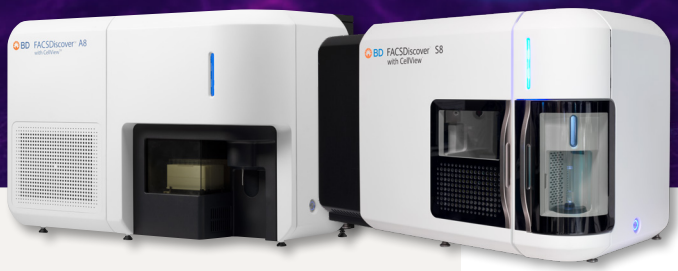
EXPAND

Analyze and sort cell-cell interactions in real-time using spectral flow cytometry to accelerate drug discovery in immuno-oncology



Key takeaways:

The BD FACSDiscover™ Platform integrates spectral flow cytometry with spatial and morphological insight to unlock complex cell-cell interactions in flow cytometry. Learn how BD CellView™ Image Technology powers your immunotherapy research in the following ways:

- Enables precise identification of immunological synapses
- Automatically generated imaging parameters reveal overlapping synapses
- Combines label-free and fluorescence imaging to distinguish true synaptic events from coincident events

Decoding cell-cell interactions is key to making progress in immunotherapy

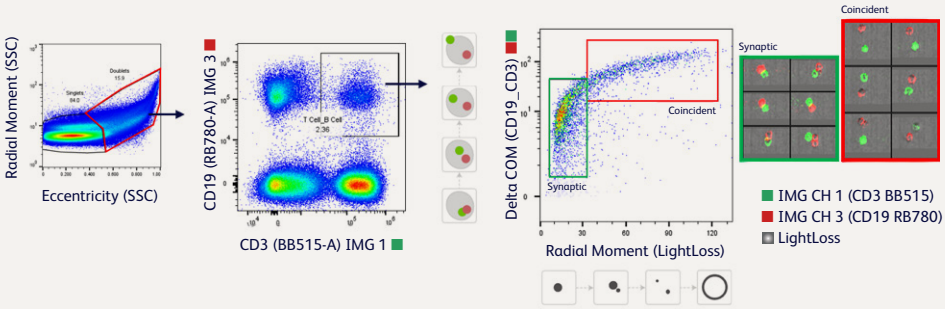
Immune cells engage in coordinated communication by forming **immunological synapses**, specialized junctions where receptors, ligands and signaling molecules converge to mediate responses critical for therapeutic development. These transient yet decisive interactions govern how T cells recognize and kill tumor cells, how macrophages polarize and how tumors evade immune attack. In immuno-oncology, decoding these interactions reveals the cellular choreography behind activation, suppression and therapeutic response, providing critical insight that guides drug and biomarker discovery.

The BD FACSDiscover™ Platform overcomes the limitations of traditional flow cytometry and enables real-time analysis and sorting of synaptic events

In traditional flow cytometry, discriminating between coincident events, associated cells without biological relevance, and synaptic events is a major challenge. Because of this, doublets are often believed to be technical artifacts and are discarded. This has also made sorting synaptic events with confidence impossible. By combining spectral flow cytometry with real-time imaging, the BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter now facilitate the identification of important cell-cell interactions in following ways.

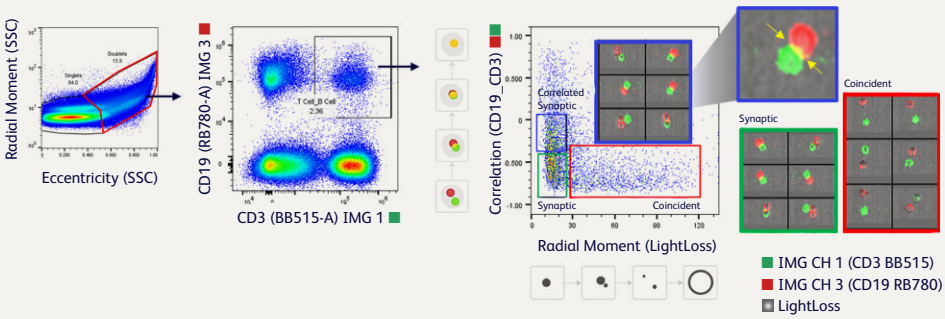
1. **Confident identification of biologically relevant synapses using real-time imaging flow cytometry:** Using a combination of label-free imaging parameters (Radial Moment of Light Loss) and imaging parameters derived from fluorescence signals on target cells (Delta Center of Mass), the BD FACSDiscover™ Platform enables the discrimination of coincident and synaptic events (Figure 1). This is particularly powerful given that the frequency of synaptic events is associated with disease states.[1]

FIGURE 1. Plotting radial moment (light loss) vs. the delta center of mass permits confident discrimination of coincident and synaptic events.



2. **Identification of overlapping synapses with automatically generated parameters:** Using automatically generated imaging parameters with the correlation of two target fluorescent imaging channels (a slight deviation from the approach utilized for Figure 1 above) supports not only the identification of coincident and synaptic events but also overlapping synapses, which are indicated with a higher correlation value (Figure 2).

