Lab-on-a-chip microdevices for manipulation and separation of microparticles using dielectrophoresis

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Lab-on-a-chip Microdevices for Manipulation and Separation of Microparticles using Dielectrophoresis

Ming LI

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ABSTRACT

The development of lab-on-a-chip (LOC) over the past decade, has attracted more and more interest, and aims to achieve the miniaturisation, integration, automation, and parallelisation of biological and chemical assays. One of the applications, the ability to effectively and accurately manipulate and separate micro and nano-scale particles in an aqueous solution is quite appealing in biological, chemical and medical fields. Among the technologies developed and implemented in microfluidic microsystems (i.e. mechanical, inertial, hydrodynamic, acoustic, optical, magnetic, and electrical methodologies), dielectrophoresis (DEP) may prove to be the most popular method for manipulating and separating particles. This is because of its great advantages, such as label-free nature, compatibility with LOC devices, ability to manipulate neutral bio-particles, easy and direct interface with electronics, and analyse with high selectivity and sensitivity. The required spatial electric non-uniformities for the DEP effect could be generated by applying alternating current (AC) fields to microelectrodes (either 2D or 3D) embedded within microchannels, or placing insulating obstacles within a microchannel and curving microchannels, while direct current (DC) or DC-biased AC fields are applied via external electrodes located at the inlet and outlet reservoirs. The major objective of this work is to develop methods of micro-fabrication and DEP-based microdevices to manipulate and separate particles.

A novel method for fabricating dielectrophoretic microdevices with top-bottom patterned microelectrodes has been proposed, which utilised a laser-patterned polydimethylsiloxane (PDMS) layer which acts as both a working and bonding layer. This method is simple and cost-effective because it eliminates the demand for a template and the corresponding fabrication process, facilities, and consumables in high-standard clean rooms. Another method has been developed to fabricate DEP-based microsystems with arc-shaped extruded microelectrodes on channel sidewalls using metal alloy microspheres. This fabrication method offers many advantages such as good conductivity, simplicity, low cost, and an improvement in the design of topological electrodes. The capabilities of these methods were demonstrated by
fabricating and testing DEP-based microfluidic chips with either top-bottom or sidewall patterned microelectrodes functioning as a micro-concentrator/separator.

Two DEP-based microsystems with bi-layer microelectrode configurations were designed and constructed using the proposed fabrication method. The structure of the first 3D electrode structure consists of a funnel-shaped focusing unit, a parallel aligning unit and a crescent-shaped trapping unit in series, which improves the integrated functionalities as concentration of single particle population in a continuous flow, separation of particle-particle mixture according to size, and separation of particle-cell mixture according to their dielectric properties. The second microdevice consists of 13 individual microchannels fixed in a radial direction and top-bottom patterned arrowhead-shaped microelectrodes, which aims to collect and separate particles in a high-throughput manner. The performance was demonstrated by dielectrophoretically collecting polystyrene (PS) particles, yeast cells, and *E. coli*, and separating live and dead yeast cells.

Unlike electrode-based DEP microdevices, insulator-based ones are mechanically robust, chemically inert, and simple to fabricate. A waved microchannel consisting of consecutive curved S-shaped channels in series was developed for continuous particle focusing. How the effects of applied electric field, particle size, and medium concentration affect the focusing performance was studied both experimentally and numerically by continuously focusing polystyrene particles of various sizes, and yeast cells. In addition, a curved S-shaped microchannel embedded with multiple round hurdles was developed to manipulate and separate particles. It combines the effect of obstacle and curvature for spatial electric non-uniformities, allowing greater control of electric field distribution and hence the particle motion. Both experiments and numerical simulations were conducted to demonstrate the controlled trajectories of particles, and the separation of polystyrene particles according to size by adjusting the voltages applied at the inlet and outlets. It is anticipated that the proposed designs will integrate with different components and functionalities into a single LOC device for widespread use in the field of biology, chemistry, and medicine.
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PUBLICATIONS


# TABLE OF CONTENTS

ABSTRACT .................................................................................................................. i  
ACKNOWLEDGEMENTS ........................................................................................ iii  
PUBLICATIONS ........................................................................................................ iv  
TABLE OF CONTENTS ............................................................................................. v  
LIST OF FIGURES .................................................................................................... ix  

Chapter 1 Introduction ................................................................................................. 1  
  1.1 Background and motivation ......................................................................... 1  
  1.2 Objectives ..................................................................................................... 3  
  1.3 Outline of the thesis ..................................................................................... 3  

Chapter 2 Literature review ......................................................................................... 7  
  2.1 Particle manipulation technologies .............................................................. 7  
    2.1.1 Mechanical ............................................................................................... 8  
    2.1.2 Inertial ...................................................................................................... 9  
    2.1.3 Hydrodynamic ........................................................................................ 10  
    2.1.4 Acoustic .................................................................................................. 11  
    2.1.5 Optical .................................................................................................... 12  
    2.1.6 Magnetic ................................................................................................. 13  
    2.1.7 Electrical ................................................................................................ 14  
  2.2 Dielectrophoresis (DEP) ............................................................................ 15  
  2.3 Micro-fabrication techniques used in dielectrophoretic applications ...... 20  
    2.3.1 2D planar electrodes ............................................................................... 20  
    2.3.2 Extruded electrodes ................................................................................ 22  
    2.3.3 Side-wall patterned electrodes ............................................................... 25  
    2.3.4 Insulator structures ................................................................................. 27  
  2.4 Various DEP microdevices for particle manipulation ............................... 30  
    2.4.1 Electrode-based DEP microdevices ....................................................... 30  
    2.4.2 Insulator-based DEP microdevices ........................................................ 39  
  2.5 Conclusions ................................................................................................ 44  

Chapter 3 A simple and cost-effective method for fabricating dielectrophoretic devices with top-bottom patterned microelectrodes ................................................. 46  
  3.1 Introduction .................................................................................................. 46
3.2 Fabrication process............................................................................................. 48
  3.2.1 Processing the layer of PDMS ..................................................................... 49
  3.2.2 Microfluidic channel patterning................................................................. 49
  3.2.3 Processing the substrate ............................................................................. 50
  3.2.4 Bonding ...................................................................................................... 50
3.3 Results and Discussion....................................................................................... 51
  3.3.1 PDMS surface treatment ............................................................................ 51
  3.3.2 Surface characterisation ............................................................................ 52
  3.3.3 Controlling the thickness of the transferred PDMS layer ......................... 53
  3.3.4 Optimisation of laser parameters ............................................................... 54
  3.3.5 Bonding examination and testing ............................................................... 55
  3.3.6 Integrated DEP-based electronic-microfluidic microdevice ..................... 56
3.4 Conclusions........................................................................................................ 60

Chapter 4 A novel method for constructing dielectrophoretic microdevices with 3D arc-shaped microelectrodes at the sidewalls .......................................................... 62
  4.1 Introduction..................................................................................................... 62
  4.2 Materials and methods .................................................................................. 63
    4.2.1 Fabrication process.................................................................................... 64
    4.2.2 Sample preparation................................................................................... 68
    4.2.3 Experimental setup.................................................................................. 68
  4.3 Results and discussion ................................................................................... 69
    4.3.1 Comparison of electric field distribution for different electrode configurations.......................................................... 69
    4.3.2 Microfluidic chip fabricated by the method described ......................... 70
    4.3.3 DEP responses of different particle types.............................................. 73
    4.3.4 Separation of polystyrene particles based on size................................. 77
    4.3.5 Separation of polystyrene particles and yeast cells based on dielectric properties.......................................................... 78
  4.4 Conclusions................................................................................................... 79

Chapter 5 A 3D dielectrophoretic chip integrating focusing, aligning and trapping for improved concentration and separation of particles.............................................. 81
  5.1 Introduction..................................................................................................... 81
  5.2 Theory ........................................................................................................... 83
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>Numerical simulation</td>
<td>83</td>
</tr>
<tr>
<td>5.4</td>
<td>Materials and methods</td>
<td>86</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Chip layout and fabrication</td>
<td>86</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Microelectrode configuration</td>
<td>88</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Sample preparation</td>
<td>89</td>
</tr>
<tr>
<td>5.4.4</td>
<td>Experimental setup</td>
<td>90</td>
</tr>
<tr>
<td>5.5</td>
<td>Results and discussion</td>
<td>90</td>
</tr>
<tr>
<td>5.5.1</td>
<td>Concentration mechanism</td>
<td>90</td>
</tr>
<tr>
<td>5.5.2</td>
<td>Concentration of polystyrene microparticles</td>
<td>92</td>
</tr>
<tr>
<td>5.5.3</td>
<td>Concentration of yeast cells</td>
<td>94</td>
</tr>
<tr>
<td>5.5.4</td>
<td>Separation of polystyrene microparticles based on size</td>
<td>96</td>
</tr>
<tr>
<td>5.5.5</td>
<td>Separation of yeast cells and polystyrene microparticles based on dielectric properties</td>
<td>98</td>
</tr>
<tr>
<td>5.6</td>
<td>Conclusions</td>
<td>100</td>
</tr>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>102</td>
</tr>
<tr>
<td>6.2</td>
<td>Materials and methods</td>
<td>103</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Fabrication of the DEP-based microfluidic chip</td>
<td>104</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Sample preparation</td>
<td>107</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Experimental setup</td>
<td>107</td>
</tr>
<tr>
<td>6.3</td>
<td>Results and discussion</td>
<td>108</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Characteristics of designed electrode</td>
<td>108</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Particles and cells choice</td>
<td>109</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Capture of polystyrene particles</td>
<td>110</td>
</tr>
<tr>
<td>6.3.4</td>
<td>Capture and release of yeast cells</td>
<td>112</td>
</tr>
<tr>
<td>6.3.5</td>
<td>Capture and release of <em>E. coli</em></td>
<td>114</td>
</tr>
<tr>
<td>6.3.6</td>
<td>Separation of live and dead yeast cells</td>
<td>116</td>
</tr>
<tr>
<td>6.4</td>
<td>Conclusions</td>
<td>119</td>
</tr>
<tr>
<td>7.1</td>
<td>Introduction</td>
<td>121</td>
</tr>
<tr>
<td>7.2</td>
<td>Theory and mechanism</td>
<td>123</td>
</tr>
<tr>
<td>7.3</td>
<td>Materials and methods</td>
<td>126</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1-1 Flow chart of the overall work. ................................................................. 3
Figure 2-1 Mechanical technique: (a) weir-type [24], and (b) pillar-type filter [25]. 8
Figure 2-2 Inertial technique: (a) slanted obstacles induced rotational flows for  
hydrophoretic separation [35], (b) and (c) the combined effect of shear-gradient-  
induced and wall-induced lift force for particle separation in a massively parallel  
and multi-orifice microchannel [37], (d) the inertial self-ordering process in a curved microchannel [38], (e) particle motion in a spiral  
microchannel under the combined effect inertial lift and Dean drag forces [39],  
and (f) soft inertial separation in a microchannel having a curved and focused  
sample flow segment [40]. .................................................................................. 9
Figure 2-3 Hydrodynamic technique: (a) hydrodynamic filtration [45], (b) pinched  
flow fractionation [46], and (c) deterministic lateral displacement [47]. .......... 10
Figure 2-4 Acoustic technique [54]: (a) a particle suspension passing over the  
transducer, which moves toward the centre of the separation channel, and (b) the cross section of separation channel ......................................................... 11
Figure 2-5 Optical technique [59]: (a) the concept of optical fractionation, and (b)  
size-dependent separation of capsules with 2 μm (black) and 4 μm (white) in  
diameter............................................................................................................. 12
Figure 2-6 Magnetophoretic technique: (a) a magnetophoretic microsystem for the  
separation of blood cells [69], and (b) lateral displacement and magnetophoretic  
force of a cell passing over a slanted ferromagnetic wire [70]. ......................... 13
Figure 2-7 Principle of dielectrophoresis: (a) the behaviour of particles (charged and  
neutral) in a uniform electric field, and (b) the behaviour of neutral particles in a  
non-uniform electric field, which experience net forces................................. 15
Figure 2-8 The real part of CM factor versus frequency [14]: (A) \( \sigma_p > \sigma_m, \varepsilon_p < \varepsilon_m \);  
and (B) \( \sigma_p < \sigma_m, \varepsilon_p > \varepsilon_m \) .............................................................................. 18
Figure 2-9 A schematic illustration of travelling wave dielectrophoresis (twDEP) of a  
spherical particle. .............................................................................................. 19
Figure 2-10 The fabrication process for planar electrodes................................. 20
Figure 2-11 The fabrication process for the extruded metal electrodes [85] ......... 22
Figure 2-12 The fabrication process for a DEP-based microdevice with carbon electrodes [86] .................................................................................................................. 23
Figure 2-13 (a) fabrication process flow for a DEP-based device with silicon electrodes, and (b) schematic diagram of chip configuration [87] ..................... 24
Figure 2-14 The fabrication process for a microfluidic device with electroplated 3D electrodes embedded in the sidewalls [88] ...................................................... 25
Figure 2-15 The fabrication process for with 3D conducting PDMS composite electrodes in sidewalls [89]. ........................................................................... 26
Figure 2-16 The fabrication process for the micro-scale copper electrodes [90]. .... 27
Figure 2-17 The fabrication process for a PDMS-based microfluidic device [92].... 28
Figure 2-18 The fabrication process for a microfluidic device using etched silicon wafers as the master for PDMS casting [97]. ................................................. 29
Figure 2-19 Planer microelectrode structures: (a) polynomial [99], (b) castellated [100], (c) interdigitated [101], (d) slanted [102], and (e) curved [103] ............ 31
Figure 2-20 Top-bottom patterned microelectrode array [149]: (a) schematic showing of design and mechanism, and (b) illustration of size-dependent particle separation. ........................................................................................................ 35
Figure 2-21 Extruded microelectrode structure: (a) gold cylindrical electrodes arranged trapezoidally [159], (b) 3D carbon microelectrode array [160], and (c) highly doped silicon pillars [161]. ................................................................. 37
Figure 2-22 Side-wall patterned microelectrode structures: (a) vertically interdigitated on both sides [168], (b) conducting silver PDMS composite [89], (c) copper electrodes [169], and (d) liquid microelectrodes [170] ................. 38
Figure 2-23 Arrays of insulating posts for the concentration and separation of live and dead bacteria [177]. ......................................................................................... 40
Figure 2-24 Insulating obstacle-based structures: (a) single rectangular hurdle [188], (b) a pair of rectangular hurdles [95], (c) multiple rectangular hurdles [189], (d) oil droplet [190], and (e) ridge [191]. ................................................................. 41
Figure 2-25 Channel geometry modification: (a) saw-tooth [199], (b) serpentine [200], (c) spiral [201], (d) circular [98], and (e) pore [202] .................. 42
Figure 2-26 Schematic illustration of contactless DEP microdevice design and configuration [212] ........................................................................................................ 44
Figure 3-1 A schematic view of the fabrication process. (Diagram not to scale)..... 48
Figure 3-2 Contact angle measurements. Water Contact angles measured on the surfaces of (a) bare PDMS, and (b) Teflon-treated PDMS.

Figure 3-3 XPS analysis results of (a) bare PDMS, and (b) Teflon-treated PDMS.

Figure 3-4 Picture showing the effect of spin speed on the thickness of transferred PDMS layer. PDMS thickness versus spin speed with the spin time fixed at 30 s, the inset picture is a microscopic photo showing a 36 µm-thick PDMS layer prepared by spinning at 2000 rpm.

Figure 3-5 Microscopic pictures showing the influence of laser parameters on cutting profiles. Lines were cut on a 36 µm-thick PDMS film by setting laser power, scanning speed and PPI at (a) 0.25W, 2.25mm/s, 1000; (b) 0.50 W, 0.75mm/s, 1000; (c) 0.25W, 0.75mm/s, 500; and (d) 0.25W, 0.75mm/s, 1000.

Figure 3-6 SEM images of bonding (a) glass-PDMS-glass, and (b) silicon-PDMS-glass.

Figure 3-7 Schematic view of the fabrication process flow for an integrated electronic-microfluidic device. Insets show top view of sputter-coated electrodes, laser-cut microchannel and final fabricated chip. (Diagram not to scale)

Figure 3-8 Diagram of the electrodes sputter-coated on the glass substrate, which consisted of two units: focusing and trapping. Wiring connections to external power supply are not shown.

Figure 3-9 Photograph of micro-fabricated DEP-based microdevice with 3D electrodes. Two glass substrates containing electrodes were bonded together by a laser-patterned PDMS layer. Wires and tubing were joined for electrical and fluidic connection, respectively.

Figure 3-10 Pictures showing microparticles (10 µm) were focused by slanted electrodes. (a) Miro-particles were distributed uniformly without electric field, and (b) micro-particles moved in the middle of the channel when an AC electric field at 10 kHz and 10 Vp-p was applied.

Figure 3-11 Pictures showing the microparticles (10 µm) trapped by arc electrodes. (a) t=0 s, (b) t=10 s, (c) t=20 s, and (d) t=30 s.

Figure 4-1 Schematic of the fabrication process including: a) SU-8 mould fabrication, b) planar electrodes patterning, c) fabrication of microchannel with metal alloy...
microsphere position (PDMS recesses are highlighted by red squares), d) plasma bonding, and e) thermal post-treatment. .......................................................... 64

Figure 4-2 Comparison of the arc-shaped electrodes at the sidewall of microchannel after annealing at (a) 75 °C for 10 min, and (b) 60 °C for 10 min. .................. 68

Figure 4-3 Comparison of square of electric field ($E^2$) with different electrode configurations at the sidewall of microchannel: (a) line electrodes, (b) rectangular protrusions, and (c) arc-shaped electrodes. The largest protrusion is 30 µm from the edge of the channel. The width of channel is 100 µm. Both of them are the same as the real picture in our experiment. The applied voltages are +5 V and -5 V, respectively, on these two adjacent electrodes. .................. 70

Figure 4-4 Picture of the fabricated microfluidic device with 3D arc-shaped electrodes at the sidewall of channel. ................................................................. 71

Figure 4-5 Picture showing the configuration of microchannel and microelectrodes. ................................................................................................................... 72

Figure 4-6 (a) An SEM picture of 3D electrodes surrounded by PDMS and in contact with the planar Pt electrode; (b) and (c) are the enlarged pictures highlighted by red dashed rectangles in (a). ................................................................. 73

Figure 4-7 A schematic illustration of the working principle of a fabricated DEP-based device. Particle behaviours (a) without, and (b) with electric fields applied to the 3D electrodes are compared. ............................................................... 74

Figure 4-8 Comparison of the trajectories of 10 µm PS particles (a) without and (b) with electric signals applied to the 3D electrodes. The applied AC signals were 15 V at 20 kHz, and the flow rates for two inlets were fixed at 0.1 µl/min. The microparticles were marked by red circles to illustrate the trajectories. .............. 76

Figure 4-9 Comparison of the trajectories of yeast cells (a) without and (b) with electric signals applied to the 3D electrodes. The applied AC signals were 25 V at 100 kHz, flow rate for two inlets are fixed at 0.1 µl/min. The yeast cells were marked by red circles to illustrate the trajectories. .................................................. 76

Figure 4-10 Separation of 10 and 5 µm PS particles depending on size. AC sinusoidal signal with amplitude of 15 V at 20 kHz was used. 10 and 5 µm PS particles are marked with black and red dashed circles, respectively, to indicate the separation process. ................................................................. 77
Figure 4-11 Separation of 5 µm PS particles and yeast cells depending on dielectric properties. AC sinusoidal signal with amplitude of 20 V at 200 kHz was used. 5 µm PS particles and yeast cells are marked with red and green dashed circles, respectively, to indicate the separation process. ................................................ 78

Figure 5-1 Simulation results showing the distribution of electric field (\(E\), contours) and direction of negative DEP force (black arrows) in a vertical plane of microchannel patterned with electrodes on the top and bottom surfaces. The channel is 30 µm high, and the voltage applied to the electrodes is 5 V. .......... 83

Figure 5-2 Comparison of triangular (left column), rectangular (middle column), and crescent (right column) microelectrodes: (a1-a3) the distribution of electric field, \(E\), in the area formed by the geometry of microelectrodes, and (b1-b3) the variation of the square of electric potential, \(\phi^2\), along the vertical centreline (indicated by black-dash lines) across the microelectrodes. The field applied to the electrodes was remained the same at 5 V................................. 84

Figure 5-3 A schematic diagram of the proposed micro-concentrator and separator using dielectrophoresis................................................................. 86

Figure 5-4 (a) Photograph of the fabricated microfluidic chip used in the experiment for manipulating particles. (b) Magnified image indicating the structure of microelectrode array, which is consists of three units from right to left: focusing, aligning and trapping........................................ 87

Figure 5-5 Schematic dynamics of the concentration of target particles as they move in a continuous through-flow ................................................................. 91

Figure 5-6 Concentration of polystyrene particles of different sizes: (a1, a2) 5, (b1, b2) 10, and (c1, c2) 15 µm at 10 (left column) and 40 seconds (right column). Target particles are sequentially focused, aligned and trapped by applying 8 Vp-p, 10 kHz signal voltage at the flow rates of sample and buffer both of 0.3 µl/min................................................................. 92

Figure 5-7 Threshold voltages for trapping polystyrene particles with diameters of 5, 10, and 15 µm at the flow rate of sample ranging from 0.3 to 0.8 µl/min........ 93

Figure 5-8 Concentration of yeast cells at the trapping unit of crescent electrodes under a field of 12 Vp-p, 10 kHz and flow rates of sample and buffer both at 0.2 µl/min over 100 seconds. Images were taken at 10 sec intervals .................... 94
Figure 5-9 (a) The effect of applied voltage on the trapping efficiency of the cell at a fixed flow rate of the sample and field frequency of 0.3 µl/min and 10 kHz, respectively. (b) The effect of sample’s flow rate on cell-trapping efficiency at a fixed signal field of 9 Vp-p and 10 kHz................................................................. 95

Figure 5-10 Schematic dynamics of particle separation based on size; large particles are focused, aligned, and finally trapped by the microelectrode array, and are therefore isolated from the small particles carried away by continuous flow.... 96

Figure 5-11 Separation of 5 and 10 µm particles at a sample and buffer flow rate both of 0.3 µl/min with an applied sinusoidal voltage of 6 Vp-p and 10 kHz: (a) the overall response of the particle mixture at the microelectrode array, and magnified images showing the response of particles at the units of (b) trapping and (c) focusing................................................................. 97

Figure 5-12 Separation of 5 and 15 µm particles at a sample and buffer flow rate both of 0.3 µl/min with an applied sinusoidal voltage of 5 Vp-p and 10 kHz: (a) the overall response of the particle mixture at the microelectrode array, and magnified images showing the response of particles at the units of (b) trapping, and (c) focusing................................................................. 97

Figure 5-13 Separation of yeast cells (white dots) and 5-µm diameter polystyrene particles (black dots) at a sample and buffer flow rate both of 0.2 µl/min with an applied sinusoidal voltage of 7 Vp-p and 300 kHz. The response of the mixture at the trapping unit over 100 sec was shown at: (a) t=10 s, (b) t=25 s, (c) t=50 s, and (d) t=100 s. The yeast cells were seen moving to the outlet with continuous through-flow, while PS particles were successfully trapped within the inner crescent-shaped electrodes, and the area of concentrated particles increased over time................................................................. 99

Figure 5-14 Percentage of each population of particles at the outlet in separation experiments: (I) size-based separation of 5 and 10 µm PS particles, (II) size-based separation of 5 and 15 µm PS particles, and (III) dielectric properties-based separation of yeast cells and 5 µm PS particles........................................ 100

Figure 6-1 Fabrication process flow of the 3D integrated microfluidic chip: the lower and upper channels as well as the chip are fabricated separately before being plasma bonded together................................................................. 104
Figure 6-2 Microfluidic chip pictures. (a) Real image of fabricated microfluidic chip containing 13 channels in a small area; (b) Optical microscope image of the electrodes configuration in the region indicated by red-dashed square in 5-2a. The arrowhead shaped electrode is designed for trapping particles by AC signal, while the circle shaped electrodes are used for manipulating particles by DC signal.

Figure 6-3 Simulation results of electric field intensity. (a) Electric field intensity distribution of arrowhead 3D electrode at the middle plane between upper and lower electrode. The inset shows the schematic diagram of 3D electrode configuration. The potentials were set to be 1V and -1V for upper and lower electrode, respectively. The red and blue bar represents high and low electric field, respectively. The black lines indicate electrode edges. (b) Cross-sectional view of field distribution along the red dashed line in 5-3a. (c) Cross-sectional view of field distribution of planar electrode located on one side of microchannel.

Figure 6-4 Accumulation of 10 µm PS particles by applying an AC signal of 21 V at 50 kHz with field strength of 7×10^6 V/m on the arrowhead electrodes. (a) t=0 s, (b) t=30 s, (c) t=60 s, and (d) t=90 s. The channel depth was 30 µm.

Figure 6-5 Optical images of the whole trapping region containing 13 channels in total and several individual channels indicated by red dashed squares. It shows that every channel could be used for trapping particles.

Figure 6-6 Trapping yeast cells in four channels to demonstrate the capability of the proposed device for bio-particle pre-concentration. Pictures were taken after applying trapping signal for 2 minutes. The applied AC signal was 10 V at 50 kHz and the channel height was 15 µm. The medium conductivity was adjusted to 140 µS/cm for collection of yeast cells by negative DEP.

Figure 6-7 Capture and release of yeast cells. (a, b) Capture of yeast cells by positive DEP at medium conductivity of 2 µS/cm. Yeast cells were collected between upper electrode and lower electrode where the electric field strength was highest. Flow was driving by difference of water level between inlet and outlet. The applied AC signal was 5 V at 50 kHz and the channel height was 15 µm. (c–f) Release of yeast cells by applying DC field of 700 V/m after the collection
of yeast cells for 55 sec. Yeast cells travelled in the same direction as that of applied field................................................................. 114

Figure 6-8 Collection and release of *E. coli*. (a, b) Collection of *E. coli* by negative DEP at medium conductivity of 380 $\mu$S/cm at $t=0$ s and $t=120$ s, respectively. The AC signal of 15 V at 50 kHz was applied within the channel of 15 $\mu$m in depth; (c, d) Collection of *E. coli* by positive DEP at medium conductivity of 2 $\mu$S/cm and release of *E. coli* after trapping for 1 minute. The applied AC signal for trapping was 10V at 50 kHz, and the applied DC field for releasing was 700 V/m................................................................................................. 115

Figure 6-9 The real part of CM factor as a function of electric field frequency for live (red line) and dead (blue line) yeast cells at medium conductivity of 140 $\mu$S/cm. Positive and negative value of the real part of CM factor represents yeast cells under p-DEP and n-DEP, respectively. The violet dashed line indicates the working frequency for separating them due to the difference in DEP forces. The inset is the optical microscope image of mixture of live and dead yeast cells dyed by different two colours......................................................... 117

Figure 6-10 The separation of live and dead yeast cells. Live yeast cells experiencing larger DEP force were trapped at the tip of arrowhead electrode, while dead yeast cells exhibiting lower DEP response were move towards the outlet under electrokinetic force. The applied AC signal was 3 V at 50 kHz and DC electric field was $1.0 \times 10^3$ V/m. The channel height is 30 $\mu$m. The pictures were taken with time interval of 10 sec............................................................................. 118

Figure 7-1 Illustration of the negative dielectrophoretic focusing of particles in a waved microchannel. The distribution of the electric-field lines and contours of the electric-field strength, $E$, within the microchannel are shown (the darker region has a stronger electric field). Particles moving through the waved channel electrokinetically are exposed to negative DEP forces.............................. 125

Figure 7-2 Photograph of the microfluidic chip used in the experiment for particle focusing. The inset indicates the structure and dimensions of the waved microchannel................................................................. 127

Figure 7-3 Experimentally observed (left column) and numerically predicted (right column) results of focusing 10 $\mu$m particles in the waved microchannel at
various applied electric fields of 100 (second row), 200 (third row) and 300 V/cm (bottom row).

Figure 7-4 Experimentally observed (left column) and numerically predicted (right column) results of focusing 15 µm particles in the waved microchannel at various applied electric fields of 100 (top), and 200 V/cm (bottom).

Figure 7-5 The effects of electric field and particle size on the particle focusing performance. Both the experimental and numerical values of the measured width of the focused particle stream at the outlet are shown.

Figure 7-6 Experimentally observed (left column) and numerically predicted (right column) results of focusing 5 µm particles in the waved microchannel in the medium of 1 (top) and 10 mM (bottom) NaCl solution.

Figure 7-7 Experimentally observed (left column) and numerically predicted (right column) results of focusing yeast cells in the waved microchannel at various applied electric fields of 100 (middle row) and 200 V/cm (bottom row).

Figure 8-1 Illustration of the negative dielectrophoretic separation and manipulation of particles in a curved microchannel embedded with a round hurdle. Distribution of the electric-field lines and contours of the electric-field strength (E) within the microchannel are shown (the darker region has a stronger electric field). Particles moving through the microchannel electrokinetically are subjected to negative DEP forces (indicated by the dark blue arrows).

Figure 8-2 Photograph of the DEP-based microfluidic chip for continuous manipulation and separation of microparticles. The inset indicates the structure and dimensions of the design.

Figure 8-3 A schematic diagram of the S-shaped microchannel embedded with multiple round hurdles.

Figure 8-4 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage at inlet A on the motion of 10 µm particle at (a1-a3) entry, (b1-b3) centre and (c1-c3, d1-d3) exit region. The applied voltage at the inlet A was increased from 140 V (third row) to 340 V (bottom row), while outlet B was grounded and applied voltage at outlet C was fixed at 15 V in both cases. The red arrows indicate the direction of flow.
Figure 8-5 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage at outlet B on the 10 µm particle motion: (a1-a3) 15 V and (b1-b3) 50 V. In both cases, the applied voltages at inlet A and outlet C were fixed at 140 and 0 V, respectively. ................................................................. 147

Figure 8-6 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage at outlet C on the motion of 10 µm particles: (a1-a3) 15 V and (b1-b3) 50 V. In both cases, the applied voltages at inlet A and outlet B were fixed at 140 and 0 V, respectively. ..................................................................................................... 148

Figure 8-7 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage on the motion of 15 µm particles. The applied voltage to inlet A and outlet C were, respectively: (a1-a3) 90 and 10 V; (b1-b3) 180 and 10 V; (c1-c3) 180 and 30 V. The outlet B was grounded in all cases................................................................. 149

Figure 8-8 Continuous separation of 10 and 15 µm particles in an S-shaped microchannel embedded with multiple round hurdles, when the applied voltages at inlet A and outlet C were 180 and 18 V, respectively, and outlet B was grounded. Both the experimental (left column: snapshot; middle column: superimposed) and numerical (right column) results of particle motion in the (a1-a3) entry, (b1-b3) centre, and (c1-c3) exit region are presented. The red arrows indicate the direction of flow. ......................................................................................... 150

Figure 8-9 The percentage of 10 and 15 µm particles in the outlet reservoirs after the experiment on size-dependent separation in the developed microchannel. ..... 152
CHAPTER 1

INTRODUCTION

1.1 Background and motivation

The development of microfluidic platforms is heading towards the miniaturisation, integration, and automation of biological and chemical assays [1, 2]. Lab-on-a-chip (LOC) devices are direct representatives of such a trend. LOC devices, also known as micro total analysis systems (µ-TAS), can be defined as micro electromechanical systems (MEMS) that integrate laboratory functions on a single small scale chip [3, 4]. Their small volume reduces the time required to synthesise and analyse, as well as reduces the amount of fluid required and lowers reagent costs; the faster response of the microsystem allows for greater control of reaction process; compact devices allow for massive parallelisation and high-throughput analysis; lower fabrication costs contribute to the mass production of disposable chips. The compactness, portability, sensitivity, and parallelisation features means that LOC devices are beneficial for various applications, including point-of-care (POC) diagnostics, drug delivery, molecule and material synthesis, chemical reaction control and detection, cellular process studies and environmental monitoring. One of the existing applications is to manipulate and separate particles (both synthetic and biological), which plays an important role in the field of biology, chemistry and medicine.

