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PROPULSION OF POLYMER PARTICLES ON CAESIUM ION-EXCHANGED CHANNEL WAVEGUIDES FOR STEM CELL SORTING APPLICATIONS

by

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS
SCHOOL OF ELECTRONICS AND COMPUTER SCIENCE

Doctor of Philosophy

PROPULSION OF POLYMER PARTICLES ON CAESIUM ION-
EXCHANGED CHANNEL WAVEGUIDES FOR STEM CELL
SORTING APPLICATIONS

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Optical trapping of particles has become a powerful non-mechanical and non-destructive technique for precise particle positioning. The manipulation of particles in the evanescent field of a channel waveguide potentially allows for sorting and trapping of several particles and cells simultaneously. In the evanescent field above an optical channel waveguide, particles experience three optical forces; i) a transverse gradient force, which acts in the direction of the intensity gradient, ii) a scattering force, which acts in the direction of the wave propagation and is proportional to the surface intensity and, iii) an absorption force, which is dependent upon the complex refractive index of the particle. A particle in the evanescent field will be propelled and trapped with a dependence on the intensity gradient (a property dependent upon the physical characteristic of the waveguide). A channel waveguide producing such an evanescent field can be photolithographically defined on a glass substrate and thus has the potential to be integrated into a single chip device.

This thesis describes the studies carried out, both theoretically and experimentally, to establish optimum waveguide fabrication conditions and the experimental requirements to ultimately allow for the separation of polymer particles and mammalian cells according to their size and refractive index. Theoretical aspects of the interaction of particles and cells on surfaces were evaluated and their Brownian motion was investigated. Optical channel waveguides of different characteristics have been fabricated using caesium ion-exchange process on soda-lime substrates. The propulsion of polymer particles has been achieved and characterised against different optical parameters, waveguide conditions and particle’s characteristics on different surfaces. The propulsion of lymphoblastoma cells was demonstrated and the trapping of teratocarcinoma cells was evaluated. These results provide evidence for the potential application of the system for trapping and sorting stem cells.
## Contents

Abstract .......................................................... ii

List of tables ..................................................... vii

List of figures .................................................. viii

Declaration of Authorship .................................... xv

Acknowledgement .............................................. xvii

Nomenclature .................................................. xviii

Chapter 1  Introduction ........................................ 1

1.1 Aims and motivation ........................................ 1

1.2 Overview of stem cells .................................... 2

1.3 Overview of optical trapping and propulsion .......... 4

1.4 Interaction of light with mammalian cells .......... 6

1.4.1 Laser wavelength ...................................... 6

1.4.2 Laser power ............................................. 9

1.4.3 Laser operation ......................................... 11

1.5 Profiles of polymer particles and mammalian cells . 13

1.5.1 Polymer particles properties ......................... 14

1.5.2 Mammalian cells properties ......................... 15

1.6 Thesis structure ............................................ 19

Chapter 2  Sorting techniques for particles and cells .. 20

2.1 Introduction ................................................ 20

2.2 Conventional particle and cell sorting ............... 21

2.2.1 Mechanical sorting .................................... 21

2.2.2 Magnetophoretic technique ......................... 24

2.2.3 Electric trapping and sorting ....................... 25

2.2.4 Microfluidic sorting .................................. 28

2.3 Optical cell trapping and sorting ....................... 31

2.3.1 Laser tweezer ......................................... 31

2.3.2 Optical lattice ......................................... 34

2.3.3 Bessel beam ............................................ 37

2.3.4 Waveguide ............................................. 38
2.4 Conclusion ....................................................................................................................... 39

Chapter 3  Particles and cells on surfaces  41
3.1 Introduction ..................................................................................................................... 41
3.2 Non-optical forces ......................................................................................................... 41
  3.2.1 Drag force and Brownian motion ........................................................................ 41
  3.2.2 Gravitational and buoyancy forces ...................................................................... 45
  3.2.3 Electrostatic forces ............................................................................................... 47
    3.2.3.1 Van der Waals force .................................................................................... 47
    3.2.3.2 Double layer force ...................................................................................... 48
3.3 Thermally induced effect .............................................................................................. 51
  3.3.1 Convection current ............................................................................................... 51
3.4 Ionic concentration of medium .................................................................................... 53
3.5 Conclusion ..................................................................................................................... 57

Chapter 4  Optical channel waveguide: Theoretical and experimental evaluation  59
4.1 Introduction ..................................................................................................................... 59
4.2 Waveguide fabrication .................................................................................................. 60
  4.2.1 Glasses for ion-exchange ...................................................................................... 60
  4.2.2 Refractive index change ...................................................................................... 61
  4.2.3 Substrate selection and cleaning .......................................................................... 63
  4.2.4 Aluminium evaporation ....................................................................................... 63
  4.2.5 Photolithography ................................................................................................. 64
  4.2.6 Ion exchange ......................................................................................................... 65
  4.2.7 Polishing ................................................................................................................ 66
4.3 Waveguide theory ......................................................................................................... 66
  4.3.1 Diffusion theory .................................................................................................... 66
  4.3.2 Ray description of the optical waveguide ............................................................ 70
  4.3.3 Electromagnetic description of optical waveguide ............................................. 72
  4.3.4 Modelling of optical waveguides ......................................................................... 76
    4.3.4.1 Effective refractive index ............................................................................. 77
    4.3.4.2 Electric field and surface intensity ............................................................... 78
4.4 Waveguide characterisation .......................................................................................... 82
  4.4.1 Effective refractive index measurement ................................................................. 83
  4.4.2 Cut-off wavelength determination ......................................................................... 87
  4.4.3 Waveguide degradation ....................................................................................... 90
4.5 Conclusion ..................................................................................................................... 91

Chapter 5  Brownian motion of particles and cells  94
5.1 Introduction .................................................................................................................... 94
List of tables

Table 1.1 Light induced processes in biological cells [38] ................................................................. 10
Table 3.1 Predicted root-mean-square (RMS) displacement of a range of polystyrene particles in two dimensions after 1 second ................................................................. 44
Table 3.2 Parameters used for modelling temperature profile ............................................................. 53
Table 3.3 Constant values for materials in the DLVO calculations [179, 188, 189] ....... 54
Table 4.1 General characteristics of alkali ions and their corresponding waveguides after ion-exchanged into sodium doped silicate glasses [197] .................................................. 62
Table 4.2 Details of the waveguides fabricated using a soda-lime glass substrate. The $n_{\text{eff}}$ of the fundamental modes were measured at 632.8 nm. The expected depth is fitted according to the theoretical simulation values in Section 4.3.4.1 ....................................... 85
Table 5.1 Tabulation of particles and cells size distribution and their corresponding errors measurements. Note that the measured diameter and size deviation for polystyrene particles were provided by the manufacturer. Diameter and size distribution for the cells were measured using Zeiss Axio Observer Z1 microscope ................................................................. 99
Table 5.2 Experimental values of $\psi$ and $\Delta$ and their corresponding layer thickness based on model in Figure 5.9 ................................................................................................................ 105
Table 5.3 Several mixtures of DMEM used for the Brownian motion investigation of teratocarcinoma cells ........................................................................................................... 120
Table 6.1 Absorption for media investigated at 1064 nm wavelength ............................................. 134
Table 6.2 Estimation of laser power at various points in the laser path ........................................ 136
Table 6.3 Tabulation of particles velocity for Figure 6.8 ............................................................... 141
Table 6.4 Details of the waveguides fabricated using a soda-lime glass substrate. The $n_{\text{eff}}$ of the fundamental mode was measured at 632.8 nm. Expected depth is fitted according to the simulation values in Section 4.3.4.1 ............................................................... 146
List of figures

Figure 1.1 Schematic showing the development of stem cells from the source (adult organs and embryonic cells) into specific cells [13] ................................................................. 3
Figure 1.2 Schematic of optical forces acting on a particle on a channel waveguide ............... 4
Figure 1.3 Optical trapping mechanism of a particle on an illuminated channel waveguide ... 5
Figure 1.4 Absorption spectrum for water within the 200-2000nm window .......................... 6
Figure 1.5 Chinese hamster ovary (CHO) cell’s cloning efficiency after exposure at 88mW for wavelengths varying from 700 to 1100nm [40] ............................................................... 8
Figure 1.6 Chinese hamster ovary (CHO) cell’s cloning efficiency with varying exposure times at 88mW [40] ..................................................................................................................... 8
Figure 1.7 Formation of abnormal chromosome bridge after irradiation with a 760nm wavelength laser for 1 second. A) before irradiation, B) after 1 minute, C) after 8 minutes, D) after 11 minutes, E) after 13 minutes, F) after 28 minutes [49] .................. 10
Figure 1.8 Exposure at 458nm wavelength with 500mWcm$^{-2}$. hTERT-RPE1 cells were stained with EthD-1/Calcein AM for live or dead viability test. A) after 1 hour post exposure recovery (PER) from CW exposure, B) after 24 hours PER from CW exposure, C) after 1 hour PER from pulsed exposure, D) after 24 hours from pulsed exposure [60] ............... 12
Figure 1.9 Polystyrene particle size range as supplied by the manufacturer .......................... 14
Figure 1.10 Lymphoblastoma cells, a) in RPMI culture media, b) mixed with 0.4% Trypan blue solution. Cells coloured in blue are dead cells .............................................................. 15
Figure 1.11 Teratocarcinoma cells (TERA1) a) image from the bottom of the flask showing 90% confluence b) after the trypsinisation process c) 2 days old TERA1 cells mixed with 20μm polystyrene particles d) 3D shape of TERA1 cell on a glass surface (pseudo-hemispherical) ...................................................................................................................... 16
Figure 1.12 Schematic of the cell cycle, M=Mitosis, G1=Gap 1, G2=Gap 2, S=Synthesis, G0=Gap 0/Resting. The duration of mitosis in relation to the other phases has been exaggerated in this diagram [66-68] ................................................................................................................ 17
Figure 1.13 Schematic of a generic eukaryotic cell, showing subcellular components.
Organelles: (i) nucleolus (2) nucleus (n=1.39) (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (n=1.40) (10) vacuole (11) cytoplasm (n=1.38) (12) lysosome (13) centrioles (*) cell membrane (n=1.48) [66, 67, 70-79]. The insert shows details of cell membrane (phospholipid bilayer) with cholesterol, glycolipid and proteins ........................................ 18
Figure 2.1 a) Comb-type filter formed from an array of 120 posts (175μm long x 18μm wide) separated by 6μm channels set across a 3mm wide x 13μm deep silicon channel [95], b) Epifluorescence picture using the vital nuclear UV dye H33342 of white blood cells in the variable length array [96] ............................................................................................................. 22
Figure 2.2 a) SEM image showing the transition from the 15μm wide channels to the 10μm wide channels [98, 99] b) SEM image showing the oblong obstacle of 3.5μm high and
measuring 1.5 x 6.0μm, the gap between adjacent obstacles is 1.5μm wide. The electric
field was applied in the V direction [100].

Figure 2.3 Lateral percolation in microfabricated filter [92]

Figure 2.4 Midaxial view a) and cross-sectional view b) of the quadrupole magnetic sorter
(QMS) [90]

Figure 2.5 Schematic diagram of the micro cell sorting system based on the magnetic
particles [104]

Figure 2.6 Comparison between DEP crossover data for nine human tumor cell types and
normal peripheral blood mononuclear cells (medium conductivity 56 mS/m) [93, 106-111]

Figure 2.7 Schematic diagram of the DEP particle separator [112]

Figure 2.8 Outline of the differences between metallic and electrodeless DEP trap [119]

Figure 2.9 (a) The viscous nature of microfluidic flows is demonstrated in converging
aqueous streams (each 30 μm in width). (b) The top stream is dyed to illustrate the lack
of convective mixing that occurs under laminar conditions. (c) The outlet image
demonstrates the presence of some molecular-scale diffusion, the only form of mixing
in this system. The inlet and outlet are separated by 3000μm [126, 127]

Figure 2.10 Schematic illustration of the working principle for two-channel flow cytometer
[132, 133]

Figure 2.11 Schematic of the 3D hydro-focusing unit [135]

Figure 2.12 Demonstration of optical handle to grab and manipulate an object that is not
trapped by the laser in a fluidic channel [137]

Figure 2.13 Fluorescent confocal microscope images of an AML cell, a) placed in front of a
channel, b) immediately after single shot of optical scissors, c) 1 second after laser shot,
d) and 5 seconds after laser shot [144]

Figure 2.14 An actuated, three-way colloidal valve [127, 147, 148]

Figure 2.15 Schematic of diode laser bar trapping and imaging system [149]

Figure 2.16 The concept of optical fractionation [151, 152]

Figure 2.17 Schematic to produce an optical lattice [151, 152]

Figure 2.18 Comparison between different lattice types [151, 152]

Figure 2.19 False colour intensity maps of different optical lattices [151, 152]

Figure 2.20 Bessel beam intensity profiles, a) for a zeroth order and b) a first-order beam, c)
two lymphocytes aligned vertically in the beam centre and erythrocytes aligned in the
outer rings of the Bessel beam

Figure 2.21 Experimental setup for creating Bessel beam [158]

Figure 2.22 a) Experimental setup used for particle sorting. b) Diagram of the waveguide
junction region [31]

Figure 3.1 Diagram illustrating the forces acting on the particle

Figure 3.2 Settling time of different polystyrene particle sizes. \( \rho_p = 1.05 \text{ g/cm}^3, \rho_m = 1 \text{ g/cm}^3 \)

Note that the y-axis is set in log scale.

Figure 3.3 A schematic showing a spherical particle near a surface

Figure 3.4 Schematic of energy versus distance profiles of DLVO interaction [179]

Figure 3.5 Two dimensions schematic of the waveguide simulated in Comsol
width 4µm ( ), 5µm ( ) and 10µm ( ). Dashed lines represent TE modes whereas solid lines represent TM modes ..................................................................................................... 88

Figure 4.22 Cut-off wavelength of caesium ion-exchanged waveguides in soda-lime substrate for 6.5 hours ( ), 8 hours ( ), 10 hours ( ) and 17 hours ( ). Dashed lines represent TE modes whereas solid lines represent TM modes..................................................................................................... 89

Figure 4.23 Channel height for caesium waveguides with varying ion-exchange times .......... 90

Figure 4.24 The degradation of 17 hour caesium ion-exchanged waveguide in water at different temperatures ............................................................................................................ 91

Figure 5.1 Experimental set up for monitoring particle and cell movements ..................... 95

Figure 5.2 Cropped images of 10µm polystyrene particles from a glass slide held by a)
waveguide mount and b) vacuum holder ............................................................................. 96

Figure 5.3 The sequence of image analysis of 10µm polystyrene particles. Data initially presented in 1 dimensional array is translated into a more meaningful, a) 2 dimensional image. In order to detect particles, a threshold is applied to distinguish the position of particles b) ................................................................................................................................ 97

Figure 5.4 Image of a 10µm polystyrene particle a) prior and b) after the thresholding process ............................................................................................................................................. 98

Figure 5.5 Schematic of surface modification by self assembled monolayer of PEG chain [235] ............................................................................................................................................. 101

Figure 5.6 Schematic setup of the ellipsometry measurement............................................. 102

Figure 5.7 Schematic of FFM system. The sample is scanned using the piezo-electric scanner with the laser and the quadrant photodiode to determine the deflection of the cantilever ......................................................................................................................................... 103

Figure 5.8 a) Image from the ellipsometry measurement showing the boundary between the plain glass and the PEG layer, b) shows the friction image of the PEG layer, the variation of shades in the image constitute different friction coefficient, less contrast in the image indicates a fairly uniform friction across the PEG layer ............................................................................................................. 104

Figure 5.9 Theoretical model of ψ and ∆ values with their corresponding layer thickness according to the layer model created in Ellgraph ............................................................................................................. 105

Figure 5.10 FFM reading for plain and PEG functionalised surface in water. The relative friction coefficient can be extracted from the gradient of each plot. ............................ 106

Figure 5.11 FFM reading for plain and PEG functionalised surface in DMEM with serum. The relative friction coefficient can be extracted from the gradient of each plot........ 107

Figure 5.12 The distance travelled (squared) from one frame to a subsequent frame by particles of different sizes on a plain glass surface in water. The theoretical data (—), experimental data (+) and linear best fit line for the experimental data (—) are plotted for each particle size ............................................................................................................................................. 110

Figure 5.13 The histograms of the step size taken to travel from one frame to a subsequent frame by particles of different sizes on a plain glass surface in water. The Gaussian best fit line (—) is plotted for each experimental data ( ) alongside the theoretical model ( ) ............................................................................................................................................. 111

Figure 5.14 The distance travelled (squared) from one frame to a subsequent frame by particles of different sizes on a PEG functionalised glass surface in water. The
theoretical data (—), experimental data (+) and linear best fit line for the experimental
data (—) are plotted for each particle size .............................................................. 113

Figure 5.15 The histograms of the step size taken to travel from one frame to a subsequent
frame by particles of different sizes on a PEG functionalised glass surface in water. The
Gaussian best fit line (—) is plotted for each experimental data ( ) alongside the
theoretical model (—) ................................................................................................. 114

Figure 5.16 The distance travelled (squared) from one frame to a subsequent frame by 20µm
particles on a PEG functionalised glass surface in a) salt solution, b) salt solution with
glucose, c) DMEM, d) DMEM with serum. The theoretical data (—), experimental data
(+) and linear best fit line for the experimental data (—) are plotted for each particle
size .......................................................................................................................... 116

Figure 5.17 The histograms of the step size taken to travel from one frame to a subsequent
frame for 20µm polystyrene particles on a PEG functionalised glass surface in a) salt
solution, b) salt solution with glucose, c) DMEM, d) DMEM with serum. The Gaussian
best fit line (—) is plotted for each experimental data ( ) alongside the theoretical
model (—) .................................................................................................................. 118

Figure 5.18 The distance travelled (squared) from one frame to a subsequent frame by
a) TERA1 cell and b) NT2 cell on a plain glass surface in DMEM with serum. c) and d) are
the corresponding graphs showing the histograms of the step size taken to travel from
one frame to a subsequent frame. The best fit line (—) is plotted for each experimental
data (+ ) alongside the theoretical model (—) ............................................................. 121

Figure 5.19 The results from a single cell analysis that show a ‘matched’ category. The
distance travelled (squared) from one frame to a subsequent frame by a) TERA1 cell
(16.01µm) and b) NT2 cell (17.22µm) on a PEG functionalised glass surface in DMEM
with serum, c) and d) are the corresponding graphs showing the histograms of the step
size taken to travel from one frame to a subsequent frame. The best fit line (—) is
plotted for each experimental data (+ ) alongside the theoretical model (—) ............... 122

Figure 5.20 The results from a single cell analysis that shows the ‘mismatched’ category. The
distance travelled (squared) from one frame to a subsequent frame by a) TERA1 cell
(19.36µm) and b) NT2 cell (20.21µm) on a PEG functionalised glass surface in DMEM
with serum. c) and d) are the corresponding graphs showing the histograms of the step
size taken to travel from one frame to a subsequent frame. The best fit line (—) is
plotted for each experimental data (+ ) alongside the theoretical model (—) ............... 123

Figure 5.21 The results from the average of all cells (up to 400 cells). The distance travelled
(squared) from one frame to a subsequent frame by a) TERA1 cell and b) NT2 cell on a
PEG functionalised glass surface in DMEM with serum, c) and d) are the corresponding
graphs showing the histograms of the step size taken to travel from one frame to a
subsequent frame. The best fit line (—) is plotted for each experimental data (+ )
alongside the theoretical model (—) ...................................................................... 125

Figure 6.1 Experimental set up for particle/cell trapping and propulsion.......................... 129
Figure 6.2 Fabrication steps for PDMS reservoir................................................................ 130
Figure 6.3 Fabrication steps for microscope slide reservoir with sputtered silica as an
isolation layer ........................................................................................................... 131
Figure 6.4 The refractive index and kinematic viscosity of several culture media compared to water ........................................................................................................................................133
Figure 6.5 Absorption spectra of several culture media and water. The insert at the top left corner shows a close up at wavelength 1064nm (indicated by the red line) ..........134
Figure 6.6 Position of 10µm polystyrene particles at the beginning of the experiment (t = 0 sec) ..........................................................................................................................................138
Figure 6.7 The propulsion of 10µm particles a) Image taken at t = 0 s b) t = 33 s c) t = 66 s and d) t=100 s ..................................................................................................................................................139
Figure 6.8 Output from the particle tracking software, particle propulsion of 10µm particles are represented in , , and coloured lines. Particles moving in Brownian motion are shown in colour. Arrows beside the particle numbers indicate the direction of motion ...............................................................................................................140
Figure 6.9 The theoretical estimation of the propulsion velocity of different sized particles for varying input laser power ..................................................................................................................142
Figure 6.10 Velocity of different sized particles for varying input laser power .................142
Figure 6.11 The theoretical estimation of the propulsion velocity of 10µm particles with varying input laser power for both polarisations .............................................................144
Figure 6.12 Velocity of 10µm particles against power for both polarisations .....................144
Figure 6.13 The theoretical estimation of the propulsion velocity of a) 3µm particle and b) 6µm particle against ion-exchange time and channel width ............................................147
Figure 6.14 The propulsion velocity of a) 3µm particle and b) 6µm particle against ion-exchange time and channel width .......................................................................................148
Figure 6.15 The theoretical estimation of the propulsion velocity of different sized particles with varying channel widths .................................................................................................150
Figure 6.16 Velocity of different sized particles with varying channel widths ......................150
Figure 6.17 Theoretical model of the propulsion velocity of varying particle sizes at TM mode. The red line indicates the best fit between the two adjacent maxima and minima in the theoretical model and the green line is the estimated non-resonance propulsion estimation ................................................................................................................................151
Figure 6.18 Propulsion velocity of varying particle sizes ........................................................................................................................................................................................................................................152
Figure 6.19 a) Typical image taken in monitoring the 20µm particles. Notice that the particle, as indicated by the arrow, lies exactly on top of the illuminated waveguide. b) Light scattering was observed by removing the 1µm low-pass filter from the microscope ......153
Figure 6.20 Snapshots illustrating the lateral trapping of the 1µm particle with their corresponding line profile at time a) 0 and b) 15 minutes ........................................154
Figure 6.21 Propulsion of PMMA particles a) Image taken at t = 0s, b) t = 66s, ..................155
Figure 6.22 Output from the particle tracking software. a) PMMA particle propulsion is presented in coloured line.................................................................156
Figure 6.23 Theoretical propulsion velocity of PMMA and polystyrene particles with varying input power ..........................................................................................................................157
Figure 6.24 Propulsion velocity of 10µm PMMA particles with varying input power. Propulsion velocity of polystyrene particles of the same size is also plotted for comparison .........................................................................................................................................158
Figure 6.25 Theoretical velocity of particles with varying refractive index ........................158
Figure 6.26 Propulsion of 8µm, 10µm and 12µm polystyrene particles on PEG-functionalised and plain waveguide surfaces .......................................................... 159

Figure 6.27 Propulsion of lymphoblastoma cells a) Image taken at $t = 0s$, b) Image taken at $t = 666s$, c) Image taken at $t = 1333s$ and d) Image taken at $t = 2000s$ ........................................... 161

Figure 6.28 Propulsion of the lymphoblastoma cells is tracked in  and coloured lines 162

Figure 6.29 Propulsion of lymphoblastoma cells ($\approx 10\mu m$) with varying input power.

Propulsion of 10µm PMMA particles is also plotted for comparison .................. 163

Figure 6.30 Images showing 20µm a polystyrene particle and a TERA1 cell of similar size a) without the laser on, b) with the laser on (without filter). c) The corresponding line profile of the scattering image .............................................................. 164

Figure 6.31 Graph showing velocity of cells moving towards the illuminated channel with varying waveguide widths ................................................................. 167

Figure 6.32 Tracked motion of the TERA1 cell towards the illuminated channel in a) in the input facet direction and b) towards the output facet against the distance from the input facet .......................................................................................................................... 168

Figure 6.33 Motion of the cell towards the input facet, a) step size histograms and b) the rose plot of trajectory angle for 0µm, 5µm and 10µm distance from the channel waveguide edge ............................................................................................................. 169

Figure 6.34 Motion of the cell towards the output facet, a) step size histograms and b) the rose plot of trajectory angle for 0µm, 5µm and 10µm distance from the channel waveguide edge ........................................................................................................... 170
Declaration of Authorship

I, Mukhzeer Mohamad Shahimin, declare that the thesis entitled Propulsion of Polymer Particles on Caesium Ion-exchanged Channel Waveguides for Stem Cell Sorting Applications and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

• this work was done wholly or mainly while in candidature for a research degree at this University;

• where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

• where I have consulted the published work of others, this is always clearly attributed;

• where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

• I have acknowledged all main sources of help;

• where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

• parts of this work have been published as shown in the List of Publications.

Signed:...............................................
Date:...................................................
Dedicated to my son, Umayr...
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Nomenclature

\( a \)  \quad \text{Radius}
\( \alpha \)  \quad \text{Damping constant}
\( a_{lm} \)  \quad \text{Partial wave coefficient for the scattered field}
\( A_{lm} \)  \quad \text{Partial wave coefficient for the incident field}
\( \text{ABC} \)  \quad \text{Antibody binding capacity}
\( \text{ABT} \)  \quad \text{Arbitrary Beam Theory}
\( \text{AC} \)  \quad \text{Alternating current}
\( \text{AML} \)  \quad \text{Acute myeloid leukemia}
\( \text{ATCC} \)  \quad \text{American Type Culture Collection}
\( \beta \)  \quad \text{Propagation constant}
\( b_{lm} \)  \quad \text{Partial wave coefficient for the scattered field}
\( B_{lm} \)  \quad \text{Partial wave coefficient for the incident field}
\( \text{BCT} \)  \quad \text{Body-centred tetragonal}
\( \text{BSA} \)  \quad \text{Bovine serum albumin}
\( C \)  \quad \text{Component of a concentration}
\( c \)  \quad \text{Speed of light in vacuum}
\( \text{CCD} \)  \quad \text{Charge-coupled device}
\( C_F \)  \quad \text{Faraday constant}
\( C_G \)  \quad \text{Gas constant}
\( \text{CHO} \)  \quad \text{Chinese hamster ovary}
\( C_p \)  \quad \text{Specific heat capacity}
\( C_s \)  \quad \text{Maximum solid solubility of the diffusing substance}
\( C_{sc} \)  \quad \text{Surface charge}
\( C_{sp} \)  \quad \text{Spring constant of the cantilever}
\( \text{CTC} \)  \quad \text{Circulating tumour cell}
\( \text{CW} \)  \quad \text{Continuous wave}
\( D \)  \quad \text{Dimension}
\( d \)  \quad \text{Distance}
\( d \)  \quad \text{Depth}
\( d_f \)  \quad \text{Depth for Fermi function}
\( d_{erfc} \)  \quad \text{Depth for complementary error function function}
\( D_c \)  \quad \text{Diffusion coefficient}
\( \text{DC} \)  \quad \text{Direct current}
\( \text{DEP} \)  \quad \text{Dielectrophoresis}
\( \text{DI} \)  \quad \text{De-ionised}
\( \text{DLVO} \)  \quad \text{Derjaguin, Landau, Verwey and Overbeek}
\( \text{DMEM} \)  \quad \text{Dulbecco’s Modified Eagle’s Medium}
\( \text{DNA} \)  \quad \text{Deoxyribonucleic acid}
\( D_o \)  \quad \text{Maximum diffusion coefficient}
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE</td>
<td>Diffractive optical element</td>
</tr>
<tr>
<td>$E$</td>
<td>Electric field</td>
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<tr>
<td>$e$</td>
<td>Electron charge</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Permittivity</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Permittivity of free space</td>
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<td>EP</td>
<td>Electrophoresis</td>
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<tr>
<td>$F$</td>
<td>Force</td>
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<tr>
<td>$f$</td>
<td>Frequency</td>
</tr>
<tr>
<td>$F_{\text{abs}}$</td>
<td>Absorption force</td>
</tr>
<tr>
<td>$F_b$</td>
<td>Buoyancy force</td>
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<tr>
<td>FC</td>
<td>Fractionation chamber</td>
</tr>
<tr>
<td>$F_{\text{DL}}$</td>
<td>Double layer force</td>
</tr>
<tr>
<td>$F_g$</td>
<td>Gravitational force</td>
</tr>
<tr>
<td>$F_{\text{grad}}$</td>
<td>Gradient force</td>
</tr>
<tr>
<td>FFM</td>
<td>Friction force microscopy</td>
</tr>
<tr>
<td>$F_{\text{lateral}}$</td>
<td>Frictional force</td>
</tr>
<tr>
<td>$F_{\text{scat}}$</td>
<td>Scattering force</td>
</tr>
<tr>
<td>$F_{\text{normal}}$</td>
<td>Load applied on the cantilever</td>
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<tr>
<td>$F_W$</td>
<td>Van der Waals force</td>
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<tr>
<td>FWHM</td>
<td>Full-width-half-maximum</td>
</tr>
<tr>
<td>$G$</td>
<td>Shear modulus</td>
</tr>
<tr>
<td>$g$</td>
<td>Gravitational acceleration</td>
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<td>$H$</td>
<td>Magnetic field</td>
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<tr>
<td>$h$</td>
<td>Propagation constant in the x direction</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid</td>
</tr>
<tr>
<td>HOT</td>
<td>Holographic optical tweezers</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>$J$</td>
<td>Flux</td>
</tr>
<tr>
<td>$K$</td>
<td>Geometrical factor of the cantilever</td>
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<tr>
<td>$k$</td>
<td>Boltzmann constant</td>
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<tr>
<td>$k_0$</td>
<td>Propagating wavefront</td>
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<tr>
<td>$\kappa$</td>
<td>Debye length</td>
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<tr>
<td>$L$</td>
<td>Length of steps</td>
</tr>
<tr>
<td>$L_c$</td>
<td>Length of the cantilever</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid crystal display</td>
</tr>
<tr>
<td>LTRS</td>
<td>Laser tweezers Raman spectroscopy</td>
</tr>
<tr>
<td>$M$</td>
<td>Total number of modes</td>
</tr>
<tr>
<td>$m$</td>
<td>mass</td>
</tr>
<tr>
<td>MCA</td>
<td>Magnetically conjugated antibodies</td>
</tr>
<tr>
<td>MDR</td>
<td>Morphology Dependent Resonance</td>
</tr>
<tr>
<td>MEMS</td>
<td>Micro-electro-mechanical system</td>
</tr>
<tr>
<td>$m_n$</td>
<td>Mode number</td>
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<tr>
<td>$n$</td>
<td>Refractive index</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Viscosity</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
<td>-------------</td>
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<tr>
<td>$n_i$</td>
<td>Cover index</td>
</tr>
<tr>
<td>$n_s$</td>
<td>Core index</td>
</tr>
<tr>
<td>$n_j$</td>
<td>Substrate index</td>
</tr>
<tr>
<td>NB</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>$n_{\text{eff}}$</td>
<td>Effective index</td>
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<tr>
<td>NIR</td>
<td>Near infra-red</td>
</tr>
<tr>
<td>$n_p$</td>
<td>Refractive index of the prism</td>
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<tr>
<td>$n_s$</td>
<td>Number of steps</td>
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<tr>
<td>$\theta$</td>
<td>Angle of propagation</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$\theta_c$</td>
<td>Critical angle</td>
</tr>
<tr>
<td>$\theta_p$</td>
<td>Angle of prism</td>
</tr>
<tr>
<td>OPA</td>
<td>One-photon absorption</td>
</tr>
<tr>
<td>$P$</td>
<td>Pressure</td>
</tr>
<tr>
<td>$p$</td>
<td>Decay constants for the substrate layer</td>
</tr>
<tr>
<td>$P_d$</td>
<td>Mean diameter of a particle size</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>$\rho_i$</td>
<td>Density of molecules in the wall</td>
</tr>
<tr>
<td>$\rho_s$</td>
<td>Density of molecules in the sphere</td>
</tr>
<tr>
<td>$P_{\text{fibre}}$</td>
<td>Output power of the fibre</td>
</tr>
<tr>
<td>$\rho_m$</td>
<td>Density of surrounding medium</td>
</tr>
<tr>
<td>$\rho_{\text{oi}}$</td>
<td>Ion’s concentration at bulk</td>
</tr>
<tr>
<td>$\rho_p$</td>
<td>Density of a particle</td>
</tr>
<tr>
<td>$\rho_{xi}$</td>
<td>Ion’s concentration at position $x$</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PER</td>
<td>Post exposure recovery</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>$P_{\text{pixel}}$</td>
<td>Pixel value</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinyl pyrrolidone</td>
</tr>
<tr>
<td>$P_{\text{waveguide}}$</td>
<td>Output power of the waveguide</td>
</tr>
<tr>
<td>$q$</td>
<td>Decay constants for the cover layer</td>
</tr>
<tr>
<td>$Q$</td>
<td>Molar activation energy</td>
</tr>
<tr>
<td>QMS</td>
<td>Quadrupole magnetic sorter</td>
</tr>
<tr>
<td>$R$</td>
<td>Radius of a sphere</td>
</tr>
<tr>
<td>$r$</td>
<td>Distance travelled (2D) in Brownian motion</td>
</tr>
<tr>
<td>$R_o$</td>
<td>Refraction per mole of oxygen atoms</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>$R_q$</td>
<td>Gas constant</td>
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<tr>
<td>RMS</td>
<td>root-mean-square</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>$r_{\text{TE}}$</td>
<td>Amplitudes of the reflected light in TE</td>
</tr>
<tr>
<td>$r_{\text{TM}}$</td>
<td>Amplitudes of the reflected light in TM</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SLOT</td>
<td>Scanning laser optical trap</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
</tr>
<tr>
<td>$\tan(\psi)$</td>
<td>Amplitude ratio upon reflection</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Torque</td>
</tr>
<tr>
<td>$t_c$</td>
<td>Thickness of the cantilever</td>
</tr>
<tr>
<td>TE</td>
<td>Transverse electric</td>
</tr>
<tr>
<td>$T_k$</td>
<td>Thermal conductivity</td>
</tr>
<tr>
<td>TM</td>
<td>Transverse magnetic</td>
</tr>
<tr>
<td>$T_{\text{pixel}}$</td>
<td>Pixel threshold</td>
</tr>
<tr>
<td>TPA</td>
<td>Two-photon absorption</td>
</tr>
<tr>
<td>$T_Q$</td>
<td>Heating power per unit volume</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Coefficient of friction</td>
</tr>
<tr>
<td>$\mu_0$</td>
<td>Permeability of free space</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>$V$</td>
<td>Volume</td>
</tr>
<tr>
<td>$v$</td>
<td>Velocity</td>
</tr>
<tr>
<td>$V_o$</td>
<td>Volume per mole of oxygen atoms</td>
</tr>
<tr>
<td>VCSEL</td>
<td>Vertical cavity surface emitting laser</td>
</tr>
<tr>
<td>$W$</td>
<td>Interaction energy</td>
</tr>
<tr>
<td>$w$</td>
<td>Steepness of the refractive index profile</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular velocity ($2\pi f$)</td>
</tr>
<tr>
<td>$w_c$</td>
<td>Width of the cantilever</td>
</tr>
<tr>
<td>$x$</td>
<td>$x$ - axis</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Percentage of cations exchanged for incoming ions</td>
</tr>
<tr>
<td>$x_{ds}$</td>
<td>Deflection sensitivity</td>
</tr>
<tr>
<td>$Y$</td>
<td>Young’s modulus</td>
</tr>
<tr>
<td>$y$</td>
<td>$y$ - axis</td>
</tr>
<tr>
<td>$Y_{lm}$</td>
<td>Spherical harmonics</td>
</tr>
<tr>
<td>$\psi_l$</td>
<td>Riccati-Bessel function</td>
</tr>
<tr>
<td>$\psi_m$</td>
<td>Potential at middle position between two surfaces</td>
</tr>
<tr>
<td>$\psi_0$</td>
<td>Surface potential</td>
</tr>
<tr>
<td>$\psi_x$</td>
<td>Electrostatic potential at position $x$</td>
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<tr>
<td>$z$</td>
<td>$z$ - axis</td>
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<tr>
<td>$z_i$</td>
<td>Valency of an ion</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>Zeta potential</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>(Delta) Phase shift difference</td>
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Chapter 1

Introduction

1.1 Aims and motivation

The optical trapping technique has proven to be a very useful tool that offers a non-contact method for precise particle handling. The pioneering work by Ashkin in 1970 showed that the forces of radiation pressure from focused laser beams could be used to significantly affect the dynamics of small transparent micrometer-sized neutral particles [1]. It was shown experimentally that, using just these forces, small micrometer-sized neutral particles could be accelerated, decelerated and even stably trapped using focused laser beams. Over the years, these newly found laser trapping and manipulation techniques were found to apply over a wide range of particle types, including particles as diverse as atoms [2], molecules [3], submicron particles and microscopic dielectric particles [4] hundreds of micrometers in size. Even living biological cells and organelles within cells can be trapped and manipulated free from optical damage (at certain wavelengths and power) [5].

In view of the advancements in the technique, the following aims are outlined for this project:

- Reviewing competing technologies in trapping and sorting of particles and cells

- Theoretical evaluation of the non-optical forces; drag force, Brownian motion, gravitational, buoyancy and electrostatic force, effect on the interaction of particles and cells with the surface
• Simulation of varying optical channel waveguide parameters to produce an optimum surface intensity for achieving stable trapping and propulsion

• Fabrication and characterisation of optical channel waveguides, with varied channel widths and ion-exchange times, for investigating trapping and propulsion of particles and cells

• Brownian motion study of particles and cells on surfaces

• Investigation of the effect of optical parameters and different optical waveguide characteristics for an optimum propulsion of polymer particles

• Evaluation of the device using mammalian cells; namely lymphoblastoma and teratocarcinoma cells.

The utilisation of evanescent waves for optical trapping has so far been limited, as reviewed in Section 1.3. The most comprehensive work to date was done by Ng involving gold nanoparticles [6]. This motivates the use of the evanescent wave trapping technique to develop a novel method for sorting particles and biological cells according to their optical properties under optimised conditions. Using optical trapping and propulsion on a channel waveguide device allows the manipulation of several particles and cells simultaneously. This has a significant clinical advantage for biological applications where high throughput is required. Furthermore the channel waveguide configuration permits integration with microsystems for a lab-on-a-chip device. This is expected to pave the way for low cost, robust and simple integrated optical devices to be fabricated and optimised, allowing this technology to become applicable to a practical system. Any application depends strongly upon the physical characteristics of the waveguide for optimum control of the particles and the applicability for biological purpose; more specifically in this case, mammalian cells. Therefore this thesis explores these two aspects for caesium ion-exchanged channel waveguides.

1.2 Overview of stem cells

There is a significant potential for stem cells to be exploited for regenerative medicine. Stem cells can be defined as cells with self-renewal and pluripotency properties [7-9]. Self-renewal in this case refers to the ability to maintain undifferentiated state after numerous cell division cycles. Pluripotency, on the other hand, is the capacity to differentiate into specialised cell types. Stem cells act as a
repair system for the body by differentiating into specialised cells and replenishing cells in regenerative organs such as skin or intestinal tissues [7-9]. Hence stem cells can be utilised to develop into many different cell types in the body for medical therapies. Currently, the number of people needing a transplant for diseased or destroyed organs far exceeds the number of donated organs or tissues available for transplantation. Theoretically, stem cell therapy has the potential to dramatically change the treatment of a myriad of diseases, conditions, and disabilities including Parkinson's and Alzheimer's diseases, diabetes, leukaemia, spinal cord injuries, muscle damage and rheumatoid arthritis [10-12].

