Magnetofluidics for Enhancement of Heat and Mass Transfer in Microscale

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ABSTRACT

Magnetofluidics is the science and technology that combine magnetism and fluid dynamics to modify transport phenomena for a variety of applications. Magnetofluidics often works with conventional microfluidics to take advantage of the small size, the low cost and the low consumption of sample for chemical and biological studies. Magnetofluidics has been used for actuation and manipulation of fluid flow and suspended particles or cells in microfluidic devices.

The use of bio-compatible ferrofluids as a paramagnetic carrier fluid in the field of microfluidics has attracted great interest recently. Ferrofluid is a colloidal liquid made of ferromagnetic or ferrimagnetic nanoparticles suspended in a carrier fluid. In the presence of a magnetic field, a ferrofluid becomes strongly magnetized. Thus, a small amount of samples containing ferrofluid could be manipulated for applications such as mixing, pumping, sorting of particles and cells, enhancement of heat and mass transfer phenomena and chemical reactions. On the other hand, magnetic force can be induced wirelessly and is suitable for biological studies as it sustains cell viability. Therefore, the combination of magnetofluidics and microfluidics has proven to be a low cost, efficient and versatile technology for a number of applications.

The main challenge of designing a micro-magnetofluidic platform is achieving competitive efficiency and high throughput. A good understanding of the dominant forces involved in a micro-magnetofluidic system and their order of magnitude is vital for this purpose. Due to the effect of magnetic force on particles and fluid flow, phenomena such as positive and negative magnetophoresis and magnetically driven mass or heat transfer should be investigated. The competition between the main effective forces determines the right geometry and operating conditions of the device and leads to an optimised design. Therefore, fundamental studies on the behaviour of non-magnetic particles in a diluted ferrofluid under a magnetic field are essential.

Cell and particle separation employing magnetic forces has proven to be an effective, fast and biocompatible method. Designing a size sensitive and efficient microfluidic device with high throughput has always been a challenge. In addition, mixing is a substantial and challenging step for many Lab on a Chip (LOC) assays. A rapid, low-cost and simple way to fabricate micromixers is desirable for chemical and biological applications. Recently,
employing ferrofluids for improving heat transfer has proven to be effective. The combination of ferrofluids with magnetic field can affect and alter the convective heat transfer. Fundamental studies are required to demonstrate heat transfer modification in milli- and microchannels. The present thesis addresses the above mentioned problems as follow:

- A hydrodynamic focusing system was used, which is a circular microfluidic chamber. First, the effect of a uniform magnetic field on a stream of diluted ferrofluid containing non-magnetic microparticles was investigated. The results indicated that magnetic bulk force on the paramagnetic stream is dominant, and determines the trajectories of the particles. The second experiment was carried out using the same device and a non-uniform magnetic field created by permanent magnets. The velocity field inside the device is altered by the non-uniform magnetization of the ferrofluid, leading to a more complex velocity field. Again, microparticles mostly follow the bulk flow pattern and are less affected by negative magnetophoretic force.

- A fundamental study on mass transport improvement with the assistance of the magnetic field was carried out. For this purpose, a microfluidic focusing system was used to examine the extent of mass transfer improvement in the existence of a magnetic field. The results indicated that significant enhancement can be achieved as compared with the case without magnetic field. Using the same principle, a simple, low cost, efficient and rapid micromixer was proposed. Fluorescent signal analysis was used to evaluate mixing efficiency throughout the device. The results showed that relatively high mixing efficiency up to 90% is achievable in a short channel.

- A particle trapping platform based on magnetofluidics for capturing and concentrating non-magnetic particles was proposed. The device was equipped with two arrays of attracting magnets on both sides of the microchannel. The particles introduced into the channel have a small difference in size. The device could successfully capture and concentrate microparticles at different locations. The device also has the potential to be exploited for mixing applications, using suitable boundary conditions.

- Further, the use of magnetic force for the manipulation of heat transfer was demonstrated. A magnetofluidic device comprised of a circular chamber and a permanent magnet in the proximity of the chamber wall was employed. Convective heat transport was examined and compared for three cases: DI-water, diluted ferrofluid, diluted ferrofluid in the presence of a magnetic field. The results indicated that under the effect of the magnetic field, magnetic body force on the ferrofluid
stream works against the transport of heat through the device. Instead, the magnetofluidic scheme operates as a heat-trapping device.
To my beloved parents

for their love, endless support

and encouragement.
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STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed) ________________________  (Date) 21/02/2017

Majid Hejaj

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Included in this thesis are papers in Chapters 2, 3, 4, 5, 6, 7, and 8 that are co-authored with other researchers. My contribution to each co-authored paper is outlined in the front of the relevant chapter. For the published papers, statement regarding the copyright status of the paper is also outlined at the front of the relevant chapter. The bibliographic details for these papers including all authors are:


**Chapter 3**: Gui-Ping Zhu, Majid Hejiazan, Xiaoyang Huang and Nam-Trung Nguyen, Magnetophoresis of diamagnetic microparticles in a weak magnetic field, *Lab on a Chip*, 14 (2014) 4609. DOI: 10.1039/C4LC00885E


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CHAPTER 1 INTRODUCTION

1.1 Background and significance of the research

Microfluidics is the science and technology of manipulating small amounts of fluids in channels with dimensions of tens to hundreds of micrometres, also called microchannels. The advantages of this technology are low sample and reagent consumption, low cost, high sensitivity, fast analysis, large surface-area-to-volume ratio and laminar flow regime. Over the past decades, microfluidics has been utilised in areas such as chemistry, biology, and optics. However, the research field of microfluidics is still at an early stage of development [1, 2].

Continuous-flow microfluidics provides the capability of controlling and changing the operating conditions through online measurement of parameters. Microfluidic manipulation is categorised into passive and active methods. Passive methods depend on hydrodynamics of the flow and physical properties of suspended particles only. Active methods require more complex designs and rely on an external source of energy such as electrostatic, optic or magnetic forces. Magnetic force has the advantage of bio-compatibility as it does not alter the pH level of the solution and does not generate heat [3, 4]. Magnetofluidics is the combination of magnetism with fluid flow that has recently attracted a great deal of attention in the research community. A number of applications have been reported utilising magnetofluidics. Examples are separation of cells and particles, mixing, magnetowetting, and pumping [5].

Ferrofluids (FFs) are stable colloidal suspensions of magnetic nanoparticles (MNPs) made of ferromagnetic materials with diameters ranging from 5 to 15 nm and dispersed in a liquid carrier [6]. Application of ferrofluids in biological studies has been gaining attention due to its proven biocompatibility [7]. Ferrofluids do not conduct electric current and possess a nonlinear paramagnetic behaviour. A magnetic body force is created within a ferrofluid that is exposed to an external magnetic field. This body force could be implemented to induce convective transport of mass or heat. Magnetically assisted heat or mass transfer could be employed when conventional methods are inadequate [8].
In biological studies, rare cell isolation is a significant sample preparation step for applications such as disease diagnosis. Cell separation methods are categorised as labelled and label-free. In the labelled method, cells are tagged with magnetic particles to be separated by a magnetic field. Label-free methods rely on intrinsic properties of the cells. Negative magnetophoresis can be achieved using ferrofluid as a paramagnetic medium. Most biological species possess non-magnetic properties. Cells are repelled away from the source of a magnetic field as a result of magnetic susceptibility difference between the medium and the cells. This method has the advantage of fast sample preparation time, low cost, simple device design, and high cell viability [9]. Rapid mixing in a microfluidic platform is another important sample preparation step for applications such as chemical synthesis and biochemical sensing. Magnetofluidics can be utilised to design an efficient, simple and low cost micromixer. A ferrofluid with the appropriate concentration and an external magnetic field can obtain substantial enhancement in mass transfer as compared with the conventional methods that only rely on molecular diffusion [10].

The use of magnetic force for particle and cell separation, heat and mass transfer enhancement and mixing improvement has been reported extensively in the literature. However, a comprehensive fundamental study to identify the dominant forces is still lacking. In addition, a good understanding of the interaction between the forces and their effect on the total performance of the microfluidic system is necessary to be optimised for the above mentioned applications. Magnetofluidic techniques have proven to be of low cost, high throughput, and high efficiency for particles capture, mixing, and enhancement of heat and mass transfer.

1.2 State of the art and applications of magnetofluidic technology

1.2.1 Magnetofluidics

Magnetofluidics has a wide spectrum of applications. Magnetic field in combination with ferrofluids and microfluidics can be used for making tunable magnetic devices. This thesis investigates the effect of magnetic body force on polystyrene microparticles suspended in a diluted ferrofluid solution. In addition, the implementation of magnetofluidics for particle trapping, mass and heat transfer control, and mixing is investigated. This section discusses the current status of the literature related to the research field of the thesis.
Magnetism has been utilised for actuating conductive liquid driven by Lorentz force [11-14]. Magnetic particles coated with an affinity marker are commonly used for sorting non-magnetic particles such as cells. The interaction between magnetism and fluid flow leads to research areas such as ferrohydrodynamics (FHD) [15, 16], magnetorheology (MR) [17], and magnetophoresis (MP) [18, 19]. Magnetophoresis has been used for various particle manipulation applications such as sorting, separation [20, 21], focusing [22, 23], assembling [24, 25], stirring, mixing [26, 27] and pumping [28, 29]. Conventional magnetophoretic manipulation drives magnetic beads along a magnetic field gradient. The force acting on the magnetic particles in a non-magnetic fluid is also caused by their susceptibility mismatch. The movement of a magnetic particle towards a higher magnetic field gradient is called positive magnetophoresis. Positive magnetophoresis is suitable for separation applications, because magnetic particles are commonly used as solid support for antigens, antibodies, DNA and cells. With a functionalised coating, targeted biomolecules or cells can be labelled with magnetic particles and subsequently trapped or sorted by an external magnetic field [30]. Magnetophoresis has been used for the separation of red blood cells [31], isolation of progenitor cells [32] and separation of breast cancer cells from human blood [33]. Efforts have been devoted to improve positive magnetophoretic separation such as the selection of magnets and flow configuration. Readers may refer to review papers on the basic principles as well as the various applications of positive magnetophoresis [5, 34, 35].

Most particles in analytical and biological fields exhibit non-magnetic properties. Negative magnetophoresis is the phenomenon, where non-magnetic particles in a paramagnetic carrier migrate away from the magnetic source or a higher gradient due to the magnetic buoyancy force [36]. The magnetization of a paramagnetic carrier could be determined by the susceptibility of the liquid and the magnetic field. Ferrofluids as a paramagnetic solution with a high susceptibility suit well for the implementation of negative magnetophoresis.

Since the discovery of ferrofluid in the early 1960s, this material has been used extensively in various applications. Rosensweig [37] investigated ferrofluids and ferrohydrodynamics in details. Vekas et al. reviewed the recent achievement of the synthesis of magnetic nanoparticles [38]. Numerous research studies have been conducted to characterize ferrofluids according to particle concentration [39], magnetization [40, 41] and viscosity [42, 43].
1.2.2 Separation and concentration

Xuan and his colleagues developed a microfluidic device with embedded permanent magnets for particle manipulation [44, 45]. The device was used for concentrating [46, 47], separation [48-50] and focusing [51] of particles and cells. Mao and Kosner [14] reported mixing a ferrofluid with a fluorescein buffer using ferrohydrodynamic instabilities caused by a sudden velocity variation in the flow passing by a permanent magnet. The same group demonstrated magnetic manipulation, separation and sorting of particles and cells by using a ferrofluid [21, 52, 53]. Size-dependent manipulation of non-magnetic particles in ferrofluids was reported. Separation of non-magnetic cells was achieved with an efficiency of 100% [54, 55] and a throughput of $10^7$ cells per hour. Efforts have also been devoted to applications in particle focusing. [50, 56, 57]. In addition to experimental investigations and an analytical model, Mao's group reported the transport of nonmagnetic particles in ferrofluids under a non-uniform magnetic field. [54, 58]. Furthermore, particle assembly [24, 25] has also been reported. Friedman and Yellen reviewed the underlying principle and models for separation, manipulation and assembly of non-magnetic solids in an external magnetic field [59].

Wilbanks et al. experimentally and theoretically investigated the concentration of non-magnetic particles using two repelling or attracting magnets [47]. The effects of different magnet arrangements and flow rates on particle concentration were studied. Liang et al. [48] compared the migration behaviour of 2.85-μm magnetic and 10-μm diamagnetic particles in diluted (10%) EMG 408 (Ferrotec) ferrofluid and in an aqueous solution. Using ferrofluid as the medium increases susceptibility mismatch and significantly improves the separation throughput. Peyman et al. [60] used magnetophoretic repulsion forces to demonstrate label-free manipulation of particles. Focusing, trapping and size selective negative magnetophoretic separation of non-magnetic particles were demonstrated. Zhu et al. [61] employed an analytical model to study the trajectories of non-magnetic microparticles in ferrofluid through a microchannel in the proximity of a permanent magnet. This study concluded that higher deflection could be achieved with larger particles or at a lower flow rate.

In many biological studies, cell concentration is an important sample preparation step. Red blood cells are paramagnetic due to deoxygenated haemoglobin proteins, while other cells existing in blood are diamagnetic. Han and Frazier [62] reported label-free separation of red and white blood cells from diluted whole blood with a diamagnetic capture (DMC).
mode and a high-gradient magnetic field. Their results showed that 89.7% of red blood cells and 72.7% of non-magnetic white blood cells could be separated. Rodríguez-Villarreal et al. [63] investigated the effect of several parameters such as the particle size, type and concentration of paramagnetic salt solution, as well as the flow rate on the focusing of non-magnetic polystyrene particles and living label-free HaCaT cells. This study revealed that raising the susceptibility mismatch between particles and carrying fluid, reducing the flow rate, increasing the particle size, and increasing the residence time in the magnetic field can improve the focusing effect. The use of a background ferrofluid enables the manipulation of non-magnetic particles and cells, and their biocompatibility has been investigated in the literature.

Zeng et al. [46] also demonstrated magnetic concentration of particles and cells in a ferrofluid flow through a straight microchannel using permanent magnets. The concentration of both 5-μm polystyrene particles and live yeast cells in 0.059 EMG 408 ferrofluid was investigated. This work examined the effect of flow rate, arrangement of the two attracting magnets and the magnet-to-magnet distance on the concentration performance and flow patterns. The authors discovered that dilute ferrofluid and magnetic force have negligible influence on the viability of yeast cells. Kose et al. [21] reported the separation of live red blood cells from sickle cells and bacteria. A concentration of 40 mM citrate (stabilised with citric acid to yield a pH of 7.4) was found to be optimum for cell viability and ferrofluid stability. Au et al. [64] investigated the effects of nanoparticles on the adhesion and cell viability on astrocytes. The study suggested that the addition of nanoparticles to immature astrocytes causes inhibition of cell attachment and impedes subsequent growth. However, nanoparticles induce mitochondrial uncoupling in mature astrocytes but do not alter cell membrane integrity.

External magnetic field has been widely used for continuous separation of cells and particles based on their size or magnetic susceptibility. The capability of contactless manipulation of species inside a microchannel is the remarkable advantage of micro magnetofluidics [65]. Pamme systematically reviewed the exploitation of magnetic force in the field of microfluidics including various functions such as trapping, transport, separation, and mixing [34]. Sorting of cells and particles using magnetofluidics could be achieved by trapping, focusing or deflection. A variety of methods have been reported in the literature on the separation by deflecting and concentration by trapping of cells and particles. Separation and sorting of cells and particles by deflection has been explored most extensively. Pamme
and Wilhelm reported the use of positive magnetophoresis for continuous sorting of mouse macrophages and human ovarian cancer cells (HeLa cells) [66]. Magnetic nanoparticles were used to label the cells. The extent of deflection and consequently the effectiveness of separation depend on the size of the cells and the flow rate. Khashan et al. utilised computational fluid dynamics (CFD) to simulate the positive magnetophoretic separation of magnetically labelled cells in a microchannel [67]. Chung et al. used the so-called magnetic tweezers to separate magnetic microparticles with positive magnetophoresis technique in a microfluidic device [68]. At a relatively low flow rate, the method can achieve a separation rate as high as 81%. Combining with optical tweezers, magnetic tweezers may yield an even higher separation rate for a broader range of flow rate. Lateral separation of superparamagnetic beads and labelled breast adenocarcinoma MCF-7 cells, utilising positive magnetophoresis were achieved using an angled permanent magnet [69]. A numerical model was formulated to predict the separation phenomenon. Liang et al. employed dilute ferrofluid as the carrier fluid in order to simultaneously use positive and negative magnetophoresis for improving the separation of magnetic and non-magnetic particles [48]. An analytical model was employed to predict the deflection of particles in both ferrofluid and water.

Gelszinnis et al. reported a magnetic composite material, i-PDMS, to capture and separate paramagnetic beads with positive magnetophoresis [70]. The composite generates high local magnetic field gradients when placed between two permanent magnets. The effects of the resolution of the microstructures and the flow rate on trapping and deflection of the particles were investigated. Zhu et al. employed ferrofluid to separate a mixture of particles with different magnetic properties using both positive and negative magnetophoresis [71]. This separation method is label-free and relies only on intrinsic properties of the cells or particles. Kim and Kim numerically investigated the positive magnetophoretic separation of malaria-infected red blood cells from whole blood [72]. Malaria-infected RBCs (pRBCs) was considered as paramagnetic particles suspended in a Newtonian fluid in their model. The design parameters such as magnetic pole array pitch, channel height, channel length and the flow rate were optimized to achieve the maximum capturing efficiency. Recently, Wu et al. numerically modelled positive magnetophoretic separation of malaria-infected red blood cells (RBCs) in a two-dimensional parallel-plate system using Eulerian–Lagrangian approach [73]. Blood plasma was considered as a Newtonian fluid, while the RBCs were modelled as soft paramagnetic spheres affected by viscous drag and magnetic force, as well as their collisions. The construction of a 50-μm high and a 2-mm long diffuser could improve the overall
separation efficiency. Zhang et al. designed a customized microfluidics device using 3D printing technology [74]. Efficient and rapid extraction of spiked Human Papillomavirus (HPV) 18 plasmids in specimen transport medium was achieved. Magnetic field was applied to move pure nucleic acids across the oil layer. In addition, a kinetics model was developed for the adsorption of nucleic acids on cellulose functionalised superparamagnetic beads, using positive magnetophoresis.

Scherr et al. investigated and compared protein capture in a system with one and two magnets configuration [75]. Positive magnetophoresis was used for protein capture. The two-magnet system demonstrated a higher efficiency and more rapid extraction of target biomarkers as compared to the one magnet system. Wang et al. developed an automated microfluidic system using a low-cost technique: PMMA-tape-glass hybrid [76]. The device was utilised for the preparation of single-stranded DNA and magnetic bead-based microarray analysis. The device contains two on-chip mixing valves, a magnet, and the fluidic control system for the manipulation of magnetic beads utilising positive magnetophoresis. Detection of nine mutation loci that account for hereditary hearing loss was achieved.

Rapid label-free separation and monitoring of different cell populations based on their magnetic and density signatures are possible using levitation platforms. A pair of magnets with their same poles facing each other and magnetic field aligned with the gravitational force can focus and separate diamagnetic particles or cells suspended in a paramagnetic fluid for imaging and monitoring purposes. Knowlton et al. developed a smartphone based platform using magnetic levitation to measure densities of micro objects [77]. The system consists of permanent magnets with the same poles facing each other, a disposable microcapillary, optical components (aspheric lens, printed lens frame, LED, and diffuser), and a customised Android application running on a smart-phone. Focusing the microspheres with varying densities in a line and their separation were achieved using a pair of magnets. Furthermore, precise estimation of the particles densities was possible by calibrating their levitation heights. The team also developed a smartphone testing platform for label-free and sensitive Sickle cell detection system was developed by Knowlton et al. The platform takes advantage of the higher density of sickle red blood cells under deoxygenated conditions [78]. The sample is suspended in a paramagnetic medium, and red blood cells are levitated as a result of the equilibrium between the magnetic and buoyancy forces acting on the cells. Tasoglu et al. reported label-free separation and monitoring of cell populations utilising a magnetic levitation based device based on magnetic and density signatures [79]. The system
uses a pair of magnets with same poles facing each other to focus cells. Circulating cancer cells, sickled RBCs, and healthy RBCs suspended in gadolinium-based \((\text{Gd}^+\)) paramagnetic medium were tested with this platform. Rapid spatial separation of the cell population was achieved.

Trapping and concentrating cells and particles has potential applications in chemical and biological studies. Ahn et al. reported one of the earliest microfluidic devices for the manipulation of magnetic particles in a dilute suspension [80]. Integrated electromagnets induce a magnetic field that traps magnetic particles in the flow without physical barriers. The trapped particles were released after removing the magnetic field by turning off the electromagnet. Winkleman et al. reported a three-dimensional magnetic trap for non-magnetic particles such as living cells in a paramagnetic solution [81]. The concentration of paramagnetic salt was maintained at a low level to keep the cells viable. Because of the relatively low field strength, field gradient and susceptibility mismatch, the device can only trap particles larger than \(5\ \mu\text{m}\) in diameter. Ramadan and Gijs reported a continuous-flow microfluidic device with rotating magnetic field for trapping and releasing of magnetic particles [82]. The rotating permanent magnets periodically arranged the magnetic field for periodic trapping and releasing of particles, allowing continuous concentrating, washing and purifying of magnetic particles in the device.

Teste et al. reported a lab-on-a-chip device with a magnetic chamber packed with ferromagnetic iron beads to trap 30-nm magnetic nanoparticles [83]. The iron beads increase the local magnetic field gradient and thus improve the trapping efficiency of the magnetic nanoparticles. Watarai and Namba investigated the impact of a non-uniform magnetic field on non-magnetic micro particles suspended in a paramagnetic manganese(II) chloride solution [84]. The team observed that when the particles approach the pair of magnets, the direction and the magnitude of their velocity change significantly. The magnetophoretic buoyancy exerted on the particles creates a magnetophoretic velocity and leads to the capture of particles in the area with a minimum magnetic field. In another work [85] the authors reported an improved performance with a pair of small iron tips attached to the Nd–Fe–B magnets. The magnetophoretic velocity of 3-\(\mu\text{m}\) polystyrene microparticles in 0.6 M manganese(II) chloride solution was determined. Furthermore, the team investigated magnetophoresis of a single human blood cell in 0.1 M manganese(II) solution and estimated its magnetic susceptibility.
Aki et al. developed a microelectromagnetic system with a non-uniform magnetic field to trap nonmagnetic microparticles and yeast [86]. Polystyrene particles and yeast cells were trapped at regions with non-uniform magnetic field. Bucak et al. developed a continuous counter current separation system based on magnetophoresis principle [87]. The non-magnetic microparticles experience a magnetophoretic force in the direction of decreasing magnetic field strength. The device could successfully concentrate and separate non-magnetic microparticles suspended in ferrofluid under a nonuniform magnetic field. Zeng et al. demonstrated the concentration of polystyrene particles and live yeast cells from a ferrofluid solution using negative magnetophoresis [46]. A pair of attracting magnets creates a non-uniform field. Peyman et al. studied the possible applications that could be achieved based on negative magnetophoresis [88]. Trapping, focusing and deflection of polystyrene particles were demonstrated by changing the arrangement of magnets along the microfluidic device. Zhou et al. examined simultaneous trapping of diamagnetic and magnetic particles in different locations of a microfluidic device [89]. A T-shaped microfluidic device with a single permanent magnet was used to concentrate both particles from a ferrofluid flow. Tarn et al. reported simultaneous trapping of magnetic and non-magnetic particles with a pair of permanent magnets [90]. In an aqueous solution of manganese (ii) chloride, magnetic particles were trapped between the magnets, while non-magnetic particles were trapped outside this zone. Eisentrager et al. reported a model of a periodic array of magnetised cylinders for high-gradient magnetic separation of particles [91].

1.2.3. Mixing

Rapid mixing in microfluidic devices is an important task in synthesis of materials, chemical/biochemical analysis, and cooling [92]. Micromixers find a broad range of applications such as reactors [93-95], lab on a chip for chemical engineering [96], enhancement of chemical selectivity [97], extraction processes [98], drug discovery [99], polymer synthesis [100] and DNA amplification [101]. The challenge in designing micromixers is achieving fast and efficient mixing within a short residence time or in a short microchannel. Depending on their operation mechanism, micromixers are categorised as passive or active. Passive mixers are easy to fabricate due to the fact that they do not need external energy supply. Passive micromixers include lamination [102, 103], chaotic advection, injection, or droplet mixers [104]. Faster and more effective mixing can be
achieved using active micromixers, which require an external energy source and a more complex fabrication process. For stirring or disturbing the fluid flow, active concepts such as acoustic [105], dielectrophoretic, electrokinetic, pressure perturbation, electro-hydrodynamic, magnetic, and thermal actuations have been used in active micromixers. A number of excellent review papers were devoted to the classification and application of micromixers [106-110].

Fluid flow in a microfluidic device can be manipulated using magnetic forces [111]. Inducing magnetic force is wireless and has the advantage of providing an environment for cell viability in biological studies [112]. Several methods have been reported on using magnetic force for mixing in microscale: micro magnetic stirrers, magnetophoresis of magnetic particles, magnetohydrodynamics (MHD), and micro magnetofluidics [113]. Chaotic mixing of magnetic beads in a biological fluid was achieved using induced magnetophoresis [114]. The micromixer consists of micro conductors embedded in a microchannel. The chaotic regime was verified by the numerical analysis of particle trajectories. The driving frequency and the residence time could be adjusted to obtain effective mixing. Zolgharni et al. investigated chaotic mixing of magnetic particles in a biological fluid using Lagrangian tracking method [115]. The efficiency of capturing the target cells with magnetic particles was evaluated. Numerical simulation allows for the optimization of the operating conditions for the mixer. Bau et al. reported theoretical and experimental studies on a MHD-based micromixer [116]. An array of electrodes in the presence of a magnetic field induces a body force in the fluid, which generates a complex flow field. The concept deforms and stretches fluid interfaces to enhance mixing.

Rida and Gijs introduced a method for mixing based on the manipulation of self-assembled magnetic microbead structures held in place by a magnetic field [117]. Soft ferromagnetic structures were integrated in a Y-shaped microchannel to create local magnetic fields. Efficient mixing could be achieved as the result of the strong particle-liquid interaction. A mixing efficiency of 95% within a length of 400 µm was achieved. Adjusting the magnetic field and the liquid flow rate optimizes the mixing process. Wang et al. investigated numerically a magnetic particle driven micromixer [118]. The effect of parameters such as magnetic actuation force, switching frequency and dimensions of the microchannels were studied. Based on the numerical results, the maximum efficiency occurs at a relatively high operating frequency for large magnetic actuation forces and a narrow microchannel.
Mao and Koser achieved rapid mixing in a microchannel using magnetofluidic actuation [119]. Embedded electrodes carrying traveling magnetic waves were used to create a local magnetic field. Mixing of water-based ferrofluids with a fluorescein buffer solution demonstrates a significant enhancement in mass transport as compared with pure molecular diffusion. An alternate-current (AC) electromagnetic field can induce transient flows between a ferrofluid and a fluorescence dye solution, allowing for a simple and efficient mixing concept [120]. The magnetic field causes a significant and uniform expansion of the ferrofluid toward the dye, creating extremely fine fingering structures at the interface. High mixing efficiency of up to 95% was achieved within 2 seconds and at a distance of 3 mm from the inlet of the microchannel. Numerical simulation of the phenomenon was reported in a separate work [121]. The results from the simulation demonstrate that the magnetic body force significantly affects the mass transport process of the ferrofluid.

Zhu and Nguyen reported the mixing phenomenon and the effect of a uniform magnetic field on a paramagnetic ferrofluid [122]. A mixture of DI water and glycerol and water-based ferrofluid were introduced into a circular microchamber. As a result of magnetic susceptibility mismatch under a uniform magnetic field, instability at the interface was observed leading to rapid mixing. The effect of parameters such as magnetic flux density, flow rate ratio and viscosity ratio on mixing efficiency was investigated. Kitenbergs et al. examined the mixing process of a paramagnetic ferrofluid stream with a diamagnetic water stream under a homogeneous magnetic field in a Hele–Shaw cell [123]. Mixing enhancement through magnetoconvective transport was demonstrated.

1.3 Research objectives

For the present research, diluted ferrofluid was employed as super-paramagnetic solution, in all experiments. The use of ferrofluid solutions in microfluidic systems leads to the creation of two major forces: negative magnetophoretic force, when non-magnetic microparticles are suspended in a super-paramagnetic fluid, and magnetoconvective force towards the magnetic field source. The aim of this research is to investigate the effect of the two forces for several microfluidic applications. The main objectives of this dissertation are listed as follow:

- To review the literature concerning various methods and approaches for the problem of particle sorting with magnetic force.
- To study the impact of negative magnetophoretic force on particle sorting.
• To induce and investigate the magnetoconvecive phenomenon.
• To highlight the significant role of magnetoconvecive force in several microfluidic applications, particularly particle sorting, separation and concentration, mass transfer, mixing, and heat transfer.
• To compare the order of magnitude of the main forces involved in micro-magnetofluidic applications.
• To develop a comprehensive multi-physics simulation for micro-magnetofluidic systems, capable of predicting their behaviour under various conditions, and for different applications.

