PRINTING OF FUNCTIONAL MATERIALS

### MAGNETIC INKS FOR PRINTING FUNCTIONAL MATERIALS

# By ABDEL RAHMAN ABDEL FATTAH, B.A.Ss., M.ENG.DESIGN

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#### LAY ABSTRACT

Our obsessive pursuit of technological advancement demands the creation of a new material arsenal. Such materials will bridge the gap between future novel applications and the concepts from which they once stemmed. We draw inspiration from the natural world and find that materials are grouped into specific patterns helping in defining function. Such examples include the dragonfly's wing with its microscopic cuticle arrangement, helping in achieving optimum lift. To create readily functional materials we manipulate magnetic inks using external magnetic fields. Such ink droplets can be guided through a viscous polymer, leaving 3D trails. Once the polymer is cured, the local mechanical properties are altered. We use magnetic carbon nanotube inks to print strain, oil and biosensors in a fast fabrication technique. Similarly, cells can be easily manipulated to achieve cellular structures, creating biological functional materials. Magnetic inks thus offer a simple avenue to readily fabricate functional materials.

#### ABSTRACT

The demand for new novel materials has never been higher. This is driven by the advancement in technologies such as wearable electronics. As more novel applications emerge, the need for materials fit for the tasks follows. Many emerging fabrication techniques such as additive manufacturing strive to claim governance over new material properties. However, they remain largely subject to the use of homogeneous materials to create structures. Comparatively, natural structures, such as the cuticle arrangement in dragonfly wings dictate the functionality of the wing, lending one of the most efficient and manoeuvrable flyers on earth. Understanding that heterogeneities define function, we explore simple benchtop techniques to create readily functional materials. Such techniques involve the use of magnetic inks, which can be remotely manipulated using external magnetic fields. When miscible ferrofluid droplets are guided with a magnet through a viscous prepolymer, they trace trail patterns in their wake. It is shown that such patterns create pattern-specific reductions in the elastic modulus compared to the matrix. When the ink is comprised of magnetic carbon nanotubes, we show the simple dynamic-assembly of the ink into conductive networks. The networks are easily encased in a rubber like matrix to readily obtain functioning strain and oil sensors. In a similar manner biosensors are created using the immobilization of antibodies on the surface the nanotubes. The fabrication technique results in simple and economical sensors able to detect target biomarkers within sixty seconds. We equally explore how cells can be manipulated using engineered buffer solutions and magnetic field geometries, leading to simulations to optimize cell separation in microfluidic channels and macroscale patterning of blood and cancer cells. Magnetic inks can open an avenue to facile and economic fabrication techniques in all facets of industrial applications spanning from wearable electronics to biological sensors to tissue engineering.

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# LIST OF ABREVIATIONS

Ab	Antibody
AFM	Atomic force microscopy
Ag	Antigen
BSA	Bovine serum albumin
CNT	Carbon nanotube
CVD	Chemical vapor deposition
DI Water	Deionized Water
EGFP	Enhanced green fluorescent protein
EC	Escaped cluster
EELS	Electron Energy Loss Spectroscopy
FF	Ferrofluid
FITC	Fluorescein isothiocyanate
Gd-DTPA	Gadolinium diethylenetriaminepentaacetic acid
HGMF	High Gradient Magnetic Field
LMA	Low Melting Alloy
PDF	Powder diffraction file
mBioink	Magnetic Biological Ink
mCNT	Magnetic Carbon Nanotube
MNP	Magnetic Nanoparticle
MWNT	Mulitwalled Carbon Nanotube
NiCNT	Ni Entangles in Carbon Nanotube
PBS	Phosphate buffer saline
PD	Parent Droplet
PDMS	Polydimethylsiloxane
PLC	Programmable logic controller
RBC	Red blood cell
SEM	Scanning Electron Microscopy
SQUID	Superconducting Quantum Interference Device
TEM	Trasmission Electron Microscopy
WBC	White blood cell
XRD	X-ray diffraction

# LIST OF SYMBOLS

α	Trail width to parent droplet radius ratio
A <sub>577</sub>	Spectral absorbance at 577 nm
A <sub>630</sub>	Spectral absorbance at 630 nm
β	Antibody to CNT weight ratio
B	Resultant magnetic field

By	Y-axis magnetic field
$\mathbf{B}_{y,avg}^2$	Y-axis averaged squared magnetic field gradient
C	Channel height
d	Distance between charges
d	Channel depth
γ	Magnetization ratio
е	Electron Charge
$\epsilon$	Strain
E	Elastic modulus
5	Escaped cluster to Parent droplet radius ratio
η	Escaped cluster to Parent droplet magnetic body force ratio
$\mathbf{F}_{\mathrm{Drag}}, \mathbf{F}_{\mathrm{D}}$	Drag force
$\mathbf{F}_{D}^{EC}$	Drag force on an escaped cluster
<b>F</b> <sub>M</sub>	Magnetic force
$\mathbf{F}_{M}^{EC}$	Magnetic force on an escaped cluster
$\mathbf{f}_{M}$	Magnetic body force
$\mathbf{f}_{\rho}$	General body force
g	Gravitational acceleration
g	Microstructure gap
$g^*$	Microstructure gap aspect ratio
$\hbar$	Reduced Plank constant
h	Microstructure height
$h^*$	Microstructure height aspect ratio
$\mathbf{H}_{ext}$	External magnetic field
θ	Angular component of a polar coordinate
$i_b$	Baseline sensor current
is	Sensor current
i <sub>s,a</sub>	Averaged sensor current
L	Orbital angular momentum
$\mathbf{L}_{z}$	Orbital angular momentum (Quantum mechanical expression)
L	Initial length of sensor specimen
l	Elongation increment
$\mu_0$	Permeability of free space
$\mu_{ ext{B}}$	Bohr Magneton
μ	Fluid viscosity
Μ	Magnetization

$M_{D}$	Magnetic distortion coefficient
m <sub>e</sub>	Mass of Electron
$\mathbf{m}_l$	Orbital magnetic moment
m <sup>z</sup> <sub>tot</sub>	Total magnetic moment (Quantum mechanical expression)
$\mathbf{m}_{l}^{z}$	Orbital magnetic moment (Quantum mechanical expression)
<b>m</b> <sub>Material</sub>	Magnetic moment of a material
<b>m</b> <sub>atom</sub>	Magnetic moment of an atom
$M_s$	Saturation magnetization
<i>n</i> , <i>N</i>	Number of samples
р	Microstructure array pitch
р	Fluid Pressure
q	Charge
ρ	Fluid density
R	Radius of particle or cell
r	Radial component of a polar coordinate
r <sub>s</sub>	Stagnation point position relative to the center of a parent droplet
Re	Reynolds number
R <sub>ref</sub>	Reference resistance
Rsample	Sample resistance
S <sub>z</sub>	Spin angular momentum (Quantum mechanical expression)
S	Stability parameter
t	Time
tsonic	Ultrasonication time
U	Free stream velocity vector
U	Scalar Free stream velocity
u <sub>r</sub>	Radial component of a fluid velocity
υ <sub>θ</sub>	Angular component of fluid velocity
$\mathbf{u}_{rel}^{EC}$	Flow velocity relative to an escaped cluster
<b>∲</b> Magnetite	Magnetite volume fraction
$V_t$	Trail volume
Vo	Initial voltage
V	Material volume
V	Voltage
V	Flow velocity
V <sub>cell</sub>	Cell volume

W	Microstructure width
W	Trail width
χ	Magnetic susceptibility
$\chi_{c}$	Cell magnetic susceptibility
$\chi_m$	Medium magnetic susceptibility
$\chi_{rbc}$	RBC magnetic susceptibility
$\chi_{wbc}$	WBC magnetic susceptibility
Ψ	Streamline function
$\nabla$	Gradient

# 1. Introduction

### 1.1 A World Governed by Heterogeneities

To build a pyramid, ancient people have laid bricks in such an interlocking manner, knowing that such form will lend a stable structure able to withstand environmental changes and endure the test of time. However, to build a vertical building is a wholly different problem with its own associated hurdles. It did not take long to realise that the manner with which bricks are laid could not offer much in terms of stability for larger taller buildings, gates, and walls. Hence, the arch was born. In addition, binding agents such as early forms of concrete help stabilize bricks, while the use of wood, such as palm trees used in some of the early fort constructions, enable ductility to the structure to fight against earthquakes. It became clear that an interplay between form and material defined function.

Materials, however, until relatively recently, have been looked at as homogeneous. Homogeneity is largely a manmade perspective of the world. It allows us to translate the observable universe into well-defined sets of equations, perhaps in certain cases oversimplified ones. The Fundamental reason why the pyramids of Giza could not be built using traditional mud bricks is that mud bricks are intrinsically weaker than lime stone, and would not be able to handle the dead weight of the structure. However, to further elucidate such difference in mechanical strength, one has to look at the nanostructures and atomic arrangement of the two materials, for there lies the difference maker. Heterogeneous patterns are indeed the building blocks of functional materials.

## 1.2 Heterogeneity and Nature

Nature offers us the best examples that shows the potential of nanoscale engineered heterogeneities. For example, the wing of a humble house cricket (Acheta Domesticus Linnaeus) has different Young's modulus and hardness, which depends on both the wing location and layer [1]. Such heterogenic stiffness patterns, allow for function optimization, in this case enhancement of lift production for the wing. Similarly, microstructure patterns in bones and other biomaterials such as pelvic floors as shown by De Vita et al. [2], offer superior shock absorption and protection of organs. Likewise, fish benefit from stiffness variations in their fins and scales to provide efficient propulsion through water [3-5]. Such propulsion can in fact be passively controlled, i.e. requiring no muscular input, and relying only on the fluid-fin interaction to optimize thrust generation. Heterogeneity in the stiffness of cellular membranes can control their elasticity as reviled by Rodui et al. using atomic force microscopy (AFM) [6].

Such natural examples have largely fueled the biomimetic research in the pursuit of new novel materials. It is ever so clear that to advance our material arsenal, is to break free of current limitations and explore the boundaries of new heterogeneous functional materials and the new applications they will bring. It is therefore equally imperative to master building at smaller scales, rapidly, cheaply and with high throughput.

## 1.3 Manipulation at the Small Scale

#### 1.3.1 Building at Small Scales: Bottom-Up or Top-Down?

The pyramid example highlights one of the fundamental methods of fabrication. Such a bottom up approach creates bulk objects by adding materials such as bricks to an otherwise empty space. Precise control over the position of such bricks can subsequently change the geometry. The scale of the final resulting structure is theoretically boundless with a feature resolution limited only by the length scale of the building blocks. Perhaps in ironically close proximity to the pyramids of Giza lies another example that highlights another common method of fabrication. The Sphynx demonstrates a great top down approach where material is removed to reveal the final structure. Here the final structure is limited by the original volume, however with resolutions that depend on the smallest chisels used for sculpting and thus often can far exceed the large blocks used to build the pyramids.

By translating into smaller scales, our approach to building structures has largely remained unchanged. For example, chemical vapor deposition (CVD), and its derivatives, dominate thin film manufacturing and represent a bottom up approach. Photolithography and chemical etching techniques have been the corner stones of the silicon manufacturing industry, offering both top down and bottom up approaches. While such technologies have excellent track records, evident by the rise of the electronics industry, a continuing research in new manufacturing techniques is ever growing to provide, cheaper, scalable, simpler and faster fabrication techniques for new novel functional materials.

Some new fabrication techniques provide incremental innovations on already existing technologies. For example, Nemiroski et al. demonstrate how shadows cast by nano-sized spheres on a surface during a deposition by physical vapor deposition beams can create complex 2D patterns [7]. The patterns are then governed by the position of the sphere as well as the angle of the beams. Nano-sized spheres have been utilized by Atanasov et al to induce ablation of materials from a surface to create nano-sized holes using femtosecond (fs) laser pulses [8]. Such plasmonic particles are able to absorb photonic energy through the surface plasmon resonance (SPR) phenomena, causing high temperatures to ablate materials around the spheres. Kolloch et al. demonstrate how patterned plasmonic nanomaterials of a triangular shape can be employed to cause nano ablation on the surface of silicon using fs laser pulses [9]. Similarly, Heltzel et al demonstrated the ability to pattern silicon substrates using nanoscale ablation by SPR of gold nanoparticles [10].

The use of nanoparticles of various shapes and sizes has proved to be very beneficial in the field of photonics, which in turn clearly demonstrates how form and pattern define function at nanoscales. Shape and size in particular, which are mainly governed by the chemical synthesis of the nanoparticles, play an important role [11]. This led to a wealth of predictive equations and empirical formulae to approximate particle-light interaction such as demonstrated by Kuwata et al., in which they expand the Rayleigh approximation used for spherical particles, to approximate the plasmonic response of nanorods [12]. Kelly et al. demonstrated experimental results of the plasmonic response of nano-sized triangular prisms, which offer more degrees of control, such as angles and side lengths, than a spherical geometry, which is governed by its radius [13]. Further, Baffou et al, numerically explored the effect of aspect ratio and nanoparticle patterns on the plasmonic response of particles, showing a particular redshift that occurs in conjunction with an increase in aspect ratio, from spherical to rod shapes [14].

Such nanoscale phenomena are often interrelated and thus can be further tailored by external measures. Again light interactions offer a great example. For example, localized plasmon resonance shows dependence on an externally applied magnetic field, which in turn affects light polarisation as demonstrated by Du et al [15]. While nanomaterials can be used for their excellent intrinsic qualities as heat generators, as demonstrated by Govorov et al [16, 17], to use them as building bricks, a fabrication technique is required that enables us to transfer from the realm of nano to the world of macroscale functional materials.

To build with nano and microscale materials, is to be able to pattern at these scales, and in turn, patterns will define function. For example, a homogeneous distribution of magnetite (Fe<sub>3</sub>O<sub>4</sub>) in a thermoplastic can employ magnetic heating effect using an AC magnetic field to trigger shape-memory effects, and thus can be used for actuation [18]. While homogeneous distribution, which is a random distribution pattern, of nanoparticles can usually be achieved using proper dispersal methods in a matrix, such as sonication and mechanical mixing, other pattern geometries require more direct manipulation.

Camposeo et al. show how cadmium sulfite can precipitate using a multi-photon absorption technique or laser direct writing in a polymer matrix [19]. The resultant luminescent patterns of complex geometries can find applications in nanophotonics and other waveguiding technologies. Other patterning techniques rely on patterned nanoparticles, and subsequent modification to create the final geometry. For example, Roberts et al. demonstrate patterned gold nanoparticles, which subsequently melt under laser irradiation, forming interconnected links [20]. Such conductive paths can be utilized in the fabrication of micro circuitry and electronic devices. Other elaborate methods to pattern nanostructures, is demonstrated by Martine et al. where protein is used to produce patterned ferritin deposition [21]. While the accuracy is on the order of 10 nm, patterns can extend to several microns. The above examples demonstrate the ability to pattern at the nanoscale while extending to mesoscopic and macroscopic scales, a precursor to functional bulk materials. Despite the creation of complex patterns, the techniques described above remain subject to existing technological limitations, which involve clean rooms, vacuum chambers and lasers and depend on lithographic techniques which can pose significant hurdles and complicate the process. Alternative methods do exist and can simplify the process of fabrication.

#### 1.3.2 Field Directed Manipulation

Field directed manipulation, is more like building with smart nanobricks. Such bricks already no know how to build the intended structure based on a guiding field. In a sense, it is no longer a top down or bottom up approach but rather relies on the rearrangement of elements within a specified control volume to create the final structure. Specifically, rearrangement is induced by an imposed force acting only on the elements, which through rearrangement, will define and create a specific function associated with the bulk material at the mesoscopic and macroscopic scales. This has great promise in waveguides, photonics, biosensors, chemical sensors, and data storage applications as well as stiffness patterning for mechanical applications and electrical and thermal applications where specific conduction paths are required.

Block copolymerization has gained significant interest in recent years as a method to influence the arrangement of nanoparticles. It is found that through the copolymerization process, originally homogeneously distributed nanoparticle, are autonomously force into specific regions in the matrix structure, following a pattern [22]. However, the process does not give freedom of patterning and is limited to the structures and patterns dictated by the block copolymers used. Jiang et al. demonstrate how lithographically patterned surfaces are able to confine nanoparticles suspended in liquid mediums [23]. With various shapes and size of patterns it is possible to create dynamically-assembled nanoparticle structures of like features as the patterns that confine them. Despite its ability to provide high throughput by covering large surface areas, the process still requires lithographic techniques as is the case with many such self-assembly approaches that rely on surface pretreatment. Although not immediately evident, patterning is not restricted to nanoparticles. Patterns can define function through patterned matrix heterogeneities. For example, Kasala and Sravanamuttu demonstrate how 3D patterns can be produced in a photopolymer using optical waveguides [24]. While no nanoparticles are present, a pattern is formed due to nonlinear propagation of white light through the matrix causing a self-organizing pattern, which in turn is reflected in the degree of photopolymerisation within the matrix. This approach can have applications in wave guides and potentially stiffness patterning.

Other methods have also been explored such as using an electric field, for its ease in field projection, covering large areas, and control. Dynamic-assembly of copper nanoparticles have been demonstrated by Liu et al. using a low current electric field to improve batteries in electric vehicles [25]. And while nanoparticle are usually associated with patterning, Park et al. demonstrate an example of how carbon nanotubes can be manipulated by an alternating electric field to cause alignment and improve the dielectric properties of polydimethylsiloxane (PDMS) [26]. Alternatively, magnetic fields have been explored to manipulate ferromagnetic, paramagnetic as well as diamagnetic particles to induce pattern dependent functions. For example, Pisanello et al. show how magnetite nanoparticle chain structures dynamically-assemble in PDMS under the influence of a magnetic field, which subsequently affect the dielectric properties of the matrix [27]. In addition, magnetophoretic manipulation can equally act to chaperone bulk materials. For example, Calabro et al. demonstrate how solder material, and by extension low melting alloys (LMA), embedded with MNPs can be manipulated in their molten state [28]. This allow migration into cavities and difficult to reach locations remotely with an applied magnetic field. Subsequent cooling allows the solidification of the solder.

Other advantages of magnetophoretic manipulation relates to the mechanical properties alterations imposed on the matrix. Specifically, heterogeneities in stiffness can offer applications in mimicking naturally occurring biomaterials such as the wings of dragonflies or house crickets or other biocompatible synthetic materials such as ligands as described in section 1.2. Local stiffness control within a PDMS matrix has been shown to be governed by Fe<sub>3</sub>O<sub>4</sub> nanoparticle concentration, which in turn were patterned by an external permanent magnet, as demonstrated by Ghosh et al. [29]. Matrix mechanical strength can thus be altered and enhanced. Jestin et al. demonstrate how a matrix mechanical strength can be controlled by manipulating the orientation of the magnetic nanoparticles (MNPs) *in situ* via a weak magnetic field [30].

Magnetophoretic manipulation at the small scale is not only used to create patterns in nanocomposite materials but also finds many biomedical applications with the direct manipulation of cells in a magnetic field. For example, interest in the magnetic properties of hemoglobin and its associated oxygenation states have been investigate as early as 1936 by Pauling and Coryell [31]. Such magnetic properties can then be leveraged in microfluidic environments in order to cause cell separation, which can be vital for cell culture and diagnosis applications. As a result many separation techniques, have been investigated including magnetophoretic separation for its ease of use [32]. An example of that is the widely used magnetic cell separation system (MACS) described by Miltenyi et al. [33], where targeted cells attach to magnetic bead labels, allowing subsequent manipulation via an external magnetic field. Further, magnetic drug targeting allows the direct drug delivery to specific sites in the body, a much more accurate and less harmful way compared to current practices which often include a full body exposure to a drug, such as the case with injections [34]. While the above mentioned examples focus on cell separation, the inherit cell manipulation of such processes may open an avenue for tissue engineering applications. Thus while functional materials are often thought of as inorganic in nature, biological functional tissue engineered materials may one day rely on magnetophoretic manipulation of cells. Magnetophoresis is thus a great tool to exploit in order to create novel functional materials as well as offers many biological applications.

### 1.4 Magnetic Inks for Functional Materials Printing

The focus of this thesis is to explore simple methodologies that utilizes magnetophresis of magnetic inks to print controllable functional materials. Novel magnetic inks, specific to the required function, are hereby employed and tested for the fabrication of various functional materials.

The magnetic inks used are composed of constituent materials and a dispersing medium. Under the influence of a magnetic field, the dispersed materials reorganizes. It is through this reorganization that a functional material is created. The beginning chapter offers a theoretical investigation of particle propagation through viscous media under the influence of a global magnetic field. This is essential in highlighting the equations of motion. And while the investigation is concerned with the motion of miscible ferrofluid droplets in a viscous media, the underlying equations of motion shed light on the general behaviour of particle propagation, including cells in suspension. From this theoretical investigation, stemmed the understanding of particle propagation through viscous media as well as particle interaction with various fields such as the combined effect of hydrodynamic and magnetic forces.

This is followed by experimental investigations, exploring pattern integrity and mechanical properties, demonstrating how 3D patterns are achievable using miscible ferrofluid droplets. While using miscible ferrofluid droplets enable pattern specific mechanical property variations, due to discontinuities, the resultant patterns are unable to conduct electricity. To expand the array of functional materials, alternative routes to achieve pattern specific electrical property enhancement are herein explored.

Printing electrical properties on demand is explored by employing magnetic carbon nanotube based inks. Magnetic nanoparticles attached physically and covalently to nanotube surfaces act as chaperones, guiding the carbon nanotubes into tightly packed conductive patterns when a magnetic template is applied. To demonstrate the functionality of electrically conductive dynamically-assembled networks, an elastomeric matrix is employed to embed the patterns. Now part of a bulk material, such networks can deform under the influence of mechanical strains, altering their internal resistance, which correlates with the degree of deformation resulting in a readily functional strain gauge. Electrical responses are equally demonstrated when oil contacts the conductive pattern, lending an oil sensor. Expanding the role of readily functional sensors further into the realm of biology, magnetic biological inks are thereby explored.

Biological components, such as antibodies, can be added to already magnetized carbon nanotube surfaces to lend them biological sensing capability. An ink containing such nanotubes offers simple dynamically-assembled patterning capabilities, creating readily functional resistance based biosensors. This offers direct monitoring of specific biomarker binding kinetics while utilizing simple and affordable electrical circuitry, culminating to a semi-quantitative, efficient, and economical biosensor.

Since cell magnetitophoresis relies on the same basic equations of motion explored in chapter 3 of the thesis, 3D magnetic manipulation of cells is thus finally explored in chapter 7. When cell are the constituent materials of an ink, the resultant patterns are 3D biological materials. This offers magnetic dynamic-assembly a new realm of investigation and research concerning the economic and facile bioprinting of functional biological materials, which can be considered a precursor to largescale tissue engineering. While the mechanism of magnetophoretic transport is similar in cell manipulation compared to ferrofluid droplets, the reduced magnetic susceptibility of the cells, explained in section 2.2, requires significant attention to the magnetic properties of the medium carrying the cells, as well as the magnetic field geometry. To offer an understanding to the dynamics of magnetophoretic manipulations of cells, magnetic properties of cells and media are experimentally investigated. The magnetic field geometry optimization are obtain through simulations to offer an understanding to their impact on cell manipulation and separation techniques in a microscale environment. While the focus is cell separation, the technique can also offer a cell capture and immobilization method which can be used for tissue engineering. This eventually leads to macroscale cell patterning to obtain 3D cellular structures.

Magnetic inks, whether biological or otherwise, offer a new avenue for rapid and scalable bench top fabrication techniques of readily functional materials. The ease and lack of technical complexity of the methods render them attractive alternatives to established fabrication methodologies. While not a complete replacement of conventional techniques, the use of magnetic inks can see applications in mechanical property patterning, the fabrication of strain, oil and biological sensors as well as the creation of 3D cellular structures. The thesis herein offers a glimpse of the potential of this methodologies, which remain in need of further research.

Each of chapters 3 to 7 offers background information and literature review on the corresponding topic. The following section provides an outline of the materials covered in the chapters.

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## 1.5 Outline

#### 1.5.1 Background

The background chapter is intended to provide the reader with an overview of magnetism and fluid mechanics in preparation for subsequent chapters. In there the reader will learn mainly about magnetism and its fundamentals, followed by fluid mechanics with a particular focus on dimensionless analysis and Stokes drag equation.

#### 1.5.2 Printing with Magnetic Ink Droplets

In this chapter, background information and theoretical analysis of printing with miscible magnetic inks will be explored. Experimental investigations will be presented to validate the theoretical work. The main work and results discussed in this chapter has been previously published in a paper entitled *Printing Microstructures in a Polymer Matrix using a Ferrofluid Droplet* by Abdel Rahman Abdel Fattah, Suvojit Ghosh and Ishwar K. Puri, available online on October 30, 2015 (DOI: 10.1016/j.jmmm.2015.10.112) in the *Journal of Magnetism and Magnetic Materials*. The main author of this thesis is also the first author and main contributor of the above mentioned publication.

#### 1.5.3 Targeting and Tailoring 3D Elastic Modulus Heterogeneities

In this chapter, background information are first presented regarding matrix alterations using magnetic nanoparticles as filler materials with an emphasis on mechanical properties. Next Experimental investigations are presented, which emphasize the ability to print 3D tailored elastic modulus within an otherwise pure elastomer matrix. The main work and results discussed in this chapter have been published in a paper entitled *Printing*  *three-dimensional heterogeneities in the elastic modulus of an elastomeric matrix* by Abdel Rahman Abdel Fattah, Suvojit Ghosh and Ishwar K. Puri, available online on April 18, 2016 (DOI: 10.1021/acsami.6b0391) in the Journal of *ACS Applied Materials and Interfaces*. The main author of this thesis is also the first author and main contributor of the above mentioned publication.

# 1.5.4 Magnetophoretic Dynamic-Assembly of Carbon Nanotubes Conductive Networks for Soft Sensor Applications

The background information presented in the chapter discusses the various methods which magnetization of carbon nanotubes (CNTs) can take place. This is followed by applications of patterned CNTs in various fields as mechanical, chemical, and biosensors. Experimental investigations are presented showing a new, inexpensive, rapid and facile benchtop fabrication technique for readily functional soft sensors by employing magnetic manipulation of ferromagnetic nickel nanoparticle and CNTs conjugate materials. The main work and results discussed in this chapter has been previously published in a paper entitled *Nickel Nanoparticles Entangled in Carbon Nanotubes: An Inexpensive Ink for Nanotube Printing* by Abdel Rahman Abdel Fattah, Tahereh Majdi, Ahmed M. Abdalla, Suvojit Ghosh and Ishwar K. Puri, available online on January 5, 2016 (DOI: 10.1021/acsami.5b11700) in the *Journal ACS Applied Materials and Interface*. The main author of this thesis is also the first author and main contributor of the above mentioned publication.