Stem cells can be harvested from adult organs such as bone marrow or from a blastocyst, as shown in Figure 1.1. Cells derived from a blastocyst are called embryonic stem (ES) cells. Another alternative to pluripotent stem cell resource is embryonic germ (EG) cells [14]. EG cells are derived from primordial germ cells found in the gonadal ridge of a late embryo. Since the development of embryonic stem cell lines by Thompson [15] in 1998, scientists have been able to manually isolate, grow and carry out experiments on the stem cells. However, the only existing successful stem cell therapy so far is to treat leukaemia and related bone/blood cancers through bone marrow transplants [16]. Apart from the policy restrictions, the researches conducted were unable to differentiate stem cells into usable cells (with or without transplant rejection) [17]. The recent reversal in policy regarding ES cells trials on humans is expected to give a significant impact in the research field [18].

Figure 1.1 Schematic showing the development of stem cells from the source (adult organs and embryonic cells) into specific cells [13]
There has been extensive research on stem cell therapy, yet remarkably little is known about the molecular mechanisms that underlie the pluripotency of stem cells. Hence, there is a need for an approach to provide a pure population of stem cells that is free from mechanical (fluid shear stress, cyclic stretch and pressure), electrical (field induced) or chemical (need for labelling) induced cellular response. Such an approach will be able to provide the effective characterisation and study of different stem cell populations and ultimately clearer strategies for regenerative medicine. Optical trapping and propulsion is seen as a potential candidate as a sorting technique that avoids detrimental effects on the stem cells.

1.3 Overview of optical trapping and propulsion

The optical manipulation of microscopic particles is a powerful non-mechanical, non-destructive and highly precise technique with extensive practical applications. Radiation pressure is the key point in the optical manipulation of particles and cells. From a theoretical electromagnetic approach, Maxwell was able to confirm the concept of radiation pressure, introduced by Kepler [19]. It was known then that light had a linear and angular momentum, and therefore could exert radiation pressure and torque on physical objects. These effects were however found to be very small and not easily detected. Nichols, Hull [20] and Lebedev [21] were the first to succeed in experimentally detecting radiation pressure on macroscopic objects and absorbing gases. It was not until the invention of the laser [22], that the study of radiation pressure and optical trapping developed tremendously. Since 1970, when Arthur Ashkin and co-workers first demonstrated optical trapping [1], extensive research has been carried out involving various particles such as gold spheres [23], latex [24], bacteria [25], yeast cells [26] and silica [27].

![Figure 1.2 Schematic of optical forces acting on a particle on a channel waveguide](Figure 1.2 Schematic of optical forces acting on a particle on a channel waveguide)
A particle in the optical field experiences three main forces, an axial scattering force, $F_{\text{scat}}$, a gradient force, $F_{\text{grad}}$, which can act in both the axial (due to losses along the propagation of light) and the radial direction (due to variation in the intensity profile), and an absorption force, $F_{\text{abs}}$, which is dependent upon the complex refractive index of the particle. A schematic of the optical forces is illustrated in Figure 1.2. There are different methods utilised to manipulate these optical forces for trapping particles. Svoboda and Block [3] trapped 36nm gold particles and 38nm latex particles in a single beam trap (SBT) method with 1047nm wavelength radiation. The ratio of the magnitude of the polarisabilities and the trapping strength were similar for both particles. Metal particles used by Sato, Harada and Waseda [28] were found to trap only when the laser beam focus was located near the bottom of the particle. Furukawa and Yamaguchi [29] demonstrated that gold particles moved towards the focus of the single beam gradient force used in their experiments. These experiments led to the utilisation of evanescent field, as shown by Kawata and Sugiura [27], produced using a sapphire prism. Latex and glass particles were found to be propelled using their experimental setup. Kawata later showed with Tani [23] that latex, gold and platinum particles, with diameters from 0.5 to 5µm, can be trapped and propelled at up to 14µms$^{-1}$ in the evanescent field of a waveguide with 80mW modal power at 1047nm wavelength. Tanaka and Yamamoto [24] compared the velocity of 4µm latex particles in different excited modes, showing a reduction in speed as the mode number increases. More recently, Ng [6] showed that 40nm gold particles could also be propelled and demonstrated the effects of optical parameters on the speed of the particles. Ng managed to propel 10 to 40nm gold particles at up to 8µms$^{-1}$ and concluded that transverse magnetic (TM) polarised light consistently propels particles at higher velocities.
Grujic [30] presented work on propulsion of microspheres using caesium ion-exchanged waveguides and observed the formation of particle chains as well as particle binding. Grujic later advanced her work on particle propulsion by demonstrating particle sorting using a Y-branched optical waveguide [31]. Continuing work by Ng and Grujic, Hole [32, 33] showed a statistically significant velocity distribution of 250nm gold particles in an evanescent field. Gaugiran used a silicon nitride waveguide to show the propulsion of yeast cells and erythrocytes at 1 ums⁻¹ for a laser power of 60mW and 40mW respectively [4, 34]. In light of the above review, it is quite plain to see that in spite of the effort used to propel multiple types of particles and cells on optical channel waveguides, optimisation (in terms of channel waveguide width or depth) for microsystem integration have not been explored. In addition, experimentations involving adherent cells, such as stem cells, in channel waveguide system have not been investigated. Hence, this project is intended to fill in the gap of knowledge and move a step closer to the realisation of microsystem integration for lab-on-a-chip devices.

1.4 Interaction of light with mammalian cells

Optical trapping and propulsion utilising evanescent fields has been successful on metallic and polymer particles. However, in order to apply the same method for stem cells, it is essential to review the effect of light on the viability and structure of the cells. In this section, the interaction was investigated of three aspects; wavelength, laser power and laser operation on mammalian cells.

1.4.1 Laser wavelength

The size of a mammalian cell typically ranges from 10µm to 30µm in diameter [35] with organelles such as nuclei (3 – 10µm in diameter), mitochondria (1 – 4µm in length, 0.3 – 0.7µm in diameter) and lysosomes (0.2 – 0.5µm in diameter) [36, 37]. Therefore, mammalian cells and the organelles within the cell can be smaller or much larger than the wavelength of light used. The interaction of mammalian cells and light can lead to scattering and also absorption. Light can be absorbed and scattered by various organelles within the cell. Proteins for instance, absorb at a wavelength around 240nm [38], however protein may also contain other molecules such as chromophore (heme group) in haemoglobin (in red blood cells - RBC). RBCs have strong absorption peaks around 280nm, 420nm, 540nm and 580nm [38]. Carbohydrates, that are located mainly in membrane structures, absorb at a wavelength below 230nm; while DNA absorbs at 230-300nm [38]. When the energy
from the light that is absorbed by these components reaches its tolerance limit, the absorbed energy is converted into heat. The resultant local heating may cause, primarily, the breaking of the intermolecular bonds around the heated components and the local denaturation of the membranes surrounding the protein. DNA was shown to be damaged when it absorbed light at a wavelength 230-300nm, due to the absorption by the purine and pyrimidine bases [38].

![Absorption Spectrum for Water](image)

**Figure 1.4 Absorption spectrum for water within the 200-2000nm window**

Due to the high concentration in biological cells, water is considered as the most important components in investigating the interaction of light with cells. Figure 1.4 shows the absorption spectrum of water over the wavelength range 200-2000nm. Between 200nm and 900nm, there exists a region of relatively low absorption. Above 900nm, the absorption increases fairly rapidly to a peak at about 970nm, and following a minor trough, continues to increase at longer wavelengths into the mid-infrared region. The region of low absorption acts as a window of a good transparency (700-1300nm), allowing near infrared lasers to be used. Wavelength ranges from 780-1100nm have been commonly used for the handling and manipulation of cells. However it should be noted that two-photon absorption (TPA) may occur within this wavelength range that can cause cell damage [39]. TPA is the rate of absorption of light by a molecule that depends on the square of the light’s intensity. This is different from one-photon absorption (OPA), where the rate of absorption is linear with respect to input intensity.
Figure 1.5 Chinese hamster ovary (CHO) cell's cloning efficiency after exposure at 88mW for wavelengths varying from 700 to 1100nm [40].

Figure 1.6 Chinese hamster ovary (CHO) cell's cloning efficiency with varying exposure times at 88mW [40].
Liang [40] have shown the wavelength dependency in cell cloning efficiency. Cloning efficiency refers to the number of live offspring expressed as percentage of the total number of initial cells. A 100% cloning efficiency, for example after exposed to certain wavelength for a certain period, indicates that the ability of the cells to maintain genome without mutations, proliferate and form clones (unaffected by the laser exposure). Any significant damages to the organelles or the cell as a whole will be detrimental in the proliferation process. Figure 1.5 illustrates the results from experiments by [40] where the nuclei of Chinese hamster ovary (CHO) cells were exposed to a range of wavelengths with different exposure time. The cloning efficiency was tabulated and presented in Figure 1.5. Two wavelength ranges have been suggested to minimise damage, which are 800-850nm (the radiation wavelengths often used in phototherapy) and 950-990nm, as shown in Figure 1.5. The wavelengths are also observed in literature, as shown in [39, 41, 42]. These wavelengths have been shown to work through the subsequent cloning of trapped cells (human melanoma cells and Chinese hamster ovary (CHO) cells) [40, 43].

Figure 1.5 also illustrated that laser manipulation on biological cells operating around 740-760nm and 900nm should be avoided. The reason behind the clonal growth behaviour in the 700-1064nm wavelength range is expected as a result from a combination of both types of photon absorption that is detrimental in the proliferation process [40]. Other laser wavelengths, particularly 1064nm which will be used in this project, may be permissible, given that lower laser powers and/or shorter exposure times are employed (refer to Figure 1.6).

1.4.2 Laser power

Although the laser power used is commonly in the miliwatts region, the intensity of a tightly focussed laser means that the technique is dealing with power densities of more than megawatts per square centimetre (>10^6 Wcm^-2). Laser absorption in this case, can be high enough to lead to cell damage ('optocution'). This may occur with both, one (linear) and two-photon (non-linear) absorption (TPA) processes. There are two major effects from the absorption process; localised thermal heating and photochemical induced processes. Light can be converted into local heating by internal conversion (from absorption) and molecular vibrational relaxation in biological cells. Such conversion induces several detrimental processes such as protein denaturation due to conformational changes, loss of enzymatic activities and water vapourisation at strong heating leading to a disruption of cellular structure.
This effect can be seen in [40, 44] where increasing laser power from 88mW to 176mW was already decreasing the clonal growth by at least 40%. Although heat shock proteins (HSPs) may be expressed in response to the exposure of elevated temperature, they only provide a partial protection. Increased cellular tolerance due to HSPs may only be beneficial for non-lethal temperature increases [45-47]. Since most of the absorption leading to thermal heating occurs at short wavelengths, the near-infrared (NIR) spectral region is preferred to avoid cellular heating and competitive thermal forces. As a result, a large number of trapping studies have been conducted with Nd:YAG or Ytterbium doped fibre lasers operating at 1064nm. With the increased availability of tuneable Ti:Sapphire and compact diode laser sources, there has been substantial interest in developing optical traps at shorter NIR wavelengths than 1064nm, at which water absorption can drop by as much as 55% (at 830nm) [48].

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photofragmentation</td>
<td>Decomposition of chemical molecule into smaller chemical fragments by the cleavage of a chemical bond. For example, riboflavin molecule when exposed to short ultra violet (UV) light</td>
</tr>
<tr>
<td>Photoaddition</td>
<td>Combination of chemical molecule such as from cysteine (in protein) to thymine (in DNA), which leads to photochemical cross-linking</td>
</tr>
<tr>
<td>Photooxidation</td>
<td>Addition of an oxygen molecule from surroundings, such as photooxidation of cholesterol molecule</td>
</tr>
</tbody>
</table>

Table 1.1 Light induced processes in biological cells [38]

Apart from localised thermal heating, photochemical induced processes may be apparent due to the non-linear two-photon absorption. Chemical molecule such as riboflavin can be decomposed into smaller chemical fragments by the cleavage of a chemical bond when exposed to short UV light [38]. This process is called photofragmentation. Other light induced processes are listed in Table 1.1. Some of these processes can be detrimental to the biological cell, as demonstrated in [49]. An abnormal chromosome bridge was observed in mitotic potorous tridactylus (PtK2) cells when irradiated with a 130mW laser of wavelength 760nm for about 1 second, as illustrated in Figure 1.7. The study of the chromosome bridge effect demonstrated the damage potential due to absorption. Mitotic cells were hindered from dividing and hence they reduce the capability of clonal growth. This agrees with Figure 1.5 where no clonal growth was observed for wavelengths around 760nm. Several groups have experimented using 532nm wavelength lasers. This
wavelength is not suitable for biological applications as the wavelength have shown to cause mitochondrial alterations to CHO cells [50] and mammalian cell lysis [51, 52]. However, the wavelength does avoid heating in water, and hence is suitable for colloidal studies [53, 54].

![Figure 1.7 Formation of abnormal chromosome bridge after irradiation with a 760nm wavelength laser for 1 second. A) before irradiation, B) after 1 minute, C) after 8 minutes, D) after 11 minutes, E) after 13 minutes, F) after 28 minutes [49]](image)

### 1.4.3 Laser operation

There are extensive reports on exposure of biological cells for a range of wavelengths using pulsed (mode-locked) and continuous (CW) lasers. Studies conducted by [55] showed that both, CW and pulsed lasers (76MHz), have similar effects on primate animal models (retina) for approximately the same average power. Laser thresholds for minimal visible damage were found to be 23.6mW and 23.4mW (average power) for pulsed and CW exposures respectively. With exposure durations of about 0.25 seconds and about 23.5mW average laser power at 800nm wavelength, this study agrees with American National Standard for Safe Use of Lasers (ANSI Z136.1-2000) [56]. Given that the average laser power is low and the exposure time is short, pulsed and CW lasers can be construed to have similar effects on biological cells, as illustrated in Figure 1.8.
It should be noted that these results point to a thermal damage mechanism within the experimental exposure duration. The different effects from CW and pulsed laser can be observed when photochemical damage is taken into context. Research conducted by [57] looked into the photochemical damage of a pulsed (200 femtosecond) laser at 800nm wavelength on DNA strands. This study, using potorous tridactylus (PtK1) cells and human cystic fibrosis pancreatic adenoma carcinoma (CFPAC-1) cells, indicates that no visible damage appeared when cells were exposed to a low irradiance ($2.1 \times 10^6 \text{ Wcm}^{-2}$) for 20ms. Damage of the cells however was evidenced at high irradiance ($6.1 \times 10^6 \text{ Wcm}^{-2}$) when exposed for 20ms. A pulsed laser in this case can induce spatially confined DNA/chromatin damage. This damage is probably due to a multiphoton and plasma induced mechanism of ablation. Using a similar optical setting, research by [58] found that optical damage occurred on red blood cells even when the average power density of the pulsed laser was low ($~2.5 \times 10^6 \text{ Wcm}^{-2}$). No optical damage was observed at the same average power for a CW laser. A similar trend was also observed by [59] where at fixed average power (70-88mW), Chinese hamster ovary (CHO) cells survived longer after CW laser exposure compared to pulsed (360MHz) laser output from a multimode laser. Using a wavelength of 760nm, cells appeared to be damage (died or no clonal growth) after 20seconds pulsed laser exposure for CHO cells. Cells exposed to a CW laser on the other hand, appeared to last longer (300 seconds for CHO cells).

In conclusion, cell damage upon exposure of light is highly dependent on three major factors; laser wavelength, power/exposure time and laser operation. It was shown in [40] that within the 700-1300nm wavelength, there are two optimal wavelength range to minimise cell damage; 800-850nm and 950-990nm. However, other wavelengths are also permissible for cell handling and manipulation given that lower laser powers and/or exposure times are used. Water absorption actually peaked around 970nm within the 700-1100nm window. Hence, although 990nm is optimal for minimising damage, at high powers this wavelength may be detrimental as a thermal mechanism takes effect. Harmful thermal effects on biological cells may be less apparent at other wavelengths due to low water absorption.

Differences between pulsed and CW laser operation is apparent when photochemical damage and two-photon absorption is taken into account. CW laser exposure might have a higher damage threshold compared to pulsed laser exposure at a similar average power. This difference may exist due to the maximisation of the non-linearity effect (two-photon absorption) on chemical reaction over single-photon reaction. Although for the time being, American National Standard for Safe Use of Lasers (ANSI Z136.1-2000) and several other papers [55, 56] shows that CW and pulsed lasers have no different effects on biological cells, it should be noted that
these research studies are pointing to a thermal damage mechanism for a quarter second exposure. Pertaining to the above explanation, a CW laser operating at 1064nm was utilised for this project. The laser power to the waveguide was maintained at 0.5W (unless stated otherwise) giving a maximum surface intensity of $1.9 \times 10^6 \text{Wcm}^{-2}$ (refer Section 4.3.4.2); which is within the safety limit for biological applications ($3 \times 10^6 \text{Wcm}^{-2}$ at 1064nm [40]).

Figure 1.8 Exposure at 458nm wavelength with 500mWcm$^{-2}$. hTERT-RPE1 cells were stained with EthD-1/Calcein AM for live or dead viability test. A) after 1 hour post exposure recovery (PER) from CW exposure, B) after 24 hours PER from CW exposure, C) after 1 hour PER from pulsed exposure, D) after 24 hours from pulsed exposure [60]

### 1.5 Profiles of polymer particles and mammalian cells

In investigating optical trapping and propulsion, several types of polymer particles or cells are used according to the objective to be achieved in individual experiments. The polymer particles used in this project are polystyrene and polymethylmethacrylate (PMMA) particles. These particles are usually diluted in water, unless stated otherwise. Meanwhile the mammalian cells that are used for
the experiments are lymphoblastoma and teratocarcinoma cells. Lymphoblastoma and teratocarcinoma cells are diluted in culture media specific for each cell type. The same media is also used for the culturing process.

1.5.1 Polymer particles properties

The polystyrene particles used are monodisperse polystyrene microspheres supplied by Polysciences Inc. These particles are bought in different sizes; 1µm, 3µm, 6µm, 10µm, 15µm and 20µm in diameter (with a standard deviation of up to 2.6µm). Two more size ranges were bought later, 8µm and 12µm (with a standard deviation of less than 0.1µm). A smaller size deviation requires a more rigorous filtering by the manufacturer and hence the particles cost almost triple the price of a standard particle size. The reason behind the additional two particle sizes is to fill the size gap observed between 6-10µm and 10-15µm particles. Smaller standard deviation particles were purchased in the attempt to reduce the experimental errors. PMMA particles were bought from Microparticles GmbH of sizes 1µm, 3µm, 6µm and 10µm (with a standard deviation up to 0.2µm). All polymer particles used in this project were spherical in shape.

![Polystyrene particle size range as supplied by the manufacturer](image)

**Figure 1.9** Polystyrene particle size range as supplied by the manufacturer
The synthesis process for these particles causes a slight anionic charge from the sulfate ester on the particle's surface in order to reduce particle agglomeration. The particles are packed typically in a 2.5% solid in water suspension and can be prepared without surfactant. Shown in Figure 1.9 is the distribution of the polystyrene particles with individual mean particle diameter, $p_d$. The refractive index of the polystyrene and PMMA particles are $n = 1.59$ and $n = 1.49$ respectively at 633nm wavelength [61-63]. The density of polystyrene particles is 1.05g/cm$^3$ and 1.19g/cm$^3$ for the PMMA particles [61, 62].

### 1.5.2 Mammalian cells properties

There are several characteristics needed to be investigated in order to ensure the compatibility of the system for optical trapping and propulsion of stem cells. For this purpose, characteristics of a mammalian cell type were listed. The cells should be non-adherent, at least pseudo-spherical and, if possible, have a high refractive index. Several potential cell lines have been identified namely lymphoblastoma (Cell line code in American Type Culture Collection (ATCC): HS 602), retinoblastoma (Weri-RB1) and gastric carcinoma (KATO II). These potential cell lines are established human cell line and they are malignant cancer cells. Lymphoblastoma cells were seen as a good candidate not only because the cells are non-adherent and pseudo-spherical in shape, but also they are readily available and easy to culture. The investigation of optical trapping and propulsion on lymphoblastoma was carried out first before applying the technique to teratocarcinoma cells, the cell type that is targeted for the system application. Teratocarcinoma cells are a germ cell tumour; the closest equivalent to stem cells [64, 65]. Apart from readily available, teratocarcinoma cells are useful for testing the feasibility of the system for handling stem cells. There are two teratocarcinoma cell lines used in this project, namely TERA1 (a stable, undifferentiated cell line) and NT2 (prone to cell differentiation).

![Figure 1.10 Lymphoblastoma cells, a) in RPMI culture media, b) mixed with 0.4% Trypan blue solution. Cells coloured in blue are dead cells.](image)
Lymphoblastoma cell line (malignant white blood cell) and teratocarcinoma cell lines (testicular germ cell tumour) were obtained from Southampton General Hospital. The lymphoblastoma cells were placed in a Roswell Park Memorial Institute (RPMI-1640) medium, while the teratocarcinoma cells were placed in Dulbecco’s Modified Eagle’s Medium (DMEM). Each medium is formulated specifically for the cell type (refer to Appendix A for the media formulations). Both culture media were supplemented with 10% heat inactivated foetal bovine serum to supply a variety of proteins for cell growth and 1% of penicillin-streptomycin for prevention and elimination of bacterial contaminants in the cell culture. Phenol red or phenolsulfonphthalein was added to the culture medium as a pH indicator. Phenolsulfonphthalein is a weak acid that changes colour from red to yellow as an indication of pH transition from 6.6 to 8.0. As both cells lines prosper at blood’s pH, which ranges from 7.35 to 7.45, such a colour code is an essential indication to maintain the cell line to be grown close to this physiological pH. As an alternative, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) can be added instead of phenol red to maintain pH of the culture media outside the incubator. HEPES is
an organic chemical buffering agent that is widely used in cell culture to maintain physiological pH.

The lymphoblastoma cells were cultured in suspension and in a grape-like structure. Figure 1.10 a) illustrates the grape-like structure and individual lymphoblastoma cells. On the other hand, teratocarcinoma cells adhered to the flask floor and formed a layer of cells in a network of proteins as shown in Figure 1.11 a). Trypsin solution, which is an enzyme found in the digestive system, was utilised to break down the proteins that hold the cells together. Pipetting the cell solution up and down helps to disperse agglomerated cells into single cells. A complete protocol for cell culture processes is given in Appendix B.

![Cell Cycle Diagram](image)

**Figure 1.12 Schematic of the cell cycle, M=Mitosis, G1=Gap 1, G2=Gap 2, S=Synthesis, Go=Gap 0/Resting. The duration of mitosis in relation to the other phases has been exaggerated in this diagram [66-68]**

The size of the lymphoblastoma cells varies from 8µm to 12µm diameter while teratocarcinoma cells size ranges from 16µm to 20µm. The variation in the cell size corresponds to the stages in the cell cycle [69]. There are four distinct phases of cell cycle, as illustrated in Figure 1.12 [66-68]. G₃ phase is where various enzymes are synthesized for the DNA replication and G₂ phase occurs when a significant number of proteins are synthesized, which is required for mitosis (cell division). S phase shows that the amount of DNA is effectively doubled and M phase marks the process of nuclear and cytoplasmic division (mitosis). G₀ phase signifies that the cell is no longer in the cell cycle, indicating that the cell is either fully differentiated, damaged (necrosis) or about to go through apoptosis (cell death). The number of sub-cellular components fluctuates according to the cell stage. Figure 1.13 shows the refractive index of each component. The refractive index of cells used in this project was assumed to be ≈1.39. The density of the cell was also assumed to be ≈1.2g/cm³.
The cells can be determined of their viability by mixing them with a 0.4% Trypan blue solution (toxic). Live cells or tissues with intact cell membranes are not coloured with the blue solution. Since live cells are very selective in the compounds that pass through the membrane, in a viable cell Trypan blue is not absorbed, however it traverses the membrane in a dead cell. Hence, dead cells are shown as a distinctive blue colour under a microscope, as illustrated in Figure 1.10 b). A healthy cell will have a pseudo-spherical shape while the dead ones will usually look ‘ruptured’. Figure 1.13 also shows the cell membrane. The cell membrane is locally rough due to protruding proteins and glycolipids [66, 67]. The locally rough cell membrane and ruptured cell may introduce more friction and adhesion compared to polymer particles. The information given in this section provides the basis of the theoretical models presented in later chapters.

Figure 1.13: Schematic of a generic eukaryotic cell, showing subcellular components. Organelles: (1) nucleolus (2) nucleus (n=1.39) (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (n=1.40) (10) vacuole (11) cytoplasm (n=1.38) (12) lysosome (13) centrioles (*) cell membrane (n=1.48) [66, 67, 70-79]. The insert shows details of cell membrane (phospholipid bilayer) with cholesterol, glycolipid and proteins.
1.6 Thesis structure

This thesis is separated into several chapters covering different aspects of the research. An overview of different techniques and procedures currently used for sorting is presented in Chapter 2. Conventional particle and cell sorting techniques such as mechanical and electrical trapping are discussed in this chapter. Optical sorting methods such as laser tweezers, optical lattice and waveguide are also thoroughly reviewed. Disadvantages and advantages of each conventional and optical method are discussed towards the end of each section. This chapter highlights the knowledge gap in the waveguide technology and the direction of the project.

The theoretical aspects of the project are tackled in two separate chapters. The investigations of the interaction of particles and cells on surfaces are presented in Chapter 3. This chapter examines the effect of non-optical forces acting on the particles, namely drag force, Brownian motion, gravitational force, buoyancy and electrostatic forces. The effects of thermal heating (convection current) and ionic concentration on the particles and cells are discussed towards the end of the chapter. A detailed theoretical description of the optical waveguides is described in Chapter 4. The theoretical evaluation is used to model the channel waveguide in order to achieve optimised waveguide characteristics. This chapter also highlights the fabrication steps as well as the methods used to characterise waveguides with their corresponding experimental results.

The experimental works were presented in the next two chapters. The investigation of Brownian motion of particles and cells is described in Chapter 5. The effect of different media and surfaces on Brownian motion of particles and cells is discussed. The works presented in this chapter describe the conditions and limitations to allow particles and cells to move freely in Brownian motion on surface. The investigation of particle trapping and propulsion forms the main subject in Chapter 6 where the technique and materials used are described in detail. The effect of optical parameters and waveguide conditions on particles of different sizes and refractive index is systematically discussed in this chapter. The investigation also covers the effects on different surfaces. The chapter continues with the evaluation of the optimised system on mammalian cells, namely lymphoblastoma and teratocarcinoma cells. Chapter 7 summarises and concludes the overall work done throughout this project. This chapter also provides a brief outline of potential future directions. Additional materials are compiled in the Appendix section.
Chapter 2

Sorting techniques for particles and cells

2.1 Introduction

Cell sorting and separation is essential in biomedical and biotechnological fields to isolate a specific cell population from a heterogeneous mixture of cells. Techniques for sorting cells can be utilised for various biochemical experimentation and analysis of living cells, such as cell cycle analysis for detecting cancerous precursors [80], stem cell research [81-83], microbiology [84-87] and genome analysis [88, 89]. In order to obtain reproducible data on the cell analysis, a reliable and non-destructive purification is necessary.

Depending on the technique employed, cell selection can often be based on multiple criteria at the same time, for example the cell size, cell shape or cell membrane characteristics. The current methods commonly used in biological laboratories for manipulation, concentration and separation of cells and particles include optical tweezers, fluorescence or magnetically activated cell sorting [90, 91], centrifugation, mechanical forces [92], filtration, and electric field based manipulation and separation [93]. Some of these methods are capable of manipulating large numbers of cells and particles simultaneously in a compact system.

These techniques, used in sorting and manipulating cells and particles, hugely benefit from microfabrication. Most, if not all, techniques for manipulating particles and cells can be integrated into microfluidic systems for standard analytical operations, as micromechanical devices are very well capable of manipulating single
objects with cellular dimensions (10-100µm). Attachment of monoclonal antibodies (mAbs) to a variety of substrates has also led to the development of highly sophisticated sorting tools capable of detecting and isolating a large numbers of cells based on a number of different criteria. Each cell type has its own complement of surface proteins (antigens) specific to that cell type. Antibodies can be produced which recognise these surface antigens and bind specifically to them. It should be noted that other molecules can be used to recognise other surface antigens, for example lectins bind to carbohydrates in the cell membrane. If the antibodies are attached to a substrate such as a fluorescent molecule or polystyrene particles, their binding to a cell surface allows a variety of separation schemes to be used.

2.2 Conventional particle and cell sorting

There are several techniques that can be utilised for trapping and sorting particles and cells. In this chapter these techniques are categorised into two types; non-optical and optical techniques. This section discusses the non-optical techniques, namely mechanical, electrical, magnetic and microfluidic sorting.

2.2.1 Mechanical sorting

Trapping biological cells mechanically was one of the earliest techniques used for sorting. Sorting in a microchip poses challenges due to the complex physical properties of biological cells such as different shape, size and surface properties. The basic concept of mechanical sorting is to use a regular lattice of asymmetric obstacles to interrupt the motion of the particles so that species of different sizes or different surface adhesion follow different trajectories through the device. Hence, a mixture of particles or cells injected in a fine stream would then be sorted continuously. Microfabricated mechanical filters have been described for trapping different cell types from blood [94-96]. These filters were usually made of comb-like silicon arrays of rectangular, parallel pillars on a chip, spaced between 5µm to 7µm apart using a wet etch process, as shown in Figure 2.1 a). These structures prevented particles larger than the channels from flowing through; hence separating particles of different sizes. Lillehoj [94], Bakajin [95] and Carlson [96] used hydrodynamic forces to move biological cells through a set of pillars that were strategically placed and fabricated in silicon. Mouse embryoid bodies (EBs) of different sizes were shown to be separated in [94] with 100% success. Meanwhile in [95, 96], erythrocytes (red blood cells) readily penetrated and passed through the lattice, while the white blood cells were greatly retarded and eventually adhered to the
surface. Figure 2.1 b) shows dyed white blood cells adhered to the surface at varied distances. Using a similar filter design, Anderson [97] used deep reactive ion etching to fabricate silicon pillars and arranged the design such as to create a reaction chamber. This confined microreactor chamber was used for trapping and for performing chemical reactions on particles.

Figure 2.1 a) Comb-type filter formed from an array of 120 posts (175μm long x 18μm wide) separated by 6μm channels set across a 3mm wide x 13μm deep silicon channel [95], b) Epifluorescence picture using the vital nuclear UV dye H33342 of white blood cells in the variable length array [96]

Figure 2.2 a) SEM image showing the transition from the 15μm wide channels to the 10μm wide channels [98, 99] b) SEM image showing the oblong obstacle of 3.5μm high and measuring 1.5 x 6.0μm, the gap between adjacent obstacles is 1.5μm wide. The electric field was applied in the V direction [100]

Mohamed [98, 99] has shown the isolation of cancerous cells, such as neuroblastoma (NB) and circulating tumour cell (CTC) when mixed with whole blood, based solely on size characteristics. This was achieved by fabricating a series of massively parallel microfabricated pillars using polydimethylsiloxane (PDMS), as shown in Figure 2.2 a). Cancerous cell have been seen to be isolated consistently in different channel widths, depending on the cell size, while blood cells migrated to
the output reservoir. A slightly different design for directed sorting by Chou [100] has the pillars arranged at an angle such that the probability of a particular particle to pass over one channel to the right ($p^+$) is more likely than the probability to pass over one channel to the left ($p^-$). The structure, as illustrated in Figure 2.2 b), was shown to separate DNA molecules of different lengths.

![Figure 2.3 Lateral percolation in microfabricated filter [92]](image)

The type of filtration method that has been discussed so far is known as axial percolation filtration as the filter acts along the flow axis. He [92] demonstrated another type of filtration; lateral percolation, a filtration method that commonly occurs in nature. The filter was fabricated using anisotropic gas phase etching in quartz consisting of a network of intersecting 1.5μm x 10μm channels and of about 10μm deep as shown in Figure 2.3. This network of channels was fabricated at the bottom of a reservoir, allowing particles to percolate through it while excluding larger, selected particles. This filter was shown to be efficient in sorting eukaryotic cells and *Escherichia coli*. A similar structure, fabricated in a silicon wafer using electrochemical etching in hydrofluoric acid, was shown by Tokranova [101]. The macroporous silicon structure was designed as an optimised filtration method for cell sorting and positioning in a micro-electro-mechanical system (MEMS) device. Sorting of particles and cells using mechanical methods has been successful. However, a major drawback of mechanical sorting is clogging. Apart from the size of the particles and cells, the characteristic used in mechanical trapping and sorting is surface adhesion. Hence, the method works until congestion of particles or cells stops the sorting flow. The problem may be rectified by reversing the flow to unclog the particles; however this solution might be difficult for adherent cells.
2.2.2 Magnetophoretic technique

Magnetic separation, as demonstrated by McCloskey [90], has exploited antibody binding capacity (ABC). ABC allows magnetically conjugated antibodies to bind to the targeted cells hence increasing the magnetophoretic mobility of the cells. The technique used by McCloskey has been shown to isolate different subpopulations of human hematopoietic progenitor cells (CD34) continuously and rapidly with low shear stress using a quadrupole magnetic sorter (QMS). A continuous particle sorting method based on differences in magnetophoretic mobility was also developed by Moore [102]. Instead of using ABC, Moore has opted to use magnetic monodisperse microspheres. Using the QMS system, Moore managed to sort, continuously, red blood cells and magnetic particles into two fractions from a feed of typically two populations; magnetic and nonmagnetic. Figure 2.4 shows the QMS system.

Mirowski [103] developed a micromachined fluid cell platform that consists of patterned magnetic thin films acting as magnetic tweezers. The key operation of this device was creating a field gradient near the magnetic elements to sufficiently trap magnetic particles that were separated from patterned films by a thick nitride membrane. The device was projected to be able to trap and sort magnetic particles tethered to biological molecules with high location specificity according to their size and magnetic susceptibility. The force exerted on the particles can be adjusted by changing the applied magnetic field.
Another device utilising magnetic sorting was developed by Suzuki. Suzuki combined a microfluidic chaotic mixer and a magnetic field to facilitate the mixing of magnetic particles and cells in a microchannel with low Reynolds number (as low as $10^{-2}$) [104]. The concept of this device is illustrated in Figure 2.5. Both the magnetic particles functionalised with antibody and a combination of cells was mixed in the microchannel. A magnetic field was applied to this microchannel to induce a chaotic mixing and later separate the tagged cells. The chaotic mixing strategy was capable of working at a Reynolds number much less than one and can be used for other external forces such as dielectrophoresis (DEP) and electrostatic force. Similar to the method used by Suzuki, Wang [105] also developed a magnetic separation using the antigen-antibody reaction. Wang developed immunomagnetic latex from magnetic core-shell composite polymer latex to assist in the cell separation process. Antibody tagging is the key process in magnetic separation. Thus, any limitation in the available antibody, its specificity and suitability may directly influence the effectiveness of magnetic separation. Apart from that, antibody tagging requires an incubation period for the antibody to bind to the specific antigen. This increases the time taken for the sorting process.

2.2.3 Electric trapping and sorting

Different types of electric field can be applied for the manipulation of cells and particles. An electric field generated from direct current (DC) can be used for electrophoresis (EP) of charged particles while a non-uniform field can be used for dielectrophoresis (DEP) of polarisable (charged or neutral) particles. Electrophoresis has been used in conjunction with electro-osmosis for the electrokinetic transportation and separation of molecules and cells in microchannels [106, 107]. However, most biological cells have similar electrophoretic mobilities; electrophoresis for the manipulation of cells has limited
applications and is almost exclusively used for pumping (electroosmotic flow) [106, 107].

On the other hand, DEP has been successfully applied to manipulate and separate a variety of biological cells including bacteria, yeast and mammalian cells [93, 108-111]. When an inhomogeneous alternating current (AC) electric field is applied to a particle or a cell, a DEP force arises depending on the particle’s dielectric properties. Therefore, cells or particles having different dielectric characteristics will experience differential DEP forces when subjected to such a field. Figure 2.6 shows the dielectrophoretic crossover frequency for normal cells and cancerous cells. Cell separations can be achieved by electronically tune to the crossover frequency of a specific cell types, by superimposing different signal frequencies onto the microelectrodes. The isolated cells can then be used for additional biochemical, physical, or genetic analysis.

A cell separation and positioning system based on the DEP has been demonstrated by Holmes [112]. The microfabricated system used non-uniform electric fields produced by arrays of microelectrodes and operates in two different modes. The system can concentrate a single cell type from a heterogeneous mixture or separate different cell types along the length of the device. Figure 2.7 shows how a binary mixture of two cell types was first focused into the central plane and then the cells
followed distinct trajectories banding at different positions along the channel length. Separating different cell types has been investigated using DEP, for example breast cancer cells from normal cells [108, 113], bacteria from erythrocytes, viable from nonviable yeast cells [114] and erythroleukemia cells from erythrocytes [115]. As an enhancement of the technique used by Holmes, Fatoyinbo [116] and Müller [117] have constructed a 3D microsystem consisting of layers of electrode structures. Fatoyinbo constructed the electrodes using multiple drilled laminated structures to form channels bearing electrodes. While in Müller’s system, the electrodes, which are driven by an AC electric field, were positioned to create a funnel, an aligner, a cage and a switch. These systems were designed for the separation of eukaryotic cells (Jurkat) (flow rate up to 300µm/s) [117], polystyrene particles (flow rate up to 3500µm/s) [117], and viable/nonviable yeast cells (flow rate up to 22000µm/s) [116]. Similar techniques were also used by Fiedler for separating living mammalian cells (L929 mouse cells) from polystyrene particles [110] with flow rates up to 10000µm/s.

![Figure 2.7 Schematic diagram of the DEP particle separator [112]](image)

Recently, Chou [118] has reported an alternative way to construct DEP traps by patterning geometrical constriction in an insulating substrate, such as quartz, instead of employing metallic electrodes. The device demonstrated concentration and patterning of both single-strand and double-strand DNA. Similar ideas have been used by Masuda to perform cell fusion at the field constriction [119]. The constriction was used in a microfluidic channel to compress the electric field in a conductive solution such as ionic buffer. This creates a high field gradient with a local maximum, as illustrated in Figure 2.8. In contrast to metallic DEP electrodes, this technique can generate a very high electric field without gas evolution due to the electrolysis taking place at low frequencies (<1kHz).

The combined use of AC and DC fields, however, can complement the limitations of EP and DEP and potentially provides an integrated method for manipulating and separating not only biological cells but also macromolecules [109, 118, 120-124]. For example, in a sequential fashion, DEP was first used to isolate *Escherichia coli*
bacteria on a microchip from whole blood, followed by electronic lysis of the isolated *Escherichia coli* to release the genomic DNA/RNA. EP was then used to enhance hybridisation of the released DNA/RNA on a separate microchip [123, 124]. As AC and DC electric devices exploit intrinsic cellular properties in both the characterisation and sorting criterion, they offer the advantage that cell modification processes such as staining of antibodies are unnecessary. This has advantages from a research standpoint, where unmodified cells are desirable, and from a clinical standpoint, where specific antibodies or markers might not be available. Conversely, electric trapping still presents several disadvantages, including fabrication complexity, electrophoretic damage to sensitive biological cells, elevated temperature and formation of gas bubbles at the electrode surface.