1.4 Research scope and original contributions

Magnetofluidic technology is able to manipulate tiny quantities of liquid by means of external magnetic fields. The common way of implementing this technology is employing ferrofluids as a paramagnetic medium fluid. Paramagnetic iron oxide nanoparticles experience positive magnetophoresis and move towards the field maxima. In a multi-stream system with a mismatch in magnetic susceptibility between the streams, a body force is exerted on the ferrofluid stream. The body force creates a secondary flow towards the field maxima and could be taken advantage of for heat or mass transfer control. In the case with no magnetic susceptibility mismatch between streams and under a non-uniform magnetic field, a concentration gradient is created in the ferrofluid. This concentration gradient leads to a magnetic susceptibility mismatch and consequently a secondary flow within the ferrofluid. The phenomenon can be utilised for applications such: heat transfer control or mixing. If particles or cells are introduced into a magnetofluidic system, and the magnetic susceptibility of the particles or cells is less than that of the medium fluid, negative magnetophoretic force is substantial. This force, depending on the operating conditions and magnet arrangement, could be used for applications such as: particle capture, particle focusing, particle separation by deflection, and particle aided mixing.

The present thesis includes fundamental studies based on experimental observations reported in peer-reviewed journal papers. Chapter 2 is a review paper on particle and cell sorting. Chapter 3 reports the effect of magnetic bulk force on non-magnetic particles under a uniform magnetic field. Chapter 4 utilises the same device with a circular chamber to investigate the effect of a non-uniform magnetic field with no magnetic susceptibility.
mismatch between the streams. **Chapter 5** presents mass transfer enhancement of fluorescent dye due to magnetoconvective flow. **Chapter 6** reports the design, fabrication and test of a particle trapping platform based on magnetofluidics. Taking advantage of magnetically driven mass transport, **Chapter 7** proposes and characterises an efficient mixer. **Chapter 8** examines the impact of magnetic bulk force on convective heat transfer of a ferrofluid. Figure 1 provides an overview on magnetofluidic phenomena and their possible applications. The chapter numbers are noted in the chart indicating which areas are covered by this thesis.

Based on the phenomena and type of the working fluid, magnetofluidics can be categorised into four research areas: magnetohydrodynamics (MHD), ferrohydrodynamics (FHD), magnetorheology (MR) and magnetophoresis (MP) [5]. The research presented in this thesis can be subcategorised under magnetophoresis and ferrohydrodynamics.

As the work was published in several stand-alone papers, the following terminologies need to be defined for consistency throughout the thesis:

- **Magnetoconductive mass transfer (magnetoconvection):** convective mass transfer aided by magnetic force. Magnetoconvection is caused by a magnetic susceptibility mismatch in the system, or a magnetic field gradient.

- **Non-magnetic particles (diamagnetic particles):** Particles that are not attracted by magnetic field are called non-magnetic particles. The term diamagnetic is also used to describe the particles in the literature.

- **Negative magnetophoresis (diamagnetophoresis):** when magnetic susceptibility of suspended particles is less than that of the fluid medium, particles are pushed away from magnetic field maxima. Both negative magnetophoresis and diamagnetophoresis are commonly used in the literature.
The original research contributions reported in this thesis can be summarised as follow:

- The effect of magnetic body force on suspended micro-particles under uniform and non-uniform magnetic fields is investigated. In most studies, negative magnetophoresis for polystyrene particles was reported. We investigate the capability of altering the velocity field by magnetic field and deflecting non-magnetic particles towards field maxima, which was not reported before in the literature.

- The capability of mass transfer improvement is examined using ferrofluid and a non-uniform magnetic field. A qualitative and quantitative evaluation of the phenomenon was carried out for the first time to demonstrate the extent of mass transfer improvement.
transfer enhancement. The comparison between a conventional molecular diffusion based microfluidic system and our magnetofluidics based system demonstrates the enormous enhancement in mass transport of a non-magnetic species.

- For the purpose of particle and cell separation, a magnetofluidic device was designed and tested for particle trapping. A total analysis of the balance between the dominant forces is provided to determine the quality of particle capture. This systematic approach was not reported before in the literature. This study elucidates effective design parameters and different possible regimes in a magnetofluidic system.

- A simple low-cost microfluidics system based on micro-magnetofluidics for mixing application is proposed. Fluorescent image analysis was carried out to evaluate and quantify mixing efficiency as well as to determine the optimum operating conditions. This quantitative work has not been done before for a micro-magnetophoresis based micromixer. Rapid and efficient mixing was achieved in a relatively small microchannel.

- Furthermore, we employed the magnetofluidic concept to examine the effect of magnetically driven body force on heat transfer. The phenomenon of heat trapping due to magnetic driven convection was demonstrated.

1.5 Thesis outline

Addressing the problems highlighted in the previous section, the thesis is devoted to the fundamental study and applications of magnetofluidic technology. Controlling forces are identified and the effect of interaction between the forces on the total performance and efficiency of the devices is investigated. The main aim of the present thesis is to demonstrate the application of magnetic body force on diluted ferrofluids for common microfluidic tasks such as: particle trapping, mixing, and heat transfer control. The key research areas and general outline of this thesis are described in greater detail in the following paragraphs.

Given that particle sorting is the most frequent application of microfluidics in biological studies, Chapter 2 presents a review on continuous-flow magnetic particle and cell separation. This review discusses the fundamental physics involved in using magnetic force to separate particles, and identifies the optimisation parameters and corresponding methods for increasing the magnetic forces. The review highlights the state-of-the-art techniques and
applications of continuous magnetic separation of cells in a microfluidic device. The review reports separation of cells and particles in microfluidic devices with various geometries, arrangements and size of magnets.

Chapter 3 focuses on the fundamental investigation of the behaviour of non-magnetic micro-particles in a diluted ferrofluid stream under a uniform magnetic field. The fluorescent microparticles are suspended in the ferrofluid core stream that is sandwiched between two non-magnetic cladding streams in a circular chamber. A custom-made electromagnet was used to generate a relatively weak uniform magnetic field across the chamber. Due to the difference in magnetic susceptibility between the core and the sheath streams, a body force was exerted on the core stream, which consequently expands towards the higher magnetic field strength. Non-magnetic particles are expected to travel towards the opposite direction and be focused along the centre line of the chamber. However, due to the bulk force on the core stream, a secondary flow makes non-magnetic microparticles follow the same direction as the core flow expands. The negative magnetophoresis force acting on particles competes with the body force exerted on the core stream. In this case, the body force is dominant. The effects of different magnetic field strengths, flow rates and concentrations of non-magnetic particles were studied in details.

Using the same circular chamber device, Chapter 4 further investigates the manipulation of non-magnetic particles suspended in a diluted ferrofluid under a non-uniform magnetic field. Non-magnetic microparticles were introduced into the device from the middle inlet, while the cladding streams are the same diluted ferrofluid. The particles in the sandwiched core flow are expected to be deflected away from the permanent magnets due to negative magnetophoresis. A simplified two-dimensional numerical simulation was carried out and showed that the particles are deflected following a curved trajectory. Experimental results demonstrate that the deflected particles form a band instead of a line. A secondary flow in the opposite direction of the main flow was also observed. This behaviour is a result of non-uniform magnetisation of the ferrofluid. Therefore, the core flow is deflected away from the magnets, creating a complex velocity field. Non-magnetic microparticles were affected by both negative magnetophoresis force and magnetically driven convective flow, and deflected away from the magnets. The extent of the deflection was examined for different magnetic field magnitudes, particle sizes and flow rates. At low flow rate ratios and relatively high magnetic field strengths, the secondary flow is strong enough to mix the core flow with the
upper sheath flow. This phenomenon has potential applications for size selective sorting and capture of particles or cells, as well as magnetic mixing of two streams in microfluidic chamber.

Using a simple flow-focusing microfluidic system, Chapter 5 examines the mass transport of fluorescent dye molecules. The core stream was diluted ferrofluid mixed with fluorescent dye, which was sandwiched between two non-magnetic streams. In the absence of a magnetic field, the paramagnetic core flow was not affected and mass transport of dye molecules relies on molecular diffusion only. Under a non-uniform magnetic field, substantial improvement in mass transfer was observed. The enhanced mass transfer was caused by the bulk force on the paramagnetic fluid and magnetic nanoparticles migrating towards the permanent magnets. As a result, the flow field was altered and the core stream was mixed with the upper cladding flow. Non-magnetic dye molecules follow the secondary flow field and experience improved mass transport. This phenomenon has the potential to be used for designing gradient generators or micromixers.

Chapter 6 reports the design and fabrication of a magnetofluidic device for concentrating and trapping of non-magnetic particles. The particles were suspended in diluted ferrofluid and fed into a straight channel surrounded by two arrays of permanent magnets. Attracting magnets create several magnetic field minima along the channel. Due to negative magnetophoresis, non-magnetic microparticles are trapped in the field minima. On the other hand, convective mass transport of nanoparticles induced by magnetic force creates a complex velocity field in the channel. Depending on the flow rate and the concentration of the ferrofluid, different behaviours were observed. The competition between positive and negative magnetophoresis, the magnetic bulk force on the ferrofluid and the hydrodynamic force of the flow determines the shape and size of the capture zones and the quality of particle trapping. Sorting of particles with a size difference of less than 2 microns was achieved. Results from this study could be applied for designing a particle aided or magnetically-actuated micromixer, or a particle separation device.

Using magnetofluidic actuation and taking advantage of magnetically aided mass transport, Chapter 7 reports the design and test of a micromixer. The device is a simple straight microchannel with permanent magnets inserted on one side. Two streams were fed into the channel: non-magnetic deionized (DI) water and paramagnetic diluted ferrofluid. Fluorescent dye was mixed with the ferrofluid for tracing the paramagnetic stream. Due to
the magnetically aided convective mass transfer of the nanoparticles, the ferrofluid was expected to mix with the DI-water stream. Non-magnetic fluorescent dye molecules follow the secondary flow and mix with the DI-water stream. This chapter analyses the fluorescent signal to evaluate the mixing efficiency under different operating conditions and concentrations of the ferrofluid. The micromixer has advantages such as simplicity low cost. In addition it could achieve high throughput with mixing efficiency up to 90% in a relatively short channel.

Chapter 8 demonstrates the use of magnetic force for the manipulation of heat transfer. A magnetofluidics device comprised of a circular chamber and a permanent magnet next to a circular chamber is employed for this purpose. Convective heat transport is examined and compared for three cases: DI-water, diluted ferrofluid, and diluted ferrofluid in the presence of magnetic field. The results indicated that under the effect of the magnetic field, magnetic body force on the ferrofluid works against the transport of heat through the device. The magnetofluidics scheme can serve as a heat trapping device.

Finally, Chapter 9 summarises the key results achieved throughout the study, and provides recommendations for future work.

1.6 List of publications

List of papers constituting this thesis following the order of the chapters:

- Majid Hejazian, Weihua Li, and Nam-Trung Nguyen, Lab on a chip for continuous-flow magnetic cell separation, Lab on a Chip, 15 (2015) 959. DOI: 10.1039/C4LC01422G

- Gui-Ping Zhu, Majid Hejazian, Xiaoyang Huang and Nam-Trung Nguyen, Magnetophoresis of diamagnetic microparticles in a weak magnetic field, Lab on a Chip, 14 (2014) 4609. DOI: 10.1039/C4LC00885E


- Majid Hejazian, Dinh-Tuan Phan and Nam-Trung Nguyen, Mass transport improvement in microscale using diluted ferrofluid and a non-uniform magnetic field, RSC Advances, 6 (2016) 62439. DOI: 10.1039/C6RA11703A
• Majid Hejazian and Nam-Trung Nguyen, Magnetofluidic concentration and separation of non-magnetic particles using two magnet arrays, Biomicrofluidics, 10 (2016) 044103. DOI: 10.1063/1.4955421

• Majid Hejazian and Nam-Trung Nguyen, A Rapid Magnetofluidic Micromixer Using Diluted Ferrofluid, Micromachines 2017, 8(2), 37; DOI:10.3390/mi8020037


1.7 References

[52] T. Zhu, R. Cheng, Y. Liu, J. He, L. Mao, Combining positive and negative magnetophoreses to separate particles of different magnetic properties, Microfluid Nanofluid, (2014).


[71] T. Zhu, R. Cheng, Y. Liu, J. He, L. Mao, Combining positive and negative magnetophoreses to separate particles of different magnetic properties, Microfluidics and Nanofluidics, 17 (2014) 973-982.


This chapter presents a critical literature review on magnetic separation of cells and particles with microfluidics. The review discusses the physics and identifies the dominant forces involved in magnetic separation. Several methods for increasing the magnitude of magnetic force were compared and analysed. Finally, applications of these techniques in biology were presented.
Statement of Contribution to Co-authored Published Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored published paper, including all authors, are:

Majid Hejazian, Weihua Li, and Nam-Trung Nguyen, Lab on a chip for continuous-flow magnetic cell separation, Lab on a Chip, 15 (2015) 959. DOI: 10.1039/C4LC01422G

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My contribution to the published paper involved:

- Literature review
- Manuscript preparation
- Discussion and preparation of figures

(Date) 21/02/2017

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21/02/2017

-Trung Nguyen
Lab on a chip for continuous-flow magnetic cell separation

M. Hejazi, W. Li and N. Nguyen, Lab Chip, 2015, 15, 959
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Lab on a chip for continuous-flow magnetic cell separation

Majid Hejazian,a Weihua Li b and Nam-Trung Nguyen*a

Separation of cells is a key application area of lab-on-a-chip (LOC) devices. Among the various methods, magnetic separation of cells utilizing microfluidic devices offers the merits of biocompatibility, efficiency, and simplicity. This review discusses the fundamental physics involved in using magnetic force to separate particles, and identifies the optimisation parameters and corresponding methods for increasing the magnetic force. The paper then elaborates the design considerations of LOC devices for continuous-flow magnetic cell separation. Examples from the recently published literature illustrate these state-of-the-art techniques.

Introduction

The separation and concentration of rare cells for sample preparation are primary steps in many biological studies such as disease diagnosis.1 Microfluidic lab-on-a-chip (LOC) devices have proven to be a promising platform for this application, owing to a number of merits such as small size, low cost, low sample and reagent consumption, portability, as well as fast analysis time.2,3 The previous decade has witnessed an increasing trend of using LOC devices for preparing samples, and isolating and analysing cells. Cells are separated based on their unique hydrodynamic, dielectrophoretic, immunochemical and magnetophoretic signatures, or a combination of these signatures.4 Due to its non-contact nature, magnetic separation can maintain cell viability and suit well with biological investigations. On the other hand, the continuous-flow separation has a high throughput with no limits on its capacity. Other advantages include the possibility of continuous monitoring and adjusting the separation parameters, the lateral separation of sample components, and the high potential for system integration.5

A number of reviews exist in the literature that partially includes the continuous-flow magnetic separation of cells. Radisic et al.6 reviewed general cell separation concepts using...
micro- and nanoscale technologies. This paper briefly discussed magnetic separation with a number of examples related to cell separation. The review by Pammé3 focused on continuous-flow magnetic separation in microfluidic devices by describing methods such as continuous-flow separation of magnetically susceptible materials and magnetically labelled cells. Tsutsui and Ho7 discussed cell separation methods according to various non-inertial forces, with one of the subcategories covering the continuous-flow separation of magnetically tagged cells. Liu et al.4 described the physics of magnetic cell sorting and mentioned the use of LOC platforms. Bhagat et al.1 reviewed several techniques and applications of microfluidic cell separation, where the concepts were categorized into passive and active techniques, and then further divided into the types of force used for separation. Magnetic cell sorting was briefly discussed through some examples. Lenshof and Laurell8 summarised different continuous-flow separation techniques for cells and particles in microfluidic devices, including a review of the magnetic method. Gossett et al.9 limited their review to label-free cell separation in microfluidic systems, where magnetic sorting as well as other several techniques was discussed. Gijs et al.10 gave a comprehensive overview of the manipulation of magnetic beads in microfluidic systems and their applications in biological and chemical analysis. Zbowski and Chalmers11 described the separation and analysis of rare cells by magnetic sorting by focusing on separation of circulating tumor cells (CTC). Another recent paper by Pammé12 reviewed the application of magnetic particles in bioanalysis and bioprocessing within a LOC platform, which are recent developments in the manipulation of magnetic particles. Both magnetically functionalised droplets and magnetically labelled cells were discussed. Hyun and Jung13 critically reviewed the microfluidic enrichments of circulating tumor cells. Several techniques for CTC isolation including magnetic separation were mentioned and categorized in this review. Chen et al.4 discussed the isolation, enrichment and analysis of rare cells from an engineering perspective. Different methods based on the force used for separation, including isolation based on the magnetophoretic signature, were introduced and illustrated by examples from recent published studies.

The above reviews indicate that despite the significance and broad impact of microfluidic continuous-flow cell separation based on magnetic force, none of them focused on or addressed the unique features of different magnetic separation techniques. To fill this gap, our present paper will give a concise review that focuses only on continuous-flow magnetic cell separation using microfluidic devices. The fundamental physics behind magnetic separation is first discussed, followed by an analysis on techniques that could enhance the efficiency and throughput of magnetic microfluidic cell separation. Finally, a variety of applications for these techniques will demonstrate the uniqueness and usefulness of continuous-flow magnetic separation.

**Physics of magnetic separation**

Microfluidic magnetic separation is a subfield of micro-magnetofluidics, a research field that involves the interaction between magnetism and fluid flow on a microscale.14 This section focuses on key forces that may affect the particle trajectory while passing through the microfluidic device. We first need to understand the force balance acting on the particles to identify the optimisation parameters of the separation devices to increase the yield and throughput of the separation process. The order of magnitude of each force is first estimated to identify the dominant forces and their related design parameters for effective separation. The trajectory of a magnetic particle in a laminar flow through a microchannel is determined by the balance of many forces, Fig. 1A. According to Newton’s second law, the force balance on a moving particle is:

\[
m_p \frac{du_p}{dt} = F_m + F_d + F_s + F_b + F_L
\]

(1)

where \(m_p\) is the mass of particles, \(u_p\) is the particle velocity, and \(F_m, F_d, F_s, F_b,\) and \(F_L\) are the magnetic force, drag force, gravity force, Brownian force, and lift force, respectively.

**Magnetic force**

The force acting on a magnetic particle within a magnetic field is:

\[
F_m = \frac{V_p \Delta \chi_p (B \cdot \nabla) B}{\mu_0}
\]

(2)

where \(F_m\) is the magnetic force (N), \(V_p\) is the volume of the particle (m³), \(\Delta \chi_p = \chi_p - \chi_f\) is the difference between magnetic susceptibilities of the particles \(\chi_p\) and the base fluid \(\chi_f\) (dimensionless), \(B\) is the magnetic induction, and \(\mu_0 = 4\pi \times 10^{-7}\) (T m\(^{-1}\)) is the permeability of the vacuum. The above equation indicates that a gradient in the magnetic field and a
susceptibility difference are required to induce a magnetic force on a particle. A torque can be generated by a uniform magnetic field, but no motion can be achieved.\textsuperscript{14,16} Eqn (2) assumes a uniform magnetisation of the bead. Considering non-uniform magnetisation, Shevkoplyas \textit{et al.}\textsuperscript{17} proposed a more general expression for the magnetic force:

$$F = \rho V V M B V B$$

where $\rho$ is the density of the bead (kg m$^{-3}$) and $M_0$ is the initial magnetization of the bead (A m$^2$ kg$^{-1}$). When the magnetization is completely saturated, the magnetic moment of the particle does not vary in space ($\nabla \cdot m = 0$). In the case of strong spatial field variations or a Janus particle, which has different properties in each half,\textsuperscript{18} the magnetic moment of the particles is not constant when moving in space ($\nabla \cdot m \neq 0$); thus, eqn (3) should be considered.\textsuperscript{19} Macrosopic permanent magnets and electromagnets can produce magnetic fields that are sufficiently strong (>0.5 T) to saturate the magnetization of superparamagnetic beads. Eqn (2) is suitable for relatively high magnetic field strengths with the order of magnitude of saturation field strength of the magnetic bead.\textsuperscript{17} Furthermore, eqn (2) can be applied for paramagnetic and superparamagnetic particles, where soft magnetism approximation was considered for the particles, considering the fact that these particles have no magnetic memory.\textsuperscript{20,21} In an external magnetic field, the nanoparticles in a ferrofluid have a ferromagnetic behaviour at room temperature. Their average magnetization is zero in the absence of an external magnetic field. There is a critical diameter for nanoparticles below which the material is superparamagnetic and eqn (2) is applicable.\textsuperscript{16,22}

### The gravitational force

Considering buoyancy, the gravitational force can be expressed as:

$$F_g = -V \rho (\rho_p - \rho_f) g$$

where $F_g$ is the gravitational force, and $V$, $\rho_p$, $\rho_f$ and $g$ are the volume of the particle, the density of the particle and fluid, and the acceleration due to gravity, respectively.

### Drag force

For a particle suspended in a fluid flow under conditions of low Reynolds numbers, the drag force is estimated from the Stokes’ law and the relative velocity:\textsuperscript{23}

$$F_d = 6 \pi d_c \eta (u_f - u_p)$$

where $\eta$ is the dynamic viscosity of the fluid, and $u_f$ and $u_p$ are the velocities of the fluid and the particles, respectively. The apparent diameter of the composite particle $d_c$ can be estimated based on the different scenarios of the relative size ratio between the magnetic particles and the biological particles attached to them through affinity, Fig. 1B. In the case of magnetophoresis, which is the motion of the particle under a magnetic force, the drag force consists of two components. One is caused by the fluid flow and opposing the flow direction. The other one is opposing the magnetic force. The net drag force opposes the resulting particle motion, Fig. 1A.

### Lift force

Spherical particles experience a hydrodynamic lift force, which results in a velocity component that is perpendicular to primary streamlines. There are two types of lift forces on particles depending on the position of the particle. The first one is the shear gradient-induced lift force:\textsuperscript{24}

$$F_{L_s} = \frac{2}{8} \rho_p \omega d_p^3$$

Fig. 1 Dominant forces on a particle in a flow field: (A) representative illustration of force and velocity components (the directions of $F_m$ and $F_g$ are arbitrary); (B) relative sizes between magnetic particles (blue) and diamagnetic (biological) particles (red); (C) orders of magnitude of different forces as functions of characteristic particle diameters (at a typical velocity of 1 mm s$^{-1}$ and channel width and depth of 100 $\mu$m).
The second one is the boundary layer lift force:

\[
F_{Lb} = 9.22 \left( \frac{9 U^2}{4 h^2} \right) \rho \frac{d^4}{v^4},
\]

(7)

where \( F_{Ls} \) and \( F_{Lb} \) are the shear gradient induced and boundary layer lift forces, respectively, \( d_p \) is the diameter of the particle, \( \rho_f \) is the density of the fluid, \( \omega \) is the vorticity of the flow, \( \text{Re}_p = \frac{U d_p}{v} \) is the Reynolds number of the particle, \( v \) is the kinematic viscosity of the fluid, \( U \) is the average velocity of the particle, and \( h \) is the channel height. For a uniform laminar flow field, the vorticity can be estimated as

\[
\omega = \frac{\partial u}{\partial y} - \frac{U}{h}.
\]

The inertial force is proportional to the flow velocity and the particle size relative to the channel length. At high Reynolds numbers \( \text{Re}_p \ll 1 \), the inertial lift force becomes dominant and can be used for lateral separation of particles. At low Reynolds numbers \( \text{Re}_p \gg 1 \), the viscous drag force is more significant. Relatively high velocity and large particle size are required for the lift force to have a magnitude comparable with magnetic force. Thus, a separation application cannot benefit from both magnetic and inertial lift forces at the same time due to the fact that magnetic separation and inertial separation are working in different ranges of velocity.\(^{25}\)

**Brownian force**

Random collisions of molecules of the fluid with the suspended particles cause a random movement called Brownian motion. The Brownian force can be estimated as:

\[
F_B = \zeta \sqrt{\frac{6\pi k_B T d_p}{\Delta t}}
\]

(8)

where \( k_B \) is the Boltzmann constant, \( \eta \) is the fluid viscosity, \( T \) is the absolute temperature, \( r_p \) is the radius of the particle, and \( \Delta t \) is the magnitude of the characteristic time step. The parameter \( \zeta \) is a Gaussian random number with zero mean and unit variance. Brownian motion can affect the movement of particles if the radius of particles is less than the threshold diameter estimated based on the following relationship:\(^{27}\)

\[
|F|d_p \leq k_B T
\]

(9)

where \(|F|\) is the magnitude of the total force acting on the particle. For particles with a diameter smaller than this critical radius, trajectories cannot be evaluated by Newton’s eqn (1).\(^{23}\)

**Other forces**

In addition to the forces discussed above, other forces acting on a particle in a laminar flow also exist, such as particle–particle interaction forces, van der Waals attraction force, thermophoretic force, lift force, and magnetic and electrostatic interaction forces between particles.\(^4\) Depending on the type of separation phenomena, these forces can be added to eqn (1), and would result in a complex model, which can only be solved by numerical simulation.

**Significant forces and optimisation parameters for cell separation**

Many of the mentioned forces could be ignored for cell separation applications, depending on the size of the particles and the magnitude of the magnetic field strength. Particle–particle and particle–fluid interactions can be ignored for particle suspensions with small particle volume concentration \((c \ll 1)\).\(^{23}\) Fig. 1C illustrates schematically the order of magnitude of forces acting on a magnetic particle, based on typical conditions used in most of magnetic separation problems. Fig. 1C indicates that in a relatively weak magnetic field typically generated by an integrated electromagnet such as a microcoil and a line conductor, drag force is dominant, while gravitational and magnetic forces have a comparable order of magnitude. But in a relatively high magnetic field typically generated by permanent magnets, drag force and magnetic force are the most dominant forces, and other forces could be ignored.\(^{10}\)

Most of the studies reported in the literature only consider the two most significant forces: the drag force and the magnetic force. Considering a single spherical magnetic particle with a radius \( r_p \) in a quiescent fluid, balancing the magnetic force and drag force results in the magnetophoretic velocity:\(^{20}\)

\[
u_p = \frac{d^2 (X_p - X_t) (B \cdot V) B}{18 \mu_B \eta}
\]

(10)

This equation reveals the key parameters for designing a microfluidic device to separate cells by a relatively strong magnetic force, namely, the size, the magnetic susceptibility difference, the magnetic field and field gradient and the viscosity of the surrounding fluid.

**Methods to improve magnetic separation**

The main objective of continuous-flow separation is to attract or repel particles or cells from their regular trajectory in a fluid flow, and to guide them to a specific outlet for collection. The main challenge in designing a microfluidic device for this purpose is achieving both high efficiency and throughput. Various techniques have been applied to improve the magnitude of magnetic force relative to other forces as highlighted in the previous section. These techniques are classified and reviewed as follows.
Techniques to increase the magnetic field gradient

Eqn (2) shows that the magnetic gradient ∇B is one of the key parameters for creating a larger magnetic force. Various methods for increasing the gradient have been reported in the literature. Xia et al.28 used micro-comb and micro-needle structures to generate a non-uniform magnetic field, Fig. 2A. Red blood cells and *E. coli* cells labelled with magnetic nanoparticles were separated with high efficiency and throughput. The results showed that the micro-needle geometry concentrates the magnetic field to a single position along the channel, while the micro-comb geometry can create a gradient over a longer channel and thus provide the particles with a longer residence time in the field gradient.

A high field gradient can also be achieved with small integrated electromagnets. Liu et al.29 suggested using current-carrying conductors to create a higher gradient to separate particles with two different sizes, Fig. 2B. Their experimental and simulation results revealed that the combined effect of magnetic fields provided by conductors and an additional uniform external field led to higher deflections of the particle trajectories. Jung and Han30 utilized a ferromagnetic wire array to create a high-gradient magnetic field for improving separation efficiency (up to 93.9% for RBCs and 89.2% for WBCs), Fig. 2C. They demonstrated a lateral-driven method for continuous magnetophoretic separation of RBCs and WBCs from peripheral whole blood, using their intrinsic magnetic properties. A reasonable efficiency was achieved with the flow rate of 20 l h⁻¹ and an external magnetic flux of 0.3 T. Micromachined ferromagnetic strips (MFS) were used by Adams et al.31 to continuously and simultaneously sort two types of labelled cells (three different types of *E. coli* MC1061 cell labelled target buffers) into two outlets, Fig. 2D. The high magnetic field gradient created by the MFS arrays...
in the microchannel made it possible to control and balance the drag force and the magnetophoretic force and to guide the target cells to two outlets, with purity higher than 90% for multiple bacterial cell types, and a throughput of $10^9$ cells per hour.

Because of the relatively large current needed for the electromagnet, a thick conductor is preferable for the electromagnet. Derec et al.\textsuperscript{32} introduced a copper-etched microchip, where the electric current passed through a line conductor parallel to the microchannel and generated a tuneable magnetic field inside the channel, Fig. 2E. Numerical and experimental data showed that the chip was capable of performing a satisfactory extraction of tumour cells labelled with magnetic nanoparticles. The main disadvantages of this device were the heat generated by the copper conductor when exposed to an electrical current, and being unable to reduce the size of the channel any further.

