

Particle Interactions in a Magnetophoretic System



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Abstract

The continuous flow separation of magnetic particles from a mixture of particles could improve the performance of magnetic bead based assays but the formation of agglomerates limit the separation efficiency. Bead agglomerates are formed as a result of magnetic binding forces while the hydrodynamic fluid environment strongly influences their movement. The ability to predict the interaction between nearby beads will help to determine a threshold separation distance which will be recommended for use when obtaining measurement within a magnetic bead assay for a specified time interval.

The introductory part of this thesis explored the development of a two dimensional numerical model in Matlab which predicts the trajectory pattern as well as magnetic induced velocities between a pair of super-paramagnetic beads suspended in water within a uniform field.

The movement of a bead pair interacting due to both magnetic and hydrodynamic forces within a magnetophoretic system was recorded using an optical system; the beads' movements were compared with the simulated trajectories and gave a good agreement. The model was used to predict the shortest agglomeration time for a given separation distance which is of practical benefit to users of bead based assays.

The concluding part of this thesis expanded the simulation into a three dimensional model to predict the interactions among three super-paramagnetic beads within a magnetophoretic system. In order to determine the height of the magnetic beads, a Huygens-Fresnel model was implemented in Matlab which was compared with off-focused diffracted images of the beads viewed under an optical system. A good comparison was obtained by comparing the simulated three-dimensional trajectories with experimental data.

CONTENTS

<i>Abstract.....</i>	<i>2</i>
<i>Figure Index</i>	<i>9</i>
<i>Research Output</i>	<i>15</i>
<i>Nomenclature</i>	<i>16</i>
<i>Chapter 1 Introduction.....</i>	<i>19</i>
1.1 <i>Aims and Objectives</i>	<i>19</i>
1.2 <i>Motivation.....</i>	<i>20</i>
1.3 <i>Properties of Super-Paramagnetic Bead.....</i>	<i>24</i>
1.4 <i>Magnetic Particle Separation.....</i>	<i>26</i>
1.5 <i>Particle Interactions in a Magnetophoretic System</i>	<i>33</i>
1.6 <i>Experimental Approximation.....</i>	<i>39</i>
1.7 <i>Structure and Outline of Thesis</i>	<i>40</i>
<i>Chapter 2 Bead Pair Interaction within a Magnetophoretic System</i>	<i>42</i>
2.1 <i>Overview.....</i>	<i>42</i>
2.2 <i>Super-Paramagnetism</i>	<i>43</i>

2.3	<i>Magnetic Force Interaction</i>	45
2.3.1	Dipole Approximation.....	45
2.3.2	Theory on Magnetic Force Interaction	46
2.3.3	Derivation	47
2.4	<i>Long Range Hydrodynamic Interaction</i>	52
2.4.1	Stokes Flow past an Isolated bead.....	53
2.4.2	Method of Reflection.....	56
2.5	<i>Short Range Hydrodynamic Interaction</i>	62
2.6	<i>Sedimentation Forces</i>	64
2.6.1	Gravity	64
2.6.2	Buoyancy	64
2.6.3	Sedimentation	65
2.7	<i>Brownian Motion</i>	66
2.7.1	Introduction	66
2.7.2	Theory on Brownian Motion	66
2.8	<i>Numerical Model</i>	67
2.8.1	Forces and Velocities of Particles	67
2.8.2	Numerical Method.....	70
2.8.2.1	Finite-Time-Finite-Difference scheme	70
2.8.2.2	Predictor Corrector Method	71
2.8.3	Implementation of Numerical Method	72

2.9	<i>Conclusion</i>	72
<i>Chapter 3 Experimental Investigation of Bead Pair Interaction in a Magnetophoretic System</i>		74
3.1	<i>Overview</i>	74
3.2	<i>Experimental Setup</i>	75
3.2.1	Microfluidic Apparatus.....	75
3.2.1.1	Hydrostatic Fluid Channel	75
3.2.1.2	Preparation of solution of one-micron chemicell beads	75
3.2.1.3	Preparation of solution of 2.8 microns Dynal Beads	77
3.2.1.4	Preparation of solution of one-micron Dynal Beads	78
3.2.2	Magnetic Configuration	78
3.2.3	Experiment Setup	80
3.2.4	Uncertainties associated with Experimental Setup	82
3.3	<i>Evaluation of Brownian motion</i>	83
3.3.1	Description of Brownian Motion Experiment.....	83
3.3.2	Discussion of Results	84
3.4	<i>Experiment investigating bead pair Interaction using one-micron chemicell beads in a Magnetophoretic system</i>	86
3.4.1	Description of Bead Pair Interaction Experiment	86
3.4.2	Comparison of Experimental data with simulation results for one-micron chemicell beads	87
3.4.3	Discussion of Results	93

3.5	<i>Experiment investigating bead pair interaction using 2.8 microns Dynal Beads (M-280) in a Magnetophoretic system</i>	94
3.5.1	Description of Bead pair Interaction Experiment using M-280 beads	94
3.5.2	Comparison of Experimental data with simulation results for 2.8-microns Dynal beads	95
3.5.3	Discussion of Results	103
3.6	<i>Interaction between Different sized beads</i>	105
3.6.1	Theoretical principle on the Interaction between different sized magnetic beads	105
3.6.2	Description of Different Sized Bead Pair Interaction Experiment	107
3.6.3	Discussion of Results	107
3.7	<i>Agglomeration Time as a function of the separation distance</i>	109
3.7.1	Simulation predicting the relationship between Agglomeration Time and Separation distance	110
3.7.2	Theoretical Analysis predicting the Agglomeration Time beyond 15 μ m	112
3.7.3	Discussion of Results	113
3.8	<i>Tolerances in Bead parameters</i>	114
3.9	<i>Conclusion</i>	114
Chapter 4 Many Beads Interaction in a Magnetophoretic System		119
4.1	Overview	119
4.2	<i>Three dimensional Model for many particles interacting within a Magnetophoretic System</i>	120
4.2.1	Magnetic Force Interaction	120
4.2.1.1	Direct Interaction	121

4.2.1.2 Indirect Interaction	121
4.2.2 Hydrodynamic Interaction.....	123
4.2.2.1 Description of Blocking	126
4.2.3 Modelling and Simulation	133
4.3 <i>Fourier Diffraction Model for height determination</i>	134
4.3.1 Review	134
4.3.2 Theory on Light Propagation.....	136
4.3.2.1 Fresnel Diffraction	137
4.3.2.2 Fraunhofer Diffraction	138
4.3.2.3 Propagation through a thin Lens	139
4.3.2.4 Defocus	140
4.3.2.5 Abbe theory of Imaging	142
4.3.2.6 Optical Model	144
4.3.3 Experiment for Height determination.....	145
4.3.4 Comparison of Simulation Results with Experimental Data.....	146
4.4 <i>Experiment investigating interactions among many beads within a Magnetophoretic System</i>	151
4.4.1 Preparation of solution of one-micron my-one beads	151
4.4.2 Magnetic Configuration.....	152
4.4.3 Many Beads Interaction Experiment	152
4.4.3.1 Description of Many Beads Interaction Experiment	152
4.4.3.2 Comparison between Experiment and Simulation	153

4.5	<i>Results and Discussion</i>	154
4.6	<i>Experiment investigating Sedimentation</i>	163
4.7	<i>Extension of the three dimensional model predicting interaction among three super-paramagnetic beads</i>	163
4.7.1	Discussion of Results	165
4.7.2	Limitation of Simplified Model	169
4.8	<i>Conclusion</i>	170
Chapter 5 Summary and Conclusion.....		173
5.1	<i>Summary</i>	173
5.2	<i>Conclusion</i>	175
5.2.1	Future work	175
Appendix.....		180
A1		180
A2		181
A3		183
References.....		186

Figure Index

Figure 1.1 1 μ m super-paramagnetic bead from Chemicell [25] showing the inner magnetic core (comprised of iron-oxide nano particles) surrounded by a silica shell with an hydrodynamic diameter .	25
Figure 1.2 Magnetic beads separated continuously from non-magnetic beads within a microfluidic device by the use of a permanent magnet	29
Figure 1.3 Continuous separation within a microfluidic device using an electromagnet [29]	30
Figure 1.4 (a) Magnetophoretic separation showing permanent magnets placed above and below a microsystem, the micro-system becomes magnetized by the permanent magnet (b) An enlarged view of the magnetized elements [27]	31
Figure 1.5 (a) A perspective, (b) side and (c) top view of a quadrupole used for continuously separating magnetically labelled cells from unlabelled cells [48, 52]	32
Figure 2.1 Schematic showing the relationship between coercivity and size distribution of magnetic particles [30]	44
Figure 2.2 A pair of super-paramagnetic beads placed in a uniform magnetic field (in the x direction) separated by a position vector $\mathbf{r}$, within a rectangular coordinate system (Oxy)	48
Figure 2.3 Two beads separated by the position vector $\mathbf{r}$, within an x-y coordinate system moving within a viscous fluid with velocities $\mathbf{u}_1$ and $\mathbf{u}_2$ respectively	58
Figure 2.4 Two beads with radii a_1 and a_2 separated by a minimum gap distance d_o approaching one another with a relative velocity and interacting due to lubrication force within a viscous fluid under the special case when the beads are approaching along their line of centre	63
Figure 3.1 A relationship between the magnetisation, $\mathbf{M}$ and the applied flux density, $\mathbf{B}$ of a volume containing a large number of 1 μ m sized super-paramagnetic bead obtained from Chemicell, at room temperature within the range ± 0.25 T. Inset figure shows the magnetisation versus applied flux density characterization within the range ± 7 T	76
Figure 3.2 Variation of applied flux density distribution along the horizontal axis; this distribution is symmetrical, either side of the centre-line indicated in the figure	79

Figure 3.3 Schematic showing the experimental set up where images and videos of the movement and interaction between a pair of magnetic beads suspended in a static fluid placed within a uniform magnetic field are captured by the microscope's camera	81
Figure 3.4a Root Mean Square distribution of a 1 μ m sized super-paramagnetic bead undergoing Brownian motion when suspended in water at room temperature	84
Figure 3.4b Comparison of the root-mean square displacement of a one-micron bead at various time steps obtained using the theoretical predictions (in red continuous line) and experimental data (in black circles with error bars). Error bars represent the standard deviation above and below the mean value	85
Figure 3.5a Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale	88
Figure 3.5b Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale	89
Figure 3.5c Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale	90
Figure 3.5d Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale	91

Figure 3.6 The average approach velocity of $1\mu\text{m}$ diameter bead interacting with another $1\mu\text{m}$ diameter bead in a uniform magnetic field (in the direction of the black arrow) suspended in water; experimental data (shown by blue crosses) and simulation (depicted by the red continuous line) are compared to validate the model. The blue and green arrows indicate the direction of velocity increase as the beads approach one another92

Figure 3.7a Trajectory plot of a $2.8\mu\text{m}$ diameter bead (indicated in red) moving towards another $2.8\mu\text{m}$ diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The dotted circles show the starting point of the magnetic beads while the continuous circle show the end point which occurs when the beads are separated by $2.8\mu\text{m}$. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale96

Figure 3.7b Trajectory plot of a $2.8\mu\text{m}$ diameter bead (indicated in red) towards another $2.8\mu\text{m}$ diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale97

Figure 3.7c Trajectory plot of a $2.8\mu\text{m}$ diameter bead (indicated in red) towards another $2.8\mu\text{m}$ diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale98

Figure 3.7d Trajectory plot of a $2.8\mu\text{m}$ diameter bead (indicated in red) towards another $2.8\mu\text{m}$ diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale99

Figure 3.7e Trajectory plot of a $2.8\mu\text{m}$ diameter bead (indicated in red) towards another $2.8\mu\text{m}$ diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale100

Figure 3.8 The resolved velocity of a 2.8 μ m diameter bead along the direction of magnetic field interacting with another 2.8 μ m diameter bead in a uniform magnetic field suspended in PEG; experimental data (shown by the blue crosses) and simulation results (illustrated by the red and black continuous lines corresponding to magnetic susceptibility values of 0.65[84] and 0.8[79] respectively) are compared to validate the model	102
Figure 3.9 A schematic showing the magnetic interaction force $\mathbf{F}_m$, between a pair of different sized super-paramagnetic beads (a_1 and a_2 respectively) displaced by a position vector $\mathbf{r}$, within a uniform magnetic field (the direction of the magnetic field is illustrated by the arrow at the top). The beads are suspended in water and sediment under the action of force $\mathbf{F}_s$	106
Figure 3.10 Trajectory plot of two super-paramagnetic beads, 1 μ m and 2.8 μ m sized, (indicated by red and blue) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction suspended in water. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model	108
Figure 3.11 Relationship between the shortest agglomeration time and the separation distance for a pair of super-paramagnetic beads (Chemicell Simag in blue, Myone T1 Dynal bead in red and M-280 Dynal bead in black) positioned along the direction of the magnetic field	111
Figure 4.1 Schematic showing direct magnetic force interaction ($\mathbf{F}_{m12}$) on a reference super-paramagnetic bead (1) due to the presence of a neighbouring bead (2) within a uniform magnetic field and an Indirect magnetic force Interaction ($\mathbf{F}_{m32}$) between the two neighbouring beads (2, 3) which affects the movement of the reference bead (1) due to the fluid environment; bead 1 is separated from beads (2, 3) by position vectors $\mathbf{r}_{12}$ and $\mathbf{r}_{13}$ respectively	122
Figure 4.2a On the verge of Blocking	128
Figure 4.2b Partial Blocking	128
Figure 4.2c Full Blocking	129
Figure 4.3 A schematic demonstrating how a blocked bead (bead 3) appears when viewed from bead 1. The shadow of the blocked bead (bead 4) is projected to the same plane as bead 2, creating bead 4	130
Figure 4.4 Propagation of light from a point source through an aperture	137
Figure 4.5 Propagation of light from the input plane through a lens to the output plane	139

Figure 4.6 Post-processed images of $1\mu\text{m}$ super-paramagnetic beads (myone Dynal Beads Streptavidin T1 [26] obtained from Thermo Fisher Scientific) viewed under the optical microscope at different axial positions	147
Figure 4.7 Normalized Intensity distribution of a $1\mu\text{m}$ super-paramagnetic bead (myone Dynal Beads Streptavidin T1 [26] obtained from Thermo Fisher Scientific) viewed under the optical microscope at focus and various defocus distances	148
Figure 4.8 Normalized Intensity distribution of a $1\mu\text{m}$ super-paramagnetic bead obtained from the optical model in Matlab at focus and various defocus distances	149
Figure 4.9 Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. The starting points of the beads are indicated by the dotted circles while the end points are shown by the continuous circles. The direction of the magnetic field is indicated by the black arrow on the plot. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model	155
Figure 4.10 Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. The starting points of the beads are indicated by the dotted circles while the end points are shown by the continuous circles. The direction of the magnetic field is indicated by the black arrow on the plot. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model	157
Figure 4.11 Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized, suspended in water (indicated by red, blue and black) under the same conditions as figure 4.9 ..	158
Figure 4.12 (a) Three-dimensional (with height)	159
Figure 4.12 (b) Two dimensional projected trajectory plot (along x-y plane and x-z plane, for illustrative purposes) of three super-paramagnetic beads, under the same conditions as figure 4.9. The starting points of the beads are indicated by the thin continuous circles while the end points are shown by the thick continuous circles. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model	160
Figure 4.13 (a) Three-dimensional (with height)	161
Figure 4.13 (b) Two dimensional projected trajectory plot (along x-y plane and x-z plane, for illustrative purposes) of three super-paramagnetic beads, under the same conditions as figure 4.9. The starting points of the beads are indicated by the thin continuous circles while the end points	

are shown by the thick continuous circles. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model162

Figure 4.14 Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model. The starting points of the beads are indicated by the dotted circles while the dash circles represent the point at which a bead pair cluster is formed and the end points are represented by continuous circles. The first set of experimental trajectories is represented by red, blue and black crosses while the second set is represented by magenta and green crosses. The first set of simulated trajectory is represented by red, blue and magenta lines while the second set is represented by black and red lines. The direction of the magnetic field is indicated by the black arrow on the plot166

Figure 4.15 Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model. The starting points of the beads are indicated by the dotted circles while the dash circles represent the point at which a bead pair cluster is formed and the end points are represented by continuous circles. The first set of experimental trajectory is represented by red, blue and black crosses while the second set is represented by magenta and green crosses. The first set of simulated trajectory is represented by red, blue and magenta lines while the second set is represented by black and red lines. The direction of the magnetic field is indicated by the black arrow on the plot168

Figure 5.1 (a) The attachment of a pair of red blood cell ($6-8\mu\text{m}$) to a $1\mu\text{m}$ sized super-paramagnetic bead and (b) subsequent magnetic manipulation in the presence of an external field, demonstrated in two stages to allow for aggregation between the red blood cells178

Research Output

- [1] O. Oduwole, D. T. Grob, S. Sheard, “Comparison between simulation and experimentally interactions between two magnetic beads in a fluidic system”, *JMM*, vol. 407, pp. 8-12, June 2016.
- [2] O. Oduwole, S. Sheard, “Comparison between simulation and experimentally interactions between two different sized magnetic beads in a fluidic system”, *ICNBE*, Madrid, March 2016.
- [3] O. Oduwole, S. Sheard, “Three-dimensional mathematical modelling of magnetic bead interactions within a magnetic separation system”, *JAMM*, 2016 (In Progress).

Nomenclature

This table provides information on all the symbols used in this thesis.

Symbols	Name	Definition of Unit	Unit
a	Radius	metre	m
a_g	Geometric mean of radii	$(a_1 \times a_2)^{1/2}$ in metre	m
a_h	Harmonic mean of radii	$(2 \times a_1 \times a_2) / (a_1 + a_2)$ in metre	m
$\mathbf{A}$	Magnetic Vector Potential	Volt second per metre	Vs/m
A	Area	metre-square	m ²
b	Blocking Factor	Dimensionless	Dimensionless
$\mathbf{B}_{\text{ext}}$	Applied Flux density	Tesla	T
C	Courant Number	Dimensionless	Dimensionless
d	Centre-to-centre distance	metre	m
$d_o = d - 2a$	Gap-distance	metre	m
df	Defocus distance	metre	m
d_r	Minimum Resolved distance	metre	m
D	Diameter	metre	m
D_f	Diffusion coefficient	metre-square/second	m ² /s
f	Focal length	metre	m
$f()$	Function	Dimensionless	Dimensionless
f_o	Frequency	Per second (Hertz)	1/s (hz)
F	2D Fourier Transform	Dimensionless	Dimensionless
$\mathbf{F}$	Force	Newton	N
g	acceleration due to gravity	metre/second square	m/s ²
G	Oseen Tensor	Dimensionless	Dimensionless
h	Channel depth	metre	m
$\mathbf{H}_c$	Coercive field	Ampere per metre	A/m
$\mathbf{H}_{\text{ext}}$	Magnetic-field Strength	Ampere per metre	A/m
$\mathbf{I}$	Unit Tensor	Dimensionless	Dimensionless
$\mathbf{J}$	Current Density	Ampere per square metre	A/m ²

Symbols	Name	Definition of Unit	Unit
k	Boltzmann constant	1.38×10^{-23} Joule per kelvin	J/k
k_o	Wave number	metre	m
k_a, k_b	Hydrodynamic Constants	Dimensionless	Dimensionless
k_x, k_y	Spatial Frequency	Per metre	m^{-1}
K_b	Anisotropy Constant	Joule per metre cubed	J/m^3
L	Langevin Function	Dimensionless	Dimensionless
m	mass	Kilogram	kg
$\mathbf{m}$	Magnetic moment	Ampere square metre	Am^2
$\mathbf{M}$	Magnetisation	Ampere per metre	A/m
n	Refractive Index	Dimensionless	Dimensionless
$\mathbf{n}$	Normal	Dimensionless	Dimensionless
n_d	Number of single domains	Dimensionless	Dimensionless
NA	Numerical Aperture	Dimensionless	Dimensionless
p	pressure	Newton per square metre	N/m^2
P	Pupil Function of lens	Dimensionless	Dimensionless
$\mathbf{r}$	Position vector	metre	m
r	Distance between beads	metre	m
R	Propagation distance	metre	m
t	Time	second	s
T	Temperature	Kelvin	k
$\mathbf{u}$	Velocity of bead	metre per second	m/s
$\mathbf{v}$	Velocity of fluid	metre per second	m/s
v_f	Visibility factor	Dimensionless	Dimensionless
V	Volume	metre per cubed	m^3
z	Axial distance	metre	m
ρ	Density	kilogram/metre-cubed	kg/m^3
χ	Intrinsic magnetic bead susceptibility	Dimensionless	Dimensionless
μ	Medium permeability	Henry per metre	H/m
μ_r	Relative permeability	Dimensionless	Dimensionless
η	Viscosity	kilogram per metre-	kg/ms

		second	
μ_0	Vacuum permeability	$4\pi \times 10^{-7}$ Henry/metre	H/m
λ	Wavelength	Metre	m
δt	Time step	Second	s
δx	Spatial step	Metre	m

Chapter 1

Introduction

1.1 AIMS AND OBJECTIVES

Magnetic cell separation involves binding biomolecules to magnetic beads and their continuous separation from either their host solution or non-magnetic beads using a magnetic device in microfluidic channels. However, the undesirable formation of bead clusters (agglomerates) within the microfluidic channel limits the separation efficiency.

Bead agglomerates are formed due to magnetic binding forces while hydrodynamic interactions strongly influence the beads' movement. Bead agglomerates cause channel clogging, surface deposits which obstructs the flow of fluid within the separation channel and alter the magnetic field properties; all reducing the separation efficiency of the magnetic separator [1, 2, 3, 4]. For example, Choesmel et al. reported in their work [2] that they experienced a problem due to bead agglomeration which led to the blockage of magnetic columns.

Furthermore, when bio-markers occur with a cluster of beads, they become less reliable for a biomedical assay. This is because the bound magnetic beads make it difficult to visualize the attached bio-molecules when viewed under an optical detector [1]. After separation, cells are sometimes analysed using optical detectors; clusters of cells produce a greater forward scatter (FS) or side scatter (SS) when compared to a single cell in isolation. The greater FS or SS may lead to erroneously discarding bio-markers bound to bead clusters during the analysis of the final results [5]. In addition, experimental observation reveals that once the super-paramagnetic beads have become agglomerated by the magnetic field within the channels or walls of separation devices, it is

possible that they will not be easy to separate once the magnetic field is removed due to other attractive intermolecular surface forces e.g. van der Waals force [3].

The main objective of this project is to understand particle interactions within a magnetophoretic system and present recommendations that will improve the separation efficiency of magnetic separator devices. This will open the way for the design of more efficient biomedical magnetic separators which will not be limited by the formation of agglomerates and for which particle behaviour can be easily predicted. Such design depends on the knowledge of particle interactions, trajectory pattern, behaviour and properties within the magnetic field and hydrodynamic environment.

Furthermore, a knowledge of the properties of magnetic beads can help predict their behaviour when manipulated within a biological medium; this will pave the way to the study and understanding of individual interaction and biophysical mechanisms (e.g. cell-cell interaction) often hidden within an ensemble model. This will be discussed in greater detail in the last chapter.

1.2 MOTIVATION

This work was initiated in collaboration with the research group and clinicians at the John Radcliffe Hospital [6] towards the design of a continuous flow magnetic separator for the separation and removal of a specific vesicle from a mixed vesicle sample obtained from pre-eclampsia patients.

During research by other workers in the field (Grob et al. [105]) on the design of a continuous flow magnetic separator, a high density of $1\mu\text{m}$ chemicell beads ($\geq 9 \times 10^6$ beads/ml) was used

during evaluation in order to obtain a visible bead output after separation. Under this condition, the magnetic beads were typically separated by no more than 5-8 μ m and thus experienced the same magnitude and gradient of the magnetic field. This would lead to interaction among beads, eventually causing bead clusters and agglomerates.

Once a bead cluster is formed, it starts to attract other beads. Gradually, agglomerates grow in size within the separator. One of the effects of agglomerates formation (as discussed in the last section) is the blockage of the walls and channel surfaces, thus hindering the separation process. Other undesirable effects of agglomerates have been discussed in the last section.

The above limitation led to the development of this work to understand bead interactions within a magnetophoretic system. Once understood, an upper limited of bead density can be recommended to avoid agglomerate formation.

Pre-eclampsia is one of the leading causes of maternal mortality in both developed as well as developing countries. It leads to the death of approximately 50,000 women annually. It is a life threatening condition to both mother and child. It results in other symptoms such as high blood pressure, stroke, protein in urine, clotting disorder and a non-functional placenta which would affect the nutritional requirements of the baby [7, 8].

The key to surviving this condition is early detection and diagnosis. In addition to a diagnosis based on patient symptoms, it is also possible to monitor certain biomarkers and biological cells within the body which would serve as a signal, for example, the variations in the physical structure of nano-vesicles (30-100nm) and micro-vesicles (0.1-1 μ m), found in the blood plasma can serve

as indicators of diseases such as pre-eclampsia, cardiovascular disease, cancer, sepsis, HIV and diabetes [7].

The isolation, detection and characterization of these vesicles will provide more insight on these diseases and help in providing appropriate treatment methods which will save lives and further prevent spread of disease to healthy organs. However, these biomarkers occur in low concentrations, for example, placental derived cellular vesicles occur in 1 per 2.2 trillion per ml in the blood [7]; this presents a challenge for light scattering techniques to detect these cells.

Furthermore, cellular vesicles and lipid vesicles are of the same dimensions and the latter occurs in 1 per 108 trillion per ml in the blood; this makes it difficult to use physical based separation method for this application. Currently, ultracentrifugation is used and the procedure is time consuming and relies on the use of complicated and expensive equipment often handled by specialized and trained personnel [7, 9].

Furthermore, the discovery of biomarkers such as the sST2 biomarker in pregnant women suffering from pre-eclampsia [8] has facilitated the use of immuno-magnetic assays. Immuno-magnetic assays can be used to separate vesicles from the blood of pre-eclampsia patients by binding biotin antibodies to a magnetic bead streptavidin coated with antigen [7]; this can then be manipulated by an external magnetic field for separation and detection. After separation, the vesicles are still retained in a healthy state without any form of alteration to their properties and appropriate tests can be carried out to discover better treatment methods for patients.

Ma et al. [9] demonstrated the use of immuno-magnetic assays to detect and separate lung cancer cells from patients using magnetic particles bound to an antigen which targets the cancer markers

in the presence of a magnetic field. They were able to capture the cancer cells within a short amount of time (1 hour) using low cost and simple equipment compared to the time consuming and costly monitoring of cancerous cells in clinical samples using density gradient separation. The use of immuno-magnetic assays helped them to detect cancer cells in 7 out of 10 patients achieving 70% sensitivity; higher than the 60% sensitivity achieved using cytological examination.

Despite the low concentration of pathogens in the blood of septic patients (1 to 100 cells/ml), Cooper and his co-authors [1] were able to use immuno-magnetic assays to capture rare pathogenic cells from blood. They achieved a high capture efficiency in less than 3 hours compared with days using current technology (blood culture test).

Inglis et al. [10] demonstrated the separation of leukocytes from whole blood in their device; this was achieved by first binding the cells to magnetic particles. The bound complex were then trapped over a micro-fabricated magnetic strip with the use of high magnetic field gradients. The micro-device can be integrated with other components to form a microfluidic total analysis system.

The above benefits have led to an increased use in immuno-magnetic assays and magnetic cell separation for various diagnostic applications such as the detection, isolation and separation of biomarkers (found in low concentration) in diseased conditions such as breast cancer [2], sepsis [1], lung cancer [9] etc., the separation of red blood cell and white blood cell from whole blood [10, 11, 12], the capture of pathogens from saline and blood [1], as well as pathogenic detection in water purification system [13].

1.3 PROPERTIES OF SUPER-PARAMAGNETIC BEAD

Super-paramagnetic beads are made of a core which consists of nano-particles (magnetite or maghemite) embedded within a spherical matrix (micron-scale) as depicted in figure 1.1. Magnetic iron oxide particles are preferred within the core because of their large magnetic moment, stability against oxidation and the material is non-toxic and biodegradable [16, 17, 18, 19].

The large surface to volume ratio of super-paramagnetic beads allows for easy attachment to specific bio-molecules such as amino acid, carboxyl group etc. through specific binding sites on the surface of the bead [20, 18]. They can also be tailored to a certain application through chemical modification of their surface (coating) to allow for hydrophilicity, colloidal stability and easy dispersion [21]. For instance, the hydrophobic surface of polystyrene-coated magnetic particles results in a large amount of unspecific binding to proteins [18, 19], and this can be further chemically modified [22]. Coating also allows for a high selectivity and specificity when used as tagging agents [9].

Besides, these beads have physical dimensions comparable to some relevant biological entities such as red blood cells (6-8 μ m), the malaria parasite (1-1.2 μ m), *Escherichia coli* (2 μ m) etc. [17, 23]. They are widely available in different sizes and with varying magnetic properties from different manufacturers [24, 25, 26].

The inherent magnetic properties of magnetic beads enable easy manipulation within a magnetic field even when functionalized with bio-molecules. This manipulation is also facilitated by the low relative permeability (μ_r) of the fluid which enables strong interaction between the magnetic field and the magnetic-tagged particle [1, 14, 23, 27].

These numerous benefits have promoted their usage in biological and biomedical applications such as drug delivery and drug targeting [16, 19, 23], high gradient separation [8, 10, 11, 16, 19], cell transport [27], cell sorting and counting [3, 28], cell capture [16, 27], hyperthermia [16, 19, 23] etc.

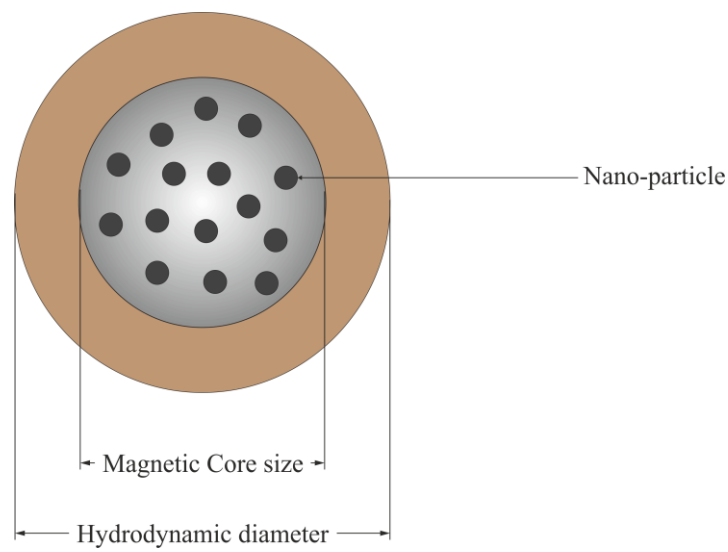


Figure 1.1 1 μ m super-paramagnetic bead from Chemicell [25] showing the inner magnetic core (comprised of iron-oxide nano particles) surrounded by a silica shell with an hydrodynamic diameter.

1.4 MAGNETIC PARTICLE SEPARATION

Here, the concept of magnetism is combined with microfluidics for use in magnetic particle separation; this separation technique has become increasingly popular within the biomedical industry over the last two decades [16, 27].

The concept of magnetic particle separation has been utilized in various diagnostic applications such as immuno-magnetic assays, where a target bio-molecule (cells, proteins, antibodies, bacteria, DNA, RNA etc.) is bound to a magnetic particle; the targeted bio-molecule is then isolated from a mixture using the magnetic properties of the complex.

Magnetic cell separation is typically achieved using a test-tube arrangement but there is growing interest in using microfluidics for this application due to (i) commercially available magnetic beads coated with specific bio-molecules e.g. dextran, carboxyl, amine groups, (ii) the ability to handle low sample volume, (iii) the potential for faster processing time, (iv) recent advances in microfluidic technology and (v) magnetic interactions are unaffected by surface charges, temperature and ionic concentration [16, 18, 27]. Furthermore, the small sizes of these microfluidic devices allow improved integration with other components such as pumps, valves and switches to form a micro total analysis system [10, 16, 27].

During continuous-flow magnetic separation, a mixture of particles is continuously introduced into a flow chamber in the presence of a magnetic field, which could be in the form of permanent magnet, integrated magnetic strip or electromagnet, depending on the application (see figures 1.2-1.5). Particles experience a magnetic force depending on their inherent magnetic properties, magnetic field gradient and physical dimensions as well as a hydrodynamic force which resists

their motion. The resultant force leads to the movement of particles in different directions within the chamber from whence they are separated and collected.

The properties of the magnetic bead and the magnetic field gradient needed to generate a high magnetic force are important factors that need to be considered when designing for a high separation or capture efficiency, transport and detection, fast separation time etc. The superparamagnetic bead has been discussed in the last section. This section will discuss the variety of magnetic configurations used by different authors for separation purposes. For instance, permanent magnets (see figure 1.2) are in-expensive, readily available and are able to produce a strong magnetic field. However, permanent magnets do not give the flexibility of varying the strength of the magnetic field within the micro-channel. Electromagnets (see figure 1.3), on the other hand, can produce high magnetic gradient, can be switched on/off and give the added flexibility of varying the magnetic field strength within the channel. They are however limited by bulkiness and Joule heating [3, 16, 29].

Pamme et al. [28] continuously separated magnetic particles (sized $2\mu\text{m}$ and $4.5\mu\text{m}$ with magnetic susceptibilities $1.12 \times 10^{-4} \text{ m}^3/\text{kg}$ and $1.6 \times 10^{-4} \text{ m}^3/\text{kg}$ respectively) from a mixture of both magnetic and non-magnetic particles ($6\mu\text{m}$ polystyrene beads) using their inherent magnetic properties in the presence of a cylindrical magnet within a rectangular microfluidic chamber.

Whereas, Furlani et al. [27] demonstrated the transport and capture of magnetic particles ($0.5\mu\text{m}$ diameter) within a magnetophoretic microsystem by using an array of integrated soft magnetic elements magnetized by a bias field (permanent magnets above and below the micro-system, see figure 1.4) and embedded in a non-magnetic substrate beneath the microfluidic channel. When the

magnetic elements become magnetized, they give rise to a non-uniform field distribution which exerts force on the magnetic particles within the micro-system (see figure 1.4).

Plouffe et al. [29] proposed the design of a magnet cell separation device comprising a disposable microfluidic channel and a reusable electromagnet (see figure 1.3). Zborowski et al. [48, 52] used a quadrupole configuration to continuously separate magnetically labelled cells (lymphocytes) from unlabelled cells in their proposed magnetic separator (see figure 1.5). The quadrupole magnetic configuration exerted a radially outward force (and 0.174T/mm gradient in the radial direction) on the magnetically labelled cells within the flow-through cylindrical column. The mixed cell suspension is introduced in inlet a' while fluid is introduced in inlet b' (see figure 1.5); the splitter is omitted within the magnetic field region in order to allow for interaction with the magnetically labelled cells. Hence, the radially directed magnetic force leads to the movement of the labelled cells in the radial direction while the unlabelled cells are unable to move in the radial direction. At the end, the flow splitter separates the flow into two outlets, a labelled flow in outlet b and the non-labelled in outlet a, leading to separation (see figure 1.5).

Afshar et al. [31] demonstrated both static and continuous separation of different sized beads (1 μ m and 2.8 μ m sized with magnetic susceptibilities of 1.38 and 0.84 respectively) in a microfluidic channel placed close to soft-magnetic micro-tips which generates a high magnetic field gradient when magnetized by an external electromagnet.

Other authors have also designed a continuous flow magnetophoretic separator by placing a ferromagnetic wire within a uniform magnetic field along the microfluidic channel [11], using an array of micro-fabricated magnetic stripes [10], and permanent magnets in an alternating spatial arrangement [12] for the separation of whole blood into its constituent blood cells.

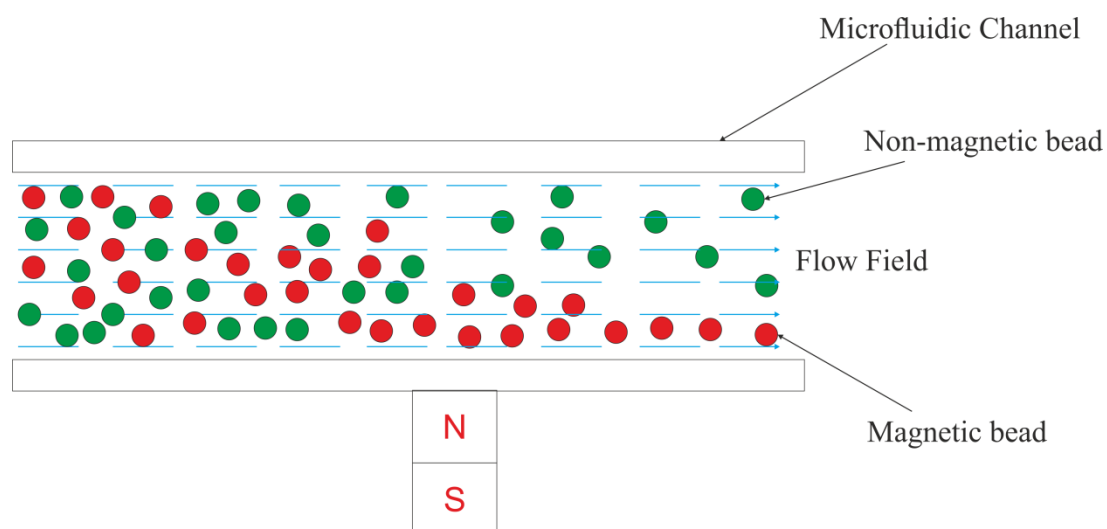


Figure 1.2 Magnetic beads separated continuously from non-magnetic beads within a microfluidic device by the use of a permanent magnet. The permanent magnet attracts the magnetic beads whereas the non-magnetic beads are not attracted, thus leading to separation.