To date, a variety of technologies have been developed to manipulate and separate particles in an aqueous solution, including mechanical, inertial, hydrodynamic, acoustic, optical, magnetic, and electrical methodologies [5-7]. Among these available methods, dielectrophoresis (DEP) has attracted most attention due to its great advantages [8, 9]. Unlike other techniques, DEP depends on the dielectric properties, which represent the structural, morphological, and chemical characteristics of bio-particles, allowing highly selective and sensitive analysis. DEP manipulation is straightforward and fully controllable by varying the electric conductivity of the suspending medium or the frequency and magnitude of the electric field applied. DEP is also easily and directly interfaced to conventional electronics, and can be used in the fabrication of LOC devices. In addition, DEP enables contact-free manipulation of particles (both charged and neutral) with lower sample consumption and fast speed.
DEP, first adopted by Pohl [10], is a phenomenon that occurs due to a translational force exerted on a dielectric particle in a non-uniform electric field. A dielectric particle and the suspending medium become electrically polarised in an electric field, which separates the electric charge at the interface between solid and liquid. Depending on the relative polarisability of the particle and suspending medium, a net dielectrophoretic force will attract the particle towards the region of higher electric field gradient (positive DEP) or push the particle towards the region of lower electric field gradient (negative DEP) [11]. DEP has proven to be a versatile mechanism for manipulating various micro/nano scale bio-particles (i.e. cells, viruses, bacteria, DNA and protein) in microfluidic systems [11-13].

There are two main strategies used to generate the electric field gradient required for the DEP effect: microelectrodes embedded within microchannels and insulator structures. Traditionally, high-frequency alternating current (AC) electric fields were applied via two-dimensional (2D) or three-dimensional (3D) microelectrodes embedded within the microchannels. The 2D planar microelectrodes were normally patterned on the bottom floor of the microchannel by the photolithograph technique, which could affect the movement of particles close to the surface of the electrode [11]. Alternatively, 3D microelectrodes [14] such as extruded vertical electrodes on the bottom, top-bottom patterned, and sidewall patterned microelectrodes have been developed and fabricated using more complicated techniques, which could generate DEP forces over a larger volume within the microchannel. When creating inhomogeneous electric fields by insulator structures [15], insulating obstacles are embedded within the microchannel or the geometry of the insulating microchannel is modified, and direct current (DC) or DC biased low-frequency AC electric fields are applied via external electrodes placed in the inlet and outlet reservoirs. Both electrode-based and insulator-based dielectrophoretic microdevices have been successfully used to manipulate and separate particles. The micro-fabrication techniques currently available have been applied to build dielectrophoretic microdevices that incorporate with microelectrodes and/or micro insulator structures. In recent years, the development of integrated, intelligent and advanced DEP-based microsystems has attracted great interest.
1.2 Objectives
Manipulating micro-/nano-particles within microfluidic systems using the dielectrophoretic effect has great advantages, so our ultimate goal is to develop the methods of micro-fabrication and DEP-based microdevices to manipulate and separate microparticles. The major objectives of our work are:

1) Development of simple and cost-effective micro-fabrication approaches for DEP-based microdevices with 3D microelectrodes, which enable a more effective and flexible control of particle motion within the microchannel.

2) Design, optimisation and fabrication of novel dielectrophoretic microdevices with 3D microelectrode structures that allow for integrated functionalities (i.e. focusing, trapping and sorting, etc.) and improved performance.

3) Design, optimisation, and fabrication of insulator-based DEP microdevices with enhanced and controllable local electric fields, which could more accurately and effectively manipulate and separate microparticles.

1.3 Outline of the thesis

Figure 1-1 Flow chart of the overall work.

Figure 1-1 shows the overall work presented in this thesis, which is consist of three main sections: micro-fabrication methods of dielectrophoretic microdevices.
(Chapters 3 and 4), electrode-based DEP microdevices (Chapters 5 and 6), and insulator-based DEP microdevices (Chapters 7 and 8). The complete thesis is organised as follows:

Chapter 1 provides the background and motivation of this work. A short summary of the latest development and application of LOC devices are presented, along with the method of manipulating particles using DEP. The objectives of the work are also discussed.

Chapter 2 summarises and compares the techniques used to manipulate particles. The theories of DEP, the fabrication techniques applied in DEP-based microdevices, and the state-of-the-art of dielectrophoretic microsystems for various applications are included.

Chapter 3 introduces a method for fabricating dielectrophoretic microdevices with microelectrodes patterned on both the bottom floor and top ceiling of the microchannel. A laser-plotted polydimethylsiloxane (PDMS) layer with channel structures is sandwiched between two electrode-patterned substrates to act as both a working and bonding layer. This method has many advantages such as simple and fast fabrication process, low cost, easy integration of electronics, strong bonding strength, chemical and biological compatibility, etc. As an application, a DEP-based microdevice with top-bottom patterned microelectrodes to focus and trap particles has been fabricated and tested.

Chapter 4 describes a novel method for fabricating dielectrophoretic devices with arc-shaped extruded microelectrodes located at microchannel sidewalls using metal alloy microspheres. These sidewall-patterned microelectrodes offer an excellent non-uniform electric field distribution in the lateral direction of the channel, enabling great control of particle motion using the DEP effect, and improvement of designing topological microelectrodes on the sidewalls. Compared to other fabrication techniques for 3D microelectrodes, this method does not require severe experiment conditions, cumbersome processes and expensive equipment. The fabricated DEP-based device for switching and sorting particles demonstrates its application.
Chapter 5 presents a DEP-based microdevice equipped with 3D microelectrode arrays patterned on both the top and bottom surfaces of the microchannel. The 3D microelectrode structure consists of a funnel-shaped focusing unit, a parallel aligning unit and a crescent-shaped trapping unit in series, which enables integrated functionality such as concentration of particles in a continuous flow, as well as separating the mixture of particles according to their size and dielectric properties. The performance of the proposed micro-concentrator is demonstrated by concentrating polystyrene (PS) microparticles and yeast cells dynamically flowing in the microchannel. Moreover, the separation function has been verified by sorting PS microparticles based on size, and isolating 5 µm PS particles from yeast cells based on their dielectric properties.

Chapter 6 reports another dielectrophoretic microdevice with top-bottom patterned microelectrode structures and multiple (i.e. 13) working microchannels in a radial direction for high-throughput performance. Both DC and AC signals are utilised in the proposed microdevice by taking advantage of DC electrophoresis, electroosmosis and AC dielectrophoresis for collecting and separating particles. The performance of the design is evaluated by collecting polystyrene particles, yeast cells, and *E. coli* according to their responses to electric field gradient, and live and dead yeast cells is also achieved.

Chapter 7 presents a waved microchannel to continuously focus microparticles and cells using negative DC DEP. The waved channel consists of consecutive curved S-shaped channels in series to generate spatial non-uniformities for the DEP effect. Particles that are electrokinetically transport through the waved channel will be gradually directed toward the central region of the microchannel. The experimental and simulated results both indicate that the focusing performance improved as the applied voltage, particle size and medium concentration increased. Compared to previous designs, the waved microchannel eliminated any regions of stagnation and locally amplified electric fields due to sharp turns, and reduced the magnitude of applied DC fields and the total length of the curved section.
Chapter 8 proposes an S-shaped curved microchannel embedded with multiple round hurdles for continuous manipulation and separation of particles using negative DC DEP. The effects of obstacle and curvature are combined in such a design for the electric field gradient, which eliminates the drawbacks of using each effect individually, and increases the controllability of localised electric fields, and hence the trajectories of particle motion. Both the experiments and numerical simulations have been conducted, and the results showed that microparticles can be directed into distinct outlets, positioned at different locations along the width of the outlet, and also focused into streams of various widths by adjusting the applied voltages at the inlet and outlets.

Chapter 9 finishes with conclusions on the thesis, its major findings and contribution, and also discusses directions for future studies.
The ability to accurately and effectively manipulate and separate nano-/micro-particles in an aqueous solution is essential to various applications in the field of biology, medicine, and biochemistry. Different approaches have been developed and used in microfluidic devices to manipulate particles, including mechanical, inertial, hydrodynamic, acoustic, optical, magnetic, and electrical methodologies. Of these existing methods, dielectrophoresis (DEP) may be the most popular one due to its many advantages. The theory of DEP is presented along with the micro-fabrication technologies in dielectrophoretic applications, and DEP-based microfluidic devices aimed towards different applications (i.e. particle separation, characterisation, positioning, focusing and trapping, etc.).

2.1 Particle manipulation technologies

Manipulating and separating particles is a critical activity in a variety of biological, medical, and biochemical applications. However, traditional techniques are labour intensive and require multiple additional tags or labels to identify target particles. Fluorescence-activated cell sorting (FACS) [16-18] and magnetic-activated cells sorting (MACS) [19-21] utilise fluorophore-conjugated antibodies and magnetic beads conjugated with antibodies to label cell of interests, respectively. Although they both offer high-throughput screening with rich data outputs, they are costly in terms of system setup, operation and reagent, and are prone to damage on cell fate and function when fluorophores and antibodies are used. Moreover, centrifugation is a common technique to separate particles in bulk based on their differences in size and density [22], but this manual, macroscale, multistep technique needs laboratories dedicated to these functions and may introduce contamination during the process [23]. Recently, there has been an interest in label-free techniques that take advantage of intrinsic properties of particle populations, such as size, shape, electrical polarisability, and density. The non-contact techniques developed to accomplish particle/cell manipulation and separation involve mechanical, inertial, hydrodynamic,
acoustic, optic, magnetic, and electrical methodologies, which are summarised and discussed as follows.

2.1.1 Mechanical

![Figure 2-1 Mechanical technique: (a) weir-type [24], and (b) pillar-type filter [25].](image)

Manipulating cells mechanically based on size is achieved by fabricating constriction structures within the microchannels. Wilding et al [24] utilised a series of weir-type structures (Fig. 2-1a) to isolate the white blood cells (WBCs) from blood. Weir-type filters were also used to separate plasma from whole human blood [26], as well as separate and collect blood cells [27]. Mohamed et al utilised micropillar arrays (Fig. 2-1b) to separate fetal nucleated erythrocytes (fNRBs) from maternal cells in cord blood [25], and circulating tumour cells (CTCs) from blood [28]. Micropillar arrays were placed perpendicular to the flow direction to separate white blood cells (WBCs) [29, 30] and plasma [31] from whole blood, and enrich the population of neonatal rat cardiac cells [32]. Yang et al [33] utilised membrane filters with circular, hexagonal and rectangular through holes to trap particles, while Zheng et al [34] proposed using a parylene membrane micro-filter device for the capture and electrolysis of circulating tumour cells (CTCs). These microfluidic filter devices had several advantages due to their purely physical method of separation, such as high labelling efficiency and reproducibility, but they did face many challenges including the heterogeneity of cell sizes within a population and clogging and fouling, in addition, their use for biological assay was limited by poor selectivity.
2.1.2 Inertial

Figure 2-2 Inertial technique: (a) slanted obstacles induced rotational flows for hydrophoretic separation [35], (b) and (c) the combined effect of shear-gradient-induced and wall-induced lift force for particle separation in a massively parallel [36] and multi-orifice microchannel [37], (d) the inertial self-ordering process in a curved microchannel [38], (e) particle motion in a spiral microchannel under the combined effect inertial lift and Dean drag forces [39], and (f) soft inertial separation in a microchannel having a curved and focused sample flow segment [40].

Inertial lift forces including shear-induced and wall-induced lift forces that inherent to particle movement in the microchannel flows have been utilized for particle manipulation. Choi et al [35, 41] proposed the use of rotational flows induced by obstacles patterned within microchannels to separate particles (Fig. 2-2a). Due to the lateral pressure gradient generated by the embedded obstacles, particles can be deflected and arranged along the lateral flow. Mach and Carlo [36] proposed a microchannel consisting of a focusing, expansion and collection region where the size-dependent inertial lift forces are utilised to separate pathogenic bacteria cells from diluted blood (Fig. 2-2b). Park et al [37] focused particles in a multi-orifice microchannel using hydrodynamic inertial forces (i.e. tubular pinch effect and wall effect), where they were deflected away from the walls and placed at specific lateral positions (Fig. 2-2c). Gossett and Carlo [42] utilised the secondary flow induced in curved microchannels to focus particles, such that the focusing position depends on the ratio of inertial lift to drag forces. This mechanism was also extended to
asymmetrically curved microchannels (Fig. 2-2d) [38, 43], which were used to separate polystyrene particles based on size and sort platelets from blood. Seo et al [44] and Kuntaegowdanahalli et al [39] proposed the use of spiral channels (Fig. 2-2e), and demonstrated the separation of triple polystyrene particles having different sizes, and the separation of neuroblastoma and glioma cells. The separation of bacteria (i.e. *E. coli*) from human blood cells has been done by Wu et al [40] using the inertial force induced by sheath flows and channel geometry (Fig. 2-2f). Although inertial separation offers high volumetric flow rates, it requires dilution either before injection or within the device to eliminate particle-particle interactions, and a long channel to focus the particles because of increased viscosity.

2.1.3 Hydrodynamic

![Figure 2-3 Hydrodynamic technique: (a) hydrodynamic filtration [45], (b) pinched flow fractionation [46], and (c) deterministic lateral displacement [47].](image)

The hydrodynamic technique presumes that particles will follow the fluid streamlines at a low Reynolds number. Hydrodynamic filtration (Fig. 2-3a) was proposed by Yamada and Seki [45] to separate and concentrate particles based on size in a microfluidic device, where particle suspension is introduced into a microchannel with multiple side branch channels which could continuously withdraw liquid from the main stream. Particles of different sizes hence are aligned along the sidewalls, and filtered out from different locations. This technique has been applied to
selectively enrich leukocytes from blood [45], and separate liver cells according to size [48]. The group also introduced pinched flow fractionation to continuously separate particles according to size (Fig. 2-3b) [46], and where particle suspension is introduced into a microchannel with a pinched segment. Particles that are initially aligned to one sidewall by another liquid flow are dispersed to different locations over the width of the main channel depending on their sizes. This technique was used to separate different-sized polystyrene particles [46], sort emulsion droplets according to size [49], and enrich erythrocytes from a blood suspension [50]. Another technique used for continuous hydrodynamic particle manipulation is based on so-called deterministic lateral displacement (DLD) method proposed by Huang et al (Fig. 2-3c) [47], which uses the asymmetric bifurcation of laminar flow through micro-obstacle arrays. Smaller particles follow the streamlines and the net migration is in the average flow direction, which is called ‘zigzag mode’; in contrast, larger particles are repeatedly bumped and displaced at each obstacle, which is called ‘displacement mode’. DLD has been applied for the separation of different-sized polystyrene particles [47], leukocytes from erythrocytes by size [51], blood plasma from the blood cells [52], and healthy lymphocytes from malignant lymphocytes [53].

2.1.4 Acoustic

Figure 2-4 Acoustic technique [54]: (a) a particle suspension passing over the transducer, which moves toward the centre of the separation channel, and (b) the cross section of separation channel.

When cells or particles suspended in fluid are exposed to an ultrasound, they will experience an acoustic radiation force, which can be utilised to manipulate particles
by generating a standing wave over the cross section of a microfluidic channel. As shown in Fig. 2-4, Petersson et al [54, 55] utilised an ultrasonic standing wave field combined with laminar flow to separate different-sized particles and also erythrocytes, platelets and leukocytes. Lenshof et al [56] proposed a separation chip based on an ultrasonic standing wave able to produce plasma fractions from whole blood, and detect PSA from whole blood by combining with protein microarray technology. Hawkes and Coakley [57] proposed a continuous flow particle filter capable of directing particles toward the centre of the acoustic path length while leaving the clarified suspending phase close to the wall of the filter. The performance was verified by polystyrene latex particles of different sizes and yeast cells. Moreover, sorting particles based on size and sorting blood cells from plasma have been achieved by Kapishinkov et al [58].

2.1.5 Optical

![Image]

Figure 2-5 Optical technique [59]: (a) the concept of optical fractionation, and (b) size-dependent separation of capsules with 2 μm (black) and 4 μm (white) in diameter.

Optical tweezers could tether biological molecules to particles, and then capture the particles at the focal point of an electric field gradient, which has been widely used as a tool to manipulate and trap [60-62]. Umehara et al [63] utilised optical tweezers to capture single bacterial cells in an on-chip single-cell micro-cultivation assay. Although optical tweezers offer high resolution for single cell trapping, the area for manipulation is limited. Vertical cavity surface emitting lasers (VCSEL) have been used for optical trapping and active manipulation of living cells and microspheres [64, 65], but it has a relatively low output power and thus causes a lower trapping
strength. MacDonald et al [59] proposed the use of an extended, interlinked, threedemonstrational (3D) optical lattice to sort particles, and successfully separated protein microcapsule drug delivery agents and colloidal particles by their size and refractive index, respectively (Fig. 2-5). Chiou et al [66] proposed using optoelectronic tweezers (OET) which utilise direct optical images to create high-resolution dielectrophoretic electrodes for the parallel manipulation of particles. This OET-based optical manipulation method has been used to separate live and dead B cells [66], and also separate Hela and Jurkat cells due to polarisability and size [67]. Shah et al [68] reported a microfluidic device which combines electrowetting-on-dielectric (EWOD) and optoelectronic tweezers (OET), and demonstrated its capability by automatically isolating and manipulating live HeLa cells. Although optical manipulation can provide various advantages, its application in microfluidics is still limited due to its complicated setup, complex operation, and expensive instrumentation.

2.1.6 Magnetic

Figure 2-6 Magnetophoretic technique: (a) a magnetophoretic microsystem for the separation of blood cells [69], and (b) lateral displacement and magnetophoretic force of a cell passing over a slanted ferromagnetic wire [70].
Magnetic manipulation is a passive, label-based method commonly used to sort and purify cells in microfluidic devices, the basic concept is to selectively bond cells with magnetic beads, and then separate them by applying a magnetic field. However, the intrinsic magnetophoretic properties of particles or cells have been used for separation, which avoids the need of additives such as magnetic tagging and inducing agent. Zborowski et al measured the magnetophoresis of ferritin-labeled lymphocytes [71] and red blood cells [72], which indicated differential cell migration when exposed to a high magnetic field. Han and Frazier [69, 73, 74] proposed the use of a high gradient magnetic separation (HGMS) method to sort red and white blood cells from diluted whole blood, based on their native magnetic properties (Fig. 2-6). Furlani [75] presented a model for the direct and continuous separation of red and white blood cells in plasma, which is capable of predicting the motion of blood cells under a non-uniform magnetic field within the microchannel, by taking the magnetic, fluidic and buoyant forces into account. Based on a combination of pump-driven lateral flow and magnetophoresis, the separation of red and white blood cells from whole blood was demonstrated by Jung and Han [70]. The magnetic field gradient required was caused by an array of ferromagnetic wire placed at an angle to the direction of flow. Pamme and Wilhelm [76] achieved a continuous separation of living cells loaded with magnetic nanoparticles (i.e. mouse macrophages and human ovarian cancer cells) using free-flow magnetophoresis that depends on the magnetic moment, cell size, and the flow rate applied.

2.1.7 Electrical

Two main kinds of electrical forces, i.e. Coulomb force and dielectrophoretic force, have been used to manipulate particles. A Coulomb force is generated by interaction between the net charge on the particle and the applied electric field, and the induced movement of the charged particle is known as electrophoresis (EP). Several studies have demonstrated the biological applications of EP, and were reviewed by Korohoda and Wilk [77]. However, for biological particles that are electrically neutral, EP may not applicable due to the absence of net charge, whereas a DEP force that arises from the interaction between the particle’s dipole and a non-uniform electric field, can be used to manipulate neutral particles. With the recent and rapid
development of lab-on-a-chip (LOC) devices, DEP has been widely used for sorting, focusing, patterning and positioning particles, because of its many advantages.

2.2 Dielectrophoresis (DEP)

When an electrically charged and an electrically neutral particle are placed in a uniform electric field (Fig. 2-7a), they behave differently due to different ion/charge dispersion processes. The former is surrounded by a diffuse layer which contains an excessive number of mobile ions with an opposite charge to the like charge, which forms an well-known electric double layer (EDL) [6]. The charged particle moves towards the electrodes of opposite electrical polarity due to the Coulomb force (or electrophoretic force). With the neutral particle, electric charge is redistributed within the dielectric particle and in the liquid side of the solid-liquid interface, building up a dipole moment. However, the Coulomb forces on each half of the dipole moment are equal in magnitude and opposite in direction, resulting in a zero net force on the particle. As shown in Fig. 2-7b, when the neutral particle is placed in a non-uniform electric field, the Coulomb forces on either sides of the dipole moment can be different, resulting in a net force being exerted on the particle, which is known as dielectrophoretic force. The direction of the force depends on the relative polarisabilities of the particle and the suspending medium, which induces the
particle move towards or against the region of electric field maxima, such a motion is called dielectrophoresis (DEP) [10].

The dipole moment of a spherical particle is given by [78, 79]

\[ p = 4\pi\varepsilon_m r^3 \text{Re}[K(\omega)] E \]  \hspace{1cm} (2-1)

where the bold letters refer to a vector quantity, \( \varepsilon_m \) is the absolute permittivity of the suspending medium, and \( r \) represents the particle radius, \( \text{Re} \) indicates the real part. \( K(\omega) \) is the Clausius-Mossotti (CM) factor depends on the complex permittivities of the particle and the suspending medium, as well as the frequency of the external electric field:

\[ K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \]  \hspace{1cm} (2-2)

where \( \varepsilon^* = \varepsilon - i\sigma/\omega \) ( \( i = \sqrt{-1} \) ) is the complex permittivities, \( \sigma \) is the electrical conductivity, and \( \omega \) is the frequency of the electric field. The subscripts \( p \) and \( m \) denote the particle and suspending medium, respectively. The complex permittivities are presented because both the particles and suspending medium exhibit dielectric and conductive properties in the presence of an electric field.

In terms of the dipole moment, \( p \), the time-average dielectrophoretic force acting on a spherical particle in a nonuniform electric field can be written as [78, 79]

\[ \mathbf{F}_{\text{DEP}} = p \nabla E = 2\pi\varepsilon_m r^3 \text{Re}[K(\omega)]\nabla E^2 \]  \hspace{1cm} (2-3)

Equation 2-3 implies that the DEP force depends on a variety of parameters, such as particle size, the permittivities and conductivities of the particle and suspending medium, and the spatial non-uniformity of the electric field. Moreover, the CM factor plays an important role in DEP force since it represents the dielectric properties of the DEP environment from the contribution of the particle and
suspension medium. It also shows that the DEP effect depends on the frequency of external electric fields, which is considered to be an excellent feature of this phenomenon. If the particle is more polarisable than the suspending medium (Re\[K(\omega)] > 0), the particle is pushed towards the region of strong electric field, and such a motion is termed positive DEP (p-DEP). In contrast, if the suspending medium is more polarisable than the particle (Re\[K(\omega)] < 0), the motion of the particle is away from the region of strong electric field, which is called negative DEP (n-DEP) [80].

The frequency dependence of the DEP force is shown by investigating the CM factor of a homogeneous spherical particle, i.e. Eq. 2-2. The factor can vary from -0.5 (i.e. \(\varepsilon_p^* << \varepsilon_m^*\)) to +1 (i.e. \(\varepsilon_p^* >> \varepsilon_m^*\)), which means a positive DEP force can be twice as strong as negative DEP force. Moreover, at low and high frequency limits, the CM factor is reduced to the following forms, respectively

\[
K(\omega) = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}, \text{ for } \omega \to 0
\]

\[
K(\omega) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}, \text{ for } \omega \to \infty
\]

Therefore, at low frequencies the DEP force depends on the conductive properties of the particle and the suspending medium, while the permittivity values govern the DEP effect at high frequencies. At intermediate frequencies, both the dielectric and conductive properties of the particle and suspending medium determine the magnitude and polarity of the DEP force.
Figure 2-8 The real part of CM factor versus frequency [14]: (A) $\sigma_p > \sigma_m$, $\varepsilon_p < \varepsilon_m$; and (B) $\sigma_p < \sigma_m$, $\varepsilon_p > \varepsilon_m$.

In Fig. 2-8, the electric conductivity of particle is fixed and the DEP responses for different medium conductivities are plotted. In both cases, and at a range of frequencies, the particle experiences a positive DEP while exhibiting a negative DEP response at another range of frequencies. The point where the response switches from p-DEP to n-DEP or from n-DEP to p-DEP is called the crossover frequency. At this frequency the complex permittivity of the particle is equal to that of suspending medium and the DEP force will be zero ($\text{Re}[K(\omega)] = 0$). The shift of the crossover frequency could be achieved by changing the permittivity and conductivity of the particle and suspending medium.

The conductivity of a spherical particle can be expressed as the sum of its bulk and surface conductivity,

$$\sigma_p = \sigma_{\text{bulk}} + \frac{2K_s}{r} \quad (2-5)$$

where $\sigma_{\text{bulk}}$ is the bulk conductivity of the particle, $r$ is the radius of the particle. $K_s$ represents the surface conductance, which is induced due to the charges in the diffuse layer and stern layer [81].

In Eq. 2-3, the DEP force induced in DC fields and stationary AC fields have been considered, but if the phase of the AC field has a spatial variation, the time-averaged DEP force arising from the interaction of the induced dipole moment and the phase of the various field components should be taken into account. Hence, the time-averaged DEP force can be rewritten as

$$\mathbf{F}_{\text{DEP}} = 2\pi \varepsilon_0 r^3 \text{Re}[K(\omega)] \nabla E^2 + 4\pi \varepsilon_0 r^3 \text{Im}[K(\omega)] \sum \mathbf{E}^2 \nabla \phi \quad (2-6)$$

where $\text{Im}$ indicates imaginary part, $\phi$ is the phase component of the AC field. The second term relates to the imaginary part of the CM factor and the spatial nonuniformity of the field phase, which is called a ‘travelling wave DEP (twDEP)’. This force directs the particle against or along the direction of travel of field according to the polarity of $\text{Im}[K(\omega)]$ [82]. As shown in Fig. 2-9, a series of raised
planar electrodes with potentials having a 90° phase variation between the adjacent ones induces a co-/anti-field twDEP response which directs the particle towards/ off the smaller phase regions.

![Diagram of travelling wave dielectrophoresis (twDEP) of a spherical particle.](image)

Figure 2-9 A schematic illustration of travelling wave dielectrophoresis (twDEP) of a spherical particle.

When a DC field (the frequency is zero) is applied, the DEP force expression (Eq. 2-6) remains the same, but the CM factor only depends on the electrical conductivities of the particle and the medium, and is simplified into Eq. 2-4a [83, 84]. The particle suspended in liquid also experiences an electrokinetic (EK) effect which is a combination of fluid electroosmosis (EO) and particle electrophoresis (EP) [78]. The resulting motion is determined by the electrokinetic velocity of the particle, which is linearly proportional to the local electric field and leads the particle being transported along the electric lines. Therefore, the motion of the particle in a DC electric field is governed by both the linear electrokinetic effect and the nonlinear dielectrophoretic effect.

Unlike electrophoresis, which relies on the net electrical charge of particles, dielectrophoresis depends on their dielectric properties which enables it to manipulate neutral bio-particles [10]. As the dielectric properties represent many structural, morphological and chemical characteristics of the particles, DEP offers a more selective and sensitive analysis of biological samples [80]. DEP manipulation is also straightforward and fully controllable by varying the electrical conductivity of the suspending medium or the frequency and magnitude of the applied electric field. This means that a label-free manipulation of particles with lower sample
consumption and faster speed can be achieved [8]. In addition, DEP can be easily used in the fabrication of LOC devices for various applications [9].

2.3 Micro-fabrication techniques used in dielectrophoretic applications

Researchers have generated spatial non-uniformities by using microelectrode arrays within microchannels, and in some cases insulator structures to manipulate and separate particles. Some basic micro-fabrication techniques, such as photolithography, thin-film deposition, etching, electroplating, soft lithography, and substrate bonding, etc., have been used to build dielectrophoretic microdevices. The main micro-fabrication techniques are summarised and presented below based on their applications in DEP-based devices having different electrode or insulator structures.

2.3.1 2D planar electrodes

Figure 2-10 The fabrication process for planar electrodes.
Traditionally, spatial non-uniformity is generated by applying AC electric fields via microelectrode arrays patterned within microchannels. The fabrication of 2D planar metal electrodes always required processes that include photolithography, thin-film deposition, and lift-off and/or etching. As shown in Fig. 2-10a, the mask with the defined microelectrode patterns is designed using AutoCAD or L-edit, after which a positive photoresist is deposited onto a substrate (i.e. glass and silicon) and spin-coated to a final thickness that depends on the spin speed. After the coating, soft-bake is conducted to remove the solvent and improve adhesion to the substrate. The substrate is then aligned to the mask and exposed to a UV source, which is followed by development to obtain the microelectrode lines. As the photoresist is positive, UV-exposed regions will be removed from the substrate, leaving inverse patterns uncovered with photoresist where the target metal material is to be located. This is so-called photolithograph technique, where geometric microelectrode patterns are transferred from a mask to a light-sensitive photoresist. The next process is thin-film deposition where metal of various materials (i.e. gold, platinum, and chromium) is deposited onto the whole substrate using the evaporation or sputtering technique. In the evaporation technique, the substrate is placed in a vacuum chamber, where a piece of metal is heated, evaporated, and deposited on top of the substrate. In sputtering, due to the bombardment of energetic inert ions, atoms or clusters are ejected from the target material, and then directed towards the substrate. After the metal has been deposited, the substrate is submerged in a solvent (i.e. acetone) to wash off the sacrificial photoresist layer together with the target material on its surface. After lift-off, only the target material remains in the regions having a direct contact with the substrate, creating a defined array of microelectrodes. As an alternative (see Fig. 2-10b), the target metal material is first evaporated or sputtered onto the top of the whole substrate, and after the thin-film deposition, a photoresist layer is patterned using the standard photolithography technique mentioned above, followed by wet/dry etching to selectively remove materials from the substrate. Wet etching always utilises potassium hydroxide (KOH), ethylene diamine pyrochatechol (EDP), and tetramethyl ammonium hydroxide (TMAH), and shows high material selectivity, whereas dry etching techniques such as plasma etching, reactive ion etching, and ion milling, is advantageous in terms of a smaller undercut and higher anisotropicity, which contribute to smaller pattern lines and structures with a high
aspect ratio, respectively. Besides, a metal layer could be directly patterned using laser ablation or deposition via a stencil or shadow mask, in which case the photolithograph and etching process are not required.

2.3.2 Extruded electrodes

![Diagram of extruded electrodes process](image)

Figure 2-11 The fabrication process for the extruded metal electrodes [85].

The electroplating technique has been used to build extruded metal electrodes inside the channel volume, where the metal ions in a solution are moved by an electric field to coat a conductive metal layer: the part to be plated and the metal needed are the cathode and anode of the circuit, respectively, both of which are immersed in an electrolyte solution and connected to an external supply of direct current. When a direct current is applied, the metal atoms comprising the anode will be oxidised and dissolved in the solution, which then moves toward the cathode and finally plates out onto the cathode. Figure 2-11 shows the fabrication process of extruded gold electrodes, the details of which can be found in the work of Voldman et al [85]. Generally, the overall process starts by a deposition of two metal layers onto a glass substrate (A), which is followed by etching the Au layer to define the electrical interconnects (B). After SU-8 mould patterning (C) and gold posts electroplating (D), the SU-8 layer and Ti layer were removed (E-F). Then, a second SU-8 layer for a flow channel was patterned using the photolithograph technique, holes for fluid access were drilled, and the whole microdevice was enclosed by glue-bonding with a coverslip.
Figure 2-12 The fabrication process for a DEP-based microdevice with carbon electrodes [86].

The technique utilised to fabricate extruded carbon microelectrodes is called pyrolysis, in which a previously patterned organic precursor is carbonised. Pyrolysis refers to the thermochemical decomposition of organic materials at high temperatures in the absence of oxygen. During this technique organic materials are transformed into gases or liquid, leaving a solid residue known as glassy carbon, which contains carbon and ash. Martinez-Duarte et al [86] provided the details of fabricating carbon electrodes for dielectrophoresis applications. As shown in Fig. 2-12, an SU-8 precursor is patterned by photolithography in two steps: in the first a planar layer is fabricated for connection leads to the base of the volumetric electrodes, and in the second a pillar array is fabricated for 3D carbon electrodes. The SU-8 patterns are then carbonised in a furnace under constant nitrogen flow in two stages: removal of residual oxygen in the chamber at 200 °C, which is then increased to 900 °C where it remains for 1 hour. The microfluidic network is fabricated separately using plotted double-sided adhesive and polycarbonate (PC) with holes drilled beforehand.
Another type of extruded microelectrodes is silicon electrodes which are formed by a heavily doped silicon wafer acting as the channel walls at the same time. The details of fabricating a dielectrophoretic chip with 3D silicon electrodes can refer to the work by Iliescu et al [87], where the fabrication process involves anodic bonding, deep reactive ion etching (DRIE), wet etching and thin-film deposition. The fabrication process of this silicon-electrode-based DEP microdevice is shown in Fig. 2-13a. A diamond bit is used to drill holes in a Pyrex glass wafer for fluidic access. The silicon-glass anodic bonding of a P-type, heavy doped silicon wafer to a Pyrex glass wafer is performed at 305 °C, 1000 V, and 1000 mbar, after which, the electrodes and microfluidic channel are simultaneously defined within the silicon wafer using the DRIE technique. In this technique, the photoresist is first patterned onto the silicon using a photolithograph, followed by a stop-etch on the glass substrate using SF6/O2 in the etching step and C4F8 in the passivation step. After the photoresist layer has been removed, a second anodic bonding is conducted for the previously prepared glass substrate with drilled holes and the patterned silicon wafer at 450 °C, 1500 V, and 2000 mbar. Holes are required at the bottom of the glass substrate for electrical connection. All other front sides of the process wafer are protected with using wax and the bottom glass is reduced to 100 μm by dipping it into a HF (49%)/HCl (30%) 10:1 solution. The Cr/Au layers are deposited and
patterned by wet etching to serve as the mask for creating the through holes. After the holes have been fabricated by dipping in the HF/HCl solution again, the metal mask is removed using classical chromium and gold etchants. Finally, metal leads are fabricated by depositing chromium and gold, patterning the photoresist as a mask and wet etching to obtain metal patterns. A schematic illustration of the device configuration is shown in Fig. 2-13b.

