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BD CELLVIEW™ IMAGE TECHNOLOGY:
Bringing New Images to Flow

Fluorescence-activated cell sorting (FACS) and microscopy have long been trusted methods for scientists to study single cells within a population. Previous attempts to combine the two technologies have been met with the challenge of speed, or rather the lack thereof. Current camera-based image technologies are not fast enough to support high-throughput cell sorting at speeds ranging from thousands to tens of thousands of events per second.

The novel BD CellView™ Image Technology overcomes this challenge by using fluorescence real-time imaging with radiofrequency-multiplexed excitation. Combining BD CellView™ Image Technology and high-throughput cell sorting unlocks new and diverse applications beyond immunology, including oncology, cell biology, plant biology, microbiology, and genomics.[1,2]

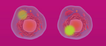
BD CELLVIEW™ IMAGE FEATURES[3]

BD CellView™ Image Technology enables imaging using scatter and fluorescent detectors to reveal the internal and external spatial characteristics of a cell, without the use of a traditional camera.

In flow cytometry, fluorochrome stained cells pass through laser beams one at a time, producing signals that are collected as electronic pulses. With BD CellView™ Image Technology, the blue laser beam is directed through an acousto-optic deflector and split into 100 separate beams. The unique optics cause each beam to blink at its own frequency. As the cell passes through the array of beams, each beam generates a signal that is collected onto a PMT. Frequencies are mathematically distinguishable, making it possible to separate each beam from the complex combined signal generated by a passing cell. The frequencies are constructed to produce a complete 2D image of the cell.

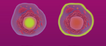
With BD CellView™ Image Technology, traditional flow parameters like scatter and fluorescence can be combined with image features such as eccentricity, total intensity, size, and many more. In addition, a light loss detector collects the incident light as it passes through a pinhole to produce images similar to brightfield images collected on a standard microscope.

ECCENTRICITY is the ratio of the shortest and longest axis of a particle. Low eccentricity indicates that the axes are close to the same length, while high eccentricity represents a signal with variable axes lengths.



MAX INTENSITY is measured as the brightest pixel in an image.

SIZE is the number of pixels that are brighter than the defined background brightness.



RADIAL MOMENT is the average distance of the signal from the center of the region of analysis.

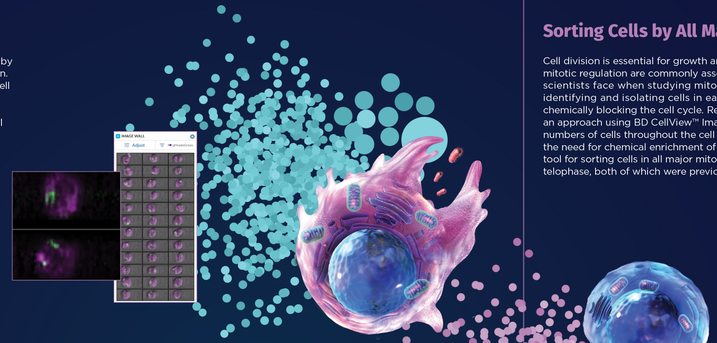
When measuring the location of two fluorescent signals relative to one another, **CORRELATION** is the degree of signal overlap.

DELTA CENTER OF MASS is the distance between the two fluorescent signals.

DIFFUSIVITY is a measure of how concentrated or spread out a signal is within the cell.

Spatial Sorting: Phagocytosing Cells

Antigen Presenting Cells (APCs) engulf pathogens such as bacteria by phagocytosis, which is crucial for immune surveillance and activation. After phagocytosis, APCs present pieces of the pathogen on their cell surface to activate T cells for a strong immune response. Detecting phagocytosis by flow cytometry has been technically challenging because a signal on the cell surface is indistinguishable from a signal within the cell by traditional technologies. BD CellView™ provides confirmation of signal location to accurately visualize phagocytosis, distinguishing between bound and internalized particles



- Scientists studied the phagocytosis of pHrodo™ Green *E. coli* BioParticles™ with BD CellView™ Image Technology. After incubating the green particles with the fibroblasts (NIH3T3), the researchers evaluated cells based on features including **LIGHT LOSS**, **RADIAL MOMENT**, and **ECCENTRICITY**. Phagocytosis of pHrodo Green *E. coli* BioParticles by fibroblasts was confirmed with real-time imaging features to identify fibroblasts containing green fluoresence.

THE SCIENTISTS BEHIND THE RESEARCH[4]
This work was carried out at VIB Flow Core by Dr. Tania Løve Aaes (Postdoctoral Researcher, VIB-UGent Center for Inflammation Research) ,Julie Van Duyse (Flow Cytometry Expert, VIB Core) and Gert Van Isterdael (Head of Flow Core, VIB).

