What's the smallest particle we can detect: A practical approach to determine the limit of detection of flow cytometers

*An internship position for Bachelor or Master students (Physics, Medical Natural Sciences, Biomedical Sciences) is available at the **Amsterdam Vesicle Center** of the Academic Medical Center. In our group, new treatment and diagnostic procedures based on innovative physical techniques are developed.*

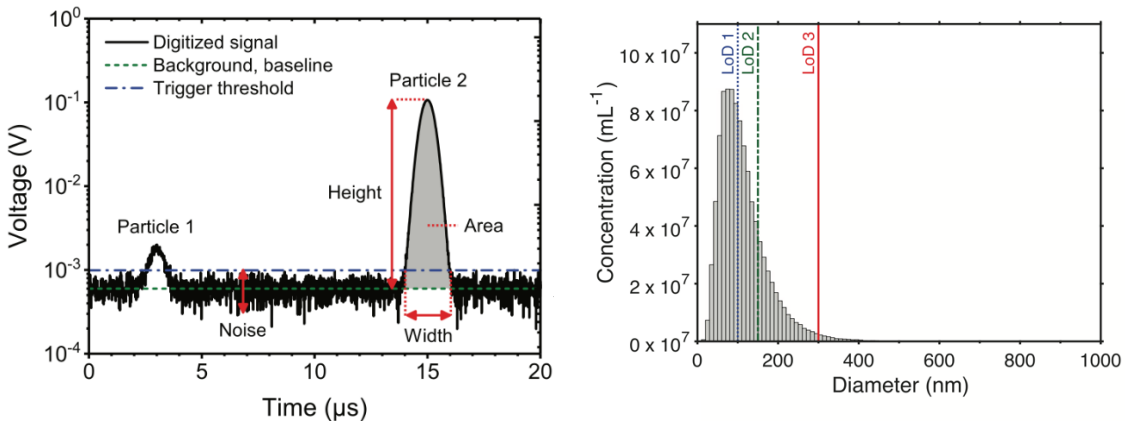

Figure 1. (A) Simulated electrical signal of the flow cytometer and the background (green line) that dominates the signal of small particles and thereby determines the limit of detection (LoD) of the flow cytometer. (B) Image of a size distribution of EVs and the determined LoD. Different LoDs, indicated by the colored vertical lines, cause a different measured concentration of particles, underlining the importance of accurate LoD determination. (Both figures copied from [1])

Background.

All body fluids contain extracellular vesicles (EVs), which are nanoparticles released by cells. EVs have the potential to be a biomarker to distinguish between health and disease, but EV measurements need more standardization before they can be used in the clinic. Flow cytometry is the preferred method for measuring EV concentrations in the clinic as it can characterize a large number of particles in a short time. However, flow cytometers are limited in measuring the smallest EVs due to a background signal of the cytometer that dominates weak signals of small particles (Fig. 1A). The largest detectable signal above the background noise is called the limit of detection (LoD).

Problem.

Small differences in the LoD between flow cytometers greatly affect the measured concentration (Fig. 1B). Therefore, an accurate determination of the LoD is crucial for a standardized and reproducible measurement of the concentration of EV samples. However, a definition of the LoD and a practical procedure to determine the LoD of a flow cytometer are currently lacking.

Solution.

Your goal is to design a practical approach, usable by all flow cytometry users, to determine the LoD of their machine. This will greatly impact the flow cytometry field beyond the application of EVs.

Proposed Project.

The first step in the project is to formulate a definition of the LoD and test this definition by (1) analyzing direct detector output, (2) analyzing flow cytometry electronics output, and (3) analyzing particle size distribution. We have a custom setup with a flow cytometer on an optical bench connected to an oscilloscope that is well-suited for this experiment. As not every flow cytometry user has access to such a setup, we need to translate these measurements to a method that is available for all flow cytometry users. After that, we can compare our approach to already existing methods and possibly translate our new approach to other devices like MRPS or NTA.

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Sources:

1. Welsh, J. A., Arkesteijn, G. J., Bremer, M., Cimorelli, M., Dignat-George, F., Giebel, B., ... & van der Pol, E. (2023). A compendium of single extracellular vesicle flow cytometry. *Journal of Extracellular Vesicles*, *12*(2), e12299.