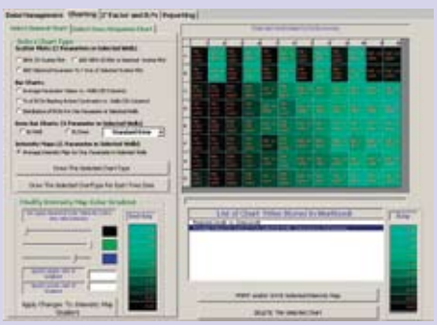
Capabilities for Advanced Bioimaging

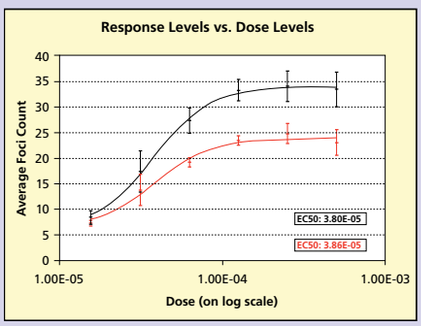
Advanced users can develop novel applications unique to their experimental needs. An intuitive macro builder allows researchers to choose from hundreds of instrument settings to meet their precise imaging requirements. The user can then experiment with a variety of settings before selecting those to apply. Once defined, these settings can be saved for running the advanced applications across any number of plates for improved results and reproducibility.