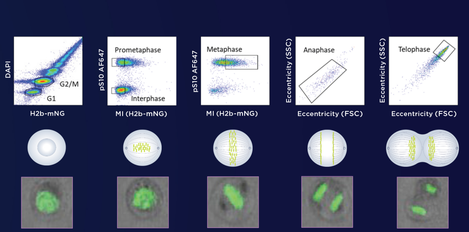
This approach has important implications for future infectious disease research. BD CellView™ Image Technology provides the tools to detect and enumerate phagocytosis in both classical (e.g. macrophages) as well as nonclassical (e.g. fibroblasts) phagocytes, and also sort these cells for downstream applications including single cell RNA sequencing and cytokine/chemokine secretion studies of function and plasticity.

Sorting Cells by All Major Mitotic Phases

Cell division is essential for growth and development, and defects in mitotic regulation are commonly associated with cancer. One challenge scientists face when studying mitosis with traditional methods is identifying and isolating cells in each stage of division, without chemically blocking the cell cycle. Researchers at EMBL developed an approach using BD CellView™ Image Technology for sorting large numbers of cells throughout the cell cycle. This novel assay bypasses the need for chemical enrichment of cell cycle phases and provides a tool for sorting cells in all major mitotic stages, including anaphase and telophase, both of which were previously inaccessible by any method.[1]

Researchers engineered cells expressing the histone H2B tagged with a fluorophore called mNeonGreen (H2B-mNG). This tagged histone enables visualization of changes to the chromatin as a cell progresses through mitosis. The scientists identified G2 and mitotic cells (G2/M) based on total DNA content, which has historically been the extent of cell cycle resolution using flow cytometry. Based on **FSC** and **SSC** images, the researchers derived **RADIAL MOMENT** and **ECCENTRICITY** features that provided information about cell morphology to further differentiate cells in anaphase and telophase. The image-enabled **MAXIMUM INTENSITY** of H2B-mNG fluorescence helped distinguish cells in different mitotic phases based on chromatin compaction and distribution within the cell.[1]

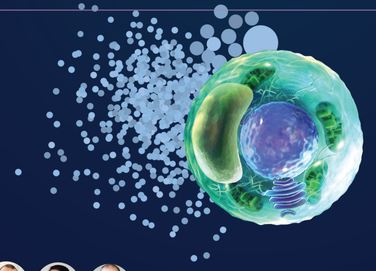
Image-enabled visualization of cell morphology and chromosomes, combined with high-speed sorting, allowed researchers to purify populations of cells at each step of mitosis without the need for chemical cell cycle blockers. Cells were sorted with BD CellView™ Image Technology by visualizing stage-specific subcellular molecular changes, such as chromosome segregation, which traditional flow cytometry methods cannot capture.[1] Sorted cells can be analyzed for downstream applications, for example evaluation of the transcriptome, proteome, or epigenome. High-speed enrichment of cells based on imaging features provides a powerful new tool for basic research, cell-based diagnostics, cell atlas efforts and high-content image screening.



THE SCIENTISTS BEHIND THE RESEARCH[1]
This work was carried out at EMBL by Dr. Daniel Schraivogel (Research Staff Scientist, EMBL) and Dr. Terra Kuhn (Postdoctoral Researcher, EMBL)

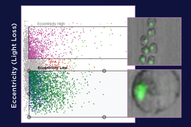
Achieving Cleaner Resolution of Solid Samples

From the discovery of new cell types to unraveling complex signaling networks that shape root development, single cell genomics is redefining plant biology research. However, scientists have not widely adopted single cell genomics in plants due to sample preparation challenges. Clearing plant cell wall debris is essential for high quality single cell experiments. Historically, researchers have relied on cell sorters that use light scattering to distinguish single cells from debris, but larger plant cells can be similar in size to cell doublets and debris, making it difficult to cleanly isolate plant cells of different sizes.[4,5]



THE SCIENTISTS BEHIND THE RESEARCH[4]
This work was carried out at VIB Flow Core by Dr. Moritz Nowack (Professor, VIB-UGent Center for Plant Systems Biology), Dr. Rafael Buono (Postdoctoral Researcher, VIB-UGent Center for Plant Systems Biology) and Gert Van Isterdael (Head of Flow Core, VIB).

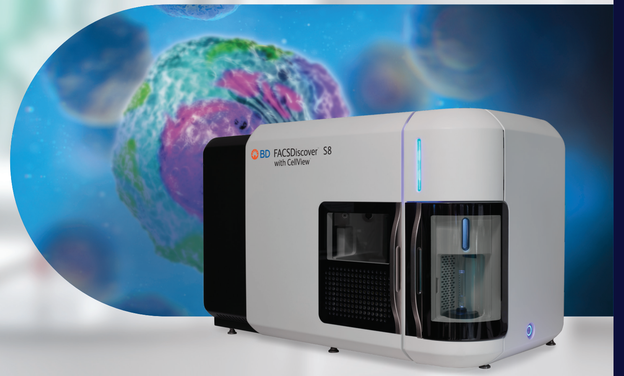
BD CellView™ Image Technology can assist plant researchers with sample clean-up challenges in their single cell genomics experiments. By combining traditional fluorescent parameters with image-based features such as **ECCENTRICITY** and **SIZE**, scientists were able to identify and sort both large and small *Arabidopsis* root protoplasts while excluding cell doublets and debris. Low **ECCENTRICITY** protoplasts were further enriched for eGFP expression to isolate cells of a specific lineage, developmental stage, or gene editing status. With BD CellView™ Image Technology, these researchers were able to make rapid and confident sorting decisions, confirmed by real-time images of each gated population during sorting.[4]



