Application Note

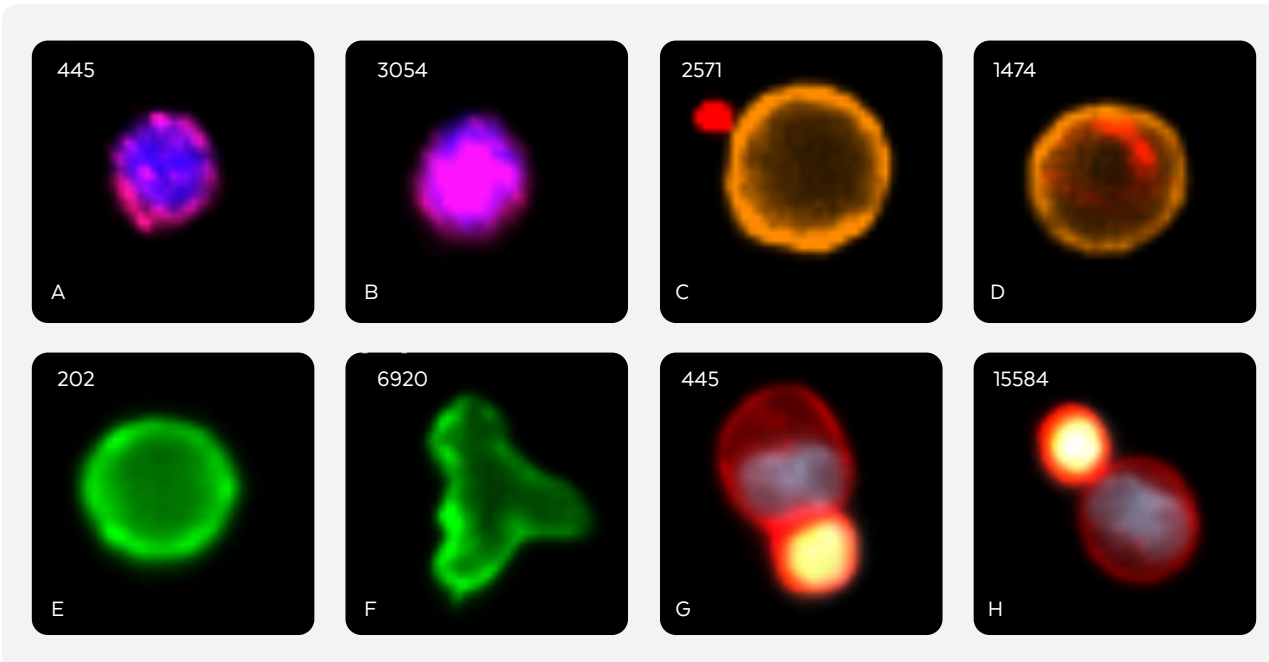
Measurement of Immune Cell Function Using Cytek® Amnis® ImageStream® Imaging Flow Cytometry

Abstract

Cells of the innate and adaptive immune system defend the host from pathogens while remaining tolerant to self-antigens. Dysregulation of these immune functions can result in infection or autoimmunity. Immune cells sense their environment by binding to and processing soluble mediators of inflammation, or through direct contact with other cells, which results in signal transduction and activation, or suppression of function.

Many assays for studying the mechanisms of immune function require imaging, but immune cells present significant challenges to image-based analysis due to their rarity and the need for simultaneous, multispectral immunophenotyping, making statistically robust quantification difficult. Because the Cytek® Amnis® ImageStream® imaging flow cytometer (IFC) is able to quantify images taken from large populations of cells, it is ideal for performing high throughput immune function assays. This application note shows several examples of immune function assays that can be performed using the ImageStream® IFC, including NF-κB translocation in whole blood cells (**Figures 1A-B**), internalization and trafficking of viral DNA within plasmacytoid dendritic cells (pDCs; **Figures 1C** and **1D**), chemokine-induced monocyte shape change (**Figures 1E** and **1F**), and T cell–APC immune synapse formation (**Figures 1G** and **1H**). These results demonstrate the unique capabilities of the ImageStream IFC to perform a wide variety of immune function assays.