# 1.5.5 Magnetophoretic Dynamic-Assembly of Carbon Nanotubes Conductive Networks for Biosensor Applications

The background information presented in this chapter is meant to brefily inform the reader about the conventional method in detecting certain biomarkers and provide certain limitations of current state of the art technologies. The results and discussion section highlights the synthesis of the biological ink. This is followed by experimental investigation of the printing process of the sensors using the biological ink, followed by a presentation of the sensor test results with various samples. The chapter shows that the simple synthesis and fabrication technique leads to an economic solution in detecting biomarkers. The main work and results discussed in this chapter have been submitted to the journal of *Nature Nanotechnology*. The main author of this thesis is also the co-first author and main contributor of the above mentioned publication.

#### 1.5.6 Label-Free Cell Magnetophoretic Separation

In this chapter, the background information is given concerning the history of magnetophoretic cell separation. Magnetophoretic manipulation in this biological context allows for label free magnetophoretic cell separation. Super Quantum Interference Device (SQUID) is utilized in order to investigate the magnetic susceptibility variations in red blood cell (RBC), white blood cells (WBC) and plasma. This is followed an investigation in optimizing cell magnetic susepitibility to enhance separation, buffer design and a numerical investigation in magnetic field geometry. Sections of this chapter has been published in a paper entitled *High Gradient Magnetic Field Microstructures for Magnetophoretic Cell Separation* by Abdel Rahman Abdel Fattah, Suvojit Ghosh and

Ishwar K. Puri, available online on May 27, 2016 (DOI: 10.1016/j.jchromb.2016.05.046) in the *Journal of Chromatography B*. The main author of this thesis is also the first author and main contributor of the above mentioned publication.

## 1.5.7 Conclusions

This final chapter provides a summary of the entire thesis with an emphasis on the contributions of each chapter. These concluding remarks reconnect the various chapters and works conducted in the thesis emphasizing the usefulness of magnetic inks in the fabrication of future materials.

# 2. Background

## 2.1 Magnetism

The field of magnetism and magnetic materials is concerned with the study of attractive and repulsive forces that occur through the phenomenon of magnetism between materials. While evidence of magnetic materials discovery dates back for many thousands of years, the underlying subatomic theoretical models have only recently been formulated. That is mainly due to the surge in technological reliance on magnets from our largest power stations to the smart phones we carry on our person.

#### 2.1.1 Origin of Magnetism

The magnetic properties of a piece of iron bar finds its roots in billions of intra and interatomic interactions. In its simplest form, magnetism is the result of 1) electron orbital motion and 2) electron spin [35, 36]. The electron orbital motion can be easily understood from the relation between moving chargers and magnetism [35]. When a charged particle, such as an electron, rotates in an orbit, it mimics a current loop. By extension, when a current rotates in a loop it generates a magnetic field, as depicted by Figure 1. The magnetic moment generated can be described in simple terms as two equal charges separated by a distance **d**, such that

$$\mathbf{m}_l = q\mathbf{d} = -\frac{e\mu_0}{2m_e}\mathbf{L} \qquad (2.1),$$

where  $\mathbf{m}_l$  is the magnetic moment vector, e = q is the electron charge,  $\mathbf{L}$  is the angular momentum created by the orbiting electron,  $m_e$  is the electron mass, and  $\mu_0$  is the vacuum permeability [37]. The spin magnetic moment can equally be expressed, however using the quantum mechanical expression,

$$\langle \mathbf{m}_{s}^{z} \rangle = -2 \frac{\mu_{\mathrm{B}}}{\hbar} \langle \mathbf{s}_{z} \rangle$$
 (2.2),

where  $\langle \mathbf{m}_s^z \rangle$  and  $\langle \mathbf{s}_z \rangle$  are the spin magnetic moment, and spin angular momentum expectation values,  $\hbar$  is the reduced Plank's constant, and  $\mu_{\rm B} = (e\mu_0\hbar/2m_e)$  is the bohr magneton. The spin of an electron can either be +1/2 or -1/2 according to Pauli's exclusion principal [38]. This results in a spin quantum number  $s = \pm \hbar/2$  [37].



#### Figure 1: Origins of Magnetism.

Electron orbital magnetic moment results from the electrons orbital motion. The spin magnetic moment results from the electron spin. Both components contribute to the total magnetic moment of the electron.

Equation 1.3 below combine the quantum mechanical form of equation 1.1 and

1.2 to obtain the total magnetic moment from an electron [35, 37].

$$\langle \mathbf{m}_{tot}^{z} \rangle = \langle \mathbf{m}_{s}^{z} \rangle + \langle \mathbf{m}_{t}^{z} \rangle = -\frac{\mu_{\mathrm{B}}}{\hbar} (2 \langle \mathbf{s}_{z} \rangle + \langle \mathbf{L}_{z} \rangle)$$
 (2.3)

After taking into account the electron configurations, according to Hund's law and Pauli's exclusion principal, for the shells and subshells that are filled, a cancelation of magnetic moments is found [35, 37]. This leaves unfilled shells to contribute to the net magnetic moment of an atom. Similarly, the total magnetic moment in a volume of material, is the summation of all magnetic moments within this volume, such that  $\mathbf{m}_{Material} = \sum \mathbf{m}_{atom}$ . The magnetization can then be defined as the total magnetic moment per unit volume *V*,  $\mathbf{M} = \mathbf{m}_{Material} / V$  [37, 39]. On any magnetic diploe, a force can be created,

$$\mathbf{F} = (\mathbf{m} \cdot \nabla) \mathbf{H}_{ext} \tag{2.4}$$

where  $\mathbf{H}_{ext}$  is an external magnetic field [37, 40]. The force on the dipole helps align the magnetic moment vector with the external magnetic field line. Since the magnetization  $\mathbf{M}$  is a vector summation of all magnetic moments within a volume, the more alignment of the magnetic moments  $\mathbf{m}$  the higher the magnetization. This leads to the famous relation,

$$\mathbf{M} = \boldsymbol{\chi} \mathbf{H}_{ext} \quad (2.5),$$

where  $\chi$  is the magnetic susceptibility of the material [35-40]. The magnetic field flux density **B** relates to any field strength as,

$$\mathbf{B} = \boldsymbol{\mu}_o(\mathbf{H} + \mathbf{M}) \qquad (2.6),$$

where  $\mu_o = 4\pi \times 10^{-7}$  is the vacuum permeability and **H** is the applied magnetic field [35].

#### 2.1.2 Kinds of Magnetism

While the previous section explored the fundamentals of magnetism, it is the electron configuration and crystallographic structure of the material that play the major role in the type magnetism manifested, which largely describes the behaviour of the material in the presence of an externally applied magnetic field.

#### 2.1.2.1 Diamagnetism

Diamagnetism is a magnetic phenomenon that is exhibited by all materials, however, it is often overwhelmed by the other kinds of magnetisms discussed below. An atom with filled valance shells and subshells undergoes complete magnetic moment cancelation such that the net magnetic moment is null [35]. However, in the present of an external magnetic field  $\mathbf{H}_{ext}$ , the orbital motion of the electrons in the material is affected. A force is generated by the external magnetic field on the orbital magnetic moment. This subsequently would cause physical change to the orbital motion of electrons, affecting the angular momentum  $\mathbf{L}$ . Much like a spinning top opposes gravity via a force vector in the opposite direction, an opposite magnetic field is generated in the opposite direction to that of the field due to the change or precession of the electron orbital motion. In that way, such a material will be repulsed by a magnet rather than attracted. The material would then have a negative magnetic susceptibility  $\chi$ . Diamagnetism does not exhibit any temperature dependence and thus is unaffected by thermal fluctuations in the material.

#### 2.1.2.2 Paramagnetism

When an atom has free and unpaired electrons in its valence shell, a non-zero net magnetic moment is created. Each atom would thus have a net magnetic moment of its own, however, they are unable to couple together [35, 38]. That is because the individual magnetic moments are too weak and the thermal fluctuations,  $k_{\rm B}T$  where  $k_{\rm B}$  is the Boltzmann constant, in the material are able to break any coupling between dipoles. This results in a net magnetization of the material of zero. When an external magnetic field is applied, a force is exerted on each individual dipole, which helps align the magnetic moment created by the dipole, with the external magnetic field lines, see Figure 2. A higher magnetic field would result in better overall dipole alignment and thus the total magnetic moment of the materials (sum of all magnetic moments) would be higher, finally resulting in a higher magnetization. Once the applied field is removed, thermal fluctuations would destroy any alignment. Unlike diamagnetism, paramagnetic behavior is affected by temperature and follows the well know Curie-Weiss inverse temperature law, which states that a paramagnetic materials has its susceptibility linearly varying with the inverse of temperature.


### Figure 2: Paramagnetism.

With the existence of an external magnetic field, dipoles align. In the absence of a magnetic field, thermal fluctuations help destroy any alignment

### 2.1.2.3 Ferromagnetism

Much like paramagnetic materials, ferromagnetic ones rely on the incomplete cancelation of electron spin [35]. Electron spin alignment in these metals such as iron, cobalt, nickel and some rare earth metals can extend over large volumes or domains, which results in a net magnetic moment in the absence of an applied magnetic field.

### 2.1.2.4 Anitferromagnetism and Ferrimagnetism

Antiferromagnetic materials rely on particular alignment of magnetic moments. This occurs due to the specific arrangement of electron spins induced by the interaction of metals and oxygen. Such as the example of manganese oxide. In this case, while oxygen has no net magnetic moment, magnetise ions do assume a magnetic moment, however the arrangement is antiparallel and equal, causing a total cancellation of moments, and resulting a net magnetic moment of zero.

Ferrimagnetic materials on the other hand, although antiparallel, the moments are not equal, such that there is a net magnetic moment. They typically assume the form  $MFe_2O_4$ , where M represents a metallic element [35, 36]. A famous example is magnetite or  $Fe_3O_4$ , which contains  $Fe^{2+}$  and  $Fe^{3+}$  ions, offering different magnitude magnetic moments, and thus do not cancel out in an antiparallel arrangement. A preferred magnetic moment orientation can occur over a material domain, before a different orientation is found, creating another domain. Between magnetic domains there are domain walls, a transition region between magnetic orientations. While a net magnetic moment can exist, an external field is required to help align the different magnetic moments found in the domains, through domain wall movement. Once all possible domains are aligned, the material is said to have reach its saturation magnetization  $M_s$  at a certain **H**. The removal of the field **H** only partially reverses the process such that a remanence magnetization  $M_r$ is found.

### 2.1.2.5 Superparamagnetic Nanoparticles

Superparamagnetism can occur when ferrimagnetic materials are created with volumes that are less than domain sizes in their bulk counterparts. This typically occurs at sub 15 nm diameter for example for magnetite nanoparticles. In such cases, an applied **H**, helps align the magnetic moments of the different nanoparticles, reaching  $M_s$ , however, once removed, thermal fluctuations can destroy coupling between adjacent nanoparticles,

such that there is no remanence magnetization  $M_r$ . The process is similar to paramagnetic materials in Figure 2.

The proposed work is concerned with nanoscale magnetism and thus expects superparamagnetic behaviours from the nanoparticles. On the other hand, biological components are expected to largely follow diamagnetic behaviours, with some paramagnetism exhibited by red blood cell due to the presence of iron ions in their constituent hemoglobin molecules.

### 2.2 Small Scale Fluid Mechanics

Fluid mechanics is a large and extensive field focusing on the study of fluid flows at different scales and geometries. The work presented focuses on microscale applications, where most geometries can be simplified to flows around spheres. Such small scales entail different governing laws than macroscopic ones.

### 2.2.1 The Momentum Equation

When analysing fluid mechanics problems, the Navier-Stokes (N-S) momentum equations is almost always used. With appropriate boundary conditions, solving the N-S equations allows for an understanding of the fluid flow velocities in and around the geometry given, from which shear stresses and forces on surfaces can be derived. Although an exact solution is often not found, software is usually employed to obtain solutions through iterative approaches. Exact solutions, however, do exist for simple problems such as flow in a channel or pipe or around a sphere. The N-S equation is presented below,

$$\rho \frac{D\mathbf{V}}{Dt} = \rho \mathbf{g} \cdot \nabla p + \mu \nabla^2 \mathbf{V} (2.7),$$

where  $\rho$ , and  $\mu$  are the fluid density and viscosity respectively,  $D\mathbf{V}/Dt$  is the material derivative of the velocity field and also represents the momentum convective term, **g** is the gravitational acceleration,  $\nabla_P$  is the pressure gradient, and  $\mu \nabla^2 \mathbf{V}$  is the momentum diffusive term [41]. In simple solutions, assumptions are often employed to simplify Equation 2.7 and obtain a simple exact solution for the flow field.

### 2.2.2 Dimensionless Numbers

Dimensionless analysis is a familiar subject to fluid mechanics problems. By rendering a problem non-dimensional, a solution for geometrically and dynamically similar problems can be obtained. This avoids solving each individual problem, such as the case with small scale airfoils and aircrafts in wind tunnels, which help find solutions for their full sized counterparts. An important dimensionless number, which is often encountered, is the Reynolds number,

$$Re = \frac{\rho VD}{\mu} \quad (2.8),$$

where D is the characteristics length of the geometry being investigated and V is the fluid flow velocity. Different objects with similar *Re* are said to be dynamically similar. As an example, if V is held constant in Equation 2.8, flow around a large sphere submersed in water, would be of the same characteristics as the flow around a smaller sphere in a less viscous and less dense fluid such as air. Reynolds number is often used to validate assumptions of turbulent versus laminar flow conditions.

Other dimensionless numbers can also be obtained such as force and velocity ratios as will be described in the next chapter. This often benefits the characterisation the problem and offers predictive tools to the behaviour of the system.

#### 2.2.3 Microscale Fluid Mechanics

At very small scales, the characteristic length, for example of a spherical nanoparticle, is such that  $Re \ll 1$ . This defines the realm of fluid mechanics called Stokes or creep flow. Dynamically similar macroscopic scenarios would experience extremely slow or creeping fluid flows. As a consequence of Stokes flow and low Re, the mode of momentum transfer is dominated by diffusion rather than convection. Thus, by ignoring gravity and the convective term, Equation 2.7 can be rewritten as,

$$\nabla p \approx \mu \nabla^2 \mathbf{V}$$
 (2.9).

### 2.2.4 Stokes Flow around a Sphere

In most cases, simplifying a geometry of a nanoparticle or an agglomeration of nanoparticles to a spherical geometry often simplifies the system's governing equations. That is because well understood exact solutions can be found for 3D Stokes flow around a sphere. The streamline function of stokes flow around a sphere can be described in polar coordinates as,

$$\Psi = \frac{1}{4} U R^2 \sin^2 \theta \left( \frac{R}{\mathbf{r}} - \frac{3\mathbf{r}}{R} + \frac{2\mathbf{r}^2}{R^2} \right)$$
(2.10),

where, *U* is the potential flow velocity, *R* the radius of the sphere, **r** and  $\theta$  are the radial and tangential vector components. The velocity components,  $\mathbf{u}_{\mathbf{r}}$  and  $\mathbf{u}_{\theta}$  can then be derived as,

$$\mathbf{u}_{\mathbf{r}} = \frac{1}{\mathbf{r}^2 \sin \theta} \frac{\partial \Psi}{\partial \theta} (2.11)$$
$$\mathbf{u}_{\theta} = -\frac{1}{\mathbf{r} \sin \theta} \frac{\partial \Psi}{\partial \mathbf{r}} (2.12).$$

The derived governing equations of motion play an important role in analysing the behaviour of the system. A spherical particle undergoing Stokes flow will be subjected to forces due to the shear stress and pressure development, which act on its surface. Ultimately, the combination of stress and pressure based forces result in a total drag force described, by solving Equation 2.9, as,

$$\mathbf{F}_{\mathrm{Drag}} = 6\pi\mu \mathbf{U}R\,(2.13),$$

from which,  $2\pi\mu RU$  and  $4\pi\mu RU$  are associated with pressure and shear stress induced drag respectively.

In the case presented in chapter 3, evidence of shear was observed due to the lack of internal droplet rotation. Thus equation 2.13 is considered a good drag force approximation.

### 3. Printing with Miscible Ferrofluid Droplets

### 3.1 Introduction

In this chapter, theoretical and experimental investigations will be presented to highlight the technique of printing 3D structures using miscible ferrolfuid droplets. Part of the results and discussion section 3.5 is reprinted and adapted from the *Journal of Magnetism and Magnetic Materials*, *Printing Microstructures in a Polymer Matrix using a Ferrofluid Droplet*, 401, Abdel Rahman Abdel Fattah, Suvojit Ghosh and Ishwar K. Puri, Copyright 2016, with permission from Elsevier. The chapter will provide a literature review which is discussed in the background information section, followed by excerpts from the above mentioned paper including additional unpublished content to emphasize some of the concepts disused below.

### 3.2 Background Information

Micro and Nano composite materials have attracted considerable research interest in the last decade for their integration in applications such as photonics, plasmonics, electronics, and information storage as well as biological applications [42, 43]. Such interests stem from the ability to pattern nano and micro scale structures and geometries, causing local physical property heterogeneities, thereby influencing bulk material properties [29, 42, 44]. Much of matter that is obtained naturally has intrinsic prosperity heterogeneities that optimizes the function.

The current practices for creating complex structural heterogeneities, such as conventional lithography, nano imprint lithography, CVD and others, are extensive, multistep processes. Emerging additive manufacturing (AM) technologies such as 3D printing make possible unprecedented customization of manufactured products and flexibility of manufacturing processes. However, despite its disruptive potential, even the most sophisticated 3D printers used for AM can only print objects with a relatively homogeneous material, producing limited variations in mechanical strength and color. In sharp contrast, most usable objects consist of multiple materials, each of which serves a desired function by virtue of its inherent properties. For instance, a mobile electronic device is typically enclosed in a plastic casing that adds aesthetics and mechanical strength, and includes copper circuitry to carry signals, silicon chips for computation, as well as a glass screen that provides a transparent display shield. Since such a diversity in material properties cannot yet be completely printed, AM is largely restricted to printing mock-ups of a product to evaluate its aesthetics and ergonomics.

The next leap in AM will involve the printing of prescribed spatial heterogeneities in material properties [45-48]. Recent efforts have focused on the development of (1) functional materials in a printable form and (2) print-heads that deposit these materials in a prescribed pattern. Most such printers employ a conventional liquid-extrusion method for printing. When a single nozzle extrudes multiple inks in one pass, the corresponding flows must behave similarly, i.e., the inks must have comparable viscosities [49]. Thus, complex products that violate this condition require multi-stage printing. For instance, to print an object containing circuitry, a plastic substrate with a desired topology is first printed. Next, features utilizing solid metals [50, 51] or conductive liquids [52-54] are printed upon the substrate. This process limits the circuitry to a surface with the consequence that building in the third dimension requires the stacking of several layers [46]. A workaround is to print a 3D conductive liquid trace, such as Gallium Indium (GaIn) low melting point alloy, in an elastomer precursor base fluid [55], accomplished by moving an extrusion nozzle through the matrix. The high viscosity of the matrix preserves the printed features initially. The precursor is finally cured, usually through the application of heat, to produce a solid object containing embedded circuitry. However, the movement of the nozzle through the base fluid can distort the printed features.

The field of nanoparticle assembly offers new way of complex microstructure formation. Magnetic nanoparticles (MNPs) specifically can be remotely manipulated with an externally applied magnetic field, thus they offer a flexible means for patterning heterogeneities [42, 43]. Field-directed dynamic-assembly of MNPs in liquid pre-polymers enables the fabrication of microstructures of desired morphology [43, 56]. Subsequent polymer curing hardens the composite sample, locking the position of MNPs within it [42, 43, 56]. In contrast to 3D printing methods mentioned above, which use the lack of Brownian motion to stabilize a certain printed feature, curing occurs while the magnetic field is applied thereby keeping the formation of MNPs in place. Prior work on the use of magnetic fields to organize MNPs has produced heterogeneities, e.g., in mechanical properties in [29] where MNP concentrations governed the reduced elastic modulus. Including MNPs impeded local crosslinking of the polymer affecting the mechanical properties locally [29]. The reduced elastic modulus was shown to vary by as much as  $\sim 9$ MPa. Heterogeneities in magnetic properties were also theoretically demonstrated where magnetic remanence was achieved to chain structures of MNP superparamagnetic assemblies [57]. Other experiments have been conducted using Low Melting Alloys (LMAs) as host materials and demonstrate altered mechanical, electrical, and magnetic properties [28]. Applications for such magnetic LMAs can include guided soldering in electronics for difficult to reach locations.

Despite the great amount of literature on nanoparticle synthesis and assembly, the complexity of dynamically-assembled embedded microstructures is still somewhat limited and restricted to patterns induced by magnetic templates [29, 58, 59]. For example in [59], magnetic domains from a computer hard disc are programmed in such a way to create a desired pattern. Such magnetic domains have very large magnetic field gradients, which help guide magnetite nanoparticles to settle, recreating the magnetic domain patterns. Template techniques are however, highly two dimensional. Alternatively, quasi-linear filaments of MNPs that are aligned with the applied field limits possible geometries [42, 43, 60-63]. For example in [43], the MNPs are guided into dynamically-assembled chain filaments, restricted in geometry by the imposed orientation of the magnetic field.

MNPs have been heavily studied in the field of ferrofluids (FF). FF in general have several applications such as dynamic seals for rotating machines in order to isolate impurities, heat dissipaters, e.g., with loud speaker coils, and as inertial and viscous dampers in stepper motors [64, 65]. Biomedical fields equality benefit from the use of ferrofluid applications such as the case with drug targeting, which exploits the remote manipulation characteristics of MNP clusters to deliver drugs to specific locations in the body [65]. Another example is magnetic fluid hyperthermia, which uses magnetic heating of MNP clusters to locally heat a targeted tumor [65].

The propagation behaviors of MNP loaded droplets (i.e. ferrofluids) in nonmagnetic medium with phase interface have been heavily studied numerically and experimentally [66-68]. Such droplet manipulation depends on the magnetic field strength as well as its temporal variation, which govern the trajectory of the droplet. For example, FF droplet shape stability under uniform magnetic fields [67, 68], and FF droplet shape evolution in a non-uniform magnetic field have been demonstrated experimentally [69]. New translation approach for ferrofluid droplets using rotating magnetic fields has been shown, allowing travel on substrate surfaces [70]. Despite the extensive FF droplet research, the focus has mostly been on immiscible conditions in a nonmagnetic medium with a phase interface, and therefore non mixing conditions. While most research focus on droplet manipulations for microfluidic and biomedical applications, some materials applications show the manipulation of FFs to obtain micro and nanostructures patterns. For example, limited research was found on miscible conditions focusing on interface stability have been reported in a rotating Hele-Shaw flow with perpendicular magnetic fields forming labyrinth patterns, as well as some magnetically assisted capillary investigations [71-75]. FF offer 3D manipulations while miscibility can offer materials deposition. Thus it is theoretically possible to increase the complexity of patterns and extend it into the true 3D realm. That is to say transition from patterns that are function only of  $f(\mathbf{B})$  to  $f(\mathbf{B}, d\mathbf{B}/dt)$ .

We propose a novel approach to deposit functional materials in an elastomer matrix. Instead of employing an extrusion nozzle, we use a ferrofluid (FF) droplet as the print head. The FF comprises magnetic nanoparticles (MNPs) dispersed in a carrier liquid. The carrier is selected to be miscible with the base prepolymer. The FF droplet is moved along a prescribed path in the prepolymer by applying an external magnetic field [66, 67, 69, 76]. The printed trail is created by the dynamics between the hydrodynamic forces that act on the miscible droplet as it is pulled through the viscous medium by a magnetic force. When the droplet moves through the prepolymer matrix, regions of higher upstream and lower downstream pressure act on the front and back surfaces of the droplet [67]. The higher upstream pressure produces the familiar droplet shape. Since the droplet is miscible, there is negligible surface tension. Hence, the lower downstream pressure allows loosely bound nanoparticle clusters to escape from the main body of the droplet. As they depart from the droplet, these nanoparticles follow the flow streamlines, forming a trail in its wake [77-80]. The relatively high viscosity of the prepolymer and low diffusivity of the nanoparticle clusters allow the printed trail to be retained within the matrix. By employing out-of-plane three-dimensional variations in the applied magnetic field, 3D curvilinear trail patterns are readily printed in the base prepolymer, as illustrated in Figure 3. After deposition, the prepolymer is crosslinked to preserve the printed structures [77, 80-82].



### Figure 3: Illustration of the printing method.

(a) A ferrofluid droplet is injected in liquid PDMS. A magnetic field is used to move the droplet along a prescribed path whereupon it sheds a trail of nanoparticles in its wake. (Insets: Photomicrographs showing the stationary and moving droplets). (b) Curvilinear lines of a desired geometry can be printed by changing the direction of magnetic field gradient over time in 3D [83].

### 3.3 Theory

The mixing process of two miscible incompressible fluids is a well-explored problem [84]. Under high Péclet numbers, the presence of a concentration and density gradients between the two fluids can be thought to create a surface tension like stress known as a Korteweg stress [73]. In all cases of miscible droplet propagation, the emission of a tail is always evident [84]. The formation of fingering and labyrinth patterns has been shown under high Péclet numbers and different Korteweg coefficients [73, 85-87]. If diffusion is considered too slow, it may be ignored and for small propagation distances and relatively short time scales, Korteweg stresses can also be neglected and thus the problem can be considered as a free surface problem [84].

In our analysis the medium is considered an incompressible fluid and thus will be governed by the following governing equations,

$$\nabla \mathbf{.u} = 0 \quad (3.1)$$

$$\rho \frac{\mathbf{D}\mathbf{V}}{\mathbf{D}t} = \rho \mathbf{g} - \nabla \mathbf{p} + \mu \nabla^2 \mathbf{V} \quad (3.2)$$

where Equation 1 is the continuity equation and Equation 3.2 is the well-known Navier-Stokes equation, where  $\rho$  is the fluid density, **V** is the velocity vector,  $\nabla p$  is the normal stress,  $\mu$  is the fluid viscosity, and  $\mu \nabla^2 \mathbf{V}$  depicts the viscous dissipation effect. A simple body force evaluation on the ferrofluid droplet yields the following generalized equation of motion,

$$\mathbf{F}_{Net} = \mathbf{F}_M - \mathbf{F}_D \quad (3.3),$$

where an FF droplet experiences a magnetic force  $\mathbf{F}_M = 4/3\pi R^3 \mathbf{f}_M$ , where *R* denotes the droplet radius and  $\mathbf{f}_M = \nabla(\mathbf{M} \cdot \mathbf{B})$  the magnetic body force, which is assumed to be uniform throughout the droplet, hence  $\nabla \mathbf{F}_M = 0$ . **M** and **B**, respectively, denote the average magnetization of the droplet and the externally applied magnetic field it experiences. Due to the microscopic size, Stokes flow conditions (Re  $\ll$  1) apply the drag is approximated by Stokes formula for flow over a rigid sphere from Equation 2.13 [84, 88]. Subsequently, this reduces Equation 3.2 to the following to Equation 2.9.

