MAGNETIC PARTICLE BASED MICROFLUIDIC SEPARATION OF CANCER CELLS FROM WHOLE BLOOD FOR APPLICATIONS IN DIAGNOSTIC MEDICINE

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ABSTRACT

Metastasis, or the process in which tumor cells spread and grow from a primary tumor site to a distant secondary site, is a significant problem in cancer research today. Metastases have also been shown to cause 90% of all cancer-related deaths, i.e. half-a-million people in the US each year. Detection of circulating tumor cells (CTCs) in whole blood demonstrates that there is a connection between the primary tumor and metastases. Therefore, there is a need to create technologies to enable biological CTC studies. This could contribute to understanding of the spreading of cancer and development of various new drugs and strategies.

As means to isolate these rare cells conventional magnet-activated cell separation (MACS) is carried out at the macroscale, with a large external magnet surrounding a flow channel. This technology uses labeling with antibody-coated magnetic microparticles and extraction by attractive magnetic forces in order to effectively isolate the cells of interest. In recent years, there has been tremendous interest in miniaturizing the MACS process to harness the traditional advantages of microfluidic systems, namely the ability to process microliter-size sample volumes economically and portably. However, recent device designs have typically required large permanent magnets or electromagnets.

These approaches have typically followed an empirical, experimental- and device-centric approach. By contrast, this dissertation represents a “bottom-up” effort to design a microfluidic MACS system where physical force balance calculations coupled, with measurements of particle and cell parameters, lead to elements of device design. This design includes external magnet design, flow channel layout, and manipulation of multiphase flows. This approach has led directly to a prototype microfluidics MACS
system that overcomes the current limitations on external magnetic field sources. In addition, the designed microfluidic platform achieved throughputs better than the state of the art, and efficiencies and purity comparable or better than the standards in separation today.

Concurrent with the rational optimization an effort to investigate the feasibility using magnetic nanoparticle as a substitute for the microparticle and sub-micron tags currently used in MACS was conducted. Magnetite (Fe$_3$O$_4$) particles were synthesized using traditional thermal decomposition methods, followed by a ligand exchange using the biocompatible surfactant dopamine. Although it was ultimately determined that labeling with magnetic nanoparticles would required applied magnetic fields beyond the constraints of the mathematical optimization, an interesting increase in magnetic moment was observed following this ligand exchange. Additionally, whilst characterizing the synthesized nanoparticles’ particle diameter and distribution, a novel quantitative evaluation model of the nanoparticle ensemble was outlined solely from temperature-dependent magnetization measurements. These new insights into the characteristics of nanoparticles may allow for better understanding of the synthesized ensembles for implantation in bio-nanotechnological applications.
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1.0 INTRODUCTION

In 2010, there were over 1.3 new diagnoses of carcinoma, or cancer of the epithelial cells in a specific organ, and more than 500,000 deaths due to some form of carcinoma in the US alone. Additionally, about 2 to 4 percent of all cancer patients have a cancer whose primary site is never identified, known as cancer of unknown primary (CUP) origin [1]. This outcome is due mostly to the fact that early-stage cancers can be asymptomatic and are not detected at an early stage for treatment. However, survival rate with cancer was reported to significantly increase when surgical resection of the primary tumor is performed at an early stage (i.e. Stage I); as an example the 5-year survival rate for lung cancer improved by 70% with early detection [2, 3]. A major contributor to these poor survival rates is due to ability of cancer to metastasize, whereby tumor cells spread and grow from the late stage, primary tumor site to distant, secondary organs. It has been established that more than 80% of all cancers, such as lung, breast, prostate, and colon cancer, originate from the epithelium (i.e. carcinomas) [2]. These epithelial cells which break off from the tumor into the peripheral blood of patients with metastatic disease [4] are defined as circulating tumor cells (CTCs). Therefore, technologies that capture CTCs for early detection may improve the survival rates and quality of life of patients [5].

Molecular cytogenetic analysis indicates that CTCs of various cancer types have chromosomal abnormalities of their original tumor, indicating CTCs originate from known primary tumors [6]. It is recognized that CTCs are created by a recent proliferating tumor due to their ~24 hr half-life-span [7]; therefore, CTCs could provide important information about the clinical status of patients, including assessing patient
prognosis over time. Moreover, results obtained from these analyses may lead to new developments in the detection of cancer relapse at an early stage [8].

Recent advances in cancer biology have enabled the isolation of these cancer cells in leukemia, and in solid tumor cancers, such as breast cancer and other tumors in the central nervous system [9-17]. These studies suggest that only a small subset of tumor cells can create metastatic sites. Thus, the ability to non-invasively isolate CTCs and significant subsets of CTCs from whole blood of cancer patients has important diagnostic and therapeutic implications. Unfortunately, the use of very rare CTCs in clinical assays has not been accomplished due to the technological necessity to reliably isolate CTCs with frequencies as low as 1 CTC in $10^9$ cells in whole blood [18]. To solve the technological challenge associated with the development of such a clinical assay, there is a need to develop a method to effectively separate rare CTCs. In addition, this separation platform would ideally have separation rates of 1 to 10 million cells per s for reasonable processing. Furthermore, a non-invasive test to analyze CTCs captured from whole blood for mutations and resistance to various drugs would be valuable in the clinical management of cancer. It may even allow for monitoring of early responses to therapy. Moreover, there has been increased number of new drugs coming out for cancer treatment; more than 200 new drugs are at various phases of clinical trial, using new approaches such as monoclonal antibodies. These treatments can cause thousands of dollars for a 6 month treatment [19]. Here, a technology that can selectively capture rare CTCs would provide a point-of-care device which could determine the effectiveness of these various treatments and open a new path for effective and inexpensive evaluation of the effect of these drugs on patients. This dissertation describes the development of such
a CTC capture technique that could significantly contribute to the clinical management of cancer patients.

As a comparison, the current state of the art in rare cell separation is magnet-activated cell separation (MACS) which relies on the tagging, or labeling, of a specific cell population in a heterogeneous mixture with magnetic beads. These magnetic tags can be tailored to target specific antigens, allowing for magnetic capture of these entities within an applied magnetic field either in a separation column or on integrated microchips. Magnet-based techniques have been shown to separate target cells at high throughputs (~$10^{11}$ cells hr$^{-1}$) while maintaining high-purity [20]. MACS has been broadly utilized in basic research for many years and over the last decade has become extensively utilized in clinical medicine as well [21-29]. An important advantage of MACS over other affinity-based separation systems is the ability to collect the magnetically-tagged target cells and/or the ability to control their movement within fluidic systems. This ability provides tremendous flexibility in downstream sub-cellular analysis and/or the utilization of cells for applications such as bio-sensing and tissue engineering. Although a valuable tool in bench-top cell separation, current MACS designs require large aliquots of whole blood to be drawn from patients, potentially resulting in significant harm to these patients. This fact is especially true of pediatric patients, who generally require much lower volume blood draw.

Reducing the process volumes required of current magnetic based cell separation designs using microfluidics may therefore be a viable approach to the isolation of CTCs in the clinic [30]. Microfluidics deals with the manipulation of fluids that are geometrically constrained to a small, sub-millimeter, scale. Microfluidic devices are
fabricated using lithographic techniques originally developed in the semiconductor industry. The advent of soft lithography resulted in a process whereby an elastomeric polymer (typically poly(dimethylsiloxane); PDMS) can be poured onto a lithographically patterned mold, cured, and then peeled off. The patterned polymer can then be bonded to glass slides to create functional fluidic devices. The ability to create replicas in this manner is termed “rapid prototyping” and is widely recognized for its cost effective and facile nature [31-33]. Microfluidic devices fabricated by this process can range from simple parallel plate flow chambers to highly complex systems with features such as sieves, valves, and electrical and optical interfaces [34-42]. The primary advantages of microfluidic separation systems relative to macroscale approaches include the ability to handle small (microliter order) sample volumes and operate with minimal external instrumentation. Microfluidic systems can also be manufactured at relatively low cost, making them attractive for point-of-care clinical diagnostic applications.

Thus, a combination of microfluidics and MACS would provide such a powerful platform for high efficiency rare cell isolation while maintaining clinically manageable volumes of whole blood. In the past five years substantial work has been carried out in the area of microfluidic MACS [26, 43-49]. Optimization of these designs has traditionally been a “top-down” approach, where a device is fabricated and the experimental parameters are then manipulated. Changes in the efficiency and purity of the collected cell population are tracked and parameters are optimized through design of experiment approaches. An alternate approach has been utilized by several groups where optimization has been carried out via modeling of microfluidic MACS processes [50-53]. Details of the prior work in the field of microfluidic MACS system will be described in
Section 2 of this dissertation. These two approaches, experimental optimization and microfluidic modeling, still have shortcomings due to two outstanding challenges in this field: (i) achieving miniaturization of the entire separation apparatus without the need for large electromagnets and (ii) blood component separation; a rational device design approach that takes into account the non-Newtonian characteristics of blood from the outset. This dissertation addresses both of these challenges with a rigorous and potentially transformative “bottom-up” approach to design a microfluidic circulating tumor cell MACS system. Starting with a mathematical model of a displacement based device (Chapter 4), followed by experimental validation and optimization of the model (Chapter 3 and 5), a finalized microfluidic magnetic-based design was developed.

In addition to developing a functional microfluidic magnetophoretic platform, the feasibility of using nanomaterials was probed. In general, magnetic biomaterials, such as nanoparticles, provide the ability for cells to be directed and concentrated with a suspension by means of external magnetic field. Additionally, magnetic nanoparticles display the phenomenon of superparamagnetism, or magnetization only in the presence of an applied magnetic field, thus offering the advantage of reduced risk of particle aggregation [54]. Also, they have sizes that place them at dimensions comparable to those of a virus (20 ± 500 nm), a protein (5 ± 50 nm) or a gene (2 nm wide and 10 ± 100 nm long). Moreover, the main advantage of using particles of sizes smaller than 100 nm is their higher effective surface areas (i.e. easier attachment of ligands) and lower sedimentation rates which enhance the stability [55]. Another advantage of using nanoparticles is that the magnetic dipole–dipole interactions are significantly reduced because they scale as $r^6$, where $r$ is the particle radius [56-58]. Although several review
articles [59-65] have speculated on the prospects of nanotechnology in cell separation there remains a lack of understanding of the influence of nanoparticle properties (such as size distribution and chemical affects on magnet moment) that has hindered the implementation of nanoparticle as labeling entities. Therefore, in addition to addressing the development of the bottom up approach towards the design of a microfluidic separation platform, this dissertation also focuses on investigating the magnetic characteristics and sizing attributes of an iron-oxide nanoparticle suspension. The results are incorporated into the rational design to study the feasibility of actually using nanoparticles in microfluidic-continuous flow mammalian cell separation.
2.0 CRITICAL LITERATURE REVIEW

Immunomagnetic cell separation relies on the ability to selective isolate chosen cells from a heterogeneous cell suspension via labeling the target cells with magnetic micro- or nanoparticles. A number of magnetic particles are currently commercially available, possessing a wide variety of size, shape, and magnetic properties [66]. These variations can have a significant effect on both the mode of magnetic separation (e.g. permanent magnets versus electromagnets) and the efficiency of separation [66]. The goal of the following critical assessment is to first describe some of the current knowledge on methods of determining nanoparticle properties. This analysis is followed by a discussion of traditional cell separation techniques used in the clinic, with specific focus on the application of magnetic tags for the manipulation and collection of target cell populations. Overall this review aims to illustrate the current shortcomings in present cell separation approaches and motivate the need for new and better developed micro-scale separation technologies via a priori characterization and computation optimization.

2.1 Magnetic Nanoparticles in Biomedicine

Magnetic nanoparticles (MagNPs) are a valuable class of nanomaterials with unique properties that remain distinct from those that manifest in their bulk counterparts. MagNPs typically are in the size range from a few nanometers up to tens of nanometers, which places them at dimensions that are smaller than or comparable to those of biological entities, such as cells (10-100 μm) and proteins (5-50 nm). As the magnetic particle size descends from the micron scale to the 10–20 nm size range, depending upon specific composition, the magnetic configuration passes from the multi-domain state
through the high-coercivity single-domain regime to the superparamagnetic regime. At this size range the volume magnetic anisotropy energy is of the same order of magnitude as the thermal energy [67]. A useful characteristic of superparamagnetic particles is that they exhibit a large magnetic moment and a high susceptibility in the presence of a small field; upon removal of the field they ideally release all residual magnetization. In this condition the particles exhibit zero coercivity, \textit{i.e.} zero resistance to magnetic reversal, which makes them highly manipulatable. The completely reversible magnetization response to an applied magnetic field allows superparamagnetic particles to be harnessed for application in a variety of fields, including medicine [59-64]. For example, MagNPs have been demonstrated to be an excellent \textit{in vivo} contrast-enhancing agent for magnetic resonance imaging [68]. They have also been used as drug and energy delivery vehicles for therapeutics and theranostics [65, 69], and are under investigation as cell separation systems for \textit{in vitro} diagnostics [70]. This section describes the lack of understanding currently in the literature on the influence of ligand exchange on the properties of MagNPs. Secondly this section describes the traditional techniques currently used for the assessment of MagNP size and discusses the need for new methodology in the determination of the particle size attributes \textit{(i.e.} the average diameter and size distribution). Overall this section aims to illustrate the two critical properties that must be better understood prior to implementation of nanotechnology in biomedicine and, more specifically, cell separation.

\subsection{Impact of Ligand-Exchange}

MagNPs in the form of biocompatible iron oxide nanoparticles are key components of next-generation drug delivery systems, MRI contrast agents and cell
separation systems for disease diagnosis [60-62]. However, most synthesis methods create hydrophobic nanoparticles that must be subjected to ligand exchange procedures to become hydrophilic so as to be soluble in aqueous media and rendered suitable for further conjugation with specific molecules for biomedical applications [71, 72]. Optimization of MagNPs for these applications requires thorough understanding of the effects of ligand exchange on the magnetic attributes of iron oxide nanoparticles. The ligand exchange process generally occurs at room temperature and results in no observed change in the physical size or structure of the nanoparticles, typically confirmed by TEM, XRD, and/or elemental analysis. Moreover the functionalization process has been shown to alter the surface magnetic state of the MagNPs such that the reported magnetic “dead” layer [73, 74] with a canted, zero-moment surface structure is restored, to some degree, to a collinear ferromagnetic structure [75, 76] of non-zero moment. A similar phenomenon has been reported by Crespo et al. [77] for Au nanoparticles, where thiol-derivatized Au nanoparticles were shown to exhibit ferromagnetic magnetization versus alkyl protected gold particles which showed a diamagnetic behavior. Recently, Daou et al. [78] reported an increase in magnetization induced by changes to ‘dead’ magnetic layers upon exchanging a hydrophobic oleic acid stabilizer (carboxylate structure; C) with a stilbene molecule (phosphonated structure; P) on nanoparticle surfaces (as shown in Figure 2.1). The magnetic “dead” layer is thus hypothesized to be “rejuvenated” by ligand exchange, resulting in a higher magnetization and an assumed larger magnetic diameter. Unfortunately, to date, no literature on the influence ligand exchanges using bio-functional molecules on the properties of MagNPs has been reported. In addition to exchanging the stabilizing moiety, from hydrophobic to hydrophilic, the resulting MagNP
must possess a functional group that can be subsequently manipulated for the binding of select biomolecules.

Figure 2.1: Schematic representation of the surface of as-prepared (NP) and C and P nanoparticles in the presence of an applied magnetic field. Arrows depict the resultant ferrimagnetic moment. Adapted from Daou et al [78].

In general, the surfactant requires a free amine (-NH₂), carboxylate (-COOH), or thiol (-SH), for binding of the biomolecule. Again, there is no literature to date that has probed the influence of such chemical surfactants on the recovery of the inherent magnetic dead layer. Therefore, there is a need to investigate such an effect prior to the development of any nanobiotechnological application.

2.1.2 Determination of Particle Size Attributes

In addition to the effects of ligand exchange, optimal deployment of MagNPs in various biomedical applications also requires a good understanding of the average size and size distribution of the particles. It is important to note that size generally refers to diameter. It has been shown that polymodality, defined as a population with a broad range of size, shape and mass characteristics, in MagNPs may preclude their use in certain biomedical applications and assays [79]. The magnetic properties of a sample of
nanoparticles with a wide distribution of diameters likewise possesses a wide variation of coercivities and a much reduced and non-uniform response to an applied magnetic field, rendering their functional response difficult to control. Some examples of biomedical assays that are highly sensitive to MagNP physical characteristics include Brownian relaxation-based immunoassays [80-84], on-chip separations of biomolecules [85] and cells [86], biochemical assays [87], and magnetoresistance biosensors [88]. It has been demonstrated that the MRI contrast effect obtained from MagNPs (4-12 nm diameter) varies drastically with size [89], further emphasizing the importance of monodispersity for ultrasensitive biomedical assays. Another clinical application where nanoparticles (NPs) size and distribution affect implementation is therapeutic hyperthermia for cancer ablation [90, 91]. Magnetic hyperthermia relies on the cyclical response of an ensemble of MagNPs in suspension to an alternating current (AC) field operating in the kHz range [92]. In this method, NPs relax to the zero magnetization state from the magnetized state either by a physical Brownian rotation of the particle within a fluid or by the rotation of the magnetic spins within the particle itself, i.e. Néel relaxation. Brownian relaxation depends on the hydrodynamic diameter of the particle and the properties of the fluid whereas Néel relaxation depends on the anisotropy energy relative to the thermal energy. In both cases the particle size and its distribution determine the efficacy of the process, rendering it necessary to select MagNPs of the right size but also to reduce the size polydispersity to maximize heat transfer.

There are a number of useful techniques available to determine the size distribution of a population of MagNPs, but each possesses specific drawbacks to consider. The most widely-utilized, and most accurate, instrumentation for the size characterization of
synthesized MagNPs is the transmission electron microscope (TEM) [93]. Sample preparation for TEM imaging can be laborious and artifacts may be introduced during the preparation procedure, such as aggregation of MagNPs from a liquid suspension on a TEM sample grid that can result in the formation of clumps or multiple layers of particles. It is also possible to damage the MagNPs during high-resolution imaging, especially in the case of MagNPs smaller than 5 nm [93]. Furthermore, it is extremely time-consuming to obtain an accurate and representative assessment of the size attributes of the population of MagNPs with TEM. The complementary technique of dynamic light scattering (DLS), also known as photon correlation spectroscopy or quasi-elastic light scattering, has also been employed for NP size determination [94]. In this technique, the scattering properties of a suspension of NPs dispersed in a suitable solvent are measured and analyzed to determine the hydrodynamic size of the particles. Due to the presence of the organic surfactant typically found on the surface of NPs synthesized from wet-chemistry processes, the average particle size obtained from the DLS method tends to be larger than that determined by TEM, as the organic surfactant component is not electron-dense. Moreover, if the surfactant used during NP synthesis is a highly charged molecule, additional solvent molecules and salts can bind to particle surface, further increasing the hydrodynamic diameter of MagNPs.

The correlation of the magnetic response $m(H)$ as a function of applied field $H$ with the size of a distribution of MagNPs has been performed using the Langevin function [95] and the Chantrell equation [96]. While these approaches are useful for the determination of a close approximation of the average particle size characteristics, these functions typically do not account for interparticle interactions; MagNP size distributions
attained from these methods typically assume the presence of a log-normal distribution function of sizes [97], which may or may not match actual particle population characteristics. Furthermore, as these functions assume zero coercivity and zero remanence, these models are not able to effectively map low temperature measurements accurately [96]. Moreover, while the above-described analysis methods provide an average particle size under specific assumptions, they do not yield a particle size distribution. It is therefore desirable to use a simple and accessible method for the determination of average particle size and distributions that can accurately replicate the relevant characteristics of NPs samples for a variety of applications, including biomedicine.

2.2 Cell Separation Techniques

Taking a look at the other key aspect of this dissertation, the isolation of pure populations of cells from heterogeneous cell suspensions is an essential part of clinical as well as basic research. For example, the diagnostic test for HIV infection relies on the separation of human lymphocytes from whole blood prior to enumeration of ones white blood cell count. Furthermore, cell separation and enrichment is an essential part of ongoing research efforts aimed at understanding fundamental aspects of biology and disease [30, 98, 99]. In the last five to ten years, miniaturized cell separation devices have been shown to offer many advantages compared to conventional separation techniques (e.g. density gradient centrifugation) such as small sample volumes, portability, low cost, improved sterility, and potential for integration with analysis techniques. Many of these new technologies are derived from the semiconductor industry and/or from advances in synthetic chemistry. This review aims to provide an overview of
the current state of cell separations and illustrate the need to reduce the size of the analyses. The goal of this critical assessment to first describe the traditional techniques used in the clinic for the collection of cancer cell populations followed by comparing these approaches to the new micro-scale cell isolation methods. Overall this review aims to illustrate the current shortcomings in present cell separation approaches and motivate the need for new and better developed micro-scale separation technologies.

### 2.2.1 Traditional Cell Separation Techniques

Traditional cell separation techniques use the various differences among cells such as size, density, expression of cell membrane antigens, and osmolarity to selectively isolation the target cell(s) of interest. Several of these techniques suffer from laborious manual sample preparation steps (i.e. density gradient centrifugation, rinsing, lyses and labeling), inconsistent results and increased cost [30]. In addition these techniques require significant processing volumes (>7.5 mL) which limits there application in clinical environments.

One common modality is the use of specific target antibodies for cell membrane antigens as a way to separate cell populations on the basis of affinity. In fluorescence activated cell sorting (FACS), antibodies tagged with fluorescent dyes are attached to cells in mixed suspensions via receptor-ligand binding. The cells are then sorted individually based on fluorescence and light scattering. Although this technique can provide highly pure (95% or higher) cell populations, it requires expensive equipment (>80,000) and has a limited throughput (~10⁷ cells hr⁻¹) [20, 100]. An alternative to FACS uses antibody-coated magnetic beads along with magnetic collectors as a means of separating target cells populations. This technique, called magnet-activated cell sorting
(MACS), allows target cells to be processed in parallel, allowing faster separation ($\sim 10^{11}$ cells hr$^{-1}$) of high-purity cell populations [20]. A common element of both FACS and MACS is the need for 30-60 mins of pre-processing incubation to attach the various tags (fluorescent dyes and magnetic beads, respectively) onto cell surfaces. This step can be avoided by immobilizing antibodies onto surfaces or substrates; this is the principle behind cell-affinity chromatography (CAC). CAC systems can provide satisfactory throughput ($10^8$-$10^9$ cells hr$^{-1}$) with yields and purity comparable to FACS and MACS [100, 101]. Most of these systems have a packed bed design, which maximizes surface area per unit volume, but also results in long residence times (on the order of 1-2 hrs for effective separation) [100-102]. Furthermore, CAC systems are capture based approaches and there currently exist few viable platform for the release of the adhered cells for further analyses and culturing[103], a key aspect in clinical research.

A classic technique is density gradient centrifugation. A density gradient is established along a tube subjected to a centrifugal force. Cells move in the tube under the action of the centrifugal field and stop at the position where their density is equal to the density of the surrounding medium [104]. This technique requires $a$ priori knowledge of the density of the target cell (or cells) and a solution capable of generating a density gradient must be used as the medium. Generally several steps are required to effectively isolate one target cell from heterogeneous suspension and density gradient centrifugation is again non-systematic, similar to size-based techniques described previously.

In addition to affinity-based techniques, several separation techniques involve size-based approaches. These size-based approaches are traditionally utilized in the case of unknown surface markers or large differences in either size of one particular cell type
in suspension. A commonly sized-based separation technique is the use of cell strainers [105-108]. Here, the cell suspension is separated according to size, where the smaller cells are allowed to pass through pores of well-defined size where the larger cells then remain in the strainer. This technique is often non-systematic and prone to non-specific cell isolation and a lack of controllable process parameters.

Purity and throughput are two important measures for a separation process, and often one is sacrificed for the other rendering an optimal separation impossible. All of the size-and density-based techniques have both a limitation of purity and throughput. In the case of FACS the purity is sufficient, but there is a lack of high throughput. MACS, on the other hand, maintains both high purity and throughput, but requires large expensive equipment and relatively high sample volumes [20]. These inherent advantages and disadvantages of MACS-based cell separation illustrate the need for new and innovative magnetic based separation technique capable of both the current high throughput and high purity enrichment of conventional instrumentation while being economical and able to process low volumes. More detail on the specifics of conventional MACS along with some of work in the last five years to address this shortcomings of high cost and large sample volumes which will be explored in the next section.

2.2.2 MACS Approach in Circulating Tumor Cells Isolation

Several magnetic-based isolation methods (also call immunomagnetic methods) have been developed to isolate CTC from blood samples. As described earlier magnet-activated cell sorting (MACS) requires pre-process incubation of antibody coated magnetic beads with the cell suspension of interest. There are two different approaches that exist: negative selection (depletion of non-target cells from suspension) and positive
selection (isolation of CTCs from blood). For negative selection, magnetic beads are coated with antibodies specific to hematopoietic (or blood) cells (such as antibodies against CD45) [98-100], therefore the non-selected cell fraction contains the CTCs. The advantage of this technique is that the resulting cells are unlabelled and therefore unaffected by the tagging procedure. For positive selection of CTCs, magnetic particles are used that are either coated with antibodies against transmembrane proteins, such as epithelial cell adhesion molecule (EpCAM) [6, 109-112], or intracellular proteins such as cytokeratin (CK) [113-116], which results in labeling of the target CTCs and subsequent capture and isolation. Many times both negative and positive selection is used to enrich the isolation and ensure a pure population of CTCs in suspension, where the blood cells are first depleted via negative selection followed by positive selection of the target CTCs population. This technique minimizes non-specific collection of contaminating blood cells and significantly increases purity.

