MoFlo Astrios EQ Enhanced Forward Scatter: Simultaneous Sorting of Micro- and Macro- Samples

Introduction

The MoFlo Astrios EQ series provides solutions to investigate biological materials between 200 nm and 30 µm. It allows for populations of beads in this range to be clearly discriminated from each other and instrument noise (Figure 2). The MoFlo Astrios EQs is the flagship sorter in the series, while the MoFlo Astrios EQ offers simultaneous light collection through two FSC modules (Figure 1), and seven different FSC masks (shown below) that allow submicron particle characterization that exploits unique cellular morphologies.

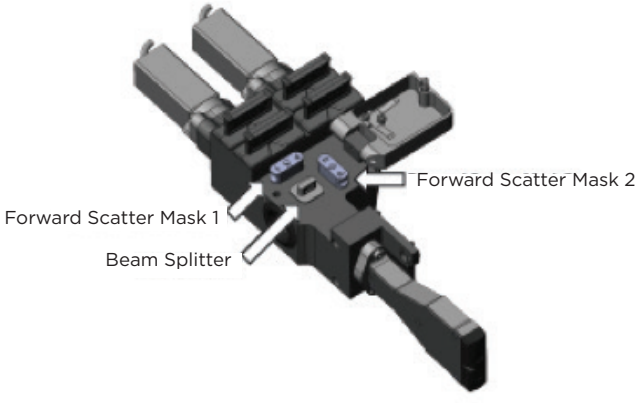


Fig. 1. MoFlo Astrios EQ forward scatter module. The enhanced FSC module on the MoFlo Astrios EQ allows the placement of 2 separate masks improving the characterization and detection of populations.

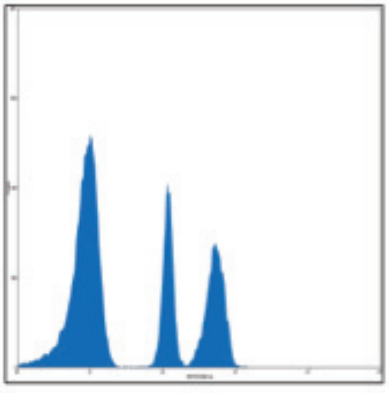
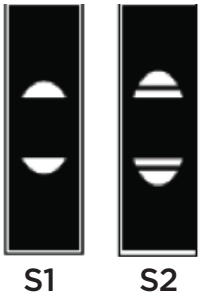


Fig. 2. Example of 200 and 300 nm polystyrene bead resolution from each other and from noise.

S - Separation

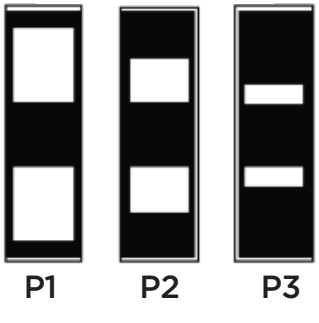


Separation (S):
Reduce sensitivity to different particle types. Good for separating small differences in similar particles.

M - Materials

Materials (M):
Designed to characterize particles of different matter material types. Allows for better relation to particle size over large ranges of particles.

P - All-Purpose



All-Purpose (P):
Overall general performance. Best when matched against M or S masks to pull out details.

MOFLO ASTRIOS EQ™
FLOW CYTOMETER SYSTEM

BECKMAN COULTER
Life Sciences

This application note examines minimally prepared whole blood samples studied and sorted. The goal of the exercise was to understand the difference between freshly collected and stabilized blood and to develop a standardized procedure for verifying the wide range of Astrios EQ features using cellular samples.

Materials

- Fresh whole blood collected in yellow top tube (BD 364606)
- IMMUNO-TROL (Beckman Coulter 6607077)
- CD45-Pacific Blue (Beckman Coulter A74765, 405-448/59)
- CD42b-PE (Beckman Coulter IM1417U, 561-579/16)
- CD235a-PC7 (Beckman Coulter A71564, 488-795/70)
- Reference 200, 300, and 1000 nanometer polystyrene beads (Bangs Laboratories NT07N, NT08N, NT15N)
- Ultra Rainbow Calibration Beads (Spherotech URFP-30-2)
- IsoFlow Sheath (Beckman Coulter 8546859)
- Sterile filtered (0.2 µm) phosphate buffered saline (PBS)

Methods

A minimal panel of three antibodies conjugates (CD45-Pacific Blue, CD42b-PE, and CD235a-PC7; 20 µl each) were added to 100 µl of whole blood (either fresh or stabilized) and incubated for 15 minutes at room temperature in the dark. Following the incubation, 4 mL of sterile filtered PBS was added to the tubes. For a relative size reference, 1000 nm beads were added to the preparation.

