Submicrometer particles separation in acoustofluidic devices

Early diagnosis has become one of the main tasks for modern medical research, in particular for genetic hereditary diseases and tumours. One of the most used approaches to perform a liquid biopsy is the blood test, in spite of two main limitations. Firstly, the knowledge about specific biomarkers is weak. The second limitation regards not just the type of biomarker, but also its concentration. Since a great amount of information about the body is carried inside the blood, an efficient blood analysis represents a powerful tool in healthcare strategy. Recently, attention turns to cell released vesicles called Extacellular Vesicles. Due to their role as carrier of information for intercellular communication Extracellular Vesicles represent a feasible way to support early diagnosis. Thus, their biogenesis, biostructure and efficient isolation have gained interest. One of the main expanding areas of research focuses on exosomes, which are lipid bilayer membrane vesicles released by almost every mammalian cell type. Different laboratory protocols which exploit conventional techniques, such as ultracentrifugation, are widely used to separate exosomes/nanovesicles from biological fluids. However, despite their incredibly high efficiency, they are expensive, time consuming, technician dependent and they present low yield. Lab on a Chip technologies have emerged as alternative techniques to overcome these drawbacks.

Different microfluidic systems have been developed based on the most varied strategies for separating nanoparticles, such as acoustophoresis, dielectrophoresis, magnetophoresis, deterministic lateral displacement. This thesis presents the design and optimization of devices that can improve the separation of nanoparticles, e.g. exosomes, in a blood sample. There is heterogeneity in particles dimensions inside the blood, e.g. red blood cells, white blood cells, platelets and extracellular vesicles. Therefore, This thesis first focuses on a pre-cleaning stage, designing a "size-exclusion" device that separate bigger particles through a pillar structured filter. The trapezoidal shaped arrays were optimized using a numerical approach to obtain homogeneous mass flow rate over the filtering modules and low pressure drop along the channel. This led to developing a device that could efficiently stop particles bigger than 2 $\mu$m. Subsequently a more pioneering technique, called acoustophoresis, was adopted with the aim of separating smaller particles. In this technique, ultrasound acoustic waves are used to generate a force for manipulation of particles inside a microfluidic channel. The main aim here was to increase the separation efficiency of the acoustophoretic systems and thus decrease the critical size of the particles, which is main limit of this technique. The first aspect that was attended to was the influence of the aspect ratio of the channel cross-section on the focusing efficiency of the particles. A 2D cross-section of a microfluidic channel was considered and validated with a 3D model implemented using the limiting velocity theory. The results show an increase in the focusing efficiency of the particles with higher aspect ratio of the channel cross-section, including sub-micrometer diameters.

Subsequently, the thesis investigates the effect of inhomogeneous fluid on the separation of sub-micrometer particles. "Stabilized concentration profile" was compared to an "acoustic fluid relocation". Both strategies were analysed using a finite element method numerical approach. The percentage of cells col-

lected in the central outlet was computed using a pseudo 3D model with a plug flow assumption. A mixture of white blood cells and platelets was considered. Promising results were obtained for a stabilized concentration profile, showing an increasing separation efficiency with higher flow rates. On the other hand it was not possible to identify a trend for the fluid relocation approach, since the separation is strictly dependent on the switching position of the two fluids along the channel.
The insights gained from this thesis may be of assistance to further research about particles separation. In particular the novel microfluidic approaches presented in this study can pave the way to the development of diagnostic devices for Extracellular Vesicles isolation from a blood sample. Moreover, this work contributes to existing knowledge of acoustophoresis by providing a preliminary understanding about the influence of channel aspect ratio and of inhomogeneous fluid for particles separation.