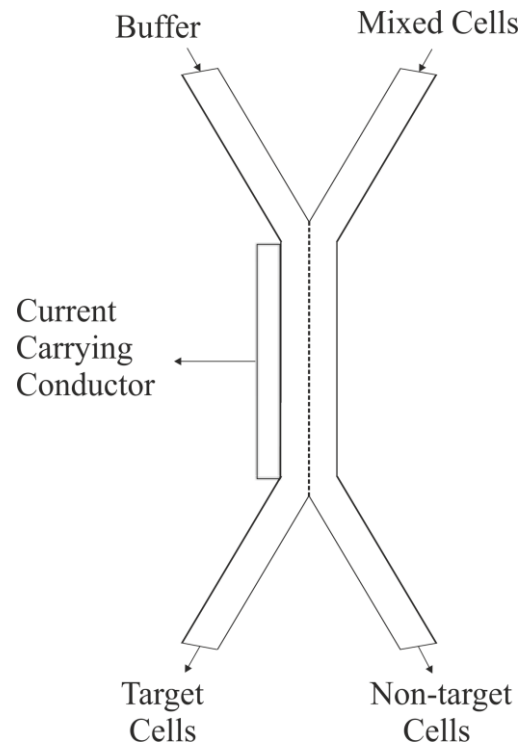


Figure 1.3 Continuous separation within a microfluidic device using an electromagnet [29]. When current is passed through the electromagnet, this gives rise to a magnetic field which attracts the target cells, giving rise to the deflection and movement of cells in different outlets.

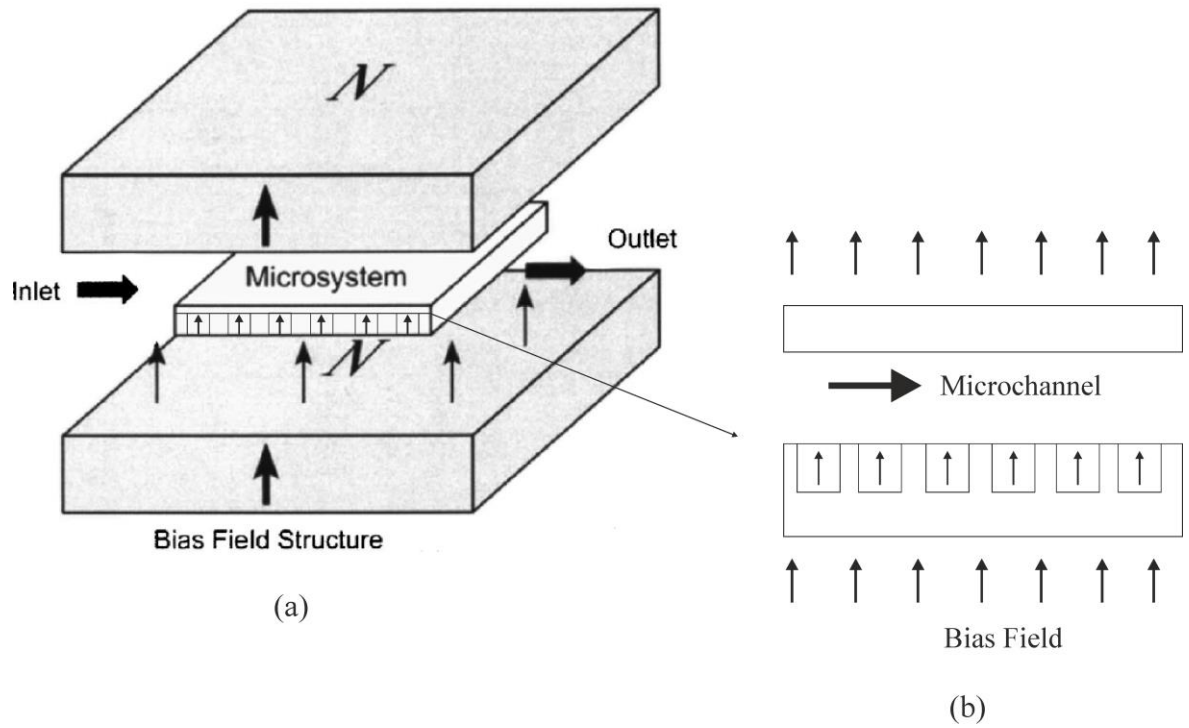


Figure 1.4 (a) Magnetophoretic separation showing permanent magnets placed above and below a microsystem, the micro-system becomes magnetized by the permanent magnet (b) An enlarged view of the magnetized elements. The magnetized elements are magnetized by the bias field and in turn exert magnetic force on the magnetic particles which leads to the continuous capture and transport of magnetic particles within the micro-system [27]. Figure 1.4a has been reproduced from [27].

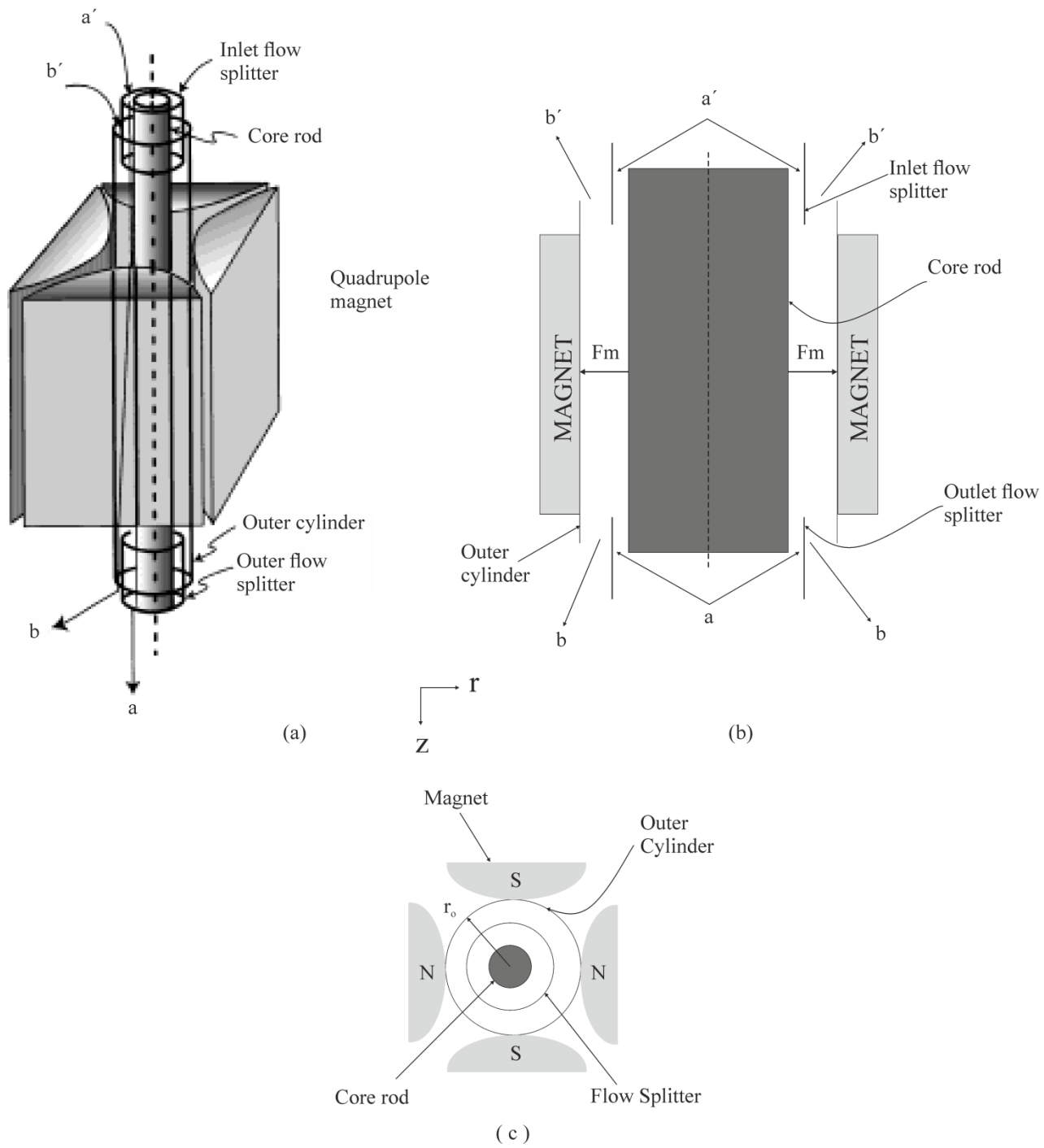


Figure 1.5 (a) A perspective, (b) side and (c) top view of a quadrupole used for continuously separating magnetically labelled cells from unlabelled cells [48, 52]. A cell suspension is introduced into inlet a' and fluid in inlet b' , the magnetically labelled cells experience a radially directed outward force and cross the fluid stream-lines while the unlabelled cells do not experience the radial force. This leads to separation at the outlet and as the magnetically labelled cells are collected in outlet b and the unlabelled cells are collected at outlet a . Figure 1.5a has been reproduced from [52].

1.5 PARTICLE INTERACTIONS IN A MAGNETOPHORETIC SYSTEM

Bead agglomerates are formed due to particle-particle interactions within the magnetic separation system. The magnetic force interaction between the beads leads to an in-homogeneity in the uniform field distribution whereas the fluid acts as a medium that transmits the reflected velocity field due to the movement of suspended particles. (In this work, bead and particle are used interchangeably).

Various research articles have been published on the concept of magnetic cell separation and its diverse use for biological and biomedical applications; however the study of particle interactions within a magnetophoretic system is limited and may have important influence on the separation capability of these channels. It is the need to understand particle interactions that has driven this work.

Ten published papers of relevance to the modelling of magnetic and hydrodynamic interactions within a magnetophoretic system are discussed here.

One of the most common methods for modelling the magnetic interactions between a pair of superparamagnetic beads has been based on the dipole approximation (see chapter two) as demonstrated by Mikkelsen et al., Banerjee et al., Sinha et al., Liu et al. [32, 33, 34, 35] in their work.

Mikkelsen et al. derived the magnetic interaction force on a bead using superposition by taking into account all forms of perturbations due to the presence of a neighbouring bead within the external field as given by equation 1.1 while Banerjee et al., Sinha et al. and Liu et al. used the same starting approach but expressed the equations in a different way. They evaluated the induced dipole due to the presence of other dipoles (equations 1.2 and 1.3) and then calculated the force interaction using equation 1.4.

$$\mathbf{F}_1 = \varphi_0 \int ((\mathbf{M}_1 + \mathbf{dM}_1) \cdot \nabla) (\mathbf{H}_{\text{ext}} + \mathbf{dH}_2) dV \quad (1.1)$$

$$\mathbf{H}_1 = \mathbf{H}_{\text{ext}} + \sum_{j=2}^n \mathbf{dH}_j \quad (1.2)$$

$$\mathbf{dH}_j = \frac{-\mathbf{m}_j}{r_{1j}^3} + \frac{3(\mathbf{m}_j \cdot \mathbf{r}_{1j})\mathbf{r}_{1j}}{r_{1j}^5} \quad (1.3)$$

$$\mathbf{F}_1 = (\mathbf{m}_1 \cdot \nabla) \mathbf{B}_1 \quad (1.4)$$

where the subscript j represents neighbouring bead.

However, Schaller et al. [20] used similar approach but a different starting point to evaluate the dipole-dipole force interaction by differentiating the interaction energy between two dipoles and using the induced moments of the bead derived by using the Langevin function, L , as given in equations 1.5 and 1.6 respectively.

$$\mathbf{F}_1 = \frac{3\mu_o}{2\pi} \frac{(\mathbf{m}_1 \cdot \mathbf{m}_j)}{r_{1j}^4}, \quad 1 \neq j \quad (1.5)$$

$$\mathbf{m}_1 = n_d \mathbf{M}_s V_d L \frac{\mathbf{M}_s V_d \mathbf{B}_1}{kT} \quad (1.6)$$

where subscript 1 represents the first bead, j represents the neighbouring bead(s), $\mathbf{F}_1$ is the interaction magnetic force on bead 1 due to the neighbouring bead(s) in N, $\mathbf{M}$ and $\mathbf{M}_s$ represent the magnetisation and intrinsic magnetisation of single domains of magnetite in A/m, V and V_d represent the volume of the bead and single domain in m^3 respectively, n_d is the number of single domains in the particle and $\mathbf{B}$ is the applied flux density in T. Other parameters have been defined within the nomenclature section.

Previous studies have modelled and studied hydrodynamic interactions between two spheres suspended in viscous fluid [36, 37], bubbles [15], micro-organisms [40], micro-machines [41], a sphere and a plane wall [39, 42] and more recently magnetic beads in a microsystem [20, 32, 33, 34].

The different methods discussed in this review used to model the hydrodynamic interactions between magnetic beads in a microsystem all rely on the assumption that a point force acts at the centre of the bead. Nasser et al. [41] reviewed the hydrodynamic interactions between two micro-machines at low Reynolds' number using the method of reflection (see chapter two). Using this method, they obtained the velocities as well as drag force for two spheres translating along their line of centres as given below.

$$-\frac{\mathbf{F}}{6\pi\eta a} = \frac{u_x \hat{i}}{1 + \frac{3a}{4d}} + \frac{u_z \hat{k}}{1 + \frac{3a}{2d}} \quad (1.7)$$

On the other hand, the induced force method, which relies on the use of the Oseen tensor [43] and external force to calculate the velocity and pressure fields, was used by authors in [37, 44] to predict the hydrodynamic interactions between particles as depicted in equation 1.8 below.

$$\mathbf{u}(\mathbf{r}) = \mathbf{G}(\mathbf{r} - \mathbf{r}').\mathbf{F}(\mathbf{r}') \quad (1.8)$$

Schaller et al. [20] also modelled hydrodynamic interactions between magnetic particles and incorporated Brownian dynamics through the use of an inter-particle diffusion tensor which assumes that the particles act as point forces (equations 1.9 and 1.10 below).

$$D_f = \frac{kT}{8\pi\eta r} \left(\mathbf{I} + \frac{\mathbf{r}\mathbf{r}}{r^2} \right) \quad (1.9)$$

$$\mathbf{u} = \frac{D_f \mathbf{F}}{kT} \quad (1.10)$$

Other authors in [32, 34] have also used the drag force (equation 1.11 below) as a simple way of accounting for the hydrodynamic interaction between the magnetic particle and the local fluid environment.

$$\mathbf{F} = -6\pi\eta a(\mathbf{v} - \mathbf{u}) \quad (1.11)$$

When the beads approach each other at a distance comparable to their dimensions within the fluid, Banerjee et al. and Liu et al. used an empirical equation which repels the beads from coming into closer contact and has a smooth variation to zero at a cut off distance. The repulsion force on bead j due to bead k is given by

$$\text{when } \mathbf{r}_{jk} < 2.1a, \mathbf{F}_{\text{contact}} = -1.02 \frac{\mathbf{r}_{jk}}{2a} \left(\frac{(2.1a)^2 - \mathbf{r}_{jk}^2}{0.044a^2} \right)^3 \quad (1.12a)$$

$$\text{else } \mathbf{F}_{\text{contact}} = 0 \quad (1.12b)$$

On the other hand, the lubrication approximation can be used to model such interactions at such close separation as described in [37, 38, 45, 46, 47] at the expense of increased computational resources. Yang et al. [38] determined the hydrodynamic interaction between two unequal spheres within the lubrication regime; however, as their surfaces approach one another, non-hydrodynamic colloidal forces become dominant over the lubrication force [38]. Chan et al. [46], on the other hand, derived the lubrication force between a curved surface and a flat plate from first principles in their paper.

Interestingly, the authors in [42] compared the method of reflection at a large gap width and lubrication approximation at small gap width with the limited experimental data available in the literature and obtained a good agreement. This confirmed the use of the method of reflection and lubrication approximation to model the hydrodynamic interactions between the magnetic beads in this work.

Experimental investigations [20, 32] have also been conducted on the interactions of magnetic beads within a micro system, for example, Banerjee et al. [32] conducted an experiment to investigate multiple bead aggregation using a higher concentration of beads and found that the aggregation time depends linearly on a group of parameters while Schaller et al. [20] demonstrated through their experiment (using higher bead concentration) that aggregated particles leads to a lower separation time and the acceleration of particles.

This review shows that theoretical predictions of particle interactions in a magnetophoretic system are abundant in the literature. Experiments that, however, exist in the literature are much more relevant to assays that require a high density of beads. So far, there has been no experimental study that predicts the trajectory, interaction of magnetic beads, agglomeration time etc. for assays that demand a low density of beads. It is this research gap that this work seeks to address.

This thesis has taken the study of particle interactions in a magnetophoretic system further by presenting experimental validation of bead pair interactions, three beads interactions, validation of theoretical model with experimental data and the prediction of a threshold distance to avoid agglomeration in a magnetic bead assay [102].

1.6 EXPERIMENTAL APPROXIMATION

In this work, bead pair interaction and three-beads interactions were experimentally investigated by observing the movement of beads suspended in a static fluid (within a solution chamber) placed in a uniform magnetic field. Bead displacement are then compared with simulated trajectories to verify the numerical model.

The use of a uniform magnetic field and static field in this study is assumed to be appropriate for investigating bead interactions in a continuous flow magnetic separator due to the following assumptions.

On any point within a continuous flow magnetic separator where magnetic beads are sufficiently close to cause magnetic interaction, the magnitude and gradient of magnetic field experienced by the beads will be effectively same and differences due to magnetic induced drift can be neglected; then a uniform magnetic field can serve as an approximate representation of such a magnetic environment.

Similarly, the use of a static fluid (during experimental observations) to serve as a representation of the moving fluid (in continuous flow magnetic separation) is justified by the fact that laminar flow conditions apply in both flow conditions. This means that due to the proximity of the beads, the velocities of the beads within a static fluid will be equivalent to the relative velocities of the beads within a moving fluid and thus the difference in flow is negligible.

1.7 STRUCTURE AND OUTLINE OF THESIS

This thesis is structured into a theoretical chapter and two experimental chapters.

Chapter two lays the theory behind particle interactions within a magnetophoretic system and introduces the concept of magnetic interaction and hydrodynamic interaction. The magnetic force interaction between a pair of magnetic beads was derived using the dipole approximation from first principles following the steps of Mikkelsen et al. While the hydrodynamic interactions between the beads was derived using the method of reflection and lubrication approximation to obtain the reflected velocity fields in a vector form. A numerical model was implemented in Matlab which predicts the interaction of a pair of super-paramagnetic beads (equal or different sized) within a magnetophoretic system.

The third chapter starts with a description of the apparatus used in obtaining experimental data. Bead pair interactions (using both $1\mu\text{m}$ and $2.8\mu\text{m}$ sized beads) were studied, observed and recorded within a magnetophoretic system. The simulated results are compared with the experimental data. The verified results helped to predict the relationship between the agglomeration time and the separation distance for beads in an assay.

Chapter four starts by extending the existing model into a three dimensional vector analysis to predict the interaction as well as sedimentation on three super-paramagnetic beads suspended in water within a uniform magnetic field. This is followed by an experimental investigation of the interactions among three super-paramagnetic beads within a magnetophoretic system. A Huygens Fresnel model was developed to predict the axial information of the bead and the model results are compared with off-focused images of the beads viewed under an optical imaging system; the

comparison gave reasonable agreement. The three dimensional bead trajectories obtained from the simulation are compared with the experimental data.

Chapter five starts with a summary of all the study undertaken in this thesis. This is then followed by a concluding section as well as a discussion of the future work which considers the application of the magnetic bead interaction method (and magnetic manipulation) to understanding intercellular interaction between biological molecules (e.g. red blood cell, malaria parasite, vesicle).

Chapter 2

Bead Pair Interaction within a Magnetophoretic System

2.1 OVERVIEW

Bead agglomerates, which occur due to bound magnetic beads interacting with one another, have been previously observed within the channel of magnetic separation devices [4]. These bead agglomerates refer to a bead pair or cluster of magnetic beads; they are formed as a result of magnetic binding forces while the hydrodynamic fluid environment strongly influences their movement. Agglomerates cause surface deposits, obstruct the separation channels and limit the separation efficiency of magnetic separation devices [1, 3].

Predicting the interactions between nearby beads will help to determine the density of beads that will avoid agglomeration over a fixed time and improve the separation efficiency of these devices. This chapter presents the theory behind magnetic and hydrodynamic interactions between a pair of super-paramagnetic beads suspended in water placed in a uniform magnetic field. The derived equations are used to develop an interaction model in Matlab based on the Finite-Time-Finite-Difference (FTFD) scheme and the Predictor Corrector Method (PCM).

Within a uniform magnetic field, the magnetic force interaction on a magnetic bead arises due to induced magnetisation and perturbed external field due to the presence of a second magnetic bead. This was derived from first principles in this chapter.

Hydrodynamic interaction occurs due to the movement of a particle in fluid which generates a velocity field that is transmitted through the fluid, this leads to a change in the velocity of suspended particles within the fluid. This was derived from the Navier-Stokes' equation using the

method of reflection for the long range interaction and lubrication approximation for the short range interaction.

The numerical model is able to predict the trajectory pattern as well as magnetic induced velocities of a pair of super-paramagnetic beads (equal sized or different sized) suspended in water within a uniform magnetic field for magnetic separation purposes. The implementation of the numerical model is discussed and the results will be compared with experimental predictions in the next chapter.

2.2 SUPER-PARAMAGNETISM

Domain walls are formed in ferrimagnets and ferro-magnets (FM) in order to minimize the magnetostatic energy. At a critical size, a threshold is reached where a wall cannot be formed within the particle. The particle then becomes single domain with a uniform magnetisation (see the left arrow on figure 2.1) [3, 17, 49].

As the size is reduced further, another threshold is reached when the remanence and coercivity of the particle becomes zero (see the red line on figure 2.1). At this point, the FM nano-particle becomes super-paramagnetic (SP) if the thermal energy is large enough to overcome the anisotropic energy and lead to a flip in the magnetisation state of the particle [17, 23, 32].

In the presence of an external magnetic field, the magnetic moments of super-paramagnets align in the direction of the applied field. When the field is removed, this alignment is lost due to thermal fluctuations [49].

The time taken to achieve zero magnetisation after the removal of the external magnetic field is called the relaxation time given by [23, 32, 49, 50]

$$t_r = t_o e^{\left(\frac{K_b V}{kT}\right)} \quad (2.1)$$

where t_r is the relaxation time in seconds and t_o depends on the material and is of the order (10^{-9} - 10^{-12}) seconds. The particle can be said to be in either a blocked state or super-paramagnetic state depending on whether the measurement time is less or greater than the relaxation time.

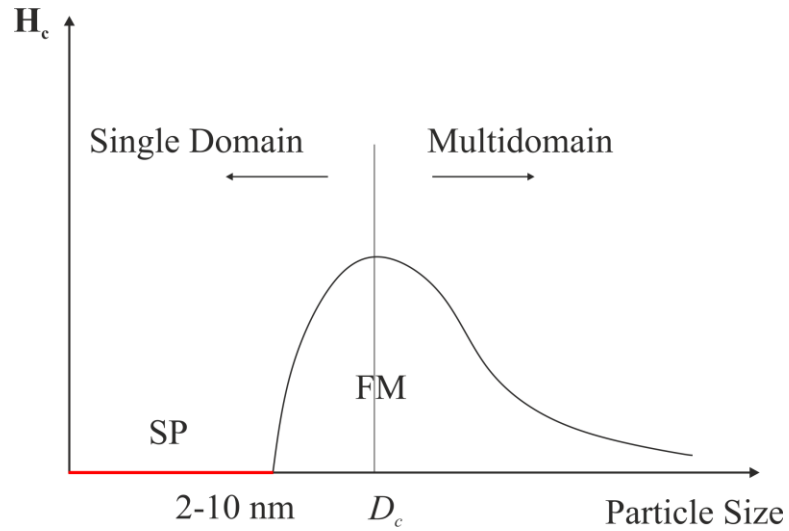


Figure 2.1 Schematic showing the relationship between coercivity and size distribution of magnetic particles [30]. The maximum coercivity corresponds to a threshold size D_c at which the particles become single domain. The super-paramagnetic state (red line) is reached when the thermal energy is able to overcome the anisotropic energy, a situation of zero coercivity and remanence.

The magnetic moments and susceptibility values achieved in super-paramagnets are much larger than that of paramagnets [49].

2.3 MAGNETIC FORCE INTERACTION

2.3.1 DIPOLE APPROXIMATION

The external field produced by a sphere with uniform magnetisation is dipole in nature [51, 101]. Though, the one-micron super-paramagnetic bead has a different structure (see section 1.3), here it is assumed that the uniform magnetised sphere can serve as a reasonable approximation.

Assuming a spherical super-paramagnetic bead lies within an external magnetic field, it can be approximated as a dipole [51, 101] with magnetisation given by

$$\mathbf{M} = \frac{3}{\mu_o} \left(\frac{\mu - \mu_o}{\mu + 2\mu_o} \right) \mathbf{B}_{\text{ext}} \quad (2.2a)$$

Equation 2.2a is equivalent to

$$\mathbf{M} = 3\chi_{\text{eff}} \mathbf{H}_{\text{ext}}, \quad \chi_{\text{eff}} = \frac{\chi}{\chi + 3} \quad (2.2b)$$

The magnetic field of a dipole [51, 101] at a position vector $\mathbf{r}$, is then expressed as

$$\mathbf{H}(\mathbf{r}) = \frac{1}{4\pi} \left[\frac{3(\mathbf{m} \cdot \mathbf{r})\mathbf{r}}{r^5} - \frac{\mathbf{m}}{r^3} \right] \quad (2.3)$$

$$\mathbf{m} = \mathbf{M}V \quad (2.4)$$

2.3.2 THEORY ON MAGNETIC FORCE INTERACTION

When a super-paramagnetic bead is placed in an inhomogeneous magnetic field, it experiences a torque aligning it in the direction of the magnetic field and it is acted upon by a translational magnetic force which causes it to move in the direction of the magnetic field; this magnetic force arises as a result of the gradient in the magnetic field [33].

In general, the magnetic force acting on a super-paramagnetic bead due to the gradient of the magnetic field is given by

$$\mathbf{F}_m = \mu_0 \int (\mathbf{M} \cdot \nabla) \mathbf{H}_{\text{ext}} dV \quad (2.5)$$

where $\mathbf{M}$ represents the magnetisation of the bead in A/m, $\mathbf{H}_{\text{ext}}$ is the external magnetic field in A/m, μ_0 is the vacuum permeability and V is the volume of the bead.

However, when the bead is placed in a uniform magnetic field, it only experiences a torque which aligns the dipole moments in the direction of the magnetic field; no translational force is experienced due to the zero gradient of the magnetic field.

$$\mathbf{F}_m = 0, \nabla \mathbf{H}_{\text{ext}} = 0 \quad (2.6)$$

This chapter is restricted to considering a uniform magnetic field only, such that the magnetic force on an isolated magnetic bead would be zero.

Figure 2.2 shows in the first instance, a super-paramagnetic bead, (bead 1), placed within a uniform magnetic field (x direction) with its magnetic moment pointing along the direction of the magnetic field; if in isolation, it would experience no translational force.

If a second super-paramagnetic bead, (bead 2 in figure 2.2), is introduced into the uniform magnetic field, it also becomes magnetized and aligns its magnetic moment in the direction of the external field.

The presence of bead 2 leads to an induced magnetisation and a perturbed external field which changes the local magnetisation and external field of bead 1. This in-homogeneity in the uniform field leads to an interaction force which causes the beads to move towards one another [33].

2.3.3 DERIVATION

The magnetic interaction force is obtained using the principle of superposition by taking into consideration the induced magnetisation and perturbed external field due to the presence of a second bead.

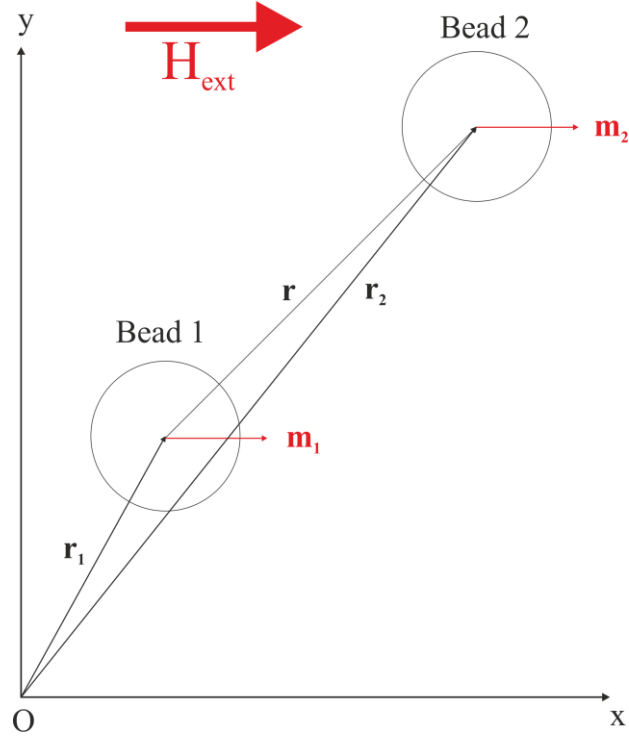


Figure 2.2 A pair of super-paramagnetic beads placed in a uniform magnetic field (in the x direction) separated by a position vector $\mathbf{r}$, within a rectangular coordinate system (Oxy). They become magnetized and their magnetic moments, $\mathbf{m}_1$ and $\mathbf{m}_2$ point in the direction of the magnetic field. The presence of bead 2 within the uniform magnetic field leads to an induced magnetization and a perturbed external field which leads to a force interaction dependent on position vectors $\mathbf{r}_1$ and $\mathbf{r}_2$.

The interaction force on bead 1 is given by [33]

$$\mathbf{F}_m = \varphi_0 \int ((\mathbf{M}_1 + d\mathbf{M}_1) \cdot \nabla) (\mathbf{H}_{\text{ext}} + d\mathbf{H}_2) dV \quad (2.7)$$

Using superposition, equation 2.7 can further be expressed as

$$\mathbf{F}_m = \int \varphi_0 [(\mathbf{M}_1 \cdot \nabla) \mathbf{H}_{\text{ext}} + (\mathbf{dM}_1 \cdot \nabla) \mathbf{H}_{\text{ext}} + (\mathbf{M}_1 \cdot \nabla) \mathbf{dH}_2 + (\mathbf{dM}_1 \cdot \nabla) \mathbf{dH}_2] dV \quad (2.8)$$

$$\mathbf{F}_m = \mathbf{F}_{11} + \mathbf{F}_{12} + \mathbf{F}_{13} + \mathbf{F}_{14} \quad (2.9)$$

The force exerted on the first bead in the absence of the second bead, primarily due to the gradient in the magnetic field is represented by $\mathbf{F}_{11}$ while the interaction force due to the presence of the second bead (arising from induced magnetisation and induced external field terms) is given by the sum of $\mathbf{F}_{12}$, $\mathbf{F}_{13}$ and $\mathbf{F}_{14}$.

During this derivation, two assumptions have been made and they are

- i. The perturbation in the applied field is small such that the particle susceptibility is unaltered.
- ii. The beads are spherical with a uniform magnetisation over its diameter such that the induced field is that of a dipole.

With the aid of equations 2.2-2.4, the induced magnetic field at $\mathbf{r}$ due to the presence of bead 2 can be expressed as

$$\mathbf{dH}_2(\mathbf{r}) = \chi_{eff} \frac{a^3}{r^3} \times \left(\frac{3(\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r})\mathbf{r}}{r^2} - \mathbf{H}_{\text{ext}}(\mathbf{r}_2) \right) \quad (2.10)$$

where $\mathbf{r} = \mathbf{r}_1 - \mathbf{r}_2$, is the position vector.

The interaction force components for a bead pair can then be expressed as [33]

$$\mathbf{F}_{11} = \varphi_0 V (\mathbf{M}_1 \cdot \nabla) \mathbf{H}_{\text{ext}}(\mathbf{r}_1) \quad (2.11)$$

$$\mathbf{F}_{11} = 3\chi_{eff}\mu_0 V (\mathbf{H}_{\text{ext}}(\mathbf{r}_1) \cdot \nabla) \mathbf{H}_{\text{ext}}(\mathbf{r}_1) \quad (2.12)$$

Equation 2.12 represents the force acting on the first bead due to the gradient of the external magnetic field, which is zero, in this case.

$$\mathbf{F}_{12} = \varphi_0 V ((\mathbf{dM}_1 \cdot \nabla) \mathbf{H}_{\text{ext}}(\mathbf{r}_1)) \quad (2.13)$$

$$\mathbf{F}_{12} = 3\varphi_0 V (\chi_{eff})^2 \frac{a^3}{r^3} \times \left\{ \frac{3(\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r})(\mathbf{r} \cdot \nabla) \mathbf{H}_{\text{ext}}(\mathbf{r}_1)}{r^2} - (\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \nabla) \mathbf{H}_{\text{ext}}(\mathbf{r}_1) \right\} \quad (2.14)$$

Equation 2.14 represents a significant interaction force of third order with distance. In this work, $\mathbf{F}_{12}$ is also zero since it depends on the gradient of the uniform field.

$$\mathbf{F}_{13} = \varphi_0 V ((\mathbf{M}_1 \cdot \nabla) \mathbf{dH}_2(\mathbf{r}_1)) \quad (2.15)$$

$$\begin{aligned} {}^1\mathbf{F}_{13} = & \frac{9(\chi_{eff})^2 a^3 \varphi_0 V}{r^5} \\ & \times \left\{ (\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r}) \mathbf{H}_{\text{ext}}(\mathbf{r}_1) + (\mathbf{H}_{\text{ext}}(\mathbf{r}_1) \cdot \mathbf{H}_{\text{ext}}(\mathbf{r}_2)) \mathbf{r} \right. \\ & \left. - 5 \frac{(\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r})(\mathbf{H}_{\text{ext}}(\mathbf{r}_1) \cdot \mathbf{r}) \mathbf{r}}{r^2} + (\mathbf{H}_{\text{ext}}(\mathbf{r}_1) \cdot \mathbf{r}) \mathbf{H}_{\text{ext}}(\mathbf{r}_2) \right\} \end{aligned} \quad (2.16)$$

Equation 2.16 represents an interaction force of fourth order with distance and is very significant in evaluating the magnetic interaction between the two beads within a uniform magnetic field. This force interaction depends on the direction of the position vector as well as the external magnetic field.

$$\mathbf{F}_{14} = \varphi_0 V (\mathbf{dM}_1 \cdot \nabla) \mathbf{dH}_2 \quad (2.17)$$

$$\begin{aligned} \mathbf{F}_{14} = & 9\varphi_0 V (\chi_{eff})^3 \frac{a^6}{r^8} \\ & \times \left\{ (\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r}) \mathbf{H}_{\text{ext}}(\mathbf{r}_2) - (\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{H}_{\text{ext}}(\mathbf{r}_2)) \mathbf{r} \right. \\ & \left. - \frac{4(\mathbf{H}_{\text{ext}}(\mathbf{r}_2) \cdot \mathbf{r})^2 \mathbf{r}}{r^2} \right\} \end{aligned} \quad (2.18)$$

¹ The authors in [33] left a missing term out when deriving $\mathbf{F}_{13}$ from first principle.

Equation 2.18 represents a weak induced dipole to dipole interaction force and is of seventh order with distance.

2.4 LONG RANGE HYDRODYNAMIC INTERACTION

When a single bead is suspended in fluid and allowed to move, it experiences a resistance due to the fluid environment. The resistance offered by the fluid to the motion of the body along the flow direction is called drag. The drag force depends on the viscosity of the liquid; viscosity leads to resistance in the fluid and arises as a result of internal friction in the fluid due to the force between the atoms and molecules of the fluid [40].

