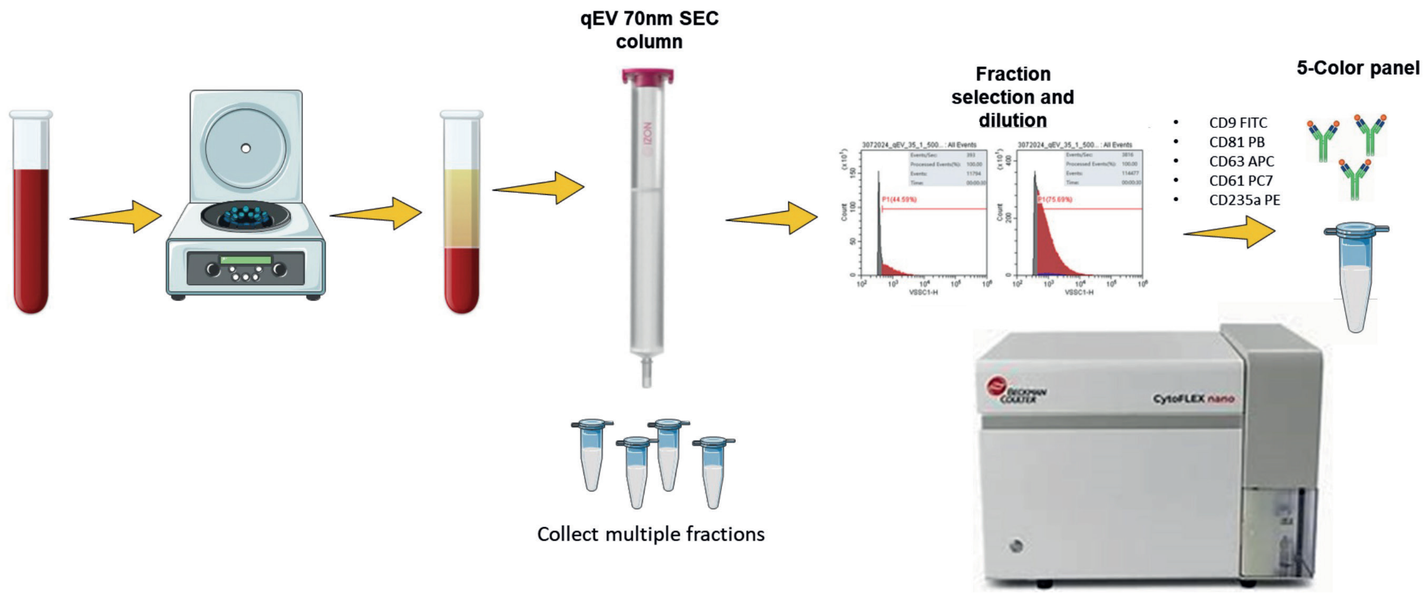
A flow cytometry approach for the characterization and isolation of extracellular vesicles

Author: Fanuel Messaggio[1], Katily Ramirez[1], Jen Bon Lui[1], Milan Popovick[1], James Tung[1], Anne-Claire Giai[1], Alfonso Blanco[2], Irene Castrosin[3], Margaret McGee[3], Anis Larbi[4]
Affiliation: [1]Research and Development, Beckman Coulter Life Sciences, Miami, FL 33196; [2]UCD Conway Institute. University College Dublin, Ireland; [3]School of Biomolecular and Biomedical Science. UCD Conway Institute. University College Dublin, Ireland; [4]Global Medical and Scientific Affairs, Beckman Coulter Life Sciences

Flow cytometry and extracellular vesicles (EVs)

The world of extracellular vesicles (EVs) is highly diverse, with variations in size, internal composition, and membrane-bound proteins. Understanding this diversity and its significance is crucial for unraveling the physiological role of EVs. To assess the potential of flow cytometry in detecting, characterizing, and isolating EVs, we employed nano flow cytometry for analysis and sorting.
The analysis of these EVs is challenging due to their heterogeneity, size, refractive index, and complexity. In this work we have used the CytoFLEX nano analyzer,* an RUO flow cytometer with high sensitivity capable of detecting nanoparticles via violet side scatter as small as 40 nm, as well as providing better sensitivity and resolution for the available fluorescent detection channels. To show the potential of the CytoFLEX flow cytometry platform we have also tested the possibility of sorting small particles using the CytoFLEX SRT benchtop cell sorter.*