The highly enriched protoplasts obtained with BD CellView™ Image Technology are ideal for downstream single cell and bulk genomic applications, such as transcriptomics and functional genomic screening. BD CellView™ Image Technology has opened the gates to isolating cleaner and less biased samples from plant tissues than traditional cell sorters.[4]

REAL-TIME SINGLE CELL IMAGING WITHOUT CAMERA LIMITATIONS

Once sorted using BD CellView™ Image Technology, cells can be analyzed for specific molecular changes such as transcriptomic, proteomic, or epigenetic profiling. BD CellView™ Image Technology enhances FACS methods with live visual inspection of target cells and novel gating strategies based on real-time images and the spatial distribution of fluorescence signals, a unique capability among cytometers. Combining the spatial information of images with the speed of flow cytometric cell sorting has broad implications for dreaming up new sorting-dependent experimental strategies[1-3]



BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology

- Obtain insights on cell populations and characteristics that can be **visually confirmed in real time** during analysis and sorting
- Attain **high-dimensional analysis** with up to five lasers and 78 fluorescent detectors, six image detectors on the blue laser, FSC and SSC detectors
- **Flexible sorting:** 6-way 5-mL sort, index sorting and additional format options including 96-well and 384-well plates and slides
- **Index flow and cell imaging data** correlates immunophenotyping, imaging and downstream assay results
- Enhance spectral flow cytometry with **spatial and morphological insights** to interrogate and sort cell types that previously could not be identified or isolated
- Combine **image features with flow parameters** for subset classification for statistical analysis and sorting

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ABOUT BD

As pioneers of the fluorescence activated cell sorting (FACS) technology, BD Biosciences has provided flow cytometry expertise, world class support and continuous innovation over more than 45 years and enabled the routine or complex FACS analysis needed to propel the goals of laboratories around the globe. Our work and our technology are currently changing how the world looks at an individual cell. Every day we partner with our customers to help reveal the identity and function of billions of cells, one at a time.

True to BD's mission of advancing the world of health™, we continue to be at the forefront of discovery to shape the future with new innovations in informatics, genomics, proteomics and image-based sorting, and we proudly offer a large and extensive portfolio of products to address the needs of our customers.

REFERENCES

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2. D. Schraivogel, L.M. Steinmetz, "Cell sorters see things more clearly now," *Mol Syst Biol*, e11254, 2023. doi:10.15252/msb.202211254
3. "Take a Behind-the-scenes Look at BD CellView™ Image Technology," *BD Biosciences*, https://www.bdbiosciences.com/en-us/learn/campaigns/cell-view-image-technology#Technology, accessed August 11, 2023.
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5. J.R. Wendrich et al., "Vascular transcription factors guide plant epidermal responses to limiting phosphate conditions," *Science*, 370(6518):eaay4970, 2020. doi:10.1126/science.aay4970

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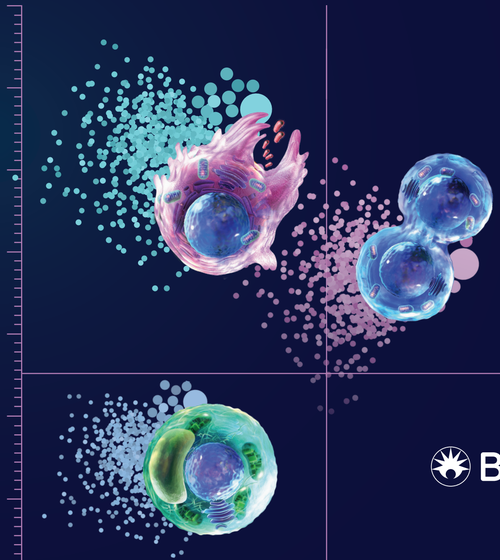

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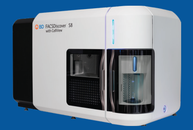
See what you sort
Sort what you see



Reveal cell populations with real-time image-enabled gating and sorting

Open up new areas of discovery. Amplify the power of flow cytometry with innovative technology—cells can finally be sorted with spatial and morphological insights on the first real-time imaging, spectral flow cytometer. The BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology empowers researchers to study cells that were previously impossible to identify or isolate. Be part of the next era of breakthroughs enabled by these advanced technologies.

Check out the poster inside and submit your BD CellView™ Image Technology Application idea for a chance to run it on the BD FACSDiscover™ S8 Cell Sorter.

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