New AC Electro-kinetic Tools for Laboratories-on-a-Chip

By

Henry O. Fatoyinbo BEng, MSc, AMIChemE

Centre for Biomedical Engineering
School of Engineering, University of Surrey

July 2006

© Henry O. Fatoyinbo 2006
Acknowledgments

I would like to express my gratitude to everyone who has aided me directly and indirectly throughout the preparation of this thesis.

I would first like to thank my supervisor, Dr. Michael P. Hughes for his invaluable wisdom, guidance and support throughout this work. His patience, encouragement and friendly talks contributed significantly in all aspects of this thesis. Thank you!

Thanks are also due to Dr Kai Hoettges for his continual advice and suggestions on technical matters; Dr David Ewins for his help and support as Head of the Biomedical Group; Dr Subrayal Reddy for helpful discussions on biosensors and the use of his laboratory; Mr David Gould for his technical assistance. I would also like to thank the EPSRC for funding and Drs Geoff Hill and Rob Airey of the University of Sheffield for supplying the electrodes.

Thanks to friends and colleagues in the Biomedical Group, in particular to Dr Fatima Labeed, Dr Salim Ghoussayni, Mr David Moser, Mr Filipe Horta, Miss Paola Catalfamo (MATLAB expert!), Dr Karla Bustamantes, Mr Issam Flaich, Mr Fei Shao, Miss Yvonne Hübner, Mr Lionel Broche and Mr Tom Wells for their stimulating conversations and company when breaks were needed. To Mr Dan Hawkins who has kept me amused throughout my time in Guildford and Captain James Graham-Smith who regularly makes sure I am still in Guildford!

I would like to say a big thank you to my brothers and sister, Dr C.B. Fatoyinbo, Bunmi, and Kayode who have been very supportive and encouraging. Finally, the two people who should take all the credit in everything I achieve are my parents. Without their moral guidance and tolerance I would never have reached this far. Lots of love, Seyi!
Summary

AC electro-kinetics involves the manipulation of particles in non-uniform alternating electric fields. Micro-systems consisting of microelectrode arrays generate the field non-uniformities which can produce several observed phenomena, including lateral particle displacements (dielectrophoresis), particle rotation (electro-rotation) and induced fluid flow (electro-hydrodynamics). These effects are influenced primarily by the dielectric properties of the particle and the suspending medium, the frequency of the applied electric field and the electrode designs.

In this thesis, novel processes involving the use of ac electro-kinetics in micro-systems are developed. Using a quasi-three dimensional dot micro-system, dielectric properties of dielectrophoretically manipulated bioparticle suspensions are shown to be determined through the use of image analysis. Significant factors contributing to the speed and accuracy of the process were found to be dependent on the particle concentration and electrode dimensions. These dependencies showed a phenomenon which has hardly been used before in ac electro-kinetic particle characterisation, the process of spontaneous particle re-dispersion in the micro-system.

The first integrated micro-system coupling ac electro-kinetic particle manipulation and piezoelectric mass detection simultaneously is described. The electrode design used enhances particle collection on to the surface of the electrode, through induced fluid flow, where detection occurs. The dynamic response of the system has shown that nano-particles are more suited for this system, with the rate and amplitude of detection shown to correspond to the concentration of particles.

Pre-concentration of biological particles in micro-fluidic systems using dielectrophoresis is a useful upstream process which can be employed prior to characterisation or detection processes. However, exposure of biological particles to high field gradients can lead to cellular damage. A comparison of dielectrophoretic and electro-hydrodynamic forces as a means of particle retention in micro-fluidic flow has shown that particles can be trapped at different electrode regions. These regions correspond to the high and low electric field gradients in the electrode vicinity. At increased flow-rates, hydrodynamic forces are seen to have a significant influence on the trapping efficiencies using electro-hydrodynamic forces. Although, at lower flow-rates the number of viable of cells eluted from the micro-fluidic chamber is significantly greater than those exposed to conventional dielectrophoretic forces.
Table of Contents

Table of Figures.................................................................................. viii

Chapter 1
Introduction & Aims of Work

1.1 Introduction .................................................................................. 1
1.2 Aims and Objectives ....................................................................... 4
1.3 References ....................................................................................... 6

Chapter 2
AC Electro-kinetics: Theory, Application and Integration

2.1 Introduction .................................................................................. 8
2.2 Dielectrics ....................................................................................... 9
  2.2.1 Polarisation ................................................................................ 10
  2.2.2 Dielectric Constant of Heterogeneous Systems ....................... 11
  2.2.3 Dielectric Behaviour of Dispersed Systems ............................. 13
2.3 AC Electro-kinetics ......................................................................... 15
  2.3.1 Dielectrophoresis (DEP) .............................................................. 16
  2.3.2 Electro-rotation (ROT) ................................................................. 20
  2.3.3 Theory of the ‘Multi-shelled Model’ ........................................ 21
  2.3.4 Electro-hydrodynamics (EHD) ..................................................... 25
2.4 Microelectrodes and Micro-fluidic Strategies ................................ 28
  2.4.1 Micro-fluidic Processes ................................................................. 28
    2.4.1.1 Differential DEP affinity ......................................................... 29
    2.4.1.2 Field Flow Fractionation (FFF) ............................................. 30
    2.4.1.3 Stepped Flow Separation ..................................................... 31
    2.4.1.4 Travelling-wave Dielectrophoresis (twDEP) ......................... 32
  2.4.2 Micro-fabrication Techniques .................................................... 32
  2.4.3 Microelectrode Geometries ......................................................... 34
2.5 Applications of AC Electro-kinetics to Biological Particles ............ 36
  2.5.1 Bioparticle Characterisation ....................................................... 36
  2.5.2 Separation and Concentration of Bioparticles ........................... 37
  2.5.3 AC Electro-kinetic Integrated Micro-bioprocesses .................... 38
2.6 Biosensors ...................................................................................... 39
  2.6.1 Acoustic Sensors: Piezoelectric Crystals (Quartz Crystal Microbalances) .......................................................... 41
    2.6.1.1 Properties of QCM ............................................................... 42
    2.6.1.2 Quartz Crystal Theory ......................................................... 43
  2.6.2 Optical Sensors: Surface Plasmon Resonance (SPR) .................. 46
2.7 Conclusions .................................................................................... 49
2.8 References ...................................................................................... 50
### Chapter 3

**Evaluation of a Dot Micro-system for Rapid Dielectrophoretic Characterisation of Biological Particles using Digital Image Processing Techniques**

3.1 Introduction ................................................................ ........................................61
3.2 Materials and Methods ..................................................................................64
   3.2.1 Microelectrode Fabrication ........................................................................64
   3.2.2 Micro-system and Experimental Set-up ....................................................66
   3.2.3 Biological Particles ...................................................................................67
   3.2.4 Optimising Particle Concentration and Redistribution against Dot Size ..............................................................................................................67
   3.2.5 Dielectrophoretic Characterisation of Biological Cells ..........................68
3.3 Experimental Results: Phenomenology ..........................................................69
3.4 Data Processing and Simulation Methodologies ..............................................73
   3.4.1 Image processing of Obtained Data ...........................................................73
   3.4.2 Compartmental Analysis of Dot Regions ...................................................74
   3.4.3 Finite Element Modelling (FEM) ...............................................................75
3.5 Image Processing Results ..............................................................................76
   3.5.1 Development of Graphical User Interfaces (GUI) .....................................76
   3.5.2 Cumulative Modal Intensity Shift (CMIS) ...............................................80
   3.5.3 Statistical Description of Particle Redistribution ......................................86
   3.5.4 Rapid Dielectrophoretic Characterisation using Image Processing ..........90
   3.5.5 Determination of Dielectric Properties of Homogeneous Populations using the Dielectrophoretic Spectrum Data Obtained from Image Analysis ..........................................................86
3.6 Discussion .........................................................................................................107
   3.6.1 Image Processing for Determining the Clausius-Mossotti Factor. .............107
   3.6.2 Effect of Particle Concentration vs. Dot Size ...........................................111
   3.6.3 Electrostatic Field Distribution in Dot Micro-system ...............................112
3.7 Conclusions ......................................................................................................115
3.8 References ........................................................................................................116

### Chapter 4

**An Enhanced Dielectrophoretic-Quartz Crystal Microbalance (DEP-QCM)**

4.1 Introduction .......................................................................................................121
4.2 Methods and Materials ...................................................................................122
   4.2.1 Electrode Design and Fabrication .............................................................122
   4.2.2 Design and Specifications of DEP-QCM Flow-cell ....................................124
   4.2.3 Frequency Modulation Interface Circuit ..................................................125
   4.2.4 Experimental Setup ...................................................................................125
   4.2.5 Preparation of Particles ............................................................................126
   4.2.6 Experimental Procedure ...........................................................................127
   4.2.7 Dielectrophoretic Modulated Signal .........................................................128
4.3 Results ...............................................................................................................129
   4.3.1 Impedance and Phase Angle Measurements of DEP-QCM .......................129
   4.3.2 Continuous Scans of Dry DEP-QCM .........................................................134
Table of Contents

4.3.3 Effect of Medium Conductivity on DEP-QCM ........................................ 138
4.3.4 Spot Depositions on DEP-QCM ......................................................... 142
4.3.5 Manipulation of Micrometer Sized Particles ..................................... 145
4.3.6 Anti-phase Modulated DEP signal ...................................................... 148
4.3.7 Manipulation of Nano-particles ......................................................... 152
4.3.8 Quantification of Rate of DEP-QCM Response ............................... 153
4.4 Discussion ................................................................................................ 158
4.4.1 Dual Active Area .............................................................................. 158
4.4.2 DEP-QCM System Dynamics ............................................................ 159
4.4.3 Sensitivity .......................................................................................... 160
4.4.4 Response to Particle Manipulation ................................................... 161
4.5 Conclusions ............................................................................................ 164
4.6 References ............................................................................................. 166

Chapter 5
Micro-fluidic Pre-concentration of Biological Cells:
‘DEP vs. EHD’

5.1 Introduction ............................................................................................ 170
5.2 Materials and Method ............................................................................ 171
5.2.1 Considerations of Micro-fluidic System ............................................. 171
5.2.2 Interdigitated Microelectrode Array .................................................. 172
5.2.3 Sample Preparation .......................................................................... 172
5.2.4 Determination of Viable and Non-viable Yeast ................................. 172
5.2.5 Continuous-flow Process Design ....................................................... 173
5.2.5.1 Particle retention using p-DEP ..................................................... 173
5.2.5.2 Particle retention using EHD flow ............................................... 174
5.2.5.3 Particle elution ............................................................................ 175
5.2.6 Detailed Design of Micro-fluidic System ......................................... 175
5.2.6.1 Bottom component ..................................................................... 175
5.2.6.2 Top component ........................................................................... 175
5.2.6.3 Micro-fluidic chamber ................................................................. 176
5.2.6.4 Electrical connections ................................................................. 176
5.2.6.5 Ports, connectors and tubing ....................................................... 177
5.2.7 FEM Model of Micro-fluidic Chamber ............................................. 180
5.3 Results and Discussion .......................................................................... 180
5.3.1 Growth Curve ................................................................................... 180
5.3.2 Process Calculations ......................................................................... 181
5.3.3 Experimental Observations ............................................................... 184
5.3.5 Electric Field Distribution ................................................................. 191
5.3.6 Force Calculations ............................................................................ 194
5.4 Conclusions ............................................................................................ 204
5.5 References ............................................................................................. 206

Chapter 6
Conclusion and Future Work

6.1 Conclusion ............................................................................................. 209
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>Future Work</td>
<td>211</td>
</tr>
</tbody>
</table>
Table of Figures
Figure 2.1 Positive dielectrophoresis of a spherical polarisable particle ...............17
Figure 2.2 Negative dielectrophoresis of a spherical polarisable particle .............17
Figure 2.3 Dielectrophoretic spectra of homogeneous latex beads of different radii and surface conductance ................................................................. 19
Figure 2.4 Representation of a particle experiencing electro-rotation in an electric field with each sinusoidal signal out of phase by 90° ........................................... 20
Figure 2.5 Real part of the Clausius-Mossotti factor for yeast in different medium conductivities using the multi-shelled model ........................................... 23
Figure 2.6 Imaginary part of the Clausius-Mossotti factor for yeast in different medium conductivities using the multi-shelled model ........................................... 23
Figure 2.7 Steps involved using the multi-shelled model to obtain the effective permittivity of a heterogeneous particle .......................................................... 24
Figure 2.8 Direction of ac electro-osmotic fluid flow created by coplanar electrodes 26
Figure 2.9 Direction of forces acting on a particle in a dielectrophoretic micro-fluidic system ............................................................................................................. 30
Figure 2.10 a) Quartz crystal in its natural form with axis showing; b) A quartz crystal microbalance (QCM) ............................................................................................. 42
Figure 2.11 Modes of crystal operation a) flexural b) extensional c) thickness shear 42
Figure 2.12 Representation of crystals cut with respect to their axes ..................... 43
Figure 2.13 Equivalent electric circuit of an AT-cut crystal in a vacuum ............... 44
Figure 2.14 Typical observed responses of an excited QCM .................................. 44
Figure 2.15 Equivalent electrical circuit for a coated crystal in liquid .................. 45
Figure 2.16 SPR system developed by BIAcore for commercial use in industry and academia .................................................................................................... 48
Figure 3.1 Representation of an array of dots' on a gold electrode ....................... 63
Figure 3.2 Array of dots etched on bottom electrode and mounted unto strip board.67
Figure 3.3 Single 500μm dot with yeast cells (a) experiencing no external field; (b) experiencing positive DEP; (c) experiencing negative DEP .................. 70
Figure 3.4 Particle velocity as a function of DEP force and dot size with experimental parameters of voltage = 6Vpp, frequency = 10 kHz (+DEP), 1MHz (-DEP) and $\sigma_m = 0.2\text{mSm}^{-1}$ ........................................... 71
Figure 3.5 Yeast cells of $10^8$ cells per ml magnitude in a 150μm dot with (a) no field applied; (b) experiencing positive DEP; (c) rapid redispersion over dot aperture; (d) random distribution; (e) experiencing negative DEP .................. 72
Figure 3.6 Image Analysis and Image Processing Graphical User Interface for redistribution analysis and Dielectrophoretic Spectra determination........ 77
Figure 3.7 Graphical User Interface for determining the dielectric properties and crossover frequencies of experimental and theoretical DEP spectra........... 77
Figure 3.8 GUI ‘load image’ button process flow diagram ..................................... 78
Figure 3.9 Process flow diagram for Image analysis and Image processing GUI ...... 79
Figure 3.10 Before and after images for full captured images and regional (centre) processed images ................................................................. 81
Figure 3.11 Before and after images for full captured images and regional (outer) processed images ................................................................. 82
Figure 3.12 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 100 Hz ........ 83
Figure 3.13 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 30 kHz ........ 83
Figure 3.14 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 7.8 MHz. 83
Figure 3.15 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 100 Hz. 84
Figure 3.16 Before and after histograms of the dot centre region. Yeast cells are suspended in distilled water with an applied frequency of 30 kHz. 85
Figure 3.17 Before and after histograms of the dot centre region. Yeast cells are suspended in distilled water with an applied frequency of 7.8 MHz. 85
Figure 3.18 Images of part of the 150μm dot array experiencing positive distribution at t = 0sec until t = 7sec where the signal is switched off. Spontaneous re-dispersion of the particles is shown over a 53 sec period. 87
Figure 3.19 Redistribution curve of yeast cells suspended over a 500μm dot array with an initial negative dielectrophoretic force exerted on the cells. 88
Figure 3.20 Redistribution curve of yeast cells suspended over a 500μm dot array with an initial positive dielectrophoretic force exerted on the cells. 88
Figure 3.21 Dielectrophoretic spectra of yeast cells (11.3μS cm⁻¹ KCl) 10 seconds after field applied at each frequency point. 92
Figure 3.22 Dielectrophoretic spectra of yeast cells (11.3μS cm⁻¹ KCl) 118 seconds after field applied at each frequency point. 92
Figure 3.23 Dielectrophoretic spectra of yeast cells in different media conductivities 118 seconds after field applied at each frequency point on 500μm dot. 93
Figure 3.24 Dielectrophoretic spectra of yeast cells in 280mM of mannitol using inner and outer regions for analysis of a 150μm dot. 93
Figure 3.25 Dielectrophoretic spectra of red blood cells in 3mSm⁻¹ KCl solution determined using 150μm dot centre region. 94
Figure 3.26 Dielectrophoretic spectrum of yeast cells suspended in conductivity media of 5mSm⁻¹; Experimental data points obtained from images are defined by the black circles (O) and the best fit lines are plotted against these points using the algorithm based on the multi-shell theory and Equation 3.8. 96
Figure 3.27 2-D model of a 150μm dot as used in electric field simulations. 98
Figure 3.28 Distribution of electrical potential for dot diameter of 150μm. 100
Figure 3.29 Distribution of electrical potential for dot diameter of 250μm. 100
Figure 3.30 Distribution of electrical potential for dot diameter of 350μm. 100
Figure 3.31 Distribution of electrical potential for dot diameter of 500μm. 101
Figure 3.32 Electric field gradient distribution within 150μm dot. 101
Figure 3.33 Electric field gradient distribution within 150μm dot. 102
Figure 3.34 Electric field gradient distribution within 250μm dot. 102
Figure 3.35 Electric field gradient distribution within 350μm dot. 103
Figure 3.36 Electric field gradient distribution within 500μm dot. 103
Figure 3.37 Electric field gradient distribution within 500μm dot. 104
Figure 3.38 Electric field gradient distribution within 500μm dot around the gold electrodes. 104
Figure 3.39 Electric field gradient distribution within 250μm dot around the gold electrodes. 105
Figure 3.40 Electric field gradient distribution within 350μm dot around the gold electrodes. 105
Figure 3.41 Electric field gradient distribution within the 500μm dot at a gold electrode edge. 106
Table of Figures

Figure 3.42 Electric field gradient distribution within the 500µm dot around a gold electrode edge ................................................................. 106
Figure 3.43 A 3x2 array of 150µm dots, with particles experiencing negative DEP 110
Figure 4.1 Quartz crystal microbalance coated with gold electrodes on top- side (a) and bottom side (b) ............................................................... 123
Figure 4.2 Image of DEP-QCM flow-cell ................................................................................................................................................. 124
Figure 4.3 Experimental set-up used in DEP-QCM experiments ....................................................................................................................... 126
Figure 4.4 Frequency modulation circuit diagram ............................................................................................................................................. 128
Figure 4.5 (a) Simulated anti-phase frequency modulated signals; (b) Section of anti-phase signals showing in-phase high frequency crystal resonating signal .... 129
Figure 4.6 Different views of the zipper electrode geometry fabricated on the quartz crystal microbalance ........................................................................ 130
Figure 4.7 Un-etched QCM scan of phase angle and impedance with two spring loaded pins driving the QCM at the tag positions ........................................ 131
Figure 4.8 Un-etched QCM scan of phase angle and impedance with one spring loaded pin driving the QCM at the tag position ........................................ 131
Figure 4.9 Typical plot of DEP-QCM's (after etching zipper array) magnitude and phase angle of impedance ........................................................................ 132
Figure 4.10 Comparison of phase angle measurements based on types of electrical connections made to the DEP-QCM ................................................................. 133
Figure 4.11 Comparison of magnitude of impedances based on types of electrical connections made to the DEP-QCM ................................................................. 133
Figure 4.12 Effects of medium conductivity on DEP-QCM series frequency, with no ac electro-kinetic signal applied ................................................................................................. 134
Figure 4.13 DEP-QCM stability of dry system when (a) one electrode on the topside is resonated, (b) both electrodes on topside are resonated and (c) when both electrodes are resonated with one electrode possessing a modulated 10V_{PP} 1 kHz signal ........................................................................................................................................ 135
Figure 4.14 Series resonant frequency of dry DEP-QCM with 10V_{PP} modulated anti-phase signals of different frequencies applied ........................................................................................................................................ 135
Figure 4.15 Minimum impedance of dry DEP-QCM with 10V_{PP} modulated anti-phase signals of different frequencies applied ........................................................................................................................................ 136
Figure 4.16 Series resonant frequency of dry DEP-QCM with 5V_{PP} modulated anti-phase signals of different frequencies applied ........................................................................................................................................ 137
Figure 4.17 Minimum impedance of dry DEP-QCM with 10V_{PP} modulated anti-phase signals of different frequencies applied ........................................................................................................................................ 137
Figure 4.18 Shift in series frequency over time for varied medium conductivities with no DEP signal ........................................................................................................................................ 138
Figure 4.19 Shift in minimum impedance over time for varied medium conductivities with no DEP signal ........................................................................................................................................ 139
Figure 4.20 Typical impedance shifts observed for loaded (blue line) and unloaded (green line) DEP-QCM ........................................................................................................................................ 140
Figure 4.21 Graphs showing the shifts of the DEP-QCM's series resonant frequency response to electro-hydrodynamic forces based on variations in medium conductivity ........................................................................................................................................ 140
Figure 4.22 Graphs showing the shifts of the DEP-QCM's series resonant frequency response to static fluid based on variations in medium conductivity ........................................................................................................................................ 141
Figure 4.23 Positions of spot depositions of PVC-THF on the DEP-QCM ........................................................................................................................................ 143
Figure 4.24 Series frequency response of the spot depositions of PVC-THF solutions ........................................................................................................................................ 144
Figure 4.25 Minimum impedance response of the spot depositions of PVC-THF solutions..........................................................................................................145
Figure 4.26 Minimum impedance shift of DEP enhanced collection for yeast of varied concentrations with signal applied before sample introduction .............................................146
Figure 4.27 Time lapse images of particle collection for a sample volume of $10^8$ yeast cells per ml ......................................................................................................147
Figure 4.28 Series frequency shifts of DEP enhanced collection for yeast of varied concentrations with signal applied after sample introduction ...............................................................................148
Figure 4.29 Time lapse images of particle collection for a sample volume of $10^4$ yeast cells per ml ......................................................................................................149
Figure 4.30 Time lapse images of particle collection for a sample volume of $10^6$ yeast cells per ml ......................................................................................................150
Figure 4.31 Time lapse images of particle collection for a sample volume of $10^8$ yeast cells per ml ......................................................................................................150
Figure 4.32 Minimum impedance shifts of DEP enhanced collection for latex beads (6.2µm diameter) of varied concentrations with signal applied after sample introduction ...............................................................................151
Figure 4.33 Series frequency shifts of DEP enhanced collection for latex beads (6.2µm diameter) of varied concentrations with signal applied after sample introduction ...............................................................................151
Figure 4.34 Series frequency shifts of DEP enhanced collection for nano-spheres (0.11µm diameter) of varied concentrations with signal applied after sample introduction ...............................................................................152
Figure 4.35 Minimum impedance shifts of DEP enhanced collection for nano-spheres (0.11µm diameter) of varied concentrations with signal applied after sample introduction ...............................................................................152
Figure 4.36 Total change in DEP-QCM frequency response to nano-sphere collection through DEP and EHD forces as a function of particle concentration ...............................................................................154
Figure 4.37 Rate of crystal’s series frequency response to DEP/EHD manipulation of varied nano-sphere concentrations ...............................................................................154
Figure 4.38 Total change in DEP-QCM impedance response to nano-sphere collection through DEP and EHD forces as a function of particle concentration ...............................................................................155
Figure 4.39 Rate of crystal’s minimum impedance response to DEP/EHD manipulation of varied nano-sphere concentrations ...............................................................................155
Figure 4.40 DEP-QCM detection rates for micro-spheres as a function of concentration for a) frequency shifts and b) impedance shifts ...............................................................................157
Figure 4.41 Diagonal cross-section across adjacent interlocking pads illustrating DEP/EHD forces on yeast (green) and micro-spheres (black); The different sized particles demonstrate the difference in rigid packing capable at the electrode surface while the fluid lines indicate the fluid motion occurring tangentially and orthogonal to the electrode surface ...............................................................................163
Figure 5.1 Dielectrophoretic collection of particles (black) in hydrodynamic flow showing characteristic regions of accumulations at the electrode (grey) edge. ...............................................................................174
Figure 5.2 Electro-hydrodynamic collection of particles (black) in hydrodynamic flow showing characteristic regions of accumulations on the electrode (grey) surface. ...............................................................................174
Figure 5.3 a) Top and Bottom components of micro-fluidic device with an interdigitated electrode array fabricated on a 36x25mm slide sitting in bottom component; b) Underside of top component zoomed in on the top surface of the chamber surrounded by the Nitrile O-ring cord. On the outside of the O-ring are
the spring loaded contacts making electrical contact with the array terminals on either side of the flow chamber. ................................................................. 178
Figure 5.4 a) Assembly of micro-fluidic device; b) Visualisation of the array position within the assembled micro-fluidic device. ................................. 179
Figure 5.5 Growth and death rates of yeast cells in 280mM D-Mannitol ............... 181
Figure 5.6 Process flow diagram of a continuous flow DEP/EHD retention and purging process ............................................................................. 181
Figure 5.7 Particle residence time as a function of the fluid mean velocity within the micro-fluidic chamber. ......................................................... 183
Figure 5.8 Particle retention over time (a=20mins; b=90mins) using EHD forces against a chamber flow-rate of 188μms⁻¹ ........................................... 184
Figure 5.9 Particle retention over time (a=20mins; b=90mins) using DEP forces against a chamber flow-rate of 188μms⁻¹ ........................................... 185
Figure 5.10 Images of EHD forces competing against hydrodynamic forces at a mean chamber velocity of 755μms⁻¹; a= 1min, b= 15mins, c= 25mins, d= end ...... 185
Figure 5.11 Effect within 3minutes of removing the 755μms⁻¹ fluid velocity from the chamber with EHD forces still applied. The particles are able to collect along the centre of the electrodes surface. ................................................................. 186
Figure 5.12 Comparison of elution efficiencies for both DEP and EHD trapped cells as a function of length of time exposed to the electric field. ............ 187
Figure 5.13 Fractions of viable and non-viable cells passing through the device with EHD and DEP forces applied as a function of chamber velocity .......... 188
Figure 5.14 Fractions of viable and non-viable cells eluted from the device after subjected EHD and DEP forces applied as a function of chamber velocity. .... 188
Figure 5.15 Retention efficiencies of DEP and EHD forces as a function of residence time and chamber velocity ......................................................... 190
Figure 5.16 Surface plot of electric potential over coplanar electrodes with electrode potentials of ±10V, electrode width = 500μm, electrode thickness = 2μm, inter-electrode gap = 250μm and chamber height= 250μm ....................................... 191
Figure 5.17 Magnitude of V|E| as a function of chamber height in 25μm intervals starting in the plane of the electrode (blue line 1) .................................... 192
Figure 5.18 Surface plot of V|E| distribution for modelled dimensions .......... 192
Figure 5.19 V|Ex| distribution in the inter-electrode gap as a function of height, taken at 10μm intervals from electrode plane to chamber surface .......... 194
Figure 5.20 Variation of V|Ex| along a diagonal line in the inter-electrode gap starting from the electrode edge and ending at the chamber surface .......... 195
Figure 5.21 Horizontal dielectrophoretic force exerted on a viable yeast cell as a function of chamber height ................................................................. 195
Figure 5.22 Vertical dielectrophoretic force exerted on a viable yeast cell as a function of chamber height ................................................................. 196
Figure 5.23 Representation of the x-component of the velocity profile of a yeast particle due to dielectrophoresis as a function of chamber height from electrode plane (2μm) to chamber centre plane (125μm) ............................................ 197
Figure 5.24 Logarithmic scale of the y-component of the velocity profile of a yeast particle due to dielectrophoresis as a function of chamber height from electrode plane (2μm) to chamber centre plane (125μm) ............................................ 197
Chapter 1

Introduction & Aims of Work

1.1 Introduction

A rapidly evolving area of engineering with applications to industry sectors such as medical, pharmaceutical, biotechnology and defence is that associated with micro-engineering and nano-engineering. This is primarily due to the application of processes developed in the semi-conductor industries enabling the miniaturisation of components for implantation, monitoring of processes or for in vitro analysis devices. With the advent of photolithography processes traditionally used to manufacture computer chips, printed circuit boards (PCB) and micro-electromechanical systems (MEMS) it is now possible to fabricate microelectrode structures on silicon substrates capable of generating high electric field gradients needed to move bioparticles [1]. The application of these manufacturing processes has led to devices known as laboratory-on-a-chip (LOC), micro-Total Analysis Systems (μTAS) and biochips being developed. The interest stimulated in the advancement of these technologies is due to the potentially massive impact on the future of health care, drug discovery, biotechnology and bio-terrorism. As technological advances continue the possibilities of developing reliable, quick, disposable and cheap devices have become more of a reality.

An increasingly popular branch of science which works on the scale of these micro-devices is that of ac electro-kinetics. As the name suggests, it covers general processes of particle manipulation through alternating electric fields. It has been known for centuries that a particle in an electromagnetic field is subjected to forces, which causes the particle to move. It was not until the late 1960s when it was realised that biological particles were able to be manipulated in non-uniform ac fields as described by Pohl [2], but initial experiments were carried out using macro-electrode structures which required extremely high voltage supplies [3]. Some techniques which fall within ac electro-kinetics offer huge benefits to LOC systems. These include dielectrophoresis (DEP), electro-rotation (ROT) and ac electro-osmosis. Dielectrophoresis is a phenomenon that is most significant on the micrometer scale with the capability of also manipulating sub-micrometer particles.
and particles up to a millimetre in size [4]. The technique is sensitive to medium properties and the properties of the particle under examination. This has allowed scientists to use this technique as a form of cell-based assaying on diluted suspensions [5-8]. The simplicity of a typical dielectrophoretic set-up i.e. an anti-phase ac signal to coplanar microelectrodes, has led to dielectrophoresis receiving a lot of attention. In addition to this, advantages include the reductions in reagent and sample volumes and costs, length of sample preparation, cost of microelectrode fabrication and system size.

Electro-rotation is also sensitive to the properties of particles and the surrounding medium and posses similar benefits as dielectrophoresis. The main differences to dielectrophoresis is that it requires the generation of $n \geq 3$ alternating current signals applied simultaneously to a polynomial electrode array which are sequentially out of phase by $\frac{360^\circ}{n}$ and this would be used for single-particle assay at any one time.

The most important criterion for the manipulation of dielectric particles using ac fields is the generation of non-uniform electric fields within the micro-system. This will cause the particle to become polarised and with the imbalance of Coulombic forces on opposite sides of the particle, created by the electric field gradient, there is a net force imparted on the particle creating motion of the particle. The motion of the particle is largely based on the morphology of the ac field gradient generated from the microelectrode geometry used, but the two main motions may be described as being translational and rotational belonging to DEP and ROT forces respectively. The dielectrophoretic forces observed for a particle in solution is based on the dielectric properties of both medium and particle and on the frequency of the applied field. This dependency allows us to predict what type of dielectrophoretic force we may expect to see. These are more commonly termed positive and negative dielectrophoresis. These two conventional forms of dielectrophoresis are observed when particles are repelled (negative) or attracted (positive) to the electrode edges through translational motion. Although these forces occur for anti-phase signals, it has been observed that particles can rotate at electrode edges, which has lead to theoretical interpretations of inter-dependencies between DEP and ROT based on empirical studies [9, 10]. Comparison of these two techniques for particle-based assaying is discussed in more detail in Chapter 2.
Another useful role dielectrophoresis offers is concerned with particle manipulation in micro-fluidic flow. A factor which determines whether the system is performing conventional dielectrophoresis is the effect the ac electric field has on the fluid over the microelectrode. Electrical body forces have been shown to arise as a result of varying the frequency of the applied ac signal between coplanar electrodes. Collectively these forces are described as electro-hydrodynamic (EHD) in nature owing to the movement of fluid due to induced gradients in the medium properties and falling into this category is that of ac electro-osmosis. The effect of ac electro-osmosis is less sensitive than that of dielectrophoresis because the fluid forces are having more of an effect on the particles than the dielectrophoretic forces. These forces tend to direct particles away from the electrode edge and concentrate them on the electrode surface. This generally leads to similar particle behaviours exhibited for very different species of intrinsic dielectric make-up and size.

Being able to understand and visualise the generated electric fields from the microelectrode structures in micro-systems, predominantly by finite element modelling (FEM), allows us to predict the movement of the particle when in the vicinity of the energised electrode. This provides us with the capability of introducing novel approaches to micro-bioprocesses on single chips using microelectrodes designed to have 'specific influences' on particles of interest. This can be extended to include multiple dielectrophoretic flow-cell units cascaded and programme-controlled to perform separate or similar bioprocesses based on the requirements of the end user.

A technique closely similar to dielectrophoresis is that of electrophoresis, in that they both require the application of a voltage signal to enable movement of particles in an electric field. However, electrophoresis uses a direct current (dc) electric field of uniform intensity. The observed movements of the particles results from the Coulomb force exerted due to the net electrical charge on the particle surface. It can thus be said that a uniform dc electric field will cause a charged particle to migrate towards the electrode of opposite polarity, while a neutrally charged particle or a particle at their isoelectric point will not exhibit any net movement in a direct current uniform field.
### ELECTROPHORESIS (dc) vs DIELECTROPHORESIS (ac)

<table>
<thead>
<tr>
<th></th>
<th>ELECTROPHORESIS (dc)</th>
<th>DIELECTROPHORESIS (ac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurs on charged particles</td>
<td>Occurs on polarisable particles</td>
<td></td>
</tr>
<tr>
<td>Non moving liquid or gel</td>
<td>Stationary or moving liquid</td>
<td></td>
</tr>
<tr>
<td>Particle speed depends on the magnitude of electric field, viscosity and particle size</td>
<td>Particle speed depends on electric field magnitude, medium viscosity and relative polarisability of medium and particle.</td>
<td></td>
</tr>
<tr>
<td>Electric field acts in one and sometimes in 2 directions</td>
<td>Acts in 2 directions and sometimes in 3 directions</td>
<td></td>
</tr>
<tr>
<td>Constant electric field magnitude; Direct Current (dc)</td>
<td>Alternating Current electric field (ac)</td>
<td></td>
</tr>
<tr>
<td>Uniform electric field</td>
<td>Non-uniform electric fields</td>
<td></td>
</tr>
<tr>
<td>Particle migrates towards electrode of opposing polarity</td>
<td>Particle can be directed towards or away from electrodes with high field gradients</td>
<td></td>
</tr>
<tr>
<td>Longer times and more reagents required for separation</td>
<td>Less time and less reagents needed for separations</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1 Differences between dc and ac electric field particle manipulations

### 1.2 Aims and Objectives

The aim of this thesis is to use ac electro-kinetic techniques in a novel manner with the development of processes which can be applied to LOC systems.

Obtaining the dielectric properties of biological materials is a useful parameter which gives valuable information for discriminating between materials of similar phenotypes. The collective name of techniques for obtaining these values is dielectric spectroscopy. For a given material, the impedance is measured at different frequencies as a function of the applied voltage and current values, which in turn allows us to derive the permittivity and loss factors of the material in question. Electro-rotation has shown to be a useful method of determining the dielectric properties of single cells, and has been extensively used as an ac electro-kinetic technique amongst researchers in the field of particle manipulation. However, dielectrophoresis has also shown to be useful in obtaining the dielectric parameters of biological particles by extraction of values from the dielectrophoretic spectrum [11]. Traditionally, acquiring the dielectrophoretic spectrum involves tedious experimental methodologies which can be time consuming and often incomplete due to the electrode geometry. In this work a novel electrode micro-system will be assessed and applied as a tool for the dielectrophoretic characterisation of biological particles. The
objective of the work is to overcome the short-comings of previous electrode micro-systems used for dielectrophoretic characterisation, with the development of a new process which can significantly enhance the time taken, without compromising the accuracy, to obtain the dielectrophoretic spectrum and hence the dielectric properties of biological particles.