REYNOLDS' NUMBER

The Reynolds' Number is a dimensionless number that gives the ratio of the inertial terms to the viscous terms [37, 40, 53]. At low Reynolds' number, viscous effects dominate and the flow is slow, a highly ordered motion and without mixing; it is also called creeping flow. When the Reynolds' number becomes high, the flow becomes turbulent; inertial effects are more dominant and the flow has velocity fluctuations and a highly disordered motion [40, 54].

The Reynolds' number is given by the equation below [37, 40].

$$Re = \frac{\rho \mathbf{u} l}{\eta} \quad (2.19)$$

Typically, in a microfluidic channel, the Reynolds' number is low. In this work, the fluid is not flowing; this means that to determine the Reynolds' number, the bead parameters (in equation 2.19, l is the bead's radius and $\mathbf{u}$ is the bead's velocity in this application) are more relevant than the fluid channel dimensions. When calculated, the Reynolds' number is of the order of 10^{-5} to 10^{-6} . This formed the basis of our assumption that creeping flow conditions apply in this study.

2.4.1 STOKES FLOW PAST AN ISOLATED BEAD

In an incompressible fluid where density is assumed constant, the continuity equation is expressed as [53, 54, 55, 56].

$$\nabla \cdot \mathbf{v} = 0 \quad (2.20)$$

The Navier Stokes equation is a combination of the continuity equation and equation of linear momentum for a fluid; for an incompressible Newtonian liquid, it is expressed below as [55, 56]

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\nabla p + \eta \nabla^2 \mathbf{v} + p \mathbf{F} \quad (2.21)$$

where p is the pressure in N/m^2 .

The boundary conditions used to solve these equations are the no slip condition at the surface of the particle and the unbounded nature of the fluid at infinity.

Neglecting inertia and the body force component, Equation 2.21 is then reduced to

$$0 = -\nabla p + \eta \nabla^2 \mathbf{u} \quad (2.22)$$

This is known as Stokes equation or creeping flow equation [54, 56].

Let's consider an isolated bead moving with constant velocity through a uniform stream, we can obtain the velocity of the bead by solving equations 2.20 and 2.22 using the spherical coordinate system.

Assume the velocity field of the bead is of the form [57, 58]

$$u_r = \mathbf{u} \cos \theta \left(1 + \frac{b}{r} + \frac{c}{r^2} + \frac{d}{r^3} \right) \quad (2.23)$$

$$u_\theta = -\mathbf{u} \sin \theta \left(1 + \frac{e}{r} + \frac{f}{r^2} + \frac{g}{r^3} \right) \quad (2.24)$$

The boundary conditions are expressed below.

$$\text{when } r = a, \quad u_r = u_\theta = 0 \quad (2.25a)$$

$$\text{when } r \rightarrow \infty, \quad u_r = \mathbf{u} \cos \theta, \quad u_\theta = -\mathbf{u} \sin \theta \quad (2.25b)$$

Solving equations 2.20 and 2.22 (in spherical coordinate system) using equations 2.23 - 2.25, we obtain the velocity field of the bead to be [55, 57]

$$u_r = \mathbf{u} \cos \theta (1 - k_p) \quad (2.26)$$

$$u_\theta = -\mathbf{u} \sin \theta (1 - k_n) \quad (2.27)$$

$$k_p = \frac{3a}{2d} - \frac{1}{2}\left(\frac{a}{d}\right)^3, \quad k_n = \frac{3a}{4d} + \frac{1}{4}\left(\frac{a}{d}\right)^3 \quad (2.28)$$

The drag force for an isolated bead suspended within fluid is then obtained from the total stress (viscous and pressure) and integrating over the area of the bead [55, 56, 57, 58].

$$\mathbf{F}_d = -6\pi\eta a(\mathbf{v} - \mathbf{u}) \quad (2.29)$$

When two or more beads are suspended in fluid, an interaction exists between the beads mediated by the fluid, called the long range hydrodynamic interaction. This interaction is dominant when the beads are separated by a gap distance greater than their physical dimensions.

The long range hydrodynamic interaction is described thus: when a single bead moves in a fluid, the fluid in near contact with the body is dragged along at its speed while creating a disturbance to the original un-disturbed flow pattern which is transmitted through the fluid stream.

This leads to a change in the velocity and flow pattern of the fluid; thereby leading to a change in the effective velocity of any bead within the fluid. This interaction is restraining in nature and leads to a reduction in the velocity of the other bead if the two beads are approaching each other.

The long range hydrodynamic interaction is obtained by solving the Navier Stokes equation (which depends on the continuum hypothesis [53, 54]) using the method of reflection (in a vector format) under a low Reynolds' assumption and the boundary conditions given above.

As the gap-distance between the pair of beads approach the molecular scale, the continuum hypothesis breaks down [39, 53].

2.4.2 METHOD OF REFLECTION

The method of reflection is used to determine the velocity fields and force between a pair of beads due to the hydrodynamic interactions between them. It is only applicable when the beads are separated by a gap distance greater than their physical dimensions.

It assumes that when a bead (e.g. bead 1) moves within fluid, the velocity field generated by this movement is transmitted through the fluid; this transmitted velocity field is assumed to be reflected by the other bead (e.g. bead 2) and thus this affects the movement of bead 1.

The resultant velocity at each bead is then obtained by summing the incident (undisturbed) field with the reflected velocity fields.

This method relies on two assumptions which are:

- i. A point force acting at the centre of the particle would produce approximately the same velocity field as that of the particle.
- ii. The drag force obtained from the reflected field at a given particle can be approximated to be equivalent to a uniform velocity whose magnitude and direction is the same as what would exist at the centre of the particle, if it were absent [41, 55].

Using the principle of linearity, it subdivides the velocity and pressure terms into a sum of terms that satisfies the Navier Stokes equations as well as the boundary conditions [41, 55]. Here, superscripts represent reflected fields while subscripts represent the beads.

$$\mathbf{v} = \mathbf{v}^1 + \mathbf{v}^2 + \mathbf{v}^3 + \mathbf{v}^4 + \dots (2.30a)$$

$$p = p^1 + p^2 + p^3 + p^4 + \dots (2.30b)$$

The first term in equation 2.30a ($\mathbf{v}^1$) represents the velocity field in the absence of hydrodynamic interactions while the subsequent terms are the disturbed velocity fields (seen as reflections) and due to the presence of the other particle [83].

Now, let's consider two beads (with radii a_1 and a_2) moving with velocities $\mathbf{u}_1$ and $\mathbf{u}_2$ in an unbounded fluid, which is stationary at infinity as depicted on figure 2.3 below.

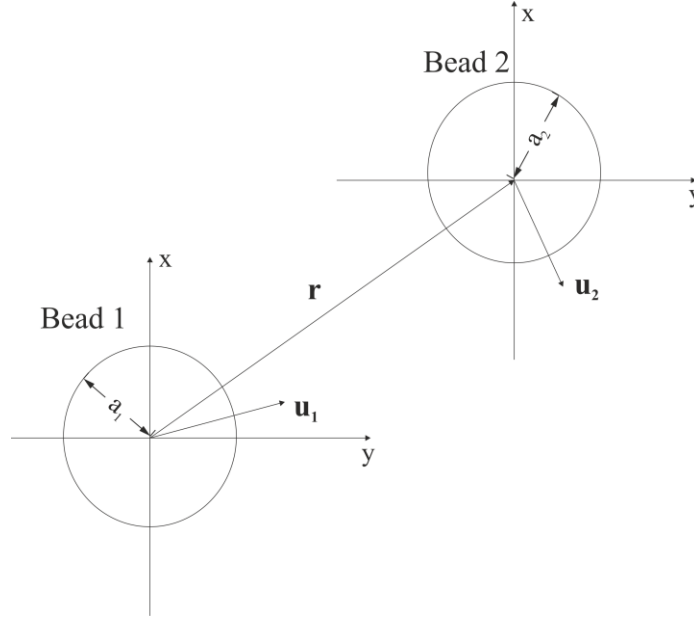


Figure 2.3 Two beads separated by the position vector $\mathbf{r}$, within an x-y coordinate system moving within a viscous fluid with velocities $\mathbf{u}_1$ and $\mathbf{u}_2$ respectively. When bead 1 moves within the fluid, the fluid stream is reflected towards bead 2 and thus affects its movement. The hydrodynamic interaction between the two beads is resolved using the method of reflection.

At Bead 1, the no slip boundary condition [41, 55] is given by

$$\mathbf{v}^1 = \mathbf{u}_1 \quad (2.31a)$$

At Bead 2, we assume the fluid stream due to the movement of bead 1 is reflected towards bead 2, this gives

$$\mathbf{v}^2 = \mathbf{u}_2 - \mathbf{v}^1 \quad (2.31b)$$

At any arbitrary bead q , the reflected velocity field can be expressed as

$$\mathbf{v}_q^2 = \mathbf{u}_q - \mathbf{v}^1 \quad (2.31c)$$

Successive reflections are taken at both beads and we have

$$\mathbf{v}^3 = -\mathbf{v}^2 \quad (2.31d)$$

$$\mathbf{v}^4 = -\mathbf{v}^3 \quad (2.31e)$$

The resultant velocity and drag force for each of the beads is then obtained by summing all the reflected velocities as well as drag forces that satisfied the boundary conditions at the bead's surface [41, 55].

$$\mathbf{F}_{h1} = \mathbf{F}_{h1}^1 + \mathbf{F}_{h1}^3 + \mathbf{F}_{h1}^5 + \dots \quad (2.32)$$

where $\mathbf{F}_h$ is the long-range hydrodynamic force. Here, we use the Navier Stokes equations and the boundary conditions outlined in equations 2.31 to solve for the reflected velocity fields and drag force between the two beads (see figure 2.3).

At Bead 1, using Stokes' equation, we have

$$\mathbf{F}_{h1}^1 = -\eta k_a \mathbf{u}_1, \quad (2.33a)$$

where

$$k_a = 6\pi a_1, k_b = 6\pi a_2 \quad (2.33b)$$

$$k_1 = \frac{k_a}{8\pi r}, k_2 = \frac{k_b}{8\pi r}, k_3 = \frac{k_a}{4\pi r}, k_4 = \frac{k_b}{4\pi r} \quad (2.33c)$$

Assuming this is equivalent to a point force acting at the centre of bead 1, we can calculate the translational velocity at distances away from the bead by solving the Navier Stokes equation for a point force.

$$0 = -\nabla p(\mathbf{r}) + \eta \nabla^2 \mathbf{v}(\mathbf{r}) + \mathbf{F}_h \delta(\mathbf{r}) \quad (2.34)$$

Using the Fourier Transform method [43, 58], we arrive at the fluid velocity given by

$$\mathbf{v}(\mathbf{r}) = k_1 (\mathbf{u}_1 + \mathbf{u}_1^r) \quad (2.35a)$$

$$\mathbf{u}_1^r = \frac{(\mathbf{u}_1 \cdot \mathbf{r})\mathbf{r}}{r^2}, \quad \mathbf{u}_2^r = \frac{(\mathbf{u}_2 \cdot \mathbf{r})\mathbf{r}}{r^2} \quad (2.35b)$$

We obtain the velocity field at bead 2 after considering the first reflection from bead 1 as

$$\mathbf{u}_2^2 = \mathbf{u}_2 - k_1 (\mathbf{u}_1 + \mathbf{u}_1^r) \quad (2.36)$$

The hydrodynamic force at bead 2 is then given by

$$\mathbf{F}_{h2}^2 = -\eta k_b \mathbf{u}_2^2 \quad (2.37)$$

Using equation 2.31d, we can then write

$$\mathbf{u}_1^3 = k_1 k_2 [(\mathbf{u}_1 + 3\mathbf{u}_1^r)] - k_2 [(\mathbf{u}_2 + \mathbf{u}_2^r)] \quad (2.38)$$

and

$$\mathbf{u}_1^5 = (k_1)^2 (k_2)^2 [(\mathbf{u}_1 + 15\mathbf{u}_1^r)] - k_1 (k_2)^2 [(\mathbf{u}_2 + 7\mathbf{u}_2^r)] \quad (2.39)$$

By summing all the odd numbered velocity fields to infinity, we can write the velocity of bead 1 due to the long range hydrodynamic interaction as

$$\frac{\mathbf{F}_h}{\eta k_a} = \frac{\mathbf{u}_1 - k_2 \mathbf{u}_2}{1 - k_1 k_2} + \frac{-k_1 k_2 \mathbf{u}_1^r}{1 - k_1 k_2} + \frac{k_3 k_4 \mathbf{u}_1^r}{1 - k_3 k_4} + \frac{k_2 \mathbf{u}_2^r}{1 - k_1 k_2} - \frac{k_4 \mathbf{u}_2^r}{1 - k_3 k_4} \quad (2.40)$$

This can further be simplified as

$$\frac{\mathbf{F}_h}{\eta k_a} = \frac{\mathbf{u}_1 - k_2 \mathbf{u}_2}{1 - k_1 k_2} + \frac{3k_1 k_2 \mathbf{u}_1^r}{(1 - k_1 k_2)(1 - k_3 k_4)} + \frac{-k_2(1 + 2k_1 k_2) \mathbf{u}_2^r}{(1 - k_1 k_2)(1 - k_3 k_4)} \quad (2.41)$$

This is consistent with results obtained in [39, 41] derived in a non vectorial form. Equivalent expressions from equation 2.41 can be derived to account for the hydrodynamic interaction on bead 2 when bead 1 moves within the fluid.

The hydrodynamic interactions depend on the physical dimensions, relative positions and velocities of the beads; it is restraining in nature as it results in the decrease of the velocity of the bead.

When the beads are separated by a sufficiently large distance where the effect of the hydrodynamic interaction becomes negligible, the hydrodynamic constants approach zero and we have

$$\mathbf{F}_h = -\eta k_a \mathbf{u}_1 \quad (2.42a)$$

where

$$d \rightarrow \infty, k_1 = k_2 = k_3 = k_4 = 0 \quad (2.42b)$$

The hydrodynamic force on bead 1 becomes equal to the drag force.

2.5 SHORT RANGE HYDRODYNAMIC INTERACTION

When the beads are separated by a gap distance comparable to their physical dimensions, as shown in figure 2.4, the lubrication force is used to account for the hydrodynamic interaction. Lubrication effects come into play when the two beads are nearly in contact and the squeezed fluid in between them sets up a high velocity gradient and pressure distribution that prevents them from coming into close contact. During lubrication, inertia effects are insignificant [37].

Figure 2.4 shows two beads approaching one another with velocities $\mathbf{u}_1$ and $\mathbf{u}_2$ at a gap distance comparable to their physical dimensions within a viscous fluid.

The lubrication force is analyzed using the thin gap approximation [58] as the gap distance is far less than the radius of the beads. The velocity fields are obtained by solving the continuity equation and Navier-Stokes' equation for an incompressible fluid using the lubrication

approximation [46, 58]; the lubrication force is then evaluated by integrating the total stress over the area of the bead.

The lubrication force experienced by the beads when they move in a viscous fluid with a relative velocity $\mathbf{u}_r$, towards each other, is given below in equation 2.43 [45, 46, 59, 66].

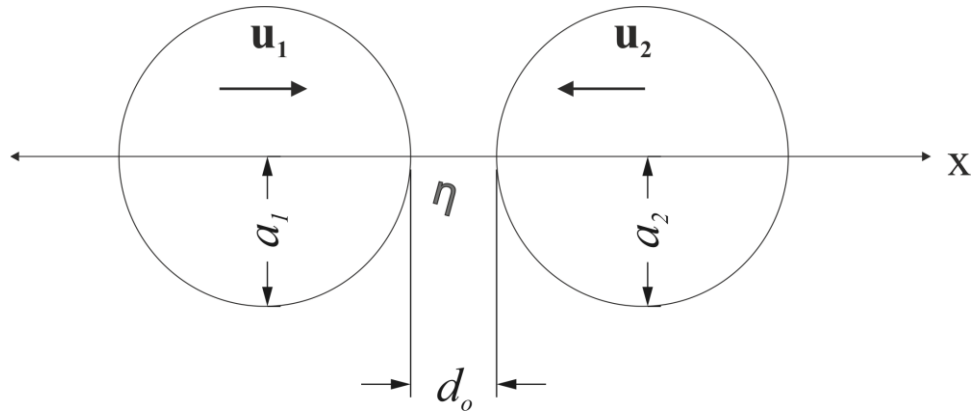


Figure 2.4 Two beads with radii a_1 and a_2 separated by a minimum gap distance d_o approaching one another with a relative velocity and interacting due to lubrication force within a viscous fluid under the special case when the beads are approaching along their line of centre.

$$\mathbf{F}_{\text{lub}} = -\frac{3\pi\eta a_h a_g \mathbf{u}_r}{d_o} \quad (2.43)$$

$$d_o = |d| - (a_1 + a_2) \quad (2.44)$$

The lubrication approximation depends on the continuum hypothesis [53, 54] and any deviation from this would affect the accuracy of the lubrication model; surface roughness and the presence of non-hydrodynamic forces such as surface attractive forces at very small scale dimensions will also limit the accuracy of the lubrication model [58].

2.6 SEDIMENTATION FORCES

2.6.1 GRAVITY

The weight of a particle is given by the product of its mass and the acceleration due to gravity. The gravitational force acts downwards in the vertical direction as given in the equation below.

$$\mathbf{F}_g = -\rho_p V_p g \hat{\mathbf{k}} \quad (2.45)$$

where the subscript p represents the particle.

2.6.2 BUOYANCY

Buoyancy is the upward force exerted by the fluid on the particle to oppose its weight. It acts vertically upwards and is given by the weight of fluid displaced in the equation below.

$$\mathbf{F}_b = \rho_v V_p g \hat{\mathbf{k}} \quad (2.46)$$

where the subscript v represents the fluid.

2.6.3 SEDIMENTATION

Sedimentation is the process by which the particles travel through the depth of the chamber within a fixed time under the action of gravity and buoyancy. The sedimentation velocity is then given by

$$\mathbf{u}_s = \frac{(\rho_v - \rho_p)V_p g \hat{\mathbf{k}}}{6\pi\eta a} \quad (2.47)$$

The sedimentation time is given by

$$t_s = \frac{h}{\mathbf{u}_s} \quad (2.48)$$

where h is the channel depth. The sedimentation velocity for a 1 μm diameter bead ($\rho_p = 2.25\text{g/cm}^3$) suspended in water ($\eta = 1 \times 10^{-3} \text{ kgm/s}$) and 2.8 μm diameter bead ($\rho_p = 1.3\text{g/cm}^3$) suspended in Poly Ethylene Glycol, PEG ($\eta = 2.01 \times 10^{-3} \text{ kgm/s}$) was calculated as 0.69 $\mu\text{m/s}$ and 0.64 $\mu\text{m/s}$ respectively. The sedimentation time of the 1 μm and 2.8 μm beads through a channel depth of 100 μm when suspended in water and PEG respectively was found to be 2.42 minutes and 2.6 minutes respectively.

2.7 BROWNIAN MOTION

2.7.1 INTRODUCTION

An observation of the motion of the super-paramagnetic beads suspended in water suggests some form of randomness in their movement; this random movement occurs due to the bombardment of the beads by the water molecules and is termed ‘Brownian motion’. The beads are hit by the water molecules in different directions and move in a random manner [3, 60].

2.7.2 THEORY ON BROWNIAN MOTION

The diffusion coefficient is given by the Stokes’ Einstein relation [60, 62]

$$D_f = \frac{kT}{6\pi\eta a} \quad (2.49)$$

Brownian displacement is measured using the mean square displacement of the particles; this displacement is expressed in two dimensions below [60, 62, 63].

$$\langle \Delta r^2(t_s) \rangle_{2D} = \langle \Delta x^2 + \Delta y^2 \rangle \quad (2.50)$$

The Brownian displacement is related to the diffusion coefficient as expressed below [60, 62, 63, 64]

$$\langle \Delta r^2(t_s) \rangle_{2D} = 4 D_f t_s \quad (2.51)$$

where t_s is the time between steps.

The diffusion coefficient can be obtained from a linear fit of the mean square displacement against time curve. A particle experiencing a constant diffusivity with respect to time is said to be undergoing Brownian motion [63].

2.8 NUMERICAL MODEL

This section deals with the implementation of the numerical model which describes the trajectory pattern as well as magnetic induced velocities of the magnetic beads in Matlab.

In order to achieve this, the velocity of the bead is expressed in terms of the resultant force; the positions of the bead are then updated over a number of time steps using the finite-time-finite difference scheme and predictor corrector method.

2.8.1 FORCES AND VELOCITIES OF PARTICLES

The forces that will be considered in this study are

1. Gravity
2. Buoyancy
3. Magnetic Force including its interaction
4. Drag Force
5. Long Range Hydrodynamic Interaction
6. Short Range Hydrodynamic Interaction

When super-paramagnetic beads move in a viscous fluid within a uniform magnetic field, their motion can be analyzed using Newton's second law of motion.

Newton's second law of motion states the force acting on a bead is equal to the product of its mass and acceleration. This is stated below.

$$\mathbf{F} = m \frac{d\mathbf{u}}{dt} \quad (2.52)$$

Due to the small mass of the bead and the assumption that the bead reaches a terminal velocity quickly [40], we can re-write the above equation by neglecting the inertial component as

$$\sum \mathbf{F} = 0 \quad (2.53)$$

This model is based on the assumption that there are two beads in the x-y plane; then we can assume that the forces will be resolved within this plane.

If the resultant force acting on the bead under consideration is expressed as $\mathbf{F_r}$. Then there is need to convert the force equations into a velocity equation because the magnetic and sedimentation forces are represented as force components while the hydrodynamic interactions are expressed in terms of the velocity fields.

We first define a unit normal to the position vector as

$$\hat{\mathbf{n}} = \begin{bmatrix} 0 & -1 \\ 1 & 0 \end{bmatrix} \hat{\mathbf{r}} \quad (2.54)$$

We then resolve the resultant force along the position vector and normal in order to obtain the velocity of bead 1 (using equations 2.40 and 2.41) as

$$\begin{aligned} \mathbf{u}_1 = & \frac{\frac{(\mathbf{F}_h \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\eta k_a} (1 - k_3 k_4) + \left[k_4 + \left(\frac{a_p}{2d_o} \right) (1 - k_3 k_4) \right] (\mathbf{u}_2 \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\left[1 + \left(\frac{a_p}{2d_o} \right) (1 - k_3 k_4) \right]} \\ & + \frac{(\mathbf{F}_h \cdot \hat{\mathbf{n}})\hat{\mathbf{n}}}{\eta k_a} (1 - k_1 k_2) + k_2 (\mathbf{u}_2 \cdot \hat{\mathbf{n}})\hat{\mathbf{n}} \quad (2.55) \end{aligned}$$

where

$$a_p = \frac{a_h a_g}{a} \quad (2.56)$$

For a moving fluid, the velocity of the beads becomes their relative velocity with respect to the fluid velocity.

2.8.2 NUMERICAL METHOD

This section introduces the numerical methods used in the numerical simulation.

2.8.2.1 *FINITE-TIME-FINITE-DIFFERENCE SCHEME*

This is a leap frog method [65] which involves the use of discrete variables in time and space to replace derivatives [61, 67]. It involves expressing the variables in terms of their future and past states.

At a given time step, one of the variables is determined at a future time while other variables are in the past; subsequently the other variable is determined at a future time while the pre-determined variable becomes a past value [65].

It is a simple method that is computationally expensive in terms of time and memory. A major factor that needs to be determined is the time step; if this value becomes too large, it produces unstable results.

The Courant number can be used to determine the appropriate time step for a simulation [61, 65].

Using FTFD scheme, the positon vector can be expressed as

$$\mathbf{r}(n + 1) = \mathbf{r}(n) + \mathbf{u}(n) dt \quad (2.57)$$

The Courant number is expressed below as

$$C = \frac{\mathbf{u}\delta t}{\delta x} \leq 1 \quad (2.58)$$

where δt is the time step in seconds and δx is the spatial step in metre.

In a system of many beads interacting, the velocity of the fastest bead will be used to determine the Courant number.

2.8.2.2 **PREDICTOR CORRECTOR METHOD (PCM)**

This is an improvement over Euler's method and is subdivided into the following steps:

- i. It predicts using Euler's forward method.

$$\mathbf{r}^1(n) = \mathbf{r}(n) + \mathbf{u}(n) dt \quad (2.59)$$

- ii. It corrects the prediction by taking an average of the predicted and initial value [67, 68].

$$\mathbf{r}(n+1) = \mathbf{r}(n) + \frac{1}{2} \left(f(\mathbf{r}_n, t_n) + f(\mathbf{r}_n^1, t_n^1) \right) dt \quad (2.60)$$

where $f(\mathbf{r}, t)$ represents the initial and predicted velocity as a function of bead position, $\mathbf{r}$ and time, t .

This method is of order 2; this means the relative error depends on the square of the step size length. It gives more accurate results when compared to Euler forward method but requires a longer time [67, 68].

2.8.3 IMPLEMENTATION OF NUMERICAL METHOD

When a pair of super-paramagnetic beads is placed in a uniform magnetic field, an interaction magnetic force is set up due to the induced magnetisation which perturbs the external field. Hydrodynamic interactions also exist between the beads due to the fluid environment and these depend on their velocity fields.

The numerical model is implemented in Matlab using both the FTFD scheme and the PCM. A change in force due to interaction leads to a change in the velocity of the beads. The new velocity is then used to update the position of the beads at each time step. This process is then repeated until the beads touch (See Appendix A1 for more information).

2.9 CONCLUSION

This chapter presents the theory behind particle interactions in a magnetophoretic system while the derived equations are used to implement a numerical model in Matlab. The model is able to predict the trajectory pattern and magnetic induced velocities of a pair of super-paramagnetic beads (equal sized or different sized) suspended in water for a uniform magnetic field. Results will be compared with experimental predictions in the next chapter.

The magnetic interaction force between a pair of magnetic beads placed within a uniform magnetic field can be estimated using equation 2.16 without going back to first principles.

The long range hydrodynamic interaction between a pair of beads has been derived in a vector format using the method of reflection.

We can therefore conclude that agglomeration occurs when the attractive magnetic interactions has sufficient time to work against the restraining hydrodynamic force between beads suspended in fluid for a uniform magnetic field. Hence, to avoid agglomeration over a fixed time frame, there needs to be an upper bead density frame which will be determined in the next chapter.

Chapter 3

Experimental Investigation of Bead Pair Interaction in a Magnetophoretic System

3.1 OVERVIEW

This chapter starts with a description of the experimental apparatus used in obtaining the data. This is then followed by an experiment evaluating the impact of Brownian motion on the super-paramagnetic beads suspended in water at room temperature.

The next experiment investigates a bead pair interaction within a magnetophoretic system using super-paramagnetic beads obtained from two different manufacturers. One-micron beads from Chemicell [25] were used in the first experiment and this exhibited Brownian displacement. Due to the impact of random displacement on determining the trajectories of the beads, the experiment was repeated with 2.8 μ m Dynal beads from Thermo Fisher Scientific [24].

The trajectory plots of the beads and magnetic induced velocity along the direction of the greatest change in magnetic energy density were obtained from the simulation and experimental data; these plots were compared against each other in order to validate the model and a good agreement was obtained.

Magnetic beads vary widely in terms of physical and magnetic properties. The next section describes a pair interaction between different sized beads within a magnetophoretic system; simulation results gave a good match when compared with the experimental data.

Using the verified numerical model, the agglomeration time (i.e. the time it takes for the pair of beads to come together) as a function of the initial separation distance was predicted. This relationship will help to predict the threshold distance (or bead concentration) to use when

avoiding agglomeration within a magnetic bead-based assay over a fixed time interval. The reason a fixed time interval needs to be stated for a given concentration is because the beads would eventually come together by action of their magnetic interaction.

3.2 EXPERIMENTAL SETUP

This section introduces the experimental apparatus used in obtaining measurement data and these include the microfluidic apparatus, magnetic apparatus and imaging system.

3.2.1 MICROFLUIDIC APPARATUS

3.2.1.1 *HYDROSTATIC FLUID CHANNEL*

Counting slides (Fast Read 102 from Immune systems), 85mm by 40mm, with depth 0.1mm were carefully machined into ten separate hydrostatic fluid channel units. Each unit holds a sample solution of volume 10 μ l. This unit serves as the hydrostatic fluid channel for the experiment. Solutions delivered by a pipette are drawn into the fluid channel by capillary action.

3.2.1.2 *PREPARATION OF SOLUTION OF ONE-MICRON CHEMI CELL BEADS*

Super-paramagnetic beads, (SiMAG-Silanol), 1 μ m sized, were purchased from Chemicell (Berlin, Germany). Following the description given in section 1.3, this magnetic bead consists of a core composed of maghemite super-paramagnetic particles (nano-metre sized) embedded within a silica

matrix. The matrix is further enclosed within an outer shell. The matrix is further expanded by growth of silica to form an outer shell, giving an overall dimension of $1\mu\text{m}$.

The magnetic response of the beads was measured using a SQUID magnetometer (Quantum Design 7T Magnetic properties Measurement System (MPMS) XL) as shown on figure 3.1.

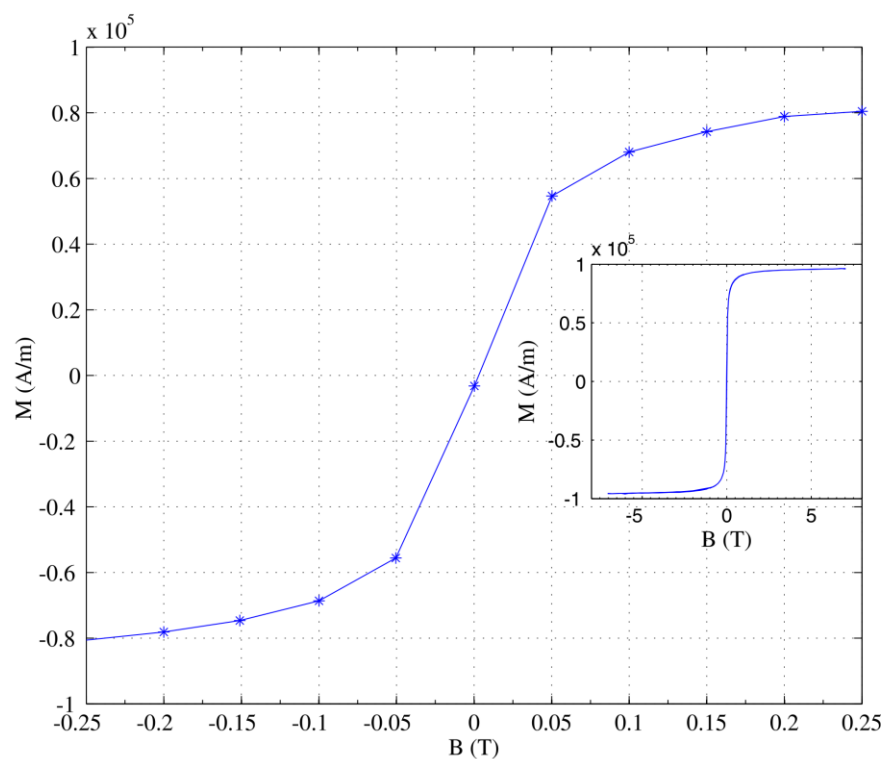


Figure 3.1 A relationship between the magnetisation, $\mathbf{M}$ and the applied flux density, $\mathbf{B}$ of a volume containing a large number of $1\mu\text{m}$ sized super-paramagnetic bead obtained from Chemicell, at room temperature within the range $\pm 0.25\text{T}$. Inset figure shows the magnetisation versus applied flux density characterization within the range $\pm 7\text{T}$.

A linear interpolation within the applied flux density range ($\pm 50\text{mT}$) was used to estimate the magnetic susceptibility of the bead ($\chi = 1.33$) at 11mT due to the limited data points (See

Appendix A3 for more information). Alternatively, in order to calculate the magnetic susceptibility at 11mT, a Langevin curve was fitted to figure 3.1 and this gave 1.25 as the value of the magnetic susceptibility at 11mT, see Appendix A3 for more information. The difference in magnetic susceptibility values using the two methods mentioned above is not significant and does not change any of the conclusions in this work.

The Chemicell beads [25] were obtained with a density of 2.25g/cm^3 at an initial concentration of 1.8×10^{12} beads/g (9×10^{10} beads/ml). A serial dilution of this concentration a thousand times yields a concentration of 9×10^7 beads/ml. From this concentration, further dilutions were made for the experiment.

The sedimentation time due to gravity and buoyancy for the Chemicell beads across the $100\mu\text{m}$ channel depth was evaluated as 2.42 minutes (see section 2.6.3).

3.2.1.3 *PREPARATION OF SOLUTION OF 2.8-MICRONS DYNAL BEADS*

Dynal Beads M-280, $2.8\mu\text{m}$ sized particles [24] with an initial density of 10mg/ml were obtained from Thermo Fisher Scientific [24]. A serial dilution of this concentration a hundred times yields a concentration of 6×10^6 beads/ml. From this concentration, further dilutions were made for the experiment.

The sedimentation time for the M-280 beads ($\rho_p = 1.3\text{g/cm}^3$, $\chi = 0.65[84], 0.8[79]$) in PEG ($\eta = 2.01 \times 10^{-3} \text{ kgm/s}$) through the $100\mu\text{m}$ channel was found to be 2.6 minutes; when suspended in water, a bead sediments in 1.3 minutes (see section 2.6.3).

3.2.1.4 **PREPARATION OF SOLUTION OF ONE-MICRON DYNAL BEADS**

Super-paramagnetic beads (Dyna Beads myone Streptavidin T1 [26]), 1 μ m sized, were obtained from Thermo Fisher Scientific, with an initial density of 10mg/ml and a concentration of $7-10 \times 10^9$ beads/ml. This was then diluted to a concentration of $7-10 \times 10^6$ beads/ml for the experiment. This magnetic bead has a magnetic susceptibility of 1.4 [31] and a density of 1.7g/cm³. The sedimentation time for this bead when suspended in water through the channel is 4.4 minutes (see section 2.6.3).

3.2.2 MAGNETIC CONFIGURATION

A uniform magnetic field was achieved by placing two rectangular (20mm by 10mm by 5mm N42 Neodymium Magnets) with magnetic field strength of 0.58T separated by a distance of 55mm in a position of attraction. Care was taken to remove any magnetic materials from the vicinity of the experiment to avoid distortion of the uniform field between the two magnets.

The applied flux density of the uniform magnetic field was measured using a Hall Effect Gauss/Tesla Meter (5100 Series Sypris Test & Measurement F.W.Bell 12/04) and gave a value of 11 ± 0.5 mT. This is consistent with field values obtained by modelling the distribution in Ansys Maxwell as depicted on figure 3.2.

The applied flux density has a symmetrical distribution over the horizontal axis and figure 3.2 depicts the variation in the applied flux density from one edge of the magnet (320mT) to the centre of the magnets (11mT). This variation is due to the rapid change in flux lines at the corners of the magnet where the magnetic flux is very strong. Therefore, the edges of the magnet will be avoided

as they will introduce non-uniformity while the centre of the magnet will be used as this gives an approximately uniform field distribution.

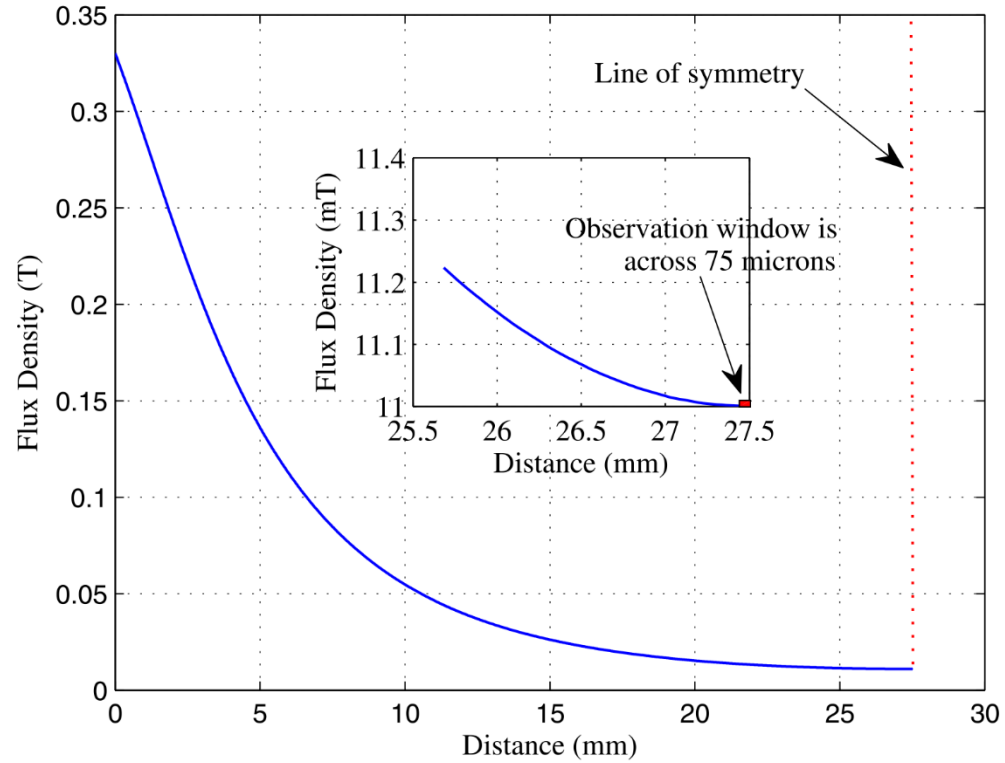


Figure 3.2 The variation of the applied flux density distribution along the horizontal axis produced by using both magnets to form a uniform magnetic field; this distribution is symmetrical, either side of the centre-line indicated in the figure. Inset shows the applied flux density variation 1.9 mm away from the observation window.