A variety of antibody-coated magnetic particles, ranging from macro-scale iron beads to colloidal iron (ferrofluids; 50-250 nm), are commercially available [117]. The advantage of the positive-selection immunomagnetic separation technique is the high enrichment, ranging from $10^3$ to $10^5$-fold, which leads to improved tumor cell recovery and increased sensitivity of detection [118]. However, non-malignant epithelial cells or blood cells expressing EpCAM could also be enriched (0.1 cells mL$^{-1}$) [22]. In addition some CTCs are negative for EpCAM, such as cells associated with melanoma [119]. For this reason, some investigators prefer negative selection, where only leukocytes are depleted and the enrichment of tumor cells is not dependent on the expression of epithelial-specific antibodies. Based on the literature, however, positive selection
technology is still currently superior to current negative selective methods (56% versus 23% detection) [6, 111]. Hence there is some utility in using a two step, negative followed by positive selection, isolation approach.

In addition to traditional MACs approaches, several groups have developed alternative macroscale magnetic approaches. Chalmers and co-workers [120] engineered an electromagnetic negative depletion, enrichment process for the isolation of CTCs as illustrated in Figure 2.2 The authors first lyse red blood cells, a standard first step in most MACS processes, followed by CD45+ cell depletion by negative selection. Their efficiency, defined as the number of target cells captured from the initial suspension, was over 99% with a purity, defined as the number of target cells divided by the total number of cells isolated, of 95%; better than commercial positive selection techniques (e.g. CellSearch systems), with efficiencies and purities of ≥85% and 90%, respectively [22, 121]. Although a promising tool in CTC enrichment, this approach requires 10-18.5 mL of blood for analyses and pre-processing of the red blood cells.

Figure 2.2 Electromagnetic apparatus utilized by Chalmers and co-workers [122].

In many cases drawing this volume of blood from a cancer patient would ultimately be deleterious to the health of the patient. Therefore, an ideal separation process would require minimal volumes of blood and required little pre-processing beyond particle
incubation. In addition, as illustrated in Figure 2.2 the separation apparatus, although more economical than commercial products, used by the authors requires large bench spaces and is not a feasible design for point-of-care diagnostics.

An alternate and more simplistic approach to CTC isolation was recently reported by Davis and co-workers [123], in which the authors isolated circulating epithelial cells from whole blood using a “magnetic sweeper device” (Figure 2.3). Their technique uses round-bottom neodymium iron boron permanent magnetic rods covered with non-biofouling plastic sheaths. Epithelial cells were labeled with commercial magnetic microparticles and captured by the magnetic rods from a well-plate filled with 1 mL whole blood.

![Figure 2.3](image)

**Figure 2.3** Pre-labeled cells are loaded into capture wells. The magnetic rods covered with plastic sheaths are swept through the well. After sweeping, the sheathed magnets are washed in a circular loop to remove loosely bound contaminating cells. The rods then immersed into new buffer solution and disengage from plastic covers. The external magnets located under wells facilitate release of labeled cells and excess magnetic particles. Adapted from Talasaz *et al.* [123].

Non-specifically bound cells are rinsed off and captured epithelial cells are released. This reported technique has the capability to process 9 mL of blood per hr, double to triple the processing rate of standard MACS instrumentation. The authors report an efficiency of 60-80% isolation while achieving close to 50-100% purity of the target
cells. The large range that was reported can be attributed to differences in the number of background cells in suspension, due to patient to patient variation. Large populations of non-target cells result in higher non-specific binding causing interferences. Although there is still a need to improve both the efficiency and purity of associated with this device, the overall simplicity and throughput of the device illustrates that complex devices are not needed to separate cells effectively.

All these aforementioned macroscale approaches effectively reduce the cost of instrumentation but still all fail to reduce the process volumes to that needed in the clinic. In order to successful reduce required volumes for cell separation the entire analysis chamber volume needs to be decrease. The following sections details how several groups have achieved a reduction in process volumes via microfluidic devices.

2.2.3 Microfluidic Cell Separation Methods in Circulating Tumor Cell Isolation

As a means of miniaturizing the analysis microfluidic devices have recently emerged as effective tools for cell separation. Since cells are on the order of microns in diameter and usually handled in aqueous suspensions, micro-scale cell separation offers the advantages of requiring significantly smaller sample volumes versus that required by conventional separation techniques. Additionally, microfluidic devices are portable, low cost and can be easily integrated with numerous analytical techniques (e.g. fluorescence microscopy, polymerase chain reaction (PCR), and genomic analyses).

Microfluidic devices are traditionally fabricated using lithographic techniques developed in the semiconductor industry. The advent of “soft lithography”, a technique for fabricating or replicating structures using elastomeric stamps, molds, and conformable photomasks, resulted in a process whereby an elastomeric polymer (typically
poly(dimethylsiloxane), PDMS) can be poured onto a lithographically patterned mold, cured, and then peeled off [31, 33, 102]. The soft lithography process allows the fabrication of a near limitless supply of patterned (Figure 2.4) polymers that may be bonded directly to glass slides to create functional fluidic devices. This ability to create replicas of a specific pattern, termed “rapid prototyping”, has been widely recognized for its cost effective and simple nature [31, 33, 102].

Figure 2.4 Schematic illustration of the procedure for casting PDMS replicas from a master patterned wafer. This process is called soft lithography. Adapted from Xia and Whitesides [124].

This fabrication process allows a range of microfluidic devices, from simple parallel plate flow chambers to highly complex systems (such as sieves, valves, and even electrical and optical interfaces) to be formed [34-42]. This versatility allows for simultaneous manipulation of various parameters during fabrication and experimentation.

The systematic nature of microfluidic cell separation is exemplified by the availability of parameters that can be manipulated to influence the separation process in terms of purity and throughput, a limitation of conventional cell separation. These parameters include flow rate and feature dimensions in the case of the size-based
approach, and shear stress and surface composition in the case of adhesion-based separation. These features and their effects are discussed in the following sections.

2.2.3.1 Size-Based Microfluidic Separation

Several unique size-based cell separation techniques have been integrated into micro- and nano-scale fluidic devices [30]. In most cases, the devices force a fluid containing a heterogeneous cell population through a series of channels or obstacles of varied heights and widths. Unlike macroscale size-based separation approaches, the microscale geometry of the flow channels results in low Reynolds’ numbers (<1). This characteristic, in turn, results in predictable and reproducible cell movement by laminar fluid flow, where the cells follow the momentum vector along the transverse direction within the fluid stream. The main advantage of the size-based cell separation approach is that it does not require the presence of cell specific markers or proteins to achieve cell separation. Hence this approach can be used to separate cells whose marker are not fully characterized, such as progenitor cells and other rare cells. Furthermore, since the microfluidic devices do not contain any biological markers or proteins, they have a long shelf-life and are easily transportable. However, the nature of these devices makes it difficult to maintain cell viability as the cells pass through the microfluidic device due to mechanical stresses. Furthermore, size based technique are limited to a narrow set of applications as most cells have similar morphology and diameters.

Mohamed et al. [125, 126] designed a simple size-based separation device to isolate metastatic cancer cells from peripheral blood, or the blood distant to the heart. Four successively narrow arrays of parallel channels ranging from 20 µm down to 2.5 µm in thickness were fabricated into the device as illustrated in Figure 2.5.
essentially acts like a sieve-based separation device, trapping larger cells such as mononuclear cells and neuroblastoma cells further upstream in the larger channels, and allowing smaller cells such as erythrocytes to pass through unimpeded. The large cell are then rinsed out and collected. This approach may only be applied to separation systems of mixed cell populations provided that the two cell types are sufficiently different in terms of their size.

![Figure 2.5](image.png)

**Figure 2.5** Size-based separations using a cell sieve using a series of channels with successively smaller width. Flow is parallel to the channels as illustrated by the arrow [125].

This technique has an additional significant limitation in the form of non-specific cell adhesion to the channel walls resulting in clogging of the sieves and loss of target cell isolation purity. Viability of the cells along with efficiency and purity were not reported. No further development in this device design has been reported by the authors in literature.

Similar to the method described above, Tan *et al.* [127, 128] developed a label-free microdevice capable of isolating CTCs from whole blood via differences in cell diameter (cancer cells are 15-30 µm whereas blood cells are 4-10 µm). They were able to achieve an efficiency of approximately 80%. Analogous to the device of Mohamed and
co-workers, their device is based on a sieve design, where target cells are captured by narrow sieves and non-target cells continuously flow through the device. A shortcoming of this chamber, however, is the low flow rate that must be utilized in order to capture these dynamic cell populations. Deformation of the cells due to mechanical stresses can easily dislodge the cells from capture and thus high throughput can not be achieved within this particular design.

One of the most promising size-based approaches towards isolation of CTCs from whole blood was reported by Lin et al. [129]. As shown in Figure 2.6, the device is conducted of membrane filter between two polymer slabs. The larger CTCs are trapped by the 8 micron pores, while the blood cells are able to pass through the membrane.

![Diagram of device assembly](image.png)

**Figure 2.6** Illustration of device assembly by Lin and coworkers[129]. A, schematic drawing of a functional microdevice consists of parylene membrane filter sandwiched between rectangular PDMS slabs and clamped in between acrylic jigs with inlet and outlet for syringes. B, bright field image of an optically transparent parylene filter with uniformly shaped and spaced 8-μm pores. C, scanning electron microscope image of single cultured tumor cell captured on the membrane.
One of the main advantages of this particular device is the extremely high processing rate of over 4 mL per min via manual syringe injection; well above any throughput currently reported. Furthermore, this capable of >90% recovery with high enrichment factor (7 logs), and is superior to the FDA-approved method currently available (CellSearch systems).

As described above, size-based approaches are limited to cell population with differing physical properties such as diameter. Although this is generally the case for CTCs, where the cancer cells are distinguishably larger than the surrounding leukocytes and erythrocytes populations, in some cases the CTCs from specific primary tumors have been found to be on the order of leukocytes and thus could not be separated by size-based means. Furthermore, these techniques are commonly susceptible to non-specific binding of both target and non-target cells, and loss of viability due to the mechanical stress associated with trapping of the cells. To avoid many of these limitations researchers reduce the flow rate, thus reducing stresses, but this results in decreases in throughput and renders the size-based techniques unfeasible. These shortcomings illustrate the need for a more specific and broad separation modality for the isolation of cells. Affinity separation technologies (based on cell membrane chemistries) allow for such cell enrichments without non-specific cell collection and mechanical disruption of cell viability. These techniques will be described in more detail in the following two sections.

2.2.3.2 Adhesion-Based Microfluidic Separation

Adhesion-based cell separation systems are akin to chromatography columns whereby a mixture is passed through a column packed with beads or other materials capable of binding to the selected constituents of the feed thus immobilizing these
constituents. In the case of cell separation, antibodies immobilized on surfaces are used for binding. An important advantage of this technique is that it may be used to separate cell populations with similar size and/or density, such as subpopulations of human lymphocytes. Another advantage of this approach is that there is no need for pre-processing incubation of the starting cell mixture with fluorescent or magnetic antibody tags, as described earlier. This step is especially important in separations where it is essential to minimize cell activation, which may result in gene expression and phenotype changes [130]. Macroscale separation designs also minimize residence times inherent in macroscale CAC techniques (on the order of 1-2 hr) [100-102], reducing throughput. Microfluidic CAC systems provide high surface areas per unit volume but their small overall fluid volume keeps residence times short (order of mins or less) [130, 131].

A well known example of adhesion-based microfluidic separation was presented by Toner and co-workers [132-136]. The authors have developed a microfluidic platform capable of CTC capture with EpCAM-antibody-coated microposts (Figure 2.7(a)).

![Figure 2.7](image)

**Figure 2.7** Scanning electron micrograph (a) of a capture CTC (pseudo-colored red) on a post-array design. The inset shows a magnified view of the cell. Adapted from Nagrath *et al.* [134]. Fluorescence (b) and bright-field image (c) of captured CTCs (green) and non-target white-blood cells (red) on a herringbone device. Adapted from Stott *et al.* [137]

The “CTC-chip” successfully identified CTCs in over 99% of cancer patients in a comparable throughput as traditional separation techniques. This device was reported to
identify cells in concentrations as low as 5 cells/mL, similar to conventional approaches [109]. Although a unique and effective tool for CTC identification, the authors recover a low proportion (~65%) of target cells from a known spiked sample and the captured cell population was less than 50% pure (i.e. half of the cells captured were not target cells). In addition, the number of target cells captured is determined by fixation with formalin and immunostaining for cytokeratin followed by manually counted by raster scanning the device on a fluorescence microscope. Although this procedure allows for identification of the CTCs versus non-target cells within the chamber, it is cumbersome and ultimately terminates all further live cell experimentation. Therefore, in order to develop an effective monitoring tool for therapeutic use, the recovery and purity must be improved relative to that of traditional methods (FACS and MACS), >95% and >90%, respectively [20, 100]. Also a new enumeration methodology needs be developed that will allow for further cellular experimentation and that does not require the additional fixation and staining steps. As a method to improve capture efficiency and purity, a novel herringbone geometry was employed by the same group [137]. As shown in Figure 2.7(b,c), a zig-zag pattern in fabricated into the device. This pattern causes mixing of the flowing blood sample and thus results in higher wall collisions and higher capture. Although the authors illustrated that the herringbone geometry allowed for lowest capture thresholds, i.e. capture of very low concentrations of cells in the blood, the purity of the captured cell population was ~10-25%. This immediately precludes these devices as a viable platform for proteomic and genomic analysis. Unfortunately, this new design still fails to address the need for a new enumeration method, which requires fixing, staining and manual counting.
Another adhesion-based technique that was recently developed by Adams et al. [138] uses an EpCAM antibody similar to that employed in the above study but they were able to significantly improve the purity of the isolated cells. Work by the same group then conducted a similar study using prostate specific membrane antigen (PSMA) for the capture of prostate cells from whole blood [139]. After immunochemical capture, the cells were released via enzymatic digestion and enumerated by a label-free solution conductivity route in which the differences in impedance associated with the CTCs versus blood cells were probed. This method was able to distinguish between cancer cells and blood cells by size and thus improve the purity of the cell count. This method is also much easier, quicker, and more accurate than manual counting. While a unique and interesting approach to improving purity and improving efficiency (97%) versus that developed by Nagrath et al. [134], enzymatic exposure can cause morphological changes due to a disruption of the cell membrane and glycocalyx, leading to losses in cellular activity and viability. Furthermore, enzymatic digestion has been shown to directly affect both the behavior and chemical makeup of the cells themselves [140, 141]. Integration of a platinum electrode, utilized as the conductance probe in this study, can increase cost and add complexity to the overall design, especially in the case of blood based cell capture methodologies.

Xu et al. [142] developed an adhesion-based device using an aptamer-based approach, in contrast to EpCAM used by other groups. Aptamers have been the focus of much recent work in cancer related research and development especially in potential diagnosis and therapy. Briefly, aptamers are selected by an in vitro process known as cell-SELEX, or Systematic Evolution of Ligands by EXponential enrichment [143], from
a pool of DNA or RNA candidates. Aptamers bind with high specificity and sensitivity to a variety of molecular or cellular targets. One major drawback of current affinity-based microfluidic systems is that few highly selective and high-affinity antibodies or ligands towards cancer cells are known beyond EpCAM. By contrast, however, one key benefit of using aptamers derived from cell-SELEX is precisely that these aptamers can be created without knowing the explicit molecular signature information that differentiates cancer cells from healthy cells. Because a healthy cell is used as a control, and then the target cancer cell is used to subtract the DNA sequences which bind to its cell-surface markers from the DNA library, cell-SELEX aptamers can be used for detection and enrichment before the corresponding antibody has even been developed for a specific cancer. Unfortunately, the cell-SELEX process used to obtain these aptamers is a very complex and tedious process. The authors report target cell capture efficiency comparable to that of prior studies (65-97%) [20, 132-134, 136-139]. In this study the cells were released via mechanical disruption of the aptamer-cell bond and subsequently cultured to validate viability and function; collecting only 47% of the capture cells for culture. This efficiency is too low for a clinical setting or for accurate diagnostics. Also, the effectiveness of the device for whole blood samples still needs to be investigated along with assessment of non-specific binding of healthy leukocytes (a problem encountered by Nagrath et al. [134]). In a similar study by Wan et al. [144] aptamers against epithelial growth factor receptor (EGFR) were used to capture glioblastoma cells with an efficiency of 62%. In this particular case the captured cells were not released, but again a capture efficiency of 62% is much lower than ideal.
As a means of improving the capture efficiency and enhance the purity of the captured CTCs within the context of immunoaffinity-based approach, Hong and co-workers [145], co-immobilized with EpCAM and selectins (P- or E-selectin). By adding the selectin molecules to the surface of a straight channel microfluidic device the authors could guide the cells via dynamic cell rolling queues. It was shown that the immobilization of E-selectin had a three-fold enhancement in cell capture and yielded capture efficiencies of 35-40% after the cells had cycled through the device five times at a flow rate upwards of 100 µL min⁻¹. Considering that cells were run through the device multiple times (up to 5) a capture efficiency of 40% is still well below the many of the aforementioned conventional or microfluidic techniques.

Another example of an adhesion-based CTC isolation was presented by Cheung et al. [146] who employed anti-N-cadherin as the capture ligand within the device as a means of capturing breast and prostate cancer cells from homogeneous cell suspensions from buffer. This design had the ability to capture ~100% of all the cells in approximately 10 min of static incubation. The authors then mechanically detached the cells under various flow rates and accelerations, detaching nearly half of the captured cells with a viability of 80 ± 5%. Similar to the study by Xu et al. [142] this number is too low for clinical applications. Furthermore, the study was conducted under static conditions in a buffer solution, an unrealistic design. Static capture do not allow for differentiation of specific binding and non-specific binding due to cellular surface remodeling.

In total, these studies illustrate that for selective cell capture, adhesion-based designs are a viable platform for diagnostics and clinical applications. But most clinical
and biological assays require release and collection of CTCs. Therefore, the shortcomings of current microfluidic techniques lie in the non-specific binding of the non-target cell populations and difficulty in detachment for further experimentation. Furthermore, immunoaffinity-based systems are limited by the heterogeneity of expression of the target antigens, and are also limited to the types of tumors expressing these antigens. Several different enumeration techniques and/or detachment methods have been implemented with varying degrees of success, however, comparable separations to that achieved by traditional FACS and MACS isolation technology have yet been achieved.

2.2.3.3 Microfluidic MACS

As a means of circumventing the cell binding and detachment difficulties previously discussed, magnetic particles have been utilized for application in the field of microfluidic cell separation. Several microfluidic MACS approaches have been described in recent years and can be broadly classified into two categories: (a) capture within magnetic traps followed by release, and (b) separation by displacement within a flow channel. In the latter category, displacement can be achieved by either external permanent magnets or magnetic field guides. To date there have been no magnetic based microfluidic CTC separation devices, therefore the following review will focus on current developments in microfluidic MACS which can then be paralleled over to applications in rare cell separation. In MACS, prior to cell separation, suspensions are incubated with magnetic particles coated with antibodies specific to the target antigens. Similar to traditional MACS described earlier, the tagged cells can then be enriched in the presences of an applied field.
In trap-based MACS, magnetic moieties are attracted to a magnetic element, which is either a low coercive, “soft” magnetic material such as permalloy [53, 147-150] or current-carrying conductors [151, 152]. Both the passive and current-carrying magnetic elements can be fabricated into arrays with scalable trapping abilities (Figure 2.8). The trajectory dynamics of magnetic trappings can be studied to optimize the specific cell capture [44, 45].

However, the traps are generally fabricated to discriminate between magnetic and non-magnetically labeled cells and cannot discriminate between particles are varying magnetic potentials. In addition, these traps not likely to capture large amounts of cells, because once a trap is occupied by a cell it becomes difficult or unable to capture a second cell due to steric hindrance. Depending on channel geometry and constraints large particle aggregates can form and clog the channel minimizing capture efficiency. Another shortcoming that may arise is non-specific binding of the cells to the magnetic pillars or traps causing a reduction in cell release.

An alternative to trapping of magnetically-labeled cells is displacement-based MACS. In this approach, cells are continuously flowed into a channel while being
exposed to an applied magnetic field. This applied field results in phoretic displacement of the magnetically-labeled target cells across the channel laterally due to a magnetic force in the direction of the magnetic field gradient according to the equation:

\[ \vec{F}_m = \frac{V_p \Delta \chi}{\mu_0} (\vec{B} \cdot \nabla)\vec{B} \]

Eq. [2.1]

where volume of the particle is \( V_p \), \( \mu_0 \) is the permeability of vacuum equal to \( 4\pi \times 10^{-7} \) Tm A\(^{-1}\), \( \Delta \chi \) represents the difference in magnetic susceptibility between the particle (\( \chi_p \)) and the surrounding fluid medium (\( \chi_{med} \)), and the magnetic flux density is \( \vec{B} \) (details of the derivation of this equation are described in Section 3.2.1). One approach by Pamme and Wilhelm [49] utilized a permanent magnet (NdFeB) positioned alongside the microfluidic channel to displace magnetically tagged cells populations perpendicular to the flow direction. This group was able to effectively displace two different cell types by tagging them with beads with differing magnetic susceptibilities. The larger moment particles were displaced a further lateral distance compared to the lower moment particles. Therefore, this technique allows for differential separation of populations within a single device. The integration of a magnet requires no complicated microfluidic construction, as the magnet can be simply placed outside of the system but lacks the precise tunability and on/off capabilities. Furthermore, as the size of the permanent magnetic is orders of magnitude larger than the particles, the resultant field gradient is low compared with other approaches.

Instead of a single large permanent magnet, micromagnetic structures can be fabricated and employed as a means to increase local magnetic field and applied field gradient, subsequently increasing the magnetic force on the labeled cells. These
micromagnets can be comprised of either of ferromagnetic materials or of current-carrying conductors. In one example, Inglis et al. [46, 47] placed parallel nickel strips at the bottom of a microfluidic channel. The nickel strips were magnetized by an external magnetic field and produce a periodic magnetic field gradient. This approach allows for directed displacement of the tagged cells along the nickel strips. A similar approach was employed by Soh and co-workers [43] to separate two different populations of bacteria by manipulation of the viscous force along with magnetic force on particle by using two different diameter particles, resulting in different magnetic moments. However, the magnetic field gradient was fixed in these cases and, in general, is less controllable unless adjusted by an external field. In addition, the choice of geometry is also limited because of magnetic shape anisotropy [48]. Martin et al. [26] were able to overcome the anisotropy limitation by placing a series of current-carrying wires within the device at an angle relative to the flow. These current-carrying conductors allowed for tuning of the magnetic field and allow for more freedom in channel geometry. Despite this advantage electrical currents consume a considerable amount of energy and the Joule heating must also be factored into any device design, as magnetic field is linear with the current (I) but heating is proportional to $I^2$.

In summary, there are several approaches to cell separation for diagnostics with either a permanent magnetic or electromagnetic, having many advantages and disadvantages. Any one of the above approaches could be extended to a rare cell separation chamber with the correct optimization of the magnetic field, flow rate, and tagging ligand. Unfortunately, flowing whole blood into the devices developed by Soh et al., Inglis et al and Martin et al would result in a biohazardous chamber and would render
the device an expensive one time use device. Therefore the analysis chamber and magnetic field source should be kept separate to minimize channel fabrication cost, while maintaining a tunable applied field for separation applications.

2.2.4 Model-Based Magnetic Microfluidic Design

As a means to fabricate an optimal microfluidic MACS separation system various parameters must be simultaneously manipulated. To avoid the expenses associated with design of experiment and trial and error approaches, a mathematical and computational modeling method allows for optimization on paper prior to designing an experimental system. As described above there remains two outstanding challenges in the field of microfluidic MACS. First, miniaturization of the entire separation apparatus without the need for large permanent or electromagnetic. Second, prior work has focused entirely on buffer solution analytes where a successful clinical device would need to process untreated blood; a rational device design approach that takes into account the non-Newtonian characteristics of blood from the outset needs to be undertaken.

Several groups have developed mathematical models for particle (micro- and nano-scale) movement in either a buffer [53, 153-158] and blood system [45, 51, 52, 153], but little work has been done on cell separation modeling in fluidic systems. It is not sufficient to merely extend particle models to cell displacement as the viscous drag that is present in the case of cells is an order of magnitude or more higher than the sub-micron particles alone. In addition, a cell will have several particles attached to its surface, influencing the magnetic forces experienced by it.

A recent study by Baier et al. [50] took a computation fluid dynamics approach to developing a microfluidic cell isolation device. The authors used a first principles
approach derived from Navier-Stokes and the mass continuity equations, but model the magnetic field as a localized dipole, \textit{i.e.} a magnetic field generated from a 1-D point in space. This assumption is not a realistic approach to a functional device as any applied magnetic field that will be found in experimentation is at least 2-D in nature. Additionally, the authors assume a maximum number of 8 particles attached to the cell, whereas in a realistic system there could be upwards of 1000 particles attached to the cell [18]. The authors allude to this fact in their work and claim that this limitation does not severely affect the results, but the authors’ computation model illustrates a linear relationship with respect to particle number. A difference of nearly 1000 particles will in fact limit the final computation result.

2.3 Summary

In summary, the above critical literature review illustrates the need for better \textit{a priori} understanding of MagNP properties during synthesis, especially chemical effect and size distribution analysis. Both characteristics factor into the evaluation of a viable magnetic-microfluidic cell separation platform and, as described in Section 4 and 5, both these parameters directly factoring into the “bottom-up” rational design formulated. Thus precise measurement and understanding can strengthen the accuracy of the described computational optimization.