2.3.3 Side-wall patterned electrodes

![Diagram of side-wall patterned electrodes](image)

Figure 2-14 The fabrication process for a microfluidic device with electroplated 3D electrodes embedded in the sidewalls [88].

The electroplating technique was also utilised by Wang et al [88] to place arrays of vertical electrodes onto the sidewalls of the microchannel. Figure 2-14 schematically illustrates the fabrication of a microfluidic device with 3D electrodes electroplated onto the sides of the channel. Briefly, fabricating a microfluidic device with sidewall-patterned electrodes commences by spin-coating a thin layer of SU-8 and then flood the UV exposure to crosslink it. This thin layer of SU-8 could improve the adhesion of the channel layer to the substrate, and also serve as an insulating layer for metal deposition. Thin films of Ti/Au are then deposited and patterned to define electrical connection pads, leads, and seed layers for subsequent electroplating. A relatively thick layer of photoresist is photo-patterned with holes to serve as the mould for electroplating electrodes. After that, the electroplating process is carried out by dipping the device into a solution of gold electroplating with controlled stir rates and current densities, where the exposed and developed holes fill with gold.
After electroplating, the layer of photoresist is removed and another layer of SU-8 is patterned as a microchannel which is finally sealed with a PDMS sheet and a thin layer of SU-8 used as adhesive.

![Fabrication process diagram](image)

Figure 2-15 The fabrication process for with 3D conducting PDMS composite electrodes in sidewalls [89].

Conductive polydimethylsiloxane (PDMS) composites have been utilised as electrodes on the sidewalls. Lewpiriyawong et al [89] provided the fabrication process of a dielectrophoretic device with conducting silver-PDMS composites embedded at the sidewall (Fig. 2-15). A layer of SU-8 is first defined for the microfluidic network, and then a layer of AZ resists is patterned around the SU-8 using a standard photolithograph technique. The synthesized AgPDMS composites made out from mixing PDMS gel and silver powders at a weight ratio of 85% is filled the AZ cavities, and excessive AgPDMS is cleaned by a blade. After the composites have been cured, the AZ is removed leaving SU-8 patterning and composite structures on the substrate. Pure PDMS gel is poured onto the mould, cured, and then peeled off together with AgPDMS composites. The PDMS piece is punched to create holes for microfluidic access, treated with oxygen-plasma, and bonded to a glass substrate to form enclosed microchannels.
Another type of sidewall-patterned electrodes is micro copper electrodes, the fabrication steps of which are shown in Fig. 2-16. A layer of SU-8 is spin-coated onto the copper-clad laminated with a layer of polyimide, which is subsequently patterned using the photolithography technique. The copper sheet partly covered with patterned photoresist is then dipped into the copper etchant to remove any uncovered copper. The copper electrodes with the desired patterns are released in a bath of NaOH (30 %) at 130 °C, while the polyimide substrate and the photoresist are peeled off simultaneously. The microelectrodes are embedded manually into the electrode cavities of a PDMS slab with microchannel patterns, and then the whole component is finally bonded with a glass substrate via plasma treatment. With liquid microelectrodes [91], planer metal electrodes are patterned onto a substrate by photolithography of the resist, thin-film deposition and lift off processes, and then a layer of SU-8 with the desired microchannel structures is fabricated onto the top of the microelectrodes using the standard photolithography process. Finally, a PDMS sheet with via-holes and reservoirs is bonded to the pyrex substrate with electrode and microchannel patterns.

2.3.4 Insulator structures

One approach used to fabricate insulator structures is to glass using the wet etching technique. When etching glass isotropically, the etchant attacks the glass in every
direction simultaneously, which results a semi-circular profile under the mask. Photoresist can serve as the mask for glass etching, but metal masks are always utilised instead to minimise the adverse effect of pin holes on the etching profile. The fabrication process of metal masks includes the thin-film deposition of metal and wet/dry etching through a photoresist mask. After etching glass using wet etchants, the mask layer is removed and thermal bonding is performed in an inert atmosphere such as nitrogen and vacuum, where the etched glass hence is sealed to another piece of glass to form a microfluidic network. Insulator-based DEP devices with posts, silica beads, and ridges have been fabricated using the process.

Figure 2-17 The fabrication process for a PDMS-based microfluidic device [92].

Another type of insulator structure is based on polymer, which has advantages over glass in terms of flexibility and the low-cost of fabrication. Fabricating the patterns and structures with polymer is called soft lithography based on injection moulding or embossing. In this technique, patterned elastomeric stamps, moulds, or conformable photomasks are prepared first, and then a pre-polymer of the elastomer is poured over the master which has relief structures on its surface, cured and peeled off [93]. Among those polymers applied for fabricating microfluidic devices,
polydimethylsiloxane (PDMS) is particularly appealing due to its transparency, flexibility, low costs and compatibility [92, 94]. After the patterned PDMS slab has been fabricated, it must be bonded with glass, silica, or another piece of PDMS to form an enclosed microfluidic network. The sealing could be achieved by simply oxidising the PDMS surface, without the need for adhesive. Figure 2-17 illustrates the fabrication process of a microfluidic device based on PDMS, and the fabricating details could be found elsewhere [95, 96]. Briefly, the fabrication process started with patterning photoresist using the standard photolithography technique to create a positive relief on the surface of a silicon wafer. Once a master has been fabricated, a channel is formed in PDMS by replica moulding, which generates a negative replica of the master in PDMS. Liquid PDMS prepared by mixing Sylgard 184 and curing agent at a weight ratio of 10:1 is poured over the master, degassed, and cured in a vacuum oven. The negative PDMS cast with the microchannel pattern is then peeled off the master, and via-holes for fluidic access or reservoirs for the buffer are created. After plasma treatment, the channel side of the PDMS and a substrate (i.e. glass, silicon and PDMS piece) are bonded to form the desired microchannels. DEP devices with obstacles extended from the sidewalls and curved microchannel geometries are fabricated using this technique.

As an alternative, etched silicon wafers are used as masters for PDMS casting, which requires an additional reactive ion etching (DRIE) step compared to those using patterned resist for the master. The details of fabricating these devices can be found elsewhere [97, 98]. As shown in Fig. 2-18, a silicon master mould is first fabricated.
by DRIE using photolithography-patterned resists as a mask. The surface roughness caused by scalloping effect during DRIE is reduced by growing silicon oxide on the silicon master, and an anti-stiction layer (i.e. Teflon) is coated onto the master mould before being used for casting PDMS. Liquid PDMS is poured onto the master, degassed, cured, and removed. The PDMS replica with punched holes finally bonded to a clean glass slide via the oxygen-plasma treatment.

2.4 Various DEP microdevices for particle manipulation

There are two main approaches to create an inhomogeneous electric field required for the DEP effect: (1) microelectrode arrays patterned within the microchannel, which are connected with external high-frequency AC electric fields; and (2) insulating obstacles placed in the microchannel or channel geometry modification, while DC (or DC-biased AC) electric fields are applied to electrodes placing at the inlet and outlet reservoirs. Depending on various configurations of microelectrodes or insulators, DEP devices are classified in the following main categories: electrode-based (including planar, top-bottom patterned, extruded, and sidewall-patterned, etc.), and insulator-based (including obstacles, curved microchannels, and contactless) dielectrophoretic microdevices.

2.4.1 Electrode-based DEP microdevices

A variety of two-dimensional (2D) and three-dimensional (3D) microelectrode structures have been developed to induce an electric field gradient. The 2D microelectrodes of different geometries, such as polynomical, castellated, interdigitated, slanted and curved, are commonly patterned on the bottom surface of the microchannel. Alternatively, 3D microelectrodes are produced by fabricating planar electrodes on both top and bottom surfaces of the microchannel, extruded electrodes on the bottom, or vertical electrodes along the sidewalls of the microchannel.
2.4.1.1 2D planar microelectrodes

Polynomial
Morgan et al [99] trapped and sorted tobacco mosaic virus (TMV) and herpes simplex virus (HSV) type 1 in a polynomial electrode (Fig. 2-19a). This design generates high electric fields along the edges of the electrode, and a low electric field at the centre. Therefore, HSV-1 experiencing a negative DEP is trapped at the centre of the electrode array, while TMV experiencing a positive DEP is collected at the edge of the electrode. Asokan et al [104] utilised the electrode design to pattern actin on a substrate and direct its motion on myosin substrates along electric field lines. The patterning and collection of mammalian virus (HSV-1) by both positive and negative DEP at different frequencies was accomplished by Hughes et al [105], as well as trapping protein molecules [106] and bacterial spores [107] in a triangular polynomial electrode.

Castellated
Another design is castellated electrode arrays (Fig. 2-19b) which generate an electric field gradient directly above their surfaces such that there are regions of high electric

Figure 2-19 Planer microelectrode structures: (a) polynomial [99], (b) castellated [100], (c) interdigitated [101], (d) slanted [102], and (e) curved [103].
fields located at the tips of the castellations and regions of low electric fields in the
bays between the tips. Morgan et al [99] utilised this design to separate submicro
latex spheres by size, and separate carboxylate-modified and protein-functionalised
latex sphere by surface charge. Other research work on trapping cells and silica
beads [100], sorting nano-[108] and micro-particles [109], and sorting cancel cells
from blood [110, 111] based on this design have also been reported. Choi et al [112]
used this design to separate porcine oocytes based on the difference of
dielectrophoretic velocity, which provides a new method for in vitro fertilisation. By
integrating the electrode array with a serpentine micro-pump and pneumatic micro-
valves, viable and non-viable cells were separated and collected, and the nucleus was
collected after cell lysis [113]. Ramadan et al [114] achieved lysing human white
blood cells (WBCs) and murine clonal cells (MN9D) in a continuous flow, and
trapping silica beads that were selectively bonded to DNA at the same time. Zhang et
al [115] utilised a similar structure with sharp tips to separate multi-walled carbon
nanotubes (MWCNTs) and polystyrene microparticles according to their dielectric
response: the MWCNTs exhibiting a positive DEP response were trapped at the
region of a high electric field and were thus isolated from the polystyrene particles
exhibiting a negative DEP response, and which moved towards the regions of a low
electric field. Yasukawa et al [116] optimised the design to rectangular features with
different widths and gaps, and the manipulation and separation of microparticles
depending on their size has been demonstrated.

Interdigitated
As shown in Fig. 2-19c, interdigitated electrodes have been developed and used to
capture DNA [117], bacteria [118], polystyrene beads [119, 120] and blood
eythrocytes [119], separate live/dead *Listeria innocua* [101] and yeast cells [121],
micro-/nano- particles [99, 119], and pattern colloid particles [122]. Park and Beskok
[123] proposed a theoretical model to predict the AC electrokinetic motion of
microparticles, which was validated by parametric experiments of colloid particles,
including polystyrene and gold particles, and *C. sporogenes* bacterial spores with
interdigitated electrodes. Crews et al [124] developed an equation to calculate the
electric gradient term above interdigitated electrodes, which is a function of the
width and gaps of the electrode, the applied voltage and height above the surface of
electrode. Suehiro et al [125, 126] proposed a method of detecting biological cells or bacteria called dielectrophoretic impedance measurement (DEPIM), which utilises the positive DEP force to capture biological particles in suspension onto an interdigitated microelectrode array, and the transient variation of the electrical impedance to quantitatively evaluate the concentration of particles. The interdigitated electrodes also integrated with other on-chip components for various applications. Gadish and Voldman [120] developed a micro-concentrator by combining interdigitated electrodes with a chaotic mixer, which achieved a concentration of particles (i.e. beads, spores and B. subtilis) with high throughput. Vykoukal et al [127] proposed a dielectrophoretic field-flow fractionation (DEP-FFF) microseparator with a flex interdigitated electrode array to enrich stem cells from enzyme-digested adipose tissue, which was fabricated using a scalable and low-cost hybrid flex-circuit method. Lagally et al [128] developed an integrated microsystem consisting of an interdigitated electrode array, PDMS micro-valves and an optical molecular beacon capable of concentrating bacterial cells (i.e. E. coli) and sequence-specific genetic detection.

Slanted
In addition, electrode arrays placed at an angle to the direction of the flow have been utilised to separate particles in a continuous-flow manner (Fig. 2-19d). Kralj et al [129] used an array of slanted, interdigitated electrodes to separate polystyrene microspheres based on size. Larger particles are deflected more than the smaller particles due to the volume-dependent DEP force, which means that different-sized particles are fractionated to distinct streams as they run down the length of the microchannel. By combining similar slanted electrode arrays with a diffusive mixer, Vahey and Voldman [130] separated polystyrene particles based on surface conductance and also separated live and dead yeast cells in an electrical conductivity gradient. Kim et al [131] developed a micro-sorter with two sets of interdigitated electrode arrays placed at different angles to the direction of flow to isolate and separate multiple target cells from mixtures into distinct fractions. Using a funnel-shaped electrode array, Pommer et al [132] demonstrated the separation of platelets from diluted whole blood, Han and Frazier [102] achieved the separation of red
blood cells (RBCs) and white blood cells (WBCs) in both divergent and convergent interdigitated electrode arrays.

Curved
Han et al [133] developed a piecewise curved electrode array placed at an angle to the direction of the flow to separate polystyrene particle from a heterogeneous mixture according to their size. Khoshmanesh et al [134] developed a curved funnel-shaped electrode array (Fig. 2-19e), which created a strong electric field gradient over the tips and maintained it over a large portion of the structure, and the induced electric field increase smoothly due to the curved structure. Based on this design, the manipulation and separation of two-sized polystyrene particles [134], and the tunable multiplex separation of polystyrene particles at different frequencies [103] were demonstrated. By combining two sets of curved electrode arrays, the separation of live and dead yeast cells at different conductivities of suspending medium has been achieved, along with assessing the sorting performance on the downstream boomerang-shaped electrodes [135]. The authors also reported the patterning of multi-walled carbon nanotubes (MWCNTs) between curved microelectrodes to trap polystyrene particles with different surface modifications [136], and sort particles of different sizes [137]. The applied MWCNs have high conductivities that can coat the surface of the microparticles, inducing their trapping at high frequencies, while also serving as a nano-electrode extension of the microelectrode after deposition.

Other planar designs
Negative DEP micro-wells consisting of a square electrode and a line electrode have been utilised to pattern single particles, such as polystyrene beads of various sizes [138], and adherent (HeLa), and non-adherent (HL60) cells [139]. Jang et al [140] combined quadrupole and micro-well electrodes to manipulate and trap single particles (i.e. polystyrene microsphere and HeLa cell). The motion of the particle is manipulated by the alternating-current electrothermal effect (ACET) induced flow, and after the particle is positioned inside the capture range, the outer quadrupole electrodes responsible for manipulation are turned off while the micro-well electrodes for trapping are actuated. Using an array (or matrix) of circular electrodes coated with porous hydrogel layers, Krishnan et al [141] separated polystyrene
particles and DNA-derived nanoparticles under high-conductive conditions. Moreover, individual living cells (i.e. tumour cells [142], yeast cells [143]), and droplets [143, 144] has been positioned, and individual live cells have been sorted from an unlabelled population [145] in integrated circuit chips with a grid pattern of electrode arrays. Ring-shaped electrodes [146], and a multi-layered array and grid (MLAG) electrodes [147] composed of two metal layers and a sandwiched dielectric layer were used to trap and pattern polystyrene particles with negative DEP. Additionally, ring-dot-shaped electrodes were developed by Taff and Voldman [148] to trap and sort individual silver-coated beads and HeLa cells with positive DEP.

2.4.1.2 3D microelectrodes

Although 2D planar microelectrodes commonly patterned on the bottom of the microchannel were developed for various dielectrophoretic applications, using this structure for some practical applications can be problematic, because the electric field gradient decreases rapidly as the distance from the electrodes increase, so that only the motion of particles close to the electrode surface can be directed, which greatly reduces the efficiency of the microsystems. Moreover, adhesion of the particles onto the surface of electrode or channel wall is always an issue. These limitations can be avoided by fabricating 3D electrodes.

Top-bottom patterned

![Diagram](image)

Figure 2-20 Top-bottom patterned microelectrode array [149]: (a) schematic showing of design and mechanism, and (b) illustration of size-dependent particle separation.

A straightforward approach to overcome the limitations of 2D planar electrodes is fabricating planar microelectrodes on the floor and ceiling of the microchannel, the
particles hence can be focused and forced to move round the centre of the channel in the height direction. Slanted electrode arrays have been patterned on both the top and bottom surfaces of the microchannel (Fig. 2-20) [149-152], which have been demonstrated for the manipulation, accumulation and size-dependent separation of micro- and nanoparticles. Yasukawa et al [153] used arrays of electrodes consisting of navigators, caps and collector electrodes for immunoassays, allowing the manipulation and capture of polystyrene microparticles modified with anti-mouse IgG. Cheng et al [154] developed an integrated electrode array consisting of interdigitated, funnel-shaped, slanted and curved microelectrodes, which is enable to sort and concentrate heterogeneous populations including polystyrene microparticles, yeast cells and bacteria. Asymmetric microelectrode arrays which induce continuously varied electric fields along the transverse direction of a channel have been developed by Park et al [155], and the capability has been demonstrated by sorting mouse P19 embryonic carcinoma (EC) and red blood cells (RBCs). Quadrupole electrodes have also been patterned on top and bottom surface of the microchannel, and have managed to accumulate hepatitis A viruses [156], and capture and cultivate a single yeast cell [157]. Yu et al [158] utilised a microelectrode array patterned on the circumference of an elliptical microchannel to focus micro-beads and human leukemia HL60 cells towards the centre of the channel.

Extruded
Another method for improving the volume where particles experience a strong DEP effect and efficient manipulation is fabricating extruded microelectrodes. Hunt et al [162] utilised an array of gold electrode posts for to trap and manipulate a single polystyrene particle using positive and negative DEP, respectively. As shown in Fig. 2-21a, Voldman et al proposed the use of extruded gold posts in a quadrupolar arrangement fabricated by electroplating, and were able to trap polystyrene microparticles [163] and HL60 cells [159]. Moreover, extruded 3D carbon (Fig. 2-21b) electrodes have been developed. Park and Madou [160] proposed using extruded carbon electrodes on the bottom substrate fabricated by carbonising patterned SU-8 structures to improve the throughput. Using this structure, Jaramillo et al [164] trapped \textit{E. coli}, and separated \textit{E. coli} from a mixture with \textit{B. cereus} bacteria, while Duarte et al [165] trapped yeast cells from the mixture with polystyrene particles. Another 3D electrode structure is made from heavily doped silicon pillars [161].
silicon wafers, which simultaneously served as the walls of a microfluidic channel (Fig. 2-21c). This 3D silicon electrode structure exhibits constant DEP forces along the height of the microfluidic channel, and achieves high trapping efficiency and high-throughput manipulation performance, which has been utilised to trap yeast cells [166], and separate live and dead yeast cells [161, 167].

Sidewall patterned

![Figure 2-22 Side-wall patterned microelectrode structures](image)

Figure 2-22 Side-wall patterned microelectrode structures: (a) vertically interdigitated on both sides [168], (b) conducting silver PDMS composite [89], (c) copper electrodes [169], and (d) liquid microelectrodes [170].

Vertical microelectrode arrays have been also patterned along the sidewalls of the microchannel, which could extend the region for the DEP effect and induce non-uniform electric fields in the direction of channel width. Wang et al proposed using two vertical interdigitated electrode arrays embedded in both sidewalls of the microchannel (Fig. 2-22a), and successfully switched polystyrene particles and mouse neural stem cells [171], separated human kidney cells (HEK293) from N115 mouse neuroblastoma cells, and also separated polystyrene microbeads from modified HEK293 cells [168]. Lewpiriyawong et al developed a novel microfluidic device with conducting PDMS composites as sidewall electrodes (Fig. 2-22b), and separated microparticles by size [89], and also separated yeast cells and *E. coli* from similar-sized latex particles as well as separating live and dead yeast cells [172]. The conductive PDMS composite are made by mixing silver (Ag) powder with PDMS gel. By utilising the combined effect of insulating hurdles and micro copper
electrodes embedded along the sidewalls (Fig. 2-22c), Kang et al [90] separated mixtures of polystyrene particles of different sizes, and also separated yeast cells with polystyrene particles; Cetin and Li [169] manipulated particles with different DEP responses and separated white blood cells (WBCs) from 10 µm latex particles. Cetin et al [173] utilised two asymmetric copper electrodes embedded along the sidewalls for an electric field gradient, and then separated latex particles based on size and also separated yeast cells and white blood cells (WBCs). Moreover, distant and large metal electrodes (so-called liquid electrodes) patterned in access channels on the side of the main channel (Fig. 2-22d) have been utilised as a lateral electric gradient. Two arrays of liquid electrodes on both sides were used to focus polystyrene microparticles [91], sort viable and non-viable yeast cells [174], and separate the normal from the \textit{B. bovis} infected erythrocytes [175]. Moreover, liquid electrode arrays patterned on a single side were also utilised to detect micro-/nano-particles [176], and separate platelets from other blood cells [170].

2.4.2 Insulator-based DEP microdevices

As an alternative to DEP devices with microelectrode arrays patterned within microchannels, insulating structures have also been used to generate the required electric gradient by placing remote electrodes at the end of the microchannel. Insulator-based DEP devices have several advantages over traditional electrode-based DEP microdevices, such as: (1) without the metal deposition process, injection moulding and hot embossing are relatively simple and applicable techniques for mass production, (2) as there are no electrodes embedded in the channel, the device with insulating structures is robust and chemically inert, accompanying by reduced surface fouling and no gas evolution due to electrochemical reactions, and (3) particle movement taking advantage of the combined electrophoretic and electroomotic effect eliminates the need for additional hydrodynamic flow. There are two main insulating structures utilized to generate spatial electric field non-uniformities for the DEP effect: (1) single or multiple insulating obstacles embedded in microchannels, and (2) curved microchannels or channel geometry modification. In addition, contactless DEP devices have been developed where electrode channels are isolated from the main channel by thin insulating barriers.
2.4.2.1 Obstacles

Due to the dielectrophoretic effects induced in constricted regions between rows of posts, an array of circular or diamond-shaped insulating posts (Fig. 2-23) were embedded in the microchannels to manipulate a variety of particles, such as DNA [178-180], protein [181], virus [182], live and dead bacteria [177, 183]. Here the shape of the post and angle of the array with respect to the electric field applied play an important role in the patterns of filaments and trapped zones [184], and the conductivity and PH value of the suspending medium can affect its performance [185]. Iliescu et al [186, 187] used round insulating beads in a filter chip to trap particles. As the maximum electric field is generated at the points of contact between beads, particles experiencing a positive DEP force would be attracted near surface of the bead, while particles with a negative DEP would move to the gaps between the beads and then be flushed out with fluid flow.

Figure 2-23 Arrays of insulating posts for the concentration and separation of live and dead bacteria [177].
Rectangular and triangular obstacles have been used to sort and focus particles. Kang et al [192, 193] designed a rectangular insulating hurdle within a straight microchannel to separate particles by size (Fig. 2-24a). As the DEP force is proportional to the cube of the radius of the particle, particles of different sizes will experience different repulsive DEP forces at the hurdle of the hurdle, and hence can be deflected into distinct flow streams. The separation of biological cells based on size was achieved using rectangular and triangular hurdles [188], and the triangular one was developed to reduce the region with the constriction gap and its accompanying adverse effect on live cells. A pair of rectangular insulting hurdles was used to focus particles (Fig. 2-24b), and DC-biased AC electric fields were applied to improve the focusing efficiency [95]. Lewpiriyawong et al [189] utilized multiple rectangular insulating hurdles within an H-shaped microchannel to manipulate and separate particles (Fig. 2-24c), which enhanced the DEP forces experienced by particles, and the controllability of particle motion. Instead of insulating solid obstacles, an oil droplet (Fig. 2-24d) [190] and oil meniscus [194] were used to create spatial non-uniformities of electric field. By adjusting the size of droplet, the radius and penetration of the meniscus, the electric field gradients, and hence the induced DEP forces, became easily controllable and adjustable to various configurations of separation. Moreover, the insulating structures (i.e. ridges [191, 195]) in the depth of the microchannel were used for spatial non-uniformities of the electric fields (Fig. 2-24e). As the motion of a particle depends on the ratio between the electrokinetic effect (the combined effect of electrophoresis and electroosmosis) and the DEP effect, such structures successfully trapped and deflected nano-
and micro-scale [195] polystyrene particles, and selectively concentrated and separated *B. subtilis* from 20-nm-diameter polystyrene particles [191]. Jen et al [196-198] utilised X-patterned insulating structures in the microchannel to squeeze the electric fields, which could generate a high field gradient at the constricted regions, when AC fields are applied via planer microelectrodes placed at the sides of the microchannel. These structures have successfully focused polystyrene particles [196] and HeLa cells [197], and selectively trapped dead HeLa cells from mixed viable cells [198].

2.4.2.2 Curved microchannels

![Channel geometry modification: (a) saw-tooth [199], (b) serpentine [200], (c) spiral [201], (d) circular [98], and (e) pore [202].](image)

Besides embedding insulating obstacles in the straight microchannel, a variety of curved microchannels have also been used to manipulate particles. A saw-tooth channel (Fig. 2-25a) was developed to separate bacteria (i.e. *E. coli*, *B. subtilis* and *S. epidermidis*) [199] and capture blood cells [203], which generates a series of gradually stronger field gradient along the length of the channel. Chen et al [204] studied the movement of particles in a non-converging sawtooth channel, and the results indicated that particles can be selectively trapped by varying the geometry of...
the channel. Xuan’s group developed a serpentine channel (Fig. 2-25b) where the electric field becomes non-uniform at each U-turns, and the maximum and minimum values are obtained at the inner and outer corner, respectively. This design has been used to focus polystyrene particles [200, 205] and cells [206], separate polystyrene particles based on size [207, 208] and dielectrophoretic response [205], and separate yeast cells from *E. coli* [206] and polystyrene particles[207]. Zhu et al (Fig. 2-25c) developed a spiral microchannel to focus particles[209], separate particles depending on their size [201] and charge [210]. Due to variation in the length of path length in the width of channel, the electric field applied becomes uniform and attains maximum and minimum value near the inner and outer wall, respectively. Zhang et al [211] developed a circular channel to generate field gradients towards the centre of circle where particles experiencing different DEP responses will be directed to different locations along the width of the channel, and thus be separated into different outlets. A series of circular microchannels (Fig. 2-25d) [98] were utilised to concentrate particles, which could focus the externally applied electric field stepwise: the field upstream is relatively lower and the electrokinetic effect responsible for continuously transporting particles is dominant, while the field downstream is higher and the dielectrophoretic effect responsible for trapping particles is dominant. Another novel design was presented by Cho et al [202], where micro-fabricated plastic membranes with honeycomb-typo pores (Fig. 2-25e) were utilised to concentrate bacteria (i.e. *E. coli*). The bacteria could be captured at the edge of the pores when the applied AC field was turned to exhibit positive DEP effect.
2.4.2.3 Contactless

Shafiee et al [97, 212] developed a technique called contactless DEP to manipulate particles (Fig. 2-26) where the electrodes are not in direct contact with the sample, but are inserted in a high conductive solution which is isolated from the main channel by thin insulating barriers. These insulating barriers exhibit a capacitive behaviour while producing an electric field in the main channel when AC electric fields are applied across the barriers. This technique trapped THP-1 human Leukemia monocytes and MCF-7 breast cancer cells [97], and also sorted live and dead THP-1 cells [213]. Moreover, this technique was utilised to selectively concentrate human breast cancer cells [214], isolate human leukemia cells from erythrocytes [215], separate tumour initiating cells (TICs) from non-TICs [212], and mouse ovarian surface epithelial (MOSE) cells from fibroblasts and macrophages [216].

2.5 Conclusions
A review of the state-of-the-art of dielectrophoresis (DEP), along with various DEP-based microdevices and corresponding micro-fabrication techniques has been
presented in this chapter. With the rapid development of lab-on-a-chip (LOC) devices, different technologies have been utilised to manipulate and separate particles in solution, including mechanical, inertial, magnetic, acoustic, optical and electrical methodologies. Of those existing technologies used in microfluidic devices, DEP may be the most popular one due to its label-free nature, ability to manipulate neutral particles, compatibility with LOC devices, easy and direct interface with electronics, and analysis of high sensitivity and selectivity. Different microfabrication techniques have been used for dielectrophoretic applications, such as photolithography, thin-film deposition, wet/dry etching, electroplating, soft lithography, and substrate bonding, etc. In addition, a variety of dielectrophoretic microdevices (i.e. electrode-based and insulator-based structures) have been developed, which are capable of wide applications such as particle (including biological ones) sorting, focusing, patterning, and switching, etc.

Although great advancements have been achieved over the last decades, there are still some limitations of DEP-based microdevices in the following aspects: (1) for microdevices with top-bottom patterned microelectrodes, current methods always require standard photolithograph technique to construct microfluidic channel, and exact control of the adhesive thickness for bonding, which are relatively complicated and time-consuming; (2) for those with side-wall patterned microelectrodes, it is hard to construct extruded electrodes with un-vertical profiles and the experimental conditions are rigorous; (3) the throughput of DEP devices with 2D planar electrodes is limited, as only particles close to the electrode surface can be manipulated, and only a single microchannel and electrode array work simultaneously; (4) obstacle-induced insulator-based DEP devices easily generate extremely strong local electric fields, inducing adverse effects on biological samples, also, curvature induced insulator-based DEP devices always require long curve sections and large applied voltages. Therefore, in the following chapters of this thesis, the work will focus on the development of relatively simple and cost-effective methods for fabricating dielectrophoretic devices with 3D microelectrode structures, and integrated dielectrophoretic microdevices (either based on electrode or insulator) with multiple functionalities and improved performance in terms of efficiency, throughput and reliability.
CHAPTER 3

A SIMPLE AND COST-EFFECTIVE METHOD FOR FABRICATING DIELECTROPHORETIC DEVICES WITH TOP-BOTTOM PATTERNED MICROELECTRODES

A simple and cost-effective method for fabricating DEP-based devices with microelectrodes patterned on both the top and bottom surfaces of the microchannel is described in this chapter. A CO₂ laser plotter was used to write patterns directly onto a layer of transferred polydimethylsiloxane (PDMS), which serves as both a bonding and a working layer. Integrating microelectrodes into the microdevices is achieved by an alignment bonding top and bottom microelectrode-patterned substrates fabricated with conventional lithography, sputtering, and lift-off techniques. The processes of the developed method of fabrication were illustrated. Major issues associated with this method such as PDMS surface treatment and characterisation, controlling the thickness of the transferred PDMS layer, and laser parameters optimisation were discussed, along with the examining and testing the bonding with two representative materials (i.e. glass and silicon). The capability of this method was further demonstrated by fabricating a DEP-based microfluidic chip with sputter-coated electrodes on the top and bottom substrates. The device functioning as a microparticle focusing and trapping chip was verified experimentally. It is confirmed that the proposed method has many advantages, including simple and fast fabrication process, low cost, easy integration of electronics, strong bonding strength, and chemical and biological compatibility, etc.