Within the observation window ($75\mu\text{m} \times 56\mu\text{m}$) in which the bead pair interaction is observed (see figure 3.2), the magnetic field is approximately uniform.

When the centre of the solution chamber is placed at the line of symmetry (27.5mm on figure 3.2), the drift experienced by the beads is zero due to the uniform magnetic field.

However, during the experiment, the camera is moved to a window to observe interaction between beads. If for example, the solution chamber is translated to 26.5mm (see inset on figure 3.2), from a knowledge of the gradient, it is possible to evaluate the drift velocity which is as small as $0.0026\mu\text{m/s}$. This drift velocity is negligible.

Furthermore, due to the proximity of the magnetic beads, any drift velocity experienced by the beads would be the same; hence the magnetic induced velocities of the beads would be the relative velocities.

3.2.3 EXPERIMENT SETUP

The hydrostatic fluid channel was placed within the uniform magnetic field distribution along the centre line of the magnets. The fluid channel and magnetic field were mounted on the object stage of a Leitz Wetzlar inverted microscope [69] which illuminates the solution chamber using a low voltage lamp from above the channel [69] as shown below in figure 3.3. A long working distance objective lens (comar-LWD 5.8mm, NA 0.3, focal length 8.19mm, length 39.2mm) [70] was mounted in the microscope and is beneath the solution chamber to provide a 20x magnification of images. A USB digital camera (UEye UI-2230SE, IDS, Germany [71]) was coupled to the inverted microscope via an adjustable length of extension tube to increase the image magnification and connected to a computer to record images and videos of the movement of the beads.

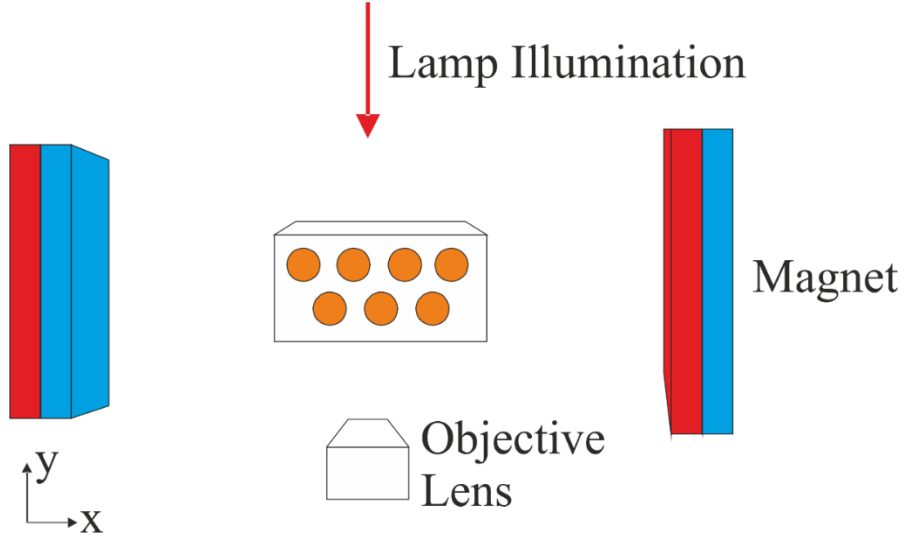


Figure 3.3 Schematic showing the experimental set up where images and videos of the movement and interaction between a pair of magnetic beads suspended in a static fluid placed within a uniform magnetic field are captured by the microscope's camera.

IMAGE ANALYSIS

Images were captured by the digital camera at a frame rate of 33 frames per second (fps) within a video window of 1024 by 768 pixels. The images were analyzed using particle tracking software (imageJ [72]).

In order to determine the image scale per pixel of the camera, a calibration slide (L4078, Agar Scientific) with a 1mm graticule, 0.01mm subdivisions and accuracy of $2\mu\text{m}$ was mounted on the stage of the inverted microscope and the image was analyzed using imageJ [72]. The conversion scale from camera pixels to μm was obtained as $1 \text{ pixel} = 0.073\mu\text{m}$.

When the video frames were loaded to imageJ [72], it was cropped to the area of interest where good candidate beads could be observed. The image was then filtered to remove background noise and the beads were tracked on a frame by frame basis.

3.2.4 UNCERTAINTIES ASSOCIATED WITH EXPERIMENTAL SETUP

The experiment aims to record the beads' movement and interaction within a magnetic separation system. This requires finding good candidate beads within an observation window within the uniform magnetic field.

During such video recording, thermal gradients were occasionally observed which produced a drift of as significant as $16\text{-}18\mu\text{m/s}$. The thermal gradients arise from the heat generated by the low voltage lamp of the microscope and can be avoided by waiting until the apparatus had thermally stabilised. When drift due to a thermal gradient was observed the recorded data was discarded.

Fast interaction between a pair of beads at gap distances less than $2\mu\text{m}$ for the $1\mu\text{m}$ sized beads and $3.6\mu\text{m}$ for the $2.8\mu\text{m}$ sized beads could not be observed accurately due to the frame rate resolution of the camera (33fps).

Any non-uniformity in the magnetic field, when observing away from mid-centre between the magnets, would lead to a gradient which affects the beads' movements. The uncertainties associated with the uniform magnetic field used in the setup has been described in section 3.2.2.

The tracking software (Image J [72]) has a rms tracking error of $\pm 0.2\mu\text{m}$. This rms error is of the same order as the Brownian displacement of the beads.

The height of the beads could only be estimated by correlation to de-focused images of a single bead taken at discrete steps using a translation stage; this process is expected to determine the

relative height of the beads with approximately 0.5 μ m accuracy. Also, beads beyond the depth of focus could not be accurately resolved. The working distance of the microscope allowed for translation of the observation window plane within the channel.

Beads stuck to the bottom of the channels were avoided during experimental observations by ensuring that the plane of the observation window lies within the middle of the channel.

In conclusion, some of the uncertainties have been avoided where possible. However, the uncertainties do not affect the validity of measured data as the overall impact is comparable to the randomness due to Brownian motion.

3.3 EVALUATION OF BROWNIAN MOTION

3.3.1 DESCRIPTION OF BROWNIAN MOTION EXPERIMENT

A solution of super-paramagnetic beads in deionized water (Chemicell [25]) was freshly prepared at a concentration of 1×10^4 beads/ml, this was placed in an ultrasonic bath for 2 minutes to remove aggregates and fully suspend the particles. A volume of 6 μ l of the solution was transferred to the hydrostatic fluid channel using an appropriate pipette.

The channel was placed on the object glass on the translation stage of the inverted microscope and videos of the movement of the beads were recorded for typically 20 seconds using the digital camera (see figure 3.3 above) in the absence of the magnets to record the random displacements due to Brownian motion. The video frames were analysed using imageJ [72].

3.3.2 DISCUSSION OF RESULTS

The displacements of the beads were recorded over 660 frames at 33fps. The changes in the random displacements were calculated for all the frames. These displacement values were averaged to obtain the mean squared displacement. From figure 3.4a, the root mean square (rms) displacement of an isolated bead is found to be $0.299\mu\text{m}$. A good confidence level was achieved as the number of analysed frames was increased, as shown on figure 3.4a.

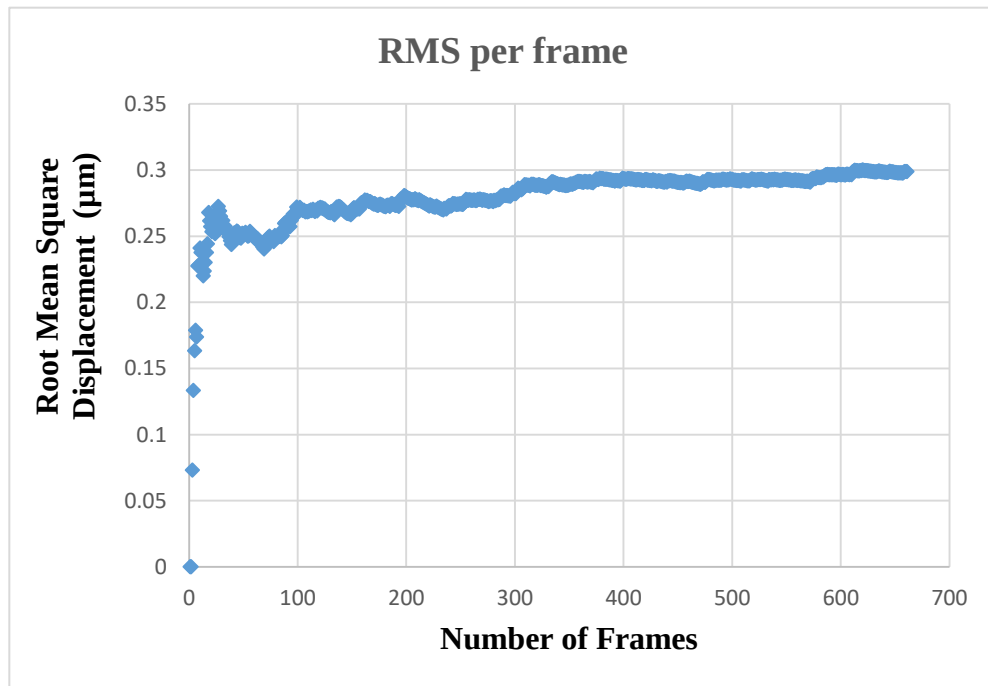


Figure 3.4a Root Mean Square distribution of a $1\mu\text{m}$ sized super-paramagnetic bead undergoing Brownian motion when suspended in water at room temperature.

The rms displacement was calculated for eight beads using the data obtained from the Brownian motion experiment; these values were compared with the predicted values. The average rms displacement of the super-paramagnetic bead is shown below in figure 3.4b. The experimental rms values deviate from the theoretical predictions and these deviations could be attributed to the reduced displacement of the bead due to the deviation in the radius of the bead ($0.5 \pm 0.115 \mu\text{m}$).

These results however suggest that when the beads are suspended in water, they are subjected to Brownian motion, rather than vibration, for example.

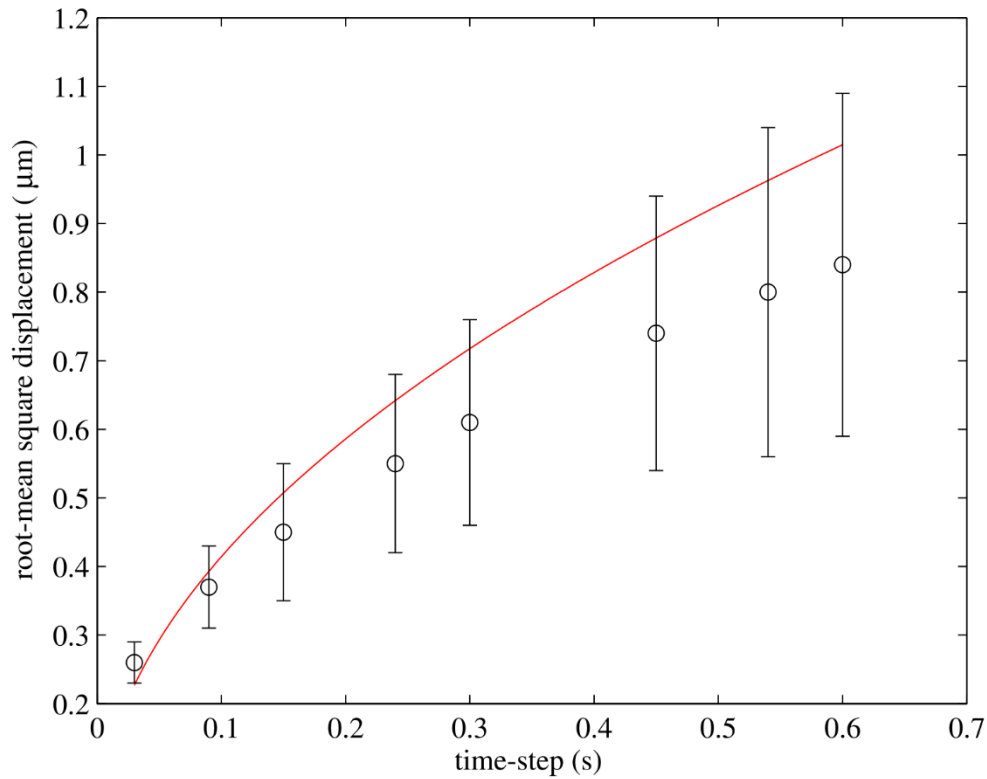


Figure 3.4b Comparison of the root-mean square displacement of a one-micron bead at various time steps obtained using the theoretical predictions (in red continuous line) and experimental data (in black circles with error bars). Error bars represent the standard deviation above and below the mean value.

3.4 EXPERIMENT INVESTIGATING BEAD PAIR INTERACTION USING ONE-MICRON CHEMICELL BEADS IN A MAGNETOPHORETIC SYSTEM

3.4.1 DESCRIPTION OF BEAD PAIR INTERACTION EXPERIMENT

A solution of super-paramagnetic beads in deionized water (Chemicell [25]) was freshly prepared at a concentration of 9×10^3 beads/ml, this was placed in an ultrasonic bath for 2 minutes to remove aggregates and fully suspend the particles.

A volume of 6 μ l of the solution was transferred to the hydrostatic fluid channel using an appropriate pipette. The channel was placed within the uniform magnetic field along the centre line of the magnets.

Interaction between a pair of isolated magnetic beads was observed within a video window of 1024 by 768 pixels (1 pixel = 0.073 μ m) such that interaction with other beads can be neglected.

The bead motion was recorded for 2.5 minutes using the set up described in section 3.2.3.

The experiment was repeated for a higher concentration (9×10^4 beads/ml) and the results are analysed and discussed in the next section.

The trajectories of the beads were tracked on a frame by frame basis using imageJ [72] and these showed a large amount of Brownian displacement which made it difficult to compare experimental data with simulation results.

Due to this limitation, bead velocity was chosen as another parameter for comparison of results.

3.4.2 COMPARISON OF EXPERIMENTAL DATA WITH SIMULATION RESULTS FOR ONE-MICRON CHEMI-CELL BEADS

A bead pair with displacement values equivalent to the first frame of the video was simulated using the numerical model. The trajectory plots of the bead pair and the velocity resolved along the direction of the magnetic field were obtained from the simulation. The resolved velocity along the direction of the magnetic field was used as the parameter for comparison in order to simplify the analysis whilst capturing the essential features of the interaction.

The trajectory plot from the simulation result was compared with the trajectory of the beads obtained from the experiment.

The velocities of the approaching beads were continuously calculated over all the frames until the beads approached one another. When the velocity values of the beads over all the frames was obtained, the randomness associated with the Brownian displacement made it difficult to determine the magnetic induced velocity.

Due to this randomness, the velocity values were averaged over a binning gap interval of $1.2\mu\text{m}$ to remove the fluctuations due to Brownian displacement. The binning gap interval was carefully chosen so that no less than three data points were averaged in each bin.

The velocity plot obtained from the simulation result was then compared to the average velocity (along the direction of the magnetic field) obtained from the experiment.

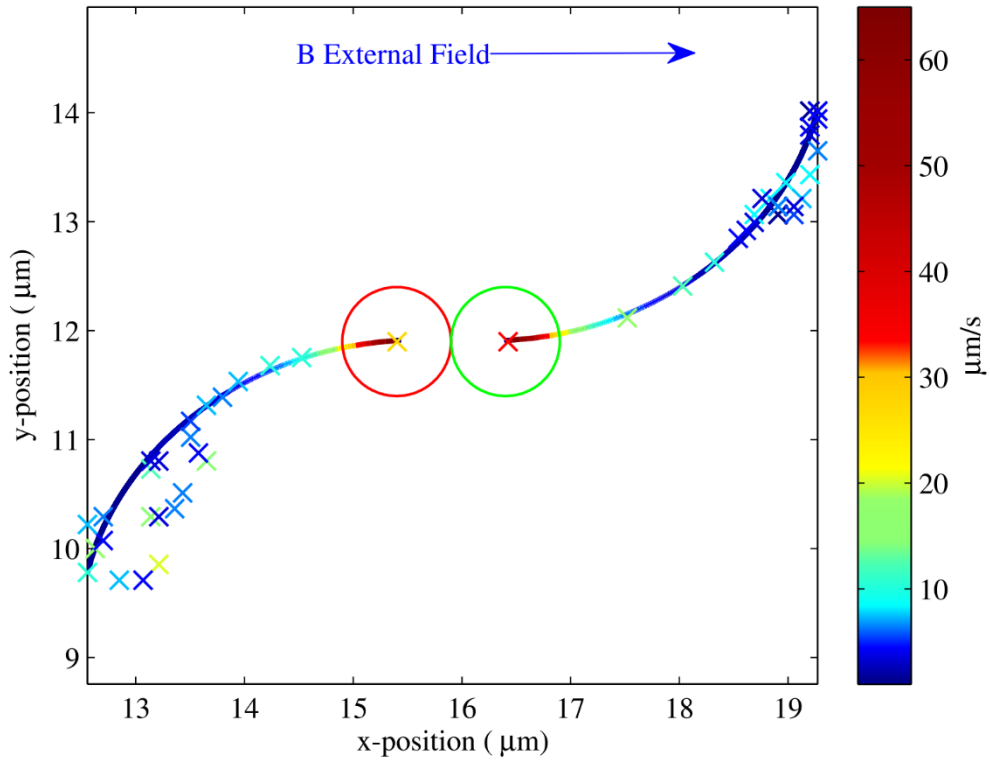


Figure 3.5a Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

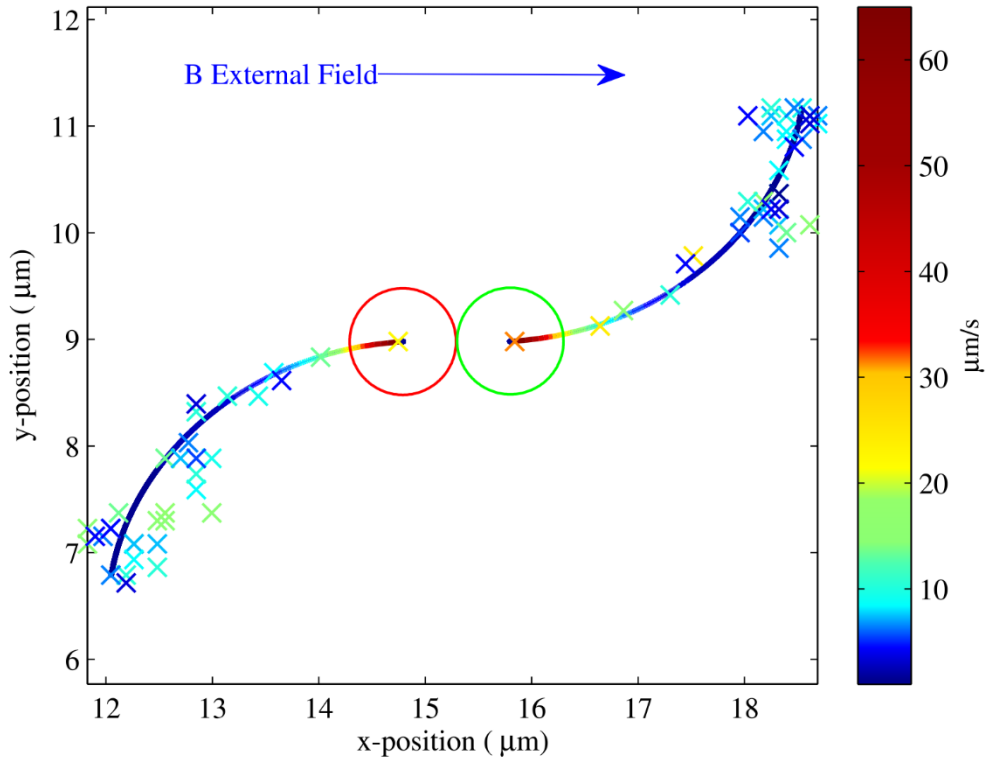


Figure 3.5b Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

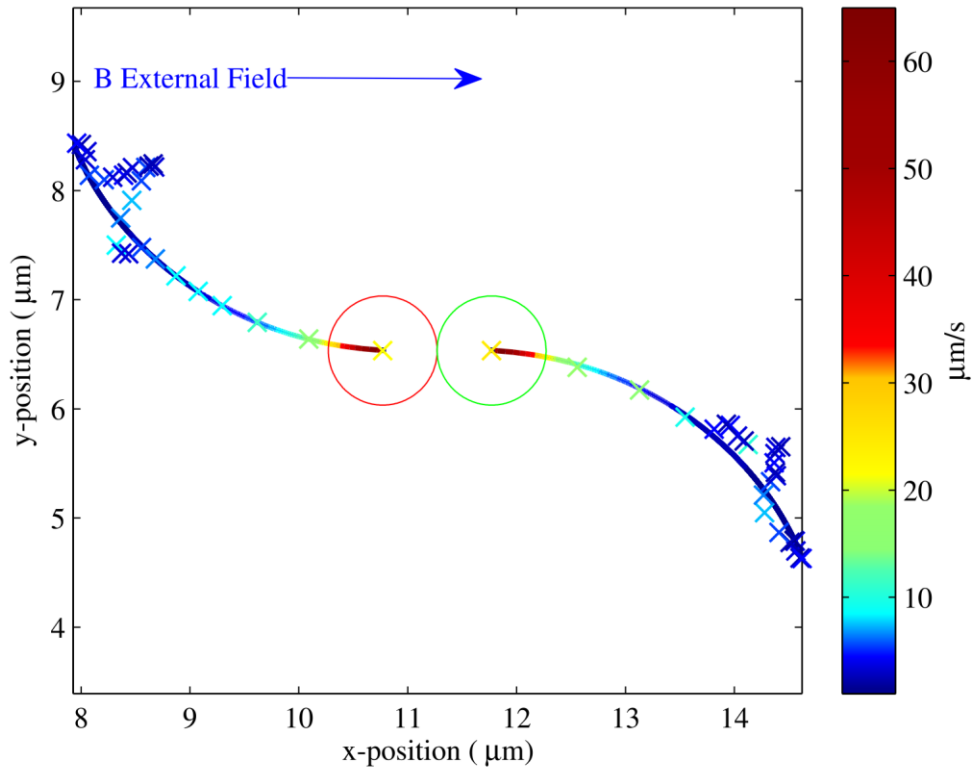


Figure 3.5c Trajectory plot of 1μm diameter bead (indicated in red) moving towards another 1μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

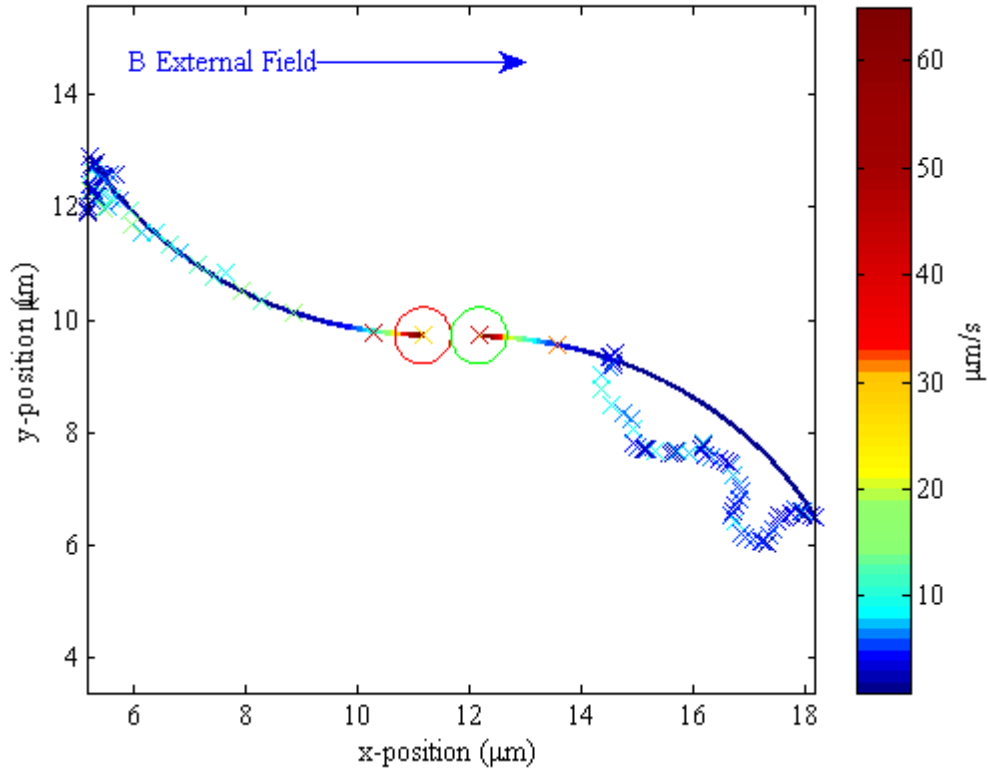


Figure 3.5d Trajectory plot of 1 μ m diameter bead (indicated in red) moving towards another 1 μ m diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in water; experimental data (crosses) and simulation results (continuous line) are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

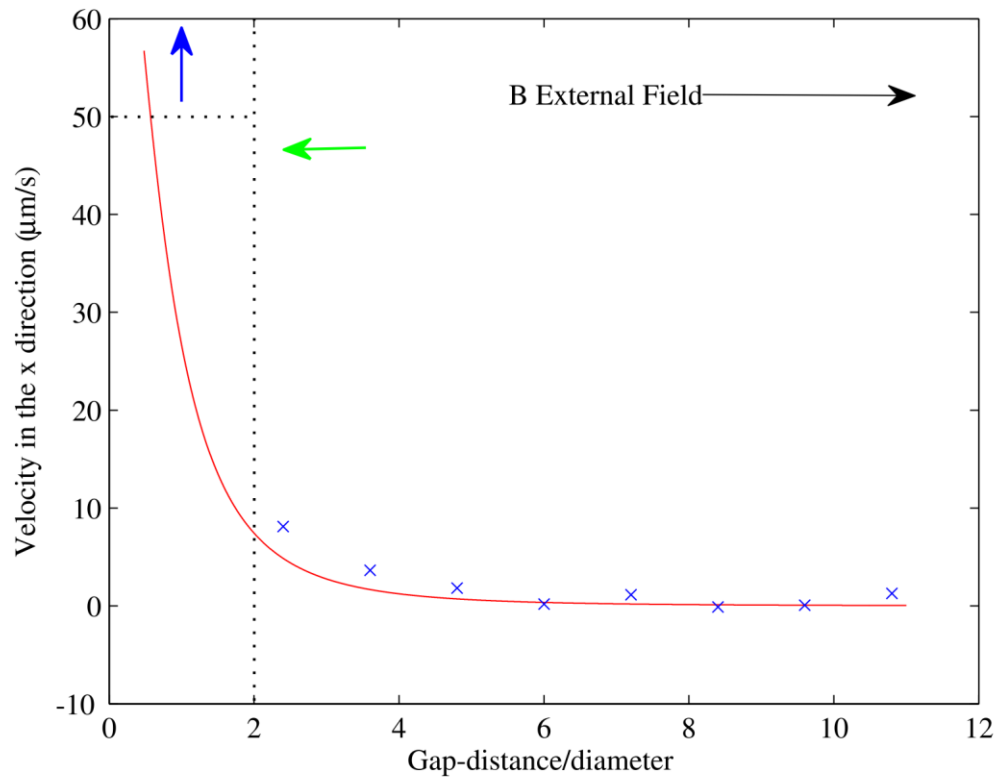


Figure 3.6 The average approach velocity of $1\mu\text{m}$ diameter bead interacting with another $1\mu\text{m}$ diameter bead in a uniform magnetic field (in the direction of the black arrow) suspended in water; experimental data (shown by blue crosses) and simulation (depicted by the red continuous line) are compared to validate the model. The blue and green arrows indicate the direction of velocity increase as the beads approach one another.

3.4.3 DISCUSSION OF RESULTS

Figures 3.5 show the trajectory paths followed by a pair of magnetic beads obtained from experimental data and compared with the simulation results.

When the beads are separated by a distance greater than six times their diameter, the interaction is weak and the beads are Brownian displaced due to the bombardment from the water molecules.

Independent observations also show that the beads are Brownian displaced at this distance.

As the beads approach one another, the interaction becomes stronger and they align in the direction of the magnetic field (x-direction) before approaching to form agglomerates.

The colours of the trajectory in figures 3.5 show the beads' velocity during interaction; a comparison of the colour coded simulated and experimental velocities show that there is a statistical variation in the velocities when the beads are Brownian displaced.

During the middle of the trajectory, the velocity of the beads gradually increases due to the higher force interaction, for example, figure 3.5c shows that at a gap-distance of 1.5-3.4 μm , the beads attain an average velocity of 2-15 $\mu\text{m/s}$ from the simulation while the data predicts an average velocity of 8-20 $\mu\text{m/s}$.

At the end of the trajectory (when the gap distance is reducing to zero), there is a deviation between the simulated velocity and the experimental velocity. The experimental velocity represents a lower limit of the velocity because of the limited capture rate of the camera (33fps) used in recording the data. Furthermore, the deviation in velocity values could be attributed to the statistical variation in the properties of the magnetic beads. For example, section 3.8 shows that the impact of physical and magnetic susceptibility variation of the 1 μm chemicell bead [25] could lead to a variation as large as 2.15 times for the agglomeration time.

Referring to figure 3.6, when the beads are separated by a distance greater than six times their diameter, the interaction is small and the average velocity arises due to Brownian displacement. As the beads approach one another, the average velocity increases due to the increased value of the magnetic force interaction set up between them as depicted by the direction of the green arrow. At a gap-distance less than two times their diameter, it is not possible to obtain reliable data due to the high speed at which the beads move. This is limited by the capture rate (33fps) of the camera. At these distances, the data predicts the velocity of the bead to be greater than or equal to $66\mu\text{m/s}$ (as highlighted by the direction of the blue arrow). The simulation shows that at such distances, the bead velocity approaches $60\mu\text{m/s}$ (see the blue arrow), a reasonable agreement with the data. There is therefore a good correspondence between the simulation results and experimental data; we can therefore conclude that the model is a good approximation to predicting particle interactions and their trajectory pattern within a magnetic separation system.

3.5 EXPERIMENT INVESTIGATING BEAD PAIR INTERACTION USING 2.8-MICRONS DYNAL BEADS (M-280) IN A MAGNETOPHORETIC SYSTEM

3.5.1 DESCRIPTION OF BEAD PAIR INTERACTION EXPERIMENT USING M-280 BEADS

In order to reduce the impact of Brownian motion, the experiment was repeated using $2.8\mu\text{m}$ Dynal beads [24] suspended in Poly Ethylene Glycol (PEG) with viscosity of $2.01 \times 10^{-3} \text{ kgm/s}$ at a concentration of $6 \times 10^5 \text{ beads/ml}$.

The procedures outlined in section 3.4.1 were repeated; new data were obtained before the bead sedimentation time elapsed, analysed and compared with the simulation results.

The beads' trajectories were tracked on a frame by frame basis using imageJ [72] and the velocities were calculated for all the frames.

3.5.2 COMPARISON OF EXPERIMENTAL DATA WITH SIMULATION RESULTS FOR 2.8-MICRONS DYNAL BEADS

A pair of beads with initial displacement set to be the same as in the first frame of the experiment data was simulated using the numerical model.

The trajectory of the approaching beads within the uniform field and the resolved velocity along the direction of the magnetic field were recorded from both the simulation and experimental data.

Figure 3.7 compares the trajectory pattern of the beads obtained from the simulation with the experimental data.

The experiment data are indicated by the crosses, simulation results are shown by the continuous line while the direction of the uniform field is illustrated by the blue arrow as shown on figures 3.7 below. The colour of the trajectory of the beads represent their velocities during interaction as indicated by the colour scale.