The magnetic field can be concentrated using ferromagnetic structures placed next to the microchannel. Lee et al.\textsuperscript{33} devised a high-speed RNA microextractor by utilizing a lateral ferromagnetic wire array for isolating RNA from human blood lysate using magnetic oligo-dT. A ferromagnetic wire array was placed at an angle proportionate to the direction of flow under an applied external magnetic field. The ferromagnetic wire array was able to produce a high-gradient magnetic field that directed the tagged particles to the collecting outlet. This device could separate more than 80% of the magnetic beads with a flow rate up to 20 ml h$^{-1}$ in only one minute. Shen et al.\textsuperscript{34} placed a ferromagnetic wire beneath a microchannel to generate a magnetic gradient from an external uniform magnetic field, Fig. 2F. The device successfully separated red and white blood cell whole blood based on their native magnetic properties.

Most reported studies used a stationary permanent magnet to induce the magnetic field into the microfluidic device. A time-dependent magnetic field is able to induce a time-varying particle velocity, and thus an additional inertial force for faster trapping and separation. Verbarg et al.\textsuperscript{35} utilised a spinning magnetic trap to make a “MagTrap” device, Fig. 2G. A rotating magnet wheel enables a magnetic gradient to trap, mix and release the targeted cells. The device was capable of performing automated target capture, efficient mixing with reagents, and separation in a single microfluidic channel.

The external magnetic field can be further optimized by arranging the permanent magnet so the position of the maximum field gradient in the microchannel can be adjusted. Wilbanks et al.\textsuperscript{36} conducted an experimental and theoretical study to investigate the effects of magnet arrangement on trapping of diamagnetic particles in a ferrofluid flow through a straight rectangular microchannel. Positioning the magnets around a microchannel asymmetrically increases the rate for particle trapping.

If the deflection of particles through magnetophoresis is not large enough, cascading multiple separation units could bring better separation results. Jung et al.\textsuperscript{37} developed a six-stage cascade paramagnetic mode magnetophoretic separation (PMMS) system for the separation of red blood cells from human whole blood, using their native magnetic properties, Fig. 2H. The cascade ferromagnetic structures created a high magnetic gradient allowing a high throughput up to 50.4 μL h$^{-1}$ and an efficiency of 86.2%. Processing a 5.0 μL blood sample only needs 6.0 min. Lee et al.\textsuperscript{38} used magnetic nanoparticles (MNPs) modified with bis-Zn-DPA to remove both Gram-negative bacteria and endotoxins from blood. By using multiple microfluidic devices in series, the MNPs bound to Escherichia coli were successfully removed from bovine whole blood, with almost 100% clearance. Khashan et al.\textsuperscript{39} proposed a microfluidic design for the separation of magnetically labelled bio-particles based on numerical simulation. Integrated soft-magnetic elements intersecting the flow were considered to overcome the disadvantage of short-range magnetic force and the limitation of channel size. The proposed scheme improved the capture efficiency quite significantly compared to systems with magnetic structures embedded in the channel wall.

Many microfluidic devices are made of polydimethylsiloxane (PDMS). Thus, mixing magnetic materials with PDMS to form a microstructure with higher magnetic susceptibility would allow integrating field gradient enhancing structures into a microfluidic device made of PDMS. Gelszinnis et al.\textsuperscript{40} reported magnetophoretic manipulation in the microsystem using I-PDMS microstructures, which is made of carbonyl iron microparticles mixed in a PDMS matrix. The magnetic composite structures generated locally high magnetic field gradients when placed between two permanent magnets, and are suitable for capturing and sorting of magnetic species with different magnetic properties.

**Techniques to increase the susceptibility mismatch**

According to eqn (2), the susceptibility mismatch is another significant parameter that practically can lead to higher separation efficiency and/or throughput. Modifying either the susceptibility of the particle or the surrounding fluid can create the desired susceptibility mismatch. Labelling the target cells with particles of high magnetic susceptibility and modifying the susceptibility of the medium are two possible approaches to increase the susceptibility difference. In order to magnetically separate cells, which are commonly diamagnetic, modification of their magnetic properties is required. Attaching cells to magnetic beads is an established method that has been well reported in the literature. The size of the magnetic particle and the effective size of the composite particle are important parameters for effective separation. As illustrated in Fig. 1B, three cases could be considered based on the relative size ratio of the beads and the cells. (i) If the size of the magnetic bead is significantly larger than the size of cells, and a single bead is surrounded by many cells, the volume for a spherical bead ($V_p = \frac{1}{6}πd_p^3$) can be used in eqn (2) to
evaluate the magnetic force. (ii) If the diameters of the cell and the bead are comparable, the magnetic force exerted on the bead is calculated by eqn (2), while the movement of the cell–bead complex is affected by counteracting drag force on the composite particle, and is more complicated to predict.\(^4^1\) (iii) If the size of the cell is significantly larger than the size of the magnetic beads, the magnetic force is small relative to the drag force. A strong magnetic field gradient or a higher concentration of tagged magnetic particles is needed for effective separation.

Kim et al.\(^4^2\) devised an immune-magnetophoresis (IMP) cell sorting chip to separate T-cells from biological suspensions using magnetic particles as tags. The cells and antibody coated magnetic particles are introduced via two separate inputs and bind together as they move through the microchannel. The labelled cells are then attracted to a permanent magnet and separated from the solution. Both binding and separation were executed in a simple and straight microchannel, Fig. 3. Pamme and Wilhelm\(^4^3\) investigated the continuous magnetic sorting of mouse macrophages and human ovarian cancer cells (HeLa cells) that were internally labelled with magnetic nanoparticles. The cells were separated from each other depending on their size and magnetic loading. The theoretical prediction agreed with the experimental data.

Shih et al.\(^4^4\) examined the separation of bacteria bound to magnetic sugar-encapsulated nanoparticles. They concluded that the flow rate and the strength of the magnetic field were significant variables affecting efficiency. The most efficient sorting they could achieve was more than 90% with a selectivity of about 100%. Forbes and Forry\(^4^5\) performed a numerical, analytical and experimental analysis of the lateral magnetophoretic deflection of magnetically labelled breast adenocarcinoma MCF-7 cells on a chip. Using the design tool, the necessary beads, magnet configuration (orientation), magnet type (permanent, ferromagnetic, and electromagnet), flow rate, channel geometry, and buffer to achieve the desired level of magnetophoretic deflection or capture could be identified and used for optimizing the separation process. This paper introduced a dimensionless magnetophoresis number to characterize the transition between the hydrodynamically dominated regime and the magnetically dominated one.

Tagging cells with magnetic particles required affinity binding reaction and the preparation of particles; a separation process without the need of magnetic tags would be simpler and at a lower cost to implement. Thus, modifying the susceptibility of the surrounding fluid also increases the susceptibility difference and results in a higher magnetic force. Shen et al.\(^4^6\) utilised biocompatible gadolinium diethylene triamine pentaacetic acid (Gd–DTPA) for this purpose. In their experiment, label-free U937 cells were separated from red blood cells (RBCs) with a purity higher than 90% and a throughput of \(1 \times 10^5\) cells h\(^{-1}\) using a 40 mM Gd–DTPA solution.

Adding a ferrofluid, a liquid with suspended magnetic nanoparticles, to the sample also increases the mismatch in magnetic susceptibility. Zeng et al.\(^4^6\) investigated the magnetophoretic separation of diamagnetic polystyrene particles and live yeast cells in a ferrofluid solution, Fig. 4A. The predicted trajectory of cells and particles from numerical simulation agreed well with the experimental results. Furthermore, the viability test for the yeast cells after separation demonstrated the reasonable biocompatibility of the diluted ferrofluid.

The increase in susceptibility of the carrier liquid such as the ferrofluid allows both positive and negative mismatch making magnetophoretic separation of both diamagnetic and magnetic particles possible. Liang et al.\(^4^7\) investigated the simultaneous positive and negative magnetophoresis of magnetic and diamagnetic particles in a ferrofluid, Fig. 4B. Particle transport in both ferrofluid- and water-based separations was experimentally and analytically investigated. Using a T-microchannel, ferrofluid-based magnetic separation can offer a significantly higher particle throughput than the water-based separation due to an induced negative magnetophoresis of diamagnetic particles in the ferrofluid.

Zhu et al.\(^4^8\) used water-based ferrofluids to increase the susceptibility difference to separate diamagnetic particles of different sizes. A minimum throughput of \(10^5\) particles per hour and close to 100% separation of microparticles were achieved, Fig. 4C. Zhu et al.\(^4^9\) developed an analytical model, along with experimental verifications, of transport of nonmagnetic spherical microparticles in ferrofluids in a microfluidic system that consists of a microchannel and a permanent magnet, Fig. 4D. Larger particles were further
deflected perpendicular to the flow. The deflection of particles could be increased by lowering the flow rates in the microchannel.

Cheng et al.\textsuperscript{50} developed a 3D analytical model to study microfluidic motions of diamagnetic particles in magnetic fluids. The model could be used to study the trajectories of the particles in the channel. The effects of flow rate, susceptibilities of particles and the base fluid, as well as different geometrical parameters of the system on the magnitude of particle deflection were investigated. Zhu \textit{et al.}\textsuperscript{51} suggested a new separation method that combines both positive and negative magnetophoresis based on ferrofluids for separating magnetic and diamagnetic particles, as well as particles with different magnetizations, Fig. 4E. The basic concept is to use a ferrofluid with susceptibility between those of the particles.

Liang \textit{et al.}\textsuperscript{52} experimentally and theoretically investigated diamagnetic particle deflection in a ferrofluid flow through a rectangular microchannel. It is found that diamagnetic particles can be moved both outwards and downwards over the channel cross-section to form a focused particle stream. Particle deflection across the channel width could be increased with the decreasing flow rate, increasing ferrofluid concentration and increasing particle size. Liang and Xuan\textsuperscript{53} suggested continuous sheath-free magnetic separation of particles in a U-shaped microchannel. Magnetophoresis focuses polystyrene particles of different sizes in a diluted ferrofluid and separates them into two branches of a U-shaped microchannel.

Zeng \textit{et al.}\textsuperscript{54} presented three-dimensional magnetic focusing of particles and cells in a ferrofluid flow through a straight microchannel. Two symmetrically repulsive permanent magnets were embedded adjuvant to a straight rectangular microchannel in a PDMS-based microfluidic device. Magnetic focusing of polystyrene particles in ferrofluid was observed three-dimensionally, with both top- and side-view visualizations. The effects of flow speed and particle size on the effectiveness of particle focusing were studied. Diamagnetic particle focusing in ferrofluid is enhanced with decreasing flow speed and/or increasing particle size.

Besides ferrofluid, some salt solutions can also be used as the paramagnetic carrier fluid for diamagnetic separation. Zhu \textit{et al.}\textsuperscript{55} developed on-chip manipulation of diamagnetic particles in paramagnetic solutions using embedded permanent magnets. Manganese(II) chloride (MnCl\(_2\)) solutions as a carrier fluid create enough susceptibility difference for sorting of polystyrene particles. The effects of particle position (relative to the magnet), particle size, MnCl\(_2\) salt concentration, and fluid flow velocity on the horizontal magnetophoretic deflection are examined using a combined experimental and theoretical approach.

Negative magnetophoresis can be extended to particle focusing and concentrating, where diamagnetic particles suspended in a magnetic fluid are trapped at places with the lowest magnetic field gradient. Zeng \textit{et al.}\textsuperscript{56} investigated magnetic concentration of polystyrene particles and live yeast cells in a ferrofluid flow through a straight rectangular microchannel using negative magnetophoresis. Two attracting permanent magnets placed on the top and bottom of the planar microfluidic device and held in position by their natural attractive force were used to create a magnetic field gradient. They could successfully separate yeast cells, without significant biological harm, from the polystyrene particles. Some of the main techniques for increasing magnetic and/or susceptibility mismatch are summarized in Table 1.

### Hybrid techniques

If other non-magnetic properties of the particles are considered, other methods could be combined with separation based on the magnetophoretic properties to further increase efficiency and throughput. Kim and Soh\textsuperscript{57} utilized an integrated Dielectrophoretic–Magnetic Activated Cell Sorter (iDMACS) to take advantage of dielectrophoresis and magnetophoresis forces to sort multiple bacterial cell types in a single pass, Fig. 5A. Three different bacterial clones of the \textit{E. coli} MC1061 strain were used in these separation experiments. The use of two distinct force fields completely eliminated any cross-contamination of target cell types between the two outlets. The use of both force fields has the benefit of removing the cross-contamination of target cell types between the two outlets leading to a high purity separation. Up to 3000-fold enrichment of tags and a 900-fold enrichment of bacterial cells at a throughput of 2.5 \texttimes 10\(^7\) cells h\(^{-1}\) were achieved.

Seo \textit{et al.}\textsuperscript{58} proposed a hybrid cell sorter that exploits both hydrodynamics and magnetophoresis for sorting Jurkat cells, and red and white blood cells. The classification efficiency of Jurkat cells and white blood cells dropped with the hybrid

### Table 1 Summary of various methods for increasing the magnetic field gradient and susceptibility mismatch

<table>
<thead>
<tr>
<th>Magnetic field gradient</th>
<th>Susceptibility mismatch</th>
<th>Type of cells</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper conductor</td>
<td>Magnetic tags</td>
<td>Tumour cells</td>
<td>32</td>
</tr>
<tr>
<td>Array of magnetized elements</td>
<td>NA</td>
<td>RBCs and WBCs</td>
<td>16</td>
</tr>
<tr>
<td>Ni wire</td>
<td>Paramagnetic salt</td>
<td>U937 cells from RBCs</td>
<td>34</td>
</tr>
<tr>
<td>Ferromagnetic wire array</td>
<td>Magnetic tags</td>
<td>RNA</td>
<td>33</td>
</tr>
<tr>
<td>Rotating magnets</td>
<td>Magnetic tags</td>
<td>\textit{E. coli}</td>
<td>35</td>
</tr>
<tr>
<td>Ferromagnetic strips</td>
<td>Magnetic tags</td>
<td>\textit{E. coli}</td>
<td>31</td>
</tr>
<tr>
<td>NA</td>
<td>Water-based ferrofluid</td>
<td>Live yeast cells</td>
<td>40</td>
</tr>
<tr>
<td>Permanent magnets</td>
<td>Magnetic tags</td>
<td>\textit{E. coli}</td>
<td>37</td>
</tr>
<tr>
<td>Magnetized NiFe microcomb</td>
<td>Magnetic tags</td>
<td>\textit{E. coli} and RBC</td>
<td>28</td>
</tr>
</tbody>
</table>

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scheme. Yet, an increase from 75.2 to 86.8% in efficiency was observed for the red blood cells, with the hybrid design. Siegrist et al. 59 exploited centrifugal and magnetophoretic forces to separate magnetic particles of different sizes, as well as magnetic and diamagnetic particles of the same size. They achieved an average separation of 75% of the larger magnetic particles. Mizuno et al. 60 introduced a microfluidic system that utilizes both hydrodynamic and magnetophoretic forces to sort the tagged JM cells and HeLa cells, based on their size and magnetophoretic properties, Fig. 5B. In the first stage, the cells were focused on one sidewall by the hydrodynamic effect, and sorted into different outlets based on their size difference. In the second stage, the outlets were exposed to a permanent magnet, and cells were separated through multiple outlet branches on the basis of their magnetophoretic characteristic. A high efficiency of 90% was achieved.

Sajay et al. 61 proposed a microfluidic platform for negative enrichment of circulating tumour cells (CTC). A two-step depletion process was used. Upstream immunomagnetic depletion first separates CD45-positive WBCs. And then a microfabricated filter membrane performs chemical-free RBC depletion and target cell isolation. The micro-slit membrane was designed to allow a selective passage of RBCs and platelets while retaining nucleated cells. This method was able to separate WBCs and RBCs with more than 90% WBC depletion and more than 90% recovery of CTCs.

Applications of continuous-flow magnetic separation

Since cells in a sample are often rare and needed to be isolated for subsequent processes, an efficient separation should not jeopardize their viability. Magnetic separation techniques are biocompatible and gentle, so any damage caused by forces acting on the cells is negligible. 4 For this reason, microfluidic magnetic separation techniques for biological particles have recently become a hot research topic. As already discussed in the previous section, various methods to achieve a higher efficiency for continuous cell per particle separation are available. This section introduces the remaining work in the literature on continuous-flow cell separation using magnetic force, to highlight the potential impact of the magnetic separation technique on biological studies.

Blood cells

About 45% by volume of mammalian blood consists of red blood cells (RBC, erythrocytes), white blood cells (WBC, leukocytes), and platelets (thrombocytes). The remaining 55% of the volume consists of a liquid medium called plasma. 62 Separating and enriching cells in blood could be used to detect several diseases such as cancer, HIV, malaria etc. Knowing the properties of blood cells is therefore crucial for designing a separation device. Deoxygenated haemoglobin proteins make RBCs paramagnetic, allowing them to be separated without magnetic labels, whereas other blood cells are diamagnetic. The physical properties of blood components are presented in Table 2. 63

Magnetic separation of blood cells using microfluidic devices have been reported previously. For instance, Furlan et al. 63 proposed a label-free continuous method for sorting red and white blood cells in plasma, using a microdevice and magnetic force. A mathematical model was also developed to predict the transport and separation of blood cells. In two separate studies, Seo et al. 58, 64 presented a hybrid method to separate RBCs and WBCs, using hydrodynamics and magnetophoresis. The experimental results revealed that the separation efficiency can be tuned by the magnetophoretic force.

Han and Frazier 65 reported the use of continuous-flow magnetophoretic separation to separate RBCs and WBCs from whole blood in a microfluidic device. Analytical model, numerical simulation, and experimental data confirmed that the concept is practical. In another work, 66 the same team compared the diamagnetic and paramagnetic capture modes. Both methods were able to extract WBCs from whole blood with a high concentration.

Cancer cells

The presence of circulating tumour cells (CTCs) in the blood stream is a sign of either primary tumour or metastases. Spreading of CTCs could lead to creation of tumours in other organs. CTC capture is a significant step in primary diagnosis and to discover personalized drugs. 13, 67, 68 To separate CTCs magnetically, it is often necessary to label them with magnetic beads. Plouffe et al. 69 devised a microfluidic device for separating cancer cells from suspension as well as high-purity isolation of spiked cancer cells directly from whole blood. The device was able to isolate hematopoietic stem cells and endothelial progenitor cells from whole blood. The device was reported as a viable platform for high purity,
efficient, and rapid sorting of rare cells directly from whole blood samples.

Hoshino et al.\textsuperscript{70} examined the magnetic separation of cancer cells labelled with magnetic nanoparticles in a microdevice, where capture rates of 90% and 86% for COLO205 and SKBR3, respectively, were reported. A non-labelled method has been reported by Han et al.\textsuperscript{71} Continuous paramagnetic capture mode (PMC) of magnetophoretic microseparator first separate RBC from peripheral blood using a 0.2 T external permanent magnet. The remaining nucleated cells are subsequently detected by electrical impedance spectroscopy. About 94.8% of breast cancer cells from a sample of spiked blood were successfully separated and detected.

**Bacteria**

*Escherichia coli* (*E. coli*) is a Gram-negative bacterium which causes a serious and deadly disease and is highly infectious. The four strains of *E. coli* that cause the disease are enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E. coli* and enterohemorrhagic *E. coli*. Magnetic isolation of *E. coli* requires tagging them with magnetic beads.\textsuperscript{31,72} Zhu et al.\textsuperscript{73} devised a microfluidic device to separate two species of cells, including *E. coli* and Saccharomyces cerevisiae, as well as fluorescent polystyrene microparticles, with a throughput of $10^7$ cells h$^{-1}$, and an efficiency close to 100%. Yassine et al.\textsuperscript{74} developed a magnetic microfluidic chip, which enabled the trapping and isolation of *E. coli* by tagging them with superparamagnetic beads. Soft ferromagnetic disks were used for trapping of the tagged cells in a microchannel. The particles were subsequently separated into two side chambers.

**Other cell types**

Continuous magnetic separation has been used to sort and isolate a variety of rare cells. This section discusses related studies reported in the literature to demonstrate the broad application of continuous-flow magnetic separation. Rodriguez-Villarreal et al.\textsuperscript{75} reported a microfluidic device based on diamagnetic repulsion to focus label-free HaCaT cells in a continuous flow. Focusing living cells was achieved using diamagnetic repulsion forces provided by paramagnetic MnCl$_2$ solution and simple permanent magnets. Robert et al.\textsuperscript{76} studied the sorting of monocytes and macrophages which internalise nanoparticles to different extents based on their endocytotic capacity. Five subpopulations of narrow iron loading distributions were successfully sorted with a purity of more than 88% and an efficacy of more than 60%.

Malaria parasite digests haemoglobin in RBC and produces an insoluble crystalline byproduct called hemozoin. Infected RBCs containing hemozoin have paramagnetic characteristics and can be separated by magnetophoresis. Nam et al.\textsuperscript{77} proposed a label-free method to separate not only late-stage but also early-stage malaria infected RBCs. An efficiency of approximately 99.2%, a recovery rate of approximately 98.3% for late-stage infected RBCs, and a recovery rate of 73% for early-stage infected RBCs were achieved.

Most other cells need to be tagged with magnetic beads to be handled with an external magnetic field. A centrifugo-magnetophoretic purification separation system for sorting an HIV/AIDS relevant epitope (CD4) using magnetic beads as tags from whole blood was proposed by Glynn et al.\textsuperscript{78} An efficiency of up to 92% was achieved with the system. Karlel et al.\textsuperscript{79} demonstrated the continuous extraction and purification of *E. coli* DNA bound to magnetic beads in a microfluidic platform. All of the essential unit operations (DNA binding, sample washing and DNA elution) were integrated onto one single chip. The magnetic beads were separated and transported using a rotating permanent magnet.\textsuperscript{80} Mizuno et al.\textsuperscript{81} demonstrated a microfluidic system for sorting of JM cells (human lymphocyte cell line) using anti-CD4 immunomagnetic beads. The device could achieve a throughput of approximately 100 cells s$^{-1}$, and high purification ratios of more than 90%. Han et al.\textsuperscript{82} presented an on-chip integrated RT-PCR microchip for integration of mRNA extraction, eDNA synthesis, and gene amplification. Implementing the lateral magnetophoretic technique with magnetic oligo-dT beads allowed the mRNA from small sample quantities of lysate to be extracted within one minute.

Durdik et al.\textsuperscript{83} used the finite element method to propose and analyse an integrated microfluidic system for combined magnetic cell separation, electroporation, and magnetofection. The numerical simulation indicates that the proposed method could be used to separate two types of magnetic particles: magnetically labelled cells and magnetically labelled genetic materials, e.g. plasmid DNA or siRNA. Sousa et al.\textsuperscript{84} presented a microfluidic device for magnetic separation of undifferentiated mouse embryonic stem (ES) cells from neural progenitor cultures. Their model could be used for the direct application in the purification of a human neural progenitor’s population of cells from pluripotent tumorigenic cells. A purity ranging from 95% to 99.5% was achieved.
Jung et al.\textsuperscript{85} designed a microfluidic device for continuous magnetic sorting of the heterogeneous cancerous cells (head and neck cells lines 212LN and 686LN-M4E), tagged with magnetic nanoparticles. From their experiment, at flow rates of 100 μL h\textsuperscript{-1} and 200 μL h\textsuperscript{-1}, 86.3% and 79.0% of tagged cancer cells were attracted toward the centre outlet, respectively, while 95.1% and 87.2% of non-tagged cells remained in the side outlets.

Conclusion and perspectives

This review highlights the state-of-the-art techniques and applications of continuous magnetic separation of cells in a microfluidic device. Due to distinctions in the type of cells, geometry of microchannels, and different arrangements and size of magnets, a quantitative comparison of the efficiency of these methods is not possible. Handling cells tagged to a magnetic bead is an established method. The availability of a wide range of immunomagnetic beads makes separation with the help of the magnetic bead an easy option. With further optimization, the separation systems introduced in this paper could lead to reasonably high efficiency, throughput and purity. We recommend a numerical study as a proper guide for an effective design before fabricating the microfluidic device and a tool to optimise and save time and costs for experiments. Identifying the significant optimisation parameters introduced in this paper will lead to a higher magnetic force, and consequently a higher efficiency and throughput of assays relying on magnetic beads.

If the cost of labelled magnetic beads is a factor to be considered, label-free magnetic separation is an attractive option. Although initial studies have been reported, the migration of diamagnetic microparticles in a magnetic fluid, also called diamagnetophoresis or negative magnetophoresis, has not been fully exploited. The use of a magnetization gradient for separation of diamagnetic particles may provide huge application potential. A magnetization gradient in the carrier fluid can be easily formed by controlling the concentration distribution of paramagnetic particles or ions in a carrier such as ferrofluid or MgCl\textsubscript{2} solution. On the one hand, the combination with other diamagnetic techniques to increase the force on cells could further improve the device performance. On the other hand, magnetic methods can complement other high-throughput techniques such as inertial microfluidics for a more precise control and higher efficiency. In inertial microfluidics for instance, magnetic force can be used to switch particles from one equilibrium position to another for better separation results. The knowledge of several magnetic separation techniques and their possible applications presented here could assist the development of new ideas for future research in this field.

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Notes and references

Chapter 2 indicates that magnetophoresis is a key phenomenon for the separation of magnetic particles. The same phenomenon also applies to non-magnetic particles if the surrounding medium is paramagnetic. In this chapter we devise an experiment to determine the impact of magnetic force on non-magnetic microparticles suspended in a diluted ferrofluid. A uniform magnetic field was used in the experiment to discriminate the effects of magnetic susceptibility gradient from that of magnetic field gradient. We first observed the significance and potential of magnetoconvective force in manipulating paramagnetic streams and the particles in this experiment. The results demonstrated that magnetoconvective force can outweigh negative-magnetophoretic force and attract the non-magnetic particles towards magnetic field maximum.
Statement of Contribution to Co-authored Published Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored published paper, including all authors, are:

Gui-Ping Zhu, Majid Hejazian, Xiaoyang Huang and Nam-Trung Nguyen, Magnetophoresis of diamagnetic microparticles in a weak magnetic field, Lab on a Chip, 14 (2014) 4609. DOI: 10.1039/C4LC00885E

Appropriate acknowledgements of those who contributed to the research but did not qualify as authors are included in the paper.

Note: There is a typo in family name of the second author. The correct family name is: Hejazian.

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My contribution to the published paper involved:

- Part of the experiments (investigation of the effect of the magnetic field strength on spreading of the core flow)
- Data analysis, discussion and preparation of figures
- Manuscript preparation: results and discussions, conclusion.

Gui-Ping Zhu contribution to the published paper involved:

- Preparation of the experimental setup
- Experiments including: magnetic flux density measurement in the air gap, and initial experiments demonstrating the spread of core flow.
- Manuscript preparation: introduction, experimental setup description, and materials and methods, results and discussions.
CHAPTER 3

(Date) 21/02/2017

First author: Majid Hejazian

(Sign) ___________________________ 21/02/2017

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Supervisor:
Magnetophoresis of diamagnetic microparticles in a weak magnetic field

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CHAPTER 3

Lab on a Chip

PAPER

Magnetophoresis of diamagnetic microparticles in a weak magnetic field

Gui-Ping Zhu, a Majid Hejiazan, b Xiaoyang Huang a and Nam-Trung Nguyen a b

Magnetic manipulation is a promising technique for lab-on-a-chip platforms. The magnetic approach can avoid problems associated with heat, surface charge, ionic concentration and pH level. The present paper investigates the migration of diamagnetic particles in a ferrofluid core stream that is sandwiched between two diamagnetic streams in a uniform magnetic field. The three-layer flow is expanded in a circular chamber for characterisation based on imaging of magnetic nanoparticles and fluorescence microparticles. A custom-made electromagnet generates a uniform magnetic field across the chamber. In a relatively weak uniform magnetic field, the diamagnetic particles in the ferrofluid move and spread across the chamber. Due to the magnetization gradient formed by the ferrofluid, diamagnetic particles undergo negative magnetophoresis and move towards the diamagnetic streams. The effects of magnetic field strength and the concentration of diamagnetic particles are studied in detail.

