Technical Overview

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Advanced Detector Technology in Agilent NovoCyte Flow Cytometers

Introduction

The quality of flow cytometry data depends on the resolution of positive signals from the background. Many factors determine the sensitivity of a flow cytometer and resolution of the stain. These factors include reagent, biological, and instrument components such as fluorescence intensity of the antibody, effect of compensation, laser power, and detector sensitivity. This technical overview describes advancements in detector technology, and highlights examples where optimal signal resolution is essential for acquiring quality data.

Silicon photomultiplier (SiPM) detector technology gives outstanding signal resolution

There are four types of detectors used in flow cytometers, photodiodes (PD), avalanche photodiodes (APD), photomultiplier tubes (PMT), and the newly integrated silicon photomultipliers (SiPM). PD detectors are typically used only for light scatter, forward scatter, and sometimes side scatter because the signal is great enough to not require amplification. PMT, APD, and SiPM are photodetectors with adjustable intrinsic gain to enhance the fluorescence signal when it is converted to digital. Previously used in PET scanners and LiDAR, SiPMs are solid-state, silicon-substrate-based, photon-level-sensitive semiconductor devices, with a 7.2 log dynamic range (Figure 1). Consisting of a compact array of APDs operating in unison, the SiPM is a compact detector with photon counting capability. The innovative optics designed into the Agilent NovoCyte Advanteon, NovoCyte Quanteon, and NovoCyte Penteon flow cytometers incorporate up to 30 independent SiPM detectors, which collect and process signals for each of the fluorescence channels.

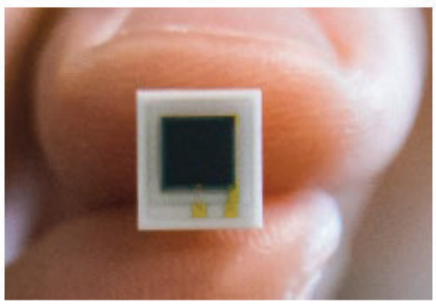

Figure 1. Image of Agilent NovoCyte flow cytometer SiPM detector.

Optimal fluorescence signal resolves the dimmest signals

A voltage titration experiment was performed to demonstrate the fluorescence sensitivity capabilities of NovoCyte flow cytometers. Fluorescence conjugated CD11c BUV compensation particles consisting of three populations with different binding capacity (blank, low binding, and high binding) were acquired at variable detector voltage settings. Results were compared on the NovoCyte Penteon flow cytometer and a competitor instrument (Figure 2). Increasing voltage can increase the resolution of the positive signal from the background. However, there are limits where the separation will no longer improve and can even diminish as the background fluorescence increases. The maximum stain index obtained, which quantifies the resolution of the signal, was used to compare the UV sensitivity of the two flow cytometers. Both the low (dim) and high binding (bright) beads were resolved better on the NovoCyte Penteon than a competitor instument. The highest obtained stain index for the NovoCyte Penteon for the bright, 442, and dim bead populations, 4.59, were both separated better from the negative population than the bright, 194, and dim, 4.59, bead populations on a competitor instrument. These data demonstrate the sensitivity on the NovoCyte Penteon and the power of SiPM detectors to achieve better resolution and detect even the dimmest of signals.

High-resolution detector easily identifies rare cells in heterogeneous cell populations

CD34 is a marker for hematopoietic stem cells (HSC). The ability to resolve CD34 positive cells from the CD34 negative population is important for identifying the number of HSCs that are present within blood. Flow cytometry is commonly used to quantify the numbers of circulating CD34+ cells after cytokine-induced stem cell mobilization and predict the total of isolated CD34+ cells from a sample. Using the NovoCyte Penteon, CD34+ cells are easily identified from other leukocyte cell populations