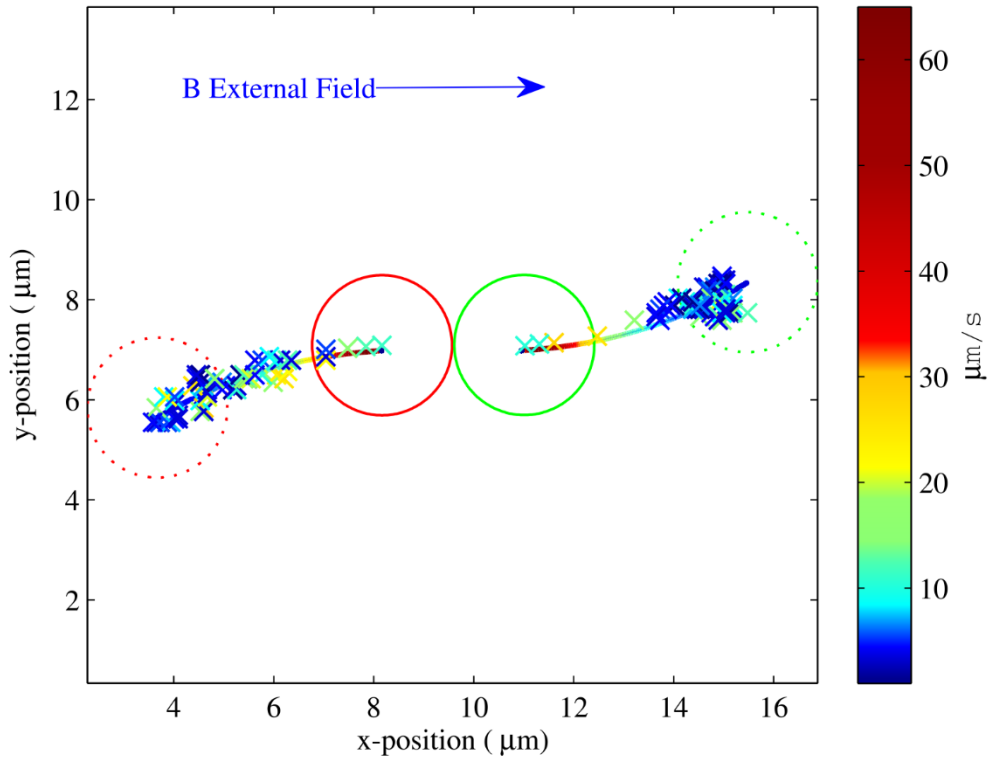


Figure 3.7a: Trajectory plot of a 2.8μm diameter bead (indicated in red) moving towards another 2.8μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The dotted circles show the starting point of the magnetic beads while the continuous circle show the end point which occurs when the beads are separated by 2.8μm. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

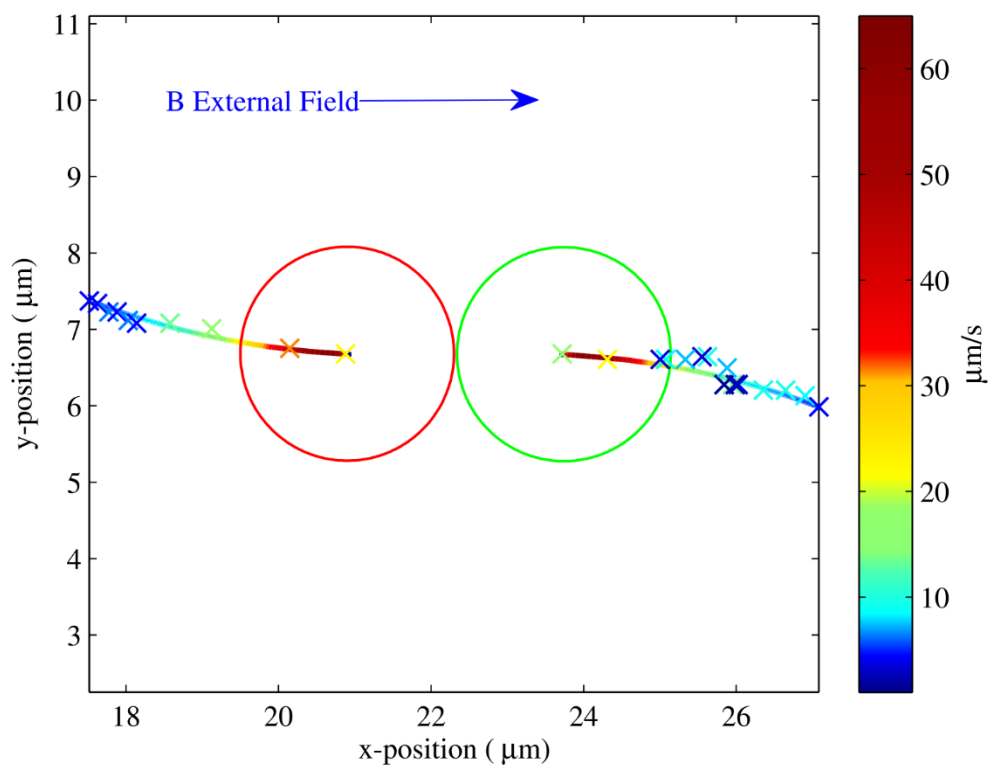


Figure 3.7b: Trajectory plot of a 2.8μm diameter bead (indicated in red) towards another 2.8μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

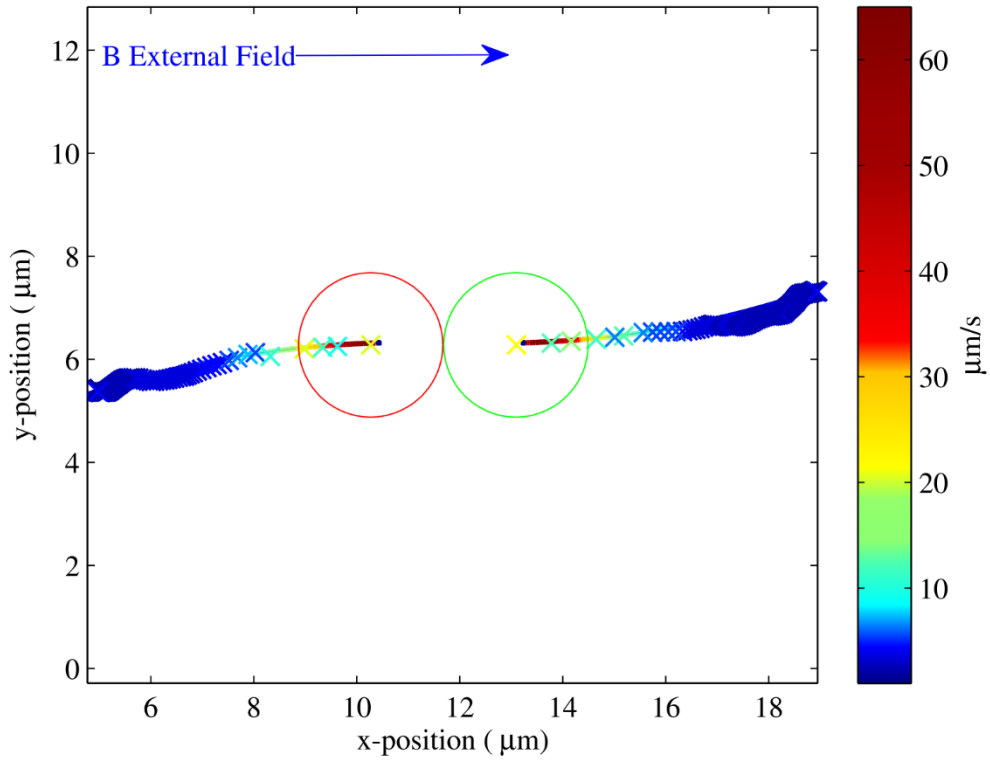


Figure 3.7c: Trajectory plot of a 2.8μm diameter bead (indicated in red) towards another 2.8μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

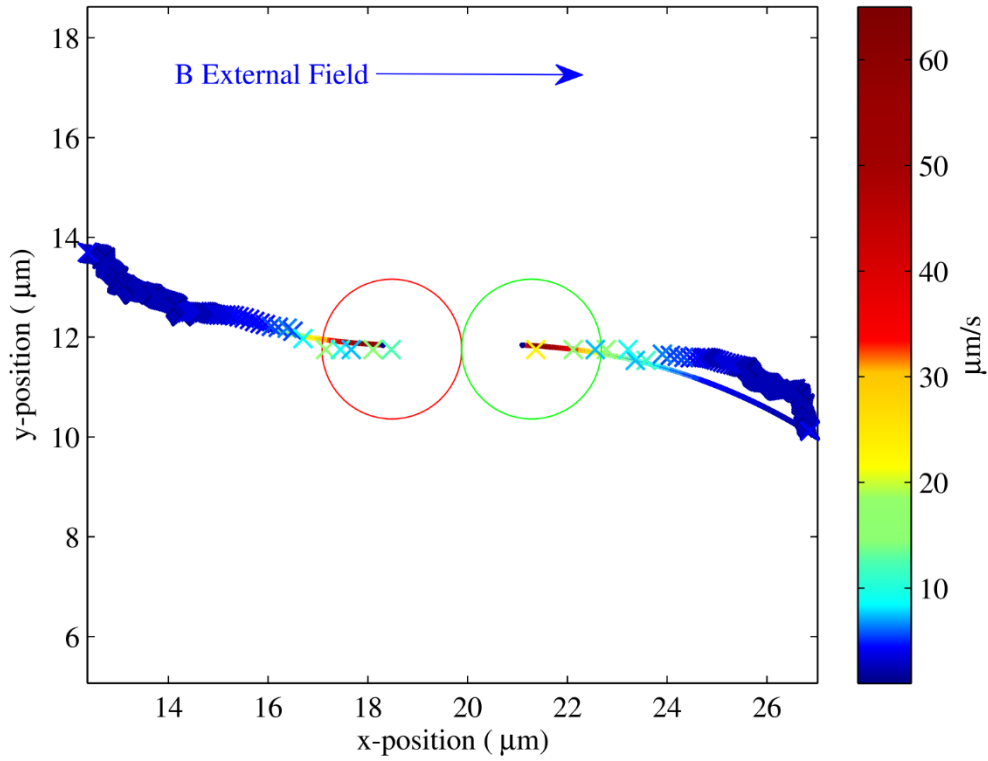


Figure 3.7d: Trajectory plot of a 2.8 μm diameter bead (indicated in red) towards another 2.8 μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

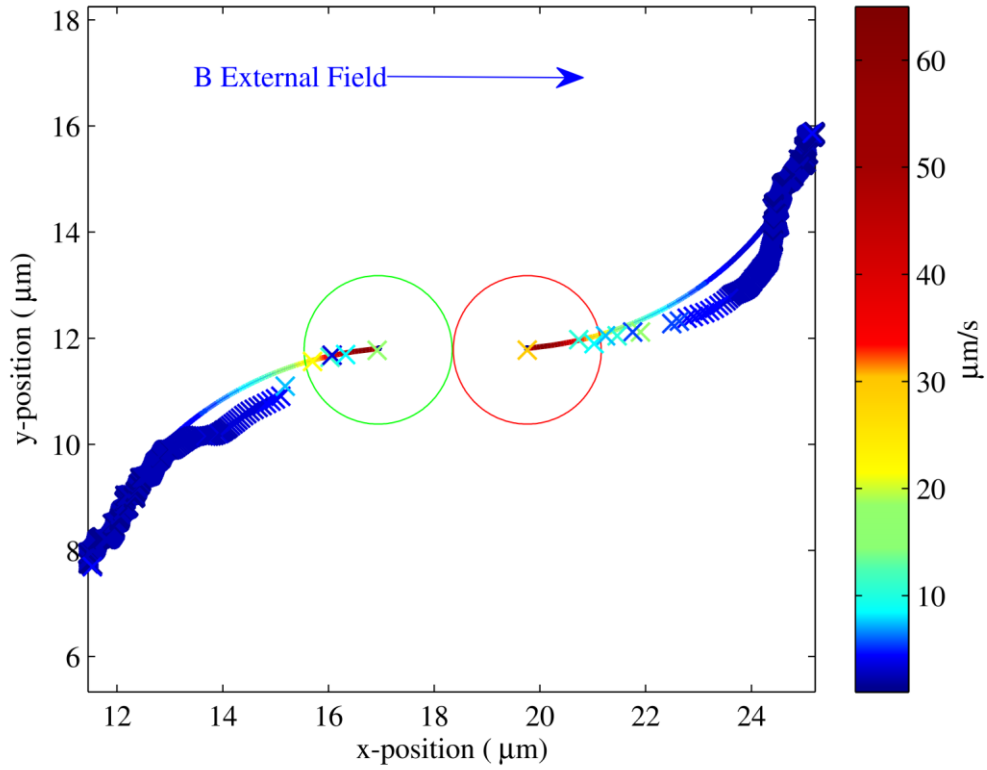


Figure 3.7e: Trajectory plot of a 2.8μm diameter bead (indicated in red) towards another 2.8μm diameter bead (indicated in green) under the influence of both magnetic and hydrodynamic interactions within a uniform external magnetic field in PEG; experimental data and simulation results are compared to validate the model. The velocities of the beads as they approach one another are indicated by the colour of the trajectories (crosses for experimental data and continuous line for simulation respectively) as indicated on the colour bar scale.

Figure 3.8 compares the simulated velocity with the average velocity obtained from the experiment due to the randomness associated with the tracking software (discussed in detail below).

Due to the uncertainty associated with the magnetic susceptibility value of the 2.8 μ m Dynal bead, the simulation was run using two magnetic susceptibility values obtained from the literature (0.65[84],0.8[79]) as indicated by the red and black continuous lines on figure 3.8 respectively.

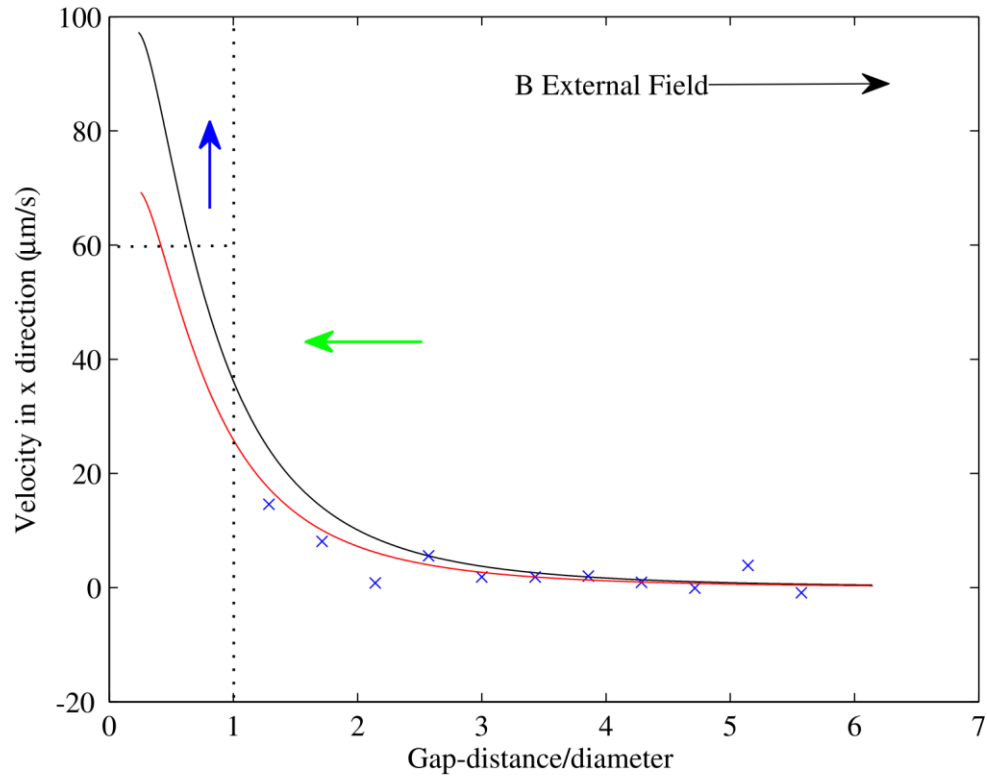


Figure 3.8 The resolved velocity of a $2.8\mu\text{m}$ diameter bead along the direction of magnetic field interacting with another $2.8\mu\text{m}$ diameter bead in a uniform magnetic field suspended in PEG; experimental data (shown by the blue crosses) and simulation results (illustrated by the red and black continuous lines corresponding to magnetic susceptibility values of 0.65[84] and 0.8[79] respectively) are compared to validate the model. The blue and green arrows indicate the direction of velocity increase as the beads approach one another.

3.5.3 DISCUSSION OF RESULTS

Figures 3.7 present the trajectory pattern of two magnetic beads and show a comparison of the experimental data with the simulation results.

The trajectory plots (see figure 3.7) show some randomness characteristic (of the order of $0.2\mu\text{m}$) which is not only due to the Brownian displacement in this case but also as a result of the error introduced by the tracking software.

This has been investigated by comparing data from tracked objects and manual observation of the movement of the particles; the data investigation shows that the automatic tracking software [72] has an rms error of $\pm 0.2\mu\text{m}$. This tracking error is consistent with the randomness observed on the trajectory plots.

From figures 3.7, we see that the magnetic beads interact with one another by first aligning themselves in the direction of the magnetic field (x-direction) before they approach and then subsequently agglomerate.

The colour on the trajectory plots in figures 3.7 represent the beads' velocity during interaction; when the beads are far apart (at a gap-distance of $10\text{-}11\mu\text{m}$), the beads approach one another with a low velocity (as indicated by the dark blue colour of both the simulation and data at the lower end of the colour bar scale). Within this region, some randomness occur during the interaction (see figure 3.7a) which is due to the Brownian displacement and tracking error (as discussed above).

As the beads approach one another, the velocity of the bead rises due to the larger interaction force, as depicted by the colour change from dark blue. Within this region, for example, figure 3.7c show that the beads attain a simulated velocity of $15\text{-}31\mu\text{m/s}$ while the data predicts that the beads have a velocity of $12\text{-}22\mu\text{m/s}$, at a gap distance of $2.5\text{-}4\mu\text{m}$.

When the gap distance approaches zero, there is a deviation between the simulated velocity and the experimental velocity because there is a 38% variation in the agglomeration time due to the dispersity of the physical and magnetic properties of the $2.8\mu\text{m}$ bead (see section 3.8) and the limited capture rate of the camera (as discussed in section 3.4.3).

Figure 3.8 shows that when the beads are separated by a distance greater than $6\text{--}7\mu\text{m}$, the interaction is small and the average displacement arises due to Brownian displacement. The Brownian displacement for this size of bead is of the same order as the tracking error. Hence, at this distance where the interaction is small, the randomness arises due to both Brownian motion and tracking error.

As the beads approach one another, the average velocity increases due to the increased value of the magnetic force interaction set up between them as indicated by the direction of the green arrow.

At a gap-distance less than $3.6\mu\text{m}$, it is not possible to obtain reliable data due to the higher speed at which the beads move. This is limited by the frame rate of the camera. At this point, the simulation predicts the velocity of the bead to be greater than or equal to $121\mu\text{m/s}$, indicated by the blue arrow. The simulated velocities (red and continuous lines) indicate that the bead velocity approaches $60\mu\text{m/s}$ to velocity values higher than $100\mu\text{m/s}$, depending on the magnetic susceptibility value, which is consistent with the data prediction.

The results above show consistency between the experiment data and simulation results; hence, the analytical model is a good approximation to predict the trajectory pattern of magnetic beads suspended in fluid for a uniform magnetic field.

3.6 INTERACTION BETWEEN DIFFERENT SIZED BEADS

Magnetic bead users face a diversity of choice due to the wide range of super-paramagnetic beads available from different manufacturers. The different manufacturing techniques lead to differences in the magnetic properties as well as physical properties of these beads.

This section presents the magnetic force induced velocities as well as interaction between a pair of different sized magnetic beads suspended in fluid moving within a uniform magnetic field.

3.6.1 THEORETICAL PRINCIPLE ON THE INTERACTION BETWEEN DIFFERENT SIZED MAGNETIC BEADS

Consider a pair of different sized super-paramagnetic beads with diameter, $1\mu\text{m}$ and $2.8\mu\text{m}$, densities 1.7g/cm^3 and 1.3g/cm^3 respectively separated by a position vector, $\mathbf{r}$, suspended in water and placed within the uniform magnetic field as depicted on figure 3.9.

Figure 3.9 shows the two forces acting on the bead pair; the sedimentation force acts downwards while the magnetic interaction force acts in the direction indicated on figure 3.9.

The resultant force therefore allows for either sedimentation or interaction. If the sedimentation force outweighs the interaction force, then the beads sediment; when otherwise, the beads interact due to the magnetic force.

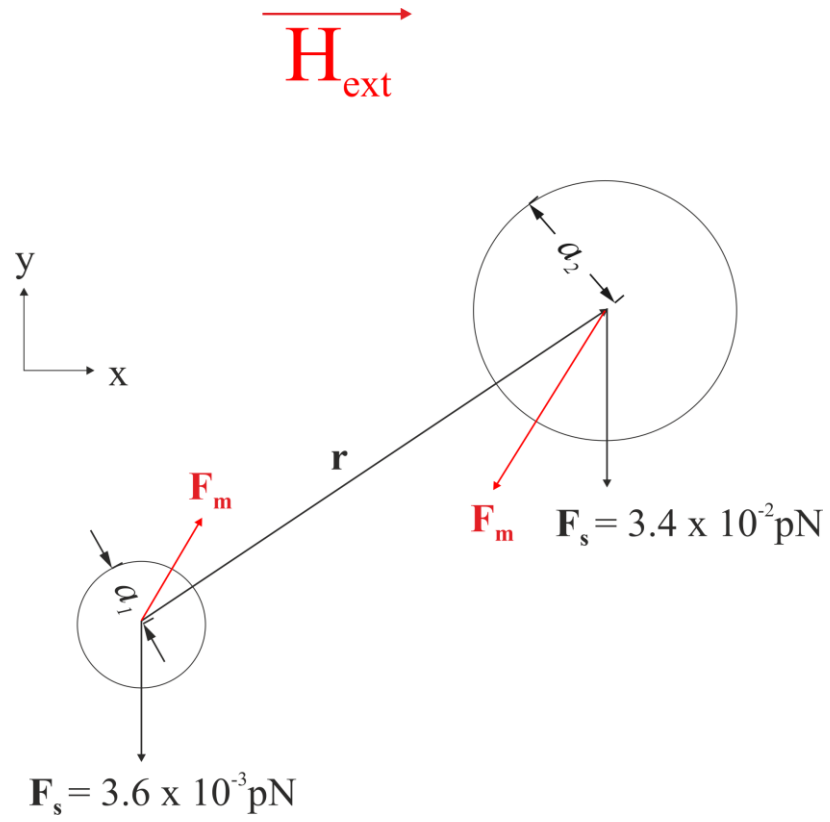


Figure 3.9 A schematic showing the magnetic interaction force $\mathbf{F}_m$, between a pair of different sized super-paramagnetic beads (a_1 and a_2 respectively) displaced by a position vector $\mathbf{r}$, within a uniform magnetic field (the direction of the magnetic field is illustrated by the arrow at the top). The beads are suspended in water and sediment under the action of force $\mathbf{F}_s$.

3.6.2 DESCRIPTION OF DIFFERENT SIZED BEAD PAIR INTERACTION EXPERIMENT

A solution of super-paramagnetic beads of two different sizes (Dynal 1 μ m Myone T1 [26] and 2.8 μ m M-280 [24]) was suspended in water at a concentration of 7×10^6 beads/ml and 7×10^5 beads/ml respectively. The experimental procedures have been outlined in section 3.4.1.

3.6.3 DISCUSSION OF RESULTS

The trajectories of the beads from the simulation were compared with the experimental data following the steps in section 3.4.2.

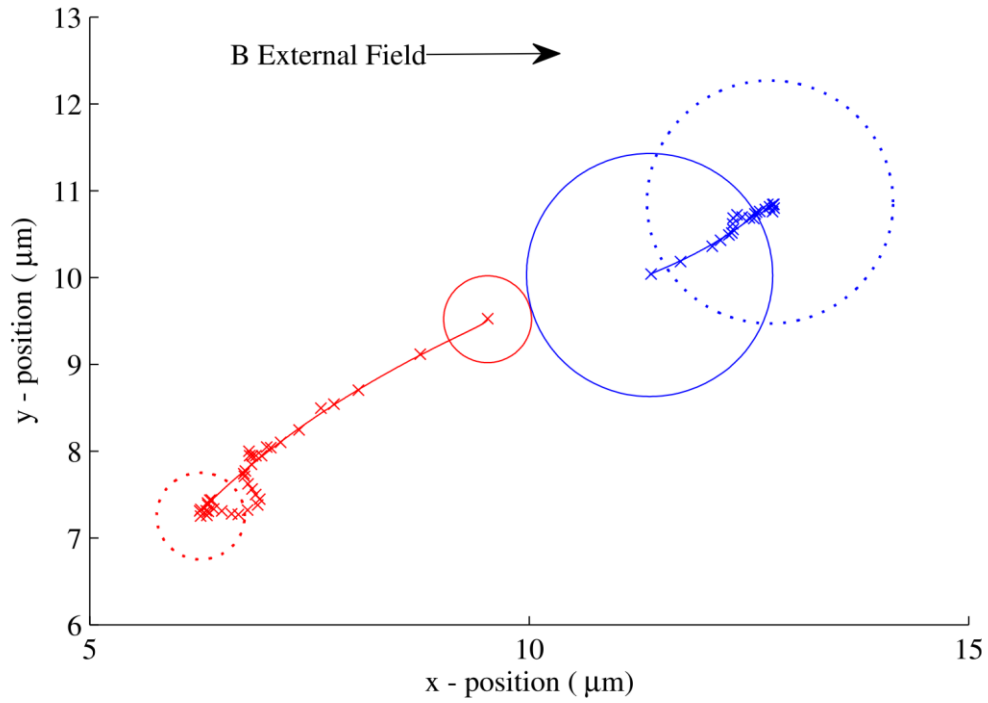


Figure 3.10 Trajectory plot of two super-paramagnetic beads, $1\mu\text{m}$ and $2.8\mu\text{m}$ sized, (indicated by red and blue) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction suspended in water. The starting points of the beads are indicated by the dotted circles while their end points are shown by the continuous circles. The direction of the magnetic field is indicated by the black arrow on the plot. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model.

The $1\mu\text{m}$ and $2.8\mu\text{m}$ super-paramagnetic beads are observed to be separated by a distance of $7.4\mu\text{m}$. At this distance, the magnetic force interaction outweighs the sedimentation force; this causes the beads to approach themselves in the direction of the magnetic field and then form an agglomerate.

The ratio of the average magnetic velocities of the small bead to the big bead is 2.2; however, the simulation predicts this ratio to be 2.8. Hence, the experimental data deviates from the simulation by a factor of 0.8. There is evidence that the value of magnetic susceptibility (2.8 μ m Dynal bead) varies by a factor of 0.8 (using the magnetic susceptibility values available from literature (0.65-0.8[79, 84]); hence the difference in velocities could be attributed to this variation in magnetic susceptibility value.

Given the uncertainties, we can therefore conclude that the model is a good approximation that can be used to predict the magnetic force induced velocities as well as interaction between magnetic beads with varying physical dimensions.

3.7 AGGLOMERATION TIME AS A FUNCTION OF THE SEPARATION DISTANCE

This section presents the relationship between the agglomeration time and separation distance for three different types of super-paramagnetic beads (from different manufacturers as highlighted on table 3.2).

Three beads were chosen for this study in order to illustrate the dependence of the agglomeration time on the magnetic and physical properties of the super-paramagnetic beads. The time-distance relationship will help in determining the threshold separation distance which will avoid agglomeration in a bead assay over a fixed time interval.

3.7.1 SIMULATION PREDICTING THE RELATIONSHIP BETWEEN AGGLOMERATION TIME AND SEPARATION DISTANCE

Considering two nearest neighbour beads (of three different types as displayed on table 3.2) positioned along a line in the direction of the magnetic field (x-axis), the simulation predicts the time it takes for the beads to agglomerate as a function of the initial separation distance, as shown on figure 3.11.

Table 3.2 Magnetic and Physical properties of super-paramagnetic beads.

Super-paramagnetic Bead	Simag-Silanol Chemicell	Myone Streptavidin T1 Dynal	M-280 Dynal
Diameter	1 μ m	1 μ m	2.8 μ m
Magnetic Susceptibility	1.3	1.4 [31]	0.65 [84]
Density	2.25g/cm ³	1.7g/cm ³	1.3g/cm ³

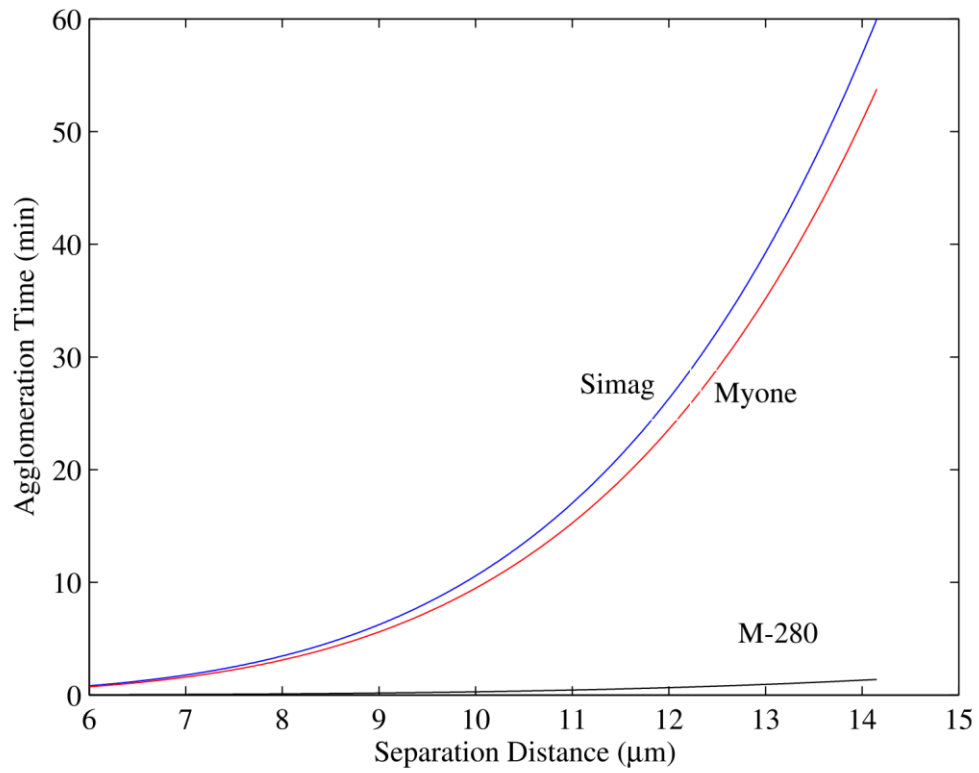


Figure 3.11 Relationship between the shortest agglomeration time and the separation distance for a pair of super-paramagnetic beads (Chemicell SiMag in blue, Myone T1 Dynal bead in red and M-280 Dynal bead in black) positioned along the direction of the magnetic field.

3.7.2 THEORETICAL ANALYSIS PREDICTING THE AGGLOMERATION TIME BEYOND 15µm.

This section presents an approximate expression for the magnetic force interaction, drag velocity and agglomeration time for a pair of beads sufficiently separated such that hydrodynamic interactions can be neglected. The goal is to explore the bead-pair interaction dynamics at large separations (beyond 15µm) where the particle interaction model becomes simplified into an analytical expression.

If a pair of super-paramagnetic beads is positioned along the direction of the uniform magnetic field, the significant interaction force component (see section 2.3.3) is simplified below in equation 3.1, by neglecting the weak induced dipole to dipole interaction force (of seventh order).

$$\mathbf{F}_m = -\frac{24\pi(\chi_{eff})^2 a^6 \phi_0}{x^4} \mathbf{H}_{ext}^2 \hat{\mathbf{i}} \quad (3.1)$$

Equation 3.1 shows that the magnetic force interaction depends on the distance to the order of four. If we assume that the pair of beads are sufficiently separated at distances beyond 15µm along the direction of the magnetic field such that the hydrodynamic interactions can be neglected, then we can work out the velocity of the bead using the drag approximation. At such large distances (>15µm), the shortest agglomeration time (which is desirable for measurement purposes within a bead assay) is expressed below by integrating the drag velocity expression.

$$t = \frac{\eta x^5}{20a^5 \chi_{eff}^2 \mu_o \mathbf{H}_{ext}^2} \quad (3.2)$$

Equation 3.2 shows that at distances beyond 15µm, the approximated agglomeration time depends on the distance to the order of five.

From equation 3.1, we can establish a relationship between the force interaction, agglomeration time and the physical and magnetic characteristics of the magnetic beads which will be used in discussing figure 3.11 and now outlined below (in equations 3.3 and 3.4 below).

$$\mathbf{F_m} \propto (\chi_{eff}^2)(a^6) \quad (3.3)$$

$$t \propto \frac{1}{(\chi_{eff}^2)(a^5)} \quad (3.4)$$

3.7.3 DISCUSSION OF RESULTS

Figure 3.11 and Table 3.2 show that an increase in the magnetic susceptibility value from 1.3 to 1.4 (see table 3.2) would lead to a shorter estimate of the agglomeration time by a factor of 1.2 (red and blue lines) for beads with the same diameter (Simag-Silanol and Myone T1 Dynal Beads). This is consistent with the approximations provided by equation 3.4 which predicts a shorter time by the same factor as a result of the change in magnetic susceptibility value.

Figure 3.11 and Table 3.2 also show that a pair of M-280 beads (black line) would agglomerate in a shorter time interval by a factor of 30 – 45 when compared with a pair of Chemicell beads (blue line). The fast agglomeration time is due to its larger physical dimension and lower magnetic susceptibility value; this result is also consistent with the theoretical approximations provided by

equation 3.4 which estimates a time increase by a factor of 40 - 60 (reduced susceptibility value and an increased radius). In estimating the magnetic susceptibility value of the magnetic beads, there is an uncertainty as confirmed by the range of values available in the literature (see section 3.2.1.3). The comparison is reasonable and lies within the range (of literature values) considering the impact of Brownian motion and uncertainty in the magnetic susceptibility values of this bead.

At distances beyond 15 μ m, it was possible to verify that the agglomeration time (Figure 3.11) follows a fifth order dependence, as predicted in section 3.7.2 when the beads are positioned along the direction of the magnetic field.

In conclusion, Figure 3.11 can be used to estimate the agglomeration time for a given concentration.

3.8 TOLERANCES IN BEAD PARAMETERS

The magnetic beads used in this work have tolerances in their physical and magnetic properties. In this section, the tolerances will be quantified and their effects on the results presented in this work will be discussed.

The tolerances in the properties of the magnetic beads would lead to a change in the agglomeration time reported in this work. For example, Figures 3.5-3.8 show that an equal sized bead pair (from the same manufacturer) placed in a uniform magnetic field approach one another with different velocities; these differences are due to the poly-dispersity in the physical and magnetic properties of the beads. Also beads of different properties arising from the statistical variation would come together at different trajectory points.

The tolerances in the parameters of the magnetic beads used in this work are summarised below.

Table 3.3 Physical and Magnetic dispersity values of three super-paramagnetic beads and impact on agglomeration time.

Super-paramagnetic Bead	Diameter (μm)	Radius (μm)	Magnetic Susceptibility (χ)	Effective Magnetic Susceptibility ($\chi_{eff} = \chi / \chi + 3$)	$\Delta t/t$	t_{new}/t
Chemicell [25]	1 $\pm$ 0.23 [104]	0.5 $\pm$ 0.115	1.33-0.08	0.302-0.013	115%	2.15
Myone [26]	1 $\pm$ 0.23 [79]	0.525 $\pm$ 0.01	1.377 $\pm$ 0.003 [31, 79]	0.315	9.55%	1.1
M-280 [24]	2.8 $\pm$ 0.04 [79]	1.4 $\pm$ 0.02	0.65 $\pm$ 0.15 [79, 84]	0.178 $\pm$ 0.033	37.76%	1.4

Assume a pair of super-paramagnetic beads is aligned in the direction of the magnetic field, equation 3.2 gives the shortest agglomeration time it takes for the pair to come together.

A sensitivity analysis on radius and effective magnetic susceptibility is performed on equation 3.2 and reveals that the squared fractional change in agglomeration time is given by

$$\left(\frac{dt}{t}\right)^2 = 25\left(\frac{da}{a}\right)^2 + 4\left(\frac{d\chi_{eff}}{\chi_{eff}}\right)^2 \quad (3.5)$$

Equation 3.5 shows that the square of the fractional error in time is approximately six times more sensitive to the square of the fractional error in radius when compared with to the square of the fractional change in the effective magnetic susceptibility.

The result shows that the uncertainty associated with the physical properties of the 1 μ m Chemicell beads [25] leads to a wide spread in the calculated agglomeration time and may thus hinder the use of the agglomeration time (as a group parameter) to predict its behaviour within the magnetic separation system. However, the results show that agglomeration time remains a useful parameter for predicting the behaviour of tighter tolerance beads such as the 1 μ m myone [26] and 2.8 μ m M-280 Dynal beads [24] within a magnetic separation system.

In conclusion, the results from the above table show that the impact of the tolerances in parameters would be a 10% variation in agglomeration time for the 1 μ m myone [26] beads, as much as 38% for the 2.8 μ m M-280 beads [24] and for the 1 μ m Chemicell beads [25], where the spread in parameters is most significant, the variation in time can be as large as 2.15 times. In practice, this suggests that if a precise knowledge of the agglomeration time is required the recommendation would be to avoid beads manufactured by Chemicell [25].

3.9 CONCLUSION

A theoretical analysis of the interactions between a pair of magnetic beads in a magnetic separation system has been investigated; this led to the development of a two dimensional numerical model in Matlab.

An experiment was designed to investigate particle interactions in a magnetophoretic system; for the $1\mu\text{m}$ sized particles, Brownian motion displacement were observed and studied.

The examples illustrate that the simulation predicts the trajectory pattern of the beads due to magnetic and hydrodynamic interactions within a uniform magnetic field with sufficient accuracy; the simulated trajectories were consistent with the data obtained from the experiment.

A conclusion can be reached that the model is a good approximation for particle interactions in a magnetophoretic system based on the assumption that the magnetic field is sufficiently small that a linear relationship exists between the magnetisation and the external magnetic field. Within the non-linear region, the magnetisation of the bead would have to be determined using a non-linear relationship. As the pair of beads approach each other, their induced fields interact and lead to a perturbation in the local field (and magnetic susceptibility). Assuming the beads approach along a line in the direction of the magnetic field, it is possible to estimate the impact of not considering this change in the magnetic susceptibility value. For a centre-to-centre distance greater than $5\mu\text{m}$, there is less than 0.1% impact on the accuracy of the magnetic force calculation that ignores perturbation in the susceptibility whereas at a centre-to-centre distance of $2\mu\text{m}$, the impact is estimated to be 2%.

Furthermore, the model is able to predict the trajectory pattern of magnetic beads that may differ in physical dimensions or magnetic properties (equal sized or different sized) when suspended in fluid for a uniform magnetic field.

It can therefore be concluded that agglomeration begins when the attracting magnetic interaction has sufficient time to work against the restraining hydrodynamic force between beads suspended in a fluid. This study shows that the magnetic attraction dominates Brownian motion when the

Chemicell beads (SiMAG-Silanol 1101-1) become separated by a gap-distance of less than six times their diameter with an agglomeration time of 2s.

For the 2.8 μ m Dynal beads (M-280), the attractive magnetic force becomes significant when the beads become separated by a gap-distance of less than 6-7 μ m with an agglomeration time of 0.04-0.07s respectively.

It is also possible to approximate the shortest agglomeration time as a function of the separation distance which will help users of bead-based assays to estimate the agglomeration time for a given concentration.

This model has been extended to a three dimensional model with more particles interacting within a magnetic chamber which will increase our knowledge of interaction as well as sedimentation within the magnetic separation system.

This knowledge can then help with recommendations that will improve the separation efficiency of existing magnetic separators as well as aid in the micro-magnetic manipulation of magnetic beads when attached to a biological entity (discussed in detail in Chapter 5).

Chapter 4

Many Beads Interaction in a Magnetophoretic System

4.1 OVERVIEW

The formation of agglomerate, which limits the separation efficiency of magnetic separators, in most cases, begins with a bead pair interaction and this has been studied in previous chapters. The bead pair then interacts with close neighbouring beads to either form ‘three beads’ or ‘many beads’ cluster, depending on the concentration.

When data is obtained in three dimensions, it allows for an accurate interpretation of the measured data and helps in evaluating the impact of the forces along the axial direction (sedimentation). Here, we present a three dimensional vector analysis of the interactions among three super-paramagnetic beads suspended in fluid within a uniform magnetic field.

A three dimensional vector model was developed from first principles to account for the magnetic and hydrodynamic interactions among the three beads. This model was implemented in Matlab and is able to predict the trajectory pattern of the magnetic beads.