3.1 Introduction

2D planar electrodes that are commonly patterned on the bottom surface of the microchannel always suffer from limitations which prevent them from being used in practical applications: the DEP force which depends on the electric field gradient will decrease quickly as the distance from the surface of the electrode increases, and particles are prone to adhere onto the surface of the electrode or channel wall. In order to improve the DEP effect within the microchannel, microdevices comprising
two layers of microelectrode arrays patterned on both the bottom floor and top ceiling of the microchannel have been developed. The corresponding fabrication process generally involves: manufacturing a microfluidic channel structure of SU-8 photoresist and applying adhesive for bonding. However, such a fabrication requires a standard photolithograph technique to pattern the microfluidic channels and exact control of the thickness of adhesive to avoid contaminating the channel and coating the surfaces unevenly, which are relatively complicated, expensive and time-consuming. Significant advances in the approaches used to fabricate microfluidic chips have recently been made with the introduction of soft lithography. Among those applied polymers, polydimethylsiloxane (PDMS) is particularly appealing due to its great advantages such as transparency, flexibility, replicability, low cost, biocompatibility and conformity [92, 94], and thus has been widely applied in microfluidic systems [96, 217].

PDMS could be utilised to combine multiple existing structured microfluidic parts together. Wu et al [218] used a thin layer of PDMS prepolymer as an adhesive to microfluidic devices from various materials, but this method requires exact control of the thickness of PDMS prepolymer layer, because any reflow may deform the initial geometry of the channel or even block narrow channels if the layer of adhesive is relatively thick. Zhang et al [219] reported using transferred PDMS layers to fabricate PDMS multilayer microfluidic chips, but did not discuss the materials used apart from PDMS. Moreover, this method requires repetitive mould-replica fabrication process for every patterned PDMS layer, and is not suitable to integrate electronic components in microfluidic chips. Pleis and Chen [220] proposed the use of a plasma-treated PDMS layer treated with plasma to bond two glasses with SiO₂ and Si₃N₄ coating on the surfaces, but constructing the microfluidic channel requires photolithography for the sacrificial template, and additional wet etching or reactive ion etching (RIE) to remove the residual PDMS layer, which are associated with strict conditions and not suitable for a common laboratorial environment.

In order to overcome the drawbacks mentioned above, we proposed the use of a laser-patterned PDMS layer serving as a microchannel structure and adhesive simultaneously to fabricate dielectrophoretic microdevices with bi-layer
microelectrodes. Open channels are defined on a thin transferred layer of PDMS using a direct-write, mask-free laser ablation technique, followed by transferring, aligning, and bonding the laser-patterned PDMS layer onto electrode-patterned substrates to form enclosed microfluidic channels. This method is simple and cost-effective because it eliminates the demand for a template and corresponding fabrication process, facilities, and consumables in a clean-room. The key fabrication processes have been described, along with the validation of this method by examining and testing the bonding of glass-PDMS-glass and glass-PDMS-silicon. As an example, a DEP-based chip with top-bottom patterned microelectrodes for focusing and trapping microparticles was fabricated and tested.

3.2 Fabrication process

Figure 3-1 shows a schematic flow of the fabrication process, which is made possible by the transfer process of a laser-patterned layer of PDMS and an oxygen-plasma bonding method to combine PDMS with two substrates. The overall approach for fabricating an integrated electronic-microfluidic device consists of four major steps: (1) processing layers of PDMS, (2) patterning the microfluidic channel, (3) processing the substrate, and (4) bonding with plasma.

Figure 3-1 A schematic view of the fabrication process. (Diagram not to scale)
3.2.1 Processing the layer of PDMS

The first step involves preparing two layers of PDMS on a glass substrate: one bulk layer serves as a transferring layer after surface treatment, while the other thin layer is a working component of the device, which is also used as an adhesive for bonding with the substrates. The transparent and flexible transferring layer of PDMS ensures effective alignment and minute deformation of the thin layer of PDMS. A bulk PDMS slab is produced by Sylgard 184 silicone elastomer mixture (Dow Corning Corporation, Miland, USA) at a weight ratio of base: curing agent = 10 : 1. A certain amount of this mixture was poured onto a level glass surface, placed in a vacuum chamber to eliminate the bubbles, and then put into an oven at 60 °C for about 2 hours. After solidification, the upper surface of the bulk PDMS slab was treated by spin-coating a layer of Teflon AF solution (Dupont, Grade 601S1-100-1), after which the slab was relaxed at room temperature for about 5 min. A thin layer of PDMS was generated by coating PDMS pre-ploymer onto the prepared bulk PDMS with Teflon covering the surface, followed by heating on a hotplate at 60 °C for curing. Thin layers of PDMS of different thicknesses can be made by varying the speed of the spin coater. At the end of this step, a glass substrate with a two-layer PDMS slab having a Teflon interlayer was ready for laser cutting.

3.2.2 Microfluidic channel patterning

A CO₂ laser plotter (Versa Laser System, model VR3.50, Universal Laser System, Ltd.) was used to make structures on the thin PDMS layer. It has the maximum power of 25 W, maximum pulse per inch (PPI) of 1000, and scanning speed ranging from 0.25 mm/s to 25 mm/s. A standard 2.0” focus lens comes with an estimated focal spot size of around 50 µm comes with this system. The designed pattern was drawn by AutoCAD software (Autodesk Inc., USA), after exporting the drawing to the drive which controls the movement of laser, resetting the scan mode and the parameters, adjusting the focal point, the pattern was plotted directly onto the PDMS layer. Parameters such as the laser power, scanning speed, and pulses per inch (PPI) were optimised to cut through the thin PDMS layer and obtain patterns with an ideal profile. The unwanted part of the thin PDMS layer was peeled off from the PDMS bulk having Teflon-coated surface using a tweezer, after which the PDMS was
cleaned with isopropanol to eliminate ashes and debris created by the CO₂ laser. In this step, open channel regions were defined onto the transferred layer of PDMS, which was later bonded to suitable substrates to form enclosed microchannels. This photoresist and residue-free step eliminates clean room facilities, which were unlikely to be accomplished by other bonding methods mentioned earlier.

3.2.3 Processing the substrate

This step consists of patterning the top and bottom substrates. These materials can be silicon, glass, plastic or others that can be well bonded to PDMS after surface pretreatment with plasma. Metal for the electrodes, such as Cu, Au, and Pt, can be grafted onto the surface for different applications. Wet etching or the reactive ion etching (RIE) technique can also be used to construct defined patterns on the substrate. Holes serving as the inlet and outlet of the fluid were drilled in the top substrate by a drilling machine (DREMEL 220 workstation), and then the substrate was cleaned in an ultrasonic bath of acetone, rinsed in DI water and isopropanol, and then dried under a flow of nitrogen. In this step, microelectrodes can be integrated into the fabricated microfluidic devices, which is helpful for further dielectrophoretic applications.

3.2.4 Bonding

The bonding step between the PDMS layer containing microfluidic channels and the top and bottom patterned substrates was finally carried out. The patterned substrate and the whole structure prepared after peeling the unwanted part off from the laser-patterned PDMS layer were treated with oxygen plasma in a plasma cleaner (Harrick Plasma PDC-002) for 2 min, after which the surface was covered with methanol which acted as a lubricant for effective alignment, and then aligned under a microscope (Meiji Techno, 43660) before making face to face contact. After heating at 90°C and pressing with 50kgm⁻² for 5min, the transferring PDMS slab was removed, leaving the thin patterned PDMS layer well attached to the substrate. One key point with this bonding process is handle the thin laser-patterned PDMS layer with bulk PDMS slab and substrate, rather than a single thin film. Plasma bonding
ensures there is a good adhesion between the substrate and the transferred PDMS layer, whereas adhesion between two layers of PDMS is decreased by the layer of Teflon. Hence, the patterned PDMS layer has stuck strongly to the substrate, thus preserving the dimensions of the pattern. The same plasma-treatment process was used to bond the reverse side of the patterned PDMS layer was bonded with the other substrate. Thus, the construction of the PDMS acted as a layer of adhesive was converted to a microfluidic device integrating microelectrodes and formed an enclosed microfluidic system.

The fabrication approach discussed here used two relatively recent advances in the micro-fabrication of microfluidic devices: (1) a modified transfer process, where a transferred layer of PDMS is processed on the top of Teflon-treated bulk PDMS as if it was an independent layer, which is then aligned, bonded, and released to chip; (2) laser ablation of the polymer where microfluidic channels were directly and quickly constructed on the PDMS. This fabrication technique has taken advantage of the transfer process and the laser through-cutting approach for integrating microelectrodes into microfluidic devices. By repeating the microfluidic channel patterning and bonding steps, many patterned PDMS layers can be combined with the substrates. This method provides a simple and fast technique for packaging integrated microfluidic devices having multiple layers and microelectrode structures.

### 3.3 Results and Discussion

#### 3.3.1 PDMS surface treatment

A critical requirement for the transferring bulk PDMS slab is that the patterned PDMS layer can easily be removed after bonding with the substrate. To detach two layers of PDMS, the adhesion strength at their interface should be lower than the bonding force of plasma-treated PDMS and substrates, so to reduce the bonding force between two layers of PDMS, Teflon AF solution was spun onto the surface of the transferring bulk PDMS slab. The reasons for choosing Teflon for surface treatment are: (1) its transparency makes effective alignment possible, and (2) surface treatment using Teflon can be achieved by spin-coating it onto the surface required, because it evaporates quickly and its limited solubility (3%-15%) allows an
ultra-thin coating in the submicron range to remain on the surface. This process is simple, time-saving, and does not need any other complicated equipment like encapsulated chamber used by Zhang et al. [219] for PFOCTC deposition.

3.3.2 Surface characterisation

The contact angle was measured and X-ray photoelectron spectroscopy (XPS) analysis was performed to ensure that Teflon AF was coated on the surface of the transferring layer of PDMS. The water contact angles were measured on the surfaces of bare PDMS and Teflon-treated PDMS by the sessile drop technique, and were then analysed using the drop shape analysis software Image J. Three measurements were taken and then averaged. As shown in Fig. 3-2, the measured contact angles of bare PDMS (Fig. 3-2a) and Teflon-treated PDMS (Fig. 3-2b) were 101.1° and 111.9°, respectively. The contact angle increased after surface treatment because of the higher hydrophobicity and roughness of Teflon; this increase in the contact angle after surface treatment confirms that Teflon has successfully been coated onto the PDMS surface.

![Figure 3-2 Contact angle measurements. Water Contact angles measured on the surfaces of (a) bare PDMS, and (b) Teflon-treated PDMS.](image)

In additional, an XPS analysis was conducted using XPS spectra (PHI 5600, Physical Electronics Corp.) to analyse the surface elemental composition. Figure 3-3a and b show the results of XPS analysis of bare PDMS and Teflon-treated PDMS, respectively. The overall element peaks detected on the surfaces as well as corresponding atomic concentrations were presented. After surface treatment using Teflon the element F appears with a relatively high concentration, and it known that PDMS is a polymer comprised mainly of C, Si, H, and O, and only Teflon contains
F, therefore we conclude that could be a residual layer of Teflon remaining on the surface of PDMS.

![XPS analysis results of (a) bare PDMS, and (b) Teflon-treated PDMS.](image)

Figure 3-3 XPS analysis results of (a) bare PDMS, and (b) Teflon-treated PDMS.

### 3.3.3 Controlling the thickness of the transferred PDMS layer

After the transferring layer of PDMS was surface-treated with Teflon, PDMS pre-polymer was spun onto its surface with a spin coater. By varying the spin speed, transferred PDMS layers of different thicknesses can be obtained, and since the transferred PDMS layer is laser-patterned and serves as a working part of the microfluidic chip, its thickness will affect the performance of the device. Various spin speeds as well as corresponding thickness of cured transferred PDMS layers were tested. Figure 3-4 shows the effect of spin speed on the thickness of the transferred layer. As shown, by increasing the spin speed from 500 to 5000 rpm, the thickness of the transferred PDMS layer decreased from about 220 µm to around 20 µm. This feature enables complicated microfluidic devices with multiple layers of various depths to be fabricated.
3.3.4 Optimisation of laser parameters

There are three main parameters controlling the settings of the laser cutting machine: laser power, scanning speed, and pulses per inch (PPI), which work together to determine the depth and smoothness of the cut patterns. Different combinations of these three parameters can result in different spatial printing resolutions. The relationship between the laser parameters and cutting profile of PDMS pattern was studied by Liu and Gong [221]. These three parameters of pulsed CO₂ laser were optimised to cut PDMS layers of different thickness, and patterns with an ideal profile were formed if they were set to a suitable level. In order to optimise the laser cutting parameters, various tests were conducted at different settings. Four profiles of cutting lines on a thin PDMS film with a thickness of 36 µm are shown in Fig. 3-5. The fourth setting produced a line with a smoother edge and fewer residues of dust. Although the resolution of CO₂ laser cutting is not as high as that of photolithography using expensive and hard chromium masks, it is comparable to prototyping processes using printed transparent masks and is quite adequate for fabricating microfluidic devices for many applications [222]. Moreover, in comparison to the conventional soft lithography process required to construct patterns on PDMS, the laser cutting method is simple, fast, and low cost, which have great advantages in the fabrication of microfluidic chips.

![Figure 3-5: Four profiles of cutting lines on a thin PDMS film with a thickness of 36 µm.](image)
Figure 3-5 Microscopic pictures showing the influence of laser parameters on cutting profiles. Lines were cut on a 36 µm-thick PDMS film by setting laser power, scanning speed and PPI at (a) 0.25W, 2.25mm/s, 1000; (b) 0.50 W, 0.75mm/s, 1000; (c) 0.25W, 0.75mm/s, 500; and (d) 0.25W, 0.75mm/s, 1000.

3.3.5 Bonding examination and testing

The flexibility and compatibility of PDMS enables it to conform to substrates such as silicon, glass, or another piece of PDMS, even those with a non-uniform topography. After treatment with oxygen plasma, Si-O-Si bonds are created and an irreversible assembly is created. In order to study the bonding of the transferred layer of PDMS and substrates surface-treated by oxygen plasma, two different material substrates (glass and silicon) were bonded together with a thin patterned layer of PDMS using our method. The bonded micro-structures were examined under a scanning electron microscope (SEM, JSM-6300). The cross section of the sample was sputter-coated with gold before the SEM imaging. Figure 3-6a and b shows the bonding of glass-PDMS-glass and glass-PDMS-silicon, respectively. The PDMS layer is 36 µm thick and the channel contained in the PDMS layer is 800 µm wide. It was found that the patterned layer of thin PDMS can be well bonded to glass and silicon using the fabrication process described above.

The bonding strength was examined by a simple leakage testing, which was performed by introducing an ink solution at various flow rates. A layer of PDMS containing a microchannel was sandwiched between two substrates (both glass and silicon substrates have been tested). Two holes were drilled into the top substrate to
act as a fluid inlet and outlet. Fittings and Teflon tubing (Tygon Tubing) was connected to the devices using UV curable adhesive (NEA 121, Norland Products, NJ). Red ink was used for visualisation. A syringe pump (kdScientific, 781200) was used to introduce an ink solution into the channel at five selected rates of 0.001, 0.01, 0.1, 1, and 10 ml/h. There was no leakage in our experiment at any flow rate.

In addition, the pressure that the chip can sustain without failure by rupture or delamination of the intermediate layer of PDMS, and the top or bottom substrate is one important indicator of the robustness of this fabrication process and chip operation. The bonding strength was tested using a similar method reported by Eddings et al [223]. A digital pressure sensor ‘Druck DPI 104’ (GE Druck, Leicester, UK) was used to monitor the pressure applied. A 1 mm diameter hole was drilled through the top substrate for connection to a gas tank which acted as the pressure source. The pressure was increased until the sample broke, and this value of pressure was recorded. It is found that both glass-PDMS-glass and glass-PDMS-silicon chips remained one integral unit when the pressure was increased to 200 KPa, which is close to the results of the oxygen plasma bonding of PDMS represented in the previous reference. This has proved that the bonding method developed is adequate for microfluidic applications.

3.3.6 Integrated DEP-based electronic-microfluidic microdevice

Using the fabrication method we developed, a DEP-based microfluidic chip with microelectrodes on the top and bottom surfaces was fabricated and its ability to manipulate microparticles was demonstrated. A laser-patterned layer of PDMS containing a microfluidic channel served as an adhesive, and was sandwiched between two glass substrates with sputter-coated microelectrodes after oxygen-plasma treatment. The fabrication process is illustrated in Fig. 3-7. Patterned HPR 507 was generated on both the top and bottom substrates using the standard photolithograph technique. An electrode layer of 10nm Ti/ 100nm Pt was deposited using sputtering technique and then patterned with the lift-off process (see Fig. 3-8). Due to the flexibility of PDMS, electrodes with a thickness at a nanometer level can be patterned on the substrate without affecting the efficiency of bonding. Two 1 mm
diameter holes were drilled in the top substrate. By setting laser power, scanning speed and PPI at 0.25 W, 0.75 mm/s and 1000, respectively, an excellent channel configuration 36 µm deep by 800 µm wide was obtained. The transferred layer of PDMS containing channel for confining fluid was bonded with the electrode-coated substrates after the oxygen-plasma treatment. Figure 3-9 shows a completed dielectrophoretic microsystem comprising 3D electrodes fabricated using the above process.

Figure 3-7 Schematic view of the fabrication process flow for an integrated electronic-microfluidic device. Insets show top view of sputter-coated electrodes, laser-cut microchannel and final fabricated chip. (Diagram not to scale)
Figure 3-8 Diagram of the electrodes sputter-coated on the glass substrate, which consisted of two units: focusing and trapping. Wiring connections to external power supply are not shown.

Figure 3-9 Photograph of micro-fabricated DEP-based microdevice with 3D electrodes. Two glass substrates containing electrodes were bonded together by a laser-patterned PDMS layer. Wires and tubing were joined for electrical and fluidic connection, respectively.

The performance of the fabricated chip was evaluated by dielectrophoretically focusing and trapping red fluorescent polystyrene spheres with a diameter of 10 µm (Invitrogen, CA, USA). Before use, the particles were re-suspended in DI water at a final concentration of $10^5$ beads/ml. The sample was injected continuously into the microchannel by a syringe pump (kdScientific, 781200). A function generator (HP 33120A) was used for voltage supply. The microparticles were monitored under a microscope (Olympus IX71, Tokyo, Japan), and recorded by a CCD camera (Olympus DP70) as they travelled. When an electric field was applied, the motion of a particle in the flow is determined by the combined effect of two forces: DEP force and hydrodynamic force. The theory of this experiment was similar to that described by Chen et al [151]. In our test, the particles were first focused by the funnel structure, after which they could move in a narrow stream in the middle of the channel until they were finally trapped by aggregating at the edge of the arc electrodes. Figures 3-10 and 3-11 show the movement of particles at the flow rate of 0.03 ml/h from left to right with a 10 KHz, 10 V_{pp} sinusoidal voltage applied, at which particles experienced negative DEP. The particles were uniformly distributed in the microchannel when no electric field was applied (Fig. 3-10a), but they were focused to move in a narrow stream in the middle of channel when the AC electric fields was applied (Fig. 3-10b). Figure 3-11 shows that the particles were successfully trapped by the arc electrodes. The number of aggregated particles
increased along with the variation in time. In our experiment, there was no leakage when the flow rate the fluid was increased from 0.001 ml/h to 10 ml/h, and maximum pressure in the channel can reach as high as 200 KPa. Depending on the application of electrodes patterned the top and bottom of the microchannel, the fabricated microfluidic structure accomplished dielectrophoretic focusing and trapping of microparticles, which has potential applications in continuous bio-particle manipulation and flow cytometry.

Figure 3-10 Pictures showing microparticles (10 µm) were focused by slanted electrodes. (a) Miro-particles were distributed uniformly without electric filed, and (b) micro-paticles moved in the middle of the channel when an AC electric field at 10 kHz and10 Vp-p was applied.
3.4 Conclusions

In this chapter, we developed a simple and cost-effective approach for the fabrication of dielectrophoretic microdevices with bi-layer microelectrode structures. With this method, complicated and laborious soft lithography processes for fabricating templates, which are usually conducted in a high-standard clean room were avoided, which can greatly reduce the time and cost of manufacture. Incorporating micro-machined structures with microelectrodes on the top and bottom surfaces of microfluidic channels was easily achieved using a laser-patterned layer of PDMS as an adhesive after oxygen-plasma treatment. To the best of my knowledge, it is the first time that taking advantage of transfer process and laser through-cutting techniques for integration of microelectrodes in microfluidic devices. This approach can be extended to fabricate integrated multi-layer microfluidic devices such as those
with multiple channels of different configurations or depths, by transfer-bonding many laser-patterned layers of PDMS together. In addition, the design and surface modification of microfluidic devices are diversified because strict fabricating conditions such as high temperature and voltage, ultra-clean and ultra-flat surface, and specific chemical compositions are no longer required. This proposed simple and cost-effective fabrication method is expected to be widely used to manufacture dielectroporetic devices with 3D microelectrodes and even integrated multi-layer microfluidic devices combining electrical and microfluidic functionalities.
In this chapter, we described a novel method for the fabricating DEP-based microdevices with three-dimensional (3D) microelectrodes at the sidewalls of the microchannel by utilising low-melting-point metal alloy. The fabrication process involves conventional photolithography and sputtering techniques to fabricate planar electrodes, positioning Bismuth (Bi) alloy microspheres at the sidewall of PDMS channel, plasma bonding, and low temperature annealing to improve the electrical connection between the metal microspheres and planar electrodes. Compared to other methods of fabricating 3D electrodes, this method does not require severe experimental conditions, cumbersome processes and expensive equipment. A numerical analysis of the electric potential distribution with different electrode configurations was presented to verify the unique field distribution of arc-shaped electrodes. Such 3D microelectrodes with high-conductivity alloy microspheres have unique properties in the application of particle/cell manipulation and separation using dielectrophoresis, which was demonstrated by switching and sorting polystyrene (PS) particles and yeast cells. The proposed technique offers alternatives to construct 3D electrodes from 2D electrodes. More importantly, the simplicity of the process provides easy and fast ways of fabricating extruded arc-shaped electrodes and also other different geometries at the sidewall of the microchannel.

4.1 Introduction

In traditional dielectrophoretic microdevices, 2D planar microelectrodes are always patterned on the bottom surface of the microchannel, but the electric field decays dramatically with distance away from the surface of the electrode. Thus, the DEP force which is proportional to the gradient of the electric field decreases along the height of the channel, reducing the efficiency of DEP manipulation in microfluidic devices, especially when the microchannel is relatively deep. In order to induce strong electric gradients and hence DEP forces within the entire microchannel, 3D...
microelectrodes, such as bi-layer planar electrodes on both the bottom floor and top ceiling of the microchannel, heavily doped silicon electrodes, electroplated metal pillar electrodes, carbonised SU-8 electrodes, and sidewall-patterned electrodes have been fabricated and implemented in DEP-based microdevices to manipulate and separate particles. However, the fabrication processes based on the techniques of electroplating, pyrolysis, and etching, are cumbersome, and typically require stringent conditions (i.e. high temperature and/or voltage, and an irritative solution). The vertical microelectrodes on the sidewalls are extended along the entire height of the microchannel, which increases the DEP effect within the microchannel. Particles flowing through the microchannel can be deflected laterally to different positions in the width of the channel regardless of their height. Moreover, the height of the microchannel could be increased without losing the strength of the electric field, allowing for high-throughput DEP-based microdevices. Although electroplated microelectrodes, conductive PDMS composites, and photo-patterned micro copper electrodes have been developed as sidewall microelectrodes, the fabrication process remains relatively expensive and time-consuming. Therefore, the development of a simple and cost-effective method for fabricating DEP-based microdevices with 3D microelectrodes on the sidewalls is quite appealing.

We therefore proposed to construct 3D arc-shaped microelectrodes at the sidewall of microfluidic channels using a low melting point Bismuth (Bi) metal alloy. The fabrication process involves patterning an SU-8 mould for microchannels, patterning planar microelectrodes, constructing microchannels with micro-spheres positioning at the sidewall, plasma bonding, and thermal post-treatment. The functionality of fabricated 3D electrodes was demonstrated by dielectrophoretic manipulation and separation of particles. This fabrication method offers many advantages such as good conductivity, low cost, simple fabrication and time-saving since the process resembles the method used to fabricate planar electrodes, which was favourable in DEP application due to the large electric field gradient produced by the arc-shaped 3D electrodes, and improving the design of topological electrode.

4.2 Materials and methods
4.2.1 Fabrication process

Figure 4-1 shows the fabrication of a microfluidic chip with 3D arc-shaped electrodes embedded at the sidewall of the microchannel. The major steps include: a) fabricating the SU-8 mould, b) patterning the planar electrodes, c) fabricating the microchannels with microspheres positioned at the sidewall, d) plasma bonding, and e) thermal post-treatment.

4.2.1.1 Fabricating the SU-8 mould

The first step involves conventional photolithography: negative photoresist SU-8 2050 was spin-coated at a speed of 1300 rpm for 30 sec on a clean Silicon (Si) wafer, resulting in a 100 µm thick film of SU-8, followed by a pre-bake at 65 °C for 15 min
and 95 °C for 20 min, a UV exposure of 40 sec using ABM #2 (ABM, San Jose, CA), a post-bake at 65 °C for 5 min and at 95 °C for 10 min, and then developing the photoresist.

4.2.1.2 Patterning the planar electrodes

Standard photolithography, sputtering, and lift-off process were used to pattern planar electrodes onto the glass substrate. The glass substrate was pre-cleaned with RCA-1 solution, a mixture of deionized (DI) water, ammonium hydroxide (NH₄OH) and hydrogen peroxide (H₂O₂) in 5:1:1 ratio, followed by rinsing in DI water three times. The glass substrate was then processed in sequence by spin-coating positive photoresist HPR 507 at 2000 rpm, soft-baking on a hotplate at 110 °C for 2 min, UV exposure by Mask Aligner (ABM #2) for 20 sec, photoresist developing using FHD-5 for 60 sec, and post-baking on a hotplate at 110 °C for 2 min. The planar electrodes made from 15 nm-Ti/150 nm-Pt were then sputtered and patterned using the lift-off process.

4.2.1.3 Fabricating the microchannels with microspheres positioned at the sidewall

Bismuth (Bi)-based 117 alloy purchased from Rotometals (U.S.A) is composed of Bi 40.63%, Pb 22.1%, In 18.1%, Sn 10.65% and Cd 8.2%. The melting point of the alloy is documented at 46.5 °C (data provided by the company - Rotometals). The conductivity of this kind of metal alloy is 2.23×10⁶ S/m which is comparable to most kinds of metals (data provided by Rotometals). Bi alloy microspheres were formed by a droplet generator consisting of a small robotic arm and a glass capillary connected to a Venturi tube. It is similar to a commercial product – Nano-Plotter(GeSiM). A small drop of molten Bi alloy was picked up by the robotic arm and then placed into a cooling bath. The droplet will automatically form a spherical shape due to surface tension. The size of the Bi alloy microsphere is controlled by the amount of molten alloy. The time that glass capillary submerged in the molten alloy determines the alloy amount, and a longer time period leads to a microsphere of larger size. By study the relationship of the diameter of microsphere and time of absorbing molten alloy, Bi alloy microsphere of desirable diameters could be
obtained. This method could be used to achieve mass production of metal spheres. It is capable of producing more than 1000 spheres in one hour.

Microchannels with deep recesses (highlighted by red squares in Fig. 4-1) at the sidewall for locating 3D microelectrodes were made of polydimethylsiloxane (PDMS) using standard soft lithography technique. PDMS (Dow Corning Corporation, Miland, USA) mixture with a 10:1 weight ratio of base:curing agent was first applied to the fabricated SU-8 mould. After curing in an oven at 60 °C for about 2 h, the PDMS slab was peeled off and metal microspheres of 110 µm which is 10 µm larger than the size of recesses, was manually positioned in the deep recesses of the PDMS layer using an optical microscope (Olympus SZX16). The purpose of this design is to increase the adhesion between Pt electrodes and alloy spheres, and then obtain a good electrical connection. The position process consists of three steps: (1) metal alloy spheres were picked up with tweezers with a sharp tip; (2) they are transfer to the PDMS recesses where the spheres would automatically drop when they are moved around; (3) wipe off excess spheres that are not positioned in the PDMS recesses. All these processes were conducted under an optical microscope. PDMS recesses are used to position spheres precisely.

4.2.1.4 Plasma bonding

The layer of PDMS containing microchannels with metal microspheres was bonded to the bottom substrate patterned with planar electrodes. PDMS slab with fluidic inlets and outlets, and Pt electrodes patterned glass substrate were treated in a plasma cleaner (Harrick Plasma PDC-002) for 1 min, after which the surface was sprayed with methanol which acted as a lubricant for latter alignment using an optical microscope. In order to evaporate the methanol and improve the bond strength between the PDMS and glass substrate after aligning, the microfluidic chip was heated on a hotplate located near the microscope at 45 °C for 5 min, an annealing temperature which was not high enough to melt the metal microspheres. Methanol will evaporate very fast (in less than 30 sec) at room temperature, so the position when the chip is moved to the hotplate is almost fixed. This heating process is used
to help the methanol evaporate completely and increase the adhesion between the PDMS and glass substrate after plasma.

4.2.1.5 Thermal post-treatment
In order to improve the electrical contact between the metal microspheres and planar Pt electrodes, the chip was cured in an oven at 60 °C for 10 min. Bi microspheres will bond to Pt electrodes after this thermal treatment. The accuracy of the curing temperature is critical because the solid microspheres might melt and collapse if the temperature is too high, and there is no improvement in the electrical contact if the temperature is too low. Figure 4-2 compares the effect of the annealing temperature on the shape of 3D electrodes. The microfluidic chip in Fig. 4-2a was cured in an oven at 75 °C for 10 min. It is clearly seen that the spheres in the side wall melted and collapsed which may destroy the main channel. Figure 4-2b shows the microfluidic chip after annealing at 60 °C for 10 min. Compared with Fig. 4-2a, the alloy spheres have maintained their shape and have good electrical contact with the planar electrodes underneath, as proved by the following experiments.
Figure 4-2 Comparison of the arc-shaped electrodes at the sidewall of microchannel after annealing at (a) 75 °C for 10 min, and (b) 60 °C for 10 min.

4.2.2 Sample preparation

Cells of *Saccharomyces cerevisiae* (yeast cells) and two types of polystyrene (PS) particles were used in this study: 10 μm fluorescent polystyrene particles (Invitrogen, CA, USA) and 5 μm polystyrene particles (Sigma-Aldrich). The 5 μm particles were similar in size to the yeast cells. The cells and particles were suspended in a pH 7.0 buffer solution composed of disodium hydrogen phosphate and potassium dihydrogen phosphate (Radiometer Copenhagen, Denmark) before the DEP experiments. The conductivity of the suspending media was adjusted to be 380 μS/cm, and the yeast cells were cultured directly on an agar plate. To prepare the yeast suspensions, yeast colonies on a yeast extract (YE) agar plate were scratched down and transferred to the pH 7.0 buffer solutions.

4.2.3 Experimental setup

Two sample solutions were prepared for particle separation experiments: (1) separation of 10 μm and 5 μm polystyrene particles in a 380 μS/cm buffer solution based on the size effect on DEP, and (2) live yeast cells and 5 μm polystyrene particles in a 380 μS/cm buffer solution. Each sample solution was injected into the microchannel by a syringe pump (KDScientific, 781200) to maintain continuous flow. There are two inlets in our device which is why two syringe pumps were used to inject the sample solutions. A function generator (HP 33120A) and an AC amplifier (Stereo Power Amplifier 216THX) were used as the power supply. The waveform of the input signal was monitored by an oscilloscope (Tektronix TDS 2012B). Soldering technique was used to connect power supply to the device. The motions of the cells and polystyrene particles were monitored using an inverted optical microscope (Olympus IX71, Tokyo, Japan), and recorded by a CCD camera (Olympus DP70, Tokyo, Japan). Scanning electron microscope pictures were taken with a JSM-6390 (JEOL).
4.3 Results and discussion

4.3.1 Comparison of electric field distribution for different electrode configurations

In order to characterise the electric field effect related phenomenon of different electrode configurations, a finite element numerical simulation (COMSOL 4.2) was performed to study the topological effect of electrode on electric field distribution. A simplified 2D geometry was used for comparing the electric field distribution for line, rectangular protrusion and arc-shaped electrodes. Here we briefly describe how to perform a simulation by COMSOL 4.2 (COMSOL Inc., Burlington, MA). First, a 2D model, electrostatics (es) in AC/DC section and stationary studies were selected, and then, the geometry of the channel and the electrodes were defined in the graphics section, followed by selecting appropriate materials for each domain and setting the electrical potential on the boundary of each electrode. A computation was then performed with the default setting of mesh. After that, the results were shown by the surface and contours of the square of the electric field which is determined by the expression as \(-\text{es.Ex}^2+\text{es.Ey}^2\). As illustrated in Fig. 4-3, a non-uniform electrical field can be generated across the width of the channel (in a lateral direction) when the electrodes are located at the sidewall, and the generated DEP force can be used to deflect cells and particles in a lateral direction. Since many microfluidic devices are based on planar electrodes which only have a large DEP force near the electrodes in a vertical direction, lateral electrodes generating a large DEP force in the whole region along the height direction are more desirable for on-chip analysis, manipulating particles, and parallel processing of samples.