Detection of particulates as small as a couple of ng/ml has been achieved through adsorption studies on to quartz crystal microbalances (QCM) [12]. It is believed that with dielectrophoretic and electro-hydrodynamic forces, adsorption of particulates in solution can be accelerated and the detection rate of particulate collection by a dielectrophoretic quartz crystal microbalance (DEP-QCM) obtained. The accumulation/detection of particles on the electrode surface through dielectrophoresis can be measured through the frequency shift of the crystal. Comparison of different sample concentrations over time may give information as to how effective the dielectrophoretic forces are in concentrating particulates from the bulk of the solution. This would greatly enhance the usefulness of particle manipulation in areas of point-of-care diagnostic as it could specifically direct particles of interest to the surface of the electrode, where the majority of biosensing techniques rely upon surface detection technologies.

Dielectrophoresis has also been shown to be effective in particle separation [3, 13-15] and concentration [16, 17] in micro-fluidic systems and this has many beneficial uses. However, there are factors which have not allowed it to be used more widely. These include design considerations for the micro-system; looking at the effects of flow-rates, voltages and electrode structures on the particle concentration over time. This could be due to ineffective designs of flow-cells which can vary in dimensions for each experiment. This will be overcome by designing a flow-cell that is ideally suited to easily replaceable electrodes which are not bound to the flow cell by adhesives and can make multiple contacts on a single slide having individual electrode terminals. A comparison of electro-hydrodynamic and dielectrophoretic particle retention and elution in laminar flow regimes will be conducted to assess the influences of the different force types on biological samples and against different flow-rates.
1.3 References


Chapter 2

AC Electro-kinetics:
Theory, Application and Integration

2.1 Introduction

The force one electric charge exerts on another electric charge was first explained by Charles-Augustin Coulomb (1736-1806). This understanding of charge interaction was a major contribution to the understanding of electromagnetism. Coulomb’s studies yielded what we now refer to as Coulomb’s Law which states ‘the force that one particle exerts on another particle is directly proportional to the product of their charges and inversely proportional to the square of their separation distance.’

More precisely Coulomb showed that the force \( F \), acting along a vector running through the centre of two charges in a vacuum, can be expressed as:

\[
F = \frac{q_1 q_2}{4\pi\varepsilon_0 d^2}
\]

Equation 2.1

where \( q_1 \) and \( q_2 \) are the net charges of particles one and two respectively, \( d \) is the vector distance between the particles and \( \varepsilon_0 \) is the permittivity of free space \( (8.85 \times 10^{-12} \text{ Fm}^{-1}) \). Also known as the inverse square law, the force between a pair of charges is unaltered by the presence of other charges in the neighbourhood. Hence, a charged body will experience a force of attraction or repulsion present when other charges are within its vicinity. The strength of an electric field \( (E) \), force per unit charge, for a charged particle is also defined by Coulomb’s Law. A theoretical relationship between the electric field of a charged particle and its magnetic field was first studied by James Maxwell based on experimental works of Michael Faraday. It was not until after Maxwell’s death that what we now know as Maxwell’s equations, mathematically describing the relation between the electric field and magnetic field, to the charge density \( (\rho) \) and current density \( (J) \) respectively, was simplified into a set of rules. They describe how the electric and magnetic fields can be found given the charge and current densities in general the electric field is determined solely by

* The electronic charge \( (e) \) on a proton or a electron has a value of \( \pm1.602 \times 10^{-19} \text{ C} \).
charge density and the magnetic field is determined solely by current density. For the static situation Maxwell's equations for the electric field are shown in Equation 2.2 and Equation 2.3, where \( \nabla \) is the field gradient operator Del or grad.

\[
\nabla \times E = 0
\]

Equation 2.2

\[
\nabla \cdot E = \frac{\rho}{\varepsilon_0}
\]

Equation 2.3

The curl operator in Equation 2.2 acting on the vector field \( E \) results in another vector field while the divergence operator in Equation 2.3 acts on the vector field \( E \) resulting in a scalar field. The work done to move a unit charge between two points of differing electric field values is the potential difference (\( \mathcal{V} \)). Since the electric field is a vector quantity, i.e. it has a magnitude and direction, it can be written with respect to its 3-dimensional coordinate system,

\[
E = \left[ i \frac{\partial V}{\partial x} + j \frac{\partial V}{\partial y} + k \frac{\partial V}{\partial z} \right]
\]

Equation 2.4

or,

\[
E = -\nabla \mathcal{V}
\]

Equation 2.5

At any point grad(\( \mathcal{V} \)) lies along the direction in which \( \mathcal{V} \) is most rapidly increasing and its magnitude is equal to the slope of \( \mathcal{V} \) in that direction. Hence the electric field can be described as the force per unit charge, pushing positive charges towards regions of lower potential energy.

### 2.2 Dielectrics

Electrically, materials can be classified as being a conductor, a dielectric, or a mixture of both. For example, biological tissues, cells and to some extent proteins and DNA have the properties of conductors and dielectrics simultaneously [1]. A primary
requirement of a dielectric is that their conductivity is very low \(<10^{-10} \text{ Sm}^{-1}\) compared to those of conductors, semiconductors and electrolytes.

2.2.1 Polarisation

The process of charge redistribution in an electric field, whereby negative and positive charges are localised at different locations is known as polarisation. The polarisation process takes a finite time to occur and charges take a finite time to accumulate. On removal of the electric field, the charges take a finite time to return to the state of equilibrium. This is known as the relaxation time \((\tau)\).

The polarisation \((P)\) induced in an insulating material is a function of the electric field. For a simple isotropic material that can be characterised by an electric susceptibility \((\chi_e)\), the displacement vector \((D)\) is shown by Equation 2.6, where

\[
\varepsilon = 1 + 4\pi\chi_e
\]

is the dielectric constant or relative permittivity.

\[
D = E + 4\pi P
\]

\[
= E + 4\pi\chi_eE
\]

\[
= \varepsilon E
\]

Equation 2.6

The dielectric constant of an isotropic material is a measure of the electric flux density to the electric field. Hence, within a region where the dielectric constant does not vary Equation 2.2 applies. That is the electric field \((E)\) satisfies the electrostatic field equations (Equation 2.3 and Equation 2.2) in this region, except that the source is modified to \(1/\varepsilon\) times the true charge density. Because the charges cannot move freely through the dielectric medium, under an electric field the charges are displaced by a distance of molecular order. As a whole the dielectric is polarised. For a homogeneous dielectric, the field cannot disturb the local electro-neutrality within the dielectric volume.

\[
A\sigma_i = \frac{Ae\psi_i}{4\pi d}.
\]

Equation 2.7
Therefore a dielectric material placed between two conducting plates of area \( A \) and true charge per unit area \( \sigma_t \), the total charge on the plate is found by Equation 2.7. The capacitance has increased by a factor of \( \varepsilon \) by the presence of the dielectric. Hence, the total charge per unit area \( \sigma_t \), existing at the interface between the positive conductor and dielectric is given by Equation 2.8, where \( E_n \) is the normal component of the electric field.

\[
\sigma_s = \frac{E_n}{4\pi} = \frac{\varphi_+}{4\pi d}
\]

Equation 2.8

The polarisation charge per unit area is therefore given as

\[
\sigma_p = \sigma_t - \sigma_s
\]

\[
= \frac{\varphi_+ - \varepsilon \varphi_+}{4\pi d}
\]

\[
= -(\varepsilon - 1) \frac{\varphi_+}{4\pi d}
\]

Equation 2.9

indicating that some of the true charge density is balanced by the polarisation charge, with the value of \( P \) being the quantitative characteristic of dielectric polarisation. In effect, more true charge is needed to be introduced in order to produce the same potential drop between the plates and therefore the capacitance increases due to the dielectric. Bearing in mind the sources of the electric field are charges there is no polarisation in a vacuum.

2.2.2 Dielectric Constant of Heterogeneous Systems

Dielectric loss due to interfacial polarisation is now commonly known as the Maxwell-Wagner effect. In 1873 James Clerk Maxwell described how when two materials in contact, with an ac electric field applied across both of them, another type of dielectric dispersion occurred at the interface of the two materials. This was later refined by K.W Wagner in 1914. This two layer system has been treated by other authors [2, 3]. It can be characterised by the materials permittivities \( \varepsilon_1 \) and \( \varepsilon_2 \),
conductivities ($\sigma_1$ and $\sigma_2$) and thicknesses ($d_1$ and $d_2$). By considering each layer to be a resistance $R$ and capacitance $C$, the admittance $G$ of material 1 is:

$$G_1 = \left( \frac{1}{R_1} \right) + i\omega C_1 = \left( \frac{\sigma_1 A}{d_1} \right) + i\omega \left( \frac{\varepsilon_0 \varepsilon_1 A}{d_1} \right)$$

Equation 2.10

where $i = \sqrt{-1}$, $A$ is the surface area of the system and $\varepsilon_0$ is the permittivity of free space. By applying the same approach to material 2, the total admittance of the system can be found to be

$$G = A \left( \frac{\sigma_1 \sigma_2 + \varepsilon_0 (\varepsilon_1 \sigma_2 + \varepsilon_2 \sigma_1) i\omega - \varepsilon_0^2 \varepsilon_i \varepsilon_2 \omega^2}{(\sigma_1 d_2 + \sigma_2 d_1) + \varepsilon_0 (\varepsilon_1 d_2 + \varepsilon_2 d_1) i\omega} \right)$$

Equation 2.11

It is possible to derive the permittivity ($\varepsilon'$) and the loss factor ($\varepsilon''$) from the displacement current density, and the electric displacement ($D$) is related to the electric field as shown in Equation 2.6, where $\varepsilon = \varepsilon_0 (\varepsilon' - i\varepsilon'')$. These quantities are defined by the Debye equations named after Peter Debye. The permittivity equals

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon'_s - \varepsilon_\infty}{1 + \omega^2 \tau^2}$$

Equation 2.12

and the loss factor is

$$\varepsilon'' = \frac{(\varepsilon'_s - \varepsilon_\infty) \omega \tau}{1 + \omega^2 \tau^2}$$

Equation 2.13

where $\varepsilon_\infty$ and $\varepsilon_s$ are the limiting values of the relative permittivity at infinite and zero frequencies respectively. The limiting values of the relative permittivity are a function
of conductivities, permittivities and thicknesses of the component materials [2]. The characteristic time \( (\tau) \) is described by

\[
\tau = \frac{\varepsilon_0 (\varepsilon_1 d_2 + \varepsilon_2 d_1)}{\sigma_1 d_2 + \sigma_2 d_1}
\]

Equation 2.14

where the loss factor has a maximum at a frequency \( f_m \), which is related to the characteristic time by \( 1/2\pi f_m \).

Dielectric loss can be understood as at very low frequencies the polarisation easily follows the alternating field and contributes maximally to the dielectric constant with no loss occurring. At very high frequencies the field alternates too fast for polarisation to occur and there is no contribution to the dielectric constant with losses occurring.

2.2.3 Dielectric Behaviour of Dispersed Systems

Based on the theories of interfacial polarisation, a comprehensive study with derived formulae on the behaviour of dispersed systems has been described by Dukhin [3]. A dispersed system can be represented as being composed of dielectrically homogeneous subsystems, located in a continuous phase. The dielectric constant remains unchanged within each subsystem and undergoes an abrupt change at the boundary between subsystems and continuous phase. Allowing for the absence of true charge in the medium bulk or at the interfaces of the subsystem, the divergence of the electric displacement vector \( (D) \) is equal to zero. Due to no true charges on the inter-phase of the non-conducting subsystems, the normal component of the electric displacement vector \( (D_n) \) at the interface should not undergo change on passing the inter-phase boundary. Hence for a homogeneous electric field, the electrostatic potential is found to be equal on both sides of the interface.

Consider a dielectric spheroid in a homogeneous electric field \( (E) \). The boundary conditions normal to the interface is such that, at an infinitesimally small distance away from the interface inside and outside of the spheroid the electric potential is the same. The solution of Laplace’s equation inside and outside the spheroid is shown in Equation 2.15 and Equation 2.16, where \( r \) is the distance to the centre of the spheroid and \( \theta \) is the angle between the radius vector \( \mathbf{r} \) and the direction of the electric field.
\[ \phi_1(r, \theta) = -Er \cos \theta + \frac{\varepsilon_2 - \varepsilon_1}{2\varepsilon_1 - \varepsilon_2} a^3 E \frac{\cos \theta}{r^2} \]

Equation 2.15

\[ \phi_2(r, \theta) = -\frac{3\varepsilon_1}{2\varepsilon_1 + \varepsilon_2} Er \cos \theta \]

Equation 2.16

The change in potential due to the polarisation of the spheroid is given by the second term in Equation 2.15. The field produced by the polarised sphere, also known as the dipole, is known to be equal to the field of the dipole oriented along the axis \( \theta = 0 \), having a dipole moment \( P_{dipole} \) equivalent to

\[ P_{dipole} = 4\pi \varepsilon_0 \varepsilon_1 \frac{\varepsilon_2 - \varepsilon_1}{2\varepsilon_1 + \varepsilon_2} a^3 E \]

Equation 2.17

The field inside the sphere can be shown (from Equation 2.15 and Equation 2.17) to be homogeneous.

The dielectric constant of a dispersed system characterises the macroscopic electrical properties, averaging the electrical field with respect to volume of the dispersed particles. The presence of dispersed particles can be compared to a homogeneous equivalent with a dielectric constant of \( \varepsilon \) (dispersed phase only) expressed through the parameters of the dispersed system. Various authors have expressed the dielectric constant of dispersed particles based on properties of the dispersion, such as volume fraction, shape of particles and the dielectric properties (permittivity and conductivity) of the dispersed phase and continuous phase. The creation of dipoles can lead to mutual polarisation of the dispersed particles, but has been generally accepted that for dilute dispersions mutual polarisation can be neglected in deriving \( \varepsilon \). Maxwell’s method of deriving \( \varepsilon \) assumes a medium with dielectric constant \( \varepsilon_1 \) containing a large number of spherical particles of radius \( a \) with dielectric constant \( \varepsilon_2 \) randomly filling a spherical volume of radius \( b \), with the average distance between each spherical particle substantially larger than their size. Under the influence of a homogeneous
external field, neglecting mutual polarisation, all particles will become polarised acquiring a dipole moment ($P_{\text{dipole}}$) and producing a field with potential

$$\phi_1 = \frac{P_{\text{dipole}} \cos \theta}{4 \pi \varepsilon_0 \varepsilon_1 r^2}$$

Equation 2.18

The spherical volume of particles may now be considered as a single spherical body polarised under the influence of an external field characterised by $\varepsilon$. Using Equation 2.17, replacing $\varepsilon_2$ with $\varepsilon$ and the radius of the particle ($a$) with the radius of the spherical container ($b$), the dipole moment of the dispersed volume ($P_{\text{dipole}}$) is obtained, showing that $P_{\text{dipole}} = m P_{\text{dipole}}$, where $m$ is the number of particles in the spherical volume, leading to Maxwell’s equation for the dielectric constant, where $\lambda$ is the volume fraction of the dispersed phase.

$$\varepsilon = \frac{3 + 2[(\varepsilon_i/\varepsilon) - 1](-1 - \lambda)}{3 + 2[(\varepsilon_i/\varepsilon) - 1](2 + \lambda)} \varepsilon_i$$

Equation 2.19

This derivation of the dielectric constant neglects the mutual polarisation of particles and only holds true for values of $\lambda$ which are sufficiently small. It can be shown that Maxwell’s derivation of the dielectric constant changes only on allowing polarisation of the introduced spheres and does not take into account the polarisation of the medium and indeed the particle shape and size are not accounted for. Theoretical formulae taking into consideration these factors has been published by various authors and is listed elsewhere [4].

### 2.3 AC Electro-kinetics

Various authors have described the processes involved for the manipulation of biological particles using alternating current electrical fields [5-7]. Unlike the well established methods of electrophoresis which uses direct current supplies for the manipulation of proteins and DNA, ac electro-kinetics offers the precise manipulation of particles in the micrometer and sub-micrometer scale.
With the advent of micro-fabricating techniques well established in the semiconductor industry, ac electro-kinetics has attracted a plethora of interest as a promising technology capable of performing diagnostic testing, separation and concentration of biological matter. The reasons for the interest shown are the ability to reduce the sizes of the devices, the cheap fabrication of microelectrodes needed, the reduction of reagents and samples and the non-destructive procedures which can be employed on biological samples.

Some of the more common modes which fall under ac electro-kinetics include dielectrophoresis (DEP), electro-rotation (ROT), travelling-wave dielectrophoresis (twDEP), electro-osmosis and electro-orientation. The principle similarity with all these modes is the use of alternating electric fields. The frequency of the alternating field, phase shift of multiple signals and the electrode array used all influence the mode of ac electro-kinetics and particle motion one may observe.

2.3.1 Dielectrophoresis (DEP)

A neutrally charged spherical particle suspended in a low conductive medium, influenced by a non-uniform alternating electric field will become polarised. This induced dipole will experience a dielectrophoretic force ($F_{\text{DEP}}$) that is proportional to the volume of the particle ($r^3$), the permittivity of the suspending medium ($\varepsilon_m$), the gradient of the electric field squared ($\nabla E^2$) and the real part of the polarisability factor of the particle, also known as the Clausius-Mossotti factor ($\text{Re}[K(\omega)]$).

$$F_{\text{DEP}} = 2\pi r^3 \varepsilon_0 \varepsilon_m \text{Re}[K(\omega)] \nabla E^2$$

Equation 2.20

Equation 2.20 shows the expression of the dielectrophoretic force in the absence of phase variations. Dielectrophoresis occurs when there is an imbalance in Coulomb forces on the induced dipole of the particle, generated by non-uniform electric fields, which leads to either a positive or negative net force on the particle [6, 7]. The force exerted on the particle by the dipole interaction with the electric field induces at first an orientating effect then a translatory motion. This was first observed by Pohl (1951) who coined the term dielectrophoresis, combining the words ‘dielectric’ meaning two charges and the Greek word ‘phoresis’ meaning force [5]. Other force components
which can be incorporated with the DEP force equation are quadrupole and higher order poles which have been derived elsewhere [8]. A particle experiencing a dielectrophoretic force has been observed to travel towards or away from regions of high field gradients.

Figure 2.1 Positive dielectrophoresis of a spherical polarisable particle

Figure 2.2 Negative dielectrophoresis of a spherical polarisable particle

Figure 2.1 shows an induced dipole travelling up the field gradient, where there is a region of field intensity maxima. By making the medium more polarisable than the particle, the electric field lines become distorted and warp around the particle, pushing the particle down the field gradient, to regions of field intensity minima, as seen in Figure 2.2. The phenomenon can be explained by examining the real part of the Clausius-Mossotti factor of Equation 2.20. The dielectrophoretic force for homogeneous spheres, with the transient regime taken into account has been shown to be important for an ac field [9]. The Clausius-Mossotti factor is a complex variable, which is dependent on the particle and suspending medium conductivity (σ_{p,m}) and
permittivity ($\varepsilon_{p,m}$) and also on the angular frequency ($\omega$) of the non-uniform electric field. This can be expressed as

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}.$$  \text{(Equation 2.21)}

Where $\varepsilon_p^*$ and $\varepsilon_m^*$ are the complex permittivities of the particle and the medium respectively. The complex permittivity of the particle or medium can be expressed by

$$\varepsilon_{(p,m)} = \varepsilon_{(p,m)} - i \frac{\sigma_{(p,m)}}{\varepsilon_0 \omega}.$$  \text{(Equation 2.22)}

Taking the real part of the Clausius-Mossotti factor the relative polarisability of the particle at a particular frequency can be realised. In general, the range for the real part of the Clausius-Mossotti factor is defined between $-0.5 < \text{Re}[K(\omega)] < 1$. If the suspending medium is more polarisable than the particle then the particle moves towards regions of low field gradients ($\text{Re}[K(\omega)] < 0$). If the particle is more polarisable than the medium then the particle moves towards regions of high field gradients ($\text{Re}[K(\omega)] > 0$). The terminology applied to these motions are positive dielectrophoresis (+DEP or p-DEP) for motion to high field gradients (Figure 2.1) and negative dielectrophoresis (-DEP or n-DEP) for motion to low field gradients (Figure 2.2). When a particle exhibits no movement, the net dielectrophoretic force on the particle is zero. The term applied to this situation is known as the particle’s crossover frequency ($f_c$), and it has been used as a parameter in the determination of single-shelled particle’s membrane capacitance and conductance [10] and the surface conductivity of homogeneous particles such as latex beads [11]. The conductivity of a homogeneous particle may be described as the sum of the bulk conductivity ($\sigma_b$) and the surface conductance ($K_s$).

$$\sigma_p = \sigma_b + \left( \frac{2K_s}{r} \right).$$  \text{(Equation 2.23)}
Figure 2.3 shows the real part of the Clausius-Mossotti spectrum for homogeneous dielectric spheroids ($\varepsilon_p = 2.5$), calculated using Equation 2.21, Equation 2.22 and Equation 2.23. It can be seen that there is a single dielectric dispersion for both particles, which corresponds to the differences in the dielectric properties of the suspending medium ($\varepsilon_m = 78$, $\sigma_m = 15\text{mS}\text{m}^{-1}$) and particle at different frequencies. The spectra shows the expected particle motions at the mentioned parameters, indicating that below 1MHz the relative polarisability of both particles is positive, but stronger for the larger particle. A decrease in the relative polarisability of the particles begins at 1 MHz and ends 2 decades later at 100 MHz. A single crossover frequency, for both particles are seen at different frequencies indicating differences in particle properties. At frequencies greater than the crossover frequencies, the particles continue to experience negative dielectrophoresis.

The dielectrophoretic force defined by Equation 2.20 shows a number of interesting aspects. A spherical particle with a small radius will require a higher non-uniform electric field gradient to attain sufficient dielectrophoretic force for motion to occur. This can be achieved by either applying a higher potential to the electrode array or by reducing the size of the electrode structures. Also, the square of the electric field is
used indicating that the induced motion of the particle is independent of electrode polarity. This is useful in dielectrophoresis as the ac potential difference between two electrodes can be applied in any arrangement, with the same particle motion observed.

### 2.3.2 Electro-rotation (ROT)

Electro-rotation is concerned with the torque (\(\Gamma\)) experienced by a particle in a rotating electric field [6]. The generation of this rotating field is commonly provided by quadrupole electrodes that are each out of phase by \(90^\circ\) per frequency.

If a single particle is placed accurately in the middle of the field, the particle will rotate on that spot with or against the field, depending if the induced dipole lags behind or is in front of the field. The torque can be found by taking the cross-product of the electric field and the dipole moment, which shows that the torque is based on the imaginary component of the Clausius-Mossotti factor, \(\text{Im} [K(\omega)]\), and the square of the electric field [6].

\[
\Gamma = m \times E
\]

\[
= -4\pi^3 \varepsilon_0 \varepsilon_r \text{Im}[K(\omega)]E^2
\]

**Equation 2.24**

![Figure 2.4 Representation of a particle experiencing electro-rotation in an electric field with each sinusoidal signal out of phase by 90°](image)
The importance of the direction of the particle's rotation is connected to \( \text{Im} \left[ K(\omega) \right] \). When the particle rotates with the electric field's direction \( \text{Im} \left[ K(\omega) \right] \) is negative and when the particle rotates opposite to the fields direction \( \text{Im} \left[ K(\omega) \right] \) is positive. These are known as anti-field and co-field rotation respectively. Interaction of polarised particles with the electric field means dielectrophoresis and electro-rotation can occur simultaneously with values of force and torque commensurable to the size of their respective values. This is observed by seeing cells spinning at electrode edges after being attracted to the electrodes under positive dielectrophoresis. It was reported by Crane and Pohl that spinning speeds for cells were dependent on cell age and voltage [12]. But the understanding of the phenomenon was explained by Arnold and Zimmerman [13]. It has since been used by many researchers to characterise biological particles based on spinning speeds [14-21].

2.3.3 Theory of the ‘Multi-shelled Model’

Analysis of the real or imaginary part of the Clausius-Mossotti spectrum for a particle can give valuable information of that particle's biophysical properties. Following on from work done by Jones and Kallio [22], Pastushenko et al [23], Pethig et al [24] and Fuhr [25] where the field induced dipole moment of a cell were concluded to be a linear response of the applied electric field and the ROT torque gave complex expressions for a multi-shelled model of a cell, a more practical and useful method was developed. This method is based on the dielectric theories of Fricke [26], Hanai [27] and Irimajiri et al [28] and later refined by Huang et al [29] for concentric spherical compartments or ‘shells’.

Classical expressions of the effective dipole moment, the dielectrophoretic force, electro-rotational torque and Clausius-Mossotti factor (Equation 2.17, Equation 2.20, Equation 2.21 and Equation 2.24) for a homogeneous spherical particle were used to describe particles that had heterogeneous compartments by simply replacing the frequency independent permittivity and conductivity parameters with frequency-dependent values.

The approach used by Huang et al for deriving the effective complex permittivity of a ‘multi-shelled’ cell \( (\varepsilon_{\text{eff}}^*) \), namely the smeared-out sphere approach, consisted of dividing the model into \( L \) concentric circles each having their own characteristic permittivity, conductivity and radius. Figure 2.7 shows the process of using the multi-
shelled model. Starting with the innermost circle (1) and the circle immediately around it (2), the effective complex permittivity \( (\varepsilon_{\text{eff}}^*) \) for both shells can be represented by a single expression. This effectively removes a concentric circle from the model and replaces it with a combined shell. The process is carried out again with the combined shell and the next shell immediately around it to derive the effective complex permittivity \( (\varepsilon_{2\text{eff}}^*) \) of the three ‘smeared-out’ concentric spheres. This process is continued until the all the spheres have been included for \( \varepsilon_{\mu\text{eff}}^* \), giving

\[
\varepsilon_{\mu\text{eff}}^* = \varepsilon_{L+1}^* \left( \frac{R_{L+1}}{R_L} \right)^3 + 2K(\varepsilon_{L-1\text{eff}}^*, \varepsilon_{L+1}^*)
\]

\[
= \varepsilon_{L+1}^* \left( \frac{R_{L+1}}{R_L} \right)^3 - K(\varepsilon_{L-1\text{eff}}^*, \varepsilon_{L+1}^*)
\]

Equation 2.25

Figure 2.5 and Figure 2.6 show the real and imaginary spectra of a live yeast cell suspended in a number of different conductivity media respectively. The spectra was obtained using the multi-shelled method described above with shell properties as described by Huang et al [29] for yeast cells having a ‘double shell’, that is three concentric shells. It can be seen that as the suspending medium conductivity increases there is an obvious change to both real and imaginary spectra. At low frequencies the relative polarisability of the cell decreases with increasing conductivity, with a gradual shift to the right of the lower cross-over frequency \( (f_c) \). This causes the dielectric dispersions to occur at different frequencies for low frequencies, but at higher frequencies the dielectric dispersions occur at the same frequencies with all high frequency crossover \( (f_c) \) points occurring at the same frequency.

This approach has been used in determining the dielectric properties of known single-shelled particles and double-shelled particles [10, 20, 30, 31]. It has also been used as a simulation tool for modelling the expected responses of multi-shelled particles (real and imaginary) and for obtaining best fit curves for experimental data [16, 32-36]. The model itself has been found to correlate well with experimental data of studies performed and provides a valuable numerical approach for extracting dielectric data.
of unknown particles through iterative computing processes such as the Nelder-Mead Simplex approach [15, 37].

Figure 2.5 Real part of the Clausius-Mossotti factor for yeast in different medium conductivities using the multi-shelled model

Figure 2.6 Imaginary part of the Clausius-Mossotti factor for yeast in different medium conductivities using the multi-shelled model
Figure 2.7 Steps involved using the multi-shelled model to obtain the effective permittivity of a heterogeneous particle
2.3.4 Electro-hydrodynamics (EHD)

The use of non-uniform ac electric field for manipulation of particles (separation or concentration) in aqueous media can give rise to fluid motion, which in turn results in viscous drag on the particle. Studies into the forces which arise in micro-systems have been published by authors [38-40]. Some forces which originate within these micro-systems include electro-thermal, electro-osmotic, gravitational, Brownian and viscous forces. Depending on the conditions within the micro-system and the particle type, the magnitude of each force on a particle can vary.

\[ F_{\text{TOTAL}} = F_{\text{DEP}} + F_{\text{VISCOUS}} + F_{\text{OTHER}} \]

Equation 2.26

In general a particle can be seen to experience a number of forces simultaneously as described in Equation 2.26, where \( F_{\text{OTHER}} \) is a combination of the electro-hydrodynamic (EHD) forces generated by the electric field on the medium. First observed by Pethig et al in the early 1990’s, cells were seen to collect on the upper surface of interdigitated castellated electrodes at low frequencies (< 500 Hz) and referred to this as ‘anomalous dielectrophoresis’, later being redefined as EHD forces [24]. Ramos et al later revealed that owing to the large non-uniform electric fields generated by the microelectrodes, as high as \( 5 \times 10^6 \) V m\(^{-1}\), heat was dissipated in the medium termed Joule heating [39]. The highly non-uniform power density developing in the medium as a result of the field non-uniformities causes local changes in density, permittivity and conductivity of the medium giving rise to forces on the fluid termed electro-thermal forces. The temperature gradients produced from Joule heating are dependent on the boundary conditions and for a simplified system such as in Figure 2.8, where the electrodes are assumed to be at a constant temperature the analytical solution for an incremental temperature rise [38] is given by Equation 2.27 where \( k \) is the thermal conductivity of the medium.

\[ \Delta T = \frac{\sigma V^2}{2k} \left( \frac{\theta}{\pi} - \frac{\theta^2}{\pi^2} \right) \]

Equation 2.27
Where $\theta$ is the angle measured from one electrode, $V$ is the amplitude of the ac signal applied between the electrodes and $\sigma$ is the conductivity of the medium.

The application of ac voltages to a pair of coplanar microelectrodes has been shown to generate fluid flow, which has a velocity dependency on frequency and voltage amplitude [41, 42]. Known as ac electro-osmosis, the force is purported to originate due to the interaction of free charge in the electrical diffuse double layer between electrode-electrolyte and the tangential electrical field (Figure 2.8). Charge accumulated in the double layer is moved by field components orthogonal and tangential to the electrode surface, creating the electro-osmotic flow. Electro-osmotic flow is a well known phenomena in direct current electric fields, its velocity ($v_{EO}$) is proportional to the tangential electrical field ($E_t$) and the zeta potential of the surface ($\zeta$). The zeta potential or the electro-kinetic potential can be described as the distance between a stationary solid boundary and the moving fluid. This region, effectively a stagnant layer, is also known as the slip plane and has an electric potential. The approximate value of the zeta potential is linearly proportional to the surface charge density in the diffuse double layer $\lambda_{dl}$ so that

$$v_{EO} = \frac{E_t \lambda_{dl}}{K \eta}$$

Equation 2.28
where \( \kappa \) is the reciprocal Debye length and \( \eta \) is the medium viscosity. As the applied voltage is constant, \( E_t \) too is constant throughout the medium and tangential to the surface. For a non-uniform ac field, double layer polarisation effects mean the magnitude of the tangential component of the electric field and surface charge density of the double layer varies with time. Measurements of potential drop across the electrolyte (tending to zero at high frequencies) and double layer (tending to zero at low frequencies) on microelectrode arrays show that at high frequencies the flow will stop, while at low frequencies the flow will also stop [41].

For an incompressible fluid, liquid motion is governed by Navier-Stokes equation [43]:

\[
-
\nabla p + \eta \left( \nabla^2 \nu \right) + \rho_b = \rho \nu
\]

Equation 2.29

with,

\[
\nu = \frac{\partial \nu}{\partial t} + \nu \cdot \nabla \nu
\]

Equation 2.30

where \( p, \eta, \nu \) and \( \rho_b \) are the pressure, viscosity, velocity and bulk medium density respectively. Coupling the time average electrical force and gravitational force observed in a micro-system and ignoring the convective term represented in Equation 2.30 since the Reynolds number (Re) shows laminar flow for typical micro-system velocities, the average fluid velocity can be found by [40]

\[
0 = -\nabla p + \eta \left( \nabla^2 \nu \right) + \Delta \rho_b g + f(E)
\]

Equation 2.31

where \( f(E) \) is the time average electrical force on the liquid. It is known that for an isothermal fluid there is no free charge and no permittivity gradients. But for small temperature rises, commonly due to localised Joule heating, gradients in permittivity and conductivity and mass density arise and along with gravitational effects, leads to
fluid motion. The time average electrical forces contributing to the fluid motion is expressed by [38, 40, 44, 45]

\[
\langle f_E \rangle = \frac{1}{2} \text{Re} \left( \left( \frac{\sigma \nabla \varepsilon - \varepsilon \nabla \sigma}{\sigma + i\omega \varepsilon} \right) \cdot E \right) E_0^* - \frac{1}{4} E_0 \cdot E_0^* \nabla \varepsilon.
\]

Equation 2.32

It can be seen from Equation 2.32 that the electrical force is frequency dependent, where if the angular frequency is greater than the conductivity-permittivity ratio the dielectric force dominates. If the angular frequency is much smaller than the conductivity-permittivity ratio the Coulomb force dominates.

2.4 Microelectrodes and Micro-fluidic Strategies

Prior to the early 1980s, research activities involving ac electro-kinetic manipulation of biological particles was not an attractive field. This was largely due to the large voltage supplies needed to generate electric field gradients with magnitudes of \( \sim 10^{12} \) V m\(^{-3} \) for particle sizes of the order of cells and bacteria [46]. In the late 1980’s Price et al was one of the first groups to use the processes developed in the microelectronics industry to fabricate microelectrode structures.

2.4.1 Micro-fluidic Processes

Fabrication of micrometer sized vessels for transporting various sorts of fluids into and out of micro-devices such as flow-cells, micro-reactors for chemical and biochemical reactions, chromatography columns for separations and other such devices where a specific reaction or process is to take place are useful as process times are reduced. The processes for fabricating such enclosures on the micron scale are predominately chemical based which can lead to expensive production costs per unit.

Mechanical based productions of flow-cells are a common method to enable fluidic flow through a chamber containing arrays of microelectrodes for ac electro-kinetic based applications. This is primarily because the planar nature of the microelectrodes, which are usually fabricated on wafer substrates such as glass, need to be enclosed for flow over the microelectrodes with provisions for electrical connections to supply the ac voltages within the micro-fluidic chamber. Owing to the exponential decrease in
the voltage magnitude as a function of distance from the electrode edge, chamber heights have been no more than 1mm, but preferably <500µm.

An attractive aspect of ac electro-kinetics is the ability to discriminate between particles based on their dielectric make-up as would be seen in the DEP spectra of live and dead yeast cells for example [29]. A binary mixture of particles can simultaneously exhibit negative DEP for one population and positive DEP for the other if appropriate conditions are applied. In 1968, Pohl et al were the first to use dielectrophoresis for the separation of particles using macro-electrodes [5]. Microelectrodes were first exploited by Gascoyne et al in the early 1990s for the separation of healthy and leukaemia mouse blood cells [47]. There has since been a lot of research devoted to bioparticle dielectrophoretic separation. Different methods for separation of bioparticles on lab-on-a-chip systems have been suggested and a review of strategies has been carried out, some of which are highlighted below [48, 49].