Rewriting Equation 3.3 and assuming a spherical geometry for the droplet,

$$\mathbf{M}\mathbf{a} = \frac{4}{3}\pi \mathbf{R}^{3}\mathbf{f}_{\rho} - 6\pi \mathbf{U}\mathbf{R}\boldsymbol{\mu} \quad (3.5),$$

where, M and **a** are the mass and acceleration of the droplet respectively, R, U and  $\mathbf{f}_{\rho}$  are the radius, velocity and force density of the droplet respectively. Realizing that M =  $(4/3)\pi R^3 \rho$ , where  $\rho$  is the droplet density, Equation 3.5 can be rearranged to the following nonhomogeneous second order PDE.

$$\frac{d^{2}x}{dt^{2}} + \frac{9\mu}{2R^{2}\rho}\frac{dx}{dt} = \frac{\mathbf{f}_{\rho}}{\rho}(3.6).$$

Solving the particular and complementary solutions of Equation 3.6 yields the following equations of motion.

$$x(t) = \left[ \left( \frac{2R^2}{9\mu} \right)^2 \mathbf{f}_{\rho} \rho \right] \left( e^{-\frac{9\mu}{2R^2\rho}t} - 1 \right) + \frac{2R^2 \mathbf{f}_{\rho}}{9\mu} t \quad (3.7),$$
$$x'(t) = \frac{2R^2 \mathbf{f}_{\rho}}{9\mu} \left( 1 - e^{-\frac{9\mu}{2R^2\rho}t} \right) \quad (3.8).$$

Operating conditions are expected to be on the micro scale and thus inertial forces are ignored thereby simplifying the equations of motion by ignoring the exponential terms. This can be verified by evaluating the term  $9\mu/2R^2\rho$  in equations 3.7 and 3.8 for  $R \ll$ 1. Upon further analysis of the equations of motion, an important dimensionless parameters,  $\Pi_1$ , is derived,

$$\Pi_1 = \frac{9\mathbf{U}\mu}{2\mathbf{R}^2\mathbf{f}_{\rho}} (3.9),$$

where,  $\Pi_1$  is the ratio between the drag and magnetic driving forces acting on the droplet found in Equation 3.5. For Stokes flow conditions, a droplet maximum terminal velocity is directly derived by assuming a steady state condition (i.e.  $\Pi_1=1$ ), i.e. assuming that LHS of Equation 3.5 is negligible.

$$\mathbf{U} = \frac{2\mathbf{R}^2 \mathbf{f}_{\rho}}{9\mu} \ (3.10).$$

Equation 3.10 shows that the droplet radius largely affects its terminal velocity. By extension large clusters of magnetic nanoparticles, have considerably more effective volume than smaller ones and thus would tend to travel faster.

As a droplet propagates through the medium in Stokes flow conditions, surface tension counteracts the negative pressure at the tail of the droplet, and while satellite droplets can break off at high magnetic field gradients, the droplet remains largely intact [89]. By eliminating the surface tension, particles are allowed to escape form the parent droplet (PD) at any velocity (i.e. magnetic field gradient). Escaped clusters (EPs) will then be subject to the velocity field created by the propagation of the PD though the viscous medium, see Figure 4.





The velocity field around the PD can be approximated using the streamline function equations in polar coordinates derived from Stokes' flow approximation, Equations 2.11 and 2.12. Since they contain MNPs, the ECs that are shed in the wake of the PD continue to be influenced by the magnetic field. When the influence of this field produces substantial motion, it disturbs and distorts the printed features. The magnetic and drag forces experienced by an EC govern its motion. They are, respectively,

$$\mathbf{F}_{M}^{EC} = \frac{4}{3}\pi(\zeta R)^{3}\eta \mathbf{f}_{M}(3.11)$$
$$\mathbf{F}_{D}^{EC} = 6\pi\mu\zeta R\mathbf{u}_{rel}^{EC}.(3.12)$$

Here,  $\zeta$  denotes the ratio of EC and PD radii, and  $\eta$  the ratio of their respective magnetic body forces, which is the ratio of their respective MNP volume fractions  $\phi_{EC}$  and  $\phi_{PD}$ . The FFs used here have limited MNP agglomeration so that  $\eta \approx 1$ . Again, due to negligible inertia,  $\mathbf{F}_{M}^{EC} = \mathbf{F}_{D}^{EC}$ . Thus, the velocity of the EC relative to the surrounding flow,

$$\mathbf{u}_{rel}^{EC} = \frac{2(\zeta R)^2 \eta \mathbf{f}_M}{9\mu} = M_D \mathbf{U} \quad (3.13),$$

where  $M_D = \eta \zeta^2$  denotes a magnetic distortion coefficient which quantifies the integrity of the printed features as a function of EC and PD properties, since high  $M_D$  values implies significant motion of the trail after deposition.

Despite ample homogenization, in some instances relatively large ECs can still be shed by the PD. Size-dependent differences in the motion of the ECs leads to self-regulation of the printing process. Whereas smaller ECs escape the PD and become deposited in its wake, a PD recaptures larger ECs. This is explained by analyzing the EC dynamics relative to the flow of the surrounding base prepolymer.

For ECs to be deposited in the wake of the PD, their magnetophoretic motion must be insignificant as compared to that of the surrounding fluid flow, i.e.,  $u_{rel}^{EC} \ll u(r,\theta)$ . Here,  $u(r,\theta)$  is obtained from Stokes solution for steady flow past a small sphere. When  $u_{rel}^{EC}$  is comparable to  $u(r,\theta)$ , the EC trajectory deviates from the surrounding flow. The ratio,

$$S = \frac{u(r,\theta)}{M_{\rm D}U} \quad (3.14),$$

is a dimensionless scalar field that maps locations where the trajectory of an EC is likely to deviate from the flow. The smaller the value of *S*, the more significant is the EC deviation. Colour maps can help identify regions of strong deviations depending on the corresponding EC  $M_D$  value, see Figure 5.



### Figure 5: S maps for different values of $\eta$ and $\zeta$ .

The colour maps show the S field downstream of the PD. As  $M_D = \eta \zeta^2$  increases toward the top right of the plot, lower S values can be observed throughout the wake, indicating large deviations from the streamlines. However, for decreasing values of M<sub>D</sub>, the corresponding high S values indicate conformation of the ECs to the streamlines and thus formation of a well-defined trail.

*S* is smallest immediately downstream of a PD, where the ECs have the highest propensity to deviate from the flow. Faaway from the PD, i.e. in a section of the deposited trail where the *ECs* are far from the PD body,  $u_{rel}^{EC} \sim U$ , and thus  $S \sim M_D^{-1}$ , hence smaller ECs, corresponding to smaller  $M_D$ , exhibit smaller deviations. Thus, downstream of the PD, the smaller an EC is, the more likely that it is locked into the Stokes flow while larger ECs deviate from it. The condition  $u_{rel}^{EC} = u(r, \theta)$ , i.e., S = 1, identifies a stagnation point at  $(r = r_s, \theta = 0)$  where the influence of the magnetophoretic velocity on the ECs counteracts their fluid drag. Upstream of this stagnation point,  $u_{rel}^{EC} > u(r, \theta)$  where ECs travel back towards the PD and are recaptured. Downstream, where  $u_{rel}^{EC} < u(r, \theta)$ , the ECs are deposited in the PD wake in the form of a trail. From Stokes streamline function Equation 2.10,

$$M_D = 1 - \frac{3}{2} \frac{r_s}{R} + \frac{1}{2} \left(\frac{r_s}{R}\right)^2 (3.15),$$

i.e.,  $r_s$  increases with EC size and thus  $M_D$ , see Figure 6 below.



Figure 6: Change of the stagnation point position as a function of  $M_D$ .

An asymptote is seen at  $M_D = 1$ , indicating that an EC with  $M_D < 1$  cannot be deposited if it breaks away from the PD. However, because ECs escape from the entire downstream facing half of the parent droplet, they are able to bypass the  $r_s$ , which result in material deposition and trail formation. The further the stagnation point (i.e. large  $r_s$ ) from the PD tail, the more chance corresponding EPs will be recaptured. However, in the range of  $1 < r_s < 5$ ,  $M_D$  rises sharply, and since  $r_s/R$  is small, the chances of deposition for relatively high value  $M_D$  EPs can be significant. If deposited, such EPs will cause trail instability and deformation. Therefore in optimizing the deposition process,  $M_D$  should be as small as possible so as not to cause trail instability, also resulting in minimal  $r_s/R$  values which in turn increases the ease of deposition.

Thus, larger ECs (corresponding to larger  $M_D$ ) are shed into a more expansive recapture region. In contrast, since the recapture zone is much diminished for smaller ECs, they are readily deposited in the PD wake. The Figure below shows a  $\mathbf{u}_{rel}^{EC}$  phase portrait for 4 different  $M_D$  values and the corresponding changes and  $r_s$ .



## Figure 7: Phase portraits showing the variation in $\mathbf{u}_{rel}^{EC}$ and stagnation point $r_s$ (yellow X) as function of the $M_D$ .

In the case where  $M_D = 1 \ge 10^{-3}$ ,  $r_s$  is located very close to the surface of the droplet with radius *R*. As  $M_D$  increases, so does  $r_s$  and  $r_s/R = 1.089$  and 1.37 for  $M_D = 1 \ge 10^{-2}$  and  $1 \ge 10^{-1}$  respectively, allowing for recapture of the ECs with that particular  $M_D$  value. For the case where  $M_D = 1$ ,  $r_s$  extends to infinity, i.e. ECs will eventually be recaptured by the droplet, regardless where ECs are located in the PD wake.

### 3.4 Methodology

Magnetite nanoparticle (2.5% v.f.) kerosene based ferrofluid was synthesized using a simple Massart coprecipitation technique [90, 91]. Iron (II) Chloride tetrahydrate, FeCl<sub>2</sub>•4H<sub>2</sub>O (ASC Grade 97-102%, A16327, Alfa Aesar), and Iron (III) Chloride hexahydrate, FeCl<sub>3</sub>•6H<sub>2</sub>O (98%, 12497, Alfa Aesar) were diluted in distilled water with the application of heat using a hot plate. Ammonium hydroxide, NH<sub>4</sub>OH, was slowly added to the solution followed by an immediate precipitation of magnetite, Fe<sub>3</sub>O<sub>4</sub>. Oleic acid was then added as a surfactant to the solution with vigorous mixing using a mechanical mixer for 3 hours while applying heat using a hot plate to help evaporate excess ammonia fumes. Finally kerosene was added to the solution with vigorous mixing for 2 hours. The oleic acid, which is hydrophobic, coats the MNPs and with the addition of kerosene, help drive the MNPs to the kerosene phase. Sodium chloride was added to the solution and mixed well until sodium chloride can be seen at the bottom of the beaker as an indication of saturation. The sodium chloride will help the buoyancy driven separation of the remaining water and ferrofluid by increasing the density of water. Upon separation, the kerosene based ferrofluid was decanted and tested for colloidal stability. Figure 8, shows the resultant magnetite nanoparticles prior to the application of oleic acid and kerosene.



#### Figure 8: TEM image of magnetite magnetic nanoparticles.

The Figure shows nanoparticles prior to the application of the oleic acid surfactant and kerosene. The nanoparticles are shown to range from 10 to 20 nm.

In creating the miscible solution, the ferrofluid is added to Polydimethyl siloxane (PDMS) (Sylgard 184 Silicone Elastomer Base, 3097366-1004, Dow Corning) (1:1 v/v). The mixture was mixed vigorously then sonicated using a probe sonicator sonication

(QSonica Q500) pulsating at 3 seconds ON and 7 seconds OFF at 30% to avoid over heating of the sample for 30 seconds. The solution was inspected using an upright microscope to ensure little or no agglomeration occurred.

The matrix was prepared using a mixture of pure PDMS and curing agent (Sylgard 184 Silicone Elastomer Curing Agent, 3097358-1004, Dow Corning) (10:1 w/w). The mixture was degassed three times to ensure no air bubbles are retained after the mixing process. 35 x 10 mm petri dishes are used as sample holders. A volume of 4.8 mL of the uncured matrix is poured into one petri dish for each sample and allowed to reach a uniform temperature of ~ 80°C over 4 min using a water bath and a hotplate. This allows convective currents to take place and thermally stabilize the matrix prior to deposition.

Upon reaching a uniform temperature, the matrix is taken out of the water bath and a droplet of the miscible solution is manually added via micropipette into the matrix. To apply a global magnetic field, permanent rare earth neodymium magnets were used (N52, K&J Magnetics). In the experimental setup, the petri dish remains fixed at the origin position, while predefined positions mark the permanent magnets locations such that the surface of the magnet is 15 mm away from the PD at the time of the recording. In order to change the orientation of the magnetic field, magnets are removed or added to the defined position around the petri dish. A DSLR camera is used in combination with an inspection microscope to record the position of the droplet as it propagates through the matrix. The patterning process is limited to 2 min to minimize cooling of the matrix. If the sample cools too much, convection currents can distort the pattern upon its return for the final curing step.

Once the pattern is complete, the petri dish is returned to the water bath for an additional 15 min of curing time, at the end of which the matrix hardens locking the pattern into place.

3.5 Results and Discussions.

In this section we demonstrate the patterning ability the miscible PD propagating in a straight line. A miscible solution is prepared as described above using ferrofluide-PDMS (1:1 v/v) yielding a 1.25% magnetite concentration. A droplet was inserted into the matrix after the preheating step. The magnetic field was then oriented to guide the parent droplet in a strait path. **Figure 8**, shows a miscible PD propagating in a straight path. The yellow arrow shows the direction of the magnetic field vector. Using ImageJ software, the parent droplet diameter was measured to be 450  $\mu$ m, and the trail diameter measures 85  $\mu$ m close to the parent droplet. The trail is found to hold a constant diameter value of 42.3  $\mp$  5.8  $\mu$ m at a distance >5R. The observed trail diameter consistency validates the accuracy of deposition.



### Figure 9: EP deposition using a miscible ferrofluid PD propagating in a straight line under a global magnetic field.

EC diameters are measured to be on the order of 10  $\mu$ m yielding  $M_D = 4.9 \times 10^{-4}$  indicating the good integrity of the trail with little distortion. The *S* map for the above experiment is presented in Figure 8 for 10  $\mu$ m diameter EPs. Figure 2 shows high *S* values near the shoulders of the parent droplet indicating no divergence from the streamlines. *S* values are shown to increase further downstream form the parent droplet indicating negligible divergence from the streamlines. This is validate by experimental observations in Figure 8, where the trail forms a slender straight line, following the parallel streamlines formed downstream of the PD. The velocity of the droplet, from observation, is calculated to be 62.5  $\mu$ m/s. With a PDMS density of 965 kg/m<sup>3</sup> and a viscosity  $\mu = 60$  Pa s, the droplet's *Re* from Equation 2.8 is *Re* = 4.52 x 10<sup>-7</sup>. This validates the creep flow assumption upon which the equations of motion are based.

To illustrate how  $M_{D}$  influences print integrity, consider the two U-shaped features produced by two different FF droplets, as shown in Figure 10. The PD in Figure 10(a) has  $R = 190 \,\mu\text{m}$  and sheds fine ECs, yielding  $M_D \approx 6.2 \times 10^{-4}$ , while Figure 10(b) shows a PD with  $R = 425 \ \mu m$  that sheds coarser ECs with  $M_D \approx 1.9 \times 10^{-1}$ . The size of the ECs are different because the two FFs are homogenized differently during their preparation. For the PD with the smaller  $M_D$ , the ECs are locked into the elastomer matrix once the PD has passed. This is best observed by considering the displacement of the EC encircled in green in Figure 10(a) at times t = 31 s and 61 s. In this  $\Delta t = 30$  s interval, the EC travels only 3.1  $\mu$ m downward although the corresponding PD travels 505.6  $\mu$ m in the same direction. Thus, the displacement of the EC is less than 1% of that of the PD. In contrast, for the larger  $M_D$ , the ECs continue to experience significant magnetophoresis far downstream of the droplet, which disturbs and distorts the printed trail. The EC encircled in cyan in Figure 10(b) travels a longer 175.4  $\mu$ m between t = 19 s and 35 s. This is significant relative to the 723.2  $\mu$ m downward travel of the corresponding PD during the same interval. Thus, to prevent distortion of the printed features by magnetophoresis, a smaller value for  $M_D$  is essential, i.e., the ECs must be much smaller than the PDs.



### Figure 10: To prevent the distortion of printed features by magnetophoresis, a small magnetic distortion coefficient $M_D$ is critical,

The nanoparticle clusters contained in the ferrofluid droplet must be small in size relative to the droplet. This is illustrated by printing with two different ferrofluids that have (a)  $M_D \approx 6.2 \times 10^{-4}$  and (b)  $M_D \approx 1.9 \times 10^{-1}$ . The clusters shed by the droplet with a lower  $M_D$  (a) are locked into the base prepolymer once the parent droplet has moved away. For instance, when the parent droplet translates 505.6  $\mu$ m downward in the interval between t = 31 s to t = 61 s, the cluster encircled in green translates only 4.1  $\mu$ m, i.e., <1% of the displacement of its parent droplet. In contrast, for the higher  $M_D$  (b), the printed features are distorted because the clusters continue to undergo significant magnetophoresis after the droplet has

passed. For instance, the cluster encircled in cyan translates 175  $\mu$ m downwards from time t = 19 s to t = 35 s when its parent droplet translates 723.2  $\mu$ m.

For illustration of the control and use of *S* values in predicting the trajectory behaviour of ECs, consider the trajectories of the two ECs that are almost simultaneously shed from the PD shown in Figure 11(a). That PD has a radius of 201  $\mu$ m, while the two ECs that are shed have radii of 22.6  $\mu$ m ( $M_D = 1.3 \times 10^{-2}$ ) and 2.4  $\mu$ m ( $M_D = 1.4 \times 10^{-4}$ ). Their two trajectories are highlighted in cyan and yellow, respectively. The smaller EC is deposited in the wake of the PD, whereas the PD recaptures the larger EC.



### Figure 11: The printing process is self-regulating.

This ensures that only smaller nanoparticle clusters may escape their parent droplet while larger clusters shed by the droplet are recaptured subsequently. The trajectories of two escaped clusters are examined in the wake of the droplet. (a) The larger cluster (LC), with

 $M_D = 1.3 \times 10^{-2}$  (cyan), is recaptured by the droplet. The smaller cluster (SC), with  $M_D = 1.4 \times 10^{-4}$  (yellow), escapes and is deposited in the wake. (b) The phase portrait of the LC reveals a location where  $u_{rel}^{EC} = u(r, \theta)$ , which is a stagnation point, marked with an X. As the LC approaches this point,  $\mathbf{u}_{rel}^{EC}$  becomes substantial enough in comparison to  $\mathbf{u}(r, \theta)$  so that it moves towards the parent droplet and is subsequently recaptured by it (magenta trajectory). The SC does not encounter such a stagnation point and it is therefore deposited in the trail ejected from its parent droplet (green trajectory). (*Inset*: Values of *S* are relatively large everywhere for the SC but smaller (*S*~1) for the LC immediately downstream of the parent droplet, allowing the LC to deviate from  $\mathbf{u}(r, \theta)$  in this region so that it is recaptured).

The *S* map of Figure 11(b) shows that for the EC with  $M_D = 1.3 \times 10^{-2}$ , recapture occurs in the region that corresponds to smaller *S* values that is situated immediately downstream of the PD. In contrast, for  $M_D = 1.4 \times 10^{-4}$ , the deposition is influenced by larger *S* values. The trajectories of both ECs relative to their PDs are predicted by examining their velocities  $\mathbf{V} = \mathbf{u}_{rel}^{EC} + \mathbf{u}(r,\theta)$ , presented as the phase portraits in Figure 11(b). Observation of the directions of **V** for the larger EC reveal a location where  $u_{rel}^{EC} = u(r,\theta)$ , i.e., the stagnation point. As the larger EC approaches this point, the magnitude of  $\mathbf{u}_{rel}^{EC}$  becomes substantial in comparison with  $\mathbf{u}(r,\theta)$ . Hence, the cluster moves towards its parent droplet and is subsequently recaptured (magenta trajectory). The smaller EC does not encounter the stagnation point and is therefore deposited in the trail, ejected from the parent droplet (green trajectory).

The coupling of magnetophoresis with Stokes flow provides control over the thickness w of the printed lines, where w is proportional to the PD radius R due to the geometric similarity of the flow fields. Since Stokes flow is time-independent, the trail

width should not depend on the PD velocity. Indeed, Figure 11 shows that the wakes of four PDs created with the same FF but with different initial radii yield the relatively constant ratio  $\alpha = w/R \approx 0.18$ .



Figure 12: The width *w* of the printed trail is proportional to the droplet radius *R*. This is verified by using four different parent droplets of varying sizes in the experiments, yielding a constant ratio  $\alpha = w/R \approx 0.18$ . (Height of error bars = 2  $\sigma$ , *N* = 10)

### 3.6 Conclusion

We report the ability to print 3D curvilinear features of magnetic nanoparticles in an elastomer matrix using a ferrofluid droplet as the print head. This new method overcomes limitations on complexity placed by the nozzle-extrusion additive manufacturing methods. By providing controlled printing in 3D, the method also expands the well-known field-directed dynamic-assembly of magnetic nanoparticles, which has hitherto yielded structures of limited complexity. By dispersing functional nanomaterials, e.g., carbon nanotubes, silver and copper nanoparticles, in the ferrofluid, patterning of such materials can be achieved. Therefore the method can be used to print functional features in an elastomer matrix, e.g., to produce a conductive circuit in a soft wearable device.

# 4. 3D Heterogeneities in the Elastic Modulus of an Elastomeric Matrix.

### 4.1 Introduction

In this chapter, experimental investigation will be presented to highlight impact of the printing technique presenting in chapter 3 to create 3D heterogeneities in the elastic moduls of elastomeric matrices. This chapter is reprinted and adapted with permission from Abdel Fattah, A.R., S. Ghosh, and I.K. Puri, *Printing three-dimensional heterogeneities in the elastic modulus of an elastomeric matrix*. ACS Appl Mater Interfaces, 2016 (DOI: 10.1021/acsami.6b0391). Copyright 2016 American Chemical Society. URL: http://pubs.acs.org/doi/abs/10.1021/acsami.6b03091 [92]. The chapter will provide a literature review which is discussed in the background information section, followed by excerpts from the above mentioned submitted manuscript including additional unpublished content to emphasize some of the concepts disused below.

### 4.2 Background

The additive manufacturing (AM) technology enables the manufacturing of a wide spectrum of products in varying shapes and sizes, and therefore promises integration in multiple industries and applications. Despite the unique advantages it offers, AM largely remains limited to building objects with homogenous materials applied through moving nozzles. In contrast, much of the naturally occurring materials have intrinsic prosperity anisotropies that optimizes functions. For example, the wing of a house cricket (Acheta Domesticus Linnaeus) has three dimensional variations in its reduced Young's modulus, which help optimize lift during flight [93]. Similarly, fish benefit from stiffness variations in their fins and scales to provide efficient and passive propulsion through water [4, 94, 95]. The translation of such natural technologies and control over 3D material property anisotropy are key to creating future materials as they would improve and refine material functionalities and expand the spectrum of applications[45-48].

AM demonstrate 3D control over object geometry, and a workaround their associated limited homogeneous materials has been demonstrated by including additives such as carbon fibers in epoxy-based inks used in 3D printers, which in turn locally increase the mechanical strengths of such composite materials [96]. However, nozzle movement can disrupt the pattern during printing. Magnetic nanoparticles (MNPs) have been shown to have an opposite effect, by reducing the stiffness of elastomeric matrices [29]. The use of magnetic nanoparticles is attractive as it offers remote manipulation. By using an external magnetic field, MNPs that are homogeneously disperse in a liquid prepolymer, migrate towards regions of higher magnetic field, such as the surface of a magnet. The assembled formations are then locked in place by curing of the polymeric matrix, where they locally induce changes in the properties the matrix [29, 30]. However, producing materials anisotropy using MNPs have been largely limited to 2D patterns using a magnetic template [29, 58, 59] or quasi 1D chains produced by the applied field [42, 60, 63, 97].

To overcome such limitation, we have recently demonstrated the ability to print 3D curvilinear patterns in an elastomeric matrix using miscible ferrofluid droplets guided by a magnetic field [98]. As the droplet progresses through prepolymer, regions of high pressures develop upstream, which help form the droplet shape. Meanwhile, negative

pressure downstream of the droplet is created, which when combined with the lack of surface tension, allows nanoclusters of magnetite to escape the droplet as it progresses through a viscous prepolymer matrix, forming a trail. Out of plane variation in the applied magnetic field allows for 3D droplet trajectories and thus 3D trails, see Figure 13. The high viscosity retains the printed patterns during printing. They are then locked in place as the matrix cures. Here we explore the ability for such patterns to induce localized, pattern specific stiffness anisotropy in a polydimethylsiloxane (PDMS) matrix. The presence nanoclusters in the printed trails during curing, induces a change in the Young modulus of the matrix inside the trail compared to a pure matrix without nanoclusters outside the trail. Such 3D targeting of anisotropy is further tailored by changing the volume fraction of magnetite in the droplet solution. This unprecedented fast, precise and controllable method of printing true 3D mechanical anisotropy in a polymer matrix promises applications in soft robotics and perhaps the synthetic biocompatible materials [2].



### Figure 13: Printing Method.

(a) A ferrofluid ink droplet is inserted into liquid PDMS and then traversed along a desired path using an external permanent magnet. The droplet sheds a trail, marking its trajectory, thus printing an ink contour in the matrix. The PDMS is then cross-linked, which preserves the printed trail. By suitably varying the temporal and spatial positions of the magnet,

desired 3D curvilinear geometries, e.g., (b) a spiral and (c) a figure-eight knot, can be printed.

### 4.3 Methodology

#### 4.3.1 PDMS Matrix Preparation

The PDMS matrixes, where trail printing occurs, are prepared by variously mixing the PDMS and curing agent (Sylgard 184 kit, Dow Corning) (10:1 w/w) for 5 min. The mixture is then placed in a desiccator under vacuum for 30 min to eliminate any air bubbles resulted from the previous mixing step. A total of seven petri dishes (10 mm in height and 35 mm in diameter), one for each experiment, each received 3.0 mL of the mixture.

### 4.3.2 Ferrofluid Synthesis

Mangetite nanoparticles were synthesized using Massart coprecipitation[91], and using oleic acid (Alfa Aesar) as a surfactant, they subsequently dispersed in kerosene (Fisher Scientific). This yielded a stable collide with  $\phi_{Magnetite} = 2.5\%$ . 10 mL of the magnetite colloid was added to 9.75 mL of PDMS. The mixture was homogenized using pulsed probe sonication sonication (QSonica Q500, 15 min at 30% power). After transfer to a drying dish, subsequent heating on a hotplate at 90°C helped evaporate the kerosene from the mixture, yield the final form of the ferrofluid with  $\phi_{Magnetite} = 2.5\%$ . Serial dilutions with additional PDMS yielded four additional ferrofluids with  $\phi_{Magnetite} = 2.0\%$ , 1.25%, 0.625%, and 0.3125%. One part PDMS curing agent (Sylgard 184 kit, Dow Corning) was then added to each ten parts of PDMS found in each ferrofluid mixture. Adding the curing agent to the ferrofluids with the same weight ratio as in the preparation of the PDMS matrix,
ensured similar properties of PDMS prior to curing are found in the trail and the matrix, and that any variation is due to the presence of the magnetite clusters in the trail.