The topology of electrodes is crucial for applications based on a non-uniform electric field such as DEP. Figure 4-3 compared the results from the numerical simulation of three types of electrode configurations: (a) line electrodes, (b) a rectangular protrusion, and (c) arc-shaped electrodes aligned on the edge of the microchannel. To present the field distributions better, the region containing two middle electrodes are illustrated in this figure. Note that the DEP force is proportional to a divergence of the square of the electric field, and it is represented by the density of contours shown in Fig. 4-3 (the higher density, the larger DEP force). The channel is 100 µm wide, the protrusion of electrical potential lines from the sidewall toward the centre of channel is 30 µm and the centre distance between the two electrodes is 200 µm.
Figures 4-3b and 4-3c indicate that a rectangular protrusion and arc-shaped electrodes resulted in a higher electric field and thus a large gradient of electric field in the lateral direction than line electrode configurations shown in Fig. 4-3a, owing to the protrusion of electrodes into the channel region. Compared to the rectangular protrusion (b), the arc-shaped electrode (c) has a smoother electric field in region 1 and region 2, as illustrated in Fig. 4-3b and Fig. 4-3c. The arc-shaped electrodes therefore distribute a better non-uniform electric field due to the larger effective area upon which the DEP force was acting. The major drawbacks of a rectangular protrusion configuration are the difficulties in fabrication and divergence in the electric field induced by sharp corners which may affect the viability of the cells. Therefore, an arc-shape electrode is better for DEP applications because it produces a large DEP force in regions 1 and 2 (specified in Fig. 4-3).

Figure 4-3 Comparison of square of electric field ($E^2$) with different electrode configurations at the sidewall of microchannel: (a) line electrodes, (b) rectangular protrusions, and (c) arc-shaped electrodes. The largest protrusion is 30 µm from the edge of the channel. The width of channel is 100 µm. Both of them are the same as the real picture in our experiment. The applied voltages are +5 V and -5 V, respectively, on these two adjacent electrodes.

4.3.2 Microfluidic chip fabricated by the method described

Based on the results of the above simulation, a new microfluidic chip with 3D arc-shaped electrodes was fabricated. The ability to fabricate 3D electrodes has many
advantages that cannot be achieved by simple planar electrodes, especially when manipulating particles with an electric field. We have developed a process of patterning electrodes at the sidewall of the microchannel which integrates them with the sidewalls of the channel, so they will not interfere with flow within the channel. In this method, a single layer of photoresist (SU-8) photolithography, metal deposition, and positioning of Bismuth alloy microspheres were used to make the microchannel and 3D electrodes, respectively. The effect of Joule-heating in our device was also reduced because the gradient of the electric field is large and the driving voltage is relatively small compared to other types of electrodes used to manipulate particles.

Figure 4-4 Picture of the fabricated microfluidic device with 3D arc-shaped electrodes at the sidewall of channel.
Figure 4-5 Picture showing the configuration of microchannel and microelectrodes.

Figure 4-4 shows the fabricated microfluidic chip with a 100 μm wide by 1900 μm long main fluidic channel with four deep recesses filled with metal microspheres connected to planar Pt electrodes (black blocks). The microfluidic chip was equipped with two sets of inlets (inlet 1 and inlet 2) and outlets (outlet 1 and outlet 2). Figure 4-5 shows the detailed design of the main microchannel, arc-shaped 3D electrodes and the planar Pt electrodes, which is also an enlarged picture of red dashed line circled in Fig. 4-4. All the microchannels are 100 μm deep, including the deep recesses where the 3D electrodes located. Each 3D electrode was designed to be 100 μm apart. This fabrication technique utilised the unique property of low melting point metal alloy to construct the 3D arc-shaped electrodes protruding from the sidewall of the PDMS microchannel. Compared to most metals with a melting temperature higher than 500 °C, the proposed alloy could easily be made to have good electric contact with the Pt planar electrodes, but if other metals were used, the annealing process may change the property of PDMS due to the high temperature (higher than 500 °C for most metals). However, in our experiment the annealing temperature for Bi metal alloy was 60 °C, which did not affect the other materials we used.
To investigate the shape of the 3D electrode, a fabricated chip was cut along the centreline of channel and SEM pictures are shown in Fig. 4-6. Figures 4-6b and c are the enlarged pictures of highlighted areas indicated by red dashed lines. It is clearly seen that the sphere maintains its original shape and the surface is smooth enough for most applications in a microfluidic chip. Particles within the channels are therefore subjected to a non-uniform electric field generated by 3D electrodes. Compared to conventional planar electrodes that are either at the top or the bottom of the microchannel, electrodes at the sidewalls allow for better visualisation of particle motion without the need for transparent electrodes and easier manipulation of particles in a continuous flow.

4.3.3 DEP responses of different particle types

In order to confirm the application of fabricated microfluidic chip, manipulation of particles was conducted using dielectroporesis. Given by Eq. 2-3, the DEP force depends on a variety of parameters, such as particle size, the permittivities and conductivities of the particle and suspending medium, and the spatial non-uniformity of the electric field. Particle will move towards the regions of strong electric field if it is more polarised than the surrounding medium, this motion is called positive dielectrophoresis (p-DEP). Alternatively, if the particle is less polarisable than the
suspension medium, it will move away from the regions of strong electric field, and the resulting motion is called negative dielectrophoresis (n-DEP). Particles of different sizes and/or different dielectric properties will have different DEP forces, and this mechanism is used to manipulate and separate particles in the experiment.

In order to get rid of electrolysis and adjust the DEP response for different particles and cells, AC signals with different amplitudes and frequencies were applied to the 3D arc-shaped electrodes. Electrolysis is induced by passing an electric current through an ionic substance. The frequency of AC electric field and the ion concentration of the medium are determined by the voltage applied to the electrodes. In our experiment with a medium conductivity of 380 \( \mu \text{S/cm} \), we found that a voltage higher than 35 V will induce electrolysis and the increment of frequency will reduce this effect. Thus, one has to choose a suitable amplitude and frequency according to the desired DEP force to avoid potential electrolysis. Moreover, Lewpiriyawong et al
[172] have studied the variation of the CM factor with respect to the electric frequency for a yeast cell and a latex particle in 380 μS/cm medium, and we utilized these data as the reference when choosing appropriate AC signals for the DEP responses of different particle samples. All the AC signals applied would not induce any noticeable electrolysis in our experiment. Figure 4-7 is a schematic illustration of the working principles of a fabricated DEP-based microdevice with 3D microelectrodes at the sidewall. The trajectories of the particles with and without an applied electric field were compared. As shown in Fig. 4-7a, particles introduced via inlet 2 were pushed towards outlet 2 by hydrodynamic force when no electric field was applied to the electrodes, but the particles were deflected away from the sidewall with electrodes due to the negative DEP force acting on them if an AC electric signal is applied to the electrodes as illustrated in Fig. 4-7b. Under the influence of a negative DEP force, the particles were repelled to the upper channel and collected at outlet 1. This kind of configuration could be applied to cell/particle manipulation in a continuous flow by adjusting the applied AC signal.
Figure 4-8 Comparison of the trajectories of 10 μm PS particles (a) without and (b) with electric signals applied to the 3D electrodes. The applied AC signals were 15 V at 20 kHz, and the flow rates for two inlets were fixed at 0.1 μl/min. The microparticles were marked by red circles to illustrate the trajectories.

Figure 4-8 compares the response of 10 μm PS particles with (a) and without (b) applied electric fields when they passed through an array of 3D electrodes. As shown in Fig. 4-8a, the particles moved close to the sidewall with 3D arc-shaped electrodes hydrodynamically, and were finally collected from outlet 2 without applied electric fields. In contrast, when electrical signals are applied, an electric field gradient and DEP forces are induced in the width of the channel, therefore, particles were deflected way from the sidewall with patterned 3D electrodes due to negative DEP force, and then collected from outlet 1 (see Fig. 4-8b). The applied AC signal was 15 V at 20 kHz, and the flow rates for the inlets were fixed at 0.1 μl/min.

Figure 4-9 Comparison of the trajectories of yeast cells (a) without and (b) with electric signals applied to the 3D electrodes. The applied AC signals were 25 V at
100 kHz, flow rate for two inlets are fixed at 0.1 µl/min. The yeast cells were marked by red circles to illustrate the trajectories.

A similar phenomenon was observed for manipulating and switching yeast cells (see Fig. 4-9). The behaviours of yeast cells are different with (a) and without (b) applied electric fields are different: without electric field, the yeast cells move close to the electrode-patterned sidewall due to hydrodynamic force and were collected from outlet 2 (see Fig. 4-9a); when electrical signals are applied, the yeast cells were deflected away from the electrodes under the effect of negative DEP force, and then collected from outlet 1 (see Fig. 4-9b). The applied AC signal was 25 V at 100 kHz, and the inlet flow rates were still fixed at 0.1 µl/min. The particles and cells in the figures were marked by red dashed circles to better illustrate their trajectories. Our chip successfully manipulated or switched the flow of PS particles and cells between inlets and outlets. It was noticed that the amplitudes and frequencies were chosen in the region where particles exhibit strong DEP force to redirect them between two outlets. The DEP response of particles were consistent with other previous work [172].

4.3.4 Separation of polystyrene particles based on size

Figure 4-10 Separation of 10 and 5 µm PS particles depending on size. AC sinusoidal signal with amplitude of 15 V at 20 kHz was used. 10 and 5 µm PS particles are marked with black and red dashed circles, respectively, to indicate the separation process.
Based on a prediction that the DEP force is proportional to the cube of the particle radius according to Eq. 2-3, particles of different sizes will have different DEP forces. Therefore, under a negative DEP force, larger particles will be deflected further away from the electrodes than smaller particles. This mechanism is utilised to separate particles based on size. The separation of different sized particles was subsequently demonstrated in Fig. 4-10, where the 10 μm PS particles were marked with black dashed circles and the 5 μm PS particles were marked with red dashed circles. In the absence of an electric field, a mixture of 5 and 10 μm diameter particles were both hydrodynamically focused near the wall of the channel where the 3D electrodes were located. When an AC sinusoidal signal of 15 V at 20 kHz was applied to the two pairs of electrodes, the 10 μm PS particles were deflected towards the upper channel (outlet 1) while the 5 μm PS particles were directed to the lower channel (outlet 2). It is noted that the 10 μm PS particles experienced a larger repulsive negative DEP force and hence were deflected further away from the arc-shaped electrodes, while the 5 μm ones experienced a proportionally smaller DEP force and moved closer to the electrodes. Therefore, the device successfully demonstrated that it could separate PS particles of different sizes.

4.3.5 Separation of polystyrene particles and yeast cells based on dielectric properties

![Figure 4-11 Separation of 5 μm PS particle and yeast cell](image)

Figure 4-11 Separation of 5 μm PS particles and yeast cells depending on dielectric properties. AC sinusoidal signal with amplitude of 20 V at 200 kHz was used. 5 μm
PS particles and yeast cells are marked with red and green dashed circles, respectively, to indicate the separation process.

Given by Eq. 2-3, the DEP force depends on the dielectric properties of a particle, hence particles with different dielectric properties will have different DEP forces. The function of separation based on the dielectric properties was examined by sorting yeast cells and 5 µm PS particles. 5 µm PS particles were used because they are similar in size to the yeast cells, which eliminated the effect of size on the separation performance. According to the previous work of Lewpiriyawong et al [172], 5 µm PS particles experienced a larger negative DEP force than yeast cells, and the separation was demonstrated. Figure 4-11 shows a still image of the process of the particle-cell separation under an AC signal of 20 V at 200 kHz, and a co-flow rate of 0.1 µl/min (the flow rate for two inlets are both 0.1 µl/min). The yeast cells stayed close to the side of the channel where the electrodes were located and were directed to outlet 2 in the downstream. On the other hand, 5 µm PS particles were deflected further from the electrodes and finally moved to outlet 1. In order to indicate the separation process, the 5 µm PS particles and yeast cells were marked with red and green dashed circles, respectively.

Our experimental results have shown the controllable performance of the fabricated microfluidic chip, in which particle-particle and particle-cell separations were successfully demonstrated based on AC-DEP. The process of particle separation was performed in a continuous-flow manner which allows parallel and in-situ processing of sample mixture. The separation efficiency was larger than 95% as calculated by statistical method in two outlets, and the flow-rate could be increased to get a higher throughput. The device was reliable after being worked repeatedly over several days. It is expected that this method of fabrication could be used for other designs and applications in microfluidics.

4.4 Conclusions

A novel method for fabricating DEP-based microfluidic devices with 3D electrodes at the sidewalls using metal alloy microspheres was described in this chapter.
Compared to other techniques, the method used to construct the 3D arc-shaped electrodes is unique, and could provide excellent non-uniform electric field distribution in the lateral direction of the channel. The geometry of the electrodes is crucial for applications requiring non-uniform electric field. Arc-shaped electrodes are preferable at manipulating particles and cells using DEP because they can generate stronger lateral DEP forces acting on the particles/cells in continuous-flow. In addition, a reduction in the divergence of the electric field is another advantage of this design because electrodes have no sharp edges. The device fabricated by the method presented demonstrated its application in particle/cell manipulation under the influence of AC DEP. The device successfully deflected 10 µm PS particles and yeast cells, separated 10 µm PS particles from 5 µm PS particles based on the size, and also separated 5 µm PS particles from yeast cells according to their difference in dielectric properties. This novel technique could also be used to construct 3D electrodes of different sizes and geometries on the sidewall of the microchannel by applying varying-shaped metal alloys, e.g. cylinder or asymmetric shape, as long as they are fabricated in different ways. These proposed 3D electrodes are guaranteed by the fact that the low temperature annealing step will maintain the original shape of manually positioned electrodes, as stated in the section describing the method of fabrication.
CHAPTER 5
A 3D DIELECTROPHORETIC CHIP INTEGRATING FOCUSING, ALIGNING AND TRAPPING FOR IMPROVED CONCENTRATION AND SEPARATION OF PARTICLES

A DEP-based microfluidic device with a three-dimensional (3D) microelectrode configuration for concentrating and separating particles in a continuous through-flow is presented in this chapter. The structure of the 3D electrode, where an array of microelectrodes is patterned on both the top and bottom surfaces of the microchannel consists of three units: focusing, aligning, and trapping. As particles flow through the microfluidic channel, they are focused and aligned by the funnel-shaped and parallel electrode array, respectively, before being captured at the trapping unit by a negative DEP force. For a mixture of two populations of different size particles or dielectric properties, with a careful selection of suspending medium and applied field, the population with a stronger negative DEP is manipulated by the microelectrode array and separated from the other population which is easily carried towards the outlet by hydrodynamic force. The functionality of the proposed microdevice was verified by concentrating different-sized polystyrene (PS) microparticles and yeast cells dynamically flowing in the microchannel. Moreover, separation based on size and dielectric properties was achieved by sorting the PS microparticles and isolating the 5 µm PS particles from the yeast cells, respectively. The performance of the proposed micro-concentrator and micro-separator was also studied, including the threshold voltage at which particles begin to be trapped, any variation in the efficiency of cell-trapping with respect to the applied voltage and flow rate, and the efficiency of the separation experiments.

5.1 Introduction

Dielectrophoresis (DEP) occurs due to a translational force exerted on a polarisable particle when it is subjected to a non-uniform electric field [10]. As mentioned in Chapter 2, a variety of 2D planar microelectrodes normally patterned on the bottom surface of a microchannel were developed to generate the required inhomogeneous electric field for the DEP effect, including parallel or interdigitated, castellated,
oblique, curved, quadruple, and matrix ones. Although 2D microelectrodes have been successfully equipped in various DEP-based microdevices for manipulating bio-particles (i.e. DNA, protein, bacteria, virus, mammalian and yeast cells), the use of such a configuration can be problematic for some practical applications due to a decrease in the DEP force away from the microelectrodes and/or particle adhesion on the surface of the electrode and the channel wall. As an alternative, 3D electrode structures were built on both the top and bottom surfaces of the channel, where effective DEP forces are created over a larger volume of the microchannel, and the particles are focused and located around the centre of the channel in a vertical direction. Thus the aforementioned problems of 2D planer microelectrodes, such as insufficient holding force, relatively low trapping/sorting efficiency, and particle adhesion are avoided. Several dielectrophoretic microdevices with top-bottom patterned microelectrodes have been developed [149, 152-154], but they were always single-functional, and complex in terms of fabrication and operation. Although other 3D microelectrode configurations formed by building extruded metal and carbon electrodes onto the bottom substrate, doping silicon wafers, and patterning vertical electrodes along the sidewalls have been used in DEP-based microdevices, their fabrication techniques are more complicated.

Based on the fabrication method described in Chapter 3, a DEP-based microfluidic device equipped with 3D microelectrode arrays (i.e. bi-layer planar microelectrode arrays patterned on both the bottom and top surfaces of the microchannel) was designed and fabricated. The 3D electrode structure consists of a funnel-shaped focusing unit, a parallel aligning unit and a crescent-shaped trapping unit in series, which enables integrated functions such as the concentration of particles in a continuous flow, and separation of mixed particles according to their size and dielectric properties. Numerical simulations were performed to optimise the design of electrodes, and experiments were conducted to demonstrate its integrated functionalities such as concentration and separation based on size and dielectric properties. Compared to previously developed dielectrophoretic microdevices with top-bottom patterned microelectrodes, the proposed one has various advantages, including multi-functionality, improved manipulation efficiency and throughput, easy fabrication and operation, etc.
5.2 Theory

As indicated in Chapter 2, the time-averaged DEP force acting on a spherical particle is given by Eq. 2-3, which depends on a variety of parameters such as particle size, the conductivity and permittivity of particles and the suspending medium, and the frequency of the external electric field. According to positive/negative the real part of the CM factor (Re[\(K(\omega)\)]), particle will move toward/away the regions of high electric field.

The hydrodynamic force applied on a particle can be defined as

\[
F_{HD} = 6\pi \eta rv
\]

(5-1)

where \(\eta\) and \(v\) represent the viscosity and velocity of the fluid, respectively, and \(r\) is the radius of the particle. The hydrodynamic force is linearly proportional to the velocity of the fluid and the size of the particle, and the movement of the particle is determined by the combined effect of the DEP force and hydrodynamic force. In order to direct and trap the particles, the DEP force in the direction of flow must be stronger than the hydrodynamic force.

5.3 Numerical simulation

Figure 5-1 Simulation results showing the distribution of electric field (\(E\), contours) and direction of negative DEP force (black arrows) in a vertical plane of microchannel patterned with electrodes on the top and bottom surfaces. The channel is 30 \(\mu\)m high, and the voltage applied to the electrodes is 5 V.
Figure 5-2 Comparison of triangular (left column), rectangular (middle column), and crescent (right column) microelectrodes: (a1-a3) the distribution of electric field, $E$, in the area formed by the geometry of microelectrodes, and (b1-b3) the variation of the square of electric potential, $\phi^2$, along the vertical centreline (indicated by black-dash lines) across the microelectrodes. The field applied to the electrodes was remained the same at 5 V.

The key point in achieving a desirable DEP effect is the generation of an electric field gradient by the electrode array. Numerical simulations were performed using the commercial software COMSOL 4.0 (COMSOL Inc., Burlington, MA). In the simulation, a 2D model was used for simplicity without considering the effect of the channel wall on the distribution of the electric field and a voltage was applied to the edge of the electrode. Assuming a medium of constant electric conductivity, the electric potential field is governed by the Laplace equation expressed as $\nabla^2 \phi = 0$, and the electric field is obtained by calculating the derivative of the electric potential field $\mathbf{E} = -\nabla \phi$ (where $\phi$ and $E$ are electric potential and electric field strength, respectively). The maximum and minimum size elements were about $10^{-5}$ and $10^{-7}$, respectively, and the density of the elements around the tips of the microelectrode was chosen with more attention. Figure 5-1 depicted the distribution of the electric field (contours) and direction of the negative DEP force (black arrows) in a vertical
plane in a 30-µm high microchannel, when a voltage of 5 V is applied to the microelectrodes patterned on the top and bottom surfaces. The numerical results indicated that a non-uniform electric field symmetric about the centreline of the channel was generated, with the maximum strength obtained at edge of the electrode, and strong electric field covers almost the whole height of the microchannel. Particles can be directed toward the centre of microchannel under the effect of negative DEP force, pointing from the region of the electrodes to the centre of the channel. Therefore, a 3D electrode configuration addresses the problems encountered by 2D planar electrodes, such as insufficient DEP force and particle levitation.

In order to optimise the design of an electrode structure, the distribution of the electric field and electric potential in three types of electrode geometries were compared: (1) triangular, (2) rectangular, and (3) crescent shaped. Both the width of the microelectrodes and the spacing of the electrode at the aperture were set at the same values of 40 and 80 µm, respectively. Figure 5-2a show the contours of the electric field ($E$) within the region formed by different-shaped electrodes at an applied voltage of 5 V. The electric potential applied to the microelectrodes generates a non-uniform electric field in every case. It is known that the electric field is constricted at the corner, thus, a stronger and highly non-uniform electric field is created at the sharp corners (the dark blue region corresponds to a strong electric field). Due to the curved shape, the crescent-shaped microelectrode created a smoothly varied electric field within the microchannel which eliminated any locally amplified electric field due to its sharp corners. Compared to triangular (a1) and rectangular (a2) microelectrodes, where electric fields are locally concentrated at the sharp corners (marked by red-dash circles), the electric field generated is more uniform within the region of the crescent microelectrode (a3), which avoids undesirable motions at the sharp corners and minimises the risk of biological damage from a high-intensity electric field.

Figure 5-2b depicts the variation of the square of electric potential along the vertical centrelines (black-dash lines in Fig. 5-2a) across the microelectrodes of different structures. The blue and red curves correspond to triangular and rectangular geometries, respectively, while the green curve corresponds to crescent geometry.
Along all lines, the magnitude of electric potential decreases with the distance away from the edges of the electrode, which obtains its maximum and minimum values at the edge of the electrode and the centre of its electrode structure, respectively. However, the electric field in the crescent microelectrodes varied more evenly than the two other electrode shapes (the potential magnitude decreases slowly as the distance from the edge of the electrode increased), and was maintained over a longer distance (120 µm), compared to triangular (62 µm) and rectangular (80 µm) ones. This suggests that a crescent structure can increase the effective area inside the microelectrodes for the DEP effect, and therefore more particles/cells can be accumulated within the microelectrode structure, producing a higher concentration yield. According to the numerical results, the crescent-shaped microelectrode eliminated the high-intensity electric field and improved the concentration yield.

5.4 Materials and methods

5.4.1 Chip layout and fabrication

Figure 5-3 A schematic diagram of the proposed micro-concentrator and separator using dielectrophoresis.

A schematic representation of the dielectrophoretic chip with a 3D microelectrode configuration to manipulate particles is illustrated in Fig. 5-3. It consists of two glass substrates with patterned microelectrode arrays, a polydimethylsiloxane (PDMS)
layer containing a microchannel sandwiched between the substrates, and a top PDMS lid with inlet and outlet for sample and buffer introduction. The proposed microchip was fabricated using the relatively simple and cost-effective method described in Chapter 3. Briefly, a microelectrode (20 nm Ti/100 nm Pt) was made by standard photolithography and lift-off techniques, while microchannel was created by patterning onto a layer of PDMS via laser ablation (VLS 2.50, Versa Laser System, Universal Laser System Ltd.). After constructing holes through the top glass substrate and PDMS lid, multiple layers (i.e. PDMS lid, PDMS-based microchannel, and microelectrode-patterned glass substrates) were bonded together using oxygen-plasma treatment (PDC-002, Harrick Plasma, NY, USA). Among all the steps required to fabricate micro-chips, an accurate alignment of the top and bottom electrodes with respect to each other as well as to the sandwiched microchannel may be the most important one because misalignment may cause an adverse effect on the DEP force and performance of the microchip [224]. In order to achieve accurate alignment, double cross-shaped marks (larger outer masks for rough aligning and smaller inner ones for precise aligning are involved) were previously defined on the sides of the electrode array and microchannel while they were being fabricated, which were used along with methanol acting as a lubricant in the following bonding step.

Figure 5-4 (a) Photograph of the fabricated microfluidic chip used in the experiment for manipulating particles. (b) Magnified image indicating the structure of
microelectrode array, which is consists of three units from right to left: focusing, aligning and trapping.

Figures 5-4a and 5-4b present the micro-fabricated dielectrophoretic micro-concentrator/separator, and a top view of the designed microelectrode array, respectively. The microchannel is 1200 µm high by 30 µm wide. The width of the electrodes and the gap between adjacent ones are both 40 µm, the length of the straight and oblique electrodes (placed at angle of 30° to the direction of the flow) inside the PDMS-based microchannel are 800 and 1000 µm, respectively. The radius of the innermost crescent is 60 µm. An electric potential was applied through 3 x 3 mm pads placed on both the top and bottom surfaces of the microchannel.

5.4.2 Microelectrode configuration

As indicated in Fig. 5-4b, the structure of a 3D electrode where microelectrodes are patterned on both the top and bottom surfaces of the microchannel, was labelled into three units from right of left: focusing, aligning and trapping. The first microelectrode array takes advantage of the funnel-shaped electrodes to focus target particles. Under a combination of dielectrophoretic and hydrodynamic force, target particles are driven laterally along the microelectrodes, and forced towards the centre of the microchannel as they pass through. This electrode array can also serve as a sorting unit for mixture of particles because target particles experiencing a stronger DEP force are deflected and directed toward centre of the channel by the electrodes, while non-target particles experiencing a weaker DEP force will pass over the electrodes and be moved along with the continuous flow toward the outlet.

The second array comprising parallel microelectrodes positioned symmetrically about the centreline of the channel serves to align target particles. The negative DEP forces from electrode arrays at both sides of the centreline can balance each other, and therefore focused target particles were further directed and forced to the centre of the channel dielectrophoretically. As a result, target particles run with the flow in the direction parallel to the electrodes, and a confined and concentrated stream of particles is formed along the centreline of the channel within the spacing between the inner straight microelectrodes. The upstream focusing and aligning units are
essential, and are designed to line the particles into a confined stream prior to entering the unit of trapping, resulting in improved trapping yield.

The final unit consists of crescent-shaped microelectrodes created by intersecting concentric circles and was used to trap and concentrate target particles after they were focused and aligned. Due to the negative DEP force, target particles were slowed down, retained, and finally prevented from passing the microelectrodes. The intensity of trapped particles within the inner crescent-shaped electrodes increases over time as the sample continuously flows through the microchannel. The captured target particles can be released and then collected at the outlet by turning off the applied electric field, making the proposed chip suitable for further detection and characterisation of target particles.

Compared to a single electrode structure, the electrode structure with two layers (an inner and an outer layer) allows for a higher operating flow rate and more efficient manipulation of particles, because both layers can generate electric field non-uniformities and DEP effects. Particles that fail to be focused, aligned, or trapped by the inner electrode array will be manipulated further by the outer one. Moreover, the use of a buffer leads to particles being confined in two streams near the sides of the channel, which demonstrates the integrated functionality of the particles: for single-particle concentration, the particles could be deflected and focused by moving from the side of the channel to the centre region along the oblique electrodes, whereas for double-particle separation, one particle population move with the continuous flow near the side of the channel and is isolated from the other population that is deflected and finally stopped at centre of the channel.

5.4.3 Sample preparation

Polystyrene particles of 5 (Sigma-Aldrich, USA), 10 and 15 µm diameters (Fluosphere, Invotrogen, CA, USA) were selected because these sizes are comparable to biological cells such as yeast and red/white blood cells. The original 10 and 15 µm particle suspensions were diluted by deionized (DI) water in volume ratio of 1:10, while 5 µm particles that were originally suspended in pure water were re-suspended in a solution of 15 mM NaCl at a concentration around $10^7$ beads per
millilitre. Baker’s yeast cells (Saccharomyces cerevisiae) were cultured at 37°C in the YEP broth (MP Biomedicals, LLC.). After about 24 hours, the cells were diluted with DI water three times and then re-suspended in 380 μS/cm NaCl solution at a concentration of about 10^7 cells per millilitre. For separation experiments, the original 5 μm particle solution was mixed with the diluted 10 and 15 μm particle solutions, and prepared yeast cells solution at a volume ratio of 1:1000, respectively.

5.4.4 Experimental setup

The sinusoidal voltages to manipulate the particles came from a function generator (33120A, Agilent Technology, CA, USA), and a voltage amplifier (Stereo Power Amplifier 216THX). Two syringe pumps (PHD 2000, Harvard Apparatus, Holliston, MA) were used to continuously inject the sample and buffer into the microchannel. Two syringes were connected to the inlets through stainless steel tubes (OD: 0.9 mm) and Tygon tubing (ID:1/32 inch, OD:3/32 inch, Teflon®FER, Upchurch Scientific, USA). The motion of the particles and cells were observed and recorded using an inverted fluorescence microscope (IX71, Olympus, Tokyo, Japan) equipped with a CCD camera (DP 70, Olympus, Tokyo, Japan), and a computer with Olympus DP controller image software. The particles were counted and the concentration was calculated using a Neubauer hemocytometer.

5.5 Results and discussion

5.5.1 Concentration mechanism
Figure 5-5 Schematic dynamics of the concentration of target particles as they move in a continuous through-flow.

The presented microfluidic chip with a 3D electrode configuration can be used to trap and concentrate particles and cells by using a force balance between negative DEP force and hydrodynamic force. With an appropriate flow rate and sinusoidal voltage, the target particles will be sequentially processed by the funnel-shaped, parallel and crescent-shaped microelectrodes, which are responsible for focusing, aligning and trapping, respectively. Figure 5-5 illustrates the overall dynamics of the target particles as they continuously flow through the microchannel. The net force resulting from a combination of dielectrophoretic and hydrodynamic force will determine the direction in which the particles will be moved. The movement of target particles in the four regions indicated by sequence numbers I, II, III, and IV, from right to left are distinct: (I) Before approaching the electrode array, particles are driven into two streams close to the sides of the channel due to the buffer flow. (II) When the particles reach the edge of the funnel-shaped electrodes, they are deflected due to a negative DEP force and moved along the length of the electrode under the combined effect of two forces and gradually focused towards the region of channel centre. (III) After travelling the length of the oblique electrodes, the particles continue through the channel within the spacing between the inner parallel electrodes. Due to the negative DEP forces generated by the two sets of straight electrodes placed on either side of the centreline of the channel, particles reach equilibrium positions where the DEP forces from these two sets of electrodes are equal, and the particles are in a confined stream along the centreline. (IV) The hydrodynamic force is overcome by the negative DEP force generated by the crescent-shaped microelectrodes, therefore, the particles are slowed down, stopped and finally captured. As time goes by, the trapped particles accumulated into a cluster, filling the region inside the inner crescent-shaped electrodes.
5.5.2 Concentration of polystyrene microparticles

Figure 5-6 Concentration of polystyrene particles of different sizes: (a1, a2) 5, (b1, b2) 10, and (c1, c2) 15 µm at 10 (left column) and 40 seconds (right column). Target particles are sequentially focused, aligned and trapped by applying 8 Vp-p, 10 kHz signal voltage at the flow rates of sample and buffer both of 0.3 µl/min.

Figure 5-6 shows the overall movement of 5 (top row), 10 (middle row) and (bottom row) 15 µm polystyrene particles a field of 8 Vp-p and 10 kHz, when the flow rates of the sample and buffer are both 0.3 µl/min. At this frequency, all the particles exhibited a negative DEP effect and were focused, aligned, and trapped sequentially by the microelectrode array. As shown in the figure, the particles carried by the continuous flow running from right to left were deflected by the funnel-shaped electrodes and moved along the oblique electrodes, and then transported within the spacing between the inner parallel electrodes, and were finally trapped and accumulated at the area inside the inner crescent-shaped electrodes. The area of
concentrated particles at the trapping unit after a 40 seconds electric field was applied (right column) is greater than after 10 seconds (left column), indicating a reliable performance of particle concentration at a continuous flow.

Figure 5-7 Threshold voltages for trapping polystyrene particles with diameters of 5, 10, and 15 \( \mu m \) at the flow rate of sample ranging from 0.3 to 0.8 \( \mu l/min \).