2.4.1.1 Differential DEP affinity
A mixture of particles is flowed through the separation chamber containing an electrode array. There is a single inlet and a single outlet, with the fluid flow being provided by an external source such as a syringe pump. The chamber is energised with an electric field, and depending on the conditions one population experiences positive DEP (attracted to electrodes where fields are maxima), while the other experiences negative DEP (repulsion from electrodes where fields are minima). The population experiencing positive DEP are trapped at the electrodes, while those experiencing negative DEP will continue with the fluid flow to the outlet. This process, termed differential DEP affinity by Becker et al [126], operates based on the crossover frequencies of the particles being different. Particles with dissimilar dielectric properties and hence having different crossover frequencies can be separated from each other. Multiple passes can be employed where the electric field penetration is poor, hence increasing the effectiveness or the efficiency of the separator. Strong electric field penetration may cause field 'walls', having an effect of trapping repelled particles in the chamber counter to the external pumping force. Careful design of electrode geometry can overcome these problems.
2.4.1.2 Field Flow Fractionation (FFF)

This method is utilised for a more heterogeneous population of particles. Three modes of field flow fractionation, namely normal, steric and hyperlayer have been described by authors [127-128]. Normal FFF involves thermal diffusion profiles of submicrometer sized particles. Steric FFF uses positive DEP forces, particle sedimentation forces and hydrodynamic lift forces in fluid flow. Due to the laminar flow regime present in micro-fluidic systems, hydrodynamic lift, a process which has the tendency to lift particles away from the wall into faster velocity gradients, tends to occur spontaneously in micro-channels. The velocity profile of an aqueous sample of low viscosity is typically parabolic and can be described as in Equation 2.33, where \( U \) is the chamber mean velocity; \( H \) is the chamber height and \( u_p \) is the velocity of the particle at height \( h \).

\[
    u_p(h) = 6U \frac{h}{H} \left(1 - \frac{h}{H}\right)
\]

Equation 2.33

Hyperlayer FFF was developed to overcome the disadvantages of particle-particle interaction and multiple equilibrium heights associated with steric FFF. Using negative DEP, particles are levitated away from the electrode array by the imposed field and balanced by the gravitational forces. Force fields acting on the particle (e.g. viscous and electrical) position them characteristically at different heights above the electrode plane in the fluid stream. The effect being particles travel at different speeds.
according to the distance from the surface, with a net result of heterogeneous particles exiting the chamber at different time intervals according to their reaction with the imposed electric field, gravity, temperature gradients and dielectric properties of the particle in a cross-flow. For a spherical particle of radius \((r>1\mu m)\) and mass density \((\rho_p)\), suspended in a fluid of mass density \(\rho_f\), the gravitational settling force \(F_g\) is given by

\[
F_g = \frac{4}{3} \pi r^3 (\rho_f - \rho_p) g,
\]

Equation 2.34

where \(g\) is the gravitational acceleration vector. Particles will essentially experience forces due to both buoyancy and dielectrophoresis, and when they are at equilibrium the particle remains at a stable position affecting the velocity at which it flows to the outlet.

2.4.1.3 Stepped Flow Separation

This process uses two port fluid entry and exit, and has been shown to be effective for the separation of bacteria, yeast and plant cells. Devised by Markx et al [50], it utilises the formation of clusters as part of the separation regime. A mixture is introduced onto the electrode array via one of the entry ports. The mixture is distributed across the electrode array and an electric field is introduced subjecting populations to negative and positive dielectrophoresis in clusters. Populations subjected to negative dielectrophoresis are held in clusters weakly, so that re-introduction of fluid flow displaces them towards the outlet. The electric field is removed and flow introduced in the opposite direction, with a net effect of collection of sub-population that experienced positive dielectrophoresis nearer the inlet port and the sub-population that experienced negative dielectrophoresis are collected nearer the outlet ports. This technique is slow and complex, but the repeated action of trapping and displacement increases the likelihood of perfect separation efficiency.
2.4.1.4 Travelling-wave Dielectrophoresis (twDEP)

This method relies on the translational motion of a particle parallel to an electrode array, rather than to a specific point. It as analogous to a conveyor belt but uses electrostatic forces with phase gradients. Interdigitated parallel electrodes are energised with 3 or more periodic sine or square waves of equal phase relationships summing up to 360°. Traditionally, 4 sequential electrodes are repeatedly out of phase by 90° (i.e. 0°, 90°, 180°, 270°), which imposes a phased electric field on a particle, enabling a controlled linear motion without the need for fluidic flow [51]. Particle separation using this method was described by Huang et al, demonstrating yeast fractionation according to size [52]. Separation was shown to be enhanced by interchanging between conventional dielectrophoresis and travelling wave dielectrophoresis on the same array [53, 54]. Development of a spiral array with four electrodes (out of phase by 90°) looping around each other until it the ends meet at the centre have shown to be able to concentrate particles from a large electrode area. Particles can be moved in either direction (i.e. towards the centre of the array or away from the centre), with the ability to detect and characterise particles of differing dielectric make-up.

It is also possible to use travelling wave dielectrophoresis to separate a heterogeneous mixture of particles into sub-populations in a micro-channel by introducing particles to the array at one end and moving them down the array by twDEP. The rate of particle motion is governed by the dielectric properties of the particles. Hence, particles will collect at the end of a sufficiently long array in order of their relative values of the imaginary component of the Clausius-Mossotti factor as described by Equation 2.21. This was demonstrated by Morgan and others using 1000 parallel electrodes, able to separate red and white blood cells from each other by a considerable distance. Travelling wave dielectrophoresis has also been thought to work in fluid flow conditions, where the electrode array is arranged orthogonal to the flow. Application of the appropriate travelling wave electric field can induce a motion on the particle allowing particles to exit the chamber at different spatial outlet locations.

2.4.2 Micro-fabrication Techniques

Photolithography, literally meaning to write with light on stone, is a process which enables the fabrication of endless geometries on substrates such as silicone wafers [7].
The substrate, a dielectric material i.e. silicone or glass, is coated with a thin film of conductive material such as gold through processes such as sputtering or evaporation. These gold coated substrates are then spun coated with a special chemical known as photo-resist. Two forms of photo-resist are widely used, positive-resist and negative resist. They dictate the photolithographic process about to be undertaken, with the main difference being the mask used.

The mask is a template which has the patterns or designs made up of dark and clear regions we wish to transfer to the gold coated layer. Mask structures obtained in the semi-conductor industries have typically had resolutions <100nm, but these are very expensive. The mask allows ultra-violet light to pass through the clear regions and blocks the passage of light in the darker regions. Depending on the type of photo-resist used the ultra-violet light will have an opposite effect on it. In general the use of positive photo-resist, when exposed to ultra-violet light will weaken the chemical bonds allowing it to dissolve in developer solution. The use of negative photo-resist will harden areas exposed to ultra-violet light and dissolve non-exposed areas of resist with developer. The ultra-violet light effectively removes areas of photo-resist that is not wanted, leaving behind a copy of the mask (positive photo-resist) or a reverse copy of the mask (negative photo-resist).

The process of removing the exposed layer beneath the photo-resist is known as etching. Chemical reactions with the layer to be removed are done with specific chemicals known as etchants. The reactions can be done in liquid solvents (wet etching) or with reactive ionised gases (dry etching). Wet etching is an isotropic process and the simpler of the two processes as the reactions can be done in traditional laboratory environments. Dry etching requires more sophisticated equipment capable of bombarding surfaces with reactive plasmas or gases which can be ionised by glow discharge. The process of bombarding the surfaces with these species comes about by their response to an applied electric field. With an applied radiofrequency across the chamber in one direction, the direction of the required etching process, the ions bombard the surface along the electric field lines. Hence dry etching is an anisotropic process and typically used for fabrication of vertical structures, multi-layer coated substrates or very thin coated substrates where the horizontal etching, found in wet etching, is undesirable.
2.4.3 Microelectrode Geometries

The use of microelectrode structures in ac electro-kinetics has proved to be one of the most important and beneficial factors in the advancement of research and development, and the understanding of mechanisms involved in particle manipulation. A vast majority of microelectrode geometries used to date are co-planar electrode arrays, ~110nm thick of gold with a titanium or chromium seed layer on top of a glass substrate. This has been very useful for the direct observation of particles manipulated in ac electric fields through microscopic inspection. In 1988 Price et al used a castellated interdigitated electrode array, fabricated using photolithographic techniques, to obtain the dielectrophoretic behaviour of micro-organisms using optical absorbance measurements [55]. Field strengths in the plane of the electrode array were found to vary between 0.8 MV m\(^{-1}\) and 80kV m\(^{-1}\) between the electrodes. Gascoyne et al showed that the same microelectrode structure could be fabricated and used on printed circuit boards [47]. Many electrode designs have since found their way into laboratories with elaborate and sometimes useful applications being developed. As previously mentioned, the generation of non-uniform electric fields is very important for the manipulation of dielectric particles. This non-uniformity is largely based on the geometry of the electrode structure and the electrical connections associated with the geometry. In the past pin-type or tapered edge electrodes have been used to carry out dielectrophoretic experiments [22, 32, 56-58]. The simple geometry of this electrode type, where the pin tips face each other a fixed distance apart, has meant they need not be fabricated on wafer substrates and can be submerged in a Petri dish suspension. Quantification of positive dielectrophoresis with this geometry type is based on cell counts at the electrode tips over a set period of time. On the other hand particles repelled from the electrode tip have been found more difficult to quantify. Huang and Pethig described a procedure for designing polynomial electrodes exhibiting negative dielectrophoresis by defining analytical expressions for the spatial variations of the factor \(\nabla |\mathbf{E}|^2\) [59]. They showed that yeast cells could be collected in the centre region of the electrodes with well defined geometries which stabilises the particle motion. Hughes and Morgan used the polynomial design to dielectrophoretically trap single sub-micrometer bioparticles [60]. Hydrodynamic forces and high frequency electric fields were used to trap both sub-micrometer and
micrometer particles in a quadrupole field trap [61]. Green *et al* presented a technique for fabricating large area multi-layered electrode arrays for DEP fractionation using travelling wave dielectrophoresis [62]. A combined travelling wave dielectrophoresis and electro-rotation microelectrode device, resembling a spiral, was fabricated and applied to concentrating and viability assaying of *Cryptosporidium parvum* oocysts [63]. Dielectrophoretic manipulation of a single chlorella cell with a dual-microdisk electrode of ultra-fine wires (~1μm radius) was shown to exert both a negative and positive force when the disks were placed side by side [64]. The use of insulating post arrays between two electrodes of ac voltages created non uniform ac fields with high field gradients near and around the insulating posts [65]. More complex geometries have been designed and placed in micro-systems allowing fluid flow to occur. For example Fielder *et al* created a micro-system which had multiple electrical connection pads and allowed the dielectrophoretic sorting of particles through funnelling [66]. Cheng *et al* (Nanogen) fabricated a bioelectronic chip where separation by dielectrophoresis and cell lysis all occurred on a 5 x 5 checkerboard addressed array [67]. This showed great potential for an integrated assaying system. Hoettges *et al* have demonstrated using coplanar interlocking circular electrodes, particle collection on the electrode surface was achieved at low frequencies, with potential applications to biosensor enhancements [68]. A step forward in microelectrode geometries was provided when 3-dimensional microelectrode arrays were fabricated. Suehiro and Pethig used a three dimensional grid system to position biological cells [69]; Müller *et al* created a 3-D microelectrode system for handling and caging single cells and particles [70]; Dürr *et al* created a micro-system with 3-D microelectrode arrays to deflect particles in micro-fluidic channels [71]. These 3-D microelectrode arrays were all fabricated using photolithographic techniques, but it has been shown by Fatoyinbo *et al* that microelectrodes can be fabricated by micromachining bores through laminated conductive and insulating layers [72]. This geometry showed that the magnitude of the electric field penetrating the cylindrical volume was highly dependent on the bore radius and the insulating layer thickness. The creation of a high gradient electric field system has recently been developed, showing that high gradient strength electric field non-uniformities can be created within a polystyrene bead filled column by cylindrical electrodes [73].
2.5 Applications of AC Electro-kinetics to Biological Particles

The use of non-uniform electric fields for separating materials in a useful manner dates back to the nineteenth century when in 1891 Lowden patented a device for removing metal particles from used lubricating oils. In the following 40 years processes in which non-uniform electric fields were used were patented mainly within the petroleum industries for removing large molecules and refining petroleum. In 1943 Pohl was able to demonstrate its usefulness for analyzing suspended pigments, but the phenomenon was only fully explored in a series of papers on non-biological materials. In 1966 Hawk and Pohl described the first biological applications of dielectrophoresis in the separation of live and dead cells. Since then the use of dielectrophoresis has been demonstrated to work on a wide range of biological particles including bacteria, algae, yeast, blood cells, cancerous cells, chloroplasts, mitochondria, viruses, DNA and proteins [15, 24, 31, 32, 35, 56, 57, 74-83].

2.5.1 Bioparticle Characterisation

The frequency dependency of particle movement in non-uniform electric fields has allowed researchers to differentiate between biological particles by obtaining their respective frequency-dependent spectrum. The spectrum of a particle is a 'fingerprint' describing the biophysical properties of that particle. The two main ac electro-kinetic techniques used to obtain the spectrum are electro-rotation and dielectrophoresis.

Dielectrophoresis requires that the ac non-uniform field is anti-phase (180°) with only two electrodes generating the electric field non-uniformity. This allows the translatory motion of particles to or away from the electrode edges. The number of particles collected at the electrode edge over a set period, at different frequencies has long been the basis of obtaining the dielectrophoretic spectrum and dielectric properties of particles using polynomial electrodes. Burt et al used the interdigitated castellated electrode array to perform low-frequency measurements using optical spectrometry on a number of colloidal suspensions [84]. They revealed previously un-reported effects with the microelectrode array that were associated with the particles surface conductivity and surface charge. The dielectrophoretic collection spectra of normal and cancerous cells using the same electrode array were analysed using an automated image analysis system, revealing differences in membrane capacitance and conductance during differentiation [34]. Gascoyne et al detected changes in
erythrocyte membranes following malarial infection using differences in cross-over frequencies, demonstrating the potential use of the different spectra as a tool for cell sorting [78]. Differences in membrane dielectric properties of multiple cultivated cell lines were determined from dielectrophoretic cross-over measurements [85]. The sensitivity of dielectrophoresis has also been used to distinguish between leukaemic cells and its multi-drug resistance (MDR) strain [32]. Due to investigations conducted into the relationship between dielectrophoresis and electro-rotation both techniques have proved useful in particle characterisation [29, 86]. Electro-rotation, a more popular method of ac electro-kinetic particle characterisation among researchers has been used to determine the dielectric properties of yeast cells [36]; peripheral blood mononuclear cells and trophoblasts [31]; liposomes [15]; changes in rat kidney membranes [18]; and changes in human T-lymphocytes following mitogenic stimulation [10]; An automated system has also been developed to capture the real-time rotation of cells [16].

Both techniques have limitations: electro-rotation is a more accurate technique, but involves the precise placement of single cells in the centre of a quadrupole electrode. If not accurately positioned the cells may experience lateral drift towards the electrodes invalidating spinning times. Depending on the electrode geometry the dielectrophoretic spectrum of a population of homogeneous particles can only be quantified for frequencies at which the particles are experiencing positive dielectrophoresis alone and these are for suspensions low in concentration so mutual polarisation effects can be neglected.

2.5.2 Separation and Concentration of Bioparticles

AC electro-kinetics has found numerous applications in micro-systems for suspensions of both heterogeneous and homogeneous populations in fluid flow (continuous or semi-continuous process) and in absence of flow (batch process). Using the interdigitated castellated electrode array introduced by Pethig’s group, dielectrophoretic separation has been shown for viable and non-viable yeast cells [87]; human leukaemic cells have been removed from blood in an electro-affinity column [88]; sub-micrometer particles using both dielectrophoresis and electro-hydrodynamics [11]; and different forms of cancer from blood [35]. The well defined regions of electric field minima and maxima made the electrode array an attractive option for using in particle separation. Talary et al. also used the same electrode array.
to separate and enrich CD34+ cell subpopulations from bone marrow and peripheral blood cells, with a six-fold enrichment [89] and Markx et al showed how bacteria could be separated using a conductivity gradient [90]. A quantitative assessment of micro-fluidic retention and separation using this electrode design has also been carried out [91].

Interdigitated electrode arrays have been used in other separation and concentration processes such as DEP levitation and hyper-layer field flow fractionation of latex beads and red blood cells [81]. Particle motion, segregation, inter-particle polarization and effects of medium electro-rheological properties in micro-fluidic flow have been studied [92-96]. These studies validated theories of dielectrophoretic phenomena of dilute suspensions in micro-fluidic flow.

2.5.3 **AC Electro-kinetic Integrated Micro-bioprocesses**

Combinations of different microelectrode arrays on single chips, which perform different operations, are becoming increasingly popular. These systems are commonly referred to as lab-on-a-chip systems (LOC) or micro-Total Analysis Systems (μ-TAS). AC electro-kinetics has been shown to be an important technique when integrated in to these systems. Although processes such as capillary electrophoresis are finding potential uses in micro-systems [97], precise manipulation of neutrally charged particles to areas on a chip can not be achieved through these processes. For a truly integrated micro-system device various processes on a single chip, including sampling, sample pre-treatment (i.e. filtration, mixing, concentration) and detection need to be fulfilled [98]. The dimensions of systems used are on the scale of microns hence due to scaling laws the process times are scaled too making processes such as mixing a great deal faster. The possibilities of a range of biochemical applications, detection techniques, and other useful processes with ac electro-kinetics have been demonstrated and are highlighted below.

One of the first integrated systems developed with applications to ac electro-kinetics was an optical system measuring the behaviour of micro-organisms to dielectrophoresis through light absorbance [55], and this was extended to take into account variations in motions around the electrode regions [84, 99]. Image analysis techniques were later introduced as form of motion tracker for separating mammalian cells [47], off-line characterisation between healthy and cancerous cells [34] and
automated electro-rotation with real time motion estimation [16]. Hughes and Morgan described how negative dielectrophoresis could be used to measure the thrust of bacterial flagellar [100]. Single cells and particles have been directed and caged in micro-fluidic flow, enabling the possibility of single particle characterisation and filtering down stream [70]. Studies of colloidal particles using evanescent-field techniques allowed dielectrophoretic collection rates to be determined and subsequently the dielectric properties [101]. Brown et al developed a system to differentiate between particles in a suspension and perform particle counts using dielectrophoresis [76]. By the end of the 1990’s, micro-systems employing micro-fluidic flow and ac electro-kinetics were becoming widespread. Nanogen showed the separation and isolation of metastatic human cervical carcinoma cells from blood cells in a chip cartridge with fluid flow using dielectrophoresis [67]. Dielectrophoresis has been used in the registration of thousands of cells on large microelectrode arrays capable of simultaneously trapping and registering thousands of individual cells [102]. In early 2000, Cui et al fabricated an ac electro-kinetic micro-fluidic system integrated with optical fibres to count and measure particle velocities [103]. Monitoring of physiological changes in cells has been achieved with a cell profiling system employing dielectrophoresis [104]. The selective detection of bacteria using dielectrophoretic impedance measurement combined with antigen-antibody reaction was successfully achieved [105]. Dielectrophoresis has been used as an immobilisation tool for latex probe beads in a micro-system, as a precursor for affinity assays [106] and also for micro-patterning monolayers to glass surfaces [107]. More recently microfabricated 3-D plates, known as DEP well-plates, have shown the possibilities of parallelisation of dielectrophoretic characterisation experiments [108].

2.6 Biosensors

Sensors are devices which receive and respond to a signal or a stimulus. It is feasible to look at the human body as having a wide assortment of sensors all linked to a processor (central and peripheral nervous systems). These wide-ranging types of sensors include the mechanoreceptors, thermoreceptors, nociceptors, chemoreceptors and the senses of hearing and sight. These sensors are very advanced in their operation, such that man-made sensors are often modelled on their mode of operation to achieve maximum sensitivity with minimum noise/error.
In 1962, Clark and Lyons adopted the term enzyme electrode which was the first description of a biosensor. In this first enzyme electrode, an oxido-reductase enzyme was held next to a platinum electrode in a membrane sandwich. The platinum anode polarised at +0.6V responded to the peroxide produced by the enzyme reaction with substrate. The primary target substrate for this system was glucose:

\[ \text{glucose} + O_2 \rightarrow \text{glucose oxidase} \rightarrow \text{gluconic acid} + H_2O_2 \]

Equation 2.35

There have since been numerous variations as to the definition of what a biosensor is. Arnold and Meyers (1988) proposed two definition of what they considered a biosensor to be [109]:

1. “a self contained analytical device that responds selectively and reversibly to the concentration or activity of chemical species in biological samples”
2. “an analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting- reversibly and selectively- the concentration or activity of chemical species in any type of sample”

The majority of traditional bio-analytes are based on a photometric method, where the biorecognition reaction is linked to a colorimetric, fluorescent or luminescent indicator molecule. On the other hand, potentiometric pH electrodes and the amperometric Clark type oxygen electrode are available. The unique feature is that it incorporates a biological sensing element in close proximity or integrated with the signal transducer, to give a reagent less sensing system specific for the target analyte. Immobilised in the vicinity of a transducer, minute quantities of biological recognition molecules are needed but reliability of the sensor in the final instance requires:

1. A high degree of specificity
2. A good stability to operating conditions (e.g. temperature, pH, ionic strength, electric field)
3. Retention of biological activity in immobilised state.
4. No undesirable sample contamination.
The advancement of effective dielectrophoretic processes for diagnosing or detection can be enhanced by the integration of suitable sensing devices for the required operation. Possible sensing techniques and their integration with DEP will be discussed in the following sections.

2.6.1 Acoustic Sensors: Piezoelectric Crystals (Quartz Crystal Microbalances)

Piezoelectric crystals, more commonly known as quartz crystal microbalances (QCM) are mass sensitive devices, and have been widely used in a variety of environments for the detection of surface loading of solutes adsorbing unto the crystal surface. Current theoretical foundations for the relation to mass and frequency changes in a piezoelectric QCM is attributed Lord Rayleigh (1885), but a more detailed investigation was performed by Jacques and Pierre Curie (1880). They observed that a pressure exerted on a small piece of quartz caused an electrical potential between deformed surfaces, and that application of a voltage effected its’ physical displacement; the piezoelectric (‘pressure electric’) effect was discovered. Piezoelectric materials are used extensively as electromechanical transducers [110]. Quartz has the smallest piezoelectric coefficient out of the crystalline solids (ammonium dihydrogen phosphate, potassium dihydrogen phosphate and ethylene dihydrogen tartrate), but can still attain mass sensitivities of the order of pg/cm² [111]. Sauerbrey (1959) suggested using a quartz crystal oscillator as a sensing device for measuring the thickness of thin films [112-118]. By the 1960s, frequencies could be measured to a precision of 1 part in \(10^{10}\) and the stability to better than a part per billion could be obtained for weeks. The sensitivity to mass change was in line of becoming outstanding and today these parameters, which are still of great importance, have been developed fully and integrated with other circuitry. Stockbridge and Warner (1961) showed that vacuum bake-outs to 300°C could be tolerated by crystals without electrodes at the active centre. The frequency measurements corresponding to a mass sensitivity of 10pg/cm² were possible and a stability of \(\pm 40\)pg/cm² could be maintained for up to 10 hours. Quartz crystal microbalances have been applied to many areas of science and technology. The most common application is that of measuring the growth of thin films. This has led to numerous other applications in areas including interferometric studies, humidity sensing, chemisorption studies,
corrosion studies of metals films, polymerization of molecules, and detection of specific molecules by surface modified electrodes [119].

2.6.1.1 Properties of QCM

A piezoelectric crystal is a precisely cut slab of quartz from its natural or synthetic crystal of quartz. It can be seen in its perfect form in Figure 2.10a, with its axes assigned. QCMs are thin quartz disks, usually a couple hundred micro-metres thick, coated on both sides with a very thin layer of gold electrodes (~100nm) as can be seen in Figure 2.10b. An application of an external electrical potential to the crystal produces internal mechanical stresses. If the electric potential is time-variant, an oscillating electric field in the crystal induces an acoustic wave which propagates through the crystal and meets minimum impedance when the thickness of the device is a multiple of a half wavelength of the acoustic wave.

Figure 2.11 Modes of crystal operation a) flexural b) extensional c) thickness shear
A crystal is able to operate in three different modes, namely flexural and shear, extensional and twist. The most sensitive is the thickness shear mode (TSM), and is a more accurate description than QCM as the resonant frequency change is a measure of the thin layer mass attached to the crystal surface [120, 121].

The Y-cut family represents the group of crystals which operate in TSM, where the two major surface of the resonator are always anti-nodal. Belonging to the Y-cut family are AT-cut and BT-cut (Figure 2.12), with the thickness being the most important dimension. Only the thickness shear mode can be electrically excited because the coupling factor for the other two modes are zero. This means that there is only one fundamental resonant and only one fundamental anti-resonant piezoelectric frequency. Also due to the high coupling factor of AT-cut crystals, the device to be electrically excited can be done so with relatively low voltages applied to the electrodes. Operating the crystal near room temperatures give the crystal a temperature coefficient of frequency of zero (T = 25°C), making it very stable under changing temperatures.

![Figure 2.12 Representation of crystals cut with respect to their axes [129]](image)

2.6.1.2 Quartz Crystal Theory

The electrical equivalent of an AT-cut quartz crystal in vacuum is shown in Figure 2.13, where C1 is the mechanical elasticity of vibrating body combined; C0 relates to the static parallel capacitance (disk capacitor) formed by the electrodes of the quartz and the substrate as dielectric material; L1 is motional inductance measuring vibrating mass; R1 is total loss of mechanical energy due to internal friction and energy dissipated to surrounding medium and supporting structures. An equation for the
electrical oscillation of the series components (L1, C1, R1) is shown in Equation 2.36 where $q$ is the charge and $V$ is the applied voltage.

$$L_1 \frac{d^2 q}{dt^2} + R_1 \frac{dq}{dt} + \frac{q}{C_1} = V$$

Equation 2.36

Converting the electrical model to a mechanical model by using an electromechanical coupling factor it can be shown that the inductance (L1), capacitance (C1) and resistance (R1) correspond to mass, compliance, and friction factors respectively. The static parallel capacitance (C0) is the only genuine electrical parameter [122].

Figure 2.13 Equivalent electric circuit of an AT-cut crystal in a vacuum

Figure 2.14 Typical observed responses of an excited QCM
By considering the crystals equivalent circuit of Figure 2.13, a series resonant frequency \( f_s \) and a parallel resonant frequency \( f_p \) can be identified. They can be expressed as

\[
f_s = \frac{1}{2\pi \sqrt{LC_1}}, \quad f_p = \frac{1}{2\pi \sqrt{LC^*}} \quad \text{where} \quad C^* = \frac{C_1C_0}{C_1 + C_0}
\]

Equation 2.37

From Figure 2.14, it can be seen that the blank quartz impedance is inductive (phase shift approx. +90°) between \( f_s \) and \( f_p \), but is capacitive (phase shift approx. -90°) outside this range.

The equivalent electrical circuit for a coated crystal in liquid is shown in Figure 2.15, with the altered electrical equivalent parameters depending on the physical properties shown. For non-conductive liquids the parallel capacitance (\( C_1 \)) must be considered,

![Figure 2.15 Equivalent electrical circuit for a coated crystal in liquid](image)

and if totally immersed the dielectric constant of the liquid leads to an increase in parallel capacitance (\( C_0 \)) [123]. Once the crystal is immersed in a liquid the oscillations become strongly damped with the distance between series and parallel resonance decreasing more and more as parallel capacitance increases.

The relation between mass and resonant frequency is considered to be proportional to added mass on the device surface, and has been expressed by the Sauerbrey equation:

\[
\Delta f_s = -\frac{2f_0^2}{\sqrt{\mu_D\rho_Q}} \frac{\Delta m}{A}
\]

Equation 2.38
Other workers have approached the theoretical aspect of QCM operations in liquid and a summary of their mathematical models can be found in [123]. Quartz operating in liquid is much harder than it operating in vacuum, because due to the damping on the crystal of the liquid as previously mentioned, there is a high loss of the quality factor \( Q = \frac{\omega_L}{R_i} \) and a decrease of the phase gradient, meaning in general stability is not as good as in a vacuum. Also, the observed frequency shift is not only caused by mass effects, but by viscous effects, surface roughness, liquid properties (conductivity and permittivity) and instrumentation.

A proposed theoretical relationship of frequency shift of QCM in contact with fluids was put forward by Kanazawa and Gordon [124]. Their model took into consideration the material properties of the quartz and the fluid and could be used for systems where one side of the crystal is in contact with the fluid.

\[
\Delta f = -\sqrt{\frac{\eta}{R_0^3}} \left[ \frac{(\eta \rho)}{\pi\mu Q P_0} \right]
\]

Equation 2.39

The deposition of microelectrode disks on quartz is based on very similar techniques as described in 2.4.2. With the development of suitable electrode geometries, etching protocols, and ancillary equipment, ac electro-kinetic techniques can be used to immobilise biomolecules on the electrode surface. This would create an integrated micro-system of manipulation and detection with the potential of speeding up bioprocessing detection.

### 2.6.2 Optical Sensors: Surface Plasmon Resonance (SPR)

The change in the refractive index of sensing surfaces and its immediate vicinity are the basis of a technique known as surface plasmon resonance (SPR). This highly sensitive detection method is based on the collective excitement of electrons in a metal film on a substrate such as glass, leading to a total absorption of light at a particular angle of incidence [125]. The efficiency of the coupling is angle dependent, hence the plot of reflectivity vs. incident angle of light reveals a dip characteristic of the optical constants of the materials at or very near the interface. Optical biosensors have been used in a number of applications including DNA hybridisations, enzyme
reactions and enzyme-linked antibody-antigen interactions. The technology of SPR has been developed into a commercial biosensor capable of analysing a variety of reactions and processes (Pharmacia Biosensor, BIAcore).

**SPR Theory**

Nylander et al (1982) first suggested the idea of using surface plasmon resonance as a sensor for halothane gas. This led to an increase interest for using SPR for sensing as it was seen to be an ideal sensor. The technique meant there was no need for electronics (passive) and it was non-destructive, it could probe hazardous environments (e.g. high temperatures or pressures), and the specificity could be controlled by the choice of overlayer material. The sensitivity of the technique is considerable too and can be enhanced by the immobilisation of receptor molecules.

When photons are made incident upon an interface of a metal-dielectric, excitation occurs. At that surface an induced resonant charge density oscillation develops, creating a propagating wave. This wave, consisting of perturbation of the almost-free electron plasma is defined as surface plasmon resonance (SPR) or more accurately surface plasmon polariton (SPP). The wave will propagate over distances of approximately a few microns in the visible range, showing exponentially decaying optical E-fields perpendicular to the interface, which penetrate the adjacent media to a distance comparable to the wavelength of the incident light. It is the E-fields which make the behaviour of the SPP so sensitive to its surrounding.

The above diagram shows the behaviour of plane parallel monochromatic radiation incident upon an interface (metal/glass), assuming both media are lossless and nonmagnetic. Part of the light striking the interface at an angle \( \theta \) is transmitted into the next medium, while the remainder of photons are specularly reflected. The refractive indices of the media are related to the angles of the reflected and transmitted light, with the transmission angle \( \phi \) being calculated from Snell’s law,

\[
    n_1 \cdot \sin(\theta) = n_2 \cdot \sin(\phi)
\]

Equation 2.40

where \( n_1 \) and \( n_2 \) are the refractive indices of the media. The permittivities of the media are associated with the refractive indices by the square of their respective indices,
assuming that the media are lossless (not absorbing light). The change in permittivity across the interface causes a change in $E_z$ from one medium to the other (Maxwell-Wagner interfacial polarisation). The light shone at the interface is linearly polarised with its electric field vector ($E$) either parallel or perpendicular to the plane of incident. This is known as p- (TM) or s- (TE) polarized respectively and can be a sum of both cases. At the interface there is a discontinuity of polarization charge meaning for the p-polarised light there is a build up of charge at the boundary. s-polarised light does not have a component of $E_z$ so there is no charge build up at the interface, hence cannot be used to excite surface charge oscillation. The main reason for SPR occurring at a dielectric-metal interface is due to the fact that the technique operates in the surface bound mode, involving charge densities that are trapped at the boundary. The excitation mode requires momentum in excess of the maximum supported by medium one, hence incident light can not be coupled directly to SPP. The extra momentum can be obtained by utilising a prism or by distorting the surface (grating). The overall sensitivity and selectivity of SPR for biosensing is enhanced by coating the metallic layer with biological receptors (e.g. antibodies) for the binding of their receptive molecules (e.g. antigens). Figure 2.16 shows the SPR system developed by BIAcore.

![Optical detection Sensorgram](image)

Figure 2.16 SPR system developed by BIAcore for commercial use in industry and academia

It is known that a majority of bioparticles have antigenic receptors on their surfaces, hence surface modification techniques such as self assembling monolayers (SAM) and Langmuir-Blodgett films of immobilised surfaces (e.g. latex beads, silicon
substrates) have allowed particles to be captured in flow through regimes were particle-particle interaction or trapping occur through mass transfer, sedimentation, concentration gradients or some other physical process. The use of dielectrophoresis for biological manipulation of cells, viruses and proteins can be beneficial with regards to immuno-sensors. With careful surface modification techniques employed and appropriate microelectrode geometries designed, particles with antigenic moieties can be directed to areas where receptor molecules are immobilised. After particle trapping through dielectrophoresis, whether it is flow conditions or batch conditions, reversal of the trapping process through frequency change or reintroduction of flow can release particles which did not interact with the immobilised molecules. With sensor technologies such as SPR or any other optical method, the integration of dielectrophoretic techniques has the potential to enhance current immuno-sensing processes with minimal costs. Also, with the advancement of micro-dispensing systems and miniaturisation of photo-optic equipment, this has serious potential application for rapid diagnostic tools in the primary health care environment.

2.7 Conclusions

Micro-engineering procedures have contributed significantly to the development of micro-devices and micro-systems which have tremendous benefits to research advancement in biotechnology and medicine. In particular, microelectrodes have allowed greater steps in ac electro-kinetics research, with demonstrations of its useful applications in manipulation of biological particles. Dielectric theories developed over a century ago and continually validated have enabled ac electro-kinetic techniques to be used to characterise a vast range of biological particles, differentiating between similar particle types with the potential for separating, detecting and diagnosing diseases.

Design of suitable microelectrode geometries is an important factor for development of useful μ-bioprocesses and integration of sensing devices with ac electro-kinetic micro-systems. This offers the potential of developing automated systems for particle characterisation, development of point-of-care devices for primary healthcare and micro-fluidic processes for particulate concentration/separation with a final detection or quantification step.
2.8 References


Chapter 3

Evaluation of a Dot Micro-system for Rapid Dielectrophoretic Characterisation of Biological Particles using Digital Image Processing Techniques

3.1 Introduction

Dielectrophoresis (DEP) has been used to characterise the dielectric properties of a number of biological particles [1-5]. A suspension of homogeneous or heterogeneous particles is subjected to a dielectrophoretic force which creates lateral movement of the particles towards or away from the microelectrodes creating the non-uniform electric field. A number of methods determining the dielectrophoretic spectrum of a particle have been based on a microscopy technique developed by Pohl [6]. These methods are laborious and time consuming, as the individual is required to continually observe particle motion over a set length of time collecting at the electrode edge, for a set of frequency points. Methods whereby photographs of the run experiments are taken at intervals and analysed later have been employed. Unfortunately, there were no concrete methods to quantify the effects of the electric fields repelling particles from the electrode edges.

Owing to problems associated with the interpretation of DEP collection data, electro-rotation (ROT) became the more favoured method determining the dielectric properties of cells [7-10]. It is known that ROT and DEP have a strong inter-relationship from analysis of their respective spectra [11-13]. A drawback associated with ROT is the characterisation of a single particle in a rotating electric field. This requires that the particle of interest is accurately positioned in the middle of the polynomial electrode geometry which can be difficult to attain. If not positioned accurately there is a strong possibility of the particle experiencing lateral drift [14]. Also, as the spectrum for ROT is obtained by counting the revolutions of a particle over a pre-determined period, the need for real time processing or post-experimental processing by an individual is required. This can contribute to extra time needed in determining a particle's dielectric properties with the possible introduction of errors.
due to human interference or inadequate computing processing memory needed for a more robust image processing methodology.

