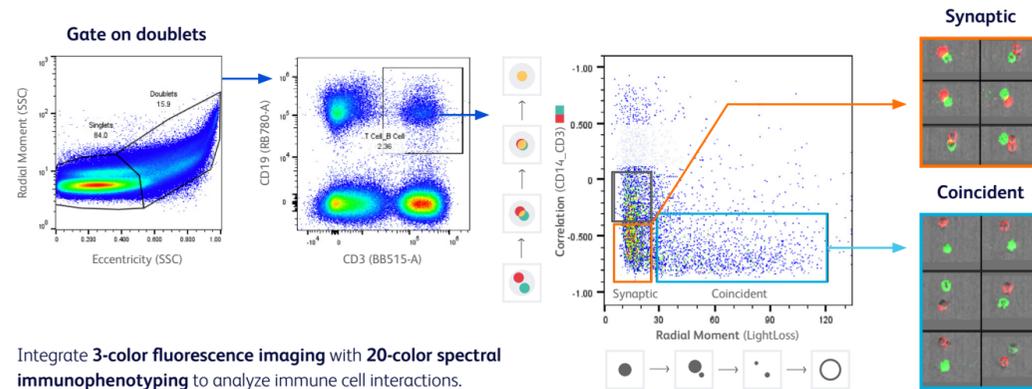


Examples of BD CellView™ Image Technology parameters in action

Cell-cell interactions: Use image parameters to define synaptic immune complexes

Reveal true synaptic cell-cell interactions with **Correlation** and **Radial Moment** metrics that identify doublets.

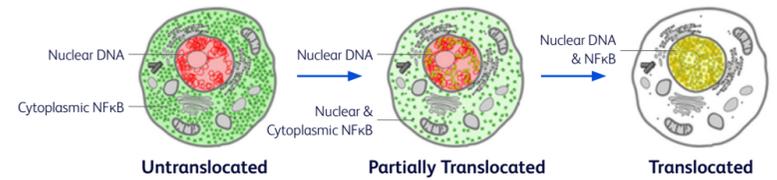
- IMG CH 1 (CD3 BB515)
- IMG CH 2 (CD14 PE-CF594)
- IMG CH 3 (CD19 RB780)
- LightLoss



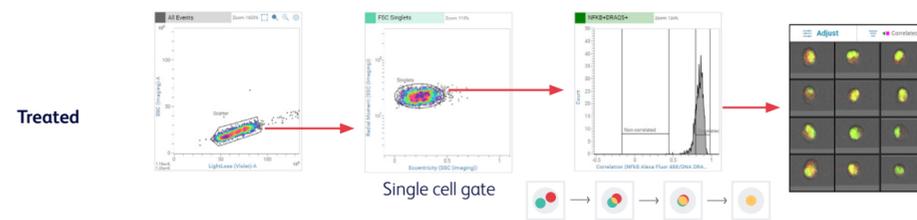
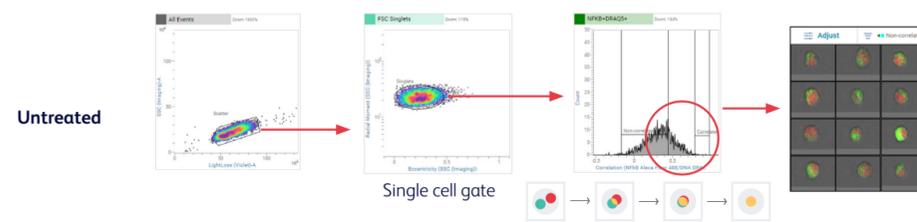
Integrate 3-color fluorescence imaging with 20-color spectral immunophenotyping to analyze immune cell interactions.

Nuclear translocation: Measure signal co-localization using NFκB nuclear translocation assay

Correlation of fluorescent tag and DNA dye can identify target protein localization into the nucleus.