Consider a particle transport through the microfluidic channel where the DEP force generated by the microelectrode array competes against the hydrodynamic force. Within relatively low voltage range, the DEP force is weaker than the hydrodynamic force, and the particle will be carried by the continuous flow toward the outlet. At a fixed flow rate, there exists a threshold voltage at which particles begin to be held by the electrode array. The threshold voltage required to successfully trap different size polystyrene particles at varying flow rates of sample (in the range between 0.3 and 0.8 \( \mu l/min \)) was measured and plotted in Fig. 5-7. It was determined by gradually increasing the applied voltage and stopping when the particles begin to be captured at the electrode trapping unit. The inset shows the concentration of 5, 10, and 15 \( \mu m \) particles at the trapping unit under a field of 8 Vp-p, 10 kHz, and with flow rates of sample and buffer both at 0.3 \( \mu l/min \) over 3 min. The region inside the inner crescent microelectrode was seen to be full of high-density captured particles. The same experiment was conducted three times for each data point and the average values of the measurements were used. It can be found that the threshold voltage increased with the rise of flow rate, and was higher for smaller particles. According to Eqns. 2-
3 and 5-1, the DEP force and hydrodynamic force are proportional to the gradient of the square of the electric field and flow rate, respectively. An increased flow rate determines larger hydrodynamic force, and therefore, a larger electric field and correspondingly larger DEP force were required to counter the increased hydrodynamic force needed to trap particles with fixed sizes. Moreover, the DEP force is proportional to the cube of the particle radius, so a higher voltage is required for smaller particles to exhibit the same dielectrophoretic effect as larger particles.

5.5.3 Concentration of yeast cells

Figure 5-8 Concentration of yeast cells at the trapping unit of crescent electrodes under a field of 12 Vp-p, 10 kHz and flow rates of sample and buffer both at 0.2 µl/min over 100 seconds. Images were taken at 10 sec intervals.

The performance of the proposed micro-concentrator was also examined by constantly trapping yeast cells flowing in the microfluidic channel. The overall motion of yeast cells with an application of 12 Vp-p, 10 kHz sinusoidal voltage, 0.2 µl/min sample and buffer flow rates is similar to polystyrene particles, because yeast cells experiencing a strong negative DEP forces were sequentially focused, aligned and trapped by the electrode array. Figure 5-8 shows consecutive images of the trapping unit captured at intervals of 10 seconds. The area where the yeast cells accumulated increased over time till after 100 seconds, it almost filled the region within the inner crescent-shaped electrodes.
Figure 5-9 (a) The effect of applied voltage on the trapping efficiency of the cell at a fixed flow rate of the sample and field frequency of 0.3 µl/min and 10 kHz, respectively. (b) The effect of sample’s flow rate on cell-trapping efficiency at a fixed signal field of 9 Vp-p and 10 kHz.

In addition, the cell-trapping efficiency was analysed with respect to voltage applied and the flow rate of the sample. The yeast cells in the sample taken from the outlet were counted with a hemocytometer after the electric fields were applied. This method was also used to measure cell concentration at the inlet. Hence, the trapping efficiency in each operation condition can be calculated by \((1-\frac{C_o}{C_i})\times100\%\) (\(C_o\) and \(C_i\) are the concentration of yeast cells at the outlet and inlet, respectively). In Fig. 5-9a, the trapping efficiency was plotted as a function of applied voltage in the range between 7 and 12 Vp-p, with the sample flow rate and the field frequency kept constant at 0.3 µl/min and 10 kHz, respectively. It can be found that the trapping efficiency depends almost linearly on the applied voltage at a relatively low flow rate. This was mainly attributed to the fact that the DEP force is proportional to the gradient of the square of the electric field (see Eq. 2-3), so an increased voltage leads to a larger field gradient, and hence a larger DEP force to capture cells, which improves the trapping efficiency at a relatively low flow rate. Figure 5-9b presents the variation of the cell-trapping efficiency with respect to the flow rate of the sample for a fixed applied sinusoidal voltage of 9 Vp-p and 10 kHz. The trapping efficiency decreases with the rise of the flow rate. As shown by Eq. 5-1, the hydrodynamic force is linearly proportional to the flow rate, so an increased flow rate determines the correspondingly larger hydrodynamic force, which counteracts...
the effect of the DEP force responsible for capturing cells, the therefore the trapping efficiency decreases.

5.5.4 Separation of polystyrene microparticles based on size

Figure 5-10 Schematic dynamics of particle separation based on size; large particles are focused, aligned, and finally trapped by the microelectrode array, and are therefore isolated from the small particles carried away by continuous flow.
As shown by Eq. 2-3, the DEP force is proportional to the cube of the particle radius, thus larger particles will experience a stronger negative DEP force than smaller particles, and could be manipulated by microelectrode array more easily. The overall response of a mixture of particles of different sizes in the micro-sorter being proposed is schematically illustrated in Fig. 5-10. The mixture of particles was introduced into the microfluidic channel and hydrodynamically pre-confined into two streams near the sides of the channel. As they move close to the microelectrode array, particles of different sizes were separated: large particles experiencing large negative DEP force will be deflected by the oblique electrodes, and sequentially
aligned and trapped by the straight and crescent microelectrodes, respectively; whereas small particles will pass over the funnel-shaped array of electrodes and flow to the outlet with continuous through-flow. Figure 5-11 shows the separation of 5 and 10 \( \mu m \) particles, when an AC signal of 6 Vp-p and 10 kHz, and the flow rates of the sample and buffer were both at 0.3 \( \mu l/min \). As expected, the 10 \( \mu m \) particles were focused, aligned, and trapped by the microelectrode array, while 5 \( \mu m \) particles passed through the focusing unit of the funnel-shaped microelectrodes with minor deflection. Similar results were obtained when a mixture of 5 and 15 \( \mu m \) PS particles were separated and examined with a 10 kHz, 5 Vp-p sinusoidal voltage. As shown in Fig. 5-12, the smaller particles (5 \( \mu m \)) passed over the focusing unit and moved along with the flow, leaving the larger ones (15 \( \mu m \)) to be successfully manipulated by the electrode structure.

5.5.5 Separation of yeast cells and polystyrene microparticles based on dielectric properties

(a) \( t=10 \) sec  (b) \( t=25 \) sec  (c) \( t=50 \) sec  (d) \( t=100 \) sec
Figure 5-13 Separation of yeast cells (white dots) and 5-µm diameter polystyrene particles (black dots) at a sample and buffer flow rate both of 0.2 µl/min with an applied sinusoidal voltage of 7 Vp-p and 300 kHz. The response of the mixture at the trapping unit over 100 sec was shown at: (a) t=10 s, (b) t=25 s, (c) t=50 s, and (d) t=100 s. The yeast cells were seen moving to the outlet with continuous through-flow, while PS particles were successfully trapped within the inner crescent-shaped electrodes, and the area of concentrated particles increased over time.

Besides separation depending on size, separation based on dielectric properties was achieved by successfully separating 5-µm diameter PS microspheres from the yeast cells. The size of the microspheres approximates to that of the yeast cells, eliminating the effect of size on the performance of separation. The sorting principle is that yeast cells and 5 µm particles exhibit a different DEP response in a 380 µs/cm NaCl solution at an AC signal of 300 kHz [172]: 5 µm particles have a stronger negative DEP force due to larger real part of CM factor, while yeast cells are near the crossover frequency (Re[K(ω)] ≈ 0), which corresponds to a weak DEP force. Therefore, yeast cells can pass over funnel-shaped microelectrode with continuous flow due to hydrodynamic force, while 5 µm particles will be deflected by the electrode array and finally concentrated at the trapping unit. Figure 5-13 illustrates the separation of yeast cells (white dots) and 5 µm PS particles (black dots) at the trapping unit over 100 seconds, under an applied 300 kHz, 7 Vp-p voltage and 0.2 µl/min flow rates of the sample and buffer, respectively. As the polystyrene particles were being focused and aligned, they were trapped and concentrated in the inner crescent microelectrodes. The area of concentrated particles increased as time went by, preferring to fill the circular region at the trapping unit. 5 µm PS particles, were therefore sorted from the yeast cells, which were seen to be moving to the outlet in two streams near the side of both channels.
Figure 5-14 Percentage of each population of particles at the outlet in separation experiments: (I) size-based separation of 5 and 10 $\mu$m PS particles, (II) size-based separation of 5 and 15 $\mu$m PS particles, and (III) dielectric properties-based separation of yeast cells and 5 $\mu$m PS particles.

The performance of the proposed dielectrophoretic micro-separator was further evaluated in terms of relative percentage. After applying an electric field, the sample from the outlet was collected, and the number of each type of particle or cell was counted using a hemocytometer. The same procedure was repeated three times for each separation experiment, and both the average value and standard deviation were obtained. Figure 5-14 shows the percentage of: (I) 5 and 10 $\mu$m particles, (II) 5 and 15 $\mu$m particles, and (III) 5 $\mu$m particles and yeast cells at the outlet, indicating that the particles which were not processed by the electrode array were very pure. Moreover, the particles for the sample from the inlet were counted, and the separation efficiency was calculated by $n_r / n_i \times 100\%$, or $(1 - n_w / n_i) \times 100\%$, where $n_i$ is the number of target particles at the inlet, and $n_r$ and $n_w$ are the number of target particles rightly and wrongly processed, respectively. The results showed a separation efficiency of above 90% was achieved in all experiments.

5.6 Conclusions

A DEP-based microfluidic device with a 3D top-bottom patterned electrode configuration for continuous concentration and separation of particles has been presented in this chapter. For the concentration of single particles, target particles
running along with continuous flow were sequentially focused, aligned, and trapped by the electrode array; for double-particle separation, particles exhibit a stronger negative DEP effect were deflected, manipulated, and isolated from the other population of particles moving towards outlet due to hydrodynamic force. A microdevice serving as both a micro-concentrator and a micro-separator was demonstrated using different-sized PS particles and yeast cells. The threshold voltage obtained for PS particles with diameters of 5, 10, and 15 µm, depends on such factors as particle size and flow rate. The experimental results also indicated that cell-trapping efficiency can be improved by increasing the signal voltage and decreasing the operating flow rate of the sample in a certain range. Moreover, an high efficiency above 90% was obtained in all separation experiments.

The proposed DEP-based microfluidic device has many advantages: (1) compared to planar electrodes only patterned on the bottom surface of the microchannel, the 3D electrode configuration generates stronger DEP force over a larger volume of the microchannel for particle deflection, allowing for a shorter microchannel and higher operating flow rate, which leads to increased throughput; (2) the upstream units of focusing and aligning serve as pre-treatment components, which direct target particles running with the flow towards the given trapping area, the capture efficiency hence improved; (3) large numbers of particles were trapped at a defined and unique location, rather than at discrete locations along the microchannel, which facilitates post-processing, such as particle collection, detection and analysis; (4) versatile capabilities can be achieved, including the concentration of single particles, and the separation of double particles depending on their size and dielectric properties; (5) it is easy in terms of fabrication and operation, because bonding the top and bottom electrodes can be achieved by plasma treatment using a PDMS-based microchannel, and single-channel AC power supply. The DEP-based microdevice presented is expected to be widely used for concentrating and separating particles of different sizes and dielectric properties in biological, medical, and environmental fields.
A high-throughput microfluidic device for enriching and separating particles and cells by applying hydrodynamic, electrophoretic, electroosmotic, and dielectrophoretic forces is presented in this chapter. This process involves generating fluid flow by a pressure difference and electroosmosis (EO), trapping particles by dielectrophoretic force, and redirecting particles by electrokinetic force (a combination of electrophoretic and electroosmotic force). Both DC and AC signals were applied in this DEP-based microdevice to take advantage of DC electrophoresis, electroosmosis and AC dielectrophoresis for on-chip bio-particle manipulation. The proposed chip with 13 individual channels in a radical direction and top-bottom patterned microelectrodes can operate in a high throughput manner for enrichment and separation of particles. We evaluated our approach by collecting polystyrene particles, yeast cells, and E. coli bacteria according to their responses to an electric field gradient. Live and dead yeast cells were successfully separated, which validated that our device can separate highly similar cells according to dielectric properties. The results showed that the fabricated chip could achieve fast cell pre-concentration and cell separation based on viability. Hydrodynamic, electrophoretic and electroosmotic forces were used in combination instead of a syringe pump to achieve sufficient fluid flow and continuous particle movement. By eliminating bulky mechanical pumps and performing on-chip separation using electric field variation, this kind of integrated chip has potential applications in diagnostic kit for in situ sampling.

6.1 Introduction

As mentioned in Chapter 2, DEP-based microdevices with 2D planar microelectrodes always suffer from weak DEP forces from the microelectrode surface and particle adhesion, which greatly reduces the efficiency and throughput of these microdevices. Alternatively, 3D microelectrode structures are built on both the bottom floor and top
ceiling of the microchannel, and could generate effective DEP forces over a larger volume of the microchannel, and also focus particles around the centre of the channel in a vertical direction. However, these devices still have a relatively low processing speed and require mechanic pumps for sample introduction, preventing the portability available for point-of-care (POC) sample diagnosis and analysis.

The throughput or productivity of these systems has been highly improved by the use of parallel multiple modular microfluidic (M3) reactors operating simultaneously [225], by which a continuous generation of polymer particles has been achieved. Gagnon and Chang [226] proposed the use of combined AC electroosmotic flow and DEP effects to collect, separate and manipulate bacteria in suspension. Moreover, mechanical syringe pumps could be replaced by electroosmotic and electrophoretic techniques to generate fluid flow and particle motion [227-229]. Inspired by these approaches, we developed a high-throughput DEP-based microdevice consisting of 13 individual microchannels with top-bottom patterned microelectrodes arranged in a radical direction, which are capable for parallel operation. This means that mechanical pumps are eliminated when hydrodynamic and electrokinetic forces are used. This proposed microsystem features easy loading and minimal consumption of samples, and also allows particles to be trapped and sorted based on a combination of hydrodynamic, electrokinetic and dielectrophoretic forces. During the trapping experiments, fluid flow driven by the pressure difference between inlet and outlet and AC DEP force were utilised for continuous fluid flow and particle trapping, respectively; both electrophoretic and electroosmotic forces were used to move particles in these releasing and sorting experiments. To assess its performance, three different sets of samples were used in the device, namely polystyrene particles (colloidal sample), yeast cells (eukaryotic cell) and *E. coli* (prokaryotic cell). The effects of AC dielectrophoresis, DC electrophoresis, DC electroosmosis and hydrodynamic force are discussed and validated through these experiments, which also indicate the potential of the proposed device.

### 6.2 Materials and methods
6.2.1 Fabrication of the DEP-based microfluidic chip

Figure 6-1 shows the process of fabricating a highly integrated microfluidic chip for cell pre-concentration and separation. It has a 3D microelectrode structure patterned on both the top and bottom surfaces of the microchannel, which was fabricated by the method described in Chapter 3. Herein, we briefly describe the fabrication of each layer and its assembly to form enclosed microfluidic channels. The major steps in this fabrication include: patterning the electrodes, polydimethylsiloxane (PDMS) thin film preparation, laser cutting the lower and upper channel, and plasma bonding.

6.2.1.1 Patterning the electrodes

Standard photolithography, sputtering and lift-off process were used to pattern planar electrodes on the glass substrate which had been pre-cleaned with RCA-1 solution, a mixture of deionized (DI) water, ammonium hydroxide (NH₄OH) and hydrogen peroxide (H₂O₂) in the volume ratio of 5:1:1, followed by rinsing in DI water for
three times. The glass substrate was then processed by spin-coating a positive photoresist HPR 507 at 2000 rpm for 30s, soft-baking on a hotplate at 110 °C for 2 min, UV exposure via Mask Aligner (ABM #2) for 20 sec, the photoresist was developed using FHD-5 for 60 sec, and then post-baking on a hotplate at 110 °C for 2 min. Pt/Cr electrodes (150 nm/15 nm thick) were deposited onto this glass plate with an Explorer 14 (Denton Vacuum) sputtering machine, followed by the lift-off process to obtain glass plates patterned with electrodes.

6.2.1.2 Construction of PDMS microchannels
PDMS thin film was spin-coated onto a substrate treated with Teflon (Dupont, Grade 601S1-100-1). PDMS gel was produced by Sylgard 184 silicone elastomer mixture (Dow Corning Corporation, Miland, USA) at a weight ratio of base: curing agent = 10:1. The spin speed was set at 2000 rpm for 30 sec and 3500 rpm for 30 sec to get a uniform film with a thickness of 30 µm and 15 µm to process different particles with different dimensions. A 2 mm PDMS slab was also prepared by mixing PDMS base and curing agent together, and pouring it into a container. After curing the PDMS gel in an oven at 60 °C for 2 h, the PDMS thin film and PDMS slab were laser cut with a CO2 laser plotter (Versa Laser System, Model VLS3.50, Universal Laser System, Ltd.). The cutting parameters were also optimised to get a smooth edge [230]. The lower channel was cut directly with 0.5 W, at 0.75 mm/s, and 1000 pulse per inch (PPI), which produced a channel around 200 µm wide, while the upper channel was etched with 2.5 W, at 0.75 mm/s, and 1000 pulse per inch (PPI), which created a channel 900 µm wide and 500 µm deep.

6.2.1.3 Plasma bonding
The bonding step was performed three times during the fabrication process. The first step was to bond PDMS thin film to glass substrate patterning electrodes. The second step was to construct an outlet on the upper layer of the channel. The last step was to bond the upper layer of electrodes to the patterned PDMS structure. The enclosed microfluidic channel was formed to create a leak-tight channel at this stage. The plasma bonding in each step was facilitated by a plasma cleaner (Harrick Plasma PDC-002, Harrick Plasma, USA) for 4 min, followed by placing two substrates in
contact by checking the alignment under an optical microscope (Olympus SZX16, OLYMPUS, Japan). Before each experiment, the chip was passivated by introducing 7% bovine serum albumin (BSA) solution for 2 h to prevent adhesion between the channel surface and bio-particles. Then excess BSA was pumped out by introducing solutions of 10 mM NaCl 5 times.

![Figure 6-2 Microfluidic chip pictures. (a) Real image of fabricated microfluidic chip containing 13 channels in a small area; (b) Optical microscope image of the electrodes configuration in the region indicated by red-dashed square in 5-2a. The arrowhead shaped electrode is designed for trapping particles by AC signal, while the circle shaped electrodes are used for manipulating particles by DC signal.](image)

Figure 6-2a shows the densely packed chip containing 13 channels in a 2×2 cm² area. The number of channels was the most possible with the laser cutting technique currently available, while the channels were designed and configured for rapid processing for cell pre-concentration compared with other chips which had only one channel. In order to perform all the experiments with low voltage power supplies, the depth of the channel is varied according to the relative DEP forces for different particles. Thus, the channel for trapping polystyrene particles was 30 µm deep, and the channels for trapping yeast cells and *E. coli* were both 15 µm deep. The inlet was defined by the big reservoir for loading samples and the outlet was constructed by laser etching on two sides of this chip. Figure 6-2b is the optical image of the bottom electrodes configuration in the region indicated by red-dashed square. The central electrode is used to apply AC signal for trapping, while the electrodes lying at two ends of the microfluidic channel were used to impose the DC signal to generate electrophoretic and electroosmotic force.
6.2.2 Sample preparation

Particles studied in our demonstrations are fluorescent polystyrene (PS) beads, yeast cells and *E. coli* bacteria. Yellow green fluorescent 10 µm polystyrene particles (Invitrogen, CA, USA) were diluted into DI water at a ratio of 1:100, which gave a concentration of 3.6×10⁴ particles/ml and NaCl concentration of 1.5 mM. Cells of *Saccharomyces cerevisiae* (yeast cells) were cultured on a yeast extract (YE) agar plate. After incubating at 37 °C for 2 days, the yeast colonies were scratched and transferred to NaCl solution of 19.6 mM with a conductivity of 140 µS/cm. Yeast cell suspensions for positive DEP experiment were prepared by transferring yeast colonies into NaCl solution of 0.28 mM with a conductivity of 2 µS/cm. Samples of dead yeast cell were prepared by heating the solution at 80 °C in water bath for 30 min. Bacterial viability kits (LIVE/DEAD BacLight, Invitrogen, CA, USA) were dropped into the suspension at a volume concentration of 0.1% to distinguish between live and dead yeast cells mixing at a ratio of 2:1. Before being spread onto agar plate, *E. coli* (cell strain K12) that express Green Fluorescent proteins by modifying at the chromosomal level were taken from stock at -20 °C with a toothpick. They carry a gene expressing GFPµ2 fused to lacZ and are controlled by the Lac repressor [231]. An expression of GFP was induced by the addition to Luteria broth of isopropylthio-β-galactoside (IPTG) at 1 µM concentration. Cultures were grown overnight at 37 °C in an incubator in Luteria broth (LB) medium. Then, *E. coli* were cultured on a LB agar plate at 37 °C for 12 h to get enough *E. coli* colonies which were then transferred into two NaCl solutions: (1) conductivity of 380 µS/cm (53.2 mM) for negative DEP (n-DEP), and (2) conductivity of 2 µS/cm (0.28 mM) for positive DEP (p-DEP).

6.2.3 Experimental setup

A function generator (HP 33120A) and an AC amplifier (Stereo Power Amplifier 216THX) were used to supply AC signals, and the waveform of the input signal was monitored by an oscilloscope (Tektronix TDS 2012B). A DC power supply – Topward DUAL-TRACKING DC power supply 6303D (Topward Electric Instruments Co., Ltd., Taiwan) was applied to provide an output voltage from 0 V to
30 V. The motions of organic particles and cells were monitored using an inverted optical microscope (Olympus IX71, Tokyo, Japan), and recorded by a CCD camera (Olympus DP73, Tokyo, Japan).

6.3 Results and discussion

6.3.1 Characteristics of designed electrode

![Simulation results of electric field intensity.](image)

Figure 6-3 Simulation results of electric field intensity. (a) Electric field intensity distribution of arrowhead 3D electrode at the middle plane between upper and lower electrode. The inset shows the schematic diagram of 3D electrode configuration. The potentials were set to be 1V and -1V for upper and lower electrode, respectively. The red and blue bar represents high and low electric field, respectively. The black lines indicate electrode edges. (b) Cross-sectional view of field distribution along the red dashed line in 5-3a. (c) Cross-sectional view of field distribution of planar electrode located on one side of microchannel.

The intensity of the electric field is a key factor in determining DEP related phenomena. In order to demonstrate the advantage of a 3D electrode fabricated by our method, a finite element simulation was performed using COMSOL 4.2 software. A 3D model, electrostatics and stationary analysis were selected for finite element simulation, the geometry was then defined using the parameters of the fabricated chip. Parallel arrowhead-shaped electrodes on both the top and bottom surfaces were constructed with a 30 µm channel in between. The electrode was set to be 40 µm wide. The view plane was set to be the slice in the middle of microchannel and parallel to the electrodes. Finally, the intensity of the electric field was plotted as the square of the electric field strength ($E^2$). Figure 6-3 is presented to illustrate the
variation in the intensity of the electric field whose gradient is directly related to the DEP force near the electrode region. A 3D simulation was performed for this arrowhead geometry when the electric potential of the upper electrode was set at 1 V and the potential of the lower electrode was set at -1 V. Then the middle plane was chosen to reveal the field intensity for the top view in Fig. 6-3a. The black lines represent the edges of the electrode. DEP force near the electrode region is directly related to the electric field gradient. We can see that the field intensity is almost uniform in the region between two electrodes and it decays very fast near the edges, which resulted in a DEP force perpendicular to the electrode. Figure 6-3b shows the cross-sectional view of the intensity of the electric field along the red dashed line in Fig. 6-3a. It is seen that particles will experience strong DEP force along the entire channel. To outline benefits brought by 3D design in comparison to 2D design, we calculated the electric field intensity and DEP force of 2D planar electrode and gave the result in Fig. 6-3C. DEP force was normalized by logarithm function with base of 10 to avoid large variation caused by the sharp corner. Owing to the symmetrical electrode configuration along channel depth, the electric field holds a much larger effective region for 3D electrodes compared with its counterpart—planar electrodes which generate DEP force only in a small area near the electrode and DEP force decreases rapidly away from the electrodes. In addition, particles will experience levitation force along the channel when they pass through the 2D planar electrodes, resulting in decrease of trapping efficiency.

Compared to other 2D planar electrode designs, the main advantages of this 3D electrode configuration include an increase in the trapping efficiency and a reduction of Joule heating [232] and electrolysis. Joule heating coming from the reaction of ions under an electric field is very strong for planar electrodes because a higher electric field has to be applied to balance the hydrodynamic force. Therefore, it is preferable for dielectrophoretic applications.

6.3.2 Particles and cells choice

The response of particles to different forces depends on their size, geometry, structure and content. In this study, we selected three particles/cells with different
properties, including polystyrene particles (10 µm in diameter), yeast cells (4-5 µm in diameter), and *E. coli* (0.5 µm in diameter). The structure of a eukaryotic yeast cell is different from prokaryotic *E. coli*, thus it responds differently to an electric field gradient. All three samples represent common entities encountered in colloidal systems and biological detections, therefore our technique may demonstrate its ability to manipulate different kinds of microparticles.

6.3.3 Capture of polystyrene particles

![Figure 6-4 Accumulation of 10 µm PS particles by applying an AC signal of 21 V at 50 kHz with field strength of 7×10⁶ V/m on the arrowhead electrodes. (a) t=0 s, (b) t=30 s, (c) t=60 s, and (d) t=90 s. The channel depth was 30 µm.](image)

The capability of this technique was first demonstrated by trapping fluorescent PS particles. The chip with a 30 µm deep channel and a maximum height of 2 mm between the inlet and outlet reservoirs was used to collect 10 µm PS particles at the tip of the electrode with an applied AC signal. The buffer solution with the same ion concentration as the sample solution was first used to infiltrate microfluidic channels. Then, 20 µl sample solution containing 10 µm fluorescent PS particles was added into the inlet reservoir with a pipette. Figure 6-4 illustrates the accumulation of...
particles in a single channel over time. It is seen that the number of particles trapped at the tip of arrowhead-shaped microelectrodes has increased from t=0 s (Fig. 6-4a) to t=90 s (Fig. 6-4d). The AC voltage applied between the upper and lower electrode is 21 Vp-p at a frequency of 50 kHz, which indicates that the maximum field strength between two electrodes is $7 \times 10^6$ V/m.

Figure 6-5 Optical images of the whole trapping region containing 13 channels in total and several individual channels indicated by red dashed squares. It shows that every channel could be used for trapping particles.

After 90 sec of trapping particles, optical images of individual trapping regions were taken and some of them are shown in Fig. 6-5. The one in the centre is the full view of the chip containing 13 channels in all. These channels could function together to trap particles, which reduces the processing time compared to a single channel. The radical design of the channel layout favoured sample solution loading. The vertices are indicated by the red-dashed squares. Florescent PS particles were trapped at the tips of the electrodes, where the gradient of the electric field reaches its maximum value. Note that the amplitude of the AC signal varies according to the velocity driven by the difference in height of the solution in the inlet and outlet reservoirs. A
higher voltage was applied in this case to ensure that all the particles were trapped when they travel at the maximum velocity.

6.3.4 Capture and release of yeast cells

Figure 6-6 Trapping yeast cells in four channels to demonstrate the capability of the proposed device for bio-particle pre-concentration. Pictures were taken after applying trapping signal for 2 minutes. The applied AC signal was 10 V at 50 kHz and the channel height was 15 \( \mu \text{m} \). The medium conductivity was adjusted to 140 \( \mu \text{S/cm} \) for collection of yeast cells by negative DEP.

Trapping bio-particles was first demonstrated by collecting yeast cells in our device. Trapping cells is important for a variety of applications, including drug testing, toxicology and basic cell biology. Yeast cells experienced negative DEP in a buffer solution with conductivity of 140 \( \mu \text{S/cm} \) at 50 kHz according to the relatively complex permittivities of cells and the suspending medium, which determines the CM factor. 20 \( \mu \text{l} \) yeast suspensions were added into the inlet reservoir by a pipette. During the collecting process, the magnitude of the driving signal was increased from 0 to 15 Vp-p, and the frequency was kept the same as that in the PS particles trapping experiment to minimise the power supply for future fabrication of a portable diagnostic kit. It was found that 10 Vp-p AC signal at frequency of 50 kHz was enough to trap almost all the cells in the channel. When the signal was imposed on the electrodes, cells began to stop near the vertices of the inner electrodes, where the electric field gradient was the highest.
Collecting yeast cells by a negative DEP was shown in Fig. 6-6, where the pictures taken after running the device for 2 min are presented. To analyse different conditions faced in detecting pathogens, we tested our approach to deal with media of different conductivities. Cells will exhibit positive DEP in the medium with lower conductivity (i.e. 2 μS/cm in our experiment). In order to demonstrate the whole process of manipulating yeast cells, AC and DC signals were applied to trap and release of cells, respectively. The method comprises two steps: (1) yeast cells were isolated from the sample solution and trapped between the upper and lower electrodes by a positive DEP when an AC electric field was applied; and (2) yeast cells were released by combined electrophoretic and electroosmotic forces, when a DC electric field was applied. Figure 6-7 shows the pre-concentration and release of yeast cells. Yeast cells were first suspended in a solution having a lower conductivity – 0.28 mM NaCl solution (2 μS/cm), followed by loading 10 μl sample solutions into the inlet reservoir. Figure 6-7a and 6-7b are two snapshot pictures taken at t=0 s and t=10 s, respectively. We can see that the entire channel region was divided into two sub-domains by electrodes: the one on the left distributes cells while the other does not. When a 5 Vp-p signal at 50 kHz was applied between the upper and lower electrodes, yeast cells tend to aggregate at the highest electric field region because yeast cells are more polarisable than the suspending medium (positive DEP).

After collecting yeast cells for 55 sec, the solutions in the inlet and outlet were at the same level and there was no hydrodynamic force in the channels. Then the AC signal was turned off and a DC electric field was applied in the direction from inlet to outlet. Due to an electrokinetic (the combined electrophoretic and electroosmotic) force under an electric field of 700 V/m, the yeast cells were moving towards the outlet (Figs. 6-7c and 6-7d). The electric field also assisted in directing cells back to the inlet reservoir by switching the direction of the field from outlet to inlet, as shown in Figs. 6-7e and 6-7f. After moving to the outlet or the inlet, the yeast cells could be collected by pipette for further analysis at a much higher concentration.
Figure 6-7 Capture and release of yeast cells. (a, b) Capture of yeast cells by positive DEP at medium conductivity of 2 µS/cm. Yeast cells were collected between upper electrode and lower electrode where the electric field strength was highest. Flow was driving by difference of water level between inlet and outlet. The applied AC signal was 5 V at 50 kHz and the channel height was 15 µm. (c – f) Release of yeast cells by applying DC field of 700 V/m after the collection of yeast cells for 55 sec. Yeast cells travelled in the same direction as that of applied field.

6.3.5 Capture and release of *E.coli*

Detecting pathogens is a prerequisite precursor in the prevention and diagnosis of infectious diseases, drug discovery, clinical research, biological warfare, as well as safe food administration. *E. coli* is a type of bacteria that naturally occurs in the intestinal tracts of human and warm-blooded animals to produce vitamins, but one particular pathogenic strain, *E. coli* O157:H7, produces toxins that damage the lining of the intestine, causing gastroenteritis, anaemia and serious complications such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). The detection and identification of foodborne pathogens and other contaminants in raw food materials in processing and assembly lines, ports of entry and in drinking water supplies and hospitals, relies largely on conventional culturing techniques. But
culturing is always a high cost, time-consuming process that requires bulky equipment, so it is not suitable for detecting and analysis on-site pathogens, whereas a deployable bio-sensor is portable, easy to use, reagent free, and fast processing.

Figure 6-8 Collection and release of *E. coli*. (a, b) Collection of *E. coli* by negative DEP at medium conductivity of 380 $\mu$S/cm at $t=0$ s and $t=120$ s, respectively. The AC signal of 15 V at 50 kHz was applied within the channel of 15 $\mu$m in depth; (c, d) Collection of *E. coli* by positive DEP at medium conductivity of 2 $\mu$S/cm and release of *E. coli* after trapping for 1 minute. The applied AC signal for trapping was 10V at 50 kHz, and the applied DC field for releasing was 700 V/m.

