Chapter 6

Summary and Future Perspectives

In this thesis, a new particle handling technique is presented. Pressure-driven (PF) and electro-osmotic flow (EOF) are opposed in a microfluidic channel, resulting in a bi-directional flow with PF and EOF dominating in the center core and near the walls of the channel, respectively. Application of this bidirectional flow in a microchannel that expands at both ends results in a recirculating flow. Using this hydrodynamic effect combined with the electrokinetic properties of the particles themselves results in a particle trapping phenomenon, which we have termed *flow-induced electrokinetic trapping* (*FIET*). Lettieri *et al*. first demonstrated FIET for trapping and preconcentration of particles and performed a model immunoassay.[1] This is reminiscent of work by Culbertson and Jorgenson, in which a pressure-induced counterflow was used in capillary electrophoresis to actively retard, halt, or reverse an analyte's electrokinetic migration through a capillary.[2] Here we've demonstrated that FIET can be used to separate particles having the same size but different charge or vice versa. The separation can be carried out in a batch format or continuously and has the advantage that one type of particle can be trapped without the use of physical barriers.

As described in **Chapter 3**, separation based on charge was studied using particles having the same size but different negative charge (expressed in zeta potential, ζ_p, of the particle). In the study presented, 3-μm polystyrene particles with $\Delta\zeta_p$ on the order of tens of mV were separated. Two different types of channels were used to study the separation mechanism using opposed PF and EOF. Firstly, in a 3-cm-long microchannel with uniform cross-section, separation was studied without trapping. Secondly, a channel with diverging and converging geometry was used to trap one particle type according to its zeta potential, while particles with higher or lower zeta potentials were flushed away with the pressure-driven or electro-osmotic flow components, respectively. Theoretical analysis confirmed that trapping occurs when electrokinetic and pressure-driven particle velocities are equal and opposite throughout the diverging and converging geometries.

When particles have the same charge (same zeta potential), they experience the same electrophoretic mobility. However, they can be separated based on size differences by the hydrodynamic effect present in the FIET system, as described in **Chapter 4**. This mechanism is reminiscent of Taylor-Aris dispersion[4-6] and hydrodynamic chromatography,[7-10] where particles are separated in a pressure-driven laminar flow by the combination of velocity variations across the flow profile and diffusion between the

streamlines. Exploiting FIET for hydrodynamic chromatographic separation was studied with polymethylmethacrylate particles with diameters between 2.33 and 2.82 μm. While trapped and recirculating, the particles have a net drift velocity towards the low-pressure end of the channel. By tuning the electric field with respect to the applied PF, the sign of the net drift velocity reverses and particles can escape the trapping channel in the direction of EOF. Larger particles exhibit a larger net drift velocity in the PF direction, so that the sign of the net drift velocity of the smaller particles flips first, resulting in their escape from the trapping channel. The separation of a sample containing 2.33 μm and 2.82 μm polymer particles was performed with an analytical resolution comparable to base-line separation in chromatography. One of the main advantages of exploiting FIET for hydrodynamic chromatography to size separate particles is that this method can be applied in quite short (few mm) channels, when one considers that hydrodynamic chromatography of fluorescently-labeled nanospheres and 10 kDa dextran required 80-mm-long, 1-μm-deep channels.[11, 12]

With an eye to the development of applications for biological particles based on FIET, such as assays and continuous flow separations to synchronize cell cycle phases, a number of aspects should be addressed. For instance, the buffers used in this research are often different from to physiological solution containing bacteria and cells. However, in the literature, multiple examples of capillary electrophoresis systems are described, in which bacteria and other microbes are analyzed using concentrated buffers at high pHs.[15-17] Two options can easily be tested to overcome these problems. Trapping and separation exploiting FIET could be tested using more physiologically relevant solutions, or cell viability in the currently used buffers could be tested. In the literature,[18] we find ζ_p values for cells which range from -3.7 mV to -51 mV.[19] When subpopulations of bacteria are studied,[20] differences as small as 8 mV can be found. Thus, to work with these types of biological particles it is important to understand the factors influencing the selectivity, efficiency and resolution of FIET with respect to charge and size. Furthermore, the minimum and maximum charges and sizes of the particles that are suitable for FIET should be determined.

Another interesting question is whether a sample containing particles of different sizes and/or charges can be separated, with each particle type being collected in separate trapping channels in one device. Dodge *et al*. demonstrated that modifying the surface results in different electro-osmotic mobilities.[24] Modifying the channel walls

can thus be used to obtain different EOF's and may therefore result in different trapping conditions. This can in future work be used to collect different species in various traps in one channel.

The sizes of the polymer particles used in this work are comparable to the microparticles used in other flow-inspired techniques like hydrodynamic filtration[21] and pinched flow fractionation.[22] These techniques were subsequently applied to cells with similar sizes as the polymer particles, such as erythrocytes in blood[21, 22] and mixtures of *Escherichia coli* and yeast cells.[23] To demonstrate the potential of FIET for biological applications, three different biological particle types were trapped for proof-of-principle studies (**Chapter 5**), namely yeast cells, blood cells and DNA strands of two different lengths. It was observed that all these non-spherical biological particles could be trapped with FIET, although different trapping conditions were required in each case. Furthermore, it was observed that biological particles do not behave like the well-characterized microspheres used to study the separations based on charge[13] or size[14] differences.

