How to differentiate EVs from lipoproteins: multi-wavelength Mie theory to determine the refractive index and size of nanoparticles

An internship position for Bachelor or Master students (Physics, Medical Natural Sciences) is available at the ***Amsterdam Vesicle Center*** *of the Amsterdam University Medical Center. In our group, new treatment and diagnostic procedures based on innovative physical techniques are developed. Our group is part of the department of Biomedical Engineering and Physics and the Laboratory of Clinical Chemistry in the AMC.*

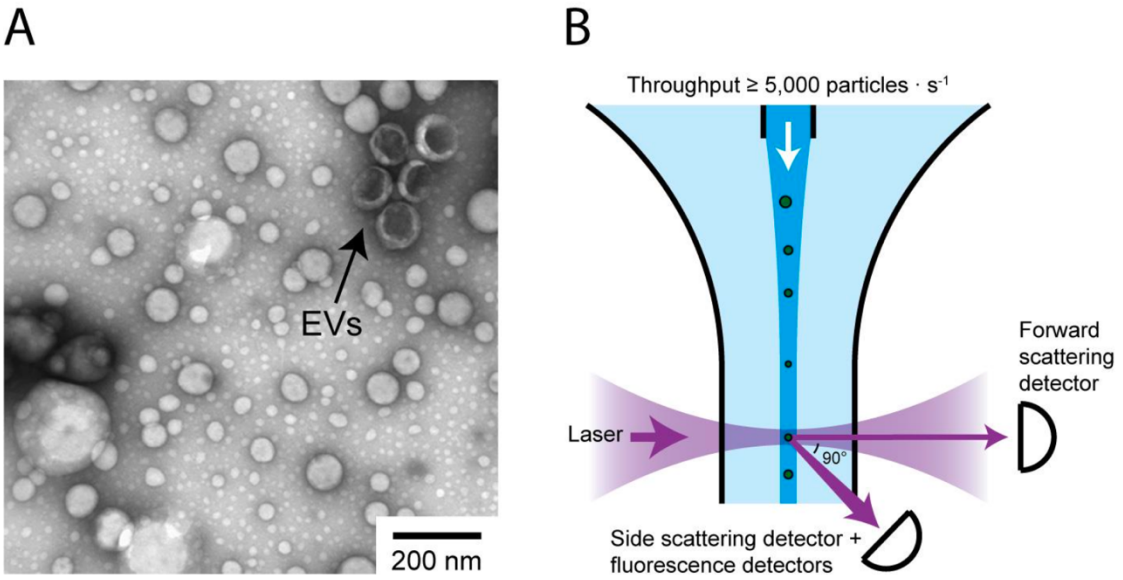

Figure 1: Extracellular vesicles (EVs) and flow cytometry. *(A) Transmission electron microscopy image of EVs (arrow) and lipoprotein particles (all other particles) from human blood plasma. (B) Schematic representation of a flow cytometer.*

Background

All body fluids contain extracellular vesicles (EVs), which are nanoparticles released by cells (Fig. 1A). EVs have the potential to be a biomarker to distinguish between health and disease. Flow cytometry is the preferred method for measuring EV concentrations in the clinic, as it can reliably characterize a large number of particles in a short time (Fig 1B). Lipoproteins have the same size as EVs but are clinically less interesting. The high abundance of lipoproteins in plasma makes measuring EVs challenging. As EVs and lipoproteins have a different refractive index, refractive index measurements can be used to differentiate between EVs and lipoproteins.

For example, our lab developed a procedure called Flow-SR to estimate the diameter and refractive index of particles using the forward and side scattering signals measured by flow cytometry [1]. The signals measured

in arbitrary units can be related to diameter and refractive index using Mie theory. The refractive index can subsequently be used to separate EVs from lipoproteins.

Problem

Flow cytometers measure the forward and side scattered light of particles in a sample stream. Flow-SR uses the ratio between the forward and side scattered light of particles to obtain their size and refractive index. The sensitivity of the forward scattering detector is limited, and therefore, Flow-SR can only be applied to particles > 200 nm. Since the side scattering detector has higher sensitivity, replacing the forward scatter detector with a second side scatter detector of a different wavelength might allow the estimation of the size and refractive index of particles < 200 nm.

Solution

The goal of this internship is to measure the diameter and refractive index of particles < 200 nm. This will greatly impact the field, since no method is currently available to quickly acquire the size and refractive index of EVs and lipoproteins < 200 nm.

Proposed Project

The first step in the project is to understand the theory of the current Flow-SR application and replicate results obtained with that application. The next step is to apply Mie theory to explore the requirements of using two side scattering detectors of different wavelengths. The challenge is to find a combination of wavelengths that have a unique solution for each particle with a certain size and refractive index. Subsequently, we can apply the theory with measurements on two flow cytometers that we have in the lab. Both flow cytometers have two side scattering detectors of different wavelengths and thus are in theory suitable for these experiments. Finally, we could explore the benefit of having a flow cytometer with side scattering detectors operating at more than two wavelengths.

Preferred Qualifications

- Programming experience in either Python or Matlab.
- Internship duration of at least four months

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Sources:

1. van der Pol, E., de Rond, L., Coumans, F. A., Gool, E. L., Böing, A. N., Sturk, A., ... & van Leeuwen, T. G. (2018). Absolute sizing and label-free identification of extracellular vesicles by flow cytometry. Nanomedicine: Nanotechnology, Biology and Medicine, 14(3), 801-810.
2. de Rond L, Libregts SFWM, Rikkert LG, et al. Refractive index to evaluate staining specificity of extracellular vesicles by flow cytometry. J Extracell Vesicles. 2019;8:1643671.