Introduction

Continuous-flow microfluidics manipulates particles both passively and actively. Passive methods rely purely on hydrodynamics in microchannels and the physical properties of particles to be manipulated. Depending on applications, the efficiency and throughput of passive methods are limited. Active methods require externally induced forces such as electrical,1-3 thermal,4 optical,5,6 and magnetic7,8 forces. Most active concepts require a complex design for inducing the force field. Active concepts utilizing electrical and optical inputs often generate unnecessary heat, which together with the required ionic concentration is often harmful to sensitive samples. Magnetic concepts can overcome the above problems and gain new functionalities in the microfluidic environment. The interaction between magnetism and fluid flow provides a truly wireless approach for microfluidic manipulation that is not affected by heat, pH level or ion concentration. Magnetic concepts have been employed for conductive liquid driven by Lorentz force.9-11 Magnetic particles coated with an affinity marker are commonly used for sorting diamagnetic particles such as cells. The interaction between magnetism and fluid flow leads to research areas such as ferrohydrodynamics (FHD),12,13 magnetorheology (MR),14,15 and magnetophoresis (MP).16,17 Magnetophoresis has been used for various particle manipulation applications such as sorting and separation18,19 focusing,20,21 assembling,22,23 stirring, mixing24,25 and pumping.26,27

In conventional magnetophoretic manipulation, magnetic beads are driven along a magnetic field gradient. The force acting on the magnetic particles in a diamagnetic fluid is also caused by their susceptibility mismatch. The movement of a magnetic particle towards a higher magnetic field gradient is called positive magnetophoresis. Positive magnetophoresis is suitable for separation applications, as magnetic particles are commonly used as solid support for antigens, antibodies, DNA and cells. With a functionalized coating, targeted biomolecules or cells can be labelled with magnetic particles and subsequently trapped or sorted by an external magnetic field.28 Magnetophoresis has been used for separation of red blood cells,29 isolation of progenitor cells30 and separation of breast cancer cells from human blood.31 Efforts have been devoted to improve positive magnetophoretic separation such as the selection of magnets and flow configuration. Readers may refer to review papers on the basic principles as well as the various applications of positive magnetophoresis.32-34

Most particles in analytical and biological fields exhibit diamagnetic properties. Negative magnetophoresis is the phenomenon where diamagnetic particles migrate away from the magnetic source or a higher gradient due to the magnetic buoyancy force in a paramagnetic carrier.35 The magnetization of a paramagnetic carrier could be determined by the susceptibility of the liquid and the magnetic field. Ferrofluids as a paramagnetic solution with a high susceptibility suit well for the implementation of negative magnetophoresis. A ferrofluid is a stable colloidal suspension of ferromagnetic...
nanoparticles with a diameter of less than 10 nm. The particles are well dispersed in a diamagnetic carrier fluid. The magnetic particles are coated with a surfactant to prevent agglomeration. Since the discovery of ferrofluid in the early 1960s, this material has been used extensively in various applications. Readers may refer to Rosensweig\textsuperscript{36} for further details on ferrofluids and ferrohydrodynamics. Vékás et al. have reviewed the recent achievement of the synthesis of magnetic nanoparticles.\textsuperscript{37} Numerous research studies have been conducted to characterize ferrofluids according to particle concentration,\textsuperscript{38} magnetization\textsuperscript{39,40} and viscosity.\textsuperscript{31,42} Xuan and his colleagues developed a microfluidic device with embedded permanent magnets for particle manipulation.\textsuperscript{53,54} The device has been used for concentrating particles,\textsuperscript{45,46} separation\textsuperscript{47-49} and focusing\textsuperscript{50} of particles and cells. Mao and Koser\textsuperscript{51} reported mixing a ferrofluid with a fluorescein buffer by using ferrohydrodynamic instabilities caused by a sudden velocity variation in the flow passing by a permanent magnet. The same group demonstrated magnetic manipulation, separation and sorting of particles and cells by using a ferrofluid.\textsuperscript{19,51,52} Size-dependent manipulation of diamagnetic particles in ferrofluids has been realized. Separation of diamagnetic cells has been achieved with an efficiency of 100%\textsuperscript{53} and a throughput of 10\textsuperscript{7} cells per hour.\textsuperscript{54} Efforts have also been devoted to applications in particle focusing.\textsuperscript{55-57}

In addition to experimental investigations, an analytical model was reported by Mao’s group on the transport of nonmagnetic particles in ferrofluids under a non-uniform magnetic field.\textsuperscript{52,53} Furthermore, particle assembly\textsuperscript{52,53} has also been reported. Friedman and Yellen reviewed the underlying basic principle and models for separation, manipulation and assembly of the solid diamagnetic phase using an external magnetic field.\textsuperscript{59} In all reported studies on negative magnetophoresis, a non-uniform magnetic field with a high gradient is required to maximise the induced magnetic force. This magnetic field often comes from a bulky permanent magnet. None of the previous studies used a weak uniform magnetic field for manipulating diamagnetic particles.

We demonstrate here the negative magnetophoresis of diamagnetic microparticles in a ferrofluid with a relatively weak external uniform magnetic field. Instead of using a magnetic field with high strength and gradient, our concept only requires a uniform magnetic field with a strength of only few milliteslas (mT), two or three orders of magnitude lower than those of most cases reported in the literature. We also experimentally investigate the influence of magnetic field strength and the concentration of diamagnetic microparticles.

**Experimental setup and results**

We fabricated a microfluidic device that was specifically designed for the negative magnetophoresis experiments. The device has three inlets, one outlet and a circular observation chamber for better visualization of the ferrofluid and the diamagnetic particles (Fig. 1(a)). The circular chamber has a height $H$ of 50 $\mu$m and a diameter $D$ of 1 mm. The inlet and outlet channels have a height $H$ of 50 $\mu$m and a width $W$ of 200 $\mu$m. The device was made of polydimethylsiloxane (PDMS) using the standard soft lithography technique. Readers may refer to Song et al. for the detailed fabrication procedure.\textsuperscript{60} The PDMS device was peeled from the mold, and access holes were created with a 0.75 mm puncher. The device was then treated with oxygen plasma and bonded to another flat PDMS piece to create a closed microfluidic device. The device was then trimmed to fit into the air gap of the electromagnet as described below.

Fig. 1(b) depicts the experimental setup with the circular chamber inserted in the uniform field generated by a custom-made electromagnet. The electromagnet was modified from a transformer whose ferromagnetic core was cut to form an air gap of 12 mm. The uniform magnetic field in the air gap applies across the microfluidic device. Demagnetization of the electromagnet is necessary to eliminate the residual magnetization after each experiment. Demagnetization was achieved by applying a reversed current for 5 to 10 minutes using the highest current value of the previous experiment. The uniformity of the magnetic field was examined with a current of 0.2 A. As the 1 mm diameter of the chamber is relatively small, calibration was only carried out in the 4 mm space around the center of the air gap. Fig. 2 shows that the center of the gap has the lowest flux density. The flux density increases slightly toward the magnetic poles. As the difference in the flux density was less than 5% within the 4 mm space, the magnetic field can be assumed to be uniform in the 1 mm chamber of our experiments. The PDMS device was inserted into the air gap of the electromagnet and therefore thermally insulated from the electromagnet.
Two precision syringe pumps (KD Scientific Inc., USA) delivered the liquids to the microfluidic device. The whole setup was placed on a Nikon (Eclipse TE2000-S) inverted microscope equipped with a digital camera (HiSense MkII). A laboratory DC power supply (GPS-3030D) provides current to the electromagnet. The microfluidic device was slotted into the air gap for testing. A maximum magnetic flux density up to 53 mT could be generated by tuning the supply current up to 2.0 A. The magnetic flux generated at different currents was measured and calibrated using a commercial Gaussmeter (Hirst, GM05, UK).

A water-based ferrofluid (EMG707, Ferrotec) was used for the core stream. The ferrofluid has a saturation magnetization of 11 mT, a density \( \rho_{FF} \) of 1.1 \( \times \) 10^3 kg m\(^{-3} \), a viscosity \( \eta_{FF} \) of 5 mPa s (at 27\(^\circ\)C), a magnetic particle concentration of 2% vol, and an initial susceptibility \( \chi_{FF} \) of 0.36. The magnetization characteristics of this ferrofluid were described in our previous work.\(^6\) Green fluorescent diamagnetic polymers microparticles with a diameter of 1.0 \( \mu \)m (Duke Scientific, 1% solids) were mixed with DI water and the ferrofluid at different concentrations. Solutions with four different concentrations were used in the experiment and termed as sample I, II, III and IV (Table 1).

The diamagnetic liquid is a mixture of DI water and glycerol (16371, Affymetrix). Glycerol has a density \( \rho_G \) of 1.26 \( \times \) 10^3 kg m\(^{-3} \) and a viscosity \( \eta_G \) of 1410 mPa s at 20 \(^\circ\)C. In order to obtain a viscosity comparable to that of the ferrofluid, a water-glycerol mixture was used with a viscosity of 5 mPa s at 25 \(^\circ\)C (50 wt% DI water and 50 wt% glycerol). The corresponding density of the liquid at 25 \(^\circ\)C is 1.13 \( \times \) 10^3 kg m\(^{-3} \).

In the absence of a magnetic field, a clear interface is formed between the ferrofluid/particle (FP) stream and the DI water/glycerol (WG) stream as shown in Fig. 1(b). At a temperature \( T \) of 300 K, the diffusion coefficient of the magnetic nanoparticles \( d_p = 10 \) nm into the water-glycerol mixture is estimated by Einstein’s model as \( D = k_BT/(3\pi\eta_{WG}d_p) = 8.79 \times 10^{-12} \) m\(^2\) s\(^{-2}\).

The experiments were carried out with an FP stream acting as the core that is sandwiched between two WG streams. Different flow rate ratios were used in the experiment to study both the migration of magnetic nanoparticles and the negative magnetophoresis of diamagnetic microparticles. The FP suspensions were delivered into the middle inlet at a constant flow rate of 0.5 ml h\(^{-1}\). The WG solution served as the cladding stream with three different flow rates of 0.25, 0.5 and 0.75 ml h\(^{-1}\). Based on the properties of the WG solution, the Reynolds number range was determined as \( Re = \rho_{WG}UD_p/\eta_{WG} = 0.896 \times 10^{-1} \) to 1.49 \( \times \) 10\(^{-1}\). Using the estimated diffusion coefficient of magnetic nanoparticles towards the WG solution, the Pélet number range is calculated as \( Pe = WU/D = 4.74 \times 10^{2} \) to 7.90 \( \times \) 10\(^{2}\). The small magnitude of Reynolds number implies a laminar flow inside the chamber. Inertial effects such as recirculation at the sudden expansion are negligible. The large Pélet number means that diffusion is negligible.

### Results and discussions

For a fixed weight ratio in the FP suspension, the stable migration of the streams without a magnetic field was investigated for the effect of the flow rate ratio \( Q_{WG}/Q_{FF} \). The recorded images were processed, and the intensity profile across the chamber was plotted. As the images were recorded both through light and using an epi-fluorescent filter (Nikon B-2A, excitation filter for 450–490 nm, dichroic mirror for 505 nm and an emission filter for 520 nm), the migration of non-fluorescent magnetic nanoparticles and the negative magnetophoresis of fluorescent diamagnetic microparticles can be distinguished. Without diamagnetic microparticles, the behavior of magnetic particles under a uniform magnetic field was systematically studied and reported previously.\(^8\) The continuum models are still applicable as the diamagnetic microparticles are at least 2 orders of magnitudes larger than the magnetic nanoparticles.

Fig. 3 shows that the ferrofluid spreads towards the diamagnetic solution, even in the relatively weak magnetic field generated across the circular chamber. With fixed liquid properties (sample II), the flow rate ratio between cladding (WG) and core (FP) streams determines the initial distribution of the liquids inside the circular chamber (Fig. 3(a)).
A larger flow rate ratio leads to a higher gradient in the final distribution of magnetic particles (Fig. 3(b)). The role of fluid flow in this behavior is similar to that in the convective/diffusive transport. The enhanced particle migration through magnetophoresis could possibly be characterized by an effective diffusion coefficient.

The effect of diamagnetic microparticles inside the ferrofluid on migration performance was subsequently investigated. As indicated in Table 1, the concentration of magnetic nanoparticles is the same for all samples. The flow rate ratio between the cladding and core streams was fixed at 0.5.

Fig. 4 shows the migration of magnetic particles in samples I and IV. The data were plotted with normalized intensity and normalized position along the chamber. Without the magnetic field, both samples show a similar migration behavior, which is determined by hydrodynamic migration only. However, as the magnetic field strength increases, the presence of diamagnetic microparticles contributes to a stronger migration of the magnetic nanoparticles (Fig. 4). Besides the magnetic force, an additional hydrodynamic force enhances the migration of magnetic nanoparticles:

$$u_p = u + u_{mag} = u + F_{mag}/6\pi\eta W r_p$$

where $u_p$ is the velocity of the particles, $u$ is the flow field, $r_p$ is the radius of the magnetic nanoparticles, and $F_{mag}$ is the magnetic force on each magnetic nanoparticle. The secondary flow field induced by the motion of diamagnetic microparticles contributes to the improved migration.

The redistribution of magnetic nanoparticles induces the migration of the FP stream leading to a concentration gradient of magnetic nanoparticles away from the center of the chamber. This concentration gradient in turn leads to an increasing magnetization gradient. Driven by the negative magnetophoretic force, diamagnetic microparticles move towards the lower magnetization gradient, e.g. in the same direction as the magnetic nanoparticles. For the same magnetic field strength, the size of diamagnetic microparticles and the concentration of magnetic nanoparticles determine the magnitude of the magnetophoretic force on the microparticles:

$$F_{diam} = V_p\rho_h V_x |H|^2 C_m$$

where $V_p$ and $C_m$ are the volume of diamagnetic microparticles and the concentration of magnetic nanoparticles (volume fraction), respectively; $H$, $\rho_h$ and $\mu_o$ are the magnetic field strength, the susceptibility of liquid and the permeability of free space which has a constant value of $4\pi \times 10^{-7}$ N A$^{-2}$. To distinguish the microparticles from the nanoparticles, the same recording was done for the results shown in Fig. 3, but with the epi-fluorescent filter. Fig. 5 shows the distribution of diamagnetic microparticles with a magnetic field strength ranging from 0 mT (Fig. 5(a)) to 32 mT (Fig. 5(b)). The diamagnetic microparticles move towards the WG stream.
Fig. 5 Negative magnetophoresis of fluorescent diamagnetic microparticles presented by their intensity profile across the circular chamber: (a) no magnetic field, the initial core width is determined by the flow rate ratio; (b) with a magnetic flux density of 32 mT.

at a relatively low flux density of 32 mT. Following the behavior of magnetic nanoparticles, a higher flow rate ratio leads to a larger concentration gradient of the microparticles, indicating the role of convective transport.

To confirm that the migration behavior of diamagnetic microparticles follows that of magnetic nanoparticles, their distributions are plotted in the same graph. Fig. 6 shows the distribution of diamagnetic microparticles and magnetic nanoparticles across the chamber at 0 mT and 32 mT, respectively. The flow rate ratio $Q_{WG}/Q_{TP}$ between the cladding and core streams was fixed at 0.5. The intensity profile confirms that the diamagnetic microparticles migrate together with magnetic nanoparticles. However, since the magnetic forces on the magnetic and diamagnetic particles differ, their migration velocities may be different. Thus, various magnetic flux densities are employed to study the field dependence of the observed phenomenon. Different concentrations of microparticles were tested to understand the role of particle concentrations.

Using sample II as the core stream, the distribution of diamagnetic microparticles across the circular chamber was examined. The motion of diamagnetic microparticles was then characterized by a normalized fluorescence intensity distribution at various magnetic field strengths (Fig. 7). The flow rate ratio $Q_{WG}/Q_{TP}$ between the cladding and the core stream was also fixed at 0.5. Initially, the distribution of fluorescent diamagnetic microparticles shows a sharp interface between the two fluids. With a relatively small magnetic field, the microparticles start to move towards the WG stream. The magnetic field strength was then slowly tuned up to promote the motion of the diamagnetic microparticles. Magnetic flux densities beyond 10 mT show a saturated state. No significant enhancement of negative magnetophoresis was observed. This agrees well with the saturation magnetization of 11 mT of the ferrofluid in use.

Fig. 6 Migration of non-fluorescent magnetic nanoparticles (top) and fluorescent diamagnetic microparticles (bottom) with magnetic flux densities of 0 and 32 mT.

Fig. 7 Normalized intensity distribution across the chamber at various magnetic flux densities (sample IV). A stronger field leads to stronger migration. The distribution remains unchanged with a flux density beyond 10 mT, corresponding to the saturated magnetization of the magnetic nanoparticles.
Table 1 indicates that samples II, III and IV have the same weight percentage of ferrofluid. Because of the initial uniform distribution, the concentrations of magnetic particles are expected to be the same in all samples. With a fixed flow rate ratio and magnetic field strength, we can then examine the effect of the concentration of diamagnetic microparticles on negative magnetophoresis. The flow rate ratio $Q_{WO}/Q_F$ between the cladding and the core stream was again fixed at 0.5. The magnetic flux density was set at 4 mT. Fig. 8 shows the negative magnetophoresis of diamagnetic microparticles with different concentrations of diamagnetic microparticles in the core stream. Magnetophoretic migration is weaker at a higher concentration of the microparticles. A strong interaction between microparticles is expected at a higher concentration. This interaction may affect and limit the migration of the microparticles.

**Conclusions**

Magnetophoretic force was utilized to achieve migration of diamagnetic microparticles in a surrounding ferrofluid under a weak uniform magnetic field. A three-stream flow was generated in a circular chamber for better visualization of the migration effect. The cladding diamagnetic streams consist of DI water and glycerol. The core stream consists of ferrofluid and diamagnetic microparticles. A uniform magnetic field was generated using a custom-made electromagnet. Upon activating the magnetic field across the chamber, the magnetic nanoparticles in the ferrofluid migrate towards the diamagnetic cladding stream. As result, diamagnetic microparticles also move towards the same direction due to negative magnetophoresis caused by the generated magnetization gradient. The migration of microparticles and magnetic nanoparticles were studied for different flow rate ratios between cladding and core streams. Up to the saturation limit, a stronger magnetic field leads to stronger migration of both magnetic nanoparticles and diamagnetic microparticles. A higher flow rate ratio leads to a higher concentration gradient of both particle types, indicating the limiting role of convective transport. Finally, a higher concentration of diamagnetic microparticles leads to weaker migration due to their strong interaction.

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**Notes and references**

Chapter 3 demonstrated that non-magnetic microparticles can be manipulated with an external magnetic field based on the phenomenon of negative magnetophoresis. Using a uniform magnetic field, the effect of the magnetic susceptibility gradient was clearly demonstrated. The same effect would occur with a magnetic field gradient and a medium of homogenous susceptibility. This chapter investigates the phenomenon of negative magnetophoresis in a non-uniform magnetic field. We eliminated the difference in magnetic susceptibility to demonstrate that the magnetic gradient across the device is sufficient to create magnetophoretic and magnetoconveective forces. Depending on the operating conditions, magnetoconveective force could overcome hydrodynamic force and create a secondary flow perpendicular to the direction of the flow. The experiment proved that the use of magnetoconveective force has the potential for manipulation of streams and the suspended particles or cells.
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My contribution to the published paper involved:

- Literature review
- The experiments
- Data analysis, discussion and preparation of figures
- Manuscript preparation

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CHAPTER 4

Negative magnetophoresis in diluted ferrofluid flow

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Negative magnetophoresis in diluted ferrofluid flow

Majid Hejazian and Nam-Trung Nguyen*

We report magnetic manipulation of non-magnetic particles suspended in diluted ferrofluid. Diamagnetic particles were introduced into a circular chamber to study the extent of their deflection under the effect of a non-uniform magnetic field of a permanent magnet. Since ferrofluid is a paramagnetic medium, it also experiences a bulk magnetic force that in turn induces a secondary flow opposing the main hydrodynamic flow. Sheath flow rate, particle size, and magnetic field strength were varied to examine this complex behaviour. The combined effect of negative magnetophoresis and magnetically induced secondary flow leads to various operation regimes, which can potentially find applications in separation, trapping and mixing of diamagnetic particles such as cells in a microfluidic system.

Introduction

The utilization of magnetic force for microfluidic applications has recently attracted great attention from the research community. Magnetic force has been used in a variety of microfluidic applications such as pumping, separation, mixing, magnetowetting, manipulation of cells labelled with magnetic beads, and diamagnetic particles. Particle manipulation using magnetophoresis could be categorized into positive magnetophoresis and negative magnetophoresis. The later is also often called diamagnetophoresis. In positive magnetophoresis, magnetic particles migrate toward a higher magnetic field gradient. Magnetic particles or cells labelled with magnetic beads can be manipulated with this method. Diamagnetic particles could also be manipulated under relatively high magnetic field gradients due to the mismatching magnetic susceptibilities. The particles experience a repulsive force and move away from the magnetic source. Using a paramagnetic medium, the repulsive effect can be achieved at a lower magnetic field strength and for micron sized objects. Considering the fact that most of the living cells possess diamagnetic properties, diamagnetophoresis could potentially be significant for label-free cell separation.

Concentration of diamagnetic particles using two repulsive or attracting magnets was experimentally and theoretically investigated by Wilbanks et al. In this study, the effects of different magnet arrangements and flow rate on particle concentration were studied. Liang et al. compared the migration behaviour of 2.85 μm magnetic and 10 μm diamagnetic particles in diluted (10%) EMG 408 (Ferrotec) ferrofluid and in an aqueous solution. Using ferrofluid as the medium increases susceptibility mismatch and significantly improves the separation throughput. Peyman et al. used diamagnetic repulsion forces to demonstrate the potential applications in label-free manipulation of particles. Focusing, trapping and size selective diamagnetophoretic separation of diamagnetic particles were demonstrated. Zhu et al. employed an analytical model to study the trajectories of non-magnetic microparticles in ferrofluid through a microchannel in the proximity of a permanent magnet. This study concluded that a larger deflection could be achieved with larger particles or at a lower flow rate.

In many biological studies, cell concentration is an important step for sample preparation. Red blood cells are paramagnetic due to deoxygenated haemoglobin proteins, while other cells existing in blood are diamagnetic. Han and Frazier reported label-free separation of red and white blood cells from diluted whole blood with a diamagnetic capture (DMC) mode and a high-gradient magnetic field. Their results showed that 89.7% of red blood cells and 72.7% of diamagnetic white blood cells could be separated. Rodriguez-Villarreal et al. investigated the effect of several parameters such as the particle size, type and concentration of paramagnetic salt solution, as well as the flow rate on the focusing of diamagnetic polystyrene particles and living label-free HaCaT cells. This study revealed that raising the susceptibility mismatch between particles and carrying fluid, reduction of flow rate, increasing the particle size, and increasing residence time in the magnetic field can improve the focusing effect.

The use of a background ferrofluid enables the manipulation of non-magnetic particles and cells, and their
biocompatibility has been investigated in the literature. Zeng et al.\textsuperscript{11} also demonstrated magnetic concentration of particles and cells in a ferrofluid flow through a straight microchannel using permanent magnets. The concentration of both 5 μm polystyrene particles and live yeast cells in 0.059 EMG 408 ferrofluid was investigated. The work examined the effect of flow rate, arrangement of the two attracting magnets and the magnet-to-magnet distance on the concentration performance and flow patterns. The authors discovered that dilute ferrofluid and magnetic force have negligible influence on the viability of yeast cells. Kose et al.\textsuperscript{12} reported separation of live red blood cells from sickle cells and bacteria. 40 mM citric acid concentration (stabilized with citric acid to yield a pH of 7.4) is found to be optimum for cell viability and ferrofluid stability combined. Au et al.\textsuperscript{13} investigated the effects of nanoparticles on the adhesion and cell viability on astrocytes. The study suggests that the addition of nanoparticles to immature astrocytes causes inhibition of cell attachment and impedes subsequent growth. However, in mature astrocytes, nanoparticles induce mitochondrial uncoupling but do not alter cell membrane integrity.

In addition to separation, mixing is another important task in microfluidics. Mixing is required in many biochemical analyses such as cell activation, enzyme reaction and protein folding. Micromixers are categorized as passive and active types. In passive micromixers external energy is not needed, while active micromixers rely on an external field such as a magnetic field to induce mixing disturbances.\textsuperscript{14} Wang et al.\textsuperscript{15} studied the performance of a magnetic particle driven micromixer, consisting of a microchannel and a pair of electromagnets. Varying the design parameters such as the applied magnetic actuation forces, they could demonstrate that the optimum switching frequency depends on the lateral dimension of the channel and the applied magnetic force. Roy et al.\textsuperscript{16} reported a droplet-based microfluidic system with magnetic stirring. The work investigated the impact on the overall mixing performance of parameters such as the rotational speed of the magnetic field, viscosity of the droplet liquid, and the concentration of magnetic particles in the liquid. Ganguly et al.\textsuperscript{17} examined \textit{in situ} immunochemical binding of biotinylated oligonucleotides on streptavidin-coated magnetic beads through magnetophoresis-aided cross-stream mixing in a microfluidic channel. The effect of various ratios of the fluid streams and viscosities of the background fluid was evaluated on the extent of immunochemical binding. For the augmentation of mixing of a magnetic based reaction, Berenguel-Alonso et al.\textsuperscript{18} designed a simple and low-cost magnetic actuator, consisting of a CD-shaped plastic unit with embedded magnets to trap and move the magnetic beads through a microfluidic chamber. Sensitivity was enhanced with the use of the magnetic actuator. The device was used for the amplification reaction of an enzyme-linked fluorescence immunoassay to detect \textit{Escherichia coli}.

In our previous work, \textsuperscript{19} the migration of diamagnetic microparticles in a ferrofluid core stream sandwiched between two diamagnetic streams under a relatively weak and uniform magnetic field was investigated. Under the uniform magnetic field, provided by a custom-made electromagnet, the core stream containing magnetic nanoparticles expanded into the surrounding diamagnetic streams. Diamagnetic particles suspended in the core stream also followed the same direction due to diamagnetophoresis effect.

Most of the studies reported previously by others used relatively narrow and straight channels together with a high field gradient generated by a permanent magnet. In this paper, we utilise the extreme combination of a large fluid chamber and a non-uniform and relatively stronger magnetic field generated by a permanent magnet. This unique combination creates the opportunity to investigate the effect of a high magnetic field gradient in a relatively large chamber on deflection of non-magnetic micro-particles. The numerical simulation predicted a deflection of the diamagnetic particles away from the magnet. However, experimental results showed a more complex phenomenon. We observed a secondary flow field in the upper half of the chamber opposite the direction of the main hydrodynamic flow, as a result of the non-uniform magnetization of the ferrofluid. We first investigated the impact of field strength through changing the distance of the permanent magnet to the chamber, the sheath flow rate, and the size of the diamagnetic particles on the extent of the secondary flow. We then repeated the experiment with a fluorescent dye to prove that the secondary flow is caused by non-uniform magnetization of the ferrofluid. This interesting phenomenon has potential for use in both mixing and trapping/sorting of diamagnetic particles based on size.

Materials and methods

Fig. 1 shows a schematic of the microchannel and magnet setup. The circular chamber has two inlets and one outlet. The inlet and outlet channels have a depth of \(H = 50 \, \mu\text{m}\) and a width of \(W = 200 \, \mu\text{m}\). The circular chamber has a depth of \(H = 50 \, \mu\text{m}\) and a diameter of \(D = 1 \, \text{mm}\). The microchannel was fabricated in polydimethylsiloxane (PDMS) using a standard soft lithography technique. Detailed information about the fabrication procedure was reported by Song et al.\textsuperscript{20} The

![Schematic of the microchannel and magnet setup.](image-url)
moulded PDMS part was bonded to another flat PDMS base after treating both with oxygen plasma.

Two precision syringe pumps (SPM100, SIMTech Microfluidics Foundry) were used to deliver the fluids into the microfluidic device. A camera (Edmund Optics, Germany) attached to an inverted microscope (Nikon Eclipse TE100) and connected to a desktop computer was used for visualization and image recording. The paramagnetic liquid used in our experiment is a water-based ferrofluid (EMG707, Ferrotec) diluted to 5% vol. of the off-the-shelf solution by DI water. Two solution samples were made by suspending 3.1 and 4.8 μm fluorescent polystyrene particles (ThermoScientific Inc.) in the diluted ferrofluid. The diluted ferrofluid without particles was introduced into the circular chamber (Fig. 1) as the sheath streams to focus the core stream with suspended particles. Fluorescein sodium salt (acid yellow, Sigma-Aldrich Co.) was used for tracing the core fluid in a further experiment to confirm that the secondary flow is caused by the non-uniform magnetization of the ferrofluid, and the diamagnetophoresis does not play a significant role in the phenomenon.

A 3.2 mm³ neodymium–iron–boron (NdFeB) permanent magnet (B222, K&J Magnetics Inc.) provided the magnetic field for this experiment. The PDMS device was cut to place the magnet on one side of the circular chamber. The experiments were carried out with three different distances (2, 3 and 4 mm) between the magnet front and the edge of the chamber. The magnetic field of the permanent magnet was measured and calibrated using a gaussmeter (Hirst Magnetic Instruments Ltd). Fig. 2 shows the measured magnetic flux density as a function of the distance from the edge of the magnet. The stream containing particles was maintained at a constant flow rate of 1 μL min⁻¹ throughout the experiments, while the sheath flow rate was varied from 1 to 50 μL min⁻¹ to observe the particle deflection and the secondary flow as a function of the flow rate ratio and total flow rate.

Theoretical background and numerical simulation

Force balance should be considered to determine the trajectory of a particle in a magnetic field. In our study, diamagnetophoretic deflection of polystyrene microparticles is dominated by the drag force and the magnetic force. The magnetic force acting on a point-like particle in magnetic induction B can be estimated as:

\[ F_m = \frac{V \Delta \chi}{\mu_0} (\mathbf{B} \cdot \nabla) \mathbf{B} \]  

(1)

where \( F_m \) is the magnetic force (N), \( V \) is the volume of the particle, \( \Delta \chi = \chi_p - \chi_f \) is the magnetic susceptibility mismatch between particles \( \chi_p \) and base fluid \( \chi_f \) (dimensionless), \( \mathbf{B} \) is the magnetic flux density, and \( \mu_0 = 4\pi \times 10^{-7} \text{ (T mA}^{-1}) \) is the permeability of the vacuum. The drag force for the suspended particles in a flow with relatively low Reynolds number can be determined using the Stokes’ law and the relative velocity:

\[ F_t = 3\pi \eta d_p (u_t - u_p) \]  

(2)

where \( \eta \) is the dynamic viscosity of the fluid, \( d_p \) is the diameter of the particles, and \( u_t \) and \( u_p \) are the velocities of the fluid and the particles, respectively.