#### 4.3.3 Trail Printing

Prior to the printing step, the PDMS matrix in each petri dish was heated in a water bath at 80°C for 3.5 min. This increased matrix viscosity through partial crosslinking of the PDMS, which ensured the more stable print[98]. After the preheating step the petri dish is removed from the water bath and the printing process is begun. All experiments used a micropipette to introduce a 1.2  $\mu$ L ferrofluid droplet to the matrix surface. Under the influence of gravity, the droplet become completely submerged within ~30 second. The petri dish is then positioned such that the droplet is at a distance of 2.5 cm from the fixed magnet's surface (NdFeB, Grade N52, K&J Magnetics Inc.). The magnet is oriented such that the magnetic field lines and thus the trajectory of the droplet is parallel to the surface. As the droplet propagates, hydrodynamic forces impose a positive pressure upstream and a negative pressure downstream of the droplet. The former helps in creating the familiar droplet shape, while the latter allows magnetite nanoclusters to escape into the printed trail[98]. It is found that for all  $\phi_{\text{Magnetite}}$  a fully developed trail is produced at a distance >3 mm from the droplet's original position. Thus all measurements and analysis exclude the first 3 mm of the trail. The printing processes should be ceded when the droplet is at a minimum distance of 5 mm from the edge of the petri dish to help reduce edge effects, which can distort the droplet shape and thus the trail. This also helped prevent rapid acceleration of the droplet as it approaches the magnet, which can distort the trail width to droplet radius ratio. The petri dish is finally returned to water bath at 80°C for at an extended period of time to ensure complete crosslinking of the PDMS in the trail and matrix. The resultant print is linear in shape. For section 2.2 the examined trail area measured ~1.3 cm in length starting from the droplet center. For section 2.3, the droplet for each  $\phi_{Magnetite}$  experiment was stopped at a distance of 7 mm from the magnet surface, leaving a trail length of 1.5 cm for analysis.

#### 4.3.4 Microtoming for Transmission Electron Microscopy

In preparation for TEM, a 1 mm thick manually cut sample was taken from a trail printed by a droplet with  $\phi_{Magnetite} = 2.5\%$  at a distance of 1 cm from the droplet center. The sample exposed the cross sectional area perpendicular to the trail length. The same sample was cut again through the center of the trail such that a second sample exposed the longitudinal cross section of the trail. Both samples were frozen in liquid nitrogen and placed into a pre-cooled cryochamber (Reichert-Jung Wien, FC 4E) at -100°C, which is attached to an ultramicrotome (Reichert-Jung Wien, Ultracut E). The sections were subsequently cut with a diamond knife to produce 100 - 150 nm thick samples, which were placed onto Formvar coated TEM copper grids and allowed to warm to room temperature prior to TEM imaging. TEM is then used to image the perpendicular and longitudinal trail cross sections. ImageJ software was used to measure magnetite nanoparticle and cluster sizes observed in the TEM images.

### 4.3.4 Atomic Force Microscopy

Atomic force microscopy (AFM) (Bruker, Multimode 8 with ScanAsyst) was used to map the local elastic modulus in sections 2.2 and 2.3 of this report. The AFM head and sample holder do not have magnets so as to not interfere with measurements over the regions with magnetite nanoparticles. A double sided tape was used to hold the test samples onto the scanner. The tip (Scanasyst-Air) was calibrated relative to a standard PDMS test sample (PDMS-SOFT-2-12M, Bruker test sample kit), with a known elastic modulus of 3.5 MPa.

Using a precision knife, two manually cut 1 mm thick samples are made to expose the trail's perpendicular (across the trail width) and longitudinal (along the trail length) cross sections ~10 mm from the droplet's center. A 2  $\mu$ m x 2  $\mu$ m scan area is specified for figure 15(b) and (e), in the center and middle of the trail for the perpendicular and longitudinal cross sections respectively. A 30  $\mu$ m x 30  $\mu$ m and 8  $\mu$ m x 40  $\mu$ m scan areas were specified for the longitudinal (Figure 15(c)) and perpendicular (Figure 15(f)) cross sections respectively, highlighting the PDMS elastic modulus transition from the matrix to the trail.

For each  $\phi_{Magnetite}$  in section 4.4.3, three 1 mm thick samples are manually cut at a distance of 5 mm, 10 mm and 15 mm away from the center of the droplet. The samples expose cross sections perpendicular to the trail's length. For each sample five equally spaced 2  $\mu$ m x 2  $\mu$ m scan areas are specified between the trail's center and edge. For each scan area, an average *E* value is obtained, excluding magnetite cluster areas where *E* > 10 MPa. Finally for each  $\phi_{Magnetite}$  a total of 15 data points are used to calculate an average and a standard deviation found in Figure 16(e).

# 4.4 Results

#### 4.4.1 Microstructure Formation

A ferrofluid ink containing magnetite nanoparticles (at a volume fraction  $\phi_m = 2.5\%$ (v/v)) is moved by guiding the magnetically responsive droplet with an external magnetic field to print a trail in a pure PDMS matrix. Then, the PDMS matrix is cured, as illustrated in Figure 14(a). Two 150 nm thick microtomed samples of the trail are extracted for which TEM images are shown in Figure 14(b) and (c) for longitudinal (along the trail length) and lateral (across its width) cross-sections, respectively. The nanoparticles (5 to 15 nm in diameter) in both figures appear as separated agglomerates having arbitrary shapes with either smaller 50-150 nm or larger 0.5-1.5  $\mu$ m characteristic dimensions. The cluster orientations surmised from the directions of their respective long axes have no coherent coordination, suggesting that cluster alignment with respect to the magnetic field lines did not occur. This random orientation indicates that any mechanical effect due to the presence of these clusters inside the trail is homogeneously distributed. The MNPs within the trails tend to cluster, thereby losing homogeneity in their spatial distribution. The clustering leads to microscale regions within the trail that contain either pure PDMS or a cluster-PDMS mixture. Therefore, in addition to the substantial composition difference between the matrix and printed trail, the trails also contain morphological differences.



## Figure 14: Trail Morphology.

(a) A straight line is printed in a PDMS matrix with a ferrofluid ink droplet that contains an MNP volume fraction  $\phi_m = 2.5\%$ . The printed contour appears continuous when viewed under an optical microscope with 4× magnification. However, when viewed using TEM, microtomed sections along the (b) longitudinal and (c) lateral cross-sections of the trail, which are cut at a 10 mm distance from the droplet center, reveal distinct MNP clusters. The inter-cluster spacing is devoid of MNPs, and thus likely consists of pure PDMS matrix. The two longitudinal and lateral cross-sections are visually indistinguishable from each other.

The shape of a printed trail is quasi-cylindrical so that its volume  $V_t \approx \pi R^2 L$ , where R and L denote the trail radius and length, respectively. For a representative printed trail, R

~ 96.5  $\mu$ m and L ~ 1.14 cm, i.e.,  $V_t = 3.34 \times 10^8 \ \mu$ m<sup>3</sup>. The droplet for this case is roughly

spherical with an initial radius  $R_{d,i} \sim 655 \ \mu$ m, i.e., its volume  $V_{d,i} \approx 1.18 \times 10^9 \ \mu$ m<sup>3</sup>. After the trail has been printed, the final droplet radius  $R_{d,f} \sim 650 \ \mu$ m, i.e., the volume  $V_{d,f} \approx 1.15 \times 10^9 \ \mu$ m<sup>3</sup>.

## 4.4.2 Targeted Elastic Modulus Heterogeneity

The elastic modulus is measured using atomic force microscopy (AFM) for a  $\phi_m = 0.625\%$  ferrofluid ink droplet. Two samples that expose longitudinal and lateral crosssections of the printed trail are interrogated with AFM, see **Figure 15(a)**. The image in Figure 15(b) shows that the elastic modulus, over a 2  $\mu$ m square longitudinal cross-section,  $E \sim 2$  MPa is spatially constant. Figure 15(c) images a 30  $\mu$ m square area across the matrixtrail transition for another longitudinal cross-section. Large local departures of E > 10 MPa are associated with the harder magnetite clusters. The sizes of areas vary between 800 nm and 1.8  $\mu$ m, which are similar to the cluster dimensions obtained from the TEM images of Figure 14. The PDMS matrix has relatively minor spatial variations with  $E \sim 3.4$  MPa. Areas within the trail that lie between clusters also have smaller  $E \sim 2.02$  MPa, suggesting that these regions essentially consist of softer PDMS in agreement with the TEM image.

The change in *E* between the outer matrix and printed trail is further visualized along five 30  $\mu$ m line sections traced at 0, 7.5, 15, 22.5 and 30  $\mu$ m, as shown in Figure 15(d). Figure 15(d) for these sections shows that *E* varies from 3.4 MPa for  $x < 2 \mu$ m in the matrix to 2.2 MPa inside the trail when  $x > 14 \mu$ m. These values indicate that the transition zone dimension is ~12  $\mu$ m with a ~0.1 MPa/ $\mu$ m gradient. Inside the trail, *E* varies between 2.0-2.4 MPa for the PDMS matrix, again with large departures when magnetite clusters are encountered.

Figure 15(e) presents AFM measurements for a 2  $\mu$ m square lateral cross-section of the trail. Regions containing PDMS alone have  $E \sim 1.89$  MPa. Figure 15(f) shows that an 8  $\mu$ m × 40  $\mu$ m area at the trail edge again contains clusters of 500 nm to 1.5  $\mu$ m characteristic dimension and relatively large E > 10 MPa. Five 40  $\mu$ m line sections traced at 0, 2, 4, 6 and 8  $\mu$ m to further elucidate these variations. Figure 15(g) shows the transition between the pure matrix where  $E \sim 3.6$  MPa for  $x < 9 \mu$ m, and the trail for which  $E \sim 2.2$ MPa when  $x > 22 \mu$ m. Similar to results for the longitudinal cross-section presented in Figure 15(d), the transition zone has a ~13  $\mu$ m dimension and a 0.108 MPa/ $\mu$ m gradient.





(a) 1 mm thick longitudinal and lateral cross-sections are obtained at a 10 mm distance from the droplet center for a  $\phi_m = 0.625\%$  straight trail. (b) A 2  $\mu$ m × 2  $\mu$ m AFM elastic modulus map for a longitudinal cross-section has an average  $E \approx 2.02$  MPa. (c) A similar 30  $\mu$ m × 30  $\mu$ m section at the trail edge reveals variations in *E*. (d) Five line sections from

(c) highlight changes in *E* across the matrix (~3.4 MPa) and trail (~2.2 MPa). Similarly, the lateral cross-section in (e) reveals E = 1.89 MPa that is comparable to (b), while the transition for *E* in (f) and (g) is in agreement with the longitudinal counterparts in (c) and (d).

#### 4.4.3 Tailored Elastic Modulus Heterogeneity

The variation in elastic modulus can be further tailored by changing the magnetite concentration in the ferrofluid solution  $\phi_m$ . Straight ~1.5 cm trails are separately printed in a PDMS matrix with five ferrofluid inks that have  $\phi_m = 2.5, 2.0, 1.25, 0.625$ , and 0.3125% v/v. While  $\phi_m > 2.5\%$  inks were synthesized, these ferrofluids could not print trails since, due to interparticle interactions, the magnetite clusters remained within the droplets.

AFM images of the five cured 2  $\mu$ m square lateral cross-sections for these concentrations elucidate their modulus variations across the pure matrix and trail, as shown in **Figure 16**. Inside the trails, on average,  $E \approx 1.30, 1.35, 1.49, 1.97$  and 2.60 MPa in order of decreasing  $\phi_m$ , i.e., the more magnetite clusters there are, the lower *E* is. Since these clusters diminish crosslinking in the polymer, the PDMS modulus in the trail decreases from  $E \sim 2.61$  MPa to 1.51 MPa as  $\phi_m$  increases from 0.3125 to 1.25%, and it plateaus at *E*  $\sim 1.31$  MPa after  $\phi_m > 2.0\%$ . While ink composition changes the trail properties, it does not influence the pure PDMS matrix modulus, which remains relatively constant at  $E \sim 3.64$  MPa regardless of  $\phi_m$ . The reduction in *E* between the PDMS contained in the trail and the matrix is 29.1, 47.1, 58.1, 62.1 and 64.0% for  $\phi_m = 0.3125, 0.625, 1.25, 2$  and 2.5% respectively.



Figure 16: Dependence of *E* on  $\phi_m$ .

Representative 2  $\mu$ m × 2  $\mu$ m spatial map of *E* for  $\phi_m =$  (a) 2.5% (b) 2.0%, (c) 1.25%, (d) 0.625%, and (e) 0.3215% in the trail. MNP clusters are encountered in regions where *E* > 10 MPa, while *E* < 3 MPa indicates PDMS matrix. For each value of  $\phi_m$ , three lateral cross-sections are sampled, each at locations, for a total of *N* = 15 samples for each datapoint. (f) *E* decays rapidly with an increase in  $\phi_m$  up to  $\phi_m = 1.25\%$ , and plateaus thereafter.

## 4.5 Discussion

Variations in the applied magnetic field, e.g., by changing the position of an external magnet, create curvilinear droplet trajectories, as shown in Figure 13. The ink droplet acts as an intrinsic print head, offering greater geometrical flexibility while printing complex patterns within a soft material, which is advantageous over extrinsic nozzles that cause disturbances in the print. In our experiments, hydrodynamic forces due to droplet motion influence trail sections that are less than  $10R_d$  removed from the droplet center. The resulting droplet trajectories, thus the printed trails, are curvilinear rather than ones with sharp corners. The results demonstrate that 3D heterogeneity can be printed in the matrix, i.e., *E* varies at small scales in the material.

Instead of assembling into chain and filament structures[42, 60, 63], TEM images in Figure 14(b) and (c) show that MNPs agglomerate in the printed trail. The agglomeration occurs through dipole-dipole interactions between nanoparticles as well as the hydrodynamic forces acting on clusters that escape from a droplet into its trail. As ink nanoparticles agglomerate, they vacate regions that subsequently contain only PDMS, as shown in Figure 14(a) and (b). The initial and final droplet radii do not reflect the ink volume lost to the trail, i.e.  $\Delta V_d < V_t$ , where  $\Delta V_d = V_{d,i} - V_{d,f}$ . This suggests that PDMS from the prepolymer matrix infiltrates the trail during printing. The clusters are randomly oriented in both the longitudinal (Figure 14(a)) and lateral (Figure 14(b)) cross-sections. They do not align with the direction of the applied field due to the high PDMS prepolymer viscosity, which resists cluster rotation during alignment.

Magnetite clusters have relatively large E but are known to reduce the stiffness of PDMS since they inhibit curing and obstruct polymer chains [29]. Hence, their presence in the trail diminishes the elastic modulus of the PDMS that lies between clusters, as shown in Figure 15. The spatial variations in E are similar for the longitudinal and lateral crosssections. Inside the trail, average values of E lie in the range 2.0-2.4 MPa, as shown in Figures 15(d) and (g), whereas in the matrix,  $E \sim 3.6$  MPa. As the bulk material deforms, this difference, i.e. the transition zone, is favorable for matrix-trail stress transfer. Although magnetite clusters have higher E, they are spatially separated so that stress transfer in the material depends upon the PDMS inside the trail.

We not only show the ability to change a material's elastic modulus, but a method to also tailor the degree to which that change occurs. Figure 16 shows that the PDMS elastic modulus inside the trail can be reduced by 29-64% relative to the matrix PDMS by varying  $\phi_m$ . While lowering it does not reduce cluster sizes, it decreases the numbers of agglomerates that are formed in that trail. Since these clusters inhibit PDMS polymerization [29] and disturb polymer chain formation, changing their numbers influences the relative reduction in the PDMS modulus for the trail. The dependence of *E* on  $\phi_m$  observed from Figure 16 agrees with recent experiment data, where high nanoparticle concentrations result in softer bulk material properties and *vice versa* [29].

The ink and printing methodology can be readily integrated to enhance inkjet and 3D printing. For example, inkjet nozzles can be used to deposit multiple droplets on the

surface of a liquid prepolymer, with the droplets subsequently patterned through magnetic manipulations. This step could be followed by printing additional sets of droplets with varying nanoparticle contents and patterns, thus rapidly tailoring E at the microscale.

# 4.6 Conclusion

We present a novel, rapid and controllable method to print 3D elastic modulus variations in PDMS with magnetically guided ferrofluid ink droplets. As the droplets propagate through the liquid prepolymer, trails of magnetite nanoclusters are printed in their wake. Upon curing of the matrix, these patterns are locked in place. The nanocluster distribution inside a trail imposes pattern-specific variations in the mechanical properties of the material such that the PDMS contained in the trail has a lower elastic modulus than the surrounding pure matrix. The volume fraction of magnetite in the ink influences the degree to which the PDMS elastic modulus is lowered. The methodology described makes possible the direct, rapid, inexpensive and tailored 3D printing of material heterogeneities. The ferrofluid ink can be readily integrated to enhance existing additive manufacturing technologies, such as inkjet and 3D printing. The printed material heterogeneities can enable the ready production of novel soft functional materials that require complex 3D stiffness patterns.

# 5. Magnetic Dynamic-Assembly: A Facile Fabrication for Sensors

## 5.1 Introduction

In this chapter, experimental investigations will be presented to highlight a different technique of creating conductive networks by employing magnetized carbon nanotube dynamic-assembly technique. The network can be easily encased in an elastomeric matrix, which lends its flexible characteristics to readily create functional flexible sensors. This chapter is reprinted and adapted with permission from Abdel Fattah, A.R., et al., Nickel Nanoparticles Entangled in Carbon Nanotubes: Novel Ink for Nanotube Printing. ACS Appl. Mater. & Interfaces, 2016. 8(3): p. 1589-93 (DOI: 10.1021/acsami.5b11700). Copyright 2016 American Chemical Society. (URL: http://pubs.acs.org/doi/abs/10.1021/acsami.5b11700) [99]. The chapter will provide a literature review which is discussed in the background section, followed by excerpts from the above mentioned paper including additional unpublished content to emphasize some of the concepts disused below.

# 5.2 Background

Carbon nanotubes (CNTs) continue to play an integral role in novel and emerging devices due to their wealth of functionalities [100]. CNTs have been integrated into electrochemical sensing applications, such as oil degradation monitoring, as well as biomedical applications such as monitoring of glucose levels [101-103]. Selective absorption capabilities rendered CNTs central to oil contamination applications, while its electrical and thermal conductive capacities landed them an important role in heat transfer

and electronics applications [104, 105]. CNTs have also been used in biosensors again due to their selective adsorption and absorption. For example, when antibodies are immobilized on the surface of CNTs, the CNT network becomes sensitive to the antibody-antigen interaction [106, 107]. Such methods will largely depend on the manipulation of CNTs in such a way to form circuitry and sensors. However CNT manipulation can present a problem since they are mostly in powder form. Electrical manipulation have been explored where CNTs are aligned using an electrical field, however usually result in large resistances in the MOhm range [108]. In addition, CNTs require a matrix to hold the patterned network in place.

Particular attention has been given to magnetic functionalization of CNTs so as to offer means of magnetic manipulation. CNTs are diamagnetic in nature but when functionalized with magnetic nanoparticles, the CNT-nanoparticle conjugates become inherently magnetic. Magnetic CNTs benefit from the physical properties offered by CNTs while allowing for remote manipulation via an external magnetic field. This enables, for example, the alignment of CNTs in polymer matrices, thereby enhancing certain electrical properties such as capacitance, or the migration of magnetic CNTs to regions of higher magnetic fields (such as the surface of a magnet, or magnetic template) [109]. Remote manipulation thus offers an avenue to pattern CNTs to desired features for example to create an electrical circuit or sensor. Such field assisted assembly offers a simpler less expensive approach compared to other patterning techniques, which often require dies and stamps, thereby reducing the coast of fabrication [110]. Further, embedding such assembled circuitry and sensors into polymers, enables the integration of CNTs in emerging markets such as the wearable and soft electronics industries [111-113]. Diamagnetic manipulation of CNTs using templates have been demonstrated with good success, while functionalized CNTs have been used to magnetically assemble onto electrodes for sensing applications, and for remote targeting and manipulation of bulk oil sensors [104, 114, 115]. There are several methods to magnetize CNTs, e.g., by encapsulating MNPs,[116-118] plating the nanotubes with a ferromagnetic metal[119-121] or decorating them with MNPs.[122-124]. These methods typically require a lengthy and complex sequence of chemical syntheses or physical depositions. Often, expensive reagents and apparatus and special skill sets are also needed. A need for a simpler, faster and reliable patterning method of CNTs is thus required for the mass production of CNT based devices [104].

Attempts have been made to deposit CNTs using the technique discussing in chapters 3 and 4. However, due to the longer CNTs structures compared to nanoparticles, entanglement rendered it very difficult for any deposition, i.e. trail formation, to take place. And an alternative method was investigated to offer targeted electrical modification of an elastomeric matrix.

Herein, we explore a simple noncovalent method to magnetize CNT bundles, allowing for magnetophoresis based patterning and the production of simple sensor design. We demonstrate that simple mechanical mixing of nickel (Ni) nanoparticles with raw multiwalled carbon nanotubes (MWCNTs) provide the means for simple magnetization of MWCNT bundles through Ni-MWCNT entanglement. Ni nanoparticles thus act as chaperons allowing MWCNT mobility in a magnetic field to form patterns of conductive networks. We further embed such patterned features into polydimethylsiloxane (PDMS) polymers, forming nanocomposite networks, and explore their ability to function as sensors for mechanical deformation and oil detection.

## 5.3 Methodology

Nickel nanoparticles (Nickel Nanoparticles / Nanopowder, Ni 99.9%, 40 nm, US Research Nanomaterials Inc.) and MWCNTs (MWCNTs >95% OD: 20-30 nm, US Research Nanomaterials Inc.) with 0.5-2  $\mu$ m length (1:2 w/w for a total of 3.0 g) are added to 7.0 mL of Kerosene (Fisher Scientific). The solution is then probe sonicated (QSonica Q500, 15 min at 30% power). The sonication allows MWCNT bundles to wrap around and entangle Ni nanoparticle clusters Superconducting quantum interference devise (SQUID) magnetometry readings were conducted (Magnetic Property Measurement System, Quantum Design, San Diego, CA, USA) to investigative the magnetic response of dried Ni-MWCNT (1:2 w/w). To effectively from dense networks under a magnetic field, the resultant Ni-MWCNT is not intended to form a stable colloid and so within an hour of sonication most the conjugates settle. Subsequent sonication or shaking of the solution can easily re-disperse the Ni-MWCNT conjugates into kerosene. A steel template (A 1.15 mm in diameter wire formed into a U shape with a 2.7 mm radius semicircle connecting two 7.0 mm long straight features) is present below a glass substrate (22 mm x 22 mm x 0.15 mm), see. A magnetic field is applied by an external magnet (K&J Magnetics) placed  $\sim 1.5$ cm above the substrate. The applied magnetic field H induces magnetization M in the template resulting in a high gradient magnetic field  $\mathbf{B} = \mu_0 (\mathbf{H} + \mathbf{M})$ , where  $\mu_0$  is the permeability of free space. The original solution was further diluted (1:10 v/v) in kerosene. Droplets of the solution where deposited on the glass substrate to cover the iron template

using a micropipette. Within a period of 20 to 30 seconds a network of Ni-MWCNT was formed following the geometry of the template due to the presence of the magnetic field B. Meanwhile, the kerosene is allowed to evaporate by placing the template and glass substrate on a hotplate at 100°C for 5 minutes or until the Ni-MWCNT network is visibly dry. The substrate is then place in a petri dish, where a mixture of PDMS and its curing agent (Sylgard 184 kit, Dow Corning) (10:1 w/w) is poured on top of the substrate and Ni-MWCNT network to form a uniform thickness of 1.75 mm. The PDMS mixture was previously degassed under vacuum for 20 min. The sample is allowed to cure for 1 hour in an oven at 69°C. Once cured, the sample can be peeled out of the petri dish, and excess PDMS, if present, can be cut with scissors.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are both used to observe the Ni-MWNT network structure before and after the introduction of PDMS. To measure the electrical response of the sensor, a simple ohmmeter design was fabricated (Arduino UNO, Somerville, MA, USA), while alligator clips are used as probes to connect the patterned features to the ohmmeter circuit, which is supplied by a 5 VDC connection from a computer unit used to log the changes in resistance through the sample at 10 Hz.

To measure the response of the sensor to mechanical strain, both ends of the sensor sample are fixed to a custom stage. Once the desired strain is reached, the sample is immediately allowed to relax to its original position. As the sensor undergoes strain and relaxation cycles, the change in resistance is logged by the ohmmeter and computer unit. The ohmmeter probes are positioned 2.0 mm apart, connecting a straight pattern. An additional mechanical test is performed where the sample is fixed at one end. The 7 mm cantilever configuration allows the same custom stage used in the strain test to move the free end of the sample a desired distance, after which the sample is immediately relaxed to is original position. As the sensor bends and relaxes, the change in Ni-MWCNT network resistance is logged by the ohmmeter and computer unit. The ohmmeter probes in this case are positioned 3.5 mm apart, connecting a straight pattern.

For the oil detection test, the sensor sample is partially submerged in 150 mL of deionized water contained in a 250 mL glass beaker, so that the middle of the sensor is located at the liquid air interface. 1 mL of Oleic acid (Alfa Aesar) is then added to the water, where it comes into contact with the sensor, inducing a change in resistance, which is logged by the ohmmeter circuit and computer unit. The ohmmeter probes are positioned 3.5 mm apart, connecting a straight pattern.

## 5.4 Results and Discussion

While developing a chemical synthesis method, we serendipitously discovered an inexpensive and rapid alternative that is schematically illustrated in Figure 17. We disperse 1 g of nickel nanoparticles (NiNPs, 40 nm diameter, US Research Nanomaterials Inc.) and 2 g multi-walled CNTs (>95% purity, 20-30 nm outer diameter and 0.5-2  $\mu$ m length, US Research Nanomaterials Inc.) in kerosene (Fisher Scientific) with probe sonication (QSonica Q500, 15 min, 1 s pule for every 6 s, at 30% power using 1/4" probe). This

magnetized the CNTs, i.e., the entirety of the dispersed phase containing NiNPs and CNTs was attracted by a permanent magnet (Figure 17(a)). This was in sharp contrast with our expectations, which were that the NiNPs would be attracted towards the magnet while the CNTs would not. A TEM image of the NiNP-CNT conjugated material, hereinafter referred to as NiCNTs, shows how the NiNPs are enmeshed in CNT bundles (Figure 17(b)). While sonication physically entangles NiNPs in CNTs, the high surface energy of NiNPs is likely responsible for NiNPs clustering and  $\pi$ -interactions which bonds them to CNT surfaces. This bonding allows NiNPs to chaperone CNTs by mangnetophoresis. Application of a strong magnetic field gradient did not separate these NiNPs from the conjugated material. Although multi-walled CNTs were used in these experiments, we expect similar results with the use of single and double-walled CNTs.