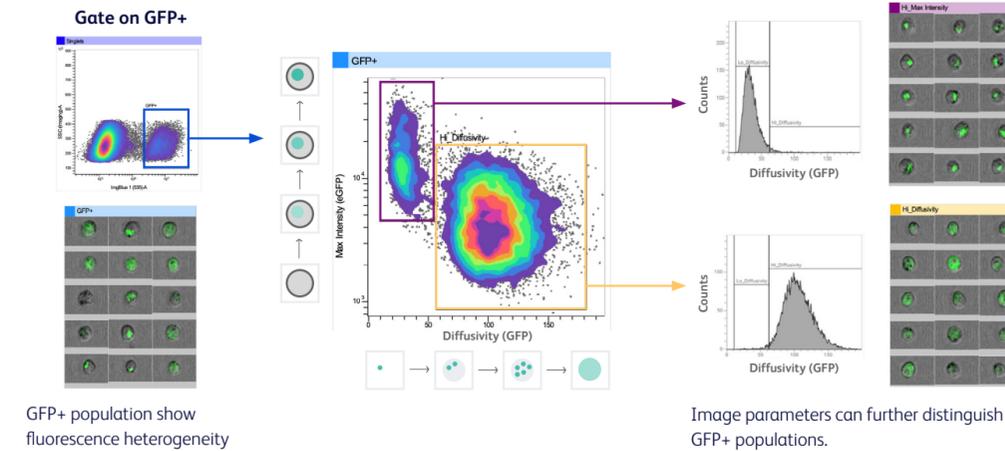


See how activation of NFκB signaling pathway results in nuclear translocation of NFκB transcription factors. Use Correlation of NFκB (AlexaFluor™488) and DNA (DRAQ5™) to observe nuclear translocation of NFκB.



Protein localization: Visualize heterogeneity of GFP+ populations

Diffusivity and **Max Intensity** can be used to distinguish spatial distribution of GFP (Green Fluorescent Protein) signal within the cell.

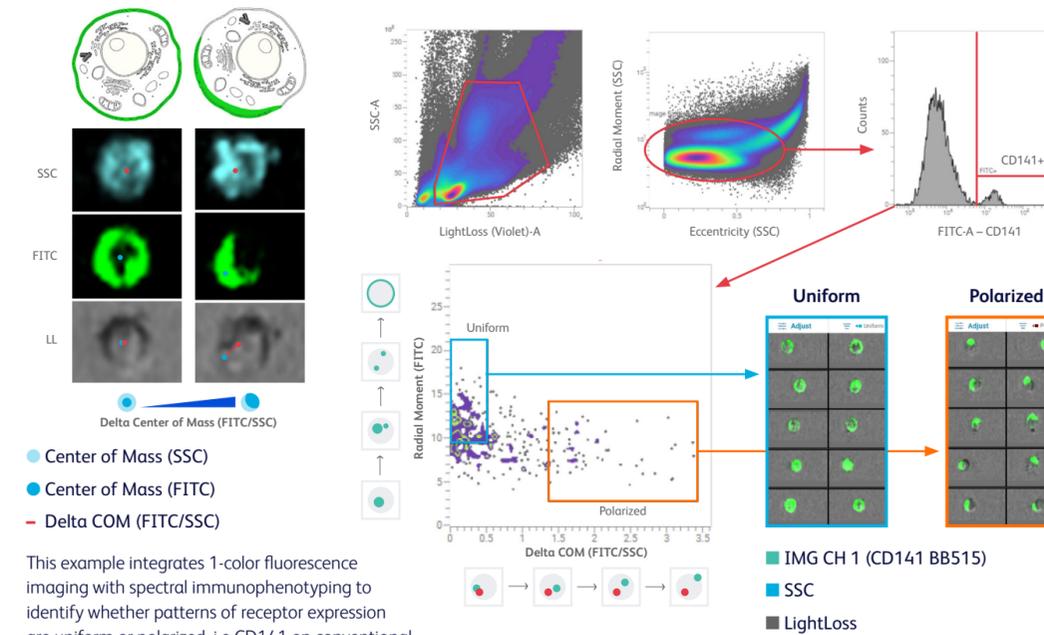


GFP+ population show fluorescence heterogeneity

Image parameters can further distinguish true GFP+ populations.