Instrument Setup

To minimize background sheath and air contamination, the Astrios EQ is equipped with a 0.04 µm inline sheath filter and 0.2 µm air filter. For this study, the 561 nm laser was set as the forward scatter laser and M2 (materials) and S1 (separation) FSC masks were installed with a 1.0 absorbing neutral density filter in front of the S1 mask. PBS threshold gating was used to eliminate noise during setup (Figure 3).

Flow Cytometry

Astrios EQ was set to trigger on the 640 nm laser side scatter parameter and threshold was set to allow 500–1000 eps of instrument, fluidic, and optical noise. Sheath and sample pressures were set to allow the samples to run at approximately 50,000 eps at 60 psi. For sorting, the stream frequency, amplitude voltage, and drop delay were set with Intellisort II and sorting occurred in purify mode with a drop window of 1 to 2 drops. Because of the nature of the sample and the high event rate, the efficiency rate ranged from 50% to 60% during the sort.

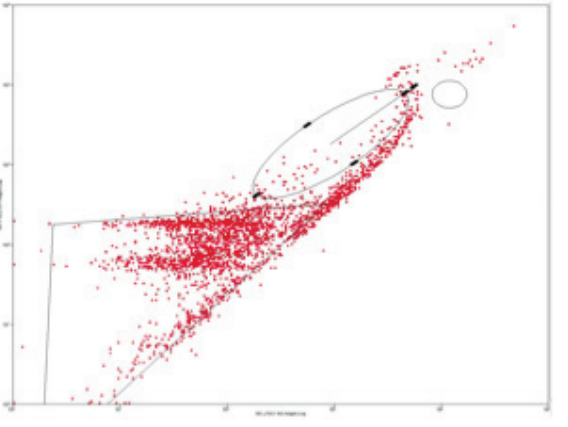


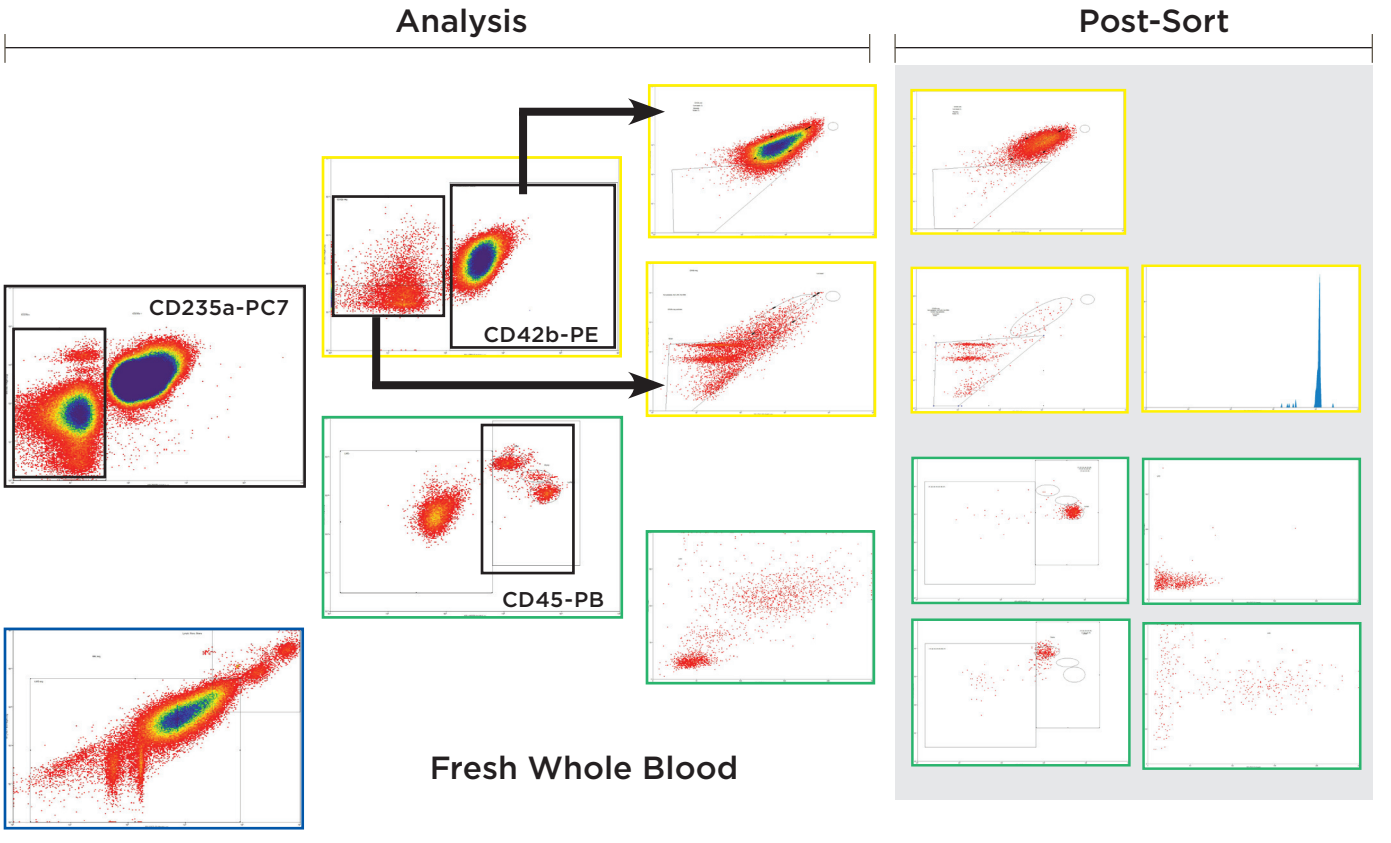
Fig. 3. Understanding noise, which is inherent in suspension buffers, is critical for sorting submicron populations. In the example above, PBS was run on the instrument to confirm noise population gating. This minimized the contribution of noise to submicron populations sort.