Figure 2.8 Outline of the differences between metallic and electrodeless DEP trap [119]

### 2.2.4 Microfluidic sorting

The use of microfluidics as a versatile and powerful research tool is becoming a ubiquitous trend within diverse technological disciplines. Due to their size, microfluidic devices are able to exploit unique transport properties and provide the capability of parallel processing and high throughput. One example of these microfluidic devices for cell sorting applications was shown by Brody [125]. The H-shaped device exploited the rapid diffusion of small particles to exit through a different outlet from the larger particles that diffused more slowly. A comparable idea, that took advantage of the passive diffusion, was also applied by Oakey to create a laminar-flow-based device to separate *Escherichia coli* cells and polystyrene particles [126, 127]. Observations of the convergence between two laminar aqueous microfluidic streams showed an apparent lack of mixing and the onset of some molecular scale diffusion at the end of the channel, as shown in Figure 2.9. Using this method, the separation of human lymphocytes (CD4 cells) [128], cervical cancer cells (HeLa cells) [129] and rat basophilic leukaemia cells (RBL-1 cells) [130] have been demonstrated.
Figure 2.9 (a) The viscous nature of microfluidic flows is demonstrated in converging aqueous streams (each 30 μm in width). (b) The top stream is dyed to illustrate the lack of convective mixing that occurs under laminar conditions. (c) The outlet image demonstrates the presence of some molecular-scale diffusion, the only form of mixing in this system. The inlet and outlet are separated by 3000μm [126, 127]

An unconventional approach was shown by Sin who utilised antibody coated microfluidic chambers to deplete undesired cell types [131]. This technique completely eliminates the problem that occurred in conventional methods, such as magnetic sorting, where the processing time for intensive antibody pre-incubation step was too long. Using human lymphocyte cell lines, MOLT-3 and Raji cells, the device examined the dynamic cell binding behaviour on antibody coated surfaces under shear flow to obtain enriched cell subpopulations at the outlet. From a balanced mixture of MOLT-3 and Raji cells, the device recovered 100% MOLT-3 cells with anti-CD19 coated chambers. Utilising multiple streams from hydrodynamic focussing, Lee demonstrated the sorting of red blood cells and polystyrene particles in a two-channel flow cytometer device [132, 133]. Multiple sample streams were achieved through one inlet port to provide stable flow control and uniform flow rates, as illustrated in Figure 2.10. The actual particle/cell identification and sorting used optical sensing and DEP forces for directing particles and cells into corresponding outlet ports. A 3D concept for the hydrodynamic focussing has been introduced by Goranovic [134] using a chimney structure. This idea has been further developed by Yang in designing and fabricating a 3D hydro-focusing microcell sorter for his micro-flow cytometry device [135]. The structure was fabricated using SU-8, placed on a silicon substrate and bonded with a glass plate. The 3D microstructure, as shown in Figure 2.11, was used for coaxial sheathing and has been demonstrated to sort labelled tanned sheep erythrocytes via optical detection.

Another method that utilises hydrodynamic focussing is flow cytometry. Flow cytometry allows multiparametric analysis of the physical and chemical characteristics of single particles/cells flowing through a detection apparatus (optical or electronic). Hinterkörner used fluorescent detection flow cytometry to sort two different CHO cell lines according to their glucose uptake rates [136].
Fiedler on the other hand, uses dielectrophoresis for his miniaturised flow cytometry to sort polystyrene particles and L929 mouse cell [110].

![Schematic illustration of the working principle for two-channel flow cytometer](image)

The advantage of using flow cytometry is the ability to analyse thousands of particles/cells per minute (a very high throughput) of multiple characteristics. However, there will be limitation whenever antibody binding is used and the possibility of inducing cellular response from the chemical tagging, electric field or fluidic shear stress that can be detrimental to mammalian cells. Based on the examples shown in this section, microfluidic devices using passive diffusion and gravitational force seem to be ineffective for sorting particles and cells with their efficiencies never reaching 100% separation. However, by combining the technique with other active sorting methods such, as DEP or optical trapping, microfluidic devices are able to provide a fast, reliable and high throughput sorting mechanism.

![Schematic of the 3D hydro-focusing unit](image)
2.3 Optical cell trapping and sorting

Conventional cell sorters, as described previously, can be used for the purification of individual cells, but are not ideal techniques and have a number of disadvantages. Mechanical trapping and sorting on a microchip poses challenges because of the complex physical properties of biological cells and the techniques have only separated a limited number of cells before the captured cells altered the flow through the device. The magnetophoretic technique, on the other hand, is limited by the availability of antibody tagging that is needed for the sorting of different populations of particles and cells. Trapping and sorting techniques using an electric field or microfluidics have the possibility of physically damaging biological cells from the charging effects of the electric field and fluidic pressure. Approaches using light to separate cells from complex mixtures are particularly attractive because they avoid physical contact between cells and surfaces and reduce the damage potential. Cell membranes are highly deformable and excessive shear stresses induced during rough sorting can cause rupture of the membrane or even destruction of the cell. The optical method also avoids the usage of antibody tagging or any marker which is an advantage in a research perspective where unmodified cells are preferred.

2.3.1 Laser tweezer

Optical trapping for the manipulation and sorting of individual cells can be readily achieved by the use of laser tweezer-type devices. Optical tweezer arrays have been generated in several ways including the use of a rapid scan device [138], diffractive gratings [139] or a spatial modulator [140]. Optical tweezers work by capturing these...
cells or particles at the focal point of a laser beam. Depending on the index mismatch of the cell or particle with the surrounding, the optical tweezer can selectively manipulate the object without mechanical contact. Although cell handling can be very precise, only one cell at a time can be manipulated [25, 26, 141, 142]. Illustrated in Figure 2.12 is a sequence of pictures taken to demonstrate an optical handle as developed by Ozkan [137]. Several 2µm polystyrene particles were trapped using optical tweezers and used as a handle to collect a larger 5µm polytyrene particle that is not trapped by the laser beam.

The optical tweezer technique has also been studied using a femtosecond laser, as demonstrated by Mao [143]. The trapping capability of the system was determined by measuring the escape speed of the trapped targets versus the average trapping power. However, the results from the experiment showed that the minimum trapping power was the same for a conventional continuous wave (CW) laser and the femtosecond laser. Hence there was no significant advantage of the femtosecond laser over CW generated optical tweezers. Munce has developed a single cell analysis device that operate using a combination of optical tweezers generated by a Nd-YAG laser and optical scissors comprised of a pulsed nitrogen laser [144]. Optical tweezers were implemented to select a single acute myeloid leukemia (AML) cell, move it to a desired location and cause the cell to undergo lysis (cell disintegration) using optical scissors. Propidium iodide was used to test the membrane integrity after the laser scissors pulse. Figure 2.13 shows images of the AML cell using a fluorescent confocal microscope.

![Figure 2.13 Fluorescent confocal microscope images of an AML cell, a) placed in front of a channel, b) immediately after single shot of optical scissors, c) 1 second after laser shot, d) and 5 seconds after laser shot [144]](image)

Recently, Raman spectroscopy has been combined with optical tweezers to form a laser tweezers Raman spectroscopy (LTRS). In a LTRS system, the same laser beam that formed a laser trap was used to excite Raman spectra that contained intrinsic
molecular vibration properties. Using this technique, Xie has shown that biological cells in different physiological states can be identified by their Raman spectral signatures and then selectively manipulated to a clean collection chamber [145]. Although no sample preparation was required, the LTRS sorting method can only be used with a small sample and the system can be very time consuming. Scaling up the separation for millions of cells at a time, like those in blood samples, would be very challenging using such a system.

Advancement to a single optical tweezer, a technology called a rapidly scanning laser optical trap (SLOT) has been implemented where a single laser beam was used to trap multiple particles simultaneously and is controlled by a piezoelectric mirror. At rates as high as 1200 Hz, the beam jumped from one particle to another in a cycle, overcoming the Brownian motion to take place and significantly dislocates the particles. Introduced by Mio [146], the technique has been exploited to create an optical cell sorter [127] and microfluidic pumps and valves [127, 147, 148]. An example of this system is shown in Figure 2.14 where the technology was used to act as a colloidal valve. Despite its utility, SLOT was restricted by the piezoelectric elements that translate the mirror and hence has limited scalability.

![Figure 2.14 An actuated, three-way colloidal valve [127, 147, 148]](image)

A comparable method for manipulating multiple particle systems was demonstrated by Dufresne using holographic optical tweezers (HOTs) [139]. HOTs utilised a single beam and passed it through a diffractive optical element (DOE), usually an LCD. A computer wrote patterns to the DOE, which in turn induced phase and amplitude modulations in the beam [139]. An objective focused the altered beam and as a result, the modulations created a substantial number of traps within the sample. This technique has proven its ability to manipulate many particle arrays in an area.
of the order of 100um by 100um, in 2D as well as 3D [147]. However, HOTs require the operator to have complex computational skills and optics expertise.

Microfluidic systems, using a diode laser bar (by Applegate [150]) and a vertical cavity surface emitting laser (VCSEL) (by Ozkan [149]) have been demonstrated for the separation of cells and particles based on their size. While larger cells were deviated when passing through the laser's enlarged beam, smaller cells were not, and consequently, the separation of large and small particles can be achieved. This technique managed to overcome the scaling limitations of conventional scanning systems and avoid altering the basic laser mode. A schematic of the scanning system is shown in Figure 2.15. The system has demonstrated trapping and sorting of polystyrene particles of different sizes and also bovine erythrocytes.

### 2.3.2 Optical lattice

Akin to HOTs technique, an optical lattice, produced as an interference pattern, was used to separate particles and cells from mixtures based on size and refractive index.
The key difference to HOTs is that the technique is dynamically reconfigurable and can be exploited to influence different optical lattice configurations on matter to enforce a strong lateral separation for continuous processing. As illustrated in Figure 2.16, normally all particles would flow from chamber B to chamber D with the actuator. By introducing a three dimensional optical lattice, which is shown in Figure 2.16 to be a body-centred tetragonal (BCT) lattice, one type of particle was selectively pushed into the upper flow field in the fractionation chamber (FC). The device has demonstrated the separation between polymer and silica spheres; presented as blue and red dots respectively in Figure 2.16. The separation was projected to be able to distinguish different particle sizes and refractive indices.

The optical lattice was produced using a single laser beam that was passed through a diffractive beam splitter, producing four beams diverging from the central point in a cross shape. Collimating optics provided independent control to these beams before co-focussing it through an aspheric lens. This produced a large, 3D optical lattice through multi-beam interference, as shown in Figure 2.17. More recently, the same principles of creating a tuneable optical lattice have been used to separate erythrocytes from leukocytes with more than 95% efficiency [153]. This type of device is reconfigurable by adjusting the interference pattern and, because it has no narrow channels, it is less prone to clogging during its use. Figure 2.19 shows different interference patterns that can be produced and Figure 2.18 shows a comparison between different lattice types in terms of flow velocity and efficiency. These figures demonstrated that the linked BCT lattice has both the highest efficiency and the highest throughput when fractionating at 45° angle.
Another method of creating an optical lattice is by counter-propagating laser beams as shown by Bain [154-156]. The symmetry and dynamics of these 2D arrays in the evanescent field depends on the polarisation of the two laser beams. The creation of these 2D arrays can be exploited for particle and cell sorting by trapping a specific particle size in a flowing solution of different particle populations. The ability of the arrays to be dynamically changed via different configurations of the laser’s polarisation means that the system can be used to trap particles of different size in
the same experiment. However in order for the system to be used as an effective particle and cell sorting method, the system has to be combined with microfluidics system.

### 2.3.3 Bessel beam

Diffraction is a phenomenon fundamentally linked to the wave nature of light and occurs when a wave encounters an obstacle. In this process, the wave may be altered in amplitude and phase and diffraction takes place. A Bessel light beam on the other hand appears to have immunity to diffraction and hence is an attractive alternative to the conventional Gaussian beam. Figure 2.20 a) and b) show intensity profiles for the Bessel beam.

![Bessel beam intensity profiles](image)

**Figure 2.20** Bessel beam intensity profiles, a) for a zeroth order and b) a first-order beam, c) two lymphocytes aligned vertically in the beam centre and erythrocytes aligned in the outer rings of the Bessel beam

Using vertically oriented Bessel beams, the separation of erythrocytes and lymphocytes has been demonstrated [157, 158]. In this technique, biological cells were shown to reside within the static optical landscape that has been created using a Bessel light beam. The light beam offers an optical field in the form of a 2D circularly symmetric pattern where each ring has approximately equivalent power. This beam was generated by passing a Gaussian output beam through an axicon (a conical lens). A telescope was used to demagnify the generated Bessel beam to a smaller core radius and shorter propagation distance. Figure 2.21 illustrates the experimental setup to generate Bessel beams. At powers above 400mW, erythrocytes were observed to vertically align to the outer rings of the Bessel beam while lymphocytes continue to move to the centre of the beam [158], as shown in Figure 2.20 c). At lower powers, both types of cells transported slowly towards the central core of the Bessel beam. None of these behaviours were seen when a Gaussian beam was substituted in the experimental setup. Recently, the difference
between the ability of femtosecond and CW Bessel light beams to trap and guide particles has been investigated by Little [159]. Little has been using particles in the Mie regime for his experiments. Similar to observations by Mao [143], both types of waves, however, were observed to induce the same behaviour with regard to particle trapping and particle velocities.

![Figure 2.21 Experimental setup for creating Bessel beam [158]](image)

### 2.3.4 Waveguide

The optical trapping and separation of particles systems discussed so far operated using technique based on free space optics, where interaction length is small and limited via the focal length. Spatial separation of particles with different optical characteristics is proportional to the length of the interaction [160]. Hence the efficiency of free space optics for precise spatial separation is limited. This can be rectified by trapping and separating particles via an evanescent field on a waveguide surface, where the interaction length can be increased according to the waveguide length. Using a laser light propagating in a Y-branched caesium doped channel waveguide, Grujic has reported sorting of polystyrene particles [31]. In this system, the laser was launched through a single mode polarisation-maintaining fibre in to the channel waveguide, as illustrated in Figure 2.22. Changing the fibre position relative to the waveguide input facet changed the field distribution at the junction and dictated how the power was distributed between the two output waveguide branches. Adjusting the input fibre position clearly allows the selection of the relative power and hence the distribution of particles in the two output branches.

The extension of this method to biological cells, however, requires the cells to be relatively the same size as the particles used and to have a sufficiently high refractive index relative to the surrounding medium due to the weak evanescent field. Alternatively, small biological molecules could be attached to polystyrene particles and hence could be manipulated by the optical field.

The trapping and sorting method has been limited to high refractive index particles due to the weak evanescent field and thus low optical power. However, silicon
nitride waveguides yield a stronger evanescent field as shown in [4, 34]. Silicon nitride waveguides were simulated to have a factor of 100 increment in optical forces compared with potassium doped waveguides. This simulation was calculated from the radiative forces using Stokes law and a modified expression for the viscosity which accounts for the fact the particle was close to the surface [161, 162]. Yeast cells and erythrocytes were demonstrated to propel at 1um/s for 60mW and 40mW, respectively, using these waveguides. The compatibility of this type of waveguide to fibre however proves to be complicated due to the thin layer of silicon nitride.

Mandal has demonstrated the spatial separation of 3µm polystyrene particles with different laser powers using a liquid core waveguide operating in the vertical position [160]. By utilising a photonics crystal fibre, the light was confined within the low index core due to the photonic band gap effect in the cladding. It was also observed that particles were concentrated into distinct floating bands, where the scattering force was balanced by the weight of the particle. The banding was observed at an excitation power as low as 50mW. This technique has great potential in optical chromatography systems to separate particles based on their optical and physical characteristics. However, the suitability of Mandal’s device for integration to a lab-on-a-chip device proved to be a problem due to its vertical orientation.

2.4 Conclusion

Several technologies have been developed in order to sort and separate cells for biomedical and biotechnological applications. Advancement in microfabrication techniques has a direct impact in the development of cell sorting technology.
Among the earliest approaches were sorting particles and cells mechanically and using microfluidic techniques. Both of these technologies are easy to implement with fairly straightforward fabrication processes. The techniques have shown success in sorting and manipulating particles and cells. However, drawbacks such as clogging limit the usability of the method and directly reduce the output rate.

Improvements in microfabrication also trigger the usage of electric fields for trapping and sorting particles and cells. A DC electric field was among the first method used for sorting. Electrophoresis, an electrokinetic phenomenon arising from the use of a DC electric field, unfortunately has limited capability for sorting biological cells. This is due to the fact that most biological cells have similar electrophoretic mobilities. Thus dielectrophoresis was developed by using a non uniform AC electric field. Dielectrophoresis has been successfully applied to separate and manipulate biological cells as different cells have different dielectric characteristics. The ability to increase magnetophoretic mobility of biological cells by exploiting magnetically conjugated antibodies (MCA) allows the use of a magnetic field for sorting cells. Working in a similar manner as dielectrophoresis, biological cells tagged with MCA can be separated and manipulated according to their magnetophoretic mobility. The limitation in magnetic sorting lies in the antibody tagging system, as a restriction in the availability of antibodies and their suitability has a direct influence on the technique’s effectiveness. The disadvantage in dielectrophoresis, on the other hand, lies in the fabrication complexity caused by having extra fabrication steps for the electrodes. Apart from that, charging effects on biological cells and the formation of gas bubbles at electrode surface shows that there is a trade-off in employing DEP technique for sorting application.

Similar to DEP, cell and particle sorting using the optical approach is seen as a contactless approach. However, the technique avoids the charging effects and hence reduces the probability of damaging the cells physically. Furthermore, sorting particles and cells optically do not need any sort of tagging or complex fabrication methods. Disadvantages of the optical techniques as presented in this chapter are based on complexity of the optical setup and the limitation of applicability as seen in optical tweezer method. Hence this project proposes the use of optical channel waveguides for sorting biological cells. The optical channel waveguide have all the advantages of optical sorting yet simple to fabricate and to operate. Furthermore, optical waveguides can be integrated easily with microsystem devices. However, there is an apparent lack of knowledge, where the application of channel waveguides to trap and sort stem cells, was yet to be demonstrated. Subsequent chapters in this thesis will discuss about the advantages, problems and feasibility of using caesium ion-exchanged waveguides towards the stem cell sorting application.
Chapter 3

Particles and cells on surfaces

3.1 Introduction

The interaction of particles and cells, suspended in a liquid medium, with a surface, involves several non-optical forces. The investigations of these forces not only help the understanding of particles and cells behaviour on surface but also justify the trapping and propulsion behaviour of particles and cells by the optical forces in later chapters. These forces are namely Brownian motion, drag force, gravitational force, buoyancy, electrostatic forces and convection current. In this chapter each of the forces involved specifically in this project is discussed and the projected theoretical behaviour is presented for comparison with experimental works in later chapters. Several surface interactions are also investigated in this chapter. Surface functionalisation and ionic concentration are also presented in this chapter, which serves as overview for the experimental actions taken in later chapters.

3.2 Non-optical forces

3.2.1 Drag force and Brownian motion

From the kinetic theory of fluids, all molecules in a liquid medium move at random. When a particle or cell is placed in the liquid, it will receive a random number of impacts of random strength, and from random directions, in any short period of time. This random bombardment by the molecules of the fluid would cause a sufficiently small particle and cell to move in the way described by Brown [163]. In statistical physics, this movement can be described by the Langevin equation [164]. The equation is a stochastic differential equation describing Brownian motion in a
potential which is similar to the situation experienced by the particle and cell in a PDMS reservoir. This can be written as,

\[ m \frac{dv}{dt} = -\alpha v + F(t) \]  

Equation 3.1

where \( m \) is the mass of the particle, \( v \) is the velocity, \( F(t) \) is the randomly fluctuating force due to molecular collisions and \( \alpha \) is the damping constant. The damping constant can be deduced from the translational flow of a liquid medium around the particle or cell (which is assumed to be a perfect sphere for simplification). The flow needs to be at low Reynolds number [165, 166] and in an unbounded liquid medium [167]. This is best described by the Stokes’ drag force [168] which is given by,

\[ \alpha = 6\pi a \eta \]  

Equation 3.2

where, \( a \) is the radius of the particle and \( \eta \) is the dynamic viscosity. This condition satisfies the relationship for a particle or cell in the plane of the substrate. However, perpendicular to the substrate plane, the drag force acting on the particle is taking place in a bounded fluid. Hence this increases the effective local viscosity of the fluid up to three times, due to the reduction in diffusion coefficient of the particle [165, 166, 169]. Consequently, particles or cells are expected to move with a lower velocity than the predicted value for an unbounded medium (depending upon the distance of the particle/cell from the surface). Shorter distances between the surface and particle/cell also increase the drag force, as observed experimentally in [170] and the van der Waals force, as explained in Section 3.2.3.

Combining the damping constant in the Langevin equation, Equation 3.1 can be rewritten into

\[ m x \frac{dv}{dt} = -\alpha xv + xF(t) \]

\[ m \left[ \frac{d(xv)}{dt} - v^2 \right] = -\alpha xv + xF(t) \]

\[ m \left\langle \frac{d(xv)}{dt} \right\rangle = m \left\langle v^2 \right\rangle - \alpha \left\langle xv \right\rangle + \left\langle xF(t) \right\rangle \]  

Equation 3.3

Equation 3.3 shows the time average of Equation 3.1. As \( F(t) \) varies randomly irrespective of the velocity, \( v \) and the position of the particle, \( x \), \( F(t) \) can be simplified to be
Equation 3.4  \[ \langle xF(t) \rangle = \langle x \rangle \langle F(t) \rangle = 0 \]

and also

\[ \text{Equation 3.5} \quad \frac{1}{2} m \left\langle v^2 \right\rangle = \frac{1}{2} kT \]

where \( k \) is the Boltzmann constant and \( T \) is the temperature. Substituting Equation 3.4 and Equation 3.5 into Equation 3.3,

\[ m \left( \frac{d \langle xv \rangle}{dt} \right) = kT - \alpha \left\langle xv \right\rangle \]

\[ \text{Equation 3.6} \quad \frac{1}{2} m \frac{d^2 \left\langle x^2 \right\rangle}{dt^2} + \frac{\alpha}{2} \frac{d \left\langle x^2 \right\rangle}{dt} = kT \]

In order to solve Equation 3.6, let

\[ \text{Equation 3.7} \quad \frac{d \left\langle x^2 \right\rangle}{dt} = w \]

and

\[ \text{Equation 3.8} \quad \gamma = \frac{\alpha}{m} \]

Substituting these into Equation 3.6,

\[ \text{Equation 3.9} \quad \frac{dw}{dt} + \gamma w = \frac{2kT}{m} \]

Let

\[ \text{Equation 3.10} \quad w = \frac{2kT}{\alpha} + ce^{-\gamma t} \]

and substitute Equation 3.10 in Equation 3.9,

\[ \text{Equation 3.11} \quad \frac{dw}{dt} + \gamma w = -c \gamma e^{-\gamma t} + \frac{2kT \gamma}{\alpha} + \gamma ce^{-\gamma t} = \frac{2kT}{m} \]
Equation 3.10 is a particular solution for Equation 3.9. Assuming that the time (in minutes) is large and the mass of the particle is small, the solution reduces to

\[ w = \frac{2kT}{\alpha} \]  

Equation 3.12

Substituting back Equation 3.7 to Equation 3.12 and solving for \( \langle x^2 \rangle \)

\[ \frac{d\langle x^2 \rangle}{dt} = \frac{2kT}{\alpha} \]

\[ \langle x^2 \rangle = \frac{2kT}{\alpha} t \]

Equation 3.13

As the distance travelled, \( \langle r^2 \rangle = 2 \langle x^2 \rangle \) in two dimensions, Equation 3.13 can be written as

\[ \langle r^2 \rangle = \frac{2kT}{3\pi \alpha \eta} t \]

Equation 3.14

as given originally by Einstein [171, 172].

<table>
<thead>
<tr>
<th>Boltzmann constant (JK(^{-1}))</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Temperature (K)</td>
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</tr>
<tr>
<td>Viscosity (Pas)</td>
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</tr>
<tr>
<td>Polystyrene particle diameter (µm)</td>
<td>RMS displacement (µm) after 1 second</td>
</tr>
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<td>1.71</td>
</tr>
<tr>
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<td>0.57</td>
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<td>20</td>
<td>0.09</td>
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</tbody>
</table>

Table 3.1 Predicted root-mean-square (RMS) displacement of a range of polystyrene particles in two dimensions after 1 second
Substituting values in Equation 3.14 with constants stated in Table 3.1, the root-mean-square (RMS) displacement of polystyrene particles in unbounded medium can be calculated. It can be seen from Table 3.1 that as the size of the particle increases, the displacement after one second decreases. The actual magnitude of the particle displacement however is expected to be less due to the boundary condition and variation in the actual particle size. Small particles such as the 1µm particles show large displacement, 1.71µm. While large particles such as 20µm particles only move for 0.09µm. The prediction shows that over a longer duration, smaller particles travelled for longer distances compared to larger particles. However this estimation does not consider other forces that also acting on the particles which may affect the distance travelled by each particle size. It should also be noted that when a particle or a cell is very close to the surface, the assumption changed from displacement in unbounded medium to bounded medium. Hence, this has a significant effect where mobility of particles and cells is expected to be significantly reduced [173, 174].

3.2.2 Gravitational and buoyancy forces

The investigation of optical trapping and propulsion involves the particle or cell of interest suspended in a liquid medium. Two main non-optical axial forces, namely gravitational and buoyancy play an important role in determining the position of the particle or cell with respect to the surface plane. Medium viscosity, density of the particle/cell and its surrounding medium are among the factors that influence these forces. Investigating these forces paved an understanding towards the effect of particle/cell position on optical trapping and propulsion behaviour.

Figure 3.1 Diagram illustrating the forces acting on the particle
The gravitational force acting on the particle/cell is directed downward and is given by

**Equation 3.15**

\[ F_g = \rho_p V g \]

where \( g \) is the gravitational acceleration, and \( \rho_p \) and \( V \) are the density and volume of the particle respectively. On the other hand, the buoyancy force acts upwards, as illustrated in Figure 3.1. The force can be written as

**Equation 3.16**

\[ F_b = \rho_m V g \]

where \( \rho_m \) is the density of the surrounding medium. The resultant force can be worked out from the difference between Equation 3.15 and Equation 3.16. As the density of the particle of interest is much greater than the density of the medium used, the resultant force is always acting downwards. Density of the cell as mentioned in Section 1.5.2, is assumed to be larger than the medium. In later chapters, this assumption will be proven correct as the cell is observed to settle to the bottom of the reservoir. From the calculation of resultant force acting on the particle, the resultant force for each particle size can be estimated.

![Figure 3.2 Settling time of different polystyrene particle sizes. \( \rho_p = 1.05 \) g/cm\(^3\), \( \rho_m = 1 \) g/cm\(^3\). Note that the y-axis is set in log scale.](image)

46
The PDMS reservoir used is approximately 150µm deep. Assuming that the particle's initial position is at the very top, the maximum settling time is calculated and plotted in Figure 3.2. The exact density of the cell is unknown; however the particle size variation gives a rough idea of how fast the cell will be settling to the bottom of the reservoir. The resultant force decreases exponentially with the size of the particle. Hence a larger particle (i.e. 20µm) is estimated to experience a shorter settling time. Note that particle less than 1µm do not settle to the bottom due to the Boltzmann distribution [175].

3.2.3 Electrostatic forces

The dispersion stability of particles or cells in a solution relies upon the balance between two opposing electrostatic forces. The forces; attractive van der Waals forces and repulsive electrical double layer force that exist between the particles/cells and particle/cell-surface interaction can be explained through Derjaguin, Landau, Verwey and Overbeek (DLVO) theory [176-181].

3.2.3.1 Van der Waals force

The van der Waals force plays an important role when considering the interaction between microscopic particles on a surface. Consider a volume of molecules in a sphere of radius, \( R \) at a distance, \((D + z)\) from a planar surface as illustrated in Figure 3.3, the interaction energy, \( W \) can be represented as [179]

\[
W(D) = -\frac{\pi^2 C \rho_1 \rho_2}{6} \int_{z=0}^{z=2R} \frac{(2R - z)z}{(D + z)^3} dz
\]

where \( \rho_1 \) and \( \rho_2 \) is the number density of molecules in the wall and the sphere respectively. Given that the distance \( D \) is much smaller than \( R \), hence only small values of \( z \) contribute to the integral; \( W \) for van der Waals forces can be written as

\[
W(D) = -\frac{\pi^2 C \rho_1 \rho_2 R}{6D}
\]

Differentiating Equation 3.18 with respect to the distance from the surface, van der Waals force is
Equation 3.19

\[ F_w = \frac{\pi^2 C \rho \rho_2 R}{6D^3} \]

with the term \( \pi^2 C \rho \rho_2 \) is known as the Hamaker constant. Equation 3.19 shows that the van der Waals force is proportional to \( 1/D^3 \) which means that, as the distance between the particle and the surface increased, the attractive force decreases.

3.2.3.2 Double layer force

Interaction that involves van der Waals force alone is restricted to situations such as an interaction in a vacuum where charges in the surroundings are not present. However, considering particles suspended in water, charging of the unmodified glass surface in water can occur due to hydrogen ions from water penetrating into the glass and replacing the alkali ions, as shown in [182, 183]

Equation 3.20

\[ \equiv Si - ONa + H_2 O \iff Si - OH + Na^+ + OH^- \]

From Equation 3.20, silanol groups (-OH) are formed and deprotonated to negatively charge the surface (given that the pH is neutral, 7). The particles are also
negatively charged during fabrication to prevent agglomeration, as stated in Section 1.5.1. This condition causes the surface to repel similar charged ions in the solution and attract oppositely charged ions. At equilibrium, the chemical potential is uniform throughout and can be represented by the Boltzmann distribution,

\[ \rho_{si} = \rho_0 e^{-\frac{z_i e \psi_x}{kT}} \]

where \( \rho_{si} \) and \( \rho_0 \) is the ion’s concentration at position \( x \) and at bulk respectively, \( z_i \) is the ion’s valency, \( e \) is the electron charge and \( \psi_x \) is the electrostatic potential at position \( x \). The net excess charge density at position \( x \) can be obtained from Poisson equation,

\[ z_i e \rho_{si} = -e\varepsilon_0 \frac{d^2 \psi_x}{dx^2} \]

Combining these equations, the Poisson-Boltzmann equation is obtained

\[ \frac{d^2 \psi_x}{dx^2} = -\frac{z_i e \rho_0}{e\varepsilon_0} e^{-\frac{z_i e \psi_x}{kT}} \]

Integrating Equation 3.23, the potential gradient can be presented by

\[ \psi_x \approx \frac{4kT}{e} \gamma e^{-\kappa'x} \]

where \( \gamma = \text{tanh} \left( \frac{ze\psi_0}{4kT} \right) \) and \( \kappa' \) is the Debye screening length, which is the characteristic decay length of the electric field, at the assumption of \( (ze\psi_0/kT) << 1 \). Given two parallel surfaces separated by distance, \( D \), with the same surface charge, \( C_{sc} \), the repulsive pressure between the surfaces is

\[ P = \frac{e^2 \psi_m^2 \rho_0}{kT} \]

where \( \psi_m \) is the middle position potential between the two surfaces. Substituting Equation 3.24 to Equation 3.25, the repulsive pressure can be simplified into

\[ P = 64kT \rho_0 \gamma^2 e^{-\kappa'D} \]
Integrating this formula with respect to the distance from the surface, the energy per unit area between the two surfaces can be written as

\[
W(D) = \frac{64kT\rho_0\gamma^2}{\kappa} e^{-x_D}
\]

Equation 3.27

In the case for force between a sphere and a surface, another condition needs to be taken into account which is the force for such geometry [179],

\[
F(D) = 2\pi RW(D)
\]

Equation 3.28

which is known as Derjaquin approximation. Including this condition into Equation 3.27, the expression for the double layer force between a sphere and a surface is given by

\[
F_{DL} = \frac{128\pi RkT\rho_0\gamma^2}{\kappa} e^{-x_D}
\]

Equation 3.29

In this case, as the distance increases, the force decays exponentially. The force increased proportionally with increasing particle size.

Combining the van der Waals force and the double layer force, five possible scenarios can be expected from the net force, as illustrated in Figure 3.4. These scenarios depend on the combination of electrolyte concentration and surface charge density [179]. For a highly charged surface in dilute electrolyte, the energy remained positive and particles were repelled from the surface. Except for the highly improbable case where the particles overcome the energy barrier, usually between 1nm to 4nm distance from the surface, the particles are strongly attracted to the surface, as illustrated in Figure 3.4 (a) lower inset. In a more concentrated electrolyte, there is a minimum in the energy curve which is known as the secondary minimum, usually beyond 3nm from the surface. If the secondary minimum is weak (\(W_0\) is small), particles remained dispersed in the medium; a condition called kinetically stable is reached, as shown in Figure 3.4 (b) lower inset. If the energy barrier is much lower as in the case of surface with low charge density, this will lead to slow particle approach to the surface, as in Figure 3.4 (c) lower inset. Above critical coagulation concentration and when the energy barrier falls below zero, particles will approach the surface unobstructed, as in Figure 3.4 (d) lower inset. When the surface charge approaches zero, the interaction curve is similar to pure van der Waals interaction; all particles rapidly come into contact with the surface (Figure 3.4 (e) lower inset).
3.3 Thermally induced effect

3.3.1 Convection current

In order to trap and propel particles and cells, a laser, which operates at 1064nm, will be used. The laser is fixed to supply a modal power of 500mW to the waveguide. Energy from the laser will be absorbed and converted into heat by the glass substrate and liquid medium in the reservoir. The optical absorption by the liquid medium induces localised heating which leads to a density change and hence, convection currents [184, 185]. A simulation using a multiphysics modelling tool, Comsol, was carried out to investigate the temperature profile due to glass and liquid medium absorption. A general heat transfer module was used in Comsol with the channel waveguide (of dimension 1.4µm x 4.5µm) sandwiched between glass substrate (silica glass) and liquid medium (water) as illustrated in Figure 3.5. The
initial temperature was set to be at room temperature (20°C). The module uses the following equation to describe the heat transfer in 2D \[164\],

\[
\nabla \cdot (-T_k \nabla T) = T_0 - PC_p \cdot \nabla T
\]

where \(T_k\) represent the thermal conductivity, \(C_p\) is the specific heat capacity and \(T_0\) is the heating power per unit volume. Assuming that the propagation loss of the waveguide is 1dB/cm due to absorption and a 500mW modal power, the rate of heat generated per area was calculated to be \(1.6 \times 10^{-12}\) W/m². The boundary conditions used here are the heat is lost from top and bottom surfaces to the surrounding air and at the sides of the model to similar materials. All media surrounding the model are assumed to be at 20°C. The heat lost to the air is given by use of a film coefficient with a value of 3Wm⁻²K⁻¹ \[186\].

Using this calculated value and the parameters listed in Table 3.2, the temperature profile caused by the heat generation due to the laser absorption was simulated. Figure 3.6 illustrates the temperature profile from the vertical cross section through the middle of the waveguide. The peak temperature simulated was increased by 12.5°C for 500mW modal power. The rate of temperature drop was greater in glass compared to water due to higher thermal conductivity. The heat flux at the water-waveguide interface induces a convection current in the water in order to reach equilibrium. Note that due to propagation loss, there is also reduction in temperature along the waveguide (in z-axis). Hence this creates a gradient of heat flux; resulting a convection current towards the input facet as observed in [185, 187].

![Figure 3.5 Two dimensions schematic of the waveguide simulated in Comsol](image)

The temperature increment simulated in Comsol was computed under the assumption that the propagation loss in the waveguide was the upper limit of the power loss. The laser power used was also not inclusive of the insertion losses as observed in the experiments in Chapter 6. Under these circumstances, the temperature increase may be lower than the simulated value. The convection
current created due to the light absorption may be beneficial in terms of drawing particles and cells towards the illuminated channel waveguide laterally. This allows overlapping of evanescent field on the particles and cells. However, the convection current was against the propagation direction of the light, which indirectly creates another opposing force to the optical propulsion.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water</th>
<th>Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific heat capacity, Jkg⁻¹K⁻¹</td>
<td>4184</td>
<td>703</td>
</tr>
<tr>
<td>Thermal conductivity, Wm⁻¹K⁻¹</td>
<td>0.61</td>
<td>1.38</td>
</tr>
<tr>
<td>Density, kgm⁻³</td>
<td>998</td>
<td>2203</td>
</tr>
</tbody>
</table>

Table 3.2 Parameters used for modelling temperature profile

![Figure 3.6 Temperature profile due to laser absorption. The graph represent a vertical cross section through the channel waveguide](image)

**3.4 Ionic concentration of medium**

The DLVO theory describes in Section 3.2.3 proposes that an energy barrier resulting from the repulsive force prevents flocculation of particles. However, there is also a possibility of a secondary minimum, as shown in Figure 3.4, where a weaker and potentially reversible adhesion exists between particles and particle-surface. Thus, in order to preserve the dispersion stability of the particle or cell in the
solution, the dominant force in the system must be the repulsive forces; the double layer force. This condition can be created by two mechanisms; steric repulsion and charge stabilisation. Van der Waals forces decrease as the distance increases; hence this relation can be exploited by increasing the particle-surface distance. There are two ways to achieve this, by coating the particles or by functionalising the waveguide surface. As the system is used for multiple types of particles and cells, it is best to functionalise the surface with a hydrophobic coating. This coating prevents particles or cells coming into close contact with the surface and effectively reduces the van der Waals force. Surface functionalisation is discussed in detail in Section 5.2.4. Another method is by stabilisation of electrostatic charge. This can be achieved by manipulating the distribution of charge species in the system by adding ions into the solution (for example by adding sodium chloride).

<table>
<thead>
<tr>
<th>Material</th>
<th>NaCl concentration, M</th>
<th>Hamaker constant, $A$, $\times 10^{-20}$ J</th>
<th>Debye length, $\kappa^{-1}$, nm</th>
<th>Zeta potential, $\zeta$, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>-</td>
<td>6.6</td>
<td>-</td>
<td>-85</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>3.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glass</td>
<td>$5 \times 10^{-5}$</td>
<td>6.5</td>
<td>43.0</td>
<td>-35</td>
</tr>
<tr>
<td>Glass</td>
<td>$5 \times 10^{-4}$</td>
<td>6.5</td>
<td>13.6</td>
<td>-30</td>
</tr>
<tr>
<td>Glass</td>
<td>$1 \times 10^{-4}$</td>
<td>6.5</td>
<td>30.4</td>
<td>-23</td>
</tr>
<tr>
<td>Glass</td>
<td>$1 \times 10^{-3}$</td>
<td>6.5</td>
<td>9.6</td>
<td>-22</td>
</tr>
</tbody>
</table>

Table 3.3 Constant values for materials in the DLVO calculations [179, 188, 189]

The DLVO theory can be utilised to estimate the effect of ion concentration on the particle/cell and surface interaction. The continuum theory suggests that the dispersion stability of particles/cells in a solution is given by the total free energy of van der Waals force (which is always attractive for two similar surfaces) and an electrostatic force due to compression of the electrical double layer (which is repulsive for two surfaces carrying the same charge) [176-181]. From the van der Waals theory in Section 3.2.3.1, only the Hamaker constant, $A$ is required for the simulation. The value can be approximated from the permittivity of all the individual components in the system; glass, water and polystyrene, as shown in Table 3.3. Note that all Hamaker constants are against vacuum.