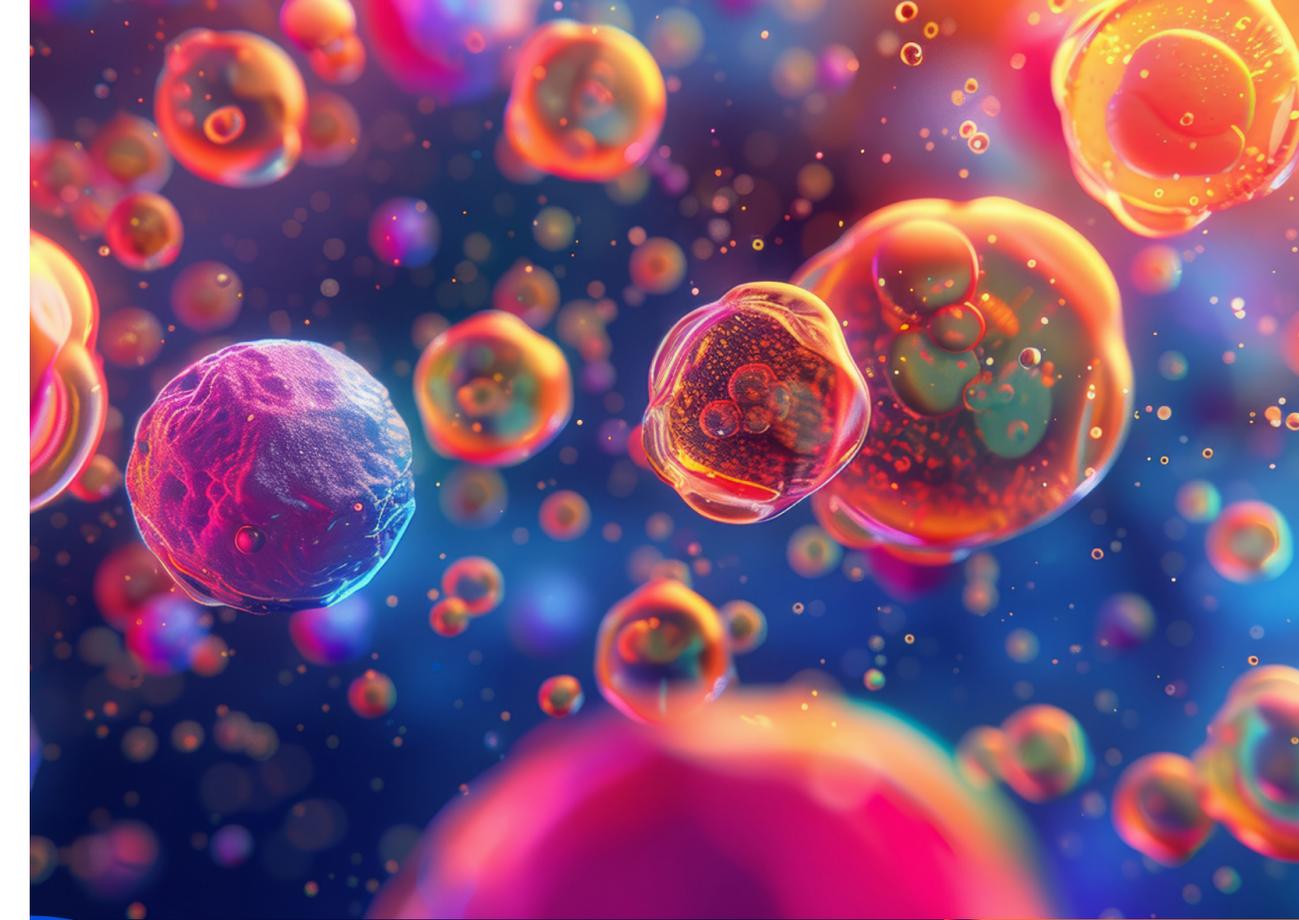
Receptor distribution: Understanding different patterns of receptor expression on the cell surface

Radial Moment and **Delta Center of Mass** can be used to assess receptor distribution on a cell.



- Center of Mass (SSC)
- Center of Mass (FITC)
- Delta COM (FITC/SSC)

This example integrates 1-color fluorescence imaging with spectral immunophenotyping to identify whether patterns of receptor expression are uniform or polarized, i.e CD141 on conventional Dendritic cells (cDC)



BD CellView™ Image Technology parameter guide
Discover what others can only imagine



BD CellView™ Image Technology

Image Parameters

Image parameters are numerical measurements of different aspects of the spacial features such as size and shape. Image parameters are extracted in real time and analyzed by firmware components within the cytometer. Much like traditional fluorescence, extracted image parameters can be used to generate dot plots to identify populations of interest which can then be gated and sorted.

BD flow cytometers are Class 1 Laser Products. For Research Use Only. Not for use in diagnostic or therapeutic procedures.
 BD Biosciences, Milpitas, CA 95035, USA | bdbiosciences.com
 BD, the BD Logo, BD CellView, BD FACSDiscover and BD SpectralFX are trademarks of Becton, Dickinson and Company or its affiliates. © 2025 BD. All rights reserved. BD-167207 (v1.0) 1225



IMAGE PARAMETERS AND APPLICATIONS

EXPLANATION

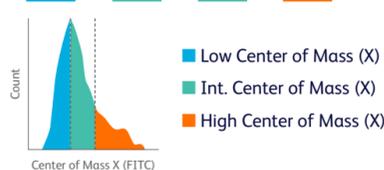
EXAMPLE DIAGRAMS AND HISTOGRAM

Center of Mass (X) (Scatter and fluorescence)

- » Internalization
- » Cell-cell interaction
- » Intracellular trafficking

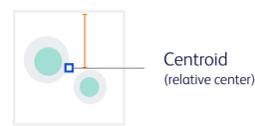


Center of Mass (X): Distance between the left edge of the field of view (→) and relative center (centroid) (□) of signal distribution



Center of Mass (Y) (Scatter and fluorescence)