Figure 1. Representative images of immune function assays



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Plasmacytoid dendritic cell (pDC) binding of double-stranded viral DNA results in a potent antiviral response that triggers the secretion of copious amounts of IFN-α. The internalization (y-axis) and trafficking (x-axis) of Cy5-labeled CpGB in human pDCs was measured using the ImageStream system (**Figure 2**). BDCA+ gated events were analyzed. With time, CpGB accumulated inside the pDCs (as indicated by increasing internalization scores), trafficking to the endosome first (as indicated by high Similarity Bright Detail scores; **Figures 2A-2C**), and then to the lysosome (**Figures 2D-2F**). Representative images of cells from the indicated regions are shown (**Figures 2G-2I**).

Figure 2. Internalization and trafficking of CpGB within human plasmacytoid dendritic cells

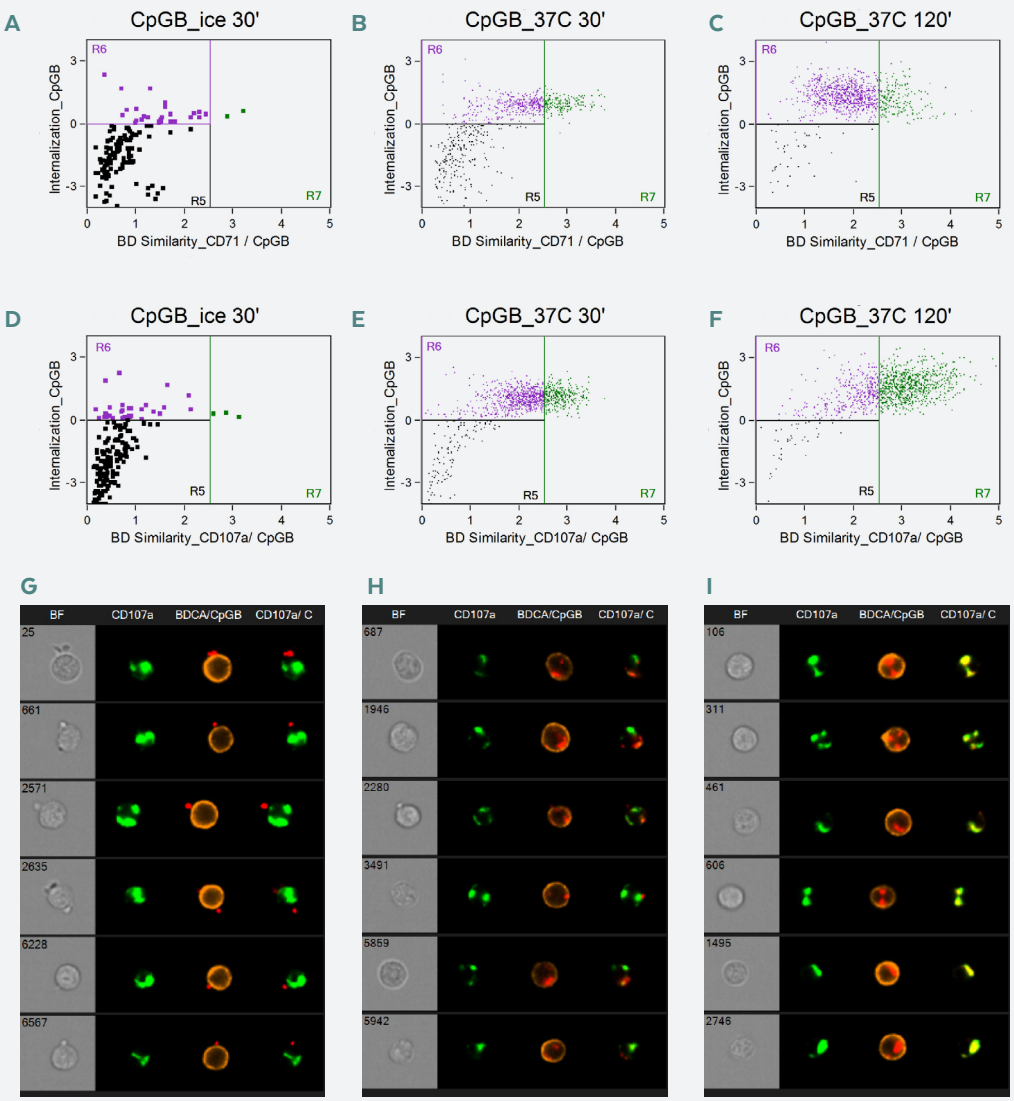

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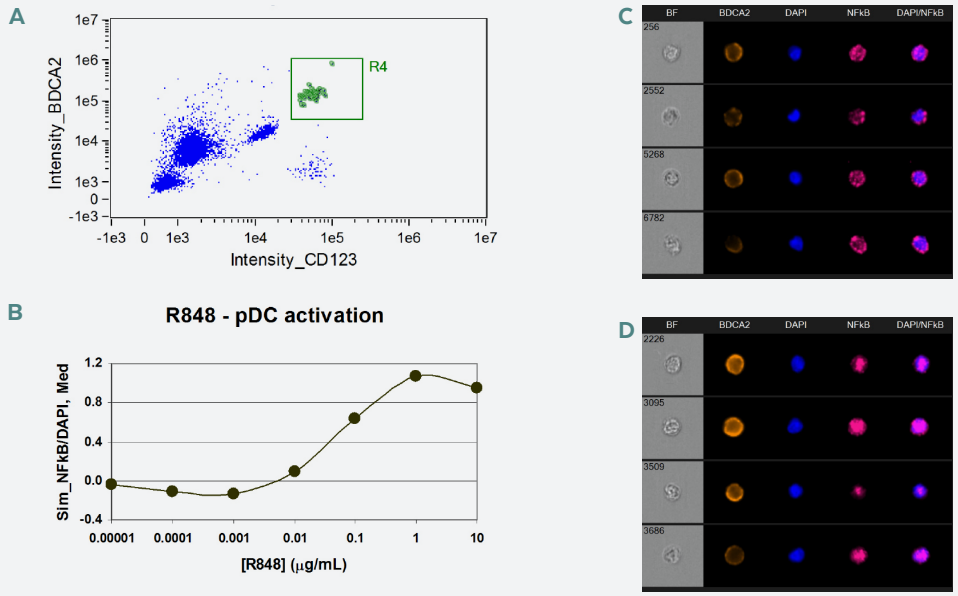
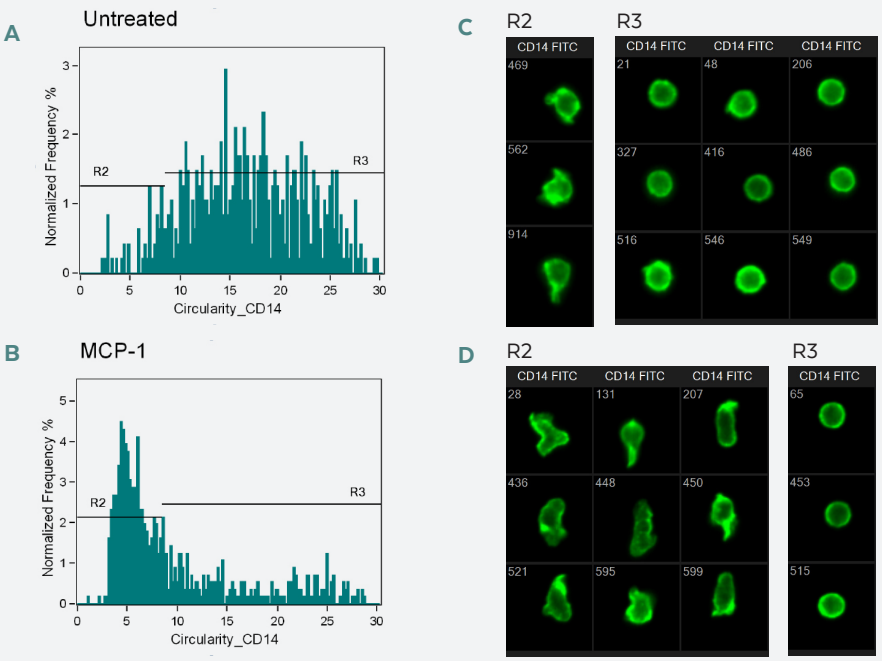
Figure 3. Measurement of NF-κB activation in whole blood plasmacytoid dendritic cells



