INTRODUCTION

There is great interest in both medical and scientific communities in submicron cell-derived particles, termed microparticles or microvesicles. Examples of tissues that shed fragments of their plasma membrane (or nanoparticles) into the circulation include platelets, endothelial cells, leukocytes, and erythrocytes. There is increasing evidence that these submicron fragments have important physiological roles. Although many techniques have been used with limited success for the identification and characterization of nanoparticles, flow cytometry remains the most accurate and reproducible. Traditionally however, the most challenging element has been the availability of sufficient forward scatter (FSC) resolution to delineate target populations from background signal and instrument noise.

The Beckman Coulter new MoFlo Astrios EQ (Astrios EQ) sets a new standard in forward scatter and fluorescence dynamic range performance for cell sorting – delivering enhanced functionality and sensitivity. Accommodating as standard, a 488 nm 200 mW diode laser with a conditioned flat-top shape beam profile, the Astrios EQ offers a patent pending FSC assembly capable of discriminating 200 nm to 30 μm particles on the same dynamic range. Designed for researchers who desire high productivity with more analytical capability, the Astrios EQ combines nano-scatter resolution with 5 decade fluorescence sensitivity on a 4-9 decade scale for the detection of submicron cell-derived particles. Therefore, we propose a patent pending innovative Forward Scatter detection module that will not only improve accuracy, but also allow for validation of the process by recovery of nanoparticles. The current study will present independent testing of the Astrios EQ demonstrating use of its two-parameter head-on PMT-based FSC optical assembly technology to identify and recover nanoparticles for downstream analysis.

MATERIALS AND METHODS

Optical and Hardware Design for Astrios EQ

The Astrios EQ is equipped with two FSC PMT pathways separated by a beam splitter. FSC1 is a direct laser beam pathway, with the FSC2 being directed at an angle from the beam splitter. Seven different and unique masks are provided to optimize particle identification and focus laser light to the PMT’s. Due to this specific design, particles from 200nm to 30μm can be identified (together or individually).

Gating and analysis

In this study, a methodology will be constructed to identify nanoparticles and to isolate them by sorting. Both Bangs Laboratories Inc. Dragon Green Beads (PN 20037) 520nm and 780nm, and Beckman Coulter’s PCS Control Labeled Beads Mixed Kit (PN 6602336) 100nm, 200nm, 300nm, and 500nm were acquired to check for uniformity along different size ranges and materials, with or without fluorescence respectively. In addition, biological nanoparticle samples from whole blood, ovarian cancer cells, and myocardial cells were analyzed. Finally, Dragon Green Beads and myocardial exosomes were sorted.

Instrument Set-up and alignment

Initial alignment was done by acquiring a bead range of 200nm down to and including 200nm particles (data not shown). After establishing the dynamic range of the Astrios EQ, the beam splitter was removed and the instrument optimized on the FSC1 for small particle detection. All subsequent measurements were acquired with threshold set to 40nm at 0.02-0.05 no neutral density filter. PCS Mixed Kit was acquired to establish differing size populations and ability to differentiate from noise (signal noise and drop drive noise). All seven masks were processed to establish best mask for nanoparticle detection. 200nm polystyrene beads from different manufacturers were compared to show mask sensitivity. Dragon Green Beads measuring 520nm and 780nm were acquired and sorted for isolation accuracy measurements. Finally, biological nanoparticles were acquired and sorted.

RESULTS

CONCLUSIONS

Many cells, including platelets, endothelial cells, leukocytes, and erythrocytes, shed fragments of their plasma membranes into the circulation. There is increasing evidence that these submicron fragments, termed nanoparticles, have important physiological roles. Although many techniques have been derived for the identification and characterization of nanoparticles, flow cytometry is still deemed to be the most accurate and reproducible. The Astrios EQ, with its dual FSC PMT and interchangeable mask system, has shown the ability to identify and recover nanoparticles (<200nm->20um) for further analysis. The Astrios EQ sorting system allows for advanced applications with nanoparticles and their cell of origin. This will aid in research studies and help define the process of nanoparticle formation. In addition, the Astrios EQ can be modified to differentiate different nanoparticle populations and separate these populations. Potentially, this could lead to understanding nanoparticle function and role in the immune system.

In this study, we have shown the Astrios EQ’s ability to distinguish a variety of submicron populations ranging from 100nm to 750nm. In addition, the ability to sort and recover with high purity has been demonstrated. The use of different masks has allowed for greater resolution of populations and dynamic low-end range.