Equation 3.31

$$A_{\text{Total}} = \left( \sqrt{A_{\text{Polystyrene}}} - \sqrt{A_{\text{Water}}} \right) \left( \sqrt{A_{\text{Glass}}} - \sqrt{A_{\text{Water}}} \right)$$
Equation 3.31 was used to calculate the total Hamaker constant which is $0.37 \times 10^{-20}$ J. On the contrary, estimating the double layer force is more complicated due its dependency on specific conditions. From the formula derived in Section 3.2.3.2, among the unknown components is the Debye length, the distance where significant charge separation can occur. This can be represented by,

$$\kappa^{-1} = \frac{\varepsilon_0 C_G T}{\sqrt{2 C_F \rho_{\text{oi}}}}$$

where $C_G$ and $C_F$ are the gas constant and Faraday constant respectively, $\rho_{\text{oi}}$ is the ion's concentration at bulk and $T$ is the temperature. The equation also shows that the magnitude of the Debye length solely depends on the properties of the solution used. Debye lengths tabulated in Table 3.3 were calculated corresponding to the various sodium ion's concentrations.

Another parameter that needs to be taken into consideration is the surface potential, $\psi_0$. This value is unfortunately not directly measurable but it can be substitute with zeta potential, $\zeta$. Zeta potential, also known as shear potential, represents the electrokinetic potential at a small distance ($\approx 1$ nm) away from the surface. Zeta potentials, listed in Table 3.3, are highly dependent on the pH of the...
water and also on the ion concentration. In this numerical simulation, the pH of the water is assumed to be fixed at pH 7. From these parameter values, the net force acting on the particles can be calculated against particle size and ionic concentration. The simulation was carried out with the sodium chloride (NaCl) concentration at $5 \times 10^{-5}$ M. NaCl was chosen for several reasons; it is monovalent, it dissociates totally and it maintains the pH at 7. NaCl was also chosen due to the fact that it does not introduce new ions in the solution, as Na\(^+\) dissociates from the glass surface, albeit in very small quantities.

![Figure 3.8 Net force acting on a 10µm polystyrene particle with an ion concentration of $5 \times 10^{-5}$ M (■) and (—) represents the van der Waals force while (--) represents the double layer force. Net forces for other concentrations are also plotted, $5 \times 10^{-4}$ M (■) 1x10^{-4}M (■) and 1x10^{-3}M (■)](image)

Figure 3.7 shows a plot of the net force acting on polystyrene particles of various sizes. The plot demonstrates that the net force increases proportionally to the particle size, as expected from the theory in Section 3.2.3. The repulsive force also starts decreasing above 3nm separation (between the surface and the particle) for all particle sizes investigated. Note that the van der Waals force is against vacuum; hence the values observed in Figure 3.7 are expected to be lower. Figure 3.8 illustrates that the increment of ion concentration causes the repulsive force to rapidly decrease and thus minimise the energy required for the particle to approach the surface [190]. This trend is observed beyond the 8nm separation between the surface and particles. Increasing the ion concentration however, can have its limits
as observed experimentally by [170, 191], where the particle is observed to be adhered on the surface. Particles were observed to immediately adhere to the surface in a NaCl solution of $3 \times 10^{-3}$ M concentration, while at $0.6 \times 10^{-3}$ M virtually no particles adhered to the surface.

In conducting the investigation of DLVO theory, several factors such as the gravitational force (pulling the particles closer to the surface than estimated here), the ion correlation effect (where the highly polarisable, conducting double layer forces may have attractive forces) and the finite ion steric effect (which enhances the repulsive force) are not taken into account in the simulation [179]. Furthermore, applying DLVO forces on biological cells was found to be much more complex. Apart from unknown parameters, it was found out that the repulsive hydration and steric forces may dominate the interaction depending on the hydrophilicity and thermal motion of the surface groups on the biological membrane [179]. In addition, the biological membrane is locally rough due to protruding proteins, as indicated in Section 1.5.2. Such conditions further complicate the application of DLVO theory to mammalian cells. Hence, this simulation is aimed to indicate the trends that can be observed in conducting experiments with various particle/cell sizes and media with different ionic concentrations.

3.5 Conclusion

In this chapter, the non-optical forces acting on particles and cells on the surface were discussed. The RMS displacement of the particles was estimated from the Brownian motion theory, derived from the Langevin equation. It shows that the increment in the RMS displacement is inversely proportional to the particle size. This theory however, was developed with the assumption that the particle motion is in an unbounded medium. As the particles approach the surface, this assumption is no longer valid and hence increased the effective viscosity of the fluid up to three times. The RMS displacement is expected to be lower in a bounded medium depending on the surface-particle distance. From the discussion on gravitational and buoyancy forces, it was shown that larger size particles ($20\mu m$) settles much faster than smaller ones ($1\mu m$). The Brownian motion in this case, has to overcome the increased viscosity (bounded medium) and hence, the drag force acting against the motion. This indicates that the RMS displacement for larger particles will be much lower than for smaller ones. The theoretical overview of the Brownian motion can be used to estimate the distance travelled by particles and cells of different sizes. The investigation of heat absorption shows that there will be a convection current, in the liquid medium, towards the input facet. This current, along with
Brownian motion and gravitational forces, can be exploited to allow particles and cells to overlaps with the evanescent field laterally (Brownian motion) and axially (Brownian and gravitational force). However the magnitude of the optical forces should be higher than these forces in order to stably trap and propel particles and cells on the channel waveguide.

The balance in the electrostatic forces determines the dispersion stability of the particles or cells in a solution. It was concluded that, in order for the particles to be able to approach the surface, the repulsive force needs to be as low as possible. At the same time, the repulsive force should not be too low to make the attractive force dominant which causes the particles to adhere to the surface. Hence there is a range of ionic concentrations that is needed to assist particles to be overlapped with the evanescent field and at the same time to avoid the particles adhering to the surface. The theoretical evaluation of various ionic concentrations can be used to determine the suitability of the ionic concentrations in the investigations of Brownian motion and propulsion of particles and cells on caesium ion-exchanged waveguides.
Optical channel waveguides: Theoretical and experimental evaluation

4.1 Introduction

Optical waveguides are devices made using a dielectric material with a high permittivity surrounded by a material with a lower permittivity. Optimisation of trapping stability and particle propulsion can be achieved through fine-tuning of certain design parameters in the optical channel waveguides. Waveguide width and depth are the parameters that can be controlled through the fabrication process. The fabrication process is described in details in this chapter along with the discussion on the selection of glass host and ions for the ion-exchange process. In order to fully understand the optical waveguide and its optimisation for the trapping and propulsion process, it is necessary to describe the basic theory of waveguides, starting with the diffusion theory which is related to the ion-exchange step in the fabrication process and this is covered here. This chapter continues with the ray description to introduce the physical quantities of optical waveguides using the readily understood ray picture. The electromagnetic approach is also described in this chapter explaining the full solution to the waveguide equation. Both descriptions follow the general work of Yariv [192] and Lee [193]. A combination of BeamPROP, a beam propagation design tool and Matlab, a numerical computing simulator was utilised for the modelling of optical channel waveguides and to obtain the optimum waveguide depth and width. These values were compared to the fabricated waveguides to determine the waveguide with the highest surface intensity for trapping and propulsion experiments. The characterisation
experiments conducted for optical waveguides are described and discussed at the end of this chapter.

4.2 Waveguide fabrication

Several channel waveguides were fabricated to be used for the trapping and propulsion experiment. Channel waveguides are usually made using silica glass as the substrate material. Glass is chosen as it is cheap and has excellent transparency. Moreover, certain types of glass, such as silicate and borosilicate glasses, have a refractive index close to that of optical fibre, hence reducing coupling loss (Fresnel reflection and modal mismatch). Waveguides can be fabricated using various techniques involving sputtering [194], ion implantation[195], ion-exchange [196-198] and chemical vapour deposition [199]. The technique of exchanging alkali ions between a substrate and a molten salt bath has been a very commonly used technique, since reported by Izawa [200]. The reason behind this is due to the simplicity and reproducibility of the technique [196-198]. In this section, an overview of types of glasses and alkali ions to be used for the ion-exchange process will be discussed. The fabrication process will also be described in detail with particular interest to the Cs⁺-Na⁺ ion exchange. All waveguide fabrication steps were conducted in a cleanroom environment.

4.2.1 Glasses for ion-exchange

Choosing the right host glass can minimise the losses inherent within an ion-exchanged waveguide [197]. Any glass containing monovalent ions such as sodium, potassium, lithium is suitable to be used as host glass for ion-exchange process. Nevertheless, there are several factors need to be considered such as scattering losses from inhomogenities in the glass and absorption from glass impurities prior to fabrication. The glass host also needs to be able to withstand ion-exchange temperatures. All of these factors can drastically affect the waveguide characteristics.

In view of the above factors, there are two commonly selected glasses for ion-exchange process; soda-lime glass and BK7 glass. Soda-lime glass, commonly used as microscope slides, is regularly used in ion-exchange process. This is due to the fact that soda-lime glass (Menzel-Gläser) is rich in sodium content, low cost and readily available [197]. Waveguide fabricated using soda-lime glass however tend to be lossy due to the variations in the material composition and metallic impurities [197].
Apart from that, there are also commercial optical glasses such as BK7 that are used as substrate. BK7 glasses have excellent transmissive properties in the visible and near infrared region and a more consistent elements composition. BK7 glasses have been used in integrated optics applications with losses down to 0.1dB/cm [196]. As illustrated in Figure 4.1, soda-lime glass has significantly more sodium content than BK7. Due to this fact, soda-lime glass is proven to be able to produce a higher $\Delta n$ than BK7 although the ion-exchange time is the same [191]. This is beneficial not only from cost point of view but also saves time in fabricating a high $\Delta n$ layer in the substrate.

![Figure 4.1 Elements composition in the glasses used in the project a) soda-lime, b) BK7. The elements are Si ( ), Ca ( ), Mg ( ), Na ( ), B ( ) oxides and others ( ) [191, 201]

### 4.2.2 Refractive index change

Different types and chemical compositions in a glass network produce different refractive index. Although the cations (the glass network modifiers) are swapped in the ion-exchange process, the oxygen anions remain fixed in the silicon-oxygen network, leaving the glass basic structure unaffected [202]. This can be applied for fabricating waveguide structure in silicate glass substrate [197, 198]. The refractive index change in glass depends on three major factors; molar volume or ionic size, stress due to ion substitution and ionic polarisability [197, 203]. The refractive index value can be approximated using a model derived from the Gladstone-Dale equation [204],

\[ \Delta n \approx \frac{\chi}{V_0} \left( \Delta R - R_0 \frac{\Delta V}{V_0} \right) \]
where \( \chi \) is the percentage of cations exchanged for incoming ions, \( V_0 \) is the volume per mole of oxygen atoms, \( R_0 \) is the refraction per mole of oxygen atoms and \( \Delta R \) and \( \Delta V \) are the changes in these quantities resulting from ion-exchange. Equation 4.1 provides method of calculation for the density, refractive index and dispersion of a glass expressed in terms of the weight percentage of oxide constitute in its compositions. The first terms in the equation demonstrates that substituting ions for another with higher polarisability increases the refractive index. Thus substituting sodium with silver, thallium or caesium ions will raise the index of the substrate [198]. Meanwhile the second term of Equation 4.1 represents the contribution of changes in molar volume to the refractive index of the glass. The glass matrix contracts as smaller ions replace the larger ones, yielding a densely packed structure. This phenomenon can be seen when lithium ions replace sodium in a glass [198]. The Gladstone-Dale model however neglects the stress induced due to ion-exchange process [203]. For example, the refractive index calculated from Equation 4.1 was shown in [203] to be two orders of magnitude lower than the measured index for potassium ion-exchanged waveguides. Potassium ions are larger than sodium ions, hence the size difference causes the swelling effect and creates compressive stresses to the substrate surface. Consequently, the refractive index of the compressive layer increases and the index in the tensile region decreases [202].

<table>
<thead>
<tr>
<th>Ion</th>
<th>Polarisability Ionic radius</th>
<th>Salt</th>
<th>Melting point (°C)</th>
<th>( \Delta n )</th>
<th>Waveguide depth (µm)</th>
<th>Losses (dB cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>0.41</td>
<td>0.95</td>
<td>NaNO(_3)</td>
<td>307</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K(^+)</td>
<td>1.33</td>
<td>1.33</td>
<td>KNO(_3)</td>
<td>334</td>
<td>0.009</td>
<td>15-20</td>
</tr>
<tr>
<td>Ag(^+)</td>
<td>2.40</td>
<td>1.26</td>
<td>AgNO(_3)</td>
<td>212</td>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td>Tl(^+)</td>
<td>5.20</td>
<td>1.49</td>
<td>TlNO(_3)</td>
<td>206</td>
<td>0.2</td>
<td>12</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>0.03</td>
<td>0.65</td>
<td>LiNO(_3)</td>
<td>264</td>
<td>0.012</td>
<td>10-70</td>
</tr>
<tr>
<td>Cs(^+)</td>
<td>3.34</td>
<td>1.65</td>
<td>CsNO(_3)</td>
<td>414</td>
<td>0.04</td>
<td>8</td>
</tr>
<tr>
<td>Rb(^+)</td>
<td>1.98</td>
<td>1.49</td>
<td>RbNO(_3)</td>
<td>310</td>
<td>0.02</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4.1 General characteristics of alkali ions and their corresponding waveguides after ion-exchanged into sodium doped silicate glasses [197]

Table 4.1 shows the properties of commonly used alkali ions for the ion-exchange process. A high waveguide index contrast was shown experimentally to increase optical forces and hence propulsion velocity in [4, 34]. Lithium, silver and thallium ions can cause a large change in the refractive index and also create a low loss multimode waveguide in a reasonably short time. Although lithium ions are highly mobile in glasses, it can cause the glass structure to have volumetric changes and consequently collapse. Thallium on the other hand is toxic and silver ions can easily
form silver metal within the glass to cause additional impurities and scattering losses. Caesium ions and potassium ions are seen to be potential candidates for fabricating the optical waveguide. However, potassium ions can only produce a small refractive index change. Hence, caesium ions are selected to be used for waveguide fabrication in this project.

### 4.2.3 Substrate selection and cleaning

All waveguides were fabricated using soda-lime glass substrates of dimension 50x50x1 mm³ (Menzel-Gläser). Each of the glass slides was inspected for any apparent scratches on the surface. The glass substrates were pre-treated to remove any organic contaminants in order to provide a clean surface for the aluminium to adhere. This is carried out by immersing the substrate in a piranha solution (hydrogen peroxide (H₂O₂):sulphuric acid (H₂SO₄) = 1:4) in a preheated ultrasonic bath at 80°C for 15 minutes. The substrates were rinsed thoroughly with de-ionised water and blow-dried with compressed nitrogen. In order to make sure that the substrate’s surfaces were totally water-free, the substrates were baked for 20 minutes in an oven at 120°C.

### 4.2.4 Aluminium evaporation

![Figure 4.2](image)

**Figure 4.2 Surface preparation for the photolithography process, a) aluminium evaporation and photoresist coating on a substrate**

A masking layer is created by aluminium evaporation, as illustrated in Figure 4.2 a), using a thermal evaporator (Edwards 306). Aluminium is used as it adheres well to a glass slide without any primer and has the ability to withstand the furnace temperature (450°C). Furthermore, there is a good aluminium etchant available. Aluminium evaporation is achieved using a vacuum chamber with a 1mm thick aluminium wire coiled around a tungsten filament. The evaporation rate is maintained at 1nm/s by controlling the current through the tungsten filament. The aluminium wire was totally melted before the chamber shutter is released to avoid
uneven deposition on the glass slide. A thickness of 250nm ± 10nm of aluminium was deposited on the glass substrate by vacuum evaporation (10⁻⁶ bar).

### 4.2.5 Photolithography

A syringe with a 0.2μm filter was used to put SU813 photoresist (Shipley) on top of the aluminium layer on the substrates. The substrates were spun at a speed of 6000 rpm for 60 seconds to achieve a uniform 1μm thickness as illustrated in Figure 4.2 b). The photoresist layer was inspected to ensure that no bubbles were formed before being soft-baked for 30 minutes at 90°C. A chromium mask with a waveguide pattern (Rutherford Appleton Labs) was used to transfer the pattern to the substrate using a mask aligner (EVG). The mask is inverted, aligned with the substrate, and forced into hard contact with the photoresist layer. This setting is then exposed to ultraviolet (UV – 365nm, 18mW/cm²) light for 4.5 seconds. The substrate is directly immersed after UV exposure into MF-319 developer solution (Shipley) for 45 seconds.

![Figure 4.3 Photolithography steps](image)

Figure 4.3 Photolithography steps, a) pattern transfer via UV light exposure, b) photoresist development, c) aluminium etching and d) photoresist removal

This develops the waveguide pattern in the photoresist layer. The waveguide pattern consists of 3 groups of waveguide channels varying from 1μm to 10μm. All waveguide width in this report refers to the mask width. The substrate is then washed thoroughly in deionised water. The substrate is hard-baked for 30 minutes at 120°C. The substrate is left to cool to room temperature while the aluminium etchant (MicroChemicals) is heated to 50°C. The substrate is put in the aluminium etchant for 90 seconds. The waveguide features are examined under the microscope to
determine whether there is a need for any further etching. The substrate is washed with acetone and isopropanol to remove the photoresist layer. Before being blow-dried, the substrate is cleaned with de-ionised water. Photolithography steps are illustrated in Figure 4.3.

### 4.2.6 Ion exchange

The ion exchange process is performed by immersing the substrate into molten salts containing monovalent cations such as Cs\(^+\). During the ion-exchange process, the cations (the glass network modifiers) are swapped while the oxygen anions remain fixed in the silicon-oxygen network of the glass substrate [202]. By this process, the monovalent cations in the molten salts replace alkaline ions, such as Na\(^+\), existing at the glass surface region resulting in the change of glass composition and therefore of the refractive index at a localised area. Note that the aluminium mask (with the waveguide pattern etched on it) only covers the front surface of the glass substrate. This left the entire back surface ion-exchanged with monovalent cations creating a slab waveguide.

![Figure 4.4 Ion-exchange process before a) and after b) aluminium layer removal](image)

For the ion-exchange process, a beaker containing 500g of caesium nitrate (CsNO\(_3\)) salt (Aldrich) was placed in a furnace and the temperature was increased to 450°C. The substrate was first suspended over the salt to avoid temperature stress. The furnace was left for about 60 to 90 minutes to stabilise. The substrate was lowered gradually into the salt melt and left for the period needed. The ion-exchange time for the individual waveguides was varied between 4 to 17 hours. The substrate was slowly lifted out from the melt to avoid any formation of microcracks and the furnace was turned off to cool. The substrate was removed from the furnace when it reached room temperature. A cleaning treatment with de-ionised water was given to the substrate to remove the excess salt. The substrate was then blow-dried. Aluminium layer was left on the substrate to protect the ion-exchanged waveguides during polishing process.
4.2.7 Polishing

A metal body was used to hold the substrate for polishing. The metal body, substrate and microscope glass slides were heated to 100°C. The ion-exchanged substrate, covered on both sides with normal glass slides, was set in a bonding wax. The substrate was secured in the metal body and left to cool. After aligning the substrate, the substrate was cut passing through the edge of the aluminium layer. During the process, a mixture of 15% ethanediol (C$_2$H$_6$O$_2$) and 85% water was used as a coolant and a lubricant. The substrate was then positioned for polishing. The substrate was initially lapped with 9μm aluminium oxide (Al$_2$O$_3$ - Logitech) particles to flatten the surface.

This process further removed 1mm and the substrate was inspected under the microscope. 9μm Al$_2$O$_3$ leaves a coarse surface of the substrate when seen under the microscope, hence 3μm Al$_2$O$_3$ (Logitech) was used for lapping the substrate for the second time. 50μm was removed and the substrate was re-examined for roughness. The substrate was chemically polished with syton solution (SF1 - Logitech) approximately one hour. The syton treatment gives a lower number of defects and provides an enhanced planarity to the surface. After polishing, the substrate was removed from the metal body and separated from the glass slides. The substrate was immersed in eco-clear polishing liquid (Logitech) at 60°C to remove all the wax. The aluminium layer was removed by placing the substrate in aluminium etchant (MicroChemicals) heated to 50°C. The substrate was cleaned using acetone, isopropanol and de-ionised water in sequence before being blow-dried with compressed nitrogen.

4.3 Waveguide theory

4.3.1 Diffusion theory

The channel waveguides used in this project were produced using the ion-exchange technique. Ion-exchange has been used as the basis for a diverse range of optical fibre compatible planar waveguide devices in glass, from passive waveguide structures [205, 206], and sensors [207] to active laser and amplifier devices [208-210]. In the ion-exchange process, sodium ions from the soda-lime glass substrate and the caesium ions from the molten caesium salt are exchanged by diffusion [211-213]. Diffusion is a passive transport phenomenon where particles move from a higher chemical concentration to a lower chemical concentration. This kinetic
process leads to the homogenisation of a material with different chemical elements. A mass flux exists in a diffusion process due to the concentration gradient. This process may continue to reach a dynamic equilibrium where the concentration gradient is minimised. Such a process is governed by Fick’s First and Second Laws.

Fick’s First Law states that the flux, $J$, of a component of concentration, $C$, going from a region of high concentration to lower concentration is proportional to the concentration gradient. Assuming an isotropic material, this can be written as

$$J = -D_c \nabla C$$

where $D_c$ is the diffusion coefficient and the multi-dimension gradient operator, $\nabla C$ can be expressed as

$$\nabla C = i \frac{\partial C}{\partial x} + j \frac{\partial C}{\partial y} + k \frac{\partial C}{\partial z}$$

where $i$, $j$ and $k$ is the coordinate in 3D. Assuming now a one dimension problem, Equation 4.2 can be simplified further to the form

$$J = -D_c \frac{dC}{dx}$$

Figure 4.5 A schematic drawing of the effect of Fick’s First Law of diffusion

By considering an arbitrarily small volume in a diffusing system, there is a mass accumulation rate as there is a difference of inflow and outflow of mass. This is limited by the conservation of mass,
Equation 4.5
\[-\nabla J = \frac{\partial C}{\partial t}\]

Equation 4.5 shows that if there is a divergent flow, which means that \( \nabla J > 0 \), then the concentration decreases with time. Substituting Equation 4.5 into Fick’s First Law, Equation 4.4, Fick’s Second Law can be obtained,

Equation 4.6
\[\frac{\partial C}{\partial t} = D \nabla^2 C\]

Fick’s Second Law states that the rate of change of concentration is proportional to the rate of change of concentration gradient at that point in the field. In one dimension, the equation simplifies to

Equation 4.7
\[\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}\]

By taking into consideration the Arrhenius equation, this allows the temperature dependence to be included in the diffusion coefficient. The diffusion coefficient can be described by

Equation 4.8
\[D_c = D_0 e^{-\frac{Q}{RgT}}\]

where \(Q\) is the molar activation energy, \(R_g\) is the gas constant, \(T\) is the temperature and \(D_0\) is the maximum diffusion coefficient. While the Arrhenius equation is remarkably accurate in a wide range of circumstances including in this diffusion problem, the equation fails at high temperature, \(T > 0.7T_M\), where \(T_M\) is the melting point of the substrate. The diffusion system starts showing non-Arrhenius behaviour due to the fact that the equation does not take into account the phase change of the substrate. The solution of the diffusion equation for unlimited diffusion source is a complementary error function (erfc) [175]. The general solution to the diffusion profile can be written as

Equation 4.9
\[C(x, t) = C_i \left[1 - \int \exp \left(-\frac{x^2}{4D_c t} \right) dx\right]\]

or

Equation 4.10
\[C(x, t) = C_i \text{erfc} \left(\frac{x}{2\sqrt{D_c t}}\right)\]
where $C_s$ is the maximum solid solubility of the diffusing substance at the temperature of the diffusion. Equation 4.1 shows that the refractive index change according to the percentage of diffused Cs\(^+\) ions. Hence the refractive index profile will follow the diffusion profile; the complementary error function. However, Equation 4.7 in the theory assumes that the salt melt molecule concentration has no effect on its diffusion rate. However the process of caesium ions diffusing into the soda-lime glass, has a significantly varying diffusion constant (as a function of caesium and sodium local ions concentration), as stated by Neuman in [214] (higher ion concentration increases the diffusion). This condition is known as ‘double alkali effect’. Hence the refractive index profile was found to closely follow the Fermi function, as shown in Figure 4.6.

![Figure 4.6 The refractive index profiles established using the Fermi function (■) and the complementary error function ( ) with increasing depth. The parameter $w$ used in Equation 4.11 is 2\(\mu\)m (selected arbitrarily)](image)

Below are the equations used to simulate Fermi function [212, 214],

**Equation 4.11**

$$n(x) = n_s + \Delta n \left[ 1 + \exp \left( \frac{x - d_i}{w} \right) \right]^{-1}$$
and the complementary error function,

\[
Equation \ 4.12 \quad n(x) = n_3 + \Delta n \ erfc \left( \frac{x}{d_{erfc}} \right)
\]

where \( n_3 \) is the substrate index, \( \Delta n \) is the peak change in the refractive index, \( d_f \) and \( d_{erfc} \) are the depth of the waveguide for Fermi and complementary error function respectively and \( w \) is the steepness of the refractive index profile centred at depth, \( d \). Figure 4.6 shows that the refractive index remained almost the same at the waveguide surface and decreased with depth. This is confirmed by the waveguide profile data, provided by Dr Olav Hellesø (of the University of Tromsø) and analysed in [191]. For simplification purposes, the ray and electromagnetic description that will be discussed in later sections, assumes that the index profiles follow a step function, an approximation to the Fermi function.

4.3.2 Ray description of the optical waveguide

Physical quantities of optical waveguide are easier to be understood from a ray optics approach. An asymmetric slab waveguide is considered in this section in lieu of channel waveguide in order to simplify the explanation. An asymmetric configuration is chosen to represent the waveguides used in this project. In a slab waveguide structure, the high index layer acts as the active layer or core (\( n_2 \)), where the light is confined. The core layer is packed in between two lower refractive index layers; cover (\( n_1 \)) and substrate (\( n_3 \)) respectively, as shown Figure 4.7, with a step index profile.

![Figure 4.7 Light propagating in optical waveguide](image)

The condition for light to propagate down the core layer is that the light undergoes total internal reflection when incident upon the two boundaries. This indicates that
\( n_1, n_3 < n_2 \) and the angle of propagation, \( \theta \), as illustrated in Figure 4.7, at each interface must be greater than the critical angle, \( \theta_c \). As \( \theta_c \) is normally larger for the substrate-core interface (due to low index contrast) compared to the core-cover interface, the value of the critical angle, \( \theta_c \), is taken to be [192, 193, 215],

\[
\sin \theta_c = \left( \frac{n_3}{n_2} \right)
\]

The light is travelling in a confined mode when \( \theta \) is greater than \( \theta_c \). However, it is more convenient to work in terms of propagation constant, \( \beta \), which is the \( z \)-component of the confined propagating wavefront \( (k_z) \). The propagation constant can be given by,

\[
\beta = k_0 n_2 \sin \theta
\]

where

\[
k_0 = \left( \frac{2\pi}{\lambda_0} \right)
\]

The quantity \( n_2 \sin \theta \) in Equation 4.14 is often known as the effective index, \( n_{\text{eff}} \). Thus Equation 4.14 can also be represented by \( \beta = k_0 n_{\text{eff}} \). The propagation constant in the \( x \) direction can be represented by,

\[
h = k_0 n_2 \cos \theta
\]

Combination of Equation 4.14 and Equation 4.16 provides the association of the propagation constant in the \( x \) and \( z \) directions,

\[
h = \sqrt{n_2^2 k_0^2 - \beta^2}
\]

In order for the wavefront to remain continuous, the propagation constant in the \( z \) direction, \( \beta \), must also be the same in the cover and the substrate layer. Instead of representing a genuine propagation constant for confined modes, light travelling in the cover and the substrate layer represents the decay constant. The decay constants for the cover, \( q \) and the substrate layer, \( p \) are given by

\[
q = \sqrt{\beta^2 - n_1^2 k_0^2}
\]
\[ p = \sqrt{\beta^2 - n_1^2 k_0^2} \]

To facilitate a confined mode, the value of \( \theta \) must be greater than \( \theta_c \) at both interfaces, this indicates that

\[ n_1 \leq n_3 \leq n_{\text{eff}} \leq n_2 \]

which defines the range of possible effective indices that the confined mode can have. In deriving equations in Section 4.3.2, it is assumed that there is a continuous number of modes for the range \( \pi > \theta > \theta_c \). In fact, there is only a discrete number of modes within this range which will propagate when considering the phase shift requirement for a constructive interference. However, in the scope of this project, the solution for the waveguide equations is more straightforward using electromagnetic approach.

### 4.3.3 Electromagnetic description of optical waveguide

The ray optics or geometric optics approach, described in the previous section, provides rules for propagating these rays through an optical system. However, this is a significant simplification of optics and does not include optical effects such as diffraction and polarisation. Thus, in solving the wave equation, the electromagnetic approach is commonly taken. Taking into account the vector identities, the Maxwell equations can be simplified and re-expressed as the Helmholtz wave equation, given by [192, 193, 215]

\[ \left( \nabla^2 + \omega^2 \varepsilon_0 \mu_0 \right) E = 0 \]

where \( \omega \) is the angular velocity, \( \mu_0 \) is the permeability of free space and \( E \) is the electric field. Solutions for this equation for the core, cover and substrate layer are expected to be a travelling wave solution where the electromagnetic waves maintain their transverse spatial distribution. The electric field is in the form of

\[ E(x, y, z, t) = E(x, y) \exp i(\omega t - \beta z) \]

while the magnetic field takes the form of

\[ H(x, y, z, t) = H(x, y) \exp i(\omega t - \beta z) \]
where \( \omega = 2\pi c/\lambda \), and \( c \) is the velocity of light in a vacuum and \( \lambda \) is the wavelength of light in a vacuum. Several simplifications can be made when considering that the waveguide is in planar form. There are no field changes in the \( y \)-direction and hence \( \delta/\delta y = 0 \), \( \delta/\delta z = -i\beta \) and \( \delta/\delta t = -i\omega \) for either field. Equation 4.22 and Equation 4.13 can be further simplified into,

**Equation 4.24**
\[
\nabla \times E = -\mu \frac{\delta H}{\delta t}
\]

**Equation 4.25**
\[
\nabla \times H = -\varepsilon \frac{\delta E}{\delta t}
\]

Thus, solutions for an isotropic, ideal dielectric medium are,

**Equation 4.26**
\[
E_y = -i \omega \mu \beta H_x
\]

**Equation 4.27**
\[
\frac{\delta E_y}{\delta x} = -i \omega \mu H_z
\]

for a transverse electric (TE) mode and

**Equation 4.28**
\[
H_y = \frac{\omega \varepsilon}{\beta} E_x
\]

**Equation 4.29**
\[
\frac{\delta H_y}{\delta x} = -i \frac{\omega \varepsilon}{i} E_z
\]

for transverse magnetic (TM) mode. The two self consistent solutions correspond to the two possible orthogonal polarisation states. The first is the transverse electric (TE) mode which means the electric field vector is confined to the \( y \)-direction. Likewise the second solution is called the transverse magnetic (TM) mode since the magnetic field vector is confined to the \( y \)-direction, as illustrated in Figure 4.8.

Further examining the TE mode, Equation 4.26 can also be written as

**Equation 4.30**
\[
E_y(x,z,t) = \xi_y(x) \exp i(\omega t - \beta z)
\]
In a planar waveguide, the power is concentrated in the guiding layer and decays in either of the boundary regions. This can be written as

\begin{align*}
\text{Equation 4.31} & \quad \zeta_y(x) = A_1 \exp(-qx) \\
& \quad 0 \leq x < \infty, \text{ cover} \\
\text{Equation 4.32} & \quad \zeta_y(x) = A_2 \exp \left[ \cos(hx) - \frac{q}{h} \sin(hx) \right] \\
& \quad -d \leq x \leq 0, \text{ core} \\
\text{Equation 4.33} & \quad \zeta_y(x) = A_3 \left[ \cos(hd) + \frac{q}{h} \sin(hd) \right] \exp \left[ p(x + d) \right] \\
& \quad -\infty < x \leq -d, \text{ substrate}
\end{align*}

where \( d \) is the thickness or depth of the core. Equation 4.31 to Equation 4.33 are the solutions for each layer. The coefficient needs to be chosen such that \( \zeta_y \) and \( \delta \zeta_y/\delta x \) are continuous at \( x = 0 \) and \( x = -d \). Noting this, and matching Equation 4.31 to Equation 4.33 with the boundary equations, yields the same quantities as given in the ray description. The expression for the wavenumbers \( q, h \) and \( p \) in the cover, core and substrate respectively are given by,

\begin{align*}
\text{Equation 4.34} & \quad q = \sqrt{\beta^2 - n_1^2 k_0^2} \\
\text{Equation 4.35} & \quad p = \sqrt{\beta^2 - n_2^2 k_0^2} \\
\text{Equation 4.36} & \quad h = \sqrt{n_2^2 k_0^2 - \beta^2}
\end{align*}

The condition that \( \delta \zeta_y/\delta x \) is continuous at \( x = -d \) gives the guidance equation for the discrete modes,
and can be re-written to this form in order to find all the possible solutions for all the waveguide modes,

\[ \tan(hd + m_n\pi) = \frac{p + q}{h - \frac{pq}{h}} \]

where \( m_n \) denotes the mode number. The propagation constant of the possible modes can be calculated from Equation 4.38 if the refractive indices and the thickness of the waveguide layer are known. The refractive indices have magnitudes obeying \( n_1, n_3 \leq n_{\text{eff}} \leq n_2 \) in order to have a confined mode. The solutions now have a discrete, finite number of modes starting with a zero order mode, closest to \( \theta = 90^\circ \), having the highest effective index. Then, with increasing values of each mode, lower values of \( n_{\text{eff}} \) are obtained and values of \( \theta \) decrease until the critical angle is reached.

If the material of the structure exhibits no loss or gain, then these modes will propagate without loss or gain indefinitely within the structure. Hence, the effective indices of the confined modes are purely real, as illustrated in Figure 4.9.

\[ \text{Equation 4.37} \quad \tan(hd) = \frac{h(p + q)}{h^2 - pq} \]

\[ \text{Equation 4.38} \quad \tan(hd + m_n\pi) = \frac{p + q}{h - \frac{pq}{h}} \]

Figure 4.9 Plot of the effective refractive index and wave representation for the three mode types that can be found within a waveguide [192]
As the $n_{eff}$ approaches $n_3$, the velocity of light is near to the velocity in $n_3$ which causes the light to be no longer confined and once this point is past, the light propagates into the substrate. This phenomenon is known as cut-off and it limits the number of confined modes that may propagate. The light propagation into the substrate is referred to as a substrate mode or is also known as leaky mode. The real part of the effective indices of the substrate mode is in the range of $n_1 \leq n_{eff} \leq n_3$. In the substrate modes, as illustrated in Figure 4.9, all modes have negative imaginary parts to the effective indices that represent loss. Since at cut-off $n_{eff} = n_3$, this serves as the limit for the light to remain confined and Equation 4.17 for $h$ can now be written as,

Equation 4.39

$$h = k_0 \sqrt{n_z^2 - n_3^2}$$

At cut-off, the phase shift is at maximum and $h$ must be an integer multiple of $\pi$ for constructive interference to occur. The total number of modes, $M$, of the same polarisation that the waveguide can support is

Equation 4.40

$$M = \frac{2d}{\lambda} \sqrt{n_z^2 - n_3^2}$$

For a monomode waveguide, the $M$ value should be equal to 1. The values of $n_z$ and $n_3$ can be obtained from the glass substrate and the index change from the salt melt used in the fabrication. The variable $d$, the thickness of the core, for a monomode waveguide can then be estimated and correlated to the ion-exchange time in the waveguide fabrication. This equation is further explored in the next section.

### 4.3.4 Modelling of optical waveguides

The diffusion of ions in the glass substrate has so far been assumed to occur in the depth or $x$-direction only. In fact, for an ion-exchanged channel waveguide, the diffusion also occurs laterally. Although a numerical solution of three-dimensional diffusion using the electromagnetic approach is possible, the technique is very time-consuming and difficult. Hence a commercial software package, BeamPROP, utilising the beam propagation method (BPM) in the model is chosen. BPM is an approximation technique where it simulates the propagation of light using the required starting field and approximates the consecutive fields in the $z$-direction of the optical waveguide at regular steps. A numerical simulator such as Matlab was used to simulate slab waveguide effective index. Equations in Section 4.3.1 to Section 4.3.3 are employed in the slab waveguide simulation. This section determines the
optimum waveguide width and depth theoretically (highest surface intensity) for trapping and propulsion of particles and cells.

### 4.3.4.1 Effective refractive index

Considering the diffusion to occur only in $x$-direction of the glass substrate, the effective refractive index of a slab waveguide against the waveguide depth is simulated using Matlab. Equations in Section 4.3.2 and 4.3.3 were used for this purpose. Estimation of the monomode to multimode boundary with respect to the depth of the waveguide based on Equation 4.40 [216, 217] was also carried out. A step function index profile is assumed in all simulation (unless stated otherwise) with the refractive index of the soda-lime substrate and the caesium ion-exchanged waveguide taken to be 1.50 and 1.54 respectively at 1064nm [191, 197, 198, 201]. A maximum refractive index increment of 0.04, for a caesium ion-exchanged, is observed in several review papers [197, 198].

![Figure 4.10 The effective refractive index of a slab waveguide. Red line indicates effective index at 632.8nm and blue line indicates effective index at 1064nm](image)

Simulation of $n_{\text{eff}}$ for slab waveguide is essential for comparison with waveguide characterisation process discussed in Section 4.4.1. Figure 4.10 shows that the
effective index increases with depth of the waveguide as more ions from the salt melt diffuse into the substrate. The increment reaches a plateau as the effective index is approaching 1.54. The waveguide remains as a single mode waveguide for depths up to 0.907µm and 1.526µm at 632.8nm and 1064nm respectively. Channel waveguides were simulated using BeamPROP using the step index profile. The effect of varying channel widths and waveguide depths was investigated and the simulation results are plotted in Figure 4.11. A similar increment trend is also observed in channel waveguides, where the effective index increases with waveguide depth. The investigation reveals that effective index also increases with channel width. In this case, the effective index simulated for slab waveguide serves as the maximum index for channel waveguides (given that the waveguide depth is fixed). For example, the effective index for a slab waveguide (1µm deep) is 1.507 and the maximum index for a similar waveguide depth, with the width of 10µm, is 1.5045. The index increment illustrated in Figure 4.11 of the channel waveguides reaches a plateau as the waveguides become wider.

![Figure 4.11 The effective refractive index of channel waveguide modes for varying depths at 1064nm wavelength](image)

### 4.3.4.2 Electric field and surface intensity

For a given value of wavelength and waveguide width and depth, BeamPROP can generate the electric field distribution. Illustrated in Figure 4.12 is the electric field
distribution for a waveguide of 4.5µm width and 1.4µm depth at 1064nm wavelength. The corresponding effective index value is 1.5075. The input power to the waveguide is normalised to 1W. Figure 4.12 b) illustrates that across the channel width, the electric field for the fundamental mode peaked at the centre of the waveguide and decrease exponentially from that point towards the core-substrate boundary symmetrically (Refer Figure 1.2 for realisation on channel waveguide schematic). This decay rate is different when looking at the cross-section of the waveguide, as illustrated in Figure 4.12 c). The different decay rate between the core-substrate boundary and the core-cover boundary observed corresponds to Equation 4.31 to Equation 4.33 due to differences in the refractive index.