*E. coli* are difficult to collect due to their small size and relatively small DEP force. 3D electrode configuration may be a suitable approach for collecting *E. coli* because it can produce a region of a high electric field along the whole height of the channel, as discussed in the simulation part. Here we demonstrate the capture of *E. coli* by n-DEP and p-DEP. A 10 $\mu$l sample solution was loaded into the inlet reservoir for trapping *E. coli*, the concentration of NaCl solution was adjusted to 53.2 mM (380 $\mu$S/cm) for n-DEP trapping and 0.28 mM (2 $\mu$S/cm) for p-DEP trapping. The AC signal applied was 15 Vp-p at 50 kHz and 10 Vp-p at 50 kHz for n-DEP and p-DEP respectively, and the channel was 15 $\mu$m deep. Figures 6-8a and 6-8b with a green background showed that *E. coli* moving from left to right was trapped by n-DEP at
the tips of the arrowhead microelectrodes, where the electric field gradient was the largest. Figure 6-8c with a brown background depicted the trapping of *E. coli* by p-DEP. *E. coli* was seen collecting along the edges of the upper and lower electrodes. By removing the AC field while imposing a DC field of 700 V/m, *E. coli* was released from the surface of the electrode (see Fig. 6-8d).

6.3.6 Separation of live and dead yeast cells

Prior to the analysis of a biological cell at the molecular level like PCR, and immunoassay, separation of different types of cells is required. Separating live and dead cells is much more difficult because they have similar content and dimensions. We assumed that our device could perform most separation experiments after successfully separating live and dead yeast cells. This process is critical at the early diagnostic stage for efficient drug screening [233]. Cell viability is also a determination of live or dead cells, based on a total cell sample. Viability measurements are used to determine whether the cancer cells are alive and to assess transplant rejection. These tests also evaluate the effectiveness of a pesticide or insecticide, and assess the environmental damage due to toxins.

In order to characterise the DEP response of live and dead yeast cells under an AC electric field, numerical calculation of the real part of the Clausius-Mossotti (CM) factor \( \text{Re}[K(\omega)] \) was performed by FORTRAN (Compaq visual fortran 6). According to Eq. 2-2, the CM factor is determined by the relative permittivities of the particle and the suspending medium, and the frequency of the external electric field. Three shell models of cell wall, cytoplasm and cell nucleus, developed by Huang et al [234], was used to calculate the CM factor. The geometrical and electrical properties of a yeast cell were taken from literature [172]. Briefly, the following properties of live and dead yeast cells were used based on optimised data: radii of 2.5 μm for the wall, 2.36 μm for the cytoplasm, and 2.35 μm for the nucleus; with conductivities of 140 μS/cm for the wall of a live cell, 15 μS/cm for the wall of a dead cell, 0.0025 μS/cm for the cytoplasm of a live cell, 1.6 μS/cm for the cytoplasm of a dead cell, 2000 μS/cm for the nucleus of a live cell, 140 μS/cm for the nucleus of a dead cell; and relative permittivity of 60 for the wall, 6 for the cytoplasm, 50 for the nucleus of a live cell, 78 for the nucleus of a dead cell. The
conductivity and permittivity of the suspending medium was set to 140 μS/cm and 78 respectively.

Figure 6-9 The real part of CM factor as a function of electric field frequency for live (red line) and dead (blue line) yeast cells at medium conductivity of 140 μS/cm. Positive and negative value of the real part of CM factor represents yeast cells under p-DEP and n-DEP, respectively. The violet dashed line indicates the working frequency for separating them due to the difference in DEP forces. The inset is the optical microscope image of mixture of live and dead yeast cells dyed by different two colours.

Figure 6-9 depicts the variation of the real part of the CM factor with respect to the frequency of the external AC fields applied for live (red) and dead (blue) yeast cells. The upper part of this graph above the transverse line (Re[K(ω)] = 0) indicates a positive CM factor, resulting in a positive DEP force, while the lower part indicates a negative CM factor, resulting in a negative DEP force. The violet dashed line represents the working frequency for separation experiments. The inset was an optical image of live and dead yeast cells after staining with a bacterial viability kit described in the previous section on ‘sample preparation’. Live yeast cells are marked with green, while dead yeast cells tend to exhibit an orange colour. The figure shows that the value of the real part of the CM factor at a working frequency of 50 kHz is different for live and dead yeast cells, giving rise to different DEP
forces (proportional to the real part of the CM factor, Eq. 2-3) for separating yeast cells based on vitality.

The electrophoretic force is identical for live and dead cells, which implies that a DC signal cannot be used to separate live and dead cells. However, complex permittivity depends on a cell’s viability and thus induces different dielectrophoretic forces and therefore, DEP could be applied to separate cells according to their vitality. Here, both DC and AC signals were applied for separation by taking advantage of identical DC electrophoretic, electroosmotic forces and different AC dielectrophoretic forces.

![Figure 6-10 The separation of live and dead yeast cells. Live yeast cells experiencing larger DEP force were trapped at the tip of arrowhead electrode, while dead yeast cells exhibiting lower DEP response were move towards the outlet under electrokinetic force. The applied AC signal was 3 V at 50 kHz and DC electric field was 1.0×10³ V/m. The channel height is 30 µm. The pictures were taken with time interval of 10 sec.](image)

To separate cells, we punched a hole at the channel end for platinum (Pt) wires. The reason why Pt wires were utilised to replace the existing planar electrodes is that they could generate an almost uniform electric field along the height of the channel. The water level at the inlet and outlet were balanced carefully to set to be the same, and then a 20 µl buffer solution without cells was loaded into the outlet reservoir, followed by the injection of 15 µl yeast suspensions with live and dead cells into the inlet reservoir. After the water had reached the same level again, a DC signal with an electric field strength of 1.0×10³ V/m was applied and an AC signal with a frequency
of 50 kHz at which both live cells and dead cells experienced a negative DEP was imposed on the upper and lower electrodes. When cells passed through the converging electrodes, the amplitude of the AC signal was adjusted from 0 V to 10 V. It was found that 3 Vp-p was enough to separate them under an apparent cell velocity of around 15 μm/s. Figure 6-10 shows the process of separation via snapshot pictures taken at 10 sec intervals. Note that live yeast cells experiencing larger negative DEP forces were trapped at the edge of the converging electrodes, as predicted in Fig. 6-9, whereas the dead yeast cells travelled towards the outlets. The separation efficiency was higher than 90% after compiling the statistics of live and dead yeast cells in the outlet reservoir.

6.4 Conclusions

We have demonstrated and tested the performance of a densely packed dielectrophoretic device with simple operation requirements for a sample pretreatment that included capture, concentration, and separation of particles and cells. The proposed microdevice consists of 13 individual microchannels in a radical direction and 3D microelectrode structures patterned on both the top and bottom surfaces of microchannels to achieve on-chip manipulation of particles in a high-throughput manner. Another contribution relies on the combination of DEP with other electrokietic forces for the whole process: hydrodynamic force was used to generate fluid flow, a DEP force was applied to trap particles, and combined electrophoretic and electroosmotic forces were used to redirect and release particles. Parameters such as the pressure inside microchannels, dielectrophoretic, electrophoretic and electroosmotic forces are adjustable according to the properties of different sample matrices. The performance of the portable, integrated, and pump-free microdevice was demonstrated by trapping PS particles, capturing and releasing yeast cells and *E. coli*, and sorting live and dead yeast cells. The ability of the device to distinguish an electrokinetic force from a dielectrophoretic force enables convenient tuning of applied electric fields and suspending medium to isolate different targeted particles. Our system was successfully tested with cells and particles of properties representative of a disparity of biological samples that may contain many components. The separation of yeast cells with different vitality shows
that it is capable of discriminating between cells that have close properties. We have thus proven that the device has the potential to serve as a pathogen detector and analyser for similar applications to flow cytometry, and sample preparation for further analysis.
In this chapter, we present a waved microchannel for continuous focusing of microparticles and cells using negative direct current (DC) dielectrophoresis. The waved channel consists of consecutive curved S-shaped channels in series to generate the electric field gradient required for the dielectrophoretic effect. When particles move electrokinetically through the channel, the negative dielectrophoretic forces alternate directions within two adjacent semi-circular microchannels, which lead to a focused continuous-flow stream along the centreline of the channel. Both the experimentally observed and numerically simulated results of this focusing performance have reported, and they coincide acceptably in proportion to the specified dimensions (i.e. the inlet and outlet of the waved channel). How the electric field applied, particle size, and medium concentration affect the performance of the waved microchannel were studied by focusing various sized polystyrene microparticles. As an application in the field of biology, the focusing of yeast cells in the waved microchannel was tested. This waved microchannel shows a great potential for micro-flow cytometry applications and is expected to be widely used before different processing steps in Lab-on-a-chip devices with integrated functions.

7.1 Introduction

As mentioned before in Chapter 2, there are two main strategies to generate the inhomogeneous electric fields required for a DEP effect: microelectrodes and insulating topographical structures. Due to the elimination of in-channel microelectrodes, insulator-based DEP devices have advantages over electrode-based DEP microdevices, in that they are easier to fabricate, mechanically robust and chemically inert, there is no gas evolution due to electrolysis, and fouling is reduced. Researchers have generated spatial non-uniformities using in-channel insulating obstacles, including posts, a pair of oil droplets, and rectangular hurdles (or constriction) within the microchannel to focus microparticles. However, these
designs suffer from a locally amplified electric field, significant shear stress, and trans-membrane voltage and Joule heating which cause adverse effects on the biological samples. Furthermore, the probability of the device fouling due to particle clogging or adhesion at the manufactured obstacles in the channels is relatively high. These drawbacks are relieved or even overcome by curving the microfluidic channel (i.e. a serpentine microchannel with 90° turns and spiral microchannel) to create non-uniformities of the electric field. However, the area where an electric gradient can be generated in a serpentine microchannel is limited, that is only within the corner of channel turns, while the electric field is uniformly distributed in the straight section of the microchannel. In a spiral microchannel, it is necessary that either the applied DC electric field is sufficiently large (~1000 V) or the curved channel is sufficiently long (5 cm), especially for effectively focusing smaller particles. Moreover, this design is more sensitive to contaminations or particle adhesion on the wall of the channel, as particles are deflected and focused towards the outer sidewall of the microchannel.

In this chapter, we propose using a waved microchannel to focus microparticles and cells using negative DC dielectrophoresis in a continuous flow. This design results in continuous pumping and focusing by taking advantage of the electrokinetic and dielectrophoretic effects which are responsible for the streamwise transport of particles and their cross-stream deflection, respectively. The overall result is the movement of particles in a confined and concentrated stream along the centreline of the channel at the outlet of the curved section. In comparison with the serpentine channel with 90° turns, this design extends the region creating the DEP effect to the full width of the waved microchannel, which gives a greater control over particle motion. More importantly, the stagnation regions and locally amplified electric field due to sharp turns are eliminated, which protects the sidewalls from particle adhesion to sidewalls and damage to cell, respectively. This design also has advantages over spiral channels, in that it focuses particles in the centre of the channel, and the magnitude of applied DC fields and the total length of curved channel required for desirable deflection are reduced. In addition, the proposed waved microchannel is more flexible in its structural design, in that the large/ small radius of the curvature, length and width of the microchannel could be optimised depending on the focusing
performance required, even the entire microchannel could be wound up to reduce its footprint. The effects of an applied DC electric field, particle size and medium concentration on the focusing performance are examined. Moreover, the functionality of the device was demonstrated by focusing yeast cells continuously and effectively. Numerical simulations were also performed to predict and verify the focusing behaviour of both microparticles and cells, the results of which indicate a reasonable agreement with the experimentally obtained particle trajectories.

### 7.2 Theory and mechanism

As mentioned in Chapter 2, suspended particles experience an electrokinetic (EK) effect in DC electric fields, which is a combination of fluid electroosmosis (EO) and particle electrophoresis (EP). The resulting motion is determined by the electrokinetic velocity written as

\[
\mathbf{u}_{\text{EK}} = \mathbf{u}_{\text{EO}} + \mathbf{u}_{\text{EP}} = \mu_{\text{EO}} \mathbf{E} + \mu_{\text{EP}} \mathbf{E} = (\mu_{\text{EO}} - \mu_{\text{EP}}) \mathbf{E} = \mathbf{u}_E
\]  

(7-1)

where \( \mu_{\text{EK}} \) is electrokinetic mobility, \( \mu_{\text{EO}} = -\varepsilon_m \zeta_w / \eta \), and \( \mu_{\text{EP}} = -\varepsilon_m \zeta_p / \eta \) are electroosmotic and electrophoretic mobility, respectively. \( \varepsilon_m \) is the permittivity of the suspending medium, while \( \eta \) is the dynamic viscosity of the suspending medium. \( \zeta_w \) and \( \zeta_p \) represent the zeta potentials of the channel wall and particle, respectively, and \( \mathbf{E} \) is the electric field vector. Equation 7-1 shows that the electrokinetic velocity is linearly proportional to the local electric field and directional flow in the field lines.

If the electric field is non-uniform, the motion of a suspended particle is also affected by dielectrophoretic force, which results in dielectrophoretic velocity, \( \mathbf{u}_{\text{DEP}} \), given by

\[
\mathbf{u}_{\text{DEP}} = \mu_{\text{DEP}} (\mathbf{E} \cdot \nabla \mathbf{E}) = (\varepsilon_m d^2 f_{\text{CM}} / 6\eta) \cdot (\mathbf{E} \cdot \nabla \mathbf{E})
\]  

(7-2)

where \( d \) is the particle diameter, \( f_{\text{CM}} = (\sigma_p - \sigma_m) / (\sigma_p + 2\sigma_m) \) is known as the Clausius-Mossotti (CM) factor, \( \sigma_p \) and \( \sigma_m \) are the electric conductivities of particle and the suspending medium, respectively.
Unlike traditional electrode-based DEP devices, where non-uniform electric field required for the DEP effect is created by applying an AC electric field via the metallic microelectrodes embedded inside the microfluidic channel, insulator-based DEP devices create spatial non-uniformities of electric field by insulating topographic structures (i.e. posts, hurdles, constriction in channels, and curved channels), and a DC or DC-biased AC electric field is applied via remote electrodes outside the microchannel. In the case of insulator-based DEP devices exploiting channel geometrical modification, the applied electric field is confined within the electrically non-conducting channel walls, thus the magnitude and gradient of the local electric field is defined by the geometry of the channel. A refined design of channel geometry contributes to the desired distribution of the electric field as well as associated effects, such as electroosmotic flow (EOF), electrophoresis (EP) and dielectrophoresis (DEP). In this study, we proposed a waved microchannel that consists of curved S-shaped channels in series to continuously focus particles into the centre region of the channel. A schematic illustration of the focusing mechanism is shown in Fig. 7-1. The electric field lines (or equivalently the streamlines with arrows indicating the direction) and the contours showing the strength of the electric field (the darker the stronger) in one S-shaped channel that consists of a lower counter-clockwise semi-circular channel, followed by an upper clockwise semi-circular channel are represented. It is found here that the uniformity of the electric field is disturbed by curving the channel wall, which results in the local electric fields obtaining their maximum and minimum values at the inner and outer wall of each semi-circular channel, respectively. Consider a particle passing through the curved channel by the combined effect of EOF and EP, where dielectrophoretic forces are exerted onto the particle all along its movement.
Figure 7-1 Illustration of the negative dielectrophoretic focusing of particles in a waved microchannel. The distribution of the electric-field lines and contours of the electric-field strength, $E$, within the microchannel are shown (the darker region has a stronger electric field). Particles moving through the waved channel electrokinetically are exposed to negative DEP forces.

In the proposed curved S-shaped channels, the particle experiencing negative DEP force will be deflected toward the region with the lowest local electric field, namely the outer wall of each semi-circular microchannel. As the inner and outer wall alternate between the counter-clockwise and clockwise semi-circular microchannels, the negative dielectrophoretic force exerting on the particle (always from the inner wall to the centreline of the channel) also alternates directions as shown in Fig. 7-1. Moreover, the repulsive negative DEP force is stronger if the particle is closer to the inner wall where the electric field gradient is larger. At appropriate and moderate electric fields, the particles that electrokinetically move through the waved microchannel will be gradually deflected towards the centre region of the channel under the influence of such a negative DEP force, leading to a focused particle stream along the centreline of the channel at the exit region. However, if the applied electric field is very high, the induced negative DEP forces will be so strong that deflect particles past the centreline to the outer wall of each semi-circular microchannel. Therefore, the particles will be forced to bounce between the sidewalls until they finally move out of the waved section close to one sidewall. This phenomenon has also been demonstrated by Church et al [208] for separating particles based on size in a serpentine microchannel.

Three features are combined in the design of a waved microchannel to control the motion of particles using negative DEP forces: (1) a curved channel (or semi-circular channel) generates an electric field gradient across the streamline when a DC electric field is applied; (2) an S-shaped channel causes the induced negative DEP forces alternating directions between two adjacent semi-circular channels; (3) a waved channel accumulates the focusing effect of negative dielectrophoretic forces in each S-shaped section.
7.3 Materials and methods

7.3.1 Device fabrication

The microchannels patterned in polydimethylsiloxane (PDMS) were fabricated using standard photo- and soft-lithography techniques. The layout of the microdevice was designed and drawn using AutoCAD (Autodesk Inc., USA), and printed onto a transparent plastic thin film with a dark field at a resolution of 20,000 dpi to serve as the mask. Photoresist (SU-8 2025, MicroChem Corp., Newton, MA) was spun on a clean 4-inch silicon wafer in a two-step coating cycle (with the setting of 800 rpm for 15 sec and 2000 rpm for 30 sec), which results in a film that is nominally 30 µm thick. After relaxing in air at room temperature for 2 h to level the photoresist, a two-step soft bake (65 °C for 10 min and 95 °C for 5 min) was carried out on the hotplates. Contact lithography was then conducted by 25-sec exposure to UV light through the printed mask using a mask aligner system (ABM, San Jose, CA). Following another two-step hard bake (65 °C for 5 min and 95 °C for 6 min), the wafer was immersed and gently vibrated in a solution of SU-8 developer for 4 min and rinsed with isopropyl alcohol (IPA), leaving a positive replica (exposed photoresist) of the microdevice pattern on the wafer. After being developed, the SU-8 master was treated by trichlorosilane (97%, Sigma-Aldrich, USA) in an encapsulated chamber under a pressure of about -0.5 bar to deposit a mono-layer of silane onto the surface. The purpose of this treatment was to reduce the adhesion of polydimethylsiloxane (PDMS) to the SU-8 master surface.

PDMS gel prepared by mixing Sylgard 184 and its curing agent (Dow Corning, Midland, MI) at a weight ratio of 10:1 was poured over the master, and then placed inside a vacuum pump to remove bubbles. Following degassing, it was put into an oven at 70 °C for 1.5 h to solidify the PDMS gel, and then the cured negative PDMS cast containing the microdevice pattern was cut and removed from the master. Two holes were punched through the PDMS cast to serve as the inlet and outlet reservoirs. Immediately after 2 minutes of oxygen plasma treatment in a plasma cleaner (PDC-002, Harrick Plasma, Ossining, NY), the channel side of the PDMS and a clean glass slide were bonded together which resulted in an enclosed microchannel. Once sealed, the channel was filled with a working buffer by capillary action to maintain its surface properties.
Figure 7-2 Photograph of the microfluidic chip used in the experiment for particle focusing. The inset indicates the structure and dimensions of the waved microchannel.

Figure 7-2 shows the structure and dimensions of the microfluidic device consisting of a waved microchannel at the centre, inlet/outlet reservoirs at each end, and two straight connecting microchannels. The device was filled with oil dyed red to ensure clear demonstration. The microchannel has a uniform width and depth of 100 µm and 30 µm, respectively. The 1 cm-long waved section at the centre is consists of 17 units of identical S-shaped channels in series, each of which has a small and large curvature of 100 µm and 200 µm, respectively, as shown in the inset. The total length of the microchannel (including the straight section) is 2 cm, and the diameter of the two reservoirs is 6 mm.

7.3.2 Preparation of the microparticle and yeast cells

Polystyrene particles 10 and 15 µm in diameter (Fluosphere, Invotrogen, CA, USA) were used in our experiments since these sizes are comparable with red and white blood cells. Both particles, which were originally suspended in a solution of 0.15 M NaCl, were diluted by deionized (DI) water 150 times. 5 µm-diameter polystyrene
microparticles (Singma-Aldrich, USA) that originally suspended in pure water were re-suspended in either 1 or 10 mM NaCl solution, and the final concentration was around $10^6$ beads per millilitre. Baker’s yeast cells (*Saccharomyces cerevisiae*) were cultured at 37°C in YEP broth (MP Biomedicals, LLC.). After about 24h, the cells were diluted with DI water and then re-suspended in a phosphate buffer solution (PBS, pH 7.0, Radiometer Analytical A/S, Denmark) diluted 5 times at a concentration of about $10^6$ cells per millilitre. The yeast cells were around 5 µm in diameter on average. The suspensions of particles and yeast cells were introduced into the inlet reservoir using a pipette, while the outlet reservoir was filled with the corresponding working solution.

### 7.3.3 Experimental setup

The electric field was generated by a DC power supply (SL10P300/200, Spellman High Voltage Electronics Corp., Hauppauge, NY), and was used to drive the fluid flow via two platinum electrodes submerged into each reservoir. The motion of particles through the waved microchannel was monitored by an inverted microscope (Olympus IX71, Tokyo, Japan) and recorded by a CCD camera (DXC-390P, Sony, Japan). The camera was run in the video mode at 25 frames per second. The digital images had a resolution of 576 X 720 pixels, which represented an approximated 1 mm-long channel for a 10X objective. All the videos and images were post-processed by MATLAB R2011b (Mathworks Inc., Natick, MA), and the images showing the trajectory of the particles were obtained by superimposing consecutive images converted from videos.

### 7.4 Modelling

In order to study and predict the effect of negative DC DEP on the particles/cells transported through the waved microchannel, a numerical model first developed by Kang et al [192] was used, this has also been applied by other researchers to simulate the dielectrophoretic (i.e. DC, DC-biased AC) focusing in different channel structures [95, 200, 209]. A correction factor, $c$, was introduced to account for the perturbation of particle size, and coupled particle-particle interaction, etc. on the dielectrophoretic velocity, which decreased with the increase in particle size, and
depends on the channel geometry, but is insensitive to the electric field applied [192, 200, 209]. By using this correction factor, the particle velocity can be rewritten as

\[ u_p = u_{\text{EK}} + c u_{\text{DEP}} = \mu_{\text{EK}} E + c\mu_{\text{DEP}}(E \cdot \nabla E) \]  

In the modelling, the electrokinetic mobility, \( \mu_{\text{EK}} \), was determined by Eq. 7-1 with zeta potential values obtained from references [236-239]. The dynamic viscosity \( \eta = 0.9 \times 10^{-3} \text{ kg/(m} \cdot \text{s)} \), and permittivity, \( \varepsilon_m = 6.9 \times 10^{-10} \text{ C/(m} \cdot \text{m)} \) for pure water at 25°C were used to calculate the electrokinetic and dielectrophoretic mobility by Eqns. 7-1 and 7-2. The CM factor, \( f_{\text{CM}} \), which depends on the electric conductivities of the particle/cell and the suspending medium, was set at -0.5. The reason for this is the electrical conductivities of polystyrene particles and live yeast cells at a DC electric field are much smaller than the suspending media used in our experiments (i.e. 1 or 10 mM NaCl solution, and PBS). The velocity in Eq. 4 was used in a streamline function in the electrostatics module of COMSOL 4.0 (COMSOL Inc., Burlington, MA) to simulate the particle trajectories. As the dielectrophoretic focusing of particles remained the same along the depth of the channel, a 2D model was used. The boundary conditions include the DC voltages applied at the ends of the microchannel, and the insulating condition on the sidewall of the channel and the edge of the hurdle. Assuming that the medium of constant electrical conductivity, and the electric potential field is governed by the Laplace equation expressed as \( \nabla^2 \varphi = 0 \), and the electric field is obtained by calculating the derivative of the electric potential \( E = -\nabla \varphi \) (where \( E \) and \( \varphi \) are the electric field strength and electric potential, respectively). At the inlet, particles were assumed massless and distributed uniformly along the width of the channel. The correction factor, \( c \), was determined by matching the predicted particle trajectories to the width of focused stream in the exit region in the experiment.

### 7.5 Results and discussion

Following the experimental and numerical approach presented previously, a parametric study was performed to understand how well the particles focussed in the proposed waved microchannel. The motion of particles in insulator-based DEP microfluidic devices depends on the three effects of EP, EOF, and DEP. Thus, the effects of a variety of parameters, including the applied electric field, particle size,
the zeta potentials of particles and suspending medium on the motion of particles were studied via both experiments and simulations. In addition, the focusing of yeast cells under an electric field with magnitudes of 100 and 200 V/cm was examined.

7.5.1 Effect of electric fields

Experimentally observed (left column) and numerically predicted (right column) results of focusing 10 µm particles in the waved microchannel at various applied electric fields of 100 (second row), 200 (third row) and 300 V/cm (bottom row).

Figure 7-3 Experimentally observed (left column) and numerically predicted (right column) results of focusing 10 µm particles in the waved microchannel at various applied electric fields of 100 (second row), 200 (third row) and 300 V/cm (bottom row).

Experimentally observed trajectories of the 10 µm particles under the effect of applied DC electric fields are presented in Figs. 7-3a-d. In order to demonstrate the effect of focusing as the particle is transport through the waved channel, the superimposed particle image at the inlet of the waved channel (Fig. 7-3a) is included, from which it was found that particles were not focused and almost covered the entire width of the straight microchannel before entering the waved channel.
Figures 7-3b-d are superimposed images of streams of focused particles at the outlet of the waved channel which were obtained under nominal DC electric fields which varied from (b) 100 to (c) 200 and (d) 300 V/cm, and which correspond to the actual applied potentials across a 2-cm-long microchannel of 200, 400, and 600 V, respectively. In every electric field, the particles were focused to move in a tight stream along the centreline of the channel at the outlet of the waved microchannel. By comparing the width of the focused stream, it is obvious that 10 µm particles obtained a better focusing performance when the electric field magnitude is increased, as the width of the stream becomes narrower at the outlet. This was mainly due to the repulsive dielectrophoretic motion that directs the particles away from the inner wall to the central region of the channel. According to Eqns. 7-1 and 7-3, electrokinetic motion is linearly proportional to the electric field, while dielectrophoretic motion depends on the gradient of the square of electric field, thus dielectrophoretic motion grows faster than electrokinetic motion as the magnitude of the electric field increases, which helps focus the particles better.

Figures 7-3e-h shows the numerically predicted trajectories of 10 µm particles at the inlet and outlet of the waved section in the same conditions as those in the experiments. In the simulations, the zeta potential values of the particles and PDMS-glass channel wall were set to -33 and – 89 mV, respectively [238, 239]. The correction factor was set at 0.5 by matching the width of the simulated stream with the experimentally obtained result at 200 V/cm, which remained constant in every case. In general, the simulated trajectories show a good agreement with the particle focusing behaviour obtained experimentally.
7.5.2 Effect of particle size

Figure 7-4 Experimentally observed (left column) and numerically predicted (right column) results of focusing 15 µm particles in the waved microchannel at various applied electric fields of 100 (top), and 200 V/cm (bottom).

Figure 7-5 The effects of electric field and particle size on the particle focusing performance. Both the experimental and numerical values of the measured width of the focused particle stream at the outlet are shown.

In order to study how particle size affects focusing, the negative dielectrophoretic focusing of 15 µm particles through the waved channel was examined. The left column of Fig. 7-4 shows the experimentally obtained superimposed images of focused particles at the outlet of the waved channel under a nominal electric field of
(a) 100 and (b) 200 V/cm. The width of the focused particle stream decreases with an increase in the electric field applied, indicating that 15 µm particles focused better when the electric field was higher, which was similar to the 10 µm particles. Additionally, focusing larger particles is more effective than smaller particles. This was demonstrated by comparing the width of the stream of focused 10 and 15 µm particles under the same conditions (see Fig. 7-5): the experimental and numerical values of the width of the focused stream of 15 µm particles were smaller than the 10 µm particles in all three different applied electric fields. As shown by Eq. 2-3, the DEP force responsible for transposing the particles cross-stream depends on the cube of the particle radius. In comparison with smaller particles, larger particles experiencing larger repulsive negative DEP forces tend to be deflected further towards the centre of the channel, which results in a narrower focused stream along the centreline of the channel. Figure 7-5 shows that focusing with 15 µm particles at applied electric field strength of 200 and 300 V/cm (the measured stream widths were around 20 and 16 µm, respectively) was excellent. However, the numerical results of measured stream widths were narrower (less than 15 µm). This discrepancy may be attributed to neglect the effect of particle size. The numerically predicted trajectories for 15 µm particles in the same experimental conditions are depicted in Figs. 7-4c and 7-4d. The zeta potentials of the 15 µm particles and wall with values of -33 and – 89 mV, respectively, were the same as the 10 µm particles given before. The correction factor was set at 0.4.
7.5.3 Effect of medium concentration

Figure 7-6 Experimentally observed (left column) and numerically predicted (right column) results of focusing 5 µm particles in the waved microchannel in the medium of 1 (top) and 10 mM (bottom) NaCl solution.

Figures 7-6a and 7-6b compare the 5 µm particles focused in the waved microchannel with two different medium solutions: (a) 1 and (b) 10 mM NaCl solution under the same applied electric field of 200 V/cm. It was observed that increasing the medium concentration improves the focusing, because the width of the stream of particles in 10 mM NaCl solution is narrower than in the 1 mM solution. This was mainly due to the difference in electroosmotic mobility on the overall electrokintic mobility of particles: the higher the ionic strength of the suspending medium, the more the double layer is compressed, so the lower the resulting electroosmotic flow [240]. This increase in the concentration of medium reduces the electroosmotic flow, which in turn decreases the electrokinetic mobility. At a lower electrokinetic velocity, the time for the DEP force to deflect particles as they progress through the waved channel is longer and therefore the focusing performance is enhanced.

In the simulations to predict the trajectories of focused particles, the zeta potential values of the microchannel walls in 1 and 10 mM NaCl buffer solutions were set at -89 and -54 mV, respectively [239]. The correct factor, $c$, was determined by matching the simulated results to experimental ones, the value of which was fixed at
0.8. The numerically predicted cell trajectories (Figs. 7-6c and 7-6d) are in reasonable agreement with the experimentally obtained superimposed images (Figs. 7-6a and 7-6b) for both concentrations of medium.

7.5.4 Focusing of yeast cells

![Diagram](image)

Figure 7-7 Experimentally observed (left column) and numerically predicted (right column) results of focusing yeast cells in the waved microchannel at various applied electric fields of 100 (middle row) and 200 V/cm (bottom row).

The focusing of yeast cells suspended in 0.2X PBS in the waved channel at different applied electric fields was studied. Both the experimentally observed (a-c) and numerically predicted (d-f) images under two different nominal electric fields with a magnitude of 100 (middle row) and 200 V/cm (bottom row) are presented in Fig. 7-7. In both conditions, the yeast cells that were not initially focused and almost uniformly distributed (covering almost the channel width) at the inlet of the waved channel (Fig. 7-7a) were moved into focused streams along the centreline of the channel at the exit region. By comparing the width of the stream within the straight section at the outlet, it was found that the cell focusing improved with the rise in the field magnitude. This expected trend is the same as that obtained previously for the 10 and 15 µm particles, and because of a faster growing dielectrophoretic motion as
well. The simulated results in the right column of Fig. 7-7 show a reasonab
agreement with the superimposed images obtained experimentally, where the zeta
potentials of the channel wall and yeast cells were set at -46 and -16 mV,
respectively [236, 237], and the correction factor was set at 0.8 in both conditions.

In the experiments of focusing the yeast cell, DC electric fields not stronger than 200
V/cm were applied because of the concerns about Joule heating of the buffer solution
and shear stress on the cell membrane associated with high DC voltage, both of
which may destroy the yeast cells. The viability of cell was examined using a
methylene blue (MB) staining method where living cells can produce the enzyme
which breaks down methylene blue and leaves the cells colourless, while non-viable
cells cannot produce this enzyme and appeared to be coloured. By staining samples
of the yeast cells from both the inlet and outlet reservoirs, it was found that more
than 85% of them were still alive after the experiment was conducted with the
highest applied DC electric field of 200 V/cm.

7.6 Conclusions

We described a waved microchannel which could continuously and effectively
focusing polystyrene microparticles and yeast cells into a confined and concentrated
stream along the centreline of the channel. This design utilises the cross-stream
negative dieletrophoresis force induced by the electric field gradient in the waved
channel which consists of successive semi-circular microchannels with alternative
directions. Particles that are continuously transported through the waved channel by
the electrokinetic effect are gradually directed toward the central region of the
microchannel. The effects of parameters, including applied electric field, particle size
and medium concentration, on the focusing efficiency were investigated
experimentally and numerically. The results indicated that the focusing performance
increases with a rise in the applied electric field, particle size, and medium
concentration.