Secondly, the above evaluation reinforces the need for a model based “bottom-up” approach to the development of two part (analysis chamber and magnetic field source) microfluidic magnetic particle cell separation device. The distinct advantaged of microfluidics in the clinical environment is the ability to use small volumes of whole blood. If an economical microfluidic device can be developed with comparable
throughputs and efficiency to the current state of art technique of MACS, a clinically viable cell separation platform can be achieved. As there is currently a lack of microfluidic modeling literature, there exists a need to better understand microfluidic MACS and the forces involved in optimization before development. Overall the combined probing of the nanoparticle properties (i.e. chemical ligand exchange influences and sizing attributes) along with detailed rational design of microfluidic design will allow for the fabrication of a robust CTC separation device.
3.0 MATERIALS AND METHODS

This section outlines the experimental details associated with the two main objectives of this dissertation. As described in Sections 1.0 and 2.0, this dissertation is made up of two separate components that interface at the development of a magnetophoretic microfluidic rare-cell isolation device. First, Section 3.1 describes the synthesis of iron-oxide nanoparticle suspensions, followed by the characterization of the resulting biocompatible iron-oxide nanoparticle ensemble. Simultaneously, a magnet-based microfluidic design was computationally optimized, where as part of the rational design, several parameters were experimental determined, as described in Section 3.2, to enhance the robustness of the resulting device. Sections 3.3 and 3.4 described the experimental methods associated with the validation of the computational design and the adaptation of the device for blood samples, respectively.

3.1 Magnetic Nanoparticle Synthesis and Exchange

There were three objectives in this particular section of the dissertation. The first objective was to synthesize well dispersed, narrowly distributed iron oxide nanoparticles; the second was to characterize the influence of conducting a ligand exchange on the resulting hydrophobic nanoparticles; and the third was to development a facile determination technique to determine the size attributes of the nanoparticles. Overall the goal was to fabricate a usable nanoparticle collection for implementation into a magnetophoretic microfluidic cell separation device.

3.1.1 Nanoparticle Synthesis

In method I, ferrous chloride (FeCl₂) and ferric chloride (FeCl₃) were co-precipitated in a sodium hydroxide solution, known as the Massart method [159]. The
ferrous chloride and ferric chloride was mixed in a molar ratio of 1:2 in deionized water at a concentration of 0.1 M iron ions in a round-bottom flask under constant agitation. A highly concentrated solution of sodium hydroxide (10 M) was then added to the solution drop wise to form a dark black iron-oxide nanoparticle suspension (as shown in Figure 3.1(a)).

![Diagram](image_url)

**Figure 3.1** Iron oxide synthesis methods. (a) Schematic illustration of the ferrous and ferric chloride co-precipitation to form magnetite. (b) Schematic illustration of the thermal decomposition method, where iron salts are decomposed to form iron oxide nanoparticle stabilized with oleic acid and oleylamine. Images were adapted from images kindly provided by Dr. Dattatri Nagesha.

In method II, a high temperature thermal decomposition method was used to synthesize Fe$_3$O$_4$ nanoparticles as described by Sun *et al.* [160]. Synthesis was carried out in two steps: (i) synthesis of seed particles and (ii) growth of larger particles from the seed particles. The seed particles were synthesized by mixing iron (III) acetylacetonate (Fe(acac)$_3$, 2 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and phenyl ether (20 mL) and magnetically stirring at room temperature under argon (as shown in Figure 3.1(b)). The mixture was first heated from room temperature
to 473 K and maintained at this temperature for 30 mins. Heating was then increased to reflux temperature of $T = 538$ K for an additional 30 mins. After cooling to room temperature, the nanoparticle seeds were collected and re-dispersed in hexane.

3.1.2 Seed Mediated Growth

Larger particles were then grown from the seeds described in method II above by mixing 50 mg (dry weight) seeds in hexane with the initial chemical mixture described above to produce a hydrophobic particle mixture consisting of larger iron oxide nanoparticles. The seed-mediated growth is hypothesized to be necessary as the seed particle from literature are approximately 4-6 nm in diameter and too small for cell separation techniques.

To achieve the goals of the study described in Section 5.2, three particle size distributions were synthesized via MagNP growth on top of seeds of different sizes, including narrow, monodispersed magnetite populations and two different polydispersed, multimodal MagNPs population. It is recognized that the size of synthesized MagNPs can be approximated by the ratio of iron salt to surfactant in the reaction mixture, the reaction temperature and time, and the choices of salt, surfactant and solvent compositions. These parameters, combined with rapid stirring of the reacting mixture during heating, ensure the formation of multiple, simultaneous nucleation sites leading to a narrow MagNP size distribution and a uniform size. These particular reactions follow a growth model known as LaMer and Dingar growth model [161]. As shown in Figure 3.2, in a homogeneous precipitation, a short single burst of nucleation occurs when the concentration of constituent species reaches critical supersaturation. Then, the nuclei obtained are allowed
to grow uniformly by diffusion of solutes from the solution to their surface until the final size is attained.

![Graph showing nanoparticle growth stages](image)

**Figure 3.2** Schematic representation of the different mechanisms of nanoparticle growth in the LaMer and Dinegar growth model. Schematic courtesy of the Advanced Colloidal Materials Group at the Instituto de Ciencia de Materiales de Madrid (http://www.icmm.csic.es/gsc/sol.htm)

To achieve monodispersity, these two stages must be separated and nucleation should be avoided during the period of growth (curve I). Another mechanism proposes that uniform particles can also be obtained as a result of aggregation of much smaller subunits rather than continuous growth by diffusion (curve II), which was not investigated in this dissertation. However, uniform particles have also been obtained after multiple nucleation events, in this case through a self-sharpening growth process called Ostwald ripening (curve III). This is the case that was investigated in these experiments. When the same chemical reagents are subsequently mixed with previously-synthesized MagNPs, which act as seed particles, and then are subjected to the same reaction
conditions, iron salt decompose on the surface of MagNPs present in the solution, resulting in the formation of larger particles via seed-mediated growth. It is important to note that the ratios of all reagents should be selected to ensure that iron salt decomposition occurs only on the surface of the original MagNPs and does not independently decompose to form new individual MagNPs. When the chemical reagents are mixed into a solution containing two differently-sized NPs, thermal decomposition of iron salt may occur on the surface of both the smaller seed and the larger seed-mediated NPs, creating a multi-modal size population. If the reaction conditions are not properly chosen, a non-uniform particle size distribution can result, containing unreacted seed MagNPs as well as larger-sized MagNPs growing on surface of the seed NPs.

3.1.3 Ligand Exchange

As the resulting oleic acid stabilized nanoparticles were hydrophobic there is a clear need to exchange the surfactant to a more amendable hydrophilic ligand. Therefore, a room temperature ligand exchange reaction using dopamine was investigated to convert the nanoparticles from the hydrophobic to the hydrophilic state [162] as shown in Figure 3.3.

![Figure 3.3](image)

**Figure 3.3** Schematic illustration of ligand exchange from oleic acid to dopamine surfactant on Fe₃O₄ nanoparticles.
Dopamine has amine functionality upon exchange and would provide a viable platform for biomolecule attachment. Briefly, 10 mg of dopamine was dissolved in dichloromethane; 10 mg of nanoparticles, also in dichloromethane, was then added and shaken overnight to facilitate ligand exchange. Dichloromethane was evaporated, dopamine-exchanged particles rinsed with hexane to remove excess surfactant and resuspended in 1X phosphate buffer solution (pH 7.4).

3.1.4 Characterization

For the ligand exchange studies described in Section 5.1 and Section 3.1.2, X-ray diffraction was used to confirm the structure and phase purity, and inductively coupled plasma-optical emission spectroscopy (ICP-OES) was employed to determine the iron content of the MagNP. In the case of ligand exchange this will allow for validation of iron losses before and after exchange. The size and morphology of the nanoparticles was characterized using a JEOL-JEM 1000 transmission electron microscope (TEM) instrument operated at 75 kV. The particle size distribution was measured by taking the largest internal diameter of ~250 particles. Magnetic studies were carried out using a superconducting quantum interference device (SQUID, Quantum Design MPMS XL-5) magnetometer. Field-cooled and zero-field-cooled (FC/ZFC) curves collected from 2 K - 300 K at the applied field $H_{\text{appl}} = 100$ Oe and hysteresis curves measured at temperatures of 6, 10, 50, 150, 200, 250, and 300 K in the field range $-5 \text{kOe} \leq H_{\text{appl}} \leq 5 \text{kOe}$. The saturation magnetization $M_S$ can be estimated from the measured hysteresis loops using the $1/H$ law approach [163] while the particle diameter determined by applying the Langevin function to the data; the Langevin function will need to be modified to include
a paramagnetic contribution previously shown to be present in similar hysteresis measurements [164]:

$$M(H) = M_S \left( \coth \left( \frac{\mu_p H}{k_B T} \right) - \frac{k_B T}{\mu_p H} \right) + \chi_a H$$  \hspace{1cm} \text{Eq. [3.1]}

where $\mu_p$ is the magnetic moment per particle provided as $\mu_p = \frac{M_S \pi D^3}{6} \text{(emu)}$ with $D$ equal to the particle diameter in centimeters, $k_B$ the Boltzmann constant and $\chi_a$ the high field susceptibility attributed to the organic stabilizer and hypothesized paramagnetic surface contributions in the iron oxide nanoparticles. All data was normalized to the mass of iron present in each sample, as determined from elemental analysis.

On the other hand, for the size distribution study described in Section 5.2 and Section 3.1.1, the physical size, distribution, and morphology of the three MagNP populations were characterized using a TEM instrument operated at 75 kV. Samples were prepared by drying a 5 µL droplet of dilute stock solution (1:100 hexane:stock) on a formvar-coated copper TEM grid. The particle size histogram was obtained from measurements of approximately 250 NPs, with the internal diameter recorded for spherical particles and corner-to-corner distance recorded for cuboids. Magnetization studies were carried out on the aforementioned particle samples using the Quantum Design SQUID magnetometer. For magnetic measurements, the samples were dried onto small 3 × 3 mm Si wafers with approximate weights of 0.005 g. The temperature dependence of the zero-field-cooled (ZFC) magnetic moment, $m_{ZFC}(T)$, was collected in the temperature range of 3.5 K - 300 K in an applied field $H_{appl} = 50$ Oe. As iron-oxide MagNP used in both of the studies were produced using the same synthesis methods, the
structure and phase purity result from the ligand exchange (Section 5.1) investigation was paralleled to the sizing studies (Section 5.2).

### 3.2 Experimental Methods for Quantification of Key Device Parameters

In parallel with the MagNP investigations, the microfluidic component of the overall goal was investigated. As described in Section 4.2, the device was designed via a computational rational approach. In conjunction with a mathematical optimization a parameteric analysis of key characteristics of the cells and tagging particle was conducted. The derivation of the motion of a cell-particle complex in the described microfluidic design must be completed by determination of realistic values for applicable parameters within the mathematical expressions. To this end, the attainment of reasonable values for cell radius ($R_c$), magnetic susceptibility ($\chi$), and particle binding characteristics ($\phi$) was achieved via experiments conducted on a breast cancer cell line that served as a model for metastatic tumor cells. As it was determined from theoretical calculations that the aforementioned magnetic nanoparticles are not feasible in the context of the specific separation device described herein (see Section 5.3.3 for details), Dynabeads® MyOne™ superparamagnetic microbeads (Invitrogen, Carlsbad, CA) were used as the model tagging particles to bind to the cells. These beads are composed of highly cross-linked polystyrene with superparamagnetic magnetite nanoparticles embedded within their matrices. These microbeads are coated with carboxylic acid (-COOH) groups that allow for the conjugation of biomolecules to their outer surface. According to the manufacturer, the magnetic particle diameter is $1.05 \pm 0.03 \, \mu m$ ($R_p=0.525 \pm 0.015 \, \mu m$), the magnetic mass susceptibility is $85 \times 10^{-5} \, m^3 \, kg^{-1}$, and the density is $1800 \, kg/m^3$, which corresponds to a volume magnetic susceptibility $\chi = 1.5$. 


An approximate particle concentration value of $7-12 \times 10^9$ particles per mL of stock particle suspension was also provided by the manufacturer. However, it should be noted that all these parameters may vary across lots and must be validated experimentally, as described below, to ensure a realistic rational device design. Quantitative results of the key parameters necessary to determine the device performance are provided in the last part of this section.

### 3.2.1 Model Cell Culture Conditions and Characteristics

MCF-7 human breast adenocarcinoma cells (ATCC, Manassas, VA) were cultured in 75 cm$^2$ tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO$_2$ and 95% air. The cells were incubated in Eagle’s Minimum Essential Medium (EMEM; ATCC) supplemented with 10% fetal bovine serum, 100 U mL$^{-1}$ penicillin, 100 µg mL$^{-1}$ streptomycin and 0.01 mg mL$^{-1}$ bovine insulin. Cells were grown to pre-confluence and isolated for experiments by trypsinization using a 0.25% Trypsin-EDTA solution.

The average cell radius was determined via electronic volume using a Coulter counter (Cell Lab Quanta™ SC; Beckman Coulter, Brea, CA) and compared to size-calibration beads (Flow-Check™ Fluorospheres; Beckman Coulter). The resulting average radius was also validated by bright-field microscopy with manual assessment of the radius of a number of the cells.

### 3.2.2 Magnetic Particle Diameter and Characteristics

The magnetic microbead radius was determined via field emission-scanning electron microscopy (FE-SEM; Hitachi S4800, Peoria, IL) of a dried particle suspension. Dried stock suspension was mounted on aluminum stubs and sputter-coated with gold-palladium to $\sim 2$ nm thickness to provide a connection path for electron density in FE-
SEM examination. The experimentally-determined particle concentration of the stock solution was verified against the concentration provided by the manufacturer. The stock suspension of particles was diluted 10,000× and counted using a hemacytometer and a Nikon TE2000 Inverted Microscope employing Nikon Elements Advance Research software.

The magnetic susceptibility of the polymer/magnetite beads was confirmed via superconducting quantum interference device (SQUID; Quantum Design MPMS XL-5, San Diego, CA) magnetometry. A 2 µL droplet of stock suspension was dried on a formvar-coated copper transmission electron microscopy grid (Electron Microscopy Science, Hatfield, PA). Magnetic hysteresis loops were measured at 300 K in the field range $-5 \text{kOe} > H > 5 \text{kOe}$. The moment of a blank grid was also measured and subtracted from the measured data; data were normalized to the mass of particles. The magnetic character obtained from three replicates was averaged and the volumetric susceptibility was determined using the density values for the particles provided by Invitrogen.

### 3.2.3 Particle-Cell Attachment Density

A binding assay was conducted to determine the number of particles that can attach to MCF-7 cells. DynaBeads® MyOne® Carboxylic Acid particles were modified with antibodies against the epithelial cell adhesion molecule (anti-EpCAM; Santa Cruz Biotechnology, Santa Cruz, CA) using standard carbodiimide chemistry [165] in ratios suggested by the carbodiimide coupling reagent manufacturer (1:1 molar ratio of beads to protein; Pierce Biotechnology, Rockford, IL). Modified particles were incubated with approximately $1 \times 10^6$ cells for 30 min. in 1 mL EMEM at concentrations of 0.1 mg mL⁻¹.
1, 0.5 mg mL$^{-1}$, and 1 mg mL$^{-1}$. Following incubation, the cell-particle complexes were removed from suspension using a permanent magnet and were then incubated with a fluorescently-labeled antibody against EpCAM (anti-EpCAM-FITC; Santa Cruz Biotechnology) for 30 min. at a concentration of 1:100 (v/v). A cell suspension containing zero particles was also incubated with anti-EpCAM-FITC at the same concentration for comparison. In both cases cells were separated from suspension via centrifugation at 190 × g for 5 min. The supernatant was retained and dried in order to assess the mass of particles that remained after tagging, representing the unbound particles. All fluorescently-tagged cells were resuspended in phosphate buffered saline (PBS) and subsequently analyzed for available receptor densities using flow cytometry. Results concerning the number of free receptors were compared with an antibody binding capacity calibration curve (Quantum™ MESF Beads; Bang Laboratory, Fishers, IN) to determine the initial number of available receptors on each cell (no particle attachment) and the number of receptors remaining after particle incubation (post-particle attachment). Comparison of the unbound particle mass, along with data obtained from untagged particle densities and the remaining receptor number after particle incubation, allows for estimation of the average particle density on an individual cell.

3.2.4 Fabrication of a Validation Design

Three different device layouts (Figure 3.4) were drawn using AutoCAD software and printed with high resolution on a transparency (FineLine Imaging, Colorado Springs, CO). Each channel length and height was maintained at 50 mm and 50 μm, respectively. Only the widths of the devices were varied to allow for model validation at three different displacements (250 μm, 500 μm, and 1000 μm). The resulting photomask was used to
generate a negative master for device fabrication at the George J. Kostas Facility for Microfabrication at Northeastern University.

![Figure 3.4](image)

**Figure 3.4** Schematic of the three device geometries fabricated from the rational design. The three device layouts are (a) 1000 μm, (b) 500 μm, and (c) 250 μm in width and are designed in order to form three separate streams. The outside streams initially contain the target cells labeled with magnetic beads, which are displaced into the center over the length of the channel.

Silicon wafers were coated with SU-8-50 photoresist to a thickness of approximately 50 μm and then exposed to ultraviolet light (365 nm, 11 mW/cm²) with the mask overlaid using a Quintel 2001 mask aligner. Unexposed photoresist was removed using SU-8 developer and feature height was measured using a Dektak surface profilometer (Veeco Instruments, Santa Barbara, CA).

To generate PDMS replicas, a mixture of silicone elastomer and curing agent (10:1 ratio) was poured over the master wafers, degassed, and allowed to cure overnight in an oven at 65 °C. The cured PDMS was then cut using a scalpel and pulled off the wafers. Inlet and outlet holes were punched on each PDMS replica prior to bonding. The bonding process consisted of exposing the PDMS replicas and the glass slides (60 (L) × 24 (W) × 0.15 (H) mm³) to an oxygen plasma (50 mW with 8% oxygen for 30 sec.) in a
PX-250 plasma chamber (March Instruments, Concord, MA), immediately bringing the PDMS replicas and the glass slides in contact, and then baking the device for 5 min at 65 °C. This process creates an irreversible bond between the PDMS replicas and the glass slides and also prepares both surfaces for chemical surface modification. See Figure 2.4 for a general schematic of the fabrication process.

Wire arrays were designed using PCB123® printed-circuit board design software and ordered from Sunstone Circuits (Mulino, OR). The wire dimensions were set to provide a gap encompassing the width of the device microfluidic channel; the height and width of the all of the wires were set to 35 µm and 178 µm, respectively. Teflon-insulated 18G copper wires were soldered to the ends of each of the printed circuit board arrays and the arrays were connected to a DC power supply (Elenco Electronics XP-4, Wheeling IL) that provided three fixed-current setting at 0.25 A, 0.50 A and 1.00 A via standard alligator clip connectors. The PDMS channels and wire arrays were then visually aligned.

3.2.5 Validation of Sheath Flow

To ensure that the two sample streams and the center collection stream (Figure 3.5) do not mix a simple validation experiment was devised. A 1:10 diluted suspension of Texas Red IgG solution (Vector Labs, Burlingame, CA) was flowed into the center inlet at 120 µm min⁻¹ whilst a PBS buffer solution was flowed into the sample stream at 240 µm min⁻¹.
Figure 3.5 Schematic illustration of the device. The current carrying wires are placed to the outside edges, beneath the channel, and the target and non-target cells are displaced by the applied magnetic field generated by the wires.

These flow rates represent the least laminar flow-like conditions within the rational design model described in Chapter 4. Fluorescent images were taken at the entrance, middle, and exit of the microfluidic device under a Nikon Eclipse TE2000 inverted microscope.

3.2.6 Homogeneous Cell Suspension Validation

Preliminary validation studies of the sheath device were conducted with a homogeneous suspension of MCF-7 carcinoma cells (describe above in 3.2.1) in phosphate-buffered saline at a cell concentration of $1 \times 10^5$ cell mL$^{-1}$, incubated with the optimized magnetic microparticle concentration (described in 3.2.2). The cells were injected at a controlled flow rate using a Harvard Apparatus PHD2000 syringe pump (Holliston, MA). Several combinations of applied current, flow rate, and channel widths were investigated. By selecting specific combinations of these three variables it is possible to probe designs which should separate all cells, designs that should not separate the cells, and which should separate some of the cells.

A second validation of the rational design was then probed as a means to test cell concentration influences on the efficiency of separation for a single set of device parameters. A device made with optimal design parameters, as determined from the
model and experimental preliminary validation, was employed to test the efficiency of cell isolation as a function of MCF-7 concentration in buffer (10 – 10,000 cell mL⁻¹).

3.3 Separation of Cancer Cells from Heterogeneous Suspension

It should be noted that separation of a cell population from a homogenous cell suspension still remains too ideal to conclude that the presented device design optimization will successfully process to a heterogeneous suspension analyte. All biologically-relevant cell suspensions have several additional characteristics that are anticipated to affect the separation character of the device, such as cell-particle migration under shear conditions, flow and collision-induced aggregation, and unintentional labeling of non-target cells with magnetic beads. To test the influence of non-target cells on separation a model heterogeneous suspension with MCF-7 cells spiked into a dense suspension of Raji B-lymphocytes was prepared.

3.3.1 Spiked Cell Experiments

Human mature naïve B-lymphoblast (Raji; ATCC) cells were cultured in 75-cm² tissue culture flasks at 37°C in a humidified atmosphere with 5% CO₂/95% air. The cells were incubated in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin. The cell suspension was centrifuged at 190 × g for 5 min and then resuspended in PBS to remove dead cells and cell debris. The cells were resuspended at a concentration of approximately 10⁶ cells mL⁻¹ (measured using a hemacytometer).

Several different total numbers of MCF-7 cells (1000, 100, and 10 cells) were spiked into the Raji cell suspension prior to mixing with the Dynal® MyOne™ EpCAM-functionalized magnetic microbeads. This will allow the EpCAM specific beads to
interact with both the target cancer cells and non-target lymphocytes thus assess (i.) the total number of non-target cells separated from the heterogeneous suspension due to non-specific binding of the magnetic particles. Mixing of the two populations with also allow for the assessment (ii.) of high concentration of non-target cells (as obstructions) on the efficiency in which MCF-7 cells are separated. In addition, to further test if non-target cell concentration plays a role in the purity of the isolated sample the initial Raji concentration was varied from $1 \times 10^6$ to $1 \times 10^4$ cells mL$^{-1}$. The flow rates of the sheath fluid in the experiments were also varied within the constraints set by the calculated rational design (Section 4.2) and homogeneous validation described above (Section 3.2.6).

To count the number of target (MCF-7) and non-target (Raji) cells that were separated a flow cytometry protocol, based on the distinct size difference of these two cells, was created. The cells were gated by their electronic volume and the total number of cells within the recovered suspension was assessed.

### 3.3.2 Viability of Recovered Cells

Cell viability was examined for cells isolated from the microfluidic devices. For viability analysis, the isolated cells were incubated with a 4 μM solution of EthD-1 (dead cell indicator) in PBS and a 2 μM solution of calcein (live cell indicator). Live and dead cells were counted using flow cytometry. Electron volume and side scattering gates were established using homogeneous suspensions of MCF-7 cells. Gating for live cells was established via incubation of freshly cultured cells in calcein. To determine the dead cell gate, cells were incubated in 70% ethanol for 15 min., followed by incubation with EthD-1.
To verify the health of the recovered cells, the cells from the target stream were centrifuged (along with any unbound particles displaced) and pipetted into a 96-well plate. The triplicate wells were then inspected and imaged 24 hr later to assess if the cells were healthy. As a comparison, untagged target cells were run through the device and collected in order to assess if the high flow rate, *i.e.* high shear forces, affects the cells ability to adhere and spread on the surface. The influence of particles was also investigated by adding tagged cells which were not run through the device. Finally, a control sample used to compare the state of the cells after 24 hr. All four conditions were examined with ~1000 cells per 250 μL and run in triplicate.

3.4 Separation of Spiked Cancer Cells from Whole Blood

As a further improvement on the heterogeneous suspension experiments described above and to more closely mimic the clinical setting, MCF-7 cells were spiked directly into whole blood. The ultimate goal of this dissertation is to develop a whole blood-based separation platform which would best suit clinical applications. Whole blood was drawn from healthy volunteers and collected in EDTA-coated Vacutainer® tubes (Becton Dickinson, Franklin Lakes, USA). Approval from the Northeastern University Institutional Review Board was obtained for this purpose.

3.4.1 Determination of the Blood-Buffer Interface

Prior to validation experiments, the location of the interface that forms between the injected blood (η ~ 2-4 cP) and buffer (η ~ 1 cP) was evaluated. As blood is a non-Newtonian, shear thinning fluid, it behaves differently from cells in buffer solutions and thus the required displacement for effective isolation is changed. The results of this evaluation influence the rational design optimization described in Chapter 4. To examine
test this interface, blood and buffer were injected into a sheath-based microfluidic
displacement device at equivalent flows (started at 120 μL min⁻¹) and subsequently tuned
as to provide comparable displacements to the buffer-buffer system previously tested.
The interface was monitored under an inverted microscope under brightfield illumination.
Three sections of the channel were monitored: the entrance for sufficient hydrodynamic
focusing of the buffer stream, the center of the device (in the y-direction) to determine the
width of the blood streams and buffer stream, and the exit outlet to ensure no cells from
the blood are traveling into the collection stream where only targets should be collected.

