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**DEVELOPMENT OF MAGNETIC STRUCTURES BY MICRO-
MAGNETOFLUIDIC TECHNIQUES**

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Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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Date

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Abstract

Microfluidics is a branch of fluid dynamics which deals with investigations of fluid behavior at the micron scale, characterized by low Reynolds number, which results in laminar flow. The field of microfluidics is of high significance due to its broad spectrum of applications, including as a Lab-on-a-Chip (LoC) platform. For example, droplet microfluidics offers a large increase in the multiplex capability desired for most LoC; applications the droplets act as individual pl to nl volume range containers.

Integration of magnetofluidics with microfluidics can provide control of droplets in the flow to develop a practical LoC device. The resulting domain of droplet micro-magnetofluidics (MMF) offers the excellent capability for the wireless and remote control of droplets, leading to versatile droplet micro-magnetofluidics (DMMF) applications. Magnetic fluids in the ferrohydrodynamic regime (termed as ferrofluids), exhibit liquid magnet like behavior. Ferrofluids exhibit a significant response to both uniform and hybrid magnetic fields. Hence, the behavior of ferrofluid droplets (FD) and magnetic Janus droplets (MJD) exposed to uniform and hybrid magnetic fields have been investigated in a microfluidic environment.

A MMF setup by integrating microfluidics and magnetofluidics have been developed. Various designs of microfluidic chips were employed for LoC droplet generation at different length scales for DMMF investigations. A droplet MMF numerical model was developed to elucidate the dynamic behavior of ferrofluid droplets in uniform as well as hybrid magnetic fields. This model simulated droplet generation, droplet deformation, and merging of droplets in uniform magnetic fields.

Experimental and numerical investigations for continuous flow MMF and investigations of MMF spreading of a ferrofluid core stream clad by diamagnetic streams have been performed. A water-based ferrofluid was utilized as the magnetic phase and aqueous glycerol as the diamagnetic phase. The effect of applied uniform magnetic field was investigated for a range of flow rates, flow rate ratio, viscosity, magnetic particle concentration and ferrofluid susceptibility. The role of diffusion of particles, drift velocity of magnetic particles, and convective diffusion was studied. We found that difference in magnetic susceptibility between the core and diamagnetic streams led to a distortion of the

magnetic field, resulting in a magnetic force acting on the ferrofluid. Spreading was observed mainly near the channel walls due to the lower flow velocity near the walls. Convective diffusion is the main factor for MMF spreading, which was favored at low flow rates, low flow rate ratio and high magnetic field.

After investigating continuous flow behavior, the studies were extended to DMMF and demonstrated LoC droplet merging and fabrication of magnetic Janus particles.

The combination of FD and uniform magnetic field offers wireless, programmable remote control, which is useful for LoC applications. LoC experiments and numerical studies were performed to investigate FD behavior in uniform magnetic fields. The dynamic behavior of FD was evaluated by investigating droplet size, aspect ratio, droplet velocity and droplet spacing. The size, shape, velocity and inter-droplet spacing of these droplets could be controlled by tuning magnetic field strength, ferrofluid susceptibility, viscosity and flow rates. The droplet micro-magnetofluidic numerical model found to be in good agreement with experimental results.

Controlled LoC merging of droplets is a challenge. This challenge was addressed by numerical modeling and experimental studies of uniform magnetic field induced merging of ferrofluid based droplets; control of droplet velocity and merging was achieved. Merging and mixing of composite droplets, such as color dye+ magnetite, was demonstrated. Our numerical results were found to be in good agreement with experiments. These studies are useful for wireless and programmable droplet merging as well as mixing relevant to biosensing, bioassays, microfluidic-based synthesis, reaction kinetics, and magnetochemistry.

These experimental and simulation findings were utilized to develop a DMMF platform for magnetic Janus particle (MJP) fabrication. This system is capable of magnetically controlled, selective LoC polymerization, resulting in the synthesis of MJP. Since the method is wash-less and does not use oils or surfactants, MJP are “ready to use”. The effect of flow rate and magnetic field on the particle size, magnetization, and polymeric properties of the MJP was investigated. Particle synthesis at high flow rates of 4 ml/h was demonstrated. The properties of synthesized MJP were assessed and the application of the particles for protein detection was demonstrated.

The development of MMF and droplet MMF techniques relevant to LoC applications have been demonstrated. These studies are useful for the development of next-generation technology with multiplexing, wireless, programmable, and remote control capabilities. The DMMF investigations demonstrate control of magnetic droplets on a LoC platform using uniform magnetic fields, which overcomes the limitations of nonuniform magnetic field based systems. DMMF was utilized to develop magnetically controlled LoC Janus particle synthesis. Such particles are useful for bioassays, tagging of particles or cells and protein detection.

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Abbreviations

1) Current Studies

Acronym	Expansion
μF	Microfluidics
B_m	Magnetic Bond Number
cap-μF	Capillary Microfluidics
CMF	Continuous Flow Microfluidics
CMMF	Continuous Flow MMF
CP	Continuous Phase
DMF	Droplet Microfluidics
DMMF	Droplet Micro-magnetofluidics
DP	Dispersed Phase
FD	Ferrofluid Droplets
FHD	Ferrohydrodynamics
H	Applied Magnetic Field
H_{no}	Applied Nonuniform Magnetic Field
H_o	Applied Uniform Magnetic Field
LoC	Lab-on-a-Chip
MD	Magnetic Droplets
MF	Magnetofluidics
MHD	Magnetohydrodynamics
MJD	Magnetic Janus Droplets
MMF	Micro-Magnetofluidics
MMFS	Micro-Magnetofluidic Spreading
RIT	Relative Interfacial Tension
SDMF	Smart Droplet Micro-magnetofluidics
SMF	Smart Micro-Magnetofluidics
STC	Surface Tension Coefficient

2) Materials

Acronym	Expansion
EO-TMPTA	Ethoxylate Trimethylolpropane Triacrylate
AA	Acrylic Acid
AgNP	Silver Nanoparticle
AuNP	Gold Nanoparticles
BSA	Bovine Serum Albumin
DNA	Deoxyribonucleic Acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EG	Ethylene Glycol
F-BSA	FITC tagged BSA
FITC	Fluorescein Isothiocyanate
HDOil	Hexadecane Oil
HMOil	Heavy Mineral Oil
HMOil	Heavy Mineral Oil
HMP	2-Hydroxy-2-Methylpropiophenone
LMOil	Light Mineral Oil
MNP	Magnetic Nanoparticle
MOil	Mineral oil
NHS	N-Hydroxysuccinimide
PAA	Poly (Acrylic Acid)
PAACAA	Poly (Acrylamide-Co-Acrylic Acid)
PC	Photonic Crystal
PEGDA	Poly (Ethylene Glycol) Diacrylate
PMMA	Poly (Methyl Methacrylate)
PNP	Plasmonic Nanoparticle
SHP	Super-Hydrophobic
SNP	Silica Nano Particle
SOil	Silicon oil

3) Methods, Techniques, and Terms

Acronym	Expansion
BFI	Bright Field Imaging
ELISA	Enzyme Linked Immunosorbent Assay
emag	Electromagnet
FSI	Fluorescent Imaging
FTIR	Fourier Transform Infrared Spectroscopy
GMR	Giant Magnetoresistance
HLB	Hydrophilic-Lipophilic Balance
HRTEM	High Resolution Transmission Electron Microscopy
LS	Level Set
NS	Navier-Stokes
PCR	Polymerase Chain Reaction
pmag	Permanent Magnet
SAED	Selected Area Electron Diffraction
SEI	Secondary Electron Images
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
tmag	Magnetic Tweezers
VoF	Volume of Fluid
XRD	X-ray Diffraction
mw	Molecular Weight
PCC	Phantom Camera Control

Chapter 1

Introduction

*Micro-magnetofluidics (MMF) is the integration of magnetic field with microfluidics for the wireless, programmable, remote control of magnetic fluids. The combination of magnetic droplets with MMF [1-5] results in the field of droplet micro-magnetofluidics (DMMF), which is a versatile tool for Lab-on-a-chip (LoC) applications. Specifically, enclosed, small volumes of magnetic droplets offer wireless magnetic control of materials for cell encapsulation, biosensing, biochemical assay, cargo-delivery or as a microreactor to produce novel particles. However, control of such magnetic fluids and magnetic droplets on LoC platform is a challenge, which was addressed by the approach of **uniform** and **hybrid** magnetic fields. The study is summarized by stating the background, aim, objectives, hypothesis, significance and novelty in the following sections.*

1.1 Introduction

Microfluidics, the investigation of fluid behavior at the microscale, is characterized by low Reynolds number. Microfluidics is a powerful platform to integrate operations which are typically performed in a lab (termed as Lab-on-a-Chip) e.g., performing reactions, merging, mixing, separation, detection, sensing, counting, cell-tissue culture and material synthesis. Droplets are versatile tools to carry out such operations on a microfluidic platform, droplets act as isolated small containers, and are useful for a range of applications such as cell encapsulation, single cell analysis[3], DNA detection[6, 7], protein detection,[8, 9] bioassays [10], immunoassay, drug discovery[1, 11], disease detection,[2] and novel materials synthesis[12-17]. A promising application of droplet microfluidics is the synthesis of symmetric and asymmetric structures of metamaterials, multifunctional particles, and multiphase *Janus* particles. Janus particles are a special class of multifunctional particles, where the phases within the particle are accessible for use but are separated from each other.

However, control of microfluidic droplets in a LoC environment and the synthesis of Janus particles is a challenge. Utilizing uniform magnetic fields offer a unique approach to wireless, programmable, and remote control of droplets in a LoC platform. Such control requires an understanding of the dynamic behavior and motion of the droplets. Magnetic droplet behavior was investigated in uniform magnetic fields, performed LoC merging, mixing to demonstrate control and finally demonstrated synthesis of Janus particles by our droplet micro-magnetofluidics (DMMF) technique.

1.2 Background

DMMF consist of three components: (i) microfluidics, (ii) magnetofluidics and (iii) magnetic fields. Each of these contains subcategories (**Figure 1.1**). The background, scope, and future of the micro-magnetofluidic technique, as well as the scope and objectives of this research, are summarized in **Figure 1.1**. Studies performed consist of (i) development of MMF, (ii) DMMF and (iii) Janus particle synthesis. These three categories include experimental and simulation components. A brief background of these categories is provided in the following subsections, a detailed discussion is elucidated in the following Chapters.

Figure 1.1: Micro-magnetofluidics: scope and objectives of the research work. The scope of the research work in this thesis focused on “droplet micro-magnetofluidics”, highlighted in yellow. The hybrid magnetic field indicate the uniform magnetic field (H_o) superimposed on a non-uniform magnetic field (H_{no}).

1.2.1 Microfluidics

Microfluidics is the investigation of fluid behavior at the micron scale, typically $\leq 750 \mu\text{m}$. Flow in this regime is characterized by low Reynolds numbers, resulting in laminar flow in the microchannels. Active and passive methods have been used to perform various LoC operations, such as mixing, sorting, trapping/capture, sensing, and multiplexing. Microfluidics can be divided into continuous flow and droplet microfluidics. Continuous flow microfluidics deals with the investigations of continuous flow on a microfluidic platform, which utilizes miscible phases of the fluids.

Droplet microfluidics is the focus of this research work. Droplet microfluidics[4, 18] deals with immiscible phases (e.g., oil and water). leading to generation and control of droplets

in the volume range of pL to μL . Various active and passive strategies are utilized for droplet control, depending on the LoC application.

1.2.2 Magnetofluidics (MMF)

Ferrofluids are suspensions of magnetic nanoparticles (particle size, $d \sim 10\text{ nm}$) which are sterically stabilized in a diamagnetic medium (water or oil). At room temperature, ferrofluids exhibit superparamagnetic behavior since the thermal energy of individual MNP exceeds the magnetocrystalline anisotropy energy. Under an applied magnetic field, ferrofluids show interesting “liquid magnet” like behavior, due to the aligned magnetic dipoles along the magnetic field direction. This behavior offers versatile applications. Uniform and hybrid magnetic fields can be used for programmable, wireless and remote control of ferrofluids.

Magnetofluidics is the investigation of the behavior of magnetic fluids in the presence of a magnetic field. The response of the magnetic fluids depends on the properties of the magnetic fluid, which in turn, depends on the size of the magnetic particles. Based on the magnetic particle size, magnetic fluids are categorized into three regimes (**Figure 1.1**). The FHD regime is the focus of this research work.

1.2.3 Magnetic Fields

Different types of magnetic fields can be utilized for the control of magnetic fluids (**Figure 1.1**). There are two main types of magnetic fields, viz., time-dependent and static. Rotating magnetic fields are time varying magnetic fields, with time-dependent changes in the magnitude of the field strength and/or direction of the magnetic fields.

Static magnetic fields are magnetic fields which are constant in time. There are three types of static magnetic fields, viz., uniform, non-uniform, and hybrid (uniform magnetic field superimposed on nonuniform magnetic fields).

1.2.4 Micro-Magnetofluidics and Droplet Micro-Magnetofluidics

Micro-magnetofluidics (MMF) is a combination of microfluidics and magnetofluidics and deals with studies of magnetic fluid behavior at the micron scale under an applied magnetic

field. MMF can be further categorized to (i) continuous flow MMF (CMMF) and (ii) droplet MMF (DMMF). The focus of the current studies is DMMF for LoC applications.

DMMF based studies involve two immiscible phases (e.g., oil and water), one of the two phases is magnetic. The two phases are termed as the continuous phase (CP) and dispersed phase (DP). The DP is the phase corresponding to the droplets, which is surrounded by the continuous phase. Water based magnetic fluids as the DP and oils of different viscosities as the CP were utilized for most of the investigations.

1.3 Janus Structures

Janus indicates two dissimilar materials combined in one particle. The word *Janus* is of Roman origin and represent a god with two faces, one looking to the future and the other to the past. Janus structures can be in the form of fibers, particles or sheets, and at macro, micro or nano- scale. A range of dissimilar properties can be combined in a single *Janus* particle as separate phases, e.g., hydrophobic+ hydrophilic; organic+ inorganic, magnetic+ photonic; magnetic+ plasmonic and magnetic+ fluorescent. Both phases are available at the same time, opening new possibilities for multifunctional capability. Specifically, the use of the magnetic phase in Janus can lead to structures which can be used for the control of the particle motion, position, orientation and configuration of the other phase. Such practical functionality can be useful for sorting, aligning, and sensing.

Though there is a vast range of applications offered by Janus structures, practical control of the two phases inside the Janus particles is a challenge. DMMF offers control of the magnetic properties of the synthesized Janus particles and wireless, contact-free control of the Janus particles. Hence, the focus of our studies is the development of magnetic based *Janus* structures by micro-magnetofluidic techniques. The microfluidic platform offers precise control for the fabrication of such structures, along with the opportunity to study the rich physics of *Janus* droplets and *Janus* particles. These studies will be helpful to optimize the properties *Janus* structures e.g., for biomedical applications, such as protein detection.

1.4 Problem Statement

A combination of the magnetic droplets and magnetic fields can be utilized for wireless, programmable droplet control. However, conventional use of the nonuniform magnetic field limits the offered advantages. This research work addresses those problems by the combining uniform, hybrid magnetic fields with droplet microfluidic platform (Table 1.1).

Table 1.1: Problem statement.

Sr	Problems/Limitations	Solution
1.	Wireless, programmable droplet control	⇒ Magnetic Droplets + Magnetic Fields
2.	Limitations of nonuniform magnetic fields: (i) limited magnetic force (ii) limited programmable operations, (iii) large coils or permanent magnets, (iv) complex fabrication techniques	⇒ Use of uniform magnetic fields (H_0) (i) Large field strengths, high susceptibility ferrofluids (ii) Controlled by current (iii) Applied externally
3.	Droplet Merging by Magnetic fields	⇒ Experiments + Simulations of droplet dynamics in H_0 at different flow rates, fluidic properties, magnetic properties
4.	Janus Synthesis After Mixing in Droplets	⇒ Hybrid fields to mix and separate
5.	Synthesis of Ready to use Janus Particles	⇒ Droplet micro-magnetofluidics for wash-less, surfactant-free, rapid synthesis

1.4.1 Choice of Uniform Magnetic Fields

The rotating and nonuniform magnetic fields on a LoC platform for the control of magnetic fluids or magnetic droplets is limited due to: (i) magnetic force is limited on a microfluidic platform due to smaller FD size, hence control at lower magnetic fields is difficult and challenging; (ii) requirement of a large magnetic field gradient; (iii) lack of programmable operations with permanent magnets; (iv) the large size of the coils or permanent magnets utilized for magnetic field gradient generation makes it difficult to integrate with a LoC platform; (v) magnetic field gradients are sensitive to location and hence difficult to control; (vi) complex and expensive techniques are necessary for the fabrication of micro-coils on

a LoC platform. These factors limit the advantages of using magnetic fields for droplet manipulation in microfluidics.

A static uniform magnetic field which overcome all the problems/limitations, described above. Hence, static uniform magnetic field is advantageous for LoC applications offering programmable, wireless droplet control and merging.

1.4.2 Janus Synthesis in Hybrid Magnetic Fields

It is difficult to perform Janus particle synthesis after mixing inside droplets by conventional Janus particle synthesis techniques. Droplet micro-magnetofluidics when integrated with hybrid magnetic fields ($H_o + H_{no}$) offers mixing and magnetic separation inside droplets useful for a surfactant free, wash-less, and rapid Janus particle synthesis.

1.5 Aims and Objectives of Research

Magnetofluidics offers remote, contact-free control on a microfluidic platform. Magnetofluidics coupled with droplet microfluidics offers a novel approach for droplet control and manipulation by an applied magnetic field. State-of-art techniques can be developed by the integration of droplet microfluidics with magnetofluidics for LoC applications and fabrication of symmetric and asymmetric multifunctional magnetic Janus structures.

1.5.1 Aim

To develop and investigate micro-magnetofluidic techniques for the control of magnetic droplets for LoC (lab-on-a-chip) applications and the fabrication of magnetic based Janus structures.

1.5.2 Objectives

The objectives of the research work are as listed below.

- To develop micro-magnetofluidic techniques for the experimental and numerical investigations of micro-magnetofluidic spreading (MFS) on a LoC platform in an

applied uniform magnetic field for a range of magnetic fields, flow rates, and flow rate ratios.

- To develop droplet micro-magnetofluidic techniques to investigate magnetic droplet behavior in uniform magnetic fields.
- To experimentally study the effect of magnetic field, flow rates, flow rate ratio, and the viscosity on the size, shape, and motion of the ferrofluid droplets.
- To develop a DMMF based numerical model for the simulation of magnetic droplet behavior in uniform magnetic fields.
- To investigate and demonstrate magnetic droplet for merging and mixing under the influence of uniform magnetic fields for a range of interfacial tensions, flow rates, flow rate ratios, and droplet size.
- To investigate the effect of oil-ferrofluid tension on droplet merging and velocity during droplet merging.
- To develop a DMMF based LoC platform to synthesize bifunctional *Janus* structures and investigate the role of flow rates, flow rate ratios, viscosity and surface tension on the synthesis.
- To perform LoC fabrication and characterization of *Janus* particles with magnetic + fluorescent functional phases for protein detection.
- To perform modeling, simulation of *Janus* droplet formation in a magnetic field and to compare these results with experimental findings.

1.6 Hypothesis

Magnetic droplets are versatile tools for a range of LoC applications. The control of magnetic droplets on LoC platform is a challenge.

The integration of uniform and hybrid magnetic fields with DMMF offers a novel approach to address the challenge of controlling magnetic droplets in a microfluidic platform under uniform magnetic fields. Development of such DMMF techniques is useful for LoC applications requiring wireless, remote and programmable magnetic control of magnetic based structures.

Successful implementation of this hypothesis will contribute to LoC droplet control and the fabrication of novel *Janus* structures useful for proteins detection.

1.7 Significance and Novelty

Droplet microfluidics has recently gained importance for a range of LoC applications and the synthesis of symmetrical-asymmetrical particles. Specifically, novel *Janus* structures have been synthesized. Applications of *Janus* structures are summarized in the next chapter.

The literature survey (Chapter 2) delineates the impact of these studies.

References

- [1] Z. Zhu and C. Y. J. Yang, "Hydrogel Droplet Microfluidics for High-Throughput Single Molecule/Cell Analysis," *Accounts of Chemical Research*, vol. 50, pp. 22-31, 2017.
- [2] G. Y. Huang, M. X. Li, Q. Z. Yang, Y. H. Li, H. Liu, H. Yang, and F. Xu, "Magnetically Actuated Droplet Manipulation and Its Potential Biomedical Applications," *Acs Applied Materials & Interfaces*, vol. 9, pp. 1155-66, 2017.
- [3] L. Armbrrecht and P. S. Dittrich, "Recent Advances in the Analysis of Single Cells," *Analytical Chemistry*, vol. 89, pp. 2-21, 2017.
- [4] R. J. Yang, H. H. Hou, Y. N. Wang, and L. M. Fu, "Micro-magnetofluidics in microfluidic systems: A review," *Sensors and Actuators B-Chemical*, vol. 224, pp. 1-15, 2016.
- [5] G. Katsikis, J. S. Cybulski, and M. Prakash, "Synchronous universal droplet logic and control," *Nature Physics*, vol. 11, pp. 588-96, 2015.
- [6] B. Bruijns, A. van Asten, R. Tiggelaar, and H. Gardeniers, "Microfluidic Devices for Forensic DNA Analysis: A Review," *Biosensors-Basel*, vol. 6, 2016.
- [7] S. W. Jung, C. H. Choi, C. S. Lee, and H. M. Yi, "Integrated fabrication-conjugation methods for polymeric and hybrid microparticles for programmable drug delivery and biosensing applications," *Biotechnology Journal*, vol. 11, pp. 1561-71, 2016.
- [8] A. Reece, B. Z. Xia, Z. L. Jiang, B. Noren, R. McBride, and J. Oakey, "Microfluidic techniques for high throughput single cell analysis," *Current Opinion in Biotechnology*, vol. 40, pp. 90-96, 2016.
- [9] M. Maeki, H. Yamaguchi, M. Tokeshi, and M. Miyazaki, "Microfluidic Approaches for Protein Crystal Structure Analysis," *Analytical Sciences*, vol. 32, pp. 3-9, 2016.
- [10] L. Cao, X. Y. Cui, J. Hu, Z. D. Li, J. R. Choi, Q. Z. Yang, M. Lin, H. L. Ying, and F. Xu, "Advances in digital polymerase chain reaction (dPCR) and its emerging biomedical applications," *Biosensors & Bioelectronics*, vol. 90, pp. 459-74, 2017.
- [11] N. Shembekar, C. Chaipan, R. Utharala, and C. A. Merten, "Droplet-based microfluidics in drug discovery, transcriptomics and high-throughput molecular genetics," *Lab Chip*, vol. 16, pp. 1314-31, 2016.
- [12] T. Nisisako, "Recent advances in microfluidic production of Janus droplets and particles," *Current Opinion in Colloid & Interface Science*, vol. 25, pp. 1-12, 2016.
- [13] G. C. Le Goff, R. L. Srinivas, W. A. Hill, and P. S. Doyle, "Hydrogel microparticles for biosensing," *European Polymer Journal*, vol. 72, pp. 386-412, 2015.
- [14] Y. J. Zhao, L. R. Shang, Y. Cheng, and Z. Z. Gu, "Spherical Colloidal Photonic Crystals," *Accounts of Chemical Research*, vol. 47, pp. 3632-42, 2014.

- [15] W. Wang, M. J. Zhang, and L. Y. Chu, "Functional Polymeric Microparticles Engineered from Controllable Microfluidic Emulsions," *Accounts of Chemical Research*, vol. 47, pp. 373-84, 2014.
- [16] S. Lone and I. W. Cheong, "Fabrication of polymeric Janus particles by droplet microfluidics," *RSC Advances*, vol. 4, p. 13322, 2014.
- [17] X. G. Wang, E. Bukusoglu, and N. L. Abbott, "A Practical Guide to the Preparation of Liquid Crystal-Templated Microparticles," *Chemistry of Materials*, vol. 29, pp. 53-61, 2017.
- [18] Z. Z. Chong, S. H. Tan, A. M. Ganan-Calvo, S. B. Tor, N. H. Loh, and N. T. Nguyen, "Active droplet generation in microfluidics," *Lab on a Chip*, vol. 16, pp. 35-58, 2016.

Chapter 2

Literature Review

Droplet micro-magnetofluidics is the integration of magnetically response ferrofluid droplets, microfluidics, and magnetic fields. DMMF studies start with the MMF investigations of magnetic fluids and magnetic droplets in the presence of applied magnetic fields. Droplet merging was demonstrated on a LoC platform. Finally, the DMMF technique was used to fabricate magnetic Janus particles. The literature review is structured accordingly, describing basics of magnetofluidics, literature on micro-magnetofluidic investigations, droplet micro-magnetofluidic studies, LoC droplet merging, and Janus fabrication by droplet microfluidics.

2.1 Introduction

The basics of FHD are summarized in the second section, including background and the previous literature. The third section highlights the basics of magnetofluidics and the transition to micro-magnetofluidics. The fourth section summarizes magnetic droplet investigations on a microfluidic platform. The fifth section highlights the range of LoC applications offered by DMMF, specifically for droplet merging and mixing. Development of various *Janus* particles are summarized in the sixth section, including crucial findings and reviews published in the literature. The impact, novelty, and applications of our work are also delineated.

2.2 Historical Background of Magnetofluidics

Many discoveries and inventions turn out to be ahead of their time, e.g., the invention of magnetic fluids and the development of magnetofluidics on a microfluidics platform. Papell (patent filed 1963, accepted 1965) performed the synthesis of low viscosity colloidal magnetic particles several decades ago for use in the space shuttle[1]. A ferrofluid is a colloidal state of monodomain ferromagnetic nanoparticles in a nonmagnetic carrier fluid. Brownian motion prevents sedimentation due to gravity or an applied magnetic field, surface passivation facilitating repulsion, prevents agglomeration. Ferrofluids are characterized by their "liquid magnet" like behavior, exhibiting a strong, attractive force in the presence of an applied magnetic field.

Rosenweig et al. (1964) developed a continuum model to explain phenomena relevant to ferrofluids. It can be applied to the microfluidic platform, giving rise to the field of micro-magnetofluidics.

2.2.1 Highlights in the development of magnetofluidics:

The following points summarize crucial findings and research articles for magnetofluidics.

- The invention of low viscosity ferrofluid by Papell et al.[1]

- The term ferrohydrodynamics (FHD) coined by Rosensweig et al.[2] for magnetically polarizable fluids, encompassing both ferromagnetic and ferroelectric case. This continuum treatment forms the basis of theoretical understanding of various FHD cases. They elaborated the phenomena and applications of ferrofluids[3-5].
- A theoretical model to elucidate FHD surface phenomena, described by Gogosov et al. [6]. They assumed several phases and components, with its own localized temperature and localized magnetization.
- Stiles et al. studied FHD Couette-Taylor instability [7]. They elaborated the influence of an axial magnetic field on magnetic fluids in a cylindrical Couette flow with reference to critical wavelength and critical Taylor number.
- Shliomis et al[8] summarized the impact and theoretical development of FHD.
- A paper in *Science* by Rosensweig et al. elucidated the novel magnetofluidic phenomena of "negative viscosity" observed in magnetic fluids [9]. The reduction of total viscosity is due to the conversion of a part of the alternating magnetic field energy to the fluid's kinetic energy.

2.2.2 Transition to micro-magnetofluidics (MMF)

Integration of magnetofluidics with microfluidics leads to MMF (**Figure 2.1**). This interdisciplinary approach of MMF enhances the range of applications offered by magnetofluidics. Hence, there is an increasing trend of publications and citations in micro-magnetofluidics (**Figure 2.1**).

Figure 2.1: Publication reports and analysis. ref.: Web of Science database, dated 21-Dec-2016 (data collected with quoted search string). **(a-c)** “(magnetic) AND (microfluidics)”: (a) publications in each year, (b) citations in each year, and (c) research areas. **(d-f)** “(synthesis) AND (microfluidics)”: (d) publications in each year, (e) citations in each year, (f) research areas.

2.3 Micro-magnetofluidics (MMF)

Magnetic fluids are suspensions of nanoparticles dispersed in a carrier medium. Incorporating magnetic particles and magnetic fluids with microfluidics provides a range of applications with programmable manipulation capabilities on a μF and DMF platform [10-21] (**Figure 2.2**).

Significant improvement in LoC applications was reported by micro-magnetofluidic techniques, due to the wireless, remote, contact-free and programmable control capabilities of magnetofluidics. The significance of magnetofluidics can be further enhanced by droplet microfluidics. Use of magnetic droplets offers a several-fold increase in the efficiency of multiplexed operations (**Figure 2.2**).

Figure 2.2: Significance of micromagnetofluidics (MMF) for a range of LoC applications. MMF is a combination of magnetic fluids and magnetic fields on a microfluidic platform.

2.3.1 Basics of Micro-Magnetofluidics

A range of studies has been performed in the field of MMF, which are summarized in the following table. Evidently, these studies start from magnetofluidic investigations in the FHD regime.

Table 2.1: MMF studies form the basis of the DMMF. **H** indicates the type of applied magnetic field, e.g., $\mathbf{H}_0$ (uniform magnetic field) and $\mathbf{H}_{no}$ (Non-uniform magnetic field). Notation: ferrohydrodynamics (FHD), **pm**- $\mathbf{H}_{no}$ ($\mathbf{H}_{no}$ applied by a permanent magnet), **rot**- $\mathbf{H}_{no}$ (rotating magnetic field $\mathbf{H}_{no}$), magnetohydrodynamics (MHD), giant magnetoresistance (GMR).

Sr	Group [ref.]	Focus (Year)	Research Highlights	H
1	Rosensweig RE [2]	FHD (1964)	Continuum approach, treating ferrofluids as “liquid magnets”	$\mathbf{H}_0$
2	McTague JP [22]	Magnetoviscosity (1969)	Investigated magnetoviscous effects by experiments and modeling	$\mathbf{H}_0$

3	Shliomis MI [23]	Magnetoviscosity (1971)	magnetoviscous effect attributed to hindrance of particle rotations	H_o
4	Rosensweig RE [5]	Review: FHD (1988)	Continuum based FHD for the role of stress, instability, and two-phase flow; useful for rotary shaft sealing, drop on demand printing.	H_o ; H_{no}
5	Rosensweig RE [9]	Negative Viscosity (1996)	Reported reduction in viscosity (<i>‘negative magnetoviscosity’</i>) caused by alternating field energy adding to fluid kinetic energy.	$rot-H_{no}$
6	Gijs MAM [10]	Review: MMF (2004)	Microfluidic magnetic control, applications in MRI contrast agents, heterogeneous assays, labels for detection.	$pm-H_{no}$
7	Pamme N [11]	Review: MMF (2006)	Integration of magnetism and microfluidics for MMF applications biochemical reactions, microstirrer, trapping, immunoassays	H_o ; H_{no}
8	Kang S [12]	Review on μ Mixing (2010)	Microfluidic mixers classified by designs, types of forces (mechanical, electrical, magnetic), mixing index, and residence time.	H_o ; H_{no}
9	Nguyen NT [24]	MMF mixing (2012)	H_o to control rapid mixing in a microfluidic chamber, attributed to the instability due to a mismatch in magnetization at the interface	H_o
10	Nguyen NT [14]	Review: MMF (2012)	Physical and engineering aspects of MMF interactions, elaborated by constituent equations and dimensionless numbers	H_o ; H_{no}
11	Ramanujan RV [25, 26] (this work)	MMF spreading (2015)	MMF spreading; numerical modeling without a correction factor, attributed to cross-sectional convection due to magnetic forces. Spreading control by H_o and flow rates	H_o
12	Ramanujan RV [27]	MMF instability (2016)	New MMF instabilities in H_o : transient, permanent and spreading, controlled by magnetic fields, flow rates, and flow rate ratios.	H_o
13	Ramanujan RV [28]	MMF Bacteria Trapping (2016)	LoC trapping of nonmagnetic bacteria in by H_o , attributed to the gradient in magnetic susceptibility at a microfluidic island.	H_o

The foundation of MMF is magnetofluidics, was studied by Rosensweig in 1964 [2]. In the FHD regime, Rosensweig’s theoretical treatment using the continuum approach is still applicable for MMF and DMF. This approach treats the ferrofluid as a continuous magnetic liquid, which results in the addition of a magnetic volume force term in the Navier-Stokes (NS) equation[2]. Depending on the focus of study, magnetic field strength, type of magnetic fields and properties of ferrofluid, contributions from magnetoviscous effects and drag force also need to be incorporated in the NS equation. McTague [22] and Shliomis [23] investigated magnetoviscous effects in ferrofluid flow under applied uniform magnetic fields. Under various flow conditions, device geometries and properties of ferrofluids, interesting instabilities [5] and ‘negative viscosity’ [9] in ferrofluids were reported by Rosensweig. Even though rich in physics, and visually appealing, the complete potential of magnetofluidic investigations was realized only after integration with microfluidics.

Reviews by Gijs [10] and Pamme [11] elaborated the scope of MMF. Gijs described the manipulation of magnetic particles on microfluidic platforms, applications in separation, immuno-assay, MRI, and drug delivery. The physics of magnetic particles was elaborated by Pamme et al. [11] using the theory of magnetism and the force on magnetic particles exposed to inhomogeneous magnetic fields. Applications of ferrofluids and/or magnetic particles for manipulation, trapping, magnetohydrodynamic(MHD) pumping (with ferrofluids), micromixing (with magnetic particles), self-assembly and patterning, superconducting quantum interference device (SQUID), sensing were summarized. Magnetic manipulation for contact-less control of matter inside microchannels and the need for on-chip integration for pre-treatment, isolation, separation, detection and labeling were discussed for biomedical applications. The effect of magnetic fields on bio-reactions, cells, self-assembly of particles in 3D structures, crystal growth and film growth was also elucidated.

Micromixing is a critical requirement to realize various applications on microfluidics platform, however comparing the micromixing of two microfluidic systems is challenging. Kang et al [12] provided metrics to compare the micromixing capabilities of microfluidic systems, based on designs and mechanical, electrical, and magnetic driving forces. Micromixing performance was evaluated by mixing index, residence time, chaotic advection, Poincare section, and the Lyapunov exponent. They concluded that droplet-based LoC mixing is preferred due to a broad range of application, and low fabrication cost.

A critical review by Nguyn et al. [14] elaborated physical and engineering aspects of MMF interactions. FHD, magnetorheology, and magnetophoresis regimes were described. Investigation of magnetofluidic mixing in a microfluidic chamber controlled by H_0 was performed [24]. Rapid mixing was attributed to magnetic instability due to a mismatch in magnetization at the interface. Mixing efficiency was analyzed at different flow rates and viscosities[24]. Applications of magnetic fluids for magnetic actuation, pumping, magnetic sorting, micromixing were summarized [14, 29-36].

The Ramanujan research group established various experimental and simulation techniques as well as demonstrated potential applications. This research group mainly focused on the use of the uniform magnetic field for MMF investigations and applications.

Experimental study of MMF spreading was performed and simulated numerically without a correction factor [25, 26]. Spreading was attributed to the cross-sectional convection caused by the magnetic forces. Spreading increases with increasing magnetic fields, decreasing flow rates, and increasing magnetic particle concentration. In their study on MMF instabilities [27] in uniform magnetic fields, they reported new types of instabilities viz., transient, permanent and spreading. The role of magnetic fields, flow rates, and flow rate ratios was determined. These instabilities induced by low magnetic fields are useful for wireless, remotely controlled mixing in practical microfluidic devices. A practical device application of MMF was reported [28] by LoC trapping of nonmagnetic bacteria in a ferrofluid by uniform magnetic fields. This trapping was attributed to the distortion of magnetic susceptibility near the diamagnetic island.