As shown in Figure 3, pDCs express pattern recognition receptors that transmit activating signals upon ligand binding. Translocation of NF-κB was measured as a marker for TLR7-induced activation in whole blood pDC. NF-κB translocation was measured using the Similarity score[1] for the gated pDC from whole blood samples exposed to a range of R848 doses (**Figure 3B**). Images of representative cells from the 1 ng/mL (**Figure 3C**) and the 1,000 ng/mL (**Figure 3D**) samples are shown.

Figure 4. Measurement of chemokine-induced monocyte shape change



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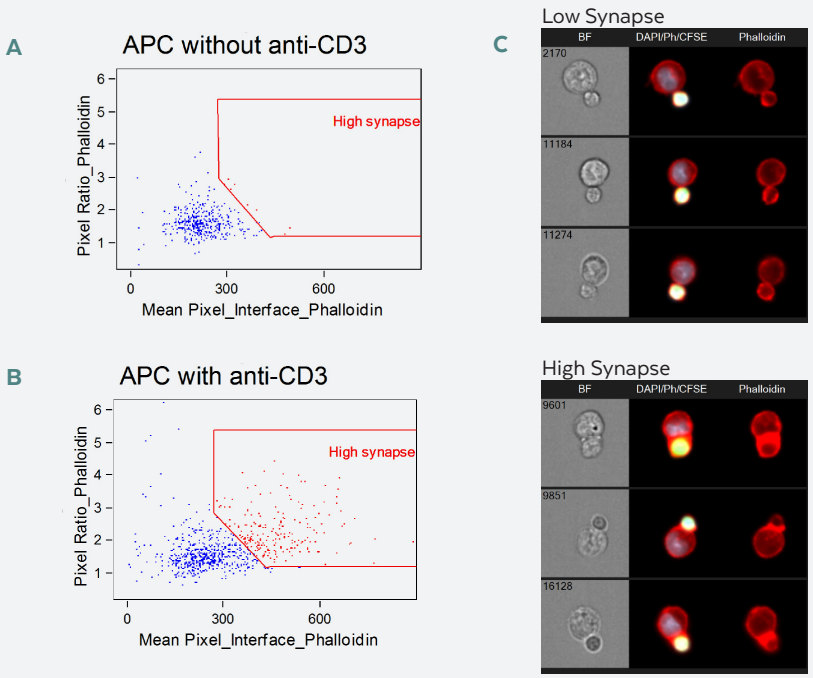
Circulating monocytes rapidly change shape when exposed to chemokine gradients. To measure chemokine-induced shape change, human PBMCs were incubated with MCP-1 for 30 minutes, stained with FITC anti-CD14, and RBC-lysed samples were analyzed for circularity (**Figure 4**). Representative images of CD14+ monocytes from regions R2, elongated monocytes, and R3, circular monocytes, are shown (**Figure 4C** and **4D**).

Figure 5. Immune synapse formation



Murine lymph node cells (LNCs) were incubated with control- (**Figure 5A**) or anti-CD3- (**Figure 5B**) coated, artificial antigen presenting cells (APCs), and conjugates were analyzed for actin accumulation at the immune synapse. Representative images are shown of conjugates without an immune synapse, and conjugates with apparent immune synapse formation from the anti-CD3 plots (**Figure 5C**).

References

1. George T, Fanning S, Fitzgerald-Bocarsly P, et al. Quantitative measurement of nuclear translocation events using similarity analysis of multispectral cellular images obtained in flow. J Immunol Methods. 2006 Apr 20; 311(1-2): 117-129. 2006 Mar. doi: 10.1016/j.jim.2006.01.018.

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