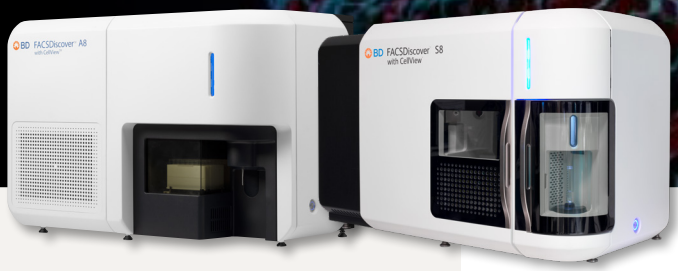
EXPAND

Supercharge your CAR T cell research in flow cytometry by integrating morphological and spatial insights in real-time



Key takeaways:

The BD FACSDiscover™ Platform integrates spectral flow cytometry with imaging to unlock spatial and morphological insights into CAR T cell function with precision. Learn how BD CellView™ Image Technology can advance your CAR T cell research in the following ways:

- Generates morphological insights to identify, quantify and sort CAR T cell subsets beyond the resolution of traditional flow cytometry
- Reveals CAR spatial organization automatically with fluorescence imaging parameters
- Enables deeper exploration into how CAR morphology and spatial organization shapes functional diversity in CAR T cells

CAR spatial organization is critical for CAR T cell function

CAR T cells, donor T cells engineered with antigen-specific chimeric antigen receptors (CARs), represent a breakthrough in cancer treatment, particularly for hematologic malignancies.[1] These personalized therapies harness a patient's own T cells, reprogramming them to recognize and eliminate tumor cells. While their precision and efficacy are promising, therapeutic efficiency is challenged by multiple factors, including CAR design and microenvironmental cues.[2]

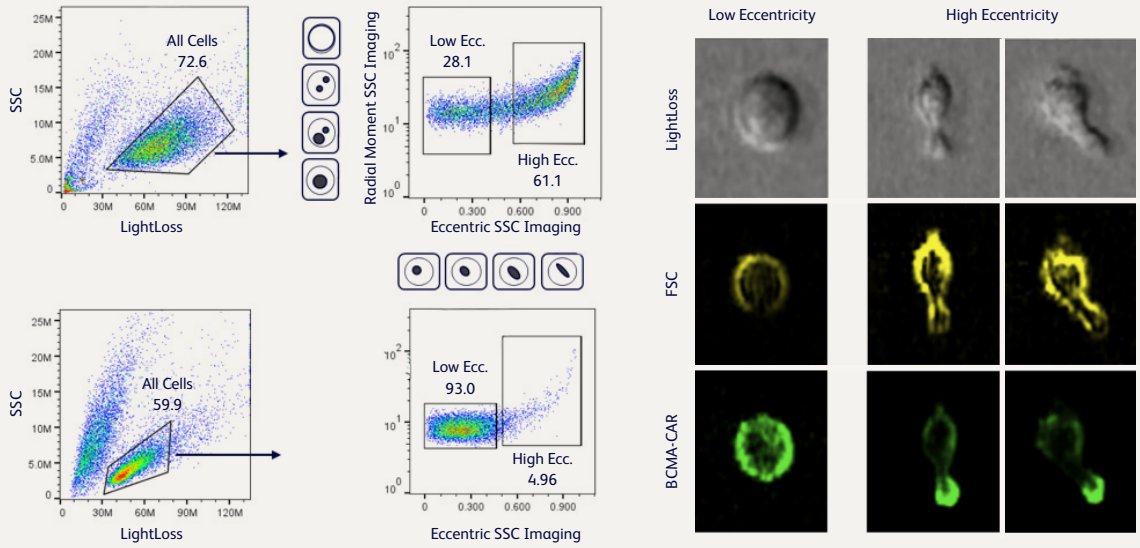
Like native T cell receptors, synthetic CARs exhibit dynamic movement across the cell surface. Imaging technologies have shown that CAR spatial organization correlates with antigen recognition, T cell signaling strength, killing potential, and even post-infusion persistence.[3,4] Imaging flow cytometry enables differentiation of cell subsets based on CAR spatial patterns, enhancing the ability to characterize and optimize next-generation CAR T therapies.