3.4.2 Whole Blood Cancer Cell Isolation Experiments

As an experimental test to determine the capabilities of the fabricated microfluidic
channel for rare-cell isolation, a concentration of 50 MCF-7 cells per mL was spiked into
blood, followed by mixing in the Dynal® MyOne™ EpCAM functionalized magnetic
microbeads. This experiment was conducted at the pre-determined flow rates for
efficiency segregation of the blood-buffer and allows for the assessment of (i.) the total
number of non-target cells separated from the heterogeneous suspension due to non-
specific binding of the magnetic particles to red blood cells, platelets, and/or white blood
cells and (ii.) the influence of high concentration of non-target cells (as obstructions) on
the efficiency in which MCF-7 cells are separated. Furthermore, this spiked blood
suspension closely mimics the clinical situation and should provide the most accurate
assessment of the robustness of the device and if this device is a viable platform in CTC
isolation.
4.0 THEORETICAL FORMULATIONS

The analytical analysis described in Section 4.1 was formulated as a means to develop a simple and accessible method for the determination of average particle size and distributions that can accurately replicate the relevant characteristics of nanoparticles (NPs) samples for a variety of applications, including biomedicine. To this end, a convenient method developed by DiPietro et al. [166] was applied for determining the magnetic volume or size attributes of an ensemble of magnetic nanoparticles (MagNPs), which is based on temperature-dependent magnetic (thermomagnetic) measurements. This work is distinct from previous reports of particle ensemble attributes attained from magnetic characterization [97, 167-169] as it describes conversion of the measured magnetic moment directly into a particle size function representing the probability distribution of magnetic particle size, containing both the average particle size as well as the distribution of sizes. The described approach, while similar to previous work on nanostructures reported by DiPietro et al. [166], focuses on magnetite MagNP suspensions. As data are analyzed within the framework of the superparamagnetic response, this analysis additionally provides quantitative insight into the effective magnetocrystalline anisotropy constant characterizing the MagNP distribution. Magnetocrystalline anisotropy, which is derived from the electronic spin-orbit interaction [67], is highly sensitive to the local atomic environment, and is thus anticipated to be different in bulk and nanostructured materials. Recently-published work employed this thermomagnetic technique to determine particle diameter and size distribution of self-assembled MnAs nanoparticles in MnAs-GaAs composite films as a means of determining the effects of annealing temperature on particle formation and the effective
anisotropy of the resulting particles [166]. In the current work, the model is applied to Fe₃O₄ MagNPs and compared to results derived from electron micrographs of MagNP populations, in order to determine the size distribution of a given MagNP ensemble and provide the ensemble anisotropy characteristics. By simply determining the systems’ magnetic characteristics as a function of temperature, it is demonstrated that the size attributes of narrow, monodispersed particle collections as well as complex, multimodel particle ensembles may be evaluated with good accuracy.

The methodology described in Section 4.2 applies a rational computational design approximation to the development of a magnetophoretic cell isolation chamber. In contrast to prior microfluidic designs which employ static magnetic separation [158], adhesion-based separation [142] or dielectrophoresis technology [170], this dissertation describes a continuous-flow, magnetic displacement-based design, wherein cells labeled with magnetic particle tags are separated from a sample stream under the influence of a magnetic field without mechanical disruption and/or post-process analysis (e.g. immunohistochemical identification or colony-form unit assays). The applied magnetic field of this rational design is generated by an integrated electromagnet (current-carrying wire) located below the microfluidic channel. Electromagnets have two distinct advantages over designs that utilize permanent magnets [49, 171-173]: firstly, they can be easily switched on/off to facilitate cell capture and release, and secondly the strength of the resultant magnetic field may be tuned by varying current. In the microfluidic device context, electromagnets have seen limited use because they typically produce weak magnetic fields and they generally require at least two steps of lithography that must be repeated in the fabrication of each device. In addition, bulkiness of the electromagnet
and potential Joule heating derived from large currents flowing through the electromagnet coil can quickly become problematic [171]. The device design described in Section 4.2 addresses these limitations by creating a new microfluidic device design derived from first-principles and rational design parameters.

As the ultimate aim of the device described herein is for application in diagnostics and regenerative medicine, additional constraints and conditions exist that need to be addressed within the model. Point-of-care diagnostic devices typically utilize a biological fluid sample analyte, such as blood, interstitial fluid, saliva, vaginal fluid or cellular material, or nasal fluid. As a means of minimizing contact with the analyst, or of contaminating further tests, the microfluidic chamber should be disposable [35, 174]. Thus it is desirable to separate the microfluidic component of this system from the reusable electromagnetic components of the design. In addition to addressing biohazard considerations, this arrangement will significantly reduce cost associated with device manufacture and implementation.

In contrast to prior models of continuous flow magnetic-microfluidic separation devices [28, 85, 158, 175], the specific advance articulated in this paper is the development and implementation of a realistic rational design based on practical experimental constraints (as described in Section 3.2 and Section 5.3) and desired need for a microfluidic system capable of delivering both high efficiency and high purity. The described approach directly accounts for variations in key parameters within the cells, tagging particles, and device and addresses several key parameters which may advance this device to clinical and bench-top applications. Furthermore, the rational design allows for direct assessment of the feasibility of implementing nanotechnology (as
described in Section 5.3.1) into a simple, electromagnetic-based microfluidic cell separation platform. Although many modeling approaches have been well established in the literature [28, 85, 158, 175], these examples fail to address all of the requirements of a clinically usable cell separation platform. The magnet-based cell separation device presented here aims to incorporate clinical diagnostic considerations ab initio by constraining the device microfluidic channel dimensions to a practical scale (i.e., that of a microscope slide) and incorporating disposable and non-disposable components (fluidic part and magnetic part, respectively) in the device as a means of reducing cost. Furthermore, the incorporation of a tunable electromagnet (relative to state-of-the-art on-chip designs that employ permanent magnets[85, 175]) maximizes versatility in addition to reducing device cost. In addition, the design presented here accounts for drag forces experienced by cells tagged with hundreds of magnetic beads. This approach is more realistic for continuous-flow cell separation compared to that described by prior theoretical/computational models that only consider the manipulation of magnetic micro- or nanoparticles in the absence of cell attachment [85, 158, 175], as cells are generally much larger in size relative to the particles. The device model described here also introduces a new and unique sheath-based design in which a system of two electromagnets acts co-operatively to displace cells within a central microfluidic channel.

4.1 Thermomagnetic Analysis Derived from the Size-Dependent Response of the Magnetic Nanoparticles to Thermal Fluctuations

A thermomagnetic analysis was derived from consideration of the size-dependent response of the MagNPs under study [166]. Superparamagnetic particles are nanometer-sized ferromagnetic/ferrimagnetic entities with large magnetic moment and a moment orientation that is unstable with respect to thermal fluctuations when the ambient thermal
energy exceeds the magnetic anisotropy energy [176]. Each individual MagNP possesses a thermal blocking temperature, $T_B$, which marks the transition from a kinetically-responsive magnetic moment to a kinetically-unresponsive moment with decreasing temperature ($T$). For $T < T_B$, thermal fluctuations in the orientation of the moment of each particle are slower than the measuring times, so the particle moment remains fixed and coercive. For $T > T_B$, thermal fluctuations allow the moments to align with a small measuring field, where they exhibit large-moment paramagnetism with a $1/T$ behavior given by [177]:

$$m(T) = \frac{H m_S^2}{3 k_B T}$$

Eq. [4.1]

where $H$ is the applied magnetic field, $m_S$ is the saturation magnetization, $k_B$ is the Boltzmann constant. The magnetic moment (or equivalently, the magnetic susceptibility) $m(T)$ of the MagNPs ensemble is essentially zero for $T < T_B$, but increases as particles are thermally unblocked for $T > T_B$. This phenomenon results in a peak in $m_{ZFC}(T)$ near $T_B$. Figure 4.1 shows the measured temperature dependence of the magnetic moment of Fe$_3$O$_4$ NPs for ZFC conditions in a small measuring field of $H_{appl} = 50$ Oe. The ZFC data display a maximum at $T_{MAX} = 30$ K. Due to interparticle interactions and system inhomogeneities, $T_{MAX}$ may not coincide with the blocking temperature $T_B$ of the particular MagNP ensemble.
Figure 4.1 Magnetic moment versus temperature of Fe₃O₄ nanoparticles in the seed particle sample. The solid line represents the magnetic moment measured for zero-field-cooling condition at applied field of 50 Oe. The dashed curve shows the blocking temperature distribution derived from the ZFC cooling data and Eq. [4.4].

The procedure that allows determination of particle diameters from thermomagnetic data is based on the Néel relaxation model [178, 179] which describes the relaxation of magnetization in the absence of an applied field for non-interacting, single-domain MagNPs which possess anisotropy. In a uniaxial anisotropy system a double-well potential energy diagram, with each well representing a stable spin direction, may be constructed with the wells separated by an energy barrier, $E_B$. Thermal activation will assist the reversal of the magnetization from one spin direction to the other over the barrier with a relaxation time of:

$$
\tau = \tau_o \exp\left( \frac{E_B}{k_B T} \right)
$$

Eq. [4.2]
where $1/\tau_o$ is the reversal attempt frequency and $E_B = KV$, where $K$ is the effective uniaxial anisotropy and $V$ is the particle volume. As the typical measurement time for a SQUID magnetometer is on the order of $10^2$ sec and the attempt time is estimated $10^{-9}$ sec [67, 180], the ratio of anisotropy energy to thermal energy is:

$$\frac{KV}{k_B T_B} = 25.$$  

Eq. [4.3]

It should be noted that there exist several limitations to this procedure that must be considered for application: (i) the magnetic field applied for ZFC measurements must be small compared to the anisotropy field so that the effective barrier height is not extrinsically lowered [181, 182], (ii) the model neglects particle-particle dipolar interactions, which increases the apparent blocking temperature [183], and (iii) lower-symmetry (i.e. cubic) magnetocystalline anisotropy, as found in the iron oxides of this study, causes a reduction in the effective barrier height [67]. To address considerations (i) and (ii) in this work, a small applied field of $H_{\text{appl}} = 50$ Oe was applied following zero-field cooling, which is well below the anisotropy field of iron oxide $H_K = 1200-1400$ Oe [184, 185]. To address consideration (ii) interparticle dipole interactions and (iii) cubic anisotropy in the iron oxide NPs, an effective anisotropy constant $K_{\text{eff}}$ was utilized that includes several anisotropy contributions including magnetocrystalline anisotropy, shape anisotropy, surface anisotropy, and dipole-dipole interactions between MagNPs; the effective anisotropy concept is commonly employed in the context of MagNP ensembles [186-188]. With these considerations in mind, it is possible to apply the thermomagnetic characterization method to the materials of this study and obtain useful information.

It should be noted that the Néel relaxation model described in Eq. [4.3] is useful for determining the magnetic volume of particles when the value of $K$ or $K_{\text{eff}}$ is known.
Conversely, if the average particle diameter is known, a value for $K_{eff}$ of the particle may be determined. Note that Eq. [4.3] is derived for a single particle volume with a unique blocking temperature $T_B$. If an ensemble of MagNPs possesses a very narrow size distribution and a relatively uniform anisotropy constant $K$, then $m_{ZFC}(T)$ will exhibit a sharp peak at $T_B$. However, $m_{ZFC}(T)$ data typically peak at $T = T_{MAX}$ ($T_{MAX} > T_B$), due to the width of the MagNP size distribution and interparticle interactions.

While it is possible to obtain an approximate distribution of particle diameter $f(D)$ by fitting the moment $m(H)$ vs. field $H$ data at a given temperature to the Langevin function and assuming that the particle diameters adhere to a log-normal distribution [96], improvement on this analysis may be achieved by incorporating the temperature-dependent magnetization of a MagNP ensemble, $m(T)$. As each particle of volume $V$ has a corresponding blocking temperature $T_B$ as described by Eq. [4.3], the MagNP size distribution may be obtained by converting the distribution in blocking temperature, $f(T_B)$, into a particle diameter distribution, $f(D)$, using the relationships shown in Eqs. [4.1] and [4.3], in addition to applying the assumption of spherical particle geometry.

The distribution of blocking temperatures $f(T_B)$ may be evaluated [189], under the simplification that temperature dependence of $m_s$ may be neglected for materials with Curie temperature $T_C >> T_B$.

$$f(T_B) = \frac{3k_B}{Hm_s^2} \frac{d}{dT} \left(T \cdot m_{ZFC}(T)\right)$$

Eq. [4.4]

A particle volume distribution function, $f(V)$, can then be obtained by converting the $f(T_B)$ into $f(V)$ via the relationship described in Eq. [4.3]. Assuming spherical particles \( V = \frac{4}{3}\pi D^3 \), the distribution in particle diameter is given as $f(D) = f(T_B) \frac{dT_B}{dD}$. 

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Therefore the ZFC thermomagnetic data $m_{ZFC}(T)$ may be directly mapped to a size distribution function that represents both the average magnetic diameter of the particles and the distribution in the particle size. Moreover, the effective anisotropy constants of known particle ensembles can also be obtained by employing a value of $K_{\text{eff}}$ that allows coincidence of the calculated distribution with the experimentally-derived distribution; in effect this shifts the peak location of the distribution function linearly.

4.2 Computational Design Approximation Towards the Development of a Magnetophoretic Cell Isolation Chamber

Concurrent with the parametric analysis of the MagNP a computation optimization of a magnet-based microfluidic platform was investigated. In the following sections, two magnetophoretic microfluidic device designs are presented, with optimized dimensions and operating conditions determined from a force balance equation that considers two dominant and opposing driving forces exerted on a magnetic particle-tagged cell, namely magnetic and viscous drag. Quantitative design criteria for an electromagnetic field displacement-based approach are presented, wherein target cells labeled with magnetic particles flowing in a sample stream are shifted laterally into a collection stream. Furthermore, the final device design is constrained to fit on standard rectangular glass coverslip (60 (L) × 24 (W) × 0.15 (H) mm$^3$) to accommodate small sample volume and point-of-care design considerations.

4.2.1 Device Geometries

This section describes the geometrical layout of the two microfluidic chambers investigated in this dissertation. In both cases, the analyte is a heterogeneous cell suspension containing cells tagged with magnetic particles in addition to non-target cells.
The design objective is to displace the target population from the mixed suspension in continuous flow.

Figure 4.2  Schematic illustration of the Generation I cell separation device design: (a) buffer stream is injected on side closest to current-carrying wire and sample stream injected to the far-side with respect to the current-carrying wire. The device length required for the target cell to displace from the sample stream to the buffer stream is $l_{ch}$. (b) Mathematical configuration of single current-carrying wire located at $(0,0)$ with current flowing in the positive $y$-direction (out of the page). The particle positioned at height above of the wire ($z=150$ µm) and distance away ($x$) from the current-carrying wire. The magnetic force vector ($\vec{F}_m$) perpendicular to direction of the magnetic field vector ($\vec{B}$). (a,c) The Generation I cell separation device displaces target cells from sample stream to buffer stream; non-target cells remain in sample stream. The distance required for complete displacement of target cells from the far-edge to interface of the sample and buffer streams is half the width of device ($x= \frac{w}{2}$).
The first-generation design (Generation I) incorporates a single current-carrying wire of rectangular cross-section located adjacent to the microfluidic channel (Figure 4.2). The generated magnetic field deflects the cell-particle complex within the biological fluid sample, which is initially located at the far-side with respect to the wire, towards the buffer stream, which is at the near-side (Figure 4.2(a,c)). The current-carrying wire is located below the microchannel and is separated from the fluidic separation channel to minimize fabrication costs (only the fluidic component needs replacement with each analysis); the wire and support may be reused for subsequent samples. This design feature requires the flow channel of the microfluidic device, and hence the cell-particle complexes within the channel, to be raised up by the thickness of a glass coverslip, or approximately 150 microns, above the current-carrying wire in the vertical \( \hat{z} \) direction (perpendicular to the plane of the chamber) as shown in Figure 4.2(b).

The incorporation of a second conducting wire in parallel alignment with the first wire allows tagged-cell displacement in both positive and negative lateral \( \hat{x} \) direction towards a center stream of buffer. This design, designated as Generation II, reduces the displacement distance required for cell isolation by increasing the magnetic forces experienced by the cell-particle complex. As shown in Figure 4.3, the current-carrying wires of the Generation II device are located equidistant from the center of the flow channel and the two currents are assumed to run anti-parallel in the \( \hat{y} \)-direction, parallel to the fluidic flow of the chamber. Here again, the microfluidic device is separated from the current-carrying wire array by a vertical distance of approximately 150 microns.
**Figure 4.3** Schematic illustration of the Generation II cell separation design: (a) injected sample split into two streams that sheath central buffer stream (c). (b) Cross-sectional illustration of magnetic flux lines resulting from anti-parallel dual-wire configuration driving cell-particle complexes to middle of device; here central rectangle represents fluid flow channel and shaded rectangles current-carrying wires.

### 4.2.2 Theoretical Formulation: Forces on the Cell-particle Complex

This section describes the derivation of an expression for the displacement of a magnetic particle in a channel subjected to both magnetic $\vec{F}_m$ and Stokes $\vec{F}_s$ forces. The magnetic force and the Stokes force contributions are considered separately; note that gravity and buoyancy forces are negligible and are thus not considered here. The effect of Joule heating is also considered in this section. Prior to derivation of the expected cell displacement under these two driving forces, the potential role of diffusion was
examined. Using the Stokes-Einstein relation [190], 
\[ D_{AB} = \frac{k_B T}{6\pi \eta R_c} \]
and the random walk theory for displacement in one dimension [191], 
\[ \Delta x \approx (2D_{AB}t)^{0.5} \]
the diffusivity \( D_{AB} \) of an average cell in buffer can be derived, as well as the transverse displacement \( \Delta x \) within the microfluidic device. It can be shown that a cell would have a diffusive constant \( D_{AB} \) on the order of \( 10^{-15} \) m\(^2\) sec\(^{-1}\) at room temperature. Assuming a channel with dimensions of \( 5 \times 0.2 \times 0.05 \) cm and a suspension flow rate of \( 10 \mu\)L min\(^{-1}\), the residence time \( t \) of a cell within the channel is 2.5 min, with lateral \( \Delta x \) diffusion of less than 300 nm. Therefore, it was concluded that the effect of diffusion may be ignored within the described design.

4.2.2.1 Magnetic Force Determination

The trajectory of a magnetically-labeled cell in the proposed microfluidic device is modeled by evaluating the forces on the cell generated by motion through the fluid under the attractive action of a magnetic field. Prior to derivation of forces on a cell-particle complex, the forces specific to a single magnetic particle are determined. In the following discussion, the particle is initially located at position \((x,y,z)\), subjected to a magnetic field \( \vec{B} \) originating from a current-carrying wire at \((0,0,0)\), as shown in Figure 4.2(b). The particle is fixed at \( z = 150 \mu\)m and moves laterally in the \(-x\)-direction, towards the current-carrying wire independent of the \( y \)-component.

A single magnetic particle is idealized as a magnetic sphere of uniform moment density. The magnetic force exerted on the particle \( \vec{F}_m = (\vec{m} \cdot \nabla)\vec{B} \), may be evaluated from the total moment on the particle \( \vec{m} = V_p \vec{M} \) which depends on the volume of the particle \( V_p \) and the volume magnetization \( \vec{M} \). Here, \( \vec{M} = \Delta \chi \vec{H} \) and \( \Delta \chi \) is the volumetric...
magnetic susceptibility difference between the particle (χ₂) and the surrounding buffered fluid medium (χ₃). The overall response of a magnetic particle in a fluid to a magnetic field is then determined by the strength and gradient of the applied magnetic field (\(B = \mu_0 H\)), yielding [152, 192]:

\[
\vec{F}_m = \frac{V_p \Delta \chi}{\mu_0} (\vec{B} \cdot \nabla) \vec{B}
\]

Eq. [4.5]

where \(\mu_0\) is the permeability of vacuum equal to \(4\pi \times 10^{-7}\ T\ m\ A^{-1}\). It should be noted that a complementary form of this equation can be determined by applying the Maxwell equation \(\nabla \times \vec{B} = 0\) to the following mathematical identity:

\[
\nabla (\vec{B} \cdot \vec{B}) = 2\vec{B} \times (\nabla \times \vec{B}) + 2(\vec{B} \cdot \nabla) \vec{B} = 2(\vec{B} \cdot \nabla) \vec{B}
\]

Eq. [4.6]

Therefore, Eq. [4.5] can alternatively be expressed as:

\[
\vec{F}_m = V_p \Delta \chi \nabla \left( \frac{\vec{B}^2}{2\mu_0} \right)
\]

Eq. [4.7]

Although Eq. [4.7] is a valuable relationship for visualizing the magnetic force operative in the system, all subsequent \(\vec{F}_m\) analyses are derived from relationships expressed in Eq. [4.5]. As the magnetic susceptibility of the surroundings is typically 5-6 orders of magnitude lower than that of the particles [171], \(\Delta \chi\) is determined primarily by the susceptibility of the particle, \(\chi_p\). By way of example, the magnetic susceptibility of phosphate buffer saline is on the order of \(10^{-7}\) and that of blood is on the order of \(10^{-6}\), while the susceptibility of commercial magnetic oxide particles is generally on the order of \(10^0 - 10^{-1}\) [193, 194]. Furthermore, it should be noted that the magnetic susceptibility of materials commonly used in the construction of a microfluidic channel has also been found to be several orders of magnitude smaller (approximately \(10^{-5} - 10^{-6}\)) [195] than
that of the magnetic beads, and thus the effect of the device itself may also be assumed to be negligible in this analysis.

With these considerations, Eq. [4.5] can then be expanded in explicit form to yield:

\[
\vec{F}_m = \frac{V_p \chi_p}{\mu_0} (\vec{B} \cdot \nabla) \vec{B} = \frac{V_p \chi_p}{\mu_0} \begin{bmatrix}
B_x \frac{\partial B_x}{\partial x} + B_y \frac{\partial B_y}{\partial y} + B_z \frac{\partial B_z}{\partial z} \\
B_x \frac{\partial B_x}{\partial x} + B_y \frac{\partial B_y}{\partial y} + B_z \frac{\partial B_z}{\partial z} \\
B_x \frac{\partial B_x}{\partial x} + B_y \frac{\partial B_y}{\partial y} + B_z \frac{\partial B_z}{\partial z}
\end{bmatrix}
\]  

Eq. [4.8]

The above equation can be simplified by assuming the current-carrying wire located at the side of the device is infinitely long in the \( \hat{y} \)-direction, thus allowing the spatial derivatives of the magnetic field vanish:

\[
\frac{\partial \vec{B}}{\partial y} = 0 \Rightarrow \left\{ \frac{\partial B_x}{\partial y} = 0, \quad \frac{\partial B_y}{\partial y} = 0, \quad \frac{\partial B_z}{\partial y} = 0 \right\}
\]  

Eq. [4.9]

Furthermore, the symmetry of the device design (Section 4.2.1) dictates that there are no off-axis components of the magnetic field gradient:

\[
\left\{ \begin{array}{l}
\frac{\partial B_y}{\partial x} = 0, \\
\frac{\partial B_y}{\partial z} = 0
\end{array} \right.
\]  

Eq. [4.10]

The first device design (Generation I) that was investigated incorporates a single, rectangular current-carrying wire placed at the periphery of the device (Figure 4.2). The wire is situated below the microchannel and is kept separate from the fluidic separation channel.