A large difference of the refractive index, in this case it refers to the core-cover boundary, causes a faster decay rate compared to the core-substrate boundary. The field penetrates deeper into the substrate (core-substrate boundary) due to the small (0.04) index difference. Considering only the electric field on the surface (channel depth = 0) and at the centre of the waveguide (channel width = 0), the surface intensity of the specific waveguide in Figure 4.12 can be calculated. The intensity is given as [218, 219],

Figure 4.12 Electric field plot for a single channel waveguide of 4.5µm width and 1.4µm depth, a) 3D view, b) against channel width, c) against channel depth in TM mode
where $c$ is the speed of light, $\varepsilon_0$ is the permittivity of free space, $n$ is the refractive index of the waveguide and $E$ is the electric field. Figure 4.13 shows the computed beam profile from Beamprop for a 1µm, 3µm, 6µm and 10µm waveguide width. The corresponding surface intensity for a range of waveguide widths is shown in Figure 4.14 for a fixed depth of 1.4µm and fixed input power of 1W. The corresponding output power is also plotted on the same figure for comparison. The output power in this case is not always 1W due to the modal mismatch simulated in BeamPROP.

The power into the waveguide increases as the waveguide width is increased, yet the field is spread across a larger area for the wider width waveguide. Hence the surface intensity, as indicated in Figure 4.14, decrease as the waveguide width is wider.
(given that the input power is fixed). When the channel’s width become smaller, the field spread across smaller area and thus, increasing the surface intensity. However, as the waveguide width becomes too small (~1µm), the light is no longer propagating into the substrates in the confined mode as shown from the beam profile in Figure 4.13. Hence there is an optimum operating point in the surface intensity distribution for the range of waveguide widths investigated. This is simulated to be a 4.5µm wide channel, as illustrated in Figure 4.14.

In order to get an optimum point (highest value) of the surface intensity, the simulation is extended to cover a range of waveguide depths as well as waveguide widths. Figure 4.15 is generated from the effective index values in Section 4.3.4.1 and the corresponding electric field plot from BeamPROP for each waveguide with a specific depth and width. The surface intensity peaked at 4.5µm width regardless of the waveguide depth, as illustrated in Figure 4.15 b) for a fixed 1W input power. The waveguide of depth 1.4µm has the highest calculated surface intensity from the range tested in the simulation. From the simulations and analysis carried out, the optimum waveguide parameter for a strong and stable trapping depends on the highest surface intensity is observed at 4.5µm width and 1.4µm depth. It is assumed that a high surface intensity will results in a stronger evanescent field to trap and propel the particles and cells.

Figure 4.14 Simulated surface intensity and output power distribution of a waveguide with varying channel widths
The surface intensity of a waveguide also varies according to the propagating mode inside the waveguide [220-223]. The corresponding surface intensity of other propagating modes can be obtained by extending the modelling of surface intensity, as presented above. Figure 4.16, which was developed with the help of Dr Fan Zhang, shows surface intensity for other modes. This analysis is also presented in several papers [220-228]. The fundamental mode is concluded to produce the highest surface intensity for depths up to about 1.6µm. Figure 4.16 illustrates that the peak intensity for each consecutive mode lessens as the mode number increases. In addition, in all the modes simulated in Figure 4.16, the TM mode is seen to produce a slightly higher surface intensity compared to the TE mode.

Figure 4.15 Intensity of the waveguide surface with varying depths and widths, a) 3D view, b) against channel width, c) against channel depth

4.4 Waveguide characterisation

The previous section discussed the fabrication processes involved in making caesium ion-exchanged waveguide. Section 4.3.4 dealt with theoretical aspects of optical waveguide and gives the parameter guidelines such as the effective index for determining the highest surface intensity of a caesium ion-exchanged waveguide. This section describes the methods for measuring these parameters and the characterisation results for the fabricated caesium ion-exchanged waveguides. It is
intended that the waveguide, with the parameters matched to the optimised theoretical value, will be used for the trapping and propulsion experiments. However, the optimised theoretical values serve only as a guideline and not necessarily as the exact value. Note that the theoretical model was calculated from the step index model and not the gradient index (Fermi) model.

![Figure 4.16 Surface intensity of TM and TE modes for step index waveguides of $n_1 = 1.33$, $n_2 = 1.54$, $n_3 = 1.50$ at 1064nm for a fixed 1W input power](image)

### 4.4.1 Effective refractive index measurement

The effective refractive index of waveguide modes is known to be a critical parameter in characterising waveguides. The prism coupling method [229] highlights the effect of different ion-exchange times by giving the number of modes excited at a given wavelength and their corresponding effective refractive index. The method measures the effective index of the excited mode via the angle of incident light onto the prism facet.

In order to measure the incident angle, the micrometer (Thorlabs, PT1), the waveguide and the prism were mounted on a rotational stage run by a motor controller (Thorlabs, CR1-Z7E) with an accuracy up to 0.001°. The waveguide was clamped to the prism using the micrometer. The HeNe laser ($\lambda = 632.8$nm, Thorlabs, HRP050) was directed to the point of contact between the prism and the waveguide.
The motor controller was put in a reset mode, where the angle of incident light and the reflected light from the input face of the prism is at zero degree. The stage was rotated until the light was coupled into the waveguide. The condition for the light to couple into the waveguide was when the evanescent field in the gap between the prism base and the waveguide has the same phase velocity. The present of coupled light indicated that a mode was excited, noticeable by a line profile in the screen, and the angle was recorded. Note that a gap of the order of half a wavelength is required for sufficient light to couple into the waveguide [229]. A power meter was used to assist the detection of excited modes by measuring the intensity of the coupled light. There was a range of intensities of the coupled light; the highest intensity in that range corresponds to the incidence angle.

![Geometrical setup for calculating the effective refractive index](image1)

**Figure 4.17 Geometrical setup for calculating the effective refractive index**

![Laser path through the prism](image2)

**Figure 4.18 Laser path through the prism**

From the ray tracing technique, the relationship of \( n_{\text{eff}} \) and the incident angle, \( \theta_m \) can be determined using Snell’s law [230].
where \( n_p \) is the refractive index of the prism and \( \theta_p \) is the angle of prism. Using Equation 4.42, the incident angle can be used to estimate the effective refractive index of each excited mode.

\[
\text{Equation 4.42} \quad n_{\text{eff}} = n_p \sin \left( \theta_p + \left( \arcsin \left( \frac{\sin \theta_p}{n_p} \right) \right) \right)
\]

<table>
<thead>
<tr>
<th>Ion-exchange time (h)</th>
<th>Fundamental ( n_{\text{eff}} )</th>
<th>Expected depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.5</td>
<td>1.5151</td>
<td>1.03( \mu )m</td>
</tr>
<tr>
<td>8</td>
<td>1.5189</td>
<td>1.39( \mu )m</td>
</tr>
<tr>
<td>10</td>
<td>1.5219</td>
<td>1.52( \mu )m</td>
</tr>
<tr>
<td>17</td>
<td>1.5280</td>
<td>2.45( \mu )m</td>
</tr>
</tbody>
</table>

Table 4.2 Details of the waveguides fabricated using a soda-lime glass substrate. The \( n_{\text{eff}} \) of the fundamental modes were measured at 632.8nm. The expected depth is fitted according to the theoretical simulation values in Section 4.3.4.1

A set of caesium ion-exchanged waveguides have been fabricated using soda-lime glass slides. These waveguides follow the fabrication process, as described in Section 4.2, with varying ion-exchange time from 4 to 17 hours. Each of the waveguides was characterised using the prism coupling method and their measured effective index of the fundamental mode is tabulated in Table 4.2. The channel waveguides were very small, ranging from 1\( \mu \)m up to 10\( \mu \)m, and coupling light from a prism to a small channel was very complicated using the setup shown in Figure 4.17. As the ion-exchange process also create a slab waveguide on the back of the glass substrate, the measurement of effective index was done on the slab waveguide modes instead. In this case, the effective index measured serves as the upper limit of the effective index of the modes in the channel waveguide. The range of the effective index of the modes in the channel waveguide is between the \( n_{\text{eff}} \) of the slab and the substrate’s refractive index. From the characterisation measurement, the effective index for the 4 hour ion-exchange time waveguide was unobtainable due to the inability to couple light into the slab waveguide. This may suggest that the waveguide depth is too shallow to permit light propagation in a confined mode. The effective index for the fundamental mode and waveguide depth of the rest of the waveguides shows an increment as the ion-exchange time is longer.

It was also observed that the effective index measured does not necessarily correspond to the ion-exchange time. For example, the fundamental mode effective
index of a 10 hour caesium ion-exchanged slab waveguide, in Table 4.2, is 1.5219. However, another waveguide fabricated using the same step and same batch of soda-lime glass, only needed 6 hour of ion-exchanged time to give an effective index of 1.5221, as illustrated in Figure 4.19. Lack of correlation between ion-exchange time and the effective refractive index was also observed in [191, 231]. This may be due to the fluctuation of the furnace temperature during the ion-exchange process or a variation in the sodium composition of each soda-lime glass substrate [191, 231].

In order to rectify this problem, a commercial optical glass, BK7 was used as a substrate. BK7 glasses, as mentioned in Section 4.2.1, have a more consistent composition. Figure 4.19 shows a set of caesium ion-exchanged waveguides using both soda-lime and BK7 glass substrates. In order to observe the effect of time and material used against the effective index, both type of glass substrate were ion-exchanged at the same time using the same caesium salt melt and furnace for a varying time duration. Figure 4.19 is a plot of the effective index difference of waveguide modes ion-exchanged for between 6 to 20 hours. The refractive index of soda-lime and BK7 substrate is 1.5098 and 1.5170 respectively at 632.8nm [197, 198]. From Figure 4.19, the $\Delta n_{eff}$ in BK7 substrates is demonstrated to be much less than soda-lime substrates despite similar ion-exchange time. For example, a BK7
substrate ion-exchanged for 20 hours only produce a maximum of 0.018 $\Delta n_{eff}$ compared to 0.023 for soda-lime substrate ion-exchanged for 6 hours. This is due to the fact that soda-lime glass possesses a higher concentration of sodium oxide ($\text{Na}_2\text{O}$) than BK7 substrates [191, 197], as mentioned in Section 4.2.1. Hence, all experimentation using optical channel waveguides in this project used caesium ion-exchanged waveguide made with a soda-lime glass substrate. As the measurements in this section were done using slab waveguide modes, it is expected (from Section 4.3.4.1) that, for channel waveguides modes, the effective index increases with width and that wider channel widths excite more modes [191, 232]. From the prism coupling method, a caesium ion-exchanged waveguide with the closest effective refractive index to the theoretical model was the waveguide with 8 hour ion-exchange time (expected depth 1.39µm).

### 4.4.2 Cut-off wavelength determination

The spectral attenuation measurement is essential not only to determine that the waveguide to be used is operating in single mode at the required wavelength, but also the wavelength limit for the waveguide operation. Above this wavelength limit, the waveguide will cease to support any mode at which point the theoretical loss will be infinite. This wavelength is called the cut-off wavelength. The spectral attenuation measurement was made by coupling light from a broadband white light source (OSRAM) into the waveguide as shown in Figure 4.20. The white light source was focussed into a standard single mode (SM) fibre through a 20x lens. The fibre was then aligned to the waveguide to give a maximum output.

![Figure 4.20 Experimental setup for cut-off wavelength measurement](image)

The output from the waveguide is focused to a computer-controlled monochromator (Princeton Instrument). A polariser and a chopper are positioned...
between the waveguide and the monochromator to obtain correct field alignment and to provide a reference signal for the lock-in amplifier (LIA, Boston Electronics). The LIA amplified the periodic square wave signal that was generated by the light source through the chopper. The monochromator then scanned in steps through a wavelength range defined in the computer. The output power spectrum observed was compared with the power spectrum from the fibre to determine the spectral loss due to the presence of the waveguide. Loss as a function of wavelength can be determined by \[30, 191, 232\],

\[
\text{Equation 4.43} \quad \text{Loss}_{\text{db}}(\lambda) = -10\log_{10} \left( \frac{P_{\text{waveguide}}(\lambda)}{P_{\text{fibre}}(\lambda)} \right)
\]

where \(P_{\text{waveguide}}\) and \(P_{\text{fibre}}\) are the output power of the waveguide and fibre respectively. There are two types of detectors used in this experiment depending on the expected cut-off wavelength of the waveguide. It will be either a silicon detector for the range 190-1100nm or an indium gallium arsenide (InGaAs) detector for the range 800-1600nm. The cut-off wavelength was defined as when the loss rises to 3dB higher than the background loss.

Figure 4.21 Typical waveguide spectral loss for caesium ion-exchanged waveguide. This plot represents a soda-lime substrate ion-exchanged for 8 hour with a nominal waveguide width 4µm ( ), 5µm ( ) and 10µm ( ). Dashed lines represent TE modes whereas solid lines represent TM modes.
Illustrated in Figure 4.21 is the typical loss spectra observed using the monochromator. The loss was observed to maintain approximately 22dB before suddenly increasing; indicating the cut-off wavelength. A high loss detected in the measurements reflects the losses in the light path; from the source to the fibre, waveguide, chopper, polariser and finally to the monochromator. Figure 4.21 also shows that, as the waveguide width increased, the corresponding cut-off wavelength also increased. This also applies to other caesium waveguides ion-exchanged at different durations as illustrated in Figure 4.22. Moreover, the cut-off wavelength also shows an increment for longer ion-exchange times. This corresponds to more modes being observed for longer ion exchange times as shown in Figure 4.19. There is also a general trend that the TE mode cut-off wavelengths are significantly higher than the TM mode in the caesium ion-exchanged waveguides. This indicates that the caesium ions produce a birefringent waveguide and this may be due to the extra stress caused by the large caesium ions [191, 197, 211, 214, 232]. The cut-off wavelength measurement is conducted for each fabricated waveguides in order to determine that the waveguide is operating in single mode at intended wavelength, which in Section 4.3.4.2 indicates to have the highest surface intensity. The 8 hour ion-exchanged waveguide, which has the closest effective refractive index to the theoretical model, is shown to be operating in single mode at 1064nm.

![Cut-off wavelength of caesium ion-exchanged waveguides in soda-lime substrate for 6.5 hours ( ), 8 hours ( ), 10 hours ( ) and 17 hours ( ). Dashed lines represent TE modes whereas solid lines represent TM modes](image)

Figure 4.22 Cut-off wavelength of caesium ion-exchanged waveguides in soda-lime substrate for 6.5 hours ( ), 8 hours ( ), 10 hours ( ) and 17 hours ( ). Dashed lines represent TE modes whereas solid lines represent TM modes.
4.4.3 Waveguide degradation

A refractive index change in ion-exchanged glass depends on three major factors; molar volume or ionic size, stress due to ion substitution and ionic polarisability [197, 203]. Caesium ions are larger than sodium ions; hence the size difference causes a swelling effect and creates compressive stresses on the substrate surface [203]. The size difference also swells the waveguide surface, creating a height increase. The channel height, which was measured using a surface profiler machine (KLA Alpha Step 500), was observed to increase with the ion-exchange time, as shown in Figure 4.23. It was also observed that height of the waveguide degrades in water. This effect was more enhanced in heated water. Figure 4.24 shows the height of the channel waveguide submersed in water at different temperatures and over various periods of time.

![Figure 4.23 Channel height for caesium waveguides with varying ion-exchange times](image)

The measurement was conducted on a caesium waveguide ion-exchanged for 17 hours with an initial channel height of 237nm. For the first stage, the waveguide was submersed in water at 20°C for 48 hours. On the second stage, the temperature was increased to 50°C for the first 24 hours using the same waveguide. The temperature was increased again to 80°C at the 24th hour and maintained at that temperature for
8 hours. The height of the waveguide was measured periodically during the investigation. The channel height reduced very slowly (0.08nm/h) at room temperature (20°C) but shows a significant reduction in height (31.25nm/h) at 80°C. The loss measurements conducted on the waveguide after the experiments showed that the waveguide failed to support any mode. Such a phenomenon has several impacts on the experiments conducted using the caesium ion-exchanged waveguide. The input power at which the waveguide can be used for trapping and propulsion experiments is limited. As the waveguide heats, through absorption, the water in the reservoir also heats up through conduction, causing the waveguide to degrade. This also indicates that the waveguide will degrades as more experiments are conducted on the same waveguide, albeit at slower rate given that the temperature was maintained at 20°C.

![Figure 4.24 The degradation of 17 hour caesium ion-exchanged waveguide in water at different temperatures](image)

### 4.5 Conclusion

Waveguide fabrication steps were described in this chapter with particular interest to the Cs⁺-Na⁺ ion-exchange process. Prior to the fabrication steps, selection of glass substrates and available ions for the ion-exchange process was discussed. Soda-lime glass substrates were chosen due to their ability to produce a high $\Delta n_{\text{eff}}$ layer as a result of high sodium content, readily available and low cost. Caesium ions are
chosen for the waveguide fabrication because of the high index change. Other ions, such as thallium and silver ions, can also produce high index change, but they are either toxic or can easily form metal impurities within the glass that can cause further scattering losses.

This chapter has also described the basic principles of optical waveguides starting with the diffusion theory. The theory is strongly related to the ion-exchange step in the fabrication process that leads to the increment of the refractive index to form optical waveguides. The theory also predicts that the refractive index profile will follow the Fermi function, as indicated in [191, 212, 214]. A ray description is discussed in this chapter to introduce the physical quantities of the optical waveguide using the readily understood ray picture. A step index profile is assumed in the waveguide structure to simplify the waveguide simulations. The solution to the waveguide equations is given in Section 4.3.3 using the electromagnetic approach. There are three possible types of mode in an optical waveguide; confined, substrate and cover modes. The confined mode is when the light is propagating inside the waveguide while the substrate and cover modes (also known as the leaky modes) are when the light is propagating in the substrate and cover respectively. As $n_{\text{eff}}$ values approach the refractive index of the substrate or the cover, the velocity of light is close to the velocity in $n_3$ or $n_1$. This causes the light to be no longer confined and hence the light propagates into the substrate or the cover layer. From the description in Section 4.3.1 to Section 4.3.3, modelling of the optical waveguides is carried out. The effective refractive index is obtained for a slab waveguide with different depths and the boundary of monomode to multimode operations is identified. The simulation is extended further to cover the effect of varying channel widths on the effective refractive index. Section 4.3.4.2 showed the electric field distribution of a waveguide in the fundamental mode and information from these plots is later used with Equation 4.41 to obtain the surface intensity of the waveguides with varying depths and widths. Figure 4.16 illustrated that, within the range of interest of waveguide depths, the fundamental modes give the highest surface intensity.

Waveguides were characterised for their effective refractive index and the cut-off wavelength. It was found from the characterisation process that there was a lack of correlation between the ion-exchange time and the effective refractive index, as observed in [191, 231]. Apart from that, the waveguides were also found to degrade when submersed in water and the effect seems to be enhanced as the temperature increases. Hence, the individual characterisation of fabricated waveguides and the periodic examination of surface profiles are essential to distinguish the properties of the waveguide used. Results from these characterisation methods have shown a
reasonable agreement with the theory, as presented in Section 4.3.4. The theoretical optimum waveguide parameters are 4.5µm channel width and 1.4µm channel depth. The closest fabricated waveguide to these parameters can be obtained from 8 hour ion-exchanged waveguide (estimated depth of 1.39µm) and on 4µm nominal channel width (assuming lateral diffusion). This shows that a waveguide optimised for trapping and propulsion is within the range of waveguides fabricated and thus feasible.
Chapter 5

Brownian motion of particles and cells

5.1 Introduction

The interaction of particles and cells on a surface are investigated via their Brownian motion. In this chapter, the continuous stochastic motion of particles and cells, suspended in various liquid media and on two different surfaces, was observed. All experimental and data processing techniques are discussed in the first section. The investigation of Brownian motion on polystyrene particles and teratocarcinoma cells is presented in later sections. Different aspects such as particle size, functionalisation of surface, viscosity and ionic concentration of media are then examined with relation to Brownian motion on the surface. The investigation of Brownian motion is aimed to give information towards understanding the behaviour of polystyrene particles and teratocarcinoma cells on surfaces that can be utilised for propulsion experiments. The conditions and limitations to allow particles and cells to move freely in Brownian motion on surface are described in this chapter.

5.2 Techniques and materials

5.2.1 Image acquisition

Brownian motion of polystyrene particles and teratocarcinoma cells was investigated through a series of images that were analysed using a combination of LabView and IgorPro software programs. In order to capture these images, a Nikon
optical stereomicroscope (Universal Epi-illuminator 10), equipped with a cooled CCD camera (QImaging, Monochrome Retiga 1300) was used to observe the particles and cells. Image acquisition was also assisted by the microscope’s ability to move in three dimensions which allows the observation of different positions without moving the sample. The microscope, which is supplied with 20x (focal length = 1.2mm) and 10x (focal length = 10.5mm) microscope objective lenses (Nikon), is capable of operating in bright or dark field mode. Bright field mode is the operation mode where all transmitted and scattered light from the sample is observable by the microscope. In contrast, dark field mode only allows scattered light to be collected by the condenser lens; resulting in an enhanced contrast to the image with dark background and bright objects.

![Diagram](image.png)

**Figure 5.1 Experimental set up for monitoring particle and cell movements**

The solution containing particles or cells was pipetted into a reservoir (CoverWell, Z379077, Sigma Aldrich) placed on a soda-lime glass slide, as depicted in Figure 5.1. The glass slide used was of the same type as the glass slide used for waveguide fabrication (Menzel-Gläser glass). ‘Plain’ notation was used for pre-treated glass slides, which indicates that the glass has been clean in diluted piranha solution and oven dried as described in Section 5.2.4. ‘Functionalised’ or ‘PEG-functionalised’ glass slides denotes that the glass surface was modified by having a self-assembled monolayer created from 4% PEG-silane solution, as explained in Section 5.2.4. The glass slide is positioned using either a waveguide mount (Thorlabs, HWMO03) or a vacuum holder (Thorlabs, HWV001). Images captured using both holders are shown in Figure 5.2. Images captured from vacuum held glass slides were found to be clearer, with higher contrast. This is due to the fact that the region of interest on the
glass slide is suspended on one end, thus eliminating the scattering from the typical waveguide holder surface. Since images in this project were captured in dark field mode, utilising a vacuum holder avoid the unnecessary scattering (noise) from the background. The microscope was adjusted in a way that it only focused on the surface of the glass slide, to ensure that only particles close to the surface were observed.

![Image 1](image1.png)  
*Figure 5.2 Cropped images of 10µm polystyrene particles from a glass slide held by a) waveguide mount and b) vacuum holder*

### 5.2.2 Image analysis

Typically up to 10,000 images were taken for each Brownian motion experiment. Each of these images covers 30 to 90 particles or cells (for a concentration of $1 \times 10^6$ particles/cells per ml) using the 20x objective lens. The experiments were repeated at least three times which makes every data point presented in this chapter an average of up to 270 measurements for particles and up to 400 for cells (due to more experimental cycles). Each of the experimental cycles is tagged with information such as the frame rate and binning level. Binning level refers to the resolution of the image. A bigger binning level corresponds to a poorer resolution. On the other hand, frame rate refers to the integration time of the CCD camera where an electronic shutter opens to capture light. Solid particles such as polystyrene particles reflect more light and hence require a shorter integration time compared to the mostly transparent, teratocarcinoma cells. Each image captured by the camera, unfortunately, is not instantaneously stored to the computer. Large image files such as from the highest image resolution (Binning 1, file size 1.28MB), take longer to transfer to the computer as there is also limitation in the data carried by the FireWire connector. Thus the frame rate has to take into consideration the integration time of the camera and also the level of binning required.
All the images are presented by one dimensional hexadecimal matrix. Experimental settings, such as frame rate and binning, are specified at the beginning of each data analysis. Images are loaded into the program and translated into two dimensional matrices for image analysis. The output is presented in black and white images, for example as illustrated in Figure 5.3 a). The main problem in identifying the particle is its boundary, as this is usually in a range of values corresponding to the grey shade. Hence a threshold level is made in order to identify particles and distinguish the background value to be 255 (black) and the particle to be 0 (pure white) as indicated in Figure 5.3 c).

![Figure 5.3 The sequence of image analysis of 10µm polystyrene particles. Data initially presented in 1 dimensional array is translated into a more meaningful, a) 2 dimensional image. In order to detect particles, a threshold is applied to distinguish the position of particles b).](image)

The threshold value is usually taken to be the average pixel value. However, depending upon the quality and the illumination of the images taken, the threshold value is adjusted by adding the standard deviation of the pixel value.

**Equation 5.1**

\[
T_{\text{pixel}} = \left\langle P_{\text{pixval}} \right\rangle + 2 \times \sigma(P_{\text{pixval}})
\]

where \(T_{\text{pixel}}\) is the pixel threshold and \(P_{\text{pixval}}\) is the pixel value. Equation 5.1 is used for determining the pixel threshold and hence detecting polystyrene particles, although the smaller particle size (<3µm) has a larger grey area (the particles image is unfocused in the threshold image due to variation in z-axis position). The area of all particles is tabulated to establish the range of pixels covered by a single particle of a specific size. This discards any motion of agglomerated particles within the image. The region of interest is specified within the image, to monitor an individual particle or multiple single particles. The distances travelled by each particle are also...
analysed, specific for each particle size, to discard any static particles, extreme sudden motion and particles moving in and out of the region of interest. The distance travelled by each particle, for each frame, is tabulated for subsequent graphical representations.

5.2.3 Error estimation

Errors occurring during the data collection and analysis of the Brownian motion are inevitable. Most of the errors usually originated from a physical limitation such as the microscope illumination brightness and camera detector sensitivity. However, identification of such errors and taking actions to minimise them can significantly reduce the inaccuracies. Three types of error are described in this section; random, systematic and dynamic errors.

Figure 5.4 Image of a 10µm polystyrene particle a) prior and b) after the thresholding process

One of the major contributors to errors during the data collection process is from the camera noise. This random error originates from the statistical fluctuation in the number of photons detected by the camera. Such fluctuations still exist even in an ideal condition, due to the nature of the microscope illumination. Error from the camera noise can be minimised by using a brighter illumination although this is limited by the detector saturation level. Any variation in the photons detected by the camera consequently gives a variation in the size detected by the computer. In order to estimate the error from camera noise, the variation of size from one frame to another for a specific particle is tabulated. Their corresponding error in
determining their exact position for each frame is calculated and averaged. Table 5.1 shows the errors from camera noise for a specific particle size and cell.

<table>
<thead>
<tr>
<th>Polystyrene particles/Cells</th>
<th>Measured diameter (µm)</th>
<th>Size standard deviation (µm)</th>
<th>Error from camera noise (x 10^{-3} µm)</th>
<th>Error from thresholding process (x 10^{-3} µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20.99</td>
<td>1.24</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>2.56</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>10</td>
<td>9.606</td>
<td>0.763</td>
<td>2.4</td>
<td>4.0</td>
</tr>
<tr>
<td>6</td>
<td>5.658</td>
<td>0.305</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>2.799</td>
<td>0.129</td>
<td>5.1</td>
<td>5.9</td>
</tr>
<tr>
<td>1</td>
<td>0.989</td>
<td>0.02</td>
<td>8.8</td>
<td>8.7</td>
</tr>
<tr>
<td>NT2</td>
<td>18.91</td>
<td>3.12</td>
<td>6.9</td>
<td>21.7</td>
</tr>
<tr>
<td>TERA1</td>
<td>17.73</td>
<td>2.46</td>
<td>5.4</td>
<td>24.4</td>
</tr>
</tbody>
</table>

Table 5.1 Tabulation of particles and cells size distribution and their corresponding errors measurements. Note that the measured diameter and size deviation for polystyrene particles were provided by the manufacturer. Diameter and size distribution for the cells were measured using Zeiss Axio Observer Z1 microscope.

The thresholding process during the data analysis and the limitation arising from the camera detector can cause pixel biasing in the image identification. The camera was unable to focus to the boundary of all the particles and cells due to the variation in the z-axis position and the focal length of the lens; this results in an ill-defined boundary as shown in Figure 5.4 a). This is a typical systematic error when capturing a 3D object in a 2D image. The thresholding process helps in the identification of the object by setting a sharp boundary, however in exchange it causes pixel biasing to the object, as illustrated in Figure 5.4 b). Error due to the thresholding process is calculated by comparing the size of the threshold image and the measured size for each particle. The bias in determining the exact position of the particle is tabulated and averaged. The errors from threshold process for a specific particle size and cell are shown in Table 5.1. The error can be reduced by using a higher magnification or a better camera detector, although both solutions incur a higher cost in the experimental setup. The thresholding process is more difficult in detecting mammalian cells due to the fact that cells have a higher transmission coefficient (low reflectance). This property permits the passage of light through the cells and consequently makes the cells appear more transparent than polystyrene particles. A low contrast at the cell-background boundary inevitably introduces more errors in determining the cell position, as illustrated in Table 5.1.
The errors shown in Table 5.1 correspond to the bias in determining the exact position of a particle or a cell from one frame to another. Although the value is small, the errors become significant when taking into account the number of frames analysed in each experiment.

Apart from the above, there are several other sources of error. A phenomenon such as drifting is common when securing particles or cells in solution in a reservoir such as CoverWell. Drifting is a phenomenon where there is a current flow that drawn the particles together in a clump. An imbalance of water pressure due to uneven surfaces, the existence of air bubbles or thermal expansion arising from illumination can all be possible factors that cause drifting. Furthermore, there is also vibration from the laboratory environment to consider and also the measurement of Brownian motion in 2D (despite the fact that the particles and cells are moving in 3D) can contribute towards errors for the Brownian motion. However such a dynamic error can be minimised through a detrending analysis [233], which is a computational correction for global error. This technique however has the possibility of reducing the distance travelled due to Brownian motion. Errors from drifting, for instance, can also be detected just by the monitoring of live data from the microscope by an experienced observer. Corrective action such as levelling the glass slide or switching off the microscope light for a while to prevent overheating will minimise the drift effect.

5.2.4 Surface functionalisation

The mammalian cells involved in this project are lymphoblastoma and two types of teratocarcinoma cells; TERA1 and NT2 cells. Lymphoblastoma cells grow in suspension whilst teratocarcinoma cells form a monolayer in a culture medium. A notable property of teratocarcinoma cells, as mentioned in Section 1.5.2, is that they are adherent cells. Polyethylene glycol (PEG) based surface passivation techniques have been demonstrated to prevent cell adhesion [234-238] due to the properties of the oligomer. The oligomer of PEG is hydrophilic and non-structure-forming which act against the association of proteins. Adhesion due to the dominance of van der Waals force can be reduced by physically increasing the distance between the particles/cells and the surface. Surface functionalisation is also one of the ways to achieve the surface separation. Reduction of adhesion is crucial for the Brownian motion analysis and propulsion experiment using the channel waveguide. Apart from that, polymer surface functionalisation was shown to reduce the surface friction [170, 234-241]. This section will discuss the frictional force measurements on PEG-functionalised surface and the thickness of the fabricated PEG layer.
5.2.4.1 Fabrication

The chemical structure of the PEG coating is shown in Figure 5.5. OH groups are created on the glass slide surface to be reacted with the silane group in the PEG chain. A spacer is included between the PEG chain and silane group as a steric protection against unwanted side reactions. The PEG layer used in this project is fabricated by cleaning the glass slide in a weak piranha solution; which is a solution of ammonium hydroxide, hydrogen peroxide, and DI water in a ratio of 1:1:5. This cleaning process creates the OH group on the glass surface. After washing with deionised water and drying, the glass slide is soaked in derivatisation solution made of 4% PEG-silane (Gelest – SIM6492.7) in toluene. The glass slide is left for 24 hours before cleaning consecutively with anhydrous toluene and ethanol. The glass slide is then cured for 30 minutes at 100°C in an oven.

![Chemical structure of PEG coating](image)

Figure 5.5 Schematic of surface modification by self-assembled monolayer of PEG chain [235]

5.2.4.2 Ellipsometry and friction force microscopy

The fabricated PEG layer was characterised using ellipsometry and friction force microscopy (FFM). Ellipsometry is an optical technique that measures the change of polarisation upon reflection or transmission to investigate the refractive index and the thickness of a film. The light incident upon the film can be decomposed into TM and TE component (Refer Figure 4.8 for components orientation). Ellipsometry measures the ratio of amplitudes for each component after reflection and normalised to their initial value.
The relation between the amplitudes can be presented as \[242\],

\[
\frac{r_{TM}}{r_{TE}} = \tan(\psi) e^{i\Delta}
\]

where \(r_{TM}\) and \(r_{TE}\) are the amplitudes of the reflected light in TM and TE components respectively. The first half of Equation 5.2 (\(\tan(\psi)\)) represents the amplitude ratio upon reflection while \(\Delta\), in the second half of the equation, is the phase shift difference. In general, the measured \(\psi\) and \(\Delta\) value cannot be converted directly into the refractive index or the thickness of the film. Hence, a layer model must be established, which in this case, by fixing the refractive index of the film. The refractive index of the PEG layer is 1.403 and of the glass slide is 1.50 \[243\]. Combining the refractive index value, the phase shift and amplitude ratio of TM and TE component of the reflected light from the ellipsometry measurements, the thickness of the PEG layer can be estimated using the ellipsometry simulator, Ellgraph (Optichem).

FFM is a variant of atomic force microscopy that is used for the investigation of material surfaces; in this case frictional force is measured. In this project, frictional force values on the surface also provide an estimation of the resistive force that is opposing the optical force. Hence, given that the optical force is fixed, a lower friction coefficient surface signifies lower opposing force acting on the particle or cell. Thus, this will give faster particles or cells propulsion compared to a high friction coefficient surface. The coefficient of friction can be measured by employing quadrant photodiode and laser setup on FFM system as illustrated in Figure 5.7.
The coefficient of friction can be calculated from [244, 245],

\[ \mu = \frac{F_{\text{lateral}}}{F_{\text{normal}}} \]

where \( F_{\text{normal}} \) is the load applied on the cantilever and \( F_{\text{lateral}} \) is the frictional force. The friction measurements were performed in contact mode and by recording the lateral deflection of the cantilever. This scanning motion produces friction image as shown in Figure 5.8 b). In order to interpret the friction image, the cantilever needs to be characterised.

The spring constant of the cantilever is given by [244, 245],

\[ C_{sp} = \frac{Ywct_c^3}{4L_c^3} \]

where \( Y \) is the Young’s modulus, \( w_c \) is the width, \( t_c \) is the thickness and \( L_c \) is the length of the cantilever. The cantilever in general, is also regarded as highly anisotropic, which leads to the distortion of the friction image. In order to overcome this problem, deflection sensitivity of the cantilever should also be accounted. The deflection sensitivity can be estimated from [244, 245]
where $T_c$ is the torque, $G$ is the shear modulus and $K$ is the geometrical factor of the cantilever. The characteristics of the cantilever (i.e. spring constant and deflection sensitivity) play an important role in determining the friction coefficient. The contrast between different functionalities on the surface may be enhanced via a tip with a stronger perpendicular force. Nevertheless, such a modification has the potential to cause damage to the substrate. Note that the PEG layer measurements were done using a FFM tip deflection sensitivity of 57.61 nm/V and the normal spring constant of 0.063 N/m.

### 5.2.4.3 Thickness and friction coefficient of PEG layer

In order to measure the thickness of the PEG layer, the laser in the ellipsometry setup is focused on the unfunctionalised, plain surface. Figure 5.8 a) shows an ellipsometry image of the PEG area (white) and the plain glass surface (black). Differences in colour indicate the difference in the refractive index. The $\psi$ and $\Delta$ values were measured on the plain and PEG surfaces and tabulated in Table 5.2. The measured values were compared to the theoretical data based on the layer model created in Ellgraph, as shown in Figure 5.9. The layer model is created by fixing the refractive index of the PEG layer and the glass slide to 1.403 and 1.50 respectively.

![Figure 5.8](image-url)

**Figure 5.8** a) Image from the ellipsometry measurement showing the boundary between the plain glass and the PEG layer, b) shows the friction image of the PEG layer, the variation of shades in the image constitute different friction coefficient, less contrast in the image indicates a fairly uniform friction across the PEG layer.
From the experimental data, the PEG layer was estimated to be between 3.7nm to 4.8nm. As a comparison, an advanced chemical drawing tool, ACD ChemSketch, was used to predict the length of the PEG chain. Depending upon the chemical structure configurations, the length of the PEG chain is estimated to vary from 4.3nm to 7.6nm. The range of thickness obtained from the ellipsometry measurements overlapped the range of PEG length. Note that due to the experimental setup, the ellipsometry measurements were conducted on a dry glass surface. This may explain the slight discrepancy between the estimated thickness from ChemSketch and from Ellgraph as the PEG layer may not maintain its brush-like structure in dry condition [246, 247].

Figure 5.9 Theoretical model of ψ and Δ values with their corresponding layer thickness according to the layer model created in Ellgraph

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental data (Degrees)</th>
<th>Thickness (nm) (based on model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Minimum</td>
<td>3.321</td>
<td>3.7</td>
</tr>
<tr>
<td>Δ Maximum</td>
<td>4.344</td>
<td>4.8</td>
</tr>
<tr>
<td>Δ Average</td>
<td>3.803</td>
<td>4.2</td>
</tr>
<tr>
<td>ψ Minimum</td>
<td>5.232</td>
<td>3.7</td>
</tr>
<tr>
<td>ψ Maximum</td>
<td>5.243</td>
<td>4.6</td>
</tr>
<tr>
<td>ψ Average</td>
<td>5.238</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 5.2 Experimental values of ψ and Δ and their corresponding layer thickness based on model in Figure 5.9
The relative friction coefficient of the PEG layer was obtained from the FFM measurements. The load, or $F_{\text{normal}}$ in Equation 5.5, was varied for each surface and in each medium and the corresponding lateral force was measured. The experiment was repeated several times on different locations on the surface. The results from FFM measurements are illustrated in Figure 5.11. Each point in the graph represents the average data measured from four experiments and the error bar corresponds to the standard deviation of the data. The relative friction coefficient is the gradient of the data, as described in Equation 5.5. The FFM measurement reveals that the relative friction coefficient of the PEG layer in a water solution is 0.83 compared to 2.77 for a plain surface under the same condition, as illustrated in Figure 5.10. Conducting FFM measurements for both surfaces in DMEM with serum showed a significant reduction (49%) in the relative friction coefficient for the plain surface. Conversely, the relative friction coefficient reading on a PEG-functionalised surface remains relatively constant, as illustrated in Figure 5.11. These results show that DMEM with serum reduces the frictional force by lubricating the surface, although not as low as observed on PEG surface.

![Figure 5.10 FFM reading for plain and PEG functionalised surface in water. The relative friction coefficient can be extracted from the gradient of each plot.](image)

There are several methods for increasing the probability of suppressing cell adhesion on PEG coated surfaces. Among these, the simplest technique is to
increase the surface density or to use a thicker layer of PEG coating \[246\]. A denser PEG layer is achievable by having a longer derivatisation period or by increasing the percentage of PEG in the solution. Opting for a longer PEG chain can increase the layer thickness given that the chain length maintains the brush-like structure \[246, 247\]. Apart from PEG, several other polymers may be used to achieve a similar effect. Polymers such as polypropylene, polytetrafluoroethylene, methyl-oxirane polymer (Pluronic), methyl substituted alkylsilane, hydroxylpropyl methylcellulose (HPMC) and fluorinated alkylsilane are among the potential candidates \[129, 243, 248, 249\]. The fouling resistance of PEG polymer is often explained by a steric repulsion interaction, resulting from the compression of PEG chains as proteins approach the surface. These alternative polymers present sterically closer structures that minimise van der Waals contact and hence may potentially serve as a better non-fouling material. Compatibility with biological materials and the current optical system however needs further investigation.