Connecting CAR spatial phenotypes with function

Powered by high-throughput, we can now effortlessly analyze CAR T morphological attributes and CAR spatial distribution alongside additional phenotypic and functional markers, as demonstrated in the following study with CAR T cells targeting the B-cell maturation and activation receptor (BCMA):

1. **Identification of morphological attributes in CAR T cells with label-free imaging:** In addition to traditional side-scatter (SSC) and forward-scatter (FSC) parameters, BD CellView™ Image Technology introduces a unique LightLoss parameter to generate label-free images. Together with the Eccentricity feature, these three parameters enable subcategorization of cells based on shape and size, allowing the distinction between round, elongated, large, small, single or clustered cells. Leveraging these insights, observed striking morphological changes in cultured BCMA-CAR T cells over time: cells became markedly elongated four days post-activation and lentivirus transduction, then returned to a rounded morphology by Day 9.

FIGURE 1. BD CellView™ Image Technology enables monitoring of CAR T cell morphological changes during culture. In the early activation phase, most CAR T cells exhibited an elongated shape with BCMA-CAR concentrated at the tips of cellular extensions, revealing dynamic spatial organization beyond the capabilities of traditional flow cytometers.



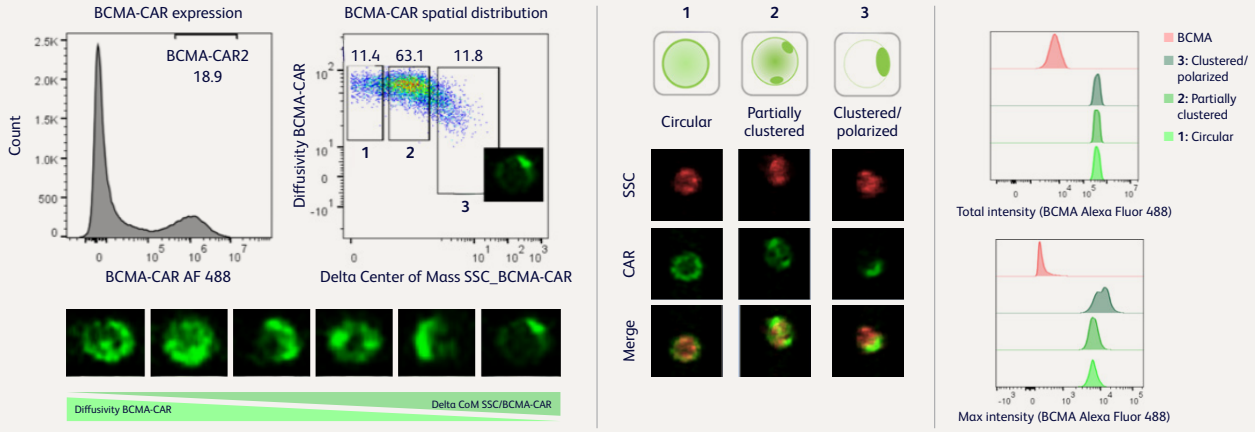
2. **CAR spatial organization revealed automatically with fluorescence imaging parameters:** Morphological features can also be extracted from up to three fluorescence imaging channels, expanding the ability to empirically determine protein maker localization and spatial distribution while preserving the speed and efficiency of flow cytometry-based phenotypic analysis. Staining the CAR T cells with the BD™ Alexa Fluor™ 488 BCMA CAR Detection Reagent enabled the distinction of CAR T cell subsets solely based on CAR morphology. Because the AF488 BCMA-CAR fluorescence signal is expected to localize on the cell surface, we assessed the Delta Center of Mass between the side scatter parameter and the AF488 BCMA-CAR parameter to visualize CAR localization. Additionally, we leveraged the Diffusivity parameter to assess the spatial distribution of the AF488 BCMA-CAR signals around the cell surface. Together, these parameters helped us to categorize the CAR T cells into three subsets:

   a) **Circular:** Highly diffuse and uniform CAR distribution around the cell membrane

   b) **Partially clustered:** Partially diffuse CAR distribution with intermittent CAR clusters