Employing the Biot-Savart Law [196] the magnetic field \( \vec{B} \) at a distance \( r \) from the current-carrying wire can be determined in cylindrical coordinates as:
\[
\vec{B} = \frac{\mu_0 I}{2\pi r} \quad \text{Eq. [4.11]}
\]

For a single current-carrying wire located at the origin (0,0,0) in Figure 4.2 with current flowing in the positive \( \hat{y} \)-directions (out of the page), Eq. [4.11] is explicitly written in Cartesian coordinates as:

\[
B_x = -\frac{\mu_0 I}{2\pi r} \frac{z}{r} = \frac{\mu_0 I}{2\pi} \frac{z}{\sqrt{x^2 + z^2}} \quad \text{Eq. [4.12a-b]}
\]

\[
B_z = \frac{\mu_0 I}{2\pi r} \frac{x}{r} = \frac{\mu_0 I}{2\pi} \frac{x^2}{\sqrt{x^2 + z^2}}
\]

The magnetic force in the tangential direction is provided as:

\[
F_{m,x} = \frac{V_p \chi_p}{\mu_0} \left[ B_x \frac{\partial B_x}{\partial x} + B_z \frac{\partial B_x}{\partial z} \right] \quad \text{Eq. [4.13]}
\]

The \( x \)- and \( z \)-components of the gradient of the magnetic field are listed below:

\[
\frac{\partial B_x}{\partial x} = \frac{\mu_0 I}{2\pi} \frac{2zx}{\left[ x^2 + z^2 \right]^2} \quad \text{Eq. [4.14a-b]}
\]

\[
\frac{\partial B_x}{\partial z} = -\frac{\mu_0 I}{2\pi} \frac{x^2 - z^2}{\left[ x^2 + z^2 \right]^2}
\]

and are substituted back into Eq. [4.13] to obtain the expression for the magnitude of the magnetic force exerted on a magnetic particle under the above stated conditions:

\[
F_{m,x} = -\frac{V_p \chi_p}{\mu_0} \left[ \left( \frac{\mu_0 I}{2\pi} \right)^2 \frac{x^2}{\left[ x^2 + z^2 \right]^2} \right] \quad \text{Eq. [4.15]}
\]
4.2.2.2 *Hydrodynamic Resistance Force Determination*

In addition to the magnetic force $F_m$ acting on the magnetic particles, there exists a viscous drag force $F_s$ acting on the particle in the direction opposite to the particle motion [190]. This drag force, or Stokes force $F_s$, is a function of the suspension medium viscosity ($\eta$), the radius of the particle ($R_p$), and the velocity $\vec{v}$ of the particle in the direction of the magnetic force. The inertial effects on the particles suspended in the fluid are negligible, as the Reynold’s number is less than unity due to the geometric constraints of the microfluidic chamber. This result implies that while the fluid exerts drag on each particle, the particles exert no force on the fluid [197, 198]. Furthermore, the number of particles within the suspension is assumed dilute, thus there exist no short-range inter-particle dipolar interaction. As the magnetic particle is carried by the flowing solution in the $\vec{y}$-direction, within the context of laminar flow, the hydrodynamic forces only act in the $\vec{x}$-direction (direction perpendicular to flow). This force can be expressed as:

$$F_s = -6\pi \eta R_p v \vec{x}$$  \hspace{1cm} \text{Eq. [4.16]}

4.2.2.3 *Joule Heating*

Resistive heating of the wires with respect to time ($\dot{q}$), or Joule heating, may limit the applied current range and geometry of the wires. Furthermore, this effect can adversely affect the flow and character of the fluid and may even degrade the device, rendering it non-reusable. Joule heating is determined by both the current conductor geometry and by the time duration of the applied current [196]; the heat generated in this manner is given by:
\hat{q} = I^2 R \tag{4.17}

Assuming no dissipative cooling, the electric resistance is equal to \( R = \frac{\rho R_{lw}}{A} \) for small wires, where \( l \) is the length of the wire, \( A \), the cross-sectional area, and \( \rho_R \), the electrical resistivity. But in a real system, there exist heat losses which greatly influence the build up of thermal energy. This heat loss is dependent upon wire surface area, the thermal properties of materials of construction, and the properties of the ambient surroundings. Therefore, while current flow is constantly heating the wire as described in Eq. [4.16], the wire is simultaneously cooling through heat transfer to the surroundings (via radiation, convection, and conduction through its surface and to the surroundings). Although all three heat transfer phenomena are occurring simultaneously, most of the energy transfer is via conduction through the substrate containing the wires and through the microfluidic device above the wires. Hence, the general Fourier’s law of heat conduction equation (or heat loss) appropriate for this situation is that mimicking an anisotropic medium, which is given as:

\[ k_x \frac{\partial^2 T}{\partial x^2} + k_y \frac{\partial^2 T}{\partial y^2} + k_z \frac{\partial^2 T}{\partial z^2} + \hat{q} = \rho_M C_p \frac{\partial T}{\partial t} \tag{4.18} \]

where \( k_x, k_y, \) and \( k_z \) are the thermal conductivities of the substrate in the \( \hat{x} \)-, \( \hat{y} \)- and \( \hat{z} \)-direction, respectively, \( \hat{q} \) is the heat generation term (or Joule heating), and \( \rho_M \) and \( C_p \) are the density and heat capacity of the substrate, respectively.

Under the assumption that the width and length of the substrate are much larger than the height, the heat transfer in the \( \hat{x} \)- and \( \hat{y} \)-direction can be ignored, thus \( \frac{dT}{dx} = \frac{dT}{dy} = 0 \). Combining Eq. [4.17] and Eq. [4.18] yields:
\[-k_z \frac{\partial T}{\partial z} = \dot{q}_o g(t) f(x) \]  
Eq. [4.19]

where \( g(t) \) defines the time varying characteristics of the current that generates the heat, \( f(x) \) is a spatial function that defines the wire locations along the \( \hat{x} \)-direction, and \( \dot{q}_o \) is the Joule heating provided at a reference current.

![Diagram showing the cross-sectional view of a printed circuit board electromagnetic array along with a PDMS microfluidic device.](image)

**Figure 4.4** Schematic illustration of cross-sectional of printed circuit board electromagnetic array along with PDMS microfluidic device used in evaluation of Joule heating constraints within rational device design. First both FR-4 and PDMS assumed to have thermal conductivity of \( \sim 0.2 \text{ W (m K)}^{-1} \) and heat transfer assumed in \( z \)-direction only. The glass coverslip employed in investigation only 150 \( \mu \text{m} \) thick (\( h \)), and assuming minimal contact with air, thermal resistance above copper wires (brown) assumed equal to PDMS alone.

Both \( g(t) \) and \( f(x) \) are controlled by the current characteristics and wire geometry, respectively. It should be noted that for the case of direct current (DC), \( g(t) = 1 \) and \( \dot{q}_o = \dot{q} \). To complete the problem statement, it is assumed that the initial temperature is set equal to room temperature (298 K). To obtain the steady-state solution for a set temperature rise as a function of current, \( I \), and wire cross-sectional area, \( A \), a classical Fourier series method is needed. As this calculation is well established and is beyond the scope of this dissertation, details of the exact solution can be found in Ref. [199].
For the Joule heating calculation, the following physical characteristics are assumed for the device. Briefly, the overall layout of the cell separation device consists of a thick poly(dimethylsiloxane) (PDMS) slab bound to a thin glass coverslip, which is mounted on thin current-carrying copper wires deposited on a printed circuit board (PCB) substrate (Figure 4.4). Traditionally, PCB copper wire arrays are mounted onto substrates composed of a material known as FR-4, which is a woven fiberglass cloth bound with an epoxy resin. For the purposes of this device model, the thermal conductivities of the PDMS [200] and FR-4 [201] were assumed to be equal to 0.2 W (m K)$^{-1}$ and of equal thicknesses (1.5 mm), with the thermal resistance of the glass coverslip ignored. Solving Eq. [4.18] at the steady state condition, the current carrying capacity, or the maximum current $I_{\text{max}}$ which results in a specified temperature increase, was computed to be approximately $I_{\text{max}} \approx 2\text{A}$ for a nominal temperature rise of 20 K and for a standard PCB copper wire array cross-section of 35 µm $\times$ 178 µm. Therefore, in the present experimental set-up, Joule heating only becomes a concern at high currents ($I > 2$ A) and/or if the wire cross-sectional area is significantly reduced.

4.2.3 Optimization of the Channel Length Device Designs

Utilizing the viscous drag $\vec{F}_s$, magnetic force $\vec{F}_m$, and Joule heating results obtained from earlier sections, it is now possible to explore the performance of the device subjected to realistic parameter values. It is desired to create a microfluidic cell separation device that delivers the greatest lateral displacement in the shortest possible channel, i.e. maximize $x$ and minimize channel length $l_{\text{ch}}$. The overall force on the magnetic particle is the sum of the magnetic force $\vec{F}_m$ (Section 4.2.2.1) and the
hydrodynamic force $\vec{F}_h$ (Section 4.2.2.2) that lends a constant velocity to the particle, which explicitly sets the acceleration equal to zero:

$$\vec{F}_m + \vec{F}_s = 0$$  \hspace{1cm} \text {Eq. [4.20]}

Eq. [4.21] yields the overall equation representing the force in the $x$-direction exerted on a magnetic particle:

$$-\frac{V_p \chi_p}{\mu_o} \left[ \left( \frac{\mu_o I}{2\pi} \right)^2 \frac{x^2}{(x^2 + z^2)^2} \right] - 6\pi \eta R_p v_x = 0$$  \hspace{1cm} \text {Eq. [4.21]}

The force balance can now be rearranged and solved for an optimized channel geometry to obtain a magnetophoretic microfluidic device design, under the assumption of fully-developed fluid flow.

4.2.3.1 Generation I Microfluidic Device Design

Rearranging Eq. [4.21] and assuming spherical geometry for the magnetic particles $\left( V_p = \frac{4}{3} \pi R_p^3 \right)$ allows determination of the velocity $\vec{v}$, normal to the magnetic field $\vec{B}$, in the $\hat{x}$-direction, of a magnetic particle flowing in the device channel. This process also allows an estimate of the time $t$ for the particle to traverse a given distance across the device channel width. In accordance with the geometry of the system, the velocity of the particle in the $\hat{x}$-direction is provided as:

$$v_x = \frac{dx}{dt} = -\frac{R_p^2 \chi_p \mu_o I^2}{18\eta \pi^2} \left[ \frac{x}{\left( x^2 + z^2 \right)^2} \right]$$  \hspace{1cm} \text {Eq. [4.22]}

Reiterating, the variables in Eq. [4.22] are: $R_p$ the radius of the magnetic particle, $\chi_p$ the volumetric susceptibility of the particle, $I$ the current applied to the wire, $\eta$ the viscosity of the carrier solution, and $z$ the gap distance fixed by the glass coverslip. It should be
noted that realization of an actual microfluidic magnetophoresis device requires the successful attachment of a large number of magnetic particles to the target cells in order to convey the largest magnetic moment possible the travelling complex. In the case of such a cell-particle complex, the above analysis is altered slightly to account for the drag forces on the cell itself, a body that is one to two orders of magnitude larger than the microparticles alone, depending on the actual tagging cell-particle characteristics. Therefore, for this case the viscous drag force on the cell-particle complex is now calculated using the larger cell radius \( R_c \), allowing the magnetic particle radius to be neglected \( \left( R_c \gg R_p \right) \).

As previously described by Chalmers et al. [18], the actual magnetic force exerted on a labeled cell derived from the magnetic field of the current-carrying wire is equal to the magnetic force on one particle (as described in Eq. [4.15]) multiplied by the number of particles attached to the cell \( \phi \), or \( \vec{F}_m \cdot \phi \). Substitution of these parameters into Eq. [4.22] allows an estimate for the displacement time of the cell-particle complex out of the stream into the buffer fluid, attracted to the current-carrying wire:

\[
\nu_x = \frac{dx}{dt} = -\frac{R^3_p \phi \chi_p \mu_0 I^2}{18 R_c \eta \pi^2} \left[ \frac{x}{\left(x^2 + z^2\right)^{3/2}} \right]
\]

Eq. [4.23]

The above differential equation may be solved analytically to obtain a solution of the form \( x = f(t) \), where \( f(t) \) is the residence time of a cell of radius \( R_c \) the microfluidic chamber. In this manner, the lateral displacement \( x \) in the device of the magnetic cell-particle complex for any given value of time \( t \) can be calculated. This quantitative estimation of the physical parameters of the cell-particle complex flowing in a laminar fashion through the designated microfluidic device allows determination of the optimal
dimensions of the device. In particular, specification of the cell-particle complex displacement time \( t \) required to traverse the channel width \( w \) permits determination of the minimum length \( (l_{ch}) \) of channel. Input of specific known parameters such as the input volumetric flow rate \( (\dot{V}) \) of the carrier fluid, the device channel height \((h)\) and width \((w)\) of the carrier fluid stream leads to calculation of the optimum length of the proposed device. The calculated residence time \( t \) may then be translated to a calculation of the lengthwise displacement \( l_{ch} \) of the cell-particle complex as the volumetric flow rate, \( \dot{V} \), is given by:

\[
\dot{V} = \frac{V}{t} = \frac{l_{ch} \cdot w \cdot h}{t}
\]

where \( l_{ch} \) represents the distance traveled along the channel along the \( \dot{y} \)-direction (lengthwise). Again, the microfluidic device design objective is to deliver the greatest lateral displacement in the shortest possible channel, or in other words, to maximize \( x \) whilst minimizing \( l_{ch} \). To this end, the solution of Eq. [4.24] for residence time \( t \) and subsequent incorporation of Eq. [4.23] yields a relationship for the lateral particle displacement as a function of the distance traveled along the channel, \( x = f(l_{ch}) \), under the influence of an applied magnetic field, with the trajectory provided below:

\[
\frac{dx}{dl_{ch}} = -\frac{R_p^3 \phi \nu h \chi_p \mu_o I^2}{18R_c \dot{V} \eta \pi^2} \left[ \frac{x}{(x^2 + z^2)^{3/2}} \right]
\]

\text{Eq. [4.25]}

4.2.3.2 Generation II Microfluidic Device Design

To determine the resultant magnetic field generated by the two conducting strips in the Generation II design, improving upon the single-wire design of Generation I, an array of conductors is considered where one conductor is positioned at far edge of the
microfluidic channel with current flowing in the negative \( \hat{y} \)-direction and the other conducting wire at the alternate edge of the microfluidic channel with current in the positive \( \hat{y} \)-direction, as shown in Figure 4.3. The magnetic field components at any point \((x, z)\) resulting from current flowing through the two conductors, set a distance equal to 2 \( X \) apart, are given as:

\[
B_x(x, z) = B^o_x(x - X, z) - B^o_x(x + X, z)
\]

Eq. [4.26a-b]

\[
B_z(x, z) = B^o_z(x - X, z) - B^o_z(x + X, z)
\]

where \( B^o_x \) and \( B^o_z \) are the field components determined for the single-wire (Generation I) configuration as specified in Eq. [4.11a-b] (as described in Section 4.2.3.1). Insertion of Eq. [4.26a-b] into the magnetic force equation (Eq. [4.13]) yields the expression for the magnitude of the attractive force exerted on a magnetic particle in the double-wire sheath (Generation II) device design configuration:

\[
F_{m,x} = \frac{V_p X_p}{\mu_o} \left[ \left( B_x(x - X, z) - B_x(x + X, z) \right) \left( \frac{\partial}{\partial x} \left( B_x(x - X, z) - B_x(x + X, z) \right) \right) + \left( B_z(x - X, z) - B_z(x + X, z) \right) \left( \frac{\partial}{\partial z} \left( B_z(x - X, z) - B_z(x + X, z) \right) \right) \right]
\]

Eq. [4.27]

Substituting this new equation into Eq. [4.20], which describes the overall force balance, and rearranging as outlined above in Eq. [4.21-4.25], yields an equation for displacement as a function of channel length in the double-wire (sheath) device design:

\[
\frac{dx}{dl_{ch}} = \frac{R_p \phi \omega h X_p \mu_o I^2}{18 R_c \eta \pi^2} \left[ \left( \frac{z}{(x - X)^2 + z^2} - \frac{z}{(x + X)^2 + z^2} \right) - \frac{2z(x - X)}{[(x - X)^2 + z^2]^2} + \frac{2z(x + X)}{[(x + X)^2 + z^2]^2} \right] + \frac{2z(x - X)}{[(x - X)^2 + z^2]^2} \left( \frac{(x - X)}{(x - X)^2 + z^2} - \frac{(x + X)}{(x + X)^2 + z^2} \right) \left( \frac{(x - X)^2 - z^2}{[(x - X)^2 + z^2]^2} - \frac{(x + X)^2 - z^2}{[(x + X)^2 + z^2]^2} \right)
\]

Eq. [4.28]
The resultant differential equation cannot be solved analytically. However, a solution of the form $l_{ch} = f(x)$ can be obtained by numerical integration using the 5th-order Runge-Kutta method and appropriate solver software, such as MATLAB®. Results of this calculation are presented in Section 5.4.3.

To reinforce the advantages of the two-wire array (i.e. Generation II) in accordance with the derived equations, it can be shown that by adding one additional wire to the array increases the displacement by at least a factor of four. Using Eq. [4.7] and the geometry illustrated in Figure 4.3, it can be seen that the magnitude of the magnetic field $\vec{B}$ would be doubled, increasing the magnetic force $\vec{F}_m$ by a factor of four. Additionally, the optimized geometry of the device results in a reduction of the displacement distance $x$ and thus, intuitively, allows a further decrease in the required channel length $l_{ch}$ versus that provided by the Generation I design.

4.3 Tuning of the Magnetophoretic Design for Application with Whole Blood Samples

To address the added complexity of using Non-Newtonian fluids, such as blood, the model framework must be augmented. The primary parameter that impacts the overall implementation of the magnetophoretic device described above for a blood-based design will be the viscosity of the carrier fluid $\eta$ in which the target cells are located. Therefore, an apparent blood viscosity ($\eta_{\text{blood}}$) as a function of height was determined [202]. This viscosity component plays a critical role in the drag force $\vec{F}_d$ experienced by the cell during displacement and may impact the interface location (i.e. blood-buffer), causing a readjustment in the displacement parameter $x$ compared with the buffer-based displacement design.
To further complicate the determination of $\eta_{\text{blood}}$ there is a known microcirculatory phenomenon called the Fahraeus-Lindqvist effect [203] that leads to a reduction in the proportion of blood volume occupied by red blood cells (or hematocrit, $H_c$) in small arterioles less than 200 microns in diameter and capillaries relative to the hematocrit of large feed arteries. This decrease in hematocrit in these flow vessels reduces the relative blood viscosity in the small vessels, which helps to offset the increase in viscosity that can occur because of reduced velocity in these same vessels. The net effect of these changes is that blood flow in the microcirculation has a lower viscosity than what is predicted by in vitro blood viscometer measurements.

Therefore, to best estimate the viscosity of blood a parametric description of apparent blood viscosity relative to the viscosity of plasma ($\eta_{\text{plasma}} = 1.5$ cP) as a function of channel height ($h_{ch}$, in microns) and hematocrit, $H_c$, will be used which was established from a collection of 18 separate studies as reported by Pries et al. [202]. Assuming velocities higher than 50 channel heights per s, the apparent viscosity was empirically determined to be:

$$\eta_{\text{blood}} = \eta_{\text{plasma}} \left[ 1 + (\eta_{0.45} - 1) \left( \frac{(1 - H_c)^C - 1}{(1 - 0.45)^C - 1} \right) \frac{h_{ch}}{h_{ch} - 1.1} \right]^2 \times \left( \frac{h_{ch}}{h_{ch} - 1.1} \right)^2 \text{ Eq. [4.29]}$$

where:

$$\eta_{0.45} = 6e^{-0.085h_{ch}} + 3.2 - 2.44e^{-0.06h_{ch}^{0.645}} \text{ Eq. [4.30]}$$

$$C = \left( 0.8 + e^{-0.075h_{ch}} \left( \frac{1}{1 + 10^{-11}h_{ch}^{12}} - 1 \right) + \frac{1}{1 + 10^{-11}h_{ch}^{12}} \right) \text{ Eq. [4.31]}$$

Here, the variable $\eta_{0.45}$ describes the apparent viscosity of at a phenotypical hematocrit ($H_c=0.45$) as a function of height (in microns). This empirical expression is a necessary
aspect to designing a realistic model of cell displacement from a blood medium to the buffer stream. By including this parameter at the onset allows for a single fabrication of a device without experimentation. To assess the location of the interface of blood and buffer a validation experiment will be needed. Details of this assessment are described in 3.4.1
5.0 RESULTS AND DISCUSSION

The results presented in Section 5.1 detail the resulting characteristics of the synthesized hydrophobic magnetic nanoparticles (MagNP) compared along side those nanoparticles which had undergone a subsequent ligand exchange as described in Section 3.1. The resulting particles have yet to be conjugated to a relevant biomolecule but an interesting increase in magnetization was shown to occur upon exchanging the oleic acid surfactant to a dopamine coating. Section 5.2 describes the development of a novel sizing technique for magnetic nanoparticles. As described in chapter 2, there exists a need for a simple, yet precise, method for determining both average particle diameter and particle distribution. The results presented in Section 5.2 aims to address this need by using the analytical analysis described in Section 4.1 applied to various MagNP ensemble.

Section 5.3.1 details the feasibility of using MagNP in cell separation using the computational analysis presented in Section 4.2. It can be shown that nanoparticles are not a viable tagging moiety in the context of the presented design. Although it is shown that nanoparticles can be used, the sample throughput is sacrificed by using MagNPs. Therefore, Section 5.3 describes the resulting rational designed derived from the computation analysis present in Section 4.2 using superparamagnetic microparticles. These commercial particles still have the advantage of superparamagnetism but allow for much higher throughputs due to their larger volume and higher susceptibility. Finally, the rational design was validated using homogeneous suspension (Section 5.3.4) and heterogeneous suspensions (Section 5.3.5) of an immortalized cancer cell line. As a final proof of principle, the rational design was re-formulated for the injection of whole blood
spiked with cancer cells. Section 5.4 details the implementation of the computationally optimized device with whole blood.

5.1 **Functionalization-induced Improvement in Magnetic Properties of Fe₃O₄ Nanoparticles for Biomedical Applications**

Uniform, spherical, nanoparticles were synthesized using the thermal decomposition method (method II from Section 3.1). The particles were shown to be dispersed by the oleic acid surfactant and no cluster was seen in the resulting nanoparticle collection as illustrated by TEM in Figure 5.1(a).

![TEM micrographs and size histograms](image)

**Figure 5.1** TEM (a,c) micrographs and size histograms (b, d) of (a, b) as-synthesized oleic acid stabilized nanoparticles and (c, d) dopamine stabilized nanoparticles.
On the other hand, it was shown that the co-precipitation of ferric and ferrous salts in sodium hydroxide (method I from Section 3.1), yielded large iron oxide nanoparticle clusters as shown in Figure 5.2. Therefore all subsequent experiments were conducted with the nanoparticles synthesized via method II. Interestingly, it was observed that the particles remained dispersed (Figure 5.2(c)) and average particle sizes were conserved upon ligand exchange from oleic acid to dopamine (described in Section 3.1.3). Figure 5.1(b,d) shows a bimodal particle size distribution approximated by log-normal distribution functions that yields an average particle diameter of 10 nm for both oleic acid- and dopamine-stabilized nanoparticles. It is apparent that a small population (< 10 %) of the 5-nm nanoparticles used as the seed for the growth method remained after exchange, indicating insufficient reaction time resulting in minimal Ostwalt ripening.

Figure 5.2 TEM micrograph of nanoparticles resulting from the co-precipitation method. Particles aggregate into large 100-200 nm clusters.

X-ray diffraction (XRD) (Figure 5.3) confirmed attainment of cubic spinel-type Fe₃O₄ with a particle size of approximately 9.3-nm derived from Scherrer broadening...
analysis of the (311) Bragg peak [180]; the calculated lattice parameter of \( a = \) of 8.416 ± 0.026 Å agrees well with literature values [204].

**Figure 5.3** X-ray diffraction pattern of nanoparticles synthesized using the thermal decomposition method (black). The pattern was compared with the known spectra of Fe₃O₄ (red), confirming the composition of the resulting nanoparticles.

There is good agreement between the size determined by XRD analysis and the TEM micrographs. The iron content was determined by ICP-OES analysis to be 54.1 ± 0.8 % and 54.7 ± 0.1 % by mass for the as-synthesized and the dopamine-stabilized nanoparticles, respectively, confirming no loss of iron upon ligand exchange. The ligand exchange process was monitored by FT-IR analysis of the surfactant molecules on the surface of as-synthesized and dopamine-exchange nanoparticles. Figures 5.4(a) and (b) show the FT-IR spectra of oleic acid and oleic acid-stabilized NPs with the characteristic \( \text{CH}_2 \) asymmetric (2922 cm\(^{-1}\)) and symmetric (2852 cm\(^{-1}\)) stretch. Note the C=O stretch at ~1710 cm\(^{-1}\) is significantly decreased Figure 5.4(b) indicating the binding of oleic acid through these carboxyl group on the nanoparticle surface. Upon ligand exchange with
dopamine, Figure 5.4(d), the characteristic peaks of oleic acid have completely disappeared indicating successful and complete exchange of oleic acid with dopamine.

![FT-IR spectra](image)

**Figure 5.4** FT-IR spectra of (a) pure oleic acid, (b) Fe₃O₄ nanoparticles coated with oleic acid, (c) pure dopamine, and (d) Fe₃O₄ nanoparticles coated with dopamine.

Figure 5.5(a) displays the field cooled/zero field cooled (FC/ZFC) magnetization trends with temperature obtained from both the as-synthesized (seed and seed-mediated growth) and dopamine-stabilized nanoparticles. All samples exhibited superparamagnetic behavior with zero remanence and zero coercivity for $T > T_B$ and significant remanence and coercivity ($\sim 200$ Oe) at $T < T_B$, where the blocking temperature $T_B$ marks the delineation between the coercive and the superparamagnetic states. It is noted that $T_B$ increases with increasing particle size from 8 K (5-nm seed) to 30 K (10-nm particle). Unexpectedly, a consistent increase in $T_B$, up to $T_B = 50$ K, was
obtained upon functionalization of the nanoparticles with dopamine, despite the fact that the physical particle size did not change.

Figure 5.5 Magnetic characterization of iron oxide nanoparticles as function of temperature. (a) FC/ZFC curves of as-synthesized 5-nm seed(- ▲ -), 10-nm seed-mediated(- □ -), and 10-nm dopamine coated(- ● -) Fe₃O₄ particles, (b) saturation magnetization using 1/H law approach at six different temperatures and (inset) shows hysteresis curves at 300 K for the three particle samples. Langevin function represented by curve fit of seed-mediated and dopamine hysteresis data.

This phenomenon also manifest in magnetization measurements (Figure 5.5(b)) that indicate the temperature-dependent saturation magnetization Mₛ(T) attains a much higher
value for the dopamine-exchanged nanoparticles as compared to the as-synthesized oleic acid-stabilized particles. The net $M_S(T)$ value increases from 38 emu/g Fe for 10 nm oleic acid-stabilized nanoparticles to 60 emu/g Fe for dopamine-exchanged 10 nm iron oxide nanoparticles. Application of the Langevin function to the 300 K $M(H)$ data (Figure 5.5(b), inset) yields a room-temperature $M_S$ value of 3.81 $\mu_B$/Fe (as-synthesized particles) and 3.87 $\mu_B$/Fe atom (dopamine-functionalized particles), close to the bulk value of 4 $\mu_B$/Fe atom in Fe$_3$O$_4$ [205]. The Langevin fit yielded a calculated magnetic particle diameter $D \sim 7$ nm for both the seed-mediated and the dopamine-coated nanoparticles that is smaller than the physical diameter of 10 nm. This result of comparable magnetic diameters was not expected, but it should be noted that the paramagnetic contribution $\chi_a$ determined from the Langevin fit provided a paramagnetic contribution from the seed-mediated sample ($1.94 \times 10^{-7}$ emu/Oe) that was over 50% larger than that obtained from the dopamine-coated sample ($1.25 \times 10^{-7}$ emu/Oe). Thus the resulting 7 nm diameter can be attributed to the fit and the paramagnetic contributions from the stabilizing surfactant.