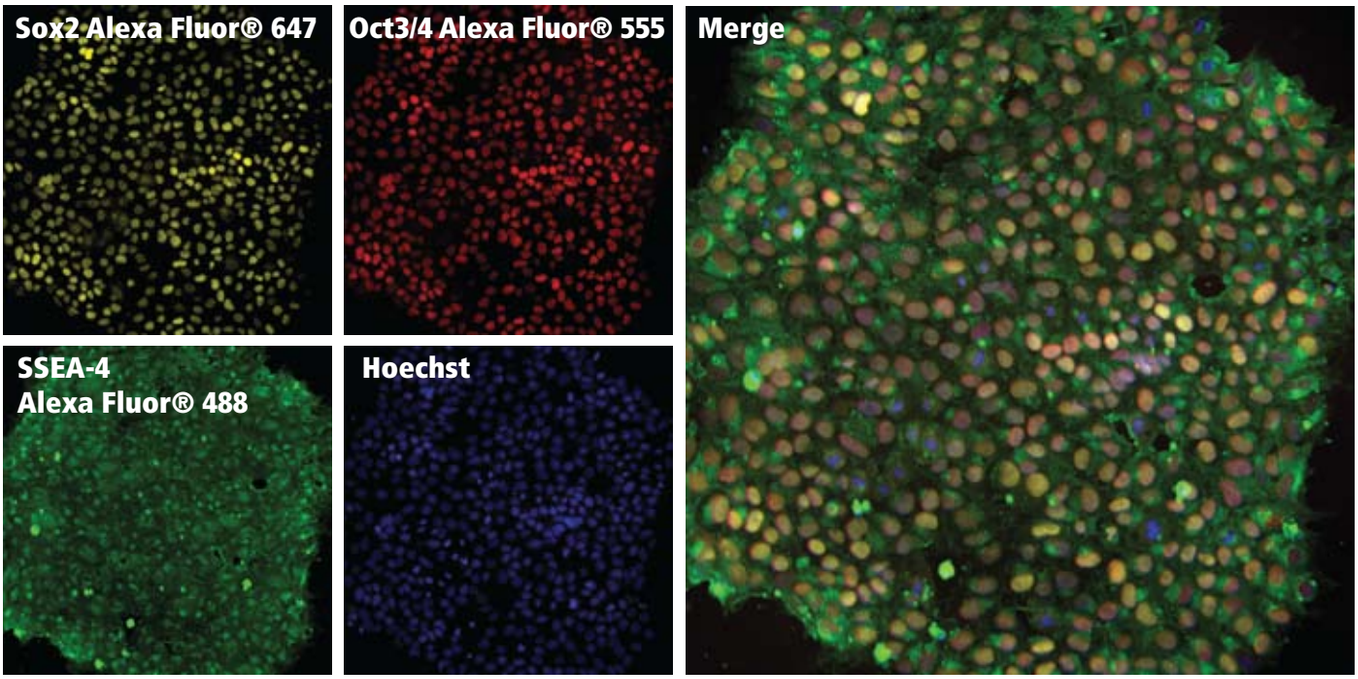
Data Analysis and Visualization Tools

Analysis of Dose-Response Levels

The heatmap display (left) shows the measurement parameter on a well-per-well basis, providing an excellent overview of a drug's dose-response activity. Multiple dose-response curves can be plotted (right) for comparison between different experimental conditions.

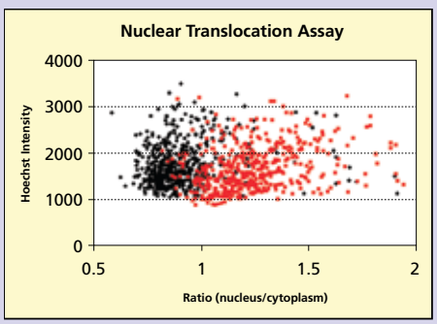


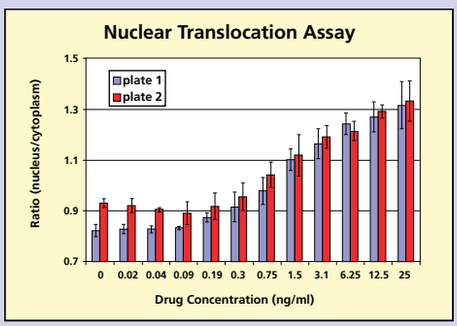




Embryonic stem cells cultured under feeder-free conditions remain pluripotent.

Human embryonic stem cells (H9) were cultured in mTeSR1 maintenance medium (StemCell Technologies) on BD Falcon™ 96-well imaging plates (Cat. No. 353219) that were coated with BD Matrigel™ hESC-qualified matrix (Cat. No. 354277). Cells were fixed with 4% paraformaldehyde, followed by BD Perm/Wash™ buffer (Cat. No. 554723). Multicolor cell staining was performed with the following antibodies: Sox2 Alexa Fluor® 647 (Cat. No. 560302) pseudocolored yellow, Oct3/4 Alexa Fluor® 555 (Cat. No. 560306) pseudocolored red, and SSEA-4 Alexa Fluor® 488 (Cat. No. 560308) pseudocolored green. Cell nuclei were counter-stained using Hoechst 33342 pseudocolored blue. Small images on the left show individual antibody staining. The larger panel on the right represents the merged image. The cells were imaged on a BD Pathway 435 bioimager using a 10x objective.





Analysis of Nuclear Translocation Assay

The scatter plot (left) shows the comparison between the area of the cell and rate of rise parameters. The bar chart (right) compares the response levels of two data sets.

Analysis and Presentation

Powerful and Flexible Analysis Tools

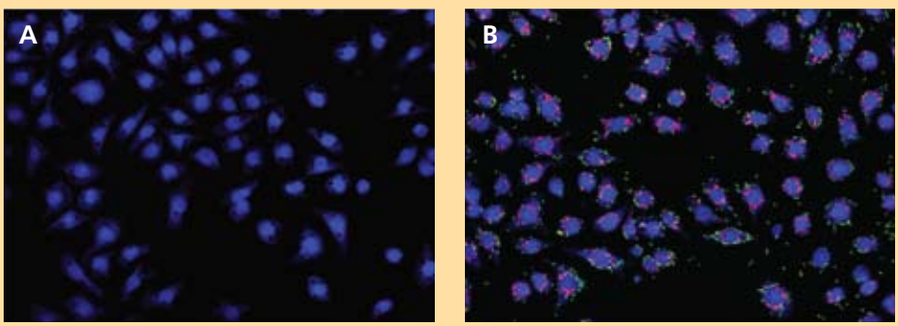
The BD Pathway system provides analysis and visualization tools for high-content image data. This data is analyzed using visualization tools such as bar charts, scatter plots, dose-response curves, heatmaps, cell-by-cell or well-by-well analysis, cell scoring (percentage of cells responding), and z'-factor. Both endpoint and kinetic data can be analyzed using the BD Pathway system.