In order to track the magnetic beads in three dimensions, a Huygens’ Fresnel diffraction model was developed to predict the axial information of a bead in Matlab. This was then compared to recorded images obtained by viewing magnetic beads under an optical microscope at different defocus distances.

The trajectory of the three super-paramagnetic beads suspended within fluid for a uniform magnetic field was observed and recorded on video using an optical imaging configuration. The video data was analysed using a tracking software and then compared with the predictions from the vector model; this gave a good agreement.

4.2 THREE DIMENSIONAL MODEL FOR MANY PARTICLES INTERACTING WITHIN A MAGNETOPHORETIC SYSTEM

Here, we used the derived equations from chapter two to evaluate the magnetic interaction force between a bead pair placed within a uniform magnetic field and introduced the concept of direct and indirect interaction forces to approximately account for all forms of perturbations that may lead to a change in the movement of a bead.

We also resolved the hydrodynamic velocities into three vector components using the method of reflection technique and lubrication approximation derived from chapter two to account for the hydrodynamic interaction among the beads.

The use of blocking was introduced in the model as a simplification to approximately predict the hydrodynamic velocity due to little or no reflected fluid field at the surface of the bead. The numerical model was implemented in Matlab.

4.2.1 MAGNETIC FORCE INTERACTION

The magnetic interaction on a reference bead 1, due to a neighbouring bead 2, placed within a uniform magnetic field (see figure 2.1) which arises as a result of induced magnetisation and a perturbation in the external magnetic field (see chapter 2) can be written as

$$\mathbf{F}_{m12} = \varphi_0 \int ((\mathbf{M}_1 + \mathbf{dM}_1) \cdot \nabla) (\mathbf{H}_{\text{ext}}(\mathbf{r}_1) + \mathbf{dH}_2) dV_p \quad (4.1)$$

4.2.1.1 *DIRECT INTERACTION*

This is the force that leads to a change in the magnetisation and external field of the reference bead as a result of its direct interaction with neighbouring beads within the field. For example, $\mathbf{F}_{m12}$ is a magnetic interaction force on bead 1 caused by interacting directly with bead 2 as depicted on figure 4.1.

4.2.1.2 *INDIRECT INTERACTION*

If three super-paramagnetic beads are displaced within a uniform magnetic field (see figure 4.1), at a reference bead 1, the interaction force on bead 2 due to the presence of bead 3, $\mathbf{F}_{m32}$, would lead to a change in the movement of both beads 2 and 3. The movement of beads 2 and 3 within the fluid would lead to a velocity field which would be transmitted through the fluid. The velocity field would be reflected at the surface of bead 1 and would lead to a change in its local velocity, hence $\mathbf{F}_{m32}$ indirectly influences the movement of bead 1.

This is an interaction force that arises between neighbouring beads and leads to a change in the movement of the reference bead.

In order to evaluate the total interaction on the reference bead, the principle of superposition is applied by evaluating all the direct and indirect interaction independently and then superposing these results.

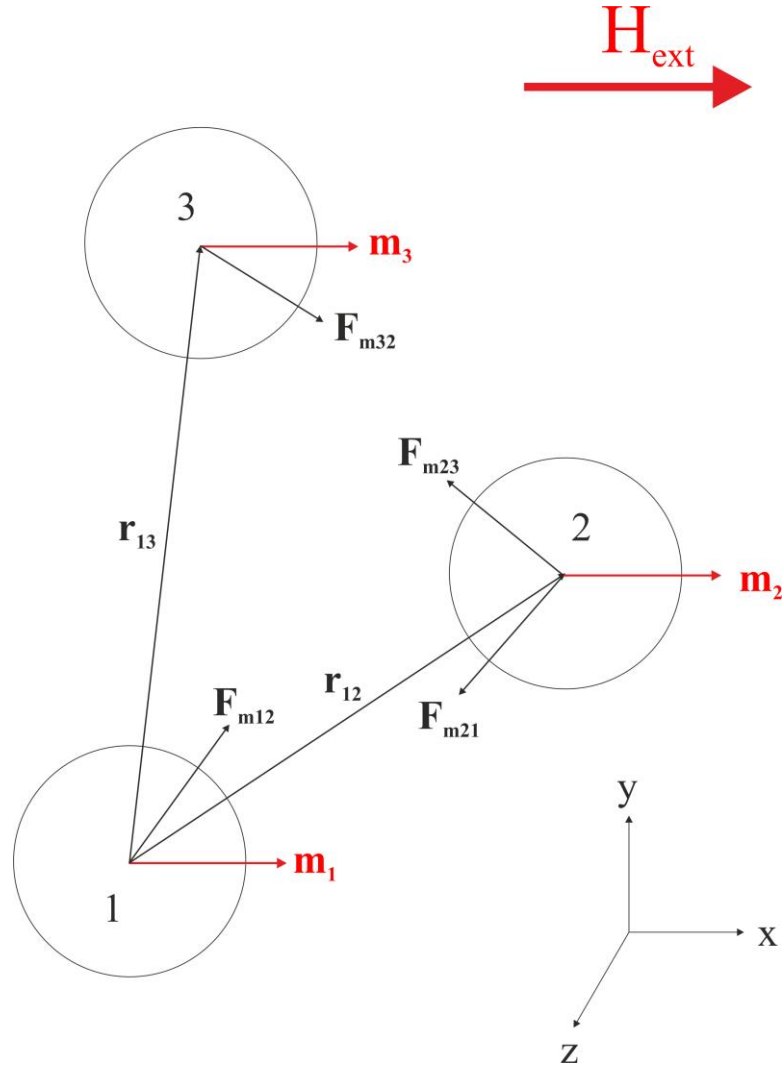


Figure 4.1 Schematic showing direct magnetic force interaction ($\mathbf{F}_{m12}$) on a reference super-paramagnetic bead (1) due to the presence of a neighbouring bead (2) within a uniform magnetic field and an Indirect magnetic force Interaction ($\mathbf{F}_{m32}$) between the two neighbouring beads (2, 3) which affects the movement of the reference bead (1) due to the fluid environment; bead 1 is separated from beads (2, 3) by position vectors $\mathbf{r}_{12}$ and $\mathbf{r}_{13}$ respectively. The magnetic moments of the beads, $\mathbf{m}_1$, $\mathbf{m}_2$, $\mathbf{m}_3$ are aligned in the direction of the uniform magnetic field (the direction is illustrated by the arrow at the top).

4.2.2 HYDRODYNAMIC INTERACTION

The theory behind this interaction as well as full derivation has been given in chapter two. When a bead moves in fluid, it disturbs the fluid stream and perturbs neighbouring beads within the fluid leading to a change in their velocity fields.

Using the derived equations (from chapter two) based on the method of reflection [41, 55], we can write the force acting on a reference bead (e.g. bead 1) suspended in fluid, disturbed by the movement of a neighbouring bead (e.g. bead 2) as

$$\frac{\mathbf{F}_h}{\eta k_a} = \frac{\mathbf{u}_1 - k_2 \mathbf{u}_2}{1 - k_1 k_2} + \frac{-k_1 k_2 \mathbf{u}_1^r}{1 - k_1 k_2} + \frac{k_3 k_4 \mathbf{u}_1^r}{1 - k_3 k_4} + \frac{k_2 \mathbf{u}_2^r}{1 - k_1 k_2} - \frac{k_4 \mathbf{u}_2^r}{1 - k_3 k_4} \quad (4.2a)$$

where

$$\mathbf{u}_1^r = \frac{(\mathbf{u}_1 \cdot \mathbf{r})\mathbf{r}}{r^2}, \quad \mathbf{u}_2^r = \frac{(\mathbf{u}_2 \cdot \mathbf{r})\mathbf{r}}{r^2} \quad (4.2b)$$

$$k_a = 6\pi a_1, k_b = 6\pi a_2 \quad (4.2c)$$

$$k_1 = \frac{k_a}{8\pi r}, k_2 = \frac{k_b}{8\pi r}, k_3 = \frac{k_a}{4\pi r}, k_4 = \frac{k_b}{4\pi r} \quad (4.2d)$$

Here, the position vector, $\mathbf{r}$ represents the displacement from the reference bead to the neighbouring bead. For instance, in figure 4.1, $\mathbf{r}$ will be represented by $\mathbf{r}_{12}$ if the reference bead is bead 1 and the neighbouring bead considered is bead 2 etc.

Equation 4.2a shows that the long range hydrodynamic force acts along the position vector and velocities. This force is valid only when the beads are separated by a distance greater than their physical dimensions. When this is not valid, lubrication approximations hold. This has also been described in chapter two.

When two beads are separated by a thin gap and approach one another with velocities, $\mathbf{u}_1$ and $\mathbf{u}_2$ respectively in a liquid with viscosity η , bead 1 experiences a lubrication force given by

$$\mathbf{F}_{\text{lub}} = -\frac{3\pi\eta a_h a_g}{d_o} [\mathbf{u}_1^r - \mathbf{u}_2^r] \quad (4.3)$$

In order to obtain the hydrodynamic velocities of the particles in three dimensions, equations 4.2a and 4.3 will be resolved along the position vector and two normals.

The first normal (acting within the plane) is obtained by first rotating the positional vector by 90 degrees along the x axis.

$$\mathbf{n}_0 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & -1 \\ 0 & 1 & 0 \end{bmatrix} \mathbf{r} \quad (4.4)$$

Using Gram-Schmidt Algorithm [100], a vector orthonormal to the positional vector $\mathbf{n}_1$, is obtained by projecting $\mathbf{n}_0$ onto $\mathbf{r}$.

$$\mathbf{n}_1 = \mathbf{n}_0 - \frac{\mathbf{n}_0 \cdot \mathbf{r}}{|\mathbf{r}|^2} \mathbf{r} \quad (4.5a)$$

$$\hat{\mathbf{n}}_1 = \frac{\mathbf{n}_1}{|\mathbf{n}_1|} \quad (4.5b)$$

The second normal (out of the plane) is obtained through a cross product of the position vector and the first normal.

$$\widehat{\mathbf{n}}_2 = \frac{\hat{\mathbf{r}} \times \widehat{\mathbf{n}}_1}{|\hat{\mathbf{r}} \times \widehat{\mathbf{n}}_1|} \quad (4.6)$$

Resolving the velocity of the reference bead (e.g. bead 1) as a result of its hydrodynamic interaction with a neighbouring bead (e.g. bead 2) along all three vectors ($\mathbf{r}$, $\mathbf{n}_1$ and $\mathbf{n}_2$) gives the equation below. For the special case under consideration, $\mathbf{F}_h$ will be represented by $\mathbf{F}_{m12}$ (see figure 4.1) in the equation below.

$$\mathbf{u}_1 \cdot \hat{\mathbf{r}} = \frac{\frac{\mathbf{F}_h \cdot \hat{\mathbf{r}}}{\eta k_a} (1 - k_3 k_4) + \left[k_4 + \left(\frac{a_p}{2d_o} \right) (1 - k_3 k_4) \right] \mathbf{u}_2 \cdot \hat{\mathbf{r}}}{\left[1 + \left(\frac{a_p}{2d_o} \right) (1 - k_3 k_4) \right]} \quad (4.7a)$$

where

$$a_p = \frac{a_h a_g}{a} \quad (4.7b)$$

$$\mathbf{u}_1 \cdot \widehat{\mathbf{n}}_1 = \frac{\mathbf{F}_h \cdot \widehat{\mathbf{n}}_1}{\eta k_a} (1 - k_1 k_2) + k_2 (\mathbf{u}_2 \cdot \widehat{\mathbf{n}}_1) \quad (4.8)$$

$$\mathbf{u}_1 \cdot \widehat{\mathbf{n}}_2 = \frac{\mathbf{F}_h \cdot \widehat{\mathbf{n}}_2}{\eta k_a} (1 - k_1 k_2) + k_2 (\mathbf{u}_2 \cdot \widehat{\mathbf{n}}_2) \quad (4.9)$$

The resultant velocity is then obtained by summing equations 4.7a, 4.8 and 4.9 together.

$$\mathbf{u}_1 = (\mathbf{u}_1 \cdot \hat{\mathbf{r}})\hat{\mathbf{r}} + (\mathbf{u}_1 \cdot \hat{\mathbf{n}}_1)\hat{\mathbf{n}}_1 + (\mathbf{u}_1 \cdot \hat{\mathbf{n}}_2)\hat{\mathbf{n}}_2 \quad (4.10)$$

This gives

$$\begin{aligned} \mathbf{u}_1 = & \frac{\frac{(\mathbf{F}_h \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\eta k_a}(1 - k_3 k_4) + \left[k_4 + \left(\frac{a_p}{2d_o} \right)(1 - k_3 k_4) \right](\mathbf{u}_2 \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\left[1 + \left(\frac{a_p}{2d_o} \right)(1 - k_3 k_4) \right]} \\ & + \frac{(\mathbf{F}_h \cdot \hat{\mathbf{n}}_1)\hat{\mathbf{n}}_1}{\eta k_a}(1 - k_1 k_2) + k_2(\mathbf{u}_2 \cdot \hat{\mathbf{n}}_1)\hat{\mathbf{n}}_1 \\ & + \frac{(\mathbf{F}_h \cdot \hat{\mathbf{n}}_2)\hat{\mathbf{n}}_2}{\eta k_a}(1 - k_1 k_2) + k_2(\mathbf{u}_2 \cdot \hat{\mathbf{n}}_2)\hat{\mathbf{n}}_2 \quad (4.11) \end{aligned}$$

4.2.2.1 *DESCRIPTION OF BLOCKING*

The method of reflection, as previously discussed, has been used to evaluate the velocity field due to the movement of one of the beads within the fluid. This method relies on the reflection of the velocity fields from the surface of the beads.

Blocking is an approximation used in this model to describe a situation where one of the beads lies within the view of the other bead(s) such that the reflected velocity fields are unable to reach its surface.

Experimental observations reveal that beads form agglomerate when they lie within the same plane. This observation justified the use of circles to model the beads during blocking and the use of the circle-circle intersection formula to predict the blocking factor in this model.

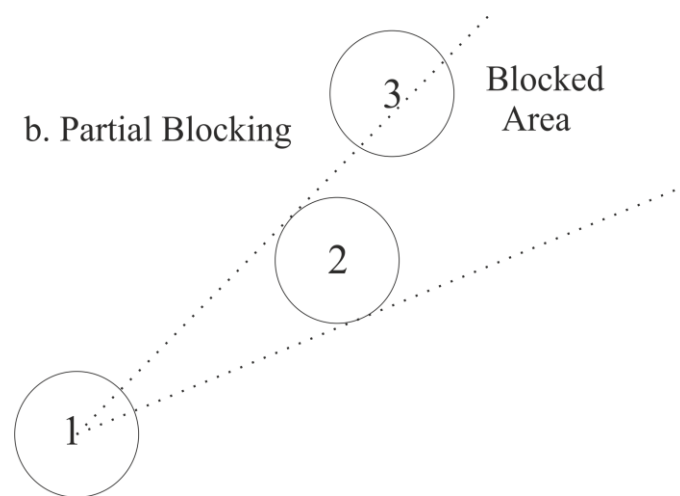
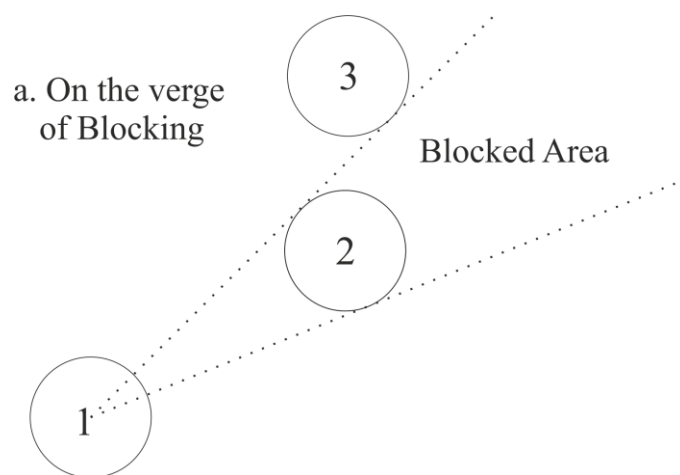
Figure 4.2 demonstrates three cases: from being on the verge of blocking, partially blocked to fully blocked. When the third bead is on the verge of being blocked (figure 4.2a) or not blocked, the reflected fluid stream that arises as a result of the movement of bead 1 within the fluid will reach its surface.

But when the third bead becomes partially blocked (figure 4.2b), some of the reflected fluid stream due to bead 1's movement will be blocked by bead 2.

While figure 4.2c shows that when bead 3 is fully blocked by bead 2, the reflected fluid velocity due to the movement of bead 1 will have little or no effect on it; In this work, we have assumed that there is no interaction from a bead that is fully blocked.

Blocking therefore leads to an interruption in the long range hydrodynamic field velocity. This model incorporates the use of a simple blocking factor which depends on the ratio of the blocked cross-sectional area to the total area of the bead; this is then combined with equation 4.11 to evaluate the hydrodynamic velocity.

Schematic of three beads displaced within fluid demonstrating the concept of: on the verge of Blocking, partial Blocking and full Blocking respectively.



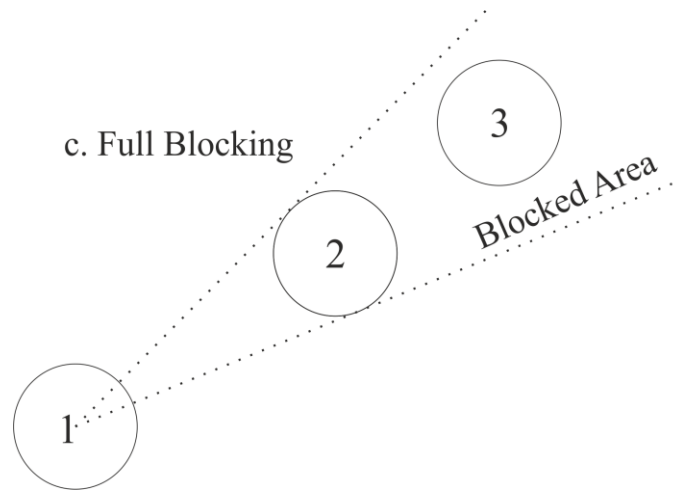


Figure 4.2: Three beads suspended within fluid demonstrating three blocking concepts namely (a) the verge of blocking (b) partial blocking (c) full blocking.

Demonstration of partial blocking: bead 3 is partially blocked by bead 2 when viewed from bead 1. Bead 4 is the image of bead 3 projected to the plane of bead 2.

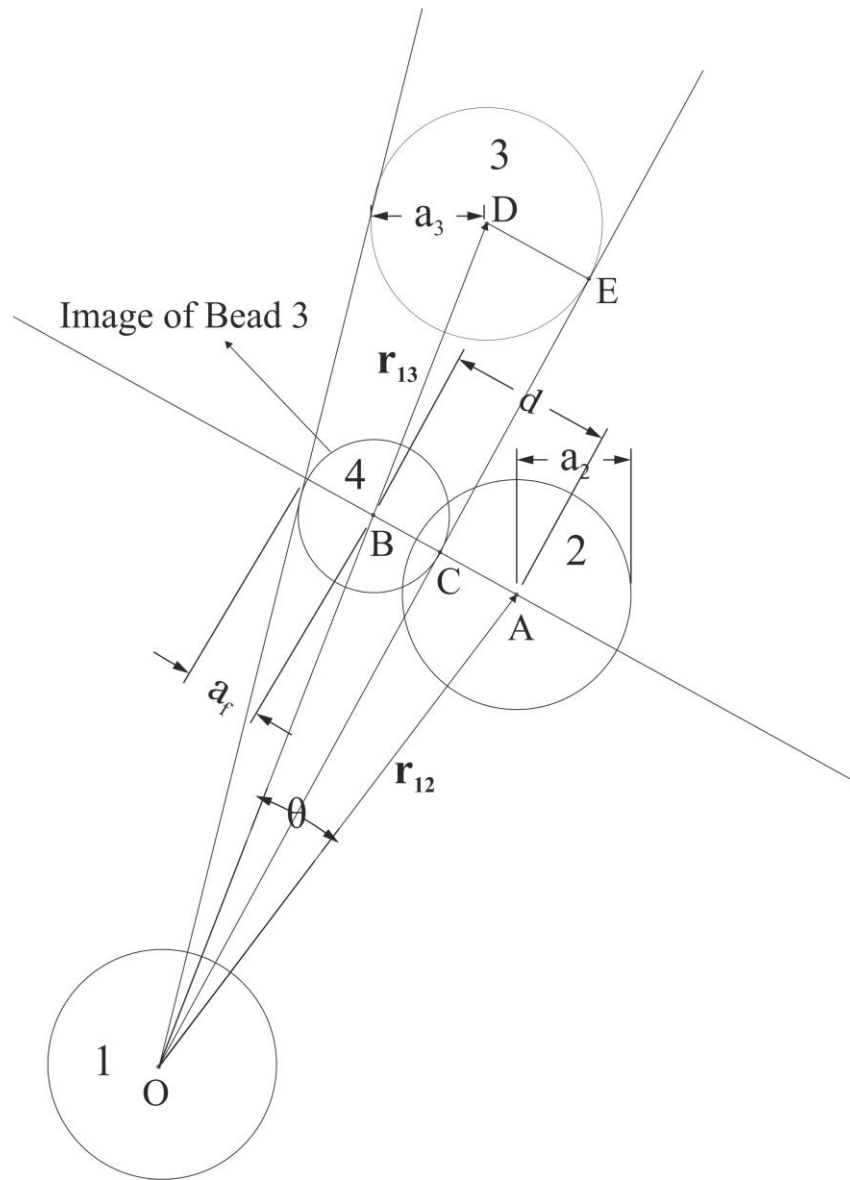


Figure 4.3 A schematic demonstrating how a blocked bead (bead 3) appears when viewed from bead 1. An image of the blocked bead (bead 3) is projected to the same plane as bead 2, creating bead 4.

Figure 4.3 shows three beads (1, 2, 3) suspended in fluid and separated by position vectors $\mathbf{r}_{12}$ and $\mathbf{r}_{13}$ respectively displaced in a configuration used to calculate the blocking parameters, where bead 4 represents a shadow of bead 3 projected to the same plane as bead 2. The line OCE is tangential to the surface of bead 3.

Triangle OAB forms an Isosceles triangle. Using the cosine rule, the distance, d , between the beads is obtained as

$$d = |r_{12}| \sqrt{2(1 - \cos \theta)} \quad (4.12)$$

Also, using similar triangles (OBC and ODE), we can write

$$\frac{a_f}{a_3} = \frac{|r_{12}|}{|r_{13}|} \quad (4.13)$$

When there is no blocking, $d > (a_f + a_2)$.

However, when the condition above is not satisfied, we know there is blocking. In order to calculate the blocking factor, the area of intersection [80] between the beads is first calculated using equation 4.14 below.

$$A_{int} = a_f^2 \cos^{-1} \left(\frac{d^2 + a_f^2 - a_2^2}{2da_f} \right) + a_2^2 \cos^{-1} \left(\frac{d^2 + a_2^2 - a_f^2}{2da_2} \right) - \frac{1}{2} \sqrt{(-d + a_f + a_2)(d + a_f - a_2)(d - a_f + a_2)(d + a_f + a_2)} \quad (4.14)$$

$$v_f = \frac{A_{int}}{A_{tot}} \quad (4.15a)$$

$$b = 1 - \frac{A_{int}}{A_{tot}} \quad (4.15b)$$

where A_{int} is the area of intersection, A_{tot} is the total area of bead 3 (in this example), v_f is the visibility factor and b is the blocking factor.

The visibility factor varies from 1 (fully blocked) to 0 (no blocking) while the reverse is true for the blocking factor.

When the visibility factor is one (blocking = 0), the long range hydrodynamic interaction becomes negligible (equation 4.16) as we assume that the reflected velocity fields can no longer reach the surface of the blocked bead and hereby influence its movement; however when the visibility factor is zero, the long range hydrodynamic interaction influences the movement of the bead through the reflected velocity fields.

In order to evaluate the hydrodynamic velocity during blocking, equation 4.11 is re-modified (from first principles) as

$$\begin{aligned} \mathbf{u}_1 = & \frac{\frac{(\mathbf{F}_h \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\eta k_a}(1 - b^2 k_3 k_4) + \left[b k_4 + \left(\frac{a_p}{2d_o} \right) (1 - b^2 k_3 k_4) \right] (\mathbf{u}_2 \cdot \hat{\mathbf{r}})\hat{\mathbf{r}}}{\left[1 + \left(\frac{a_p}{2d_o} \right) (1 - b^2 k_3 k_4) \right]} \\ & + \frac{(\mathbf{F}_h \cdot \hat{\mathbf{n}}_1)\hat{\mathbf{n}}_1}{\eta k_a}(1 - b^2 k_1 k_2) + b k_2 (\mathbf{u}_2 \cdot \hat{\mathbf{n}}_1)\hat{\mathbf{n}}_1 \\ & + \frac{(\mathbf{F}_h \cdot \hat{\mathbf{n}}_2)\hat{\mathbf{n}}_2}{\eta k_a}(1 - b^2 k_1 k_2) + b k_2 (\mathbf{u}_2 \cdot \hat{\mathbf{n}}_2)\hat{\mathbf{n}}_2 \quad (4.16) \end{aligned}$$

When the bead is not blocked ($v_f = 0$, $b = 1$), equation 4.16 remains the same as equation 4.11 for which the hydrodynamic interaction influences the bead movement. And when the bead becomes partially blocked ($v_f < 1$, $b < 0$), the bead's movement is partially influenced by the hydrodynamic interaction. However, when the bead become fully blocked ($v_f = 1$, $b = 0$), equation 4.16 reduces to the drag force and lubrication equation.

4.2.3 MODELLING AND SIMULATION

The numerical model was implemented in Matlab using the FTFD scheme given below (see chapter two), i.e.

$$\mathbf{r}(n + 1) = \mathbf{r}(n) + \mathbf{u}(n) dt \quad (4.17)$$

Three super-paramagnetic beads ($2a = 1\mu\text{m}$, $\chi = 1.4$ [31, 79], $\rho_p = 1.7\text{g/cm}^3$ [79]) are suspended in water ($\rho_v = 1\text{g/cm}^3$, $\eta = 10^{-3}\text{kgm/s}$) and placed within a uniform magnetic field ($\mathbf{B}_{\text{ext}} = 11\text{mT}$ along the x direction) at room temperature using a time step of 10^{-8} .

The presence of neighbouring beads within the magnetic and fluid environment leads to direct and indirect magnetic interactions which are evaluated at each bead; these forces are then converted into a velocity field using equation 4.16 (whilst checking for blocking). The positions of the beads are then updated using the obtained velocity fields [equation 4.17] at a given time step. (See Appendix A2 for more information).

4.3 FOURIER DIFFRACTION MODEL FOR HEIGHT DETERMINATION

4.3.1 REVIEW

Off-focused images contain information that can be extracted to predict the axial position of a particle. This is possible because the off-focused images depend on the axial distance [73, 74].

Various experimental studies based on submicron three dimensional particle tracking have used different characteristics of the diffracted images such as its central spot size [75, 87], intensity pattern [74], image shape and orientation [73], radius of the outer ring [76, 77], radius vector [78] etc. to predict the height of a particle with respect to the in-focus object plane.

Similarly, analytical methods based on theories like Lorentz-Mie, Huygens-Fresnel [74, 75, 87] and scalar diffraction etc. have been used in previous studies to determine the axial position of a particle.

Luo and co-authors [74] were able to track the three dimensional Brownian displacement and velocities of $0.5\mu\text{m}$ particles by comparing the diffracted image pattern obtained from using the scalar diffraction theory and their experiments. Results show that the determined axial positions had an accuracy of $0.2\text{-}0.3\mu\text{m}$.

On the other hand, Kao et al. [73] introduced a weak cylindrical lens in the optical path in order to create astigmatism and then used the shape and orientation of the particle to determine the axial position. At focus, particles appeared circular; when defocused, the particles appeared ellipsoidal in shape. A knowledge of the axial position helped in tracking the particles in three dimensions when suspended in solutions. A limitation associated with this method is that the astigmatism degrades the transverse (x,y) resolution of the microscope.

While Guerrero et al. [87] developed a model based on the Lorentz-Mie theory and Huygens-Fresnel propagation to develop a matrix array that measured the central spot size (diameter of the first diffraction ring) of various particles, where columns and rows represent the radius and defocus positions. An experiment was also designed to evaluate the central spot size (CSS) of particles through the application of an unknown defocus. The experimental data (CSS) is then searched for within an array of spot sizes and the defocus position corresponds to the value with minimum deviation from the obtained data. Their results suggested a relative error of 10%-32% associated the determination of the defocus position.

Speidel et al. [76] measured the position of a fluorescent nano-particle in three dimensions with sub nano-metre precision by fitting a Gaussian ring to the intensity of the obtained image and subsequent determination of the radius of the outer ring.

The outer bright ring of the off-focused images was also used by Wu and co-authors in their work [77] to track the three-dimensional trajectories of bacteria (1-3 μ m).

Zhang et al. [78] presented a three dimensional particle tracking algorithm with sub nano-meter resolution in their paper; they achieved tracking by converting the off-focused two dimensional image into a radius vector and subsequent comparison with a calibrated model to estimate the height of the particles.

In this study, we used the Full Width Half Maximum (FWHM) of the intensity distribution of the image as the parameter to predict the axial position of a 1 μ m magnetic bead because it is easy and less-ambiguous, especially when combined with visual observation.

Herein lies two approaches used to determine the height of the bead, an analytical model based on Huygens' Fresnel Diffraction technique and an experiment that records the distorted images obtained from an optical set up when the bead is moved in and out of focus.

4.3.2 THEORY ON LIGHT PROPAGATION

This section introduces Huygens' Fresnel Diffraction technique and the propagation of light through an imaging system. The Point Spread Function (PSF) of a lens is derived in terms of the 2D Fourier Transform. The Image of the object is then obtained by convolution and we discuss the change in the intensity pattern of the image as the object is moved out of focus.

4.3.2.1 FRESNEL DIFFRACTION

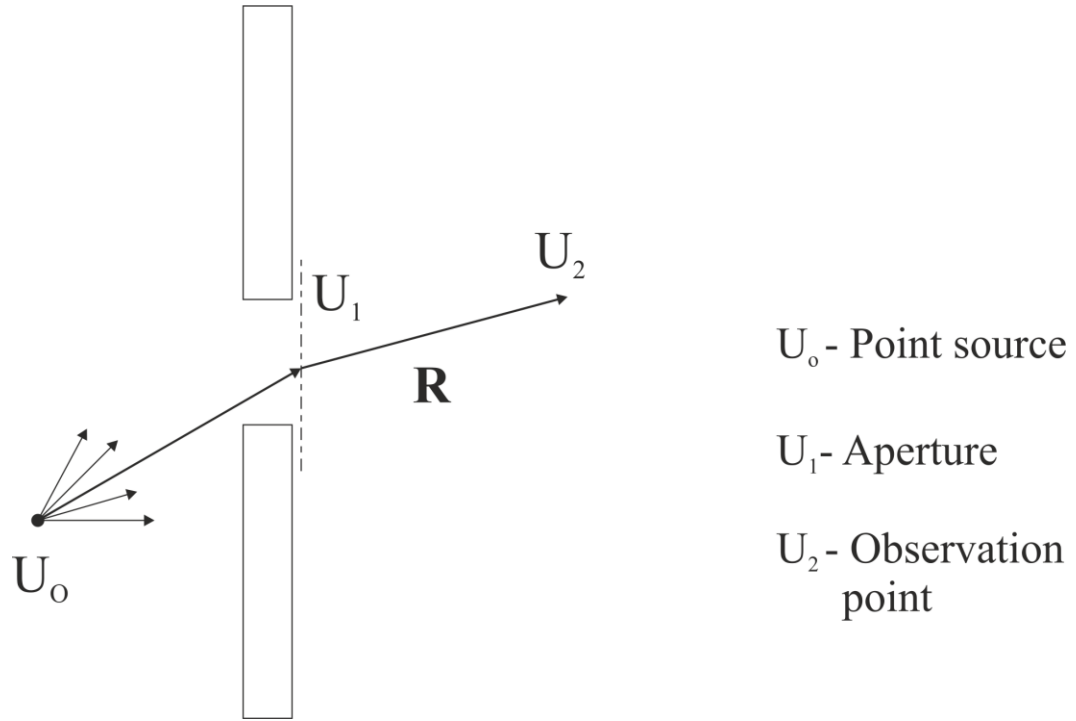


Figure 4.4: Propagation of light from a point source through an aperture.

In scalar diffraction theory, the Kirchhoff diffraction Integral [81, 82] can be used to describe the optical field propagated through an aperture given that the properties of the incident field are known.

Assume an aperture is illuminated by a single spherical wave arising from a point source, U_0 (see figure 4.4), then the propagated light at an observation point, U_2 can be expressed using the Kirchhoff diffraction Integral [81, 82] as

$$U_2(x_2, y_2) = \iint_{-\infty}^{\infty} \frac{1}{j\lambda R} U_1(x_1, y_1) e^{-jk_o R} dx_1 dy_1 \quad (4.18)$$

Assuming the paraxial approximation is valid, the Fresnel diffracted light [81, 82] propagated over a distance, z_1 (see figure 4.5.) can be written as

$$U_2(x_2, y_2) = \frac{e^{-jk_0 z_1}}{j\lambda z_1} e^{-\frac{jk_0(x_2^2 + y_2^2)}{2z_1}} F \left[U_1 e^{-\frac{jk_0(x_1^2 + y_1^2)}{2z_1}} \right] (k_x, k_y) \quad (4.19)$$

where the F (in equation 4.19) represents the 2D Fourier Transform. The spatial frequency components (k_x, k_y) serve as scaling conversion factors from the physical domain to the spatial domain.

4.3.2.2 **FRAUNHOFER DIFFRACTION**

If the observation distance becomes sufficiently large (far-field), then we can make another assumption [equation 4.19] that

$$z_1 \gg \frac{1}{2} k_0 (x_1^2 + y_1^2)_{max} \quad (4.20)$$

The quadratic phase factor then becomes approximately unity over the aperture and we can then rewrite equation 4.19 as

$$U_2(x_2, y_2) = \frac{e^{-jk_0 z_1}}{j\lambda z_1} e^{-\frac{jk_0(x_2^2 + y_2^2)}{2z_1}} F[U_1](k_x, k_y) \quad (4.21)$$

Hence, at such distances, it is referred to as Fraunhofer diffraction (far-field) which is simply the Fourier transform of the incident light [81, 82] as depicted above.

4.3.2.3 PROPAGATION THROUGH A THIN LENS

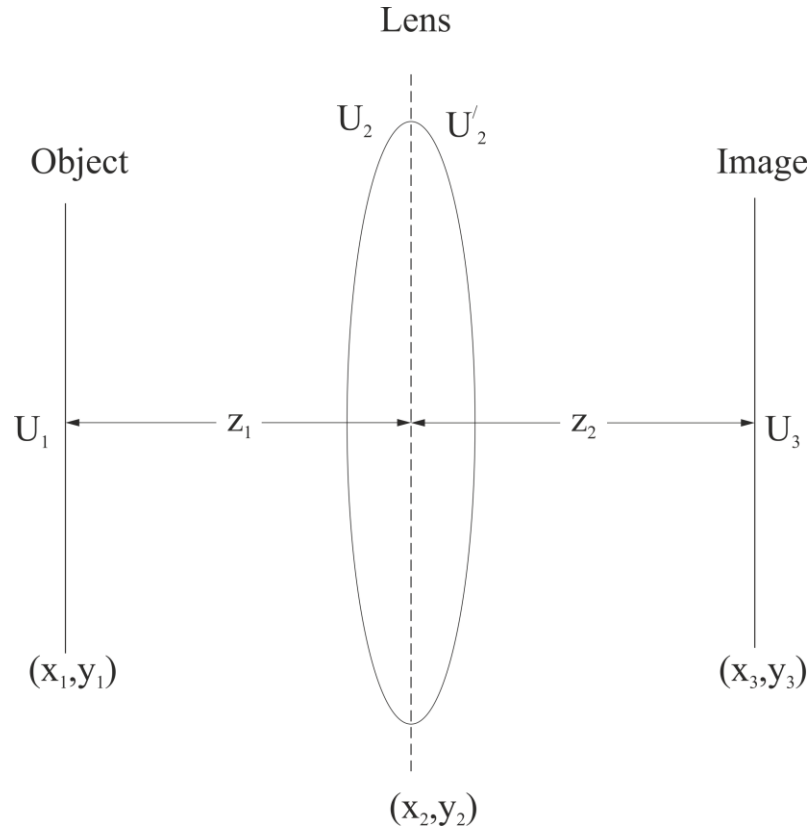


Figure 4.5: Propagation of light from the input plane through a lens to the output plane.