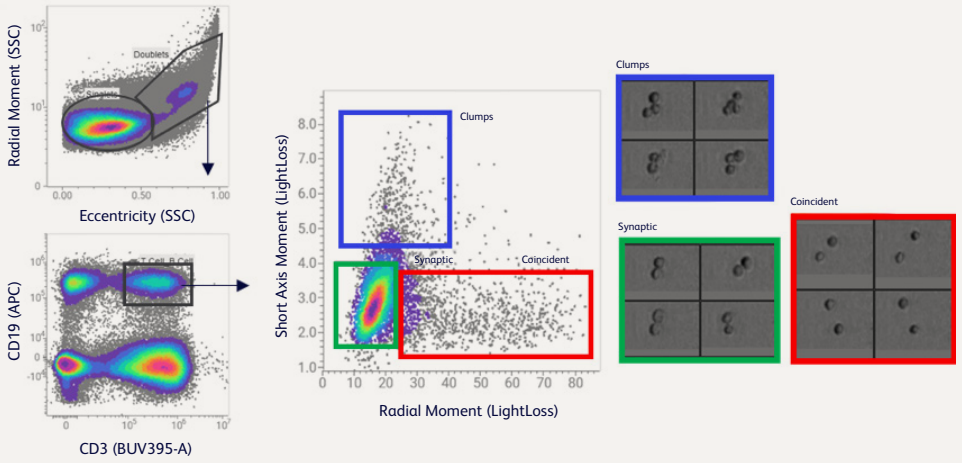
FIGURE 2. Using the correlation of two target fluorescent imaging channels supports the identification of coincident events, synaptic events and overlapping synapses, which are identified via a higher correlation value (driven by the overlap). Overlapping synapses appear yellow due to the overlap of red CD19 and green CD3.



Radial Moment is an imaging feature that is automatically applied to images generated on all the 6 imaging channels on the instrument, making it a quantitative imaging parameter. It is defined as the average distance of the pixels from the centroid within the region of analysis. In this application highlight we used Radial Moment to detect cell-cell interactions (immunological synapses) and paired Radial Moment with another imaging feature, Eccentricity, to discriminate doublets.

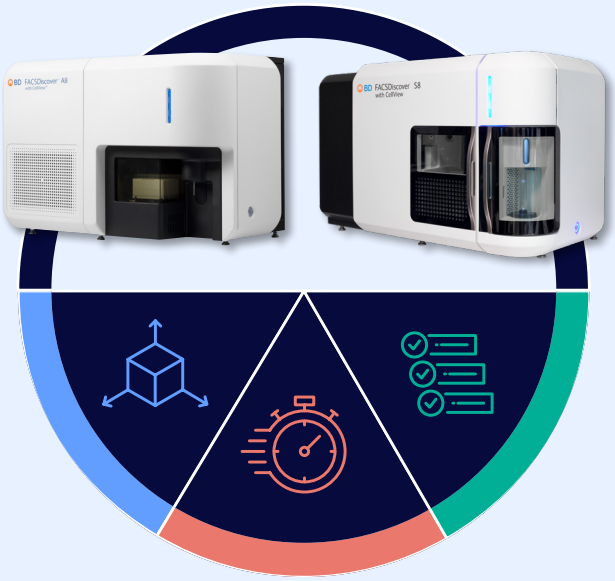
3. **Identification of synaptic events with label-free imaging:** In cases where fluorescence cannot be detected on target cells or when fluorescence reagents are preferentially used for markers of interest expressed by the target cells (e.g., activation makers, immune checkpoint markers), users can identify synaptic events with label-free imaging. In the below example (Figure 3), where no fluorescence is used for synaptic markers, a combination of lineage-specific spectral reagents and a double positive gating strategy are leveraged to identify synaptic events using label-free imaging.

FIGURE 3. Lineage-specific spectral reagents (CD3 BUV395 and CD19 APC) were used to define gates. A double positive was used to determine the phenotype of the doublet and label-free imaging features were used to refine the analysis to only include synaptic events. Radial Moment of Light Loss was used to exclude coincident events while the Short Axis Moment was used to exclude unwanted triplets or clumps (see blackbox in set at bottom left).



	Clumps	Synaptic Doublets	Coincident Doublets
Raw Image			
Radial Moment	RM= 17	RM= 14	RM= 54
Short Axis Moment	SA=6.5	SA=2.8	SA=2.6

These examples unequivocally demonstrate that the BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter with CellView™ Image Technology can be used to identify and quantify important immune cell synaptic complexes. This provides researchers in immuno-oncology with a powerful new tool for visualizing and interrogating immune cell complexes to advance the development of the next generation of immunotherapies.



Immuno-Oncology Discovery **Reimagined**

The BD FACSDiscover ™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter with BD SpectralFX™ Technology and BD CellView™ Image Technology are transforming immuno-oncology drug discovery by uniquely integrating spectral flow cytometry, real-time spatial information and image-enabled cell sorting. This unprecedented combination empowers researchers to de-risk and accelerate the development of next-generation cancer therapies.

Choose the BD FACSDiscover™ Platform to:

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your discovery potential with a new dimension

ACCELERATE
and de-risk your discovery timelines

ACHIEVE
reproducible results with out-of-the-box standardization

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SCAN TO REIMAGINE YOUR RESEARCH

References

1 Burel JG, Pomaznoy M, Lindestam Arlehamm CS, et al. Circulating T cell-monocyte complexes are markers of immune perturbations. *eLife*. 2019; 8:e46045. doi: 10.7554/eLife.46045.