2.4 DMMF for LoC Applications

Extension of MMF studies to DMMF is challenging mainly due to specific requirements of the microfluidic chip design for droplet generation, incorporation of two immiscible phases, tuning of fluidic properties (viscosity, surface tension, flow rates, and flow rate ratios) to enable droplet generation, applying magnetic fields, imaging, and analysis. Depending on the focus of the studies, modifications of DMF studies performed in the literature is also required. Investigation of DMMF requires simulation techniques, which can be developed from an understanding of the physics, theory and its integration in modeling. The following subsections provide information on experiments, modeling, simulations, and applications of DMMF.

2.4.1 Basics of DMMF

Studies performed by various research groups, which forms the basis of DMMF, are summarized in **Table 2.2**.

Table 2.2: DMMF studies. **H** indicates type of applied magnetic field, e.g., **H_o** (uniform magnetic field), **H_{no}** (Non-uniform magnetic field) and scale indicates the length scale (L) utilized in the studies. Notation: ferrohydrodynamics (FHD), **pmag-H_{no}** (**H_{no}** applied by permanent magnet), **emag-H_{no}** (**H_{no}** applied by electromagnet), **rot-H_{no}** (rotating magnetic field **H_{no}**), Ferrofluid Droplet (FD).

S r	Group, Yr [ref.]	Focus	Research Highlights	H	Scale (L)
1	Bacri JC, 1982, [37, 38]	FD Instability	Droplet shape instability at a threshold magnetic field	H_o	~1 mm
2	Widom, 1999, [39]	FD Elongation	Droplet elongation in parallel plates along the field direction	H_o	~1 mm
3	Ismagilov, 2006, [16]	Review DMF for reactions	DMF for miniaturized reactions compared by criteria, applications, and miniaturizations.	Not used	~100 μ m
4	Whitesides, 2006, [40]	Scaling Law	Scaling law for droplet, bubble size in the squeezing regime ($Ca < 10^{-2}$).	Not used	~500 μ m
5	Haeberle, 2007, [41]	DMF for bioassays	DMF biochemical assays enables miniaturization, integration, and automation.	Not used	~300 μ m
6	Kumar, 2008, [18]	Review: DMF for synthesis	DMF for nanomaterials synthesis requires μ F device design, mechanism of nanoparticle formation, scaling of synthesis.	Not used	~300 μ m
7	Lee AP, 2008 [42]	Review: DMF	DMF designs for droplet generation, merging, mixing, sorting, and detection	Not used	~500 μ m
8	Baroud, 2010, [20]	Review: DMF	Examined the role of surfactants and dimensionless numbers on droplet formation, transport and merging	Not used	~400 μ m
9	Huck, 2010, [21]	DMF for biochemistry	Chemical, biological applications for isolation of species or reactions at extremely small volume consumption.	Not used	~400 μ m
10	Renardy, 2010, [43]	FD Deformation	biocompatible hydrophobic FD deformation by volume-of-fluid and numerical shape to deduce interfacial tension.	H_o	~1 mm
11	Edd, 2013, [44]	Review: Single Cell Analysis	Metrics of single cell analysis: cell encapsulation, droplet sensing, actuation, trapping, sorting, labeling, and indexing.	Not used	~500 μ m
12	Kokalj, 2015, [45]	Review bioassay	State-of-the-art of device integration, commercialization, and biological applications	pmag-H_{no}	≤ 1 mm
13	Pit AM, 2015, [46]	DMF control	Active: mechanical, electrophoresis, magnetic, acoustics, optical. Passive methods: drag force, viscous force, geometric structures.	H_{no}	≤ 1 mm
14	Prakash, 2015, [47]	FD logic operations	Algorithmic operations by magnetic and hydrodynamic interactions for mesoscale material processing.	rot-H_{no}	~1 mm
15	Di Carlo, 2016, [48]	FD Formation	Pumpless magnetically driven FD generation, rate control by position of magnet and size control by channel geometry.	pmag-H_{no}	~500 μ m

Various research groups contributed to the development of DMF. Those developments are summarized in the following, encompassing the design of the DMF chips, a method of analysis, droplet behavior, functions of DMF devices and applications. The Baroud [20] research group in their review of droplet microfluidics examined factors affecting droplet

formation, transport, and merging. Droplet motion in the absence of lateral walls or in the presence of obstacles was discussed. Chemical and biological applications were reviewed by the Huck research group[21]. They showed the importance of functionalities offered by a droplet microfluidic platform, i.e. isolation of species or reactions, monodispersity, extremely small volume consumption for analysis, hence the suitability for quantitative studies and high-throughput experiments. They pointed out the possibility of integrating NMR spectroscopy for droplet characterization. Droplet microfluidics for nanomaterials synthesis was reviewed by the Kumar research group [18]. The need for investigations of the design of the microfluidic device, mechanism of nanoparticle formation, scaling of the fabrication process, the material of construction and control were highlighted. The Haeberle [41] research group reviewed microfluidic approaches for biochemical assays to facilitate miniaturization, integration, and automation. Various droplet microfluidic operations were discussed to realize the Lab-on-chip concept, concluding with the need for cost-effective technologies. Song [16] reviewed reactions inside the droplets. They discussed the criteria, applications, and outlook of miniaturization of chemical reactions by droplet microfluidics. They pointed out the challenges with device fabrication and fluidics control.

Magnetofluidic aspects of FD have been reported. The research group of Bacri[37, 38] performed experimental investigations of FD in uniform magnetic fields. Ferrofluid droplet instability was studied by droplet shape evolution; the ellipsoid shape becomes unstable for a threshold magnetic field. After a critical magnetic field, the FD shape jumps to a more elongated shape. This threshold was attributed to a balance between magnetic energy and interfacial tension energy. Widom [39] et al. reported an experimental and theoretical study of FD behavior confined in parallel plates under a weak uniform magnetic field parallel to the plates. They observed FD elongation along the field direction to attain equilibrium shape. An applied uniform magnetic field leads to an equilibrium elongated shape of FD which was attributed to the balance between magnetic forces the surface tension force. They also observed logarithmically increasing FD elongation with aspect ratio, in contrast to the behavior of unconfined droplets. Investigations performed by Renardy research group [43] are useful for numerical simulations of FD shape. They reported an experimental and numerical investigation of biocompatible hydrophobic FD deformation

in uniform magnetic fields. They utilized a volume-of-fluid scheme for numerical studies and used it to deduce interfacial tension from an optimal fit with the experimental shape.

Potential applications of DMMF have been reported by various research groups. Kokalj et al. [45] in their critical review elaborated various applications of DMF based bioassays. They described the state-of-the-art of device integration, commercialization and challenges of DMF devices. The potential of integrated DMF platforms for DNA amplification, sequencing, genotyping, immunoassays, cell analysis was described in detail. Pit et al. [46] elaborated passive and active techniques for droplet manipulation on the DMF platform. Various active techniques were described, including pneumatic membrane, dielectrophoresis, potential wells, pre-charging, magnetic manipulations, surface acoustic waves, ultrasonic acoustophoresis, and optical methods. The Prakash research group [47] experimentally demonstrated algorithmic logical operations of FD by rotating magnetic fields. They utilized magnetic and hydrodynamic interactions to develop various logical operations useful for LoC operations and mesoscale material processing. Di Carlo [48] et al. reported an experimental pumpless technique for magnetically driven FD generation useful for LoC mixing. They utilized the position of the magnet to control FD generation and channel geometry to control FD size.

2.4.2 DMMF Investigations in Nonuniform Magnetic Fields (H_{no})

DMMF investigations performed for the case of non-uniform magnetic field H_{no} are elaborated in this subsection. **Table 2.3** summarize recent DMMF studies performed by H_{no} . The focus of these studies, type of nonuniform magnetic field used, magnetic field strength, device geometry, the length scale of performed studies, significant findings, applications, and possible improvement are described.

Table 2.3: Nonuniform magnetic field based DMMF studies. H_{no} is type of applied non-uniform magnetic field, e.g., H_{no} applied by permanent magnet (pmag), electromagnet (emag), magnetic tweezers (tmag). L is the length scale of droplet generation. Notation: super-hydrophobic (SHP). Data collected with search string “(magnetic field) AND (droplet microfluidics)”, from Web of Science database, dated 21-Dec-2016. Articles related to the present work were selected.

Sr	1 st Author (Year) ref.]	Focus	H_{no}	Device geometry (~L)	Findings	Applications	Limitations
1	Tan S (2010) [49]	FD control on DMF	pmag: 0.03 T	T-junction (50 μ m)	FD dynamics dependence on H_{no} strength & gradient	FD size control by H_{no}	Limited magnetic force
2	Misuk V (2013) [50]	cap- μ F droplet control	pmag: 0.3 T	T-junction (1 mm)	Continuous multicore droplet generation	Droplet control on cap- μ F	Limited to ionic liquids
3	Teste B (2013) [51]	Immune-agglutination Assays	tmag: 0.1 T	cap- μ F (600 μ m)	Low cost, multimodal, high throughput magnetic bead based assay	Biosensing @ 300 assays/h, 100 pM detection limit	Lower Sensitivity than ELISA.
4	Irajizad P (2015) [52]	Magnetic dispenser of FD	pmag: 0.15 T	Porous Membrane (300 μ m)	Magnetic dispenser for continuous FD generation in range 0.1-1000nL	Continuous MD generation	Large pmag size
5	Yan Q, (2015) [53]	FD LoC Control	emag: 0.1 T	Flow Focusing (200 μ m)	Magnetic field controlled FD generation and dynamics.	Wireless active droplet size control	Limited magnetic force
6	Yang Y (2015) [54]	Particle Synthesis	pmag: 0.1 T	cap- μ F Coflowing (200 μ m)	Janus synthesis by side-by-side capillaries, size: 500 μ m.	drug delivery	Large particle size with only flow control
7	Zhu T (2015) [55]	Magnetic DMF synthesis	pmag: 0.5 T	Flow Focusing (200 μ m)	H_{no} for polymer particle synthesis by shape, droplet assembly.	Shape tunable particle synthesis	Size tuning only by flow rates
8	Ahmadi A (2016) [56]	Macroscale MHD	pmag: 1.3 T	2D planar (10 mm)	MHD droplet transport, merging, mixing on 3D printed channel	Macroscale bi-oassays	Not compatible with LoC platform
9	Al-Kaidy H (2016) [57]	Macro-scale MD control	pmag	2D planar (1 mm)	Magnetic shell microreactor control by overlapping magnetic fields.	Laccase A activity measurements	Large droplet size, No dynamic control
10	Biswas S (2016) [58]	FD magnetic control	emag: (pull~ 15 kg)	Polymer Film, (1 mm)	MD control by magnetically controlled elastomeric film.	Biochemical reactions, PCR	Complex fabrication
11	Huang JP (2016) [59]	cap- μ F droplet Merging	pmag: 0.7 T	coflowing, (0.7 mm)	Paramagnetic ionic liquid droplet size control by H_{no}	Paramagnetic droplet merging cap- μ F platform	Large pmag size
12	Jo Y (2016) [60]	Single Cell DMF	rotating	T-junction (500 μ m)	Magnetophoretic sorting of droplets with cells	Single cell sorting	very sensitive to H_{no} gradient
13	Khaw MK (2016) [61]	Magnetic Liquid Marble	pmag: 0.35 T	2D planar (1 mm)	Magnetic actuation of floating liquid marbles & role of magnetic, frictional forces	Bioreactor for cell growth, tissue culture.	Limited to specific fluid combinations
14	Rigoni C (2016) [62]	FD Magneto-wetting	pmag: 1 T/cm	2D planar (1 mm)	FD wetting control by H_{no} , susceptibility	FD control on flat surfaces	No dynamic control

The control of FD by nonuniform magnetic fields can be categorized on the basis of length scale (macroscale and microscale), or device type (capillary microfluidics, continuous microfluidics or digital microfluidics). Though studies performed at the macroscale are limited by large droplet size, offering only a small window of applications, they are rich in physics and offers possibilities for integration with a microfluidic platform. Misuk et al.[50] investigated continuous multicore droplet generation and merging of organic and ionic droplets by magnetic fields. Ahmadi et al.[56] performed MHD droplet manipulation on a 3D printed SHP channel, and demonstrated applications for droplet transport, merging, and mixing. Biswas et al.[58] demonstrated MD control of an elastomeric film by magnetically controlled surface curvatures. Huang et al.[59] studied magnetic control of paramagnetic ionic liquid droplets. They investigated the role of magnetic and viscous forces by modeling droplet behavior in magnetic fields. Khaw et al.[61] demonstrated magnetic actuation of floating liquid marbles and investigated the role of magnetic and frictional forces.

The use of nonuniform magnetic fields on a microfluidic LoC platform is now described. Tan et al. [49] demonstrated FD size control using a permanent magnet. FD shape evolution was investigated with and without a magnetic field. FD dynamics were studied at various flow rates, magnetic field strengths, field gradients and ferrofluid magnetizations. Teste et al.[51] developed a low-cost method for multimodal, high throughput assay by combining magnetic bead, DMF, and magnetic tweezers. Their DMMF was able to perform 300 assays per hour with 100 pM detection limit. Irajizad et al.[52] demonstrated a pumpless magnetic dispenser for continuous FD generation. The FD generation was investigated by experiments and modeling. Droplet volume in the range of 0.1-1000nl can be used for magnetic based structures. Yan et al. [53] developed a magnetic field controlled FD generation system. They studied FD dynamics and generation by experiments and described the role of magnetic drag force. Yang et al.[54] developed an experimental method for Janus particle synthesis using side-by-side capillaries. They demonstrated particle synthesis in size range of 500 μm . Zhu et al.[55] demonstrated magnetic field assisted polymer particle synthesis with control of the shape and droplet assembly. Al-Kaidy et al.[57] showed magnetic shell microreactor control by overlapping magnetic fields. They investigated mass transport by simulation in magnetic fields. Jo et al.[60]

performed magnetophoretic cell sorting for sorting of droplets with cell from droplets which did not contain cells. Rigoni et al.[62] performed FD wetting studies in the magnetic field. They demonstrated control of magneto-wetting by applied magnetic fields and susceptibilities.

The above approach, of using nonuniform magnetic fields requires integration of permanent magnets or coils on a microfluidic platform, is challenging because of the (i) large size of magnets compared to the size of microchannels; (ii) low magnetic volume force; (iii) complex microcoil fabrication techniques on a microfluidic platform; (iv) heating due to on-chip microcoil use; (v) sensitivity of the gradient field to the position of the magnet; (vi) lack of programmable control due to use of magnets.

2.4.3 DMMF Investigations in Uniform Magnetic Fields (H_0)

Realizing LoC by uniform DMMF is challenging. DMMF based on *uniform magnetic fields* (DMMF@ H_0) is a potential candidate to overcome the challenges mentioned in the last subsection. In addition, DMMF@ H_0 offer wireless, programmable remote control capabilities, which enhances the range and applicability of LoC devices. However, the potential of DMMF@ H_0 has not been explored in detail.

This subsection describes DMMF investigations performed for the case of the uniform magnetic field H_0 . **Table 2.4** summarize recent DMMF studies performed by H_0 . Various aspects are elaborated in terms of their focus, type of uniform magnetic field, magnetic field strength, device geometry, length scale, findings, applications, and possible improvement.

Table 2.4: Uniform magnetic field based DMMF studies. H is the applied magnetic field. H_o is the uniform magnetic field. H_{no} is a nonuniform magnetic field applied by a permanent magnet (pmag), electromagnet (emag), magnetic tweezers (tmag). The scale indicates the length scale (L) of the performed studies. Notation: ferrohydrodynamics (FHD), Ferrofluid droplet (FD). Data collected with search string “uniform magnetic field AND magnetic droplet”, from Web of Science database, dated 21-Dec-2016. Articles related to present work then selected.

Sr	1 st Author (Year) [ref.]	Focus	H	Device geometry (~L)	Findings	Applications	Limitations
1	Arkhipenko (1979) [63]	FD shape in H_o	H_o : 500 mT	FD (4 mm)	Analytic explanation FD shape in H_o	FD shape control by H_o .	Role of viscosity not studied
2	Bacri JC (1982) [38]	FD elongation	H_o : 1 mT	2D planar (2 μ m)	Sudden FD elongation after a critical field. Surface tension calculations from FD shape.	FD shape control by H_o .	FD dynamics not studied
3	Banerjee S (1999) [39]	FD elongation	H_o : 5 mT	2D planar (130 μ m)	Experiments, modelling of confined FD shape in H_o .	FD shape control by H_o .	No description of FD dynamics.
4	Afkhami S (2010) [43]	FD shape	H_o : 80 mT	2D planar (2 mm)	Hydrophobic FD shape at different size. Interfacial tension from simulation.	magnetic control of wettability	Large FD size
5	Liu J (2011) [33]	FD dynamics	emag- H_o : 100 mT	Flow focusing (100 μ m)	FD formation in H_o , increasing droplet size with increasing H_o	Simulation of FD generation.	No description of droplet motion.
6	Zhu G (2011) [64]	FD shape	H_o : 350 mT	2D planar (1 mm)	FD Deformation on SHP surface, elaborating role of B_m	magnetic control of wettability	Large FD size
7	Shi D (2014) [65]	Falling FD shape	H_o : 10 mT	Air (1 mm)	VoF+LS numerical simulation of falling FD shape	Numerical modeling of FD.	Does not describe LoC behavior of FD
8	Lohrasebi (2015) [66]	FD dynamics	rot- H_{no} ; H_o : 100 mT	2D planar (100 μ m)	Spring-mass model for MD shape in H_o . H_{no} to control droplet motion.	MD shape, motion control	No experimental, studies
9	Chen C (2015) [67]	FD rotation	rot- H_o : 10 mT	2D planar (2 mm)	FD shape, self-alignment control by rot- H_o .	Mixing of two fluids.	Large droplet size
10	Ghaffari (2015) [68]	Falling FD shape	H_o : 10 mT	Air (5 mm)	Determined role of field, STC, FD size by VoF+ LS	Falling FD merging	Role of viscosity not studied
11	Wu Y (2015) [69]	FD breakup	H_{no} ; H_o : 100 mT	T-junction (600 μ m)	FD breakup: longer time in H_o ; asymmetrical breaking in H_{no} .	LoC control of FD breakup	Sensitive to magnetic field gradient.
12	Ody T (2016) [70]	FD control	emag- H_o 100 mT	2D planar (15 mm)	FD control by surface tension gradient and H_o .	biomedical and LoC applications	No multiplexing, simulation
13	Padovani JI (2016) [71]	FD actuation	emag+ pmag 50 mT	Flow Focusing (80 μ m)	Negative magnetophoresis for droplet actuation up to 300 μ m/s.	Water droplet displacement	Lacking dynamic study of FD.

Though the theoretical foundation of magnetofluidics was established by Rosensweig in 1964, experimental studies of magnetofluidic droplet behavior in uniform magnetic fields were reported much later. Arkhipenko et al. [63] performed an experimental investigation of FD shape in H_0 . The experimental shape was described using dimensionless numbers. Analytic modeling of FD shape in H_0 was performed. However, the role of other important factors was not investigated in detail, e.g., the effect of surface tension, viscosity, and FD size. Bacri et al.[38] investigated FD elongation in H_0 and reported a sudden increase in FD aspect ratio beyond a critical magnetic field. Surface tension was determined from the deformation of FD shape in the uniform magnetic field. However, they did not elaborate FD dynamics and the effect of different FD size not studied. Banerjee et al. [39] studied elongation of confined FD in H_0 for the case of ferrofluid water-surfactant emulsions. They performed experimental and theoretical modeling of the observed FD behavior. Their study was limited due to the lack of study of FD dynamic motion and hence was not useful for LoC based investigations.

Afkhami et al.[43] investigated deformation of falling hydrophobic FD in H_0 . They successfully determined interfacial tension from the simulations. However, investigation of dynamic motion of FD and FD behavior on microscale was not performed. Liu et al.[33] performed a numerical and experimental study of FD formation at the flow focusing geometry in H_0 and the effect of H_0 on FD size. The role of various competing forces (surface tension, magnetic, hydrodynamic) was described by numerical modeling of FD generation. However, their study did not describe the complete process of droplet generation in the uniform magnetic field and the deformation of FD caused by uniform magnetic field. Zhu et al.[64] investigated the deformation of FD on a superhydrophobic (SHP) surface in mineral oil (MOil). They studied the role of magnetic control of FD deformation and magnetic bond number (B_m) by experiments and simulation. Shi et al.[65] used the volume of fluid (VoF) method combined with the level-set (LS) numerical approach to simulate the FD shape in a diamagnetic medium. They investigated the role of magnetic field strength and surface tension on FD shape deformation. However, their method did not describe the behavior of FD on the microscale or on a LoC platform.

Lohrasebi et al. [66] reported simulation results of MD elongation along H_0 , simulated by the spring-mass model. They studied the effect of various factors, such as radius, susceptibility, and magnetic field strength. However, the reported method does not encompass droplet generation on the microfluidic platform and was not supported by experimental results. Chen et al.[67] studied visually appealing elongation and self-alignment of FD in rotational H_0 . The observation showed a dependence on the field strength, uniformity of the magnetic field and the number of FD. The studies were not performed on a LoC platform, and the dynamic motion of FD was not studied. Ghaffari et al.[68] reported detailed numerical simulation of FD shape and elongation using the VoF method combined with the LS method and determined the effects of the field, STC, and FD size.

Interesting experimental studies have been reported in the literature for LoC control of FD by uniform magnetic fields. Wu et al.[69] experimentally investigated FD breakup at T-junction in the presence of a magnetic field. Longer breakup time was observed in the presence of H_0 and asymmetrical breaking was observed in nonuniform magnetic fields. Ody et al. [70] studied FD control by surface tension gradient and H_0 . FD flow was studied on metal surfaces in the presence of H_0 . Padovani et al.[71] used the phenomena of negative magnetophoretic forces to induce droplet actuation in hybrid magnetic fields. They experimentally demonstrated droplet actuation with droplet velocities up to 300 $\mu\text{m/s}$.

2.5 DMMF for Janus Synthesis

A range of *Janus* particles containing photonic and/or magnetic phase have been studied. Some highly-cited articles are summarized in **Table 2.5**.

2.5.1 DMMF Reviews: Microfluidics for *Janus* Structures

Microfluidics offers control of fluidics at the micron scale. Control and flow can be used to develop multifunctional *Janus* structures with a well-defined geometry. This progress is summarized with the aid of some highly cited review articles.

Table 2.5: Published articles with high citations. Ref: Web of Science database, search string "(magnetic *Janus*) OR (photonic *Janus*)", dated 17 Oct 2016.

Sr	1 st Author; Journal, Year	Title	Cit'n	Janus System	Approach or Findings	Application
1	Dendukuri D [72]; Nat Materials, 2006	"Continuous-flow lithography for high-throughput microparticle synthesis"	357	PEG-DA: Rhodamine	One Phase Photolithography	Synthesis of controlled structures
2	Lattuada M [73]; J Am Chem So, 2007	"Preparation and controlled self-assembly of <i>Janus</i> magnetic nanoparticles"	104	PAA: Magnetite	pH sensitivity	Self-Assembly
3	Chen T [74]; J Am Chem So, 2008	"Controlled assembly of eccentrically encapsulated gold nanoparticles"		aunp: Polymer	Salt Induced Aggregation	Structure control
4	Kim S [75]; Adv Mater, 2008	"Optofluidic Synthesis of Electroresponsive Photonic <i>Janus</i> Balls with Isotropic Structural Colors"	81	Silica: Carbon Black	UV irradiation	Reflective colour pigments
5	Chen C [76]; Adv Mater, 2009	"Microfluidic Assembly of Magnetic Hydrogel Particles with Uniformly Anisotropic Structure"	79	mnp: Hydrogel	Double emulsion	Micro-mixing
6	Yuet K [77]; Langmuir, 2010	"Multifunctional Superparamagnetic <i>Janus</i> Particles"	77	PEG: mnp	Photo-polymerization	Tissue Eng, Self-assembly
7	Berger S [78]; Macromolecules, 2008	"Stimuli-Responsive Bicomponent Polymer <i>Janus</i> Particles by Grafting from/Grafting to Approaches"	73	Silica: Polymer	Stimuli Aggregation	pH sensing
8	Isojima T [79]; ACS Nano, 2008	"Reversible clustering of pH- and temperature-responsive <i>Janus</i> magnetic nanoparticles"	72	PAA: mnp(magnetite)	Self-assembly at low pH	Temp responsive <i>Janus</i>
9	Kim S [80]; J Am Chem So, 2008	"Optofluidic encapsulation of crystalline colloidal arrays into spherical membrane"	72	ETPTA: Crystalline Colloids	Optofluidic Polymerization	Electrophoresis
10	Smoukov, S [81]; Soft Matter, 2009	"Reconfigurable responsive structures assembled from magnetic <i>Janus</i> particles"	69	Iron: Polystyrene	Chain formation	Self-assembly
11	Zhao Y [82]; J Am Chem So, 2011	"Microfluidic Generation of Multifunctional Quantum Dot Barcode Particles"	64	QD: mnp in ETPTA	Double droplets polymerization	Barcodes
12	Rasch M [83]; Nano Lett, 2010	"Hydrophobic Gold Nanoparticle Self-Assembly with Phosphatidylcholine Lipid: Membrane-Loaded and <i>Janus</i> Vesicles"	60	aunp: Phosphatidylcholine	Dialysis process	Nano-particle vesicle
13	Yoshida M [84]; Biomaterials, 2007	"Short-term biocompatibility of biphasic nanocolloids with potential use as anisotropic imaging probes"	55	PAA: PAACAA	Electrified Jetting	Surface Marker for cells
14	Yan J [85]; Nature, 2012	"Linking synchronization to self-assembly using magnetic <i>Janus</i> colloids"	52	Ni film: Silica Particle	Self-assembled Oscillations	Structure control
15	Yin S [86]; Adv Materials, 2011	"Versatile Bifunctional Magnetic-Fluorescent Responsive <i>Janus</i> Supraballs Towards the Flexible Bead Display"	51	CdS QD: mnp (Fe ₃ O ₄)	Magneto responsive fluorescence	Bead display

Multifunctional micrometer-sized polymeric particles with nonspherical shapes have attracted significant research for fundamental studies on self-assembly, suspension rheology, they find applications from medical diagnostics to photonic devices. Dendukuri et al. [87] provide a good summary of microfluidic approaches used for the synthesis of such particles. They summarized three approaches, i.e., droplet, flow-lithography and particle assembly in microfluidic devices, classifying particles on the basis of morphology, chemical anisotropy, and internal structure. They also summarized emerging applications of such particles.

Baah et al. [88] (2014) reviewed the role of photocrosslinkable materials for particle synthesis by light-initiated cross-linking approach on a microfluidic platform. Three types of polymeric particles (spherical, spheroidal and *Janus*) were discussed, they highlighted the need for high particle throughput. They also summarized applications of these particles in drug delivery, security, abrasives, rheology, and catalysis. Kim et al. [89] (2014) reviewed various droplet microfluidic approaches used for the synthesis of novel microparticles, with added functionality. They summarized the key ideas, functionalities, applications of various microparticles (isotropic, engineered, hybrid) which can be synthesized on a microfluidic platform.

Song et al. [90] (2014) reviewed particle functionalization for the fabrication of structurally asymmetrical particles. They summarized approaches and important properties of nanometre-sized *Janus* particles for a range of applications e.g., electrochemistry, sensing, and catalysis. They pointed out the difficulty in scaling up the preparation of *Janus* particles and the urgent need for the development of effective synthetic protocols. Sun et al. [91] (2014) reviewed microfluidic-based fabrication methods of multifunctional particles used in analytical and bio-analytical chemistry. They pointed out the need for new strategies and approaches for fabrication of particles on a microfluidic platform. Also, there is a need to develop the fabrication of other promising materials, e.g., metals, carbon-based materials which possess superior performance for catalysis, sensing, and markers.

Yang et al.[92] (2012) reviewed various fabrication methods for *Janus* particles on a microfluidics platform for biomedical applications. They pointed out the opportunity to resolve various issues related to disease monitoring, high-throughput bioassays,

therapeutic delivery in biomedicine using these particles. New applications of drug design, synthesis, and analysis which can be integrated into a microfluidic platform were also discussed. Zhang et al. [93] (2013) explored materials innovation and processing of drug delivery systems using *Janus* particles.

2.5.2 Articles: Important Findings and Applications

This section summarizes important articles and approaches for the synthesis and applications of novel *Janus* particles.

Park et al.[94] demonstrated full-color spectrum, independent of viewing angle with photonic pigment prepared by the colloidal assembly on a microfluidic platform. The synthesized microcapsules contain a dense amorphous packing of core-shell colloidal particles. These findings provide a new approach for the design and synthesis of materials with structural colors on a microfluidic platform. Zhao et al. [95] reported a novel "barcode" system of multiple magneto-photonic crystals for the enhancement of throughput and quality of multiplexed assays, prepared on a microfluidic platform. They used ethoxylated trimethylolpropane triacrylate (ETPTA) as cores, which provides photonic properties to the particles. A shell of polyethylene glycol (PEG) hydrogel provides a bio-compatible part, which can be easily functionalized. With magnetic control, these barcodes possess the capability to increase the sensitivity and simplicity of bio-assays.

Appleyard et al.[96] demonstrated assays as well as scanning for protein detection, using *barcoded* micro-hydrogels synthesized on a microfluidic platform. Lee et al.[97] demonstrated the use of free-floating color tunable magnetic structures for the enhancement in bioassay throughput with a large number of distinct identification codes on assay particles. A novel readout method was employed on free-floating probe microparticles by color tunable magnetic particles providing multi-axis rotational control. They demonstrated its capabilities with a DNA hybridization assay. Sun et al [98] demonstrated colloidal crystal bead fabrication by a drop breaking technique for bioassays. The droplets were converted to photonic beads by heating at 60°C for 12 h. They pointed out the effect of evaporation rate on bead structure formation. They demonstrated applications of the photonic beads for bioassay with mouse IgG, rabbit IgG and human IgG.

Contact angles play an important role in *Janus* particle configurations and geometry as described by Hasinovic et al. [99] . These studies are helpful to tune the morphology and configurations of *Janus* particles.

Yu et al. [100] (2012) reported photonic crystal fabrication on a triphasic microfluidic platform. Their approach of directed self-assembly of polystyrene beads in a photopolymerizable polymer matrix provided a novel approach for *Janus* fabrication. Shape control was achieved by optimization of the interfacial energies of the three phases. They demonstrated applications of these *Janus* particles, with magnetic and photonic phases for bead display. Zhao et al. [82] demonstrated the fabrication of an active macroporous ferrogel scaffold, with 70 % volume reduction on command by applying a magnetic field, to deliver various biological agents. They proposed its application in tissue engineering and cell-based therapies to trigger and enhance release of various drugs. Ji et al. [101] reported quantum dot (QD) embedded optical barcode microparticles with precise ratios in a polymeric matrix to realize high throughput multiplexed biological assays. The fabrication was performed on a microfluidic platform.

Seiffert et al. [102] (2010) developed a microfluidic platform to separate polymer synthesis from particle gelation, useful for control of material properties and morphology of microgel particles. They used a microfluidic-based emulsification of poly(N-isopropylacrylamide) from pre-fabricated precursor polymers, leading to fabrication of sensitive polymer microgels. They demonstrated the fabrication of monodisperse, thermoresponsive microgel particles useful for hollow gel shells, anisotropic microgels or multi-layered microgel capsules.

2.5.3 Plasmonics for Biosensing

Table 2.6 summarizes applications of various plasmonic particles for biosensing. Many novel approaches and applications are compared. These novel approaches open new possibility for *Janus* systems consisting magnetic phase for manipulation.

Table 2.6: Published articles with highest citations. Ref: Web of Science database, search string "(Plasmonic) and (Biosensing)", Refined by: Document Types: (Article); dated 01 Dec 2016. #Fano resonance is a type of resonant scattering phenomenon that gives rise to an asymmetric lineshape.

Sr	Author; Journal, Year	Title	Cit'n	Plasmonic system	Approach or Findings	Application
1	Kabashin A [103]; Nat Mater 2009	"Plasmonic nanorod metamaterials for biosensing"	322	Plasmonic Metamaterial of Au Nanorod Assembly	Enhanced sensitivity to refractive index variations of the medium between the rods	Label-free plasmonic detection of streptavidin-biotin
2	Yu C [104]; Anal Chem, 2007	"Multiplex biosensor using gold nanorods"	268	Nanorods of Au with different aspect ratio	Distinct response of the plasmon spectra of the gold nanorods to binding events	Multiplex Biosensing
3	Chen J [105]; Nature, 2012	"Optical nano-imaging of gate-tunable graphene plasmons"	262	Graphene nanostructures	Launch and detection of propagating optical plasmons using near-field scattering microscopy	Active subwavelength-scale optics for biosensing
4	Liu N [106]; Nat Mater, 2008	"Nanoantenna-enhanced gas sensing in a single tailored nanofocus"	211	Pd nanoparticle - Au nanoantenna	Hydrogen detection by dark field microscopy	Plasmonic sensing
5	Yanik [107]; 2011	"Seeing protein monolayers with the naked eye through plasmonic Fano [#] resonances"	91	plasmonic nanohole devices	Direct detection of a single monolayer of biomolecules with the naked eye	Label -free sensing of proteins

Kabashin et al. [103] reported a plasmonic metamaterial of Au nanorod assembly with enhanced sensitivity used for the application of label-free plasmonic detection of streptavidin-biotin. The reported enhanced refractive index sensitivity was more than 30,000 nm per refractive index unit. Yu et al. [104] reported a multiplex biosensor of gold nanorods fabricated by seed-mediated growth. They used gold nanorods with different aspect ratio for distinct plasmon responses. Multiplex detection was demonstrated for three targets viz. goat anti-human IgG1 Fab, rabbit antimouse IgG1 Fab, rabbit anti-sheep IgG (H+L).

Chen et al. [105] reported a graphene nanostructure for a field tunable resonant plasmonic cavity with real-time images of plasmon fields. This strongly enhanced light-matter interaction, enabling the development of optoelectronic devices for biosensing. Liu et al. [106] demonstrated enhanced hydrogen gas sensing by a gold nanoantenna and Pd nanoparticle. Pd nanoparticles were placed near the tip of the gold nanoantenna, which detects changes in optical resonance due to the dielectric constant of the material in the nanofocus. Yanik et al. [107] experimentally demonstrated visible detection of monolayers

of protein by a plasmonic nanohole device, utilizing plasmonic *Fano* resonance (a Fano resonance is a type of resonant scattering phenomenon that gives rise to an asymmetric lineshape due to interference between a background and a resonant scattering). This label-free platform offers a sensitive detection technique for sensing biomolecules, useful for point-of-care devices.

2.6 Impact and Significance of DMMF

MMF is a versatile interdisciplinary field offers a range of LoC applications in diverse research areas (**Figure 2.1**). DMMF extends the scope of MMF by multiplexing, wireless, programmable, and remote-control capabilities (**Figure 2.2**). A range of LoC operations was reported in the literature using non-uniform magnetic fields, however certain affected by certain limitations (**Table 2.3**). Our DMMF investigations demonstrate magnetic droplet control on a LoC platform using uniform magnetic fields, which overcomes most of the limitations of nonuniform magnetic field based studies (**Table 2.3**). DMMF findings were utilized to develop a magnetically controlled *DMMF based* Janus particle synthesis system.