![Figure 5.11 FFM reading for plain and PEG functionalised surface in DMEM with serum. The relative friction coefficient can be extracted from the gradient of each plot.](image)

### 5.3 Brownian motion of polystyrene particles

Brownian motion investigations were carried out using the polystyrene particles, described in Section 1.5.1. Six particle sizes were used, 1µm, 3µm, 6µm, 10µm, 15µm
and 20µm unless stated otherwise. These particles were diluted in water to a concentration of \(1 \times 10^6\) particles per ml, before being pipetted into the reservoir. The experimental setup, as described in Section 5.2.1, was used for all experiments in this section. A combination of a 250ms frame rate, binning 2 (632 x 504 pixels) and 20x optical lens were used to provide a clear and manageable output from the experimental setup. 7000 frames (per cycle) were taken for each particle size. The experiments were repeated for three cycles which enables each point in the graph to represent an average value of up to 270 particles. Two controllable factors that can influence Brownian motion, as discussed in Section 3.2.1, are viscosity and temperature. Viscosity was maintained by using samples from the same particle solution for each data acquisition run. The fluctuation in the temperature was minimised through the restriction of the microscope illumination to avoid heating the solution in a temperature moderated laboratory. In this section, Brownian motion was investigated for a range of particle sizes, on different surfaces and in different liquid media.

### 5.3.1 Investigation of different particle sizes

The theoretical representation of the Brownian motion, as derived in Section 3.2.1, can be interpreted in practice as [250-253]

\[
\langle r^2 \rangle = L n_s(t)
\]

Equation 5.6

which means that the mean square distance, \(\langle r^2 \rangle\), of any particle is equal to the number of steps taken, \(n_s\), of a specific time, \(t\), multiplied by the length, \(L\), of each step. From this interpretation, the distance travelled by each identified particle was analysed by extracting the position difference from one frame to a subsequent frame. The distance travelled was tabulated and a graphical representation of the particle motion is illustrated in Figure 5.12. This is the typical presentation of Brownian motion, as can be seen in several papers [250-253]. Note that the investigation of particles moving in Brownian motion has so far been made using particles of about 1µm diameter or less. The figure shows the mean distance travelled for a given frame rate (250ms). From the series of graphs in Figure 5.12, the data points sometimes jumps or falls drastically from one frame to another resulting a ‘ripple’ motion in the measurements. This type of particle motion is non-physical and is most likely to arise from errors such as from the thresholding process, as described in Section 5.2.3. The best fit linear line, included in each graph, is regarded as the true representation of the particle motion. The slope of the linear fit line reflects the dependency on the size of the particle and can be compared with
the theoretical plot (Equation 3.14), which included in the graphs. Figure 5.12 demonstrates two distinct trends. The linear fit line for particles of size 1µm, 3µm, and 6µm varies within 18% with the theoretical gradient. Particles of larger size (10µm, 15µm and 20µm) however show a significant gap between the experimental fit and theoretical fit. A reduction in gradient, of 69% in average, was observed for these particle sizes.

In order to understand such motion behaviour, more information needs to be extracted from the data. Data presented in Figure 5.12 is re-plotted in a histogram format by compiling the frequency of distance travelled between each frame. Figure 5.13 shows a series of histograms for all polystyrene particles under consideration. All histogram plots are fitted with a Gaussian profile and their corresponding theoretical Gaussian profile. The theoretical projection of distance travelled by the particles was calculated (refer to Section 3.2.1) from the measured particle diameter and the corresponding size distribution as listed in Table 5.1. The values from this calculation were used to generate the theoretical Gaussian plot. The Gaussian width or full width half maximum (FWHM) was calculated from equation,

\[ \text{FWHM} = 2\sqrt{2\ln 2}\sigma \]

where \( \sigma \) denotes the standard deviation. The peak frequency of the Gaussian profile was made equal to the experimental Gaussian plot for comparison.

There are several points that can be extracted from the histograms; the peak value (and the frequency of the peak point) and the width of the experimental Gaussian profile. The peak value of the Gaussian fitted line on the experimental data is equivalent to the average particle size. The width of the Gaussian profile corresponds to the size distribution of the particle as well as the errors in the data acquisition/analysis and the interaction between the particle and the surface. Figure 5.13 a), b) and c) show the experimental Gaussian peaks are within 10% of the theoretical peak. This means that the average distance travelled by the particles (1µm, 3µm, and 6µm) deviate by 10% from the theoretical distance prediction, which explains the gradient difference as observed in Figure 5.12. It is also observed that there are discrepancies between the width of the theoretical Gaussian profile (which is calculated from errors tabulated in Table 5.1) and the experimental data for the histogram plots in Figure 5.13. The width of the experimental Gaussian profile, for Figure 5.13 a) for instance, is 85% larger than the theoretical Gaussian profile despite taking into account all quantifiable errors.
Figure 5.12 The distance travelled (squared) from one frame to a subsequent frame by particles of different sizes on a plain glass surface in water. The theoretical data (—), experimental data (+) and linear best fit line for the experimental data (—) are plotted for each particle size.
Figure 5.13 The histograms of the step size taken to travel from one frame to a subsequent frame by particles of different sizes on a plain glass surface in water. The Gaussian best fit line (—) is plotted for each experimental data (■) alongside the theoretical model (—)
Inconsistency between the theoretical and experimentally observed Brownian motion especially for the larger size particles can be explained from different aspects. Van der Waals force between the surface and the particles, as described in Section 3.2.3.1, indicates that the force is proportional to the particle size and inversely proportional to the separation distance. This means that the attractive force between the surface and the particle increases as the particle size in consideration increases. Furthermore the gravitational force is also proportional to particle size, hence increasing the attractive force via the reduction of separation distance for larger particles. Reduction in separation distance also changes the boundary condition for the Brownian motion theory, as explained in Section 3.2.1 and in [173, 174, 191, 254]. The drag force in this case is expected to increase as the effective local viscosity is increased. Hence larger particles (10µm, 15µm and 20µm) were observed to move significantly more slowly than predicted by the theory. Note that the Brownian motion theory considered here assumed that the mass of the particle is small and that the particle is moving in 2D in an unbounded medium. Movement in 3D is more significant for a smaller particle (1µm, 3µm and 6µm). Hence, these factors introduce more error in the image analysis and are consequently translated into a wider width of the Gaussian profile.

5.3.2 Brownian motion on functionalised surfaces

The previous section discussed the Brownian motion behaviour of polystyrene particles on a plain, unfunctionalised surface. One of the factors that affect the Brownian motion is the separation distance between the particles and the surface. The separation distance influences the drag force and the attractive van der Waals force which opposes the Brownian motion. As described in Section 5.2.4, this condition can be improved by surface functionalisation. Surface functionalisation is also required to prevent adhesion of cells. Functionalisation of the glass used in the previous section was carried out as described in Section 5.2.4. The distance travelled for each particle size was averaged and presented in a graphical format, as illustrated in Figure 5.14.

For particles of size 1µm, 3µm, and 6µm, the figure indicates that the motion on a functionalised surface is similar to that on a plain glass surface. In comparison, particles with larger sizes (10µm, 15µm and 20µm) were observed to demonstrate 21% longer distances travelled compared to particles of the same size on a plain surface. Evaluating the gradient of the experimental data with the theoretical prediction, however, indicates a 16% and a 47% decrease for smaller (1µm, 3µm, and 6µm) and larger (10µm, 15µm and 20µm) size particles respectively.
Figure 5.14 The distance travelled (squared) from one frame to a subsequent frame by particles of different sizes on a PEG functionalised glass surface in water. The theoretical data (—), experimental data (+) and linear best fit line for the experimental data (—) are plotted for each particle size.
Figure 5.15 The histograms of the step size taken to travel from one frame to a subsequent frame by particles of different sizes on a PEG functionalised glass surface in water. The Gaussian best fit line (—) is plotted for each experimental data (■) alongside the theoretical model (—).
As indicated in Section 5.3.1, larger particles were affected by different forces, such as the van der Waals and drag force that add up and eventually hinder Brownian motion. Smaller particles in contrast were observed to be less affected by the surface changes due to small mass that affects the settling time and allows the particles to move in 3D. This demonstrates that functionalising the surface with a PEG layer helps to improve the Brownian motion especially for the larger size particles.

Representing the experimental data in a histogram format reveals more information regarding Brownian motion on a functionalised surface. Histograms of particle motion, as shown in Figure 5.15 a), b) and c), reveal that the peak value varies to within 5%. This is 50% improvement compared to Brownian motion of a similar size particle on a plain surface. Apart from that, the histograms illustrate an overall improvement in the Brownian motion from the maximum frequency and the width of the Gaussian profile extracted for all particle sizes. The maximum frequency of the step size taken, within the frame rate, is increased on average by 20% while the width decreased by 15% and 49% for small (1µm, 3µm, and 6µm) and large (10µm, 15µm and 20µm) particles respectively. \( R^2 \), which is known as the correlation of determination, is the statistical evaluation of the goodness of fit of a model vis-à-vis experimental data. An \( R^2 \) of 1.0 indicates that the Gaussian profile line perfectly fits the data. The \( R^2 \) value for the histograms in Figure 5.15, on average, is 0.92. In comparison, the \( R^2 \) value for histograms in Figure 5.13 is 0.89. The frequency increment, narrower width and higher \( R^2 \) value indicate that PEG functionalisation improves the Brownian motion of particles on a surface by minimising the effect of the van der Waals and the drag force that act against the motion.

### 5.3.3 Brownian motion in different media

Apart from the surface functionalisation, the medium, in which the particles are dispersed to, also affects the Brownian motion. Two aspects of the medium, viscosity and ionic concentration, are investigated with respect to the Brownian motion of the particles. The increment of viscosity, based on theory in Section 3.2.1, has a proportional relation to the drag force and Brownian motion is expected to be slower in a more viscous medium. Meanwhile, ionic concentration influences two opposing electrostatic forces; double layer force and van der Waals, as mentioned in Section 3.2.3. A high ionic concentration is essential to allow less energy to be needed for the particles to approach the surface (especially the small particles). However, too high an ionic concentration increases the attractive van der Waals force, causing the particles to adhere to the surface. Thus, the ionic concentration needs to be balanced in order to satisfy both conditions.
Polystyrene particles were investigated in two media, DMEM and salt solution, with the option of several additives (serum and glucose), on PEG functionalised glass slides. The media to be used for cells in subsequent work were studied in this section with the polystyrene particles in order to isolate the effect of these media on the Brownian motion. DMEM is selected as it is the medium used for culturing cells. 10% of foetal bovine serum (FBS) is added as an option, as it was observed to allow cells to be more mobile, as explained in Section 5.4.1. The salt solution used consists of 0.64g of sodium chloride (NaCl) and 0.37g of sodium bicarbonate (NaHCO₃) diluted in 100ml of water. The salt solution is the minimum isotonic medium.
required to maintain the osmotic pressure and pH in order for the cell to survive in the medium. 0.45g of glucose is added as an option, as cells were observed to maintain viability longer [255].

Figure 5.16 illustrates the distance travelled by 20µm particles in 4 different media. Each of the plots in Figure 5.16 was fitted with theoretical model based on the Brownian motion expected in the specific medium (varied viscosity). Particles in all media show average distance travelled less than 2µm² (except in DMEM with serum, which about 3µm²) after 125 seconds. All experimental data shows a hindered mobility compared to theoretical model. Figure 5.16 shows the average distance travelled for the 20µm particle in DMEM shows a significant drop in comparison to the Brownian motion in water. After 125 seconds, on average, the distance travelled by the particles was only about 1µm². Histograms of particle motion in DMEM also show that the average of the step sizes has shifted towards zero vis-à-vis motion in water. This behaviour can be construed to be for several reasons. One of the reasons is a higher viscosity of DMEM compared to water, as illustrated in Figure 6.4 and as pointed out in Equation 3.14. Apart from that is the introduction of additional ions in the solution. As mentioned in Section 1.5.1, the polystyrene particles were negatively charged in order to avoid agglomeration of particles in the solution. The negative charge however may attract additional ions in the solution which leads to surface adhesion. The introduction of 10% serum in DMEM acts as a lubricant, which also reduced the viscosity of the medium, as demonstrated in Figure 6.4. Reduction in viscosity also translated into a change in the increment of distance travelled by the particles. Nevertheless, the distance increment was still less than for the Brownian motion observed in water (34% shorter). The histogram in Figure 5.17 d) reflects the effect of the serum, as there is a closer match between the experimental Gaussian peak and the theoretical expectation. The width of the experimental Gaussian profile, however, shows a similar trend as in Figure 5.13 f). This shows that although the motion was on a PEG-functionalised surface, the behaviour indicates a similarity as observed on a plain surface.

The result for Brownian motion of particles in DMEM was not as expected, as the mobility of particles was reduced (virtually static) even though they were on a PEG-functionalised surface. Hence, salt solution (of concentration 0.11M) was used as the minimum concentration required for the cell with the option of glucose to be added. Nevertheless, Brownian motion in either the salt solution with glucose, or just the salt solution was observed to be the same as particle motion in DMEM. Such an effect was discussed in Section 3.4 and it was suggested that a salt concentration of $0.6 \times 10^{-3}$ M should be used in order to avoid particles becoming stuck to the surface. The concentration suggested, however, is too dilute for the
targeted application as it will cause hypotonicity to the mammalian cells. Hypotonicity happens in a low osmotic pressure solution that causes cells to swell until they burst. Hence, the investigation in this section demonstrated that Brownian motion of polystyrene particles cannot be directly compared with mammalian cells using the same medium. This is due to the characteristic of the particles (negatively charged) that interact with the medium and causes the particles to adhere to the surface.

Figure 5.17 The histograms of the step size taken to travel from one frame to a subsequent frame for 20µm polystyrene particles on a PEG functionalised glass surface in a) salt solution, b) salt solution with glucose, c) DMEM, d) DMEM with serum. The Gaussian best fit line (—) is plotted for each experimental data (■) alongside the theoretical model (—)
5.4 Brownian motion of mammalian cells

The previous section discussed the Brownian motion behaviour of polystyrene particles of various sizes on a plain and on a functionalised surface. These investigations show that the distance travelled by Brownian motion is increased on functionalised surface. Applying a similar method to teratocarcinoma cells, namely TERA1 and NT2, was not as straightforward. The shape and surface characteristic (adherent) of the cells, for instance, are very different from polystyrene particles.

Teratocarcinoma cells were prepared for the Brownian experiments by trypsinisation process and re-suspended in a fresh DMEM solution (with phenol red and 10% serum unless stated otherwise) with a concentration of $1 \times 10^6$ cells per ml (refer to Appendix B for cell culture protocols). The cells were pipetted on the surface of the glass slide, using the setup as defined in Section 5.2.1, directly after re-suspension on a fresh culture medium. Images for the Brownian analysis were taken in binning 2 (632 x 504) with an image depth of 8 bit. The frame rate acquired was 250ms using a 20x lens. Illumination was adjusted so that the image of the cells was not saturated. The motion of each cell was analysed, as described in Section 5.2.2, from the frames taken. More frames were taken for this experiment (up to 10,000 frames for a single experimental cycle) particularly targeted for better cell motion detection and to increase the probability of detecting each cell. It was also noticed that fewer single cells were observed compared to polystyrene particles due to agglomeration (imperfect trypsinisation). Hence, each experiment was repeated up to five cycles in order to achieve reliable number of cells to be investigated (comparable to average number of polystyrene particles). The cell solution is changed every 1 hour to maintain the same cell condition for all data acquired. All experimental data are compared with the theoretical model, as derived in Section 3.2.1.

5.4.1 Brownian motion in different culture media

Brownian motion of teratocarcinoma cells was investigated initially on a plain glass slide by suspending them in DMEM. Several additives were mixed to DMEM in order to investigate the effect of these additives to the Brownian motion of cells. The additives used are tabulated in Table 5.3. Results from these initial experiments show that almost 90% of the cells were adhered to the surface (for medium number 1, 4, 9, 10, 11, 12 in Table 5.3). The cells, however, were seen to be more mobile in a culture medium with serum. It was noted in a previous section that adding the serum to the culture media (DMEM) reduces the viscosity. The percentage of
reduction, however, depends on the percentage of serum and other accompanying additives in the media. In DMEM with phenol red for instance, the viscosity reduces by 32% from 10% serum addition compared to 4% for DMEM with HEPES, as illustrated in Section 6.2.3. However, serum is generally ill-defined and unquantifiable as the serum varies from batch to batch and from manufacturer to manufacturer [256].

<table>
<thead>
<tr>
<th>No.</th>
<th>Culture media</th>
<th>Foetal bovine serum, %</th>
<th>Bovine serum albumin (BSA), %</th>
<th>Polyvinyl alcohol (PVA), %</th>
<th>Polyvinyl pyrrolidone (PVP), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMEM + Phenol red</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>DMEM + Phenol red</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>DMEM + Phenol red</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>DMEM + HEPES</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>DMEM + HEPES</td>
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<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>DMEM + HEPES</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>DMEM + HEPES</td>
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<td>-</td>
</tr>
<tr>
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<td>DMEM + HEPES</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 5.3 Several mixtures of DMEM used for the Brownian motion investigation of teratocarcinoma cells

Several other lubricating agents were also investigated, namely bovine serum albumin (BSA), polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP). The cells were observed to move in culture media with BSA although the motion was less than in media with serum. In trials made to mimic the effect of serum with other more quantifiable materials, such as BSA, PVA and PVP, they were found not to reduce the viscosity nor reduce cell adhesion significantly. The Brownian motion investigation of teratocarcinoma cells suspended on these media shows similarities to motion of particles in salt solution, salt solution with glucose and DMEM (refer to Section 5.3.3). Observation of Brownian motion on a plain surface indicates that the cells are adhered to the surface. The investigations carried out on PEG-functionalised glass slide for polystyrene particles of sizes similar to teratocarcinoma cells were demonstrated, in Section 5.3.2, to improve on average by 21% (in terms of the distance travelled). Hence the mobility of cell is expected to improve on PEG-functionalised surface.
5.4.2 Brownian motion of single cells

This section describes the Brownian motion of teratocarcinoma cells investigated on PEG functionalised surface. It was observed, in experiments described in Section 5.4.1, that cells was found to be more mobile in DMEM medium supplemented with 10% serum. Hence, the investigation was carried out with teratocarcinoma cells dispersed in DMEM with serum. Analysis of individual cells moving in Brownian motion on a functionalised surface shows three distinct categories.

Figure 5.18 The distance travelled (squared) from one frame to a subsequent frame by a) TERA1 cell and b) NT2 cell on a plain glass surface in DMEM with serum. c) and d) are the corresponding graphs showing the histograms of the step size taken to travel from one frame to a subsequent frame. The best fit line (—) is plotted for each experimental data (+■■) alongside the theoretical model (—).
Figure 5.19 The results from a single cell analysis that show a 'matched' category. The distance travelled (squared) from one frame to a subsequent frame by a) TERA1 cell (16.01µm) and b) NT2 cell (17.22µm) on a PEG functionalised glass surface in DMEM with serum, c) and d) are the corresponding graphs showing the histograms of the step size taken to travel from one frame to a subsequent frame. The best fit line (—) is plotted for each experimental data (+ ■) alongside the theoretical model (—).

One of the categories is illustrated in Figure 5.19 a) and b) which illustrates the distance travelled by the TERA1 and NT2 cells respectively. Note that after 125 seconds, the cells on average moved about 2.8µm. At this rate, the motion of the cell matches the motion of a 20µm polystyrene particle moving on a plain surface. Figure 5.19 c) and d) shows the corresponding histograms where the experimental peak value of the Gaussian best fit line matched (within 10%) the theoretical peak. On a functionalised surface, 63% of all the cells observed were in this category. The peak value corresponds to the size of the cell. Hence, this category shows that the
cell is moving in Brownian motion, as expected from the theory, in the majority of the frames analysed. However, the widths of the experimental histogram do not match the theoretical prediction. This may be due to the fact that not all the errors can be quantified. The experimental width is always 40% to 50% wider than the theoretical model.

Figure 5.20 The results from a single cell analysis that shows the 'mismatched' category. The distance travelled (squared) from one frame to a subsequent frame by a) TERA1 cell (19.36µm) and b) NT2 cell (20.21µm) on a PEG functionalised glass surface in DMEM with serum. c) and d) are the corresponding graphs showing the histograms of the step size taken to travel from one frame to a subsequent frame. The best fit line (——) is plotted for each experimental data (+■) alongside the theoretical model (——)

Discrepancy above 10% between the experimental and theoretical peak value, as illustrated in Figure 5.20 c) and d), is regarded as the mismatch category. This
category indicates that the movement of the cells is rather restricted, signifying that
the cell is slightly adhering to the surface. 28% of the cells monitored demonstrated
this behaviour. Notice that the size of cells for this category is slightly bigger (19%)
than in the previous category. Such behaviour may be due to the fact that there is a
sub-population in the solution (i.e. different cell stage, hence size), showing
different characteristics of cell membrane due to a biological process such as
apoptosis or patches in the PEG layer. Figure 5.20 a) and b) shows that the cell on
average moved approximately 2.2µm, slightly shorter vis-à-vis Figure 5.19 a) and b).
This complements the corresponding histograms (Figure 5.20 c) and d)) where the
majority of the step size is shown to be shorter than the theoretical models. Another
9% of the cell population demonstrates a total mismatch with the theoretical
Gaussian plot, akin to Figure 5.18 c) and d)). The histogram shows a very high
frequency of no movement (zero step size) in the frames taken. The Gaussian profile
failed to be fitted to the experimental data and there was a 100% mismatch with the
theoretical prediction. A cell falling in this category can be regarded as virtually
static due to the cell physically adhering to the surface. This behaviour is also
observed when monitoring a cell after more than 4 hours which may indicate that
the cell is started to die (apoptosis). Homogeneity of the motion of cells on a plain
glass slide may also indicate that the friction force between the surface and cell
surpasses the Brownian motion. It may be deduced from these observations that
apoptotic (and maybe necrotic) teratocarcinoma cells adhere to the surface and can
be detected from their Brownian motion behaviour.

5.4.3 Brownian motion of a group of cells

Analysing teratocarcinoma cells individually reveals properties specific to the cell
stage, surface characteristics and cell size. Analysing the whole population of cells
has the benefit of reducing errors within each single data point. Cells that were
considered to be adhered to the surface were systematically neglected for the
averaging process. Figure 5.21 a) and b) show the distribution of distance travelled
by TERA1 and NT2 cells respectively. The ripple motion, as mentioned in Section
5.3.1, is still apparent. However the variance was significantly reduced due to the
increased number of cells investigated and neglecting the adhered cells. Figure 5.21
d) illustrates the histogram of the step size taken in each frame for NT2 cells.
Comparing this graph to Figure 5.21 c) shows that the distribution of step sizes of
NT2 cells is broader than for TERA1 cells. As the experimental setup and the data
collection process for both cell types was the same, a wider histogram width for NT2
might indicate a larger size distribution. This agrees with the measurements
tabulated in Table 5.1 which shows a wider standard deviation for NT2 cells. Both
cells have approximately the same average size; 19µm for NT2 and 18µm for TERA1 cells as estimated from the Gaussian best fit profile and these values concur with the average cell size measurements using the microscope. The width of the theoretical Gaussian profile is still slightly smaller than the experimental value for both cells. Width difference for TERA1 is about 29% and for NT2 is 34%. The $R^2$ value for the Gaussian fit in Figure 5.21 c) and d) is 0.94 and corresponds to the reduced variance in the plot of the distance travelled. This is an 8% improvement compared to Gaussian fit for single cells.

Figure 5.21 The results from the average of all cells (up to 400 cells). The distance travelled (squared) from one frame to a subsequent frame by a)TERA1 cell and b) NT2 cell on a PEG functionalised glass surface in DMEM with serum, c) and d) are the corresponding graphs showing the histograms of the step size taken to travel from one frame to a subsequent frame. The best fit line (—) is plotted for each experimental data (+■) alongside the theoretical model (—)
5.5 Conclusion

An experimental investigation of Brownian motion on a surface was carried out using polystyrene particles and teratocarcinoma cells. The apparatus used in this exercise was described along with the data processing technique. The image analysis takes into consideration the possible errors in the Brownian motion investigation; random, systematic and dynamic errors. Most of the errors came from physical limitations such as the microscope illumination brightness and the camera detector sensitivity. However, methods for minimising such errors were performed to ensure reliable data. Quantifiable errors, such as from camera noise and the thresholding process, were noted for each particle and cell type. An overview of the surface functionalisation was shown in this chapter, discussing the suitability, fabrication and characterisation of the PEG layer. Several other materials for surface functionalisation were also suggested as options for future work. Surface functionalisation in this case, is advantageous not only in reducing adhesion but also to reduce the drag force that opposing the motion of particles and cells.

Brownian motion analysis for polystyrene particles was carried out with 1µm, 3µm, 6µm, 10µm, 15µm and 20µm particle sizes. This exercise was performed on plain and PEG functionalised glass surfaces. The distance travelled by Brownian motion reduces as the particle size increases, regardless of the surface condition. In fact, the discrepancies in the distance travelled after 125 seconds between the theory and experimental data is more pronounced for large sizes (10µm, 15µm and 20µm) of polystyrene particles. Functionalising the surface improves the mobility of the particles, especially for the 10µm, 15µm and 20µm particles. A 21% improvement in terms of the distance travelled, was observed for the large size particles and the Gaussian peaks of smaller particles (1µm, 3µm and 6µm) were within 5% of the theoretical peak. Conducting the Brownian motion studies in DMEM and salt solutions, does not show any improvement of particle mobility despite using the PEG-functionalised surface. The high ionic concentration of the medium interacted with the negatively charged particles which caused them to adhere to the surface, as expected from Section 3.4.

The initial Brownian motion investigation of teratocarcinoma cells on a plain glass surface demonstrated that the cells were adhering to the surface. Using various lubricating agents, such as BSA, PVA and PVP, the motion of teratocarcinoma cells was still unapparent due to the cell adhesion. Subsequent experiments involving teratocarcinoma cells were conducted on a PEG-functionalised surface. Three distinct categories were observed in this exercise according to their comparison with a theoretical model. 63% of the cells investigated showed the peak of the
experimental Gaussian profile within 10% of the theoretical prediction. The majority of the step sizes taken in each frame, by cells in this category, were regarded to move in synchronous with the theoretical model. In spite of this, the distance travelled by the cells was observed to be comparable to the distance travelled by particles on a plain surface. In contrast, 9% of the cell population investigated showed immobility which reflects a 100% mismatch between the experimental and theoretical peak of the Gaussian profiles. This occurrence was also observed for cells on plain surfaces which suggest that these cells were adhered to the surface. In between these two extremes, there were 28% of cells that were >10% mismatched, indicating a slight adhesion. This may be due to cells of different stages in the cell cycle that exist in the population. Analysing cell and particles population as a whole provides an average size of the particles and the size distribution with reduced errors.

The investigation in this chapter reveals the conditions for particles and cells to move freely in Brownian motion and the limitation of their mobility. It was found that PEG functionalisation of the glass surface improves the particles and cells mobility. Thus the propulsion on channel waveguide will benefit from the surface functionalisation; especially when experimenting with the naturally adherent teratocarcinoma cells. Note that Brownian motion of teratocarcinoma cells on PEG functionalised surface was observed to be similar to motion of 20µm particles on plain surface. This chapter also shows that DMEM with serum increases the mobility of cells. This medium will be used for the propulsion experiment of teratocarcinoma cells described in the next chapter.
Chapter 6

Channel waveguide trapping and propulsion of particles and cells

6.1 Introduction

The investigation of Brownian motion in Chapter 5 shows the conditions for polystyrene particles and teratocarcinoma cells to be feasible for optical trapping and propulsion using channel waveguides. The aim of this chapter is to characterise trapping and propulsion of particles and cells on theoretically optimised caesium ion-exchanged waveguides. The experimental setup, data analysis and materials used in this investigation are described in the beginning of the chapter. Propulsion characterisation of polystyrene particles against the laser properties and the waveguide conditions is discussed in this chapter and compared to theoretical evaluations in Chapter 4. Additional characterisation to accommodate the system for the trapping and propulsion of mammalian cells is also discussed. The investigation includes the variation in cell size, refractive index and the use of different surfaces. Finally, the investigation of trapping and propulsion of lymphoblastoma and teratocarcinoma cells is presented and discussed.

6.2 Techniques and materials

6.2.1 Optical arrangements

The propulsion of particles and cells in the evanescent field of a channel waveguide potentially allows for the detection and separation of stem cells via differences in
propulsion velocity. In order to optically propel particles and cells, a solution of particles or cells is placed in a reservoir on top of the waveguide, as illustrated in Figure 6.1. The trapping and propulsion is powered by a diode pumped ytterbium doped fibre laser (IPG Photonics) operating at 1064nm. The wavelength is used as the laser source is readily available and it was proven to work for polystyrene particles propulsion on caesium ion-exchanged channel waveguides [30]. The laser source, which is linearly polarised and produce continuous wave, is capable of supplying power up to 5W with a Gaussian output beam of 1.6mm diameter. The laser source is directed to a built-in isolator in order to provide a collimated beam and to avoid the backscatter reflection that can damage the laser source. Output from the isolator is coupled into a single mode polarisation maintaining (PM) fibre (Fibercore, HB980-T) via a 20x objective lens (loss ≈ 5dB).

The PM fibre is used to adjust the input field to the optical waveguide into different polarisation modes. The PM fibre is aligned in three dimensions to give maximum output, using a fibre holder with a nanopositioner (Thorlabs - MAX313), and set to the correct polarisation using a nanorotator (Thorlabs - HFR003) attached to the fibre holder. Scattering due to Fresnel reflection is minimised by cleaving the fibre using a fibre cleaver (PK FK11). A coupling efficiency, between the fibre and the waveguide input facet, of approximately 4dB is achieved using this setup. An optical microscope was used to place the PM fibre in close proximity to the waveguide input facet. The coupling was optimised by monitoring the output of the waveguide.

6.2.2 Reservoir

In order to confine the particle/cell solution, a reservoir is placed on the surface of the waveguide. There are several limitations to the designs of the reservoir used in this project. The most important aspect is that the material used to fabricate the
reservoir must be of a lower refractive index than that of the core layer. This is due to the fact that the light will leak to the reservoir (if a higher refractive index material is used) and hence making the waveguide excessively lossy. The reservoir also must not be affected by the wavelength used. The material used can suffer from laser damage such as material burn (indicated by discoloration) which increases the amount of loss. In addition, the reservoir needs to be transparent for imaging the particle during trapping and propulsion. These conditions limit the number of materials that can be used to fabricate the reservoir. Most photoresists, glues, tapes and coatings are incompatible with the requirements. Approaches considered in this project are the use of commercially available reservoir, as well as fabricated reservoirs using polydimethylsiloxane (PDMS) \( (n \approx 1.45) \) and sputtered silica \( (n \approx 1.45) \) [257].

There are several commercially available reservoirs such as Vacu-cell (C&L Instruments), OptiCell (Nunc), CoverWell (Grace Bio-Labs) and PCP-1 (AutoMate Scientific). The best option for the project is the CoverWell perfusion chambers. CoverWell is a press-to-seal cover which forms a single or multiple cell chambers when pressed to glass coverslips or microscope slides. There are dual access ports on the chambers which makes it easy to add or remove a solution containing particles or cells. The dimensions of the reservoir are 32mm × 19mm with a thickness of 0.5mm. The reservoir, which is made from polycarbonate films, is also bio-inert and suitable for live cell imaging [258]. This is important as the reservoir is also used for investigating mammalian cells. CoverWell, however, added approximately 1.5dB/cm loss when used with the waveguide. The loss is quite large due to the fact that the
refractive index of the CoverWell is slightly higher ($n \approx 1.586$) than the refractive index of the core layer [258].

Another approach uses a reservoir made from PDMS (Dow Corning – Sylgard 184). PDMS seals well to the waveguide surface and can be fabricated rapidly and easily. The material is also bio-inert, optically transparent and suitable for handling biological cells [257]. The reservoir is made by thoroughly mixing a 10:1 ratio of elastomer base and curing agent for approximately 10 minutes. The solution is then degassed in a weak vacuum for at least one hour. The solution is poured over a mould and degassed again for a further 15 minutes, as shown in Figure 6.2 b). The mould is made from a coverslip of dimensions 10mm x 30mm x 0.2mm glued on top of a microscope slide. The solution is inspected to make sure that it is free of air bubbles before being baked at 80°C for 30 minutes. After baking, the reservoir is cut with a scalpel and peeled off the mould. Two access ports are punched-in at both ends of the reservoir. Teflon tubes of dimensions 1mm for inner diameter and 1.8mm for outer diameter may be inserted into these ports. These tubes can be attached to a peristaltic pump to add solution to the reservoir. Hence, the PDMS reservoir can potentially facilitate the easy change of solutions by cleaning it with deionised water pumped at high speed. This avoids the need to remove the reservoir to accommodate new solutions and realign the waveguide. Due to the low refractive index, the losses using PDMS reservoir are minimised. In order for the PDMS reservoir not to collapse and be strong enough to avoid tear, a thickness of at least
2mm is required. At this thickness, only a 10x objective lens can be used and hence it is not ideal for imaging particles smaller than 6µm.

Another method for making the reservoir is using a silica (SiO$_2$) isolation layer, defined with photoresist, on top of the waveguide, as illustrated in Figure 6.3 a). The isolation layer of thickness 1.5µm is sputtered before being washed in acetone to reveal an area for imaging. A Parafilm (Pechiney) is cut to make a reservoir and placed strategically to secure the revealed area, as shown in Figure 6.3 c). A microscope slide with two access ports is placed on top of the Parafilm. The waveguide is then heated up to 100°C to bond everything together. Although the isolation area is found to produce less loss, several disadvantages are also discovered. Apart from the lengthy fabrication process, the area for imaging is limited to the area uncovered by the silica. As this process is irreversible, propulsion of particles at different points along the waveguide cannot be monitored. Thus the PDMS reservoir is the preferred technique to use for observing particle and cell trapping and propulsion in this project.

### 6.2.3 Media characterisation

Optical trapping and propulsion of particles and cells is affected by the properties of the medium on top of the optical waveguide. Features such as the refractive index of the medium determine how the particle or cell interacts with the evanescent field. A larger refractive index contrast between the medium and particle/cell increases the optical force on the particle/cell. Furthermore, a more viscous medium increases the drag force (Equation 3.14) acting against the particle/cell motion and consequently reduces the propulsion velocity. As the medium temperature also affects its viscosity, the absorption spectrum of the medium was also investigated. Unless stated otherwise, all studies of propulsion of polymer particles in this project were conducted in water while experiments on the mammalian cells were conducted in their specific culture media as described in Section 1.5.2.

The refractive index, kinematic viscosity and the absorption spectrum of all media used were investigated and compared to water. The refractive index of the medium was measured using a refractometer (Kyoto Electronics) with $1 \times 10^{-5}$ refractive index accuracy at 589.3nm. The refractometer was maintained at a working temperature of approximately 20°C and calibrated before measuring the refractive index of the medium. Kinematic viscosity of the medium was measured using an Ostwald viscometer (PSL SLSC) in a temperature controlled laboratory (20°C). The time taken for the medium to flow between two marked levels on the capillary of the
The viscometer was measured using a stopwatch (accuracy down to milliseconds). The viscosity of each medium was measured six times. Results for the refractive index and kinematic viscosity measurements are shown in Figure 6.4.

![Graph showing refractive index and kinematic viscosity of several culture media compared to water](image)

**Figure 6.4** The refractive index and kinematic viscosity of several culture media compared to water

There is an increase in the refractive index observed in all culture media compared to water. DMEM with added HEPES shows the highest index increment (0.2% increase from the refractive index of water) compared to DMEM with phenol red or RPMI with phenol red. All culture media with added serum demonstrate an increasing refractive index as the percentage of serum is increased (0.3% increase for 10% serum). However, increment in the refractive index for all media was considered insignificant vis-à-vis index contrast with the particle or cells. The viscosity of the media was observed to decrease with increasing serum percentage. This is believed to be due to the fact that the serum acts as a lubricating agent in the medium, as observed in Section 5.3.3 and Section 5.4.1. DMEM with phenol red and 10% serum was found to have the lowest viscosity compared to other serum added media. Several other additives were also investigated, namely bovine serum albumin (BSA), polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) to investigate their
lubrication property. Media with these lubricants added were found to be more viscous compared to DMEM with phenol red and 10\% serum. In conducting the propulsion experiments, a balance between the refractive index and the viscosity of the medium is needed. However in this case, the refractive index was found to be similar to water. Hence DMEM with phenol red and 10\% serum was concluded to be the best medium for the experiments after water, as the viscosity was shown to be the lowest compared to other media.

![Figure 6.5 Absorption spectra of several culture media and water. The insert at the top left corner shows a close up at wavelength 1064nm](image)

Table 6.1 Absorption for media investigated at 1064nm wavelength

<table>
<thead>
<tr>
<th>Media</th>
<th>Absorption at 1064nm (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.069470</td>
</tr>
<tr>
<td>RPMI + phenol red</td>
<td>0.060463</td>
</tr>
<tr>
<td>RPMI + phenol red + 10% serum</td>
<td>0.062723</td>
</tr>
<tr>
<td>DMEM + phenol red</td>
<td>0.062098</td>
</tr>
<tr>
<td>DMEM + phenol red + 5% serum</td>
<td>0.061334</td>
</tr>
<tr>
<td>DMEM + phenol red + 10% serum</td>
<td>0.064413</td>
</tr>
<tr>
<td>DMEM + HEPES</td>
<td>0.065737</td>
</tr>
<tr>
<td>DMEM + HEPES + 5% serum</td>
<td>0.063817</td>
</tr>
<tr>
<td>DMEM + HEPES + 10% serum</td>
<td>0.065661</td>
</tr>
</tbody>
</table>
A spectrophotometer (Varian CARY 500) was used to carry out absorption spectrum measurement of the media. A range of 200-2000nm was set on the spectrophotometer and an empty cuvette was used as a reference. The absorption spectra of water and the culture media are shown in Figure 6.5. The measurement of absorption spectra of water was found to be in agreement with values commonly found in the literature [259-263]. All culture media used in this project generally followed the same declining trend as water at 1064nm. It was also observed that the absorption at higher wavelengths (>1500nm) was greater than that to water for all culture media. At lower wavelengths, the absorption spectrum of the culture media increased due to the existence of inorganic salts, amino acids and vitamins, depending on the additives, as observed in [259-263]. Note that all culture media with phenol red show a peak at 557nm. The peak lessens as the percentage of serum is increased. As the laser to be used in the propulsion experiments is operating at 1064nm, absorption of media at this wavelength is important. Table 6.1 shows absorption of all media at 1064nm. All media shows a general trend of having a lower absorption than water. A low absorption indicates a low heat generated by the laser due to energy conversion, as described in Section 3.3.1. Consequently, this may minimise the effect of convection current on the particles or cells. RPMI with phenol red was observed to have the lowest absorption. DMEM with phenol red and 10% serum, on the other hand, shows 5% lower absorption than water. Variation of all media from water was concluded to be insignificant, albeit lower. Hence, from the characterisation processes conducted in this section, it can be concluded that the medium to be used when investigating teratocarcinoma cells was DMEM with added phenol red and 10% of serum, unless stated otherwise.