The magnetic data derived from FC/ZFC and hysteresis measurements performed on the as-made and dopamine-exchanged nanoparticles attest to an improvement in the magnetization and an increase of the blocking temperature of the iron oxide nanoparticles upon functionalization to become hydrophilic. The ligand exchange process occurs at room temperature and produces no observed change in the physical size or structure of the nanoparticles, as confirmed by TEM, XRD, and elemental analysis. It is thus hypothesized that the dopamine functionalization process alters the surface magnetic state of the particles such that the reported magnetic “dead” layer [73, 74] with a canted, zero-moment surface structure is restored, to some degree, to a collinear ferromagnetic
structure [75, 76] of non-zero moment. A similar phenomenon has been reported by Crespo et al. [77] for Au nanoparticles, where thiol-derivatized Au nanoparticles were shown to exhibit ferromagnetic magnetization versus alkyl ammonium protected gold particles which showed a diamagnetic behavior. Recently, Daou et al. [78] reported a similar increase in $M_s$ induced by changes to a ‘dead’ magnetic layer upon varying the functional groups that are bound to nanoparticle surfaces. These conclusions are supported by the results obtained from the Langevin fitting to the magnetic data that indicate the paramagnetic contribution of the particles, hypothesized to originate from the oxide surface layer as well as from the surfactants, decreases upon nanoparticle functionalization with dopamine. The magnetic “dead” layer is thus hypothesized to be “rejuvenated” by dopamine functionalization, resulting in a higher magnetization and an assumed larger magnetic diameter.

The origin of the improvement in the magnetic properties of the Fe$_3$O$_4$ nanoparticles upon functionalization is hypothesized to lie in the steric interaction between the surfactant molecules (oleic acid and dopamine), arising from their strongly covalent interaction with the Fe atoms on the oxide nanoparticle surface to form a chelating bidentate bond. It has been reported that removal of a ligand bound to surface Fe atoms, as during the exchange process, can also remove the bound Fe atom [206]. It is expected that this process would lead to complete rearrangement of the nanoparticles’ surfaces and result in a significant decrease in particle size. As this effect was not observed in the current study, the phenomena reported in this paper are attributed to collinear realignment of canted surface spins. Additionally, the chemical composition of
surfactant and its ability to bind to nanoparticle surface is hypothesized to play an important role in modifying these canted-surface spin layers.

5.2 Thermomagnetic determination of Fe$_3$O$_4$ magnetic nanoparticle diameters for biomedical applications

The properties of three different populations of nanometer-scaled magnetite particles, fabricated as described in Section 3.1.2, were investigated. The thermomagnetic responses of these three MagNP ensembles (original seed particles and two seed-mediated growths) were measured to determine $m_{ZFC}(T)$ and subsequently the particle diameter distribution function $f(D)$; this distribution was compared to that obtained from the analysis of TEM images of the three types of particle populations.

Figure 5.6 Magnetic moment versus temperature of Fe$_3$O$_4$ nanoparticles in the seed particle sample. The solid line represents the magnetic moment measured for zero-field-cooling condition at applied field of 50 Oe. The dashed curve shows the blocking temperature distribution derived from the ZFC cooling data and Eq. [4.4].
As mentioned in Section 4.1, Figure 4.1 (re-illustrated as Figure 5.6) displays representative $m_{ZFC}(T)$ data with the application of a small field ($H_{app} = 50$ Oe) for a monodispersed magnetite particle ensemble. The $m_{ZFC}(T)$ data exhibit a peak at $T_{\text{MAX}} = 30$ K followed by $1/T$ paramagnetic behavior; this data was analyzed using Eq. [4.4] to compute the distribution in MagNP blocking temperature $f(T_B)$. From this translation of $m_{ZFC}(T)$ to $f(T_B)$, the obtained maximum peak shown in $f(T_B)$ at $T = 23$ K (Figure 5.6) represents the most likely blocking temperature for this ensemble of NPs. The relationship between anisotropy and thermal energy is often quantified using the expression from the Néel model, Eq. [4.3]. However, as the Néel model was derived for single particle volume with a unique $T_B$, it fails to account for two major effects that shift the peak of the measured $m_{ZFC}(T)$ data, (i.) the finite width in the MagNP size distribution [182], and (ii.) interparticle dipole-dipole interactions. The thermomagnetic model presented here directly accounts for the width of the size distribution by calculating the blocking temperature distribution function, which subsequently allows for calculation of the particle size distribution. However, it is recognized that interparticle interactions may result in increasing the apparent blocking temperature in a manner that is difficult to include analytically.

One can compare the $m_{ZFC}(T)$ data for the seed and seed-mediated MagNP collections to obtain qualitative size attributes, particularly the distribution in particle diameters. As described by Jiang and Mørup [167] the characteristics of the ZFC curve can indicate the distribution profile of MagNP diameters within an ensemble, where a sharp peak with a well-defined $T_{\text{MAX}}$ indicates a nominally monodispersed population. On the other hand, broad $m_{ZFC}(T)$ peaks with no distinct $T_{\text{MAX}}$ are common in ensembles
with no well-defined particle size average and wide distributions. Application of these descriptive guidelines to the ZFC data shown in Figure 5.7(a-c) allow immediate conclusions regarding size characteristics of the MagNPs, in advance of any subsequent TEM imaging or computational size distribution analysis.

Figure 5.7 Magnetic moment verses temperature of Fe₃O₄ nanoparticles. (a) ZFC curves of as-synthesized seed, (b) 1x seed-mediated, and (c) 2x second seed-mediated particles. The seed particle profile in (a) displays a sharp peak with T_{MAX} = 18 K, the 1x seed-mediated particle profile in (b) exhibits a well defined peak at T_{MAX} = 32 K, and the 2x second seed-mediated particles in (c) have no well-defined peak and a broad width.

The MagNP seed sample (Figure 5.7(a)) has a sharp peak at T_{MAX} = 18 K that is indicative of a narrow particle size distribution, whereas the 2x seed-mediated growth sample (Figure 5.7(c)) has a broad peak with no distinct T_{MAX}. The data obtained from the 1x seed-mediated growth sample (Figure 5.7(b)) has a distinct T_{MAX} peak at 32 K that is broader than the corresponding m_{ZFC}(T) peak of Figure 5.7(a), indicating the wider particle size distribution compared to that of the original seed synthesis. These qualitative conclusions were confirmed by analysis of TEM micrograph, as follows.

After collection of thermomagnetization data from all three three MagNP ensembles, the particles were analyzed for physical size characteristics using TEM. Micrographs of the three MagNP populations are shown in Figure 5.8(a-c) along with their respective physical size histograms in Figure 5.8(d-f).
Figure 5.8 Representative TEM micrographs of Fe$_3$O$_4$ nanoparticles for: (a) as-synthesized seed particles, (b) 1x seed-mediated particles, and (c) 2x second seed-mediated particles. Physical size histograms (d-f) were determined from multiple micrographs for approximately 250 NPs, and fit with log-normal distribution functions shown by the solid curves. The seed particle histogram (d) illustrates a narrow distribution with an average particle diameter of 4.2 ± 1.3 nm. In (e) the 1x seed-mediated regrowth from the seed particles illustrates a bimodal distribution with two distinct peaks in the histogram at 4.2 ± 1.4 nm and 10.5 ± 1.8 nm. When the 1x seed-mediated particle are used as seeds for the 2x second seed-mediated particles, the resulting size histogram (f) becomes broad and has two peaks at 4.8 ± 2.1 nm and 11.7 ± 4.9 nm.

The physical size histograms are fitted to a log-normal distribution function, with the seed particles (Figure 5.8(a)) having a mean diameter of 4.2 ± 1.3 nm, illustrating a narrow size distribution as predicted by the visual analysis of $m_{ZFC}(T)$ characteristics. On the other hand, the synthesis of (1x) seed-mediated MagNPs (Figure 5.8(b)) resulted in particles with mean diameter of 10.5 ± 1.8 nm in a mixture that most certainly contained an excess concentration of the initial 4-nm diameter seed MagNPs. As described in
Section 3.1.2, it is hypothesized that in this sample the total amount of chemical reagents far exceeded the total amount of small seed MagNPs added to the reaction mixture, resulting in the presence of unreacted seed MagNPs along with the large seed-mediated MagNPs. Therefore, it appears that an excess concentration of reagents, including salts and/or surfactants, were added to the initial seed population in the solute resulting in a small population of seed particles that remain in the final suspension. Per the LaMer growth mechanism shown in Figure 3.2, the particle growth and size are a direct function of solute concentration and time. Therefore, another explanation is that excess salts may drive the solute to saturation too quickly causing the formation of new particles while forming the larger particles from the seeds. This particular phenomenon has been described recently by Huang et al. [207], where the authors observed that higher concentrations of iron salts caused formation of small particles in competition with as-prepared seeds. To further emphasize this point, the bimodal 1x seed-mediated suspension, shown in Figure 5.8(b), was used for the second (2x) seed-mediated growth step which resulted in a further broadening of the particle size distribution compared to that of the previous reaction. The resulting MagNP ensemble is shown by the TEM micrograph in Figure 5.8(c) and in the histogram of Figure 5.8(f). The log-normal fit is again bimodal with mean diameters of 4.8 ± 2.1 nm and 11.7 ± 4.9 nm in the mixed-particle sample. The physical size histograms for the three MagNP collections support the predictions introduced earlier: the initial seed ZFC-curve had a narrow profile with a distinct peak, whereas the first seed-mediated growth had a broader distribution with an average peak at ~ 10 nm and the 2x seed-mediated growth showed a broadened distribution with no well-defined average particle diameter. It should be noted, that these
unique particle collections were intentionally synthesized to test the ability of the model to predict abnormal distribution. These complex ensembles were not intended for any specific application, rather just a proof-of-concept of the robustness of the analytical fits.

The distribution of blocking temperatures $f(T_B)$ was computed by applying Eq. [4.4] to the magnetic data obtained from the measured $m_{ZFC}(T)$ curves shown in Figure 5.7(a-c). The thermomagnetic distribution of diameters $f(D)$ was then computed by translating $f(T_B)$ under the assumption of spherical particles and employing Eq. [4.3]; $f(D)$ is indicated by the solid circular data markers in Figure 5.9(a-c).

![Figure 5.9](image)

Figure 5.9 The size distribution function $f(D)$ of Fe$_3$O$_4$ nanoparticles obtained from thermomagnetic data for the three nanoparticle ensembles is overlaid with the physical histogram from the TEM images for comparison. Results are shown for (a) seed particles, (b) 1x seed-mediated particles, and (c) 2x second seed-mediated particles. The thermomagnetic-derived size distribution function $f(D)$ is represented by solid markers (●) and is fit to a log-normal distribution function shown by the solid curves. The $f(D)$ data shows comparable average diameter values to those represented by the TEM histograms. For the 2x second seed-mediated particles shown in (c), the size distribution function does map the broad distribution, but fails to overlay the smaller seed particle particles present in the population.

The thermomagnetic distributions were made to overlap the measured particle size histograms (Figure 5.8(d-f)) by selecting an appropriate value for the effective anisotropy constant ($K_{eff}$). It should be noted that $K_{eff}$ was assumed to be a fixed value for all the MagNPs within an ensemble and throughout the volume of each individual particle. As
illustrated in Figure 5.9(a), there is excellent agreement between the physical size distribution and the computed $f(D)$ for the seed particles. The solid curve fit to the data in Figure 5.9(a) represents the log-normal distribution function with a MagNP diameter of $4.2 \pm 0.8$ nm; these values compare well with the log-normal distribution values of $4.2 \pm 1.3$ nm determined from the TEM micrographs of the physical size distribution. Figure 5.9(b) illustrates $f(D)$ for the 1x seed-mediated particle ensemble that was computed from the $m_{ZFC}(T)$ data of Figure 5.7(b). Overlaying the 1x seed-mediated $f(D)$ with the physical size distribution shows some agreement with the average particle diameter, whereas the log-normal functions describe a bimodal distribution with mean diameters of $5.8 \pm 2.3$ nm and $9.7 \pm 2.8$ nm. Within statistical error these values compare well with those derived from physical size measurements ($4.3 \pm 1.4$ nm and $10.5 \pm 1.8$ nm). However, close inspection of the 1x seed-mediated overlay shows that the $f(D)$ calculated from the thermomagnetic data failed to sufficiently reproduce smaller diameters of the seed particles within the MagNP collection, instead they only donated a small inflection or shoulder in the distribution at the lower average particle diameter. Finally, analysis of the second 2x seed-mediated growth ensemble, shown in Figure 5.9(c), illustrates that $f(D)$ of this distribution does indeed reproduce a large percentage of the particles but fails to overlay the remaining seed particles in the ensemble. The log-normal function of the 2x seed-mediated growth sample shows a bimodal distribution with mean diameters of $12.8 \pm 3.8$ nm and $9.4 \pm 2.6$ nm. Comparison of $f(D)$ with the physical size histograms derived from TEM micrograph analysis shows agreement with the large particle diameter ($<f(D)> = 11.7 \pm 3.8$ nm vs. $<\text{TEM}> = 12.8 \pm 3.8$ nm), but fails to match the remaining small-diameter MagNP population ($4.8 \pm 2.1$ nm) that is documented in the TEM.
micrographs. It should be noted that a size distribution with such a non-uniform
distribution of particle diameters $f(D)$ would ultimately exclude the 2x seed-mediated
MagNP population from most biomedical applications. Although $f(D)$ does not perfectly
overlay the entire physical diameter histogram of the bimodal (1x and 2x) seed-mediated-
grown ensembles, it is recognized that computational approaches employing only the
hysteresis curve $m(H)$ at given temperatures [95, 96], as described earlier, would be even
less effective in providing the average MagNP diameter and diameter distribution of
these complex MagNP populations. Therefore, it can be seen that this sizing
methodology derived from thermomagnetic data is a valuable tool in the determination of
average diameters and distributions of a MagNP collection, including cases of complex,
bimodal MagNP ensembles.

In addition to characterizing the average diameter and distribution of the
diameters of MagNP ensembles, the derived thermomagnetic distribution function $f(D)$
can also be employed for the determination of an effective anisotropy constant $K_{\text{eff}}$ by
aligning the derived distribution $f(D)$ with the physical distribution histograms attained
from analysis of the electron microscopy images. As the illustrated in Table 1, the value
of $K_{\text{eff}}$ appropriate to the distribution functions shown Figure 5.9(a-c) was between 2.2-
3.9 × 10$^5$ erg cm$^{-3}$ for the three MagNP collections described in this investigation. The
anisotropy constant of bulk Fe$_3$O$_4$ is included in Table 1 for comparison. The effective
anisotropy constant used for the seed ensemble and 2x second seed-mediated growth
ensemble $f(D)$, shown in Figures 5.9(a) and 5.9(c), were comparable, whereas a
significantly smaller value of 2.2 × 10$^5$ erg cm$^{-3}$ was appropriate for the 1x seed-mediated
bimodal ensemble $f(D)$ (Figure 5.9(b)). It is believed that these differences may be a
result of the more cuboidal shape of the MagNP as shown in the TEM micrographs. Recent work by Salzar-Alvarez et al. [208] described the influence of shape on the effective anisotropy of $\gamma$-Fe$_2$O$_3$. In their report, the authors concluded that the rounded morphology of spherical particles increases the surface disorder of the individual particle and thus increases the effective surface anisotropy. This increase manifested itself in a higher overall effective anisotropy. It remains unclear why the synthesis of the 1x seed-mediated particle collection resulted in particles with a cubic morphology, whereas the 2x seed-mediated MagNP, which were grown from the cubic particle, returned to a spherical geometry. As described earlier, several parameters may directly affect the characteristics of the particles including temperature and reagent concentrations. One possible explanation may be attributed to diol concentration; Xie et al. [162] have illustrated that higher diol concentrations result in the formation of non-spherical MagNPs.

<table>
<thead>
<tr>
<th>Particle Ensemble</th>
<th>$&lt;D&gt; \pm \sigma_D$ (nm)$^a$</th>
<th>$K_{\text{eff}}$ ($\times 10^5 \text{ erg cm}^{-3})^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>4.2 $\pm$ 0.8</td>
<td>3.8</td>
</tr>
<tr>
<td>1x Seed-Mediated (bimodal)</td>
<td>5.8 $\pm$ 2.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>9.7 $\pm$ 2.8</td>
<td></td>
</tr>
<tr>
<td>2x Seed-Mediated (bimodal)</td>
<td>9.4 $\pm$ 2.6</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>12.8 $\pm$ 3.8</td>
<td></td>
</tr>
<tr>
<td>Bulk Fe$_3$O$_4$</td>
<td>------</td>
<td>1.1-1.3</td>
</tr>
</tbody>
</table>

$^a$ Obtained from distribution function with a log-normal fit in this work

$^b$ Approximated from an aligned fit of $f(D)$ to physical histogram derived from TEM micrographs

Table 5.1 Average particle diameter ($<D>$) and standard deviation ($\sigma_D$) in particle diameter, and the best fit effective anisotropy constant ($K_{\text{eff}}$) for the three Fe$_3$O$_4$ nanoparticle collections, obtained from ZFC data in Figure 5.6 and illustrated in Fig. 5.9. Data for bulk Fe$_3$O$_4$ is listed for comparison [180].

It should be noted though, that the $K_{\text{eff}}$ values in Table 5.1 are all higher than bulk magnetite values (1.1-1.3 $\times 10^5$ erg cm$^{-3}$) [180] and are comparable to literature values for MagNPs (1-3 $\times 10^5$ erg cm$^{-3}$) [181, 183, 209-213]. Of course, if the anisotropy constant $K_{\text{eff}}$ of a particular MagNP ensemble is already known, the distribution in
particle diameters \( f(D) \) may be directly determined without conducting a detailed analysis of TEM micrographs via manual measuring and enumeration. The anisotropy constant is an important materials parameter in MagNPs and allows for determination of the applicability of such MagNPs as MRI contrast agents and for magnetic hyperthermia protocols. As introduced earlier, \( K_{\text{eff}} \) plays a role in the magnetic relaxation attributed to the ensemble; thus quantification of \( K_{\text{eff}} \) will provide insight into the magnitude of the thermal energy barriers that separate blocked from unblocked behavior. A larger anisotropy constant \( K_{\text{eff}} \) translates into a larger energy barrier and thus dictates higher magnetic field requirements for applications.

5.3 Computational Design Optimization for Microfluidic Magnetophoresis

Section 5.3 details the resulting magnetophoretic microfluidic device parameters. Prior to developing the finalized microfluidic design the possibility of using MagNP as the tagging moiety was tested. As described in Section 5.3.1, within the context of the two-component, electromagnetic, continuous flow displacement-based separation proposed, MagNP do not provide sufficient magnetic force to enable a high throughput separation. Therefore, the final device design was formulated using commercial superparamagnetic microparticles from Invitrogen (details of particle provided in Section 3.2). While ultimately the Generation I design proved to be ineffective in achieving the stated device design goals, the successful Generation II device design makes use of the force balances developed for the Generation I device design. These two designs were determined by computational efforts based on a force balance equation that considers the two driving forces exerted on a magnetically-tagged cell moving through a Newtonian liquid. The main forces considered are the magnetic forces originating from a current-
carrying wire located adjacent to the device to draw a tagged particle towards a desired location and the Stokes force that opposes the motion of the particle. The variables associated with the described derivations are defined in Section 8.0. To attain a more realistic device design, an experimental analysis of several of the key parameters associated with magnetophoretic devices was performed (i.e. magnetic particle and cell properties, and cell-particle binding characteristics) and directly accounted for in the resulting computation. In the course of this investigation it was determined that the Generation I (single wire) device design was not feasible as it yielded significantly inferior particle deflection as compared to that provided by the Generation II (dual wire/fluid sheath) device design. Several different width (Generation II) designs were fabricated and experimentally validated against the derived optimization, whereas applied current and flow rates were tuned and compared with the rational design, as to provide a functional cell separation platform.

5.3.1 Feasibility Assessment of Magnetic Nanoparticles in Magnet-Based Microfluidic Cell Separation

Prior to evaluation of a computationally optimized magnet-based microfluidic cell separation device, the practicability of nanoparticles as the tagging bead was assessed. The three variables related that need to be investigated to best evaluate the possibly of using a nanometer sized particle are the impact of particle radius $R_p$, the approximate number of particle that can attached to a particular cell $\phi$, and the magnetic susceptibility $\chi$. As particle radius has an inverse effect on the displacement and particle radius is cubed, small changes in particle radius have large implications on the effective displacement on-chip as re-illustrated in Eq. [5.1]:
First, in the case of this investigation, it was assumed that a 100 nm diameter iron-oxide nanoparticle was used for labeling. This constraint is set by the standards defined by the National Nanotechnology Initiative, where particles with a diameter less than or equal to 100 nm are classified as “nanoparticles” and larger particles are defined as “sub-micron” particles. Secondly, the magnetic susceptibility was set to unity, which was directly extrapolated from the dopamine particles described earlier (Section 5.1). Third, to determine the maximum number of particles that can physically bind to a cell Eq. [5.2] was applied to the cell-particle complex.

\[
\phi_{\text{MAX}} = \frac{S_{\text{AC}}}{A_p} = \frac{4\pi R_p^2}{\pi R_p^2} = 4
\]

Eq. [5.2]

Briefly, \( \phi_{\text{MAX}} \) is determined from the surface area of the cell \( S_{\text{AC}} \) divided by the cross-sectional area of the nanoparticle \( A_p \). Assume the average radius of an MCF-7 cell (7.5 \( \mu \text{m, Table 5.2} \) as the cell radius and a 50 nm as the particle radius, the maximum number of particles that can physically fit onto an average cell is approximately 90,000 particles per cell. Inserting the values for \( R_p \), \( \phi_{\text{MAX}} \), and \( \chi \), and assuming a 100 micron channel with a flow rate of 25 \( \mu \text{L min}^{-1} \) (1.5 mL hr \(^{-1} \); comparable to the state of the art) yields a length of approximately 10 cm. As initial defined, the final device design is constrained to fit on a standard rectangular glass coverslip (60 (L) \( \times \) 24 (W) \( \times \) 0.15 (H) mm\(^3\)) to accommodate small sample volume and point-of-care design considerations. Therefore, the implementation of a nanoparticle of 50 nm radius with reasonable susceptibility...
results in a device length outside the initial constraints. By reducing the flow rates to 10 μL min⁻¹ (600 μL hr⁻¹) a device which fits within the sizing constraints (~ 4.5 cm) is possible but the throughput is significantly sacrificed in this proposed case. Clearly this evaluation does not exclude nanoparticles from cell separation; rather it merely excludes nanoparticles from being used as labeled entities in the context of the described device design, set size constraints, and practical throughputs requirements. From this point onwards, the nanoparticle work was suspended and the rational design was evaluated with commercial available superparamagnetic microparticles of approximately 1 micron diameter. All further experiments were conducted with the commercial microbeads, including validation of the computation design with homogenous and heterogeneous cell suspensions.

5.3.2 Quantitative Determination of Key Device Parameters

To ensure a robust microchannel device, several measurements of each parameter were performed to obtain meaningful values for key parameters associated with the proposed device designs. These average values are presented in Table 5.2. The average cell radius was determined to be \( R_c = 7.5 \pm 1.3 \, \mu m \), which compares well with previously reported radius values for the MCF-7 cell line (7.5–15 μm [214]). The average microbead particle radius was determined via SEM to be 0.525 ± 0.050 μm, consistent with the manufacturer’s specifications of 0.525 ± 0.015 μm. Furthermore, the volumetric magnetic susceptibility was determined to be 1.10 ± 0.19, comparable to the manufacturer’s reported value of 1.5.

Approximation of the particle binding density per cell, \( \phi \), was then investigated. Under the ideal circumstances of complete surface area coverage of the cell surface, the
maximum binding density of magnetic microparticles to one cell is determined to be $816 \pm 161$ particles per cell. However, determination of the actual particle-binding character of the cell must consider biological characteristics such as the number of possible binding sites \((i.e.\, receptors)\) available on the cell surface and clustering of these binding sites.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average</th>
<th>Error$^a$</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap Distance</td>
<td>$z$</td>
<td>150</td>
<td>20</td>
</tr>
<tr>
<td>Cell Radius</td>
<td>$R_c$</td>
<td>7.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Particle Radius</td>
<td>$R_p$</td>
<td>0.525</td>
<td>0.070</td>
</tr>
<tr>
<td>Volumetric Magnetic Susceptibility</td>
<td>$\chi$</td>
<td>1.10</td>
<td>0.19</td>
</tr>
<tr>
<td>Binding Density$^b$</td>
<td>$\phi$</td>
<td>794</td>
<td>280</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation
$^b$ Obtained from flow cytometry reading

**Table 5.2** Measured cell and particle characteristics for determination of effective magnetophoretic displacement of cell-particle complex within microfluidic channel.

