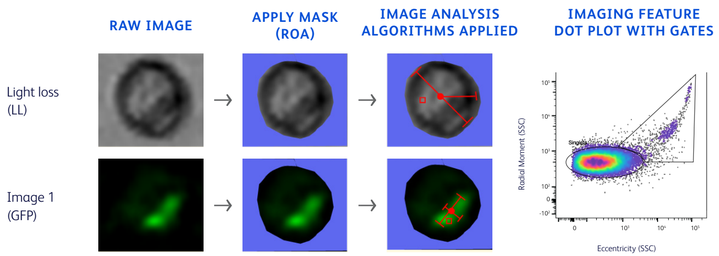
Real-time image-enabled cell sorting

using BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology

Real-time imaging enhances fluorescence activated cell sorting (FACS) with live visual inspection of target cells and novel gating strategies based on image features. It enables access to detailed information about cells that was previously invisible in traditional flow cytometry experiments and can be used to answer complex biological questions, such as how cells grow, function and interact, or to study exact locations of viruses or proteins within a cell, all at a highly accelerated pace.

Image Feature Extraction

Image feature extraction with BD CellView™ Image Technology occurs in real-time as raw images are analyzed by firmware components within the cytometer. Image features are numerical measurements of different aspects of the image such as size and shape and are calculated based on the Region of Analysis (ROA). Much like traditional fluorescence, image features can be used to generate dot plots to identify populations of interest which can then be gated and sorted.



Learn how image features are generated using BD CellView™ Image Technology.

Click on the video thumbnail at the right or scan the QR code.

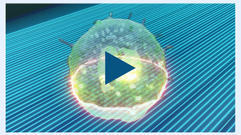


Image Features

FEATURE AND APPLICATIONS	EXPLANATION	EXAMPLE DIAGRAMS AND HISTOGRAMS
Eccentricity (Scatter and fluorescence) » Morphology » Cluster identification » Doublet discrimination	**Eccentricity =** Long axis / Short axis	Low Eccentricity Int. Eccentricity High Eccentricity Count Eccentricity (FITC)
Radial Moment (Scatter and fluorescence) » Morphology » Cluster identification » Doublet discrimination	Centroid (relative center) **Radial Moment =** – Median distance of all pixels from the centroid	FITC Signal Centrally Concentrated FITC Signal Radially Spread Count Radial Moment (FITC)
Size (Scatter and fluorescence) » Morphology » Label-free sorting » Cell cycle	**Size =** □ Pixel count	Small Cells Large Cells Count Size (SSC)
Total Intensity (Scatter and fluorescence) » Morphology » Imaging FMOs	**Total Intensity =** □ Additive intensity of all pixels	Low Total Intensity Int. Total Intensity High Total Intensity Count Total Intensity (FITC)

Image Features (continued)

FEATURE AND APPLICATIONS	EXPLANATION	EXAMPLE DIAGRAMS AND HISTOGRAM
Correlation (Fluorescence: Any two channels) » Nuclear translocation » Co-localization » Intracellular trafficking	Co-localized pixels **Correlation =** □ Score based on the percentage of pixels from two fluorescence channels that occupy the same space	Uncorrelated Correlated Count Correlation
Delta Center of Mass (Fluorescence: Any two channels) » Intracellular trafficking » Cell-cell interaction	Centroid of red fluorophore (relative center) Centroid of green fluorophore (relative center) **Delta Center of Mass =** – Distance between the centroids of two fluorescence channels	Low Delta Center of Mass Int. Delta Center of Mass High Delta Center of Mass Count Delta Center of Mass
Max Intensity (Scatter and fluorescence) » Punctate fluorescence » Phagocytosis assay » Cell cycle analysis	**Max Intensity =** ▪ Brightest pixel	Dim Fluorescent Cells Int. Fluorescent Cells Bright Fluorescent Cells Count Max Intensity (FITC)
Diffusivity (Scatter and fluorescence) » Punctate fluorescence » Cell morphology » Phagocytosis	**Diffusivity =** Fluorescence-A / Max Intensity	Low Diffusivity Int. Diffusivity High Diffusivity Count Diffusivity