Dielectrophoresis has a range of advantages over traditional particle characterisation methods such as flow cytometry and electrophoresis. There is no need for expensive reagents, the fabrication of electrode geometries is cheap and there is less scope for erroneous results due to the labelling of particles which may alter their biophysical make-up [4, 15]. Other techniques like image analysis and fluorescent labelling have been used to study the behaviour of biological particles under the influence of inhomogeneous fields [16-19].

One of the principal disadvantages of DEP for the acquisition of biophysical properties is the difficulty in obtaining spectrum information automatically. An optical technique employing dielectrophoresis was used with castellated microelectrodes to study the behaviour of micro-organisms as a function magnitude and frequency of the applied electric field [20]. The response of particles to low frequency electric fields were also studied using an optical method, with the aim of taking into account the influences of electrode polarisation effects [21]. Talary and Pethig found that using an interdigitated castellated microelectrode geometry, cells were being collected at the electrodes under both positive and negative dielectrophoresis, prompting them to create an optical system for measuring both negative and positive dielectrophoresis using a dual beam laser [22]. However this system used lasers shining through the entire inter-electrode space, allowing only one measurement to be taken at a time. A need still exists for an electrode geometry allowing rapid determination of polarisability for many samples simultaneously.

It is important to take into consideration the electrode geometry when performing any dielectrophoretic process, including characterisation. In this chapter a microelectrode system is described to tackle the problems associated with determining the different magnitudes of the Clausius-Mossotti factor using dielectrophoresis on a homogeneous suspension of particles. The design consists of parallel electrode plates facing each other, so particles are suspended between them. The bottom electrode creating the required inhomogeneous electric fields, using an array of circular apertures we have termed ‘dots’. In addition to the characterisation efficiency of the dot array, the array has a larger surface area providing an electrical transmission line which does not attenuate the signal easily, giving a voltage of similar potential ($V_0$) at the edges of the
all dots in an array. This reduces the inconsistencies associated with supply voltages across the microelectrode.

The dot micro-system consists of micro-arrays of circular apertures etched through a thin layer of conductive material such as gold (Au), indium tin oxide (ITO) or platinum (Pt), deposited on a transparent dielectric substrate such as a microscopic glass slide. The etched circles reveal a path through which visible light or any other light source can pass through so that suspension density can be detected via some form of photo/optical detector. A counter electrode situated a distance away from the dot electrode is used to contain the dispersion of dielectric particles in low conductive media. The counter electrode of preference used is a thin film of indium tin oxide (ITO), a transparent semiconductor material deposited on a glass substrate. Indium oxide ($\text{In}_2\text{O}_3$) doped with tin is reported as having a decreased electrical resistivity [23, 24]. In addition, the optically transparent properties of ITO thin films commonly used for optoelectronic devices makes this material a suitable choice for the visualisation of particle movement under an inhomogeneous electric field. Cadmium stannate (CTO) is a possible alternative material which could be used as a counter electrode as it possesses properties comparable to ITO as first described by Nozik [25].

![Figure 3.1 Representation of an array of 'dots' on a gold electrode](image-url)
Figure 3.1 shows a 3D representation of a section of the dot array which makes up the bottom part of dot micro-system. An electrical connection is made at any point near the bottom of the dot electrode via wire bonding. The counter electrode is an ITO coated microscope slide cut to size so it is situated over the dot micro-array. The electrical connection is made at the corner so both electrodes are as close as possible to each other. The limiting factor associated with the distance of the electrodes from each other should be based solely on the spacer placed between the electrodes. The spacer should ideally be no more than 300µm in thickness and be electrically insulating so not to short the system.

3.2 Materials and Methods

3.2.1 Microelectrode Fabrication
Photolithographic processes were used to fabricate the microelectrode structures after they had been designed on a CorelDraw Select Edition package. This package is inexpensive compared to traditional CAD packages used to design masks where resolutions down to a couple of microns and sub-micron structures are needed. However, a process described by Hoettges et al [26] utilises office printers and photographic reduction for mask production capable of obtaining feature resolutions as low as 25µm. This procedure is low in cost for mask design and the masks can be prepared within 24 hours of design conception.

CorelDraw was used to design various electrode dot diameters ranging from 150µm to 500µm. The electrode geometries were created within a 192x288 mm frame on the software page. This was equivalent to exactly 8-times the size of a single film exposure (24mmx36mm). The dimensions used in drawing the electrode arrays on the software were in millimetres and 8-times the final desired size.

The designs were printed off on an office printer (HP Laser Jet 1200), with the black and white sections of the print representing the glass and gold sections respectively.

Print reduction was carried out in the Micro-engineering Laboratory (Duke of Kent Building), with a SLR camera (Olympus-OM2) and stand for the camera and the required chemicals needed to develop black and white film. The black and white film used was Kodak Technical Pan (TP 135-36; Jessops) as it is able to achieve a high contrast over other films, critical for mask production. To set the exposure, a page was printed with 25% greyscale pattern and metered automatically by the camera. The
designs were exposed to the same settings. Once the roll of film was finished the film was developed within the laboratory.

Microscopic slides were sputtered with gold (100nm thickness) at the University of Sheffield, Electronic Engineering Department (courtesy of EPSRC). A seed layer of titanium (10nm) was deposited on the slide before sputtering for improved adhesion of the gold to the slide. The gold (Au) coated slides, cut to 36×25 mm, were cleaned in an ultrasonic bath for 1 hour and submerged in sodium hydroxide overnight then washed with 80% acetone and distilled water three times each. They were then completely dried using an Ultra-jet duster (RS, UK) before use.

Clean Au coated slides were placed on the chuck of the photo-resist spinner (Headway) situated in our Micro-engineering Laboratory. Three-fourths of the surface was covered with positive photo resist (Microposit S1800), and spun at 5000rpm for 60secs. Suppliers have indicated the average coat thickness being 1.2nm at those conditions. After spinning, the slides were soft-baked (115°C) on a hotplate for a further 60secs and stored in a dark area for use within 24hours. The developed film (mask) with the required geometry and the surface of the UV light-box were cleaned with an air duster to minimise defects in the irradiation process. The positive photo-resist coated Au substrates were also air sprayed to remove any dust particles and aligned with the mask in the UV light-box. The photo-resist coated Au slides were exposed to near-UV light, 436nm (recommended spectral output range of 350nm-450nm for the photo-resist) through the transparent regions of the mask for 50 seconds. After exposure, the substrates were immersed in diluted developer (3:1) for approximately 10-15 seconds. Then the substrates were rinsed with water and hard-baked in the oven (Memmet) at 90°C for 45 minutes.

Gold etchant (KI (10% w/w), I₂ (2.5% w/w)) was poured into a Petri dish and the UV irradiated Au slides were completely submerged in the etchant for 2 minutes. The slides were removed from the gold etchant and dropped in sodium thiosulphate (Na₂S₂O₃) solution then rinsed in with distilled water. Due to the presence of the Ti seed layer, hot diluted hydrochloric acid was used to etch away the exposed titanium seed layer then rinsed with distilled water. After rinsing the slides with distilled water the layer of photo-resist still present was removed with photo-resist stripper and thoroughly washed with distilled water and dried with an air duster.
3.2.2 Micro-system and Experimental Set-up

Prototyping strip boards of pitch 2.54mm were used to mount the patterned gold slides. A window was sawn out of the board large enough for the area of interest on the slide to be visible from beneath, and then mounted using a heat curing or UV curing epoxy (e.g. Araldite). Half of a dual-in-line (DIL) plug was soldered onto an end of the board providing a connection to a function generator via a coaxial cable made with the other half of the DIL plug. An ac signal from the function generator to the micro-system, passed through a high pass filter enabling high end frequencies to pass with minimal attenuation, via an insulated copper runner attached to the dot electrode with silver epoxy. The coaxial cable had a ground wire attached to a separate pin on the DIL plug which was run through soldered wires on the strip board and attached to the ITO electrode at the appropriate site. All electrical connections were tested using a digital multi-meter (Precision Gold, PG 017) to ensure no short or open circuits were present. Figure 3.2 shows a dot array mounted on a strip board. The top and bottom electrodes were separated by heat treated parafilm 125 μm in thickness. The dashed circle (A) shows the region where the dot arrays are situated.

The complete micro-system was placed in a purpose built micro-system holder easily adjoined on to a Nikon Eclipse 400 microscope platform allowing easy handling and more importantly stability. Insulating clamps on the side of the holder were designed to provide a downward pressure on the ITO slide, bringing the electrodes as close as possible to each other and keeping them at a constant distance and position. The objective lens of the microscope was focussed in the plane of the gold electrode and using phase contrast microscopy movements displayed by the particles in the suspension were easily monitored. The microscope was connected to a CCD (charge-coupled device) camera (Photonic Science, Cambridge), a PC, a video recorder and television monitor, allowing experiments to be visualised on screen with simultaneous recording. Image acquisition software (PhotoLite) on the PC was used to capture images as seen on the television monitor and saved automatically or manually as a tagged image file format (TIFF) on the PC for subsequent image analysis.
3.2.3 Biological Particles

Yeast pellets (Allinson Dried Yeast) were cultured in sterile YPD media (10ml) and incubated at 30°C for periods of 18-24 hours. The cells were then re-suspended in distilled water centrifuged and washed (×3) before being finally re-suspended in appropriate conductivity medium. Cell counts were performed on a haemocytometer. Suspensions of different cell concentrations were made by re-suspending calculated aliquots from the stock solution into the appropriate media solution.

Red blood cells (Lorne Laboratories, UK) type ‘O’ were made up to a concentration of ~10^8 cells per ml and re-suspended in a medium conductivity of 3 mSm⁻¹ potassium chloride (KCl).

3.2.4 Optimising Particle Concentration and Redistribution against Dot Size

Yeast cells with an average size of approximately 5±1 μm in diameter were used at different concentrations suspended in 1mSm⁻¹ potassium chloride solution (KCl) to evaluate and characterise the behaviour of particles in different dot sizes. Suspensions of yeast cells in distilled water were pipetted (5μl) on to a dot micro-array quadrant, enclosed by a circular parafilm spacer, 4cm in diameter, and covered with the ITO counter electrode. Once securely clamped down in the system holder, the microscope objective was focussed into the plane of the gold electrode, enabling the cells to be visualised within a single dot; for a 150μm dot the objective lens was ×20, while all other dot sizes used a ×10 objective lens. The signal generator (TG120 20MHz function generator) was subsequently switched on, with a 10V_pp, 100 kHz sinusoidal signal applied to the system, which was slowly ramped up to 20MHz, the upper limit of the signal generator. Observation of the cells behaviour with varying the applied frequency experimentally enabled us to characterise the field distribution within this
novel microelectrode system. On satisfactorily being able to describe the behaviour of the cells within the dot geometry, 5µl aliquots of yeast cells from stock concentrations of 3.1x10^6, 7.8x10^7 and 1.05x10^8 cells per ml were examined on dot diameters of 150µm, 200µm, 300µm and 500µm. This allowed an investigation into how concentration effects influence particle behaviour in the varying dot diameters. On applying a 6Vpp, 10 kHz and 1 MHz signal to the electrodes, a stopwatch was started simultaneously and snaps of the dots were taken after 1 minute, 2 minutes and 5 minutes had elapsed.

Optimal concentrations were found for spontaneous particle redistribution across the dot micro-array on removal of the electric field. Quantification of the times taken for redistribution carried out over a 1 minute period for the 150µm, 300µm and 500µm dots were also deduced. Their respective concentrations (5.13x10^8, 2.4x10^9 and 1.4x10^10 cells/ml for the 150µm, 300µm and 500µm dots respectively) of yeast cells were suspended in potassium chloride with a conductivity of 5.09mS/m (Jenway 470 portable conductivity meter, Barloworld Scientific, ±0.5%, ±2 digits). 12 experiments (6 for positive DEP and 6 for negative DEP) were performed on each dot with images captured every second. A 20Vpp signal was applied to each microarray with a frequency of 5MHz and 100 kHz for an initial positive and negative dielectrophoretic force respectively. The forces experienced by the particles were kept to 7 seconds (± 2 seconds) before the signal was switched off and particles began to spontaneously redistribute. For each set of dot experiments, the cells were withdrawn from the same stock solution so as to keep the errors in concentration gradients to a minimum.

3.2.5 Dielectrophoretic Characterisation of Biological Cells

Yeast (suspended in 5mSm^-1 KCl, 280mM mannitol and in distilled water) and red blood cells (RBC; suspended in 3mSm^-1 KCl) having a concentration range of 5±3x10^8 cells/ml were used on an array of dots 150µm in diameter. 20µl aliquots of cells in suspending medium were pipetted onto the array and covered with the ITO counter electrode. The objective lens (x20) of the microscope (Nikon Eclipse 400) was focussed into the plane of the cells, such that the disperse system of cells was clearly visible within a single dot. A 10 V.pp AC signal was applied to the to the system for different lengths of time, 20, 10 and 5 seconds, over a frequency range of 1kHz - 10 MHz at 5 points per decade. Without interacting with or disturbing the
system so as to make the conditions constant for the duration of the experiment, images were taken immediately before a signal was applied and immediately before the signal was turned off for each frequency point. Yeast cells with concentrations ranging between $2.4\pm1 \times 10^9$ and $1.4\pm1 \times 10^{10}$ cells/ml were used on the array of dots 300µm and 500µm in diameter. An x10 objective was used for visualising single dots. Conductivities of 1.1, 5.09 and 41.7 mSm$^{-1}$ were used to obtain the dielectrophoretic spectra of the cells from a starting frequency of 1 kHz to an ending frequency of 10MHz at 5 points per decade. At each frequency point for the 500µm dot the signal remained on for 2 minutes with images captured every 2 seconds. At each frequency point for the 300µm dot the signal remained on for 1 minute with images captured every second.

### 3.3 Experimental Results: Phenomenology

**Optimisation of Dot Micro-system vs. Particle Concentration**

Figure 3.3 shows a single 500µm diameter dot with a suspension of the order of $10^8$ yeast cells per ml dispersed between the top and bottom electrodes. Figure 3.3(a) shows the cells in a randomly distributed order prior to an external field being applied. Figure 3.3(b) shows the characteristic alignment of the spherical cells under the influence of an external field and frequency at which the cells experience positive dielectrophoresis. Figure 3.3(c) shows the aggregation of the cells at the centre of the dot under the influence of a frequency where they are experiencing negative dielectrophoresis. It can also been seen that cells are still visible near the electrode edge.

Table 3.1 and Table 3.2 show data obtained at 10 kHz and 1 MHz for cell concentrations of $3.1 \times 10^6$ and $7.8 \times 10^7$ cells per ml against the different dot sizes. It shows how particle concentrations influence the speed in which a particle travelled towards or away from the electrode edge when situated either at the electrode edge or at the centre of the dot region (see Figure 3.4) for different dot sizes. For the concentrations used, cells were clearly visible allowing direct observation of single particles. Tracking a single particle’s movement in the electric field, under the mentioned conditions, allowed an estimate of the average particle speed in directions for +DEP and −DEP. Hence it was calculated that the clearance ratio, defined as the amount of particles removed from the dot region after a period of time under +DEP,
increased as the cell concentration increased together with a decrease in dot size. As the concentration was increased to $1.05 \times 10^8$ cells per ml, the ability to accurately discriminate between two adjacent cells became increasingly difficult as can be seen from Figure 3.3(a-c).

A single dot with a diameter of 150µm and a 5µl aliquot of yeast cells, with no external applied field, taken from a stock solution with a concentration in the range of $10^8$ cells per ml can be seen in Figure 3.5(a). Figure 3.5b-e shows the same cell suspension experiencing positive dielectrophoresis (Figure 3.5b); on removing the applied electric field the cells are in the process of spontaneously redistributing over the dot aperture (Figure 3.5c).

![Figure 3.3 Single 500µm dot with yeast cells (a) experiencing no external field; (b) experiencing positive DEP; (c) experiencing negative DEP](image)

<table>
<thead>
<tr>
<th>Dot radius</th>
<th>Area</th>
<th>No. of cells before signal applied</th>
<th>No. cells alive 1 minute</th>
<th>After 2 minutes</th>
<th>After 5 minutes</th>
<th>% of cells cleared in 1 min</th>
<th>% of cells cleared in 5 mins (±DEP)</th>
<th>from radius in mins (±DEP)</th>
<th>to radius in mins (±DEP)</th>
<th>Average speed from radius to radius (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.50E-05</td>
<td>1.77E-08</td>
<td>25</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>68.0</td>
<td>100.0</td>
<td>1.33</td>
<td>2.15</td>
<td>9.40E-07</td>
</tr>
<tr>
<td>1.00E-04</td>
<td>3.14E-08</td>
<td>39</td>
<td>17</td>
<td>8</td>
<td>6</td>
<td>56.4</td>
<td>79.5</td>
<td>3.15</td>
<td>8.05</td>
<td>5.29E-07</td>
</tr>
<tr>
<td>1.50E-04</td>
<td>7.07E-08</td>
<td>63</td>
<td>24</td>
<td>13</td>
<td>10</td>
<td>61.9</td>
<td>79.4</td>
<td>4.43</td>
<td>16.5</td>
<td>5.64E-07</td>
</tr>
<tr>
<td>2.50E-04</td>
<td>1.96E-07</td>
<td>134</td>
<td>53</td>
<td>31</td>
<td>24</td>
<td>60.4</td>
<td>76.9</td>
<td>8.34</td>
<td>22.35</td>
<td>5.00E-07</td>
</tr>
</tbody>
</table>

Table 3.1 Data obtained for yeast cells with a concentration of $3.1 \times 10^6$ cells per ml
Table 3.2 Data obtained for yeast cells with a concentration of $7.8 \times 10^7$ cells per ml

<table>
<thead>
<tr>
<th>Dot radius</th>
<th>Area</th>
<th>No. of cells before signal applied</th>
<th>No. of cells after 1 minute</th>
<th>After 2 minutes</th>
<th>After 5 minutes</th>
<th>% of cells cleared in 1min</th>
<th>% of cells cleared in 2min</th>
<th>from radius in mins (+DEP)</th>
<th>to radius in mins (-DEP)</th>
<th>Average speed from radius (+DEP)</th>
<th>Average speed from radius (-DEP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.50E-05</td>
<td>1.77E-08</td>
<td>48</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>89.6</td>
<td>100.0</td>
<td>0.44</td>
<td>2.01</td>
<td>2.84E-06</td>
<td>6.21E-07</td>
</tr>
<tr>
<td>1.00E-04</td>
<td>3.14E-08</td>
<td>71</td>
<td>24</td>
<td>11</td>
<td>7</td>
<td>66.2</td>
<td>84.5</td>
<td>2.37</td>
<td>6.29</td>
<td>7.01E-07</td>
<td>2.64E-07</td>
</tr>
<tr>
<td>1.50E-04</td>
<td>7.07E-08</td>
<td>97</td>
<td>34</td>
<td>8</td>
<td>7</td>
<td>64.9</td>
<td>91.8</td>
<td>4.02</td>
<td>13.12</td>
<td>6.22E-07</td>
<td>1.90E-07</td>
</tr>
<tr>
<td>2.50E-04</td>
<td>1.96E-07</td>
<td>254</td>
<td>79</td>
<td>33</td>
<td>22</td>
<td>68.9</td>
<td>87.0</td>
<td>9.41</td>
<td>23.4</td>
<td>4.43E-07</td>
<td>1.78E-07</td>
</tr>
</tbody>
</table>

Figure 3.4 Particle velocity as a function of DEP force and dot size with experimental parameters of voltage = 6$V_{pp}$, frequency = 10 kHz (+DEP), 1MHz (-DEP) and $\sigma_m = 0.2 \text{mSm}^{-1}$

The redistributed cells were observed to acquire the same sort of dispersion as seen prior to the application of an external field within 10 seconds (Figure 3.5d). On applying a 10MHz frequency negative dielectrophoresis was observed, characterised by the distinct darker region formed at the centre of the dot in Figure 3.5e. On removing the external electric field, the cells were seen to redistribute outwards from the centre of the dot to a state similar as in Figure 3.5d in the same characteristic time as previously observed.
Concentration Dependency on Dot Size

Yeast particles were used to determine how accurately and rapidly the dielectrophoretic spectra of a homogenous suspension of cells could be obtained. Results of experiments carried out using the 500μm, 300μm and 150μm dot arrays showed that over a micro-array, 3.14μl in volume for each quadrant, a 20μl aliquot of cells experiencing a dielectrophoretic force could spontaneously and randomly redistribute themselves over the micro-array upon removal of the electric field, which was similar to the cell distribution observed before the electric field was applied to the array. This observation was found to be concentration dependent. Cell concentrations of ~6×10^8, ~3×10^9 and ~1×10^10 cells per ml were used as stock solutions for obtaining the 20μl aliquots deposited on the 150μm, 300μm and 500μm dot micro-arrays respectively. Taking the radius of a yeast cell to range between 2 and 3μm, the volume (V_{yeast}) is found to be:
the amount of cells able to occupy the micro-array (quadrant) volume was calculated to range between $3 \pm 1.5 \times 10^9$ cells. Volume fractions based on the amount of cells in the micro-array taken from each stock solution is shown in Table 3.3. It was calculated that the fraction of cells occupying a single dot ranged between 0.11% and 0.55% for all dots. Observation of the redistribution times indicated that there was a significant difference in times particles took to disperse over the dot electrode upon removal of the electric field. The re-dispersion times were seen to be a factor of both dot size and particle concentration.

<table>
<thead>
<tr>
<th>Dot size (μm)</th>
<th>Single Dot Volume ($m^3$)</th>
<th>Aliquot concentration (cells per 20μl)</th>
<th>Number of cells able to occupy a dot</th>
<th>Volume in % occupied by cells over whole micro-array</th>
</tr>
</thead>
<tbody>
<tr>
<td>150μm</td>
<td>$2.2 \times 10^{-12}$</td>
<td>$1.2 \times 10^7$</td>
<td>$42,570 \pm 23,000$</td>
<td>$0.54 \pm 0.25$</td>
</tr>
<tr>
<td>300μm</td>
<td>$8.8 \times 10^{-12}$</td>
<td>$6.8 \times 10^7$</td>
<td>$170,439 \pm 91,100$</td>
<td>$3 \pm 1.5$</td>
</tr>
<tr>
<td>500μm</td>
<td>$2.5 \times 10^{-11}$</td>
<td>$2.0 \times 10^8$</td>
<td>$483,753 \pm 262,500$</td>
<td>$11 \pm 7$</td>
</tr>
</tbody>
</table>

Table 3.3 Comparison of dot size and number of yeast particles occupying the microarray based on the cellular concentrations used for spontaneous redistribution

Estimations of particle re-dispersion times from experiments performed suggest that for dot sizes of smaller diameters i.e. 150μm, a concentration at the upper end of the Beer Lambert region, $\sim 10^8$ per ml, gave the most rapid response, which could be interpreted in terms of optical transmission.

3.4 Data Processing and Simulation Methodologies

3.4.1 Image processing of Obtained Data

The acquired images, captured over time for each frequency point, were imported into MATLAB 7.0 and with aid of the Image Processing Toolbox (IPT), algorithms were
written to analyse the images. The images were first read into the MATLAB workspace as a 3-D matrix. The values were of class unsigned 8-bit integers (uint8) corresponding to the intensity of each pixel \( P \) in a 2-D matrix, ranging between \( 0 \leq P \leq 255 \). To decrease associated errors with subsequent processing of the images a threshold value corresponding to the edges of the gold electrode was used to find the cardinal perimeter co-ordinates. The cardinal co-ordinates were then used to find the centre co-ordinate of the dot. As all the experiments were conducted with stringently controlled conditions, these co-ordinates need only be found for the first image per frequency or group of images and applied to subsequent images of their respective frequency or group of images. A script was written to ensure all first images had similar pixel radii. If any of the radii of the first images did not fall within a certain pixel range then any image out of that range was deemed to have an area significantly different from the rest. To overcome that, the images falling out of the pixel range were automatically brought on to the screen for interactive selection of the cardinal points, and subsequent calculation of the proper radius value. This procedure dramatically reduced errors in subsequent regional analysis and calculations.

3.4.2 Compartamental Analysis of Dot Regions

From pre-analytical observations of particle movement within the dots, it was deduced that there were 2 distinct compartamental regions where useful information could be obtained. These regions are

- **Region 1**: The **centre region** where the centre co-ordinate of the dot is located.
- **Region 2**: The **outer region** close to the perimeter of the dot, where the electrode edge is located.

It was proposed that for negative dielectrophoresis to occur, the centre region would get darker in pixel intensity i.e. the cumulative pixel intensity value of the after image \( I_A \) in the centre region would decrease compared with that of the reference image \( I_B \) intensity. The opposite is expected for positive dielectrophoresis in the centre region, that is, the cumulative pixel intensity value for \( I_A \) will increase compared to \( I_B \). The outer region of the dot will display differing effects. As positive dielectrophoresis is occurring cells will be pushed to the electrode edge where the field gradient is highest, hence \( I_A \) will decrease in cumulative pixel value compared to
For negative dielectrophoresis, analysis of the image at the outer region should show the opposite effect.

### 3.4.3 Finite Element Modelling (FEM)

Using commercial finite element software (FEMLAB 3.1) the electrode system was modelled in 2 dimensions (cross-section) for single dot diameters of 150μm, 250μm, 350μm and 500μm. The spacing between the top and bottom electrode was given a constant dimension of 128μm. The bottom electrodes of the model represents the gold electrode surface encircling the dot, and expands to half the diameter of the dot/aperture region in both directions, i.e. for a dot diameter of 150μm, the bottom electrodes either side of the dot region will span 75μm in length. The thicknesses of the electrodes were 2μm. With the Electromagnetic Module- Electrostatics of FEMLAB, the electric field distribution in the electrode system was obtained. The electric potential, under static conditions is defined by Equation 2.6. Combining this with constitutive equation relating the dielectric induction (D) to the electric field (E), and the equation of continuity, Gauss’s law can be represented by Poisson’s.

\[
-\nabla \cdot (\varepsilon_0 \nabla V - P) = \rho
\]

**Equation 3.2**

Where \( P \) is the electric polarisation, \( \varepsilon_0 \) is the permittivity of free space and \( \rho \) is the space charge density. Material properties defined for the model were the relative permittivity and conductivity which were taken to be isotropic (see Table 3.4). The boundary conditions for the bottom and top electrodes were defined as the 10V and -10V respectively. The boundary conditions of the medium in contact with the glass substrate and the side of the model where defined by their surface charge equivalent to zero.

<table>
<thead>
<tr>
<th>Material type</th>
<th>Conductivity ( \Omega^{-1} m )</th>
<th>Relative Permittivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>45454545</td>
<td>1</td>
</tr>
<tr>
<td>ITO</td>
<td>10000000</td>
<td>10</td>
</tr>
<tr>
<td>Medium</td>
<td>5e-3</td>
<td>78</td>
</tr>
</tbody>
</table>

**Table 3.4 Material properties used in the finite element modelling**
3.5 Image Processing Results

3.5.1 Development of Graphical User Interfaces (GUI)

Using MATLAB’s Graphical User Interface Development Environment (GUIDE), a user interface for relatively quick and easy analysis of large image data was developed. By incorporating developed scripts and functions with other MATLAB and MATLAB Image Processing Toolbox (IPT) functions a user friendly interface was created allowing large image data files to be read into the MATLAB workspace and data manipulation of each image. For obtaining dielectrophoretic spectra, the user is required to choose the analysis type from the menu bar and enter the file extension, start and end frequencies, points per decade, number of images per frequency and the duration of the applied electric field per frequency. The user is then prompted to enter the filename. The script using MATLAB’s imread function locates the files in the current directory folder and stores all the images as a 3-D matrix in a structure array G, with a name G.image. The frequency points are also calculated and stored in the same structure array, named G.frequency. A process flow diagram of the GUI button, ‘Load Images’, is shown in Figure 3.8. The inputted data and calculated data, along with the field name G.perim (radius and centre coordinates) can be saved as a ‘.mat’ file from the save option on the menu bar. This allows for the data to be loaded directly into the workspace at a later date. Typically each image file is 432 kilobytes with an image resolution of 576x768 pixels. The process flow diagram for the image analysis and data processing GUI is shown in Figure 3.9.

After loading the images, binary masks are created for each first image by entering the number of concentric circles or square regions needed. A range of options are available to view the images i.e. montages, movie clip or single images. After creating the masks, the images are processed by choosing the analysis type required. The cumulative modal intensity shift was chosen as the preferred method as is was able to manipulate the image pixel values rapidly for a large amount of images, sometimes in excess of 1500 images for one experiment. The dielectrophoretic spectra were plotted on the GUI axes present, along with any other type of plot or image. The Dielectric Properties GUI (Figure 3.7) was created to determine the biophysical properties of particles and crossover frequencies using the images. Data from the image analysis GUI i.e. shift in greyscale intensity for specific region and frequency points were imported into the GUI.
Figure 3.6 Image Analysis and Image Processing Graphical User Interface for redistribution analysis and Dielectrophoretic Spectra determination

Figure 3.7 Graphical User Interface for determining the dielectric properties and crossover frequencies of experimental and theoretical DEP spectra
Depending on the particle type, a number of options for determining the biophysical properties are available including number of shells and the geometry of the particle. A database for particle types was created to add/store initial properties for easy access.

LOAD IMAGES GUI BUTTON

Figure 3.8 GUI 'load image' button process flow diagram
PROCESS FLOW DIAGRAM OF IMAGE ANALYSIS GUI

Figure 3.9 Process flow diagram for Image analysis and Image processing GUI
3.5.2 Cumulative Modal Intensity Shift (CMIS)

An image may be continuous with respect to the x- and y- coordinates, and also in amplitude. An image converted to digital form requires that the coordinates and the amplitudes be digitized. Digitizing the coordinate values is known as sampling, and digitizing the amplitude is known as quantization. Hence when x, y and amplitude values of g, where g is an image, are all finite, discrete quantities the image is a digital image. Assuming g(x, y) is sampled, we are able to obtain a matrix of size M-by-N i.e. row-by-column, with the origin of g defined as being (0, 0) and the next coordinate being (0, 1), that is the second sample along the first row. The notation used in image processing textbooks for digital images instead of x and y are r and c denoting the rows and columns, with the origin of the coordinate system being (1, 1). These are more commonly known as image elements, picture elements, pixels or pel [27].

The value of each pixel for a digital image is represented by a discrete value representing the amplitude. The value of the amplitude is dependent on the colour spectrum used i.e. RGB, greyscale e.t.c. The amplitude, also known as the intensity value can be visualised by the image’s histogram. In a histogram of a digital image in the range [0, G] is defined as a discrete function

\[ h(r_k) = n_k \]

Equation 3.3

Where \( r_k \) is the kth intensity level in interval [0, G] and \( n_k \) is the number of pixels in the image whose intensity level is \( r_k \). For a greyscale image of class unsigned integer-bit, the minimum intensity value corresponding to black is zero, while the maximum intensity value is 255 corresponding to white. If a smaller range of intensity levels is used the maximum value will still be white and the minimum value black, with all other values between scaled accordingly. Hence it is possible to see the ratio of pixels in an image which have a particular intensity value, or to see how many pixels of an image fall within specific intensity intervals. This is commonly known as binning the pixels into intensity levels.

The two main compartmental regions analysed for each individual image acquired are the outer region and the central region of the dot. Histograms of these regions were analysed by scripts written, which took the greyscale intensity values of the pixels and
binned similar values in the same group. Hence for any image with a specific compartment being analysed, the number of bins can be any value between 1 and 256 with a range of pixel intensities between 0 – 255. For a more accurate analysis a default bin size of 256 was used.

Figure 3.10 and Figure 3.11 show typical before and after images of the outer and centre regions and its corresponding full image. The cells in the after images are a population of yeast cells exhibiting positive dielectrophoresis, as demonstrated by the clearance of cells from the centre region (Figure 3.10) and the collection of cells in the outer region (Figure 3.11).
Figure 3.12 and Figure 3.13 show pairs of histograms for the outer region of a dot, with a suspension of yeast cells in distilled water, for frequencies of 100 Hz and 30 kHz applied for 20 seconds. The pair of histograms for the 100 Hz frequency point (see Figure 3.12) where no movement of cells were expected, does not show a significant difference in their intensity spread, apart from a slight increase at ~P(35) and a slight decrease at around ~P(100). In comparison, the pair of histograms for the 30 kHz frequency point where +DEP is expected (see Figure 3.13) shows a striking difference in terms of their intensity spread.

At the lower end of the intensity spectrum, there is a distinct increase in the cumulative pixel numbers in the after image. This would suggest that the higher end of the intensity spectrum would possess fewer brighter value pixels as the number of overall pixels being analysed in the image remains constant.
Figure 3.12 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 100 Hz.

Figure 3.13 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 30 kHz.

Figure 3.14 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 7.8 MHz.
At 7.8 MHz, where –DEP is expected to be seen, (Figure 3.14) there is a distinguishable difference between the other pairs of histograms. At the lower end of the intensity spectrum the after image has a markedly reduced cumulative pixel number over a range of intensities, while at the higher end of the intensity spectrum, especially in the last bin there is a huge increase in the number of pixels. This suggests that there are less cells present and light can pass through un-impeded and with minimal adsorption of light waves, indicating negative dielectrophoresis.

The centre regions of the dot in the reference images (IB) show only one characteristic peak at an intensity value of around P(125) as can be seen in Figure 3.15 to Figure 3.17. The after image at 100 Hz for the centre region (Figure 3.15) shows a little increase in the width of the histogram. Figure 3.16 shows a narrowing of the width with an overall shift to the right-end of the spectrum indicating, that there is more light passing through the aperture. The opposite transition is observed for the after image at 7.8 MHz, which shows a big decrease in pixel numbers at the right-end of the intensity spectrum with respect to its before image (Figure 3.17).

To obtain the dielectrophoretic spectrum based on the images taken at each frequency point, manipulation of each regional-based histogram into a characteristic and representative value was required. The modal intensity value, $P_{\text{modal}}$, for the before images were found for each frequency point.

The same intensity value was then assigned to the after images (IA) as a point of reference to the before images. With respect to the outer region, the cumulative sum of pixels ($P_\Sigma$) from intensity values 0 to $P_{\text{modal}}$ were computed for each image in turn and the

![Figure 3.15 Before and after histograms of the dot outer region. Yeast cells are suspended in distilled water with an applied frequency of 100 Hz](image-url)
difference ($G_{\text{DIFF}}$) between the after image and before image gave us a representation as to the magnitude of change in light transmittance.

For the centre region the modal intensity value was also found, but the cumulative sum of pixels from the intensity value of $P_{\text{modal}}$ to 255 was used instead, as more activity could be seen occurring at the higher end of the intensity spectrum. The differences of the after and before image were also taken. To scale the values down, the frequency point with the maximum value of $G_{\text{DIFF}}$ was found and used as the divisor for all other values. The scaled values of $G_{\text{DIFF}}$ were then plotted against the experimental frequency points to give the dielectrophoretic spectrum.
3.5.3 Statistical Description of Particle Redistribution

Based on the cumulative modal intensity shift method, the image analysis GUI developed allowed analysis of the length of time taken for the cells to redistribute to within 50, 75, 90, 95 and 99 percent of the original intensity value on removal of the electric field and were calculated for the 150\(\mu\)m, 300\(\mu\)m and 500\(\mu\)m dots. Typical re-dispersion plots are shown below for particles experiencing both positive and negative dielectrophoresis for a 500\(\mu\)m dot. Analysis of the redistribution images were based on the size of the region of interest. The centre region of all the dots were analysed at the mentioned percentage levels. Each centre region was analysed for sizes of the regions one-third, one-half and one complete multiple of the calculated radius.

