CytoFLEX nano Flow Cytometer: the new frontier of nanoscale Flow Cytometry

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CytoFLEX nano Flow Cytometer provides high sensitivity and high resolution, enabling detection and characterization of nanoparticles at least as small as 40nm (based on polystyrene beads), accurately distinguishing particles of similar size within 10 nm difference (based on silica beads), and the ability to characterize low-abundance targets in a heterogeneous population.

Introduction

Nanoscale flow cytometry has numerous research applications, and it holds promise in the development of diagnostic tools and targeted drug delivery systems. The ability to analyze nanoparticles based on their characteristics opens new possibilities for personalized medicine and nanomedicine.

Although nanoscale flow cytometry is a powerful technique with numerous advantages, it also has limitations, including detection sensitivity and size resolution. Researchers have been trying to solve this problem by stretching available flow cytometers to their lower limit of detection, improving cleaning techniques and adding cleanup steps in sample preparation, as well as multiple experimental controls and orthogonal check points.

CytoFLEX nano Flow Cytometer brings nanoscale flow cytometry to a new level, allowing flexibility to design experiments, thanks to 5 side scatter parameters and 6 fluorescence channels. Performance consistency is ensured via detailed QC and a fluorescence Sensitivity Monitor tool, as well as multiple options of on-board cleaning. Here we show an example of 5-color panel on PPP (Platelet Poor Plasma) EVs isolated from healthy donors.

Protocol

Platelet Poor Plasma EVs were prepared from whole blood collected in K3-EDTA vacutainers through a centrifugation at 160 x g for 5 min, followed by a 0.2 µm filtration step and size exclusion chromatography (SEC) using qEV single 70 nm (Izon). Fractions 4 to 12 were tested on the CytoFLEX nano analyzer, and one was selected to proceed based on the amount of extracellular vesicles present, to help ensure a high enough dilution post staining, and to remove antibody aggregates and other contaminants. Antibodies were spun at 20,000 x g for 10 min at room temperature to eliminate aggregates, and single antibody titration was run and adjusted for fluorescent spillover. Compensation was applied to the sample, using the compensation matrix.

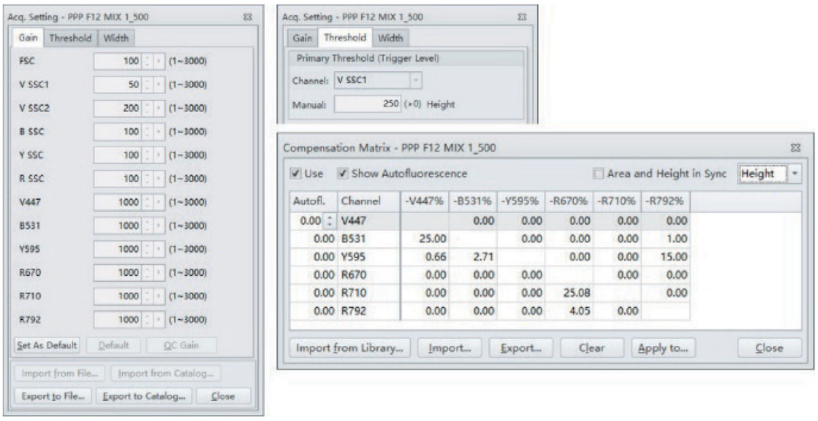

Figure 1. CytExpert nano software acquisition and compensation matrix settings.

Results

CytoFLEX nano Flow Cytometer enables researchers to effectively detect and resolve plasma-derived EVs as low as 70 nm (as defined limit by SEC qEV single 70 nm from Izon). This analyzer detects and distinguish more than one FL signal on single PPP EV particles, including PB, FITC, PE, APC, and PC7, allowing users to see even small positive populations for markers that are low in expression, like CD63 tetraspanin and CD235a for erythrocyte contamination. It allows clear detection without interference by antibody background noise. This level of sensitivity can greatly benefit EV research, as most flow cytometers have been known to be unable to detect EVs smaller than 100nm in diameter.