If the Fresnel diffracted light [equation 4.19] is passed through a thin lens with a pupil function $P(x_1, y_1)$ as depicted on Figure 4.5, the lens introduces a quadratic phase change. This quadratic phase change causes a plane wave to be directed towards or away from the focal point of the lens. It is worth remembering that a thin lens is an approximation for which light enters and exits at the same coordinates.

The pupil function of the lens can be used to account for its optical properties such as absorption, reflection etc. and is usually unit magnitude within the pupil and zero elsewhere [82].

The image after passing through the lens is given by [82]

$$U_2'(x_2, y_2) = U_2(x_2, y_2) P(x_2, y_2) e^{\frac{jk_o(x_2^2 + y_2^2)}{2f}} \quad (4.22)$$

The Fresnel diffracted image [82] propagated over a distance z_2 , after passing through the lens becomes

$$U_3(x_3, y_3) = \frac{e^{-jk_o z_2}}{j\lambda z_2} e^{\frac{-jk_o(x_3^2 + y_3^2)}{2z_2}} F \left[U_2' e^{\frac{-jk_o(x_2^2 + y_2^2)}{2z_2}} \right] (k_x, k_y) \quad (4.23)$$

4.3.2.4 **DEFOCUS**

If an object with transmittance U_1 , is illuminated with light at a distance z_1 , behind a lens, we can write the amplitude of the image at a distance z_2 , away from the lens using the approximations above (see figure 4.5) as

$$U_3(x_3, y_3) = \frac{1}{\lambda^2 z_1 z_2} \iint_{-\infty}^{\infty} U_1(x_1, y_1) dx_1 dy_1 \iint_{-\infty}^{\infty} P(x_2, y_2) e^{\frac{-jk_o(x_2^2 + y_2^2)}{2q}} e^{\frac{jk_o\{(x_3 - x_1)x_2 + (y_3 - y_1)y_2\}}{z_1}} dx_2 dy_2 \quad (4.24)$$

where

$$\frac{1}{q} = \frac{1}{z_1} + \frac{1}{z_2} - \frac{1}{f} \quad (4.25)$$

Using the 2D Fourier Transform, equation 4.24 can be rewritten as

$$U_3(x_3, y_3) = \frac{1}{\lambda^2 z_1 z_2} \iint_{-\infty}^{\infty} U_1(x_1, y_1) F \left\{ P(x_2, y_2) e^{\frac{-jk_o(x_2^2 + y_2^2)}{2q}} \right\} \left(\frac{((x_3 - x_1), (y_3 - y_1))}{\lambda z_1} \right) dx_1 dy_1 \quad (4.26)$$

This is a convolution of the object transmittance represented by U_1 with the PSF of the lens. The PSF is the impulse response of the imaging system.

If we consider a point object at focus, the image gives the PSF of the lens as presented below.

$$U_4(x_3, y_3) = F \{ P(x_2, y_2) \} \left(\frac{((x_3 - x_1), (y_3 - y_1))}{\lambda z_1} \right) \quad (4.27)$$

But if we consider a defocused point object, where z_1 becomes the sum of $2f$ and the defocus distance, we see that the phase term becomes dominant and a change in the defocus distance leads to a variation of this phase term. The image is then represented as the 2D Fourier Transform of both the PSF and a phasor term given below.

$$U_5(x_3, y_3) = F \left\{ P(x_2, y_2) e^{\frac{-jk_o(x_2^2 + y_2^2)}{2q}} \right\} \left(\frac{((x_3 - x_1), (y_3 - y_1))}{\lambda z_1} \right) \quad (4.28)$$

4.3.2.5 **ABBE THEORY OF IMAGING**

The image of a point source object when viewed under a microscope produces a central spot surrounded by diffraction rings. The central spot, formed as a result of passing an electromagnetic wave through an aperture, is called the Airy Disk [85]. The Airy Disk is simply the PSF of a circular lens which can be obtained from equation 4.27.

When the object appears out of focus, the diameters of these rings become enlarged leading to the formation of a complex intensity pattern [equation 4.28].

The Numerical Aperture, NA of a lens describes the collection angles of light that can be captured by the objective lens [85].

$$NA = n \sin \theta \quad (4.29)$$

where n is the refractive index of the medium between lens and specimen and θ is the half angle of cone of the light accepted by the lens.

The NA can further be expressed as

$$NA = \frac{D}{2f} \quad (4.30)$$

where D is the diameter and f is the focal length of the lens.

An image is formed by the collection of different Airy Disk patterns. Each point source of the object can be considered as a source of overlapping Airy Disks. When two disks are very close, then it is possible that their central spots will overlap, it then becomes difficult to distinguish between two objects. The ability of the microscope to either resolve or distinguish between two objects is called the resolving power, which is dependent on the NA. The resolution of an imaging

system can be conveniently defined as the minimum distance between two point sources at which they are distinctly observable.

The Rayleigh criterion [85] is used to determine this resolving power and occurs when the first minimum of the Airy pattern of one object overlaps with the maximum of the Airy pattern of the second object, given by

$$d_r = \frac{0.61\lambda}{NA} \quad (4.31)$$

where d_r is the minimum resolved distance in m, λ is the wavelength in m. This resolution can also be termed to be the distance from the centre of the Airy disk to the first dark ring [86].

When a sample is observed under an optical microscope, light is first scattered by the sample, the scattered light is collected in the objective and then focussed in the image plane.

Abbe theory states that the image is formed as a result of interference between the non-deviated light and deviated light at the image plane; the higher the order of interference patterns captured by the system, the more resolved the object appears [85].

4.3.2.6 **OPTICAL MODEL**

An optical model was implemented in Matlab which can be used to approximately predict the intensity distribution of different sized beads either in focus or out of focus. The optical model can be used to approximately predict the relative height of a $1\mu\text{m}$ super-paramagnetic bead using the intensity distribution of the images with respect to the plane of focus.

The pupil function of a 3mm diameter circular lens with NA of 0.58 was represented by a unit cylindrical function. Care was taken to ensure that the array size was sufficiently large for the purpose of the Fast Fourier Transform (FFT) window size chosen and to avoid spectral leakage. A phase function was generated using equation 4.28 (or 4.26) to account for the variation in phase due to defocus. These expressions were Fourier transformed to give the PSF while the k space vectors served to convert from the physical domain to the spatial domain.

It is helpful to compare the simulation with the equation predicted by the Rayleigh criterion. The minimum resolved distance using light of wavelength $0.5\mu\text{m}$, according to Rayleigh Criterion (equation 4.31) gave a value of $0.53\mu\text{m}$ while the minimum resolved distance predicted by the simulation was obtained as $0.51\mu\text{m}$. The discrepancy in values could be attributed to the approximations embedded in both the simulation and the Rayleigh criterion.

The internal composition of the magnetic bead has been described in section 3.2.1.2. The diffraction model assumes that the bead is flat-circular and has a uniform transmittance from the core to the edge of the shell.

The intensity distribution of the image was obtained by first mapping the object's transmittance to the image plane (using the k space vectors) and then convolving with the intensity distribution of the PSF of the lens using light of wavelength $0.5\mu\text{m}$.

The intensity distribution of the image was normalised based on Parseval's theorem of conservation of energy within the system. The obtained results are discussed in section 4.3.4.

4.3.3 EXPERIMENT FOR HEIGHT DETERMINATION

A solution of super-paramagnetic bead (obtained from Thermo Fisher Scientific) was prepared at a concentration of 9×10^3 beads/ml. 6 μ l of this solution was placed on a microscope glass slide (iMED) using an appropriate pipette.

This was then viewed under an inverted microscope (Leitz Wetzlar, Germany) with a long working 20 $\times$ objective lens (Comar Optics, Cambridge, UK) for magnification of the images. The images were then recorded using a USB digital camera (UEye UI – 2230SE, IDS, Germany).

The stage vernier scale was used to determine the defocus position in μ m. The microscope sample stage was moved vertically until a single bead appeared in focus, translated up and down for the various defocus distances. The focussed and defocused images were then recorded. The recorded images were post-processed using image J tracking software.

In order to improve the quality of recorded images, the images were exported into Matlab and the following smoothing procedure was undertaken, following the steps of Luo et al in their paper [74]. It is noticed that the small beads are imaged over a small number of camera pixels. The centroid was determined by manual observation. A line was projected from the centroid across the image; the line was divided into a number of points. Each line point was used to produce three hundred and sixty line segments with same length, radiating from the centroid. The intensity distribution for each line point was then obtained by averaging the intensities over the 360 lines. The images are displayed in figure 4.6 below.

The intensity distribution of the images at focus and various defocus distances were obtained and recorded for comparison with the simulation results (see figure 4.7). These will be discussed in the next section.

4.3.4 COMPARISON OF SIMULATION RESULTS WITH EXPERIMENTAL DATA

This sub section compares the Intensity distribution of the images obtained from the optical model developed in Matlab with the diffracted images viewed under the optical microscope. The data presented here depict the axial distribution for conditions in which the bead appears above focus only (for simplification purposes). The intensity distributions have been normalized by the intensity value of the bead at focus and brightness of the image background as presented in figures 4.7 and 4.8 below.

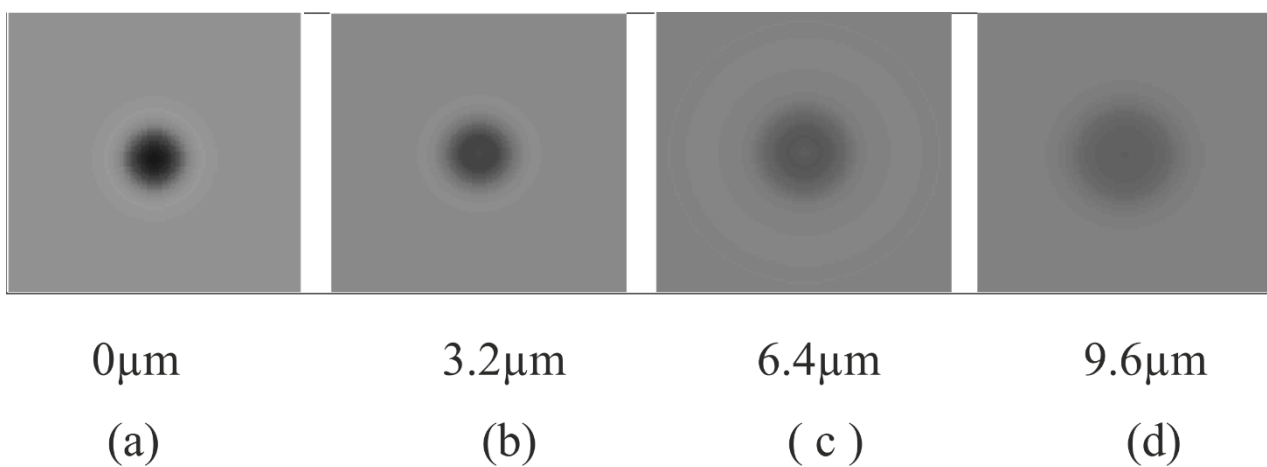


Figure 4.6 Post-processed images of 1µm super-paramagnetic beads (myone Dynal Beads Streptavidin T1 [26] obtained from Thermo Fisher Scientific) viewed under the optical microscope at different axial positions corresponding to a) in focus b) 3.2µm defocus c) 6.4µm defocus and d) 9.6µm defocus distance respectively.

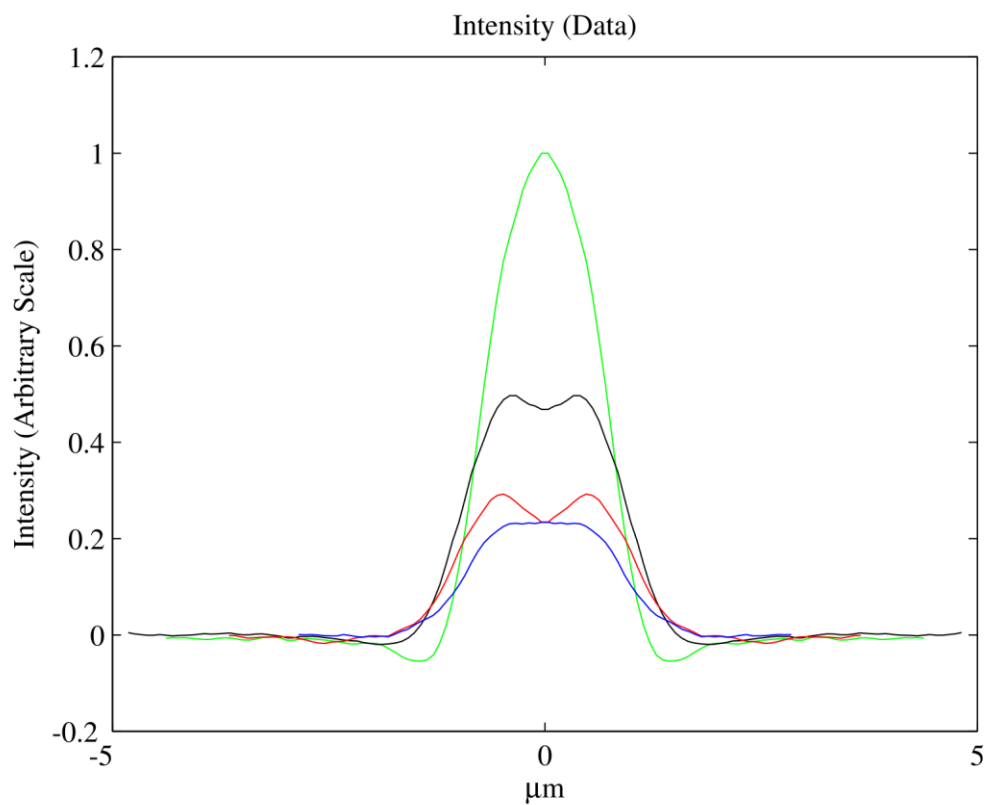


Figure 4.7 Normalized Intensity distribution of a 1 μm super-paramagnetic bead (myone Dynal Beads Streptavidin T1 [26] obtained from Thermo Fisher Scientific) viewed under the optical microscope at focus (in green) and various defocus distances (3.2 μm in black, 6.4 μm in red and 9.6 μm in blue).

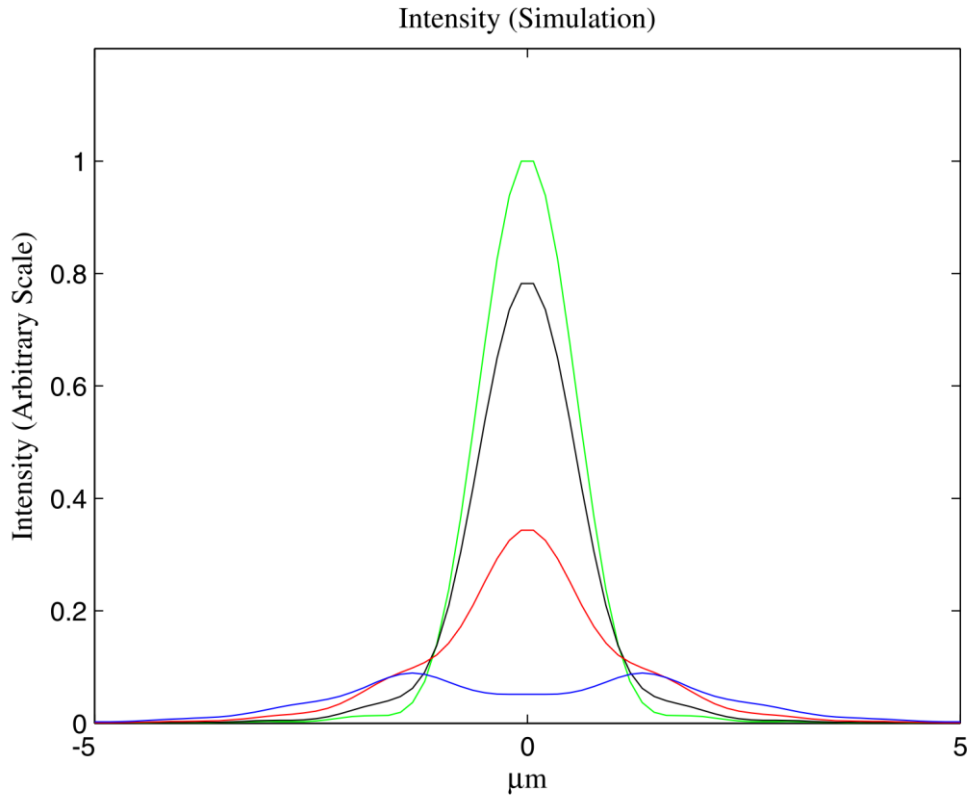


Figure 4.8 Normalized Intensity distribution of a 1 μm super-paramagnetic bead obtained from the optical model in Matlab at focus (in green) and various defocus distances (3.2 μm in black, 6.4 μm in red and 9.6 μm in blue).

Figure 4.6 shows the different image patterns as the bead is moved from focus towards higher defocus distances. At further defocus, the intensity of the bright rings reduces and becomes more spread out (blurred).

Figures 4.7 and 4.8 compares the normalized intensity distribution of a 1 μm super-paramagnetic bead when viewed at focus and for various defocus distances from both the recorded optical images and the optical model in Matlab.

Figure 4.7 shows the different features in the intensity distribution of the bead as it is moved from focus towards higher defocus distances (approximately Gaussian in shape, showing dips and a broad distribution). Figure 4.8 show some features of figure 4.7 in terms of the relative height of the intensity distribution of the bead with respect to its intensity distribution at focus. Hence, the comparison is reasonable and shows that the simple technique used in the optical model to predict the height distribution of the 1 μ m magnetic bead is promising.

However, there are differences in the intensity distributions of the bead presented in both figures 4.7 and 4.8; these differences could be attributed to the fact the optical model designed in Matlab is a simple one that differs from the optical setup used to obtain experimental data. The differences also show that further complexity is required in the model.

The simple optical model can be further improved in the future by taking into consideration the following points.

- i. By considering the angular divergence of the light which was apparent during use but has not been measured. This could be included in the model as an angular spectrum originating from the object (as an additional phase term) and would increase the image spreading through the focus.
- ii. The effect of scattering and light absorption at the imperfect spherical silica surface. This model assumes that the bead has a uniform transmittance from the core to the shell which neglects the deviation of light due to the internal structure of the beads.
- iii. The complex nature of the intensity distribution of the bead at higher defocus distance which cannot be accurately predicted by a simplified lens arrangement.
- iv. The design of the optical model using optical software like Zemax [103].

If some of these issues are addressed in the future, then the model will be able to predict a more accurate intensity distribution of the bead for the various defocus distances which can be used to predict the axial distribution of the bead.

Hence, for the purpose of this work, the diffracted images of the bead viewed under the optical microscope (figure 4.7) offers an easier method to predict the height of the 1 μ m bead accurately. Hence, the Full Width Half Maximum (FWHM) will be used as the parameter to predict the axial distribution of a magnetic bead when recording the video (see section 4.4.3.2 for a detailed approach). The FWHM will be cross-referenced to figure 4.7 to determine the three-dimensional position of the 1 μ m magnetic bead within the magnetophoretic system.

4.4 EXPERIMENT INVESTIGATING INTERACTIONS AMONG MANY BEADS WITHIN A MAGNETOPHORETIC SYSTEM

This section describes the experiment pre-processing and post-processing steps as well as procedures taken during the experiment.

4.4.1 PREPARATION OF SOLUTION OF ONE-MICRON MY-ONE BEADS

Super-paramagnetic beads (Dyna Beads myone Streptavidin T1 [26]), 1 μ m sized, were obtained from Thermo Fisher Scientific, with an initial density of 10mg/ml and a concentration of $7 \cdot 10^9$ beads/ml. This was then diluted to a concentration of $7 \cdot 10^6$ beads/ml for the experiment.

These beads have a magnetic susceptibility of 1.4 [31] and a density of 1.7g/cm^3 . The sedimentation time for this bead when suspended in water through the channel is 4.4 minutes (section 2.6.3).

4.4.2 MAGNETIC CONFIGURATION

A uniform magnetic field was achieved by placing two rectangular magnets in an attractive position; this magnetic arrangement has been described in detail in section 3.2.2.

The applied flux density of the uniform magnetic field was measured using a Hall Effect Gauss/Tesla Meter (5100 Series Sypris Test & Measurement F.W.Bell 12/04) and gave a value of $11\pm 0.5\text{mT}$. This is consistent with field values obtained by modelling the distribution in Ansys Maxwell.

4.4.3 MANY-BEADS INTERACTION EXPERIMENT

4.4.3.1 *DESCRIPTION OF MANY-BEADS INTERACTION EXPERIMENT*

The set up for the experiment has been described in section 3.2.3.

A solution of super-paramagnetic beads (Dynal Beads myone [26]) was freshly prepared at a concentration of $7\text{-}10 \times 10^6$ beads/ml. The particles were fully re-suspended and aggregates were separated by placing the solution in an ultrasonic bath for 2 minutes.

A volume of $6\mu\text{l}$ was transferred using an appropriate pipette to the hydrostatic fluid observation channel (see section 3.2.1.1). This was then placed within the uniform magnetic field distribution by avoiding the edges of the magnets.

The motion and interaction among the beads were recorded using the experiment setup described in section 3.2.2 following the procedures outlined in section 3.4.1.

The camera image was moved until a close interaction among just three magnetic beads could be observed such that interaction with other beads can be neglected and subsequently recorded. The trajectories of the beads were tracked frame by frame using the Image J software [72].

4.4.3.2 *COMPARISON BETWEEN EXPERIMENT AND SIMULATION*

The video data obtained from the experiment gives the beads' trajectories in two dimensions only. In order to obtain the approximate height of the beads, the first step is to determine the bead(s) at focus which would then serve as a reference ($z = 0$). The focused bead(s) is determined by visual observation and comparing the FWHM of the intensity distribution with figure 4.7.

The next step estimates the height of the defocused bead(s) relative to the bead(s) at focus. This is achieved by comparing the FWHM of the intensity distribution of the defocused bead(s) for each frame with figure 4.7 manually.

This relative height information is added to the x-y displacement values (from the video data) to predict the beads' trajectories in three dimensions.

In order to compare the three dimensional experimental data with the simulation, three magnetic beads with properties similar to those used in the experiment were displaced at distances corresponding to the starting points of the beads. The interaction of these beads as well as their trajectory patterns were then obtained from the simulation and compared to the three dimensional data.

4.5 RESULTS AND DISCUSSION

This section presents and discusses five trajectory examples which shows the interaction among three super-paramagnetic beads due to both magnetic and hydrodynamic forces when suspended in fluid within a uniform magnetic field. It compares the simulated trajectories with the beads' movement captured on video.

These examples illustrate an interaction among three beads in a line along the direction of the magnetic field and for which two of the beads are separated from the third bead by an approximately similar distance (figure 4.9), a strong interaction between two closer magnetic beads and a weak interaction by the bead further apart (figure 4.10), three beads undergoing Brownian displacement until the interaction between a pair of beads become stronger (figure 4.11), a three dimensional trajectory of three magnetic beads which allows for an accurate interpretation of the beads displacement within the magnetic field (figure 4.12) and magnetic beads being attracted and repelled within the magnetic field (figure 4.13).

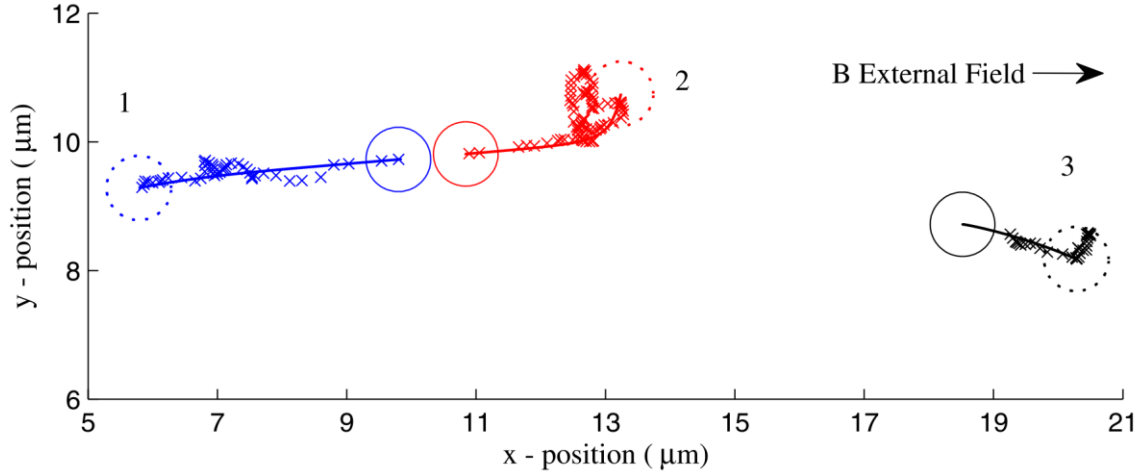


Figure 4.9: Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. The starting points of the beads are indicated by the dotted circles while the end points are shown by the continuous circles. The direction of the magnetic field is indicated by the black arrow on the plot. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model.

Figure 4.9 shows three super-paramagnetic beads observed initially to be at $(5.9, 9.3, 0)$, $(13.2, 10.7, 0)$ and $(20.3, 8.2, 0)\mu\text{m}$ which gives a separation distance of $7.4\mu\text{m}$ between the blue and the red magnetic beads and $7.5\mu\text{m}$ between the red and black magnetic beads.

The three magnetic beads are approximately aligned in attractive positions in the direction of the magnetic field, as shown on Figure 4.9. At the start of the simulation, the red bead interacts with both the blue and black beads, as evident from the curvature of the starting trajectory of the red bead.

The interaction force between the red and blue bead becomes stronger due to the closer separation distance between these beads; they approach themselves to form an agglomerate. On the other hand, the force of interaction between the red and black beads becomes weaker as they move apart.

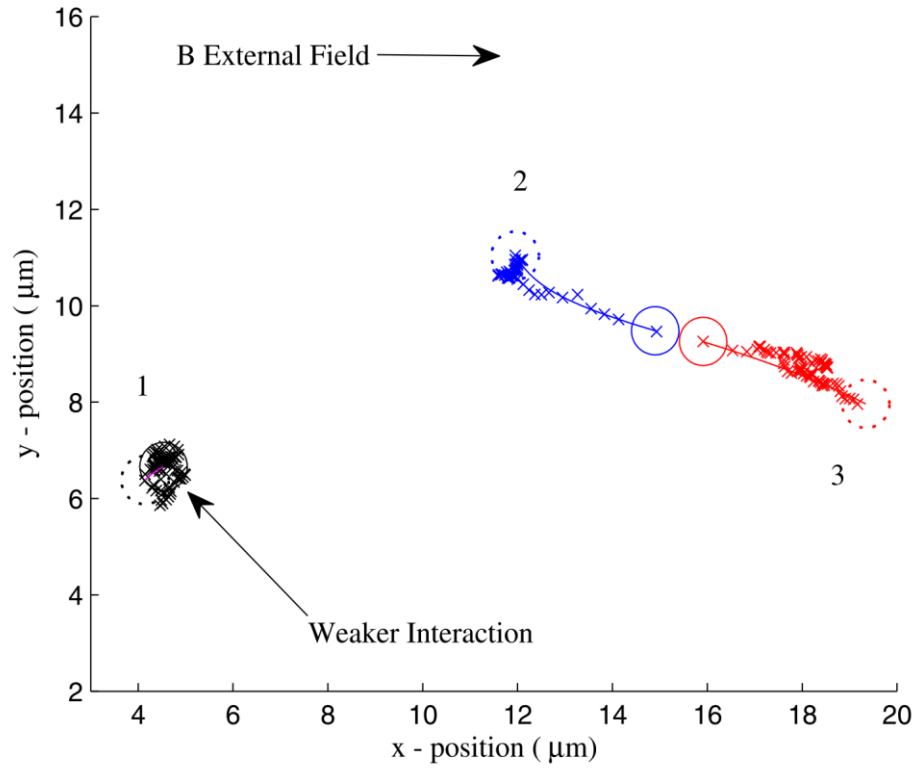


Figure 4.10: Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. The starting points of the beads are indicated by the dotted circles while the end points are shown by the continuous circles. The direction of the magnetic field is indicated by the black arrow on the plot. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model.

Figure 4.10 depicts a strong interaction between two magnetic beads which are closely separated ($7.6\mu\text{m}$) while the third bead is at a further distance ($9.1\mu\text{m}$ from the blue bead).

This leads to a strong interaction force between the red and blue magnetic beads; they come together approximately in alignment with the magnetic field to form an agglomerate while the interaction force on the black bead becomes weaker.

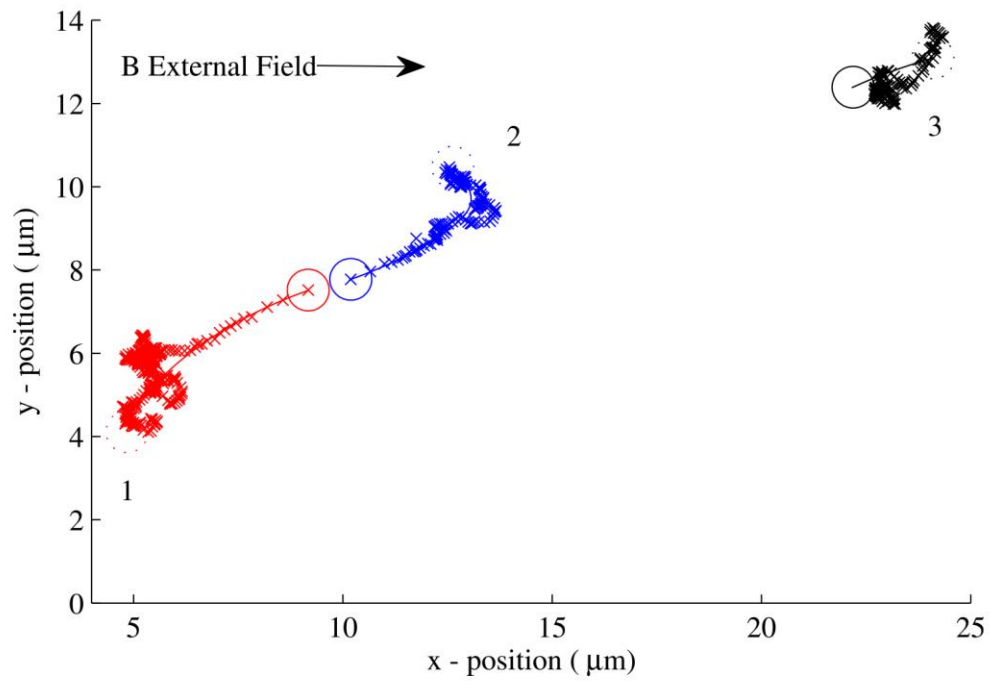
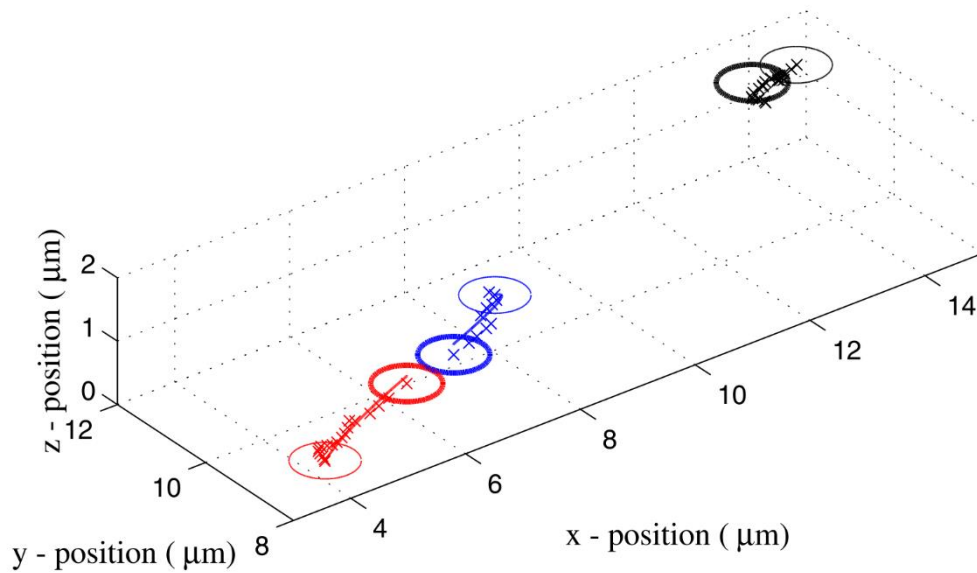


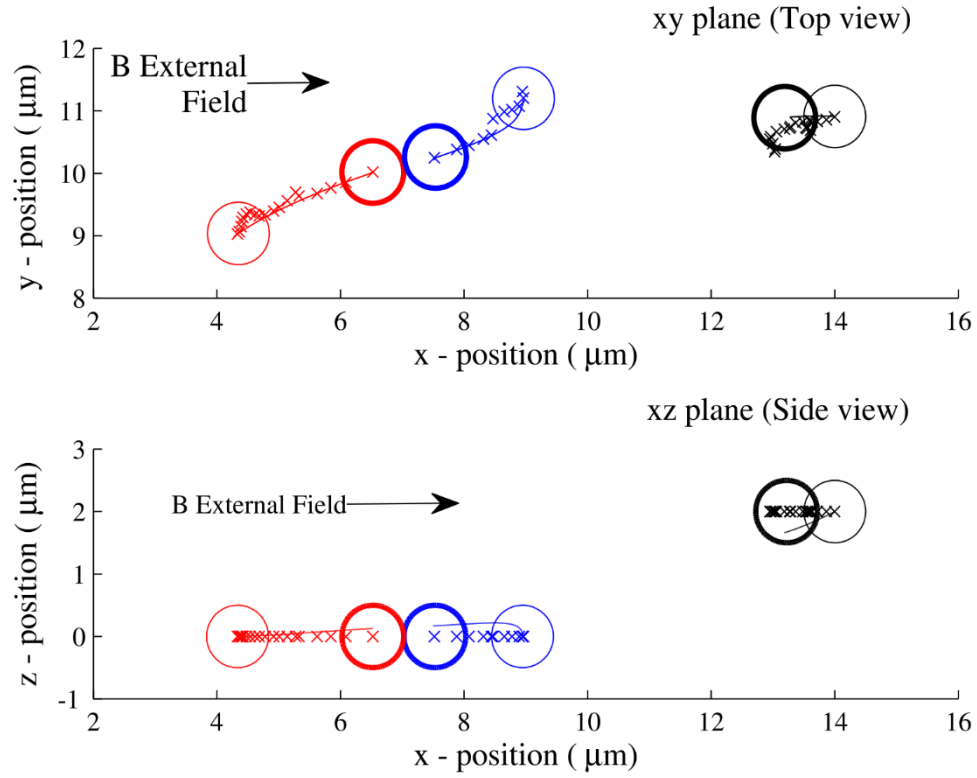
Figure 4.11: Two-dimensional trajectory plot of three super-paramagnetic beads, 1μm sized, suspended in water (indicated by red, blue and black) under the same conditions as figure 4.9.

The red bead is observed to be Brownian displaced and gives the black bead the opportunity to interact closely with the blue bead at a large separation distance of $11.7\mu\text{m}$. This is depicted on the plot (see figure 4.11) by the curved trajectory of the blue bead at the start of the simulation.

However, the force interaction between the red and blue beads becomes stronger as they approach each other; this reduces the effect of Brownian displacement on the red bead. Conversely, a weaker interaction force is experienced by the black bead.



