Quantitative Flow Cytometry (qFCM) protocols for end-to-end optimization of cross-platform extracellular vesicle studies

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We use this collection and it's working

Translational Nanobiology Section

Jennifer Jones

DISCLAIMER

This protocol summarizes key steps for a specific type of method, which is one of a collection of methods and assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

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ABSTRACT

Flow cytometry (FCM) is a common extracellular particles (EPs), including viruses and extracellular vesicles (EVs), characterization method. Frameworks such as MIFlowCyt-EV exist to provide reporting guidelines for metadata, controls, and data reporting. However, tools to optimize FCM for EP analysis in a systematic and quantitative way are lacking. Here, we demonstrate a cohesive set of methods and software tools that optimize FCM settings and facilitate cross-platform comparisons for EP studies. We introduce an automated small particle optimization (SPOT) pipeline to optimize FCM fluorescence and light scatter detector settings for EP analysis and leverage quantitative FCM (qFCM) as a tool to further enable FCM optimization of fluorophore panel selection, laser power, pulse statistics, and window extensions. Finally, we demonstrate the value of qFCM to facilitate standardized cross-platform comparisons, irrespective of instrument configuration, settings, and sensitivity in a cross-platform standardization study utilizing a commercially available EV reference material.

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FILES

Protocol

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Resource 1: Scatter Detector Setting Incrementation for FCMPASS

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Resource 2: Fluorescence Detector Setting Incrementation for FCMPASS

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Resource 5: rEV Scatter Detector Setting Incrementation

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Resource 6: rEV Fluorescent Detector Setting Incrementation

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Resource 7: rEV immunophenotyping

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