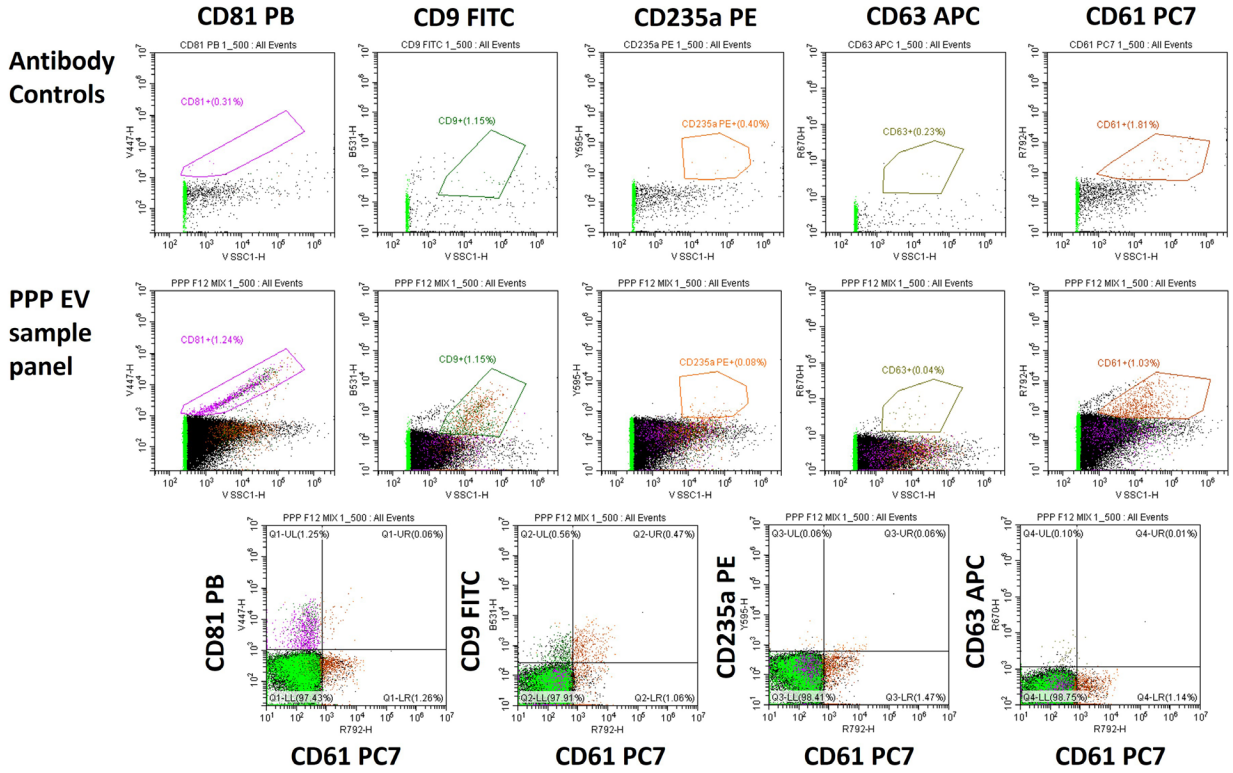

Figure 2. 5-color panel on PPP EVs isolated from a healthy donor. A. single color control for CD81 PB, CD9 FITC, CD235a PE, CD63 APC and CD61 PC7. **B.** sample stained with panel showing CD81, CD9 and a low CD63 positivity. CD235a shows a minimal contamination by erythrocytes and CD61 defined platelet derived EVs.

While flow cytometry is a tool that provides unique sensitivity and single particle characterization, the data is expressed in arbitrary units. As the use of flow cytometry evolves in this space, researchers will require data to be expressed in absolute units such as nanometers and mean equivalent soluble fluorescence.To accomplish this each researcher will need to select an appropriate method to determine the size and abundance of fluorescence from each detected particle. Thankfully, robust tools such as FCMpass[1] already exist to aid the researcher in creating data that can be compared across time and multiple orthogonal methods.

REFERENCE

1. Welsh, J. A. *et al.* FCM_{PASS} Software Aids Extracellular Vesicle Light Scatter Standardization. *Cytometry A* **97**, 569–581 (2019).

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