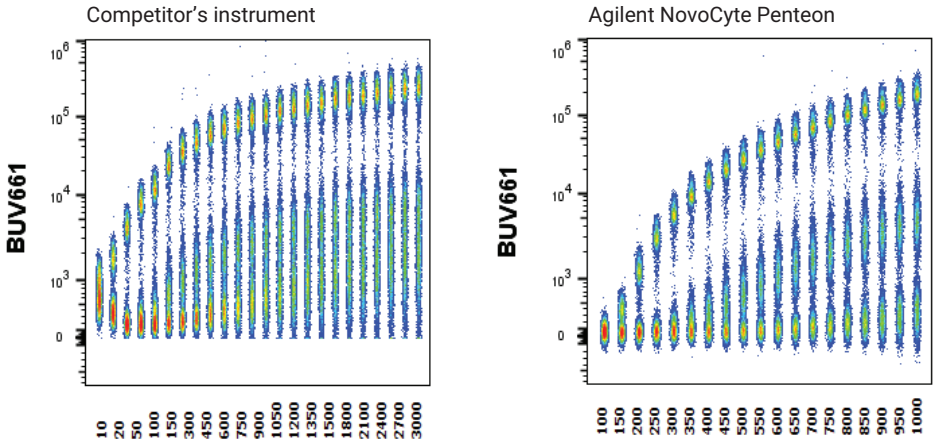

Figure 2. Voltage titration on the Agilent NovoCyte Penteon flow cytometer and a competitor instrument. COMPTrol particles (Spherotech, Inc.) stained with CD11C BUV661 antibody (Becton, Dickinson and Company) were analyzed on the NovoCyte Penteon flow cytometer and a competitor instrument. These particles have three populations: blank, low antibody binding, and high antibody binding. Stain resolution was calculated by the stain index at each voltage.

with sensitive SiPM detectors (Figure 3). High resolution of CD34+ cells from the large number of other blood cells in the same sample helps in accurately gating this rare cell population and ensuring correct enumeration.

High-performance signal detects distinct peaks of cell proliferation for generational analysis

Cell proliferation is one of the most fundamental processes in biology. Quantitative analysis of cell proliferation is crucial for studies of cell growth and differentiation. It is used to evaluate compound toxicity and inhibition of tumor cell growth during drug development. Flow cytometry assays are ideal for measuring the proliferation of individual cells and can identify different cell types in a single sample. Generational analysis determines how many times a given cell has divided and can measure heterogeneous growth within the same population of cells.

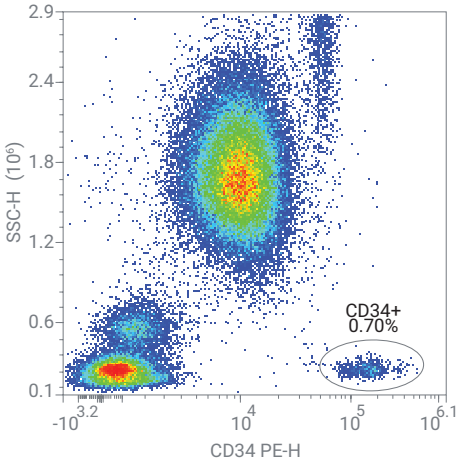

Figure 3. Resolution of CD34+ populations to identify hematopoietic stem cells. Blood cells were stained and analyzed according to International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines for CD34+ cell determination. Detection of CD34+ cells was based on FSC, SSC, a viability stain (7-AAD), and antibody staining of CD45 and CD34.

This assay is performed by measuring the dilution of a cell-tracking dye as a cell proliferates, where the labeling dye is equally partitioned between the two daughter cells. Through this method, several distinct successive generations (up to 10) of cell division can be labeled. Each peak represents a generation of cells. Algorithms used to quantify the amount of proliferation rely on the resolution between each peak. Anti-CD3 antibody activated T cells were stained with ViaFluor488, CFSE, or CellTrace violet and examined five days after activation (Figure 4). These cells were compared to the unstimulated control (red histogram). The cell proliferation analysis module in Agilent NovoExpress software can resolve seven distinct peaks from ViaFluor488 and eight peaks from both CFSE and CellTrace violet. Therefore, high-performance signal detection using the NovoCyte flow cytometers allows optimal resolution of each generation upon cell proliferation, which enables better quantification.