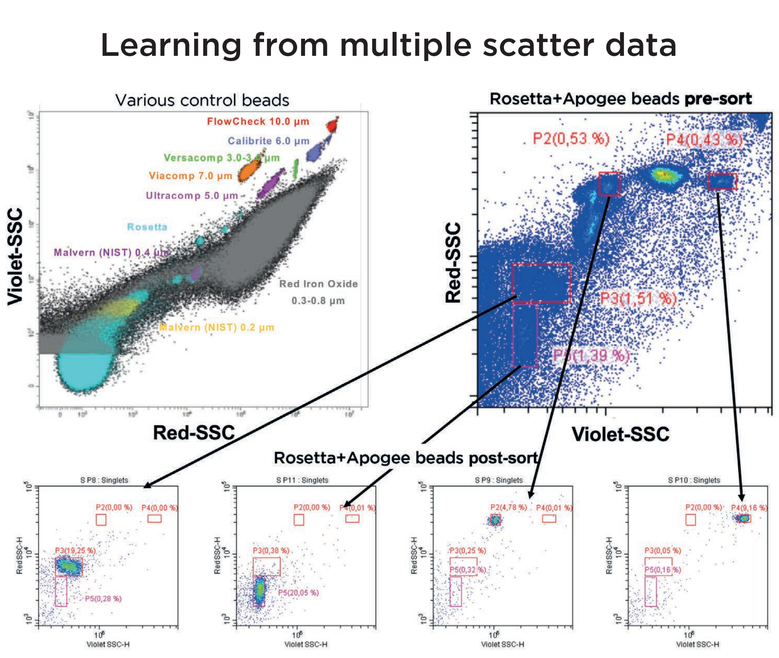
Protocol: Analysis of Platelet-poor plasma (PPP)*



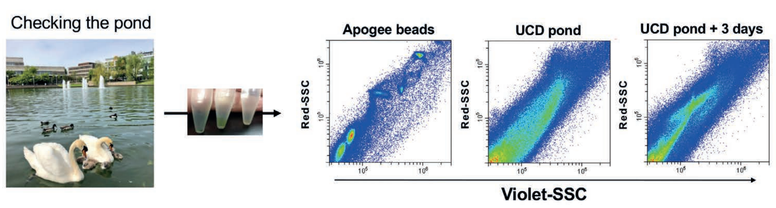
Protocol: Sorting of extracellular vesicles*

Defining the right population of EVs could be performed using the CytoFLEX nano flow cytometer. Hence, we sought to test the feasibility to sort certain classes of EVs by cell sorting. We used the advent of the CytoFLEX SRT optical bench configuration (V-SSC) and sensitivity for this purpose.

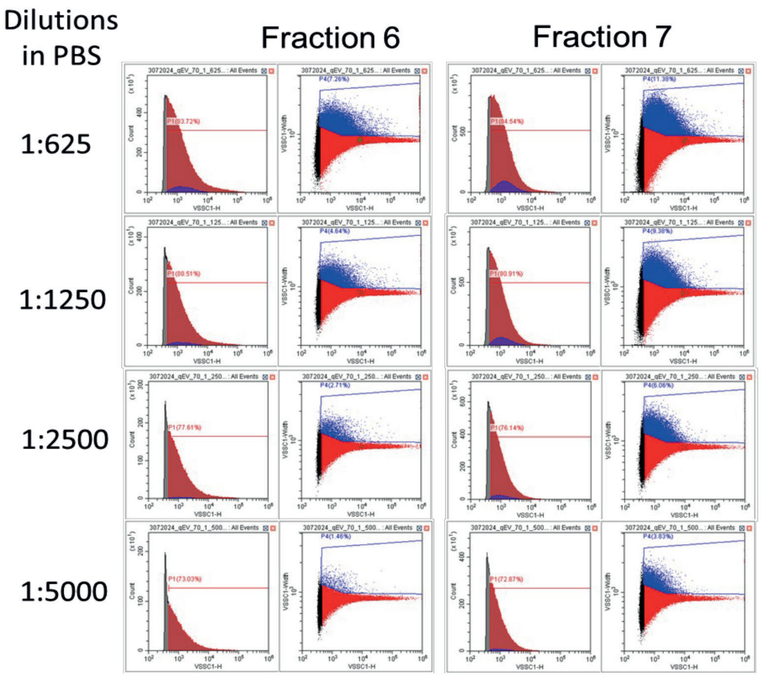
Platelet-poor plasma (PPP) EVs were prepared from fresh human blood as follows: 3mL of whole blood was centrifuged for 5 min at 200xg. 1 mL of the plasma was removed from the top and filtered through a 200 nm syringe. PPP was purified using iZON qEV 70nm size-exclusion columns (SEC). Selected fractions were screened and used for single-color stain and multi-color stain for immunophenotyping. The selected PPP fractions were then stained with the following antibodies: CD81 PB, CD9 FITC, CD63 APC, CD61 PC7 and CD235a PE. Samples were acquired on the CytoFLEX nano flow cytometer and analyzed in CytExpert nano software.*

