Unraveling T cell mitochondrial dynamics using imaging flow cytometry

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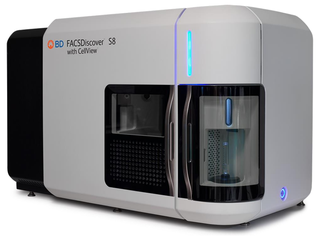
Abstract

Mitochondria are highly mobile, dynamic subcellular organelles that mediate energy generation in mammalian cells. In response to changes in energy and stress, mitochondria can either join or divide, a process known as fusion or fission, respectively. Mitochondrial morphology plays a critical role in the metabolic state of T cells and can affect T cell function in health and disease. Despite increased interest in mitochondria dynamics, a robust and efficient method to simultaneously image mitochondria morphology, analyze T cell phenotypes and sort cells has not been feasible. Here, we used the state-of-the-art BD CellView™ Image Technology on the BD FACSDiscover™ S8 Cell Sorter to detect changes in mitochondria morphology with high resolution and profile T cell phenotypes associated with specific mitochondrial shapes. With the imaging capabilities of the BD FACSDiscover™ S8 System, we visualized live mitochondria and nuclei using three-color staining in a high-throughput manner. We differentiated effector T cells in vitro and imaged increased fission-like mitochondria in these cells. With the cell sorting feature of the BD FACSDiscover™ S8 System, we sorted effector T cells based on their cell surface phenotype and distinct mitochondrial characteristics and evaluated their mitochondrial gene signature. Thus, we provide an advanced and comprehensive workflow to unravel mitochondrial dynamics and isolate cells with a particular phenotype for downstream in-depth characterization enabled by the BD FACSDiscover™ S8 System. As many cellular processes are linked to mitochondrial dynamics, this technique provides a powerful tool to understand how this critical organelle is regulated in a variety of human diseases.

BD FACSDiscover™ S8 Cell Sorter

- 6-way tube sorting
- Plate sorting
- Spectral sorting



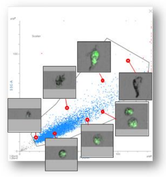
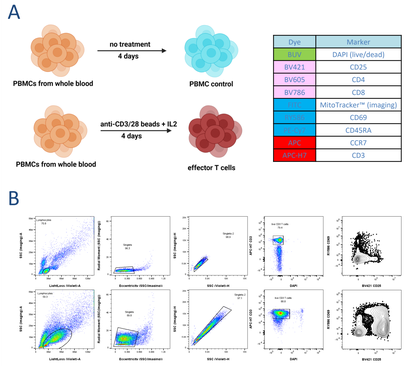


Image Cytometry:
Real-time image viewing in BD FACSChorus™ Software

Advanced spectral system

Methods



Dye	Marker
BUV	DAPI (live/dead)
BV421	CD25
BV605	CD4
BV786	CD8
[illegible]	MitoTracker™ (imaging)
[illegible]	CD69
[illegible]	CD45RA
AQC	CCR7
AF647	CD3

Figure 1. Establishing a cell culture system to study mitochondria morphology using the BD FACSDiscover™ S8 System. (A) PBMCs were treated with anti-CD3/28 beads and IL2 to differentiate effector T cells. (B) Flow cytometry plots showing CD69 and CD25 expression in gated live CD3+ T cells from the stimulated versus the non-stimulated sample using a 9-color flow cytometry panel (Table 1).

Results (1)