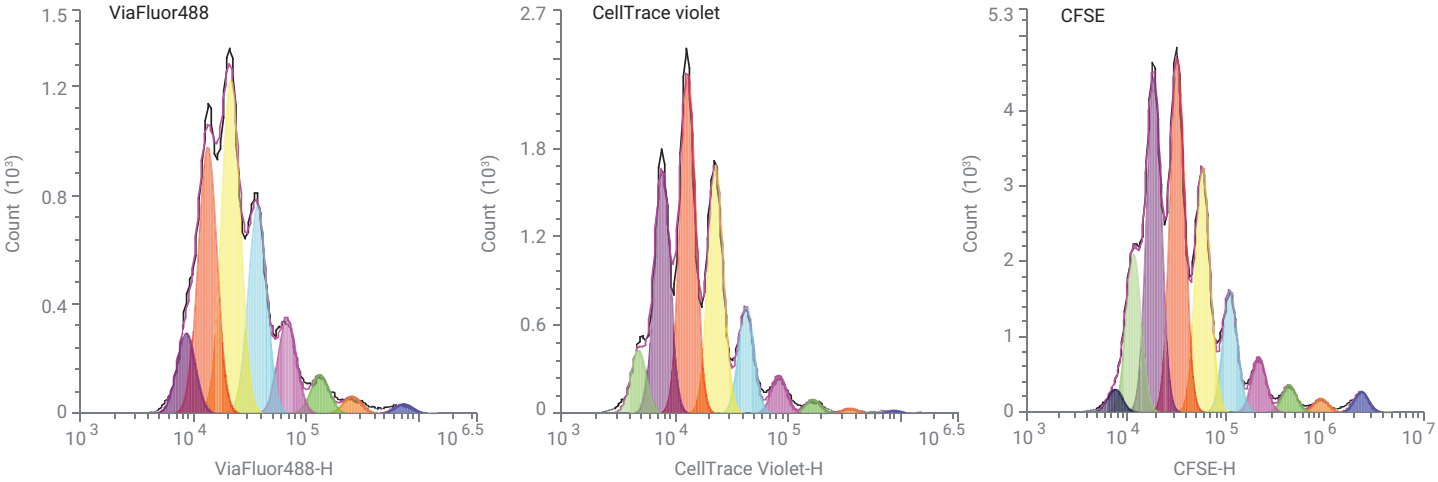

Figure 4. Cell proliferation module identification of cell proliferation generations using different commercially available dyes. Peripheral blood mononuclear cells (PBMCs) were activated with plate-bound anti-CD3 antibody (5 µg/mL) for 1 hour and washed with PBS prior to addition of cells. PBMCs were stained with 1 µM of indicated dye (CFSE, ViaFluor488, or CellTrace violet) and seeded at 2 × 105 cells/well in complete RPMI media and 100 ng/mL IL-2. After five days of stimulation, cells were stained with anti-CD3 ECD, anti-CD4 PE-Cy7, and CD8 APC antibodies. Cells were then analyzed on the Agilent NovoCyte Quanteon flow cytometer for proliferation. See Agilent application note 5994-1851EN for more information.

Highly sensitive laser scatter detection resolves small particles

For the detection of small particles, it is also important to have sensitive laser scatter detection as well as fluorescence detection. The NovoCyte Advanteon, NovoCyte Quanteon, and NovoCyte Penteon are equipped with APD side scatter (SSC) detectors. The detection optics and signal processing electronics have been optimized to resolve particles down to 0.1 µm in size (Figure 5). These features unlock the possibility to investigate small particles such as extracellular vesicles (EVs), which have previously been difficult to resolve due to their small diameter and variable composition. EVs are cell-derived membranous vesicles released from cells under normal and pathological conditions. EVs include exosomes, microvesicles, and apoptotic bodies according to their cellular origin and size ranging from 50 nm to 1 µm. With such highly sensitive resolution, platelets, bacteria, and various submicron particles can be readily identified and analyzed.

