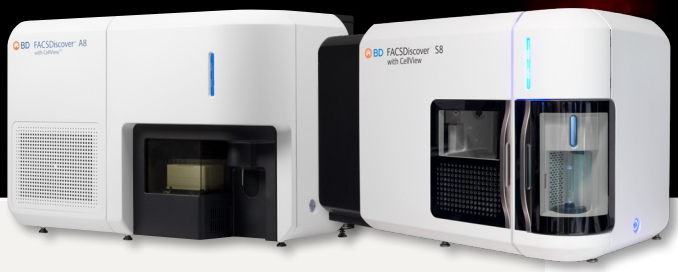
EXPAND

Remove blindspots in flow cytometry by identifying nuclear translocation of transcription factors with BD CellView™ Image Technology



Key takeaways:

The BD FACSDiscover™ Platform integrates spectral flow cytometry with real-time spatial imaging to reveal dynamic signaling events, such as nuclear translocation, that would otherwise go unseen with traditional flow cytometry. Learn how this platform can bring clarity to your research in the following ways:

- Powers deep analysis of nuclear translocation of NF-κB that cannot be captured using traditional flow cytometry
- Enables plate-based assays to analyze various levels of translocation across conditions, facilitating dose-response or time-course experiments
- Provides highly reproducible data, reducing or eliminating the need for secondary microscopy validation

Understanding NF-κB translocation, a critical readout in immuno-oncology

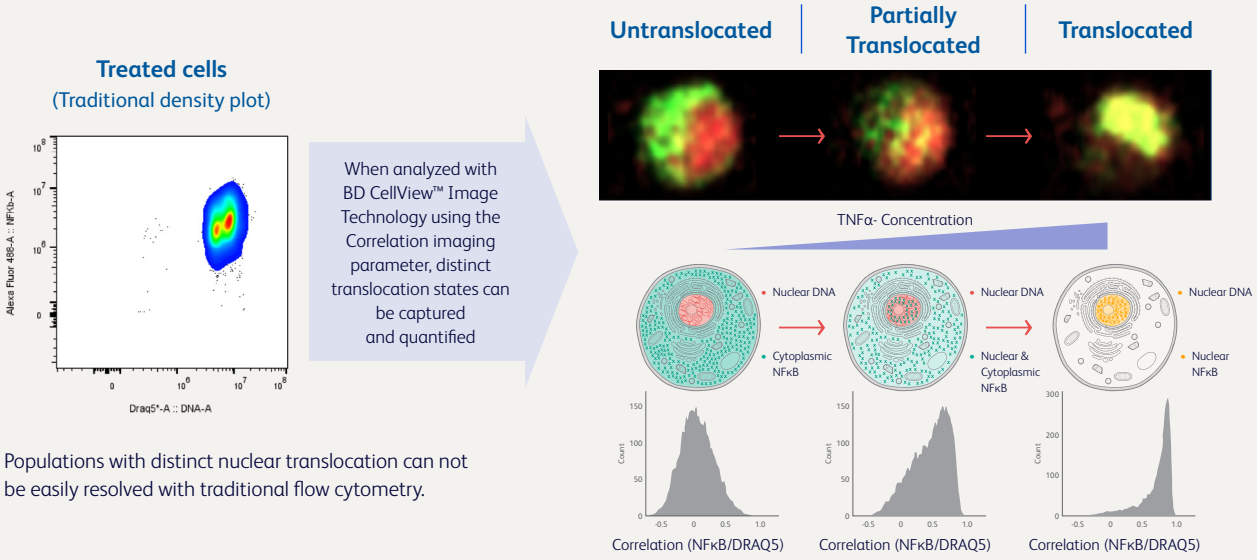
Nuclear factor-κB (NF-κB) is a group of transcription factors that regulate the immune response, inflammation and cell survival.[1] Upon stimulation by cytokines like TNF-α, NF-κB moves from the cytoplasm into the nucleus. There, it can drive expression of inflammatory and immune-regulatory genes.[2] When NF-κB activity is dysregulated, a variety of diseases, including cancer, cardiovascular diseases and immune-related diseases, can develop.[3] Thus, NF-κB has been a target for therapeutic development for many indications.[4] Measuring NF-κB translocation provides a functional readout of how cells respond to signaling cues, offering insight into pathway activation, therapeutic responsiveness and mechanisms of immune evasion.

Studying NF-κB translocation in real-time at the single-cell level

Traditional flow cytometry cannot resolve nuclear versus cytoplasmic localization, limiting researchers' ability to measure pathway activation at scale. BD CellView™ Image Technology, integrated into the BD FACSDiscover™ A8 Cell Analyzer and the BD FACSDiscover™ S8 Cell Sorter instruments overcomes this barrier by adding real-time spatial imaging to spectral flow cytometry — enabling functional single-cell insights previously accessible only through microscopy or dedicated imaging cytometers that are slower.

1. **Visualizing NF-κB localization:** Unlike traditional flow cytometry which gives you the intensity for an entire cell, BD CellView™ Image Technology separates fluorescent intensity based on cellular location in the cell. The Correlation imaging parameter on the BD FACSDiscover™ Platform allows scientists to determine and quantify the overlap in fluorescent signals in the same location of an image. To assess NF-κB localization, NF-κB is visualized using antibody staining with Alexa Fluor 448 (AF448) and the nucleus is stained with DRAQ5.