Visualization of High-Content Analysis
Using the BD Pathway system, bioresearchers collect and view data to gain insights into results at the cellular and subcellular level. BD Pathway software provides powerful image preprocessing filters for segmentation, including background correction, morphological, and convolution filters. These filters improve the extraction and identification of cells or cellular regions of interest for improved image analysis. The software also enables researchers to quickly and accurately split objects using sophisticated algorithms to optimize data analysis.

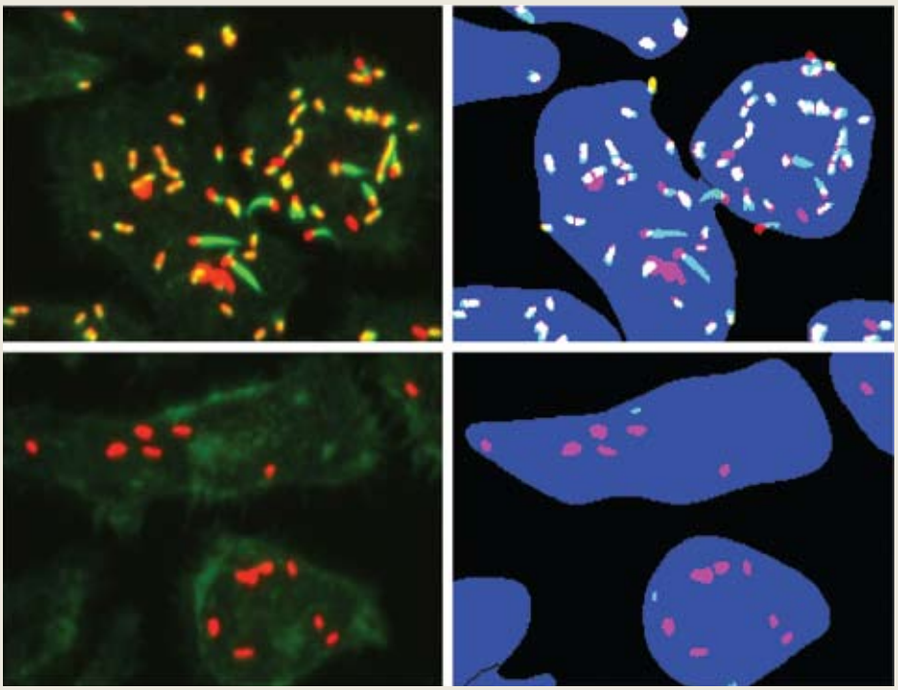
Additional Cellular and Subcellular Advances
Intracellular imaging and analysis evaluating the cell nucleus are supported by band segmentation output, including erosion of the nuclear region. This output enables the BD Pathway system to deliver better results for plasma membrane translocation and additional subcellular region analysis. Exclusion regions can be created around the perinuclear region for improved signal to noise in other nucleus/cytoplasm translocation assays.