During the yeast experiments, it was observed that smaller yeast cells form a more diffuse gradient, with more small cells located towards the converging geometry, than larger yeast cells. This result is a good indication that in future work, FIET can be used to synchronize cells, exploiting the relationship between cell size and phase in the cell cycle as Choi *et al*. did using another microfluidic approach, hydrophoresis.[24]

One of the drawbacks of the yeast cell experiments presented in this thesis is the formation of clusters. Zheng and Yeung investigated the formation of aggregates of rod-shaped bacteria. They reported that the electric field, buffer type used and the ionic strength influence cluster formation.[25] These factors should be kept in mind when further investigating the cluster formation in the FIET system. When performing cell experiments with FIET, it is important to know if the cells are and remain viable when used. This is for example important when transferring an assay like the yeast androgen bioassay, developed by Bovee *et al*.[26-28], into a lab-on-a-chip format. Viability can be studied by staining the cells with traditionally used methylene blue or fluorescent dyes.[29] Non-viable cells are colored, whereas viable cells will remain colorless. Lettieri *et al.* already described that the net flux through the FIET system can be used to perfuse the trapped particles with another medium, as shown for a model im-

munoassay.[2] This technique can be used to develop different assays where the analyte will interact with the trapped particles. When, as a consequence of the interaction, the charge or size changes dramatically, separation can also directly be performed.

For switching between different fluids or reagents, one can think of a pressure-driven injection system using a cross-injector located before the trapping channel. The different channels that meet at the cross-injector can hold different fluids in their reservoirs. Altering the height of the fluid columns in the reservoirs of each channel can be used to change the resistance of the channel and will determine from which reservoir the fluid will flow towards the outlet reservoir. However, one should keep in mind the net flow through the system. When the net flow is in direction of EOF a method to switch between solutions should be located after the trapping channel. A system with multiple outlets can then be used and switching can be performed by switching the anode from one outlet to another.

Preliminary experiments with blood in the FIET system (**Chapter 5**) demonstrated that blood cells could be trapped. However, it has been described in the literature that biconcave discoid erythrocytes behave differently than solid microspheres.[21, 22] Our preliminary results yielded similar behavior, as red blood cells tend to align themselves against the wall, with their longest axis parallel to the flow, when redirected into the trapping channel by FIET. Further investigation of blood cell trapping and this alignment should be performed with samples containing one type of blood cell instead of the dilute whole blood used for the proof-of-principle study presented here. Staining blood cells gives the possibility to use bright-field microscopy and investigate the movement, rotation and alignment of the cells near the channel walls while recirculating in the trapping channel.

When working with FIET, it should not be forgotten that electric fields are used. Electric fields are also used to reversibly change the permeability of the cell membrane to introduce small molecules for gene therapy, a process called electroporation.[30] However, exposure to extreme conditions can result in a permanent change of permeability and cell lysis.[31] Cell lysis due to high electric fields might have happened when flushing the channels filled with blood cells, as described in **Chapter 5**. It was observed that the cells lost their spherical or biconcave shape, and an EOF could not be generated anymore. It has been reported in the literature that proteins and peptides

from the sample and lysed cells can adsorb to the channel walls and result in decay of the EOF.[24] Working at lower applied pressures will result in lower applied potentials/electric fields to trap particles. This can be advantageous to prevent cell lyses and related protein and peptide adsorption and should be considered for future work. Furthermore, it was described (**Chapter 3**) that high-ζ_p beads formed "pearl chains" at high electric fields. Therefore, lower applied pressure-driven flow and electric fields could be used to trap high-ζ_p beads without the formation of these chains.

On the other hand, short pulses of high electric fields can be used to lyse trapped cells for further on-chip analysis of the cell content. If the pulse is short enough, the cells will not escape from the trapping channel, however, adsorption of the cell content remains an issue that should be addressed. The fact that not all cell types lyse at the same electric field[32] can be used to lyse and analyse the different cell types sequentially in the same device. In future work, FIET can be used to separate and/or lyse the different blood cells to develop diagnostic applications. One can also think of diagnostics according to the behavior of the non-spherical cell in the flow. The deformability and shape of the cell might influence the behavior in the flow and can change due to disorders[33] such as sicklecell disease.[34]

It was shown that different lengths of DNA strands could be trapped with FIET. When the concentration increases during trapping, the DNA forms clusters that are flushed with the EOF. It is well-known that DNA is a coiled strand that can be stretched under the influence of flow. Velocity gradients in the direction of flow can stretch DNA strands due to the shear on the molecule.[35, 36] Different studies have reported on the stretching of DNA using shear and elongational flows,[37, 38] including studies performed in microchannels.[39, 40] Zheng and Yeung concluded that DNA can be treated not as rigid but as deformable particles when studying radial migration.[41] Reese and Zimm studied the stretching and breaking of DNA when passing through a 130-µm-wide converging orifice.[42] Stretching of DNA was reported in electric field comparable to field strengths used in FIET experiments.[43-45] Furthermore it has been reported that hydrodynamic shear stresses associated with standard laboratory procedures like stirring, centrifugation, filtration and vial filling can induce breaking of DNA[46, 47] and especially large plasmid strands (>5 kbp) are increasingly sensitive to degradation.[48] Conventional techniques like gel electrophoresis can be used to confirm DNA degradation prior or during FIET experiments. Further investigation is also required to

clarify if DNA stretching occurs and causes DNA clustering. It was observed during the experiments that the rate at which clusters were formed at the diverging geometry and transported with the EOF was higher after the initial concentration of the experiment increased.

It has been shown that FIET can be used to trap and separate particles. The results in this thesis suggest that it could be possible to develop a multi-FIET system to combine cell trapping, lysis and DNA extraction into a single device. However, further research is needed to accomplish this goal.

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