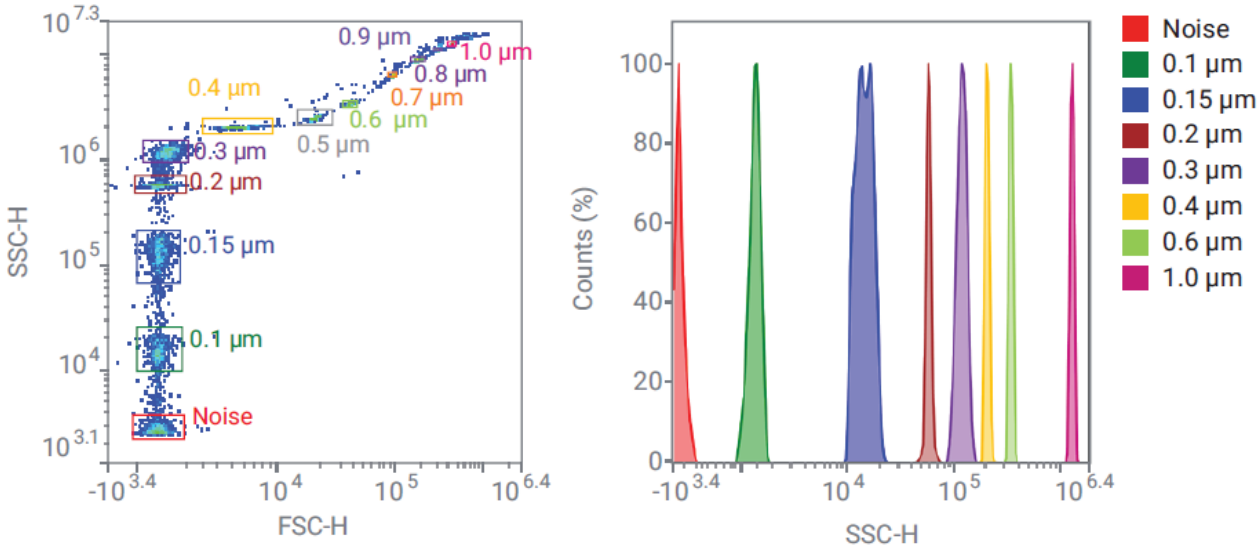

Figure 5. Small particle bead detection on the Agilent NovoCyte Advanteon flow cytometer. NIST traceable size standards (Bangs Laboratories, Inc.) commercially available small particle beads were used to demonstrate the high sensitivity of the NovoCyte Advanteon flow cytometer side scatter detection (SSC). Particles as small as 100 nm were easily resolved from the background. Instrument settings: SSC-H gain 1,000, FSC-H gain 1,000, threshold SSC-H greater than 1,000. See Agilent application note 5994-1457EN for more information.

Wide dynamic range simultaneously identifies eosinophils and lymphocytes

NovoCyte flow cytometers provide both sensitivity and stability, and they also incorporate a wide dynamic range of detection. Dim and bright signals can be collected and gated in the same view, thus eliminating the need for laborious trial and error detector adjustments. To demonstrate, blood leukocytes were analyzed on the NovoCyte flow cytometer and a competitor instrument. Data analysis was performed with NovoExpress software. The wide dynamic range of detection (7.2 logarithmic decades) provided by the NovoCyte makes it easy to identify the large eosinophils and small lymphocytes in the same plot (Figure 6). In contrast, the limited dynamic range of the competitor instrument restricts the ability to identify these two cell types in the same plot. Adjusting voltages to accurately identify the lymphocyte population resulted in poor resolution of the eosinophils and vice versa. The NovoCyte also enabled clear distinction of monocytes from granulocytes showing that this flow cytometer-based assay offers rapid, accurate, and unbiased identification and quantitation of eosinophils in human peripheral blood. It is not always possible to achieve similar results with other instrumentation without the need to make manual adjustments.