Table 2.5 summarize several applications of *Janus* particles for multiplex detection, biomarkers and self-assembly. Specifically, for *Janus* particles with magnetic and photonic phases, applications such as optical barcodes, which are used in bioassay, tagging of particles or cells, protein detection can be envisaged. Some of the work on *Janus* particles, summarized in **Table 2.5** and **Table 2.6**, have been published in prestigious journals (Nature [95, 97, 108] and others [82, 94, 98, 100, 102]); similarly, publications related to “plasmonics biosensing” have been published in Nature [103, 105, 106] and other journals[104, 107].

Hence, the developed CMMF and DMMF are significant both from fundamental understanding and application perspective to develop LoC devices and *Janus* structures of magnetic+photonic and/or plasmonic phases.

Table 2.7: Review articles with highest citations. Ref: Web of Science database, search string "(magnetic *Janus*) OR (photonic *Janus*)", dated 17 Oct 2016.

Sr	Author; Journal, Year	Title	Total Cit'n	Focus	Comments / Remarks
1	Pawar, A [109]; Macromol, 2010	"Fabrication, Assembly, and Application of Patchy Particles"	211	Modeling and Properties of <i>Janus</i> particles	Theoretical descriptions as predictive tools
2	Boker, A [110]; Soft Matter, 2007	"Self-assembly of nanoparticles (nP) at interfaces"	187	Assembly of nP at liquid-liquid interfaces	Entropy to control the spatial distribution of the nP
3	Li, F [111]; Angew Chem Int Edit, 2011	"Colloidal Assembly: The Road from Particles to Colloidal Molecules and Crystals"	176	Development in experiments and theory of colloids	Optical metamaterials fabrication by colloidal arrays
4	Salgueirino-Maceira, V et al. [112]; Adv Mater, 2007	"Increasing the complexity of magnetic core/shell structured nanocomposites for biological applications"	158	Magnetic core/shell structured nanoparticles	Need enhancement in individual and collective properties for low-cost production
5	Yang, S [113]; J Mater Chem, 2008	"Synthesis and assembly of structured colloidal particles"	154	state-of-the-art in colloidal assemblies and structures	Need enhancement in self-assembled mask to enhance applicability

References

- [1] P. S. Stephen, "Low viscosity magnetic fluid obtained by the colloidal suspension of magnetic particles," USA Patent 3,215,572, 1965.
- [2] J. L. Neuringer and R. E. Rosensweig, "Ferrohydrodynamics," *Physics of Fluids*, vol. 7, p. 1927, 1964.
- [3] R. E. Rosensweig, "Directions in Ferrohydrodynamics," *Journal of Applied Physics*, vol. 57, pp. 4259-64, 1985.
- [4] R. E. Rosensweig, "Magnetic fluids," *Annual review of fluid mechanics*, vol. 19, pp. 437-61, 1987.
- [5] R. E. Rosensweig, "An Introduction to Ferrohydrodynamics," *Chemical Engineering Communications*, vol. 67, pp. 1-18, 1988.
- [6] V. V. Gogosov, V. A. Naletova, N. G. Taktarov, C. Z. Binh, and G. A. Shaposhnikova, "Surface Phenomena in Ferrohydrodynamics," *Acta Astronautica*, vol. 7, pp. 489-97, 1980.
- [7] P. J. Stiles, M. Kagan, and J. B. Hubbard, "On The Couette-Taylor Instability in Ferrohydrodynamics," *Journal of Colloid and Interface Science*, vol. 120, pp. 430-38, 1987.
- [8] M. I. Shliomis, T. P. Lyubimova, and D. V. Lyubimov, "Ferrohydrodynamics - an Essay on the Progress of Ideas," *Chemical Engineering Communications*, vol. 67, pp. 275-90, 1988.
- [9] R. E. Rosensweig, "'Negative viscosity' in a magnetic fluid," *Science*, vol. 271, pp. 614-14, 1996.
- [10] M. A. M. Gijs, "Magnetic bead handling on-chip: new opportunities for analytical applications," *Microfluidics and Nanofluidics*, vol. 1, pp. 22-40, 2004.
- [11] N. Pamme, "Magnetism and microfluidics," *Lab Chip*, vol. 6, pp. 24-38, 2006.

- [12] Y. K. Suh and S. Kang, "A Review on Mixing in Microfluidics," *Micromachines*, vol. 1, pp. 82-111, 2010.
- [13] J. F. Zhang, "Lattice Boltzmann method for microfluidics: models and applications," *Microfluidics and Nanofluidics*, vol. 10, pp. 1-28, 2011.
- [14] N. T. Nguyen, "Micro-magnetofluidics: interactions between magnetism and fluid flow on the microscale," *Microfluidics and Nanofluidics*, vol. 12, pp. 1-16, 2012.
- [15] A. Karimi, S. Yazdi, and A. M. Ardekani, "Hydrodynamic mechanisms of cell and particle trapping in microfluidics," *Biomicrofluidics*, vol. 7, p. 21501, 2013.
- [16] H. Song, D. L. Chen, and R. F. Ismagilov, "Reactions in droplets in microfluidic channels," *Angew Chem Int Ed Engl*, vol. 45, pp. 7336-56, 2006.
- [17] S. Haeberle and R. Zengerle, "Microfluidic platforms for lab-on-a-chip applications," *Lab Chip*, vol. 7, pp. 1094-110, 2007.
- [18] Y. Song, J. Hormes, and C. S. Kumar, "Microfluidic synthesis of nanomaterials," *Small*, vol. 4, pp. 698-711, 2008.
- [19] S. Y. Teh, R. Lin, L. H. Hung, and A. P. Lee, "Droplet microfluidics," *Lab Chip*, vol. 8, pp. 198-220, 2008.
- [20] C. N. Baroud, F. Gallaire, and R. Danga, "Dynamics of microfluidic droplets," *Lab Chip*, vol. 10, pp. 2032-45, 2010.
- [21] A. B. Theberge, F. Courtois, Y. Schaerli, M. Fischlechner, C. Abell, F. Hollfelder, and W. T. Huck, "Microdroplets in microfluidics: an evolving platform for discoveries in chemistry and biology," *Angew Chem Int Ed Engl*, vol. 49, pp. 5846-68, 2010.
- [22] J. P. McTague, "Magnetoviscosity of magnetic colloids," *The Journal of Chemical Physics*, vol. 51, pp. 133-36, 1969.
- [23] M. Shliomis, "Effective viscosity of magnetic suspensions," *Soviet Journal of Experimental and Theoretical Physics*, vol. 34, p. 1291, 1972.
- [24] G. P. Zhu and N. T. Nguyen, "Rapid magnetofluidic mixing in a uniform magnetic field," *Lab on a Chip*, vol. 12, pp. 4772-80, 2012.
- [25] Z. M. Wang, V. B. Varma, H. M. Xia, Z. P. Wang, and R. V. Ramanujan, "Spreading of a ferrofluid core in three-stream micromixer channels," *Physics of Fluids*, vol. 27, p. 052004, 2015.
- [26] Z. M. Wang, V. B. Varma, Z. P. Wang, and R. V. Ramanujan, "Tuning magnetofluidic spreading in microchannels," *J Micromech Microeng*, vol. 25, p. 124001, 2015.
- [27] X. H. Wang, Z. M. Wang, V. B. Varma, Z. P. Wang, A. Ray, W. S. Lew, and R. V. Ramanujan, "Instability-Induced Mixing of Ferrofluids in Uniform Magnetic Fields," *IEEE Magnetics Letters*, vol. 7, pp. 1-5, 2016.
- [28] Z. M. Wang, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Trapping of Bacteria at Low Magnetic Fields," *Sci Rep*, vol. 6, p. 26945, 2016.
- [29] G. P. Zhu and N. T. Nguyen, "Magnetofluidic spreading in microchannels," *Microfluidics and Nanofluidics*, vol. 13, pp. 655-63, 2012.
- [30] G. P. Zhu and N. T. Nguyen, "Rapid magnetofluidic mixing in a uniform magnetic field," *Lab Chip*, vol. 12, pp. 4772-80, 2012.
- [31] N. T. Nguyen, S. H. Tan, and J. Liu, "Magnetically Mediated Formation of Ferrofluid Emulsion," *Proceedings of the Asme 9th International Conference on Nanochannels, Microchannels and Minichannels 2011, Vol 2*, pp. 405-12, 2012.
- [32] N. T. Nguyen, "Special issue on magnetic-based microfluidics," *Microfluidics and Nanofluidics*, vol. 13, pp. 527-28, 2012.
- [33] J. Liu, S. H. Tan, Y. F. Yap, M. Y. Ng, and N. T. Nguyen, "Numerical and experimental investigations of the formation process of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 11, pp. 177-87, 2011.

- [34] N. Nguyen, Y. Sun, Y. Kwok, H. Tan, and W. Loke, "Lab on a Chip Application in Life Sciences," in *The Third International Conference on the Development of Biomedical Engineering in Vietnam*, 2010, pp. 303-07.
- [35] A. Beyzavi and N. T. Nguyen, "One-dimensional actuation of a ferrofluid droplet by planar microcoils," *Journal of Physics D-Applied Physics*, vol. 42, 2009.
- [36] N. T. Nguyen, K. M. Ng, and X. Y. Huang, "Magnetic actuation for microfluidics based on ferrofluid droplets - art. no. 64140T," *Smart Structures, Devices, and Systems III*, vol. 6414, pp. T4140-T40, 2007.
- [37] J. C. Bacri and D. Salin, "Instability of Ferrofluid Magnetic Drops under Magnetic-Field," *Journal De Physique Lettres*, vol. 43, pp. L649-L54, 1982.
- [38] J. C. Bacri, D. Salin, and R. Massart, "Study of the Deformation of Ferrofluid Droplets in a Magnetic-Field," *Journal De Physique Lettres*, vol. 43, pp. L179-L84, 1982.
- [39] S. Banerjee, M. Fasnacht, S. Garoff, and M. Widom, "Elongation of confined ferrofluid droplets under applied fields," *Physical Review E*, vol. 60, p. 4272, 1999.
- [40] P. Garstecki, M. J. Fuerstman, H. A. Stone, and G. M. Whitesides, "Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up," *Lab on a Chip*, vol. 6, pp. 437-46, 2006.
- [41] S. Haeberle and R. Zengerle, "Microfluidic platforms for lab-on-a-chip applications," *Lab on a Chip*, vol. 7, pp. 1094-110, 2007.
- [42] S. Y. Teh, R. Lin, L. H. Hung, and A. P. Lee, "Droplet microfluidics," *Lab on a Chip*, vol. 8, pp. 198-220, 2008.
- [43] S. Afkhami, A. J. Tyler, Y. Renardy, M. Renardy, T. G. St Pierre, R. C. Woodward, and J. S. Riffle, "Deformation of a hydrophobic ferrofluid droplet suspended in a viscous medium under uniform magnetic fields," *Journal of Fluid Mechanics*, vol. 663, pp. 358-84, 2010.
- [44] T. P. Lagus and J. F. Edd, "A review of the theory, methods and recent applications of high-throughput single-cell droplet microfluidics," *Journal of Physics D-Applied Physics*, vol. 46, p. 21, 2013.
- [45] T. Kokalj, E. Perez-Ruiz, and J. Lammertyn, "Building bio-assays with magnetic particles on a digital microfluidic platform," *New biotechnology*, vol. 32, pp. 485-503, 2015.
- [46] A. M. Pit, M. H. Duits, and F. Mugele, "Droplet Manipulations in Two Phase Flow Microfluidics," *Micromachines*, vol. 6, pp. 1768-93, 2015.
- [47] G. Katsikis, J. S. Cybulski, and M. Prakash, "Synchronous universal droplet logic and control," *Nature Physics*, vol. 11, pp. 588-96, 2015.
- [48] S. Kahkeshani and D. Di Carlo, "Drop formation using ferrofluids driven magnetically in a step emulsification device," *Lab on a Chip*, vol. 16, pp. 2474-80, 2016.
- [49] S. H. Tan, N. T. Nguyen, L. Yobas, and T. G. Kang, "Formation and manipulation of ferrofluid droplets at a microfluidic T-junction," *Journal of Micromechanics and Microengineering*, vol. 20, p. 045004, 2010.
- [50] V. Misuk, A. Mai, K. Giannopoulos, F. Alobaid, B. Epple, and H. Loewe, "Micro magnetofluidics: droplet manipulation of double emulsions based on paramagnetic ionic liquids," *Lab on a Chip*, vol. 13, pp. 4542-48, 2013.
- [51] B. Teste, N. Jamond, D. Ferraro, J. L. Viovy, and L. Malaquin, "Selective handling of droplets in a microfluidic device using magnetic rails," *Microfluidics and Nanofluidics*, vol. 19, pp. 141-53, 2015.
- [52] P. Irajizad, N. Farokhnia, and H. Ghasemi, "Dispensing nano-pico droplets of ferrofluids," *Applied Physics Letters*, vol. 107, p. 191601, 2015.
- [53] Q. Yan, S. Xuan, X. Ruan, J. Wu, and X. Gong, "Magnetically controllable generation of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 19, pp. 1377-84, 2015.
- [54] Y.-T. Yang, J. Wei, X. Li, L.-J. Wu, Z.-Q. Chang, and C. A. Serra, "A side-by-side capillaries-based microfluidic system for synthesizing size-and morphology-controlled

- magnetic anisotropy janus beads," *Advanced Powder Technology*, vol. 26, pp. 156-62, 2015.
- [55] T. Zhu, R. Cheng, G. R. Sheppard, J. Locklin, and L. Mao, "Magnetic-Field-Assisted Fabrication and Manipulation of Nonspherical Polymer Particles in Ferrofluid-Based Droplet Microfluidics," *Langmuir*, vol. 31, pp. 8531-4, 2015.
- [56] A. Ahmadi, C. M. McDermid, and L. Markley, "Magnetohydrodynamic actuation of droplets for millimetric planar fluidic systems," *Applied Physics Letters*, vol. 108, p. 4, 2016.
- [57] H. Al-Kaidy and N. Tippkotter, "Superparamagnetic hydrophobic particles as shell material for digital microfluidic droplets and proof-of-principle reaction assessments with immobilized laccase," *Engineering in Life Sciences*, vol. 16, pp. 222-30, 2016.
- [58] S. Biswas, Y. Pomeau, and M. K. Chaudhury, "New Drop Fluidics Enabled by Magnetic-Field-Mediated Elastocapillary Transduction," *Langmuir*, vol. 32, pp. 6860-70, 2016.
- [59] J. P. Huang, X. H. Ge, J. H. Xu, and G. S. Luo, "Controlled formation and coalescence of paramagnetic ionic liquid droplets under magnetic field in coaxial microfluidic devices," *Chemical Engineering Science*, vol. 152, pp. 293-300, 2016.
- [60] Y. Jo, F. Shen, Y. K. Hahn, J.-H. Park, and J.-K. Park, "Magnetophoretic Sorting of Single Cell-Containing Microdroplets," *Micromachines*, vol. 7, p. 56, 2016.
- [61] M. K. Khaw, C. H. Ooi, F. Mohd-Yasin, R. Vadivelu, J. St John, and N.-T. Nguyen, "Digital microfluidics with a magnetically actuated floating liquid marble," *Lab on a Chip*, vol. 16, pp. 2211-18, 2016.
- [62] C. Rigoni, M. Pierno, G. Mistura, D. Talbot, R. Massart, J.-C. Bacri, and A. Abou-Hassan, "Static magnetowetting of ferrofluid drops," *Langmuir*, vol. 32, pp. 7639-46, 2016.
- [63] V. Arkhipenko, I. D. Barkov, and V. Bashtovoi, "Shape of a drop of magnetized fluid in a homogeneous magnetic field," *Magnitnaia Gidrodinamika*, vol. 14, pp. 131-34, 1979.
- [64] G. P. Zhu, N. T. Nguyen, R. V. Ramanujan, and X. Y. Huang, "Nonlinear Deformation of a Ferrofluid Droplet in a Uniform Magnetic Field," *Langmuir*, vol. 27, pp. 14834-41, 2011.
- [65] D. X. Shi, Q. C. Bi, and R. Q. Zhou, "Numerical Simulation of a Falling Ferrofluid Droplet in a Uniform Magnetic Field by the VOSET Method," *Numerical Heat Transfer Part a-Applications*, vol. 66, pp. 144-64, 2014.
- [66] A. Lohrasebi, "Computational Modeling of the Effect of an External Magnetic Field on a Ferromagnetic Fluid Droplet," *Journal of Superconductivity and Novel Magnetism*, vol. 28, pp. 777-80, 2015.
- [67] C. Y. Chen, H. C. Hsueh, S. Y. Wang, and Y. H. Li, "Self-assembly and novel planetary motion of ferrofluid drops in a rotational magnetic field," *Microfluidics and Nanofluidics*, vol. 18, pp. 795-806, 2015.
- [68] A. Ghaffari, S. H. Hashemabadi, and M. Bazmi, "CFD simulation of equilibrium shape and coalescence of ferrofluid droplets subjected to uniform magnetic field," *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, vol. 481, pp. 186-98, 2015.
- [69] Y. N. Wu, T. T. Fu, Y. G. Ma, and H. Z. Li, "Active control of ferrofluid droplet breakup dynamics in a microfluidic T-junction," *Microfluidics and Nanofluidics*, vol. 18, pp. 19-27, 2015.
- [70] T. J. Ody, M. Panth, A. D. Sommers, and K. F. Eid, "Controlling the motion of ferrofluid droplets using surface tension gradients and magnetoviscous pinning," *Langmuir*, 2016.
- [71] J. I. Padovani, S. S. Jeffrey, and R. T. Howe, "Electropermanent magnet actuation for droplet ferromicrofluidics," *Technology*, pp. 1-10, 2016.
- [72] D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, "Continuous-flow lithography for high-throughput microparticle synthesis," *Nat Mater*, vol. 5, pp. 365-9, 2006.

- [73] M. Lattuada and T. A. Hatton, "Preparation and controlled self-assembly of Janus magnetic nanoparticles," *J Am Chem Soc*, vol. 129, pp. 12878-89, 2007.
- [74] T. Chen, M. Yang, X. Wang, L. H. Tan, and H. Chen, "Controlled assembly of eccentrically encapsulated gold nanoparticles," *J Am Chem Soc*, vol. 130, pp. 11858-9, 2008.
- [75] S. H. Kim, S. J. Jeon, W. C. Jeong, H. S. Park, and S. M. Yang, "Optofluidic Synthesis of Electroresponsive Photonic Janus Balls with Isotropic Structural Colors," *Advanced Materials*, vol. 20, pp. 4129-+, 2008.
- [76] C.-H. Chen, A. R. Abate, D. Lee, E. M. Terentjev, and D. A. Weitz, "Microfluidic Assembly of Magnetic Hydrogel Particles with Uniformly Anisotropic Structure," *Advanced Materials*, vol. 21, pp. 3201-04, 2009.
- [77] K. P. Yuet, D. K. Hwang, R. Haghighi, and P. S. Doyle, "Multifunctional superparamagnetic Janus particles," *Langmuir*, vol. 26, pp. 4281-7, 2010.
- [78] S. Berger, A. Synytska, L. Ionov, K.-J. Eichhorn, and M. Stamm, "Stimuli-Responsive Bicomponent Polymer Janus Particles by "Grafting from"/"Grafting to" Approaches," *Macromolecules*, vol. 41, pp. 9669-76, 2008.
- [79] T. Isojima, M. Lattuada, J. B. Vander Sande, and T. A. Hatton, "Reversible clustering of pH- and temperature-responsive Janus magnetic nanoparticles," *ACS Nano*, vol. 2, pp. 1799-806, 2008.
- [80] S. H. Kim, S. J. Jeon, and S. M. Yang, "Optofluidic encapsulation of crystalline colloidal arrays into spherical membrane," *J Am Chem Soc*, vol. 130, pp. 6040-6, 2008.
- [81] S. K. Smoukov, S. Gangwal, M. Marquez, and O. D. Velev, "Reconfigurable responsive structures assembled from magnetic Janus particles," *Soft Matter*, vol. 5, pp. 1285-92, 2009.
- [82] Y. Zhao, H. C. Shum, H. Chen, L. L. Adams, Z. Gu, and D. A. Weitz, "Microfluidic generation of multifunctional quantum dot barcode particles," *J Am Chem Soc*, vol. 133, pp. 8790-3, 2011.
- [83] M. R. Rasch, E. Rossinyol, J. L. Hueso, B. W. Goodfellow, J. Arbiol, and B. A. Korgel, "Hydrophobic gold nanoparticle self-assembly with phosphatidylcholine lipid: membrane-loaded and janus vesicles," *Nano Lett*, vol. 10, pp. 3733-9, 2010.
- [84] M. Yoshida, K. H. Roh, and J. Lahann, "Short-term biocompatibility of biphasic nanocolloids with potential use as anisotropic imaging probes," *Biomaterials*, vol. 28, pp. 2446-56, 2007.
- [85] J. Yan, M. Bloom, S. C. Bae, E. Luijten, and S. Granick, "Linking synchronization to self-assembly using magnetic Janus colloids," *Nature*, vol. 491, pp. 578-81, 2012.
- [86] S. N. Yin, C. F. Wang, Z. Y. Yu, J. Wang, S. S. Liu, and S. Chen, "Versatile bifunctional magnetic-fluorescent responsive Janus supraballs towards the flexible bead display," *Adv Mater*, vol. 23, pp. 2915-9, 2011.
- [87] D. Dendukuri and P. S. Doyle, "The synthesis and assembly of polymeric microparticles using microfluidics," *Advanced Materials*, vol. 21, pp. 4071-86, 2009.
- [88] D. Baah and T. Floyd-Smith, "Microfluidics for particle synthesis from photocrosslinkable materials," *Microfluidics and Nanofluidics*, vol. 17, pp. 431-55, 2014.
- [89] J. H. Kim, T. Y. Jeon, T. M. Choi, T. S. Shim, S. H. Kim, and S. M. Yang, "Droplet microfluidics for producing functional microparticles," *Langmuir*, vol. 30, pp. 1473-88, 2014.
- [90] Y. Song and S. Chen, "Janus nanoparticles: preparation, characterization, and applications," *Chem Asian J*, vol. 9, pp. 418-30, 2014.
- [91] X. T. Sun, M. Liu, and Z. R. Xu, "Microfluidic fabrication of multifunctional particles and their analytical applications," *Talanta*, vol. 121, pp. 163-77, 2014.

- [92] S. Yang, F. Guo, B. Kiraly, X. Mao, M. Lu, K. W. Leong, and T. J. Huang, "Microfluidic synthesis of multifunctional Janus particles for biomedical applications," *Lab Chip*, vol. 12, pp. 2097-102, 2012.
- [93] Y. Zhang, H. F. Chan, and K. W. Leong, "Advanced materials and processing for drug delivery: the past and the future," *Adv Drug Deliv Rev*, vol. 65, pp. 104-20, 2013.
- [94] J. G. Park, S. H. Kim, S. Magkiriadou, T. M. Choi, Y. S. Kim, and V. N. Manoharan, "Full-spectrum photonic pigments with non-iridescent structural colors through colloidal assembly," *Angew Chem Int Ed Engl*, vol. 53, pp. 2899-903, 2014.
- [95] Y. J. Zhao, Z. Y. Xie, H. C. Gu, L. Jin, X. W. Zhao, B. P. Wang, and Z. Z. Gu, "Multifunctional photonic crystal barcodes from microfluidics," *Npg Asia Materials*, vol. 4, p. e25, 2012.
- [96] D. C. Appleyard, S. C. Chapin, R. L. Srinivas, and P. S. Doyle, "Bar-coded hydrogel microparticles for protein detection: synthesis, assay and scanning," *Nature Protocols*, vol. 6, pp. 1761-74, 2011.
- [97] H. Lee, J. Kim, H. Kim, J. Kim, and S. Kwon, "Colour-barcoded magnetic microparticles for multiplexed bioassays," *Nat Mater*, vol. 9, pp. 745-9, 2010.
- [98] C. Sun, X. W. Zhao, Y. J. Zhao, R. Zhu, and Z. Z. Gu, "Fabrication of colloidal crystal beads by a drop-breaking technique and their application as bioassays," *Small*, vol. 4, pp. 592-6, 2008.
- [99] H. Hasinovic, S. E. Friberg, I. Kovach, and J. Koetz, "Janus Emulsion Drops: Equilibrium Calculations," *Journal of Dispersion Science and Technology*, vol. 34, pp. 1683-89, 2013.
- [100] Z. Yu, C. F. Wang, L. Ling, L. Chen, and S. Chen, "Triphase microfluidic-directed self-assembly: anisotropic colloidal photonic crystal supraparticles and multicolor patterns made easy," *Angew Chem Int Ed Engl*, vol. 51, pp. 2375-8, 2012.
- [101] X. H. Ji, W. Cheng, F. Guo, W. Liu, S. S. Guo, Z. K. He, and X. Z. Zhao, "On-demand preparation of quantum dot-encoded microparticles using a droplet microfluidic system," *Lab Chip*, vol. 11, pp. 2561-8, 2011.
- [102] S. Seiffert and D. A. Weitz, "Microfluidic fabrication of smart microgels from macromolecular precursors," *Polymer*, vol. 51, pp. 5883-89, 2010.
- [103] A. V. Kabashin, P. Evans, S. Pastkovsky, W. Hendren, G. A. Wurtz, R. Atkinson, R. Pollard, V. A. Podolskiy, and A. V. Zayats, "Plasmonic nanorod metamaterials for biosensing," *Nat Mater*, vol. 8, pp. 867-71, 2009.
- [104] C. Yu and J. Irudayaraj, "Multiplex biosensor using gold nanorods," *Anal Chem*, vol. 79, pp. 572-9, 2007.
- [105] J. Chen, M. Badioli, P. Alonso-Gonzalez, S. Thongrattanasiri, F. Huth, J. Osmond, M. Spasenovic, A. Centeno, A. Pesquera, P. Godignon, A. Z. Elorza, N. Camara, F. J. Garcia de Abajo, R. Hillenbrand, and F. H. Koppens, "Optical nano-imaging of gate-tunable graphene plasmons," *Nature*, vol. 487, pp. 77-81, 2012.
- [106] N. Liu, M. L. Tang, M. Hentschel, H. Giessen, and A. P. Alivisatos, "Nanoantenna-enhanced gas sensing in a single tailored nanofocus," *Nat Mater*, vol. 10, pp. 631-6, 2011.
- [107] A. A. Yanik, A. E. Cetin, M. Huang, A. Artar, S. H. Mousavi, A. Khanikaev, J. H. Connor, G. Shvets, and H. Altug, "Seeing protein monolayers with naked eye through plasmonic Fano resonances," *Proc Natl Acad Sci U S A*, vol. 108, pp. 11784-9, 2011.
- [108] D. C. Appleyard, S. C. Chapin, R. L. Srinivas, and P. S. Doyle, "Bar-coded hydrogel microparticles for protein detection: synthesis, assay and scanning," *Nat Protoc*, vol. 6, pp. 1761-74, 2011.
- [109] A. B. Pawar and I. Kretschmar, "Fabrication, assembly, and application of patchy particles," *Macromol Rapid Commun*, vol. 31, pp. 150-68, 2010.
- [110] A. Boker, J. He, T. Emrick, and T. P. Russell, "Self-assembly of nanoparticles at interfaces," *Soft Matter*, vol. 3, pp. 1231-48, 2007.

- [111] F. Li, D. P. Josephson, and A. Stein, "Colloidal assembly: the road from particles to colloidal molecules and crystals," *Angew Chem Int Ed Engl*, vol. 50, pp. 360-88, 2011.
- [112] V. Salgueirino-Maceira and M. A. Correa-Duarte, "Increasing the complexity of magnetic core/shell structured nanocomposites for biological applications," *Advanced Materials*, vol. 19, pp. 4131-44, 2007.
- [113] S. M. Yang, S. H. Kim, J. M. Lim, and G. R. Yi, "Synthesis and assembly of structured colloidal particles," *Journal of Materials Chemistry*, vol. 18, pp. 2177-90, 2008.

Chapter 3

Experimental and Simulation Methodology

A micro-magnetofluidic setup for experimental CMMF and DMMF investigations was developed. Various microfluidic chips were also fabricated for MMF and DMMF studies. Specifically, for DMMF investigations a range of microfluidic chip designs were developed to facilitate LoC droplet generation by different geometries at different length scales. A DMMF numerical model was developed to analyze the dynamic behavior of FD in both uniform and hybrid magnetic fields. The model was used to simulate generation, deformation, and merging of FD and the results were compared with the experimental observations. These findings were utilized to develop a DMMF Janus fabrication system. The system is capable of selective LoC polymerization, resulting in the synthesis of magnetically controllable Janus particles for protein detection. The properties of these particles were evaluated by various characterization techniques. These findings are delineated in the following sections.

3.1 Introduction

As described in earlier chapters, MMF is the combination of magnetofluidics and microfluidics. The development of the experimental methods starts with the integration of microfluidics and magnetofluidics. A MMF setup was fabricated for both CMMF and DMMF investigations. The components of the MMF setup are described in the *Development of MMF Experimental Setup* section. The microfluidic chips and design of chip holder are described in Appendix A.

The dynamic behavior of FD on our LoC platform was also studied by DMMF simulations, using the same parameters used for the experiments. The effect of the uniform magnetic field on FD size, deformation, motion, and FD spacing was simulated. The model was extended to delineate the droplet generation, consequent deformation, and merging in uniform magnetic fields. The effect of variation in the velocity and surface tension were studied. These findings are described in the *Simulation Methodology* section.

After studying the FD behavior on a LoC platform, the findings were then utilized for DMMF Janus synthesis. The novel synthesis of magnetic Janus particles by selective polymerization on a LoC platform using wireless, programmable, remote control by the hybrid magnetic fields was studied. The section *Janus fabrication unit* describes the experimental setup.

The properties of the synthesized Janus particles were controlled by tuning flow rates, flow rate ratios, device geometry and magnetic fields. These results are summarized in the final section.

3.2 Development of MMF Experimental Setup

Analysis of micro-magnetofluidic phenomena on a LoC platform requires an electromagnet system to apply uniform magnetic fields, precision syringe pumps to deliver and maintain flow rates and a high-speed camera coupled with high magnification optics for imaging. CMMF and DMMF are differentiated by microfluidic chip design, flow regime, magnetic field strength, and materials. The experimental analysis also requires microfluidic measurements of viscosity and density. The dimensions of the microfluidic

chip are input parameters for numerical simulations. These details are mentioned in the following sections.

3.2.1 Components of Developed MMF Setup

The main components of our MMF experimental setup are described in this subsection, viz., (i) magnetic field unit, (ii) high-speed imaging system, (iii) precision syringe pumps, and (iv) precision syringes.

3.2.1.1 Magnetic Field Unit

A uniform magnetic field (uniformity of ± 0.1 % over an area of 5 mm width $\times$ 10 mm length) was generated by a DEXING Electromagnet System (DXSB-178), it consists of an electromagnet, a DC power supply and a chiller (**Figure 3.1**). It can produce a 2.5 T magnetic field for an air gap of 5 mm. The largest air gap is 178 mm, which is useful for a variety of microfluidic chip sizes. A Hall probe is mounted at the center of the pole piece, close to the pole face. The Hall probe measures and provides a feedback loop to maintain uniformity of the magnetic field. The feedback loop lowers the current if the measured magnetic field is higher than the set value. The electromagnet and power supply are water cooled by a chiller. The important specifications of the electromagnet system are summarized in **Table- E.1** (Appendix E).

Appendix E

3.2.1.2 High-Speed Imaging System

The high-speed imaging system consists of a Phantom Miro (Model: M320s) high-speed camera, high magnification optics (Navitar Zoom 6000) and an image acquisition system (**Figure 3.1**). It is capable of full resolution imaging (1980 x 1600) at the frame rate of 1300 fps. For imaging on a microfluidic platform, this high-speed camera is coupled with high magnification zoom optics. Key parameters of the high-speed imaging system are summarized in **Table- E.2** (Appendix E).

A calibration scale (Olympus Microscope Calibration Scale, 1 mm with least count of 10 μm) was used to calibrate and quantify the MMF measurements. The high magnification optics offers a resolution of $\pm 4 \mu\text{m}$ at maximum magnification.

The intensity illumination of is important for high-speed imaging since the high-speed imaging system requires higher illumination for larger frame rate. The metal halide light source showed flickering at high frame rates. The flickering was solved by using a ring LED source, mounted coaxially with the adapter of the high magnification optics.

A typical system operation utilize a frame rate of 200 fps at the resolution of 640×1200 pixels.

3.2.1.3 Precision Syringe Pumps

Various types of high precision syringe pumps were utilized to control flow rates in the microfluidic channels. Appropriate syringe pumps were utilized as per the required flow rates for the experimental investigations. For a flow rate range of 5 $\mu\text{l/h}$ to 10 ml/min, KDS Scientific Double Syringe Pump (Gemini 88) and a NEW ERA (NE-1010) syringe Pump were used. The NEW ERA syringe pump (NE-1002x) can produce flow rates down to few nl/h, and was used for flow rates less than 1 $\mu\text{l/h}$. For most of our investigations, flow rates in the range of 8 to 10 ml/h were utilized. The specifications of syringe pumps are summarized in **Table- E.3**(Appendix E).

3.2.1.4 Precision Syringes

Exmire and Hamilton gastight syringes with volumes of 0.25, 1, 2.5 and 5 ml were utilized. Low volume syringes (0.25 ml or 1 ml) were used for greater precision, to extend the life of syringe pumps and for high viscosity (viscosity $\geq 10 \text{ mPa.s}$) or/and low flow rates (flow rates $\leq 50 \mu\text{l/h}$).

Figure 3.1: Micro-Magnetofluidic SETUP. (a) MMF setup. (a1-a3) Components of MMF setup: (a1) electromagnet, (a2) high-speed camera, (a3) high precision syringe pump. (b) Mounted and aligned microfluidic chip. (c) The design of microfluidic chip and schematic of the setup. For

droplet generation, the continuous phase (CP) of oil and dispersed phase (DP) of ferrofluids enters through two inlets (I/L) and droplets are generated in the outlet (O/L).

3.2.2 Microfluidic Chip, Connectors and Tubing

All microfluidic chips were designed in AutoCAD software, designs are given in Appendix A. The standard template size of 25 mm × 75 mm, with six inlets and three outlets for chip design was used. A compatible chip holder (37.4 mm in width) was also fabricated using a nonmagnetic material (PMMA) to facilitate fluidic connection to the chip, chip alignment and mounting of the chip in the uniform magnetic field. Precision M6 size connectors were used to connect the tubing with the chip holder. Microfluidic chip dimensions are in the size range of 80 to 800 μm width and 30 to 600 μm height.

The microfluidic chips and the holder were fabricated in PMMA by a micro-milling technique. Thermal bonding was performed to seal the two parts of the chips (one flat with holes and the other with microchannels). To reduce channel deformation, low-temperature and low-pressure thermal bonding [1] (below the glass transition temperature of 105 °C for PMMA) was performed by Specac (model: Atlas 15T, with heating assembly). Parameters were optimized to a temperature of 95°C, duration of 16 min and a load of 50 kg.

