Optimization of FCM-based microparticle analysis protocols for cytometers using small-angle forward scatter for size measurements.

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Background:

Microparticles (MP) are submicron vesicles released from cell membranes. Their relative measurement in plasma is needed due to recognized pathophysiological roles in thrombosis, inflammation and cancer. Their low size range (0.1–1 µm) creates a difficult challenge for detection, sizing and counting by flow cytometry (1). A standardized protocol for FMP counts in the limited size range of 0.5 to 1.0 µm (2) had been developed on Beckman-Coulter (BC) FC5000 cytometers (FCMs), using submicron beads (Megamix, BioCytex, Marseille, F).

The MP gate was thus reproducibly set between:

- 0.5 µm beads-equivalent (eq.) in FS, as the lower limit defined by FS threshold,
- 0.5 µm beads: eq. in FS, as the upper limit defined by the end of 0.9 µm beads

Recent studies (3) showed that this strategy, optimized on BC FCMs measuring Forward Scatter (FSC)/SSC at high solid angle (1°), did not fully fit with FC500. Other BD FCMs only discriminated 0.5 µm from 0.9 µm beads, as did FC500. The aim was to test if settings more adapted to BD FCMs, using submicron beads, always fully discriminated MP.

Methods:

Multi-instrument comparison includes BC Galileo (left), FACS-CANTO II (middle left), LSRII (middle right and far right) and LSR-FORTESSA (right) with FSC detection by photodiode or PMT.

Beads: Prototype version of Megamix enriched with 2 smaller submicron fluorescent beads: i.e. 0.3 µm and 0.1 µm (Megamix-Plus prototype), and alternatively a 3rd 0.34 µm bead (see far-right plots).

1st threshold is done on FL1 to avoid noise. Then switched to FSC or SSC or both depending on FCM:

- How to best set up SSC threshold on BD instruments without loosing MP?
- How to best set-up SSC threshold on BD instruments without loosing MP?

Results:

Galileo (B.C.): Increased FS resolution via MP option (see poster 33G, CYTO 2011). All large PMPs resolved from 0.1 µm to 0.9 µm, with major gap from 0.3 to 0.5 µm beads.

FACS-Canto II (B.D.): Best possible FSC resolution shown here (see poster 33F, CYTO 2011), but limited to 0.5–0.9 µm:

- Clear SS resolution. All beads of Megamix-Plus resolved from 0.1 µm to 0.9 µm, with major gap from 0.3 to 0.5 µm beads.

LSR II (B.D.): Typical FSC resolution shown here but limited to 0.5–0.9 µm. Clear SS resolution. All beads of Megamix-Plus resolved from 0.1 µm to 0.9 µm, with major gap from 0.3 to 0.5 µm beads.

 Ballard (B.D.): “Red” FSC with photodiode resolution limited to 0.5 µm (comparable to FC500).

Conclusion:

SSC may be used as size-related parameter on BD FCMs provided that similar reference beads are used (0.24 µm seems ad hoc). 0.9µm beads set at upper SSC.

Abstract

Aim: Our study looked for alternative MP settings more adapted to BD FC5ms.

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