- » Internalization
- » Cell-cell interaction
- » Intracellular trafficking

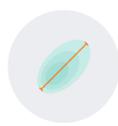


Center of Mass (Y): Distance between the top edge of the field of view (→) and relative center (centroid) (□) of signal distribution

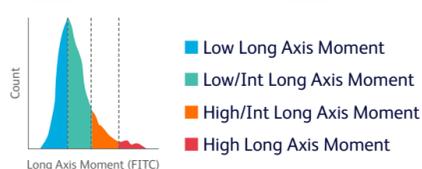


Long Axis Moment (Scatter and fluorescence)

- » Cellular morphology
- » Cluster identification
- » Doublet discrimination



Long Axis Moment: The longest axis across the distribution of signal (→)



Short Axis Moment (Scatter and fluorescence)

- » Cellular morphology
- » Cluster identification
- » Doublet discrimination



Short Axis Moment: The shortest axis across the distribution of signal (→) that is perpendicular to the long axis moment



IMAGE PARAMETERS AND APPLICATIONS

EXPLANATION

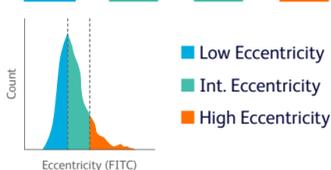
EXAMPLE DIAGRAMS AND HISTOGRAMS

Eccentricity (Scatter and fluorescence)

- » Morphology
- » Cluster identification
- » Doublet discrimination

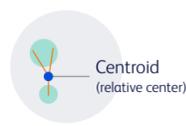


Eccentricity:
 Long axis (→)
 Short axis (→)

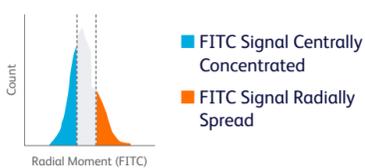


Radial Moment (Scatter and fluorescence)

- » Morphology
- » Cluster identification
- » Doublet discrimination



Radial Moment:
 Median distance of all pixels from the centroid (→)

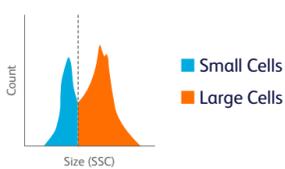


Size (Scatter and fluorescence)

- » Morphology
- » Label-free sorting
- » Cell cycle



Size: Pixel count (□)



Total Intensity (Scatter and fluorescence)

- » Morphology
- » Imaging FMOs



Total Intensity:
 Additive intensity of all pixels (□)

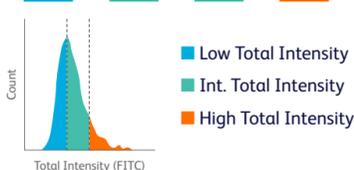


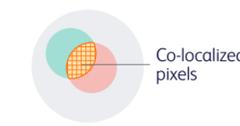
IMAGE PARAMETERS AND APPLICATIONS

EXPLANATION

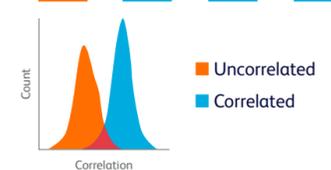
EXAMPLE DIAGRAMS AND HISTOGRAM

Correlation (Fluorescence: Any two channels)

- » Nuclear translocation
- » Co-localization
- » Intracellular trafficking

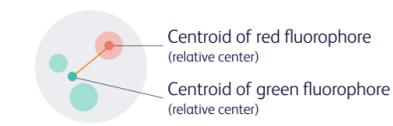


Correlation: Score based on the percentage of pixels from two fluorescence channels that occupy the same space (□)

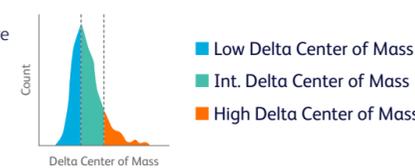


Delta Center of Mass (Fluorescence: Any two channels)

- » Intracellular trafficking
- » Cell-cell interaction



Delta Center of Mass:
 Distance between the centroids of two fluorescence channels (→)

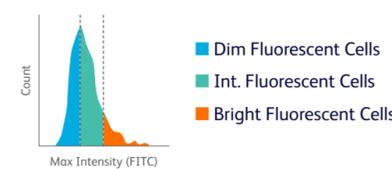


Max Intensity (Scatter and fluorescence)

- » Punctate fluorescence
- » Phagocytosis assay
- » Cell cycle analysis



Max Intensity: Brightest pixel (□)



Diffusivity (Scatter and fluorescence)

- » Punctate fluorescence
- » Cell morphology
- » Phagocytosis



Diffusivity:
 $\frac{\text{Fluorescence-A}}{\text{Max Intensity}}$ (□)

