ACCELERATE

Enhance your research with spatial flow cytometry by going beyond fluorescence: analyze and sort cells based on protein localization

Key takeaways:

The BD FACSDiscover™ Platform integrates spectral flow cytometry with imaging to unlock spatial and morphological insights into complex protein dynamics. Learn how BD CellView™ Image Technology can power your immunotherapy research in the following ways:

- Ability to evaluate Golgi-related drug targets that disrupt protein trafficking, by analyzing and sorting cells based on subcellular protein localization in real-time
- Quantifiable spatial imaging parameters in addition to fluorescence intensity
- High-throughput screening potential to measure the impact of specific therapeutics on protein trafficking across experimental variables and determine ideal conditions

Deciphering Golgi-mediated protein trafficking may unlock novel immunotherapies

Numerous proteins, including immune checkpoint proteins (e.g., PD-L1), cytokine receptors, tumor antigens and exosomal proteins, play a key role in cancer etiology and are popular therapeutic targets. These proteins are synthesized, folded and processed in the endoplasmic reticulum (ER) and then transported to the Golgi apparatus for further modifications before translocated to their final location, such as the cell surface or extracellular environment.

Disruptions to this pathway can lead to the accumulation of misfolded proteins or incorrect protein translocation, contributing to the development and progression of diseases such as cancer. In the context of drug discovery and development in immuno-oncology, these disruptions may reveal novel druggable vulnerabilities and biomarkers.[1,2]

Accelerate discovery with real-time insights on protein trafficking phenomena

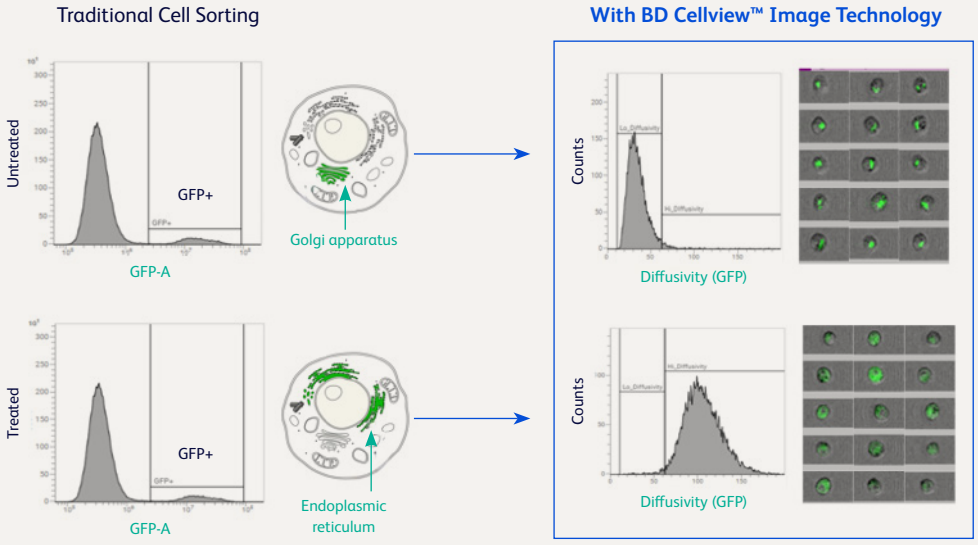
Green Fluorescent Protein (GFP) detection by flow cytometry for intracellular protein expression is for tracking localization in immuno-oncology drug discovery and development as it enables real-time, high-throughput measurement of GFP intensity in live cells. Nevertheless, traditional flow cytometry cannot identify GFP localized to specific intracellular locations, beyond total expression. This limits the potential for understanding the biology underpinning protein trafficking and disease etiology, slowing efforts to identify novel therapeutic targets and biomarkers associated with Golgi-mediated protein trafficking and modification.

By integrating imaging with spectral flow cytometry, GFP intracellular localization can be quantified, enabling real-time assessment of drug effects on protein trafficking and sorting of cell populations solely based on protein localization within specific organelles.

Using a cell line with a GFP-tagged Golgi protein and treated with Brefeldin A to block protein transport from the ER to the Golgi apparatus, we demonstrated the following capabilities unique to the BD FACSDiscover™ S8 Cell Sorter:

1. **Visualization of intracellular protein compartmentalization and sorting:** Real-time visualization of GFP localization in Brefeldin A-treated cells using the diffusivity image parameter enabled rapid differentiation of cells with GFP localized to the ER from cells with GFP localized to the Golgi apparatus (see Figure below). Cells could then be isolated and sorted for downstream analysis based on intracellular GFP localization.