The advantages of the microfluidic device proposed herein are obvious: (1) the
induced inhomogeneous electric fields covers the whole waved microchannel,
resulting in a decrease in the total length of the curved section and the applied electric field necessary for the focusing performance required; (2) the regions of stagnation due to the sharp turns are eliminated, and the particles are focused along the centreline of the channel, which reduces the possibility of them adhering on the wall of the channel; (3) the focusing performance of particles flowing through the waved channel is controllable by adjusting the applied electric field or the concentration of the suspending medium. Furthermore, the waved channel (i.e. its length, width, radius, and configuration) can be optimised according to the requirement of various applications; (4) without external pressure pumping, in-channel microelectrodes and insulating obstacles, the proposed microfluidic device is simple in terms of operation and fabrication, and enables any surface fouling and chemical reaction to be controlled better; (5) this design eliminates locally amplified electric field, and induced large shear stress and Joule heating, indicating that it has a great potential for biological applications, such as manipulating cells. The waved channel proposed for continuous particle and cell focusing is anticipated to integrate different functions, such as detection, calculation, separation and analysis, into a single LOC device for widespread use in the field of biology, medicine, and chemistry.
CHAPTER 8
AN S-SHAPED MICROCHANNEL EMBEDDED WITH MULTIPLE ROUND HURDLES FOR CONTINUOUS MANIPULATION AND SEPARATION OF PARTICLES

This chapter presents a novel dielectrophoresis (DEP)-based microfluidic device incorporating round hurdles within an S-shaped microchannel for continuous manipulation and separation of microparticles. Local non-uniform electric fields are generated due to the combined effects of obstacle and curvature, which in turn induce negative DEP forces exerting onto the particle that transports electrokinetically throughout the microchannel. Experiments were conducted to demonstrate the controlled trajectories of polystyrene (PS) particles fixed in size (i.e. 10 or 15 µm), and the separation of 10 and 15 µm particles based on size by adjusting the voltages applied at the inlet and outlets. Numerical simulations were also performed to predict the trajectories the particles and they showed reasonable agreement with the experimentally observed results. Compared to other microchannel designs that make use of either obstacle or curvature individually for inhomogeneous electric fields, the microchannel developed here offers advantages such as improved controllability of particle motion, lower applied voltage required, and reduced fouling and particle adhesion, etc.

8.1 Introduction

As mentioned in Chapter 2, two main approaches have successfully utilised in insulator-based DEP microdevices to generate the required non-uniform electric fields. The first one is the use of electrically insulated obstacles embedded in straight microchannels, including posts, rectangular/triangular hurdles, ridge, oil droplet, and oil menisci. However, these obstacle-based designs have limitations such as locally amplified electric fields around obstacles, large trans-membrane voltages and shear stresses on cells, and significant induced Joule heating. Moreover, the device is prone to fouling due to particle clogging or adhesion [241]. Alternatively, curved insulating microchannels were employed for spatial non-uniformities, such sawtooth,
serpentine, circular, and spiral ones. Although this curvature-based method avoids local high-intensity electric fields, it does require a sufficiently large applied DC voltage (i.e. 600 V in the spiral design) and/or a long curved section (i.e. 5 cm in the spiral design) to perform effectively which induces complexities in terms of operation and fabrication. In addition, this device is more sensitive to contamination (i.e. particle adhesion on the channel wall), because a long channel increases the possibility of surface inhomogeneity, which could disturb the electroosmotic flow for particle transport [242].

In this chapter, we describe a novel design coupling the effects of obstacle and curvature to generate the electric field gradients required for the DEP effect, where multiple round hurdles were embedded within an S-shaped microchannel to achieve continuous particle manipulation and separation. By using hurdles with a curved channel for the spatial non-uniformities, the aforementioned adverse effects of using each approach individually (i.e. locally applied electric fields, particle clogging, and large applied voltage, etc.), have been significantly reduced. Moreover, the necessary functionality of the microfluidic device is facilitated due to an increase of approach for particle manipulation, as well as the integration of pre-focusing and post-manipulating processes. How applied voltages at the inlet and outlets affect the trajectory of particle motion was studied by manipulating 10 or 15 \( \mu \text{m} \) polystyrene (PS) particles flowing through the microchannel. Moreover, the separation function of the design was verified by simultaneously directing 10 and 15 \( \mu \text{m} \) PS particles into distinct outlets simultaneously. Both the experimental and numerical results are presented, indicating an acceptable coincidence with each other.

8.2 Theory and mechanism

As stated in Chapter 7, particles suspended in an electrically conducting liquid under the influence of an external electric field are subjected to electrophoretic, electroosmotic and dielectrophoretic effects. This combination of fluid electroosmosis (EO) and particle electrophoresis (EP) is termed electrokinetic (EK)
flow, and it results in the electrokinetic velocity of particles written as Eq. 7-1, while the induced dielectrophoretic velocity is expressed as Eq. 7-2.

Figure 8-1 Illustration of the negative dielectrophoretic separation and manipulation of particles in a curved microchannel embedded with a round hurdle. Distribution of the electric-field lines and contours of the electric-field strength ($E$) within the microchannel are shown (the darker region has a stronger electric field). Particles moving through the microchannel electrokinetically are subjected to negative DEP forces (indicated by the dark blue arrows).

Here, we utilised the effect of both obstacle and curvature to generate a local electric field gradient throughout the microchannel, which contributes to a novel technique for the continuous control of particle movement in a microfluidic chip by the DEP effect. The mechanism of the proposed design is illustrated schematically in Fig. 8-1, and it shows a semi-circular microchannel embedded with a round hurdle along with the electric field lines (or equivalently the streamlines with black arrows indicating the direction) and contours of the strength of the electric field (the darker the stronger). The overall distribution of the electric field is as follows: a relatively weaker and slightly non-uniform electric field is generated in the width direction of the curved channel with uniform cross sections; while a stronger and highly non-uniform electric field is created near the edge of the hurdle (the electric field obtained maximum value at the edge of the hurdle and decayed as distance away from the hurdle increased). If we consider a particle subjected to a negative DEP effect passing though the microchannel under the combined effect of EOF and EP, the repulsive DEP forces (dark blue arrows, which are relatively weak in the curved section and strong in the constricted region) are exerted on the particle all along its movement.
Incorporating hurdles with a curved channel is a novel design and can be advantageous because these two methods are integrated to generate electric field non-uniformities (or DEP forces) to control the motion of particles in an insulator-based DEP device: (1) curved channels could generate an electric field gradient across the streamlines due to variations in the length of the path of electric current; (2) embedding hurdles from the sidewall concentrates the electrical field in the gap formed between the hurdle and the wall of the channel, which increase the electric field and field gradient near the edge of the hurdle. As mentioned above, spatial non-uniformity induced by either obstacles or curvature has its own limitations. However, the optimal design of a curved microchannel with embedded hurdles that makes the best use of both approaches for an electric field gradient could achieve greater control over particle motion.

8.3 Materials and methods

8.3.1 Microchannel fabrication and layout

Figure 8-2 Photograph of the DEP-based microfluidic chip for continuous manipulation and separation of microparticles. The inset indicates the structure and dimensions of the design.

The polydimethylsiloxane (PDMS) microfluidic channel was fabricated using standard soft-lithography technique, the details of which can be found in Chapter 7. As shown in Fig. 8-2, the microfluidic chip consist of two semi-circular channels
integrated with three round hurdles from the inner wall, and with one inlet (A) and
two outlet (B and C) reservoirs, and three straight connecting microchannels. Both
semi-circular channels are 300 µm wide, which have a large curvature of 600 µm,
while a small curvature of 300 µm. The round hurdles in the first and second curved
channel have radius of, respectively, 150 µm and 170 µm, which create 40 µm and
60 µm-wide gaps between the hurdle and the outer wall, respectively. All three
straight connecting channels are 300 µm wide and 1 mm long, and the microchannel
is 40 µm deep. All three reservoirs at the inlet and outlets are 3 mm high and 6 mm
in diameter.

![Figure 8-3 A schematic diagram of the S-shaped microchannel embedded with
multiple round hurdles.](image)

As indicated in Fig. 8-3, the proposed microchannel consists of two units and takes
advantage of curvature and hurdle for continuous particle manipulation and
separation. The first (or clockwise) unit connects to an inlet, while the second (or
counter-clockwise) unit connects to two outlets with an intersecting angle of 90
degrees. Both units incorporate a semi-circular microchannel with three round
hurdles on the inner wall. Multiple round hurdles were chosen to enhance the DEP
effect and improve particle manipulation and separation [189]. Moreover, Kang et al
[193] stated in their previous work that the incoming position of particles could
affect the particle trajectory shift after passing the block, which is why the first
curved channel with embedded round hurdles was designed to pre-focus particles
close to the hurdle within the second curved unit for subsequent manipulation, which increases the efficiency of switching and sorting particles.

Once particles have moved into the first curved unit, they experience negative DEP forces and will be focused into a squeezed stream in the gap region between the hurdle and outer wall. When they enter into the second curved unit, particles that pre-confined to the edge of the hurdle were still subjected to a negative DEP effect, but in the opposite direction due to the alternating inner and outer walls between the two semi-circular microchannels. Under the effect of repulsive negative DEP forces, particles will be deflected away from the edge of the hurdle, and finally moved out of the microchannel in a focused stream. According to Eq. 2-3, different applied voltages (or electric field gradient) will cause different DEP forces for particle deflection, while the deviation in their trajectory due to a repulsive DEP force will be different for particles of varied sizes and hence they can be separated into distinct outlets. Based on this principle, the proposed design was studied by continuously manipulating and sorting particles under different applied voltages at the inlet and outlets.

8.3.2 Microparticle preparation

In the experiment, we used two types of particles of different sizes (Fluospheres, Invotrogen, CA, USA): yellow-green fluorescent 10 µm and blue fluorescent 15 µm polystyrene (PS) microspheres. These particles were selected because their sizes are comparable to red and white blood cells. Both particles originally suspended in 0.15 M NaCl solution were diluted by deionized (DI) water 15 times. For separation experiment, the diluted 10 and 15 µm particle solutions were mixed at a volume ratio of 1:1. The particle solutions were gently vibrated before being introduced into the inlet reservoir with a pipette. Meanwhile, the outlet reservoirs were filled with the corresponding working solution, and the level of liquid in each reservoir was carefully balanced before the DC voltages were applied.

8.3.3 Experimental setup
The electric field was generated by a DC power supply (SL10P300/200, Spellman High Voltage Electronics Corp., Hauppauge, NY), and applied via three platinum (Pt) electrodes submerged in the inlet and outlet reservoirs. The motion of the particles was monitored and recorded by an inverted microscope (Olympus IX71, Tokyo, Japan) equipped with a CCD camera (DP 70, Olympus, Tokyo, Japan), and a computer having Olympus DP controller image software. The camera was run in the video mode at a speed of 15 frames per second and the digital images acquired had a resolution of 680 x 512 pixels. All the videos and images were post-processed by MATLAB (Mathworks Inc., Natick, MA), and images of the particle trajectory were obtained by superimposing consecutive images converted from videos.

8.3.4 Numerical modelling

To predict the trajectories of particle motion throughout the developed microchannel, we used a two-dimensional (2D) numerical model described in Chapter 7. Briefly, a correction factor, \( c \), was intruded to account for the effects of particle size, and particle-particle interaction, etc., on the dielectrophoretic velocity. In the simulation, the electrokinetic mobility, \( \mu_{\text{EK}} \), and the dielectrophoretic mobility, \( \mu_{\text{DEP}} \), were calculated by Eqns. 7-1 and 7-2, respectively. The zeta potential values of the PS particles and channel wall in a solution of 10 mM NaCl were set at -33 and -54 mV, respectively [238, 239]. The dynamic viscosity, \( \eta = 0.9 \times 10^{-3} \text{ kg/(m}\cdot\text{s}) \), and permittivity, \( \varepsilon_m = 6.9 \times 10^{-10} \text{ C/(}\nu\cdot\text{m}) \) for pure water at 25°C were also used. The CM factor, \( f_{\text{CM}} \), was set at -0.5, because the electric conductivity of polystyrene particles in a DC electric field is much smaller than the suspending medium (i.e. 10 mM NaCl solution) used in the experiments. The correction factor, \( c \), was obtained by matching the simulated particle trajectories to the superimposed experimental results in the same conditions, which has already been shown to decrease with the rise in particle size and depend on the geometry of the channel [193, 200, 209].

8.4 Results and discussion

As mentioned above, the electrophoretic, electroosmotic, and dielectrophoretic effects which are combined to determine particle motion depend on parameters such
as the electric field and particle size. Since the electric field in the microchannel is obtained by applying voltages through electrodes placed in the inlet and outlet reservoirs, experiments and numerical simulations were conducted to study how the applied voltage affects the trajectory of particle motion. Moreover, the separating function of the developed microchannel was examined by sorting particles (i.e. 10 and 15 µm PS particles) according to their difference in size.

8.4.1 Continuous manipulation of particles

8.4.1.1 Effect of applied voltage at the inlet

Figure 8-4 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage at inlet A on the motion of 10 µm particle at (a1-a3) entry, (b1-b3) centre and (c1-c3, d1-d3) exit region. The applied voltage at the inlet A was increased from 140 V (third row) to
340 V (bottom row), while outlet B was grounded and applied voltage at outlet C was fixed at 15 V in both cases. The red arrows indicate the direction of flow.

Figure 8-4 shows the comparison between the experimentally observed (left column: snapshot; middle column: superimposed) images and numerically predicted (right column) results of the movement of 10 µm particles under varied inlet voltages. As shown in Figs. 8-4a1, a2, particles in the entry region were uniformly distributed and covered almost the entire width of the channel. In the first curved unit, particles that were not initially focused were confined in the gap regions, and forced to move closer to the hurdle region in the second curved unit (see Figs. 8-4b1, b2). The trajectories of the particle at the exit region are presented in Figs. 8-4c and 8-4d: at a lower inlet voltage (c: 140 V), all the particles were moved out of the microchannel from outlet B, while the particles were directed to a confined stream in outlet C at a higher applied voltage (d: 340 V). Moreover, by comparing the width of the particle stream within the outlet in the experiment (the measured widths were 145 and 74 µm for inlet voltages of 140 and 340 V, respectively), it could be found that the 10 µm particles were focused better at a higher applied voltage, which agrees with the findings in chapter 7 that the focusing increased with the rise in applied electric field. Since the DEP force depends on the gradient of the square of the electric field (see Eq. 2-3), particles with fixed sizes will experience a larger repulsive DEP effect and tend to be deflected further away from the hurdle edge at a larger inlet voltage. The right column of Fig. 8-4 represents the numerically predicted trajectories of 10 µm particles, which showed a reasonable agreement with the experimentally observed ones when the correction factor was set at 0.5. These results indicated that particles could be focused and directed to distinct outlets depending on the inlet voltage.
8.4.1.2 Effect of applied voltage at the outlet

Besides the effect of inlet voltage, we also studied experimentally and numerically how applied voltages at the outlets affect the movement of 10 µm particles. Figure 8-5 shows the trajectory of the particles at the exit region, when the applied voltages at outlet B was (a1, a2) 15 and (b1, b2) 50 V, respectively, while the applied voltages at inlet and outlet C were fixed at 180 V and 0 V, respectively. In both cases, particles were observed to move in outlet C, but the particle stream was close to the left-side wall (a1, a2) and almost at the centre of the channel (b1, b2) with applied voltages of 15 and 50 V, respectively, indicating that particles could be repelled further toward the right-side wall of the outlet C by increasing the applied voltage at outlet B. Moreover, the particles moved in a narrower stream at higher applied voltage (the measured widths of the stream were, respectively, 117 and 65 µm for the voltage of 15 and 50 V), indicating that focusing could increase with the rise of applied voltage at outlet B.
Figure 8-6 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage at outlet C on the motion of 10 µm particles: (a1-a3) 15 V and (b1-b3) 50 V. In both cases, the applied voltages at inlet A and outlet B were fixed at 140 and 0 V, respectively.

Additionally, different shifts in the trajectory can also be achieved by varying applied voltage at outlet C. As shown in Figure 8-6, by increasing the voltage at outlet C from (a1, a2) 15 V to (b1, b2) 50 V, 10 µm particles were not only shifted towards the left-side channel wall of outlet B, but also focused into a tighter stream. By comparing Fig. 8-5a2 with Fig. 8-6a2, and Fig. 8-5b2 with Fig. 8-6b2, we could conclude that particles would be directed to distinct outlets by switching the applied voltages at the outlets, because the outlet voltages would determine the flow streams at the bifurcation, and hence direct particle movement after passing the curved section, this has also been stated in previous work by Kang et al [188]. Moreover, a larger applied voltage causes a higher electric field non-uniformity, which generates larger DEP forces for particle deflection in both constricted and curved regions. The numerically predicted trajectories for 10 µm particles at the exit are presented in the right column of Figs. 8-5 and 8-6, and seem to match the experimental results closely when the correction factor is fixed at 0.5.
Figure 8-7 Experimental (left column: snapshot; middle column: superimposed) and numerical (right column) demonstration of the effect of applied voltage on the motion of 15 µm particles. The applied voltage to inlet A and outlet C were, respectively: (a1-a3) 90 and 10 V; (b1-b3) 180 and 10 V; (c1-c3) 180 and 30 V. The outlet B was grounded in all cases.

In a similar analysis, we studied experimentally and numerically how the applied voltages at inlet and outlets affected the movement of 15 µm particles. Figure 8-7 represents the experimentally observed (left column: snapshot; middle column: superimposed images) trajectories of 15 µm particles at the exit of the microchannel under different combinations of inlet and outlet voltages: (a1, a2) when applied voltages at inlet A, outlet B, and outlet C were, respectively, 90, 0, and 10 V, the particles moved out of the microchannel in a confined stream from outlet B; (b1, b2) by increasing the inlet voltage to 180 V but retaining the outlet voltages, the particles were pushed further away from the round hurdles and directed to outlet C in a narrower stream due to larger repulsive DEP force; (c1, c2) by increasing the outlet C voltage to 30 V, but fixing outlet B to be grounded, particles were diverted to move from outlet B again due to the re-distributed flow at the bifurcation. By
comparing Fig. 8-7a2 with Fig. 8-7b2, and Fig. 8-7b2 with Fig. 8-7c2, it can be found that both applied voltages at the inlet and outlets could affect particle trajectory, and 15 µm particles can be directed into either outlet B or outlet C depending on the inlet and outlet voltages. In addition, with an increase in the inlet and/or outlet voltage, 15 µm particles were focused better (i.e. forced into a narrower stream), which corresponds to our previous finding in Chapter 7 that particle focusing could be improved by increasing applied electric field. By setting the correction factor at 0.4 for 15 µm particles, the numerically predicted results (right column) show a good agreement with the experimentally obtained superimposed images. All these results indicated that the location and trajectory of particles within the microchannel could be reasonably controlled by adjusting the applied voltages at the inlet and outlets. These actions demonstrated the integrated focusing-switching functionality of the proposed design.

8.4.2 Continuous size-dependent separation of particles

Figure 8-8 Continuous separation of 10 and 15 µm particles in an S-shaped microchannel embedded with multiple round hurdles, when the applied voltages at
inlet A and outlet C were 180 and 18 V, respectively, and outlet B was grounded. Both the experimental (left column: snapshot; middle column: superimposed) and numerical (right column) results of particle motion in the (a1-a3) entry, (b1-b3) centre, and (c1-c3) exit region are presented. The red arrows indicate the direction of flow.

According to Eq. 2-3, the DEP force is proportional to the cube of the particle radius, and therefore, larger particles are subjected to larger DEP forces, and tend to be deflected further away from the edge of the hurdle and the sidewall compared to smaller ones. This mechanism is utilised in the proposed microchannel to continuously separate particles based on size. A typical case of separating mixed 10 µm (bright) and 15 µm (dark) PS particles with applied voltages at inlet A, outlet B and outlet C of 180, 0, and 18 V, respectively, is shown in Fig. 8-8. Here the electric fields within the microchannel were numerically computed using COMSOL. The highest electric field occurred at the top edge of the hurdle in the first semi-circle and was around 500 V/cm, and the average value across the entire microchannel and was around 130 V/cm. Initially, a mixture of particles was introduced and it covered almost the whole width of the inlet microchannel (see Fig. 8-8a1, a2). Two types of particles were focused into a single stream in the gap in the first curved unit, which lead to a confined stream of particles close to the edge of the hurdle before entering the second curved unit for subsequent separation (see Fig. 8-8b1, b2). As shown in Fig. 8-8c1, c2, there were two distinct streams of particles in the exit region, as the 10 and 15 µm particles were sorted and moved in a focused stream from outlet B and outlet C, respectively. The overall process was also reasonably predicted by numerical simulation (see right column of Fig. 8-8), where the yellow and blue lines represent the trajectories of 10 and 15 µm particles, respectively, and the correction factors were fixed at 0.5 and 0.4, respectively, for the 10 and 15 µm particles.
In order to test the efficiency of this separation, the numbers of each type of particle (i.e. bright 10 and dark 15 µm PS particles) in both outlet reservoirs were counted. Three discrete regions within each reservoir were selected and a total of over 300 beads were counted. The separating efficiency was calculated by $n_r/n_t \times 100\%$ or $(1-n_w/n_t)\times 100\%$, where $n_t$ is the total number of particles counted, and $n_r$ and $n_w$ are the numbers of particles flowing into the right and wrong outlet, respectively. Figure 8-9 shows the percentages of two populations of particles in the reservoirs of both outlet B and outlet C, indicating a separating efficiency of above 95%. With a proper selection of inlet and outlet voltages, the effective and continuous separation of particles according to size was successfully achieved, and little particle fouling was observed during the experiment.

8.5 Conclusions

In this chapter, an S-shaped microchannel embedded with multiple round hurdles for continuous manipulation and separation of microparticles using negative DC DEP was presented. In such design, the effect of obstacle and curvature were combined to induce non-uniform electric fields, which contributes to a novel technique for the continuous control of particle movement in DEP-based microfluidic devices. Both the experimental and numerical results indicated that by adjusting applied voltages at
the inlet and outlets, microparticles can be directed into distinct outlets, positioned at different locations along the width of the outlet, focused into streams of various widths, and separated according to their size.

Since the design utilised both obstacle and curvature to generate spatial non-uniformities, there are more parameters (i.e. the width of the gap, and configuration of the hurdle and curved channel, etc.) which can be optimised according to the requirement of various applications, and the controllability of particle motion has increased compared to those devices taking advantage of only obstacle or curvature. More importantly, the problems that occur in a device that utilizes either obstacle or the curvature effect individually for electric field gradients, including locally amplified electric fields, adverse effects on cell viability, particle clogging or adhesion, and large applied voltage, are greatly relieved, and thus enable the performance of the device to be improved. Furthermore, in the present design, the upstream curved unit, a semi-circular microchannel combined with round hurdles, serves as a pre-treatment component for driving particles into a confined stream close to the hurdle within the downstream curved unit, which facilitates the subsequent process of manipulating and separating particles. Due to these aforementioned benefits, the novel design with its demonstrated integrated ability to focus, switch and sort microparticles has great potential of being used in LOC or μTAS devices for biological, chemical, and medical applications.
CHAPTER 9
CONCLUSIONS AND FUTURE WORK

9.1 Conclusions

The ability to effectively and accurately manipulate and separate particles are essential requirement for a variety of biological, chemical and medical applications. Microfluidic-based lab-on-a-chip devices provide excellent platforms for handling and analysing biological and chemical samples, because they have numerous advantages such as increased portability, low costs, high resolution and sensitivity, and a short time for analysis. Different techniques have been developed for manipulating and separating particles within microfluidic devices, among which dielectrophoresis is a non-invasive, non-destructive, and label-free methodology. Dielectrophoresis (DEP) refers to the motion of a particle in an inhomogeneous electric field with the demonstrated capability of focusing, trapping, sorting, patterning, and characterising different particles (i.e. cells, viruses, bacteria, DNA and protein). Although various electrode- and insulator-based DEP microdevices have been developed, more research effort is still needed for micro-fabrication techniques of 3D microelectrode structures, and integrated dielectrophoretic microsystems with improved performance. The overall objective of the work is to develop methods of manufacture and DEP-based microdevices to manipulate and separate particles. The results of the work are summarised as follows:

9.1.1 Methods of micro-fabrication

A novel method to fabricate dielectrophoretic microdevices with top-bottom patterned microelectrodes using a laser-patterned PDMS layer has been developed (Chapter 3). With this method, the transferred PDMS layer is defined and machined using a direct-write, mask-free laser ablation technique, and then transferred, aligned and bonded to substrates patterned with electrodes to form enclosed microfluidic channels. With this method, the needs for a template and corresponding fabrication processes, facilities, and consumables in a clean-room are eliminated. The fabricated chip was able to focus and trap polystyrene particles dielectrophoretically, thus
demonstrating the capability of the proposed method. The advantages of this method involve a simple and fast fabrication process, low cost, easy integration of electronics, strong bonding strength, and chemical and biological compatibility, etc.

This simple and cost-effective fabrication method is expected to be widely used to manufacture integrated multilayer DEP-based microfluidic devices with complicated 3D microelectrode structures, and integrated electronic-microfluidic devices with advanced functionalities. Another method for fabricating DEP-based microdevices with arc-shaped extruded microelectrodes on the sidewall of the microchannel using metal alloy microspheres was proposed (Chapter 4). The overall fabrication process involves manufacturing SU-8 mould, patterning planar microelectrodes, microsphere placement, plasma bonding, and thermal post-treatment. Switching and sorting polystyrene particles and yeast cells was achieved by the fabricated dielectrophoretic chip with two pairs of sidewall-patterned arc-shaped microelectrodes. This method has many advantages such as good conductivity, simplicity, low cost, and improving the design of the topological electrode. In addition, this presented technique could also be used to construct sidewall-patterned 3D electrodes of different sizes and geometries using metal alloys of various shapes, i.e. cylindrical or asymmetrical.

9.1.2 Electrode-based DEP microdevices

Two DEP-based microdevices with 3D microelectrode arrays placed on both the bottom floor and top ceiling of the microchannel were designed, fabricated, and tested. One 3D microelectrode structure consists of a funnel-shaped focusing unit, a parallel aligning unit, and a crescent-shaped trapping unit in series (Chapter 5). Its integrated functionalities as concentration of particles in a continuous flow, and separation of particle mixtures according to size and dielectric properties were demonstrated by concentrating PS particles and yeast cells, separating different-sized PS particles and isolating 5 µm PS particles from the yeast cells. This proposed microdevice has various advantages, including multi-functionality, improved manipulation efficiency and throughput, easy fabrication and operation, etc. The other microdevice has parallel multiple arrowhead-shaped microelectrode arrays, and 13 independent microchannels arranged in a radial direction, to manipulate particles in a high-throughput manner (Chapter 6). Combined hydrodynamic, electrokinetic
and dielectrophoretic forces, and both AC and DC signals were utilised for the performance. Its functionalities were demonstrated by capturing polystyrene particles, collecting-releasing yeast cells and *E. coli*, and separating live and dead yeast cells. Based on the parallel operation and elimination of bulky mechanical pumps, this proposed microdevice has wide applications for in-situ sample preparation, detection, and analysis.

9.1.3 Insulator-based DEP microdevices

Two insulator-based dielectrophoretic microdevices were developed. One is a waved microchannel consisting of repetitive S-shaped microchannels in series to focus particles along the centreline of the microchannel (Chapter 7). When particles move electrokinetically through the channel, the negative DEP force will gradually direct the particles towards the centre of the channel, and focused them into a continuous-flow stream along the centreline of the channel. Compared to the serpentine channel with 90° turns, *this design eliminates the regions of stagnation and locally amplified electric field due to sharp turns*, moreover, *the magnitude of the applied DC fields and the total length of curved channel required for desirable deflection are reduced* compared to spiral channels. Its functionality was studied experimentally and numerically using different-sized polystyrene particles and yeast cells, with the results indicating that the focusing improved with a rise in the applied electric field, particle size and medium concentration. The other device is an S-shaped microchannel incorporated with multiple round hurdles for manipulating and separating particles (Chapter 8). *The effects of obstacle and curvature are combined to generate local electric field gradients throughout the microchannel*, which contribute to a novel technique for controlling particle motion dielectrophoretically in insulator-based microdevices. This design eliminates the adverse effects of using either approach individually, while increases the controllability of particle motion. Both the experimental and numerical results indicated that by adjusting the applied voltages at the inlet and outlets, microparticles can be directed into distinct outlets, positioned at different locations along the width of the outlet, focused into streams of various widths, and separated according to size. It is anticipated that both designs
would integrate different components into a single LOC or µTAS device for wide biological, chemical and medical applications.

Specially, my major contribution to this work involves vast majority of a fabrication method for DEP devices with top-bottom patterned microelectrodes (Ch. 3), development of an integrated microelectrode array (Ch. 5), a waved microchannel (Ch. 7), a curved microchannel embedded with multiple round hurdles (Ch. 8), while the fabrication method for DEP devices with sidewall-patterned microelectrodes (Ch. 4), and development of parallel multiple electrode arrays (Ch. 6) are the outcome of a joint research undertaken collaboratively.

9.2 Future work

This thesis describes the development of micro-fabrication methods and DEP-based microdevices to manipulate and separate particles. The application of the methods and functionalities of these microdevices have been demonstrated. Based on the results achieved in this study, further research work would focus on the following aspects:

9.2.1 Fabrication techniques

DEP-based devices with microelectrodes embedded within microchannels offer several benefits. For example, the Joule heating effect is avoided due to weak electric field strength, and the microdevice is more compatible with integrated circuits and is also suitable for on-site analysis and characterisation. However, micro-fabricating the interior metal microelectrodes require relatively complex and expensive manufacturing techniques, such as photolithography, thin-film deposition (i.e. evaporation and sputtering) procedures that are not suitable for massive production. It therefore makes sense to invent alternative technologies for fabricating microelectrodes inside microchannels. Conductive polymer-based materials provide a solution to this issue, and could be used as electrodes and built in microfluidic devices by milling, drilling, and moulding techniques. Novel micro-fabrication
approaches for dielectrophoretic microdevices with conductive polymer-based electrodes within microchannel systems may make inexpensive and mass fabrication.

9.2.2 Device design and optimisation

Although a variety of dielectrophoretic microsystems have been developed over the past decades, further study of their design or optimisation should be carried out. With electrode-based DEP microdevices, the structure and configuration of the microelectrodes determines the distribution of the electric field and hence the DEP force. The development of integrated, intelligent, and advanced microelectrode arrays will achieve greater control of particle motion. In insulator-based DEP microdevices, as the applied field is confined within the non-conducting channel walls, local electric fields are defined by the morphology of the channel. Therefore, optimising or designing insulating microchannel structures with controllable localised electric fields is needed to increase the performance of DEP-based microsystems. Moreover, although the microdevices for DEP-based manipulation are simple, the instruments required to operate the system are still bulky and complex, and the throughput of DEP-based is relatively low compared to traditional techniques. DEP-based microdevices could be integrated with different functionalities (i.e. detection, calculation, separation, and concentration), and different components (i.e. pumps, mixers, valves) into a single all-in-one chip. Both electrode-and insulator-based DEP microdevices hence will be developed to improve their efficiency, throughput, integration, and portability.

9.2.3 Applications

At present, our proposed microsystems are mostly utilised for manipulate polystyrene microparticles, so it would be valuable to extend the work to biological species. However, polarisation mechanisms for different bio-particles must first be understood, and extreme care must be taken to consider the proper DEP model and experimental conditions when applying DEP to biological systems and components, because bio-particles vary greatly in shape, size, membrane and wall properties, organelle composition, each of which results in different electrical properties. In addition, the viability and functionality of bio-particles should not be affected by
being exposed to electric fields. Several prospective applications of the DEP technique involve: manipulation of micro- or nano-scale bio-particles such as cells, bacteria, viruses, protein and DNA, etc.; single-cell analysis (SCA) for investigating and elucidating cellular functions; conducting complicated biochemical assays and reactions; and drug delivery. Therefore, the applications of proposed DEP-based microfluidic devices will be explored by addressing needs in the biological, chemical, and medical fields.

In conclusion, further work towards the invention of micro-fabrication techniques and advanced DEP-based microdevices is required to achieve real LOC systems for sample analysis and characterisation. DEP-based microsystems will be further improved in terms of manufacturing and performance, and used in ever wider applications.
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170