A Superconducting Quantum Interference Device (SQUID) magnetometer (Quantum Design) was used to determine the magnetic response of the NiCNTs at room temperature. They were found to possess a strong saturation magnetization  $M_s = 14.61$  emu/g, as shown in Figure 17(c). This is substantially higher than that of CNTs ( $M_s < 0.1$  emu/g)[125] and is consistent with the 33% weight fraction of NiNPs ( $M_s = 44.33$  emu/g).[126] The sample is mildly ferromagnetic, with a remanence of 1.72 emu/g. While the NiNPs are individually superparamagnetic, the mild ferromagnetism is attributed to the inter-NiNP magnetostatic interactions that are expected between adjacent particles that are in close proximity, yielding superferromagnetic behavior.[127]





(a) Nickel nanoparticles (NiNPs) and carbon nanotubes (CNTs) are dispersed in kerosene by probe sonication. The CNTs entangle the NiNPs, enabling the latter to act as magnetic chaperones without detachment or separation in a strong gradient magnetic field. (b) TEM images show clusters of NiNPs enmeshed in CNTs. (c) SQUID magnetometry shows that the NiCNTs, containing 33% (w/w) NiNPs, have a saturation magnetization Ms = 14.61 emu/g which is commensurate with the mass fraction of nickel in the sample.

To investigate the effectiveness of sonication time,  $t_{sonic}$ , in creating NiCNTs, the mass of an ink sample,  $M_o$ , is measured using a scale. When a magnet (K&J Magnetics, N42, 1/2" x 1/2" x 2") is suspended above the sample, 5.5 cm above the scale substrate, NiCNT conjugates are pulled upwards such that the new observed ink mass  $M < M_o$ . The relative reduction in mass  $M_R = (M_o - M)/M_{NiCNT}$ , reflects the amount of NiCNTs created through sonication, where  $M_{NiCNT}$  is the maximum observed mass reduction, occurring when all NiCNTs (i.e all NiNPs and CNTs in the ink) phase separate as they are pulled to the top surface of the ink. When  $M_R \sim 100\%$ , sonication has effectively allowed sufficient NiNP entanglement to mobilize all CNTs. Figure 18(a) shows  $M_R$  dependence on NiCNT  $t_{sonic}$  (30% power using 1/4" probe) for three samples, 1) 1 hour CNTs pre-sonication in kerosene, 2) 1 hour NiNPs pre-sonication in kerosene, and 3) no pre-sonication. Samples 1 and 2, were added with NiNPs and CNTs respectively after the pre-sonication step so that each of the three samples contained CNTs (0.014 g), NiNPs (0.007 g) and Kerosene (1.4 mL). Each sample was repeated three times to obtain an average  $M_R$  value for each  $t_{sonic}$ . Figure 18(a) shows that while CNTs pre-sonication increases entanglement for  $t_{sonic} = 1$  s and 10 s, all three samples quickly converge to  $M_R \sim 100\%$  for  $t_{sonic} > 60$  s. Further, TEM images in Figure 18(b-d) shows no observable difference between the three cases in the formation of NiCNT conjugates for  $t_{sonic} = 120$  s.

The final ink batch used in the following experiments contained 2 g of CNTs, 1 g of NiNP, probe sonicated (30% power using 1/4" probe, without pre-sonication) in 70 mL of kerosene for 250 s (15 min, 1 s pulse for every 6 s). The resultant ink, after sonication,

is black in color. The NiCNTs tend to settle within 1 hour, which requires printing to immediately follow sonication.



### Figure 18: Effect of sonication time on NiNP-CNT entanglement.

(a)  $M_R$  values reflect the amount of NiCNTs created at various sonication times,  $t_{sonic}$ , relative to the maximum mass reduction observed,  $M_{NiCNT}$ , when all NiCNTs are pulled to the surface of the ink.  $M_R \sim 100\%$  for  $t_{sonic} > 60$  s for all cases. 1 hour of CNT pre-sonication slightly improves entanglement for  $t_{sonic} < 10$  s. b) TEM of NiCNT for  $t_{sonic} = 120$  s and 1

hour of CNT pre-sonication. c) TEM of NiCNT for  $t_{sonic} = 120$  s and 1 hour of Ni presonication. d) TEM of NiCNT for  $t_{sonic} = 120$  s without pre-sonication. There are no observed differences in the NiCNT formed between the cases.

Next, we examined how the NiCNTs could be used to print circuitry embedded within an elastomeric matrix, as illustrated in Figure 19. A soft iron wire (diameter d = 1.15 mm) was bent into a U-shape to create a magnetic template and then affixed on a glass coverslip (VWR, No. 1). A 100  $\mu$ L drop of kerosene containing dispersed NiCNTs was placed on the other side of the glass substrate so that it covered the iron template. A permanent magnet (K&J Magnetics, N42, 1/2" x 1/2" x 2") was placed ~1.5 cm above the coverslip to produce a magnetic field. The field magnetized the template, which caused the NiCNTs to settle on the glass substrate immediately above it within ~30s. Thus, the pattern of the template was transferred above the glass in the form of a structure made entirely with NiCNTs. The kerosene was carefully evaporated by heating on a hot plate (100°C, 5 min). This yielded a dense network of CNTs with intertwined clusters of NiNPs, as seen in the SEM images (Figure 19a)).





(a) An U-shaped soft magnetic wire is used as a template. A glass coverslip is placed over it, a drop of kerosene containing dispersed NiCNTs is deposited on the coverslip and the

wire is then magnetized using a permanent magnet. The wire produces localized gradients in the magnetic field, which settles the dispersed NiCNTs on the coverslip surface immediately adjacent to it. The kerosene is then evaporated by heating, yielding a U-shaped dense network of CNTs intertwined with a few clusters of NiNPs, as observed by SEM. The NiCNT U network is placed inside a petri dish, covered with liquid PDMS, and heated to cure the PDMS. The PDMS infiltrates the printed structure, and after curing, it lends a polymer matrix to the NiCNTs. (b) When the PDMS is peeled off, it forms a flexible and stretchable membrane with an embedded NiCNT structure. (c) SEM image of a crosssection of such a structure reveals a ~2.5  $\mu$ m thick NiCNT-PDMS composite network embedded in pure PDMS. The smooth texture of the top surface suggests full PDMS infiltration of the NiCNT network resulting a thin PDMS top layer.

The coverslip containing the NiCNT print was placed in a petri dish, then covered with polydimethylsiloxane (PDMS, Sylgard 184 kit, Dow Corning) to form a ~1.75 mm film. The PDMS infiltrated the NiCNT network, likely due to its low surface energy, as seen from the SEM images (Figure 19(b)). This lent an elastomeric matrix to the otherwise powdery network of NiCNTs. The sample was then heated (70°C, ~1 hour) in an oven (Across International, AccuTemp) to crosslink the PDMS. Once cured, the PDMS film containing the magnetically printed NiCNT structure embedded within it was peeled off. Figure 19(c) illustrates a U-patterned NiCNT network.

Flexible circuits are alternatives to otherwise rigid conductive pathways such as wires, and better suited for wearable electronic devices where flexibility is key.[128] Through environmental triggers such as mechanical deformations or chemical changes, flexible electronics rely on sensors to convey valuable information such as strain,[110] or the presence of specific chemicals[102] and biological[103] species. The fabrication of flexible sensors can be simplified by implementing a rapid, facile, and inexpensive fabrication method.

The NiCNT network forms a ~2.5  $\mu$ m thick conductive path, as evidenced when the top thin layer of PDMS is penetrated with two multimeter probes. Further, the matrix housing the NiCNT network, i.e., PDMS, is quite flexible. These features motivated us to evaluate the method for printing a strain gage. A voltage divider was supplied with a steady 5V DC and the voltage drop, V, across the sensor was measured (Arduino UNO, 10 samples per second) (Figure 20(a)) when the PDMS block was subjected to bending (Figure 20(b)) and elongation (Figure 19(c)). For bending, a 7 mm specimen was cantilevered by fixing one end of the sample and displacing the other by 1.5 mm with a translation stage, and then relaxing the deformation instantaneously. The normalized voltage drop,  $V/V_{o}$  reaches a maximum of 102.5%  $\pm$  0.1% (n = 4), where  $V_o = V$  at the sample's neutral position. For elongation, a 7mm long specimen was extended incrementally  $l = 100 \ \mu m$ , 150  $\mu m$ , 200  $\mu$ m and 250  $\mu$ m, each of which was followed by relaxation. A linear transduction is observed and  $V/V_{\rho} = 2.4\epsilon + 1$  where  $\epsilon = l/L$ . In both cases, relaxation is gradual, likely due to the viscoelastic nature of PDMS. However,  $V/V_o$  always reached 99.9%  $\pm 0.2\%$  (n = 4), indicating that there is no permanent offset. The sensor response to bending and relaxation mimics the stress-strain response that is expected of a viscoelastic polymer, [129] in this case through its electrical response to changes in strain.



Figure 20: The use of a Magnetically Printed NiCNT structure as a sensor.

(a) A PDMS block containing a printed NiCNT structure is placed in a voltage divider. The voltage drop V across the structure rises when the block is subjected to (b) bending by displacement of the free end by 1.5 mm or (c) elongation of  $l = 100 \ \mu\text{m}$ , 150  $\mu\text{m}$ , 200  $\mu\text{m}$  and 250  $\mu\text{m}$ . In both configurations, V increases almost instantaneously when a load is applied, but shows a gradual relaxation when the load is removed. (d) When the sensor is held at the air-water interface in a beaker and ~1 mL of oleic acid is dropped on the water, V rises when sensor contacts the oil.

The correlation between the electrical response of the sensor and the viscoelastic properties of PDMS may be explained through the number of NiCNT interconnections. As the sensor undergoes strain, the PDMS matrix is subjected to deformations, specifically linear elongations, which in turn change the cross-sectional area of the sample. These matrix deformations cause the relatively hard NiCNTs to reorient and move apart so that there are fewer interconnections in the strained sample than in the unstrained configuration, with an increase in the voltage drop across the sample.[110, 129] As the sample returns to its neutral position after the elongation strain is released, the stress relaxes more slowly due to the viscoelasticity of PDMS. This slow relaxation gradually increases the NiCNT interconnections, which would produce a corresponding slow decrease in  $V/V_o$ .

Owing to the oleophilic nature of PDMS and the porous structure of the PDMS-NiCNT composite, the conductive network is likely to draw oils by capillary action. This is known to cause swelling in PDMS when it is exposed to oils. Such swelling will also disturb the interconnects of the NiCNTs, thus changing the conductivity. Hence, we next examined the use of this method in sensing oil floating on water. A linear structure printed by magnetic patterning of NiCNTs was connected to two leads, then partially immersed in water (Figure 20(d)) contained in a 250 mL glass beaker. 1 mL of Oleic acid (Alfa Aesar) was then added to the water. As soon as the network came into contact with the oil, ( $t \sim 83$  s),  $V/V_o$  started to rise. Here  $V_o = V$  in the absence of the oil. The normalized voltage drop gradually exceeded 110% ( $t \sim 200$  s) at which time the experiment was ceased.

Overall, the printed circuit produces repeatable strain sensitivity up to 3.6% strain. The use of other silicone rubbers, e.g., Ecoflex, as the matrix supporting the NiCNT network will facilitate the ability to ready up to 500% strain[110], useful high strain wearable electronics applications. Further, while oil sensing capabilities of CNTs have been well explored, e.g., for engine oil monitoring[101] and detecting oil-dissolved gases[102], the physical manipulation[101] or functionalization of CNTs[102] required to assemble CNT based sensors can be overcome by the rapid and inexpensive dynamic-assembly technique using NiCNT inks.

The method described here allows for upscaling by integrating the NiCNT ink and assembly technique with such technologies as inkjet and 3D printing, providing a simple direct writing approach to soft circuitry, thus offering more complex geometries. However, fast settling of NiCNTs would require constant sonication of ink to ensure homogeneity. In addition, tuning the ink viscosity must be employed to ensure proper flow through nozzles and tubing. Furthermore, as the nozzle travels, means to ensure previously deposited NiCNTs are not distorted must be adopted, such as immediate evaporation of the solvent, allowing deposited NiCNTs to adhere to the printing surface.

# 5.5 Conclusion

We have readily magnetized and patterned carbon nanotubes into a sensor network by entangling them with nickel. While conventional magnetic functionalization of carbon nanotubes is based on forming strong covalent bonds between carbon nanotubes and magnetic nanoparticles, this method requires considerable volumes of reagents, is time consuming and includes acid treatment which impairs carbon nanotube properties. Instead, simply entangling nickel nanoparticles with multi-walled carbon nanotube bundles, allows the magnetic nanoparticles to behave as mobile chaperons that can be moved to pattern their entangled carbon nanotubes. The movement of the NiCNT nanomaterial through magnetophoresis makes the directed assembly of conductive nanocomposite networks possible, thereby offering a more rapid and inexpensive fabrication process that can be tailored for mass production as compared to the use of stamps and dies. The assembled networks are functional and can be successfully integrated into PDMS to fabricate strain and oil sensors. Readily encasing these networks in a thin polymer layer enables flexibility, which is desirable for wearable and soft electronic devices.

# 6. Magnetic Dynamic-Assembly: A Facile Fabrication forBiosensors

# 6.1 Introduction

In this chapter we explore how the same technique of magnetic dynamic-assembly can help in the facile fabrication of biological sensors. Decorating the carbon nanotubes with certain biological species lends great biological sensitivity with selective biomarkers. Magnetizinging carbon nanotubes further help in manipulating the resultant magnetic biological ink enabeling the printing of sensor strips. This chapter is adapted from a manuscript entitled *Biosensor Printed with a Magnetized Carbon Nanotube Biological Ink* by Abdel Rahman Abdel Fattah, Ahmed M. Abdalla, Sarah Mishriki, Elvira Meleca, Suvojit Ghosh, Fei Geng, and Ishwar K. Puri, submitted to the journal of *Nature Nanotechnology* for possible publication.

## 6.2 Background

Modified into a biosensor,[107, 130-134] a carbon nanotube (CNT) can detect the electrical response of an antigen (Ag) when it interacts with an antibody (Ab) that is immobilized on the nanotube surface.[133, 135, 136] When a magnetic nanoparticle is bonded to the CNT surface, it acts as a chaperone that can be remotely manipulated with a magnetic field to transport the attached nanotube.[34, 76, 80, 83, 92, 99, 109, 136-139] Here, we describe a method to synthesize a magnetic biological ink and then print a biosensor with it.[107, 140] The ink contains CNTs that are first functionalized with magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticle chaperons,[141] then with immobilized Abs and thereafter

dispersed in DI water. Using a magnet, the ink is printed in the form of a sensing strip that is integrated into a biosensor. When samples containing picomolar amounts of c-Myc are deposited on the strip, the sensor detects real-time current changes analyzing the sample within 60 s (Figure 21). Since the c-Myc oncogene is expressed in many human cancers, including breast, prostate, gastrointestinal cancer, lymphoma, melanoma and myeloid leukemia,[142, 143] it is a potential biomarker of malignant cancer phenotypes.[144, 145]



Figure 21: Synthesis of the magnetic biological ink (mBioink) and sensor fabrication. As manufactured CNTs are treated with nitric acid and subsequently magnetized using low weight ratios of Fe<sub>3</sub>O<sub>4</sub> to CNT ( $\gamma$ ), yielding extra carboxylic groups. These remaining carboxylic groups subsequently react with amine groups present on anti-c-Myc Abs and form strong covalent bonds. The mBioink is magnetically manipulated to print an electrically conducting strip, which is subsequently integrated into the sensor assembly. The introduction of a sample containing the target c-Myc Ags on the strip increases its electrical resistance and reduces the current.

The magnetic biological ink is synthesized by first treating CNTs with nitric acid to produce carboxylic groups on the nanotube surfaces, which then covalently bond with Fe<sub>3</sub>O<sub>4</sub> nanoparticles.[141] Next, the surfaces are bio-functionalized through Ab immobilization, as follows. If the Fe<sub>3</sub>O<sub>4</sub> yield is low enough during magnetization, the remaining active carboxylic groups on a CNT surface provide sites for the covalent immobilization of Abs. These Abs can also be adsorbed on that surface through physical bonding. The CNT-Fe<sub>3</sub>O<sub>4</sub>-Ab complex is dispersed in DI water containing 0.1% Tween 20 (polysorbate 20), which acts as a surfactant and, as a blocking agent, also prevents nonspecific Ag-Ab binding. The reagents used, the method of CNT functionalization and magnetization, and material characterization (using X-ray diffraction, transmission electron microscopy and super quantum interference device magnetometry) are discussed in supplementary information S1-5. [99, 107, 140, 141, 146]

# 6.3 Methodology

### 6.3.1 Materials and Reagents

Multiwall CNTs produced by CVD with purity > 95%, outside diameters of 20-30 nm, inside diameters of 5-10 nm and lengths between 0.5-2.0  $\mu$ m were purchased from US Research Nanomaterials. Other reagents used included ferric chloride hexahydrate

(FeCl<sub>3</sub>· 6H<sub>2</sub>O, 97-102%, Alfa Aesar), ferrous chloride tetrahydrate (FeCl<sub>2</sub>· 4H<sub>2</sub>O, 98%, Alfa Aesar), nitric acid (HNO<sub>3</sub>, 68-70%, CALEDON), and ammonium hydroxide (NH<sub>4</sub>OH, 28-30%, CALEDON). C-Myc Ag (Abcam, Cambridge, Massachusetts, USA) had a molecular weight of 49 kDa (49,000 g mol<sup>-1</sup>). Bovine serum albumin (BSA) (Sigma Aldrich, Oakville, Ontario, Canada) was used as a negative control. All reagents were used as received without further purification. The NdFeB, Grade N52 magnets were purchased from K&J Magnetics Inc (25.4 × 6 × 6 mm). The electrode support was fabricated using polydimethylsiloxane (PDMS) and a curing agent (Sylgard 184 kit, Dow Corning). The coverslips (Fisher Scientific, 12-540-B) had dimensions of  $22 \times 22 \times 2$  mm.

#### 6.3.2 Characterization Methods

X-Ray Diffraction (XRD) analysis of CNT and magnetized CNT powder samples was performed using a Bruker D8 Discover instrument comprising a DavinciTM diffractometer operating at 35 kV and 45 mA using Co-K $\alpha$  radiation ( $\lambda_{avg} = 1.79026$  Å). Bruker's DIFFRAC.Eva V3.1 and TOPAS softwares were used for the analysis and semiquantitative estimation of the sample composition. Transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS) spectroscopy were conducted with a JEOL 2010F field emission microscope, where the samples were suspended in ethanol, dripped on to a TEM grid and then wicked off with a tissue-wipe. Optical and fluorescence (Enhanced Green Fluorescent Protein, EGFP) microscopy were conducted using a Zeiss Axio Observer.Z1. Magnetization measurements were performed using a Quantum Design superconducting quantum interference device (SQUID) magnetometer at room temperature.

#### 6.3.3 Double Functionalization of Carbon Nanotubes

The first step in producing the magnetic biological ink requires treatment of CNTs with concentrated HNO<sub>3</sub>. This creates surface sites where reactive molecules, such as COOH, C=O, and C–OH, form covalent bonds with the CNT scaffold and subsequently nucleate magnetite nanocrystals (Fe<sub>3</sub>O<sub>4</sub>). [141] When the MNP yield is low enough, antic-Myc covalently bonds to the surface through a condensation reaction between the amine groups of the Ab and the remaining carboxylic groups[107] without additional reagents.[140]

#### 6.3.4 Carbon Nanotubes Functionalization and Magnetization

The details of the various sample preparations are reported elsewhere.[141] Briefly, 1 g of MWNTs was first activated by dispersing it in 200 mL of concentrated nitric acid and sonicated for 4 h in a sonication bath (VWR International, Model: 97043-936). The activated CNTs (aCNTs) were subsequently washed several times with deionized (DI) water, filtered, washed again and finally dried in a vacuum oven. Magnetite nanoparticles were co-precipitated onto the aCNTs by stoichiometric calculations to obtain a Fe<sub>3</sub>O<sub>4</sub>:aCNTs magnetization weight ratio  $\gamma = 0.1, 0.2, \text{ and } 0.4$  (w/w). For  $\gamma = 0.4$ , a mixture of 0.92 g of FeCl<sub>3</sub>·6H<sub>2</sub>O and 0.36 g of FeCl<sub>2</sub>·4H<sub>2</sub>O was first dissolved in 160 mL of degassed DI water and followed by ultrasonic dispersion of 1 g of the aCNTs for 10 mins with a probe sonicator (Qsonica, Model: Q500 with 1/4" micro-tip at 35% power) and subsequently for 50 minutes in a sonication bath at 50°C. A 2 ml of 30% ammonia solution was slowly introduced as a precipitant to increase the pH to 9. The magnetized CNTs produced were washed several times until pH ~ 7, filtered and then dried in a vacuum oven
for 1 hr. In the case of the adsorbed MNPs on the surface of the CNTs, a previously reported methodology[99] was followed to entangle magnetite and MWNTs yielding a  $\gamma = 0.4$ .

#### 6.3.5 Characterization of Magnetized Multi-Walled Carbon Nanotubes

XRD analysis of dried magnetized CNTs was conducted for three Fe<sub>3</sub>O<sub>4</sub>:CNT weight ratios  $\gamma = 0.1$ , 0.2, and 0.4. Figure 22 shows that all samples consisted only of the intended magnetite and carbon phases. The powder diffraction file (PDF) database, available through the Eva software, qualitatively confirmed that all three samples contain hexagonal carbon (carbon nanotubes, PDF No. 00-058-1638) and the spinel magnetite phase (Fe<sub>3</sub>O<sub>4</sub>, PDF No. 01-071-6336). The average size of the Fe<sub>3</sub>O<sub>4</sub> nano-crystals was calculated through the Scherrer equation applied for the highest diffraction peak (311). For all three samples, the MNP sizes lie in a narrow 8.6 - 10.3 nm range. Using Bragg's Law,[141] calculations of the average lattice parameters of Fe<sub>3</sub>O<sub>4</sub> are 8.403, 8.396 and 8.404 Å for  $\gamma = 0.1$ , 0.2, and 0.4, values that agree with those for magnetite (8.394 Å, JCPDS No. 79-0417; and 8.400 Å, COD card No. 1011084). For all three cases, TEM images at various magnifications in Figure 23 show that the CNT surfaces are successfully decorated with MNPs with high crystallinity and a narrow crystallite size distribution around ~10 nm. Increasing the value of  $\gamma$  improves the decoration density.

Because of their small size, the magnetite nanoparticles are superparamagnetic[146] with a high saturation magnetization  $M_s = 60-80$  emu/g. Conjugating the MNPs with diamagnetic CNTs retains their superparamagnetic behavior, as shown in Figure 24. The measured hysteresis loops for all of the cases indicate that there is no remanence or coercive field. The magnetic saturations  $M_s = 3.03$ , 7.79, 15.09 emu/g for  $\gamma = 0.1$ , 0.2 and 0.4. A higher value of  $M_s$  indicates that the conjugate material exhibits a stronger magnetic response, which is more helpful for magnetic printing.



#### Figure 22: X-Ray Diffraction Analysis of mCNTs.

The XRD (Co K<sub> $\alpha$ </sub>,  $\lambda$ =1.79 Å) patterns for magnetite to CNTs weight ratios  $\gamma$  = 0.1, 0.2, and 0.4 which confirm the presence of a magnetite (Fe<sub>3</sub>O<sub>4</sub>) phase and a hexagonal carbon phase from the carbon nanotubes.



# Figure 23: TEM images of magnetic CNTs samples at various magnetization weight ratios $\gamma = 0.1$ to 0.4

The figures confirm that all samples are successfully decorated with highly crystalline MNPs that are synthesized within a narrow size distribution around ~10 nm.



#### Figure 24: Magnetic Characterization.

Magnetic hysteresis curves show that all magnetized CNT samples exhibit superparamagnetic behavior, but have different saturation values  $M_s$  depending on their magnetite content ( $\gamma$ ). The greater this content, the stronger is the material response to a magnetic field.

#### 6.3.6 Production, Purification and Quantification of anti-c-Myc Monoclonal Antibodies

9e10 hybridoma cells were used to produce anti-c-Myc antibodies (Abs). Anti-c-Myc Abs were then purified with a centrifugal filter to remove fetal bovine serum from the cell culture media (Amicon Ultra, 3k with 3000 nanomolecular weight limit and 100k with 100,000 nanomolecular weight limit). Purified anti-c-Myc Abs were analyzed with SDS-PAGE. Quantification of anti-c-myc Abs was performed with Qubit 2.0 Fluorometer. Concentration of anti-c-Myc Ab suspension resulted in a 0.5 mg/mL concentration.

#### 6.3.7 Immobilization of anti-c-Myc Antibodies on mCNTs

Following activation and magnetization of the CNTs, Ab immobilization was performed. For each mg of MWNTs contained in the magnetized CNTs, 2 mL of deionized water was used as media to disperse the precursor magnetic biological ink in solution with a probe sonicator (15 seconds, 30% amplitude). Corresponding to the amount of CNTs, an anti-c-Myc to MWNT weight ratio  $\beta = 2.5 \times 10^{-4}$  value was selected and an appropriate amount of Ab (0.5 mg mL<sup>-1</sup>, 0.5 µL) was added to the magnetized CNTs (1.4 mg) in solution. The mixture was incubated for 1 hour at room temperature, inverted gently every five minutes to maintain the suspension, or when sedimentation of the magnetic biological ink was observed. Following incubation, the supernatant was removed.

A blocking procedure was performed following incubation with Ab in order prevent non-specific binding of Ag molecules to the magnetic biological ink. Blocking of the CNT surface ensures that the detected signal is directly related to the specific Ag-Ab interaction, reducing noise that may originate due to adsorption of non-specific molecules.[107, 147] For every 1 mg of activated CNTs, 2 mL of blocking solution (0.1% Tween-20 in deionized water) was added to the magnetic biological ink.[132] The ink was then blocked for a half hour at room temperature, inverted gently every five minutes to ensure saturation of the ink surface. Following incubation with the blocking solution, the blocked ink was washed three times in deionized water and a final concentration of 10 mg/mL was obtained by adjusting the amount of DI water. The same approach was followed for the case when MNPs and Ab were adsorbed on the CNT surfaces.

#### 6.3.8 Procedure for Fluorescent Imaging

The Immobilization of fluorescein isothiocyanate (FITC) labeled Abs on the surface of magnetic CNTs follows the procedure explained in the above section, except for the blocking step. Samples were prepared for fluorescent imaging with a Zeiss, EGFP instrument.

#### 6.3.9 Sensor Printing

A neodymium N52 magnet was used as a magnetic template to print the sensor. A glass coverslip was first positioned over the magnet. A 10  $\mu$ L volume of the magnetic biological ink was deposited with a micropipette over a 7 mm length on a glass coverslip directly above one of the magnet edges. The edge locally concentrated the magnetic field, which helped to create a dense conductive network of CNTs. After printing, each sensor was left on the magnet for 20 minutes for the dispersing medium (DI Water) to naturally evaporate at room temperature. The printed and dried mBioink sensor had lengths of ~7 mm and widths of ~1.5 mm. Once dried, the magnet was removed and the mBioink network was held in place on the coverslip by Van der Waals and electrostatic forces. Each sensor comprised of ~100  $\mu$ g of CNTs, ~40  $\mu$ g of Fe<sub>3</sub>O<sub>4</sub> and ~25 ng of anti-c-Myc Ab.