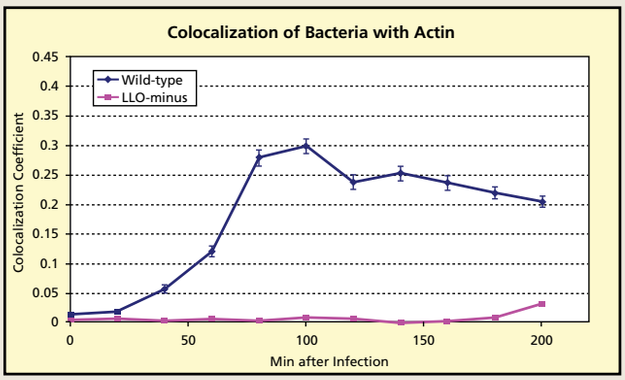
Morphometric, intensity, and positional measurements support more precise characterization and analysis of cellular and subcellular regions or phenotypes. The BD Pathway software also counts objects within objects, which is a useful tool for applications such as DNA damage, colocalization, and spot counting.

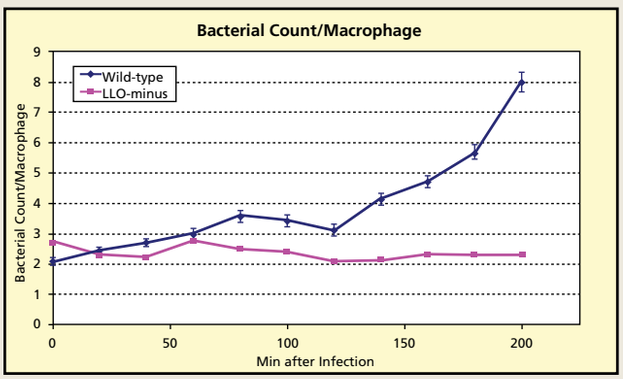
Phagocytosis Assay



Phagosomal Escape Assay to Quantify Bacterial Count





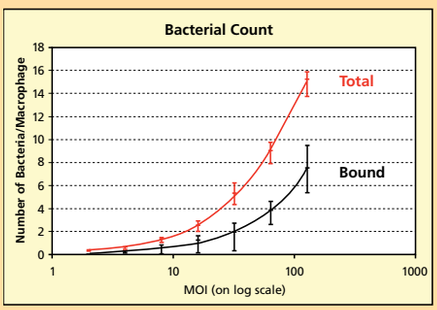


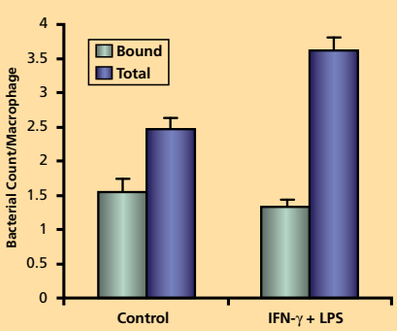
Sub Object Counting

These confocal collapsed stack images (40x, 0.9 NA) were taken of macrophages infected with wild-type (top) and LLO-minus mutant *L. monocytogenes* (bottom). The images were pseudocolored, merged, and cropped. Macrophage actin is green, bacteria are red, and colocalized signals appear as merges of these color channels. Images on right show segmentation masks generated after Sub Object counting from images on the left. The upper right plot shows time-dependent colocalization coefficients of wild-type and LLO-minus mutant bacteria with actin and vice versa, respectively (n = 4 wells). The lower right plot shows time-dependent quantification of bacterial count per macrophage (n = 4 wells).

Activation of Macrophages

To the left are single field confocal collapsed stack images (40x, 0.9 NA) of (A) uninfected macrophages and (B) macrophages infected with *L. monocytogenes* at an MOI of 128. The images were pseudocolored and merged. Macrophages are blue, bound bacteria are green, total bacteria are red, and colocalized signals appear as merges of these color channels. The log scale plot shows the effect of increasing MOI on bound and total bacterial count (number of bacteria per macrophage) in red, and total and average bacterial area per macrophage from replicate wells (n = 12 wells) in black. The bar chart shows the effect of activation of macrophages by IFN-γ plus LPS on the average bound and total bacteria per macrophage (n = 4 wells).





Tools to Support Imaging Applications

Instrumentation, visualization, data analysis, and data management are critically important for high-content analysis. Equally important are microplates and reagents to optimize bioimaging applications. BD offers optimized conjugated and purified antibodies, as well as specially designed microplates, to facilitate a variety of cell analysis applications.