(a)

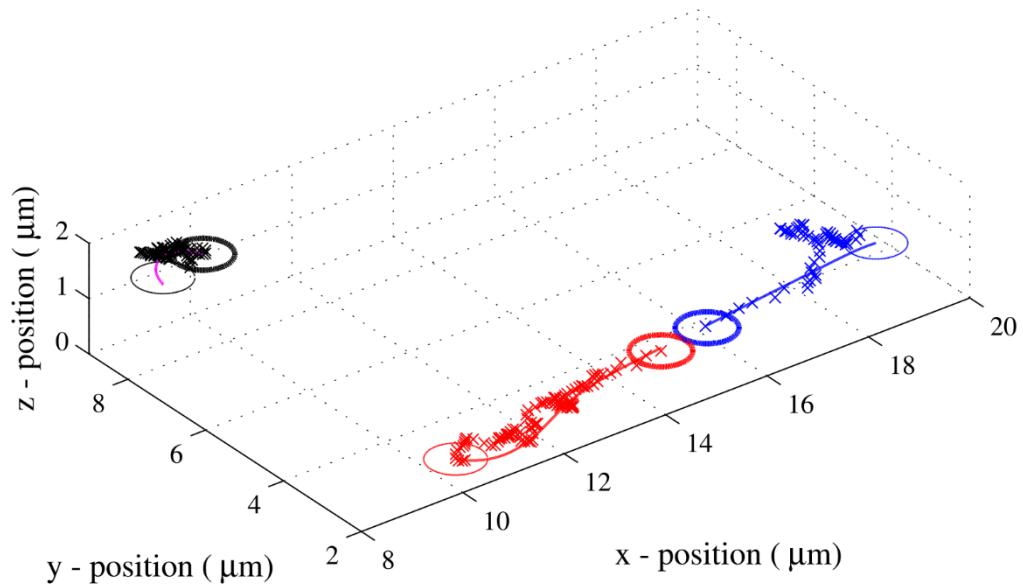


(b)

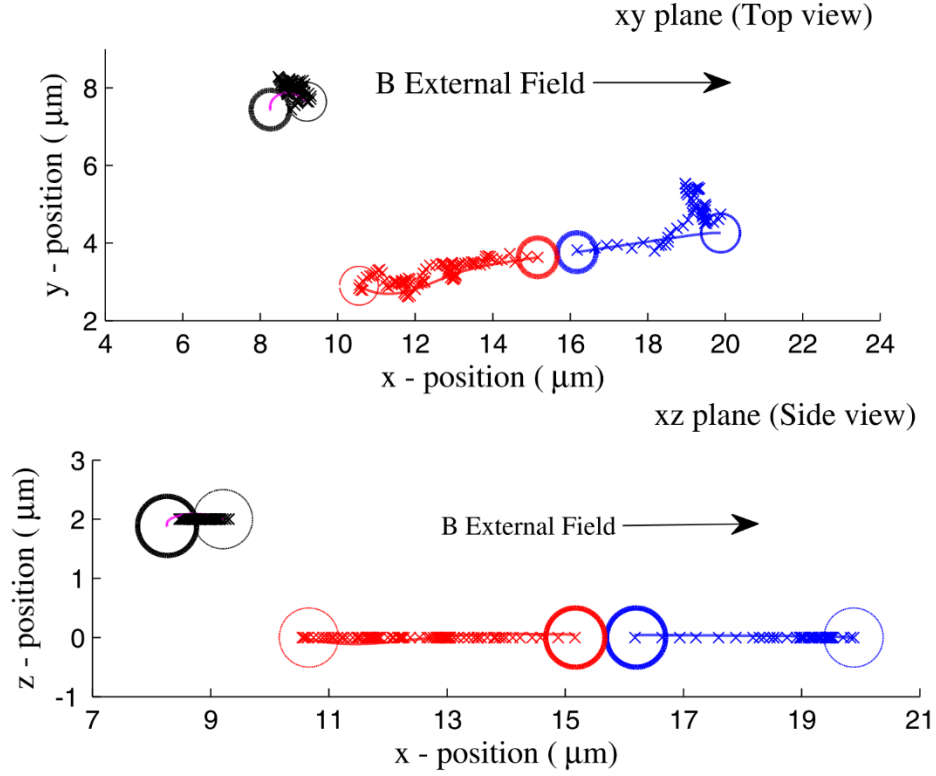
Figure 4.12 (a) Three-dimensional (with height) and (b) two dimensional projected trajectory plot (along x-y plane and x-z plane, for illustrative purposes) of three superparamagnetic beads, under the same conditions as figure 4.9. The starting points of the beads are indicated by the thin continuous circles while the end points are shown by the thick continuous circles. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model.

Figure 4.12b (i) depicts a two dimensional trajectory plot of three magnetic beads on the x-y plane; this figure gives the illusion that the blue bead is closer to the black bead than the red bead; this would have suggested a stronger interaction between the blue and black beads.

However, figure 4.12a and 4.12b (ii) shows a three dimensional trajectory plot and the height displacement of the three beads and we see that the blue bead is actually closer to the red bead and further away from the black bead. This leads to a stronger interaction between the red and blue beads and a weaker interaction between the blue and black beads.



(a)



(b)

Figure 4.13 (a) Three-dimensional (with height) and (b) two dimensional projected trajectory plot (along x-y plane and x-z plane, for illustrative purposes) of three superparamagnetic beads, under the same conditions as figure 4.9. The starting points of the beads are indicated by the thin continuous circles while the end points are shown by the thick continuous circles. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model.

The red and the black beads are separated by a lower distance of $5.7\mu\text{m}$ but are repelled along the direction of their magnetic field (see figure 4.13). The black bead moves away from the red bead in order to align itself to be in an attractive position with the red bead. However, the red bead is

attracted by the blue bead even though they are separated by a higher distance of $9.44\mu\text{m}$ due to their attractive position in the direction of the magnetic field. They come together to form an agglomerate.

4.6 EXPERIMENT INVESTIGATING SEDIMENTATION

This section investigates the sedimentation rate of the $1\mu\text{m}$ Myone beads (from Life-Technologies) using theoretical predictions as well as experimental observations.

Using equations 2.45-2.48, the sedimentation time of the $1\mu\text{m}$ Myone beads at various depth was evaluated and is presented in table 4.1 below.

A description of the sedimentation experiment is hereby given. $10\mu\text{l}$ of solution of $1\mu\text{m}$ Myone beads suspended in water (at a concentration of $7\cdot 10^6$) was placed in the hydrostatic fluid channel. The sedimentation of the beads through the fluid channel (with depth $100\mu\text{m}$) was observed under the optical microscope and subsequently recorded. This was repeated a number of times.

In order to evaluate the relative height of the beads in the video, with respect to an arbitrary focal plane, the intensity distribution of the beads was compared to intensity profiles taken over a range of microscope de-focus positions (z-height) for a single stationary bead; similar to the profiles in figure 4.7.

By best-correlation of the intensity profiles, the sedimenting rate of the $1\mu\text{m}$ Myone beads is compared with the model predictions of downward drift of the beads; the results are presented in table 4.1 below.

Table 4.1: Sedimentation time for the 1 μ m Myone beads (from Life-Technologies) when suspended in water at various depths.

Depth (μ m)	Experiment Sedimentation Time (s)	Calculation Sedimentation Time (s)
2	10 $\pm$ 2	12
4	22 $\pm$ 3	24
6	36 $\pm$ 4	35.9

There is a good agreement between the estimated sedimentation time and model predictions for beads sinking to depths of 2, 4 and 6 microns. Table 4.1 predicts that the 1 μ m myone beads (from life-technologies) sediment at the rate of 0.2 $\pm$ 0.03 μ m/s when suspended in water.

Result from chapter three suggest that magnetic interaction becomes dominant when the 1 μ m sized beads are separated by a distance of 6 μ m.

Experimental observations show that the agglomeration-interaction among three beads within a magnetophoretic system typically takes an average time of 5-6s. During such interaction, the beads sediment to a depth of just 1-1.2 μ m.

This sedimentation depth cannot be readily observed simultaneously with a situation of two or three beads agglomerating, and estimates of z-displacement would not be sufficiently reliable using the current measurement technique where de-focused images are recorded.

Furthermore, all three beads sediment at the same rate. This means that at any point in time, the beads displacement would be equivalent to their relative displacement.

4.7 EXTENSION OF THE THREE DIMENSIONAL MODEL PREDICTING INTERACTION AMONG THREE SUPER-PARAMAGNETIC BEADS

This section describes an extension to the three-dimensional interaction model among three super-paramagnetic beads ($1\mu\text{m}$ sized) suspended in water within a magnetophoretic system. During experiment, the bead pair cluster is observed to interact with the third bead to form a three bead agglomerate. Experiments have been described in section 4.4.3.1. The movements of the beads during interaction are then compared with the simulated trajectories.

This interaction is modelled using the three dimensional analytical method described in this work (which consists of magnetic interaction, long range hydrodynamic interaction and short range interaction), see section 4.2. The bead pair cluster is represented as a bead with twice the original physical dimension (i.e. $2\mu\text{m}$ diameter) and the same magnetic susceptibility as the $1\mu\text{m}$ sized bead (i.e. 1.33).

4.7.1 DISCUSSION OF RESULTS

This section discusses two further trajectory results showing interaction among three super-paramagnetic beads ($1\mu\text{m}$ sized) within a magnetic separation system.

In the first instance, two of the beads come together to form a bead-pair agglomerate; during the next phase of interaction, the bead-pair agglomerate interacts with the third bead to form a three-bead agglomerate.

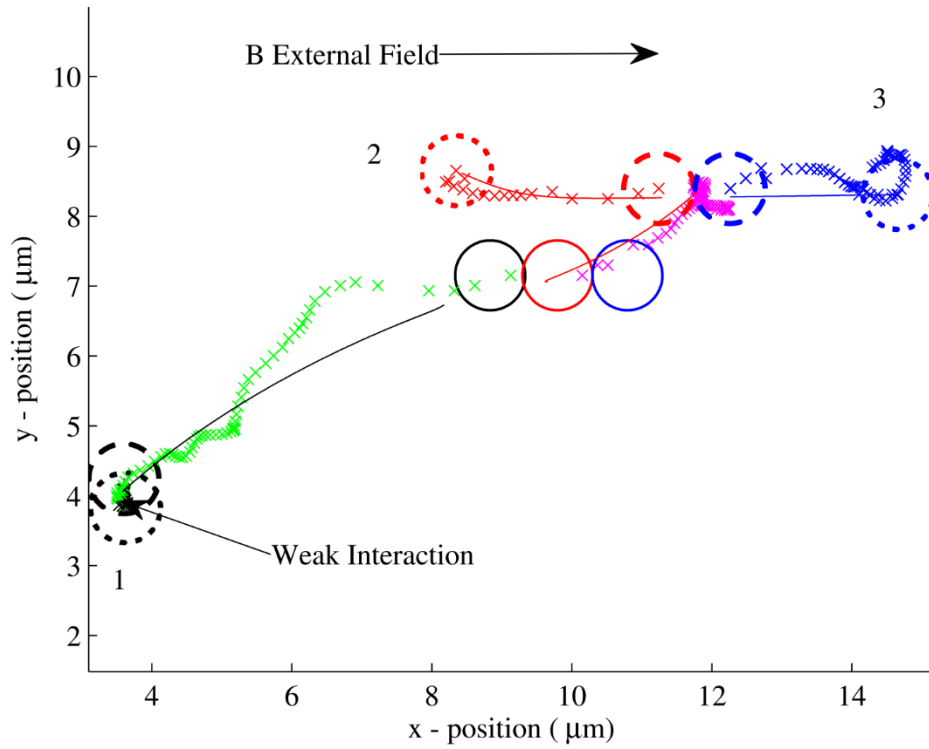


Figure 4.14: Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model. The starting points of the beads are indicated by the dotted circles while the dash circles represent the point at which a bead pair cluster is formed and the end points are represented by continuous circles. The first set of experimental trajectories is represented by red, blue and black crosses while the second set is represented by magenta and green crosses. The first set of simulated trajectory is represented by red, blue and magenta lines while the second set is represented by black and red lines. The direction of the magnetic field is indicated by the black arrow on the plot.

Figure 4.14 shows that the red and blue beads were initially separated by a distance of $6.4\mu\text{m}$ in a position approximately aligned to the direction of the magnetic field while the black bead is a distance of $6.8\mu\text{m}$ to the left of the red bead. The red and blue beads interact to form a bead pair agglomerate due to their closer separation, representing a lower energy configuration. During this phase of the interaction, there is a much weaker interaction experienced by the black bead due to the larger separation, highlighted by an arrow in the figure.

Subsequently, the bead pair agglomerate and third bead interact and come together over a separation distance of $6.8\mu\text{m}$, such that all three beads align in the direction of the magnetic field.

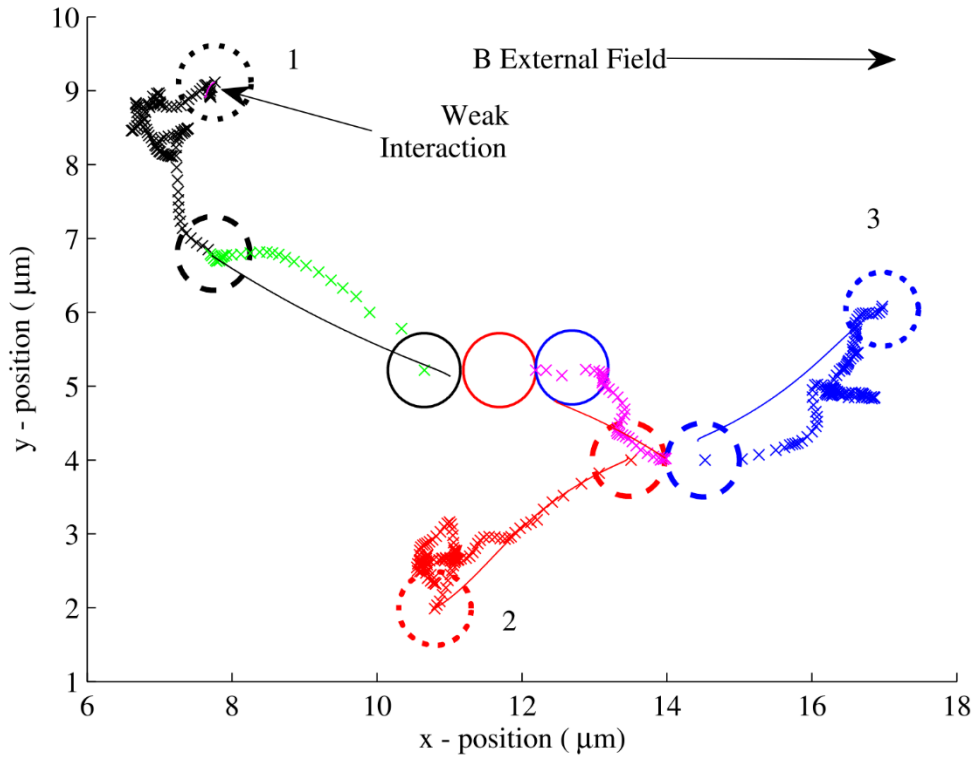


Figure 4.15: Two-dimensional trajectory plot of three super-paramagnetic beads, $1\mu\text{m}$ sized suspended in water (indicated by red, blue and black) under the influence of both magnetic and hydrodynamic interactions within a uniform magnetic field of 11mT in the x direction. Experimental data (crosses) are compared with simulation results (continuous line) to validate the model. The starting points of the beads are indicated by the dotted circles while the dash circles represent the point at which a bead pair cluster is formed and the end points are represented by continuous circles. The first set of experimental trajectory is represented by red, blue and black crosses while the second set is represented by magenta and green crosses. The first set of simulated trajectory is represented by red, blue and magenta lines while the second set is represented by black and red lines. The direction of the magnetic field is indicated by the black arrow on the plot.

Referring to Figure 4.15, initially the red bead interacts weakly with both beads as evidenced by the random movement at the start of the trajectory. However, the blue and red beads, at a distance of $7.72\mu\text{m}$ experience a stronger interaction and eventually come together in the direction of the magnetic field to form a bead pair agglomerate aligned with the magnetic field.

There is a deviation between the model and the data in simulating the movement of the third bead during the formation of the bead pair agglomerate. This is possibly due to the statistical nature of the Brownian displacement of the third bead; this random behaviour cannot be modelled by the simulation.

The above results generally show that there is a deviation between the simulation results and the experimental data in predicting the end point of the interaction between the bead pair agglomerate (blue and red) and the third bead (black) as well as a deviation in compared trajectories. Some factors responsible for the deviation in results are discussed in the next section.

In conclusion, the above results show that when a bead agglomerate is formed, it attracts other beads within a close separation distances ($<6-7\mu\text{m}$) to form larger bead clusters within the separation system. As mentioned in the first chapter, once a bead cluster is formed, it attracts more beads and gradually leads to an increase in the formation of agglomerates within the magnetic separation system.

4.7.2 LIMITATION OF SIMPLIFIED MODEL

In this model, the bead agglomerate has been simplified to represent a spherical bead with diameter of $2\mu\text{m}$ and to have the same magnetic susceptibility as the $1\mu\text{m}$ sized bead; deviation in results suggest that further work needs to be done to accurately predict the interaction between the

bead pair agglomerate and third bead. Some of the reasons for the deviation in results are given below.

The model is a simplified representation and an approximation used to predict the interaction between the bead pair cluster and third bead. There is an uncertainty in predicting the magnetic properties of the bead pair cluster. It is also anticipated that the bead pair cluster would have different drag forces in different directions due to its axial structure, however the model used in the simulation does not take this into account; instead treating the bead pair as simply an enlarged single bead.

Furthermore, the trajectories were simulated with the agglomerate modelled as a single bead with varying diameter ranging from 1.26-2 μm until the best fit with the experimental data was obtained. Justification for restricting simulations to this range of diameters is because a diameter of 1.26 μm provides a single bead with the same volume as a pair of beads (lower limit) and 2 μm as it is the largest span across two touching beads. Within this range, a diameter of 2 μm gave the closest fit.

4.8 CONCLUSION

A three dimensional vector model has been developed in Matlab for studying magnetic, hydrodynamic interactions as well as highlighting the effect of sedimentation among three super-paramagnetic beads placed in a uniform magnetic field.

A Fourier diffraction optics model was developed in Matlab to predict the axial positions of the magnetic particles using off-focused images. This model was compared with recorded images of

the beads obtained at various defocus distances using the optical setup; the comparison gave a reasonable approximation. Results further show that obtaining and recording the diffracted images of the bead under the optical system offers a simple way to predicting the axial displacement of the bead. The optical model can be improved further by considering the angular divergence of the illumination, lens and bead characteristics; this would allow for a more accurate determination of the height of the bead.

An experiment was designed to capture the three dimensional trajectory pattern as well as interactions among beads using a higher density [$7-10 \times 10^6$ beads/ml] of $1\mu\text{m}$ sized super-paramagnetic particles [myone beads] suspended in a static fluid within a uniform magnetic field on video.

Figure 4.12 shows that a two dimensional projection does not always provide accurate and sufficient information to predict the interactions among beads which could be sedimenting within a micro-channel. And a three dimensional model helps us to understand the impact of axial forces on magnetic bead interaction.

A good comparison was obtained between the theoretical predictions and video data through the presentation of different bead configurations; therefore, the model is a good approximation for predicting a three dimensional trajectory pattern of magnetic particles interacting within a uniform magnetic field for a higher density ($7-10 \times 10^6$ beads/ml). It also improves our understanding of the complex process involved when more than two beads interact simultaneously within a magnetic field.

Furthermore, the results showed that a three bead system effectively reduces to a bead pair when the magnetic interaction force between a bead pair outweighs the Brownian force on the beads.

This happens when the $1\mu\text{m}$ beads are separated by a gap distance less than or equal to $6\mu\text{m}$, which is consistent with our observations in Chapter three.

However, the model, in its present form, cannot be used to study interaction between agglomerates once formed; however one step would be to include in the model extensions that deal with agglomerates modelled as cylinders [4] and larger agglomerates modelled as larger spheres.

Magnetic beads are used in a variety of biomedical applications such as cell transport, separation, sorting, capture, manipulation etc. as discussed in the introductory chapter; hence the model is of practical use to designers and users of magnetic bioassays in tracking, predicting and understanding the interactions among magnetic particles (with varying physical and magnetic properties) within a magnetic micro-system.

The model also provides a knowledge for designing magnetic separator and other micro-system where it is important to understand the build-up of agglomerates from single magnetic particles.

Furthermore, the determination of the magnetic characteristics of these beads within a magnetic environment will aid their magnetic manipulation and help predict their behaviour when suspended within a biological fluid (discussed in the next chapter).

Chapter 5

Summary and Conclusion

5.1 SUMMARY

The formation of bead agglomerates within a magnetophoretic system due to interaction between magnetic beads was studied in this thesis. Magnetic binding forces lead to an attractive force between the magnetic beads while hydrodynamic interactions strongly influence the beads' movement.

This study began with the development of a theoretical model to predict the trajectory pattern as well as magnetic induced velocities between a pair of super-paramagnetic beads (equal sized or different sized) suspended in fluid within a uniform magnetic field.

The magnetic interaction on a super-paramagnetic bead was derived from first principles following the work of Mikkelsen in [33] by accounting for the induced magnetisation and perturbed external field due to the presence of a second super-paramagnetic bead within a uniform magnetic field.

The hydrodynamic interaction was developed by solving the Navier-Stokes' equation using the method of reflection [55] in a vector form to account for the transmitted velocity field due to the movement of a bead suspended in fluid. The lubrication approximation [45, 46] was included in the hydrodynamic interaction equations to account for the high velocity gradient and pressure which occurs when the beads approach themselves at a gap-distance less than their physical dimensions.

Next, an experiment was designed to investigate bead pair interactions within a magnetophoretic system using $1\mu\text{m}$ and $2.8\mu\text{m}$ sized magnetic beads; as anticipated, observations showed that the $1\mu\text{m}$ magnetic bead were Brownian displaced when suspended in water at room temperature. The trajectory plots as well as magnetic induced velocities of these beads were obtained from the experiment and compared with the simulation results and these gave good agreement.

The agglomeration time was obtained as a function of separation distance for three super-paramagnetic beads (obtained from different manufacturers); this relationship can be used to predict the threshold distance when using magnetic beads in an assay that avoids agglomeration over a fixed time interval.

This model was then extended to study the interactions among three super-paramagnetic beads suspended in fluid within a uniform magnetic field projected onto a three dimensional plane. This allows us to evaluate the effect of sedimentation on the beads and helps in understanding the complexity of the agglomeration process when more than two beads interact strongly within a magnetic separation system.

The developed analytical model was extended in three dimensions in Matlab to account for the magnetic interaction, hydrodynamic interaction and sedimentation force among the three super-paramagnetic beads displaced in water within a uniform magnetic field.

An experiment was designed to predict the trajectories as well as interactions among the three super-paramagnetic beads within a uniform magnetic field using an optical imaging configuration. In order to predict the axial information of the super-paramagnetic bead, a Huygens Fresnel model was developed in Matlab which was then compared to off-focused recorded images of the magnetic beads viewed under the optical microscope.

A good agreement was obtained when the three-dimensional simulated trajectories were compared with the experimental data.

5.2 CONCLUSION

A conclusion can be reached that the developed model is a good approximation for predicting the trajectory pattern of super-paramagnetic beads suspended in fluid and interacting within a uniform magnetic field, including the action of bead sedimentation.

The developed model is of practical use to designers and users of magnetic bioassays (involving the use of magnetic beads) in tracking, predicting and understanding the interactions among magnetic beads (with varying physical and magnetic properties) as well as their sedimentation within a magnetophoretic system.

An area of potential application for the interaction study (demonstrated throughout this thesis) is in the micro-magnetic manipulation of magnetic beads to understand and study biophysical mechanisms such as molecular interaction processes (discussed in detail below) often times concealed within an ensemble model.

5.2.1 FUTURE WORK

There is often the need to understand and study molecular interaction processes such as cell-cell, ligand-receptor, protein-DNA, protein-protein, other bio-polymers interaction etc., and obtain quantitative information during such complex biological processes.

The obtained information can reveal individual processes that may be hidden in the ensemble averaging process, aid medical experts as well as help the pharmaceutical industry come up with innovative drugs for combating various medical conditions (e.g. a knowledge of erythrocyte-malaria parasite interaction can help in the development of malaria vaccines or inhibitors [95]).

In order to achieve these objectives, some biological systems have to be artificially manipulated, e.g. using optical tweezers and traps [89, 93, 94].

Optical tweezers and traps rely on the use of a highly focused laser with an objective lens of high NA to trap and manipulate small dielectric spherical particles which experience an optical force due to the momentum transfer from the scattering of incident photons. The optical force is further subdivided into a scattering force and gradient force [96, 99].

The scattering force depends on the optical intensity and points in the direction of light propagation. While the gradient force depends on the gradient intensity and points in the gradient direction; it is due to the interaction between the dipole and the in-homogenous electric field. Both the scattering and gradient forces depend on the size of the particle and the wavelength of the light beam [96, 99]. For stable trapping the gradient force pulling the particle towards the focal region must be greater the scattering force pushing it away from the region [99].

Here, we reviewed some biological applications based on the use of optical traps/tweezers. For example, Bronkhorst et al. [93] manipulated and brought together two red blood cells (6-8 μ m) using a pair of optical traps in order to understand the aggregation process which involved bridge formation during binding while Thoumine et al. [88] used an optical tweezer to study the adhesion/detachment process during a protein-protein interaction.

Crick et al. [95] used optical tweezers to deliver malaria-parasite (merozoite, 1.5 μ m length, 1 μ m width) to erythrocytes in order to study the attachment, invasion and deformation processes as well as obtained a measurement of the strength at contact; they went a step further by studying the merozoite-erythrocyte interaction in the presence of invasion inhibitors.

Prada et al. [97] positioned individual extracellular vesicles (<1000nm) on cell membranes using optical tweezers in order to monitor vesicle-cell dynamics in vitro. Also the authors in [98] used an optical tweezer to bind an influenza virus coated microsphere to a target cell in order to understand the binding process.

Other biophysical properties such as mechanical properties of proteins bound to a stretched DNA molecule [94], translocation force measurement during a DNA-RNA interaction [91, 92] have been observed and experimentally investigated using an optical trap.

However, the authors in [88] report that their results may have been modified by optical effects such as scattering, interference and photo-activation, despite the use of a low photon energy infra-red laser; these are some of the limitations associated with the optical trap and tweezer method.

Other drawbacks associated with this method involve the large intensity at the laser focus which can result in local heating and then damage to biological cells. The generated heat can also lead to a change in the properties of the medium (e.g. viscosity, enzymatic activity) which can affect the validity of the measured results [90, 96]. Trapping durations were kept short (<10s) to minimize the effect of local heating in some applications (e.g. malaria parasite-erythrocyte interaction).

Following the work in this thesis, it is proposed that magnetic bead binding to cells and their magnetic manipulation be extended into a further study of the intercellular molecular interaction processes highlighted above.

For example, following the work of Bronkhorst et al., the aggregation process between two red blood cells (6-8 μm) can be replicated using the magnetic interaction system developed in this thesis and which produces pico-Newton forces (commonly applied to biomolecules) over nanometre distances within 2-8s.

In the first instance, the pair of red blood cell are attached to a pair of magnetic beads (see figure 5.1 below). In the second step, the super-paramagnetic beads become magnetized in the presence of the external magnetic field; they are then magnetically manipulated and steered in a way that allow the cells to come into closer contact to allow for aggregation.

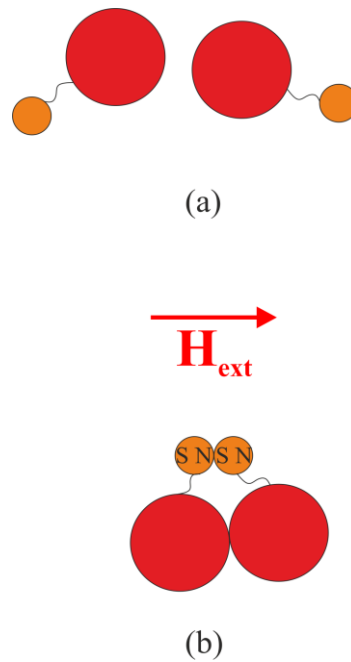


Figure 5.1 (a) The attachment of a pair of red blood cell (6-8 μm) to a 1 μm sized super-paramagnetic bead and (b) subsequent magnetic manipulation in the presence of an external field, demonstrated in two stages to allow for aggregation between the red blood cells.

After the aggregation process within 2-8s, the external magnetic field can be either removed (in the case of permanent magnet) or switched off (for electromagnets). This, of course, leads to the super-paramagnetic bead losing their magnetisation.

Bronkhurst et al. also reported that the exact trapping force was not known in their work. This could easily be determined using the magnetic interaction system by measuring the magnetic induced velocities of the magnetic complex.

A second example is the use of magnetic bead to study the interaction between malaria parasite (merozoites) and erythrocytes, following the work of Crick and co-authors.

Repeating the steps in figure 5.1, a pair of super-paramagnetic bead can be attached to the merozoite and erythrocyte. In the second step, the merozoite and erythrocyte are brought together by manipulating the magnetic beads within the external magnetic field.

Using the magnetic bead method, erythrocytes and merozoite can be kept for as long as desired; Crick et al. mentioned that trapping durations were kept short (<10 s) to minimize the effect of local heating. Hence, the magnetic bead successfully avoids this limitation.

In conclusion, the magnetic bead interaction method offers an alternate viable way to understanding intercellular interaction, obtaining quantitative results and successfully avoid the limitations associated with optical trapping technique such as heating, scattering etc.

Appendix

A1

The following steps are taken at each time step for the numerical model in Matlab. The model predicts the trajectory pattern and magnetic induced velocities of a pair of super-paramagnetic beads suspended in water for a uniform magnetic field.

- i. The beads are assumed stationary, $\mathbf{u}_1 (n = 1) = 0$, $\mathbf{u}_2 (n = 1) = 0$, $\mathbf{u}_1^1 (n = 1) = 0$, $\mathbf{u}_2^1 (n = 1) = 0$; $n = 1$.
- ii. Calculate the magnetic interaction force on bead 1 due to bead 2.
- iii. Repeat calculation for the magnetic interaction force on bead 2 due to bead 1
- iv. Calculate $\mathbf{u}_1 (n)$ and $\mathbf{u}_2 (n)$ using $\mathbf{u}_2 (n - 1)$ and $\mathbf{u}_1 (n - 1)$ respectively.
- v. Calculate the predicted position of the beads, $\mathbf{r}_1^1 (n)$ and $\mathbf{r}_2^1 (n)$ using equation 2.59.
- vi. Repeat steps ii to iii using $\mathbf{r}_1^1 (n)$ and $\mathbf{r}_2^1 (n)$.
- vii. Calculate $\mathbf{u}_1^1 (n)$ and $\mathbf{u}_2^1 (n)$ using $\mathbf{u}_2^1 (n - 1)$ and $\mathbf{u}_1^1 (n - 1)$ respectively.
- viii. Using equation 2.60, calculate $\mathbf{r}_1 (n)$ and $\mathbf{r}_2 (n)$.
- ix. Increment n .
- x. Repeat from step ii for the next time step until the beads touch.

The numerical model was implemented in Matlab for three super-paramagnetic beads suspended in water for a uniform magnetic field using the finite-time-finite-difference scheme.

The simulation steps at a given time step are outlined below.

- i. Assume a reference bead, say bead 1.
- ii. The beads are assumed stationary, $\mathbf{u}_1(n) = 0$, $\mathbf{u}_2(n) = 0$, $\mathbf{u}_3(n) = 0$, $n = 1$.
- iii. Calculate the magnetic interaction force on bead 2 due to bead 3, $\mathbf{F}_{m23}(n)$ (indirectly influences the movement of bead 1).
- iv. Using bead 2 as a sub-reference, calculate the blocking factor on bead 3 due to bead 1.
- v. Calculate the velocity, $\mathbf{u}_{23}(n)$, by substituting $\mathbf{F}_{m23}(n)$ and the blocking factor into equation 4.16.
- vi. Update the velocity at bead 2 ($\mathbf{u}_2(n) = \mathbf{u}_{23}(n)$).
- vii. Repeat steps iii – vi using bead 3 as sub-reference to obtain $\mathbf{F}_{m32}(n)$ and update the velocity of bead 3 ($\mathbf{u}_3(n) = \mathbf{u}_{32}(n)$).
- viii. Calculate the magnetic interaction force on bead 1 due to bead 2, $\mathbf{F}_{m12}(n)$ (direct magnetic interaction on bead 1).
- ix. Using bead 1 as a reference, calculate the blocking factor on bead 2 due to bead 3.
- x. Obtain the velocity at bead 2, $\mathbf{u}_{12}(n)$ using equation 4.16.
- xi. Obtain the velocity at bead 3, $\mathbf{u}_{13}(n)$, by repeating steps viii – x with bead 1 as reference.

- xii. Calculate the velocity at bead 1, $\mathbf{u}_1^1(n) = \mathbf{u}_{12}(n) + \mathbf{u}_{13}(n) + \mathbf{u}_s(n)$ and save this value.
- xiii. Now repeat steps iii – x using beads 2 and 3 as references to obtain $\mathbf{u}_2^1(n)$ and $\mathbf{u}_3^1(n)$ while alternating the roles of the other beads to serve as sub references in each case.
- xiv. Update the new velocities of the beads as ($\mathbf{u}_1(n) = \mathbf{u}_1^1(n)$, $\mathbf{u}_2(n) = \mathbf{u}_2^1(n)$, $\mathbf{u}_3(n) = \mathbf{u}_3^1(n)$).
- xv. Calculate the new position of the beads ($\mathbf{r}_1(n+1)$, $\mathbf{r}_2(n+1)$, $\mathbf{r}_3(n+1)$) using equation 4.20.
- xvi. Increment n.
- xvii. Repeat steps iii – xiv until two of the beads touch.

A3

The value of the magnetic susceptibility of the 1µm Chemicell [25] bead was obtained using two methods, linear interpolation of figure 3.1 and by fitting a Langevin curve to figure 3.1.

LINEAR INTERPOLATION

The magnetisation of the bead at various applied flux density values were obtained from figure 3.1 and are summarised on Table A3.1 below.

Table A3.1 Magnetisation and applied flux density values of 1µm Chemicell [25] bead obtained from Figure 3.1.

B (mT)	M (A/m)×10 ⁴
50	5.467
-50	-5.562

By linearly interpolating between these values, the magnetisation at 11mT was obtained as 1.167×10⁴A/m. The ratio of the magnetisation of the bead to its magnetic field strength (at 11mT) gave the magnetic susceptibility value of 1.33.

LANGEVIN FIT

The magnetisation of a bead can be expressed as

$$\mathbf{M}(\mathbf{H}) = n_p \mathbf{m} L\left(\frac{\mathbf{B} \cdot \mathbf{m}}{kT}\right) \quad (\text{A3.1})$$

where n_p is the density of nanoparticles in the sample and other parameters have been defined within the nomenclature section.

The Langevin distribution (from equation A3.1) was fitted to figure 3.1 as depicted below.

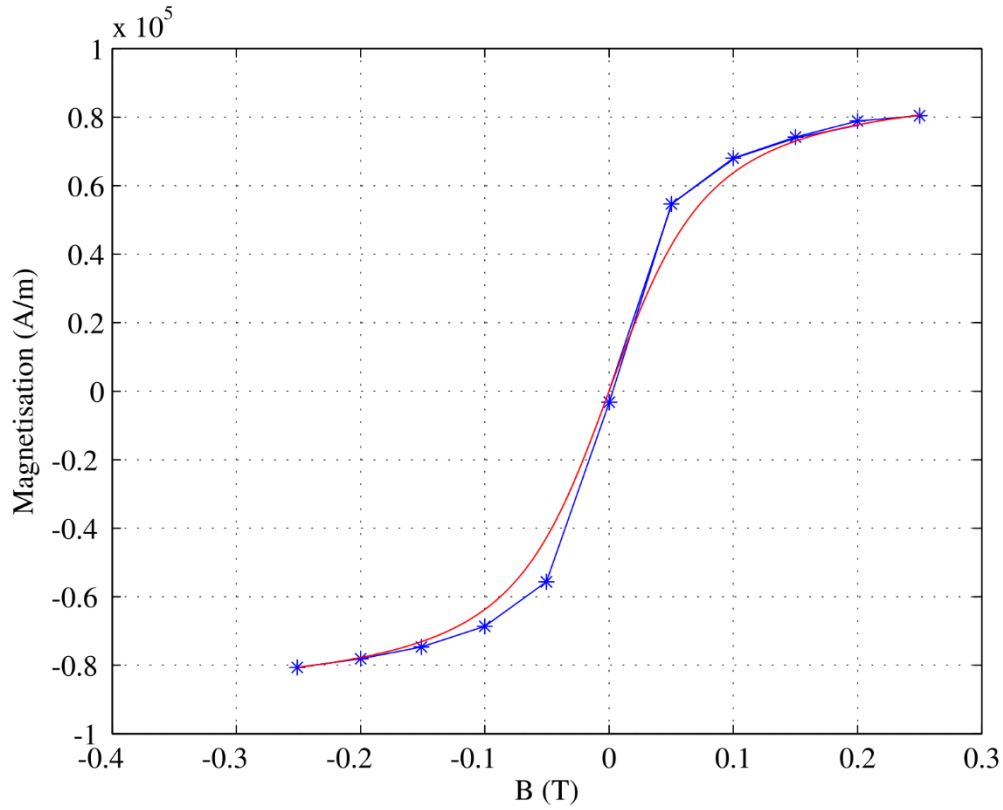


Figure A3.1 Fitting the M-B Characterisation of the 1 μ m sized super-paramagnetic bead obtained from Chemicell, at room temperature within the range ± 0.25 T (in blue) with a Langevin distribution (in red).

Based on figure A3.1, the magnetisation of the bead was obtained at 11mT from the Langevin distribution (in red) and the ratio of the magnetisation to the magnetic field strength gave a value of 1.25.

The magnetic susceptibility of the 1 μ m Chemicell [25] bead was therefore evaluated as 1.33-0.08 at 11mT. The difference in magnetic susceptibility values using the two methods discussed here is not significant and does not change any of the conclusions in this thesis.

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