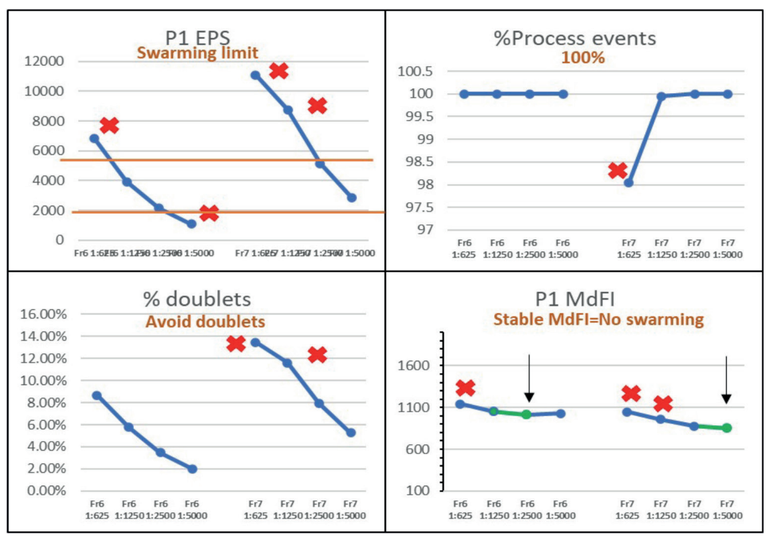


*We analyzed multiple control beads on the SRT to understand its large **dynamic range**. We **sorted** various bead sizes based on the differences between the side scatter derived from red and violet laser excitation to confirm that their differences are not an artifact.*



With the same settings, we also analyzed water from a pond and we used side scatter derived from red and violet laser excitation, which proved to be helpful with the identification and sorting of populations.



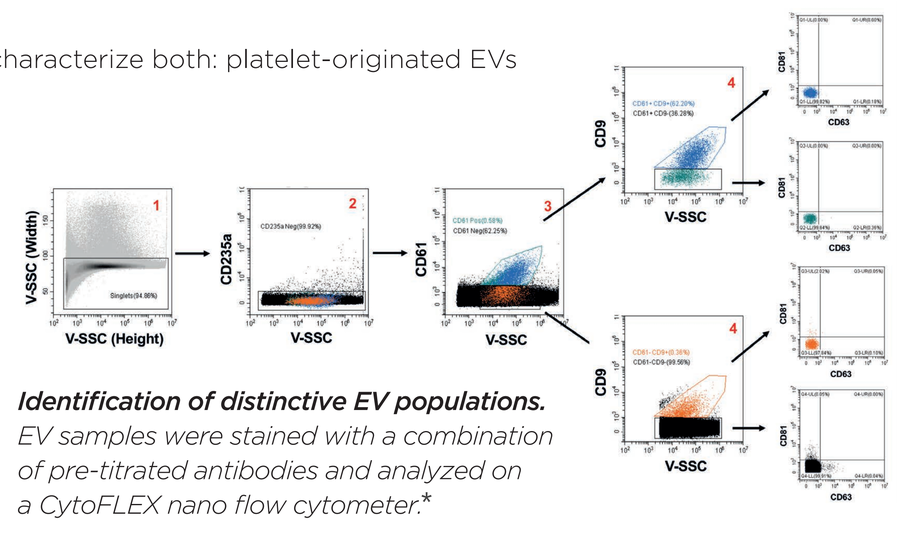


***Swarming analysis.** Sample dilution was chosen for non-swarming condition (<6000 eps), 100% processed events, and stable MdFI within the range of dilutions.*

Gate strategy. Identify erythrocyte-negative EVs, and characterize both: platelet-originated EVs and non-platelet-originated EVs

1. Gate on Singlets
2. Gate out CD235+ (possible aggregates)
3. From CD235-, identify CD61+ (Platelet-originated EVs) and CD61-
4. Further identify CD9+ and CD9-

The last plots help to identify CD81+ and CD63+ from these subpopulations



***Identification of distinctive EV populations.** EV samples were stained with a combination of pre-titrated antibodies and analyzed on a CytoFLEX nano flow cytometer.**

Conclusion

CytoFLEX nano flow cytometer was able to detect and distinguish more than one fluorescence signal on single PPP EV particles, including PB, FITC, PE, PC7 and APC. At the proper antibody concentration and with the use of proper controls, we avoided antibody aggregates and antibody background interference (data not shown). Moreover, we demonstrated that the system was able to compensate the spillover between different fluorescent dyes allowing analysis of the 5-color staining simultaneously. Finally, we demonstrated the ability to perform small particle sorting using the CytoFLEX SRT.
The field of EVs is rapidly advancing, necessitating a deeper understanding of their heterogeneity. This knowledge is crucial for comprehending their physiological roles and involvement in diseases. In this study, we successfully applied flow cytometry to analyze EVs, employing various tools. Further research will enable the development of tailored flow cytometry protocols for EV characterization.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com
2024-GBL-EN-105518-v1

*For research use only. Not for use in diagnostic procedures.