Fluorinated ethylene propylene (**FEP**) tubing with an outer diameter of ~1.6 mm and inner diameter ranging from 250 μm to 1000 μm was used. A tubing size higher than the width of the test channel was selected to minimize flow resistance. Incheck valves were utilized for all inlets to prevent backflow in the tubing. All precision connectors and tubing were purchased from IDEX Health & Science, Singapore. Exmire (Science Team Services, Singapore) and Hamilton (Achema Pte Ltd, Singapore) precision glass syringes were used to feed fluids to the tubing and were connected via a Luer lock.

3.2.3 MMF Materials

Water based ferrofluid series EMG 507, EMG 607, EMG 707, and EMG 807 (Ferrotec, Singapore) were used in our experiments. Hexadecane (HDOil), light mineral oil (LMOil), and heavy mineral oil (HMOil) were used as the continuous phase to obtain a range of viscosities. Silicone oils (SOil) belonging to the KF-96 series with viscosity of 6, 100, 350, and 1000 cS were purchased from Shin-Etsu, Japan, supplier: Crane Wright Pte. Ltd, Singapore. Surfactants, such as Tween- 20, 80, 85 and Span -80, 85 were utilized to tune hydrophile-lipophile balance (HLB) values to facilitate droplet generation. Glycerol was utilized to tune the viscosity of the water. 1-Octanol and 1-Butanol were used for solvent extraction. Most of the chemicals are purchased from Sigma-Aldrich, Singapore, unless otherwise specified.

For Janus synthesis PEGDA (molecular weight, mw 575 and mw 700) were used as hydrophilic polymers. EO-TMPTA was used as a hydrophobic polymer. 2-Hydroxy-2-Methylpropiophenone (HMP) was used as a photoinitiator. Acrylic acid (AA) and ethylene glycol (EG) were used to tune the properties of the synthesized Janus particles. Ethyl(dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were used for protein immobilization experiments. EDC/NHS protocol was used for covalent immobilization of protein on Janus particles. Bovine serum albumin lyophilized powder (BSA) and FITC tagged BSA (FITC-BSA) were used as model proteins. Rhodamine-B and rhodamine-6G were used as dyes for visualization.

3.2.3.1 Properties of ferrofluids

Water based ferrofluids with a range of physical properties were utilized to investigate various CMMF and DMMF phenomena. The measured properties and the properties provided by the supplier are summarized in **Table 3.1**.

The viscosity of the ferrofluids was measured by a Brookfield DV3T Rheometer (CPA-40Z Spindle, for a 0.5 ml sample volume). The densities of the ferrofluids were determined using the micro-syringe based method reported by Burrough et al.[2].

Table 3.1: Properties of water-based ferrofluids (dispersed phase). Note: *Properties as provided from supplier data sheet (Ferrotec Singapore), #Measured properties. Viscosity measurements were performed by Brookfield DV3T rheometer using a CPA-40Z Spindle, for a 0.5 ml sample volume. Density measurements were performed by the technique reported by [2].

Sr.	Quantity	Not'n	EMG 507	EMG 607	EMG 707	EMG 807
1	#Viscosity (mPa·s)	η_{dp}	1.44±0.01	2.00±0.01	1.78±0.02	1.80±0.02
2	#Density at 25 °C (×10 ³ kg/m ³)	ρ_{dp}	1.118±0.004	1.104±0.006	1.110±0.002	1.105±0.003
3	*Saturation Magnetization (mT)	M_s	11	11	11	11
4	*Initial Magnetic Susceptibility (SI)	χ_o	1.63	1.63	1.51	1.88
5	*Magnetic Particle Conc. (% vol)	c_v	2	2	2	2
6	*Particle Diameter (nm)	d_p	10	10	10	10
7	*pH	pH	8-9	9-10	8-9	6-7
8	*Nature of Surfactant	-	Anionic	Cationic	Anionic	Anionic

3.2.3.2 Viscosity Measurements

The viscosity of all samples was determined using a Brookfield DV3T Rheometer (CPA-40Z Spindle, for a 0.5 ml sample volume).

Table 3.2: Viscosity measurements performed using a Brookfield DV3T rheometer (spindle= CPA-40Z, sample volume= 0.5 ml).

Sr.	Sample	Viscosity mPa.s	Error ±	Speed RPM	Torque %
1	DI water	1.01	0.01	250	90
2	DI Water+ 60% (v/v) Glycerol	13.42	0.03	20	96
3	EMG 807+1% (v/v) Tween 80	1.89	0.02	160	98
4	EO-TMPTA + 5% (v/v) HMP	63.50	0.50	5	91
5	Ethanol absolute, 99% Pure	1.22	0.01	250	99
6	Ethylene Glycol	18.57	0.04	15	91
7	Ferrofluid- APG 311 (Oil Based)	83.73	0.60	3.3	90
8	Ferrofluid -EMG 911 (Oil Based)	23.20	0.05	13	98
9	Ferrofluid-EMG 507 (Water based)	1.44	0.01	200	94

10	Ferrofluid-EMG 607 (Water based)	2.00	0.01	140	88
11	Ferrofluid-EMG 707 (Water based)	1.78	0.02	170	98
12	Ferrofluid-EMG 807 (Water based)	1.80	0.02	170	96
13	Heavy Mineral Oil	147.20	1.50	2	96
14	Hexadecane	3.31	0.02	90	97
15	Light Mineral Oil	25.78	0.01	11.2	94
16	1-Octanol	8.27	0.03	35	94
17	PEGDA 575+5% (v/v) HMP	44.90	0.50	8	90
18	[PEGDA 575+5% (v/v) HMP] +10% (v/v) EMG507	48.50	0.60	7	88
19	Silicone OIL- KF-96-100CS	105.40	1.10	2.9	99
20	Silicone OIL- KF-96-350CS	372.10	3.80	0.8	97
21	Silicone OIL- KF-96-6CS	5.95	0.06	50	97

3.3 DMMF Experimental Methodology

As mentioned earlier, the DMMF methodology uses different microfluidic chip designs compared to the CMMF for droplet generation and the immiscible flow system. For droplet generation, two geometries were utilized, viz., T-junction and flow focusing. The immiscible phases used are termed as continuous phase (CP) and dispersed phase (DP). The experimental strategy consists of droplet flow (x direction), magnetic field (y direction), and high-speed imaging (z direction).

High-speed imaging was performed in the range of 30 fps to 1000 fps at resolutions ranging from 640×1200 to 1920×600 pixels. Various packages *ImageJ*, *PCC*, *GIMP*, and *Inkscape* software were used for image acquisition, processing, analysis, and presentation.

Microfluidic chips were fabricated by the process described earlier. The droplet system i.e. oil in water (o/w) or water in oil (w/o), droplet size and the fluid must be chosen. A microfluidic device for droplet generation can then be designed[3-5]. A typical design consists of two inlets to feed the CP and the DP. Droplet size is determined by device geometry, device cross-section where the two phases meet, and flow rates. Our initial experiments also indicated a dependence of droplet generation on other parameters, e.g., surface modification, viscosity, and temperature. Surface tension was found to be the most important property in determining droplet generation. A combination of hydrophobicity

and hydrophilicity in the device, obtained by surface modification, can lead to more complex droplet systems, e.g., o/w/o or w/o/w.

Figure 3.2: Experimental setup used for DMMF investigations (a) schematic, (b) outline. Two immiscible fluids were used for droplet generation, viz., dispersed phase (DP) droplets of a water-based ferrofluid and a continuous phase (CP) of oil. A typical device geometry for droplet generation is shown for the case of a microfluidic T-junction. Droplet flow is along the x direction, uniform magnetic field along the y direction, and high-speed imaging was performed along the z direction. (not to scale).

The studies are focused on water based FD in oil, hence two designs were mainly utilized: (i) T-junction and (ii) Flow Focusing. To facilitate droplet generation, a design strategy was followed, using a smaller cross-section at the location the CP and the DP phases meet (at the position of T-junction or at flow focusing). A small amount of surfactant was added to facilitate droplet generation or to improve droplet uniformity, the specific surfactant used depends on the fluidic system, ferrofluid type, and type of DP. The addition of surfactant also enhanced the response of FD to the uniform magnetic field due to a reduction in the interfacial tension. Complex behavior of droplet alignment during motion, alignment of three or more droplets and very large deformation of moving droplets was also observed by the addition of surfactants.

3.4 Simulation Methodology: Development of DMMF Numerical Model

The Rosensweig [6] continuum model explains the behavior of ferrofluids in the FHD regime, this behavior is attributed to the "liquid magnet" like behavior of ferrofluids. The DMMF model was developed using a continuum approximation and simulated the response of FD in uniform magnetic fields. The model is delineated in **Figure 3.2**, with FD moving along the positive x direction, under the influence of an applied uniform magnetic field in the positive y direction. The dynamic behavior of the FD was investigated at various values of flow rates, magnetic fields, droplet magnetization, interfacial tension, and viscosities. This model is described in the following subsections.

3.4.1 Dimensionless Parameters

DMMF is a multiphysics phenomena, governed by magnetic, hydrodynamic, inertial forces, viscous, and the surface tension forces. Due to the microfluidic length scale, gravitational force is insignificant compared the previously described forces, hence it can be neglected. The role of surface tension force was only considered for the case of immiscible fluid systems.

Visualizing these forces from the relevant 3D (three dimension) equations is challenging, hence it is difficult to assess the dominant force directly. Dimensionless numbers are very

handy to understand the dominant force, use of dimensionless numbers helps in understanding, analysis, and interpretation of the process.

The following subsections summarize various dimensionless numbers useful for CMMF and DMMF[4].

3.4.1.1 Weber number (We)

Weber number is a measure of competition between the inertial force due to fluid flow and the surface tension force due to the immiscible fluids [7]. The Weber number is proportional to the square of fluid velocity and inversely proportional to the surface tension (Eq. (3.1)).

$$We = \rho v^2 d_j / \sigma \quad (3.1)$$

where, v = velocity of fluid, d_j = jet diameter and σ = interfacial tension

The transition between jetting and dripping regimes can be categorized by We . At small We , the surface tension force dominates over the inertial forces, leading to the dripping regime of droplet formation. When $We > 1$, inertial forces dominate over surface tension force, leading to a jetting regime of droplet formation[7].

3.4.1.2 Capillary number

The Capillary number explains the dominance of viscous force over the surface tension force and is mathematically expressed by the following equation (Eq. (3.2)).

$$Ca = \eta_{cp} v_{cp} / \sigma \quad (3.2)$$

where, η_{cp} and v_{cp} are the viscosity and the velocity of the CP, respectively.

The Ca is proportional to viscosity, velocity of the continuous phase and inversely proportional to interfacial tension. Hence capillary number increases with flow rate and viscosity of the CP. The droplet size decreases with increasing capillary number [7].

Ca is the most important dimensionless number for DMF based studies. Ca was used to calculate the microfluidic chip dimensions for chip design, choice of flow rates (CP and DP), and the amount of surfactant needed to reduce the interfacial tension to sufficiently

low values. The squeezing regime of droplet generation is defined by $Ca \leq 10^{-2}$. The following simplified equation was used to estimate Ca (Eq. (3.3)),

$$Ca = \left(\frac{1}{3.6} \right) \frac{\eta_{cp} [mPa.s]}{\sigma [mN/m]} \cdot \frac{Q_{cp} [\mu l/h]}{w [\mu m] \cdot h [\mu m]} \quad (3.3)$$

Estimating Ca for typical experimental values, $Q_{cp}=500 \mu l/h$, $w=500 \mu m$, $h=100 \mu m$, $\sigma=10 mN/m$ and $\eta_{cp}=10 mPa.s$

$$Ca \sim 0.0028 \text{ i.e., } Ca < 10^{-2}.$$

Hence, our experiments and experimental designs are in the squeezing regime.

3.4.1.3 Magnetic bond number

The magnetic Bond number is important for resting droplets and is defined as the ratio of magnetic body force to interfacial tension[8], Eq. (3.4)

$$B_m = \mu_0 \chi_f V^{\frac{1}{3}} H^2 / (2\sigma) \quad (3.4)$$

where χ_f = susceptibility of ferrofluid, V = volume of ferrofluid droplet, H =magnetic field strength and σ = interfacial tension.

The magnetic bond number explains the deformation of the droplets due to an applied magnetic field strength for a given magnetic susceptibility and interfacial tension.

3.4.2 Equation of motion

The Rosensweig continuum model is the Navier-Stokes equation with an additional magnetic volume force term, $\mathbf{F}_m$. The equation of motion and for ferrofluid droplets under the influence of force $\mathbf{F}_m$ is given by Eq. (3.5) [6, 9]. Eq. (3.6) is the *continuity equation*.

$$\underbrace{\rho(\partial \mathbf{u} / \partial t) + \rho(\mathbf{u} \cdot \nabla) \mathbf{u}}_{F_I} = - \underbrace{\nabla p}_{F_p} + \underbrace{\nabla \cdot [\eta(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)]}_{F_\eta} - \underbrace{\sigma \kappa \delta_s \nabla \phi}_{F_\sigma} + \mathbf{F}_m \quad (3.5)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (3.6)$$

where, $\mathbf{F}_m$, $\mathbf{u}$, p , σ , and κ are the magnetic volume force, velocity, pressure, surface tension, and curvature, respectively. δ_s is the smoothed delta function (δ_s is zero everywhere except at the droplet interface). ϕ is the level set function[6, 9, 10]. $\mathbf{F}_I$, $\mathbf{F}_p$, $\mathbf{F}_\eta$ and $\mathbf{F}_\sigma$ are the inertial force per unit volume, net force due to pressure per unit volume, viscous force per unit volume and surface tension force per unit volume, respectively. $\mathbf{F}_I$ is the sum of two forces, viz., the force due to time-dependent acceleration (first term in Eq. (3.5)) and the convective force term (space-dependent, the second term in Eq. (3.5)). The droplet behavior is determined by the competition between these five force terms, viz., $\mathbf{F}_I$, $\mathbf{F}_p$, $\mathbf{F}_\eta$, $\mathbf{F}_\sigma$, and $\mathbf{F}_m$.

Weighted average interpolation was used to define the density ρ and viscosity η by the following equations (Eq. (3.7) and Eq. (3.8)),

$$\rho = \rho_{cp}(1-\mathcal{C}) + \rho_{dp} \mathcal{C} \quad (3.7)$$

$$\eta = \eta_{cp}(1-\mathcal{C}) + \eta_{dp} \mathcal{C} \quad (3.8)$$

where, $\mathcal{C}$ is the volume fraction of the ferrofluid ($\mathcal{C}=1$ represents the DP).

3.4.3 Ferrofluid magnetization

The ferrofluid magnetization follows a non-linear behavior at large magnetic fields, as described by the Langevin function. Hence, the FD magnetization at different applied magnetic field $\mathbf{H}$ was defined by the Langevin function $L(\gamma H)$ [6, 11] as (Eq. (3.9)),

$$M(H) = M_s L(\gamma H) = M_s \coth\left(\gamma H - \frac{1}{\gamma H}\right) \quad (3.9)$$

where, $\gamma=(3\chi_o / M_s)$, χ_o , and M_s denote the initial magnetic susceptibility, and saturation magnetization of the ferrofluid, respectively.

For an applied uniform magnetic field $\mathbf{H}$, the magnetic susceptibility takes the form[6, 11], (Eq. (3.10))

$$\chi_H = \frac{M_s}{H} L(\gamma H) = \frac{M_s}{H} \coth\left(\gamma H - \frac{1}{\gamma H}\right). \quad (3.10)$$

3.4.4 Magnetic volume force

The magnetic field was defined by Maxwell's equation for magnetostatics (Eq.(3.11) and Eq.(3.12)),

$$\nabla \cdot \mathbf{B} = 0. \quad (3.11)$$

$$\mathbf{B} = \mu \mathbf{H} = \mu_0 \mu_r \mathbf{H} = \mu_0 (1 + \chi_{ff}) \mathbf{H} = \mu_0 (\mathbf{H} + \mathbf{M}). \quad (3.12)$$

The ferrofluid permeability and relative permeability are defined by $\mu = \mu_0 \mu_r$ and $\mu_r = (1 + \chi_{ff})$, respectively.

The dynamic behavior of the FD in uniform magnetic fields $\mathbf{H}_0$ is due to the sum of two magnetic volume force terms: (i) $\mathbf{F}_{m1}$, which acts at the interface of FD and the CP. This force is responsible for FD deformation in the field $\mathbf{H}_0$. (ii) $\mathbf{F}_{m2}$, acting on the total volume of the FD and which determine FD motion.

3.4.4.1 Magnetic Volume Force $\mathbf{F}_{m1}$

The magnetic stress tensor formulation was utilized to determine the magnetic force $\mathbf{F}_{m1}$ [6]. The FD deformation in the uniform magnetic field is attributed to this force. The magnetic stress tensor for uniform magnetic $\mathbf{H}_0$ is defined by the following equation (Eq. (3.13)):

$$\boldsymbol{\tau}_m = -(1/2)\mu H_0^2 \mathbb{I} + \mu \mathbf{H}_0 \mathbf{H}_0 \quad (3.13)$$

where “ $\mathbb{I}$ ” is an identity matrix. The tensor $\boldsymbol{\tau}_m$, in indicial form is given by, (Eq.(3.14)).

$$\tau_{ij} = -(1/2)\mu H^2 \delta_{ij} + \mu H_i H_j. \quad (3.14)$$

The magnetic volume force responsible for FD deformation is given by (Eq.(3.15)),

$$\mathbf{F}_{m1} = \nabla \cdot \boldsymbol{\tau}_m = -(1/2)\mathbf{H}_0^2 \nabla \mu. \quad (3.15)$$

The smoothed delta function (δ_s) implies that the force acts only at FD-oil interface [12] (Eq. (3.16)).

$$\mathbf{F}_{m1} = -(1/2)\mathbf{H}_0^2 \mu_{har} \delta_s \nabla \phi \quad (3.16)$$

where the magnetic permeability of the medium is determined by the harmonic mean (μ_{har}) [13, 14], (Eq. (3.17)):

$$\frac{1}{\mu_{\text{har}}} = \frac{1 - \mathcal{C}}{\mu_{\text{cp}}} + \frac{\mathcal{C}}{\mu_{\text{dp}}} . \quad (3.17)$$

The permeability of the CP was taken as $\mu_{\text{cp}} = \mu_0$ and $\mu_{\text{dp}} = (1 + \chi_H)$. Evidently, magnetic permeability μ_{har} is the magnetic permeability of the ferrofluid inside the droplet (for $\mathcal{C}=1$) and outside it is the magnetic permeability of oil ($\mathcal{C}=0$), i.e. μ_0 .

3.4.4.2 Magnetic Volume Force $\mathbf{F}_{m2}$

The force $\mathbf{F}_{m2}$ acts on the total volume of the FD, it defines the motion of FD within the microchannel in the presence of a magnetic field $\mathbf{H}$ [15, 16]. This force determined by the total volume of the FD. The magnetic volume force $\mathbf{F}_{m2}$ is given by (Eq.(3.18)),

$$\mathbf{F}_{m2} = \mathcal{C}\chi_H (\mathbf{B} \cdot \nabla \mathbf{B}) / \mu_o . \quad (3.18)$$

As defined earlier, $\mathcal{C}$ is ferrofluid volume fraction and χ_H is the FD susceptibility.

3.4.5 Drag Force

The drag force on a droplet with a radius r_d is given by (Eq.(3.19)) [17],

$$\mathcal{F}_{dd} = 6\pi r_d v_{\text{cp}} \eta_{\text{cp}} \frac{1 + 2\eta_{\text{cp}}/3\eta_{\text{dp}}}{1 + \eta_{\text{cp}}/\eta_{\text{dp}}} \quad (3.19)$$

where η_{cp} , η_{dp} , and v_{cp} are the CP viscosity, DP viscosity, and CP velocity, respectively. For the hard sphere approximation ($\eta_{\text{dp}} \gg \eta_{\text{cp}}$), equation (11) can be expressed as $\mathcal{F}_{dd} = 6\pi r_d v_{\text{cp}} \eta_{\text{cp}}$. For the air bubble approximation ($\eta_{\text{cp}} \gg \eta_{\text{dp}}$) this leads to $\mathcal{F}_{dd} = 4\pi r_d v_{\text{cp}} \eta_{\text{cp}}$. The viscosity of the continuous phase is comparable ($\eta_{\text{cp}} \approx \eta_{\text{dp}}$) to that of the dispersed phase. Hence, for this intermediate regime Eq. (3.19) can be expressed as (Eq.(3.20)),

$$\mathcal{F}_{dd} = 5\pi r_d v_{\text{cp}} \eta_{\text{cp}} . \quad (3.20)$$

3.4.6 Numerical Methodology

COMSOL Multiphysics software was used to perform the numerical simulations. A level set laminar *two-phase* flow method was selected in the *fluid dynamics module* for the simulations. *no currents method* in AC/DC module was used to simulate the uniform magnetic field $\mathbf{H}_0$. Extra-fine meshing was used for the whole geometry. Triangular elements of size $\sim 0.2 \mu\text{m}$ were used for microchannel meshing and $\sim 10 \mu\text{m}$ for magnetic field domain meshing.

Osher et al. [18] reported level set method for the numerical computations of the interface tracking for time varying objects on a fixed Cartesian grid without the need of parameterizations, hence found to be relatively easy to implement. The principle of the level set method [18, 19] is the use of a level set function $\phi(x,t)$ at the interface (x indicating the coordinate at time t). After the initialization of the function at time t_0 , the approximate value of the function $\phi(x,t)$ over small time step is determined by the numerical methodology. Hence, the propagation of the interface with time can be tracked by the level set function $\phi(x,t)$. Osher et al. originally defined a level set function $\phi(x,t)$ as the signed distance function, $\phi=0$ at the interface, positive ($\phi>0$) for one side of the interface, and negative ($\phi<0$) for the other side of the interface.

Olsson et al.[20] proposed a conservative level set method, where $\phi = 0.5$ was defined at the interface, $\phi = 0$ defined for the continuous phase (outside the droplet), and $\phi = 1$ was defined for the dispersed phase (inside the droplet). We have used this modified level set method.

Our DMMF numerical model was utilized to simulate the complete process: (i) FD generation (ii) FD deformation in a uniform magnetic field (iii) magnetically induced merging on LoC. The role of other factors such as deformation and consequent merging was also determined using our simulation methodology. The FD deformation was used to quantitatively validate the model (**Figure 3.3**). More complex simulations are then performed, as elaborated in the following chapters.

A DMMF platform magnetically controlled by hybrid magnetic fields $\mathbf{H}_0 + \mathbf{H}_{no}$ was developed for LoC synthesis of Janus particles. The main components of the system are (i) Janus droplet generation unit, (ii) magnetic control unit, (iii) polymerization unit, (iv) high-precision stage, and (v) collection unit. The above components are described in the following subsections.

LoC fabrication of Janus particles is challenging due to the state-of-the-art requirements for the microscale control of position and rate of polymerization. The development phase of the system utilized various approaches for LoC polymerization. UV polymerization experiments were performed with a large UV light source offering a larger area of

polymerization. At the low intensity of this light source, droplets did not polymerize. On the other hand, at high-intensity polymerization occurs in all inlets, leading to blockage and permanent failure of the microfluidic device. At high flow rates, the intensity was insufficient to polymerize droplets.

Integration of magnetic control for Janus fabrication is another challenge, requiring control of fluidics, magnetic fields, and LoC polymerization. Magnetic Janus droplets (MJD) exhibit very low transmittance, hence polymerization of such droplets is difficult.

3.5.2 Janus droplet generation unit

Two platforms were developed for Janus droplet generation, i.e., LoC based flow focusing design and capillary microfluidic-based micro flow focusing. Both designs offer certain advantages. LoC platform offers good control of the droplet phases, but limited range of droplet size. If the blockage occurs due to stray polymerization, the device has to be replaced with a new one.

The capillary-based design was found to be cheap, simple, and flexible offering a broad range of flow rates (hence a broad range of droplet sizes), compatible with the polymers used. Flow rates up to 10 ml/h can be utilized for droplet generation and particle fabrication. This device was found to be handy in the case of blockage since it can be recovered by changing the tubing.

Different photopolymers were used, such as Polyethylene glycol diacrylate (PEGDA) and ethoxylate trimethylolpropane triacrylate (EO-TMPTA) with 2-Hydroxy-2-Methylpropiophenone (HMP) as the photoinitiator. For most of our experiments, Janus droplets containing PEGDA+ 5 to 13 % (v/v) HMP were used for magnetic and non-magnetic phases, respectively.

3.5.3 Magnetic Control Unit

Two Nd-Fe-B permanent magnets with dimensions 20 mm×10mm×10mm (length× width× height) were utilized. By tuning the distance between two magnets, hybrid magnetic fields can be created. A magnetic field H_0 of 440 mT was generated, at 1 cm distance between two magnets.

Figure 3.4: The Experimental Setup for DMMF Janus Synthesis. The main components of the system are (i) Janus droplet generation unit, (ii) magnetic control unit, (iii) polymerization unit, (iv) high-precision stage, and (v) collection unit.

3.5.4 LoC Polymerization Unit

The polymerization unit offers LoC polymerization of the Janus droplets. The unit was developed by integrating a UV LED with a 20X objective. A high-intensity UV-LED of 4 mm beam width was utilized as the UV light source (Agiltron UV LED, Model: SUVA-011111021) with the following configuration **Table- E.4** (Appendix E).

3.5.5 High-Precision Stage

Controlling the location of polymerization is challenging. This issue was solved by integrating a microscope with a high precision control stage with the setup. The stage offers high precision control along x and y directions. The UV light incident along the z direction was focused by using the z-adjustment in the microscope. Hence, this setup offers precise, 3D control of polymerization point.

3.5.6 Collection Unit.

The fabricated particles were collected at the end in antistatic weighing dishes/pouring boats. The final unit resulted in a novel magnetically controlled LoC polymerization unit utilizing MJD for the fabrication of magnetic Janus particles. The challenge of polymerization of MJD at high flow rates (1 to 5 ml/h) was solved by using a slightly higher amount of PEGDA and HMP, which further enhanced the flow rate range of the synthesis by utilizing a high amount of HMP (up to 15%). The final system is capable of LoC synthesis of particles in the size range of 50 μm to 1000 μm up to flow rates of 10 ml/h (without ferrofluid) and 5 ml/h (with ferrofluid).

3.6 Characterization Techniques

Synthesized Janus particles were characterized by optical microscopy, VSM, FTIR, DSC and SEM techniques. Image processing and analysis was performed by Image J.

3.6.1 Optical Microscopy

Olympus IX73 was used for optical microscopy. Janus droplets and particles were imaged in bright field and fluorescence mode to characterize size and distribution. TRITC filter was used for fluorescent imaging.

3.6.2 ImageJ Analysis

ImageJ software was used for image processing and analysis of captured images. Videos in .cine format were imported by cine plugin in ImageJ and then converted to a virtual stack. A calibrated reference scale was entered in ImageJ to measure droplet or particle size.

3.6.3 Vibrating Sample Magnetometer (VSM) Measurement

VSM characterization technique was employed to determine magnetic moment of synthesized Janus particles. The VSM Lakeshore 7400 was used for the measurement. Measurements were performed at 20kOe magnetic field.

3.6.4 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy technique was employed to determine polymerization of particles by detecting changes in the functional groups. FTIR spectrum was recorded using a Perkin Elmer *Spectrum* spectrometer. Samples were prepared in a KBr matrix. Background, CO₂ and H₂O corrections were performed after recording the FTIR spectrum and intensities were normalized. The obtained spectrums were then compared with the literature to determine the presence of functional groups before polymerization and the absence of the specific bands after polymerization.

3.6.5 Differential Scanning Calorimetry (DSC)

The DSC characterization technique was employed to determine glass transition temperature (T_g) and crystallization temperature of synthesized particles. Measurements were performed on a DSC TA instrument (model: Q10). ~5 mg of sample was placed in the aluminum pan and used for DSC measurements. The DSC scan was operated in nitrogen ambient at a heating rate of 15°C/min from -80°C to 300°C.

3.6.6 Thermogravimetric Analysis (TGA)

TG analysis was performed (TA instruments, model: Q500) to determine the percentage of nanoparticles in the Janus microparticles. ~5 mg sample was taken in a platinum pan and

used for the measurement. Nitrogen ambient at a flow rate of 20 ml/min and a heating rate of 10°C/min from room temperature to 1000°C was employed.

3.6.7 Scanning Electron Microscopy (SEM)

The SEM characterization technique was employed to analyze the surface morphology of the Janus particles. The SEM instrument used was SEM JEOL 5410A operated at 5keV. Particles were placed on a carbon tape and then coated with gold at 1 min intervals as the particles were initially not conductive. The spot size and the working distance were set at 10 mm and 8-9 mm respectively.

3.6.8 Viscosity Measurements

The viscosity of all fluids was measured using a BROOKFIELD DV3T Rheometer. All measurements were performed at 25°C. The viscosity was determined with the Spindle CPA-40Z (viscosity measurement range from 0.1 cP to 3000 cP) for a sample volume of 0.5 ml. Torque was optimized ($\geq 85\%$) by adjusting the speed of the spindle. The results of the measurements are summarized in **Table 3.2**.

References

- [1] J. H. Li, D. Chen, and G. Chen, "Low-temperature thermal bonding of PMMA microfluidic chips," *Analytical Letters*, vol. 38, pp. 1127-36, 2005.
- [2] J. E. Burroughs and C. P. Goodrich, "Rapid method for determining densities of liquids using micro syringes," *Analytical Chemistry*, vol. 46, pp. 1614-15, 1974.
- [3] S. Y. Teh, R. Lin, L. H. Hung, and A. P. Lee, "Droplet microfluidics," *Lab on a Chip*, vol. 8, pp. 198-220, 2008.
- [4] C. N. Baroud, F. Gallaire, and R. Dangla, "Dynamics of microfluidic droplets," *Lab on a Chip*, vol. 10, pp. 2032-45, 2010.
- [5] A. B. Theberge, F. Courtois, Y. Schaerli, M. Fischlechner, C. Abell, F. Hollfelder, and W. T. Huck, "Microdroplets in microfluidics: an evolving platform for discoveries in chemistry and biology," *Angew Chem Int Ed Engl*, vol. 49, pp. 5846-68, 2010.
- [6] R. E. Rosensweig, *Ferrohydrodynamics*: Dover Publications, Inc. New York, 2013.
- [7] A. S. Utada, A. Fernandez-Nieves, H. A. Stone, and D. A. Weitz, "Dripping to jetting transitions in coflowing liquid streams," *Physical review letters*, vol. 99, p. 094502, 2007.
- [8] G.-P. Zhu, N.-T. Nguyen, R. V. Ramanujan, and X.-Y. Huang, "Nonlinear deformation of a ferrofluid droplet in a uniform magnetic field," *Langmuir*, vol. 27, pp. 14834-41, 2011.
- [9] A. Ghaffari, S. H. Hashemabadi, and M. Bazmi, "CFD simulation of equilibrium shape and coalescence of ferrofluid droplets subjected to uniform magnetic field," *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, vol. 481, pp. 186-98, 2015.

- [10] Z. M. Wang, V. B. Varma, H. M. Xia, Z. P. Wang, and R. V. Ramanujan, "Spreading of a ferrofluid core in three-stream micromixer channels," *Physics of Fluids*, vol. 27, p. 052004, 2015.
- [11] G. P. Zhu, N. T. Nguyen, R. V. Ramanujan, and X. Y. Huang, "Nonlinear Deformation of a Ferrofluid Droplet in a Uniform Magnetic Field," *Langmuir*, vol. 27, pp. 14834-41, 2011.
- [12] D. X. Shi, Q. C. Bi, and R. Q. Zhou, "Numerical Simulation of a Falling Ferrofluid Droplet in a Uniform Magnetic Field by the VOSET Method," *Numerical Heat Transfer Part a-Applications*, vol. 66, pp. 144-64, 2014.
- [13] S. Afkhami, A. J. Tyler, Y. Renardy, M. Renardy, T. G. St Pierre, R. C. Woodward, and J. S. Riffle, "Deformation of a hydrophobic ferrofluid droplet suspended in a viscous medium under uniform magnetic fields," *Journal of Fluid Mechanics*, vol. 663, pp. 358-84, 2010.
- [14] J. Liu, S. H. Tan, Y. F. Yap, M. Y. Ng, and N. T. Nguyen, "Numerical and experimental investigations of the formation process of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 11, pp. 177-87, 2011.
- [15] Q. A. Pankhurst, J. Connolly, S. K. Jones, and J. Dobson, "Applications of magnetic nanoparticles in biomedicine," *Journal of Physics D-Applied Physics*, vol. 36, pp. R167-R81, 2003.
- [16] Z. M. Wang, V. B. Varma, Z. P. Wang, and R. V. Ramanujan, "Tuning magnetofluidic spreading in microchannels," *Journal of Micromechanics and Microengineering*, vol. 25, p. 124001, 2015.
- [17] F. M. White and I. Corfield, *Viscous fluid flow* vol. 3: McGraw-Hill New York, 2006.
- [18] S. Osher and J. A. Sethian, "Fronts Propagating with Curvature-Dependent Speed - Algorithms Based on Hamilton-Jacobi Formulations," *Journal of Computational Physics*, vol. 79, pp. 12-49, 1988.
- [19] S. Bashir, J. M. Rees, and W. B. Zimmerman, "Simulations of microfluidic droplet formation using the two-phase level set method," *Chemical Engineering Science*, vol. 66, pp. 4733-41, 2011.
- [20] E. Olsson and G. Kreiss, "A conservative level set method for two phase flow," *Journal of Computational Physics*, vol. 210, pp. 225-46, 2005.

Chapter 4

Controlling Ferrofluid Droplets in Microchannels by Uniform Magnetic Fields[~]

A range of Lab-on-a-chip (LoC) applications can be realized with magnetic droplets. These versatile capabilities of wireless and programmable manipulation are due to the integration of uniform magnetic field and magnetic droplets. The influence of uniform magnetic fields (H_0) on ferrofluid droplets (FD) was investigated by microfluidic experiments and simulations. Droplet size, aspect ratio, droplet spacing and droplet velocity were used to examine the dynamic behavior of the flowing FD. The influence of ferrofluid susceptibility, flow rates, and viscosity of the carrier medium was studied to demonstrate control of size, shape, velocity, and spacing of FD. A droplet-based micro-magnetofluidic numerical model was developed. The results of our simulations and experiments are in good agreement. These studies are useful for understanding magnetic droplet behavior and can be applied to mixing in a LoC environment using a uniform magnetic field.

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4.1 Introduction

Droplet microfluidics (DMF) [1, 2] is a versatile platform to carry out multiple reactions in many small isolated containers in the form of droplets. Microfluidics provide multiplexing capabilities by miniaturization of laboratory operations, hence simultaneous execution of several parallel processes is possible on this Lab-on-a-Chip (LoC) platform. Such platforms can be useful for sensing [3, 4], fabrication of microparticles [5-7] and combinatorial chemistry for drug discovery [8-10]. Sensing has been performed for disease detection [8], protease activity [11], enzyme kinetics [12], high throughput bioassays, and bacteria trapping [13]. A flexible fabrication technique on a LoC platform for controlled particle fabrication could also be realized by the DMF approach[6] e.g., fabrication of Janus particles[7], anisotropic particles[14], polymeric particles[15], controlled encapsulation for drug delivery [16] and multidimensional optical barcoding [17].