Considering these dominating and most effective forces on particles, a numerical model for the prediction of particle trajectories could be established with the magnetic field, the single phase laminar fluid flow through the microchannel, and particle tracing in COMSOL (COMSOL Inc., USA). Our numerical model considers three distinct domains: the permanent magnet, the microfluidic channel and the surrounding PDMS substrate. Circular magnetic insulation boundary conditions with a diameter of 2 cm were applied around the system to bind the magnetic field and to achieve an accurate field distribution. The two-dimensional model consists of three inlets, a circular chamber and one outlet. The permanent magnet is considered as a square (3.2 mm × 3.2 mm) placed in a distance of 2 mm from the edge of the chamber. The working fluid for all the inputs is ferrofluid diluted with DI water (with a volume fraction of 5%), and it is considered as incompressible. Two streams were introduced into the chamber; the middle stream is set with a flow rate of 1 μL min⁻¹, while the other two inlets for the sheath streams have equal flow rates varying between 1 and 7 μL min⁻¹. Steady state conditions are applied for simulation of the magnetic field and fluid flow. The calculated flow and magnetic field were used to evaluate the trajectories of the particles using a time-dependent particle tracing method.

For modelling the laminar flow of diluted ferrofluid inside the microchannel, continuity equation:

![Image](Fig. 2 Magnetic flux density versus distance to the permanent magnet. The inserted image shows the characterisation setup. The distance was adjusted using the linear stage of a syringe pump.)
∇(ρfut) = 0

and Navier–Stokes equation:

ρf(ut∇)ut = −∇P + ∇(η∇ut)

were solved, where P is the pressure, ut is the fluid velocity, and ρf and η are the density and dynamic viscosity of the fluid, respectively. The normal inflow velocities are set for the three inlets, no slip conditions were considered at the walls, and pressure has no viscous stress at the outlet.

A scalar magnetic potential was used to solve the magne-tostatic problems, in the absence of electric currents:

∇(μ₀μrH) = 0

where μ₀ is the relative permeability and H is the magnetic field strength (A m⁻¹). Magnetic insulation is considered around the whole system.

The magnetic susceptibility of various PS particles is

−(7.93 ± 0.16) × 10⁻⁶.²²,²³ Effective susceptibility of diluted ferrofluid emulsions could be estimated by the Maxwell-Garnett formula:²⁴

μeff = μw + 3μp

where μw is the susceptibility of water, μp is the susceptibility of polystyrene particles, and μeff is the effective susceptibility of diluted ferrofluid.

Particle trajectory was obtained by considering the balance of the dominant forces:

m_p du_p/dt = F_m + F_d

where m_p is the mass of the particle, u_p is the velocity of the particle, F_m is the magnetic force on the particle, and F_d is the drag force. The initial position in which the particles are released is set to be the middle inlet, Fig. 1. Stick boundary conditions (u_p = 0) were used for the microchannel walls and the outlet, meaning that the particles will stop when they reach the wall. The velocity field and the magnetic field in the flow domain, which has been calculated in the steady state study, were used to solve the force balance equation and to predict the trajectory of the particles. The time step for the time dependent particle tracing study is set as 10⁻⁴ s to achieve an accurate and more detailed particle trajectory, Fig. 4.

For the numerical solution of the above equations, finite element discretization was based on quadratic basis functions for the magnetic field and second order functions for velocity, while the pressure field is described by linear basis functions. Fig. 3 shows the grid independency for the simulation. Three structured non-uniform meshes (finer close to the walls where large gradients exist) have been
evaluated for the velocity profile of pure water along a line parallel to the y-axis and crossing the centre of the chamber. Fig. 3(a) shows that the velocity profile is not dependent on the number of elements. A test is also done for the magnetic flux density along a line parallel to the x-axis and crossing the centre of the chamber. Fig. 3(b) indicates that the number of finite elements has no effect on the value of the magnetic flux density norm. The measured data from Fig. 2 deviates within 5% from the simulated data shown in Fig. 3. In this study, 1644 elements were selected for meshing the geometry. Two-dimensional simulation was employed for this study, because the flat geometry of the chamber requires much less computational time compared to three-dimensional (3D) simulation. The two-dimensional (2D) model considers the depth-averaged velocity.

The numerical simulation was carried out for two particle diameters (3.1 and 4.8 μm) and for various sheath flow rates between 1 and 15 μL min⁻¹, while the flow rate of the core flow containing the particles is held constant at 1 μL min⁻¹. The results are illustrated in Fig. 5. As can be seen from these trajectories, the deflection for the diamagnetic particle increases with the decreasing sheath flow rate and the increasing particle size.

Results and discussion

In our experiments, the non-uniform magnetic flux was exerted on the system using the permanent magnet at variable distances of 2, 3 and 4 mm to the edge of the chamber. In order to study the effect of sheath flow rate on the deflection, the particle flow is held constant while the sheath flow varies between 1 and 50 μL min⁻¹. Two particle sizes, 3.1 and 4.8 μm, were used to study the impact of the particle size on deflection of the particle stream. The experimental results are summarized in Fig. 6 and 7.

Effect of sheath flow rate

The first interesting observation at low flow rates is the existence of a secondary flow opposing the main hydrodynamic flow. The secondary flow can be seen in the upper half of the chamber, and circulation of particles in some locations is also apparent. We hypothesize that this phenomenon is induced by the non-uniform magnetization of the ferrofluid, because of the non-uniform magnetic field distribution across the chamber. Due to magnetic attraction, the magnetic nanoparticles migrate towards the magnet. The magnetic nanoparticles of the ferrofluid accumulate in the lower half of the chamber, while the nanoparticles in the upper half are blocked by the core flow. Because of this blockage, a higher

Fig. 5  Simulation results showing the variation of magnetic deflection in different sheath flow rates for one particle only (distance, 2 mm).

Fig. 6  Experimental results of 3.1 μm particles for three different magnet-to-chamber distances (2, 3, and 4 mm).
concentration of the magnetic nanoparticle exists just above the core flow. As an example, for a flow rate ratio of 7 and a magnet distance of 2 mm, the core flow is expanded into the upper half of the chamber, Fig. 6(a). As the flow rate ratio decreases, this difference in the concentration of the ferroparticles causes a flow in the opposite direction of the main flow, strong enough to compete with the upper sheath flow, and appears as a secondary flow. On the other hand, the particles near the edge of the fluorescent particle stream, at low sheath flow rates, depart from the core stream, because of the diamagnetophoresis effect, and are dragged into the secondary flow path. As the sheath flow rate increases, this secondary flow starts to fade away. The numerical simulation was unable to model and demonstrate the secondary flow, because a uniform concentration was considered for the base flow throughout the system.

Another noticeable phenomenon is that the deflected particles do not follow a focused line. Instead, a wide band of particles is observed. The deflected core flow is not integrated and it could be divided into two streams; the lower one is the main stream and flows to the chamber outlet, while the upper and less dense band of particles travels a longer path to reach the outlet. The particles in the upper band are more deflected and travel in a lower velocity compared to the particles in the lower band. This behavior occurs because of the velocity distribution in the z-axis along the height of the chamber, which has its maximum in the center and decreases when approaching the walls. The extent of diamagnetic deflection depends on the balance between magnetic force in y-direction and hydrodynamic force in x-direction. The particles closer to the middle of the chamber height have a higher velocity and less deflection, because of the larger hydrodynamic force. This behavior also fades with increasing sheath flow rate.

The S-shape of the particle trajectory also deviates from the ideal case of the numerical simulation. Near the entrance, the trajectory bends downwards, while close to the outlet it bends upwards as expected with the simulation (Fig. 5). The downward bend is caused by the sudden change in concentration of magnetic nanoparticles due to the downward migration discussed above.

**Effect of magnetic field strength**

The experiment was repeated for three different positions of the permanent magnet, 2, 3, and 4 mm from the edge of the chamber, to investigate the effect of magnetic field strength. The results are shown in Fig. 6 and 7. Considering the fact that the secondary flow opposes the main hydrodynamic flow, the upper sheath flow competes with the secondary flow induced by the magnetic field. A weaker magnetic field leads to a weaker magnetization of the ferrofluid. Therefore the secondary flow appears at lower flow ratios and a shorter magnet distance. Also, by decreasing the magnetic field strength, the difference between the upper and lower bands of the deflected particles becomes less apparent, and the stream of particles becomes more uniform. The reason lies in the diamagnetophoresis effect. A lower magnetic field leads to weaker magnetic force and less particle deflection; therefore the core stream mainly follows the flow path caused by the magnetization of the ferrofluid.

**Effect of particle size**

The experiments were carried out with two particle sizes (3.1 and 4.8 μm) to observe the effect of particle size. For the small particles, at a flow rate ratio of 1 and a magnet to chamber distance of 4 mm, the effect of secondary flow on particles could be avoided. Meanwhile, larger particles under the same conditions still show significant secondary flow. Having smaller particle size leads to a lower hydrodynamic force caused by the secondary flow according to eqn (2), and therefore the small particles exit the chamber unaffected by the secondary flow.

A flow ratio of 6 was taken for comparison to compare the effect of particle size on the particle band width. The images in Fig. 6 and 7 show that small particles cause more spreading and a wider band. The reason can be justified by the fact that larger particles are more concentrated in the middle of the channel and less in the vicinity of the channel walls. Therefore, larger particles have a larger mean velocity that leads to a narrower particle band.
In our experiment, the diamagnetophoresis effect could not be distinguished clearly because of the existence of the secondary flow. However, it can be noticed by comparing the extent of deflection for the bottom of the particle stream, between two particle sizes. At low flow rate ratios (less than 6) more deflection could be observed at the bottom of the particle streams. Fig. 8 shows an operation map of the range of conditions, in which secondary flow occurs.

Experiment with a fluorescein dye
We repeated the experiments using fluorescein sodium salt dissolved in ferrofluid in the core stream without microparticles. The purpose of this experiment is to prove that negative magnetophoresis plays no role in the observed recirculation of the particles, as they just follow the induced secondary flow. Fig. 9 shows the fluorescent image of the chamber under the same experimental conditions in Fig. 6 and 7. The results show that the secondary flow occurs at almost the same conditions as observed previously with the particles. A deflection of the fluorescent dye towards the upper half of the chamber could be observed. The secondary flow and mixing of the fluorescent dye become stronger with decreasing sheath flow rates. The S-shape deflection could also be observed in Fig. 9. The downward migration near the inlet is caused by the ferrofluid concentration difference in that location, as the magnetic nanoparticles move towards the magnet. The upward migration happens before the chamber outlet as the result of the secondary flow.

Conclusions
This paper reports the behaviour of non-magnetic particles in diluted ferrofluid under the effect of a magnetic field in a circular chamber. The effect of sheath flow rate and magnetic field strength was examined for two particle sizes and a fluorescein dye. Interestingly, a secondary flow in the opposite direction of the main flow was observed. At low flow rate ratios and relatively high magnetic field strengths, the secondary flow is strong enough to mix the core flow with the upper sheath flow. Results from the experiment with a fluorescein dye prove that the secondary flow is caused by the non-uniform magnetization of ferrofluid, and the non-magnetic particles mostly follow the path of this flow. This phenomenon has potential applications for particle or cell capture based on their size difference and also for magnetic mixing of two streams in a microfluidic chamber. A possible approach for simulating the secondary flow observed and reported in this paper is described as follows. The migration and consequently the concentration distribution of magnetic nanoparticles can be modelled by adding the magnetophoretic migration term to the convective/diffusive transport equation as reported previously by our group. The distribution of magnetic susceptibility of the ferrofluid can then be determined based on the concentration distribution. The term of bulk magnetic force is then added to the momentum equation (eqn (4)), which can then be solved iteratively together with the mass conservation equation and the convective/diffusive transport equation.

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Notes and references

CHAPTER 5 MASS TRANSPORT IMPROVEMENT IN MICROSCALE USING DILUTED FERROFLUID AND A NON-UNIFORM MAGNETIC FIELD

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The experimental results in Chapter 4 show that not only magnetophoresis, but also magnetoconvection, the bulk flow of diluted ferrofluid, plays an important role in the transport processes in a microchannel in the presence of a magnetic field. Magnetic convection can occur with both magnetic susceptibility gradient and magnetic field gradient. This chapter demonstrates that two streams with different magnetic susceptibilities can be mixed by taking advantage of magnetofluidic phenomena. Magnetoconvective force could be used for the transport of non-magnetic particles and fluorescent dye. Mass transport was compared between two cases: pure molecular diffusion and magnetofluidic techniques. The latter case demonstrated a significant enhancement in mass transfer. We already demonstrated the feasibility of transporting microparticles using magnetoconvective force in Chapters 3 and 4. The experiment in this chapter shows that the magnetoconvective force is capable of transporting much smaller particles such as dye molecules.
CHAPTER 5

Statement of Contribution to Co-authored Published Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored published paper, including all authors, are:

Chapter 5: Majid Hejazian, Dinh-Tuan Phan and Nam-Trung Nguyen, Mass transport improvement in microscale using diluted ferrofluid and a non-uniform magnetic field, RSC Advances, 6 (2016) 62439. DOI: 10.1039/C6RA11703A

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My contribution to the published paper involved:

- Literature review
- The experiments
- Data analysis, discussion and preparation of figures
- Manuscript preparation

(Date) 21/02/2017

First author:

21/02/2017
CHAPTER 5

Mass transport improvement in microscale using diluted ferrofluid and a non-uniform magnetic field

M. Kejazian, D. Phan and N. Nguyen, RSC Adv., 2016, 6, 62439
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Mass transport improvement in microscale using diluted ferrofluid and a non-uniform magnetic field

Majid Hejazian, a Dinh-Tuan Phanb and Nam-Trung Nguyena* a

This paper demonstrates that a non-uniform magnetic field and diluted ferrofluid can improve mass transport of non-magnetic solutes in a microfluidic device. A hydrodynamic flow-focusing configuration was employed to test this hypothesis. The system consists of a core stream and two sheath streams. The core stream contains a diluted ferrofluid and a fluorescent dye. Without a magnetic field, both magnetic nanoparticles of the ferrofluid and the fluorescent dye in the core stream rely on molecular diffusion to spread transversally. The susceptibility mismatch between the core and focusing fluids results in a magnetoconvective secondary flow and subsequently the mass transport of the non-magnetic fluorescent dye. We observed a significant enhancement in mass transport of the fluorescent dye. The platform presented here could be used as a microfluidics-based gradient generator or micromixer.

1. Introduction

Over the last two decades, microfluidics has proven to be a technology that provides highly effective tools for biological and chemical research.1–2 This technology has been exploited for designing a diverse range of fluid handling devices in the microscale such as micropumps,3 microvalves,4 micromixers,5 and microseparators.6–7 Micro gradient generator and micromixer are the two typical microfluidic devices that rely on effective mass transport in the microscale, but are currently limited to molecular diffusion due to the predominant laminar flow in this size scale.

Recently, microfluidics-based concentration gradient generators8 have been used for cell migration studies. A concentration gradient generator is capable of creating a gradient of biochemical signals such as growth factors, hormones and chemokines.9 Pressure-driven gradient generator is one of the most common types in continuous-flow microfluidics, where mass transport is governed by molecular diffusion and convection through a network of microchannels. Lung cancer chemotherapy resistance, for instance, has been studied using this device.10 An integrated microfluidic device having an upstream gradient network and downstream cell culture chambers was used for this purpose. Other type of gradient generators relies solely on molecular diffusion. An example of this type is a device for bacterial chemotaxis analysis, the movement of cells in a channel induced by the cells chemotactic response.11 The diffusion of a chemical through a porous membrane adjacent to the channel wall formed a static linear chemical gradient in the microchannel.

Micromixers are other microfluidic devices that play an important role for biological and chemical research. Micromixers can be categorised in passive and active groups.7 Passive micromixers solely rely on diffusion or chaotic advection for mixing, while active micromixers take advantage of an external energy field, such as temperature, magnetic field, electric field and acoustic field. Parallel lamination micromixers are a type of passive micromixers that has been exploited to study diffusive mixing in microchannels. Wu et al. developed a analytical model and used a diluted fluorescent dye to evaluate the concentration distribution across a microchannel width.12 The authors further examined analytically and experimentally diffusive mixing in hydrodynamic flow-focusing configuration with three fluid streams.13 The concentration was measured using recorded images of a fluorescent dye, while the velocity distribution was determined with micro particle image velocimetry (micro-PIV).

Active mixing employs externally induced disturbance to improve mass transport. For instance, magnetofluidic micromixers utilize a magnetic field and magnetic particles suspended in a fluid to enhance mass transport through magnetophoresis, the migration of magnetic particles. Wang et al. investigated numerically a magnetofluidic micromixer using magnetic beads for fluid agitation.14 Tsai et al. and Fu et al. used a permanent magnet and ferrofluid, a liquid with a suspension of magnetic nanoparticles, to improve the performance of a Y-mixer.15,16 The non-uniform magnetic field re-distributes the magnetic nanoparticles in the microchannel and consequently induces magnetoconvective flow. Mixing efficiency between water and ferrofluid can be higher than...
90%, which is significant compared to the value of below 15% for pure molecular diffusion in the same microchannel. Azimi et al. utilised ferrofluid and a stationary magnetic field to enhance mixing in an Y-micromixer.\textsuperscript{17} The mixing efficiency can reach up to 70% with an optimal concentration of magnetic nanoparticles. Mao et al. reported the design and simulation micro-mixer utilizing paramagnetic ferrofluid.\textsuperscript{18} Using a low-voltage excitation, the device could achieve significant improvement in mass transport and consequently complete mixing within seconds. Wen et al. reported a DC-driven electromagnet for mixing ferrofluid and Rhodamine B in a micro device.\textsuperscript{19} The magnetic field causes the ferrofluid to expand into the non-magnetic flow. The mixing efficiency of 95% was achieved within 2.0 s. Zhu et al. investigated the mixing phenomena in a circular chamber using ferrofluid and under a uniform magnetic field.\textsuperscript{20} Full mixing was achieved at a relatively low magnetic flux density up to 10 mT as a result of susceptibility mismatch between the paramagnetic ferrofluid and non-magnetic shear flow. Wen et al. reported mixing of ferrofluid and deionized (DI) water using an electromagnet driven by DC or AC power to induce transient interactive flows.\textsuperscript{23} The magnetic force results in a body force that causes the ferrofluid to move into the non-magnetic flow. Extremely fine finger structures along the direction of local magnetic field lines at the interface were observed and verified both numerically and experimentally. Our group previously exploited an uniform magnetic field and the susceptibility gradient between a diamagnetic fluid and a ferrofluid to induce mixing in a circular chamber.\textsuperscript{22–23} The gradient of magnetic susceptibility led to instability at the liquid–liquid interface and consequent mixing. In a recent work, we demonstrated that even without the existence of a magnetic susceptibility gradient, mixing could be achieved with a non-uniform magnetic field.\textsuperscript{24} The field of a permanent magnet induces a body force on the ferrofluid inside the chamber, resulting in a secondary flow called magnetoconvection. The magnetoconvective transport was traced by both fluorescent dye and non-magnetic particles.

As discussed above, mixing of ferrofluid and water has been reported, but mass transfer enhancement for other non-magnetic species such as fluorescent dye through magnetic field have not been studied and reported before. In the present work, we employed permanent magnets in a simple hydrodynamic focusing device to create a non-uniform magnetic field. The transport process of the fluorescent dye was evaluated based on the recorded fluorescent images. The concentration field was analysed for different flow rates to compare the relative significance of hydrodynamic transport and the secondary magnetoconvective transport induced by the magnetic field. A numerical study was also carried out to simulate the concentration field and velocity field in the absence of magnetic field. Magnetically induced mass transfer was observed and discussed based on our experimental results. The enhancement in mass transfer is benchmarked by the convective/diffusive flow-focusing system that relies on molecular diffusion only.

### 2. Materials and methods

Fig. 1(a) shows the schematic of the hydrodynamic focusing device. The microchannel has a depth of $H = 50 \mu m$, a width of $W = 500 \mu m$, and a length of $L = 12 \text{ mm}$. The device was fabricated in polydimethylsiloxane (PDMS) using the standard soft lithography technique. Detailed information about the fabrication procedure can be summarized as follows.\textsuperscript{25,26} First, photoresist SU-8 with a thickness of 50 \( \mu m \) was coated evenly and patterned on a Si wafer by a standard photolithography process. A mixture of Sylgard 184 base and a curing agent (Dow Corning Inc.) at 10 : 1 ratio by weight was poured onto the SU-8 mould and subsequently degassed for one hour in a vacuum chamber. A 2 hour baking at 80 °C in the oven followed. Subsequently, the top PDMS layer was peeled off from the SU-8 mould and the 1 mm access holes were punched. The PDMS microchannel was bonded to another flat PDMS base with the help of oxygen plasma treatment and underwent another post-baking process at 80 °C for 10 minutes to enhance the bonding strength.

Three precision syringe pumps (SPM100, SIMTech Microfluidics Foundry) delivered the fluids into the microfluidic device. The whole setup was placed on an inverted microscope (Nikon Eclipse TS 100) equipped with a high-speed camera (Photron 120K-M2) for visualization. The commercial water-based ferrofluid (EMG707, Ferrotec) containing 2% vol. magnetic nanoparticles, was used to make a diluted paramagnetic solution for the core stream. For this purpose, 0.05 g of fluorescein sodium salt (acid yellow, Sigma-Aldrich Co.) was dissolved in 20 mL of DI-water. Then the commercial ferrofluid was diluted with the fluorescent dye and DI-water solution to

![Fig. 1](image-url)

**Fig. 1.** (a) Schematic of the setup with a microchannel and a set of permanent magnets. (b) Characterisation of magnetic field. The inset shows the setup used for magnetic field measurement. The distance was adjusted using the linear stage of a syringe pump ($x^* = 2x/W$, $y^* = 2y/W$).
20% vol. Three identical 3.2 mm$^3$ neodymium–iron–boron (NdFeB) permanent magnets (B222, K&J Magnetics Inc.) provided the magnetic field for this experiment. DI-water served as the liquid in both sheath streams. The experiments were carried out with a constant flow rate ratio, for three different flow rates (30 : 2.5 : 30, 60 : 5 : 60, 120 : 10 : 120 μL min$^{-1}$). The magnetic field of the permanent magnets was measured and calibrated using a gauss meter (Hirst Magnetic Instruments Ltd). Fig. 1(b) shows the measured magnetic flux density as a function of the distance from the edge of the magnet. The distance between the magnets to channel wall was 1 mm. Across the microchannel width, the magnetic flux density drops from approximately 250 mT to 175 mT.

3. Numerical simulation

We used diffusive/convective transport as the reference for the magnetoconvective/convective transport that is characterized later experimentally. A numerical model with COMSOL (COMSOL Inc., USA) simulated the diffusive/convective transport in the flow-focusing configuration to predict the distribution of fluorescent dye in the microchannel, Fig. 1(a). The two-dimensional model consists of three inlets, a straight rectangular channel and one outlet. Because of the low aspect ratio between channel height and channel width, the velocity is averaged in the z-axis. All streams were considered as incompressible. Steady-state conditions were applied. The model solves the continuity equation:

$$\nabla(\rho u_i) = 0 \quad (1)$$

and the Navier–Stokes equation:

$$\nabla \left[ -p_i I + \mu_i \left( \nabla u_i + (\nabla u_i)^T \right) \right] - 12 \mu_i \nabla^2 u_i = 0 \quad (2)$$

where $u_i$ is the fluid velocity, $p_i$ the pressure (Pa), $\nabla$ the divergence operator, $\nabla(\cdot)$ the gradient operator, $I$ the identity matrix, $\mu_i$ the fluid dynamic viscosity (Pa s), and $H$ is the height of the channel.

The normal inflow velocities are set for the three inlets, no slip conditions were considered at the walls, and pressure has no viscous stress at the outlet. Using single phase model, the density of the ferrofluid is calculated by:27

$$\rho_i = (1 - \varphi) \rho_{water} + \varphi \rho_{np} \quad (3)$$

where $\rho_{np}$ and $\varphi$ are the density of nanoparticles and volume fraction, respectively. The effective dynamic viscosity of the ferrofluid is estimated as:28

$$\mu_i = \mu_{water} \frac{1}{(1 + \varphi)^{n_{27}}} \quad (4)$$

Diffusive/convective transport of a solute is obtained by solving:

$$-\nabla(-D \nabla c + cu) = 0 \quad (5)$$

where $c$ (mol m$^{-3}$) is the concentration, and $D$ (m$^2$ s$^{-1}$) is the diffusion coefficient of the solute. The concentrations for the middle inlet and sheath inlets were set to 4 mol m$^{-3}$ and 0 mol m$^{-3}$ respectively. For the numerical solution of the above equations, finite element discretization was based on second order functions for velocity, while the pressure and concentration fields are described by linear basis functions. We used the fine mesh consisting of 64 324 domain elements and 1976 boundary elements. As mentioned above, the two-dimensional (2D) model considers the depth-averaged velocity. The numerical simulation was carried out for three flow rates at a fixed ratio as used in the later experiments. Fig. 2 shows the representative results for flow field and concentration field.

4. Results and discussion

The experiments were carried out for a constant flow rate ratio between the sheath and core streams but different total flow rates, to vary the strength of convective transport. Diluted ferrofluid mixed with fluorescent dye serves as the fluid of the core stream. DI-water works as the sheath fluids sandwiching the core stream in the microchannel. Our aim is to investigate the transport and spreading of the core flow in response to the magnetic field. Without a magnetic field, the width of the core flow (measured as the width between the maximum concentration gradients) is expected to remain constant, as the flow rates and the viscosity determine this width only. We also aim to evaluate the effect of magnets on the mass transport of the fluorescent dye. A relatively high concentration of diluted ferrofluid, 20% vol., was selected to observe the phenomenon thoroughly: the expansion of the ferrofluid stream, movement toward the magnet and then accumulation of the ferrofluid near the magnet. Lower concentrations of the ferrofluid could not create high enough magnetic susceptibility mismatch to manifest this effect. Six images were taken at different locations along the channel: $x = 0, 4, 5.75, 7.5, 10, 12$ mm. The magnets were located between $x = 4$ mm to $x = 7.5$ mm. We normalised the length scale by half of the channel width $W/2 \left(x^* = 2x/W, y^* = \frac{y}{y_{channel}}\right)$.
= \frac{2y}{W}\). Half channel width W/2 was used as the characteristic length because we later use the location \(y^* = 0\) as the centre line of the microchannel. The negative space \((y^* < 0)\) is next to the magnet and exposed to the field of the permanent magnets.

Fig. 3 shows the simulated concentration distribution at locations along the channel length for different flow rates as used later in the experiments. Without a magnet, the concentration has its maximum value at the centre line of the channel \((y^* = 0)\). The profiles for different flow rates are almost overlapping because the flow rate ratio was kept constant. A diffusion coefficient of \(D = 1 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}\) was used in our simulation.\(^{29}\)

We also carried out a calibration of fluorescent intensity, to understand the light-blocking role of magnetic nanoparticles on the fluorescent intensity. The intensity calibration corrects the intensity value and converts it into the dimensionless concentration \(c^*\) of the fluorescent dye. Three streams comprising DI-water mixed with fluorescein dye, ferrofluid mixed with fluorescein dye, and ferrofluid are fed into the microchannel with identical flow rates. Fig. 4 depicts the normalized intensity across the channel width. Fig. 4 shows that the ratio between the intensity of ferrofluid/fluorescent dye to DI-water/fluorescent dye mixture is around 0.55.

Fig. 5 compares the experimental results at \(x^* = 40\) and at the lower flow rate of 2.5 \(\mu\text{l min}^{-1}\) and 30 \(\mu\text{l min}^{-1}\) for the core and sheath flows, respectively. In the absence of a magnetic field, experimental and numerical data agree relatively well. In the presence of a magnetic field, substantial transport of fluorescent dye toward the magnets was observed. The mass transport of fluorescent dye was obviously enhanced in the presence of the permanent magnets. The magnetic nanoparticles move towards the magnets as result of the susceptibility mismatch between the sheath flows and the core stream magnetic susceptibility gradient lead to a body force that causes the so-called magnetocative secondary flow. This secondary flow transports along the dye molecules, which are too small to be affected by negative magnetophoresis effect.

Next, we evaluated the fluorescent intensity across the channel width, to examine the extent of the transport of fluorescent dye. Fig. 6 shows the normalised intensity distribution of all positions along the \(x^*\) axis for the three different total flow rates mentioned above. The results consistently show that the dye molecules moved toward the permanent magnet, and followed the same path of the magnetic nanoparticles. At low and medium total flow rates, the dye reached and accumulated at the top channel wall \((y^* = -1)\). As the total flow rate increases, the pressure-driven hydrodynamic flow competes with magnetically driven secondary flow, and the intensity gradient becomes steeper. At the higher flow rate, the fluorescent dye could not reach the top channel wall.

