Optical detection of 50 nm vesicles

Inline scatter detector

Bachelor/Master project

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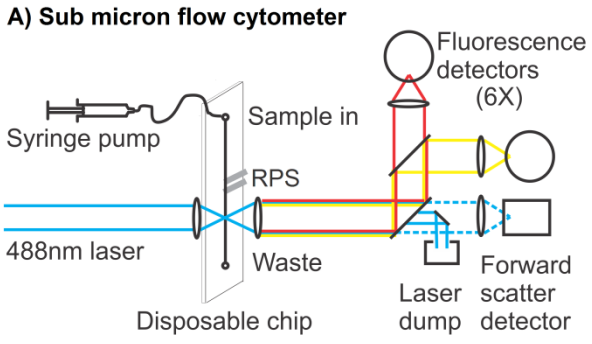
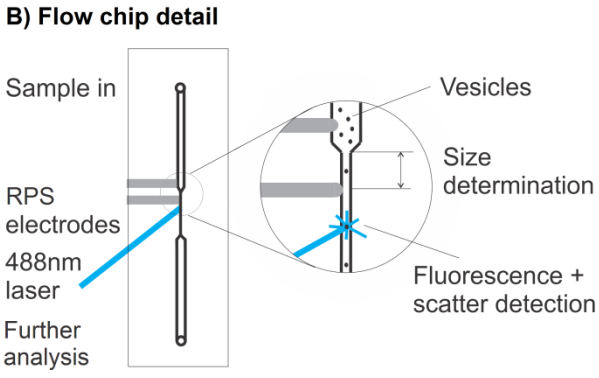
Project background

Microparticles and exosomes are cell-derived vesicles that are present in all body fluids. Their concentration is associated with the development of venous thromboembolism, cardiovascular disease, preeclampsia and cancer.
Consequently, the concentration, size distribution and origin of these vesicles are currently explored for diagnoses, prognosis, and monitoring of treatment. Typically, the concentration of vesicles is 10^{10} /mL and their diameter range between 30 nm to 1,000 nm. Because their median diameter (~60 nm) is ~100-fold smaller than cells, the utility of instruments optimised for analysing cells is limited. These instruments either measure vesicles in bulk, or measure a single parameter (size, biochemical composition, charge).
The current gold standard in single cell analysis, the flow cytometer, is capable of measuring concentration, size and multiple fluorescence signals on thousands of individual cells per second, but lacks the sensitivity to detect single vesicles. Furthermore, measurement of the concentration and size of the vesicles is severely hampered by the "large" detection volume of ≈50 pL and by the unknown scattering properties of single vesicles.
In this project, we will develop and evaluate the "Exoflow": a flow cytometer tailored to detect and characterise sub-micron vesicles by (1) reducing the excitation volume from 50 pL to 10 fL, (2) replacing the flow chamber with exchangeable chambers, (3) miniaturizing the exchangeable flow chamber and (4) implementing resistive pulse sensing on the exchangeable flow chamber. Finally, this new instrument will perform measurements on beads, viruses, and vesicles from urine samples and plasma samples to evaluate performance of the Exoflow.





Your challenge:
To convert a commercial flow cytometer from an orthogonal scatter detection setup to an inline setup.

In most commercial flow cytometers, the laser light is eliminated from the sensitive scatter detector by placing this detector orthogonal to the laser beam. Because we need to use a micro fluidic chip to reduce the detection volume, this orthogonal orientation is no longer possible. Instead we will block the low angles of laser light and collect the higher angles of scattered light (see figure below). To evaluate and optimize this design we will use commercially available 20 µm channel micro fluidic chips.

In this project you will:

- Design and build an inline scatter detector for a flow cytometer
- Optimize the design to minimize laser light in the scatter detector