Enhancement of these capabilities require movement and shape control of the ferrofluid droplet (FD). An applied magnetic field (uniform: H_o and/or non-uniform: H_{no}) provides remote manipulation and wireless control of FD. Motion of the continuous phase (CP) relative to the dispersed phase (DP) of FD can be categorized as: (i) stationary CP (sCP): FD behavior investigated in magnetic field when there is no motion of the CP (zero velocity of the CP) and (ii) dynamic CP (dCP): FD behavior investigated when they are in motion due to the flow of the CP (nonzero CP velocity).

Several studies of FD behavior have been reported for the case of the stationary continuous phase. Chen et al. reported novel planetary motion of FD arrays in a rotating magnetic field [18]. They correlated the morphology with the motion of the FD and determined its surface tension from the ratio of the major and minor axis of the stretched ellipsoid droplets in the presence of H_o . They also explored the effect of the rotating magnetic field on the FD. They attributed local spinning of the droplets to the simultaneous realignment of dipoles along the magnetic field lines and global planetary motion to magnetic torque acting on the aligned point dipoles, which results in phase lag between the aligned dipoles and the magnetic field [18]. Poesio et al. studied wetting transitions of ferrofluid droplets on superhydrophobic surfaces and demonstrated a magnetic force driven Cassie-Wenzel transition near the resonant frequency [19]. This wetting transition is due to the increased

Laplace pressure arising from the large deformation near the resonant frequency [19]. Lee et al. experimentally demonstrated magnetic field driven formation of hexagonal and square patterns of ordered magnetic droplet arrays [20]. Chen et al. experimentally investigated the breakup patterns of a large ferrofluid droplet in the presence of a uniform magnetic field [21]. Numerous uniformly sized, small FD were formed by the breakup of a large droplet. Pattern formation initiates from the center of the large droplet, which was attributed to the low surface tension at the center of FD. Lohrasebi reported numerical modeling for FD deformation in a magnetic field by considering (a) interactions between neighboring magnetic nanoparticles at the droplet interface and (b) between magnetic particles and the center of the droplet [22]. The motion of a deformed droplet was studied for the two-dimensional case using a combination of magnetic field gradient and uniform magnetic field.

For the case of dynamic continuous phase a few studies have been reported, which provide insight into FD manipulation when the CP is in motion. Various operations have been performed by FD, e.g., breakup [23], ordered arrangement [24], dynamics by field gradient H_{no} [25, 26] and numerical simulations [22, 27, 28]. Wu et al. demonstrated FD breakup in a T-junction by a combination of two permanent magnets [23]. They investigated the influence of H_o on stretching, breakup of FD and H_{no} on asymmetrical breakup, leading to different FD sizes. The average droplet size decreases at higher CP flow rates. Lee et al. investigated the effect of applied magnetic fields on the generation and distribution of FD in a microfluidic T-junction [20]. They experimentally demonstrated change in generation rate and size of FD for applied magnetic fields in out-of-plane direction. They observed a smaller spatial density of FD for stronger magnetic fields. The effect of different types of magnetic field on FD have been reported, e.g., FD kinematic behavior in magnetic fields generated by planar coils [25], formation and manipulation in the magnetic field of a permanent magnet [26] and formation under the influence of uniform magnetic fields [28]. Numerical and experimental studies describe FD formation as a function of viscous drag, pressure drop, and interfacial tension. FD deformation decreases with increasing interfacial tension, leading to increased viscous drag and pressure drop, which increases the force on the FD. Applied magnetic field along the same direction as the viscous drag adds to the

volume force. This force acts against interfacial tension (at higher magnetic Bond number and higher ferrofluid susceptibility), resulting in larger FD size[28].

It is clear from the literature that the effect of a *uniform magnetic field* (H_o) on *FD behavior* has not been explored in detail for the case of *dynamic continuous phase*. Further, LoC control of FD by a H_o has not been demonstrated on a microfluidic platform.

This study report the effect of a H_o on the size, shape and the motion of FD generated by a microfluidic T-junction on microfluidic platform. The effect of flow rate ratio (Q_r), viscosity (η), and ferrofluid susceptibility (χ) on FD behavior under the influence of H_o was studied by experiments and simulations. The flow rate ratio (Q_r) is defined as the ratio of the CP flow rate (Q_{cp}) to the DP flow rate (Q_{dp}). These studies are useful for wireless, remote and programmable control of magnetic droplets and magnetic Janus particle synthesis[7], relevant to drug delivery, reaction kinetics, and biosensing applications.

4.2 Experimental Section

The experimental investigations were performed by the MMF setup described in the earlier *Section 3.2*. The methodology and technical details of various components of this MMF setup are described in *Section 3.2*. The following subsections describe the specific experimental details and parameters utilized to investigate the dynamic behavior of FD in an applied uniform magnetic field (H_o).

In brief, FD were generated by a T-junction inside a CP of oil (**Figure 4.1**). Uniform magnetic field H_o was applied along the y direction (**Figure 4.1** and **Figure 4.2**). The FD flow was along x direction. High-speed imaging was performed along z direction. The FD behavior was determined by the perimeter of the droplets (POD), the aspect ratio of the droplets (ARD), velocity of the droplets (VOD), and inter-droplet spacing (IDS).

4.2.1 Microfluidic Chip

The FD generation was performed by a Poly(methyl methacrylate) (PMMA) microfluidic chip, designed in AutoCAD 2015. A micro milling technique was used for the fabrication. The microfluidic chip design includes two inlets (I/L) and one outlet (O/L), all with the

cross-section of $500\mu\text{m}$ width $\times$ $250\mu\text{m}$ height. A T-junction with a cross-section of $250\mu\text{m}$ width $\times$ $250\mu\text{m}$ height was used for droplet generation.

Figure 4.1: (a) Schematic of droplet generation under uniform magnetic field. The dispersed phase (DP) droplets of water-based ferrofluid are generated in a continuous phase (CP) of oil by a microfluidic T-junction (not to scale). (b) Outline of the experimental setup used for micromagnetofluidic (MMF) investigations.

Figure 4.2: Numerical model compared with experimental geometry. Simulation results (scale in mm,) are shown for the set S1-Qr50. Scale bar = 500 μm . For experiments, the dispersed phase (DP) is in black color and the continuous phase (CP) is transparent. For simulations, DP is denoted by blue color and CP is denoted by red color.

4.2.2 Materials

Two oils with different viscosity were used to investigate the effect of viscosity, viz., n-hexadecane (3 mPa.s) and light mineral oil (18 mPa.s). 1 % (v/v) SPAN 80 was added to the oil phase to prevent droplet coalescence. Two types of ferrofluids were used to study the effect of magnetic susceptibility, viz., EMG 607 and EMG 807 (properties are summarized in **Table 3.1**).

4.2.3 Experimental setup

The scheme of the experimental setup to study the effect of uniform magnetic field on FD is shown in **Figure 4.1**, it is described in *Section 3.2*. The setup consists of (i) FD generation, (ii) uniform magnetic field (H_0) and (iii) high-speed imaging with image processing. A dual rate syringe pump (model: KDS, Gemini 88) connected to the microfluidic chip was used for droplet generation. IDEX tubing with 0.50 mm inner and 1.59 mm outer diameter, connected to two independent 1 ml Exmire Luer lock gastight syringes were used to feed the CP and the DP.

A DEXING electromagnet system with an air gap of 4 cm was used to apply the uniform magnetic field. High-speed imaging was performed (Phantom Miro Camera, M320s) at ~ 50 fps and ~ 30 fps at resolution 640×1200 pixels. ImageJ software was used to measure FD shape, size, and spacing (**Figure 4.3**). The PCC software was used to measure the velocity of 10 consecutive FD. The data points denote mean values of the measurement. The error bar denotes the standard error of the mean [29].

Figure 4.3: (a) Experimental micrographs showing the effect of uniform magnetic field on ferrofluid droplets for sets S1-Qr10 and S1-Qr50. (b)–(c) Droplet deformation in uniform magnetic fields, experimental (b) are compared with simulation (c). For experiments (a, b), black color denotes dispersed phase (DP) and continuous phase (CP) is transparent. For simulations (c), DP and CP are denoted by blue and red colors, respectively.

4.2.4 Experiment Parameters

Experiments were performed with flow rate ratios of $Q_r = 10$ and 50 , the range of parameters explored in the DP susceptibility and the CP viscosity are shown in **Table 4.1**. Uniform magnetic fields of $H_0=0, 10, 25, 50, 100, 250, 500$ mT were externally applied.

Table 4.1: Parameters (Oil was used as continuous phase (CP) and ferrofluid was used as dispersed phase (DP)).

SET	Viscosity of the CP of oil (mPa.s)		Susceptibility of DP of ferrofluid (SI units)		Flow Rate Ratio	Notation
S1-Qr10	Hexadecane	$\eta_1 = 3$	EMG 607	$\chi_1=1.63$	$Q_1=10$	$\eta_1\chi_1Q_1$
S2-Qr10	Hexadecane	$\eta_1 = 3$	EMG 807	$\chi_{high}=1.86$	$Q_1=10$	$\eta_1\chi_{high}Q_1$
S3-Qr10	Mineral Oil	$\eta_2 = 18$	EMG 607	$\chi_1=1.63$	$Q_1=10$	$\eta_2\chi_1Q_1$
S1-Qr50	Hexadecane	$\eta_1 = 3$	EMG 607	$\chi_1=1.63$	$Q_2=50$	$\eta_1\chi_1Q_2$
S2-Qr50	Hexadecane	$\eta_1 = 3$	EMG 807	$\chi_2=1.86$	$Q_2=50$	$\eta_1\chi_2Q_2$
S3-Qr50	Mineral Oil	$\eta_2 = 18$	EMG 607	$\chi_1=1.63$	$Q_2=50$	$\eta_2\chi_1Q_2$

4.3 Physics of Numerical Model and Numerical Methodology

The physics of the numerical model was described in the earlier *Section 3.4.2* by Eq.(3.5) to Eq. (3.17), Eq. (3.19) and Eq. (3.20).

The constitutive relations given in *Section 3.4* were used to develop a droplet micro-magnetofluidic numerical model in COMSOL Multiphysics software. A laminar two-phase level set method (fluid dynamics module) was used for droplet simulation. No currents method (AC/DC module) was used to simulate the uniform magnetic field. Meshing was performed by triangular elements. The element size range of $0.25 \mu\text{m}$ to $21.7 \mu\text{m}$ was used for the microchannel with FD. For the magnetic field domain, element size range of $1.1 \mu\text{m}$ to $100 \mu\text{m}$ was used. Extra-fine meshing was performed by 30778 total elements (19582 elements for the microchannel with FD and 11196 for magnetic fields).

4.4 Results and Discussion

The influence of a field H_0 on FD was studied. Investigations were performed for flow rate ratio $Q_1=10$ and $Q_2=50$ at a constant Q_{dp} of 10 $\mu\text{l/h}$ and at CP viscosities $\eta_1=3$ mPa.s, and $\eta_2=18$ mPa.s (Section 4.2). The role of the magnetic properties of FD was investigated at different ferrofluid susceptibilities ($\chi_2=1.86$ and $\chi_1=1.63$, SI units) (**Table 3.1**). Initially, spherical FD are generated at the T-junction. FD elongate along the field direction (**Figure 4.2** and **Figure 4.3** (a-c)) under the influence of a uniform magnetic field, since the magnetic volume force described in Eq.(3.16) acts against the force of surface tension.

4.4.1 Perimeter of Droplets (PoD)

Figure 4.4 shows the variation of the FD size (i.e. POD) under an applied magnetic field H_0 . At a low flow rate ratio of 10 (set S2-Qr10, $\eta_1\chi_2Q_1$), the POD increases. For high ferrofluid susceptibility, increasing field H_0 leads to an increase in magnetic volume force at the T-junction. Due to the low flow rate ratio of 10, there is a smaller hydrodynamic force from the CP flow.

Consequently, the effect of magnetic volume force is high at the T-junction and increases with increasing field H_0 , resulting in FD breakup at higher POD. For set S2, at a lower flow rate ratio of 10, a decrease in droplet size was observed with increasing uniform magnetic field from 0 to 50 mT. Droplet size increases with increasing field H_0 from 50mT to 250mT and saturates beyond 250mT.

However, at higher Qr (Qr=50), the FD decreases with increasing field H_0 , due to higher drag force from the CP. For sets S1 and S3 (sets with χ_1), changes in the droplet size are smaller than that in the set S2. The droplet size generally decreases with increasing field H_0 at lower viscosity (set S1) for both Qr (Q_1 and Q_2). However, for set S1 this behavior was not observed at 50 mT, Q_1 and at 100 mT, Q_2 . This is due to the higher variation in the droplet size, evident from the larger error bars. These variations are due to the dynamic competition between magnetic force, hydrodynamic force and the surface tension. The magnetic force and surface tension force contributes to smaller droplet size at lower hydrodynamic force (i.e. at Q_1). At higher hydrodynamic force (at Q_2) increasing magnetic force contributes to the slightly higher droplet size.

Figure 4.4: Experimental results showing variation in the Perimeter of Droplets (POD) with increasing uniform magnetic field. (Refer **Table 4.1** for notations).

The droplet size generally increases with increasing field at higher viscosity and lower Qr (set S3-Qr10). However, this behavior was not observed at 25 mT. As mentioned earlier this deviation is responsible for dynamic balance between the magnetic, surface tension and hydrodynamic forces, resulting in slightly higher variation in droplet size, hence larger error bar. With increasing field from 0 to 100 mT, the droplet size increases at higher Qr (for set S3-Qr50). The droplet size decreases beyond 100 mT and stabilizes after 250 mT. Evidently, with increasing field, high ferrofluid susceptibility (set S2-Qr10) demonstrated significant variation in the droplet size at lower Qr, compared to the low susceptibility ferrofluids (set S1-Qr10 and S3-Qr10). Hence, the droplet size can be more easily controlled by a higher ferrofluid susceptibility, lower Qr (Qr=10) and a lower CP viscosity.

4.4.2 Aspect Ratio of Droplets (ARD)

The variations in ARD with increasing uniform magnetic field is shown in **Figure 4.5**. Under an applied uniform magnetic field, the FD elongates along the field direction, leading to an increase in the ARD. The magnetic volume force (Eq. (3.16)) is responsible for this increase in the aspect ratio. For a high ferrofluid susceptibility (set S2, **Figure 4.5**) the ARD increases with increasing field at both Qr (Qr10 and Qr50). However, more significant ARD increase was observed at Qr10 compared to Qr50, for fields higher than 50 mT. This significant ARD increase is due to domination of magnetic volume force at low Qr for a high ferrofluid susceptibility. With increasing field from 0 to 100 mT, the ARD increases for low ferrofluid susceptibility at low Qr (set S1-Qr10 and set S3-Qr10). Above 100 mT, the increase in the ARD stabilizes. The ARD decreases with increasing field, at a high Qr (Qr50) and low ferrofluid susceptibility (set S1-Qr50 and set S3-Qr50).

Figure 4.5: Experimental results showing variation in the Aspect Ratio of Droplets (ARD) with increasing uniform magnetic field strength (Refer **Table 4.1** for notations).

The ARD variations at low and high Q_r is an outcome of competition between surface tension and magnetic volume force. At the droplet interface (interface between oil and ferrofluid), magnetic susceptibility increases towards FD and decreases towards the oil phase. The magnetic volume force acts at the location of the susceptibility change, i.e., at the droplet interface. At a constant uniform magnetic field, with increasing droplet size the ARD increases [30, 31]. For the set S1 at high Q_r (Q_{r50}) the droplet size ($1000\ \mu\text{m}$) is smaller than the size ($1200\ \mu\text{m}$) at low Q_r (Q_{r10}). Droplet size also decreases with increasing field H_0 and CP viscosity (**Figure 4.4**). Hence, with increasing field, the ARD increases at high ferrofluid susceptibility, low Q_r and low viscosity. At high Q_r with increasing field, POD and the ARD both decrease.

4.4.3 Simulations: Aspect Ratio of Droplets (ARD)

Simulations were performed in a 2D Cartesian coordinate system with the experimental variables tabulated in **Table 3.1**. Experimental values of FD diameter and droplet spacing were used as the initial condition. The field H_0 was simulated and applied to the flowing FD. Movies of the simulations were exported and analyzed in ImageJ to determine the ARD and the VOD. The experimental and simulated variation in the ARD with increasing magnetic field strength are compared in **Figure 4.6**. The simulated ARD were found to be in good agreement with the experimental results. The simulated ARD for the set S1- Q_{r10} exhibit an increasing trend with increasing field.

At zero magnetic field, FD are spherical to minimize surface tension since the spherical shape has a minimum surface area for a given volume. Any change in this shape increases its surface tension due to increased surface area. Droplet shape change to ellipsoidal shape results in higher ARD. This ellipsoidal shape exhibit higher surface tension compared to a spherical droplet. An external force is required to maintain this non-equilibrium ellipsoidal shape, which is supplied by the magnetic volume force [30, 32].

Hence, under the influence of an applied magnetic field, the change in surface tension can be correlated to the ARD change (**Figure 4.6**). However, change in surface tension with an applied magnetic field is difficult to determine by conventional experimental methods [33].

Surface tension was determined, numerically by fitting the experimental ARD with the simulated results[33]. An increase in surface tension with increasing field was observed (**Figure 4.6**) for low Qr[33]. However, a high ARD was observed for high Qr (set S1-Qr50 in **Figure 4.6**) at low H_0 (10mT), below the saturation magnetization of the ferrofluid. The observed higher ARD is due to the ferrofluid susceptibility attaining higher value, leading to higher magnetic body force. For magnetic fields above the saturation magnetization, variation in ARD follow the usual trend with increasing magnetic fields at higher flow rate ratio.

Figure 4.6: Experimental and simulation results showing Aspect Ratio of Droplets (ARD) as a function of uniform magnetic field strength.

4.4.4 Inter Droplet Spacing (IDS) and Velocity of Droplets (VOD)

The variation of droplet spacing and droplet velocity with increasing field is shown in **Figure 4.7(a-b)**. With increasing magnetic fields, changes in the VOD and the IDS were observed. A competition between magnetic volume force (Eq. (3.16)), drag force acting on FD (Eq. (3.19)), and surface tension (Eq.(3.5)) leads to the observed variations. The drag force increases with increasing droplet size, CP viscosity, and CP flow rate. The magnetic volume force increases with increasing field, leading to ARD increase. The increase in magnetic volume force increases ARD (as discussed earlier), leading to an increase in surface tension.

The competition between the above forces leads to an increase in VOD with increase in Q_r , droplet size and CP viscosity. Conversely, with increasing magnetic field strength, drag force on the FD decreases due to increase in the ARD, leading to lower VOD. The reduction in VOD with increasing H_o results in IDS reduction.

At Q_r50 (set S3- Q_r50 corresponding to $\eta_2\chi_1Q_2$), the VOD generally increases with increasing field (**Figure 4.7 b**). However, at 100 mT this behavior was not observed. The drag force dominates at higher Q_r and at high viscosity, leading to the observed increase in VOD for set S3- Q_r50 . The VOD decreases with increasing field strength from 10mT to 500mT, as observed for sets S1 Q_r50 and S2- Q_r50 . The drag force on FD reduces with increasing magnetic field due to the ARD increase. Hence, the VOD decreases with increasing field H_o . For set S1 at Q_r10 , there is an anomaly of VOD increase with increasing H_o . This is due to increase in FD size with increasing H_o , leading to larger drag force. Hence, the VOD increases for set S1- Q_r10 , with increasing H_o .

(a) Inter Droplet Spacing (IDS)

(b) Velocity of Droplets (VOD)

Figure 4.7: Experimental results showing the change of (a) Inter Droplet Spacing and (b) Velocity of Droplets as a function of uniform magnetic field strength.

4.4.5 Simulation: Velocity of Droplets (VOD)

The variation in v_{dim} with increasing magnetic field H_o is shown in **Figure 4.8**. v_{dim} is the ratio of the droplet velocity (v_{drop}) to the CP velocity (v_{cp}) and termed as dimensionless droplet velocity. v_{dim} is mathematically defined as, $v_{\text{dim}} = v_{\text{drop}}/v_{\text{cp}}$. Experimental and simulation results in **Figure 4.8** show VOD decrease, with increasing H_o . The simulation results of VOD are in good agreement with the experimental results. A mismatch was observed at 500mT. This mismatch is due to an increase in non-uniformity of H_o at magnetic fields ≥ 500 mT.

Figure 4.8: v_{dim} as a function of magnetic field H_o . v_{dim} is termed as the dimensionless droplet velocity and mathematically defined by $v_{\text{dim}} = v_{\text{drop}}/v_{\text{cp}}$. Notations: droplet velocity (v_{drop}) and continuous phase velocity (v_{cp}).

4.5 Conclusion

The shape, size and motion of ferrofluid droplets in a flowing immiscible continuous phase were investigated in a Lab-on-a-Chip environment under the influence of a *uniform* magnetic field. The magnetic field was applied to the ferrofluid droplets along the plane of the microchannels perpendicular to the flow of the continuous phase. The effect of viscosity of continuous phase, magnetic susceptibility of ferrofluid and flow rate ratio were studied. The major conclusions are:

- Ferrofluid droplet size and aspect ratio increases with increasing magnetic field at high susceptibility and low flow rate ratio.
- Ferrofluid droplet spacing and velocity increases at high flow rate ratio and high viscosity of continuous phase.
- Ferrofluid droplet motion, shape, size can be readily controlled by uniform magnetic fields at high susceptibility, low viscosity, and low flow rate ratio.
- A droplet micro-magnetofluidic numerical model was developed, the simulation results are in good agreement with the experimental findings. The experimental and numerical studies performed can be useful for the development of programmable, wireless capabilities for droplet microfluidic Lab-on-a-chip devices.

References

- [1] R. Seemann, M. Brinkmann, T. Pfohl, and S. Herminghaus, "Droplet based microfluidics," *Rep Prog Phys*, vol. 75, p. 016601, 2012.
- [2] T. P. Lagus and J. F. Edd, "A review of the theory, methods and recent applications of high-throughput single-cell droplet microfluidics," *Journal of Physics D-Applied Physics*, vol. 46, p. 21, 2013.
- [3] L. Malic, D. Brassard, T. Veres, and M. Tabrizian, "Integration and detection of biochemical assays in digital microfluidic LOC devices," *Lab Chip*, vol. 10, pp. 418-31, 2010.
- [4] S. Badilescu and M. Packirisamy, "Microfluidics-Nano-Integration for Synthesis and Sensing," *Polymers*, vol. 4, pp. 1278-310, 2012.
- [5] Y. Zhao, L. Shang, Y. Cheng, and Z. Gu, "Spherical colloidal photonic crystals," *Acc Chem Res*, vol. 47, pp. 3632-42, 2014.
- [6] W. Wang, M. J. Zhang, and L. Y. Chu, "Functional polymeric microparticles engineered from controllable microfluidic emulsions," *Acc Chem Res*, vol. 47, pp. 373-84, 2014.
- [7] S. Lone and I. W. Cheong, "Fabrication of polymeric Janus particles by droplet microfluidics," *Rsc Advances*, vol. 4, pp. 13322-33, 2014.

- [8] H. Zec, D. J. Shin, and T. H. Wang, "Novel droplet platforms for the detection of disease biomarkers," *Expert Rev Mol Diagn*, vol. 14, pp. 787-801, 2014.
- [9] G. T. Vladislavljevic, N. Khalid, M. A. Neves, T. Kuroiwa, M. Nakajima, K. Uemura, S. Ichikawa, and I. Kobayashi, "Industrial lab-on-a-chip: design, applications and scale-up for drug discovery and delivery," *Adv Drug Deliv Rev*, vol. 65, pp. 1626-63, 2013.
- [10] O. J. Dressler, R. M. Maceiczky, S. I. Chang, and A. J. deMello, "Droplet-based microfluidics: enabling impact on drug discovery," *J Biomol Screen*, vol. 19, pp. 483-96, 2014.
- [11] C. H. Chen, M. A. Miller, A. Sarkar, M. T. Beste, K. B. Isaacson, D. A. Lauffenburger, L. G. Griffith, and J. Han, "Multiplexed protease activity assay for low-volume clinical samples using droplet-based microfluidics and its application to endometriosis," *J Am Chem Soc*, vol. 135, pp. 1645-8, 2013.
- [12] S. L. Sjostrom, H. N. Joensson, and H. A. Svahn, "Multiplex analysis of enzyme kinetics and inhibition by droplet microfluidics using picoinjectors," *Lab on a Chip*, vol. 13, pp. 1754-61, 2013.
- [13] Z. M. Wang, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Trapping of Bacteria at Low Magnetic Fields," *Scientific Reports*, vol. 6, p. 26945, 2016.
- [14] Y. Cheng, C. Zhu, Z. Xie, H. Gu, T. Tian, Y. Zhao, and Z. Gu, "Anisotropic colloidal crystal particles from microfluidics," *J Colloid Interface Sci*, vol. 421, pp. 64-70, 2014.
- [15] Q. Xu, M. Hashimoto, T. T. Dang, T. Hoare, D. S. Kohane, G. M. Whitesides, R. Langer, and D. G. Anderson, "Preparation of monodisperse biodegradable polymer microparticles using a microfluidic flow-focusing device for controlled drug delivery," *Small*, vol. 5, pp. 1575-81, 2009.
- [16] W. J. Duncanson, T. Lin, A. R. Abate, S. Seiffert, R. K. Shah, and D. A. Weitz, "Microfluidic synthesis of advanced microparticles for encapsulation and controlled release," *Lab Chip*, vol. 12, pp. 2135-45, 2012.
- [17] G. Lin, D. Makarov, M. Medina-Sanchez, M. Guix, L. Baraban, G. Cuniberti, and O. G. Schmidt, "Magnetofluidic platform for multidimensional magnetic and optical barcoding of droplets," *Lab on a Chip*, vol. 15, pp. 216-24, 2015.
- [18] C. Y. Chen, H. C. Hsueh, S. Y. Wang, and Y. H. Li, "Self-assembly and novel planetary motion of ferrofluid drops in a rotational magnetic field," *Microfluidics and Nanofluidics*, vol. 18, pp. 795-806, 2015.
- [19] P. Poesio and E. N. Wang, "Resonance induced wetting state transition of a ferrofluid droplet on superhydrophobic surfaces," *Experimental Thermal and Fluid Science*, vol. 57, pp. 353-57, 2014.
- [20] C. P. Lee, S. T. Yang, and Z. H. Wei, "Ordered magnetic microdroplets array on magnetic films," *Journal of Applied Physics*, vol. 111, p. 07B331, 2012.
- [21] C.-Y. Chen and C. S. Li, "Ordered microdroplet formations of thin ferrofluid layer breakups," *Physics of Fluids*, vol. 22, p. 014105, 2010.
- [22] A. Lohrasebi, "Computational Modeling of the Effect of an External Magnetic Field on a Ferromagnetic Fluid Droplet," *Journal of Superconductivity and Novel Magnetism*, vol. 28, pp. 777-80, 2015.
- [23] Y. N. Wu, T. T. Fu, Y. G. Ma, and H. Z. Li, "Active control of ferrofluid droplet breakup dynamics in a microfluidic T-junction," *Microfluidics and Nanofluidics*, vol. 18, pp. 19-27, 2015.
- [24] C. P. Lee, T. S. Lan, and M. F. Lai, "Fabrication of two-dimensional ferrofluid microdroplet lattices in a microfluidic channel," *Journal of Applied Physics*, vol. 115, p. 17B527, 2014.

- [25] N. T. Nguyen, A. Beyzavi, K. M. Ng, and X. Y. Huang, "Kinematics and deformation of ferrofluid droplets under magnetic actuation," *Microfluidics and Nanofluidics*, vol. 3, pp. 571-79, 2007.
- [26] S. H. Tan, N. T. Nguyen, L. Yobas, and T. G. Kang, "Formation and manipulation of ferrofluid droplets at a microfluidic T-junction," *Journal of Micromechanics and Microengineering*, vol. 20, p. 045004, 2010.
- [27] J. Liu, Y. F. Yap, and N. T. Nguyen, "Numerical study of the formation process of ferrofluid droplets," *Physics of Fluids*, vol. 23, p. 072008, 2011.
- [28] J. Liu, S. H. Tan, Y. F. Yap, M. Y. Ng, and N. T. Nguyen, "Numerical and experimental investigations of the formation process of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 11, pp. 177-87, 2011.
- [29] M. L. Boas, *Mathematical methods in the physical sciences*, 3 ed.: Wiley New York, 2006.
- [30] S. Banerjee, M. Fasnacht, S. Garoff, and M. Widom, "Elongation of confined ferrofluid droplets under applied fields," *Physical Review E*, vol. 60, p. 4272, 1999.
- [31] A. Ghaffari, S. H. Hashemabadi, and M. Bazmi, "CFD simulation of equilibrium shape and coalescence of ferrofluid droplets subjected to uniform magnetic field," *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, vol. 481, pp. 186-98, 2015.
- [32] G.-P. Zhu, N.-T. Nguyen, R. V. Ramanujan, and X.-Y. Huang, "Nonlinear deformation of a ferrofluid droplet in a uniform magnetic field," *Langmuir*, vol. 27, pp. 14834-41, 2011.
- [33] S. Afkhami, A. J. Tyler, Y. Renardy, M. Renardy, T. G. St Pierre, R. C. Woodward, and J. S. Riffle, "Deformation of a hydrophobic ferrofluid droplet suspended in a viscous medium under uniform magnetic fields," *J Fluid Mech*, vol. 663, pp. 358-84, 2010.

Chapter 5

Droplet Merging on a Lab-on-a-Chip Platform by Uniform Magnetic Fields[~]

Droplet microfluidics offers a range of applications. However, wireless and programmable manipulation of such droplets is a challenge. This challenge was addressed by experimental and modeling studies of uniform magnetic field induced merging of ferrofluid based droplets. Control of droplet velocity and merging was achieved through the uniform magnetic field and flow rate ratio. Conditions for droplet merging with respect to droplet velocity were studied. Merging and mixing of color dye+ magnetite composite droplets was demonstrated. Our experimental and numerical results are in good agreement. These studies are useful for wireless and programmable droplet merging as well as mixing relevant to biosensing, bioassay, microfluidic-based synthesis, reaction kinetics, and magnetochemistry.

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5.1 Introduction

Droplet microfluidics (DMF)[1, 2] is a versatile tool for the manipulation of matter[3-5] on a microfluidic platform. Applications of DMF require droplet manipulation in order to perform operations such as droplet merging, mixing, breakup, and sorting. Droplet manipulation on a LoC platform has been used for the synthesis of novel materials[6] relevant to biosensing[7], three-dimensional (3D) cell culture[8], photonic crystals[9], Janus structures[10], anisotropic particles[11], functional polymeric structures[12-14], controlled encapsulation for drug delivery[15] and multidimensional optical barcoding[16]. Specifically, droplet merging and mixing has been used for a broad range of biomedical applications[17], such as bioassays[18, 19], biomaterials[20], single cell analysis[21], cell sorting[22, 23], population transcriptomics[24], disease detection[25], and diagnostics[26]. Merging and mixing of droplets can also lead to miniaturized volume conditions (nl to pl), reduced operation time and several fold increase in screening of chemical reactions[27], which opens a new domain of DMF chemistry[28, 29], drug discovery [30-32], high-throughput molecular genetics[33], interfacial studies[34] and ‘on water reaction’[35].

In previous reports, various strategies were utilized to merge droplets e.g., hydrodynamic flow focusing[36], surface acoustic waves[37], dielectrophoresis[38], surfactant-hydrodynamic flow focusing[39], droplet velocity-lipid concentration[18], electrorheological fluids[40], cavitation bubble[41], electro-coalescence[42] and liquid phase flow[43]. Most of the above strategies require one or more of the following undesirable attributes: direct contact with the liquid, complex microfabrication techniques, changes in the channel geometry, or changes in the flow rates.

Hence, contact-free, wireless and programmable manipulation capabilities for droplet breakup, merging and mixing will be advantageous. In the literature, generally a combination of non-uniform magnetic field (H_{no}) and ferrofluid droplets (FD) was used to demonstrate: (i) FD breakup by H_{no} applied by a permanent magnet in a microfluidic Y junction[44], flow focusing devices[45, 46], a straight channel[47] and at a T-junction[48]. (ii) Nguyen et al. investigated FD size control and FD formation by the field H_{no} of a permanent magnet, one (1D)[49] and two (2D)[50] dimensional manipulation by planar coils, deformation by two pairs of planar coils[51], FD actuation by a coil-magnet

combination[52] were demonstrated. (iii) Chen et al. investigated the effect of a rotational field on self-assembly of FD[53] and ordered FD formation[54]. (iv) Di Carlo et al. demonstrated magnetic droplet generation and size control by a gradient magnetic field of a permanent magnetic [55]. (v) Sanders et al. demonstrated magnetic transport-release[56] by a permanent magnet.

Recent reports of the use of a magnetic field to perform FD merging include: (i) Xiao et al.[57] used a spin torque oscillator to demonstrate stationary droplet merging by a combination of applied current and magnetic field. However, those studies were not performed on a microfluidic platform; (ii) Ahmadi et al.[58] reported a magnetohydrodynamic method for actuation and merging of millimetric droplets. The role of the magnetic force on FD manipulation was also investigated; (iii) Teste et al.[59] demonstrated a ferromagnetic rail based manipulation system to control FD motion and merging.

From the literature it is evident that the integration of non-uniform magnetic fields on a microfluidic platform is limited by the (i) requirement of a large magnetic field gradient; (ii) high sensitivity of FD control to the position of the gradient along the microchannel[60]; (iii) larger size of permanent magnets than the microchannel size; (v) lack of programmable operations with permanent magnets; (vi) complex designs, fabrication techniques required for micro-coil integration with the microfluidic chip; and (vii) magnetic force is limited on a microfluidic platform due to smaller FD size, hence manipulation at lower magnetic fields is difficult and challenging. These factors limit the advantages of using magnetic fields for droplet manipulation in microfluidics. The above challenges can be addressed by a combination of *magnetic fluids* and *uniform magnetic fields*, which offers a *wireless*, *programmable* and *remote* method to perform microfluidic operations. In the literature a uniform magnetic field (H_0) was used on microfluidic platforms to investigate ferrohydrodynamic instabilities in uniform magnetic field[61, 62], FD formation[63, 64], non-linear deformation of FD[65], magnetic trapping of bacteria[66], magnetofluidic mixing[67], and spreading[68].

Uniform magnetic fields (H_0) can be used for contact-free, wireless, programmable and precise manipulation of magnetic fluid droplets. However, the use of a uniform magnetic

field for the wireless and programmable merging of moving magnetic fluid droplets on a DMF based Microfluidic platform has not yet been investigated in detail.

Hence, the present work reports for the first time, *uniform magnetic field induced* merging of moving droplets on a Microfluidic platform. The merging of ferrofluid droplets at various magnetic field strengths and flow rate ratios were investigated. The control of ferrofluid droplet merging was demonstrated experimentally and numerically. A micromagnetofluidic numerical model was developed to investigate droplet merging. The merging of color dye + ferrofluid composite droplets was demonstrated. These studies can be useful for wirelessly controlled merging, droplet mixing, Janus particle formation, reaction kinetics, and biosensing.

5.2 Methodology

Most of the technical details and methodology of experimental setup, the microfluidic chip fabrication, are physics of the numerical approach are summarized in *Section 3.2*. Details specific to the research work performed for this chapter are summarized in this section, covering material properties, parameters, and the simulation methodology.