For the 500\(\mu\)m dot, the total number of images reaching 50% of the original image intensity was found to be 50.0% and of the remaining not able to reach 50% of the original value, 61.1% were able to reach 40% of the original intensity value. The number of images found to reach 50% of the original image intensity value for the 300\(\mu\)m dot was found to be 55.6% and of the remaining not reaching that value only 37.5% were able to reach 40% of the original image value. In comparison all of the images for the 150\(\mu\)m dot were capable of reaching 40% of the original image intensity value, with 97.2% of the images also reaching 50% of the original image intensity value. As the threshold level was increased, at 75% of the original image intensity value 41.7%, 16.7% and 80.6% of the total images analysed reached the threshold for the 500\(\mu\)m, 300\(\mu\)m and 150\(\mu\)m dots respectively. At 90% of the original image intensity level, 19.4%, 0% and 61.1% of the total images analysed reached the threshold value for the 500\(\mu\)m, 300\(\mu\)m and 150\(\mu\)m dots respectively. At 95% of the original image intensity level, 13.8% and 50% of the total images reached the threshold value for the 500\(\mu\)m and the 150\(\mu\)m dots respectively. At 99% of the original image intensity level, 13.8% and 41.7% of the total images reached the threshold value for the 500\(\mu\)m and the 150\(\mu\)m dots respectively. The percentage of images reaching 75% of the original intensity value after experiencing positive dielectrophoresis was found to be 66.7%, 16.7% and 72.2% for the 500\(\mu\)m, 300\(\mu\)m and 150\(\mu\)m dots respectively.
Initial particle distribution (time =0) +DEP signal switched off (time = 7sec)

3 seconds after signal is off 6 seconds after signal is off

40 seconds after signal is off 53 seconds after signal is off

Figure 3.18 Images of part of the 150μm dot array experiencing positive distribution at $t = 0$sec until $t = 7$sec where the signal is switched off. Spontaneous re-dispersion of the particles is shown over a 53 sec period.
Chapter 3

Figure 3.19 Redistribution curve of yeast cells suspended over a 500μm dot array with an initial negative dielectrophoretic force exerted on the cells.

Figure 3.20 Redistribution curve of yeast cells suspended over a 500μm dot array with an initial positive dielectrophoretic force exerted on the cells.
H. O. Fatovinbo

Chapter 3

<table>
<thead>
<tr>
<th>Dot Size</th>
<th>Percentage of total experiments redistributing to within % of its original image intensity value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>150μm</td>
<td>97.2</td>
</tr>
<tr>
<td>300μm</td>
<td>55.6</td>
</tr>
<tr>
<td>500μm</td>
<td>50.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dot Size</th>
<th>Percentage redistributed to within 75% of original image after positive DEP</th>
<th>Percentage redistributed to within 75% of original image after negative DEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>150μm</td>
<td>72.2</td>
<td>88.9</td>
</tr>
<tr>
<td>300μm</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>500μm</td>
<td>66.7</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Table 3.5 Percentage of experiments reaching threshold value of original image from cell redistribution

While the percentage of images reaching 75% of the original intensity value after experiencing negative dielectrophoresis was found to be 16.7%, 16.7% and 88.9% for the 500μm, 300μm and the 150μm dots respectively. To quantify the results further, the original images of each experiment were analysed to obtain the intensity values of each region analysed.

The average cumulative pixel values for the centre regions possessing 3 concentric circles was found to be 2240.08, 11459 and 7995.8 for the 150μm, 300μm and 500μm dots respectively. The standard deviations were 466.28, 2297 and 2907.1 for the 150μm, 300μm and 500μm dots respectively. As the number of concentric circles decreased the centre region analysed increases in size. For 2 concentric circles the average cumulative pixel values for the centre regions were found to be 4789.58, 26714 and 16670 with standard deviations of 1341.4, 5889.5 and 5861.7 respectively for the 150μm, 300μm and 500μm dots respectively.
<table>
<thead>
<tr>
<th>Dot size</th>
<th>Positive Dielectrophoresis</th>
<th>Negative Dielectrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>150µm</td>
<td>23.4</td>
<td>32.25</td>
</tr>
<tr>
<td></td>
<td>(83.3)</td>
<td>(66.7)</td>
</tr>
<tr>
<td>300µm</td>
<td>38.5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>(33.5)</td>
<td>(0)</td>
</tr>
<tr>
<td>500µm</td>
<td>21</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>(83.3)</td>
<td>(67.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dot size</th>
<th>Redistribution times (sec) of dots for centre region with 2 concentric circles</th>
</tr>
</thead>
<tbody>
<tr>
<td>150µm</td>
<td>25.67</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
</tr>
<tr>
<td>300µm</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>(16.7)</td>
</tr>
<tr>
<td>500µm</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(66.7)</td>
</tr>
</tbody>
</table>

Table 3.6 Redistribution times for dot sizes and the respective percentage of experiments able to reach the threshold level

3.5.4 **Rapid Dielectrophoretic Characterisation using Image Processing**

The Beer-Lambert law is a linear relationship between absorbance and concentration of an absorber of electromagnetic radiation. Experimentally, the transmittance of light \((T)\) is related to the measured absorbance \((A)\) by:

\[
A = -\log(T) = -\log\left(\frac{P}{P_0}\right)
\]

Equation 3.4

where \(P\) is the power of light after passing through the sample and \(P_0\) is the initial light power. It has been demonstrated using the Beer-Lambert law that a relationship can be found for a shift in particle concentration over time and the Clausius-Mossotti
factor (by correspondence, Broche, 2005). This relation assumes that at time zero, a uniformly dispersed collection of particles within the Beer-Lambert region has an initial light intensity \((I_0)\). A dielectrophoretic force applied to the suspension will either attract or repel the particles. Depending on the path of the light source, over time the concentration shifts imposed on the particles by the dielectrophoretic force will tend to increase or decrease the light intensity \((I)\), defined by:

\[
I = I_0 e^{-\Delta \alpha C}
\]

Equation 3.5

where \(C\) is the concentration, \(A\) is area and \(\Delta z\) an infinitesimally small path length. It can then be found that over time the concentration difference is proportional to the particle velocity, which in turn is proportional to the dielectrophoretic force and the Clausius-Mossotti factor, i.e.

\[
d\ln \frac{I}{I_0} \propto |F_{DEP}| \propto \text{Re}[K(\phi)]
\]

Equation 3.6

Images were taken for each frequency point, before applying the field and after the external electric fields were applied for 2, 5 and 10 seconds for the 150\(\mu m\) dot. For the 300\(\mu m\) and 500\(\mu m\) dots the electric fields (10\(V_{pp}\)) were applied for 60 and 120 seconds respectively per frequency, with images captured every second and 2 seconds respectively. The dielectrophoretic spectra of yeast cells suspended in 11.3 \(\mu S \text{ cm}^{-1}\) potassium chloride over the 500\(\mu m\) micro-array are shown below for different time bases. At time \(= 0\) the cells are at rest, and no external field is applied. When the field is applied movement of the cell suspension creates a shift in the image greyscale intensity as shown in Figure 3.21 and Figure 3.22. All of the dielectrophoretic spectra obtained for the 500\(\mu m\) dot were based on the cumulative modal intensity shift within the centre region. The region of interest was obtained by specifying the dot be divided into three concentric areas based on the radius of the dot in pixels. The dielectrophoretic spectra obtained for yeast cells in conductive media of 11.3 \(\mu S \text{ cm}^{-1},\)
50.9 μScm⁻¹, 417 μScm⁻¹ and 1.48 mS cm⁻¹ is shown in Figure 3.23. The dielectrophoretic spectrum of yeast suspended in 280 mM of D-Mannitol is shown in Figure 3.24 and shows the frequency response of yeast cells obtained from analysing the centre (blue) and outer region (red) of the 150μm dot.

Figure 3.21 Dielectrophoretic spectra of yeast cells (11.3μScm⁻¹ KCl) 10 seconds after field applied at each frequency point

Figure 3.22 Dielectrophoretic spectra of yeast cells (11.3μScm⁻¹ KCl) 118 seconds after field applied at each frequency point
Chapter 3

Figure 3.23 Dielectrophoretic spectra of yeast cells in different media conductivities 118 seconds after field applied at each frequency point on 500μm dot.

Figure 3.24 Dielectrophoretic spectra of yeast cells in 280mM of mannitol using inner and outer regions for analysis of a 150μm dot.
The dielectrophoretic spectrum of red blood cells suspended in 3mSm⁻¹ KCl solution is shown in Figure 3.25 as determined using the imaging method at the centre of the 150μm dot.

### 3.5.5 Determination of Dielectric Properties of Homogeneous Populations using the Dielectrophoretic Spectrum Data Obtained from Image Analysis

Given the dielectric properties of a continuous suspending medium which has a dispersion of particles similar in biophysical makeup, the dielectric properties of the dispersed phase can be found by analysis of the dielectrophoretic spectrum [2, 28-31]. The frequency at which a particle changes from being negatively polarised to positively polarised or vice versa is known as the cross-over frequency ($f_c$). At this frequency the net magnitude of the dielectrophoretic force is zero, hence particles will not exhibit any movement. The lower cross-over frequency point has been used to determine the dielectric properties of cellular membranes [2, 32], where the cross over frequency is written as a function of membrane capacitance and membrane conductance according to:
Equation 3.7

\[ f_c = \frac{\sqrt{2}}{8\pi r C_{\text{mem}}} \sqrt{\left(4\sigma_m - rG_{\text{mem}}\right)^2 - 9r^2G_{\text{mem}}^2} \]

where \( r \) is the particle radius, \( C_{\text{mem}} \) is the membrane capacitance, \( G_{\text{mem}} \) is the membrane conductance and \( \sigma_m \) is the conductivity of the suspending medium. This formula has yielded good results for particles with a single shell covering the cytoplasm. But for particles possessing a double shell around the cytoplasm, such as yeast cells, the cellular wall which has quite distinct dielectric properties to that of the cellular membrane, has been found not to yield accurate results.

The multi-shelled model of Irimajiri et al and Huang et al [33, 34] was used to define the frequency dependency of the dispersed phase (yeast cells) suspended in low conductive media. The model is based on dielectric theories whereby the electrical properties of spherical cells can be described in terms of concentric spheres that are “smeared” out. That is for a spherical cell of \( N_i \) heterogeneous concentric compartments, the multi-shell describes that particle, in terms of the dielectric properties of the concentric compartments, as a homogeneous particle.

The dielectrophoretic spectrum obtained for the yeast cells using a 150µm dot with 3 concentric regions and the centre region being analysed is shown in Figure 3.26. The best fit curve was obtained using the fminsearch function of MATLAB, which is based on the Nelder-Mead Simplex method [35, 36]. The function minimises the error of several variables, based on the function inputted to be minimised [7, 34, 37]. The error function to be minimised based on starting values of the cells cytoplasm, cell wall and cell membrane dielectric properties is

\[ \sum_{i=1}^{N} \left( R_{\text{sim}}(\omega_i) - \alpha R_{\text{exp}}(\omega_i) \right)^2 = 0 \]

Equation 3.8

where \( N \) is the number of experimental frequency points based on angular frequency \( \omega_i \), \( R_{\text{sim}} \) is the simulated value of the real part of the Clausius-Mossotti factor, \( R_{\text{exp}} \) is the arbitrary value of the experiment and \( \alpha \) is the weight given to the experimental values in the iterative minimisation procedure. The initial starting values for the curve fitting procedure were taken to be 50, 6 and 60 for the relative permittivity of the cell
cytoplasm, cell membrane and cell wall respectively. The conductivity of the cytoplasm, cell membrane and cell wall were taken to be 0.2 Sm\(^{-1}\), \(2.5 \times 10^{-7} \) Sm\(^{-1}\) and 0.01 Sm\(^{-1}\) [34]. Although the cell radius was found to vary, it was kept constant at 4\(\mu\)m as used in [34], with the cell membrane and cell wall being 8nm and 0.22\(\mu\)m respectively. Parameters set for this iterative procedure were set to a maximum function evaluation of 3000, a maximum iteration of 5000 and an error tolerance of \(1 \times 10^{-25}\). This yielded final values of 0.031 Sm\(^{-1}\), 0.1 Sm\(^{-1}\) and \(1.43 \times 10^{-7} \) Sm\(^{-1}\) for the cell wall, cytoplasm and cell membrane respectively. The relative permittivities at the end of the minimisation procedure were 1.62, 43 and 8.6 for the membrane, cytoplasm and cell wall respectively. The value of the error function was 1.023 with an initial weighting of 1 and a final weighting of 0.59.

The spectrum of the best fit curve with different initial permittivity values is also shown in Figure 3.26(-). Initial starting values for the curve fitting procedure were set at 50, 10 and 50 for the relative permittivity of the cell cytoplasm, cell membrane and cell wall respectively. The conductivity of the cytoplasm, cell wall and cell membrane were left the same. The end values were 10, 2 and 59 for the relative permittivity of the cell cytoplasm, cell membrane and cell wall respectively; and 0.029 Sm\(^{-1}\), 0.094Sm\(^{-1}\) and \(3.42 \times 10^{-7} \) Sm\(^{-1}\) for the cell wall, cytoplasm and cell membrane respectively. The final weight given to the values was 0.62 and the value of the error function was 1.01.

Figure 3.26 Dielectrophoretic spectrum of yeast cells suspended in conductivity media of 5mSm\(^{-1}\); Experimental data points obtained from images are defined by the black circles (O) and the best fit lines are plotted against these points using the algorithm based on the multi-shell theory and Equation 3.8.
From the experimental data, a lower crossover frequency point was found to be at 91.36 kHz. The curve fitting data was able to calculate the Clausius-Mossotti factor at higher frequencies, hence a lower crossover frequency was found at 102 kHz and a high crossover frequency was found at 16 MHz for both best fit curves.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obtained results</th>
<th>Published results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic conductivity (μScm⁻¹)</td>
<td>900 - 1000</td>
<td>5500±500 [38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2300 [39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2200-3500 [40]</td>
</tr>
<tr>
<td>Membrane conductivity (μScm⁻¹)</td>
<td>1.47×10⁻³ - 3.42×10⁻³</td>
<td>≤ 2.5×10⁻⁴ - 4.5 [38]</td>
</tr>
<tr>
<td>Cell wall conductivity (μScm⁻¹)</td>
<td>295 ± 5</td>
<td>100 - &gt;500 [38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 [41];</td>
</tr>
<tr>
<td>Scaling factor</td>
<td>0.6 ± 0.2</td>
<td>0.81±0.05 (sc) [38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5-0.7 (bc) [38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 [41]</td>
</tr>
</tbody>
</table>

Table 3.7 Comparison of biophysical properties of yeast cellular compartments determined by electro-rotation spectra of other authors and the DEP spectra obtained using imaging techniques; sc=single cell and bc=budding cells.

Table 3.7 shows a summary of the conductivity of the cellular compartments obtained using the imaging techniques described for a suspension of yeast cells experiencing dielectrophoresis. The published literature for these compartments, were predominantly obtained using electro-rotation and shows a variation in values between authors. Our results show that our values lie close or between published results, indicating the method used here is a viable technique for acquiring the biophysical properties of cells.

3.5.6 Electric field Distribution of the Dot Micro-system

Electrostatic simulations conducted with finite element modelling package FEMLAB allowed visualisation and quantification of the field distributions within the modelled systems. Four differing dot diameters were modelled in 2-D, with a constant height between top and bottom electrodes of 128μm. The electrodes were given a constant thickness of 2μm (whereas in reality the fabricated electrodes are < 1μm in thickness)
Figure 3.27 2-D model of a 150μm dot as used in electric field simulations

in all of the models. Figure 3.27 shows a typical geometrical model of the dot system represented in 2-D for the simulations. Regions 1 and 5 represent the glass substrate; region 2 and 6 represent gold electrodes; region 3 represents the suspending medium with no particles; region 4 represents the ITO electrode. The x and y-coordinate system represents the width (x) and the height (y) of the model, i.e. the centre of the dot for all models is found at (0,0) which is the point midway between the gold electrodes, on the surface of the glass substrate.

Conditions used for solving the problem along with the global meshing parameters used for all simulations were left constant and are presented below. Table 3.8 shows the results of the refined meshing for each simulated model, including the number of degrees of freedom, the number of elements in the model and at the boundaries and the minimum quality of the elements.

Figure 3.28 to Figure 3.31 show the electrostatic potential distributed within the simulated model for all dot diameters. With the height of the system left constant with respect to the dot diameters, the electric potential at the gold electrode can be seen to be at its maximum (7.07V) while the minimum is located at the ITO electrode (-7.07).
- FEMLAB ELECTROMAGNETIC MODULE SIMULATION PARAMETERS

**Global meshing parameters**

- Predefined mesh size: Normal
- Maximum element size scaling factor: 1
- Element growth rate: 1.3
- Mesh curvature factor: 0.3
- Mesh curvature cutoff: 0.001
- Mesh geometry to: subdomains
- Refinement method: Regular

**Solver Parameters**

- Stationery Linear solver: Direct (UMFPACK)
- Solution form: Coefficient
- Pivot threshold: 0.1
- Memory allocation factor: 0.7

<table>
<thead>
<tr>
<th>Dot diameter ($\times 10^6$ m)</th>
<th>Extended mesh: Number of D.O.F</th>
<th>Base Mesh: Number of elements</th>
<th>Number of boundary elements</th>
<th>Minimum element quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>241282</td>
<td>120501</td>
<td>1615</td>
<td>0.4521</td>
</tr>
<tr>
<td>350</td>
<td>197971</td>
<td>98704</td>
<td>3198</td>
<td>0.4817</td>
</tr>
<tr>
<td>250</td>
<td>55995</td>
<td>27910</td>
<td>977</td>
<td>0.5029</td>
</tr>
<tr>
<td>150</td>
<td>94008</td>
<td>46937</td>
<td>550</td>
<td>0.5236</td>
</tr>
</tbody>
</table>

Table 3.8 Refined meshing information on all systems modelled
Figure 3.28 Distribution of electrical potential for dot diameter of 150\(\mu\)m

Figure 3.29 Distribution of electrical potential for dot diameter of 250\(\mu\)m

Figure 3.30 Distribution of electrical potential for dot diameter of 350\(\mu\)m
It can be seen that as the dot diameter increases the electric potential at the centre of the dot decreases from a positive potential to a more negative potential. This effect is also displayed laterally at the surface of the glass substrate and just above the surface. By taking the square of the gradients of the electric fields in the x and y directions $\nabla E^2$ is obtained. Surface plots of the electric field gradient for all diameter dots can be seen in Figure 3.32 – Figure 3.42. With the voltage used in the model the value of the field gradient at the electrode edges was in the magnitude range of $1 \times 10^{12} \text{ V}^2 \text{ m}^{-3}$. 

**Figure 3.31 Distribution of electrical potential for dot diameter of 500\(\mu\)m**

**Figure 3.32 Electric field gradient distribution within 150\(\mu\)m dot**
The field gradient value at the co-ordinate (0, 0) was found to be $4.38 \times 10^7$, $1.65 \times 10^{10}$, and $1.09 \times 10^8$, $1.63 \times 10^8$ V$^2$ m$^{-3}$ for the 150μm, 250μm, 350μm and 500μm diameter dots respectively. In the plane of the dot electrodes at the centre of the dot (0, 2 × 10$^{-6}$), the field gradient was found be lower in magnitude for all dot sizes compared to that at the surface, with the 150μm, 250μm, 350μm, and 500μm diameter dots having values of $8.44 \times 10^6$, $6.45 \times 10^7$, $1.20 \times 10^8$, $1.68 \times 10^8$ V$^2$ m$^{-3}$ respectively. For x = 0 at any height y in the system, above the plane of the electrodes there is a general increase in the magnitude of the field gradient at any of those points. This can be easily visualise by the surface plots of Figure 3.33, Figure 3.34,
Figure 3.35 Electric field gradient distribution within 350μm dot

Figure 3.36 Electric field gradient distribution within 500μm dot

Figure 3.35 and Figure 3.37, where the field gradients at the centre regions for the dots have been scaled so they are displayed on the colour bar more effectively.

It was found that at the centre of system for all models where x = 0 and y = 1.25 × 10⁻⁴ m, 5μm away form the counter electrode the field gradient was greater for the smallest diameter dot and decreased as the dot diameter increased. Values determined at that point were 5.99 × 10⁹, 2.51 × 10⁹, 1.09 × 10⁹ and 4.42 × 10⁸ V² m⁻³ for the 150μm, 250μm, 350μm and 500μm diameter dots respectively. At the surface of the ITO electrode (0, 1.3 × 10⁻⁴ m) the field gradient dropped significantly (~2 × 10⁶ V² m⁻³) with respect to the field gradient in the bulk of the medium in the same x-plane for all models.
The characteristic field gradient distribution can be seen to adopt a dome-like geometry at the centre of the dot. The dome shape is more pronounced for smaller diameter dots, but as the diameter increases the dome’s sides begin to slope at an angle as the centre is approached, contributing to a more triangular field gradient distribution as seen in Figure 3.36 and Figure 3.37. The field gradient is also shown to increase as you approach the edges of the gold electrode. Figure 3.38 to Figure 3.42 show the field gradient is at its maximum closest to the electrode edge with values of $\sim 2 \times 10^{12}$ V$^2$ m$^{-3}$ at the electrode tip.

![Surface plot of Electric field gradient $E^2$ within a 500µm diameter dot](image)

Figure 3.37 Electric field gradient distribution within 500µm dot

![Surface plot of Electric field gradient $E^2$ within a 150µm diameter dot](image)

Figure 3.38 Electric field gradient distribution within 150µm dot around the gold electrodes
Around the electrode edge the field gradient rapidly reduces by a magnitude of 1, approximately one-fifth away from electrode edge as the centres of the dots are approached. By the mid-point of all simulated models, i.e. the point in the plane of the gold electrodes (\(y = 2 \times 10^{-6}\) m) and midway between the centre of the dots, the field gradient was found to decrease by a magnitude of 3 for the 150\(\mu\)m, 250\(\mu\)m, 350\(\mu\)m diameter dots and by a magnitude of 4 for the 500\(\mu\)m diameter dot. In comparison, with respect to height at the midway-point in both x and y planes from the tip of the
electrode, the magnitude of the field gradient was found to be higher by a magnitude of 1 for the 500μm diameter dot, with no change in magnitude for the smaller diameter dots when compared to the mid-point in the plane of the gold electrode. Figure 3.41 shows the tip of the electrode, magnified so as to visualise the field gradient present around the vicinity of the electrode.

Figure 3.41 Electric field gradient distribution within the 500μm dot at a gold electrode edge

Figure 3.42 Electric field gradient distribution within the 500μm dot around a gold electrode edge
3.6 Discussion

3.6.1 Image Processing for Determining the Clausius-Mossotti Factor

Studies have been carried out on the 'dot' electrode to determine whether the dielectrophoretic response of a particle can be acquired through image processing techniques. A number of workers have used similar methods for quantifying the responses of particles to electric field gradients by observing their motion over electrode arrays [18, 42], while others have used optical methods for the direct measurement of a particle response to varied frequencies and suspending media conductivities [17, 21, 22]. The method used in this study is based on a similar principle associated with the optical methods mentioned.

As previously discussed, the characteristic motion of particles experiencing a positive or negative dielectrophoretic force is either towards or away from the electrode edge respectively. The simple design of the dot micro-system gives a definite area of observation that is easily identified. The area used was generally a single dot from an array of dots. The volume above a single dot can be considered to be of a cylindrical whose volume varies depending on the thickness of the spacer and to a lesser extent the thickness of the gold coating on the dot electrode. With a spacer between top and bottom electrodes of 125μm in thickness, the volume percentage occupied by yeast cells (2.5 ± 0.5μm in radius) over a single dot of 150μm, 300μm and 500μm able to produce rapid red-dispersions over the dot aperture ranged between 0.11% and 0.55%.

As shown earlier, at a combined particle concentration and dot size it is possible to observe particles spontaneously redistributing over the aperture region upon removal of the applied electric field. This redistribution process provided a major advantage for rapid characterisation of particles using the dot micro-system. The dependency of concentration for redistribution made location and segregation of individual particles almost impossible from captured images. The method developed for analysing the digital images stemmed from analysis of the image's histograms. By defining a region of interest (ROI) within the aperture region of the dots, the value of the pixels showed a spread corresponding to particle structures and the background luminance. For instance the histograms obtained for the centre region are different than the outer regions. The reference images of the outer region show 2 distinct peaks, which likely correspond to the illuminated surface of the gold electrode at around P(50) and the boundary structures of the cell at around P(125).
The centre region of the aperture was preferred as the ROI because it was easier to locate for reproducible and constant sizes; the spread was more uniform than the edges and errors were markedly reduced by concentrating the analysis region to an area that hardly gave false positive results i.e. if the analysis region was nearer the electrode edges, the likelihood of including the electrode into the analysis region would be very high, also, depending on the width of the concentric analysis region at the electrode edge particles experiencing negative dielectrophoresis may not totally clear from the region, incorrectly indicating a collection of particles. In opposition to that event, particles experiencing positive dielectrophoresis collect at the electrode edge, but may ‘overshoot’ the electrode edges and indicate a region with a higher number of bright pixel values, again indicating a wrong result. These artefacts have been shown to be related to concentration occur more frequently at the outer region. If the particle concentration is not accurately obtained for whichever dot size used the possibility of obtaining errors in the determination of the dielectrophoretic spectrum is greatly increased. The ability to attain concentrations of the order of $10^8$ cells per ml with a high degree of confidence was a factor in choosing the 150μm dots as a good size for obtaining the dielectrophoretic spectra of particles, approximately 4μm in radius. Also, the concentration value was close to the Beer-Lambert region and occupied a total volume fraction of <1%, validating the method used.

The spectra of yeast cells (118 seconds after the field is applied) suspended in a range of conductivities obtained using a 500μm dot is shown in Figure 3.23. The ROI had a radius one-third the size of the original dot radius in pixels. Figure 3.21 and Figure 3.22 show the spectra of the same dot with the particles suspended in 1.13 mSm$^{-1}$ KCl solution at 10 seconds and 118 seconds respectively. It is seen that the form of the spectra for that dot size starts to be realised at 10 seconds, but becomes more defined at a higher time base. The spectra of Figure 3.23 show some inconsistencies in their spectra patterns which indicate that larger size dots and higher concentrations may introduce errors in the analysis. These may include:

- Errors in attaining suspension concentrations leading to inadequate redistribution of particles.
- Prolong exposure to the electric field gradients could damage or burst cells
• Sedimentation of particles within dot cavity further away from the electrode edges
• Slow particle movement over larger dot area
• Deviation from using the Beer-Lambert Law due to high concentrations
• Increase in medium conductivity due to extended particle residence time

A majority of these errors can be reduced or eliminated by using a combination of a lower particle concentration with a smaller dot size which also leads to a compromise as to which factors are less likely to contribute significantly to errors in experiments. The dielectric dispersions exhibited by a particle in an alternating electric field are due to complex interactions of particle and medium conductivity and permittivity and the frequency of the applied field [43-45]. The dielectrophoretic spectrum of a bioparticle can be described as the particle’s fingerprint, indicating its current biophysical make-up. The spectrum can be seen to differ based on the number of interfacial polarisation regions, the geometry and size of the particle, the frequency range and the dielectric properties of the medium and the particle [12]. Over a preferred frequency range of 1 kHz – 10MHz the experiments carried out on yeast cells show spectra which varied due to changes in media conductivities. Figure 3.21 and Figure 3.22 show the dielectrophoretic spectra of yeast cells in 1.13mSm⁻¹ KCl obtained using the 500μm dot. The relatively flat line at 0 between 100Hz and 1 kHz indicate here that there was an infinitesimally small shift in cell position due to dielectrophoresis. This frequency range is believed to manifest electrophoretic forces more than the dielectrophoretic ones which become masked [46]. Between 1 kHz and approximately 150 kHz, the spectra show a negative shift in greyscale intensity, corresponding to the magnitude of the polarisation effects experienced by the particles. A crossover frequency is present at about 180 kHz after which a positive value for the polarisation becomes apparent. At about 1MHz a plateau begins to develop up until 10 MHz. The comparison of the 2 figures show that at the mentioned suspension conductivity the formation of the DEP spectrum for the 500μm dot actually becomes apparent within 10 seconds, but is fully formed 118 seconds after the field is applied. A very different spectrum was obtained for red blood cells in 3 mSm⁻¹ of KCl over a 150μm dot. It shows that at low frequencies (<100 kHz) the cells are experiencing negative dielectrophoresis, and start to experience positive at
180 kHz. There seems to be a distinct maximum polarisation value at 630 kHz, gradually falling back to 0 at 10 MHz. The DEP crossover frequency for red blood cells in isotonic solution has been determined with a range of media conductivity and shows to be within a range comparable to the frequency determined here [47]. Figure 3.24 shows the spectrum of yeast cells for the outer and inner regions of a 150μm dot. Both curves show a striking similarity, with similar magnitudes of response on change of frequency. An interesting observation is the high end cross over frequency (f_c) found at ~5MHz for both curves. The low end of the frequency range shows a little discrepancy at 3 kHz, but continues to exhibit similar rises and falls afterwards.

By applying the appropriate theoretical formulae it was possible to deduce the dielectric properties of a suspension of biological particles using the iterative process. The dielectrophoretic spectrum of yeast cells (double-shelled) suspended in 5mSm⁻¹ KCl has been found using the 150μm dot (Figure 3.26). Using the dielectric properties GUI, the best fit curves were found with initial biophysical values inputted. The first iteration procedure gave final biophysical values which closely matched the initial values. By changing the values of the initial permittivity values, the iteration procedure gives a second best fit curve which had a lower error value than the first procedure. The final dielectric properties were also found to be in good correlation with the final values of the first iterative procedure, particularly with the particle’s conductive components.

Figure 3.43 A 3x2 array of 150μm dots, with particles experiencing negative DEP
3.6.2 Effect of Particle Concentration vs. Dot Size

Particles experiencing a dielectrophoretic force were observed moving either towards the electrode edge (+DEP) or towards the centre of the aperture (-DEP). Single yeast cells surrounded by homogeneous particles of the same dielectric makeup were monitored as they travelled towards or away from the electrode edge. Concentrations of $\sim 7 \times 10^7$ and $\sim 3 \times 10^6$ cells per ml were used as starting concentrations as the ability to distinguish between individual cells was very clear. As the concentration increased to $\sim 1 \times 10^8$ cells per ml, suspensions over the 150µm dot array were over-crowded to an extent that distinguishing a cell from another became very difficult. When positive dielectrophoresis occurred, a single particle located at the centre of the aperture begins to travel to the electrode edge with all other particles. The velocities for that single particle suspended in a concentration of $10^7$ cells per ml were calculated to be 0.44µms$^{-1}$, 0.70µms$^{-1}$, 0.66µms$^{-1}$ and 2.84µms$^{-1}$ for dot diameters of 500µm, 300µm, 250µm and 150µm respectively. A particle located at the electrode edge, travelling towards the centre of the dot aperture, in the same cell concentration, was found to have distinctly lower velocities for all dot diameters examined. Dot diameters of 500µm, 300µm, 250µm and 150µm had velocities of 0.18µms$^{-1}$, 0.19µms$^{-1}$, 0.26µms$^{-1}$ and 0.62µms$^{-1}$ respectively. The velocities were approximately 6.5 times faster for the 150µm dot over the 500µm dot under positive dielectrophoresis and 4.5 times faster for the 150µm diameter dot under positive dielectrophoresis when compared to negative dielectrophoresis. A reduction in cell concentration revealed a drop in the overall velocities for both types of dielectrophoretic forces apart for the 500µm diameter where there seemed to be negligible differences for both force types on the particle. This observation should not affect obtaining an accurate dielectrophoretic spectrum. As long as all measurements are carried out with the same suspension density the magnitude of the response is relative to the polarisability of the particles at varying frequencies with concentration shifts from a reference point commensurable to the dielectrophoretic force experienced by the particles.

It was observed that as the concentration of cells increased and the size of the dot decreased the clearance rate of the suspended cells under positive dielectrophoresis was rapid. If we define the clearance rate as the time taken to remove cells from the aperture region of the dot, then for a concentration of $\sim 3 \times 10^6$ cells per ml, 68% of the cells were cleared from the centre of the 150µm dot diameter within 1 minute and
100% within 2 minutes. Whilst for a concentration of \( \sim 7 \times 10^7 \) cells per ml 87% of the cells were cleared within 1 minute and 100% within 2 minutes. In comparison, for the 500\( \mu \text{m} \) dot it took 2 minutes to clear 77% and 87% for cell concentrations of \( 10^6 \) and \( 10^7 \) cells per ml respectively. It was established that an optimal concentration of yeast cells to use for rapid characterisation was a concentration of \( 10^8, 10^9 \) and \( 10^{10} \) cells per ml for the 150\( \mu \text{m} \), 300\( \mu \text{m} \) and 500\( \mu \text{m} \) dots, as the cells once collected under positive DEP, redistributed rapidly to a uniform static state upon removal of the ac electric field. This may be due to the negative surface charge on the particles causing them to repel each other and move away from the electrodes, or to diffusion.

The time taken for the particles to redistribute showed to be dependant on a number of variables including dot size i.e. region of interest, experimental conditions, threshold-level settings and crucially on the dielectrophoretic force initially applied. A general and reproducible trend across all dot sizes and for any particular intensity threshold level was based primarily on the relative size of the region being analysed in relation to the actual dot size. As the centre region being analysed is increased in size, the time it takes for the particles to redistribute to a particular threshold level decreases if the initial dielectrophoretic force on the particles is positive. If the initial dielectrophoretic force on the particles is negative the redistribution time increases as the size of the region being analysed is increased. This was particularly obvious for the 150\( \mu \text{m} \) dots, where the set of redistribution experiments for a threshold level of 75% showed a high degree of confidence in reaching their final values. With an initial positive dielectrophoretic force on the cells, the average time taken for the particles to redistribute over the array to within 75% of the original image intensity value was found to be 32.25 seconds (66.7%) and 35.67 seconds (50%) for regions of analysis one-third and one-half in radius of the dot radius. In contrast, for the same 150\( \mu \text{m} \) dot an initial negative dielectrophoretic force on the cells gave a redistribution time of 11.2 seconds (100%) and 16 seconds (83.3) for regions of analysis one-third and one-half in radius of the dot radius.

3.6.3 Electrostatic Field Distribution in Dot Micro-system

Computer simulations of the 2D electric field potentials and gradients distributed within a single dot were done using FEMLAB 3 a commercially available finite element modelling software which solves the partial differential equations (PDE)
associated with the electromagnetic module. The use of computer simulations is a useful way to visualise the electric field distributions within an electro-kinetic micro-system. For dielectrophoresis the electric field gradient distribution is of particular importance when visualising the electric field simulations [10, 48-51]. The electric field gradient squared at any point in the system is directly related to the dielectrophoretic force a particle experiences at that point less all other forces such as gravitational, viscous, thermal and hydrodynamic [52-54].

Field gradients of the dot micro-system were found to be greatest at the edges with a value of $2 \times 10^{12} \text{ V}^2 \text{ m}^{-3}$ characterised by a 10V RMS signal applied to the dot electrode surface. It is anticipated that particles experiencing a positive dielectrophoretic force would be attracted to these regions. These values are in good correlation with previous simulations of other electrode geometries of similar dimensions [54, 55]. For a particle of around 1μm in radius suspended in an aqueous medium the dielectrophoretic force should be at least 10 times larger than the gravitational and Brownian forces each having a value of around $2 \times 10^{-15} \text{ N}$ [15]. Hence it is possible to observe particles such as latex beads and smaller cells experience translational motion due to the dielectrophoretic forces.