Fig. 6 also indicates that the magnetic field has no effect on the concentration distribution of the dye at the inlet \((x^* = 0)\). The distribution of the fluorescent dye is similar to that numerically simulated and previously reported.\(^{12}\) The mass transport of the dye in the channel width direction relies on molecular diffusion only. The saddle shape on top of the intensity profiles at \(x^* = 0\), is the result of the different diffusion coefficients of fluorescent dye and magnetic nanoparticles.\(^{38}\) The smaller dye molecules, having a larger diffusion coefficient, migrate more into the sheath streams of DI-water, while
magnetic nanoparticles with a diffusion coefficient of $D = 4.29 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, three orders of magnitude smaller than that of fluorescent dye do not diffuse significantly. As a result, the centre of the core stream appears darker than its border.

A further interesting phenomenon could be observed from the intensity profiles in Fig. 6. As the maximum intensity is expected at the channel inlet, where species in the core stream have not diffused into the sheath streams. However, at $x^* = 16$ (medium flow rate) and $x^* = 16, 23$ (high flow rate), the intensity peak exceeds the maximum intensity of the inlet, and moves away from the magnets. This phenomenon could be explained by the migration of the dark iron oxide nanoparticles toward the magnets due to positive magnetophoresis. The migration dilutes the ferrofluid at the positions in the proximity of the interface between the core and the sheath streams. Consequently, the remaining fluorescent dye appears to be brighter without the light-blocking magnetic nanoparticles.

At positions near the outlet, two intensity peaks can be observed. The lower one at $y^* > 0$ represents a more concentrated fluorescein line at the interface. The difference between diffusion coefficients of fluorescent dye and magnetic nanoparticles at the inlet and the absence of blocking magnetic nanoparticles created this bright line. As more magnetic nanoparticles moved toward the permanent magnet in downstream locations ($x^* = 16, 23, 30$), this peak appears to move to the left and approaches $y^* = 0$.

The second peak on the left side ($y^* < 0$) is higher compared to the right peaks. This peak appears next to the wall on the side of the magnets. As mentioned above, fluorescent dye is transported toward the magnet by magnetoconvection secondary flow and accumulates on the channel wall. The spread of fluorescent dye on the left side ($y^* < 0$) confirms that magnetoconvection transport induced by the magnetic field is the dominant mass transfer mechanism in this flow configuration. Fluorescent dye does not spread to the right side ($y^* > 0$), due to the insignificant molecular diffusion relative to the pressure-driven convective transport.

We further evaluated the spreading width of the core stream. Fig. 7(a) describes how the spreading width is determined from

![Fig. 6 Normalized intensity versus $y^*$ axis for six $x^*$ positions along the channel ($x^* = 0$: blue, $x^* = 16$: green, $x^* = 23$: red, $x^* = 30$: cyan, $x^* = 40$: purple) and three flow different flow rates: (a) 30-2.5-30; (b) 60-5-60; (c) 120-10-120.](image)

![Fig. 7 Evaluation of the width of the core stream: (a) the width is evaluated as the distance between the gradient peak to the centreline ($y^* = 0$); (b) spreading width at $y^* > 0$ along the channel; (c) spreading width at $y^* < 0$ along the channel. Low flow rate: red, medium flow rate: blue, high flow rate: green.](image)
the maximum intensity gradients at the interface between core and sheath streams. Fig. 7(b) plots the width $a$ at $y^* > 0$ along the channel length $x^*$. Fig. 7(c) shows the width $b$ at $y^* < 0$ along the channel length $x^*$. Fig. 7(c) indicates that at the lower flow rate the spreading width for the left part ($y^* < 0$) quickly reaches the value of the half width $W/2$. As the flow rate increases, the spreading width $b$ decreases, as the pressure-driven hydrodynamic flow dominates over the magnetoconvective flow.

5. Conclusions

We used a flow focusing system with three streams to investigate the mass transport of fluorescein dye in a microchannel using diluted ferrofluid and an external non-uniform magnetic field. The sheath streams were non-magnetic DI water, while the core stream was diluted ferrofluid. Fluorescein dye was dissolved in the core stream to trace the mass transport of the secondary magnetoconvective flow. Various phenomena were involved in the transport process of the fluorescent dye: magnetoconvective secondary flow due to the magnetic susceptibility difference, molecular diffusion, and pressure-driven hydrodynamic flow. The susceptibility gradient of the fluid together with the magnetic field of the permanent magnet causes a bulk force acting on the fluid and consequently the magnetoconvective flow. As shown in our experiments, the transport process of fluorescent dye caused by magnetoconvective secondary flow is comparable to that of the pressure-driven hydrodynamic flow and significantly higher than molecular diffusion. The significance of the observed phenomenon is that transport of a non-magnetic sample transversal to the main pressure-driven flow could be improved by orders of magnitude using permanent magnets and diluted ferrofluid. The observed phenomenon has potential applications in designing more efficient micro-mixers or gradient generators.

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Notes and references

CHAPTER 6 MAGNETOF LIQUIDIC CONCENTRATION AND SEPARATION OF NON-MAGNETIC PARTICLES USING TWO MAGNET ARRAYS

*Biomicrofluidics, 10 (2016) 044103. DOI: 10.1063/1.4955421*

Chapter 3, 4 and 5 demonstrate the ability of magnetofluidics for transporting particles with different sizes ranging from fluorescent dye molecules to larger microparticles. The observed effects are a combination of transport phenomena such as pressure driven flow, magnetophoresis and magnetoconvection. If these phenomena are engineered in a way that their effects are cancelling each other, trapping and concentrating of particles are possible. This chapter demonstrates a particle sorting platform technology based on magnetofluidics. This device allows for the direct observation of the competition between the dominant transport phenomena: magnetoconvection, negative-magnetophoresis, and hydrodynamics. Depending on the flow rate and concentration of the diluted ferrofluid, non-magnetic microparticles could be captured in different zones. This experiment also demonstrates the significance of magnetoconvective force in particle separation. The results provide readers a good insight into the interactions between main forces and their effects on the separation performance.
CHAPTER 6

Statement of Contribution to Co-authored Published Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored published paper, including all authors, are:

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My contribution to the published paper involved:

- Literature review
- The experiments
- Data analysis, discussion and preparation of figures
- Manuscript preparation

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CHAPTER 6

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Magnetofluidic concentration and separation of non-magnetic particles using two magnet arrays

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The present paper reports the use of diluted ferrofluid and two arrays of permanent magnets for the size-selective concentration of non-magnetic particles. The micro magnetofluidic device consists of a straight channels sandwiched between two arrays of permanent magnets. The permanent magnets create multiple capture zones with minimum magnetic field strength along the channel. The complex interaction between magnetic forces and hydrodynamic force allows the device to operate in different regimes suitable for concentration of non-magnetic particles with small difference in size. Our experimental results show that non-magnetic particles with diameters of 3.1 µm and 4.8 µm can be discriminated and separated with this method. The results from this study could be used as a guide for the design of size-sensitive separation devices for particle and cell based on negative magnetophoresis.

I. INTRODUCTION

Magnetofluidics is a research area that utilises the phenomena caused by magnetism and fluid flow leading to novel applications in the microscale. External magnetic field has been widely used for continuous separation of cells and particles based on their size or magnetic susceptibility. The capability of contactless manipulation of species inside a microchannel is the remarkable advantage of micro magnetofluidics—the combination of magnetism and microfluidics. Pamme systematically reviewed the exploitation of magnetic force in the field of microfluidics including various functions such as trapping, transport, separation, and mixing. Sorting of cells and particles using magnetofluidics could be achieved by trapping, focusing, or deflection. A variety of methods have been reported in the literature on the separation by deflection and concentration by trapping of cells and particles, taking advantage of magnetic force.

Separation and sorting of cells and particles by deflection have been explored extensively. There are two basic concepts for separation of particles and cells using magnetic field: positive and negative magnetophoresis. Positive magnetophoresis is the migration of particles with a magnetic susceptibility higher than that of the surrounding fluid. With positive magnetophoresis, the particles are attracted towards regions with higher magnetic field strength. On the other hand, negative magnetophoresis occurs when magnetic susceptibility of the surrounding fluid is higher than that of the particles. With negative magnetophoresis, the particles move to regions with lower magnetic field strength.

Pamme and Wilhelm reported the use of positive magnetophoresis for continuous sorting of mouse macrophages and human ovarian cancer cells (HeLa cells). Magnetic nanoparticles were used to label the cells. The extent of deflection and consequently the effectiveness of separation depend on the size of the cells and the flow rate. Khashan et al. applied computational fluid dynamics (CFD) to simulate the positive magnetophoretic separation of magnetically labelled cells in a microchannel. Chung et al. used the so-called magnetic tweezers to separate magnetic microparticles with positive magnetophoresis technique in a microfluidic device. At a
relatively low flow rate, the method can achieve a separation rate as high as 81%. Combining with optical tweezers, magnetic tweezers may yield an even higher separation rate for a broader range of flow rate. Lateral separation of superparamagnetic beads and labelled breast adenocarcinoma MCF-7 cells, utilizing positive magnetophoresis were achieved using an angled permanent magnet. A numerical model was formulated to predict the separation phenomenon. Liang et al. employed dilute ferrofluid as the carrier fluid in order to simultaneously use positive and negative magnetophoresis to further improve the separation of magnetic and non-magnetic particles. An analytical model was employed to predict the deflection of particles in both ferrofluid and water.

Gelszinnis et al. reported a magnetic composite material, i-PDMS, to capture and separate paramagnetic beads with positive magnetophoresis technique. The composite generates high local magnetic field gradients when placed between two permanent magnets. The effects of the resolution of the microstructures and the flow rate on the trapping and deflection of the particles were investigated. Zhu et al. employed ferrofluid to separate a mixture of particles with different magnetic properties using a combination of positive and negative magnetophoresis. This separation method is label-free and relies only on intrinsic properties of the cells or particles. Kim and Kim numerically investigated the positive magnetophoretic separation of malaria-infected red blood cells from whole blood. Malaria-infected was considered as paramagnetic particles suspended in a Newtonian fluid in their model. The design parameters such as magnetic pole array pitch, channel height, channel length, and the flow rate were optimized to achieve the maximum capturing efficiency. Recently, Wu et al. numerically modelled positive magnetophoretic separation of malaria-infected red blood cells (RBCs) in a two-dimensional parallel-plate system using Eulerian–Lagrangian approach. The blood plasma was considered as a Newtonian fluid, while the RBCs were modelled as soft paramagnetic spheres affected by drag and magnetic forces, as well as their collisions. Using a constriction of 50-μm high and a 2-mm long diffuser could improve the overall separation efficiency. Zhang et al. designed a microfluidic device using 3D printing technology. Efficient and rapid extraction of spiked Human Papillomavirus (HPV) 18 plasmids was achieved. In addition, a kinetics model was developed for adsorption of nucleic acids on cellulose functionalized superparamagnetic beads using positive magnetophoresis. Scherr et al. investigated protein capture using positive magnetophoresis and compared the performance of one and two magnets configurations. The two-magnet configuration demonstrated a higher efficiency and more rapid extraction of target biomarkers compared to the one magnet system. Wang et al. developed an automated and low-cost PMMA-tape-glass microfluidic system. The device prepares single-stranded DNA for magnetic bead-based microarray analysis using two on-chip mixing valves, a magnet for the manipulation of magnetic beads with positive magnetophoresis.

Magnetic levitation can enable label-free separation and monitoring of different cell populations based on their magnetic and density signatures. Knowlton et al. used a pair of magnets with the same poles facing each other aligns the magnetic field with the gravitational field, allowing diamagnetic particles or cells suspended in a paramagnetic fluid to be focused and separated. Calibrating the levitation heights may estimate the densities of the particles precisely. Furthermore, the authors used a smartphone platform for label-free and sensitive detection sickle cells. The separation concept takes advantage of the higher density of sickle red blood cells under deoxygenated conditions. Tasoglu et al. reported label-free separation and monitoring of different cell types utilizing magnetic levitation. The system utilizes a pair of magnets with same poles facing each other to focus the cells. Circulating cancer cells, sickled RBCs, and healthy RBCs suspended in gadolinium-based paramagnetic medium were tested. Rapid spatial separation of the different cell populations was achieved. Trapping and concentrating of cells and particles has potential applications in chemical and biological studies. Ahn et al. reported one of the earliest microfluidic devices for the manipulation of magnetic particles in a dilute suspension. Integrated electromagnets induce a magnetic field that traps magnetic particles in the flow without physical barriers. The trapped particles were released after removing the magnetic field by turning off the electromagnet. Gooneratne et al. reported a three-dimensional magnetic trap for non-magnetic particles such as living cells.
in a paramagnetic solution. The concentration of paramagnetic salt was maintained at a low level to keep the cells viable. Because of the relatively low field strength, field gradient, and susceptibility mismatch, the device can only trap particles larger than 5 μm in diameter. Ramadan and Gijs reported a continuous-flow microfluidic device with rotating magnetic field for trapping and releasing of magnetic particles. The rotating permanent magnets periodically arranged the magnetic field for periodic trapping and releasing of particles, allowing continuous concentrating, washing, and purifying of magnetic particles in the device.

Teste et al. reported a lab-on-a-chip device with a magnetic chamber packed with ferromagnetic iron beads to trap 30-nm magnetic nanoparticles. The iron beads increase the local magnetic field gradient and thus improve the trapping efficiency of the magnetic nanoparticles. Watarai and Namba investigated the impact of a non-uniform magnetic field on non-magnetic micro particles suspended in a paramagnetic manganese(II) chloride solution. They observed that when the particles approach the pair of magnets, the direction and the magnitude of their velocity change significantly. The magnetophoretic buoyancy exerted on the particles creates a magnetophoretic force in the direction of decreasing magnetic field strength. The device could successfully concentrate and separate non-magnetic microparticles suspended in ferrofluid under a nonuniform magnetic field. Zeng et al. demonstrated the concentration of polystyrene particles and live yeast cells from a ferrofluid solution using negative magnetophoresis. A pair of attracting magnets creates a non-uniform field. Peyman et al. studied the possible applications that could be achieved based on the negative magnetophoresis. Trapping, focusing, and deflection of polystyrene particles were demonstrated by changing the arrangement of magnets along the microfluidic device. Zhou et al. examined simultaneous trapping of diamagnetic and magnetic particles in different locations of a microfluidic device. A T-shaped microfluidic device with a single permanent magnet was used to concentrate both particles from a ferrofluid flow. Tarn et al. reported simultaneous trapping of magnetic and non-magnetic particles with a pair of permanent magnets. In an aqueous solution of manganese (ii) chloride, magnetic particles were trapped between the magnets, while non-magnetic particles were trapped outside this zone. Eisen et al. reported a model of a periodic array of magnetised cylinders for high-gradient magnetic separation of particles.

We previously demonstrated the effect of bulk magnetic force on diluted ferrofluids. We showed experimentally that a body force caused by susceptibility mismatch and magnetic field gradient can compete with the hydrodynamic drag force of the flow and the negative-magnetophoretic force in the transport process of suspended non-magnetic particles. The present work systematically studies the relative roles of these forces to identify the dominating force in a wide range of operation conditions. The dominance of each force leads to a trapping regime that potentially leads to the separation and concentration applications. For this purpose, a magnetofluidic device was fabricated. Two arrays of attracting magnets are placed next to the straight microchannel. The device was tested for a range of ferrofluid concentrations and flow rates to assess the competition between the magnetically induced phenomena and hydrodynamic drag. Our device was able to separate non-magnetic polystyrene (PS) particles with a size difference of only 1.6 μm. Required susceptibility mismatch could be created by adjusting the concentration of the diluted ferrofluid for different purposes for different sizes of the particle. Furthermore, the results from this study could be applied in designing a separation system based on deflection, since we report the effect of balance between dominant forces on particle
manipulation. Considering the fact that the device works based on the negative magnetophoresis effect, trapping of both magnetic and non-magnetic particles is possible. The experimental results form an operation map to guide future research in this area and could lead to a variety of applications such as cell separation, concentration, and sample preparation.

II. MATERIALS AND METHODS

Figure 1 shows the schematic of the channel and the magnet arrays used in our experiments. The straight channel has a width of \( W = 1 \) mm and a height of \( H = 500 \mu m \). We used a laser engraving machine (Trotec/Rayjet) to cut the channel through a clear 500-\( \mu m \) thick double-sided adhesive tape. The channel was then sandwiched between a glass slide and a poly(methyl methacrylate) PMMA slide. The slots for the magnets were cut through the PMMA slide before bonding to the tape. Neodymium–iron–boron (NdFeB) permanent magnet cubes with a volume of 3.2 mm\(^3\) and grade of N42 (B222, K&J Magnetics, Inc.) were placed in two arrays on both sides of the channel, with 11 magnets on each side. The magnetic field magnitude of the individual permanent magnet versus distance from the magnet was measured and reported previously.\(^{34}\) A precision syringe pump (SPM100, SMTech Microfluidics Foundry) delivers the fluid into the channel. The flow rate in the later experiments ranges from 1 to 400 \( \mu l/min \). A charge-coupled device (CCD) camera (Edmund Optics, Germany) attached to an inverted microscope (Nikon Eclipse TE100) and connected to a desktop computer was used for visualization and image recording. Diluted water-based ferrofluid (EMG707, Ferrotec) was used as the paramagnetic liquid in the experiments. The commercial ferrofluid has an initial magnetic susceptibility of 0.12, a magnetic particle concentration of 2% vol., and a saturation magnetization of 110 G. The ferrofluid was further diluted by Deionized Water (DI-water) into eight different concentrations: 0.25, 0.5, 0.75, 1, 2, 5, 20, and 50% vol. Fluorescent polystyrene (PS) particles (ThermoScientific, Inc.) with different sizes and colours (3.1 \( \mu m \), red) and (4.8 \( \mu m \), green) were suspended in the diluted ferrofluid samples.

III. THEORETICAL BACKGROUND

Three dominant forces are involved in manipulating non-magnetic particles in a paramagnetic liquid: negative magnetophoretic force caused by the magnetic field on the particles which moves the particles towards magnetic field minimum, hydrodynamic drag force caused by the fluid flow and in the direction of the flow, and magnetoconvective drag force. Magnetoconvective flow is the result of a bulk force induced by the magnetic field on a paramagnetic fluid, in the direction of magnetic field maximum. The competition between these forces determines whether the particles could be trapped at a given position or transported along a particular trajectory. The magnetic energy of a spherical magnetic particle exposed to a magnetic field is estimated as\(^{1}\)

\[
E_m = \frac{\pi}{6} d^3 MB, \tag{1}
\]

where \( E_m \) is the magnetic energy, \( M \) is the magnetization of the particle, \( d \) is the diameter of the particle, and \( B \) is the magnetic flux density. For suspended particles in a flow, frictional energy can be estimated as

\[
E_f = 3\pi\mu d^2 u, \tag{2}
\]
where $E_f$ is the frictional energy and $u$ is the velocity. We use the ratio between magnetic and frictional energies to evaluate the stability of the particles. For this purpose, the dimensionless characteristic number is magnetophoretic stability number

$$S_mp = \frac{E_m}{E_f} = \frac{1}{18} \frac{MBd}{\eta u}. \quad (3)$$

According to Equation (3), the magnetophoretic stability number is a function of microparticles diameter, magnetic susceptibility and viscosity of the ferrofluid. The value of magnetophoretic stability number is highly affected by ferrofluid concentration and flow rate. On the other hand, the intensity of magnetic field varies with distance from the magnets. Evaluating this number at capture zones and relating the values to the experimental data lead to an operation map, which can provide the required values of concentration and flow rate to achieve particle capture.

We used COMSOL (COMSOL, Inc., USA) to simulate the distribution of magnetic flux density created by the magnet array. Magnetic insulation is considered around the whole system. Figure 2(a) shows the typical simulated magnetic field in the microchannel between a pair of magnets. The simulation was carried out with water inside the channel, only to illustrate the distribution of the magnetic field. The simulation result indicates that the actual field maxima are located outside the channel, between the edges of the magnets. Within the channel, periodic traps of field maxima and minima are at the channel walls facing the magnets and between the magnet poles, respectively. The trapping behaviour in these locations will be investigated in detail.
IV. RESULTS AND DISCUSSION

Ferrofluid samples with various concentrations from highly diluted to highly concentrated content of magnetic nanoparticles were prepared. Two sizes of fluorescent PS particles with different colours were suspended in all ferrofluid samples. Each sample was tested with a wide range of flow rates. The different concentrations lead to different susceptibilities, which in turn tune the magnitudes of magnetophoretic and negative magnetophoretic forces. Also by varying the flow rates, the magnitude of hydrodynamic forces can also be adjusted. The images from the experiment were taken after a few minutes, when the system reaches a steady state condition.

A. The trapping phenomenon and concentration modes

Two arrays of 11 magnets create 10 simultaneous spherical plugs of trapped non-magnetic particles along the channel. At a given flow rate and ferrofluid concentration, large particles are trapped in the gap between the two neighbouring magnets. The small non-magnetic particles accumulate on the wall next to the magnet poles, Fig. 2(b). Thus, size-selective concentration of non-magnetic particles can be achieved. Large non-magnetic particles are repelled from the regions with a higher magnetic field, because of the significant negative magnetophoresis force. Consequently, large particles cannot enter the region between two attracting magnets. The negative magnetophoretic force is large enough to trap them at locations with a minimum field strength and form spherical particle plugs along the channels.25 The negative magnetophoretic force is dominant for larger particles. The magnitude of the stability numbers is evaluated and presented later for each condition.

As mentioned above, large particles are captured at locations of field minima. Each particle plug forms an internal recirculation, which is created by the equilibrium between the negative magnetophoretic force acting on the particles, the magnetoconvection force on the ferrofluid and the hydrodynamic drag force of the main flow. Particles that escape the internal circulations of each plug feed the next one downstream. The escaped particles are focused into a line at the centre of the channel until they reach the next location of field minimum. Particles circulate around the minimum field zone, until they become almost stationary and trapped at its centre, Fig. 2(a).

This trapping pattern continues with the formation of the 10th circular trapping zone where the remaining particles exit the channel in a focused line. Meanwhile, small non-magnetic particles accumulate on the walls near the magnet poles, along with paramagnetic nanoparticles of the ferrofluid. These particles form a butterfly shape on each channel side with maximum field, Fig. 2(a). Escaped small particles bypass the trapping regions and feed into their next trap at the magnet poles.

As mentioned above, small red micro-particles are captured on the magnets poles, mixed with a pile of Iron oxide nanoparticles. Being in a close proximity of the magnet poles where strong magnetoconvection force exists, the small red particle plugs are not affected significantly by increasing flow rate. On the other hand, large green particles form clear circular capture zones and are very sensitive to the change of the flow rate. As a result, the green particle plugs are better representatives of the quality of particle capture and sorting in the system.

B. Effect of flow rate

We used a wide range of flow rates to observe the trapping behaviour with each ferrofluid concentration. An optimum flow rate exists for each concentration where a maximum number of particles were captured. At a given trapping zone and for a fixed concentration of 1% vol., a relatively low flow rate of 5 µl/min allows the large particles to slowly accumulate at the field minimum trap. The particle plug grows until it reaches a steady state condition and the diameter of the spherical plug becomes constant. This behaviour occurs as the result of the balance between negative magnetophoretic, magnetoconvection, and hydrodynamic forces. Smaller particles accumulated as expected on the walls next to the magnet poles.
As the flow rate increases to 10 $\mu$l/min, we observe better trapping of the large particles. More particles are trapped as more particles are supplied with the higher flow rate. As a result, a larger particle plug is formed in the steady state condition.

As the flow rate increases significantly up to 50 and 100 $\mu$l/min, the number of captured particles reduces and the size of the plug becomes smaller. A large flow of recirculation with two major vortexes next to the plug was observed at higher flow rates. Large particles cannot reach the force equilibrium, while fewer particles become stationary. Most particles escape the field-minimum trap and exit the channel, as the strong hydrodynamic force is dominant. This experiment indicates that there is an optimal flow rate for which the number of captured particles for size based concentration reaches its maximum. Figure 3 shows the relationship between the optimal flow rate and the ferrofluid concentration. The two particle types of our experiment could be separated with these optimal flow rates.

C. Effect of ferrofluid concentration

We prepared diluted ferrofluids with eight different concentrations ranging from 0.25% to 50% vol. Figure 4 summarises the effect of concentration on particle trapping for three different flow rates. Three significant forces are involved in the trapping phenomenon: two drag forces and the negative magnetophoretic force. The drag forces are caused by the primary hydrodynamic flow and the secondary magnetoconvective flow, which is the magnetic body force exerted on the ferrofluid. The balance between the three forces determines whether a particle with a given size could be separated and collected at the field minimum traps or at the field maximum traps. Furthermore, the balance between the forces can explain the shape and size of the trapping zones. Fig. 4 shows that highly diluted ferrofluids such as 0.25% vol. sample only forms particle plugs at lower flow rates. At higher flow rates, the negative magnetophoretic force is not strong enough to compete with the hydrodynamic drag force to keep the particles stationary. At high flow rates, the resultant force keeps the particles moving in a circle at the trapping zone. Another interesting phenomenon observed with the lowest ferrofluid concentration is that both small and large particles are captured at the field minimum zones. Some large particles are even captured at the field maximum zones next to the magnet poles. However, the number of large particles trapped at the field maximum zones decreases significantly with increasing ferrofluid concentration.

The above observation proves that the concentration of ferrofluid should be high enough to yield a negative magnetophoretic force strong enough to trap large particles. Therefore,
ferrofluid concentrations of less than 1% vol. are not suitable for capturing 4.8-μm particles, while concentrations lower than 1% vol. are appropriate for trapping the 3.2-μm particles.

The reason for large particles being trapped on the walls next to the magnet poles could be the magnetoconvective flow at the field maximum zone. A low ferrofluid concentration leads to a smaller magnetic susceptibility of the fluid and a weaker magnetoconvective force elsewhere in the flow. However, magnetic nanoparticles accumulate at the field maximum zones creating both high magnetic susceptibility and susceptibility gradient and consequently a strong local magnetoconvective secondary flow. The large particles are dragged by the flow and enter the regions with maximum magnetic field. The negative magnetophoretic force is weaker than the drag force caused by the local magnetoconvective flow preventing them from escaping the maximum-field zone. As a result, some of the large particles are trapped at the field maximum zone. On the other hand, negative magnetophoretic force is dominant for small particles at low concentration allowing them to be trapped at field minimum zones.

At a concentration between 1% and 20% vol., large particles are captured at the field minimum zones, while smaller particles are trapped at field maximum zones. Size-selective concentration can be achieved in this regime, as particles of different sizes were simultaneously captured at separate zones. The reason for trapping small particles at field maximum zones with a high ferrofluid concentration is the dominant magnetoconvective secondary flow in these zones. Negative magnetophoretic force acting on large particles is dominant at field minimum zones. As the concentration reaches 50%, both particles are captured at field minimum zones at a rapid rate. The high magnetic susceptibility results in a powerful negative magnetophoretic force. Accumulation of particles at the field maximum zones was not observed.

Figure 4 shows another interesting phenomenon of different shapes and sizes of trapping zones under the various sets of flow rate and ferrofluid concentration. The balance between magnetoconvective force and hydrodynamic force determines the size of the trapping zones. Magnetoconvective force expands the circular zone, while the hydrodynamic force compresses it. Consequently, a higher flow rate leads to a smaller zone. On the other hand, the hydrodynamic force also competes with the negative magnetophoretic force. Increasing flow rate leads to more particles circulating around the trapping zones and less stationary particles. Another noticeable phenomenon is the shape of a hollow plug under certain conditions. The empty
region at the centre of the circular plugs is the result of the competition between magnetocon-
vective force that moves particles out of the zone, and negative magnetophoretic and hydrody-
namic forces that push the particles into the centre of the trapping zone.

**D. Trapping zones**

As mentioned above, the magnet arrays create 10 field minimum zones along the channel of our device. In order to compare the different trapping zones, we fixed the ferrofluid concentration and the flow rate at 2% vol. and 20 µl/min, respectively. Figure 5 shows the images of the captured green 4.8-µm particles in the 10 zones along the channel. The flow is from right to left. The zones closer to the inlet can trap more particles. Further downstream, both magnetic nanoparticles of the ferrofluid and non-magnetic particles are depleted. Lower concentration of magnetic nanoparticles downstream lead to weaker magnetophoretic and magnetoconvective forces making the capture zone appears to be larger. Fewer non-magnetic particles make the capture zone looks hollow.

![Images of trapping zones](image-url)

**FIG. 5.** All trapping zones (flow is from right to left, 1: close to the inlet, 10 close to the outlet) at 2% vol. and 20 µl/min.
E. The operation map

We plot different trapping and separation regimes on an operation map with the Reynolds number and the magnetophoretic stability number as the two dimensions of the space. The Reynolds number is the relative ratio between inertial force and friction force of the flow and represents the strength of the hydrodynamic force on the particles. The magnetophoretic stability number was defined in Equation (3) as the ratio between magnetic and frictional energies representing the strength of magnetophoretic force. The present dimensions do not include the magnetoconvective effect.

Figure 6 shows the $S_{mp}$-Re space as the operation map of our device. In our present work, trapping of both particle sizes happens in the range of $40 < S_{mp} < 400$. For $S_{mp} < 40$ and $Re < 1$, particles are not captured but circulate around the trapping zone. For $S_{mp} < 40$ and $Re > 1$, magnetic force no longer has any impact on the particles. Particle sorting by hydrodynamic force and inertial force is feasible in this region and the operation moves into the regime of inertial microfluidics. For $S_{mp} > 400$ and $0.1 < Re < 10$, negative magnetophoretic and magnetoconvective forces are dominant. This region is suitable for size-selective concentration of particles. The size of non-magnetic particles should be considered to find the appropriate flow rate to achieve separation, as there is a competition between negative magnetophoretic and magnetoconvective forces in this region. More diluted ferrofluids and lower flow rates are needed for the separation of small particles. A higher concentration of ferrofluids would trap small particles at field-maximum zones close to the magnet. On the other hand, more concentrated ferrofluid and a high flow rate are appropriate for separating large particles.