5.2.1 Materials

Two immiscible phases for the generation of droplets: silicone oil (KF96A-100CS, Shin-Etsu, Japan) as CP and water-based ferrofluid EMG 807 (Ferrotec, Singapore) as DP were used. A small amount of surfactant 0.3 % (v/v) Tween 20 (Sigma-Aldrich, Singapore) was added to the DP for uniformly sized droplet generation. The measured viscosities (Brookfield rheometer, model: LV-DV3T) of silicone oil and ferrofluid (with surfactant) were 105 ± 0.10 mPa.s and 1.50 ± 0.01 mPa.s, respectively. The measured densities[69] of silicone oil and ferrofluid were $0.965 \pm 0.005 \times 10^3$ kg/m³ and $1.100 \pm 0.004 \times 10^3$ kg/m³, respectively. Other properties and notations used for the DP of ferrofluid (EMG 807) are summarized in **Table 3.1** and silicone oil KF96A-100CS in **Table 3.2**.

Yellow and blue food dyes (Star Brand food color, FairPrice, Singapore) were used to visually demonstrate merging and mixing. 10 % (v/v) blue food dye solution was prepared in deionized (DI) water. Yellow food dye solution was used without dilution. 10 % EMG

807 ferrofluid was mixed in both solutions, along with 0.5 % (v/v) Tween 20 for uniform droplet generation.

5.2.2 Microfluidic Chip

Two microfluidic chips were designed for the experiments (**Figure 5.1**). For ferrofluid droplet merging, a microfluidic chip with two inlets and one outlet was used (**Figure 5.1a**). One inlet microchannel was used for the CP and other for the DP, each with a cross-section of $500\mu\text{m}$ width $\times$ $150\mu\text{m}$ height. A T-junction with a cross-section of $150\mu\text{m}$ width $\times$ $150\mu\text{m}$ height was used for the droplet generation. The outlet microchannel was with a cross-section of $500\mu\text{m}$ width $\times$ $150\mu\text{m}$ height. For color dye droplet merging, a cross-junction chip was used to simultaneously generate two droplets with blue and yellow color (**Figure 5.1b**). The middle inlet was used for the CP (silicone oil) and DP1 was used for blue and DP2 for yellow dye droplets. Flow rates and flow rate ratios were tuned to obtain uniformly sized droplet generation. Chip dimensions are described in **Figure 5.1b**.

5.2.3 Experimental setup

The micromagnetofluidic (MMF) setup [68, 70] was used for the experimental studies, which consists of (i) microfluidic droplet generation, (ii) uniform magnetic field and (iii) high-speed imaging. The technical details and methodology of the MMF are already described in the earlier *Section 3.2.1*.

A KDS Gemini 88 dual rate syringe pump, 2.5 ml Exmire Luer lock gastight syringes and IDEX tubing (0.50 mm inner diameter, 1.59 mm outer diameter) were used for droplet generation. The uniform magnetic field H was applied by DEXING electromagnet at an air gap of 4 cm. Videos are recorded at ~ 100 fps and ~ 200 fps (resolution 640×1200 pixels). ImageJ and Phantom Camera Control (PCC) software were used for image processing and analysis. Ferrofluid droplet size was measured by ImageJ software. The PCC software was used to determine the velocity of 10 consecutive ferrofluid droplets. Points in all graphs denote the mean of the measurements and the error bar represents the standard error of the mean [71] for the corresponding data points.

5.2.4 Experimental Parameters

Droplet merging was investigated at four flow rate ratios, $Q_r = 2, 3, 4, 5$ and at different uniform magnetic field strengths, $H_o = 0, 50, 100, 500, 1000$ mT. Sets and notations are summarized in the following table.

Table 5.1: Sets and notations (Silicone oil was used as continuous phase (CP) and ferrofluid was used as dispersed phase (DP)). Where, Q_r : flow rate ratio= Q_{cp}/Q_{dp} .

Set	Flow rate ratio			Sets at an applied uniform magnetic field, H_o (mT)				
	Q_r	CP ($\mu\text{l/h}$)	DP ($\mu\text{l/h}$)	0	50	100	500	1000
Q2	2	200	100	Q2H0	Q2H50	Q2H100	Q2H500	Q2H1000
Q3	3	300	100	Q3H0	Q3H50	Q3H100	Q3H500	Q3H1000
Q4	4	400	100	Q4H0	Q4H50	Q4H100	Q4H500	Q4H1000
Q5	5	500	100	Q5H0	Q5H50	Q5H100	Q5H500	Q5H1000

5.3 Physics of Numerical Model and Numerical Methodology

The physics of numerical model is described in the earlier *Section 3.4.2* by Eq.(3.5) to Eq.(3.20).

A droplet micro-magnetofluidic numerical model was developed, using the above constitutive relations. Numerical simulations were performed by COMSOL Multiphysics software, using the laminar two-phase flow, a level set method in fluid dynamics module. The uniform magnetic field was modeled with no currents method in AC/DC module. Extra-fine meshing was performed for whole geometry with 34046 total elements. The meshing of the microchannel domain was created using 20066 triangular elements with a size of $0.25 \mu\text{m}$ (minimum) to $21.7 \mu\text{m}$ (maximum). Meshing for magnetic field domain was created by 14265 triangular elements of size $1.1 \mu\text{m}$ (minimum) to $150 \mu\text{m}$ (maximum).

Interestingly, our micro-magnetofluidic numerical model simulates the: (i) generation of ferrofluid droplets according to the experimental flow rates and flow rate ratios under the influence of magnetic field (ii) deformation of the droplets in a uniform magnetic field (iii) magnetically induced merging of ferrofluid droplets. Droplet generation, deformation, and consequent merging show a dependence on interfacial tension between ferrofluid and silicone oil. At zero magnetic field, interfacial tension between silicone oil and ferrofluid was 12 mN/m[64], the effective interfacial tension changes with magnetic field[72-74]. The magnetic volume force $\mathbf{F}_{m1}$ acting on the interface changes the interfacial tension, leading to droplet deformation, which was evident from our experimental results and our numerical simulations. From our simulated results, the dependence of droplet merging on interfacial tension was determined (**Figure 5.5**).

5.4 Results and Discussion

Our experimental studies of uniform magnetic field induced droplet merging can be divided into a) Generation and merging of ferrofluid droplets under applied uniform magnetic field (H) and b) Generation and merging of color dye + ferrofluid composite droplets. Two designs were used for droplet generation (**Figure 5.1**), using two immiscible phases: oil as the continuous phase (CP) and water-based ferrofluid or water based dye solution as the dispersed phase (DP). Uniform magnetic fields were applied perpendicular to droplet flow, and high-speed imaging was performed using our micro-magnetofluidic setup[68, 70].

Our studies start with the generation of droplets at various flow rate ratios, Q_r . The effect of flow rate ratio and an applied uniform magnetic field on droplet size (before merging) is described in the first subsection. The second subsection describes magnetically induced merging of ferrofluid droplets at various flow rate ratios. The process of merging is then elaborated by investigating the variation of droplet velocity with time. The experimental setup, notations, and definitions are described in the *methodology section*.

Figure 5.1: Schematic of uniform magnetic field(H_0) induced merging of (a) Ferrofluid droplets (150 μm channel height). Under the influence of field H_0 , droplet 'a' (with velocity V_a) merges with droplet 'b' (with velocity V_b), yielding a droplet 'ab' (with velocity V_{ab}). (b) Color dye + magnetite (ferrofluid) composite droplets (100 μm channel height). (c) Schematic of micromagnetofluidic setup[68, 70] (i) microfluidic droplet generation along the x-direction, (ii) magnetic field, H along y-direction (iii) high-speed imaging along the z-direction. As delineated in (a-c), inlets (I/L) contain continuous (CP), dispersed (DP) phases and outlet (O/L) contain generated droplets in the CP. $d=150\text{ }\mu\text{m}$, $W=500\text{ }\mu\text{m}$, $d'=100\text{ }\mu\text{m}$ and $W'=300\text{ }\mu\text{m}$ (not to scale).

5.4.1 Generation of Ferrofluid Droplets at Different Flow Rate Ratio

Ferrofluid droplets were generated using a T-junction configuration on a LoC platform (**Figure 5.1**). Droplet formation for all of our experiments is in the 'squeezing' regime since the capillary number [75] ($Ca=\eta_{cp}v_{cp}/\sigma$) is less than 10^{-2} , where η_{cp} , v_{cp} and σ are the viscosity of the CP, velocity of the CP and surface tension, respectively. At a constant DP flow rate, droplet formation proceeds in the following steps (**Figure 5.2a**): (i) The ferrofluid enters the main channel and forms a paraboloid. (ii) A neck is developed as the paraboloid grows in size. (iii) Neck elongation starts as the paraboloid advances. (iv) The paraboloid confines the flow of the CP, resulting in the 'squeezing of the neck' due to increased upstream pressure. (v) The squeezing of the neck proceeds at a rate which is proportional to the flow rate of the CP, this decides the droplet size. (vi) Finally, the neck breaks and the droplet detaches from the DP stream. When a magnetic field was applied

($H=500\text{mT}$, **Figure 5.2c**) the droplet generation time was 200ms, which was smaller than the generation time of 250ms without a magnetic field ($H_0=0\text{mT}$, **Figure 5.2b**). This decrease in droplet generation time is due to the additional force contributed by the applied magnetic field (i.e., the magnetic volume force).

Figure 5.2: Generation of ferrofluid droplets in a uniform magnetic field H_0 . (a-c) Droplet generation at a flow rate ratio of 2 (Q_2). (a) Schematic. (b-c) Experimental micrographs at (b) $H_0=0\text{ mT}$ and (c) $H_0=500\text{ mT}$, scale bar= $250\text{ }\mu\text{m}$. (d) Droplet size vs flow rate ratio (Q_r) at magnetic field $H_0=0, 50, 100, 500, 1000\text{ mT}$. Solid lines denote polynomial fits and the dotted line shows a linear fit. The inset shows the graph for the scaling law $D/d=1+1/Q_r$ [75] at $H=0\text{mT}$, for droplet diameter D , channel width d and flow rate ratio $Q_r=Q_{cp}/Q_{dp}$. The purple arrow indicates the direction of the CP flow (x-direction). The magnetic field is in the y-direction.

At zero magnetic field, a linear decrease in droplet size was observed with increasing flow rate ratio Q_r (**Figure 5.2d**). Since the droplets are formed by the T-junction in the squeezing regime ($Ca < 10^{-2}$), the scaling law $D/d=1+1/Q_r$ is followed at zero fields for droplets with size D , channel width d and flow rate ratio $Q_r=Q_{cp}/Q_{dp}$ [75]. With increasing magnetic field, an increase of $\sim 15\text{ }\mu\text{m}$ in droplet size was observed. This increase was caused by the magnetic volume force, which increases the DP flow, leading to increased droplet size.

However, this behavior was not followed for 1000 mT, where a decrease in the droplet size was observed the size at 500 mT. The non-uniform component of magnetic field $\leq 1\%$ implies that (i) for a field of 500 mT, the nonuniformity of ± 5 mT and (ii) for 1000 mT, the nonuniformity is ± 10 mT. With increasing uniform magnetic field, the elongation of the ferrofluid droplets increases and hence the surface tension of the ferrofluid droplets is higher [72, 73, 76, 77]. The observed decrease in droplet sizes at 1000 mT compared to that for 500 mT is the result of increased surface tension and the non-uniformity of ± 10 mT in the magnetic field.

5.4.2 Droplet merging

Droplet merging under the influence of a magnetic field have been quantified through a study of the variation of merging length (L_m) with magnetic field (H_o). The merging distance L_m is defined as the distance from the T-junction to the center of the droplet at the point of droplet merging (**Figure 5.1a**). Droplet merging under the influence of a magnetic field is the result of competition between the force due to fluidic pressure (which varies with flow rate ratio), surface tension force and magnetic volume force (F_m) [78]. *Droplet merging will occur if the magnetic volume force exceeds the combined forces due to fluidic pressure and surface tension.* This competition divides droplet behavior into three regimes (**Figure 5.3** and **Figure 5.4a**):

Regime 1: Multiple droplet merging, at magnetic field $H_o = 0, 50$ mT for $Q_r \leq 3$ and $H_o = 100$ mT for $Q_r = 2$ (red filled squares in **Figure 5.3a**, experimental micrograph of **Figure 5.3b** and region below the red dotted line in **Figure 5.4a**).

Regime 2: Two droplet merging at $H_o \geq 500$ mT, for all Q_r (blue filled circles in **Figure 5.3a**, experimental micrographs in **Figure 5.3c** and region between red and blue dotted line in **Figure 5.4a**).

Regime 3: No merging, at $Q_r \geq 4$ for magnetic field $H_o \leq 100$ mT. Black filled triangles in **Figure 5.3a**, experimental micrograph of **Figure 5.3d** and region above blue dotted line in **Figure 5.4a**, denoted by dashed arrow lines.

Figure 5.3: (a) Droplet merging map depicting various droplet merging regimes. (b-d) Experimental micrographs (scale bar= 500 μm) for droplet merging regimes: (b) Multiple droplet merging (c) Two droplet merging (d) No merging. Q2, Q3, Q4 and Q5 denotes flow rate ratios. The purple arrow indicates the direction of the CP flow (x-direction). The magnetic field H_o is in the y-direction.

5.4.2.1 Regime 1 and Regime 3

Regime 1, which is the region below the red dotted line in **Figure 5.4a**, shows *multiple droplet merging* (**Figure 5.3b**). It is caused by close spacing of the droplets at low flow rate ratios, viz. $Q_r = 2, 3$ for H_o less than 100 mT. Two droplet merging was observed at 100 mT for a flow rate ratio of 3 (**Figure 5.3a**). The other extreme is regime 3, i.e., the *no merging regime* (**Figure 5.3d**), which is shown by dashed arrow lines in **Figure 5.4a**. This corresponds to a larger merging distance ($L_m \geq 6.5$ mm) for Q_r greater than 3 and H_o less than 500 mT (**Figure 5.3a**). The fluidic pressure force at higher flow rate ratios ($Q_r = 3, 4$) dominates regime 3.

5.4.2.2 Regime 2 (Two Droplet Merging)

Two droplets merging (**Figure 5.3c**) is shown in regime 2 of **Figure 5.4a**, which is the region between the two horizontal (red and blue) dotted lines. The merging distance L_m increases linearly with increasing flow rate ratio at $H_o = 500, 1000$ mT. The magnetic

volume force due to magnetic field $\geq 500\text{mT}$ dominates at all Q_r . Two droplet merging was observed at 500 mT and 1000 mT at all Q_r (**Figure 5.3a**). Two droplet merging was also observed at 100 mT for $Q_r=3$ (**Figure 5.3a**). The merging distance is larger at Q4H1000 than that for Q4H500, which may be due to increased droplet velocity for set Q4H1000 (**Figure 5.4a**). A linear behavior of droplet merging distance with flow rate ratio was observed (**Figure 5.4a**).

Figure 5.4: Variation in droplet merging distance L_m with increasing flow rate ratio Q_r under a uniform magnetic field H (a) Experimental results for $H_0 = 0, 50, 100, 500, 1000$ mT. (b) Experiments vs simulations at $H_0 = 500, 1000$ mT.

5.4.2.3 Transitions between various regimes

The transitions between various regimes can be explained by the competition between three forces: (i) hydrodynamic force (ii) magnetic volume force and (iii) surface tension force. The merging length, L_m was used to quantify these transitions: (i) $L_m \leq 2\text{mm}$ results in multiple droplet merging, indicating low hydrodynamic force (resulting in close spacing) and insufficient magnetic volume force. (ii) $L_m > 6.5\text{ mm}$, indicating high hydrodynamic force (resulting in greater droplet spacing), dominating over magnetic volume force. (iii) $2\text{mm} < L_m \leq 6.5\text{mm}$ leads to controlled merging due to the dominating effect of magnetic volume force over both hydrodynamic and surface tension force.

5.4.3 Simulation of Dependence of L_m on Interfacial tension

At zero magnetic field, the ferrofluid droplets are spherical to minimize surface area i.e., minimum ferrofluid-oil interfacial tension. Under the influence of a *uniform magnetic field*, the ferrofluid droplet *elongates*, deviating from its spherical shape. With increasing magnetic field, the elongation of ferrofluid droplet increases, implying increased ferrofluid-oil interfacial tension[72-74].

Moving ferrofluid droplets in a magnetic field exhibit *pairing of ferrofluid droplets* before merging. The ferrofluid droplets attract each other, form pairs, travel some distance together, and then merge to form a single droplet. This process depends on (i) strength of the magnetic field, (ii) volume of ferrofluid droplet, (iii) flow rates of CP, DP, and (iv) interfacial interactions. Interfacial interactions are due to (i) surfactants and (iii) increased ferrofluid-oil interfacial tension due to droplet elongation in the magnetic field. A *resultant interfacial tension* (RIT) between the ferrofluid and the silicone oil was used to calculate the droplet merging distance in our simulations. The droplet merging distance is a function of magnetic field strength, *resultant interfacial tension* and flow rates of CP, DP. **Figure 5.5** shows the variation in droplet merging distance with RIT at a magnetic field of 500 mT and flow rate ratio of 3. The merging distance increases slowly for $RIT \leq 10$ mN/m and rapidly for RIT greater than 10 mN/m. For RIT greater than 20 mN/m no merging was observed, indicating that interfacial forces dominate over the magnetic field. The modeled merging distance at RIT of 15 mN/m matches with an experimental value for $Q_r=3$ and $H_o=500$ mT (**Figure 5.4b**). This value is higher than the value of 12 mN/m for ferrofluid-silicone oil interfacial tension without magnetic field[64].

5.4.4 Experimental and Simulation Results of Droplet Merging at $H \geq 500$ mT

Figure 5.4b shows the experimental and simulation results for the variation of merging distance with increasing flow rate ratio. These micromagnetofluidic numerical simulations were performed for regime 2, which corresponds to the magnetic field induced merging at 500 mT and 1000 mT. Evidently, from **Figure 5.4b**, the simulation and experimental results are in good agreement. However, deviation was observed at lower flow rate ratios for $H_o=500, 1000$ mT, which shows a linear variation compared to our experimental results.

The observed mismatch may be due to non-linear effects in the experimental flow rates, which become significant at lower flow rate ratios compared to the magnetic volume force.

Figure 5.5: Simulations of the dependence of merging distance on the *resultant interfacial tension* at magnetic field $H_0=500$ mT and flow rate ratio $Q_r=3$. At $RIT \leq 20$ mN/m merging was observed.

5.4.5 Process of Droplet Merging

To study the process of droplet merging, the variation in droplet velocity was investigated with time at a constant flow rate ratio and $H_0=500$ mT, for sets Q2H500 and Q5H500 (Table 5.1). Two droplets traveling with velocity V_a and V_b were assumed (Figure 5.1a): V_a indicates the velocity of droplet a, which enters first in the microchannel, and V_b is the velocity of the droplet b, which follows droplet a. V_{ab} is the velocity of the merged droplet ab (Figure 5.1a). Figure 5.6 shows the variation in droplet velocity with time. Negative times indicates velocity variation before merging, positive time indicates velocity variation after merging. The region between the two vertical dash-dotted red lines indicates the droplets merging region (DMR) (Figure 5.6 and Figure 5.7). Before DMR, droplets exist

in the non-merged state; after DMR the droplets have merged and travel as a single entity. The transition merging points (TMPs) were assigned a time equal to 0s. The merged interface length of the droplets a and b is denoted by MIL. A value of MIL greater than half of the droplet circumference (HDC) denotes complete merging of the droplets. High-speed micrographs of the DMR revealed various states of droplet merging with time (**Figure 5.7a**) viz. (i) at time of - 40ms, droplet interfaces come in contact (ii) MIL is less than HDC [for time < 0ms] (iii) MIL is equal to HDC [for time = 0s] (iv) MIL is greater than HDC [for time > 0ms] (v) complete merging of the droplet interfaces, at the end of DMR [for time > 0 ms] corresponds to a minimum velocity V_{ab} (**Figure 5.6** and **Figure 5.7b**).

Figure 5.6: Experimental variation in droplet velocity at $H_0=500$ mT for Q2 and Q5. (a) Velocity variation before merging (V_a , V_b) and after merging (V_{ab}). (b) Variation in differential velocity $V_b - V_a$ with time, implying the necessary condition for droplet merging of $V_b - V_a > 0$.

Assuming that the direction of CP flow is the positive direction of velocity, there are three possible cases with respect to the variation of droplet velocity V_a and V_b with time, which result in merging of two moving droplets: (i) V_a , V_b travelling in the flow direction [$V_b > V_a$] (ii) V_a and V_b travelling in the opposite flow direction [$-V_a > -V_b$] (iii) both droplets travel towards each other. The required condition for droplet merging, that the differential velocity, $V_b - V_a$ should be positive. It is evident from **Figure 5.6b** that near DMR, the differential velocity increases, satisfying $V_b - V_a > 0$ for both sets Q2H500 and

Q5H500. At TMP, $V_b - V_a$ reaches to a maximum value, resulting in the merging of droplet a and droplet b.

Figure 5.7: Time variation of droplet velocity during merging at $H_0 = 500\text{mT}$ and flow rate ratio Q5. (a) Experimental micrograph of DMR, states of the droplet merging with respect to time are encircled, with TMP at 0ms. The purple arrow indicates the direction of the CP flow (x-direction). The magnetic field is in the y-direction. (b) Simulations vs Experiments. Purple, blue, and black arrows are to guide the eye for V_a , V_b , and V_{ab} , respectively.

It is interesting to note that our simulation results for the time variation of velocity are in close agreement with experiments (**Figure 5.7b**). The droplet velocity V_a decreases and V_b increases for both experiments (**Figure 5.6a** and **Figure 5.7b**) and simulations (**Figure 5.7b**). Close to the TMP, the droplet velocity V_b attains a maximum value, which was also observed in our simulations. The simulated V_b was found to be slightly higher than the experimental V_b . At TMP, droplets merge and beyond TMP, the merged droplet travels with a velocity V_{ab} , which initially falls (**Figure 5.7b**). V_{ab} increases beyond DMR and then saturates. The simulated droplet velocity profile V_{ab} was found to be slightly higher than the experimental V_{ab} (**Figure 5.7b**). This mismatch may be due to modeling, which neglects interparticle interactions of magnetic particles.

5.5 Application of Magnetically Induced Merging

Merging and mixing of droplets on a LoC platform was demonstrated using color dye + magnetite (ferrofluid) composite droplets (**Figure 5.1b** and **Figure 5.8**). Two composite droplets were used, both containing 10% loading of ferrofluid in (i) 10 % (v/v) blue dye in deionized water (ii) yellow dye solution. A schematic of the experiment is shown in **Figure 5.1b**. These composite droplets were generated at flow rates of (i) CP = 120 μ l/h (ii) DP1 (blue droplets) = 20 μ l/h (iii) DP2 (yellow droplets) = 10 μ l/h.

Evidently (**Figure 5.8**), no merging occurs when the magnetic field was not applied. When a magnetic field of 1000mT was applied, blue droplets merge and mix with yellow droplets, yielding green droplets. The mixing of blue and yellow composite magnetic droplet is evident from the green color (**Figure 5.8**).

Figure 5.8: Magnetically induced merging of composite droplets (a) No merging at $H_0 = 0 \text{ mT}$ (b) Merged green droplets at $H_0 = 1000 \text{ mT}$. The CP flow is along the x-direction. The magnetic field is in the y-direction. Composite droplets were generated at flow rates of (i) CP = $120 \mu\text{l/h}$ (ii) DP1 (blue droplets) = $20 \mu\text{l/h}$ (iii) DP2 (yellow droplets) = $10 \mu\text{l/h}$.

5.6 Conclusions

Droplet merging in a Lab-on-a-Chip environment under the influence of a *uniform* magnetic field was investigated. The effect of applied magnetic field and flow rate ratio was investigated. Control of the droplet merging distance L_m was demonstrated, L_m increases with increasing flow rate ratio. A droplet micro-magnetofluidic model was developed, our numerical results are in good agreement with experimental results. The condition for droplet merging was found to be that the differential velocity of two droplets a and b, $V_b - V_a$ should be positive; this was verified experimentally and numerically. Droplet merging was studied through variation of the droplet velocity with time under the influence of a uniform magnetic field. Merging and mixing of composite magnetic droplets was demonstrated under the influence of a magnetic field. The present work is useful for wireless, programmable merging and mixing of droplets on a LoC platform, which finds applications in biosensing, bioassay, microfluidic-based synthesis, reaction kinetics, and magnetochemistry.

References

- [1] R. Seemann, M. Brinkmann, T. Pfohl, and S. Herminghaus, "Droplet based microfluidics," *Rep Prog Phys*, vol. 75, p. 016601, 2012.
- [2] S. Y. Teh, R. Lin, L. H. Hung, and A. P. Lee, "Droplet microfluidics," *Lab on a Chip*, vol. 8, pp. 198-220, 2008.
- [3] M. Joanicot and A. Ajdari, "Applied physics. Droplet control for microfluidics," *Science*, vol. 309, pp. 887-8, 2005.
- [4] G. Katsikis, J. S. Cybulski, and M. Prakash, "Synchronous universal droplet logic and control," *Nature Physics*, vol. 11, pp. 588-96, 2015.
- [5] N. J. Ciria, A. Benusiglio, and M. Prakash, "Vapour-mediated sensing and motility in two-component droplets," *Nature*, vol. 519, pp. 446-50, 2015.
- [6] J. Puigmarti-Luis, "Microfluidic platforms: a mainstream technology for the preparation of crystals," *Chemical Society Reviews*, vol. 43, pp. 2253-71, 2014.
- [7] S. Badilescu and M. Packirisamy, "Microfluidics-Nano-Integration for Synthesis and Sensing," *Polymers*, vol. 4, pp. 1278-310, 2012.
- [8] S. B. Lowe, V. T. Tan, A. H. Soeriyadi, T. P. Davis, and J. J. Gooding, "Synthesis and high-throughput processing of polymeric hydrogels for 3D cell culture," *Bioconjug Chem*, vol. 25, pp. 1581-601, 2014.
- [9] Y. Zhao, L. Shang, Y. Cheng, and Z. Gu, "Spherical colloidal photonic crystals," *Acc Chem Res*, vol. 47, pp. 3632-42, 2014.
- [10] S. Lone and I. W. Cheong, "Fabrication of polymeric Janus particles by droplet microfluidics," *Rsc Advances*, vol. 4, pp. 13322-33, 2014.
- [11] Y. Cheng, C. Zhu, Z. Xie, H. Gu, T. Tian, Y. Zhao, and Z. Gu, "Anisotropic colloidal crystal particles from microfluidics," *J Colloid Interface Sci*, vol. 421, pp. 64-70, 2014.
- [12] W. Wang, M. J. Zhang, and L. Y. Chu, "Functional polymeric microparticles engineered from controllable microfluidic emulsions," *Acc Chem Res*, vol. 47, pp. 373-84, 2014.
- [13] Q. Xu, M. Hashimoto, T. T. Dang, T. Hoare, D. S. Kohane, G. M. Whitesides, R. Langer, and D. G. Anderson, "Preparation of monodisperse biodegradable polymer microparticles using a microfluidic flow-focusing device for controlled drug delivery," *Small*, vol. 5, pp. 1575-81, 2009.
- [14] D. Dendukuri and P. S. Doyle, "The Synthesis and Assembly of Polymeric Microparticles Using Microfluidics," *Advanced Materials*, vol. 21, pp. 4071-86, 2009.
- [15] W. J. Duncanson, T. Lin, A. R. Abate, S. Seiffert, R. K. Shah, and D. A. Weitz, "Microfluidic synthesis of advanced microparticles for encapsulation and controlled release," *Lab on a Chip*, vol. 12, pp. 2135-45, 2012.
- [16] G. Lin, D. Makarov, M. Medina-Sanchez, M. Guix, L. Baraban, G. Cuniberti, and O. G. Schmidt, "Magnetofluidic platform for multidimensional magnetic and optical barcoding of droplets," *Lab on a Chip*, vol. 15, pp. 216-24, 2015.
- [17] D. T. Chong, X. S. Liu, H. J. Ma, G. Y. Huang, Y. L. Han, X. Y. Cui, J. J. Yan, and F. Xu, "Advances in fabricating double-emulsion droplets and their biomedical applications," *Microfluidics and Nanofluidics*, vol. 19, pp. 1071-90, 2015.
- [18] B. Schlicht and M. Zagnoni, "Droplet-interface-bilayer assays in microfluidic passive networks," *Sci Rep*, vol. 5, p. 9951, 2015.
- [19] L. Malic, D. Brassard, T. Veres, and M. Tabrizian, "Integration and detection of biochemical assays in digital microfluidic LOC devices," *Lab Chip*, vol. 10, pp. 418-31, 2010.
- [20] D. Barata, C. van Blitterswijk, and P. Habibovic, "High-throughput screening approaches and combinatorial development of biomaterials using microfluidics," *Acta Biomater*, vol. 34, pp. 1-20, 2016.

- [21] T. P. Lagus and J. F. Edd, "A review of the theory, methods and recent applications of high-throughput single-cell droplet microfluidics," *Journal of Physics D-Applied Physics*, vol. 46, p. 21, 2013.
- [22] L. Dong, D.-W. Chen, S.-J. Liu, and W. Du, "Automated Chemotactic Sorting and Single-cell Cultivation of Microbes using Droplet Microfluidics," *Scientific Reports*, vol. 6, p. 24192, 2016.
- [23] J. C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, J. J. Agresti, D. R. Link, D. A. Weitz, and A. D. Griffiths, "Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity," *Lab on a Chip*, vol. 9, pp. 1850-8, 2009.
- [24] C. Plessy, L. Desbois, T. Fujii, and P. Carninci, "Population transcriptomics with single-cell resolution: a new field made possible by microfluidics: a technology for high throughput transcript counting and data-driven definition of cell types," *Bioessays*, vol. 35, pp. 131-40, 2013.
- [25] H. Zec, D. J. Shin, and T. H. Wang, "Novel droplet platforms for the detection of disease biomarkers," *Expert Rev Mol Diagn*, vol. 14, pp. 787-801, 2014.
- [26] K. V. Kaler and R. Prakash, "Droplet microfluidics for chip-based diagnostics," *Sensors (Basel)*, vol. 14, pp. 23283-306, 2014.
- [27] J. Atencia and D. J. Beebe, "Controlled microfluidic interfaces," *Nature*, vol. 437, pp. 648-55, 2005.
- [28] M. J. Jebrail, M. S. Bartsch, and K. D. Patel, "Digital microfluidics: a versatile tool for applications in chemistry, biology and medicine," *Lab on a Chip*, vol. 12, pp. 2452-63, 2012.
- [29] A. B. Theberge, F. Courtois, Y. Schaerli, M. Fischlechner, C. Abell, F. Hollfelder, and W. T. S. Huck, "Microdroplets in Microfluidics: An Evolving Platform for Discoveries in Chemistry and Biology," *Angewandte Chemie-International Edition*, vol. 49, pp. 5846-68, 2010.
- [30] P. Neuzi, S. Giselbrecht, K. Lange, T. J. Huang, and A. Manz, "Revisiting lab-on-a-chip technology for drug discovery," *Nat Rev Drug Discov*, vol. 11, pp. 620-32, 2012.
- [31] O. J. Dressler, R. M. Maceiczky, S. I. Chang, and A. J. deMello, "Droplet-based microfluidics: enabling impact on drug discovery," *J Biomol Screen*, vol. 19, pp. 483-96, 2014.
- [32] G. T. Vladislavljevic, N. Khalid, M. A. Neves, T. Kuroiwa, M. Nakajima, K. Uemura, S. Ichikawa, and I. Kobayashi, "Industrial lab-on-a-chip: design, applications and scale-up for drug discovery and delivery," *Adv Drug Deliv Rev*, vol. 65, pp. 1626-63, 2013.
- [33] N. Shembekar, C. Chaipan, R. Utharala, and C. A. Merten, "Droplet-based microfluidics in drug discovery, transcriptomics and high-throughput molecular genetics," *Lab Chip*, vol. 16, pp. 1314-31, 2016.
- [34] A. Hibara, M. Fukuyama, M. Chung, C. Priest, and M. A. Proskurnin, "Interfacial Phenomena and Fluid Control in Micro/Nanofluidics," *Anal Sci*, vol. 32, pp. 11-21, 2016.
- [35] D. Guo, D. Zhu, X. Zhou, and B. Zheng, "Accelerating the "On Water" Reaction: By Organic-Water Interface or By Hydrodynamic Effects," *Langmuir*, vol. 31, pp. 13759-63, 2015.
- [36] D. H. Yoon, A. Jamshaid, J. Ito, A. Nakahara, D. Tanaka, T. Akitsu, T. Sekiguchi, and S. Shoji, "Active microdroplet merging by hydrodynamic flow control using a pneumatic actuator-assisted pillar structure," *Lab on a Chip*, vol. 14, pp. 3050-55, 2014.
- [37] M. Sesen, T. Alan, and A. Neild, "Microfluidic on-demand droplet merging using surface acoustic waves," *Lab on a Chip*, vol. 14, pp. 3325-33, 2014.

- [38] M. Viefhues, R. Eichhorn, E. Fredrich, J. Regtmeier, and D. Anselmetti, "Continuous and reversible mixing or demixing of nanoparticles by dielectrophoresis," *Lab on a Chip*, vol. 12, pp. 485-94, 2012.
- [39] L. Mazutis and A. D. Griffiths, "Selective droplet coalescence using microfluidic systems," *Lab on a Chip*, vol. 12, pp. 1800-06, 2012.
- [40] M. Y. Zhang, L. M. Wang, X. Wang, J. B. Wu, J. X. Li, X. Q. Gong, J. H. Qin, W. H. Li, and W. J. Wen, "Microdroplet-based universal logic gates by electrorheological fluid," *Soft Matter*, vol. 7, pp. 7493-97, 2011.
- [41] Z. G. Li, K. Ando, J. Q. Yu, A. Q. Liu, J. B. Zhang, and C. D. Ohl, "Fast on-demand droplet fusion using transient cavitation bubbles," *Lab on a Chip*, vol. 11, pp. 1879-85, 2011.
- [42] M. Zagnoni and J. M. Cooper, "On-chip electrocoalescence of microdroplets as a function of voltage, frequency and droplet size," *Lab on a Chip*, vol. 9, pp. 2652-58, 2009.
- [43] L. H. Hung, K. M. Choi, W. Y. Tseng, Y. C. Tan, K. J. Shea, and A. P. Lee, "Alternating droplet generation and controlled dynamic droplet fusion in microfluidic device for CdS nanoparticle synthesis," *Lab on a Chip*, vol. 6, pp. 174-8, 2006.
- [44] H. J. Li, Y. N. Wu, X. D. Wang, C. Y. Zhu, T. T. Fu, and Y. G. Ma, "Magnetofluidic control of the breakup of ferrofluid droplets in a microfluidic Y-junction," *Rsc Advances*, vol. 6, pp. 778-85, 2016.
- [45] T. T. Fu, Y. G. Ma, and H. Z. Li, "Breakup dynamics of slender droplet formation in shear-thinning fluids in flow-focusing devices," *Chemical Engineering Science*, vol. 144, pp. 75-86, 2016.
- [46] W. Du, T. T. Fu, C. Y. Zhu, Y. G. Ma, and H. Z. Li, "Breakup Dynamics for High-Viscosity Droplet Formation in a Flow-Focusing Device: Symmetrical and Asymmetrical Ruptures," *Aiche Journal*, vol. 62, pp. 325-37, 2016.
- [47] Y. N. Wu, T. T. Fu, C. Y. Zhu, X. D. Wang, Y. G. Ma, and H. Z. Li, "Shear-induced tail breakup of droplets (bubbles) flowing in a straight microfluidic channel," *Chemical Engineering Science*, vol. 135, pp. 61-66, 2015.
- [48] Y. N. Wu, T. T. Fu, Y. G. Ma, and H. Z. Li, "Active control of ferrofluid droplet breakup dynamics in a microfluidic T-junction," *Microfluidics and Nanofluidics*, vol. 18, pp. 19-27, 2015.
- [49] A. Beyzavi and N. T. Nguyen, "One-dimensional actuation of a ferrofluid droplet by planar microcoils," *Journal of Physics D-Applied Physics*, vol. 42, 2009.
- [50] A. Beyzavi and N. T. Nguyen, "Programmable two-dimensional actuation of ferrofluid droplet using planar microcoils," *Journal of Micromechanics and Microengineering*, vol. 20, p. 8, 2010.
- [51] N. T. Nguyen, A. Beyzavi, K. M. Ng, and X. Y. Huang, "Kinematics and deformation of ferrofluid droplets under magnetic actuation," *Microfluidics and Nanofluidics*, vol. 3, pp. 571-79, 2007.
- [52] N. T. Nguyen, K. M. Ng, and X. Y. Huang, "Manipulation of ferrofluid droplets using planar coils," *Applied Physics Letters*, vol. 89, p. 052509, 2006.
- [53] C. Y. Chen, H. C. Hsueh, S. Y. Wang, and Y. H. Li, "Self-assembly and novel planetary motion of ferrofluid drops in a rotational magnetic field," *Microfluidics and Nanofluidics*, vol. 18, pp. 795-806, 2015.
- [54] C.-Y. Chen and C. S. Li, "Ordered microdroplet formations of thin ferrofluid layer breakups," *Physics of Fluids*, vol. 22, p. 014105, 2010.
- [55] S. Kahkeshani and D. Di Carlo, "Drop formation using ferrofluids driven magnetically in a step emulsification device," *Lab on a Chip*, vol. 16, pp. 2474-80, 2016.
- [56] J. S. Sander, R. M. Erb, C. Denier, and A. R. Studart, "Magnetic transport, mixing and release of cargo with tailored nanoliter droplets," *Adv Mater*, vol. 24, pp. 2582-7, 10, 2012.