Moving away from the edge of the dot electrode the field gradient gradually drops in magnitude until it reaches the centre of the dot aperture. The value of the electric field gradient at the centre of the dot aperture on the substrate surface was found to be within the same order of magnitude for all dot diameter sizes (150μm, 300μm and 500μm) apart from the 250μm diameter dot which was 2 orders of magnitude higher. In this region it is anticipated that particles experiencing negative dielectrophoresis will be confined and trapped within this area. As you approach the top counter electrode from the surface of the substrate located at the centre of the aperture there is a decrease in the field gradient magnitude for all sizes up until the plane of the electrode is reached, 2 μm from the surface of the substrate. As the half-way point between the centre of the dot aperture and the top counter electrode is approached, a general increase in the magnitude of the electric field gradient is seen. The magnitude of the field gradient is highest for the smaller diameter dot (150μm) 5μm from the top counter electrode, showing a value of 1order of magnitude larger than the 500μm diameter dot at the same position, but a value of less than 3 orders of magnitude lower than the field gradients created at the electrode edges of all models.
The characteristic field gradient distribution of the dot micro-system shows one similar of a dome-shape. Figure 3.32 to Figure 3.37 show the characteristic electric field gradient distribution of the 150μm, 250μm, 350μm and 500μm dot diameters. It can be seen that the 2D representation of the field gradients are grouped in bands of arcs over the aperture of the dot. The arcs are more pronounced for the 150μm diameter dot, with very distinct bands of similar field gradient magnitudes. At the middle of the aperture there is a well defined arc corresponding to the low field gradient region. Gradually, as the edges of the bottom electrode and the top electrode are approached the bands increase in field gradient strength. As the size of the dot diameter increases there becomes a more tapered form to the sides of the arc at the middle of the aperture (Figure 3.35). Approaching the edges of the bottom electrode and the top electrode the characteristic dome-like distribution gradually disappears replacing it with bands of field gradients from the bottom of the electrode straight up to the top electrode (Figure 3.37). This suggests that there may be an optimal spacing distance which can be used between the top and bottom electrode depending on the dot size and there is a threshold dot size which once passed the ability to exert a dielectrophoretic force on a particle situated at the centre will not be effective.

A general observation for the field distribution within the electrode system is the strong field gradient obtained around the electrode edge, which if looked at in three dimensions would resemble a donut structure. The electric field lines entering the suspending medium from the electrode edge show components parallel and perpendicular to the electrode surface. This suggests that a dielectric particle located at the centre of the dot at a specific height \(h_i\) above the plane of the dot electrodes in the electrode system will experience a DEP force that is stronger in magnitude than a particle of the same dielectric makeup, situated the same x distance away from the electrode edge into the aperture area, but at a lower height than that of \(h_1\) and overall a shorter distance away from the electrode edge. It is then believed that a suspension of concentrated particles suspended within this electrode system will, under positive dielectrophoresis, collect at the dot perimeter with particles in the bulk of the solution exhibiting rapid movement compared to those which are closer to and in a lower plane to those in the bulk of the medium. Under negative dielectrophoresis, the low field gradient region is situated at the centre of the dot in the plane near the glass substrate. This suggested that a suspension of particles experiencing a negative force will
occupy regions available in the centre of the dot which would lead to cells piling up above each other.

3.7 Conclusions

A novel dielectrophoretic micro-system has been evaluated and shown that it is possible to obtain the Clausius-Mossotti factor using image processing techniques. Experimental evaluation of the system showed a suspension of homogeneous particles experienced spontaneous re-dispersion upon removal of the external field. This phenomenon was found to be a function of particle concentration and electrode diameter size, with a smaller electrode diameter (150μm) possessing favourable experimental parameters in terms of higher percentage of particles re-dispersing upon the removal of the external field and generally in much faster times. The use of lower particle concentrations reduces errors in image processing and the application of shorter electrical fields to the suspension also reduces overall experimental times and chances that the particles are not significantly affected by undesirable external factors such as heating.

The method used to process the images was found to give good correlations with published literatures spectrum for yeast and blood cells. The correlation seemed to improve as the dot size was reduced owing to a combination of lower particle concentration required and increased particle velocities.

Using the 150μm dot array, it has been calculated that the entire dielectrophoretic spectrum, at 5 points/decade (1 kHz – 10MHz) could be realistically obtained within 15 minutes, with an external field applied for 5 seconds and a re-dispersion time of 30 seconds, significantly longer than measured times. This can be significantly reduced by up to a factor of 180 by introducing multi-dots (see Figure 3.43) where the dots are individually connected to same voltages but different frequencies for the same length of time and images are captured all at once. This would allow multiple experiments of the same suspension in a couple of minutes and could be incorporated at the end of a flow system as a form of optical/imaging detection array on a single chip, after the upstream processing (i.e. concentration or separation) has taken place.
3.8 References


Chapter 4

An Enhanced Dielectrophoretic-Quartz Crystal Microbalance (DEP-QCM)

4.1 Introduction

Biosensors offer the ability to selectively detect particles of varied sizes as small as DNA and as large as cells. Selectivity of bio-molecules are largely based on surface modification techniques as there exists natural [1] and artificial [2, 3] molecular entities which specifically bind to domains or antigenic sites of these bio-molecules. The binding of molecules stimulate an appropriate transducer, which in turn sends a signal to a detector. A quartz crystal microbalance (QCM) is a non-specific transducer in its un-modified form but has been shown to possess highly specific biosensing capabilities when it has its surface modified [4]. The mass sensitivity of a quartz crystal microbalance operating in thickness shear mode (TSM) provides a good measure of thin film growth on the crystal surface. The sensitivity of the quartz crystal is very high (~10 pg cm$^{-2}$ in a vacuum) giving quantifiable results for mass changes at the solid interface [5]. The quantitative nature of a QCMs response to mass change for rigid layers is absolute, meaning there is no need for calibration unlike surface acoustic wave (SAW) devices. However, when non-rigid layers are formed, such as visco-elastic films the system requires careful investigation.

In 1964, King first used QCM devices for gas analyses [6, 7], demonstrating it to be a useful tool for measuring thin film deposition in solution-phase analytical electrochemistry [8, 9]. With suitable modifications, the use of the QCM as a biosensor has received a lot of interest in recent years particularly because of its usability in liquid environments. It has been used as a transducer for immuno-sensing in static and continuous flow systems, as a viscometer in the food industry, detection of cells and viruses with artificial receptors, changes in microtubule alterations in living cells and for the long term monitoring of biofilms [3, 4, 10-12]. Mass loading of the crystal at the solid-solution interface is related to a change in the crystals frequency [13]. For simultaneous liquid damping and mass loading there is a force difference on the crystal attributed to the properties of the liquid and the adsorbed
load. Kanazawa and Gordon (1985) derived the frequency shift of a crystal loaded on one side with liquid and determined the shift was due to viscous and density properties of the liquid [14].

AC electro-kinetics offers a range of phenomena with possible applicability to QCM, particularly the precise movement of particles towards electrodes from bulk solutions [15-17]. In the early 1990’s Pethig et al discovered the collection of particles on the upper surface of microelectrodes when subjected to low frequency voltages [18]. This was later reported to be a phenomena caused by fluid motion termed ac electro-osmosis [19, 20]. Collection of different types of particles on the surfaces of coplanar microelectrodes, due to dielectrophoretic and electro-hydrodynamic forces has been observed by a number of groups [21-24].

Thus far only one microelectrode geometry, known as the “zipper array”, has been designed specifically to concentrate particles from the bulk solution on to the electrode surface by ac electro-osmosis for a range of particle types [21, 25]. The microelectrodes are coplanar separated by a gap of a constant width. The gap between the electrodes meanders, forming alternating electrode pads on either side of the coplanar electrodes. The pads are almost circular in nature, but possess a neck at each meander, leading to the formation of interlocking pads.

In this chapter, an enhanced dielectrophoretic quartz crystal microbalance (DEP-QCM), based on the zipper phenomenon is suggested to possess a superior advantage over current applied uses of the QCM for biosensing. By applying ac electro-kinetic forces to particles in the bulk solution, particles are rapidly dragged to the surface of the electrode and concentrated in specific locations. Detection of this phenomenon is thought possible with appropriate signal modulating, and filtering of crystal and DEP signals.

4.2 Methods and Materials

4.2.1 Electrode Design and Fabrication

AT-cut quartz crystals (8mm diameter, 160µm thick) were obtained through Dr Subrayal Reddy (School of Biomedical and Molecular Sciences, University of Surrey). A mask template was made in the School of Engineering workshop for evaporation purposes. The mask template (brass) was designed such that the crystals sat in a bottom plate having 32 seats, each revealing 6mm diameter of the crystal and
a “tag” extending to the edge of the crystal. A top plate was screw-tightened to the bottom plate, holding the crystals in place. The top plate also possessed 32 holes (6mm in diameter) but had 2 tags at opposite ends extending to the edge of the crystals (see Figure 4.1). This facilitated the electrical connections to the top part of the electrode with minimal interference in the active region of the crystal. Before setting the crystals in the mask template, the crystals were ultra sonicated in ethanol for 30mins, washed in water and acetone (×2) and rinsed with iso-propyl alcohol. The crystals were then allowed to dry on clean foil with a lamp shining on them evaporating all fluid from the crystal surface.

Layers of gold and titanium were evaporated onto the crystals at the EPSRC National Centre for III-V Technologies (Electrical and Electronic Engineering Department, University of Sheffield). Gold and titanium wires were degreased in heated acetone before loading the materials onto their respective boats in the evaporator. The template mask was positioned below the boats, but above the film thickness sensor. Roughing of the evaporator, to remove the air from the chamber took 15mins and then pumping down to achieve a vacuum took a further hour and a half. On achieving the vacuum (10⁻⁵ Torr) electrical current was passed through the boat containing titanium first, and then through the boat containing gold. The chamber was admitted air and allowed to cool down (~20mins), then the mask template was turned over and the procedure was repeated for the other side. Zipper geometries were designed using CorelDraw as described in 3.2 of Chapter 3. They were all designed within a 7mm diameter (actual
size) region. The photolithography steps (also described in Chapter 3) remained the same except for the etching of the top gold surface of the quartz crystal. Instead of submerging the crystal into the gold etchant, the crystals were sat upon a glass slide, topside up, and droplets of gold etchant were micro-pippetted on to the surface forming a stable hemispherical fluid in contact with the gold surface. After 2 minutes the crystals were submerged in sodium thiosulphate solution to neutralise the iodine, and the rest of the photolithography process continued as previously described.

4.2.2 Design and Specifications of DEP-QCM Flow-cell

A QCM holder was designed, based on a modification of a traditional QCM holder. The objective of the design was to create a structure capable of accommodating a combination of detecting the crystals resonant frequency and also applying a sinusoidal frequency to the crystal for manipulating particles depending on the electrode geometry on the topside of the crystal.

To eliminate rough handling and increase re-usability of the crystal, a holder with spring loaded contacts was designed to provide good electrical connections and crystal stability when positioned in the holder. A 6mm thick Perspex top plate had two spring loaded pins glued into the drilled-out bores. The spring loaded pins chosen (PD4JS; CODA Systems Ltd, Essex, UK) had a hemispherical tip, which was chosen to minimise surface scratching of the QCM electrodes and provided good electrical contacts to the QCM electrode surface. The width of the tip (1.14mm) and the spring force (initial = 43g; working = 85g) were chosen to minimise the pin-electrode contact area on the tags and the force exerted on the crystal, but to maintain a pressure sufficient to hold the crystal in place and connect the bottom electrode of the crystal to the brass pad situated on the bottom plate, so not to allow fluid leakage and contact with the bottom electrode.

Figure 4.2 Image of DEP-QCM flow-cell
A 200μm deep and 8mm diameter recess was created in the bottom Perspex component of the holder to allow the crystal to sit in. The brass pad, 7.5mm in diameter and approximately 1cm thick, was concentrically placed within this area, with the surface of the brass pad being in the plane of the 200μm deep crevice and acting as the surface the bottom of the crystal is to be in contact with. A screw made contact to the brass pad from the side of the bottom half of the flow-cell. Around the 8mm crevice, a groove 1.5mm deep and 10mm inner diameter was created to hold a 1.6mm thick O-ring. Top and bottom components of the QCM holder were tightened together by two finger screws creating a chamber thickness, with a crystal mounted, of 150μm. Fluid inlet and outlet pipes (1mm diameter) allows continuous flow or batch operation.

4.2.3 Frequency Modulation Interface Circuit
The AT- cut quartz crystal, with a resonant frequency of 10MHz, required the two separate top halves to resonate in phase with respect to the bottom grounded electrode. At the same time, the dielectrophoretic/electro-hydrodynamic forces, with a typical low frequency band-with of 1-100 kHz was to originate from the top halves of the electrode array. This low frequency signal was supplied by a function generator (TTi Thurlby Thandar Instruments, TG120 20MHz FG) with all circuit measurements performed with a 20MHz oscilloscope (HAMEG HM203.3). Simulations of the modulating circuit were carried out on electronic workbench software (MultiSim 2000) to ensure the appropriate specification was met.

4.2.4 Experimental Setup
An impedance analyzer (HP 4194A, Agilent) was used to excite the QCM at its resonant frequency. The impedance analyser was connected to a PC which recorded the magnitude and phase angle of the crystal during experiments (single scan or continuous scan) with a program written by Dr Reddy. The output signal from the impedance analyzer was connected to the interface circuit box via a 4mm banana socket, and the ground directly to the QCM screw contact with the brass pad of the flow-cell.
A power supply was used to power the operational amplifier (TL 081HC, NI), with a gain of -1. A TTL signal generator was used to supply the ac signal through a coaxial
cable to the interface box. Two banana jacks were connected to the interface box at separate banana sockets, with the other end of the leads having crocodile clips. The leads carried the modulated inverted and non-inverted signals to the spring loaded contact electrodes of the flow cell. The maximum interface output signals measured with an oscilloscope was $10 \text{ V}_{pp} (\pm 200 \text{mV})$ up to 100 kHz for both signals.

Six single-pole single-throw switches (SPST) were situated on the interface circuit, each corresponding to a 4mm banana socket with a 7th banana socket for ground. The input signal from the signal generator was connected via the side through a BNC. Of the 6 banana sockets 5 were active (two were used to power the op-amp, one was for the input signal from the impedance analyzer and two used for the modulated output signals to the QCM), while all switches were used to control the desired signalling mode to the crystal. Images were acquired using a QX3 microscope (Intel).

![Figure 4.3 Experimental set-up used in DEP-QCM experiments](image)

### 4.2.5 Preparation of Particles

Latex beads, styrene divinylbenzene (mean diameter = 6.2μm) were obtained from Sigma-Aldrich (UK), 10% solids w/w in 2ml in pure water. Suspensions of beads ranging from $10^4$-10$^8$ beads per ml were prepared by serial dilutions of a 5μl aliquot of the stock solution into 4995μl low conductive electrolyte, KCl at 1.7mSm$^{-1}$. All suspensions had the same conductivity. Before the serial dilutions, the stock solution was ultrasonicated in a water bath for 1 hour to ensure particle dispersion.

Yeast pellets (Allinson Dried Yeast) were cultured in sterile YPD media (10ml) and incubated at 30°C for a period of 18hours. The cells were then re-suspended in
distilled water centrifuged and washed (×3) before being finally re-suspended in 1.7mSm⁻¹ KCl. Cell counts were performed on a haemocytometer. Suspensions of different cell concentrations (10⁴-10⁸ cells per ml) were made by re-suspending calculated aliquots from the stock solution into the appropriate media solution.

Stock solution of carboxylate-modified micro-spheres (Molecular Probes, Oregon USA) 0.11μm in diameter was sonicated for 45 minutes re-suspended in 2.2mSm⁻¹ KCl solution with particle concentrations ranging between 10⁴-10⁹ spheres/ml. To determine the effect of deposition position on the DEP-QCM, 0.1g of polyvinyl chloride (PVC) was dissolved in 5ml of Tetrahydrofuran (THF) solution and 1μl spot samples were pipetted at various regions of the zipper array.

4.2.6 Experimental Procedure

Using software written in Qbasic, frequency and impedance measurements detected by the impedance analyser were logged and stored on a PC. The sweep rate of the impedance analyser across the crystal was 3 seconds and a sample rate of 5 seconds was used for all continuous experiments involving the test particles.

Measurements of the dry crystals were performed in the flow-cell in continuous and single scan mode for a number of conditions. In continuous mode, measurements of the blank crystal were always started before introducing the suspension. Before the introduction of the suspensions into the flow cell, the suspensions of particles were vortexed for 30 seconds. Suspensions were manually introduced into the flow cell via a 1ml syringe until a droplet of solution was present at the outlet. This ensured that the fluid fully occupied the chamber. Experiments were allowed to run for a minimum of 30 minutes from the time the impedance analyzer started to take measurements of the blank crystal. After each experiment the crystals were taken out of the flow cell and washed with acetone and rinsed with distilled water (×3) to remove any adhered particles from the electrodes. At the end of each experimental run the measurements of the quartz crystal were exported to MATLAB for data analysis.
4.2.7 Dielectrophoretic Modulated Signal

An interface circuit (Figure 4.4) containing low-pass and high pass filters was constructed to interface between the crystal’s oscillator (HP-4194A impedance analyzer) and the signal generator.

The signal generator (V1), with an output impedance of 50Ω, had its signal divided in two, with one signal passed through an inverted op-amp (TL 081HC, ±15V) with a gain of -1. The two signals were then passed through low pass filters, $f_{3db} = 194.1$ kHz. The output signal from the impedance analyzer (V2) was measured at $1V_{pp}$ with an output impedance of 50Ω. The signal was split in two and passed through high pass filters, $f_{3db} = 958.8$ kHz. The pair of signals was mixed after the filtering stages producing two modulated waveforms, a low-pass signal carrying a high-pass signal. The only difference being the two low pass signals were out of phase by 180°, but the high-pass signals riding the low pass signals were in phase (V3 & V4) as can be seen from Figure 4.5.

The interface circuit was designed such that by including single pole switches different configurations of the signals could be used. Using a power supply (Isotech IPS112, 0-20V@1/4A) of -15V and +15V supplied to the operational amplifier, the maximum signal input to the interface box from the function generator was $20V_{pp}$ giving outputs to the DEP-QCM electrodes of $10V_{pp}$ each.

The measured output signal from the impedance analyser showed a high frequency pulsating signal with a maximum voltage of $1V_{pp}$. When modulated with the inverted
and non-inverted signals, the high frequency amplitude (impedance analyser signal) dropped at the output to 400mV ± 50mV.

![Image of oscilloscope graphs]

Figure 4.5 (a) Simulated anti-phase frequency modulated signals; (b) Section of anti-phase signals showing in-phase high frequency crystal resonating signal.

Using a high-impedance probe an amplitude signal of 40mV at 1 kHz was detected at the interface box input terminal of the impedance analyser when the function generator switch on the interface box was in the ‘on’ position. In the ‘off’ position there was no stray low frequency signal present at the impedance analyser input terminal.

4.3 Results

4.3.1 Impedance and Phase Angle Measurements of DEP-QCM

Figure 4.6 shows the electrode geometry on the top face of the quartz crystal. The pad sizes were measured as being 490μm wide, with an inter-electrode gap of 78μm. The calculated surface area of the top electrode, excluding the tags, was calculated to be 18.85mm² before etching the zipper array. After etching the electrodes surface area was reduced to 15.07mm². Owing to the positioning of the coplanar array on the quartz crystal, nearer the centre, removal of the gold electrode by etching was performed at the active centre of the crystal, reducing the active centre to what may be considered to be two separate halves. The bottom electrode of the QCM had an entire surface coverage of 18.85mm² and was found to overlap with the top electrodes providing the sensing regions.
Single scans of gold coated crystals were performed in order to obtain the magnitude (|Z|) and phase angle (θ) of impedance of the 10MHz TSM sensor, before etching the zipper pattern. The un-etched quartz crystal microbalances were placed in the flow cell and screwed close to ensure good electrical contact. A typical plot obtained for the magnitude and phase angle of impedance for the crystal are shown below for a crystal driven by two spring contacts (Figure 4.7) and one spring contact (Figure 4.8) on the top of the QCM. The magnitude of the impedance, $|Z| = \sqrt{R^2 + X^2}$, for the QCM with 2 spring loaded contacts was found to be $|Z|_{\text{max}} = 95,820 \Omega$ and $|Z|_{\text{min}} = 252 \Omega$. In comparison, for a QCM with one spring loaded contact, $|Z|_{\text{max}} = 194,300 \Omega$ and $|Z|_{\text{min}} = 231.9 \Omega$. 

Figure 4.6 Different views of the zipper electrode geometry fabricated on the quartz crystal microbalance
Figure 4.7 Un-etched QCM scan of phase angle and impedance with two spring loaded pins driving the QCM at the tag positions

Figure 4.8 Un-etched QCM scan of phase angle and impedance with one spring loaded pin driving the QCM at the tag position
Figure 4.9 Typical plot of DEP-QCM's (after etching zipper array) magnitude and phase angle of impedance

A major difference in the phase angle measurements was observed near the parallel resonant frequency. Instead of a smooth roll-off observed for the crystal driven by 2 spring loaded pins, an upward spike (>100 deg) was seen near the parallel resonant frequency, with a vertical drop at the parallel resonant frequency, crossing the zero phase angle and approaching -170 deg, before rapidly increasing to -90 deg again.

Magnitude and phase angle plots of an etched QCM driven by the impedance analyzer are shown in Figure 4.9. It can be seen that there is a decrease in the magnitude of impedance; when the QCM is etched, removing some of the coated gold layer, $|Z|_{\text{max}} = 29,200 \Omega$ and $|Z|_{\text{min}} = 186.8 \Omega$. Figure 4.10 and Figure 4.11 shows the effects of phase and magnitude of impedance depending on how the crystal is being driven and with an AC electro-kinetic force applied. When only one side of the zipper electrode is being driven, a second peak with a non-zero phase angle becomes apparent at higher frequencies. The importance of the TSM sensor possessing a frequency at phase angle zero is that the series and parallel resonant frequencies are measured at that angle. It can be seen that there were no variations in the parallel resonant frequency based on the connections to the QCM, but there are shifts in the series resonant frequency. The series resonant frequency decreases ($\Delta f_s = 4750 \text{ Hz}$), when both halves of the top
electrode are being driven. A small increase occurs when the AC electro-kinetic force is applied to the electrode modulating the signal.

Figure 4.10 Comparison of phase angle measurements based on types of electrical connections made to the DEP-QCM.

Figure 4.11 Comparison of magnitude of impedances based on types of electrical connections made to the DEP-QCM.
The effect of medium conductivity on the quartz crystal was examined and showed a decrease in series resonant frequency with increasing conductivity and application of a DEP signal (Figure 4.12). It was also observed that over the range of medium conductivities examined a reduction in the magnitude of minimum impedance occurred. This is expected for the resonator in contact with solution. The value of the reduction with respect to a dry crystal was substantial, with values ranging between 30,000 ± 200Ω.

### 4.3.2 Continuous Scans of Dry DEP-QCM

Measurements of a new clean DEP-QCM over time were performed for blank crystals with different electrical connections to the electrodes. Figure 4.13(A) shows the crystal’s response over time with only one top half being driven by the impedance analyser. Figure 4.13(B) shows the response over time when both top electrodes are being driven by the impedance analyser. Figure 4.13(C) shows the response of one side with a modulated signal (10Vpp) and the other side un-modulated, but being driven by the impedance analyser.
Figure 4.13 DEP-QCM stability of dry system when (a) one electrode on the topside is resonated, (b) both electrodes on topside are resonated and (c) when both electrodes are resonated with one electrode possessing a modulated $10V_{pp}$ 1 kHz signal.

Figure 4.14 Series resonant frequency of dry DEP-QCM with $10V_{pp}$ modulated anti-phase signals of different frequencies applied
Depending on the connection type it can be seen that there are variations in the series resonant frequencies of the etched crystal over time. With only one side of the crystal being driven by the impedance analyser, there are random shifts in the series resonant frequency around 9651670 ± 75 Hz. As was observed in the single scans, there was a drop in the series resonant frequency when both top electrodes were being driven by the impedance analyser, but the stability of the resonant frequency was significantly improved with a constant value of 9,647,350 Hz over a 1 hour period.

When one side was modulated with a 10V$_{pp}$ 1 kHz signal the series resonant frequency had random shifts around 9,648,250 ± 150 Hz. Figure 4.14 and Figure 4.15 shows the series frequency and impedance response, respectively, over time for a dry DEP-QCM modulated with anti-phase dielectrophoretic signals of varying amplitudes and frequencies sampled at 2 seconds.

![Graphs showing series frequency and impedance response over time for a dry DEP-QCM modulated with different frequencies.](image.png)

Figure 4.15 Minimum impedance of dry DEP-QCM with 10V$_{pp}$ modulated anti-phase signals of different frequencies applied
Figure 4.16 Series resonant frequency of dry DEP-QCM with 5V\text{pp} modulated anti-phase signals of different frequencies applied.

Figure 4.17 Minimum impedance of dry DEP-QCM with 10V\text{pp} modulated anti-phase signals of different frequencies applied.
With the application of a 10 kHz dielectrophoretic signal applied to both top halves of the quartz crystal the change in series resonant frequency was seen to shift around a base value with a value of approximately ± 30 Hz. A 1 kHz and 100 kHz signal also exhibited random fluctuations around a base series resonant frequency, with a shift corresponding to approximately ± 60Hz. With the exception of the 100 kHz, 10V<sub>pp</sub> dielectrophoretic signal, all series impedances fluctuated over time but by no more than 2 Ω.

4.3.3 Effect of Medium Conductivity on DEP-QCM

Fluid motion has been shown to be a major factor in driving particles to the surface of a coplanar electrode when a low frequency potential is applied to the electrodes [19]. The vortices created by this signal were studied for a number of low-conductivity media on the DEP-QCM to determine whether the crystal’s response was influenced by the fluid motion rather than the collection of particles on to the surface of the electrodes. Figure 4.18 and Figure 4.19 show the response of the crystal over time for KCl conductivities ranging between 0.26mS<sup>-1</sup> – 15.5mS<sup>-1</sup> with no DEP signal applied to the electrode array.

![Figure 4.18 Shift in series frequency over time for varied medium conductivities with no DEP signal](image-url)
As the medium conductivity increased, the shift in the crystal’s series resonant frequency decreased, upon crystal loading. Over time, experiments showed that the crystal response was shown to rapidly achieve a constant value for all conductivities, apart for 2.6mSm⁻¹ where the value varied by approximately 10% between 1000sec and 1200 sec, after which it levelled off again. Impedance measurements at the series resonant frequencies showed an increase in impedance when a solution is loaded on to the DEP-QCM when no DEP signal was applied. There was no noticeable correlation in the impedance measurements with media conductivity. Figure 4.20 shows the impedance measurements for a loaded crystal (blue) and a blank crystal (green). An observation with regards to the impedance measurements was the periodicity of peaks for both blank and loaded crystals. For blank crystals, the impedance shift was found to be 3 Ω while for a loaded crystal the impedance shift was 6Ω. The periodicity of the peaking was found to be in the same range for both loaded and unloaded crystals, with frequencies ranging between 4.4-5.5 mHz.

Application of a DEP signal to electrodes with same conductive media loading were measured to compare the responses of the DEP-QCM with the results of no DEP.
Figure 4.20 Typical impedance shifts observed for loaded (blue line) and unloaded (green line) DEP-QCM

Figure 4.21 Graphs showing the shifts of the DEP-QCM's series resonant frequency response to electro-hydrodynamic forces based on variations in medium conductivity
signal. With the application of a 1 kHz, 10V_{pp} DEP signal, the DEP-QCM's response of the fluid in motion can be seen in Figure 4.21.

Closer examination of the DEP-QCM's response to different conductivities, with no DEP signals, can be seen in Figure 4.22. It can be seen that the series frequency shift for the crystal varies significantly when the DEP signal is applied to the electrodes in comparison to no signal. There is also a significant difference in the range of frequency shift based on the conductivity of the medium. The higher the medium conductivity, the larger the frequency shifting range, while the range of frequency shifting for the DEP-QCM with no signal applied showed to be

![Graphs showing the shifts of the DEP-QCM's series resonant frequency response to static fluid based on variations in medium conductivity](image)

**Figure 4.22** Graphs showing the shifts of the DEP-QCM's series resonant frequency response to static fluid based on variations in medium conductivity
<table>
<thead>
<tr>
<th>Conductivity (mSm⁻¹)</th>
<th>Range of frequency shift for DEP-QCM with no DEP signal (Hz)</th>
<th>Range of frequency shift for DEP-QCM with a 10V_{pp}, 1kHz DEP signal (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.26</td>
<td>300</td>
<td>673</td>
</tr>
<tr>
<td>2.67</td>
<td>300</td>
<td>875</td>
</tr>
<tr>
<td>6.51</td>
<td>300</td>
<td>1947</td>
</tr>
<tr>
<td>9.91</td>
<td>300</td>
<td>2227</td>
</tr>
<tr>
<td>15.1</td>
<td>300</td>
<td>2358</td>
</tr>
</tbody>
</table>

Table 4.1 Range of frequency shifts observed for static fluid and fluid motion for DEP-QCM as a function of medium conductivity

largely invariant with respect to changes on the medium conductivity, with a range of 300Hz which is very similar to the shifts associated with varying the electrical connection to the crystal.

4.3.4 Spot Depositions on DEP-QCM

To determine the effect of the crystal response based on position of the adsorbate Polyvinyl chloride dissolved in tetrahydrofuran solution was deposited on various areas of the zipper array. Figure 4.23 shows the positions of the depositions on the DEP-QCM. Each 1μl spot deposition had an average area coverage of 7.1mm², with the THF solution evaporating within 1 minute of exposure to air. The surface coverage of PVC on the DEP-QCM after THF evaporation was a thin continuous film. Impedance and frequency measurements of the spot deposition of PVC can be seen in Figure 4.24 and Figure 4.25 respectively. It shows that on loading the crystal with a micro-drop at specific locations, there is a difference in the crystals response. The initial frequency response on loading is a drop in frequency, with the largest shift seen for depositions away from the zipper pads (-11450 ± 350Hz). When the solution evaporated, the frequency was seen to increase across the quartz crystal, with the highest frequency shift seen for the deposition at the centre of the array (+31,800 Hz).
A characteristic trend for the frequency shifts for all depositions apart from at the centre of the zipper array is the immediate sharp fall in frequency after the initial frequency rise. There is then a gradual rise in frequency. No such trend is seen for deposition at the centre of the zipper array, where after the initial rise the frequency remains level for the duration of the experiment. The impedance measurements (Figure 4.25) show that for all spot depositions there is an increase in impedance, with the maximum shift shown for the deposition at the centre of the zipper array. After the initial rise in impedance, there is a gradual fall over time for all spot depositions apart from the deposition at the centre of the zipper array, to a level just above the unloaded crystal. This level ranges between 50-170Ω, while for the deposition at the centre of the zipper array, the initial fall in impedance is approximately 1100Ω above the unloaded crystal and over time there is an overall increase in impedance to 1250Ω.
Figure 4.24 Series frequency response of the spot depositions of PVC-THF solutions
4.3.5 Manipulation of Micrometer Sized Particles

A DEP-QCM was used to detect and quantify the collection of yeast cells at the sensor surface, at concentrations of between $10^8$ and $10^6$ cells per ml suspended in 1.1mSm$^{-1}$ KCl solution. The electrical connection used was such that only one top half of the zipper array had a modulated dielectrophoretic signal with 10V$_{pp}$ amplitude and a frequency of 1 kHz, but both top electrodes were used as a sensing region, due to both being connected to the impedance analyser. Before introducing the samples into the flow cell, both impedance analyser and signal generator were switched on. The introduction times for the both samples were measured from the impedance and

---

Figure 4.25 Minimum impedance response of the spot depositions of PVC-THF solutions
frequency measuring software on the PC. There was a delay of approximately 180 seconds before introduction of the samples into the flow-cell. Figure 4.26 shows the series frequency shift and the minimum impedance shift for both concentration samples. It can be seen that from the point of introduction of the sample, there is an increase in impedance and a decrease in frequency, with the frequency decrease for the $10^8$ cell/ml being very steep during the first 100 seconds, whilst the $10^6$ cell/ml sample shows a continuous, gradual slope, throughout the run time. Another noticeable feature with the $10^8$ cell/ml sample is a small rise in the frequency slope at around 500 seconds which begins to steadily decrease at 550 seconds, with a second dip observed at 1050 seconds.

![Figure 4.26 Minimum impedance shift of DEP enhanced collection for yeast of varied concentrations with signal applied before sample introduction](image)
Figure 4.27 shows images of the DEP-QCM zipper array suspended with a $10^8$ cell/ml sample of yeast cells captured with a QX3 Intel microscope at different time points during the experiment. As can be seen the characteristic particle motion of the zipper array is to concentrate particles on the pads in a teardrop fashion, while at the same time pushing particles away from the edges of the electrode (the black meander is the inter-electrode gap). From the images shown here and observations throughout the experiments, a front is built up a distance away from the outer electrode edge, and while this occurs particles are also dragged from this area through the pads neck unto the pad, clearing the area of any particles. It can be also seen that the force originating from the left electrode (un-modulated) is not as strong as the right, as it has been observed that the created front is of the same size when a DEP signal is applied to the zipper array.
4.3.6 Anti-phase Modulated DEP signal

Figure 4.28 shows the frequency shift for yeast cells ($10^4$-$10^8$ cells/ml) suspended in 17.2μS/cm KCl solution directed on to the surface of the DEP-QCM. The vertical drop in series frequency for each graph shows the DEP-QCM being loaded with the samples. All the graphs apart from the $10^4$ cell/ml sample (DEP signal switched on at 146 seconds) show no variation in response to the sample as soon as it comes into contact with the DEP-QCM, apart from the shift in frequency. As soon as the DEP signal is switched on for the $10^4$ cell/ml sample there was a small but sharp decrease in the resonant series frequency (~700Hz). There were no obvious effects observed when the DEP signals were switched on for the $10^5$ and $10^6$ cell/ml samples (326 and 271 seconds respectively). When the sample from the $10^8$ cell/ml solution was added the frequency drop was found to be 3910 Hz, larger than the other samples frequency shifts. On application of the DEP signal at 481 seconds, an immediate increase in the frequency by 1235 Hz was seen. This was immediately followed by a gradual decrease to its stable frequency range over a 160 second period, with continual reduction. Clearer detection may be seen from the impedance measurements obtained in Figure 4.29.

![Figure 4.28](image-url)
It shows a gradual increase in impedance for the 2 lowest sample concentrations whilst showing an immediate effect on the 2 highest sample concentrations upon application of the DEP signal.

Minimum impedance shifts of DEP enhanced collection for yeast of varied concentrations with signal applied after sample introduction

291 sec 676 sec 776 sec

876 sec 1056 sec 1153 sec

(figure 4.29 continued on next page)
Figure 4.29 Time lapse images of particle collection for a sample volume of $10^4$ yeast cells per ml

Figure 4.30 Time lapse images of particle collection for a sample volume of $10^6$ yeast cells per ml

Figure 4.31 Time lapse images of particle collection for a sample volume of $10^8$ yeast cells per ml
Minimum impedance measurements for latex beads collected on the DEP-QCM surface shows that a slope develops over time which seems to be concentration dependent. In contrast, the series frequency shift does not indicate any form of detection over time for concentrations less than $10^8$ beads/ml. The magnitude of the initial frequency shift of the crystal upon loading of different sample concentrations is seen to increase as the concentration increases, with a significant increase over time for $10^8$ beads/ml sample concentration.

![Graphs showing minimum impedance shifts and series frequency shifts for different sample concentrations.](attachment:image.png)

**Figure 4.32** Minimum impedance shifts of DEP enhanced collection for latex beads (6.2μm diameter) of varied concentrations with signal applied after sample introduction.

**Figure 4.33** Series frequency shifts of DEP enhanced collection for latex beads (6.2μm diameter) of varied concentrations with signal applied after sample introduction.
4.3.7 Manipulation of Nano-particles

On establishing that there is a response to the zipper array being loaded with micrometer sized particles of various concentrations, the response of the DEP-QCM loaded with nano-particles was investigated using 0.11µm nano-spheres. The crystal’s response to loading of various concentrations can be seen in Figure 4.35 and Figure 4.36.