To this end, the number of receptors available on the cells was measured via flow cytometric analysis. A cell suspension was incubated with the fluorescently-labeled antibody against EpCAM, a known antigen found on carcinoma cells, and subsequently analyzed via flow cytometry to yield a receptor number of $251,250 \pm 51,382$ (approximately $\pm 20\%$ variation) EpCAM binding sites per cell, comparable to previous reports of $222,100 \pm 13,700$ EpCAM receptors per cell [214]. A second suspension of cells ($26.25 \times 10^4$ total cells) was then incubated with magnetic particles functionalized with anti-EpCAM at a concentration of $1\, \text{mg}\, \text{mL}^{-1}$ for 30 mins; the tagged cells were then removed via centrifugation. A concentration of $1\, \text{mg}\, \text{mL}^{-1}$ magnetic microbeads in EMEM was demonstrated from flow cytometry experimentation with various particle suspensions to provide the maximum number of particles binding onto the cells. To
ensure complete separation of the cells from the magnetic beads in suspension, centrifugation of the entire suspension was performed. Centrifugation ensures that all cells in suspension (untagged and tagged) will be subsequently analyzed, while separating free magnetic microparticles from the bound cells. The cells recovered following centrifugation were incubated with anti-EpCAM-FITC, which will bind to any free, unoccupied receptors remaining on the cell. These suspended cells were then analyzed for the number of available receptors free of particles. After incubation the number of unoccupied EpCAM receptors was 6,898 ± 1,218 EpCAM antigens per cell, which corresponds to an approximate coverage of 97% of the cell surface and an overall binding density of 794 ± 280 microparticles per cell. To provide an independent confirmation of this value, the unbound microparticle suspension remaining after the centrifugation step was dried and weighed. A mass analysis was carried out, where the initial mass of particles incubated with 26.25 × 10^4 cells was determined to be 1.1 ± 0.1 mg and the remaining mass of particle after incubation was determined to be 0.9 ± 0.1 mg. As determined by hemacytometry, the approximate microparticle concentration of particles per mL of liquid stock is 8.45 ± 1.33 × 10^9 particles mL^−1, which translates to 8.45 ± 1.33 × 10^8 particles per mg of dry magnetic microparticles. Therefore, by mass conservation, a particle binding density of 644 ± 338 beads per cell was determined, which is nearly 20% smaller than the coverage determined by flow cytometry but may be accounted for by dilution errors, hemacytometer errors, and the inaccuracies of the balance. However, comparison of the results attained from flow cytometry and mass conservation calculations illustrates that, within the margin of error, these two techniques yield consistent binding densities. This information is used to provide realistic constraints
to the design of the microfluidic isolation device. Furthermore, understanding of the
distribution in cell radius, magnetic microbead particle radius, and binding densities
ensures that the resulting device is sufficiently robust enough to isolate all the cells of
interest.

5.3.3 Microfluidic Device Design Optimization

Employing the expression derived in Section 4.2 for the displacement of a cell-
magnetic particle complex under the influence of a magnetic field and utilizing the values
derived in Section 5.3.2 (Table 5.2) for the key parameters in the expression, it is now
possible to computationally investigate the physical behavior and appropriate dimensions
of the Generation I and Generation II cell separation device designs. Solving Eq. [4.25]
for the length $l_{ch}$ of the Generation I device with a reasonable lateral cell-particle complex
displacement ($\Delta x$) of 100 µm and employing the commercial microbeads described
earlier indicates that the applied current ($I$) would need to be greater than 10 A to produce
a field sufficient to ensure that the length of the channel $l_{ch}$ remained below 1 m. Not
only is this applied current value well above the constraints set by minimizing the Joule
heating contribution (described in Section 4.2.2.3), the derived device length of 1 m is
unrealistic. This result motivated the Generation II design, as derived in Eq. [4.28],
which was designed to (i.) reduce the device length required for cell displacement and
isolation relative to the Generation I design and (ii.) increase the magnetic forces
experienced by the tagged target cells by virtue of its double current-carrying wire design
that produces double the magnetic field. An average maximum cell displacement for the
Generation II device, using the earlier-described parameters, was determined from Eq.
[4.28]; as before, the intended design was envisioned to consist of a disposable
microfluidic component and a re-usable electromagnetic component, with the length of
the device set by the length of commercially-available glass slides (60 mm). To compute
the lateral displacement of the cell-particle complex in the device, two current-carrying
wires of equal dimension are placed at the outer edges of the device, the height of the
channel ($h$) is assumed to be 50 µm, and the distance between the channel midpoint and
the current-carrying wire array is set equal to the thickness of a #1 glass slide ($z = 150$
µm). This lateral displacement was determined as a function of current and volumetric
flow rate using a rearrangement of Eq. [4.28]. Volumetric flow rates are directly
impacted by changes in channel width or starting position of the cell-particle complex;
therefore derivation of the cell-particle complex displacement as a function of volumetric
flow rate is non-linear and these two variables must be solved for simultaneously in the
calculation. It should be noted that the calculated complex displacement is defined as the
distance from the outside edge of the channel, near the wire, to the long axis of the
microfluidic channel (Figure 4.3(c)); therefore the width of the entire channel is
equivalent to twice the displacement, as shown in Figure 4.3(c).

The surface plot shown in Figure 5.10 illustrates the maximum displacement of an
average cell-particle complex from channel edge to channel center as a function of
current $I$ and volumetric flow rate $\dot{V}$ for the Generation II device, as determined from Eq.
[4.28]. The displacement maxima, defined as the largest distances that the cell-particle
complex traverses to reach the device center within a length of 50 mm, are below 2100
µm (or 2.1 mm), a width significantly less than that of a standard coverglass slide (24
mm). The device length was set to 50 mm to account for the integration of the channel
outlet and inlets to create a hydrodynamic focusing of the buffer stream, as illustrated in
Figure 4.3(a). As expected, higher current through the device provides greater maximum cell displacement, as the magnetic force increases as $I^2$. The linear velocity of the cell in the $\hat{x}$-direction increases with increasing volumetric flow rates, causing a less drastic displacement as the particle travels down the microfluidic channel ($\hat{y}$-direction).

![Figure 5.10](image)

**Figure 5.10** Surface plot illustrating maximum displacement achievable as function of volumetric flow rate ($\mu$L min$^{-1}$) and current (A) derived in Eq. [4.28]. All parameters fixed at average values in Table 5.2 and length of device constrained to 50 mm. Current varied from 0.1-1.2 A and volumetric flow rate varied from 10-120 $\mu$L min$^{-1}$. Maximum displacement increases with increasing current and decreases with increases in flow rate.

Figure 5.11 depicts the surface plot from Figure 5.10 along with intersecting planes that represent sample volumetric flow rates utilized with magnet-activated and non-magnetic cell separation systems described in the literature. Other researchers who have isolated cells using commercial [100] or microfluidic [128, 134, 138, 142, 145, 172, 215] systems have employed volumetric flow rates in the range of 6.3 µl min$^{-1}$ - 100 µL min$^{-1}$. 

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Figure 5.11 illustrates that this simple design can not only effectively meet but also exceed the processing speeds or throughputs of both commercial macroscale systems (green plane) and microfluidic devices (red planes).

<table>
<thead>
<tr>
<th>Current (A)</th>
<th>Flow Rate (µL/min)</th>
<th>Displacement (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µL min⁻¹</td>
<td>60 µL min⁻¹</td>
<td>32 µL min⁻¹</td>
</tr>
<tr>
<td>16 µL min⁻¹</td>
<td>11.7 µL min⁻¹</td>
<td>6.3 µL min⁻¹</td>
</tr>
<tr>
<td>18 µL min⁻¹</td>
<td>11.7 µL min⁻¹</td>
<td>6.3 µL min⁻¹</td>
</tr>
</tbody>
</table>

**Figure 5.11** The surface plot of displacement of cell-particle complex on standard glass coverslip (60 (L) × 24 (W) × 0.15 (H) mm³) compared with current literature values of isolation of target cell populations. Intersecting planes drawn at average throughputs for commercial magnet-based separation (green; 60 µL min⁻¹) and microfluidic cell separation devices (red; 11.7 – 100 µL min⁻¹). The 3-dimensional plot illustrates rational design yields comparable throughputs, and narrow channel widths allow for greater throughputs than state-of-the-art separators.

This is a relevant comparison for the present work because any new separation device must have at least the same throughput as similar, state-of-the-art systems. Furthermore, control of the applied current and channel widths in this device allow for cell throughputs higher than those currently reported in the literature for other comparable devices [128, 134, 138, 142, 145, 172, 215]. Although the higher cell throughput provided by the current design is advantageous, it is still unclear what the anticipated effect of device
parameters on purity of collected target cells will be as there remains a dearth of literature addressing the effect of flow rates on efficiency and purity of the isolated cell suspension from heterogeneous suspensions. A review of the literature on magnetic-bead-based device designs (both commercial and microfluidic designs) shows that no correlation between purity, efficiency, and flow rate has yet been illustrated. Therefore, to assess this relationship extensive experimental validation of the presented design with biologically-relevant mixed cell population, described in Section 5.4.4 and 5.4.5, is required.

So far, all presented calculations were derived from average particle and cell characteristics shown in Table 5.2, whereas distributions in the values of these particular parameters were not considered. To address these parameter variations, the cell and particle diameter distributions shown in Table 5.2 were factored into the computational device design. This analysis demonstrates a need to design the Generation II system according to the lower bound of the maximum cell-particle complex displacement as illustrated in Figure 5.12, rather than target the average displacement of the complex as described earlier (Figures 5.10 and 5.11). To assess the worst-case scenario, or the lowest maximum displacement, the least favorable bounds of each of the parameters were inserted into the design equation (i.e. $R_p = 0.455 \, \mu m$, $R_c = 8.8 \, \mu m$, $\phi = 514 \, \text{particles cell}^{-1}$, $\chi = 0.91$). As it is desired that all cells be displaced from the sample stream into the collection stream located in the center of two sheath fluids (i.e. 100% recovery), the lower bound surface models the movement of a large cell with minimal magnetic-particle binding densities and thus minimal magnetic force experienced. Thus, the lowest surface plot shown in red in Figure 5.12 represents the most conservative rational design criterion.
that should be followed for subsequent design of a magnetic-based cell separation platform.

**Figure 5.12** Consideration of distribution in cell and particle parameters constrains the true maximum displacement achievable. The average maximum displacement in Figure 4 re-plotted, shown in the center transparent white surface plot. The upper bound plot (as shown in transparent white) represents case of highly mobile cell-particle complex, *i.e.* small cells maximally labeled with magnetic particles or best-case scenario \((R_p = 0.595 \ \mu m, \ R_c = 6.2 \ \mu m, \ \phi = 1074 \ \text{particles cell}^{-1}, \ \chi = 1.29)\). Case where larger-than-average cells experiencing high drag, tagged with small particles with low susceptibility with minimum binding density (*i.e.* worst-case scenario) shown in red represents true realistic parameter space \((R_p = 0.455 \ \mu m, \ R_c = 8.8 \ \mu m, \ \phi = 514 \ \text{particles cell}^{-1}, \ \chi = 0.91)\).

The parametric analysis conducted in this study illustrates that particle binding characteristics, as well as the individual cell and particle properties, do play an integral role in the separation efficiency. Unfortunately, most commercial MACS systems have been generalized for separation of a wide variety of cell population and are not tuned to account for these variations in particle-cell binding character. Therefore, this standardization of the commercial system may result in the low yields currently found for
magnet-based cell isolations. Moreover, the total number of particles bound to a particular cell is a direct function of the cell type, the particle type, and the specific marker of interest. Therefore, prior to any experimentation the number of particles bound to the cell $\phi$ must directly measured as described in Section 3.2.3 and subsequently included in the validation studies presented below. Ultimately, assessment of the effects of particle binding density, or poor labeling efficiency, on cell separation efficiency of the presented design requires experimental validation with different cells populations.

5.3.4 Validation of Derivation with Homogenous Suspensions

Following determination of the rational design criteria, preliminary validation studies of the sheath device were conducted. First, the sheath characteristics of the device were validated with fluorescent dye illustrating a clear delineation of the cell samples streams and the central buffer stream. This was followed by a homogeneous suspension of MCF-7 carcinoma cells in phosphate-buffered saline at a cell concentration of $1 \times 10^5$ cell mL$^{-1}$. Several combinations of applied current, flow rate, and channel widths were investigated. By selecting specific combinations of these three variables it is possible to probe designs which should separate all cells (below the red surface plot in Figure 5.12), designs that should not separate the cells (above the upper bound in Figure 5.12), and design which should separate some of the cells (between the upper and lower bounds in Figure 5.12). As shown in Table 5.3, the combinations predicted to separate all of the cells had efficiencies of approximately 100 % within the margin of error, whereas combinations which forced the design outside of the optimized region yielded very low separation of labeled cells from the buffer solution. Interestingly, those parameters which intersect between the upper and lower bounds on Figure 5.12 illustrate
that only a percentage of the cells were actually separated from the fluid stream. Furthermore, as the parameter intersection approached the lower bound (or worst case-scenario) the percentage of cells increases towards 100 % efficiency separation. This result illustrates that the surface plots shown in Figure 5.12 accurately represent the available device design space, and given one or two parameters an optimized functional device can be attained.

<table>
<thead>
<tr>
<th>$\dot{V}$</th>
<th>$I=0.25$ A</th>
<th>0.50 A</th>
<th>1.00 A</th>
<th>$w=250$ μm</th>
<th>$w=500$ μm</th>
<th>$w=1000$ μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μL min$^{-1}$</td>
<td>93.0 ± 2.7</td>
<td>⋮</td>
<td>⋮</td>
<td>92.1 ± 1.6</td>
<td>⋮</td>
<td>⋮</td>
</tr>
<tr>
<td>50 μL min$^{-1}$</td>
<td>97.7 ± 2.7</td>
<td>⋮</td>
<td>⋮</td>
<td>71.1 ± 7.3</td>
<td>⋮</td>
<td>⋮</td>
</tr>
<tr>
<td>120 μL min$^{-1}$</td>
<td>97.0 ± 4.0</td>
<td>98.5 ± 10.6</td>
<td>97.0 ± 1.5</td>
<td>6.3 ± 4.2</td>
<td>76.0 ± 2.6</td>
<td>98.6 ± 1.6</td>
</tr>
</tbody>
</table>

Table 5.3 Capture efficiency for an initial cell concentration of $5 \times 10^4$ cell mL$^{-1}$. The efficiency was determined for three different microfluidic channel width ($w = 250 \mu$m, $500 \mu$m, and $1000 \mu$m) injected at three different flow rates ($\dot{V} = 10 \mu$L min$^{-1}$, $50 \mu$L min$^{-1}$, and $120 \mu$L min$^{-1}$) and influenced by an electromagnetic field at three different currents ($I = 0.25$ A, 0.50 A, and 1.00 A).

A second validation of the rational design was then probed as a mean to test cell concentration influences on the efficiency of separation for a single set of device parameters. A device made with optimal design parameters, as determined from the model and experimental preliminary validation ($w = 250 \mu$m, $\dot{V} = 120 \mu$L min$^{-1}$ and $I = 0.25$ A), was employed to test the efficiency of cell isolation as a function of MCF-7 concentration in buffer ($10 – 10,000$ cell mL$^{-1}$). As shown in Figure 5.13, in this instance the efficiency of cell separation remains around 100 % as the total number of cells injected is lowered from 10,000 cells to as low as 10 cells. Overall, the results of these validation tests illustrate that the device design optimized according to the derived
computational model can effectively isolate (~100%) a magnetic-particle-tagged population of cells from a general cell suspension, even in low abundance.

Figure 5.13 The capture efficiency was shown to be nearly 100% for homogenous samples of 10 – 10,000 MCF-7 cells injected. As determined from the experiments shown in Table 2, all experiments were conducted with a 250 μm wide microfluidic channel at a flow rate 120 μL min⁻¹ and a current of 0.25 A. The four solid points represent five replicates experiments (n = 5) for each respective cell suspension and the dotted line represents 100 % efficiency of cell capture. Error bars represent the standard error of both the input cell number and collected cell number (n = 5)

It should be noted that separation of a cell population from a homogenous cell suspension still remains too ideal to conclude that the presented device design optimization will successfully extend to a heterogeneous suspension, such as whole blood. All biologically-relevant cell suspensions have several additional factors that are anticipated to affect the separation character of the device, such as cell-particle migration
under shear conditions, elastic collisions of cells, flow and collision-induced aggregation, and unintentional labeling of non-target cells with magnetic beads. Unfortunately, each of these factors is highly dependent on the constraints of the suspension (e.g. cell-particle binding densities and carrier solution characteristics) and thus difficult to generalize for inclusion of such factors into the presented homogeneous cell suspension analysis.

Another question that has yet to be evaluated is the effect of concentration (target cell plus non-target cell totals) on the efficiency of target cell isolation. The recovery or yield of rare cell isolation (subpopulations of <1%) [26, 215] techniques reported in the literature for MACS systems over the past ten years remain too low (20-80%) [216-221] for possible application in clinical diagnostics. A possible explanation of the low cell recoveries currently reported in the literature may be a result of a lack of a rational optimization approach towards device design which has been addressed within this investigation and/or the non-idealities of heterogeneous suspensions described above, including cell-cell collisions [222]. As the target cells move across the flow stream within the described design there will be numerous collisions with neighboring cells, causing interference in the lateral displacement and possible deviation away from the displacement stream. As with flow rate effects on cell separation purity and efficiency, this effect must be evaluated via further experimental validation, as described below in Sections 5.3.5 and 5.4.

5.3.5 Heterogeneous Suspensions

To investigate the influence of non-target cell populations on the overall efficiency, as well as to determine the estimated purity of the effluent stream, various heterogeneous cell suspensions were prepared. The first variable that was tested was the
influence of target cell suspension (MCF-7 cancer cells) in a set non-target cell suspension (Raji B-lymphocytes) of \(1 \times 10^6\) cells per mL. The cancer cells spiked into a high concentration of B-cells represent a valid heterogeneous model for metastasis found in red cell depleted blood. This will allow for the assessment of (i) the possibility of non-target cell labeling which would manifest itself in separation of the non-target cells along with target cells (or low purity yields); (ii.) the influence of non-target/target cell elastic collisions causing a reduction in efficiency or mechanical shifting of the non-target cells into the collection stream. As shown in Figure 5.14(a) the efficiency of isolation (shown in white) was above 95% for all conditions. On the other hand, it was observed that the purity of capture (defined as the percentage of non-target cells in the collected target stream) decreased with increases in the total number of MCF-7 cells spiked into the Raji cell suspension. It should be noted though that the number of Raji cells collected was conserved at approximately 12 cells (~0.001%). To test if this decreased purity is result of either the target or non-target cells in suspension, experiments with differing number of non-target cells, where the target cell number is held constant at approximately 10 cells, were conducted. As shown in Figure 5.14(b), when the number of target cells (MCF-7 cells) are held at 13 cells total and the non-target cell (Raji cells) number is decreased the percent purity of the isolated suspension increases to near 100%. This increase confirms that the purity is not dependent on the target cells that are spike, within the range of cell numbers investigated, but rather a direct function of the number of non-target in the sample stream. Of course, considering \(1 \times 10^6\) non-target cells were initially in suspension, only 0.001% of the Raji cells were inadvertently isolated from suspension.
Figure 5.14 The capture efficiency was shown to be nearly 100% for heterogeneous samples of MCF-7 cells spiked into Raji cells injected into the Generation II device. As determined from the experimental values shown in Table 2, (a, b) experiments were conducted with a 250 μm wide microfluidic channel at a sheath and sample flow rate of 120 μL min⁻¹ and a current of 0.25 A. The influence of (c) current and (d) flow rate was also investigated. As shown in (a) the efficiency (white bars) remained above 95% for all concentrations, the purity significantly decreased when the total number of MCF-7 cells was reduced, but the total number of non-target cells remained the same. (b) To test the influence on the purity when the number of non-target cells is decreased, 13 MCF-7 cells were spiked into different suspensions (10⁴-10⁶ Raji cells). As the total number of Raji cells was decreases the purity was increased, as less non-target cells were present in the collection stream. Overall, it was observed that (c) current and (d) flow rate had no influence on the efficiency and/or purity.
It is not exactly known why this is occurring, but with the context of the devised experiments, these results are very promising, especially considering the near 100% efficiency of separation of ~10 cells in $10^6$ non-target cells.

To test if changing the parameters may result in higher or lower efficiencies and/or purities two additional experiments were devised. In the first experiment, the concentration of Raji cells were fixed at approximately $10^6$ with ~ 10 MCF-7 cells spiked into suspension; the flow rate was fixed at 120 $\mu$L min$^{-1}$ for both sample streams (a 240 $\mu$L min$^{-1}$ injection flow rate bifurcated into two side streams) and the applied current was tuned from 1.0 – 0.25 A. As illustrated in Figure 5.14(c), the efficiency and purity was not significantly changed, even though the magnetic force applied to the tagged cells was much greater. Conversely, in the second set of experiments, the concentrations were again held constant at $10^6$ Raji and 10 MCF-7 cells, with a constant current of 0.25 A; the flow rate $\dot{V}$ of the sample streams was now changed from 120 – 10 $\mu$L min$^{-1}$. It was observed, as shown in Figure 5.14(d), that the flow rate also had no effect on the isolation efficiency and purity.

Overall, the experiments with heterogeneous suspensions have illustrated that the rationally designed magnetophoretic separation platform is capable of high efficiency isolation down to the level of ~ 10 target cells in a 1 mL sample. Furthermore, it was illustrated that the purity within this device is a function of the number of non-target (or interfering) cells in the suspension. These results confirm that the low isolations or recoveries reported in the literature [216-221] can be attributed more to the lack of a rational design and less to the elastic collisions occurring with the separation platform.
Of course, to get a true estimate of the capability of this device, a realistic sample must be run through the device, such as a tissue digest or a whole blood sample.

![Figure 5.15](image)

**Figure 5.15** Behavior of cells in culture to assess impact of cell separation process. (a) A population of ~1000 MCF-7 cells per 250 μL was plated as a comparative control. The control cells were judged against (c) cells incubated with particles but not run through the device, (b) cells run through device without particle attachment (no displacement), and (d) cells tagged with magnetic microbeads and displaced within the device. Scale bars represent 50 μm. Dark spots can be seen in (c, d) which indicate residual microparticles on the cells surface during culture and spreading.

In addition to testing the influence of non-target cells on recovery and purity, a cell viability assay, using EthD-1 and calcein, conducted on the outlet population shows that the cells remain about 87.5 ± 2.5% viable; within the range of the injected cell viability of 91.0 ± 3.2%. This confirms that the magnet-based cell separation platform presented does not adversely affect the cells. To further confirm that the particle tagging
and/or high shear rates do not affect the growth and spreading of the cells, cells were plated for 24-hr in 96-well plates, followed by imaging and comparison with controls. As shown in Figure 5.15, no clear difference can be seen in the control, Figure 5.15(a), and the displaced cells, Figure 5.15(d). Furthermore, the particle and the high flow rates have no visual influence on the behavior of the cells in culture. All four conditions exhibit clustering and large spread-out cell morphology, both typical of epithelial cancer cell cultures. It is evident from Figure 5.15(c-d) that the microparticles remain on the cells (dark spots) and there remain excess particles in culture, but no visual difference in cell behavior can be seen. This does not confirm that the microbeads have no influence on the cells, but does illustrate that labeled cells do spread in a similar fashion as controls.

5.4 Isolation of a Magnetically-Labeled Cell from Whole Blood within a Microfluidic Channel

As a final proof-of-principle of the capabilities of the described magnet-based microfluidic cell separation device, the model breast cancer MCF-7 cells were spiked into whole human blood at a concentration of 50 cells mL$^{-1}$. To determine the available device design space, such as possible flow rates and applied currents, which computationally allows for efficient separation of the spiked cell from the blood, several additional parameters were re-evaluated. As described earlier in Section 4.3, blood behaves as a shear thinning fluid and within the context of microfluidic channel height range utilized in this study the red blood cell concentration (or hematocrit) causes a reduction in the apparent viscosity of the whole blood carrier fluid (Fahraeus-Lindqvist effect [203]). As the empirical model (Eqs. [4.29-4.31]) formulated by Pries et al. [202] is predicated on the assumption that blood’s linear velocities are higher than 50 channel heights per sec within the channel, a calculation of the flow velocities in the channel is
required prior to computing the viscosity of whole blood. The device utilized throughout
this dissertation is ~50 microns in height thus for implementation of the empirical model
described the linear velocity of the blood must be in excess of 2500 \( \mu \text{m}^3 \text{ sec}^{-1} \). Calculations for a 1000 micron width channel (the largest channel in the study) with a flow rate of 10 \( \mu \text{L min}^{-1} \) (the lowest volumetric flow rate in this study) yield a fluid linear velocity down each side channel of 10^4 \( \mu \text{m}^3 \text{ sec}^{-1} \). This value is significantly higher than the fluidic velocity assumption defined in the model and thus this empirical model can be used to estimate the apparent blood viscosity present in the microfluidic device. Therefore, the constants \( C \) from Eq. [4.31] and \( \eta_{0.45} \) from Eq. [4.30] were determined with \( h_{ch} = 50 \) microns to be 0.7765 and 2.13 respectively. Inserting these values into Eq. [4.29] at an average phenotypical hematocrit (\( H_c \)) of 0.45 yields an apparent blood viscosity \( \eta_{blood} \) of 2.75 cP. Substituting this viscosity value for the \( \eta \) in Eq. [4.28] yields a new surface plot as shown in Figure 5.16(a) which illustrates the maximum displacement of an average cell-particle complex from edge to channel center as a function of applied current \( I \) and volumetric flow rate \( \dot{V} \) for the Generation II device described in 5.4.3. Similar to the analysis conducted for the buffer system described in Section 5.4.3, Figure 5.16(a) illustrates the maximum computed displacement derived from average particle and cell characteristics in red (Table 5.2), as well as the best-case (pink) and worse-case scenario (dark red).
Figure 5.16 (a) Surface plot of maximum displacement with consideration of distribution in cell and particle parameters constraining the true maximum displacement achievable for a blood-based device ($\eta_{\text{blood}}=2.75$ cP). The average maximum displacement shown in the center transparent red surface plot. The upper bound plot (pink) represents case of highly mobile cell-particle complex, *i.e.* best-case scenario. The case where larger-than-average cells experience high drag and are tagged with small particles with low susceptibility with minimum binding density (*i.e.* worst-case scenario) is shown in dark-red. (b) The blood-based device is compared to the buffer-based device (Figure 5.12) illustrating a clear delineation of the blood from the buffer device.
As described for the device design analysis used in both the homogenous and heterogeneous validation experiments, the worst-case scenario, or the lowest maximum displacement, is the desired design criterion that should be followed for subsequent experiments as it ensures displacement of those cells which exhibit the least favorable drag and magnetic bounds. To illustrate the drastic shift in the maximum displacement constraint (Figure 5.16 (b)) imposed by the apparent viscosity of blood ($\eta_{\text{blood}} = 2.75$ cP) versus the much lower buffer viscosity ($\eta = 1$ cP) the graph in Figure 5.16(a) was superimposed with the previously presented Figure 5.12. Overall, it can be seen that there is a significantly smaller design space for cell separation involving isolation from blood versus conventional water-based isolations.

