Optical detection of 50 nm vesicles

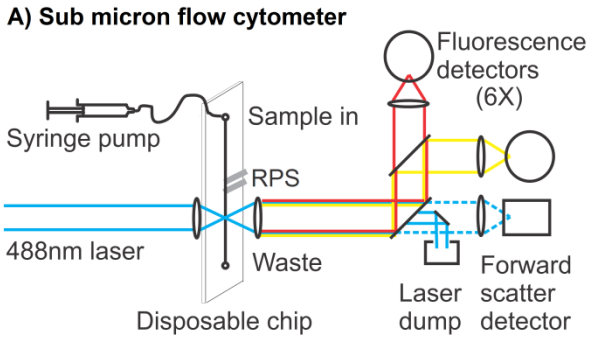
Micro chip for flow cytometry

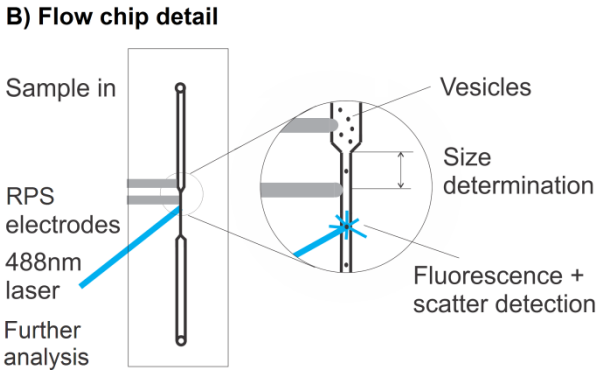
Bachelor/Master project

More info: Frank Coumans
020-5668977
f.a.coumans@amc.uva.nl

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 make a micro fluidic chip suitable for flow cytometry of micro vesicles.

This chip will need a channel that is 2 μm wide with low scattering from the channel walls, and an optically clear front and back. Two electrodes need to be placed on the chip for Coulter size detection. Preferably this chip can be replicated outside of the cleanroom, for example with PDMS based soft lithography techniques.

In the project you will:

1. Make an overview of available micro fabrication techniques
2. Design a chip
3. Build a prototype and prove it works