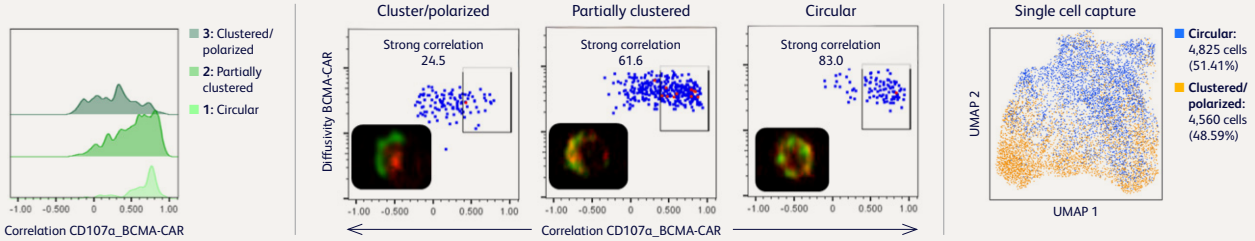
   c) **Clustered/polarized:** CAR distribution is punctate, often polarized to one direction, increasing the delta center of mass between SSC versus CAR signals

FIGURE 2. BD CellView™ Image Technology reveals distinct CAR T subsets that traditional flow cytometers cannot resolve. Although these subsets may exhibit similar total CAR expression (total intensity), differences in CAR morphology create distinct spatial phenotypes, such as cells with clustered versus uniform CAR distribution. Highly aggregated or polarized CAR subsets can be additionally distinguished by their punctate AF488 BCMA-CAR signals, highlighted by comparing max AF488 intensity across the subsets. These findings demonstrate that CAR spatial organization is independent of total CAR expression and contributes to CAR T cell population heterogeneity.



3. **Insights into CAR T cell function based on CAR spatial organization:** Beyond traditional flow cytometer capabilities, BD CellView™ Image Technology allows detailed visualization and quantification of marker localization and physical interactions, providing deeper insight into the dynamics of cellular responses. For instance, stimulation of BCMA-CAR T cells with the multiple myeloma cell line U266, which expresses BCMA, resulted in CAR T activation as evidenced by CD107a externalization. Strikingly, we observed a strong spatial overlap between CD107a and BCMA-CAR signals in cells displaying the circular or partially clustered BCMA-CAR rearrangements. These observations raised questions about the significance of CAR spatial phenotypes in relation to CAR T cell function, which we further explored by sorting cells with the different spatial phenotypes for whole transcriptome analysis using the BD Rhapsody™ HT Xpress System. Initial results revealed that cells exhibiting a circular CAR rearrangement were transcriptionally distinct from those with clustered or polarized morphology, as indicated below by the partial overlap between these groups.

FIGURE 3. CAR spatial phenotypes may predict CAR T cell function. BCMA-CAR and CD107a fluorescence signals showed strong correlation in circular and partially clustered phenotypes, suggesting colocalization of these markers during CAR T cell degranulation. In contrast, the polarized phenotype exhibited lower correlation indicating that these cells are likely less responsive to stimulation. The BD OMICS-One™ WTA Next Assay and BD Cellismo™ Data Visualization Tool reveal transcriptionally unique populations as seen in the UMAP plot.



Together these findings demonstrate the transformative potential of the BD FACSDiscover™ Platform with BD CellView™ Image Technology for high resolution CAR T cells analysis. This capability enables researchers to resolve structural differences that may influence therapeutic efficacy, supporting the development of improved CAR T cell therapies.

Eccentricity is an imaging feature that is automatically applied to images generated on the instrument's 6 imaging channels, making it a standardized quantitative parameter. It is a ratio of the shortest to the longest axis of the identified particle (as identified by the region of analysis). The inverse of Eccentricity can be utilized as circularity. Similarly, Delta Center of Mass is defined as the distance between two fluorescent signal sources in any two imaging channels within a particle as defined by the region of analysis.

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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.

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