### 6.2.4 Data analysis

The propulsion of particle and cells was imaged, as detailed in Section 5.2.1. The microscope was adjusted in a way so that it focuses on the surface of the channel waveguides to ensure that only particles close to the surface are observed. As a result, channel waveguides with particles on the surface can be seen using the microscope. The scattered laser light was blocked by using a 1µm wavelength low pass filter. Output from the CCD camera was logged in a computerised LabView environment. The region of interest was specified and fixed at the start of each experiment, with the desired rate at which the images should be recorded. Up to 10,000 images are recorded for a single data point. Each experiment was repeated, typically for three cycles or more, especially when investigating mammalian cells. Images of particle/cell trapping and propulsion were analysed using in-house generated particle tracking software in Matlab [191]. A threshold level was set in
order to identify the particles or cells. Images were brightened and averaged out to discard all static particles and cells before the propulsion was analysed. A matrix in the particle tracking software was used to calculate the displacement of each particle and to extract the propulsion velocity of the particles. The propulsion of particles and cells were analysed against the theoretical particle velocity evaluated from the force exerted by the evanescent field, as given in [161, 162].

6.3 Particle propulsion characterisation

This section discusses the propulsion of polymer particles on caesium ion-exchanged waveguides. The first half of the section examines the theoretical model of the optimum waveguide condition for propulsion, as detailed in Section 4.3.4. The second half describes the propulsion characterisation of polymer particles that are close to the characteristics of mammalian cells. All polymer particle solutions used in this section were prepared by diluting the particles in deionised water until a concentration of $1 \times 10^6$ particles per ml was reached. The concentration was such as to achieve a low density of particles in order to avoid the formation of long particle chains [191, 232]. Observation of particle chains is explained further in Section 6.3.1.

<table>
<thead>
<tr>
<th>Laser power (mW)</th>
<th>Power out from fibre (mW)</th>
<th>Power into waveguide (mW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>126</td>
<td>50</td>
</tr>
<tr>
<td>450</td>
<td>142</td>
<td>57</td>
</tr>
<tr>
<td>500</td>
<td>158</td>
<td>63</td>
</tr>
<tr>
<td>550</td>
<td>173</td>
<td>69</td>
</tr>
<tr>
<td>600</td>
<td>189</td>
<td>75</td>
</tr>
<tr>
<td>650</td>
<td>205</td>
<td>81</td>
</tr>
</tbody>
</table>

Table 6.2 Estimation of laser power at various points in the laser path

Unless stated otherwise, the waveguide channel used for the particle propulsion investigation has a nominal width of 4µm and is single mode at 1064nm. The optical arrangement described in Section 6.2.1 was used with a similar imaging setup as detailed in Section 5.2.1. The laser power was set at 500mW using the TM mode unless stated otherwise. The TM mode was selected to achieve a higher surface intensity as discussed in Section 4.3.4.2 and Section 6.3.3. There are reductions of laser power at various points due to the coupling loss as indicated in Figure 6.1.
Table 6.2 shows the laser power at several points in the laser path. Loss in the waveguide is estimated to be approximately 0.75dB/cm.

Each of the experimental data was compared with a theoretical model. The theoretical model was simulated by adapting the Arbitrary Beam Theory (ABT) developed by [264, 265] (Refer Appendix D for ABT derivation). The simulation was made using the same model as in [161, 162] and carried out with the help of Dr Hitesh Jaising and Dr Olav Hellesø, of the University of Tromsø, Norway. Unless stated otherwise, the waveguide parameters used in the simulation were a 4.5µm channel width, a substrate index of 1.50, a waveguide index of 1.54 and a particle index of 1.59 dispersed in water (index of 1.33). The wavelength used in the simulation was 1064nm with laser power set at 500mW (63mW into the waveguide). Note that the model assumed that there is no power loss due to propagation loss along the channel waveguide and lossless spherical particles. Furthermore, it is also assumed that the propulsion of particles is not affected by any non-optical forces.

Based on the waveguide characterisation in Section 4.4, the optimised caesium waveguide was found to be fabricated with 8 hour ion-exchange time. Experiments in Section 6.3.4 were carried out using this set of waveguides. Several other sets of waveguides were fabricated in response to the results obtained in Section 6.3.4. However, due to an unfortunate fire incident, all waveguides fabricated were lost in the fire. The rest of the experiments were carried out using waveguides provided by Dr Olav Hellesø; a 10 hour and a 12 hour caesium ion-exchanged waveguide with $n_{\text{eff}}$ of 1.5202 and 1.5221 respectively at 632.8nm. The 10 hour caesium ion-exchanged waveguide was used (closest to the theoretical optimum $n_{\text{eff}}$) for the experiments until it showed degradation as indicated in Section 4.4.3. At this point, the 12 hour caesium ion-exchanged waveguide was used as a substitute.
6.3.1 Positional analysis

The investigation of the propulsion of polystyrene particles and their positional analysis was conducted using the 10 hour caesium ion-exchanged waveguide. Figure 6.6 shows the distribution of 10µm polystyrene particles randomly dispersed on the surface of the caesium ion-exchanged waveguide. The dotted line in Figure 6.6 shows the position of the waveguide. The image was compressed to facilitate the longer lateral (x-axis) distance. Four particles were observed above the channel waveguide of interest at the beginning of the experiments. These particles were marked with #1, #2, #3 and #4 for further reference.

Images from Figure 6.7 were taken at 33 second intervals for 100 seconds duration. Within that period, all four particles, located on top of the waveguide, were observed to be propelled in the direction of light propagation. Particle #1 and #4 move at the same average speed, $1.4 \pm 0.19\mu m/s$ as tabulated in Table 6.3. It is also noticed that the velocity of the particle propulsion fluctuates, as particle #4 moves further in the first 66 seconds compared to the rest. Particle #1 was observed to drift away from the waveguide at time $t = 66s$, as shown in Figure 6.7 c) and consequently it moved in Brownian motion. Particle #3 on the other hand was slightly off the waveguide initially and started to move towards the illuminated
channel after several seconds. It took several seconds to stably trap in the evanescent field and particle #3 travelled for 80µm within the duration (100s) observed. Particle #2 was monitored propelling at an average velocity of 1.8 ± 0.07µm/s. Fluctuations in propulsion velocity of individual particles can be attributed to several factors. Their position relative to the illuminated channel waveguide, in both the lateral direction and height above the waveguide, can influence the overlapping of the evanescent field on the particles. Varying surface friction and slight differences in particle size can also be responsible for these variations.

![Figure 6.7 The propulsion of 10µm particles ](image)

During this experiment, situations were also observed where there were two particles propelled very close together to each other. Particle chains (two or more particles) such as these were shown to be propelled much faster than single particles. This phenomenon, which was also observed in literature [30, 266] can be
explained by hydrodynamic coupling [267]. Hydrodynamic coupling has a
kinematical effect that exists between the propelled particles and the waveguide
surface as well as between the particles themselves. Particle motion is stalled near
the waveguide surface in air but particles moving through a fluid excite flows at
their surfaces. These flows couple distant particle motion, thus the dynamic of each
particle depends on the particular configuration of the entire collection. Collective
diffusion coefficients are hence enhanced by hydrodynamic coupling as fluid that is
displaced by one particle entrains other [267]. Analysis of particle propulsion in
later sections will not be taking into account any propulsion of particle formations.

Figure 6.8 Output from the particle tracking software, particle
propulsion of 10µm particles are represented in    and   coloured
lines. Particles moving in Brownian motion are shown in   colour.

Arrows beside the particle numbers indicate the direction of motion

In order to prove that particles #1, #2, #3 and #4 were propelled by the laser;
neighbouring particles off the waveguide were also monitored at the same time, as
illustrated in Figure 6.8. Throughout the duration of 100 seconds, those
neighbouring particles were moving in Brownian motion. The speed of this
movement (≈0.18µm/s), however, is not comparable to particles #1, #2, #3 and #4.
Figure 6.8 shows the Brownian motion of neighbouring particles as large blue dots.
The transverse motion of propelled particles was reduced when compared to the
particles moving in Brownian motion. This is due to the fact that the transverse motion was reduced by the restoring force from the lateral gradient of the evanescent field. Several parameters have been identified to give a significant effect on particle trapping and propulsion. The parameters can be divided into two categories, optical and waveguide properties. The optical properties investigated are the laser power and polarisation, while for the waveguide properties they are the ion-exchange time (which corresponds to waveguide depth) and the waveguide width.

<table>
<thead>
<tr>
<th>Particles</th>
<th>Velocity (µm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>1.4 ± 0.19</td>
</tr>
<tr>
<td>#2</td>
<td>1.8 ± 0.07</td>
</tr>
<tr>
<td>#3</td>
<td>0.8 ± 0.11</td>
</tr>
<tr>
<td>#4</td>
<td>1.4 ± 0.19</td>
</tr>
</tbody>
</table>

Table 6.3 Tabulation of particles velocity for Figure 6.8

6.3.2 Effect of laser power

This section discusses the laser power dependency of the particle propulsion velocity. The waveguide used in investigating the effect of laser power was the 10 hour caesium ion-exchanged waveguide. A similar experimental setup as described in Section 6.2.1 was used with the laser set to TM mode. The laser power was varied from 400mW to 600mW by adjusting the supply current through the laser controller (corresponding to 50mW to 75mW input power to the waveguide). Three polystyrene particle sizes were used; 3µm, 6µm and 10µm. All particles were diluted with deionised water and pipetted into the PDMS reservoir. A series of experiments with varying laser power were conducted before changing to another particle size. The experiments were repeated 3 times for each particle size.

It is clear from the theory described in Section 4.3.4 that, as the laser power is increased, the intensity of the evanescent field is also increased linearly. Hence, the optical forces acting in the direction of the wave propagation increase with the laser power; resulting a higher mean particle velocity, as illustrated in Figure 6.9. Figure 6.10 consistently shows the increment of the particle velocity in relation to the laser power supplied to the 4µm channel width for all particle size used. The laser power, however, was not increased over 650mW in order to avoid burning any of the components along the optical path as well as to preserve the waveguide’s lifetime.
Figure 6.9 The theoretical estimation of the propulsion velocity of different sized particles for varying input laser power

Figure 6.10 Velocity of different sized particles for varying input laser power
An over exposure to high laser power has been recorded as the major factor in deteriorating the lifetime of the waveguide [191] as discussed in Section 4.4.3. There are discrepancies between the velocity observed experimentally and from the theoretical model due to limitation in the model as described in Section 6.3. However, the experimental data shows the same trend as expected from the model where the propulsion velocity increases with particle size. The highest velocity observed in the range tested was 3.44µm/s, obtained for a 10µm particle at 78mW. The velocity then decreased as the power coupled to the waveguide was decreased. At 51mW, the 10µm particles moved at 1.14µm/s and at 0.53µm/s for the 6µm particles.

6.3.3 Effect of laser polarisation

Another optical parameter that plays an important role in the particle trapping and propulsion is the laser polarisation. There are two polarisation modes, transverse electric (TE) or transverse magnetic (TM). TE mode signifies that the electric field vector is directed along the $y$-direction (parallel to the waveguide channel), as illustrated in Figure 4.8. Likewise, TM mode signifies that the magnetic field vector is directed along the $y$-direction. In determining the effect of polarisation on particle velocity, a 10 hour caesium ion-exchanged waveguide was used. A 10µm polystyrene particle solution was pipetted to the reservoir and the propulsion on a 4µm nominal channel width was monitored. The nanorotator attached to the fibre holder and the utilisation of PM fibre allows control of the polarisation of the propagating light in the waveguide. A polariser was used to set the light to the correct mode before the beginning of each experiment. The nanorotator was set by fixing the polariser, for example to the TE mode. The nanorotator was rotated until the minimum output power was detected from the power meter. The laser polarisation was now set to TM mode. Output power from the PM fibre of both polarisations was recorded and a polarisation extinction ratio of about 30dB was obtained.
Figure 6.11 The theoretical estimation of the propulsion velocity of 10µm particles with varying input laser power for both polarisations.

Figure 6.12 Velocity of 10µm particles against power for both polarisations.
Propulsion velocity of particles for each polarisation was investigated by varying the laser power coupled to the waveguide. Figure 6.12 illustrates a significant difference for the velocity of particles according to the polarisation. Particles propelled in the direction of wave propagation were shown to be propelled at a higher average velocity in the TM mode as predicted in Section 4.3.4.2 and from the theoretical model in Figure 6.11. For example, the velocity of particles propelled at 66mW was 1.92µm/s in the TM mode and 0.87µm/s in the TE. The velocity for TM and TE modes was in accordance with the results observed in [191, 232]. The greater overall gradient for the TM mode indicates that the mode exhibits a higher surface intensity, as expected from Section 4.3.4.2 and several published papers [6, 32, 33, 161, 162, 232, 268]. Hence, the TM mode was used for all subsequent experiments unless stated otherwise. Note that if the gradients for both polarisations are extrapolated, both cross the x-axis at approximately 38mW. This may indicate that 38mW was the threshold power needed for creating the surface intensity to propel 10µm polystyrene particles. A similar extrapolation on Figure 6.10 shows a possible threshold power for 3µm and 6µm particles of about 44mW and 50mW respectively. A higher threshold power is needed for smaller particles and this may be due to the fact that smaller particle motion is more influenced by Brownian motion. Thus stronger gradient force is needed to stably trap smaller particles.

6.3.4 Effect of waveguide ion-exchange time

Several waveguides have been fabricated to investigate the effect of ion-exchange time on the particle propulsion. By varying ion-exchange time, the waveguide depth is also varied. The ion-exchange time was varied from 4 hours to 17 hours. Two polystyrene particles, 3µm and 6µm were used for this investigation. Each particle size was diluted in deionised water before being pipetted into the reservoir. The same optical and imaging setup was used as described in previous sections.

Details of the waveguides fabricated are tabulated in Table 6.4 with the expected waveguide depth taken from simulation data in Section 4.3.4.1. Out of all 5 waveguides tested in this investigation, the 4 hour ion-exchanged waveguide shows no guiding at all. This indicates that the ion-exchange time was not enough to create a waveguide depth that permits light to propagate in a confined mode. The 17 hour ion-exchanged waveguide has the highest effective refractive index and was observed to show a considerably higher loss. No propulsion of 3µm and 6µm particles was observed on this waveguide. Hence these observations signify that there is an optimal waveguide effective index (and hence ion-exchange time) for particle propulsion. The 17 hour ion-exchanged waveguide was determined to be...
multimoded from the simulation data and the rest were single mode waveguides when operating at 1064nm wavelength.

<table>
<thead>
<tr>
<th>Ion-exchange time (h)</th>
<th>Fundamental $n_{\text{eff}}$</th>
<th>Expected depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.5</td>
<td>1.5151</td>
<td>1.03µm</td>
</tr>
<tr>
<td>8</td>
<td>1.5189</td>
<td>1.39µm</td>
</tr>
<tr>
<td>10</td>
<td>1.5219</td>
<td>1.52µm</td>
</tr>
<tr>
<td>17</td>
<td>1.5280</td>
<td>2.45µm</td>
</tr>
</tbody>
</table>

Table 6.4 Details of the waveguides fabricated using a soda-lime glass substrate. The $n_{\text{eff}}$ of the fundamental mode was measured at 632.8nm. Expected depth is fitted according to the simulation values in Section 4.3.4.1

In order to establish the optimal waveguide effective index, based upon the fabrication condition used, the propulsion velocity of the 3µm and 6µm particles was compared on all fabricated waveguides. Mesh plots of particle velocity against ion-exchange time, for both the 3µm and 6µm particles, are illustrated in Figure 6.14. The maximum velocities achieved for the 3µm and 6µm particles using the 4µm channel width were 0.75µm/s and 2.16µm/s respectively at 66mW input. Both of the maximum velocities were observed using the 8 hour ion-exchanged waveguide; which translated into a waveguide depth of about 1.39µm, based on the simulated value in Section 4.3.4.1. It is expected, in Section 4.3.4 that the maximum surface intensity will be observed on a 1.4µm waveguide depth. The theoretical propulsion velocity distribution as illustrated in Figure 6.13, also shows maximum velocity on 8 hour ion-exchanged waveguide. The maximum velocity for different channel width is also shown in the figures; this will be discussed further in the next section. Using the longer ion-exchange time waveguides, the velocity of both particles is lower. This corresponds to the shorter range of the evanescent field of deeper waveguide depth and hence reduces the intensity on the waveguide surface. Reduction in velocity was also apparent on the short ion-exchange time waveguide. Such an effect is due to the fact that the field penetrates deeper into the substrate layer and consequently lowers the surface intensity. From these general observations, it can be concluded that there is an optimum ion-exchange time for producing the maximum surface intensity for the propulsion of particles, as predicted in Section 4.3.4.2. From the range of waveguides tested, the 8 hour ion-exchange waveguide is the optimum sample, as expected from Section 4.4.
Figure 6.13 The theoretical estimation of the propulsion velocity of a) 3µm particle and b) 6µm particle against ion-exchange time and channel width.
Figure 6.14 The propulsion velocity of a) 3µm particle and b) 6µm particle against ion-exchange time and channel width
6.3.5 Effect of waveguide channel width

Another waveguide property that plays an important role in particle trapping and propulsion is the waveguide channel width. The laser power was coupled into the 12 hour caesium ion-exchanged waveguide channels with the nominal width varying from 3µm to 10µm. The same waveguide was used to ensure that the velocity of the particle’s propulsion is affected by the channel width alone. Particles of diameter 3µm, 6µm and 10µm were used in a series of experiments to investigate the effect of channel width on the particle propulsion.

Experimental results for each particle size were compiled and are illustrated in Figure 6.16. On average, the propulsion velocity increases with size for all waveguide widths as observed from previous sections. All particle sizes show a peak in the propulsion velocity on the 4µm nominal channel width. Smaller particle sizes (3µm and 6µm) however show a less significant peak. As indicated in Section 3.2.2, smaller particle sizes have longer settling times and they are easily affected by Brownian motion. Hence the optical force acting on the particle is believed to cause the particle to move in three dimensions away from the evanescent field. Consequently, this reduces the propulsion velocity. Nonetheless, the experimental observation agrees strongly with the waveguide characteristic to produce the optimum surface intensity, as suggested in Section 4.3.4. At waveguide widths smaller than the optimum point, the velocity reduces with decreasing width, indicating a near cut-off point where the channel is no longer guiding and the light is totally propagating in the substrate layer.

The trend of particle velocity against the waveguide widths follows the simulation conducted in Section 4.3.4.2 and theoretical model illustrated in Figure 6.15. There is also a general trend of increasing particle velocity as the channel widths approach 4.5µm although these velocities fluctuated. Note that 4µm nominal width waveguide used in the experiment is the width of the mask during fabrication process (without the enlargement of width due to lateral diffusion during ion-exchanged process). Discrepancy from the simulation can be due to several reasons, such as inconsistent surface friction, a variation in particle size distribution or a fluctuation in the supplied input power. From the experiments discussed so far, it can be shown that the optimum channel width in the range tested is 4µm on an 8 hour caesium ion-exchanged waveguide, as shown theoretically in Section 4.3.4.2, and from waveguide characterisation in Section 4.4. In terms of optical properties, propulsion was observed to be higher with a higher laser power and at the TM mode, as indicated in the simulation.
Figure 6.15 The theoretical estimation of the propulsion velocity of different sized particles with varying channel widths

Figure 6.16 Velocity of different sized particles with varying channel widths
6.3.6 Effect of particle size

In order to apply the current technique in the trapping and propulsion of particles to mammalian cells, certain characteristics of the mammalian cells can be investigated using particles of a similar property. One of the characteristics that can be investigated using particles is the cell size. The diameter of the cell used varies depending on its type and the state of the cell cycle. Lymphoblastoma cells can vary from approximately 8µm to 12µm, while teratocarcinoma cells vary from 15µm to 23µm. In order to evaluate the effect of varying cell size, six different polystyrene particle sizes, ranging from 1µm to 20µm diameter, were used. The propulsion velocity of each particle size was measured using a 12 hour caesium ion-exchanged waveguide.

![Figure 6.17 Theoretical model of the propulsion velocity of varying particle sizes at TM mode. The red line indicates the best fit between the two adjacent maxima and minima in the theoretical model and the green line is the estimated non-resonance propulsion estimation.](image)

Figure 6.17 illustrates the theoretical model of the propulsion velocity for varying particle sizes. The theoretical plot shows velocity resonance, an effect that is observed in total scattering and extinction cross-sections for Mie particles [269, 270]. These resonances originate from the electromagnetic modes of a sphere and are known as Morphology Dependent Resonances (MDRs) [269, 270]. With
increasing radius, the loss for the internal field decreases, the internal field accumulates and resonances occur [161]. Note that the resonances become narrower with particle size. This indicates that the condition for resonance is harder to satisfy for larger particle size. There are a few limitations for the theoretical model to be met experimentally. The model assumes a lossless particle where the particle is assumed to be a perfect sphere and of high quality factor, $Q$. These particle characteristics are not met with the particles used in this experiment. Furthermore, the laser is assumed to be an ideal laser of a single frequency linewidth.

The experimental data is illustrated in Figure 6.18. The propulsion velocity was observed to increase with particle size. However, the 15µm particles show reduction in velocity and no propulsion observed for 20µm particles. An increase in the particle size increases the polarisability of the particle and the optical force. The scattering image from a 20µm particle, as shown in Figure 6.19, proves that there was optical force acting on the particle although no propulsion was observed. There was also no apparent propulsion for 1µm particles observed in the experiments, mostly due to the limitation of the lenses available to monitor and track the 1µm particles. However, lateral trapping of the particles can be seen from Figure 6.20. The increment in the intensity line profile, as shown from Figure 6.20 a) to Figure 6.20 b), indicates an increasing number of 1µm particles trapped in the illuminated channel with increasing time. As indicated in Section 3.2.1, the Brownian motion is
more apparent as the size of the particles decreases. The 1µm particles were seen to move randomly in Brownian motion. However the motion was directed to the illuminated channel, indicating a strong lateral trapping.

Figure 6.19 a) Typical image taken in monitoring the 20µm particles. Notice that the particle, as indicated by the arrow, lies exactly on top of the illuminated waveguide. b) Light scattering was observed by removing the 1µm low-pass filter from the microscope.

There are some discrepancies between experimental velocities and the predicted velocity in the theory. Apart from the reasons stated previously, the standard deviation of the particles used was observed to be higher than the distance between two adjacent maxima and minima of the velocity resonance. For example, the standard deviation for a 10µm particle is 0.763µm while the difference between maximum and minimum near 10µm particle size is 0.06µm in the theoretical plot. In addition, small deviations from spherical symmetry are known to affect the quality factor of the narrowest resonances [271]. Therefore this may indicate that the particles that were used in the experiments are not perfectly spherical. Hence, the experiments did not observed a wide range of propulsion velocities as expected from the theory. In addition, estimation of mammalian cell propulsion using the theoretical model is also invalid. Mammalian cells were observed to be hemispherical on surface, as shown in Section 1.5.2 which indirectly increases the frictional force due to a larger contact area. However, assuming non-resonance propulsion estimation, as illustrated in Figure 6.17, the trend of particles propulsion observed in the experiments follows the theory. This might be a better approximation to estimate the propulsion velocity according to size. The theory should also taken into consideration the reduction of laser power due to modal mismatch, scattering and Fresnel reflection.
6.3.7 Effect of refractive index of particles/cells

Apart from differences in size, density, structure and surface properties, cells also have a low refractive index compared to the polystyrene particles. A variation in the refractive index changes the propulsion behaviour of the cells. The refractive index of the polystyrene particles used in all experiments is $n=1.59$, as stated in Section 1.5.1. Exact values of the refractive index of lymphoblastoma cells or teratocarcinoma cells on the other hand could not be found in the literature. However, as most of the cell structure consists of cytoplasm, it is fair to assume that the refractive index will be close to $n=1.39$, as indicated in Section 1.5.2 [66, 67]. Prior to manipulation of cells, propulsion of particles with the refractive index close to the cell index was
investigated in order to predict the propulsion behaviour of a low index material. Listed in Appendix C are the potential particles with different refractive indices.

![Image of PMMA particle movement](image-url)

**Figure 6.21 Propulsion of PMMA particles** a) Image taken at $t = 0s$, b) $t = 66s$, c) $t = 133s$ and d) $t = 200s$

The lowest refractive index available commercially is obtained from silica spheres, which is approximately 1.46. The refractive index is the closest to the assumed refractive index of mammalian cells; unfortunately the particle is quite dense with the density of 2.5gcm$^{-3}$. A high density particle has a larger mass (hence, more friction force) and preliminary propulsion experiments using silica spheres show no motion at all. Hence, 10µm diameter polymethylmethacrylate (PMMA) particles with a refractive index of 1.489 were chosen [61]. The same optical and waveguide setup as in Section 6.3.6 was used to carry out trapping and propulsion analysis of PMMA particles. A series of images of PMMA particles propulsion is shown in Figure 6.21. The images were compressed (in the $x$-direction) in order to accommodate the distance travelled by the particle. The sequence of images in Figure 6.21 shows that the PMMA particle moved with a mean velocity of...
0.23±0.07µm/s. As illustrated in Figure 6.22, Brownian motion was more pronounced in the PMMA particle propulsion vis-à-vis the observation in Section 6.3.1 for the polystyrene particles. This is due to the lower refractive index of the PMMA particles. This investigation was extended by varying the input power supplied to the 4µm nominal width channel waveguide.

Figure 6.24 shows the velocity of 10µm PMMA particles on the 12 hour caesium ion-exchanged waveguide. The input power was varied from about 400mW up to 620mW by adjusting the current supplied to the laser (corresponds to 50mW to 78mW input power to the waveguide). Propulsion of a 10µm polystyrene particle on the same waveguide is also plotted on the same graph for comparison. Theoretical model of both, polystyrene and PMMA particles is shown in Figure 6.23 as reference. The propulsion for PMMA particles is clearly much less than for the polystyrene particle in all ranges of input power tested, as expected from the theoretical model. PMMA particles were also observed to be unable of propulsion at about 50mW input power. This may indicate the minimum threshold power needed for PMMA particle’s propulsion. At similar input powers, polystyrene particles propel, on average, at a velocity of 0.93µm/s. PMMA and polystyrene particles propel with a mean velocity of 0.24µm/s and 1.58µm/s respectively with an input power of approximately 66mW.

![Figure 6.22 Output from the particle tracking software. a) PMMA particle propulsion is presented in coloured line](image-url)
In order to understand the effect of different refractive indices of particles, a simulation using the same model as in [161, 162] was carried out. The same optical and waveguide arrangement, as described earlier in this section, was set in the simulation. The simulated velocity for polystyrene particles was normalised to the experimental values in order to have a more realistic estimation of the propulsion velocity of PMMA particles. The predicted particle velocity against the particle's refractive index is illustrated in Figure 6.25. The simulation indicates that higher propulsion velocity is achieved at the TM mode compared to the TE mode for the particles under consideration (refractive index less than $n=1.6$) as predicted in Section 4.3.4. The velocity for the PMMA particle is expected to be around 0.2266µm/s due to a lower effective index which approximately the same as observed experimentally. The observations on varying particle size and refractive index suggest that the propulsion of mammalian cells of interest, lymphoblastoma and teratocarcinoma cells was indeed feasible. The size range of the mammalian cells overlaps the size range of particles that were able to be propelled on the caesium ion-exchanged waveguide. The theoretical model, as illustrated in Figure 6.25, also shows the predicted velocity for cells of refractive index near 1.39, which is about 6.273nm/s.

![Input Power vs Velocity Graph]

**Figure 6.23** Theoretical propulsion velocity of PMMA and polystyrene particles with varying input power
Figure 6.24 Propulsion velocity of 10 µm PMMA particles with varying input power. Propulsion velocity of polystyrene particles of the same size is also plotted for comparison.

Figure 6.25 Theoretical velocity of particles with varying refractive index.
6.3.8 Effect of surface functionalisation

Mammalian cells involved in this project are lymphoblastoma and two types of teratocarcinoma cells; TERA1 and NT2 cells. Although lymphoblastoma grows in suspension and is less likely to adhere to the surface, teratocarcinoma cells adhere to the surface and form a monolayer in culture. Thus a method to reduce adhesion and promote the dominance of a double layer repulsive force, as mentioned in Section 3.2.3, is by functionalising the waveguide surface. A 12 hour caesium ion-exchanged waveguide was PEG-functionalised, as detailed in Section 3.4.1. Prior to the functionalisation step, propulsion experiments of 8µm, 10µm and 12µm polystyrene particles were carried out on the waveguide for comparison. Figure 6.26 shows results from the propulsion experiments on both PEG-functionalised and plain surfaces.

It can be observed in the propulsion experiments that particles of all sizes tested show a faster propulsion on the PEG-functionalised surface. From Figure 6.26, propulsion of polystyrene particles is on average 25% faster than propulsion on a plain surface. Apart from preventing cell adhesion [234, 235], the faster propulsion observed also indicates a reduction in the frictional force that is opposing the particle motion, as discussed in Section 5.2.4.3. This is beneficial in terms of
applying the system to propel mammalian cells since a low refractive index is expected to reduce the propulsion velocity dramatically, as shown in Section 6.3.7. Any minimisation of forces that hinder the propulsion (frictional force and cell adhesion) is definitely seen as an advantage.

6.4 Cell trapping and propulsion

The investigation of mammalian cell's trapping and propulsion on a caesium ion-exchanged waveguide is discussed in this section. Mammalian cells used in this section were prepared fresh for every experiment and diluted in their specific culture media to a concentration of $1 \times 10^6$ cells per ml. The optical arrangement described in Section 6.2.1 was used with a similar imaging setup as detailed in Section 6.2.1. Unless stated otherwise, a nominal channel waveguide width of 4µm on a 12 hour caesium ion-exchanged waveguide was used with laser power set to 500mW and in the TM mode. Due to the nature of the culture media that provide a healthy environment for culturing cells including bacteria and viruses, extra safety measures were carried out. All manipulation involving the culture medium was conducted in the laminar flow cabinet to avoid any unnecessary exposure. This includes pipetting culture media containing mammalian cells to the PDMS reservoir. The optical bench, computer and optical equipment were also wrapped with fresh polythene sheets. The working surfaces were routinely disinfected once a week and after each experiment involving cell cultures, using 95% ethanol solution to avoid any contamination to the cell culture.

6.4.1 Lymphoblastoma propulsion analysis

Prior to the experimentation using lymphoblastoma cells, a 10µL sample was taken from the culture flask and tested with a 0.4% Trypan blue solution (refer to Section 1.5.2). This was carried out to ensure that the samples have plenty of healthy cells for the experiment. The testing was also done after each experiment to make sure that the experimental environment is plausible for maintaining healthy cells and to monitor the effect of mechanical stress (due to optical propulsion) on the cell membranes. Lymphoblastoma cells were dispersed in RPMI medium with 10% serum. The cell solution was pipetted into the PDMS reservoir and propulsion of cells was monitored on the waveguide channel.
A series of snapshots of an approximately 10µm lymphoblastoma cell being propelled is illustrated in Figure 6.27. The cells have much less contrast than the polymer particles as the cell has a lower refractive index and hence provides less light scattering. This increases the time taken in analysing propulsion of each cell as the pixel threshold has to be fine-tuned for cell identification. Such steps, unfortunately, raised other problems. Fragments from ruptured cells and artefacts in the culture medium, moving with Brownian motion, are also recognised and this increased the background noise for the cell identification process.

The lymphoblastoma cell (indicated by red arrow in Figure 6.27) was detected to move for approximately 35.7µm in the direction of the light propagation with an average velocity of 23±3nm/s. The motion of the cell was tracked and presented in Figure 6.28 by the blue line. The cell was observed to move in Brownian motion towards the illuminated channel before being stably trapped and propelled. The motion of the cell in positive x-direction suddenly stopped and continued moving in
Brownian motion in the positive $y$-direction. This sudden motion may be due to higher surface friction or uneven surface height. It is crucial to note that the shape of the lymphoblastoma cell, or any biological cell, is not spherical and the surface characteristics are different from a polystyrene particle. The density of the cell is also inconsistent from one side to another which may point out a variation in refractive index. These physical differences may consequently affect the consistency of the cell propulsion. The transverse movement of the cell cannot be accounted by the drift effect, as another cell located exactly on top of the waveguide (indicated by green arrow in Figure 6.27) was observed to propel in the direction of light propagation as shown by the pink line in Figure 6.28.

The propulsion velocity was observed to be faster than the predicted value in Figure 6.25. This may indicate that the refractive index of lymphoblastoma cells is slightly higher ($n = 1.42$ from Figure 6.25) than the assumption ($n = 1.39$). This investigation was repeated with varying input power. Lymphoblastoma propulsion was not observed with a power lower than 56mW. This may be due to the inadequate input power available to overcome the forces hindering forward propulsion such as frictional force. A subsequent increment of power shows an increase in the propulsion velocity, albeit not as fast as observed for PMMA particles.
maximum power supplied, of about 78mW, only managed to propel lymphoblastoma cells at a mean velocity of 38±5nm/s. Lack of velocity increment of the lymphoblastoma cells compared to the PMMA particles is mostly due to the low refractive index of the cell, in addition to the cell shape and lack of rigidity.

Figure 6.29 Propulsion of lymphoblastoma cells (=10µm) with varying input power. Propulsion of 10µm PMMA particles is also plotted for comparison

6.4.2 Investigation using teratocarcinoma cells

The investigation of teratocarcinoma cell (TERA1 cell) propulsion on channel waveguides was carried out using a 12 hour caesium ion-exchanged waveguide. The waveguide was functionalised with PEG-silane in the same method as described in Section 5.2.4. The OH group was generated by dipping the channel waveguide in a diluted piranha solution for 20 minutes. The corrosion of the channel waveguide has been identified as a potential problem, as discussed in Section 4.4.3, hence the surface profile was measured prior to and after the piranha solution treatment. The measurements show an average decrease of about 2nm in thickness of the channel waveguide. Dipping in a piranha solution of ratio 1:1:5 for 20 minutes is considered safe to maintain the caesium channel waveguide structure. Propulsion of TERA1 cells was monitored for channel waveguides varying from 3.5µm to 10µm width with input power of 66mW. The experiment was repeated at least three times with a
Propulsion of lymphoblastoma cells, as described in Section 6.4.1, provides evidence to show the capability of the caesium ion-exchanged waveguide to propel cells, albeit with a low velocity. Applying the same technique on teratocarcinoma cells, however, do not show similar behaviour. TERA1 cells were seen to move in Brownian motion, as observed in Section 5.4. However, there was no propulsion of cells in the direction of light propagation even on cells directly on top of the waveguide channel.
waveguide and with the input power increased to 1W and 2.5W. The experiment was repeated again without serum (FBS) in the cell solution in order to increase the refractive index contrast between the medium and the TERA1 cells, yet similar results were observed.

The inability to propel TERA1 cells on caesium ion-exchanged waveguide needs to be investigated in several aspects in order to develop rectifying solutions. The obvious physical difference between TERA1 cells and lymphoblastoma is the cell size, as TERA1 cells are almost twice the diameter (≈20µm). Furthermore, polystyrene particles of similar size (20µm) were also observed not to be propelled even under higher power (up to 78mW) using the caesium ion-exchanged waveguide. Such an observation is concluded to be attributed to the mass of the particle (and hence the frictional force on the surface) that overcomes the optical forces acting on it. In contrast to lymphoblastoma cells, teratocarcinoma cells are adherent cells. As teratocarcinoma cells are not naturally held in suspension, the membrane surface is expected to promote cell adhesion on the waveguide surface. This is obvious from the initial experiments conducted alongside the Brownian investigation on caesium ion-exchanged waveguides. Surface functionalisation was observed to improve the mobility of teratocarcinoma cells as shown in Section 5.4. Nevertheless, the reduction of cell adhesion was not significant enough to allow optical propulsion of TERA1 cells.

Another property that may contribute to the inability to propel TERA1 cells on caesium ion-exchanged waveguide is the refractive index of the cells. As mentioned in Section 1.5, the refractive index of the polystyrene particles and the teratocarcinoma cells is 1.59 and ≈1.39 respectively. The scattering of light can be used to illustrate the index contrast between the particles/cells and the surrounding medium. The scattered-light image can be obtained from the optical setup (refer to Section 6.2.1) by removing the 1µm low-pass filter. Figure 6.30 b) shows the scattering of a 20µm polystyrene particle and a TERA1 cell. Image analysis on these scattering images shows an average of 35% less scattering on the TERA1 cell vis-à-vis the polystyrene particle of similar size. It can be generally implied that scattering from the particle/cell is proportional to the relative optical forces acting on the particle/cell. Given that the shape of the particle and cell is a perfect sphere, reduced scattering on the cell will indicates less optical force. This is largely due to the significantly lower refractive index of the TERA1 cell compared to the polystyrene particle. Note that the shape of the cells is not spherical and thus has an effect on the scattering of light. Analysis of light scattering of cells however is beyond the scope of this project.
6.4.3 Trajectory of TERA1 cells towards the illuminated channel waveguide

In spite of the inability of TERA1 cells to be propelled on the channel waveguides, the cells were found to move in Brownian motion towards the illuminated channel. Hence, the trajectory angle of the motion of the cells was investigated to distinguish the factors that affect such behaviour. The trajectories of TERA1 cells were investigated using a 12 hour, PEG-functionalised caesium ion-exchanged waveguide. The investigation was carried out on channel waveguides varying from 3.5µm to 10µm width with 66mW input power. TERA1 cells were dispersed on DMEM with serum and were prepared fresh for every experiments. The cells were monitored for 30 minutes on different positions along the channel waveguides.

The velocity of the motion of the cells towards the illuminated channel is plotted in Figure 6.31. On average, the cells were observed to move at 0.023±0.005µm/s on all channel waveguide widths. The motion is comparable with Brownian motion as observed in Section 5.4. The trajectory of the motion of all cells were tabulated and categorised into three groups; towards the input facet, towards the output facet and perpendicular to the waveguide. The trajectory of a cell can be a combination of two or more groups, thus cell motion is categorised via the majority (>50%) of the trajectory angles. For example, 60% of the trajectory angle of a cell is towards the input facet while another 40% is perpendicular to the waveguide. This particular cell is categorised into trajectory towards the input facet. The probability of the cell to move towards the laser input facet of the channel waveguide is 48%, and towards the output facet is 19% and perpendicular to the waveguide is 33%.

The TERA1 cell motion towards the illuminated channel was investigated further by taking two cases representing motion towards the input facet and the output facet. Illustrated in Figure 6.32 is the cell motion for both cases. The red bar in the figure indicates the position of the channel waveguide. Figure 6.32 a) shows that the cell moved towards the input facet of the illuminated channel waveguide. The initial position of the cell was ≈70µm away from the waveguide. As the cell moved closer towards the illuminated waveguide, the angle of trajectory changed towards zero (perpendicular to the waveguide). The trajectory behaviour may correspond to the compound effect of convectional current, which drew the cell towards the input facet, and lateral trapping (via evanescent field) that changed the trajectory angle. Figure 6.32 b), on the other hand, shows the motion of a cell towards the output facet of the waveguide. The cell initially positioned ≈15µm away from the waveguide and approximately 300µm from the input facet. The trajectory angle of the cell remained the same as the cell moved towards the output facet. Note that the direction of the motion of the cell was along the direction of light propagation. The
opposite direction of motion compared to Figure 6.32 a) may be due to the distance farther away (300µm) from the input facet which reduced the effect of convection current. The cell however was not observed to propel in the direction of light propagation once positioned on top of the channel waveguide.