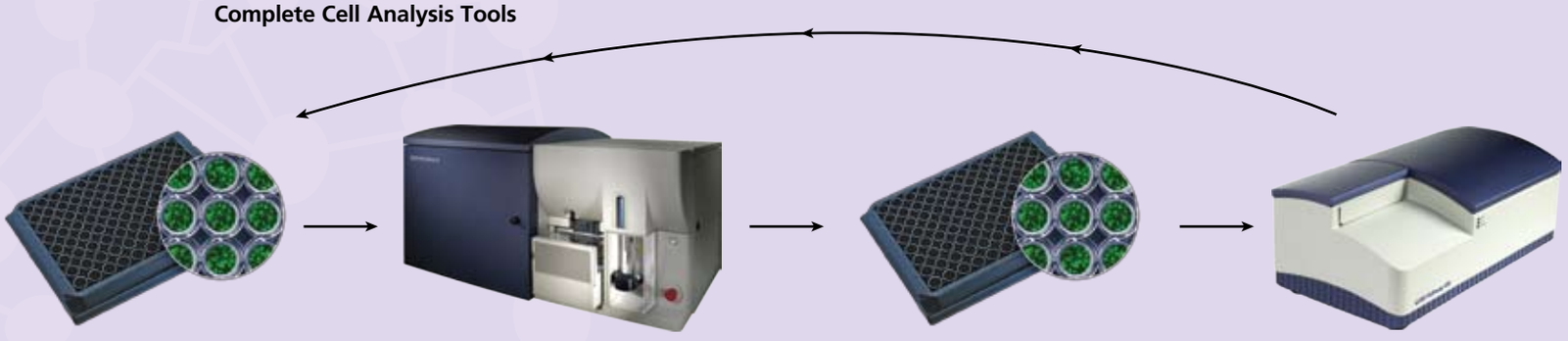
Microplates for Bioimaging

To minimize well-to-well crosstalk and maximize resolution during imaging applications, BD Falcon™ and BD BioCoat™ plates are available for use with BD Pathway systems. These plates feature black well walls and a thin, clear underside specially developed for bioimaging. The BD BioCoat product comes in a 384-well plate, and BD Falcon products come in 96-well and 384-well configurations.