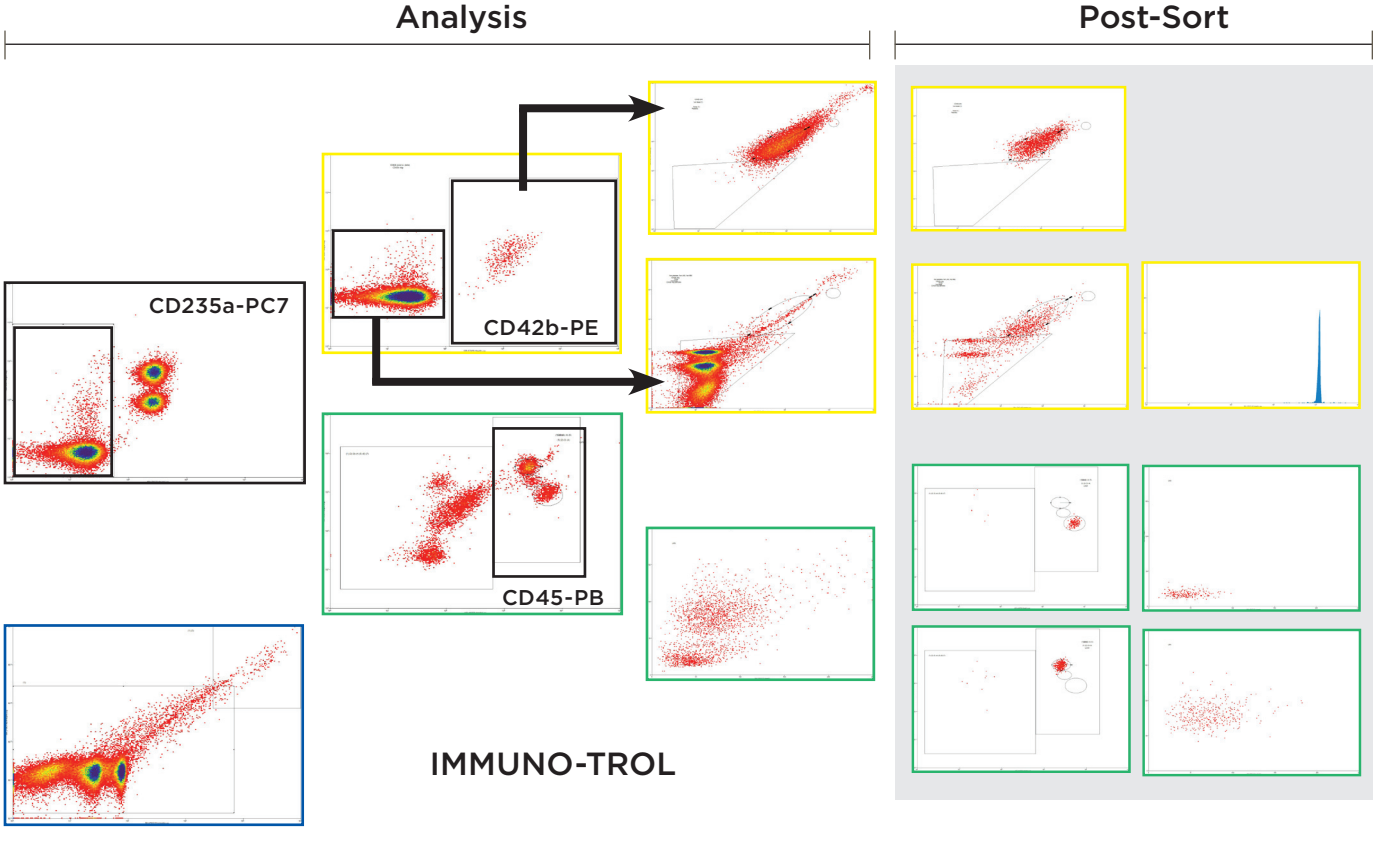
Analysis and Sorting Strategy

The top data set demonstrates results from running freshly drawn whole blood (Figure 4). The bottom data set demonstrates results from running Beckman Coulter's IMMUNO-TROL.

To eliminate the vast number of erythrocytes from the analysis, cells negative for CD235a were selected. These cells were further selected using CD45 to study lymphocyte, monocyte, and granulocyte populations (green path) or CD42b to examine platelets and other uncharacterized CD42b negative populations (yellow path). These populations were further evaluated using the S1 forward scatter mask to enhance separation of leukocyte populations and M2 for examination of submicron populations. Representative cellular populations and 1000 nm beads were sorted and reanalyzed. Notice that the populations can be analyzed by utilizing only log forward scatter parameters (blue path). It was important to understand and mark noise populations by running PBS during analysis and sorting.

Fig. 4. Analysis and Sorting Strategy.





Discussion

This protocol successfully created a minimalistic assay allowing the analysis of both micron and submicron populations present in whole blood. The unique features of the Astrios EQ allowed for the high-speed analysis and sorting of these populations. While IMMUNO-TROL populations are not as "clean" compared to freshly drawn blood, the exercise demonstrates that these cells can be used as a standard to set up and test the system. This standard method can aid in separating and characterizing various submicron and large populations in whole blood using the advanced optics of the Astrios EQ system.

Conclusions

Astrios EQ system's enhanced forward scatter module (patent pending) enables users to visualize and sort submicron and large populations simultaneously.

The method presented in this application note demonstrates a standard process for preparing and testing the Astrios EQ for studies of widely disparate populations in whole blood.

Forward scatter masks used on the Astrios EQ provide users the opportunity to enhance the separation and characterization of various populations based on material composition, size, and morphology in many different sample types.

Authors

Adapted from a poster created by:

Alan Dean, Ph.D.

Carley Ross, Ph.D.

Kerry Weinberg

Blake McCarty

Robin Morris

Mike Morell

Beckman Coulter Life Sciences, Flow Cytometry, Indianapolis, IN, USA

BECKMAN COULTER

Life Sciences

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at www.astrioseq.com

FLOW-377APP08.14-A