- [57] D. Xiao, Y. W. Liu, Y. Zhou, S. M. Mohseni, S. Chung, and J. Akerman, "Merging droplets in double nanocontact spin torque oscillators," *Physical Review B*, vol. 93, p. 5, 2016.
- [58] A. Ahmadi, C. M. McDermid, and L. Markley, "Magnetohydrodynamic actuation of droplets for millimetric planar fluidic systems," *Applied Physics Letters*, vol. 108, p. 4, 2016.
- [59] B. Teste, N. Jamond, D. Ferraro, J. L. Viovy, and L. Malaquin, "Selective handling of droplets in a microfluidic device using magnetic rails," *Microfluidics and Nanofluidics*, vol. 19, pp. 141-53, 2015.
- [60] N. Pamme, "Magnetism and microfluidics," *Lab Chip*, vol. 6, pp. 24-38, 2006.
- [61] C. Y. Chen, W. K. Tsai, and J. A. Miranda, "Experimental study of a hybrid ferrohydrodynamic instability in miscible ferrofluids: Droplet size effects," *Magnetohydrodynamics*, vol. 45, pp. 3-14, 2009.
- [62] C. Y. Chen and Z. Y. Cheng, "An experimental study on Rosensweig instability of a ferrofluid droplet," *Physics of Fluids*, vol. 20, p. 054105, 2008.
- [63] J. Liu, Y. F. Yap, and N. T. Nguyen, "Numerical study of the formation process of ferrofluid droplets," *Physics of Fluids*, vol. 23, p. 072008, 2011.
- [64] J. Liu, S. H. Tan, Y. F. Yap, M. Y. Ng, and N. T. Nguyen, "Numerical and experimental investigations of the formation process of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 11, pp. 177-87, 2011.
- [65] G. P. Zhu, N. T. Nguyen, R. V. Ramanujan, and X. Y. Huang, "Nonlinear Deformation of a Ferrofluid Droplet in a Uniform Magnetic Field," *Langmuir*, vol. 27, pp. 14834-41, 2011.
- [66] Z. M. Wang, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Trapping of Bacteria at Low Magnetic Fields," *Sci Rep*, vol. 6, p. 26945, 2016.
- [67] G. P. Zhu and N. T. Nguyen, "Rapid magnetofluidic mixing in a uniform magnetic field," *Lab on a Chip*, vol. 12, pp. 4772-80, 2012.
- [68] Z. M. Wang, V. B. Varma, H. M. Xia, Z. P. Wang, and R. V. Ramanujan, "Spreading of a ferrofluid core in three-stream micromixer channels," *Physics of Fluids*, vol. 27, p. 052004, 2015.
- [69] J. E. Burroughs and C. P. Goodrich, "Rapid method for determining densities of liquids using micro syringes," *Analytical Chemistry*, vol. 46, pp. 1614-15, 1974.
- [70] Z. M. Wang, V. B. Varma, Z. P. Wang, and R. V. Ramanujan, "Tuning magnetofluidic spreading in microchannels," *Journal of Micromechanics and Microengineering*, vol. 25, p. 124001, 2015.
- [71] M. L. Boas, *Mathematical methods in the physical sciences*, 3 ed.: Wiley New York, 2006.
- [72] S. Afkhami, A. J. Tyler, Y. Renardy, M. Renardy, T. G. St Pierre, R. C. Woodward, and J. S. Riffle, "Deformation of a hydrophobic ferrofluid droplet suspended in a viscous medium under uniform magnetic fields," *Journal of Fluid Mechanics*, vol. 663, pp. 358-84, 2010.
- [73] S. Banerjee, M. Fasnacht, S. Garoff, and M. Widom, "Elongation of confined ferrofluid droplets under applied fields," *Physical Review E*, vol. 60, p. 4272, 1999.
- [74] D. X. Shi, Q. C. Bi, and R. Q. Zhou, "Numerical Simulation of a Falling Ferrofluid Droplet in a Uniform Magnetic Field by the VOSET Method," *Numerical Heat Transfer Part a-Applications*, vol. 66, pp. 144-64, 2014.
- [75] P. Garstecki, M. J. Fuerstman, H. A. Stone, and G. M. Whitesides, "Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up," *Lab on a Chip*, vol. 6, pp. 437-46, 2006.
- [76] V. B. Varma, A. Ray, Z. M. Wang, Z. P. Wang, R. G. Wu, P. J. Jayaneel, N. M. Sudharsan, and R. V. Ramanujan, "Control of Ferrofluid Droplets in Microchannels by Uniform Magnetic Fields," *IEEE Magnetics Letters*, vol. 7, pp. 1-5, 2016.

- [77] C. P. Lee, S. T. Yang, and Z. H. Wei, "Field dependent shape variation of magnetic fluid droplets on magnetic dots," *Journal of Magnetism and Magnetic Materials*, vol. 324, pp. 4133-35, 2012.
- [78] A. Ghaffari, S. H. Hashemabadi, and M. Bazmi, "CFD simulation of equilibrium shape and coalescence of ferrofluid droplets subjected to uniform magnetic field," *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, vol. 481, pp. 186-98, 2015.

Chapter 6

Synthesis of Magnetic Janus Particles by Droplet Micro-magnetofluidic Techniques[~]

Synthesis of Janus particles with two accessible functionalities is a challenge. The DMMF technique with wireless, programmable and remote-control capabilities offers an approach to address this challenge. The DMMF technique, employing hybrid magnetic fields was used for the synthesis of magneto-polymer Janus particles. This method is useful for the controlled mixing, separation, and polymerization on LoC and capillary microfluidic platforms. The capillary microfluidic platform was found to be more advantageous due to its adaptability, flexibility and low cost. The magnetization, particle size, and configuration of the Janus particles can be tuned by a combination of magnetic fields and flow rates. An application of synthesized Janus particles was demonstrated for protein detection, with BSA as a model protein.

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6.1 Introduction

Droplet microfluidics[1-4] (DMF) is a useful technique for the manipulation of matter[5] on a microfluidic platform. Each droplet acts as an isolated reaction container on the microscale, with a wide volume range (μl to pL) and multiplexing capabilities[3, 6]. Hence, DMF is a very promising technique for a range of applications. DMF utilized for various biochemical assays such as enzyme inhibition assays[7], biochemical analysis[8], microbiology[9]. DMF based single cell analysis was performed for the detection of protein expression[10], cell culture, cell lysis, drug efficacy studies [11], single cell transcriptomics[12], stem cell engineering[13], and single-cell barcoding[14]. DMF techniques employed for cancer research include DNA, protein marker detection[15], single molecule analysis[16]. DMF is also recognized as a novel platform for materials science and engineering for the synthesis of hydrogel microparticles[17], photocrosslinkable materials[18], polymer particles [19], multifunctional particles[20], and particles for drug delivery applications [21, 22] with control on morphology [23] and mechanical properties [23] of particles.

Specifically, there is increasing interest in research and development of DMF techniques for the synthesis of Janus particles, progress is summarized in various review articles[24-26]. The word Janus is coined from the Roman God Janus who has two faces, one face looking to the past and other face looking towards the future. Janus particles are a class of multifunctional particles, where the constituent phases are accessible and available for use. Janus particles overcome the limitation of the core-shell structure, where one phase is surrounded by another phase, decreasing the net advantage. In contrast to the core-shell structure, Janus particles provide anisotropic control on the constituent phases with direct access to the phases, e.g., hydrophilic-hydrophobic, magnetic-diamagnetic, magnetic-optical, magnetic-plasmonic, and magnetic-photonic.

However, synthesis of Janus particles with control of the constituent phases is challenging. The significance and challenges in the synthesis of Janus particles are reviewed by various research groups: (i) Heida et al.[27] reviewed mechanical properties, control on functionalization and crosslinking density. (ii) Zhao et al.[28] reviewed photonic crystal based Janus particles. (iii) Nisisako [24] reviewed progress and trends of microfluidic

approaches utilized for Janus droplets and Janus particles fabrication. (iv) Lone et al.[25] summarized DMF approaches for the fabrication of Janus particles. (v) Kim et al.[29] reviewed the current state of the art of DMF based synthesis utilized for functional microparticles. The synthesis begins with droplet generation, which requires an understanding of the relevant dimensionless numbers, and selection of either an active or a passive method for droplet generation [1-4].

The next stage is the integration of various components for Janus particle synthesis. In the literature, different strategies were outlined for the synthesis of Janus particles (**Table 6.1**). Um et al.[30] used an electric charge concentration (ECC) method for droplet generation, control, and fabrication of Janus particles of size ~ 1 mm. Their method was operated in three modes (i.e., attaching, uniform, and bursting) by tuning flow rates, voltages, and oil surface-nozzle distances. They combined their ECC with UV polymerization to fabricate polyethylene glycol diacrylate (PEGDA) based Janus particles containing CaCl_2 : alginate. Lan et al.[31] used a flow focusing configuration for the fabrication of alginate based Janus particles of magnetite: CdSe/ZnS quantum dots. The fabrication was performed by ionic crosslinking of the alginate phase by the continuous phase containing Ca^{2+} . These Janus particles were then utilized for DNA assays. Hessberger et al.[32] utilized a capillary-based microfluidic setup integrated with UV polymerization to fabricate actuating Janus particles. The Janus system was composed of a hydrophobic liquid crystalline part and a hydrophilic polyacrylamide network, with particle size of ~ 700 μm .

Zarzar et al.[33] demonstrated dynamic control of complex emulsions and utilized it for Janus particle synthesis by tuning the interfacial tensions of the constituent phases. They photopolymerized the four-phase configuration, resulting in magnetite-based Janus particles. They suggested application of the method for controlled release of drugs at tumors, camouflage, tunable lenses, and sensors. Takimoto et al.[34] used the inverse suspension polymerization approach to fabricate molecularly imprinted submillimeter microgels. They used water-in-oil (W/O) droplets containing water-soluble monomer, formed by a microchannel and photopolymerized using UV light. The synthesized molecularly imprinted microgels were then utilized for the detection of BSA and human serum albumin(HSA). Li et al.[35] fabricated Janus microspheres of carbon black: PTFE

using a simple capillary microfluidic device by drying the droplets in an oven at 90°C for 24 h. The optical and electrical anisotropy of the particles was controlled by tuning flow rates and flow rate ratios. Detailed investigation of the electro-responsive properties was also performed. Hu et al.[36] utilized off-chip ionic cross-linking for fabrication of shape controllable alginate: pNIPAAm Janus microgels. Droplet generation was performed by a microfluidic coflowing configuration. Ionic cross-linking was performed by a hot aqueous solution of glycerol + barium acetate, at various concentrations. The shape and surface morphology of the particles was tuned by changing initial droplet size and glycerol concentration in the crosslinking solution.

Kim et al.[37] used the DMF approach combined with directional UV curing for the synthesis of asymmetric porous Janus particles. Their Janus structures were prepared with poly(tripropylene glycol diacrylate) poly(TPGDA)) as the matrix, containing a smooth structure in one part and porous structure in the other part. They used a flow focusing geometry for droplet generation and polymerized particles using a directional UV light, resulting in Janus particles of size $\sim 100\ \mu\text{m}$. Chen et al.[38] utilized a single emulsion DMF approach for the fabrication of Janus particles. Their Janus system composed of branched polymers containing colloidal particles (PMMA microspheres, TiO_2 particles, or magnetite nanoparticles). The Janus configuration was achieved by separation under magnetic field and gravity. They used a coflowing geometry for droplet generation, combined with evaporation of water, resulting in Janus particles of $\sim 500\ \mu\text{m}$ size.

Yu et al.[39] synthesized photonic Janus particles by photopolymerization of EOTMPTA based polystyrene: magnetite nanoparticles. They used a balance between interfacial forces to control the geometry between biphasic droplets. Swelling and photonic properties (colors) of the particles was controlled by acrylic acid. Application of the particles was demonstrated for multicolor patterns controlled by a magnetic field. Xie et al.[40] synthesized Janus particles useful for drug delivery applications containing a hydrophilic: hydrophobic drug. They developed a fluidic nanoprecipitation system for one-step fabrication of PLGA based sub-micrometer Janus particles. They used a T-junction configuration with two parallel inlets for dispersed phases, resulting in a Janus particle size of $\sim 400\ \text{nm}$.

Table 6.1: Comparison of various methods utilized for the synthesis of Janus particles. Notations: Dp: particle size, PPMZ: photopolymerization, DNA: deoxyribonucleic acid, PEGDA: poly(ethylene glycol diacrylate), HSA: human serum albumin, PTPGDA: poly(tripropylene glycol diacrylate), μ PMMA: PMMA microparticles, nano-TiO₂: TiO₂ nanoparticles, or nano-Fe₃O₄: magnetite nanoparticles, PNIPAAm: poly(N-isopropylacrylamide).

Research Group	Approach/ Method	D _p (μm)	Janus Components	Applications/ Advantage
S.J. Lee ³⁰	PPMZ+ electric charge concentration	1000	CaCl ₂ : alginate in PEGDA	electrostatic self-assembly
Z.K. He ³¹	Ionic cross-linking of the alginate by Ca ²⁺ solution	60	magnetite: CdSe/ZnS quantum dots	DNA assays
R. Zentel ³²	PPMZ+ capillary microfluidic	700	hydrophobic liquid crystals: hydrophilic polyacrylamide	liquid crystals
T.M. Swager ³³	PPMZ+ emulsions tuned by interfacial tensions	100	four-phase configuration with nano-Fe ₃ O ₄	drug release at tumours, sensors camouflage,
T. Takeuchi ³⁴	Inverse suspension PPMZ	≥ 1000	molecularly imprinted microgels	human serum albumin detection
Z.Q. Chang ³⁵	Drying the droplets at 90°C for 24 h	160	carbon black: PTFE	Optical, electrical control
M. Ardekani ³⁶	ionic cross-linking by hot glycerol + barium acetate	140	alginate: PNIPAAm	shape controlled particle fabrication
S.Y. Park ³⁷	PPMZ on DMF platform	100	Smooth: porous PTPGDA	self-assembly of particles
S. Bon ³⁸	DMF + separation in magnetic field, gravity	500	μ PMMA: nano-TiO ₂ , or nano-Fe ₃ O ₄	Temperature, pH based entrapment and release
S. Chen ³⁹	PPMZ + control of interfacial forces	150	photonic, polystyrene: nano-Fe ₃ O ₄	magnetic multicolour patterns
J.W. Smith ⁴⁰	Fluidic nano-precipitation	0.5	hydrophilic: hydrophobic	drug delivery
P.S. Doyle ⁴¹	Continuous flow lithography	130	PEGDA+ rhodamine	shape controlled particles
P.S. Doyle ⁴²	Stop-flow lithography	250	porosity controlled PEGDA	transferrin detection

The Doyle group used continuous flow lithography[41] and stop flow lithography[42] for the fabrication of barcoded Janus particles. For the continuous lithography approach, they used a single phase based synthesis of morphologically complex multifunctional particle synthesis. They investigated particle size control by flow rates and particle shape control by lithography masks. For the stop flow lithography approach, they used PEGDA as the hydrogel medium containing constituent Janus components. They described the protocol utilized for fabrication, barcoding, mask, functionalization, and scanning procedure. Application of the synthesized Janus barcodes was demonstrated for protein detection.

Though various approaches have been utilized in the literature (**Table 6.1**), magnetic field was not explicitly used for dynamic control of Janus droplets and for synthesis of Janus particles. Our droplet micro-magnetofluidic (DMMF) technique offers a versatile approach

with wireless, programmable and remote control of droplets need to synthesize Janus particles.

A DMMF technique combined with hybrid magnetic fields was developed for the synthesis of magnetic Janus particles (MJP). The hybrid magnetic field ($H=H_o+H_{no}$) is a combination of uniform magnetic field (H_o) and non-uniform magnetic field (H_{no}). This method is useful to perform controlled polymerization on LoC and capillary microfluidic platforms. The capillary microfluidic platform was found to be more adaptable, flexible and can be developed at low cost. Hence, a capillary DMMF platform was used for the current studies. The effect of flow rates and applied magnetic field on mixing, separation of magnetic phase inside droplets, as well as magnetization, particle size and configuration of the magnetic part in MJP was investigated in detail. The properties of MJP were studied by VSM, FTIR, DSC, TGA and SEM techniques and protein detection demonstrated with BSA as a model protein.

6.2 Experimental Methodology

The schematic (**Figure 6.1** and **Figure 6.2**), details of materials used in the experiments, the microfluidic chip, and experimental parameters are summarized in this section.

Figure 6.1: (a) Schematic of DMMF based Janus particle synthesis. Magnetic Janus droplet (MJD) generation was performed by two configurations of flow focusing geometry: (i) LoC configuration consisting of a PMMA microfluidic chip, with dimensions: $a=100\ \mu\text{m}$, $W=300\ \mu\text{m}$. (ii) Capillary

DMF configuration consisting of a micro flow focusing device with dimensions: $a=150\ \mu\text{m}$, $W=250\ \mu\text{m}$. The continuous phase (CP) consists of hexane and the dispersed phase (DP) consists of co-flowing streams of magnetic polymeric phase (MPP) and a functional polymeric phase (FPP). (b) Magnetic field distribution along $\pm x$ direction. The hybrid magnetic field ' $H=H_o+H_{no}$ ' was generated by two Nd-Fe-B magnets facing each other. Janus particle synthesis was performed at 440 mT by photopolymerization under an UV LED. MJD flow is along the x direction. UV LED and the hybrid magnetic fields are along the y direction. The position $x=0\ \text{mm}$ was assigned to the magnetic field center, denoted by the dotted red line.

6.2.1 Materials

Immiscible fluids were used for magnetic Janus droplet (MJD) generation, viz. hexane as the continuous phase (CP) and a Janus polymeric phase (JPP) as the dispersed phase (DP) (**Figure 6.1** and **Figure 6.2**). The JPP consists of (i) a magnetic polymeric phase (MPP) with EMG 507 ferrofluid (Ferrotec, Singapore) for magnetic control and (ii) a functional polymeric phase (FPP) for protein detection (**Table 6.2**). Two photopolymerizable solutions (PPS) of polyethylene glycol diacrylate (PEGDA, MW 575 and MW 700) containing 2-hydroxy-2-methylpropiophenone (HMP) as the photoinitiator were prepared, viz., (i) PPS5= PEGDA 575 + 13 % v/v HMP and (ii) PPS7= PEGDA 700 + 13 % v/v HMP.

Ethyl(dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were used for protein immobilization experiments. Bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC) tagged BSA (FITC-BSA) were used as model protein. All chemicals were purchased from Sigma-Aldrich, Singapore, unless otherwise specified. The MPP consists (**Table 6.2**) of 2.5 ml PPS5 + 4.5 ml PPS7 + 2 ml EMG 507 + 2.5 ml DI water + 1 ml 1% (v/v) Tween 20 (in DI water). The composition of FPP was optimized for enhanced protein binding, the details are described in the results section.

Table 6.2: Compositions and viscosities of liquid phases. Brookfield rheometer (model: LV-DV3T, spindle: CPA 40z, sample volume of 0.5 ml) was used to measure viscosity. Density measurement was performed by the rapid micro-syringe method[43]. (i) PPS5= PEGDA 575 + 13 % v/v HMP and (ii) PPS7= PEGDA 700 + 13 % v/v HMP. Polyethylene glycol diacrylate (PEGDA, MW 575 and MW 700) containing 2-hydroxy-2-methylpropiophenone (HMP) as the photoinitiator was used as the photopolymerizable solution (PPS).

Sr.	Description	Composition	Viscosity (mPa.s)	Density (10^3kg/m^3)
1	Ferrofluid	EMG 507 ferrofluid	1.44±0.01	1.118±0.004
2	Continuous Phase (CP)	n-Hexane	0.31±0.01	0.667±0.007
3	Functional Polymeric Phase (FPP)	[5 ml PPS5+ 2.5 ml AA + 2.5 ml EG] + 0.5 mg/ml rhodamine 6G	35.83±0.36	1.102±0.005
4	Magnetic Polymeric Phase (MPP)	PPS5 +4.5 ml PPS7 +2 ml EMG 507+2.5 ml DI water +1 ml 1% (v/v) Tween 20 (in DI water)	22.25±0.23	1.133±0.002
5	Dispersed Phase (DP)	0.25ml FPP+0.25ml MPP	31.20±0.31	1.119±0.006

6.2.2 EDC/NHS protocol for protein immobilization

The EDC/NHS protocol was used for covalent immobilization of protein (BSA and FITC-BSA as model protein) on the synthesized MJP or hydrogel surface. The coupling protocol was performed by adding 2mM EDC and 5mM NHS to MJP in PBS 1X buffer for 3 h. After 3 h, the particles were washed 3 times in PBS 1X buffer and protein solution (in PBS 1X) was added immediately and kept for 3 h at 4°C. The particles were then thoroughly washed 3 times by PBS 1X to remove weakly bonded or physically entrapped protein.

A fluorescent microscope (Olympus IX73) integrated with TOUPCAM camera (ToupTek photonics, Model: UCMOS03100KP, P/N:TP603100A) was used to capture fluorescent images of the particles in FITC mode and ImageJ was used to quantify the measurements. Viscosity measurement of liquid phases was performed by a Brookfield rheometer (model: LV-DV3T, sample volume: 0.5 ml, spindle: CPA 40z), the results are summarized in **Table 6.2**. The measured density[43] of the ferrofluid was $1.118\pm0.004\times10^3 \text{ kg/m}^3$. The properties of the water-based ferrofluid EMG 507 are summarized in **Table 3.1**.

Figure 6.2: Experimental setup of DMMF based MJP synthesis, consist of (a) Magnetic Janus droplet generation unit, (b) magnetic control unit, (c) polymerization unit, (d) high-precision control stage, and (e) collection unit. For droplet generation two designs can be used, (i) PMMA microfluidic chip based LoC flow focusing design ($100\mu\text{m} \times 100\mu\text{m}$ cross-section), (ii) capillary microfluidic design ($150\mu\text{m}$ thru hole diameter).

6.2.3 Experimental Parameters

The effect of flow rates, magnetic field, and synthesis of MJP was performed at four flow rate ratios (Ψ). The flow rate ratio (Ψ) was defined as the ratio of flow rate of continuous phase (Q_{cp}) and the flow rate of the dispersed phase (Q_{dp}). The flow rate Q_{dp} is the combined flow rate of the MPP (Q_{MPP}) and the FPP (Q_{FPP}). Competition between the magnetic volume force and the hydrodynamic force on the MPP and Janus droplets was investigated at a constant magnetic field of 440mT through the effect of different flow rate ratios. The parameters used for investigation and synthesis are summarized in **Table 6.3**.

Table 6.3: Parameters and notations. (Refer **Table 6.2** for compositions and notations). MJD: magnetic Janus droplets and MJP: magnetic Janus particles. Q_{cp} , Q_{MPP} , and Q_{FPP} are flow rates of CP, MPP, and FPP, respectively. The flow rate ratio (Ψ) was defined by $\Psi=Q_{cp}/Q_{dp}$, with $Q_{dp}=Q_{MPP}+Q_{FPP}$.

Sr	Flow rate ratio (Ψ), $\Psi=Q_{cp}/(Q_{MPP}+Q_{FPP})$	Q_{cp} ($\mu\text{l/h}$)	DP ($\mu\text{l/h}$)		Analyzed Set Notations	
			Q_{MPP}	Q_{FPP}	MJD	MJP
1	2.5	1000	200	200	MJD1000	MJP1000
2	5	2000	200	200	MJD2000	MJP2000
3	6.25	2500	200	200	MJD2500	MJP2500
4	7.5	3000	200	200	MJD3000	MJP3000
5	10	4000	200	200	MJD4000	MJP4000
6	6.25	5000	400	400	MJD5000	MJP5000

6.3 Experimental Setup

A DMMF platform was developed for the LoC synthesis of MJP, magnetically controlled by hybrid magnetic fields ($\mathbf{H}_o+\mathbf{H}_{no}$). The main components of the developed system are (i) Janus droplet generation unit, (ii) magnetic control unit, (iii) polymerization unit, (iv) high-precision control stage, and (v) collection unit. The above components are described in the following subsections.

6.3.1 Magnetic Janus droplet generation unit and analysis

Two platforms were developed for MJD generation, viz., (i) LoC based flow focusing design and (ii) capillary microfluidic-based micro flow focusing. Both designs have their own advantages.

6.3.1.1 LoC Flow Focusing Design

Poly(methyl methacrylate) (PMMA) based LoC flow focusing design was prepared by a micro-milling technique and bonded by thermal bonding [44] at 95°C under a load of 50 kg for a duration of 16 min. The dimensions and schematic of the setup are described in **Figure 6.1**. The LoC platform offers good control on droplet size; however, it is limited by the narrow range of flow rates (**Figure 6.3**) and low droplet generation rate (due to flow rates ≤ 100 $\mu\text{l/h}$). Moreover, the LoC platform is difficult to clean and re-use on blockage e.g., blockage due to stray polymerization; on blockage, LoC is required to be replaced with a new one. Hence, we found that the LoC platform was less suitable for our experiments.

Figure 6.3: LoC Magnetic Janus droplet generation (a) MJD micrograph (scale bar= 500 μm) and (b) droplet size v/s flow rate ratio. Where, CP= continuous phase, DP= dispersed phase, Q_{cp} =continuous phase flow rate, Q_{dp} =dispersed phase flow rate. Q_{cp} and Q_{dp} are in $\mu\text{l/h}$.

6.3.1.2 *Capillary-based Droplet Microfluidic Platform*

The capillary droplet microfluidic platform consists of a micro flow focusing connector with 150 μm thru-hole (P-891-microcross PEEK, IDEX, Singapore) connected by fluorinated ethylene propylene (FEP) microcapillaries, which are chemically inert to most of the solvents. FEP microcapillaries were used as inlet (500 μm diameter, P/N: 1548, IDEX, Singapore) and outlet microchannels (250 μm diameter, P/N: 1527, IDEX, Singapore).

The capillary-based design was found to be affordable, simple, and flexible design; offering a broad range of flow rates (hence a broad range of droplet and particle size), and compatible with all the chemicals used for the MJP synthesis. Flow rates up to 10 ml/h can be utilized for droplet generation and particle fabrication by this capillary droplet microfluidic platform. This device found to be very handy for cleaning of blockages, which can be recovered simply by purging at high flow rates or changing the FEP microcapillary.

6.3.1.3 *Syringe Pump*

KDS (model: Gemini 88 dual rate) and New Era (model: NE-1010 and NE-1002x, Achema, Singapore) syringe pumps were used to control the flow rates. Exmire Luer lock gastight syringes (volume: 2.5 ml, P/N: MS*GLL250) were used to feed the flow to microchannels. FEP microcapillaries were used for connections.

6.3.1.4 *High-Speed Imaging and analysis*

A Phantom Miro Camera (Model: M320s) integrated with Olympus IX73 microscope was used for high-speed imaging. High-speed imaging was recorded at the frame rate of 1000 fps and a resolution of 1920 $\times$ 600 pixels. Image acquisition and analysis of recorded videos was performed by ImageJ and Phantom camera control (PCC) software. Points in all graphs denote the mean of the measurements with the error bar denoting the standard error of the mean[45].

6.3.2 Magnetic Control Unit

Two Nd-Fe-B permanent magnets with dimensions 25mm×12.5mm×12.5mm (length×width×height) were used to generate a hybrid magnetic field. The strength and distribution of the hybrid magnetic field can be controlled by tuning the distance between two magnets. The magnetic field distribution was measured along the $\pm x$ direction by a LakeShore Gaussmeter (model: 410). The distribution of experimental and simulated hybrid magnetic field is shown in **Figure 6.1b**, with H_0 field of 440 mT in the region $-8 \text{ mm} \leq x \leq 8 \text{ mm}$.

6.3.3 LoC Polymerization Unit

The polymerization unit offers LoC polymerization of the Janus droplets. The unit was developed by integrating a UV LED with a 20X objective. A high-intensity UV-LED of 4 mm beam size was utilized as the UV light source (Agiltron UV LED, Model: SUVA-011111021) with the configuration described in **Table- E.4** (Appendix E).

6.3.4 High-Precision Stage

Control of the location of polymerization is a challenge, which was addressed by the high precision control stage by control along x, y directions. The UV light was incident along the z-direction and focused by using the z-adjustment of the microscope. Hence, this setup offers precise, 3D control of polymerization.

6.3.5 Collection Unit.

The fabricated particles were collected at the end in antistatic weighing dishes/pouring boats. The final unit resulted in novel magnetically controlled LoC polymerization unit utilizing MJD for the fabrication of MJP. This system was capable of LoC Janus particle synthesis of in the size range of 50 μm to 1000 μm up to flow rates of 10 ml/h (without ferrofluid) and 5 ml/h (with ferrofluid).

6.4 Physics of Numerical Model and Numerical Methodology

The physics of the numerical model is described in the *Section 3.4.2* by Eq.(3.5) to Eq.(3.20).

The MJD behavior in a hybrid magnetic field was simulated by a DMMF numerical model developed by the above equations. COMSOL software was used to perform the simulations. The following Multiphysics modules (i) Laminar flow in microfluidic regime, (ii) Two-phase level set method, and (iii) magnetic fields with no currents were utilized for simulations. The complete geometry consists of 89890 triangular elements. The microfluidic domain consists of 44138 triangular elements with 0.9851 as average element (size range: 0.16 to 15.3 μm). The magnetic field domain consists of 44728 triangular elements with average element quality of 0.9600 (size range: 3.75 to 500 μm).

6.5 Results and Discussion

The development of DMMF experimental setup utilized for the MJD control and the synthesis of MJP has been described earlier. Our studies, comprising MJD control and fabrication of MJP are categorized as: (i) *Optimization of FPP* composition to enhance protein binding, (ii) *Generation of MJD*, (iii) *Magnetic Control of MJD* for mixing and magnetic separation of the magnetic part inside MJD, (iv) *Characterization of MJP*, (v) *Application for Protein Detection*. The following subsections describe our investigations in the above sequence.

6.5.1 Optimization of FPP composition to enhance protein binding,

The FPP was optimized for protein binding by the addition of acrylic acid (AA) and ethylene glycol (EG). Bradford assay was used as a rapid method for direct observation of protein binding. The suitable composition of the FPP was determined in the following steps; (i) synthesize cylinders with 4 mm length, 1 mm diameter; (ii) perform EDC/NHS coupling protocol at a fixed concentration of BSA in PBS 1X buffer; (iii) wash 3 times in PBS 1X buffer; (iv) add 0.5 ml of Bradford reagent; (v) wait 5 min and wash again 3 times by PBS 1X buffer; (vi) observation of blue color indicates the amount of protein binding.

AA was added to the FPP to enhance covalent bonding in the FPP part of the MJP, which was found to increase BSA binding. Addition of EG was also found to improve protein binding. The optimized composition of FPP was determined (**Table 6.2**) as 10 ml PPS5+ 2.5 ml AA + 2.5 ml EG and found to significantly improve covalent bonding, resulting in higher binding of protein. 0.5 mg/ml rhodamine 6G was added to the FPP to track mixing, separation of magnetic part during the MJP synthesis, and protein detection.

Figure 6.4: Optimization of FPP composition. A: Direct observation of protein binding (a) PPS5, (b) 10 ml PPS5+2.5 ml AA, (c) 10 ml PPS5+2.5 ml AA + 2.5 ml EG. B: Quantitative measurements of mean gray value of (a-c). Acrylic acid and ethylene glycol were added to the FPP to enhance protein binding. Bradford assay was used as a rapid method for visible and quantitative determination of protein binding from the developed blue color. The decreasing mean gray value (or increasing contrast) denotes increasing amount of protein binding. The composition (c) was the optimized composition of the FPP, as confirmed by the intense blue color (lowest mean gray value) indicating higher protein binding compared to (a) and (b). Scale bar= 1 mm. Dimensions of the synthesized cylinder: 1 mm diameter and 4 mm length.

6.5.2 Generation of MJD

To facilitate MJP formation, simulations were performed for various dimensions of the flow focusing device as well as different sizes of outlet microchannel (O/L). The design was optimized for the sufficient separation between MJD, so as to incorporate surfactant-free synthesis of MJP at a high flow rate. MJD were generated by a flow focusing configuration under hybrid magnetic fields. For the utilized experimental parameters, droplet formation is governed by the ‘*squeezing regime*’ as $Ca \leq 10^{-3}$. The capillary number [46] is given by $Ca = \eta_{cp} v_{cp} / \sigma$, for a given CP viscosity (η_{cp}), CP velocity (v_{cp}) and surface tension (σ).

The experimental and simulated sequence followed for MJD formation in a flow focusing configuration is summarized as (a): (i) entry of Janus phase resulting in the formation of a paraboloid, (ii) increase in the paraboloid size with the forward motion of the JPP, (iii) formation of a neck as paraboloid gets squeezed in the flow focusing by the flow of CP, (iv) neck elongation starts with progressive motion of CP and DP, (iv) a critical point is reached as the paraboloid confines the CP flow, resulting in ‘squeezing’ and thinning of the neck, (v) breaking of the neck and droplet formation.

Figure 6.5b shows the quantitative comparison between the simulated and experimental MJD size. The performed simulations match closely with the experimental results.