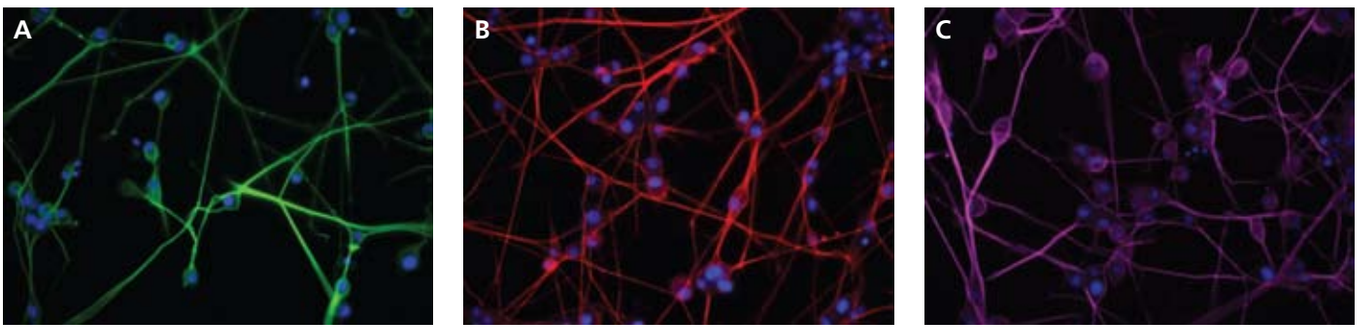
Beyond Bioimaging

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

Complete Cell Analysis Tools



Cell population | Analyze, enrich, and sort | Culture | Image

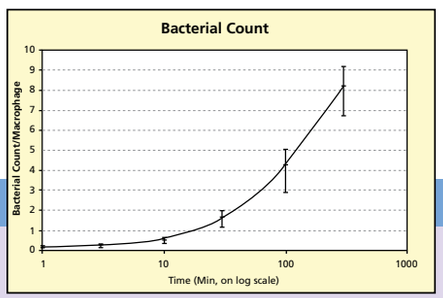


Representative Images Using BD Bioimaging Certified Primary Conjugated Antibodies

Pseudocolored merged images of nerve growth factor treated PC12 cells. Nuclei are pseudocolored blue in all images. Neurites were stained with: (A) Alexa Fluor® 488 conjugated anti ß-tubulin antibody (green), (B) Alexa Fluor® 555 conjugated anti ß-tubulin antibody (red), and (C) Alexa Fluor® 647 conjugated anti ß-tubulin antibody (magenta). Images were acquired using a 20X objective (0.75 NA).

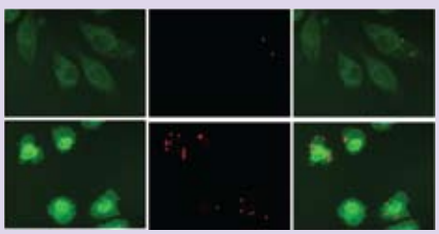
High-Content Imaging Reagents

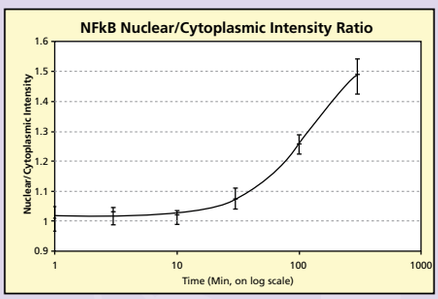
BD Biosciences Bioimaging Certified Reagents provide high performance conjugated and purified antibodies based on an internal qualification program that ensures optimal signal intensity and localization. BD reagents can be used for a full range of multiplexed bioimaging applications including: cell cycle analysis by quantification of cells in M and S phase using a one-step staining reaction; assessment of genotoxic effects in cultured cells using the micronucleus assay; and exploring organelle vectors with fluorescent proteins for monitoring protein trafficking, gene activation, cellular differentiation, and more.



Multiplexing NF-κB and Bacterial Replication Assays

Representative pseudocolored cropped confocal collapsed stack images (40x, 0.9 NA). NF-κB protein is green, bacteria are red, and colocalized signals appear as merges of these color channels. The log scale plot at the upper right shows measurements of bacterial count per macrophage (n = 3 wells). The log scale plot at the lower right shows the ratio of NF-κB intensity in the nucleus (n = 3 wells) over the NF-κB intensity of the cytoplasm.





Technical Expertise from BD

Service and Support

BD Biosciences is fully committed to the success and satisfaction of its customers. BD Pathway systems are backed by a world-class service and support organization with unmatched experience in cell science. Since 1974, BD has innovated cell analysis for optimal performance, ease of use, and improved workflow. This expertise is made available to BD Pathway customers through comprehensive training, applications and technical support, and expert field service.

Technical Application Support
BD Biosciences technical applications support specialists are available to provide field- or phone-based assistance and advice. They are well equipped to address customer needs in both instrument and applications support, including input on high content imaging reagents such as the BD™ Cell Cycle Kit, BD Gentest™ Micronucleus Assay Kit, BD Pharmingen™ FP organelle vectors, and BD™ Ratiomax Calcium Reagent Kit.

Field Service
When instrument installation or service is required, a BD Biosciences Technical Field Service Engineer can be dispatched to the customer site. BD Biosciences field service engineers are located across the world. On-site service and maintenance agreements are available to provide long-term support for BD Pathway systems.

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