#### 6.3.10 Electrical Circuitry, Sensor Assembly and Sensing

To investigate the sensing capability of the magnetic biological ink, a voltage divider circuit was used that had a reference resistance of  $R_{ref} = 100 \text{ k}\Omega$ . A square PDMS ( $2.5 \times 2.5 \times 0.3 \text{ cm}$ ) section was used to support two aluminum foil electrodes. The PDMS was fabricated using a mold with a mixture of the PDMS precursor and PDMS curing agent (10:1 w/w), which was subsequently degassed for 20 min and cured at 70°C in an oven. The electrodes were fixed using double-sided tape to the PDMS support with a separation of 5 mm. A cutout (1 x 0.5 cm) was created through the PDMS support and centered between the electrodes to allow sample deposition on the magnetic biological ink strip. The electrodes were wrapped around this support to provide electrical access with alligator clips. The PDMS electrode assembly was positioned on the top of the sensor, while alligator

clips mechanically held the sensor assembly together. This ensured a good connection between the ink network and the aluminum electrodes. Each electrode covered a ~1 mm section of the sensor, leaving another 5 mm ink strip exposed for purpose of sample deposition and Ag detection. The printed sensor, of resistance  $R_s$ , was connected in series with  $R_{ref}$ . A PLC (Arduino Uno) was used to supply the circuit with a 5 V DC power supply, while an analog feedback voltage allowed the PLC and computer unit to interpret and sample the current *i* passing through the circuit at a frequency of 10 Hz. After each test, the electrodes were wiped with ethanol (100%) and left to dry to ensure no cross contamination.

#### 6.4 Results and Discussions

We use anti-c-Myc monoclonal Abs to detect c-Myc protein (supplementary information S6). The binding efficacy of Ab molecules to CNTs is visualized through fluorescent imaging. Fluorescein isothiocyanate-conjugated Donkey anti-Mouse IgG H&L is added to an ink containing magnetized and bio-functionalized CNTs for which the Ab:CNT weight ratio  $\beta = 2.5 \times 10^{-4}$ . Supplementary information S7[107, 132, 147] and S8 provide information about the Ab immobilization protocol and fluorescent imaging. No fluorescence occurs in the absence of Ab conjugation, i.e., when  $\beta = 0$ , or in the supernatant for any sample. The fluorescence is independent of magnetization since its intensity remains unchanged when the Fe<sub>3</sub>O<sub>4</sub>:CNT weight ratio  $\gamma$  is varied, as shown in Figure 25(a)–(c). Hence, CNTs magnetized at  $\gamma = 0.4$ , which exhibit a robust magnetic response, are used to fabricate the biosensor.



Figure 25: Brightfield and fluorescent images of magnetized CNTs conjugated with FITC-labeled Ab (unblocked, 40× magnification).

(a) The mBioink with  $\gamma$  0.1,  $\beta$  2.5×10<sup>-4</sup>; (b) Ink,  $\gamma$  0.2,  $\beta$  2×10<sup>-4</sup>; (c) Ink,  $\gamma$  0.4,  $\beta$  2.5×10<sup>-4</sup>; and (d) adsorption,  $\gamma$  0.4,  $\beta$  2.5×10<sup>-4</sup>). (e) STEM and EELS micrographs showing C, O and N concentrations for CNTs, magnetite and anti-c-Myc, respectively.

The fluorescence images for CNT samples with covalently bonded Abs in Figure 25(a)–(c) are virtually indistinguishable from those in Figure 25(d) for Abs that are physically bonded onto as-manufactured nanotube surfaces. Thus, while covalent functionalization is required to conjugate Fe<sub>3</sub>O<sub>4</sub> nanoparticles with CNTs, the immobilization of Abs on nanotube surfaces does not require reagents. Since anti-c-Myc Abs are inherently non-fluorescent, an electron energy loss spectrum (EELS) is performed to identify Abs on the CNT surfaces. Figure 25(e) depicts a scanning transmission electron microscopy micrograph and the corresponding EELS spectrum for the ink. It highlights the presence of C, O and N, showing a general structure consisting of a CNT-Fe<sub>3</sub>O<sub>4</sub>-Ab network, where the CNT mesh produces the electrical path and the Abs are Ag receptor sites. Of the ink components, since N originates solely from the Abs, its map reveals anti-c-Myc locations.

The specificity and sensitivity of the device is investigated by depositing 10  $\mu$ L of anti-c-Myc conjugated magnetic ink on a cover slip, see Figure 26. A magnet placed edgewise underneath the cover slip concentrates the deposited ink and prints it in the form of a compact strip with typical dimensions of 7 ± 0.9 mm length and 1.5 ± 0.2 mm width (based on n = 25 samples). The print is dried at room temperature for 20 min after which two alligator clips are attached to provide power. Supplementary information S9 includes details about the biosensor printing method.



#### Figure 26: Printing Technique and Sensor Assembly.

10  $\mu$ L of the mBioink are deposited on top of a coverslip that is positioned on a magnet. The magnet edge concentrates the field, aiding the dynamic-assembly of a dense ink print, which is dried at room temperature. The current passing through the sensor assembly decreases when a sample containing c-Myc is introduced on the dried ink strip and logged. The STEM micrograph visualizes the mBioink print where MNPs and anti-c-Myc are identified.

A circuit measures the current changes as samples are deposited on the biosensor, see Figure 27(a). Supplementary information S10 provides details about the electrical circuit, and the biosensor assembly and response. Two types of tests are performed: (1) a

sample is deposited once on the surface of the printed biosensor strip, and (2) equal amounts of the sample are deposited repeatedly at specific intervals on that surface. Figure 27(b) presents the temporal responses when 2  $\mu$ L of (1) purified c-Myc with 40, 20, and 10 pM concentrations, (2) DI water, and (3) bovine serum albumin (BSA) with a 40 pM concentration are placed on the biosensor. The BSA is a nonspecific Ag and a negative control. Three tests are performed with every sample, each with a newly printed biosensor.



#### Figure 27: Biosensor transitent response.

(a) A voltage divider is used with a reference resistor  $R_{ref} = 100 \text{ k}\Omega$ , to determine the response of the biosensor to various samples by monitoring current changes. (b) The transient response of the sensor to 2 µL samples, where DI water and BSA samples quickly

level out after an initial reduction in current when the sample is introduced. All c-Myc concentrations reduce the current in the period 30 s < t < 60 s as c-Myc reaches anti-c-Myc binding sites in the mBioink print. Normalizing the average current for that period alone reveals the quasi-linear transient response shown in (c). (d) Provides linear correlations between the normalized current gradients in (c) and the corresponding c-Myc concentrations.

Figure 27(b) shows that, with an initially dry biosensor, the baseline current  $i_b \approx 47$   $\mu$ A decreases rapidly after a sample is deposited on it (at time t = 2s) and then steadies. Both DI water and BSA samples induce similar decreases in the biosensor current. In contrast, the temporal current  $i_s$  does not level off as quickly after c-Myc is deposited on the strip, but continues to decrease below the eventual values for DI water and BSA. That the biosensor responds differently to c-Myc is due to the Ag-Ab binding kinetics. The interaction of anti-c-Myc Ab with the specific c-Myc Ag increases the electrical resistance of the sensor, decreasing  $i_s$ .[148, 149] Due to the relatively slow binding kinetics of c-Myc Ag-Ab, the current reduction is gradual. The nonspecific interactions of anti-c-Myc with DI water and BSA do not decrease  $i_s$  as significantly below  $i_b$ . The biosensor response shown in Figure 27(b) emphasizes the specificity with which the ink detects c-Myc.

Increasing Ag concentration enhances Ab binding and thus the electrical resistance, leading to larger current reductions and steeper temporal current gradients  $di_s/dt$ , as shown in Figure 27(b). The values of  $i_s$  at t = 60 s for the 40, 20, and 10 pM c-Myc concentrations are 34.6 ± 0.5, 38.8 ± 0.4, and 40.9 ± 0.1 µA, respectively, confirming that current reduction scales with Ab concentration. During 30 < t < 60 s, the gradient  $di_s/dt$  is constant and it also correlates with c-Myc concentration. Figure 27(c) presents the ratio  $i_{s,a}/i_{s,a,30s}$ , where  $i_{s,a}$  is the temporal current at time t averaged over three repetitive tests for a sample and  $i_{s,a,30s} =$   $i_{s,a}$  at t = 30 s. Figure 27(d) provides linear correlations between the current gradients obtained from Fig, 4(c) and c-Myc concentrations. These results are in agreement with responses reported for CNT-based biosensors.[107, 135, 140] They show that by simply monitoring the sensor's transient response rapid identification of c-Myc positive samples is possible along with a quantitative response to different concentrations. Thus, Ag monitoring is possible without using additional reagents and the sophisticated electrical equipment that is typically used with other biosensors.[140]

While Ab-Ag interactions at defect sites where Abs are immobilized restrict current transport, [148, 149] similar interactions at the ends of the multiwall nanotubes constrain current across several concentric CNT layers. Therefore, the Ab-Ag interactions at the ends of the nanotubes are likely responsible for the significant current reductions observed in tests. Thus, when the anti-c-Myc Ab is not covalently bonded to a CNT, its binding kinetics cannot be detected with the biosensor. The response of the biosensor to successive 1 µL sample depositions is presented in Figure 28 for three types of biosensor strips. These strips are printed using (1) covalently magnetized CNTs that have not yet been functionalized with Abs (ink 1), (2) CNTs that contain only adsorbed  $Fe_3O_4$  nanoparticles and anti-c-Myc Abs on their surfaces (ink 2), and (3) CNTs covalently magnetized with  $Fe_3O_4$ nanoparticles and containing adsorbed anti-c-Myc Abs on their surfaces (ink 3). The functionalized CNTs for the three inks are dispersed in DI water containing 0.1% Tween 20. Biosensors printed with inks 1 and 2 produce similar responses for 40 pM c-Myc Ag and 40 pM BSA, i.e., they do not discriminate between these two samples. Thus, positive c-Myc detection is not possible by printing biosensor strips with these inks. Differences between specific (c-Myc) and non-specific (BSA) samples become apparent with biosensors printed with ink 3. The 40 pM c-Myc sample produces the largest current reduction, followed by the 20 pM and 10 pM c-Myc samples. When a 5  $\mu$ L sample is deposited on the biosensor in 1  $\mu$ L increments, the successive sample additions keep decreasing the current.



#### Figure 28: Biosensor response to successive 1 µL sample addition.

Functionalized magnetized CNT cannot distinguish between BSA (40 pM) and c-Myc (40 pM) samples, black dashed and solid curves respectively. When anti-c-Myc is immobilized on CNT surfaces through adsorption, there is again insufficient discrimination between BSA (40 pM) and c-Myc (40 pM) samples, yellow dashed and solid curves respectively. The mBioink sensor clearly distinguishes the BSA sample from the c-Myc samples. The BSA and DI water samples quickly level off while c-Myc samples reduce the current gradually and with each successive sample volume addition. The current reductions for the c-Myc samples are proportional to their c-Myc concentrations. The mBioink print amplifies Ag-Ab binding kinetics, improving c-Myc detection.

## 6.5 Conclusion

In summary, a biological ink containing Abs that are immobilized on the surfaces of magnetized CNTs that are dispersed in DI water containing 0.1% Tween 20 is used to print a biosensor to detect specific Ags. Ink synthesis is straightforward and does not involve complex chemistry. The ink can be readily printed as a compact electrical biosensor strip with the assistance of a magnetic field. After it is integrated into an electrical circuit, the biosensor detects picomolar c-Myc concentrations within 60 s and is able to distinguish between c-Myc and BSA samples of different concentrations. Current decreases, which occur due to real-time specific Ab-Ag binding kinetics, are greater for higher Ag concentrations. This is a simple, rapid, economical and sensitive method that has potential applications to detect pathogens and diagnose diseases.

# Magnetophoretic Manipulation: A Tool for Cell Separation and Patterning

## 7.1 Introduction

To understand how cell magnetophoresis can be leverage in order to create new materials, in this chapter, an overview of cell magnetic properties will be given in the background section. The focus of the chapter will target blood cells, since white blood cells present magnetic properties similar to other types of cells present in the body, i.e. diamagnetic in nature. This will also facilitate exploration of red blood cells (RBCs) presenting an alternative yet interesting cell type. Since RBCs have paramagnetic properties, the study will highlight possible ways of magnetophoretic manipulations. The techniques explored here are all transferable to other cell types. In addition to exploring cell magnetic properties, leveraging such characteristics demonstrates applications of magnetophoretic manipulation through 1) label free cell separation, and 2) large scale cell patterning. Section 7.3 is reprinted and adapted from *Journal of Chromatography B*, *High Gradient Magnetic Field Microstructures for Magnetophoretic Cell Separation*, 1027, Abdel Rahman Abdel Fattah, Suvojit Ghosh and Ishwar K. Puri, Copyright 2016, with permission from Elsevier

## 7.2 Magnetic Properties Tailoring of Cell Magnetophoresis

#### 7.2.1 Background

Medical diagnostics play a pivotal role in disease control, management and prevention. Blood fractionation is at the center of the diagnostics industry with ever

increasing innovations to detect diseases quickly, and with greater accuracy [150, 151]. Despite its importance, conventional methods of blood fractionation are laborious, multistep, batch processes that involve several manual handling and centrifugation stages [152]. The automation of cell sorting would have the benefit of reducing human error and continuous fractionation instead of conventional batch process, all while providing easier diagnostics. The field of cell therapy would readily benefit from such devices. In cell therapy, T lymphocytes or T-cells are isolated from whole blood. Subsequently, they are reprogramed via viruses to selectively target specific tumors that are otherwise left undetected. Once reintroduced into the body, T-cells attack and destroys targeted tumors. The isolation process begins with whole blood fractionation into its constituents, i.e. red blood cells (RBCs), white blood cells (WBCs) and plasma. As conventional methods prove laborious, several other processes have been developed. Cell labeling is a proven method to negatively or positively select a targeted cell type. Magnetic beads are used to bind to specific cell types in a medium, and upon the application of a magnetic field, only cells bound to the beads will propagate through magnetophoresis towards a higher magnetic field, i.e. towards the magnet, allowing separation. Cell manipulation in such a manner can, however, damage cellular membranes, while requiring post processing such as washing, in addition to the high cost of magnetic beads. A workaround is the use of label free techniques, of which the use cell intrinsic properties, such as magnetic susceptibilities are used [151].

The magnetic susceptibility of hemoglobin, present in RBCs, in its different states including oxygenated and deoxygenated has been heavily studied [153-158]. Each

hemoglobin molecule contains four heme subgroups having one Fe atom each. The Fe atom in each heme subgroup is present in its ferrous  $Fe^{2+}$  state. In its deoxygenated state, i.e. not bound to oxygen,  $Fe^{2+}$  is present with four unpaired electrons in it 3d<sup>6</sup> orbital. The unpaired electrons provide Red Blood Cells (RBCs) with paramagnetic properties compared to the diamagnetic White Blood Cells (WBCs). To transport oxygen from the lungs to the rest of the body, each  $Fe^{2+}$  can bind to one oxygen molecule O<sub>2</sub> thereby leaving the now oxygenated hemoglobin without unpaired electrons, therefore resulting in a diamagnetic RBC.

Using superconducting quantum interference device (SQUID) measurements, and MRI techniques, there has been multiple reports of the different RBC magnetic susceptibilities, which have been describe as heavily depended on the hemoglobin state [156, 158, 159]. Slight differences in reported magnetic susceptibilities of oxygenated and deoxygenated RBCs are mainly attributed to the measurement difficulty, which currently requires multiple manual steps such as centrifugation, washing, and pipetting, each of which can introduce a potential source of error. In addition, SQUID measurement techniques for blood samples, have not been completely standardized given the many varying preparation techniques found in the literature. For example, carbon monoxide hemoglobin is reported in [159] to be of similar susceptibility as oxygenated hemoglobin and so used in its place for susceptibility measurements as it is far more stable due to higher hemoglobin affinity to carbon monoxide than oxygen. In producing deoxygenated hemoglobin, oxygen can be stripped from hemoglobin by either, introducing N<sub>2</sub> bubbles through a medium of suspended RBCs, or simply passing N<sub>2</sub> gas upon an RBC suspension,

and relying on diffusion of N<sub>2</sub> through the air-liquid interface to strip the oxygen [156, 158, 159]. Despite the different preparation protocols however, the majority of reports describe Oxygenated hemoglobin as having a magnetic susceptibility of ~ -4.83 x 10<sup>-7</sup>, while that of deoxygenated hemoglobin is ~ -7.49 x 10<sup>-7</sup>. The difference in susceptibility between oxygenated and deoxygenated hemoglobin, which can reach ~ 2.7 x 10<sup>-7</sup> as reported in [159], is largely attributed to the level of saturated oxygen in hemoglobin, i.e. oxygenated or deoxygenated hemoglobin. It has also been reported that variation in hemoglobin content in RBCs due to hemoglobin deficiency in certain RBCs, which ultimately impacts the number of oxygen binding sites in an RBC, affects the susceptibility of RBCs [160].

The magnetic susceptibility of WBCs have been reported in lesser numbers compared to RBCs. WBC susceptibility has been reported to be similar to plasma or water  $\sim 7.19 \times 10^{-7}$ . The susceptibility contrast between RBCs and WBCs can be further emphasized by altering the hemoglobin state in RBCs reaching a maximum of  $\sim 2.4 \times 10^{-7}$ . The tween WBCs and Deoxygenated hemoglobin. Therefore the difference in magnetic susceptibilities between RBCs and WBCs can be leveraged to provide magnetic separation, i.e. blood fractionation under the influence of global magnetic field.

Although most investigations are pertained to blood cells, it will become clear in the next section, that when in a highly paramagnetic medium, a cell's magnetic susceptibility becomes negligible compared to that of the medium. This some of the techniques presented in this chapter to become fully transferable to other cell lines [161].

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#### 7.2.2 Results and Discussion

#### 7.2.2.1 Magnetic properties of cells

To investigate magnetophoretic manipulation of cells, it is imperative to introduce the equation magnetic force equation below. Unlike MNPs, which can often reach large values of magnetic saturation under low applied magnetic fields, biological species are mainly diamagnetic and have relatively low magnetic susceptibilities magnitudes. Thus other important parameters, such as the magnetic susceptibility of the medium, often ignored in the MNPs cases, are thereby highly considered. The magnetic force on a cell can be defined as [162-164],

$$\mathbf{F}_{M} = \left( \left( C_{cell} - C_{m} \right) V_{cell} / 2m_{o} \right) \nabla \left| \mathbf{B} \right|^{2}, \tag{7.1}$$

where  $\chi_{cell}$  and  $\chi_m$  denote the magnetic susceptibilities of the cell and fluid medium respectively,  $V_{cell}$  and  $\mu_o$  the cell volume and permeability of the free space respectively, and  $\nabla |\mathbf{B}|$  the magnetic field gradient. From Equation 7.1, the magnetic force depends on the (1) difference in the magnetic susceptibilities between the cell and fluid medium, (2) cell volume, and (3) magnetic field gradient. Although the magnetic field can be varied to alter the resultant magnetic force, we first focus consider  $\chi_{cell}$  and  $\chi_m$ .

The majority or susceptibility studies are reported on oxygenated or deoxygenated RBCs. We hereby conduct an experimental investigation of as-is RBCs from 7 different individuals to observe the variation in magnetic susceptibilities between each sample. Whole blood was drawn from each individual and underwent several centrifugation and washing steps to finally obtain 200  $\mu$  L of pelleted RBCs inside polycarbonate capsules.

SQUID measurements were later performed to obtain susceptibility measurements in Figure 29(a). All measurements were corrected for magnetic the susceptibility signal of the capsules.



**Figure 29: RBC, WBC and plasma magnetic susceptibility measurements.** (a) The magnetic moment of RBC samples shown to vary significantly while exhibiting paramagnetic behavior. (b) The magnetic moment of plasma and WBC samples showing less variation and a diamagnetic response.

By conducting the test at low temperatures from 5 K to 30 K, paramagnetic behavior within the RBC samples is emphasized, as the reduced thermal fluctuations  $k_BT$  allows the sample to become more magnetic, i.e. better dipole alignment. This is observed as an increase in susceptibility as the temperature decreases. Using the Curie-Weise law and the obtained data points, magnetic susceptibility then can be extrapolated to more realistic operating temperatures, ~300 K. The test concludes that as-is RBCs have high magnetic susceptibility variability and does not exclude the effect the individual's condition, i.e. diseases or other conditions, has on such susceptibility.

Figure 29(b) presents the results of a similar test conducted for the WBCs and plasma, however, with only 3 individuals. The diamagnetic characteristics can be clearly observed by the flat susceptibility signals obtained from WBCs and plasma.

#### 7.2.2.2 Modification of Cell Magnetic Susceptibility

In this section, a method is presented that highlights possible way of altering the magnetic susceptibilities of cell. When cells are diamagnetic in nature, i.e. the majorities of cell lines, there is very little to be done to intrinsically alter their respective magnetic susceptibilities. Hence, we resolve to labeling cell membrane with superparamagnetic particles, which act as chaperones to magnetically manipulate such cells. However, with RBC, it is possible to alter the susceptibility to, which will help manipulation and possibly fractionation.

The variability of WBC susceptibility is not as evident in Figure 29(a) compared to RBCs, and may be attributes to RBC and plasma contamination of the sample. Despite possible contamination, the flat signals confirm that the samples are overwhelmingly diamagnetic. From Figure 29(a) and (b), the susceptibility difference between RBCs and WBCs is strikingly large.

RBCs from different patients will have varying behaviors under the same magnetic field, due to the high degree of variability in  $\chi_{rbc}$ . As mentioned above, to increase the magnetic susceptibility contrast between RBCs and WBCs, hemoglobin must be in its deoxygenated state. This can be achieve by exposure to nitrogen gas. A workaround that is to oxidize hemoglobin to its ferric, Fe<sup>3+</sup>, state which results in methemoglobin. Methemoglobin has 5 unpaired electrons and cannot bind to oxygen. Although the

methemoglobin state can be reversed by methemoglobin reductase enzymes, it is more stable than deoxygenating hemoglobin where the chance of oxygen diffusion is present allowing iron to bind to oxygen molecule and reducing the magnetic susceptibility contrast between RBCs and WBCs.

We therefore start by investigating the oxidization of hemoglobin by creating as specially designed Phosphate Buffer Saline (PBS)-Sodium Bisulfite, NaNO<sub>2</sub>, buffer solution. PBS is often used in cell washing and suspension, as it is an isotonic solution, and the dissolved NaNO<sub>2</sub> oxidized the iron in hemoglobin to produce methemoglobine. 50 mM of NaNO<sub>2</sub>was dissolved in 100 mL of PBS to create the oxidizing buffer. 50  $\mu$  L of pelleted RBCs were subsequently mixed with 5 mL of the buffer solution and allowed to incubate at 37°C for 1 hour. In parallel, 50  $\mu$  L of pelleted RBCs were mixed in a PBS only buffer, i.e. no oxidization of iron, and incubated for 1 hour. After transferring samples to a 96 well plate, spectral analysis were performed to provide insight on the amount of methemoglobin and oxyhemoglobin present in the samples. Oxyhemoglobin and methemoglobin differ significantly in their spectral absorption at both 577 nm and 630 nm, which allows for measurement of their respective concentrations in each sample [165], see Figure 30(a).



# Figure 30: Hemoglobin and Methemoglobin absorption readings and magnetic moment readings.

(a) Absorption spectra for hemoglobin in PBS only and PBS-NaNO<sub>2</sub> buffer solutions after 1 hour of incubation at 37°C. Oxyhemoglobin has a characteristic peak at 577 nm while a characteristic peak appears at 630 nm for methemoglobin. (b) Magnetic moment measurement for PBS only and PBS-NaNO<sub>2</sub> buffer solutions.

The absorption results follow a similar trend in Figure 30. The samples also differ visually with PBS only buffer being redish in color while that of the PBS-NaNO<sub>2</sub> has a rusty tint. Using the Winterbourn equation,

$$[Oxyhemoglobin] = 66A_{577} - 80A_{630}$$
  
[Methemoglobin] = 279A\_{630} - 3.0A\_{577}, (7.2,7.3)

where A is the absorption at a specific wavelength, the concentrations in  $\mu$  M of both methemoglobin and oxyhemoglobin can be calculated [165]. For the PBS only buffer sample, oxyhemoglobin accounts for 35.1  $\mu$  M while that of methemoglobin is 23.0  $\mu$  M for a total of 58.1  $\mu$  M. For the PBS-NaNO<sub>2</sub> buffer sample, oxyhemoglobin measured at -2.4  $\mu$  M while that of methemoglobin is 62.8  $\mu$  M for a total of 60.4  $\mu$  M. While a negative concentration does not have any physical significance, the dramatic reduction in oxyhemoglobin and resulting increase in methemoglobin ensures the oxidation process of hemoglobin has taken place with high efficacy. It should be noted that the total concentration of both samples should be roughly the same, since a similar amount of RBCs are present in each well. It is also worth mentioning that RBCs were not oxygenated prior to the experiment and were exposed to  $CO_2$  in the incubator, both can account for the unusually low oxyhemoglobin count in the PBS only sample of ~60%.

RBCs from both buffers were pelleted and transferred to capsules for magnetic susceptibility measurements using a Physical Property Measurement System (PPMS) shown in Figure 30(b). The magnetic susceptibility for the PBS only buffer is calculated to be -6.43x10<sup>-7</sup> while that of the PBS-NaNO<sub>2</sub> is calculated to be -5.35x10<sup>-7</sup>. Both values fall within the RBC susceptibility envelope of -4.83x10<sup>-7</sup> for deoxygenated RBCs and -7.49x10<sup>-7</sup> for oxygenated RBCs. Therefore treating RBCs with NaNO<sub>2</sub>, allows for a more paramagnetic sample, thereby increasing the susceptibility contrast between RBCs and WBCs.

#### 7.2.2.3 Magnetic Tailoring or Buffer Solutions

To further increase cell magnetophoresis, the magnetic susceptibility of the buffer solution in which they are suspended can be altered [166]. Cell magnetophoresis largely depends on the difference between its magnetic susceptibility and that of the medium in which it is suspended,  $\Delta \chi = \chi_{cell} - \chi_{medium}$ . Therefore by properly choosing  $\chi_{medium}$ ,  $\Delta \chi$  can be positive, i.e. cell becomes paramagnetic in the medium, or negative, i.e. cell becomes diamagnetic in the medium.

Gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) is a biocompatible paramagnetic salt used as a contrast or broadening agent in MRI operations [167]. Being a lanthanide, a gadolinium ion Gd<sup>3+</sup> paramagnetic properties are offered by seven free and unpaired electrons in the 4f shell. By dissolving Gd-DTPA in a buffer, the medium is rendered more paramagnetic than the cells. This would result in cells being repulsed by a magnetic field. In [168], Gd-DTPA was used in a microfluidic environment to increase the separation distance between two differently sized cells, namely U937 cells and RBCs. While both cells are diamagnetic in the Gd-DTPA medium, their difference in size, and the increase of  $\Delta \chi$ , allows for easier separation. Gd-DTPA concentrations were varied from 0 to 80 mM [168]. Gd-DTPA with 100 mM concentration was also tested on human sperm in toxicity test for up to 120 min with  $\sim$ 83% viability and was deemed nontoxic [167]. One drawback of this method is the reliance on cell size. It is known that cell size do differ within a certain population of cell type. For example regular RBCs are relatively larger than RBCs with hemoglobin deficiency [160]. Although the concept is very well introduced in [168], it may be hindered by such size variability in the cell population.

As Gd-DTPA is an effective susceptibility contrast agent, we hereby investigate its toxicity on fresh which blood cells. Using conventional fractionation techniques (centrifugation, and ficoll separation), the buffy coat was separated thereby isolating the WBCs. Four different concentrations of Gd-DTPA in PBS solution were used test for WBC toxicity, 0.2 M, 0.1 M, 0.05 M and 0.025 M obtained through serial dilution. The pH of the solutions was initially acidic ~1.7, but was brought back to isotonic of ~ 7.4 pH, conditions by adjustments with NaOH and HCL. WBCs where then suspended in each solution and

incubated for 30 min, 90 min and 150 min. A reference PBS only sampe was also used. At each time interval, the viability was tested using the Nexcelom Cellometer Auto 2000 Cell Viability Counter and the results are present in Figure 31(a).



**Figure 31: Cell Viability testing and Gd-DTPA susceptibility measurements.** (a) Cell viability testing for WBCs in different Gd-DTPA concentrations. (0 M refers to the case with only PBS.) Testing was done for 30 min, 90 min and 150 min, and shows not significant dependence on concentration or time. (b) Magnetic moment measurements for Gd-DTPA with concentrations or 0.2 M, 0.1 M, 0.05 M, 0.025 M and 0 M using SQUID.

High salt concentrations can increase the osmotic pressure from its isotonic value of ~ 310 mOsm [169] and cause cells to lyse [166]. However, from Figure 31(a), it can be seen that the toxicity of Gd-DTPA even at the highest tested concentration of 0.2 M is not distinguishable from that of PBS. The time dependent toxicity does not seem to be of significance for the duration of the test of 150 min. By dissolving Gd-DTPA in PBS, the resultant solution gains paramagnetic properties. 100  $\mu$  L of each sample was used to conduct SQUID measurements for the four different Gd-DTPA concentrations used in the viability test above, including a reference PBS only sample, see Figure 31(b). The paramagnetic behavior increases along with concentration of Gd-DTPA. Therefore, careful engineering of a buffer solution will tailor cells magnetic response, i.e. paramagnetic or diamagnetic. Such buffers can then increase the efficiency of cell sorting devices.

It is therefore clear that for cell magnetiphoretic manipulations in a highly paramagnetic medium, the cell susceptibility can be negligible compared to that of the medium. This allows such manipulation to be transferable to different cell lines.

Next We present a parametric study on rectangular magnetic arrays, which are often encountered in literature, describing the effects of microstructure geometries on the magnetic field generated in a microchannel.

### 7.3 Magnetic Field Tailoring for Cell Magnetophoresis Manipulation

#### 7.3.1 Background

In the previous section we have explored the magnetic properties of cells and how manipulation of the medium can help control the magnetic force. In this section we explore the  $\nabla |\mathbf{B}|^2$  term in Eq. 1, in order to help manipulate and pattern cells magnetically. To obtain a better understanding of how magnetic field affect cells we first look at cell separartion techniques, which have been heavily explored.

Medical diagnostics to detect, control, manage and prevent disease use blood fractionation [150, 151]. Conventional methods of fractionation are slow and laborious, involving multistep batch processes that include several manual handling and centrifugation stages. These stages slow down fractionation and introduce multiple sources of error [152]. Magnetic cell labeling is a workaround that binds magnetic beads to targeted cells [170-172]. Upon application of a magnetic field, cells that are bound to the beads

separate from the remainder of the medium through magnetophoresis and propagate towards regions that experience the highest magnetic field strength (such as a magnetic surface) [34, 173]. However, magnetic beads are relatively expensive and, when the cell-bead conjugates are washed, the magnetic pull that the beads exert on cell membranes can damage the cells.

Continuous label-free methods leverage the intrinsic properties of cells that allow cell separation from a host medium [151]. We investigate label-free separation due to differences in the magnetic susceptibilities of different cell types. Diamagnetism is exhibited by all materials. In general such materials are repelled by a magnet. However, diamagnetic behavior can be overshadowed by the presence of free electrons, which lends for paramagnetic, ferromagnetic, ferrimagnetic and antiferromagnetic behaviors. Iron ions found in hemoglobin molecules are responsible for oxygen transport through the body. Each hemoglobin molecule contains four heme groups each with one Fe<sup>+2</sup> iron ion which is able to bind to one oxygen molecule. In its deoxygenated state, RBCs contain no oxygen and thus the Fe<sup>+2</sup> ions contains free electrons. The spin of these free electrons are responsible for the paramagnetic behavior observed in RBCs. By comparison, the iron ions contained in oxyginated RBCs lack free electrons due to oxygen binding. This lends for a diamagnetic behavior. The iron ions in RBCs can be oxidized to form methemoglobin,  $Fe^{+3}$ . In such state, methemoglobin is unable to bind to oxygen and is also responsible for paramagnetic RBC behavior. Certain oxidizing agents can be used to intentionally oxidize hemoglobin to methemoglobin, ensuring that RBCs are to remain deoxygenated. In doing so, a larger magnetic susceptibility contrast is ensured between RBCs and white blood cells (WBCs), leading to a more efficient separation at the cost of additional reagents. Since WBCs contain no iron ions or free electrons, they exhibit a diamagnetic behavior. The magnetic susceptibility of hemoglobin, which provides red blood cells (RBCs) with paramagnetic properties, has been extensively investigated in its oxygenated and deoxygenated states [153-158]. Paramagnetism increases the magnetic contrast of RBCs with white blood cells (WBCs), which are diamagnetic. The resulting difference in the susceptibilities of the RBCs and WBCs can be leveraged to fractionate blood.

Microfluidics has advanced magnetic blood fractionation by making integrated miniaturized devices possible [162, 174], such as those containing microstructures fabricated from ferromagnetic materials [163]. These materials are suitable for separating blood cells using both label and label free methods [152, 163, 164, 166, 169, 175-180]. The magnetic force imposed on a cell depends on the gradient of the magnetic field, the cell's magnetic susceptibility and its volume. A microstructure that is integrated into a microchannel focuses the local magnetic lines of force imposed by an external magnet, thereby producing a high gradient magnetic field (HGMF). This reoriented field increases the magnetic force experienced by a cell and therefore the separation efficiency.

Analytical solutions are available for the magnetic force produced by inserting an array of rectangular microstructures into a microfluidic channel and also for the resultant force on the cells that are bound to paramagnetic beads [181, 182]. Despite their promise, the geometries of the embedded microstructures, which range from periodic arrays to continuous ferromagnetic micro wires, have not yet been optimized. We examine rectangular magnetic microstructure arrays, which are often encountered in literature, to

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characterize how microstructure geometry variations influence the ensuing magnetic field in a microchannel and thus the blood fractionation efficiency.

#### 7.3.2 Methodology

Two dimensional simulations are conducted using Finite Element Magnetic Method software (FEMM, version 4.2) to determine separation with an array of rectangular nickel microstructures of height *h*, width *w* and separation gap *g*, as shown in Figure 32. The magnetic field is simulated for  $12.9 \times 5.8$  mm NdFeB 52 magnets. These magnets sandwich the 300  $\mu$ m high microchannel and the microstructure that adheres to one of the sides of the channel. The base of the microstructures lies at a 50  $\mu$ m displacement from the magnet. All unfilled spaces assume the magnetic properties of air. Material properties are obtained from the FEMM software library. The influence of different array configurations on RBC trajectories in a plasma medium is simulated using MATLAB (R2014b, The MathWorks, Inc.) software along with the magnetic fields generated by the FEMM software.



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# Figure 32: Microstructure array of width w, height h and separation gap g placed within a microchannel and within a double magnet configuration.

The array pitch p = w + g, the channel height  $c = 300 \ \mu$ m, and the array and bottom magnet are separated by 50  $\mu$ m. Magnetic properties are obtained from the FEMM material library (Finite Element Magnetic Method Version 4.2 November 15, 2013).

#### 7.3.3 Results and Discussion

#### 7.3.3.1 Effect of Magnet Configuration on the Generated Magnetic Field

From Equation 7.1, the cell behavior will change dramatically depending on the cellular content. When a medium susceptibility is chosen such that,  $\chi_{rbc} > \chi_m > \chi_{wbc}$ , for an RBC, Eq. 1 will result in a positive force indicating paramagnetism, i.e. attraction towards the magnet. Comparatively, the same magnetic field will also magnetize WBCs, however, resulting in a diamagnetic behavior, i.e. repulsion by a magnet, since  $\chi_m > \chi_{wbc}$ . The host media equally plays a significant role in the magnetic behavior of cells. For example a heaviliy paramagnetic media can be prepared such that  $\chi_m > \chi_{rbc}$  and  $\chi_m > \chi_{wbc}$ . Such susceptibility values, from Equation 7.1, would indicate a magnetic repulsion of both RBCs and WBCs [183]. In this way, while RBCs are still independently paramagnetic, they act as diamagnetic materials similar to WBCs. For the purpose of this study however, we assume that  $(C_{cell} - C_m)V_{cell}/2m_o$  is constant and focus instead on the influence of the gradient in the cross-channel direction  $\partial |\mathbf{B}_y|^2/\partial y$ .

The literature commonly considers configurations where (1) a single bar magnet is placed in close proximity to the microchannel [164, 176, 184], and (2) two bar magnets are placed on either side of the microfluidic channel such that their opposite poles face each

other [177, 181, 182]. First, we investigate the difference between use of these single and double magnet configurations for a single  $w=50 \ \mu m$  and  $h=80 \ \mu m$  nickel microstructure. Figure 33 shows the variation of  $\partial |\mathbf{B}_y|^2 / \partial y$  for different configurations across the channel along the centerline of the structure moving away from its top surface.



Figure 33: Variation of the magnetic field gradient  $\partial |\mathbf{B}_y|^2 / \partial y$  across a channel. A single 50 × 80  $\mu$ m nickel microstructure for the double and single magnet configurations is simulatied. The largest gradient occurs for Case A, which is a double magnet configuration. The lowest gradient for a microstructure occurs for Case B that involves a single magnet. Case C provides intermediate results, which is also a single magnet configuration but where the volume of the bar magnet is now two times that of Case B and equal to the combined volume of the two magnets placed on either side of the microchannel for Case A. Case D contains no microstructures and yields the lowest overall  $\partial |\mathbf{B}_y|^2 / \partial y$ .

Case (A) in Figure 33 represents a double magnet configuration with single NdFeB 52 bar magnets placed on each side of the microchannel. Single magnet simulations are reported for Case (B) where a bar magnet is placed only on the side closest to the microstructure. Case (C), which is similar to Case (B) but the magnet volume is doubled.

Case (A) provides in the largest value of  $\partial |\mathbf{B}_{\mathbf{y}}|^2 / \partial y$  at any location, followed by results for

Case (C) and then Case (B). Doubling the magnet volume for the single magnet in Case (C) increases  $\partial |\mathbf{B}_y|^2 / \partial y$  near the microstructure surface over that for Case (B). However, despite using the same overall magnet volume, the gradient fails to reach the values generated for Case (A). Case (D) in Figure 33 yields the smallest  $\partial |\mathbf{B}_y|^2 / \partial y$  for a single magnet without microstructures. Beyond 150  $\mu$ m in the cross-channel direction, the differences between all cases are negligible. The higher  $\partial |\mathbf{B}_y|^2 / \partial y$  is, the greater is cell magnetophoresis and overall separation within the device.

#### 7.3.3.2 Effect of Microstructure Geometry on the Generated Magnetic Field

#### 7.3.3.2.1 Periodic Microstructure Arrays

Adding microstructures to form an array is expected to alter  $\partial |\mathbf{B}_y|^2 / \partial y$  due to the anticipated interactions between these microstructures. Hence, we consider a double magnet configuration and a microstructure array with  $w = 50 \ \mu$ m, and vary *h* from 25 to 200  $\ \mu$ m and *g* from 25 to 200  $\ \mu$ m. All other dimensions are held constant. Invoking geometric similarity, we use the dimensionless parameters  $h^* = h/w$  and  $g^* = g/w$ . The magnitude of **B** is largest at the microstructure surface due to a local field concentration effect, but its value decays rapidly moving away from the microstructure surface into the microchannel till it reach a constant value of 0.78 T. This value depends upon the strength of the imposed magnetic field for the particular case. Increasing **B** at the microstructure surface increases  $\partial |\mathbf{B}_y|^2 / \partial y$  across the channel, thus improving cell separation.

Figure 34 presents values of **B** for arrays of different dimensions at the center of the microstructure surfaces. **B** at the microstructure surface increases with increasing  $h^*$ . This is consistent with the magnetic shape anisotropy principle, where a slender  $(h^* \gg 1)$  microstructure placed with its long axis parallel to an external field **H** induces a higher **B** above the microstructures. Since  $\mathbf{B} = \mu_o(\mathbf{H} + \mathbf{M})$ , the increase in **B** occurs due to the higher magnetization **M** of the microstructure. However, when multiple microstructures placed in close proximity are magnetized along the same parallel axes, their local magnetic fields interfere, reducing overall **B**.



**Figure 34: Magnitude of B on the surfaces of nickel microstructure arrays with different g\* and h\*. As h\* increases so does the surface magnetic field B.** However, microstructure interference also increases with increasing h\*, which diminishes

the surface **B**. Since the source of interference is the microstructure geometry, as  $g^*$  increases the interference diminishes accordingly. This allows the microstructures to reach a maximum surface **B** with the imposed magnetic field. Reducing  $g^*$  increases microstructure interference, decreasing the surface **B**.

For the examples presented in Figure 35, the magnetic field is measured at the microstructure surface for different separations g = 25, 50, 100 and 200  $\mu$ m, and with  $w = h = 50 \ \mu$ m along a single array pitch p = w + g. Because the microstructures are themselves
sources of interference for the magnetic field, increasing g reduces this interference, which increases **B**. The interference leads to field minima within the gaps between microstructures, yielding 0.58, 0.64, 0.69 and 0.72 T for g = 25, 50, 100, and 200  $\mu$ m respectively. The local field minimum influences the cell trajectories.



Figure 35: Magnitude of B along the pitch p = w+g for g = 25, 50, 100, and 200  $\mu$ m for  $w = h = 50 \ \mu$ m and measured at the microstructure surface.

Magnetic field minima occur in the gaps between adjacent microstructures, yielding 0.58, 0.64, 0.69, and 0.72 T for g = 25, 50, 100, and 200  $\mu$ m respectively. Smaller values of g enhance microstructure interference and reduce the overall **B**.

Above a microstructure, the local field strengthens since the magnetic field becomes more focused. Hence, while **B** is lowered in the gaps between microstructures, diminishing the local attraction of RBCs, the more focused and stronger magnetic field on the surfaces of the structures attracts these cells. For diamagnetic cells with a negative susceptibility the effect should be opposite, i.e., cell repulsion will be greater along the microstructure surfaces than within the gaps between adjacent teeth. This periodic variation in the magnetophoretic force produces an undulating cell trajectory as the cell travels through neighboring fields that differ in their magnitudes. For a specified value of  $g^*$ , increasing  $h^*$  also increases the interference discussed above. This is evident through Figure 35, which shows that, after a threshold value of  $g^*$  is reached, further increases in  $h^*$  no longer produce a significant augmentation of **B**. When  $h^*$  is fixed, increasing  $g^*$  weakens microstructure interference, allowing **B** to increase until it reaches a maximum value. This is evident from the plateaus in Figure 35, which point to an optimum  $g^*$  for a particular  $h^*$  beyond which **B** is relatively constant.

Since microstructure arrays are responsible for enhancing the magnetic separation force experienced by RBCs, a reduction in  $g^*$  necessitates a denser array, leading to the inclusion more microstructures per unit length. Figure 35 shows how this occurs at the expense of significantly decreasing **B**, thereby reducing the separation force per unit length. In contrast, increasing  $g^*$  also increases the surface value of **B** but reduces the array density. The optimal separation is determined from the surface integral,

$$\mathbf{B}_{y,avg}^{2} = \left(1/2c\left(g+w\right)\right) \int_{0}^{g+w} \int_{0}^{c} \left(\partial \left|\mathbf{B}_{y}\right|^{2}/\partial y\right) dy dx, \qquad (7.4)$$

where  $\mathbf{B}_{y,avg}^2$  denotes the average value of  $\partial |\mathbf{B}_y|^2 / \partial y$  over an array pitch. Values of  $\mathbf{B}_{y,avg}^2$  can be used to compare the separation effectiveness of a microstructure array. Higher values indicate larger force densities per array pitch, which lead to greater separation.

The variation of  $\mathbf{B}_{y,avg}^2$  with changing  $h^*$  and  $g^*$  is presented in Figure 36(a). Although reducing  $g^*$  increases microstructure interference, leading to lower surface values for **B** as shown in Figure 34, Figure 36(a) shows that  $\mathbf{B}_{y,avg}^2$  increases as  $g^*$  decreases because a denser array induces a larger average force per unit length. However, when  $g^* < 1$ , microstructure interference is more prominent and  $\mathbf{B}_{y,avg}^2$  decreases rapidly, resulting in a local maximum at  $g^*=1$ . When  $g^*=0$ , the microstructures join together to form a flat plate so that there is no more magnetic field focusing and, as a result,  $\mathbf{B}_{y,avg}^2$  is minimized. When  $g^*$  is increased, microstructures are scarcer, lowering the value of  $\mathbf{B}_{y,avg}^2$  for all  $h^*$ . For the  $h^*$  and  $g^*$  values considered, the maximum value of  $\mathbf{B}_{y,avg}^2$  occurs for  $h^*=2$  and  $g^*=1$ .



Figure 36: Variation of  $\mathbf{B}_{y,avg}^2$  for periodic and continuous microstructures (a) The variation of  $\mathbf{B}_{y,avg}^2$  for a nickel microstructure array with varying  $g^*$  for specified  $h^*$ . Increasing  $g^*$  results in scarcer microstructure elements, reducing  $\mathbf{B}_{y,avg}^2$ . Upon decreasing  $g^*$ , the microstructure element density and  $\mathbf{B}_{y,avg}^2$  both increase. When  $g^* < 1$ ,  $\mathbf{B}_{y,avg}^2$  decreases rapidly with further decreases in  $g^*$  since the microstructures now behave

as a flat plate. Hence, there is no more field concentration. (b) The variation of  $\mathbf{B}_{y,avg}^2$  for continuous nickel microstructures with varying  $h^*$ . Increasing  $h^*$  also increases  $\mathbf{B}_{y,avg}^2$ . For small  $h^*$ , the microstructure acts as a flat plate with no magnetic field concentration and thus  $\mathbf{B}_{y,avg}^2$  approaches 0 T<sup>2</sup>m<sup>-1</sup>. From interpolation,  $\mathbf{B}_{y,avg}^2$  for a continuous microstructure becomes comparable to that of a periodic array when  $h^* \sim 0.04$ .

#### 7.3.3.2.2 Continuous Microstructures

In contrast to the undulating trajectory of a paramagnetic cell in the presence of a periodic microstructure array, due to the absence of microstructure interference a cell passing over a continuous microstructure (with  $g^* = 0$ ) does not encounter repetitive repulsion. The attraction force exerted by the microstructure in this case remains undisturbed along the microchannel. Figure 36(b) shows the dependence of  $\mathbf{B}_{y,avg}^2$  on  $h^*$  for continuous microstructures with  $h = 50 \ \mu$ m. For such a continuous and non-periodic microstructure, the width *w* is assumed along the microchannel depth *d* rather than in the stream-wise direction, which was earlier employed to examine an array element in a periodic microstructure. As  $h^*$  increases,  $\mathbf{B}_{y,avg}^2$  plateaus since the microstructures become magnetically saturated. When  $h^*$  approaches zero, the geometry of the microstructure approaches that of a flat plate for which the field concentration is negligible and consequently  $\mathbf{B}_{y,avg}^2$  also becomes negligible.

In a blood separation application, increasing the flowrate of separated cells is beneficial for reducing the processing time. For a specified inlet flow velocity V, channel height c, and channel length L, increasing the channel depth d should increase the volumetric flowrate and thus the number of cells processed through the system. If the microchannel depth is increased, the width of a continuous microstructure must also increase, such that  $w \ge d$ , to ensure uniform separation across the depth of the entire channel. However, increasing the width of a continuous microstructure decreases  $h^*$  and thus  $\mathbf{B}_{y,avg}^2$ , reducing the separation efficiency. In contrast, since  $h^*$  is independent of channel depth for periodic microstructure arrays,  $\mathbf{B}_{y,avg}^2$  is unaffected by an increase in the channel depth, conserving separation efficiency. Interpolating from Figure 36(b), for  $h^* \sim$ 0.04,  $\mathbf{B}_{y,avg}^2 \sim 50 \text{ T}^2 \text{m}^{-1}$ , a value that is equivalent to that for periodic microstructure arrays. Thus, periodic microstructure arrays should be implemented if the increase in *d* for a continuous microstructure case results in  $h^* \leq 0.04$ , otherwise continuous microstructures are more efficient.

The separation of blood cells can be affected by biochemical changes in the blood such as iron traces in endogenous biochemicals. However, because such biochemical changes affect cell behavior in a magnetic field, they can be leveraged to identify and detect diseases. For example, a hemoglobin deficiency will hinder the separation efficiency of RBCs since they will be less susceptible to a magnetic field. This, however, can be leveraged to separate hemoglobin deficient RBCs from an RBC population for assessment [185].

#### 7.3.3.3 Cell Trajectory Simulations

Next, RBC trajectories are simulated for different microstructure array configurations. Here,  $\chi_c = -4.83 \times 10^{-7}$  and  $\chi_m = -7.14 \times 10^{-7}$  [157], the viscosity of the water

medium  $\mu$ = 1.0 mPa s, and an average flow velocity of 200  $\mu$ m/s is assumed. The RBCs are assumed to be spherical with diameters of 10×10<sup>-6</sup> m [180]. The simulated trajectories trace the movements of their respective centers.

Figure 37(a) presents trajectories for RBCs entering a microchannel at a displacement  $y = 20 \ \mu\text{m}$  above microstructures with  $w = g = 50 \ \mu\text{m}$  but varying *h*. The streamwise position where a cell first comes in contact with the microstructure array and becomes stationary is taken as a measure of the separation effectiveness. The earlier this occurs along the length of the microchannel, the more effective separation is assumed to be. Again,  $g^* = 1$ ,  $h^* = 2$  provides the most effective separation, which is highlighted through the corresponding RBC trajectory in Figure 37(a). This trajectory terminates at a stream-wise distance of 7.85 mm in comparison to the trajectories for  $h^* = 1$  and 4, which conclude at 8.03 and 9.11 mm, and that for  $h^* = 0.5$ , which ceases at 15.95 mm. This ordering is consistent with the values of  $\mathbf{B}^2_{y,avg}$  shown in Figure 37.

Similarly, Figure 37(b) presents RBC trajectories above microstructures with  $w = 50 \ \mu\text{m}$ ,  $h = 100 \ \mu\text{m}$  and varying g. When  $h^* = 2$  and  $g^* = 1$ , the RBC trajectory ends at a stream-wise distance of 7.85 mm, while for  $g^* = 2$  and 4, the trajectories cease at 10.25 and 19.2 mm, respectively, and for  $g^* = 0.5$ , the trajectory terminates at a relatively long 28.8 mm. Continuous microstructures provide significantly better separation effectiveness. For these microstructures, the RBC trajectories end at streamwise distances of 1.05 mm, and 1.03 mm for  $h^* = 0.5$  in Figure 37(a) and  $h^* = 2$  for Figure 37(b) respectively.



Figure 37: RBC trajectories for periodic and continuous microstructures.

(a) RBC trajectories over periodic microstructures of constant width w, gap g and varying height h, and over a continuous microstructure with g = 0. (b) RBC trajectories over periodic microstructures of constant width w, height h, and varying gap g and over a continuous microstructure with g = 0. Both simulations show that continuous microstructures are more effective separators than multi-element microstructures. The best separation with a periodic microstructure array occurs for  $h^* = 2$  and  $g^* = 1$ , as predicted by the surface integral equation.

## 7.3 Paramagnetic Buffers for Macroscale Cell Patterning

As previously discussed in section 7.2.2.3, paramagnetic buffers can significantly affect the magnetic force imposed on the cell. In this case all cells act as diamagnetic materials due to the large differences between their respective susceptibilities and that of the medium or buffer. While diamagnetophoresis has been previously investigated to pattern cells on the microscale, it has been deemed necessary to use HGMF microstructures to enable patterning, even in reported usage of paramagnetic Gd-DTPA buffers [161, 186].

And thus the patterning technique is restricted to the microscales without fluidic momentum diffusion considerations, i.e. convection effects.

Briefly presented here is a proof of concept of macroscale cellular patterning with non-adherent whole blood cells to provide different patterning shapes. Reported diamagnetic patterning methodologies rely of fluid flow to transport cells to low magnetic field regions, where they will become stationary (diamagnetic materials migrate to low magnetic field levels). The presented methodology relies purley on diamagnetophoresis alone for cell transport. The motion of cells, however, triggers fluidic motion through momentum transfer and diffusion, causing recirculation. This allows cells to be transported into the low magnetic field zone by diamagnetophoresis, while a certain number cells are forced out of the zone by fluid recirculation, the former being lager in numbers than the latter. This eventually causes a buildup of cells in the low field zone and an equivalent depletion elsewhere in the medium. This depletion causes a reduction in diamagnetophoretic transport, since now most cells are in the low field zone. As a result, fluidic momentum diffusion is reduced along with the weakening and eventual halt of circulation at which time patterning is considered complete.

This magnetically induced convection cell patterning technique can be leverage to produce various types of large scale cell patterns. A buffer solution used is made of 3 mL of PBS-Gd-DTPA [200 mM]. 3  $\mu$ L of whole blood were mixed in with the solution using a micropipette in a circular class vial. Neodymium N52 magnets were used in two different configurations to create a magnetic field with a low energy field in the center of the vial, 1) double magnets 180° pole angle, and 2) triple magnets with 120° pole angles. Simulations

were also conducted using the same program and material library described in section 7.3.2. Figure 38(a), shows an initially homogeneous distribution of blood cells in the buffer solution. Upon subjection to the magnetic field, diamagnetophoresis transport occurs and magnetically induced convection patterning begins. After 2 hours, the resultant cell pattern is little changed and the experiment is concluded.