Figure 6.5: Magnetic Janus droplet (MJD) generation. (a) Simulated MJD generation. Blue denotes the dispersed phase (magnetic Janus phase, MJP) and red indicates the CP of n-hexane. Refer **Table 6.3** for the MJD set notations. Scale bar=500 μm. (b) Comparison of experiments and simulations of variation of MJD diameter with flow rate ratio. Scale bar=250 μm.

6.5.3 Magnetic Control of Magnetic Janus Droplets

Interesting behavior was obtained when MJD travels through hybrid magnetic fields **Figure 6.6**(a-d). The forward motion of droplet results in the circulation of Janus phase in each half (in opposite direction to each other) of the droplets, resulting in mixing. This mixing is evident from the uniform color of rhodamine in the droplets. At 0 mm, the uniform magnetic field acts on the magnetic nanoparticles (MNP), resulting in chain formation along the y direction.

However, competition between the magnetic and hydrodynamic forces determines the net force on the MNP. At a flow rate ratio less than 7.5, magnetic volume force dominates, resulting in greater chain formation. The non-uniform field component (H_{no}) of H acts on the MNP with the highest value at $x=13$ mm, resulting in a net force on the magnetic phase. This magnetic separation of magnetic phase is significant at low flow rate ratios, viz., 2.5 and 5, as evident from **Figure 6.6**(a-d).

Since, significant separation was obtained at $x=13$ mm, synthesis of Janus particles was performed at the same location by focusing the UV beam at the point $x=13$ mm. The following subsections describe DMMF control of the properties of the synthesized MJP.

Figure 6.6: Dynamic magnetic control of magnetic phase in microchannels. (a-d) MJD generated at CP flow rate of 1000, 2000, 3000, 4000 $\mu\text{l/h}$, for a constant $Q_{MPP}=Q_{FPP} = 200 \mu\text{l/h}$. Refer **Table 6.3** for the MJD set notations. Scale bar= $250 \mu\text{m}$. Droplet flow is along +x direction, in the direction of the blue arrow. Magnetic field is along y direction.

6.5.4 Characterization of MJP

Synthesis of MJP was performed at $x=13$ mm, with the parameters summarized in **Table 6.3**. Following characterization was performed on the MJP.

6.5.4.1 Optical Microscopy

Optical microscopy analysis of the MJP was conducted. **Figure 6.7(a-b)** confirm the formation of the Janus configuration, as evident from the separated magnetic phase in the form of particle chains. Mixing of the FPP and MPP is evident from the fluorescent images in DF field mode with TRITC filter.

Figure 6.7: Optical micrographs of (a)MJP2500 and (b)MJP4000. Where, BF indicate bright field imaging mode and DF TRITC indicate dark field imaging mode with TRITC filter. Refer **Table 6.3** for the MJD set notations. Scale bar=100 μ m.

6.5.4.2 VSM Measurements

VSM measurements were performed by the VSM Lakeshore 7400. Measurements were performed for 10 to 15 mg of the synthesized MJP to enhance the signal from the MJP and to reduce diamagnetic contributions. All the synthesized batches demonstrated coercivity less than 10 Oe, hence all samples exhibited soft magnetic behavior.

As shown in **Figure 6.8**, the magnetic moment of the particles increases with decreasing flow rate ratios (or increasing MJP size). MJP2500 demonstrated good magnetic properties

and good stability in comparison to other samples. Though MJP5000 shows high magnetic moment (due to the higher DP flow rates), it possesses poor polymerization (hence, poor stability) caused by the high MJD velocity, resulting in less time in the polymerization zone.

Figure 6.8: Magnetic property measurement by VSM. Refer **Table 6.2** and **Table 6.3** for notations. MJP2500 exhibited better magnetic response, compared to other samples. MJP5000 possess high moment, but poorly polymerized due to higher MPP content and less time in polymerization zone.

6.5.4.3 FTIR Analysis

FTIR analysis revealed polymerization of synthesized MJP (**Figure 6.9**). Signature peaks at 1790 cm^{-1} , 1720 cm^{-1} , 1630 cm^{-1} , 1610 cm^{-1} , 1400 cm^{-1} and 800 cm^{-1} indicate the non-polymerized state of PEGDA samples, viz. PPS5, FPP, and MPP in liquid form[47].

After UV polymerization, bands at 810, 1960, 2080, and 2880 corresponding to PEGDA either vanishes or decrease significantly, indicating cross-linking.

Figure 6.9: FTIR analysis. Refer **Table 6.2** and **Table 6.3** for notations.

6.5.4.4 DSC Analysis

The DSC plots of samples are depicted in **Figure 6.10**. DSC plot corresponding to PEGDA reveals an endotherm near -20°C which is attributed to its glass transition (T_g). An exotherm at 170°C corresponds to the crystallization temperature of PEGDA.

The synthesized samples exhibited a T_g near 160°C , higher than that for PEGDA. This shift of T_g to higher temperatures is due to the presence of Fe_3O_4 nanoparticles. Poor polymerization of MJP5000 is evident from the DSC profile, which shows lower values of endotherms and exotherms compared to other samples. The MJP2500, MJP3000 and MJP4000 samples exhibit similar DSC profiles. T_g for MJP2500 > MJP3000 > MJP4000, indicating higher degree of polymerization of MJP2500. Hence, superior polymerization was observed at lower flow rate ratios, corresponding to the set MJP2500.

Figure 6.10: DSC analysis. Refer **Table 6.2** and **Table 6.3** for notations.

6.5.4.5 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (**Figure 6.11**) was performed to determine % weight of MNP. EMG 507 was used as the source solution of MNP (Fe_3O_4). The TGA profile of EMG 507 exhibited 23 % w/w of MNP, which matches with the supplier data sheet. For the synthesis of MJP different liquid phases were utilized, such as FPP and MPP. The FPP only contains polymers, which decomposes near 450°C . The MPP exhibited 5.5 % w/w of MNP. Interestingly, all of the synthesized MJP exhibited higher weight % of MNP (8.4% for MJP2500, 7.8% for MJP3000, and 8.8% for MJP4000) compared to MPP, which is attributed to the effect of the hybrid magnetic field.

Hence, the use of hybrid magnetic field for the synthesis of Janus structures not only separates the magnetic phase, but also increase the amount of magnetic phase.

Figure 6.11: Thermogravimetric analysis. Refer **Table 6.2** and **Table 6.3** for notations.

6.6 Application for Protein Detection

Application of the magnetic Janus particles (MJP2500) was demonstrated for protein detection. FITC-BSA was used as a model protein at concentrations of 200 and 20 $\mu\text{g/ml}$. Covalent immobilization of FITC-BSA was performed by the EDC/NHS coupling protocol, described earlier in the *experimental methodology section*.

Figure 6.12 (a-b) shows the fluorescent micrographs of MJP2500 after protein binding. Qualitative observation demonstrates high fluorescent intensity at protein concentration of 200 $\mu\text{g/ml}$ compared to 20 $\mu\text{g/ml}$. Quantitative analysis was performed by measuring the corrected total fluorescence per unit area ($\text{CTF}/\mu\text{m}^2$) by ImageJ software. Measurements were repeated for 10 different particles and averaged to obtain mean CTF. Obtained mean CTF values were ~ 120 and ~ 55 at protein concentrations of 200 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$, confirming the good protein binding and detection capabilities of MJP2500.

Figure 6.12: (a-b) Fluorescent images of MJP2500 particles showing protein (FITC-BSA) binding, at protein concentrations (a) 200 $\mu\text{g/ml}$ and (b) 20 $\mu\text{g/ml}$. Scale bar=100 μm . (c) Quantitative measurement of protein binding, determined from the corrected total fluorescence intensity (CTF) per unit area (mean CTF or fluorescence/ μm^2).

In the literature, Janus particle synthesis is performed by separated phases. The separation of the magnetic phase from the polymer offers better control of the particle orientation. These capabilities can be used to reduce the washing stage and the Janus particles can be used as individual platforms for detection, similar to a well in the 96 Well plate. Hence, such magnetic Janus particles offer a potential replacement to perform operations on a microfluidic platform which are typically performed in a 96 Well plate. Here, this study adds one more facet to the development of Janus particles: mixing $\Rightarrow$ separation of magnetic part $\Rightarrow$ photopolymerization, utilizing a simple, wash-less and rapid method. Our approach is advantageous and increase the functionality, e.g., (i) chemical reactions can be performed just before photopolymerization and the resultant product properties can be observed in the Janus particles, (ii) antibodies dispersed in one phase can be mixed with the other phase prior to photopolymerization, and (iii) a stable suspension can be used as one phase to mix with incompatible polymers prior to photopolymerization.

6.7 Conclusions

A simplified approach with wireless, programmable and remote control of droplets, useful for the synthesis of Janus particles has been demonstrated by the combination of a DMMF technique with hybrid magnetic fields. A capillary microfluidic platform was utilized in the present technique, which was found to be advantageous due to its adaptability, flexibility and low cost. DMMF simulations were performed at high flow rates to optimize the geometry for surfactant-free, wash-less synthesis. The experiments and simulations are in good agreement. The effect of flow rates and applied magnetic field on mixing, separation of magnetic phase inside droplets, as well as magnetization, particle size and configuration of the magnetic part in MJP was investigated. Significant separation of the magnetic phase was obtained at 400 mT for flow rate ratios less than 7.5 and CP flow rates less than 3000 $\mu\text{l/h}$. The center of the magnetic field was found to contribute to particle alignment and the highest magnetic field gradient contribute to the dominating effect of magnetic volume force, resulting in net separation of the magnetic phase. Magnetic Janus particle synthesis was performed at the point of highest field gradient for significant separation of magnetic phase after mixing. The functionality of the polymeric phase was

enhanced for protein detection by addition of acrylic acid and ethylene glycol. The properties of synthesized magnetic Janus particles were examined by various characterization technique and control of the properties of MJP by DMMF parameters was demonstrated. Application of synthesized Janus particles was demonstrated for protein detection, with BSA as a model protein. Quantitative analysis demonstrated significant change in the fluorescent intensity, confirming protein binding and detection.

References

- [1] P. Zhu and L. Wang, "Passive and active droplet generation with microfluidics: a review," *Lab Chip*, vol. 17, pp. 34-75, 2017.
- [2] T. P. Lagus and J. F. Edd, "A review of the theory, methods and recent applications of high-throughput single-cell droplet microfluidics," *Journal of Physics D-Applied Physics*, vol. 46, p. 21, 2013.
- [3] R. Seemann, M. Brinkmann, T. Pfohl, and S. Herminghaus, "Droplet based microfluidics," *Rep Prog Phys*, vol. 75, p. 016601, 2012.
- [4] S. Y. Teh, R. Lin, L. H. Hung, and A. P. Lee, "Droplet microfluidics," *Lab Chip*, vol. 8, pp. 198-220, 2008.
- [5] G. Katsikis, J. S. Cybulski, and M. Prakash, "Synchronous universal droplet logic and control," *Nature Physics*, vol. 11, pp. 588-96, 2015.
- [6] X. Casadevall i Solvas and A. deMello, "Droplet microfluidics: recent developments and future applications," *Chem Commun (Camb)*, vol. 47, pp. 1936-42, 2011.
- [7] L. F. Cai, Y. Zhu, G. S. Du, and Q. Fang, "Droplet-based microfluidic flow injection system with large-scale concentration gradient by a single nanoliter-scale injection for enzyme inhibition assay," *Anal Chem*, vol. 84, pp. 446-52, 2012.
- [8] E. Y. Basova and F. Foret, "Droplet microfluidics in (bio)chemical analysis," *Analyst*, vol. 140, pp. 22-38, 2015.
- [9] T. S. Kaminski, O. Scheler, and P. Garstecki, "Droplet microfluidics for microbiology: techniques, applications and challenges," *Lab Chip*, vol. 16, pp. 2168-87, 2016.
- [10] A. Huebner, M. Srisa-Art, D. Holt, C. Abell, F. Hollfelder, A. J. de Mello, and J. B. Edel, "Quantitative detection of protein expression in single cells using droplet microfluidics," *Chem. Commun. (Cambridge, U. K.)*, pp. 1218-20, 2007.
- [11] A. Rakszewska, J. Tel, V. Chokkalingam, and W. T. S. Huck, "One drop at a time: toward droplet microfluidics as a versatile tool for single-cell analysis," *NPG Asia Mater.*, vol. 6, p. e133, 2014.
- [12] J. P. Junker and A. van Oudenaarden, "Single-Cell Transcriptomics Enters the Age of Mass Production," *Mol. Cell*, vol. 58, pp. 563-64, 2015.
- [13] S. Allazetta and M. P. Lutolf, "Stem cell niche engineering through droplet microfluidics," *Curr Opin Biotechnol*, vol. 35, pp. 86-93, 2015.
- [14] R. Zilionis, J. Nainys, A. Veres, V. Savova, D. Zemmour, A. M. Klein, and L. Mazutis, "Single-cell barcoding and sequencing using droplet microfluidics," *Nat. Protoc.*, vol. 12, pp. 44-73, 2017.
- [15] I. V. Smolina and N. E. Broude, "Ultrasensitive detection of DNA and protein markers in cancer cells," *Cancer Biol Med*, vol. 12, pp. 143-9, 2015.

- [16] D.-K. Kang, M. Monsur Ali, K. Zhang, E. J. Pone, and W. Zhao, "Droplet microfluidics for single-molecule and single-cell analysis in cancer research, diagnosis and therapy," *TrAC Trends in Analytical Chemistry*, vol. 58, pp. 145-53, 2014.
- [17] Z. Zhu and C. J. Yang, "Hydrogel Droplet Microfluidics for High-Throughput Single Molecule/Cell Analysis," *Acc. Chem. Res.*, vol. 50, pp. 22-31, 2017.
- [18] D. Baah and T. Floyd-Smith, "Microfluidics for particle synthesis from photocrosslinkable materials," *Microfluidics and Nanofluidics*, vol. 17, pp. 431-55, 2014.
- [19] C. A. Serra and Z. Chang, "Microfluidic-assisted synthesis of polymer particles," *Chem. Eng. Technol.*, vol. 31, pp. 1099-115, 2008.
- [20] X.-T. Sun, M. Liu, and Z.-R. Xu, "Microfluidic fabrication of multifunctional particles and their analytical applications," *Talanta*, vol. 121, pp. 163-77, 2014.
- [21] W. J. Duncanson, T. Lin, A. R. Abate, S. Seiffert, R. K. Shah, and D. A. Weitz, "Microfluidic synthesis of advanced microparticles for encapsulation and controlled release," *Lab Chip*, vol. 12, pp. 2135-45, 2012.
- [22] C. X. Zhao, "Multiphase flow microfluidics for the production of single or multiple emulsions for drug delivery," *Adv Drug Deliv Rev*, vol. 65, pp. 1420-46, 2013.
- [23] Z. Kang, T. Kong, L. Lei, P. Zhu, X. Tian, and L. Wang, "Engineering particle morphology with microfluidic droplets," *J. Micromech. Microeng.*, vol. 26, pp. 075011/1-11/8, 2016.
- [24] T. Nisisako, "Recent advances in microfluidic production of Janus droplets and particles," *Curr. Opin. Colloid Interface Sci.*, vol. 25, pp. 1-12, 2016.
- [25] S. Lone and I. W. Cheong, "Fabrication of polymeric Janus particles by droplet microfluidics," *RSC Adv.*, vol. 4, pp. 13322-33, 2014.
- [26] D. Dendukuri and P. S. Doyle, "The Synthesis and Assembly of Polymeric Microparticles Using Microfluidics," *Advanced Materials*, vol. 21, pp. 4071-86, 2009.
- [27] T. Heida, J. W. Neubauer, M. Seuss, N. Hauck, J. Thiele, and A. Fery, "Mechanically Defined Microgels by Droplet Microfluidics," *Macromol. Chem. Phys.*, p. Ahead of Print, 2016.
- [28] Y. Zhao, L. Shang, Y. Cheng, and Z. Gu, "Spherical colloidal photonic crystals," *Accounts of chemical research*, vol. 47, pp. 3632-42, 2014.
- [29] J. H. Kim, T. Y. Jeon, T. M. Choi, T. S. Shim, S. H. Kim, and S. M. Yang, "Droplet microfluidics for producing functional microparticles," *Langmuir*, vol. 30, pp. 1473-88, 2014.
- [30] T. Um, J. Hong, D. J. Im, S. J. Lee, and I. S. Kang, "Electrically Controllable Microparticle Synthesis and Digital Microfluidic Manipulation by Electric-Field-Induced Droplet Dispensing into Immiscible Fluids," *Scientific Reports*, vol. 6, p. 10, 2016.
- [31] J. W. Lan, J. Y. Chen, N. X. Li, X. H. Ji, M. X. Yu, and Z. K. He, "Microfluidic generation of magnetic-fluorescent Janus microparticles for biomolecular detection," *Talanta*, vol. 151, pp. 126-31, 2016.
- [32] T. Hessberger, L. B. Braun, F. Henrich, C. Muller, F. Giesselmann, C. Serra, and R. Zentel, "Co-flow microfluidic synthesis of liquid crystalline actuating Janus particles," *Journal of Materials Chemistry C*, vol. 4, pp. 8778-86, 2016.
- [33] L. D. Zarzar, V. Sresht, E. M. Sletten, J. A. Kalow, D. Blankschtein, and T. M. Swager, "Dynamically reconfigurable complex emulsions via tunable interfacial tensions," *Nature*, vol. 518, pp. 520-24, 2015.
- [34] K. Takimoto, E. Takano, Y. Kitayama, and T. Takeuchi, "Synthesis of monodispersed submillimeter-sized molecularly imprinted particles selective for human serum albumin using inverse suspension polymerization in water-in-oil emulsion prepared using microfluidics," *Langmuir*, vol. 31, pp. 4981-87, 2015.

- [35] X. Li, Y.-T. Yang, L.-J. Wu, Y.-C. Li, M.-Y. Ye, Z.-Q. Chang, D.-Q. Meng, and C. A. Serra, "Fabrication of electro- and color-responsive CB/PTFE Janus beads in a simple microfluidic device," *Materials Letters*, vol. 142, pp. 258-61, 2015.
- [36] Y. Hu, S. Wang, A. Abbaspourrad, and A. M. Ardekani, "Fabrication of shape controllable janus alginate/pNIPAAm microgels via microfluidics technique and off-chip ionic cross-linking," *Langmuir*, vol. 31, pp. 1885-91, 2015.
- [37] J. Kim, J. Joo, and S. Y. Park, "Preparation of asymmetric porous Janus particles using microfluidics and directional UV curing," *Particle & Particle Systems Characterization*, vol. 30, pp. 981-88, 2013.
- [38] Y. Chen, G. Nurumbetov, R. Chen, N. Ballard, and S. A. Bon, "Multicompartmental Janus microbeads from branched polymers by single-emulsion droplet microfluidics," *Langmuir*, vol. 29, pp. 12657-62, 2013.
- [39] Z. Yu, C. F. Wang, L. Ling, L. Chen, and S. Chen, "Triphase microfluidic-directed self-assembly: anisotropic colloidal photonic crystal supraparticles and multicolor patterns made easy," *Angew Chem Int Ed Engl*, vol. 51, pp. 2375-8, 2012.
- [40] H. Xie, Z. G. She, S. Wang, G. Sharma, and J. W. Smith, "One-step fabrication of polymeric Janus nanoparticles for drug delivery," *Langmuir*, vol. 28, pp. 4459-63, 2012.
- [41] D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, "Continuous-flow lithography for high-throughput microparticle synthesis," *Nat Mater*, vol. 5, pp. 365-9, 2006.
- [42] D. C. Appleyard, S. C. Chapin, R. L. Srinivas, and P. S. Doyle, "Bar-coded hydrogel microparticles for protein detection: synthesis, assay and scanning," *Nat Protoc*, vol. 6, pp. 1761-74, 2011.
- [43] J. E. Burroughs and C. P. Goodrich, "Rapid method for determining densities of liquids using micro syringes," *Analytical Chemistry*, vol. 46, pp. 1614-15, 1974.
- [44] J. H. Li, D. Chen, and G. Chen, "Low-temperature thermal bonding of PMMA microfluidic chips," *Analytical Letters*, vol. 38, pp. 1127-36, 2005.
- [45] M. L. Boas, *Mathematical methods in the physical sciences*, 3 ed.: Wiley New York, 2006.
- [46] P. Garstecki, M. J. Fuerstman, H. A. Stone, and G. M. Whitesides, "Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up," *Lab on a Chip*, vol. 6, pp. 437-46, 2006.
- [47] L. Zhao, L. Pan, K. Zhang, S. Guo, W. Liu, Y. Wang, Y. Chen, X. Zhao, and H. Chan, "Generation of Janus alginate hydrogel particles with magnetic anisotropy for cell encapsulation," *Lab on a Chip*, vol. 9, pp. 2981-86, 2009.

Chapter 7

Conclusions and Future Work

Our droplet micro-magnetofluidic studies of magnetic droplets in the presence of uniform and hybrid magnetic fields, consist of experiments and multiphysics numerical simulations. Those studies were performed for a range of flow rates, flow rate ratios and magnetic field strengths. The influence of various fluidic properties such as, viscosities, magnetic susceptibility and interfacial tension on droplet behavior, control and merging was also studied. The development of the experimental setup and simulation model are described in the first section. Major conclusions and findings of the research work are stated in the second section. The third section describes future perspectives originating from this research work.

7.1 Development of Experimental Setup and Numerical Methodology

DMMF investigations were performed by experiments and simulations. A droplet micromagnetofluidic (DMMF) experimental setup and simulation model was developed to perform those studies, as described below.

DMMF methodology requires integration of microfluidics and magnetofluidics. A DMMF experimental setup was successfully developed to study the ferrofluid droplet (FD) behavior under the influence of an applied uniform magnetic field. The useful range of flow rates, flow rate ratios, and magnetic fields were determined for the experimental investigations. Different experimental methodologies and protocols were developed for high-speed imaging, image acquisition, and analysis for quantification of experimental results.

Simulation methodology was utilized for qualitative and quantitative understanding of the FD behavior in uniform and hybrid magnetic field. A DMMF numerical model was developed to perform simulations. The developed model was then utilized to simulated the process of droplet generation, deformation and merging in uniform magnetic field. These numerical simulations were also utilized to design a suitable geometry for Janus particle synthesis.

A rapid, wash-less Janus particle synthesis method was developed on a capillary microfluidic platform. This method was successfully utilized for the mixing, separation, and synthesis of magnetic Janus particles. It was found to be cheap, flexible, adaptive and compatible with solvents.

7.2 Conclusions

The conclusions of the research work are categorized as per the logical trend of DMMF methodology, viz., (i) DMMF investigation of FD behavior in uniform magnetic field, (ii) droplet merging in uniform magnetic field and (iii) Janus particle synthesis.

7.2.1 Droplet Micromagnetofluidic Investigations

Investigation of the shape, size, and motion of FD in a flowing immiscible continuous phase (CP) under the influence of a uniform magnetic field (H_0) perpendicular to FD flow were performed. The effect of viscous forces was studied for different viscosity values of CP. The effect of magnetic volume force was studied by changing the ferrofluid susceptibility and magnetic field strength. The effect of hydrodynamic forces was studied by changing flow rate ratio and flow rates. The major conclusions are:

- At high susceptibility and low flow rate ratio, the FD size and aspect ratio increases with increasing H_0 . Greater spacing and velocity was observed for FD, at high flow rate ratio and high viscosity of the CP.
- Superior control of the motion, shape, and size was observed at high susceptibility, low viscosity, and low flow rate ratio, under the influence of a uniform magnetic field.
- Our DMMF simulation results were in good agreement with the experimental findings.

7.2.2 Droplet Merging

After investigating the FD behavior, control of magnetic droplet behavior was then demonstrated by droplet merging under the influence of a *uniform* magnetic field. The experimental and simulation results demonstrated control of the droplet merging distance (L_m). The major conclusions are:

- At an applied uniform magnetic field, L_m found to increase with increasing flow rate ratio. Our numerical results showed quantitative agreement with experimental results.

- The process of droplet merging was studied experimentally and numerically through variation of the droplet velocity with time under the influence of a field H_0 .
- A map was constructed to identify different merging/non-merging regimes.
- The merging of composite blue and yellow magnetic droplets was demonstrated experimentally at 1000 mT and mixing was confirmed from the green color developed after merging.

7.2.3 Janus Particle Synthesis

After investigating control of magnetic and composite magnetic droplets, the studies were extended to develop a simplified approach for the synthesis of Janus particles with wireless, programmable and remote control of droplets by a combination of a DMMF technique with hybrid magnetic fields. The utilized capillary microfluidic platform was found to be advantageous due to its adaptability, flexibility and low cost. DMMF simulations were performed to optimize the geometry for surfactant-free, wash-less synthesis at high flow rates, and found to be in good agreement with experiments. The major conclusions are:

- The mixing, separation of magnetic phase, as well as magnetization, particle size and configuration of the magnetic part in magnetic Janus particle was investigated for high flow rates and constant applied field.
- We found that for flow rate ratios less than 7.5 and CP flow rates less than 3000 $\mu\text{l/h}$, significant separation of the magnetic phase can be obtained, with the center of the magnetic field contributing to particle alignment and the point of maximum magnetic field gradient contributing to the net separation of the magnetic phase. The synthesis was performed at the same point for significant separation of magnetic phase after the mixing.
- The properties of synthesized magnetic Janus particles was studied by various characterization technique and control of their properties by the DMMF parameters was demonstrated.

- The efficiency of the polymeric phase for protein detection was increased by the addition of acrylic acid and ethylene glycol by utilizing Bradford assay for direct visual observation.
- Application of the synthesized Janus particles was demonstrated for protein detection, with BSA as a model protein. Quantitative analysis demonstrated significant change in the fluorescent intensity, confirming protein binding and detection.

7.3 Future Perspectives

Future perspectives of the present work can be characterized as, (i) applications of DMMF method for LoC applications and (ii) utilization of developed DMMF method for the synthesis of complex Janus particles.

7.3.1 LoC Applications

The wireless, programmable control of droplets demonstrated by uniform and hybrid magnetic fields for merging and mixing of droplets on a LoC platform, finds applications in biosensing, bioassay, microfluidic-based synthesis, reaction kinetics, and magnetochemistry. The merging and resulting magnetic tagging of droplets are useful for LoC based multiplexed sensing applications, such as biochemical sensing for luminol-based chemiluminescence blood detection, a biochemical assay for measuring glucose in human serum at physiologically relevant levels, and multiplex protein detection. Perhaps the most promising application of the present method can be in magnetic immuno-agglutination assays when combined with a GMR-based magnetofluidic device.

Integration of various disease markers on such DMMF leads to miniaturized, wireless and programmable LoC devices. Hence, future work of our DMMF platform is in the development of LoC devices with multiplexed capabilities.

7.3.2 Complex Janus Particles

In the literature, Janus particles with separated phases are synthesized. The method demonstrated by our research work performs mixing and then magnetic separation of the phases, which can be utilized for the development of complex Janus particles, as summarized in **Figure 7.1**. These complex structures can be useful for multidrug delivery, multiplex protein detection, and multiplex disease detection.

Figure 7.1: Future perspectives of DMMF based magnetic Janus particles fabrication.

7.4 Publications

Based in part on this thesis research, the following articles were published.

- [1] **V. B. Varma**, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Janus Particles Synthesized by Droplet Micro-magnetofluidic Techniques for Protein Detection," *Lab on a Chip*, 2017 (In Press).
- [2] **V. B. Varma**, A. Ray, Z. M. Wang, Z. P. Wang, and R. V. Ramanujan, "Droplet Merging on a Lab-on-a-Chip Platform by Uniform Magnetic Fields," *Sci Rep*, vol. 6, p. 37671, 2016.
- [3] **V. B. Varma**, A. Ray, Z. Wang, Z. Wang, R. Wu, P. J. Jayaneel, N. M. Sudharsan, and R. V. Ramanujan, "Control of Ferrofluid Droplets in Microchannels by Uniform Magnetic Fields," *IEEE Magnetics Letters*, vol. 7, pp. 1-5, 2016.
- [4] A. Ray, **V. B. Varma**, P. J. Jayaneel, N. M. Sudharsan, Z. P. Wang, and R. V. Ramanujan, "On demand manipulation of ferrofluid droplets by magnetic fields," *Sensors and Actuators B: Chemical*, vol. 242, pp. 760-768, 2017.
- [5] A. Ray, **V. B. Varma**, Z. Wang, Z. Wang, P. J. Jayaneel, N. M. Sudharsan, and R. V. Ramanujan, "Magnetic Droplet Merging by Hybrid Magnetic Fields," *IEEE Magnetics Letters*, vol. 7, pp. 1-5, 2016.
- [6] Y. Wang, R.G. Wu, **V. B. Varma**, Z. M. Wang, Y. P. Seah, Z. P. Wang and R. V. Ramanujan, "Trapping of Label-free Bacteria in Flowing Ferrofluids by Small Magnetic Fields", *Lab on a Chip*, 2017 (In Review).
- [7] C. Pai, V.B. Varma, S. Radha, R. Nagarajan, and R.V. Ramanujan, "Control of Magnetofluidic Laser Scattering of Aqueous Magnetic Fluids," *IEEE Magnetics Letters*, 2017 (In Press) doi:10.1109/lmag.2017.2735946.
- [8] Z. M. Wang, **V. B. Varma**, H. M. Xia, Z. P. Wang, and R. V. Ramanujan, "Spreading of a ferrofluid core in three-stream micromixer channels," *Phys Fluids*, vol. 27, p. 052004, 2015.
- [9] Z. M. Wang, **V. B. Varma**, Z. P. Wang, and R. V. Ramanujan, "Tuning magnetofluidic spreading in microchannels," *J Micromech Microeng*, vol. 25, p. 124001, 2015.
- [10] X. Wang, Z. Wang, **V. B. Varma**, Z. Wang, A. Ray, W. S. Lew, and R. V. Ramanujan, "Instability-Induced Mixing of Ferrofluids in Uniform Magnetic Fields," *IEEE Magnetics Letters*, vol. 7, pp. 1-5, 2016.

Appendix

Appendix A Microfluidic Chip and Holder Design

The fabrication of microfluidics chip holder and microfluidic chips were carried out in PMMA by a standard micro milling technique. AutoCAD designs used for the fabrication are summarized in **Fig. A.1** and **Fig. A.2**

(a) Chip Holder Design

The microfluidic chip holder was designed to connect microfluidic connectors with the standard 75 mm $\times$ 25 mm chip size. Chip holders fit along each 25mm side of the microfluidic chip. Each side of chip holder has six ports to connect the microfluidic tubing with the chip inlets and outlets by standard M6 connectors. The present chip design utilized six inlets and three outlets of the microfluidic chips. The chip holder dimensions are 38 mm (length) $\times$ 37.4 mm (width) $\times$ 16 mm (height) (**Fig. A.1**). The cavity dimensions which hold the microfluidic chip are 28mm (depth) $\times$ 25.4mm (breadth) $\times$ 5mm (height).

Fig. A.1: Design of chip holder utilized to mount the microfluidic chips (all dimensions are in mm) in uniform magnetic fields.

(b) Microfluidic Chip Design

The microfluidic chip design consists of six inlets and three outlets. The holes are separated by 9 mm from neighboring holes and by 9.5 mm from the chip boundary. The hole diameter is 0.5 mm. Other details are summarized in the relevant chip designs. Different types of chips were designed and tested to explore the range of flow rates for magnetofluidic spreading, droplet generation and droplet micro-magnetofluidic (DMMF) investigations of magnetic droplets. The designs are summarized in **Fig. A.2**.

(a) Chip for MMF Spreading

(c) Chip for DMMF: JF4

(d) Chip for DMMF: MX5

Fig. A.2: Designed microfluidics chip used for investigations of micro-magnetofluidic (MMF) spreading and droplet generation (all dimensions are in mm).

Appendix B Chip Designs for Droplet Microfluidic Studies

DMMF investigations of ferrofluid droplets was performed by the chips designs summarized in **Fig. B.1(a-c)**. Chip design (c) was found to be suitable for the utilized range of flow rates.

Fig. B.1: (a-c) Designed droplet microfluidic chips used in DMMF investigations of ferrofluid droplets (all dimensions are in mm). Design (c) was found to be suitable for DMMF investigations.

Appendix C Chip Designs for Droplet Merging and Janus Particle Synthesis

Droplet merging (**Fig. C.1a**) and Janus particle synthesis (**Fig. C.1b**) was performed by utilizing two designs of DMF chips. Design (**Fig. C.1a**) was also utilized as flow focusing configuration for Janus particle synthesis.

Fig. C.1: Designed droplet microfluidic chips for (a) two colored droplet generation and (b) Janus particle fabrication (all dimensions are in mm).

Appendix D Capillary Microfluidics: Outline

The capillary microfluidic setup consists of a micro-T Junction or micro-cross Junction with the interior through hole diameter of $150\ \mu\text{m}$ (**Fig. D.1**). Continuous phase inlet consists of $500\ \mu\text{m}$ capillary tubing (interior diameter) and the dispersed phase consists of $250\ \mu\text{m}$ capillary tubing (interior diameter). The outlet capillary tubing consists of $500\ \mu\text{m}$ capillary tubing (interior diameter). The setup is mounted on a glass slide.

Fig. D.1: Outline of the Capillary Microfluidic Setup for droplet generation.

Appendix E Specifications

Table- E.1: Specifications of Electromagnet System

Sr	Parameter	Value/Details
1	Model	Dexing Electromagnet System DXSB-178
2	DC Magnetic Field	2.5 T for 5 mm and 0.47 T for 130 mm
3	Pole Pieces	76 mm (diameter) with Optical Access
4	Power Supply	DC, 9 kW with current range 90 A
5	Current Stability	2 mA/hr, 10 mA/24 hr
6	Chiller	Cooling of Power supply and Electromagnet with 15 l/min flow rate

Table- E.2: Specifications of High-Speed Imaging System

Sr	Parameter	Value/Details
1	Camera model	Phantom MIRO 320s, COLOR, 6GB RAM
2	Maximum Resolution	1920 × 1200
3	Frame Rate	1380 fps at Maximum Resolution
4	Throughput (Gpx/s)	3.2 Gpx/s
5	Sensor Size	19.2 mm × 10.8 mm
6	Minimum Exposure	1 μs
7	Image Acquisition System	Intel(R) Core(TM) i7-3540M CPU @ 3.00 GHz, 8 GB RAM, 64 bit OS
8	Analysis Tool	Phantom Camera Control Software Ver 2.5
9	Optics	Navitar Zoom High Magnification Optics
10	Light Source	Moritex 250 W Metal Halide Light Source

Table- E.3: Specifications of Syringe Pumps

Sr	Model	Syringes	Accuracy %	Force (N)	Flow rates (min, max)
1	KDS Gemini 88	2	0.35	254	(0.0004 μl/h, 106.6 ml/min)
2	NEW ERA 1010	1	1	445	(0.001 μl/h, 166 ml/min)
3	NEW ERA 1002x	1	1	445	(0.008 nl/h, 166 ml/min)

Table- E.4: Specifications of UV LED Source (Supplier datasheet: Agiltron UV LED, Model: SUVA-011111021)

Sr	Parameter	Description	Unit
1	Wavelength	365±5, 385±5	nm
2	UV irradiance	4.0 – 5.0	W/cm2
3	Cure time Max	60 s	
4	UV spot size	Φ4	mm
5	UV cure distance	20	mm
6	Cooling method	air blowing	-