V. CONCLUSIONS

We designed, fabricated, and characterised a magnetofluidic device for highly size-selective particle trapping and concentration. The device consists of two arrays of attracting magnets, which exert a non-uniform magnetic field on the ferrofluid flow inside the channel. We examined the effect of concentration and flow rate on the quality of particle trapping. Various concentrations of diluted ferrofluid were tested. The behaviour of the trapped particles is determined by three force types: negative magnetophoretic, magnetoconvective, and hydrodynamic forces. The impact of their competition was analysed and discussed. The balance between these forces determines the shape and size of the trapping zones, as well as the feasibility of particle separation. We used the magnetophoretic stability number and Reynolds number to describe the effect of the force balance quantitatively. The magnitude of these numbers determines the regimes of size-selective separation by particle trapping, particle deflection, or no separation. Furthermore, the operation map from this study can also be used to extend the study and predict the possibility of capture and sorting particles with different sizes. The key
significance of the presented method is the capability of separating non-magnetic particles with a relatively high size-selectivity. The results presented here could be used as a guide for designing devices for separation of non-magnetic particles such as cells with small size difference using diluted ferrofluid.

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Besides trapping, concentrating and separation of particles as demonstrated in Chapter 6, mixing is also an important task in a microfluidic system. This chapter presents the use of magnetoconvectional force in mixing, one of the most common tasks of microfluidics. The micromixer works based on the concept investigated and discussed in Chapter 5. The results show the potential of the concept for designing and implementing a low-cost and efficient micromixer.
CHAPTER 7

Statement of Contribution to Co-authored Published Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored paper, including all authors, are:


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My contribution to the paper involved:

- Literature review
- The experiments
- Data analysis, discussion and preparation of figures
- Manuscript preparation

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A Rapid Magnetofluidic Micromixer Using Diluted Ferrofluid

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Abstract: Effective and rapid mixing is essential for various chemical and biological assays. The present work describes a simple and low-cost micromixer based on magnetofluidic actuation. The device takes advantage of magnetoconvective secondary flow, a bulk flow induced by an external magnetic field, for mixing. A superparamagnetic stream of diluted ferrofluid and a non-magnetic stream are introduced to a straight microchannel. A permanent magnet placed next to the microchannel induced a non-uniform magnetic field. The magnetic field gradient and the mismatch in magnetic susceptibility between the two streams create a body force, which leads to rapid and efficient mixing. The micromixer reported here could achieve a high throughput and a high mixing efficiency of 88% in a relatively short microchannel.

Keywords: magnetic; microfluidics; mixing; magnetoconvection; ferrofluid

1. Introduction

Rapid mixing in microfluidic devices is an important task for material synthesis, chemical/biochemical analysis, and cooling [1]. Micromixers have a broad range of applications such as for reactors [2–4], lab on a chip for chemical engineering [5], enhancement of chemical selectivity [6], extraction processes [7], drug discovery [8], polymer synthesis [9], and DNA amplification [10].

The challenge in designing micromixers is to achieve fast and efficient mixing within a short residence time or in a short microchannel. Depending on their operation mechanism, micromixers are categorized as passive or active. Passive mixers are easy to fabricate due to the fact that they do not need an external energy supply. Passive micromixers include lamination [11,12], chaotic advection, injection, or droplet mixers [13]. Faster and more effective mixing can be achieved using active micromixers, which require an external energy source and a more complex fabrication process. For stirring or disturbing the fluid flow, active concepts such as acoustic [14], dielectrophoretic, electrokinetic, pressure perturbation, electro-hydrodynamic, magnetic, and thermal actuations have been used in active micromixers. A number of excellent review papers were devoted to the classification and application of micromixers [15–19].

Fluid flow in a microfluidic device can be manipulated using magnetic forces [20]. Inducing a magnetic force is wireless and has the advantage of providing an environment for cell viability in biological studies [21]. Several methods have been described which use magnetic forces for mixing on a microscale: micro magnetic stirrers, magnetophoresis of magnetic particles, magnetohydrodynamics (MHD), and micro magnetofluidics [22]. Chaotic mixing of magnetic beads in a biological fluid was achieved using induced magnetophoresis [23]. The micromixer consists of micro conductors embedded in a microchannel. The chaotic regime was verified by the numerical analysis of particle trajectories. The driving frequency and the residence time could be adjusted to obtain effective mixing. Zolgharni et al. investigated chaotic mixing of magnetic particles in a biological fluid using the
Lagrangian tracking method [24]. The efficiency of capturing the target cells with magnetic particles was evaluated. Numerical simulation allows for the optimization of the operating conditions for the mixer. Bau et al. reported theoretical and experimental studies on a MHD-based micromixer [25]. An array of electrodes in the presence of a magnetic field induces a body force in the fluid, which generates a complex flow field. The concept deforms and stretches fluid interfaces to enhance mixing.

Rida and Gijs introduced a novel method for mixing based on the manipulation of self-assembled magnetic microbead structures held in place by a magnetic field [26]. Soft ferromagnetic structures were integrated in a Y-shaped microchannel to create local magnetic fields. Efficient mixing could be achieved as a result of the strong particle-liquid interaction. A mixing efficiency of 95% within a length of 400 μm was achieved. Adjusting the magnetic field and the liquid flow rate optimizes the mixing process. Wang et al. numerically investigated a magnetic particle driven micromixer [27]. The effect of parameters such as magnetic actuation force, switching frequency, and dimensions of the microchannels were studied. Based on the numerical results, the maximum efficiency occurs at a relatively high operating frequency for large magnetic actuation forces and a narrow microchannel.

With manipulation of an entire ferrofluid stream using a magnetic field, Mao and Koser achieved rapid mixing in a microchannel using magnetofluidic actuation [28]. Embedded electrodes carrying traveling magnetic waves were used to create a local magnetic field. Mixing of water-based ferrofluids with a fluorescein buffer solution demonstrates a significant enhancement in mass transport as compared with that of pure molecular diffusion. An alternate-current (AC) electromagnetic field can induce transient flows between a ferrofluid and a fluorescence dye solution, allowing for a simple and efficient mixing concept [29]. The magnetic field causes significant and uniform expansion of the ferrofluid toward the dye, creating extremely fine fingering structures at the interface. High mixing efficiency of up to 95% was achieved within 2 s and at a distance of 3 mm from the inlet of the microchannel. Numerical simulation of the phenomenon was reported in a separate work [30]. The results from the simulation demonstrate that the magnetic body force significantly affects mass transport of the ferrofluid.

Zhu and Nguyen reported the mixing phenomenon and the effect of a uniform magnetic field on a superparamagnetic ferrofluid [31]. A mixture of deionized (DI) water, glycerol and water-based ferrofluid were introduced into a circular microchamber. As a result of magnetic susceptibility mismatch under a uniform magnetic field, instability at the interface was observed leading to rapid mixing. The effect of parameters such as magnetic flux density, flow rate ratio, and viscosity ratio on the mixing efficiency was investigated. Kitenbergs et al. examined the mixing process of a superparamagnetic ferrofluid stream with and the diamagnetic water stream under a homogeneous magnetic field in a Hele-Shaw cell [32]. Mixing enhancement through magnetoconvective transport was demonstrated.

Using fluorescent dye, the extent of mass transport through magnetofluidic phenomena can be investigated [33]. We demonstrated in our previous work [34] the expansion of a ferrofluid stream containing non-magnetic particles under the effect of the magnetic body force created by a uniform magnetic field. The difference in magnetic susceptibility created a strong enough body force to carry along the diamagnetic micro particles in the same direction as the magnetic nanoparticles. We further demonstrated that without a susceptibility mismatch between the streams and only utilizing a nonuniform magnetic field, diamagnetic microparticles in a ferrofluid can be deflected [35]. Recently, we utilized a simple hydrodynamic focusing system to investigate mixing of ferrofluid and water [36]. The core stream was ferrofluid mixed with diamagnetic fluorescent dye. We demonstrated the spreading of the core stream under a non-uniform field of permanent magnets. Compared to passive diffusion, significant improvement in mass transfer was observed.

In the present paper, we propose a simple, efficient, and low-cost micromixer. We examined the mixing of a ferrofluid stream into a water stream in a non-uniform magnetic field at different flow rates and concentrations. Furthermore, we evaluated the mixing efficiency using the recorded images of a fluorescent dye. This simple device has the advantage of exploiting both susceptibility mismatch and
non-uniform magnetic fields, resulting in a strong mixing effect. On the other hand, using low-cost permanent magnets allows for the simple design and fabrication of the device. Our mixer could be integrated as a part of a lab on a chip system for chemical or biological applications.

2. Materials and Methods

Figure 1a depicts a schematic of the micromixer. The micromixer is a straight rectangular channel with two inlets and one outlet. The microchannel has a depth of $H = 50 \, \mu m$, a width of $W = 500 \, \mu m$, and a length of $L = 12 \, mm$. The device was fabricated out of polydimethylsiloxane (PDMS) using the standard soft lithography technique, which was described briefly in our previous work [36]. Two precision syringe pumps (SPM100, SIMTech Microfluidics Foundry, Singapore) were used to feed the fluids into the microchannel. The microfluidic device was placed on an inverted microscope (Nikon Eclipse TS 100, Nikon Corporation, Tokyo, Japan) equipped with a high-speed camera (Photon 120K-M2, Photron, Tokyo, Japan) for visualization. Deionized (DI) water was fed into the microchannel as the diamagnetic stream. The commercial water based ferrofluid (EMG707, Ferrotec, Santa Clara, CA, USA), containing 2% volume concentration ($\phi = 2\%$ vol.) magnetic nanoparticles, was used to make a diluted superparamagnetic solution for the second stream. Nominal nanoparticle diameter was 10 nm. Initial magnetic susceptibility of the commercial ferrofluid was 1.51 (International System of Units (SI)). For visualization purposes, 0.05 g of Fluorescein sodium salt (acid yellow, Sigma-Aldrich Co., St. Louis, MI, USA) was dissolved in 20 mL of DI water. The commercial ferrofluid was then diluted with the fluorescent dye solution to make $\phi = 2\%$ and $\phi = 20\%$ vol. ferrofluid concentrations. Three identical 3.2 mm$^3$ neodymium–iron–boron (NdFeB) permanent magnets (B222, K&J Magnetics Inc., Pipersville, PA, USA) were used to create the non-uniform magnetic field for the mixing experiments. A slot was cut in the PDMS to embed the magnets into the device. The position of this slot was approximately in the middle of the microfluidic channel. For this experiment, the magnets were positioned in a way that allowed us to observe the following: the secondary flow, the magnetic stream approaching the upper wall, and mixing of the streams before and after the magnets. The experiments were carried out with the constant flow ratio of 1, for a wide range of flow rates from 2 to 300 $\mu L/min$ and two concentrations of the ferrofluid (2, 20% vol.). The magnetic field of the permanent magnets was measured as a function of distance and calibrated using a Gauss meter (Hirst Magnetic Instruments Ltd., Falmouth, UK) and reported in our previous work [36]. The distance between the magnets to the channel wall was 1 mm. Across the microchannel width, the magnetic flux density drops from approximately 250 mT to 175 mT.

![Figure 1](image.png)

**Figure 1.** Schematic of the experiment (a) Experimental setup ($x^* = x/W$, $y^* = y/W$); (b) Deflection of superparamagnetic ferrofluid stream towards the magnets due to magnetoconvection, based on experimental observation.
3. Results and Discussion

The micromixer reported here takes advantage of the non-uniform magnetic field provided by permanent magnets placed next to the microchannel. Six images were taken at different locations along the channel: $x = 0, 4, 5.75, 7.5, 10$, and $12$ mm. The magnets were located between $x = 4$ mm to $x = 7.5$ mm (Figure 1a). We normalised the length scale by the channel width $W$ ($x^* = x/W$, $y^* = y/W$). The distance between the magnets to the upper wall of the channel ($y^* = -0.5$), and the ratio between flow rates of the two streams was held constant for all experiments. In order to investigate the effect of magnetic susceptibility on mixing efficiency, two diluted ferrofluid samples with the concentration of 2% and 20% vol. of the commercial solution were prepared. The stream next to the magnets was DI-water. Mixing of the two streams is only possible if the magnetic solution is introduced through the lower channel. This configuration is best to demonstrate the secondary flow perpendicular to the direction of main flow (Figure 1b) caused by the susceptibility mismatch between the streams. The magnetic stream travels half of the channel width to approach the permanent magnets.

We hypothesise that magnetic susceptibility difference between the two streams creates a magnetoconvective secondary flow [36,37] in the direction of the maximum of the magnetic field. Our aim is to take advantage of the strong, secondary, magnetoconvective flow to achieve efficient mixing of the two streams. For this purpose, different flow rates were tested for each concentration to observe the effect on mixing efficiency. In addition, an extra experiment on testing a wide range of flow rates while holding other parameters constant revealed the optimum operating conditions for our mixer. Diluted ferrofluid was mixed with fluorescent dye for tracing the ferrofluid stream and for evaluating mixing efficiency. The images from the experiment were taken a few minutes after changing the flow rate to make sure that the system has reached a steady state condition.

3.1. Fluorescent Signal and Qualitative Analysis

We analysed the fluorescent intensity $I$ to investigate the effect of flow rate and concentration on the quality of mixing and to demonstrate the phenomenon. The strength of the fluorescent signal is representative for the concentration of the dye. For this purpose, we use normalized intensity [33]:

$$I^* = \frac{I - I_{\text{min}}}{I_{\text{max}} - I_{\text{min}}}$$

The measured dimensionless intensity is assumed to be the same as the normalized concentration of the dye molecules $c^* (I^* = c^*)$. $I_{\text{max}}$ and $I_{\text{min}}$ were measured at the inlet ($x^* = 0$) where the intensity distribution of the fluorescent dye is not affected by the magnetic field. The measurement of the normalized intensity is made on a line across the channel width. The fluorescent signal is evaluated at six positions along the channel’s length ($x^* = 0, 8, 11.5, 15, 20, 24$). Figure 2 shows the normalised intensity distribution of all positions along the $y^*$ axis for three total flow rates of 2, 20, and 200 $\mu$L/min and for the lower ferrofluid concentration of $\varphi = 2\%$ vol. Superparamagnetic iron oxide nanoparticles deflect towards the magnets and create a mismatch in magnetic susceptibility and a secondary flow. As a result, the non-magnetic dye molecules are transported and follow the same path of the nanoparticles. Therefore, the mixing efficiency can be evaluated by analysing the fluorescent signal.

Figure 2 indicates that for all flow rates, the superparamagnetic stream at the inlet ($x^* = 0$) was not affected by the magnetic field, and the intensity profile suddenly drops from $I^* = 1$ to $I^* = 0$ at the interface of the two streams. The effect of the magnetic field on the intensity distribution becomes apparent as the fluid flow approaches the magnets at $x^* > 0$. For the lowest flow rate of 2 $\mu$L/min, the intensity profile becomes flat, Figure 2a. A flat intensity profile means a uniform concentration distribution across the width of the channel and complete mixing. As the flow rate increases to 20 $\mu$L/min, the hydrodynamic force acting in the flow direction becomes dominant and prevents mixing. As a consequence, the deflected superparamagnetic stream cannot reach the upper wall.
(\(y^* = -0.5\)). This effect is more apparent at the higher flow rate of 200 \(\mu\)L/min, where the effect of the magnetic field and mixing are almost negligible.

Another interesting phenomenon that can be observed from the intensity profiles is that the relative intensity values may exceed the value of 1 for the value of \(y^* > 0\). The higher intensity values are the result of magnetophoresis of the magnetic nanoparticles making the fluorescent superparamagnetic stream appear brighter. At the lowest flow rate of 2 \(\mu\)L/min, the magnetoconvective force is dominant and carries along the dye molecules until they reach the upper wall. If the flow rate increases, the hydrodynamic force outweighs the magnetoconvective force. Dye molecules, following the flow field, mostly travel in the direction of the pressure-driven main flow. On the other hand, superparamagnetic nanoparticles travel towards the interface due to the positive magnetophoresis effect. The ferrofluid becomes more diluted at \(y^* > 0\) as the flow approaches the outlet, and, consequently, the superparamagnetic stream becomes brighter. The effect is shown as subplots in Figure 2.

Figure 2. Normalized intensity across the normalised channel width \(y^*\) for six positions of \(x^*\) along the mixing channel with a ferrofluid concentration of 2\% vol. (\(x^* = 0\): blue, \(x^* = 8\): green, \(x^* = 11.5\): red, \(x^* = 15\): cyan, \(x^* = 22\): purple, \(x^* = 24\): black) and three flow different flow rates: (a) 2 \(\mu\)L/min; (b) 20 \(\mu\)L/min; (c) 200 \(\mu\)L/min.

Figure 3 shows the relative intensity profiles for the higher concentration of 20\% vol. At the lowest flow rate of 2 \(\mu\)L/min the residence time of the streams was longer, and the positive magnetophoresis effect on superparamagnetic nanoparticles was significant. Despite the fact that good mixing is
achieved at the outlet, concentration distribution is not uniform: The ferrofluid is more diluted next to $y^* = 0.5$ and more concentrated next to $y^* = -0.5$. More homogeneous mixing was achieved at the higher flow rate of 20 μL/min. The high flow rate and the high magnetic susceptibility mismatch between the two streams allow for fast mixing. At the highest flow rate of 200 μL/min, as a result of the stronger hydrodynamic force in the flow direction, the superparamagnetic stream cannot reach the upper wall. Compared to the lower ferrofluid concentration of $\varphi = 2\% \text{ vol.}$, the interface of $\varphi = 20\% \text{ vol.}$ is closer to the upper wall ($y^* = -0.5$). The images corresponding to the position of $x^* = 11.5$ in Figure 3 demonstrate the secondary flow on the permanent magnets. At a lower flow rate of 2 μL/min (Figure 3a) the magnetic stream reaches the upper wall, and nanoparticle agglomeration occurs. At a flow rate of 20 μL/min, nanoparticle agglomeration disappears due to the higher hydrodynamic force. For the higher flow rate of 200 μL/min, secondary flow is not strong enough to reach the upper wall.

![Figure 3](image)

**Figure 3.** Normalized intensity across the normalized channel width $y^*$ for six positions of $x^*$ along the mixing channel with a ferrofluid concentration of 20% vol. ($x^* = 0$: blue, $x^* = 8$: green, $x^* = 11.5$: red, $x^* = 15$: cyan, $x^* = 22$: purple, $x^* = 24$: black) and three different flow rates: (a) 2 μL/min; (b) 20 μL/min; (c) 200 μL/min.

### 3.2. Mixing Efficiency, Probability Distribution, and Qualitative Analysis

The homogeneity of the mixed fluid indicates good mixing. Therefore, the distribution of the intensity values of an image can be used for evaluating the degree of mixing. By normalizing the pixel number of each intensity value by the total number of the pixels in the evaluated region, the probability values can be obtained [38]:

$$ P(I) = \frac{N(I)}{\sum_{I=0}^{I_{max}} N(I)} $$
We used a customised MATLAB (MathWorks) code to evaluate the probability distribution from the grayscale intensity images. If two intensity peaks appear on the probability graph (probability distribution function versus normalized intensity or concentration), then it means no mixing. In contrast, the existence of a single intensity peak indicates full mixing. Furthermore, the standard deviation can be normalized by the mean concentration to obtain the mixing efficiency:

\[
\eta_{\text{mixing}} = 1 - \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left( \frac{I_i - \bar{I}}{\bar{I}} \right)^2}
\]  

(3)

where \( \eta_{\text{mixing}} \) is the mixing efficiency, \( I_i \) is the intensity value at a given position (pixel), and \( \bar{I} \) is the average of intensity values of the region of interest. The mixing efficiency varies from 0 (no mixing) to 1 (full mixing).

Effective magnetic permeability of diluted ferrofluid emulsions could be estimated by the Maxwell-Garnett formula [39]:

\[
\mu_{\text{ef}} = \mu_w + 3\mu_w \frac{\varphi \frac{\mu_p - \mu_w}{\mu_p + 2\mu_w}}{1 - \varphi \frac{\mu_p - \mu_w}{\mu_p + 2\mu_w}}
\]  

(4)

where \( \mu_w \) is the magnetic susceptibility of water, \( \mu_p \) is the magnetic susceptibility of iron oxide particles, and \( \mu_{\text{ef}} \) is the effective magnetic susceptibility of diluted ferrofluid.

The images from experiments were taken at the six positions along the microchannel as mentioned above. The customized MATLAB code evaluated the mixing efficiency and probability distribution graphs for each position. Figure 4a shows the results of the analysis for concentrations of \( \varphi = 2\% \) vol. and \( \varphi = 20\% \) vol. at a constant flow rate of 20 \( \mu \text{L/min} \). The results show the change of mixing efficiency from the inlet to the outlet. At a lower concentration of \( \varphi = 2\% \) vol., the mixing efficiency remains around a low value of 0.2. The probability function also shows agreement with the mixing efficiency, suggesting no mixing occurred along the microchannel. With a higher concentration of \( \varphi = 20\% \) vol., the mixing efficiency increases from about 0.3 at the inlet to around 0.8 at the outlet, indicating good mixing. The larger mismatch in magnetic susceptibility between the two streams and the high concentration of superparamagnetic nanoparticles created a strong secondary flow in the direction of the higher magnetic field. The magnetoconvective secondary flow causes a significant rise of mixing efficiency when the flow passes the permanent magnet region. The probability distribution graph also shows good mixing at the outlet for a higher concentration.

Figure 4b illustrates the effect of the flow rates (2, 20, 200 \( \mu \text{L/min} \)) at a constant concentration of 20\%. For the lowest flow rate of 2 \( \mu \text{L/min} \), a major drop of mixing efficiency can be observed at the location next to the magnets. The reason for this sudden decrease is the agglomeration of magnetic nanoparticles. The pile of accumulated nanoparticles creates a dark area with lower intensity. However, the mixing efficiency at the outlet of the device reaches a value of more than 0.7. The curve for the flow rate of 20 \( \mu \text{L/min} \) and a concentration of \( \varphi = 20\% \) vol. was already discussed; a higher mixing efficiency was achieved as agglomeration problems do not exist. No mixing occurs at the higher flow rate of 200 \( \mu \text{L/min} \) due to the high hydrodynamic force which eliminates the magnetoconvection effect. Figure 4a,b indicate that best mixing result was obtained at the concentration of \( \varphi = 20\% \) vol. and a flow rate of 20 \( \mu \text{L/min} \).
3.3. Effect of Flow Rate and the Optimum Operating Conditions

Figure 4 indicates that a lower concentration of \( \varphi = 2\% \text{ vol.} \) does not create a strong enough magnetoconvective secondary flow for mixing. On the other hand, there must be an optimum flow rate for the highest mixing efficiency while at the same time avoiding nanoparticle accumulation. The mixing efficiency for the low flow rate of 2 \( \mu \text{L/min} \) shows a sudden decrease in the region between \( x^* = 8 \) to \( 15 \) because of nanoparticle agglomeration next to the magnets. The pile of accumulated nanoparticles is dark and has a low intensity close to zero, Figure 3a. At this flow rate, the secondary flow is strong enough to fully mix the two streams. After \( x^* = 15 \), where there is no agglomeration next to the upper wall, mixing efficiency rises to a higher value. We carried out mixing experiments for a wide range of flow rates, from 2 \( \mu \text{L/min} \) to 300 \( \mu \text{L/min} \) at the constant concentration of \( \varphi = 20\% \text{ vol.} \), to examine the effect of flow rate on mixing efficiency and to determine the optimum range for flow rate.

Figure 5 shows the mixing efficiency values at the outlet versus flow rate. The probability graphs and mixing efficiency data show that low flow rates between 2 and 5 \( \mu \text{L/min} \) provide good mixing. The reason is the longer residence time of the superparamagnetic stream in the microchannel allowing for a better distribution of dye molecules across the channel width. Despite the high mixing efficiency, agglomeration of magnetic nanoparticles exists in that range of flow rates.
As the flow rate increases up to 20 μL/min, the mixing efficiency drops. At these medium flow rates, a concentrated substream of ferrofluid occurred near the upper wall ($y^* = -0.5$). This sub-stream next to the upper wall is negligible at lower flow rates, and almost disappears at higher flow rates. At a flow rate of 45 μL/min, the device achieves the most homogenous mixing. At this flow rate, mixing was not affected by the accumulation of the nanoparticles or the concentrated stream next to the upper wall. As the flow rate increases beyond 45 μL/min, the high hydrodynamic force in the flow direction causes a drop in mixing efficiency. Although the probability graphs show one single peak for a wide range of flow rates, this tool was unable to identify a specific point for the optimum flow rate. Therefore, mixing index is a better indicator of efficient mixing for our device.

4. Conclusions

We proposed and investigated a simple micro-mixer based on magnetofluidic transport phenomena. The device takes advantage of a non-uniform magnetic field provided by permanent magnets. The magnetic susceptibility difference between a non-magnetic stream and a superparamagnetic stream leads to a magnetoconvective secondary flow toward a magnetic field maximum. The competition between this secondary flow and the pressure-driven hydrodynamic flow determines the extent of mixing. The superparamagnetic stream was diluted ferrofluid mixed with fluorescent dye. By analysing the fluorescent image, mixing was evaluated both qualitatively and quantitatively. Comparing the two concentrations of 2% vol. and 20% vol. indicates that the higher concentration delivers more efficient mixing. The effect of flow rate on mixing efficiency was also studied. An optimum flow rate range was determined to achieve efficient mixing and at the same time to avoid agglomeration of magnetic nanoparticles. The flow rate of 45 μL/min and the concentration of 20% vol. resulted in a mixing efficiency of 88% in our device. Considering the low cost, simplicity, and ability to achieve rapid mixing in a short microchannel, our micromixer has the potential to be implemented in lab-on-a-chip devices for chemical and biological studies. However, it is worth mentioning that the commercial ferrofluid used in this study contains surfactants, which contaminates fluids under mixing. Surfactants are hard to remove after the mixing, which changes the properties of the fluids under mixing. In addition, the effects of ferrofluid on chemical reactions is unknown. More work with customized ferrofluids is essential to discover the effect of surfactants on mixing efficiency and chemical reactions. Furthermore, studies to identify biocompatible ferrofluids are also needed for biological studies.
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Author Contributions: M.H. performed the experiments, analysed the data. N.T.N. designed and supervised the research. M.H. and N.T.N. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References


Chapter 7 demonstrated that magnetofluidic phenomena can be utilised to enhance mass transport in a microchannel. As mass transport is linked to heat transfer, this chapter considers heat transfer within the context of magnetofluidics. A simple heat sink based on forced convective heat transfer was used in the experiment. Three cases were examined and compared: DI water as the operating fluid, ferrofluid as the operating fluid, ferrofluid as the operating fluid under the effect of a non-uniform magnetic field. The results showed the capability of manipulating the outlet temperature with the external magnetic field. The chapter also presents a full numerical simulation of the phenomenon. The simulation results support the better understanding of the key parameters affecting magnetically induced heat transfer. The multi-physics numerical simulation could be employed for future studies in magnetofluidics.
Statement of Contribution to Co-authored Journal Paper

This chapter is in the form of a co-authored published paper. The bibliographic details of the co-authored published paper, including all authors, are:


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My contribution to the published paper involved:

- Literature review
- The experiments
- Numerical simulation
- Data analysis, discussion and preparation of figures
- Manuscript preparation

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INTRODUCTION

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GENERAL TERMS
Magnetofluidics for manipulation of convective heat transfer

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**Abstract**

We report heat transfer manipulation and control in a magnetofluidic device. The device consists of a circular chamber with a heat source on top and is positioned next to a permanent magnet that creates a non-uniform magnetic field. Convective heat transfer was evaluated and compared for three cases: DI-water, ferrofluid, and ferrofluid under the magnetic field. Experimental results indicate enhancement of convective heat transfer with the use of diluted ferrofluid as the working fluid. However, we observed a reduction in outlet temperature and Nusselt number in the presence of a non-uniform magnetic field. In addition, we report a full simulation of transport phenomena in the system explaining the physics of this phenomenon. The simulation results agree with the experimental data, and show the same trends.

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**1. Introduction**

Heat transfer enhancement is the main challenge for various applications in electronics and industrial cooling systems. Ferrofluids offer promising advantages for heat transfer augmentation. Ferrofluids are stable suspensions of paramagnetic nanoparticles, typically magnetite, in non-magnetic base fluids such as water and oil. Several studies have reported improvement of thermophysical properties of the base fluid using nanofluids [1–21].

Utilizing paramagnetic ferrofluids in the presence of a magnetic field for the control of heat transfer has attracted growing interest of many researchers recently [22–27]. Using this method in systems with contentious forced convection, fluid flow and thermophysical properties of the ferrofluid can be altered due to the migration of paramagnetic nanoparticles under an external magnetic field. As a result, temperature distribution and convective heat transfer can be controlled locally in a magnetofluidic based device.