#### Figure 38: Magnetically induced convection cell patterning.

(a) A homogeneous cell distribution is first introduced to the magnetic field. Once subject to the magnetic field, diamagnetophoresis of cells occurs, triggering fluid recirculation. (b) With various magnet configuration, it is possible to tune fluid recirculation and subsequent cell transport to easily pattern various geometries such as a line or a three pointed star. Simulation also complement the experimental result.

It is shown that the magnetic field can be tailored to tune the induced circulation of the buffer, thereby obtaining different geometries, Figure 38(b). The conducted simulation, however, do not take in consideration application fluid recirculation or cell-substrate adhesion, and thus would predict that all cell patterning, regardless of magnetic field configuration, would from a spherical or circular pattern as they all converge to the lowest magnetic field zone. This is clearly not the case in the experimental results. This effect can potentially be attributed to cell recirculation with the induced convective currents which weakens over time, and cell settling due to gravity.

Other magnetic field geometries can help create cell spheroids. Unlike the configurations in Figure 38(b), the magnetic arrangement in Figure 39 is favorable, i.e. magnetically stable. A simple tilt of the cuvette containing the whole blood creates a spatially varying magnetic field strength at different locations in the cuvette. This leads to the formation of blood droplets of different sizes. The higher the magnetic field strength the smaller the spheroids will become, since they are subject to a more magnetic force, compared to those in lower magnetic fields. Such control to in the formation of cell spheroids cannot be easily matched for example using the hanging drop method to create spheroids. Different size spheres equally translates to a control in cell density of the spheroids, which again surpasses the capability of the hanging drop method. This equally lends the capacity to induce mechanical stresses on demand on a spheroidal cell culture by varying the magnetic-substrate separation distance. Subjecting mechanical stresses can mimic internal physiology conditions, presently not possible using conventional methods.



### Figure 39: Blood spheroids with size gradient.

A homogenous solution of 3  $\mu$ L whole blood cells is first inserted in a cuvette and mixed with 500  $\mu$ L PBS with 150 mM Gd-DTPA solution. Once the cuvette is placed on the magnet bank, diamagnetic patterns forces cells into spheroidal formations. Because the cuvette is tilted, the magnetic field strength varies spatially. Since spheroids in relatively weak magnetic fields (larger magnet bank separation gap) are not under high diamagnetic forces, they are larger than their smaller counterparts located in higher magnetic field regions (small magnet bank separation gap). This simple magnet arrangement provides a facile rout to control spheroid size and cell density.

To demonstrate the ability for this cell patterning methodology to adherent cells,

MCF7 breast cancer cells are hereby tested. The cells are first cultured and subsequently

centrifuged into a small pellet. The pellet was put into 400  $\mu$ L DTM media containing 75

mM of Gd-DTPA at 7.8 pH. Repetitive pipetting of the pellet ensure re-dispersion of the cells in the media. When the vial containing the cells and media is placed onto a magnet bank diamagnetic patterning immediately begins to form a spheroid, see Figure 40.



### Figure 40: MCF7 adherent cells diamagnetic patterning

When a homogeneous suspension of MCF7 breast cancer cells in a paramagnetic medium are subject to a bank of magnets to from a spheroid. The cells immediately respond by diamagnetiphoresis to form a spherical structure. The inset shows a circular cell concentration with a radially decreasing cell density. Cells adhering to the bottom of the glass vial can also be seen. The diamagnetic pattern formed within 5 min.

The pattern in Figure 40 formed within 5 minutes, compared to ~1 hour for whole

blood cells. To understand why such a difference exists we look at the equation of motion

of cells under a magnetic field while subjected to drag forces. Equation 3.10 can be

rewritten to express the terminal of cell undergoing magnetophoresis in a host fluid.

$$\mathbf{U} = \frac{2\mathbf{R}^2 \mathbf{f}_c}{9\mu} \quad (7.5)$$

where  $\mathbf{f}_{e}$  is the magnetic body force on the cell. From Equation 7.1,  $\mathbf{f}_{e}$  can be expressed

such that

$$\mathbf{f}_{c} = \left(\frac{\chi_{cell} - \chi_{m}}{2\mu_{o}}\right) \nabla |\mathbf{B}|^{2} \quad (7.6).$$

Assuming that the host medium is heavily paramagnetic such that  $\chi_{cell} - \chi_m \approx -\chi_m$ , using Equation 7.6, Equation 7.5 can be rewritten as,

$$\mathbf{U} = -\frac{R^2 \chi_m}{9\mu\mu_o} \nabla \left| \mathbf{B} \right|^2 (7.7).$$

Assuming all else is constant in Equation 7.7, a variation in *R* indicates that the terminal velocity of a cell scales with  $R^2$ , i.e.  $\mathbf{U} \sim L^2$ . For a comparison between the terminal velocity of RBC,  $\mathbf{U}_{RBC}$ , versus that of MCF7,  $\mathbf{U}_{MCF7}$  for which the Radii are  $R_{RBC} \sim 9 \ \mu \text{m}$  and  $R_{MCF7} \sim 24 \ \mu \text{m}$  respectively, the ratio of velocities are thus  $\mathbf{U}_{MCF7}/\mathbf{U}_{RBC} = R_{RBC}^2/R_{MCF7}^2 = 7.1$ . The MCF7 cells are thus 7.1 times faster than RBCs under the same conditions. In addition because MCF7 are adherent cells, as they come in contact they couple together into small clusters. However since these clusters now act as one, unlike blood cells, they can increase their terminal velocity since they now together have a bigger equivalent radius. The above mathematical expressions confirm observations of rapid patterning of MCF7 cells compared to whole blood cells.

## 7.4 Conclusions

In summary, as a first step to cell manipulation, the governing magnetic force equation is analyzed. To do so, an investigation on whole blood cells help clarify a range of magnetic properties. While mainly diamagnetic in nature, cell types such as RBC can exhibit paramagnetic behaviours. First by analysing the magnetic properties of cells, magnetometry measurements on as-is RBC samples shown that a patient depended variation does indeed occur and may be due to differences in the amount of oxygenated and deoxygenated RBCs in the sample as the blood was drawn. Using NaNO<sub>2</sub> in a PBS buffer solution, highlights a method to intrinsically alter the magnetic properties of cell. This resulted in the oxidation of hemoglobin to methemoglobin in RBCs and the subsequent increase in sample paramagnetic properties. More work is still however needed to shows that RBCs from different patients reach the same level of paramagnetic properties when oxidized. However, when cell exhibit diamagnetic properties, altering their magnetic response is let to labeled methods. An additional way to alter the magnetic response of cells is by engineering the buffer solution, such as with the addition of Gd-DTPA, a highly paramagnetic salt. This allows all cells to behave diamagnetically in the medium, thereby insinuating the behaviour of other cell lines, such as cancer cell.

The chapter also presents and investigation into how magnetic field geometries affect cell magnetiphoresis by focusing the study on the established field of HGMF cell separation. The study present criteria for the geometries of high gradient magnetic field microstructures that optimize continuous label free whole blood separation in microfluidic channels. While the literature reports numerical and experimental blood separation analyses, it largely ignores the influence of microstructure geometry. Through a comparative study of different rectangular periodic array geometries we show that blood separation is best with arrays that have aspect and gap ratios  $h^* = 2$  and  $g^* = 1$ . The simulated trajectories of red blood cells show that the greatest separation occurs with continuous microstructures that produce a seven-fold increase in separation over comparable periodic microstructure arrays. However, for large microchannel depths, periodic arrays are more appropriate since, unlike continuous microstructures, their separation efficiency is independent of depth. Our results can guide the fabrication of microstructures that produce high gradient magnetic fields in microfluidic whole blood separation devices.

To apply the lesson learned thus far, an experimental and numerical investigation shows that combining highly paramagnetic buffer solutions, different magnetic field geometries, large scale cell patterning is possible. The mechanisms at play prove that labelfree cell patterning using diamagnetiphoresis is possible without the use of HGMFs as well as different magnetic field geometries govern the final cell pattern. Due to the large magnetic susceptibility differences between the whole blood cells and the medium, this mechanism is transferable to other cell lines. However, further viability studies would be necessary. This technique can potential be employed to create 3D cell cultures for other than spherical geometries, or in tissue engineering applications.

# 8. Conclusions

With the ever increasing progression of smart technologies, the demand for materials that can do more, for less has never been higher. This drives the pursuit of new materials that can fulfill newly formed knowledge gaps, interest, and applications. By increasing the complexity of our material arsenal, more design parameter help in tailoring such materials for specific applications. The demand for such complexity has resulted in a surge in various fabrication methodologies that claim governance over the resultant material properties. In turn, the properties of the materials finally define the function.

It could be easily seen that lithographic fabrication techniques, perfected over many decades of silicon based manufacturing, offer a wide spectrum of uses, including electronics, MEMS, NEMS, photonics, and biological applications. In fact microfabrication is largely responsible for the surge seen in consumer electronics and the continuing advancement in that field. Microfabrication techniques give an excellent example to two main established routes used in building objects that date back since early civilization. Bottom up approaches entails the addition of materials in order to obtain the final object. The precision of such methodologies are mainly governed by their constituent building blocks. An advantage of such method is that different type of building blocks can be combined together in order to construct heterogeneous materials and fabricate a particular surface effect with a specific function, such as an electrical circuit. On the other hand, top down approaches involves the removal of materials to finally expose the resultant structure. Much like sculpting, the precision of such methods is restricted by the tools used

to remove materials. The characteristics of the final object in this case is mainly governed by the nature of the bulk material, which renders it difficult to obtain different material heterogeneities. However, unlike bottom up, top down allows for the creation of welldefined bulk objects, which is a cornerstone for some MEMS devices.

However, while attractive, usual hurdles for such fabrication techniques include the use of cleanrooms, expensive materials and an array of fabrication steps. Many researches have attempted to explore different fabrication techniques. Much of the effort has been focusing on finding new ways to pattern materials to fulfill specific targeted applications. For example, patterning certain nanoparticles on a substrate can help with some photonic applications. The applications of such surface modifications has largely been the focus of many researches, and many still rely in part or fully on derivatives of laborious techniques. This can result in non-scalable fabrication methods, such as in the case of laser tweezing nanoparticle patterns. The two-dimensional nature of these surface modifications, makes it no surprise that many of the resultant application are heavily related to photonics. Despite the promising results from previous researches, reliance on 2D patterns limits the functionality of materials to specific applications.

To begin with, we explore field directed assembly (dynamic-assembly) of magnetic inks for three-dimensional patterning of materials. Magnetiophoretic transport have recently gained attention as a result to increased integration of magnetic nanoparticles in several mechanical, electrical, chemical and biological applications. This equally sparked research in magnetiophretic dynamic-assembly as a method to pattern materials and thus leverage heterogeneities to create functional materials. Leveraging dynamic-assembly is analogous to building with smart nano or micro sized bricks. By employing a specific magnetic field geometry, such brick simply dynamically-assemble into desired patterns. Magnetic nanoparticles are excellent candidate for such tasks. Their superparamagnetic properties allow them excellent magnetic responses without significant remanence, which can complicate patterning techniques. However, thus far, magnetic dynamic-assembly of nanoparticles has yielded quasi one-dimensional filaments or chains locked in a particular direction in polymeric matrices. While conclusive evidences reveal how the presence of magnetic nanoparticles locally affect material properties, such as the elastic modulus, electrical and thermal conductivities and magnetic properties, such pattern specific changes in properties remain largely two-dimensional. To expand the simplicity of dynamicassembly techniques to the realm of three dimensions would pave the way to facile fabrications of functional materials and biomaterials and validating the role dynamicassembly techniques can play in future materials.

In chapter 3, printing three-dimensional patterns in an elastomer matrix was theoretically and experimentally investigated. Ferrofluid propagation under a magnetic field and in a viscous medium has been thoroughly investigated with several researches reporting immiscible droplet shape evolution under steady and unsteady conditions. However, the use of miscible ferrofluid droplets presents a new approach of material deposition directly into the body of the matrix. When a droplet progresses through a viscous fluid, the positive pressure upstream help create the familiar droplet shape, while the negative pressure downstream allows clusters to escape from the main body of the droplet. In general, the escaped clusters are in turn focused by the flow streamlines, created by the moving ferrofluid droplet, into a well-defined trail. Theoretical analysis demonstrates how the escaped clusters have to be small in size compared to the ferrolfuild droplet in order to be properly deposited in the downstream trail. That is because the bigger such magnetic clusters are, the more deviations will occur from the flow streamlines, caused by the global magnetic field. Experimental observations, however, show how such deviations can be beneficial in certain cases where large clusters accidentally breakoff the main body of the droplet. In such a case, larger clusters would be recaptured by the droplet, preventing the development of severe irregularities in the trail. The printing method is thus self-regulating. Once in the trail, the clusters have negligible propagation speeds relative to the droplet. This allows for variations in the magnetic field direction governing the path of the droplet and thus the trail pattern in three dimensions. In addition, the large viscosity of the medium subdues diffusive effects, preserving the trail shape. The printing technique thus allows three dimensional printing using ferrofluid droplets as the print heads. While the methodology is not a replacement of 3D printers, it may provide an avenue for dynamicassembly techniques to compete in such a market. For example, the ability to deposit magnetic field concentrators along a prescribed path (Trail) for use in cell separation or trapping devices, can be easily done using such a technique without the use of a layer by layer approach, which can be time consuming. In addition, stiffness patterns and high thermal conductivity paths, can be easily created, using various sets of droplets and magnetic field variations. Trail width can also be easily controlled by varying the droplet diameter as seen by the experimental results, offering additional design parameters. The absence of nozzles during printing would allow for more geometrical freedom. Finally, while only magnetic nanoparticles where investigated, it is possible for the ferrofluid to contain non-magnetic materials, such as copper. This allows the printing of multi-materials trails. However such materials must be investigated for their colloidal distribution and stability.

The ability to produce 3D patterns directly into polymeric matrices, can only be validated through a demonstration of functionality. Thus, to expand the printing methodology further, chapter 4 investigates the effect of trails on the local elastic modulus of the bulk elastomeric matrix. Upon investigating the trail using transmission electron microscopy, it was found that nanoparticles agglomerated into micron and submicron cluster islands. This discontinuity prevents electrical continuity if the magnetic nanoparticles were made of a conductive material such as nickel. However, through atomic force microscopy it was found the matrix inside the trail (space between cluster islands) was made of softer PDMS than the bulk matrix, which hosts the trail. Further, by varying the volume fraction of magnetite in the original ferrofluid ink, it is possible to control the elastic modulus variation that occurs. Thus the 3D nozzle-free printing allows the creation controllable pattern-specific stiffness variations in the elastomeric matrices faster than current commercial 3D printing technologies. While, not a replacement of 3D printing technologies, the printing mechanism can excel in specific applications requiring stiffness patterns. Because of the ink format, the ferrofluid can be integrated in ink jet printers, which would enable printing for several sets of droplets at a time on a surface of polymeric material. They can be subsequently guided into different patterns via magnets. Different droplet sets can be deposited at different times, and guided differently.

Patterning through thick matrices is possible, although, a limit exists where the magnetic field becomes too weak due to the droplet's first position. For large surfaces and membrane patterning, however, the technique is anticipated to perform the well. This can, for example, print three-dimensionally varying stiffness arrangement of fish fin mimicking membranes, which can be mounted on submersible robots and used for optimized propulsion. It can equally be used to generate stiffness variation in synthetic biocompatible materials.

Further investigations focused on using the printing technique discussed in chapters 3 and 4 to investigate the electrical properties of the printed trails using nickel and copper nanoparticles as well as carbon nanotubes with the ferrolfuid ink. However, as expected, cluster formation creates discontinuities preventing direct electrical current from conducting through the trail. Alternating current was used to investigate pattern specific capacitance variations when subjected mechanical deformation for possible use as strain sensors. However, is was significantly hard to confidently obtain a meaningful measurement. Many researches demonstrate electrical property enhancements of polymeric materials containing carbon nanotubes, however, modified resistances are often described in M $\Omega$ . While magnetic dynamic-assembly was investigated to concentrate carbon nanotubes dispersed in PDMS, the resultant dense network proved to be unconducive when supplied with a direct current. Partially due to the coating of carbon nanotubes with the

nonconductive polymeric matrix, which subsequently prevents electrical continuity even after dynamic-assembly.

The pursuit of electrically conductive dynamically-assembled networks would facilitate the simple fabrication of electrical circuitry and sensory components, adding to the achievable functionalities of magnetic dynamic -assembly techniques. In Chapter 5, during the investigation of carbon nanotube patterning, it was discovered that nickel nanopartiles, when sonicated in a medium with the presence of carbon nanotubes, formed nickel-carbon nanotube magnetic ink in a matter of minutes. This is done through the entanglement of nickel within bundles of carbon nanotubes, due the high surface energy of nickel nanoparticles and  $\pi$ -interactions with the surface of the nanotubes. The conjugate material is durably magnetic and can therefore be guided by magnetic field to print, through dynamic-assembly, certain geometrical features such as electrical circuitry. This magnetization technique is relatively simple compared to functionalization and chemical magnetization of carbon nanotubes. Such methods often use several reagents, acidic treatment, which can impair nanotube properties, is time consuming and labor intensive.

Leveraging the magnetic properties of the ink, circuits were printed using a magnetized template. This demonstrates the ability for the ink to be patterned into various geometries, allowing design freedom for different applications. Once the dispersing medium is evaporated, it was found that the printed features conduct electricity. The dry powdery form of the features cannot be further manipulated without the use of a host matrix. PDMS again offers an excellent host matrix due to its low surface energy, allowing

it to infiltrate through the conductive network giving it mechanical integrity. Further, PDMS lends its flexible properties to allow mechanical deformation of material. When supplied with an electrical current, such deformations induce electrical changes in the network, which can be interpreted by an external circuit. Effectively a strain sensor, the electrical response correlates with the degree of deformation during elongation and bending tests. Additionally the sensor readily senses oil. Such applications demonstrate the ability of magnetic dynamic-assembly techniques and the employment of magnetic inks in creating simple bench top fabrication of strain and oil sensors. The methodology foregoes the heavy use of reagents as well as complex printing techniques thus saving time and cost.

In order to expand the application of magnetic CNTs, chapter 6 discusses how CNTs can be used in biological sensors. Early pathogen detection is critical for containing and preventing the spread of infectious diseases. Time consuming and labor intensive methods, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are not always applicable for early detection. In chapter 6 we describe a sensor based on a new bio-nanomaterial that uses functionalized carbon nanotubes to detect biomarkers, specifically c-Myc antigens. Recognized by the anti-c-Myc primary antibody, our target c-Myc antigen is over-expressed in cancerous tumour cells and is a potential biomarker of malignant phenotypes. While carbon nanotubes show an electrical response to certain biological species, their sensing affinity towards specific biomarkers can be enhanced by attaching antibodies to their surfaces that bind with specific antigens, in this case c-Myc antibodies are used.

We prepare a magnetic Bioink (mBioink) consisting of multi-walled CNTs, first functionalized with magnetite magnetic nanoparticles and subsequently with immobilized Abs, that are dispersed in DI water. The reported synthesis process offers a simpler and faster alternative compared to previously reported use of intermediary reagents. By employing a magnet, the ink can be easily printed into inexpensive dense conductive networks, avoiding the need for lithographic techniques, while providing sensing capability through a simple electrode system. When a current is supplied to the network, the presence of c-Myc antigens is transiently detected. The sensing relies on real-time specific Ab-Ag binding kinetics, which cause a decrease in current over time and resolving picomolar c-Myc concentrations within sixty seconds. This offers magnetic biosensors a simple, direct and fast alternative methodology compared to previously reported cyclic voltammorgam analysis. The magnetic mBioink sensors provide enough contrast between c-Myc and BSA samples of different concentrations. This is possibly due to the increased chance of covalently bonded Abs in the mBioink. The reported mBioink can be tailored for different Ag detection, and allows for rapid and economical benchtop printing of highly sensitive biosensors, finding valuable applications in pathogen detection and diagnosis of diseases.

While magnetic nanomaterials are often employed in magnetophoretic dynamicassembly printing, such as demonstrated in chapters 3-6, biological species can equally be magnetically manipulated. In chapter 7 we investigated the magnetic properties of whole blood cells, identifying the importance of cell magnetic susceptibility, size, and magnetic field geometry in helping to manipulate cells. We equally highlight how the host medium can be engineered to amplify and govern cell manipulation. SQUID magnetometry revealed a large contrast in the magnetic behaviour of red blood cell, which are largely paramagnetic, to white blood cells which are diamagnetic. Such contrast can be leveraged in whole blood magnetic label-free fractionation. Additionally the host medium can be chemically modified to increase the paramagnetic characteristics of red blood cells thereby increasing the magnetic separation efficiency from whole blood. Label free separation of whole blood requires high gradient magnetic field, achievable only through the use of magnetic microstructures. While literature describes multiple use of such microstructures in microfluidic channels, no consensus as to what microstructure geometries should be used to optimize separation efficiency. In chapter 7, simulations show how rectangular arrays, with a height and gap aspect ratios of 2 and 1 respectively, help optimize the manipulation of red blood cells in a microfluidic channel. Moreover, non-periodic and continuous microstructures offer a seven fold increase in the separation efficiency compared to periodic ones.

While such magnetic manipulation offers a viable avenue for cell separation and capture, it equally paves the way to label free cell patterning. Patterning cells can help facilitate tissue engineering, produce cell on chip devices or even create 3D cell culture for drug screening. 3D cell culture offers a better understating to tissue drug response than convention monolayer cell cultures. Creating 3D cell cultures often rely on the hanging drop methodology and lately 3D Bioprinters. The former limits the achievable size of spheroids, due to limitations of droplet size and the latter requires expensive tools. While such techniques prove effective, the hanging drop is limited to the creation of spheroids, and 3D bioprinters rely on slow cell level manipulation. Magnetic manipulation of cells

may provide a faster alternative to achieve simple yet impactful 3D cell patterns for use in applications such high through put drug screening.

In chapter 7 we show a proof concept of engineered buffer mediums and their ability to diamagnetically pattern cells. The patterning of strips, three-pointed stars, and large spheroids of blood cells demonstrate the success of the patterning methodology in creating 3D cellular structures with non-adherent cells. Adherent MCF7 cancer cell were successfully patterned into large tumor sized spheres in under 10 min. The methodology offers a fast and easy route to achieve 3D cellular structure. This culture technique can be easily implemented to create, for example, banks of tumor spheres, only to be later cultured and tested as an alternative to mouse models.

Magnetic inks offer a promising path to achieve simple, scalable benchtop fabrication techniques of functional materials. Whether such materials target mechanical, electrical or even biological applications, magnetic ink manipulations require minimal equipment and can easily complement other existing systems. While more research is still required, the work presented in this thesis can help act as a starting point for various research directions and applications.

# 9. Future Directions

Drawing inspiration from nature, we see countless examples of function defined by complex material arrangements. For example, a dragonfly's wing is composed of soft and hard materials, which allow for optimum generation of lift, lending one of the best fliers in the natural world. Similarly fish fins have complex stiffness patterns allowing for passive propulsion through fin-water hydrodynamic interactions. Such interactions require no muscular input and thus save on energy. This natural technology can be directly translated into manmade objects such as submersible robots and micro fliers where energy saving is key to prolonged deployment. It is therefore imperative to leverage material heterogeneities to create future functional bulk materials. In achieving this, we must equally explore fabrication techniques to control the spatial heterogeneities of materials. This will enable us to effectively print materials with designed performances and a multitude of properties such as mechanical, electrical, magnetic and thermal ones. Such an expansion in the array of available materials will thrust the exploration of new applications.

Control over such heterogeneities can come in many forms, and here we explored the use of magnetic inks with nanomaterials and their dynamic-assembly into predefined patterns. The choice of nanoscale building blocks is an obvious one, since we have reached the ability to synthesize materials at that scale. This can therefore increase the resolution at which we can pattern and modify material properties. However, creating macroscale materials using nanoscale patterns remains a challenge mainly due to the largely unexplored manipulation techniques of such nanomaterials. The coupling of theoretical, simulative and experimental investigations is currently lacking for dynamic-assembly techniques aimed at tuning material properties.

Thorough understanding of the theory and mechanisms of assembly and equations of motion must be validated with experimental observations. Theoretical models should have the capacity to treat large number of individual elements or particles, which is bound to pose significant mathematical hurdles. In turn, sound theoretical studies are to be used in the building of simulation tools. It is equally imperative for such simulation tools to carefully expand current approximation approaches such as the overuse of flow around spherical geometries to simplify fluid drag experienced by nano and microparticles. Such simulation tools should interrogate realistically achievable patterns while equally predicting, with high accuracy, pattern-specific changes in material properties. The simulation algorithms coupled with mathematical models should be as efficient as possible in order not to demand large computational needs. Thus new approaches to simulate large amounts of interacting dynamically-assembling particles may be required. Simulation results in turn should validate, predicts and complement existing and new experimental investigations. Such coupling between 1) theoretical models, 2) simulation tools, and 3) experimental investigations will ameliorate the level of trust in dynamic-assembly techniques as a viable and fast fabrication method.

The impact of dynamically-assembled nanoparticles on the mechanical properties of materials have been relatively well explored. That is mainly due to the readily available tools with which one is to investigate such alterations. Much less research has been given to dynamically-assembled electrode systems or circuitry, which can be problematic when probing 3D geometries. Such research, however, may help create multi-axis sensor systems and 3D soft circuitry to accelerate the field of wearable electronics. And although unsuccessful attempts were made to create 3D circuitry, such investigations were not exhaustive enough to discredit the potential of miscible magnetic inks for that purpose. Thus, further research in that direction is needed.

Further research can also benefit the creation of more accurate and robust biosensors. For example, the creation of biocompatible scaffolds that can offer protection to printed biomaterials and biosensors. Equally important is the exploration of new kinds of biomaterial-nanomaterial combinations that will simplify dynamic-assembly while increasing bio-sensitivity to an expanded array of biomarkers. Such research can offer dynamic-assembly techniques a solid foundation in the diagnostics field.

While synthetic nanomaterials have been extensively used to tune material properties through dynamic-assembly patterning, few researches are concerned with the patterning of biological materials. Doing so can pave the way to a facile and rapid bioprinting methodology. A buffer solution can be engineered to have a vastly different magnetic susceptibility than the cells which it hosts. This will allow significant magnetophoresis when the system is subjected to a magnetic field. Such cell movement can create 3D cellular structures, which can be beneficial to tissue engineering and drug screening applications. However, extensive cell and media magnetic property investigations are required, and may be performed using SQUID magnetometry. In addition, thorough viability assays must be conducted to ensure cellular functions are preserved after patterning compared to ideal conditions. The application of dynamicallyassembled cellular structures must also be expanded and validated by venturing into the territories of tissue engineering, such as vasculature formation, cell culture, and drug discovery. This direction, however, will impose its own hurdles through heavy regulations and diverse collaborations with multiple disciplines and experts in the various involved fields.

Dynamic-assembly fabrication techniques are generally tailored for simplicity, scalability, cost effectiveness, and efficiency. This requires future researches in the field to hold true to such characteristics, while providing an exhaustive comparison against conventional techniques.

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