Subsequent to confining the flow rate and current according to Figure 5.16(a) a second constraint, which must be determined experimentally, was applied to further refine the possible design space required for high purity separation. In order to maintain a continuous parabolic flow profile transversely across the channel, the location of the blood and buffer interface within the stream, sheath fluid flow pattern must be determined. To ensure complete separation of the target cells from the blood stream at the outer parameter of the device to the center collection, buffer stream the required displacement $x$ must be well characterized and repeatable. Furthermore, to maintain the purity of the collection stream it is imperative that the blood stream not flow into the collection outlet. As illustrated in Figure 5.17, by tuning the buffer flow rate (from 120 $\mu$L min$^{-1}$ to 200 $\mu$L min$^{-1}$) to compensate for the higher viscosity of the blood, the three streams can run parallel without mixing and allows for buffer collection stream to flow out of the center outlet only.
Figure 5.17 Bright field micrographs illustrating the blood-buffer three stream, sheath flow that manifests with a side channel flow of 120 \( \mu \text{L min}^{-1} \) and a center buffer stream flow rate of 160 \( \mu \text{L min}^{-1} \). (a) These specific flow rates result in a hydrodynamic focusing of the buffer between the two blood streams, which leads in (b) the buffer stream is approximately 100 microns, whilst the blood streams are both 75 microns in width. (c) At these flow rates, the blood streams are segregated from the collection outlet, which allows for only the target cells to be isolated.

It was determined via visual inspection that flow rates greater than 160 \( \mu \text{L min}^{-1} \) are required to have (i.) three separate side-by-side streams and (ii) no “leaking” of the blood stream into the collection outlet (as shown in Figure 5.17). Therefore, for all further experiments blood was injected at 240 \( \mu \text{L min}^{-1} \), which results in two blood streams of 120 \( \mu \text{L min}^{-1} \), and the buffer stream was injected at 160 \( \mu \text{L min}^{-1} \). The three stream widths then were measured to determine the required displacement for a labeled target cells to travel from the device edge to the long axis of the magnetophoretic device. As
shown in Figure 5.17(b), the target cells would need to travel a total distance of 75 µm to enter the buffer stream for separation and isolation from the blood. Interestingly, when the three streams (at a buffer flow rate of 150 µL min⁻¹) had approximately equivalent widths of 83 µm, there were blood cells exiting the center channel outlet. Therefore, the flow rate was increased to 160 µL min⁻¹ to ensure that pure populations were isolated. This phenomenon was not visualized in the heterogeneous validation experiments and thus was attributed to the high density of cells in blood and its unique viscous properties.

Following augmentation of the rational design criteria for use with whole blood, validation studies of the sheath device were conducted. First the sheath characteristics of the device were validated via bright field imaging to determine a working range of flow rates and ratios of flow rates. This was followed by a suspension of MCF-7 carcinoma cells spiked into whole human blood at a cell concentration of 50 cell mL⁻¹. Three different applied currents were investigated. By selecting an applied current which fits within the determined design space it was shown that separation efficiencies above 95% could be achieved. As shown in Table 5.4, the applied currents (0.5 A and 1.0 A) which are predicted to separate all of the cells had efficiencies of approximately 90% within the margin of error. Whereas the current which predicts a lowest bound from Figure 5.16(a) of 66.7 µm, less than the measured width of the blood stream of 75 µm, yielded lower separation of labeled cells from the blood (~87%). Interestingly, it was observed that as the applied current was increased the purity of capture inversely decreased. Although, initially this was an unexpected result, literature has shown that in oxygenated blood red blood cells are paramagnetic and white blood cells are diamagnetic [223], which may explain the increase in non-target cells in the collection stream. With higher currents,
higher magnetic fields are produced, causing the white blood cells, due to their diamagnetic behavior, to remain in the blood stream and to even be attracted to the edges of the channel. Furthermore, in vitro experiments assessing the radial distribution of white blood cells in small glass tubes (69 µm to 200 µm diameter) have shown that white blood cells marginate in a tube depending on rheological factors such as hematocrit, blood suspension medium and shear stress [224]. White blood cell margination has also been shown in large rectangular channels (3 mm wide and 300 µm deep), also dependant on blood rheology [225, 226]. Conversely, the paramagnetic behavior of the red blood cells, in the presence of the applied magnetic field, would result in a very small population displacing from the blood stream to the buffer stream.

<table>
<thead>
<tr>
<th>Current</th>
<th>Lowest Bound from Figure 5.16(a)</th>
<th>Efficiency (%)</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I = 0.25 A</td>
<td>66.7 µm</td>
<td>87.5 ± 3.2</td>
<td>81.0 ± 2.6</td>
</tr>
<tr>
<td>I = 0.50 A</td>
<td>308.5 µm</td>
<td>93.2 ± 4.5</td>
<td>78.4 ± 4.6</td>
</tr>
<tr>
<td>I = 1.0 A</td>
<td>361.6 µm</td>
<td>94.7 ± 3.4</td>
<td>55.6 ± 5.2</td>
</tr>
</tbody>
</table>

Table 5.4: Capture efficiency and purity with a spiked concentration of 500 MCF-7 cells mL⁻¹ in whole human blood. The lowest bound from the computation optimization is also shown along side the efficiency and purity.

To provide some perspective on how small a population is shifting from the side stream to the center stream, blood is composed of ~10⁹ red blood cells per mL and only 45-60 red blood cells are found in the collection stream. Referring back to the heterogeneous suspensions shown in Figure 5.14, it is apparent that the diamagnetic behavior of white blood cell did not seem to have an influence on the purity of the collected stream with changes in applied currents. Overall, these results illustrate that to isolate a pure population lower currents are required but efficiency will be sacrificed in doing so.
Depending on the desired end results, either high current should be used for high efficiency or low current for high purity.
6.0 CONCLUSIONS

Metastases are a significant problem in cancer research, since metastasis from the primary tumor to distant organs causes 90% of cancer-related deaths, i.e. half-a-million people in the US. Detection of circulating tumor cells (CTCs) in whole blood demonstrates that there is a connection between the primary tumor and metastases. Therefore, there is a need for creation of enabling technologies to enable biological CTC studies. This could contribute to understanding of the spreading of cancer and development of various new drugs and strategies.

The overall goal of this dissertation was to develop a point-of-care, disposable microfluidic device capable of efficient and rapid isolation of rare CTCs at a throughput rate on the order of millions of cells per sec. However, the CTCs are very rare in whole blood, i.e. ~1-3,000 in one billion cells. In addition to isolating low concentrations of cells from blood, the separation platform must work within the constraints of the clinical setting (i.e. consideration of the device as a biohazard) while remaining cost effective for both the clinicians and patients. To address these needs an inexpensive, novel, disposable, user-friendly technology for efficient capture of CTCs from whole blood. The significance of the described approach is that it would provide a unique platform technology to detect cancer and capture circulating tumor cells. Moreover, this new microfluidic tool to capture CTCs could lead to the discovery of targets for further basic and clinical research.

In this dissertation it was proposed that a device which integrates nanotechnology, biology and microfluidics can be design to isolation pure populations of CTCs directly from whole blood, with a focus on breast cancer. To achieve this objective, two specific
aims were pursued: (i.) Development and characterization of a biocompatible nanoparticle ensemble and (ii.) develop a point-of-care, high-throughput microfluidic CTC isolation technology. Iron-oxide nanoparticles were synthesized via a thermal decomposition of iron salts at high temperature. The resulting nanoparticles were hydrophobic, thus a ligand exchange to render them soluble in water (hydrophilic) was conducted. This exchange of surfactant from oleic acid to dopamine resulted in an increase in magnetic moment without changes in composition, size, or phase. Another key parameter that must not only be understood but also well characterized particle size. To investigate this particular characteristic, a thermomagnetic sizing methodology was developed. It was demonstrated that the size distribution of an ensemble of magnetite MagNPs can be determined with reasonable accuracy via this analytical analysis of the thermomagnetic response of the population. The significance of this approach is that it is an alternative to TEM. The fabricated and characterized MagNP were then evaluated concurrent with the development of a microfluidic cell separation device.

A mathematical rational model was formulated for the design of a magnet-activated microfluidic cell isolation device. The model was based on a first-principles force calculation for spherical, uniform cells labeled with superparamagnetic beads. The feasibility of using the above magnetic nanoparticles within the computationally optimized design was investigated and the design was validated with homogenous and heterogenous suspensions of MCF-7 breast cancer cells in concentration of less than 1%. The rational optimization was then tested with spiked samples in whole blood as a means to truly evaluate the potential of the microfluidic CTC isolation device.
6.1 Characterization of Nanoparticle Properties Give Insight into Key Parameters for Microfluidic Device Designs

First, it was shown that the exchange of the oleic acid hydrophobic stabilizing ligand to a dopamine hydrophilic ligand more conducive for nanobiotechnological application results in an augmentation of the magnetic moment. This result is of significance because biomedical devices employing magnetic nanoparticles[30], especially those that target field-driven cell separation systems, are more sensitive and more efficient with higher magnetization values. The results presented here provide a means to understand and ultimately tailor the surface of magnetic oxide nanoparticles towards optimal states for biomedical usage.

Along with understanding the influence that exchanged stabilizing ligands have on a nanoparticle suspension, another key parameter that was investigated was the size attributes of a particle magnetic nanoparticle (MagNP) suspension. As a means to evaluate this parameter in the most facile way, the size distribution of MagNP diameters was obtained from the temperature-dependent zero-field cooled (ZFC) moment, $m_{ZFC}(T)$. The size distribution was extracted from $m_{ZFC}(T)$ via a computation translational from the Néel relaxation model and Curie’s Law. The resultant distribution of particle diameters $f(D)$ exhibited reasonable agreement with the distribution of the physical diameters obtained from TEM. The value for the low temperature effective anisotropy constant ($K_{eff}$) representing each sample was determined by simply scaling $f(D)$, and the results were shown to be comparable to literature values for iron oxide MagNPs. Use of this thermomagnetic model, where ZFC magnetic data can be translated to an analytical representation of $f(D)$, may minimize the need for characterization particle size
distribution using electron microscopy and is a facile alternative to conventional average size and distribution determination techniques.

The ability to understand the magnetic properties of a nanoparticle collection as the ligand is exchanged from hydrophilic to hydrophilic allows (i.) tuning of the magnetic properties and (ii.) a fundamental understanding of a key parameter within the context of nanotechnology as a whole, and more specifically nanomedicine. Secondly, the ability to determine the average size and size distribution allows for a valuable understanding of the limitations or advantages of a synthesized MagNP ensemble. Within the context of this dissertation, both the average size and the distribution in size were shown to play an critical role in cell separation device design, validation, and realization.

6.2  Effective Microfluidic Displacement-based Separation can be Achieved with Single-Wire Electromagnets

As a complement to the nanoparticle analysis, a mathematical rational model based on a first-principles force calculation for spherical, uniform cells labeled with superparamagnetic particles was created for the specific enrichment of rare cell populations from a sample stream. Two geometric layouts were investigated; the first design incorporated a single current-carrying wire located adjacent to a microfluidic channel with simple straight channel geometry and a single displacement direction (Figure 4.2). The second design considered two current-carrying wires with currents running anti-parallel, allowing for displacement in both the negative and positive lateral direction into a central buffer stream (Figure 4.3). The two-wire array, which included a hydrodynamic focusing of the separation stream, was shown to be preferable over the single wire based design, and allowed for the complete separation platform to fit on a standard glass coverslip (60 (L) × 24 (W) × 0.15 (H) mm³). With a completed device the
practicability of utilizing nanoparticles as the tagging moiety was studied. Within the constraints set by fitting the device onto a standard rectangular glass coverslip (60 (L) × 24 (W) × 0.15 (H) mm³) the implementation of a nanoparticle of 50 nm radius with reasonable susceptibility results in a device length far larger than the dimensions of the coverslip. It should be noted, however, that this evaluation does not exclude nanoparticles from cell separation, rather it merely eliminates nanoparticles from being used as labeled entities in the context of the defined device design and its associated constraints. Therefore, commercial superparamagnetic microbeads were substituted as the tagging beads. The microbead and cell parameters such as diameter, magnetic susceptibility, and particle binding characteristics were experimentally measured and the resultant values were directly inserted into the design equations to ensure realistic cell displacement estimates. Furthermore, key variables including current $I$ and volumetric flow rate $\dot{V}$ were investigated and compared with those accompanying state-of-art separation devices currently available and described in the literature. The initial work illustrated that effective displacement-based separation with homogenous suspensions can be achieved with electromagnets driven by reasonable and tunable currents as opposed to the more expensive and constant field permanent magnets approaches currently in use.

Following validation with a homogeneous cell suspension the influence of non-target cells on separation efficiency as well as the purity of the separated cells were investigated. As described in Section 5.4.2, as the target cells move across the flow stream within the described design there will be numerous collisions with neighboring non-target cells, causing interference in the lateral displacement and possible deviation
away from the displacement stream. Additionally, by mixing two populations of cells with the microbeads, the possibility of the anti-EpCAM coated beads either binding significantly lower than the experimentally determined binding densities or binding to the non-target cells can be also be evaluated. Probing of the effluent stream using flow cytometry illustrated that the capture efficiency was nearly 100% for heterogeneous samples of ~10 – 1,000 MCF-7 cells injected. As determined from the optimization experiment conducted with homogenous MCF-7 cell suspensions (Table 5.3), separation experiments were conducted with a 250 μm wide microfluidic channel, with a sheath and center flow rate of 120 μL min⁻¹ and an applied current of 0.25 A. Overall, it was shown that the efficiency remained above 95% for all concentrations investigated (13, 125, and 1150 cells) and the purity of the collected stream significantly decreased when the total of MCF-7 cells was reduced, but the total number of non-target cells remained the same. Due to this interesting observation a second set of experiments was performed to test the influence of non-target cell concentration on the purity when the number of non-target cells is decreased, ~13 MCF-7 cells were spiked into different suspensions (10⁴-10⁶ Raji cells). As the total number of Raji cells was decreased the purity of separated target cells was increased, as less non-target cells were present in the collection stream. Furthermore, the viability and behavior of the collected cells were tested. The collected cells had comparable viability to the inlet cell viability and the cells adhered, grew and spread similar to control cells.

A final validation of the capabilities of the rationally designed magnetophoretic microfluidic device was conducted with spiked whole blood samples. First, the computational optimization illustrated in Figure 5.12, was updated for an empirically
determined apparent blood viscosity. As illustrated in Figure 5.16, the possible device design space is significantly decreased but still remains within the constraints of the initially fabricated microfluidic channel and wire array design (as described in Section 3.2.4). Upon determination of the theoretical design restrictions, experimental determination of the blood-buffer interface was performed (Figure 5.17) and a flow rate ratio of 3:2 (blood:buffer) yielded a functional work space for the implementation of a cell separation platform directly from whole blood. Overall, these it can be concluded that to isolate a pure population from whole blood lower currents are required, but this results in slightly lower efficiencies. Depending on the desired end results, either high current should be used for high efficiency or low current for high purity. If measuring number of CTCs in a blood sample is desired as a means of diagnosing and tracking cancer, high currents should be used. On the other hand, if one needs a pure population for proteomics and genomic analysis lower currents should be used.

In conclusion, it was shown that described cell separation device design could serve as a valuable tool for continuous flow cell separation device design for implementation in the fields of diagnostic and regenerative medicine. Cells remained viable after separation as well as adhered and spread comparable to non-separated cells in static culture. Cells were isolated with efficiencies of over 95% and purities over 80%, comparable to and better than the start of the art in cell separation [22, 26, 113, 115, 116, 121, 125, 133-138, 145, 170, 215, 227].
7.0 RECOMMENDATIONS

This dissertation presents the design and development of a magnet-based microfluidic cell separation platform for the high efficiency and purity isolation of circulating tumor cells, including a rational computation optimization of a simple disposable separation chamber. This dissertation also investigated the feasibility of using biocompatible magnetic nanoparticles as tagging moieties for the manipulation of the circulating tumor cells in suspension, along with new novel characterization and understanding of these nanoparticle for application the rational design. The recommendations for future research, based on the presented results, are as follows:

1. Continue to investigate the influence of ligand exchange on the magnetic moment of superparamagnetic nanoparticles.

2. Investigate the affect of tagging on the biology of the target cells. So far viability has been shown to remain high and the growth behavior unaffected by the tagging of the cells with microbeads. Next the protein and gene profiles of the cells should be investigated.

3. Probe the possibility of using the developed magnetophoretic device for other diseases, such as cardiovascular diagnostics, and for tissue engineering. Will real clinical samples mimic the results from the spiked samples?

4. Build a final device which integrates the disposable microfluidic chamber, wire array, and power source.

**Recommendation 1: Functionalized induced changes in magnetic moment.** The first recommendation that is suggested is the continuation of work presented in Section 5.1.
To date only the influence of exchanging the oleic acid with dopamine has been investigated. There are several research groups who have presented new ligand exchange modalities but have failed to investigate if changes in magnetic moment occur upon changing the stabilizing surfactant. In addition, the work presented in Section 5.1 described the improvement in magnetization, but intentionally omitted data on stability as the dopamine-functionalized particles failed to remain in aqueous solution for over 24 hr. As this shortcoming will eliminate dopamine as a viable candidate for ligand exchange, other chemistries should be investigated in a similar fashion. These new chemistries would then be characterized in a similar fashion to investigate the influence of stabilizing moieties on the character of the hydrophilic nanoparticle. This would include, again, obtaining percent iron content from ICP-MS, sizing from the thermal response of the magnetic moment (as presented in Section 5.2), sizing and morphology from TEM, magnetization as a function of field using SQUID, and confirmation of ligand exchange using FTIR.

**Recommendation 2: Influence of immunomagnetic labeling on protein and gene expression.** The work presented in Section 5.3.4-5.3.5 clearly illustrated that tagging the breast cancer cells with the commercial microparticles did not adversely affect the viability or growth behavior of the cells. Of course, there may less obvious influences on the cells such as changes in gene expression and/or protein expression. In a 2005 paper by Woelfle and coworkers [228], the authors investigated the influence of anti-EpCAM- and anti-HER2/neu-coated microparticle labeling on the gene expression of MCF-7 and BT474 (both breast cancer cell lines). For both cell lines, it was shown that expressed
genes in antibody-treated versus untreated cells was within the range observed in the duplicate experiments. In the B474 cell line 38 genes were strongly differentially expressed (<3% of 1176 genes probed) in antibody-treated versus untreated cells. Interestingly, most of these genes play a role in extracellular matrix remodeling, signal transduction and replication, as well as repair and transcription. On the other hand, MCF-7 cells differentially expression of 31 genes (<3% of genes probed) differentially expressed genes with a ratio of over 3. Although a similar group of genes were affected, only 3 common genes (CDC7, SGI and KIR) were differentially expressed in both cell lines after antibody incubation. The authors’ results suggest that the two antibodies against EpCAM and HER2/neu used for the immunomagnetic selection process did not considerably influence the gene expression pattern of the enriched cells. Surprisingly, to the best of the author’s knowledge no other literature exists on this topic yet immunomagnetic cell separation is one of the standard methodologies used in the lab and clinic. This paucity of literature illustrates that better understanding of the influence this particular device, with it’s labeling and high fluid stresses, has on the expression of key genes and proteins would greatly enhance the potential of the presented magnetophoretic device.

**Recommendation 3: Application of device with clinical samples and investigation of other rare-cell populations for application in diagnostic medicine and tissue and regenerative medicine.** A third recommendation for this works is the realization of clinical samples with the presented device. This dissertation only went as far as spiked MCF-7 cells in whole human blood with anti-EpCAM-coated microbeads. Until the
device has been validated with clinical samples and a correlation of effectively isolation rate is determined, the device will not be adopted into a clinical setting. Even prior to translating the current device to clinical samples, other antibodies and cell lines should be probed. MCF-7 cells have over 250,000 EpCAM surface receptors (as described in Section 5.3) which allows for high binding density ϕ within the context of the rational design. Other epithelial cancer cell lines, such as PC3 cells express relatively low levels of cell-surface EpCAM (51,667 EpCAM molecules per cell) [229] and would make a more appropriate choice for optimization of the cell isolation platform. Furthermore, new antibodies need to be investigated as not all CTCs express EpCAM, one example is melanoma cells which do not EpCAM [119]. Secondly, as the tumor cells break-off from the primary tumor and enter the blood stream they begin to undergo a process called epithelial to mesenchymal transition (EMT), by which adherent epithelial cells acquire migratory cell fates. Although EpCAM-directed antibody-mediated labeling of CTCs is clearly effective, it selects for cells expressing this epithelial marker, which is down-regulated during the process of EMT [132].

As designed, this particular magnetophoretic microfluidic cell separation device can and should be used for the isolation of other rare-cell populations from blood (e.g. endothelial progenitor cells, circulating mature endothelial cell, fetal red blood cells, and hematopoietic stems cell) and tissue digestions (e.g. intestinal stem cells, follicle stem cell, and corneal stem cells). Furthermore, this device can be extended to the separation of cancer cells present in fine needle aspirates. Using the computationally optimized design equations and some a priori information on the particular system of interest one can design a cell separation platform for any number of applications. As described
earlier in this dissertation, the ability to enumeration cell populations within a heterogeneous suspension may have diagnostic value and tracking of cell concentration allows for therapeutic monitoring of patients as a means of confirming a treatment is or is not working. Furthermore, if one can effectively isolate pure population of key cell subtypes from the blood or digested tissue, and then culture these cells there may have a significant impact on the field of tissue engineering and regenerative medicine.

**Recommendation 4: Construction of a final, functional cell separation device.** A final recommendation for this project is the design and fabrication of a final separation platform. The current device, although a great design for research purposes, more steps are still needed to make the device field-deployable for clinical use. The device is currently visually aligned on a large PCB, cells injected with two separation syringe pumps, and collected into separate microcentrifuge tubes. For this research-based device to have a chance of making an impact in the general laboratory or clinical setting, all the device components should be designed into one complete (and enclosed) separation platform. The separation chamber should also be moved from a PDMS-based chamber to a more manufacturable economic polymer like poly(methyl methacrylate), cyclic olefin copolymer, or poly(carbonate).
8.0 NOMENCLATURE

\( a = \) Lattice parameter
\( h = \) Height
\( k = \) Thermal conductivity
\( k_B = \) Boltzmann constant
\( l = \) Length
\( m, M = \) Magnetic moment
\( m_S, M_S = \) Saturation magnetization
\( \dot{q} = \) Joule heat
\( t = \) Time
\( w = \) Width
\( x = \) Distance
\( A = \) Cross-sectional area
\( B = \) Applied magnetic field
\( D = \) Diameter
\( E_B = \) Energy barrier (or Activation energy)
\( F = \) Force
\( H = \) Coverslip height, Magnetizing field (or applied magnetic field)
\( H_C = \) Hematocrit
\( H_k = \) Anisotropy applied field
\( I = \) Current
\( K = \) Anisotropy
\( K_{\text{eff}} = \) Effective anisotropy
$L = \text{coverslip length}$

$M_R = \text{remanent magnetization}$

$R = \text{radius}$

$SA = \text{surface area}$

$T = \text{temperature}$

$T_B = \text{blocking temperature}$

$T_C = \text{curie temperature}$

$\dot{V} = \text{volumetric flow rate}$

$W = \text{coverslip width}$

$\chi = \text{susceptibility}$

$\chi_a = \text{high field susceptibility}$

$\eta = \text{viscosity}$

$\mu_o = \text{permeability in vacuum}$

$\mu_B = \text{bohr magnetons}$

$\mu_p = \text{magnetic moment per particle}$

$\rho_M = \text{density}$

$\rho_R = \text{electrical resistivity}$

$\phi = \text{number of particle attached to cell}$

$\tau = \text{neel relaxation}$

$\tau_o = \text{attempt frequency}$

**Subscripts**

$0.45 = \text{phenotypical hematocrit}$

$\text{appl} = \text{applied}$
blood = Whole blood

c = Cell

ch = Channel

inj = Injection

m = Magnetic

p = Particle

plasma = Blood plasma

s = Viscous drag (Stokes Law)

w = Wire

x = vector in the x-direction

y = vector in the y-direction

z = vector in the z-direction

ZFC = Zero-field cooled

MAX = Maximum
9.0 REFERENCES


