Conventional, high-resolution and imaging flow cytometry

potentials, pitfalls and solutions for EV characterization

Botha, Jaco; Rasmussen, Rikke Wehner; Sanden, Mathilde ; Handberg, Aase

Published in:
Journal of Extracellular Vesicles

DOI (link to publication from Publisher):
10.1080/20013078.2019.1593587

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

fluorescence signals in terms of molecules of equivalent soluble fluorochrome, (3) export calibrated channels to new .fcs files, (4) recognize unstable flow rates, (5) determine fluorescence thresholds, (6) apply gates, (7) create PDFs with scatter plots and (8) report statistics. We are using clinical studies to validate and apply the software.

Results: Compared to manual thresholding, automatic thresholding results in a systematic decrease in counts of 10% and a maximum difference of 14% (n = 5). Using a high-end laptop, data processing takes typically a minute or several seconds per .fcs file with or without PDF reporting, respectively. Flow rate monitoring is useful for 61% of the data. The platelet marker CD61 stains 7% of the events with an RI >1.42, which are lipoproteins, and the concentration of these lipoproteins differed 4000-fold between individuals.

Summary/Conclusion: We have developed software to automate calibration and processing of flow cytometry data in clinical studies, thereby reducing analyses time, preventing human mistakes and providing new insights. For example, non-specific labelling of antibodies to lipoproteins together with variations in lipoprotein concentrations emphasize the relevance of fasting before venipuncture. Our next step is to extend the software with machine learning.

Funding: NWO-TTW VENI 15924.

OWP2.03=PS08.10

Conventional, high-resolution and imaging flow cytometry: potentials, pitfalls and solutions for EV characterization

Jaco Botha, Rikke Wehner Rasmussen, Mathilde Sanden and Aase Handberg

Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark

Introduction: Flow cytometry (FCM) has long been a preferred method for characterizing EVs, however their small size have limited the applicability of conventional FCM to some extent. Thus, high-resolution and imaging FCMs have been developed but not yet systematically evaluated. The aim of this presentation is to describe the applicability of high-resolution and imaging FCM in the context of EV characterization and the most significant pitfalls potentially influencing data interpretation.

Methods: (1) First, we present a side-by-side comparison of three different cytometry platforms on characterising EVs from blood plasma regarding sensitivity, resolution and reproducibility: a conventional FCM, a high-resolution FCM and an imaging FCM. (2) Next, we demonstrate how different pitfalls can influence the interpretation of results on the different cytometry platforms. (3) Finally, we propose controls, solutions or workarounds for understanding and limiting the influence of each of these pitfalls.

Results: (1) High-resolution FCM and imaging FCM displayed greater sensitivity and resolution compared to conventional FCM when measuring a mixture of nanospheres. Equally, both methods could detect larger concentrations of specific EV phenotypes than conventional FCM, where imaging FCM outperformed high-resolution FCM. Within day variability (n = 20 aliquots) was similar for conventional and high-resolution FCM, while imaging FCM had a markedly larger variability. Between day variability ($n = 5 \times 5$ aliquots) was similar for all three platforms. (2) The three most substantial pitfalls variably influencing interpretation of results on the three platforms are non-specific binding of labels, antibody aggregates, and entities in the sample (i.e. lipoproteins) binding EV-defining dyes. (3) The most important strategies for circumventing these pitfalls are stringent matching, gating and comparison of antibodies and isotype controls, high-speed centrifugation of antibodies and labels prior to staining, and the use and interpretation of stained buffer controls and detergent treated samples.

Summary/Conclusion: High-resolution and imaging FCM hold great potential for EV characterization. However, increased sensitivity also leads to new artefacts and pitfalls. The solutions proposed in this presentation provide useful strategies for circumventing these.

OWP2.04=PS08.11

Convolutional neural networks for classification of tumour derived extracellular vesicles

Wooje Lee[a], Aufried Lenferink[a], Cees Otto[b] and Herman Offerhaus[a]

[a]University of Twente, Enschede, Netherlands; [b]Medical Cell Biophysics, University of Twente, Enschede, Netherlands

Introduction: Raman spectroscopy probes molecular vibration and thus reveals chemical information of a sample without labelling. This optical technique can be used to study the chemical composition of diverse extracellular vesicles (EVs) subtypes. EVs have a complex chemical structure and heterogeneous nature so that we need a smart way to analyse/classify the obtained Raman spectra. Machine learning (ML) can be a solution for this problem. ML is a widely used strategy in the field of computer vision. It is used for recognizing patterns and images as well as classifying data. In this research, we applied ML to classify the EVs' Raman spectra.