The motion of the cells was analysed in more detailed based on the angle of their trajectory and the step size taken from one frame to another as an indication of the cell velocity. The angle was measured, as shown in Figure 6.32, for each frame. Similarly to processing step size data into histograms, the trajectory angle is tabulated before being represented in a rose plot form (an angle histogram plot). These measurements were categorised by the distance from the edge of the channel waveguide. Figure 6.33 a) shows the motion of the cell towards the input facet. Histograms of the step size, taken from one frame to another, reveal a reduction in the movement speed as the cell move closer to the waveguide. The average velocity was around 1.28µm/s at 10µm away from the waveguide edge and was shifting towards zero as the cell moved towards the waveguide. A similar effect was also observed in the motion of the cell towards the output facet, as illustrated Figure 6.34 a). It is anticipated that increasing evanescent field intensity, as the distance to the waveguide edge shortens, will increase the velocity of the motion. The velocity of the motion of the cells however was shown to decrease as the cell move closer to
the waveguide. Contradicting behaviour observed in the cell motion may suggest that the motion was induced by the convection current created by the heat from the laser, as discussed in Section 3.3.1, but prevented from moving upwards due to the cell's high density.

Figure 6.32 Tracked motion of the TERA1 cell towards the illuminated channel in a) in the input facet direction and b) towards the output facet against the distance from the input facet
The heat generated by the laser is at maximum at the input facet of the channel waveguide. Hence the flow of the convection current is also at maximum at the input facet. The rose plot in Figure 6.33 b) however shows that the trajectory angle was biased towards zero degrees as the cell was getting closer to the illuminated waveguide. In fact, the trajectory angle, if totally induced by the convection current, should remain the same. On the other hand, Figure 6.34 b) shows the trajectory angle totally opposite the flow of the convection current as the cell moved closer towards the channel waveguide. Considering the forces involved, as illustrated in Figure 6.32, the motion of the cell based on the trajectory angle of both towards the input facet and the output facet, might suggest that the movement is influenced by the evanescent field.

Figure 6.33 Motion of the cell towards the input facet, a) step size histograms and b) the rose plot of trajectory angle for 0µm, 5µm and 10µm distance from the channel waveguide edge
The behaviour of the cell movement signifies that the motion may be a result of a combination of forces from the convection current and the evanescent field. The observations deduce that motion due to the optical field exists and were more pronounced when considering the trajectory angle towards the output facet. However, the optical forces is not significantly large enough to counter the motion due to the convection current or other factors as described in Section 6.4.2. A quick remedy to reduce the convection current is by moderating the temperature increase due to the heating. This can be achieved by using a thermoelectric cooler (Thorlabs – TEC3.6) with a dedicated heater controller (Thorlabs – TC200). A lower temperature was achieved when the thermoelectric cooler positioned underneath the waveguide substrate. However, the convection current was still apparent and no propulsion of TERA1 cells observed.

Figure 6.34 Motion of the cell towards the output facet, a) step size histograms and b) the rose plot of trajectory angle for 0µm, 5µm and 10µm distance from the channel waveguide edge.
A lower input power may be favoured to reduce the convection current. Note that this will also reduce the intensity of the evanescent field for the trapping and propulsion of cells. The small index contrast between the cell and its surroundings may worsen the condition. Hence, these results suggest for the need of a new waveguide approach that can provide a strong evanescent field with a long penetration depth. A detailed account of the new waveguide approach is presented in the next chapter.

6.5 Conclusion

The optical trapping and propulsion of polymer particles, namely polystyrene and PMMA particles and mammalian cells, lymphoblastoma and teratocarcinoma cells were presented in this chapter. The experimental arrangement for trapping and propulsion is explained in the beginning of the chapter. This includes the optical setup for coupling light into the waveguide, the fabrication of the reservoir for securing the particle/cell solution and data acquisition utilising LabView and Matlab for propulsion analysis. Characterisation of the culture medium is also investigated to compare its physical properties, in terms of the refractive index, kinematic viscosity and absorption spectrum, to water. This is to ensure that the system is capable of handling mammalian cells in culture media without any significant alteration to the system.

Characterisation of polystyrene propulsion against optical and waveguide parameters is used to investigate the optimum parameters for propulsion on caesium ion-exchanged waveguide. The effects of laser power and laser polarisation upon propulsion were investigated. 10µm polystyrene particles were observed to propel at a rate of 1.1x10^{-2} \mu m/s\cdot mW^{-1} as laser power increased and in the TM polarisation. The rate of propulsion velocity of 10µm particles in the TE polarisation is 5.6x10^{-3} \mu m/s\cdot mW^{-1}. This observation confirms the theoretical evaluation and experimental findings of several papers [30, 32, 161, 162]. Experimental characterisation of the waveguide parameters of caesium ion-exchanged waveguides was also carried out. Propulsion of polystyrene particles was conducted on several waveguides, investigating the effect of waveguide width and the waveguide ion-exchanged time (which is related to the waveguide depth). Results from this research found that the optimum propulsion is observed on an 8 hour caesium ion-exchanged waveguide ($n_{\text{eff}}=1.5189$) which is theoretically deduced to correspond to a 1.39µm depth. The optimum depth, simulated in Chapter 4, is 1.4µm. Variation in propulsion velocity of particles with different channel widths concludes that the optimum propulsion is observed on a 4µm nominal caesium ion-exchanged channel waveguide width (normalised to modal output power). Propulsion on a 10µm
channel width shows the highest output power. The work carried out here determined the optimum optical and waveguide parameters to be further exploited for trapping and sorting of biological cells on caesium ion-exchanged channel waveguides.

Biological cells not only vary in terms of size and refractive index but also their surface characteristics. Hence, the propulsion of polymer particles of varying sizes and refractive indexes were investigated initially on plain and functionalised surfaces of caesium ion-exchanged waveguides. It was found that as the refractive index of the particles decreased, the propulsion velocity was also observed to decrease, as demonstrated by the PMMA particles (propulsion rate of $3.9 \times 10^{-3} \mu m s^{-1} mW^{-1}$). The investigation of different surfaces showed that the propulsion velocity of particles on a PEG-functionalised surface increased on average by 25%. The investigation was further carried out using lymphoblastoma cells. Propulsion of lymphoblastoma cells of size approximately 10µm on a caesium ion-exchanged waveguide was successfully demonstrated for the first time. The propulsion velocity of lymphoblastoma cells was found to be 23nm/s. Theoretically, the propulsion velocity was expected to be 6.3nm/s, given that the refractive index of lymphoblastoma cells is 1.39. However the discrepancy observed in the experimental data might suggest that index of 1.42 fit the experimental data best. Applying a similar technique to teratocarcinoma cells, however, does not show comparable results. Several factors, such as the larger cell size (which is almost twice the size of lymphoblastoma cells), surface characteristics (cell adhesion) and low refractive index, may all contribute to the observed outcome. Further investigations on the trajectory pattern of teratocarcinoma cells shows that convection current also led cells moving towards the input facet; against the propagation of light. Cells located further away from the input facet ($\approx 300\mu m$) shows trajectory towards the illuminated channel along the propagation of light. This may suggest that applying analogous optical and waveguide parameters on a waveguide, that can provide a strong evanescent field with a long penetration depth, would give better results by reducing the effect of the convection current and at the same time increase the surface intensity.
Chapter 7

Conclusion and future work

7.1 Conclusion

The isolation of specific cells from background population is imperative, with application ranging from pathology, clinical diagnosis to stem cell research. Cell sorting has been a method to select a desired population of cells of known characteristics for further investigation. A reliable and non-destructive purification of cell population is necessary to obtain reproducible data on the cell analysis. In order to establish the state-of-the-art, a review of the cell/particle trapping and sorting techniques has been presented, covering work done by other research groups. Electrical trapping [110, 272, 273], microfluidics [129, 133, 274], mechanical [275-277] and magnetophoresis [90, 104, 105] techniques are among the conventional non-optical methods used for trapping and sorting cells and particles. Optical trapping techniques such as laser tweezers [149, 150], optical lattice [151, 152], Bessel beam [157, 158, 278] and waveguide [31, 160] have been compared and discussed. Advantages and disadvantages of each method are also presented. The review indicates that there are areas of research that are completely unexplored, especially regarding the optimisation of channel waveguides and their application for mammalian cell sorting which this thesis addresses.

Interaction of particles and cells on surfaces are analysed theoretically in Chapter 3 in terms of the non-optical forces namely drag force, Brownian motion, gravitational force, buoyancy and electrostatic forces. It is well known that the increment in the Brownian motion displacement is inversely proportional to the particle size. However, as the particles approach the surface, the model is no longer valid as the particle motion is in a bounded medium. Reduction in surface-particle distance increases the effective viscosity and hence further reduces the particle
displacement. This effect is more apparent for larger particles (≈20µm) as the combination of gravitational and buoyancy forces help them to settle and reduce the surface-particle distance. Another non-optical force that is taken into consideration is the heat generated from the laser (as used in the propulsion experiments). The heat induces convection current in order for the medium to reach temperature equilibrium. The convection current is beneficial in terms of drawing the particles and cells towards the illuminated channel. However, the flow of the convection current opposes the propagation direction of light, which will oppose the propulsion of particles and cells on the channel waveguides. Besides that, the balance in the electrostatic forces determines the dispersion stability of particles or cells in a solution. The repulsive double layer force needs to be as low as possible in order to assist the particles to approach the surface and to be overlapped with the evanescent field. At the same time, the repulsive force should not be too low (making the attractive force dominant) since this causes the particles to adhere to the surface. Hence from the theoretical evaluation, there is a range of ionic concentrations that helps to maintain particle dispersion stability. This effect is simulated and discussed as a way to reduce the dominance of the attractive van der Waals force.

The basic principles of optical waveguides are described in Chapter 4 covering diffusion theory, the ray and electromagnetic description of the optical waveguide. From the diffusion theory, the refractive index profile of the caesium ion-exchanged waveguides was approximated to follow the Fermi function. The step function, an approximation to the Fermi function, was used to simplify the ray and electromagnetic description of optical waveguides. Modelling of optical waveguides was carried out to generate a theoretical model of waveguide parameters which can be compared experimentally and also to predict the optimised surface intensity of optical channel waveguides. Based on the optical waveguide theory, the fabrication and characterisation of caesium ion-exchanged waveguides are discussed following the optical waveguide simulations. Optimisation for strong trapping and propulsion forces has been emphasised in order to overcome random influences such as the Brownian motion and to achieve stable propulsion. All optical waveguides used in the project have been characterised and the results show a reasonable agreement with the theory. Hence, an optimised waveguide is within the range of the waveguides fabricated and thus feasible for further experimentation.

The interaction of particles and cells was investigated on different surfaces via Brownian motion. Quantifiable error contributions (random, systematic and dynamic) were taken into consideration during the analysis. The functionalisation of surface using PEG-silane was described along with the characterisation process.
The frictional coefficient was determined for both plain and PEG-functionalised surfaces. The observation of Brownian motion shows that the distance travelled is reduced as the particle size increases, regardless of the surface condition. However, the mismatch between the theory and experimental data was more pronounced for polystyrene particles of larger sizes (10µm, 15µm and 20µm). PEG-functionalising of the surface improves the mobility of the particle, as expected from the theory. A 21% improvement, in terms of the distance travelled, was observed for the large size particles and the histogram peaks of the step size (from the Gaussian distribution) of smaller particles (1µm, 3µm and 6µm) were within 5% of the theoretical step size histogram peak. Changing the solution from water to DMEM or salt solution (media used for teratocarcinoma cells) shows adhesion of the particles to the surface due to the high ionic concentration, albeit on a PEG-functionalised surface. Similar findings were demonstrated for Brownian motion of teratocarcinoma cells on plain surfaces. Using various lubricating agents, such as BSA, PVA and PVP, motion of teratocarcinoma cells was still unapparent due to the cell adhesion. Utilising a functionalised surface, three distinct Brownian motion categories were revealed when analysing individual cells. 63% of the cells investigated showed a peak of the experimental Gaussian profile matched within 10% of the theoretical prediction, 9% showed a total mismatch and 28% of the cells were >10% mismatched. Variation in the Brownian motion of teratocarcinoma cells may be due to apoptotic or necrotic cells and a sub-population of cells that may arise from different stages in the cell cycles. This investigation opens the possibility of the identification of particle size and cells. Applications directed towards cell’s identification however need further research, for example using staining markers such as 7-Aminoactinomycin-D (detect necrotic cell) and propidium iodide (detect apoptotic cell) [279, 280]. Analysing cell and particle populations as a whole provides an average size of the particles and the size distribution at reduced errors.

Particle trapping and propulsion is observed from the series of experiments conducted throughout the project. The position of the particle on top of the channel plays an important role in order to ensure a secure trapping and hence a steady propulsion. This can be seen from the example in Section 6.3, where particles on top of the illuminated channel were propelled as soon as the laser light was coupled to the waveguide. Lateral trapping was also evident in the experiment as particles initially positioned several microns away from the waveguide were later drawn to the illuminated channel. It took several seconds for the particle to be stably trapped before being propelled in the direction of the laser. The velocities obtained for the propulsion, in the series of experiments carried out, is comparable to the ones obtained in the literature [30, 32, 161, 162]. The investigations of the effect of optical parameters and waveguide conditions have been performed on polystyrene
particles. Propulsion of particles was shown to be much faster on a TM polarisation, confirming the expected trend from theory. As the laser power was increased, the velocity of the propulsion of particles also increased linearly. Different particle sizes however showed a different velocity gradient, with a greater gradient observed as the particle size was increased. The optimum waveguide condition was observed from the particle propulsion experiments, as expected from theory. In the experiments with varying waveguide ion-exchange time, particles were shown to propel at their highest velocity on an 8 hour caesium ion-exchanged waveguide. Likewise in the experiments against waveguide channel width, a 4µm nominal channel width shows the highest particle velocity. These behaviours were observed on all particle sizes tested. Other ion-exchange times and channel widths show lower and significantly different propulsion velocity. As far as the literature is concerned, the optimum waveguide condition investigation carried out was the first reported. The propulsion characterisation was shown to complete the work done by [191, 232]. Six different particle sizes; 1µm, 3µm, 6µm, 10µm, 15µm and 20µm, were used in order to predict the propulsion behaviour of mammalian cells. The propulsion of the varying diameter particles fits soundly with the predicted velocities from theoretical calculations. For the first time, particles of a low refractive index, PMMA particles and lymphoblastoma cells, were shown to trap and propel using a caesium ion-exchanged waveguide. The propulsion is compared to the theoretical evaluation in [161, 162] and the experimental data agrees with the simulated values. Applying a similar technique to teratocarcinoma cells, however, does not show comparable results. Several factors, such as the larger cell size (which is almost twice the size of lymphoblastoma cells), surface characteristics (adherent cells) and low refractive index may all contribute to the observed outcome. Further investigations on the trajectory pattern of teratocarcinoma cells shows that convection current also led cells moving towards the input facet; against the propagation of light. Cells located further away from the input facet (∼300μm) shows trajectory towards the illuminated channel along the propagation of light.

These results show that there is a need of a new waveguide approach which can provide a high surface intensity and a long penetration depth of the evanescent field. A high index contrast waveguide, such as silicon nitride or tantalum pentoxide can provide a higher surface intensity. However, these waveguides also have a shorter field penetration depth; hence less evanescent field overlapping the particle or cell. As the cell of interest is about 20μm in size, the field need to overlaps in a longer range in order to provide a significant optical force. Multimode waveguides are seen favourable in this aspect as they provide a longer penetration depth. The current system was also observed to have a high coupling loss. If the coupling loss can be reduced, for example by coupling the laser and the waveguide in free space
using a high quality lens, this is expected to give a higher surface intensity. The laser power can be increased as long as the surface intensity is lower than $30 \times 10^6$ W/cm$^2$ (1064nm) \cite{40}. Thus the usage of multimode waveguides, with a new method of coupling the laser to the waveguide, is expected to be a better alternative. Given that a high surface intensity and a longer evanescent field penetration depth are obtained with the new waveguide approach, the theoretical particle propulsion might be achievable. In this case, suitable launching optics should be used to excite predominantly the higher-order mode with the optimum evanescent field penetration into the superstrate. In addition, the theoretical model should be modified to take into account the optical charateristics of the higher order mode. Let say the input power to the waveguide is fixed at 1W, the expected velocity for a 20µm polystyrene particle is about 97.6µm/s (estimated from non-resonant plot line in Figure 6.17). Assuming a 1cm waveguide length, the throughput worked out to be 1.7 minutes per particle. This is significantly slower that the commercial flow cytometry device (MoFlo™ XDP Cell Sorter – Beckman-Coulter \cite{281}) which have a throughput of up to 70,000 cells or particles in less than a second using an opto-electronic sensor chip \cite{281,282}. The device can analyse and identify various cells and particles based on multiparametric immunophenotyping or immunophenotyping in combination with functional characteristics of the cells/particles. However, the device is potential to induce cellular response from the electric field (used for sorting), mechanical stress (pressure, fluid sheer stress) and fluorescent tagging upon the biological cells. Hence applicability to stem cells is still questionable, unless the detrimental effect can be remedied. In this case, advantages and disadvantages of such high throughput devices against the optical waveguide approach need serious considerations in order to get a clearer strategy for stem cell sorting.

In summary, the work carried out in this thesis has demonstrated, theoretically and experimentally, the optimum channel waveguide fabrication conditions for the propulsion of polymer particles and mammalian cells on caesium ion-exchanged waveguide. Theoretical aspects of the channel waveguide property have been investigated and fabricated to produce the optimum surface intensity for stable trapping and propulsion. The interaction of particles/cells on surface and their estimated propulsion velocity were also evaluated. The propulsion of polymer particles with different optical configurations and waveguide parameters on different surfaces were demonstrated and characterised in detail for the first time. The propulsion of lymphoblastoma cells was also shown for the first time and the trapping of teratocarcinoma cells on caesium ion-exchanged waveguide was evaluated. The outcome from this thesis demonstrated the potential of applying the
system to be integrated in lab-on-chip devices for trapping, sorting, characterising and purification of a specific cell population.

### 7.2 Future work

The investigation carried out in this project has laid a good foundation in characterising the behaviour of polystyrene particles of different sizes on optical waveguides with varied configurations. The system was also shown to be capable of handling low refractive index particles/cells such as the PMMA particles and lymphoblastoma cells. The velocity of the propulsion differs depending upon the size of the particles or cells and their refractive index. These experiments provide support for the potential separation of particles and cells according to their optical and physical properties.

The Brownian motion investigation, discussed in Chapter 5, opens the possibility of identifying polystyrene particles and teratocarcinoma cells. Identification of sizes can be done based on the distance travelled within a determined time as well as via the histograms of the step size taken for each frame. The teratocarcinoma cell type (TERA1 and NT2) may also be identified through the width of the histogram, from differences in their size distribution. The cell stage or cell sub-population that corresponds to each category of individual cell motion can be investigated using flow cytometry coupled with a staining marker such as 7-Aminoactinomycin-D (detect necrotic cell) and propidium iodide (detect apoptotic cell). Using a staining marker can further enhance the identification of teratocarcinoma cells, for instance apoptotic cells, and relate to their motion behaviour. Note that such a step will incur more time for preparation and analysis apart from requiring a totally different microscope setup as described in this thesis. However, this opens a possibility for a different and simple approach in cell identification.

As the propulsion of PMMA particles and lymphoblastoma cells were found to be slow, there are a number of possibilities that should be further investigated to increase the propulsion velocity. One of the main solutions to this problem would be to change the type of waveguides used. Other types of waveguide such as silicon nitride waveguides \[4\] and tantalum pentoxide waveguides (which are currently being developed within the research group) is expected to provide a higher surface intensity. However, these waveguides have a short evanescent field penetration depth. Hence a multimode waveguide with a better coupling between the laser and channel waveguide is expected to improve the propulsion performance and ultimately be used to facilitate cell sorting. The usage of different wavelength (800-
850nm or 950-990nm) is also favourable to minimise the possibility of damaging the cells at high intensities. Apart from that, the existing technology used in the project could be merged with other methods, for instance hydrodynamic focusing [133, 283], to assist particle/cell propulsion and selection. Hydrodynamic focusing also allows the alignment of particles/cells directly on top of the waveguide and potentially to improve the throughput significantly.

In order to facilitate particle positioning on the illuminated channel, the next step is to research fluidic channels with hopper structures that will be incorporated in the reservoir. From the observation of the particle trapping and propulsion, it can be seen that any particles that were close to each other formed a particle chain when propelled along the waveguide. This particle chain was shown to move at a faster velocity compared to single particles. Therefore, having hopper structures on the reservoir gives control over the number of particles propelled along the waveguide and also avoids the formation of particle chains. Besides having optical force acting in the direction of the light propagation, there is also optical force acting laterally along the waveguide. This force will attract particles near to the illuminated waveguide. Particles attracted to the waveguide laterally will disturb the motion of already propelled particles. Hence the propelled particles will be stopped, slowed down, or knocked off the waveguide by particles attracted from the side. Hopper structures will avoid this particle interference by having two reservoirs to separate propelled particles from static ones. Thus, particles coming out from the hopper will be propelled without the interference from particles trapped laterally.

Manipulation of mammalian cells can also benefit from some improvements to the current system. For instance, the culture cells prosper at a temperature of 37°C in a 5% carbon dioxide humidified atmosphere. Hence, if the optical setup could be arranged in a controlled environment, it would prolong the lifetime of the cells and also avoid the limitation of the time taken to conduct the experiments. The culture media in the PDMS reservoir can also benefit from the controlled environment to maintain the pH of the solution. The studies carried out have laid the foundations for developing a potentially accurate sorting for particles and mammalian cells. Practical improvements to the system, made by selecting a totally new type of waveguide or incorporating other technologies to improve the system performance, indicate that the system has a great potential and that this work will lead to many exciting avenues of research in the future.
Appendix A

Medium formulation

A.1  DMEM formulation (mgL⁻¹)

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<td>Sodium Hydrogen Carbonate</td>
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### A.2 RPMI formulation (mgL⁻¹)

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Appendix B

Cell culture protocol

B.1 Thawing and culture of cell lines

- Pre-warm the culture medium to 37°C in the water bath.
- Start flow hood and spray well with 95% ethanol. Prepare a large beaker with Virkon solution in the flow hood. Label the required number of culture flasks and 15 ml centrifuge tubes (one of each per aliquot of cells to be thawed).
- Remove 1 ml aliquots of frozen cells from the liquid nitrogen storage tank.
- Warm the vials in the water bath set at 37°C until thawed; spray vials with 95% ethanol.
- Using a disposable 5 ml pipette and a pipettor, draw up 7ml of pre-warmed culture medium, put 6ml of culture medium into the centrifuge tube, add the remaining 1 ml of culture medium to the vial of cells.
- Remove the suspension of cells and culture medium from the cryovial and add to the 6ml of culture medium in the centrifuge tube.
- Rinse the cryovial again with the diluted cell solution from the centrifuge tube.
- Centrifuge the tube at 1000 rpm for 4 minutes. Spray the centrifuge tube with ethanol before returning to flow hood.
- Remove the supernatant with a disposable Pasteur pipette, discard supernatant in the Virkon solution.
- Draw-up 10ml of pre-warmed culture medium in a new sterile 10 ml pipette, place 5ml into the clean labelled cell culture flask, add the remaining 5ml to the pellet of cell.
- Re-suspend cell pellet by gently pipetting up and down.
• Transfer suspended cells to the flask containing culture medium.
• Ensure that the bottom of the flask is completely covered by culture medium.
• Place the flask in the incubator at 37°C, 5 % CO₂.
• For teratocarcinoma cells, check for cell attachment to the flask; once cells are attached to substrate replace existing culture medium with fresh, pre-warmed culture medium to ensure that all dimethyl sulfoxide (DMSO) has been removed. DMSO was used as cryoprotectant to preserve cells.
• Culture the cells until at correct confluency for passaging or harvesting.

B.2 Cryopreservation and storage of cell lines

• After initial thawing and culture of cell lines to establish growth, cryopreservation should be considered at the earliest opportunity. From an initial single 75 cm² flask of cells seeded from one cryovial, grow and passage cells until there are 9 x 75 cm² flasks at 70 -80 % confluency (3 x passages). From these 9 flasks of cells, freeze the contents of 8 flasks while continuing to split and grow on the contents of the remaining flask.
• Pre-warm trypsin solution (0.05 % trypsin + ethylenediaminetetraacetic acid (EDTA)), phosphate buffer saline (PBS - sterile) and culture medium (+10% foetal bovine serum (FBS) + Penicillin/streptomycin) in water bath set at 37°C. Thaw cell freezing medium.
• Start the flow hood, spray with 95% ethanol, minimise all equipments in the hood – try to only have essential items in the flow hood.
• Place a selection of pipettes next to the flowhood, put large plastic beaker containing 150ml Virkon into the hood (spray beaker with ethanol before it enters the hood). Spray pipettor with ethanol before use. Dry off any bottles of culture medium/PBS/trypsin solution etc. and spray with ethanol before putting in the flow hood. Make sure there is a suitable repository for any waste – both contaminated (which must be autoclaved or treated with Virkon) and uncontaminated.
• Remove the flasks of cells from the incubator, open in the flow hood and using a 10 ml disposable pipette remove the culture medium (teratocarcinoma cells remain attached to bottom of flask). Discard culture medium into beaker of Virkon.
• Rinse the cell layer with pre-warmed PBS – 7 mls/75 cm² flask, discard the PBS into the beaker of Virkon.
• Cover the cell layer with a pre-warmed trypsin solution – 3 ml/75 cm² flask, return the flasks to the incubator and set the timer for 3 minutes. Look at
the cell layer after 2 minutes on the microscope, cells should be rounding up and detaching from surface, bang the flask sharply to lift cell layer.

- Once the cells have been detached from the surface of the flask, stop the action of the trypsin by adding culture medium containing serum – 7 mls/75 cm² flask. Transfer the suspended cells into pre-labelled 50ml centrifuge tubes (1 per 4 flasks).
- Centrifuge at 1000rpm for 4 min.
- Whilst centrifuging, label the cryovials with the cell type, passage number, frozen date and initials.
- Remove supernatant from the pellet of cells. Resuspend cells in freezing medium (1 ml of freezing medium per flask of cells trypsinised).
- Place 1 ml aliquots of the re-suspended cells into each cryovial, close the lid.
- Clip the cryovials to the cryocanes, approximately 10 cm from the bottom. Fill the vacuum flask with liquid nitrogen so that the bottom of the flask is covered with a depth of 3 – 4 cm. Place cryocanes into the liquid nitrogen so that the cryovials are suspended in the liquid nitrogen vapour, approximately 7 cm above the surface of the liquid nitrogen. Replace the lid on the vacuum flask and set the timer for 30 minutes.
- After 30 minutes, remove the cryovials from the canes and plunge into liquid nitrogen.
- Label the cryocanes with the details of the cell line, clip the frozen cryovials to labelled cryocanes, cover with cryosleeve, place into the final storage location within storage dewar. (Once frozen do not allow cryovials to warm-up until ready to thaw and continue culturing). Record location of cells within storage dewar (name of dewar + number of canister + number of cryovials/cryocane).
- *If there is an access to a - 80°C freezer, the cryovials may be frozen using a rate controlled freezing device, for example “Mr Frosty” from Nalgene. Follow the instructions on the device (usually fill base with isopropanol and place cryovials in rack), place the device containing cryovials overnight in the - 80°C freezer. Transfer cryovials to storage dewar next morning as above.

B.3 Passaging and continued growth of cell lines

- The cell lines require splitting (passaging) before overgrowing the culture area i.e.: becoming more than 100% confluent. The cells should be split when they achieve 70-80 % confluency. (When flasks are viewed under the
microscope patches of cells occupy 70 - 80 % of the observed area, with the gaps between them forming approximately 30 – 20 %)

- Different cell lines have different passaging requirements.
- Split NT2 cells and TERA1 cells 1:3 at reaching 70 – 80 % confluency, cells that are only 60 % confluent may be split 1:2. Cells from a 75 cm² flask are trypsinised, before being resuspended and divided between 3 new 75 cm² flasks or 2 new 75 cm² flasks, 3 of the small 25 cm² flasks are equivalent to one 75 cm² flask. Use the large flasks for continued growing of the cell lines either prior to cryopreservation or continued propagation. Small flasks provide suitable aliquots of cells for a single experiment.

- Pre-warm the trypsin solution (0.05 % trypsin + EDTA), PBS (sterile) and the culture medium (+10% FBS + Penicillin/streptomycin) in water bath set at 37°C.
- Start the flow hood, spray with 95% ethanol and minimise all the equipments in the hood – try to only have the essential items in the flow hood.
- Place a selection of pipettes next to the flow hood, put a large plastic beaker containing 150 ml Virkon into the hood (spray the beaker with ethanol before it enters the hood). Spray the pipettor with ethanol before use. Dry off any bottles of culture medium/PBS/trypsin solution etc. and spray with ethanol before putting in the flow hood. Make sure there is a suitable repository for any waste – both contaminated (which must be autoclaved or treated with Virkon) and uncontaminated.
- Remove the flask of cells from the incubator, open in the flow hood and using a 10 ml disposable pipette, remove culture medium (cells remain attached to bottom of flask). Discard PBS into the beaker of Virkon.
- Rinse the cell layer with pre-warmed PBS – 7ml for a 75 cm² flask, 4ml for a 25 cm² flask and discard PBS into the beaker of Virkon.
- Cover the cell layer with a pre-warmed trypsin solution – 3ml for a 75 cm² flask, 1.5ml for a 25 cm² flask, return the flask to the incubator and set the timer for 3 minutes. Look at the cell layer after 2 minutes on the microscope, cells should be rounding up and detaching from surface, bang the flask sharply to lift cell layer.
- Once the cells have been detached from the surface of the flask, stop the action of the trypsin by adding culture medium containing serum – 7ml for a 75 cm² flask, 3.5ml for a 25 cm² flask. Transfer the suspended cells into a 15 ml centrifuge tube.
- Centrifuge at 1000rpm for 4 min.
• Whilst centrifuging cells, label each new flask with details of the cell line, passage number, date and initials. Add 7ml of pre-warmed culture medium to each 75 cm\(^2\) flask and 4ml to each 25 cm\(^2\) flask. Use the large flasks for continued growing of the cell lines. The small flasks provide suitable aliquots of cells for a single experiment.

• After centrifuging, remove supernatant from the cell pellet. Re-suspend cells in pre-warmed culture medium by gently pipetting up and down (exact volume of culture medium depends upon the number and size of flasks the cells are being split into. 3 mls/75 cm\(^2\) flask, 1 ml/25 cm\(^2\) flask)

• Transfer appropriate volume of cell suspension to culture medium in each clean flask.

• Ensure that the bottom of the flask is completely covered by the culture medium.

• Place the flask in the incubator at 37°C, 5 % CO\(_2\).

• Check cell growth and confluency approximately every 24 – 48 hours.

• Do not grow cell lines past passage 40. Cell characteristics might change with repeated passaging.

B.4 Trypsinisation of cells

• Pre-warm the trypsin solution (0.05 % trypsin + EDTA), PBS (sterile) and the culture medium (+10% FBS + Penicillin/streptomycin) in the water bath set at 37°C.

• Start the flow hood, spray with 95% ethanol and minimise all equipments in the hood – try to only have essential items in the flow hood.

• Place a selection of pipettes next to the flow hood, put a large plastic beaker containing 150 ml Virkon into the hood (spray beaker with ethanol before it enters the hood). Spray the pipettor with ethanol before use. Dry off any bottles of the culture medium/PBS/trypsin solution etc. and spray with ethanol before putting in the flow hood. Make sure there is a suitable repository for any waste – both contaminated (which must be autoclaved or treated with Virkon) and uncontaminated.

• Remove the flask of cells from the incubator, open in the flow hood and using a 10 ml disposable pipette remove the culture medium (cells remain attached to bottom of flask). Discard the culture medium into the beaker of Virkon.

• Rinse the cell layer with a pre-warmed PBS – 7ml for a 75 cm\(^2\) flask, 4ml for a 25 cm\(^2\) flask, discard PBS into the beaker of Virkon.
• Cover the cell layer with a pre-warmed trypsin solution – 3ml for a 75 cm² flask, 1.5ml for a 25 cm² flask, return the flask to the incubator, set timer for 3 minutes. Look at the cell layer after 2 minutes on the microscope, cells should be rounding up and detaching from surface, bang the flask sharply to lift the cell layer.

• Once cells have been detached from the surface of the flask stop the action of the trypsin by adding culture medium containing serum – 7ml for a 75 cm² flask, 3.5ml for a 25 cm² flask. Transfer the suspended cells into a 15 ml centrifuge tube.

• Centrifuge at 1000rpm for 4 min.

• Remove supernatant. Re-suspend cells in the solution appropriate for their use.
Appendix C

Particle’s profile

C.1 Profiles of different refractive index particles

<table>
<thead>
<tr>
<th>Product</th>
<th>Material/Shape</th>
<th>Diameter (µm)</th>
<th>Density (g/cm³)</th>
<th>Refractive index</th>
<th>Wavelength (nm)</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical Glass Materials</td>
<td>Silica, Sphere</td>
<td>1-515</td>
<td>2.5</td>
<td>146</td>
<td>589</td>
<td>Duke Scientific</td>
</tr>
<tr>
<td>Polymethylmethacrylate (PMMA)</td>
<td>Polymethylmethacrylate (PMMA), Sphere</td>
<td>0.001-200</td>
<td>1.190</td>
<td>1.489</td>
<td>589</td>
<td>Microparticles GmbH</td>
</tr>
<tr>
<td>Soda-lime Glass Microspheres (9000 series)</td>
<td>Soda-lime, Sphere</td>
<td>30-2000</td>
<td>2.4-2.5</td>
<td>1.51</td>
<td>589</td>
<td>Duke Scientific</td>
</tr>
<tr>
<td>9000</td>
<td>Borosilicate Glass, Sphere</td>
<td>2-20</td>
<td>2.50-2.55</td>
<td>1.56</td>
<td>589</td>
<td>Duke Scientific</td>
</tr>
<tr>
<td>Spherical Polymer Materials</td>
<td>Polystyrene, Sphere</td>
<td>1-750</td>
<td>1.05</td>
<td>1.59</td>
<td>589</td>
<td>Polysciences</td>
</tr>
<tr>
<td>Melamine Resin Particles</td>
<td>Polymethylenemelamine, Sphere</td>
<td>0.3 - 2</td>
<td>1.51</td>
<td>1.68</td>
<td>589</td>
<td>Microparticles GmbH</td>
</tr>
<tr>
<td>GLS microspheres</td>
<td>Gallium lanthanum sulphide, Sphere</td>
<td>1-500</td>
<td>4.04</td>
<td>2.4</td>
<td>1014</td>
<td>In house</td>
</tr>
</tbody>
</table>
Appendix D

Arbitrary Beam Theory (ABT)

D.1 Derivation of ABT

Calculation of radiation forces on a micrometer-sized spherical particle requires the use of rigorous Mie theory. A generalisation of Mie theory was developed by Barton, Alexander and Schaub [264, 265], which is sometimes referred to as Arbitrary Beam Theory (ABT). The theory has been applied by several papers [30, 161, 162, 284] to calculate the guiding velocities for spherical particles in the evanescent field. The theory uses the following expression for force $F$ on a dielectric body [285]

\[
F = \oint_{\partial S} \epsilon E' E' + H' H' - \frac{1}{2} \left( \epsilon E'^2 + H'^2 \right) da
\]

where the integrand in Equation D.1 is the Maxwell’s stress tensor and this is integrated over the surface $S$ enclosing the dielectric body. In order to give a complete description of the fields outside the body, scattering due to the dielectric body have to be taken into account. The total fields outside the body are therefore $E' = E^{(i)} + E^{(s)}$ and $H' = H^{(i)} + H^{(s)}$, where $E^{(i)}$ and $H^{(i)}$ are the incident fields, while $E^{(s)}$ and $H^{(s)}$ are the scattered fields. In ABT, which is a generalisation of Mie theory for scattering by a sphere, the incident and scattered fields are expanded in terms of the spherical harmonics $Y_{lm}(\theta, \varphi)$. The radial parts of the incident fields are given below [264],

\[
E_r^{(i)} = \frac{E_0}{r^2} \sum_{l=1}^{\infty} \sum_{m=-l}^{l} l(l+1) A_{lm} \psi_l(k_r) Y_{lm}(\theta, \varphi)
\]
Equation D.3

\[ H^{(i)}_r = \frac{H_o}{r^2} \sum_{l=1}^{\infty} \sum_{m=-l}^{l} (l+1)B_{lm}\psi_l(k_r r)Y_{lm}(\theta, \varphi) \]

where \( k = 2\pi n / \lambda \) is the wave vector for the cover region having refractive index \( n \), \( \lambda \) being the wavelength of the incident wave, \( l \) and \( m \) are the spherical mode numbers and \( E_o \) and \( H_o \) are the field amplitudes. The partial wave coefficients \( A_{lm} \) and \( B_{lm} \) are the matrices which are derived by using the orthogonality properties of the spherical harmonics \( Y_{lm} \) [264]. \( A_{lm} \) and \( B_{lm} \) are given by the following expressions,

Equation D.4

\[ A_{lm} = \frac{1}{E_o l(l+1)\psi_l(k_r a)} \int_\Omega E^{(i)}_r(a, \theta, \varphi)Y^*_l(\theta, \varphi) d\Omega \]

Equation D.5

\[ B_{lm} = \frac{1}{H_o l(l+1)\psi_l(k_r a)} \int_\Omega H^{(i)}_r(a, \theta, \varphi)Y^*_l(\theta, \varphi) d\Omega \]

which \( d\Omega = \sin \theta d\theta d\varphi \) and \( a \) is the radius of the spherical scatterer. The partial wave coefficients \( A_{lm} \) and \( B_{lm} \) for TM polarisation are obtained when the following expressions for the evanescent field of the waveguide are inserted in Equation D.4 and Equation D.5,

Equation D.6

\[ H^{(i)}_r = H_o e^{-\sigma z} e^{jk_z z} \sin \theta \sin \varphi \]

Equation D.7

\[ E^{(i)}_r = E_o e^{-\sigma z} e^{jk_z z} \left( -k_z \sin \theta \cos \varphi + j\sigma \cos \theta \right) \]

where \( x = a \sin \theta \cos \varphi, \ z = a \cos \theta, k_z \) is the propagation constant for the waveguide and \( \sigma = \sqrt{k_z^2 - k_r^2} \) is decay constant of the evanescent wave in the cover medium. The origin of the axes is at an arbitrary point on the waveguide cover interface. \( E_o \) and \( H_o \) are the field amplitudes which depend on the power in the waveguide as well as on whether the waveguide is symmetric or asymmetric. The optical force on the sphere, \( F \), is then given by a nested sum of binary products of the partial wave coefficients for the scattered field \( a_{lm} \) and \( b_{lm} \).

The explicit expressions for the optical force on the sphere is [284],

\[ \frac{F}{\varepsilon_o E_0 a^2} = -\alpha^2 \sum_{l=1}^{L} \sum_{m=-l}^{l} \left[ \frac{(l-m+1)(l+m+1)}{(2l+1)(2l+3)} \right]^{1/2} \]

\[ l \frac{1}{(l+2)} \left[ 2n_z^2 a_{l+1,m} a_{l,m} + n_z^2 a_{l+1,m} A_{l,m} + n_z^2 B_{l+1,m} B_{l,m} + 2b_{l+1,m} B_{l,m} + B_{l+1,m} B_{l,m} \right] + n_z^2 \left( a_{l,m}^* b_{l,m} + a_{l,m}^* B_{l,m} + A_{l,m}^* b_{l,m} \right) \]
List of publications


Conferences attended


Annual Bio-Dielectric Conference - Theories, Mechanisms and Applications, Leicester, United Kingdom (10th-12th April 2006)

The 17th Annual Workshop on Micromachining, Micromechanics and Microsystems, Southampton, United Kingdom (3rd-5th September 2006)

Nanoscale Physics and Technology: the Interface with Medical and Biological Sciences, Southampton, United Kingdom (26th-27th March 2007)
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