![Figure 4.34 Series frequency shifts of DEP enhanced collection for nano-spheres (0.11µm diameter) of varied concentrations with signal applied after sample introduction](image1)

![Figure 4.35 Minimum impedance shifts of DEP enhanced collection for nano-spheres (0.11µm diameter) of varied concentrations with signal applied after sample introduction](image2)
The crystal’s frequency response to an initial sample loading shows an initial shift in frequency of approximately -4000Hz with sample loadings of all concentrations. On application of a 10V\text{pp}, 1 kHz dielectrophoretic signal the frequency response of the crystal varies in magnitude over time for the different sample concentrations used. At the end of each experimental run the final series frequency of the crystal was found to have changed -4400Hz, -6000Hz, -6500Hz and -6800Hz for sample concentrations of $10^5$, $10^6$, $10^7$ and $10^8$ nano-spheres per ml respectively, indicating a relationship between frequency shift and the mass loading of the nano-particles. Minimum impedance responses of the crystal for various sample concentrations show that upon initial introduction of the sample into the flow-cell, an increase in impedance of 170±10Ω. Over time the impedance increased at various rates, with final values of 189.9Ω, 257.8Ω, 279.6Ω and 294.6Ω for sample concentrations of $10^5$, $10^6$, $10^7$ and $10^8$ nano-spheres per ml respectively.

4.3.8 Quantification of Rate of DEP-QCM Response

From the impedance and frequency response curves of the DEP-QCM it is possible to obtain the rate of detection per sample concentration due to particle manipulation effects contributed by ac electro-osmotic flow, in particular for sub-micrometer particles. By obtaining data measurements at specific points in the course of an experiment, the rate of the crystals response to loading can be determined. The first time point (initial response) corresponds to the point at which the introduced sample is in contact with DEP-QCM. The second time point (early stage) is the time at which the dielectrophoretic signal was applied to the zipper array. The third time point corresponds to 240 (middle stage) seconds after the introduction of the samples into the flow-cell, while the fourth time point corresponds to 480 (late stage) seconds after the samples were introduced into the flow cell until the end. The final time point corresponds to the final value reached at the end of the experiment.

Analysis over time of the crystal’s response after the application of the dielectrophoretic signal shows that there is a difference in the rate the crystal reaches a stable impedance or frequency value. This rate is seen to be concentration dependent and shows that as the concentration increases the time it takes for the crystal to reach a stable value decreases. Figure 4.36 and Figure 4.37 shows graphs of the cumulative frequency change over time and the rate of the frequency response when samples of
nano-particles are loaded on to the DEP-QCM through electro-osmotic flow. It can be seen that the crystals response to fluid contact upon introducing the suspension varies.

Figure 4.36 Total change in DEP-QCM frequency response to nano-sphere collection through DEP and EHD forces as a function of particle concentration

Figure 4.37 Rate of crystal’s series frequency response to DEP/EHD manipulation of varied nano-sphere concentrations
After the samples were introduced to the flow-cell the DEP signal was applied 100 ± 5 seconds later. Hence at approximately 135 seconds and 375 seconds after the DEP signal was applied the response of the crystal was sampled.

Figure 4.38 Total change in DEP-QCM impedance response to nano-sphere collection through DEP and EHD forces as a function of particle concentration

Figure 4.39 Rate of crystal’s minimum impedance response to DEP/EHD manipulation of varied nano-sphere concentrations
The cumulative change in frequency over the duration of the experiment show that the sample concentrations of $10^5$, $10^6$, $10^8$ and $10^9$ nano-spheres per ml have increased to 1.6 Hz s$^{-1}$, 14.2 Hz s$^{-1}$, 15.0 Hz s$^{-1}$ and 18.3 Hz s$^{-1}$ respectively. Similarly, final impedance values over time (Ωs$^{-1}$) increased as the sample concentration increased with values of 0.03 Ωs$^{-1}$, 0.55 Ωs$^{-1}$, 0.66 Ωs$^{-1}$ and 0.88 Ωs$^{-1}$ for sample concentrations of $10^5$, $10^6$, $10^8$ and $10^9$ nano-spheres per ml respectively. The rate of detection based on frequency and impedance measurements were calculated and are shown in Table 4.2 and Table 4.3 respectively. When the DEP signal is applied to the electrodes, the crystal’s response rate is seen to increase for all sample concentrations.

<table>
<thead>
<tr>
<th>Sample concentration (particles/ml)</th>
<th>Initial response to sample load (s$^{-1}$)</th>
<th>Early stage of ac electro-osmotic flow (s$^{-1}$)</th>
<th>Middle stage of ac electro-osmotic flow (s$^{-1}$)</th>
<th>Late stage of ac electro-osmotic flow (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3$</td>
<td>0.00023</td>
<td>0.00000</td>
<td>0.00010</td>
<td>0.00004</td>
</tr>
<tr>
<td>$10^6$</td>
<td>0.00179</td>
<td>0.00078</td>
<td>0.00022</td>
<td>0.00006</td>
</tr>
<tr>
<td>$10^8$</td>
<td>0.00090</td>
<td>0.00140</td>
<td>0.00038</td>
<td>0.00000</td>
</tr>
<tr>
<td>$10^9$</td>
<td>0.00093</td>
<td>0.00205</td>
<td>0.00013</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

Table 4.2 Rates of detection based on DEP-QCM series frequency measurements at varied stages

<table>
<thead>
<tr>
<th>Sample concentration (particles/ml)</th>
<th>Initial response to sample load (s$^{-1}$)</th>
<th>Early stage of ac electro-osmotic flow (s$^{-1}$)</th>
<th>Middle stage of ac electro-osmotic flow (s$^{-1}$)</th>
<th>Late stage of ac electro-osmotic flow (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^5$</td>
<td>0.00014</td>
<td>0.000004</td>
<td>0.00002</td>
<td>0.00003</td>
</tr>
<tr>
<td>$10^6$</td>
<td>0.00157</td>
<td>0.00072</td>
<td>0.00034</td>
<td>0.00010</td>
</tr>
<tr>
<td>$10^8$</td>
<td>0.00106</td>
<td>0.00138</td>
<td>0.00034</td>
<td>0.00001</td>
</tr>
<tr>
<td>$10^9$</td>
<td>0.00124</td>
<td>0.00219</td>
<td>0.00021</td>
<td>0.00004</td>
</tr>
</tbody>
</table>

Table 4.3 Rate of detection based on DEP-QCM minimum impedance measurements at varied stages
The magnitude of the rate increase varied depending on the period analysed, but was generally found to be greatest immediately after the application of the DEP signal. The early stage response of the crystal to the DEP signal show that the rate at which the crystal is responding to mass loading increases almost linearly as the sample concentration increases, with typical rates ranging between 1-2 milliseconds. This gradually reduces in the middle stage and almost diminishes by the end of the late stages. These trends can be seen in Figure 4.40a and Figure 4.40b.

![Figure 4.40 DEP-QCM detection rates for micro-spheres as a function of concentration for a) frequency shifts and b) impedance shifts](image_url)
4.4 Discussion

4.4.1 Dual Active Area

Quartz crystals operating in thickness shear mode have in the past been used as a quantitative mass measuring sensor [26-29]. The active area of a quartz crystal microbalance (QCM) resides in the region where there is an overlap of electrodes on the major faces of the crystal [30]. According to Sauerbrey's equation, loading the QCM with a uniform thin film across the active area produces a decrease in the crystals resonant frequency which is proportional to the mass of the load [13].

In the work presented here, we have developed a novel integrated dielectrophoretic QCM (DEP-QCM) whereby the top electrode is divided into two equal regions by etching away approximately 75µm in width near the centre and across the diameter of the electrode. This predefined geometry, known as the zipper array [25], has effectively created two active areas on a single quartz crystal with a common reference electrode at the bottom.

Excitation of one half of the top electrode with no mass load showed 3 characteristic peaks (Figure 4.10) of which 2 had phase angle peaks below zero degrees. This indicated that for the electrical connection used the crystals series and parallel resonant frequencies were not in that region. The third peak had a phase angle maximum >60 degrees, with the rise and fall passing zero degrees at ~9.654MHz (series resonant frequency) and ~9.667MHz (parallel resonant frequency) respectively. With both halves excited, a decrease in series resonant frequency was observed (~9.648 MHz) with no change in parallel resonant frequency and only one characteristic peak (>70 degrees) as typically seen for a quartz crystal microbalance operating in thickness shear mode [31]. This possibly indicates that when one half of the crystal is excited, the shear deformation of the crystal is occurring on one side of the crystal with most of the energy being trapped in the smaller overlapping area. The un-excited electrode overlapping with the bottom electrode interferes with and attenuates the crystal oscillation. The application of a second excitation signal allows the whole crystal to oscillate more or less in unison with the presence of the inter electrode gap and hence has the energy trapped in a larger area between the top electrodes.

The response of the dry crystal over time with a dielectrophoretic signal superimposed (5 second sampling) showed fluctuations between set intervals for the series
resonant frequency. It has been previously described using Mindlin’s Theory that frequency interference between two adjacent QCMs can occur [32]. This was described for a dual QCM with separate overlapping top and bottom electrodes fabricated on a single quartz crystal. Factors affecting the frequency interference and hence the mass sensitivity were found to depend on the separation distance and electrode width distance, with larger electrodes reducing interference.

4.4.2 DEP-QCM System Dynamics

The oscillations of a perturbed quartz crystal microbalance allows real time mass detection of adsorbates at the electrode surface [3, 33, 34]. In the liquid phase, the dynamic response of the crystal at the interface has also been shown to be affected not only by the thin mass added on the surface but also by the viscoelastic and density properties of the liquid [14, 35], the dielectric constant of both quartz and solution, solution conductivity and acousto-electric coupling [36].

Electro-hydrodynamic (EHD) forces originating from coplanar electrodes with applied low frequency voltages manipulate particles in bulk solution so as to concentrate them on the electrode surface [18]. Using these effects, for the first time the zipper array dielectrophoretic-QCM has quantified the rate of particle collection from the bulk solution using ac electro-kinetics, in real time. The dynamics of particles manipulated by EHD, specifically ac electro-osmosis, has been described to be influenced by the induced motion of the fluid surrounding the particle [19, 37]. Due to the double layer polarisation effects, see Figure 2.8, the time dependent tangential electric field and the time dependent excess charge mean the velocity of the fluid varies with applied frequency changes. The time average electrical force is found by Equation 2.32, and it shows it to be dependent on a number of variables including the medium conductivity and permittivity and the applied frequency. The conductivity-permittivity ratio dictates which property of the fluid has more of an influence on fluid motion. With an angular frequency of 6283.19 rad/sec used in our experiments, much less than the ratio, it was concluded that the electrical forces were predominantly due to the conductivity gradients. The dynamic response of the DEP-QCM to varying conductivity media showed that when no dielectrophoretic signal was superimposed on the crystal’s excitation signal the ± range in series resonant frequency shift was found to be constant at ~3 00Hz. With the application of a 1 kHz 10V_{pp} dielectrophoretic signal the fluid was set into motion, with crystal oscillations
increasing in range as the medium increased in conductivity. The medium conductivity is purported to influence the velocity of the fluid convection [38], which may also have an influence on the magnitude of the crystals response, whereby the fluctuating hydraulic pressure on the crystal increases with an increase in conductivity [39].

4.4.3 Sensitivity
A decrease in series resonant frequency was seen when suspensions of particles were introduced into the flow cell. This is the typical response of a traditional QCM loaded with a liquid [14]. Spots of polyvinyl chloride in tetrahydrofuran solution were deposited at various locations on the zipper array DEP-QCM (Figure 4.23). The results of these depositions show that the etching process did not affect the sensitivity of the crystal with the dual active area. On spotting, the initial shift in series resonant frequency was seen to decrease as usual with the liquid loading. Evaporation of tetrahydrofuran occurs when exposed to air. This process was observed to take place within 1 minute of exposure, leaving behind a thin film of polyvinyl chloride. On evaporation of the liquid a sharp increase in series resonant frequency was seen for all depositions. The positive increase in frequency was greatest for the spot deposition at the centre of the DEP-QCM, with a final resting frequency greater than the unloaded crystal resonant frequency. As the spot depositions approached the edge of the DEP-QCM, the final resting frequencies were also greater than the initial unloaded crystal resonant frequency, but approximately 18,850±150 Hz less than the DEP-QCM crystal with a thin film of polyvinyl chloride at the centre.

The sensitivity of a thickness shear vibrating crystal has its maximum value at or close to the centre of the quartz plate and diminishes as the edges are approached. This has been supported by studies carried out where evaporating small spots of solids, dragging the tip of a wire across the electrode and electrodepositing spots along the crystal diameter have been conducted [5, 30, 40]. The frequency shift of the crystal varies along the crystal diameter, following a Gaussian distribution, where there exists a differential mass sensitivity of both solids and liquids which has its maximum at the crystal centre [41]. This has been verified here by the magnitude of the frequency shifts observed.

Positive increases in frequency shift have previously been observed for a loaded QCM [42-45]. It has been suggested that the increase in frequency comes about due to a
change in viscosity with the establishment of a coupled resonance. On the evaporation of THF, the polyvinyl chloride residue increases in cohesion with the electrode surface. This increases the mechanical stiffness of the adsorbate in the absence of liquid. The dielectric constant of polyvinyl chloride depending on formulation is \( \sim 3.405 \) and for quartz, 4.52. They are both piezoelectric materials, and the formation of a new 'coupled resonator' on complete evaporation of the liquid could explain the observed frequency decreases to a final resonant frequency just above the crystal original resonant frequency.

4.4.4 Response to Particle Manipulation

When oscillating in thickness shear mode, the crystal surface induces an exponentially decaying shear wave into the liquid. The decay length of the shear wave (\( \delta \)) is the distance into the liquid were the amplitude of the shear wave has fallen by a factor \( e \) and is defined by,

\[
\delta = \sqrt{\frac{2\eta_f}{\omega \rho_f}}
\]

Equation 4.1

Where \( \eta_f \) is the dynamic viscosity of the medium, \( \rho_f \) is the density of the medium and \( \omega \) is the angular frequency of the crystal's resonant frequency. For a 10MHz crystal, with \( \eta_f = 1.002cP \) and \( \rho_f = 998.29 \text{ kg m}^3 \) at 20°C, the decay length is 5650nm and for a crystal with a resonant frequency of 5MHz this reduces to 2520nm.

On inspection of the series resonant frequency and minimum impedance responses of the DEP-QCM with anti-phased modulated signals for micrometer sized particles, the application of an ac electro-kinetic force did not seem to produce a noticeable shift in frequency for sample concentrations less than or equal to \( 10^7 \) cells/ml. Observed was an initial shift in frequency for all sample concentrations, which may again correlate to the DEP-QCM detecting the viscosity/density of the medium. It was observed that the higher the sample concentration the higher the shift in series resonant frequency upon loading. For sample concentrations of \( 10^8 \) beads or yeast cells per ml, on application of the electric field, there is an initial positive increase in series resonant frequency which could be due to the initial close packing of the cells onto the
electrode surface or some form of initial dielectric coupling of the particles with the quartz interface near the electrode edges. The close packing reduces the areal density that the particles occupy on the crystal surface hence causing an increase in the series frequency. Also concentrating these large diameter particles in a smaller region mean that the shear wave propagating in the fluid only ‘sees’ a fraction of some particles as more pile up on top of each other. As this occurs, the re-circulating fluid carrying the particles introduce more particles to within the crystal’s decay length and more particles begin to travel tangential to the electrode surface collecting at centre of the electrode pads. This could explain the apparent negative shift in resonant frequency subsequently seen. Shift in DEP-QCM minimum impedance for the manipulation of micrometer particles showed that over time, increases were observed for sample concentrations as low as $10^4$ particles per ml, with immediate but unpredictable effects occurring for $10^8$ particles per ml.

The DEP-QCM response to the manipulation of submicron particles on the application of the ac electro-kinetic signal provided a smoother transition over time for sample concentrations as low as $10^5$ particles per ml. This indicated that the collection of smaller sized particles on the electrode surface was detected with more sensitivity than the larger sized particles. For a 10MHz crystal with one side in contact with a fluid the shear wave propagating in to the fluid is about 565nm. This value indicates the upper limit distance above the crystal surface where mass loading is detected by the crystal and is reflected by the shift in frequency. Figure 4.41 shows a schematic diagram of yeast particles (green) and the micro-spheres (black) modelled in the bulk solution experiencing EHD and collecting on the DEP-QCM electrode surface. It shows that on a single pad ($\sim$500μm diameter) the number of particles in contact with the electrode-solution interface is dramatically reduced as the diameter increases. This causes regions on the electrode surface which become vacant of mass as particle size increase.

Collection of polystyrene micro-spheres (0.11μm mean diameter), have previously been demonstrated using the zipper array of the dimensions used here and at the same frequency [21]. Direct observation of these particles being manipulated were done by fluorescent microscopy and showed instant effects. Due to the size of the particles the ability for the quartz crystal to detect a whole particle near the surface is increased
and the propagation of the shear wave extends 4 times the radius of the particle into the medium.

![Figure 4.41 Diagonal cross-section across adjacent interlocking pads illustrating DEP/EHD forces on yeast (green) and micro-spheres (black); The different sized particles demonstrate the difference in rigid packing capable at the electrode surface while the fluid lines indicate the fluid motion occurring tangentially and orthogonal to the electrode surface.](image)

Also, the close packing of the particles provide more of a rigid layer as the size and number of vacant spaces within the area density will be significantly reduced compared to those created by yeast cells. These effects are evident by the smooth trends observed for series resonant frequency shifts as a function of concentration on application of the ac electro-kinetic field. When the sample concentration increases, the magnitude of the frequency shift is observed to also increase. On application of the ac field, varied time-based responses were seen to occur. These too were concentration based, but seemed to happen fastest for higher particle densities. Values of detection rates observed for when the saturation frequency shifts of the DEP-QCM were reached range from \(2.5 \times 10^{-4}\) to \(3 \times 10^{-3}\) Hz \(s^{-1}\), with similar rate changes calculated for the minimum impedance shifts. The response rates were found to be linear with concentration on the initial application of the ac electro-kinetic signal. Experiments carried out for the micro-spheres with no ac electro-kinetic signal applied and allowed to settle over time did not show similar trends as previously seen. Higher particle concentrations showed small frequency shifts while the lower concentrations hardly showed a shift. This is likely due to the size and density of the particle which has buoyancy and with gravitational forces acting on these size particles, the terminal settling velocity is very slow. Previous studies with nano-spheres (~\(10^{10}\) per ml) electro-statically attracted to the QCM surface showed that steady state responses were reached after a period of 30 minutes [46]. It has also been
reported that real time detection of *L. monocytogenes* at concentrations of $1 \times 10^8$ cells/ml with antibody coated crystals gave a response rate greater than 1 Hz s$^{-1}$ [33]. This is significantly slower than what we have achieved here, with particles substantially smaller in volume, we were able to achieve responses due to ac electro-kinetic manipulation of nano-spheres greater than 18 Hz s$^{-1}$, and reaching steady-state 5 times faster than other QCM adsorption techniques.

### 4.5 Conclusions

By integrating ac electro-kinetic techniques with a 10MHz AT-cut bulk acoustic wave (BAW) sensor, mass changes at the electrode-solution interface have been detected for submicron particles. The mechanism of detection is caused by the rigid packing of particles at specific locations close to the crystal’s centre, on the electrode surface, which causes the resonant frequency of the crystal to decrease with added mass. Particles greater than one micron in diameter were found to be less sensitive to collection on the electrode surface because of the lesser areal coverage these particles exhibited with respect to the decay length of the crystal.

Images of particle manipulation were observed for yeast cells while simultaneously, series and impedance measurements with little change were being collected. This indicated that the DEP-QCM set-up did in fact allow the crystal to operate with minimal deviation from its resonant frequency. The electrode geometry used has been observed to produce spurious frequency shifts at the resonant frequency, but remain to be within a predictable or reproducible range close to the resonant frequency. Signal modulation did not affect the crystal response over time, but due to the ac electro-osmotic effects imparted by the low-frequency field, gradients in fluid permittivity and conductivity gave rise to fluid motion. This motion is detected by the crystal and is believed to vary as the conductivity rises. The increase in conductivity produces shifts in resonant frequencies, and the range of the shifts increase with the rise in solution ionic concentration. Hydrostatic pressures and electrical body forces arising from the fluid motion may be the reason for the range of shifts observed because it has been reported that fluid motion is dependent on the applied frequency and the solution conductivity.
This novel biosensing system has been shown to increase particle detection rates by up to 6 times faster than other modified-QCM techniques and enhances the effects of gravitational and sedimentation forces.
4.6 References


Chapter 5

Micro-fluidic Pre-concentration of Biological Cells:
‘DEP vs. EHD’

5.1 Introduction

The generation of non-uniform electric fields to separate or pre-concentrate particles in fluid streams is an attractive alternative to methods such as liquid-liquid extraction, size exclusion chromatography, membrane filtration and high pressure liquid chromatography. This is primarily due to the non-destructive, yet extremely sensitive manner in which the electric fields can interact with the particles of interest [1-4]. Also, the technique is a low cost alternative to the techniques previously mentioned. It has been shown that fluid flowing over electrode arrays of various geometries and dimension are able to attract or repel particles suspended in the fluid [5-8]. In addition, the field gradients created by certain electrode geometries can redirect particles to certain areas for subsequent processing. Some common strategies developed for dielectrophoretic processing of particles in fluid flow include flow separation, dielectrophoretic field-flow fractionation (DEP-FFF), stepped flow separation, travelling wave dielectrophoresis (twDEP) [9, 10]. The general principle behind all these strategies is that flow is introduced into the chamber housing the electrode array and depending on the nature of the applied electric field particles can be attracted (positive DEP) or repelled (negative DEP) from the electrode edge. Other phenomena which can occur are the particles being levitated at different heights in the fluidic chamber (all experiencing negative DEP) according to their density and polarisability [11]. The flow within the chamber takes on a parabolic profile and the velocity of the fluid flow will vary across the height of the chamber. This has the effect of transporting particles towards the outlet port at varying spatial times depending on their position. Travelling-wave dielectrophoresis uses the change in phase of the imposed electric field on the particle. First described by Batchelder [12], the force exerted on a particle can be made to act in a direction parallel to the electrode surface. This method, unlike the others described, requires the need for interdigitated electrodes to be periodically energised with sine or square wave signals of equal phase relationships totalling 360°. This poses the problem of supplying
power of the type required to each electrode in the array. But it has been successfully shown to induce controlled motion of red blood cells [13], pollen and cellulose particles [14] and separation by fractionation of yeast cells [15, 16]. This method effectively pumps the content of a suspension without actually moving the medium itself. Although, in fluid flow the particles can be redirected against fluid flow (depending on flow rate and particle properties) by pumping the particle in the opposite direction, hence increasing the residence time in the chamber for particles of interest and collecting them at different outlets.

The work presented here describes a designed ac-electro-kinetic flow cell which was fabricated to perform pre-concentration experiments on yeast cells. Assessment of trapping efficiencies for dielectrophoretic (DEP) and electro-hydrodynamic (EHD) forces against different flow-rates were studied and the effects of exposure time to the electric field gradients on these particles were evaluated.

5.2 Materials and Method

5.2.1 Considerations of Micro-fluidic System

A micro-fluidic system housing a microelectrode array was designed for the experiments carried out. The design of the system was heavily based on the dimensions of the substrate on which the electrode array was fabricated on. The size of the substrate (half a microscope slide) was measured to be 36×26×1 mm. The length of the chamber should be as long as possible to accommodate as many electrode bars as possible. This increases the residence time of the particle in the chamber and its exposure to the field gradients along the chamber. The height of the chamber should be set so as to balance between the size and concentration of particles to be investigated and the penetration of the electric field gradient into the chamber. For particles with an average radius of 4 μm chamber heights >100μm but < 500μm were deemed appropriate. The setting of this condition reduces the possibilities of trapped particles at the electrodes blocking the path of flow of other particles higher up in the flow stream. The size of the channels leading to and exiting the chamber need to be chosen carefully so blockage is avoided. This can be alleviated by reducing the overall volume of the system i.e. piping to and from the micro-system, which will reduce the residence time in the channels and dead spaces around the system.

The easy access for replacing or cleaning electrodes is preferable, allowing...
reproducible experimental procedures to be carried out. Also the application of voltages to the electrode array needs to be taken account with regards to the electrode arrangement, the number of voltage signals potentially needed and the ease of aligning electrical connectors to the electrode pads. These concerns will be addressed in more detail in the results section.

5.2.2 Interdigitated Microelectrode Array
The microelectrode array used in this study was an interdigitated non castellated array. Gold coated microscope slides, courtesy of the EPSRC National Centre for III-V Technologies (Electrical and Electronic Engineering Department, University of Sheffield), were cut in half with a glass cutting machine (Dr. Brian LePage, Civil Engineering Department, University of Surrey). The array was designed and fabricated using the technique described in Chapter 3 (3.2.1).

5.2.3 Sample Preparation
Before every experiment, yeast cells were freshly harvested in a 20ml Sterilin centrifuge tube with sterile YPD broth for an average of 16 hours at 37° C. Before taking a sample from the stock solution, the tube was centrifuged for 30 seconds and a 1ml aliquot was taken out and re-suspended in a micro-centrifuge tube. The sample was spun down in a micro-centrifuge for 5 minutes at 1400 rpm and the supernatant was decanted with a pipette. The settled cells were then washed with 4.1mSm⁻¹ of KCl solution buffered with 280mM D-Mannitol then agitated with a mixer for 30 seconds. This procedure was carried out 3 times to remove waste products and non-viable cells and to attain the required medium conductivity. Final concentrations (viable and non-viable) were determined before each experiment

5.2.4 Determination of Viable and Non-viable Yeast
A rapid technique for determining viable and non-viable yeast cells was described by Lee et al [17]. A solution containing methylene blue (MB) dye was made up to 200ml in a sterile bottle. Aliquots of MB were mixed accordingly with cell samples before cell counts took place in the haemocytometer. Cell counts were taken before injection into the micro-system and for the two collections downstream, i.e. cells not retained and cells eluted after retention.
5.2.5 Continuous-flow Process Design

The collection of particles in a micro-system under non-uniform electric fields is frequency dependent and can occur at three different locations:

- At the electrode edge due to positive dielectrophoresis
- Away from the electrode region due to negative dielectrophoresis
- On the electrode surface due to electro-hydrodynamic effects

To assess the effectiveness of particle retention under fluid flow, two types of processes will be used. These are positive dielectrophoresis (p-DEP) and electro-hydrodynamic (EHD) flow. These two processes are distinct from each other with respect to the frequencies used to collect the particles in the electrode vicinity. Ideally, the process of particle collection under conventional positive dielectrophoresis aligns particles at the edges of the electrodes (Figure 5.1), with pearl chains forming between particles into the inter-electrode gap. Under electro-hydrodynamic flow, particles are ideally collected on the surface of the electrode array (Figure 5.2).

5.2.5.1 Particle retention using p-DEP

Freshly prepared and counted samples with a total concentration of \( \sim 10^6 \) cells per ml were loaded into a 5ml plastic tapered-end syringe. The syringe was placed in a syringe pump (Model A-99, Razel Scientific Instrument) and connected to inlet port 1 through a blunt-ended syringe needle and 10mm PDMS inlet tubing. Using another 5ml syringe, buffer solution (4.1mSm\(^{-1}\) KCl, 280mM D-Mannitol) was manually injected into the micro-fluidic chamber via inlet port 2 to eliminate air bubble formation.

A 10\( V_{pp} \) 1MHz signal was subsequently applied to the electrode array and the feed solution from inlet port 1 was manually fed carefully to the junction where the two channels met. Inlet port 2 was closed off with a plug and the syringe pump started, pumping 2ml volumes of feed through micro-system. The effluent stream was collected in a receptacle placed at outlet port 1, with outlet port 2 plugged.
Figure 5.1 Dielectrophoretic collection of particles (black) in hydrodynamic flow showing characteristic regions of accumulations at the electrode (grey) edge.

5.2.5.2 Particle retention using EHD flow

The same process steps were used for this process, but instead of using a 1MHz signal, a 1 kHz 10V_{pp} signal was used. This low frequency signal has been observed to produce particle collection on the surface of the electrode. The expected region and pattern of particle retention in fluid flow in the micro-fluidic chamber can be seen in Figure 5.2. Voltage and frequency were supplied by a TTL function generator and continuously monitored using a digital oscilloscope.

Figure 5.2 Electro-hydrodynamic collection of particles (black) in hydrodynamic flow showing characteristic regions of accumulations on the electrode (grey) surface.
5.2.5.3 Particle elution
After the required amount of feed had been injected in the micro-system, the syringe pump was stopped and the feed syringe connected to inlet port 1 was replaced with a 5ml syringe containing 4ml of buffer solution. The receptacle at outlet port 1 was replaced with a new receptacle for collection of the elution. The electric field was removed from the micro-system and the buffer solution was manually injected into the system to flush out the cells.

5.2.6 Detailed Design of Micro-fluidic System
The micro-fluidic device housing the electrode array was designed based on the dimensions of the gold coated slides. The device primarily consists of 2 parts, a top part through which electrical connections are made and a bottom part where the slide with the electrode array sits. Both parts are joined together by six screws, three equally spaced either side on the long-side of the device, which gives a well distributed pressure around the device enabling retention of the fluid in the fluidic chamber, when flow is introduced.

5.2.6.1 Bottom component
The bottom component is a 52×50×6 mm (length×width×thickness) slab of Perspex, cut to size to snugly hold the slide. A 26×15×6 mm window was cut through the Perspex allowing the passage of light from beneath to the electrode array to observe particle manipulation. A recess was then cut into the slab from the top to enable the slide to sit in place with minimal movement. The recess was exactly 1mm thick (slide thickness), 37 mm long, 26mm wide and was made at the centre of the window by making the 1mm recess extend out 5mm in all directions.

5.2.6.2 Top component
The top component was made up of two separate Perspex units. The larger of the two units was 52×50×5 mm, and the smaller unit was 30×12×2 mm. At the centre of the larger unit, in-line with the centre of the bottom component, a window was cut out (20×8×2 mm) with a recess having the same dimensions as the smaller unit created. The smaller unit, which makes up the top wall of the fluidic chamber, had an oval-shape groove mechanically etched out (1.35mm deep) to hold a Nitrile O-ring cord (1/16" diameter). The smaller unit was carefully glued in the larger unit's recess,
groove-side facing away from the larger unit, creating a single component. A 0.25mm recess was machined out of the smaller unit forming the micro-fluidic chamber and on assembly of the top and bottom components the height of the fluidic chamber was 250μm.

5.2.6.3 Micro-fluidic chamber
The actual micro-fluidic chamber has one inlet and one outlet (1mm diameter bore) positioned centrally at either end of the chamber. The length between the inlet centre and outlet centre is 22.5 mm with a chamber length of 27 mm. The chamber height and width are 0.25mm and 6mm respectively, giving a chamber volume of approximately 40.5mm³. Horizontal channels leading to the inlet and outlet bores are 3.25mm above the chamber floor. The channels have an inner diameter of 1mm.

5.2.6.4 Electrical connections
To provide for ease of device use and electrode array reusability, voltages supplied to microelectrodes were via gold plated spring loaded pins positioned around the device. They are always in contact with the slide surface, depending on the electrode geometry to be used and process to be carried out, enabling multi-signal applications. Use of the spring loaded contacts meant that they would not interfere with the setting of the chamber height, but will give a good electrical contact to the electrode pad with careful electrode design and pad positioning. The spring contacts chosen were 2.3mm radius flat head, medium spring obtained from Coda Systems Ltd (Essex, UK). The large surface area ensured contacts to electrode pads that may have been misaligned. Based on the chosen fluid chamber width (8mm) the distance between spring contacts across the chamber width was 13mm. Maximising on the use of the remaining slide area on either side of the flow chamber, maximum separation distances between each adjacent contact head were calculated to accommodate the maximum number of contacts. It was found that by staggering the pins between two columns on either side of the flow chamber, 13 pins could be comfortably positioned on either side of the fluid chamber, in contact with the slide surface, without interfering with each other. Pin sockets were drilled out to snugly fit the pins and with fast curing epoxy the pins remained in place when a pressure was applied to the spring heads. Wires were
soldered onto the shell of the spring contacts to facilitate electrical connectivity with a signal generator.

### 5.2.6.5 Ports, connectors and tubing

Four outer ports were created on the micro-fluidic device, two on the inlet side and two on the outlet side. Both inlet and outlet ports were designed so that they converge at a point just before entering/exiting the fluid chamber. The design resembles a ‘V’-shaped arrangement owing to the angles of the channels where they meet. This design was chosen to allow medium feed to be pumped into the flow cell to remove air bubbles prior to introduction of the particle solutions via the secondary inlet port through a syringe pump, resulting in a laminar flow profile throughout the fluid chamber. The outlet ports allow collection of particulates into separate receptacles based on temporal separation/concentration strategies. Outlet or inlet ports not in use are plugged giving a simple in and out flow through system. Each port has an external diameter of 2.5mm and an internal diameter of 1mm. The internal diameter of the ports form pipes at an angle of 60°, leading to the single inlet or outlet pipe (1mm diameter) which flows in to the fluidic chamber.

PDMS tubing (2mm inner diameter) were used to transport fluid in to and out of the flow cell. The length of the tubing from the syringe to the inlet port was 10mm and the length of the tubing from the outlet port to the receptacle was 20mm. These lengths were found to be the optimal for connecting the syringe pump with the micro-system and collection of the effluent stream off the microscope stage. Owing to the elasticity of the tubing, the tubing was connected to the outer ports by wrapping the inner diameter of the tubing around the outer diameter of the port. A syringe needle tip was fitted at one end of the tubing with the other end of the needle connected to a 5ml volume tapered end syringe.
Figure 5.3 a) Top and Bottom components of micro-fluidic device with an interdigitated electrode array fabricated on a 36x25mm slide sitting in bottom component; b) Underside of top component zoomed in on the top surface of the chamber surrounded by the Nitrile O-ring cord. On the outside of the O-ring are the spring loaded contacts making electrical contact with the array terminals on either side of the flow chamber.
Figure 5.4 a) Assembly of micro-fluidic device; b) Visualisation of the array position within the assembled micro-fluidic device.
5.2.7 FEM Model of Micro-fluidic Chamber

Using a commercial finite element modelling package (Comsol Multi-physics) a 2-D section of the electrostatic field distribution in the micro-fluidic chamber was modelled. The dimensions of the model were taken to be as close to possible of the fabricated micro-system. The length of the system was 1.5mm, the width of the electrode was 500μm, the inter-electrode gap was 250μm and the height of the chamber was 250μm. The thickness of the gold-titanium layer coated on the glass substrate was 110nm, but for ease of processing power the electrode thickness was taken to be 2μm.

Using the constitutive equation described in Equation 2.6, the electric field distribution was found by setting the sub-domain and boundary properties of the model. The area between the top of the chamber and the electrodes on the bottom substrate was modelled as the fluid region, with a relative permittivity of 78. The relative permittivity of the glass substrate (top and bottom) and the gold electrodes were set at 4.2 and 1 respectively. The voltages applied to the coplanar electrodes were ±10V, the continuity equation was applied at the interface of the fluid and substrate while the outer boundary of the model was grounded.