FIGURE 1. Using the Correlation imaging parameter, the overlap in fluorescence between NF-κB and the nucleus could be determined. We treated cells with different concentrations of TNF-α. Since DRAQ5 stains double stranded DNA in the nucleus, high correlation between DRAQ5 and AF488 indicates nuclear localization of NF-κB while low correlation indicates cytoplasmic retention of NF-κB. The image features clearly resolve low, intermediate and high translocation states — populations that could not be distinguished using fluorescence intensity alone.

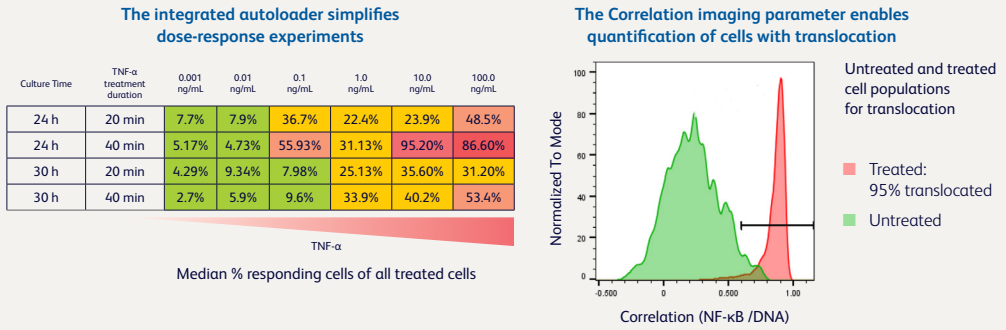


Populations with distinct nuclear translocation can not be easily resolved with traditional flow cytometry.

Correlation is an imaging feature that is automatically applied to images generated on the three fluorescence imaging channels on the instrument. It is a quantifiable imaging parameter, derived from the combination of any two imaging channels. Correlation is defined as the degree to which the location of two imaging channels are the same within the region of pixels defined by the Region of Analysis. It is paramount to determining nuclear translocation signaling pathways in cell analysis.

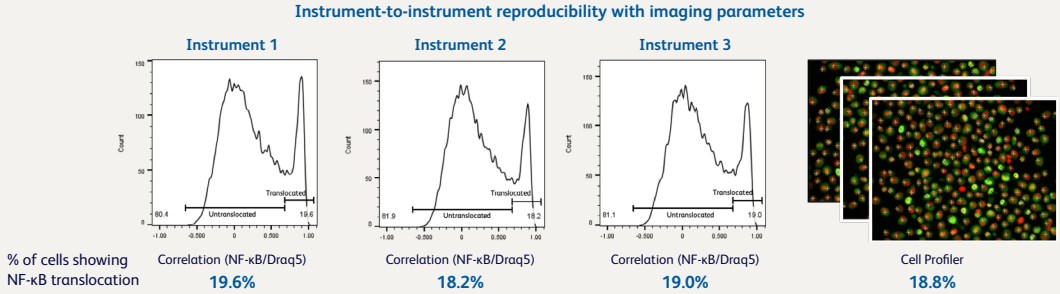
2. **High-throughput and high-confidence screening:** The BD FACSDiscover™ A8 Cell Analyzer integrated autoloader enables efficient plate-based assays, acquiring ~8,500 events per well and generating thousands of images per condition. This accelerates dose-response and time-course experiments without compromising sample integrity.

FIGURE 2. The integrated autoloader provides the flexibility to test numerous experimental variables, which is particularly helpful in dose response studies. In this example, 192 variables: (24 + 8 controls) x 2 (treated and untreated) x 3 replicates were tested in a 96-well plate. The most robust response was observed at 10 ng/mL, 24-hour culture and 40-minute TNF-α treatment duration: 95.2% of the treated cells showed nuclear translocation as measured by BD CellView™ Image Technology.



3. **Reproducible and scalable functional biology:** Next-Gen QC on the BD FACSDiscover™ Platform delivers out-of-the box data consistency day-to-day and instrument-to-instrument. Across multiple instruments, NF-κB translocation measurements demonstrated high reproducibility, aligning closely with independent microscopy-based analysis, which could reduce or eliminate the need for secondary microscopy validation.

FIGURE 3. Mixed populations of translocated and non-translocated cells were analyzed on three different instruments that delivered remarkable instrument-to-instrument reproducibility. The results are corroborated using microscopy on the Cell Profiler image analysis platform equipped with an automated translocation classifier. Note that analyzing 531 cells using microscopy and the Cell Profiler required 8 hours whereas >10,000 cells could be analyzed on the BD FACSDiscover™ A8 Cell Analyzer in one hour.



In summary, the BD FACSDiscover™ Platform can be used to analyze nuclear translocation accurately and efficiently in flow cytometry. While the BD FACSDiscover™ A8 Cell Analyzer enables fast, high-throughput analysis of cells, the BD FACSDiscover™ S8 Cell Sorter can sort cells based on localization of specific proteins for downstream analysis. Together, these instruments enable researchers to uncover previously unseen correlations between protein localization rapidly and with high reproducibility, facilitating the study of new signaling mechanisms and advancing drug discovery for immuno-oncology.

References

1. Oeckinghaus A, & Ghosh S. The NF-kappa B family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol.* 2009;1(4):a000034. doi: 10.1101/cshperspect.a000034
2. Hayden M, & Ghosh S. Regulation of NF-κB by TNF Family Cytokines. *Semin Immunol.* 2014;26(3):253–266. doi:10.1016/j.smim.2014.05.004
3. Yu H, Lin L, Zhang Z, Zhang H, & Hu H. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther.* 2020;5(1),209. doi: 10.1038/s41392-020-00312-6
4. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T, & Zhang J. NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduct Target Ther.* 2024;9(1),53. doi: 10.1038/s41392-024-01757-9