Goharkhah et al. recently investigated the laminar forced convective heat transfer of ferrofluid in a long uniformly heated parallel plate channel under an external magnetic field [28,29]. Increasing the flow rate and concentration of the ferrofluid enhanced the convective heat transfer. The authors reported an increase of 16.4% in heat transfer by the use of ferrofluid and in the absence of a magnetic field. Exposure to a constant and alternating magnetic field achieved an improvement of 24.9% and 37.3%, respectively. In the absence of the magnetic field, the application of ferrofluid improved the average convective heat transfer up to 13.5% compared to DI-water. Under a constant and alternating magnetic field, this value grows up to 18.9% and 31.4%, respectively.

Yarahmadi et al. performed an experimental study on the effect of ferrofluid and a magnetic field on convective heat transfer in a tube under a constant heat flux [33]. The authors reported that compared with distilled water convective heat transfer improves in the absence of a magnetic field. Furthermore, an oscillatory magnetic field enhances the convective heat transfer through the circular tube, while heat transfer decreased with a constant magnetic field. Lajvardi et al. reported heat transfer enhancement for forced convective ferrofluid flow in a heated copper tube in the presence of magnetic field [34]. The effect of ferrofluid concentrations and magnet position on heat transfer was also examined. Change of thermophysical properties of the ferrofluid under the influence of the applied magnetic field was mentioned as the reason for heat transfer augmentation. Choefani et al. reported heat transfer enhancement with a ferrofluid flow inside a circular copper tube in the presence of an alternating magnetic field [35]. The interaction between the magnetic field and the nanoparticles created a...
complex convection regime that led to heat transfer augmentation. The alternating magnetic field proved to enhance the convective heat transfer rate. Heat transfer improved with increasing frequency of the alternating magnetic field and increasing concentration of the ferrofluid. At low Reynolds numbers, a maximum increase of 27.6% in the convection heat transfer was reported. Recently, Asfer et al. experimentally examined the effect of magnetic field on ferrofluid flow in a circular stainless steel channel [36]. The authors used infrared thermography (IRT) to investigate the temperature profile. The authors reported that the magnetic force could have a positive or negative effect on convective heat transfer coefficient. The transport of heat increases with increasing magnetic field gradient as in case of double-inline arrangement of magnets.

Our present work reports a numerical simulation and experimental verification of convective heat transfer of ferrofluid in a microchannel in the presence of an external magnetic field. As reviewed above, the effect of an external magnetic field on forced convective heat transfer has been reported in the literature. Governing equations for momentum, heat and mass transfer were solved using COMSOL to gain a better understanding of the phenomena, which is missing in the literature. In addition, we examine the effect of magnet arrangement, direction of the magnetic field gradient, concentration of the ferrofluid, and flow rate.

2. Materials and methods

Fig. 1 shows a schematic of the geometry employed in this study. The inlet and outlet channels have a depth of $H = 500 \mu m$ and a width of $W = 2 mm$. The circular chamber has a depth of $H = 500 \mu m$ and a diameter of $D = 6 mm$. We used a laser engraving machine (Trotec/Rayjet) to cut the channel through a clear 500 $\mu m$ thick double-sided adhesive tape (source, brand of the tape). The upper wall of the circular chamber was closed with a thermostatic cooler (TEC1-00701010 KJLP (SHENZHEN) CO.). The remaining area of the channel was bonded to poly(methyl methacrylate) PMMA slides. Two thermocouples (RS Pro K Type Thermocouple, RS Components Pty Ltd) measure the inlet and outlet temperatures. The slots for the inlet, outlet and thermocouples were cut through the upper PMMA slide before bonding to the tape. A neodymium–iron–boron (NdFeB) permanent magnet cube (Trotec/Rayjet) was placed next to the chamber, in order to study the effect of the magnetic field. The magnetic field magnitude of the individual permanent magnet versus distance from the magnet was measured and reported previously [37]. Diluted water-based ferrofluid (EMG707, Ferrotec) was fed into the device as a paramagnetic fluid. The commercial ferrofluid has an initial magnetic susceptibility of 0.12, a magnetic particle concentration of 2% vol. and a saturation magnetization of 110 G. The ferrofluid was further diluted by deionized water (DI-water) into 20% vol. concentration. A precision syringe pump (SPM100, SIMTech Microfluidics Foundry) delivers the fluid into the device. The flow rate in this experiment ranges from 50 to 400 $\mu L/min$.

3. Numerical analysis

We perform a full simulation including transport of heat, mass and momentum. The numerical model with COMSOL (COMSOL Inc., USA) simulated the transport phenomena in the circular chamber. The two-dimensional model consists of one inlet, a circular chamber and one outlet. The shallow channel approximation was applied to consider the height of the channels. The fluid flow was considered as an incompressible. Steady-state conditions were applied for the simulation. Three separate domains were modelled: the permanent magnet, the channel and the surrounding PMMA layer. A circular magnetic insulation boundary conditions with a diameter of 100 mm were applied around the system to bind the magnetic field and to achieve an accurate field distribution.

The laminar flow of diluted ferrofluid inside the chamber is governed by the continuity equation:

$$\nabla \cdot (\rho_f \mathbf{u}_f) = 0 \quad (1)$$

and the Navier–Stokes equation:

$$\nabla \left[ -p_f I + \mu_f \left( \nabla \mathbf{u}_f + \left( \nabla \mathbf{u}_f \right)^T \right) \right] - 12 \frac{\mu_f H_f}{H^2} + \left( \mathbf{M} \cdot \nabla \right) \mathbf{B} = 0 \quad (2)$$

where $\mathbf{u}_f$ is the fluid velocity, $p_f$ the pressure, $\nabla \cdot$ the divergence operator, $\mathbf{I}$ the identity matrix, $\mu_f$ the fluid dynamic viscosity, $\mathbf{B}$ magnetic flux density, $\mathbf{M}$ magnetization vector, and $H$ is the height of the channel. No slip conditions were applied at the walls, and the pressure has no viscous stress at the outlet.

The magnetic field in the absence of electric currents is described by:

$$\nabla \cdot \left( \frac{\mathbf{H}_f \mathbf{H}_f \mathbf{M}}{\chi_m} \right) = 0 \quad (3)$$

where $\mu_r$ is the relative magnetic permeability and $\mu_0$ is magnetic permeability constant.

The energy equation is formulated as:

$$\rho_f c_{pf} (\mathbf{u}_f \cdot \nabla T) = k_f \nabla^2 T - \mu_f \frac{\partial M}{\partial T} \left( \frac{\mathbf{u}_f \cdot \nabla |\mathbf{M}|}{\chi_m} \right) \quad (4)$$

where $T$ is the temperature, $c_{pf}$ the heat capacity of the ferrofluid, $k_f$ thermal conductivity of ferrofluid, and $\chi_m$ the total magnetic susceptibility of the fluid.

The diffusive/convective transport of a solute is obtained by solving:

$$-\nabla \cdot \left( -D \nabla c + \mathbf{u}_f c \right) = 0 \quad (5)$$

where $c$ (mol m$^{-3}$) is the concentration, and $D$ (m$^2$/s) is the diffusion coefficient of the solute.

Using single phase model, the density of the ferrofluid is calculated by:

$$\rho_f = (1 - \varphi) \rho_{\text{water}} + \varphi \rho_{\text{np}} \quad (6)$$

where $\rho_{\text{np}}$ and $\varphi$ are the density of nanoparticles and volume fraction, respectively. The effective dynamic viscosity of the ferrofluid is estimated as:

$$\mu_f = \mu_{\text{water}} \left( \frac{1}{1 + \varphi} \right)^{0.25} \quad (7)$$
Fig. 2. Simulation results for flow rate of 50 μL/min, and under magnetic field: (a) magnetic field distribution; (b) velocity field; (c) concentration distribution; (d) temperature profile.
The effective thermal conductivity of the ferrofluid was determined by [17]:

\[
\frac{k_{ff}}{k_f} = 1 + 64.7 \phi^{0.7640} \left( \frac{d_f}{\alpha_f} \right)^{0.3690} \left( \frac{K_f}{K_p} \right)^{0.7476} Pr_f^{0.955} Re_f^{1.2321}
\]  

(8)

The Prandt number \(Pr_f\) and the Reynolds number \(Re_f\) are defined as:

\[
Pr_f = \frac{\mu_f}{\rho_f \alpha_f}
\]

(9)

\[
Re_f = \frac{\rho_f \mu_f T}{3 \pi \mu_f / \alpha_f}
\]

(10)

where \(k_b = 1.3807 \times 10^{-23} \text{ J/K}\) is the Boltzmann constant, \(\lambda_f\) and \(\alpha_f\) is the mean path of fluid particles given as 0.17 nm. This model considers effects of temperature and nanoparticle size for the temperature ranging from 21 to 70 °C.

The heat capacity of the ferrofluid is evaluated as:

\[
\frac{(\rho c_p)_{ff}}{(\rho c_p)_f} = (1 - \phi) \frac{(\rho c_p)_f}{\phi (\rho c_p)_f} + \phi (\rho c_p)_{np}
\]

(11)

where \(\phi\) is volume fraction of the solid particles, and subscripts \(f, ff\) and \(np\) stand for base fluid, ferrofluid and magnetic nanoparticles, respectively.

The flow and magnetic fields were coupled first to obtain the velocity field of the ferrofluid under the effect of magnetic field. The diffusive/convective transport of nanoparticles and the energy transport equation were solved using the velocity field of the ferrofluid to achieve concentration and temperature field. Finite element method was employed for the solution of the above equations. Finite element discretization was based on the second-order functions for velocity, quadratic functions for concentration, and linear functions for the pressure and temperature fields. We utilized user controlled meshing in COMSOL, consisting of 98,808 domain elements and 1461 boundary elements. The numerical simulation was carried out for seven flow rates at fixed heat flux, magnetic field magnitude and ferrofluid concentration. Fig. 2 shows the representative results for magnetic field, velocity field, concentration field, and temperature distribution for the flow rate of 50 \(\mu L/min\) of ferrofluid under the effect of magnetic field.

4. Results and discussion

The experiments were performed for a constant heat flux, from the top of the chamber, and a constant magnitude of the magnetic field. We apply different flow rates to vary the strength of hydrodynamic flow. The experiment was carried out with DI-water, diluted ferrofluid, and diluted ferrofluid under the effect of the magnetic field. Our aim is to investigate the effect of the iron oxide nanoparticles to the DI-water, and the effect of magnetic field on heat transfer performance. A relatively high concentration of diluted ferrofluid, 20% vol., was selected to increase the effect of magnetic field on both thermophysical properties and bulk force on convective heat transfer.

Fig. 2 Illustrates the representative simulation results, with ferrofluid and under the effect of magnetic field. The magnitude of magnetic field decays with increasing distance from the permanent magnet. The non-uniform magnetic field distribution is depicted in Fig. 2(a). The velocity field inside the chamber is depicted in Fig. 2(b). The accumulation of paramagnetic nanoparticles next to the permanent magnet indicated by the higher concentration can be seen in Fig. 2(c). This phenomenon is caused by the positive magnetophoresis of the magnetite nanoparticles towards the magnet. The movement creates a secondary flow in the direction of magnetic field maxima leading to a longer residence time in the chamber. Fig. 2(d) shows the distribution of the temperature inside the chamber. A higher temperature gradient exists at the centre of the chamber due to the higher velocity, shorter resident time, and more convective heat transfer compared with the areas away from the centre.

Fig. 3 compares the outlet temperature obtained from the experiments and the simulation. Three cases were considered: DI-water as the operating fluid, diluted ferrofluid as the operating fluid, and ferrofluid under the effect of magnetic field. The experimental data demonstrate that with an increasing flow rate, the outlet temperature decreases due to the higher convective heat transport. Comparing DI-water and ferrofluid with no magnetic field indicate that the presence of nanoparticles increases the outlet temperature considerably. The main reason for convective heat transfer enhancement with ferrofluid is the improvement of thermophysical properties of a nanofluid, according to Eqs. (8) and (11). In addition, movement of nanoparticles can lead to a stronger energy exchange in the fluid and agitate the thermal boundary layer and consequently increase the temperature gradient between the operating fluid and the heat source. However, the outlet temperature drops significantly to the values even less than that of DI-water with the presence of the magnetic field in the system. This phenomenon is caused by the secondary flow, which is perpendicular to the direction of main flow and increases the residence time of the ferrofluid in the chamber.

According to the experimental data, at flow rates higher than 300 \(\mu L/min\) the outlet temperature approaches that of the case with ferrofluid and no magnetic field. However, this effect is more distinguishable in the simulation, Fig. 3(b). The value of outlet temperature is the highest in the case of ferrofluid and no magnetic field for the whole range of flow rates. By adding the magnetic field to the system, the outlet temperature substantially drops to values even lower than that of DI-water. This trend continues for the flow rates as high as 350 \(\mu L/min\). For flow rates higher than 350 \(\mu L/min\) the hydrodynamic force of the main flow overcomes the effect of the secondary flow and the value of the outlet temperature come close to that of ferrofluid with no magnetic field.

Fig. 4 displays the variation of the total Nusselt number \(Nu\) versus Reynolds number \(Re\). Convective heat transfer grows with the increase of flow rate for all operating fluids. According to experimental data (Fig. 4(a)), addition of nanoparticles to DI-water enhances the total convective heat transfer in the device. This improvement is more pronounced with higher \(Re\) numbers. The addition of magnetic field to the system at low flow rates, where magnetocative secondary flows dominate.
flow is significant, has a negative effect on the total convective heat transfer of the device. With a higher Reynolds number Re, the negative effect of secondary flow starts to disappear due to the dominant hydrodynamic force of the main flow. As a result, the Nusselt number Nu for the case with magnetic field tends to approach the values with ferrofluid and no magnetic field. Fig. 4(b) shows the simulations results, which indicate the same effect as in the experiment: the magnetic field leads to trapping of heat in the circular chamber.

5. Conclusions

We report magnetic control of heat transfer in a circular chamber experimentally and numerically. We fabricated a microfluidic device for this purpose. Three cases were examined and compared: DI-water, diluted ferrofluid, and diluted ferrofluid in a magnetic field. Using diluted ferrofluid as the operating fluid results in heat transfer augmentation. Experimental data indicate that the presence of a magnetic field causes heat trapping at a relatively low flow rate. In the presence of a magnetic field, paramagnetic nanoparticles move towards the magnet. Convective heat transfer reduction is a result of magnetoconvective secondary flow of the ferrofluid towards the permanent magnet. Increasing flow rate reduces the negative effect of the induced secondary flow. As a result, convective heat transfer and Nu number improves at higher flow rates. A full simulation of the phenomenon was performed, considering momentum, heat and mass transport in the system. Simulation results clearly illustrate the observed phenomenon and agree well with experimental data. Employing this simulation for different geometries and operating conditions could assist designing heat transfer devices for various applications such as micro-reactors and cooling devices for electronics.

Acknowledgements

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References


Fig. 4. Nusselt number versus Reynolds number: (a) experimental data; (b) simulation results.


CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

Magnetofluidic techniques have the potential to be employed in various microfluidic applications. The combination of ferrofluid and an external magnetic field provides the capability of manipulating fluidic samples in microscale. The research presented in this thesis provided the proof of magnetofluidic concepts and demonstrated their application in two common microfluidic functions: particle sorting and mixing. Moreover, the capacity of this technique in adjusting heat and mass transfer was also demonstrated. The outcome of the research of this thesis is summarised as follows:

The competition between negative magnetophoretic and magnetoconvective forces was first examined under a uniform magnetic field. A circular chamber with a three-stream configuration was utilised for this purpose. The core stream consists of non-magnetic microparticles suspended in a diluted ferrofluid. Two non-magnetic cladding streams focus the ferrofluid flow. The magnetoconvective force on acting on the iron oxide particles result in an expansion of the core flow. Increasing the concentration of ferrofluid led to a higher magnetoconvective force and more expansion. The suspended microparticles also travelled towards the magnetic field maximum despite their non-magnetic properties. This phenomenon illustrated the significance of magnetoconvective bulk force, which should be considered in designing micro-magnetofluidic devices.

The effect of a non-uniform magnetic field on non-magnetic microparticles suspended in diluted ferrofluid was investigated in a circular chamber with the sheath and core flows of the same susceptibility. The core flow consists of non-magnetic microparticles suspended in paramagnetic diluted ferrofluid. The same concentration of ferrofluid was used for the cladding flows. The magnetic field gradient generated by a permanent magnet located on one side of the chamber produced a magnetoconvective secondary flow toward the lower magnetic field strength. The secondary flow was strong enough to transport microparticles from the core flow. The experiment with florescent dye confirms that the secondary flow is a result of...
magnetoconvective force instead of magnetophoretic force on the non-magnetic microparticles.

Using flow-focusing configuration in a straight microchannel, the effect of magnetic field on mass transport enhancement was examined. The sheath flows were non-magnetic fluids and the core flow was diluted ferrofluid mixed with fluorescent dye. A combined effect of magnetic field gradient and magnetic susceptibility difference between the fluids was observed. The core stream mixes well with the sheath stream. A stronger magnetoconvective force was generated with a higher ferrofluid concentrations and a lower flow rate. This experiment demonstrates the capability of improving mass transfer of non-magnetic species with orders of magnitudes higher than that of molecular diffusion. Using the same concept, a micromixer was developed based on the magnetofluidic phenomena. Rapid and efficient mixing of a paramagnetic and non-magnetic stream was achieved in a relatively short channel.

Employing two arrays of attracting magnets, highly size-selective trapping of non-magnetic microparticles was achieved. The arrangement of the magnets creates multiple magnetic maximum and minimum zones. Depending on the flow rate and concentration of the diluted ferrofluid, the microparticles could be captured in either zone. The device operates based on the balance between the three dominant forces: magnetoconvective force, negative magnetophoresis, and hydrodynamic force of the pressure-driven main stream. The experiments were carried out for a wide range of flow rates, concentrations of ferrofluids and two microparticle sizes. The experimental results were summarised in an operating map, which may serve as a guide for future research in this area. Furthermore, strong recirculations were observed at certain operating conditions, indicating that this method is suitable for designing a micromixer.

The phenomenon of magnetoconvection was subsequently used to examine heat transfer in a circular chamber. A heat source was positioned on the top of the chamber to induce heat into the working fluid. The results showed that compared to DI-water as the working fluid diluted ferrofluid improves forced convective heat transfer. However, with the addition of a permanent magnet to the system, the outlet temperature decreased to values even less than that of the DI-water case. The non-
uniform magnetic field of the permanent magnet results in a magnetoconvective secondary flow perpendicular to the direction of the main flow, and consequently trapping the heat in the chamber. Further, a simulation of mass, heat and momentum transfer under the effect of magnetic field was developed. The simulation results showed the same trend of the experimental data and confirmed the trapping of heat in the channel. This heat-trapping phenomenon shows the possibility of rapid manipulation of outlet temperature using magnetofluidic technique and could be employed for chemical and biological applications.

9.2 Research limitations

The limitations, issues and challenges encountered during this research are discussed as follow:

- **Negative magnetophoresis in diluted ferrofluid flow**: The deflection of non-magnetic particles was mainly affected by the secondary flow, caused by magnetoconvective force. However, the extent of the deflection also depends on the size of the micro-particles. This difference was hard to study, considering the fact that the deflected particles travel in a wide band, instead of a line. Two types of microparticles were available in our experiments 3.2 and 4.8 µm. Having the same green colour, it was impossible to introduce both particles to the device for this purpose. Comparing the deflected bands of particles, it was discovered that the two bands almost overlapped due to small difference in size of the particles and the geometry of the device. In order to properly study the effect of size, micro-particles with a larger difference in size and different colours is needed. The geometry and design of the device are also not suitable for particle separation purpose, as it is circular and only has one outlet.

- **Device fabrication**: the microfluidic devices used for our study were made of PDMS. Considering the elastic property of PDMS, it cannot firmly hold the magnets in the desired position. This matter might have caused inaccuracies in the studies reported in chapter 4, 5, and 7. This issue also led to using PMMA for making devices for the magnetic concentration study (chapter 6), and the heat transfer study (chapter 8). Micro sized channels could not be fabricated using PMMA and the laser engraver in our lab. Hence, the fluidic channels in chapter 6 and 8 are larger in size and the devices are not really microfluidic devices. Another problem concerning the fabrication step was the limitation in approaching the magnets to the fluidic channel’s
wall: the closer the magnet is to the channel’s wall, the higher is the risk of leakage. More efficient devices could be achieved by decreasing the distance of the magnets to the channel’s wall. New fabrication methods are needed to overcome these problems.

- In all experiments, plastic tubes were used to connect syringes to the microfluidic devices. In addition, the syringe pumps available in our lab are old and needed to be serviced. As a result, inconsistencies and sudden variations in the flow rate were observed during the experiments. Considering the fact that hydrodynamic force is one of the main forces in microfluidic applications, a slight change in flow rate could result in incorrect results. Although the experiments were repeated several times and inaccuracies were reported, the use of non-faulty syringe pumps and more rigid tubing for similar experiments are recommended.

- Agglomeration of the ferrofluid on channel walls and close to the magnets caused problems such as: blockage, variations in flow rate, and local changes in magnetic susceptibility and magnetic field gradient. The use of other super-paramagnetic solutions could overcome this issue in magnetofluidic studies.

- The multi-physics simulation evolved gradually through the study. Therefore, for most of the research, lack of a comprehensive simulation is noticeable. Magnetoconvective force was added to the simulation at final stages of the candidature, and is reported in chapter 8. Considering the importance of this force throughout this research, proper simulation of other chapters using the developed multi-physics model might be considered as future work.

9.3 Suggestions for future research

Magnetofluidics is a promising, highly efficient, biocompatible and low-cost approach for designing lab-on-a-chip platforms. Magnetoconvective bulk force created by the interaction between magnetic field and paramagnetic carrier fluid enables manipulation of cells and particles and adjustment of transport phenomena. The following suggestions could be a guide for future research in this area:

- In the present thesis, stationary uniform and non-uniform magnetic fields generated by permanent magnets and a commercial ferrofluid (water-based ferrofluid EMG 707) as the paramagnetic working fluid were employed. With the application of a
controllable magnetic field produced with an electromagnet, the effect of magnetic field magnitude variations could be studied more comprehensively and conveniently. Moreover, bio-compatibility of the paramagnetic fluid is important for implementation of magnetofluidics in biological studies. Using a customised ferrofluid instead of a commercial one enables more research in this area.

- Chapter 4 discusses the possibility of deflecting non-magnetic micro-particles using a combination of negative-magnetophoresis and magnetoconvective forces. The same device was tested, with the same experimental setup, for separating healthy and infected red blood cell. The blood cell sample was mixed in bovine serum albumin and 1 % vol. of ferrofluid (EMG 707) and fed into the device as the core flow. Bovine serum albumin was used as non-magnetic cladding flows. As illustrated in Figure 9.1, both infected and healthy red blood cells behaved as non-magnetic particles and deflected away from the permanent magnet. Although separation of healthy and infected red blood cells was not achieved, this experiment indicates the feasibility of employing magnetofluidics for biological studies and disease diagnosis.

- The particle trapping platform introduced in Chapter 6 was capable of sorting non-magnetic microparticles with small difference in size. The sensitivity of this device could be used to separate cells with a small mismatch in size and susceptibility such as: cancer cells from healthy cells, and malaria infected red blood cells from healthy red blood cells. Experiments with biological particles are required to test the capability of this technique. The device can also be used as a magnetofluidics-based multi-stage mixer for applications such as labelling of cells with magnetic microparticles, or as a reactor. In addition, a rapid, efficient, and low cost micromixer based on magnetofluidics was introduced in Chapter 7. Further experiments with cells are needed to discover the suitability of the device for biological applications.
Fig. 9.1 Deflection of red blood cells in a circular chamber as a result of negative magnetophoresis and magnetoconvective force.

- A heat transfer control system was introduced along with a complete simulation of the phenomena. Both experiment and simulation indicated the possibility of controlling transport phenomena using magnetofluidics. Considering the governing equations and experimental evidence it can be concluded that the velocity of the flow, concentration of paramagnetic and non-magnetic species, and the temperature of the operating fluid could be manipulated or controlled by an external magnetic field. Further research could focus on designing a micro-reactor based on magnetofluidics for temperature sensitive reactions. On the other hand, the simulation allows the investigation of a variety of geometries and designs and optimises the operating conditions before carrying out the experiments.
Appendix A

Additional information for chapter 3

Non-magnetic fluorescent micro-particle (1 µm) was used for the experiment in Chapter 3. The particles were considered spherical, and the Stokes-Einstein relation could be employed to calculate the diffusion coefficient [1]. The specifications of the micro-particle could be found in the online catalogue of ThermoFisher [2]. According to the catalogue, the size distribution of the non-magnetic particles is:

Catalogue number: G0100

Diameter: 1 µm

Mean diameter: 1 µm

Nominal diameter: 1 µm

Uniformity: <5%

References


Appendix B

Additional information for chapter 4:

Flow rate control: The microfluidic device in chapter 4 has 3 inlets. Each inlet was connected to a separate syringe. Three syringe pumps were used in this experiment. The syringe pumps are controlled by the software, which adjusted the flow rates in (μL/min).

Size distributions for the 3.1 and 4.8 μm non-magnetic particles:

3.2 μm particles [1]:
Catalogue number: 5320B
Diameter: 3.2 μm
Mean diameter: 3.2 μm
Nominal diameter: 3.2 μm
Uniformity: <5%

4.8 μm particles [2]:
Catalogue number: G0500
Diameter: 4.8 μm
Mean diameter: 4.8 μm
Nominal diameter: 4.8 μm
Uniformity: <5%

Appendix C

Additional information for chapter 6

Steady state condition:

Steady state condition was confirmed visually for this experiment: After a few minutes, the size of the capture zones remained unchanged, which is considered as steady state condition.

The number of particles:

The number of particle was not measured in this study. Instead “the size of the captured zones” was compared and discussed. The number of particles is proportional to the size of the capture zones, which can be inferred qualitatively from experimental observations.

Figure 3:

The optimal flow rate for each volume concentration was obtained based on a visual comparison between the capture zones.
Appendix D

Additional information for chapter 7

The accuracy of intensity measurement:

The black and white images were introduced to the MATLAB code. In the code, the maximum and minimum values for the intensity are set in a way that the values for normalised intensity become between 0 and 1. For this experiment, $C_{\text{max}}=220$ and $C_{\text{min}}=10$. The resolution of the normalised intensity (or normalised concentration) measurement is $1/(C_{\text{max}}-C_{\text{min}})$, which is 0.005. Regarding the fact that all images for this experiment were taken at the same software settings and room light, the comparisons between the curves are reliable.

The customised MATLAB code for evaluation of the probability distribution:

```matlab
%Evaluation of mixing image using probability function
%Copyright Dr. N. T. Nguyen, Nanyang Technological University, 2005
%Here are the file names for evaluation
file1='3';
%thix max and min values are taken at the inlet
%cmax=106;
cmin=79;
A = imread(file1,'jpg');
%cmax=220;
cmin=10;
image_handle=figure;
%A=wiener2(A,[5 5]);
%cmax=220;
cmin=10;
image_handle=figure;
%A=wiener2(A,[5 5]);
%display gray scale image with improved contrast
imshow(A,[cmin cmax]);
%display color-coded image
imagesc(A,[cmin cmax]);
%Defining the evaluation surface by drag a rectangle
rect=getrect(image_handle);
title('Drag to select a rectangle for probability evaluation');
AA = imcrop(A,rect);
figure
imagesc(AA,[cmin cmax]);
figure
Anorm=(double(AA)-double(cmin))/(double(cmax)-double(cmin));
N=double(cmax)-double(cmin);
for i=1:N+1
    edges(i)=(i-1)/N;
end
```
imagesize = size(Anorm);
for i = 1:imagesize(1)
    for j = 1:imagesize(2)
        if Anorm(i, j) < 0
            Anorm(i, j) = 0;
        end
        if Anorm(i, j) > 1
            Anorm(i, j) = 1;
        end
    end
end
Cnorm = reshape(Anorm, imagesize(1)*imagesize(2), 1);
distribution1 = HISTC(Cnorm, edges) / imagesize(1) / imagesize(2);
plot(edges, distribution1);
Appendix E

Additional information for chapter 8

Correction:
In page 106, the second paragraph, the word “contentious” is a typo. The correct term is “Continuous”.

The heat flux from the thermoelectric heater:
The voltage of 0.8 V and current of 4 A (DC) was used for this experiment. It equals 3.2 W.

The size of the two type K thermocouples:
Two RS Pro K Type Thermocouples, 1/0.3mm Diameter, were used for this experiment.

Effect of specific heat on heat transfer enhancement with ferrofluids:
In page 109, results and discussion, paragraph 3: it is worth mentioning that: Specific heat has no part to play in steady-state heat convection.

The deviations between experimental results and simulation (Fig. 3):
The fluidic device was fabricated using PMMA. The heat loss due to thermal conductivity of PMMA was not considered in the simulation. In addition, considering the large size of the device, the position of the permanent magnet and its location cannot be exactly the same as in the simulation. Further, the size of the heat source was larger and it covered the area around the chamber as well, which was not considered in the simulation. Therefore, the deviations between experimental and simulation results are significant, but as stated in the paper it could predict the phenomenon of heat transfer successfully.

Nusselt number in Figure 4:
The Nusselt number in Fig. 4 is an average Nusselt number.