Mitochondrial visualization using mitochondrial dye MitoTracker™

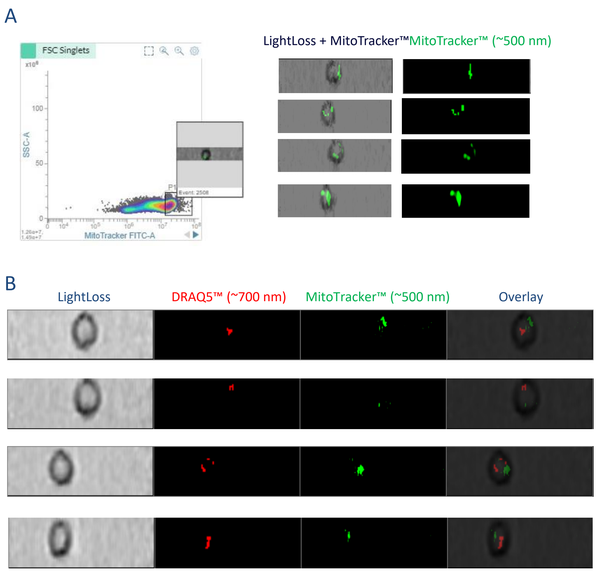


Figure 2. Visualization of mitochondria in unstimulated peripheral blood mononuclear cells (PBMCs) on the BD FACSDiscover™ S8 System prototype instrument. (A) Mitochondria imaging using the mitochondrial dye MitoTracker™. PBMCs were prepared with fresh whole blood and stained with MitoTracker™. (B) Visualization of both nuclei and mitochondria by co-staining PBMCs using nuclei dye DRAQ5™ and MitoTracker™.

Radial moment is associated with unique mitochondrial structure

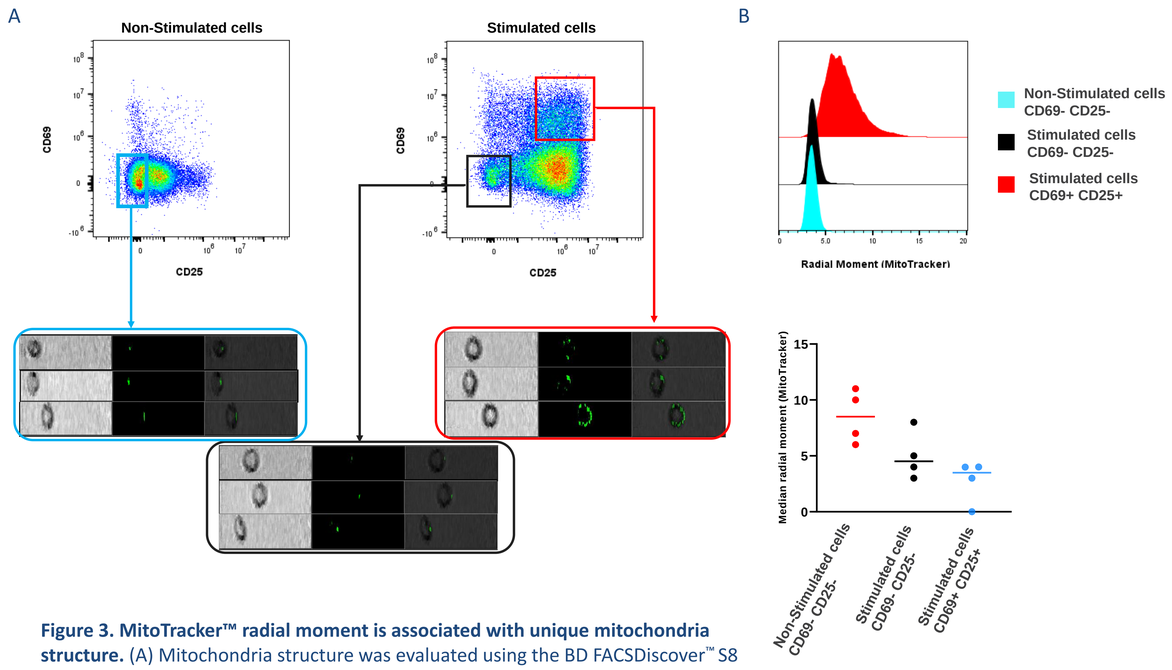


Figure 3. MitoTracker™ radial moment is associated with unique mitochondria structure. (A) Mitochondria structure was evaluated using the BD FACSDiscover™ S8 System. Compared to $CD69^-CD25^-$ T cells in the non-stimulated sample (blue) and $CD69^-CD25^-$ T cells from the stimulated sample (black), $CD69^+CD25^+$ stimulated T cells (red) showed increased punctate-like mitochondria. (B) Histogram and graph showing increased MitoTracker™ median radial moment in the $CD69^+CD25^+$ T cells from the stimulated sample (red) compared with $CD69^-CD25^-$ T cells from the stimulated sample (black) and $CD69^-CD25^-$ T cells from the non-stimulated sample (blue).