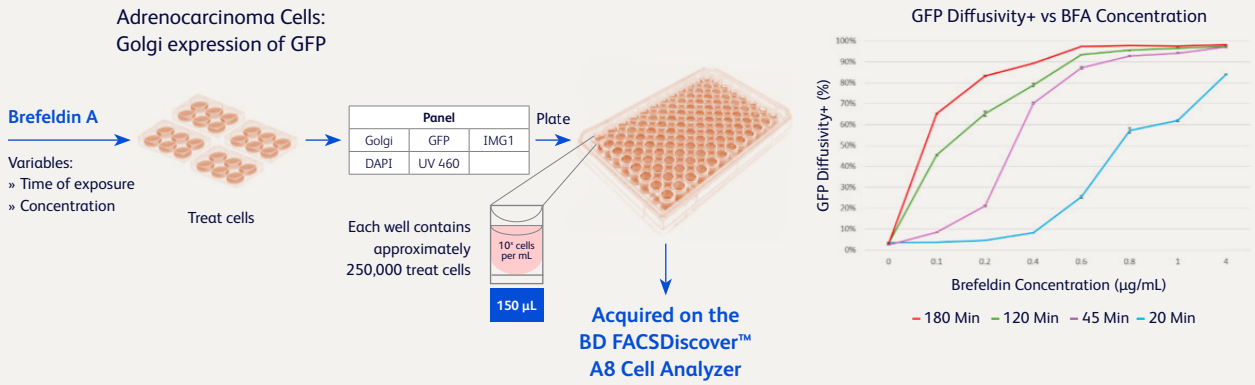
FIGURE 1. The BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology allows researchers to differentiate and sort cells based on GFP intracellular localization (ER or Golgi apparatus).



Now we call out the unique aspect of the BD FACSDiscover™ A8 Cell Analyzer, which is loader and throughput screening, different from the speed associated with the BD FACSDiscover™ S8 Cell Sorter or other imaging flow cytometers:

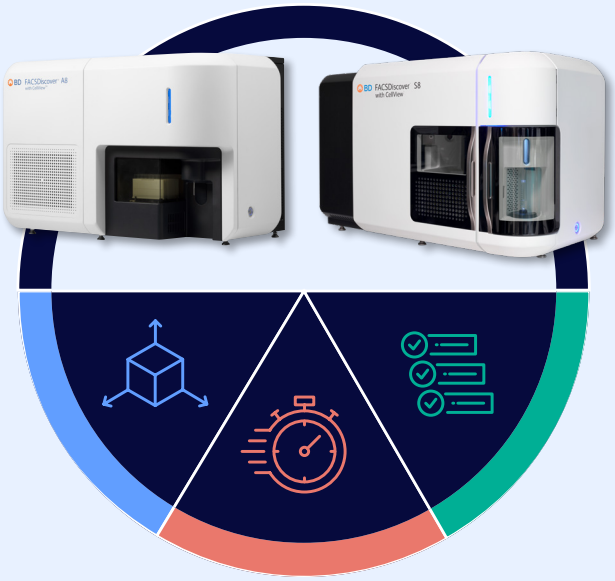
2. **High-throughput analysis of the impact of experimental variables on protein localization in cells**: The high-throughput imaging and analysis capabilities of the BD FACSDiscover™ A8 Cell Analyzer allowed us to capture 1,500 images, encompassing 30,000 events from each well of a 96 well plate. Such information can be used to reveal the optimized conditions of Brefeldin A concentration and incubation time to identify cells that are limiting protein trafficking.

FIGURE 2. The BD FACSDiscover™ A8 Cell Analyzer facilitates high-throughput analysis of the impact of experimental variables on GFP diffusivity.



Together, this data demonstrates the BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology can be used to visualize and sort cells based on subcellular protein localization and analyze the impact of experimental conditions on protein translocation in high-throughput.

Diffusivity is an imaging feature that is automatically applied to images generated on all the 6 imaging channels on the instrument, making it a quantifiable imaging parameter. The ratio of the Total Intensity to the Maximum Intensity, which are both imaging features as well. Total Intensity is the sum of the intensities of all pixels within the Region of Analysis, and Maximum Intensity is the intensity of the brightest pixel in the image. Diffusivity helps determine "diffused or punctate fluorescence," in assays beyond the example highlighted here, such as in phagocytosis and cell cycle analysis.



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:

EXPAND
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

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2 Bajaj R, Warner AN, Fradette JF, et al. Dance of The Golgi: Understanding Golgi Dynamics in Cancer Metastasis. Cells. 2022;11(9):1484. doi: 10.3390/cells11091484