5.3 Results and Discussion

5.3.1 Growth Curve

Yeast cells re-suspended in 280mM D-Mannitol solution were monitored over a 4-hour period to calculate the growth and death rate in these conditions. Both viable and non-viable cell counts were performed every hour. It was found that the over a 1 hour the total number of cells in the culture increased by a factor of 1.02, with viable cells increasing by a factor of 0.97 and non-viable cells increasing by a factor of 1.07. Over a 2 hour period (1-3 hours) the growth of cells exceeds the death of cells, representing its exponential growth phase. The overall death and growth factors were found to be equal at the end of the 4 hours with a value of 1.89. The growth curve of viable and non-viable yeast cells enables correction factors to be used when determining the efficiency of the different ac electro-kinetic forces in the micro-system for particle retention.
**5.3.2 Process Calculations**

From the process flow diagram, it can be seen that the performance of a continuous-flow dielectrophoretic system is a function of a number of variables. These include the mean chamber velocity ($\bar{u}$), the root mean square voltage of the applied signal ($V_{\text{rms}}$), the real part of the Clausius-Mossotti factor ($\text{Re}[K(\omega)]$), the height ($h$) and length ($l$) of the micro-fluidic chamber, the particle size ($r$) and concentration ($C_x$) and the inter-electrode gap ($d_{\text{electrode}}$) for interdigitated electrodes.

**Figure 5.6 Process flow diagram of a continuous flow DEP/EHD retention and purging process**
Assuming the system is operating in steady-state before the introduction of the feed into the DEP unit, then the rate of momentum input is equal to the rate of momentum output. As there is no generation of momentum the rate of accumulation is equal to zero.

\[
\begin{bmatrix}
\text{Rate of momentum input} \\
\end{bmatrix} - \begin{bmatrix}
\text{Rate of momentum output} \\
\end{bmatrix} + \begin{bmatrix}
\text{Rate of generation = sum of forces} \\
\end{bmatrix} = \begin{bmatrix}
\text{Rate of momentum accumulation} \\
\end{bmatrix}
\]

\[\text{Equation 5.1}\]

\[
\rho_1 u_1^2 A_1 - \rho_2 u_2^2 A_2 + \sum \vec{F} = \frac{dP}{dt}
\]

\[\text{Equation 5.2}\]

\[
\text{Steady-state: } \frac{dP}{dt} = m \frac{du}{dt} = 0
\]

\[\text{Equation 5.3}\]

Volumetric flow-rates \((Q)\) ranging between 0.508 – 4.08 ml hr\(^{-1}\) were used to pump the feed solution through the micro-system. Based on the dimensions of the micro-fluidic chamber (~40.5mm\(^3\)), the average velocity within the micro-fluidic chamber can be calculated using the continuity equation applicable to incompressible fluids. This allows for an estimation of the residence time \((\tau)\) a particle experiences within the micro-fluidic chamber. This assumption is valid as the velocity of the particle in the bulk medium is taken to be equal to that of the fluid due to similar density properties, low Reynolds number \((2.3 \times 10^{-8} - 19 \times 10^{-8})\) and the high drag coefficient, \(C_D\), defined in Equation 5.4 for low Reynolds number [18].

\[
C_D = \frac{1}{Re}
\]

\[\text{Equation 5.4}\]
Chapter 5

At low Reynolds number the flow through channels and pipes are laminar. The velocity profile is parabola with the velocity at the walls equal to zero. This boundary condition is known as the no slip condition, and the velocity at distances away from the wall to the centre can be found using Equation 2.33.

The application of an ac electric field for the retention of particles in a continuous-flow process can be expressed as a balanced equation, where the number of particles per unit volume entering the system is effectively removed from the bulk solution along the chamber length. Hence, for particles experiencing positive dielectrophoresis in a DEP unit and assuming conditions are so that the Clausius-Mossotti factor is at its maximum, the amount of particles trapped can be said to be proportional to the residence time in the unit for a given electric potential over the electrode array in the y-direction and a given electrode dimension.

\[
\frac{dC}{dx} \propto \tau
\]

Equation 5.5

Figure 5.7 Particle residence time as a function of the fluid mean velocity within the micro-fluidic chamber.
The efficiency ($Eff$) of a micro-system in trapping particles can thus be calculated by
the output concentration ($C_{out}$) of particles which have passed through the system with
respect to the input concentration ($C_{in}$) and is defined by Equation 5.6, where $f$ is a
factor describing the growth/death rate of particles over a set period of time.

$$Eff_{retention} = 1 - \left( \frac{C_{out}}{C_{in} \times f} \right)$$

Equation 5.6

5.3.3 Experimental Observations

From the images in Figure 5.8 and Figure 5.9 it can be seen that the particles are
collected at different positions along the electrode array. Particle motion arising from
EHD forces, tend to redirect the particles away from the electrode edge on to the
surface of the electrodes, concentrating them at the centre of the electrodes. Particles
manipulated by DEP forces are seen to be collected along the electrode edge. As the
flow-rate increases the EHD forces are counter-acted by the lateral flow forces,
inhibiting particle collection along the centre of the electrode. This can be seen in
Figure 5.10 for a volumetric flow-rate of 4.08 ml hr$^{-1}$.

Figure 5.8 Particle retention over time (a=20mins; b=90mins) using EHD forces against a
chamber flow-rate of 188μm$^{-1}$. 
Figure 5.9 Particle retention over time (a=20mins; b=90mins) using DEP forces against a chamber flow-rate of $188\mu m/s$.

Figure 5.10 Images of EHD forces competing against hydrodynamic forces at a mean chamber velocity of $755\mu m/s$; a= 1min, b= 15mins, c= 25mins, d= end
Figure 5.11 Effect within 3 minutes of removing the 755 μm s⁻¹ fluid velocity from the chamber with EHD forces still applied. The particles are able to collect along the centre of the electrodes surface.

5.3.4 Elution and Retention

Cells eluted from the micro-system were performed manually after the electric field was removed. The purging efficiency was found to vary for cells collected by EHD forces and in comparison to the cells collected by DEP forces were lower in percentage across all flow-rates. A higher percentage of cells collected by DEP forces were found to be eluted, with a general increase with less time exposed to the electric field. The elution efficiency of the system is expressed as:

$$Eff_{elution} = 1 - \left( \frac{C_{purged}}{(C_{in} \times f) - C_{out}} \right)$$

Equation 5.7

where $f$ is a factor describing the increase in total particle concentration over a set period of time, for both viable and non-viable cells.

Viable and non-viable yeast cells entering and leaving the micro-system were also determined and showed significant differences based on the electric forces used to manipulate the particles. Fractions of viable and non-viable cells were determined for each flow experiment and elution step.
From Figure 5.13 it can be seen that the percentage of viable cells passing through the DEP field increases as the flow-rate increases, whilst viable cells passing the EHD field decreases with an increase in flow-rate. The elution process showed that particles subjected to shorter periods of DEP electric fields, had a higher fraction of viable cells eluted from the micro-system and decreased as the exposure increased. High fractions of viable cells exposed to EHD electric fields were eluted from the micro-system and decreased as exposure time decreased.
Figure 5.13 Fractions of viable and non-viable cells passing through the device with EHD and DEP forces applied as a function of chamber velocity

Figure 5.14 Fractions of viable and non-viable cells eluted from the device after subjected EHD and DEP forces applied as a function of chamber velocity
The ratio of viable to non-viable cells passing through the electric field and eluted from the micro-system for DEP and EHD applied forces are shown in Table 5.1 and Table 5.2 respectively. It shows that the concentration of viable cells trapped by positive DEP at low flow-rates reduces with respect to the fraction entering the system. Also, the application of the DEP force is selectively trapping viable cells and allowing non-viable cells to pass through the system. This is clearly seen by the significant reduction in ratio for cells not collected with respect to the cells entering the system, although the ratio gradually increases with increased volumetric flow-rates. Cells subjected to EHD fields have shown that at different flow-rates the ratio...
of viable to non-viable cells trapped and passed through varies. At the lowest flow-rate the ratios are greater with respect to the ratio entering the system, but tend to be less than that entering the system as the flow-rate increases.

Yeast cells with a total concentration averaging $3 \times 10^6$ cells per ml for all experiments were flowed through the micro-system at varying flow-rates with EHD forces (1 kHz, $10V_{pp}$) and DEP forces (1MHz, $10V_{pp}$) applied. From the fractions of viable and non-viable cells entering the system total cell concentrations were determined. The number of cells exiting the micro-system as a function of chamber velocity for both DEP and EHD forces was found to increase as the volumetric flow-rate increased. This is conveniently described as the retention efficiency of the system described previously in 5.3.2.

The retention efficiency is >90% at low chamber velocities (94$\mu$m s$^{-1}$) corresponding to a residence time of ~5mins in the DEP micro-fluidic chamber for both frequencies used. This reduces gradually as the residence time decreases. A distinct difference in the retention efficiency between DEP and EHD forces as function of chamber velocity exists at higher flow-rates. The retention efficiency using EHD forces reduces by 50% when the flow-rate increases from 188$\mu$m s$^{-1}$ to 385$\mu$m s$^{-1}$. In comparison the retention efficiency using DEP forces reduces by 20% with a flow-rate increase from 188$\mu$m s$^{-1}$ to 755$\mu$m s$^{-1}$.

![Comparison of Particle Trapping using EHD and DEP Torso as a function of lateral flow rates](image)

Figure 5.15 Retention efficiencies of DEP and EHD forces as a function of residence time and chamber velocity
5.3.5 Electric Field Distribution

Finite Element Modelling (FEM) is a powerful tool used to visualise the electric field distributions in micro-systems, especially where non-uniform fields are present. A surface plot of the electric potential (V) can be seen in Figure 5.16, showing the variation in voltage across the micro-fluidic chamber.

![Surface plot of electric potential over coplanar electrodes](image)

**Figure 5.16** Surface plot of electric potential over coplanar electrodes with electrode potentials of ±10 V, electrode width = 500 μm, electrode thickness = 2 μm, inter-electrode gap = 250 μm and chamber height = 250 μm

The field gradient squared (V|E^2|) distribution along the modelled section is plotted in Figure 5.17 for 25 μm height intervals starting at the electrode plane (line 1, blue) and ending at the chamber surface (line 10, red). V|E^2| has its maximum intensity in the plane of the electrode at the electrode tip (4.5 ± 1 x 10^14 V^2 m^-3) approximately 5 times greater than the same x-position 25 μm above the electrode plane. Dividing the voltage by the length of the system in the x-direction and squaring the value, the electric field squared distribution can be obtained as seen in Figure 5.18.
Figure 5.17 Magnitude of $\nabla|E^2|$ as a function of chamber height in 25$\mu$m intervals starting in the plane of the electrode (blue line 1)

Figure 5.18 Surface plot of $\nabla|E_x^2|$ distribution for modelled dimensions
Further increase in height reduces the intensity of the field gradient, but does not reach a zero value within this system at the x-position corresponding to the edge of the electrode. For different x-positions along the chamber, in the plane of the electrode surface, as we approach the centre of the 500μm width from the electrode edge there is a sharp fall in field gradient intensity which tends to zero at ~175μm in from the electrode edge. Going towards the centre of the inter-electrode gap a ‘kink’ is observed ~25μm in which increases the field gradient magnitude a little, but then an almost linear approach to a zero field gradient intensity is seen at the centre. It is also noticed that as height position from the electrode plane is increased as we approach the x-position in the plane of the electrode centre, the broader field intensity slopes mean that it takes longer for the intensity to converge a zero value. The zero value is approached quicker as we tend to the inter-electrode gap centre.

Analysis of the field gradient squared across the length of the inter-electrode gap as a function of chamber height shows a symmetrical profile centred in the middle of the inter-electrode gap. Field values were determined at height a spacing of 10μm, starting at the electrode surface plane (2μm) and ending at the top of the chamber. It can be seen in Figure 5.19 that $V|E_x^2|$ has a range in the middle of the inter-electrode gap of $\sim 8 \times 10^8 - 2.5 \times 10^9$ V$^2$ m$^{-3}$ for the electrode plane and chamber surface respectively. As the edges of the electrodes are approached an exponential increased in field gradient magnitude is seen along the plane of the electrode surface. Overshooting the inter-electrode gap by 5μm on either side causes the field gradient magnitude to begin decreasing as seen by the tail ends on the curves. As you move up the chamber the characteristic exponential increase becomes broader until eventually a height is reached were the field gradient intensity value becomes more or less uniform. The onset of this trend was found to lie at about 82μm above the electrode plane, which is 32.8% of the chamber height.

Inspection of $V|E_x^2|$ along a diagonal line within the inter-electrode gap from the electrode edge up to the chamber surface an exponential decrease is observed. The drop in magnitude was of the order of one across the height of the chamber, showing the field magnitude in the x-direction doesn’t fall to zero within the inter-electrode gap.
The distribution of $\nabla|E|^2$ is critical for the determination of the dielectrophoretic force exerted on a particle at a specific location within a micro-system. With the knowledge of process parameters i.e. $\text{Re}[K(\omega)]$, $\rho_m$, $\eta_m$ it has been described how bio-processing conditions i.e. flow-rates can be approximated to optimise process trapping efficiencies using dielectrophoresis for a cylindrical micro-fluidic structure [19].

5.3.6 Force Calculations

For yeast particles (4μm radius) suspended in a micro-system with the same geometrical dimensions as above, and with the medium properties set so that the Clausius-Mossotti factor is one, the vertical and horizontal dielectrophoretic force exerted on a cell, as described by Equation 5.8, at different locations can be seen in Figure 5.21 and Figure 5.22.

$$F_{\text{DEP}(x,y)} = 2\pi r^3 \varepsilon_m \text{Re}[K(\omega)]|\nabla|E(x,y)|^2|$$

Equation 5.8
Figure 5.20 Variation of $\nabla |E|^2$ along a diagonal line in the inter-electrode gap starting from the electrode edge and ending at the chamber surface.

Figure 5.21 Horizontal dielectrophoretic force exerted on a viable yeast cell as a function of chamber height.
From Figure 5.21 it can be seen that the maximum $F_{\text{DEP-x}}$ value ($\sim 4.2 \times 10^{-11}$ N) lies around the electrode edge in the electrode plane. A second peak is seen in the same plane, approximately 25 μm from the electrode edge, going towards the electrode centre. The force decreases by one order of magnitude at a height of 50 μm from the electrode plane at approximately the same length from the electrode edge, tending to zero at plane heights greater than 102 μm. $F_{\text{DEP-y}}$ at all heights were found to have the same characteristic shape, resembling a V-shape with its minimum value ($F_{\text{DEP-y}}=0$) found at some distance within the inter-electrode gap. The maximum value was not at the electrode edge but showed slight increases from the electrode edge approaching the electrode centres. At heights of 30 μm $F_{\text{DEP-y}}$ approached values of $2 \times 10^9$ N decreasing by one order of magnitude with a 45 μm increase in height. At half the chamber height the vertical DEP force was reduced by 5 orders of magnitude, rapidly tending to zero at heights above that plane.

Figure 5.22 Vertical dielectrophoretic force exerted on a viable yeast cell as a function of chamber height.
Figure 5.23 Representation of the x-component of the velocity profile of a yeast particle due to dielectrophoresis as a function of chamber height from electrode plane (2μm) to chamber centre plane (125μm)

Figure 5.24 Logarithmic scale of the y-component of the velocity profile of a yeast particle due to dielectrophoresis as a function of chamber height from electrode plane (2μm) to chamber centre plane (125μm)
It is clearly seen that the vertical DEP force on a particle is stronger in magnitude than the horizontal force. The sedimentation force due to gravity for a spherical particle is given in Equation 5.9, where \( r \) is the particle radius, \( \rho_p \) is the particle density, \( \rho_m \) is the medium density and \( \dot{g} \) is the gravitational acceleration constant.

\[
F_g = \frac{4}{3} \pi r^3 \left( \rho_p - \rho_m \right) \dot{g}
\]

Equation 5.9

The force on a yeast particle due to gravity is calculated to be approximately \( 5 \times 10^{-13} \) N, which is comparable to the x-component of the dielectrophoretic force. The velocity at specific points in space due to the dielectrophoretic force (\( u_{DEP} \)) can be approximated using Stokes Law [20]. Assuming the viscosity of the bulk medium for D-mannitol being 0.00135Ns m\(^{-2}\) @ 20°C [21] for dilute suspensions of yeast cells, the velocity components of the particle can be found (Figure 5.23 and Figure 5.24). The x-component of the particle’s velocity is seen to have negligible influence with respect to the total particle velocity, indicating that the x-component of a particle’s velocity in fluid flow with an applied orthogonal electric field is primarily influenced by hydrodynamic flow.

The terminal settling velocity (\( u_g \)) of a spherical particle due to gravity, for a dilute suspension is given by Stokes Law as [22]

\[
u_g = \frac{\left( \rho_p - \rho_m \right) r^2 \dot{g}}{18 \eta_m}
\]

Equation 5.10

At significant heights close to the electrode plane and when the chamber’s mean velocity is significantly greater than that due to gravity, i.e. \( u_g << \overline{u}_{chamber} \), sedimentation forces are taken to be negligible.

From simulations carried out the dielectrophoretic velocity of a particle in the y-direction near the centre plane of the chamber above the electrode regions were calculated to be \(~120\mu m s^{-1}\), rapidly increasing as the plane comes closer to the electrodes. Process calculations have shown that the hydrodynamic mean chamber velocity at the centre of the micro-fluidic chamber, based on the experimental
volumetric flow-rates, range between 94μms⁻¹ to 755μms⁻¹. As the dielectrophoretic force becomes negligible at chamber heights greater than the centre plane, and due to the parabolic velocity profile of the flow, the total force on a particle will vary considerably depending on the height position within the chamber. At heights greater than the chamber’s centre plane gravitational forces become more significant than DEP forces, but depending on the flow rate particles will settle over time into the fast flowing stream which may not be enough time for effective particle trapping using conventional dielectrophoresis.

5.3.7 Electro-hydrodynamic vs. Dielectrophoretic Trapping

Dielectrophoresis has often been used for selective retention and separation of biological particles based on their dielectric properties in fluid flow conditions [11, 23-25]. Particles passing through ac electric fields become polarised and depending on the polarisability factor, particles are repelled from or attracted to electrode edges. Experiments performed here have shown that viable yeast cells are collected at the edges of interdigitated electrode arrays with an applied frequency of 1MHz. At frequencies below 10 kHz, the polarisability factor of viable yeast cells is negative in low conductivity medium (<5mSm⁻¹) as depicted in Figure 3.26. This would have the effect of repelling particles from the electrode edge and ideally levitating the particles at some plane in the chamber above the electrode plane. Yet we have observed low frequency electric fields trapping particles on the electrode surface. These low frequency field effects have been reported previously and are said to occur from electro-hydrodynamic (EHD) forces originating from coplanar microelectrodes [26-28].

In the presence of hydrodynamic flow, with ac electric fields applied so the cells experience either EHD or DEP forces, dilute suspensions of cells have been found to have different trapping efficiencies influenced by the lateral fluid velocity. At low a volumetric flow-rate (0.508 ml hr⁻¹) and based on the dimensions of the micro-fluidic chamber, the calculated residence time in the chamber approaches 5 minutes. Application of conventional dielectrophoretic forces and electro-hydrodynamic forces showed total particle trapping efficiencies of 97% and 91% respectively. As the fluid flow-rate increased to 4.02 ml hr⁻¹ the trapping efficiency based on EHD forces decreased at a quicker rate than that based on conventional DEP forces to 11% and 55% respectively. At low chamber flow-rates the number of viable cells passing
through the DEP field was found to be considerably less than non-viable cells. As the flow-rate increased the ratio of viable to non-viable cells increased, indicating that the DEP trapping efficiency of the micro-system begins deteriorating (i.e. < 75%) at a chamber velocities greater than 565μms⁻¹. In contrast, the ratio of viable to non-viable cells passing through the EHD field was found to vary across all flow-rates strongly indicating the non-selective nature of EHD forces for particle retention. It was also observed that the efficiency of EHD trapping began to deteriorate at chamber velocities greater than 188μms⁻¹.

Elution of DEP trapped particles after approximately 4 hours of subject to high field gradients saw a higher fraction of non-viable cells, which decreased significantly with less time exposed to high field gradients. This suggests that cells may be damaged by prolonged high field exposures. It has been cited that particles retained by DEP forces are subjected to high field gradients which may cause cell bursting rendering them non-viable [29]. Regions of electric field minima were found to lie above the electrode surface (Figure 5.17), and rapidly decreased in magnitude at the centre of the electrode. At low flow-rates particles trapped by EHD forces are seen to collect at the centre of the electrode surface indicating that the cells were not subjected to high field gradients. On elution of EHD trapped particles after ~4 hours of retention, the fraction of viable cells was found to be 93.5% in comparison to the 47.6% for DEP trapped cells. As exposure time decreased the ratio of viable to non-viable cells eluted after EHD trapping was found to vary, with very little deviations from the original input ratios.

5.3.8 Particle Displacement

Dielectrophoretic particle manipulation occurs due to the imposed non-uniform ac electric field which induces a dipole causing the particle to move up or down the field gradient. Typically the DEP forces are significantly larger than gravitational forces so can be ignored [20]. It can then be assumed that the motion of a particle in a resting fluid follows the electric field lines for conventional dielectrophoresis, with particle being collected at the electrode edge.

When the fluid is set into motion via an external pump, for a laminar regime (i.e. Re < 1) the momentum of a fluid volume element is not changing and travels at a constant speed. The hydrodynamic forces can be written as in Equation 5.11, where the first term on the left-hand side is the viscous force per unit volume and the second term is
the net force per unit volume in the downstream direction which is positive and the pressure gradient is independent of x-position \[18\].

\[
\mu \frac{\partial^2 u}{\partial y^2} \delta x \delta y \delta z - \frac{\partial P}{\partial x} \delta x \delta y \delta z = 0
\]  

Equation 5.11

Rearranging Equation 5.11 and integrating across the chamber by setting the boundary conditions so that the velocities at the walls of the chamber are zero (i.e. the no slip condition) we find the velocity profile varies as a function of height and follows a parabolic profile. The hydrodynamic force on a particle varies as a function of height with the maximum at the centre decreasing as the walls are approached. When the hydrodynamic force is greater than the dielectrophoretic force at heights within the chamber, the particle’s displacement will follow that of the fluid flow. Over time gradual negative displacements of the particle in the y-direction, below the chamber’s centre, due to dielectrophoretic forces occur bringing the particle into slower fluid streams. In these streams, the dielectrophoretic forces increase in magnitude and generally become more effective than the hydrodynamic forces in holding the particle at the electrode edge.

The application of ac voltages between coplanar electrodes to a resting fluid has been described to set the fluid into motion \[30, 31\]. The flow is convective in nature and arises due to externally introduced temperature variations between the boundary and the bulk medium. Temperature variations give rise to variations in properties of the medium i.e. density, viscosity, permittivity and conductivity. Both electrical and gravitational body forces are present when the medium properties are inhomogeneous, and it is the combined actions of these forces which causes the fluid motion. Since the electrical force is oscillatory, as defined in Equation 2.32, with a non-zero average, the fluid motion also has an oscillatory component and a non-zero time-average. The non-zero time-averaged velocity of the convective flow is dominated by either Joule-heating or ac electro-osmosis and approximate calculations of velocities due to both mechanisms have been described \[20, 32\]. Steady fluid flow velocity depends on both the applied potential and frequency and by considering the potential drop across the double layer the slip velocity above the electrodes, produced by the tangential ac field on the oscillating induced charges in the double layer, an expression describing the
surface stresses has been derived which takes into account for the ratio of the voltage drop across the diffuse double layer to the total drop across the double layer. It is this slip velocity produced by the tangential electric field at the electrolyte-electrode interface which displaces particles from the electrode edge to the electrode centre. The circulatory motion of the fluid above the electrodes displaces particles in the bulk of the fluid orthogonally and tangentially to the electrode. Depending on the particles position in the convective streams, the velocity of the vortexes influences the displacement of the particle. The introduction of hydrodynamic forces makes the prediction of particle trajectories more complicated, due to the oscillatory displacements in the vortexes meaning particles are continually being displaced laterally into upward vortex streams and then into faster flowing hydrodynamic streams. As the hydrodynamic flow rate increases the velocity near the electrode surface counteracts the slip velocity making particle aggregation at the centre of the electrode surface difficult to attain (see Figure 5.10).

5.3.9 Micro-fluidic System and Process Design

The dimensions of the micro-fluidic system contribute significantly to the efficiency of the process. For the interdigitated electrode array used here, these include the size of the electrodes (width and thickness), the inter-electrode gaps and the height and length of the micro-fluidic chamber housing the electrode array. It is possible to increase the strength of the electric field gradient penetrating the chamber volume by reducing the height of the chamber or by increasing the applied voltage. These can have adverse effects on the processes too. An increase in voltage increases the chances of cells collected at the electrode edge to be susceptible to damage from very high electric field gradients [29]. Depending on the size distribution and concentration of particles flowing through the micro-system, a reduction in chamber height can lead to regions upstream where particle aggregation across the chamber height can lead to a build up in pressure, inhibiting steady-state flow along the chamber length and in some cases cease of flow altogether. This effect can also occur when the electrode array is not suitably positioned an appropriate distance away from the inlet bore. It was observed in initial experiments that if the first electrode of the array is situated directly below the inlet bore particles flowing into the chamber at slow to medium flow-rates are readily trapped by dielectrophoresis and over time concentration build up near the inlet contributed to the pressure increases ceasing fluid flow. Positioning
the array <1mm away from the inlet bore and introducing a concentrated cell population (~10^8 cells per ml) at a volumetric flow-rate of 1.02ml hr^{-1} also affected the steady-state flow. To overcome these problems the first electrode from the array was situated 3mm away from the inlet bore, which was decided to be an acceptable distance for the flow-rates and yeast cell concentration (~10^6 cells per ml) used. The length chosen was a balance between reducing the number of particles at low flow-rates settling under the influence of gravity before reaching the electric field regions and the ability of the fluid on entrance into the chamber spreading out over the substrate and attaining its mean velocity before reaching the electric field regions. This was critical as the entry length from the feed tank into the micro-fluidic chamber was designed to be as short a distance as possible, with a mean velocity much greater than that in the micro-fluidic chamber, meaning the probability of pipe blockages due to particle build up were dramatically reduced.

The design of the micro-fluidic chamber also took into consideration the possibilities of dead-space forming at corners of the chamber. Attempts to overcome this were done by creating an oval-shaped chamber with the intention of re-directing flow at the walls to the far ends of the chamber. Positioned at these ends were the inlet and outlet bores enabling flow to converge and exit. This was thought to aid in the elution efficiency of the micro-system as when the chamber is purged, the non-existence of corners mean there are no dead-spaces for particles to accumulate. The height of the chamber (250μm) was chosen as the upper limit value because of the size of bioparticles used for experiments. Depending on the collection regime of trapped particles (~8μm), particularly if they pile up on each other, chamber heights <50μm may interfere with fluid flow rates and block up the chamber, especially for large volumes of dilute suspensions. Also, for incremental increases in particle concentration there are increases in bulk solution viscosity. This had the effect of practically inhibiting the convective flow of the suspension by ac electro-osmosis as it was observed that at particle concentrations of ~10^8 cells per ml, the yeast cells in slow hydrodynamic flow-rates were not being directed to the centre of the electrode surface. As described by Equation 2.29, the velocity of the convective flow is also influenced by the viscosity of the medium and it could be due to the inability of the electric fields to influence the medium properties within the highly viscous solution that EHD trapping was not observed.
Possible causes of errors in the processes carried out were largely thought to have occurred with slow flow-rates i.e. \( u_{\text{chamber}} < 188 \mu \text{m s}^{-1} \). The ability of introducing the feed solution into the chamber at steady-state was easier as the volumetric flow-rate increased. At smaller volumetric flow-rates, the manual introduction of buffer solution into the chamber was essential before introducing the feed solution. As the buffer stream was being introduced, it was pertinent that the feed stream join the buffer stream. This was important because at the low flow-rates within the chamber the pressure was not sufficient enough alone to create a uniformly laminar flow in the large chamber volume. Air bubbles at the entry position and over the electrode array were observed without the buffer stream. Hence, the likelihood of cells exiting the chamber is likely due to the buffer stream and not the actual pump volumetric flow-rate at lower flow-rates, meaning the trapping efficiency could be higher than experimentally found.

Also, micro-system purging was carried out manually as typical maximum syringe pump flow-rates were seen to not exert enough pressure to carry cells from the walls of the chamber. A constant flushing method was employed for each purging step which was seen to remove cells from the wall and electrode surface. It was also observed that there were non-specific binding of cells to the electrode surface and edges after removing the electric field and flushing at high flow-rates. This indicated that procedures for effectively eluting cells for practical purposes are needed if particle trapping in micro-system is to be used as an upstream separation, pre-concentration or filtration step.

5.4 Conclusions

A mechanically fabricated micro-fluidic system for ac electro-kinetic applications has been designed specifically for assessing the performances of EHD and DEP retention in hydrodynamic flow. The micro-fluidic system allows easy cleaning, reusing and quick inter-changing of electrode arrays for different flow applications. Spring contact pins were used to electrically connect to aligned electrode terminals on the outside of the fluidic chamber making the use of conductive epoxy obsolete. The design is practical and can be scaled to any size required, especially and crucially for chamber heights which may need to be of the order of tens of micro-metres.
Particle trapping efficiencies for dielectrophoretic and electro-hydrodynamic forces have both shown to decrease as a function of flow-rate, which based on the dimensions of the micro-fluidic chamber, was seen to reflect the residence time of particle. The rate of decrease in trapping efficiency was faster for the EHD regime than the DEP regime. This is believed to be due to the nature of particle displacement in a combined hydrodynamic and convective flow regime. Detailed modelling of particle trajectories for both regimes will need to be carried to assess the effects of the different sized forces on a particle.

The viability of cells exposed to high field gradients at the electrode edge (DEP) decreased as length of exposure time increased. Particles trapped in low field gradients at the centre of the electrode surface (EHD) were found to have a higher fraction of viable cells. Elution of trapped particles were seen to vary for both collection regimes, but was less than perfect as cells were observed to be still bound to the electrodes.
5.5 References


Chapter 6

Conclusion and Future Work

6.1 Conclusion

Manipulation of biological particles using ac electro-kinetics has been demonstrated here to be a versatile technique capable of dielectric characterisation, integration with sensing elements and pre-concentration and separation of viable and non-viable cells in a micro-fluidic system.

The design of micro-systems used to perform any ac electro-kinetic bioprocess is a critical factor influencing process efficiency and accuracy. Other important factors influencing the performance of a micro-system include particle size, suspension concentration, electrode geometry, electrode dimensions, and the magnitude and type of ac electro-kinetic force applied within the micro-system. Hence, when a specific process is to be carried out, whether it is a batch or continuous process, these factors should be taken into consideration when designing the micro-system.

Assessment of a new electrode geometry operating in batch mode has revealed dependencies on both electrode dimensions and suspension concentrations with respect to particle re-suspension times. Particle motion in the dot micro-system exhibited collection at the edges of the dot electrode (positive dielectrophoresis) and accumulation at the centre of the aperture, on the substrate surface (negative dielectrophoresis). Particle velocity was found to correlate with dot radius and suspension concentrations for both positive and negative dielectrophoresis. As the size of the dot size decreased and suspension concentration increased the particle velocity increases. At certain concentration values for each dot size, the micro-system becomes saturated and the application of an electric field does not produce particle motion. At optimal concentrations it was observed that particles were capable of re-suspending themselves on removal of the electric field. This unique property of the dot micro-system was used as a principle factor for the rapid dielectric characterisation of homogeneous particles. The method here uses a devised algorithm which calculates intensity shifts of captured images as a function of applied frequency over time and has proved to be effective in determining the magnitudes of both
positive and negative dielectrophoresis over a wide frequency bandwidth which has not been previously described. This property is largely attributed to the quasi-three dimensional structure of the electrode system, which for low frequencies reduces errors in particle collection commonly associated with electro-hydrodynamic forces typical found with coplanar electrodes.

Electro-hydrodynamic (EHD) forces are observed manipulating particles on to the electrode surfaces of coplanar electrode geometries. A system has been engineered demonstrating the integration of ac electro-kinetics with acoustic sensing elements. The dielectrophoretic-quartz crystal microbalance (DEP-QCM) has shown the importance of the electrode geometry for surface based detection. This micro-system uses the zipper array to concentrate particles from the bulk solution on to the surface of the electrode using ac electro-osmosis. By super-imposing the crystals resonant frequency (10MHz) on to the applied ac electro-kinetic frequency (1 kHz) the crystal’s response to surface mass loading by EHD forces were monitored in real time. The size of particles used in this micro-system was found to influence the crystal’s response to the mass attached to the electrode surface. Also, the sensitivity of the crystal was found to be greater for particles less than 1μm in diameter, dramatically decreasing in sensitivity at an average diameter of 8μm. Surface detection of sub-micron particles using the DEP-QCM was found to correlate with particle concentration on initial application of the ac electro-kinetic signal. The time taken for the crystal’s response to reach steady-state increased with increasing concentration. This system could prove to be a useful tool in developing on current theoretical models on EHD and applied as a rapid biosensor device.

Separation or pre-concentration of biological particles in a continuous-flow process using ac electro-kinetic forces can be achieved in micro-fluidic systems, and the efficiency of the process is influenced on a number of system parameters including applied frequency and magnitude of signal and the volumetric flow-rate. A micro-fluidic system was designed and fabricated by precision mechanical engineering. This micro-fluidic system differs from other fabricated ac electro-kinetic micro-fluidic systems because of the easy access of the microelectrode array for replacement or cleaning, which eliminates the need for epoxy resin for electrical connection and chemical etching and bonding techniques for micro-chamber formation.
Differences in dielectrophoretic and electro-hydrodynamic particle retention and elution in a micro-fluidic system have been observed for viable and non-viable cells. The polarisability factor of particles showing negative dielectrophoresis at low frequencies is cancelled out when the frequency is applied to coplanar electrodes. Electro-hydrodynamic forces become more prominent than the dielectrophoretic forces and fluid convection above the electrodes drive all particles to the electrode surface. Particle retention using EHD is not as selective as DEP, but strongly indicates that the prolonged exposure to the electric fields causes less damage to biological particles. The efficiency of particle retention was found to be related to the residence time within the micro-system, increasing with longer exposure to the electric fields. The effect of increased flow rates in the micro-fluidic chamber had a strong effect on particle retention using EHD. Increases in lateral hydrodynamic forces showed that the convective forces arising from EHD reduces in magnitude, making surface collection difficult and particles collected remaining at the electrode edge. On removal of the lateral forces, particles were seen to accumulate as usual at the centre of the electrode surface.

6.2 Future Work

Performance analysis of the dot micro-system was carried out with particles having an average radius of ~4μm. Particle motion has also been observed for fluorescent nano-spheres with the same micro-system dimensions used for manipulating cells. The devised algorithm for dielectric characterisation using digital imaging was not applied to the nano-spheres. This was because concentration values would exceed those that are applicable to the Beer-Lambert law. A reduction of the micro-system dimensions (height and dot diameter) along with a reduction of nano-sphere concentrations could, with more sensitive photo-optic sensors, enhance the efficiency of the process for nano-particles. The reverse is applicable to larger cells such as cancer cells, where an increase in the system dimensions i.e. height may produce the desired effects of particle re-suspension upon electric field removal. Investigations into optimal micro-system dimensions and concentrations for different particle sizes are needed to find whether there are limitations of this process. This can then be used for devising a process whereby multiple frequencies could be applied to an array of individual dots, simultaneously capturing the particles response to the applied ac electric field in a
single process. With correct system dimensions for particle sizes and concentrations, particle re-suspension will aid in performing multiple analysis of the same sample in a short space of time which would dramatically reduce errors.

Integration of ac electro-kinetics with biosensors can be applied to both the dot micro-system and the DEP-QCM for selective detection of particles of interest. Surface modification techniques can aid in the development of integrated ac electro-kinetic biosensors which could enhance detection rates by particle manipulation to site specific immobilised antibodies. By designing appropriate micro-fluidic systems, these integrated systems can be incorporated together as a single device for the development of μ-total analysis systems (μ-TAS) using ac electro-kinetic forces.