Results (2)

Distinct radial moment and mitochondria morphology can be detected within a stimulated T cell population

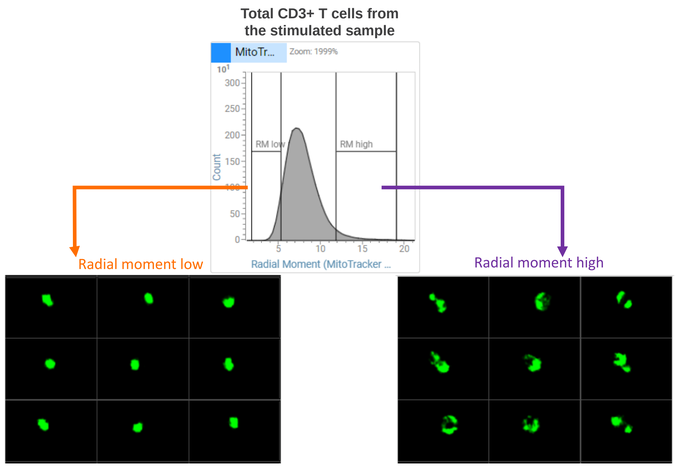


Figure 4. MitoTracker™ radial moment is associated with unique mitochondria structure within total stimulated T cells. Histogram and images showing distinct mitochondria morphology in radial moment high versus low cells from total gated $CD3^+$ T cells from the stimulated sample.

Gene expression analysis on radial moment hi versus lo sorted cells

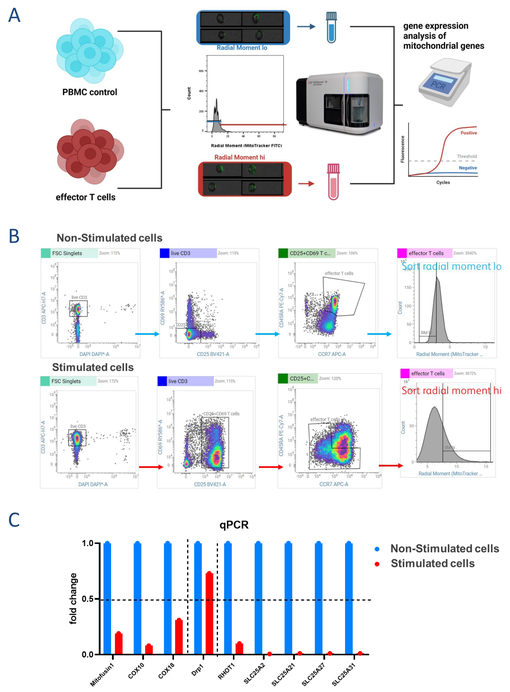


Figure 5. Gene expression analysis of mitochondria-associated genes. (A) Graphical illustration of experimental workflow. (B) Gating strategy for cell sorting using the BD FACSDiscover™ S8 System to isolate populations of interest. (C) Gene expression analysis on sorted cells. mRNA was isolated from the two sorted populations followed by quantitative PCR analysis of several mitochondria-associated genes.

Conclusions

- Intracellular organelle mitochondria can be visualized using the state-of-the-art BD CellView™ Image Technology on the BD FACSDiscover™ S8 Cell Sorter.
- Mitochondria in $CD69^+$ $CD25^+$ stimulated T cells showed unique structure as visualized on the BD FACSDiscover™ S8 System.
- The imaging parameter radial moment on the BD FACSDiscover™ S8 System was associated with unique mitochondrial structure.
- Cell sorting was performed to isolate cells with different mitochondrial structure based on the imaging parameter radial moment.
- Gene expression changes were observed between stimulated T cells with high radial moment compared to non-stimulated T cells with low radial moment.