Conclusion

The Agilent NovoCyte flow cytometers are highly sensitive instruments that employ new SiPM detector technology to obtain the highest signal resolution. In addition to fluorescence detection, NovoCyte instruments have highly sensitive laser scatter detector resolution making it possible to distinguish extremely small particles. Simultaneous resolution of both dim and bright fluorescence signals, as well as small and large particles using light scatter, is possible in part due to the wide dynamic range of detection. This dynamic range not only allows the user to see more, it also eliminates the need for routine detector adjustments, which saves both time and money.

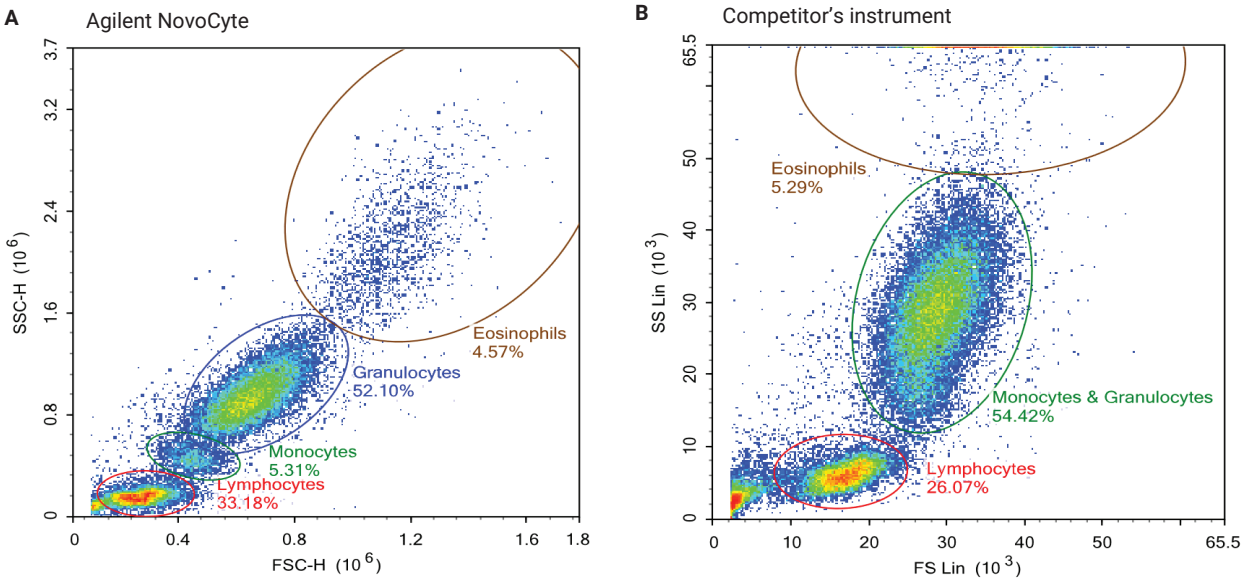

Figure 6. Simultaneous analysis of eosinophils and other leukocytes in whole blood. Human peripheral blood leukocytes were used for acquisition on the Agilent NovoCyte flow cytometer and a competitor flow cytometer. Data were analyzed using the Agilent NovoExpress software. The population highlighted in brown represents the eosinophils present in each sample. The full range scale was displayed for the data acquired on the competitor instrument (5-decade dynamic range). Two different leukocyte populations representing monocytes and granulocytes were also clearly distinguished for the NovoCyte flow cytometer data (A), but not for the competitor's flow cytometer (B). See Agilent application note 5994-2119EN for more information.

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RA44377.6142013889

Printed in the USA, August 11, 2021
5994-3658EN

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